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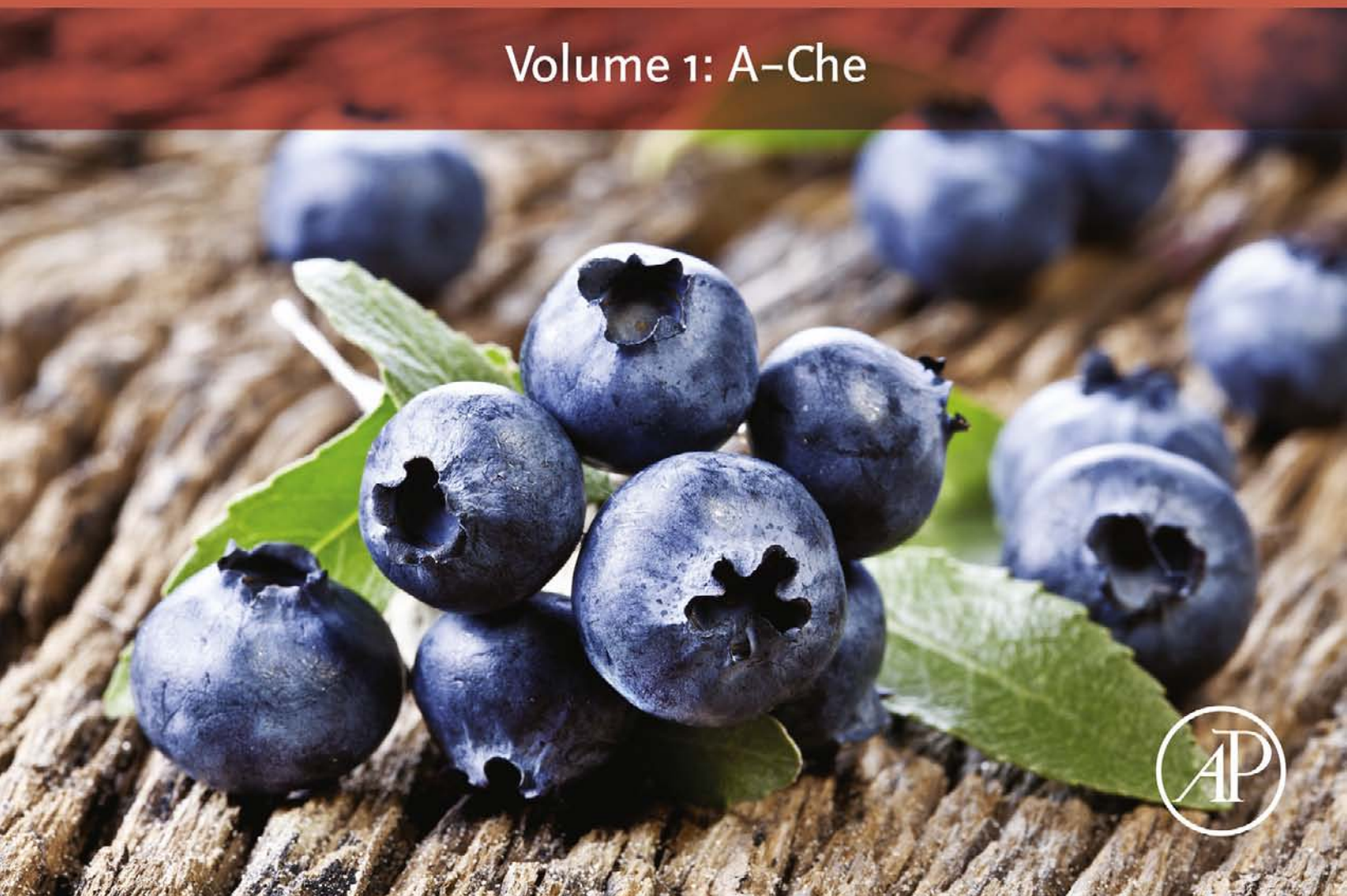

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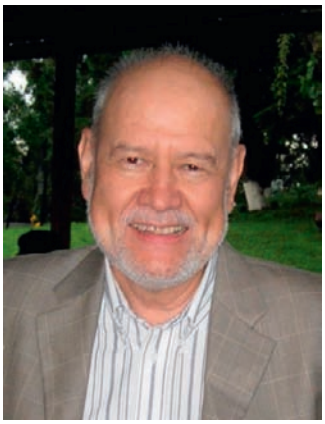


Benjamin Caballero is professor of International Health and of Maternal and Child Health (Bloomberg School of Public Health), and professor of pediatrics (School of Medicine) at Johns Hopkins University.

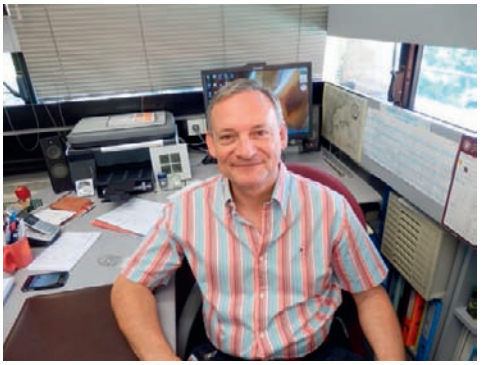
He obtained his MD from the University of Buenos Aires, his MSc in biochemistry from the University of San Carlos, and his PhD in neuroendocrine regulation from MIT, in Cambridge, MA. He started his academic career as assistant professor of pediatrics at Harvard Medical School and director of the Nutrition Unit of Boston Children's Hospital, and subsequently became the founding director of the Center for Human Nutrition at Johns Hopkins University, in Baltimore.

Prof. Caballero has focused his research on child nutrition and health in developing countries. In particular, he has explored the combination of undernutrition and overweight that has become increasingly prevalent in low- and middle-income countries. He was a member of the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences, USA, and of a number of expert panels created by the Institute, including the Dietary Reference Intakes (DRI) Committee, the Expert Panel on Macronutrient Requirements, and the Childhood Obesity Task Force. He was also a member of the Dietary Guidelines for Americans Advisory Committee, of the Scientific Advisory Board of the Food and Drug Administration (FDA), and of a number of advisory committees of the National Institutes of Health (USA).

He is the editor-in-chief of the *Encyclopedia of Food Sciences and Nutrition*, a 10-volume work on food production, consumption and biological effects. He is also editor-in-chief of the *Encyclopedia of Human Nutrition*, which received the Book of the Year Award from the British Medical Association. His *Guide to Dietary Supplements* summarizes the current scientific basis for the use of mineral and vitamin supplements. His book *The Nutrition Transition: Diet and Disease in the Developing World* explored the impact of demographic and economic development on diet- and lifestyle-related diseases in developing countries. His book *Obesity in China* summarizes research conducted in rural and urban China to track the impact of socioeconomic development on health outcomes. He is also coeditor of a widely used textbook on human nutrition, *Modern Nutrition in Health and Disease*.

He is a member of the Spanish Academy of Nutritional Sciences, and a Fellow of the American Society for Nutrition and of the Royal Society of Medicine (UK). Recent awards include the Donald Medearis Lectureship from the Massachusetts General Hospital/Harvard Medical School, the Mataix Prize for lifetime achievements in nutrition science from the Spanish Academy of Nutritional Sciences, the Ancel Keys Prize for achievements in international public health, and the Thompson–Beaudette Lectureship from Rutgers University.

Paul Finglas joined the Institute of Food Research in 1981 and is currently head of the Food Databanks National Platform and Research Leader in Food and Health at the Institute (http://www.ifr.ac.uk/science/platform/FD/default.html). He has, for most of his science career, been involved in a wide range of research in food composition and analysis, and the nutritional effects of micronutrients in food and health research. Paul has considerable experience of co-coordinating both national and international projects (e.g., EuroFIR, TDS-EXPOSURE, Bacchus and QualiFY (all EU FP7), and is currently of the spin-out EuroFIR AISBL, a non-profit international association based in Belgium, from one of these projects. Paul has a broad range of experience in science publishing and is currently editor of the journals *Food Chemistry* and *Trends in Food Science and Technology*, and was one of the coeditors for the *Encyclopedia of Food Science and Nutrition* (2nd Ed.). Paul has a degree in chemistry from Aston University in Birmingham and has published over 150 publications on a wide range of topics in food science and nutrition.



Fidel Toldrá holds a BSc in chemistry (1980), high degree on food technology (1981) and PhD in chemistry from the University of Valencia (1984). Professor Toldrá was a Fulbright postdoctoral scholar at Purdue University in West Lafayette (US, 1985–86) and visiting scientist at the University of Wisconsin-Madison (1991 and 1995), and the Institute of Food Research-Bristol (UK, 1987). Currently, he is research professor at the Instituto de Agroquímica y Tecnología de Alimentos (CSIC), in Paterna, Valencia (Spain). He is also associate professor of food technology at the Polytechnical University of Valencia.

Prof. Toldrá has focused his research on food biochemistry and its relationship with nutrition, quality and safety. He has filed 12 patents, directed 22 PhD thesis and published over 245 manuscripts in recognized scientific journals and more than 115 chapters of books. His h-index is 41. Prof. Toldrá has authored two books and edited/co-edited more than 30 books for major publishers like CRC Press, Wiley-Blackwell, Elsevier and Springer.

Prof. Toldrá is the European editor of *Trends in Food Science and Technology* (2005–) and associate editor of *Meat Science* (2014–); he was the editor-in-chief of *Current Nutrition & Food Science* (2005–2012), section editor of the *Journal of Muscle Foods* (2009–2010) and guest editor of 12 special journals issues. He is a member of the editorial boards of *Food Chemistry*, *Food Analytical Methods*, *Journal of Food Engineering*, *Journal of Food and Nutrition Research*, *The Open Nutrition Journal*, *The Open Enzyme Inhibition Journal*, *Recent Patents in Agriculture*, *Food and Nutrition*, *Food Science & Nutrition* and *Current Opinion in Food Science*.

He has been a member of the Scientific Panel on food additives, flavorings, processing aids and materials in contact with foods (periods 2003–2008) and the Scientific Panel on flavorings, enzymes, processing aids and materials in contact with foods (2008–2015) of the European Food Safety Authority (EFSA) acting as Chairman of the Working groups on Irradiation (2009–2010), Processing Aids (2011–2014) and Enzymes (2010–2015). He was a member of FAO/WHO group of experts to evaluate chlorine-based disinfectants in the processing of foods (2008–2009). He was a member of the Executive Committee of the European Federation of Food Science and Technology (EFFOST, 2002–2009). He is a Fellow of the International Academy of Food Science and Technology (IAFOST, 2008) and of the Institute of Food Technologists (IFT, 2009–). He received the Iber Award on Food and Cardiovascular Diseases (1992), the Institute Danone award in Food, Nutrition and Health (2001), the International Prize for Meat Science and Technology from the International Meat Secretariat (2002), GEA award on R+D activity from the Valencian Community (2002), and the Distinguished Research Award (2010) and Meat Processing Award (2014), both from the American Meat Science Association.

EDITORIAL ADVISORY BOARD

Siân Astley has worked extensively with individuals and organizations throughout Europe from a variety of disciplines including research, food and biotech industries, and the media. She is the author of more than 300 popular science articles for magazines and trade publications as well as 25 peer-reviewed papers, and she was awarded her Diploma in Science Communication in 2009 (Birkbeck University of London). After 14 years as a bench-scientist, Siân became communications manager for NuGO, one of the first FP6 networks of excellence, and was the European communications manager for the Institute of Food Research in Norwich (UK) until April 2012. Currently, she is the training and communications manager for the European Food Information Resource (EuroFIR AISBL) supporting training within EU-funded research projects and networks, and communication of research activities.

David J. Baer is a supervisory research physiologist with the US Department of Agriculture's Beltsville Human Nutrition Research Center located in Beltsville, Maryland. He serves as the research leader for the Center's Food Components and Health Laboratory and also serves as the director of the Center's Human Studies Facility.

Dr. Baer conducts controlled dietary intervention studies to investigate the relationship between diet and the risk for chronic degenerative diseases, especially cardiovascular disease, cancer, and diabetes in people. His research also includes studies on the health impacts of weight gain and determining the calorie content of foods. Some of the dietary interventions he has investigated include the effects of different types of protein, fats and fatty acids, fiber, margarine, butter, plant sterols, salad dressings, nuts, whole grains, berries, alcohol, and tea on overall nutrition and health. In addition to dietary intervention studies, Dr. Baer is involved in research studies to validate food survey methodologies and in developing new methods for dietary assessment.

Dr. Baer earned his bachelor's degree from the University of Illinois and his master's and doctorate in nutrition from Michigan State University.

Marina Carcea was awarded a master degree in agricultural sciences at the University of Pisa, Italy "cum laude" in 1980, and a PhD in food science also "cum laude."

She is currently a senior researcher in the Research Center on Food and Nutrition of the Council for research in agriculture and analysis of agricultural economy (CRA-NUT formerly INRAN National Research Institute on Food and Nutrition) and she was the director of the Cereals Research Programme in INRAN. CRA-NUT is a primary research institute in Italy under the egis of the Ministry of Agriculture. Dr. Carcea joined INRAN in 1989 after having worked in Italian and English universities (Queen Elizabeth College, King's College, and University of London) and after a two-year contract with the Food and Agriculture Organization (FAO) of the United Nations (UN), Rome.

She has a vast experience in the field of research on foods, cereals in particular. In recent years, her main research interests have been: chemical characterization and study of the functional properties of cereal components; study of the interactions between components and of the interrelationships between the biochemical properties of components and the technological properties of the raw material and derived products; development of new, cereal-based products; development of methods to assess technological parameters of the raw material; nutritional value of cereals; and developments of protocols for quality assurance of cereals, food authenticity.

She has taken part and/or co-ordinated several research projects within national or international programs (European Commission, FAO) involving several institutions. She is the author of more than 160 scientific publications, mostly in international journals, eight book chapters, and two scientific books. She delivered lectures on her research activity at about 150 national and international congresses and she seats in several national and international committees (Italian Ministry of Agriculture, Codex Alimentarius, and European Commission) regarding food and nutrition topics. She is also a member of the editorial board of scientific journals.

From 1994 to 2006, she has also been a lecturer of food science and technology at the University of Tor Vergata, Rome, Italy.

She is a founding member of AISTEC, the Italian Association of Cereal Science and Technology. Since 1996, she is an elected member in the Executive Committee of the same association and since 2009, president of the association.

Since 2000, she is the Italian National Delegate of the International Association for Cereal Science and Technology (ICC) and she was also the president of the same association for 2011–2012.

In 2004, she was the first woman to be awarded the International Harald Perten Prize for her excellent research achievements in the field of cereal science and technology.

She is also a member of the Georgofili Academy in Florence, Italy.

Lawrence J. Cheskin graduated from Dartmouth Medical School and completed a fellowship in gastroenterology at Yale–New Haven Hospital. He is an associate professor of health, behavior, and society at the Johns Hopkins Bloomberg School of Public Health, with a joint appointment in International Health–Human Nutrition, and in medicine (GI) at the Johns Hopkins University School of Medicine. Dr. Cheskin is also a founder and director of the Johns Hopkins Weight Management Center, a comprehensive treatment program for obesity.

In his research, Dr. Cheskin has studied the effects of medications on body weight, the gastrointestinal effects of olestra, how cigarette smoking relates to dieting and body weight, and the effectiveness of lifestyle and dietary changes in weight loss and weight maintenance.

He is also the author of four books: *Losing Weight for Good*, *New Hope for People with Weight Problems*, *Better Homes and Gardens' 3 Steps to Weight Loss*, and *Healing Heartburn*. Dr. Cheskin has appeared on television news programs and lectured to both professional and lay audiences on the topics of obesity and weight control.

Nigel Cook is a graduate of the University of Dundee. After postdoctoral research in the Universities of Aberdeen and Leicester, he moved to the Central Science Laboratory (now the Food and Environment Research Agency (FERA)) at the Food Science Laboratory, Torry, Aberdeen in September 1994, before relocating to new facilities in York. At FERA, he studies the transmission of pathogens, particularly enteric viruses, through foods and the environment. He has a visiting professorship at the Katholieke Universiteit Leuven in Belgium. He is a councilor of the International Association for Food and Environmental Virology. He is a project leader within the standardization working group ISO TC34 SC9 WG6, currently developing a standard for detection of *Cryptosporidium* and *Giardia* on berry fruits and leafy green vegetables. He was a coordinator of the European Framework 7 project "Integrated monitoring and control of foodborne viruses in European food supply chains (VITAL)," and a chair of COST Action 929 "A European Network for Environmental and Food Virology" from 2006 to 2010. Between 2009 and 2014, he was a member of various European Food Safety Authority's Working Groups preparing opinions on the risk of foodborne viruses, and represented the European Communities on the Codex Committee on Food Hygiene Working Group developing *Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food*. He was a member of the UK Advisory Committee on the Microbiological Safety of Food's Viral Infections Subgroup. He was the founding editor of the journal *Food and Environmental Virology*, published by Springer.

Luca Simone Cocolin graduated in 1994 in food science with a grade of 110/110 and remark followed by food biotechnology PhD studies from 1995 to 1998. In February 1999, he defended his thesis acquiring the title of PhD in food biotechnology. From 1998 to 2001, he received a scholarship from the Friuli Venezia Giulia region (Italy). From November 1, 2001, he was an assistant professor at the University of Udine, Faculty of Agriculture, Food Science Department, Italy, and in October 1, 2006, he became an associate professor at the University of Torino, Italy. In January 2014, he had the habilitation for full professor and from June 2015, he is the full professor in food microbiology at the University of Torino.

From September 2008, he is an executive board member of the International Committee on Food Microbiology and Hygiene (ICFM) part of the International Union of Microbiological Societies (IUMS) (http://www.icfmh.org/). From January 2008, he is the editor-in-chief of the *International Journal of Food Microbiology* and he is a member of the editorial board of *Applied and Environmental Microbiology*, *Food Analytical Methods*, *Frontiers in Food Microbiology*, and *Frontiers in Nutrition and Food Science Technology*. He regularly reviews paper for *Food Microbiology*, *Meat Science*, *Journal of Applied Microbiology*, and *Letters in Applied Microbiology*. He is a co-author of more than 180 papers on national and international journals and he attended national and international congresses with oral and poster presentations. On Scopus (www.scopus.com, consulted on March 2015) he has 172 documents reviewed, which were cited 3520 times, resulting in an h index of 33.

His scientific interests comprise:

development, optimization, and application of molecular methods for the detection, quantification, and characterization of foodborne pathogens;
study of the microbial ecology of fermented foods (mainly sausage, cheese, and wine) by using culture independent and dependent methods;
bioprotection: molecular characterization of bacteriocin production and its study *in vitro* and *in situ*;
selection of new putative probiotics from artisanal fermented foods; and
study of the human microbiome and its influence on human health.

Christopher Duggan, for the past 25 years, has been performing clinical trials in the fields of pediatric nutrition, gastroenterology, and global health. His early work centered on the management of diarrheal diseases in children, including trials that demonstrated the feasibility and efficacy of oral rehydration solutions (ORS) for diarrhea management in the United States and globally. In collaboration with colleagues at Harvard TS Chan School of Public Health and Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania, Dr. Duggan and colleagues are evaluating the efficacy of micronutrient supplementation in infants and young children born to women with or at risk of HIV infection. Recent studies include the development of new biomarkers of environmental enteric dysfunction as well as the evaluation of nutritional status on neurodevelopment. With colleagues at St John's Research Institute in Bangalore, India, he is evaluating the efficacy of maternal vitamin B12 supplementation on biochemical and clinical parameters during pregnancy and infancy. He is a course co-director of the Bangalore, Boston Nutrition Collaborative (http://bbnc.globalhealth.harvard.edu). Past and present research support has come from the National Institutes of Health, the Gates Foundation, and the World Health Organization.

In addition to his global health research interests, he is a pediatric gastroenterologist and a nutrition physician at Boston Children's Hospital where he directs the Center for Nutrition (http://www.childrenshospital.org/nutrition). He is a medical director of the Center for Advanced Intestinal Rehabilitation, one of the largest centers in the United States for the care of children with intestinal failure/chronic diarrhea syndromes (http://www.childrenshospital.org/cair). He is also the course co-director of an inaugural Harvard College course "Nutrition and Global Health" and mentors undergraduate, graduate, and postdoctoral students at HMS and HSPH (http://www.hsph.harvard.edu/nutrition-and-global-health/).

He is a professor of pediatrics at Harvard Medical School and a professor in the Department of Nutrition at the Harvard TS Chan School of Public Health (http://www.hsph.harvard.edu/faculty/christopher-duggan/).

Jed William Fahey's current research concerns elucidating the mechanisms of how plants protect themselves against unfavorable and stressful conditions, and how this understanding can be translated to chemoprotection of eukaryotic mammalian systems. This work draws on elements of natural product chemistry, enzymology, nutritional epidemiology, and clinical research in order with isothiocyanates (e.g., sulforaphane) and glucosinolates. His work led to the discovery that broccoli sprouts are an exceptionally rich and consistent source of phytochemicals that induce the detoxification of carcinogens, and to the development of methods for their detection and for assessing their metabolism in humans. He discovered that one of the inducers, sulforaphane, has potent antibiotic activity against *Helicobacter pylori*, a causative agent of peptic ulcer and stomach cancer, and followed up with trials in animals and in *H. pylori*-infected humans. Ongoing collaborations examine the effects of broccoli, Moringa, and the other plants and their phytochemicals against a range of chronic diseases. Dr. Fahey has for years taught courses in chronic disease prevention and nutrition at both medical and public health schools.

Manuel Franco is an associate professor at University of Alcalá in Madrid (Spain) where he leads the social and cardiovascular epidemiology research group (http://www3.uah.es/cardiosocialepi/). He is also an adjunct professor at Johns Hopkins University (Baltimore).

Prof. Franco's work focuses on the social determinants of cardiovascular diseases and its major risk factors as diet. His methodological interests include the measurement of the urban environment and large social and economic changes in relation to cardiovascular health. He is the lead investigator of the Heart Healthy Hoods, study funded by the European Research Council, that will study the urban environment in relation to cardiovascular health in Madrid (http://hhhproject.eu/). This longitudinal study will be collecting neighborhood level data (via audits, Google Street view, photovoice, and qualitative methods) and linking them to clinical outcomes collected from patients enrolled at the City of Madrid primary healthcare clinics. Prof. Franco trained in Spain and Germany to obtain his MD and obtained his PhD from Johns Hopkins Bloomberg School of Public Health working with Dr. Ana Diez-Roux in the MESA study on food environment and dietary patterns. He has published over 30 international high impact articles and collaborates with universities in the United States, Europe, and Latin America.

Maria Glibetic is a research director of Centre of Research Excellence in nutrition research, Institute for Medical Research in Belgrade, University of Belgrade, Serbia, and member of executive board of directors for food data association EuroFIR AISBL. She is an experienced basic and nutritional scientist with over 250 scientific publications and presentations. Maria has considerable experience in leading national and international projects and since 2006, she participated in nine EU funded projects including EuroFIR, EURRECA, BaseFOOD, CHANCE, BACCHUS, and ODIN. Maria and her team are responsible for the creation of the first online national food database, for designing food data management system, and for the development of different nutritional tools for intake analysis. She was a principal leader of many nutrition intervention studies evaluating the plant bioactive component effects on human cardiovascular health. She leads postgraduate department for integrated nutritional sciences at University of Belgrade, where she teaches two courses.

Linda Harvey obtained her PhD from the University of East Anglia, UK. She is currently the head of the Human Nutrition Unit at the Institute of Food Research, Norwich, UK. Her research interests include micronutrient requirements, bioavailability, and metabolism.

Ronald Jackson received his bachelor's and master's degrees from Queen's University and doctorate from the University of Toronto. His time in Vineland, Ontario, and subsequent sabbatical at Cornell University, redirected his interest in *Botrytis* toward viticulture and enology. As part of his teaching duties at Brandon University, he developed the first wine technology course in Canada. For many years he was a technical advisor to the Manitoba Liquor Control Commission, developing sensory tests to assess candidates of its sensory panel, and was a member of its external tasting panel. He is the author of *Wine Science: Principles and Applications, 4th edition* (2014), *Wine Tasting: A Professional Handbook, 2nd edition* (2009), *Conserve Water, Drink Wine*, and chapters and technical reviews in other multiple books and encyclopedia. He is retired in Bronte, Ontario, but remains active writing, cycling, doing yoga, and traveling, as well as being a fellow in the Cool Climate Viticulture and Oenology Institute, Brock University, St. Catharines, Ontario, Canada.

Joe P. Kerry is a senior college lecturer and the head of the food packaging research group in the School of Food and Nutritional Sciences at University College Cork (UCC). He received his doctorate in microbiology at University College Galway in 1995. Prof. Kerry is also a qualified member of the Institute of Packaging. He is very involved in national and international research projects both at fundamental and applied levels. Primary research interests address various aspects of food packaging, shelf-life stability, food composition, and numerous aspects of food quality, particularly in relation to muscle foods. He also has very strong links with industry and his research team assists companies in relation to many aspects of new food product development. He has over 220 publications in peer-reviewed international journals, over 300 presentations at major international conferences, along with several other significant publications. His expertise includes use and manipulation of modified atmosphere packaging systems for use with foods, use of extrusion technology for the manufacture of food products/packaging materials, and applications and sensor/new technology developments within the area of food packaging, especially in the area of smart packaging.

Frédéric Leroy, after studying bio-engineering at Ghent University, obtained a PhD in applied biological sciences at the Vrije Universiteit Brussel in 2002, where he continued his academic career at the research group of Industrial Microbiology and Food Biotechnology (faculty of sciences and bio-engineering sciences). As associate professor, his lecturing activities include courses in food science and technology (i.e., "Nutrition," "Technology of animal products," "Food microbiology and ecology," and "Quantitative and predictive microbiology"). Dr. Leroy's research primarily deals with the ecology and functional roles of bacterial communities in (fermented) foods, in particular with respect to the generation of quality, safety, and/or nutritional and health advantages. Focus is mostly on meat products, but other food systems are also being studied, including fermented milks and sourdough breads. In addition, his research interests relate to elements of tradition and innovation in foods, both from a technological and societal point of view.

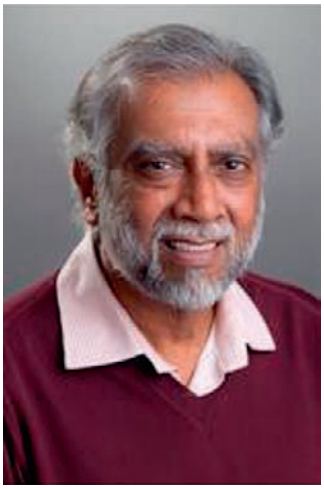
Catherine M. Logue completed her undergraduate and postgraduate degrees in Ireland and earned a PhD in meat microbiology from the University of Ulster, UK in 1996. Dr. Logue was a faculty member at North Dakota State University from 1999 to 2011 rising through the ranks of assistant to associate and full professor. In 2011, she re-located to Iowa State University's College of Veterinary Medicine and is a professor of veterinary microbiology and preventive medicine. Dr. Logue is also the director of faculty and staff advancement and equity for the college. Her research interests focus on foodborne pathogens of food animals and the contamination of meat and meat products destined for human consumption. Her research studies the detection, isolation, and characterization of a range of foodborne pathogens such as *Salmonella*, *Campylobacter*, *Listeria*, *Escherichia coli*, and methicillin-resistant *Staphylococcus aureus* (MRSA) in poultry, bovine, and swine. She also focuses her research on antimicrobial resistance in commensals and pathogens of production animals. She has been an author and a co-author on more than 90 peer-reviewed papers and book chapters as well as more than 150 abstracts and presentations at national and international meetings.

F. Xavier Malcata graduated in chemical engineering in 1986 from the University of Porto (Portugal), received a PhD in chemical engineering/food science from University of Wisconsin, Madison (USA) in 1991, and his habilitation in food science and engineering by Portuguese Catholic University in 2002. He was the dean of College of Biotechnology of Portuguese Catholic University, the chairman of Portuguese Society of Biotechnology, Portuguese representative at VI and VII European Union Framework Programs of research and development, expert for European Food Safety Agency, and a co-ordinator of Portuguese Engineering Accreditation Board in chemical engineering for Northern Region. He is currently a full professor at University of Porto.

His major research interests have focused on technological improvement of traditional Portuguese foods and upgrade of byproducts thereof, development of nutraceutical ingredients and functional foods, design and optimization of enzymatic reactors for edible oil processing, characterization of plant proteases toward cheese and whey cheese manufacture, production of starter and nonstarter cultures from adventitious microflora, and optimized application of unit operations to food processing.

With an academic career of independent research and teaching for more than two decades, Prof. Malcata published more than 450 papers in refereed journals worldwide, wrote 11 books, and prepared more than 45 chapters for edited books. Among many international distinctions, he was

recipient of Ralph H. Potts Memorial Award in 1991 by American Oil Chemists' Society (AOCS, USA), Foundation Scholar Award – Dairy Foods in 1998 by American Dairy Science Association (ADSA, USA), Young Scientist Research Award in 2001 by AOCS, Canadian/International Constituency Investigator Award in physical sciences and engineering in 2002 and 2004 by Sigma Xi (USA), Danisco International Dairy Science Award in 2007 by ADSA, Scientist of the Year Award in 2007 by European Federation of Food Science and Technology (the Netherlands), Samuel C. Prescott Award in 2008 by Institute of Food Technologists (IFT, USA), International Leadership Award in 2008 by International Association for Food Protection (IAFP, USA), Elmer Marth Educator Award in 2011 by IAFP, Distinguished Service Award in 2012 by ADSA, and William V. Cruess Award in 2014 by IFT. He has been elected for the honor societies of food science (Phi Tau Sigma, USA), scientific research (Sigma Xi, USA), and engineering (Tau Beta Pi, USA). He was also elected for fellow of IFT, ADSA, AOCS, and International Academy of Food Science and Technology.



Gopinadhan Paliyath is a professor at the Department of Plant Agriculture, University of Guelph, and the research program director for "Food for Health," under the UG/OMAFRA partnership. Dr. Paliyath is a biochemist and has an interest in various aspects of fruits and vegetables, specifically the nutraceutical components and their mechanism of action. He obtained his BSc Ed degree (botany and chemistry) from the University of Mysore, MSc degree (botany) from the University of Calicut, and PhD degree (biochemistry) from the Indian Institute of Science, Bangalore. Subsequently, he did postdoctoral work at Washington State University, University of Waterloo, and University of Guelph. Dr. Paliyath's research is focused on the biochemistry of plant senescence, specifically pertaining to postharvest biology and technology of fruits and vegetables. Investigations on the role of phospholipase D (PLD) in membrane homeostasis and signal transduction have led to advances in the understanding of the mechanism of membrane deterioration that occur during stress and senescence. Another aspect of his research is focused on understanding the mechanism of action of food components in disease prevention. The efficacy, bio-accessibility, bioavailability, and molecular mechanisms of action of nutraceuticals in fruits and processed products in relation to their cancer-preventive and anti-inflammatory actions are being investigated using mammalian cell lines, and animal model systems.

Dr. Paliyath has developed technologies and products for enhancing the shelf life and quality of fruits and vegetables based on PLD inhibition. R&D activities relevant to the industry sector include: (1) optimization of an enhanced freshness formulation for application to various fruits, vegetables, and flowers; (2) developing methods for nutraceutical carriers that would enhance the functional food quality and delivery (e.g., stabilizing lycopene in tomato juice, sauce, etc., for health beneficial effects); and (3) developing novel technologies to enhance the cancer-preventive ingredients in fruit products, etc. Patents awarded include: (1) # 6,514,914 (US) and 2,298,249 (Canada); (2) #7,198,811 (USA), 4141387-1 (Japan), 260738 (Mexico), 1469736 (Turkey), 028 284763 (China), and 223077 (India). The patents describe the use of nanoformulations based on hexanal and other generally regarded as safe (GRAS) ingredients for enhancing the shelf life and quality of fruits, vegetables, and flowers by pre or postharvest treatments. These technologies are currently being evaluated for extending shelf life and quality of mango in India and Sri Lanka with the assistance of the Canadian International Food Security Research fund. The collaboration involves researchers from Canada, India (Tamil Nadu Agricultural University), and Sri Lanka (Industrial Technology Institute).

Dr. Paliyath is also the research program director for the food and health theme-related activities under the OMAF/MRA/University of Guelph research partnership. He serves on the editorial board of several journals. He is a member of American Chemical Society and Canadian Society of Plant Biologists.

(Total-refereed publications in journals – 92; patents and intellectual properties – 2; disclosures – 4; chapters in books – 27; nonrefereed contributions – 10; research reports – 28; conference proceedings – 88; edited books – 9; book reviews – 6 (Google Scholar: h index – 31, i10 index – 68, citations – 4332; RG score – 35.63)).

Yolanda Picó is a full professor of nutrition and food science at the University of Valencia since 1998. She is currently the head of the research group on food and environmental safety of the University of Valencia. Her research interests are the development of new analytical methods to determine organic contaminants in food and the environment, identification of unknown compounds by liquid chromatography–mass spectrometry, micro-extraction separations, and environmental and food safety. To the date, she is the author of nearly 200 peer-reviewed papers, 170 scientific papers in journals of SCI, 25 book chapters, and editor of four books on food and environmental safety.

Vieno Piironen is a professor of food chemistry at the Department of Food and Environmental Sciences, University of Helsinki, Finland. She received her PhD in food chemistry at the University of Helsinki in 1987 and has approximately 35 years of experience in food research and education on bachelor, master, and PhD levels. She has participated actively in international research and education projects and networks. Her research has focused especially on chemical and nutritional properties, reactions and analysis of lipids, vitamins, and other bioactive compounds. Research on vitamins has been active from the beginning of 1980s. She has studied both lipid- and water-soluble vitamins; their chemical and nutritional properties and importance in foods and diets as well as factors influencing vitamin levels. In addition, development of analytical methods for different vitamers as well as validation and harmonization of the methods through international collaboration have been among the priorities. Recent collaboration projects have focused on enhancing vitamin contents in cereal-based foods by plant breeding, utilization of vitamin-rich grain fractions, and bioprocessing. Currently, the research focus lies on investigating microbial *in situ* synthesis of folate, vitamin B12, and other B vitamins in cereal and legume matrices as a means to improve nutritional quality of foods and to develop new food applications. In lipid research, different lipid classes and their chemical and enzymatic reactions in food matrices are studied. Diverse methods are used to study proceeding of oxidation from primary products to monomeric oxides, volatiles, and polymerization products and to study possibilities to control oxidation. Controlling enzymatic reactions leading to off-flavors in cereal and legume matrices is also among the interests. Phytosterols and their conjugates have been studied as natural food components belonging to the dietary fiber complex. On the other hand, questions related to sterol enrichment such as oxidation susceptibility and mechanisms as well as factors affecting oxidation reactions have been of interest. She has also studied nutrients and anti-nutrients in legumes and more recently started research on utilization of high value components in microalgae. She has approximately 160 papers in international journals and a number of other publications.

David Rodríguez-Lázaro is a doctor in veterinary medicine (DVM), specialized in food science (BSc and MSc) and molecular microbiology (PhD). He is a senior scientist at ITACyL and an assistant professor of microbiology at the University of Burgos. He has performed research stays in the Danish Institute for Food and Veterinary Research (Denmark), the University of Prague (Czech Republic), the Food and Environmental Research Agency (UK), and the University of Bristol (UK). He was a Leverhulme visiting professor in the Institute of Advanced Sciences in the University of Bristol during the years 2004 and 2005 and Marie Curie research fellow in the faculty of medical and veterinary sciences in the University of Bristol (UK) until 2007.

His research interest is focused on the establishment of reliable, quantitative molecular strategies for detection of important food-borne pathogens from environmental sources and various types of foodstuffs, the characterization of the prevalence of the main foodborne pathogens in food and food-related environments, and the development of emergent food preservation processes and their effects in the microbial virulence. He has participated in a number of coordinated EU-funded projects such as PROMISE, BASELINE, VITAL, FOOD-PCR, SACROHN, and MONI-QA, having established active links with the leading European research groups working in food safety.

He has published more than 100 international scientific papers and book or book chapters regarding food safety. He is currently a member of the editorial board of *Applied and Environmental Microbiology*, *International Journal of Food Microbiology*, *Food and Environmental Virology*, and *International Journal of Food Contamination* and the editor-in-chief of the journal *Food Analytical Methods*. He was awarded with the XV Jaime Ferrán Award in 2013 by the Spanish Society for Microbiology for his promising scientific career in microbiology.

Turid Rustad is a professor and the head of the food science group at the Department of Biotechnology, Norwegian University of Science and Technology. The main research focuses on the biochemistry of marine raw materials, the relationship between biochemistry and quality, and changes in raw material properties during processing. Studies of enzymatic activities in different raw materials have been linked to studies of changes in the biochemistry of these raw materials. She has worked with characterization of composition and enzymatic processes in a wide range of different raw materials, such as fish, fish by-products, and zooplankton in relation to different storage and processing methods such as chilling, heating, superchilling, and frozen storage.

Noel W. Solomons has lived and worked in Guatemala for 40 years. He was born and educated in Massachusetts in the United States. As a young child, he became an amateur naturalist and was a nature counselor at various summer camps; this would guide him to a career in science. In his young adulthood, he would participate in the civil rights and anti-war movements, only to become disillusioned by the intractable nature of the injustice elements in the fabric of American society. As a physician by graduate training, he performed his university studies at Harvard College and Harvard Medical School; it was during overseas electives in his medical training that he visited Peru and Colombia and committed to an expatriate life trajectory outside of his homeland. Clinical training included a residency in internal medicine and infectious diseases at the Hospital of the University of Pennsylvania and specialization in gastroenterology and clinical nutrition at the University of Chicago. He became a resident of Guatemala in 1974 as an affiliated investigator at the Institute of Nutrition of Central America and Panama. He would later commute for eight years to a faculty position in the Department of Nutrition and Food Science of the Massachusetts Institute of Technology. Assuming a full-time Guatemala commitment in 1985, he co-founded the Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM) where he remains its scientific director. Over 40 local university theses have been completed by Central American students in that institution as well as an equal number of master's degree research projects from international students from Europe, and North and South America. He has supervised doctoral dissertations for 12 PhD candidates from the United States, Canada, Germany, Spain, and the Netherlands through CeSSIAM.

Dr. Solomons has 332 publications indexed on Medline. In addition, he has edited two books and contributed over 100 articles, reviews, editorials, and commentaries in nonindexed venues and over 50 book chapters. These are dedicated to the scientific and academic interests of his career including: clinical nutrition; human growth and body composition; lactose maldigestion; dietary intake, nutritional status, intestinal absorption, and food fortification related to various micronutrients (vitamins, trace elements, and essential fatty acids); complementary feeding; nutrition in aging and chronic disease; and the interaction of malnutrition and infection.

Among the honors bestowed upon Dr. Solomons are the International Nutrition Prize of the International Union of Nutritional Sciences and the Kellogg Prize of the Society for International Nutrition Research. He is a fellow of the American Society of Nutrition. He is an academic member of the Guatemalan Academy of Medical, Physical and Natural Sciences and the Spanish Academy of Nutrition and Food Science. He was the awardee of the 2010 National Medal for Science and Technology for Guatemala.

He has been a visiting professor in university courses in Mexico, Peru, Brazil, Indonesia, and Spain. He currently holds adjunct professorial appointments at the Boston University School of Public Health, and the Friedman School of Nutrition Science and Policy and the Department of Community Medicine and Public Health, both at Tufts University. He is a founding board of directors, member of the Hildegard Grunow Foundation in Munich and the Essential Nutrient Foundation of Singapore. Finally, Dr. Solomons is a coordinator for Central America of the Nevin Scrimshaw International Nutrition Foundation in Boston, and an associate editor for the Foundation's *Food and Nutrition Bulletin*. He serves on editorial boards for ten scientific journals.

Maria Tsimidou is a professor of food chemistry and the head of the Laboratory of Chemistry and Technology in the School of Chemistry at the Aristotle University of Thessaloniki (AUTh), Greece. Her teaching is food chemistry, analysis, quality control, and food legislation. Research interests are related to virgin olive oil chemistry, quality and authenticity, saffron chemistry, authenticity and quality, antioxidant activity of plant extracts and constituents, new sources of targeted bioactive compounds (squalene, carotenoids, and phenols), and analytical procedures for their determination. She has published many research papers, review articles, and contributions to scientific books and encyclopedias on the above-mentioned topics. Currently, she is an associate editor in the *European Journal of Lipid Science and Technology* and chairs the COST ACTION FA1101 "Saffronomics."

Jorge Welti-Chanes earned his degree in biochemical engineering (1976) and master of science in food engineering (1978) at Tecnológico de Monterrey (ITESM, Mexico), later he moved to Spain to perform his doctoral studies in chemistry, in the area of food technology, obtaining his degree at the University of Valencia. He is currently the national director of graduate studies at School of Engineering and Sciences at Tecnológico de Monterrey also is professor and researcher in the areas of biotechnology and food at the same institution. He started his academic activity in 1976 as a university professor of ITESM, has additionally been a full professor at the National Polytechnic Institute (IPN, Mexico) and the University of the Americas, Puebla, Mexico (UDLA). He has an experience of 37 years as a teacher and university researcher, 20 of which were spent in combination with the development of administration work in education, science and technology. In the UDLA, he was teaching in the Departments of Chemistry and Biology and Chemical Engineering and Food, in the latter was responsible for the leadership for a period of a year and subsequently became dean of the School of Engineering (1986–1988). From January 1989 to June 2002, he was an academic vice chancellor at UDLA. He has published 14 books and has over 200 scientific publications in refereed journals and books, has given more than 250 presentations at international conferences. He is an associate editor of the journals *Food Engineering Reviews* and *Journal of Food Science* and participates as a member of the editorial boards of *Journal of Food Engineering* and *Current Opinion in Food Science*. In May 2011, he received the Life Achievement Award by the International Association for Engineering and Food (IAEF), for his career as a researcher and academic worldwide, and in January 2014, the Romulo Garza Award from the Tecnológico de Monterrey for the impact of their research work and as recognition for being one of the most productive researchers in the life of Tecnológico de Monterrey. He has been the president of ISOPOW and IAEF and is the currently president of the International Society of Food Engineering (ISFE).

Peter J. Wilde graduated in biophysics at the University of East Anglia in 1985 and has been researching the colloidal and interfacial properties of food systems at the Institute of Food Research (IFR) for over 25 years. IFR is the only publicly funded UK research institute that focuses on the underlying science of food and health to address the global challenges of food security, diet, and health, healthy aging, and food waste. IFR is the one of eight institutes that receives strategic funding from the Biotechnology and Biological Sciences Research Council (BBSRC). It also receives funding from government agencies and departments, the EU, charities, and industry, from the UK and overseas.

Pete's research expertise is the interfacial behavior of proteins and other surface active components in food relevant systems. The aim is to determine how the molecular and interfacial processes control the functionality of foams and emulsions. Currently, the functional aspects of his research have focused on improving the dietary impact of emulsified foods. These include fundamental studies on how interfacial layers control emulsion rheology to develop novel fat reduction strategies; the design of interfacial structures to control lipid digestion to promote satiety or the delivery of fat-soluble nutrients and drugs; and to determine the physico-chemical role played by the salivary film in perceiving fat content in emulsions. The impact of this research will be to aid the rational design of foods with enhanced nutritional benefits to address the global challenges of obesity, type 2 diabetes, and other major diet-related conditions.

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HOW TO USE THE ENCYCLOPEDIA

All articles in the encyclopedia are arranged alphabetically as a series of entries.

1. Contents

Your first point of reference will likely be the contents. The complete contents list appears at the front of each volume providing volume and page numbers of the entry. We also display the article title in the running headers on each page so you are able to identify your location and browse the work in this manner.

2. Cross-references

The majority of articles within the encyclopedia have an extensive list of cross-references that appear at the end of each article, for example:

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Iron: Biosynthesis and Significance of Heme; Iron: Physiology of Iron.

3. Index

The index provides the volume and page number for where the material is located, and the index entries differentiate between material that is a whole article; is part of an article, part of a table, or in a figure.

4. Contributors

A full list of contributors appears at the end of volume 5.

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INTRODUCTION

Until a few decades ago, virtually all known health effects of foods were related to their content of essential nutrients. The clinical description of most diet-related illnesses mirrored the signs of essential nutrient deficiencies, such as pellagra, beriberi, and others. Consequently, the key public health concern regarding diet was ensuring that everyone consumed enough food. It was only in the past 50 years that large-scale epidemiological observations began to associate chronic diseases like diabetes and cardiovascular disease with nonessential diet constituents such as saturated fat, fiber, and cholesterol. Taking advantage of the emergence of digital informatics, these studies were able to manipulate increasingly large sets of data and provide, for the first time, a picture of the secular changes in the health of large populations and its association with what they ate regularly. These findings progressively shifted the concern from eating enough to avoiding excessive consumption of certain foods. Eating enough was replaced by eating well.

But it turned out that defining how to eat well is far more complex than defining minimum needs of essential nutrients. First, there is no single paradigm to study those relationships, given the wide variety of biological mechanisms and the long exposures involved. Second, many of the experimental models used to define essential nutrient needs are not applicable to the study of long-term effects of diets in free-living populations. And it is now clear that experiments with isolated dietary compounds do not reflect the actual effects of the complex food matrix we consume daily. Finally, while the discovery of essential nutrients and their role in health was the domain of a few specialties speaking a common language (primarily biochemists and physiologists), the study of the long-term effects of whole diets in humans must of necessity involve epidemiologists, social and behavioral scientists, food scientists, clinicians, policy experts, etc., making far more difficult the development of consensus and foundational concepts.

It is thus not surprising that today we have still not achieved a stable consensus on how to eat 'well.' Furthermore, while few nonscientists would care about the minimum requirement of a vitamin to sustain life, there are plenty of opinions among nonscientists on how to eat 'well.'

Our goal in preparing this encyclopedia has been to contribute to the understanding of that complex diet–health relationship by providing a multidisciplinary, integrative and accurate source of information. We aim to serve the needs not only of established and in-training scientists, but also of the increasingly important group of professionals who are key to disseminate and sustain the practice of science: journalists, science writers, science administrators, fund raisers, donors, and policymakers. In preparing this work, we had the enormous advantage of working with one of the publishers with the most extensive expertise in major reference works, Elsevier. This first edition builds on the impressive breadth of knowledge of over 922 authors and on the tireless work of our editorial advisory board. We are very grateful to all of them.

Benjamin Caballero
Paul Finglas
Fidel Toldrá

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A

Acesulfame-K

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Introduction

Nonnutritive sweeteners (NNSs) have been utilized since the late 1800s with a significant increase in recent decades. NNSs (also known as noncaloric sweeteners, artificial sweeteners, very low-calorie sweeteners, and intense sweeteners) are a caloric sweetener (CS) replacement, which provide minimal or no calories. NNSs have thus been an attractive option in the setting of the obesity and diabetes mellitus epidemic and are found in thousands of foods and beverages. The majority of individuals cite reduced caloric intake as a major reason for using NNS, with other common reasons including goals of weight loss and reduced glycemic load. However, there has been controversy regarding the role of NNS in weight and glycemic control, among concerns for other adverse effects. A Mintel survey accordingly indicated that 64% of individuals are concerned with the safety of NNS use, highlighting the public awareness and perception of present controversies.

To date, six NNS have been approved by the FDA: acesulfame-K (ACK) (Sunett and Sweet One), aspartame (Equal and NutraSweet), neotame, saccharin (Sweet'N Low), sucralose (Splenda), and stevia (Truvia, Pure Via, and SweetLeaf) (Table 1).

ACK, which is often used in blends with other NNSs or CSs, is one of the most commonly used NNSs. ACK was approved by the US Food and Drug Administration (FDA) in 1988 for use in specific food and beverage categories. In 1998, ACK was also approved for use in soft drinks and in 2003 as a general purpose sweetener and flavor enhancer (except for use in meat and poultry).

The goal of this article is to detail the pharmacological properties and associated controversies regarding clinical outcomes for ACK.

Table 1 Non-nutritive sweeteners with acceptable daily intake (ADI), year of FDA approval, and common serving sizes

Sweetener and chemical structure	*Common brand names*	*ADI/JECFA toxicology monograph no. (year)*	*Year FDA-approved*	*Representative amount of sweetener in 12 oz soda (mg)*	*No. of servings = to ADI for a 150 lb (68 kg) person*	*Amount of sweetener in a packet (equivalent to 2 tsp sugar) (mg)*	*No. of packets = to ADI for 150 lb (68 kg) person*
Acesulfame-K	Sweet One	15 mg kg^{-1} bw 28 (1991)	1988	40 (Blended with aspartame)	25, 12 oz servings	50	20
Aspartame	Equal NutraSweet	40 mg kg^{-1} bw 15 (1980)	1981	187	14, 12 oz servings	40	68
Neotame	Neotame	2 mg kg^{-1} bw 52 (2004)	2002	Not in carbonated beverages		No consumer product	
Saccharin	Sweet'N Low	5 mg kg^{-1} bw 32 (1993)	Before 1958	8 (Blended with aspartame)	42, 12 oz servings	40	8.5
Sucralose	Splenda	15 mg kg^{-1} bw 28 (1991)	1999	68	15, 12 oz servings	11	30
Plant-based sweeteners, Stevia	Truvia Pure Via SweetLeaf	4 mg kg^{-1} bw 60 (2009)	2008	17	16, 12 oz servings	9	30

Source: Gardner, C., Wylie-Rosett, J., Gidding, S. S., et al. (2012). Nonnutritive sweeteners: current use and health perspectives: a scientific statement from the American Heart Association and the American Diabetes Association. *Diabetes Care* **35**(8), 1798–1808.

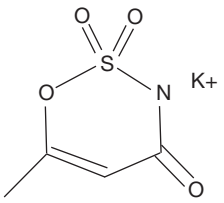

Figure 1 Chemical structure of ACK.

Sources and Production

Similar to a host of NNS previously accidentally identified, including saccharin in 1878, cyclamate in 1937, and aspartame in 1966, ACK was fortuitously discovered by Karl Claus and Harold Jensen in 1967. ACK is a potassium salt of 6-methyl-1,2,3-oxathiazin-4(3*H*)-one 2,2-dioxide (Figure 1). Initial synthesis by Claus et al. involved a base of chlorosulfonyl or fluorosulfonyl isocyanate with propyne–acetone, which was subsequently cyclized by potassium to form ACK. Alternative methods of production later included the treatment of acetoacetamide with at least two equivalents of sulfur trioxide, which is subsequently dehydrated by sulfur trioxide to form oxathiaazinone dioxide. This results in the formation of *N*-sulfoacetoacetamide, which is then neutralized by potassium hydroxide. ACK content in processed foods can be analyzed by multiple methods, such as quantitative NMR, with high accuracy.

Availability, Absorption, and Metabolism

Radiotracer studies in animal and human models, as well as autoradiographic and quantitative studies in animals, have demonstrated that ACK is completely absorbed, distributed rapidly and evenly, and excreted renally. Human studies have shown that after the administration of a single dose of 30 mg of ACK, peak blood concentrations were achieved in 1–1.5 h. ACK was rapidly eliminated with a plasma half-life of 2–2.5 h. With up to ten repeated doses, no evidence of tissue accumulation nor increase in blood levels of ACK was seen in animals. Only the parent compound was identified in serum and urine indicating lack of significant biotransformation of ACK, suggestive of a biologically inert substance. Due to concerns about poor-quality toxicity tests, ACK was nominated twice for testing in 1996 and 2006 in the National Toxicology Program bioassay program and subsequently rejected and thus has not undergone such testing.

Patterns of Consumption

NNSs are used to enhance the flavor profile of thousands of beverages and food products and their use has increased in recent decades. ACK is touted as being approximately 200-fold sweeter than sucrose, though Antenucci et al. demonstrated that it may not surpass the perceived sweetness intensities of natural sweeteners (such as sucrose, maple syrup, and agave nectar), likely a function of increasing bitterness with concentration. Some individuals find the taste to be objectionable, and interestingly, Allen et al. identified two single-nucleotide polymorphisms felt to explain 13.4% of the variance in perceived bitterness. Accurate estimates of the exact intake of NNS are difficult as there are no requirements that the amount of NNS used in drinks and food be made available on food labels or released to federal agencies. Yang et al. estimated that 6000 new products with NNS became available between 1999 and 2004 with the most popular additives, sucralose and ACK, found in 2500 and 1103 products, respectively.

Overall, the consumption of NNS by both adults and children has increased significantly, presently utilized by 15–35% of the US population. Mattes and Popkins reported that approximately 15% of the US population consumed NNS in food or beverages from 2003 to 2004, as compared to 2.5% in 1965 based on their analysis of the data from the US Department of Agriculture Nationwide Food Consumption Survey and National Health and Nutrition Examination Survey (NHANES). The proportion of consumers ingesting NNS in beverages and foods from 1989 to 2004 increased by 6.9% and 81.2%, respectively. Overall, 10.8% of the population was estimated to consume NNS in beverages and 5.8% to consume NNS in foods. Interestingly, products with added sugars during this time period did not decrease, suggesting that NNS may not be acting as a substitute for products sweetened with sugar. Sylvetsky et al. further built upon the findings of Mattes and Popkin by using the NHANES database to evaluate trends among demographic subgroups stratified by the source of NNS. They reported that in 2007–2008, the prevalence of consumption of beverages with NNS increased from 6.1% to 12.5% among children ($P < 0.0001$) and from 18.7% to 24.1% in adults ($P < 0.001$) regardless of weight, age, socioeconomic, and race–ethnicity subgroups. They reported little change in the consumption of foods with NNS. Piernas et al. also demonstrated that from 2000 to 2010, the percent of households, particularly those with children, purchasing NNS and combined NNS and CS products increased, while those purchasing CS products alone decreased. The highest percentage of CS beverage purchases was seen among African-American and Hispanic households and households with children.

Presently, the acceptable daily intake (ADI) for ACK in the United States is 15 mg kg^{-1} body weight. Internationally, studies of estimated daily intake of ACK have generally been shown to be below the ADI in populations in Korea, Portugal, Italy, the Netherlands, Denmark, Norway, Australia, and New Zealand. A Swedish study including 1120 diabetics found that the ADI was never reached in men and women. However, worst-case calculations in young children demonstrated that ACK consumption may be as high as 169%. This study was limited in that it largely reflected soft drink use, as ACK was not used as a tabletop sweetener at that time.

Health Effects

Limited studies have examined the impact of ACK on health outcomes and the following is a focussed review.

Obesity

The relationship between NNS and weight has been controversial. While it has been hypothesized that NNSs facilitate weight

loss and/or weight maintenance as a low-calorie substitute, it has also been suggested that NNS may cause weight gain via changes in metabolic signaling and ultimately food intake. Evidence from observational studies and randomized controlled trials (RCTs) has largely been conflicting. While observational studies are limited by means of NNS assessment and confounding lifestyle factors, RCTs are generally short term in duration and may be difficult to broadly apply given increased subject awareness of NNS use and, in many instances, increased individual nutritional support from study staff.

Multiple observational studies have yielded conflicting results regarding NNS use in the setting of obesity. An early study by the American Cancer Society in 1986 demonstrated that based on survey results conducted over 1 year ($n = 78\,694$; age range 50–60 years), NNS users were significantly more likely to gain weight than nonusers. However, the difference in mean weight between treatment and control groups was approximately 0.9 kg (2 lbs), likely minimally clinically relevant. The applicability of the results was further limited due to methodological design. Subsequent short-term studies ranging from 10 days to 16 weeks did not support the initial findings from the American Cancer Society. Furthermore, an inverse association was reported in some studies. In the San Antonio Heart Study, 5158 adults were initially assessed from 1979 to 1988 and subsequently 7–8 years later. A positive dose relationship was seen between baseline intake of artificially sweetened beverages and change in body mass index (BMI). Overall, BMI changes were 47% greater among individuals who used artificial sweeteners as compared to nonusers ($+1.48\ \mathrm{kg\ m^{-2}}$ vs. $+1.01\ \mathrm{kg\ m^{-2}}$, $P < 0.0001$). Nettleton et al. performed an observational study including 5011 individuals in the Multi-Ethnic Study of Atherosclerosis cohort that demonstrated that diet soda use was associated with a 36% greater relative risk of incident metabolic syndrome as compared to individuals who did not consume diet soda (HR 1.36 (95% CI 1.11–1.66)). Specifically, an association between diet soda consumption and increased waist circumference and fasting hyperglycemia was noted. Of note, metabolic syndrome was not independent of baseline measures/changes in adiposity.

RCTs have also yielded conflicting data. De Ruyter performed a large RCT including 641 normal-weight children who were followed for 18 months and stratified to receive 8 oz $\mathrm{day^{-1}}$ of an artificially sweetened beverage or a sugar-containing beverage. The study demonstrated that the group receiving artificial sweeteners had reduced weight gain (weight 6.35 kg in the sugar-free group as compared with 7.37 kg in the sugar group (95% CI for the difference, −1.54 to −0.48) and fat accumulation. The Choose Healthy Options Consciously Everyday trial stratified adults to one of two intervention groups: water intake ($n = 106$, 94% women) or diet beverage intake ($n = 104$; 82% women). The study demonstrated both intervention groups decreased absolute intakes of total daily energy, carbohydrates, fat, protein, saturated fat, total sugar, added sugar, and other carbohydrates. However, overall, the diet beverage group decreased the intake of CS significantly more than the water group did, suggesting that the former group may have had better adherence to the diet. Furthermore, at 6 months, the diet beverage group decreased their intake of desserts significantly more than the water group. Overall, this study demonstrated that short-term consumption of diet beverages, as compared to water, did not increase preferences for sweet foods and beverages.

A recent meta-analysis by Miller and Perez including 15 RCTs and nine prospective cohort studies examined the relationship between body weight and composition and low-calorie sweeteners (LCSs) (defined as ACK, aspartame, luo han guo extract, neotame, saccharin, steviol glycosides, sucralose, cyclamate, thaumatin, neohesperidin dihydrochalcone, and alitame). Analysis of the RCTs demonstrated that the substitution of sugar for LCS resulted in modest weight reduction (0.80 kg), along with a decrease in BMI, fat mass, and waist circumference. It was hypothesized that it was unlikely that replacement of LCS for sugar was solely responsible for these changes. Rather, LCSs were felt to facilitate increased adherence to weight-loss or weight-maintenance plans. Analysis of the prospective cohort studies showed a modest significant positive association with BMI, but not with weight or fat mass. The prospective observational studies were felt to be limited due to inconsistent measurement of LCS intake and inadequate control of confounding variables such as diet and lifestyle factors.

Overall, the American Heart Association and American Diabetes Association (ADA) scientific statements concluded that there is insufficient evidence to conclude whether NNS use leads to weight loss or reduction in cardiometabolic risk factors.

Role in Diabetes Mellitus

Data regarding glycemic response to NNS have also been conflicting. Sweet taste receptors not only are expressed in the taste buds where they serve a gustatory function but also have been identified in endocrine cells in the gastrointestinal (GI) tract, pancreatic beta cells, rodent hypothalamic neurons, and adipocytes where they have roles in nutrition and fuel metabolism. Rat and mouse models have shown that CS and NNS may activate enteroendocrine sweet taste receptors by upregulation and insertion of small intestine transporters, facilitating glucose absorption. Glucose absorption at the small intestines occurs through two mechanisms predominantly: The Na^+–glucose cotransporter (SGLT) is predominantly responsible for active absorption in the setting of low glucose concentrations; however, at glucose levels of >30 mM in the lumen postprandially, SGLT2 is saturated, and thus, further glucose absorption is mediated by glucose transporter 2 (GLUT2). While SGLT1 is a known mediator of GLUT2, the activation of sweet taste receptors in the enterocyte and in the enteroendocrine cells of the gut may also be important. Zheng et al. examined the role of ACK on glucose uptake in the enterocyte by preincubating Caco-2, RIE-1, and IEC-6 cells starved from glucose for 1 h and subsequently measuring glucose uptake. The study demonstrated that ACK increased glucose uptake by 20–30% with glucose concentrations >25 mM in a GLUT2-dependent mechanism. While these effects were seen at 5 min of incubation, no additional effect was seen at 10 min suggesting that cells had maximized GLUT2 translocation by this time. Furthermore, NNS may increase GLP-1 secretion by intestinal neuroendocrine cells, but interestingly, intracellular signaling patterns were different for ACK, as compared to sucralose and saccharin.

There has accordingly been concern that the combination of NNS and glucose may worsen postprandial hyperglycemia, particularly in type 2 diabetics who may have overexpression of glucose transporters at baseline. Bryant et al. examined the role of NNS (aspartame, saccharin, and ACK) and glucose in commercially relevant doses on glycemic and appetite responses in ten individuals in a pilot study. None of the sweeteners caused a change in perceptions of hunger or fullness and neither aspartame nor saccharin had an impact on blood glucose response after administration of oral glucose. However, ACK did exert a small effect (blood glucose following oral glucose administration alone peaked at 15 min at 7.6 ± 0.3 mmol l^{-1} vs. blood glucose following combined ACK and glucose peaked at 8.3 ± 0.3 mmol l^{-1}). Post hoc analysis revealed no difference between glucose and NNS conditions.

These findings are in contrast with a recently published meta-analysis including 11 studies with nine cohorts including nearly 280 000 participants, among whom 22 000 individuals had type 2 diabetes mellitus. The study examined the association of both sugar-sweetened and artificially sweetened soft drinks and type 2 diabetes mellitus. A positive association was seen for sugar-sweetened soft drinks (RR 1.20/330 ml day^{-1}, 95% CI 1.12, 1.19, $P < 0.001$) along with artificial sweeteners (RR 1.13/330 ml day^{-1}, 95% CI 1.02, 1.25, $P = 0.02$) and diabetes mellitus, but the association of the former was stronger and more consistent than the latter. Of note, the meta-analysis was limited to prospective observational studies, an important consideration in the setting of multiple possible confounding factors in examining this association. Four RCTs ranging in duration from 1 to 16 weeks have not found an association between NNS and glycemic response.

Interestingly, Suez et al. recently demonstrated that intake of noncaloric artificial sweeteners (saccharin, sucralose, and aspartame) resulted in gut microbiota alterations with subsequent dysbiosis and metabolic abnormalities in mice and humans. In an analysis of 381 nondiabetic individuals (44% males and 56% females; ages 43.3 ± 13.2), the authors reported increased weight and waist-to-hip ratio, higher fasting blood glucose, glucose tolerance test response, and elevated serum alanine aminotransferase levels (felt to be related to nonalcoholic fatty liver disease). Additionally, despite correction for BMI, a statistically significant difference in hemoglobin A1C levels was seen in the group reporting high consumption of NNS as compared to low consumers. Upon characterization of 16s rRNA in 172 randomly selected individuals, a statistically significant positive correlation was seen between multiple taxonomic entities and nonartificial sweetener consumption. Finally, a group of seven healthy volunteers (five men and two women; ages 28–36) who typically do not consume NNS were followed for 1 week. On days 2–7, the individuals consumed the maximal ADI of saccharin. Four of the seven individuals developed statistically significant poorer glycemic responses. The microbiome configuration of those with a poorer glycemic response was noted to be different than those who did not exhibit a response. Stool matter was subsequently transferred from two individuals who exhibit poorer glycemic response and two individuals who did not to mice. Germ-free mice that received stool from the former group replicated part of the donor-induced saccharin dysbiosis. Overall, these findings suggest that humans may have a personalized response to NNS, possibly stemming from differences in microbiota composition and function at baseline.

In 2010, the American Dietetic Association concluded that NNSs have a negligible effect on glycemic control in diabetic individuals. Similarly, the ADA recommends that if NNSs are used to replace CS without caloric compensation, then NNSs may be useful in reducing caloric and carbohydrate intake, though the need for more research in this arena is recognized.

Risk of Malignancy

The National Cancer Institute issued a statement in 2009 indicating that sweeteners such as ACK are safe for use and do not contribute to malignancy. The NTP performed a 9-month study in genetically modified p53 haploinsufficient mice fed with daily diets consisting of 0%, 0.3%, 1%, or 3% ACK. They found no increased carcinogenicity or neoplastic activity in these mice over 9 months. Follow-up *in vivo* cytogenetic studies in mice exposed to 15, 30, 60, 450, 1500, and 2250 mg of ACK did show increased toxicity via clastogenic effects. These results contradicted prior studies in animal cell lines performed prior to FDA approval. In sum, while ACK may contribute to clastogenic effects in animals exposed to high doses, there is no present evidence of carcinogenicity in humans.

Neurometabolic Effects

ACK has also been postulated to effect neurocognitive function. A series of *in vivo* and *in vitro* studies suggest that acute exposure to ACK may decrease intracellular ATP production and reduce cellular viability and protective activity of neuronal cells. Cong et al. also showed that chronic ingestion of ACK in mice (at doses within the expected exposure range for humans ingesting ACK) resulted in impairments in learning and memory in tasks localizable to the hippocampus. These studies based their dose calculations on appropriate animal to human dosage calculations and report a human equivalent dose of 18.7 -29.1 mg kg^{-1} day^{-1}, which is 1.2–1.9 times the estimated human ADI. Therefore, these results are hard to interpret since most humans may not be ingesting similar amounts of ACK on a daily basis. More studies are needed to verify and assess the applicability of these results.

Recommendations During Pregnancy

Most sweeteners are approved for use during pregnancy including ACK, sucralose, saccharin, and stevioside. Early animal studies have shown a possible association between saccharin exposure in pregnant rats and increased risk of bladder cancer; however, this has not been demonstrated in human studies. High doses of ACK were used in these studies, further limiting their applicability to humans. In 2010, a study using the National Danish Birth Cohort suggested that daily intake of carbonated beverages supplemented with ACK or aspartame was associated with preterm birth. A subsequent Norwegian study showed that high intake of artificially sweetened beverages (adjusted OR for >1 serving per day 1.11, 95% CI 1.00, 1.24) and sugar-sweetened beverages (adjusted OR 1.25, 95% CI 1.08, 1.45) was associated with preterm delivery. Given these are observational studies and limited to beverages, one cannot make a causal association between artificial sweeteners and preterm birth. However, these studies suggest caution should be used in choosing to consume artificial sweeteners.

Presently, the American Academy of Nutrition and Dietetics recommend intake that is limited to the FDA's ADI of 15 mg kg^{-1} during pregnancy.

Conclusion

The use of NNS has increased worldwide due in part to its appeal as a low-calorie and low-carbohydrate alternative. There are conflicting data regarding the health effects of the NNS as a class, most notably in terms of its implications in weight and glycemic control. Despite the prevalence of ACK use, limited research is available in terms of its specific role in health outcomes. Recent research has suggested that personalized responses to NNS may partly explain heterogeneity in terms of outcomes and may be a potential means of establishing who may be predisposed to a more adverse event profile with use of the supplement. Regardless, careful attention must be paid to the host of effects, most notably in terms of weight and glycemic control, associated with NNS use. There is no concern for nonmetabolic aspects.

See also: Carbohydrate: Digestion, Absorption and Metabolism; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Saccharin – How Sweet It Is; Sweeteners: Classification, Sensory and Health Effects.

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Acidophilus Milk

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Introduction

Milk is a good source of several key nutrients such as proteins (casein and whey proteins), fat, sugar (lactose), vitamins, and minerals, thus having a range of biological activities that influence digestion, growth, and metabolic response to absorbed nutrient. Digestion of milk may also result in the formation of many substances with specific biological activities, that is, bioactive peptide and fatty acid analogs. Due to its complex chemical composition, which includes a high content of water, milk is a highly perishable food; thus, preservation of its nutritional value has been an important concern in food production since ancient times. Several preservation methods are available, of which biofermentation – fermentation of milk with lactic acid bacteria (LAB) – is probably the oldest, most widely accessible, and efficient preservation method. The cumulative knowledge on LAB physiology, human health and diet needs, and processing technology has led to the development of a diversity of dairy products fermented with LAB, of which yogurts and acidophilus milk are the most known.

Sources and Production

The application of LAB to preserve milk (fermentation or biopreservation) and obtain products with specific taste and a higher shelf life is known for centuries. In fact, at the time of the Roman Empire, fermented milk was often prescribed for curing disorders of the gastrointestinal tract (GIT).

LAB have thus a long and essentially safe history of application in food processing and today are generally recognized as safe (GRAS status) for human consumption.

A number of different LAB species may be, and are indeed used and claimed to be probiotic. The European Food Safety Agency (EFSA) has proposed, however, a system for a premarket safety assessment of selected groups of microorganisms, leading to granting a 'Qualified Presumption of Safety (QPS)' status to LAB species belonging to *Lactobacillus*, *Leuconostoc*, *Bifidobacterium*, *Streptococcus*, and *Pediococcus* genera. Taxonomy and proper identification at species level is one of the main pillars of QPS.

Research anchored on previous work by Pasteur related to fermentation and Metchnikoff on the potential positive effects of fermented products in people's health has been the stimulus to the development of many fermented milk products, which are today important components or supplements in Western diet and a huge boost to the dairy industry. Specifically, a new type of milk products the so-called probiotics – also called nutraceutical, pharmafoods, or designed foods – has emerged, among which 'acidophilus milk' is one of them. Probiotic bacteria are defined as live microorganisms, which, when administered in adequate amounts, confers a health benefit on the host. Most probiotic bacteria belong to genera *Lactobacillus* and *Bifidobacterium*.

Acidophilus milk is obtained via fermentation with *Lactobacillus acidophilus*, a type of LAB commonly found in the normal digestive tract of mammals. The popularity of acidophilus milk is due to the many health effects attributed to its consumption. The name 'acidophilus milk' may be, and often is, used as a general designation for milk fermented either with *Lactobacillus acidophilus* only or with any other lactobacillus strain or even bifidobacteria. Today, food products or supplements containing strains of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* GG, or *Lactobacillus casei* Shirota, bifidobacteria, and other LAB are in the market due to a perceived positive health effect attributed to presence of these bacteria.

The processing of such food products in general requires some stringent technological processing conditions, due to specific physiological needs that these bacteria have to grow optimally and survive in the food product or supplement.

Acidophilus Milk is usually made from low-fat (partially skimmed) milk. After sterilization (120 °C 15 sec) and cooling of milk to 37 to 38 °C, *Lactobacillus acidophilus*, as a pure culture is added at the rate of 5%. The high temperature used in sterilization releases peptides from milk proteins, which helps the growth of the organism known to lack a good proteolytic system for hydrolyzing milk proteins. The inoculated milk is gently stirred to mix the inoculum, avoiding much incorporation of air, and incubated for 18 to 24 hours. When the acidity reaches 1.0%, the product is cooled to less than 7 °C, and bottled.

To effectively convey the expected health functionalities of probiotic bacteria present in acidophilus milk, it is accepted that they need to reach the lower intestinal tract in high numbers. Thus, the survival of probiotic strains during food processing and during passage in the upper and lower parts of the GIT is an important technological concern. It has been established that the minimum number of available probiotic bacteria should be in the range of 10^7–10^8 colony-forming units per ml (CFU ml^{-1}) of the product at the time of consumption. To meet these requirements, technological developments that efficiently protect probiotic bacteria from the harsh conditions present in the stomach and most parts of the upper intestinal tract have been developed, which include enteric coating and microencapsulation, directed to give higher survival rates of probiotic bacteria and increase their delivery at expected amounts in the lower GIT.

Lactobacilli and other probiotic bacteria in general grow slowly in nonsupplemented milk; therefore, technological developments targeting at creating optimal growth conditions to enhance growth and survival of the probiotic strains during processing have been developed. These include using prebiotic substances (food ingredients such as fructooligosaccharides (FOS/GOS), which stimulate the growth and activity of the desired bacteria), creating low-redox potential conditions, or

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other technological improvements that address the sensitivity of probiotic bacteria to metabolites produced during their growth alone or in combination with other LAB starter cultures. Recall also that some of the metabolites (such as acetic acid produced by bifidobacteria) may be undesirable due to the formation of off-flavors in the product. Growing probiotic bacteria in a mixed culture with more robust species such as *Streptococcus thermophilus* is a common strategy, as it has been reported that some starter cultures may enhance the growth and survival of probiotic microorganisms either because the adjuvant starter culture produces growth-enhancing metabolites or because they reduce the oxygen content in milk. For example, a study found a strain of *Bifidobacterium animalis* that grows faster in goat's milk when in coculture with *Lactobacillus acidophilus*. Also, optimum growth for probiotic bacteria specially those of human origin may occur at slightly higher temperatures than temperatures commonly used for fermentation with traditional LAB; however, in the case of mixed cultures, increasing the fermentation temperature may also result in development of undesirable flavors. Thus, another common approach is to use traditional growth temperatures for starter cultures and then add the probiotic microorganisms at the required high numbers.

Some Lactobacillus acidophilus strains are commonly found in the human intestine, thus showing an ability to survive and grow in the presence of normal levels of surface tension-depressing bile salts found in the enteric environment. To promote wider consumption of such beneficial bacteria, modifications in the delivery of the microorganisms via milk were sought. That search gave rise to a product called *Sweet Acidophilus Milk a* forerunner of Probiotic Milks widely prevalent today. *Sweet Acidophilus Milk* is made by adding a concentrated cell suspension of the organism to cold (5 °C), pasteurized milk, mixing to obtain homogenous distribution of the culture, bottling and cold storing, thus avoiding fermentation and high acidification, which were found to inhibit Lactobacillus acidophilus.

Patterns of Consumption and Regulations

There is an overall increasing pattern of consumption of all types of fermented milks in most countries. Acidophilus milk or probiotic dairy food products represent the largest segment of the functional food market in Europe, Japan, and Australia. The fermented milks in the market today represent ~€63.2 billion with North America, Europe, and Asia accounting for 77% of the market. Sales of yogurt and fermented milks also continue to expand worldwide, most noticeably in emerging markets such as China, Brazil, and Russia and in countries in the Middle East, North Africa, and Latin America. Probiotic drinks in particular have contributed to the growth of the dairy market, and together with encapsulated supplements, they allow for a constant launching of new products, making innovation in this field a very dynamic activity.

Thus, development and consumption of functional foods, or foods that promote health beyond providing basic nutrition, are on the rise, and currently, more than a 100 probiotic fermented milk products may be found in the market (**Table 1**), of which Yakult, Actimel, and LC-1 are the most known.

The beneficial health claims associated with them are the main reasons behind such popularity, which, in many cases, has even surpassed the scientific and regulatory requirements. In fact, the market pull for functional foods has in many cases been too fast to the point where it has resulted in certain knowledge gaps in the scientific understanding of their mechanism of action on the consumer (**Figure 1**).

As the global probiotic markets are expanding rapidly, harmonization of national and international regulations and guidelines is considered very important toward evaluating the efficacy and safety of probiotic bacteria and products therefrom in fulfilling essential prerequisites before being marketed, thus avoiding false claims.

Difficulties in harmonization at the international level are due to the fact that in different countries, probiotic products may be considered either as 'drugs' or as dietary

Table 1 Examples of probiotic fermented milk products in the EU market

Type of product and trade name	*Probiotic microorganism present in the product as stated by the manufacturer*
I. Nondrinkable fermented milks:	*Lactobacillus acidophilus*, *Lactobacillus acidophilus* LA5, *Lactobacillus rhamnosus* LGG, LB21 and 271, *Lactobacillus casei*, *Lactobacillus johnsonii*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Lactococcus lactis* subsp. *lactis* L1A, *B. bifidum*, *B. animalis* subsp. *lactis* BB-12, *B. animalis* subsp. *animalis*
Bifisoft, Bifidus, Bioghurt, Biofit, Biofarde Plus, Biola, Biologic Bifidus, Culture Dofilus, DUjat Bio Aktive, Ekologisk Jordgubbs Yoghurt, Fit & Active, Fysiq, Gefilus, Lc1, ProVIva, RELA, Verum, Vitality, Yogosan, Milbona	
II. Drinkable fermented milks:	*Lactobacillus acidophilus*; *Lactobacillus acidophilus* LA5; *Lactobacillus rhamnosus* LGG, LB21, and 271, *Lactobacillus casei* (F19, 431, Imunitas, Shirota); *Lactobacillus johnsonii*; *Lactobacillus plantarum*; *Lactobacillus reuteri*; *Lactobacillus fortis*; *Lactococcus lactis* ssp. *lactis* L1A; *B. bifidum*; *B. animalis* subsp. *lactis* BB-12; *B. animalis* subsp. *animalis*; *B. longum*
Afil, Actimel, Akfit, Bella Vita, Bifidus, Biofit, Biola, Casilus, Cultura, Everbody, Gaio, Lc1go, LGG+, Onalka, Probiotic drink, Proviva, Yakult, Yoco acti-vit	
III. Nonfermented dairy products:	*Lactobacillus rhamnosus* LGGG, *Lactobacillus plantarum* 299v, *Lactobacillus reuteri*
Gefilus, God Halsa, RELA, Vivi Vivo	

Lactic starter cultures microflora are not listed.
Data adapted from Tamime et al. (2005).

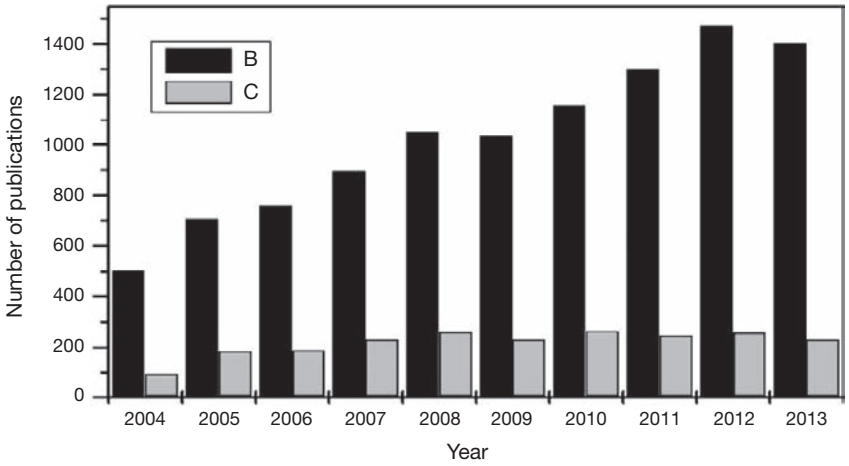

Figure 1 Number of researches and randomized trials on probiotics published in MEDLINE database in the last 10 years (2004–2013).

supplements. It is easily understood in most countries that a 'drug' or a new therapeutic agent needs to follow a regulatory process until being marketed, while a dietary supplement may not need any evaluation or approval before reaching the market.

The probiotics' 'health claims,' defined as "the statements, [which] characterizes the relationship of any substance to a disease or health-related condition," must be based upon well-established scientific evidences. For reasons in general associated with the structure of the reported scientific studies, with the exception of a few cases, most therapeutic or disease-prevention claims associated with probiotic foods have not been approved yet in the EU.

In the United States, probiotic bacteria may be regulated as a biological agent and/or dietary supplement, and in the former case, there is a need of a premarket evaluation of the safety, purity, and potency, as well as efficacy.

In Canada, the amount and quality of the data to be supplied for a claimed probiotic product depend on the claim that is sought. In any case, health products are considered as a subset of drugs and require assessment and licensing before being marketed.

In Japan, functional foods, including probiotics, have been legally defined and regulated under the 'Foods for Specified Health Uses' (FOSHU) system by the Japanese Ministry of Health, Labor, and Welfare. The FOSHU system allows several health claims for probiotic bacteria such as 'colonizes the intestines alive,' 'increases the intestinal beneficial bacteria,' and 'inhibits harmful bacteria.'

Finally, the Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria has developed and proposed guidelines for evaluating probiotic bacteria in food. The recommended guidelines included (1) using a combination of phenotypic and genotypic tests to identify the genus and species of the probiotic strain, as clinical evidences suggested that the health benefits of probiotic bacteria may be strain-specific; (2) *in vitro* testing to delineate the mechanism of the probiotic effect; and (3) substantiation of the clinical health benefit of probiotic agents with human trials.

These requirements are seen as a potential base to establishing the harmonization of regulations and standards of probiotic bacteria health claims in most countries.

It seems that two important factors associated with the increase in dairy products consumption are the growing population and the increase in per capita consumption. It is generally recognized that economic factors such as higher consumer income and declining retail prices for dairy products are the main cause of the increase in per capita consumption. Secondary factors that affect per capita consumption include demographic and socioeconomic factors (such as aging population, decreasing household sizes, urbanization, and increase in the number of working women) and food preferences and consumer attitudes (including health and nutritional issues, food safety, quality (e.g., freshness, taste, and branding), and production ethics (e.g., environment and animal welfare)). In general, the demand for innovative value-added products such as probiotic fermented milk products is replacing consumption of other dairy products (Figures 2–5).

Factors such as food legislation and measures with respect to obesity, fashions, change in the age distribution of consumers, and increasing health consciousness are also expected to have large implications for overall demand and consumption patterns for individual milk and dairy products. It is known that gender, age, educational level, and socioeconomic status are important factors determining the purchasing decisions for such products.

Health Effects

The concept of probiotic or functional milk food products (such as acidophilus milk) has evolved some 100 years ago. Ellie Metchnikoff (1907), a Russian-born Nobel laureate who was working at the Pasteur Institute in Paris, attributed the longevity of Bulgarians to their regular consumption of fermented milk products such as yogurt. Minoru Shiroma in the 1930s cultivated a beneficial *Lactobacillus* strain to survive digestion and went on to incorporate it into a fermented milk beverage known as Yakult. Yakult was sold on the Japanese market from the early 1950s, but today, it is sold in over 25

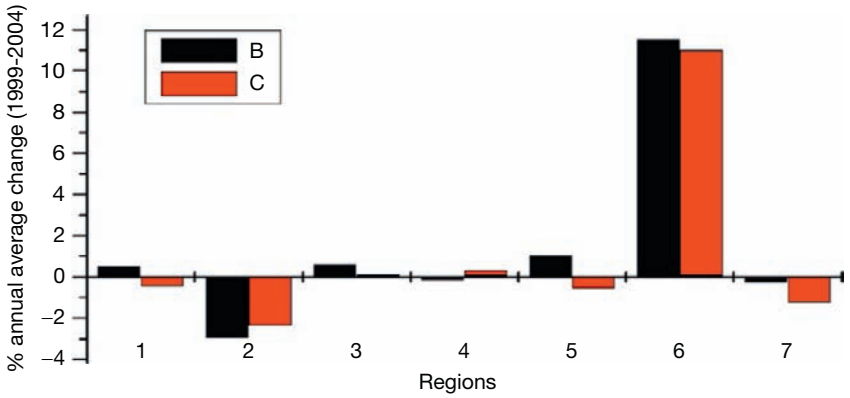

Figure 2 World regional liquid–milk consumption (% annual change, 1999–2004). B, consumption; C, consumption per capita. 1, North America; 2, South America; 3, European Union; 4, Former Soviet Union; 5, South Asia, 6, Asia; 7, Africa. Adapted from USDA-FAS, ZMP Agra CEAS calculations.

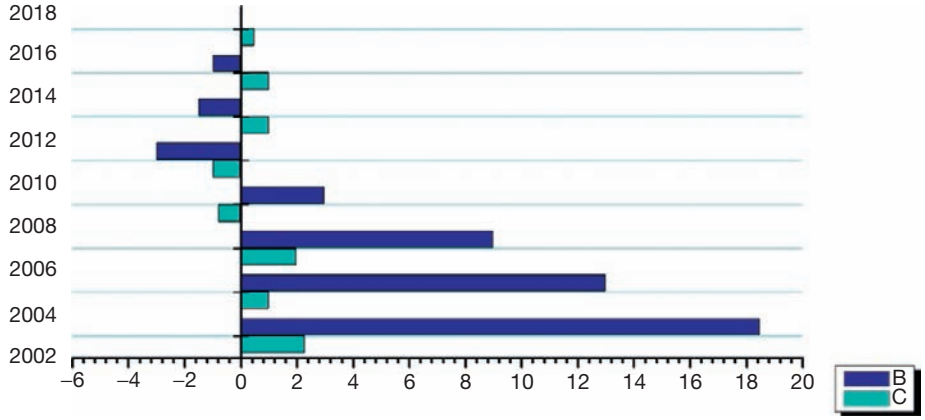

Figure 3 Annual growth prices (%) of probiotic/prebiotic (B) and nonprobiotic/nonprebiotic (C) dairy in EU. Adapted from Euromonitor, 2004 Agra CEAS calculations.

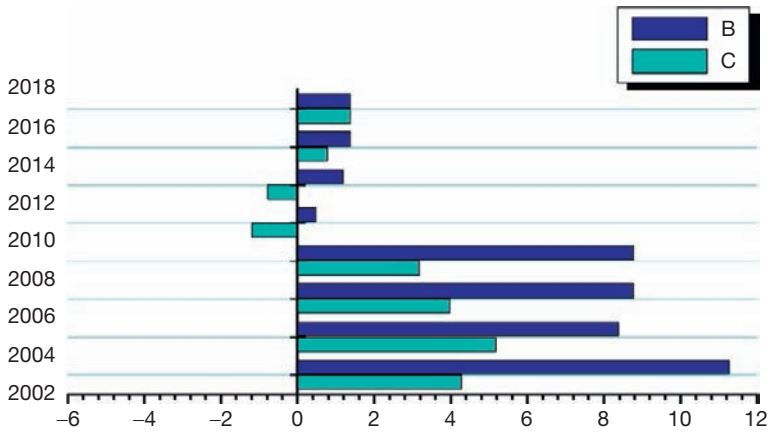

Figure 4 Probiotic (B) versus nonprobiotic (C) market annual growth. Adapted from Euromonitor, 2004 Agra CEAS calculations.

countries worldwide. Since then, considerable attention has been directed on the benefits derived from consumption of milk products containing *Lactobacillus acidophilus*. The earliest work dealt with the use of fermented acidophilus milk to treat intestinal infections, while more recent studies have focused on other aspects of health or nutritional benefits that might be derived from this organism. Such studies suggest that consumption of milk products containing *Lactobacillus acidophilus* has indeed the potential for several health benefits (Table 2) such as preventing or controlling intestinal infections,

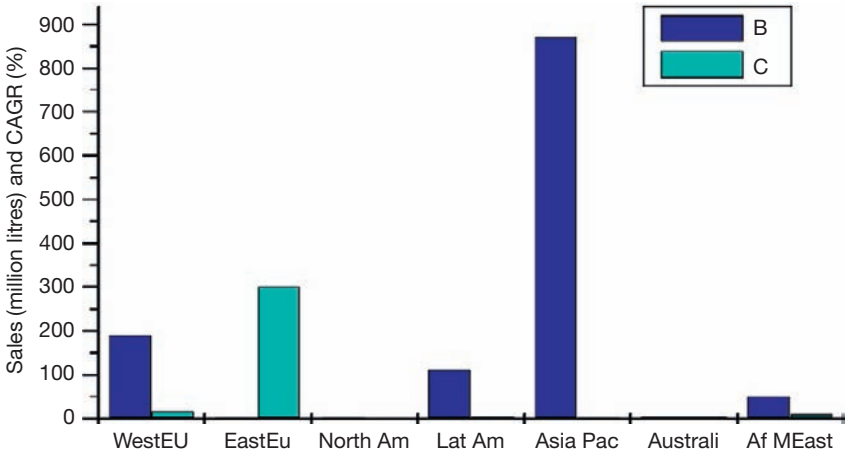

Figure 5 World fermented dairy drink sales (2003), million liters, and CAGR (%) (CAGR – compound annual growth rate 1998–2003). Adapted from Euromonitor, 2004 Agra CEAS calculations.

Table 2 Most common mechanisms for probiotic functionality

Antimicrobial activity
Colonization resistance
Immune effects
Adjuvant effect
Cytokine expression
Stimulation of phagocytosis by peripheral blood leucocytes
Secretory IgA
Antimutagenic effects
Antigenotoxic effects
Influence on enzyme activity
Enzyme delivery

Table 3 Some proposed mechanisms whereby probiotic bacteria might influence the incidence of cancer, particularly colon cancer

Enhancing host's immune response
Suppression of growth and activities of intestinal microbes that produce carcinogens and promoters by competitive colonization or production of inhibitors (short-chain fatty acids or bacteriocins)
Binding and removal of carcinogens
Production of antimutagenic compounds
Production of butyrate to stimulate programmed cell death of abnormal cells
Inhibition of the conversion of bile salts to secondary bile salts

improving lactose digestion in persons classified as lactose maldigestors, helping control serum cholesterol levels, having potential to modulate the immune system, and exerting anticarcinogenic activity (Table 3).

As the importance of the colonic microbiota in human physiology and metabolism is being recognized, due to advances in molecular techniques and fermentation technology for studying the dynamics of the normal microflora, accurate information concerning the effect of probiotics is increasing.

Consistent scientific research has been undertaken in recent years regarding isolation of LAB strains to prove their health effects potentials. Functional properties of probiotics have been studied, and a few therapeutic applications seem to have been solidly demonstrated, of which the treatment of acute diarrhea and improvement in lactose digestion are probably the most known. Today, food products or supplements containing LAB strains of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* GG, or *Lactobacillus casei* Shirota are regular components of the diet of many. There is a popular perception, eventually supported by scientific data, that these bacteria exert a positive health effect in the consumer, although the mechanism by which they exert said beneficial effects is, in many cases, not very clear yet. Some of the reasons for such situation are the current (still) very limited understanding of the activities of the intestinal microbiota; the fact that health effects of probiotics are not generalized, but instead strain-specific (see Table 4); the need to definitely determine the metabolites that impact health and provide reliable biomarkers for the selection of functional diets or ingredients such as probiotics; and the fact that it is difficult to obtain optimal physiological samples from the intestine; thus, compiling large and reliable data from human trials toward established efficacy of probiotics is difficult and expensive. In a symposium sponsored by the American Nutritional Society in 2012, a designated 'panel of independent academic scientists with proved track records in probiotics research' made the final following statement: "Regulatory agencies in US and Europe must continue to protect consumers from misleading labeling and advertising; ... these agencies currently believe that the scientific evidence for probiotics does not meet the standards for approved health claims. Therefore, investigators wishing to conduct studies that will substantiate health claims for probiotics should carefully consider the study design, study populations, and select relevant experimental outcome."

Acidophilus milk is used commonly as a dietary supplement to alter the bacterial flora of the GIT in the treatment of certain digestive disorders and is one of the many dairy products considered as ideal vehicles for delivering probiotics to the human gut. In fact consumption of acidophilus milk is often prescribed by many physicians to persons suffering from either constipation or diarrhea and also for persons who experience

Table 4 Probiotic strains and some specific clinically shown health benefits

Probiotic strain	*Clinical benefits*
Lactobacillus acidophilus NCFM	Lowers fecal enzyme activity, improves lactose absorption, and produces bacteriocin
Lactobacillus rhamnosus GG	Plays a role in the prevention of antibiotic- and rotavirus-associated diarrhea
Lactobacillus casei Shirota	Helps in preventing intestinal disturbance, balancing intestinal flora, and lowering fecal enzyme activity
Lactobacillus reuteri	Colonizes the intestinal tract, shortens the duration of rotavirus diarrhea, and helps in immune enhancement
B. animalis BB-12	Plays a role in treating rotavirus-associated diarrhea and balancing intestinal flora

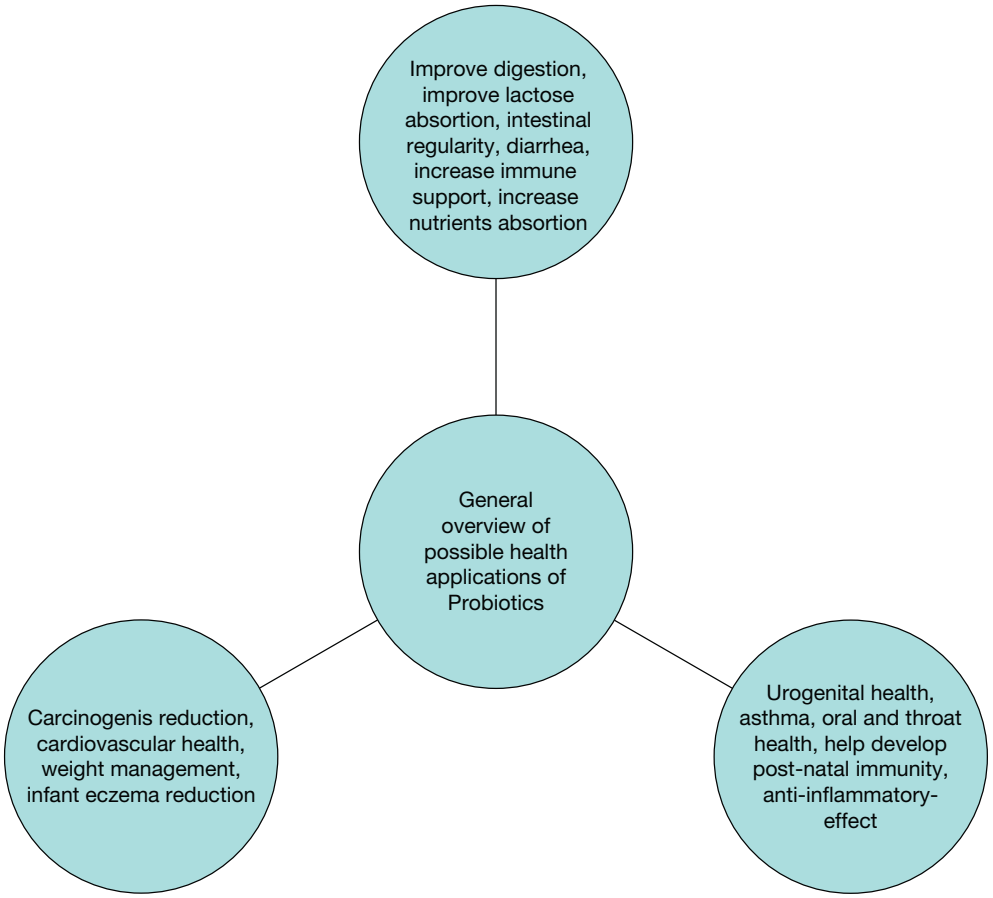

Figure 6 A broad health claims attributed to probiotic bacteria or probiotic-containing products.

intestinal distress on consuming ordinary milk, due to lactose malabsorption. Acidophilus milk is acidic in flavor as its acidity ranges from 1.5% to 2.0%.

Probiotics Use in GI Tract Conditions

The intestinal microbiota of an individual originates from the host genetics, environment factors, and microbiological influences. This culminates in a stable community of microorganisms that is unique to that individual. Although intestinal microbes are fairly stable through time, transitions occur at weaning and again in the elderly. Colonizing microbiota can be impacted by antibiotics, diet, immunosuppression, intestinal cleansing, and other factors, even though, in general, it tends to return to normal, following cessation of the disturbing factor. Probiotic research has been focused on their use in the treatment or preventative applications to solve GI health problems, due to the foreseen potential they may play in accelerating the normalization of a disturbed microbiota (see Figure 6).

The release of bacteriocins is one of positive factor associated with consumption of probiotic bacteria, as these peptides may help in protecting against proliferation of undesirable or pathogenic bacteria in GIT or even in the food product, making it safer, and many LAB bacteriocins have been so far isolated. Ingestion of *Lactobacillus acidophilus* is also expected to have a healthy effect on the consumer as it contributes to lowering the levels of undesirable bacteria in the GIT, via competition for nutrients and colonizing sites between the probiotic bacteria and the undesirable bacteria.

Lactose intolerance

The most accepted health effect of dairy products fermented with LAB is associated with their lower content in lactose as during fermentation, the bacteria feed on the lactose sugar in the milk, breaking some of it down. For lactose-intolerant people, that means that their bodies may have an easier time digesting this milk, even considering that some fermented milk may still contain milk sugar that can cause gas and bloating in more sensitive people.

Infant diarrhea

Many studies have been carried out concerning prevention or cure of infantile diarrhea, a serious problem particularly in developing countries. The best evidence of the usefulness of probiotics in the prevention of this condition comes from studies with *Lactobacillus rhamnosus* that showed that its consumption reduces the risk of infants developing diarrhea. Data from other of clinical trials also confirm a strong evidence of the role of probiotics in resolving the condition of infant diarrhea.

Crohn's disease, ulcerative colitis, pouchitis, and irritable bowel syndrome

These are inflammatory diseases associated with the GIT, whose cause in many cases is not totally understood. Research has been carried out using probiotic therapy to solve them. In general, the results regarding the efficacy of probiotics in the treatment of these conditions are regarded as either weak or essentially only promising, requiring larger-scale clinical trials.

Antibiotic-associated diarrhea and Clostridium difficile

Besides the recent worries that most antibiotics are becoming ineffective to treat many infectious diseases, oral antibiotics can and often do disturb the GI microflora, allowing for opportunistic pathogens to colonize the gut, most probably because elimination of the resident microflora by antibiotics will cause a decrease in secretion of protective antimicrobial substances, increase local pH, and allow for physical colonization by pathogens.

Clinical data seem to suggest that although there may be some benefits associated with the use of such probiotics as *Streptococcus boulardii*, *Enterococcus faecium*, and bifidobacteria, further larger-scale trials are needed to determine their efficacy in the treatment of antibiotic-associated diarrhea.

Probiotic Use in Nongastrointestinal Conditions

Urogenital infections

The majority of probiotic trials are focused on diseases related to the GI tract. However, probiotics such as *Lactobacillus*-containing supplements have been suggested for the treatment and prophylaxis of bacterial urogenital infections to restore commensal vaginal bacteria. Recent reviews found that despite enhanced cure rates reported in some studies, concerns about product stability and limited documentation of strain-specific effects prevent recommendations for the use of *Lactobacillus*-containing probiotics in the treatment of bacterial vaginosis. Also, the results of studies of lactobacilli for the prophylaxis of urinary tract infection remain inconclusive as a result of small sample sizes and the use of unvalidated dosing strategies.

Cancer prevention and treatment

There are some case-controlled studies conducted to evaluate the effects of yogurt or fermented milks on some cancer rates. An inverse relationship between frequency of fermented milk consumption and risk of breast cancer has been reported in France and the Netherlands; yogurt was found to be a protective factor in a case-controlled study of colon cancer incidence in Los Angeles County, and an intervention trial did show that the recurrence rate for superficial bladder cancer was lower for subjects receiving freeze-dried *Lactobacillus casei* Shirota than a placebo.

Several experimental animal studies demonstrated a protective effect of probiotics such as some *Lactobacillus* and *Bifidobacterium* strains or the combination of probiotics and prebiotics (oligofructose) on the establishment, growth, and metastasis of transplantable and chemically induced tumors; a 4-year study of 398 subjects found that *Lactobacillus casei* Shirota decreased the recurrence of a typical colonic polyps. The European Union (EU)-sponsored 'Symbiotic and Cancer Prevention in Humans' project tested a symbiotic (oligofructose plus *Lactobacillus rhamnosus* GG and *B. animalis* subsp. *lactis* Bb12) in patients at risk for colonic polyps. Among several intermediate end points that were used as biomarkers of colon cancer risk, the study found that the symbiotic decreased uncontrolled growth of intestinal cells.

More studies will be important in clarifying the role probiotic products play in cancer rates. For now, in a more general evaluation, a review of epidemiological studies on dairy or fermented foods in general and cancer (prostate, breast, colorectal, and others) suggests that there is no significant association (positive or inverse) between dairy food consumption and any cancer.

The positive actions of probiotics are tied to specific strains and specific doses; thus, the primary goal with any probiotic product is to deliver the right bacteria in ideal numbers to the right place in the body.

A careful selection of specific strains of *Lactobacillus acidophilus* combined with proper production and handling procedures is in general necessary to ensure that desired benefits are provided to consumers. In general, the mechanisms by which probiotics exert their effects are largely unknown but may involve modifying gut pH, antagonizing pathogens through production of antimicrobial and antibacterial compounds, competing for pathogen binding and receptor sites and for available nutrients and growth factors, stimulating immunomodulatory cells, and producing lactase, an important enzyme to digest lactose. On the other hand, in many cases, some of the beneficial effects exerted by probiotics have been so far shown to occur in animal models, thus still requiring fully human studies. Figure 6 is a schematic view of the many potential positive health effects attributed to fermented milks.

Although the physiological effects of some probiotic bacteria have been in general accepted, the health effect claims of some so-called probiotic food products are still controversial, to the point that the word 'probiotic' and descriptors such as 'live active cultures' or 'active bacteria' have been banned for

food products by some member states in the EU. Appropriately designed studies and the generation of consistent evidence for specific effects suitably to convince the regulators are required before claims can be approved. In fact, there are many confounding factors contributing to this situation, including the lack of clear direction on what research is required, exclusion of well-conducted studies because they studied patient (not healthy) populations or disease outcomes, difficulty in defining physiological benefits with healthy study subjects, and existence of studies that do not substantiate the physiological benefit (i.e., null studies). So far, the EU Nutrition and Health Claims Regulation have not granted health claims status to probiotics; that is, probiotics in general have not been accepted as having health-promoting outcomes, and while a few claims have been accepted, more than 1500 applications for claims regarding health effects of other species and strains remain unauthorized. The accepted definition of 'probiotic,' as agreed upon by the World Health Organization, is "Live microorganisms which when administered in adequate amounts confer a health benefit on the host," implying an inherent health claim. This means that if consumers are aware of this definition, they would deduce that any yogurt with 'probiotic' on the label, for example, would improve their health, even if the particular strain of bacteria in the yogurt had not been studied and proved to have benefits. One generally accepted probiotic claim is associated with lowering the lactose content of the food products, and beyond that, the term probiotic is still seen by many as describing too many and often nonproved health claims. In general, the position of the European Food Safety Authority (EFSA) is that while some specific bacterial strains have shown proved actions, those actions cannot be extended to all of the strains available in the marketplace labeled as probiotics.

In conclusion, fermented milks generally known as acidophilus milk or probiotics foods, produced via fermentation with LAB species, are now a common part of the diet in many countries. It is generally accepted that the strains used for their processing exert a variety of positive health effects, although the mechanism proposed for mediating these effects are not totally clear yet in most cases. Thus, it seems that probiotics may offer a broad range of potential health benefits, even though the extent of the effect of specific strains on the health of a generally healthy general population remains to be determined. The probiotic theory offers a complex approach to controlling negative metabolic or pathogenic activities of microbes to which we are exposed on a daily basis, representing an exciting opportunity to move toward a more preventative health-care model, expected to reduce health-care costs specially for the aged population. Scientific research and technological developments directed to the development and production of novel fermented dairy products such as acidophilus milk are on the rise. Industrial production of acidophilus milk and other probiotic products require unique processing conditions, although a wide range of fermented milks such as Yakult, Actimel, and LC-1 are already present in the market and their consumption is increasing worldwide. The key idea concerning probiotic products is that in general, more research, specially in the form of well-designed clinical trials, is needed to evaluate the efficacy and safety of probiotics. Many factors are seen as contributing to the lack of agreement on the results observed in probiotic efficacy in human health studies, that is, testing of ineffective strains, use of doses too low to be effective, and poor study design. These and the characterization of the health benefits further, the definition of the 'active principle' in probiotic preparations, and the appropriate and the posterior specific labeling concerning proved health claims are important future challenges to firmly establish acidophilus milk and other probiotic dairy products as true addition to our diets and for the consumer to make an informed consumption choice. The development of pertinent biomarkers and strain-specific genetic probes and determination of the needs of specific target groups of consumers may help meeting such challenges.

A final important remark: the GI microflora composition is unique to each individual, while regular ingestion of acidophilus milk or other probiotic supplements eventually decrease such uniqueness. Should then these supplements be consumed unless in a situation of taking advantage of their potential in accelerating the normalization of a disturbed microbiota?

See also: Fermented Foods: Fermented Milks; Functional Foods; Lactic Acid Bacteria; Probiotics.

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Acids: Natural Acids and Acidulants

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Background

Acids, or acidulants as they are also called, are commonly used in food processing as flavor intensifiers, preservatives, buffers, meat-curing agents, viscosity modifiers, and leavening agents. This article discusses the functions that acidulants have in food systems and reviews the more commonly used food acidulants.

Functions of Acidulants

The reasons for using acidulants in foods are numerous and depend on what the food processor hopes to accomplish. As outlined in the preceding text, the principal reasons for incorporating an acidulant into a food system are flavor modification, microbial inhibition, and chelation.

Flavor Modification

Sourness or tartness is one of the five major taste sensations: sour, salty, sweet, bitter, and umami (the most recently determined). Unlike the sensations of sweetness and bitterness, which can be developed by a variety of molecular structures, sourness is evoked only by the hydronium ion of acidic compounds.

Each acid has a particular set of taste characteristics, which include the time of perceived onset of sourness, the intensity of sourness, and any lingering of aftertaste. Some acids impart a stronger sour note than others at the same pH. As a general rule, weak acids have a stronger sour taste than strong acids at the same pH because they exist primarily in the undissociated state. As the small amount of hydronium ions is neutralized in the mouth, more undissociated acid (HA) molecules ionize to replace the hydronium ions lost from equilibrium (eqn [1]). The newly released hydronium ions are then neutralized until no acid remains. Taste characteristics of the acid are an important factor in the development of flavor systems:

$$HA + H_2O \rightarrow H_3O^+ + A^- HA + H_2O \rightarrow H_3O^{++}A^-. \qquad [1]$$

As pH decreases, the acid becomes more undissociated and imparts more of a sour taste. For example, the intense sour notes of lactic acid at pH 3.5 may be explained by the fact that 70% of the acid is undissociated at this pH, compared with 30% for citric acid. In addition to sourness, acids have nonsour characteristics such as bitterness and astringency, though these are less perceptible. At pH values between 3.5 and 4.5, lactic acid is the most astringent. Acids also have the ability to modify or intensify the taste sensations of other flavor compounds, to blend unrelated taste characteristics, and to mask undesirable aftertastes by prolonging a tartness sensation. For example, in fruit drinks formulated with low-caloric sweeteners, acids mask the aftertaste of the sweetener and impart the tartness that is characteristic of the natural juice. In another example, in substitutes for table salt, acids remove the bitterness from potassium chloride and provide the salty taste of sodium chloride. Other acids, such as glutamic and succinic acids, possess flavor-enhancement properties.

Because acids are rarely found in nature as a single acid, the combined use of acids simulates a more natural flavor. Two acids that are frequently blended together are lactic and acetic.

Microbial Inhibition

Acidulants act as preservatives by retarding the growth of microorganisms and the germination of microbial spores, which lead to food spoilage. The effect is attributed to both the pH and the concentration of the acid in its undissociated state. It is primarily the undissociated form of the acid, which carries the antimicrobial activity: as the pH is lowered, this helps shift the equilibrium in favor of the undissociated form of the acid, thereby leading to more effective antimicrobial activity. The nature of the acid is also an important factor in microbial inhibition: weak acids are more effective at the same pH in controlling microbial growth. Acids affect primarily bacteria because many of these organisms do not grow well below about pH 5; yeasts and molds, in comparison, are usually acid-tolerant.

In fruit- and vegetable-canning operations, the combined use of heat and acidity permits sterilization and spore inactivation to be achieved at lower temperatures; this minimizes the degradation of flavor and structure that generally results from processing.

Acidification also improves the effectiveness of antimicrobial agents such as benzoates, sorbates, and propionates. For example, sodium benzoate – an effective inhibitor of bacteria and yeasts – does not exert its antimicrobial activity until the pH is reduced to about 4.5. Blends of acids act synergistically to inhibit microbial growth. For example, lactic and acetic acids have been found to inhibit the outgrowth of heterofermentative lactobacilli.

Chelation

Oxidative reactions occur naturally in foods. They are responsible for many undesirable effects in the product, including discoloration, rancidity, turbidity, and degradation of flavor and nutrients. As catalysts to these reactions, metal ions such as copper, iron, manganese, nickel, tin, and zinc need to be present in only trace quantities in the product or on the processing machinery.

Many acids chelate the metal ions so as to render them unavailable; the unshared pair of electrons in the molecular structure of acids promotes the complexing action. When used in combination with antioxidants such as butylated

hydroxyanisole, butylated hydroxytoluene, or tertiary butylhydroquinone, acids have a synergistic effect on product stability. Citric acid and its salts are the most widely used chelating agents.

Other Functions

One of the most common reasons for adding acids is to control pH. This is usually done as a means to retard enzymatic reactions, to control the gelation of certain hydrocolloids and proteins, and to standardize pH in fermentation processes. In the first example, the lowering of pH inactivates many natural enzymes that promote product discoloration and development of off-flavors. Polyphenol oxidase, for example, oxidizes phenols to quinones, which subsequently polymerize, forming brown melanin pigments that discolor the cut surfaces of fruits and vegetables. The enzyme is active between pH 5 and 7 and is irreversibly inactivated at a pH of 3 or lower. In the second example, acidification to 2.5–3 is required for high-methoxyl pectins to form gels. Because pH influences the gel-setting properties and the gel strength obtained, proper pH control is critical in the production of pectin- and gelatin-based desserts, jams, jellies, preserves, and other products. In the final example, standardization of pH is done routinely in fermentation processes, such as wine making, to ensure optimum microbial activity and to discourage growth of undesirable microbes. Acids are also added postfermentation to stabilize the finished wine.

Acid salts function as buffers in various systems. For example, in confectionery products, acid salts are used to control the inversion of sucrose into its constituents, glucose and fructose, the latter being hygroscopic. The resulting lower concentration of fructose yields a less hygroscopic food system and a longer shelf life.

Acids are a major component of chemical leavening systems, where they remain nonreactive until the proper temperature and moisture conditions are attained. The gas evolved by reaction of the acid with bicarbonate produces the aerated texture that is characteristic of baked products such as cakes, biscuits, doughnuts, pancakes, and waffles. The onset and the rate of reaction of these compounds are controlled by such factors as the solubility of the acid, the mixing conditions for preparing the batter, and the temperature and moisture of the batter. Many chemical leavening systems are based on salts of phosphoric and tartaric acids. Acids have also been used for other purposes. For example, they are added to chewing gum to stabilize aspartame and to cheese to impart favorable textural properties and sensory attributes.

Commonly Used Acidulants

Among the most widely used acids are acetic, adipic, citric, fumaric, lactic, malic, phosphoric, and tartaric acids. Gluconoδ-lactone, though not itself an acid, is regarded as an acidulant because it converts to gluconic acid under high temperatures.

Acetic Acid

Acetic acid is the major characterizing component of vinegar. Its concentration determines the strength of the vinegar, a value termed 'grain strength,' which is equal to 10 times the acetic acid concentration. Vinegar containing, for example, 6% acetic acid has a grain strength of 60 and is called 60-grain. Distillation can be used to concentrate vinegar to the desired strength.)

Fermentation conducted under controlled conditions is the commercial method for vinegar production. Bacterial strains of the genera *Acetobacter* and *Acetomonas* produce acetic acid from alcohol, which has been obtained from a previous fermentation involving a variety of substrates such as grain and apples. Vinegar functions in pH reduction, control of microbial growth, and enhancement of flavor. Use in a variety of products, including condiments such as ketchup, mustard, mayonnaise, and relish; salad dressings; marinades for meat, poultry, and fish; bakery products; soups; and cheeses has been found. Pure (100%) acetic acid is called glacial acetic acid because it freezes to an icelike solid at 16.6 °C. Though not widely used in food, glacial acetic acid provides acidification and flavoring in sliced, canned fruits and vegetables, sausage, and salad dressings.

Adipic Acid

Adipic acid, a white, crystalline powder, is characterized by low hygroscopicity and a lingering, high tartness that complements grape-flavored products and those with delicate flavors. The acid is slightly more tart than citric acid at any pH. Aqueous solutions of the acid are the least acidic of all food acidulants and have a strong buffering capacity in the pH range 2.5–3.0.

Adipic acid functions primarily as an acidifier, buffer, gelling aid, and sequestrant. It is used in confectionery, cheese analogs, fats, and flavoring extracts. Because of its low rate of moisture absorption, it is especially useful in dry products such as powdered fruit-flavored beverage mixes, leavening systems of cake mixes, gelatin desserts, evaporated milk, and instant puddings.

Citric Acid

The most widely used organic acid in the food industry, citric acid, accounts for more than 60% of all acidulants consumed. It is the standard for evaluating the effects of other acidulants. Its major advantages include its high solubility in water; appealing effects on flavor, particularly its ability to deliver a 'burst' of tartness; strong metal chelation properties; and the widest buffer range of the food acids (2.5–6.5).

Citric acid is naturally present in animal and plant tissues and is most abundantly found in citrus fruits including the lemon (4–8%), grapefruit (1.2–2.1%), tangerine (0.9–1.2%), and orange (0.6–1.0%).The principal method for commercial production of the acid is fermentation of corn. Formerly, the acid had been obtained by extraction from citrus and pineapple juices. Citric acid is available in a liquid form, which solves processing problems related to incorporating the acid into a food system, such as predissolving citric acid crystals and caking or crystallate deposits on processing equipment. Also available are granulated forms that allow the particle size to be customized to meet the particular need.

Citric acid has numerous applications. It is commonly added to nonalcoholic beverages where it complements fruit flavors, contributes tartness, chelates metal ions, acts as a preservative, and controls pH so that the desired sweetness characteristics can

be achieved. Sodium citrate subdues the sharp acid notes in highly acidified carbonated beverages; in club soda, it imparts a cool, saline taste and helps retain carbonation. The acid is also used in wine production both prior to and after fermentation for adjustment of pH; in addition, because of its metal-chelating action, the acid prevents haze or turbidity caused by the binding of metals with tannin or phosphate. The calcium salt of citric acid is used as an anticaking agent in fructose-sweetened, powdered soft drinks, where it neutralizes the alkalinity of other ingredients that support browning, such as magnesium oxide and tricalcium phosphate.

Citric acid is used in confectionery and desserts. In hard confectionery, buffered citric acid imparts a pleasant tart taste; it is added to the molten mass after cooking, as this prevents sucrose inversion and browning. Citric acid is used in gelatin desserts because it imparts tartness, acts as a buffering agent, and increases the pH for optimum gel strength.

Low levels of the acid, ranging from 0.001 to 0.01%, work with antioxidants to retard oxidative rancidity in dry sausage, fresh pork sausage, and dried meats. Citric acid is also used in the production of frankfurters: 3–5% solutions are sprayed on the casings after stuffing and prior to smoking to aid in their removal from the finished product. Used at 0.2% in livestock blood, sodium citrate and citric acid act as anticoagulants, sequestering the calcium required for clot formation so that the blood may be used as a binder in pet foods.

In seafood processing, citric acid inactivates endogenous enzymes and promotes the action of antioxidants, resulting in an increased shelf life. Citric acid also chelates copper and iron ions that catalyze the oxidative formation of off-flavors and fishy odors associated with dimethylamine. In processed cheese and cheese foods, citric acid and sodium citrate function in emulsification, buffering, flavor enhancement, and texture development. Sodium citrate is also combined with sodium phosphate as a customized emulsification salt for processed cheese. Cogranulation of citric acid with malic and fumaric acids yields new tart flavor profiles.

Fumaric Acid

The extremely low rate of moisture absorption of this acid makes it an important ingredient for extending the shelf life of powdered food products such as gelatin desserts and pie fillings. Fumaric acid can be used in smaller quantities than citric, malic, and lactic acids to achieve similar taste effects.

Fermentation of glucose or molasses by certain *Rhizopus* spp. is the method used to produce fumaric acid commercially. The acid is also made by isomerization of maleic acid with heat or a catalyst and is a by-product of the production of phthalic and maleic anhydrides. Fumaric acid is also made in particulate form, where the acid makes up about 5–95% of the particulate, with the remainder being other acids such as malic, tartaric, citric, lactic, ascorbic, and related mixtures.

Applications of fumaric acid include rye bread, jellies, jams, juice drinks, candy, water-in-oil emulsifying agents, reconstituted fats, and dough conditioners. In refrigerated biscuit doughs, the acid eliminates crystal formations that may occur in all-purpose leavening systems. In wine, it functions as both an acidulant and a clarifying aid, although it does not chelate copper or iron.

Glucono-δ-Lactone (GDL)

A natural constituent of fruits and honey, GDL is an inner ester of D-gluconic acid. Unlike other acidulants, it is neutral and gives a slow rate of acidification. When added to water, it hydrolyzes to form an equilibrium mixture of gluconic acid and its δ- and γ-lactones. The acid formation takes place slowly when cold and accelerates when heated. As GDL converts to gluconic acid, its taste characteristics change from sweet to neutral with a slight acidic aftertaste.

GDL is produced commercially from glucose by a fermentation process that uses enzymes or pure cultures of microorganisms such as *Aspergillus niger* or *Acetobacter suboxydans* to oxidize glucose to gluconic acid. GDL is extracted by crystallization from the fermentation product, an aqueous solution of gluconic acid and GDL.

Because of its gradual acidification, bland taste, and metal-chelating action, GDL has found application in mild-flavored products such as chocolate products, tofu, milk puddings, and creamy salad dressings. In cottage cheese prepared by the direct-set method, GDL ensures development of a finer-textured finished product, void of localized denaturation. It also shortens production time and increases yields. In cured-meat products, GDL reduces cure time, inhibits growth of undesirable microorganisms, promotes color development, and reduces nitrate and nitrite requirements.

Lactic Acid

Lactic acid is one of the earliest acids to be used in foods. It was first commercially produced about 60 years ago, and only within the past two decades has it become an important ingredient. The mild taste characteristics of the acid do not mask weaker aromatic flavors. Lactic acid functions in pH reduction, flavor enhancement, and microbial inhibition. Two methods are used commercially to produce the acid: fermentation and chemical synthesis. Most manufacturers using fermentation are in Europe.

Confectionery, bakery products, beer, wine, beverages, dairy products, dried egg whites, and meat products are examples of the types of products in which lactic acid is used. The acid is used in packaged Spanish olives where it inhibits spoilage and further fermentation. In cheese production, it is added to adjust pH and as a flavoring agent.

Malic Acid

This general-purpose acidulant imparts a smooth, tart taste that lingers in the mouth, helping to mask the aftertastes of low-caloric or noncaloric sweeteners. It has taste-blending and flavor-fixative characteristics and a relatively low melting point with respect to other solid acidulants. The low melting point allows it be homogeneously distributed into food systems. Compared with citric acid, malic acid has a much stronger apparent acidic taste. As DL-malic acid is the most hygroscopic of the acids, resulting in lumping and browning in dry mixes, the encapsulated form of this acid is preferred for dry mixes.

Malic acid occurs naturally in many fruits and vegetables and is the second most predominant acid in citrus fruits, many

berries, and figs. Unlike the natural acid, which is levorotatory, the commercial product is a racemic mixture of D-isomers and L-isomers. It is manufactured during catalytic hydration of maleic and fumaric acids and is recovered from the equilibrium product mixture.

The acid has been used in carbonated beverages, powdered juice drinks, jams, jellies, canned fruits and vegetables, and confectionery. Its lingering profile enhances fruit flavors such as strawberry and cherry. In aspartame-sweetened beverages, malic acid acts synergistically with aspartame so that the combined use of malic and citric acids permits a 10% reduction in the level of aspartame. In frozen pizza, malic acid is used to lower the pH of the tomato paste without chelating the calcium in the cheese, as would citric and fumaric acids. This application improves the texture of the frozen pizza.

Phosphoric Acid

The second most widely used acidulant in food, phosphoric acid, is the only inorganic acid to be used extensively for food purposes. It produces the lowest pH of all food acidulants. Phosphoric acid is produced from elemental phosphorus recovered from phosphate rock.

The primary use of the acid is in cola, root beer, and other similar-flavored carbonated beverages. The acid and its salts are also used during production of natural cheese for adjustment of pH; phosphates chelate the calcium required by bacteriophages, which can destroy bacteria responsible for ripening. As chemical leavening agents, phosphates release gas upon neutralizing alkaline sodium bicarbonate; this creates a porous, cellular structure in baked products. The main reason for incorporating phosphates into cured meats such as hams and corned beef is to increase retention of natural juices; the salts are dissolved in the brine and incorporated into the meat by injection of brine, massaging, or tumbling. When used in jams and jellies, phosphoric acid acts as a buffering agent to ensure a strong gel strength; it also prevents dulling of the gel color by sequestering prooxidative metal ions.

Tartaric Acid

Tartaric acid is the most water-soluble of the solid acidulants. It contributes a strong tart taste that enhances fruit flavors, particularly grape and lime. This dibasic acid is produced from potassium acid tartrate, which has been recovered from various by-products of the wine industry, including press cakes from fermented and partially fermented grape juice, lees (the dried, slimy sediments in wine fermentation vats), and argols (the crystalline crusts formed in vats during the second fermentation step of wine making). The major European wine-producing countries, Spain, Germany, Italy, and France, use more of the acid than the United States.

Tartaric acid is often used as an acidulant in grape- and lime-flavored beverages, gelatin desserts, jams, jellies, and hard sour confectionery. The acidic monopotassium salt, more commonly known as 'cream of tartar,' is used in baking powders and leavening systems. Because it has limited solubility at lower temperatures, cream of tartar does not react with bicarbonate until the baking temperatures are reached; this ensures maximum development of volume in the finished product.

See also: Canning: Process of Canning.

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Acids: Properties and Determination

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Background

In very general terms, an acid is a compound that contains or produces hydrogen ions in aqueous solutions, has a sour taste, and turns blue litmus paper red. A more comprehensive definition, given by the US chemist G.N. Lewis, states that acids are substances that can accept an electron pair or pairs, and bases are substances that can donate an electron pair or pairs. This definition, applicable to both nonaqueous and aqueous systems, requires that an acid be either a positive ion or a molecule with one or more electron-deficient sites with respect to a corresponding base.

The definition most widely used to describe acid–base reactions in dilute solution is one that was proposed independently by two scientists in 1923 – the Danish chemist J.N. Brønsted and the US chemist T.M. Lowry. The Brønsted–Lowry theory defines an acid as a proton donor, that is, any substance (charged or uncharged) that can release a hydrogen ion or proton. A base is defined as a proton acceptor or any substance that can accept a hydrogen ion or proton.

This article discusses the physicochemical properties of acids and describes several methods for their analysis.

Strong Versus Weak Acids

The strength of a Brønsted–Lowry acid depends on how easily it releases a proton or protons. In strong acids, owing to their weaker internal hydrogen bonds, the protons are loosely held. As a result, in aqueous solutions, almost all of the acid reacts with water, leaving only a few unionized acid molecules in the equilibrium mixture. The reaction takes place according to eqn [1]:

$$HA + H_2O \rightleftharpoons H_3O^+ + A^- HA + H_2O \rightleftharpoons H_3O^{++}A^- \qquad [1]$$

In this equation, HA represents the undissociated acid, H_3O^+ the hydronium ion formed when a proton combines with one molecule of water, and A^- the conjugate base of HA.

Unlike strong acids, weak acids exist largely in the undissociated state when mixed with water, since only a small percentage of their molecules interact with water and dissociate. Most acids found in foods, including acetic, adipic, citric, fumaric, malic, phosphoric, and tartaric acids and glucono-δ-lactone, are classified as weak or medium strong acids.

Physicochemical Properties

Physicochemical properties, including the ionization constant, pH, the apparent dissociation constant (pK_a), and buffering capacity, are discussed in the succeeding text and are listed in Table 1.

Ionization Constant

The tendency for an acid or acid group to dissociate is defined by its ionization constant, also denoted as pK_a. The ionization constant, given at a specified temperature, is expressed as

$$K_a = \frac{[H_3O^+][A^-]}{[HA]} \qquad [2]$$

where the brackets designate the concentration in moles per liter. The ionization constant is a measure of acid strength: the higher the K_a value, the greater the number of hydrogen ions liberated per mole of acid in solution and the stronger the acid.

Acids with more than one transferable hydrogen ion per molecule are termed 'polyprotic' acids. Monoprotic or monobasic acids are those that can liberate one hydrogen ion, such as acetic acid and lactic acid. Those containing two transferable hydrogen ions are called diprotic or dibasic acids and include adipic acid and fumaric acid. Acids such as citric acid and phosphoric acid, which have three transferable hydrogens, are called triprotic or tribasic acids. Ionization of polyprotic acids occurs in a stepwise manner with the transfer of one hydrogen ion at a time. Each step is characterized by a different ionization constant.

pH

Measurement of acidity is an important aspect of ascertaining the safety and quality of foods. Such measurements are given in terms of pH, which is defined as the negative logarithm of the hydronium ion concentration (strictly, activity):

$$pH = \log_{10} \frac{1}{[H_3O^+]} = -\log_{10}[H_3O^+] \qquad [3]$$

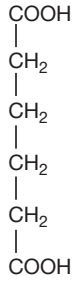
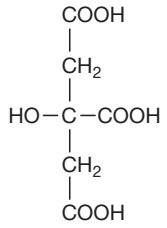
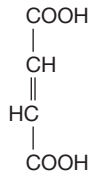
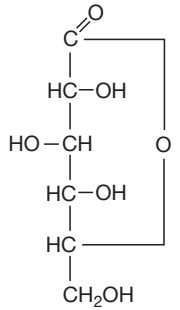
The lower the pH value, the higher the hydrogen ion concentration associated with it. A pH value of <7 indicates a hydrogen ion concentration $>10^{-7}$ M and an acidic solution; a pH value of more than 7 indicates a hydrogen ion concentration of $<10^{-7}$ M and a basic solution. When the hydronium and hydroxide ions are equal in concentration, the solution is described as neutral.

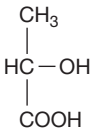
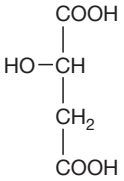
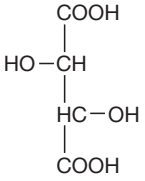
It is also important to note that, because the pH scale is logarithmic, a difference of one pH unit represents a tenfold difference in hydrogen ion concentration.

pK_a

The term pK_a is defined as the negative logarithm of the dissociation constant:

Table 1 Structure, ionization constant, pK_a, and key physical and chemical properties of acidulants

Acid	*Structure*	*Ionization constant(s)*	*pK_a*	*Physical form*	*Melting point (°C)*	*Solubility (g per 100 ml of water)*	*Hygroscopicity*	*Taste characteristics*
Acetic acid	$CH_3COOHCH_3COOH$	1.76×10^{-5} at 25 °C	4.76	Clear, colorless liquid	−8.5	Soluble	na	Tart and sour
Adipic acid		$K_1 = 3.71 \times 10^{-5}$	4.43	Crystalline powder	152	1.9 g at 20 °C	Low level of hygroscopicity	Smooth lingering tartness; complements grape flavors
		$K_2 = 3.87 \times 10^{-6}$ at 25 °C	5.41			83 g at 90 °C		
Citric acid		$K_1 = 7.10 \times 10^{-4}$	3.14	Crystalline powder			Moderately hygroscopic	Tart; delivers a 'burst' of flavor
		$K_2 = 1.68 \times 10^{-5}$	4.77					
		$K_3 = 6.4 \times 10^{-7}$ at 25 °C	6.39					
Anhydrous					153	181 g at 25 °C		
Hydrous					135–153	208 g at 25 °C		
Fumaric acid		$K_1 = 9.30 \times 10^{-4}$	3.03	White granules or crystalline powder	286	0.5 g at 20 °C	Nonhygroscopic	Tart; has an affinity for grape flavors
		$K_2 = 3.62 \times 10^{-5}$ at 18 °C	4.44			9.8 g at 100 °C		
Glucono-δ-lactone		1.99×10^{-4} (for gluconic acid)	3.7	White crystalline powder	153	59 g at 25 °C	Nonhygroscopic	Neutral taste with acidic aftertaste, when hydrolyzed

Lactic acid		1.37×10^{-4} at 25 °C	3.86	Liquid; also available in dry form	16.8	Very soluble	na	Acrid
Malic acid		$K_1 = 3.9 \times 10^{-4}$ $K_2 = 7.8 \times 10^{-6}$ at 25 °C	3.40 5.11	Crystalline powder	132	62 g at 25 °C	Nonhygroscopic	Smooth tartness
Phosphoric acid		$K_1 = 7.52 \times 10^{-3}$ $K_2 = 6.23 \times 10^{-8}$ $K_3 = 2.2 \times 10^{-13}$ K_1 and K_2 at 25 °C; K_3 at 18 °C	2.12 7.21 12.67	Liquid		Very soluble in hot water	na	Acrid
Tartaric acid		$K_1 = 1.04 \times 10^{-3}$ $K_2 = 4.55 \times 10^{-5}$ at 25 °C	2.98 4.34	Crystalline powder	168–170	147 g at 25 °C	Nonhygroscopic	Extremely tart; augments fruit flavors, especially grape and lime

na, not applicable.

Source: *Food Technology*, *Acidulants*: *Ingredients that do more than meet the acid test*. 44(1): 76–83. Chicago, IL: Institute of Food Technologists, with permission.

$$pK_a = \log_{10}\frac{1}{K_a} = -\log_{10}K_a \quad [4]$$

The pK_a corresponds to the pH value at the midpoint of a titration curve developed when one equivalent of weak acid is titrated with base, and the pH resulting from each incremental addition of base is plotted against the equivalents of hydroxide ions added.

The pH of a system is at the pK_a when the concentrations of acid (HA) and conjugate base (A^-) are equal. At the pK_a and, to a lesser extent, in the area extending to within one pH unit on either side of the pK_a, the system resists changes in pH resulting from addition of small increments of acid or base. In other words, at the pK_a, acids and their salts function as buffers.

The number of pK_a that an acid has depends on the number of hydrogen ions it can liberate. Monoprotic acids have a single pK_a, whereas diprotic and triprotic acids have two and three pK_a, respectively.

Strong acids have low pK_a values, and strong bases have high pK_a values.

Buffering Capacity

A solution of a weak acid (or a weak base) and its corresponding salt is called a buffer solution. In these systems, the hydronium ion content is not significantly changed when a small amount of acid or base is added to that solution. The reason that buffer solutions resist appreciable changes in pH can be best illustrated by an example. If a small amount of hydrochloric acid is added to a buffer solution composed of acetic acid and sodium acetate, the protons from the hydrochloric acid would associate with the acetate ions to form unionized molecules of acetic acid. As the newly formed acid molecules ionize, the equilibrium would shift toward forming more hydronium ions (eqn [1]). This would result in only a very slight increase in pH.

Similarly, the addition of a small amount of sodium hydroxide to the same buffer solution would have little effect on pH. Hydroxide ions from the sodium hydroxide would combine with hydronium ions in the equilibrium mixture, forming undissociated molecules of sodium hydroxide. More of the acid molecules would then dissociate to replace the hydronium ions lost; though a new equilibrium system would be created, it would produce only a minimal effect on pH.

The quantity of acid or base that a buffer solution is capable of consuming before a change in pH is realized is termed the 'buffering capacity.' The buffering capacity is defined as the number of moles of strong acid or base required to increase the pH by one unit in 1 l of buffer solution. The buffering capacity of a solution is greatest at its pK_a value where the concentrations of acid and conjugate base are equal.

Analytic Methods

Quantitative determinations of acidity play an important role in ensuring food product quality and stability. Information obtained on acid levels can help in detecting cases of food adulteration, monitoring fermentation processes, and evaluating the organoleptic properties of fermented foods. pH determination, titratable acidity, chromatographic methods, and capillary electrophoresis are procedures commonly employed by the food industry to determine food acids.

pH Determination

pH can be measured by two techniques: colorimetric and potentiometric. The colorimetric method involves adding a suitable indicator to a solution and matching the color of the solution to a standard solution containing the same indicator. This method can estimate pH to the nearest 0.1 pH unit.

A more accurate technique and the one most frequently employed, the potentiometric method, uses a pH meter to determine hydrogen ion concentration. The two electrodes of the meter – a calomel reference electrode and a glass indicator electrode – are immersed in the solution, of known temperature, whose pH is to be measured. The electrode potential of the indicator electrode is linearly related to changes in hydrogen ion concentration and therefore pH.

Titratable Acidity

The total concentration of acid in a solution can be determined by titration. The titration process is performed by placing in a flask a known volume of acid solution whose concentration is unknown. To the flask, a few drops of indicator, for example, phenolphthalein, which is colorless in acid solutions and pink in basic solutions, are introduced. A base solution of known concentration is then gradually added until the acid is completely neutralized. This point is indicated when the solution permanently changes color. The concentration of acid can then be calculated from the volume of base solution used.

The value obtained, called titratable acidity, is an estimate of the total acid in the solution. It accounts for both the free hydronium ions present in the equilibrium mixture and the hydrogen ions released from undissociated acid molecules. For weak acids, the titratable acidity is different from the actual acidity (hydrogen ion concentration), since these compounds exist largely in the undissociated state in solution. For strong acids, however, titratable acidity and actual acidity are virtually the same, since strong acids and their salts are completely ionized in solution.

Chromatographic Methods

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) have almost entirely replaced paper and thin-layer chromatography as methods for identifying and quantifying food acids.

Gas chromatography

GC has been used to analyze organic acids in fruit and fruit juice. Analysis involves preparing volatile derivatives such as methyl esters of the organic acids, prior to their injection into the gas chromatograph. Derivatives are chromatographed on a nonpolar stationary phase column and detected by a flame ionization detector.

By use of GC, malic acid has been shown to be a major constituent of many fruits, including apples, pears, grapes,

peaches, and nectarines, and significant levels of citric acid have been found in citrus fruits, such as orange, lemon, and grapefruit, and in noncitrus fruits, including pears, nectarines, cherries, and strawberries.

High-performance liquid chromatography

HPLC is used more extensively than GC to determine organic acids because the technique requires little or no chemical modification to separate these nonvolatile compounds. Separation is usually done on either a reversed-phase C8 or C18 column or a cation-exchange resin column operated in the hydrogen mode. Acids are detected by either refractive index (RI) or ultraviolet (UV) detectors. RI detection requires prior removal of any sugars present that potentially can interfere with quantification; sugar removal is not required for UV detection at 220–230 nm.

Adulteration of a commercial cranberry juice drink was detected using HPLC when the test yielded different results for organic acids, sugars, and anthocyanin pigments than those obtained for a standard juice drink. Atypical citric and/or malic acid contents and presence of a natural colorant, probably grape skin extract, confirmed that the drink was adulterated.

In wine making, HPLC is used to monitor concentrations of tartaric, malic, succinic, citric, lactic, and acetic acids, which contribute tartness and stability to the finished product. A common approach involves using a column containing a strong cation-exchange resin and eluting the sample with dilute sulfuric acid; the eluant is then analyzed for acids by RI detection. This column has the additional advantage of permitting the simultaneous detection and quantification of ethanol and the monitoring of wine for adulteration with methanol. Organic acids in wine can also be separated using ion chromatography with a conductivity detector.

Capillary Electrophoresis

A relatively new technique, capillary electrophoresis, is also useful for separating and quantifying organic acids in food systems. This technique utilizes an electrical field to separate molecules on the basis of their charge and size. Small volumes of sample, usually a few nanoliters, are injected on to a fused silica capillary tube, which is usually <1 m in length and 50 μm in internal diameter. The ends of the tube are placed in electrolyte reservoirs containing electrodes. A voltage in the range of 20–30 kV is delivered to the electrodes by a power supply and causes the charged molecules to move. Because organic acids are negatively charged, they migrate away from more neutral or positively charged molecules, such as sugars and phenols, respectively. Acids are detected by a UV detector, and the signal is sent to a data collector. The resulting separation is graphically represented as an electropherogram.

Enzymatic Analysis

Enzyme assays provide another means of analyzing acids. For example, an enzymatic assay of L-malic acid uses an NAD(P)-linked malic enzyme and involves spectrophotometrically measuring the absorbance of NADPH, a reaction product, at 340 nm.

See also: Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Food Fraud; pH: Principles and Measurement.

Further Reading

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Acrylamide

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Introduction

Acrylamide (2-propenamide, CAS No. 79-06-01) is a colorless and odorless crystalline solid with a molecular weight of 71.08 and a melting point of 84.5 °C. Acrylamide is soluble in water, acetone, and ethanol but not in nonpolar solvents. Acrylamide has long been used as an intermediate in the production of polyacrylamide, which has many industrial applications, from sewage and wastewater treatment to the formulation of cosmetics. Polyacrylamide is also widely used in molecular biology as a medium for the electrophoresis separation of proteins and nucleic acids. Acrylamide is known to have acute toxicity and it is classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans based on genotoxic effects in animal studies. Acrylamide has become a food safety issue since, in 2002, the Swedish National Food Administration announced the detection of a substantial amount of acrylamide in several heat-treated, carbohydrate-rich foods such as potato chips and crisps, coffee, and bread. From that moment onward, acrylamide has been the object of an extensive, still ongoing, collaborative effort to clarify several aspects of its toxicity, to establish the actual risk from its dietary exposure, and to develop and implement effective mitigation strategies of its occurrence.

Mechanism of Formation

Acrylamide forms in foods upon baking, frying, microwaving, roasting, grilling, broiling, or toasting but not in pouched, boiled, or steamed products. However, the type of cooking is not an issue per se for acrylamide formation as suitable conditions for Maillard reaction development, namely, high temperature and low water activity, are present. Experimental studies with stable isotope-labeled compounds have confirmed that acrylamide mostly forms from asparagine degradation enhanced by carbonyl compounds through Maillard reaction. Although asparagine can thermally decompose to acrylamide by deamination and decarboxylation, the yield of acrylamide formation is considerably higher when a carbonyl source is present (up to 1 mol% in aqueous model systems). Reducing sugars and other carbonyl compounds (i.e., those from oxidized fats) can all contribute to asparagine conversion into acrylamide. However, in most of the cases, reducing sugars are by far the most important contributors to acrylamide formation in potato-based and cereal-based products since they are present in higher concentration. Within the Maillard reaction scheme, several pathways and intermediates can simultaneously lead to acrylamide. Acrylamide may form from (1) the β-elimination of the decarboxylated Amadori compound (from reducing sugars), (2) the Hofmann-type elimination from the Amadori compound (from reducing sugars), or (3) the deamination of 3-aminopropionamide (3-APA; from both reducing sugars and dicarbonyls). Other minor reaction routes for acrylamide formation in foods have been postulated from (1) acrolein, (2) acrylic acid, (3) preformed 3-APA (even in the absence of a carbonyl source), and (4) pyrolysis of wheat gluten, but the practical significance of those minor pathways has not been demonstrated yet. Acrylamide content of foods can be regarded as the result of concomitant reactions of formation and elimination. It starts to form at a temperature >120 °C (248 °F) and accumulates in time since a plateau is reached after which it can decrease because of the exhaustion of (one of) the reactants and/or its elimination. Indeed, acrylamide may undergo Michael-type addition reactions to the electron-deficient vinylic double bond with nucleophiles, such as amino and thiol groups of amino acids and proteins or Diels–Alder addition and radical reactions. On the other hand, the amide group can undergo hydrolysis, dehydration, alcoholysis, and condensation with aldehydes. The acrylamide elimination phase is more evident under severe heating conditions (e.g., coffee or nut roasting).

Occurrence in Foods

Acrylamide is not present in raw ingredients but it is formed during food processing or cooking. Acrylamide typically occurs in plant-derived, carbohydrate-rich heat-treated foods. The highest acrylamide levels have been indeed found in fried and baked potato products, bread and bakery products, and coffee. Significant amounts of acrylamide can also occur in hazelnuts and almonds and sometimes also in foods that have not been subjected to severe heat treatments such as olives and dried fruits. However, the contributions of those products to the overall acrylamide dietary intake have to be considered marginal. Animal-derived heat-treated foods such as meat and fish generally exhibit low or negligible levels of acrylamide because of the low concentrations of precursors (both reducing sugars and free asparagine).

Since 2003, competent authorities and food industry from each member state submit data on the occurrence of acrylamide in food commodities to the Joint Research Centre (JRC) of the European Commission. In April 2009, the European Food Safety Authority (EFSA) published for the first time the results of the monitoring of acrylamide levels in foods in response to a request of the European Commission (Commission Recommendation 2007/331/EC). Data collected in that report concerned with foods sampled in 2007 and were submitted to the commission by 21 member states and Norway. Two additional reports based on food sampled in 2008 and 2009 were published by EFSA in the following two years on a yearly basis. This monitoring has been extended for three more years till 2012. In Table 1, a summary of the results reported by EFSA in 2010 relative to samples collected in 2009 is shown. The highest median

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Table 1 Sample size (*N*), arithmetic mean, 95th percentile (P95), and maximum for results covering foods sampled in 2009 in the framework of the acrylamide European monitoring

Food group	*N*	*Mean (μg kg^{-1})*	*P95 (μg kg^{-1})*	*Max (μg kg^{-1})*
Biscuits				
Crackers	99	195–208	865	1320
Infant	51	88–108	270	430
Not specified	330	128–140	455	2640
Wafers	90	244–246	615	725
Bread				
Crispbread	130	219–223	499	860
Soft bread	110	27–37	90	364
Not specified	84	54–76	310	1460
Breakfast cereals	153	132–142	403	1435
Cereal-based baby food	99	55–70	230	710
Coffee				
Instant	46	591–595	1009	1470
Not specified	14	551	2929	2929
Roasted	172	225–231	500	2223
French fries	469	326–328	810	3380
Jarred baby food	118	32–47	106	677
Other products				
Gingerbread	302	376–384	1682	4095
Muesli and porridge	92	53–82	250	484
Not specified	249	182–204	604	1650
Substitute coffee	34	1502–1504	3976	4300
Potato crisps	388	689–693	2329	4804
Home-cooked potato products				
Deep fried	49	234–241	729	1238
Not specified	136	257–265	914	2762
Oven-baked	72	317	1152	1665

Source: Adapted from EFSA. (2010). Results on acrylamide levels in food from monitoring years 2007–2009 and exposure assessment. *EFSA Journal*, **9**, 2133.

content in acrylamide was reported for the categories 'substitute coffee,' 'instant coffee,' 'French fries,' 'potato crisps,' and 'wafers.'

Acrylamide Dietary Exposure

Dietary exposure to acrylamide has been assessed several times for many countries and many populations or segments of the population. Dietary exposure depends on the acrylamide content of the food products and the amounts consumed. Thus, a food product that exhibits a relative low concentration of acrylamide can still be a major contributor to dietary exposure if consumed frequently or in large amounts. Sources of dietary acrylamide include a broad range of foods commonly consumed daily worldwide and produced in homes, in restaurants, and by catering services or commercially available. In 2005, the World Health Organization (WHO) estimated a dietary intake of acrylamide in the range 0.3–2.0 μg kg^{-1} body weight (BW) per day for the general population and a dietary intake of up to 5.1 μg kg^{-1} BW per day for the 99th percentile. The most recent evaluation of human consumption data and acrylamide dietary exposure has been conducted by Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2011 in eight countries and has led to very similar conclusions. The major food categories mostly contributing to the overall acrylamide intake were fried potatoes (French fries in the United States, contributing for 10–60% of the total acrylamide dietary intake), potato crisps (potato chips in the United States, 10–22%), bread and roll/toast (13–34%), and pastry/sweet biscuits (cookies in the United States, 10–15%). Other food categories (including coffee) would contribute less than 10% of the overall intake. A dietary exposure to acrylamide of 1 μg kg^{-1} BW per day has been taken as representative of the average for the general population, whereas 4 μg kg^{-1} BW per day has been considered representative of the average for consumers with the highest dietary exposure. The most recent EFSA estimation came to very similar conclusions. The mean acrylamide intake for adults (>18 years) in Europe was estimated to range between 0.31 and 1.1 μg kg^{-1} BW per day and the 95th percentile between 0.58 and 2.3 μg kg^{-1} BW per day. 'Fried potatoes' (including 'French fries'), 'soft bread,' and 'roasted coffee' were identified as the major contributors to overall adult acrylamide exposure. Higher mean and 95th percentile values were estimated for adolescent, children, and toddlers. Children have to be included in the highest percentile of the population because of their higher intake of acrylamide-rich foods (French fries and potato crisps). However, a very large variability of acrylamide concentration within the same food categories, brands of the same product, or batch of the same brand was found. This, together with the existence of profound differences in the way the foods are domestically prepared in household or by caterers and the unsuitability of the food frequency questionnaires (FFQs) for cooked foods, may further complicate the accurate estimate of acrylamide dietary intake.

Mitigation Strategies

Significant efforts at a global scale have been produced over the past years to develop strategies reducing acrylamide concentration in the main categories of concern, namely, potato products, cereal-based products (biscuits and bakery wares, breakfast cereals, bread, and crispbread), and coffee. The main problem in the mitigation of acrylamide in final products is that acrylamide form through the same reaction network, that is, Maillard reaction, which contributes to the color, flavor, and texture of the final product. Therefore, it often happens that the reduction of acrylamide concentration is paralleled by a reduction of food organoleptic quality. The mitigation efforts should therefore aim at decoupling acrylamide formation from the Maillard reaction pathways leading to the formation of desired flavor and color.

A number of mitigation strategies have been proposed, but unfortunately, some of them bring about changes in organoleptic properties of foods (excessive browning and generation of off-flavors as result of glycine addition, insufficient browning as result of the reduction in the overall heat load, etc.) that can dramatically affect the final quality and consumers' acceptance. It would be also necessary that the strategies aiming at lowering acrylamide content of foods are complemented by a risk–risk or risk–benefit analysis to reveal all the side effects and their impact on humans. For instance, prolonging yeast fermentation efficiently reduces acrylamide concentration in bread, but it brings about the increase in the levels of 3-monochloropropane-1,2-diol (3-MCPD), a harmful neoformed contaminant. Similarly, the replacement of ammonium bicarbonate with sodium bicarbonate as a raising agent for fine bakery products will reduce acrylamide levels but would concomitantly result in an increase of sodium intake, which has adverse effects on blood pressure.

The information collected by the scientific community and industry has been collated by the FoodDrinkEurope (FDE; formerly termed the CIAA (Confederation of the Food and Drink Industries of the European Union)) in a guidance document termed 'Acrylamide Toolbox.' Its last updated version was released in 2011. The FDE Acrylamide Toolbox is meant to assist manufacturers, caterers, and final consumers in implementing steps for the reduction of acrylamide levels in the final products. Many of the strategies described in the FDE Acrylamide Toolbox have been summarized by Codex Alimentarius in a 'Code of Practice for the Reduction of Acrylamide in Foods' document. Finally, the FDE and the European Commission's Directorate General for Health and Consumer Protection (DG SANCO) in collaboration with national authorities have developed the Acrylamide Pamphlets to assist small- and medium-sized enterprises in the implementation of the FDE Acrylamide Toolbox.

Cereal-Based Products

It has been widely proved that in most of the cereal products (especially those without added sugars), free asparagine rather than reducing sugar levels is the key determinant for acrylamide formation. The accurate selection of flours low in free asparagine is therefore the most obvious action to lower acrylamide levels in cereal products. Indeed, free asparagine is known to largely vary according to grain species and variety. Furthermore, asparagine content increases as the flour extraction rate (amount of bran) increases so that whole-wheat flours are richer in asparagine than more refined flours. However, the substitution of flours richer in asparagine with alternatives lower in asparagine may be not always feasible or advisable. Some products, for instance, have a particular grain as specific characteristic defining their identity, such as rye in crispbread, so that the replacement of rye with another grain species would not be possible without changing the product identity. Analogously, since whole-wheat flour contains more free asparagine, using less whole-wheat or bran compared to endosperm not only would reduce acrylamide content but also would reduce the organoleptic and nutritional properties of the resulting products (whole-wheat flour and bran are richer in dietary fiber and vitamins compared to endosperm). However, a large variation within the same grain variety is reported according to geographic and climatic conditions and, above all, agronomic practice so that it would be difficult to ensure supply of flour with consistent levels of asparagine. However, some agronomic practice has shown to have a strong impact on free asparagine content such as the level of sulfur fertilization. Asparagine level in the crop and thus the risk of acrylamide formation are inversely correlated to the level of sulfur in the soil.

Another change in the recipe that has been proposed is the replacement of ammonium bicarbonate with another leavening agent in biscuits even though the impact of the replacement on the organoleptic properties of the final product has to be assessed case by case. NH_4HCO_3 would increase acrylamide content because it would yield highly reactive dicarbonyl fragments upon baking. The addition of glycine, calcium salts, antioxidant compounds, and organic acids has been also proposed, but in most of the cases, these strategies have been only tested at lab or at pilot scale. Glycine would reduce acrylamide formation by competing with reducing sugars in the very first step of Maillard reaction. The addition of organic acids would decrease acrylamide formation because of the drop in the dough pH, which would slow down the very first step in the Maillard reaction between asparagine and sugars. However, the addition of glycine and organic acid often results in products of unacceptable quality and, in the case of organic acids, as for prolonging yeast fermentation, in products with increased 3-MCPD levels. The addition of ingredients other than flour, such as almonds and other nuts, raisins, and dried fruits, can also increase acrylamide content in cereal products.

Another obvious strategy to reduce acrylamide formation in cereal products is size dilution. Acrylamide is formed almost exclusively in the crust and the crust (area) to crumb (volume) ratio determines the quantity of acrylamide expressed on the total product. Decreasing the surface area to volume ratio by producing a larger bread loaf would therefore reduce acrylamide content in the final product. When the processing is considered, the first option would be the extension of the fermentation for bread, crackers, gingerbread, and similar products. Indeed, in fermented bakery products, acrylamide concentration is generally lower compared to nonfermented analogous products. This is because yeast rapidly assimilates asparagine, aspartic acid, and sugars and also contributes to

lower dough pH. One of the most promising technological options to reduce acrylamide in cereal products is the addition of the enzyme asparaginase (L-asparagine amidohydrolase), which is able to catalyze the hydrolysis of asparagine in aspartic acid and ammonia thus lowering the content of precursor asparagine. Gingerbread, crispbread, and short sweet biscuits and certain cereal-based snacks can be produced with the addition of asparaginase without negative impact on the final product quality. In breakfast cereals, though, the use of low moisture content matrices makes the penetration of the enzyme in the dough difficult, which results in much less efficiency in acrylamide mitigation. Finally, since acrylamide formation is related to the thermal input provided, the optimization of the time–temperature profile would be an obvious mitigation option. Since, as mentioned earlier in the text, acrylamide formation follows the same pathways leading to brown and flavor compounds, the optimization of the time–temperature profile requires the knowledge of the temperature dependence of the rate constants for acrylamide formation and for browning and flavor generation. Alternative baking technologies such as infrared heating, steam baking (during the last minutes of baking), radiofrequency, and vacuum baking (low pressure) are effective in reducing acrylamide, but the impact on sensorial properties may be quite strong.

Potato Products

In potato products, final acrylamide concentrations depend mainly on the level of reducing sugars in the raw potatoes and the intensity of the heat treatment applied. Controlling reducing sugar is therefore the primary measure implemented by the industry to reduce acrylamide levels in potato products. This can be achieved through

(1) the selection of potato varieties and lots with less reducing sugars;
(2) the growth of potato varieties best suited to the local growing conditions, selection of the appropriate fields, and adherence to good agronomic practice;
(3) processing tubers that are mature at the time of harvest because immature tubers tend to have higher reducing sugar levels;
(4) controlling tuber storage conditions, for example, storing tubers not longer than recommended for the specific variety, storing at temperature $>6\,^{\circ}C$ for long-term storage, using sprout suppressants in accordance with the law and with good agronomic practice, and reconditioning at higher temperature over a period of a few weeks.

A prolonged blanching of the potato slices or strips is another option to reduce the content of reducing sugars in the tubers even though it may result in unacceptable adverse effect of flavor and texture in potato crisps. Future opportunities are represented by breeding new potato varieties with lower reducing sugar content and/or less sensitive to cold sweetening and the optimization of agricultural practices to minimize reducing sugars and asparagine. The nitrogen fertilization regime appears to be inversely correlated to the level of reducing sugars in the raw potatoes.

On the other hand, the reducing sugar concentration of potatoes is not always directly proportional to the acrylamide concentration in the final potato product. The concentration of free asparagine and the ratio of asparagine to other free amino acids are also important. Asparagine is the most abundant free amino acid in potatoes, amounting at 0.2–4% of potato dry weight, which represents 20–60% of total free amino acids. A lower ratio of asparagine to other amino acids would lower the amount of acrylamide that forms during the heat treatment because of the competition for reactants during the Maillard reaction. From this perspective, the application of the enzyme asparaginase proved to be ineffective in potato crisps, likely because the enzyme cannot penetrate the potato matrix so as to act on asparagine and in French fries. On the other hand, it seems to be a valuable option in blanched (non-par-fried) chilled potato strips, where a longer enzyme–substrate contact time is allowed, as well as in potato-based products.

Analogous to what is observed in cereal-based products, acrylamide forms on the surface of potato-based products, and the surface area to volume ratio will have an effect on the level of acrylamide in the final product. In French fries, decreasing the surface area to volume ratio by creating thicker strips/sticks of potato could be a valuable mitigation option. However, producers have relatively little room to change the strip cut dimension, which is specified by customers. In potato crisps, which are fried to low moisture content, reducing the surface area to volume ratio can result in higher acrylamide levels as a thicker strip will require a higher thermal input to reach the optimal moisture.

However, the primary technological tool to control acrylamide formation in potato products is the optimization of the time–temperature profile during frying. Since, at low moisture contents, the activation energy for acrylamide formation is larger as compared with the activation energy for browning development, the setup of the end phase of the frying process at a lower product temperature would minimize acrylamide formation. Also, frying up to the highest moisture content, which still gives an acceptable product, is another sensible choice. For French fries, the finished frying/(oven) cooking of the prefried potato product is done by the professional end user or by the consumer at home. The par-frying step does not produce significant levels of acrylamide in the semifinished product, nor does it correlate with the acrylamide level in the final product. In the final preparation stage, it is pivotal that temperature not $>175\,^{\circ}C$ is used and that the fries are cooked to a golden yellow color rather than to a brown color. However, in some European countries, consumers prefer French fries that are cooked to a golden brown color rather than golden yellow. The use of food colorings as an ingredient in industrially produced potato products could be an option to match the consumers' expectations in terms of color in such countries. However, regulatory constraints on the use of food colorings in plain potato products can be found in many countries including the EU.

Coffee

Unlike cereal-based and potato products, few mitigation opportunities exist at the moment for reducing acrylamide content in coffee. The organoleptic properties of roasted coffee

are carefully tuned so that even limited changes in the formulation/processing may result in a product that is unacceptable to the consumers. It appears that free asparagine rather than reducing sugars is the limiting factor for acrylamide formation in such product. However, free asparagine content of green coffee beans does not vary too much so that selection of varieties having low asparagine concentration does not seem a feasible option. In coffee, acrylamide level is strongly related to the severity of the heat treatment, but the final level is inversely correlated to the total heat load being lower in dark roasted coffee. This occurs because acrylamide elimination is predominant at the end of the roasting step. However, roasting to dark color as it happens in Italy or Spain is not always an option because of consumer acceptance particularly in the Nordic countries where a light roasting is preferred.

Risk Assessment

Metabolism

Acrylamide metabolism has been thoroughly investigated by means of toxicokinetic studies in humans, rats, and mice. After ingestion, acrylamide is rapidly absorbed and distributed to many organs and tissues as well as in human placenta and breast milk. Acrylamide can be either oxidized by cytochrome P450 2E1 into the epoxide glycidamide (2,3-epoxypropionamide) or conjugated with glutathione (GSH) by glutathione S-transferases M1, T1, and P1 (GSTM1, GSTT1, and GSTP1). The extent of glycidamide formation is higher for low-dose dietary acrylamide exposure. Both acrylamide and glycidamide can bind *in vivo* hemoglobin (Hb), serum albumins, DNA, and enzymes, glycidamide being more reactive than acrylamide. Glycidamide can be further hydrolyzed by the microsomal epoxide hydrolase (EPHX1) to 2,3-dihydroxypropionamide (glyceramide) and then converted to 2,3-dihydroxypropanoic acid. GSH conjugates of acrylamide and glycidamide are further converted to mercapturic acid conjugates, *S*-(3-amino-3-oxopropyl)cysteine, *N*-acetyl-*S*-(3-amino-3-oxopropyl)-cysteine (AAMA), *N*-acetyl-*S*-(3-amino-3-oxopropyl)-cysteine and its *S*-oxide, *N*-(*R*,*S*)-acetyl-*S*-(3-amino-2-hydroxyethyl-3-oxopropyl)-cysteine (iso-GAMA), and *N*-acetyl-*S*-(1-carbamoyl-2-hydroxyethyl) cysteine, which are excreted with urines. The conversion of acrylamide to glycidamide is therefore thought as the crucial step for the toxicity of acrylamide, whereas the hydrolysis of glycidamide to glyceramide and the conjugation of acrylamide and glyceramide to GSH are regarded as detoxification pathways. Toxicokinetic studies on humans showed that approximately 60% of absorbed acrylamide is excreted in the urine mostly (86%) as GSH conjugates and to a less extent as unchanged acrylamide (4.4%), whereas only negligible amounts of unchanged glycidamide could be found in human urine. Hemoglobin adducts of acrylamide and glycidamide reflect the exposure to acrylamide over the last four months (lifetime of the erythrocytes) and can be regarded as biomarker of long-term exposure to acrylamide. On the other hand, the mercapturic acid metabolites of acrylamide and glycidamide can be regarded as biomarkers of recent acrylamide exposure (from hours up to a few days). The urinary AAMA/GAMA ratio is a measure of the extent of conversion of acrylamide to glycidamide and reflects the internal exposure to the latter. DNA adducts can be regarded as a biomarker of biologically active internal dose of acrylamide but have not been detected yet in humans so far.

Neurotoxicity

Acrylamide is neurotoxic from the high levels of exposure. Acrylamide neurotoxicity is characterized by ataxia and skeletal muscle weakness. The neurotoxicity of acrylamide is especially of concern for workers occupationally exposed to acrylamide through inhalation or dermal absorption. In rats and mice studies, the no observed adverse effect level was estimated ranging from 0.2 to 10 mg kg^{-1} BW per day and is far above dietary exposure.

Carcinogenicity

Since 1994, acrylamide is classified as probable carcinogen by the IARC (group 2A), by the US National Toxicology Program's (NTP) *Report on Carcinogens* as 'reasonably anticipated to be a human carcinogen,' and by the US Environmental Protection Agency (EPA) as 'likely to be carcinogenic to humans.' Acrylamide carcinogenicity has been tested in two early chronic oral lifetime studies in rats and one more recent two-year long study conducted on F344/N Nctr male/female rats and male/female B6C3F1 mice by the US National Center for Toxicological Research (NCTR)/National Toxicology Program (NTP). In this last study, acrylamide was administrated in drinking water ad libitum at four concentrations. The results of the study were generally in agreement with those reported in the earlier studies with a significant increase in thyroid gland adenoma and mesothelioma of the tunica vaginalis of the testes and testicular tumors in male rats and a significant increase in mammary gland fibroma or fibroadenoma, central nervous system tumors, and thyroid gland adenoma or adenocarcinoma in female rats. On the other hand, a different pattern of target organs has been observed in mice.

Currently, there is no plausible mode of action (MoA) for acrylamide-induced tumors to thyroid gland in rats and mice, but proposal on the MoA for mammary glands and testes tumors in rats has been put forward. It is widely accepted that the carcinogenicity of acrylamide would stem from its conversion in mammalians to glycidamide. Glycidamide has been shown to be mutagenic and genotoxic in bacterial and mammalian cells. The acrylamide-induced DNA adduction and consequent mutagenesis have been postulated as the key process in acrylamide carcinogenicity. In contrast, acrylamide without metabolic activation to glycidamide has not been found to be neither genotoxic nor mutagenic at biological relevant concentrations. Glycidamide is considerably more reactive toward DNA and other nucleophiles than acrylamide and may thus give numerous adducts *in vitro* and *in vivo*.

Although the results obtained *in vitro* and *in vivo* experiments support the evidence that acrylamide is genotoxic and carcinogenic, epidemiological data have not yet unambiguously proven that dietary acrylamide exposure can increase cancer risk for humans. Up to date, the epidemiological studies agree in indicating no positive association between total dietary acrylamide intake and the risk of colorectal, bladder, esophageal, prostate, oropharyngeal, laryngeal, pancreatic, gastric, and brain cancer. For renal cell cancer, ovarian cancer,

and breast cancer, the results from epidemiological studies are conflicting. One reason for the conflicting results could be that the relative risk for cancer upon acrylamide dietary exposure is so low even at high exposure levels that no epidemiological studies, albeit well designed, can detect the effect. The second reason might be the inaccurate estimation of acrylamide intake when FFQs are used to assess acrylamide dietary intake. Generally, FFQs are not specifically designed to assess the dietary exposure to acrylamide and do not take into account the way the foods are cooked or prepared at home. Moreover, the wide variability of acrylamide concentration among food categories would reduce the differences between low and high levels of exposure, thus reducing the power of the statistical tests applied.

The margin of exposure (MOE) approach has been used by EFSA and the JECFA for the risk assessment of acrylamide. The MOE is the ratio between a defined point on the dose–response curve for the adverse effect and the human intake. The BMDL (benchmark dose lower confidence limit; the lower limit of the 95% confidence interval for a dose that causes a low, but measurable response) is preferably used as a reference point on the dose–response curve. The MOE is therefore a measure of how close the intake of a specific toxic compound is to the exposure levels that are anticipated to produce adverse effects in humans. From the data provided by the recent NTP bioassay, in its latest evaluation, JECFA calculated an MOE of 310 for average consumers and of 78 for high consumers based on an acrylamide exposure of 1 $\mu g\ kg^{-1}$ BW per day (average consumers) to 4 $\mu g\ kg^{-1}$ BW per day (high consumers) and on a BMDL for the induction of mammary tumors in female rats and an MOE of 180 and 45 for average and high acrylamide exposure based on the BMDL for the Harderian gland tumor in male rats. The MOE as calculated for acrylamide is remarkably lower than the value of 10 000 that would indicate a high concern from a public health point of view and is far below those reported by JECFA for polycyclic aromatic hydrocarbons (25 000 for average consumers) and for the heterocyclic amine PhIP (260 000 for average consumers). Therefore, the dietary exposure to acrylamide for the Western population is considered a potential health risk.

Risk Management

As a genotoxic carcinogen, acrylamide is considered to have no threshold limit of exposure, that is, a single exposure to one molecule of acrylamide can trigger the biological process leading to cancer. The level of such genotoxic carcinogens in foods should conform the principle of 'as low as reasonably achievable.' Despite that, at present, no country has ever established regulatory limits to acrylamide concentration in any food category or in the diet. Risk management of acrylamide in foods has derived from the voluntary collaboration between national regulatory agencies and companies producing acrylamide-containing foods. In Germany, an acrylamide minimization strategy has been developed and implemented as of 2002. This approach is based on signal values that are established for single categories approximately as the 90th percentile within that category. If producers are found to deliver products with an acrylamide content higher than the signal value for the relevant food category, discussions are started as to which mitigation strategy to implement. The acrylamide content of foods is then reviewed annually and the signal value reduced if necessary. The European Commission has implemented a similar strategy. Since 2007, the annual monitoring of acrylamide levels in the EU has been carried out under the Commission Recommendation 2007/331/EC of 3 May 2007 subsequently extended by with a revised food categorization. Based on the occurrence data collected in these years, EC has established indicative values for ten food categories. These values do not have to be intended as safety or regulatory thresholds, but rather to indicate the need for investigating the reasons behind acrylamide levels exceeding the indicative value of the particular category.

See also: Bread: Types of Bread; Carcinogenic: Carcinogenic Substances in Food; Cereals: Types and Composition; Coffee: Types and Production; Maillard Reaction; Potatoes and Related Crops; Risk Assessment of Foods and Chemicals in Foods.

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Adipose Tissue: Structure and Function of Brown Adipose Tissue

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Histology

Brown adipose tissue (BAT) is regarded as an adipose tissue because it shares the capacity of white adipose tissue to store lipids in intracellular droplets still not counted for ectopic fat. The intracellular lipid compartment may be used for storing the excess energy from the circulation, but the storage may be also released rapidly for enhanced cellular respiration. Apart from white adipose tissue, the lipid droplets in BAT are smaller and organized in multilocular shape instead of one droplet in white adipocyte. One large lipid droplet in white adipocyte squeezes the nucleus against the cell membrane (crescent-shaped nucleus), but in brown adipocyte the nucleus appears roundish.

The size of brown adipocyte is small, in average 15–60 μm in diameter, while the size of white adipocyte is 25–200 μm in diameter, owing to the capacity of increasing its size several-fold. The appearance of white adipocyte is round and spherical, but the brown adipocytes are polygonal.

Brown adipocytes include numerous mitochondria, which in part induce the darker color of this tissue compared to white adipose tissue. In fact, rodents have clearly brown-red colored adipose tissue in the interscapular region, and their white adipose tissue is clearly white, while human BAT in the supraclavicular region is colored with orange, and white adipose tissue with light yellow.

The structure of mitochondria in brown adipocytes differs from the mitochondria in white adipocytes. In addition to higher density and number of mitochondria in brown adipocytes, the size of mitochondria is larger. The inner membrane cristae in mitochondria are densely packed, which results in great surface area of the inner membrane.

In addition to cellular histology, BAT is densely vascularized and innervated. A dense capillary network with adrenergic innervation forms the basis for rapid signal transduction and response to stimuli from other parts of the body.

Biochemical Characteristics of BAT

The most typical characteristic of BAT is uncoupling protein 1 (UCP1). The gene and protein expression of UCP1 is tightly related to the function and activation of the tissue. UCP1 function is located at the mitochondrial inner membrane, where it is able to uncouple oxidative phosphorylation from ATP synthesis by affecting proton gradient and producing heat instead of ATP. This capacity denotes that BAT is primarily energy-expending tissue instead of energy-storing tissue. Relative UCP1 expression level is several 100- or 1000-fold in human BAT samples, compared to white adipose tissue samples.

Another factor highly enriched in BAT is PR domain-containing 16 (PRDM16), which has a crucial role in the fate of brown adipocytes arising from precursor cells that express Myf5. PRDM16 controls the skeletal myoblast/brown adipocyte switch from these progenitors and activates BAT phenotype. Other functional markers for brown adipocytes may be PPARγ and PGC-1α, but they are not specific to BAT.

Adrenergic β_3-receptors are abundantly found in brown adipocytes, although other adrenergic receptors exist as well. Functional activity is mainly mediated by β_3-receptors, yet β_1-receptors may also have a minor role in binding norepinephrine released from adrenergic nerve endings during activation of the sympathetic nervous system.

Beige Adipose Tissue

Classical brown adipocytes arise from myogenic lineage under controlled programming during embryogenesis. In a newborn, this kind of BAT is found back in the neck between the shoulder blades and in the thoracic cavity in the mediastinum. Classical BAT has an important role in the regulation of body temperature in infants and small children.

Previously, BAT was thought to vanish after childhood and human adults were regarded to have no functional BAT, although existence of brown-like adipose tissue around neck arteries was shown in autopsies of outdoor workers. The confirmation about functional BAT in adult humans was made less than ten years ago. This was possible due to advanced imaging technology, namely positron emission tomography (PET) combined with computed tomography (CT). PET is a non-invasive imaging tool for the measurement of physiological function in the body, while CT provides information about anatomy and tissue density.

Rapidly, it became evident that BAT in the supraclavicular region in adults is distinct from the classic BAT found in infants. In resting state these adipocytes resemble white adipocytes, but when activated by specific stimuli the adipocytes transdifferentiate to resemble brown adipocytes. These adipocytes – called beige or brite (brown-in-white) adipocytes – are UCP1 positive, but they emerge from a non-Myf5 lineage and exist also in white adipose depots. Gene expression patterns between beige and brown adipocytes are mostly different, but in the human supraclavicular depot they are partly overlapping. Plasticity of beige adipocytes provides a functionally promising target for modification; therefore, the 'renaissance' of BAT has gained interest from obesity research.

Localization of BAT in Rodents and Humans

In rodents, such as in mice and rats, the most prominent site of BAT is located in the interscapular region. This butterfly-shaped depot may be seen with the naked eye if specific activation has been carried out. Why rodents have BAT in this location is not quite clear, but it may be partly explained by the different

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posture when compared to humans. However, rodents show a high capacity of transdifferentiation between white and brown adipose tissue, and rodents have both white and brown adipocytes mixed in several adipose depots. The fat content of the depot may be estimated with magnetic resonance imaging (MRI), and the interscapular brown adipose depot consists of a multilocular (brown) area in the deepest portion, while the surface area of the depot is mainly unilocular (white).

Human newborns have BAT in the back of the neck between the shoulder blades, and in the thoracic cavity in the mediastinum. The amount of BAT in infancy is 1–5% of the body weight, which in a full-term newborn weighing around 3500–4000 g means a mass of 35–200 g of BAT. The amount of BAT remains quite similar through the years, though the location changes.

In adult humans, the most prominent site of BAT is found in the supraclavicular region (Figure 1). This depot extends down to axillar regions and up along carotid arteries. Smaller BAT depots are found along the backbone, around big (e.g., renal) arteries, and in the adrenal area above the kidneys. Human adult BAT is regarded mostly as beige adipose tissue, at least in the supraclavicular region, which is most often the site of sampling of the tissue.

Physiological Function of BAT

Two main structural characteristics that determine the functional differences of BAT apart from white adipose tissue are (1) the multilocular organization of lipid droplets and (2) mitochondria with densely packed cristae. Organization of lipid droplets in multilocular form guarantees that the surface area for lipolysis is as high as possible. This provides a high amount of fatty acids for activated mitochondria. Similarly, densely packed cristae warrant as high a surface area as possible for uncoupling – the great surface area of the mitochondrial inner membrane is related to energy production of the mitochondria. These two characteristics interact with each other in order to rapidly optimize functional activity of the tissue.

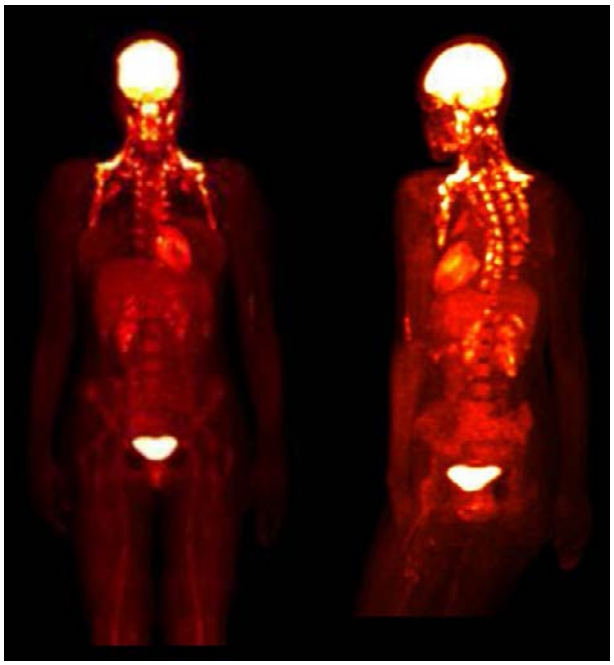

Figure 1 Human brown adipose tissue distribution illustrated with ^{18}FDG-PET/CT uptake in young female subject. Bright accumulation is found in supraclavicular and axillar regions as well as in paravertebral and slightly in adrenal regions. Metabolic activity in neck and axillar region is comparable to brain metabolic activity. The lower bright accumulation is in the urinary bladder due to excretion of the tracer through kidneys.

Thermogenesis – The Main Function of BAT

All cells in the body produce heat as a by-product in the metabolic processes. However, two tissues actively produce heat against cold, namely BAT and skeletal muscles. A cold environment increases whole-body metabolism and oxygen consumption in humans and animals, but the role of BAT is emphasized and is in optimal level during nonshivering thermogenesis, that is, when muscles are not yet producing heat by shivering.

Heat release from the activated tissue is fairly local, especially in humans, but the response may be detected rapidly after initiation of cold exposure on the skin area in the supraclavicular region. Skin temperature begins to rise within minutes, and as cold exposure continues, the skin temperature above the clavicles is not decreasing as much as on other skin areas, for example, in the legs.

The sympathetic nervous system has a crucial role in controlling and mediating the cold sensations from skin to BAT. The cold environment is sensed by skin thermoreceptors from which the signals are mediated to the lateral parabrachial nucleus and further more centrally to the thalamus and somatosensory cortex. In the hypothalamus in the preoptic area, the warm-sensitive neurons are inhibited by cold stimulus and the signal is transferred to peripheral sympathetic nerve endings, which in turn release norepinephrine. Binding of norepinephrine to β_3-adrenergic receptors in brown adipocytes initiates a signal cascade in the cell, which ends up with activation of UCP1 production and increased thermogenesis.

Thermogenic activity may be measured in animals directly from tissue samples. In adult humans, the metabolic activation of brown/beige adipose tissue reflects the activation of thermogenesis. Thus, functional in vivo imaging with PET/CT is the method of choice. With this method, it is possible to follow how much each tissue takes up glucose, oxygen, or fatty acids, and measure blood flow or oxidation rate. Imaging of the molecule of interest (e.g., glucose) requires a label with a positron-emitting radioisotope (e.g., 18-fluoro (^{18}F)), which is chemically synthesized to a glucose-like molecule and then given to the study subject by intravenous injection. The studies are most commonly performed at room temperature, but for the activation of BAT function and thermogenesis, the studies are performed during proper cold exposure.

Cold-Induced Metabolic Changes in BAT

After overnight fasting and in normal room temperature, BAT metabolism is silent and comparable to white adipose tissue

metabolic activity. Cold is one of the most potent and natural activators of BAT metabolism, and whole-body resting energy expenditure increases in line with activation of BAT. The resting metabolic rate in the whole body is increased in cold, specifically in those subjects with functionally active BAT (BAT-positive subjects).

Oxygen consumption in BAT (direct measurement with $^{15}O_2$-PET/CT) is increased during mild cold exposure and is clearly more emphasized in BAT-positive subjects. The cold-induced increment in oxygen consumption is strongly related to blood flow (measured with ^{15}O-H_2O-PET/CT) of the tissue. Therefore, measurement of blood flow may be used as an indirect measure of oxygen consumption in BAT. Blood flow increases twofold during acute cold exposure in healthy lean subjects, which supports the increased oxidative role of BAT in cold. Blood flow in BAT is closely related with glucose uptake and whole-body energy expenditure, suggesting a perfusion-dependent manner of energy expenditure in cold.

Glucose uptake by activated BAT reflects the activity of thermogenesis. This can be measured using glucose analogue ^{18}FDG and PET/CT, and the majority of the recent results by different study centers were achieved using this tracer. During cold exposure, BAT glucose uptake increases approximately tenfold in healthy lean subjects. Based on animal studies, glucose uptake by BAT is estimated to be 10% of the total energy need of the tissue during cold exposure. In animals this contribution is suggested to be significant, and in humans the contribution of glucose uptake as a part of thermogenesis is also considered remarkable – only a few tissues are able to increase their metabolic rate tenfold during short stimulation. In addition, BAT glucose uptake in cold is almost as high as cerebral glucose uptake (Figure 1).

During activated thermogenesis, such as in mild cold exposure, BAT intracellular triglycerides are rapidly hydrolyzed and utilized in activated mitochondria. Intracellular lipid content may be estimated using CT and Hounsfield units, which provide an estimate of tissue density. Cold exposure increases the radiodensity of BAT, suggesting that intracellular triglycerides are the main source of energy for increased thermogenesis. One-third of the intracellular lipid reserve in BAT may be burned in three hours of cold exposure, which corresponds to 200–250 kcal per day energy consumption.

Meal-Induced Thermogenesis

Cold is an effective activator of BAT function, but most humans do not spend a long time in a cold environment. In addition to cold, eating and feeding are related to whole-body thermogenesis, and BAT is considered important in this context. In rodents, activation of the sympathetic nervous system is integrated with feeding, and in fact, BAT thermogenesis seems to precede initiation of feeding. The term 'thermoregulatory feeding' is used to describe the role of BAT thermogenesis, not only in initiation of feeding, but also in regulation of meal size and after balancing with temperature and termination of feeding. The same phenomenon is believed to occur in newborn babies.

A single meal increases uncoupled respiration significantly in BAT during the early postprandial hours in rats. Also, the content of the meal may affect BAT thermogenesis. High-carbohydrate meals seem to increase uncoupled respiration more than equicaloric high-fat meals. However, fatty acid composition may be crucial because a diet rich in polyunsaturated fatty acids results in activation of BAT thermogenesis in mice.

In humans, whole-body energy expenditure, or meal-induced thermogenesis, increases in the postprandial state. Resting metabolic rate may be measured using calorimetry, either directly (chamber) or indirectly (canopy hood). It is questionable how much BAT could contribute to whole-body thermogenesis after a meal, and final answers remain to be seen. Two meals with high-fat and low-carbohydrate content (one in the previous evening and the other in the morning of scanning) lower ^{18}FDG uptake in BAT in humans, while a high-calorie meal rich in carbohydrates increases ^{18}FDG uptake in BAT in healthy lean men. Thus, at least ^{18}FDG (glucose) uptake as a marker of attenuated or increased thermogenesis may be affected by eating in humans. But whether fatty acid oxidation in BAT is affected by meals is not yet known in humans. One of the functional characteristics that BAT may have is clearance of triglycerides from the circulation. This has shown to occur in mice, and it could be important in the postprandial state.

Eating and feeding are related with an increase in plasma insulin concentration, along with several other neural and hormonal signals. Insulin facilitates substrate influx to skeletal muscles, adipose tissue, and the liver. The effects of insulin stimulation on the whole body may be determined using the euglycemic hyperinsulinemic clamp technique, where plasma insulin concentration is artificially elevated to postprandial levels (70–100 U l^{-1}). When ^{18}FDG PET/CT-scanning is performed during insulin stimulation, tissue-specific glucose uptake may be measured in several tissues at the same time. In health, the insulin-stimulated glucose uptake rate in BAT is fivefold when compared to the fasting glucose uptake rate. The skeletal muscle glucose uptake rate is at a similar level, suggesting that BAT is an insulin-sensitive tissue type. The effect of insulin is partly mediated via activation of the sympathetic nervous system, which subsequently activates BAT thermogenesis. Moreover, insulin may promote its effect via the central nervous system in meal-induced thermogenesis.

Regulation of BAT Function

BAT functional activity is under accurate control of the sympathetic nervous system, but the central nervous system also is involved in this regulation. Activation of the sympathetic nervous system contributes to increased lipolysis both in white and brown adipose tissue and induced uncoupling in mitochondria of brown adipocytes. Simultaneously, plasma norepinephrine and free fatty acid concentration elevate. Increased free fatty acids activate UCP1 in brown adipocytes. Activation of thermogenesis in BAT is rapid and powerful to secure warm blood flow to cold sensitive tissue such as brain.

Thyroid hormones thyroxine (T_4) and tri-iodothyronine (T_3) participate in the regulation of BAT function as brown adipocytes display thyroid hormone receptors. In animals, T_3 treatment induces hypertrophy of the tissue, while on the cellular level T_3 activates UCP1 gene expression. Thyroxine is taken up from circulation by brown adipocytes but is rapidly

converted to T_3 by the enzyme DIO2 (deiodinase type 2). However, the effect of T_3 on BAT function is dependent on the release of norepinephrine from adrenergic nerve ends in order to complete the thermogenic response. In healthy humans, plasma concentration of T_3 decreases during acute cold exposure, suggesting that activated BAT is able to utilize it. On the other hand, patients with hyperthyroidism have enhanced BAT function – glucose uptake rate as a marker of metabolic activity is threefold when compared to healthy controls.

Bone morphogenetic protein 7 (BMP7) regulates selectively brown adipogenesis and affects energy expenditure by increasing the mass of BAT in mice. In addition, BMP7 increases gene expression of PRDM16 and UCP1 in BAT. The effects of BMP7 are leptin-independent because ob/ob (obese) mice as well as diet-induced obese mice treated with BMP7 increase energy expenditure, decrease food intake and body weight, and have improvement in metabolic syndrome.

Adipokines, such as leptin, adiponectin, and resistin, affect BAT function indirectly via the central nervous system. Rodents (mice or rats) deficient either for leptin or leptin receptors have impaired BAT activity and thermogenesis, but in order to function properly, leptin requires functional β_3-receptors on brown adipocytes. In addition, the melanocortin system is involved in the effect of leptin on BAT thermogenesis. If the melanocortin system is inhibited, BAT activity decreases. Adiponectin has a suppressive effect on BAT thermogenesis, reducing protein kinase A (PKA) signaling and UCP1 expression. Resistin reduces BAT thermogenesis via hypothalamic ERK1/2 signaling pathway. In addition, the effect of estradiol on BAT thermogenesis is mediated via hypothalamic AMP-activated protein kinase.

Several brain regions are involved in the regulation of BAT function. Especially, the ventromedial hypothalamic nucleus seems essential where the peripheral signals are integrated. In humans, metabolic activity in several brain regions is increased with mild cold exposure. BAT activation coincides with brain activation in cold, but this is observed only in lean subjects.

Defective BAT Function

Dysfunctional BAT is found in obesity and type 2 diabetes. Obesity clearly decreases the probability of detecting functionally active BAT in humans. Whether obesity is a consequence of dysfunctional BAT and an inactive sympathetic nervous system or vice versa – obesity and fatty acid overflow contribute to transdifferentiation of BAT to energy-storing white adipose tissue – is not clear. Based on cross-sectional studies in humans, it is known that body mass index, body fat content, and abdominal fat mass are inversely related to BAT functional activity, which all are also related to metabolic balance in systemic glucose and lipid metabolism. However, in morbidly obese humans, the Trp4Arg mutation in β_3-receptor gene is associated with high weight gain in adulthood.

Genetically obese animals, such as fa/fa Zucker rats or ob/ob mice, have impaired BAT function. Mice lacking BAT or β-adrenergic receptors (all three subtypes 1, 2, and 3) are obese. If the mice with no β-receptors are fed a high-fat diet, they develop massive obesity.

In obesity, BAT substrate metabolism is impaired: the glucose uptake rate and blood flow in cold is half the rate found in lean subjects. In addition, the insulin-stimulated glucose uptake rate is decreased, indicating tissue-specific insulin resistance in BAT in obesity. Relation between BAT activity and cerebral activity in mild cold is lost in obesity.

Increasing age may have even more of an effect on BAT function than obesity does. The incidence of cold-activated BAT decreases with age, being only 10% in subjects ages 50–60, while in the younger population the incidence may be close to 100%.

Improvement of Dysfunctional BAT

Manipulation of BAT function is a potential target for obesity treatment. The size of the tissue is considerably low, which limits the applicability of the treatment for extensive obesity. However, the beneficial effects of functionally active BAT on glucose and lipid metabolism overcome the effect only in weight loss in kilograms per pounds.

Conventional nonsurgical weight reduction of 10% in initial body weight results in an increased metabolic activity (^{18}FDG-PET/CT) in BAT. Also, surgical gastric banding in morbidly obese subjects has a beneficial effect on BAT function one year after operation.

Cold Acclimation

Acute cold exposure is an effective tool for activation of BAT function. Prolonged cold exposure has been used to treat obese animals, and both BAT mass and activity increase. Repeated cold exposure every day for 10 days, 4 weeks, or 6 weeks in healthy lean subjects increases BAT metabolic activity and cold-induced thermogenesis. Thus, recruitment of functional BAT is possible at least in health, but whether cold acclimation improves dysfunctional BAT in obesity remains to be seen.

Browning of White Adipose Tissue

Recruitment of silent beige/brite adipocytes in white adipose tissue depots is one treatment option. Local cold exposure on the thigh increases UCP1 expression in subcutaneous white adipose tissue. Both cold and β_3-adrenergic agonists recruit beige/brite adipocytes in rodent white adipose tissue. The plasticity of beige/brite adipocytes in rodents is further emphasized by chronic treatment with PPARγ-agonists, which activate rat epididymal white adipose tissue.

The concept of browning became common when the hormone irisin was discovered. Secretion of irisin is related to exercise and cold-induced shivering by muscles, and it promotes beige/brite cell formation. The effects of irisin have been clearly shown in rodents, but human results are to date contradictory.

Expression of another interesting factor, fibroblast growth factor 21 (FGF21), is induced by cold and consequently released from BAT both in rodents and in humans. FGF21 has endocrine effects on white adipose tissue (i.e., browning) but may also have autocrine effects. It seems that FGF21 release is dependent on the presence of BAT.

Several other factors and hormones may be involved in the browning of white adipose tissue, such as BMP7, BMP8b, ANP/BNPs, and prostaglandins. The importance of these factors in human white adipose tissue browning and the role in energy balance remain to be seen.

Pharmacological Approach

Adrenergic β_3-agonists have shown to be effective in activating rodent BAT function. Most of these molecules, which were tested in the 1990s, were not, however, functional and effective in humans.

Thiazolidinediones, PPARγ-agonists induce UCP1 gene expression in brown adipocytes. Also, PPARα, which is a PPARγ-coactivator, stimulates UCP1 gene expression and lipid oxidation in brown adipocytes.

Capsinoids, including capsaicin (found in chili peppers), have shown to be effective in humans. Current evidence is from the studies in lean subjects where it was found that cold-induced thermogenesis and BAT activity are improved. Future studies will evaluate their applicability in obesity.

See also: Adipose Tissue: White Adipose Tissue Structure and Function; Appetite Control in Humans: A Psychobiological Approach; Carbohydrate: Digestion, Absorption and Metabolism; Cholesterol: Absorption, Function and Metabolism; Copper: Physiology; Energy: Intake and Energy Requirements; Energy Metabolism; Fatty Acids: Metabolism; Glucose: Metabolism and Regulation; Obesity: Causes and Prevalence; Obesity Management; Obesity: The Role of Diet; Skeletal Muscle.

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Adipose Tissue: White Adipose Tissue Structure and Function

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Definition

The development of white adipose tissue (WAT) has evolved as a physiological adaptation to preserve energy stores during periods of food deprivation. While WAT provides a survival advantage in times of starvation, excess WAT is now linked to obesity-related health problems in the current nutritionally rich environment. At the present time, it is known that the adipose tissue not only is an energy reservoir in the form of triglycerides but also functions as an insulator preventing heat lost and providing a physical protection for the organism. In addition, in the last two decades, the adipose tissue has also been classified as an endocrine organ because it secretes enzymes, hormones, growth factors, cytokines, complement factors, and matrix and membrane proteins, collectively termed adipokines.

WAT is distributed throughout the organism and is composed of two representative anatomical depots: subcutaneous WAT (sWAT) and visceral WAT (vWAT). sWAT represents >80% of total adipose tissue in the body and is located inside the abdominal cavity and underneath the skin and interspersed among skeletal muscles. The vWAT constitutes 10–20% of total body fat in men and 5–10% in women. It is located inside the peritoneum and distributed around internal organs (the liver, stomach, kidney, and intestine). Depending on the location, vWAT is subclassified in mesenteric, retroperitoneal, perigonadal, and omental adipose tissue.

The adipose tissue is a heterogeneous tissue; although by volume, adipocytes are the most prominent cells within a given white fat depot (35–70% of adipose mass in adults), by cell number, they represent approximately 25% of the total cell population. The remaining 75% comprise diverse cell types known as the stromal-vascular fraction, which include fibroblasts, macrophages, pericytes, vascular elements, nervous elements, preadipocytes, and cells with mesenchymal and hematopoietic stem cell capacity.

The adipose tissue has been classified into two types, the WAT and the brown adipose tissue (BAT) based on their different morphologies, colors, metabolic functions, biochemical features, and gene expression patterns. WAT generally constitutes 20% of the body weight of normal adult humans and is the larger energy store in the organism, whereas BAT participates in regulating body temperature by generating heat via the consumption of stored energy, thus playing an important role in body thermogenesis.

Recently, a new type of brown-like adipocyte was discovered that shows distinct gene expression patterns from those of white or brown adipocytes. These novel brown-like cells that reside within WAT, especially inguinal WAT, were termed beige or brite ('brown in white' adipocytes or inducible brown adipocytes). The relative amount of the tissues varies with age, strain, environmental, and metabolic conditions.

Origins of WAT

The development of WAT begins *in utero* but primarily occurs after birth when specialized fat storage cells are needed to provide fuel during fasting periods.

White adipocytes derive from $MYF5^-$ (myogenic factor 5) progenitors, although recent evidence has shown that WAT adipose precursors can also derive from $MYF5^+$ lineages. Moreover, WAT and BAT share a similar transcriptional cascade that establishes and maintains the stable differentiation of the adipocyte. The cascade of transcription factors is driven by the peroxisome proliferator-activated receptor (PPAR)γ, PPARγ coactivator (PGC)-1α, the three CCAAT/enhancer-binding protein family members (C/EBPα, C/EBPβ, and C/EBPδ), and the sterol regulatory element-binding protein (SREBP)-1c. This transcriptional cascade interacts with other transcription factors, coactivators, and microRNAs during the decision to adopt a white 'nonthermogenic' cell fate or a brown or beige 'thermogenic' cell fate (Figure 1). The adipogenesis of white phenotype requires the corepressor RIP140 and the coactivator TIF2. Also, it has been described that some microRNAs including miR-27 and miR-133 drive a white phenotype. miRNAs have recently been proposed as regulators of cell differentiation in WAT and BAT. miR-27 and miR-133 are negative regulators of the brown and beige adipogenic program, and they suppress the major brown fat transcriptional regulators. PPARγ has been extensively involved in the process of adipogenesis, which is the process of adipocyte formation from preadipocytes, and mice carrying adipose-specific deletion of the PPARγ gene suffered from lipodystrophy. Thiazolidinediones, which are PPARγ agonists, are capable to induce differentiation of preadipocytes. Adipogenesis in adults can still occur, and the inability of an individual to increase cell numbers by this process contributes to the development of metabolic diseases.

Structure of White Adipocytes

WAT is composed of adipocytes held together by a loose connective tissue that is highly vascularized and innervated. The main morphological characteristic of mature white adipocytes is the presence of a large 'unilocular' lipid droplet that occupies over 90% of the cell volume and a thin cytoplasm layer that contains elongated mitochondria, Golgi complex, smooth and rough endoplasmic reticulum, nucleus, vesicles, and other organelles. The mature white adipocytes have a spherical form and a diameter from a minimum of 30–40 μm to a maximum of 150–160 μm. An average adult has 30 billion of fat cells with a weight of 14 kg.

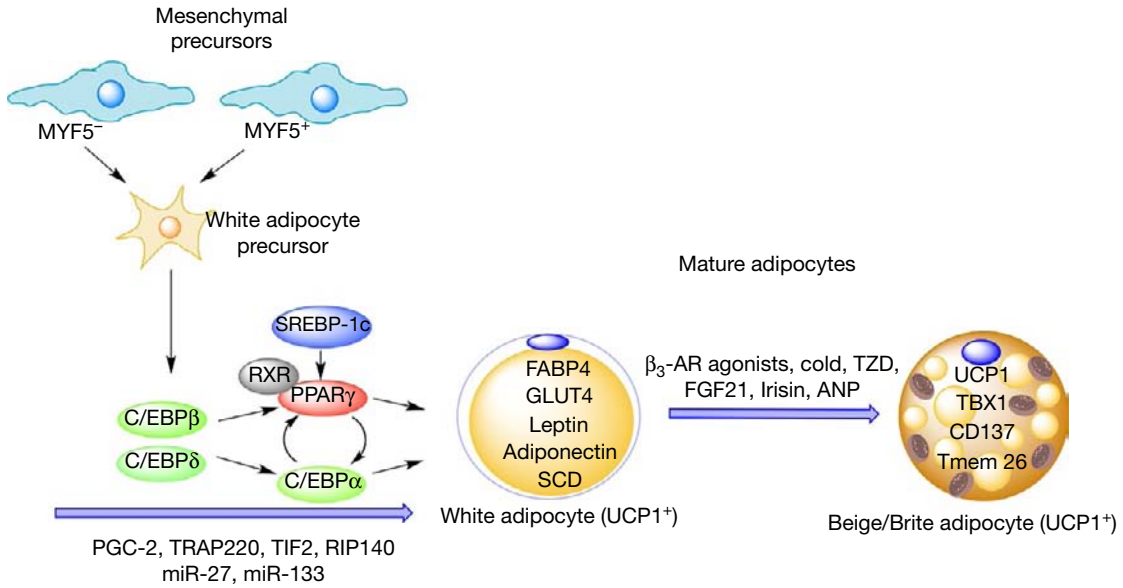

Figure 1 Origins of white adipocytes and factors regulating white adipogenesis. WAT adipocyte precursors can derive from both MYF5$^+$ and MYF5$^-$ lineages. Key transcription factors can differentiate white adipocytes and change the phenotype between white and beige adipocytes. MYF5: myogenic factor 5, C/EBPβ, C/EBPδ, C/EBPα: CCAAT/enhancer-binding protein β, δ, α, respectively. PPARγ: peroxisome proliferator-activated receptor-γ, RXR: retinoid X receptor, SREBP-1c: sterol regulatory element-binding protein 1c, FABP4: adipocyte fatty acid binding protein, GLUT4: glucose transporter type 4, SCD: stearoyl-CoA desaturase, PGC-2: PPARγ coactivator 2, TRAP220: thyroid hormone receptor-associated protein 220, TIF2: transcriptional intermediary factor 2, RIP140: receptor-interacting protein 140, miR-27: microRNA-27, miR-133: microRNA-133, β_3-AR: β_3-adrenergic receptors, TZD: thiazolidinediones, FGF21: fibroblast growth factor 21, ANP: atrial natriuretic peptide, UCP: uncoupling protein 1, Tbx1: T-box transcription factor, CD137: tumor necrosis factor receptor superfamily member 9, Tmem26: transmembrane protein 26.

Function of WAT

WAT has metabolic and endocrine functions. The metabolic functions include lipogenesis, fatty acid oxidation, and lipolysis, and the endocrine functions include the production of adipokines.

Metabolic Functions

The main functions of WAT have been described as storing and releasing highly energetic molecules, specifically fatty acids that supply fuel to the organism during fasting periods. A functional adipocyte depends on the equilibrium between lipid synthesis and fatty acid oxidation, as well as fatty acid release. These three processes are known as lipogenesis, fatty acid oxidation, and lipolysis. Lipogenesis is the synthesis of esterified fatty acids, which form triglycerides from carbohydrates or other energy sources acquired in the diet. Lipogenesis is regulated by the transcription factor sterol regulatory element-binding protein-1c (SREBP-1c). Fatty acid oxidation, also known as β-oxidation, is the process that occurs in the mitochondria to break down fatty acids into acetyl-CoA to generate energy as ATP, and it is mainly controlled by the transcription factor PPARα. Lipolysis is the release of fatty acids from triglycerides by a group of specific enzymes that hydrolyze triglycerides sequentially, which include the adipose triglyceride lipase (ATGL), the hormone-sensitive lipase (HSL), and the monoglyceride lipase (MGL). Different lipases gain access to the lipid droplet when the perilipins that are proteins that coat the vesicle are phosphorylated. Either perilipins or HSL is phosphorylated by the protein kinase A via β-adrenergic or glucagon stimulation (Figure 2).

Endocrine Functions

Adipokines. The term adipokine should refer to the proteins secreted by adipocytes; moreover, a generic concept of adipokine has been coined to include a wider range of factors, including factors released by other cell types like macrophages. WAT releases many biologically active molecules, the adipokines, that include more than 50 cytokines, hormone-like factors, and other mediators. Adipokines affect appetite and satiety, glucose and lipid metabolism, blood pressure regulation, inflammation, and immune functions (Figure 3). Precisely, they work as a network to regulate inflammation, insulin action, and glucose metabolism locally and systemically. This adipokine–cytokine networking system is altered in obesity, contributing to inflammation state and impaired adipocyte metabolism.

Within the main adipokines are adiponectin, leptin, resistin, apelin, visfatin, omentin, retinol binding protein (RBP) 4, interleukin (IL)-6, tumor necrosis factor (TNF)-α, interleukin (IL)-1, interleukin (IL)-10, monocyte chemoattractant protein (MCP)-1, angiotensinogen, and C-reactive protein (CRP), among others. A list of physiological functions of adipokines is mentioned in Figure 3. The function and physiological significance of some adipokines will be described in the succeeding text.

Adiponectin

Definition. Adiponectin is an adipokine that is specifically and abundantly expressed in WAT and BAT. Adiponectin exists in a

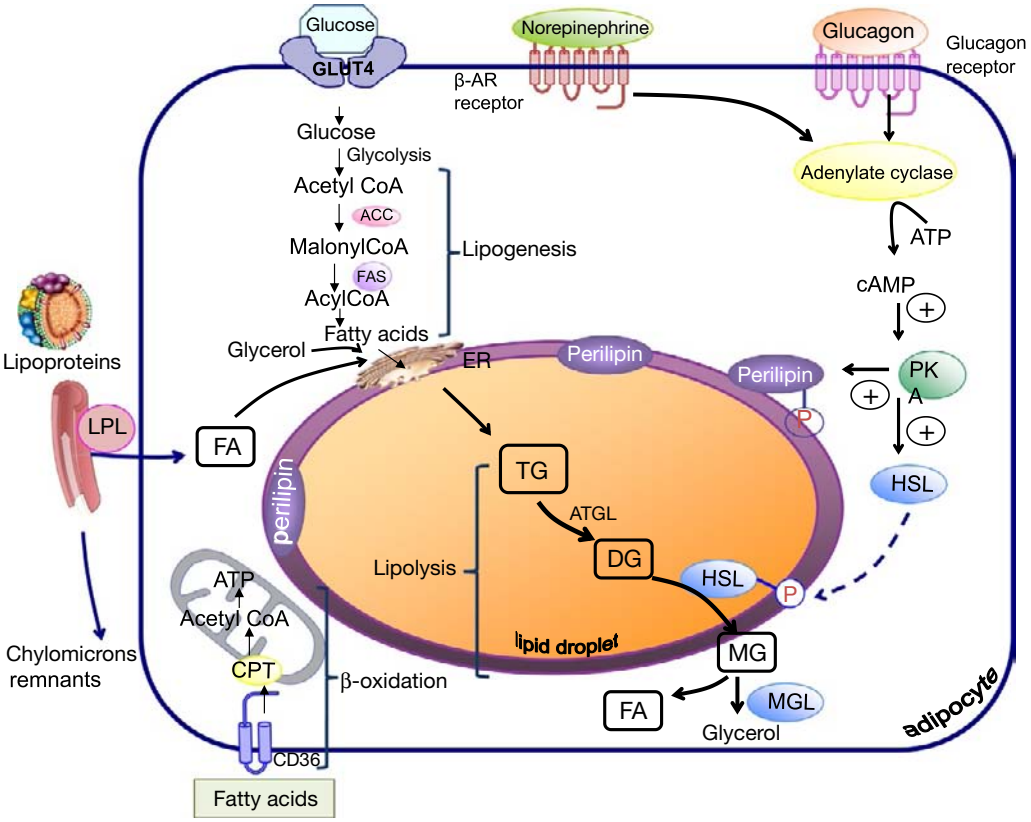

Figure 2 Metabolic functions of white adipocytes: Lipogenesis, lipolysis, and β-oxidation. LPL: lipoprotein lipase, FAs: fatty acids, ER: endoplasmic reticulum, DG: diacylglycerol, TG: triacylglycerol, MG: monoacylglycerol, ATGL: adipose triglyceride lipase, HSL: hormone-sensitive lipase, MGL: monoacylglycerol lipase, β-AR: β-adrenergic receptors, AC: adenylate cyclase, ATP: adenosine triphosphate, cAMP: cyclic adenosine monophosphate, PKA: protein kinase A, GLUT-4: glucose transporter type 4, CPT: carnitine palmitoyltransferase, ACC: acetyl-CoA carboxylase, FAS: fatty acid synthase.

wide range of multimer complexes in plasma and combines via its collagen domain to create three major oligomeric forms: a low-molecular-weight trimer, a middle-molecular-weight hexamer, and a high-molecular-weight 12- to 18-mer adiponectin.

Mechanism of action. Acute increase in the level of circulating adiponectin activates the enzyme adenosine monophosphate kinase (AMPK), triggering a transient decrease in basal glucose level by inhibiting both the expression of hepatic gluconeogenic enzymes and the rate of endogenous glucose production, indicating that adiponectin sensitizes the body to insulin. Adiponectin also increased fatty acid oxidation and energy consumption, in part via PPARα activation, which led to decreased triglyceride content in the liver and skeletal muscle and thereby a coordinated increase of *in vivo* insulin sensitivity (Figure 4).

Clinical implication. A decrease in the circulating levels of adiponectin produced by a genetic factor (adiponectin gene polymorphism) or lifestyle changes (high-fat diet and sedentary lifestyle) has been shown to contribute to the development of diabetes and the metabolic syndrome. Plasma adiponectin levels have also been reported to be reduced in obese humans, particularly those with visceral obesity, and to correlate inversely with insulin resistance (IR).

Leptin

Definition. Leptin is a circulating protein in the plasma of 167 amino acids with a molecular weight of ~16 KDa. This adipokine is secreted not only by the WAT but also by gastric epithelial cells and placenta. Leptin is involved in controlling feeding behavior and energy balance. Leptin also supports reproductive competence and immune function and contributes to the regulation of metabolic homeostasis by modulating insulin secretion, hepatic glucose production, and lipid metabolism.

Mechanism of action. Leptin acts via its cell surface receptor. There are five forms of the leptin receptor (LR) that are encoded by an alternative splicing of a single gene; however, only one has a long cytoplasmic region required for signal transduction. This receptor is located in several hypothalamic nuclei including the arcuate nucleus, ventromedial, dorsomedial, and lateral, resulting in the upregulation of the proopiomelanocortin (POMC) and downregulation of neuropeptide Y to increase energy expenditure and inhibit feeding. The actions of leptin are exerted when it binds with an LR. LR has also been located in other tissues such as the skeletal muscle, liver, kidney, intestine, immune cells, and adipose tissue, among others, and regulates the expression of genes involved in fatty acid oxidation. LR, which has a single transmembrane segment, dimerizes when leptin binds to the extracellular domains of two monomers. Both monomers are phosphorylated on a Tyr residue of their intracellular domain by a Janus kinase (JAK). The P-Tyr residues become the docking sites for three proteins that are signal transducers and activators of transcription (STATs). The docked STATs are then phosphorylated on Tyr residues by the same

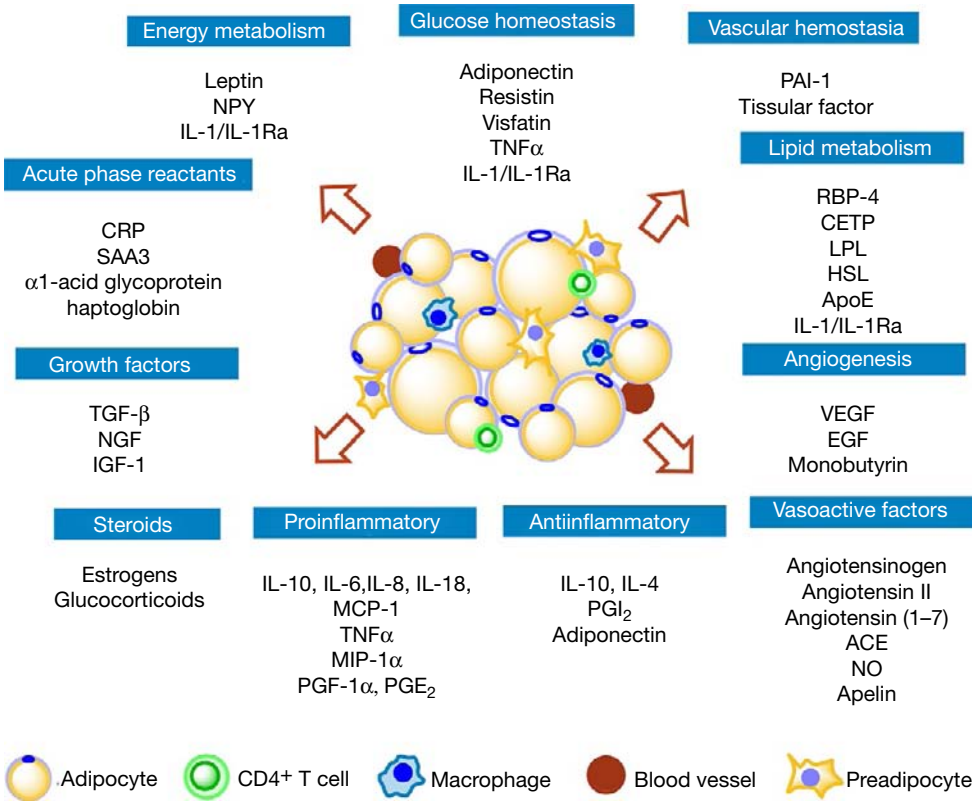

Figure 3 Adipokines released by WAT involved in metabolic and physiological processes. TNF-α: tumor necrosis factor-α, IL-1Ra: interleukin-1 receptor antagonist, RBP4: retinol binding protein 4, CETP: cholesterol ester transfer protein, LPL: lipoprotein lipase, HSL: hormone-sensitive lipase, ApoE: apolipoprotein E, PAI-1: plasminogen activator inhibitor-1, VEGF: vascular endothelial growth factor, EGF: endothelial growth factor, ACE: angiotensin-converting enzyme, NO: nitric oxide, MCP-1: monocyte chemoattractant protein-1, MIP-1α: macrophage inflammatory protein-1α, PGI2: prostacyclin, PGF2α: prostaglandin F2α, PGE2: prostaglandin E2, TGF-β: transforming growth factor-β, NGF: nerve growth factor, IGF-1: insulin-like growth factor-1, CRP: C-reactive protein, SAA3: serum amyloid A3, NPY: neuropeptide Y.

JAK. After phosphorylation, the STATs dimerize and then move to the nucleus, where they bind to specific DNA sequences and stimulate the expression of target genes, including the gene for POMC from α-melanocyte-stimulating hormone (α-MSH) (Figure 5).

Clinical implication. Circulating leptin concentrations generally reflect the status of long-term adipose tissue energy stores, and obese subjects have a greater concentration than lean subjects. When fat stores are adequate after feeding, leptin is secreted to diminish the drive to feed while enabling energy expenditure, whereas during fasting or caloric restriction, leptin concentration decreases, increasing the desire to eat and decreasing energy utilization. However, in obese subjects, hyperleptinemia is frequently observed, but the elevated levels of leptin fail to return body adiposity and insulin sensitivity to the normal range. The diminished responsiveness to leptin has been designated as 'leptin resistance.'

Resistin

Definition. Human resistin is a member of a family of resistin-like molecules, also known as the FIZZ family for 'found in inflammatory zone.' Resistin is a cysteine-rich molecule composed of 108 amino acids with a molecular weight of 12.5 kDa. It was first described in obese mice and is mainly released from WAT, particularly in adipocytes. An increase in serum resistin is linked to obesity, IR, and diabetes. Although resistin is expressed in adipocytes, in humans, it appears that macrophages are the most important source of this protein. Low levels of resistin are also detected in tissues such as the skeletal muscle, placenta, small intestine, stomach, thyroid gland, uterus, and thymus.

Mechanism of action. Human resistin is a cytokine that induces low-grade inflammation by stimulating monocytes. Resistin-mediated chronic inflammation can lead to obesity, atherosclerosis, and other cardiometabolic diseases. Recently, the receptor for resistin was identified as adenylyl cyclase-associated protein 1 (CAP1). Resistin directly binds to CAP1 in monocytes and upregulates cyclic AMP concentration, protein kinase A activity, and NF-κB-related transcription of inflammatory cytokines. Stimulation of CAP1 by resistin aggravates adipose tissue inflammation. In addition, reduction in resistin levels is associated with an increase in AMPK activity in the liver, leading to a decrease in the expression of gluconeogenic enzymes and a consequent reduction in hepatic glucose production. Conversely, elevation in resistin levels is associated with an increase in hepatic glucose production and glucose intolerance.

Resistin also is involved in various inflammatory processes, such as in the recruitment of immune cells and in the secretion of proinflammatory factors. Resistin stimulates monocytes inducing vascular inflammation and aggravating atherosclerosis. The

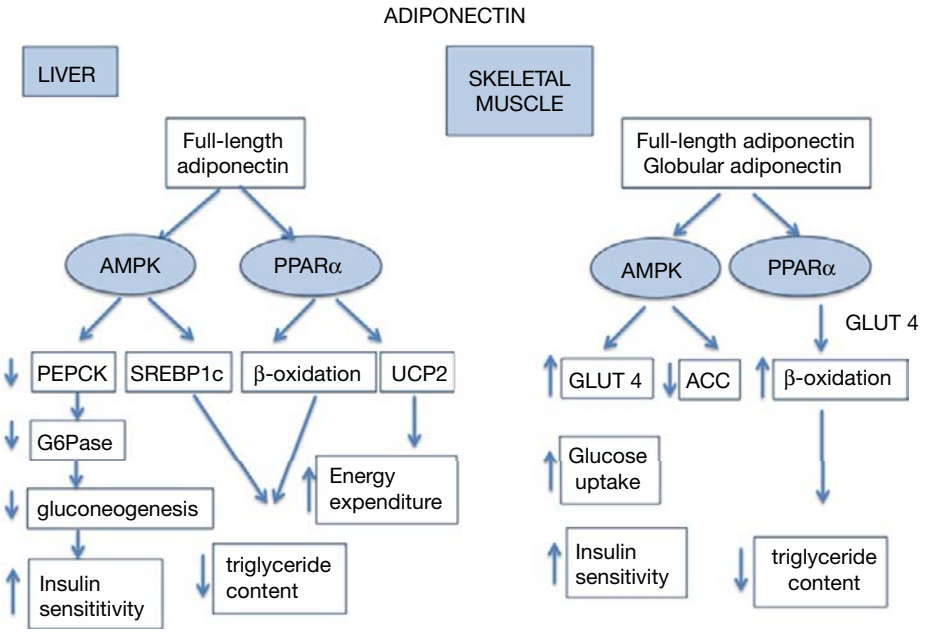

Figure 4 Adiponectin activates AMPK and PPARα on the liver and skeletal muscle, increasing insulin sensitivity and decreasing TG content. AMPK: AMP-activated protein kinase, PPARα: peroxisome proliferator-activated receptor-α, PEPCK: phosphoenolpyruvate carboxykinase, SREBP-1c: sterol regulatory element-binding protein 1c, G6PAse: glucose 6-phosphatase, UCP2: uncoupling protein-2, GLUT4: glucose transporter type 4, ACC: acetyl-CoA carboxylase.

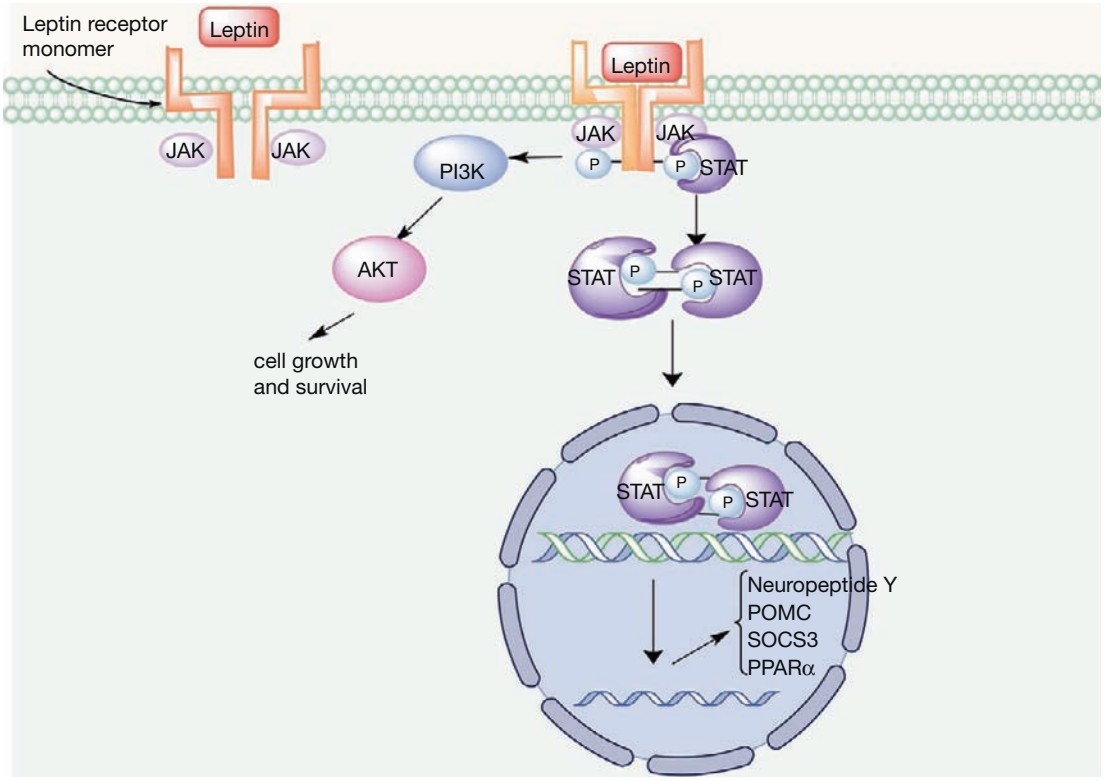

Figure 5 Leptin signaling pathway induces activation of JAK/STAT3 (Janus kinase/signal transducer and activator of transcription 3). This results in the production of NPY: neuropeptide Y, POMC: proopiomelanocortin, SOCS3: suppressor of cytokine signaling 3, and PPARα: peroxisome proliferator-activated receptor-α. Leptin signaling also activates PI3K: phosphoinositide 3-kinase for cell growth and survival.

release of resistin is often stimulated by an inflammatory process and by IL-6, hyperglycemia, and hormones such as growth hormone and gonadal hormones.

Clinical implication. Some studies in rodents have implicated that resistin is involved in the pathogenesis of obesity-mediated IR and type 2 diabetes; thus, resistin is proposed to antagonize insulin action. However, in humans, these effects remain inconclusive, partly because there are no differences in resistin expression among normal, insulin-resistant, and type 2 diabetic subjects.

Visfatin

Definition. Visfatin, also named NAMPT (nicotinamide phosphoribosyltransferase), is a protein with a molecular weight of 52 kDa coded by a gene of 34.7 kb located on chromosome 7q22.2 that is transcribed in a 2.4 kb long mRNA. Visfatin is secreted predominantly by the adipose tissue, but it is also synthesized in other tissues such as the skeletal muscle, liver, immune cells, cardiomyocytes, and brain. It is involved in the synthesis of nicotinamide adenine dinucleotide (NAD), and it has been associated with obesity development, insulin secretion, lipid profile, and inflammation, among others.

Mechanism of action. Visfatin is an enzyme involved in the synthesis of NAD, and it converts nicotinamide to nicotinamide mononucleotide (NMN), an NAD precursor. The synthesis of NMN is essential for the maintenance of NAD levels. NAD synthesis modulates insulin secretion. On the other hand, it has been suggested that visfatin can bind to the insulin receptor and is capable of stimulating the insulin signaling pathway modifying the IR. A suggested mechanism involves TNF-α, a master disruptor of insulin signaling. The mechanism suggests that TNF-α downregulates the expression of visfatin, leading to a decrease in intracellular NAD^+ concentrations. Thus, Sirt1 activity decreases because this enzyme is highly dependent of NAD^+. Inhibition of Sirt1 in adipocytes leads to a reduction in tyrosine phosphorylation of insulin receptor substrate (IRS)-1, Akt and ERK phosphorylation, and an increase of serine phosphorylation of IRS-1. Hence, the lack of Sirt1 inhibits downstream insulin signaling and decreases insulin sensitivity.

Clinical implication. A correlation of the levels of circulating visfatin with the appearance of type 2 diabetes has been shown. On the other hand, other studies have demonstrated a negative correlation between visfatin levels with the body mass index (BMI). Furthermore, it has been observed in several studies that visfatin concentration is positively associated with an increase in HDL-cholesterol concentration.

Retinol binding protein 4

Definition. RBP4 is a protein of 201 amino acids with a molecular weight of ~21 kDa. It belongs to the lipocalin family of proteins that transport small hydrophobic molecules and is the only retinol (vitamin A) transporter in the circulation. The main site of synthesis is the liver followed by the adipose tissue. In serum, 90% of circulating RBP4 is bound to retinol (holo-RBP4) and 10% is present in the unbound form (apo-RBP4). RBP4 transports retinol from the liver to the peripheral tissues.

Mechanism of action. RBP4 has an important role in regulating vitamin A metabolism and maintaining a constant and continuous supply of vitamin A to peripheral tissues for a variety of physiological processes. Circulating RBP4 coordinates cellular retinoid homeostasis through the membrane receptor stimulated by retinoic acid 6 (STRA6) and RPB4 receptor-2 (RBPR2). STRA6 mediates cellular retinol uptake from holo-RBP4. Within cells, retinoids bind to cellular retinol binding proteins or cellular retinoid-acid binding proteins and produce effects via activating retinoic acid receptors and retinoic X receptors that are involved in the regulation of adipogenesis.

Clinical implication. Epidemiological studies have demonstrated that RBP4 is a biomarker for IR, metabolic syndrome, and myocardial infarction. In these pathologies, RBP4 increases and may contribute to the development of metabolic dysfunction by impairing adipocyte differentiation and increasing secretion of proinflammatory cytokines by macrophages through Toll-like receptor 4 (TLR4) and c-Jun N-terminal protein kinase pathways. Also, the holo-RBP4 stimulates JAK2/STAT5 signaling in hepatocytes, thus contributing to the development of IR by inducing the suppressor of cytokine signaling 3 (SOCS3).

Tumor necrosis factor-α

Definition. TNF-α is a proinflammatory cytokine consisting of 157 amino acids with a molecular weight of 17 kDa. Its bioactivity is mainly regulated by soluble TNF-α–binding receptors. TNF-α is expressed and secreted by WAT, macrophages, T lymphocytes, and natural killer cells, Fibroblasts, smooth muscle cells, and tumor cells express low levels of TNF-α.

Mechanism of action. Biological functions of TNF-α are mediated by two receptors, TNF receptor-1 (TNFR-1), which is ubiquitously expressed, and TNF receptor-2 (TNFR-2), which is found in cells of the immune system. Although the affinity for TNFR-2 is five times higher than that for TNFR-1, the latter initiates the majority of the biological activities of TNF-α. TNF-α has several effects in adipocytes, for instance, inhibits adipogenesis via TNFR-1, inhibits insulin-stimulated glucose, lipogenesis, and fatty acid oxidation in differentiated human adipocytes. Other functions of TNF-α are involved in host defense and in various types of systemic toxicity. One main action of TNF-α consists in inducing apoptosis by TNFR-1 activation and by cross talk with TNFR-2 to strongly enhance TNFR-1-induced apoptosis. An early proposal was that TNF-α plays a role in immune surveillance against tumors, but in some cases, it seemed to promote tumor cell survival. Hence, TNF-α can elicit dual but opposing reactions from many different cell types.

Clinical implication. TNF-α acts as a link between adiposity and development of IR. Obese humans and mice present high levels of TNF-α in both serum and WAT. TNF-α reduces the expression of mRNA encoding the insulin-sensitive glucose transporter (GLUT-4), which is responsible for glucose uptake in the peripheral tissues. Also, TNF-α decreases the expression of insulin receptor and insulin receptor substrate-1 (IRS-1). Moreover, elevated serum free fatty acids during obesity have also been involved as potential mediators of IR, and TNF-α stimulates lipolysis and release of free fatty acids from fat cells.

WAT and Branched-Chain Amino Acids

In addition to its classical metabolic and endocrine functions, WAT plays a key role in the metabolism of branched-chain

amino acids (BCAAs). Altered regulation of BCAA has been associated with metabolic abnormalities observed during obesity.

BCAAs, leucine, isoleucine, and valine are substrates for protein synthesis via the insulin signaling pathway and precursors for the synthesis of alanine and glutamine when the minimum need for protein synthesis is met. In contrast to the other 17 amino acids, which are predominantly metabolized in the liver, BCAAs are metabolized in extra hepatic tissues by the mitochondrial branched-chain aminotransferase (BCAT2), the first enzyme in the catabolism of BCAAs in most peripheral tissues. It transfers the amino group of a BCAA to α-ketoglutarate to form glutamate and the corresponding branched-chain α-keto acid. The transamination reaction is rapid and of high capacity in muscle and WAT. The second step is regulated by the branched-chain α-keto acid dehydrogenase (BCKD) complex. The complex catalyzes the oxidative decarboxylation of the branched-chain α-keto acids, forming the branched-chain acyl-CoA derivatives, CO_2, and NADH. Because BCAT2 is not expressed in the liver, BCAAs from dietary protein bypass first the metabolism in the liver. This may contribute to the sharp rise of plasma leucine in response to a meal, thereby promoting leucine signaling in the peripheral tissues that respond to leucine.

BCAA and Obesity

WAT is second only after the skeletal muscle in its capacity to catabolize BCAAs and is capable of metabolizing significant quantities of BCAAs in rodents and humans. During the obesity, the BCAAs rise due to an alteration in BCAA catabolism. The rises in the BCAAs are of particular interest because they appear to have unique obesity-related effects. Our findings and previous work indicate that omental WAT plays an important role in BCAA homeostasis because this organ has a large capacity to catabolize BCAAs, and the presence of IR or metabolic syndrome downregulates adipose tissue BCAA pathway enzyme expression. The higher the BMI and IR, the higher the serum BCAA concentrations due to a decrease in the expression of the two key BCAA enzymes, *BCAT2* and *BCKDH E1α*, in the adipose tissue. BCAA catabolic pathway in the adipose tissue is sensitive to changes in insulin action, and IR impairs efficient BCAA catabolism in the adipose tissue. During obesity, hypertrophic adipocytes develop metabolic inflexibility, which may prevent the utilization of BCAA. It has been proposed that high tissue and blood concentrations of BCAAs in human obesity cause or exacerbate IR through mechanisms involving leucine; this amino acid promotes the activation of the mechanistic target of rapamycin (*mTOR*) in the muscle and the phosphatidylinositol 3-kinase signaling pathways. In addition, high serum BCAA (especially leucine) concentrations are associated with obesity and hyperinsulinemia, a finding that is consistent with earlier studies suggesting that BCAAs may augment the pancreatic secretion of insulin in the IR state. Studies in transgenic mice with disruption of BCATm or BCKD kinase support the evidence that dysregulation of BCAA metabolism results in sustained changes in plasma BCAA concentrations. Microarray studies have suggested that mRNA for enzymes involved in BCAA metabolism in the adipose tissue is depressed in mutant or transgenic animals with an obese phenotype.

WAT and Renin–Angiotensin System

Renin–angiotensin system (RAS) exerts a central role in blood pressure regulation and also participates in adipocytes homeostasis, specifically in growth regulation, differentiation, and metabolism. The systemic RAS starts with the release of angiotensinogen mainly from the liver. Recent findings demonstrate that human and rodent adipose tissues also contain all of the RAS components. The adipose tissue is a major contributor of extrahepatic angiotensinogen (AGT), particularly in obesity. Nutritional status, nutrient distribution, and nutrients per se modulate the RAS expression and activity in various tissues including the adipose tissue. Fasting and feeding affect AGT production. Nutrients or food components, such as fructose, lipids, and soy protein, among others, influence tissue and systemic RAS activity. Dietary fructose treatment results in hypertension, glucose intolerance, and hypertriglyceridemia in animals. This is in part because fructose feeding exacerbates the presence and activity of the RAS. On the other hand, soy protein has beneficial effects on RAS mediated by soy protein gastrointestinal digestion-derived amino acids, peptides, and isoflavones that ameliorate metabolic syndrome. Conversely, the consumption of a high-fat diet increases the components of RAS, leading to an increase in blood pressure.

WAT and Diet

The amount of dietary fat plays a central role in the development of obesity. Recent evidence demonstrated that chronic consumption of a high-fat diet regardless of the type of fat, either polyunsaturated fatty acids (PUFAs) or saturated fatty acids (SFAs), represses the expression of lipogenic, fatty acid oxidation, and thermogenic genes in the adipose tissue, leading to the accumulation of lipids in the adipose tissue and liver. However, adequate consumption of PUFAs decreases the expression of lipogenic genes in WAT and increases the expression of genes involved in fatty acid oxidation.

Not only the type of fat but also the type of protein regulates the expression of genes in the adipose tissue. This effect is mediated in part by the capacity of each type of protein to stimulate insulin secretion to a different extent. Vegetable proteins such as soy protein stimulate insulin secretion to a lesser extent than animal proteins such as casein. It has been shown that rats fed with a soy protein–high-fat diet show lower body weight gain and adipocyte size compared with rats fed with a casein–high-fat diet. In addition, dietary soy protein activates PPARγ in the adipose tissue, preventing metabolic abnormalities during obesity by stimulating adipogenesis.

Interestingly, there is evidence that shows an interaction between the type of protein and the type and amount of dietary fats that play an important role in the functionality of WAT.

See also: Adipose Tissue: Structure and Function of Brown Adipose Tissue; Amino Acids: Metabolism; Energy Metabolism; Fatty Acids: Metabolism; Obesity: The Role of Diet.

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Adolescent Nutrition

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Introduction

Adolescence is a time of major physical change. Girls gain an average of 12.5 pounds per year and boys gain an average of 20 pounds per year during puberty. Although both gain weight during adolescence, males have a decrease in body fat percentage to an average of 12% during this time, while females experience an increase to 16–27% body fat. Weight gain is only one of the countless changes a young person will experience during adolescence. The adolescent period is characterized by profound biological, psychosocial, and cognitive changes. Teens are also gaining increasing independence as they grow into young adulthood. Where previously, their parents were making the decisions about where, when, and what they would eat, a teenager starts to make some of these decisions on their own. Adolescence is a critical period in the development of lifelong health behaviors, and ideally, they have been given the skills to make healthy choices when confronted with this new-found freedom. Unfortunately, teens that develop unhealthy habits may be at risk for serious health consequences. Some problems, like obesity, might have started developing at a younger age. Others, like an eating disorder, might only come to light once puberty occurs and changes start to happen to a person's body. The choices that a teenager makes with regard to his or her diet can have lasting effects; healthy eating can reduce risk of diseases such as heart disease, cancer, stroke, and diabetes. Unhealthy eating can have the opposite effect.

In addition, being a teenager today means being exposed to media constantly. From magazines emphasizing the 'right' size for your waistline and social media sites like Facebook and Tumblr allowing teens to view 'thinspiration' posts to TV commercials and website pop-up advertisements encouraging teens to drink more soda and eat more fast food, no one can avoid being influenced by the media.

What Are Teenagers Eating?

Many teenagers are not meeting the suggested requirements for major food groups, especially fruit and vegetables. According to the Continuing Survey of Food Intakes by Individuals and the National Health and Nutrition Examination Survey, major contributors to the adolescent diet in the United States include sugar-sweetened beverages, pizza, full-fat milk, grain-based desserts, breads, pasta dishes, and savory snacks. Recent data from the Youth Risk Behavior Surveillance System (YRBSS) show that 6.6% of high school students surveyed had not eaten a single vegetable 7 days preceding the survey and 5% had not eaten fruit or had 100% fruit juice to drink. A recent study on dietary adequacy in teenage girls specifically found that they were lacking in fruits, vegetables, and diary, giving them lower than adequate intakes (AIs) of calcium, magnesium, potassium, and vitamins D and E.

The setting where adolescents eat can have an impact on the quality of their food intake. A recent review article of family meals found that adolescents who have more frequent family meals also have healthier diets. Higher frequency of family meals is also associated with reduced prevalence of overweight and obesity. Unfortunately for many families, evening meals together are not feasible given busy schedules or the lack of interest in these gatherings on the part of the adolescent.

American adolescents who eat the lunch provided at their school will be eating a meal that is nutritionally balanced and meets the nutrition standards set forth by the National School Lunch Program. The meal will have no more than 30% calories from fat and less than 10% calories from saturated fat. Each meal must provide one-third the recommended dietary allowance (RDA) of protein, vitamin A, vitamin C, iron, calcium, and calories. Additionally, recent changes to school lunch guidelines involve increasing fruits and vegetables and whole grains in school meals.

Skipping meals is prevalent among adolescents, with breakfast being the meal skipped the most often. According to the YRBSS, 13.7% of teens surveyed had not eaten breakfast 7 days preceding the survey and only 38.1% had eaten breakfast on all 7 days. Meal skipping has been associated in numerous studies with risk of overweight and obesity. Nutrition counseling can help a teenager identify quick and easy breakfast items for those who cite lack of time in the morning as the main reason that they are missing this important meal. Counseling would also be warranted for a teen who might mistakenly think that skipping a meal is an effective strategy for weight loss.

Snacking is also common among adolescents and has only increased over time. The types of snacks showing the biggest increase within this age group are nutrient-poor, energy-dense foods including desserts, candy, salty snacks, and sugar-sweetened beverages (SSBs). One study showed that more than 27% of a child's calories each day came from snacks, often three or more per day. While snacking is not necessarily unhealthy, calories should ideally come primarily from balanced meals in addition to small, nutrient-dense snacks throughout the day. See articles by Popkin and others, based on NHANES and Nielsen datasets.

Factors Influencing Food Choice

There are many factors that can influence an adolescent's food choice; some are external such as peer pressure, and others are internal such as cravings. Some are affected by cultural or religious beliefs (such as not eating meat during Lent), and others are based purely on availability. A teenager who does not have access to a car, has no nearby grocery store, and mainly shops at a neighborhood convenience store is not likely to develop a strong affinity towards fresh fruits and vegetables. Current food trends, home environment, body image, and health status are other factors that can readily contribute to the decisions that adolescents make daily regarding food choices.

What adolescents themselves identify as factors that influence their intake do differ from internal/cultural factors that a teen might not personally think are relevant factors. One focus group study showed that adolescents identify hunger/food cravings, appeal of food, time, and convenience as the most important factors influencing food choices. This same group of teenagers identified making healthy food look and taste better as the primary suggestion to increase adolescent healthy eating.

Dietary Assessment

When assessing an adolescent's diet, it is important to ask specific questions. There are several dietary assessment strategies, among which the 24 h recall is the commonly used clinically. The 24 h recall involves asking the teenager very specifically about what they ate and drank over the past day including portion sizes. Depending on the population, it might be worthwhile to provide a food frequency questionnaire where the teen can check off how many times per week they eat vegetables and how often they drink soda, for example. Some adolescents might have an easier time remembering what they ate if they are asked to take a photo of each meal or log the meal into an online tracker. The best dietary assessment strategy to use will depend on the adolescent's nutritional goals. For example, you would not necessarily want a teenager struggling with an eating disorder to track their intake using a website that listed calories.

Energy Requirements

Energy requirements for teenagers can vary greatly depending on their physical activity level (PAL) and current stage of growth. The Institute of Medicine (IOM) published estimated energy requirements (EERs) based on a global doubly labeled water database. The EER for adolescents 9–18 years of age includes the total energy expenditure, in addition to calories needed for energy deposition.

For boys, EER is calculated as follows:

$$\text{EER} = 88.5 - (61.9 \times \text{age [y]}) + \text{PA} \times (26.7 \times \text{weight [kg]} + 903 \times \text{height [m]}) + 25 \text{ kcal}$$

where PA is the physical activity coefficient:

PA = 1.00 if PAL is estimated to be ≥ 1.0 < 1.4 (sedentary).
PA = 1.13 if PAL is estimated to be ≥ 1.4 < 1.6 (low active).
PA = 1.26 if PAL is estimated to be ≥ 1.6 < 1.9 (active).
PA = 1.42 if PAL is estimated to be ≥ 1.9 < 2.5 (very active).

For girls aged 9–18 years, EER is calculated as follows:

$$\text{EER} = 135.3 - (30.8 \times \text{age [y]}) + \text{PA} \times (10.0 \times \text{weight [kg]} + 934 \times \text{height [m]}) + 25 \text{ kcal}$$

where PA is the physical activity coefficient:

PA = 1.00 if PAL is estimated to be ≥ 1.0 < 1.4 (sedentary).
PA = 1.16 if PAL is estimated to be ≥ 1.4 < 1.6 (low active).
PA = 1.31 if PAL is estimated to be ≥ 1.6 < 1.9 (active).
PA = 1.56 if PAL is estimated to be ≥ 1.9 < 2.5 (very active).

Dietary Guidelines

Various agencies and organizations around the world have published standards and guidelines for what constitutes an optimal diet. In the United States, the USDA has published the MyPlate icon, recommending that everyone eat balanced meals with half of their plates composed of fruits and vegetables and the other half divided between protein and grains with a serving of dairy at each meal (Figure 1). The Harvard School of Public Health has countered MyPlate with their own Healthy Eating Plate (Figure 2), a similar guide that provides additional guidance such as encouraging the protein to be healthy (i.e., limiting red meat and high-fat or processed protein) and grains to be whole grain (i.e., whole-wheat bread, brown rice, and whole-wheat pasta).

The USDA provides suggested servings per day of various food groups as well. See Table 1. For vegetables, the cups given are intended as cooked vegetables; if eaten raw, the amounts should be doubled. For reference, a large banana, orange, or peach counts as one cup of a fruit.

Carbohydrates

The recommended dietary allowance (RDA) of carbohydrates for adolescents of both genders is 130 g per day. Carbohydrates provide essential energy to the body, especially for the brain. Foods that contain carbohydrate include grains (cereal, bread, pasta, rice, oats, tortillas, and pita), starchy vegetables (potatoes, sweet potatoes, corn, and peas), fruit, dairy, and legumes. The body stores carbohydrate as glycogen in the muscles and liver.

Fiber

AI of fiber for males aged 9–13 is 31 g per day, for males aged 14–18 is 38 g per day, and for females aged 9–18 is 26 g per day. Fiber is found naturally in foods such as fruits, vegetables, beans, legumes, and whole grains. Fiber is essential for maintaining bowel health and for preventing constipation throughout the life span. Despite the health benefits of fiber and its availability in multiple food sources, low fiber intake is extremely common among adolescents.

Protein

Protein is essential for building and repairing muscles, in addition to other important functions in the body. The RDA for

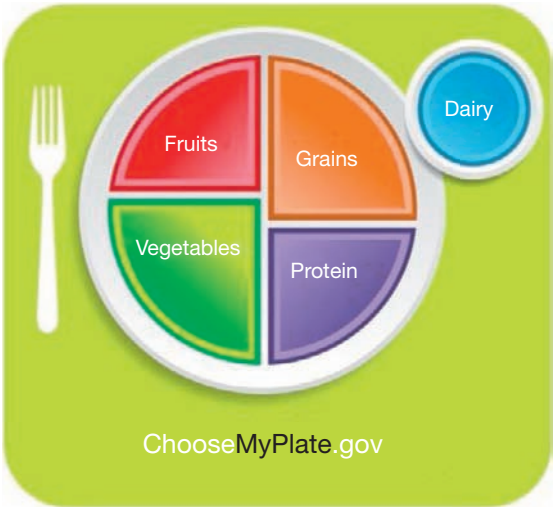

Figure 1 Choose MyPlate.gov.

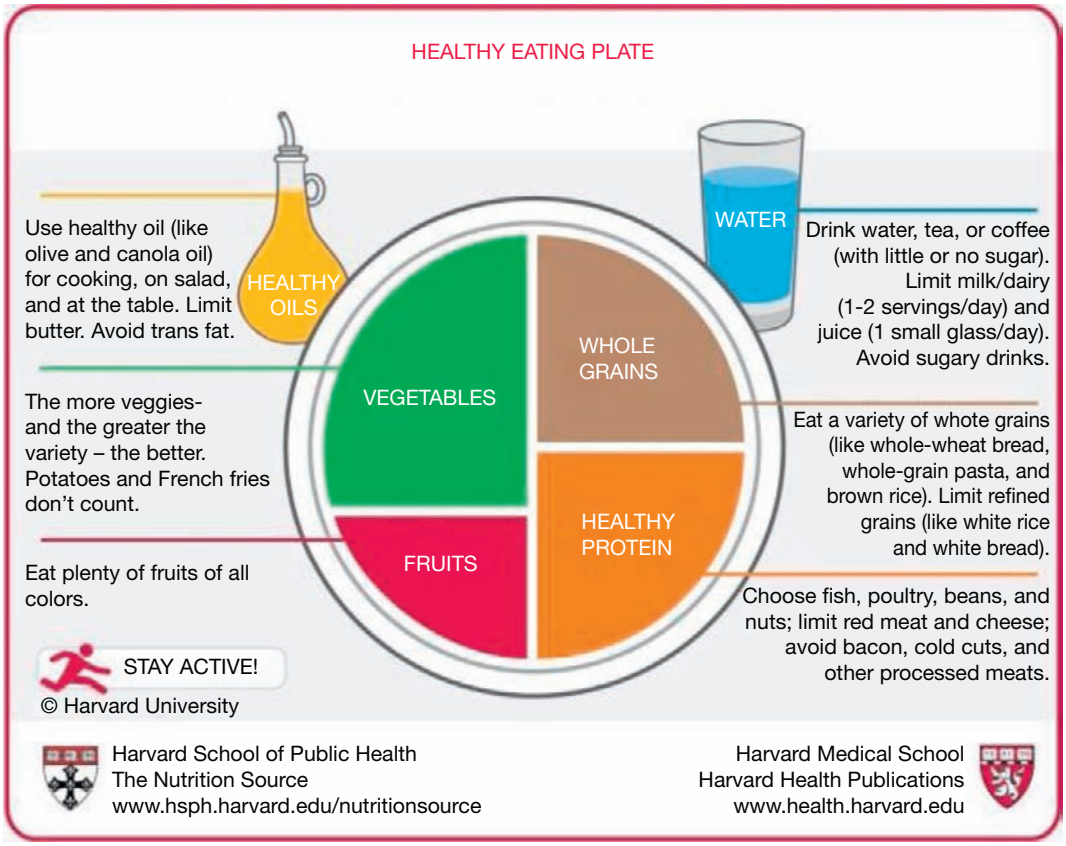

Figure 2 Harvard School of Public Health Healthy Eating Plate.

Table 1 Select micronutrients suggested intake

	Males		*Females*	
Nutrient	Aged 9–13	Aged 14–18	Aged 9–13	Aged 14–18
Vitamin A (IU per day)	600	900	600	700
Vitamin C (mg per day)	45	75	45	65
Vitamin D (IU per day)	15	15	15	15
Vitamin E (mg per day)	11	15	11	15
Vitamin K (IU per day)	60	75	60	75
Thiamin (mg per day)	0.9	1.2	0.9	1.0
Riboflavin (mg per day)	0.9	1.3	0.9	1.0
Niacin (mg per day)	12	16	12	14
Vitamin B6 (mg per day)	1.0	1.3	1.0	1.2
Folate (IU per day)	300	400	300	400
Vitamin B12 (IU per day)	1.8	2.4	1.8	2.4
Calcium (mg per day)	1300	1300	1300	1300
Iron (mg per day)	8	11	8	15
Potassium (mg per day)	4.5	4.7	4.5	4.7
Sodium (g per day)	1.5	1.5	1.5	1.5

Source: National Research Council. (2006). *Dietary Reference Intakes*: *the essential guide to nutrient requirements*. Washington, DC: The National Academies Press

adolescent boys aged 9–13 is 34 g per day and for adolescent boys aged 14–18 is 52 g per day. The RDA is 34 g per day for girls aged 9–13 and 46 g per day for girls aged 14–18. Protein is found in animal products such as meat, poultry, fish, dairy, and eggs and in beans, legumes, and nuts.

Fat

It is necessary for the diet to contain fat in order to help absorb fat-soluble vitamins (vitamins A, D, E, and K) and to provide linoleic acid and linolenic acid, essential for neurological

Table 2 Recommended fruit and vegetable intake

Gender and age	*Fruit servings*	*Vegetable servings*
Girls 9–13	1 ½ Cups	2 Cups
Girls 13–18	1 ½ Cups	2 ½ Cups
Boys 9–13	1 ½ Cups	2 ½ Cups
Boys 13–18	2 Cups	3 Cups

Source: www.choosemyplate.gov.

development and growth. The acceptable macronutrient distribution range for fat for teenagers of both genders is 25–35 g per day. Adolescents should attempt to eat as little trans fat as possible and limit the amount of saturated fat in their diet. Sources of fat in the diet include dairy, cheese, butter, oil, avocado, certain fish, certain cuts of meat, and nuts.

Vitamins and Minerals

Certain vitamins and minerals have a recommended dietary allowance (RDA), while others have only an established AI because no RDA has been established. See Table 2 for a list of select vitamins and minerals and the suggested intake levels for adolescents. Most of these nutrients can be consumed in these suggested amounts by eating a balanced, varied diet that includes fruit and vegetables. In the absence of adequate portions of these healthy foods, however, a multivitamin or other supplement may be warranted.

One particular nutrient of special importance during adolescence is calcium, which is aided in absorption by vitamin D. Adequate calcium intake during adolescence is key for preventing osteoporosis because childhood and adolescence are the time when bones are gaining strength and density that cannot be made up for later in life. Calcium can be found in the diet in beverages such as milk and soy milk and in foods such as tofu, beans, yogurt, cheese, almonds, canned seafood, leafy green vegetables, and fortified foods such as cereal and snack bars.

Hydration

The Holliday–Segar method of figuring hydration needs is used in hospitals but can also be applied to healthy adolescence. The equation is as follows:

Patient weight	*Fluid needs*
11–20 kg	1000 ml + 50 ml kg^{-1} for each kg >10 kg
>20 kg	1500 ml + 20 ml kg^{-1} for each kg >20 kg

The daily recommended intake (DRI) can also be used to determine the recommended fluid intake for teenagers. For males aged 9–13 years, the DRI is 2.4 l per day; for males aged 14–18, it is 3.3 l per day. For females aged 9–13, the DRI is 2.1 l per day; for females aged 14–18, it is 2.3 l per day. This includes all liquids consumed such as water and other beverages, in addition to liquids and moisture in foods such as soup, watermelon, and cucumber.

Supplements and Alcohol

Energy Drinks

Energy drinks such as Red Bull, 5-Hour ENERGY, and Monster Energy drink are a growing product category that seems to appeal to adolescents. Studies have shown not only that there are potential negative health effects to the energy drinks themselves but also that those adolescents who consume energy drinks are at higher risk of substance use such as smoking, drinking alcohol, and using illicit drugs. The American Academy of Pediatrics recommends that children and adolescents avoid consuming energy drinks, suggesting that they use water as their primary source of hydration.

Alcohol

According to a recent YRBSS report, 66.2% of high school students reported having had at least one alcoholic drink in their life. During the 30 days prior to the survey, 34.9% of teenagers had consumed alcohol at least once and 20.8% had had five or more drinks in one sitting, the definition of binge drinking. Teen consumption of alcohol remains a problem for many reasons. According to the American Academy of Pediatrics, alcohol can interfere with adolescent brain development, which continues into young adulthood. In addition, using alcohol during adolescence can promote the risk of alcoholism later in life, can lead to motor vehicle-related fatalities (the leading cause of death among US teens), and can lead to other mental and physical disorders. From a nutritional perspective, alcohol provides excess calories, which when consumed in large quantities can lead to overweight and obesity. Alcohol consumption is also often associated with poor dietary choices, and long-term use can affect the absorption of certain vitamins and minerals.

Obesity

The criterion for children aged 2–20 for overweight is a BMI between the 85th and 95th percentile according to Centers for Disease Control and Prevention growth charts. For obesity, the criterion is a BMI over the 95th percentile. Obesity rates among adolescents have increased significantly over the past fourteen years. An article looking at the prevalence and trends in obesity and severe obesity showed that from 2011 to 2012, 17.4% of children aged 2–19 were obese and prevalence among adolescents exceeded 20%. Prevalence of severe obesity is also growing among youth aged 2–19 with 5.9% meeting criteria for class 2 obesity (with a BMI greater than or equal to 120% of the 95th percentile or a BMI of greater than or equal to 35) and 2.1% meeting criteria for class 3 obesity (with a BMI of greater than or equal to 140% of the 95th percentile or a BMI of greater than or equal to 40).

Sugar-Sweetened Beverages

According to YRBSS data, 27% of teenagers had consumed one nondiet soda per day 30 days leading up to the survey, and even more worrisome, 19.4% had consumed nondiet soda two

or more times per day. SSBs include juice, lemonade, punch, soda, and other drinks that adolescents consume on a regular basis. These beverages (with the possible exception of juice) provide no nutritional value, but contain a large amount of calories. This is often referred to as 'empty' calories because they are providing nothing besides energy. Soda is often vilified when discussing causes of increased obesity in society. Indeed, the serving sizes have grown larger over the years, and the marketing does directly target young people. One recent study of SSBs on adolescents linked increased intake with greater waist circumference, a risk factor for metabolic syndrome. However, it is important to remember that while SSBs can contribute to excess calories, it is often only one piece of the obesity puzzle.

Screen Time

There is strong relationship between screen time and excess weight gain/obesity in children and adolescents. Whether this is due to the effects of commercials advertising to teens on television, the fact that one often mindlessly consumes calories when in front of a screen, the lack of physical activity due to screen time, or the effect that screen time has on sleep, experts agree that less screen time is beneficial to all children and teens, especially those at risk of overweight or obesity.

Extreme Dieting

According to the recent YRBSS data, 47.7% of teenagers reported that they were trying to lose weight with females being more likely to report this than males. Of concern, 13% of students reported that they had not eaten for twenty-four or more hours in an attempt to lose weight and 5% reported having taken diet pills. Additionally, 4.4% reported vomiting or taking laxatives to lose weight or keep from gaining weight. Extreme dieting does not work and often leads to a heavier weight in the long run. In addition, it can cause numerous health issues and nutritional deficiencies. For more information, see the section on '**Eating Disorders**.'

Type 2 Diabetes

Type 2 diabetes, also referred to as non-insulin-dependent diabetes as a way of differentiating it from type 1 diabetes (previously called juvenile diabetes), is an increasing problem among children and adolescents commonly caused by obesity. In the past, this type of diabetes was called adult-onset diabetes, but that name is no longer accurate due to the rising number of diagnoses in younger populations. In addition to obesity, several comorbidities such as proteinuria (protein in the urine), hypertension, dyslipidemia, nonalcoholic fatty liver disease, polycystic ovary syndrome (PCOS), and obstructive sleep apnea are seen among adolescent with type 2 diabetes. There are currently few treatments for type 2 diabetes in adolescents that include lifestyle changes (eating a healthy, balanced diet plus exercising regularly), pharmacology, and gastric bypass surgery.

Polycystic Ovary Syndrome

PCOS is a disease that affects 7–14% of adult women (depending on the diagnostic criteria used), with the onset happening mainly during adolescence. While no specific causes of PCOS have been definitively identified, childhood obesity is thought to be a contributing factor. PCOS is often associated with obesity, metabolic syndrome, and type 2 diabetes; it is characterized by irregular periods, hirsutism, acne, weight gain, and acanthosis nigricans. Weight loss can reduce some symptoms, but elevated insulin levels may make weight loss more difficult for adolescent girls who have PCOS compared with their healthy counterparts. Adolescent girls with PCOS can manage their insulin levels by decreasing the amount of refined carbohydrates they eat or drink, increasing the amount of protein and fiber in their diet, and getting plenty of physical activity.

Eating Disorders

Adolescence is a particularly hard time for a person to deal with body image issues since there are so many changes happening to the body during puberty. This can set the stage for an eating disorder that may not have been an issue previously. While any disordered relationship with food can be considered an eating disorder of concern, there are differing levels of clinical severity. The Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V), published in 2013, revised several of the previous definitions for specific eating disorders. It is important to keep in mind that just because an adolescent might not fit one of these diagnoses entirely, they may still have a disordered relationship with food that would warrant treatment.

Anorexia Nervosa

The DSM-V includes the following diagnostic criteria for anorexia nervosa (AN):

1. Restriction of energy intake relative to requirements, leading to a significantly low body weight in the context of age, sex, developmental trajectory, and physical health
2. Intense fear of gaining weight or of becoming fat or persistent behavior that interferes with weight gain
3. Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or persistent lack of recognition of the seriousness of the current low body weight

The DSM-V removed the requirement for AN that a patient have amenorrhea (not applicable to males or to females who have not yet reached menarche) and took out the specific percent ideal body weight, changing the terminology to 'significantly low' that does include some indicators in the manual. According to the DSM, prevalence for AN among young women is 0.4% in the course of 12 months. Increasing numbers of males are being diagnosed with AN, but females tend to seek treatment more often.

Bulimia Nervosa

The DSM-V includes the following diagnostic criteria for bulimia nervosa (BN):

1. Recurrent episodes of binge eating characterized by eating an amount of food that is definitely larger than what most individuals would eat in a similar period of time associated with a lack of control over eating during the episode.
2. Recurrent inappropriate compensatory behaviors in order to prevent weight gain, such as self-induced vomiting; misuse of laxatives, diuretics, or other medications; fasting; or excessive exercise.
3. The binge eating and inappropriate compensatory behaviors both occur, on average, at least once a week for 3 months.
4. Self-evaluation is unduly influenced by body shape and weight.
5. The disturbance does not occur exclusively during episodes of AN.

While AN has a prevalence of 0.4%, BN is much higher among young females at 1–1.5% according to the DSM.

Binge Eating Disorder

Binge eating disorder (BED) was not an official diagnosis until the DSM-V was released. Previously, patients who binged without purging were grouped into a category called Eating Disorder Not Otherwise Specified. The diagnostic criteria for BED are the following:

1. Recurrent episodes of binge eating characterized by eating an amount of food that is definitely larger than what most individuals would eat in a similar period of time associated with a lack of control over eating during the episode.
2. The binge eating episodes are associated with three (or more) of the following: eating much more rapidly than normal, eating until feeling uncomfortably full, eating large amounts of food when not feeling physically hungry, eating alone because of feeling embarrassed by how much one is eating, feeling disgusted with oneself, depressed, or very guilty afterward.
3. Marked distress regarding binge eating is present.
4. The binge eating occurs, on average, at least once a week for 3 months.
5. The binge eating is not associated with the recurrent use of inappropriate compensatory behavior as in BN and does not occur exclusively during the course of BN or AN.

Avoidant/Restrictive Food Intake Disorder

While many children will grow out of being picky eaters, some will continue to restrict their intake without having concerns about their weight (differentiating it from one of the other eating disorders). Clinically, this is referred to as avoidant/restrictive food intake disorder (ARFID) and is diagnosed as follows:

1. An eating or feeding disturbance as manifested by persistent failure to meet appropriate nutritional and/or energy needs associated with one or more of the following: significant weight loss, significant nutritional deficiency, dependences on enteral feeding or oral nutritional supplements, and marked interference with psychosocial functioning.
2. The disturbance is not better explained by lack of available food or by an associated culturally sanctioned practice.
3. The eating disturbance does not occur exclusively during the course of anorexia or bulimia or better explained by another medical or mental disorder.

Sometimes, adolescents with ARFID have sensory issues or it can be comorbid with the autism spectrum. Presentations differ, but a few case examples are a teenager who will eat only foods that are soft in texture such as macaroni and cheese and mashed potatoes or one who refuses to eat any fruit or vegetables and rarely eats protein-containing foods, preferring mainly white carbohydrates such as crackers, chips, bread, and rice.

Other Specified Feeding or Eating Disorder

There are some eating disorders that do not fit within the criteria for AN, BN, BED, or ARFID. These eating disorders fall into the category called Other Specified Feeding or Eating Disorder (OSFED) and include atypical AN, subthreshold BN, subthreshold BED, purging disorder, and night-eating syndrome. One example of a patient with OSFED is a teenager whose BMI goes from the 95th percentile down to the 50th percentile in a short period of time. Being at the 50th percentile would preclude them from being 'significantly low weighted,' but they might be restricting intake, hyperexercising, or using other unhealthy behaviors that will have an effect on their health.

'Orthorexia'

According to Mayo Clinic, 'orthorexia' comes from the Greek words 'orthos,' meaning straight or proper, and 'orexia,' meaning appetite. While not an official eating disorder diagnosis, people who become obsessive about eating healthy can have disordered eating patterns and thoughts that can get in the way of living a happy life. Steven Bratman is the doctor who first described and named this disorder. He differentiates healthy eating from orthorexia by the level of obsession that a person has (i.e., whether or not they allow themselves to eat foods they might think of as unhealthy in appropriate situations such as birthday cake at a party).

Other Nutritional Issues in Adolescents

Female Athlete Triad

Female teenage athletes are especially at risk for the female athlete triad. In the past, this triad was considered to be eating disorder, amenorrhea, and osteoporosis. Now, however, it is considered to be more of a continuum, with low energy availability taking the place of eating disorder, implying that the athlete does not necessarily have an eating disorder but is for whatever reason not taking in enough calories that is causing the functional amenorrhea that then causes the low bone mineral density. Female athletes should be screened for the female athlete triad on a regular basis to prevent any interference with bone growth and development. If a female athlete has amenorrhea, nutrition counseling is warranted to identify ways that she can consume adequate calories in order to resume menses.

Iron-Deficiency Anemia

During adolescence, teens have increased iron needs due to normal growth and development. Girls in particular have increased iron needs due to the blood loss during menstruation. Because of this, adolescents are more susceptible than adults to iron-deficiency anemia, which is characterized by not enough or especially small red blood cells. To prevent iron-deficiency anemia, adolescents should make sure to include iron-rich food sources in their diet including red meat, eggs, poultry, fish, legumes, and fortified breads and cereals. Girls and boys aged 9–13 need 8 mg per day, boys aged 13–18 need 11 mg per day, and girls aged 13–18 need 15 mg per day. Consuming foods rich in vitamin C (such as fruits and vegetables) in conjunction with iron-containing foods can help with the absorption of iron.

Vegetarianism/Veganism

As with any population including growing children, adolescents can eat a healthy, varied, and balanced vegetarian or vegan diet that will provide all of the necessary nutrients that they need for growth. With adolescents who might already have suboptimal nutritional intake, however, extra precautions are necessary to ensure that they are actually consuming enough of each nutrient on an animal-free diet, specifically protein, calcium, B12, vitamin D, and iron. Many products that are available to vegetarians and vegans are fortified with some of these important nutrients, but assessment and monitoring by a dietitian may be warranted. It is also important to assess why a teenager has chosen to become a vegetarian. In some cases, eliminating meat and/or dairy could be the beginning of a restrictive eating disorder. In general though, a vegetarian diet can be a healthy option for an adolescent. One study showed that vegetarian teenagers had better fruit and vegetable consumption and less total and saturated fat consumption than their meat-eating peers.

Celiac and Food Allergies

Food allergies are not specific to adolescence, and in fact, some childhood food allergies may no longer be an issue by the time the child reaches puberty. However, others will persist through childhood into adulthood and may be particularly tricky to deal with during adolescence when a teenager might not want to bring attention to himself or herself by asking about ingredients when out at a restaurant, for example, or carrying an EpiPen.

The general public has recently become much more aware of celiac disease and gluten sensitivity. For some, this awareness leads to a diagnosis of celiac disease where the only treatment is to avoid gluten. For others, the hype in the media causes them to needlessly avoid gluten altogether. While a gluten-free diet is an absolutely necessary treatment for someone with celiac disease, gluten (the protein found in wheat, barley, triticale, and rye) should not be removed from the diet without reason. Grain products provide an important source of carbohydrate in addition to being fortified with iron and often good sources of fiber.

Future Trends in Adolescent Nutrition

As teenagers across the world continue to be influenced by popular media and increasingly by various forms of social media, diet trends will likely continue to affect their foods choices, body image concerns, and health habits. Practices like juice cleansing, fasting, eating clean, consuming only organic foods, and cutting out items like sugar, gluten, and dairy without being medically advised to do so are just a few of the trends that adolescents are starting to follow. Whatever popular media decides as the next big weight loss secret or key to having clear, glowing skin will be seen before too long in the adolescent population. With any luck, these same teens will also have a caring, educated community supporting him or her to provide education on healthy, balanced, adequate nutrition.

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Appetite Control in Humans: A Psychobiological Approach; Beverage: Health Effects; Bioavailability of Nutrients; Caffeine: Consumption and Health Effects; Cystic Fibrosis, Nutrition in; Dietary Practices; Dietary References: US; Eating Disorders; Energy: Intake and Energy Requirements; Energy Metabolism; Food Allergies; Growth promoters: Characteristics and Determination; Hunger; Obesity: Causes and Prevalence; Obesity: Epidemiology of; Obesity Management; Obesity: The Role of Diet; Protein: Requirements; Satiety; Sports Nutrition; Vegetarian Diets; Vitamins: Overview.

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Aerated Foods

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Introduction

Aerated foods and drinks such as bread and other baked products, beer, sparkling wines, fizzy drinks, ice cream, whipped cream, meringues, chocolate, Swiss cheese, puffed rice, and popcorn offer novel and luxurious textures and represent the height of culinary and technological skill. A diverse range of air contents and aerated structures are achievable from aeration processes that include low-viscosity whipping and high-viscosity mixing, gas injection, and slow or rapid generation and expansion of gases within foods. Aerated foods can be characterized in terms of the gas content, bubble or gas cell distribution, texture, and stability, which together deliver novelty, luxury, and appeal.

The benefits of aerating foods include

(i) reduced density and increased volume;
(ii) improved palatability and sensory appeal (smoothness, lightness, crispness, crunch, and fizz);
(iii) creation of novel textures and structures;
(iv) reduction in the intensity of flavors;
(v) entrapment of aroma compounds and subsequent delivery for retronasal olfaction, enhancing flavor perception;
(vi) increased digestibility;
(vii) altered perceptions of satiety;
(viii) aesthetic appeal;
(ix) enhanced ability to take up sauces, due to increased surface area and capillary action;
(x) connotations of luxury;
(xi) the advertising and market appeal of bubbles.

These benefits have been exploited across a diverse array of aerated foods, which can be broadly categorized in several ways as illustrated in Table 1: food type, historical appearance, aeration processes, stability, stabilization mechanism, and the principal gases contributing to aeration. Such categorization helps to identify common themes and challenges and opportunities for cross-fertilization. Table 2 presents the primary aeration methods used across the different food types.

Tables 1–2 illustrate the wide range of aerated foods and hint at the challenges of their manufacture in industry or in the domestic kitchen. Most food processing, either domestic or commercial, is concerned with creating desirable, distinctive, or novel textures, along with pleasant flavors and an attractive appearance. Many of the most appealing foods deliver their characteristic texture and appearance by exploiting the presence of bubbles. Examples include bread, cakes, ice cream, breakfast cereals, meringues, whipped cream, waffles, soufflés, aerated chocolate bars, beer, champagne, popcorn, and many others. Bubbles in foods offer no nutritional benefit; they represent pure luxury and proclaim the skill of the chef or his or her industrial counterparts. Aeration transforms food from merely flavorsome fuel into textural novelty. The textures achieved vary from the soft but strong crumb of bread to the crispness of breakfast cereals, to the crunch of honeycomb, to the smoothness of whipped cream, to the 'melting bubbles' of aerated chocolate bars, to the tingle of carbonated beverages.

Aerated foods offer product differentiation and marketing advantage in the highly competitive, innovative, and dynamic food market. They also inspire delight and praise in the dining room. Their creation requires detailed understanding, empirical and fundamental, of the complex interactions between food chemistry and physics in the kitchen or in the manufacturing environment.

Aeration Processes and Equipment

Aerated foods are produced using one or more of the three general methods: (1) Liquid is forced around air to form bubbles, (2) gas is sparged into liquid to form bubbles, and (3) gas is generated within the food to create bubbles. The first of these can be divided into low- and high-viscosity systems, while the third method can be divided into slow and rapid generation and expansion of gas, giving a total of five categories of aeration method. Within these five broad categories, a wide range of specific processes and operations are used, including whipping cream, beating eggs, dough and paste mixing, widgets in beer, fermentation, gas injection, frying, vacuum puffing, and extrusion. Thus, the major food aeration methods are as follows:

Type 1(a)

- *Whipping*, *beating*, or *shaking* of low–medium-viscosity liquids to entrap air
- *Pressure beating* (dissolution of air or gas under pressure), for example, in a syrup, fat mixture, or chocolate, for confectionery manufacture

Type 1(b)

- *Mixing* of doughs or high-viscosity pastes, in which air bubbles are entrapped as surfaces come together. The high viscosity of the dough or paste prevents rising and disengagement of bubbles. In raised bread, the bubbles incorporated by the mixing act as nucleation sites for the CO_2 produced during fermentation. During creaming of butter and sugar, sugar crystals aid aeration; fat crystals in cake batters and ice cream act similarly, with the particle size of the crystals affecting the size and number of bubbles entrained.
- *Entrapment* of air between sheeted layers, as in pastries and croissants, or between pulled strands, as in pulled taffy and candies.

Table 1 Bases for categorizing aerated foods, with examples

Food type	*Historical appearance*	*Aeration processes (in rough order of historical appearance)*	*Stability*	*Stabilization mechanisms*	*Principal aeration gases*
Bread and baked products Chocolate and confectionery Dairy foams Egg-based foams Breakfast cereals Snack products Beverages Miscellaneous	Ancient (4000–1000 BC): bread, beer, wine Classical period and Dark Ages (1000 BC–AD 1000): few new aerated foods Medieval (1000–1492): Swiss cheese, wafers, biscuits, koumiss, popcorn (Aztecs) Age of discovery (1492–1800): cakes, waffles, pastries, crumpets, bagels, whipped cream, ice cream, egg foams, bubbly beer, sparkling wines, soda water Industrial Revolution (1800–1900): baking powders, croissants, doughnuts, ice cream, angel cake, sponge cake, sabayon, modern marshmallow, carbonated soft drinks Early modern (1900–50): ice cream cone, instant whipped cream, crema on espresso and cappuccino, pavlova, bubblegum, aerated chocolate, breakfast cereals, potato crisps, extruded cereals and snacks, extruded marshmallows, mechanically developed doughs Recent (1950–the present): Chorleywood bread process, whipped margarine, widgets, Nescafé Foam Booster	Fermentation Whipping (low viscosity) Mixing (high viscosity) Steam generation and thermal expansion during slow cooking Entrapment between layers Frying Chemical raising agents Rapid dry heating Gas injection (including steam injection) Expansion extrusion Pressure beating Puffing Vacuum expansion Sudden pressure release Gas dissolved in a glassy matrix	Seconds: champagne foams Minutes: beer foams, crema on espresso and cappuccino Hours: batters, whipped cream, milk shakes Days: bread, mousse Weeks: cakes Months: chocolate, cereals, Swiss cheese, biscuits, ice cream Years: meringues, crackers, confectionery, rice cakes	Proteins: egg foams, beer and wine foam, bakery products, crema on espresso and cappuccino Fat crystals: whipped cream Ice crystals: ice cream, frozen desserts Emulsifiers: milk shakes High-viscosity, semisolid: batters, fruit fools, dairy desserts, mousses Solid matrix ● starch/protein: bread, bakery products, ice cream cone ● sugar: meringue ● fat: aerated chocolate	Carbon dioxide ● Yeast or bacteria: bread, yeast-leavened cakes, Swiss cheese, beer, wine, ginger beer ● Chemically leavened: cakes, biscuits, batters, soda bread, wafers, waffles ● Direct injection: carbonated soft drinks, sparkling wines, pressure beating of chocolate Steam: crema on espresso and cappuccino, unleavened breads, popcorn, puff pastry, puffed rice, cornflakes Air: whipped cream, egg foams, angel food cake, bread dough, chocolate Nitrogen: widget-induced beer foam, chocolate Nitrous oxide: instant whipped cream

Table 2 Primary aeration methods used with different food types

Food type *Aeration process*	*Baked products*	*Dairy products*	*Egg products*	*Chocolate and confectionery products*	*Breakfast cereals and snacks*	*Beverages*	*Others*
Fermentation	• Breads • Crackers • Crumpets • Pikelets • Stollen • Pretzels • Bagels	• Swiss cheese			• Fermented extruded products	• Beer • Wine • Ginger beer	
Whipping or shaking	• Batters • Yorkshire puddings	• Cream • Ice cream • Mousses • Sherbet • Frozen desserts • Milk shakes • Butter • Koumiss • Whipped margarine	• Meringue • Soufflé • Omelet • Sponge cake • Angel cake • Chiffon cake • Zabaglione • Sabayon	• Frappé • Marshmallow • Foamed chocolate beverage (Aztecs)			• Fruit fool • Sorbet • Meat foams • Fish foams
Dough and paste mixing	• Bread dough • Biscuit dough	• Soft butter • Cream cheese • Creaming of butter and sugar for cakes	• Choux pastry	• Fondant • Nougat • Chocolate			• Crèmes • Icings • Peanut butter • Snack preparations • Meat doughs
Slow dry heating/ baking (steam generation and thermal expansion)	• Unleavened bread • Pancakes • Doughnuts • Pizza base • Wafers • Yorkshire puddings • Bagels		• Soufflé • Omelet • Sponge cake • Angel cake • Chiffon cake				• Micronized wheat, lentils
Rapid dry heating (steam generation and thermal expansion)					• Cornflakes • Micronized wheat • Popcorn (Aztecs) • Popped sorghum		• Crisps
Frying	• Poppadoms				• Snacks • Potato crisps		• Bubble and squeak

(*Continued*)

Table 2 (Continued)

Food type *Aeration process*	*Baked products*	*Dairy products*	*Egg products*	*Chocolate and confectionery products*	*Breakfast cereals and snacks*	*Beverages*	*Others*
Raising agents	• Cakes • Biscuits • Waffles • Soda breads • Doughnuts • Batters			• Honeycomb • Brittles • Boiled sweets	• Extruded products with added bicarbonate		
Entrapment, pulling	• Puff pastry • Croissants • Vol-au-vents			• Pulled taffy • Flaked chocolate • Cotton candy • Boiled sweets			
Gas injection				• Boiled sweets • Bubblegum		• Espresso • Cappuccino • Carbonated drinks • Widgets in canned beer	
Extrusion	• Crispbreads			• Marshmallow • Chocolate	• Breakfast cereals		• Snacks • Pet food
Pressure beating		• Ice cream		• Chocolate • Toffee • Caramel • Fillings			
Puffing					• Rice crispies • Puffed wheat		• Snacks
Vacuum expansion				• Chocolate bars • Sweets • Gums			
Sudden pressure release	• Pillsbury Doughboy	• Instant whipped cream					
Gas dissolved in a glassy matrix, released on dissolution				• Pop Rocks • Fizzing candies		• Nescafé Foam Booster	

Source: Campbell, G. M. and Mougeot, E. (1999). Creation and characterisation of aerated food products. *Trends in Food Science and Technology* **10**, 283–296.

Type 2

- *Gas injection*, for example, air or nitrogen injection in ice cream and sugar confectionery, carbon dioxide injection in soft drinks, steam frothing of espresso and cappuccino, or children blowing bubbles into milk to make it more interesting

Type 3(a)

- *Fermentation*, in which aeration is achieved through carbon dioxide production by yeast in bread, beer, and wine or by *Propionibacterium* in Swiss cheeses.
- *Steam generation* during slow to moderate cooking, baking, or frying. Steam generation is often accompanied by *thermal expansion* of the gases already in the bubbles and by evaporation of other dissolved components (e.g., CO_2 and ethanol).
- The use of *chemical raising agents* such as baking powders in cakes or sodium bicarbonate in soda bread, honeycomb, or dulce de leche.
- *Vacuum expansion*, followed by rapid cooling to set the expanded product, for example, chocolate bars.

Type 3(b)

- *Rapid dry heating* or *toasting* of small or thin products to induce blistering or slight puffing
- *Frying* in very hot oil, such that internal steam is formed rapidly, causing the product to puff
- *Expansion extrusion*, in which superheated product under pressure emerges suddenly from an extruder, such that internal moisture immediately vaporizes into steam bubbles, to produce crisp snacks, cereals, and sugar confectionery
- *Puffing*, in which products such as breakfast cereals containing superheated moisture are subjected to a sudden release of pressure
- *Popping*, in which the material (e.g., popcorn) is naturally able to retain pressure for explosive release and structure formation

Despite the wide variety of processes, aeration equipment comprises primarily mixers of various designs, extruders, or specialized puffing or expansion equipment. Most other aeration processes are achieved by heating or by chemical raising agents.

Several aeration operations may contribute together or consecutively during the process; for example, in breadmaking, bubbles are incorporated into the high-viscosity dough during mixing (a type 1b process); these bubbles are inflated slowly by carbon dioxide gas generated by yeast fermentation (type 3a) and are further inflated by steam generation and thermal expansion during baking (also type 3a) while also undergoing coalescence and rupture along with setting of the matrix structure.

Mixers for food aeration include high-speed whisks and beaters with stainless steel wire assemblies for egg foams, whipped cream and cake batters, and high- and low-speed heavy-duty mixers for doughs and pastes. Pressure beating delivers greatly accelerated aeration and produces fine foams, with air consumption of up to $1000\,l\,h^{-1}$. Blades can be mounted horizontally within the mixer bowl or, more usually, vertically from the top or through the base of the bowl. Industrial-scale high-speed whisks operate at speeds of around 200 rpm with a specific power input of around $50\,W\,kg^{-1}$. Low-viscosity mixers must use high speeds to entrain air and break up the bubbles, while in dough mixing, bubbles are entrained unavoidably simply through the action of surface renewal during mixing. In both high- and low-viscosity mixing operations, the air content and bubble-size distribution depend on the balance between entrainment and disentrainment of air, along with bubble breakup and coalescence, with viscosity, surface tension, and the presence of particles influencing these processes.

In modern breadmaking processes, the bubble structure created in the dough directly affects the baked loaf structure. Some dough mixers use pressure-vacuum mixing, in which the dough is mixed initially under high pressure to provide additional oxygen (which contributes to the development of the gluten network), followed by mixing under a partial vacuum to reduce the air content in the dough. Some batch dough mixers, for example, the BiPlex, operate initially at slow speed to blend and hydrate the ingredients and then at high speed to develop the dough.

Continuous dough mixers lack the versatility of batch mixers to modify the bubble structure in the dough and in the resulting bread. Continuous, tubular, pressurized scraped-surface aerator freezers with a residence time of about 30 s are used in ice cream manufacture to give air contents of up to 50% by volume.

Aeration Gases

The three gases of greatest importance in food aeration are carbon dioxide, steam, and air. Nitrogen may also find application in specific instances, for example, in pressure beating to produce microaerated chocolate in which the bubbles are too small to be visible or to produce a foamy head on beer. Oxygen can be bubbled through cheap wine to approximate aging, while nitrous oxide is the gas that propels instant whipped cream from a can, chosen for its similar solubility to carbon dioxide but not imparting a sour taste. However, most aerated foods employ carbon dioxide, steam, and air, separately or together, to achieve aeration.

Table 3 presents a two-dimensional categorization of aerated foods according to processing methods and the major gases used in their manufacture. Carbon dioxide can be produced biologically from bacterial or yeast fermentation, as in bread, beer, wine, and Swiss cheese. Carbon dioxide can also be produced from chemical reactions involving sodium bicarbonate and a suitable acid, as in baking powders used in cakes and honeycomb/cinder toffee, or can be directly introduced to the food or beverage as gaseous CO_2, as for carbonated soft drinks and cheap sparkling wines or in certain pressure-beating applications. Steam can be injected into, for example, milk to produce the attractive *crema* on espresso and cappuccino coffees. Slow generation of steam occurs in all baking processes, along with dissolution of gases when the temperature rises and

Table 3 Processing methods for aerated foods and the major gases involved in their creation

Processing method	*CO_2 – yeast or bacterial fermentation*	*CO_2 – chemically leavened*	*CO_2 – direct injection*	*Steam*	*Air*	*Other*
1(a). Low–intermediate-viscosity whipping			Pressure beating of chocolate		Batters Whipped cream Egg foams Mousses Frappe Angel cake, sponge cake Foamed chocolate beverage	Nitrogen – pressure beating of chocolate to produce microaerated chocolate
1(b). High-viscosity mixing or layering					Bread dough (during mixing) Pastry dough (puff pastry, Choux pastry, croissants)	
2. Gas injection			Carbonated soft drinks Sparkling wines	Cappuccino, espresso	Bubble gum	Nitrogen – widgets to produce a creamy foam on beer Oxygen – bubbled through wine to approximate aging
3(a). Slow *in situ* generation or expansion of gases	Bread dough (during proving), yeast-leavened cakes, crumpets Swiss cheese Beer, wine, ginger beer	Cakes, soda bread, biscuits, pancakes, doughnuts, wafers, waffles		Bread (during baking); baking of all baked products	Vacuum-expanded chocolate Thermal expansion during baking	
3(b). Rapid *in situ* generation or expansion of gases				Unleavened breads Popcorn Fried snacks Potato crisps Extruded snacks	Extruded marshmallow	Nitrous oxide – instant whipped cream

accompanied by expansion of the steam, air, and released gases. Meanwhile, rapid creation of steam and expansion of air and steam occur in rapid heating or rapid pressure reduction processes, giving the explosive power to create extruded snacks, rice crispies, and popcorn. Whipping is perhaps the archetypal aeration process, in which air is the relevant gas, entrained via the rapid deformation of the surface of a liquid, as in whipped cream and beaten egg whites. Air can also be entrapped more slowly via layering of pastry or slow mixing of high-viscosity materials such as bread doughs. In processes that involve heating, this air, and any other gases, undergoes thermal expansion, accompanied by the creation and expansion of steam. Alternatively, the air may be expanded without heat by reducing the pressure, either by aerating under positive pressure and releasing to atmospheric pressure or by applying a vacuum to the foamed liquid and allowing it to set.

As ever, tidy classifications are impossible, as the creation of a given food may involve several operations and different gases at each stage. Air is frequently the initial and the final aerating gas – steam and carbon dioxide may contribute intermediate roles, but frequently, the initial aeration (as the word implies) is with air, while for porous products, the final aerated structure is also filled with air. Breadmaking has, for example, been described as 'a series of aeration stages': Air bubbles are created during mixing; these bubbles are inflated with carbon dioxide gas produced by yeast during proving; there is further expansion during baking due to ongoing CO_2 production until the yeast is killed by the increasing temperature, the evaporation of water into steam, the dissolution of CO_2 from the liquid phase due to the higher temperature, and the thermal expansion of the steam, CO_2, and air; and on setting of the porous crumb structure, the steam and CO_2 are released and replaced once again by air.

Characterization of Aerated Foods

Aerated foods can be characterized in terms of their rate of aeration, air content, bubble size distribution, the resulting

texture, and the stability of the aerated structure. Foamability (the ease with which a foam is formed, in terms of rate and air content) and foam stability are usually applied to transient food foams such as beer and wine foams and beaten egg whites. Foamability may be measured by sparging gas or by rapid whipping to determine the time required to form a prescribed volume of foam, while foam stability is characterized by the half-life of the foam, the rate of foam drainage, or the change of conductivity of the foam. Foamability and foam stability are often inversely related – a greater foam volume is achieved at the expense of a less stable foam. Stability is conferred through a range of stabilization mechanisms, discussed later in the text.

For more solid aerated foods, texture is of greater importance, and rheometers and textural measurements, including sensory evaluation, are applied. Rheometers may be empirical, imitative, or fundamental. Crisp aerated foods can be characterized by calculating the apparent fractal dimension of the jagged stress–strain curve resulting from crushing. Mechanical properties of solid aerated foods depend on the mechanical properties of the matrix, the amount and distribution of the air, and whether the foam is of open or closed gas cells. Closed gas cells occur in aerated chocolate bars, for example, in which each bubble is discrete, while bread has an open-cell or sponge structure, in which the gas cells are interconnected to form a porous network. Mechanical properties of foams, such as Young's modulus, collapse stresses, crushing strength, and fracture toughness, can be related to the density of the foam by a power law model:

$$\frac{\sigma}{\sigma_m} \propto \left[\frac{\rho}{\rho_m}\right]^n$$

where σ is a general mechanical property, σ_m is the mechanical property of the matrix material in the foam, and ρ and ρ_m are the density of the foam and of the matrix material, respectively. The exponent n is usually in the range 2–3. For constant matrix properties, mechanical properties vary with foam density raised to the power of n, such that as air content increases, the foam becomes less strong.

Air contents of aerated foods range from 2% for some confectionery products to >95% for popcorn, rice cakes, and beer foam, with every air content in between. The air content of highly aerated fluids such as ice cream and whipped cream is characterized in terms of the overrun, OR, calculated as

$$\mathrm{OR} = \frac{\text{Weight of unwhipped product} - \text{weight of whipped product}}{\text{Weight of whipped product}} \times 100\%$$

where weights are measured in a container of constant volume. The overrun represents the additional air added. In aerated foods with lower air contents, the void fraction of air as a fraction of the total volume is more usually calculated as

$$\phi = \left(1 - \frac{\rho}{\rho_{gf}}\right) \times 100\% = \left(\frac{\mathrm{OR}}{\mathrm{OR} + 100}\right) \times 100\%$$

where ρ is the density of the product and ρ_{gf} is the gas-free density. Table 4 gives typical values of the density and air content of a range of aerated foods, from which the other parameters describing aeration can be calculated. Figure 1 shows typical air contents and specific volumes of a selection of aerated food products.

Table 4 Typical values of density and gas content of aerated foods (dependent in all cases on temperature, composition, and processing factors)

Food	*Density (g cm^{-3})*	*Void fraction of gas (%)*
Popcorn	<0.07	>95
Rice cakes	0.11–0.13	90–92
Puffed rice	0.13–0.17	88–90
Extruded products	0.10–0.33	75–90
Meringue	0.17–0.18	88–90
Beaten egg whites	0.15–0.20	80–85
Baked loaf	0.20–0.35	72–85
Sponge cake	0.25–0.35	70–80
Risen dough	0.25–0.40	68–80
Marshmallow	0.35–0.45	68–75
Cake	0.35–0.40	68–72
Whipped cream	0.40–0.60	40–60
Ice cream – hard	0.54–0.55	50
Cake batter	0.55–0.80	30–50
Aerated chocolate bar	0.70–0.80	40–45
Nougat	0.80–0.90	30–40
Fruit fool	0.75–0.8	25–30
Ice cream – soft	0.78–0.8	28
Milk shake	0.90–0.95	9–13
Micronized wheat	1.15–1.25	7–11
Bread dough (unrisen)	1.15–1.20	4–8
Wheat grains	1.25–1.35	2–3

Source: Campbell, G. M. and Mougeot, E. (1999). Creation and characterisation of aerated food products. *Trends in Food Science and Technology* **10**, 283–296.

Density measurements indicate the gross air content of a food, but not how that air is distributed. The size distribution of bubbles or gas cells determines the texture, appearance, and mass transfer behavior of an aerated food. The size distribution can be determined by image analysis of an aerated surface or of thin slices of the product. When thin slices of a food material are taken, the holes appearing on the slice are, on average, smaller than the bubbles from which they came. The hole size distribution measured is therefore different from the true bubble size distribution, which must be reconstructed using stereological techniques. The advent in recent years of accessible x-ray microtomography has begun to empower studies of aerated foods, in terms of more precise characterization of aerated structures and insights into the dynamic processes by which those structures are created. Figure 2 illustrates x-ray tomographic images of bread dough during proving.

Stabilization of Aerated Foods

The lifetimes of aerated food products range from a few seconds for wine foams, to minutes for beer foams and soufflés, to hours for whipped cream, to days for bread, to weeks for cakes, to several months for Swiss cheeses, cereals, chocolate bars, and ice cream. Liquid aerated systems are inherently unstable, and the bubble structure must be stabilized against collapse. In solid aerated foods such as crackers and snack and chocolate bars, stability is achieved through the solid matrix. These products are however delicate due to their fine aerated structure, and their high surface area makes them prone to oxidative deterioration or picking up atmospheric moisture, these factors ultimately

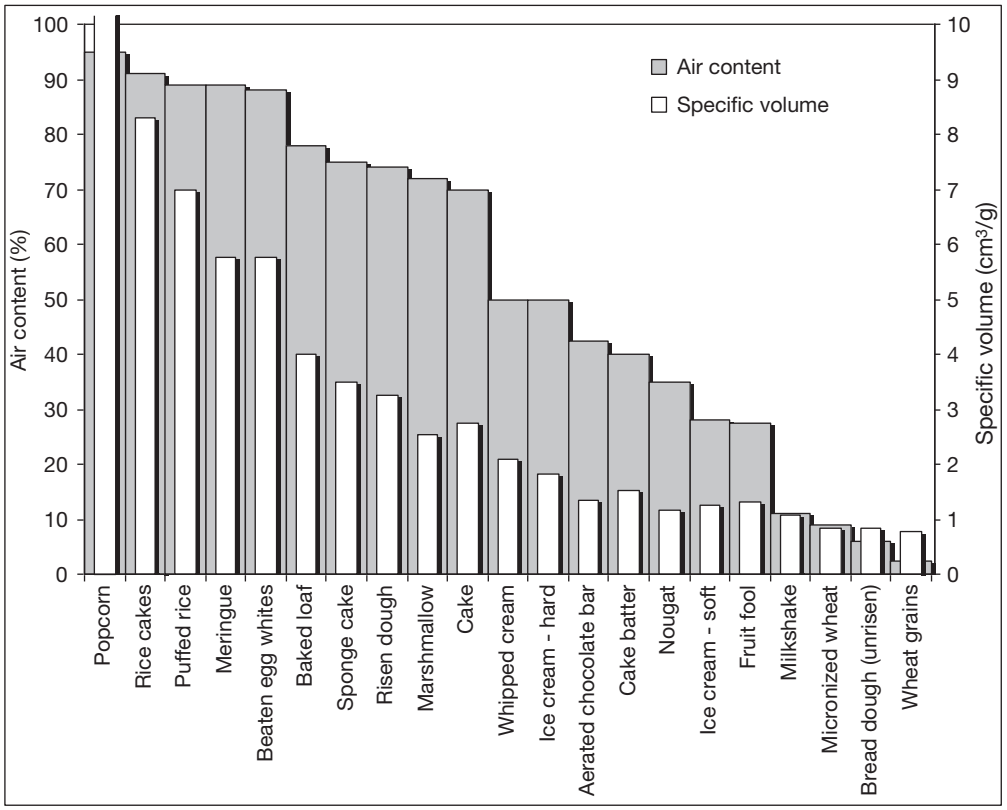

Figure 1 Typical air contents and specific volumes of a selection of aerated foods. Adapted from Campbell, G. M. and Mougeot, E. (1999). Creation and characterisation of aerated food products. *Trends in Food Science and Technology* **10**, 283–296.

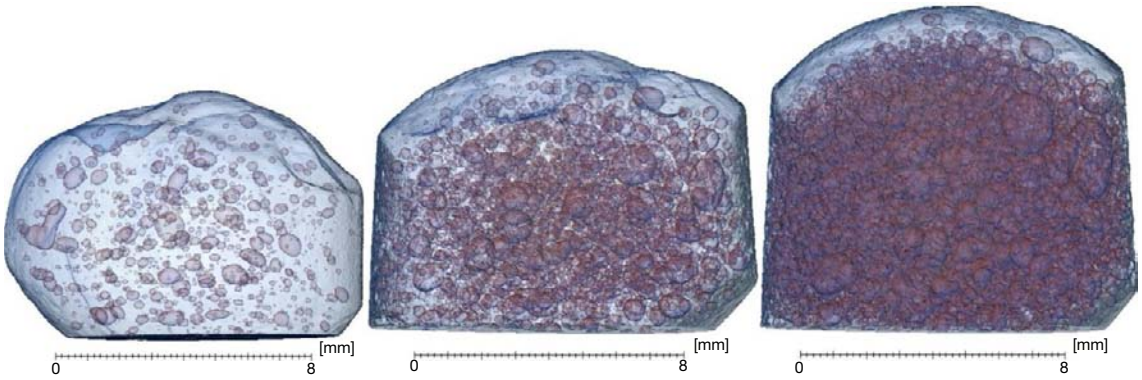

Figure 2 x-Ray tomographic images of bread dough showing the volume of dough (blue) and the bubbles within it (red) during proving of bread dough. (With acknowledgements to Linda Trinh and Peter Martin.)

limiting their shelf-life. During the formation of these products, however, and in other more liquid food foams, the bubbles must be stabilized against their thermodynamic tendency to coalesce. Stability is conferred to aerated foods through the action of indigenous or added emulsifiers, proteins (as in beer foams, egg foams, proving bread doughs, and cakes), fat or ice crystals, or other particles (whipped cream, ice cream, and puff pastry) or through a high-viscosity or solid continuous matrix (chocolate bars, meringues, breakfast cereals, Swiss cheese, and mousse).

In low-viscosity foams, three distinct mechanisms of destabilization occur: drainage, coalescence, and disproportionation. Drainage occurs when liquid flows from the thin lamellae between bubbles into the Plateau borders that form at the meeting point of three bubbles. Plateau borders form a continuous pathway through the foam, allowing liquid to drain until the bubble lamellae thin sufficiently to allow coalescence. If the foam is stable against coalescence, drainage will continue until a relatively dry polyhedral foam skeleton remains. Higher viscosity in the liquid slows both drainage and coalescence.

Coalescence between bubbles is thermodynamically favorable because of the resulting reduction in surface area. The presence of surface-active materials (e.g., emulsifiers and proteins) stabilizes against coalescence; pure liquids, free of surfactants, are unable to produce stable foams. The presence of hydrophilic particles may stabilize a foam, while hydrophobic particles destabilize foams by thinning the film between bubbles, as illustrated in Figure 3. Pickering particles, which have both hydrophobic and hydrophilic regions and so accumulate at interfaces, are the subject of much current research in food foam stabilization as they are able to confer remarkable stability.

Disproportionation in foams is the growth of large bubbles at the expense of smaller ones, equivalent to Ostwald ripening in emulsions. The gas pressure in smaller bubbles is greater than that in larger bubbles, due to the contribution of surface tension, γ, as described by the Laplace equation:

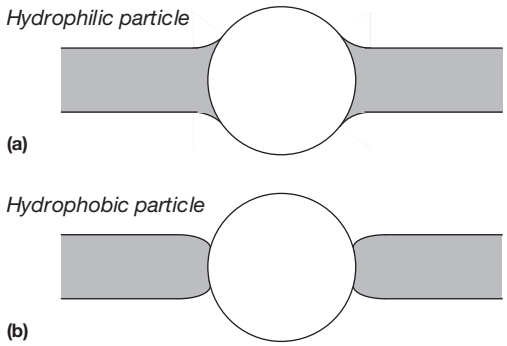

Figure 3 Effect of particles on foam stability: (a) stabilization of bubble lamellae by hydrophilic particles that draw liquid to the particle and oppose film thinning; (b) destabilization by hydrophobic particles.

$$P_b = P_\infty + \frac{2\gamma}{r}$$

where r is the bubble radius. The higher Laplace pressure in smaller bubbles causes a higher gas solubility in the neighboring region. This results in a mass transfer driving force between small and large bubbles that causes the diffusion of gas to the latter. The process is self-accelerating, as the loss of gas makes small bubbles even smaller, the Laplace pressure higher, and the gas solubility greater. Ultimately, small bubbles implode and the foam coarsens. However, the increasing concentration of surface-active components at the surface of the shrinking bubble slows down and can even arrest the process. Disproportionation is a major factor in foam stability of carbonated beverages such as beer, due to the high solubility of carbon dioxide in water. The presence of a small proportion of nitrogen in the gas phase stabilizes against disproportionation, as the loss of carbon dioxide by disproportionation from a small bubble lowers the partial pressure of carbon dioxide remaining in the bubble, lowering its concentration in the surrounding liquid, and thus ultimately removing the driving force for diffusive mass transfer.

Most aerated foods are not low-viscosity foams, and in most aerated foods, bubble coalescence is the most significant destabilization mechanism. Stability against bubble coalescence in both low- and high-viscosity systems is conferred by the presence of surface-active materials such as low-molecular-weight lipids and high-molecular-weight proteins. These two types of surfactant stabilize against coalescence via different mechanisms, as illustrated in Figure 4. Two adjacent bubbles will coalesce when the lamella between them thins and breaks as a result of drainage or mechanical disturbance (Figure 4 (a)). When the lamella thins, it experiences greater stress, which rapidly causes further thinning and breakage of the film. If a low-molecular-weight surfactant is present at the

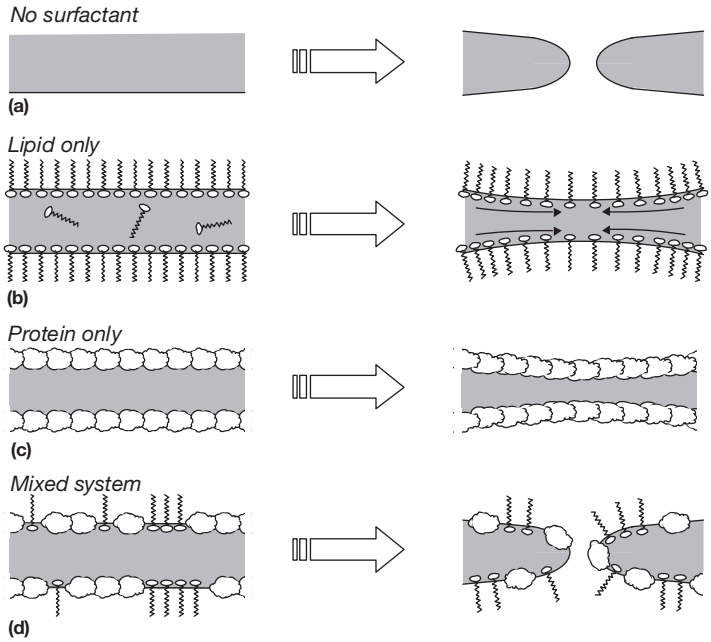

Figure 4 Illustration of film stability between bubbles with (a) no surfactant present; (b) low-molecular-weight surfactants such as lipids; (c) protein-stabilized bubbles; and (d) mixed protein–lipid systems.

surface, its concentration decreases at the point of film thinning. This creates a surface tension gradient that causes surfactant molecules from the adjacent area to diffuse rapidly to the depleted region, sweeping liquid into it and restoring the thickness of the region (the Marangoni effect) and thus safeguarding against coalescence (**Figure 4(b)**). The effectiveness of low-molecular-weight surfactants thus depends on their rates of surface lateral diffusion. Large-molecular-weight surface-active molecules such as proteins have low lateral diffusivities; they stabilize against coalescence via a different mechanism, in which they interlink to form a rigid layer at the surface (**Figure 4(c)**). If both proteins and low-molecular-weight surfactants such as lipids are present, competing for space at the bubble interface, these two stabilization mechanisms can interfere; the presence of protein prevents rapid surface diffusion of lipid molecules, while the presence of lipids prevents the formation of a rigid interlinked protein network (**Figure 4(d)**). Such mixed systems are responsible for the reduction in egg foam volume when a small amount of egg yolk is allowed in with the whites, the reduction in loaf volume when small amounts of polar lipids are added to bread dough formulations, and the disastrous effects of lipids on beer foam stability. In other cases, low-molecular-weight lipids may act cooperatively with proteins to improve foam stability.

See also: Biscuits, Cookies, and Crackers: Chemistry and Manufacture; Biscuits, Cookies and Crackers: Nature of the Products; Butter: Manufacture; Butter: Properties and Analysis; Cakes: Types of Cakes; Cereals: Types and Composition; Cheese: Composition and Health Effects; Cream: Clotted Cream; Cream: Types of Cream; Eggs: Composition and Health Effects; Extrusion Cooking: Chemical and Nutritional Changes; Extrusion Cooking: Principles and Practice; Ice Cream: Composition and Health Effects; Snack Foods: Role in Diet; Snack Foods: Types and Composition.

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Aeromonas

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Introduction

The genus *Aeromonas* belongs to the Aeromonadaceae family and comprises gram-negative, non-spore-forming, rod-shaped, facultative anaerobic bacteria that can be isolated from a very wide spectrum of environmental niches. The history and the perception of *Aeromonas* by the scientific community have evolved over 100 years, from its discovery in the late nineteenth and early twentieth centuries until nowadays. Aeromonads were first described as pathogens of poikilothermic animals. Today, they are recognized as causing severe illnesses in aquatic organisms (fish and other cold-blooded species) and also as emerging pathogens associated with several human infections and, in particular, as food-borne pathogens. In 2010, Janda and Abbott published an excellent review about *Aeromonas* spp., providing a wide and comprehensive view of the genus. However, many open questions regarding the ecology, pathogenicity, and taxonomy of aeromonads were present, and after 4 years, *Aeromonas* still represents a very complex genus.

A distinctive characteristic of *Aeromonas* has always been its controversial taxonomy. The complexity in identifying and discriminating *Aeromonas* species relies on the extremely high intra- and interspecies genetic variability. The genus was first discovered in 1891 and included in the family of Vibrionaceae together with *Vibrio* spp., *Plesiomonas* spp., and *Photobacterium* spp. This was due to the prevalence of these genera in the aquatic environments and the common phenotypic characteristics. The genus was officially created in 1943 and aeromonads were roughly divided into two major groups, based upon growth characteristics and other biochemical features. The mesophilic group, named *A. hydrophila*, consisted of motile isolates that grew well at 35–37 °C and were associated with a variety of human infections. The psychrophilic group, referred to as *A. salmonicida*, included nonmotile strains that had optimal growth temperatures of 22–25 °C and caused diseases in fish. From the mid-1970s until nowadays, an enormous explosion in the number of proposed species has been seen, and the list of species assigned to the genus is constantly changing. This is mainly due to two reasons: (1) the general and recent tendency to propose new species based upon single strains, especially in the last 5 years, and (2) the invalidity of some species names or the use of heterotypic synonyms of previously published species.

To date, there are 27 valid published species names among *Aeromonas* spp. included in the List of Prokaryotic names with Standing in Nomenclature (Table 1), but the second edition of *Bergey's Manual of Systematic Bacteriology* (*Bergey's*) recognizes far fewer. The genome sequences of 46 *Aeromonas* strains, including both draft and complete genomes, are now available in GenBank.

Aeromonas and Laboratory Identification

Aeromonas spp. can be easily isolated from clinical and environmental samples. Several media are routinely used for *Aeromonas* isolation, but their performances can vary according to the nature of samples (food, clinical, or water) and some selective agents that can reduce the recovery of some species. *Aeromonas* grow well on routine enteric isolation media (MacConkey, XLD, HE, SS, and DC media); however, the lactose-negative isolates must be differentiated from commonly isolated pathogens such as *Salmonella* and *Shigella*. The media that are frequently used for both qualitative and quantitative evaluations of *Aeromonas* spp. are listed in Table 2.

Microbiological methods are clearly needed for bacterial isolation, but their use as species identification tools can be very challenging, especially for aeromonads. For instance, it can be difficult to separate *A. veronii* bv. sobria or *A. caviae* from *A. hydrophila* or they may be confused with other genera, such as *Vibrio* and *Plesiomonas*.

In the last decade, DNA-based molecular methods have become more popular and widely acceptable for bacteria species identification due to their reproducibility, simplicity, and high discriminatory power. Several molecular methods have been applied for discriminating *Aeromonas* species. 16S rRNA gene sequencing represents the most commonly utilized molecular technique for this purpose. However, it is now recognized to be problematic for bacterial characterization mainly because of its intragenomic heterogeneity. This suggested that a single-gene-based identification approach may not be appropriate for characterizing *Aeromonas* spp. As a consequence, the multilocus sequence typing (MLST) approach became the new trend in the last 10 years. From 2011, three MLST schemes were published for *Aeromonas* spp. demonstrating the validity of this technique in discriminating aeromonads at species level. Moreover, the first *Aeromonas* MLST online database was opened (www.pubmlst.org/aeromonas) and is now available for collecting and sharing information about *Aeromonas* strains from different laboratories all over the world.

Aeromonas in the Environment

Aeromonas are described as ubiquitous bacteria. They can be isolated not only from a variety of aquatic environments and from different terrestrial ecosystems, such as food, invertebrates, plants, and slurry and fecal contents of farm animals, but also as a digestive tract symbiont of fish, leeches, and bats.

Initially, three *Aeromonas* genomospecies (*A. hydrophila*, *A. caviae*, *and A. veronii*) were considered to be related to the vast majority to human infections, while *A. salmonicida* has been

Table 1 List of the valid and proposed species in the genus *Aeromonas*

Species	*Year of proposal*	*Synonym (year of proposal)*
A. hydrophila	1943	
A. salmonicida	1953	
A. sobria	1981	
A. media	1983	
A. caviae	1984	*A. punctata* (1957)
A. veronii	1988	*A. ichthiosmia* (1991), *A. culicicola* (2002)
A. eucrenophila	1988	
A. schubertii	1989	
A. enteropelogenes	1991	*A. trota* (1992)
A. allosaccharophila	1992	
A. jandaei	1992	
A. encheleia	1995	
A. bestiarum	1996	
A. popoffii	1997	
A. simiae	2004	
A. molluscorum	2004	
A. bivalvium	2007	
A. aquariorum	2008	
A. tecta	2008	
A. diversa	2010	
A. fluvialis	2010	
A. piscicola	2010	
A. sanarellii	2010	
A. taiwanensis	2010	
A. rivuli	2011	
A. australiensis	2013	
A. cavernicola[a]	2013	

[a]Not yet included in the species with standing in nomenclature.

included as the predominant species in fish and water samples. However, *A. hydrophila* and *A. veronii* have been also recognized as involved in fish diseases, resulting in enormous economic losses. Some studies have also identified the presence of less frequently encountered species in environmental samples, such as *A. schubertii* in organic vegetables. However, although *Aeromonas* are still described as ubiquitous, the preferential association and adaptation between particular species and defined habitats have been recently highlighted. Two main different habitats were identified for *Aeromonas* species: aquatic (fish and water) and terrestrial (mainly food and human cases of disease). *Aeromonas* were first described as water bacteria, and the use of water on foods and irrigation and in human consumption could have easily contributed to their wide dispersal. The differentiation of species to a particular habitat might be the result of their adaptation over time. Species such *A. hydrophila*, *A. salmonicida*, *A. veronii*, *A. bestiarum*, *A. sobria*, and *A. allosaccharophila* are commonly isolated from the aquatic environment, while species such *A. caviae*, *A. media*, *A. enteropelogenes*, *A. jandaei*, and *A. schubertii* are described as 'terrestrial' (mainly associated with ready-to-eat food and human diseases). Unfortunately, limited data exist on the distributions of newly described species (such as *A. rivuli*, *A. taiwanensis*, *A. sanarellii*, *A. australiensis*, and *A. cavernicola*) in the environment outside their initial taxonomic description.

Aeromonas in Water

The main reservoir of the genus *Aeromonas* has always been the aquatic environment, with isolates from rivers, lakes, ponds, seawater (estuaries), drinking water, groundwater, wastewater, and sewage in various stages of treatments.

Many studies have demonstrated the ability of *Aeromonas* to survive and grow in drinking water supplies. The bacterium can resist to water treatment strategies such as rapid/slow sand filtration, hyperchlorination/direct filtration, and the use of granular activated carbon. Studies indicated that after disinfection with 1 mg l^{-1} of chlorine, 10% of the pipes had aeromonads and that *A. hydrophila* in biofilms could survive up to 0.6 mg l^{-1} of monochloramine, which could remove *E. coli* biofilms. Some studies reported that the presence of *Aeromonas* in drinking water could lead to septicemia in immunocompromised persons, although no link has been demonstrated so far. Due to the prevalence of *Aeromonas* in drinking water, the onset of new resistance mechanisms, and the presence of several virulence factors, aeromonads are included in the 'Contaminant Candidate List' by the Environmental Protection Agency. The World Health Organization lists *Aeromonas* in the third edition of Guidelines for Drinking-water Quality. On the basis of the Consumer Confidence Report Rule, public water systems are required to report unregulated contaminants, such as *Aeromonas*, when detected. Moreover, the presence of aeromonads in water supplies poses risk factors for the transmission of these bacteria to food products such as ready-to-eat vegetables. Decontamination with a lactic acid solution and not chlorine seems to show the highest potential to reduce *Aeromonas* spp. and to guarantee prolonged shelf lives of fresh-cut vegetables.

Aeromonas in Animals

Animals represent a very frequent reservoir for the transmission of *Aeromonas* species in the environment. Aeromonads are implicated in infections of both aquatic and terrestrial organisms. *A. salmonicida* causes fish furunculosis, especially in salmonids, and the disease has several presentations, from an acute form characterized by septicemia with hemorrhages at the bases of fins, inappetence, and melanosis to a chronic variety in older fish, consisting of lethargy, slight exophthalmia, and hemorrhaging in muscle and internal organs. *A. hydrophila* and *A. veronii* cause similar diseases, including hemorrhagic septicemia in carp, perch, catfish, and salmon; red sore disease in bass and carp; and ulcerative infections in catfish, cod, carp, and goby. *Aeromonas* have been implicated in several infectious processes; in seals, they can also cause 'red leg' disease in frogs, ulcerative stomatitis in snakes and lizards, septicemia in dogs and septic arthritis in calves, and seminal vesiculitis in bulls.

Aeromonas in Food

In the last 15 years, many studies were conducted to determine both the frequency and the concentration of *Aeromonas* spp. in food products (Table 2) from supermarkets and retail stores, and it has been observed that aeromonads are inhabitants of

Table 2 Isolation and characterization of *Aeromonas* spp. in food

Approach	*Medium*	*Matrix*	*N° samples*	*Species identification*[a]
Qualitative[b] and quantitative	ADA, mBIBG	Retail foods: vegetables, meat and meat products, seafood	68	130 isolates ● 73 fish (*A. hydrophila* 59%; *A. caviae* 12%) ● 41 vegetables (*A. caviae* complex 71%; *A. hydrophila* 7%; *A. bestiarum* 5%) ● 16 meat and poultry (*A. hydrophila* 37%; *A. caviae* 12%; *A. veronii* biovar *sobria* 19%)
	Ryan, SAA	Ready-to-eat foods: vegetables, cheeses, meat products, and ice creams	320	51 isolates *A. hydrophila* 53%, *A. caviae* 45%, *A. sobria* 2%
	Ryan	Minimally processed vegetables	26	46 isolates *A. hydrophila* group 72%, *A. caviae* group 28%
	Agar overlay method in BBGS	Ready-to-eat foods: meat, milk, fish Swab samples	557	74 isolates *A. hydrophila* 43%, *A. bestiarum* 3%, *A. caviae* 12%, *A. media* 1%, *A. eucrenophila* 3%, A. *sobria* 5%, *A. veronii* bv. sobria 12%, *A. veronii* bv veronii 4%, *A. jandaei* 5%, unidentified 11%
Qualitative	SAA	Organic vegetables	86	33 isolates *A. schubertii* 55%, *A. trota* 15%, *A. hydrophila* 15%, *A. caviae* 9%, *A. veronii* biovar *veronii* 6%
	BAA	Frozen–thawed fish	250	82 Isolates *A. salmonicida* 63%, *Aeromonas bestiarum* 20%, *A. veronii* bv. Sobria 5%, *A. hydrophila* 2%, *Aeromonas encheleia* 4%, others 6%
	GSP, Ryan, TCBS, EA, SCA (without ampicillin)	Raw fish	84	134 isolates *A. hydrophila* 68%, *A. caviae* 26%, *A. sobria* 6%
	ASA, ADA	Meat, seafood, dairy products, vegetables, beverage, and rice	389	72 isolates *A. sobria* 47%, *A. hydrophila* 53%
	ASDAB	Freshwater food fishes (healthy and diseased)	53	103 isolates *A. hydrophila* 48%, *A. sobria* 15%, *A. caviae* 15%, *A. jandaei* 11%, *A. veronii* 5%, *A. schubertii* 3%, and *A. trota* 3%
	Blood agar, MacConkey agar	Commercial sick chickens	2000	11 isolates *A. hydrophila* 100%
	ASDAB	Fish and fishery products (freshwater and marine fish and shellfish)	73	91 isolates *A. hydrophila* 19%, *A. sobria* 13%, *A. caviae* 7%, *A. jandaei* 4%, *A. trota* 8%, *A. schubertii* 5%

ADA, ampicillin-dextrin agar; ASA, *Aeromonas*-selective agar; ASDAB, *Aeromonas* Starch DNA Agar Base; BAA, blood agar with ampicillin; BBGS, bile salts–brilliant green starch agar; EA, enterohemolysin agar; GSP, *Pseudomonas–Aeromonas*-selective; mBIBG, modified bile salts–Irgasan–brilliant green agar; Ryan, *Aeromonas* medium base; SAA, starch ampicillin agar; SCA, standard count agar; TCBS, thiosulfate–citrate–bile salts–sucrose agar (vibrio-selective)

[a]Percentage of species among isolates.

[b]Mainly APW (alkaline peptone water) enrichment.

most types of food, from seafood to vegetables, meats (lamb, veal, pork, chicken, and ground beef), and dairy products.

Their presence in foods often leads to spoilage reactions, but in some products, such as milk, they can reach high concentrations (up to 10^8 CFU ml^{-1}) without any detectable organoleptic changes. Since their main reservoir is the aquatic environment, they have been isolated from several seafood species and the most common *Aeromonas* species found were *A. salmonicida*, *A. bestiarum*, *A. veronii*, and *A. encheleia*. Their frequent presence in these food matrices represents again a potential risk seen in the actual trend of eating raw seafood. Stratev and colleagues recently published a detailed review about the prevalence of *Aeromonas* spp. in food, but the final species description was clearly affected by the methods of isolation and identification (biochemical vs. biomolecular) (Table 2). *Aeromonas* is frequently found in vegetables, especially in ready-to-eat salads that are usually consumed without washing. The type of vegetables seems to influence the *Aeromonas* growth rate (more than the type of the atmosphere present), with more rapid growth occurring on shredded endive and lettuce than on sprouts or grated carrots. A work conducted on RTE at the University 'Federico II' of Naples on 320 food products revealed the presence of *Aeromonas* in 46% of samples, mostly vegetables (45% lettuce, 40% endive, and 15% rocket), but also on dairy products (45% ricotta cheese) and meat (25% salami and raw ham) (Table 2). *A. hydrophila* was the most common species isolated from food of animal origin, while *A. caviae* was mostly found in vegetables. Initial counts in food ranged from $<10^2$ to $>10^5$ CFU g^{-1} at 5 °C, and after 7 days at refrigeration temperature, *Aeromonas*

concentration increased one to three log as most aeromonads are psychrotolerant. In the majority of the studies, the isolates were recovered after enrichments techniques, indicating that *Aeromonas* concentrations were relatively low. However, enrichment is suggested for processed food, since food-preserving methods affect the recovery of *Aeromonas*, while for raw foods, the quantitative methods could provide a view of contamination.

Aeromonas importance in food bacteriology is due to their strong adaptive capacity, their high lipolytic and proteolytic action, and their surviving ability at wide ranges of temperatures and pH that make this genus able to grow on any food matrix. Moreover, some strains produce thermostable toxins and can survive in some processed food. *Aeromonas* strains can be recovered from foods stored at −20 °C for considerable periods (years) and it has been demonstrated that *A. hydrophila* resists to 5% NaCl at specific temperatures. Their capacity to grow at low pH values or high NaCl concentrations may represent a risk in ready-to-eat products where acidifications techniques are used for food conservation. Acetic, lactic, tartaric, citric, sulfuric, and hydrochloric acids are effective at restricting growth, and polyphosphates can also control their growth in certain foods. Overall, *Aeromonas* grow anaerobically as they do aerobically, their growth under modified atmospheres depends on the nature and number of competing microbiota, and the use of modified atmospheres to extend shelf lives of packaged meats and fresh vegetables may enable aeromonads to grow to high populations. However, *Aeromonas* spp. are readily killed by heat treatment or irradiation, but they are resistant to chlorination processes and to multiple antibiotics.

Aeromonas in Human Health

From 1954, when *Aeromonas* was first associated with the death of a 40-year-old-woman in Jamaica, to the present date, the role of this bacterium in human colonization and infection is still much debated. Although *Aeromonas* does not belong to the human enteric microbiota, it has been demonstrated that it is present in 1% of the adult people and this value increases to 3% in warm periods and up to 30% in developing nations.

Since *Aeromonas* spp. are ubiquitous bacteria, the association with humans is easily established. They are mainly acquired via contact with contaminated drinking water or through the ingestion of foods that are naturally exposed to aeromonads through irrigation processes or other 'farm-to-table' operations. Also, raw seafood represents a common way of contamination. Bivalves such as oysters and mussels are naturally bathed in estuary waters containing these bacteria, and through their filter-feeding process, they actually concentrate these bacteria within their meats. It has been also reported that recreational activities such as boating, fishing, and diving can lead to infection, although no reliable data are available.

Janda and Abbott listed a detailed survey of the incidence of *Aeromonas* infections all over the world, based on available data. In 1988, California reported that the incidence of *Aeromonas* infections was 10.6 per million. In 2006, 99 *Aeromonas* infections were reported in 70 hospitals in France; this represents a prevalence of 1.62 infections per million, a value much lower than that reported in the Californian study. In England and Wales, *Aeromonas* bacteremia is a voluntarily reportable condition and 82 cases of *Aeromonas* bacteremia were recorded in 2004. Based upon these data, it has been calculated that the incidence of *Aeromonas* septicemia in England/Wales and the United States is 1.5 per million. To date, the exact incidence of *Aeromonas* infections on a global basis is unknown as many cases may be asymptomatic or not reported.

It has been observed that *Aeromonas* species implicated as causes of human colonizations and infections are not restricted to a single genomospecies, and it seems that an association between some *Aeromonas* species and their effects on humans exists. Among the 27 species identified to date, *A. hydrophila*, *A. caviae*, *A. veronii*, and *A. jandaei* are most commonly associated with humans and account for more than 85% of all clinical isolates.

While *Aeromonas* was originally thought to be an opportunistic pathogen in immune-compromised humans, an increasing number of cases of *Aeromonas*-associated intestinal and extraintestinal disease documented worldwide seem to suggest it is an emerging human pathogen irrespective of the host's immune status. A recent study reports 91 cases of bacteremia caused by *Aeromonas* spp. recorded in a computerized database of a regional hospital in southern Taiwan and confirms this bacterium as a nosocomial pathogen. To date, it is described as bona fide enteropathogen, but it is not universally accepted as a pathogen bacterium. The proof that establishes *Aeromonas* as a true pathogen is lacking, and this is mainly due to the failure to identify a single clonally related outbreak of disease and to detect an immune-specific response in human serum. However, *Aeromonas* spp. have been isolated from several cases of human infections and are described as responsible of several intestinal and extraintestinal diseases and syndromes. *Aeromonas* are mainly the cause of gastrointestinal syndromes, but they have been also described as causing other types of infections.

Aeromonas in gastroenteritis

The gastrointestinal tract is the most common site from which aeromonads are recovered. The colonization of the human gastrointestinal tract by aeromonads is most likely a result of the consumption of food and drinking water containing *Aeromonas* spp. In recent years, the incidence of gastroenteritis due to *Aeromonas* spp. has increased significantly, affecting all age groups in both industrialized and developing nations. In industrialized countries, the frequency of *Aeromonas* in stool samples has been reported to be between 2.2% and 10%. The results recently obtained by Global Enteric Multicenter Study to identify the etiology and population-based burden of pediatric diarrheal disease in developing countries report *Aeromonas* as a common cause of diarrhea in children younger than 5 years in Pakistan and in Bangladesh.

Aeromonas infections of the gastrointestinal tract can lead to five different settings, from nondescript enteritis to more severe forms accompanied by bloody stools or chronic intestinal syndrome, traveler's diarrhea, or even cholera-like disease. The most common setting is the secretory enteritis, which has been reported to account for up to 89% of all cases of *Aeromonas* gastroenteritis. It includes fever and abdominal pain and in some cases also vomiting. The dysenteric form is more rare,

accounting for up to 22% of *Aeromonas* gastroenteritis. Moreover, there are several complications associated to *Aeromonas* gastroenteritis; they mainly include segmental colitis or the hemolytic uremic syndrome.

Despite all these data, *Aeromonas* is not officially considered a gastrointestinal pathogen, as a real proof of pathogenicity still lacks. To date, there is no animal model that can faithfully reproduce the *Aeromonas*-associated diarrheal syndrome, although many attempts have been made. However, several studies reported *Aeromonas* as the sole pathogen present in patients affected by gastroenteritis, but it was also found in the stools of 1–4% of asymptomatic individuals. Thus, their role in gastroenteritis is still problematic.

A hypothesis that seems to be the most reliable so far suggests the possibility that the pathogenicity of *Aeromonas* spp. relies on the presence of specific virulence factors in the genome and of particular conditions in hosts that favor the onset of the disease (i.e., immunocompromised patients and persons with hematologic cancers, tumors of the gastrointestinal tractor, and other underlying pathological anomalies of the alimentary canal).

Other infections

Aeromonas spp. are also associated with a variety of skin and soft tissue infections, mainly as a consequence of direct contact with contaminated water and traumatic injuries. In terms of incidence, wound infections are much less frequent than gastroenteritis and, while the overall incidence of *Aeromonas* infections in United States was ~10 per million (when reported), wound infections were estimated to be 0.7 per million. The manifestations range from mild infections of the subcutaneous tissues (cellulitis), which represent the most common symptom, to serious conditions affecting deeper tissues (necrotizing fasciitis and myonecrosis). *Aeromonas* infection was also associated with the use of medicinal leech therapy that mainly causes cellulitis. *Aeromonas* species were recognized as important pathogens in natural disaster situations; they were isolated in high concentrations (10^6–10^7 CFU ml^{-1}) after both the hurricane Katrina in New Orleans and the tsunami in Thailand in 2004.

Another disease form associated with *Aeromonas* infections is septicemia. The vast majority of cases are seen in persons who are severely immunocompromised or have underlying complications such as diabetes mellitus, renal problems, cardiac anomalies, and other hematologic conditions. However, some cases of septicemia caused by *Aeromonas* in healthy persons have been described. The most common symptoms include fever, jaundice, abdominal pain, septic shock and dyspnea. *Aeromonas* septicemia is mostly caused by traumas and direct contact with microorganisms through wounds, and, in some instances, it was associated with leech therapy. As a matter of fact, leeches harbor aeromonads symbiotically and their use in therapeutic procedures may cause infections. Currently, it is not possible to clinically distinguish *Aeromonas* bacteremia from those caused by other gram-negative bacteria such as *Escherichia coli* or *Klebsiella pneumonia*. However, a peculiar indicator of *Aeromonas* infection is the presence of ecthyma gangrenosum-like lesions in the form of petechiae or bullae.

Aeromonads are also recognized as causing intra-abdominal diseases such as peritonitis and infections of the hepatobiliary and pancreatic systems. In Southeast Asia, *Aeromonas* is the third most common gram-negative cause of peritonitis. The peritonitis caused by *Aeromonas* results mainly from extensions of infections from the biliary or gastrointestinal tract. However, the source of infections is unclear in most cases, with few medical histories suggesting an environmental origin.

Aeromonas have been associated with respiratory tract infections, not only mainly pneumonia but also with cases of empyema. The main cause was the presence of near-drowning events involving seawater and other massive aquatic exposures. Again, the presence of underlying syndromes has often been reported

Finally, *Aeromonas* species have been occasionally implicated in eye and urogenital tract infections.

Pathogenicity

As already discussed, it is presently unclear whether aeromonads can be considered proper pathogens. An animal model of infection is lacking and the attempts made to reproduce the illnesses were unsuccessful. In addition, the microbial factors responsible for the onset of the diseases are still unknown and their identification is even more clouded by the widespread presence of genes potentially implicated in microbial infections throughout the genomes of most *Aeromonas* species.

To shed light onto the role of *Aeromonas* in gastroenteritis, the use of putative gene markers for pathogenicity has been widely applied to characterize strains from different food origins (Table 2), but their presence is still only indicative of potential virulence.

The species involved in the vast majority of systemic infections in humans are *A. hydrophila*, *A. caviae*, and *A. veronii*; however, recent environmental studies extended the knowledge of 'human-related' *Aeromonas* to other taxa, including *A. jandaei* and *A. enteropelogenes*. Moreover, an ecological and genetic link has been found between species isolated from food matrices and human cases of diseases. Thus, if initially the main cause of *Aeromonas* infection was considered to be just the aquatic environment, now, the connection between human infections and the ingestion of contaminated food seems to become a more common scenario. The adaptation to specific habitats may suggest that the infectious process involves, at least in part, selection of species (or strains) with certain characteristics that favor infections. However, this has not been demonstrated so far. One of the problematic issues in understanding the pathogenicity of *Aeromonas* concerns the fact that this genus produces an impressive array of virulence factors and also the lack of consensus on standardization of terminology regarding these factors between different research groups. *Aeromonas* spp. produce several extracellular products that fall into several broad categories, including cytolytic toxins with hemolytic activity, cytotonic enterotoxins, hemolysins, lipases, proteases, leukocidins, phospholipases, fimbriae or adhesins, and the capacity to form capsules.

Several classes of genes have been identified that play important roles in the colonization of the leech digestive tract, including bacterial cell surface modifications, regulatory factors, nutritional elements, and genes involved in the secretion system

(SS) (such as T2SS and T3SS). *Aeromonas* spp. produce a wide range of proteases, which cause tissue damage and aid in establishing an infection by overcoming host defenses and by providing nutrients for cell proliferation. Lipases secreted by *Aeromonas* may also constitute virulence factors by interacting with human leukocytes or by affecting several immune functions through fatty acids generated by lipolytic activity. Two factors thought to play intimate roles especially in the colonization of gastrointestinal tract are flagella and pili. *Aeromonas* produces two types of flagella, a constitutively expressed polar flagellum (Pof) and multiple inducible lateral flagella (Laf), which are, respectively, involved in the initial attachment of bacteria to the gastrointestinal epithelium and in cell adherence, long-term colonization, and biofilm formation. Biofilm development may also be regulated by quorum sensing that appears to act in concert with T3SS to regulate the expression of the *Aeromonas* enterotoxins, as its production increases when bacterial cell density increases. The cytotonic enterotoxin Act/Asa is a pore-forming toxin, also known as aerolysin AerA: it was originally recovered from a diarrheal isolate of *A. hydrophila* and was subsequently determined to possess a variety of biological activities, including hemolysis, cytotoxicity, and enterotoxicity, and to cause lethality in mice. While it is clear that Act induces extensive host cell signaling (it stimulates proinflammatory responses by increased cytokine production through elevated tumor necrosis factor, IL-1β and IL-6 levels), it is unknown how the toxin exerts the effects. Another well-characterized toxin (AHH1) belongs to the family of β-hemolysins and has a high sequence homology to the HlyA hemolysin of *Vibrio cholerae*. These toxins are also named Act- and aerolysin-like molecules and are enterotoxigenic cytolysins. Many *Aeromonas* strains possess a surface layer (S-layer), which resists complement-mediated killing of the organism by impeding complement activation. It seems that the set of bacterial virulence factors and host responses that eventually lead to *Aeromonas*-associated diseases are ill-defined. Regarding gastrointestinal diseases, aeromonads can apparently produce diarrhea by elaboration of enterotoxigenic molecules and/or by invasion of the gastrointestinal epithelium. At least two cytotonic toxins have been identified: a heat-labile cytotonic enterotoxin (Alt) and a heat-stable cytotonic enterotoxin (Ast). Invasins have also been reported, but they are difficult to detect *in vitro*; some studies suggested that only a fraction of *Aeromonas* strains are invasive and the degree of invasion is considerably less than the observed for classic enteropathogens, such as *E. coli* and *Yersinia enterocolitica*. The gene encoding enolase was also found in *A. hydrophila* strains recovered from stools. Enolase is a glycolytic enzyme whose surface expression was shown to be important in the pathogenesis of *Streptococcus pyogenes*-associated rheumatic fever. It has been suggested that the surface expression of enolase occurs only in gram-positive bacteria, while other researchers have demonstrated the ability of this protein to bind human plasminogen, potentially indicating an important role during *Aeromonas* infections. The T3SS includes several factors with multiple biological functions, and the gene AexT, a homologue of *Pseudomonas aeruginosa* T3SS-secreted ExoT/S, was detected in some *Aeromonas* isolates, but no information is available on its role in bacterial virulence using *in vivo* models. Another well-known T3SS gene is *ascV* that codes for an inner-membrane component of the T3SS channel.

TagA has been described as a new virulence factor found in an *A. hydrophila* isolate from diarrhea and only present in pathogens as *E. coli* O157:H7 and *V. cholerae*; its role seems to be related to the inhibition of the classical complement-mediated lysis of the erythrocytes but, even in *V. cholera*, its function in pathogenesis is speculative.

There are a large number of unresolved questions regarding the role of the potential virulence factors in *Aeromonas* infections. Some genes, such as *act*, are also found in species that are infrequently associated with human diseases (*A. bestiarum*). Moreover, research on *Aeromonas* pathogenicity demonstrated the enormous complexity of the situation involving polygenic expression in both the pathogen and the host. Thus, there is still much to be learned about *Aeromonas* virulence determinants and how they combine to result in the virulent subsets within each *Aeromonas* species that causes disease. At present, it is not possible to identify the disease-causing strains because of the incomplete understanding of *Aeromonas* virulence mechanisms.

Aeromonas and Antimicrobial Susceptibility

A particularly interesting area that is receiving more attention in the last years is the susceptibility of *Aeromonas* to antimicrobial agents. The studies regarding this topic reported data on the three major species associated with human disease, *A. hydrophila*, *A. caviae*, and *A. veronii*, so we do not know yet if the available information is also valid for the other species.

The first studies recording the antibiotic susceptibility of *Aeromonas* were conducted between the mid-1980s and mid-1990s. Inducible chromosomal β-lactamases are still the major resistance mechanism for most aeromonads, although their resistance to other antibiotics is dramatically increasing. Upon characterization of the antimicrobial resistance of 94 *Aeromonas* isolates from warm- and cold-water ornamental fish species using microarray analysis and conventional PCR, a surprisingly high level of antimicrobial tolerance was identified in the strains tested. Half of the *Aeromonas* spp. isolates were tolerant to more than 15 antibiotics, representing seven or more different classes of antimicrobials. The quinolone and fluoroquinolone resistance gene was detected at high frequency, although it has been reported that *Aeromonas* strains are almost universally susceptible to fluoroquinolones. Resistance has been also observed to carbapenems, imipenem, chloramphenicol, and florfenicol. Moreover, tetracyclines were particularly widespread across all screened isolates.

The discovery of multidrug resistance in strains isolated from wild shellfish in the Adriatic Sea suggests an involvement of *Aeromonas* in the dissemination of antibiotic resistance in the environment and in seafood. The susceptibility status of *Aeromonas* isolates for therapeutically active drugs appears to be independent of species designation. While some species-specific susceptibility differences have been found, these results should be considered preliminary at present. Moreover, no connection between a specific resistance pattern and the origin of isolation has been identified so far. Certainly, more studies need to be performed in this area.

Conclusions

After more than a century from its discovery, the *Aeromonas* genus is still intricate. Indeed, great improvements have been made in the last years, as molecular genetics have led to considerable advantages in the taxonomic determination of these bacteria, which was one of the most controversial issue of the genus. Moreover, the ecology and the mechanisms of environmental adaptation have been tackled, identifying a genetic adaptation process of *Aeromonas* species toward specific habitats. However, the image of *Aeromonas* as a human pathogen is still blurred, as no evidences of a clear association exist. There is still much to be learnt about *Aeromonas* pathogenicity and virulence determinants and how they combine to result in disease, but the advent of next-generation techniques and the possibility to analyze and combine massive amounts of data make us believe it is likely to happen.

See also: Chilled Foods: Modified Atmosphere Packaging; Fish: Fish in the Human Diet; Spoilage: Bacterial Spoilage.

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Relevant Websites

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Aflatoxin: A Global Public Health Problem

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Discovery and Exposure to Aflatoxin

The aflatoxins were discovered in the early 1960s, when they were identified as causative agents of 'turkey X' disease, an epidemic involving deaths of thousands of young turkeys, ducklings, and chicks fed with diets containing certain lots of peanut meal originating in South America. Careful investigations revealed that toxicity was associated with the presence of the common spoilage mold *Aspergillus flavus* and further that extracts of cultures of the fungus isolated from toxic meal were capable of inducing the toxicity syndrome. The name 'aflatoxin' was accordingly assigned to the toxic agents. Subsequent studies on extracts of *A. flavus*-contaminated groundnut meal confirmed that these agents were capable of inducing acute liver disease in ducklings and liver cancer in rats. Detection of aflatoxins in extracts of contaminated peanut meal was facilitated by their intense fluorescence in ultraviolet light, and soon thereafter, purified metabolites with identical physical and chemical properties were isolated from *A. flavus* cultures. Structural elucidation of aflatoxin B_1 was accomplished and confirmed by its total synthesis in 1963. Development and application of fermentation technology for production of substantial quantities of aflatoxins led to the availability of purified compounds, which in turn enabled extensive investigations into their toxicology and relationships to human diseases ranging from acute liver damage to liver cancer. As a result, to date, the aflatoxins represent a limited group of ubiquitous and structurally identified environmental carcinogens for which quantitative estimates of human exposures have been systematically sought and risk assessments carried out. These efforts have produced well over 10 000 scientific publications to date, dealing with all aspects of the problem. Collectively, available data led the International Agency for Research on Cancer (IARC) to classify aflatoxins as a category I known human carcinogen. Recently, IARC published an updated monograph outlining public health-based methods that could be applied in different societies to control aflatoxin contamination and reduce human exposures.

Chemically, the aflatoxins are highly substituted coumarins containing a fused dihydrofurofuran moiety (Figure 1). Aflatoxins B_1 and B_2 (AFB_1 and AFB_2) were so named because of their strong blue fluorescence in ultraviolet light, whereas aflatoxins G_1 and G_2 (AFG_1 and AFG_2) fluoresced greenish yellow. These properties facilitated the very rapid development in the early 1960s of methods for monitoring grains and other food commodities for the presence of the toxins. AFB_1 and AFG_1 possess an unsaturated bond at the 8,9 position on the terminal furan ring, and future studies demonstrated that epoxidation at this position was critical for their carcinogenic potency. AFB_2 and AFG_2 are essentially biologically inactive unless they are first metabolically oxidized to AFB_1 and AFG_1 *in vivo*.

Human populations are exposed to aflatoxins by consumption of foods on which strains of *A. flavus* or *A. parasiticus* have grown during harvest or storage. In general, diets may contain AFB_1 and AFB_2 in concentration ratios of 1.0:0.1, and when all four aflatoxins occur, AFB_1, AFB_2, AFG_1, and AFG_2 proportions of 1.0:0.1:0.3:0.03 exist. Grains and foodstuffs found to be contaminated with aflatoxins include corn, peanuts, milo, sorghum, copra, and rice. While contamination by the molds may be universal within a given geographic area, levels of aflatoxins in the grain product can vary from less than 1 $\mu g\ kg^{-1}$ (1 ppb) to greater than 12 000 $\mu g\ kg^{-1}$ (12 ppm). Indeed, in a recent outbreak of aflatoxin-induced death of people in Kenya, the daily ingestion of AFB_1 was estimated to be 50 mg per individual.

The present action-level guideline for seizure of aflatoxin-contaminated agricultural commodities in the United States is 20 µg total aflatoxins/kg (20 ppb). However, in Europe and Japan, aflatoxin limits in foods and feeds are lower, ranging from detection limit to 10 ppb. The US Food and Drug Administration (FDA) has also set a practical action guideline of 0.5 µg aflatoxin M_1 (AFM_1) per liter (0.5 ppb) for fluid milk based in part on the demonstration that AFM_1 was about tenfold less carcinogenic than AFB_1 in rats. In recent years, based on epidemiological data, much lower tolerances for aflatoxin exposures have been advocated for people who are hepatitis B virus carriers.

Aflatoxin Carcinogenesis and Metabolism

The carcinogenic potency of AFB_1 has been well established in many species of animals, including rodents, nonhuman primates, and fish. The liver is consistently the primary target organ affected and the toxin induces a high incidence of hepatocellular carcinoma (HCC). Additionally, under certain circumstances, depending on animal species and strain, dose, route of administration, and dietary factors, significant numbers of tumors have been found at other sites, such as the kidney and colon. Indeed, no animal species has been shown to be completely resistant to aflatoxin-induced carcinogenesis. Wide cross species potency, including sensitivity of nonhuman primates, provided the initial experimental basis for proposing that this agent would contribute to human cancer.

The high experimental potency of AFB_1 provided an impetus for research to characterize the metabolism of AFB_1 and to elucidate underlying molecular mechanisms of tumor initiation by this compound. Metabolic products that have been identified are summarized in Figure 2. Aflatoxin–DNA and aflatoxin–protein addition products (adducts) have been of particular interest because they are direct products of (or

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00015-5

surrogate markers for) damage to critical cellular macromolecular genetic targets. Metabolic pathways for the formation and chemical structures of the major aflatoxin macromolecular DNA and protein adducts have been elucidated and are shown in Figure 2. The finding that the major aflatoxin–nucleic acid adduct AFB_1–N^7-guanine was excreted in the urine of exposed rats spurred interest in exploiting this metabolite as a biomarker of exposure and risk. The serum aflatoxin–albumin adduct was also employed as a biomarker of exposure. The longer half-life *in vivo* of the albumin adduct as compared with the urinary DNA adduct reflects exposures over longer time periods, and subsequent studies in experimental models have shown that levels of aflatoxin–DNA adducts in liver, excretion of the urinary aflatoxin–guanine adduct, and levels of serum albumin adduct are highly correlated. Collectively, these data led to the application of these aflatoxin metabolites as biomarkers of human exposure and risk (Figure 3).

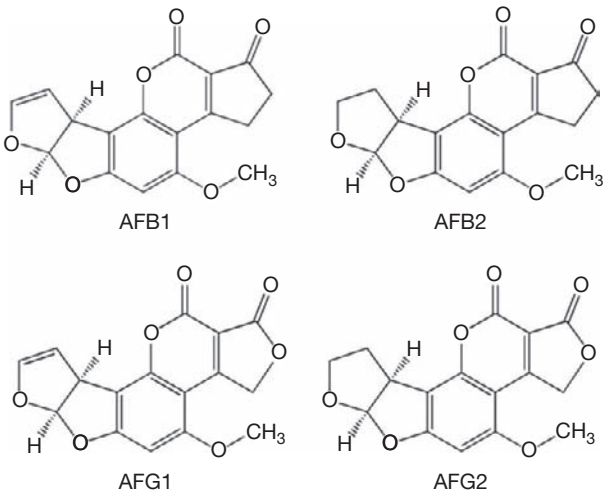

Figure 1 Structures of the four major aflatoxins.

Human Liver Cancer and Aflatoxin

Collectively, liver cancer, including HCC, accounts for 5.7% of all reported cancer cases and is the sixth most common cancer diagnosed worldwide. Globally, the incidence of liver cancer varies enormously, and the incidence of this fatal disease is much higher in economically less-developed countries of Asia and sub-Saharan Africa. Nearly 700 000 new cases and over 300 000 deaths occur annually in the People's Republic of China (PRC) alone. In contrast to most common cancers in the economically developed world, where over 90% of cases are diagnosed after the age of 45, in high-risk regions for liver cancer, onset begins in both men and women by 20 years of age, peaking between 40 and 49 years of age in men and 50 and 59 years in women. The earlier onset of HCC might be attributable to exposures that are both substantial and persistent across the life span. Gender differences in liver cancer

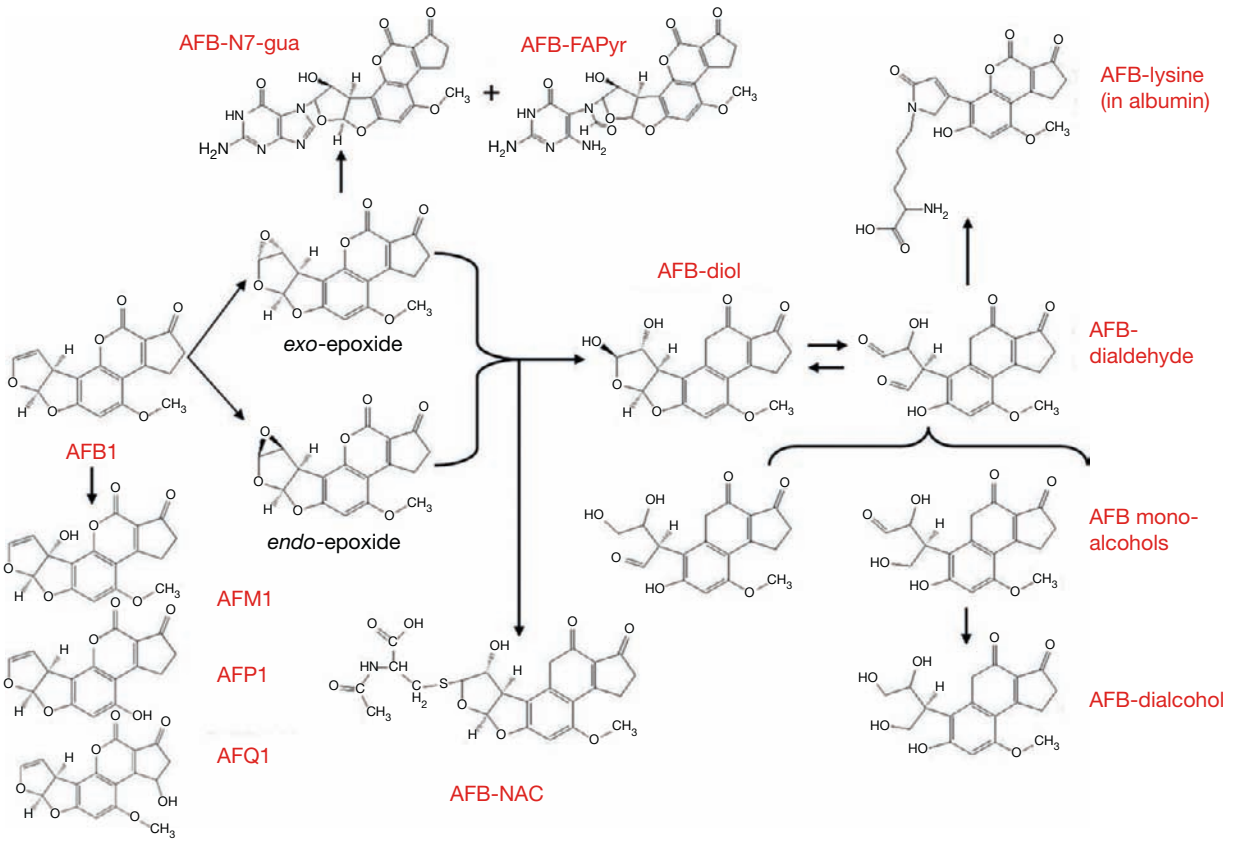

Figure 2 Major metabolites of aflatoxin B_1.

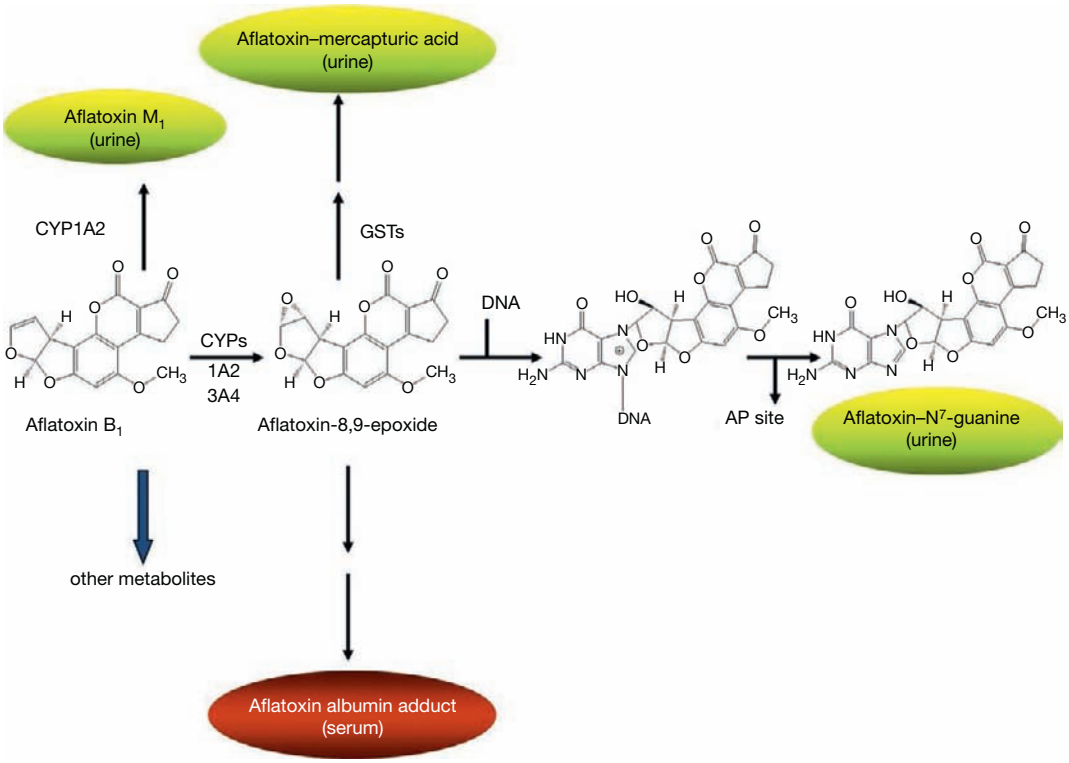

Figure 3 Aflatoxin metabolites used as biomarkers of exposure and risk.

incidence have also been described; the worldwide annual age-standardized incidence rate among men is 15.8 per 100 000 and 5.8 per 100 000 among women. These epidemiological findings are also consistent with experimental animal data, in which male rats have been found to have an earlier onset and higher tumor incidence than female animals of aflatoxin-induced liver tumors.

To date, two major cohort studies incorporating aflatoxin biomarkers have clearly demonstrated the etiologic role of this carcinogen in HCC. The first study, comprising over 18 000 men residing in Shanghai, examined the interaction of HBV and aflatoxin biomarkers as independent and interactive risk factors for HCC. The nested case-control data revealed a statistically significant increase in the relative risk (RR) of 3.4 for those HCC cases in whom a urinary aflatoxin biomarker (AFB_1–N^7-guanine) was detected. In men whose serum was HBsAg-positive but whose urine did not indicate aflatoxin exposure, the RR was 7, but in individuals exhibiting both urinary aflatoxin marker and positive HBsAg status, the RR was 59. These results strongly support a causal relationship of both biomarkers and the risk of HCC, as well as strong synergy between the presence of aflatoxin and viral-specific biomarkers and HCC risk.

Subsequent cohort studies in Taiwan have substantially confirmed the results from the Shanghai investigation. Wang et al. examined HCC cases and controls nested within a cohort and found that in HBV-infected people, there was an adjusted odds ratio of 2.8 for detectable compared with nondetectable aflatoxin–albumin adducts and 5.5 for high compared with low levels of aflatoxin metabolites in urine. In a follow-up study, there was a dose–response relationship between urinary AFM_1 levels and risk of HCC in chronic HBV carriers. As in the Shanghai cohort, HCC risk associated with AFB_1 exposure was most striking among HBV carriers with detectable AFB_1–N^7-guanine in urine.

Thus, these cohort data from two different populations demonstrate the power of validated aflatoxin biomarkers to define a previously unrecognized chemical–viral interaction in the induction of human HCC. These findings have significant public health implications. First, vaccination to prevent HBV infection will substantially ameliorate a major risk factor for HCC. Unfortunately, in most parts of the world, HBV infection is acquired before 3 years of age; consequently, worldwide elimination of HBV infection by vaccination will require much of the next century to accomplish. Second, minimizing aflatoxin exposure would also significantly reduce HCC risk. This goal could be attained through available technologies, and dose–response data from epidemiological studies indicate that, in a manner similar to reduction of lung cancer risk through smoking cessation, minimization of aflatoxin exposure during an individual's lifetime should reduce risk of HCC.

A recent report demonstrates that in China, the impact of agricultural reforms in the 1980s led to diminished maize consumption and that this change has had a major impact on PLC primary prevention. In Qidong, China, a population-based cancer registry was used to track PLC mortality, which was compared with the timeline of HBV immunization. More than 50% reductions in PLC mortality rates occurred across birth cohorts from the 1960s to the 1980s for Qidongese younger than 35 years, although all were born before universal vaccination of newborns began. Levels of aflatoxin biomarkers were determined in randomly selected archived serum samples

collected from subject cohorts from the 1980s to 2013. Median levels of the aflatoxin–albumin adduct biomarker decreased from 19.3 pg mg^{-1} albumin in 1989 to undetectable (<0.5 pg mg^{-1}) by 2009. A population-attributable benefit of 65% for reduced PLC mortality was estimated to result from a government-facilitated change of dietary staple from maize to rice; 83% of this benefit occurred in those infected with HBV. Thus, food policy reforms in China resulted in a dramatic decrease in aflatoxin exposure, which, independent of HBV vaccination, substantially reduced liver cancer risk.

Aflatoxin and *p53* Mutations

The relationship between aflatoxin exposure and development of HCC has been further highlighted by molecular biological studies on the *p53* tumor suppressor gene, the gene most commonly mutated in many human cancers. Many studies of *p53* mutations in HCC occurring in populations exposed to high levels of dietary aflatoxin have found high frequencies of G:C to T:A transversions, with clustering in the third base of codon 249.

Results from previous mechanistic studies showed that AFB_1-induced almost exclusively guanine to thymine transversions in bacteria and that aflatoxin-8,9-epoxide could bind to codon 249 of *p53* in a plasmid *in vitro*. Further, mutagenesis of the *p53* gene in human HepG2 cells and hepatocytes exposed to AFB_1 and found preferential induction of the guanine to thymine transversion in the third position of codon 249. A further study has mapped AFB_1 adduct formation to codon 249.

Additional experimental data support the role of aflatoxin as a generator of the codon 249 mutation. The primary DNA adduct of AFB_1 is AFB_1–N^7-guanine, which in double-stranded DNA is readily converted into two secondary lesions, an apurinic site and an AFB_1–formamidopyrimidine (AFB_1–FAPY) adduct. AFB_1–FAPY is detected at near maximal levels in rat liver DNA days to weeks after AFB_1 exposure, underscoring its persistence *in vivo*. Experimental mutagenesis studies revealed two striking properties of this DNA adduct: (i) $AFB(_1)$–FAPY was found to cause a G to T mutation frequency in *Escherichia coli* approximately six times higher than that of AFB_1–N^7-guanine, and (ii) one proposed rotamer of AFB_1–FAPY was a block to replication, even when the efficient bypass polymerase MucAB was used by the cell. Taken together, these characteristics make the FAPY adduct a prominent candidate for inducing both the genotoxicity of aflatoxin, since mammalian cells have similar bypass mechanisms for combating DNA damage, and mutagenicity that ultimately may lead to liver cancer.

In summary, studies of the prevalence of codon 249 mutations in HCC cases from patients in areas of high or low exposure to aflatoxin suggest that a G:C to T:A transversion at the third base is associated with aflatoxin exposure and *in vitro* data support this hypothesis. A majority of codon 249 mutations are found in liver tumors of patients infected with HBV, implicating an association. In comparisons of codon 249 mutations in regions of high HBV infection but varying levels of AFB_1 exposure, the mutation only occurs in areas of high AFB_1 exposure. HBV evidently plays an important role in mutagenesis, perhaps by causing preferential selection of cells harboring the mutation. Interpretation of the codon 249 mutation as a marker of aflatoxin exposure to aflatoxin must be done with caution until evidence has been obtained from studies measuring both AFB_1 adducts and mutations in the same individual. However, in general, available experimental data strongly support the findings of cross-sectional epidemiological studies indicating that AFB_1 is an important etiologic factor for HCC.

As illustrated earlier, detection of specific *p*53 mutations in HCC tumors has provided valuable insight into the etiology of primary liver cancer. Application of these specific mutations as biomarkers for early detection also offers great promise for HCC prevention. In a seminal study, Kirk et al. reported for the first time detection of *p*53 codon 249 mutations in plasma of liver tumor patients residing in the Gambia; however, the mutational status of their tumors was not determined. These authors also reported the presence of this mutation in the plasma of a small number of cirrhosis patients. Given the strong relationship between cirrhosis and future development of HCC, the possibility of this mutation serving as an early detection marker merits further exploration. Jackson et al. compared results obtained with short oligonucleotide mass analysis of plasma DNA with results of sequencing of DNA from 25 HCC tumors for specific *p*53 mutations. Mutations detected in plasma samples were in agreement with those found in HCC DNA by DNA sequencing. Jackson et al. further explored the temporality of detection of this mutation in plasma before and after clinical diagnosis of HCC in the same patient. This study was facilitated by availability of longitudinally collected plasma samples from a cohort of 1638 high-risk individuals in Qidong, PRC, who have been followed since 1992. Sixteen patients diagnosed with liver cancer between 1997 and 2001 from which plasma samples had been collected before and after HCC diagnosis were selected for study. Results showed that in samples collected prior to liver cancer diagnosis, 21.7% of the plasma samples had detectable levels of the codon 249 mutation, with a 95% confidence interval of 9.7–41.9%. The persistence of this prediagnosis marker was borderline statistically significant ($P = 0.066$, two-tailed). The codon 249 mutation in *p*53 was detected in 44.6% of all plasma samples following the diagnosis of liver cancer with 95% confidence intervals from 21.6% to 70.2%. This level of positive samples following liver cancer diagnosis compares with about 50% of all liver tumors in Qidong, suggesting a nearly 90% concordance between plasma and tumor *p*53 codon 249 mutation outcome. Further, persistence of this mutation in plasma once it became measurable was statistically significant ($P = 0.024$, two-tailed) in repetitive samples following diagnosis. Collectively, these data suggest that in nearly 50% of persons at high risk of HCC, this marker can be detected at least 1 year, and in one case 5 years, prior to diagnosis of clinical disease.

Children's Health

While much of the international focus on aflatoxin contamination has been framed within its carcinogenic properties, many of its other toxic effects can have both short- and long-term health consequences. In South Asia, where both maternal health status and child health status are seriously compromised, the potential effects of aflatoxin exposure are most

likely manifested in these noncarcinogenic end points. However, in the future, as early-life health status improves across this region, these potential cancer end points could become much more consequential for these populations. Nonetheless, when aflatoxin exposure is determined to be substantial, these data should be viewed as being a sentinel for overall poor quality of staple food commodities and nutritional status.

A number of studies, primarily in West Africa, have raised the potential etiologic contribution of aflatoxin to diminished child growth and development. Stunting has been calculated to affect nearly 200 000 000 children worldwide, primarily in sub-Saharan Africa and South Asia. Stunting has profound associations with a variety of growth and development deficiencies in these children; to date, the overall etiology of this problem remains obscure. Since aflatoxin has profound impacts on growth and development in a number of animal species, as has been well characterized in the veterinary literature, it is reasonable to hypothesize that similar growth and developmental effects can occur in humans. The first step in the exploration of this hypothesis will be to characterize exposures in specific high-risk communities. Aflatoxin albumin adducts, a biologically effective dose biomarker, provide an objective metric for characterizing these exposures for subsequent follow-up and potential intervention.

Summary and Perspectives for the Future

HCC is a slowly developing disease involving progressive genetic insults and their resulting genomic changes. HCC may not become evident before more than 30 years of chronic infection with HBV, HCV, and/or aflatoxin exposure. Chronic hepatitis and cirrhosis may develop only 5 years before HCC is evident; globally, 70–75% of all HCC is accompanied by cirrhosis. This genomic heterogeneity may be a reflection of different etiologies of HCC and their effect upon the molecular regulation of hepatocytes.

Over the past 25 years, the development and application of molecular biomarkers reflecting events from exposure to development of clinically recognizable disease have rapidly expanded our knowledge of the mechanisms of disease pathogenesis. These biomarkers will have increasing potential for early detection, treatment, interventions, and prevention. Biomarkers derived from toxicant/carcinogen metabolism include a variety of chemicals and their metabolites in body fluids and excreta, which serve as biomarkers of internal dose. Carcinogen macromolecular adducts such as DNA and protein adducts formed in blood and tissues or excreted in urine can be employed as specific biomarkers for various purposes: as biomarkers for exposure to assess human exposure to the complex mixture and occupational carcinogens, as biomarkers for biologically effective dose or early biological effect to measure the actual dose to the carcinogen target site, and as biomarkers for assessment of relationships between carcinogen exposure and eventual cancer formation.

The molecular epidemiology of aflatoxins and liver cancer produced one of the most extensive data sets in the field and should constitute a useful template for future investigations. Molecular biomarkers played a crucial role in this endeavor. Development of aflatoxin biomarkers required fundamental knowledge of their biochemistry and toxicology, gleaned from both experimental and human studies. These biomarkers were successfully utilized in experimental models to provide data on their modulation and in epidemiological investigations in humans under different situations of disease risk. This systematic approach demonstrates the potential power provides encouragement for preventive interventions and should serve as a template for the development, validation, and application of other biomarkers to cancer or other chronic diseases.

Acknowledgments

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See also: Antinutritional Factors in Legume Seeds: Characteristics and Determination; Carcinogenic: Carcinogenic Substances in Food; Carcinogens: Identification of Carcinogens; Mycotoxins: Classification; Mycotoxins: Occurrence and Determination; Mycotoxins: Toxicology.

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Agglomeration

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Introduction

Agglomeration is a size enlargement process in solid process engineering and describes the formation of relatively large assemblies made out of smaller primary particles. The size of primary particles varies in typical application from a few nanometers to several millimeters; the size of the formed agglomerates ranges typically from several micrometers to several centimeters. Instead of agglomeration, the terms aggregation for the process and aggregate for the formed assemblies are used synonymously. In polymer technology, also the term coagulation is used to describe this size enlargement process. Although the physical mechanisms leading to the formation may be different, in all agglomeration processes, the total number of primary particles decreases gradually, whereas fewer but larger agglomerates are formed. This situation is depicted in Figure 1 for binary agglomeration where the agglomerates are built up by pairwise assembly of particles. It can be seen that the primary particles need not to be of the same size; they can also vary in other properties, for example, shape and material.

Agglomeration processes find widespread application in many industries, for instance, in food, mining, fertilizers, and detergents, and many unit operations, for instance, in mixing, pelletization, polymerization, sintering, and granulation. The main reasons for agglomeration of fine solid materials are the following:

- Improvement of flow behavior: Very fine particles tend to caking due to interparticle forces, which reduce their ability to flow. By agglomeration, these interparticle forces are overcome and the flow behavior is improved. In practical application, this results in easier dosing and conveying, for example, dosing of food powders like instant coffee or soup mixes in vending machines.
- Improvement of rewettability: By agglomeration, particles sink easier in liquid, which penetrates by capillary suction and can disintegrate the agglomerates to the original primary particles. In this way, the rewettability is increased and the so-called instant properties are created, which are essential for the use of food and beverage powders.
- Safety and health improvement: In many applications, hazardous dust, for instance, a toxic material, is formed. Depending on their size, particles may be airborne, becoming a danger to human or animal health if inhaled. Agglomerated dust is, however, no longer airborne and the danger of inhalation is reduced significantly. Also, the handling of the dust-free product is much easier due to the improved flow behavior. By agglomeration also, the danger of dust explosion, for example, in the handling of flour, is significantly reduced, reducing the necessary amount of safety installations at an operating plant.

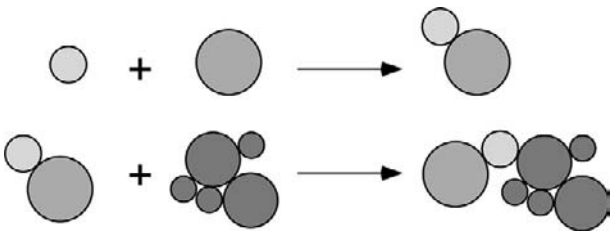

Figure 1 Scheme of binary agglomeration.

In many applications, the improved particle properties increase the economic value of the product, but agglomeration can also be an unwanted effect, for instance, in milling, screening, sifting, drying, transportation, or storage of solid materials.

Agglomeration is often accompanied by other processes, for instance, drying, depending on the formation process. Formation processes can be classified in different ways, for instance, in processes using a binding agent and processes without a binding agent.

Formation Processes

In all agglomeration processes, the particles have to come very close or in contact with each other (collision) for the individual formation mechanics to become active. The motion of the particles in an apparatus has therefore significant influence on the probability of an agglomeration event, that is, the successful formation of an assembly of particles.

Agglomeration with Binding Agent

In these processes, agglomeration is initiated by the formation of bridges between the primary particles made up of the binding agent, which is often sprayed onto the primary particles. The sprayed liquid spreads on the particle surface building up a film of a certain height and viscosity. The film needs not to cover the total surface area of the particle; discrete spots may also be sufficient. If on collision with another primary particle, the wetted area is hit and the kinetic impact energy can be absorbed by the liquid film and then a new agglomerate is formed, as none of the collision partners are able to rebound. The connection between the primary particles is established by a liquid bridge. The strength of the liquid bridges and the formed agglomerate depends on the viscosity of the liquid. The higher the viscosity, the higher the strength of the bridge and the higher the forces the agglomerate can withstand.

With increasing amount of liquid, the initially discrete liquid bridges will fully fill the interior voids between the primary particles until saturation. Capillary forces will then also contribute to the binding of the primary particles. If sufficient liquid is supplied, then the agglomerate can also be formed directly by immersion of primary particles in a liquid droplet, for example, dust in a rain droplet. The strength of the

agglomerate depends in this case on the surface tension of the liquid.

If the liquid contains also a solid material, either dissolved (solution) or dispersed (suspension), or is a melt (solid with a temperature larger than the melting temperature), then a solid bridge can be formed between the primary particles. The process is initially similar to the formation of liquid bridges: The binding agent is sprayed onto the particles and partially wets the surface area. If upon collision the kinetic energy can be absorbed, then a liquid bridge will form between the collision partners. In an additional step, the liquid from either the solution or suspension is removed, for instance, by evaporation, or the liquid melt is cooled below the melting temperature. In both cases, solidification of the bridge, possibly after a crystallization or precipitation process, will take place. In Figure 2, a scanning electron microscope picture of an agglomerate produced from glass beads by using a binding agent (hydroxypropyl methylcellulose (HPMC)) in a fluidized bed is shown, in which the solidified bridges are clearly visible. In the food industry, often, sugars, for example, glucose and maltodextrins, are used as binding agent to form solid bridges between the primary particles, for example, in the production of chocolate powder, instant coffee, or soup ingredients. The structure and morphology of the formed bridges depend significantly on the process conditions, for instance, the drying conditions under which the liquid is removed or the temperature profile for the cooling of the melt. In Figure 3, for the same material combination as in Figure 2 but different drying conditions, the formed solid bridges between the primary particles are shown. Whereas bridges solidified at low drying temperatures appear smooth and compact, bridges solidified at higher drying temperatures are visibly porous and fragmented, influencing their strength and the strength of the agglomerate as a whole.

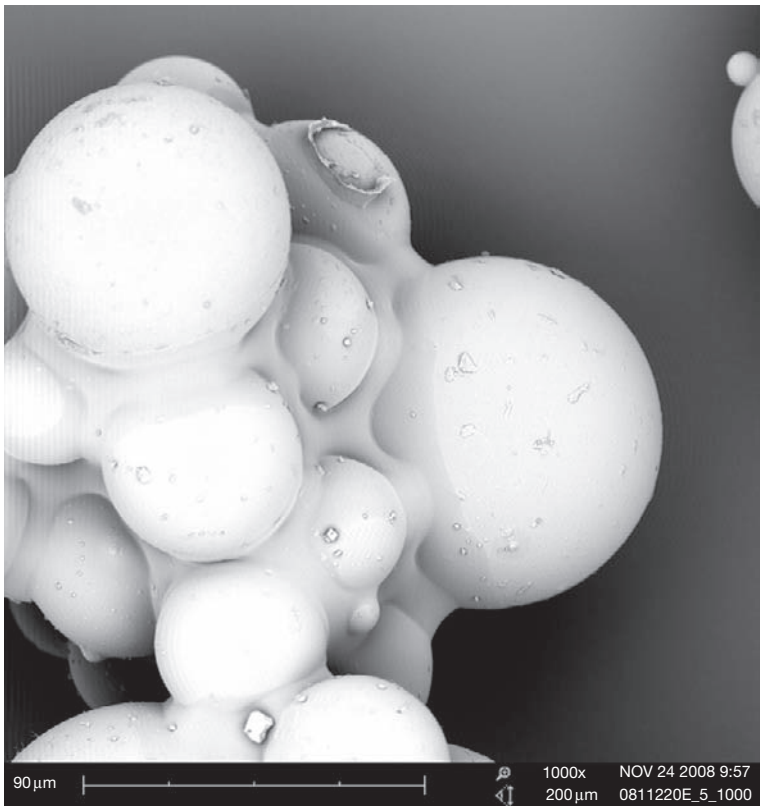

Figure 2 Agglomerate bound by solid bridges of a binding agent (glass, HPMC).

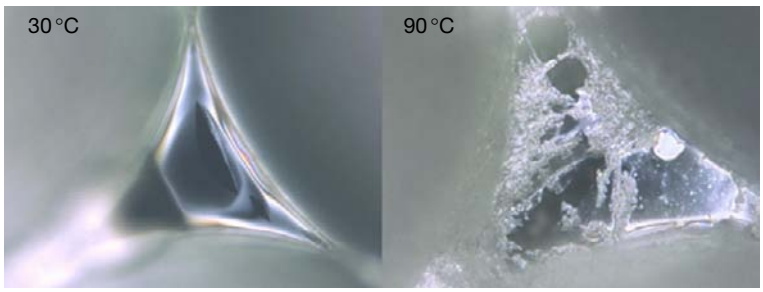

Figure 3 Differences in the structure of formed bridges due to different drying conditions.

Agglomeration Without Binding Agent

Agglomerates can be formed without a binding agent by molecular, electrostatic, or magnetic interparticle forces, by thermal effects and chemical reactions, or if the primary particles possess special surface structures that create the binding of the primary particles.

The main force acting on agglomerates from the millimeter scale upward is weight. For agglomerates and particles significantly below this size, molecular forces are dominating, as the influence of gravity decreases cubically with decreasing size. The most important among the molecular interparticle forces are the van der Waals forces, which are, for instance, for sizes of 1 μm, one million times stronger than gravity. Hence, such forces are significant in the formation of small agglomerates out of nanosized primary particles. The van der Waals forces are, however, only short-range forces, decreasing quadratically in intensity with increasing distance between two primary particles or agglomerates. A similar behavior is shown by adhesion forces, ionic or hydrogen bonds that also become only dominant for very small particles and are only active over very short ranges. Agglomerates bound by the van der Waals forces and other molecular forces can usually be broken by increasing the distance between the primary particles above a critical, material-dependent threshold, for instance, by mechanical stress.

Similarly, electrostatic forces and magnetic forces can lead to the formation of agglomerates. These too are short-range forces only effective on very small particles that are in close contact. Agglomeration due to these forces is considered unwanted in almost every application.

The group of formation processes by thermal effects consists mainly of

- sintering,
- partial melting,
- glass transition.

Sintering is a thermal agglomeration process that finds widespread application in ceramics and metallurgy for the production of solids with a predefined shape out of a powdery mixture of different ceramic or metallic components. The powder mixture is put into the desired shape and is heated, but the temperature of the mixture stays below the lowest melting temperature of the components. During heating, contacts between primary particles are transformed into solid bridges, the so-called sinter bridges, due to surface diffusion from both primary particles. The diffusion process aims at the minimization of surface energy during the formation of the bridges and the growth of the agglomerate. During this process, which can also be conducted under increased operating pressures, the volume of the mixture decreases significantly, that is, a compaction is achieved. The strength of the sintered agglomerate and many other properties can be influenced by the temperature profile used to control the sintering process. However, the compaction is generally not uniform, resulting in different agglomerate sizes and internal morphologies, which are two of the major drawbacks of sintering processes.

If a particulate material is heated to a solid temperature above the melting temperature of at least one of the components, a partial melting of the particles starting at the surface can be achieved. Upon collision of the particles with each other, liquid bridges are formed and kept in place by capillary forces if the collision energy can be absorbed. Cooling leads to solidification of the bridges and the formation of agglomerates. The necessary heat can either be supplied thermally or be released by friction or plastic deformation of the material. One particular application of this effect in food is in the production of whipped cream. Here, initially, solid fat particles begin to stabilize the air bubbles created by whipping. With increasing temperature, the solid fat particles melt partially at the surface, forming a cohesive and stable shell around the air bubble.

Glass transition of amorphous materials is an important thermal binding mechanism, especially in the food industry. In comparison with crystalline materials where the molecules, atoms, or ions are structured regularly on a grid, amorphous materials possess only a short-range order, that is, the molecules have the possibility to move inside the formed structure. This structure develops during the solidification of melts by cooling or in the case of solutions by evaporation of the solvent. The main reason is the increasing viscosity of the melt or solution and the decrease in agility of the molecules, which is further decreased by the movement of the neighboring molecules. The inner structure of the solidifying melt or liquid tends to the amorphous state, which is not thermodynamically stable but a kinetic-controlled equilibrium, that is, it depends on the process history.

Typical examples of amorphous substances in food are, for instance, maltodextrins, glucose, starch, milk powder, hydrolyzed fish protein, and vegetable pulp and powders. In the polymer industry, the important amorphous materials are, for instance, PVC, PET, PMA, and PE.

The main characteristic of amorphous substances is the glass transition. This effect takes place at a characteristic temperature, the glass transition temperature (T_g), at which the amorphous material transforms from its solid and brittle glassy state to a rubbery and viscoelastic state. This regime is bounded by the glass transition temperature from below and the melting temperature (T_m) of the solid from above. By increasing the temperature from the glass transition temperature to the melting temperature, the viscosity of the solid decreases, and it behaves increasingly like a fluid. As the diffusion coefficient depends inversely on the viscosity, the mobility of the molecules in the solid increases significantly above the glass transition temperature, amplifying chemical and enzymatic reactions. In the food industry, in order to extend the shelf life, products must therefore be stored below the glass transition temperature to avoid premature spoilage.

The glass transition temperature of a pure substance can be roughly estimated based on the knowledge of its melting temperature. For many practically important amorphous substances, the glass transition temperatures (in °C) typically range between

$$\frac{1}{2}T_m \leq T_g \leq \frac{2}{3}T_m$$

The glass transition temperature of a one-component system can be significantly changed by adding a plasticizer, which has a lower glass transition temperature than the component and thereby decreases the glass transition temperature of the mixture, or by adding a vitrifier, which has a higher glass transition

temperature and thereby increases the glass transition temperature of the mixture. Based on the mass fraction of the plasticizer in the mixture, the glass transition temperature typically behaves as shown in Figure 4. With increasing amount of plasticizer, the glass transition temperature of the mixture tends toward the glass transition temperature of the plasticizer.

If instead a vitrifier is added, then the initial component acts as a plasticizer, and the roles in the diagram have to be interchanged.

The glass transition temperature of a two-component mixture (amorphous solid and plasticizer) can be estimated based on the known glass transition temperatures of the two components, for instance, by the Gordon–Taylor equation:

$$T_g = \frac{wT_{g,p} + k(1-w)T_{g,s}}{w + k(1-w)}$$

In this equation, the subscript p denotes the plasticizer, s denotes the amorphous solid, and w denotes the mass fraction of the plasticizer in the blend; the parameter k is a mixture-dependent constant that has to be determined from measurements. In Table 1, the glass transition temperatures of various important amorphous materials in the food and polymer industries are presented. The given values correspond to the pure amorphous substance, that is, without any plasticizer. The values for the parameter k then correspond to one specific plasticizer, which is given in square brackets.

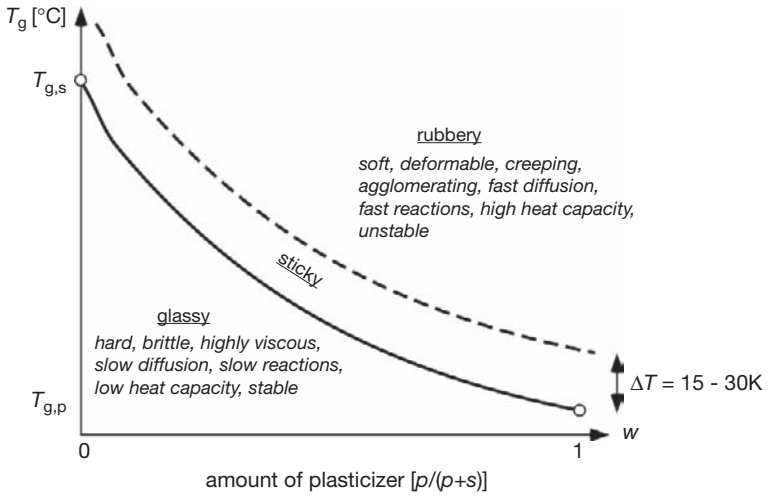

Figure 4 Diagram illustrating the glass transition phenomenon and the accompanying changes in material properties.

Table 1 Glass transition temperatures and Gordon–Taylor constants for selected food materials and polymers

Component	T_g (°C)	k	*Component*	T_g (°C)	k
Fructose [water]	5	3.8	Glucose [water]	31	4.5
Lactose [water]	101	6.7	Glucose [sorbitol]	32	0.464
Maltose [water]	87	6.2	Trehalose [sucrose]	114	0.56
Sucrose [water]	57	5.4	Polyethylene (PE)	−23	–
Maltodextrin DE 20 [water]	141	6.8	Polyvinyl chloride (PVC)	74	–
Maltodextrin DE 10 [water]	160	7	Polytetrafluoroethylene (PTFE)	127	–
Maltodextrin DE 5 [water]	188	7.7	Polyethylene terephthalate (PET)	69	–
Starch [water]	250	5.2	Poly methyl acrylate (PMA)	7	–
Water	−135	–	Poly methyl methacrylate (PMMA) [PMA]	105	0.65
Wheat flour [water]	128	–	Polyvinyl alcohol (PVA) [PE]	71	4.38
Whole milk powder [water]	101	8.6	Butyl methacrylate (BMA) [PMMA]	32	1.36
Tomato powder [water]	55	5.5	Styrene [MA]	107	0.9
Glycerol	−95	–	Polypropylene	−14	–

The Gordon–Taylor constant k refers to binary blends with the plasticizer given in square brackets, for example, [water].

Data are collected from Palzer, S. (2007). Agglomeration of dehydrated consumer foods. In: Salman, A. D., Hounslow, M. J. and Seville, J. P. K. (eds.) *Granulation*, pp. 591–671. Amsterdam: Elsevier B.V.; Schmelzer, J. W. P. and Gutzow, I. S. (2011). *Glasses and the glass transition*. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA; Penzel, E., Rieger, J. and Schneider, H. A. (1997). The glass transition temperature of random polymers: 1. Experimental data and the Gordon–Taylor equation. *Polymer* **38**, 325–337; Donth, E.-J. (1992). *Relaxation and thermodynamics in polymers – glass transition*. Berlin: Akademie Verlag; Brostow, W., Chiu, R., Kalogeras, I. M. and Vassilikou-Dova, A. (2008). Prediction of glass transition temperatures: binary blends and copolymers. *Materials Letters* **62**, 3152–3155; Seo, J.-A., Kim, S. J., Kwon, H.-J., et al. (2006). The glass transition temperatures of sugar mixtures. *Carbohydrate Research* **341**, 2516–2520.

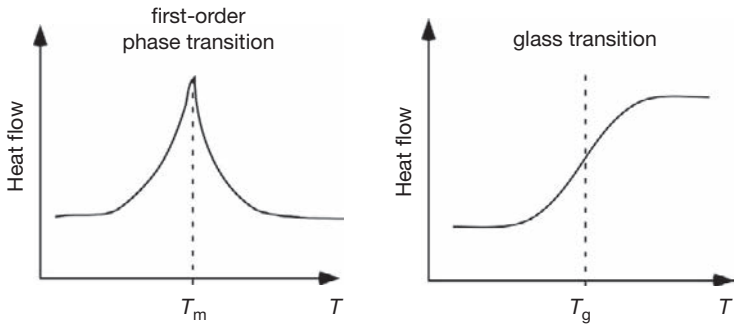

Figure 5 Detection of glass transition by differential scanning calorimetry (DSC). On the left-hand side, the change in measurement signal at the occurrence of a first-order phase transition, for example, melting, is illustrated. On the right-hand side, the change at the occurrence of glass transition is shown.

The glass transition temperature can be measured due to the fact that by glass transition, the values of many material properties change significantly, for instance, the specific heat capacity. This can be exploited, for instance, in differential scanning calorimetry where, given a specific cooling or heating rate (constant heat flow), the sample temperature is recorded. If glass transition occurs, then a stepwise change in the measured curves can be observed (Figure 5). The temperature at which this step is observed is the glass transition temperature. Its location may slightly depend on the cooling and evaporation rates, as glass transition is kinetically controlled.

The importance of glass transition in the food industry for the formation of agglomerates stems from the fact that liquid water is a powerful plasticizer with a glass transition temperature of $-135\,^{\circ}C$. Additionally, many amorphous food substances are water-soluble. If liquid water is sprayed onto a food powder, then the water integrates into the solid matrix of the amorphous material. It does not dissolve the structure as in crystalline solids but reduces the viscosity of the amorphous material, initially only at the surface of the particle. Important for application is the region where the solid temperature is $\sim 15–30\,K$ higher than the glass transition temperature as shown in Figure 4. On this curve, the viscosity is high enough that the material becomes sticky, that is, upon contact with another particle, a viscous bridge is established and an agglomerate is formed. In Figure 6, an example of agglomeration of a food powder by glass transition is shown. Water was sprayed onto maltodextrin (MD12) particles in a fluidized bed, and an agglomerate is formed without any binder. The sprayed water that initiates the formation is evaporated so that over process time, the glass transition temperature of the mixture increases again and the formation process stops. Agglomeration of water-soluble amorphous substances by glass transition is an important example of the simultaneous occurrence of particle formation and drying.

Knowledge of the glass transition temperatures can also be used to prevent the formation of agglomerates in solid processing, for example, in drying, for instance, by adding a vitrifier, such as corn starch, or by conducting the process at temperatures well below the glass transition temperature.

Chemical reactions can also lead to the creation of solid bridges between primary particles. The formation can be initiated by different components, for example, other liquids or gases in the apparatus, or by thermal conditions. Bonds created

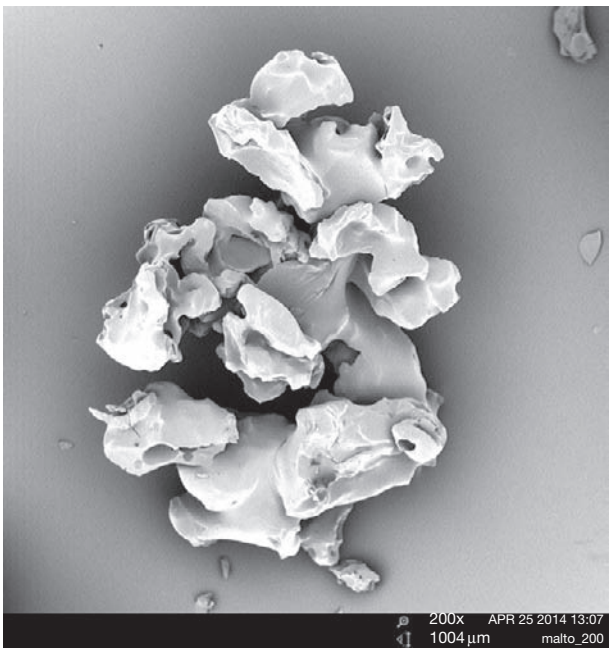

Figure 6 Agglomerate of maltodextrin (MD12), produced in a fluidized bed by glass transition using water as plasticizer.

by chemical reactions are usually very strong so that the formed agglomerates can withstand large external forces and do not break easily.

Agglomeration of particles can, moreover, be achieved by the interlocking of particle surfaces with a fibrous surface structure, for example, in felting. The strength of the agglomerate then depends primarily on the fiber length, the degree of interlocking, that is, the length of the fiber accessible for locking, and material properties, for example, the elasticity of the fibers.

Product Quality Aspects in Food Applications

By agglomeration of primary food particles by one of the described mechanisms, several application-related product properties can be modified: The optical and color appearance can be changed by varying the porosity of the formed agglomerates due to a change in light reflectance and scattering at the porous surface. This can be easily observed in cocoa powder,

which after agglomeration attains a dark brown color. The porosity of the agglomerates can also influence the shelf life of a product, for instance, in the encapsulation of antioxidants or flavors. The agglomerates build a layer around the antioxidant and can limit the oxidation. Additionally, it can also regulate the release of flavors and thus the taste intensity. Furthermore, porosity influences the rewettability of the agglomerated material, thus creating the instant behavior. However, it may occur that oils or fats migrate from the interior of an agglomerate to the surface, thereby reducing the wettability. A good wettability also poses the danger of stickiness, for example, by glass transition, forming insoluble lumps of the food powder. For the use in vending machines, a good flowability of food powders is required. This implies high agglomerate density and low porosity, which reduce the instant capabilities. The mouthfeel, which is, for instance, expressed by crunchiness, creaminess, or melting behavior, depends on various particle properties. The crunchiness can be related to the shape of the particles and to the required stress to break up the agglomerate in the mouth. The stress is applied to the agglomerate by the tongue (against the roof of the mouth) or the teeth. Similarly, the creaminess of a product, for example, thick soup, cream cheese, or ice cream, can be related to the agglomerate structure. The melting behavior, for instance, of dark chocolate, is reported to depend on particle size, with smaller size particles having a higher melting temperature. In order to produce a chocolate with a high melting point, fine particles may be preferred; however, simultaneously, the flow behavior and instant properties are reduced. In general, the product quality aspects in food are competing, and an importance ranking is necessary for each application.

Characterization of Agglomerates

Agglomerates can be characterized in many different ways, depending on the application requirements. Often, at least one of the following agglomerate properties is of interest:

- Size distribution
- Bulk density
- Porosity and pore size distribution
- Shape
- Flow behavior
- Strength, breakage, and attrition behavior
- Specific surface area
- Drying behavior
- Instant/rewettability behavior

These characteristics are not necessarily independent of each other, for instance, the specific surface area influences the instant behavior, but in many applications, only a few agglomerate properties dominate the product quality, very often the size distribution, bulk density, and agglomerate shape.

The size distribution of the produced agglomerates can be determined in various ways. The classical, standardized way is by using mechanical screens with defined gap widths. The screening process is often realized in a cascade of screens with decreasing mesh size, starting with the largest mesh size at the top of the screening tower. The size distribution is then determined from the mass fractions not being able to pass a screen with a certain mesh. Mechanical screening applies a considerable amount of mechanical stress on the agglomerates, for instance, due to the load of the screen, that is, the weight of other agglomerates, or movement of the screen, so that very brittle agglomerates may break during screening and introduce errors in the measurement of the size distribution.

The size distribution can also be determined by other measurement principles, for instance, by image-based techniques. Here, a thin free-falling curtain of agglomerates is created in front of a high-speed camera that records the fall and provides a sequence of two-dimensional projections of the three-dimensional agglomerates. By image processing, equivalent diameters, for instance, Sauter and Feret diameters, can be assigned to each projection and are used as representatives for size distribution.

Further measurement techniques, which are often used for the monitoring of an agglomeration process, are laser diffraction where the scattering of electromagnetic waves at the boundaries of an agglomerate is used to determine its size, focussed beam reflectance where the backscattering of the emitted light after having hit a particle and traveled on its surface is recorded and translated into a chord length, and spatial filter velocimetry where the size of an agglomerate is determined in the following way: Inside a measurement volume, a sequence of optical fibers with a known distance to each other is installed. These fibers are illuminated by laser light; agglomerates entering the measurement volume will subsequently pass and disrupt these rays. The total information of passing the whole set of optical fibers, which create pulse signals proportional to the velocity of the agglomerate, is used to determine a chord length of the agglomerate.

Bulk density is in many applications, especially if the handling or storage of agglomerates is involved, the second most important product characteristic. Bulk density is defined as the ratio of the mass of a randomly packed agglomerate sample to the occupied volume, incorporating the packing porosity. The porosity is closely linked to particle shape and size distribution, for example, for monosized spherical particles, the porosity of a random packing ranges between 0.38 and 0.42, but a polydisperse packing can have a significantly lower porosity. The porosity of a random packing of cylindrical objects depends heavily on the aspect ratio of the rods, allowing for a wide variety of bulk densities. Although the bulk density can be measured easily, it is quite difficult to influence directly in an agglomeration process due to the connection to other particle properties. The bulk density may be adjusted after the agglomeration process by screening the product and mixing with other agglomerate sizes.

Agglomerate shape has a large influence on the flow behavior of an agglomerate bulk. Whereas spherical agglomerates are mostly free-flowing, nonspherical agglomerates possess a reduced flowability due to the possible surface interaction. The particle shape can be measured using image-based probes where projection images of the agglomerate are taken. By image processing, the outer contour of the agglomerate is determined, and the sphericity, a measure of deviation from a sphere with, for instance, the same mean diameter, can be calculated.

The drying, rewettability, and instant behavior are closely connected to the inner morphology of the agglomerates. This morphology, characterized by the macroscopic porosity of the

agglomerates, that is, how much volume is occupied by the arrangement of primary particles, and the pore size distribution can be modified in the course of the agglomerate production process to achieve easy rewetting and a good instant behavior. The same is true for the drying of agglomerates by evaporation of liquid from their interior.

One way to investigate the inner morphology is by means of tomography, for instance, x-ray microcomputed tomography. Here, the agglomerate is subjected under different angles to a beam of x-rays, which are, after passing the agglomerate, detected. Based on the different x-ray absorption capacities of the constituents, for example, primary particles, binder, liquid, and air, two-dimensional projections of the inner morphology are obtained. These projections can be transformed into a three-dimensional representation of the agglomerate, and quantities of interest, for example, porosity, pore size, and fractal dimension, can be extracted via image processing and evaluation algorithms. The outer surface structure can be investigated by various methods, for instance, light microscopy, scanning electron microscopy, and confocal laser scanning microscopy. The latter can also be used to investigate the inner morphology of semitransparent materials of biological origin, such as food materials.

Agglomeration Equipment

To perform agglomeration on an industrial scale, many different apparatuses are available. Common in each design is that the particle bed is kept in movement to increase the probability of collision of primary particles. The means by which this particle movement is achieved differ, depending on the solid material, the formation process, that is, the active binding mechanism, and the product specifications. The throughput of the apparatuses can also vary significantly, from some kilograms per day to several hundred tons per hour.

In fluidized and spouted beds, which find widespread application in pharmaceutical, food, and, to some extent, fertilizer production, the initial powdery particle bed is subjected to a flow of heated gas, for instance, air, which yields a fluid-like, well-mixed behavior of the particles, the fluidized state. A binding agent or plasticizer, for instance, water, is sprayed from the top, from the bottom, or tangentially, and the particles are partially wetted. Due to the mixing, the particles collide and agglomerates are formed, for instance, by first establishing liquid bridges, which are then solidified by evaporation of the solvent by the heated gas flow. The intensity with which the agglomerate formation takes place depends not only on the amount of binding agent or plasticizer sprayed but also on the drying conditions, that is, the temperature and the mass flow rate of the fluidization gas. The mixing of the bed may improve at higher mass flow rate of fluidization gas, but simultaneously, the number of collisions and the mechanical stress on the agglomerates increase. The agglomerate size distribution of the product depends on the residence time and is a result of the apparatus design or implementation of the fluidized bed process. It can be performed in a single, cylindrical vessel, very similar to a continuously operated stirred tank reactor, with a very broad residence time distribution and final agglomerate size distribution, or in a cascade of single, rectangular vessels, called horizontal fluidized bed, which is similar to a plug flow reactor, yielding a narrow residence time distribution and more uniform agglomerate sizes. The latter configuration, implemented in one apparatus with weirs or baffles separating the individual vessels, can also be used for posttreatment, for instance, cooling or drying, in the same apparatus. The high flexibility of fluidized bed technology is however balanced by higher investment and operating costs as compared, for instance, with agglomeration in rotating drums. Fluidized and spouted beds are commonly used for the encapsulation of ingredients and instantizing of food powders, for example, tomato and cocoa powders, flour products, or baby foods.

Rotating drums are often used for agglomeration processes in fertilizer production, curing of ores, or concrete production. The dimensions of these cylindrical apparatuses can range up to several meters in diameter and hundreds of meters in length. The particle bed is mixed, inducing collisions, by a steady rotation of the drum. Depending on the process, a binding agent or substances initiating chemical reactions are sprayed or injected into the drum. Also, a pure thermal treatment of the solid is possible. By direct and indirect heating, temperatures well above 1000 °C can be achieved in rotating drums, realizing agglomerate formation by partial melting or sintering. Rotating drums are simple and robust apparatuses, which enable their use even under harsh environmental conditions. Due to differences in the residence time of the agglomerates in the drum, the resulting agglomerate size distribution is quite broad, necessitating a subsequent screening of the outlet of the drum and a partial recycle of the solid stream. The mechanical stress on the formed agglomerates in the drum depends, for instance, on the loading of the drum, the rotational speed, and the use of internal flights, which not only facilitate a better mixing of the bed but also exert a significant amount of stress on the agglomerates.

In high- and low-shear mixers, the primary particles and later agglomerates are moved by installed impellers, the speed of which can be manipulated externally, thus exerting high- or low-shear forces on the agglomerates. A binding agent is sprayed onto the particles and is distributed by the rotation of the impeller among them. The main formation process is the establishment of liquid bridges, which then solidify. Depending on the speed of the impeller and its geometric design, either quite compact and strong or rather porous and brittle agglomerates can be produced. A strengthening of the agglomerates can be achieved either by heating the particle bed directly or in a subsequent drying process. Low- and high-shear mixers find much application in the further processing of food powders, for example, created by spray drying, to improve the flow behavior and instant properties, for example, in dairy, the production of condiments, and flours. By the adjustment of the shear rate, the strength of the agglomerate can be influenced, which can be used to create a desired mouthfeel, as discussed in a previous section. Low- and high-shear mixers are also often used in the production of pharmaceutical intermediates before tableting.

Agglomeration in pans is similar to agglomeration in rotating drums, with the main difference that the particle bed is placed in a rotating, inclined disc, instead of a drum. The binding agent is again sprayed, usually from the top or

tangentially to the particle movement. The movement of the tray yields a segregation of agglomerates with respect to size, with smaller particles situated below the larger agglomerates. This results in a relatively uniform agglomerate size of the product so that a subsequent classification or screening of the solid stream at the outlet is not necessary.

Pressure agglomeration is an alternative if no liquid component is to be used in forming agglomerates. By increasing the pressure on the individual particles in a powdery bed, the interparticle space is reduced, and the number of contact points is increased. The van der Waals forces and sintering become dominant due to the plastic deformation of the contact points into attractive contact areas. A further improvement can be obtained by inserting a limited amount of binding agent, for instance, as a solid component. Agglomerates of high strength can be produced by pressure agglomeration; if the material behaves plastically, it becomes less efficient if the material is elastic. Pressure agglomeration is used in the food and feed industries, for instance, in the production of confectionery, flakes, pellets, or cereal bars.

In extruders, pressure agglomeration with additional heating is used to form agglomerates from a powdery plastic material, often some kind of plastic. Due to pressure and heating, a partial melting of at least one compound is achieved. This solid–liquid mixture is then pressed through a matrix, often made from steel or copper, producing strains of the agglomerated material, which are cut at the required length. The strength of the agglomerates can be improved by adding an additional binding agent; also, the viscosity of the mixtures directly at the matrix influences the strength of the agglomerate, so that by manipulation of the temperature, different product properties can be realized. Extruders are heavily used in food and feed, for example, for the production of pellets or various types of pasta. The strength of the formed extrudates, for instance, determines the cooking behavior of the pasta by controlling the water uptake and heat conduction in the material.

Equipment in which agglomeration is in most cases unwanted are

- screens,
- mills,
- spray dryers.

Agglomerate formation in screens and mills, which can result in a breakdown of the unit operation, is initiated by the load of the screen or the mill, the particle shape – which may lead to the interlocking and blocking of the transport – and the moisture content of the agglomerates. The latter is important in the milling operation: If agglomerates are surface-dry but contain a significant amount of moisture in the interior, this may be released upon breakage due to the stressing by the mill and initiate the formation of liquid bridges. The interlocking and blocking can often be reduced by adding a small amount of dispersant.

Spray drying is used to produce particles with a size from a few to about 200 μm from a solution, which is sprayed into a cocurrent or countercurrent heated gas flow, for instance, spray drying of milk in air. The evaporation of the solvent depends heavily on the drying conditions; if these are not sufficient to produce particles with a dry crust from the droplets, then agglomeration will occur in the vessel where the dried particles are collected. Furthermore, care has to be taken to keep the amount of liquid, usually water, and the temperature within bounds such that glass transition does not set in, which is another significant formation process, as many spray-dried food products are amorphous materials. However, agglomeration can also be desired in spray dryers to, for instance, obtain better flow behavior of the bulk product. Such agglomeration can be achieved by, for example, recycling fines to appropriate positions of the spray tower.

Summary

Agglomeration is a size enlargement process that can be used to tailor the properties of particulate products. The effects leading to agglomeration are manifold, also depending on the material properties and the used production equipment. Agglomeration processes find widespread application in the production of food powders to influence, for example, optical appearance, mouthfeel, instant behavior, melting properties, mechanical stability, or shelf life. The quality aspects can be competing due to the complex interplay of particle properties, for example, size and porosity, and material properties, for example, the occurrence of glass transition, making product formulation by agglomeration a challenging task in food engineering.

See also: Controlled Atmosphere Storage: Applications for Bulk Storage of Foodstuffs; Controlled Atmosphere Storage: Effect on Fruit and Vegetables; Drying: Physical and Structural Changes; Drying: Principles and Types; Fructose: Sources, Metabolism, and Health; Glucose: Properties and Analysis; Hypovitaminosis A; Lactose; Milk Powder; Milk: Processing of Milk; Starch: Structure, Property, and Determination; Storage Stability: Mechanisms of Degradation.

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Alcohol: Metabolism and Health Effects

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Introduction

Although alcohol is a nutrient containing 7.1 kcal g^{-1}, it is also one of the most abused and addictive drugs in the world and is consumed by about two-thirds of adult Americans. Most consumers of alcoholic beverages are moderate drinkers, while about 13% are alcohol abusers whose habit has resulted in risks of harm, including drunk driving, disrupted family relationships, alcohol withdrawal states, and a variety of chronic illnesses. Adult male drinkers consume about three times more alcohol than adult women drinkers. Moderate drinking can be defined as no more than two drinks per day for men or one drink per day for women, where one drink contains 12–15 g of alcohol. Heavy drinking is defined as consuming more than 15 drinks per week or 5 drinks on one or more occasions per week in men or 8 drinks per week or 4 drinks on any given day per week for women and people over 65 years of age. Chronic alcoholics, about 9% of the US population, are addicts who typically consume excessive amounts of alcohol on a daily basis and have alcohol-related health and/or social problems. Binge drinkers are chronic alcoholics who escalate their alcohol intake over weeks or months, typically to the exclusion of the essential components of their regular diets. Alcoholic beverages differ in their alcohol content, such that spirits contain about 40 g/100 ml, wine about 12 g/100 ml, and beer about 4.5 g/100 ml. Thus, the amount of alcohol in 12 oz of beer is roughly equivalent to the amount found in 5 oz wine or 1.5 oz spirits, and each is equally potentially damaging to health.

Chronic alcoholism is an underlying factor in at least one-quarter of general hospital admissions in the United States. Alcohol consumption has the potential to damage many organ systems including the liver, brain, pancreas, and heart and also increases the risk of cancers of the throat and esophagus, breast, and colon. In addition, acute alcoholism contributes to significant trauma deaths including suicide, homicide, and household and motor vehicle accidents. When consumed in moderation, alcoholic beverages protect against cardiovascular disease, but when alcohol is consumed in excess, it can become an addictive drug with potential for displacement of beneficial components of the diet. Also, the consumption of excessive amounts of alcohol contributes to generalized malnutrition, with particular effects on the availability and metabolism of both water- and fat-soluble vitamins including folate, thiamine, pyridoxine, niacin, and vitamins A and D. This article will address the risks and benefits of alcohol consumption on health including human nutritional status.

Acute Effects and Metabolism of Alcohol

The level of alcohol in the blood is dependent upon the amount and rate of alcohol consumption as well as the efficiency of its metabolism. Blood levels of alcohol determine its effects on mood and mental function. Thus, blood alcohol levels of 0.05–0.07 g dl^{-1} as typically occurs after one drink associate with euphoria, while levels of 0.08–0.10 g dl^{-1} associate with impaired judgment, reaction time, and motor skills and constitute legal intoxication in the definition of drunk driving in different locations. Blood alcohol levels of 0.10–0.20 g dl^{-1} associate with impaired judgment, balance, and memory; levels of 0.20–0.30 g dl^{-1} associate with confusion and disorientation; and levels greater than 0.35 g dl^{-1} cause stupor, disordered breathing, and ultimately coma and death. Most alcohol-related trauma occurs at legal intoxication levels or higher, while coma and death have been described in young men or women who drink excessive amounts of alcohol over a short period of time.

Although alcohol is a nutrient, it is rapidly metabolized to acetaldehyde in the human liver and negligible amounts are stored as energy in the body. There are three principal routes for alcohol metabolism, two in the liver and one in the stomach. Alcohol dehydrogenase (ADH) is present in the cytosol of hepatocytes and metabolizes the relatively low levels of alcohol that would be expected after moderate drinking. The metabolism of alcohol by ADH causes a redox change that contributes to lipid synthesis in the liver, reduces gluconeogenesis, and increases lactate production. Thus, even moderate drinking can cause fatty liver with elevated serum triglyceride levels and, in the absence of dietary carbohydrate, may result in low blood glucose levels that impair brain concentration and even consciousness. The second liver enzyme, cytochrome P4502E1 (CYP2E1), is a microsomal oxidizing enzyme that metabolizes alcohol at levels to be expected during heavy drinking. During the metabolism of high levels of alcohol, CYP2E1 utilizes adenosine triphosphate energy units and thus 'wastes' alcohol calories, with resultant potential for weight loss in heavy drinkers. At the same time, the activation of CYP2E1 contributes to the cascade of oxidative liver injury that results in alcoholic liver disease. Another form of this enzyme, gastric CYP2E1, exists in the stomach and, as the first of the three alcohol-metabolizing enzymes to encounter alcohol, accounts for about 30% of all alcohol metabolism in men, but only 10% in women. This gender difference may explain why women's tolerance to alcohol is much less than men's, hence the recognized lower 'safe' level for moderate drinking in women. The fact that CYP2E1 is induced by its substrate alcohol may account in part for increased tolerance of chronic alcoholics for alcoholic beverages, with subsequent requirement for increasing amounts of ethanol to produce its intoxicating effects.

The Potential Benefits of Moderate Alcohol Consumption

In 1992, French scientists published a report indicating that cardiovascular mortality was much less among wine-drinking residents of the Mediterranean southern provinces of France than in northern provinces where wine is less frequently

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preferred, in spite of similar overall dietary components and rates of consumption of alcoholic beverages. The initial report on the 'French paradox' attributed specific cardioprotective benefit to wine but was soon tempered by *in vitro* studies that showed that the protective effect of wine on the oxidation of low-density lipoprotein could be mimicked by constitutive antioxidant flavonoids present not only in grapes but also in many other fruits and vegetables. A comprehensive study from Copenhagen, Denmark, demonstrated decreased mortality among men and women consuming up to three drinks daily of wine, whereas equivalent amounts of beer or spirits resulted in greater mortality than abstainers. Another epidemiological study concluded that the lower mortality risk among wine drinkers compared to non-wine drinkers could be attributed in large part to a better lifestyle, including less smoking, more exercise, and better diet. The medical benefits of moderate drinking include reductions in incidences of coronary vessel occlusions and ischemic strokes, but not of hemorrhagic strokes. Whereas red wine and white wine each contain protective antioxidant flavonoids, moderate amounts of alcohol also improve the circulating lipid profile by increasing levels of high-density lipoprotein and tissue plasminogen activator while reducing platelet adhesiveness (Table 1).

The Medical Risks of Excessive Alcohol Consumption

Unlike other abused drugs, chronic alcohol in excess affects many different organ systems, which include the liver, pancreas, heart, and brain, and increases the risk of cancers of the esophagus, breast, colon, and liver. While these risks are apparent among alcohol abusers in the United States, their prevalence is generally no less in countries such as France, Italy, and Spain where drinking wine with meals is considered part of the culture. The organ damage from chronic alcoholism may impact on processes of nutrient assimilation and metabolism, as is the case with chronic liver and pancreatic disease, or may be modulated in large part by nutrient deficiencies, for example, that of thiamine with resultant effects on brain function. This section will consider specific effects of alcohol abuse on certain organs as a background for consideration of specific effects on nutritional status.

Alcoholic Liver Disease

Alcoholic liver disease is the 12th leading cause of death in the United States with similar or higher mortality rates in Western European countries where wine is considered a dietary staple. The three stages of alcoholic liver disease include, first, alcoholic fatty liver, which can occur after short-term drinking and is completely reversible with abstinence; second, alcoholic hepatitis, which is associated with inflammation and destruction of functional liver cells in about 40% of heavy alcohol abusers; and, third, subsequent alcoholic cirrhosis with advanced fibrosis of the liver in about 10–20% of chronic alcoholics . A classical study of well-nourished German male executives who agreed to undergo liver biopsies found that the incidence of alcoholic cirrhosis was directly related to the

Table 1 Benefits and risks of alcohol consumption

	Minimal amount of drinks	*Mechanism*
Benefits		
Coronary disease protection	1–2 (women), 2–4 (men)	Antioxidants
Cerebrovascular disease (nonhemorrhagic) protection		Elevated HDL lipoprotein
Risks		
Cancer		
Oropharynx and esophagus	>2 (women), >4 (men)	Unknown; higher risk in smoking alcoholics
Breast (women)	>2	Increases estrogen production
Colon	>2 (women), >4 (men)	Initiation risk increases with low folate; proliferation risk increases with excessive folate
Alcoholic liver disease		
Fatty liver	>2	Increased liver fat synthesis, decreased oxidation and export
Alcoholic hepatitis	>3 (women) × 10 years >6 (men) × 15 years	Toxicity of alcohol metabolism
Alcoholic cirrhosis	>3 (women) × 15 years >6 (men) × 20 years	Increased collagen synthesis
Pancreas		
Pancreatitis	~10 years	Acute inflammation of pancreas
Pancreatic insufficiency	~10–15 years	Loss of exocrine and endocrine pancreatic cells
Cardiomyopathy	Binge drinking	Mitochondrial damage of muscle cells or thiamine deficiency
Neurological		
Acute trauma, for example, motor vehicle accidents	1–2 in social setting	Legal intoxication
Coma and death	10–20 in rapid succession	Severe toxicity
Withdrawal syndrome	Follows binge	Neuronal hyperexcitability
Wernicke–Korsakoff syndrome	Unknown	Thiamine deficiency
Blood		
Anemia	Unknown	Folate, iron, pyridoxine deficiencies

amount and duration of alcohol consumption. Specifically, the data showed that the daily ingestion of 160 g alcohol, equivalent to that found in somewhat less than a pint of whisky, predicted a 50% risk of cirrhosis over a 15-year period. Other worldwide demographic data indicate that mortality rates from cirrhosis of the liver can be related to national per capita alcohol intake. Since a minority of chronic alcohol drinkers develop clinically significant alcoholic liver disease with hepatitis and subsequent cirrhosis, it is likely that genetic factors play a significant role in the risk of developing this disease.

Several mechanisms are implicated in the pathogenesis of alcoholic liver disease. Alcohol-induced translocation of bacterial lipopolysaccharide from the intestinal lumen initiates an inflammatory process in the liver by activating tumor necrosis factor alpha. This cytokine promotes oxidative liver injury and also has systemic effects including fever, anorexia, and weight loss. Steatosis (increased lipid deposition in the liver) is initiated by several factors. These include the effects of alcohol on methionine and adipokine metabolism that promote lipid synthesis in liver cells, while other mechanisms reduce fatty acid oxidation and the export of lipid from the liver. Altered methionine metabolism in the liver also contributes to apoptosis (programmed cell death) and reduction of antioxidant glutathione. Fibrosis results from collagen synthesis by hepatic stellate cells and is in part initiated by their incorporation of apoptotic liver cells as well as a functional switch in vitamin A storage to the production of collagen.

Among the three stages of alcoholic liver disease, fatty liver is related to the acute effects of alcohol on hepatic lipid metabolism and is completely reversible with sobriety. By contrast, alcoholic hepatitis usually occurs after a decade or more of chronic alcoholism, is associated with steatosis and inflammation of the liver with death of liver cells, and carries about a 40% mortality risk within 6 months. Alcoholic cirrhosis represents irreversible scarring of the liver as a sequel of alcoholic hepatitis. The scarring process greatly alters the circulation of blood through the liver and is associated with increased blood pressure in the portal venous circulation and shunting of blood flow away from the liver and through other organs such as varices in the esophagus. The potentially lethal complications of portal hypertension include rupture of esophageal varices, ascites or accumulation of fluid in the abdominal cavity, and hepatic encephalopathy caused by inadequate hepatic detoxification of ammonia.

Pancreatitis and Pancreatic Insufficiency

Acute pancreatitis occurs in about 10–15% of chronic alcoholics after at least 10 years of heavy alcohol abuse and is characterized by severe attacks of abdominal pain due to pancreatic inflammation, the etiology of which is unclear. Chronic recurrent pancreatitis can result from repeated acute attacks, most likely due to progressive damage to pancreatic duct outflow. This destructive process is associated with progressive scarring of the pancreas together with distortion and partial blockage of the pancreatic ducts, which limits secretion of pancreatic enzymes. Pancreatic insufficiency is a consequence of chronic pancreatitis and is associated with the destruction of exocrine pancreatic cells that secrete digestive enzymes and of endocrine cells that secrete insulin. Since the pancreas is the site of production of proteases and lipases for protein and lipid digestion and of insulin, destruction of more than 90% of the pancreas results in significant malabsorption of dietary fat with consequent steatorrhea (fat in the stool), weight loss, and adult insulin-dependent diabetes. Since the absorption of fat-soluble vitamins is dependent upon pancreatic lipase for solubilization of dietary fat, these patients are also at risk for deficiencies of vitamins A, D, and E. These patients can usually be managed by commercially available oral compounds of pancreatic enzymes, daily insulin injections, and strict abstinence from alcohol.

Heart

Although the risk of coronary heart disease may be decreased by moderate alcohol consumption, excessive alcohol use also impairs cardiac muscle function. Episodic heavy drinking bouts can lead to arrhythmias with potential for sudden death in the 'holiday heart' syndrome. Chronic alcoholics are prone to left-sided heart failure, secondary to decreased mitochondrial function of cardiac muscle cells, possibly mediated by abnormal fatty acid metabolism. A specific form of high-output heart failure, or 'wet beriberi,' occurs in association with thiamine deficiency as described in more detail in the succeeding text.

Neurological Effects

Chronic alcoholics in the Unites States are affected by neuropsychological difficulties that occur earlier than in the general population. The many neurological effects of acute and chronic alcohol abuse can be categorized as those related directly to alcohol, those secondary to chronic liver diseases, and those mediated by thiamine deficiency. The variable effects of alcohol on the brain are related to several factors including the duration and amount of drinking, the age when drinking was started, malnutrition, genetic background, and family history of alcoholism. As described earlier, the stages of acute alcohol toxicity progress upward from legal intoxication with blood levels of alcohol greater than 0.08 g dl^{-1} to coma and death with blood levels of alcohol greater than 0.35 g dl^{-1}. Automobile accidents, which account for a large portion of alcohol-related deaths, are equally, if not more, common in intoxicated pedestrians than in drunk drivers. Intoxication also leads to frequent falls and head trauma, and subdural hematoma can be present with a loss of cognition, headaches, and eventual death. Chronic alcoholics are prone to episodes of alcohol withdrawal, which can be characterized by stages of tremulousness, seizures, and delirium tremens, with hyperexcitability and hallucinations at any time up to 5 days after the last drink. This state of altered consciousness is distinct from hepatic encephalopathy in chronic alcoholic liver disease, which is associated with progressive slowing of cerebral functions with stages of confusion, loss of cognition, and eventual coma and death. Progressive altered cognition and judgment can also result from cerebral atrophy following years of heavy drinking and may also be mediated by thiamine deficiency as described in greater detail in the succeeding text.

Cancers

Chronic alcoholics are at increased risk for cancers of the oropharynx and esophagus, colon, breast, and liver as a sequel to cirrhosis. The risk of oropharyngeal cancer is greatest when heavy smoking is combined with excessive daily alcohol. Increased risk of squamous cell cancer of the esophagus is also compounded by smoking and may be associated with deficiencies of vitamin A and zinc. Breast cancer in women alcoholics is mediated in part through increased estrogen production during heavy alcohol intake. Colon cancer risk is increased among chronic alcoholics with marginal folate deficiency.

Anemia

Chronic alcoholics who substitute large amounts of alcohol for other dietary constituents are at risk for developing anemia. The causes of anemia in chronic alcoholics are multifactorial, including iron deficiency secondary to occult bleeding from episodic gastritis or other gastrointestinal sites; folate deficiency from inadequate diet, malabsorption, and increased renal excretion of folic acid; and deficiency of pyridoxine (vitamin B6) due to abnormal effects of the metabolite acetaldehyde on its metabolism. Consequently, the bone marrow may demonstrate absent iron and mixtures of megaloblastosis from folate deficiency and sideroblastosis from pyridoxine deficiency.

The Effects of Chronic Alcohol Consumption on Nutritional Status

Body Weight and Energy Balance

The effects of alcoholism on body weight are dependent upon the timing and amount of alcohol consumption in relation to meals and on the presence or absence of organ damage, in particular alcoholic liver disease. Whereas body weight is usually unaffected by moderate alcohol consumption, chronic alcoholics who substitute alcohol for other dietary constituents lose weight since alcohol is predominantly metabolized without body storage of its caloric value. Conversely, since alcohol consumption reduces dietary restraint, obese moderate drinkers on weight loss regimens are less likely to lose weight than obese dieting teetotalers.

The presence of alcoholic liver disease results in significant changes in body composition and energy balance. According to large multicenter studies, alcoholic hepatitis patients demonstrate universal evidence for protein calorie malnutrition, which plays a role in its overall mortality risk. Anorexia is universal and a major cause of weight loss in patients with alcoholic hepatitis. Furthermore, active alcoholic hepatitis contributes to increased resting energy expenditure. On the other hand, resting energy expenditure is normal in stable alcoholics with cirrhosis of the liver who are also typically underweight or malnourished in part due to preferential metabolism of endogenous fat stores. At the same time, the digestion of dietary fat and the absorptions of fat-soluble vitamins A, D, and E are decreased in cirrhotic patients due to diminished secretion of bile salts from the liver and digestive enzymes from the pancreas.

Micronutrient Deficiencies in Chronic Alcoholism

Chronic exposure to excessive amounts of alcohol is associated with deficiencies of many micronutrients, in particular thiamine, folate, pyridoxine, vitamin A, vitamin D, zinc, and iron. The frequency of these deficiencies is increased in the presence of alcoholic liver disease, which results in decreased numbers of hepatocytes for vitamin storage and metabolism. Many of the clinical signs of alcoholic liver disease are related to vitamin deficiencies (Table 2).

Table 2 Common micronutrient deficiencies in chronic alcoholic patients

Deficiency	*Cause*	*Effect*
Thiamine	Poor diet Intestinal malabsorption	Peripheral neuropathy Wernicke–Korsakoff syndrome High-output heart failure
Folate	Poor diet Intestinal malabsorption Decreased liver storage Increase urine excretion	Megaloblastic anemia Hyperhomocysteinemia and liver disease Neural tube defect Altered cognition
Vitamin B6	Poor diet Displacement from circulating albumin Promotes urine excretion	Peripheral neuropathy Sideroblastic anemia
Niacin	Poor diet	Pellagra with dermatitis, diarrhea, dementia
Pantothenic acid	Poor diet	Paresthesias 'burning feet' syndrome
Vitamin A	Malabsorption Increased biliary secretion	Night blindness May promote development of fibrosis in alcoholic liver disease
Vitamin D	Malabsorption Decreased sun exposure	Calcium deficiency Metabolic bone disease
Zinc	Poor diet Increased urine excretion	Night blindness Decreased taste Decreased immune function
Iron	Gastrointestinal bleeding	Anemia

Thiamine deficiency

Low circulating levels of thiamine, or vitamin B1, have been described in up to 80% of patients with alcoholic cirrhosis. Thiamine pyrophosphate is a coenzyme in the intermediary metabolism of carbohydrates, in particular as a coenzyme for transketolases that play a role in cardiac and neurological functions. Alcoholic beverages are essentially devoid of thiamine, and acute exposure to alcohol also decreases the activity of intestinal transporters required for thiamine absorption. The major neurological signs and symptoms of thiamine deficiency in alcoholics include peripheral neuropathy, partial paresis of ocular muscles with double vision, and wide-based gait secondary to cerebellar lesions. The presence of peripheral neuropathy is sometimes referred to as 'dry beriberi,' while the other symptoms constitute the Wernicke–Korsakoff syndrome, which is associated with severe impairment of judgment and memory loss in aging alcoholics. Whereas abnormal eye movements are an early sign of deficiency and can be treated acutely by thiamine injections, the other signs are often permanent and contribute to the dementia that often afflicts alcoholics after years of drinking. 'Wet beriberi' refers to high-output cardiac failure that can also occur in thiamine-deficient alcoholics and is responsive to thiamine therapy in addition to conventional treatment. Since endogenous thiamine is consumed during carbohydrate metabolism, acute and generalized paralysis can be precipitated by the administration of intravenous glucose to malnourished and marginally thiamine-deficient patients by depletion of remaining thiamine stores. This process can be prevented by the addition of soluble vitamins including thiamine to malnourished chronic alcoholic patients who are undergoing treatment for medical emergencies.

Folate deficiency

Folates, a family of vitamins with folic acid at its core, function in DNA synthesis and cell turnover and play a central role in methionine metabolism in the liver. While originally recognized as a cause of megaloblastic anemia, the expanding known consequences of folate deficiency are related to elevated circulating homocysteine and include increased risk for neural tube defects and other congenital abnormalities in newborns as well as altered cognition in the elderly. Prior to folate fortification of grains in the United States in 1998, the incidence of low serum folate levels in chronic alcoholics was at about 80%, but there are no data in alcoholics on the incidence of postfortification folate deficiency. Megaloblastic anemia, due to the negative effects of folate deficiency on DNA synthesis, has been described in about one-third of chronic alcoholics. Furthermore, folate deficiency may play a role in the pathogenesis of alcoholic liver disease by reducing hepatic levels of *S*-adenosyl methionine (SAM) with consequent reduction in antioxidant glutathione. Furthermore, since SAM is the principal methyl donor, its deficiency can result in decreased DNA and histone methylation with increased potential for activation of genes relevant to alcoholic liver injury. Whereas supplemental SAM prevented the ethanol-induced production of alcoholic liver disease in a small pig model, its use as a therapeutic agent in treatment of clinical alcoholic liver disease has not been successful.

The causes of folate deficiency in chronic alcoholism are multiple. With the exception of beer, all alcoholic beverages are devoid of folate, and the typical diet of the binge drinking chronic alcoholic does not include fresh vegetable sources and fortified grains. Owing to its effects on various membrane transporters, chronic alcoholism causes intestinal folate malabsorption, decreased liver folate uptake, and accelerated folate excretion in the urine. In addition, alcoholic liver disease results in decreased liver stores of folate, so the duration of time for development of folate deficiency with marginal diet is shortened.

Pyridoxine deficiency

Pyridoxine (vitamin B6) is required for transamination reactions, including the elimination of homocysteine. Pyridoxine deficiency in chronic alcoholism is caused by poor diet, whereas displacement of pyridoxal phosphate from plasma albumin by the alcohol metabolite acetaldehyde increases its urinary excretion. Low serum levels of pyridoxal phosphate are common in chronic alcoholics, and pyridoxine deficiency is manifest by peripheral neuropathy and sideroblastic anemia. In alcoholic hepatitis, the serum level of alanine transaminase (ALT) is disproportionately low compared with aspartate transaminase, due to the requirement of ALT synthesis for pyridoxine.

Vitamin B12 deficiency

The incidence of vitamin B12 deficiency in chronic alcoholism is undefined, since serum levels are often normal or increased due to the increased presence of B12 analogs in the presence of alcoholic liver disease. Nevertheless, the intestinal absorption of vitamin B12 is decreased in chronic alcoholics due to defective uptake at the ileum, and low liver levels of vitamin B12 have been described, which may contribute to abnormal hepatic methionine metabolism with elevated serum homocysteine, since this vitamin is a cofactor for methionine synthase.

Other less common water-soluble vitamin deficiencies in chronic alcoholism

Niacin deficiency is typically found in less-developed countries in association with decreased intake of the animal protein, in particular the amino acid tryptophan, which is a precursor of nicotinic acid and nicotinamide. Clinical symptoms of niacin deficiency constitute the syndrome known as pellagra, which can occasionally be found in chronic alcoholics as a component of severe malnutrition due to inadequate diet. These signs include the 'three D's' of chronic diarrhea, dermatitis including a scaly rash over sun-exposed areas such as the neck and forearms and hands, and dementia with features of disorientation, confusion, memory loss, and psychosis. In addition to this typical triad, laboratory features include low urinary *N*-methylnicotinamide excretion. Recovery from pellagra in chronic alcoholism follows treatment for protein malnutrition with supplemental niacin and abstinence from alcohol.

Pantothenic acid is a component of coenzyme A, which is involved in many reactions related to lipid and carbohydrate metabolism, and is found in animal proteins, dairy products, and whole grains. Pantothenic acid deficiency is rare and may sometimes be found in malnourished chronic alcoholics with symptoms of paresthesias that include the 'burning feet' syndrome. Its causation in chronic alcoholism is most likely related to inadequate diet and generalized malnutrition. There is no specific diagnostic test, and the deficiency

symptoms usually respond to restoration of nutrition and supplemental pantothenic acid.

Vitamin A deficiency

Although serum levels of vitamin A are usually normal in chronic alcoholics, liver retinoids are progressively lowered through the stages of alcoholic liver disease during the conversion of hepatic stellate cells from vitamin A storage to collagen synthesis.

The causes of vitamin A deficiency in alcoholic liver disease include intestinal malabsorption, which is due to decreased secretion of bile and pancreatic enzymes necessary for the digestion of dietary retinyl esters and their incorporation into water-soluble micelles prior to intestinal transport. In addition, the transport of retinol is impaired due to decreased hepatic production of retinol-binding protein. Thirdly, the metabolism of alcohol induces microsomal enzymes that promote the production of polar retinol metabolites that are more easily excreted in the bile. The signs of vitamin A deficiency include night blindness with increased risk of automobile accidents and increased risk of esophageal cancer due to abnormal squamous cell cycling. Conversely, patients with alcoholic liver disease are more susceptible to vitamin A hepatotoxicity so that supplemental doses of vitamin A should be used with caution.

Vitamin D and calcium deficiencies

Chronic alcoholic patients are at increased risk for metabolic bone disease due to low vitamin D levels and hence decreased intestinal absorption of calcium. Alcoholic liver disease increases the likelihood of vitamin D deficiency because of decreased excretion of bile required for absorption of this fat-soluble vitamin, poor diet, and often decreased sun exposure. Calcium deficiency results from low levels of vitamin D that is required to regulate its absorption and also from fat malabsorption that often accompanies alcoholic liver disease, which results in increased binding of calcium to unabsorbed intestinal fatty acids.

Zinc deficiency

Zinc is a cofactor for many enzymatic reactions including retinol dehydrogenase, is stored in the pancreas, and circulates in the blood bound mainly to albumin. Chronic alcoholic patients are frequently zinc-deficient due to poor diet, pancreatic deficiency, and increased urine excretion because of low zinc-binding albumin in the circulation. The consequences of zinc deficiency include night blindness due to decreased activity of retinol dehydrogenase, decreased taste, and hypogonadism that may result in lowered testosterone levels and increases the risk of osteoporosis in men. Since zinc is required for cellular immunity, its deficiency may contribute to increased infection risk in alcoholic patients.

Iron

Chronic alcoholic patients are often iron-deficient because of increased frequency of gastrointestinal bleeding, typically due to alcoholic gastritis or esophageal tears from frequent retching and vomiting or from rupture of esophageal varices in patients with cirrhosis and portal hypertension. The major consequence of iron deficiency is anemia, which may be compounded by the concurrent effects of folate and pyridoxine deficiencies. Conversely, increased exposure to iron, for example, from cooking in iron pots, increases the likelihood and severity of alcoholic liver disease, since increased iron promotes oxidative liver damage during the metabolism of alcohol.

See also: Alcohol: Properties and Determination; Anemia: Causes and Prevalence; Antibiotics and Drugs: Drug–Nutrient Interactions; Appetite Control in Humans: A Psychobiological Approach; Calcium: Physiology; Carotenoids: Physiology; Cirrhosis; Cobalamin (Vitamin B12): Metabolism and Disorders; Dietary Practices; Energy: Intake and Energy Requirements; Energy Metabolism; Folic acid and Folates: Physiology and Health Effects; Gin; Iron: Physiology of Iron; Malnutrition: Concept, Classification and Magnitude; Malnutrition: Prevention and Management; Mediterranean Diet; Obesity: The Role of Diet; Pantothenic Acid; Protein: Digestion, Absorption and Metabolism; Retinol: Physiology; Retinol: Properties and Determination; Thiamin: Physiology; Thiamin: Properties and Determination; Tocopherols: Physiology and Health Effects; Tocopherols: Properties and Determination; Vitamin K: Physiology; Vitamin K: Properties and Determination; Vitamins: Overview; Vodka; Whisky, Whiskey and Bourbon: Composition and Analysis of Whisky; Wines: Wine and Health; Zinc: Physiology and Health Effects; Zinc: Properties and Determination.

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Alcohol: Properties and Determination

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Alcohol Sources and Production

Alcoholic Fermentation

The most important method for alcohol production is fermentation, which is a natural process, during which microorganisms consume organic compounds under anaerobic conditions to produce gas (CO_2) and ethanol, a substance with intoxicating properties. The process of alcoholic fermentation has been known to humanity for more than 10 000 years, and it has been speculated, based on archaeological findings, that it played a significant role in driving humans to abandon nomad life, settle in permanent fertile locations in order to grow crops, and develop organized communities, thus creating civilizations. During history, various cultures have developed prejudices regarding alcohol, either due to the adverse effects of alcohol consumption (Islamic nations, prohibition era and neoprohibitionist movements in the United States, etc.) or even due to occasional poisoning effects as a result of spoilage or contamination (e.g., medieval beer witch hunting). However, the applications and benefits of alcoholic fermentation to humanity are enormous including the production of alcoholic beverages, bread, ethanol for fuel, pharmaceutical and medical uses, and acetic acid. Fermentation leads to better preservation and microbial safety of the product, as well as improved nutritional value due to degradation of complex components to more biologically available ones, enrichment in bioactive compounds, etc.

Alcoholic fermentation takes place, through the Embden–Meyerhof–Parnas (glycolytic) pathway, that is, the metabolism of hexose sugars into pyruvate. Under anaerobic conditions and high initial substrate concentration, pyruvate is decarboxylated by the enzyme pyruvate decarboxylase with thiamine pyrophosphate as cofactor, into acetaldehyde and CO_2. Acetaldehyde acts as an electron acceptor oxidizing NADH with the formation of ethanol in order to regenerate NAD^+ and allow ATP synthesis to proceed under these conditions (Figure 1). Theoretically, 1 g of sugar should yield 0.51 g of ethanol and 0.49 g of CO_2. However, the usual yield of alcoholic fermentation is about 0.46 g of ethanol and 0.44 g of CO_2, due to heat losses and other yeast metabolic activities.

The yeast *Saccharomyces cerevisiae* is the most widely used species for alcohol production because it is remarkably tolerant to high concentrations of sugar, alcohol, and SO_2 (a common preservative and antioxidant used in alcoholic beverages), low pH, low temperatures, and high pressures. It can completely utilize the sugars during beer or wine fermentations, producing low amounts of undesirable compounds such as hydrogen sulfide (H_2S), acetic acid, and urea. Because it has a low respiratory potential, it converts sugars mainly to alcohol and flavor-active compounds rather than microbial biomass in the absence of oxygen. Different strains differ regarding the production of flavor by-products and can be selected accordingly depending on the desired characteristics of the final products. Numerous pure yeast species are commercially available to cover the needs of the alcoholic fermentation industries, including brewer's, wine, and distiller's yeasts. Spontaneous fermentations, such as in traditional wine making, involve various yeast species, each of which may dominate at different stages of the process. The basic nutritional requirements for yeast growth are water, nitrogen and carbon sources, oxygen, phosphorus, magnesium, trace minerals, and vitamins. Water, free and bound, comprises about 85% of the cellular mass.

Factors That Affect Alcoholic Fermentation

The factors that affect yeast growth and alcoholic fermentation as well their regulation influence the quality of the final product. These have been extensively studied, and various theoretical models have been developed to describe the process. The principal factors are mainly the carbon and nitrogen sources, temperature and pH, oxygen and alcohol levels, and minor substances that affect yeast metabolism such as minerals and vitamins. Carbohydrates other than glucose and fructose, such as sucrose, maltose, maltotriose, raffinose, and lactose, can be fermented by yeast after hydrolysis to their monosugar constituents. The sugar concentration is directly related to ethanol production; however, above 20% sugar, fermentation is significantly retarded and yeast viability and alcohol tolerance decrease due to osmotic stress. At high sugar concentrations, the formation of flavor-important compounds is affected, for example, the production of glycerol, acetic acid, and acetate esters increases.

Fermentation is also highly affected by temperature, which is one of the easier controlled parameters during industrial processes. Below or above extreme temperature limits, yeast cells may die. At high temperatures, this effect is increased by other inhibitory factors such as ethanol concentration. At low temperatures, growth is suppressed but the cells are more tolerant to ethanol due to alterations in their membranes composition. At low temperatures, the fruit esters' synthesis and the retention of aroma volatiles are also favored.

The rate of fermentation is also highly affected by pH and ceases at values below 3. However, at low pH, the antimicrobial activity of SO_2, inhibition of spoilage microorganisms, uptake of nutrients, and ester hydrolysis are enhanced. Yeasts ferment sugar in the absence of oxygen. Nonetheless, low levels ($0.3–1.0\ mg\ l^{-1}$) may be desirable to accelerate the start-up of fermentation. Oxygen is essential for the synthesis of membrane components such as sterols and fatty acids. On the other hand, hyperoxidation may cause oxidative browning and increased synthesis of fusel alcohols, acetaldehyde, acetic acid, H_2S, urea, and ethyl carbamate (a suspected carcinogen).

Assimilable nitrogen is necessary for yeast to initiate and complete fermentation as it is required for protein and nucleic acid synthesis. High concentrations (e.g., above $400–500\ mg\ l^{-1}$ in wine fermentations) may promote cell growth and reduce ethanol yield. Low nitrogen leads to

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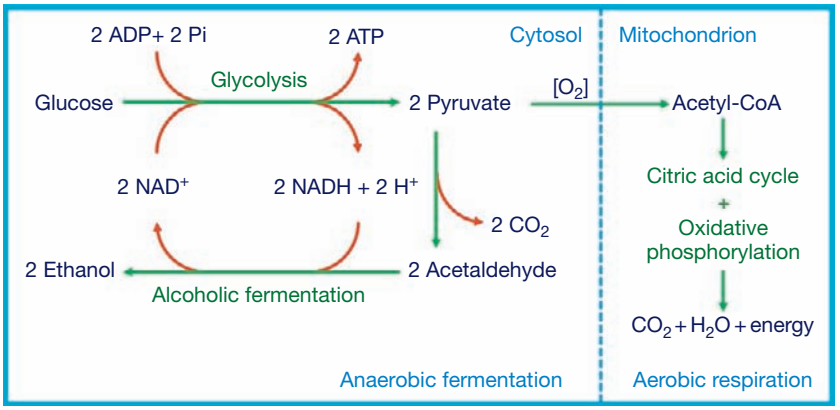

Figure 1 Overview of alcoholic fermentation.

increased production of glycerol and trehalose and enhances the release of fusel alcohols and H_2S as a consequence of restricted amino acid synthesis.

Finally, vitamins play a crucial role in yeast performance as coenzymes and enzyme precursors. A noticeable example is the reduced fusel alcohol production during fermentation by thiamine, while deficiencies in pyridoxine and pantothenic acid may result in increased H_2S synthesis.

Chemical Synthesis of Alcohol

Alcohol intended for human consumption is produced exclusively by fermentation. On the other hand, most ethanol for industrial purposes is produced by chemical synthesis from petrochemical feedstocks, mainly the acid-catalyzed hydration of ethylene (50% water/sulfuric acid; 250 °C): $CH_2{=}CH_2 + H_2O \rightarrow CH_3CH_2OH$. This reaction yields alcohol directly without need for a separate alkyl hydrogen sulfate hydrolysis step. Ethanol can also be produced from carbonyl compounds by reduction of carboxylic acids, esters, ketones, and aldehydes with specified reducing agents.

Properties of Alcohol

Physicochemical Properties

Ethyl alcohol (or ethanol) is a 2-carbon aliphatic alcohol with the structural formula CH_3CH_2OH. It is a colorless liquid with a characteristic odor and taste (sweet and burning sensation; 100 mg l^{-1} sensory threshold level in water at 20 °C). The main physical properties of ethanol are presented in Table 1.

Ethanol is a versatile solvent, miscible with water and with many organic solvents, light aliphatic hydrocarbons (up to undecane), aliphatic chlorides, and other nonpolar substances such as essential oils. The polar nature of the hydroxyl group allows ethanol to dissolve many ionic compounds, such as Na and K hydroxides and Mg, Ca, and NH_4 chlorides. Ethanol is also able to participate in hydrogen bonding (H-bonding), which renders it more viscous and less volatile than other organic compounds of similar molecular weight, such as propane. H-bonding in ethanol occurs between the oxygen of a molecule and the hydroxyl group proton of another. This type of bonding is much stronger than other types of dipole–dipole attractive forces, such as those between amine (=NH) groups. H-bonding makes ethanol highly hygroscopic to the extent that it can readily absorb water from the air. Distillation of ethanol/water solutions will not yield more than 95% concentrated ethanol because it produces an 'azeotrope' mixture (95% ethanol and 5% water) that boils at lower temperature (78.15 °C) than either its pure ingredients.

Table 1 Physical properties of alcohol

Molecular weight	46.069
Melting point	−117.3 °C
Boiling point	78.5 °C
Density	0.789 g ml^{-1}
Refractive index	1.3568 (at $\lambda = 830$ nm and 20 °C)
Triple point	150 K (−123.15 °C) at 4.3×10^{-7} kPa
Flash point	16.6 °C for pure alcohol 26 °C for spirits with 40% alcohol 52 °C for wine with 12.5% alcohol
pK_a	15.9
Dipole moment	1.69 D
Dielectric constant	24.55
Water solubility	Completely miscible
Reaction with sodium	Displaces hydrogen

Spectroscopic Properties

Ethanol, like all alcohols, has characteristic IR absorptions, associated with hydrogen-bonded O—H and C—O stretching vibrations, in the regions 3200–3650 cm^{-1} (broad absorption of moderate intensity) and 1025–1200 cm^{-1} (moderate to strong absorbance), respectively.

The ^1H NMR spectrum of ordinary ethanol, containing acidic and basic impurities, appears as a singlet for the proton of the hydroxyl group and as a quartet for the methylene (—CH_2—) group protons. No signal splitting from coupling of the hydroxyl and the methylene group protons appears, although they are on adjacent atoms. However, in the ^1H NMR spectrum of very pure ethanol, the signal of the hydroxyl group protons and that of the methylene group protons are split into a triplet and a multiple of eight peaks, respectively. The chemical shift in ^{13}C NMR spectra for the carbon of the

C—O group of alcohols (60–75 ppm) is higher than the corresponding alkanes, because of the electronegative oxygen that decreases the shield of the carbon to which it is attached. The chemical shift for the $—CH_2OH$ in ethanol is 56–58 ppm.

Regarding ultraviolet–visible spectra (UV–vis), alcohols are transparent above 200 nm, unless there are other chromophores in the molecule (double bonds, aromatic rings, etc.). The minimum absorption wavelength for the use of ethanol as a solvent in UV–vis determinations is 205 nm.

In mass spectra, the molecular ion peak of alcohols is usually small. Alcohols fragment in a way that the molecular ion loses an alkyl group from the hydroxyl-bearing carbon, forming a stable cation ($CH_2{=}OH^+$) with a prominent peak at m/z 31.

Chemical Reactions

Like all alcohols, ethanol is involved in many chemical reactions including the conversion to ethers, esters, carbonyl compounds, and carboxylic acids. In summary, the main reactions of ethanol are the following:

(1) Diethyl ether synthesis by heating in the presence of acid catalyst:

$$2CH_3CH_2OH \rightarrow (CH_3CH_2)_2O + H_2O$$

(2) Ethyl ester synthesis by reaction with (a) a carboxylic acid with acid catalyst (Fischer esterification), (b) an acyl chloride in the presence of pyridine, and (c) a carboxylic acid anhydride:
   (a) $CH_3CH_2OH + \text{R-COOH} \rightarrow RCOOCH_2CH_3 + H_2O$
   (b) $CH_3CH_2OH + \text{R-COCl} \rightarrow RCOOCH_2CH_3 + HCl$
   (c) $CH_3CH_2OH + \text{RCO-O-OCR} \rightarrow RCOOCH_2CH_3 + \text{RCOOH}$

(3) Conversion to an inorganic acid ethyl ester (ethyl nitrate, ethyl sulfate, or triethyl phosphate) by direct reaction with the acid:

$$CH_3CH_2OH + HNO_3 \rightarrow CH_3CH_2ONO_2 + H_2O$$

(4) Oxidation to acetaldehyde using pyridinium chlorochromate or dichromate in dichloromethane as oxidizing agents:

$$CH_3CH_2OH \rightarrow CH_3CHO$$

(5) Oxidation to acetic acid using acidified dichromates, chromic acid, or potassium permanganate as oxidizing agents:

$$CH_3CH_2OH \rightarrow CH_3COOH$$

Biological Oxidation and Health Effects

In humans, the main enzymes involved in ethanol oxidation are alcohol dehydrogenases (ADHs). These are mostly dependent on the coenzyme nicotinamide adenine dinucleotide (NAD^+) and convert ethanol to acetaldehyde:

$$CH_3CH_2OH + NAD^+ \rightarrow CH_3CHO + NADH + H^+$$

ADHs are abundant in the liver and present in other tissues, being possibly indirectly responsible for the toxicity or carcinogenicity of ethanol in these tissues. Ethanol oxidation may also occur via the action of cytochrome P450 enzymes and peroxisomal catalase, or it can be nonoxidatively metabolized to form cytotoxic fatty acid ethyl esters. The latter appear in human serum soon after ingestion. Several minor pathways for ethanol conversion to acetaldehyde have also been proposed, involving nitric oxide synthases, cytosolic xanthine oxidoreductase, threonine aldolase, and enzymes that have not yet been identified.

Acetaldehyde is subsequently metabolized, predominantly by NAD^+-dependent ALDHs. These are expressed in a many tissues and have broad substrate specificity for a variety of aldehydes. Chronic ethanol consumption reduces the liver's ALDH activity and increases blood acetaldehyde levels. Acetaldehyde is toxic and there is sufficient evidence of malignant human carcinogenicity, especially those deficient in ALDH.

The quality of alcoholic beverages may impact human health and mortality as a result of heavy drinking and addiction. Also, occasional poisoning may occur due to adulteration or contamination with methanol, as in the case of illegally produced and sold alcoholic beverages (unrecorded alcohol), or with other toxic substances. According to the WHO *Global status report on alcohol and health*, world consumption in 2010 was 6.2 l of pure alcohol per person, aged 15 or older. Of this, 24.8% was estimated to be unrecorded and 50.1% consumed in the form of spirits (Figure 2). More than 200 alcohol-related disease and injury conditions have been recorded, including dependence (alcoholism), neuropsychiatric disorders, gastrointestinal diseases, cancer, violence, traffic- and work-related injuries, cardiovascular diseases, pregnancy implications, and increase of sexually transmitted diseases. However, various beneficial effects of low alcohol consumption (mainly of wine and beer) have been noted, such as reduction of undesirable stress and depression effects, enhanced sociability, and cardioprotective effects. These positive effects may be attributed to a variety of compounds found in these drinks, notably antioxidant polyphenols and ethanol.

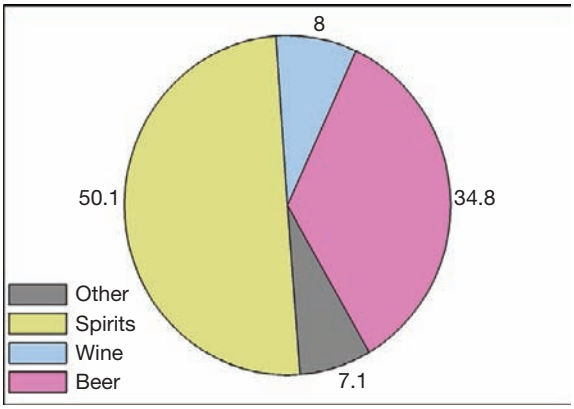

Figure 2 Proportion (%) of recorded alcohol per capita (15+) consumption consumed in the form of beer, wine, spirits, and other types of beverages in the world in 2010. World Health Organization. (2014). *Global status report on alcohol and health – 2014 ed*. Geneva: WHO Press.

Types of Alcohol

Alcoholic Beverages

According to the United Nations Statistics Division, the activity of manufacture of alcoholic products based on fermentation can be divided into three categories:

- Distilling, rectifying, and blending of spirits: distilled, potable, alcoholic beverages (whisky, brandy, gin, liqueurs, and mixed drinks), blended distilled spirits, ethyl alcohol from fermented materials, and neutral spirits
- Manufacture of wine (including sake, cider, perry, mead, other fruit wines, mixed fermented beverages, vermouth, fortified, and low-alcohol and nonalcoholic wine)
- Manufacture of malt and malt liquors (low-alcohol and nonalcoholic beer)

A general scheme for alcoholic beverages' production from various raw materials is illustrated in Figure 3.

Wine is the product of fermentation of grape juice (must), traditionally carried out spontaneously with the indigenous grape skin and/or winery yeast microflora. Cider is the product of controlled yeast fermentation of apple juice and perry is derived from pears. Beer is the alcoholic beverage made by fermentation of the wort extracted from malted cereal grains, mainly barley, and flavored with hops. Rice wines are naturally brewed alcoholic beverages made from rice, which undergo simultaneous starch hydrolysis and alcoholic fermentation by koji fungi (*Aspergillus oryzae*) and yeasts, respectively. Distilled spirits are alcoholic beverages produced by distillation of fermented broths. The distillation process carries over most of the volatile compounds to the distillate, and due to the high alcohol content, microbial spoilage is unlikely to occur. Based on the distillation process, two kinds of spirits may be defined: (a) those produced by rectification (repeated distilling) and sometimes filtration, thus removing most of the flavor characters of the raw material (e.g., vodka and gin), and (b) those produced according to specific fermentation and distillation techniques that allow retention of the unique raw material flavors (e.g., whisky, rum, brandy, and tequila). Neutral alcohol, or rectified spirit, or ethyl alcohol of agricultural origin is a highly rectified alcohol without the organoleptic properties of the raw materials. It is used for the production of spirits such as vodka, gin, aniseed-flavored drinks, and most liqueurs.

Denatured Alcohol

The term 'denatured alcohol' refers to alcohol products adulterated with toxic and/or bad tasting additives (e.g., methanol, benzene, pyridine, castor oil, gasoline, isopropyl alcohol, and acetone), making it unsuitable for human consumption. The most common additive used is methanol (5–10%), giving rise to the term 'methylated spirits.' Denatured alcohol is used as a lower-cost solvent or fuel for home-scale or industrial use, compared with the heavily taxed pure alcohol and alcohol used in beverages.

Absolute Alcohol

To produce pure alcohol, which is called absolute alcohol, various dehydration processes exist, such as azeotropic distillation, extractive distillation, adsorption with molecular sieves, and pervaporation membrane techniques. Azeotropic distillation is the most common process, involving solvents such as benzene and cyclohexane, which form a different azeotrope mixture with ethanol and water. Distillation of this mixture eventually yields absolute alcohol, which contains $<1\%$ water and trace amounts of the separation agent. Absolute alcohol is not intended for human consumption. It is mainly used as solvent for laboratory and industrial applications and as a fuel.

Composition of Alcohol Products

Ethanol

The ethanol percentage in alcoholic beverages is mostly indicated as percentage by volume (% vol) (French or Gay-Lussac

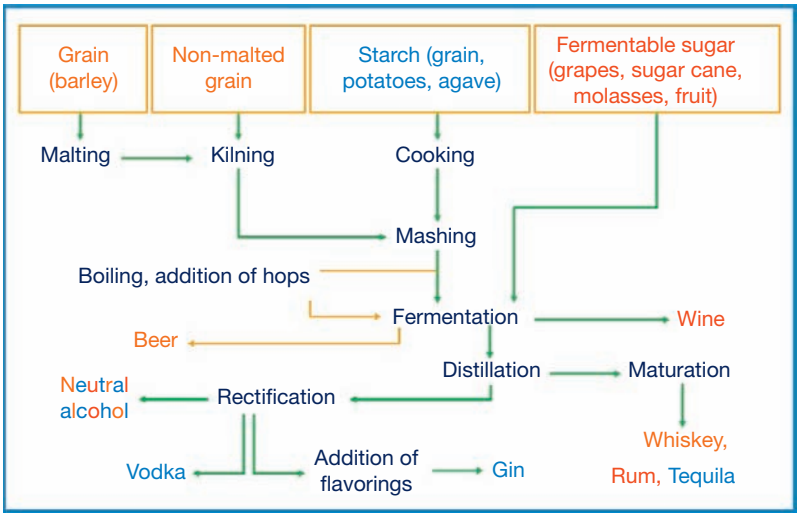

Figure 3 Raw materials and processes for alcoholic beverages production.

Table 2 Properties of neutral alcohol and some distilled spirits

	Neutral alcohol	*Scotch whiskey*	*Brandy*	*Rum*	*Gin/vodka*
Minimum actual alcoholic strength (% vol.)	96.0[a]	40.0[b]	36.0 (wine)/37.5 (fruit)[b]	37.5[b]	37.5[b]
Maximum methanol content (g hl^{-1} pure alcohol)	50[a]	–	200 (wine)–1000 (fruit)[b]	–	–
Total acidity as acetic acid (g hl^{-1} pure alcohol)	<1.5[a]	3–70[b]	5–160[b]	80–240[b]	–
Volatile acidity as acetic acid (g hl^{-1} pure alcohol)	–	9–40[b]	–	50–140[b]	–
Esters as ethyl acetate (g hl^{-1} pure alcohol)	<1.3[a]	10–85[b]	70–580[b]	40–280[b]	–
Aldehydes as acetaldehyde (g hl^{-1} pure alcohol)	<0.5[a]	2–270[b]	2–80[b]	0–40[b]	–
Higher alcohols (g hl^{-1} pure alcohol)	<0.5 (as 2-methyl-1-propanol)[a]	–	140–780[b]	100–200[b]	–

[a]WHO/IARC (2010).
[b]Kirk and Sawyer (1991).

system). In America, the proof system is usually used, which is double the % vol. The ethanol content in wines varies from 8% to 15%; concentrations above 14–15% are usually the result of fortification. Beer usually contains 4–6% alcohol although a variety of products exist with 0.5% (alcohol-free beer), 2.3% (low-alcohol beer), or even 20–40% alcohol. Spirits contain about 35–40% (or up to 74% in some cases). Neutral alcohol contains at least 96% ethanol (Table 2). Ethanol is crucial to the stability, aging, and sensory properties of alcoholic beverages, suppressing the growth of pathogenic or spoilage microorganisms, acting as solvent in extracting constituents from the raw material, participating in essential reactions leading to important volatile compounds such as esters, and affecting the overall aroma perception in fermented products.

Water

Water has a critical effect on the quality of fermented beverages. In wines, these include the solubility of flavor compounds and pigments, control of the basic flow characteristics, and chemical reactions involved in fermentation, aging, etc. In beer, the chemical composition of water exerts a strong effect on flavor, color, head retention, and clarity. In whisky, water affects the efficiency of the mashing process as well as the chemistry of the fermentation and distillation processes. The quality of mixed spirits, such as vodka or gin, is also affected by the quality of the dilution water. The addition of water in the preparation of spirits is authorized, if its quality is in conformity with legislated specifications relating to the exploitation of natural waters, quality relative to human consumption, and that it does not change the nature of the product.

Methanol

Methanol is not a fermentation by-product. It is generated from the enzymatic breakdown of pectins (galacturonic acid polymers with carboxyl groups partly esterified with methanol) by the action of pectinesterases. Therefore, methanol content is directly related to the pectin content of the raw material. The addition of pectolytic enzymes to facilitate clarification or blending with distilled spirits may increase the methanol content of alcoholic beverages. Because distillation potentially concentrates the methanol content in the distillate, it is of major concern for the quality of distilled spirits, notably in unreported and adulterated alcohol products, where quality control is largely nonexistent. The principal detrimental by-products of methanol metabolism (formaldehyde and formic acid) can cause optic nerve destruction and blindness.

Volatile By-Products (Congeners)

The formation of fermentation by-products is mainly yeast-dependent. Secondary factors include the raw materials, temperature, and pH. The main by-products of alcoholic fermentation are esters, carbonyl compounds, fusel alcohols, volatile organic acids, sulfur compounds, glycerol, and methanol. Their effect on product flavor depends mainly on their concentrations and odor detection threshold, as well as the levels of other volatile compounds, ethanol, O_2, and CO_2. Due to concentration during distillation, spirits contain higher amounts of volatile compounds unless they have been removed by rectification and filtration processes.

Esters are considered important aroma components of fermented drinks, because they impart fruity/flowery odors and have low odor threshold values. They are produced during fermentation as by-products of yeast lipid metabolism (alcoholysis of acyl CoA compounds) or by slow reactions between alcohols and fatty acids during aging (Figure 4). Important esters in fermented beverages are ethyl acetate (fruity below 150 mg l^{-1}), isoamyl acetate (banana), benzyl acetate (apple), ethyl hexanoate (apple and aniseed), ethyl octanoate (fruity, fat), and ethyl decanoate (brandy-like).

Fusel alcohols (more than two carbon atoms) are mainly produced by yeast during fermentation (Figure 4). The few exceptions, such as hexanols, benzyl alcohol, and 2-phenylethanol, are usually derived from the raw material. The most abundant alcohols are 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol. They contribute more positively to aroma complexity at low concentrations

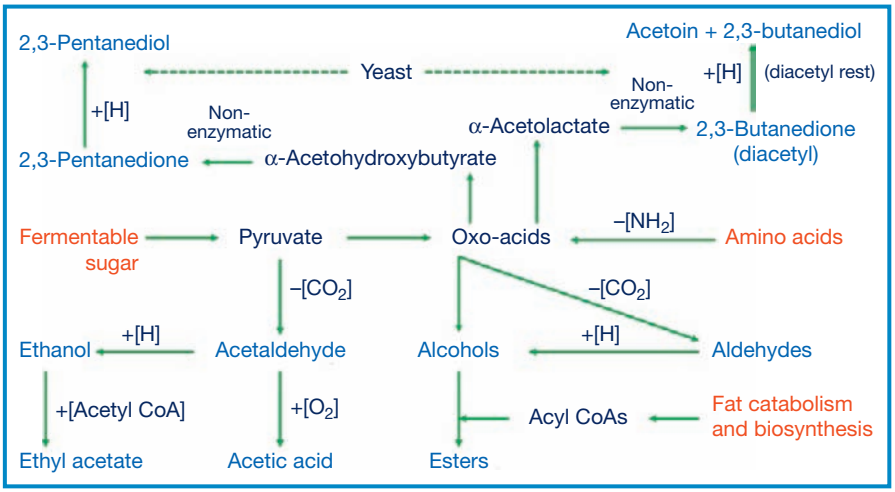

Figure 4 Formation of volatile by-products during alcoholic fermentation.

(<300 mg l^{-1}). At higher concentrations, they may musk the product aroma with their strong pungent smells.

Most aldehydes found in alcoholic beverages are produced during fermentation (e.g., acetaldehyde), processing (e.g., furfural), or extraction from oak cooperage (e.g., cinnamaldehyde and vanillin). Acetaldehyde is usually considered an off-odor above threshold values (herbaceous). Examples of ketones derived from the raw material are the norisoprenoid ketones, β-damascenone (exotic flower or rose), and α-ionone. Various ketones are also produced during fermentation. They usually have no sensory significance, with the major exception being diacetyl (2,3-butanedione). It imparts buttery, nutty, or toasty flavors at low levels (<5 mg l^{-1}). Diacetyl is a key aroma compound in beer, where it is slowly converted by yeast to derivatives of lower sensory significance during maturation at very low temperatures (~0 °C). This process, known as 'diacetyl rest,' is a time-consuming and energy-demanding process for breweries (Figure 4).

Acidity is commonly classified into two categories – volatile and fixed. Volatile acidity refers to acids that can be removed by steam distillation, whereas fixed acidity refers to poorly volatile acids. Acetic acid is the major volatile acid found in wines, but other carboxylic acids such as formic, butyric, and propionic, and longer chain fatty acids also may be involved. All volatile acids have specific odors but typically occur at detectable levels only as a result of microbial spoilage.

Finally, the main sulfur compounds in wine are inorganic sulfites due to deliberate addition of SO_2. The major, organic sulfur-containing compounds are the amino acids cysteine and methionine, the peptide glutathione, the vitamins thiamine and biotin, thiols, and a wide diversity of volatile compounds. The latter are derived from yeast metabolism and nonenzymatic reactions during fermentation, maturation, and aging postbottling. Although they are found in trace amounts, their low sensory thresholds can give them great significance.

Toxic Contaminants

Toxic substances that can be found in alcoholic beverages are ethyl carbamate, chloropropanols (CPs) (mainly 3-monochloropropane-1,2-diol), acrylamide, furan, phthalate esters (PAEs), mycotoxins, pesticides, and heavy metals. Ethyl carbamate is a by-product of fermentation. Levels up to 45 μg kg^{-1} have been reported in wine. Many chemical and biological techniques have been proposed to reduce or avoid these levels. CPs, acrylamide, and furan are formed during heat-involving processing (e.g., kilning or boiling in brewing). They are all suspected carcinogens for humans (Table 3).

PAEs are the most commonly used plasticizers for polymers such as polyvinyl chloride, polyethylene, polyethylene terephthalate, and polyvinyl acetates. High alcohol contents may cause migration of lipophilic PAEs during their contact with plastic materials. PAEs and some metabolites and degradation products can cause toxic effects in multiple human organ systems.

Other toxic contaminants that have been detected in alcoholic beverages are benzene (from contaminated industrial CO_2 or formation in the presence of sodium benzoate), monostyrene (contact with polyester tanks), halogenated acetic acids (equipment disinfectants), polydimethylsiloxanes, and polycyclic aromatic hydrocarbons.

Metals find their way into alcoholic products during all stages of production including raw materials, process type, equipment, bottling, aging, storage, and adulteration. The soil composition, environmental pollution, and agrochemical treatments all affect the mineral content and presence of toxic metals in the raw materials. Wine and beer contain much higher amounts of metals than distilled beverages.

Finally, two important classes of toxic organic compounds that may be present in alcoholic products are mycotoxins (secondary fungal metabolites) and pesticides. Mycotoxins are frequent contaminants in grain and, therefore, potential contaminants in beers. Established maximum limits for their presence in foodstuffs have been set for ochratoxin A and aflatoxins B1, B2, G1, and G2. The distillation process effectively reduces the contamination risk in distilled spirits. Regarding pesticides, various studies have shown that residues in alcoholic beverages are smaller than those in the raw material. Pesticides may pass into the distillates only if present at very high concentrations.

Table 3 List of potential carcinogens in alcoholic beverages

Substance	*Type of alcohol*	*Evaluation of carcinogenicity in humans*
Ethanol	All	Sufficient
Acetaldehyde	All	Sufficient
Formaldehyde	Wine, spirits, unrecorded	Sufficient
Aflatoxins	Beer	Sufficient
Ochratoxin A	Beer, wine	Inadequate
Patulin	Apple cider	Inadequate
Ethyl carbamate	Beer	Inadequate
Acrylamide	Beer	Inadequate
Furan	Beer	Inadequate
Benzene	Beer	Sufficient
Safrole	Liqueurs	Inadequate
4-Methylimidazole	Caramel-colored beer and whisky	Inadequate
N-Nirosodimethylamine	Beer	Inadequate
Arsenic	Beer	Sufficient
Cadmium	Beer	Sufficient
Lead	All	Limited

Analysis of Alcohol Products

Analytic procedures and quality assurance standards for alcohol products are delivered by various international, intergovernmental, national, and private organizations in the form of comprehensive and reliable collections of methods such as the European Community Reference Methods, the Compendium of International Methods of the International Organisation of Vine and Wine (OIV), the Official Methods of Analysis of the Association of Analytical Chemists (AOAC International), and the Analytica-EBC of the European Brewery Convention. Numerous analytic techniques have also been proposed at research level for the analysis of ethanol, congeners, minerals, and toxic compounds.

Determination of Ethanol

Various methods are available for the analysis of ethanol in alcoholic products, including determination by specific gravity (SG), chemical, chromatographic, and spectroscopic methods.

Determination by specific gravity

For determination of the alcohol strength of a product based on SG, distillation or steam distillation is usually required. Some samples such as straight bourbon whiskey and alcohol–water mixtures containing low amounts of volatile ingredients may not require distillation prior to analysis. Determination of alcohol in the distillate may be carried out after neutralization by an alkaline solution to avoid volatile acids to pass into the distillate or by sulfuric acid in the case of abnormal concentrations of ammonium anions. The measurement of the alcoholic strength of the distillate may be done by pycnometry, densitometry (frequency oscillation or hydrostatic balance), hydrometry, and refractometry techniques.

- For determination of alcohol by the pycnometer method, the sample is distilled using an apparatus consisting of a flask connected to a vertically assembled Liebig condenser. For samples containing 60% or less alcohol, the pycnometer is filled at the calibration temperature and the content is then quantitatively transferred into the distillation flask. The distillate is collected into the same pycnometer and the volume is completed with water at the calibration temperature. The SG of the sample and that of the distillate are determined by the ratio of weight of sample (or distillate) per weight of water, and the corresponding % vol alcohol content of the distillate at 15.56 °C (AD) is obtained using conversion tables. Samples containing more than 60% alcohol are distilled into higher volume pycnometers than the ones used for sample preparation under the same process conditions.
- The hydrometer (or alcoholmeter) method is applicable to spirits containing <600 mg extract/100 ml. Clean and dry graduated (0.1–0.2° proof) hydrometers are used. Calibration corrections are applied to both hydrometer and thermometer readings.
- In densitometric methods, a density meter is used, which determines the SG at 20 °C by measuring the frequency of oscillating U-tube filled with sample compared with that of two standards: in air (apparent SG = 0.00000) and with freshly double-distilled or deionized water (apparent SG = 1.00000).
- In refractometer methods, a specific volume of sample is measured and distilled. The distillate is diluted to an indicated volume at calibrated temperature, and the refractometer reading is obtained by immersion.

In the methods mentioned earlier, the % by volume alcohol content at 15.56 °C is obtained, after temperature corrections, using suitable conversion tables. The alcohol content by weight can also be determined following similar procedures after accurately weighing specific amounts of sample.

Chemical methods

Simple, rapid, and sensitive colorimetric/fluorometric methods for quantitative determination of alcohol have been

developed, applicable to alcoholic beverages, biofuels, basic research, and clinical studies. A common method is the use potassium dichromate to oxidize ethanol to acetic acid and then determine the unreacted oxidant by titration with ammonium iron(II) sulfate. The reaction of ethanol with ceric ammonium nitrate in acidic medium to produce an intensely red-colored complex has also been proposed. Finally, the enzyme alcohol oxidase (AOX) is a valuable tool for ethanol analysis in complex samples. Various AOX sensors have been developed based on monitoring O_2 consumption or H_2O_2 formation using amperometric electrodes. Bienzymatic systems have also been assembled using AOX coupled to another enzyme such as horseradish peroxidase.

Chromatographic methods

Gas chromatography (GC) methods usually involve the use of flame ionization detectors (FID); columns made with chemically inert and thermally stable porous polymers, such as polyethylene glycol and polyaromatic-type cross-linked resins; ethanol/water solutions as standards; and N_2 as carrier gas with pressure control. These GC systems are also suitable for the analysis of other volatile compounds such as alcohols and fatty acid esters. These methods may require headspace sampling or organic extraction of small sample volumes or even no extraction at all, followed by direct injection in the GC-FID system. Finally, ethanol can be determined by high-performance liquid chromatography (HPLC) techniques, usually involving columns packed with ion-moderated partition polymers, dilute acid or distilled water as mobile phase, and refractive index detectors.

Spectroscopic methods

Ethanol can be determined by infrared spectroscopy (IR) techniques, based on the measurement of the absorption of different wavelengths that pass though the sample. Although the equipment involved is cheaper and the methods are simpler and faster, IR techniques do not have the high resolutions of GC or HPLC and are mainly used for qualitative analysis. However, near-infrared spectroscopy has become an important quantitative tool for solvent characterization. Raman spectroscopy techniques have also been proposed for the quantification of ethanol and methanol in distilled alcoholic beverages.

Determination of Dry Extract and Inorganic Elements

The total dry extract (TDE) includes all matter that is nonvolatile under specified conditions. Gravimetric methods for TDE involve weighing of the residue left after evaporation and drying. The TDE can also be calculated indirectly based on the density of the sample from which alcohol has been removed and has been returned to the original volume by adding water. The inorganic content is represented by ash, which is defined as the amount of substances remaining after igniting (500–550 °C) the solid residue left after sample evaporation (dry ashing), in a way that all cations (except ammonium) are converted into carbonates or other anhydrous inorganic salts. Specific metals in alcohol products are often determined by atomic absorption or emission techniques as well as by colorimetric and electrochemical methods.

Determination of Volatile Congeners

Determination of other volatile substances (congeners) in alcoholic beverages, such as acetaldehyde, ethyl acetate, acetal, and methanol, can be directly analyzed or after distillation by GC-FID using suitable internal standards. Methanol can also be determined colorimetrically after oxidation to formaldehyde by potassium permanganate followed by reaction with chromotropic acid to form a violet product. Minor volatile constituents can be identified and analyzed by a variety of gas chromatography–mass spectrometry techniques (GC-MS).

Determination of Organic Contaminants

For the analysis of pesticides, several analytic methodologies have been proposed. GC coupled to tandem mass spectrometry (GC-MS/MS) is increasingly becoming the analytic tool of choice regarding pesticides in complex matrices, because it is a highly selective technique not affected by the sample matrix or coeluting interferences. LC-MS(/MS) techniques have also been recently introduced for pesticide analysis. Analytic methods for mycotoxins, such as LC with fluorescence detection and LC-MS/MS, have been developed mainly for ochratoxin A (OTA) determination in wines. Multiresidue methods for the simultaneous determination of pesticides and mycotoxins have also been proposed. Finally, the determination of phthalates in alcoholic beverages can also be done by GC-MS after extraction with a nonpolar solvent, while ethyl carbamate can be determined by GC-MS/MS.

See also: Alcohol: Metabolism and Health Effects; Beverage: Health Effects; Beverage: Patterns of Consumption; Brandy and Cognac: Consumption, Sensory and Health Effects; Fermented Foods: Composition and Health Effects; Rhum–Ron–Rum: Technology and Tradition; Tequila: Raw Material, Classification, Process, and Quality Parameters; Whisky, Whiskey and Bourbon: Composition and Analysis of Whisky; Whisky, Whiskey, and Bourbon: Products and Manufacture; Wines: Champagne and Sparkling Wines – Production and Effervescence; Wines: Madeira, Port and Sherry Fortified Wines – The *Sui Generis* and Notable Peculiarities. Major Differences and Chemical Patterns; Wines: Types of Table Wines; Wines: Wine and Health; Wines: Wine Production; Wines: Wine Tasting; Yeasts.

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Alkaloids: Properties and Determination

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Definition

The definition of alkaloids has changed throughout the years. Formerly, this class of secondary compounds was restricted to plant bases with a heterocyclic nitrogen atom. Exocyclic nitrogen bases were termed 'pseudoalkaloids.' Other definitions demanded that the skeleton of alkaloids should derive from amino acids or that these bases have explicit pharmacological activities. At present, alkaloids are defined in a more pragmatic way; they include all nitrogen-containing natural products that are not otherwise classified as peptides, nonprotein amino acids, amines, cyanogenic glycosides, glucosinolates, cofactors, phytohormones, or primary metabolites (such as purine and pyrimidine bases). Even a number of antibiotics produced by bacteria or fungi are therefore included in the group of alkaloids.

Occurrence

Alkaloids have been detected in about 15% of plants, bacteria, fungi, and even in animals. Within the plant kingdom, they occur in primitive groups such as *Lycopodium* or *Equisetum*, in gymnosperms and angiosperms. In higher plants (angiosperms), some families contain more alkaloid-containing taxa than others. Such alkaloid-rich taxa include Papaveraceae, Berberidaceae, Fabaceae, Boraginaceae, Apocynaceae, Asteraceae, Liliaceae, Gnetaceae, Ranunculaceae, Rubiaceae, Solanaceae, and Rutaceae. Also, several food plants and food items may contain alkaloids (Table 1).

It has been speculated that alkaloids evolved early in evolution and were already present at the time (*c*.200 Ma) when the angiosperms began to radiate. In general, specific alkaloid types are restricted to particular systematic units and are therefore of some importance for the systematics, taxonomy, and phylogeny of plants. For example, benzylisoquinoline alkaloids are typical for Papaveraceae, Berberidaceae, and Ranunculaceae, which seem to be phylogenetically related. Other alkaloids occur in phylogenetically unrelated plant families. Ergot alkaloids occur not only in fungi (*Claviceps*) but also in members of the Convolvulaceae; quinolizidine alkaloids (QAs) are typical for some Fabaceae but have also been detected in Berberidaceae (*Caulophyllum* and *Leontice*). It has been speculated that the genes encoding enzymes leading to the main QA skeleton are distributed much more widely in the plant kingdom but are normally switched off. Alternatively, convergent evolution, horizontal gene transfer, and the production of alkaloids by endophytic fungi (e.g., ergot alkaloids) have been shown or suggested.

Isolation and Detection

Alkaloids form free bases in alkaline solutions. The free base is usually lipophilic and mostly insoluble in water but soluble in organic solvents (ethanol, methanol, diethyl ether, and methylene chloride). At higher hydrogen ion concentrations (i.e., $pH < 7$), alkaloids occur in a protonated state and are usually soluble in water but insoluble in apolar organic solvents. The different solubilities are useful to isolate and purify alkaloids. In the laboratory, alkaloids are often solubilized from plant material by dissolving them in acidic solutions (e.g., 0.5 M HCl). Treatment of this solution with organic solvents will remove nonalkaloidal substances. The solutions are then brought to $pH > 12$ in the next step and extracted with CH_2Cl_2, ethyl acetate, or diethyl ether to yield free alkaloids.

Plant extracts are often analyzed by thin-layer chromatography (TLC) as a first screening to find out whether alkaloids are present or not. A number of reagents give typical color reactions, such as Dragendorff's or Mayer's reagent. Because alkaloids are typically present in complex mixtures consisting of two to five main and up to 20–50 minor alkaloids, TLC does not have sufficient separation capacities for a complete analysis. Better methods are high-performance liquid chromatography (HPLC) and capillary gas–liquid chromatography (GLC). The latter method is extremely useful because it has a strong separation power and is very sensitive and selective if a nitrogen-specific detector is used. Furthermore, GLC can be directly coupled with a mass spectrometer, allowing mass spectra to be obtained, even from very minor components. Since many alkaloids have been analyzed by mass spectrometry, already a large collection of mass spectra is available, making it possible to identify many of the known alkaloids unambiguously. Usually, mass spectra are recorded in the electron impact (EI) mode, which promotes fragmentation. If information on the molecular ions is needed (which can be elusive in EI-MS), other MS techniques, such as chemical ionization, field desorption, and fast-atom bombardment, are the methods of choice.

HPLC tends to be less sensitive and of lower separation capacity than capillary GLC. However, modern photodiode array detectors are very helpful to identify known metabolites by UV–VIS spectroscopy. Today, also, HPLC and capillary electrophoresis can be coupled to a mass spectrometer, thus widening the use of MS for the analysis of natural products. LC–MS has become a major technique in many analytic applications.

In addition, HPLC has a major advantage in that it is possible to isolate a compound in milligram quantities that allow structural elucidation by nuclear magnetic resonance (^1H, ^{13}C). Nuclear magnetic resonance is the method of choice if unknown structures are to be elucidated, whereas mass spectrometry is extremely useful for identifying previously described substances or substances that are slightly different to known compounds.

If small quantities (femtogram or nanogram amounts) of known alkaloids need to be detected routinely, immunologic procedures such as radioimmunoassays (RIA) and enzyme immunoassays (EIA, ELISA) should be appropriate. In order

Table 1 Alkaloids present in food plants and beverages and in addition as stimulants or hallucinogens

Substance	*Occurrence*	*Biological activity*
Alkaloids in food		
Solanine and other steroid alkaloids	*Solanum* (potato and tomato)	Membrane disruption, acetylcholine esterase inhibition, mutagenicity
Pyrrolizidine alkaloids	*Symphytum* (comfrey); honey (if bees have visited PA plants)	DNA and protein alkylation; mutagenicity, cancer
Cycasin	Cycas, other cycads	Mutagenicity
Ergot alkaloids	*Claviceps purpurea*; on rye, wheat, barley	Neuroreceptor interaction; vasoconstriction; uterus contraction
Saxitoxin	Algae; ends up in food chain (mollusks and fish)	Neurotoxin (Na^+ channel)
Lupanine and other quinolizidine alkaloids	*Lupinus*; other genistoids	Interaction with AChR[a], Na^+, K^+ channels
Pelletierine	*Punica granatum*	Interaction with AChR
Alkaloids in beverages		
Caffeine, theophylline, theobromine	*Coffea*, *Thea*, *Paullinia*, *Ilex paraguariensis*	Stimulants
Quinine	*Cinchona succirubra*	Bitter tonic; neuroreceptor and ion channel interaction
Alkaloids as stimulants and hallucinogens		
Ephedrine and related compounds	*Ephedra*; *Catha edulis*	Central stimulant
Morphine	*Papaver somniferum*, opium	Hallucinogen; analgesic
N-Methyltryptamine	Fungi, plants	Central stimulant
N, *N*-Dimethyltryptamine, 5-methoxy-*N*, *N*-dimethyltryptamine	Mimosaceae: *Anadenanthera* (syn. *Piptadenia*), *Mimosa hostilis*; Myristicaceae: *Virola*; Malpighiaceae: *Banisteriopsis*; Poaceae: *Phalaris*	Central stimulants, hallucinogen
Serotonin	Fungi (*Amanita*); stinging hairs of *Urtica*, *Laportea*, *Jatropha urens*, *Mucuna pruriens*; seeds and fruits	Local inflammation
Bufotenine	Fungi (*Amanita*); animal poisons (Cnidaria, spider, scorpions, wasps, and toads)	Central stimulant, hallucinogen
Psilocybin, psilocin	Fungi (*Psilocybe*, *Stropharia*, *Conocybe*, *Panaeolus*, *Gymnopilus*, *Psathyrella*, etc.)	Central stimulant, hallucinogen
Mescaline	*Lophophora williamsii*; other cacti	Hallucinogen
Harmaline and other β-carboline alkaloids	*Peganum harmala*, *Banisteriopsis caapi*	Hallucinogens
Cocaine	*Erythroxylon coca*	Stimulant, analgesic
Arecoline	Nuts of areca palm	Stimulant

[a]AChR, acetylcholine receptor.

to obtain specific antibodies, the alkaloid in question has to be coupled chemically to a large protein, such as albumin, usually by some sort of spacers (e.g., succinic acid). Such constructs induce the generation of specific antibodies.

recombinant DNA technology allows the production of such enzymes in microbial systems. In the future, it is likely that several of the medicinally important alkaloids could thus be produced in recombinant microorganisms.

Biosynthesis

The skeleton of most alkaloids is derived from amino acids (Figure 1 and Table 2), although moieties from other pathways, such as terpenoids, are often combined. In addition, in a number of alkaloids (e.g., steroid alkaloids), the nitrogen (deriving from glutamine or other NH_2 sources) is added near the end of a biosynthetic pathway, that is, the alkaloid skeleton does not stem from amino acids. Biosynthetic pathways have been worked out in detail for several alkaloids. For isoquinoline and monoterpene indole alkaloids, not only the enzymes but also the corresponding genes are known that catalyze individual steps in the biosynthesis. Employing

Accumulation and Storage

Although the exact site of alkaloid formation in a plant cell has been elucidated for a few species only, it is certainly correct to assume that most compounds are synthesized in the cytoplasm. Membrane-enclosed vesicles have been implied in the biosynthesis of berberine. Not only is the chloroplast the site of photosynthesis and related processes, but also it harbors a number of biosynthetic pathways, such as those for fatty acids and amino acids. In addition, QAs are synthesized in the chloroplast stroma; thus, both the alkaloid and its amino acid precursor, L-lysine, share the same compartment. QA formation is regulated by light and displays a diurnal rhythm.

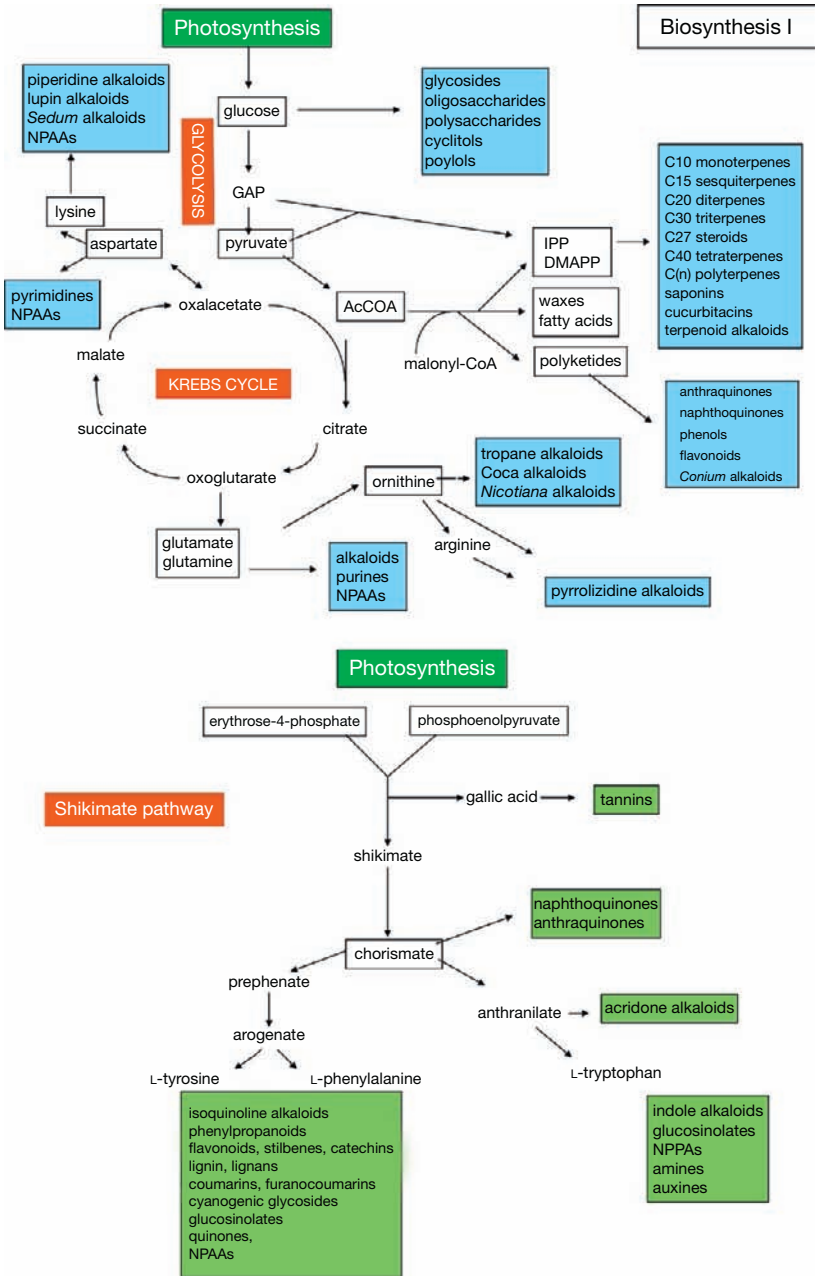

Figure 1 Schematic overview of biosynthetic pathways leading to different groups of alkaloids.

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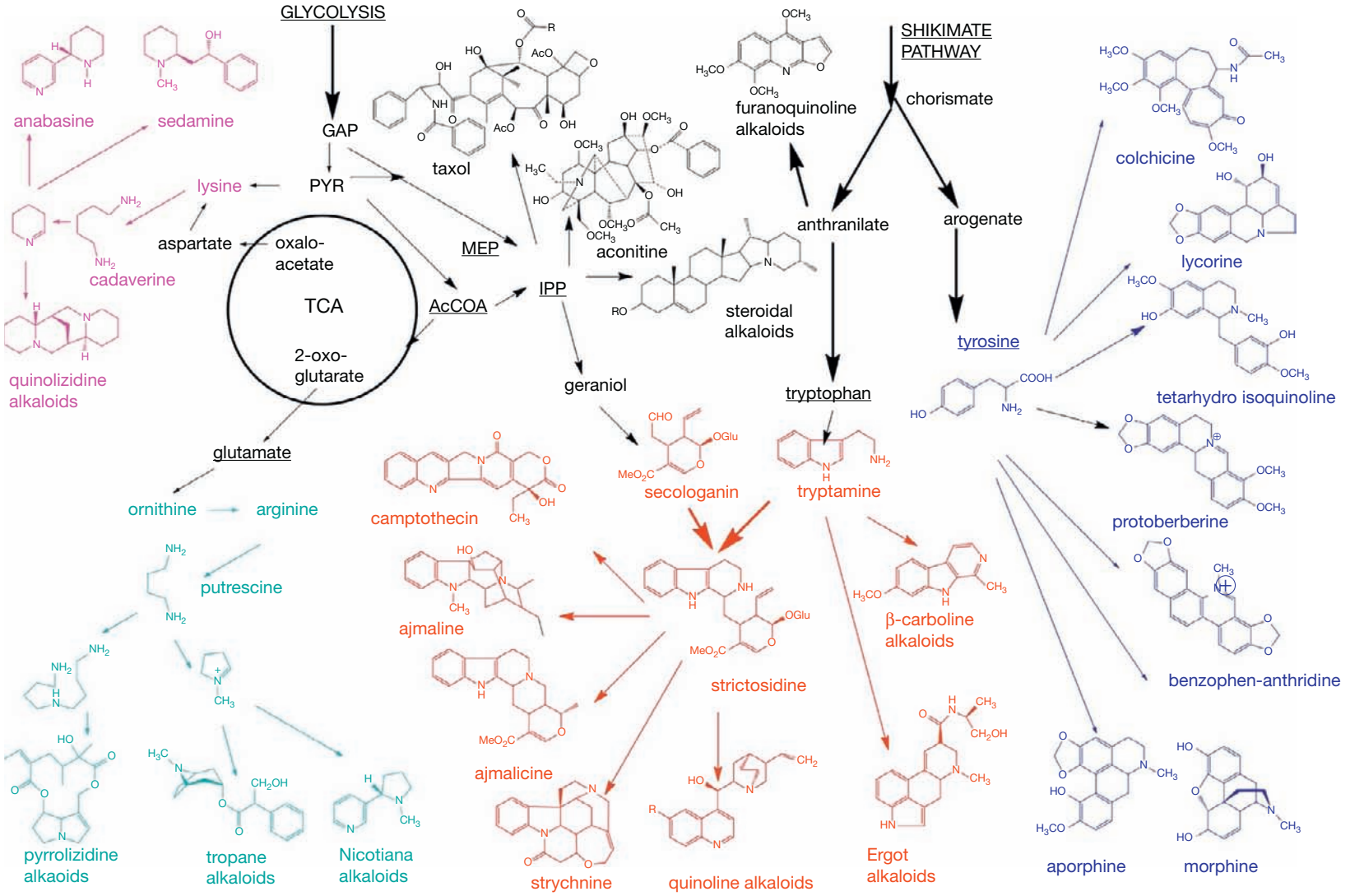

Figure 1—Cont'd

Table 2 Examples for important alkaloids and their biosynthetic precursors

Amino acid	*Alkaloid*	*Main occurrences*	*Example structure*
Lysine	Quinolizidine alkaloids	Fabaceae	Lupanine, sparteine, cytosine
	Lycopodium alkaloids	Lycopodiaceae	Lycopodine
	Piperidine alkaloids	Punicaceae, Crassulaceae	Pelletierine, sedamine
Ornithine	Tropane alkaloids	Solanaceae, Erythroxylaceae	Hyoscyamine, cocaine
	Pyrrolizidine alkaloids	Asteraceae, Boraginaceae, Fabaceae	Senecionine, heliotrine
	Nicotiana alkaloids	Solanaceae	Nicotine, anabasine
Tryptophan	Monoterpene indole alkaloids	Apocynaceae	Strychnine, vincamine, yohimbine, ajmalicine, etc.
	Simple indole alkaloids	Fabaceae	Physostigmine
	Quinoline alkaloids	Rubiaceae, Cornaceae	Quinine, cinchonine, camptothecin
	Ergot alkaloids	*Claviceps*, Convolvulaceae	Ergotamine, lysergic acid
	β-Carboline alkaloids	Loganiaceae, Zygophyllaceae	Harman, harmaline
Phenylalanine/ tyrosine	*Ephedra* alkaloids	Ephedraceae	Ephedrine
	Tetrahydroisoquinoline alkaloids	Rubiaceae	Emetine
	Benzylisoquinoline alkaloids	Papaveraceae, Berberidaceae	Papaverine
	Benzophenanthridine alkaloids	Papaveraceae	Sanguinarine
	Protoberberine alkaloids	Berberidaceae, Papaveraceae	Berberine
	Morphinan alkaloids	Papaveraceae	Morphine, codeine
	Aporphine alkaloids	Monimiaceae	Boldine
	Phenylethylisoquinoline alkaloids	Colchicaceae	Colchicine
	Aristolochia alkaloids	Aristolochiaceae	Aristolochic acid
Anthranilic acid	*Ruta* alkaloids	Rutaceae	Skimmianine, dictamine

Light regulation seems to be triggered by (1) lysine availability (it is also made during the day), (2) the change in stromal hydrogen ion concentration to pH 8 (enzymes of QA formation have a pH optimum at pH 8), and (3) the reduction of QA enzymes by thioredoxin (reduced thioredoxin is generated under illumination).

Alkaloids are not formed in the extracellular space or in the vacuole. The storage of high concentrations of alkaloids is a prerequisite for their allelochemical roles as defense compounds. Since these concentrations would interfere with the normal metabolisms, the allelochemicals are safely stored in the vacuole (**Table 3**) of often specialized cells or tissues (such as epidermis). The vacuole of these alkaloid-accumulating cells has been termed the 'toxic' or 'defense' compartment. A number of plants produce latex, which, in addition to its gluing properties (think of insect mandibles!), often contains defense chemicals, such as alkaloids (morphine and related benzylisoquinoline alkaloids in Papaveraceae, protoberberine and benzophenanthridine alkaloids in *Chelidonium*, and lobeline and other piperidine alkaloids in *Lobelia*) and terpenoids (e.g., phorbol esters). The alkaloids are selectively sequestered in small (diameter < 1 μm) latex vesicles and reach local concentrations of up to 1 M.

Storage in the vacuole or in vesicles demands that alkaloids pass the tonoplast and accumulate within the vacuole against a concentration gradient. For the passage across the tonoplast, three mechanisms are plausible:

- Simple diffusion (takes place in the case of lipophilic alkaloids, e.g., nicotine, ajmalicine, vinblastine, and colchicine)
- Carrier-mediated transport (in the case of polar and charged alkaloids, which is the rule for most alkaloids under physiological conditions, e.g., hyoscyamine, lupanine, reticuline, scoulerine, and senecionine)
- Membrane fusion (in the case of alkaloids that are formed in vesicle-enclosed compartments, e.g., berberine)

Table 3 Storage of alkaloids in the vacuole

Alkaloid	*Genus*
Leaf vacuoles	
Lupanine	*Lupinus*
Sparteine	*Cytisus*, *Lupinus*
Hyoscyamine	*Atropa*
Nicotine	*Nicotiana*
S-Coulerine	*Fumaria*
S-Reticuline	*Fumaria*
Ajmalicine	*Catharanthus*
Serpentine	*Catharanthus*
Catharanthine	*Catharanthus*
Betalains	*Beta*, *Chenopodium*
Senecionine-*N*-oxide	*Senecio*
Capsaicin	*Capsicum*
Latex vesicles	
Sanguinarine	*Chelidonium*
p-Berberine	*Chelidonium*
Morphine and other morphinane alkaloids	*Papaver*

Because alkaloids are sequestered against a concentration gradient in the vacuole or in latex vesicles, the driving force of uphill accumulation needs to be determined. In some instances, vesicles or vacuoles contain alkaloid binding or complexing compounds. For example, latex vesicles of *Chelidonium majus* contain between 500 and 1200 mM chelidonic acid, which binds or

complexes berberine and benzophenanthridine alkaloids. It has been shown experimentally that chelidonic acid acts as a trapping agent that causes the apparent uphill transport, resulting in alkaloid concentrations in vesicles of 500–1200 mM. Protonation, organic acids, and specific peptides may constitute other trapping mechanisms. There is some evidence that amino acids and some ions are transported into the vacuole with aid transporters that are fueled by proton–substrate antiport mechanisms. Protons are enriched in the vacuole, which generally has hydrogen ion concentrations of 0.001–1 mM (=pH 6–3), by proton-translocating ATPases and pyrophosphatases. In analogy, it has been assumed (supported by experimental evidence) that carrier transport of alkaloids is achieved by a proton–alkaloid antiport mechanism or with the aid of ABC transporters. There is good evidence that plants have a large number of ATP-binding cassette (ABC) transporter genes and that these pumps play a role in the distribution of alkaloids within plants and within cells.

Whereas a few alkaloids are formed ubiquitously in all plant organs, organ- or even tissue-specific formation seems to be more common (Table 4). Since most eukaryotic genes are regulated in a cell-, tissue-, and organ-specific manner, genes of alkaloid formation seem to be no exception. The corresponding promotors are presumed to be regulated by respective regulatory proteins. This conclusion is important for the interpretation of results obtained with cell suspension cultures. Whereas alkaloid formation is active in differentiated systems (root or shoot cultures), it is usually reduced, or even absent, in undifferentiated suspension cultured cells. It is likely that the corresponding alkaloid genes are just 'switched off.' The production of berberine and sanguinarine in cell cultures seems to be more the exception than the rule.

Alkaloids are stored predominantly in tissues that are important for survival and reproduction, which include actively growing young tissues, root and stem bark, flowers (especially seeds), seedlings, and photosynthetically active tissues. Alkaloid contents in storing organs can be quite high, reaching up to 10% of dry weight in some instances, which is important if the alkaloids are to function as effective defense compounds. In several herbaceous plants, alkaloids are stored in epidermal and subepidermal tissues (e.g., cocaine, colchicine, aconitine, steroidal alkaloids, nicotine, veratrine, buxine, and coniine), which have to ward off small enemies (insects and microorganisms) in the first place. In lupins and broom, QA concentrations are up to 200 mM in epidermal tissue, whereas mesophyll tissue has values below 5 mM. Some plants possess typical alkaloid-storing cells, called 'idioblasts.' These idioblasts have been detected in *Corydalis* (for corydaline), *Sanguinaria* (for sanguinarine), *Ruta* (for rutacridones), *Catharanthus* (for indole alkaloids), and *Macleaya* (for protopine). Short- and long-distance transports are often required to reach these sites of accumulation.

Alkaloid patterns usually vary between the site of synthesis and the sites of accumulation, since a number of secondary substitutions may take place in the latter tissues, or transport may be selective, distributing differing cocktails. In addition, alkaloid profiles of seeds and seedlings often differ from those of the mature plant. Both patterns and concentrations usually change during the development of plants and the annual cycle. In general, alkaloid levels are markedly reduced in senescing tissues, so that shedded leaves are often nearly alkaloid-free. Alkaloid formation and storage may be influenced by environmental stress, such as wounding or infection.

Table 4 Examples for organ-specific biosynthesis of alkaloids

Alkaloid	*Organ*	*Species*
Tropane alkaloids	Roots	*Atropa*, *Datura*, *Hyoscyamus*, *Mandragora*
Nicotine	Roots	*Nicotiana*
Senecionine and other PAs	Roots	*Senecio*
Emetine	Roots	*Cephaelis*
Sanguinarine	Roots	*Sanguinaria*
Betalains	Roots, shoots	*Beta*
Quinine	Stem bark	*Cinchona*
Berberine	Stem and root bark	*Berberis*, *Mahonia*
Caffeine	Green tissue	*Coffea*
Quinolizidine alkaloids	Leaves and other photosynthetic tissues	*Lupinus*, *Cytisus*, *Laburnum*, *Baptisia*
Steroid alkaloids	Roots, tubers, leaves	*Solanum*

Transport and Turnover

A number of alkaloids are synthesized and stored in all parts of plants, whereas others are restricted to a particular organ. Alternatively, alkaloids are synthesized but accumulated in many organs, which usually demands long-distance transport. Theoretically, this transport could proceed sym- or apoplastically. However, the utilization of established transport routes, such as xylem or phloem, seems to be more common. Because it is technically difficult to sample and analyze xylem and phloem sap, only a limited number of appropriate data are available (Table 5). Besides long-distance transport, short-distance and intracellular transports need to be reviewed. In general, alkaloids are synthesized in the cytosol or in membrane-enclosed vesicles (endoplasmic reticulum, mitochondria, and chloroplasts) but are accumulated and sequestered in the vacuole.

Table 5 Evidence for long-distance transport of alkaloids by phloem or xylem

Alkaloid	*Xylem*	*Phloem*	*Occurrence*
Lupanine, sparteine		X	*Lupinus*, *Cytisus*
Cytisine		X	*Laburnum*, *Petteria*, *Genista*, *Spartium*
Matrine		X	*Sophora*
Senecionine (*N*-oxide)		X	*Senecio*
Aconitine	?	X	*Aconitum*
Swainsonine		X	*Astragalus*
Nicotine	X		*Nicotiana*
Hyoscyamine	X		*Atropa*
Scopolamine	X		*Datura*, *Hyoscyamus*

In general, alkaloids are not end products of metabolism but can be degraded, which seems to be plausible because nitrogen is a limited nutrient for plants. As discussed previously, alkaloids stored in seeds are partly degraded during germination and seedling development, and their nitrogen is probably used for the synthesis of amino acids. Degradative pathways have not been worked out yet.

In addition to this developmentally specific recycling, there is evidence that a number of alkaloids are turned over all the time, with half-lives of between 2 and 48 h. Examples are nicotine, QAs, and tropane alkaloids. Alkaloid turnover is often quite substantial in cell cultures: *Lupinus* callus cultures are even able to live on the QA sparteine as a sole nitrogen source for more than 6 months.

How can we explain this phenomenon? A number of alkaloids are defense chemicals (allelochemicals) and affect molecular targets such as receptors of neurotransmitters (tropanes, nicotine, etc.). For this interaction, a correct stereochemical configuration is required. Because alkaloids may oxidize or give rise to racemic mixtures spontaneously, a continuous turnover would make sure that a sufficient concentration of active compounds is always available, similar to the situation of protein turnover.

Functions

Although several alkaloids and other secondary metabolites have been used by mankind for thousands of years as dyes (e.g., indigo and shikonine), flavors (e.g., vanillin, capsaicin, and mustard oils), fragrances (e.g., rose oil, lavender oil, and other essential oils), stimulants (e.g., caffeine, nicotine, and ephedrine), hallucinogens (e.g., morphine, cocaine, mescaline, hyoscyamine, scopolamine, and tetrahydrocannabinol), insecticides (e.g., nicotine, piperine, and pyrethrin), vertebrate and human poisons (e.g., coniine, strychnine, and aconitine), and even therapeutic agents (e.g., atropine, quinine, codeine, and cardenolides), their putative functions have been discussed controversially.

Alkaloid biology is tightly connected with the basic physiology of plants. Many of the features described before would make no sense if these compounds did not have a vital function for the producer. As a common theme, it has been observed that plants that produce seeds rich in energy supplies (carbohydrates, lipids, and proteins) concomitantly accumulate potent chemical defense compounds, often alkaloids, nonprotein amino acids, cyanogenic glycosides, glucosinolates, protease inhibitors, lectins, or other toxalbumins. Their presence in seeds can be mutually exclusive, that is, legume seeds store either alkaloids (e.g., quinolizidines and pyrrolizidines) or nonprotein amino acids, but not both at the same time. During germination, the breakdown of nutrient reserves is a general procedure and usually includes the nitrogenous defense compounds. They serve a double purpose, that is, that of N-storage and that of protection. They are thus degradable and toxic N-storage compounds.

The main function is obviously that of chemical defense against herbivores (insects, other arthropods, and vertebrates), which can be deduced from the fact that many alkaloids have a high affinity for receptors of neurotransmitters that are present only in animals. In some instances, alkaloids play a role (additionally) in the antimicrobial defense (against bacteria, fungi, and viruses) and even in the interaction between plants (allelopathy).

Alkaloids are certainly multipurpose compounds that, depending on the situation, may be active in more than one environmental interaction. For example, QAs are certainly the most important defense chemical in Fabaceae against insects and other herbivores, but they also influence bacteria, fungi, viruses, and even the germination of other plants. In addition, they are employed as degradable N-transport and N-storage compounds.

Alkaloids repel or deter the feeding of many animals (many have a bitter or pungent taste to humans and other vertebrates) or are toxic if ingested. In microorganisms and competing plants, a reduction of growth and antibiosis are usually the visible effects of alkaloid intoxication. How are these diverse effects being achieved? Although most compounds have not been studied in full detail, an impressive number of cellular and molecular targets have been identified that are selectively inhibited or modulated by alkaloids. As a consequence of such interactions, organ malfunctions (heart, lung, liver, kidney, and CNS disorders) result that may impair reproduction and fertility in animals and other organisms or simply kill them.

Because many alkaloids have been shaped during evolution by 'molecular modeling,' many of them are used by humans as medicinal compounds; allelochemicals may have positive effects if used at nontoxic concentrations.

Presence at the Right Concentration at the Right Time and Place

To be effective, alkaloids need to be present at the right time, site, and concentration. Alkaloid metabolism and biochemistry seem to have been optimized and coordinated in most systems to fulfill this prerequisite. An interesting variation can be seen in some plants, where alkaloid formation is enhanced by wounding or microbial attack, that is, in case of emergency, the production of defense compounds is stimulated.

How effective are alkaloidal defenses? In lupins, which normally produce high amounts of QAs, mutants have been selected (the so-called sweet lupins) that accumulate only trace amounts of alkaloids. When both alkaloid-rich and low-alkaloid lupins are planted in the field without any fences and phytoprotectives, a dramatic effect can often be seen (**Figure 2**). Whereas the low-alkaloid lupins were selectively grazed or infested by herbivores, their alkaloid-rich counterparts remained almost undisturbed. More experimental data are certainly needed, but the defensive role of alkaloids seems to be beyond doubt.

Similar to the situation in sweet lupins, in which plant breeders have eliminated the alkaloid trait, also in other crop plants, secondary metabolites, which had evolved to serve as defense compounds, have been bred away or strongly decreased. As a consequence, many crop plants have lost their original resistance to pests and herbivores. Man-made chemistry (i.e., synthetic pesticides) has to be used if these crop plants have to be cultured.

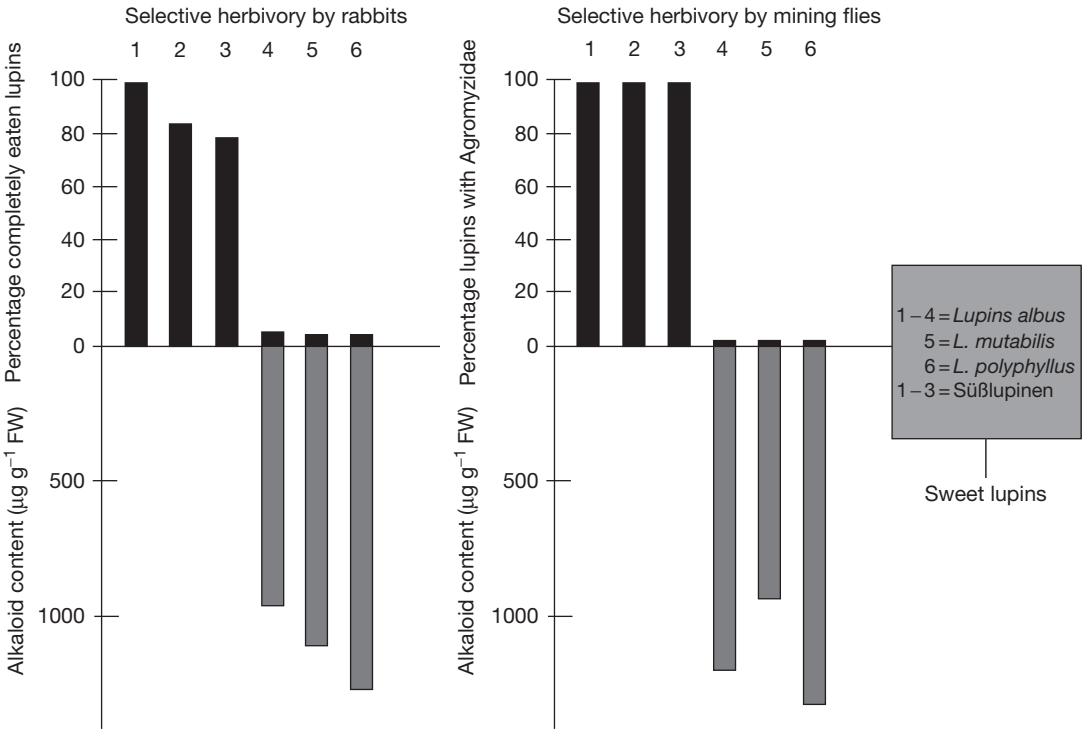

Figure 2 Selective advantage of alkaloids in lupins against herbivores (rabbits and mining flies). Lupins without alkaloids suffer heavily from herbivory, whereas alkaloid-rich lupins are widely protected.

Exceptions to the Rule: Adaptations of Specialists

No defense is absolute. Whereas chemical defense works against the majority of potential enemies, usually a few specialists have evolved during evolution that have become specialized in the toxin-protected ecological niche. This phenomenon can be clearly seen in many insects, which are often highly host plant-specific. Some of these insects take up the dietary alkaloids, store, and exploit them for their own chemical defense or that of their offspring. Others additionally transform the alkaloids into pheromones or utilize them as morphogens. Well-studied examples have been published for pyrrolizidine and QAs. Vertebrate herbivores (humans included) have effective liver enzymes that can detoxify xenobiotics. Often, substances become hydroxylated, conjugated, and then excreted via the feces or the kidney and urine.

See also: Chromatography: High-Performance Liquid Chromatography.

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Alkaloids: Toxicology and Health Effects

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Why Alkaloids Are Abundant in Plants

Apparently, most alkaloids play an important role in the ecology of plants or animals. They serve as defense chemicals against herbivores and predators. To a lesser degree, they protect against bacteria, fungi, and viruses or provide a means for plant–plant interactions. To be effective defense chemicals, alkaloids must closely interact with specific targets in herbivores, predators, microorganisms, or competing plants, that is, they must either inhibit or otherwise deregulate important processes that are vital for these organisms. For this purpose, the molecular shape of alkaloids has apparently been optimized during a million years of evolution in a process, which could be termed 'evolutionary molecular modeling.'

While the structures of more than 21 000 individual alkaloids have been reported, rather limited knowledge is available for most of them in terms of biological activities and functions. In this article, the modes of action of the better known alkaloids, especially those found in food plants, are summarized and discussed, considering interactions with organs or complete organisms first and then molecular targets. These interactions are the base for understanding the toxic or antinutritional effects that are observed if humans or animals have ingested alkaloids with their diet.

Toxic and Pharmacological Effects at the Organ Level

Many alkaloids are known for their toxic or adverse effects on animals (Table 1). In many cases, only the toxicity of an alkaloid has been reported evidencing substantial interactions, but the exact mode of action has not yet been elucidated or is rather complex, involving several molecular targets and organs.

In medicine, alkaloids are employed as local anesthetics, narcotics, analgesics, and cardiac, uterine, and respiratory stimulants or to raise blood pressure, to dilate pupils, and to relax the skeletal muscles (Table 2). The use of alkaloids as narcotics and hallucinogens causes major social problems.

Ultimately, the toxic and pharmacological effects (Tables 1 and 2) observed must be the result of interactions of alkaloids with molecular targets present in or on the cells.

Central Nervous System and Neuromuscular Junctions

A remarkable number of alkaloids interfere with the metabolism and activity of neurotransmitters in the brain and nerve cells. A disturbance of metabolism or binding of neurotransmitters and related signal pathways impairs learning and memory, sensory faculties (smell, vision, or hearing), and coordination of bodily functions or produces euphoric or hallucinogenic effects.

Muscle activity (skeletal, heart, etc.) is controlled by acetylcholine (ACh) and norepinephrine (noradrenaline). Any inhibition or overstimulation of neurotransmitter-regulated ion channels will severely influence muscular activity and thus the mobility or organ function (such as the heart, lungs, and gut). When there is inhibition, the muscles will relax; when there is overstimulation, they will be tense or in tetanus, leading to a general paralysis and/or respiratory failure (which is the effect of many of the more toxic alkaloids). Alkaloids that activate (the so-called parasympathomimetics) or inhibit (parasympatholytics) neuromuscular action are tabulated in Table 3. These compounds are usually considered to be strong poisons (Table 1).

Inhibition of the Digestive Process

Food uptake can be reduced by pungent or bitter taste in the first instance. The next step can be the induction of vomiting, which is a common reaction to the ingestion of a number of alkaloids; the alkaloid emetine already implies this activity in its name! Causing diarrhea, or the opposite, constipation, would be another activity that negatively influences the digestive system. Many intoxications with alkaloid-containing plants have diarrhea as one of the symptoms. Another way to interfere would be the inhibition of digestive enzymes or of transport proteins for amino acids, sugars, or lipids.

Modulation of Liver and Kidney Function

Nutrients and xenobiotics (such as secondary metabolites) are transported to the liver after resorption in the intestine. In the liver, the metabolism of carbohydrates, amino acids, and lipids and the subsequent synthesis of proteins and glycogen take place. The liver is also the main site for the detoxification of xenobiotics. Lipophilic compounds, which are easily resorbed from the diet, are often hydroxylated and then conjugated with a polar, hydrophilic molecule, such as glucuronic acid, sulfate, or an amino acid. These conjugates are exported via the blood to the kidney for elimination via the urine. Both organ systems are affected by a variety of secondary metabolites: pyrrolizidine alkaloids (PAs) are activated during the detoxification process and are converted into potent carcinogens, causing liver cancer. Many other metabolic inhibitors, discussed later in the text, are also liver toxins. Many alkaloids are known for their diuretic activity. Increased diuresis would also mean an increased elimination of water and essential ions. Since Na^+ ions are already limited in plant food, long-term exposure to diuresis-inducing compounds would reduce the fitness of a herbivore substantially.

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Table 1 Toxicological properties (LD_{50}) of alkaloids

Alkaloid	*Test system*	*LD_{50} mg kg^{-1}*
Alkaloids derived from tryptophan		
Brucine	Rat	p.o., 1
Cinchonidine	Rat	i.p., 206
Cinchonine	Rat	i.p., 152
Ellipticine	Mouse	i.v., 1.2
Ergocryptine[a]	Rabbit	i.v., 1.1
Ergometrine[a]	Mouse	i.v., 0.15
Ergotamine[a]	Mouse	i.v., 62
Harman	Mouse	i.p., 50
Harmine	Mouse	i.v., 38
Physostigmine	Mouse	p.o., 4.5
Psilocybin	Mouse	i.v., 285
Quinidine	Rat	i.v., 30; p.o., 263
Quinine[a]	Agelaius	p.o., 100
Reserpine	Agelaius	p.o., 100
Strychnine	Agelaius	p.o., 6
	Rat	i.v., 0.9
Vinblastine	Mouse	i.v., 9.5
Vincamine	Mouse	i.v., 75
Vincristine	Mouse	i.p., 5.2
Alkaloids derived from phenylalanine/tyrosine		
Aristolochic acid	Mouse	i.v., 38–70; p.o., 56–106
Berberine	Mouse	i.p., 23
Bulbocapnine	Mouse	p.o., 413
Canadine	Mouse	p.o., 940
Chelerythrine	Mouse	s.c., 95
Chelidonine	Mouse	i.v., 35
Codeine	Mouse	s.c., 300
Colchicine	Mouse	i.v., 4.1,
	Man	p.o., 0.1–0.3
Emetine	Mouse	s.c., 32
Galantamine	Mouse	i.v., 8; p.o., 18.7
Morphine	Mouse	i.v., 226–318
Papaverine	Mouse	i.v., 27.5; s.c., 150
Protopine	Mouse	i.p., 36–102
Sanguinarine	Mouse	s.c., 102; i.v., 16
Thebaine	Mouse	i.p., 20
Tubocurarine	Mouse	p.o., 33.2
Steroid alkaloids		
Jervine	Mouse	i.v., 9.3
Protoveratrine	Rabbit	i.p., <0.1
Samandarine	Mouse	i.p., <3.4
Solanine[a]	Mouse	i.p., 42
Veratridine	Mouse	i.p., 1.4
Tropane alkaloids		
Atropine	Rat	p.o., 750
Cocaine	Rat	i.v., 17.5
Pyrrolizidine alkaloids		
Echimidine[a]	Rat	i.p., 200
Heliotrine	Rat	i.p., 300
Jacobine	Rat	i.p., 138
Monocrotaline	Rat	i.p., 175; p.o., 71
Senecionine	Rat	i.p., 85
Seneciphylline	Rat	i.p., 77
Quinolizidine alkaloids		
Cytisine	Mouse	i.v., 1.7
13-Hydroxylupanine[a]	Mouse	i.p., 172
Lupanine[a]	Mouse	i.p., 80
N-methylcytisine	Mouse	i.v., 21; i.p. 51
Sparteine[a]	Mouse	i.p., 55–67; p.o., 350–510
Miscellaneous alkaloids		
Aconitine	Mouse	i.v., 0.17; p.o., 1
α-Amanitin	Mouse	i.p., 0.1
Arecoline[a]	Mouse	s.c., 100
Caffeine[a]	Mouse	p.o., 127–137
Coniine	Agelaius	p.o., 56
Delphinine	Rabbit	i.p., 1.5–3.0
Maytansine	Rat	s.c., 0.48
Muscimol	Rat	p.o., 45
Nicotine[a]	Agelaius	p.o., 17.8
	Mouse	i.v., 0.3; p.o., 230
Tetrodotoxin[a]	Mouse	i.p., 0.01; s.c., 0.008

i.p. = intraperitoneal; i.v. = intravenous; p.o. = oral; s.c. = subcutaneous.
[a]Encountered in food plants or food items.

Disturbance of Reproduction

Quite a number of allelochemicals are known to influence the reproductive system of animals, which will ultimately reduce their numbers (and fitness as a species). Antihormonal effects could be achieved by mimicking the structure of sexual hormones, such as coumarins that dimerize to dicoumarols or isoflavones. The next target is the gestation process itself. As outlined in the succeeding text, a number of alkaloids are mutagenic and lead to malformation of the offspring or directly to the death of the embryo. The last step would be premature abortion of the embryo. This dramatic activity has been reported for a number of allelochemicals, including many mono- and sesquiterpenes and alkaloids. Some alkaloids achieve this by the induction of uterine contraction, as do the ergot and lupin alkaloids.

Molecular Targets of Alkaloids

In the following, a number of important cellular molecular targets (Figure 1) have been addressed that are often affected by alkaloids and other plant toxins.

Biomembranes, Membrane Transport, and Neuronal Signal Transduction

Cells can only operate effectively if their biomembranes (cytoplasmic membrane and internal membranes) are intact. Biomembranes are almost impermeable for ions and polar molecules. As an exchange of these molecules must take place between cells and organs, specific membrane proteins, which can be ion channels, pores, or carrier proteins, mediate the controlled flux of these compounds across biomembranes. The biomembranes and the complex transport systems are targets of many natural products.

Steroidal alkaloids, such as solanine and tomatine, which are present in many members of the Solanaceae (including potatoes and tomatoes), can form complexes with the cholesterol present in the biomembranes. While the steroidal moiety 'dives' into the lipophilic interior of the membrane and

Table 2 Pharmacological and medicinal properties of alkaloids

Type	*Alkaloid*	*Activity*
Poison	Pyrrolizidine alkaloids (from *Senecio*, *Heliotropium*, *Crotalaria*)	Conversion to DNA- and protein-alkylating agent in the liver; causing liver cirrhosis, mutations, cancer
	Ergot alkaloids (from *Claviceps purpurea*)	Cause vasoconstrictions, hallucinogenic effects, gangrenous limps; disease named 'ergotism'
Analgesics	Aconitine (*Aconitum*)	Local anesthetic, general paralytic effect
	Morphine (*Papaver somniferum*)	Very effective pain killer (used since ancient times); with addictive properties
	Codeine (*Papaver*)	Pain and cough depression
	Cocaine (*Erythroxylon coca*)	Local anesthetic
Cardiac stimulant	Quinidine (*Cinchona* spec.)	Antiarrhythmic properties at the heart ventricle
	Sparteine (*Cytisus scoparius*)	Antiarrhythmic properties
	Ajmalicine (*Rauvolfia serpentina*)	Antiarrhythmic properties at the heart ventricle
Respiratory stimulant	Nicotine (*Nicotina*), cytisine (*Laburnum*)	Stimulation of respiration is followed by respiratory depression, asphyxia, or even respiratory failure
	Lobeline (*Lobelia* spec.)	Stimulant; used to treat bronchial asthma
	Coniine (*Conium maculatum*)	Used as a potent poison in antiquity (Socrates)
Constriction of blood vessels	Ergot alkaloids (*Claviceps purpurea*)	Used in obstetrics
	Ephedrine (*Ephedra* spec.)	Employed in the treatment of bronchial asthma, cold, sinusitis
	Scopolamine (*Hyoscyamus*, *Atropa*, and *Datura*)	Inhibitor of smooth muscles, for example, dilatator in vessels
Muscle relaxant	Tubocurarine (*Chondrodendron tomentosum*)	Block nAChR[a]; used in surgery
	Hyoscyamine (atropine, *Hyoscyamus*, *Atropa*, and *Datura*)	Antispasmodic at smooth muscles (gastrointestinal tract and bladder)
	Papaverine (*Papaver somniferum*)	Smooth-muscle relaxant
Antiparasitic and antimicrobial activity	Berberine (*Berberis*, *Mahonia*, and *Coptis*)	Intercalates DNA and inhibits parasites and microorganisms
	Emetine (*Psychotria ipecacuanha*)	Intestinal amebiasis, emetic drug
	Boldine (*Peumus boldo*)	Anthelmintic activity
	Quinine (*Cinchona succirubra*)	Antimalarial
Anti-inflammatory activity	Colchicine (*Colchicum autumnale*)	Treatment of acute gout, recurrent gout
Anti-Alzheimer's disease	Physostigmine (*Physostigma venenosum*) Galantamine (*Galanthus nivalis*)	Inhibitor of acetylcholinesterase
Eye treatments	Pilocarpine (*Pilocarpus jaborandi*)	Miotic used in the treatment of open-angle glaucoma
Cancer chemotherapy	Taxol (*Taxus brevifolia*)	Treatment of breast and ovary carcinoma; other malignancies
	Vinblastine, vincristine (*Catharanthus roseus*)	Treatment of lymphomas and other tumors
	Camptothecin (*Camptotheca acuminata*)	Cancer chemotherapy

[a]nAChR, nicotinic acetylcholine receptor.

interacts with the structurally similar cholesterol, the hydrophilic side chain remains outside and binds to external sugar receptors. Since phospholipids are in a continuous motion, a tension easily builds up, which leads to membrane disruption; transient 'holes' occur in the biomembrane, rendering the cell leaky. A similar mechanism has been postulated for saponins, a widely distributed group of natural products, to which the steroidal alkaloids may be assigned. Steroidal alkaloids can also interact with other targets, such as neuroreceptors or even with DNA; malformations have been observed in animal embryos after having been exposed to *Solanum* alkaloids.

Communication between cells is especially important for the nerve cells. Signal transduction in the central nervous system and in neuromuscular junctions is mediated by receptor proteins residing in the membrane, which are directly or indirectly coupled with ion channels. The neurotransmitters involved include, among others, norepinephrine (noradrenaline), epinephrine (adrenaline), serotonin, dopamine, histamine, glycine, γ-aminobutyric acid (GABA), glutamate, and ACh.

Neuroreceptors can be ligand-gated channels, that is, a receptor that is part of an ion-channel complex. When the neurotransmitter binds, a conformational change induces the opening of a Na^+–K^+ channel for microseconds, allowing Na^+ ions (the external concentration is about 145 mmol l^{-1}) to enter the cell following a concentration gradient (the internal Na^+ concentration is between 5 and 15 mmol l^{-1}). The ligand quickly dissociates from the receptor and, in the case of ACh, is hydrolyzed by ACh esterase (Figure 2). Glutamate (*N*-methyl-D-aspartate) and GABA receptors are also ligand-gated ion channels.

More abundant are G protein-coupled neuroreceptors. A prominent one is the muscarinic ACh receptor (AChR); norepinephrine, serotonin, and dopamine receptors also belong to this type. When ACh binds, the receptor changes its conformation, inducing a conformational change in an adjacent G protein molecule. Its α-subunit dissociates and then activates the enzyme adenylyl cyclase, which in turn produces cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). The cAMP molecule, a second messenger, activates protein kinases or Ca^{2+} channels directly.

Quite a number of alkaloids are known whose structures are more or less similar to those of endogenous

Table 3 Examples for alkaloids that bind to neurotransmitter receptors and neurotransmitter-degrading enzymes

Target	*Ligand*	*Alkaloid*	*Occurrence*
Acetylcholine receptor			
Nicotinic receptor	Acetylcholine	Nicotine	*Nicotiana*, *Duboisia*
		C-toxiferine	*Strychnos*
		Tubocurarine	*Chondrodendron*
		Coniine	*Conium*
		Cytisine and other QAs	Several legumes
		Lobeline	*Lobelia*
		Anabasine	*Anabasis*, *Nicotiana*
Muscarinic receptor	Acetylcholine	Hyoscyamine (atropine)	*Atropa*, *Hyoscyamus*, *Datura*, *Mandragora*
		Scopolamine	Several Solanaceae
		Arecoline	*Areca*
		Pilocarpine	*Pilocarpus*
		Muscarine	*Amanita*, *Inocybe*, *Clitocybe*, other fungi
		Sparteine and other QAs	Several legumes
Adrenergic receptors	Noradrenaline/adrenaline	Ergot alkaloids	*Claviceps*
		Yohimbine	Pausinystalia, *Aspidosperma*
		Rauwolscine	*Rauvolfia*
		Corynanthine	*Rauvolfia*
		Norlaudanosoline	Papaveraceae
		Ephedrine, norephedrine	*Ephedra*
Serotonin receptor	Serotonin	Ergot alkaloids	*Claviceps*
		Psilocin, psilocybin	*Psilocybe*, other fungi
		N,*N*-dimethyltryptamine	Several plants and toads
		Bufotenine	*Virola*, *Anadenanthera*
		β-Carboline alkaloids	*Banisteriopsis*, *Peganum*
		Mescaline	*Lophophora*, other cacti
Dopamine receptor	Dopamine	Ergot alkaloids	*Claviceps*
		Bulbocapnine	*Corydalis*
GABA receptor	GABA	Bicuculline	*Dicentra cucullaria*, *Corydalis* species
		Muscimol	*Amanita*
		β-Carboline alkaloids	*Peganum*, *Banisteriopsis*
Adenosine receptor	Adenosine	Caffeine	*Coffea*, *Camellia*, *Ilex*, *Paullinia*
		Theophylline, theobromine	*Theobroma*
Glycine receptor	Glycine	Brucine	*Strychnos*
		Strychnine	*Strychnos*
Opioid receptor	Endorphins	Morphine	*Papaver somniferum*
Acetylcholine esterase	Acetylcholine	Physostigmine (eserine)	*Physostigma venenosum*
		Berberine	Several Papaveraceae
		Coptisine	Several Papaveraceae
		Galantamine	Several Amaryllidaceae
		Solanine and other steroid alkaloids	*Solanum*
		Huperzine A	*Huperzia serrata*
Monoamine oxidase (MAO)	Noradrenaline, dopamine, serotonin, histamine	Harmaline, harmine	*Peganum*
		Salsolinol	Chenopodiaceae
Catechol-O-methyltransferase	Noradrenaline, adrenaline, dopamine	Tetrahydroisoquinoline	Papaveraceae

QAs, quinolizidine analogs; GABA, γ-aminobutyric acid.

Source: Wink, M. (1998). Modes of action of alkaloids. In: Roberts, M. F. and Wink, M. (eds.) *Alkaloids: biochemistry, ecology and medicinal applications*, pp. 301–326. New York: Plenum; Wink, M. (2000). Interference of alkaloids with neuroreceptors and ion channels. In: Atta-Ur-Rahman (ed.) *Bioactive natural products*, pp. 1–127. Amsterdam: Elsevier.

neurotransmitters. They can function therefore as structural analogs. In addition, several plants produce compounds that are identical to animal neurotransmitters, such as ACh and histamine in stinging hairs of *Urtica* or serotonin and dopamine in several species. Targets can be

- the receptor itself through inhibition or overstimulation (Table 3);
- the enzymes that deactivate neurotransmitters after they have bound to a receptor (Table 3);
- transport processes, which are important for the uptake of neurotransmitters into the presynapse or their storage in synaptic vesicles (Table 4); or
- enzymes involved in the biosynthesis of a neurotransmitter.

The stimulation of neurotransmitter-activated ion channels leads to a rapid influx of Na^+ ions, which in turn activates voltage-gated Na^+ and K^+ channels, which are essential for further signal transduction. These Na^+ and K^+ channels constitute another important target for alkaloids (Table 5).

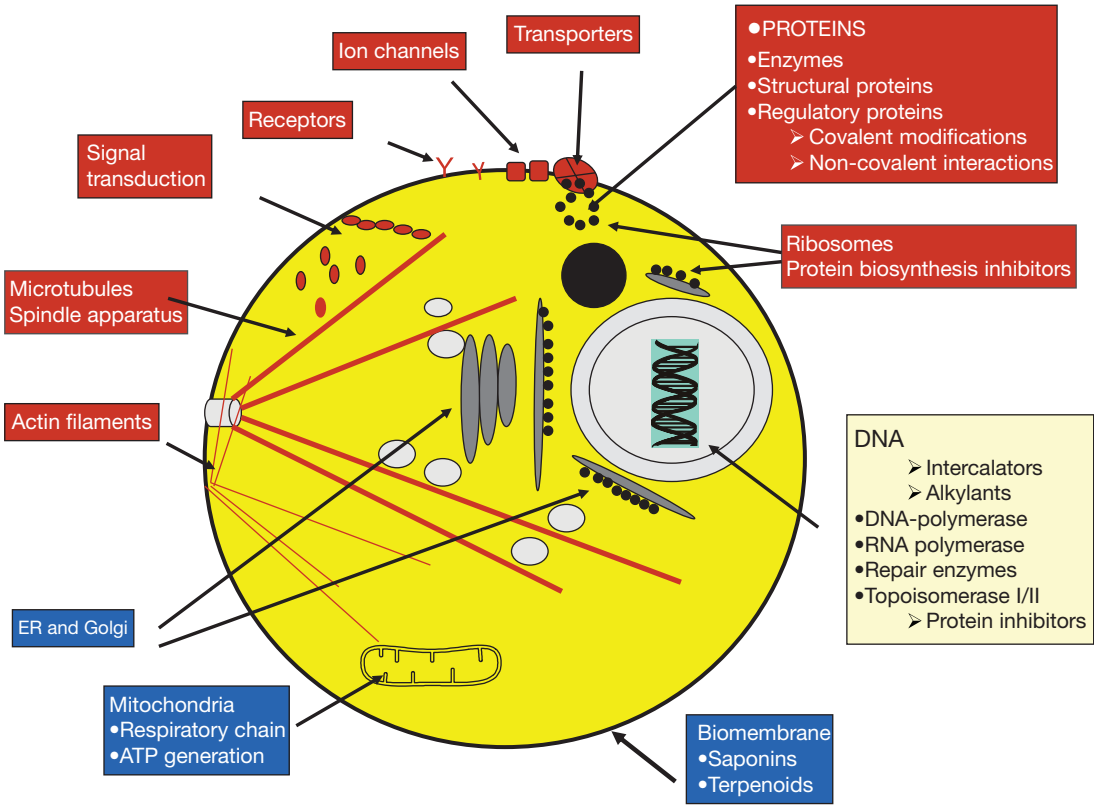

Figure 1 Molecular targets of animal cells that are affected by alkaloids.

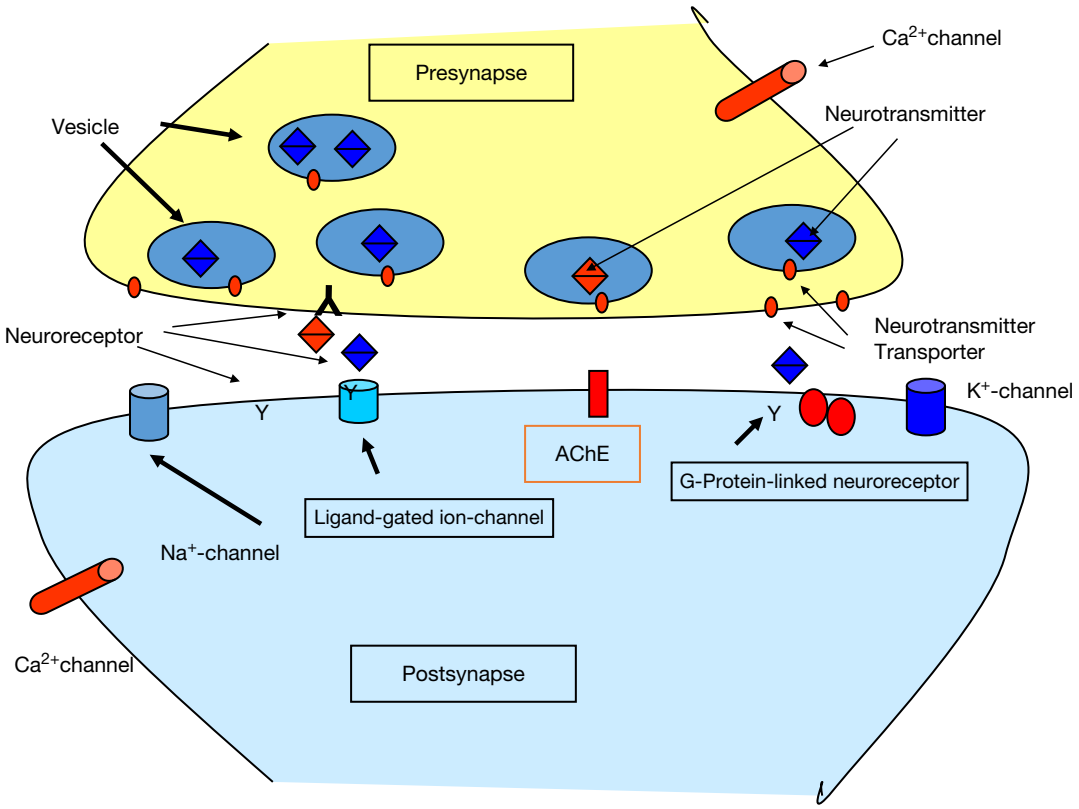

Figure 2 Signal transduction in excitable synapses.

Cells carefully control ion concentrations inside and outside of the cells with the help of specific ion channels (e.g., Na^+, K^+, Ca^{2+}, and Cl^- channels) and of active Na^+, K^+, or Ca^{2+} pumps, such as Na^+/K^+-ATPase and Ca^{2+}-ATPase. Ion gradients and ion fluxes mediated by these channels and pumps are the main elements in the active transport processes, in neuronal and neuromuscular signaling. Cardiac glycosides are potent and well-known inhibitors of Na^+/K^+-ATPase found in plants, some insects, and in the skin of certain toads. A few alkaloids such as harmaline, nitidine, sanguinarine, capsaicin, cassaine, and solenopsin (from ants) inhibit Na^+/K^+-ATPase.

Whereas receptor–ion channel interactions represent the initial part of many signal pathways, key enzymes that produce or inactivate second messengers or amplify the signal can be important targets further down the pathway (Table 6). These enzymes include

- adenylyl cyclase (making cAMP),
- phosphodiesterase (inactivating cAMP),
- phospholipase (releasing arachidonic acid or inositol phosphates), or
- several protein kinases, such as protein kinase C (which is activated by phorbol esters and the alkaloid chelerythrine) or tyrosine kinase (activating other regulatory proteins or ion channels).

Because these targets are almost exclusively found in animals but absent in plants, the development of active compounds directed to these targets appears to be advantageous for the plants producing them. They can store these compounds without risk of being intoxicated by their own toxins.

Table 4 Alkaloids as inhibitors of neurotransmitter uptake (transport into presynapse or into vesicles)

Transporter	*Alkaloid*	*Occurrence*
Norepinephrine	Reserpine	*Rauwolfia*
(Noradrenaline)	Ephedrine	*Ephedra*
Biogenic amines	Tetrahydro-β-carboline	*Peganum*
	Salsolinol	*Salsola*
	Tetrahydroisoquinoline	Papaveraceae
	Tetrahydropalmatine	Berberidaceae
Dopamine	Cocaine	*Erythroxylum*

Table 5 Alkaloids as modulators of Na^+, K^+, and Ca^{2+} channels

Alkaloid	*Occurrence (genera)*	*Action*
Na^+ and K^+ channels		
Aconitine[a]	*Aconitum*	Activation
Ajmaline[a]	*Rauvolfia*	Inhibition
Batrachotoxin[a]	Frogs (Dendrobatidae)	Activation
Harmaline	*Peganum*	Inhibition
Protoveratrines A and B[a]	*Veratrum*	Activation
Quinidine[a]	*Cinchona*	Inhibition
Quinine	*Cinchona*	Inhibition
Saxitoxin[a]	*Protogonyaulax* (algae)	Inhibition
Sparteine[a]	*Cytisus*, *Lupinus*, *Genista*	Inhibition
Tetrodotoxin[a]	Algae/fish	Inhibition
Veratridine[a]	*Veratrum*	Activation
Ca^{2+} channels		
Ryanodine	*Ryania speciosa*	Inhibition

[a]Na^+ channel.

DNA/RNA

The genetic information of most organisms is mainly encoded in DNA. Since the integrity of DNA is important for the structure and function of rRNAs, proteins, and enzymes that are important for metabolism, structure, and development of an organism, DNA is a highly vulnerable target. It is not surprising that a number of secondary metabolites became selected during evolution that interact with DNA or DNA-processing enzymes. Some alkaloids are known to bind or to intercalate with DNA (Table 7). Many of these molecules are planar, hydrophobic molecules that fit between the planar stacks of AT and GC base pairs. Other alkaloids act on the level of DNA- and RNA polymerases and DNA topoisomerases, thus impairing the process of replication and transcription.

The effects of DNA-binding or DNA-intercalating compounds can be mutations, which may result in malformations of newborn animals or in the initiation of cancer. When anabasine, coniine, or anagyrine is administered to pregnant cows or sheep, a large proportion of the offspring develop malformations of the legs – the so-called crooked calf disease. Some

Table 6 Alkaloids modulating enzymes involved in signal transduction

Enzyme	*Function*	*Alkaloid*	*Occurrence*
Adenylyl cyclase	cAMP formation	Anonaine	Annonaceae
		β-Carboline-1-propionic acid	Fabaceae
		Isoboldine	*Peumus*
		Tetrahydroberberine	Berberidaceae
Phosphodiesterase	cAMP inactivation	Papaverine	*Papaver*
		Caffeine, theobromine	*Coffea*, *Paulinia*
			Camellia, *Theobroma*
		Theophylline	*Ilex paraguariensis*
		1-Ethyl-β-carboline	*Peganum*
Protein kinases	Protein phosphorylation	Chelerythrine	*Chelidonium majus*
		Lyngbyatoxin A	Marine seaweeds

cAMP, cyclic adenosine monophosphate.

Table 7 Alkaloids interacting with DNA/RNA and related enzymes

Target	*Activity*	*Alkaloid*	*Occurrence*
DNA	Photoaddition	Dictamnine	*Dictamnus*
		Harman	*Peganum*
		Harmine	*Peganum*
	Alkylation	Pyrrolizidine alkaloids	Several Asteraceae, Boraginaceae
		Aristolochic acid	*Aristolochia*
		Cycasin	*Cycads*
	Intercalation	Ellipticine	*Ochrosia*
		Quinine	*Cinchona*
		Skimmianine	*Skimmia*
		Berberine	*Berberis*, *Mahonia*, *Thalictrum*, *Chelidonium*
		Chelerythrine	*Chelidonium*
		Coptisine	Several Papaveraceae
		Fagaronine	Rutaceae
		Sanguinarine	Several Papaveraceae
		Olivacine	*Aspidosperma*
DNA polymerase	Inhibition	Fagaronine	Rutaceae
		Hippeastrine	*Hippeastrum*
		Lycorine	Several Amaryllidaceae
DNA topoisomerase I	Inhibition	Camptothecin	*Camptotheca acuminata*
Reverse transcriptase	Inhibition	Berberine	Several Berberidaceae, Papaveraceae
		Chelidonine	*Chelidonium*
RNA polymerase	Inhibition	Vincristine, vinblastine	*Catharanthus roseus*
Transcription	Inhibition	Colchicine	*Colchicum*, *Gloriosa*
		Amanitin	*Amanita*

alkaloids of the monocot *Veratrum*, such as jervine and cyclopamine, cause the formation of a large central eye, the cyclopean eye, which was probably known to the ancient Greeks and thus led to the mythical figure of the Cyclops.

Other alkaloids are known as carcinogens, such as aristolochic acid from Aristolochia and PAs that are produced by ~3% of the higher plants, especially within the families of Asteraceae and Boraginaceae. Aristolochic acid has a nitro group that can be transformed into reactive intermediates in the intestine. If resorbed, these metabolites can alkylate DNA. PAs are not carcinogenic in their native form, but become so when they are 'detoxified' in the liver: PAs are usually present in the plant as their N-oxides, which are polar compounds that cannot pass biomembranes by simple diffusion. In the intestine, PA N-oxides are reduced by gut bacteria. The free base is then readily taken up by the gut cells and transported to the liver. There, PAs are transformed into alkylating compounds, which covalently bind to DNA and proteins. As a result, mutations and cancer can be initiated.

Protein Biosynthesis

Protein biosynthesis is essential for all cells and thus provides another important target. Indeed, a number of alkaloids have been detected that inhibit protein biosynthesis *in vitro*. Emetine from *Psychotria ipecacuanha* (Rubiaceae) is the most potent plant constituent. Other alkaloids with the same ability include harringtonine, homoharringtonine, cryptopleurine, tubulosine, hemanthamine, lycorine, narciclasine, pretazettine, pseudolycorine, tylocrepine, and tylophorine. Several alkaloids that inhibit protein biosynthesis and are also DNA-intercalating substances can induce apoptosis in cells.

Electron Chains and Other Enzyme Activities

The respiratory chain and ATP synthesis in the mitochondria or photophosphorylation in chloroplasts demand the controlled flux of electrons. These targets seem to be attacked by nicotine, sanguinarine, ellipticine, gramine, alpinigenine, capsaicin, and a few other alkaloids. A multitude of enzymes exist in animal cells and several alkaloids have been reported that interfere with at least one of them.

A recently discovered group of alkaloids are the polyhydroxyalkaloids, such as swainsonine or castanospermine, which inhibit hydrolytic enzymes, namely, glucosidase, galactosidase, trehalase (trehalose is a sugar found in some beetle cocoons and fungi that is hydrolyzed by trehalase), and mannosidase selectively.

Cytoskeleton

Microtubules, which are important for cellular movements, vesicle transport in neurons, or the separation of chromosomes during cell division, are composed of tubulin subunits. Movements and some transport processes are mediated through either the rapid assembly or disassembly of microtubules. The assembly of microtubules is inhibited by colchicine and dimeric indole alkaloids vinblastine and vincristine (important for chemotherapy of certain cancers). These alkaloids thus interrupt cell division. The diterpene alkaloid paclitaxel (Taxol; used in the treatment of ovarian and breast cancer) affects microtubules in the opposite way; the disassembly of tubulin is inhibited by Taxol. As a consequence, Taxol-induced

microtubules are very stable and dividing cells are arrested in the metaphase.

Cell stability, phagocytosis, cell–cell interactions, and cell movements are also controlled by actin filaments, which are rapidly assembled or disassembled from action monomers. Cytochalasin B and latrunculin B bind to the plus end of a growing actin filament, preventing the addition of actin monomers there. Another alkaloid, phalloidin, produced by the fatally poisonous toadstool *Amanita phalloides*, stabilizes actin filaments and inhibits their depolymerization.

Mechanisms of Allelochemical Activities in Antiviral, Antimicrobial, and Phytotoxic Interactions

Circumstantial evidence indicates that some alkaloids protect the producing plant against viruses, bacteria, fungi, and competing plants. A number of antimicrobial alkaloids such as sanguinarine, quinine, or berberine intercalate with viral and microbial DNA or bind to it. These compounds may thus inhibit processes such as DNA replication and RNA transcription, which are vital for the microorganisms. Protein biosynthesis in ribosomes is another vulnerable target, attacked by emetine. The stability of biomembranes can be disturbed by steroidal alkaloids and tetrandrine. Other targets may be electron chains or just metabolically important enzymes. Phytotoxic properties or germination inhibition, which can be observed in plant–plant interactions, can also proceed via the earlier-mentioned mechanisms. But interactions with growth hormones and their metabolism must also be considered.

Target Specificity of Alkaloids

In general, the interactions of a particular alkaloid with a molecular target (as described in the preceding text) suggest a high degree of specificity. A closer look, however, shows that many alkaloids interfere with more than one target. The phenomenon will be explained for two groups of alkaloids: ergot alkaloids and quinolizidine alkaloids (QAs).

Ergot Alkaloids

Ergot alkaloids, such as ergotamine, ergometrine, or ergoclavine, are produced by fungi of the genus *Claviceps*, which lives in close contact with many grasses (family Poaceae) such as the cereal *Hordeum vulgare*. These alkaloids can modulate several receptors of neurotransmitters, such as dopamine, serotonin, and norepinephrine. As a consequence, the pharmacological action of ergot alkaloids is rather broad, ranging from vasoconstriction and uterus contraction to hallucinations. We can explain these activities through structure similarities between the alkaloid and the different neurotransmitters.

Quinolizidine Alkaloids

QAs, such as lupanine, sparteine, or cytisine, are produced by lupins and many members of the Fabaceae. They are bitter for many animals (and plants producing them are therefore avoided as food). If ingested, QAs exhibit a broad level of toxicity: they interact with AChRs as agonists. QAs, like many other alkaloids, occur as complex mixtures in plants. Some QAs preferentially bind to the nicotinic AChR, whereas others tend more to bind to the muscarinic AChR. Some QAs exhibit a prominent cross-reactivity. Additionally, QAs such as lupanine and sparteine inhibit Na^+ and K^+ channels, thus blocking the signal transduction in the nerve cells at a second critical point. A few particular QAs, such as anagyrine, cytisine, and the bipiperidine alkaloid ammodendrine (which co-occurs with QAs in many plants), are mutagenic and lead to malformations (see earlier text).

If we accept the hypothesis that alkaloids were developed as chemical defense compounds through a process of 'evolutionary molecular modeling,' the 'cross-reactivity' described makes sense: any compound that can interfere with more than one target or with more than one group of adverse organisms is likely to be more effective and thus has a better survival value in general than a more selective allelochemical. In addition, herbivores will try to develop tolerance to or resistance against the dietary toxins. If more than one target is affected by a defense chemical, the chances of a herbivore developing specific resistances concomitantly are much smaller than in single-target situations. In conclusion, we can say that nature has obviously tried 'to catch as many flies with one clap as possible' in the selection of alkaloids during evolution.

Medicinal Applications

Because alkaloids can interfere with several molecular targets in animals and microbes, some of them can be used in medicine to treat infections, health disorders, and even cancer.

Important chemotherapeutic alkaloids, used in cancer therapy, include vinblastine and other vinca alkaloids, paclitaxel (Taxol), demecolcine, and camptothecin, Whereas vinblastine, paclitaxel (Taxol), and demecolcine modulate microtubules, camptothecin inhibits DNA topoisomerase I.

Alkaloids that interfere with ion channels can be used as analgesics (aconitine and cocaine) or antiarrhythmic drugs (ajmaline, quinidine, and sparteine).

Alkaloids that modulate receptors of neurotransmitters are interesting as stimulants (caffeine, cathine, cocaine, ephedrine, nicotine, theobromine, and theophylline), hallucinogens (tropane alkaloids, ergot alkaloids, and heroin), muscle relaxants (atropine and tubocurarine), vasodilators (papaverine, ajmalicine, and vincamine), vasoconstrictors (ergotamine), antihypertensives (rescinnamine and reserpine), antitussives (codeine, lobeline, narceine, and noscapine), glaucoma treatment (pilocarpine), or painkillers (morphine).

A few alkaloids that inhibit ACh esterase (such as physostigmine and galantamine) are of medicinal interest to treat Alzheimer's disease.

Some alkaloids have direct cytotoxic effects (because they interfere with DNA) and are used in chemotherapy or as antimicrobial or antiparasitic compounds (such as berberine, quinine, and sanguinarine).

Emetine inhibits protein biosynthesis and is used to treat amebiasis; it is also used as an emetic and secretolytic drug.

It is likely that more alkaloids or derived agents will be exploited in the future when more and diverse molecular targets, which are presently discovered by functional genomics and proteomics, will enter the pipeline of drug development. They remain interesting because they have been selected during evolution as chemical agents that can modulate a multitude of molecular targets.

See also: Alkaloids: Properties and Determination; Cereals: Dietary Importance; Lupine; Potatoes and Related Crops; Saponins; Solanaceous Fruits Including Tomato, Eggplant, and Peppers; Tea: Chemistry and Processing.

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What Is an Allergy?

Allergies are a type of adverse reaction resulting from inappropriate immune responses to a variety of environmental agents and can be classified as being either immunoglobulin E (IgE)- or non-IgE-mediated. During the course of normal immune function, the body produces immunoglobulins such as IgA, IgG, and IgM, to many environmental agents, including microbes, dusts, pollens, and dietary proteins. One type of immunoglobulin, IgE, is normally produced in response to parasitic infections like malaria, but in allergic diseases, historically classified as type I hypersensitivity reactions, this antibody repertoire is altered and the body synthesizes larger quantities of IgE to environmental agents like foods, pollens, and dusts. The process of generating an IgE response is termed sensitization and involves complex interactions between immune cells involved in presenting foreign proteins (such as dendritic cells) and those responsible for generating antibody responses (such as B cells and T cells). It takes place in individuals who have a predisposition to becoming allergic and may involve exposure to environmental agents through the skin and the lungs and also by ingestion although exposure to antigens via the gut mucosa is generally thought to induce tolerance.

The IgE becomes bound to the surface of histamine-containing cells, such as basophils and tissue-associated mast cells. On reexposure to the original sensitizing agent in a multivalent form, the cell-bound IgE becomes cross-linked, triggering release of histamine and other inflammatory mediators, which then go on to cause the symptoms of an allergic reaction. Most commonly, the signs and symptoms of an allergic reaction include the following, alone or in combination:

- Skin reactions, such as hives, redness, and swelling
- Itching or tingling in or around the mouth and throat
- Digestive problems, such as diarrhea, stomach cramps, nausea, and vomiting
- Tightening of the throat
- Shortness of breath or wheezing
- Runny nose

In rare cases, a more severe reaction, anaphylaxis, develops. This requires immediate treatment with an epinephrine (also known as adrenaline), and individuals with severe allergies are often provided with the drug in a self-injectable form. It can result in emergency admission to hospital, and in rare cases, reactions can be fatal. The signs and symptoms of anaphylaxis include combinations of the previously mentioned symptoms plus the following:

- Constriction of airways
- Swelling of the throat that makes breathing difficult
- A dramatic drop in blood pressure (anaphylactic shock)
- Rapid pulse
- Dizziness, lightheadedness, or loss of consciousness

A second type of food allergy, which involves the cellular immune system, is celiac disease (CD). First clearly described in the writings of the Greek physician Aretaeus between the first and second centuries CE, the relationship to food ingestion was firmly established by Gee in 1888. Subsequently, Dicke and coworkers established that wheat and rye were the triggers for the condition in the 1950s. An immune-mediated disorder that is largely characterized by chronic inflammation of the small intestinal mucosa, CD is generally characterized by atrophy of the villi and intraepithelial lymphocytosis, although it may also manifest itself in a variety of other ways, either during childhood or later on in adulthood. Symptoms can include diarrhea, abdominal cramping, pain, bloating, and distension. There is a strong genetic component to CD with a link to human leukocyte antigen (HLA) alleles. In particular, HLA-DQ2 and HLA-DQ8 have been shown to be highly associated with CD, although their presence alone is not sufficient for development of CD. Pathogenic T cells recognize gluten selectively in the context of these predisposing HLA molecules.

Prevalence of Food Allergy

The prevalence of food allergy reported in different studies around the world varies, with methodological issues, in particular the method of diagnosis, having a significant impact on the resulting estimates. In general, the lifetime prevalence and point prevalence of self-reported food allergy have been estimated in a recent meta-analysis as being around 17.3% (95% CI: 17.0–17.6) and 5.9% (95% CI: 5.7–6.1), respectively. However, the point prevalence of sensitization to foods is much lower, with 10.1% (95% CI: 9.4–10.8) of the population having food-specific IgE to ≥ 1 food, while sensitization indicated by skin prick testing being lower still at 2.7% (95% CI: 2.4–3.0). The prevalence of food allergy defined by food challenge was lowest of all, at 0.9% (95% CI: 0.8–1.1). There appears to be a geographic variation in prevalence of food allergy across Europe, with rates being higher in Northern Europe. In the United States, the self-reported prevalence of food allergy has been estimated at around 8.0%, with peanut, milks, and shellfish being important foods. In the United Kingdom, peanut has been estimated as causing food allergy in around 2% of school-age children, while in Australia, up to 9% of infants have been estimated to suffer from egg allergy. Infants in Australia from Asian backgrounds are more at risk of experiencing peanut allergy, with 7.7% of infants with both parents born in East Asia having a challenge-proven food allergy, while only 2.3% of infants with both parents born in Australia had a confirmed peanut allergy. As regards the prevalence of severe and fatal reactions, a recent analysis of fatalities in the United States has shown food anaphylaxis to be the least common cause of fatal anaphylaxis in the United States, with 164 fatalities being recorded between 1999 and 2010, and the rate (6.7% of all deaths due to anaphylaxis) is similar to

that defined in Australia and the United Kingdom. Allergies in infancy are dominated by egg and milk, the majority of which are outgrown by school age, and the patterns of food allergies vary around the world and may be related to patterns of food consumption.

The prevalence of CD is highest in Europe with around 1% of the population estimated to suffer from the condition, although it is heterogeneous with 1–2.4% of the population in Finland and 1% of children and adults in the United Kingdom being affected by CD. Interestingly, in the Russian Republic of Karelia, which is a close neighbor of Finland, the prevalence of CD is only 0.2%. Similarly in North Africa, Algeria has the highest reported incidence of CD worldwide of 5.6%, yet the prevalence in nearby Tunisia is one of the lowest reported worldwide at 0.3%. It seems highly probable that much of the variance in incidence relates to differences in diagnosis rather than reflecting true differences in prevalence. There also seems to have been a CD 'epidemic' in Sweden with the incidence rising to almost 3% of children born between 1984 and 1996, which was attributed to changes in infant feeding practices. One consequence of that 'epidemic' is the recommendation of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition that gluten is gradually introduced at the age of 4–7 months during breast-feeding.

Molecules Causing Allergies

In allergies, the immune responses (either IgE binding in IgE-mediated food allergies or T-cell responses in CD) are directed toward target molecules that are known as allergens, which are usually proteinaceous in nature. The sites an antibody recognizes on a protein are known as epitopes and can be either linear (both IgE and T-cell epitopes) or conformational (IgE epitopes only). In the former, only the primary sequence of a polypeptide is involved in recognition by either antibody or a T-cell receptor. However, the structure of conformational epitopes is determined by the three-dimensional structure of a protein, with a number of segments of the polypeptide chain being brought together as a consequence of its tertiary and quaternary structure. IgE responses can also be generated to posttranslational modifications such as glycans that may be attached to the proteins during synthesis. While IgE responses to such glycans are not generally considered to be effective in triggering an allergic reaction, an exception is α-galactose, which has been identified as an important determinant of allergy to meat in individuals who become sensitized to α-galactose after suffering from tick bites. While not necessarily involved in elicitation of allergic reactions, there is evidence that N-linked glycans on certain allergens may play a role in driving sensitization.

The World Health Organization and the International Union of Immunological Societies produced an official list of the molecules that can trigger IgE-mediated allergies. An allergen is termed major if it is recognized by IgE from at least 50% of a cohort of allergic individuals but does not carry any connotation of allergenic potency; allergens are otherwise termed 'minor.' The allergen designation is then based on the Latin name of the species from which it originates, with the first 3–4 letters representing the genus and the second 1–2 the species, followed by an Arabic numeral; for example, Ara h 1 relates to *Arachis hypogea* (peanuts). The numbering was originally consecutive but more recently numbering has been used to denote protein families where possible. Closely related allergen isoforms are denoted by decimals with the first two relating to isoforms with >67% homology, while the second two decimals denote allergens with >90% sequence homology.

As regards triggers for CD, a number of gluten-derived peptides have been identified, which determine the ability of trigger the condition and depend on the following:

(1) The ability of the peptide to resist gastrointestinal digestion sufficiently to allow polypeptides of 15–20 residues to survive in the gastrointestinal tract.
(2) The effectiveness of the digestion-resistant peptide to act as a substrate for TG2. TG2 appears to act in a specific manner, targeting particular gluten peptides.
(3) The binding specificity and affinity for HLA-DQ2 and HLA-DQ8 of a 9-residue peptide.

A number of databases have been developed, which contain information on food allergens and peptide motifs involved in CD.

Treatment of Food Allergy

Currently, there is no cure for either IgE-mediated food allergies or CD and individuals are advised to practice food avoidance. For those at risk of severe IgE-mediated reactions, this advice is supplemented with antihistamines and self-injectable epinephrine (in the United Kingdom, adrenaline) to be self-administered in the event that a problem food is unintentionally consumed. In order to help allergic consumers avoid their problem food, mandatory labeling of a list of certain allergens was recommended by FAO/WHO Codex Alimentarius Commission, which was subsequently implemented around the world. This has significant negative implications for quality of life for both the sufferer and, in the case of children, their parents and relatives. However, accidental exposure to allergens may be difficult to prevent, despite the improvements in food labeling. Thus, food avoidance is not a satisfactory treatment option as the risk of anaphylaxis remains.

Several therapeutic approaches are being developed for IgE-mediated allergies. Since immunotherapy injections for food allergy are associated with a high rate of allergic reactions, alternatives, such as oral immunotherapy (OIT) using foods, are being explored. OIT initially involves patient being treated in hospital with individuals being incrementally exposed to low doses of their allergenic food over the course of a week or two (dose escalation), followed by treatment at home with patients eating a problem food every day over a period of at least 3 years to achieve a fully tolerant state. Other approaches being investigated include delivering the allergens for desensitization via skin patches or using allergen molecules or peptides to desensitize individuals, while medicines based on Chinese herbal medicines may have potential for preventing or treating allergic reactions.

Major Allergenic Foods

Across various populations, allergies to over 100 different foods have now been documented. However, it has been

estimated that the majority of food allergies are caused by the so-called top 8 allergenic foods of both plant and animal origins:

- Egg
- Milk
- Crustacean and molluscan shellfish (such as crab, lobster, shrimp, clams, and squid)
- Fish (such as bass, cod, flounder, and salmon)
- Legumes including peanut, soybean, and lupin
- Tree nuts (such as almonds, cashews, and walnuts)
- Seeds (such as mustard and sesame)
- Cereals containing gluten including wheat, barley, and rye

A summary of these major allergenic foods and their allergens is given in the succeeding text, and a number of databases with more extensive information are given at the end of this article.

Animal Food Allergens

Egg

The major allergens of egg originate from the white and include ovomucoid (Gal d 1) and ovalbumin (Gal d 2), which comprise 10–11% and 50–54% of egg white proteins, respectively. Both proteins are heavily glycosylated with 25% of the mass of ovomucoid comprising carbohydrate. The IgE epitopes of ovomucoid appear to be conformational in newly acquired sensitivities to egg, with linear epitopes being more important in long-standing egg allergy. The epitopes are clustered in seven regions of the protein structure and do not include the regions that are glycosylated. The IgE epitopes of ovalbumin are also resistant to enzymatic digestion and denaturation, and seven IgE-binding regions have been identified, which tend to cluster at the N- and C-terminal regions of the protein. Ovalbumin has also been found in an immunologically active form in the blood of human subjects following consumption of raw egg and can be taken up intact in cell models of the gut epithelium. In general, cooking procedures, such as boiling and baking, have been found to reduce allergenic activity of egg. Other egg white allergens, considered to be more minor, are ovotransferrin (Gal d 3) and lysozyme (Gal d 4). Two allergens have been described in egg yolk, α-livetin (Gal d 5) and a protein of unknown function, Gal d 6. α-Livetin, the serum albumin of hens, may lead to food allergy through previous aerosensitization later in life.

Cow's milk

Both the whey and casein fractions contain allergens, the most common being β-lactoglobulin and α-casein, although other IgE-reactive proteins have been identified. β-lactoglobulin is highly resistant to proteolysis and can be taken up in an intact form by the gut epithelium in cell and animal model systems. IgE epitopes have been identified in four main regions located on the more mobile surface loops of the protein. The major protein fraction of milk, the caseins, is also the major allergens. The caseins possess a loose highly hydrated tertiary structure. As a result of this highly mobile structure, which resembles that of a denatured globular protein in its native state, the allergenic activity of casein is not modified extensively by thermal treatments compared to globular proteins. Thus, linear epitopes are equally available for IgE binding in both the native and the thermally denatured states. Thus, the casein fraction appears to contain thermostable epitopes, with IgE binding being located in around seven different regions of the protein. Boiling milk for short periods of time reduces IgE binding to caseins to only a limited extent, while heating for 2–10 min results either in no difference or in a reduction of about 50–66% of the positive reactions as compared to raw milk; similar observations have been reported with homogenized and pasteurized milk. Baking appears to reduce the allergenic activity of milk, baked goods often being better tolerated in children than liquid milk. There are also indications that hard cheeses, matured for 36 months, may have reduced allergenicity in cow's milk allergic children, possibly because of proteolytic processes. The extensive homologies between mammalian milk proteins from different sources mean that individuals with allergy to cow's milk are usually allergic to goat and sheep milk, although there are instances when individuals are tolerant to cow's milk but allergic to sheep or goat milk.

Fish

The major allergen in fish is the muscle protein parvalbumin, the β-form of which is unique to fish and amphibians. It has been shown to be conserved across fish species, a factor responsible for the cross-reactive nature of allergens in cod, salmon, mackerel, herring, and plaice, among many others. Parvalbumin binds calcium in a calcium-binding motif known as an EF-hand and functions as a calcium buffer protein in fast muscle. Several different isoforms have been identified, with the β-2 isoform of parvalbumin being the form predominantly expressed in muscle tissue, while the β-1 isoform is the predominant isoform in brain tissue, although it is found in a variety of other organs including muscle. IgE-binding epitopes have been characterized in five fish species including sea fish, Atlantic cod (Gad m 1.0101), Baltic cod (Gad C 1.01), Atlantic salmon (Sal s 1.0101), Pacific mackerel (Sco j 1.0101), and the freshwater fish carp (Cyp c 1.0101) with between 3 and 4 main IgE-binding regions having been identified. Like other calcium-binding proteins, parvalbumins are heat-stable, and thus, fish flesh tends to retain its allergenicity after cooking, although in some patients, it appears that it may result in a reduction in allergenicity. The levels of parvalbumin are higher in the flesh of white fish, such as cod, and lower in the muscle of fish such as mackerel, tuna, and swordfish. In some instances, this may mean individuals allergic to white flesh fish may be able to consume fish species such as swordfish. Other potential allergens have been characterized in fish including collagen and enzymes such as aldolase and enolase. Lastly, while not a fish allergen, individuals may react to the presence of the fish parasite, *Anisakis simplex*, to which they are sensitized.

Crustacean and molluscan shellfish

Tropomyosin is the major allergen in crustacean and molluscan shellfish. Many isoforms of this ubiquitous protein have been identified and are expressed in numerous tissues in addition to muscle, reflecting the key role played by tropomyosin in contractile fibers. The proteins have been designated 'pan-allergens' having been identified in various crustacean species including shrimp, lobster, and crab as well as a range of molluscan shellfish, octopus, and squid. The homologous nature

of the proteins explains the cross-reactive allergies frequently observed between, for example, shrimps, lobsters, crab, and inhalant insect allergies, such as those observed with dust mite and cockroaches. However, such homologies do not extend to vertebrate tropomyosins, so there is no cross-reactivity between shellfish and animal muscle tropomyosins. A number of linear IgE-binding epitopes have been identified in a variety of shellfish tropomyosins, with epitopes located in the C-terminal region appearing to be crucial for IgE binding, which are not shared with vertebrate tropomyosin. Informatic analysis has identified regions of the invertebrate tropomyosin sequence, which has a lower probability of forming α-helix than is found in the corresponding vertebrate tropomyosin, suggesting this may play a role in determining their immunogenicity and hence allergenicity. Tropomyosin is a thermostable allergen, and hence, it resists the thermal treatments typically used in food processing, although there is some evidence that Maillard modifications and novel processing approaches such as ultrasound treatment may reduce allergenicity. In addition to being found in cooked meat, tropomyosin also leaches into cooking water. Other allergens identified in crustacean shellfish include arginine kinase (another pan-allergen but thermolabile), troponin C, myosin light chain, sarcoplasmic calcium-binding protein, and triosephosphate isomerase.

Plant Food Allergens

Fresh fruits and vegetables

Allergy to fresh fruits and vegetables is frequently associated with inhalant allergies to substances like birch and grass pollen and latex. After becoming sensitized to the inhalant allergens in pollen and latex, individuals subsequently go on to develop allergies to foods. This is because the foods contain proteins, which are structurally similar to those in pollen, which means IgE developed from, for example, tree pollen proteins cross-reacts to closely related homologues in fruit. Symptoms are often mild and confined to the oral cavity and are collectively known as oral allergy syndrome (OAS), although OAS can occur in any food reaction. The geographic distribution of pollen-related fruit and vegetable allergies is related to the potential exposure to the relevant pollen A.

A major group of allergens involved in such cross-reactive allergies are those belonging to the Bet v 1 family, which includes allergens from a variety of pollens, fruits, and vegetables. Some of the most important include Bet v 1 itself, the major birch pollen allergen, and homologues from Rosaceae fruits such as apple (Mal d 1), cherry (Pru av 1), and peach (Pru p 1) and other emerging allergenic fruits such as kiwi (Act d 8). Homologues are also found in exotic fruits not generally consumed in Europe, which may pose a risk such as sharon fruit and jackfruit. Vegetables have also been identified that contain allergenic Bet v 1 homologues such as celeriac (Api g 1) and carrot (Dau c 1). The IgE epitopes on Bet v 1 and its homologues tend to be conformational in nature, and consequently, food processing procedures, which cause the allergens to unfold, generally result in a loss of IgE reactivity, although this is not so for vegetables such as celeriac. A second group of IgE-cross-reactive allergens involved in pollen–fruit cross-reactive allergies are the profilins, and while found in both plants and animals, where they are thought to play a role in the polymerization of actin, only plant profilins have been described as allergens. The clinical relevance of IgE to profilins is a matter of debate, and it may be that they play an important role in eliciting clinical reactivity only in certain plant foods.

Another kind of fruit and vegetable allergy, generally found in the Mediterranean area, is characterized by much more severe, even life-threatening allergic reactions. These allergies involve the nonspecific lipid transfer proteins (LTPs) and do not seem to be associated with pollen allergy. LTPs have been characterized as allergens in fruits such as apple (Mal d 3), peach (Pru p 3), and grape (Vit v 1) as well as vegetables such as asparagus (Aspa o 1), cabbage (Bra o 3), and lettuce (Lac s 1). They belong to the prolamin superfamily and are highly resistant to gastroduodenal digestion, and while originally identified by their ability to transfer lipids *in vitro*, it seems likely they have a different function in plants. Allergenic LTPs belong to PR group 14 and are generally located in epidermal tissues, where they may function as transporters of cutin and suberin monomers, which are then polymerized to form a waxy layer. LTPs are highly resistant to thermal processing, although removing the outer layers has been shown to reduce the allergenicity of peach products.

Another group of allergens involved in fruit allergy are those involved in the latex–fruit cross-reactive allergy syndrome. This includes the class I chitinases, a group of carbohydrases with a role in protecting plants from pathogens, found widely in plants. These have been identified as allergens in foods such as avocado (Pers a 1), banana (Mus p 1), and chestnut (Cas s 1). Another type of protein involved in IgE cross-reactive allergies between foods and latex is patatin, a storage protein from potato, which, along with other proteins from avocado and banana, has also been shown to be cross-reactive with the latex allergen Hev b 7. Food processing procedures usually, but not always, reduce the allergenicity of these foods such that they can be safely consumed by individuals with fruit/vegetable–latex allergies (Table 1).

Many other proteins have been implicated in allergies to fresh fruits and vegetables and include cupin allergens found in fruit seeds, the highly disulfide-bonded thaumatin-like proteins (TLPs), C-proteases, and a variety of lectins and Kunitz inhibitors. Other less widely found allergens include the flavin adenine dinucleotide-containing oxidase allergen of celery, Api g 5, a M_r 53–57 kDa protein, which is extensively glycosylated, which possesses cross-reactive glycans and a germin-like protein, which has been identified in bell pepper. Many have antifungal and/or antibacterial activity and therefore may have a role in plant protection. Examples of important emerging allergens in fruit include 13 allergens including a TLP (Act d 2), the thiol protease actinidin (Act d 1), and cupin and 2S albumin seed storage proteins found in the seeds of kiwi (Act d 12 and Act d 13, respectively).

Legumes

Allergenic legumes contain many of the same types of allergen that are found in fresh fruits and vegetables, such as allergenic homologues of Bet v 1, which have been identified as allergens in soybean (Gly m 4) and peanut (Ara h 8) together with LTP allergens in peanut (Ara h 9). Other members of the prolamin superfamily, the 2S albumins, are also major allergens in peanut (Ara h 2, 6, and 7), with some evidence that they are allergens in

Table 1 Summary of the attributes of major food allergen families

Protein family	*Structural attributes*	*Biological properties*	*Types of foods*	*Effects of processing*
Animal food allergens				
Tropomyosins	Two-stranded proteins containing 40 uninterrupted heptapeptide repeats that form α-helical coiled coils and form head-to-tail polymers along the length of an actin filament	Regulation of muscle contraction through interactions with actin and myosin	Crustacean and molluscan shellfish	Generally heat-stable and cross-reactive between the various crustacean and molluscan species
E-F hand	The EF-hand motif comprises a loop of 12 amino acid residues flanked on either side by a 12-residue α-helical domain. Allergenic fish β-parvalbumins comprise three EF-hands, two of which bind calcium	Present in high levels in the white muscle of many fish species, it plays a role as a calcium buffer. By binding myoplasmic calcium during contractions, β-parvalbumin reduces the calcium concentration, thus enhancing relaxation	Fish	Holo parvalbumin (which has bound calcium ions) possesses a remarkable stability to heat denaturation unlike the apo form, which is more thermolabile
Caseins	Structurally mobile proteins, caseins comprise several different types, αs1-, αs2-, β- and κ-caseins. αs1-, αs2-, and β-caseins bind calcium through clusters of phosphoserine and/or phosphothreonine residues, forming nanoclusters, which are assembled into the casein micelles found in milk, which are in turn stabilized by κ-casein	Protein source for mammalian off spring and the calcium-binding nanoclusters allow calcium levels in milk to exceed the solubility limit of calcium phosphate	Mammalian milks	The molecular mobility of caseins is not greatly altered by thermal processing and hence IgE epitopes tend to be thermostable
Plant food allergens				
Prolamin superfamily	Contains a characteristic core of eight cysteine residues including a characteristic Cys–Cys and Cys–X–Cys motif (X representing any other residue)			
2S albumins	α-Helical proteins where the pattern of disulfide bonds formed by the core cysteine motif is arranged to give a compact molecule without a lipid-binding tunnel. Synthesized as a single polypeptide chain, they are usually cleaved by proteases posttranslationally to form a large and a small subunit linked by disulfide bonds	Seed storage proteins, which may have additional roles in plant protection	Seeds, including those of legumes (including peanut), and tree nuts	Resistant to thermal processing
Lipid transfer proteins	α-Helical proteins where the pattern of disulfide bonds formed by the core cysteine motif is arranged such that a central lipid-binding tunnel is formed	Diverse functions but allergenic forms are found in epidermal tissues and may be involved in the transport of cupin monomers to the outer layers of fruits and seeds	Fresh fruit and vegetables, seeds, including legumes and cereals, and tree nuts	Resistant to thermal processing
Cereal inhibitors of α-amylase and trypsin	Contain two additional cysteine residues in addition to the conserved core cysteine motif	Inhibit amylases and proteases secreted by pests	Cereal grains	Resistant to thermal processing
Prolamin seed storage proteins	The core cysteine motif is disrupted by the insertion of a repetitive domain comprising motifs rich in proline and glutamine. The repeat motifs contain the celiac toxic motifs	Seed storage proteins	Cereal grains from the Triticeae	The repetitive domain is mobile and is unaffected by heating. Consequently, thermal processing does not modify IgE binding to epitopes located in these regions

(*Continued*)

Table 1 (Continued)

Protein family	*Structural attributes*	*Biological properties*	*Types of foods*	*Effects of processing*
Cupins	β-Sheet-rich proteins, which form a characteristic β-barrel structure			
7S seed storage globulins	Bicupin trimeric proteins, which are usually glycosylated	Seed storage proteins	Seeds, including those of legumes (including peanut), and tree nuts	Thermostable proteins, which have a tendency to form large aggregates following heating
11S seed storage globulins	Bicupin hexameric proteins comprising subunits, which are synthesized as single polypeptides, which are posttranslationally cleaved into an acidic and basic polypeptide linked by an intramolecular disulfide bond	Seed storage proteins	Seeds, including those of legumes (including peanut), and tree nuts	Thermostable proteins, which have a tendency to form large aggregates following heating
Bet v 1 family	A β-sheet-rich protein with a central lipid-binding tunnel	Has a role in plant protection through its capacity to bind plant sterols	Fresh fruit and vegetables, seeds, including legumes and cereals, and tree nuts	The scaffold is thermostable but the protein is unstable in certain plant tissues such as apple, such that its allergenic activity is lost following food processing

soy (Gly m 8) and chickpea. The 2S albumins are usually synthesized in the seed as a single polypeptide chain and then undergo posttranslational processing to yield a small and a large polypeptide chain joined by an intramolecular disulfide bond. The 2S albumins, like the other members of the prolamin superfamily, are highly resistant to thermal processing and are relatively resistant to simulated gastrointestinal proteolysis.

The other major allergens of legumes are the seed storage globulins belonging to the cupin superfamily, including the 7S seed storage globulin of soybean (β-conglycinin; Gly m 5), peanut (Ara h 1), lentil (Len c 1), and pea (Pis s 1) together with the 11S seed storage globulins of soybean (glycinin; Gly m 6) and peanut (Ara h 3). The 11S globulins, sometimes termed legumins, are hexameric proteins of M_r ~300 000–450 000. Each subunit is synthesized in the seed as a single chain of M_r about 60 000, which is posttranslationally processed to give rise to acidic (M_r about 40 000) and basic (M_r about 20 000) chains, which are linked by a single disulfide bond and are rarely, if ever, glycosylated. The 7/8S globulins, also termed vicilins, are somewhat simpler, comprising three subunits of M_r ~40 000–80 000, but typically about 50 000. There is evidence that there is IgE cross-reactivity between the different cupin allergens as a consequence of sequence homology between the proteins. Thus, serum IgE from peanut allergic individuals reacted with both Ara h 1 from peanut and conglutin-β, the 7S globulin from lupin, Lup an 1, reflecting the significant sequence homology between the two proteins. Such cross-reactivity explains clinical observations that individuals with peanut allergy can react to foods containing lupin as a hidden ingredient.

The 7S and 11S globulins are also relatively resistant to thermal processing but are susceptible to pepsinolysis, although a number of lower-molecular-weight polypeptides appear to persist following digestion and retain their IgE-binding capacity.

Other soybean allergens that have been identified include a Kunitz trypsin inhibitor and a member of the cysteine protease family, known as Gly m 1 (Glym Bd 30k). Another soybean allergen, which is of relevance in countries such as Japan, is the M_r 23 kDa protein known as Gly m 28k, which is glycosylated and contains important IgE-reactive glycans also found in a derived 23 kDa peptide. Lastly, allergenic oleosins have been identified in peanut (Ara h 10, 11) together with a lectin, peanut agglutinin. The oleosins are associated with oil bodies where they play an important role in packaging and stabilizing the oil droplet surface, having a portion of the protein structure buried in the oil phase with a second domain on the aqueous facing surface. They may be relevant when considering the allergenicity of edible oils, as they pose a risk of eliciting an allergic reaction, if they find their way into crudely refined oil, which, unlike highly refined oils, has sufficient protein to trigger allergic reactions.

Tree nuts and seeds

Allergenic Bet v 1 homologues have been identified in various nuts and seeds including Cor a 1.04 from hazelnut, while several allergenic LTPs have also been found in nuts and seeds, including those from walnut (Jug r 3) and hazelnut (Cor a 8). However, the major allergens in tree nuts are similar to those found in legume seeds and include other members of the prolamin superfamily, the 2S albumins and the cupin seed globulins. As for legumes, many tree nut and seed 2S albumin allergens comprise two polypeptide chains, including those from Brazil nut (Ber e 1), walnut (Jug r 1), cashew nut (Ana o 3), and almond as well as the seeds of oriental and yellow mustard (Bra j 1 and Sin a 1) and sesame (Ses i 1 and 2). However, the allergenic 2S albumin from sunflower, SFA-8, is a single chain albumin. In many nuts and seeds, both 11S and 7S seed storage globulins have been identified as allergens in tree nuts such as hazelnut (Cor a 11 [7S globulin] and Cor a 9 [11S

globulin]), cashew nut (Ana o 1 [7S globulin] and Ana o2 [11S globulin]), walnut (Jug r 2[7S globulin] and Jug r 4 [11S globulin]), and sesame (Ses i 3 [7S globulin] and Ses 4,5 [11S globulin]), while only the 11S globulins have been identified as an allergen in almond, also known as almond major protein AMP (Pru du 6), and mustard (Sin a 2). Other minor allergens include the oleosins that have been identified as allergens in sesame (Ses i 4,5) and hazelnut (Cor a 12,13).

Cereals

In addition to their role in triggering CD, cereal proteins can cause IgE-mediated allergies, although allergies to wheat products do not appear to be as widespread as allergies to other plant-derived foods such peanut and tree nuts. Thus, the seed storage prolamins of cereals, which can form gluten, have also been characterized as causing IgE-mediated allergies and in particular a condition known as food-dependent exercise-induced anaphylaxis. This is a severe allergic reaction that certain patients experience only if they exercise after eating a problematic food. Allergens involved in this condition include γ- (Tri a 20), α-, and ω-5 (Tri a 19) gliadins. Other seed storage prolamins have also been identified as major cereal allergens, including both the polymeric HMW and LMW subunits of glutenin, as well as the monomeric γ- and an α-gliadins. Cooking appears to affect allergenicity, and one study suggested baking may be essential for allergenicity of cereal prolamins.

Other wheat seed proteins have been implicated as food allergens including several members of the prolamin superfamily. Thus, a number of trypsin/α-amylase inhibitors have been described as allergens, one of which corresponds to a trypsin/α-amylase inhibitor, known as CM3, sensitization to which has been implicated as a cause of atopic dermatitis. Homologous proteins from other cereals have also been shown to be allergens including a M_r 16 000 barley protein found in beer and a M_r 16 000 protein allergen from maize. A number of α-amylase inhibitors with M_r of about 14 000–16 000 including one M_r ~16 000 subunit, termed RA 17, have been described as allergens in rice. Other prolamin superfamily allergens identified in cereals include the LTPs from maize (Zea m 14), spelt, and wheat (Tri a 14). While LTPs are generally resistant to food processing cooking with maize LTP retaining its allergenic activity in cooked foods like polenta, barley LTP retains its allergenicity even after brewing.

Managing Allergens in Foods

In order to support food allergic individuals practicing food avoidance, labeling of certain priority allergenic foods has been made mandatory in many parts of the world, irrespective of the level of inclusion in a recipe. This has been extended in some jurisdictions, such as the EU, from prepacked foods to those sold loose or in catered foods. In some instances, precautionary 'may contain' labels are also applied to warn customers as to the possibility of unintended presence of certain allergenic foods. Such precautionary labels are unregulated, and while best practice guidance is available, it is not always clear how they are applied and can be confusing and difficult for individuals with food allergies to interpret. Approaches are being developed and implemented to effectively manage food allergens in a factory environment, but key pieces of information and tools are still lacking. In particular, identifying levels of food allergens that represent minimal risk of eliciting an allergic reaction would help support allergen management practices. Tools are still lacking that can be effectively used to analyze allergens in foods, both to validate cleaning protocols applied in manufacturing lines and to confirm the absence of allergen residues in finished products. Methodologies have been developed for monitoring allergen levels in products, especially peanuts using immunoassays, particularly enzyme-linked immunosorbent assays, with new methods based on mass spectrometry being developed. Such technology will help support improved ways of managing allergens in factory environments, which will help to minimize the use of precautionary allergen labeling in future.

See also: Browning: Non-enzymatic browning; Casein and Caseinate: Methods of Manufacture; Cashew Nuts; Cereals: Dietary Importance; Condensed Milk; Dairy Products: Dietary and Medical Importance; Eggs: Composition and Health Effects; Eggs: Use in the Food Industry; Fish: Dietary Importance and Health Effects; Fish: Fish in the Human Diet; Fish: Processing; Food Allergies; Goat: Milk; Lupine; Milk Powder; Milk: Processing of Milk; Milk: Role in the Diet; Milk: Sources and Composition; Mustard; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Nuts: Brazil Nuts; Nuts: Health Effects; Peanuts; Protein: Food Sources; Sheep: Milk; Shellfish: Characteristics of Crustaceans and Mollusks; Shellfish: Role in the diet; Soy Beans: Dietary Importance; Soy Beans: Processing; Soy Beans: Properties and Analysis; Soy Beans: The Crop; Wheat: Grain Structure of Wheat and Wheat-based Products; Whey and Whey Powders: Fermentation of Whey; Whey and Whey Powders, Principles and Applications of Dialysis; Whey and Whey Powders: Production and Uses; Whey and Whey Powders: Protein Concentrates and Fractions.

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Aluminum: The Toxicology of

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A Brief History of Recognition of Aluminum as a Toxicant

Aluminum compounds have been used for millennia. The discovery of aluminum (Al) as an element was made in the early 1800s, when it was first isolated as a pure metal. The Hall–Héroult process to isolate elemental Al, invented in the 1880s, enabled inexpensive production of metallic Al. It became widely available in pots and pans in the early twentieth century. Some published their assurance that this was safe. However, by the late 1800s, adverse opinions had been expressed about its use in food storage and surgical applications. Near the end of the nineteenth century, it was demonstrated that Al had the potential to be toxic to the central nervous system. In the early twentieth century, it was recognized that some of Al orally consumed as Al salts is absorbed and distributed throughout the body, and the use of alum (potassium aluminum sulfate) baking powder, based on results of studies, was claimed to be dangerous. A single case report in 1921 of a 46-year-old in England who had been dipping hot metal articles in concentrated nitric acid and was experiencing persistent vomiting, slow and irregular pulse, loss of memory, tremor, jerking movements, and impaired coordination, and whose urine contained a large amount of Al, was attributed to Al poisoning. Although some, but not all, of his signs are consistent with Al intoxication, one cannot draw any firm conclusion from such uncontrolled observations. The highest documented levels of Al inhalation, as flake powder used in explosives by Germans during World War II, led to a fibrotic lung disease (aluminosis). The textbook used by the author's pharmacology course (copyright 1965) assured "Large doses of soluble [aluminum] compounds taken orally produce . . . no systemic effects. Inhalation of dusts of aluminum metals or aluminum oxides leads to no observable toxic effects in animals or man. No entity 'chronic aluminum poisoning' has been identified in human beings." This benign view of Al toxicity started to be changed in the 1970s with two observations. In 1972, patients receiving hemodialysis developed what was later termed the dialysis encephalopathy syndrome (DES), which was shown to be due to Al exposure from contaminated dialysis fluid and consumption of Al-containing phosphate binders. In 1973, a report of greater Al in the brains of victims of Alzheimer's disease (AD) led to the hypothesis that Al is a contributor to this neurodegenerative condition. The aforementioned and other exposures to Al that have resulted in toxicity are discussed in more detail in the succeeding text, clearly demonstrating the potential for Al to toxic. Aluminum has no demonstrated essential function in humans, so one might consider that its exposure only presents a possible downside. However, it is imperative to recognize that, as the father of toxicology, Paracelsus, taught us, "All things are poisons, for there is nothing without poisonous qualities. It is only the dose which makes a thing poison."

Reported Toxic Effects of Aluminum

There have been many studies ascertaining and demonstrating toxic effects produced by Al. This article focuses on results reported from *in vivo* studies of Al's effects because the intact animal and human include the many processes that can influence response to chemical exposure, such as uptake into, distribution within, biotransformation in, and excretion from the organism. It focuses on results obtained from *in vitro* studies for descriptions of the mechanisms of the effects of Al, because molecular response is better studied in reductionist systems such as single cells. It focuses on the toxicity of Al relevant to humans and therefore does not specifically discuss Al toxicity to plants or nonhuman animals.

Many studies have been conducted in animals that have demonstrated effects produced by Al, but the relevance of the results from many of these studies to humans are questionable due to the design of the studies. It is a classical approach in toxicology and experimental pathology, when assessing potential adverse effects, to use high-dose exposures to elucidate the potential effects. Exposure doses are often orders of magnitude greater than anticipated for humans. For example, based on results of a good laboratory practice conducted, double-blind, vehicle-controlled study in which rats were exposed to one of three levels of Al from *in utero* through 1 year after birth in the drinking water, the authors concluded that "concentrations of aluminum in the drinking water that are required to produce minimally detectable neurobiological effects in the rat are about 10,000 times higher than what is typically found in potable drinking water." This raises the question of the relevance of findings of many studies that utilized very large oral Al exposures. To determine if observed effects are relevant to humans, studies subsequent to the initial studies that utilize very large doses should then be conducted with doses that might relate to human exposure and multiple doses, ideally including sufficiently low doses that produce no statistically significant effects, to be able to define the boundary between exposures that produce adverse effects and those that do not. Unfortunately, many of the reported studies do not meet these criteria. Most animal studies employed exposure doses well beyond what humans would experience. Many employed single- or short-duration exposures. A justification may be that the experimental subjects (most often rats or mice) have a much shorter life span than humans, so when extrapolated to humans, the short exposure becomes a significant percentage of the human life span, for example, 1 year in a laboratory rat's life span = ~35 years of the developed world's human life span. Another shortcoming of many animal studies is that only a single dose was studied. Further limitations to many of these studies are the observation of effects at only a single time (as it is well known that short-term and long-term responses can be very different) and the use of exposure routes that are not common for humans (such as intraperitoneal injection).

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The route of Al exposure and a person's renal function status greatly affect the potential for Al-induced toxicity. As discussed elsewhere in this encyclopedia, uptake of Al into circulating blood (bioavailability) from oral consumption is ~0.1–0.4%. In contrast, in intravenous Al introduction, as might occur when Al-contaminated medications or parenteral nutrition (PN) (intravenous feeding) are given by that route, all of the Al is administered into circulating blood, creating a much greater potential for toxicity. Bioavailability from the lungs (1.5–2%) is intermediate. Aluminum injected into muscle, in vaccines, is slowly absorbed and may ultimately be 100% absorbed over weeks to months. The kidneys are responsible for eliminating >95% of Al from the body. Reduced, or absent, renal function compromises the body's ability to eliminate Al, creating the potential to accumulate more Al and developing toxicity. Aluminum contamination of dialysis fluids used in hemodialysis presents Al on the opposite side of the membrane used in dialysis, across which Al readily diffuses, and is then complexed by transferrin in the blood. This can result in Al loading of the dialysis patient, who, being unable to readily eliminate Al from the poorly or nonfunctioning kidney(s), accumulates it, to potentially develop sufficient Al levels in the body to be toxic, the DES, discussed later. As good kidney function protects against Al accumulation, when asked by someone if they should be concerned about Al poisoning, the author usually assures them that if they have good kidney function and have typical Al exposure (e.g., they are not receiving Al-contaminated solutions intravenously), they should not worry.

Aluminum Toxicity to the Nervous System

The Dialysis Encephalopathy (Dementia) Syndrome

After hemodialysis became extensively practiced in the early 1970s, some patients developed a slowly progressing encephalopathy that was fatal within 6 months, characterized by dysarthria (a motor speech disorder in which the mouth, face, and respiratory system muscles may become weak, move slowly, or not move at all), myoclonus (sudden, involuntary jerking of a muscle or group of muscles), mental changes, and hallucinations. Symptoms were intermittent and often transiently worsened immediately following dialysis. Increased Al was found in the brain and other organs of affected patients, which was attributed to Al contamination of the dialysis fluids and administration of Al-based phosphate binders. Aluminum in dialysis fluids can diffuse across the dialysis membrane into the patient's blood, where it very strongly binds to transferrin and can then be transported into tissues. Elevated phosphate is a significant problem in chronic dialysis patients, and dialysis does not very effectively remove it. Orally consumed Al, which is poorly absorbed, forms very insoluble Al phosphate in the gastrointestinal tract, enhancing fecal phosphate elimination. As dialysis patients lack good renal function, and therefore good ability to eliminate Al, they accumulate Al, leading to the DES and osteomalacia and anemia, which are discussed later. Implementation of a guideline that limits the Al concentration in dialysis fluids to $<10\ \mu g\ l^{-1}$ has eliminated reports of the DES, except in isolated instances when dialysate Al was inadvertently much higher.

Aluminum and Alzheimer's Disease

The typical neuropathologic signs of AD are neurofibrillary tangles (NFTs), senile plaques (SPs), and cerebrovascular amyloid. Early-onset AD usually has a familial link and accounts for 5–15% of the cases. No specific gene mutations have been associated with late-onset/sporadic forms of AD, which account for the remainder of AD cases. The lack of identified hereditary links for the majority of AD cases suggests environmental factors, such as Al, are likely to interact with other factors to cause this disease. Injection of Al into the brain of rabbits produces a neurofibrillary degeneration that has some similarities, but is not identical, to the NFTs of AD. The neuropathology of the DES is different from that seen in AD. A causative role for Al in AD was suggested in the early 1970s by the observation of elevated Al in the brains of AD victims, determined by graphite furnace atomic absorption spectroscopy. This was followed by many studies, some of which found up to a few-fold higher Al in the brains of AD victims than in controls and some of which did not. Many studies with microprobe techniques that are able to determine the Al concentration within a cell, NFT, or SP, as well as Al-selective stains, have also produced mixed results. Elevated Al in the AD brain would not prove that it causes AD, as the neuronal degeneration of AD may result in Al accumulation.

Many epidemiological studies have been conducted to compare the incidence of AD and other dementias in relation to the concentration of Al in drinking water. The results of some of these studies show a positive correlation; the results of some do not. However, focussing on drinking water as an Al source that might contribute to AD may be ignoring the primary source of Al for typical humans, given that ~95% of oral Al consumption comes from foods and the absorption of Al from foods is not greatly different than that from water. Only a few, generally small, published studies have addressed a potential association between Al in food and AD. A small epidemiological study showed a significantly increased incidence of AD associated with consumption of pancakes, waffles, biscuits, muffins, corn bread, and corn tortillas. These are baked goods that often have higher Al amounts than other foods due to the use of acidic sodium aluminum phosphate to make the baked goods rise. Dietary intake of Al was shown to be higher among Chinese with AD than controls. Aluminum absorption was greater among those with AD than those without. These do not demonstrate a cause–effect relationship, but like many of the animal studies, suggest there may be a link that requires more rigorous investigation. The lack of AD pathology in DES victims, lack of increased progression of DES survivors to develop AD, and lack of epidemiological studies demonstrating a positive relationship between occupational Al exposure and AD are some of the observations that have convinced most of the lack of a significant role of Al in the etiology of AD. In the opinion of this author, until all of the causative factors contributing to AD are identified, it will be very difficult to totally dismiss Al as a contributor.

Aluminum Neurobehavioral Toxicity

Many animal studies have demonstrated the ability of Al to produce neurobehavioral toxicity. Increased age has been

shown to increase susceptibility. However, as noted in the preceding text, animal studies have generally used Al doses, and sometimes routes of administration, that do not directly relate to human exposure. Such studies create models to study Al-induced toxicity and can be used by risk assessors to set exposure limits for regulatory purposes, but it is difficult to predict human response to Al from the results. Numerous studies have shown reductions in performance of many neurobehavioral and psychometric tests among those occupationally exposed to Al, such as Al foundry and production workers but particularly Al welders. Performance decrements relate well to urine Al concentration.

Aluminum Toxicity to the Musculoskeletal System

Aluminum-Induced Bone Disease

Aluminum accumulation in bone can produce a low-turnover bone disease, manifested as osteomalacia (softening of the bones due to a defect in the bone-building process) and an adynamic bone disease (characterized by a reduction of the cells that resorb bone tissues (osteoclasts) and cells that deposit new bone tissue (osteoblasts), resulting in markedly reduced bone turnover). Patients with Al toxicity who have this condition may complain of generalized bone and joint pain and muscle weakness. Aluminum interferes with parathyroid hormone synthesis and function. In osteomalacia, Al is localized at the mineralization front, where it disrupts bone mineralization by inhibiting calcium accumulation and osteoblast activity and increases bone resorption, which results in an increase of nonmineralized bone that can lead to painful fractures. In adynamic bone disease, Al decreases osteoid (a fibrous protein matrix) formation and bone mineralization (deposition of calcium into the matrix). The joint symptoms may reflect Al deposition within the joint. High Al concentrations have been observed in the synovial fluid of patients taking Al-containing phosphate binders, perhaps reflecting a general increase of Al in their bodies.

Macrophagic myofasciitis is a rare inflammation of muscle tissues manifested as a delayed onset of diffuse muscle pain and muscle weakness at the site of the intramuscular injection of Al hydroxide-containing vaccines. It is accompanied by fever, chronic fatigue, and cognitive dysfunction (memory impairment, difficulties maintaining attention, and mood disturbances). It is accompanied by formation of Al-loaded macrophages at the injection site that form a granuloma. Genetic susceptibility has been suggested to contribute to this and other similar adverse outcomes of Al-containing vaccines.

Aluminum Toxicity to the Hematopoietic System

A microcytic (small red blood cell) hypochromic (reduced hemoglobin content) anemia can occur in patients who have compromised or no renal function and have prolonged exposure to excessive Al via dialysis fluids or who consume large amounts of Al-based phosphate binders. Anemia may precede or occur with lower Al exposures than Al-induced osteomalacia or encephalopathy. This anemia is associated with Al accumulation in the bone marrow, is resistant to iron, and is due to impaired red blood cell integrity, disruption of a step in the hemoglobin synthesis pathway, and reduced iron absorption and delivery to cells. Al-induced anemia has been largely avoided by the use of non-Al-based phosphate binders and maintenance of Al concentration in dialysis fluids at $< 10\ \mu g\ l^{-1}$. Anemia has also been reported among those who practice ancestral geophagy (consumption of clay, which is primarily aluminum and calcium silicate), illustrating the ability of long-term high-dose Al consumption to induce anemia.

Aluminum Toxicity to the Respiratory System

Occupational exposure to high levels of Al has been associated with lung fibrosis from stamped Al powder, asthma from Al salts and welding fumes, and increased oxidative stress.

Exposure to Al-containing minerals that contain silica, such as bauxite (the primary natural source of Al), can lead to lung fibrosis (Shaver's disease). Stamped pure Al powder can also lead to a lung fibrosis (aluminosis) manifested as coughing, fibrosis, lung atrophy, and secondary emphysema.

Aluminum Toxicity to the Reproductive System

Daily intraperitoneal injection of Al (as the nitrate) to male mice before mating reduced the pregnancy rate and sperm counts and produced histological changes in the males when the daily dose was $100\ mg\ kg^{-1}$ (equivalent to $7\ mg\ kg^{-1}$ Al) or greater. Absorption of soluble salts, such as Al nitrate, from the peritoneal cavity is generally complete after intraperitoneal injection. The potential for Al to produce reproductive toxicity was assessed in two-generation studies in rats comparing three concentrations of Al sulfate or Al ammonium sulfate in their drinking water before and during mating and for the females, throughout pregnancy. Based on the observed reduced body weight gain and delayed sexual development in the first-generation female offspring, the lowest-observed-adverse-effect levels for Al sulfate and Al ammonium sulfate were determined to be 31 and $36\ mg\ kg^{-1}\ day^{-1}$, respectively, of Al. This is 200–500-fold greater than the typical daily oral Al intake by humans of $5–10\ mg\ day^{-1}$, which results in $< 1\%$ absorption of Al. These studies suggest reproductive toxicity from Al would not be expected from typical Al intake or intake that is several orders of magnitude greater.

Embryotoxic, Teratogenic, and Developmental Aluminum Toxicity

Oral administration of Al hydroxide to rats at doses far exceeding human Al intake did not produce significant embryotoxicity or teratogenicity. Addition of citrate, which increases Al absorption, resulted in delayed ossification, skeletal variations, and cleft palate, but the amount of Al was $\sim$1000-fold greater than typical daily Al intake. Intraperitoneal injection of Al chloride to male mice for 2 weeks produced reversible reduced fertility, decreased testes weight, and spermatogenic impairment. There were greater postimplantation losses, fetal mortality, bleeding, and decreased body weight, but no fetal abnormalities, in the

offspring. These effects were seen at doses 100-fold or greater than the typical daily Al intake, given by a route that probably eventually results in 100% absorption. Prenatal stress during intraperitoneal Al injections resulted in skeletal anomalies and defects, but this was not seen when Al was given orally.

Studies in mice that consumed a diet containing Al lactate from conception throughout lactation showed increased grip strength, which was not Al exposure-level-dependent, whereas some other studies showed reduced grip strength or no effect. A double-blind study was conducted following good laboratory practice. It exposed rats to 30, 100, or 300 mg kg^{-1} day of Al, as the citrate, in their drinking water from day 6 of gestation and to 1 year after birth and assessed multiple behavioral, cognitive function, clinical chemistry, hematology, and neuropathology endpoints. Renal pathology, impaired grip strength, and splayfoot were observed. Many end points showed no effect of the Al intake. The authors concluded that "concentrations of aluminum in the drinking water that are required to produce minimally detectable neurobiological effects in the rat are about 10,000 times higher than what is typically found in potable drinking water." A two-generation study conducted in rats given 50, 500, or 5000 ppm Al in their drinking water measured many end points of reproductive activity, offspring viability, histology, and behavior. The no-observed-adverse-effect level was calculated to be 5.35 mg kg^{-1} day^{-1} or ~50–75-fold greater than the typical daily oral Al intake. The results of these studies suggest a lack of concern for oral Al intake during pregnancy and lactation with the possible exception to avoid massive Al consumption during pregnancy.

On the other hand, intravenous administration introduces Al into the blood, ~300–1000-fold more efficiently than when taken orally, presenting a much greater risk. When this occurs in the presence of reduced ability to eliminate Al, as occurs due to the Al contamination of PN solutions given to premature infants, it presents an even greater risk. One manifestation is the potential for Al-induced developmental delay. This was demonstrated in 18-month-olds who received standard PN solutions as premature infants compared with age-matched infants who received PN solutions containing less Al, as discussed in section '**Aluminum Contamination of Intravenous Solutions**.'

Aluminum and Autism Spectrum Disorder

There has been an increase in the number of immunizations received by preschool children in the United States and diagnosis of autism spectrum disorders. The role of Al as an adjuvant in vaccines is discussed in section '**Aluminum as an Adjuvant**.' Although some have suggested that there is a cause and effect relationship between these two observations and shown positive correlation between these two increases, there is little peer-reviewed published literature supporting this relationship. The World Health Organization's Global Advisory Committee on Vaccine Safety, the US FDA, and Health Canada do not state a concern about this possible relationship. Like the proposed relationship between Al and AD, until the cause or, most likely, contributing factors to autism spectrum disorder are definitively identified, this controversy will not be resolved to the satisfaction of those who are impacted by these and similar neurodegenerative and neurobehavioral diseases and disorders.

Aluminum Toxicity to the Dermal System (Skin)

Application of Al to the skin can produce irritation, which is dependent on the chemical form (species) of Al. An animal study showed 10% Al chloride and nitrate produced epidermal changes and damage, whereas Al chlorhydrate (as used in antiperspirants), sulfate, hydroxide suspension, and basic acetate suspension did not. There is little documentation of skin irritation from occupational Al exposure. Contact sensitivity to Al can manifest as irritation, soreness, and recurrent eczema from topical antiperspirant application, which is rare, and as sensitization from Al injection (see section '**Aluminum as an Adjuvant**'). Contact sensitivity can be diagnosed using patch testing.

Aluminum Toxicity to the Liver

Numerous animal studies have shown the development of Al-induced effects indicative of liver toxicity. The effects included an increase in the blood of enzymes that are released from damaged liver cells (ALT and AST), increased triglyceride and cholesterol in the liver indicative of fatty degeneration, and increased lipid peroxidation and decreased catalase (an enzyme that catalyzes hydrogen peroxide, thereby protecting against oxidative damage), which indicate oxidative stress and organ damage. However, there are few reports of significant liver toxicity in humans. A population that is highly susceptible to Al toxicity, parenterally fed premature neonates, is prone to cholestatic hepatitis (reduced bile flow and occasionally gallstones). Elevated Al in the PN solutions (see section '**Aluminum Contamination of Intravenous Solutions**') has been associated with this, but the role of Al and other contributors to cholestatic hepatitis in this population has not been well determined.

Aluminum as an Adjuvant

Aluminum is used as an adjuvant in vaccines and hyposensitization treatments to adsorb/precipitate toxins and toxoids, to enhance their antigenic properties, and to reduce their rate of absorption from the injection site and elimination from the body. Injection of Al as an adjuvant probably induces immune activation. When injected, Al can produce immune system induction (which enhances the toxoid response), inflammation, accumulation of macrophages at the injection site, and formation of granulomas (containing macrophages and other cells, which become persistent itching nodules in ~1% of children receiving Al-adsorbed vaccines) and sterile abscesses at the injection site. These responses are more common with Al hydroxide than Al phosphate adjuvants and more common with subcutaneous than intramuscular injection.

Aluminum and Cancer

In vitro studies have shown the ability of Al to bind to DNA and to induce DNA damage and inhibit repair of radiation-induced

DNA lesions. It has been suggested that the underarm use of Al-containing antiperspirants increases the risk of breast cancer in women. However, several epidemiological studies have not found support for this suggestion. There is no good evidence of increased cancer incidence among workers occupationally exposed to Al, in the absence of known cancer risk factors. On the other hand, an increased incidence of cancer among Al production workers has been well demonstrated, which is believed to be due to non-Al compounds, particularly polycyclic aromatic hydrocarbons, some of which are known carcinogens.

Exposures Leading to Aluminum Toxicity

Aluminum Contamination of Intravenous Solutions

The population that is perhaps the most susceptible to Al accumulation and toxicity is premature infants who receive their nutrients and fluids intravenously, for example, who receive their nutrients and fluids intravenously as PN. Premature infants have immaturely developed kidneys and a high requirement for calcium for their developing skeletal system. One of the components of PN solutions is calcium, generally introduced into the PN solution as calcium gluconate. Calcium gluconate is routinely contaminated with Al, due to its storage in glass vials that contain 2–5% Al. The gluconate ion binds Al well, leaching it out of the vial over time. Aluminum accumulation in this population has been shown to cause cognitive deficits and reduced bone density and may contribute to a rickets-like condition and liver problems. The US Food and Drug Administration adopted a labeling requirement for Al in large- and small-volume parenterals used to prepare total PN solutions. This has not solved the problem because it does not inform the user of the actual Al concentration in the small-volume parenteral product at the time of its use nor demand a reduction of Al in these parenteral products.

The preparation of drugs in Al vessels for intravenous self-administration by recreational drug users and inhalation of heroin volatilized from Al foil has resulted in the signs observed in dialysis encephalopathy and elevated Al in the body and urine.

Nonintravenous Al Introduction

Implanted medical devices (prosthetics) containing Al

There are a few reports of dialysis encephalopathy-like signs and elevated brain Al following introduction of an Al-containing cement into the brain that came into contact with the cerebrospinal fluid (CSF), presumably resulting in dissolution of Al from the cement and, via the CSF, its uptake into the brain.

Bladder irrigation

Severe hemorrhagic cystitis (blood in the urine), caused by radiation, chemotherapy, cancer, or other conditions, has occasionally been treated with 1% alum irrigation (an astringent). Some patients with renal insufficiency or failure developed Al-induced encephalopathy. This illustrates the potential for massive amounts of Al administered acutely to disrupt brain function.

Occupational Exposure to Aerosolized Al

Occupational Al exposure can occur during refining and welding and in industries that use Al products and can result in exposure to airborne Al concentrations 1000-fold greater than the general population. The highest occupational Al exposure occurs for those engaged in Al powder production and Al welding.

Asthma and reversible bronchial obstruction are fairly common among those engaged in Al production (in smelters, termed potroom asthma) and production of Al salts, usually due to exposure to irritant airborne particulates and fluoride fumes.

Food and Beverages

The main source of Al intake for typical humans is the diet. Aluminum, being ubiquitous and a major component of the Earth's crust, is taken up into plants. Numerous plants, such as the tea plant, are hyperaccumulators of Al, depositing it into the leaves and other plant parts. For those who consume considerable tea, this beverage can provide up to 50% of a typical human's daily dietary Al intake. For others, a significant source of Al in the diet is added Al salts, as approved food additives, such as acidic and basic sodium aluminum phosphate (used as a leavening agent in baked goods and emulsifier in cheese, respectively) and sodium aluminosilicate (used as an anticaking agent in salt and artificial dairy creamer), among many other Al salts and applications. Although much attention has been paid to the potential link between Al in drinking water and AD and other dementias (see preceding text), drinking water typically contributes only a few percentage of the daily intake of Al in foods and beverages.

Nanoscale Al

There is much interest in nanoscale materials, including Al nanoparticles. As novel nanomaterials are being developed, some are being tested for their potential toxicity. Uptake by the lungs is the route of most concern for unintended exposures. The amount of Al nanomaterial that reaches the lungs influences the magnitude of the response. Most airborne nanomaterials form agglomerates with themselves or other airborne particles so that the size of the particles taken up is greater than their primary size, which influences how deeply they enter the lungs. The most common adverse effect of NPs is oxidative stress and inflammation. Shape and surface area are major factors influencing nanomaterial effects. As with other fiber-like materials, there is increased toxicity as the aspect ratio (length/diameter) of Al nanorods increases. The distribution of Al nanomaterials out of the lungs to other organs is very low, as is generally seen with quite insoluble nanomaterials. The absorption of nanomaterials from the gastrointestinal tract (bioavailability) is generally very low.

Nanoclays (which are primarily composed of Al and silicon), for example, montmorillonite, are used in polymers for food packaging and storage to improve water vapor and gas barrier properties by creating a tortuous path and increasing mechanical strength. The US FDA considers nanoclays as Generally Recognized as Safe. The primary concern about

their use in food packaging is migration out of the clay–plastic nanocomposite. *In vitro* studies have demonstrated the potential for nanoclays to be cytotoxic, but long-term exposure studies at concentrations relevant to human exposure are lacking.

See also: Aluminum: Properties, Presence in Food and Beverages, Fate in Humans, and Determination.

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http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=191&tid=34 – The US Agency for Toxic Substances and Disease Registry's "Toxicological Profile on Aluminum".

http://emedicine.medscape.com/article/165315-overview – Medscape "Aluminum Toxicity".

http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/aluminum/index-eng.php – Health Canada's "Aluminum".

Aluminum: Properties, Presence in Food and Beverages, Fate in Humans, and Determination

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Properties and Sources of Aluminum

Properties

Elemental aluminum (Al), which does not exist naturally, is soft, light, and malleable. Exposure to water, oxygen, and other oxidants leads to formation of Al oxide that provides a few nm thick surface film, for example, on the 0.18 mm thick household Al foil, that has high resistance to corrosion and is virtually insoluble from pH 4.5 to 8.5. In aqueous solution, in the absence of cationic ligands that bind to it, its chemical form (species) is pH-dependent. The Al^{3+} ion predominates below pH 5.5, as Al $(H_2O)_6^{3+}$. As the pH increases, $Al(OH)^{2+}$, $Al(OH)_2^+$, $Al(OH)_3$, and $Al(OH)_4^-$ predominate at pH 5.5, 6, 6.2, and above 6.2, respectively. $Al(OH)_3$ (gel) has the lowest solubility, at pH 6.2. As a Lewis acid (electron pair acceptor and electrophile), it strongly complexes with multidentate carboxylate- and hydroxyl-/keto-containing ligands, for example, carboxylic acids such as citrate. The chemical species of Al has a great impact on its kinetics and effects. The effective ionic radius of Al^{3+} is sufficiently similar to that of Fe^{3+} to engage in some of the same chemical and metabolic processes. Aluminum binds particularly strongly to phosphates, giving it the potential to bind with DNA, ATP, and many other biomolecules.

Elemental Al and its compounds have extensive applications. They are used in water purification, sugar refining, and brewing. They are incorporated in personal care and medical products including antacids/antiulcerative medications (which are also used as phosphate binders), buffered analgesics, antiperspirants, acne-treating products, toothpastes as an abrasive and to reduce sensitivity, astringents and rash products, antidiarrheal agents, vaginal douches, cosmetics, and as an adjuvant in some vaccines to increase their antigenic properties.

Natural Occurrence

Aluminum is the third most common element, and most common metal, of the Earth's crust, comprising 8%, mainly as silicates, oxides, and hydroxides. It is ubiquitously distributed throughout the environment. In contrast to its abundance in the Earth's crust, most natural waters contain very little dissolved Al. Its average concentration in lakes, rivers, groundwater, coastal sea water, and open ocean water is ~0.15, 0.4, 0.1, 0.001–0.007, and 0.001 mg l^{-1}, respectively. Increased acidity and organic matter can increase Al concentration. The World Health Organization (WHO) stated large and small water treatment facilities should be able to produce water that has $\leq$0.1 and $\leq$0.2 mg l^{-1} Al, respectively. The EU *indicator parameter* directive for Al in drinking water is 0.2 mg l^{-1}. Failure indicates there may be a problem with the supply. The US Environmental Protection Agency has as a nonmandatory standard for Al a maximum contaminant level of 0.05–0.2 mg l^{-1}. Colored water is a noticeable effect above this level. Canadian guidance levels, based on operational considerations, are $<$0.1 mg l^{-1} for conventional water treatment plants (using Al-based coagulants) and $<$0.2 mg l^{-1} for other types of treatment systems.

Aluminum is present in food naturally, as a food additive, and can be taken up through contact during food processing, preparation, and storage. The Al content of foods is highly variable, depending on the food product; the site of its growth, if the plants are Al-tolerant varieties; and food processing and storage. Contamination of food with soil that typically contains 5–10% Al can significantly increase the food Al content, for example, spinach and lettuce, although the Al may not be in a readily absorbable form. Most plants, and plant-eating animals, contain little Al, due to its very low oral bioavailability and limited transfer to eggs and milk (see the succeeding text). Some plants accumulate Al and deposit it in their leaves. The tea plant accumulates and concentrates Al, accounting for the higher Al concentration in tea infusion than other beverages. Some spices contain considerable Al, illustrated by paprika and pepper. Addition of Al as a food additive can greatly increase its food content, illustrated by baking powder, cake and pancake mixes, cakes and pancakes, and cheese containing sodium aluminum phosphate (SALP). The following discusses these points in more detail.

Aluminum in unprocessed beverages and foods

Table 1 shows median Al concentrations for representative beverages and foods. The values in Tables 1 and 2 are from studies published since 1985 and are based on multiple reported values for each entry in the table. Most unprocessed foods contain $<$5 mg kg^{-1} Al. The Al concentrations in paprika and pepper (Table 1), as examples of spices, are based on their dry weight, whereas most food Al concentrations are based on wet weight. Spices generally have much higher Al concentrations than other unprocessed foods but are consumed in much smaller amounts.

Anthropogenic Sources: The Addition of Aluminum Compounds to Beverages and Foods

Many governmental bodies permit the use of Al compounds as food additives (intentionally added to food for a functional purpose, up to a few percentage). In the United States, Al salts had been used in foods prior to 1958 when the Food Additives Amendment of the US Food, Drug and Cosmetic Act was amended to require pre-market approval of substances intentionally added to food. Exceptions were granted when the use of the substance was generally recognized as safe (GRAS) or was excepted from the definition of food additive, e.g., is a color additive. In 1959, the US Food and Drug Administration published a list of substances considered GRAS for use in foods that included Al salts. A 1975 review of the GRAS status of Al concluded "There is no evidence in the available literature

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Table 1 Median Al concentration in some representative beverages and foodstuffs

Beverage or foodstuff	*Median Al concentration (mg l^{-1} or mg kg^{-1})*
Cow milk	0.070
Human milk	0.043
Apple	0.72
Banana	0.55
Grape	0.82
Orange	1.5
Peach	2.6
Pear	0.64
Plum	1.1
Raison (sultana)	10
Strawberry	1.0
Watermelon	0.28
Apple juice	0.44
Orange juice	0.18
Pineapple juice	0.35
Tomato juice	0.67
Bean	4.0
Broccoli	1.3
Cabbage	0.31
Carrot	0.57
Cauliflower	0.80
Celery	0.90
Corn	1.5
Cucumber	2.2
Lettuce	5.5
Mushroom	2.9
Onion	0.30
Pea	2.1
Pepper (green)	0.94
Potato	2.5
Soybean	7.8
Spinach	24
Tomato	0.74
Corn flour	5.3
Oats	4.0
Rice	3.3
Wheat flour	5.6
Eggs	0.27
Beef	1.2
Chicken	1.2
Pork	2.2
Fish	0.15
Almonds	3.0
Cashews	4.6
Chestnuts	3.8
Peanuts	2.0
Pine nuts	38
Walnuts	2.3
Paprika	92
Pepper	31

on... acidic sodium aluminum phosphate [and other Al forms]... that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future." It was noted that care should be taken by patients with kidney disease when consuming food containing high levels of Al salts. This was prior to elucidation of the role of Al in dialysis encephalopathy or the controversial role of Al in Alzheimer's disease. Many governmental bodies legislated acceptable Al compounds as food additives. International Numbering System numbers are assigned by the Codex Alimentarius Commission to allow each food additive to be uniquely identified, for example, 541 for acidic and basic SALP and 554 for sodium aluminosilicate (SAS) (AKA: sodium silicoaluminate). On packaging in the EC, approved food additives are written with a prefix of 'E.'

Table 2 shows median Al concentrations for representative processed beverages and foods. The higher Al content of soy-based infant formula (Table 2) derives from the higher Al content of soybeans than cow milk (Table 1). Al-containing food additives used in the greatest amounts include acidic SALP in self-rising flour as an acidifying and leavening agent (by reacting with sodium bicarbonate to produce carbon dioxide); basic SALP in processed cheese, cheese food, and cheese spread as an emulsifying agent to provide a soft texture and easy melting characteristics; and SAS as an anticaking agent. Acidic SALP accounts for the increase of Al in biscuit, cake mix, cake, baking powder, pancake mix, and pancake; basic SALP for the increase of Al in frozen pizza cheese; and SAS for the Al in powder nondairy creamer and the increase in single-serving salt packets (Table 2). Aluminum is permitted in lakes (water-soluble artificial colors adsorbed onto alumina), which typically have an Al content of 25%. Canada, the European Union, the United Kingdom, Australia, New Zealand, India, China, Japan, Taiwan, and South Africa generally permit similar Al-containing food additives as the United States.

The contribution of processing to aluminum in beverages and foods

Many countries permit the use of Al compounds in food processing, packaging, and storage. Most countries, and the EC, do not have specific requirements for light metal alloys in contact with food. Aluminum has been used in cookware since ~1890 due to its heat conductivity. The Al surface oxidizes to form a layer of Al oxide that resists corrosion. Many studies conducted from 1890 to date have shown that Al can be solubilized by beverages and foods. Table 3 shows published results of studies that directly compared beverages and foods processed or prepared in Al compared to non-Al containers. The values are percentages to illustrate the relative magnitude of effect and as such do not inform about the absolute magnitude, for example, the amount of mobilized Al. Differences among studies that investigated similar beverages or foods and process conditions might be due to different initial Al levels. Processing and preparation of beverages and foods in Al containers routinely increased their Al content compared to steel, stainless steel, enamel, Teflon®, glass, or even containers made of clays, which are Al silicate complexes. The lack of consistency between old and new Al containers may be due to more Al release from a new container whose surface is not oxidized and more release from an older container with a pitted surface, vessel manufacture and surface coating, extent of use or cleaning prior to their study, and the food under study. Acidic and alkaline (the latter are much less common) foods mobilize more Al, which relates to increased Al solubility as the pH deviates from circumneutral pH (see section '**Properties**'). Similar to the results with canned mushy peas, tomatoes, and rhubarb, many other studies found an increase of Al in

Table 2 Aluminum concentration in some representative processed beverages and foods

Beverage or food	*Median Al concentration (mg l^{-1} or mg kg^{-1})*
Tap water	0.04
Mineral water	0.016
Cow milk-based infant formula	0.19
Soy-based infant formula	3.9
Infant foods (>50 foods, many commercial, from 15 reports)	10
Infant foods (strained) (>30 commercially purchased foods from 2 reports)	0.41
Cola	0.25
Noncola soft drinks	0.40
Black tea	3.0
Green tea	2.5
Instant tea	1.2
Herbal tea	0.31
Coffee	0.24
Beer	0.16
Wine	0.90
Distilled spirit	0.42
Butter	1.4
Cheese (nongoat)	3.8
Cheese (processed)	15
Cheese (goat)	15
Cheese (on restaurant pizza)	2.9
Cheese (on frozen pizza)	415
Yogurt	0.28
Yogurt (goat)	2.8
Margarine	1.7
Olive oil	0.043
Vinegar	0.21
Peanut butter	1.9
Bacon	2.4
Ham	0.85
Luncheon meat	3.2
Sausage	6.2
Soup	1.2
Chocolate	9.4
Honey	0.050
Sugar	1.7
Jelly and jam	4.1
Biscuit	22
Bread (white)	3.6
Bread (wheat)	4.5
Cake mix	445
Cake (not stated to contain SALP)	6.3
Cake (containing acidic SALP)	190
Cereal	1.0
Cookie	6.9
Baking powder	69
Pancake mix	100
Pancake	85
Pasta	5.5
Pickle	7.4
Nondairy creamer powder (multiple-serving container)	38
Nondairy creamer (single-serving packet)	170
Salt (multiple-serving container)	2.4
Salt (single-serving packet)	180

prepared foods compared to the food before preparation. Sodium chloride (table salt) can increase Al mobilization from Al cookware, illustrated by peas cooked with salt in an Al pan for 10, 20, and 30 min, which had 200%, 406%, and 438% as much Al as when salt was absent.

The conclusion of a review conducted in 1939 was that Al is safe and harmless as a material for cooking and household utensils in contact with food. The US FDA came to a similar conclusion in 1991. However, serum Al and urine excretion were significantly greater in people with chronic

Table 3 Aluminum in beverages and foods processed/prepared in aluminum compared with nonaluminum containers

Beverage or food processed/prepared in Al cookware	*As a percentage of non-Al cookware*
Water boiled 20 min in old Al versus stainless steel vessel	2500
Water boiled 20 min in new Al versus stainless steel vessel	267
Water boiled 15 min in Al versus enamel pot	993
Water boiled 15 min in Al versus Teflon® pot	8370
Water boiled in Al percolator versus Teflon® coffee pot	2500
Tea decocted in old Al vessel versus stainless steel	100
Tea decocted in new Al vessel versus stainless steel	142
Tea boiled for 10 min in Al versus stainless pot	104
Coffee prepared in Al versus stainless steel	251 and 314
Coffee boiled in Al percolator versus Teflon® coffee pot	987
20 coffees brewed in Al versus stainless steel	120 (median)
Milk boiled in old or new versus stainless steel vessel	100
Whole milk cooked in Al versus porcelain or stainless steel	146
Milk boiled 15 min in Al versus enamel pot	6000
Milk boiled 15 min in Al versus Teflon® pot	7680
Cottage cheese made from above milk in dark (oxidized) Al pot versus glass	2520
Cottage cheese made from above milk in light (newer) Al pot versus glass	730
Milk curds set overnight in old Al versus stainless steel vessel	100
Milk curds set overnight in new Al versus stainless steel vessel	430
Fresh (not soured) yogurt fermented in old Al versus boron glass or steel container	6000
Fresh yogurt fermented in new Al versus boron glass or steel container	2600
Yogurt (soured) fermented in old Al versus boron glass or steel container	84 000
Yogurt (soured) fermented in new Al versus boron glass or steel container	6000
Rhubarb cooked in Al versus stainless steel pot	295
Rhubarb boiled 12 min in Al versus steel pot	2060
Peeled potatoes in Al versus stainless steel pan	212
17 fruits and vegetables cooked in old Al versus stainless steel vessel	120 (median)
17 fruits and vegetables cooked in new Al versus stainless steel vessel	152 (median)
6 fruits and vegetables cooked in old Al pan versus clay pot	138 (median)
6 fruits and vegetables cooked in new Al pan versus clay pot	113 (median)
Canned mushy peas after cooking in Al versus before cooking	300
Canned mushy peas cooked in Al versus Teflon® saucepan	200
Canned tomatoes after cooking in Al versus before cooking	12 650
Canned tomatoes cooked in Al versus Teflon® saucepan	7170
Canned rhubarb after cooking in Al versus before cooking	26 250
Canned rhubarb cooked in Al versus Teflon® saucepan	11 350
Rhubarb cooked in Al versus stainless steel pot	295
Carrots cooked in Al versus stainless steel pot	175
Tomato cooked 10 min in Al versus steel pan	162
4 vegetables prepared in Al pot versus glassware	101, 195, 243, and 272
Oatmeal cooked in Al versus stainless steel pot	372
Rice cooked in old Al versus stainless steel vessel	156
Brown rice cooked in Al versus stainless steel pan	125
White rice cooked in Al versus stainless steel pan	234
Chinese noodles boiled 10 min in Al pan versus beaker	445
Japanese noodles boiled 10 min in Al pan versus beaker	91
Pasta (white) Al versus stainless steel pan	267
Pasta (whole grain) Al versus stainless steel pan	104
Pasta prepared in Al pot versus glassware	104
Quince jam cooked with sugar in old Al versus stainless steel pressure cooker	1950
Strawberry jam cooked in old Al versus steel pan for 30 min	174
Strawberry jam cooked in old Al versus steel pan for 90 min	338
Pickles prepared in Al tray versus glass pot	1015
Vegetable, salt, oil, egg mixture cooked in old Al utensil versus boron glass	688
Vegetable, salt, oil, egg mixture cooked in new Al utensil versus boron glass	219
Vegetable, salt, oil, egg mixture cooked in Al foil versus boron glass	156
Vegetable, salt, oil, meat mixture cooked in old Al utensil versus boron glass	667
Vegetable, salt, oil, meat mixture cooked in new Al utensil versus boron glass	242
Vegetable, salt, oil, meat mixture cooked in Al foil versus boron glass	106

renal insufficiency who used Al rather than stainless steel kitchen utensils for 3 months, suggesting a potential risk to this susceptible population.

The roles of aluminum in beverage and food packaging and storage

Metallic Al and its salts have many roles in food packaging and storage. Most soft drinks and beer are packaged in Al cans, which are lined with a polymeric coat to prevent Al-beverage contact. Approximately seven billion Al foil containers are produced annually, as packaging and food trays for many food types and pharmaceuticals. Aluminum borate is an antistatic and antifogging agent for olefin polymers intended as food-packaging materials. Aluminum-containing clarifying agents are used in polyethylene and polypropylene copolymers intended for food contact. Aluminum and Al oxide polymer films are used as barriers in food packaging. Nanoclays are included in the polymer matrices (e.g., polyethylene and polypropylene) in packaging intended for food contact to enhance their barrier (reducing air and water vapor passage) and mechanical (increasing strength up to 100-fold) properties. Nanoclay incorporation in polylactic acid plastics used for food packaging enhances biodegradation.

The contribution of aluminum in packaging and storage to aluminum in beverages and foods

Table 4 has examples of the influence of Al packaging and storage on the Al concentration of the packaged contents. Tap water (pH 8.2) that originally had 0.053 mg l^{-1} Al increased to 0.16 mg l^{-1} after storage for 32 h in a new Al bottle. When stored in an old Al bottle, the Al concentration increased more (Table 4). Storage of beer at room temperature in Al cans increased its Al content over time, whereas storage at 4 °C did not. Storage of tomato puree in an Al pan for 72 h at 4 °C increased the Al concentration 31-fold. Addition of salt increased the Al by 10%. Storage of tamarind in an Al pan for 72 h at 4 °C increased the Al concentration 17-fold. Addition of sugar increased the Al by 16%. The Al concentration in grapefruit juice increased 6-fold when stored at 50 °C for 12 weeks; increased ~14-fold in cola and lemon drink, ~9-fold in lemon–lime drink, and ~26-fold in orange drink stored in Al cans for 12 months; and increased ~30-fold in a soft drink

Table 4 Aluminum in beverages and foods package and/or stored in aluminum compared with nonaluminum packaging/storage materials

Beverage or food	As a percentage of non-Al storage/packaging material
Tap water stored 1 week in Al versus glass siphon at 5 °C	5260
Tap water stored 32 h in old versus new Al bottle	125
Mineral water packaged in Al can versus glass bottle	67
Milk packaged in Tetra Brik (paperboard, polyethylene, and Al) versus plastic bag	129
Milk packaged in Tetra Brik versus plastic bottle	174
Vanilla milk shake in Tetra Brik versus plastic container	75
Vanilla milk shake in Tetra Brik versus glass container	22
Cola packaged in Al can versus glass	290
Cola packaged in Al can versus plastic	365
Cola packaged in lacquered Al can versus plastic bottle	200
Fanta Orange packaged in Al can versus glass bottle	328
Fanta Orange packaged in Al versus steel can	204
Apple juice stored 22 months in 2-piece lacquered Al versus 'tin' can	723
Lemon juice packaged in Al can versus glass	203
Lemon juice packaged in Al can versus plastic	253
Orange juice packaged in Al can versus glass	222
Orange juice packaged in Al can versus plastic	275
Orange squash packaged in lacquered Al can versus plastic bottle	41
Pocari Sweat packaged in Al versus steel can	106
Pocari Sweat packaged in Al can versus glass bottle	225
Tonic water packaged in Al can versus glass bottle	132
Tonic water packaged in Al can versus plastic bottle	160
Lime blossom tea (pH 3.1) after 145 h in old versus new Al bottle	108
Japanese uron tea packaged in Al can versus glass bottle	106
Japanese uron tea packaged in Al can versus steel bottle	127
Beer from Al can versus glass bottle	149
Eleven beers from Al can versus glass bottle	151 (median)
Domestic beer from Al can versus glass bottle	151
Domestic beer from Al versus steel can	108
Imported beer from Al can versus glass bottle	182
Imported beer from Al versus steel can	151
Wine stored 2 years in Al ring pull can versus glass bottle	137
Wine stored 2 years in Al ring pull versus 'tin' can	173
Tropical fruits from Al can versus glass	84
Al contributed by Al versus steel can to tomatoes over 2.5 years after canning	261
Mushrooms stored 2.5 years in Al versus steel can	107
Mackerel in white wine stored 2.5 years in Al versus steel can	93
Liver paste, stored 2.5 years in Al versus steel can	96

stored in an Al can for 48 months. The mobilization of Al during the processing and storage in contact with Al is greatest for acidic beverages and foods (Tables 3 and 4) (e.g., tomato pH ~4.5 and rhubarb ~3.2), consistent with the chemical species of Al as a function of pH; solubility increases below pH 6.2. It has been estimated that typical food processing and storage do not contribute more than 2 mg day^{-1} of Al to daily Al intake in foods. Compared to daily Al intakes of 3.6–9 mg of Al in beverages and foods (see section 'Aluminum Consumption'), this represents ~20–50% of daily Al intake.

Aluminum Consumption

Human Al exposure is mainly from food, water, airborne dust, antiperspirants, immunizations, allergy injections, and antacids. Foods and beverages are the largest single source of Al for the typical human, in the absence of occupational exposures or chronic use of Al-containing pharmaceuticals. Food additives provide a significant percentage of daily Al intake. Among the food additives, SALPs are the main contributors. Drinking water, based on daily average consumption of 1.4 l, provides ~0.1 mg, or ~2% as much as food.

More than 50 studies conducted since the mid-1980s in Australia, Brazil, Canada, China, East Germany, Finland, France, Germany, Hungary, India, Italy, Japan, the Netherlands, Portugal, Slovenia, Spain, Sweden, Switzerland, Taiwan, Turkey, the United Kingdom, and the United States have reported daily dietary Al intakes. The intakes were based on total diet studies, market basket surveys, dietary records, and calculations based on food consumption and Al levels and duplicate diets and portions. Aluminum intake by males generally exceeded that of females. The median daily Al intake by adults in these studies was 4.8, teenagers 8.6, children 6, and infants (<2 years old) 0.7 mg day^{-1}. Reported intakes were highest in China, Japan, and Taiwan (~9 mg day^{-1}). Aluminum intake by adults was higher in the United States and Canada (8.5 mg day^{-1}) than Europe (3.6 mg day^{-1}), probably because there are fewer approved Al food additives and lower minimum permissible levels in the EU than the United States, less Al is added to cereal grain products as raising agents, and basic SALP is not permitted to be used in processed cheese. Total Al intake generally relates to total food intake, partially explaining the greater Al intake in teenagers than adults. Another contributor is food selection. Teenagers eat more prepared foods that have Al-containing food additives.

Aluminum intake in food is ~50-fold greater than from drinking water. As tea contains more Al than other beverages, its consumption can contribute 50% of the daily Al intake in those who consume considerable amounts of this beverage, when other sources do not provide larger than typical amounts of Al.

In 2008, the Agency for Toxic Substances and Disease Registry set the minimal risk level for intermediate (15–364 days) oral Al intake at 1 mg $kg^{-1}day^{-1}$. In that year, the European Food Safety Authority Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials of the European Commission lowered its tolerable weekly intake to 1 mg kg^{-1} body weight per week. The Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives in 2011 established a provisional tolerable weekly intake for Al of 2 mg kg^{-1} body weight per week, raised from the previous 1 mg kg^{-1}, which applies to all Al compounds in food, including food additives. There were recommendations from the thirty-sixth session of the Joint FAO/WHO Food Standards Programme in 2013 to eliminate some approved uses of Al as a food additive.

Aluminum Absorption, Distribution, and Elimination

Bioavailability

The typical routes of Al uptake are by inhalation and through the gastrointestinal tract. The percentages of Al absorbed from the lungs and gastrointestinal tract are 1.5–2 and ~0.1–0.4, respectively. Results from one small study suggest up to 0.012% of Al is absorbed from underarm application. Aluminum injected in vaccines is slowly absorbed as it dissolves and may ultimately reach 100%. Aluminum, as a contaminant of dialysis fluid, extensively distributes from dialysis fluid into blood due to its strong binding to the plasma protein transferrin. Limited results suggest Al might enter the brain from the nasal cavity by uptake into nerve endings involved in smell.

Absorption of Al from the gastrointestinal tract is increased by citrate, and other carboxylic acids to a lesser extent; soluble Al forms; low iron, calcium, or sodium status; and uremia. In contrast, silicon-containing compounds appear to reduce its absorption and/or enhance its elimination in urine, the primary route of excretion (see section 'Aluminum Excretion').

Aluminum Biokinetics in Blood

The upper range of the blood Al concentration in normal humans has been stated to be 3–5 μg l^{-1}. There has been considerable variability in normal values reported in the past decade, with mean values from 1 to 15 μg l^{-1}. The differences probably reflect differences in diet, environment, occupation, and sampling and analytic methods. Given this is very low and Al is ubiquitously present, much attention must be paid to collection, storage, and handling of blood samples to avoid contamination. In addition to enhancing Al absorption, citrate enhances its distribution out of the blood and into tissues and its excretion by the kidney.

Aluminum Tissue Deposition and Body Retention

Aluminum distributes unequally throughout the body. The normal human has ~60 mg of Al, with ~60%, 25%, 10%, 3%, 1%, 0.3%, 0.25%, and 0.2% in the bone (which provides a depot, discussed later in text), lung, muscle, liver, brain, heart, kidney, and spleen, respectively. Aluminum localizes at the mineralization front and in osteoid of the bone, contributing to a low turnover bone disease. Aluminum in the lung may be from particle inhalation, occupational exposure, and distribution from the blood. Insoluble particles trapped in the lung remain there a long time. With continuous intake, Al accumulates in the body, resulting in increased Al concentrations in the brain, bone, and serum with age. Hair Al concentration as an indicator of Al body burden has not been validated.

The whole-body Al half-life was estimated to be ~50 years in one human who received an intravenous injection of Al citrate. Given the large percentage of Al in bone, it probably drives the Al concentration and elimination half-life throughout the body. Increased bone turnover, in children or geriatrics, may accelerate bone Al release.

Aluminum Excretion

The kidneys provide the primary route of Al excretion, accounting for >95% of excreted Al, presumably by glomerular filtration of Al citrate. Reduced or no renal function creates the risk of Al accumulation and toxicity. Susceptible populations include those receiving dialysis, premature neonates receiving parenteral nutrition (all fluid and nutrition given intravenously), and infants receiving soy-based infant formula. Bile (feces) accounts for most of the remaining excreted Al, although it is also eliminated in saliva, sweat, semen, and hair.

Methods for Aluminum Detection and Quantification in Water; Food Constituents, Additives, and Contaminants; and Biological Samples

Aluminum ions in water can be quantified by reaction with pyrocatechol violet and spectrometric measurement of the resulting colored complex. Many methods using similar chromogenic agents have been developed to measure Al in water and simple aqueous solutions with low microgram per liter detection limits. Quantification of Al in more complex materials, such as beverages, foods, and biological samples, usually requires converting the materials to a homogenous liquid (if not originally as such). Aluminum quantification is usually conducted using inductively coupled plasma atomic emission spectroscopy (ICP-AES) or the more sensitive graphite furnace (flameless or electrothermal) atomic absorption spectroscopy (GFAAS) and inductively coupled plasma atomic mass spectroscopy (ICP-MS). Detection limits are 5 $\mu g\ g^{-1}$ and 10 $\mu g\ l^{-1}$ for ICP-AES and $< 1\ \mu g\ l^{-1}$ or $\mu g\ g^{-1}$ for GFAAS and ICP-MS, to as low as 0.01 or less.

There are numerous methods to localize Al in histological sections, at the light or electron microscopic level. For experimental purposes, the use of the rare isotope ^{26}Al, and its quantification by accelerator mass spectrometry, enables the determination of picogram per liter Al.

Biological monitoring of Al exposure and potential excessive body burden can be conducted with urine that is thought to indicate recent exposure and blood plasma that better reflects the Al body burden and long-term exposure. Reliable quantification of Al in blood is quite difficult because the blood Al concentration of the normal healthy human is very low (see preceding text) and Al is ubiquitous. Sample contamination is very easily encountered. However, neither urine nor blood is a good predictor of the Al body burden, which is better estimated by bone Al, the desferrioxamine challenge test, or combined measurement of serum parathyroid hormone and the desferrioxamine test. Desferrioxamine is a chelator that binds Al, iron, and many other trivalent metals and can increase their levels in blood serum and urine when they are present in excess.

See also: Aluminum: The Toxicology of.

Further Reading

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Amaranth

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Amaranthus

Amaranth is an edible plant that has been used by humans for over 4000 years. The origin of amaranth domestication is unknown; diverse tropical and subtropical climates possess indigenous amaranth species, which have facilitated amaranth cultivation around the world, both before and during domestication.

Amaranth is classified within the Dicotyledoneae in the *Amaranthaceae*, which includes more than 70 genera. The genus *Amaranthus* possesses more than 60 species, the majority of which are from the American continent. The large number of varieties is reflected in its common and vulgar names: *amaranto* (Spanish and Portuguese); *kiwicha, achita, coyo, achis,* and *qamaya* (Peru, Quechua); *coimi, millmi,* and *inca pachaqui o grano inca* (Bolivia); *sangorache, ataco,* and *quinua de castilla* (Ecuador); *millmi* (Argentina); *alegría* and *huanthi* (Mexico); *rejgira, ramdana,* and *eeerai* (India); and *een, choy, yin choy, in-tsai, hsien tsai,* and *xian ca* (China).

The most cultivated *Amaranthus* spp. are cited in Table 1. Species division is based on their method of utilization: into grain, vegetable, ornamental, and weedy amaranths.

Three species of amaranth are the most used for grain production, *A. cruentus* L., *A. caudatus* L., and *A. hypochondriacus* L. Vegetable amaranth are found in two major species, *A. tricolor* L. and *A. lividus*.

Leaf color can be green, yellow, orange, or red, due to the presence of betacyanin. Inflorescence morphology is very variable but is usually prominent, being displayed at the apex of the stem. The seeds, considered pseudocereals (nongrasses), are small and lenticular (disk-shaped), averaging 1–1.5 mm in diameter. One to three thousand seeds per gram are common. Seed color varies from pale ivory to black. Figures 1–3 show common amaranth species and crops in the *Cerrado*, a central region in Brazil.

Sources, Production, and Consumption

Great importance is attributed to amaranth in the pre-Hispanic period. The plant played a role in ancient religions, being used to celebrate the gods of earth, fire, and rain in Mexico and to prepare drinks associated with fertility rituals in Peru. Spanish colonization of the Americas in the 1500s banished amaranth production, in an attempt to eradicate these and other pagan ceremonies. However, by the 1700s, amaranth cultivation had spread throughout Europe, being used mainly as an herb and ornamental.

Currently, amaranth is an underutilized crop. Amaranth production levels worldwide are not known. It is more commonly cultivated in Mexico, Peru, and other Andean countries and grown on a small scale in some other South and Central American countries, aimed at grain production. Amaranth is also grown and consumed as grain or vegetable crop in Australia, Africa, and Asia and especially in Nepal and by inhabitants of mountainous regions of northern and southern India, where it is the most commonly consumed as a green leafy vegetable. Researchers have verified that amaranth is the second most preferred indigenous vegetables for the Sudanese people.

The production of amaranth has been promoted around the world, mainly due to its many interesting characteristics, such as fast growth (in about ninety days). Unlike other green vegetables, it is cultivated during the summer months, when no other market leafy vegetables are available. This makes it possible to use in grain crop rotation systems. Amaranth can also grow under varied soil and agroclimatic conditions. Amaranth culture can be employed under different production systems, ranging from direct seeding, transplanting, irrigated or rain-fed conditions, interspersed, as edges around other cultures, to monoculture, depending on the environmental conditions and location. Good results have been obtained at sea level and in tropical areas. Although the plant is susceptible to cold and excessive moisture, it is resistant to water deficit and heat. Recent research indicates that under optimal soil, moisture, and temperature culture conditions, yields can reach 5000 kg ha^{-1}, although average yields are within the range of 1000–2500 kg ha^{-1}.

Amaranth grown conventionally brings around $ 0.40 per pound, while organic amaranth may sell for $0.65 per pound or more. Thus, with high productivity, amaranth gross returns can easily surpass commodity crops. In addition, productivity keeps increasing due to improvements in available technology, derived from research, as well as new varieties, harvest mechanization, breeding, and agroindustry development. Projects worldwide are aimed at contributing to the improved livelihood of resource poor communities through increased grain amaranth production, increased use as food, and the introduction of value-added products. Currently, many farmers seem more interested in growing the crop for sale than home consumption. Nonetheless, two expensive problems involve seed cleaning and transportation to market. If the harvested seed contains considerable extraneous material, cleaning and drying should be done as fast as possible. After that, the grain should be stored at about 10–12% relative humidity.

On small, family properties, amaranth can be prepared using low-energy techniques. The grain can be boiled in water, toasted, or parched lightly, sprouted, popped, or puffed like popcorn. Milling for household consumption may pose a problem, requiring the installation of mills in communities to facilitate this task. However, industrial grinding or milling of the grain is very feasible, and its flour can partially substitute other flours to generate several products, such as biscuits, cakes, and bread. For bread production, though, adjustment is necessary because the grain has no gliadin to form a gluten structure. On an industrial scale, quality products can be

produced with amaranth flour, generating expanded products, such as snacks. Besides that, flakes, cereal bars, or fermented products, such as an alcoholic drink, can be produced. A water extract can used to produce a nonalcoholic amaranth drink. Leaves usually are used as boiled greens or in salads.

Chemical Composition and Analyses

Both the leaves and seeds of amaranth possess high nutritional value. Ash, protein lipid, fiber, and carbohydrate contents of amaranth leaves are about 14%, 18%, 5%, 9%, and 52%, respectively. They also possess appreciable amounts of vitamins and low levels of toxicants, especially after thermal processing.

The National Research Council of the United States considered amaranth grain as one of the most promising foods of the millennium, and the US National Academy of Sciences encourages commercial exploitation of the grain due to its high nutritional quality. Seed lipid content is about 6–8% and a lipid profile similar to that of cereals. Amaranth differs mainly by the presence of squalene in the unsaponifiable fraction. Starch and fiber contents are 50–60% and 8%, respectively. In addition, the seed possesses a high soluble fiber content, as compared to cereals. Another advantageous factor favoring amaranth consumption is the availability of amino acids that are limiting in cereals and legumes, such as lysine and methionine. Finally, the protein content of amaranth grain is comparatively high (13–18%) and its amino acid composition close to the optimum for human consumption.

The mineral and vitamin contents of amaranth are also considerable. Calcium, phosphorus, and iron contents are high, relative to cereals. In addition, amaranth's content of antinutritional factors such as phytate is negligible. The pseudocereal also possesses antioxidant compounds, such as tocotrienols, tocopherols, flavonoids, and other phenolic compounds.

Due to its interesting composition, several compositional and physicochemical tests have been performed on amaranth leaves, grain, and their by-products. The identification and quantification of vitamin, lipid, carbohydrate, protein, peptide, phenolic content, and other organics usually involve the use of chromatographic methods, combined with mass spectrometers. Mineral analyses involve spectrophotometric methods, such as atomic absorption and flame atomic emission.

Table 1 Most frequent species in *Amaranthus* genus and synonymous

Amaranthus *species*	*Synonymous*
A. caudatus L.	*A. edulis* Spegazzini
	A. mantegazzianus Passerini
A. hypochondriacus L.	*A. leucocarpus* S. Watson
	A. flavus L.
A. cruentus L.	*A. paniculatus* L.
A. hybridus L.	*A. quitensis* S.
A. tricolor L.	*A. gangeticus* L.
	A. tristis L.
	A. mangostanus L.
	A. melancholicus L.
A. lividus L.	*A. blitum* L.

Source: Mujica-Sánchez, Berti-Díaz and Izquierdo (1997).

Figure 1 From left to right: *Amaranth hybridus*, *A. retroflexus*, *A. caudatus*, and *A. cruentus*. Courtesy of Dr. C. Spehar – Embrapa Cerrados – Brazil.

Figure 2 A mixed crop of the three species of **Figure 1** in the 'Cerrado' region of Central Brazil. Courtesy of Dr. C. Spehar – Embrapa Cerrados – Brazil.

Figure 3 Amaranth caudatus crop in the 'Cerrado' region of Central Brazil: (a) mature crop, (b) beginning of the flowering process. Courtesy of Dr. C. Spehar – Embrapa Cerrados – Brazil.

Chemical and physicochemical analyses may be accompanied with assessment of nutrient bioavailability. In this regard, genomic, transcriptomic, and proteomic methods have been used to determine specific effects of its components on biological systems. For food safety, PCR is one of the most used methods, specifically to detect for gluten contamination.

Availability, Absorption, Metabolism, and Health Effects

Besides these nutritional characteristics, studies have shown that the introduction of amaranth into the diet may prevent or diminish the risk of diseases by presenting several biological effects, possessing antidiabetic, cholesterol-lowering, antioxidant, antitumoral, and antimicrobial effects.

Antinutritional Factors and Bioavailability

Oxalates are present in the leaves of cooked amaranth (*A. gangeticus* or *A. tricolor*) and could inhibit the absorption and bone deposition of calcium as demonstrated in young rats. Amaranth grains (*A. cruentus*, *A. hypochondriacus*, and *A. hybrid*) contain 4–5 times more oxalate than cereals and legumes, averaging 229 mg 100 g^{-1}, of which 80% were insoluble. Oxalate reduces the bioavailability of calcium and magnesium by complexing with these minerals, preventing their absorption. Moreover, this contributes to the formation of kidney stones.

In order to reduce the intake of soluble oxalate, cooking of the grain prior to consumption is essential.

Cooking and popping of *A. cruentus* and *A. caudatus* decreased the presence of antinutritional factors such as phytates, trypsin inhibitors, and increased nutrient bioavailability. In contrast, the same processes decreased concentrations of phenolic compounds, of which popping had the lesser effect.

Antidiabetic Effects

Studies with different amaranth leaves have shown positive effects in terms of reducing hyperglycemia. Methanol extracts from *A. caudatus* inhibit α-amylase under *in vitro* conditions. These extracts contain flavonoids, saponins, alkaloids, carbohydrates, proteins, peptides, amino acids, and phenolic compounds. The latter accounted for 48% of the organic constituents. The same inhibition was observed by ethanol extracts from raw and processed (bleached) *A. cruentus* leaves. Inhibition of amylases reduces the bioavailability of carbohydrates, thus contributing to the reduction of glucose levels in blood. Therefore, glycated hemoglobin decreased, as well as α-glucosidase inhibition, related to the phenolic content of amaranth leaves. A hypoglycemic effect of methanol extracts was observed, from *A. caudatus*, *A. spinosus*, and *A. viridis* leaves, on diabetic rats, depending on extract concentration. In spite of these potential beneficial effects, the antidiabetic role of amaranth is still controversial, especially because the compound class responsible for such effects has not been established.

Conflicting results have been obtained from studies concerning the potential antidiabetic effects of amaranth grain. Peptides were found in grain amaranth (*A. hypochondriacus*) that interact with dipeptidyl peptidase IV, a regulator of the glucose levels, inhibiting its action through hydrophobic interactions and hydrogen bonds. The effect of oil and grain amaranth (*A. esculentus* L.) on diabetic rats showed that supplementation with oil and grain reduces their glucose levels. The mixtures of amaranth grain with chapatti (a common Middle East bread wheat) was considered of low or medium glycemic index (GI), depending on amaranth proportion. On the other hand, the glycemic response of normal volunteers following ingestion of extruded amaranth (*A. cruentus* L.) showed an effect similar to that of white bread and a greater capacity than the control to stimulate insulin production. Although grain amaranth has high dietary fiber content, it was not enough to reduce the glycemic response. Grain processing may expose starch matrix and facilitate its gelatinization, which in turn increases susceptibility to enzymatic digestion.

Cholesterol-Lowering Effect

Several studies have evaluated the influence of diet changes on the plasma levels of total cholesterol and its fractions. For many decades, amaranth has been investigated about its beneficial effects in this regard. Initially, the fractions suspected responsible for this effect were amaranth's protein and lipid moieties. Whereas the data on amaranth protein effect have been consistent, results from the lipid fraction seem controversial. The effect of amaranth oil on patients with heart disease and both obese and hypertensive was a reduction in the blood cholesterol levels. Squalene and phytosterols in the oil might be involved in this decrease. However, studies of the effect of amaranth oil and squalene on hamsters subjected to a hypercholesterolemic diet showed that the lipid fraction of *A. cruentus* did not reduce the levels of serum cholesterol or triglycerides. On the other hand, they increased bile acid excretion in the feces, indicating a possible decrease of the solubility of cholesterol micelles. Phytosterols and their corresponding saturated sterols reduce the absorption of cholesterol through the inhibition of its solubility in the intestinal lumen and thus decrease plasma LDL-c (low-density lipoprotein cholesterol). Supplementation with amaranth oil and grain in diabetic rats was effective in lowering total cholesterol, very low-density lipoprotein, and plasma triglycerides but had no effect on high-density lipoprotein particle of cholesterol levels and LDL-c. In the liver, there was a decrease in both triglycerides and supplemental grain showed an effect in lowering cholesterol.

The first consistent studies on amaranth grain in reducing cholesterol in mammals were performed with extruded amaranth in rabbits. In these studies, amaranth without oil reduced total cholesterol of hypercholesterolemic rabbits by 50%. Pure amaranth protein, without any other major component as fiber or oil, was found to produce the equivalent reduction in hamsters. Amaranth protein was then tested in hypercholesterolemic patients, and a decrease in their plasma cholesterol levels was observed. The protein dosage was critical for this effect to be observed. When insufficient protein (< 10 g day^{-1}) is used, the effect may be not relevant. However, when it amounts to around 25 g day^{-1} or more, the effect is substantial (around 4.5% decrease in blood cholesterol) that represents a significant reduction in the cardiovascular risk.

The mechanisms of serum cholesterol reduction associated with the consumption of amaranth protein are still poorly understood. One possibility is by the inhibition of the micellar solubilization of cholesterol in the lumen due to the considerable hydrophobic amino acid content of the samples. Peptides derived from hydrophobic amino acids, such as the hypocholesterolemic IAEK (lactostatin), found in soybeans, inhibit cholesterol absorption. There is also the possibility of peptides absorbed into enterocytes and transported to the liver to inhibit the cholesterol synthesis pathway. In fact, reports on the inhibition of liver HMGR (HMG-CoA reductase), a key enzyme of cholesterol synthesis, by the ingestion of amaranth protein, and the ability of peptides from amaranth to inhibit HMGR *in vitro*, support this mechanism.

Antioxidant Effect

Oxidative stress is related to cellular aging and neurodegenerative, cardiovascular, liver, and other diseases. The antioxidant effect of methanol extracts of grain amaranth (*A. hypochondriacus*) on the liver of rats intoxicated with ethanol showed interesting results. The group that ingested alcohol, in combination with amaranth extracts, had a decrease in malonaldehyde (MAD) concentrations and an increase in the activity of superoxide dismutase (SOD). Both effects were observed in serum and in the liver. Other enzymes involved in oxidative stress, such as catalase and glutathione peroxidase (GPx), hardly changed. Stimulation of gene transcription for Cu–Zn SOD was demonstrated, and the *in vitro* extract was able to inhibit lipid peroxidation and to act as a free-radical scavenger. The addition of grain amaranth (*A. cruentus*) to the diet together with fructose reduced the concentrations of MAD, SOD, GPx3, and lipid peroxidation in

plasma and various rat tissues. The grain was more effective on lung and heart tissues.

When one compares the total phenolic content, antioxidant activity, and inhibition of lipid oxidation between raw and processed amaranth (*A. cruentus*) (extruded, roasted, boiled, and popped), it is found that the ethanol extracts from cooked amaranth were able to inhibit lipid oxidation more effectively as compared with the extracts from distinct processed cereal and pseudocereal grains. Among the processed grains, only the extracts from the roasted grains had a lower antioxidant activity index in relation to the raw grain. Although processing reduced the total phenolic content, this did not affect the antioxidant capacity, highlighting the participation of other compounds in this property.

The peptides from amaranth albumin, globulin, and glutelin (*H. mantegazzianum*) showed significant scavenging capacity, but glutelin fraction exhibited the greatest effect. Molecules smaller than 0.5 kDa had the greatest antioxidant effect. Peptides with molecular masses below 0.25 kDa showed no effect. Amaranth proteins were effective sources of antioxidant peptides when this activity is detected using ORAC and $ABTS^{+}$ methods.

Antihypertensive Effect

Inhibitors of the angiotensin-converting enzyme (ACE) have an important role in the regulation of blood pressure. The enzyme promotes the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor. On the other hand, a vasodilator acts by inactivating bradykinin.

The ACE inhibitory activity of amaranth, quinoa, and buckwheat grains was studied, and it was found that these species inhibited the enzyme's activity by 8.8%, 23.3%, and 22.8%, respectively. Even though amaranth had the least effect, its effect was higher than those of rice and wheat. This activity of amaranth is due to its bioactive peptides found in glutelin.

The ACE inhibition by peptides of amaranth 11S globulin showed that the tetrapeptides, ALEP and VIKP, were effective inhibitors of the enzyme. A three-dimensional model was built of globulin, and its peptides were mapped by computer. The inhibitory activity on ACE by peptides from the 7S globulin showed that the effect was similar to that of 11S globulin. The better results were observed with the peptides obtained with protein hydrolysis by alkalase.

Other Beneficial Effects

Amaranth can affect cell replication. The effects of a lunasin-like amaranth peptide on histone acetylation showed a dose-dependent inhibitory effect upon acetylation of the core histones H3 and H4. These histones are involved in DNA packing and unraveling, being their lysine residues' acetylation the mechanism by which unraveling and replication of DNA are promoted. Therefore, substances that inhibit this acetylation will have a potential anticarcinogenic effect. Interruption of deacetylation–acetylation processes leads to cell death. In a maximum utilized concentration of lunasin-like peptide from amaranth (1000 nM), acetylation of H3 and H4 by the histone acetyl transferase was inhibited up to 77% and 70%, respectively. Amaranth lunasin acts in various ways to inhibit uncontrolled cell replication. One of these involves competition with histone acetyltransferases on H3 and H4. This peptide presents high content of methionine and cysteine.

Amaranth peptides have antimicrobial activity. By measuring the effect of the antifungal peptide, Ar-AMP, of amaranth (*A. retroflexus*) against several funguses, the observed growth inhibition was greatest against the *Fusarium culmorum* fungus in a dose-dependent manner. Significant antihelminthic effects were also observed when using amaranth peptides. The initial methanol extracts of the species *A. spinosus*, *A. caudatus*, and *A. viridis* had the greatest effect in inducing rapid helminth mortality.

The effect of supplementation with amaranth (*A. viridis* L.) on analgesia in mice was reported. It was found that a dose of 400 mg kg^{-1} of amaranth per mouse weight reduced pain by 60.5%, similar to the effect of aspirin.

Conclusion

In conclusion, amaranth cultivation and consumption have increased in the past few decades, especially due to three factors: rediscovery of the crop, development of new agronomic technologies, and increased demand for healthy food products. Amaranth contains several compounds that act beneficially, protecting, modulating, inhibiting, or stimulating activities in human cells and tissues or even modulating human genome. Further studies are necessary to elucidate the biochemical pathways of amaranth bioactive substances and evaluate their bioavailability in processed food. In the future, data may allow the establishment of dietary references for amaranth intake and its products for consumption, improving human health.

See also: Amino Acids: Determination; Amino Acids: Metabolism; Antinutritional Factors in Legume Seeds: Characteristics and Determination; Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Bioactive Peptides in Foods; Bioavailability of Nutrients; Carbohydrate: Digestion, Absorption and Metabolism; Cereals: Dietary Importance; Cereals: Types and Composition; Chapatis and Related Products; Cholesterol: Absorption, Function and Metabolism; Cholesterol: Properties, Processing Effects, and Determination; Cooking: Domestic Techniques; Dietary Fiber: Determination; Essential Oils: Properties, Composition and Health Effects; Extrusion Cooking: Chemical and Nutritional Changes; Functional Foods; Glucose: Metabolism and Regulation; Hypertension and Diet; Legumes in the Diet; Mass Spectrometry: Principles and Instrumentation; Phenolic Compounds: Occurrence, Classes, and Analysis; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Vitamins: Overview.

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Amino Acids: Determination

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Introduction

Proteins and peptides contribute to many relevant functions in an organism and, in fact, constitute key elements for the survival of animals and humans. Proteins are naturally constituted by 23 amino acids, which act as basic components of the polymeric structure. Some proteins or peptides contain also 'nonstandard' amino acids that are formed by posttranslational modification during protein synthesis and that are essential for the function or regulation of that protein.

Once foods are ingested, its proteins are hydrolyzed and small peptides and amino acids are released by enzymatic digestion and absorbed into the body. So, protein quality in a particular food strongly depends on its amino acid content and digestibility. Amino acids participate in many biochemical pathways for growth, maintenance, and metabolic activity of cells and organs, and its requirements vary, depending on the stage of life and the quality of proteins. Thus, the knowledge of the amino acid profile or the essential amino acids contents in foods and the limiting amino acids in a protein is very important for the characterization of their biological value and how their nutritional benefits may be affected by food processing or during storage. There are many available methods for the analysis of amino acids in foods that are summarized in this article.

Methods for the Analysis of Amino Acids in Foods

The choice for the methodology for the analysis of amino acids in foods depends on many factors such as the nature of the matrix because the presence of proteins, sugars, starch, or fat could interfere with amino acid extraction or analysis. Also, the choice will depend on the amino acid concentration and the required sensitivity level, but, except in some cases, it is not a limitation since sample size is usually more than enough. Finally, the technique of choice will depend on the availability in the laboratory. The simplest aim in the analysis of amino acids is to determine the total amino acid content. This analysis mainly quantifies free amino acids and some small peptides and is based on the reaction of the α-amino group with reagents like *o*-phthaldialdehyde (OPA), cadmium–ninhydrin, fluorescamine, and trinitrobenzene sulfonic acid that are the most used. These reagents contribute to a better ultraviolet response of amino acids at a higher wavelength or confer them visible or fluorescent characteristics. This global determination has a wide number of applications such as obtaining a proteolysis index for controlling the progress of food protein hydrolyzates or controlling manufacturing processes of some food products like cheese, meat products, and anchovies. Methods for this analysis generally include the precipitation of proteins, reagent addition, and colorimetric, UV absorption, or fluorescent determination of the amine nitrogen in the supernatant.

In most cases, the goal is focussed on determining the amino acid profile by analyzing the individual content of each amino acid, but such profile will vary, depending on whether they are in free form to obtain the free amino acid profile or come from protein hydrolysis constituting the total amino acid profile that may include also free amino acids present in the sample. The three described possibilities for the analysis of amino acids in foods are summarized in Figure 1.

Sample Preparation

The treatment of samples differs depending on the analytic purpose as mentioned earlier. In the case of free amino acids, some or all of the following processes may be required: extraction, cleanup, and derivatization, before or after the amino acid separation. In the case of total amino acids, additional protein hydrolysis is required. Once these stages are performed, amino acids can be separated and quantified. In this section, sample extraction and methods for sample cleanup and protein hydrolysis are described.

Free Amino Acids

Figure 2 shows a flowchart with the steps for the determination of free amino acids. The steps are described in detail in the following.

Extraction

This stage must be performed in the case of nonsoluble solid matrices (fish, meat, cereals, etc.) and is usually achieved by homogenization of the ground or milled food sample in an appropriate solvent. A dilute solution (0.1 N) of hydrochloric acid, or even water, is the typical extraction solvent to be used. In some cases, stronger acid concentrated solutions such as 4% of 5-sulfosalicylic acid (SSA) and 5% of trichloroacetic acid (TCA) or rich alcohol-containing solutions (>75%) such as ethanol and methanol have been successfully used as extraction solvents with the additional advantage that proteins are not extracted and, then, there is no need for further cleaning up of the sample. In the case of liquid food samples like milk or drinks, in general, no extraction is needed.

Once homogenized, the solution is centrifuged (10 000 g at 4 °C) to separate extracted (supernatant) from nonextracted (pellet) materials and filtered through glass wool to retain any fat material on the surface of the supernatant.

Sample cleanup

Sample cleanup consists in the isolation of amino acids from other extracted compounds (proteins, carbohydrates, and fats) as much as possible. The most common requirement is the separation of amino acids from proteins, a process known as deproteinization, which can be achieved through different chemical or physical methods. To this aim, concentrated strong acids like SSA, perchloric acid, trifluoroacetic acid (TFA), TCA, picric acid (PA), and phosphotungstic acid and organic solvents like methanol, ethanol, and acetonitrile (by

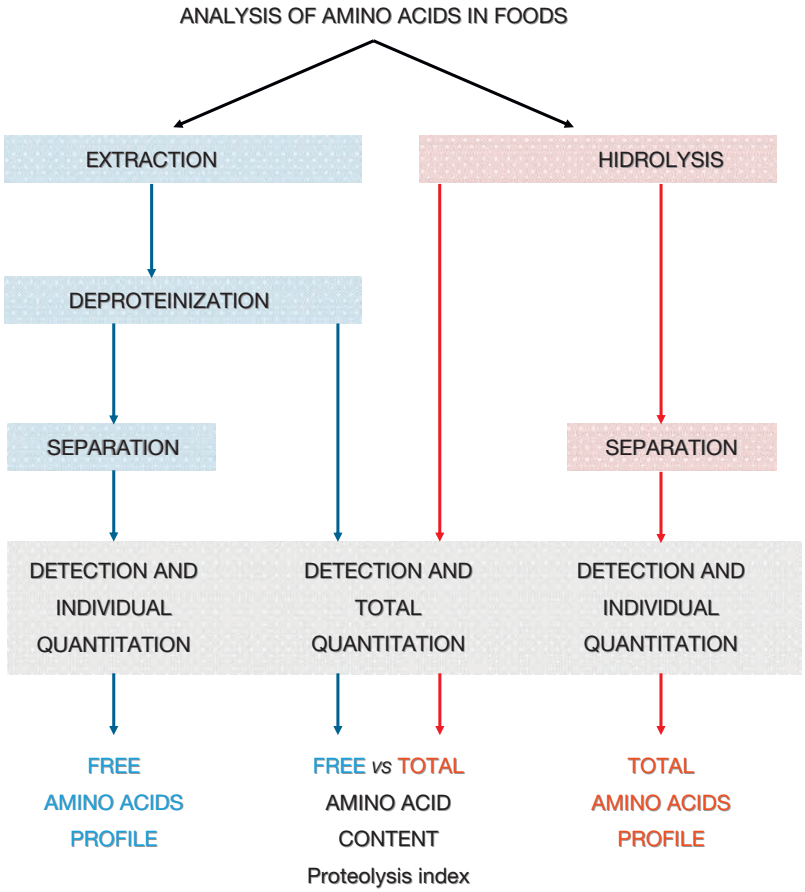

Figure 1 Flowchart showing the three general types for the analysis of amino acids in foods.

mixing two or three volumes of organic solvent with one volume of extract) are used. Under these conditions, proteins precipitate by denaturation, while free amino acids remain in solution. Some physical methods consist in the centrifugation through cutoff membrane filters (1000, 5000, 10 000, and 30 000 Da) that allow free amino acids through while retaining large compounds. All these methods give a sample solution rich in free amino acids and free of proteins.

Sample cleanups using cation-exchange resins (Amberlite, Rexyn, Biorex, Dowex, etc.) and C-18 minicolumns (Sep-Pack™ from Waters, Bond-Elut™ from Varian, etc.) have also been used. Cation-exchange supports retain amino acids under acidic conditions, while other interfering compounds, such as carbohydrates, are washed off the column. Amino acids are afterward eluted with a strong volatile base that will be removed by evaporation. C-18 minicolumns operate in a similar way to reverse-phase chromatographic columns, and thus, when the sample is percolated through the minicolumn, amino acids are eluted, while lipids, proteins, and peptides are retained. This is not a very feasible method because some hydrophobic amino acids may be retained while some polar proteins may elute together with the amino acids.

Total Amino Acids

The determination of the total amino acid profile can give information on the nutritional value of foodstuffs. Prior to the analysis, proteins must be hydrolyzed into their constituent amino acids (see Figure 1). The main hydrolysis methods are described in the following.

Acid hydrolysis that is based on acid digestion is the most common method for hydrolyzing proteins. Typically, samples are treated with concentrated (6 N) hydrochloric acid in a conventional oven (110 °C for 20–96 h or at 145 °C for 4 h) or in a microwave oven (at shorter times, usually <20 min). These temperatures in such acidic and oxidative media can degrade some amino acids, and thus, the elimination of oxygen by keeping a nitrogen atmosphere in sealed vials during hydrolysis in order to minimize degradation is strongly recommended.

Hydrolysis may be improved by optimizing the temperature and time of incubation and with the addition of amino acid oxidation protective compounds like 1% phenol and 0.1% sodium sulfite that prevent losses of the most labile amino acids like tyrosine, serine, threonine, and methionine. Indeed, protective agents improve the recovery of nearly all amino acids except tryptophan and cysteine.

Tryptophan is especially labile in samples having a high content in carbohydrates. Alternative hydrolysis with 4 N methanesulfonic or mercaptoethanesulfonic acid in samples with up to 20% of sugar content has been reported to improve tryptophan recoveries. Many authors recommend alkaline hydrolysis with 4.2 M of NaOH, KOH, LiOH, or BaOH, for 18 h at 110 °C for a better tryptophan determination, even in samples with a large amount of sugars (70–85%).

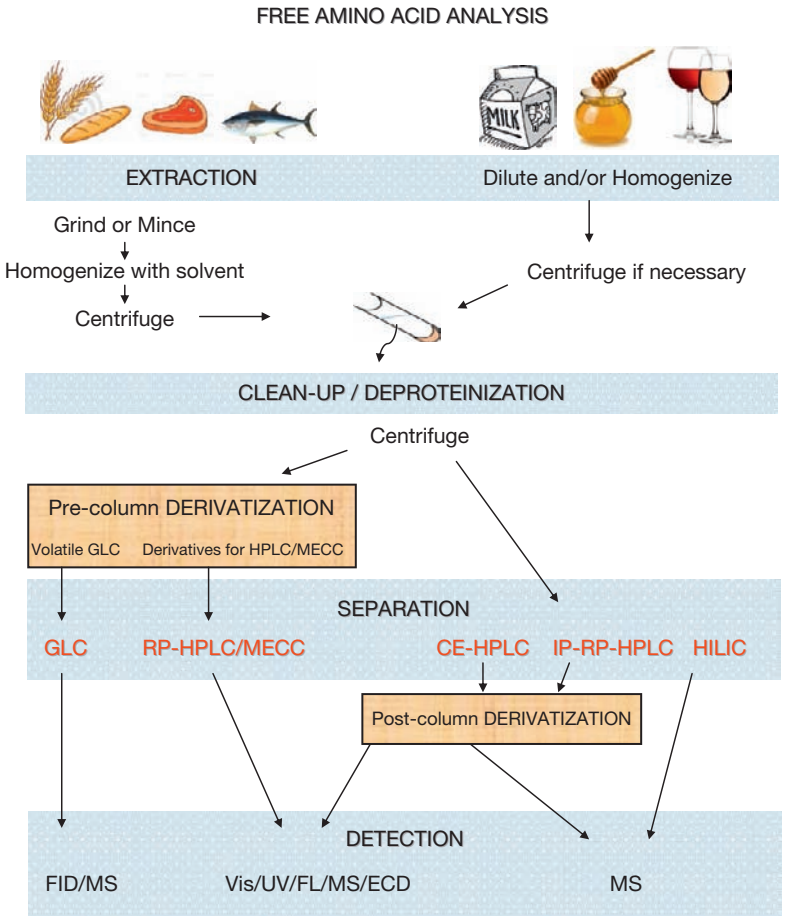

Figure 2 Flowchart showing the main steps and procedures for the analysis of free amino acids in foods.

Cyst(e)ine is partially oxidized during acid hydrolysis yielding several adducts, which makes its analysis difficult. The performic acid oxidation of cysteine to cysteic acid or the use of alkylating agents (3-bromopropylamine, 4-vinyl pyridine, iodoacetic acid, and 3,3′-dithiodipropionic acid) to stabilize cysteine before hydrolysis improves cysteine recovery.

Finally, glutamine and asparagine are deamidated during hydrolysis and are codetermined with glutamic and aspartic acids, respectively.

Derivatization

Once amino acids are extracted, cleaned up, or hydrolyzed, they must be separated from each other for their individual analysis. Before or after this separation, amino acids are usually derivatized to improve their separation or to enhance their detection. Several types of derivatives are obtained depending on the chosen separation and/or the detection technique.

Derivatives for Gas Chromatographic Separations

The conversion of amino acids into a volatile and thermostable molecule by means of substitutions in their polar moieties by nonpolar groups makes them apt to gas chromatographic (GC) analysis. Original reactions consisted in two stages: an esterification with an acidified alcohol (methanol, isopropanol, isobutanol, *n*-propanol, *n*-butanol, or isoamyl alcohol) followed by N-acylation using TFA anhydride, pentafluoropropionic anhydride, or heptafluorobutyric anhydride or silylation.

Silylation is the most prevalent derivatization technique. The products are generally more volatile and more thermally stable, and, in opposition to acylation, silylation normally does not require a purification step, and the derivatives can be injected directly into the GC. Nowadays, the silylating reactions have improved with the use of *N*,*O*-bis(trimethylsilyl) trifluoroacetamide or *N*-methyl-*N*(tert-butyldimethylsilyl)trifluoroacetamide that may permit a one-step derivatization procedure, with formed derivatives more stable to moisture. They constitute the basis of a methodology marketed by Sigma-Aldrich/Supelco (Bellefonte, PA, USA). There are a wide range of reagents available for the silylation of amino acids, which differ in their reactivity, selectivity, and secondary reactions and the nature of the reaction products. All these reactions require in more or less extent a water-free medium.

The use of alkyl chloroformates (methyl chloroformate, propyl chloroformate, etc.) allows the direct derivatization in aqueous solution even without prior removal of proteins. These derivatives are stable for up to 1 day at room temperature and for several days if refrigerated. The formed amino acid

derivatives will be afterward extracted with an organic solvent that will be injected directly into the gas or liquid chromatographs. This method is the basis of a commercially available procedure offered by Phenomenex (Torrance, CA, USA) and registered under the name EZ:faast kit.

Derivatives for Liquid-Media Separations

The derivatization of amino acids to be analyzed in liquid media like capillary zone electrophoresis (CZE) or high-performance liquid chromatography (HPLC) has another goal, that is, to enhance their detection by acquiring spectroscopic or electrochemical characteristics. By labeling the amino acids with reagents that allow ultraviolet absorption or add fluorescent properties to the molecule, not only sensitivity but also selectivity is improved in the detection.

With regard to derivatization for liquid chromatographic amino acid analysis, the derivatization reaction can be done after (postcolumn derivatization) or before (precolumn derivatization) the separation of the amino acids.

Postcolumn derivatization is achieved by the introduction of a suitable derivatizing reagent into the effluent system from the column, the flow of the combined liquids through a mixing manifold followed by a reaction coil, and the introduction of the derivatized amino acids through an on-line detector system. The reagents that have been usually employed for postcolumn derivatization are ninhydrin, for visible detection, and fluorescamine or *o*-phthalaldehyde, for fluorescence detection.

When using precolumn derivatization, the molecule formed improves sensitivity and selectivity for the detection, and also the derivatizing agent confers hydrophobicity to the amino acid molecule, making it suitable for separation by partition chromatography on a reverse-phase (RP) column. The most usual derivatizing agents are described in the following.

Phenylisothiocyanate (PITC). The methodology involves the conversion of primary and secondary amino acids to their phenylthiocarbamyl (PTC) derivatives, which are detectable by UV (λ 254 nm). The PTC amino acids are moderately stable. Sample preparation is quite laborious because it includes several drying steps. A 20 min reaction time is recommended for a complete reaction. PTC cystine shows a poor linearity that makes the quantitation of free cystine nonfeasible with this method. The selection of the column is critical for achieving a good resolved separation, especially when the analysis of physiological amino acids is pursued.

This method is available as a commercially prepackaged system named 'Pico-Tag' (Waters Associates, Milford, MA), which includes the analytic column, standards, and solvents. Until recently, PITC was one of the preferred precolumn derivatizing agents to analyze both physiological and hydrolyzed amino acids from foods and feeds by HPLC.

4-Dimethyl-aminoazobenzene-4′-sulfonyl chloride. The detection of the amino acid derivatives is by absorption in the visible range presenting a maximum from 448 to 468 nm. The high wavelength of absorption makes the baseline chromatogram very stable with a large variety of solvents and gradient systems. Derivatives are very stable (weeks) and can be formed from both primary and secondary amino acids. The reaction time is around 15 min at 70 °C. Reaction efficiency is highly matrix-dependent and variable for different amino acids, being especially affected by the presence of high levels of some salts (chloride). By-products originating from an excess of reagent must be minimized because they absorb at the same wavelength and appear in the chromatogram. The commercial 'System Gold/Dabsylation Kit' uses this technique (Beckman Instruments).

1-Dimethylamino-naphtalene-5-sulfonyl chloride (Dansyl-Cl). Dansyl-Cl reacts with both primary and secondary amines to give a highly fluorescent derivative (λ_{ex} 350, λ_{em} 510 nm), also detectable at UV (λ 250 nm). The dansylated amino acids are stable up to 7 days at −4 °C, if protected from light. The reaction time ranges from 1 h at 40 °C to 2 min at 100 °C. However, the reaction conditions (pH, temperature, and excess of reagent) must be carefully fixed to optimize the product yield and to minimize secondary reactions. This methodology reveals excellent linearity for cystine and also cystine-containing short-chain peptides.

9-Fluorenylmethyl chloroformate (F-MOC). This reagent yields stable derivatives (days) with primary and secondary amines. The derivative is fluorescent (λ_{ex} 265 nm and λ_{em} 315 nm). The reaction time is fast (45–90 s) and does not require any heating. The major disadvantage is the presence of the hydrolyzed reagent that is highly fluorescent and may interfere in the chromatogram. In order to avoid such trouble, it must be extracted with pentane or diethyl ether, as proposed in the automated AminoTag™ method (Varian Associates Ltd.), or converted into a noninterfering adduct prior to injection by reaction with a very hydrophobic amine such as 1-adamantylamine that gives a late-eluting noninterfering peak.

o-Phthaldialdehyde (OPA). This reagent reacts with the primary amine of amino acids in the presence of a mercaptan cofactor to give highly fluorescent derivatives (λ_{ex} 230 or 330 nm and λ_{em} 455 or 470 nm). OPA derivatives can be detected by UV absorption (λ 338 nm) as well. The choice of mercaptan can affect derivative stability, chromatographic selectivity, and fluorescent intensity; ethanethiol, 2-mercaptoethanol, and 3-mercaptopropionic acid are those most frequently used. The derivatization is fast (1–3 min) and is performed at room temperature. Derivatives are not stable, but this problem is overcome by standardizing through automation the time between sample derivatization and column injection. Some methods have been patented and commercially marketed (AutoTag OPA™, Waters Associates, Milford, MA, and AminoQuant™, Agilent Technologies, Palo Alto, CA). One of the main disadvantages of this procedure is the inability of OPA to react with secondary amines and thus it does not derivatize proline or hydroxyproline. This drawback can be overcome by combining OPA with other derivatization methods such as that based on F-MOC, which should take place sequentially. This is the basis of the AminoQuant system™ developed and marketed by Agilent Technologies (Palo Alto, CA). On the other hand, the cysteine yield is low and variable and several methods to improve its analysis will be described later.

6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). This reagent reacts with primary and secondary amines yielding very stable derivatives with fluorescent properties (λ_{ex} 250 nm and λ_{em} 395 nm), which are easily separated by RP HPLC. The main advantage of this reagent is that the yield and

reproducibility of the derivatization reaction are scarcely interfered by the presence of salts, lipids, and other compounds naturally occurring in food. The excess of reagent is consumed during the reaction to form aminoquinoline, which is only weakly fluorescent under the amino acid derivative detection conditions and does not cause any interference in the chromatogram. Reaction time is short (1 min), but 10 min at 50 °C would be necessary if a tyrosine monoderivative is required, because both mono- and diderivatives are the initial adducts from tyrosine. The fluorescence of the tryptophan derivative is very poor. Derivatives are very stable (1 week at room temperature). The methodology has been commercialized as a prepackaged kit by Waters Corporation as 'AccQ Tag.'

Derivatization reagents specific for cysteine are the Ellman's reagent 5,5′-dithio-bis-nitrobenzoic acid and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole, which react with cysteine giving a product showing a maximum of absorbance by 420 nm. These reagents are highly specific for cysteine and do not need a posterior chromatographic separation. Other reagent is *N*-(1-pyrenyl)maleimide, which reacts with free sulfhydryl groups to form fluorescent derivatives (λ_{ex} 230 nm; λ_{em} 376 nm) that will be separated by RP HPLC. A prederivatization sample treatment with dithiothreitol was suggested to reduce the cystine disulfide bonds and thus quantify it from the total cysteine determination.

Derivatives for laser-induced fluorescence (LIF) detection are fluorescein isothiocyanate, 3-benzoyl-2-quinolinecarboxaldehyde, and 4-fluoro-7-nitro-2,1,3-benzoxadiazole. LIF constitutes an alternative to fluorescence when looking for more selective and sensitive detectors for HPLC or micellar electrokinetic capillary chromatography (MECC), with a wide linear dynamic range (3 orders of magnitude) to cover new high sensitivity applications.

Some derivatization reagents permit the separation of racemic mixtures (D- and L-amino acids) by RP HPLC and MECC. It is the case of fluorescent chiral reagents like OPA plus *N*-isobutyryl-L-cysteine or *N*-isobutyryl-D-cysteine, OPA plus 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose, R(−)- and S(+)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(*N*,*N*-dimethylaminosulfonyl)-2,1,3-benzoxadiazoles, and 1-fluoro-2,4dinitrophenyl-5-L-alanine amide (Marfey's reagent). The quantitation of D-amino acid isomers is used as an indicator of the geographic origin of some foods, as a marker of the correct heat or alkali treatment, or as an index of microbial quality.

Derivatization reagents for electrochemical detection (ECD) are molecules with electroactive functional groups. All of them also have spectroscopic properties. The most used are OPA/mercaptoethanol or OPA/sulfite, 6-AQC, PITC, and naphthalene-2,3-dicarboxaldehyde. Sensitivity is of the order of fluorescent derivatives.

Separation

The analysis of individual amino acids needs a previous separation of each other unless a very selective detection is used. The separation of the individual amino acids in a mixture requires very efficient separation techniques as is liquid chromatography or GC or capillary electrophoresis.

Liquid Chromatographic (LC) Methods

Cation-exchange method

The separation is based on the amino acid charge. The most widely used is cation-exchange chromatography, which is based on the underivatized amino acid separation using cation-exchange resins as the stationary phase and citrate buffers as the mobile phase. After separation, amino acids react with ninhydrin, to obtain colored derivatives, or with either OPA or 4-fluoro-7-nitrobenzo-2,1,3-oxadiazole to obtain highly fluorescent derivatives with enhanced sensitivity.

There are many manufacturers (Beckman, Biotronik, Dionex, LKB, Pickering, etc.) who offer integrated commercial systems including the column, buffer system, and an optimized methodology having the advantage of the ease of use and reliability. The advantage of this method is the accurate results that make it as a reference method for amino acid analysis. Main drawbacks of this methodology are the high cost of the ion-exchange amino acid analyzer and its maintenance, the very complex mobile phase composition, and the long time of analysis.

Reverse-phase HPLC

RP HPLC separates the amino acids based on their hydrophobicity. This technique is widely used and has the advantage of requiring standard equipment able to be shared by different types of analysis. In this case, the previous amino acid derivatization not only is necessary to confer hydrophobicity to the amino acid molecule, making it apt for partition based on chromatography, but also allows the spectroscopic (ultraviolet or fluorescent) detection of amino acids.

If the choice of the derivative reaction is a challenge, the choice of the RP column is not an easy subject because of the great variability of commercially available RP columns. The most used column packaging consists of alkyl-bonded silica particles, mainly octadecylsilane. However, the selectivity obtained with this same stationary phase in columns from different manufacturers is different due to the particular chemistry employed in their manufacture.

Mobile phase composition combines an aqueous buffered phase with an organic phase constituted by acetonitrile and/or methanol and/or tetrahydrofuran. The buffer may be constituted by acetate or phosphate, but in the case of using mass spectrometry (MS) detection, volatile solvents like acetic and formic acids should be used. A finely adjusted gradient elution is often necessary when the overall amino acid profile from hydrolyzed and, especially, physiological amino acids has to be analyzed. Figure 3 shows a typical chromatogram of PTC-free amino acids from Spanish dry-cured ham.

Ion-pairing method

This procedure is applied to direct analysis of native (underivatized) amino acids. Ion-pairing (IP) technique consists in an RP HPLC where a contraion (ion with opposite charge to the amino acid ones) is added to the mobile phase in order to obtain an ion pair with charged amino acids with enhanced retention. The IP complexes are separated by RP as neutral molecules and ultraviolet or fluorescence detection after OPA or fluorescamine postcolumn derivatization. The most used ion-pairing reagent is lauryl sulfate. This technique has not

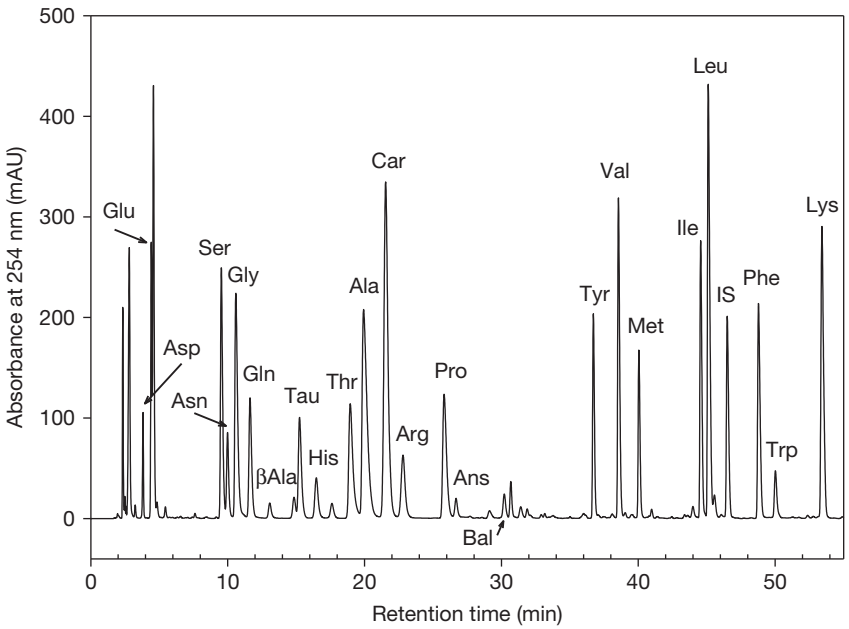

Figure 3 Reverse-phase HPLC chromatogram of free amino acids from dry-cured ham after PITC derivatization. Tau, taurine; Car, carnosine; Ans, anserine; Bal, balenine; IS, internal standard norleucine (unpublished data).

many adepts because of its complexity and lack of advantages when compared with other techniques. The current development of MS has reactivated the use of ion-pairing chromatography on amino acid analysis. Due to the requirements of MS, volatile ion-pairing reagents like perfluorinated carboxylic acids are used.

Hydrophilic-interaction chromatography

Hydrophilic-interaction chromatography (HILIC) employs polar stationary phases such as bare silica; amide, amino, hydroxyl, or zwitterionic on silica; and polymeric supports and RP-type solvents, but in this case, things work as normal phase when higher content of water implies greater elution strength. An initial mobile phase with a high content of organic solvent (mainly acetonitrile) is used to promote hydrophilic interactions between the analyte and the polar stationary phase. Under these conditions, polar solutes are retained, and with the aid of gradients in which the water amount is increasing, compounds will be gradually eluted. Volatile salts, ammonium acetate, and ammonium formate are used to provide some ionic strength to the system and to adjust the pH of the mobile phase with the advantage of being compatible with MS detection. This combination HILIC–MS, using narrow-bore columns (2.1 mm), permits the analysis of native (underivatized) amino acids as happened with IP–RP-HPLC method but with no need of IP reagents.

Micellar Electrokinetic Capillary Chromatography (MECC or MEKC)

The technique of CZE is an extremely efficient technique for separations of charged solutes. The high efficiency, the speed, and the low requirements of the sample amount make this technique very interesting when compared with classical electrophoresis and chromatographic techniques. The difficulty of separating amino acids by this technique relies on their structure. Amino acids constitute a mix of basic, neutral, and acidic constituents, and even though a particular pH can significantly improve the resolution of one kind, it is likely to cause overlap with the others. The primary limitation of CZE is its inability to separate neutral compounds each other. A modified version in which surfactant-formed micelles (sodium dodecyl sulfate, dodecyltrimethylammonium bromide, Tween 20, and octylglucoside) were included into the running buffer to provide a two-phase chromatographic system for separating neutral compounds together with charged ones has been developed. This technique has also been termed MECC. With few exceptions, it is used to derivatize the amino acids (Dansyl-Cl, PITC, phenylthiohydantoin, and OPA), to improve separation, and to enhance detection.

Nevertheless, separation of amino acids with this technique has not reached the resolutive power obtained through HPLC methods. The reported applications are limited to a reduced number of amino acids separated in the same electropherogram. For this, the use of MECC for the analysis of amino acids in food has not gained widespread being more restricted to clinical diagnosis, neuroscience, or metabolomic studies, in which very specific, feasible, and sensible methods are generally required, in which the sample amount in some cases is scarce, and where the economic cost is not so important in relation to the obtained benefits. Even though the current trend to miniaturizing analytic techniques suits well with the CE methodologies, the applications are more directed toward biomedical and space exploratory issues than to food.

GC Methods

Since the development of RP HPLC in 1990s, the use of GC for the analysis of amino acids was dropped. Recently, the emergence of new and improved derivatives of amino acids

(described earlier) and the wide dissemination of MS detection that can be interfaced efficiently with GC instruments have revived its use for analyzing complex mixtures of amino acids.

The very high-resolution capacity, especially since the capillary columns appeared, is the main advantage of GC in relation to the LC techniques. This high-resolution capacity allowed the separation of complex mixtures of protein plus nonprotein amino acids from food samples or the simultaneous analysis of amino acids and other food components like amines, organic acids, and sugars. GC is not very expensive because no solvent is used, and the equipment is very versatile and naturally available in an analytic laboratory. The connection with MS detectors greatly increases not only its specificity and efficiency but also its price.

Detection Techniques

Separated amino acids peaks should be detected for their quantification. There are some selective detectors for GC systems and others for liquid systems (flow injection analysis (FIA), HPLC, and MECC), while MS may be used with either gas or liquid systems.

Among the detectors specific for GC, the flame ionization detector (FID) is a universal detector and is the most widely used, while thermionic N-P or flame photometric detector is selective toward organic compounds containing phosphorous and nitrogen like phosphoserine, phosphothreonine, and phosphotyrosine being much more sensitive than FID for such compounds. The main advantage of these detectors is their high sensitivity and wide linear range.

Detectors more used for liquid systems are spectroscopic or electrochemical.

Amino acids, in their native form, absorb at 210 nm, which is a very unspecific detection wavelength. Only three amino acids (phenylalanine, tyrosine, and tryptophan) possess a chromophore moiety, which confers a suitable maximum absorbance for more specific ultraviolet detection (280 nm for tyrosine and tryptophan and 254 nm for phenylalanine). Tryptophan also possesses native fluorescence ($\lambda_{ex} = 295$ nm, $\lambda_{em} = 345$ nm) that facilitates a more selective detection. Apart from these, the spectroscopic detection of amino acids requires their previous derivatization to obtain a visible or ultraviolet-absorbing or fluorescent molecule. A wide explanation of the different possibilities in pre- or postcolumn derivatization was given earlier.

ECD is based upon the electrical properties of a solution of analyte when it forms part of an electrochemical cell. Only amino acids with aromatic rings or sulfur-containing side chains are enough electrochemically active to be detected by this method. Other amino acids need an electrochemically active compound to be attached to them (some of them were mentioned earlier).

MS is based in the conversion of components of a sample into rapidly moving gaseous ions that can be resolved on the basis on their mass-to-charge ratios that is characteristic of each ion and allows its identification. The identification of the proteinogenic amino acids may not be a problem, but this detector increases its value in the analysis and identification of nonprotein amino acids in complex matrices. Despite the high cost of purchase and maintenance of mass spectrometers, their use for analysis in food industry and/or food control is each time more often.

MS detectors were firstly connected to GC equipments. A good compatibility between both techniques, especially when capillary columns were available, permitted the rapid development and the onset of these complementary techniques.

Due to its high specificity, MS detectors coupled with HPLC or MECC do not require amino acids derivatization, an additional advantage, which means a minor sample manipulation and, due to its high specificity, a reduction of the problems related to the analysis of coeluted peaks. In these cases, volatile solvents and running buffers should be used. One of the main requirements for samples to be analyzed by MS is that analytes, amino acids in this case, must be ionized. The atmospheric pressure ionization by either microwave-induced plasma ionization, electrospray ionization, or chemical ionization has shown to be optimal interfaces between LC or MECC and tandem mass (MS/MS) detectors for the analysis of amino acids in biological samples.

See also: Amino Acids: Metabolism; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Food Composition Databases; Proteins: Chemistry, Characterization, and Quality.

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Amino Acids: Metabolism

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Introduction

Transcription and translation are the backbone of molecular biology that carries the information from DNA (genes) to mRNA to synthesize more than 20 000–25 000 proteins (in more than 200 different cell types) using the 20 standard L-amino acids (AA) present in proteins universally.

In living organisms, there are about 300 of nonstandard AA (not all of them are found in the proteins, but have specific functions). Almost all of that nonstandard AA emerge from posttranslational enzyme modifications of standard AA (e.g., 4-hydroxyproline and 5-hydroxylysine in the collagen, desmosine in elastin as a derivative of four lysine residues, and γ-carboxyglutamate as constituent of a blood-clotting protein).

The exceptions are two nonstandard AA, designated as 21 and 22: selenocysteine (found in archaea, eubacteria, and animals, including mammals) and pyrrolysine (found in certain archaea and eubacteria). Both selenocysteine and pyrrolysine are encoded by RNA codons that signify a command to stop translation of mRNA into protein (UGA for selenocysteine and UAG for pyrrolysine) and incorporated directly into these proteins during the normal process of translation (there is a specific tRNA for these AA).

From 1806 when the first AA (asparagine) was discovered from asparagus shoots to 1938 when Rose discovered threonine, the last of the 20 AA, the interest for AA permanently increased in chemistry, biology, and medicine, from physiology to pathology, nutrition, metabolism, and especially the role that AA play in the regulation of fundamental cellular processes.

Sources of AA and Nutritional Requirement

While most bacteria and plants can synthesize all 20 AA, mammals can only synthesize 10. Those that can be synthesized and are not needed in the diet are called nonessential AA, and those for which a synthetic pathway does not exist and must be obtained from food (Table 1) are called essential AA. The latter are also essential for maintaining nitrogen balance and are indispensable in growth, whereas the former are not essential (dispensable) to mammals, birds, and fish. In some cases, this is a rough division. The fact that a synthetic pathway exists for a given AA does not necessarily mean it will be synthesized in sufficient amounts to satisfy the normal requirement (arginine, proline, and glycine). For example, arginine biosynthetic pathways do not have the capacity to compensate for depletion or inadequate dietary supply in adult rats and humans to meet the metabolic demand under certain conditions (e.g., normal growth and development of children, pregnancy, lactation, burns, injury, infection, and stress). Thus, with regard to dietary requirements, L-arginine is classified as a semiessential or, better, a conditionally essential AA.

In different organisms, the need for AA and their sources are widely different, especially in eukaryotes. Not only can most microorganisms synthesize all AA, but also they can uptake them from the environment and use in metabolic processes, including oxidation and energy production. Plants synthesize all AA, use them for synthesizing proteins and other important biomolecules, and almost never (except in stress conditions) use as an energy source. Animals can only synthesize nonessential AA. They do not store AA as glucose or fatty acids and use them in the biosynthetic processes, depending on conditions (e.g., during starvation, skeletal muscle AA become the principal source of substrates for gluconeogenesis in the liver). Besides that, herbivores satisfy most of their energy demand (90%) through oxidizing AA, while that contribution is much smaller with carnivores, including humans (15%), and strongly depends on the presence of proteins in the diet.

Digestion and Intracellular Degradation of Proteins

Dietary proteins are an important source of AA in humans. Degradation of ingested proteins occurs in the gastrointestinal tract by the action of proteolytic enzymes (proteinase) that break the long polypeptides of proteins into shorter fragments (peptides) and finally into AA. The proteolytic digestion was initiated in the stomach (pepsin) where the proteins are only partially digested and continues in the small intestine, mainly with proteolytic enzymes secreted by acinar cells of the pancreas as inactive precursor (trypsinogen, chymotrypsinogen, proelastase, and procarboxypeptidase). After activation in the small intestine (duodenum), together with proteinases secreted by enterocytes (epithelial cells of the small intestine), pancreatic proteases convert proteins into free AA, which are easily absorbed by the cells of the intestinal epithelium.

Both enterocytes and other cells in the body uptake AA by specific transporters in the plasma membrane. The transport of AA is an energy-requiring process. The fact that there are ten transporters for twenty AA suggests that some of these carry more than one AA. Distribution and properties of transporters are highly specific for the cells and tissues.

All cellular proteins have specific half-lives, including transcription factors, structural proteins (membrane), or enzymes. Some time, the half-lives of proteins depend on the lifetime of a cell (110 days for hemoglobin in the erythrocytes) and generally vary from 30 to 60 s to a few months. In addition, all abnormal or modified (oxidatively) proteins are promptly degraded in the cells by ATP-dependent cytosolic systems, in both bacteria and eukaryotes and lysosomal–autophagic and ATP-dependent ubiquitin–proteasome systems in eukaryotic cells. This is a very important source of AA in humans, and if a variable, it contributes to the overall pool of AA of more than the amount of food ingested (adult humans normally turnover about 2% of their proteins per day).

In addition to the two earlier-mentioned sources, hydrolysis of the digestive enzymes secreted by the small intestine and

pancreas and from dead microorganisms in the intestine (especially in the colon) contributes to the total pool of AA in the organism.

Metabolism of AA: Catabolism and Anabolism

Oxidative Transdeamination of AA

The metabolism of AA is strongly coupled with the metabolism of amino groups. The main chemical characteristic of AA, the presence of the α-amino group (α-NH_2), is one of the key features in their catabolism and anabolism. In the first case, a metabolic strategy is to remove the 'excess' of amino groups because ammonia is toxic and, in the second case, to provide a source of amino groups not only for the biosynthesis of AA but also for other important biomolecules, particularly nucleotides.

Table 1 Essential and nonessential AA in humans

Essential	*Nonessential*
Arginine	Alanine
Histidine	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Lysine	Glutamate
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine

Catabolism (oxidative degradation) of AA normally occurs in our cells, and its intensity increases when we consume food rich in protein (overcomes the need for protein synthesis) or in pathological conditions when cellular proteins (e.g., muscle proteins) were used as fuel (starvation and untreated diabetes).

Different organisms have a variety of strategies for the excretion of the excess amino groups. It depends on the type of habitat and water availability. Thus, the main excretory form of nitrogen in most microbes and aquatic vertebrates is ammonia (as ammonium ion (NH_4^+)), urea for many terrestrial vertebrates and sharks, and uric acid in birds and terrestrial reptiles.

In humans, the liver is the major organ for catabolism of AA. The overall process involves three major steps: transamination in the cytoplasm of hepatocytes, oxidative deamination in the mitochondria of hepatocytes, and degradation of the corresponding α-keto acid analogs of AA (Figure 1).

The metabolic strategy of transamination reactions is to channel amino groups into only a few products. Enzymes that catalyze the transamination reactions are named transaminases (aminotransferases). All transamination reactions are freely reversible and all transaminases have the same prosthetic group, pyridoxal phosphate. The most common acceptor of α-amino groups in transamination reactions is a α-ketoglutarate. Thus, the amino groups of different AA are channeled to glutamate (e.g., alanine aminotransferase catalyze the transfer of α-amino group of alanine to α-ketoglutarate giving glutamate and pyruvate as a corresponding α-keto analog) (Figure 1).

Glutamate in hepatocytes is transported from the cytoplasm into mitochondrial matrix via the glutamate–aspartate transporter of inner mitochondrial membrane. Then, glutamate

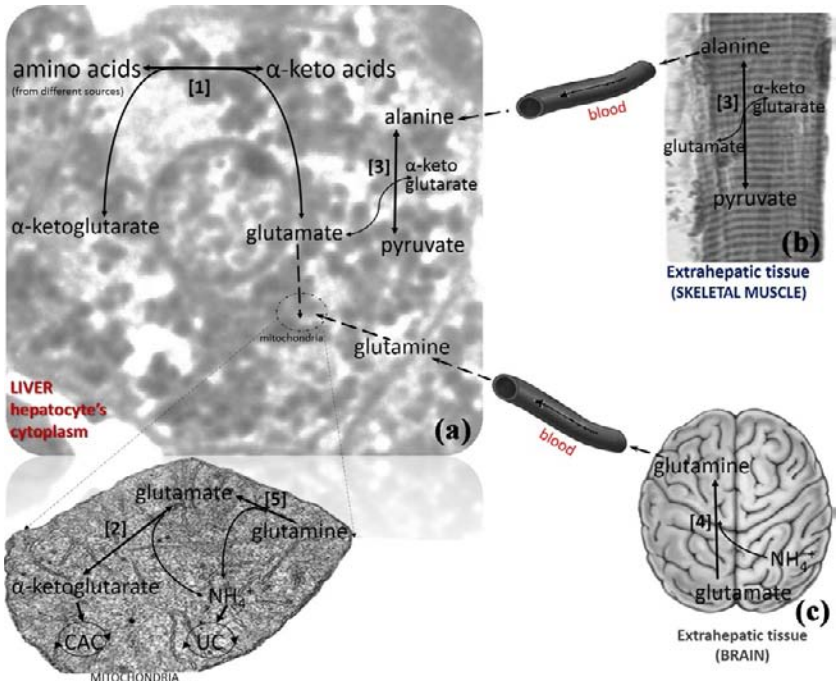

Figure 1 Metabolic flow of amino groups. (a) Transamination and oxidative deamination reactions (transdeamination) in liver cytoplasm and mitochondria (enlarge detail: liver mitochondria). Transporting route of ammonia from extrahepatic tissues in the form of (b) alanine (e.g., the muscle) and (c) glutamine (e.g., the brain). (1) Aminotransferases (transaminases), (2) glutamate dehydrogenase, (3) alanine aminotransferase, (4) glutamine synthetase, (5) glutaminase. CAC, citric acid cycle; UC, urea cycle.

undergoes oxidative deamination catalyzed by enzyme glutamate dehydrogenase. The first product of glutamate deamination is ammonium ion that enters the urea cycle (including ammonia from extrahepatic tissues and ammonia that leads to the liver via the portal vein from the intestine). Urea cycle takes place in the liver, starting in the mitochondria of hepatocytes, the only place where free ammonia is released. The second product, α-ketoglutarate, goes to the citric acid cycle, where it can be completely oxidized or be used in gluconeogenesis for the synthesis of glucose. Glutamate dehydrogenase reaction is a readily reversible (Figure 1).

Because ammonia is toxic to animal tissues, especially the brain, the excess is transported from extrahepatic tissues in the bloodstream in the form of glutamine. In the extrahepatic tissues, the enzyme glutamine synthetase catalyze the reaction of synthesis of glutamine from glutamate and NH_4^+. Glutamine is transported through the blood to the liver (also intestine and kidneys) where in the mitochondria of cells of these tissues, the amide nitrogen of glutamine is released as ammonium ion by enzyme glutaminase. In the second way of the ammonia transport in nontoxic form from the muscle and some other extrahepatic tissues to the liver, alanine serves as a carrier. Alanine aminotransferase in the muscle catalyzes conversion of pyruvate to alanine with glutamate as the amino group donor. In the liver, the same enzyme (alanine aminotransferase) catalyzes the reverse reaction, converting alanine to pyruvate with α-ketoglutarate as the amino group acceptor (Figure 1). In that way, a metabolic burden is transferred from the muscle to the liver, where the ammonia transferred from the muscles enters urea cycle, and pyruvate is used in gluconeogenesis (glucose–alanine cycle).

Pathways of AA Catabolism and Anabolism

Catabolism and anabolism of AA have some common characteristics. The same coenzymes are used in both paths. The final products of catabolic pathways and metabolic precursors of anabolic pathways are intermediates of glycolysis, citric acid cycle, and pentose phosphate pathway.

In catabolism, the next step (after deamination) is oxidative degradation of α-keto acid analogs of AA. They are oxidized to CO_2 and H_2O in the mitochondria of the liver, small intestine, muscle, and kidney. These tissues play the major role in catabolic processes, with dominant participation of the liver.

Catabolic pathways of 20 AA are a classic example of convergent metabolic pathway. All individual pathways flow into citric acid cycle across six common intermediates: acetyl-CoA, pyruvate, α-ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate. In this way, many catabolic pathways of AA are simplified, but functionally integrated with the energy metabolism of glucose and fatty acids (Figure 2).

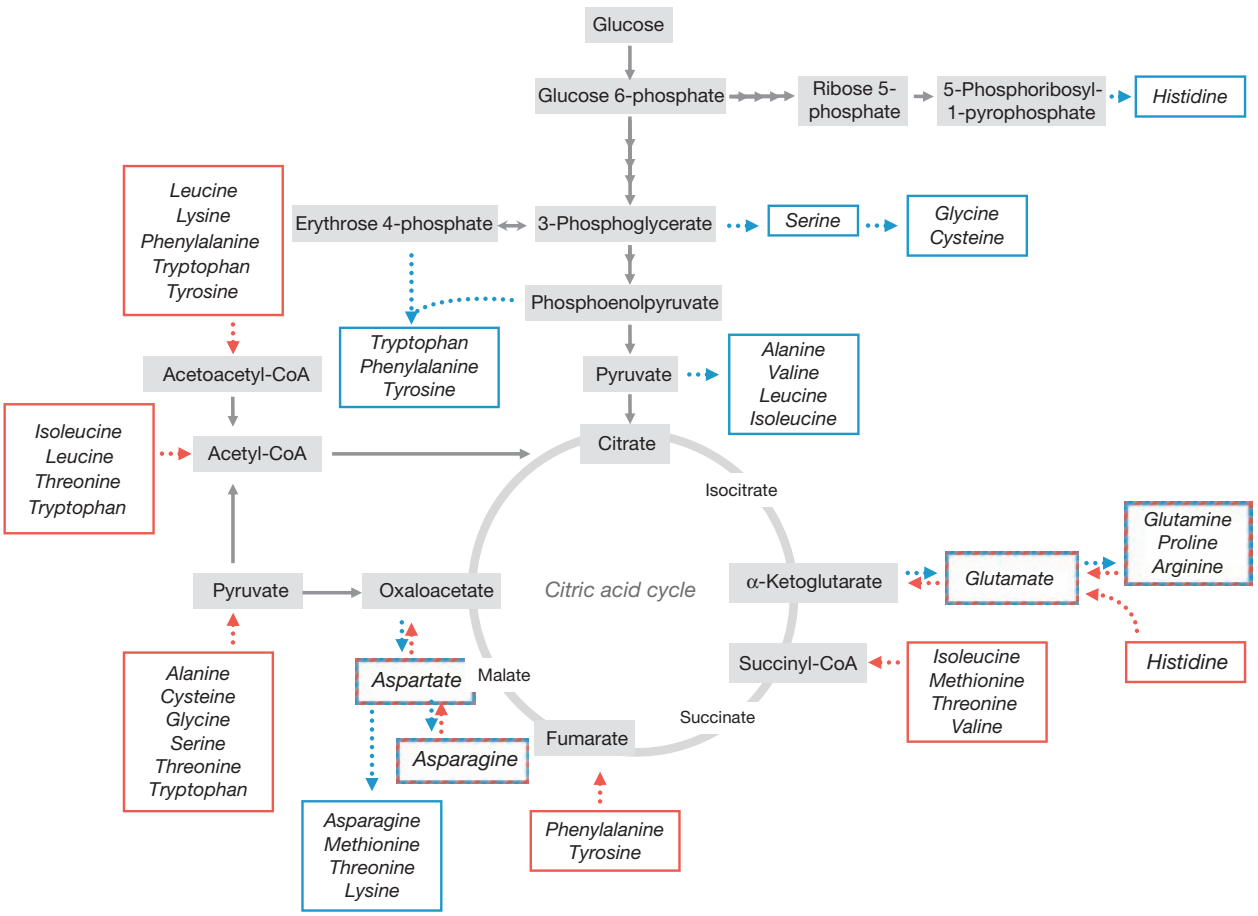

Figure 2 General scheme of AA catabolism and anabolism (red arrows represent catabolic and blue arrows anabolic pathways).

Complete oxidation of AA contributes to the production of ATP (in this case, CO_2 is the end product). This contribution is variable and depends on the presence of AA in the diet. If the amount of AA exceeds the needs for protein synthesis and ATP production, their carbon skeletons are diverted to other products: *de novo* synthesis of glucose (gluconeogenesis), fatty acids, or in specific conditions ketone bodies (ketogenesis).

Some AA gives only one final intermediate (e.g., asparagine and aspartate give only oxaloacetate and lysine and leucine only acetyl-CoA), but some more than once, reflecting different catabolisms of their carbons (e.g., phenylalanine and tyrosine give both fumarate and acetyl-CoA and threonine gives succinyl-CoA, pyruvate, and acetyl-CoA).

Those AA that are completely or partially degraded to acetyl-CoA (or acetoacetyl-CoA) can participate in ketogenesis, contributing to the synthesis of ketone bodies. These AA are referred to as ketogenic. Similarly, those AA that give the remaining five products (pyruvate, α-ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate) are called glucogenic AA, since they can be converted to glucose, by gluconeogenesis. This division is not exclusive. Some AA are also ketogenic and glucogenic. Only leucine and lysine are solely ketogenic AA, because they are broken down to acetyl-CoA only (recall that carbons of fatty acids do not contribute directly to gluconeogenesis because they break down entirely to acetyl-CoA, but indirectly contribute, providing energy in the form of ATP for glucose synthesis). In uncontrolled diabetes mellitus and prolonged starvation, tissue (muscle) proteins are degraded, and AA are used to provide precursors for gluconeogenesis and ketogenesis.

Such as the catabolism of AA is a convergent process, anabolism is an example of divergent metabolic process, with the starting points in the glycolytic (3-phosphoglycerate, phosphoenolpyruvate, and pyruvate), citric acid cycle (oxaloacetate and α-ketoglutarate), and pentose phosphate intermediates (ribose 5-phosphate and erythrose 4-phosphate) (Figure 2).

As we mentioned earlier, there are great variations in the degree of AA synthesis in different organisms. In contrast to bacteria and plants, humans can synthesize only a half of the twenty, that is, nonessential AA. Humans get the second half, essential AA, by food intake. Their synthetic pathways are complex, specific in different organisms, contrary to nonessential AA, which are synthesized in a few (often one) simple steps.

One of the important determinants of anabolism of AA is the incorporation of nitrogen (amino group) in biosynthetic intermediates. Glutamate and glutamine have major roles in these processes: glutamate via transamination reactions, with pyridoxal phosphate as a prosthetic group, and glutamine in the reactions catalyzed by enzymes glutamine amidotransferases. Please do not confuse that with glutaminase in the mitochondria that catalyzes the release of free ammonium ion and glutamate from glutamine; amidotransferases have two domains: One binds glutamine and the other domain is the acceptor of gamma-amido nitrogen of glutamine.

The second equally important determinant of AA metabolism is the transfer of one-carbon group in different oxidation states: in most oxidized state (CO_2) with biotin as a cofactor, intermediate states (methylene, methenyl, formyl, formimino, and also methyl groups) with tetrahydrofolate as a cofactor, and *S*-adenosylmethionine, which is the predominant cofactor for transfer one-carbon unit as the most reduced state – methyl group.

Organ and Interorgan Specificity of AA Metabolism

Although the liver is the central site of AA metabolism, these processes are important and occur in all other tissues. Moreover, the cooperation between the tissues is essential for the integration of the metabolism of AA. We will focus only on some illustrative cases.

Catabolism of branched-chain AA (BCAA) (leucine, isoleucine, and valine) does not primarily occur in the liver, but in the muscle, adipose tissue, kidney, and brain, because these extrahepatic tissues contain the enzyme branched-chain aminotransferase, which is absent in the liver. The products, corresponding three α-keto acids, are either oxidized in the myocytes (the branched-chain α-keto acid dehydrogenase complex catalyzes oxidative decarboxylation to CO_2 and acyl-CoA derivative) for ATP production or released into the blood and oxidized in the liver.

Quantitatively, the muscles (40% of the body mass) contribute most to the metabolism of BCAA. On the other hand, BCAA could have cell-signaling properties on protein metabolism, predominantly in the muscle. Namely, the administration of BCAA or leucine alone induces anabolic effect in humans via a reduction in protein breakdown without increasing protein synthesis.

The separation of BCAA metabolism in the muscle (and other extrahepatic tissues) allows the segregation of that part of the nitrogen metabolism, bypassing the channeling of amino groups to urea cycle in the liver. In that way, the amino group of BCAA transamination reactions in the muscles is used for the synthesis of glutamine, over α-ketoglutarate and glutamate. The same metabolic pattern of BCAA in the adipose tissue, directed to glutamine, opens a new and remarkable function of adipocytes: providing glutamine for immune cells (lymph nodes in adipose tissue) and for rapidly proliferating cells in the bone marrow (very rich in adipocytes).

The small intestine not only is a place of digestion and absorption of AA from food but also, in varying degrees, has an important role to play in their catabolism and also for uptake and catabolism of AA from the circulation. Some AA are metabolized to a great extent in the enterocytes of the small intestine, particularly glutamine, glutamate, aspartate, asparagine, proline, and arginine. The physiological significance of their catabolism is to provide ATP, not only in the small intestine but also in epithelial cells of colon. This provides support for the epithelium of the digestive tract for digestive and absorptive functions, maintenance of the cell structural integrity, and physical barriers. On the other hand, the metabolism in the small intestine provides a constant concentration of AA in circulation, especially those that are neurotransmitters (e.g., glutamate and aspartate).

Enterocytes of the small intestine have a special place in the interorgan metabolism of arginine, either taken from the lumen and circulation or synthesized from glutamine, glutamate, and proline. In enterocytes, arginine is metabolized to citrulline. Citrulline, as the only product, is released into the

bloodstream and taken up by virtually all extraintestinal cell types (except hepatocytes), where it is converted back into arginine. Quantitatively, the kidneys contribute most to this process. Arginine, synthesized in the kidney, is released into the circulation. Such interorgan arginine metabolic profile avoids the liver and degradation in the urea cycle. In this manner, a constant level of arginine is maintained in the bloodstream and is available for the other cells.

Functions of AA

Besides the well-known role they play in the regulation of the metabolism (e.g., urea synthesis, transfer of reducing equivalents from the cytoplasm to mitochondria and vice versa, nutrient transport, activation of lipolysis and glucose oxidation, methylation of DNA and proteins, and one-carbon transfer), AA are precursors of many specialized biomolecules and play an important role in the regulation of several physiological processes. In recent years, there has been growing interest in the role of AA in the regulation of growth, reproduction, immunity, digestive functions, lactation, blood flow, cardiovascular function, thermogenesis, osmoregulation, appetite, apoptosis, aging, antioxidant defense, synthesis of neurotransmitters, spermatogenesis, hormone secretion, etc. AA enhance insulin secretion from pancreatic β-cells (leucine and phenylalanine), stimulate β-cell neogenesis through the activation of the transcription factors (arginine), and participate in the activation of insulin signaling pathway (leucine).

AA achieve many of these functions by participating in cell signaling via kinases: mammalian target of rapamycin, AMP-activated protein kinase, cGMP-dependent protein kinase, cAMP-dependent protein kinase, and mitogen-activated protein kinase or G protein-coupled receptors. In addition, AA may enhance the transcription of many genes containing an AA response element (particularly in a state of deficiency), directly participate in the regulation of gene expression, alter the levels and biogenesis of micro-RNA, and alter cell redox status and synthesis and bioavailability of gasotransmitters.

AA and Redox Biology

AA play an important role in maintaining the redox balance of the cells, tissues, and whole organism. Studying oxidants, antioxidants, the redox active molecules, and redox regulation is important in both physiological and pathological conditions. With a lot of reasons, we can talk about the development of a new scientific discipline, redox biology. We will consider some important aspects of redox biology in which AA are key players.

Cysteine, Selenocysteine, Glutathione, and Glutathione Peroxidase

Cysteine is a naturally occurring AA that is found only in small quantities in most proteins. It is the only AA that contains thiol in its side group. So, cysteine is an important source of sulfur in human metabolism, and although it is classified as a nonessential AA, it may be essential for infants, the elderly, and individuals with certain metabolic diseases. Thiol group is a responsible for a number of important functions of cysteine, such as allowing the formation of disulfide bonds that are crucial to defining the structures of many proteins, stabilizing extracellular proteins, conferring proteolytic resistance, and enabling catalytic properties of enzymes.

Like most thiols, cysteine undergoes a variety of redox reactions. Oxidation by removal of hydrogen of cysteine produces the previously mentioned disulfide cysteine. This reaction is reversible, as reduction of this disulfide bond regenerates two cysteine molecules. More aggressive oxidants produce sulfinic acid or sulfonic acid. The cysteine thiol group is also nucleophilic and thus can undergo addition and substitution reactions.

Due to its ability to undergo redox reactions, cysteine has antioxidant properties and it has been shown that cysteine itself is the major extracellular antioxidant. Further examples of cysteines' critical role in redox balance can be found in other enzymatic systems including the multiple enzymes involved in the maintenance of peroxiredoxins, glutaredoxins, and thioredoxins among others. The natural role of cysteines as redox sensors is further witnessed by the observation that throughout evolution, cysteines are found in transcriptional regulators that are modulated by oxidative stress such as redox-sensitive transcriptional activator (OxyR) and the nuclear factor erythroid 2-related factor 2/kelch-like ECH-associated protein 1 (Nrf2/Keap1).

Cysteine is also a major determinant of intracellular redox conditions. Namely, along with glutamic acid and glycine, cysteine is a precursor of glutathione (GSH), which is the body's most important low-molecular-mass intracellular antioxidant.

Compared to cysteine, GSH is less susceptible to oxidation, which makes it an ideal candidate for the maintenance of the intracellular redox state. There are two forms of GSH: reduced (thiol, GSH) and oxidized (disulfide, GSSG). The reduced form of GSH is dominant in the cell, while the oxidized form makes up $<1\%$ of the total food daily intake GSH.

Biological activity of GSH is enabled by cysteine's thiol group that serves as proton donor. Apart of maintenance of cell redox milieu in reduced state, GSH–GSSG redox couple achieved an essential role in the regulation of the function of many proteins by modification of the cysteine residues. Specifically, it regulates the formation of reversible intra- and intermolecular disulfide bonds directly or indirectly through the process of protein *S*-glutathionylation. *S*-glutathionylation is a reversible posttranslational modification of proteins, achieved by the formation of disulfide bonds between cysteine motif in the protein and the cysteine in GSH, which can lead to change in protein function, localization, and stability.

Taking into account the fact that GSH is less susceptible to oxidation of cysteine, GSH plays an important role in the storage and transportation of this AA. This role of GSH is achieved through the so-called saving cycle, in which outside of the cell, γ-glutamyl transpeptidase catalyzes the transfer of γ-glutamyl GSH motif on one of the AA. The remaining cysteinylglycine may be decomposed to the extracellular cysteine and glycine by cysteinylglycine dipeptidase or transported as such in the cell, rehydrolyzed, and used for the synthesis of GSH.

Although the level of intracellular cysteine depends on the type of tissue and the diet, the level of cysteine is constantly

lower than the level of glutamate and glycine, and due to that, the cysteine is limiting AA for the GSH synthesis. The liver is unique in that it has the ability to convert methionine to cysteine in its path known as transsulfuration. Also, the liver is supplied with cysteine from the proteins in nutrition. For other tissues, a major source of cysteine represents GSH and GSSG from plasma.

The analog of cysteine having the same structure as that of cysteine, but in which sulfur atom is replaced by selenium forming selenium-containing selenol group in place of the sulfur-containing thiol group, is designed as selenocysteine. It is the twenty-first proteinogenic AA with a lower pK_a and a higher reduction potential than cysteine. These properties make it very suitable in proteins that are involved in antioxidant activity, such as glutathione peroxidases (GSH-Px) and thioredoxin reductases. It is also present in a number of enzymes like tetraiodothyronine 5′-deiodinases, formate dehydrogenases, glycine reductases, selenophosphate synthetase 1, methionine-*R*-sulfoxide reductase B1, and some hydrogenases. Proteins having more than one selenocysteine residues are called selenoproteins; those with catalytic activities that depend on selenocysteine's biochemical activity are called selenoenzymes.

There is no single free pool of selenocysteine AA that exists within cells to be used. So, it means it is an essential AA that is needed to be provided through food.

The first selenoprotein identified in mammals is GSH-Px, an enzyme that protects the cell from oxidative damage by catalyzing the reduction of H_2O_2, lipid peroxides, and other organic peroxides, using GSH as the reducing substrate. There are five types of mammalian selenium-containing GSH-Px, which catalyze oxidation of the reduced selenocysteine residue by hydroperoxide forming a selenic acid, which is further converted to a selenyl sulfide by GSH. An additional GSH reacts with the enzyme and regenerates the reduced selenol.

AA and Gasotransmitters

Gasotransmitter refers to a gaseous messenger molecule involved in any signaling process. Molecular mechanisms whereby they signal to their targets, by chemically modifying intracellular proteins and affecting cellular metabolism in an immediate fashion, are the most remarkably unique feature of gasotransmitters. The first identified gasotransmitter is nitric oxide (NO), while recently, carbon monoxide (CO) and hydrogen sulfide (H_2S) have been established as members of the gasotransmitter family. Their signaling involves a diverse range of biological targets and the cellular effects resulting from these molecular interactions are clearly seen in cardiovascular, neuronal, gastrointestinal, immune, and genitourinary systems.

NO, CO, and H_2S are produced in human cells from L-arginine, heme (glycine), and L-cysteine by specific enzymes: NO synthases (NOS); heme oxygenases (HO); and cystathionine-β-synthase, cystathionine-γ-lyase, and 3-mercaptopyruvate sulfurtransferase, respectively. It has been shown that the aforementioned (and other) AA not only act as precursors for gasotransmitter production but also regulate their production. For example, as an important substrate for arginine synthesis, glutamine has an important influence on whole body NO synthesis. Besides, glutamate and glycine activate neuronal NOS-dependent NO synthesis, through binding the different sites of *N*-methyl-D-aspartic acid receptor and increase in Ca^{2+} influx. The stimulatory effect of gamma-aminobutyric acid on NO synthesis has been shown in both hypothalamic neurons and ischemic brains. In contrast, reduction of NO synthesis has been shown by homocysteine in endothelial cells, glycine in endotoxin- or cytokine-challenged liver cells, and taurine in various cell types including hepatocytes, macrophages, and glial cells.

Apart from regulating NO synthesis, arginine is a key physiological regulator of CO production. Precisely, it was shown that dietary L-arginine increases the expression of HO3 in white adipose tissue in diet-induced obese rats and HO1 expression in vascular smooth muscle cells. Glutamate and sulfur AA increase CO production in multiple cell types, as well as the second product of glutamine catabolism, alanine. The same holds true for glutamine and taurine, whereas their stimulatory effects were seen mostly on intestinal epithelial cells and macrophages, respectively.

Considering that cysteine is a precursor for H_2S synthesis, it is reasonable that high intakes of diet enriched by cysteine increase production of this gasotransmitter. However, similar results were reported for taurine- and arginine-supplemented diet. The effects of glutamate on H_2S synthesis, at least in neuronal tissue, are bidirectional and concentration-dependent: Elevated levels of glutamate inhibit H_2S synthesis and low concentration of glutamate stimulates H_2S synthesis. In addition, intracellular glycine has been shown to modulate H_2S production, through the synthesis of GSH.

L-Arginine

Owing to more than 100-year-long physiological and nutritional studies on L-arginine, today, we know that this AA is involved in the regulation of multiple areas of human physiology. Apart from L-arginine per se, products of its metabolism such as NO, creatine, polyamines, agmatine, and urea, in addition to L-arginine per se, also play significant roles in the regulation of fundamental physiological functions. L-arginine participates in regulating protein synthesis, gene expression, micro-RNA levels, cell signaling, blood flow and capillary remodeling, nutrient transport and metabolism, antioxidative response, hormone secretion and synthesis, and mitochondriogenesis. In neural tissue, L-arginine regulates neurological functions and behavior, synthesis of neurotransmitters, and neuroprotective reactions. In the digestive system, L-arginine regulates gastrointestinal empting and the motility of the small intestine, as well as intestinal microbial growth and metabolism. Well-known roles of L-arginine in the cardiovascular system involve the regulation of blood pressure and vascular reactivity and setting of intimal hyperplasia. Importantly, it has been shown that numerous health disorders are characterized by reduced concentrations of L-arginine in plasma and/or various tissues and failure of its metabolic effects.

So, it is not surprising that dietary supplementation of L-arginine has multiple beneficial effects in the treatment of various diseases. To date, the favorable effects of exogenous L-arginine have been documented in many developmental and health problems including male and female infertility, injuries

and trauma, wound healing, immunomodulation, HIV/AIDS, athletic performance, cancer, and gastrointestinal and cardiovascular diseases. Both experimental and clinical studies clearly show favorable effects of L-arginine in diabetes. Accordingly, we reported recently that 12 days of oral supplementation with L-arginine had multiple beneficial roles in the diabetic pancreas resulting in endocrine pancreatic cell neogenesis.

L-arginine has beneficial effects on fuel homeostasis, including stimulation of glucose and fatty acid oxidation in insulin-sensitive tissues; inhibition of glucose, triacylglycerol, and LDL synthesis in target tissues (liver and adipose tissue); and enhancement of lipolysis in adipocytes. Thus, L-arginine supplementation is clearly a novel effective treatment for the prevention and treatment of obesity and metabolic syndrome.

See also: Amino Acids: Determination; Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Coenzymes and Cofactors; Energy Metabolism; Enzymes: Functions and Characteristics; Functional Foods; Maillard Reaction; Obesity: The Role of Diet; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Proteins: Chemistry, Characterization, and Quality; Proteomics: Contribution of Proteomics Techniques to Understanding the Interrelationship between Food and Health.

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Anemia: Causes and Prevalence

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Defining Anemia

Anemia is defined as an insufficient mass or altered morphology of red blood cells to meet the physiological needs of the body. Hemoglobin is the protein responsible for carrying 97% of the oxygen from the lungs to the peripheral tissues. Its decreased concentration leads to anemia, with less cellular oxygenation as a direct consequence. At a public health level, anemia is defined as hemoglobin concentration <5th percentile of the distribution of hemoglobin from a normal population, for the same sex, age, and physiological condition. The criteria to define the cutoffs for anemia were first established in the early 1960s considering population data of individuals <60 years of age. Later, in 1989, in light of new data from the Second National Health and Nutrition Examination Survey (NHANESII), the cutoffs were updated and validated to define anemia in different age groups, changing the established cutoffs for the school-age population (from 12 to 11.5 g dl^{-1}). Cutoffs established by the World Health Organization (WHO) to define anemia and its severity are still in force (Table 1). There has been no change or update of these to define anemia, despite growing evidence that the distribution of hemoglobin differs during the first 3 months of pregnancy and according to race and population >60 years of age.

For pregnant women, the increase in plasma volume as a result of hormonal changes of pregnancy demands an increased supply of iron for adequate production of red blood cells due to the increased blood volume. Several authors have reported the need to have valid cutoffs for each stage of pregnancy in order to promptly identify pregnant women at risk; however, there has been no consensus to date.

Data derived from various studies have shown that hemoglobin levels in the black population are less ($\approx$1 g l^{-1}) compared to the white population for each gender, and that this variation is not attributable to sociodemographic or economic conditions, health behaviors, or nutritional status. Anemia is three times more common in the black population than in the white population. WHO-defined anemia has been associated with an increased risk of death in both black and white populations, but hemoglobin thresholds for predicting mortality in older adults were 7 g l^{-1} below the WHO criteria in the black population, whereas in the white population thresholds were 4 g l^{-1} above the WHO cutoffs for anemia. It has been documented that at least one-third of the racial difference in hemoglobin concentration can be explained by α-thalassemia.

Epidemiology of Anemia and Iron Deficiency

Data collected by the WHO during the period from 1993 to 2005 on the status of anemia worldwide revealed that the region with the highest prevalence in the preschool population is Africa with 67.6%, followed by southeast Asia and the Oriental Mediterranean with a prevalence of 65.5% and 46.7%, respectively. A lower prevalence is reported in regions of the Americas (29%), Western Pacific (23.1%), and Europe (21.7%).

Kassebaum et al. gathered information from 187 countries to estimate the global burden of anemia in 2010, classified as mild, moderate, and severe anemia. Estimates of the prevalence of anemia were 50–80%, of which 10–20% had moderate to severe anemia. The prevalence was higher in persons with low socioeconomic status, low weight, and women who recently gave birth. In the study period (1990–2010), the prevalence decreased from 40.2% to 32.9%.

Worldwide, the three most common forms of micronutrient deficiencies are iron, vitamin A, and iodine deficiency. Together these affect at least one-third of the world population, most of whom live in developing countries. Iron deficiency is the most prevalent of the three.

Black et al. estimated an overall prevalence of anemia due to iron deficiency, with 18.1% in children <5 years of age and 19.2% in pregnant women. On the other hand, the prevalence of severe anemia was 1.5% in children <5 years of age and <1% in pregnant women.

Main Causes of Anemia in the Population

The terms 'iron deficiency' and 'iron-deficiency anemia' are often used synonymously, although they are, in fact, not the same conditions. The causes of anemia are multifactorial; the most common is iron deficiency, according to data of the Global Burden of Disease. Parasitic infections (hookworm, schistosomiasis) and malaria contribute to explain the second cause of anemia, whereas hemoglobinopathies and anemia inflammation rank as the third cause of anemia (13% of all causes), being present in higher-income countries. Table 2 summarizes the main causes of anemia in the population.

Nutritional Deficiencies

Nutritional deficiencies represent a deficit of nutrients needed for the formation of red blood cells. This group includes anemia due to vitamin B12 and folate deficiencies, both characterized by defective DNA synthesis (megaloblastic anemias); and iron-deficiency anemia in which heme synthesis is impaired.

Iron Deficiency

Iron is essential in the synthesis of hemoglobin and is also involved in many bodily functions, including the respiratory chain, phagocytosis, prostaglandin synthesis, and cytochrome P450, among others.

Due to the redox capacity of iron, it plays a crucial role in oxygen transport. Much of the intracellular iron is used in the mitochondria for heme synthesis. Heme iron is synthesized by

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Table 1 Hemoglobin levels for diagnosis and grading of anemia severity at sea level

Population group	*Age group*	*Cutoff point for diagnosis of anemia (g l^{-1})*	*Severity*		
			Slight	*Moderate*	*Severe*
Preschool children	12–59 months	<110	100–109	70–99	<70
School-age children	5–11 years	<115	110–114	80–109	<80
Nonpregnant women	12 years and older	<120	110–119	80–109	<80
Pregnant women	20–49 years	<110	100–109	70–99	<70
Males	12–14 years	<120	110–119	80–109	<80
	15 and older	<130	110–129	80–109	<80

Adapted from the World Health Organization. (2011). *Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity*. Geneva: World Health Organization.

Table 2 Causes of anemia

Direct causes	*Components (in order of importance)*
Poor, insufficient, or abnormal red blood cell production	Poor dietary intake and/or absorption of iron
	Poor dietary intake and/or absorption of vitamins (A, B12, folic acid, and possibly B6, C, and riboflavin) and copper
	Increased needs for nutrients due to growth or disease (diarrhea), HIV/AIDS
	Other infectious diseases (tuberculosis, malaria)
	Chronic inflammation
	Chronic diseases and medical treatments (cancer, HIV/AIDS, kidney diseases)
	Genetic blood diseases (sickle cell disease or trait, thalassemia)
Excessive red blood cell destruction	Malaria
Excessive red blood cell loss	Helminth (worm) infections (hookworm, schistosomiasis)
	Bacterial or viral infections (peptic ulcers, gastritis, diarrhea)
	Reproduction (excessive blood loss during menstruation, delivery, and postpartum period; multiple pregnancies, shortened postpartum amenorrhea)
	Contraceptive methods (intrauterine devices)
Contributing causes	*Components*
Knowledge and behavior	Poor knowledge among health workers about anemia, iron supplementation, and other anemia prevention and control interventions
	Poor knowledge among vulnerable groups about the importance of anemia
	Prevention and control interventions
	Cultural taboos or biases (e.g., women eating after others eat)
	Practices that restrict food intake, including poor infant breastfeeding practices and inadequate introduction of complementary foods
	Poor compliance with recommended behaviors (iron supplementation; malaria, tuberculosis, and other medication regimens; use of family planning; use of sanitation facilities; HIV prevention behaviors)
Environmental lack of access to services	Contamination due to heavy metals (lead)
	Low use of antenatal and other services providing iron supplements
	Lack of trained midwives to manage bleeding during delivery
	Lack of access to sanitation services that mitigate helminth infestation
	Lack of access to bed nets to prevent malaria transmission
Poverty	Lack of income to buy foods with adequate amounts of absorbable iron or to obtain iron supplements, malaria treatment, insecticide-treated bed nets, shoes to prevent helminth infection, and other preventive commodities or services

Adapted from Galloway, R., International Nutritional Anemia Consultative Group (INACG). (2003). *Anemia prevention and control: what works. Part I. Program guidance*. Washington, DC: USAID.

a sequence composed of eight reactions in the mitochondria and cytoplasm, with the final reaction being Fe^{2+} inserted into protoporphyrin IX by ferrochelatase. Heme iron is essential in the formation of hemoglobin. The core is fixed into the oxygen to be transported to tissues. Consequently, iron deficiency results in low red blood cell production, giving a hypochromic and microcytic appearance to erythrocytes. The most common cause of iron deficiency is the inadequate intake of iron (heme iron) coupled with a high intake of absorption inhibitors (phytates, tannins).

Deficiency of Vitamin B12 and Folic Acid

Vitamin B12 and folic acid share many metabolic pathways. Deficiency of one alters the metabolism of the other in the formation of red blood cells. Hematologic effects of deficiency

of these vitamins are identical. Macrocytic anemia is due to megaloblastic changes in the bone marrow; however, megaloblastic anemia can occur without macrocytosis. It is a common condition in elderly persons because it occurs as a result of vitamin B12 malabsorption due to impaired production of the intrinsic factor of parietal cell gastric atrophy. In malnourished individuals, it is estimated that less frequent causes of anemia are associated with vitamins A, E, B2, B3, B6, C, and zinc and copper deficiency.

Anemia Due to Malaria and Parasitism

Malaria contributes to iron-deficiency anemia by causing intravascular hemolysis, and also causes an immune response that suppresses erythropoietin through rupturing, phagocytosis, and hypersplenism. Extensive geographic overlap of hookworm and malaria yields a high prevalence of coinfection, which may increase additively the risk of anemia.

Hookworms (*Necator americanus* and *Ancylostoma duodenale*) reside in the small intestine of infected individuals, where they attach to the villi and feed on host blood. Heavily infected patients are unable to maintain adequate iron stores and become anemic. WHO currently recommends that school-age children living in areas of high prevalence of soil-transmitted helminths (hookworms, Ascaris lumbricoides, and Trichuris trichiura) receive treatment with either albendazole or mebendazole.

Inflammation; or Anemia of Chronic Disease

Human hepcidin is a 25-amino acid peptide synthesized by the liver, induced by infection and inflammation. Hepcidin regulates expression of ferroportin and ceruloplasmin. Both proteins are responsible for exporting iron from the basolateral membrane (enterocyte and polarized cells, respectively). The link of hepcidin with ferroportin causes internalization and degradation of ferroportin, affecting the export of iron from the enterocyte into the systemic circulation. Inflammatory anemia is characterized by decreased serum iron and the iron-binding capacity by transferrin, indicating an impairment of mobilization of iron reserves. The link among infections, hypoferremia, and inflammatory anemia suggests that the last two are part of the host response to infection. Because most of the iron in the transferrin compartment is intended for bone marrow, hypoferremia resulting from the excess of hepcidin reduces the amount of iron available for hemoglobin synthesis and erythrocyte production.

Anemia of Chronic Renal Disease

In the absence of disease, erythropoiesis is controlled by a negative feedback system. The rate of change of the concentration of erythrocytes is the difference between the rate of its production and destruction. Erythropoietin, along with the colony-forming units, is responsible for the differentiation and proliferation of erythroid progenitors in the bone marrow to increase red blood cell mass. This hormone is produced by the juxtaglomerular apparatus of the kidney cortex. The basal rate of red blood cell production is decreased in patients with chronic renal disease (CRD) so that anemia is proportional to the severity of renal function. Anemia of CRD develops early and worsens as renal failure progresses.

Consequences of Anemia

Iron deficiency has been shown to have adverse effects on physical capacity, work performance of adolescents and adults, immune status, and morbidity from infections of all age groups. Iron deficiency anemia reduces the body's ability to regulate the internal temperature by altering the production of hormones involved in energy metabolism, affecting the synthesis of neurotransmitters and thyroid hormones related to muscle and neurological functions involved in regulating body temperature. It also impairs cognitive performance and behavior at any age. In general, these effects are corrected by iron supplementation, but if moderate to severe iron deficiency occurs in infancy, the effects on cognition may not be reversible.

In addition to the obvious and direct health effects, the existence of iron deficiency and anemia has profound implications for economic development and productivity, particularly in terms of the potentially huge public health costs and loss of formation and quality of human capital. Anemia is a prevalent and often underdiagnosed and untreated condition, but one that has important substantial consequences for subjects with the condition, diminishing their quality of life as well as resulting in economic consequences for the health sector.

Recently, the Global Burden of Disease estimated that anemia was responsible for 68.3% of YLDS (years lived with disability) in 2010 and accounted for 8% of all disabilities, with women and preschool children carrying the highest burden.

In terms of disability-adjusted life years (DALYs), iron-deficiency anemia accounts for 25 million lost DALYs, or 2.4% of the overall total. Annually, iron-deficiency anemia leads to 134 000 deaths of children and indirectly leads to 841 000 maternal and perinatal deaths. Anemia is a risk factor for perinatal (591 000) and maternal (115 000) deaths globally; 18.4% of maternal and 23.5% of perinatal deaths are attributable to anemia. Combined with the direct impacts of iron-deficiency anemia, the total deaths are 841 000 annually. In the United States, patients with anemia have an average cost two times higher than nonanemic patients. Anemic patients most frequently use health services and represent higher costs ($14 535 vs. $9451 $p < 0.001$) when compared with nonanemic patients having the same pathological condition. Average annualized per-patient costs were $14 535 for anemic patients (55% outpatient, 33% inpatient, 13% pharmacy), 54% higher than the $9451 average cost for nonanemic patients (45% outpatient, 36% inpatient, 19% pharmacy).

Particular Effects on Women

Anemia is associated with increased mortality and adverse outcomes in a variety of physiological and pathological conditions. In women of childbearing age, iron demand is high due to menstrual losses as well as during pregnancy. Iron requirements are greater as pregnancy progresses. Approximately 1200 mg of iron are required from the time of conception until delivery throughout pregnancy, because the iron stores of the mother must meet the needs of the developing fetus and accommodate blood loss at delivery. The red cell

mass increases 350–450 ml during this period; ~80% of the maternal iron is used for the production of hemoglobin, and 30% for the accumulation of iron stores. Beyond the first year, iron intake or stores must remain sufficient to support the ongoing growth and increased red cell mass.

It has been documented that women with insufficient prepregnancy iron stores increase their risk of anemia during pregnancy and also have less capacity for physical activity, increased susceptibility for infections, and less interaction with their children. During pregnancy, iron-deficiency anemia increases the risk of premature birth and low birth weight, reducing infant iron stores.

Under conditions of no maternal anemia, the fetus accumulates iron during the last weeks of pregnancy to meet and satisfy the iron demands of the infant for the next 6 months postpartum, being fed only with breast milk during this period. Inadequate maternal iron reserves also result in low reserves of the newborn. In anemic women, the fetus will develop limited reserves of iron, therefore being likely to develop iron deficiency in the first months of life, which is characterized by rapid growth.

Pregnant women with anemia are 3.5 times more likely to die from complications during childbirth and the postpartum period than are nonanemic women. Low maternal hemoglobin level has been found as a strong predictor of perinatal mortality due to placenta previa and abruption placenta. Perinatal mortality was nearly 50%; of these were stillbirths in women from Ethiopia.

Particular Effects on Children

Anemia and iron deficiency in children <2 years of age is of great interest and concern because at this critical stage of growth, a higher intake of iron is required and is often not provided by diet. Neurobehavioral effects are of greatest concern because they persist long after treatment with iron and resolution of anemia. Iron deficiency in utero appears to have direct effects on indicators of brain maturation in premature infants.

Iron requirements during the first 2 years of life gradually increase to meet the demands of multiple roles in brain development and in the immune and endocrine systems. In this regard, there is ample evidence in the literature that iron deficiency in children is associated with adverse effects on psychomotor, cognitive, and social-emotional development, as well as increased susceptibility to infections, persisting after treatment of anemia and iron deficiency resolution.

Although the causal link between iron deficiency and adverse child development is unclear, evidence from animal studies suggests that iron deficiency may affect neurological development through brain functions, specifically myelination, dopamine and norepinephrine metabolism, and neuronal energy metabolism. The evidence of a causal effect of lack of iron on behavior comes from animal studies in which the environment is controlled and differences in iron status are experimentally induced. Studies in both monkeys and rats document behavioral differences in infancy and later on that relate directly to findings in the human infant. Recent studies of iron deficiency during brain development in nonhuman primates show different behavioral consequences depending on the timing of iron deficiency relative to brain developmental stages, and the potential for long-lasting changes in brain iron regulatory systems. Iron deficiency caused long-term diminished protein levels of four factors critical for hippocampal neuron differentiation and plasticity, including CamKII alpha, Fkbp1a (Fkbp12), Dlgh4 (PSD-95), and Vamp1 (synaptobrevin-1). Iron deficiency altered gene expression in the mammalian target of rapamycin pathway, and during late fetal and early postnatal life, iron deficiency alters the levels and timing of expression of critical genes involved in hippocampal development and function.

Iron has multiple roles in the neurotransmitter system and can affect behavior through its effect on dopamine metabolism. This interaction between iron deficiency and dopamine metabolism is highly relevant for early cognitive development because dopamine clearance has strong effects on attention, perception, memory, motivation, and motor control. Low cerebral iron may interfere with myelination and dopaminergic function, potentially disrupting sleep cycles, motor control, learning, and memory. Iron deficiency during the perinatal period is associated with altered expression of genes critical for hippocampal development and function.

Children with chronic, severe iron deficiency in infancy continue at a behavioral disadvantage relative to their peers at school entry. Sustained differences in mother/child interaction might contribute to the long-lasting behavioral and developmental alterations reported in children with chronic, severe iron deficiency in infancy.

Some studies have found that infants with chronic, severe iron deficiency compared with those with a healthy iron status are more likely to be fearful, hesitant/wary, unhappy, inactive, easily fatigued, in close contact with their mothers, or lower in vocalization. Unfortunately, the effects of anemia during the first 2 years of life are nonreversible. Neurocognitive complications of iron deficiency during critical pre- and postnatal periods of brain development are difficult to remedy, persisting into adulthood. Derived from this evidence of adverse physical and mental health effects of the child, prevention of perinatal iron deficiency and promotion of neural plasticity in individuals exposed to poor iron status during critical stages of development require more attention.

Programs for the Prevention and Control of Anemia and Iron Deficiency

Globally, there are various ways to control and treat anemia at the population level. Some of them include iron supplementation, food fortification, and control of parasitic diseases, as well as promoting increased intake of bioavailable iron, decreasing its losses, and increasing reserves in newborns by late clamping of the umbilical cord.

Iron Supplementation

Pharmacological iron supplementation can be used to treat iron-deficiency anemia in persons who suffer from the condition or to prevent anemia in susceptible groups such as children <5 years of age, women of childbearing age, adolescents, and pregnant women. The therapy generally requires a daily

dose, whereas preventive treatment is given weekly or intermittently.

The evidence shows that daily iron supplementation in children 4–23 months of age is effective in reducing iron-deficiency anemia. A systematic review of 55 clinical trials administering oral and parenteral iron or through fortified food to children found that, on average, iron supplementation improves hemoglobin concentration ($0.74\,g\,l^{-1}$) and decreases the prevalence of anemia. A systematic review carried out in 2009 showed results on the type and frequency of intermittent iron supplementation in populations where malaria is endemic. Despite the fact that intermittent supplementation was effective in reducing the risk of anemia and iron deficiency in children <12 years of age, a daily supplementation scheme was more effective. Therefore, the authors conclude that an intermittent scheme can be effective in populations where the daily scheme daily has not been implemented or has failed.

In nonpregnant women of childbearing age, a systematic review assessed intermittent iron supplementation (1, 2, or 3 times a week on nonconsecutive days), and found that it improves hemoglobin and ferritin in addition to reducing the risk of anemia by 27%. In this same review, a comparison of intermittent supplementation with daily supplementation was performed. It was found that women who received intermittent supplementation had a higher risk of anemia, although the average final concentration of hemoglobin did not differ between comparison groups.

In pregnant women, iron supplementation has been found effective in outcomes such as iron deficiency at term and hemoglobin concentrations in pregnancy; however, the pattern of daily supplementation has also been associated with side effects, especially at doses >60 mg iron. An alternative scheme is supplementation with iron compounds alone or in combination with other vitamins and minerals intermittently (1–3 times a week), as these have fewer adverse effects.

Food Fortification

Food fortification has been recognized as one of the most effective measures for the control of micronutrient deficiency at the population level, where the fortification of staple foods such as maize, wheat, and rice, or condiments like salt, curry, and soy sauces, has been proposed. In Egypt, a program of fortification of wheat flour with iron (ferrous sulfate) and folic acid implemented since 2008 in low-income groups estimated that 50 million people consume ≈12 mg iron and 600 μg folic acid daily by eating fortified bread.

There are different forms of iron for food fortification such as ferrous sulfate (water-soluble) and ferrous fumarate (soluble in weak acid), which are highly bioavailable. Ferrous sulfate is cheaper, so it is widely used for flour fortification. But it causes rancidity during storage, which is an unfortunate consequence. Ferrous fumarate may produce less damage in foods. Other iron compounds are the iron soluble in strong acids such as elemental iron, pyrophosphate, and ferric orthophosphate, which have less of an effect on the food but have minimal bioavailability in humans. Fortification of cereal, salt, and sauces has been successfully achieved by EDTA iron, which contains iron protected that enhances the absorption of dietary nonheme iron. Likewise, encapsulated iron as ferrous salt has very little reaction to food and has high bioavailability, but is a little more expensive.

The effectiveness and sustainability of fortification programs is a challenge in developing countries due to the necessity of infrastructure and various resources for their implementation. A major limitation that occurs in some countries is inadequate food fortification; for example, in Ghana and South Africa, a low addition of iron in wheat flour by millers was detected, making the desired reduction of deficiency unlikely. Another limitation of fortification is that persons who need it the most have little access to it. A clear example of this problem is the fortification of wheat flour in Guatemala. It was observed that vulnerable groups did not consume fortified products despite the availability of fortified corn flour, which is a highly consumed product. Therefore, any fortification program that is developed should take national context into account.

Iron Fortification of Foods

One strategy that has been successful in combating anemia in infants >6 months of age is home supplementation with micronutrient powders known as 'Sprinkles.' These preparations have iron encapsulated in a lipid layer that prevents adverse effects on both taste and appearance of food. This form of supplementation consists of single-dose packets of micronutrient powder and is added to childrens food at the time it is served. It has no smell or taste. In clinical trials, its effectiveness has been shown to control anemia compared with liquid supplements of ferrous sulfate. In 2011, a review of eight studies of powder micronutrient supplementation containing iron showed that home fortification with micronutrients reduced anemia by 31% and iron deficiency by 51% compared with the control group. Other studies found no difference comparing the powder micronutrient with daily iron supplementation in anemia or hemoglobin.

Control of Parasitic Infections

Deworming is a proven strategy to prevent iron deficiency caused by hookworm in children and pregnant women. In a 12-month clinical trial, 10 mg iron and/or 500 mg mebendazole were administered daily every 3 months in children 6 to 71 months of age in Zanzibar. It was found that the administered dose of iron had no effect on growth retardation, hemoglobin concentration, or mild to moderate anemia (hemoglobin <110 or $<90\,g\,l^{-1}$, respectively); however, it significantly improved serum ferritin and erythrocyte protoporphyrin. In children <24 months of age, treatment with mebendazole also reduced moderate anemia. Finally, a systematic review in 2007 showed that deworming increases hemoglobin and reduces the prevalence of anemia.

Late Clamping of the Umbilical Cord

Cord clamping is a strategy to prevent iron deficiency and is especially important in children <6 months who need enough iron stores until they start complementary feeding at 6 months of age. Delayed clamping for 2 min after birth ensures reserves,

preventing iron deficiency. It has also been associated with a lower prevalence of neonatal anemia. A review of studies of delayed umbilical cord clamping compared with early clamping found higher hemoglobin levels in infants with late clamping, but also a greater need for phototherapy for jaundice. Although hemoglobin levels were not maintained after 6 months of age, higher levels of ferritin were found at this age.

Nutritional Recommendations

One of the main causes of iron-deficiency anemia is the low consumption of foods rich in this nutrient. These foods are mainly of animal origin and include meat, fish, and poultry; therefore, poor families often consume small amounts of these foods.

In general, nutritional recommendations should be adapted to the national or local context because there may be variations in diet, food availability and access, seasonality, and so on. Bioavailability of iron in foods should be noted because bioavailability of iron in food is strongly influenced by substances that promote or inhibit its absorption.

Inhibitors of iron absorption are phytates present in cereals, refined flour, legumes, nuts, and seeds; foods rich in inositol; tannins; tea, coffee, cocoa, herbal teas, and some vegetables; and calcium in milk and dairy products. Certain methods of preparation such as fermentation can reduce the phytic acid that inhibits iron absorption.

Promoters of iron absorption are ascorbic acid or vitamin C in fruits, juices, potatoes, and other tubers, plus green leafy vegetables, cauliflower, and some fermented or sprouted foods. The heme iron in meat, poultry, fish, and seafood is absorbed by a different mechanism than nonheme iron and has a high bioavailability.

In order to improve the bioavailability of dietary iron, it is important to avoid eating inhibitors of iron absorption in conjunction with iron-rich foods.

Recommendations to improve the absorption of iron from food:

- Drink coffee or tea at least 1 or 2 h after a meal.
- Include fruit juices in the diet, such as orange juice, or other sources of ascorbic acid (vitamin C).
- Consume dairy products as snacks between meals instead of during a meal.
- Eat foods containing iron-absorption inhibitors with low iron content such as cereal, tortilla, or bread accompanied by milk, because this can provide sufficient calcium without impeding the absorption of iron.

Furthermore, exclusive breastfeeding for 6 months covers almost all the nutritional needs of children born at term; however, this recommendation is always given when the mother is well nourished. Children born with normal weight from well-nourished mothers can have adequate iron stores in the liver and therefore are at lower risk for anemia. However, children with low birth weight, premature infants, or infants born to mothers with iron deficiency (even those born with normal weight) have a higher risk of iron deficiency, hence the recommendation to improve the diet of the mother during pregnancy. A scheme has been proposed for home supplementation with micronutrient fortification in children ages 6–23 months (Table 3). In the case of children with low birth weight, supplementation is recommended starting at 6–8 weeks of age.

During the stage of weaning or supplementary feeding, fortified foods should be chosen versus iron supplementation, especially in areas where the prevalence of infectious diseases and malaria is high. It is important to note that before supplementation, the nutritional status of children <5 years old should be assessed – along with measures existing in the community for controlling iron deficiency – to ensure that the daily iron requirements are not exceeded. It must be taken into consideration that if iron supplementation is administered, adverse effects such as stunting may occur. Therefore, we must prioritize the consumption of fortified foods in order for infants to receive the necessary nutrients. Iron supplementation is recommended at a dose of 2 mg kg^{-1} body weight in children 6–23 months of age in areas where the prevalence of anemia in children <1 year of age is >40%, or where the consumption of iron-fortified foods is low. Likewise, if consumption in children (>6 months) of food of animal origin is very low or nonexistent, it is necessary to provide vitamin and mineral supplementation, particularly iron.

Women of childbearing age should be aware that pregnancy demands high amounts of iron for the development of the fetus and placenta and increased blood volume. Added to this, childbirth is accompanied by variable blood losses. When pregnancy occurs in adolescence, iron demands are even greater because it combines the demands of growth and of

Table 3 Home fortification scheme with multiple micronutrient powders for children 6–23 months of age

Dose of micronutrients	*Iron*: 12.5 mg of elemental iron, preferably with ferrous fumarate capsules (12.5 mg of elemental iron is equal to 37.5 mg of ferrous fumarate, 62.5 mg of ferrous sulfate heptahydrate of 105 mg of ferrous gluconate) *Vitamin A*: 300 μg of retinol *Zinc*: 5 mg of elemental zinc, preferably zinc gluconate
Special conditions	In malaria setting, additional pharmacological treatment is recommended
Frequency	One dose daily
Duration and interval between intervention periods	Minimum of a 2-month period followed by a period of 3–4 months without supplementation; use of micronutrients is initiated each 6 months
Target group	Children 6–23 months of age, beginning at the same time as cessation of breastfeeding
Scenario	Populations where the prevalence of anemia in children 2–5 years of age is 20% or higher

Adapted from World Health Organization. (2011). *Use of multiple micronutrient powders for home fortification of foods consumed by infants and children 6–23 months of age*. Geneva: World Health Organization.

pregnancy, resulting in a high risk for anemia. The postpartum period is the ideal time to recover iron reservoirs because amenorrhea during this time (due to breastfeeding) reduces iron loss. Hence, the promotion of exclusive breastfeeding for 6 months, followed by breastfeeding up to 2 years of age, may contribute to controlling iron-deficiency anemia in women of childbearing age. Another important measure is to allow at least 2 years between pregnancies in order for women to recover iron stores. However, one should keep in mind that iron supplementation helps to control anemia, and eating foods rich in iron is also necessary.

WHO recommends daily oral supplementation of iron and folic acid as part of prenatal care in order to reduce the risk of low birth weight, anemia, and iron deficiency (Table 4); likewise, it suggests a supplementation scheme for menstruating women (Table 5).

With respect to the preschool and school-age populations, an intermittent supplementation scheme proposed by the WHO to improve iron status and reduce the risk of anemia is shown in Table 6.

In regard to regular deworming for helminths, it is recommended that in areas where the prevalence among school children is >50%, the process should be carried out every 6 months both in preschool and school-age children as well as in women of childbearing age. Where the prevalence among school-age children is between 20% and 50%, deworming is recommended once a year. The WHO-recommended dose is 200 mg of albendazole for children <2 years of age and 400 mg for the remaining population or administration of 500 mg of mebendazole.

In the general population, if the amount of absorbable dietary iron will not immediately improve anemia, pharmacological

Table 4 Scheme of daily supplementation with iron and folic acid in pregnant women

Composition of the supplement	Iron: 30–60 (if prevalence >40%) mg of elemental iron[a] Folic acid: 400 μg (0.4 mg)
Special conditions	60 mg of elemental iron if anemia is a severe public health problem (40% or higher) Anemia in clinical setting: 120 mg of elemental iron plus 400 μg of folic acid In malaria settings: additional pharmacological treatment
Frequency	Daily
Duration	During pregnancy. Supplementation should begin as soon as possible in pregnancy
Target group	Adolescent and adult women
Scenarios	All scenarios

[a]30 mg of elemental iron equivalent to 150 mg of ferrous sulfate heptahydrate, 90 mg of ferrous fumarate, or 250 mg of iron gluconate.
Adapted from WHO. (2012). *Guideline: daily iron and folic acid supplementation in pregnant women*. Geneva: World Health Organization.

Table 5 Scheme of intermittent supplementation with iron and folic acid in menstruating women

Composition of the supplement	Iron: 60 mg of elemental iron[a] Folic acid: 2800 μg (2.8 mg)
Special conditions	Anemia in clinical setting: 120 mg of elemental iron plus 400 μg of folic acid In malaria settings: additional pharmacological treatment
Frequency	Once weekly
Duration	3 months of supplementation followed by 3 months without supplement; reinstate supplementation after this period
Target group	All menstruating adolescent and adult women
Scenarios	Populations where the prevalence of anemia among nonpregnant women of reproductive age is 20% or higher

[a]60 mg of elemental iron equivalent to 300 mg of ferrous sulfate heptahydrate, 180 mg of ferrous fumarate, or 500 mg of iron gluconate.
Adapted from World Health Organization. (2011). *Guideline: intermittent iron and folic acid supplementation in menstruating women*. Geneva: World Health Organization.

Table 6 Scheme for iron supplementation of preschool and school-age children

Target group	*Preschool children (24–59 months of age)*	*School-age children (5–12 years of age)*
Composition of the supplement	25 mg of elemental iron[a]	45 mg of elemental iron[a]
Special conditions	In malaria and hookworm endemic countries, additional pharmacological treatment is recommended at least annually	
Form of the supplement	Drops/syrup	Tablets/capsules
Frequency	Once weekly	
Duration and time interval between supplementation periods	3 months of supplementation followed by 3 months without supplement; after this period, reinstate supplementation	
Scenario	When the prevalence of anemia in preschool and school-age children is 20% or higher	

[a]25 mg of elemental iron equivalent to 75 mg of ferrous fumarate, 125 mg of ferrous sulfate heptahydrate, or 210 mg of ferrous gluconate.
Adapted from World Health Organization. (2011). *Guideline: intermittent iron supplementation for preschool and school age children*. e-*Library of Evidence for Nutrition Actions (eLENA)* (vol. 2011). Geneva: World Health Organization.

supplementation is recommended, which should focus on vulnerable groups such as children ages 6–24 months and pregnant women. These programs must be accompanied by educational messages that will provide information about the foods that should be preferred for their iron content. Also, exclusive breastfeeding should be supported for 6 months, continuing with complementary feeding (without neglecting breastfeeding). The infant should be provided with iron-rich and -fortified foods as much as possible, especially during the first 2 years of life. It is noteworthy that some breast milk substitutes, especially cow's milk, can cause gastrointestinal bleeding in infants, possibly causing iron-deficiency anemia. It is worth mentioning that iron supplementation in malaria-endemic areas should be administered together with measures for disease prevention, diagnosis, and treatment.

Conclusions

Iron-deficiency anemia affects a large number of women of childbearing age and children <5 years of age. Periodic monitoring of hemoglobin level is recommended in these age groups. If the population has a high prevalence of anemia (>50%), universal supplementation is essential. When the prevalence is lower, supplementation is recommended for specific groups. Iron compounds used for the treatment of anemia must be well selected, with a preference for water-soluble compounds, such as ferrous sulfate, or compounds soluble in weak acids, such as ferrous fumarate. Compounds with bound iron (such as bisglycinate or Fe-EDTA) represent other options. It is advisable to recommend eating foods with highly bioavailable iron such as animal tissues, mainly liver. Other foods of plant origin should be eaten along with absorption facilitators, such as vitamin C and other organic acids.

See also: Anemia: Prevention and Dietary Strategies; Bioavailability of Nutrients; Food Fortification: Rationale and Methods; Iron: Biosynthesis and Significance of Heme; Iron: Physiology of Iron; Iron: Properties and Determination; Meat: Role in the Diet.

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Anemia: Prevention and Dietary Strategies

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Introduction

Anemia is a widespread public health issue affecting people in both developing and developed countries. Anemia due to iron deficiency has been ranked by the World Health Organization in 2002 as one of the leading factors affecting the global burden of disease. Nearly one-quarter of the world's population is affected by anemia, with over one and a half billion people affected worldwide.

People living in developing countries are at increased risk of developing anemia. Populations most vulnerable to anemia include infants, children, adolescents, women of reproductive age, and pregnant women. It has been estimated that 47.4% of preschool children worldwide, 41.8% of pregnant women, and 30.2% of nonpregnant women have anemia.

Anemia (a low concentration of circulating red blood cells resulting in a reduced capacity to transport oxygen around the body) is caused by a number of factors. These may occur in isolation or simultaneously. Anemia may be caused by genetic factors (e.g., hemoglobinopathies), infections (e.g., malaria, tuberculosis, human immune deficiency virus, and intestinal helminths), blood loss (e.g., menstruation), and chronic conditions (e.g., cancer). Micronutrient deficiencies including vitamin A, vitamin B12, folate, and copper also increase the risk of anemia, but the level at which they contribute to anemia risk is not clear.

Iron deficiency is estimated to contribute to ~50% of anemia cases globally. Between two and five times the amount of individuals who have iron-deficiency anemia are affected by nonanemic iron deficiency. An individual's iron status falls on a continuum from replete iron stores to iron-deficiency anemia. Iron-deficiency anemia is usually preceded by the depletion of iron stores (reflected by a decrease in serum ferritin concentration). The depletion of iron stores may progress to iron deficiency (reflected by further reductions in serum ferritin (a reduction in iron stores); decreases in serum iron and increases in total iron-binding capacity, which results in a fall in transferrin saturation; and increases in soluble transferrin receptor and zinc protoporphyrin concentrations). In the final stage (iron-deficiency anemia), oxygen supply to the tissues is impaired and hemoglobin concentrations fall. Hemoglobin concentrations are often used as a proxy for iron-deficiency anemia, even though not all individuals with anemia are iron-deficient.

Iron-deficiency anemia can result in irreversible cognitive impairment in infants and, in children, reduced performance at school. In pregnant women, severe iron-deficiency anemia is associated with increased maternal and child mortality. Iron-deficiency anemia increases the risk of preterm delivery, low-birth-weight infants, and poor iron status of the mother, which contributes to a reduction in iron status of the infant. Iron-deficiency anemia is associated with decreased work capacity and has major social and economic implications.

Several strategies are required in the prevention and treatment of anemia, mainly due to anemia's multifactorial nature. These strategies will depend on the population group at risk, including stage of life, local conditions, and the etiology of anemia within the specific population. For example, strategies to prevent and treat anemia are likely to differ between developed and developing countries. Strategies to prevent anemia have focussed on infection control, improvement of nutritional status, and increasing dietary iron intake and bioavailability through dietary education, food fortification, and iron supplementation.

Dietary Factors and Anemia

Both dietary and nondietary factors contribute to iron deficiency and, ultimately, iron-deficiency anemia. Nondietary factors contributing to iron deficiency include genetics, physiological factors that increase iron requirements (e.g., pregnancy and growth), blood loss (e.g., menstruation), parity, and the inflammation associated with obesity, which upregulates hepcidin (a peptide hormone produced in the liver that inhibits iron absorption). Approximately half the variation in iron absorption is explained by hepcidin and other genetic or physiological factors, while an individual's iron status (increased iron absorption in states of iron deficiency) and dietary factors account for the remaining variation.

Dietary Factors Affecting Iron Absorption

Iron absorption involves the entry, movement through, and release of iron from the intestinal cell to the circulation. There are two main types of iron in the diet, absorbed through different pathways in the small intestine. Heme iron derived from hemoglobin and myoglobin is found in meat, fish, and poultry, and while heme iron only contributes ~10–15% of dietary iron intake, ~15–35% of heme iron is absorbed. Relatively few dietary factors impact on heme iron absorption. Meat and soy protein have been shown to enhance heme iron absorption, while calcium inhibits it. Heme iron is converted to nonheme iron if meat is cooked for a long period of time at too high a temperature.

The majority of dietary iron is from nonheme iron found not only in meat, fish, and poultry but also in cereals, pulses, legumes, nuts, seeds, eggs, and some vegetables. Only 2–20% of nonheme iron is absorbed by the body, and absorption of nonheme iron is affected by a number of factors. It is also likely that dietary iron is taken into the intestinal cells as ferritin, the iron storage molecule of animal and plant cells. Once in the intestinal cell, heme iron and nonheme iron enter a common pool and are treated in the same manner.

Dietary factors enhancing nonheme iron absorption act by converting the insoluble ferric form of iron (Fe^{3+}) to the more

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soluble ferrous form (Fe^{2+}) or by maintaining the iron released from food during digestion in a soluble form prior to entering the intestinal cell. Vitamin C is a strong enhancer of nonheme iron absorption, as is an unidentified factor in meat, commonly known as the meat–fish–poultry factor. Other organic acids (e.g., citric acid), alcohol, and fermented foods also enhance nonheme iron absorption. In some, but not all studies, vitamin A and carotenoids have been shown to enhance nonheme iron absorption. Nonheme iron absorption is inhibited by phytic acid (found in whole grain breads, cereals, legumes, nuts, and seeds), polyphenols (found in tea, coffee, fruit, vegetables, some cereals and legumes, and red wine), and some proteins (e.g., soy protein). These bind nonheme iron to form insoluble complexes inhibiting entry into the intestinal cell. Calcium inhibits the absorption of both heme and nonheme iron. Supplemental doses (rather than the amounts found in food) of organic elements such as zinc, manganese, and copper also compete with nonheme iron for transport into the intestinal cell. The effects of enhancers and inhibitors on iron absorption are strongest when consumed with meals containing iron. Studies conducted over several days or weeks have shown that the effects of these individual dietary factors on iron absorption may be reduced when consumed as part of a whole diet rather than being consumed as part of a single meal. Vitamin C, meat, and phytic acid appear to have the strongest influence on nonheme iron absorption when consumed as a meal containing multiple enhancers and inhibitors of iron absorption.

Dietary Factors Affecting Iron Status

Despite the number of dietary factors affecting iron absorption, it has been difficult to determine whether these factors translate to an actual effect on iron status (usually measured using serum ferritin concentrations). A large number of cross-sectional studies (observational studies analyzing population data at one time point) have shown meat intake to be associated with an increased iron status. The serum ferritin concentrations of vegetarians tend to be lower than in nonvegetarians, but still within the normal range, and vegetarians tend to have similar rates of anemia compared with nonvegetarians. Some, but not all, studies have observed an inverse relationship between calcium and dairy product intake and iron status. Studies have found either a positive association or no association between alcohol intake and iron status. Tea intake appears to only affect iron status in individuals with marginal iron status. Most cross-sectional studies have observed no relationship between iron status and intakes of iron, vitamin C, fruit and vegetables, fiber, or phytate. These studies have however had a number of limitations, including inadequate assessments of dietary intake and the use of food composition databases, which contain limited data, especially for phytic acid and polyphenol contents, varying cutoff points to determine iron deficiency and not taking into account confounders that may affect iron status (e.g., many studies have not measured markers of inflammation, also associated with increased serum ferritin concentrations). Furthermore, cross-sectional studies are unable to identify cause and effect and have tended not to take into account the timing of food consumption or the interaction or synergistic effects enhancers and inhibitors of nonheme iron absorption have on one another.

Recent cross-sectional studies have attempted to address this, by investigating associations between combinations of foods consumed and a population's iron status. A study in China found a 'healthy' dietary pattern (consisting of whole grains, fruits, and vegetables) to be inversely associated with anemia, while 'sweet tooth' (drinks and cakes) and 'traditional' (rice, vegetable, and wheat flour) dietary patterns were positively associated with anemia. In Norway, high serum ferritin concentrations were associated with a 'reindeer meat' (reindeer meat, reindeer products, moose meat, cured/salted fish, and boiled coffee) dietary pattern. In New Zealand, the likelihood of suboptimal iron status was lower in premenopausal women who followed a 'meat and vegetable' (beef, chicken, broccoli, capsicum, and lettuce) dietary pattern and increased in premenopausal women who followed a 'milk and yogurt' (milk added to food, milk as a drink, and yogurt) dietary pattern. The few studies that have investigated associations between iron status and timing of food and beverage consumption have had mixed results.

Only a few prospective studies (involving repeated observations in the same individuals over time) have investigated associations between dietary factors and iron status. Results have been mixed, but the majority have observed no associations between dietary intake and serum ferritin concentrations. The majority of randomized controlled trials (gold standard research study design in which people are randomly assigned to one of two or more treatments) have not observed an effect of dietary enhancers and inhibitors on iron status. However, these studies have largely been undertaken in iron-replete Western populations, who are less likely to absorb iron. Recent studies of more than 16 weeks' duration undertaken in iron-depleted populations have found meat intake to maintain iron status and vitamin C to increase iron status when consumed with meals that contain iron. The most effective dietary strategies for improving iron status appear to be those that use a combination of dietary strategies to improve iron status, for example, increasing the intake of iron in the diet (through iron-fortified foods and the use of cast-iron cookware), increasing the intake of enhancers of iron absorption (e.g., meat and vitamin C), decreasing the intake of dietary inhibitors of iron absorption (e.g., tea and coffee and phytic acid), and optimizing the timing of foods (e.g., consuming iron-containing foods at mealtimes alongside iron absorption enhancers and inhibitors of iron absorption in between meals). However, these changes are not usually enough to move an individual from an iron-deplete state to an iron-replete state. Other strategies to improve the iron bioavailability of foods include food processing methods such as the fermentation and germination of foods such as cereals and legumes to reduce their phytate content. For individuals with anemia, dietary strategies should be used alongside iron supplementation. Dietary strategies are useful in the treatment and prevention of iron deficiency (prior to iron deficiency developing). Other approaches for reducing iron-deficiency anemia include large-scale educational programs and promotional campaigns focussing on improving the intake and bioavailability of iron in the diet. Educational programs targeted at health workers may also be effective. For populations in developing

countries, dietary changes may however be less feasible due to limited dietary diversity and poor economic circumstances.

Recommended Iron Intakes for Different Population Groups

It is generally recognized that dietary contributors to iron-deficiency anemia include diets of limited diversity, with low dietary iron and poor iron bioavailability. These diets contain low levels of foods that enhance iron absorption (e.g., meat and fruit and vegetables high in vitamin C) and high amounts of foods that inhibit iron absorption (e.g., staple foods such as cereals that are high in phytic acid) and tend to be consumed in developing countries. People living in developed countries tend to consume diets with greater dietary diversity and higher iron bioavailability. These diets contain generous amounts of meat and foods that enhance iron absorption and low levels of foods that inhibit iron absorption.

Recommended intakes for iron have been established in several countries. The recommended intakes for iron across different life stages are displayed in Table 1. These are presented as recommended dietary allowances, recommended dietary intakes, or reference nutrient intakes (depending on country/-population), which meet the needs of 97.5% of individuals in a particular stage of life. The variation in the iron bioavailability across diets and varying levels of iron status across populations (i.e., iron-deficient individuals absorb more iron) make it difficult to estimate individual iron requirements, and this in part causes recommendations for iron intake to vary between countries. For example, recommendations for iron intake by the Food and Agriculture Organization of the United Nations and World Health Organization are based on lower levels of iron bioavailability in the diet and are therefore somewhat higher than recommendations from developed countries such as the United Kingdom, the United States, and Canada.

The iron requirements for adolescent and premenopausal females are higher than those in children and males of a similar age to account for iron losses during menstruation. Physiological requirements for iron increase during pregnancy and iron requirements increase at 6 months of age due to rapid growth and depletion of iron stores. Therefore, infants require an additional source of iron, and complementary foods are recommended by the World Health Organization from 6 months of age. The iron content of breast milk is highly bioavailable compared with the iron in infant formula. The early introduction of other fluids such as water, tea, and fruit juice may also displace breast milk in the diet. The iron content of cow's milk is low and poorly bioavailable, and its early introduction has been identified as a risk factor for the development

Table 1 Recommendations for dietary iron intake in different populations

The United Kingdom[a]		*The United States and Canada*[b]		*FAO/WHO*[c]		
Gender, age	*RNI*[d] *(mg day^{-1})*	*Gender, age*	*RDA*[e] *(mg day^{-1})*	*Gender, age*	*RNI*[d] *(mg day^{-1})*	*RNI*[f] *(mg day^{-1})*
0–3 months	1.7		–		–	–
4–6 months	4.3	0–6 months[g]	0.27		–	–
7–12 months	7.8		11.0[c]	6–12 months[h]	6.2	9.3
1–3 years	6.9		7.0		3.9	5.8
4–6 years	6.1	4–8 years	10.0	4–6 years	4.2	6.3
7–10 years	8.7		–		5.9	8.9
Males, 11–18 years	11.3	Males, 9–13 years	8.0	Males, 11–14 years	9.7	14.6
		Males, 14–18 years	11.0	Males, 15–17 years	12.5	18.8
Females, 11–18 years	14.8	Females, 9–13 years (assumption: nonmenstruating)	8.0	Nonmenstruating females, 11–14 years	9.3	14.0
		Females, 14–18 years	15.0	Menstruating females, 11–14 years	21.8	32.7
				Females, 15–17 years	20.7	31.0
Males, 19–50 years	8.7		8.0	Males, 18+ years	9.1	13.7
Females, 19–50 years	14.8		18.0	Females, 18+ years	19.6	29.4
Pregnancy	–		27.0			
Lactation, 14–18 years	–		10.0	Lactating	10.0	15.0
Lactation, 19–50 years			9.0			
50+ years	8.7		8.0	Postmenopausal females	7.5	11.3

Abbreviations: RDA, recommended dietary allowance; RNI, reference nutrient intake.

[a]Department of Health (1991).

[b]World Health Organization and Food and Agricultural Organization of the United Nations (2004).

[c]Food and Nutrition Board: Institute of Medicine (2001).

[d]Based on 15% absorption.

[e]Based on 18% absorption.

[f]Based on 10% absorption.

[g]Recommended intakes based on observed mean intakes of infants fed breast milk.

[h]Bioavailability of iron varies greatly at this time.

Adapted from Scientific Advisory Committee on Nutrition (2010). Iron and health. London: The Stationery Office.

of iron-deficiency anemia. Severe maternal iron-deficiency anemia may adversely affect the iron content of breast milk, and therefore, it is advantageous to both mother and infant that maternal iron deficiency is treated.

Iron Supplementation: Prevention and Treatment of Iron-Deficiency Anemia

In many countries, the amount of iron absorbed from the diet is not adequate to maintain iron stores. The increased iron requirements during pregnancy and in infancy mean that individual iron requirements are difficult to achieve through diet alone. Iron supplementation should therefore be considered for pregnant women and infants aged 6–24 months, due to their increased risk of developing iron-deficiency anemia and widespread public health benefits.

Iron supplementation programs during pregnancy operate in both developed and developing countries. In some countries, iron supplementation in pregnancy is only recommended for women with clinically diagnosed iron-deficiency anemia. However, in countries where the prevalence of anemia is high, diets are low in bioavailable iron, and there is no widespread fortification of foods, iron supplementation is the most practical and cost-effective way to assist women of child-bearing age to enter pregnancy with adequate iron stores. In these countries, iron supplementation should continue after the infant's delivery to ensure that adequate iron stores are achieved.

In populations where iron-fortified complementary foods for infants are not readily available, infants may benefit from iron supplementation. Iron supplementation may also benefit children, adolescents, and women of reproductive age particularly in countries where the prevalence of anemia is high. However, untargeted iron supplementation of children living in countries where malaria is endemic may be problematic, with one study observing an increased risk of severe illness and death in preschool children consuming iron and folic acid supplements. Several studies have investigated the efficacy of iron supplementation once or twice per week, as opposed to daily iron supplementation, and results in school-age children, adolescents, and nonpregnant women appear promising. Limitations of iron supplementation programs include the logistics of delivering iron supplements to the intended population and individual lack of compliance to taking iron supplements.

Where iron-deficiency anemia has already developed, dietary treatment alone is insufficient to improve iron status. The quickest and most effective way to treat iron-deficiency anemia is through iron supplementation. In populations where severe anemia is common, its early detection and treatment are essential to prevent morbidity and mortality. Heath workers need to be trained to recognize and treat (or appropriately refer) individuals presenting with anemia. As the causes of anemia are multifactorial in nature, an individual should be followed up after commencing iron supplementation to ensure the treatment is working effectively.

Fortification of Food with Iron

Fortification of food with iron involves the addition of iron to a food, in order to prevent or treat iron deficiency. Fortifying food with iron can be a cost-effective, long-term approach to combating iron deficiency in populations with a high prevalence of dietary iron deficiency and is the main approach used to improve the iron supply in the diet. Iron fortification has the advantage of not being dependent on individual motivation and compliance. However, the risks of iron overload (e.g., in people with hemochromatosis) need to be considered.

The reasons for fortification of food with iron vary. Market-driven fortification is common in developed countries and includes initiatives used by the food industry to increase sales such as adding iron to breakfast cereals. Targeted fortification is aimed at meeting the needs of specific groups (e.g., infant formulas and complementary foods) and has been shown to be effective in preventing iron-deficiency anemia in infants in Latin America and the United States. Mass fortification is usually mandatory and involves the addition of iron to foods commonly consumed by the general population. Depending on the choice of food, mandatory fortification may enable all sectors of the population to be reached. Foods that have been fortified with iron include wheat flour, cereals, milk and milk products, and condiments such as sugar, salt, fish sauce, soy sauce, and curry powder. Iron is often added to foods such as flour to replace iron lost during the milling process.

Iron compounds used to fortify foods are affected by the same factors that affect iron absorption in food (e.g., an individual's iron status, other dietary factors, and the characteristics of the iron fortificant itself). The development of an iron-fortified food involves the selection of an iron compound that is adequately absorbed but does not change the taste and appearance of the food to which it is added. The iron chosen must also be able to overcome the inhibitory effects of food components such as phytic acid on iron absorption. Water-soluble iron compounds such as ferrous sulfate are highly bioavailable and therefore desirable to use as food fortificants. However, they often lead to sensory changes (e.g., taste and color changes and rancidity), making them unsuitable for use in many foods. Ferrous sulfate is often used to fortify foods that are stored for short periods of time (e.g., infant formulas). Fortificants that are water-insoluble but soluble in acids (e.g., ferrous fumarate) tend to be well absorbed. Iron fortificants that are water-insoluble and poorly soluble in dilute acid (e.g., elemental iron powders and ferric pyrophosphate) have the lowest and most variable bioavailability. Despite this, elemental iron compounds are commonly used to fortify food products such as breakfast cereal and wheat flour, as they are relatively inexpensive and do not cause sensory changes, increasing shelf life and consumer acceptability.

Early reports suggested a lack of evidence regarding the efficacy of iron fortification of staple foods such as flour in improving the iron status of populations. This was attributed to the use of less bioavailable elemental iron powders, inadequate intakes of iron-fortified foods, and insufficient levels of iron fortificants in food. However, in a recent systematic review of randomized controlled trials, consumption of iron-fortified foods was found to significantly increase serum ferritin and hemoglobin concentrations and reduce the risk of iron deficiency and iron-deficiency anemia. More recently, selective breeding and genetic engineering have been used to increase the iron content of staple foods. More work is needed to ensure the bioavailability of this iron and that the iron content is increased to nutritionally beneficial levels.

Conclusion

Traditionally, one approach (e.g., iron supplementation, dietary education, and fortification of food) has been recommended as the main avenue to pursue in the development of iron nutrition programs. However, it is now recognized that a multitude of integrated strategies are necessary in order to effectively control iron deficiency (and anemia) in populations. The absorption of nonheme iron (the predominant form of iron in the diet) is affected by several dietary factors. Clear evidence is lacking regarding the most effective strategy for improving the iron status of populations. Intervention strategies include the widespread fortification of staple foods (and complementary foods) with iron, wider use of oral iron supplementation, education regarding strategies to improve iron intake and bioavailability in the diet, and the improvement of complementary feeding in infants. These strategies should complement other public health programs, such as infection control, deworming and other nutrition programs, and programs focussed on maternal and child health such as family planning and breastfeeding initiatives. Governments need to prioritize and commit to sustainable, long-term programs to prevent iron-deficiency anemia in vulnerable populations, particularly in countries with limited dietary diversity. All agencies and stakeholders should be involved including government agencies, the food industry, and the health sector. The prevalence of anemia and effectiveness of programs implemented to prevent anemia should be monitored using ongoing and reliable surveillance systems.

Tips to Increase Iron Absorption from Food

- Include meat, fish, or poultry in meals where possible.
- For vegetarians or in populations with limited access to meat, fish, and poultry, include foods rich in nonheme iron such as cereals, pulses, legumes, nuts, seeds, eggs, vegetables, and iron-fortified foods.
- Include vitamin C-rich food or drinks alongside meals containing nonheme iron.
- Avoid drinking tea and coffee with meals containing nonheme iron. Drink between these meals instead.
- Consume milk and milk products in between meals, rather than with meals containing nonheme iron.
- Limit the addition of foods high in phytate to main meals (e.g., the addition of extra bran to breakfast cereals).
- Infants should be exclusively breastfed for approximately six months. At 6 months of age, include complementary foods high in iron or fortified with iron where possible. Cow's milk as a drink should be avoided until at least 1 year of age.
- For individuals with iron-deficiency anemia, iron supplementation is required. Dietary treatment supplementation alone is not sufficient to treat iron-deficiency anemia, but should be used alongside iron supplementation.

See also: Anemia: Causes and Prevalence; Ascorbic Acid: Physiology and Health Effects; Ascorbic Acid: Properties, Determination and Uses; Calcium: Physiology; Calcium: Properties and Determination; Food Fortification: Rationale and Methods; Iron: Biosynthesis and Significance of Heme; Iron: Physiology of Iron; Iron: Properties and Determination; Meat: Structure; Milk: Role in the Diet; Phytic Acid: Properties, Uses, and Determination; Vegetarian Diets.

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Annonaceous Fruits

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Introduction

The family Annonaceae is composed of more than 119 genera with more than 2000 species. It is the largest family in Magnoliales. Only four genera (*Annona*, *Asimina*, *Rollinia*, and *Uvaria*) produce edible fruit. The genus *Annona* is composed of about 120 species and is the most important source of edible fruit in Annonaceae. *Annona cherimola* L., *Annona muricata* L., *Annona squamosa* L., *Annona reticulata* L., *Asimina triloba* L., and the interspecific hybrid atemoya (*A. cherimola* x *A. squamosa* Mabb.) are some of the most important species of this genus.

Sources, Production, and Fruit Morphology

Annona cherimola Mill

Annona cherimola or cherimoya is believed to have originated in the highland Andes valley between Peru and Ecuador and is considered as the best of the Annonaceae fruits. *Annona cherimola* is known as cherimoya (Spanish), cherimolier (French), anona (Mexico), and noina ostrelia (Thailand). This species is commercially grown in Spain, Bolivia, Chile, Peru, and New Zealand. Cherimoya is cultivated mainly in the Mediterranean, and Spain is the world's leading producer. It is a small, erect, spreading deciduous tree (NRC, 1989). Cherimoya is more tolerant to low temperatures than the soursop. Cherimoya fruit is normally conical, oval, or heart-shaped and the skin may be smooth with fingerprint-like markings in some varieties or covered with conical or rounded protuberances. Five botanical forms of cherimoya are seen based on the fruit shape and markings on the skin: finger-printed, smooth-skinned (cherimoya lisa), tuberculate (the most common type with heart-shaped fruit and wartlike tubercles), mammilate, and umbonate (the barbed or the spiny cherimoya). Its fruit has a thick green peel and creamy white flesh with custard-like consistency. Cherimoya fruit is fleshy and sweet and the pulp is white in color. The low-acid flesh has a delicate, rich, aromatic flavor that resembles that of pineapple and banana. The fruit has numerous black seeds. Fully mature fruit is harvested while still firm, and on ripening, the fruit's skin color changes from grayish green to yellow-green.

Annona muricata L.

Annona muricata, commonly known as graviola or soursop or guyabano, is another tropical fruit tree in the Annonaceae family. This plant probably originated in the Antilles in the Caribbean. Soursop is cultivated in many countries such as Angola, Brazil, Colombia, Costa Rica, Cuba, Jamaica, Mexico, Panama, Peru, Puerto Rico, Venezuela, and SE Asia. Mexico is the main soursop producer in the Americas. Soursop is a small perennial branched, quick-growing tree. Soursop thrives well in the humid tropical and subtropical lowlands with warm winters. Trees are sensitive to low temperature below 5 °C and to frost. Soursop has the largest fruit in the genus, weighing from 0.9 to 10 kg with an average weight of 4 kg. Soursop fruits are normally ovate, heart-shaped, or conical. The fruit is prickly and has a sour and sweet flavor. Unripe fruit is dark green that turns to light green when ripe. Soursop fruit is derived from the fusion of many fruitlets. The fruit pulp consists of white fibrous juicy segments. Numerous black or dark brown seeds are embedded in a firm fleshy, acid-sweet, whitish pulp. It has more acidic flavor than cherimoya and resembles a mixture of pineapple and mango. The aroma volatiles of soursop consist of mainly esters (80%). Fruit is harvested when it is fully mature, firm, yellow-green, and with spines apart.

Annona squamosa L.

Annona squamosa is the most widely grown *Annona* spp., and this small tropical tree originated in the New World tropics, probably in the Caribbean region. This plant is also known as sugar apple or sweetsop and has many other regional names such as custard apple (India), anon (Portuguese), and noi-na (Thailand). Sugar apple is a favorite fruit in Cuba and is also common in West Indies. Sweetsop is also widely cultivated in Taiwan. It is still grown as a backyard tree and the Philippines is considered as one of the largest producer in the world. Sweetsop tree is smaller than the cherimoya and is semi-deciduous in growth habit. It grows 3–8 m in height with a short trunk and irregularly spreading branches. Owing to small fruit size, poor shelf life, and fruit cracking at maturity, this fruit has not shown much potential for large-scale commercial cultivation. Sweetsop is a round, heart-shaped, ovate, or conical fruit weighing about 120–330 g. Fruit are greenish yellow with many outer round protuberances and covered with a white powdery bloom. The flesh is white with numerous black seeds and has a pleasant sweet–sour flavor. Fruit has high caloric value and a high sugar content of 58% (dry mass). The majority of cultivars are grown in India.

Annona reticulata L.

This plant, also called custard apple, bullock's heart, or netted custard apple, is used as a common medicinal plant in the Caribbean. It is a native of the West Indies and cultivated in the Bahamas and occasionally in Bermuda and southern Florida. *A. reticulata* is a warm tropical species, but can survive in subtropical conditions. It prefers humid atmosphere, but is not cold-hardy or drought-tolerant. This plant is a small tree often growing 3–10 m tall with an irregular canopy and widely branched near the base. Fruit are commonly heart-shaped but may be conical, ovoid, or irregular in form and weigh from 0.1 to 1.0 kg. The flesh is white and sweet with several dark coffee-colored seeds. Fruit are more or less smooth with a reddish-yellow fruit skin color and the pulp is white, granular, and aromatic. Numerous dark brown glossy seeds are present in the pulp.

Atemoya

Atemoya is a hybrid between *A. squamosa* and *A. cherimola* Mabb. (sugar apple and cherimoya) in the *Annona* family. The crosses were made by P. J. Webster in 1907 for the USDA in Florida. Natural crossing also occurred in the field in Australia in 1850 and in Palestine in 1930. This hybrid is in cultivation in Australia where it is called custard apple. It is also grown in Spain, Bolivia, Chile, Peru, Israel, Florida, and Hawaii. Atemoya is a small tree with a short trunk and low drooping branches growing to a height of 8–10 m. Atemoya thrives best in warm tropical to subtropical areas with 70–80% relative humidity and with adequate rainfall. Atemoya is frost-sensitive and destroyed by low temperatures. Trees are semideciduous and remain dormant in late winter and early spring. Most atemoya hybrids resemble cherimoya in tree vigor and habit. Atemoya fruit is a fleshy syncarp formed by the fusion of carpels and the receptacles. The fruit is pale-bluish green to pea green, heart-shaped, or conical weighing 2–2.5 kg. The fruit shape is highly variable and the fruit surface is covered with prominent angular areoles, which are either smooth or pointed. The fruit flesh is white with fewer seeds and not conspicuously divided into segments. The pulp is easily separable from the seeds. The seeds are dark brown to black and are smooth.

Asimina triloba

Asimina triloba (L.) Dunal, or pawpaw, is the only temperate plant species belonging to tropical Annonaceae. *Asimina* comprises nine species, most of which are native to southeastern regions of Florida and Georgia. The North American pawpaw, also known as the Kentucky or Hoosier or poor man's banana, is the largest fruit native to North America. Pawpaws grow wild in the forests of 25 states in the eastern United States from northern Florida to southern Ontario (Canada) and as far west as eastern Nebraska. At present, pawpaw is gaining interest as a gourmet food. Commercial cultivation of pawpaw is limited to small private orchards usually less than 1 ha in size. Currently, most pawpaw fruit for sale are collected from the wild. In the United States, pawpaw fruit are sold at the farmer's market. Pawpaw fruit have both fresh market and processing potentials. Pawpaw is a small deciduous tree or shrub attaining a height of 5–10 m. Pawpaw fruit is a berry. Fruit are oblong with green skin and occur singly or in clusters. The fruit are typically 3–15 cm long and weigh from 100 to 1000 g. Two rows of seeds are seen in the fruit that are brown-colored and bean-shaped. Ripe fruits have a strong and unique aroma and characteristic flavor that resemble a mixture of banana, mango, and pineapple. Skin and seeds of the fruit are not eaten. The pawpaw fruit is highly nutritious and is comparable to banana in dietary fiber and overall nutrient content. Fully ripe pawpaw fruit is very soft, so it should be handled with extreme care as they are easily susceptible to bruises and other physical damages. The seed endosperm contains alkaloids that impair digestion. Pawpaw allergies have been reported in some individuals.

Patterns of Consumption

Most annonaceous fruits are primarily dessert fruit and are consumed fresh when fully ripe. The fruit pulp can also be frozen and eaten like ice creams. Ripe fruits are used for making ice creams, milk shakes, jellies, or sorbets. Fruit pulp can be frozen and can also be processed into yogurt, juice, nectars, and wine. Among the various annonaceous fruits, soursop has the greatest processing potential because of its high sugar content, excellent flavor, and high recovery from the large fruit. Pulp recovery ranges from 62% to 85.5% of the fruit. Viscous soursop pulp is diluted and the pH is adjusted to 3.7 by the addition of citric acid. Sugar is also added to the pulp to create a desirable balance between acidity, sweetness, and flavor. Soursops are considered as too acid for eating fresh, but it is good for preparing refreshing drinks. Soursop is also made into strained juice and also preserved as juice blends, nectars, canned juice, jam, or jelly. In the Philippines, soft soursop fruit is used as a vegetable cooked with coconut milk. Two types of soursop were distinguished in El Salvador, the sweet variety that is eaten raw (guanaba azucaron) and the very sour (guanaba acida) used for the preparation of drinks. Immature soursop fruits are used as vegetables. In Brazil, seeds are roasted or fried. In Indonesia, soursop fruits are added to soups. An alcoholic drink called champola is prepared from processed pulp. A method for canning soursop juice was developed by blending with sugarcane juice or papaya juice. The cherimoya fruit is chilled often with salt and lemon. The fruit is also mixed with wine, milk, and yogurt and also processed into ice cream and sherbet and baked into cookies and pastries. Chilling of annonaceous fruits improves their flavor.

Postharvest Quality and Shelf Life

Annonaceous fruits are climacteric with a rise in ethylene production during ripening. Most of them have a short postharvest life. Ripe fruits are very soft and are susceptible to injury and bruises during handling. *Annona* fruits are very delicate and extreme care should be taken while handling and transportation. Annonaceous fruits are harvested while still firm and are ripened at room temperature. Immature fruits do not develop the full flavor and aroma off the tree. Optimum handling temperature for *Annona* fruits is suggested to be 8–12 °C and the fruits are sensitive to temperatures below 8–12 °C resulting in chilling injury causing mealiness, less flavor, darkening, and hardening of the skin.

Nutritional Composition and Health Effects

The pulp of annonaceous fruit is flavorful and has a reasonable content of vitamins and minerals. Cherimoya, soursop, and sugar apple are the most widely consumed fruits. The compositions of nutrients in raw annonaceous fruits (100 g edible portion) are presented in Table 1. In general, annonaceous fruits are high in sugars. Cherimoya fruit is a rich source of antioxidants such as vitamins A and C. About 60% of cherimoya fruit is edible. The sugars constitute a mixture of fructose, glucose, and sucrose. Pulp carbohydrate content of cherimoyas is generally high, but low in acidity. Furthermore, it is a good source of thiamine, riboflavin, and niacin. Fiber content is represented by cellulose, hemicellulose, lignin, and pectic substances. Cherimoya fruit is particularly high in several essential minerals such as potassium, copper, manganese,

Table 1 Compositions of nutrients of cherimoya, custard apple, soursop, and sugar apple fruits (100 g)

Components	*Unit*	*Cherimoya*	*Custard apple*	*Sugar apple*	*Soursop*
Water	g	79.39	71.50	73.23	81.16
Energy	kcal	75	101	94	66
Protein	g	1.57	1.70	2.06	1.00
Total lipid (fat)	g	0.68	0.60	0.29	0.30
Carbohydrate (by difference)	g	17.71	25.20	23.64	16.84
Fiber, total dietary	g	3.0	2.4	4.4	3.3
Sugars, total	g	12.87	nd	nd	13.54
Minerals					
Calcium (Ca)	mg	10	30	24	14
Iron (Fe)	mg	0.27	0.71	0.60	0.60
Magnesium (Mg)	mg	17	18	21	21
Phosphorus (P)	mg	26	21	32	27
Potassium (K)	mg	287	382	247	278
Sodium (Na)	mg	7	4	9	14
Zinc (Zn)	mg	0.16	nd	0.10	0.10
Vitamins					
Total ascorbic acid	mg	12.6	19.2	36.3	20.6
Vitamin B_1	mg	0.101	0.080	0.110	0.07
Vitamin B_2	mg	0.131	0.100	0.113	0.05
Niacin	mg	0.644	0.500	0.883	0.900
Vitamin B_6	mg	0.257	0.221	0.200	0.059
Folate, DFE	μg	23	nd	14	14
Vitamin A, RAE	μg	0	2	0	0
Vitamin A IU	IU	5	33	6	2
Vitamin E (α-tocopherol)	mg	0.27	nd	nd	0.08
Vitamin K_1 (phylloquinone)	μg	nd	nd	nd	0.4
Lipids					
Total saturated fatty acids	g	0.233	0.231	0.048	0.051
Total polyunsaturated fatty acids	g	0.188	nd	0.040	0.069
Total monounsaturated fatty acids	g	0.055	nd	0.114	0.090
Cholesterol	mg	0	0	0	0

IU, international units.
USDA National nutrient database for standard reference, Release 27 (http://ndb.nal.usda.gov).

and magnesium. Edible portion of cherimoya fruit is rich in numerous health-promoting phytochemicals. The pulp of soursop is high in carbohydrates and sugars. It is also a good source of vitamin C, vitamin B_1, vitamin B_2, and potassium. The edible portion corresponds to about 67.5% of the total fruit weight. Soursop fruit pulp is a potential source of dietary fiber and is the only annonaceous fruit with tannin in pulp. The sugars are represented mainly by fructose, glucose, and sucrose. The fruit also contains vitamins A and B_5. The most predominant acid is citric acid followed by low content of malic and isocitric acid. Custard apple pulp is creamy yellow and sweet with low acidity. The edible portion of the fruit is about 45%. Sugar apple flesh is slightly granular, creamy yellow, or white and is flavorful with low acidity. It is the sweetest of the *Annona* and 28–37% of the total fruit weight is edible. The fruit flesh is high in carbohydrates and sugars and also contains vitamins C, B_1, and B_2; potassium; and dietary fiber. Vitamin A content is low. Sugar apple has vitamin C slightly higher than in grapefruit. Fructose, glucose, sucrose, and oligosaccharides form the major carbohydrates of the fruit pulp. The amount of sugar is quite high (58% of dry mass). The fruit has low triglyceride content. A diterpenoid compound, kaur-16-en-18-oic acid, was also identified in the lipid fraction. Atemoya fruit contain significant amounts of vitamin C, thiamine, potassium, magnesium, and dietary fiber. The fruit also has citric and malic acids. Fructose and glucose forms the main sugar components (80–90%). Pawpaw fruit has high nutritional value and is rich in many nutraceutical compounds. Pawpaw and banana are similar in dietary fiber content and in overall nutritional composition. In particular, pawpaw fruit contains threefold more magnesium and 17-fold more manganese than banana.

Acetogenins and Their Biological Effects

Annonaceous plants contain acetogenins in parts such as the leaf, bark, fruit, and root. Annonaceous acetogenins are a unique class of C35 or C37 secondary metabolites derived from the polyketide pathway. Acetogenins are gaining much attention recently due to their diversity of biological activities and are one of the most rapidly growing classes of new natural products. Acetogenins are fatty acid derivatives with tetrahydrofuran rings and a methylated γ-lactone having various hydroxyl, acetoxyl, and/or ketoxyl groups along the hydrocarbon chains. Numerous studies on acetogenins indicate that these compounds are powerful cytotoxins and demonstrated *in vivo* antitumor, pesticidal, antipiscicidal, antihelminthic, antiviral, antimalarial, and antimicrobial properties, suggesting many potentially useful applications. The isolation of

uvaricin from the roots of *Uvaria accuminata* by Joland and his coworkers in 1982 initiated the research on acetogenins. Since then, more than 400 acetogenin compounds were identified and studied from various plants. The various biological activities of acetogenins are summarized here.

Anticancer Activity

Presently, acetogenin compounds are attracting much research attention worldwide due to their potent cytotoxic activities, which is tested *in vitro* against a large number of tumor cell lines. Annonaceous acetogenins are known as powerful inhibitors of complex I (NADH/ubiquinone oxidoreductase) in mammalian and insect mitochondrial electron transport systems. They also inhibit the NADH oxidase of the plasma membranes of cancer cells. Both these mechanism of actions deplete the ATP supply of the cells, which induces apoptosis (programmed cell death). Cancer cells have higher ATP demand than the normal cells and are better targets for the cytotoxic action of acetogenins. Acetogenins also show potent cytotoxic activity against multidrug-resistant tumors and insecticide-resistant pests.

Numerous research reports on the cytotoxic effects of acetogenins from various annonaceous plants towards different human cancer cell lines are available. Only a few relevant studies are mentioned here. Various parts of soursop tree contain an array of acetogenins that are responsible for its several different types of biological and medicinal activities. One of the acetogenin, cis-annonacin isolated from the seeds of soursop, showed very high cytotoxicity towards colon adenocarcinoma cells with a potency of 10 000 times that of Adriamycin, a drug used to treat cancer. A number of acetogenins were identified and isolated from the seeds of ripe soursop including annonacin, isoannonacin, goniothalamicin, and gigatetrocin. These acetogenins showed cytotoxicity against A-549 lung carcinoma, MCF-7 breast carcinoma, and HT-29 colon adenocarcinoma cell lines. In *in vitro* studies, solamin, an acetogenin from soursop leaves, showed cytotoxic action against KB and VERO cell lines. Murisolin (acetogenin isolated from seed of soursop) showed 105 to 106 times more potency than Adriamycin in their cytotoxic activity against human tumor cell lines.

A. squamosa are abundant in acetogenins especially in the bark and seeds. Squamotacin, the acetogenin from the bark of sugar apple, has been reported to possess extremely high cytotoxicity against human prostate tumor cell line (PC-3). Two acetogenins from seeds, squadiolins A and B, showed high cytotoxicity against human hepatocellular carcinoma (HepG2) and human breast cancer (MDA-MB-23) cells. The acetogenins contained in the bark and leaf of *A. reticulata* have been shown to have very potent anticancer properties. Two acetogenins called squamocin and annonacin, isolated from the seeds, showed strong cytotoxic activities against a number of cancer cell lines tested and showed great potential as anticancer compounds. Squamocin also arrested the T24 bladder cancer cells at the G1 phase and also induced the expression of Bax and Bad pro-apoptotic genes and triggered cell apoptosis. Various acetogenins such as annonacin, annonisin, and bullatacin were identified from the seeds of atemoya. Bullatacin was shown to inhibit proliferation of human hepatocellular carcinoma . Pawpaw contains acetogenins in its unripe fruit, seeds, roots, twigs, and bark tissues that exhibit antitumor and pesticidal properties. This plant species contains annonaceous acetogenins. Three acetogenins, namely, asimicin, bullatacin, and bullatalicin, were identified in the ripe pawpaw fruit pulp by HPLC-MS analysis.

Pesticidal and Insecticidal Effects

Acetogenins isolated from fruit pericarp of *A. squamosa* showed antileishmanial activity and were inhibitory to *Leishmania in vitro*. Acetogenins also showed pesticidal properties. Extracts of *A. muricata* and *A. cherimola* seeds showed potent antiparasitic activity against *Entamoeba histolytica*, *Nocardia brasiliensis*, and *Artemia salina*. Also, acetogenins such as annonacin, isoannonacin, and goniothalamicin isolated from the leaves showed strong antimolluscicidal activity. Apart from this, the leaves of *A. muricata* demonstrated antimalarial and antiprotozoal activities *in vitro*. A number of acetogenin-based commercial products were developed including a shampoo for treating head lice infestation, pesticidal sprays, and an ointment for the treatment of oral herpes (HSV-1) and other skin ailments. Additionally, acetogenins from Annonaceae and pawpaw showed very high insecticidal activity against a number of insects. They are particularly effective against chewing insects. Studies demonstrated that acetogenin compounds are toxic to pests including cockroaches, Colorado potato beetle, blowfly larvae, mosquito larvae, spider mites, European corn borers, melon or cotton aphids, and nematodes.

Other Medicinal/Therapeutic Properties

The stem extract of *A. muricata* showed antiviral activity against herpes simplex virus. Several kaurane compounds were isolated from *A. squamosa* fruit and some of them showed significant activity against HIV replication in H9 lymphocyte cells. Some animal studies showed the hyperglycemic activities of methanolic extracts of *A. muricata*. *Annona squamosa* is used by ethnic groups in India for the management of diabetes and its complications. Studies showed that the hot leaf extract of this plant possess hypoglycemic and antidiabetic activities. Studies conducted in animal systems demonstrated that administration of *A. squamosa* leaf extract improved the lipid profile of the treated group than the control. Studies reported that *A. squamosa* fruit pulp could reduce the total cholesterol level by 45–46% in normal and 32% in diabetic animals. Some reports are available that show the potential antithyroid activity of seed extracts of *A. squamosa* in reducing hyperthyroidism in lab animals. Eleven ent-kauranes isolated from the stem bark of *A. squamosa* showed immunomodulatory activities in leukocytes.

Adverse Effects

Chronic consumption of annonaceous fruits has also been linked to an increased risk of developing atypical Parkinsonism.

Prevalence of an atypical form of Parkinsonism among the natives of Guadeloupe in the West Indies and in New Caledonia in the Pacific was reported. Patients with severe progression of the disease often consumed the fruits and tea of leaves of the Annonaceae family (*A. muricata*, *A. reticulata*, and *A. squamosa*) particularly *A. muricata*. At first atypical form of Parkinsonism was attributed to the benzylisoquinoline alkaloids present in Annonaceae, but later, acetogenins became the suspected potential neurotoxins. Recent studies have suggested a possible link between the long-term frequent intake of Annonaceae fruit and tea by the people of Guadeloupe, New Caledonia, and the Caribbean region and the development of high rate of atypical Parkinsonism in their later life. The fruit and other parts of *A. muricata* are abundant in annonacin, an acetogenin that has been shown to be toxic *in vitro* and *in vivo* to dopaminergic neurons. A number of animal studies reported the annonacin-induced degeneration of the basal ganglia and brain stem. Scientific studies are needed to determine the risks of neurotoxicity and neurodegeneration associated with the consumption of soursop, pawpaw, and other related annonaceous plant derivatives.

Traditional Medicinal Uses

Various parts of Annonaceae plants have been used traditionally in many parts of the world for treating various ailments and complaints ranging from high blood pressure and cancer.

Annona muricata is popularly used in traditional Indian medicine for the treatment of kidney troubles, fever, and ulcers and possesses antispasmodic, antidysenteric, and parasiticidal activities. In Ayurvedic medicine, this plant is used as a tonic, as an abortifacient, as a febrifuge, for scorpion stings, for controlling high blood pressure, and as a respiratory stimulant. In Trinidad and Tobago, the leaves are used to treat hypertension. In Brazil, a tea made from the leaf is used to treat arthritis pain, rheumatism, and neuralgia. In Jamaica, Haiti, and West Indies, the fruit and/or fruit juice is used for treating fevers, parasites, and diarrhea and as a lactagogue. The bark or leaf of this plant is used as an antispasmodic and as a sedative, to control coughs and spasmodic bowel pain and help ease difficult child birth, and against asthma, hypertension, and parasites. Pulverized seeds and seed oil are found to be effective for head lice infection. Seeds, bark, root, and unripe fruit have strong astringent properties.

Crushed leaves of *A. squamosa* are inhaled for dizziness. In the Philippines, insect bites are treated by the application of juice from *A. squamosa* unripe fruit. Crushed seeds of *A. squamosa* are used as an abortifacient. Poultice of mashed leaf of *A. muricata*, *A. squamosa*, and *A. reticulata* has been used externally as compresses for swollen feat and also used to treat skin ailments such as eczema, boils, abscesses, and ulcers. Unripe fruits of *A. muricata*, *A. squamosa*, and *A. reticulata* are rich in tannins, have astringent property, and used for treating dysentery and diarrhea. Root bark of *A. reticulata* is used to relieve toothaches. Powdered seeds and seed oil from *A. muricata*, *A. squamosa*, and *A. reticulata* have insecticidal property and are effective for treating head lice. *A. reticulata*, *A. squamosa*, and *A. muricata* leaves are used internally against worms.

See also: Fruits of Tropical Climates: Biodiversity and Dietary Importance; Fruits of Tropical Climates: Dietary Importance and Health Benefits.

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Antibiotics and Drugs: Drug–Nutrient Interactions

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Introduction

The composition of patients' diet and their overall nutritional state can markedly impact both pharmacokinetic (i.e., what the body does to the drug such as absorption, distribution, metabolism, and elimination) and pharmacodynamic (i.e., what the drug does to the body such as affinities to receptors or tissues) drug properties. Many nutritional components can affect biotransformation and disposition of drugs, by impacting blood flow, gastrointestinal (GI) motility, and enzymatic activity. These effects are variable and patient-specific, and confounded by numerous other factors, and often unpredictable. In times of increased energy demands (i.e., during growth, pregnancy, and lactation), there is an increased risk for drug-induced nutrient deficiencies. Not considering these factors can result in a serious consequence such as antibiotic treatment failure that can occur when the absorption of an orally administered antibiotic is taken with food. Similarly, toxicities can occur if a nutrient inhibits the enzymes in the gut that detoxify a medication. The practitioner must consider the potential for interactions that may occur between nutritional status, disease state, and drug action and even patient age when developing a therapeutic plan. This article will review how drug therapy can impact a patient's nutritional state and how nutrition can impact drug response, with a particular emphasis on anti-infective agents.

Factors That Impact Drug Pharmacokinetics

Differences in body composition are an important consideration when determining the pharmacokinetic properties of a given drug. Both nutrients and drugs are delivered via the GI tract and go through an absorption phase before reaching the systemic circulation and the sites of action. Once absorbed, these compounds enter the portal vein from the GI lumen and via a series of complex processes eventually leading to the systemic circulation. Properties such as dissolution of the solid dosage form, the passage of the chyme along the GI tract, passive diffusion, active transport, presystemic metabolism, and the presence of GI disease may further affect the pharmacokinetics of the drug.

Influence of Medications on Nutrient Status

Nutrient Transport and Absorption

Drugs can impact the uptake of nutrients. For example, fluoroquinolones can inhibit L-carnitine transport, which may account for the toxicity of this drug class to the fetus. There are several factors that can interfere with nutrient absorption. The direct systemic effect of a drug on one nutrient may have secondary effects on another nutrient. For example, neomycin and para-aminosalicylic acid may damage intestinal mucosa and destroy intestinal villi and microvilli, resulting in an inhibition of brush border enzymes and intestinal transport systems. Similarly, isoniazid can inhibit the hydroxylation of vitamin D in the liver and kidney, resulting in a functional deficiency of vitamin D and secondary impaired calcium absorption.

Nutrient Metabolism

Medications may affect nutrient metabolism via several mechanisms. They may promote the catabolism of the nutrient or inhibit the essential intermediary metabolism of a nutrient, usually a vitamin. This is often an unwanted side effect, as in the case of pyridoxine antagonism seen with isoniazid use. Isoniazid therapy can result in pyridoxine deficiency by inhibiting pyridoxal kinase. Isoniazid depletes pyridoxine stores and subsequently the neurotransmitter gamma-aminobutyric acid, resulting in seizures. In the event of an isoniazid overdose, administration of pyridoxine can eliminate the resulting seizures and metabolic acidosis.

Many medications induce drug-metabolizing enzymes leading to enhanced enzymatic activity, with a subsequent increased demand for their vitamin cofactors. For example, patients treated with cephalosporin antibiotics may develop hemorrhagic states secondary to drug-induced vitamin K deficiency. Cephalosporins block vitamin K reductase, which is necessary for vitamin K activation. Cephalosporins may also block carboxylation of vitamin K-dependent peptides to yield Gla residues that are required for calcium binding in the conversion of vitamin K-dependent proenzymes to their active state, which are necessary in the coagulation cascade.

Drug-Associated Fluid and Electrolyte Disturbances

Several commonly used anti-infective agents can alter electrolyte balance. Amphotericin B and the antipseudomonal penicillins both can cause renal wasting of potassium, resulting in hypokalemia. Conversely, trimethoprim can cause hyperkalemia due to its weak diuretic properties coupled with potassium-sparing activity.

The impact of a drug on phosphorus balance is important in patients receiving nutritional support as the synthesis of new cells increases phosphorus requirements. Patients already at risk for refeeding syndrome are especially prone to the effects of drugs known to decrease available phosphorus stores. Medications such as sucralfate can decrease the absorption of phosphorus from the Gl tract by binding to dietary phosphate. On the other hand, patients with renal dysfunction are at risk for development of hyperphosphatemia due to the inherent phosphate content present in clindamycin phosphate injection or that contained in the phospholipid emulsifiers in intravenous fat emulsions. Hypomagnesemia as a result of renal wasting can occur in patients treated with amphotericin B, aminoglycosides,

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loop diuretics, thiazide diuretics, cisplatin, or cyclosporine. Renal wasting of magnesium can also occur in patients receiving prolonged courses of high doses of aminoglycosides. Aminoglycosides inhibit the proximal tubular transport of magnesium in the kidney, predisposing patients with already low magnesium intake to hypomagnesemia. If left untreated, magnesium deficiency can induce a transient hypoparathyroidism by reducing the secretion of parathyroid hormone (PTH) and a blunted PTH response. This leads to an inhibition of the hypocalcemic feedback loop. Management of hypomagnemia-induced hypocalcemia involves correcting the hypomagnesemia first and then replacing ongoing magnesium losses. In some cases, calcium supplementation may even be unnecessary.

Glucose

Patients with diabetes mellitus or those with insulin resistance (e.g., severe infections, catabolic stress) are susceptible to the effects of drugs known to influence glucose metabolism. Protease inhibitors have long been recognized as a cause of hyperglycemia. Conversely, hypoglycemia is the most common metabolic derangement linked to pentamidine therapy. The mechanism responsible involves a direct cytolytic effect on pancreatic beta cells, resulting in insulin release and hypoglycemia and a subsequent insulin deficiency due to a loss of beta cell function. Over time, however, pentamidine-induced pancreatic beta cell damage may result in insulin deficiency and lead to hyperglycemia, although this is considerably less frequent than hypoglycemia. Other anti-infectives that alter glucose metabolism are listed in Table 1.

Table 1 Anti-infectives that impact glucose metabolism/response

Drug	*Response*
Amprenavir	Hyperglycemia
Chloroquine	Hypoglycemia
Ciprofloxacin	Hyper/hypoglycemia
Clofazimine	Hyperglycemia
Darunavir	Hyperglycemia
Didanosine	Hyperglycemia
Dolutegravir	Hyperglycemia
Emtricitabine	Hyperglycemia
Ethionamide	Hypoglycemia
Ertapenem	Hyperglycemia
Etravirine	Hyperglycemia
Fosamprenavir	Hyperglycemia
Gemifloxacin	Hyper/hypoglycemia
Indinavir	Hyperglycemia
Lamivudine	Hyperglycemia
Micafungin	Hyper/hypoglycemia
Moxifloxacin	Hyper/hypoglycemia
Nelfinavir	Hyperglycemia
Norfloxacin	Hyper/hypoglycemia
Pentamidine	Hyper/hypoglycemia
Posaconazole	Hyperglycemia
Quinine	Hypoglycemia
Ritonavir	Hyperglycemia
Saquinavir	Hyperglycemia
Valganciclovir	Hyperglycemia
Zalcitabine	Hyperglycemia

Fat

Drug-induced lipoprotein abnormalities should be considered in individuals in whom no other causes of dyslipidemia exist (Table 2). When a drug is used for a short duration, prescribers need to be aware of the effects on the patient's baseline lipoprotein profile versus the chance that an underlying dyslipidemia has been exacerbated. Chronically used medications that cause dyslipidemia may be more problematic as they may predispose the patient to atherosclerosis.

In addition to causing hyperglycemia, protease inhibitors interfere with proteins involved in fat metabolism (i.e., cytoplasmic retinoic acid-binding protein type 1). Protease inhibitor binding to low-density lipoprotein receptor-related proteins (LRPs) impairs hepatic chylomicron uptake and triglyceride clearance via the endothelial LRP–lipoprotein lipase complex. The resulting hyperlipidemia contributes to insulin resistance and central fat deposition. Moreover, by disrupting steroid hormone production, protease inhibitors may predispose patients to lipodystrophy.

GI Complications

Many drugs can negatively impact the function of the GI tract. Most are minor and self-limiting. Others, however, can be significant, causing severe GI illness (e.g., colitis and esophagitis) that can adversely affect the tolerance to an oral or tube-fed diet. Drug-induced esophagitis (i.e., 'pill esophagitis') can

Table 2 Anti-infectives associated with dyslipidemia

Medication	*Total cholesterol*	*Triglycerides*	*Low-density lipoprotein*	*High-density lipoprotein*
Abacavir		↑		
Amprenavir	↑	↑		
Atazanavir	↑			
Darunavir	↑	↑	↑	
Didanosine		↑		
Efavirenz	↑	↑		↑
Elvitegravir, cobicistat, emtricitabine, and tenofovir	↑	↑		
Emtricitabine		↑		
Etravirine	↑	↑	↑	
Fluconazole	↑	↑		
Fosamprenavir	↑	↑		
Indinavir		↑		
Itraconazole		↑		
Miconazole (IV)	↑			
Nelfinavir				
Nevirapine		↑		↑
Rilpivirine	↑	↑	↑	
Risperidone		↑		
Ritonavir	↑	↑		
Tipranavir	↑	↑		

Adapted from Gura, K. M. (2013). Drug-nutrient interactions. In: Hendricks, K. M. and Duggan, C. (eds.) Manual of pediatric nutrition (5th ed.). Shelton, CT: People's Medical Publishing House.

occur with such antibiotics as doxycycline and tetracycline. Typically, this complication occurs in patients with left atrial enlargement or cardiomegaly as the heart impinges on the esophagus, thus increasing the transit time of the medication in the esophagus, or in adolescents who drink inadequate amounts of fluid with their medications. Switching to the liquid formulation of the medication or administering the problematic drug with plenty of water can often alleviate the situation.

Changes in Appetite

Drug-induced nutritional deficiencies may result when a medication causes appetite suppression leading to decreased food intake. In many cases, the signs and symptoms of nutrient deficiencies are nonspecific and mimic those of other disease states. Drugs that affect appetite (Table 3) may do so by either a central or a peripheral effect, including anorexia, inducing drowsiness, or triggering an adverse response when food is ingested. The primary effect usually focuses on appetite suppression, a centrally acting mechanism that includes the catecholaminergic, dopaminergic, serotonergic, and endorphin modulators, which may lead to appetite suppression. Peripherally acting mechanisms that can indirectly suppress appetite include bulking agents and those drugs that inhibit gastric emptying. A secondary response may also result when a drug induces a negative reaction to food that leads to a loss in appetite. The emetic center, located within the brain stem, is easily stimulated by the action of numerous medications. These include drugs that cause nausea and vomiting, a loss of taste, or stomatitis. Altered taste perceptions can also lead to a decrease in nutrient intake. Drugs such as metronidazole and clarithromycin have been linked with causing taste perversions. Taste is regulated by chemosensory nerves that respond to stimulation chemicals by direct receptor binding, opening ion channels, or via a secondary messenger channels using nucleotides or phosphorylated inositol. Drugs that disrupt these cellular processes can trigger a loss or distortion in taste. Likewise, xerostomia due to decreased saliva production can alter ion concentrations between saliva and plasma that results in diminished taste sensation. Another way in which drugs can cause anorexia is through nutrient depletion. Medications known to deplete folate, such as phenytoin, sulfasalazine, and trimethoprim, can result in weight loss and anorexia.

Table 3 Examples of anti-infectives that decrease appetite

Abacavir
Amantadine
Aminoglycosides
Artemether and lumefantrine
Atovaquone
Caspofungin
Cidofovir
Chloroquine
Clofazimine
Dapsone
Darunavir
Enfuvirtide
Ethionamide
Ethambutol
Griseofulvin
Hydroxychloroquine
Indinavir
Itraconazole
Lamivudine
Lopinavir and ritonavir
Metronidazole
Micafungin
Nelfinavir
Nitrofurantoin
Penicillins
Pentamidine
Posaconazole
Primaquine
Quinolones
Rifabutin
Rifampin
Rilpivirine
Ritonavir
Stavudine
Tenofovir
Tetracycline
Tipranavir
Valganciclovir
Voriconazole
Zalcitabine
Zidovudine

Adapted from Gura, K. M. (2013). Drug-nutrient interactions. In: Sonneville, K., and Duggan, C. (eds.) Manual of pediatric nutrition (5th ed.). Shelton, CT: People's Medical Publishing House.

Basic Principles of Nutrient–Drug Interactions

By definition, a nutrient–drug interaction is one that is the result of a physical, chemical, pathological, or physiological relationship between a medication and a nutrient. Such interactions are clinically relevant if they impact the therapeutic response of a drug either by decreasing bioavailability (and thereby increasing the risk of treatment failure) or by increasing bioavailability (and thus increasing the risk of toxicity) of the drug.

There are four basic classifications of drug–nutrient interactions that are based on their nature and mechanism:

1. Type I *ex vivo* bioinactivations – These occur between the medication and the nutrient, via biochemical or physical reactions, usually in the delivery device. These interactions are most commonly seen with drugs and nutrients administered intravenously or via feeding tubes. Examples include complexation, hydrolysis, neutralization, oxidation, and precipitation.
2. Type II – These interactions are characterized by a modification of the bioavailability of the medication and effect absorption. The function of the enzymes or transport systems that are responsible for drug biotransformation may be altered.
3. Type III – The systemic disposition of the drug is involved in this form of interaction and occurs after the drug or nutrient has been absorbed from the GI tract into the systemic circulation. This interaction leads to changes in

tissue distribution, transport, or penetration to a specific organ or tissue.
4. Type IV – The elimination or clearance of the drug or nutrient is impacted in this form of interaction. It may modify either the enterohepatic or the renal elimination process.

Relationship of Meal Timing and Drug Absorption

The concurrent of food and oral drug absorption is an example of a type II interaction. The rate and extent of drug absorption can both be altered (Table 4). In many cases, food will stimulate both gastric and intestinal secretions, thus aiding drug dissolution and improving in its absorption. High-fat meals can improve the intestinal uptake of highly lipophilic drugs due to the release of bile salts triggered by dietary fat. Moreover, cholecystokinin release can be stimulated by dietary fat that decreases gastric motility, thereby increasing the contact time between a drug and the intestine that can potentially increase drug absorption.

In some cases, this interaction is used for therapeutic benefit. For example, the antibiotics cefuroxime and erythromycin ethylsuccinate should both be taken with food to maximize their absorption. Conversely, food composition may hinder drug absorption by binding. In such instances, the drug should be administered on an empty stomach (i.e., 1 h before or 2 h after a meal) to allow for maximal absorption. These include protease inhibitors such as indinavir and antibiotics such as ampicillin, ciprofloxacin, and doxycycline. In some instances, the dosage form of the antibiotic will determine if such meal timing is necessary. For example, the azalide antibiotic, azithromycin, if administered in the capsule form with food, exhibits a negative effect in which the bioavailability of the azithromycin is reduced in comparison to its tablet form. This phenomenon is because the capsule form of azithromycin disintegrates more slowly than the tablet in the fed stomach, resulting in more contact time with gastric acid, thus allowing significantly more descladinose azithromycin, an acid degradation product, to form.

Table 4 Examples of anti-infectives whose absorption is altered by food

Drug absorption enhanced by food	*Drug absorption reduced/delayed by food*
Albendazole	Ampicillin
Atazanavir	Azithromycin
Atovaquone	Cefaclor
Cefuroxime	Cefixime
Clarithromycin	Cephalexin
Clofazimine	Ciprofloxacin
Darunavir	Dapsone
Efavirenz	Didanosine
Etravirine	Doxycycline
Erythromycin estolate	Dirithromycin
Erythromycin ethylsuccinate	Erythromycin stearate
Ganciclovir	Famciclovir
Griseofulvin	Indinavir
Hydroxychloroquine	Isoniazid
Itraconazole	Metronidazole
Ivermectin	Nafcillin
Ketoconazole	Nalidixic acid
Mebendazole	Norfloxacin
Nelfinavir	Ofloxacin
Nitrofurantoin	Penicillin G or V
Rilpivirine	Rifampin
Ritonavir	Tetracycline
Saquinavir	Voriconazole
Tenofovir	
Tipranavir	
Valganciclovir	
Zalcitabine	

Adapted from Gura, K. M. (2013). Drug-nutrient interactions. In: Sonneville, K. and Duggan, C. (eds.) Manual of pediatric nutrition (5th ed.). Shelton, CT: People's Medical Publishing House.

Drug and Nutrient Transport Systems

Oral drug absorption across the GI tract is highly dependent on its lipophilic properties and its affinity for membrane transporters. Most drugs are absorbed in the small intestine where transport proteins present in the enterocytes facilitate in drug absorption. These transport proteins aid in absorption by increasing the intraluminal uptake of compounds. These transporters also efflux molecules already absorbed in the cytoplasm of the enterocyte back into the intestinal lumen, thus decreasing the bioavailability of some compounds. It is thought to be an intrinsic protective mechanism by the host to minimize xenobiotic exposure. P-glycoprotein (P-gp) is an example of this type of efflux system. P-gp is part of adenosine triphosphate (ATP)-binding cassette (ABC) family of transporters that is an efflux protein widely distributed a variety of tissues, including the blood–brain barrier, intestinal epithelium, liver, and renal tubule. Hepatic transporters are membrane proteins that facilitate nutrient and drug transport into the cell via uptake transporters or pump out toxic entities via canalicular transporters.

Certain foods contain phytochemicals that have the potential to alter drug absorption. Fruits and vegetables are particularly rich in them. Recent studies suggest that phytochemicals can serve as substrates and modulators of specific members of the superfamily of ABC-transporting proteins (i.e., P-gp, MRP1, MRP2, and BCRP). The two most important limiting factors in regulating the oral bioavailability of drugs are CYP3A4 and P-gp. CYP3A4 is thought to be responsible for the metabolism of more than 50% of all medications. Another is the isothiocyanates, a class of chemotherapeutic agents derived from cruciferous vegetables (i.e., broccoli and cabbage) that influence the pharmacokinetics of substrates of these transporters and interact with ABC efflux transporters.

The Relationship between Malnutrition and Pharmacokinetic Parameters

The pharmacological effect of a medication can be modified by an individual's nutritional state. Obesity and undernutrition can alter a medication's pharmacokinetic and pharmacological properties by causing structural alterations in organs that

directly impact drug disposition. Malnutrition can affect the absorption, distribution, metabolism, and elimination of a drug that can ultimately alter its therapeutic or toxic response. Patient-specific variations in the pharmacokinetic responses can further complicate interpreting the true impact of altered nutritional status on drug disposition. This variation can be threefold to over 20-fold, depending on genetic (e.g., genetic polymorphism) and environmental factors and underlying disease. The pathological changes that occur vary with degree of malnutrition, suggesting that the severity of malnutrition can govern the body's response to a particular drug. Even short-term malnutrition can potentially have a negative impact on drug absorption and metabolism, a risk that increases significantly in patients who go without food more than 5 days as a consequence of impaired mucosal integrity. The integrity of the intestinal mucosa is dependent on a continuous intake of adequate nutrition. The turnover of enterocytes in the small intestine takes 2–3 days, while in the colonocyte of the large intestine, it is 3–5 days. Protein–energy malnutrition (PEM) may result in physiological alterations in the absorptive capacity of the GI tract, body fluid status, cardiac output, glomerular filtration rate (GFR), and plasma protein concentrations. Hormonal and metabolic changes may also occur. Malnutrition-associated tissue receptor alterations may alter therapeutic drug levels. Taken together, malnourished patients are at greater risk of developing toxicities due to a drug or its metabolite, with a subsequent risk of morbidity or mortality, making therapeutic drug monitoring and dosage adjustments crucial. Drugs with narrow therapeutic indexes or narrow dose–response curves (i.e., aminoglycosides) are particularly susceptible with even small changes in absorption, resulting in significant changes in blood levels.

Little is known about the handling of drugs in malnourished patients even though the combination of infection and malnutrition is common in many parts of the world. The high morbidity of mortality associated with malnutrition could be due in part by adverse drug reactions.

The risk of antimicrobial resistance may be enhanced in the presence of starvation. Starvation produces stress in the pathogen's host environment that can produce a multitude of adaptive responses that not only protect bacteria from the offending stress but also may change the cell that can impact innate antimicrobial susceptibility. Bacterial susceptibility to a number of antimicrobials may be influenced when the host is malnourished or experiencing a nutrient deficiency. For example, antibiotics tend to kill rapidly replicating bacteria, and in states of starvation or nutrient deficiency, reduced growth and metabolic activity may trigger antimicrobial resistance. One case of nutritional stress is PEM that results in a depletion of amino acid that activates the stringent response. Other nutritional stresses (e.g., iron deficiency, hypophosphatemia, and fatty acids deficiencies) can activate the stringent response-mediated increase in ppGpp influencing bacterial cell physiology and subsequent antimicrobial susceptibility. In times of amino acid deprivation, diminished penicillin susceptibility in *Escherichia coli* relA mutants unable to synthesize ppGpp can occur that are more susceptible to penicillin-dependent lysis. Presumably, plsB is a ppGpp target and ppGpp-dependent inhibition of phospholipid biosynthesis interferes with membrane-associated steps in peptidoglycan biosynthesis, thereby promoting penicillin resistance. Inhibition of cell wall synthesis by ppGpp has been described in other bacteria (i.e., *Streptomyces coelicolor*, *Bacillus subtilis*, and *M. tuberculosis*) where it is also linked to reduced β-lactam susceptibility. Resistance to other antimicrobials is also linked to ppGpp. *E. coli* mutants deficient in ppGpp production are more susceptible to trimethoprim and gentamicin. Conversely, increased ppGpp accumulation in mutant *E. coli* is linked to improved resistance and increased survivability in the presence of fluoroquinolones. Antimicrobials (i.e., mupirocin, vancomycin, and penicillin) have been reported to stimulate ppGpp accumulation, but this has not been associated with any increases in antimicrobial resistance except in the case of *Enterococcus faecalis*, where a mutant strain unable to synthesize ppGpp displayed an increased susceptibility to vancomycin. Similarly, recent evidence illustrates that both ppGpp and the stringent response are linked to resistance to several antimicrobials in nutrient-starved biofilm cells of *P. aeruginosa*. Nutrient-limited planktonic and biofilm cells that are defective in the genes for ppGpp production were shown to be less resistant to antimicrobials than their wild-type counterparts, with ppGpp-deficient biofilm cells presenting with increased susceptibility to several classes of antimicrobials, including aminoglycosides, β-lactams, cationic antimicrobial peptides, and fluoroquinolones. The mechanism of cell lysis is linked to the production of hydroxyl radicals. The stringent response promotes antimicrobial resistance by increasing antioxidant defenses and by limiting 4-hydroxy-2-alkylquinolines synthesis, which serve to enhance the oxidative killing of cells upon contact with an antimicrobial. These findings suggest that starved, nongrowing cells may be at greater risk from oxidative stress/killing, and to avoid it, they adapt, thereby providing protection from the oxidative killing by bactericidal antimicrobials.

Other forms of nutrient starvation can similarly impact the type and severity of antimicrobial resistance. One study of the effects of nutrient deprivation on antibiotic resistance involving *E. coli* demonstrated that phosphate starvation stimulated a transient increase in ofloxacin resistance, while amino acid starvation was associated with transient resistance to both ofloxacin and ampicillin; furthermore, the combination of amino acid and glucose starvation can lead to ampicillin and ofloxacin resistance. Likewise, decreased magnesium intake can also impact antimicrobial susceptibility in a variety of bacteria. For example, in the case of *P. aeruginosa*, there are several reports of PhoQ and pmrB mutations responsible for PXB and colistin resistance. Similarly, in *Salmonella*, the response to magnesium deficiency is mediated by the PhoPQ two-component system, where PhoQ, a sensor kinase, senses the nutrient limitation and activates the PhoP response regulator to upregulate a variety of target genes that ultimately promote adaptation to this nutrient stress.

Impact of Nutritional Status on Pharmacokinetic Properties

Absorption

The limited evidence on the impact of malnutrition on drug absorption is variable. In one case, it was shown that tetracycline absorption was significantly reduced in subjects with

malnutrition and pellagra but not in patients with vitamin B complex deficiency or in those with severe anemia. This contrasts with reports that showed the absorption rate of acetaminophen disposition in children with PEM was not altered in malnutrition.

Distribution

Drugs can distribute into various body compartments such as the extravascular space, intracellular fluids, adipose, and lean body tissue. Shifts in body composition, particularly the presence of edema, can impact the plasma clearance of a drug by changing its volume of distribution (Vd). Once in the systemic circulation, many drugs become bound to plasma proteins, such as albumin, globulins, and lipoproteins. These binding proteins play an important role in the delivery of a medication to its target organ. In the bloodstream, free drug (i.e., not bound to plasma proteins) is able to exert their pharmacological response. The degree of drug–protein binding is contingent on the drug's physicochemical properties and the concentration of plasma binding proteins. In malnutrition, albumin and lipoprotein synthesis is reduced, while globulin and a1-acid glycoprotein synthesis is increased. Medications that are extensively bound to a1-acid glycoprotein (i.e., propranolol) have a decreased percentage of unbound drug in malnourished subjects, resulting in less available active drug to exert a therapeutic response. Other drugs, for example, metronidazole, have no significant change in their volume of distribution (Vd) between malnourished and well-fed states. In the case of parenteral penicillin, the rate of absorption from the muscle mass to the systemic circulation is unchanged in malnourished versus eutrophic individuals as evidenced by no significant differences in the maximum concentration (C_{max}) when the antibiotic was administered intramuscularly. Conversely, in patients with marasmus and kwashiorkor, there was a trend toward a lower Vd for penicillin, suggesting that if the typical dosing strategies are used, the risk of overdose could be a concern.

Metabolism

In chronic starvation, the body adapts or alters various processes to keep or maintain enzyme functions. In fact, enzyme activity may even increase in states of chronic starvation. In the starved state, the conjugation and biotransformation of aromatic compounds are decreased, whereas oxidation remains dominant. Since many hormones serve as substrates for drug-metabolizing enzymes, endocrine tissue is typically affected in semistarvation. For example, in malnourished females, elevations in free cortisol can occur that may enhance the metabolism of contraceptive steroids, thus increasing the risk of contraceptive failure.

Clearance

Hepatic drug clearance may be influenced by an individual's nutritional state. PEM may result in decreased cardiac function with a subsequent reduction in renal and hepatic perfusion. Three independent factors can influence the hepatic clearance: hepatic blood flow, the amount of free drug in the blood, and hepatic clearance of the unbound drug. Drugs with high extraction ratios may experience reduced clearance due to diminished hepatic blood flow. Alterations in presystemic metabolism can occur. Enhanced drug clearance and lower serum drug concentrations can occur in states of hypoalbuminemia due to increased amounts of free drug becoming available for metabolism.

Renal elimination of a drug is closely linked with kidney function. Glomerular filtration, active tubular secretion, and passive tubular excretion are all involved in renal elimination. Nutritional status can impact those medications or their metabolites that are primarily renally filtered and excreted. Increased protein intake can increase the GFR, renal blood flow, and renal tubular function. Conversely, PEM is associated with decreased GFR and renal blood flow. Reduced renal perfusion results in less drug being available to undergo filtration by the tubules. Nevertheless, as plasma protein binding is similarly reduced, more free drug becomes available for renal excretion, thus further reducing plasma drug concentrations. Examples of medications that undergo decreased renal elimination in severely malnourished patients include tetracyclines, aminoglycosides, cefoxitin, and penicillins.

The systemic clearance of a medication can occur when an undernourished patient is refed, requiring that dosage adjustments be performed so as to maintain a therapeutic response. The protein component of enteral or parenteral nutrition (PN) appears to be the major macronutrient that improves the systemic clearance of susceptible drugs in patients transitioned from the 'unfed' to the 'fed' state. For example, the daily maintenance dose of metronidazole for pediatric patients with severe malnutrition should be 60% less of the usual pediatric dose to achieve and maintain adequate therapeutic plasma levels of the drug.

Impact of Kwashiorkor on Pharmacokinetics

The edema that occurs in kwashiorkor is due to increases in total body water, extracellular fluid volume, and plasma volume, with a decrease in intracellular water. Low protein intake results in a negative nitrogen balance that leads to decreased drug metabolism, while adequate intake of total calories but with less than optimal protein intake does not significantly impact drug metabolism.

Drug Therapy in Marasmus

Marasmus, unlike kwashiorkor, is associated with an adaptation to insufficient energy intake. There are decreased total body water and intracellular water, with increased plasma volume and extracellular fluid. In malnourished patients, an increased Vd in gentamicin is observed in comparison with well-nourished patients due to its extensive distribution into the extracellular fluid. In patients treated with metronidazole however, no difference in Vd was noted between malnourished and nutritionally rehabilitated individuals due to its large Vd. One pharmacokinetic study focussing on the role of PEM in quinine metabolism showed that the metabolism of quinine is increased in individuals with PEM, suggesting that the dosing interval should be reduced in order to obtain the same therapeutic quinine concentrations compared those seen in well-nourished patients.

Thus, normal or increased drug metabolism can occur in cases of mild to moderate malnutrition, while decreased metabolism occurs in severe malnutrition. Given the unique needs of ill children with severe acute malnutrition, special guidelines have been developed for the use of antimicrobial agents.

Obesity

Obesity (BMI >95th percentile for age and sex) is linked with physiological changes that can impact the pharmacokinetic parameters of many drugs, but for most medications, dosage recommendations fail to take into account these alterations in body composition in which there are both an increased proportion and absolute amount of adipose tissue and an increase in lean body mass, blood volume, cardiac output, and organ size. In the obese patient, increases in both CYP2E1 activity and phase II conjugation activity have been observed, although there is no difference in albumin binding of drugs. This increase in CYP2E1 activity appears to be downregulated with weight loss. Increased abdominal fat can also impact gastric emptying, which becomes altered by increased intra-abdominal pressure due to excess adipose tissue. Evidence to date suggests that drug absorption is unchanged in obese individuals. As expected, obese patients who have undergone bariatric surgery such as gastric bypass may experience alterations in drug absorption that may affect a drug's therapeutic response. Drug malabsorption is more likely to occur with the primary procedures such as jejunoileal bypass and pancreatobiliary diversion. Other surgical procedures for weight loss, such as the Roux-en-Y gastric bypass, a primary restrictive procedure with mild malabsorptive component, have similar changes in drug absorption and bioavailability that require standard drug doses and administration guidelines to be adjusted. Similarly, little is known on the impact of other procedures, such as gastric banding, on drug pharmacokinetics.

The lipophilicity of a drug determines the extent to which obesity affects the Vd and ultimately whether dosing should be based on actual or adjusted body weight. In severely obese patients, modest increases in Vd have been observed with aminoglycosides and vancomycin. This suggests that dosing of these agents should be done using an adjusted body weight rather than actual body weight to avoid toxicity. Nevertheless, the most accurate approach to adjust for the excess body mass remains unknown and may vary, depending on the characteristics of the individual compounds. Therapeutic drug monitoring is essential to optimize therapy.

The free fraction of basic drugs may be decreased with obesity although the protein binding of acidic drugs appears to be unchanged. Similarly, changes in hepatic drug clearance are variable. In obese subjects, phase I reactions and phase I acetylation appear to be unaffected, but the phase II glucuronidation and sulfation pathways are intensified. Systemic clearance of highly extracted drugs such as aminoglycosides may also be impacted by obesity.

Aminoglycosides are distributed within the extracellular fluid compartment. Early dosage recommendations were based on ideal body weight based on the premise that the drug distributed only into lean body mass. It has since been shown that when the Vd is corrected for total body weight, it is considerably smaller in comparison to normal-weight subjects. The distribution of aminoglycosides into excess body weight is estimated to be about 40% of that distributed into ideal body mass. It is suggested that in obese individuals, initial doses of aminoglycosides be calculated by adding 40% of the excess weight to the patient's ideal body weight, with any further dosage adjustments determined by serum drug concentrations and clinical status. Less is known about other antimicrobials in obesity and dosage adjustments should be done on a case by case basis, depending on initial response.

Effect of Dietary Manipulation on Pharmacokinetics

Modifying the intake of specific foods may impact a drug's therapeutic response (Table 5). Individuals at specific risk to an adverse event due to a drug–nutrient interaction include those with a chronic condition necessitating the need for multiple medications, those requiring specialized nutritional support, or those with some evidence of malnutrition.

Several dietary factors can change the rate and extent of drug absorption. These alternations in response may be the result of the effects on gastric pH, gastric emptying time, intestinal motility, and mesenteric and hepatic portal blood flow or biliary flow or the activities of the enzymes and transport proteins in the gut. The absorption of susceptible agents can be altered by direct physicochemical interactions with dietary components. Solubilization of a drug in dietary fat, binding with metal ions, and adsorption to insoluble dietary components are examples of such interactions.

The activity and expression of hepatic drug-metabolizing enzymes may be altered by dietary modifications. This can lead to changes in the systemic elimination kinetics of drugs metabolized by these enzymes, although the impact of these changes is typically minimal. The rate of drug metabolism can be enhanced by the medications themselves or via a variety of dietary factors, such as inclusion of charcoal-broiled meats or cruciferous vegetables in the diet or protein supplementation. Large intakes of polycyclic aromatic hydrocarbons contained in charcoal-broiled beef are responsible for hepatic enzyme induction. Conversely, levels of drug-metabolizing enzymes can be reduced by high-carbohydrate, low-protein diets and various vitamin and mineral deficiencies, consequently altering the rate of drug metabolism such that the serum drug concentrations decline much more slowly, leading to increased drug potency.

When orally administered drugs are taken with meals, the rate rather than the extent of absorption is more significantly altered. Food affects drug absorption by improving gastric blood flow in conjunction with delayed gastric emptying or by changing its dissolution. The absolute systemic availability of a medication can be increased and decreased, or the presence of food may have no effect on its availability. Simultaneous ingestion with food reduces the absorption of medications such as ampicillin, penicillin, and isoniazid. In some instances, food may enhance the absorption of other drugs, such as griseofulvin. Table 4 lists medications whose absorption is altered by food. In most instances, altering the rate of absorption of a medication alone without changing the total amount absorbed is not expected to impact its efficacy.

Table 5 Effect of nutrients on therapeutic drug response

Nutrient	*Impact on metabolism Decreased or Increased (↓ or ↑)*	*Proposed mechanism*
Macronutrients		
Protein	Deficiency: ↓ rate of metabolism Excess: ↑ rate of metabolism	↓ Protein synthesis ↓ Synthesis of other elements (i.e., hormones) involved in enzyme induction
Carbohydrates	Excess: ↓	May be due to ↓ protein or inhibition of CYP 450 via ↓ in supporting enzyme components
Fats	High intake of saturated fatty acids: ↓ High intake of polyunsaturated fatty acids: ↑ activity and induction of CYP enzymes Deficiency: ↓ Excess: ↑	Decreased CYP activity may be due to requirement of PUFAs in the B-position of phosphatidylcholine (lecithin), which is an essential component of the CYP system
Micronutrients		
Iron	Excess: ↑ microsomal lipid peroxidation Deficiency: ↑↓	↑ Lipid peroxidation may lead to damage to the integrity of the system
Pyridoxine	Deficiency: ↓	Impaired protein synthesis ↓ synthesis of heme
Riboflavin	Deficiency ↑↓ depending on severity	↓ Reductase activity but ↑ CYP450 activity such that drug metabolism may be altered (↑↓)
Thiamine	Excess: ↓ (both reductase and CYP 450) Deficiency: ↑ CYP450 activity	Excess may be due to decreased substrate binding ↑ Activity of specific CYP450 isoenzymes and perhaps other enzymes in deficiency (mechanism unknown)
Ascorbic acid	Deficiency: ↓ Excess: ↑ CYP 450 activity	Alterations in CYP450 and CYP 450 reductase via changes in expression of specific CYP isoenzymes depending on excess or deficiency state
Vitamin E	Deficiency: ↓	May be due to a reduction in antioxidative mechanisms (e.g., protection of the lecithin component; thus, the activities of CYP450 and reductase are unaffected)

Adapted from Raiten, D. J. (2011). Nutrition and pharmacology: general principles and implications for HIV. *American Journal of Clinical Nutrition* 94(6), 1697S–1702S. Epub 2011 November 16.

Meal composition will also alter splanchnic blood flow. A liquid glucose meal can slightly reduce blood flow, whereas a high-protein liquid meal can double it. The importance of this effect on splanchnic flow is significant for those drugs with high hepatic extraction. Continued meal intake, especially with foods with high fat content, will also slow the rate gastric emptying, which may subsequently cause a delay in drug absorption from the GI tract. The type of meal may impact gastric emptying due to the physicochemical properties of the drug. Highly viscous solutions, those rich in fat, or simply hot meals have the most significant impact on decreasing gut motility. Food has been shown to enhance the presystemic clearance of lipophilic basic drugs (e.g., amitriptyline and propranolol) but rarely alter the clearance of lipophilic acids drugs (e.g., salicylic acid and penicillin). On the other hand, food may decrease the presystemic clearance of some lipophilic basic drugs via transient, complex effects on splanchnic–hepatic blood flow. Furthermore, repeated intake of specific food contaminants (e.g., aromatic hydrocarbons) and nutrients (e.g., protein) can enhance presystemic drug clearance via enzyme induction.

Role of Specific Nutrients in Pharmacokinetics

Until recently, the perception that dietary substances could significantly alter drug response by disturbing intestinal transporter and metabolizing enzymes was viewed as immaterial. This was believed to be due to the misconception that drug absorption was a passive process and the intestine had no significant role in drug elimination. With the subsequent reports of the interaction between grapefruit juice and several medications, this commonly held belief has changed, and the importance of the diet on drug performance has been reassessed.

Carbohydrates

There evidence on the importance of carbohydrates on drug metabolism is conflicting. It is known that diets rich in carbohydrates may induce the expression of several glycolytic and lipogenic hepatic enzymes. Some suggest, however, that carbohydrates have little impact on drug metabolism. In animal models, dietary carbohydrates and fat have been shown to significantly influence hepatic drug-metabolizing enzymes. These changes may occur due to an alteration in the phospholipid composition of endoplasmic reticulum or by limiting the supply of cofactor(s) necessary for optimal functioning of CYP and UGT.

Protein

After ingesting a high-protein meal, medications that undergo extensive first-pass effect may have enhanced bioavailability due to increased hepatic blood flow. High-extraction drugs can rapidly pass through the liver, allowing higher drug concentrations in the systemic circulation. Reductions in dietary protein decrease creatinine clearance and renal plasma flow. In situations where there is low protein intake, care is needed to avoid toxicity secondary to delayed drug clearance. Specific dietary proteins may also impact medication response. A classic example is the interaction between the monoamine oxidase inhibitors (MAOIs) and the amino acid tyramine that is present

in aged cheeses, pickled/smoked meats, fermented foods, and red wines. Tyramine is an indirect sympathomimetic amine that releases norepinephrine from the adrenergic neurons, resulting in a significant hypertensive response. Normally, tyramine is metabolized by the enzyme monoamine oxidase before any significant pressor response occurs. When the enzyme becomes blocked, however, severe and potentially fatal increases in blood pressure can ensue when tyramine-rich foods are ingested. Although MAOIs are not used routinely as antidepressants, other drugs, such as isoniazid and linezolid, also possess MAOI properties, and patients should avoid ingesting large amounts of tyramine while being treated with them.

The renal tubular transport of certain compounds by dietary can also be affected by dietary protein. Dietary proteins bind to a drug that accentuates changes in bioavailability after a protein meal. For example, ciprofloxacin, when taken with milk or other dairy product, not only undergoes complexation with calcium but also adsorbs to the surface of proteins, which decreases the absorbable amount of ciprofloxacin and may increase the risk of treatment failure or resistance. It is thought that the casein component present in milk has a more pronounced effect on the amount of absorbable ciprofloxacin.

Dietary Fat

Lipids are an essential part of cell membrane structure and are involved in many of its normal enzymatic activities. Essential fatty acid deficiency or low-fat diets decrease the activity of the enzyme systems responsible for nutrient metabolism. After consumption of a high-fat meal, plasma free fatty acid levels rise, increasing the potential to become bound to plasma albumin and consequently displacing albumin bound drugs, making the risk for toxicity greater. Dietary fats along with food-stimulated secretions (e.g., bile salts) may aid in the solubilization and dispersion of lipophilic compounds. This leads to a reduction in the extent of first-pass metabolism due to improved splanchnic blood flow. High-fat diets are linked with the induction of CYP2E1. The extent this enzyme is upregulated is governed by the type of fat. Polyunsaturated fats (PUFAs) such as soybean and fish oils appear to have the greatest influence in comparison to lard or olive oils. This can result in enhanced peroxidation of the PUFA substrates and aid in free radical production. The fat content of a meal can also influence the rate of gastric emptying. Fat delays gastric emptying to a greater extent than either protein or carbohydrate.

The effect of dietary fat on drug absorption depends upon the area of the drug absorption, either portal or lymphatic. For drugs absorbed via the lymphatic system, dietary fat improves the absorption of the dissolved medications, while poorly bioavailable lipophilic drugs absorbed by the portal route (thus bypassing the lymphatic system) have their absorption enhanced by better drug dissolution. Conversely, lipophilic medications having good bioavailability will less likely to be altered by a high-fat meal. The absorption of hydrophilic medications does not appear to be influenced by fatty meals.

Fruits and Vegetables

Diets rich in fruits and vegetables may also impact drug response. Many plants contain flavonoids, isothiocyanates, and allyl sulfides that are potent modulators of the cytochrome monooxygenase system. Fruits and vegetables serve as sources of trace minerals that are contained in metalloenzymes, including several antioxidants that can influence a variety of enzymatic pathways. Cruciferous vegetables, citrus juices, spices, dietary supplements, and herbs contain phytochemicals that modulate of a variety of metabolic pathways. There are five major families of phytochemicals: alkaloids, carotenoids, nitrogen compounds, phenolics, and sulfur compounds. Typically, induction of these enzyme systems is rapid and plateaus within 5 days of continued daily ingestion of the food possessing the enzyme-inducing capacity.

Minerals

Mineral deficiencies (i.e., iodine, magnesium, potassium, and zinc) have been linked with a decrease in drug oxidation and drug clearance. Although not well understood, low dietary iron intake has been associated with an increase in some CYP activities and a decrease in other degradative functions. Foods fortified with calcium or other multivalent minerals present a new challenge. For example, any drug carrying a warning to avoid milk, dairy products, or nutritional supplements can be affected by calcium-fortified orange juice. Antibiotics are the most susceptible drug class to undergo chelation and adsorption by fortified cereals, calcium-fortified orange juice, or protein supplements. Patients need to be counseled that consuming fortified foods with susceptible antibiotics may decrease their therapeutic response, thereby increasing the risk of treatment failure.

Other Dietary Restrictions

In addition to caloric restriction, restriction of other dietary components can also influence drug response. For example, patients receiving aminoglycosides, amphotericin B, cisplatin, or radiocontrast media in conjunction with a low-sodium diet have an increased risk for hemodynamic, nephrotoxic, and ischemic acute renal failure.

Effect of Beverage Type on Drug Bioavailability

Any drinkable liquid other than plain water is commonly referred to as a 'beverage.' They are classified as alcoholic, caffeinated, fruit/vegetables juices, milk-based, or mineral waters. Depending upon the type of fluid ingested, drug absorption may be impacted. When a medication is mixed with fruit juice or other beverages to mask their taste, its absorption may be altered due to changes in gastric pH, which in turn can affect dissolution of solid dosage forms and formulations with a pH-sensitive coating. Dairy products decrease the absorption of tetracyclines and reduce their bioavailability due to the formation of insoluble chelates between the drug and the calcium present in the beverage. Soft drinks may decrease drug absorption for a number of reasons. These carbonated drinks contain phosphoric acid and that can slow gastric emptying and the tendency to serve them chilled may further reduce the rate of intestinal blood flow. Additionally, carbonation may increase mixing and possibly motility. Acidic beverages improve dissolution of tablets and capsules and thus can improve and stabilize oral bioavailability. If a drug is one

Table 6 Examples of anti-infective-nutrient interactions

	Nutritional considerations	*Possible gastrointestinal side effects*	*Comments/recommendations*
Antibiotics			
General	Gut microflora decreases vitamin K synthesis; antibiotic depletion of gut microflora can also lead to dysbiosis that can alter the digestion and absorption of nutrients	N/V/D Lactase deficiency	
Aminoglycosides	↑ Urinary excretion of potassium and magnesium May also ↓ sodium and calcium	↓ Appetite N/V Increased salivation	
Amoxicillin Amoxicillin/clavulanic acid		N/V/D Incidence of diarrhea higher with amoxicillin/clavulanic acid versus amoxicillin alone	Take with food to reduce GI upset
Cephalosporins	Possible nephrotoxicity with vitamin K deficiency	V/D GI mucosa damage	
Chloramphenicol	Decreased protein synthesis; increased need for cyanocobalamin, pyridoxine, riboflavin	V/D Stomatitis Enterocolitis Glossitis	Take on an empty stomach
Clindamycin		N/V/D Esophagitis Pseudomembranous colitis	Take with a full glass of water to avoid esophageal irritation
Fluoroquinolones Ciprofloxacin Gemifloxacin Levofloxacin Norfloxacin	Ciprofloxacin: food decreases rate, but not extent, of absorption. Enteral feedings may decrease plasma concentrations of ciprofloxacin probably by >30% inhibition of absorption Ciprofloxacin should not be administered with enteral feedings. The feeding should be discontinued for 1–2 h prior to and after ciprofloxacin administration. Nasogastric administration produces a greater loss of ciprofloxacin bioavailability than does nasoduodenal administration Gemifloxacin: may take tablets with or without food, milk, or calcium supplements Moxifloxacin: may be taken without regard to meals Norfloxacin: best taken on an empty stomach with water (1 h before or 2 h after meals, milk, or other dairy products) Ofloxacin: average peak serum concentrations may be ↓ if taken with food	N/V/D Abdominal pain ↑ Serum transaminases ↑ Gamma-glutamyl transferase	May administer with food to minimize GI upset Avoid or take ciprofloxacin 2 h before or 6 h after antacids, dairy products, or calcium-fortified juices alone or in a meal containing >800 mg calcium, oral multivitamins, or mineral supplements containing divalent and/or trivalent cations Ensure adequate hydration during therapy Gemifloxacin: should be taken 3 h before or 2 h after supplements (including multivitamins) containing iron, zinc, or magnesium Moxifloxacin: may be taken without regard to meals. Take 4 h before or 8 h after multiple vitamins, antacids, or other products containing iron, zinc, magnesium, or aluminum Norfloxacin: hold antacids, sucralfate, or multivitamins/supplements containing iron, zinc, magnesium, or aluminum for 2 h after giving dose; do not administer together. Best taken on an empty stomach with water Ofloxacin: do not take within 2 h of food or any antacids, which contain zinc, magnesium, or aluminum
Linezolid	Concurrent use with foods or beverages containing large quantities of tyramine may result in a significant hypertensive response	N/D Dyspepsia Oral moniliasis Tongue discoloration Localized abdominal pain Constipation Pseudomembranous colitis	Patients receiving linezolid and tyramine-containing foods or beverages should be monitored for significant blood pressure increases. Avoid foods containing large amounts of tyramine (<100 mg per meal) including aged cheese, sour cream, red wine

(*Continued*)

Table 6 (Continued)

	Nutritional considerations	*Possible gastrointestinal side effects*	*Comments/recommendations*
Macrolides Azithromycin Clarithromycin Erythromycin Lincomycin	Rate and extent of GI absorption may be altered depending upon the formulation Azithromycin: food does not affect bioavailability of the tablet formulation, immediate release oral suspension, or the 1 g suspension regimen; however, extended release suspension ↑ absorption when given with a high-fat meal Clarithromycin: food delays rate, but not extent of absorption; extended release, food ↑ clarithromycin AUC by ~30% relative to fasting conditions Erythromycin: serum levels may be altered if taken with food (formulation-dependent)	Abdominal pain Cramping N/V/D Stomatitis Dyspepsia	Do not crush enteric coated or delayed release products Azithromycin: immediate release suspension and tablet may be taken without regard to food; extended release suspension should be taken on an empty stomach (at least 1 h before or 2 h following a meal) Clarithromycin: immediate release tablets and oral suspension may be given with or without meals and may be taken with milk. Extended release tablets should be taken with food Erythromycin: avoid milk and acidic beverages 1 h before or after a dose; administer after food to decrease GI discomfort
Metronidazole	Food decreases peak drug concentration	N/V/D Metallic taste Xerostomia Furry tongue	Disulfiram reaction with alcohol
Neomycin	↓ Absorption of fat, MCT, vitamins A,D,K, B12, sodium, glucose, lactose, sucrose, xylose; may also deplete beta-carotene, calcium, iron, magnesium, potassium	N/V/D Colitis Cadidiasis Inactivation of bile salts GI mucosal damage ↓ activity of disaccharidases Lipase inhibition	
Penicillins	↑ Urinary potassium excretion May inactivate B6 Food ↓ drug absorption	Decreased appetite diarrhea	Administer with water on an empty stomach (1 h before or 2 h after meals); may take with food to ↓ GI upset
Quinolones	Dairy foods, mineral supplements, calcium-fortified juices ↓ drug concentrations; may ↑ caffeine concentrations	N/V/D GI bleeding Abdominal pain Anorexia Pseudomembranous colitis Dyspepsia	Administer 2 h after meals, may take with food to ↓ GI upset
Sulfonamides	↓ Synthesis of folic acid, B vitamins, vitamin K; ↓ iron absorption; ↑ urinary excretion of vitamin C; presence of food delays but does not ↓ absorption	↓ Appetite N/V Stomatitis Pseudomembranous colitis Abdominal pain	Avoid large amounts of vitamin C or acidifying agents (cranberry juice) to prevent crystalluria
Tetracyclines	Chelate divalent ions; ↓ absorption of calcium, iron, zinc, magnesium, amino acids; ↑ urinary excretion of vitamin C; absorption of tetracycline Hydrochloride ↓ by 50% when taken with milk/dairy products	N/V/D Anorexia Stomatitis Glossitis Pseudomembranous colitis Esophagitis Candidiasis	Take on empty stomach 1 h before/2 h after dose; avoid milk/dairy products, polyvalent ions with 2–3 h of dose Doxycycline and minocycline may be given without regard to meals but best to avoid concurrent administration with milk/dairy products
Trimethoprim	↓ Folate concentrations	N/V Epigastric distress	Leucovorin may be given until normal hematopoiesis is restored
Antifungals			
Amphotericin B	Possible nephrotoxicity with ↑ urinary excretion of potassium and magnesium	↓ Appetite N/V Steatorrhea/diarrhea with oral formulation	Monitor potassium, magnesium/supplement as needed

(*Continued*)

Table 6 (Continued)

	Nutritional considerations	*Possible gastrointestinal side effects*	*Comments/recommendations*
Caspofungin		N/V/D Abdominal pain Anorexia Mucosal inflammation	
Fluconazole	Food delays time of peak absorption but has no effect on total amount of drug absorbed	N/V/D Abdominal pain	
Flucytosine	Food ↓ rate but not extent of absorption; magnesium or aluminum salts delay rate of absorption	N/V/D Enterocolitis	May take with food
Griseofulvin	High-fat foods ↑ absorption rate	N/V/D Oral thrush	Give with fatty meals to ↑ absorption as well as avoid GI upset
Itraconazole	Food ↑ absorption of capsule formulation; hypochlorhydria may ↓ Absorption Grapefruit juice ↓ AUC by 30% Absorption ↑ when taken with a cola beverage	N/V/D Abdominal pain Anorexia	Take capsules with food Take oral solution on an empty stomach
Ketoconazole	Food ↑ rate and extent of absorption Administration with an acidic beverage (cola and citrus juice) enhances absorption	N/V/D Abdominal discomfort GI bleeding	Take with food to ↓ GI upset
Micafungin		N/V/D Mucosal inflammation Constipation Anorexia/dyspepsia	
Posaconazole	Adequate posaconazole absorption from GI tract and subsequent plasma concentrations are dependent on food for efficacy	N/V/D Anorexia Abdominal pain Constipation Dyspepsia	Must be administered during or within 20 min of a full meal or oral liquid nutritional supplement or may be given with an acidic carbonated beverage
Voriconazole	High-fat meals ↓ extent of absorption	N/V Anorexia Constipation Abdominal pain	Take 1 h before or 1 h after meals
Anthelmintics			
Albendazole	Bioavailability increased when taken with a fatty meal	N/V Abdominal pain	
Ivermectin	Bioavailability ↑ 2.5-fold when administered following a high-fat meal	N/D	Take on an empty stomach with water
Mebendazole	Food ↑ drug absorption	N/V/D Abdominal pain	Administer with food
Antimalarials			
Artemether and lumefantrine		N/V Abdominal pain Anorexia	Administer with a full meal for best absorption. Patients should be encouraged to take with a meal as soon as food can be tolerated. Patients who remain averse to food during treatment should be closely monitored as the risk of recrudescence increases
Chloroquine phosphate		N/V/D Anorexia Stomatitis Weight loss	Take with food to ↓ GI upset Bitter taste may be masked by mixing with chocolate syrup
Hydroxychloroquine	Food ↑ bioavailability	N/V/D Anorexia Abdominal cramps	Take with food to ↓ GI upset
Primaquine phosphate		N/V Abdominal cramps anorexia	Take with food to ↓ GI upset Drug has bitter taste

(*Continued*)

Table 6 (Continued)

	Nutritional considerations	*Possible gastrointestinal side effects*	*Comments/recommendations*
Pyrimethamine	↓ Serum folate concentrations	Anorexia Abdominal cramps V/D Atrophic glossitis	Take with meals to ↓ GI upset Leucovorin may be given until normal hematopoiesis is restored
Sulfadoxine Pyrimethamine	↓ Serum folate concentrations	Anorexia Gastritis Glossitis V/D	Take with meals; leucovorin may be given until normal hematopoiesis is restored
Antiprotozoals			
Atovaquone	Food ↑ bioavailability	N/V/D Abdominal pain Constipation Anorexia	Patients who cannot take with meals or have chronic diarrhea or GI problems at risk for drug malabsorption and treatment failure
Nitazoxanide	Food ↑ AUC	N/V/D Abdominal pain	Administer with food
Antiretrovirals			
Protease inhibitors			
Amprenavir	High-fat meals may ↓ AUC; product contains vitamin E to improve bioavailability; may increase risk of bleeding if Vitamin K deficient or on concomitant anticoagulant therapy	N/V/D Taste disorders	Fat redistribution and accumulation have been reported
Atazanavir	Bioavailability ↑ when taken with food	N/V/D	Administer with food; may cause redistribution of fat (e.g., buffalo hump, peripheral wasting with increased abdominal girth, and cushingoid appearance)
Darunavir	Absorption increased with food Take with meals	N/V/D Pancreatitis Abdominal pain ↓ Appetite ↑ Serum lipase ↑ ALT ↑ AST Abdominal distention Dyspepsia	Coadministration with ritonavir and food is required (bioavailability is increased) May cause redistribution of fat (e.g., buffalo hump, peripheral wasting with increased abdominal girth, and cushingoid appearance)
Fosamprenavir	Tablets may be taken with or without food. Adults should take oral suspension without food; however, children should take oral suspension with food	N/V/D Abdominal pain May cause transaminase elevations, hepatitis, and/or exacerbate preexisting hepatic dysfunction	Take tablets with food if taken with ritonavir. May be administered without regard to food if not taken with ritonavir Adults should take oral suspension *without* food; however, children should take oral suspension *with* food May cause redistribution of fat (e.g., buffalo hump and peripheral wasting with increased abdominal girth)
Indinavir	↓ Absorption when given with high amounts of protein or fatty foods; grapefruit juice ↓ AUC by 26%	N/V/D Abdominal pain Metallic taste	Ensure adequate hydration; take on empty stomach; if GI upset a problem, take with light meals or other liquids
Lopinavir Ritonavir	Solution should be taken with food Tablet may be taken with or without food	N/V/D Abdominal pain Altered taste Weight loss	Solution: administer with food; if using didanosine, take didanosine 1 h before or 2 h after lopinavir/ritonavir Tablet: may be taken with or without food. Swallow whole, do not break, crush, or chew. May be taken with didanosine when taken without food
Nelfinavir	Food ↑ absorption	N/V/D Abdominal pain anorexia Dyspepsia Epigastric pain Mouth ulceration GI bleeding Pancreatitis	Do not administer with acidic foods or juices (results in bitter taste)

(*Continued*)

Table 6 (Continued)

	Nutritional considerations	*Possible gastrointestinal side effects*	*Comments/recommendations*
Ritonavir	Food ↑ absorption May cause avitaminosis	N/V/D Taste perversion Abdominal pain Pancreatitis	Administer with food to ↑ Absorption; liquid formulations taste unpleasant, reserve use for tube-fed patients, or mix with chocolate milk or nutritional supplement
Saquinavir	High-fat meals maximize bioavailability Grapefruit juice ↑ saquinavir levels	N/V/D Abdominal discomfort Stomatitis	Take within 2 h of a full meal; high-calorie/high-fat meals ↑ AUC and C_{max} more than low-cal/low-fat meals
Tipranavir	Coadministration with ritonavir is required Administer with ritonavir capsules or solution without regard to meals; administer with ritonavir tablets with meals	N/V/D Abdominal pain Weight loss Dehydration Taste perversion	May cause redistribution of fat (e.g., buffalo hump, peripheral wasting with increased abdominal girth, and cushingoid appearance) May cause facial wasting Capsule contains dehydrated ethanol. Oral solution formulation contains vitamin E; additional vitamin E supplements should be avoided
Nucleoside analog reverse transcriptase inhibitor (NRTI)			
Abacavir	Food does not affect AUC	N/V/D Anorexia Pancreatitis	Fat redistribution and accumulation have been reported
Didanosine	May alter GI absorption of various nutrients due to prolonged GI transient time Food may ↓ oral bioavailability by 50%	N/V Constipation Xerostomia Dry throat Dysphagia	Buffered powder for oral solution is inactivated in acidic juices/fluids
Emtricitabine	May be administered with or without food	N/V/D Abdominal discomfort Gastroenteritis	May cause redistribution of fat
Lamivudine	Food may ↓ rate of absorption and peak serum concentrations, but does not significantly change the AUC	N/V/D Feeding problems Abdominal discomfort Pancreatitis Anorexia Stomatitis	May cause fat redistribution and accumulation
Stavudine	Food ↓ peak serum concentrations by 45%, bioavailability not changed	N/V/D Abdominal pain Anorexia Pancreatitis	Take without regard to food
Tenofovir disoproxil	High-fat meals ↑ oral bioavailability	N/V/D Flatulence Anorexia Pancreatitis	Administer with meals to improve absorption
Zalcitabine	Food ↓ rate and extent of absorption; AUC ↓ by 14%	N/V/D Oral/esophageal ulcers Dysphagia Anorexia Abdominal pain Constipation Pancreatitis Weight loss Anemia	Take on empty stomach
Zidovudine	Folate/B12 deficiency ↑ zidovudine-associated myelosuppression; rate of absorption and peak serum concentration may ↓ when taken with food	N/V/D Anorexia	May take with food; take capsules while in upright position to ↓ risk of esophageal ulceration; syrup is strawberry-flavored

(*Continued*)

Table 6 (Continued)

	Nutritional considerations	*Possible gastrointestinal side effects*	*Comments/recommendations*
Nonnucleoside reverse transcriptase inhibitor (NNRTI)			
Delavirdine	Patients with achlorhydria should take the drug with an acidic beverage	N/V/D Abdominal pain	May be taken without regard to meals Patients with achlorhydria should take the drug with an acidic beverage 200 mg tablets should be taken intact May cause fat redistribution and accumulation
Efavirenz	High-fat/high-calorie meals ↑ AUC and peak concentration and may ↑ adverse effects	N/V/D Abdominal pain	Take with water on an empty stomach preferred, tastes peppery (grape jelly may be used to improve taste) Tablets should not be broken. Some clinicians recommend opening capsules and adding to liquid or food for patients that cannot swallow capsules; however, no pharmacokinetic data are available and this is not recommended
Etravirine	Food ↑ absorption by ∼50%	N/D	Central redistribution of body fat Take after meals. May disperse tablets in glass of water; stir well prior to drinking and rinse glass several times to ensure administration of complete dose
Nevirapine	Can be given without regard to food	N/V/D Abdominal pain	Central redistribution of body fat Extended release tablets must be swallowed whole and not crushed, chewed, or divided
Rilpivirine	Administer with a normal- to high-calorie meal. Absorption ↑ by ∼40% when taken with a normal to high-calorie meal. Administration with a protein supplement drink alone does not ↑ absorption	Abdominal discomfort/pain Appetite ↓ N/V/D	May cause redistribution of fat
Entry inhibitor (fusion inhibitors)			
Enfuvirtide		D/N Weight loss Abdominal pain Appetite ↓ Pancreatitis Anorexia Xerostomia	
Maraviroc	Absorption ↓ with ingestion of a high-fat meal; however, can be given with or without food	Abdominal pain Altered appetite Constipation	
Integrase inhibitor			
Raltegravir	May be administered without regard to meals; however, clinically insignificant variability in absorption exists depending on meal type	N/D	May be taken without regard to meals. Some products may contain phenylalanine
Dolutegravir	May be administered without regard to meals Food increases the extent of absorption and slowed the rate of absorption Low-, moderate-, and high-fat meals increased dolutegravir AUC by 33%, 41%, and 66%, respectively; increased C_{max} by 46%, 52%, and 67%, respectively; and prolonged T_{max} to 3, 4, and 5 h from 2 h under fasted conditions, respectively	N/D Abdominal pain ↑ Liver function Tests Hyperglycemia	May be taken with or without food. Take 2 h before or 6 h after cation-containing antacids or laxatives, oral supplements containing iron or calcium, or buffered medications Alternatively, dolutegravir and supplements containing calcium or iron can be taken together with food Zinc salts may decrease the serum concentration of dolutegravir May cause redistribution of fat (e.g., buffalo hump, peripheral wasting with increased abdominal girth, and cushingoid appearance) Increased liver function Tests

(*Continued*)

Table 6 (Continued)

	Nutritional considerations	*Possible gastrointestinal side effects*	*Comments/recommendations*
Elvitegravir/cobicistat, emtricitabine/tenofovir		N/D ↑ AST	Administer with food Consider calcium and vitamin D supplementation in patients with history of bone fracture or osteopenia May cause redistribution of fat (e.g., buffalo hump, peripheral wasting with increased abdominal girth, and cushingoid appearance) May cause osteomalacia with proximal renal tubulopathy
Antitubercular agents			
Cycloserine	B6 antagonist; ↓ absorption of calcium, magnesium, vitamin B12; decreased folate utilization and vitamin K synthesis; may ↑ B12 and folate requirements		Some neurotoxic effects may be prevented or lessened by pyridoxine supplementation. May administer without regard to meals
Ethambutol	May ↓ copper and zinc	N/V Abdominal pain Anorexia	Take with food to ↓ GI upset
Ethionamide	Neurotoxic effects may be prevented or relieved by the coadministration of pyridoxine	N/V/D Abdominal pain Anorexia Excessive salivation Metallic taste Stomatitis Weight loss	May be taken with or without meals
Rifabutin	High-fat meal may ↓ the rate but not extent of drug absorption	N/V/D Anorexia Dyspepsia Abdominal pain Dysgeusia Flatulence	Take with food to ↓ GI upset
Rifampin	Food may ↓ or delay amount of drug absorbed	N/V/D Anorexia Stomatitis	Take on empty stomach
Antivirals			
Acyclovir	Food does not appear to impact absorption	N/V/D	May administer with food
Amantadine		N/V/D Xerostomia Anorexia Constipation	
Cidofovir		N/V/D Anorexia	
Famciclovir	Rate of absorption and/or conversion to penciclovir and peak concentration is ↓ with food, bioavailability not affected	N/V/D Constipation Anorexia Abdominal pain	May take with food to ↓ GI upset
Ganciclovir	High-fat meal ↑ AUC	N/V/D Pancreatitis	Administer with food; do not open capsules or crush tablets
Oseltamivir	May be administered without regard to meals; take with food to improve tolerance	N/V/D Pseudomembranous colitis	Administer with food to ↓ GI upset Capsules may be opened and mixed with sweetened liquid (e.g., chocolate syrup)
Valacyclovir	May administer with or without food	N/V/D Abdominal pain	May be taken with or without food. Administer with food to ↓ GI upset
Valganciclovir	Coadministration with a high-fat meal ↑ AUC by 30%	N/V/D Abdominal pain Constipation Dyspepsia ↓ Appetite	Take with food

(*Continued*)

Table 6 (Continued)

	Nutritional considerations	*Possible gastrointestinal side effects*	*Comments/recommendations*
Miscellaneous anti-infective agents			
Clofazimine	Food ↑ extent of absorption	N/V/D Abdominal pain Constipation Bowel obstruction GI bleeding Dysgeusia	Administer with meals/milk to maximize absorption
Furazolidone	Large doses or prolonged therapy ↑ risk of hypertensive effects if taken with tyramine-containing foods	N/V/D	Avoid tyramine-containing foods
Methenamine	Foods/diets that alkalinize urine pH >5.5 ↓ activity of methenamine; cranberry juice can be used to acidify urine and ↑ activity of methenamine	N/V/D Abdominal cramping anorexia Stomatitis	Administer with food
Nalidixic Acid	Food delays absorption	N/V/D Abdominal pain	
Nitrofurantoin	Food ↑ total amount absorbed Cranberry juice or other urine acidifiers enhance drug action	N/V Anorexia Pancreatitis	Administer with food or milk
Pentamidine		N/V/D Metallic taste Pancreatitis Anorexia Dyspepsia Xerostomia	

Adapted from Gura, K. M. (2013). Drug-nutrient interactions. In: Sonneville, K. and Duggan, C. (eds.) Manual of pediatric nutrition (5th edn.). Shelton, CT: People's Medical Publishing House.

that is poorly soluble at higher pH, dissolution of the tablet or capsule form in an acidic beverage can improve and stabilize its oral bioavailability. For example, the acidic pH of cola beverages can be used therapeutically to optimize the clinical responses of orally administered ketoconazole and itraconazole in patients with gastric hypochlorhydria, such as those patients with AIDS gastropathy.

Parenteral Nutrition

Mucosal atrophy due to lack of oral nutrient intake during PN can also lead to a reduction in gastric biliary, pancreatic, and intestinal secretions. As a result of the progressive decline in intestinal function due to impaired motility and depressed enzyme activity, bacterial overgrowth can occur. This may alter both the rate and the extent of absorption of various drugs as well as specific nutrients such as chloramphenicol, chloroquine, tetracycline, and rifampin and nutrients including fat, iron, peptides, and vitamins A and B12.

Examples of Drug–Nutrient Interactions in Specific Infectious Disease States

Human Immunodeficiency Virus

Patients infected by human immunodeficiency virus (HIV) are likely to develop nutritional deficiencies secondary to drug–nutrient interactions. The relationship between nutrition and antiretroviral therapies (ARTs) has been an important factor in the effectiveness of these agents in the prevention and treatment of HIV and its complications. It was noted that certain foods such as garlic and African potato altered the bioavailability of ARTs and that complementary and alternative 'traditional' medicines also impacted the efficacy of ARTs and patient compliance. Table 6 lists the common drug–nutrient interactions seen with ARTs.

Nutrient utilization can be affected by ARTs. Protease inhibitors, such as ritonavir and nelfinavir, can disrupt lipid metabolism, resulting in hypertriglyceridemia and hypercholesterolemia. In other cases, protease inhibitors have been linked with alterations in carbohydrate metabolism, leading to insulin resistance. Interestingly, kwashiorkor can be precipitated with the initiation of ARTs in HIV-infected children with preexisting marasmus. There are several potential mechanisms. First, it is thought that some immune competence is necessary to develop the edema seen with kwashiorkor. Second, the immune reconstitution inflammatory syndrome may occur after ART is initiated. Third, the edema that ensues may be the result of the refeeding syndrome caused by ART-associated appetite stimulation. It may also be simply a manifestation of ART toxicity in severely malnourished children. Regardless, current guidelines recommend initial therapeutic feedings for HIV-infected children with severe malnutrition, followed by 50–100% increased energy intake for the first 6–10 weeks of ART.

Macronutrients can also impact the bioavailability of many ARTs. Dietary fat can alter the absorption of the antiviral agent

zidovudine. When orally administered with a high-fat meal, its absorption is reduced in comparison when the drug is taken on an empty stomach. It is recommended that zidovudine be taken in the fasted state to achieve peak serum concentrations and minimize the risk of treatment failure.

Tuberculosis

Tuberculosis (TB) is one of the leading causes of death due to infection. Strict patient compliance to medication regimens and minimizing any drug–nutrient, drug–food interactions are necessary. Failure to do so can result in disease relapse, treatment failure, and the development of drug-resistant TB. GI side effects that accompany these multidrug regimens in the initial weeks of therapy (e.g., anorexia, nausea, vomiting, and abdominal pain) are a frequent cause for noncompliance. It is recommended that these medications best be taken with meals if GI intolerance persists. This practice is not without concern, however. The bioavailability of both rifampin and isoniazid is decreased when taken with food and may lead to treatment failure. High-fat meals can reduce the C_{max} and area under the curve (AUC) of isoniazid 69% and 43%, respectively. Similarly, the absorption of rifampin is reduced when taken with food, with the mean C_{max} and AUC reduced to 29% and 16%, respectively. Ethambutol, ethionamide, and pyrazinamide are less likely to be impacted by the presence of food. The time for absorption of second-line antitubercular agent cycloserine/terizidone can be delayed by 3.5 times along with a 35% reduction in maximum blood concentrations when taken with food. Conversely, food can enhance the absorption of clofazimine and para-aminosalicylic acid.

Isoniazid, in addition to having food alter its bioavailability, can also interact with foods that have high histamine and/or tyramine content (i.e., scombroid fish, cheeses, or red wine). Isoniazid indirectly inhibits the metabolism of tyramine and histamine and may predispose patients to tyramine intoxication (e.g., hypertension) or histamine poisoning (e.g., headache, palpitations, and sweating).

Conclusion

The pharmacokinetic and pharmacodynamic responses a patient may experience to a medication vary widely based on age, gender, culture, genetic makeups, and economic status. Those responses that are influenced by a component of individuals' diet or their underlying nutritional state will add an additional layer of complexity. Even within the same individual, seasonal variations will occur that impact dietary habits and ultimately drug-related effects. Although each factor may be minor alone, a much greater synergistic effect could result when combined with other dietary, environmental, or genetic factors. By limiting medication use to as short a period of time as necessary and by periodically reassessing the pharmaceutical care plan, one can potentially reduce the risk of such complications.

See also: Alcohol: Metabolism and Health Effects; Appetite Control in Humans: A Psychobiological Approach; Ascorbic Acid: Physiology and Health Effects; Beverage: Health Effects; Cooking: Domestic Techniques; Enteral Feeding; Food–Herbal Medicine Interface; Parenteral Nutrition; Tocopherols: Physiology and Health Effects; Vitamins: Overview; Zinc: Physiology and Health Effects.

Further Reading

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Antibiotics and Drugs: Residue Determination

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Introduction

Animal husbandry on a large scale has stimulated the production of drugs designed to treat diseases and to prevent their spread among animals confined in a restricted area. Only in the United States, the demand for animal health products is expected to rise 3.5% annually to almost 13 billion dollars in 2016. Antibacterials cover the larger segment of this market, but a considerable sector is also related to the volume of parasiticides consumed in the modern farming practices. Detailed data from the Department for Environment, Food and Rural Affairs of the United Kingdom indicate that the five categories of veterinary products most sold in this country in 2009 were antimicrobials (402 tons) and coccidiostats (234 tons), followed by antifungals (8 tons) and antiprotozoals (3 tons), while no sale figure is available for anti-inflammatory drugs.

Most used veterinary medicines are listed in Table 1 together with generalities, treatment details, and physicochemical properties. Besides their therapeutic, metaphylactic, and prophylactic purposes, veterinary drugs are used to control reproduction, to relieve stress, and to promote growth. Animals may be treated individually, but it is often more efficient to treat entire groups, especially in the case of poultry and fish, by medicating feed or water. The occurrence of unwanted residues in foodstuffs can be caused by either an illegal use of banned substances (group A compounds) or an inadequate withdrawal time of the authorized ones (group B compounds). The risk these chemicals pose for the public health is related to the adverse effects of the parent drug and its metabolites, which can induce subacute reactions and chronic symptoms like immunodepression, teratogenicity, mutagenicity, and carcinogenicity. Nevertheless, the antibiotic resistance is the problem of major concern because some pathogenic bacteria have become resistant to several antibiotics currently used in human medicine. This phenomenon has been associated with the selection of resistant forms in intestines of animals continuously treated with subtherapeutic doses of antibacterials. In order to reduce the consumption of antibiotics in animals by 30–50%, the EU has banned their use as growth promoters since 2006, whereas the United States and other countries have kept unchanged this kind of employment.

The regulatory agencies of many countries have introduced restrictive food-control measures to ensure a high level of safety for consumers. In this regard, the European Union (EU) has pursued a very severe policy, and among the actions taken to minimize the occurrence of drug residues in foodstuffs are the strict regulation on the use of veterinary drugs (Council Regulation 2377/90/EEC), the establishment of maximum residue limits for the allowed substances (Council Regulation 2377/90/EEC; Commission Regulation (EU) No. 37/2010), and the ban of hormones and other performance enhancers (Council Regulation 2377/90/EEC; Commission Regulation (EU) No. 37/2010; Directive 96/23/EC; Directive 96/22/EC). At the same time, the EU has encouraged the development of novel and efficient analytic methods that meet the criteria described in the Commission Decision 2002/657/EC and its implementation. This grade of flexibility is advantageous because it allows the ready adaptation to the latest technological advances and readiness to face new emergencies.

At the present moment, the control of residues is based on screening and subsequent confirmation of those suspected noncompliant samples. The screening methods, involving both immunologic and chromatographic techniques, can quickly detect an analyte or a class of analytes with high sensitivity and specificity. Some false-positives are acceptable since they will be submitted for confirmatory analysis, but the number of false-negatives must be as low as possible because samples considered compliant will not be reanalyzed. Unlike the immunologic techniques, the chromatographic techniques can perform quantitative multiresidue determinations. In particular, liquid chromatography (LC) is more suitable than gas chromatography (GC) since it can analyze veterinary drugs directly, due to their polar and nonvolatile nature. For this reason, LC has become the technique of choice for multiclass analysis, especially when coupled to mass spectrometry (MS). GC/LC-MS techniques are also indispensable to perform confirmation analyses when following the identification criteria stated in the Commission Decision 2002/657/EC.

This article illustrates the most recent advances and trends in the residue analysis of veterinary drugs in food, giving particular emphasis on the multiclass determinations. In fact, the growing interest for these methods is motivated by the incidental occurrence of residues in foodstuffs as multicomponent mixtures exhibiting synergistic effects potentially more toxic than those of the individual compounds. Table 2 shows some remarkable examples of multiclass methods published in the past 3 years, displaying a concise summary of procedures for extracting, screening, and confirming veterinary drugs, while generalities, analytic problems, and other interesting examples of multiresidue methods are illustrated in the subsequent paragraphs.

Sample Preparation

Problems Related to the Food Matrix and Physicochemical Properties of Veterinary Drugs

The scientific literature is rich with papers dealing with the analysis of veterinary drugs in milk and dairy products, tissues, eggs, honey, seafood, and, to a less extension, fruit and vegetables; in fact, it is less known that antibacterials are also used in agriculture. The proposed methods range from single-analyte methods to single-class or multiclass multiresidue methods; in every case, the sample preparation is a key step depending on the kind of food, the physicochemical properties of the interest drugs, and the number of compounds to be extracted.

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Table 1 Physicochemical properties, action mechanism, and priority uses of veterinary drugs

Pharmaceutical class	*Action*	*Uses*	*Classes (subclasses)*	*Physicochemical properties*
Antibiotics	Technically, antibiotic is a natural substance produced by bacteria or fungi that can kill or inhibit the growth of other bacteria. However, the terms antibacterial, antimicrobial, and antibiotic are often used interchangeably to represent natural, semisynthetic and synthetic anti-infectives	Therapy, metaphylaxis, auxinic use	β-Lactams (penicillins and cephalosporins) Tetracyclines Macrolides Aminoglycosides Amphenicols Sulfonamides Quinolones Nitrofurans Nitroimidazoles Polyether ionophores	300–800 mol wt range From very simple to extremely complex structures with basic and/or acid functionalities Under different pH conditions, antibiotics can be neutral, cationic, anionic, or zwitterionic Solubility and stability can be affected by the solution pH: for example, polyether ionophores and macrolides are unstable in acidic media, while β-lactams degrade in aqueous acids and bases
Anthelmintics	Anthelmintics are parasiticides, also known as endectocides, used to control nematodes and arthropods affecting livestock. Since they expel or destroy gastrointestinal worms, their more common name is dewormers	Therapy, prophylaxis of cattle, sheep, and swine	Benzimidazoles Nicotinic agonists (imidazothiazoles and tetrahydropyrimidines) Macrocyclic lactones (avermectins and milbemycins) Aminoacetonitrile derivatives	80–900 mol wt range Depending on the structure, they can be soluble in water (cambendazole and thiabendazole), alcohols (levamisole and mebendazole), and nonpolar solvents (dichlorvos) Dissimilar physicochemical properties can be recognized in the same chemical class: for example, benzimidazoles belong to a large family varying in lipophilicity and acid–base behavior
Antifungals	Antifungals are used orally or topically to treat fungal or yeast infections. They can be grouped into three classes based on their site of action: azoles, which inhibit the synthesis of ergosterol (the main fungal sterol); polyenes, which interact with fungal membrane sterols physicochemically; and 5-fluorocytosine, which inhibits macromolecular synthesis	Therapy	Azoles Polyene macrolides Flucytosine	Azoles are poorly soluble in water but can be dissolved in organic solvents. An exception is fluconazole. Imidazoles are weak dibasic agents Polyenes are poorly soluble in water and the common organic solvents. They are soluble in highly polar solvents (DMF and DMSO). They are quite unstable in aqueous, acidic, or alkaline media
Coccidiostats	Coccidiostats are used in the treatment of coccidiosis, an infectious disease caused by parasitic microbial organisms (protozoa), collectively known as coccidian	Therapy, prophylaxis of intensively reared species (poultry, pigs, cattle, and sheep)	Chemical coccidiostats; ionophores	140–940 mol wt range Their chemical structure and polarity vary greatly, particularly in the group of chemical coccidiostats
Tranquilizers	Tranquilizers are administered to sedate animals	Stress reduction during the transportation of food-producing animals (pigs)	Phenothiazine derivatives Propanediol derivates	The solubility of phenothiazine derivatives depends on the type of substituents The unsubstituted phenothiazine exhibits thermal stability only up to 100 °C The pK_a values are ~9.2–9.4
Anti-inflammatories	Anti-inflammatory drugs are used to relieve pain and inflammation, to lower fever, and to treat allergy (corticosteroids)	Management of musculoskeletal disorders and acute respiratory distress syndrome in cattle	Nonsteroidal anti-inflammatory drugs (three classes – carboxylic acids, enolic acids, and anilides and related subclasses) Corticosteroids	Most of NSAIDs are acid compounds (pK_a ~3–5), while anilides are neutral compounds with the exception of nimesulide. Their lipophilicity is related to the nature of their aryl groups and substituents Glucocorticoids are thermolabile: in particular, triamcinolone can isomerize during mild heat treatments (40–50 °C)

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Table 2 Selected multiclass methods for analyzing veterinary drug residues in foods

Screening methods

Class	*Matrix*	*Technique/type of assay*	*Remarks*	*Extraction procedure*	*Recovery (%)*	*Method limits*	*Reference*
β-Lactams, tetracyclines, sulfonamides, and quinolones Total of 26 analytes	Milk	Microbiological bioassay in microtiter plates (*Geobacillus stearothermophilus*, *Bacillus cereus*, and *Bacillus subtilis*)	Dichotomous colorimetric responses in short time (6 h); semiquantitative	No sample treatment	Not reported	LOD: 4–3240 μg l^{-1}	Nagel et al.
Tetracyclines, macrolides, sulfonamides, aminoglycosides, quinolones, β-lactams Total of 28 analytes	Honey	Microbiological kits (*Bacillus stearothermophilus*)	Validation of two microbiological kits (Eclipse 50® and Premi®Test) according to the Commission Decision 2002/657/EC	SE with CH_3CN: CH_3COCH_3 (70:30, v/v)	Not reported	Eclipse 50® CCβ: 40–1500 μg kg^{-1} and 5% false-positive results Premi®Test CCβ: 5–25 μg kg^{-1} but 14% false-positive results	Gaudin et al.
Fluoroquinolones and sulfonamides Total of 35 analytes	Milk	Dual-colorimetric ELISA (two different enzymes) (*alkaline phosphatase* and *horseradish peroxidase*)	–	Samples defatted by centrifugation and added to $Na_2[Fe(CN)_5NO]\cdot 2H_2O$ and $ZnSO_4$. Supernatant diluted with phosphate-buffered saline	67–105	LOD: 2.4–5.8 μg l^{-1}	Jiang et al.
Fluoroquinolones, sulfonamides, and phenicols	Milk	Six channels SPR biosensor	–	Samples diluted with water	Not reported	LOD: 1.7–2.1 μg l^{-1}	Fernandez et al.
Antibiotics and veterinary drugs Total of 29 analytes	Milk and infant formulas	Comparison of three screening methods: (a) UHPLC-ESI(+)-Orbitrap (b) UHPLC-ESI(+)-QqTOF (c) UHPLC-ESI(+)-QqQ	Two columns for the three methods: (a) Hypersil GOLD aQ C18 (100 × 2.1 mm, 1.7 μm). Total run time: 4 min (b) and (c) ACQUITY UPLC BEH C18 (100 × 2.1 mm, 1.7 μm). Total run time: 3 min Mobile phases: H_2O 0.05% formic acid and CH_3OH. Gradient elution Flow rate: 0.3 ml min^{-1} at 30 °C	Buffered QuEChERS extraction with CH_3CN 1% CH_3COOH and H_2O 0.1 M Na_2EDTA	Not reported	CCα: 4.1–213.3 μg kg^{-1} CCβ: 8.1–226.6 μg kg^{-1}	Romero-González et al.

Macrolides, sulfonamides, quinolones, avermectins, benzimidazoles, imidazothiazoles, and tetracyclines Total 21 analytes	Milk	UHPLC-ESI(+)-QqQ Two screening methods operating in NLS or PIS mode. Confirmation of nonnegative samples with two MRM transitions	ACQUITY UPLC BEH C18 (100 × 2.1 mm, 1.7 μm). Column temperature: 30 °C Mobile phases: CH_3OH and H_2O 0.05% formic acid. Gradient elution Flow rate: 0.3 ml min^{-1}. Total run time: 3 min for screening methods and 8.5 min for the confirmation method	QuEChERS extraction with CH_3CN 0.1% CH_3COOH and H_2O 0.1 M Na_2EDTA	63–122	Screening methods: CCβ: 0.8–97 μg kg^{-1} Confirmation method: LOQ: 0.3–10 μg kg^{-1}	Martínez Vidal et al.
Sulfonamides, tetracyclines, β-lactams, macrolides, NSAID metabolite, and benzimidazole Total of 25 analytes	Milk	HPLC-ESI(+/−)-QqTOF Nontarget screening method	YMC ODS-AQ (2 × 100 mm, 3 μm). Column temperature: 35 °C Mobile phases: CH_3CN and H_2O 0.1% formic acid. Gradient elution Flow rate: 0.25 ml min^{-1} Total run time: 16.5 min	SE with CH_3CN. Cleanup with a 3000 Da molecular weight cutoff centrifuge filter	42–154	Not reported	Turnipseed et al.
Aminoglycosides, amphenicols, β-lactams, quinolones, tetracyclines, macrolides, NSAIDs, sulfonamides Total of 38 analytes	Veal muscle	HPLC-ESI(+/−)-QqQ	Two different columns: (A) Waters Atlantis dC18 Guard (20 × 3.9 mm, 3 μm) (B) ZIC-HILIC (50 × 2.1 mm, 5 μm, 200 Å) Mobile phases: H_2O 0.1% formic acid and CH_3CN. Gradient elution Flow rate: 1.0 ml min^{-1}. Column (A). Autosampler temperature: 4 °C Two separate injections: one with positive ionization and one with negative ionization Total run time: 15 min (column B) and 16.5 (column A) min	Two consecutive extractions: with CH_3CN:H_2O (86:14, v/v), followed by heating, cooling, and addition of formic acid and with H_2O. Extract defatted with hexane	45–106	MDL: 1–41 ng g^{-1}	Martos et al.

(*Continued*)

Table 2 (Continued)

Screening methods

Class	*Matrix*	*Technique/type of assay*	*Remarks*	*Extraction procedure*	*Recovery (%)*	*Method limits*	*Reference*
Antibiotics and veterinary drugs (anthelmintics, β-lactam antibiotics, fluoroquinolones, flukicides, macrolides, nitroimidazoles, NSAIDs, thyreostats, tranquilizers, and others) Total of 113 analytes (quantified 87)	Bovine muscle	UHPLC-ESI(+/−)-QqQ	ACQUITY UPLC HSS T3 column (100 × 2.1 mm, 1.8 μm). Column temperature: 40 °C Mobile phases: 5:95 (v/v) CH_3CN:H_2O and CH_3CN (both 0.1% formic acid). Gradient elution Flow rate: 0.5 ml min^{-1} Total run time: 13 min	SE with CH_3CN:H_2O; dispersive SPE with 500 mg of end-capped C18 sorbent	70–120	Not reported	Geis-Asteggiante et al.
Veterinary drugs (tetracyclines, ionophores, coccidiostats, penicillins, cephalosporins, fluoroquinolones, sulfonamides, and mycotoxins) Total of 15 drugs	Hen eggs	HPLC-ESI(+)-QqQ	ACE C18 column (150 × 2.1 mm, 3 μm, 100 Å). Column temperature: 40 °C Mobile phases: H_2O 0.1% HCOOH and CH_3CN 0.1% HCOOH. Gradient elution Flow rate: 200 ml min^{-1} Total run time: 35 min	QuEChERS extraction with CH_3OH:H_2O:CH_3COOH 80:20:1 (v/v/v) and the addition of 0.125 g ml^{-1} CH_3COONa and 0.5 g ml^{-1} Na_2SO_4 anhydrous	>62	MDL: 0.9–27.1 μg kg^{-1} MQL: 1.3–28.7 μg kg^{-1} CCα: 11.9–246 μg kg^{-1} CCβ: 14.0–283 μg kg^{-1}	Capriotti et al.
Pesticides, antibiotics, and veterinary drugs (macrolides, quinolones, tetracyclines, sulfonamides, avermectins, nitroimidazoles, coccidiostats, penicillins, amphenicols, tranquilizers, corticoids, ionophores, NSAIDs, and others) More than 350 analytes	Honey	UHPLC-ESI(+/−)-Orbitrap MS	Hypersil GOLD aQ C18 column (100 × 2.1 mm, 1.7 μm). Column temperature: 30 °C Mobile phases: H_2O and CH_3OH (both 0.1% (v/v) formic acid and $HCOONH_4$ 4 mM). Gradient elution Flow rate: 0.3 ml min^{-1}. Total run time: 14 min	SE with 7.5 ml of CH_3CN containing 1% of formic acid (v/v)	60–120	Not reported	Gómez-Pérez et al.

Sulfonamides, quinolones, macrolides, tetracyclines, penicillins, imidazothiazoles, avermectins, and benzimidazoles Total of 40 analytes	Honey	UHPLC-ESI(+)-Orbitrap MS Posttarget screening approach and confirmation by fragmenting in the higher-energy collision-induced dissociation cell	Hypersil GOLD aQ C18 column (100 × 2.1 mm, 1.7 μm). Column temperature: 35 °C Mobile phases: H_2O and CH_3OH both 4 mM $HCOONH_4$ acidified with 0.1% formic acid. Gradient elution Flow rate: 0.3 ml min^{-1} Total run time: 10 min	Extraction with aqueous solution of Na_2EDTA (0.1 M). Turbulent flow chromatography (TFC) with a Cyclone P (50 × 0.5 mm, 60 μm; 60 Å). TFC solvents: (A) H_2O 0.05% formic acid (v/v) (B) CH_3OH:CH_3CN (1:1, v/v) with Na_2EDTA, 0.1% (C) H_2O 10 mM CH_3COONH_4 (D) CH_3CN: CH_3COCH_3:2-propanol (4:3:3, v/v/v)	68–121	LOI: 0.1–50 μg kg^{-1}	Aguilera-Luiz et al.
Sulfonamides and tetracyclines Total of 22 analytes	Fish, poultry, and porcine meat	UHPLC-ESI(+)-QqQ Two runs: one operating in precursor ion scan and one in data-dependent scan modes	Zorbax Eclipse Plus C18 (50 × 2.1 mm, 1.8 μm) Mobile phases: H_2O and CH_3CN (both 0.1% formic acid). Gradient elution Flow rate: 0.1 ml min^{-1} Total run time: 21 min	SE with CH_3OH:CH_3CN (50:50, v/v) 0.05% formic acid	30–100	CCα: 3.99–25.1 μg kg^{-1} CCβ: 6.80–37.1 μg kg^{-1}	Dasenaki and Thomaidis
Trimethoprim, sulfamethoxazole, chloramphenicol, quinolones, and fluoroquinolones Total of 8 analytes	Aquacultured species	HPLC-ESI(+/−)-QTOF Posttarget and nontarget screening methods followed by confirmation for detected analytes	YMC ODS-AQ (2 × 100 mm, 3 μm). Column temperature: 35 °C Mobile phases: CH_3CN and H_2O 0.1% formic acid. Gradient elution Flow rate: 0.25 ml min^{-1} Total run time: 18.5 min	SE with diluted acetic acid, CH_3CN, and NaCl	80–130	Not reported	Turnipseed et al.
Tetracyclines, sulfonamides, penicillins, quinolones, macrolides, and benzimidazoles Total of 34 analytes	Porcine muscle	HPLC-ESI(+)-QqQ	Zorbax Eclipse XDB C18 column (150 × 4.6 mm, 5 μm). Column temperature: 30 °C Mobile phases: H_2O: CH_3CN (95:5 v/v) and H_2O:CH_3CN (5:95 v/v) (both 0.1% formic acid). Gradient elution Flow rate: 0.3 ml min^{-1} Total run time: 18 min	SE with fast partition at very low temperature. Extraction with CH_3CN, centrifugation, and 15 s in liquid nitrogen	Not reported	CCβ:12.5–150 μg kg^{-1}	Lopes et al.

(*Continued*)

Table 2 (Continued)

Screening methods

Class	*Matrix*	*Technique/type of assay*	*Remarks*	*Extraction procedure*	*Recovery (%)*	*Method limits*	*Reference*
Antibiotics, pesticides, and mycotoxins Total of 82 analytes	Fish feed and fish fillets	UHPLC-ESI(+)-QqTOF for screening and UHPLC-ESI(+)-QqQ for confirmation of positive samples	ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm) Mobile phases: H_2O and CH_3OH (both 0.01% HCOOH and 0.1 mM NH_4Ac). Gradient elution Flow rate: 0.3 ml min^{-1} at 60 °C Total run time: 18 min	SE with CH_3CN:H_2O 80:20 v/v 0.1% formic acid	Not reported	LOI: 20–100 μg kg^{-1}	Nacher-Mestre et al.
Tetracyclines, quinolones, and sulfonamides Total of 21 analytes	Cattle and poultry meat	HPLC-ESI(+)-QqQ	Symmetry C18 (75 × 4.6 mm, 3.5 μm). Column temperature: 20 °C Mobile phases: H_2O and CH_3CN (both 0.1% formic acid). Gradient elution Flow rate: 0.4 ml min^{-1} Total run time: 17 min	MSPD with EDTA-treated sand; extraction with CH_3CN 0.1% formic acid	19–29	CCβ: 25–50 μg kg^{-1}	Bittencourt et al.
Sulfonamides, tetracyclines, macrolides, quinolones, and chloramphenicols Total of 33 analytes	Bovine, caprine, and ovine milk	UHPLC-ESI(+/−)-QqQ	ACQUITY UPLC HSS T3 column (100 × 2.1 mm, 1.8 μm) Mobile phases: H_2O and CH_3CN (both 0.1% formic acid). Gradient elution Flow rate: 0.45 ml min^{-1} at 40 °C Total run time: 12 min	SE with CH_3CN	Not reported	CCβ: 0.1–100 μg kg^{-1}	Freitas et al.
Penicillins, cephalosporins, sulfonamides, macrolides, tetracyclines, aminoglycosides, and quinolones Total of 63 analytes	Bovine meat	UHPLC-ESI(+)-LTQ-Orbitrap	Purospher STAR RP-18e (125 × 3 mm, 5 μm) Mobile phases: H_2O 1 mM HFBA and 0.5% formic acid and CH_3OH:CH_3CN (50:50, v/v) 0.5% formic acid. Gradient elution Flow rate: 0.5 ml min^{-1} at 25 °C Total run time: 15 min	Two protocols: SE with CH_3CN and SE with H_2O:CH_3CN 5% TCA followed by SPE on C18 cartridge	Not reported	LOD: 1–308 μg kg^{-1} CCβ: 25–1000 μg kg^{-1}	Hurtaud-Pessel et al.

Confirmatory methods

Class	*Matrix*	*Technique*	*Chromatographic conditions*	*Extraction*	*Recovery (%)*	*Method limits*	*Reference*
Macrolides, β-lactams, lincosamide, fluoroquinolones, and anthelmintics Total of 23 analytes	Milk	UPLC-ESI (+)-QqQ	HSS T3 (10 × 2.1 mm, 1.8 μm) Mobile phases: H_2O and CH_3CN (both 0.05% formic acid). Gradient elution Flow rate: 0.3 ml min^{-1} Total run time: 20 min	SE with CH_3CN	51.5–139.0	Not reported	Tang et al.
Penicillins and amphenicols Total of 8 analytes	Milk	HPLC-DAD	Kinetex XB-C18 (150 × 4.6 mm, 2.6 μm) Mobile phases: H_2O (0.05 M CH_3COONH_4) and CH_3CN. Gradient elution Flow rate: 0.9 ml min^{-1} Total run time: 17 min	MSPD procedure using a mixture of sorbents (Strata-X by Phenomenex plus QuEChERS by Agilent Technologies). Elution with 1 ml CH_3OH and 1 ml CH_3CN	84–102	CCα: 35.2–56.3 μg kg^{-1} CCβ: 39.9–61.9 μg kg^{-1}	Karageorgou and Samanidou
Macrolides, sulfonamides, and anthelmintics Total of 17 analytes	Cheese	UPLC-ESI(+)-QqQ	ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm). Column temperature: 30 °C Mobile phases: CH_3OH and H_2O 0.01% (v/v) formic acid. Gradient elution Flow rate: 0.3 ml min^{-1} Total run time: 8.5 min	QuEChERS procedure with 10 ml of CH_3CN 1% acetic acid, 10 ml of a solution of 0.1 M Na_2EDTA, 4 g of $MgSO_4$ anhydrous, and 1 g of sodium acetate	70–112.7	CCα: 2.3–11.3 μg kg^{-1} CCβ: 4.2–14.3 μg kg^{-1}	Gómez-Pérez et al.
Coccidiostats, antimicrobial agents, corticosteroids, and antifungal agents Total of 18 analytes	Milk	HPLC-ESI (+)-QqQ	Synergi Polar-RP (50 × 2.00 mm, 2.5 μm, 100 Å) Mobile phases: H_2O and CH_3CN (both 0.1% formic acid). Gradient elution Flow rate: 0.2 ml min^{-1} Total run time: 36 min	SE with CH_3CN; SPE with Strata-X cartridge, elution with CH_3OH	65–119	CCα: 0.1–18.7 ng ml^{-1} CCβ: 0.2–31.8 ng ml^{-1}	Nebot et al.
Antibiotics and benzimidazoles Total of 29 analytes	Milk-based infant formulas and meat-based baby food	UPLC-ESI(+)-QqQ	ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm). Column temperature: 30 °C Mobile phases: CH_3OH and 0.05% (v/v) formic acid in H_2O. Gradient elution Flow rate: 0.3 ml min^{-1} Total run time: 9.5 min	Two procedures: (A) 'Dilute and shoot': diluting samples with H_2O and extracting with CH_3CN (1% formic acid, v/v); filtration, injection (B) QuEChERS: extracting with 1% (v/v) acetic acid in CH_3CN, anhydrous $MgSO_4$, and sodium acetate; filtration, injection	(B) 70–120	(B) CCα: 0.5–16.2 μg kg^{-1} CCβ: 1.2–22.4 μg kg^{-1}	Aguilera-Luiz et al.
Veterinary drugs (agonists, benzimidazoles, β-lactams, lincosamides, nitroimidazoles, quinolones, quinoxalines, sedatives sulfonamides, tetracyclines, and thyreostats) and other contaminants Total of 255 analytes	Raw milk	UHPLC-ESI(+)-QqQ and UHPLC-ESI(−)-QqQ	ACQUITY HSS T3 column (100 × 2.1 mm, 1.8 μm). Column temperature: 40 °C Two different chromatographic runs: one for ESI + and the other for ESI −. Mobile phases: (positive) H_2O containing 0.5 mM CH_3COONH_4 and CH_3OH (both 0.1% (v/v) formic acid); (negative) H_2O containing 2.5 mM CH_3COONH_4 and CH_3OH Flow rate: 0.4 ml min^{-1}. Time of each run: 12 min	SE with CH_3CH_2OH:CH_3CN (1:5, v/v) containing EDTA	63–141	LOD: 0.05–10 μg kg^{-1}	Zhan et al.

(*Continued*)

Confirmatory methods

Class	*Matrix*	*Technique*	*Chromatographic conditions*	*Extraction*	*Recovery (%)*	*Method limits*	*Reference*
Aminoglycosides and macrolides Total of 6 analytes	Meat	HPLC-ESI (+)-QqQ	Luna C18 (250 × 4.6 mm, 5 μm) Mobile phases: H_2O and CH_3OH (both 1 mM heptafluorobutyric acid). Gradient elution Flow rate: 0.5 ml min^{-1} Total run time: 35 min	PLE using EDTA-treated sand as dispersive medium; extraction with CH_3OH at 80 °C and 1500 psi for 10 min in two cycles	70–96	CCα: 85–510 μg kg^{-1} CCβ: 98–514 μg kg^{-1}	Berrada et al.
β-Lactams and tetracyclines Total of 11 analytes	Bovine muscle	UPLC-ESI (+)-QqQ	ACQUITY UPLC BEH C18 column (50 × 2.1 mm, 1.7 μm) Mobile phases: water with 0.1% formic acid and methanol. Gradient elution Flow rate: 0.60 ml min^{-1} Total run time: 11 min	SE with H_2O and CH_3CN; dispersive SPE with C18	90–110	CCα: 30.9–371.7 μg kg^{-1} CCβ: 36.8–443.4 μg kg^{-1}	Rezende et al.
Quinolones, sulfonamides, macrolides, anthelmintics, avermectins, diamino derivatives, benzathine Total of 22 analytes	Chicken muscle	UPLC-ESI (+)-QqQ	ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm). Column temperature: 30 °C Mobile phases: CH_3CN and H_2O (both 0.1% (v/v) formic acid). Gradient elution Flow rate: 0.3 ml min^{-1} Total run time: 8.5 min	QuEChERS procedure: SE with 5.0 ml of H_2O and 10.0 ml of 1% acetic acid in a solution of CH_3CN: H_2O (80:20, v/v); addition of sodium citrate dibasic sesquihydrate, sodium citrate dehydrate, anhydrous $MgSO_4$; dispersive SPE with PSA	70–120	CCα: 17.0–411.8 μg kg^{-1} CCβ: 24.1–423.6 μg kg^{-1}	Pereira Lopes et al.
Sulfanilamide, nitroimidazoles, quinolones, macrolide antibiotics, lincosamides, and praziquantel Total of 54 analytes	Pork meat	HPLC-ESI (+)-QqQ	Zorbax Eclipse XDB C8 column (150 × 4.6 mm id, 5 μm). Column temperature: 30 °C Mobile phases: CH_3CN, CH_3OH, and H_2O (0.15% (v/v) formic acid). Gradient elution Flow rate: 0.8 ml min^{-1} Total run time: 23 min	SE with CH_3CN. SPE with Supelclean LC-18 cartridges, elution with CH_3OH	20.9–121	Not reported	Xie et al.
Aminoglycosides, β-lactams, lincosamides, macrolides, quinolones, sulfonamides, tetracycline Total of 24 analytes	Chicken muscle	UPLC-ESI (+)-QqQ	ZIC-HILIC (100 × 2.1 mm, 3.5 μm). Column temperature: 40 °C Mobile phases: 50 mM $HCOONH_4$ in H_2O at pH 2.5 and CH_3CN. Gradient elution Flow rate: 0.2 ml min^{-1} Total run time: 10 min	SE with aqueous solution – CH_3CN (1:1, v/v) 2% TCA	53–99	CCα: 58–510 μg kg^{-1} CCβ: 65–525 μg kg^{-1}	Chiaochan et al.

Aminoglycosides, macrolides, lincosamides, sulfonamides, tetracyclines, quinolones, and trimethoprim Total of 36 antibiotics	Chicken muscle	HPLC-ESI (+)-QqQ	BetaSil Phenyl/Hexyl (50 × 2.1 mm, 3 μm) Mobile phases: 1 mM HFBA + 0.5% formic acid and 0.5% formic acid in ACN/methanol (1:1, v/v) Flow rate: not specified Total run time (online cleanup + chromatographic separation): 19 min	SE with CH_3CN:2% TCA (45:55, v/v). Centrifugation, filtration, injection onto TFC-HPLC system TFC column: Cyclone P (50 × 0.5 mm, 60 μm; 60 Å) TFC solvents: same as analytic column Flow rate: 1.5 ml min^{-1}	80–120	CCα: 1.7–602.2 μg kg^{-1} CCβ: 2.2–704.4 μg kg^{-1}	Bousova et al.
Sulfonamides, diaminopyridine derivates, quinolones, tetracyclines, macrolides, penicillins, lincosamides Total of 41 analytes	Hen eggs	UPLC-ESI (+)-QqQ	Mobile phases: H_2O 0.02% formic acid and 1 mmol l^{-1} oxalic acid and CH_3CN 0.1% formic acid. Gradient elution Flow rate: 0.3 ml min^{-1} at 40 °C Autosampler temperature: 15 °C Total run time: 13 min	Static PLE extraction with CH_3CN: 0.01 mol l^{-1} succinic acid buffer pH 6.0, 1:1 (v/v) at 70 °C 1500 psi	47–320%	CCα: 0.3–232.3 μg kg^{-1} CCβ: 0.9–264.6 μg kg^{-1}	Jiménez et al.
Polyether ionophores, macrolides, lincosamide Total of 10 analytes	Eggs	LC-ESI(+)-QqQ 3 MRM transitions for each analyte	ACE C_{18} column (50 × 2.1 mm, 3 μm, 100 Å) Mobile phases: H_2O, CH_3CN, and CH_3OH (all with 0.1% formic acid). Gradient elution Flow rate: 0.3 ml min^{-1} at 35 °C Total run time: 18 min	SE with 4 ml of CH_3CN; evaporation to dryness of an aliquot of 250 μl of the supernatant, dilution to 1 ml with 5 mmol l^{-1} CH_3COONa: MeOH (70:30, v/v); filtration, injection	78.5–168.1	CCα: 0.87–229.7 μg kg^{-1} CCβ: 1.74–262.7 μg kg^{-1}	Ferraz Spisso et al.
Tetracyclines, macrolides, quinolones, sulfonamides, anthelmintics Total of 25 analytes	Eggs	UPLC-ESI (+)-QqQ	ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm^{-1}). Column temperature: 30 °C Mobile phases: CH_3OH and H_2O 0.05% formic acid. Gradient elution Flow rate: 0.3 ml min^{-1} Total run time: 9.5 min	Comparison between several extraction procedures: (1) SE with CH_3CN: 0.5 M citric acid (pH 4): 0.1 M Na_2EDTA (10:1:0.5, v/v/v) and SPE with Oasis HLB cartridge (2) QuEChERS procedure with CH_3CN 1% acetic acid and H_2O 0.1 M Na_2EDTA followed by the addition of $MgSO_4$ and CH_3COONa_2 (3) MSPD with Florisil. Elution with CH_3OH, CH_3CN, and 0.04% (v/v) of 35% NH_3 solution in CH_3OH (4) SPE with Oasis HLB washing with hexane and elution with CH_3OH, CH_3CN, and 0.04% (v/v) 35% NH_3 in CH_3OH	(1) 71–97 (2) 11–96 (not all analytes extracted) (3) 2–90 (not all analytes extracted) (4) 3–131 (not all analytes extracted)	For solvent extraction: LOQ: 0.1–5.0 μg kg^{-1} CCα: 2.1–220.8 μg kg^{-1} CCβ: 4.7–241.6 μg kg^{-1}	Garrido Frenich et al.
Macrolides, lincosamides, quinolones, tetracyclines, pleuromutilins, and diaminopyrimidine derivatives Total of 37 analytes	Honey	HPLC-ESI(+)-QqQ	AQUA C18 (150 × 2.1 mm, 3 μm). Column temperature: 30 °C Mobile phases: CH_3CN and H_2O (both 0.2% (v/v) formic acid). Gradient elution Flow rate: 0.3 ml min^{-1} Total run time: 25 min	Extraction with buffer solution. SPE with Oasis HLB cartridge, elution with CH_3OH	92–106	CCα: 7.5–12.9 μg kg^{-1} CCβ: 9.4–19.9 μg kg^{-1}	Bohm et al.

(*Continued*)

Confirmatory methods

Class	*Matrix*	*Technique*	*Chromatographic conditions*	*Extraction*	*Recovery (%)*	*Method limits*	*Reference*
Benzimidazoles, quinolones, macrolides, nitroimidazoles, penicillins and cephalosporins, sulfonamides, tetracyclines, tranquilizers, and various compounds Total of 113 analytes	Pork muscle, meat, bovine kidney, bovine liver, fish, and honey	UPLC-ESI (+)-Orbitrap MS	Kinetex Core-Shell C18 (150×2.1 mm, 2.6 μm) Mobile phases: CH_3CN and H_2O (both 0.3% (v/v) formic acid) Gradient elution Flow rate: 0.4–0.8 ml min^{-1} Total run time: 14 min	SE with CH_3CN and Na_2EDTA and $(NH_4)_2SO_4$ dissolved in succinic buffer. SPE with Evolute™ ABN cartridge. Elution with CH_3CN and DMSO	80% of all compounds were recovered with more than 50%, while one compound was recovered with more than 120%	CCα: 1.0–1181.2 μg kg^{-1} CCβ: 1.1–2337.0 μg kg^{-1}	Kaufmann et al.
Antibiotics, benzimidazoles, triphenylmethane dye, and metabolite Total of 13 analytes	Shrimp	HPLC-ESI(+)-TOF	Zorbax Eclipse XDB C18 (5×4.6 mm, 1.8 μm) Mobile phases: H_2O with 0.1% formic acid and CH_3CN Gradient elution Flow rate: 0.5 ml min^{-1} Total run time: 16 min	QuEChERS methodology: SE with CH_3CN containing 1% acetic acid; dispersive SPE with PSA	58–133	LOD: 0.06–7 μg kg^{-1} Not validated according to Commission Decision 2002/657/EC	Villar-Pulido et al.

CCβ, detection capability; CCα, decision limit; CH_3CN, acetonitrile; DAD, diode array detector; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunospecific assay; ESI, electrospray ionization; Na_2EDTA, ethylenediaminetetraacetic acid disodium salt; Evolute™ ABN cartridge, acid, basic, and neutral cartridge; HFBA, heptafluorobutyric acid; HPLC, high-performance liquid chromatography; LOD, limit of detection; LOI, limit of identification; LOQ, limit of quantitation; MRM; multiple reaction monitoring; MSPD, matrix solid-phase dispersion; MDL, method detection limit; MQL, method quantitation limit; NLS, neutral-loss scan; NSAIDs, nonsteroidal anti-inflammatory drugs; Oasis HLB cartridge, hydrophilic–lipophilic balance cartridge; PIS, product ion scan; PLE, pressurized liquid extraction; PSA, primary–secondary amine; QqQ, triple quadrupole; QqTOF, quadrupole-quadrupole time of flight; QuEChERS, quick, easy, cheap, effective, rugged, and safe; SE, solvent extraction; SPE, solid-phase extraction; SPR biosensor, surface plasmon resonance (SPR) biosensor; TCA, trichloroacetic acid; TFC, turbulent flow chromatography; TOF, time of flight; UHPLC; ultrahigh-performance liquid chromatography; XDB columns, extra dense bonding columns; ZIC-HILIC, zwitterionic hydrophilic interaction liquid chromatography.

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Single-analyte analyses are optimized for achieving the maximum recovery of the target compound in a specific matrix. In order to realize a quantitative extraction, a problem that has to be often overcome is the tight interaction of some drugs with proteins or other components of the matrix. For example, nitrofurans are rapidly converted into peptide-bound metabolites that persist for a long time in animal tissues; the sample homogenization in the presence of diluted hydrochloric acid is a common solution applied for their release. A strong noncovalent interaction is also established by nonsteroidal anti-inflammatory drugs (NSAIDs) with proteins of milk and tissues. Since the value of the drug–protein association constant increases with decreasing pH, acid deproteinization is less effective than that obtained with an organic solvent. As a matter of fact, the addition of neutral water-miscible organic solvents (acetonitrile, acetone, and ethyl acetate) to bovine milk and minced meat samples is advantageous because, decreasing the medium dielectric constant, protein molecules aggregate and precipitate, while pK_a value of NSAIDs rises steeply. Glucuronidation of NSAIDs and glucocorticoids is another problem to be faced to increase extractive yields from milk and meat; for example, carprofen and carprofen glucuronide are the marker residues in bovine muscle tissue. Usually, chemical hydrolysis with hydrochloric acid or enzymatic digestion with β-glucuronidase is a deconjugation step performed prior to a solid-phase extraction (SPE) cleanup. Enzymatic hydrolysis is also sometimes chosen to deconjugate glucuronate residues of corticosteroids potentially occurring in milk and in the liver from various animal species, while the linkage of sulfonamides to the sugars of honey is commonly solved with a step of acid hydrolysis, useful to break the *N*-glycoside linkage. The occurrence of multivalent cations in food causes the formation of strong complexes with tetracyclines, which are strongly retained in tissues. For these same reasons, tetracyclines can establish specific interactions with silanol groups of silica-based adsorbents, complicating their analysis; in order to avoid analyte losses during sample preparation and tailed peaks during LC, chelating agents like EDTA salts have been used during the sample treatment. β-Lactams are difficult to treat for other reasons: they are degraded by endogenous muscle enzymes and are unstable to heat and in alcohols. Quinolones, amphoteric and zwitterionic compounds, are poorly soluble in water and can penetrate tissues between pH 6 and pH 8. Because of these proprieties, their extraction has been often performed using an acidified organic extractant. Extraction of antibacterials, coccidiostats, and anthelmintics from eggs can be very problematic due to the high lipid and protein content, the linkage to lipoproteins, and the formation of emulsions and foams with organic solvents. In these cases, acetonitrile is considered the best extractant because it precipitates proteins and denatures enzymes that could degrade drugs during the sample treatment.

Single-Class and Multiclass Multiresidue Methods

In contrast with the single-analyte methods, single-class multiresidue methods are designed to monitor the maximum number of analytes within a particular class with an acceptable recovery. Multiclass methods have the similar but more difficult goal of maximizing the number of analytes belonging to more than one pharmaceutical class. The chemical heterogeneity of drugs, their different physicochemical properties, and their occurrence at trace level in complex matrices make very difficult to find common extraction conditions. For this reason, multianalyte protocols include generic and nonselective sample-preparation procedures that have the advantage of reducing time, cost, and complexity of the pretreatment and achieving high sample throughput. Basically, the most suitable techniques for this purpose are solvent extraction (SE), SPE, and the QuEChERS methodology.

SE without further purification is a commonly adopted solution for multiresidue analyses: the solvent is selected to obtain the maximum yield of the target drugs and, at the same time, the minimum extraction on interfering compounds from the matrix. The procedures are easy, fast, cheap, and especially suitable for the simultaneous analysis of families of veterinary drugs; Table 2 illustrates 14 examples of such methods that have succeeded in isolating a huge number of compounds (up to 255) often identified via LC-MS. Nevertheless, the coextraction of interferences is responsible for high quantitation limits, low sensitivity, poor selectivity, and stress on analytic systems (chromatographic column and/or MS). The so-called 'dilute and shoot' approach, used in the strict sense of the term only for matrices such as urine and plasma, has been sometimes applied to food matrices before/after a SE step; for example, the dilution of the final extract and a reduced injection volume into the LC-MS/MS system allow controlling matrix effect and improving method performances. Interesting variants of SE, developed in the recent years to decrease organic solvent usage, succeed in isolating only a limited number of veterinary drugs. For example, ion-paired SE can be applied to extract penicillin antibiotics using tetrabutylammonium bromide as ion-pairing agent at pH 8 (fully deprotonation of the analytes) and a binary water–acetonitrile mixture as extractant; the addition of $(NH_4)_2SO_4$ between 16 and 43 wt% decreases the acetonitrile solubility in aqueous solution, inducing phase separation and quantitative recoveries. Supramolecular solvent-based extraction is another recent proposal that uses reverse micelles of decanoic (DeA) acid; this nanostructured liquid is produced by mixing DeA, tetrahydrofuran, and a hydrochloric acid aqueous solution in proper ratios at pH 4 (i.e., at pH values lower than the DeA pK_a) and is able to establish strong hydrogen bonds with sulfonamides, guarantying high yields. Dispersive liquid–liquid microextraction (DLLME) utilizes a mixture of a high-density solvent (extractant) and water-miscible polar solvent (disperser); if applied to extract veterinary drugs (e.g., NSAIDs), acetonitrile is usually chosen as disperser and chloroform as extractant. The rapid addition of the two solvents into an aqueous medium containing the sample generates fine droplets of the extractant and, therefore, a large contact surface with analytes. After centrifugation, the droplets settle at the bottom of the centrifuge tube, facilitating the recovery. The main disadvantage of the DLLME is related to the toxicity of chlorinated solvents, while simplicity, rapidity, low cost, and high enrichment factor are the main advantages.

Conventional SPE, used singly or in combination with other extractive techniques, is still the most common approach applied for the isolation of a large number of pharmacological

molecules from solid or liquid foods. Nevertheless, due to its intrinsic characteristics of selectivity, this technique is less suitable than SE for extended multiclass methods (see examples in Table 2), and the number of extracted veterinary drugs is around 50 at the most. According to the properties of the analytes and the sample composition, the most tested phases are

- classical reversed phases (C_4, C_8, C_{18}, phenyl, and polymeric sorbents),
- ion exchangers,
- mixed-mode cation-exchange sorbents,
- normal phases (alumina, silica, and amine-bounded silica).

In general, a preliminary deproteinization/extraction step with an organic solvent comes first the cleanup on SPE cartridges for protein-rich foods, while a defatting step with hexane can be performed for fatty foods before or during SPE. Another simple solution to remove lipids is the low-temperature centrifugation of an organic extract prior and after SPE. In analyzing honey, the high content of sugar, wax, and dyes has to be removed because it is responsible for severe signal suppression during LC-MS analysis. Moreover, sugars are a real problem for the analysis of sulfonamides and ronidazole (a nitroimidazole) since they combine in a complex with these antibacterials. SPE has proved to be very effective in removing all these interfering compounds and it has been sometimes used directly after a simple dilution of honey with water. Hitherto, most published analytic methods have involved off-line SPE, but lately, online SPE coupled to LC-MS has been proposed for the multiresidue determination of antibacterials in different kinds of foods (fish, milk, meat, and eggs): Using an automatized device (an example is the Symbiosis™ Pico online SPE system, Emmen, the Netherlands), the analytes are eluted from the cartridge to the chromatographic column in backflush mode, after the 'sample loading' and 'sample cleanup' steps, while a new cartridge is ready for another extraction.

In recent years, other modes to perform SPE have been designed to improve performances of this extractive technique. For example, the membrane-protected micro-SPE uses a porous polypropylene membrane, folded into a small envelope, that contains a small amount of polymeric sorbent. This membrane avoids the sorbent blockage during the manipulation of particularly 'dirty' samples. Another variant of SPE is the dispersive SPE (dSPE). This technique, based on the uniform dispersion of the sorbent in the sample solution/suspension, is applied for single-class and multiclass determinations, being successful in isolating more than 100 veterinary drugs (two examples in Table 2). Its remarkable development is the magnetic SPE (MSPE) that uses functionalized magnetic materials (FMMs) with high adsorption ability and superparamagnetism. In the food safety field, the most used FMMs are iron oxide microparticles or nanoparticles embedded with phenyl silica sorbent or silica coated with MAA-co-EGDMA (i.e., methacrylic acid-co-ethylene glycol dimethacrylate). In a typical experiment, these materials are first dispersed in a sample solution, incubated for an appropriate time to favor the analyte adsorption, and then isolated from the solution with a magnetic lure (no centrifugation or filtration steps). Compared with conventional SPE, both dSPE and MSPE take less time, require less labor, use lower amounts of solvent and sorbent, and avoid the cartridge blockage, channeling effects and breakthrough phenomena.

QuEChERS (quick, easy, cheap, effective, rugged, and safe) is an extractive methodology that has become popular for the multiresidue analysis of veterinary drugs in food products. Figure 1 schematically illustrates a typical experiment. Practically, the homogenized sample is placed in a centrifuge tube along with a high salt content (anhydrous $MgSO_4$ plus CH_3COONa or NaCl) and an organic solvent (usually acidic acetonitrile in the presence or absence of EDTA); afterward, the tube is capped, shaken vigorously, and centrifuged. In this way, the analytes are partitioned from the sample to the organic phase via salting out. An important parameter is the salt dosage: if it is too low, the phase separation is incomplete; if it is too high, the organic extractant is less efficient due to possible adsorption phenomena of analytes to the drying agent. This stage is sometimes followed by another one based on dSPE: a portion of the organic extract from the previous step is transferred to a tube containing a combination of $MgSO_4$ and different sorbents for cleaning up unwanted sample components. Silica-based sorbents functionalized with primary/secondary amino (PSA) or C_{18} groups are the most used, alone or in combination: PSA is efficient in the removal of organic acids, whereas C_{18} retains fats and hydrophobic compounds. The coined acronym explains perfectly all the advantages of this approach, which allows preparing many samples in a few minutes and extracting a large number of structurally different compounds with good efficiencies. Table 2 illustrates eight of such examples that applied QuEChERS-like strategies prior to screening and confirmatory analyses.

In the last 3 years, other techniques that have been used for an extensive multiresidual extraction of veterinary drugs are turbulent flow chromatography (TFC), matrix solid-phase dispersion (MSPD), and pressurized liquid extraction (PLE). Table 2 shows examples for all three techniques. TFC is a size exclusion-based technique, which allows injecting a liquid sample onto a column (0.5 or 1.0 mm of internal diameter) packed with large porous particles (30–60 μm diameter; 60 Å pore size) at flow rates of mobile phase higher than 1 ml min^{-1} to obtain a turbulent flow. Under these conditions, the small molecules of analytes diffuse into the particle pores of the stationary phase, whereas high-molecular-weight compounds from matrix (polysaccharides, proteins, and lipids) are quickly flowed to the waste. After this retention on the TFC column, the analytes are eluted toward the analytic column for the chromatographic separation. The main drawbacks of this technique are the low concentration capacity and the high solvent consumption, whereas its major advantage is the speed of the analysis, maintaining acceptable levels of recovery for a good number of veterinary drugs (up to 40) after a reduced sample manipulation (Table 2). In the case of solid or semisolid samples, such as tissues and eggs, the use of a PLE, a solid–liquid extraction that uses MSPD for the sample preparation and solvents at high temperature and pressure, is quite common. Automation, low solvent consumption, and short extraction times make it a feasible technique for high-throughput multiresidue extractions.

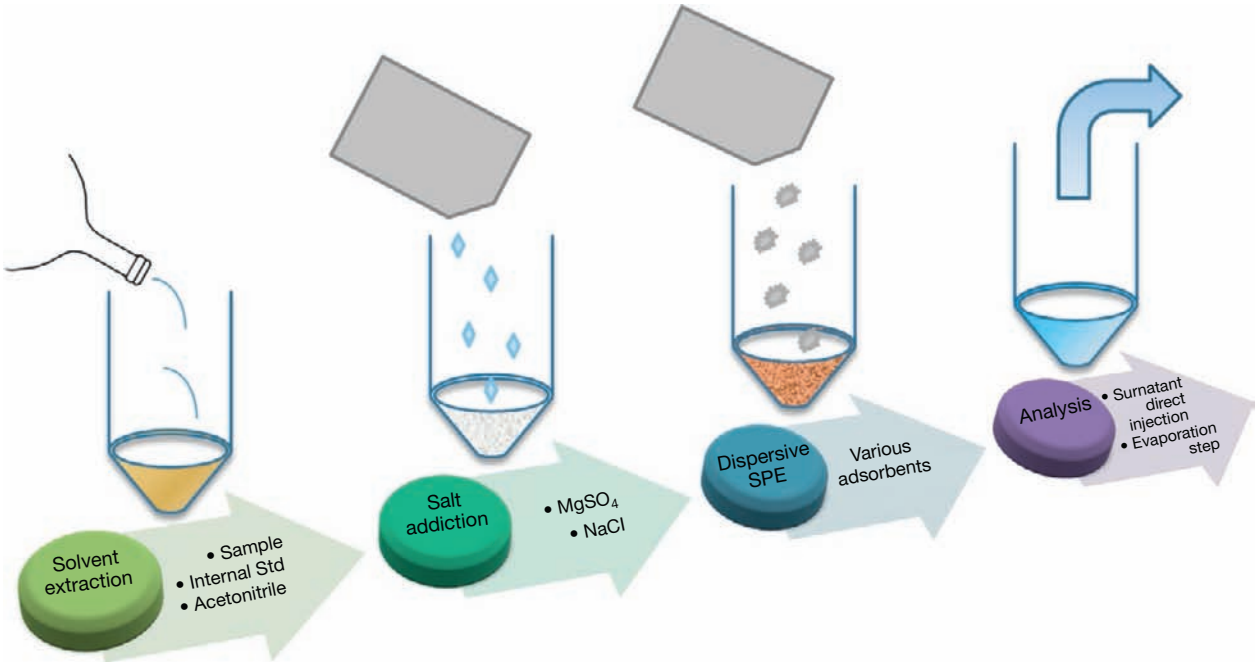

Figure 1 Graphic representation of a typical QuEChERS extraction. Usually, samples are extracted with acetonitrile using $MgSO_4$ and NaCl (to induce a better phase separation and analyte partition into organic extractant), while cleanup is conducted with dispersive SPE. Adsorbent choice depends on the matrix and on the analytes (i.e., C18, PSA, and polymeric). The supernatant is generally evaporated under nitrogen, filtered, and finally analyzed.

Screening Methods

Besides chromatographic techniques, the most used methods for screening veterinary drugs in foods relies on microbiological inhibition assay and immunoassay. For the latter, there have been rapid advances in the development of single tests that combine the usual advantages, concisely listed in Table 3, along with the main drawbacks, with the ability to detect multiple residues simultaneously within different food matrices.

Microbiological methods use susceptible microorganisms to detect antibiotics in foodstuffs. If the UV/Vis detection is applied and no residues are present in the food sample, the organic acids produced during the bacteria growth change the indicator color, permitting 'naked-eye' or photometric detection; on the other hand, if antibiotics occur over their limit of detection, either a zone of inhibition or no color change is observed. Regarding the assay format, microtiter plates and paper disks are the most used ones. Multiresidue analysis is possible using more than one plate, each seeded with a different organism. Due to cost-effectiveness and broad spectrum characteristics, they are preferred for large-scale monitoring programs and are the only rapid tests recognized by the EU for the screening of penicillins. However, long incubation times (18–24 h), short expiry dates, and difficulty in measuring the inhibitory halos are serious limitations for their use in routine analyses. In recent years, several in-house and commercial tests have been introduced and validated according to the European Decision 2002/657/EC.

Immunoassays are based on the binding properties of labeled antibodies with antigens and can be classified on the basis of the detection label. ELISA (enzyme-linked immunosorbent assay) use enzymes that, reacting with an appropriate substrate, produce a visible-sensitive chromogen. Even if ELISA usually focuses on the detection of a single analyte or a single group of analytes, multiclass immunoassay has been achieved preparing a bihapten antigen and a broad-specific antibody for simultaneous determination of penicillins and tetracyclines. Fluorescence immunoassay (FIA) and time-resolved fluorescence immunoassay (TR-FIA) are techniques that use fluorescent labels excited by the light of a suitable wavelength. Although their development has been incentivized by the necessity to overcome some drawbacks of ELISA (see Table 3), FIA is characterized by a high background fluorescence level that affects sensitivity. This problem is solved with TR-FIA that, relying on fluorophores (chelates of lanthanum, europium, etc.) with narrowband emission lines and long fluorescence half-lives, is able to reach a sensitivity superior to that of LC-MS in the screening of sulfonamides and tetracyclines.

Biosensors are analytic devices that combine a biological recognition element (enzyme, antibody, and receptor) with a transducer to produce a signal proportional to the extent of interaction between recognition element and analyte (i.e., to the analyte concentration). For the screening of drug residues in food, immunosensors with electrochemical or optical transducer systems are the most commonly used systems. Particularly, surface plasmon resonance immunosensors are able to detect fluoroquinolones, sulfonamides, and phenicols without the need for labels, allowing the monitoring of the biological interactions in real time. Functionalized magnetic beads (MBs) and screen-printed carbon electrodes constitute an attractive platform to perform immunoreactions and electrochemical detection, respectively. In particular, MBs minimize matrix effect, allow fast assay kinetics and, just after a simple dilution

Table 3 Main screening techniques and their features

	Advantages	*Drawbacks*
Microbiological methods		
– Tube tests, single or multiplates assay/diameter of inhibition zones, color change or UV/Vis detection	– Cost-effectiveness – Entire antibiotic spectrum within one test	– Lack of specificity – Long incubation periods – False-positive results
Immunologic methods		
– Enzyme-linked immunosorbent assay (ELISA) – Fluorescence immunoassays (FIAs) – Time-resolved fluorescence immunoassays (TR-FIA)	– Easy to use – Available kits for families of compound – Large number of samples per kit – Reduced analysis time (2–2.5 h) – Sensitivity – Specificity – Possibility of automation and to use within the food-processing facility	– Interferences giving some false-positives – Semiquantitative analysis – Lack of stability – Lengthy development time required for immunoreagent preparation – High background (ELISA and FIA) – Large number of incubation and washing steps (ELISA)
Biosensors	– Easy to use – Results available in short time – Multiple residues analyzed in one shot – Full automatization – High throughput	– High costs – Instability of biological-sensing component
HPLC or UPLC-MS methods		
HPLC/UPLC-triple quadrupole (HPLC/UPLC-QqQ) HPLC/UPLC-time of flight (HPLC/UPLC-ToF) HPLC/UPLC-Orbitrap	– Identification – Short run time – High selectivity and sensitivity – High throughput	– Expertise required – Need of sample preparation – High initial investment and operative costs – Time-consuming

Boove, T. F. H. and Pikkemaat, M. G. (2009). Bioactivity-based screening of antibiotics and hormones. *Journal of Chromatography A* **1216**, 8035–8050.
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Toldrá, F. and Reig, M. (2006). Methods for rapid detection of chemical and veterinary drug residues in animal foods. *Trends in Food Science & Technology* **17**, 482–489.

of milk samples, are able to detect selectively tetracyclines and sulfonamides.

Molecularly imprinted polymers (MIPs) are materials obtained by polymerizing functional and cross-linking monomers around a template molecule, which is subsequently removed, leaving a cavity complementary to the target compound. For their recognition ability, they are compared to synthetic antibodies, but without sharing their storage and stability problems. Recently, MBs were used to construct MIP-based electrochemical sensors whose operating principle is concisely explained in Figure 2. These devices can be considered as the next-generation sensors due to the high sensitivity, simple instrumentation, and excellent compatibility with miniaturization techniques; moreover, they can analyze complex samples without any pretreatment and be easily extended to detect other interest molecules by controlling the imprinted target molecules.

Due to their versatility, chromatographic techniques are the most suitable to perform large-scale screening. In particular, the hyphenation, which is continuing to gain ground, is LC coupled to low- or high-resolution MS (more than 100 residues detected in a single chromatographic run). Depending on the objective of the analysis and the available analyzer, most of the published papers deal with multiclass methods based on three different approaches: pretarget screening, posttarget screening, and nontarget screening. Table 2 reports some examples of each approach.

Pretarget screening methods use authentic standards for the ion current preselection and low-resolution (LR) mass spectrometers, such as triple quadrupole (QqQ) operating in neutral-loss scan (NLS), parent-ion scan (PIS), or multiple reaction monitoring (MRM). The MRM scan mode allows the identification of target drugs, whereas NLS and PIS modes can screen a family of compounds.

An attractive alternative is the use of full-scan MS techniques, relying on high-resolution (HR) mass spectrometers such as time of flight (TOF) or Orbitrap. These analyzers allow performing the other two screening modes; moreover, the highest resolving power of Orbitrap (up to 100 000 full width at half maximum (FWHM)) is important to avoid both false-positives and false-negatives. The posttarget screening approach provides that the m/z values of the target analytes are extracted from the full-scan total ion current (TIC) chromatogram after its acquisition using a narrow mass window (± 10 mDa); the identification is based on the retention time window and the measurement of the accurate mass (<5 ppm for Orbitrap and 5–20 ppm for TOF) and the isotopic pattern. This procedure is advantageous because it permits a retrospective data analysis, increasing the number of drugs and/or metabolites determined in a single chromatographic run.

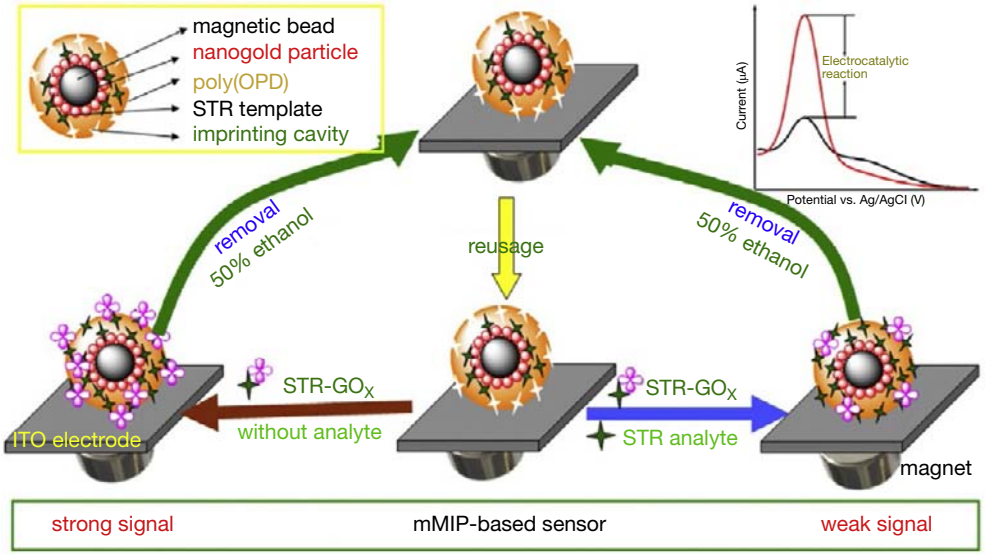

Figure 2 Schematic illustration of a novel sensor, based on redox-active magnetic molecularly imprinted polymer (mMIP) nanospheres. Nanospheres are composed of a nanogold-encapsulated poly(*o*-phenylenediamine) shell on magnetic iron oxide cores. The electrochemical detection of streptomycin residues (STR) is allowed by coupling with bioelectrocatalytic reaction of glucose oxidase (GOx) for signal amplification. When STR occurs in a food sample, the analyte competes with GOx-labeled STR (GOx-STR) for molecular imprints on the mMIP nanospheres. Thence, the conjugation amount of GOx-STR on the mMIP nanospheres decreases, leading to the change in the bioelectrocatalytic current. Reproduced from Liu, Tang, Zhang, et al. (2013). Au(III)-promoted magnetic molecularly imprinted polymer nanospheres for electrochemical determination of streptomycin residues in food. *Biosensors and Bioelectronics* **41**, 551–556, with permission from Elsevier.

Methods utilizing hybrid mass spectrometer, such as QqTOF, can collect product ion data that support the identification of the detected compounds. Nontarget screening methods evaluate the occurrence of residues after the acquisition of the full-scan TIC chromatogram by searching the detected drugs in homemade spectral libraries; here, the analyst tries to identify unknown compounds without having previous information.

Confirmatory Methods

Commission Decision 2002/657/EC has clearly established analytic techniques, requirements, and criteria to perform confirmatory analyses of organic residues in foods. With regard to this, only the chromatographic detection systems that provide structural information can be used, on their own or in combination, for the unequivocal identification of the target contaminants. Nevertheless, with the exception of MS, all the other techniques (UV/visible, fluorescence, etc.) can be applied only for the confirmation of group B substances.

Most of the current publications dealing with this matter are still based on QqQ running in MRM (see Table 2); in these cases, because the analyzer does not record a full-mass spectrum, a system of identification points (IPs) is adopted to support the analyte identification: two MRM transitions allow earning 4 IPs (1 IP for the pseudomolecular ion and 1.5 IPs for each product ion), which are enough to confirm both group B substances (IPs $\geq$ 3) and group A substances (IPs $\geq$ 4). Notwithstanding the high selectivity of this scan mode, the unit resolution of the quadrupole cannot distinguish between the target analytes and coeluting isobaric interferences, giving rise to potential false identifications and/or quantitation. This possibility can be verified by matching the ion ratio of the two selected MRM transitions with the average values obtained for the reference standard in solvent; if the problem is proved, it can be easily solved, improving the chromatographic efficiency in order to separate the intrusive peaks from those of the analytes. Another solution is to use a highly selective MRM acquisition mode allowed by an enhanced resolution QqQ: the Q1 analyzer can be set in enhanced resolution (0.1 Da at FWHM) and the Q3 analyzer in unit resolution (0.7 Da at FWHM) maintaining a good sensitivity. After years characterized by a constant focus on typical acquisition modes of LRMS, the use of HRMS is emerging for some figures of merit such as resolution, accuracy, and duty cycle. Nevertheless, as mentioned in the previous paragraph, LC-HRMS is mainly applied for screening purposes, while it could be a valuable alternative to MRM-based confirmation analysis for those contaminants that, under collision-induced dissociation, generate only one diagnostic fragment ion; as a matter of fact, the Commission Decision 2002/657/EC states that one precursor ion and one fragment ion, produced by a mass spectrometer with a resolution of 10 000 FWHM, generate a number of 4.5 IPs (2 + 2.5 IPs) enough to confirm permitted and forbidden substances. An HR instrument suitable to this end is QqTOF but, if the HR instrumentation allows using only the pseudomolecular ion, a resolution of 50 000 FWHM and a corresponding narrow window width are needed to obtain a selectivity comparable to tandem MS. Figure 3 is explanatory in this sense. A performance comparison of QqQ, TOF, and Orbitrap has demonstrated that the latter can discriminate isobaric interferences better than TOF (12 000 FWHM, but QqTOF can reach 50 000) due to its superior mass resolution. Nevertheless, a

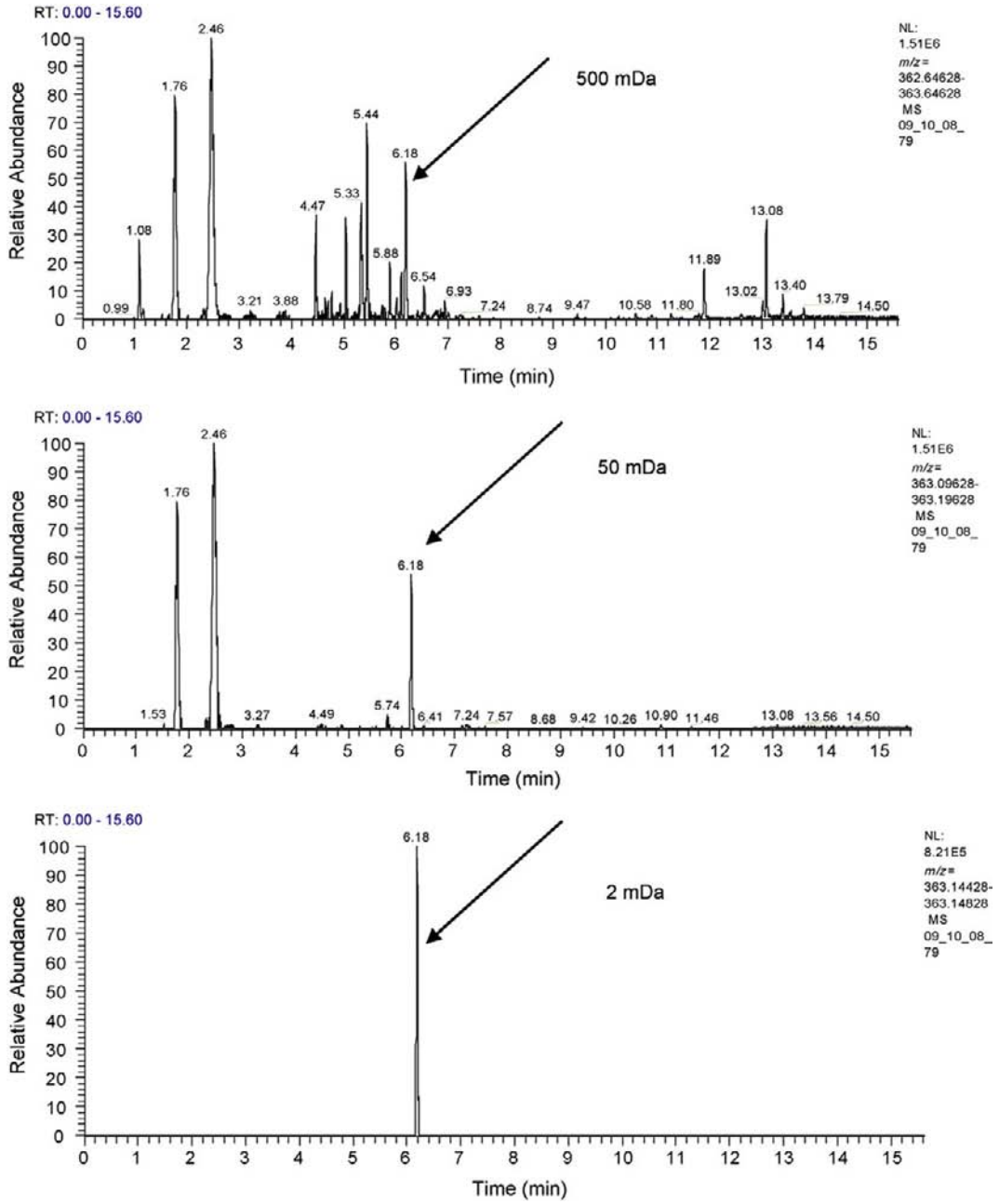

Figure 3 Effect of mass window width on selectivity of a high-resolution mass spectrometer (Orbitrap operated at a resolution of 10 000 FWHM). The example is related to the detection of the pseudomolecular ion $[M+H]^+$ of marbofloxacin at m/ 363.14628 in a liver extract (50 g l^{-1} fortification level). Several intrusive peaks from matrix are detected with a 500 mDa mass window (corresponding to unit resolution). Selectivity increases notably when the mass window is narrowed down to 2 mDa and only the analyte peak is visible. Reproduced from Kaufmann, Butcher, Maden, Walker and Widmer (2010). Comprehensive comparison of liquid chromatography selectivity as provided by two types of liquid chromatography detectors (high resolution mass spectrometry and tandem mass spectrometry): "Where is the crossover point?" *Analytica Chimica Acta* 673, 60–72, with permission from Elsevier.

cumbersome aspect of the single-stage Orbitrap is related to a phenomenon of postinterface signal suppression that affects sensitivity in detecting small-molecular-weight compounds. This event occurs when dirty extracts from protein-rich food are injected into the LC-MS system, causing entrapment of multiple-charged proteins in the C-trap (a focusing device analogous to a linear ion trap) and losses of low m/z ions.

Conclusions and Future Trends

Veterinary drugs include both registered drugs (groups B1 and B2) and banned substances (group A). Owing to the vast range of compounds, routine monitoring tasks have become time- and cost-demanding, accelerating the trend toward the simultaneous analysis of different families of veterinary drugs irreversibly. For

group B substances, the screening with inexpensive microbiological tests or portable biosensors competes with sophisticated and high-priced UHPLC-HRMS systems, which can analyze up to 300–500 substances in one chromatographic run of c. 5 min. It is exactly within this context that the scientific community is continuing to invest its efforts. On the one hand, with the aid of nanotechnology have been designed miniaturized and/or automatized biosensors with enhanced functionality and economic attractiveness. On the other hand, the superior performances of the latest generation mass spectrometers combined with a reduced sample preparation have allowed high-throughput analyses. If LC-MRM is still the gold standard method for confirming drug residues in food, flexible open strategies relying on full-scan HRMS techniques are expected to dominate in the near future. Parallel to the evolution of these approaches (i.e., targeted and untargeted LC-MS methods), there are two opposite trends related to the sample preparation. The first concerns the ongoing development of new nonselective treatments to maximize the number of compounds screened via LC-HRMS. Obviously, these procedures are less suitable for confirmatory methods since a severe matrix effect compromises detection limits, sensitivity, selectivity, and maintenance frequency. In this case, a more extensive purification is demanded to obtain a reliable quantification and, to this end, constant energies are channeled into the development of new sorbent materials and automatic devices for speeding up also routine confirmatory analysis.

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Antinutritional Factors in Legume Seeds: Characteristics and Determination

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Introduction

Plants commonly synthesize a range of secondary metabolites as part of their protection against attack by herbivores, insects, and pathogens or as a means to survive in adverse growing conditions. If farm or domestic animals or humans consume these plants, these compounds may cause adverse physiological effects. The terms antinutrient or natural toxicant have been widely employed to describe plant-defense metabolites in the food and nutrition literature. Legumes are a rich source of antinutrients in the human diet. In food legumes, the presence of some well-defined antinutritional factors has been documented. Antinutritional factors like protease inhibitors, amylase inhibitors, lectins, goitrogens, and antivitamin factors constitute heat-labile antinutritional factors, whereas nonprotein amino acids, alkaloids, cyanogenic glycosides, tannins, saponins, flavones, isoflavones, pyrimidine glycosides, and flatus-producing oligosaccharides form the heat-stable antinutritional factors. Some of the antinutritional factors are discussed below.

Protease Inhibitors

Protease inhibitors isolated from soybean fall into two main categories: the Kunitz inhibitor and the Bowman–Birk inhibitor. The Kunitz inhibitors have a molecular weight of about 21.5 KDa with two disulphide bridges and possess a specificity directed mainly against trypsin. The Bowman–Birk inhibitors have a molecular weight of about 8 kDa with a high proportion of disulphide bonds and the capability of inhibiting chymotrypsin and trypsin at independent binding sites.

The Kunitz inhibitor consists of 181 amino acid residues, with the reactive site (site directly involved in its interaction with trypsin) located at residues Arg 63 and Ile 64. This molecule combines with trypsin in a stoichiometric fashion; that is, one molecule of the inhibitor inactivates one molecule of trypsin. The complex that forms is analogous to an enzyme–substrate complex that, unlike the usual enzyme–substrate complex, is very unstable. The amino acid sequence of Bowman–Birk has two independent binding sites: a trypsin-reactive site (Lys 16 and Ser 17) and a chymotrypsin-reactive site (Leu 43 and Ser 44). In contrast to the Kunitz inhibitors, the Bowman–Birk inhibitors are very rich in disulphide bonds, possessing seven disulphide bridges. This feature is responsible for the very tight, compact, three-dimensional structure revealed by X-ray crystallography and nuclear magnetic resonance. The sequence of amino acids surrounding these two reactive sites are remarkably similar to each other, and a high degree of homology has been found between the Bowman–Birk inhibitor and a number of other low-molecular weight inhibitors that have been isolated from other legumes. In common beans, lima beans, cow peas, and lentils, protease inhibitors have been characterized as members of the Bowman–Birk family. Trypsin/chymotrypsin inhibitors from red kidney bean, Brazilian pink bean, lima bean, and soybean are closely related with high homology. Trypsin inhibitor content of some of the common legumes and underutilized legume seeds have been determined and tabulated (Table 1).

Nutritional Significance

Purified fractions from soybean, which are enriched with trypsin inhibitor activity, are in fact capable of inhibiting the growth of rats, chicks, and mice, an effect that is generally accompanied by a depression in the digestibility of protein in the diet. Furthermore, feeding rats with a raw soybean extract from which the trypsin inhibitors have been removed by affinity chromatography on immobilized trypsin showed improved growth performance when compared with control rats fed diets containing raw soybeans from which the trypsin inhibitors have not been removed. The simplest explanation for the growth inhibition produced by the trypsin inhibitor would be the fact that it interferes with the digestibility of dietary protein. The presence of protease inhibitors such as trypsin and chymotrypsin inhibitors in the diet leads to the formation of irreversible trypsin enzyme–trypsin inhibitor complexes, causing a decrease in trypsin in the intestine and a decrease in the digestibility of dietary protein, thus leading to slower animal growth. As a result, the secreting activity of the pancreas increases, which could cause pancreatic hypertrophy and hyperplasia. Although there is limited information on their specific effects in humans, it is generally considered prudent to remove these protease inhibitors from food prior to consumption. A major beneficial effect of cooking and autoclaving of legume grains is the destruction of protease inhibitors. Significant reduction of trypsin inhibitor (92–99%) content has been noticed after cooking/autoclaving of both raw and presoaked seed samples. Dry-heat treatment also reduces trypsin inhibitor activity (93%). Boiling, however, may be insufficient to deactivate these substances fully. So dry heat (roasting, toasting) is the preferred method for processing.

α-Amylase Inhibitors

α-Amylase inhibitors have been purified and partially characterized from different varieties of common beans, including white kidney beans, red kidney beans, and black kidney beans. The content of α-amylase inhibitors differs greatly among legumes, with the highest amounts found in dry beans. α-amylase inhibitors were found in common beans and runner beans (*Phaseolus coccineus*) at levels of 2–4 kg^{-1} of seed meal. Field beans, black-eyed peas, and chickpeas contain low levels of 0.1–0.2 kg^{-1} of seed meal. In lentils, soybeans, peas, winged beans, lima beans, and adzuki beans, α-amylase inhibitor

Table 1 Trypsin inhibitor, cyanogen, total free phenolic, and tannin contents of some common and underutilized legume seeds

Name of legume seeds	*Trypsin inhibitors (TIU mg^{-1} protein)*	*Cyanogen (mg $100g^{-1}$)*	*Total free phenolics (g $100g^{-1}$)*	*Tannins (g $100g^{-1}$)*
Cajanus cajan	4.13–4.75	0.5	0.35	0.23–1.4
Cicer arietinum	11.90	0.8	0.27	0.18
Cicer arietinum cv.Amits			0.14	
Dolichos lablab	2.11			
Dolichos lablab var. *vulgaris*	29.28–31.24	0.24–0.31	0.52–0.61	0.17–0.19
Glycine max	34.7–122.6			
Phaseolus aureus	15.8		0.51	0.39
Phaseolus lunatus	2.11	210.0–312.0		
Phaseolus vulgaris Roba variety	4.59			
Phaseolus vulgaris		2.2		
Phaseolus vulgaris (White beans)				1.7
Phaseolus vulgaris (Black beans)				2.1
Pisum sativum		2.3		
Pisum sativum cv. *Stratus*			0.12	
Pisum sativum cv. *Golden*			0.13	
Vicia faba	1.72–3.35			
Vigna sinensis		2.1		
Abrus precatorius	34.16–35.30	0.09–0.12	0.76–0.84	0.54–0.61
Atylosia scarabaeoides		0.14	0.28	0.54
Canavalia gladiata	25.54	0.31	1.21	0.41
Dolichos biflorus				0.15
Dolichos trilobus		0.20	1.34	0.58
Entada rheedi		0.33	3.13	0.63
Lablab purpureus			0.26	0.40
Lablab purpureus var. *lignosus*		0.46	0.26	0.40
Lens culinaris cv CDC *Richles*			0.73	
Lens esculenta			0.32	0.23
Erythrina indica	36.24	0.09	0.94	0.36
Mucuna atropurpurea	39.24–44.10	0.21–0.33	2.62–3.50	0.18–0.34
Mucuna monosperma			0.85	0.06
Mucuna pruriens var. pruriens	40.40–48.39	0.26–0.34	4.73–5.47	0.23–0.33
Mucuna pruriens var. *utilis* (Black colored seed coat)	43.20–43.70	0.21–0.24	4.06–4.24	0.18–0.31
Mucuna pruriens var. *utilis* (White colored seed coat)	44.26–46.30	0.16–0.24	3.68–3.98	0.14–0.21
M. deeringiana	46.16	0.18	2.74	0.16
Neonotonia wightii var. *coimbatorensis*		0.18	1.03	0.12
Rhynchosia cana	12.34–15.36	0.22–0.27	1.10–1.60	0.43–0.78
Rhynchosia filipes	19.39	0.22–0.25	1.98	0.66
Rhynchosia rufescens	15.48	0.30	1.88	0.56
Rhynchosia suaveolens	16.26	0.29	1.88	0.48
Tamarindus indica			3.76	0.03
Teramnus labilis			2.01	0.21
Vigna aconitifolia	28.30–31.36	0.26–0.32	1.02–1.46	0.48–0.65
Vigna bourneae	21.60	0.14	1.21	0.36
Vigna radiata subsp. sublobata	24.48	0.24	1.07–1.38	0.19–0.31
Vigna trilobata	23.19	0.18	0.78	0.23
Vigna umbellata	34.30	0.09	0.58	0.24
Vigna unguiculata subsp. *cylindrica*	26.40	0.27	0.83–2.77	0.27
Vigna unguiculata subsp. *unguiculata*	24.28–25.36	0.26–0.28	1.09–1.24	0.34–0.36
Vigna vexillata	33.20	0.12	0.98	0.19

Sangronis, E and Machado, C. J. (2007). Influence of germination on the nutritional quality of *Phaseolus vulgaris* and *Cajanus cajan*. *LWT Food Science and Technology* **40**, 116–120; Alajaji, S. A. and El-Adawy, T. A. (2006). Nutritional composition of chickpea (*Cicer arietinum* L.) as affected by microwave cooking and other traditional cooking methods. *Journal of Food Composition and Analysis* **19**, 806–812; Alector, V. A and Aladetimi, O. D. (1989). Compositional evaluation of some cowpea varieties and some under-utilized edible legumes in Nigeria. *Nahrung* **33**, 999–1007; Kalpana Devi, V. and Mohan, V. R. (2013). Nutritional and antinutritional assessment of underutilized legume *Dolichos lablab* var. *vulgaris*. *Bangladesh Journal of Industrial and Scientific Research* **48**, 119–130; Anderson, R. L. and Wolf, W. J. (1995). Compositional changes in trypsin inhibitors, phytic acid, saponins andisoflavones related to soybean processing. *Journal of Nutrition* **125**, 581S–585S; Mubarak, A. E. (2005). Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. *Food Chemistry* **89**, 489–495; Shimelis, E. A. and Rakshit, S. K. (2007). Effect of processing on antinutrients and *in vitro* protein digestibility of kidney bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. *Food Chemistry* **103**, 161–172; Makkar, H. P. S., Becker, K., Abel, H. and Pawelzik, E. (1997). Nutrient contents, rumen protein digestibility and antinutritional factors in some colour and white flowering cultivars of *Vicia faba* beans. *Journal of Science Food and Agriculture* **75**, 511–520; Tresina P. S. and Mohan, V. R. (2012). Comparative assessment on the nutritional and antinutritional attributes of the underutilized legumes,

activity was undetected. The inhibitor blocks the function of mammalian salivary and pancreatic amylases but not plant, fungal, or bacterial α-amylases.

Lectins

Lectins are proteins or glycoproteins that are widely distributed in the plant kingdom and have the unique property of binding to carbohydrate-containing molecules, with a high degree of specificity toward the sugar component. Lectins are able to agglutinate the red blood cells from various species of animals *in vitro*, and depend on their specificity and high binding affinity for a particular carbohydrate moiety on the cell surface. In 1989, Shillmark was the first to observe that a protein fraction of the castor bean, *Ricinus communis*, which he called *ricin*, was capable of agglutinating red blood cells, a property that led to the term phytohemagglutinin (PHA). In 1908, Landsteiner and Raubitcheck showed for the first time that even the seeds of the edible species of some common legumes such as navy beans, lentils, and garden peas also contain these so-called PHAs. One extremely interesting observation was the fact that the relative hemagglutinating activities of various seed extracts were quite different when tested with the erythrocytes from different animals.

In addition to being able to agglutinate red blood cells, the lectins exhibit a variety of other interesting and unusual biological properties, including interaction with specific blood group substances, mitogenesis promotion of cell adhesion, inhibition of fungal growth and an insulin-like effect on fat cells. Lectins are hardy proteins that do not break down easily. They are resistant to stomach acid and digestive enzymes. Lectins may bind to the gut wall and damage the gut lining, are not altered by digestive enzymes, and may alter gut permeability and pass through the gut into general circulation. Lectins can cause alterations in gut function that may be related to colitis, Crohn's disease, celiac sprue, irritable bowel syndrome (IBS), and gut permeability.

Hemagglutinating activity has been detected in more than 800 different plant species, of which more than 600 are in the family Leguminosae. Most lectins appear to have molecular weights ranging from 100 000 to 150 000 and are usually composed of tetramers that may or may not be identical. A smaller number of lectins, such as those isolated from the lentil and lima bean, appear to be dimmers, having about one-half of the molecular weight of the lectins. All lectins bind one transition ion, usually manganese, and one calcium ion.

Amounts of lectin in legumes vary significantly. Lectins account for about 2.4–5% of the total protein (17–23%) in kidney bean seeds, 0.8% in soybean and lima bean protein (34% and 21%, respectively), and around 0.6% of the total protein (24–25%) in garden peas.

Nutritional Significance

Some legume and cereal lectins can inhibit the growth of experimental animals and reduce the digestibility and biological value of dietary proteins. The antinutritional effects are most likely caused by some lectins that can impair the integrity of the intestinal epithelium and alter the absorption and utilization of nutrients. The lectins from the field bean (*Dolichos lablab*) are toxic when injected into rats; when fed at a level of 2.5% of the diet, they inhibit the growth of rats and cause zonal necrosis of the liver. Thus, dietary lectins have generally been considered toxic and antinutritional factors. However, many lectins are nontoxic, such as those from lentils, peas, chickpeas, and faba beans.

Several studies have suggested a strong correlation between certain lectin-binding patterns and their biological behavior in various tumors. *Vicia faba* agglutinin, a lectin present in broad beans, stimulated the morphological differentiation and reduced the malignant-phenotype colon-cancer cells. The inclusion in the diet of PHA, a lectin present in raw kidney bean, greatly reduced the growth of a murine non-Hodgkin's lymphoma tumor in the mouse. The reduced growth rate occurred in a dose-dependent manner. Lectin is also being used for the discovery of protein markers of cancer using a natural glycoprotein microarray approach. Multiple lectins can screen serum samples from patients with pancreatic cancer or pancreatitis by selective detection of glycan structures.

Inclusion of raw kidney beans in the diet of obese rats reduced lipid accumulation that was related to a decrease of insulin levels caused by lectins. However, no loss of muscle protein occurred even at high doses, as with normal rats, suggesting a possible use of lectins as therapeutic agents to

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treat obesity. The most recent EUREKA study showed that red kidney bean lectin given as an additive to 11–12-day-old piglets greatly enhanced successful weaning at 28 days. This result was achieved by stimulating the digestive tract, thereby accelerating the production of mature intestinal cells.

Lectin can be completely removed from lentil flour after 72 h of fermentation at 42 °C with a flour concentration of 76 gl^{-1}. Cooking effectively removes lectin levels of vegetable peas and significantly reduces protein and amino acid solubility.

Goitrogens

Unheated soybeans have been reported to cause marked enlargement of the thyroid gland of the rat and chick, an effect that could be counteracted by the administration of iodine or partially eliminated by heat treatment. A number of cases of goiter have also been reported in human infants fed soybean milk, a condition that could likewise be eliminated by iodine supplementation. The soybean component responsible for the goitrogenic effect of soybeans is still not known for certain. The factor responsible for goitrogenicity observed in these studies was not identified. On the other hand, the goitrogenic principle has been reported to be a low-molecular-weight oligopeptide. It has also been reported that part of the goitrogenic effect of soybeans can be attributed to soyasapogenol. A soy saponin composition provides high bodily absorption characteristics. In an embodiment, a soyasapogenol composition comprises soyasapogenol B and 3-*O*-D-glucuronopyranosyl soyasapogenol B. Some researchers, however, believe that goiter may result from an increased fecal loss of thyroxine or a simple iodine deficiency. One report showed that female rats that had been fed defatted soybeans with an iodine-free diet for 6–12 months developed thyroid carcinomas. This effect was completely prevented by iodine supplementation.

Rats fed with peanuts also develop enlarged thyroids, but in this instance, the goitrogenic principle has been identified as a phenolic glycoside that resides in the skin. It was suggested that the phenolic metabolites formed from this glycoside are preferentially iodinated and thereby deprive the thyroid of available iodine. Thus, the goitrogenicity of peanuts is effectively prevented by iodine supplementation but not by heat treatment.

Cyanogens

A number of plants are potentially toxic because they contain glycosides from which hydrogen cyanide (HCN) may be released by hydrolysis. As shown in Table 1, the *Phaseolus lunatus* (lima bean) has higher cyanide-producing potential than that of cassava, which is well known as a source of cyanide. In order to be considered acceptable for human consumption, the cyanide concentration of lima beans should fall within the range of 10–20 mg/100 g. The cyanide content of most other legumes is of such low levels that it does not constitute a risk to human health.

Cyanide in the form of HCN is released from the glycoside of lima beans (phaseolunatin, also known as linamarin) through the sequential action of two enzymes, a β-glucosidase and oxynitrilase. Hydrolysis then proceeds quite rapidly during the early stages of the cooking process, but much of the HCN is subsequently lost by volatilization.

Total Free Phenolics and Tannins

Plants produce an amazing array of phenolic compounds such as free phenolics and tannins, which have the potential to react with proteins and other cytoplasmic components. In some cases, the subsequent reaction between phenols and proteins is highly appreciated because it adds flavor, taste, and appearance to products such as tea, coffee, beer, and tobacco. However, from a nutritional point of view, the main concern with phenols in dietary proteins is the way in which they decrease their digestibility and nutritive value.

Phenolic compounds decrease the digestibility of proteins and carbohydrates and the availability of vitamins and minerals. They lower the activity of digestive enzymes such as α-amylase, trypsin, chymotrypsin, and lipase, and may cause damage to the mucosa of digestive tract and also reduce the absorption of nutrients such as vitamin B_{12}.

The negative nutritional effects of the tannins are diverse and incompletely understood, but the major effect is to cause growth depression by decreasing the digestibility of proteins and carbohydrates. This is most likely the consequence of the interaction of tannins with either protein or starch to form enzyme-resistant substrates. Interaction with the enzymes themselves may also lead to an interference with the digestibility of these substrates.

The total phenolic and tannin content of commonly consumed pulses and underutilized legumes is presented in Table 1. Phenolics and tannins are water-soluble compounds and, moreover, are concentrated in the seed coat. They can be eliminated by common processing methods such as decortication, soaking and heat treatment, or the cooking process.

The potential health benefits of common beans are attributed to the presence of secondary metabolites such as phenolic compounds that possess antioxidant properties. Pulses with the highest total phenolic content (lentil, red kidney, and black beans) exert the highest antioxidant capacity assessed by 2,2, diphenyl-1-picrylhydrazyl free radical scavenging, ferric-reducing antioxidant power, and oxygen radical absorbance capacity. Phenolics have been suggested to exhibit health-related functional properties such as anticarcinogenic, antiviral, antimicrobial, anti-inflammatory, hypotensive, and antioxidant activity.

Phytic Acid

Phytic acid (myoinositol hexaphosphate or $InsP_6$) is a major phosphorus storage form in plants, and its salts, known as phytates, regulate various cellular functions such as DNA repair, chromatin remodeling, endocytosis, nuclear messenger RNA export, and potential hormone signaling important for plant and seed development. It is often regarded as an antinutrient because of its strong mineral, protein, and starch binding properties, thereby decreasing their bioavailability. Although

the ability of phytate to influence the availability of minerals no doubt accounts for its major antinutritional effect, it is also true that phytate strongly interacts with the basic residues of proteins. As a consequence of this nonselective binding to proteins, phytate has been shown to inhibit the action of a number of enzymes important in digestion, including pepsin, trypsin, and alpha amylase. Though phytate inhibits the digestion of protein when studied *in vitro*, binding of phytate to proteins may be direct (phytate: protein) or indirect (through a cation bridge). These complex interactions vary with pH, time, and relative concentrations. At low pH (e.g., in the stomach), phytic acid forms electrostatic linkages with the basic arginine, lysine, and histidine residues, resulting in insoluble complexes.

In vivo and *in vitro* studies have demonstrated that phytic acid exhibits significant anticancer (preventive as well as therapeutic) properties. It reduces cell proliferation and increases differentiation of malignant cells, with possible reversion to the normal phenotype, and is involved in host defense mechanisms and tumor abrogation. Phytic acid delays postprandial glucose absorption, reduces the bioavailability of toxic heavy metals such as cadmium and lead, and exhibits antioxidant activity by chelating iron and copper. Dietary and endogenous phytic acid has protective effects against cancer and heart disease and may be responsible for the cancer-protective effects of high-fiber foods. The anticarcinogenic properties of phytic acid may result from numerous factors, including its ability to chelate metal ions; this depends on the phytate retaining its integrity in the colon, a profuse microbial ecosystem.

The phytic acid content of some common legumes and underutilized legume seeds is shown in Table 2. Hydration-induced reduction in phytate content in legumes may be attributed to the activity of phytase and diffusion of the products. An increase in phytase activity with a decrease in the level of phytate as a result of hydration in faba beans has been reported. Previous reports on the effects of germination on phytic acid in beans indicated that as a result of increased enzyme activity, 20–70% or more of the phytic acid is hydrolyzed during germination, depending on the type of bean and the increase in phytase activity. The reduction of phytic acid indicated that an increase in hydrolysis of phytates during germination led to the liberation of inorganic phosphates for plant growth from an organic phosphorus-containing compound (phytate). Because phytic acid has been considered one of the factors responsible for reducing mineral availability, the reduction during germination may have enhanced the nutritional quality of beans.

Saponins

Saponins comprise a large family of structurally related compounds containing a steroid or triterpenoid aglycone (sapogenin) linked to one or more oligosaccharide moieties. They are characterized by their hemolytic activity and foaming properties and are responsible for imparting a bitter taste and astringency to plant materials containing a high concentration of saponins. Saponins are very poorly absorbed. Most of the saponins form insoluble complexes with 3-*b*-hydroxysteroids and are known to interact and form large mixed micelles with bile acids and cholesterol. Although saponins were shown to lower cholesterol in some animal species, the hypocholesterolemic effects of saponins in humans are more speculative. In addition, they form insoluble saponin–mineral complexes with iron, zinc, and calcium. The most common saponins in legumes include the soyasaponins, which are classified into group A, B, and E saponins on the basis of the chemical structure of the aglycone. Field pea extracts were initially thought to contain soyasaponin I (S-I) and then soyasaponin VI (S-VI) as the only soyasaponin, but recently field pea extracts were shown to contain dehydrosoyasaponin I (D-I) minor component. This triterpenoid saponin dehydrosoyasaponin I is a natural product present in chickpeas and other legumes, and is known to be a potent calcium-activated potassium channel opener, and as such can be used for treating cardiovascular, urological, respiratory, neurological, and other disorders.

Saponins have been reported in many edible legumes. The major sources of dietary saponins are legumes, and many types of saponins can be present in the same bean. Saponin content may vary even among the same species of edible legumes (Table 3).

Recent *in vivo* studies have established that the health benefits of legume saponins are several, such as the hypocholesterolemic effect and anticarcinogenic, antioxidative, antitumor, antivirus, antihepatic, antidiabetic, and hepatoprotective properties; they can also be beneficial for hyperlipidemia. In addition, they reduce the risk of heart disease in humans who consume a diet rich in food legumes containing saponins. Epidemiological studies suggest that saponin may play a major role in protection from cancer. Research on colon cancer cells suggests that it is the lipophilic saponin cores that may be responsible for the biological activity.

Oxalate

Oxalate salts are poorly soluble at intestinal pH, and oxalate is known to decrease calcium absorption in monogastric animals. For instance, oxalate binds to calcium to form complexes (calcium oxalate crystals). These oxalate crystals prevent the absorption and utilization of calcium by the body, causing diseases such as rickets and osteomalacia. The calcium crystal may also precipitate around the renal tubules, thereby causing renal stones. The formation of oxalate crystal is said to take place in the digestive tract. Oxalate is a concern in legumes because high-oxalate diets can increase the risk of renal calcium absorption, as calcium is made unavailable to the body due to the presence of oxalate. Legumes such as lentils, red kidney beans, and white beans have been analyzed for oxalate. The highest and lowest contents are present in Anasazi beans (80 mg, 100 g, 100 g^{-1} wet weight) and black-eyed peas (4 mg, 100 g, 100 g^{-1} wet weight), respectively.

Pyrimidine Glycosides

Vicine and convicine are generally present in *Vicia faba* and belong to the group of pyrimidine glycosides, which are composed of one molecule glucose linked to one pyrimidine nucleoside. In contrast, other grain legumes (e.g., *Pisum sativum*) and

Table 2 Phytic acid and L-DOPA content of some common and underutilized legume seeds

Name of legume seed	*Phytic acid*	*L-DOPA*
Cajanus cajan	734	
Cicer arietinum	121	
Dolichos lablab var. *vulgaris*	388.21–413.26	0.87–0.91
Phaseolus vulgaris	140	
Phaseolus vulgaris (White beans)	780	
Phaseolus vulgaris (Black beans)	911	
Abrus precatorius	348.14–366.32	0.98–1.12
Atylosia scarabaeoides		2.28
Canavalia gladiata	354.30	2.50
Dolichos trilobus		1.74
Entada rheedi		0.23
Lablab purpureus	605.39	
Lablab purpureus var. *lignosus*		1.43
Erythrina indica	386.24	2.48
Mucuna atropurpurea	384.94–464.09	2.94–4.20
Mucuna monosperma	503–632	4.24
Mucuna pruriens var. pruriens	473.10–623.00	6.66–7.63
Mucuna pruriens var. *utilis* (Black colored seed coat)	496.14–634.12	6.35–7.93
Mucuna pruriens var. *utilis* (White colored seed coat)	483.00–536.20	6.08–7.97
Mucuna deeringiana	510.12	6.55
Neonotonia wightii var. *coimbatorensis*		0.88
Rhynchosia cana	348.60–431.21	0.98–1.16
Rhynchosia filipes	433.14	2.14
Rhynchosia rufescens	378.30	0.88
Rhynchosia suaveolens	410.40	1.24
Tamarindus indica		4.12
Teramnus labilis		4.52
Vigna aconitifolia	376.00–421.38	0.56–3.27
Vigna bourneae	436	1.04
Vigna mungo	1124.30–1147.03	
Vigna radiata	664.76–692.40	
Vigna radiata subsp. sublobata	394	0.44–0.62
Vigna trilobata	312	0.78
Vigna umbellata	336	0.36
Vigna unguiculata subsp. *cylindrica*	406	1.12–1.96
Vigna unguiculata	139	
Vigna unguiculata subsp. unguiculata	339.21–378.40	2.23–2.26
Vigna vexillata	414	0.74

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Table 3 Saponin content of some common and underutilized legume seeds

Name of legume seed	*Saponin content g kg^{-1} dry weight*
Cicer arietinum	3.6
Vigna mungo	2.3
Vigna aconitifolia	3.4
Vicia faba	3.7
Pisum sativum	2.5
Pisum sativum (light colored)	1.2–2.3
Pisum sativum (dark colored)	
Phaseolus vulgaris var. Roba	9.4
Phaseolus vulgaris var. Awash	11.8
Phaseolus vulgaris var. Beshbesh	11.3
Phaseolus vulgaris	13.2
Sphenostylis stenocarpa	11.5
Phaseolus lunatus	12.0
Vigna subterranea	14.0
Canavalia gladiata	17.0
Canavalia ensiformis	18.0
Cajanus cajan	13.0
Lablab purpureus	14.0
Glycine max	8.0
Cajanus cajan genotypes	4.01–4.75
Mucuna pruriens var. *pruriens*	11.5–13.1

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oil seeds contain only negligible amounts compared to faba beans. Vicine and convicine act by reducing glutathione and glucose-6-phosphate dehydrogenase activity, which may result in hemolytic anemia due to biochemical abnormalities of blood cells.

Alkaloids

Alkaloids are naturally occurring toxic amines produced by plants mainly as a defense mechanism to protect themselves against herbivores. The main toxic effects of alkaloids result in disturbances of the central nervous system, digestive processes, reproduction, and the immune system. Within grain legumes, mainly lupins are known to contain alkaloids in considerable amounts, while faba beans, peas, and oil seeds are devoid of alkaloids. The major alkaloids of *Lupinus albus* are lupanine (700 mg g^{-1} total alkaloids), albine (150 mg g^{-1} total alkaloids), and 13 α-hydrolupanine (80 mg g^{-1} total alkaloids), while predominant alkaloids of *L. luteus* were identified as lupanine (600 mg g^{-1} total alkaloids) and sparteine (300 mg g^{-1} total alkaloids). The average alkaloid content of recently harvested cultivars of low-alkaloid lupins, also referred to as sweet lupins, is below 0.288 kg^{-1}. In 1981, Dike reported the presence of bioactive compounds such as nicotine, physostinginine, and serotonin present in the *Mucuna* seeds.

Oligosaccharides

Oligosaccharides, which are common in legume seeds, are thought to be the major producers of flatus. These saccharides contain one, two, or three galactose units jointed to α-1,6 galactosidic linkages. They cannot be hydrolyzed and absorbed in monogastric animals because of the lack of α-1,6 galactosidic activity in the small intestine. Microorganisms in the large intestine utilize these sugars, leading to the production of flatus gases (H_2, CO_2, and small amounts of CH_4) and causing abnormal rumbling, diarrhea, and discomfort. Oligosaccharide content of some of the common legumes and underutilized legume seeds have been tabulated (Table 4).

Maximum reduction of oligosaccharides has been observed in legume seeds when processed by autoclaving. Soaking for 20 h in tamarind pulp extract or sodium bicarbonate solution followed by autoclaving causes loss of α-galactosides to the extent of 68–71% and 68–70%, respectively. Moreover, the oligosaccharides are also markedly reduced when subjected to the natural fermentation process (62.68%). Sprouting for 48 and 72 h seems to reduce maximum content (77–94%) of total oligosaccharides. However, among the various processing methods, the crude α-galactosides obtained from a weed, *Cassia serisea*, exhibit promising results. It almost completely removed the galactosides via raffinose (79–85%), stachyose (95–98%), and verbascose (82–83%).

Toxic Amino Acids

Several toxic nonprotein amino acids occurring in higher plants, which are especially abundant in certain legumes (Vicieae, Phaseoleae, Mimosoideae, and Caesalpinioideae), have been detected that resemble protein amino acids. If nonprotein amino acids are taken up by herbivores, microorganisms, or other plants, they may interfere with several targets. They can be accepted in ribosomal protein biosynthesis in place of the normal amino acid, leading to defective proteins (e.g., Canavanine, 3,4-dihydoxyphenylalanine, azetidine-2-carboxylic acid). They can inhibit the charging of aminoacyl-tRNA synthetases or other steps of protein biosynthesis, or they may competitively inhibit uptake systems for amino acids. In vertebrates, effects may be fetal malformations, neurotoxic disturbances, hallucinogenic effects, hair loss, diarrhea, paralysis, liver cirrhosis, hypoglycemia, and arrhythmia, among others.

Mimosine

In 1977, the National Academy of Sciences published a monograph that described the high potential value of the legume

Table 4 Oligosaccharide content of some common and underutilized legume seeds

	Oligosaccharides g 100 g^{-1}		
Name of legume seed	*Raffinose*	*Stachyose*	*Verbascose*
Vicia faba	1.00	0.70	2.10
Vigna mungo	0.32–0.36	0.34–0.42	3.05–3.16
Phaseolus aureus	0.41	1.49	–
Pisum sativum	0.83–1.18	2.61–3.69	1.67–2.22
Phaseolus vulgaris var. Roba	0.34	1.24	–
Phaseolus vulgaris var. Awash	0.29	1.84	–
Phaseolus vulgaris var. Beshbesh	0.24	1.67	–
Cicer arietinum	1.45	2.56	0.19
Sphenostylis stenocarpa	0.66	2.86	0.31
Phaseolus lunatus	0.29	2.82	0.24
Cajanus cajan	0.42	0.85	1.06
Dolichos lablab var. *vulgaris*	0.41–0.58	1.66–1.76	1.20–1.24
Canavalia ensiformis	0.28	2.29	0.24
Tamarindus indica	1.53	1.89	4.53
Mucuna monosperma	1.36–1.62	1.18–1.24	0.96–1.07
Mucuna pruriens var. *pruriens*	0.74–0.94	1.10–1.78	3.68–4.78
Mucuna pruriens var. *utilis*	1.04–1.12	1.21–1.36	3.68–4.06
Mucuna deeringiana	0.98	1.14	4.24
Mucuna atropurpurea	0.54–1.01	1.21–1.94	3.94–5.55
Canavalia gladiata	0.64	1.64	2.11
Abrus precatorius	1.21–1.38	1.08–1.12	3.34–3.96
Erythrina indica	1.24	0.98	5.86
Rhynchosia cana	0.42–0.68	1.38–1.68	1.01–1.26
Rhynchosia filipes	0.58	1.46	1.12
Rhynchosia rufescens	0.31	1.21	0.98
Rhynchosia suaveolens	0.78	1.36	0.94
Vigna aconitifolia	0.54	1.68	1.26
Vigna unguiculata subsp. *unguiculata*	0.48–0.51	1.72–1.74	1.04–1.21
Vigna trilobata	0.41	1.78	1.17
Vigna radiata var. *sublobata*	0.38	1.58	0.98
Vigna umbellata	0.78	2.01	1.76
Vigna unguiculata subsp. *cylindrica*	0.56	1.76	1.01
Vigna vexillata	0.66	2.06	1.74
Vigna bourneae	1.01	2.12	1.84

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Leucaena leucocephala as a forage crop for livestock and human feeding. One of the principle factors limiting the use of this plant is the presence of an unusual amino acid, mimosine. It comprises 3–5% of the dry weight of the protein. This amino acid is responsible for the poor growth performance of cattle when this plant provides more than one-half of the diet. This adverse effect on growth has been traced to the underproduction of thyroxine, presumably because the rumen bacteria convert mimosine to 3,4-dihydroxypyridine, which acts as a goitrogen. In nonruminants such as the horse, pig, and rabbit, the goitrogenic effect is not very marked, but the animals nevertheless do very poorly on diets containing *Leucaena*, one of the characteristic symptoms being loss of hair. In fact, it has even been suggested that mimosine might be used as a defleecing agent in sheep. Certain segments of the human population, particularly in Indonesia, are known to consume portions of *Leucaena* in their diet, and a loss of hair has frequently been observed among individuals who have eaten the leaves, pods, and seeds in the form of a soup. As far as is known, mimosine has no effect on the meat or milk of ruminants that can be harmful to humans.

In 1951, Matsumoto et al. reported that the mimosine content of the seeds and leaves of *Leucaena* could be decreased by storing the plant at temperatures in excess of 70 °C in the presence of moisture. Adding ferrous sulfate to diets containing unhealed Leucaena also reduced its toxicity to rats. The latter effect has been attributed to an interference with the absorption of mimosine from the gastrointestinal tract.

Djenkolic Acid

Consumption of *Pithecolobium lobatum* seed sometimes leads to kidney failure, which is accompanied by the appearance of blood and white, needle-like clusters in the urine. The latter substance is a sulfur-containing amino acid known as djenkolic acid, which is present in the free state in the bean to the extent of 1–4%.

Canavanine and Canaline

Canavanine, a potentially toxic arginine antimetabolic, and canaline, its primary metabolite also a toxic nonprotein amino acid, are found in seeds of *Canavalia* species. Investigation of these two in *C. ensiformis* has shown that these were synthesized from homoserine and that the degradation of canavanine and canaline was similar to the arginase-medicated hydrolysis of canavanine to canaline in the process of canavanine metabolism. The toxicity of canavanine is due to the structural similarity of arginine. The effect of canavanine is to inhibit the nitric oxide pathway and thereby affect peristalsis. Canaline is a structural analog of citrulline and hence affects the ornithine cycle. Canaline is also toxic due to its ability to react with aldehydes (vitamin B_6) to form oximes and with keto acids. In 1972, Schlueter and Bordas isolated and purified canavanine from *C. gladiata* by hot methanol extraction followed by ion exchange chromatography.

3,4-Dihydroxyphenylalanine

The nonprotein amino acid, 3,4-dihydroxyphenylalanine (L-DOPA), was first isolated by Guggenheim about 70 years ago from the fruits of *Vicia faba*. Since then, its occurrence has been observed in other plants, mostly from leguminous species. Several investigators have reported fairly high levels of L-DOPA from different species of the genus *Mucuna* (Table 2). High levels of L-DOPA in the (unboiled) seeds of *M. utilis* have been reported. The presence of high levels of L-DOPA in the uncooked seeds of *M. utilis* has been implicated to be responsible for causing skin eruptions and an increase in body temperature of the consuming Kanikkars, a South Indian hill tribe.

The L-DOPA extracted from *Mucuna* seeds is used in the preparation of a drug that is administered for the treatment of Parkinson's disease in humans. Although L-DOPA medicine is synthetically manufactured today, *Mucuna* as a source of L-DOPA continues to receive attention. Clinical trials also show higher effectiveness of *Mucuna* powder over synthetic L-DOPA. However, the administration of L-DOPA has been reported to have some serious side effects in patients treated for Parkinson's disease, such as toxic confusional state and hallucination in addition to gastrointestinal disturbances such as nausea, vomiting, and anorexia. It has also been shown to be toxic in individuals with glucose-6-phosphate dehydrogenase deficiency in their erythrocytes, and induce favism.

In 1997, Takasaki and Kawakishi reported that the oxidation product of L-DOPA conjugates with SH compounds (cystein) of proteins to form a protein bound 5-*S*-cysteinyl dopa cross-link and it leads to polymerization of proteins. This might be one of the factors that could be responsible for the lowering of protein and starch digestibility. However, L-DOPA is found to be soluble in water, and it could be possible for consumers to remove/reduce it by adopting simple house-processing methods such as water soaking with boiling. In an earlier study, it was demonstrated that the level of L-DOPA gets significantly reduced by repeated soaking and boiling of seeds. The L-DOPA content in *Mucuna pruriens* is significantly eliminated by dry-heat treatment, cooking, and autoclaving. In 2003, Janardhanan et al. demonstrated that repeated boiling of seeds in water followed by decanting this water seven times resulted in a substantial reduction in the quantity of L-DOPA.

The maximum decrease in L-DOPA level (~51–69%) was achieved by germinating the seed for 3 days, then boiling it for 1 h and dehulling it. In 2002, St. Laurent et al. reported that complete extraction of L-DOPA from *Mucuna* bean is <5 min by placing 0.1 g of powdered seed into 15 ml of distilled water (150 parts of water to 1 part of seed) and placing it in an ultrasonication bath for 5 min.

Antivitamin Factors

The inclusion of raw soybean meal in the diet of chicks may cause rickets unless higher-than-normal levels of vitamin D_3 are added to the diet. This rachitogenic response can be eliminated by autoclaving the soybean meal, but supplementation with calcium or phosphorus is ineffective. It appears that the rachitogenic effect of soy protein isolated may be simply because of phytate, although evidence on this point is inconclusive. Raw kidney beans have been reported to contain an antagonist of vitamin E, as evidenced by liver necrosis in rats and muscular dystrophy and low levels of plasma tocopherol

in chicks. The antivitamin E effect of raw kidney beans can be partially eliminated by heat treatment. Isolated soybean protein has been demonstrated to increase chick requirement for α-tocopherol, as measured by growth, mortality, exudative diathesis, and encephalomalacia. No explanation was offered for this effect, although it has been suggested, but not proven, that α-tocopherol oxidase shown to be present in soybeans may be responsible for the destruction of this vitamin.

Conclusion

Legumes contain antinutritional factors such as protease inhibitors, lectins, cyanogens, total free phenolics, tannins, phytic acid, saponins, toxic amino acids, antivitamins, and oxalate. Legumes also have complex sugars such as raffinose, stachyose, and verbascose, which are responsible for flatulence. These compounds reduce protein digestibility and availability, and are considered antinutritional factors. But some antinutritional factors in legumes have been reported to have health benefits, so these secondary metabolites are currently marked as functional food and nutraceutical ingredients. Further research may determine if they should be preserved or eliminated in each main nutritional situation.

See also: Alkaloids: Toxicology and Health Effects; Antioxidants: Role on Health and Prevention; Legumes in the Diet; Phenolic Compounds: Bioavailability and Health Effects; Phytic Acid: Properties, Uses, and Determination; Pulsed Electric Fields; Saponins; Soy Beans: Dietary Importance; Soy Beans: Properties and Analysis; Tannins.

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Antioxidants: Characterization and Analysis

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Defining Antioxidants in Foods

Plants thrive on ultraviolet (UV) light to generate energy. Significant by-products of UV exposure include free radical species that damage lipids, proteins, and DNA. Consequently, plants have adapted to manage free radicals effectively and minimize damage. A number of small molecules in plants act as scavengers of free radical species and so have been classified as antioxidants, and several methods are used to characterize antioxidant scavenging specificity and activity. These are reviewed in section '**Characterizing Antioxidant Properties**.' However, even if antioxidants are highly reducing *in vitro*, whether these molecules act as antioxidants in physiological systems has been hotly debated.

Diets rich in many of these molecules with *in vitro* antioxidant activity have been linked to good health through epidemiological studies. For example, the studies of Gey and others in the late 1980s showed that higher levels of vitamin E in plasma are associated with reduced cardiovascular risk. Yet, of the many intervention studies that followed, very few showed any beneficial effect from vitamin E supplementation, and some studies reported more harmful outcomes in those receiving the supplements than in placebo groups. This led to a number of emerging concepts, which should be considered when interpreting antioxidant intervention studies:

- A number of related molecules form vitamin E (tocopherol isoforms). Alpha-tocopherol is at the highest concentration in humans and is predicted to show the greatest antioxidant-like activity *in vivo*. Other tocopherols and metabolites have been postulated to exert effects on signaling via nonantioxidant mechanisms. However, the biological effects of vitamin E may not be due to the antioxidant properties of the molecule(s). Other classes of phenolic 'antioxidants' (e.g., flavonoids) are also widely reported to act as regulators of cell signaling.
- Antioxidant activity in tissue is affected by a combination of absorption, distribution, metabolism, and excretion. Only the metabolites of a given antioxidant may be available biologically (e.g., for flavonoids) because nutrients or bioactive dietary compounds are formed by metabolism in the gut. The efficiency of metabolism may, therefore, affect the rate of absorption, availability, and antioxidant bioactivity.
- Dietary antioxidants act in synergy with endogenously synthesized antioxidants, particularly the tripeptide glutathione, which is the major intracellular defense against free radicals. Glutathione synthesis is carefully regulated by redox state. Dietary antioxidants may suppress endogenous glutathione synthesis by altering cellular redox state and so disable the cellular adaptive response.
- Antioxidant networks that comprise molecules with similar redox potentials exist in physiological systems and regenerate reduced 'antioxidants' of lower redox potential (**Figure 1**). Disruption of the network by overloading with a single micronutrient may prevent effective regeneration networks and lead to failure to adapt to stress.
- Bioactive molecules in plant foods may exert secondary antioxidant effects through upregulating phase 2 enzymes and glutathione levels; glucosinolates (GLS) are effective phase 2 inducers.

The take-home messages from these concepts are that (1) accurate measurement of different micronutrient isoforms, where they exist, should be undertaken; (2) 'antioxidant-like' activity can be achieved by direct radical scavenging and hydrogen donation, as well as by induction of new protein expression, so evaluation of antioxidant activity should include all possibilities; and (3) knowledge of 'antioxidant' bioavailability, metabolism, and bioactivity of metabolites should also be considered.

Characterizing Antioxidant Properties

An antioxidant is, chemically speaking, a reducing agent. Reducing agents are defined as molecules that can receive electrons and/or donate hydrogen. There are a number of ways in which reducing activity can be assessed. These vary based on the nature of the radical species being scavenged. Consequently, it is appropriate to consider using several different methods for analysis when seeking to define antioxidant properties – see Prior et al. on standardized methods for antioxidant capacity measurements of phenolics, which suggests three core assays, the oxygen radical absorbance capacity (ORAC) assay and the Folin–Ciocalteu and Trolox equivalent antioxidant capacity methods that are based on electron transfer and give reducing capacity, normally expressed as phenolic contents.

The USDA published a website detailing the ORAC capacity of specific foods, but this was later withdrawn owing to the lack of evidence for value of nutrients via antioxidant effects.

Different approaches commonly used to measure antioxidant activities are reviewed in the succeeding text. These include scavenging the peroxy-radical generator 2,2′-azobisisobutyramidinium chloride (AAPH), ferric ions, and nitric oxide. Naively, these assays can be considered as a competition between indicator and antioxidant for the oxidizing source. With many of these assays, there is an important caveat, however. The real situation is more complex as the antioxidant may, in fact, reduce the oxidized indicator directly rather than interact with the oxidizing species. In many cases, the indicator is readily reducible (e.g., 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS), so any reaction rate recorded may relate to the direct reduction of the oxidized indicator.

Oxidation of ABTS and Reduction of $ABTS^{+}$

The oxidation of ABTS by AAPH results in the formation of a colored product, the ABTS cation radical that can be determined spectrophotometrically at 734 nm. Antioxidants may

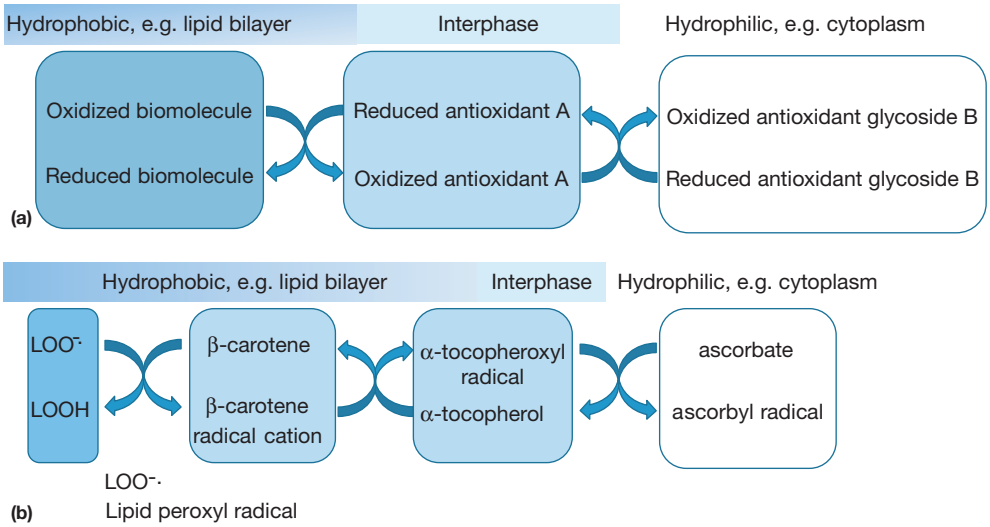

Figure 1 How an antioxidant network could work – (a) theory, (b) thermodynamically feasible reactions. Caution: dietary forms of an antioxidant, metabolized and absorbed from the gut; proximity of antioxidant to a radical; reversibility of the reactions; and relative concentrations of radicals and reducing agents will influence the biological reactions.

delay the onset of the reaction, and the duration of the delay (induction time) is proportional to the concentration and activity of the antioxidant. An alternative to this, which overcomes the caveat, is to simply quantify the reducing potential of the molecule in a reduction assay. Reduction assays are precise because they determine the difference between absorbances of the stable, colored $ABTS^{+}$ before and after addition of antioxidant. In this assay, $ABTS^{+}$ is preprepared by oxidation with potassium persulfate.

Ferric Reducing Ability of Plasma

Ferric reducing ability of plasma (FRAP) is based on the reduction of a colored ferric complex to ferrous ion at low pH, causing the colored ferrous tripyridyltriazine complex to form. Values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing blue ferrous chloride. The only antioxidant class that is insensitive in FRAP is thiol-active molecules. At low pH, the hydrogen-donating effects of thiols are poor, rendering them undetectable.

Nitric Oxide Scavenging

Nitric oxide scavenging activity can be measured by a variation of the Griess assay, which is commonly used to measure nitrite. Weakly buffered saline solution (pH 7, 40 mmol l^{-1} phosphate is a radical scavenger) and methanol containing the antioxidant are mixed with the nitric oxide donor, sodium nitroprusside. The nitrite produced on decay of sodium nitroprusside is measured at 540 nm after reacting with sulfanilamide and *N*-(1-naphthyl)ethylenediamine dihydrochloride. The greater the antioxidant capacity to scavenge nitric oxide, the lower the nitrite concentration produced.

Standard Electrode Potential Determination

In a cellular system, where multiple scavengers of radical species exist, including amino acids, polyunsaturated fatty acids, and DNA bases, it is important to understand the rates of reaction with radicals and also standard electrode potentials. Pulse radiolysis and electron paramagnetic resonance offer powerful approaches to study one-electron reduction potentials of radical cations. It is also possible to study the transfer of electrons from amino acid radicals to scavengers and vice versa. The standard electrode potential data emerging from these studies, summarized in Table 1, vary according to the solvent system used and pH. This information can provide insight into whether specific scavengers can in fact reduce an amino acid/lipid radical or if they are more likely to transfer electrons to lipids or amino acids and cause oxidation. Biologically speaking though, further important considerations include the (1) rates of the reverse reaction under the same conditions, (2) concentrations and compartments where antioxidants are found, and (3) proximity to the radical they scavenge. Figure 1 provides an overview of our current understanding of an

Table 1 Published redox potentials of biological radicals and dietary antioxidant radicals

Class of redox-active molecules	*Species*	$E^{o\prime}$ *(V)*
Lipids	Lipid peroxyl radical	1.00
Proteins	Trp radical	0.64–1.00
	Tyr radical	0.78–0.91
	Cys radical	0.13–0.27
Vitamin E	α-Tocopheryl radical	0.48
Vitamin C	Ascorbyl radical	0.28
Carotenoids[a]	β-Carotene cation radical	0.5, 0.69, 1.06
	Zeaxanthin cation radical	0.54, 1.03
	Lycopene cation radical	0.98
Polyphenols	Caffeic acid	0.54

[a]Published by a number of different sources under discrete conditions. NB: In the case of some polyphenols, metabolites and conjugates are likely to be present in the blood following dietary intake rather than parent metabolites.

antioxidant network. Newer electrochemical methods are being applied to study redox cycling and standard electrode potentials of antioxidants in foods.

Induction of Phase 2 Enzymes

At least nine classes of phase 2 enzyme inducers have been proposed. While they are chemically reactive with thiol entities in proteins, they may act as oxidizing, alkylating, or reducing agents. Phase 2 enzyme inducers are not able to participate in radical or redox reactions. However, their activity changes the redox potential of cells in favor of a reducing environment by inducing the expression of enzymes such as glutathione synthetase, glutathione transferase, and thioredoxin.

A simple reporter assay can be used to measure the potential of a molecule to act as a phase 2 enzyme inducer. Cells that take up antioxidants are transfected with the antioxidant response element (ARE) consensus sequence, which is required for the induction of phase 2 enzymes, and linked directly to a reporter gene such as luciferase. When the cell receives an inducing signal, a chemiluminescent signal is produced that is proportional to the extent of cell activation.

Persistent oxidative stress over an extended time frame tends to overcome the adaptive response, leading to maladaptation and, ultimately, chronic diseases. The ability to adapt and respond via antioxidant enzyme induction is believed to be critical for cellular survival and is thought to decline with age.

Analysis of Antioxidants

The following section reviews knowledge about the biological forms of major classes of antioxidants and antioxidant inducers. Details of analytic approaches are provided for vitamins C and E and carotenoids, about which most are known. Summary overviews of methods for polyphenols and GLS are included at the end of this article. Readers should refer to the recommended texts for further details about polyphenol analysis.

Vitamin C

Consistent with its role as an antioxidant, first identified by Szent-Györgyi in 1932, ascorbic acid is a highly labile entity that readily undergoes oxidation to dehydroascorbic acid. Recommended daily intake varies by country ranging from 40 to 90 mg day^{-1}. Uptake is either via anion transporter – in an energy-requiring process (reduced form) – or via glucose transporters (oxidized form).

Several authors have shown the food matrix does not influence the bioavailability of vitamin C. Acidification of the extraction buffer increases the stability of vitamin C after cells have been ruptured, and, under these conditions, proteins and DNA are also removed. This approach is suitable for measurement of vitamin C in plants or humans. Typically, 10% metaphosphoric acid is used as a stabilizer although others have described the use of equal volumes of ice-cold 0.54 mol l^{-1} perchloric acid. Typically, plasma from blood collected into ethylenediaminetetraacetic acid (EDTA) is analyzed for vitamin C. It is likely the addition of EDTA or DTPA – as chelators – to extraction buffers (2 mmol l^{-1}) that provides additional stability for ascorbate extracted from plant foods. Despite this acidification, ascorbate can still undergo oxidation, even at −80 °C, and is best analyzed within 1 month of sample collection. An extract suitable for analysis is generated after the addition of metaphosphoric acid and centrifugation. High-performance liquid chromatography (HPLC) analysis of vitamin C is relatively straightforward, offering sensitivity and specificity. Sufficient sensitivity can be achieved after derivatization to measure intracellular concentrations of ascorbate in mammalian cells.

Depending on the purpose of analysis (i.e., quantify total or only reduced ascorbic acid), it may be necessary to reduce existing dehydroascorbic acid. This can be achieved effectively with the addition of several compounds, including cysteine and tris(2-carboxyethyl)phosphine hydrochloride.

Several different columns have been used for vitamin C analysis. This is partly due to individual preference but is also largely driven by the detection method. The simplest approach is UV detection based on the ascorbic acid absorbance maxima at 245 and 260 nm. However, the absorbance is sensitive to pH fluctuations with absorbance lost at pH 9. It is important to include standards in each run to compensate for any error in pH. An external standard curve should also be generated from freshly prepared ascorbate serially diluted prior to spectrophotometric analysis.

Direct analysis at 245 and 260 nm, under acidic pH, favors ionization of ascorbate, meaning an amino column can be used successfully in normal phase mode with UV detection. To improve sensitivity and specificity for the analysis of dehydroascorbic acid, ortho-phenylenediamine can be derivatized prior to separation on an amino column with fluorescence detection. The optimal mobile phase has been determined as 0.2 mol l^{-1} KH_2PO_4/H_3PO_4 (pH 3) containing 2 mmol l^{-1} EDTA, delivered at 1 ml min^{-1}.

Newer detection methods favor the application of electrochemistry, such as a Coulochem array detector with +200 mV electrode potential. The array produced is unique for ascorbate and improves both specificity and sensitivity of analysis. Some methods have moved over to reverse-phase C18 columns, using acidic buffers, which are either phosphoric- or acetic acid-based and contain metal chelators (e.g., EDTA and DTPA) and freshly added paired-ion reagent. When applied to analysis of foodstuffs, Proteggente et al. showed that broccoli has an equivalent fresh weight vitamin C content to fresh orange.

Vitamin E

Lipophilic by nature, vitamin E comprises several tocopherol and tocotrienol isoforms (α, β, γ, and δ). Tocotrienols share many of the biological properties of isolated tocopherols *in vitro* and are detectable in plasma after supplementation, but little is known of their accumulation in tissues at present. The study of physiological effects of dietary tocotrienols is an emerging area for study and offers an interesting avenue for future research.

The naturally occurring tocopherol isomers are RRR, whereas synthetic forms tend to be D-isomers. α-Tocopherol is considered to be the major antioxidant in animal foods. However, although the composition is variable, the majority of plant foodstuffs have similar levels of α- and γ-tocopherol. In oils and fats, tocopherols are readily bioavailable, but bioavailability from small seeds and nut fragments is lower. Tocopherol absorption from the gut follows emulsification

and is, generally, associated with lipids. An important biological function of α-tocopherol is its ability to donate hydrogen and so reduce lipid hydroperoxides directly or via β-carotene. In doing so, the oxidized polyunsaturated fatty acids are restored and the tocopheroxyl radical is formed. Regeneration of α-tocopherol may be achieved via the reducing action of ascorbate, which in turn is converted to dehydroascorbate (Figure 1). γ-Tocopherol has been suggested to be a powerful scavenger of peroxynitrite.

Recommended daily intake of α-tocopherol varies by country with mean recommendations lying in the range 6–14 mg day^{-1}. However, there are no such criteria for the other tocopherol isoforms. More recently, attention has been focussed on cell signaling-related effects of tocopherols, and a review of this topic by Hussain et al. is recommended.

Analysis of tocopherols requires extraction from biological samples using organic solvents. Tocopherol acetate is a useful internal standard, which can be added prior to extraction to enable recovery calculations to be undertaken. For extraction, the preferred method uses hexane to which 2,6-di-*tert*-butyl-4-methylphenol (BHT) is added as an antioxidant to minimize oxidation. Phase separation of organic (tocopherol containing hexane) from biological samples is achieved by centrifugation. To improve recovery, further extraction of the remaining aqueous phase can be achieved using an equal volume of hexane. The organic phases should be combined and evaporated to dryness under reduced pressure before the pellet is dissolved in methanol for HPLC. For analysis, a reverse-phase C18 column can be used and tocopherol eluted using 100% methanol. Freshly prepared standards should be used for each analysis with standards and unknown samples determined at 292 nm.

Many laboratories undertake analysis of several lipophilic antioxidants simultaneously. This can be achieved using photodiode array with MS–MS detection, the latter being more sensitive and more suitable for small sample volumes. For MS methods, ionization of analytes is essential, and, in addition to methanol as eluent, the presence of ammonium formate improves sample ionization. One advantage of MS–MS methods is that internal standards can be exactly the same as the analytes of interest, except they are deuterated, which means losses during extraction or analysis, particularly with respect to ionization, are the same for standards as the samples of interest.

Interpretation of plasma α-tocopherol measurements also merits some consideration: given its lipophilicity, α-tocopherol is transported by lipoproteins. Individuals who are hyperlipidemic tend to have a higher plasma α-tocopherol concentration, but this may still be insufficient to protect low-density lipoproteins (LDL) from oxidation. A better index of α-tocopherol antioxidant capacity is its concentration per LDL molecule, which eliminates lipid peroxides. When expressed as α-tocopherol/LDL, concentrations are lower in hyperlipidemic individuals than in (apparently) healthy controls and correlate well with lipid oxidation.

Analysis of α-tocopherol in foods reveals that nuts, in particular almonds, have very high vitamin E content per gram. Whether this is bioavailable is less clear since this is affected by digestion, metabolism, and absorption. Nevertheless, Choudhury et al. showed in an intervention study that increased intake of dietary almonds is associated with increased plasma α-tocopherol/LDL in healthy subjects.

Carotenoids

Beyond the effects of chlorophyll in green vegetables, the color of many plant foods is due to their carotenoid content. First chemically characterized by Willstatter in 1907, the concentration of carotenoids in plants is affected by exposure to light and nitrogen supply.

Up to 600 carotenoids have been described, but the major carotenoids found in foodstuffs, plant- and animal-derived, are α- and β-carotene, lutein, lycopene, β-cryptoxanthin, and zeaxanthin. Natural forms tend to be *cis*-forms, although *trans*-forms can be found in processed foods. There are no dietary requirements laid down for carotenoids, largely because no known deficiency states exist. However, the requirements are likely to be age-dependent, and deficiency of carotenoids has been associated with both dementia and age-related macular degeneration. Guidance from many countries to eat five to seven or more portions of (colored) fruits and vegetables each day will determine the carotenoid concentrations found in plasma. A review of current concepts in carotenoids by Hammond is recommended.

Astaxanthin, one of the oxygen-containing carotenoids (xanthophylls), is attracting recent interest because it was approved for use in fish farm feed to enhance flesh color; it is via this route that it enters the human food chain where it appears to have similar properties to the other major carotenoids in the human diet.

Bioavailability of carotenoids is similar to tocopherols; due to their lipophilicity, they are released from cooked digested food to form mixed micelles, which are, subsequently, absorbed. The distribution of carotenoids in plasma is achieved by lipoproteins. The hydrocarbon carotenoids, β-carotene and lycopene, are mainly transported by LDL, while the more polar carotenoids, lutein and zeaxanthin, are carried by high-density lipoproteins.

Metabolism of β-carotene is complex, and there are many gaps in our knowledge. About 30% of the dietary vitamin A intake in industrialized countries is from β-carotene. However, for developing countries, β-carotene is the most abundant source of vitamin A and in some instances the only one. The review by Shete and Quadro covers the state of the art in carotenoid metabolism. A better understanding of β-carotene metabolism and requirements is essential to reduce risks that may be associated with high levels of intake (above normal dietary intake). Indeed, no health benefits have been reported from supplementation with β-carotene, and deleterious outcomes have been reported in heavy smokers who were also deficient in ascorbic acid. This again supports the importance of maintaining a network of antioxidants where interactions between them may be critical for outcome (Figure 1). Peroxynitrite is scavenged effectively by carotenoids, particularly lycopene.

Lycopene has attracted significant research interest, which is well described by Wang. It is a powerful inducer of phase 2 enzymes, via the transcription factor Nrf2, which activate ARE. Thus, the biological role of carotenoids as 'antioxidants' may be indirect as well as direct (radical scavenging). It is important to note that these effects are largely determined from chemical and studies *in vitro* rather than intervention studies.

Extraction from the biological matrix is necessary for the analysis of carotenoids *in vivo*. Their lipophilicity favors organic

extraction, as with tocopherols. A combination of isopropanol/hexane/dichloromethane (1:5:1) in the presence of BHT with sonication has been used successfully to release carotenoids. After centrifugation, the organic supernatant is removed and retained while the aqueous phase is reextracted. The combined organic phases can be evaporated to dryness under nitrogen before being resuspended for analysis. Others have adopted a simpler ethanol extraction approach for plasma samples with good recovery. Equal volumes of ethanol and plasma are combined, followed by sonication, phase separation, and evaporation to dryness as described earlier. Samples can be resuspended in methanol/acetonitrile/2-propanol (5:4:2) prior to HPLC. The columns used for separation are, typically, non-end-capped silica suitable for the separation of small zwitterions. Carotenoids are eluted with methanol/acetonitrile/2-propanol and determined by photodiode array or absorbance at 460 nm. Others have used reverse-phase chromatography with methanol/water as eluent.

The use of MS–MS for detection can offer advantages in carotenoid identification using precursor and product ions consistent with standards because of the many closely related isoforms that occur biologically and are formed during food processing. Electrochemical detection has shown similar levels of sensitivity to MS, but it provides less selectivity: The measured dominant oxidation potentials are not unique to individual compounds, and unknown interfering compounds may also be present.

Polyphenols

There are six major classes of polyphenols in the human diet: chlorogenic and ferulic acids, flavones and flavanols, catechins, isoflavones, and lignans. There are no recommended daily intakes for these bioactive compounds because they are not nutrients and, although associated with optimal health, it has not been possible to identify the range or extent of intake required. The polyphenols are subject to a number of metabolic reactions: many undergo oxidation, hydrolysis, and condensation in the gut. Bioavailability is affected by gut microflora, as shown in studies where radiolabeled polyphenols have been administered to animals in order to determine the fate of their metabolites. Many studies *in vitro* on the effects of polyphenols in cell culture have been criticized for failing to use the physiological (metabolized) form (without the sugar moiety) or for using concentrations in excess of those likely to be observed *in vivo*.

Extraction is critical in the analysis of polyphenols, and the methods vary according to the analyte of choice. These have been considered in an article by Inat et al. Liquid–liquid, solid–liquid, and supercritical fluid extractions are the favored approaches. Once extracted, both spectrophotometric and HPLC methods have been adopted for analysis. The Folin–Ciocalteu assay is a common spectrophotometric method albeit increasingly criticized. The majority of HPLC methods favor reverse-phase chromatography with either photodiode array or tandem mass spectrometry detection methods.

Glucosinolates

The cruciferous vegetables, which include broccoli, cabbage, and cauliflower, are an excellent source of GLS. GLS coexist in plants with myrosinase, the enzyme that controls its degradation. Isothiocyanates are the major metabolites of GLS and are formed in the gut after plant walls have been ruptured. GLS are highly reactive with thiols and preferentially bind to glutathione and albumin in the systemic circulation. Thiol interaction has been implicated in GLS biological activity as a phase 2 enzyme inducer.

Analysis of GLS frequently involves conversion to desulfo derivatives that can be more easily determined by reverse-phase HPLC with UV detection by diode array. However, standards are lacking, and the identification of GLS is difficult or even impossible to determine unequivocally. The use of MS offers a solution for a more robust identification of GLS, as with other antioxidant enzymes.

Conclusions

Advances in analytic methods, particularly around the use of mass spectrometry, have enabled a new level of confidence in analysis as well as improved sensitivity in many cases. This has led to rapid advances in studying the metabolism of dietary 'antioxidant' molecules within cells as either the parent molecule or metabolite(s). By combining this new knowledge with biological studies to explore molecular mechanisms of action, and chemical evaluation of rates of reaction in simulated systems, we are moving toward a better understanding of the role of dietary antioxidants in human health. The epidemiological data are clear: diets rich in antioxidants are good for health. To date, however, our approach has been too simplistic, and there is a need for improved understanding of why antioxidant-rich diets are good for us. This will be aided by improved analyses and systems biology approaches.

See also: Antioxidants: Role on Health and Prevention; Ascorbic Acid: Physiology and Health Effects; Ascorbic Acid: Properties, Determination and Uses; Bioavailability of Nutrients; Carotenoids: Occurrence, Properties and Determination; Carotenoids: Physiology; Mass Spectrometry: Applications; Mass Spectrometry: Principles and Instrumentation; Phenolic Compounds: Bioavailability and Health Effects; Tocopherols: Physiology and Health Effects; Tocopherols: Properties and Determination.

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Antioxidants: Role on Health and Prevention

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Oxygen is essential element for life. Human cells, as well as those of other aerobic organisms, use oxygen to break down nutrients and provide energy. In the mitochondrial energy-generating system, oxygen is reduced to water, and energy is stored inside ATP molecules. However, this process is a natural phenomenon, as it is essential for life and can be harmful to our body at the same time. Different chemical entities that contain partially reduced oxygen are called reactive oxygen species (ROS). They are continuously produced in the mitochondrial respiratory chain and some other biochemical reactions and have very important signaling role in various cellular processes. Most of them are very reactive, with great affinity to vital molecules of human cells – proteins, lipids, and deoxyribonucleic acids (DNA). Oxidative damage of macromolecules can lead to the disturbance of their function and the development of various diseases. However, in normal conditions, the levels of ROS are low, and human cells protect themselves from their action with efficient antioxidant machinery. Surprisingly, the human body can even use the deleterious action of ROS to help the immune system, destroy foreign substances, and combat infectious diseases. Undoubtedly, the delicate balance between physiological effects of ROS, as signaling molecules and efficient component of immune cells, and their pathological effects is under the control of complex system of antioxidant defense and represents a key aspect of life. However, in pathological states, production of ROS can overcome the capacity of antioxidants. This state is called oxidative stress. The precise definition given by Sies in the early 1990s describes oxidative stress as an "imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage." So, it is not just about the increase in ROS (if it induces more efficient antioxidant defense) or about the decrease in antioxidants (if the level of ROS are low); it is a disturbance in their balance that could end with the damage of our body.

ROS is a term that encompasses all highly reactive, oxygen-containing molecules, including free radicals. ROS includes superoxide ($O^{2}\bullet\ -$), hydroxyl ($\bullet OH$), peroxyl ($ROO\bullet$), lipid peroxyl ($LOO\bullet$), alkoxyl ($RO\bullet$) radicals. Reactive nitrogen species (RNS) include nitric oxide ($NO\bullet$) and nitrogen dioxide ($NO^{2}\bullet$). Oxygen and nitrogen free radicals can be readily converted to other nonradical reactive species that are also dangerous for health. Hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite ($ONOO-$), dinitrogen trioxide (N_2O_3), and lipid peroxides (LOOH) are not free radicals but generally named oxidants and can easily lead to free radical reactions in living organisms.

All ROS and RNS are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and various small molecules, resulting in cellular damage.

Mechanisms of ROS Generation

ROS can be produced from both endogenous and exogenous sources. Production of ROS in the body is continuous and a normal part of our physiology.

Endogenous sources of ROS include the following:

Mitochondrial respiratory chain (*MRC*). It is the main source of ROS, particularly $O_2^{\cdot-}$, the most crucial one as it can induce formation of several other reactive oxygen intermediates. $O_2^{\cdot-}$ is formed by reduction of molecular oxygen with electron 'leaked' from the MRC.

Respiratory burst and NADPH oxidase. Respiratory burst is the process by which phagocytic cells consume large amounts of oxygen during phagocytosis, mainly via activation of NADPH oxidase and $O_2^{\cdot-}$ release into the extracellular space or phagosomes. NADPH oxidase is an enzyme present in the plasma membrane and phagosomes of phagocytes such as monocytes, macrophages, neutrophils, and eosinophils. Relocation of the cytosolic components of NADPH oxidase to the cell membrane induces its activation. Enzyme is normally latent in phagocytes but is activated in the membrane before respiratory burst. There are six homologues of NADPH oxidase, collectively called the NOX family of NADPH oxidases.

Xanthine oxidase (*XO*). It is found on the outer surface of the plasma membrane and in the cytoplasm. It catalyzes oxidation of hypoxanthine to xanthine and, further, to uric acid as part of purine catabolism. Both reactions generate $O_2^{\cdot-}$. However, $O_2^{\cdot-}$ has a short half-life and is readily reduced to H_2O_2, so is not considered as highly reactive. Additionally, due to charged moiety of $O_2^{\cdot-}$, it cannot pass through lipid membranes. The production of xanthine and XO is greatly enhanced during ischemia, accompanied with the loss of antioxidant enzymes. O_2 is an electron acceptor and cofactor for XO, thus generating $O_2^{\cdot-}$ and H_2O_2, a major ROS in ischemia, causing damage to ischemic cells and tissues of different origins.

Lipoxygenases (*LOX*). They are nonheme iron enzymes that catalyze dioxygenation of polyunsaturated fatty acids and formation of hydroperoxyl derivatives that can be subjected to reduction and give corresponding hydroxyl derivatives, including leukotrienes and lipoxins. In humans, oxidation of arachidonic acid by LOX generates ROS. Five LOX enzymes identified in humans, named based on position of oxygenated residues, catalyze four different reactions that produce fatty acid hydroperoxides. LOX are deeply involved in the process of atherogenesis.

Myeloperoxidase (*MPO*). It is a heme enzyme localized in lysosomes of neutrophils, macrophages, and monocytes. It catalyzes the reaction of H_2O_2 to highly reactive HOCl and an oxidation of thiocyanate (SCN^-) to hypothiocyanite ($OSCN^-$).

Nitric oxide synthase (*NOS*). It is a heme-containing monooxygenase that generates NO. Three different isozymes of NOS have been identified: constitutively expressed neuronal NOS (nNOS or NOS I), endothelial NOS (eNOS or NOS III), and endotoxin- or cytotoxin-inducible NOS (iNOS or NOS II). All types of NOS catalyze the oxidation of L-arginine to an intermediate, *N*-hydroxy-L-arginine, followed by generation of L-citrulline and NO. NOS can also generate H_2O_2 within a reaction with $O_2^{\cdot-}$ at low L-arginine levels. $NO^{\cdot}$ is a weak oxidant, but in reaction with $O_2^{\cdot-}$ generates $OONO^-$. NO and $OONO^-$ can further form very stable nitrite (NO_2^-) and nitrate (NO_3^-) ions, which accumulate in cells, and form reactive intermediates: NO_2, N_2O_3, and $^{\cdot}NO$. They are capable to nitrate and nitrosate important biological macromolecules such as DNA, RNA, proteins, and lipids and disrupt their function. 8-Nitroguanine, a nitration product of DNA and RNA, is a potent prooxidant and mutagen.

Cyclooxygenase (*COX*). It is a bifunctional enzyme with both COX and peroxidase activities. COX releases arachidonic acid (AA) from membrane phospholipids and catalyzes further conversion of AA to prostanoids. There are two isoforms of COX, COX-1 and COX-2. COX catalyzes oxidation of AA to unstable cyclic hydroperoxide (PGG_2) and further its reduction of PGG_2 endoperoxide due to its peroxidase activity (PGH_2). PGH_2 is converted to stable prostanoids such as PGE_2, prostacyclins, and thromboxane A_2. The peroxidase activity of COX generates $NAD^{\cdot}$ and $NADP^{\cdot}$ radicals, which can further generate $O_2^{\cdot-}$.

Transition metals. Transition metal ions such as iron (Fe^{2+}) and copper (Cu) are involved in the Fenton reaction that generates $HO^{\cdot}$ and $OH^{\cdot}$ from H_2O_2, and they are oxidized to Fe^{3+} and Cu^{2+}, respectively. The generation of $HO\cdot$ through this pathway accelerates lipid peroxidation.

Oxidative stress can also be triggered by external factors acting as direct or indirect sources of ROS:

Radiation and chemotherapy. Ionizing radiation can produce $HO^{\cdot}$ by radiolysis of water or other ROS via secondary reactions. Particularly, susceptible systems are the cerebrovascular, gastrointestinal (GI), and hematopoietic systems. Cancer chemotherapy induces generation of ROS and decrease in vitamin E and beta-carotene levels that often cause toxic side effects.

Cigarette smoke. It is a significant contributor to oxidative stress as source of a large number of free radicals and other oxidative and aromatic agents acting as direct or indirect ROS generators.

Xenobiotics including drugs, food, and alcohol. Food can contain different ROS or compounds that can generate ROS within a human body (iron, copper, trans fatty acid, and acrylamide). Thermally treated lipids and alcohol can be significant contributors to oxidative stress. Increase in oxidative stress has been observed in postprandial states after high-fat and/or high-sugar meals. Many drugs (glucocorticoids, anesthetics, and nonsteroidal anti-inflammatory agents) and xenobiotics generate free radicals. This is also an important feature of a great number of anticancer agents, which often act as therapeutic through generation of ROS.

Mental stress. It can trigger the production of free radicals as toxic by-product of intensive metabolism. In addition, hormones that mediate stress reaction in the body (cortisol and catecholamine) decompose into destructive free radicals.

Pollutants. Air pollutants (asbestos, benzene, carbon monoxide, chlorine, formaldehyde, ozone, and toluene), chemical solvents (cleaning products, glue, paints, paint thinners, perfumes, and pesticides), and water pollutants (chloroform and other trihalomethanes) are potent generator of free radicals. Burning of organic matter during cooking, forest fires, and volcanic activities also can generate free radicals.

Antioxidant Defense System

Nature has endowed each cell of our body with adequate antioxidant mechanisms for protection against any harmful effects of ROS generated within the body or those that entered our body from the environment. Endogenous antioxidant defense system (ADS) comprises enzymatic and nonenzymatic antioxidants. Besides endogenous antioxidants, different substances or agents that can scavenge reactive oxygen metabolites, block their generation, or enhance capacity of endogenous antioxidants act as exogenous antioxidants. Dietary compounds, including vitamins, minerals, polyphenols, isothiocyanates, and carotenoids, are the most important exogenous antioxidants. The fact that they are safe, cheap, and orally bioavailable resulted in their most often use as antioxidant supplements in the prevention or therapy of stress-related diseases. Antioxidants act as radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors, enzyme inducers, synergists, or metal-chelating agents.

Endogenous Enzymatic Antioxidants

The major antioxidant enzymes in human cells are superoxide dismutases, glutathione peroxidase, glutathione reductase, and catalase. SOD and catalase provide major antioxidant defenses against ROS.

Superoxide dismutases (*SOD*) are enzymes that catalyze dismutation of $O_2^{\cdot-}$ into O_2 and H_2O_2. There are three isoforms of SOD in humans: cytosolic copper- and zinc-containing SOD (Cu-Zn-SOD), manganese-containing mitochondrial SOD (Mn-SOD), and extracellular copper- and zinc-containing SOD (EC-SOD). Mn-SOD is essential for life.

Glutathione peroxidase (*GPX*) converts glutathione into oxidized glutathione (glutathione disulfide, GSSG) and simultaneously reduces H_2O_2 to H_2O and lipid hydroperoxides (ROOH) to corresponding stable alcohols. This reaction is coupled to the reaction catalyzed with glutathione reductase (GSSG-R), the enzyme responsible for maintaining optimal reduced glutathione (GSH) levels. Neurons are characterized by very low levels of GSH and thus are particularly vulnerable to free radical damage. GPX has very important role in protecting cells from the harmful effects of peroxide decomposition. There are eight isotypes of GPX in humans, and most of them have selenocysteine residues at their active site, crucial for their action.

$O_2^{\cdot-}$ formed in the mitochondria is converted to H_2O_2 by the action of Cu-Zn-SOD of the mitochondrial intermembranous space and Mn-SOD of the mitochondrial matrix. Formed H_2O_2 is reduced by GPX present in the mitochondrial matrix. Uncharged H_2O_2 passes the mitochondrial membranes and

can be scavenged in the cytosol by cytosolic Cu-Zn-SOD or catalase.

Catalase is a heme enzyme located mainly in peroxisomes. Liver and kidney cells, as well as erythrocytes, are rich in catalase. It converts H_2O_2 to H_2O and O_2.

Glutathione reductase (*GR or GSR*) is a homodimeric flavoprotein disulfide oxidoreductase. It reduces oxidized glutathione (GSSG) to GSH. GSH is an important antioxidant, and thus, GR's main role is to protect red blood cells, hemoglobin, and cell membranes from oxidative stress.

Heme oxygenase (*HO*) catalyzes degradation of heme and formation of CO, biliverdin, and iron. It does not have a direct antioxidant enzymatic function, but indirectly, via its products, it can protect cells against oxidative stress. It exists in two isoforms, inducible HO-1 and constitutively expressed HO-2. HO-1 expression is induced by heat shock, UV radiation, or ischemia/reperfusion injury.

Endogenous Nonenzymatic Antioxidants

Glutathione (*GSH*), a tripeptide consisting of glutamate, cysteine, and glycine, is found in all eukaryotic cells and represents one of the key nonenzymatic antioxidants in the body. It is mainly present in its reduced form, GSH. It is accompanied by three enzymes involved in its homeostasis: GR, GPX, and glutathione *S*-transferases (GST). They form the glutathione system. In the gut mucosa, the GSH system serves as an antioxidative barrier.

Thioredoxin system is composed of thioredoxin (Trx) and thioredoxin reductases (TrxR). Trx is disulfide-containing oxidoreductase that modulates activity of redox-sensitive transcription factors. In its dithiol form, it scavenges ROS and recovers from the formed oxidized Trx state (Trx-S-S) via reduction catalyzed by a flavoenzyme TrxR and NADPH.

Melatonin is a hormone of the pineal gland but can be found also in the retina, lymphocytes, GI tract, and bone marrow. It neutralizes $HO^{\cdot}$ and peroxyl radicals, $CO_3^{\cdot -}$, NO_2, $O_2^{\cdot -}$, and HOCl. The characteristic of this antioxidant is that it is irreversibly oxidized, so it is often called a suicidal or terminal antioxidant. It is a valuable protector of the mitochondria against oxidative stress, as it can pass through the mitochondrial membrane, and thus, it is an important protector of the liver from oxidative stress induced by alcohol consumption.

Exogenous Antioxidants

Vitamin C or ascorbic acid is the primary antioxidant in the plasma and cells. It is a water-soluble vitamin found in all body fluids. As an essential nutrient, it needs to be taken from foods, mainly fresh fruits and vegetables. It donates two electrons from C-2 and C-3 double-bond carbons, which results in the formation of an intermediate free radical, semidehydroascorbic acid E. The resulting ascorbate free radicals reduce to a neutral ascorbate molecule. It can react with various ROS, RNS, sulfur radicals, O_3, nitrosating compounds, and HOCl.

Vitamin E and α-tocopherol, as the most active form of this vitamin, protect cell's lipids from peroxidation by scavenging ROS, but they can also act as prooxidants and reduce transition metals. The mode of action depends on the level of α-tocopherol.

Carotenoids and vitamin A are thought to be antioxidants, but they can act as either prooxidant or oxidant depending on the level of $O_2^{\cdot}$ and carotenoids.

Minerals (zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), and selenium (Se)) are antioxidant micronutrients as they are cofactors of important antioxidant enzymes. Zn, Mn, and Cu are cofactors of superoxide dismutase (Cu/Zn-SOD), and Fe is a component of catalase. Se is a major antioxidant as it is a component of selenoproteins. Se deficiency can result in toxicity through increased $O_2^{\cdot -}$, NO, and lipid peroxidation.

Bioactive plant polyphenols are secondary plant metabolites, widely distributed in fruits and vegetables. Plant polyphenols are important dietary antioxidants, and dietary intake of these compounds can be up to 1200 mg day^{-1}. Main polyphenol classes are flavonoids, phenolic acids, and procyanidins, and they all posses strong antioxidant potential confirmed in chemically based assays. However, their direct antioxidant potential is compromised by their low bioavailability and extensive metabolism, resulting in very low levels of the parent compounds (in aglycone from) and metabolites. They can also be susceptible to the metabolic transformation by intestinal microbiota resulting in a wide variety of simple compounds, which bioactivity still needs to be confirmed. Polyphenols are shown to be potent inhibitors of XO, COX, LOX, GST, microsomal monooxygenases, and NADH oxidase and are capable to chelate transition metals and indirectly suppress ROS production. Isothiocyanates are bioactives of cruciferous vegetables, present in the form of their precursors, glucosinolates. They do not exert direct antioxidant activity, but are potent inducers of antioxidant enzymes, such as GST, and induce increase in cellular glutathione levels. They can also be cytotoxic depending on their concentration and the side chain. Less lipophilic isothiocyanates enter the cell in a time-dependant manner and react with GSH, and the decrease in GSH levels is a signaling factor for GST induction, resulting in net increase of cellular GSH levels. More lipophilic ones enter the cell very fast resulting in immediate deprivation of GSH levels and induction of cytotoxic effects. Dietary levels of isothiocyanates cannot exert toxic effects on normal cells.

Levels of Antioxidant Action

An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical-scavenging property. Antioxidants interact with free radicals and terminate chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. A large number of antioxidants, acting this way, including glutathione, ubiquinol, and uric acid, are produced in the body during normal metabolism. Other antioxidants are obtained from a diet.

The ADS assumes several different acting levels such as prevention, radical scavenging, repair and *de novo* synthesis, and adaptation as the lines of defense.

The first line of defense comprises preventive antioxidants, able to suppress the formation of free radicals. GPX, GST, phospholipid hydroperoxide glutathione peroxidase (PHGPX), and peroxidase are known to decompose lipid

hydroperoxides to corresponding alcohols. PHGPX is unique as it can reduce hydroperoxides of phospholipids integrated into biomembranes. Glutathione peroxidase and catalase reduce hydrogen peroxide to water.

The second line of defense comprises antioxidants that scavenge the active radicals to suppress chain initiation and/or break the chain propagation reactions. The examples include both hydrophilic (vitamin C, uric acid, bilirubin, albumin, and thiols) and lipophilic (vitamin E and ubiquinol).

The third line of defense is the repair and *de novo* synthesis of antioxidants. The proteolytic enzymes, proteinases, proteases, and peptidases, present in the cytosol and in the mitochondria of mammalian cells, recognize, degrade, and remove oxidatively modified proteins and prevent their accumulation. The DNA repair systems also play an important role in the total defense system against oxidative damage. Various kinds of enzymes such as glycosylases and nucleases, which repair the damaged DNA, are known.

The fourth line of defense is another adaptation mechanism, where the signal for the production and action of reactive species induces formation and transport of the appropriate antioxidant to the right site of action.

Antioxidants in Human Diet

Vitamin C cannot be stored in the body and should be included in regular diet. Important sources include citrus fruits, green peppers, broccoli, green leafy vegetables, strawberries, raw cabbage, and potatoes.

Vitamin E (*tocopherols and tocotrienols*) is a fat-soluble vitamin that can be stored in the liver and other tissues. It is often indicated for a range of states, from aging delay to sunburn healing. Important dietary sources of vitamin E are wheat germ, nuts, and seeds.

Beta-carotene, as the most studied of more than 600 different carotenoids that have been discovered, protects dark green, yellow, and orange vegetables and fruits from solar radiation damage. It is hypothesized that it plays a similar role in the body. Carrots, squash, broccoli, sweet potatoes, tomatoes, kale, collards, cantaloupe, peaches, and apricots are particularly rich sources of beta-carotene.

Selenium is an important component of antioxidant defense. Selenium should be taken from foods, as large doses that can be present in supplements can exert toxic effects. Good food sources include fish, shellfish, red meat, grains, eggs, chicken, and garlic. Vegetables can also be a good source if grown in selenium-rich soils. Cereals contain selenomethionine, a naturally occurring amino acid that is the most important nutritional source of Se.

Plant bioactives. In addition to many vitamins and minerals as nutrients, plants are rich sources of nonnutritive dietary compounds and plant secondary metabolites – phytochemicals – and many of them are bioactive, influencing different processes of the human body. One of the most investigated activities is their antioxidant potential, confirmed in numerous chemically based assays. However, very often, their metabolites, which are the form of bioactives that can be found in the circulation, lose the antioxidant potential of the parent compounds. Still, they have been shown to affect redox states in the cell by acting as signaling molecules. Depending on the particular class or subclass of polyphenols, main dietary sources of these bioactive compounds are numerous. Rich sources of phenolic acids are berry fruits, kiwi, cherries, aubergine, chicory, artichoke, potatoes, corn flour, ciders and coffee. Berry fruits are also the main dietary source of anthocyanins (subclass of flavonoids) that are also present in substantial quantities in grapes, plums, and wine. Bioactives of other flavonoid subclasses can be found in onion, kale, tomato, cherry, broccoli, berry fruits, apricots, apples, green and black tea, and beans (flavonols); parsley, celery, and capsicum pepper (flavones); citrus fruits and juices (flavanones), soy and soy products (isoflavones); and chocolate, beans, apricots, grapes, peach, apple, green and black tea, and wine (monomeric flavanols). Isothiocyanates are the main bioactives of cruciferous vegetables including broccoli, cauliflower, cabbage, kale, watercress, garden salad, turnip, and horseradish.

Glutathione (*GSH*) protects cells from toxins including free radicals. The human body produces GSH from the synthesis of three key amino acids: cysteine, glycine, and glutamic acid. Food sources with the highest amounts of naturally occurring GSH include asparagus, avocado, grapefruit, squash, potato, cantaloupe, peach, zucchini, spinach, broccoli, watermelon, and strawberries. Fish, meat, and foods that yield sulfur-containing amino acids (e.g., eggs) are the preferred sources for maintaining and increasing bodily GSH levels.

Peroxidase is an enzyme occurring especially in plants, milk, and leukocytes and consisting of a protein complex with hematin groups that catalyzes the oxidation of various substances. Food sources of peroxidase are horseradish root, soybean, mango fruit, and turnip.

Cysteine is an important antioxidant in cellular systems. Cysteine is incorporated in the cellular glutathione, which works along with vitamin E to protect cells against free radical oxidant damage. Cysteine is a nonessential amino acid, synthesized in the liver from methionine. Animal proteins are better sources of sulfur amino acids compared to proteins from vegetables. Therefore, a balanced diet that includes both animal and vegetable proteins (from grains and beans) would provide adequate cysteine intake. Excessive intake of cysteine can result in liver damage, kidney stone formation, or even some forms of neurological disturbances.

Role on Health and Prevention

Oxidative Stress and Disease

In physiological conditions, the human body is in a steady state with ROS being continuously generated and quenched. In pathological conditions, in the state of 'oxidative stress,' increased levels of ROS can cause long-term damage that has been implicated in numerous degenerative diseases. Oxidative stress has been demonstrated by depressed levels of antioxidant substances (e.g., carotenoids, vitamin C, glutathione, vitamin E, and uric acid); change in overall antioxidant capacity of plasma using different chemically based assays, including total reactive antioxidant potential, the Trolox-equivalent antioxidant capacity, the ferric reducing/antioxidant power, the oxygen radical absorbance capacity, or the ferrous oxidation-xylenol orange assays; low levels of enzymes that are important parts of the ADS or their activity (SOD, GPX, and CAT); and

increased levels of oxidation products (e.g., malondialdehyde, 8-hydroxy-2-deoxy-guanosin, oxi-LDL, and protein carbonyls) or markers of macromolecular damage (comet assay for DNA degradation and skin markers for protein degradation). Oxidative stress, as a result of either excessive generation of reactive species or disturbed antioxidant defense, has been implicated as underlying cause of various diseases. Most of previous research was targeted to the elucidation of its role on the pathogenesis of cardiovascular diseases, cancer, and neurodegenerative diseases. However, the putative list of diseases and pathological conditions considered to be associated with oxidative stress should include atherosclerosis, coronary heart disease, an impaired immune system, and increased risk of infectious disease; diabetes (both noninsulin-dependent and insulin-dependent diabetes); autoimmune conditions including rheumatoid arthritis; various respiratory diseases; and eye diseases including cataracts and retinal damage leading to age-related macular degeneration.

The effects of antioxidants on the prevention of these pathologies were supported by the results of large prospective studies (Nurses' Health Study (NHS), Health Professionals Follow-Up Study (HPFS), the European Prospective Investigation into Cancer and Nutrition (EPIC), etc.), suggesting an inverse association between diet rich in fruits, vegetables, and whole grains and the incidence of major chronic diseases, including cancer and cardiovascular diseases. Evaluation of obtained data by focusing on particular class of antioxidants or particular disease or risk factors provides more precise information. However, initiated by epidemiological studies, a great number of intervention trials were conducted in the past aiming to investigate the effects of antioxidants in the prevention (primary, secondary, or tertiary) or therapy of chronic diseases, in healthy subjects or participants with the diseases. And the results of recent meta-analyses vary between supportive, inconclusive, and even disappointing, depending on the type of antioxidant and the targeted population. However, there are many gaps that still need to be filled before the final conclusion about the relevance of antioxidant activity (both direct and indirect) as a mechanism of action of dietary compounds in the prevention and therapy of various diseases. Based on their expertise and experience drawn from up-to-date evaluations, European Food Safety Authority's (EFSA's) Panel on Dietetic Products, Nutrition and Allergies (NDA), responsible for verifying the scientific substantiation of the health claims, published a guidance document on the scientific requirements for health claims related to antioxidants, oxidative damage, and cardiovascular health. Regarding the effects of antioxidants, major concerns were related to the causal correlation between certain biomarkers and health benefit (biomarkers of plasma antioxidant status), their specificity (Comet assay and plasma markers of protein oxidation), and applied methodology (ELISA). Still, numerous studies using valid biomarkers show promising results. However, meta-analyses of studies on markers of diseases instead on markers of disease risks were less supportive. Some authors share opinion that disappointing results obtained with exogenous antioxidants are not a proof of their inefficiency but rather indicate a misleading approach with the whole body antioxidant defense as a target instead of focusing on disease relevant sites, where the disturbance has been shown and correlates with the pathogenesis. Accordingly, a strategy that will include an approach similar to that in pharmaceutical research could lead to a discovery of new, advanced, and more efficient antioxidant nutraceuticals.

Antioxidants and Cardiovascular Disease

Animal and human data implicate increased levels of ROS associated with oxidative stress in the pathogenesis of cardiovascular disease. However, in numerous clinical trials, at least with small-sized antioxidants, supplementation failed to reduce cardiovascular morbidity or mortality. Even the results from German EPIC study on the associations between vitamin/mineral supplementation and cancer, cardiovascular, and all-cause mortality were unsupportive or at least inconclusive. On the contrary, recent data from the Cancer Prevention Study II Nutrition Cohort indicated an association of flavonoid consumption with lower risk of death from CVD. Surprisingly, associations were strongest with intermediate intakes, suggesting that even relatively small amounts of flavonoid-rich foods may be beneficial. Another prospective study showed inverse association between anthocyanin intake and myocardial infarction risk. This was confirmed in large number of intervention trials, showing beneficial effects of polyphenols intake on variety of CVD risk factors and biomarkers. As shown in numerous intervention studies, polyphenols in general exert pleiotropic effects on cardiovascular health. It is still questionable if these effects were a result of direct or indirect antioxidant effects or more specific interactions with relevant molecular and cellular targets. Cruciferous vegetable consumption is associated with a reduced risk of total and cardiovascular disease mortality. Results from intervention trials were promising as well, suggesting relatively new area of isothiocyanate research.

Antioxidants and Cancer

Cancer is uncontrolled cell growth, in which cells lose their natural function and spread throughout the blood in the entire body. Oxidative stress is involved in the process of the development of cancer and tumors, due to the damage of the macromolecules by generated ROS. Beyond direct effect on DNA, ROS-induced oxidation products of lipids (malondialdehyde) can cause mutations or breaks in the DNA double chain or modifications in guanine and thymine bases and sister chromatid exchanges, which can affect the activities of signal transduction, transcription factors, and gene tumor suppressors. Oxidative damage or genetic defects in enzymes that repair mutations favor age-dependent cancer. Importantly, some chemotherapeutics or radiation therapy increases ROS and decreases antioxidant content, producing a state of severe oxidative stress, causing side effects. However, it was shown that if generation of ROS is deeply involved in the mechanism of action of anticancer agents, supplementation with antioxidants could compromise the final outcome of the therapy.

Based on Cochrane reviews, there is no scientific evidence for the effects of antioxidant supplementation in the prevention of GI cancers, lung cancer, or skin cancer. On the contrary, some antioxidant supplements seem to increase overall mortality in general population and cancer patients.

Natural compounds such as polyphenols, in particular (−)-epigallocatechin gallate and resveratrol, were shown to have a promising future as antioxidants and anticarcinogenesis agents. However, at this moment, the evidence for polyphenol intake associations with cancer incidence is mostly limited to the cancers of gastrointestinal tract. In substantial number of prospective studies, intake of cruciferous vegetables was positively associated with lower risk of breast, colon (but not rectal), prostate, and lung cancers.

Generally, the discrepancies between epidemiological data and clinical trials with supplements (mainly vitamins and minerals) might be due to the synergistic effects of various bioactives in the whole food (antioxidants, vitamins, and minerals) compared to the effects of isolated compounds.

Antioxidants and Immune Function

The protective function against external pathogens carried out by the immune system is by itself a source of ROS, since activated neutrophils produce free radicals to a significant extent. Frequent claims suggest that antioxidants benefit the immune system, but recent meta-analyses indicated that the results are uncertain even for vitamin C and its beneficial effects on common cold, despite long history of use, and null for vitamins and minerals in general, as supportive therapy in pneumonia or asthma.

Antioxidants and Gastrointestinal Diseases

The GI tract is an important source of ROS. This is a putative mechanism of various GI diseases including ulcers, malignancies, and inflammatory bowel disease. Dietary antioxidants, mainly polyphenols, have been shown to exert beneficial effects on oxidative stress in GI tract. They provide support to the protective function of epithelial barrier against deleterious effects of prooxidants and proinflammatory agents in the gut lumen through their prebiotic properties on GI microbiota, anti-inflammatory effects on GI epithelial cells, and direct interaction with their lipid bilayer. In addition to the beneficial effect of plant bioactives on the prevention of GI cancers, several studies postulated an effect on other diseases of GI tract.

Antioxidants and Aging

For many people, the greatest interest is in antioxidants' anti-aging potential. Since the body's production of its own antioxidants decreases with age, few doubt the potential value of dietary sources. However, there is no evidence that supplementation with antioxidants will stop hair graying, prevent wrinkles, or provide a 'fountain of youth.' However, promising results were obtained in a study investigating the effects supplementation with vitamins and mineral antioxidants (SU.VI.MAX) on cognitive performance. A direct correlation was also shown for cognitive performance and total flavonoid intake in a prospective Nurses' Health Study. In general, cognitive function is considered as a very promising target for the action of polyphenols and is relevant, considering the rise in the percentage of aged population in developed countries.

Antioxidants and Macular Degeneration

Some positive messages were expected from studies of particular antioxidants in macular degeneration, the major cause of blindness in elderly people. The proposed mechanism is based on the fact that ROS, produced in retina during light absorption, are deeply involved in the progression of the disease. Some (but not all) studies initially suggested that specific antioxidant supplements help in the protection against further degeneration. The final conclusion drawn in the most recent Cochrane review was supporting, with the limitation that most of the data were taken from one randomize trial. The results for age-related cataract were negative, showing no effects of antioxidants (beta-carotene, vitamin E, and vitamin C) in the prevention and progression delay. There are also some evidences of benefits from vegetables and fruits rich in lutein and zeaxanthin as antioxidants. Egg yolk is also a good source of these compounds. A recent and extensive review reports no benefits of vitamin E, beta-carotene, or any antioxidant supplements for preventing age-related macular degeneration.

Antioxidants and Neurodegenerative Diseases

Oxidative processes have been implicated in the pathogenesis of neurodegenerative diseases including Alzheimer's and Parkinson's diseases. The suggestion that they are triggered, at least in part, by oxidative and nitrosative stress and sustained by inflammatory cytokine production rationalizes the protection of the central nervous system against these damaging mechanisms with antioxidants as a useful prophylactic and therapeutic approach. The results of both the Rotterdam Study and the Cache County Study supported the hypothesis that high dietary intake of vitamin C and vitamin E may lower the risk of Alzheimer's disease, but not cognitive decline and dementia in general. Additionally, based on the systematic review of the literature, other antioxidants that could be effective in the prevention of Alzheimer's disease include garlic extract, curcumin, melatonin, resveratrol, Ginkgo biloba extract, and green tea. Generally, the Kame project confirmed a hypothesis that polyphenol-rich fruit and vegetable juice intake may exert beneficial effects in delaying the onset of Alzheimer's disease, particularly among susceptible individuals. Recent studies support the use of antioxidants specifically targeted to the mitochondria as promising therapy of Parkinson's disease.

Further Reading

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Appetite Control in Humans: A Psychobiological Approach

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Defining the Issue

One of the most salient features of appetite control in humans is that we are omnivores. Unlike herbivores or carnivores, humans have the capacity to consume a larger and more diverse range of food materials. Therefore, there is no standard or uniform pattern of human food consumption. Although there is a strong biological component to the basic drive that motivates humans to seek food, there is not a strong biological control over the types of foods that humans put into their own mouths. The food selected for ingestion is heavily influenced by culture, economy, geography, climate, and social conditions – in other words, by the environment. Therefore, the human system has the capacity to adapt to many different types of diet, and humans have the ability to learn to deal with foods according to their availability. Considering the diverse types of food items consumed by humans in different parts of our planet, it can be deduced that this aspect of appetite is not heavily programmed; rather, the system is adaptable. However, once a range of foods has been presented for consumption, some clear biological principles start to exert an influence (e.g., through hedonic processes). In seeking explanations for the way in which humans seek, select, and consume (and overconsume) food, it is important to recognize the influences of biology and culture – and how they interact.

The Psychobiological System of Appetite Control

The control of appetite is based on a network of interactions that form part of a psychobiological system. This system can be conceptualized on three levels: firstly, the level of psychological events (e.g., hunger perception, hedonic sensations, and cravings) and behavioral operations (e.g., meals, snacks, macronutrient, and energy intakes); secondly, the level of peripheral physiology and metabolic events; and, finally, the level of neurotransmitter and metabolic interactions in the brain. Appetite reflects the synchronous operation of events and processes in these three levels and when appetite is disrupted – as in certain eating disorders – these three levels become desynchronized. While neural events trigger and guide behavior, each act of behavior involves a response in the peripheral physiological system; in turn, these physiological events are translated into brain neurochemical activity. This brain activity represents the strength of motivation to eat and the willingness to refrain from feeding. The lower part of the psychobiological system illustrates the satiety cascade (see Figure 1), which depicts the events that stimulate eating and motivate organisms to seek food. It also depicts those behavioral actions that actually form the structure of eating, in addition to the processes that follow the termination of eating – referred to as either postingestive or postprandial events.

Prior to food reaching the mouth, physiological signals are generated by the sight and smell of food. These events comprise the cephalic phase of appetite, and they function to anticipate the ingestion of food and are generated in many parts of the gastrointestinal (GI) tract. During and immediately following eating, afferent information (sensory stimulation from the taste and texture of food) provides the major control over appetite. Further to this, it has been noted that the afferent information from ingested food acting in the mouth provides primarily positive feedback for eating; that from the stomach and small intestine is primarily negative feedback.

Signals of Appetite Control and the Drive to Eat

Traditionally, a distinction has been made between the short-term regulation and the long-term regulation of appetite; however, the connotation of episodic and tonic is more functionally appropriate. Episodic signals of appetite control are for the most part inhibitory, although they can be excitatory, and are typically generated in response to an eating episode. These signals oscillate throughout the day in accordance with the pattern of eating, and most are closely associated with the signaling of satiety. Tonic signals arise from the body's energy stores – including the adipose tissue and fat-free mass – and exert a tonic pressure on the expression of appetite. These two sets of signals – one set responding sharply to changes in behavior (episodic) and the other providing a slow modulation (tonic) – are integrated by complex brain networks that control the overall expression of appetite (see Figure 2).

Tonic Signals

The tonic signals of appetite arise from the body's energy stores and exert a tonic pressure on the expression of appetite. When in a state of energy balance, the control of appetite is considered to be largely under the influence of homeostatic mechanisms with strong defenses to guard against substantial loss of body weight and fat mass but comparatively weak mechanisms in place that mitigate long-term increases in these variables.

Fat-free mass, resting metabolic rate, and the drive to eat

For many years, the main focus of investigations on appetite control has centered on the termination of eating; however, 50 years ago, there was an equal emphasis on the excitatory features of appetite in an attempt to explain what the driving forces were behind the motivation to seek out food. Around this time, a group of physiologists posited that energy expenditure was the main driver for appetite. However, due to the large daily fluctuation in levels of physical activity, Edholm found no within-day relationship between energy intake and energy expenditure. More recently, this proposition has been reexamined with the suggestion that resting metabolic rate

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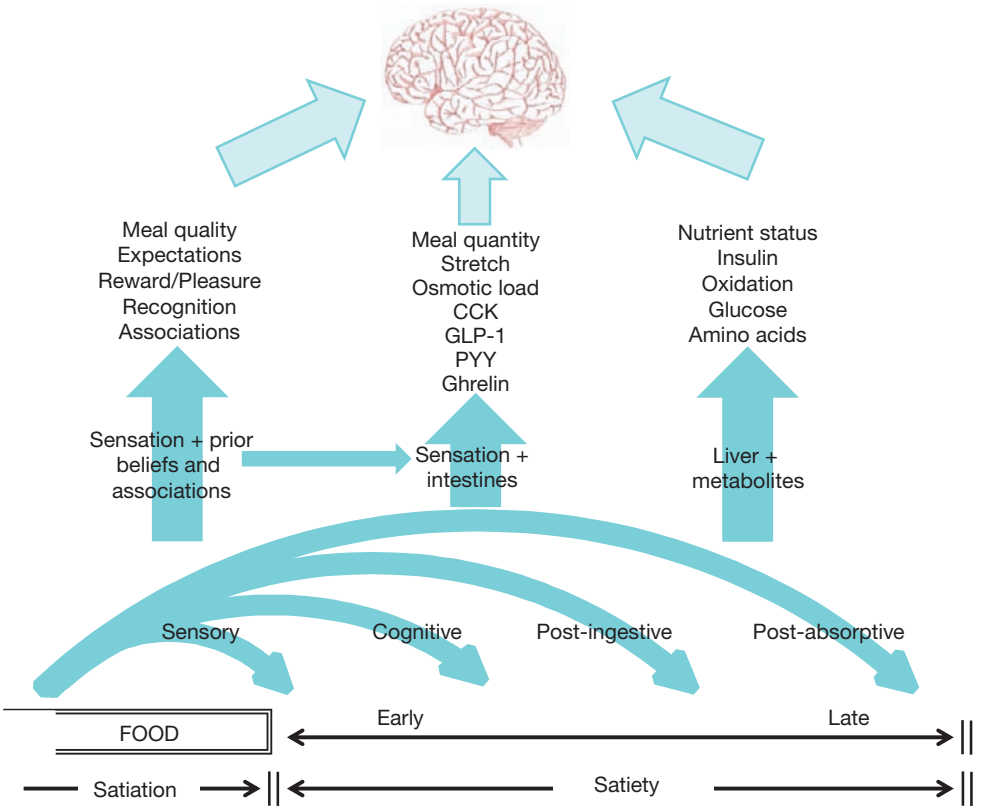

Figure 1 The satiety cascade. The satiety cascade describes the sensory, cognitive, postingestive, and postabsorptive processes. Reproduced from Blundell, J. E. (1991). Pharmacological approaches to appetite suppression. *Trends in Pharmacological Sciences 12*, 147–157.

(RMR) – the largest component of daily energy expenditure – may act as a regulator of food intake and appetite control. Blundell and colleagues examined this proposition in a sample of overweight and obese individuals using a novel energy balance framework, which included controlled measures of body composition and resting metabolism, and objectively measured within-day food intake and sensations of appetite. They demonstrated that fat-free mass was closely associated with self-determined test meal intake and overall daily energy intake whereas BMI and fat mass were not. In a follow-up study, Caudwell et al. demonstrated that RMR was a strong predictor of daily food intake and that individuals with the highest levels of RMR also reported the highest levels of hunger in the periods between laboratory test meals. These findings have subsequently been confirmed in other studies examining obese individuals.

The association between fat-free mass/RMR and the motivation to eat helps to explain why obese individuals, who carry large amounts of stored energy, still experience strong feelings of hunger and an increased drive to eat. By recognizing that obese individuals have, in addition to a large amount of adipose tissue, an increased level of fat-free mass, which results in a proportionally raised RMR and coincides with their apparent increased drive to eat. Furthermore, the enhanced drive to eat is quite compatible with, and independent of, the accumulation of fat stores and development of leptin resistance, as fat mass makes a relatively minor contribution to RMR in comparison to fat-free mass.

Signals from adipose tissue: role of leptin

While recent evidence supports a role for the activity of fat-free mass/RMR as a driver of appetite, a classic theory of appetite control concerns a signal that informs the brain about the state of the adipose tissue stores. This idea has given rise to the notion of a lipostatic or ponderstatic mechanism. Indeed, this is a specific example of a more general class of peripheral appetite signals believed to circulate in the blood reflecting the state of depletion or repletion of energy reserves that directly modulate brain mechanisms. In 1994, in a landmark study, Zhang et al. identified the mouse gene responsible for obesity. This led to the identification of a hormone termed leptin that is mainly expressed by adipocytes but is also present at lower levels in the gastric epithelium and circulates in the blood. To date, the exact association between leptin and weight regulation has not been completely determined. However, it has been reliably demonstrated that the amount of leptin in the plasma is greater in obese human and nonhuman animals compared to their lean counterparts. Therefore, while leptin serves as a signal from the adipose tissue to the brain, high levels of leptin do not prevent weight gain or obesity but rather reflects the amount of adipose tissue within the body. This suggests that obese individuals are resistant to the anorectic effects of leptin, which may be the result of slower transportation across the blood–brain barrier or defective signaling in the leptin responsive neurons. Moreover, under normal conditions of appetite control, leptin does not appear to exert any appreciable influence on food intake. This is quite different of course

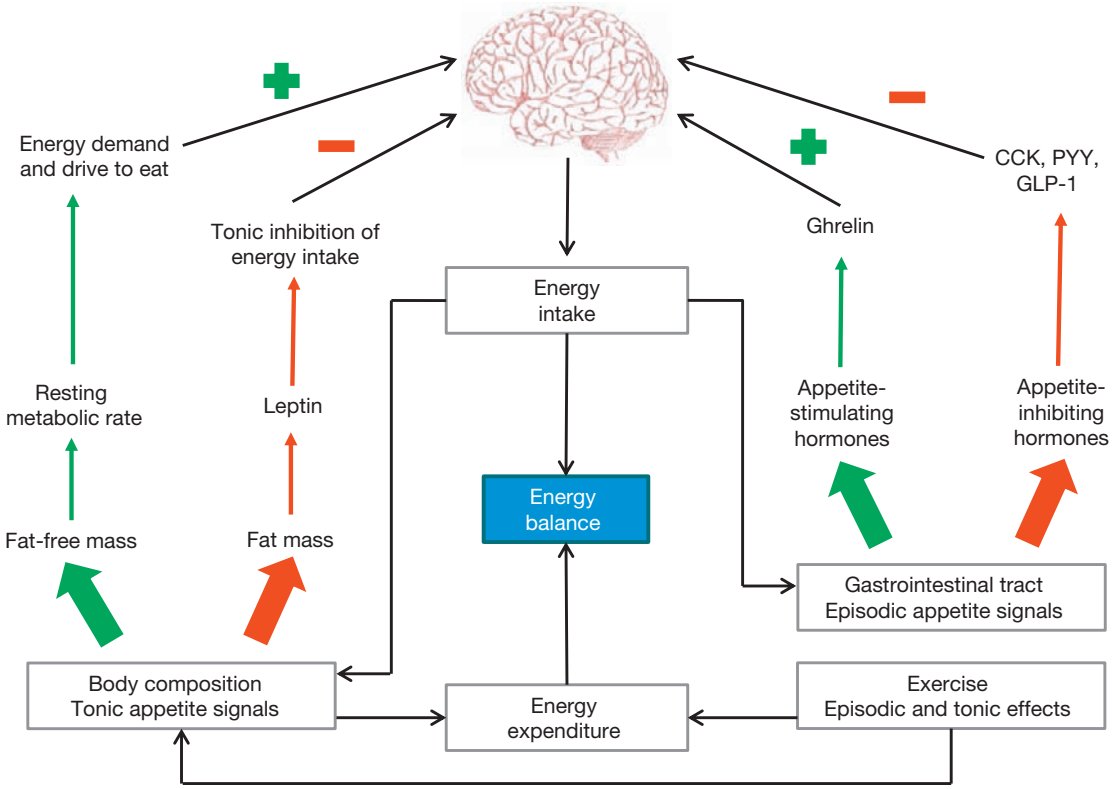

Figure 2 A new formulation for appetite control developed by Blundell et al. (2012). Role of resting metabolic rate and energy expenditure in hunger and appetite control: A new formulation. *Disease Models & Mechanisms* 5(5), 608–613.

from the effect of leptin in those rare individuals who are leptin deficient.

Episodic Signals: The Satiety Cascade

Eating in omnivores is not a single continuous process but occurs in episodes (usually called meals). The types of signals involved in terminating a meal, the process of 'satiation,' and preventing further consumption, the process of 'satiety,' can be represented by the satiety cascade. Developed more than 25 years ago, the satiety cascade provides a conceptual framework from which to examine the processes that influence the frequency and size of eating episodes and the intervals between them. The amount of food consumed in a meal (satiation) is heavily influenced by the weight and volume of the food itself and by the energy density of the food items. High-energy dense foods, such as those high in fat, lead to unnecessary intake of calories. This process is termed 'passive overconsumption.' The satiety cascade (see Figure 1) indicates the different overlapping processes – arising from eating – that influence the intensity and duration of satiety.

Brain signals of hunger

Although it seems that hunger is driven by the energy requirements of the body reflected in RMR (and FFM), this motivation must be routed through the brain and will be encoded in physiological pathways. One hormonal biomarker seems to be ghrelin. Ghrelin is primarily secreted from the stomach (although it is synthesized and secreted in many other tissues) and was the first orexigenic peptide to be identified in the periphery. Ghrelin plays a role in meal initiation with circulating levels being elevated before and then suppressed following food intake. Consistent with this, both peripheral and central infusions of ghrelin have been shown to stimulate food intake in rats and mice. In addition, postprandial ghrelin levels within physiological range have been shown to be associated with both hunger and energy intake. While predominantly an episodic hormone, ghrelin may also be considered as a tonic hormone as it has been shown to correspond with the body's energy stores. For example, Shiiya et al. demonstrated that obese individuals have lower levels of acylated ghrelin compared with their normal weight counterparts. However, contrary to expectations, they did not demonstrate that lower levels of ghrelin observed in obese individuals were associated with lower levels of hunger or desire to eat suggesting that decreased sensitivity to ghrelin signaling following weight gain may contribute to impaired appetite control. Neuropeptide Y (NPY) is expressed in both the central nervous system and the peripheral nervous system and is released from the hypothalamus during periods of fasting and in situations that demand an increase in energy (e.g., exercise). Bannon et al. demonstrated that NPY knockout mice had a lower food intake compared to wild-type mice in response to fasting supporting a role for NPY in mediating food intake.

Signals of satiety and the termination of eating

Much current thinking favors the view that hormones released from the GI tract play a role in either the termination of an eating episode (satiation) or the inhibition of eating after the

meal has finished (satiety). One celebrated hormone is cholecystokinin (CCK). CCK is released from the small intestine shortly following food consumption and has been shown to mediate satiation. Peripheral administration of CCK has been demonstrated to decrease food intake. The consumption of fat specifically has been shown to stimulate the release of CCK, and fats in the form of free fatty acids of carbon chain lengths C12 and above produce pronounced CCK releases. GLP-1 is an incretin hormone (one that stimulates the release of insulin), which is released from the gut into the blood stream in response to intestinal nutrients. Peripheral administration of GLP-1 has been found to inhibit food intake in both human and nonhuman animals, and in normal weight males, infusions of synthetic human GLP-1 during the consumption of a fixed breakfast test meal have been demonstrated to enhanced ratings of fullness and satiety when compared to a placebo. PYY is released from endocrine cells in the distal small intestine and large intestine. PYY is released predominantly after rather than during a meal and causes a decrease in gastric emptying (the so-called ileal brake) and is therefore more associated with postmeal satiety. Research has shown that peripheral administration of PYY decreases food intake in both human and nonhuman animals. More recently, reports suggest that obese individuals have an attenuated meal-stimulated PYY response across a range of caloric loads suggesting that PYY may have a role in the control of body weight.

The adaptability of the appetite system is apparent in the GI response to different types of nutrients. GI peptide release (CCK, GLP-1, insulin, and PYY) varies with the fat, protein, and CHO content of the food ingested. Consequently, different profiles of peptides can be shown to produce an equivalent degree of satiety. There does not appear to be any unique satiety peptide or any single unique pattern. The search for a biochemical identity of a satiety biomarker has been driven by the belief in a molecular explanation for behavior. Considering the inhibition of food intake in the postprandial period, the role of the stomach should not be overlooked. The characteristics for gastric distension and the mechanisms of gastric emptying constitute physiological processes, which operate to modulate the feeling of fullness and the motivation to eat (this means that they influence the intensity and time course of satiety).

Homeostatic and Hedonic Processes of Appetite Control

Energy balance depends on both what and how much food is consumed in relation to energy expenditure. The qualitative aspects of eating behavior (what to eat) depend in part on the direction of food preferences, driven by the anticipation and experience of pleasure derived from food. The quantitative aspects of eating behavior (how much to eat) reflect a general drive and inhibition of this drive to eat (the processes of satiation and satiety). This distinction between drive and direction is often framed in terms of homeostatic and hedonic systems of appetite control. The homeostatic system refers to the regulation of food intake that arises from biological need and acts to maintain the body's energy stores and internal environment. It comprises a network of brain neurotransmitters and GI peptides described previously and depicted in Figure 1. The hedonic system refers to the sensory and external examination of food intake and considers that eating behavior is motivated by external cues in the environment and does not solely arise as a result of energy need. Studies in nonhuman animals have reported that the hedonic system of appetite control is underpinned by the brain's reward circuitry and involves opioid and dopamine neurotransmission that underpins the liking (subjective pleasure experienced from food) and wanting (attribution of incentive salience to the reward and its associated cues) components of reward, respectively. While the homeostatic and hedonic systems of appetite control are underpinned by distinct neural substrates, there is considerable functional overlap between the two systems in the control of appetite.

Psychological Components of Liking and Wanting for Food

The terms 'liking' and 'wanting' are not only used to refer to the core processes of reward identified in behavioral neuroscience research but also discussed in relation to subjective states and objectives behaviors that correspond to the more everyday meaning of these terms. Like the core processes of reward identified in behavioral neuroscience, liking and wanting as psychological constructs are believed to be distinct. Liking is typically defined as the perceived or expected hedonic value of a food, the appreciation of its sensory properties, or a judgment of the degree of pleasure it elicits. In this context, liking for food appears to be a relatively enduring characteristic within an individual that varies only slightly under specific circumstances. For instance, researchers have demonstrated that ratings of liking for food are greater when individuals are in a hungry compared to a fed state. Further to this, liking for a recently consumed food has been shown to decrease in a manner consistent with sensory specific satiety. Therefore, liking is thought to be more important in establishing the range of foods eaten and in determining the motivational value of a food.

On the other hand, wanting refers to subjective states of desire and craving that are triggered by the food itself or its related cues within in the environment. Importantly, rather than being a constant drive, such as hunger, the wanting component of reward implies a target with a direction that may vary according to a number of factors, including level of hunger, time of day, and the degree of attentional resources available. To this end, wanting for food is created anew on each encounter with the food or its associated cues. Furthermore, research suggests that the target of wanting may vary from being relatively broad to being relatively focussed. For instance, it has been reliably demonstrated that, independent of BMI, in a fasted state, individuals have a greater wanting for food in general compared to when they are in a fed state. Further to this, there is some evidence to suggest that wanting may become focussed to specific food items (and at times dissociated from liking) under certain conditions in which one food is wanted to a greater extent than the available alternatives. For example, Griffioen-Roose et al. assessed the impact of a 14-day high- or low-protein dietary intervention on ad libitum energy intake (over 2.5 days) and food reward. With regard to energy intake, they found that participants following a low-protein diet consumed a significantly greater amount of protein

compared to those who adhered to a high-protein diet. Further to this, following a low-protein diet, implicit wanting (assessed using the Leeds Food Preference Questionnaire) was enhanced for high-protein foods, which was consistent with the participants' actual eating behavior but was not observed in the subjective measures of reward.

Individual Differences in the Control of Appetite

The expression of appetite involves the interaction of both homeostatic and hedonic processes. The pleasurable response to food that may stimulate consumption will be coordinated with the satiation and satiety processes that inhibit eating, and it is important to recognize that the expressed pattern of eating will be dependent upon influences from both systems and that both hedonic and homeostatic processes influence the regulation and potential dysregulation of appetite control. As follows, overeating and obesity may arise due to either some defect in homeostatic signaling that fails to inhibit the motivation to eat or due to excessive or inappropriate responding to the hedonic aspects of food.

Obesity and Weight Gain

Individual differences in the neural response to the rewarding aspects of food have been identified as a risk factor for overeating, weight gain, and the development of overweight and obesity. One theory of the role of reward in obesity proposes that obese individuals have a hypofunctioning reward system, which causes them to overeat palatable, rewarding foods as a means of compensating for this deficit. In a landmark positron emission topography study, Wang et al. demonstrated that extremely obese individuals ($BMI > 40\ kg\ m^{-2}$) had reduced striatal dopamine D2 receptor binding compared to lean individuals. In addition, functional magnetic resonance imaging studies have shown that compared to their lean counterparts, obese adolescents show less activation in the dorsal striatum in response to the consumption of a palatable milk shake versus a tasteless control solution. However, it should be noted that research regarding reduced dopamine availability and obesity has been mixed. An alternative theory for the role of reward in obesity is the reward surfeit model, which posits that individuals who experience a greater amount of reward from food are at risk of overeating and weight gain. For example, Dimitropoulos et al. found that compared to normal weight individuals, obese individuals exhibited an increased neural response to high- and low-calorie food images in brain regions associated with reward despite having low levels of hunger. Furthermore, Yokum et al. demonstrated in a sample of female adolescents that greater activation in the orbitofrontal cortex during the initial orientation of attention to palatable food images was associated with an increase in BMI at 1-year follow-up.

Satiety Responsiveness

Impaired appetite control may be attributed to a weakened satiety response to food. For example, some obese individuals report that their own eating patterns bear little or no relation to their feelings of hunger or fullness, which suggests they may have an altered or weakened recognition and response to these internal sensations. Barkeling et al. examined obese patients who reported either a 'good relationship' between their appetite and eating (i.e., start eating when hungry; stop eating when full) or 'no relationship' between their appetite and eating (i.e., never hungry before meals; never full following meals). While Barkeling et al. found no difference in sensations of desire to eat, hunger, or fullness in relation to fixed-energy test meals consumed in the laboratory, they did report that those with 'no relationship' between appetite and eating exhibited a relatively weaker suppression of prospective consumption (i.e., how much additional food could be eaten) immediately after meals. Further research by Drapeau and colleagues has identified some of the psychobiological characteristics of individuals who experience a weakened satiety response to food – termed the 'low-satiety phenotype.' The low-satiety phenotype displays a cluster of disadvantageous tendencies that can stimulate eating and prevent its termination. Individuals show many different ways of expressing appetite and there is no single dominant pattern. Humans are omnivores and an adaptable appetite system is a feature that reflects biological variability in response to environmental opportunities and challenges. An obesogenic culture confronts humans with numerous problems that can compromise healthy appetite behavior.

See also: Adipose Tissue: White Adipose Tissue Structure and Function; Energy: Intake and Energy Requirements; Gut Hormones; Hunger; Satiety.

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Apples

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Sources and Production

Apples (*Malus domestica*) are some of the most ancient, widely cultivated, and consumed tree fruits (Figure 1). The cultivation of apples can be traced back to 4000 years ago in Asia, and currently, more than 7500 different cultivars of apples are grown in all continents in the temperate and subtropical zones. Cultivated apples have been consumed fresh or processed into beverages, vinegar, sauces, jellies and jams, and used in pastry. People enjoy eating apple for its sweet and fruity aroma; its juicy tastes that vary from sour, to tart, to sweet; and its crispy and crunchy texture. In addition to the great flavor and taste, apples are also liked because of its rich nutrient content.

While many commercial varieties have been developed, only a few dozen are commercially grown. Many factors can affect the consumer preference and thus the production of different varieties. In addition to texture, taste, and flavor and nutritional attributes, preference of apple also depends on the region and cultural background. North Americans and Europeans tend to favor sour-tasting apples, while Asians generally like very sweet apples. Golden Delicious apple continues to be the most popular variety in Europe, followed by Gala, Idared, Red Delicious, and Jonagold, whereas in the United States, the top five most popular varieties are Red Delicious, Gala, Fuji, Golden Delicious, and Granny Smith and recent introductions such as Honeycrisp and Ambrosia. Most of the commercial apple cultivars are bred for fresh consumption with a few specifically for cooking or cider making. In Canada, for example, 59% of the apples were consumed fresh, 36% as juice, and 5% in processed forms.

Apples ranked number 17 of the 20 top agricultural commodities and the second globally in the fruit category after grapes. Apples are produced in every continent. According to the United Nations Food and Agriculture Organization, in the early 1960s, the United States was the leading apple producer, followed closely by mostly European countries including Italy, France, and the Union of Soviet Socialist Republics (USSR). That order changed dramatically throughout the 1970s and 1980s when the USSR became the world's largest apple producer by tripling its production (Table 1). Meanwhile, China became the third largest apple-producing country in the early 1980s, and the growth continued. In 1991, total productions of apples from USSR, China, and the United States were nearly the same. However, from the beginning of the twenty-first century, China became the sole leader of the world's apple production, producing 20 million metric tons annually in 2001 and nearly 36 million metric tons in 2011, while productions in other top five countries stayed relatively at the same level during the same period. In recent years, India has become one of the top five apple producers in the world. In 2012, China produced 37 million metric tons of apples, contributing more than 15 billion dollars to its economy, followed by the United States, Turkey, Poland, and India at 4.11, 2.89, 2.88, and 2.20 metric tons, respectively (Figure 2).

Patterns of Consumption

Apples are most often consumed fresh. The entire fruit except for the seeds in the apple core (Figure 1) is edible, although in certain cultures such as in Asia, apples are often peeled before consumption. In recent years, minimally processed fresh-cut apple slices have become increasingly popular. Eden is a newly developed (GMO) nonbrowning apple cultivar in Canada, suitable for fresh-cut market. Apples are processed by pressing or milling to produce beverages to be either consumed as is or mixed with other fruit juices. Most clear apple juices or beverages containing it are filtered and pasteurized, and packed in cans, bottles, or juice boxes, and can be kept at room temperature and usually sold in stores with shelf life extending over a year. Nonfiltered apple juice (or apple cider in North America), particularly nonpasteurized apple cider, is more flavorful but normally sold in refrigerated display cases or produce sections of stores with a shelf life of $\sim$2 weeks. Apple juice is also fermented to produce hard cider (in North America) and other alcoholic beverages and further to produce apple vinegar. Cider apples are traditionally classified according to the tannin and acid contents; however, recent advances in technology have led to the proposal of a new method of classification.

In addition to the various apple-based beverages, apples are also cooked or processed to make different products or ingredients of products. Cooking apples may include some cultivars for fresh consumption but are normally special cultivars that are tart and more acidic. These are made into applesauce or apple butter, which can be used as a condiment or dipper or served alone as a dessert. Apples are also made into treats and confectionaries such as caramel apples. Apples are also an important ingredient of baked desserts such as apple pie and apple crisp in Western culture.

Nutrient Contents of Apple

As one of the most nutritious fruits, apple is a rich source of minerals, vitamins, dietary fibers, and phytochemical antioxidants such as polyphenols. There is no better exemplification than the old English adage 'An apple a day keeps the doctor away,' which sends a clear and accurate message on the nutritious and health promotional value of apple consumption.

Apples are high in dietary fiber, low in fat, and rich in vitamins. One medium-sized apple (3″ diameter, 182 g) contains 18% of the recommended daily value of dietary fiber, 14% vitamin C, 6% potassium, 5% vitamin K, 4% vitamin B6, 3% iron, 3% riboflavin, and many other nutrients (Table 2). These

Figure 1 A typical apple variety – Gala.

components provide some of the most essential nutrients to humans and collectively contribute to the healthful properties of apples. However, these nutrients alone may not explain the health benefits of apples. Recent studies have shown that phytochemicals, particularly polyphenols, contribute significantly to the risk reduction and health-promoting effects of apples. Polyphenols in apples are mostly found in the skin. In addition to simple phenolic acids such as gallic acid and ferulic acid, flavan-3-ols such as catechin and epicatechin and their oligomers such as procyanidins B1 and B2 are found to exist in the skin, and flavonols such as quercetin and its glycosides are mostly found in the flesh. Structures of the major polyphenolic compounds found in apple and the concentrations are shown in Figure 3 and Table 3.

Availability, Absorption, and Metabolism

Reviewing and discussion on the physiological effects and mechanism of action of all nutrients in human body are beyond the scope of this article as there have been plenty of research on how essential nutrients such as vitamins and minerals are absorbed and metabolized and how the bioavailability of these nutrients is affected by various factors in humans. Instead, discussion in this section will be focussed on the most prevalent bioactive phytochemicals, the polyphenols. Emphasis will be given particularly on the stability, bioaccessibility, and bioavailability of apple polyphenols and how these compounds are metabolized and absorbed into important tissues and organs to exert various biological functions.

Just as in all plants, the biosynthesis of phytochemicals such as the polyphenols can be affected by genetics and environment. The latter factor may include geographic location (e.g., temperature, altitude, rainfall, and soil), agronomic practice, growing season, and postharvest storage conditions and processing methods (in the case of producing juice, cider, and other apple-based foods or food ingredients). All these factors are known to affect the quality and quantity of the total and individual polyphenolic contents, consequently the bioavailability of these compounds. Farming practice, that is, organic versus conventional, does not seem to affect the bioavailability of polyphenols of apple.

In general, apples have a very long shelf life compared to other fruits. Under low temperature and controlled atmosphere, apples can be stored for up to a year. Research showed that polyphenols of four different apple cultivars (Jonagold, Golden Delicious, Cox's Orange Pippin, and Elstar) and the concentrations of the major individual compounds such as flavonols, catechins, phloridzin, and chlorogenic acid and the antioxidant activities were not affected by long-term storage, both at refrigerator temperature (25 weeks) and under controlled atmosphere (52 weeks) conditions. Other studies have in fact suggested that concentrations of polyphenols such as catechin and phloridzin can be significantly increased during storage. Processing can significantly reduce the concentration of polyphenols. Studies showed that the levels of flavonoids and chlorogenic acid in conventionally produced juice were reduced to between 50% (chlorogenic acid) and 3% (catechins) compared to the respective fresh apples, because most of the polyphenols were retained in the pomace rather than being transferred into the juice. Novel production method can markedly enhance the extraction of the polyphenol antioxidants into the juice. The levels of flavonoids and chlorogenic acid in juice made by novel method were between 1.4 (chlorogenic acid) and 9 (quercetin glycosides) times higher than in conventional apple juice. The stability of polyphenols in apple juice can be affected by temperature and the presence of oxygen, but the effect was found to be minimal. Different compounds had different sensitivities to these factors; for example, phloridzin and chlorogenic acid are relatively more stable than quercetin glycosides and epicatechin. The polyphenolic content and antioxidant activity of enriched apple juice were found to be quite stable at ambient or refrigerated storage conditions up to half a year. Processing can also affect the health effect of apple. Apple juice made from fresh apples was consistently found to have better efficacy on improving serum lipid profile and other biomarkers than apple.

Polyphenols in fresh or processed apples must also be stable during human ingestion, reach the colon, and made available at certain concentrations in target tissues and organs in order for these bioactives to have favorable physiological effects. Studies have shown that polyphenols in general are not as biologically available as vitamins. The plasma concentrations of ascorbic acid (vitamin C), α-tocopherol (vitamin E), coenzyme Q10, and naringenin glycoside (a flavonoid) were found to be 72, 19, 0.4, and 0.33 μM, respectively, in young women consuming 16 oz of grape juice for 3 months. An *in vitro* model simulating gastrointestinal (GI) digestion, including dialysability, was used to assess free soluble polyphenols from apples. Results indicated that polyphenols were mainly released during the gastric phase (~65% of phenolics and flavonoids compared to <10% during intestinal digestion). Anthocyanins were only present in the gastric phase. Dialysis experiments using a semipermeable cellulose membrane showed that free soluble dialyzable polyphenols and flavonoids were approximately 20% and 30% lower than those of the GI digesta. The antioxidant capacities of dialyzable antioxidants were related to the corresponding concentrations of individual compounds. Studies have shown that polyphenols of apple are absorbed from or metabolized in the small intestine. In patients with

Table 1 The world's top five apple-producing countries

	1961		1971		1981		1991		2001		2011	
Rank	*Area*	*Production (MT)*	*Area*	*Production (MT)*	*Area*	*Production (MT)*	*Area*	*Production (MT)*	*Area*	*Production (MT)*	*Area*	*Production (MT)*
1	The United States	2584000	USSR	5080000	USSR	6334000	China	4540445	China	20014986	China	35985000
2	Italy	2167000	France	2967000	The United States	3510620	USSR	4705000	The United States	4276810	The United States	4275108
3	France	2142000	The United States	2890820	China	3006000	The United States	4402500	Turkey	2450000	India	2891000
4	USSR	1744000	Germany	2309000	Italy	1741600	Turkey	1900000	Poland	2433940	Turkey	2680075
5	Japan	955400	Italy	1697300	Turkey	1450000	Italy	1830170	France	2397000	Poland	2493078

Source: FAO: http://faostat.fao.org/site/339/default.aspx (accessed on 6 October 2014).

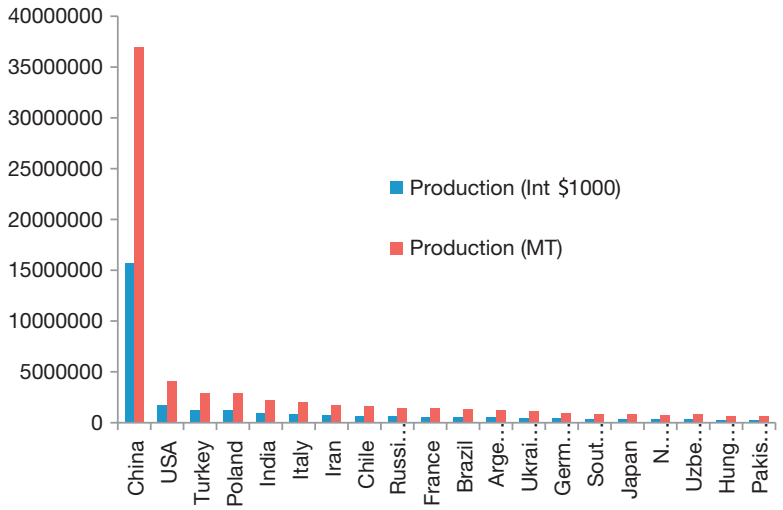

Figure 2 World apple production in year 2012. Data extracted from FAO: http://faostat.fao.org/site/339/default.aspx (accessed on 6 October 2014).

Table 2 Major nutritional components of apples, with skin (edible parts) nutritional value per 100 g

		Value per 100 g				
Nutrient	*Unit*	*Raw, with skin*[a]	*Raw, without skin*[b]	*Juice without sweetener or ascorbic acid*[c]	*Pie filling, canned*[d]	*% Recommended daily value**
Proximates						
Water	g	85.56	86.67	88.24	73.4	
Energy	kcal	52	48	46	100	
Protein	g	0.26	0.27	0.1	0.1	1
Total lipid (fat)	g	0.17	0.13	0.13	0.1	
Carbohydrate, by difference	g	13.81	12.76	11.3	26.1	8%
Fiber, total dietary	g	2.4	1.3	0.2	1	18%
Sugars, total	g	10.39	10.1	9.62	13.8	
Minerals						
Calcium (Ca)	mg	6	5	8	4	1
Iron (Fe)	mg	0.12	0.07	0.12	0.29	3
Magnesium (Mg)	mg	5	4	5	2	2%
Phosphorus (P)	mg	11	11	7	7	2
Potassium (K)	mg	107	90	101	45	6
Sodium (Na)	mg	1	0	4	47	
Zinc (Zn)	mg	0.04	0.05	0.02	0.04	1
Vitamins						
Vitamin C	mg	4.6	4	0.9	1.7	14
Thiamin	mg	0.017	0.019	0.021	0.012	2
Riboflavin	mg	0.026	0.028	0.017	0.011	3
Niacin	mg	0.091	0.091	0.073	0.035	1
Vitamin B6	mg	0.041	0.037	0.018	0.016	4
Folate, DFE	µg	3	0	0	0	1
Vitamin B12	µg	0	0	0	0	
Vitamin A, RAE	µg	3	2	0	2	2
Vitamin A, IU	IU	54	38	1	24	
Vitamin E (α-tocopherol)	mg	0.18	0.05	0.01	0.04	2
Vitamin D (D2 + D3)	µg	0	0	0	0	
Vitamin D	IU	0	0	0	0	
Vitamin K (phylloquinone)	µg	2.2	0.6	0	0.5	5
Lipids						
Fatty acids, total saturated	g	0.028	0.021	0.022	0	
Fatty acids, total monounsaturated	g	0.007	0.005	0.006	0	
Fatty acids, total polyunsaturated	g	0.051	0.037	0.039	0	
Cholesterol	mg	0	0	0	0	

A medium-sized apple (3″ diameter) is about 182 g.

[a–d]Nutrient Database (NDB) numbers 09003, 09004, 09016, 19312. Report run on 6 October 2014; http://ndb.nal.usda.gov/ndb/.

*Percent daily values are based on a 2000-calorie diet. Your daily values may be higher or lower depending on your calorie needs (values <0.5% were not included; values >0.5% were rounded).

Source: USDA National Nutrient Database for Standard Reference 27 Software v.2.0b.

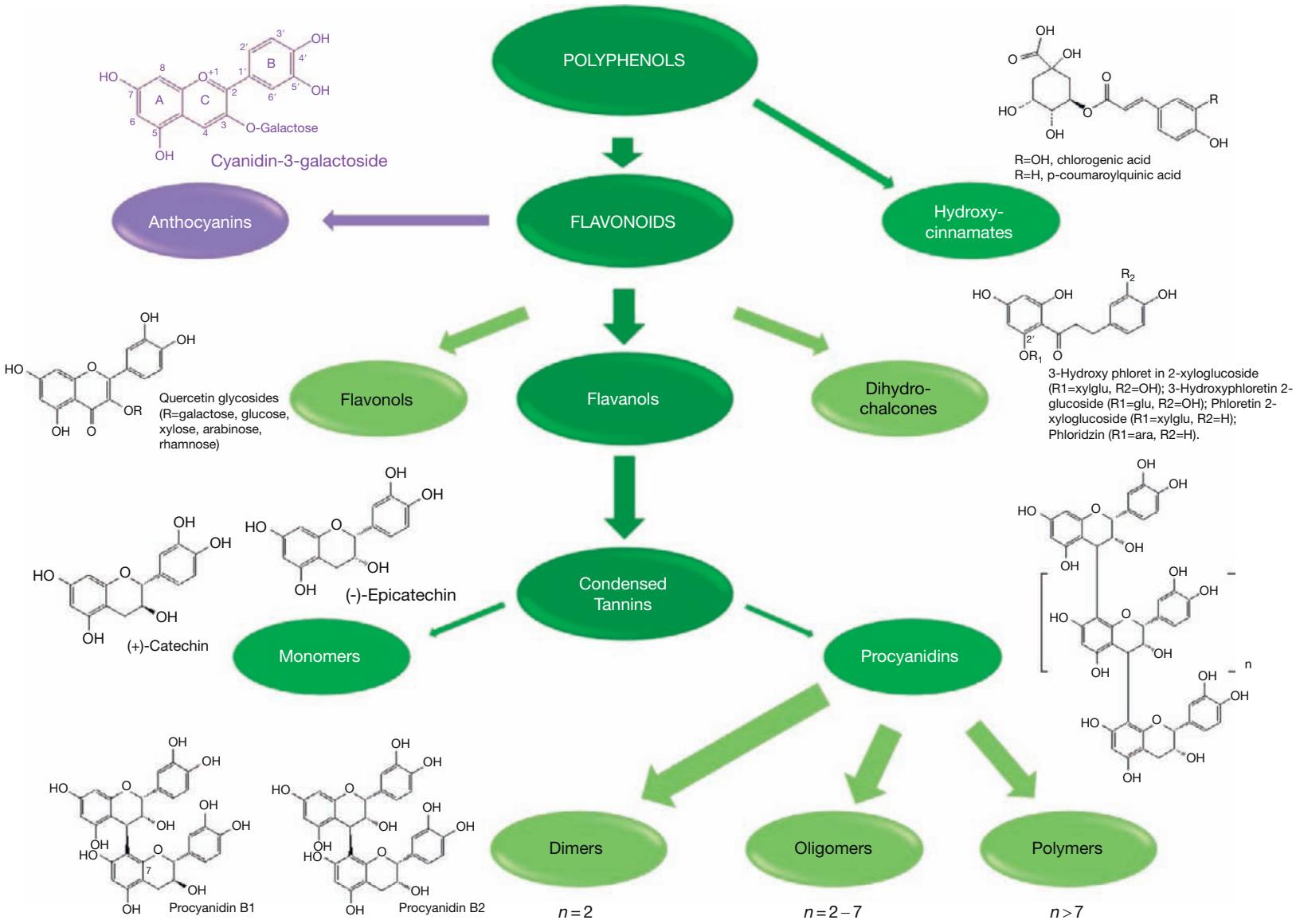

Figure 3 Polyphenols found in apples.

Table 3 Concentrations (μg g^{-1} fresh weight) of polyphenol in the peel and flesh of different apple cultivars[a]

	Empire	*McIntosh*	*Cortland*	*Mutsu*	*Red Delicious*	*Northern Spy*	*Golden Delicious*	*Ida Red*	*Mean*	*%*
Peal (μg g^{-1} fresh weight)										
Chlorogenic acid	176.9	135.6	19.3	134.3	44.6	233.6	149.0	195.3	136.1	8.5
p-Coumaroylquinic acid	5.4	33.6	14.2	7.5	5.5	13.8	9.6	9.3	12.4	0.8
Total hydroxycinnamates	182.3	169.2	33.5	141.8	50.1	247.4	158.6	204.6	148.5	9.3
Catechin	ND	112.8	123.9	43.0	81.9	112.8	38.1	79.3	74.0	4.6
Epicatechin	78.1	232.7	293.5	165.6	591.6	439.1	207.2	290.4	287.3	17.9
Procyanidin B1	ND	254.4	153.0	50.7	183.8	201.4	46.6	201.2	136.4	8.5
Procyanidin B2	73.2	196.5	251.1	205.5	468.1	460.3	276.8	269.9	275.2	17.2
Other procyanidins[b]	ND	218.9	243.8	110.1	329.4	242.8	140.0	197.1	185.3	11.5
Total procyanidins	151.3	1015.3	1065.3	574.9	1654.8	1456.4	708.7	1037.9	958.2	59.7
Cyanidin 3-galactoside	208.2	42.9	159.8	ND	148.9	17.4	ND	111.0	86.0	5.4
Total anthocyanins	208.2	42.9	159.8	0	148.9	17.4	0	111	86	5.4
Quercetin 3-galactoside	81.3	57.8	101.1	98.2	90.1	62.9	72.5	100.9	83.1	5.2
Quercetin 3-glucoside	78.0	65.8	89.2	32.6	15.2	11.8	24.9	18.0	42.0	2.6
Quercetin 3-xyloside	44.9	37.8	34.8	25.6	37.1	31.9	20.3	42.7	34.4	2.1
Quercetin 3-arabinoside	81.8	82.1	73.1	49.6	69.4	75.4	43.5	103.3	72.3	4.5
Quercetin 3-rhamnoside	63.9	57.3	35.6	67.4	32.3	91.4	59.1	44.0	56.4	3.5
Total flavonols	349.9	300.8	333.8	273.4	244.1	273.4	220.3	308.9	288.2	17.9
3-Hydroxyphloretin-2′-xylglu	6.7	4.1	ND	5.8	ND	3.8	7.7	ND	3.5	0.2
3-Hydroxyphloretin-2′-glu	16.0	ND	ND	5.6	29.3	ND	6.4	4.5	7.7	0.5
Phloretin-2′-xylglu	31.2	46.2	28.4	39.3	51.2	29.6	79.4	16.4	40.2	2.5
Phloridzin	70.9	58.0	37.6	48.5	172.0	44.7	67.5	79.2	72.3	4.5
Total dihydrochalcones	124.8	108.3	66	99.2	252.5	78.1	161	100.1	123.7	7.7
Total polyphenolics (HPLC)[a]	1016.5	1636.4	1658.5	1089.4	2350.4	2072.7	1248.5	1762.6	1604.4	100
Total phenolic content (FC)[c]	781.6	1163.4	1322.8	1016.9	2011.5	1548.3	1265.2	1478.8	1323.6	
Flesh (μg g^{-1} fresh weight)										
Chlorogenic acid	158.6	205.5	103.1	132.6	125.0	308.0	153.6	231.9	177.3	36.8
p-Coumarylquinic acid	3.8	29.9	29.1	9.0	11.7	20.0	11.0	11.1	15.7	3.3
Total hydroxycinnamates	162.4	235.4	132.2	141.6	136.7	328	164.6	243	193	40.1
Catechin	ND[d]	20.2	41.7	1.1	25.4	55.2	1.1	21.2	20.7	4.3
Epicatechin	ND	70.1	133.8	27.6	122.5	142.3	65.8	51.6	76.7	16.0
Procyanidin B1	ND	64.2	37.9	32.4	96.0	172.8	31.9	67.3	62.8	13.1
Procyanidin B2	ND	81.9	140.1	91.0	122.3	212.6	121.4	90.3	107.5	22.3
Total procyanidins	0	236.4	353.5	152.1	366.2	582.9	220.2	230.4	267.7	55.7
Quercetin 3-rhamnoside	ND	ND	ND	ND	3.7	ND	6.4	ND	1.3	0.3
Total flavonols	0	0	0	0	3.7	0	6.4	0	1.3	0.3
Phloretin-2′-xyloglucoside	3.3	7.2	4.1	5.0	3.3	6.5	7.5	2.3	4.9	1.0
Phloridzin	11.7	9.2	8	14.3	24.6	16.1	17.9	13.7	14.4	3.0
Total dihydrochalcones	15.0	16.4	12.1	19.3	27.9	22.6	25.4	16	19.3	4.0
Total polyphenolics (HPLC)[b]	177.4	488.1	497.7	313.0	534.4	933.6	416.6	489.3	481.3	100
Total phenolic content (FC)[e]	158.6	428.1	536.5	329.0	445.8	755.2	370.3	413.1	429.6	

[a]Data are average of duplicate determined by HPLC method.

[b]Sum of peaks, 2, 7, 9, and 11, calculated as procyanidin B2 equivalent.

[c]Total phenolic content measured by the Folin–Ciocalteu method.

[d]Not detectable.

[e]Calculated based on the total phenolics measured from HPLC.

Source: Tsao, R., Yang, R., Young, J. C. and Zhu, H. (2003). Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). *Journal of Agricultural and Food Chemistry* **51**, 6347–6353.

ileostomy bags, less than 33% of the oral dose of polyphenols was recovered in the bags, indicating the levels of polyphenol that may reach the colon. Bioaccessibility and bioavailability of polyphenols were found to be related to the structure, particularly to the glycosidic pattern of the polyphenolic compounds. Phloretin 2′-*O*-glucuronide as a product of polyphenol metabolism and very minor amounts of unmetabolized polyphenols were recovered in the ileostomy effluent, which would reach the colon under physiological conditions. It is likely that polyphenols that ultimately reach the colon may cause the prevention of colorectal carcinogenesis, a positive effect resulting from apple consumption. A recent study on the bioavailability and metabolism of apple polyphenols during intestinal transit has provided valuable information on how these bioactives are affected in the digestive tract. In this series of experiments, apple polyphenols were incubated under *in vitro* digestive conditions with saliva (for 5 min) and simulated gastric or duodenal juice (4 or 10 h, respectively) or separately with rat hepatocytes (4 h) under aerobic conditions and with ileostomy fluid under aerobic conditions for 10 h. The polyphenol profile in human serum (8 h later) and renal elimination in urine (24 h later) were also investigated after consumption of 1 l apple juice. The study revealed that in the presence of native saliva or ileostomy fluid, polyphenol glycosides such as those of phloretin and quercetin were hydrolyzed to produce aglycones, although the degree of hydrolysis depended on the glycosidic moiety. Oligomeric proanthocyanidins such as procyanidin B2, a dimer of two (−)-epicatechin molecules connected via a four to eight bond in the beta position, were depolymerized in simulated gastric juice (pH 1.8). Epimerization of flavan-3-ols, that is, (+)-catechin to (−)-epicatechin and (−)-epicatechin to (−)-catechin, was observed in artificial duodenal juice (pepsin, pancreatin, and bile extract, pH 7.2), but procyanidin B2 was degraded completely within 8 h because no (−)-epicatechin was detected. Under these conditions, however, the dihydrochalcones (phloretin and its glycosides) and flavonol glycosides (mainly quercetin glycosides) were stable over the entire 24 h incubation period. The released aglycone, quercetin, was found to be completely metabolized to smaller molecules including phloroglucinol, 3,4-dihydroxybenzoic acid, and 2,4,6-trihydroxybenzoic acid. Hydroxycinnamic acids, such as the *p*-coumaroylquinic acid, were transformed to its 3- and 5-isomers and their corresponding methyl esters in the lower gut duodenal juice. When these apple polyphenols were incubated with rat hepatocytes, in addition to the aforementioned metabolites, products of phase II metabolism were also found; these include two phloretin 2′-*O*-glucuronides and eight quercetin 3-*O*-glucuronides. Further *in vivo* study with five healthy volunteers who drank one liter of cloudy apple juice showed that six phenolic compounds, 5-*O*-caffeoylquinic acid, *p*-coumaroylquinic acid, caffeic acid, (−)-epicatechin, phloretin, and quercetin, were present in both serum and urine (5.3% and 3.5% of the amounts consumed, respectively) after samples were treated with β-glucuronidase/sulfatase. Bioavailability as measured by the maximum human serum concentration varied significantly among the six metabolites, with 5-*O*-caffeoylquinic acid to be the most bioavailable at 0.73 μM, followed by quercetin at 0.25 μM. (−)-Epicatechin was the least bioavailable with a maximum serum concentration of 0.05 μM. Most of these metabolites reached the maximum serum concentration between 0.7 and 2.1 h after apple juice consumption, with a half life between 2.3 and 6.0 h. Nearly 20% of the total polyphenols consumed were metabolized to more hydrophilic compounds and excreted in the urine.

Health Effects

Studies of the health beneficial effects of apples have at least partially instigated by the old adage 'An apple a day keeps the doctor away.' While the overall health benefits can be the result of a combined effect of all bioactive components in apple, including vitamins, minerals, dietary fiber, and the various phytochemicals, the contribution of the polyphenols can be of special significance. In fact, many of the health effects of apples may be triggered by the finding that vitamin C contributed nearly none to the total antioxidant capacity of apple juice. The antioxidant activity of apples was confirmed by many researchers, including the author of this article, that it was the polyphenols in apples that gave the strong antioxidant activity. It has also been found that not all apples are the same in terms of the total and individual polyphenol contents and that the majority of the polyphenols of apple are in the skin. While the antioxidant activity of apple polyphenols is an important indicator of the potential health benefits of apples, due to the relatively low bioaccessibility and bioavailability and low serum concentration of polyphenols, whether these compounds or their metabolites are the actual bioactive components *in vivo* remains unclear. Antioxidants reduce the oxidative stress caused by excess free radicals such as the reactive oxygen species. Polyphenols in apples are strong antioxidants when tested in various *in vitro* chemical-based assays because these compounds are relatively abundant in apple juice or its extract. However, most of these assays are not sufficiently sensitive; thus, when polyphenols or their metabolites are at very low physiological concentrations (nM), no significant antioxidant activities would be detected. Inflammation, on the other hand, has also been linked to the oxidative stress, and long-term inflammation has recently been considered to be the cause of various chronic diseases including immune system dysfunction, cancer, cardiovascular disease, diabetes, and neurodegenerative diseases. Research in recent years also suggests that at physiological concentrations, polyphenols and their metabolites can be actively involved in modulating biomarkers of various cell signaling pathways and therefore can potentially and effectively exert biological activities to promote health after apple consumption.

Anti-inflammation and Effects on the Immune System

In a recent study using immunorelevant human cell lines (DLD-1, T84, MonoMac6, and Jurkat), it was revealed that polyphenols from apple significantly inhibited the expression of NF-κB-regulated proinflammatory genes (TNF-α, IL-1β, CXCL9, and CXCL10), inflammatory relevant enzymes (COX-2 and CYP3A4), and transcription factors (STAT1 and IRF1) in LPS/IFN-gamma stimulated MonoMac6 cells, without significant effects on the expression of housekeeping genes. The same authors further showed that it was procyanidin B2 that was mainly responsible for the anti-inflammatory activity

of the apple polyphenol extract. This corresponds well to the early discovery that procyanidin B2 was the most likely main contributor to the antioxidant activity among apple polyphenols. It was further found that phloretin and procyanidin B1 significantly inhibited proinflammatory gene expression and repressed NF-κB-, IP-10-, IL-8 promoter-, and STAT1-dependent signal transduction in a dose-dependent manner. *In vivo* experiments with rats also showed that antioxidant polyphenols of apple had protective effect against GI damage induced by indomethacin. Previous administration of apple peel extract protected the gastric, intestinal, and colonic mucosa from induced oxidative stress as evidenced by lowered malondialdehyde concentrations and the glutathione/glutathione disulfide (GSH/GSSG) ratio. Treatment of apple polyphenols also lowered the myeloperoxidase activity, suggesting the anti-inflammatory effects were by preventing neutrophil infiltration in the mucosa. It was believed that prevention of macro- and microscopic damage and of barrier dysfunction along the GI tract by the apple polyphenols led to the protection of the indomethacin-treated animals. Similar results were observed by others using different cell lines, but the same rat model. Apple polyphenol extracts were found to lower the xanthine–xanthine oxidase or indomethacin induced injury to gastric epithelial cells by 50%, and catechin or chlorogenic acid (also the main phenolic components of apple extracts) was equally effective as apple extracts. Studies also found polyphenols of apples induced a fourfold increase in intracellular antioxidant activity, which would significantly reduce the oxidative stress, thus inflammation. Most recently, a high-flavonoid apple variety was found to significantly reduce the transcription levels of inflammation-linked genes in mice, compared with another apple variety with regular flavonoid concentration, with decreases of >2-fold for interleukin-2 receptor (Il2rb), chemokine receptor 2 (Ccr2), chemokine ligand 10 (Cxcl10), and chemokine receptor 10 (Ccr10) after a week on supplemented diet. The same study also showed that the inflammation marker prostaglandin E(2) in the plasma of mice fed with this high-flavonoid apple was reduced by 10-fold compared with a regular apple diet and the bacteria counts in the colonic microbiota was 6% higher in mice fed with the former diet after 3 weeks.

Anticancer Effect

No other commonly consumed fruits are like apples in terms of evidence in cancer risk reduction. Among the fruits and vegetables assessed in the Nurses' Health Study and the Health Professionals' Follow-up Study involving over 77 000 women and 47 000 men, apple (together with pear) showed the most significant association with lung cancer reduction in women, but not in men. Other studies have been conducted to specifically evaluate the role of apple consumption and cancer risk. In a case-control study assessing the potential protective impact of apples, it was found that the risk of colorectal cancer was inversely correlated with the daily number of apple servings, but not other fruits, and the most significant reductions were observed for an intake of one or more apple servings daily. In a recent case-control study on apple intake and risk of development of cancer involving 6000 participants, daily consumption of one or more medium-sized apples was found to be associated with a significant reduction in the risk of cancer compared with the consumption of less than one apple a day. Apple consumption particularly lowered the risk of larynx (41%, reduction rate, same for the following), colorectal (30%), esophagus (22%), breast (24%), ovary (24%), and prostate (7%) cancer.

Various animal models have been conducted to confirm these epidemiological findings and the preventative effects of apples and related products on cancer. A review of the findings of colon cancer animal models showed that apple extracts, juice, or related products could significantly reduce the genetic damage, proliferation of carcinoma, aberrant crypt foci of the colon, carcinoma incidence, carcinoma multiplicity, small intestinal adenoma, and spleen weights along other favorable health benefits. In addition to the direct antioxidant activities, apple polyphenols were also found to increase the expression of antioxidant response element-dependent genes, for example, superoxide dismutases 1 and 2 (SOD1/SOD2), glutathione peroxidases 1 and 2 (GPx1/GPx2), γ-glutamylcysteine ligase catalytic subunit to modifier subunit ratio (GCLC/GCLM), glutathione-disulfide reductase (GSR), and catalase (CAT).

Apples are a rich source of polyphenolic antioxidants, particularly in the skins. The mechanism of the anticancer action of apple polyphenols has been investigated *in vitro* in different cell models. A polyphenol-rich extract of apple peel showed significant antiproliferative effect in a dose-dependent manner against the human colon carcinoma cell line HT29. The same effect was not found in a breast cancer cell line MCF-7, suggesting different cancer cells may respond differently. Apple extract rich in polyphenols selectively inhibited the proliferation and induced apoptosis of a cancer cell line adenoid cystic carcinoma M (ACC-M) cells over a normal cell line MRC-5 (cells derived from normal lung tissue). The effects were found to be related to the downregulation of vascular endothelial growth factor receptor-2 expression and the activation of caspase-3 expression. Apple extracts containing polyphenols were found to reduce NF-κB activity, likely mediated via inhibited phosphorylation of IκBα in human umbilical vein endothelial cells.

Effect on Cardiovascular Disease

A team from the United Kingdom recently modeled the effect on vascular mortality such as heart attacks and strokes and concluded that for people over 50 years old, both an apple a day (100 g) and a statin (a cholesterol-lowering drug) a day had the potential to reduce the vascular death significantly with a similar rate of reduction. They also claimed that "the 150-year-old health promotion message is able to match modern medicine and is likely to have fewer side effects." A cohort study following 9208 Finnish men and women 15 years of age or older for 28 years showed that even though the population under study was initially free from cardiovascular disease, only those who consumed apple, a main source of the polyphenol quercetin, had significantly decreased risk of thrombotic stroke. Plasma lipid concentration and abnormal metabolism of lipids are the two most established risk factors of cardiovascular disease that can be caused by many factors, among which atherosclerosis and hypertension are the most common. Polyphenol extract containing high amount of procyanidins was found to inhibit *in vitro* pancreatic lipase activity and lowered

in vivo triglyceride absorption in mice. Polyphenol extract of apple was found to significantly reduce the total and low-density lipoprotein (LDL) cholesterols and increase the high-density lipoprotein (HDL) cholesterol in human subjects after 4 weeks. Meanwhile, atherosclerosis is related to the oxidation of LDL and apple antioxidants therefore have the potential to reduce cardiovascular risk. Apple juices and fresh apple extracts rich in polyphenols were found to significantly inhibit LDL oxidation in an *in vitro* copper-induced human LDL oxidation system. Polyphenols in apples and apple juice were also found to contribute significantly to the improvement of lipid profile and oxidative status *in vivo* in hypercholesterolemic hamsters. Hamsters given a human equivalent of ca. 2.5 large apples or 500 ml of juice daily for 12 weeks significantly improved serum lipid profile by reducing total cholesterol (11% and 24%, respectively) and the ratio of total cholesterol–HDL (25% and 38%), prevented the development of atherosclerosis, and showed favorable effects on antioxidant enzymes in the liver.

Antidiabetic Effect

Only limited information is available on the potential effect of apple or its polyphenol extracts on regulating blood glucose level and other markers related to diabetes. Phenolic extract of apple, particularly that of the peel, showed a strong inhibitory activity against α-glucosidase, a key enzyme regulating blood glucose level. Phenolic compounds in apple juices were found to significantly affect plasma concentrations of glucose, insulin, and other two hormones, glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1, in volunteers, and the result appeared to be consistent with delayed intestinal absorption of glucose. Cloudy apple juice containing higher polyphenols, particularly phloridzin, showed greater effect suggesting they may be the active components. More recently, a low-sugar, fiber- and phloridzin-enriched apple preparation showed significantly improved glucose metabolism in the oral glucose tolerance test (OGTT) in human volunteers by reducing the postprandial glucose response at 15–30 min by approximately twofold and by increasing urinary glucose excretion during the 2–4 h interval of the OGTT by fivefold. This implies that apple or its preparation containing high phenolic content may be used to reduce postprandial glycemia and to improve the health of patients with diabetes. In another most recent study, it was demonstrated that an extract and selected individual polyphenols from apple diminished sodium-coupled glucose transporter 1 mediated glucose uptake *in vitro* and *in vivo*, and the strongest inhibition was observed for phloridzin. An OGTT performed in volunteers with prior administration of the apple extract reduced venous blood glucose and plasma insulin levels. Another comprehensive 5×4-week dietary crossover human study showed that consumption of whole apples (550 g day^{-1}), apple pomace (22 g day^{-1}), and cloudy apple juices (500 ml day^{-1}) significantly lowered serum LDL concentration by 6.7%, 7.9%, and 2.2%, respectively. However, LDL cholesterol concentrations increased by 6.9% with clear juice compared to whole apples and pomace in the study. No effect was found for other biomarkers including HDL cholesterol, triglycerides, weight, waist-to-hip ratio, blood pressure, inflammation, and composition of the gut microbiota or markers of glucose metabolism. The authors suggested that in addition to the polyphenols, the fiber component is necessary for the cholesterol-lowering effect of apples in healthy humans and that clear apple juice may not be a suitable surrogate for the whole fruit in nutritional recommendations.

Summary

Apples (*Malus domestica*) are one of the most ancient, widely cultivated, and highly popular fruits in the world. Apples are produced in all continents of the world, but China is currently the world's largest apple producer, followed by the United States producing one-tenth of that of China. While apples are mostly consumed fresh, they are processed into beverages, jam, jelly, and other forms of foods. In addition to the essential nutrients of apple such as vitamins and minerals and dietary fibers, polyphenols have been found in recent years as the main bioactive components responsible for the various health-promoting effects. There are many factors from the field to fork, such as genetics, environmental and postharvest storage conditions, method of processing, and those such as microflora in the human digestive tract that can affect the stability, bioaccessibility, bioavailability of polyphenols, and the antioxidant and anti-inflammatory effects of polyphenols in apples. The consumption of apple and its processed products or extracts rich in polyphenols have been linked to reduced risk in cancer, cardiovascular disease, diabetes, and many other chronic diseases including asthma. Polyphenols exert these health effects through antioxidant and anti-inflammatory activities and by modulating biomarkers in various cell signaling pathways. While sufficient evidence has been found for some of the health beneficial effects, many of them still require further studies.

See also: Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Chilled Foods: Effects on Shelf-life and Sensory Quality; Chilled Foods: Modified Atmosphere Packaging; Chilled Foods: Principles; Cider (Cyder; Hard Cider): The Product and Its Manufacture; Controlled Atmosphere Storage: Applications for Bulk Storage of Foodstuffs; Controlled Atmosphere Storage: Effect on Fruit and Vegetables; Pectin: Properties Determination and Uses; Pectin and Health.

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Arsenic: Properties and Determination

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Atomic Properties

Arsenic (As – CAS (Chemical Abstracts Service) No. 7440-38-2) has properties of both metals and nonmetals and is properly referred to as a metalloid. Arsenic is located in the pnictogen group of the periodic table, which is group 15 (IUPAC notation) or V_A in the CAS system. It has an atomic number of 33 with an atomic weight of 74.922, an atomic radius of 1.33 Å, an ionic radius of 0.58 Å, an atomic volume of 13.1 $cm^3\ mol^{-1}$, a covalent radius of 1.2 Å, and a cross section (thermal neutron capture) σ_a/barns of 4.3. Arsenic has weak bonding between the layers and is therefore brittle and has a relatively low Mohs hardness of 3.5. The crystal structure is designated as a trigonal–hexagonal scalenohedral class. It has a space group *R*-3*m* with cell parameters $a = 3.7680$ Å, $c = 10.5740$ Å, and $Z = 6$ and a volume of 130 Å. An example of the trigonal–scalenohedral class symmetry is shown in Figure 1.

The electron configuration is $1s^2\ 2s^2p^6\ 3s^2p^6d^{10}\ 4s^2p^3$ with the following electrons per energy level: 2, 8, 18, 5. The number of protons, electrons, and neutrons is 33, 33, and 42, respectively. The electron shell model for arsenic is shown in Figure 2.

Arsenic (As) has about 30 known isotopes. Isotope ^{73}As has the longest half-life of 80.3 days, isotope ^{74}As has a half-life of 17.77 days, and isotopes ^{76}As and ^{77}As half-lives are just over 1 day. Isotope ^{75}As is stable and has no measurable half-life.

Chemical Properties

The three most common arsenic allotropes are yellow, black, and metallic gray, which is the most common. The crystalline gray arsenic has a density 5.727 g cm^{-3} (20 °C), a melting point of 817 °C, and a boiling point 613 °C. Yellow arsenic (metastable) has a density of 1.97 g cm^{-3} and a melting point of 815 °C. If yellow arsenic is exposed to light, it will quickly turn into black arsenic, which is also a metastable nonmetallic, glassy, dense mineral substance that is brittle in its pure form. It is a poor electrical conductor with properties intermediate between gray arsenic and yellow arsenic. Arsenic compounds are generally classified into one of three groups: (1) inorganic arsenic compounds, (2) organic arsenic compounds, and (3) arsine gas. Arsenic can exist in four oxidation states: (−3), (0), (+3), and (+5). The most common oxidation states are (−3) in the intermetallic compounds and (+3) in the arsenites, arsenates (III), and most organoarsenic compounds. The most common naturally occurring arsenic compounds are arsenite (AsO_3^{3-}) and arsenate (AsO_4^{3-}). The most common inorganic trivalent arsenic compounds are arsenic trioxide (As_2O_3), sodium arsenite ($NaAsO_2$), and arsenic trichloride ($AsCl_3$). Arsenic forms colorless, odorless, crystalline oxides that are hygroscopic and form acidic solutions upon exposure to water. Pentavalent inorganic compounds include arsenic pentoxide (As_2O_5), arsenic acid (H_3AsO_4), and arsenate (AsO_4^{3-}). Notable sulfur compounds of arsenic include orpiment (As_2S_3), realgar (As_4S_4), and arsenic pentasulfide (As_2S_5). The most common organic arsenic compounds include arsanilic acid ($C_6H_8AsNO_3$), methylarsonic acid (CH_5AsO_3), dimethylarsinic acid (cacodylic acid) ($C_2H_7AsO_2$), and arsenobetaine ($C_5H_{11}AsO_2$).

Occurrence

Arsenic can be found almost everywhere in the environment and is the 20th most common element in the Earth's crust with an overall concentration of about 1.5 ppm. The World Health Organization (WHO) estimates that about one-third of the atmospheric flux of arsenic is of natural origin – primarily volcanic activity. Environmental arsenic levels are a result of the weathering of the 200+ arsenic-containing natural minerals, while anthropomorphic contributions include mining, smelting of nonferrous metals, burning of fossil fuels, the use of arsenical pesticides, the leaching of wood preservatives, and disposal of industrial wastes. Because of the general environmental arsenic contamination and potential toxic exposure, the use of arsenic-containing pesticides and arsenic wood preservatives (e.g., chromated copper arsenate) has been discontinued. Commercial use of elemental arsenic is primarily as an additive for alloys. High-purity gallium arsenide (GaAs) is utilized in the manufacture of devices such as integrated circuits, infrared light-emitting diodes, laser diodes, solar cells, and optical windows. Indium arsenide (InAs) is used for the construction of infrared photovoltaic photodiode detectors for the wavelength range of 1–3.8 μm and diode lasers. Closely related organoarsenics arsanilic acid ($C_6H_8AsNO_3$), roxarsone ($C_6AsNH_6O_6$), and carbarsone ($C_7H_9AsN_2O_4$) have been used in the United States for many years as veterinary drugs and/or feed additive to promote growth and as antiprotozoal drugs to prevent or treat dysentery and as a coccidiostat in poultry and swine. In September 2013, the FDA announced that the two US manufacturers would voluntarily withdraw current roxarsone, arsanilic acid, and carbarsone from the market. Only one organoarsenic animal drug remains on the market with an FDA approval in place for use in animal food: nitarsone ($C_6H_6AsNO_5$).

The soil contents of arsenic range from 1 to 10 ppm; open ocean water averages approximately 1.6 ppm. Arsenic is also found in surface freshwaters with concentrations generally below 10 μg l^{-1}. It is present in many mineral species – primarily in its sulfide form in complex minerals containing silver, lead, copper, nickel, antimony, cobalt, and iron – the most common of which is arsenopyrite (FeAsS). The mean total arsenic concentration in air in remote areas ranges from 0.02 to 4 ng m^{-3}, while urban area samples range from 3 to 200 ng m^{-3}. Inorganic arsenic particulate matter found in urban/industrialized areas

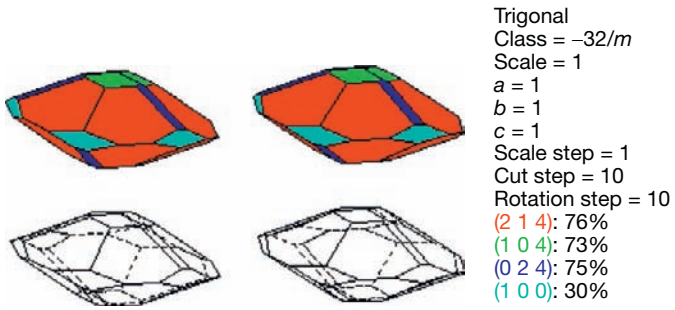

Figure 1 Example of the trigonal–scalenohedral class symmetry. From www.webmineral.com, with permission.

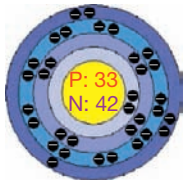

Figure 2 Shell model for arsenic. From http://www.chemicalelements.com.

includes both the tri- and pentavalent forms with the pentavalent form being the predominant species. Arsenic is found in surface freshwaters at concentrations generally below 10 $\mu g\,l^{-1}$ and in groundwater averages about 1–2 $\mu g\,l^{-1}$. High levels of inorganic arsenic can be found in groundwater used as drinking water in various locations throughout the world. Bangladesh is notorious for having some of the highest levels of groundwater arsenic in the world. Arsenic concentrations in soil range from 1 to 40 mg kg^{-1}, with a mean value of about 3 mg kg^{-1}. Organoarsenic compounds such as arsenobetaine ($C_5H_{11}AsO_2$), arsenocholine ($C_5H_{14}AsO$), tetramethylarsonium salts (C4H13As.X), arsenosugars ($C_{10}H_{21}AsO_7$), long-chain hydrocarbons such as 1-dimethylarsinoyl all-*cis*-4,7,10,13,16,19-docosahexaene, and saturated fatty acids with terminal dimethylarsinoyl moieties are all found in marine animals although some of these compounds have also been found in terrestrial species. Arsenoglycerolipids such as glycerophosphorylarsenocholine, diacylglycerophospho-2-hydroxypropyl-5-deoxy-5-(dimethylarsinoyl)-β-ribofuranoside, and arseno-glycophospholipid are found primarily in marine organisms, however, at very low levels. Interestingly, it has been shown that some of these organoarsenics have also been found in terrestrial species.

According to the EPA, bioaccumulation is the net accumulation of a chemical by aquatic organisms as a result of uptake from all environmental sources, such as water, food, and sediment, whereas bioconcentration is the uptake of a chemical by an aquatic organism through water. The level of arsenic in seawater is fairly uniform globally ranging between 0.5 and 2 $\mu g\,l^{-1}$. Marine plants and animals normally contain organoarsenic residues because they are capable of bioaccumulating arsenic both directly from inorganic forms of arsenic in ambient water and from food. The arsenic can be biotransformed from microbes or by internal mechanisms from the plants and animals themselves. Arsenic bioaccumulates in all aquatic organisms; however, the bioaccumulation factor is more prominent in saltwater organisms than in freshwater organisms as evidenced by the fact that freshwater concentrations of arsenic are usually <1 mg kg^{-1} with marine organisms containing arsenic ranging from 1 to >100 mg kg^{-1}. The levels of arsenic present in marine organisms are primarily organic. The major bioaccumulation transfer occurs between water and algae, at the lowest level of the food chain that ultimately has a significant impact on the levels of arsenic found in fish. Biomagnification has not been observed in aquatic food chains. Terrestrial biota may accumulate arsenic through the roots from the soil or by deposition of airborne arsenic particles on the leaves at concentrations usually <1 mg kg^{-1}.

Speciation of Arsenic

As previously described, arsenic exists in elemental, organic, inorganic, and gaseous (arsine) forms. Generally, metallic arsenic is nontoxic because it is insoluble in body fluids and/or water and it remains stable upon exposure to biological systems. On the other hand, inorganic arsenics are toxic and the degree to which they are toxic is dependent on valence state. The toxicity of the trivalent state (As^{3+}) exhibits greater toxicity than the pentavalent form (As^{5+}) state. The oral LD_{50} values for inorganic arsenic compounds range from 4 to 20 mg kg^{-1} body weight. Interestingly, Fowler's solution (1% solution of potassium arsenite ($LD_{50} = 14$ mg kg^{-1})) has been used to treat various diseases, including malaria, syphilis, asthma, chorea, eczema, and psoriasis. Organoarsenics also vary in toxicity; however, the degree of toxicity is minimal in most cases. Arsenobetaine and arsenocholine (with LD_{50}s of about 10 000 and 6500 mg kg^{-1}, respectively) are found in marine invertebrates and fish that contain arsenic residues ranging from <1 to more than 100 mg kg^{-1}. However, not all organoarsenics are as nontoxic as arsenobetaine and arsenocholine. Recently, two arsenosugar metabolites – dimethylarsinic acid (DMA(V), also known as cacodylic acid) (oral $LD_{50} = 644$ mg kg^{-1}) and thio-dimethylarsinic acid (thio-DMA(V)) – were shown to be toxic to cells in *in vitro* cell cultures; thio-DMA(V) was slightly more cytotoxic than arsenite. Therefore, these metabolites DMA(V) and thio-DMA(V) are toxic to humans. Arsine is a highly toxic arsenic gas, and exposure usually occurs in industrial settings when arsenic-containing ores or metals vaporize or come into contact with acidic solutions off-gassing arsine.

Historically, in most cases, arsenic exposure is evaluated by examining for the total arsenic concentration in the blood or urine sample. This is termed determination and is basically the identification of elemental arsenic in a sample, but does not

provide enough information to evaluate the effects the arsenic may have on the environment or on an organism since the bioavailability and metabolic processes depend upon the chemical form of the arsenic. The multiple valence/activity states and significant differences in toxicity following arsenic exposure make it essential to identify the specific species in order to assess the toxic impact of arsenic exposure. This process of differentiation between the various forms of arsenic is referred to as speciation. More specifically, speciation is a process of assessment of the chemical form of a compound in which arsenic occurs in both nonliving and living systems. The specific form directly relates to the bioavailability of arsenic to an organ or organism. The process can separate inorganic arsenics such as arsenite (As[III]) ($LD_{50} = 4–5$ mg kg^{-1}) and arsenate (As[V]) ($LD_{50} = 14–18$ mg kg^{-1}) as well as less toxic metabolites monomethylarsonic acid (MMA) ($LD_{50} = 1800$ mg kg^{-1}) and dimethylarsinic acid (DMA) ($LD_{50} = 2600$ mg kg^{-1}) from nontoxic arsenicals of marine food origin, namely, arsenobetaine and arsenocholine. For instance, seafood can yield a significant increase in total urine arsenic within 10 h of ingestion. Therefore, the normal consumption of marine seafood can simulate exposure to inorganic arsenic if exposure is not detected by arsenic speciation analysis. This underscores the fact that speciation of arsenic is essential to differentiate toxic inorganics from the generally nontoxic organics to understand the true risk of arsenic exposure.

A detailed discussion of arsenic speciation of water, urine, soil, and seafood is beyond the scope of this review; however, it suffices to say the topic of arsenic environmental and biological chemistry is highly complex and is still evolving. There have been several thousand scientific articles over the last two decades describing the determination and speciation of arsenic alone. In order to assess the chemical form of arsenic, one must conduct arsenic speciation, which is the assessment of the chemical form of a compound in which arsenic occurs in both nonliving and living systems.

Preparing Samples for Analysis

Speciation is commonly accomplished in three steps: (1) sample preparation, (2) species separation, and (3) detection. Sample preparations consist of the isolation of different species from the sample matrix. Biological samples are highly complex, and the arsenic species to be analyzed are usually very dilute. Sample preparation is a very critical step necessary to solubilize the sample into a solution while attempting to minimize loss by vaporization. The most commonly utilized extraction methods include solvent extraction, enzymatic hydrolysis, solid-phase extraction (SPE), solid-phase microextraction (SPME), heating blocks, and microwave extraction. According to the Association of Official Analytical Chemists, in determining arsenic levels in food, it is recommended that the sample be heated in an oven at 150 °C for several hours with HNO_3. The container is sealed with the HNO_3 and ammonium hydroxide as a tissue solubilizer. The result is that the organoarsenic compounds are decomposed to inorganic arsenate.

Solvent extraction is frequently used to determine the organoarsenic content of biological samples. This method utilizes a methanol/water mixture for the extraction of minimally polar organoarsenics. The extract is then purified via a stationary phase cartridge or centrifugation and/or filtration. The sample is subjected to gel-permeation, ion-pair, ion-exchange, and buffered ion-exchange chromatographies in order to maintain stability and decomposition. Preparation of foods can also employ tetramethylammonium hydroxide in an alkaline medium to solubilize the sample.

The use of proteolytic enzymes is another method of sample preparation. The concept is that this method promotes biomolecular hydrolysis under neutral pH and room temperature releasing the various analytes from the sample matrix without chemical species changes. It has been shown that utilizing sonification reduces the time of the extraction process and improves the extraction.

Microwave-based procedures involve sample extraction with trifluoroacetic acid/H_2O_2 and measurement of arsenate by anion-exchange HPLC/ICP-MS using aqueous malonic acid. Extraction procedures using dilute acid or organic solvents at relatively low temperatures can be augmented by focussed-microwave ovens. It has been shown that upon microwaving, food samples for organoarsenic compounds, such as MMA, dimethylarsinic acid, arsenobetaine, and tetramethylarsonium ion, all remained stable.

SPE is used for preconcentration and separation according to the partitioning between the respective liquid (sample) and solid (sorbent) phases. SPE is a highly sensitive procedure that results in either the desired analytes of interest or the undesired impurities in the sample that are retained on the stationary phase. SPME is a sample preparation technique that employs the use of a fiber coated with either a liquid or a solid that extracts arsenic from various types of media. There are many methods for the evaluation of soil samples, and other abiotic samples are extracted using aqueous solutions of varying ionic strengths/pH/redox potentials to release arsenic bound to the various mineral phases in the samples. In one method, hydrochloric acid is heated at low temperatures with the sample followed by filtration that ultimately is subjected to hydride generation atomic absorption spectroscopy.

Arsenic in water can be evaluated utilizing adsorption. Chemisorption filters activated with various ions such as Cu^{2+}, Al^{3+}, and Mg^{2+} are used to as absorbents to remove arsenic from water. Another method for the preparation of water samples in the separation of As(III) and As(V) species involves the use of two resins – a strong base anion exchange (SBAE) and a hybrid (HY). The process permits the resins to separate the As(V) from the As(III) by retaining As(V) and allowing the AsIII to pass through. Yet another procedure can be used with low levels of water arsenic that involves the coprecipitation of the arsenic with ferric hydroxide following pretreatment with potassium permanganate.

Organoarsenic compounds such as those found in seafoods are extracted with methanol or a 1:1 methanol/water mixture. Other procedures include extraction with chloroform/methanol/water followed by sonification. The extract is then purified via a stationary phase cartridge or centrifugation and/or filtration. The sample is next subjected to gel-permeation, ion-pair, ion-exchange, and buffered ion-exchange chromatographies in order to maintain stability and decomposition. Preparation of foods can also employ tetramethylammonium hydroxide in an alkaline medium to solubilize the sample.

Different Methods of Analysis

The most commonly used method for speciation of arsenic species is high-performance liquid chromatography (HPLC) in its various derivatives. As(III), As(V), MMA, dimethylarsinic acid (DMA), and arsenobetaine are generally separated by HPLC and determined online by ICP-MS. Two forms of HPLC are generally used – ion pairing and ion exchange. The detection limits for arsenic range between 50 and 300 pg. If an ICP-MS detector is used in combination with HPLC, the detection limits for arsenic species are significantly enhanced to ranges of 10–30 pg.

Previously, basic colorimetric and gravimetric methods such as Reinsch's method, Marsh's test, and Gutzeit's test were considered the 'gold standard' for use in detecting arsenic. However, these methods lack the sensitivity and quantification necessary to adequately analyze for individual arsenic species. With the development of various analytic equipment such as high-performance liquid chromatography/inductively coupled plasma mass spectrometry (HPLC/ICP-MS), it has now become one of the most frequently used method for precise arsenic determination. The advantage of the HPLC/ICP-MS versus other analysis techniques such as atomic absorption and inductively coupled plasma atomic emission spectroscopy (ICP-AES) since the accuracy and level of detection is far superior. Further, it has a higher volume throughput than the graphite furnace atomic absorption spectroscopy (GFAAS). In addition, the HPLC/ICP-MS provides isotope data for more complete analysis. An ICP-MS converts the atoms of the elements in the sample to ions. These ions are then separated and detected by the mass spectrometer.

A brief summary of some of the commonly used methods for the determination of total arsenic in biological samples is found in Table 1.

Table 2 presents brief summary of some of the commonly used methods for the determination of total arsenic in water and food samples.

Table 3 presents brief summary of some of the commonly used methods for arsenic speciation in biological samples.

Table 4 presents brief summary of some of the commonly used methods for arsenic speciation in food and water.

Certified Reference Materials

For quality control, analytical chemists employ certified reference materials (CRMs) that are also referred to as standard reference materials (SRM®) by the National Institute of Standards and Technology (NIST), the United States. NIST currently supplies industry, academia, government, and other users with over 1300 SRMs. In the EU, the European Commission's Joint Research Centre (JRC) currently provides over 800 different reference materials under the BCR®, IRMM, and ERM® brands in the fields of food and feed analysis, environmental analysis, engineering, and health applications. NIST uses the trademarked term SRM® to denote a CRM that satisfies additional specific criteria. Similarly, the term European reference material (ERM®) is a trademarked term referring to CRMs produced by the European reference materials consortium. CRMs are also produced by the following agencies:

- National Research Council of Canada (NRCC) by the Canadian Certified Reference Materials Project (CCRMP), Ottawa, Canada. The NRCC CRM program is operated by the Measurement Science and Standards portfolio and provides CRMs for environmental, biotoxin, food, nutritional supplement, and stable isotopic analysis. NRCC has about 100 CRMs available for analytic use.
- International Atomic Energy Agency (IAEA), Terrestrial Environment Laboratory, Vienna, Austria. The IAEA provides approximately 90 reference materials for various applications.
- Japan National Institute for Environmental Studies (JNIES), Tsukuba-shi, Ibaraki, Japan. The JNIES has about approximately 15 reference materials for chemistry applications.
- Korea Research Institute of Standards and Science (KRISS), Daejeon, Republic of Korea. KRISS provides almost 400 reference materials for various applications.

CRMs allow the analytical chemist to link his or her results with those of internationally recognized standards. In addition, CRMs enable the chemist to verify his or her performance with respect to comparative accuracy. The certified value of a CRM is defined by the applied method following a very strict

Table 1 Arsenic determination in biological samples for total arsenic

Analytic method	*Sample*	*Detection limits*	*References*
Hydride generation atomic absorption spectrometry (HGAAS)	Blood	0.5 μg l^{-1}	Foa et al. (1984)
	Hair	0.06 μg g^{-1}	Curatola et al. (1978)
	Nails	1.5 μg g^{-1}	Agahian et al. (1990)
Flow injection Hydride generation atomic absorption spectrometry (HGAAS)	Urine	0.1 μg l^{-1}	Guo et al. (1997)
Graphite furnace atomic absorption spectrometry (GFAAS)	Soft tissue	0.2 ppm	Mushak et al. (1977)
Colorimetric photometry	Urine	0.5 μg/sample	Pinto et al. (1976)
x-Ray fluorescence (XRF)	Urine	0.2 μg l^{-1}	Clyne et al. (1989)
Neutron activation analysis (NAA)	Serum	0.088 ng ml^{-1}	Versieck and Vanballenberghe (1985)
	Urine	40–100 ng g^{-1}	Landsberger and Simsons (1987)

Source: Agency for Toxic Substances and Disease Registry (ATSDR) (2007). Toxicological profile for arsenic. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services; Francesconi, K. A. and Kuehnelt, D. (2004). Determination of arsenic species: a critical review of methods and applications, 2000–2003. *Analyst* **129**, 373–395; Nearing, M. M., Koch, I. and Reimer, K. J. (2014). Complementary arsenic speciation methods: a review. *Spectrochimica Acta, Part B: Atomic Spectroscopy* **99**, 150–162.

Table 2 Arsenic determination in food and water samples for total arsenic

Analytic method	*Sample*	*Detection limits*	*References*
SDDC colorimetric spectrophotometry at 510 nm (silver diethyldithiocarbamate) (EPA Method 206.4)	Water	10 μg l^{-1}	EPA (1983)
Hydride generation atomic absorption spectrometry (HGAAS)	Water	2 ng l^{-1}	EPA (1998)
	Food	0.1 μg g^{-1}	Hershey et al. (1988)
Graphite furnace atomic absorption spectrometry (GFAAS)	Food	10 ng	Dabeka and Lacroix (1987)

Source: Agency for Toxic Substances and Disease Registry (ATSDR) (2007). Toxicological profile for arsenic. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services; Francesconi, K. A. and Kuehnelt, D. (2004). Determination of arsenic species: a critical review of methods and applications, 2000–2003. *Analyst* **129**, 373–395; Nearing, M. M., Koch, I. and Reimer, K. J. (2014). Complementary arsenic speciation methods: a review. *Spectrochimica Acta, Part B: Atomic Spectroscopy* **99**, 150–162.

Table 3 Arsenic speciation in biological samples

Analytic method	*Sample*	*Detection limits*	*References*
Hydride generation atomic absorption spectrometry (HGAAS)	Urine	0.08 μg l^{-1}	Norin and Vahter (1981)
Ion-exchange chromatography/hydride generation atomic absorption spectrometry (IEC/HGAAS)	Urine	0.5 μg l^{-1}	Johnson and Farmer (1989)
Hydride generation atomic absorption spectrometry/thin layer chromatography/high-resolution mass spectrometry (HGAAS/TLC/HRMS)	Urine	0.34 mg/sample	Tam et al. (1982)
High-performance liquid chromatography/hydride generation atomic absorption spectrometry (HPLC/HGAAS)	Marine	organisms	0.3–0.9 ng
López-Gonzálvez et al. (1994)			
Flow injection Hydride generation atomic absorption spectrometry (HGAAS)	Inorganic arsenic	0.045 mg kg^{-1} (dry matter)	Øygard et al. (1999)
Atomic emission	Urine	≤1 ng	Braman et al. (1977)
Gas–liquid chromatography/electron capture detector (GLC/ECD)	Blood/ tissue	0.1 mg ml^{-1}	Dix et al. (1987)
High-performance liquid chromatography/inductively coupled plasma mass spectrometry (HPLC/ICP-MS)	Blood plasma	2.5 ng As/mL	Ebdon et al. (1999)
	Marine	organisms	6–25 ng ml^{-1}
Thomas and Sniatecki (1994)			
High-performance liquid chromatography/inductively coupled plasma mass spectrometry (IEC/ICP-MS)	Urine	<0.45 μg l^{-1}	Inoue et al. (1994)
Atomic fluorescence spectrometry (AFS)	Urine/ blood/ tissue	0.03 μg l^{-1}	Gregus et al. (2000)
High-performance liquid chromatography/inductively coupled plasma atomic emission spectrometry (HPLC/ICP-AES)	Marine	organisms	19–41 ng
Benramdane et al. (1999)			
High-performance liquid chromatography/inductively coupled plasma/dynamic reaction cell™ mass spectrometry (HPLC/ICP/DRC-MS)	Urine	0.4–1.7 μg l^{-1}	Verdon et al. (2009)

Source: Agency for Toxic Substances and Disease Registry (ATSDR) (2007). Toxicological profile for arsenic. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services; Francesconi, K. A. and Kuehnelt, D. (2004). Determination of arsenic species: a critical review of methods and applications, 2000–2003. *Analyst* **129**, 373–395; Nearing, M. M., Koch, I. and Reimer, K. J. (2014). Complementary arsenic speciation methods: a review. *Spectrochimica Acta, Part B: Atomic Spectroscopy* **99**, 150–162.

analytic protocol, for example, a standard. CRMs are most commonly used as follows:

1. Pure substances or solutions to be used for calibration and/or identification: pure substances are usually certified by establishing the maximum amount, in mass fractions, of the impurities that remain in the purified substance.
2. Materials of a known matrix composition for the calibration of a specific type of comparison.
3. Matrix reference materials that represent the particular matrix being analyzed and have certified matrix content.

A number of CRMs for total arsenic are available, although only a few are available for speciation. **Table 5** lists some of the more commonly available arsenic CRMs from the agencies noted earlier.

Table 4 Arsenic speciation in food and water samples

Analytic method	*Sample*	*Detection limits*	*References*
Hydride generation atomic absorption spectrometry (HGAAS)	Urine	0.08 μg l^{-1}	Norin and Vahter (1981)
Ion-exchange chromatography/hydride generation atomic absorption spectrometry (IEC/HGAAS)	Urine	0.5 μg l^{-1}	Johnson and Farmer (1989)
Hydride generation atomic absorption spectrometry/thin layer chromatography/high-resolution mass spectrometry (HGAAS/TLC/HRMS)	Urine	0.34 mg/sample	Tam et al. (1982)
High-performance liquid chromatography/hydride generation atomic absorption spectrometry (HPLC/HGAAS)	Marine organisms	0.3–0.9 ng	López-Gonzálvez et al. (1994)
Flow injection Hydride generation atomic absorption spectrometry (HGAAS)	Inorganic arsenic	0.045 mg kg^{-1} (dry matter)	Øygard et al. (1999)
Atomic emission	Urine	≤1 ng	Braman et al. (1977)

Source: Agency for Toxic Substances and Disease Registry (ATSDR) (2007). Toxicological profile for arsenic. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services; Francesconi, K. A. and Kuehnelt, D. (2004). Determination of arsenic species: a critical review of methods and applications, 2000–2003. *Analyst* **129**, 373–395; Nearing, M. M., Koch, I. and Reimer, K. J. (2014). Complementary arsenic speciation methods: a review. *Spectrochimica Acta, Part B: Atomic Spectroscopy* **99**, 150–162.

Table 5 Commonly available arsenic CRMs

Source	*Code*	*Description*
NIST[a]	SRM® 1566b	Oyster tissue
	SRM® 1568b	Rice flour
	SRM® 1570a	Spinach leaves
	SRM® 1946	Lake superior fish tissue
	SRM® 2669	Frozen human urine
	SRM® 2976	Mussel tissue
	SRM® 3103a	Standard solution
	SRM® 2669	Frozen human urine
	SRM® 3669	Frozen human urine (elevated levels)
JRC[b]	BCR® 185	Bovine liver
	BCR® 279	Sea lettuce
	BCR® 422	Cod muscle
	BCR® 626	Arsenobetaine solution
	BCR® 627	Tuna fish tissue
	ERM®-BB184	Bovine muscle
	ERM®-BB186	Pig kidney
	ERM®-BB422	Fish muscle
	ERM®-BC211	Rice
	ERM®-CA022a	Soft drinking water
	ERM®-CA011b	Hard drinking water – UK metals
	ERM®-CA615	Groundwater
	ERM®-DB001	Human hair
NRCC CCRMP[c]	DORM-4	Fish protein for trace metals
	LUTS-1	Nondefatted lobster hepatopancreas for trace metals
	RM 8414	Bovine muscle powder
	TORT-3	Lobster hepatopancreas for trace metals
IAEA[d]	IAEA-407	Fish tissue
	IAEA-140	Seaweed
	IAEA-155	Whey powder
	IAEA-336	Lichen
	IAEA-339	Cabbage
	IAEA-392	Algae
	IAEA-436	Tuna fish flesh homogenate
	IAEA-450	Trace elements in algae
	IAEA-452	Scallops
JNIES[e]	CRM No. 18	Human urine
	CRM No. 14	Brown alga (Hijiki)
	CRM No. 15	Scallop
	CRM No. 27	Typical Japanese diet (TJD)
KRISS[f]	CRM No. 108-01-001	Rice flour (natural level)
	CRM No. 108-01-002	Rice flour (fortified level)
	CRM No. 108-04-001	Oyster tissue powder
	CRM No. 108-04-003	Oyster powder for As, Cd, and arsenobetaine
	CRM No. 108-05-001	Water dropwort powder
	CRM No. 108-01-001	Rice flour (natural level)

[a]National Institute of Standards and Technology.
[b]Joint Research Centre.
[c]National Research Council of Canada by the Canadian Certified Reference Materials Project.
[d]International Atomic Energy Agency.
[e]Japan National Institute for Environmental Studies.
[f]Korea Research Institute of Standards and Science.

See also: Arsenic: Toxicology and Health Effects; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: High-Performance Liquid Chromatography; Chromatography: Supercritical Fluid Chromatography; Infrared Spectroscopy: Applications; Mass Spectrometry: Principles and Instrumentation; Spectroscopy: Types.

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Arsenic: Toxicology and Health Effects

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Arsenic Occurrence in the Environment

Arsenic is found ubiquitously in the natural environment. In nature, arsenic ranks 20th in order of abundance in the Earth's crust, 14th in seawater, and 12th in the human body. Arsenic cannot be eliminated from the environment; however, it can change its form and attach to or separate from existing particular matter in the environment. Arsenic is a major constituent in more than 200 minerals, hence its wide distribution. The Committee on Medical and Biologic Effects of Environmental Pollutants of the National Academy of Sciences established that the average arsenic content of the Earth's crust is 2.5 mg kg^{-1} with the concentration in soil ranging from 0.1 to 50 mg kg^{-1}, which varies considerably among various regions in the world.

Arsenic is generally introduced into the environment from either naturally occurring geologic sources or anthropogenic sources such mining, smelting, petroleum refining, and numerous types of chemical manufacturing. In addition, trace amounts of arsenic also enter the soil and water through various biological sources that contain arsenic such as plants and aquatic biota. Arsenic contained in soil is usually found in larger particles. These particles settle to the ground and are subsequently separated from the particle by air or rain. Arsenic that is attached to very small particles may be windblown and remain aloft for many days and travel long distances. Many of the more common arsenic compounds can dissolve in water and therefore can be found in lakes, rivers, or underground water by dissolving in rain or snow. Ultimately, most arsenic ends up in the soil or sediment.

Another source of environmental arsenic was through agricultural use in pesticides such as Paris Green (an arsenic pesticide); herbicides such as monosodium methanearsonate (CH_4AsNaO_3), sodium arsenite ($NaAsO_2$), and disodium methanearsonate ($CH_3AsNa_2O_3$); and phosphate fertilizers. Besides elemental arsenic, these materials reportedly also release calcium arsenate, magnesium arsenate, zinc arsenate, zinc arsenite, and lead arsenate into the soil. With the exception of monosodium acid methanearsonate (MSMA), virtually all arsenic-containing pesticide has been restricted by the US government. Arsenic that accumulates in the environment can be subject to various biotransformations – reduction, oxidation, and methylation. For instance, some plants, fish, and microorganisms affect the redistribution of arsenic through their inherent bioaccumulation and biotransformation and eventual volatilization. Some transformation results in less toxic forms of arsenic. Methylation of inorganic arsenic in some bacteria under anaerobic conditions can occur. Similarly, some marine algae can transform arsenate into less toxic nonvolatile methylarsonic acid and dimethylarsenic acid in seawater. On the other hand, some fungi transform inorganic and organic arsenic compounds into highly toxic methylarsines. The arsenic concentrations in agricultural plants are reported to range from 0.007 to 7.50 mg kg^{-1} while the arsenic concentration in some fish can be as high as 100 mg kg^{-1} (in organic forms).

In addition to the normal levels of arsenic in air, water, soil, and food, human exposure to arsenic can occur because of proximity to hazardous waste sites containing high levels of arsenic, or occupational exposure to arsenic from copper or lead smelting, wood treating, and pesticide applications; use of arsenic-treated wood or arsenic-containing pesticides; or drinking water with high levels of arsenic. The elemental form of arsenic is commonly used in alloys for lead-acid batteries and cable sheaths. Arsenic compounds are also used in semiconductors and light-emitting diodes. The result is that humans are exposed to arsenic from a myriad of places including food. In fact, the primary route of arsenic exposure for the general population is via the ingestion of contaminated food or water. The daily intake of total arsenic from food and beverages is believed to be in the range of 20–300 μg per day. In 2001, the World Health Organization (WHO) determined that the daily dietary intake of total arsenic in Japan is higher than that of both Europe and the United States. WHO subsequently estimated that approximately 25% of daily dietary arsenic intake is from inorganic sources and arsenic intake is typically higher in men than in women and children. The estimated arsenic intake levels for various grouping are as follows:

- 1.3 μg per day for infants <1 year of age
- 4.4 μg per day for 2-year-olds
- 9.9 μg per day for 25–30-year-old men
- 10 μg per day for 60–65-year-old women
- 13 μg per day for 60–65-year-old men

Based on these numbers, one could speculate that a daily requirement for humans would be about 12–15 μg per day for individuals consuming approximately 2000 calories of a mixed-food diet. The Contaminants in the Food Chain (CONTAM) Panel of the European Food Safety Authority extensively examined the arsenic content of foods in the European Union and published the results in 2009. Most food categories contained low levels of arsenic (<0.02 mg kg^{-1}): some mean values include water (0.003 mg kg^{-1}), rice (0.14 mg kg^{-1}), and seafood/shellfish (5 mg kg^{-1}). The highest value reported was for a species of algae (30.9 mg kg^{-1}). The mean arsenic levels found in various food categories as determined by EFSA in 2009 are presented in Table 1.

The CONTAM Panel reviewed the provisional tolerable weekly intake (PTWI) of 15 μg kg^{-1} established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1989 and determined that dietary exposure to inorganic arsenic should be reduced. The panel calculated that people consumed 0.3–8 μg kg^{-1} per day of inorganic arsenic. EFSA reexamined the arsenic levels in food in 2014 and calculated a lower dietary exposure when compared to that published in the 2009 EFSA opinion ranging from 0.09 to 0.38 μg kg^{-1} per day. The EFSA report suggests that several factors could account for the discrepancies including the use of a more detailed codification to classify the foods (FoodEx), the exhaustive evaluation of the occurrence data carried out in the latter

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Table 1 Contribution of various food types to intake of arsenic

Food identification	*Range of arsenic (mg kg^{-1})*
Cereals and cereal products	0.0536–0.0725
Sugar, sugar products, chocolate	0.0135–0.0321
Vegetable and animal fats	0.0062–0.0205
Vegetables and nuts	0.0261–0.0366
Starch/potatoes	0.0031–0.0142
Fruits	0.0058–0.0168
Juices, soft drinks, and bottled water	0.0024–0.0053
Coffee, tea, cocoa	0.0490–0.0613
Alcoholic beverages	0.0062–0.0127
Meat and meat products, offal	0.0051–0.0150
Fish and seafood	2.3818–2.3837
Milk and dairy-based products	0.0042–0.0136
Tap water	0.0013–0.0022

Data from EFSA (2009).

report, and the different occurrence data used and how they were handled.

In all cases, EFSA found the main contributor to dietary exposure to inorganic arsenic was 'grain-based processed products (nonrice-based)' – specifically wheat bread and rolls. Other important contributors to inorganic arsenic exposure were rice, milk and dairy products, and drinking water.

Absorption

Inhalation

Inorganic arsenic occurs in the atmosphere as particulate matter, and studies reveal that these particles are absorbed into the lungs via two basic mechanisms:

1. Deposition onto the lung surface
2. Absorption of arsenic from the deposited material

It is estimated that the overall rate of absorption of inhaled arsenic is 30–34%.

Organic arsenic is also thought to be absorbed via the inhalation route; however, there are no publicly available studies that definitively address organic arsenic via the inhalation exposure route.

Oral

The major site of absorption of inorganic arsenic is the small intestine via a proton (H+) gradient. The optimal pH for arsenic absorption is 5.0. Studies in humans indicate that arsenates and arsenites are well absorbed as much as 95% across the gastrointestinal tract; however, gastrointestinal absorption may be considerably lower if highly insoluble forms of arsenic are ingested. Studies reveal that 75–85% of organoarsenics such as MMA (CH_4AsNaO_3) and DMA ($C_2H_6AsO_2$) are absorbed across the gastrointestinal tract.

Dermal

Dermal exposure leads initially to arsenic binding to the skin, and it has been shown that the bound arsenic may only very slowly be taken up into the blood although it can occur post-exposure because it is stored in the skin.

Metabolism

Because of the presence of arsenic in the environment for millions of years, living organisms have evolved a variety of resistance mechanisms to overt arsenic exposure. The most prominent of these include the following:

1. Extracellular precipitation
2. Chelation (bonding arsenic to nonmetal ions)
3. Intracellular sequestration (bonding it to another ion or molecule rendering it chemically inert)
4. Active extrusion (removal via active transport through the cell membrane)
5. Biochemical/bioelectrical transformation through reduction/oxidation reactions
6. Biochemical transformation through methylation

Inorganic Arsenicals

Although a single, clear mode of action of toxicity has not been delineated for arsenic, data suggest that arsenic may replace phosphate in various biochemical reactions throughout the cell. Metabolic processes appear to be similar whether exposure is by the inhalation, oral, or parenteral route. The metabolism of inorganic arsenic has been extensively studied in humans and animals. There are two primary metabolic processes found in higher organisms:

(1) Reduction/oxidation reactions
(2) Methylation reactions

The result of these processes is the reduction of inorganic arsenate to arsenite, methylation to MMA(V), reduction to MMA(III), and methylation to DMA(V).

Exposure of humans to either arsenates or arsenites results in increased levels of arsenite, arsenate, and MMA and DMA in urine. Greater than 75% of the absorbed arsenic dose is excreted in the urine. It is when the system is overwhelmed that the exposure then results in arsenic toxicity. DMA is the principal metabolite following long-term arsenic exposure, with lower levels of inorganic arsenite, arsenate, and MMA. Studies in humans reveal the relative proportions are approximately 40–75% DMA, 20–25% inorganic arsenics, and 15–25% MMA.

An alternative biotransformation pathway has been proposed for arsenic involving the nonenzymatic formation of glutathione complexing with arsenite yielding arsenic triglutathione. The arsenic triglutathione is subsequently methylated by arsenic (+3 oxidation state) methyltransferase (AS3MT) to form monomethyl arsenic glutathione. Depending upon the concentration of glutathione, either MMA (at low levels of glutathione) or DMA (at high levels of glutathione) is formed. Studies have shown that methylation of arsenic results in lower tissue retention of inorganic arsenic, and this process is considered to be a detoxification mechanism.

Organic Arsenicals

Organoarsenic or organic arsenicals such as MMA and DMA appear to undergo scant metabolic change when ingested by humans. On the other hand, almost 85–90% of ingested

arsenosugars are excreted within 90 h; however, arsenosugars have been shown to undergo metabolism. Arsenosugars per se are generally not toxic; however, arsenosugar degradation can result in toxic urinary metabolites such as trivalent methylated arsenic. While DMA is the common metabolite from both inorganic sugars and arsenosugars, thiolated intermediate forms of DMA can be formed and present considerably higher toxicity.

Excretion

Inhalation

Urinary excretion of arsenic appears to account for a large part (30–60%) of the inhaled dose suggesting that most of the arsenic found on respirable particles deposited in the lungs is excreted in the urine. The primary forms of arsenic found in the urine of inhalation-exposed humans are DMA and MMA. Inorganic arsenic constitutes <25% of the total urinary arsenic.

No data were available for inhaled organic arsenicals.

Oral

Urinary excretion in humans accounts for 55–87% of ingested inorganic arsenic. In contrast, ingestion of the highly insoluble arsenic triselenide (As_2Se_3) showed little urinary excretion indicating that mostly soluble forms of arsenic are found in urine. Studies measuring arsenic excretion in humans indicate that minimal inorganic arsenic is excreted in the feces and that approximately 50% is excreted in urine within 1–5 days. Arsenic is also excreted in the bile in the form of two arsenic–glutathione complexes (arsenic triglutathione and methylarsenic diglutathione).

Studies of organoarsenicals in humans indicate that ingested MMA and DMA are excreted mainly in the urine (~80%) within 24 h, and these materials are found in both the feces and urine mostly unchanged.

Dermal

No studies were available on the excretion of dermal exposure of either inorganic arsenicals or organoarsenicals.

Biochemical Function and Nutritional Essentiality

Animal studies conducted in 1975 demonstrated that arsenic deprivation increased stillbirths of rat pups and depressed the growth of those that survived. Other later studies revealed a decrease in fertility among animals deprived of arsenic in the feed. These and other nutritional studies have suggested that arsenic may be essential for reproduction and maternal milk production. Arsenic deficiency has resulted in histological changes in the mitochondria of the skeletal muscles and liver tissue. While specific intakes of arsenic are still not precise, it has been shown that dietary consumption of $<50\ \mu g\ kg^{-1}$ is arsenic-deficient. The range considered normal background for arsenic in feed is $350–500\ \mu g\ kg^{-1}$.

Arsenic active transport systems in cellular membranes are found in nearly all organisms. Interestingly, some microorganisms have evolved to be able to utilize arsenic as a metabolic energy source through either arsenite oxidation or arsenate reduction. There is evidence that a trace amount of arsenic may be necessary in several metabolic processes including methylation metabolism. Arsenocholine ($C_5H_{14}AsO$) can replace choline in phospholipids indicating that arsenocholine might have a role in the metabolism of labile methyl groups. Arsenic also appears to have a role in the conversion of methionine to various metabolites such as *S*-adenosylmethionine ($C_{15}H_{22}N_6O_5S$) and taurine ($C_2H_7NO_3S$). Since arsenic binds to sulfhydryl groups, it is possible that its presence may decrease enzymatic catalysis as is commonly noted with other metals. There is limited evidence that arsenic may be necessary along with zinc in protein synthesis and degradation and possible uric acid metabolism. Additional studies have also shown that arsenic may also control gene expression at the transcriptional level via changes in the methylation of core histones.

A recent study indicates that arsenic may be crucial for longevity. Telomeres are complexes of tandem repeats of DNA and protein that constitute the eukaryotic chromosomes. Telomeres shorten with normal aging and studies have shown that this process can be accelerated by increased oxidative stress, kinase activity, and cellular inflammation. There is significant evidence that telomere length may be affected by environmental chemicals that have frequently been associated with chronic diseases such as black carbon, benzene, toluene, polycyclic aromatic hydrocarbons (PAHs), and *N*-nitrosamines. Decreasing the length of telomeres is generally associated with advancing age and exposure to a vast array of environmental chemicals resulting in increased oxidative stress, increased kinase activity, and inflammation associated with some chronic diseases. However, short-term arsenic exposure was shown to be associated with increases in telomere length begging the question of what constitutes the underlying mechanism and whether or not it could be a factor in longevity. It should be noted, however, that there is little definitive evidence showing that arsenic is essential for humans. If arsenic is an essential element, it has a very limited range of safety, which might be from >12 to about 250 μg per day. More than this range could be toxic while less may not permit certain biochemical functions as noted in the preceding text. The only available data are from animals from which some guesstimates can be made with respect to a daily requirement for arsenic in humans. Common foods contain 11.5–40 μg total arsenic.

Acute Toxicity

Acute data are mixed showing a variety of target organs. The lethal dose of acute inorganic arsenic ranges from 100 to 300 mg, while the Risk Assessment Information System database states that "the acute lethal dose of inorganic arsenic to humans has been estimated to be about $0.6\ mg\ kg^{-1}$ per day" with death usually occurring within 24 h to 4 days. The clinical features of oral ingestion initially involve the gastrointestinal system due to a direct irritation of the gastrointestinal mucosa including nausea, vomiting, abdominal pain, and copious watery diarrhea. Other symptoms of inorganic arsenic toxicity can include excessive salivation, acute psychosis, skin rash,

cardiomyopathy, seizures, respiratory failure and pulmonary edema, renal failure, general central nervous system malfunctions with acute psychosis, multiorgan failure, and eventually death. Hematological effects have been reported to include hemoglobinuria, coagulation, bone marrow depression, severe pancytopenia, and normocytic normochromic anemia and basophilic stippling. Acute, high-dose exposure can lead to encephalopathy, with clinical signs such as confusion and hallucinations.

In survivors of arsenic exposure, bone marrow depression, hemolysis, hepatomegaly, melanosis, polyneuropathy, and encephalopathy – with clinical signs such as confusion and hallucinations – may be observed. After acute poisoning, atomic absorption spectrometry studies reveal that the highest concentration of arsenic is in the kidneys and liver. In both fatal and nonfatal inorganic arsenic exposure of humans, nausea, vomiting, and watery diarrhea are the most common symptoms.

Chronic Toxicity

Six-month dietary dog studies showed a dose-dependent decrease in feed consumption and body weights and increased levels of aspartate aminotransferase suggesting hepatotoxicity at dose levels of 4 and 8 mg kg^{-1} per day of arsenite; however, no confirmatory histopathology was noted. Conversely, histological alterations in the kidney and liver were observed in rats exposed to 50 mg sodium arsenate per milliliter for 320 days in drinking water.

In humans, clinical features of chronic inorganic arsenic toxicity reveal significant variations among individuals, population and various subpopulation groups, and geographic locations. Therefore, persons exposed to chronic arsenic poisoning exhibit a wide range of clinical features generally with an onset of nonspecific symptoms of abdominal pain, diarrhea, and sore throat. The most serious resulting effect of this exposure is an increased risk of cancer of the skin, lungs, bladder, and/or kidneys. Other effects include skin changes in pigmentation and hyperkeratosis with chronic toxicity diarrhea occurring in recurrent vomiting. Chronic exposure also causes direct myocardial injury, cardiac arrhythmias and cardiomyopathy, and hypertensive heart disease. The most frequent neurological finding is a peripheral neuropathy. The arsenic-related effects also include changes in behavior and memory loss. Increased prevalence of cerebrovascular disease has also been observed after long-term arsenic exposure in drinking water. Exposure to high concentrations of arsenic is associated with an increased risk of diabetes mellitus and neutropenia. In chronic arsenic ingestion, it has been shown that arsenic accumulates in the liver, kidneys, heart, and lungs and smaller amounts in various other tissues and organs. After about 2 weeks of ingestion, some arsenic is deposited in the keratin-rich tissues, namely, nails, hair, and skin.

Reproductive/Developmental Toxicity

Studies conducted in 1975 previously demonstrated that arsenic deprivation increased stillbirths of rat pups and depressed the growth of those that survived. Other studies showed that only 58% of arsenic-deprived goats and 62% of arsenic-deprived miniature pigs produced offspring compared with 92% and 100% of control animals. Fertility was severely depressed. These and other data suggest that too little dietary arsenic could be detrimental and there is a threshold for arsenic toxicity. Data from numerous animal studies have demonstrated that excess arsenic can produce developmental toxicity, including malformation, death, and growth retardation, in several species. Animal studies of oral inorganic arsenic exposure have reported developmental effects; however, these effects were only observed at maternally toxic concentrations. The most sensitive species is the rabbit, which presented increased resorptions and mortality with decreased viable fetuses/litter at 1.5 mg kg^{-1} per day and a developmental no observed adverse effect level ($NOAEL_{developmental}$) of 0.4 mg kg^{-1} per day of As. Oral dose studies indicate that death and growth retardation are produced by arsenic exposure. Arsenic has been shown to transfer to the fetus and produces developmental toxicity in embryo cultures. Studies have identified increased inorganic or organic arsenic exposures to result in decreased fertility and pregnancy rates in both rats and mice. When females were dosed preimplantation and during pregnancy, the primary effect of arsenic on reproduction was a dose-dependent increase in fetal mortality and in postnatal growth retardation. On balance, the animal studies suggest that arsenic exposures are primarily a risk to the developing fetus. Human data are limited but show an association with spontaneous abortion and stillbirth; however, interpretation of these studies is complicated because of multichemical exposure history. There are no definitive data on the effects of reproduction in humans experiencing arsenic exposure. However, exposure to arsenic in drinking water has been associated with adverse reproductive outcomes showing increases in spontaneous abortions in women drinking water at 0.008 mg kg^{-1} per day for 5–10 years. Chronic exposure of pregnant women to arsenic in the drinking water has been associated with low infant birth weights. Similar associations have been made between late fetal mortality, neonatal mortality, and postneonatal mortality and exposure to 0.86 mg l^{-1} of arsenic in drinking water. These data are not definitive because of lack of sufficient study controls; however, arsenic can cross the placenta and has been found in fetal tissue and in breast milk.

Genotoxicity

In vitro studies of inorganic arsenic in bacterial assays have generally been negative for gene mutations. However, *in vitro* arsenic studies in various human, mouse, and hamster cells and Syrian hamster embryo cells have been positive for DNA damage and repair and enhancement or inhibition of DNA synthesis and chromosome aberrations and sister chromatid exchanges. Arsenic has also been shown to be positive in the Comet assay. Animal and human data indicate that inhaled inorganic arsenic causes chromosomal aberrations. Chromosomal abnormalities in rats given oral doses of sodium arsenate (4 mg kg^{-1} per day) for 2–3 weeks produced an increase in the number chromosomal aberrations. In contrast, studies

in mice given sodium arsenite (50 mg kg^{-1} per day) for up to 8 weeks showed no consistent increase in chromosomal aberrations in either gonadal or somatic cells. Data from two populations of individuals exposed to mean concentrations of either 5 or 109 μg l^{-1} in drinking water found no significant differences in the frequency of chromosomal aberrations or sister chromatid. These studies suggest that ingested arsenic may cause chromosomal effects, but these data are too limited to draw a firm conclusion. While ingested arsenic studies suggest that arsenic may cause chromosomal effects, these data have yet to be confirmed.

Several studies on organoarsenicals reveal that DMA and roxarsone may be able to cause chromosome aberrations, mutations, and deoxyribonucleic acid (DNA) strand breaks. However, *in vitro* studies with MMA did not find significant increases in the occurrence of chromosome aberrations, bacterial mutations, or unscheduled DNA synthesis. Other data show reversible DNA damage in *in vivo* rat and mouse tissue at 1500 mg kg^{-1} of DMA. Interpretation of these data with respect to health risk is unclear.

Carcinogenesis

Animal studies of both inhalation and oral exposure to inorganic arsenic have not resulted in increased incidence of cancer formation in adult animals. However, there is strong evidence that inorganic arsenic exposure to pregnant mice results in their offspring showing transplacental carcinogenesis. Further, one study in mice exposed to pentavalent arsenic in drinking water at 500 mg per day for 2 years showed an increased incidence in tumors of the lungs, liver, gastrointestinal tract, and skin. The organoarsenic DMA has been shown to be carcinogenic in a 2-year drinking water study in rats.

Evidence for the association of cancer in humans exposed to arsenic in drinking water comes from studies in five areas of the world with extremely elevated levels of naturally occurring inorganic arsenic. These areas include the following:

- China/Taiwan
- Chile
- Argentina
- Bangladesh
- India

Additionally, inorganic arsenic has been shown to be a human carcinogen by both the inhalation and oral exposure routes. There is a large body of evidence that inhalation exposure of humans to arsenic significantly increases the risk of lung cancer. In addition, there is evidence that the incidence of skin, liver, and gastrointestinal tumors can occur following inhalation exposure. The strongest evidence that arsenic is responsible for the observed lung cancer comes from quantitative dose–response data relating specific arsenic exposure levels to lung cancer risk. Cumulative exposure to 0.75 mg m^{-3} over a year has been associated with an increased risk of lung tumors. Most of the study data have been from arsenic exposure at copper smelting operations. A limitation of the inhalation studies is the inability to control for exposure to other chemicals, such as sulfur dioxide, and cigarette smoking. It should also be noted that arsenic has been referred to as a 'paradoxical' human carcinogen since the animal data are mostly negative for carcinogenesis and are not judged to be reliable for determining the levels of significant human exposure. The American Conference of Industrial Hygienists (ACGIH), the USEPA, the International Agency for Research on Cancer (IARC), the Maximale Arbeitsplatz-Konzentration (MAK) of Germany, the National Institute for Occupational Safety and Health (NIOSH), the National Toxicology Program (NTP), and the Occupational Safety and Health Administration (OSHA) concur that inorganic arsenicals are human carcinogens and have officially classified them as such.

Other Effects of Arsenic Exposure

The primary incidental effect of long-term oral exposure to inorganic arsenic is a pattern of skin changes. These skin changes are also associated with changes in the blood vessels of the skin, which result in patches of darkened skin. These patches are usually accompanied by small wart-type blemishes on the palms of the hand and soles of the feet (palmar keratosis and solar keratosis, respectively). Leukonychia striata can occur with arsenic exposure. This is a condition also referred to as Aldrich–Mees' lines, which are lines of discoloration across the nails of the fingers and toes. Other incidental effects from inorganic arsenic ingestion could include a decreased production of red and white blood cells, which may ultimately cause fatigue, abnormal heart rhythm, blood vessel damage resulting in bruising, and impaired nerve function. The nerve damage can cause the sensation of 'pins and needles' in the hands and feet. Inhalation of inorganic arsenic can present throat and respiratory irritation, while dermal contact can be irritating to the skin causing both erythema and edema. Arsenic inactivates almost 200 enzymes involved in cellular energy pathways and DNA synthesis and repair. Hence, the effects are widespread.

Children residing in areas of known high natural or anthropomorphic arsenic are at higher risk because of the tendency of young children to consume nonnutritive objects (pica), specifically soil or clay (geophagy). Because of this pica activity, children are at higher risk than adults because it is an additional pathway to arsenic exposure. There is also some limited evidence that suggests that long-term exposure of developing children to inorganic arsenic may contribute to lower IQ scores. There is some evidence that transplacental exposure to arsenic during gestation and overt arsenic exposure during early childhood may increase mortality in young adults.

Therapeutic Uses of Arsenic

Historically, elemental arsenic was available as solutions, tablets, and pastes and in injectable forms and used as a healing agent after Greek physicians Galen and Hippocrates popularized its use. In the eighteenth century, Fowler's solution (1% arsenic trioxide) was used for the relief of various ailments and remained very popular for over 150 years. Fowler's solution was used to treat leukemia, skin conditions (psoriasis, dermatitis, and eczema), stomatitis and gingivitis in infants, and Vincent's angina up until the 1950s. Salvarsan (also

known as arsphenamine) is an organoarsenic with a chemical formula of $C_{12}H_{12}As_2N_2O_2$. It was the primary treatment for syphilis from its discovery by Drs. Sahachirō Hata and Paul Ehrlich in 1910. In fact, arsenicals were the drug of choice in the treatment of syphilis until the advent of penicillin during World War II. Some of the side effects from salvarsan included rashes, liver damage, and risks of life and limb and were thought to be due to the fact that salvarsan was highly unstable in air and has an IV LD_{50} range of 37–91 mg kg^{-1}. The organoarsenic melarsoprol is still used in the treatment of African trypanosomiasis, Chagas disease, and West African sleeping sickness and has toxicity similar to that of the inorganic arsenics with an IV LD_{50} range of 788–2400 mg kg^{-1}.

Based on the induction of apoptosis via the apoptosis-inducing factor (AIF), arsenic trioxide (As_2O_3) is widely used to induce remission in patients with acute promyelocytic leukemia. In China, arsenic sulfide (realgar) is used to treat psoriasis, syphilis, asthma, rheumatism, hemorrhoids, cough, and pruritus and is used as an anti-inflammatory agent. In India, some hematological malignancies are treated with arsenic-containing medications, while in Korea, arsenic is used in herbal preparations to treat hemorrhoids.

Clinical Management of Arsenic Exposure

Acute arsenic poisoning must be managed aggressively with life support monitoring along with electrolytes and fluid balance to try to minimize cerebral and pulmonary edema. Gastrointestinal irrigation is indicated in cases of arsenic poisoning. The use of activated charcoal or bentonite is sometimes contraindicated because of the nausea and vomiting associated with the arsenic exposure. Nasogastric suction can be used to remove recirculating biliary secretions. Chronic arsenic exposure is less aggressive; however, gastric decontamination with activated charcoal is indicated with oral ingestion.

The medical treatment of both acute and chronic arsenic toxicity is also generally conducted with chelating agents. These agents link the arsenic molecules forming a complex ringlike structure converting the arsenic to a chemically inert form that can be excreted without further interaction with the body. Arsenite inhibits the binding of steroids to the glucocorticoid receptor, but not other steroid receptors; therefore, binding of arsenite to protein at nonessential enzyme sites is believed to be one detoxification mechanism. Chelators have been used clinically as antidotes for acute and chronic poisoning. The most common chelators include dimercaprol, DMSA, and Dimaval. D-Penicillamine was a chelator used previously, but is no longer recommended because of serious reactions involving the hematologic system (thrombocytopenia, leukopenia, agranulocytosis, and aplastic anemia) and renal system (proteinuria, hypoalbuminemia, and nephrotic syndrome). Dimercaprol or British anti-Lewisite was developed by British scientists as an anecdote to an arsenic-containing weapon called lewisite. DMSA (meso-2,3-dimercaptosuccinic acid) was developed as a chelator for mercury poisoning. All three chelating agents bind with arsenic to form a stable 1,2,5-arsadithiolane that effectively inactivates the arsenic. Although body burden is not reduced, chelators bind free arsenic and serve to reduce the biologically active arsenic. Serious side effects occur with the administration of these agents such as nausea, vomiting, gastrointestinal distress, and hemolysis and elevated liver enzymes. Since each of these chelators has its limitations, additional research is ongoing seeking better treatment for arsenic exposure. Hemodialysis in arsenic-exposed individuals has shown that only very minimal amounts of arsenic are removed, and it is only used where there is renal failure. Dimaval of DMPS was also developed for mercury poisoning; however, it is only approved for use in Germany and Taiwan and not in the United States.

Regulations/Guidelines/Legislation

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), as amended in 1986, by the Superfund Amendments and Reauthorization Act (SARA), required that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the US Environmental Protection Agency (EPA) a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List, prepare toxicological profiles for each substance on the priority list of hazardous substances, and determine the associated acute, subacute, and chronic health effects. The ATSDR Minimal Risk Levels (MRLs) were developed as an initial response to that mandate to derive substance-specific health guidance levels for nonneoplastic end points. An MRL is defined as an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. These substance-specific estimates, which are intended to serve as screening levels, are used to identify contaminants and potential health effects that may be of concern at hazardous waste sites. While the MRLs do not have the force of law, they are highly regarded guidelines from which other regulations have been derived. Since arsenic was one of the listed hazardous chemicals found on the CERLCA National Priorities List, the following oral MRLs have been established:

- Acute oral exposure ($\leq$14 days) to inorganic arsenic: MRL of 0.005 mg kg^{-1} per day
  - No intermediate-duration oral MRL was derived for inorganic arsenic.
- Chronic oral exposure ($\geq$1 year) to inorganic arsenic: MRL of 0.0003 mg kg^{-1} per day
  - No acute-duration oral MRL was derived for MMA.
- Intermediate-duration oral exposure (15–364 days) to MMA: MRL of 0.1 mg kg^{-1} per day
- Chronic oral exposure ($\geq$1 year) to MMA: MRL of 0.01 mg kg^{-1} per day
  - No acute- or intermediate-duration oral MRLs were derived for DMA.
- Chronic oral exposure ($\geq$1 year) to DMA: MRL of 0.02 mg kg^{-1} per day
  - No acute-, intermediate-, or chronic-duration inhalation MRLs were derived for inorganic or organic arsenic compounds.

From about 1850 to the introduction of organic pesticides in the 1940s, inorganic arsenic compounds were the primary pesticides used in the United States. These included calcium

Table 2 Arsenic guidelines and regulations

Regulating body	*Standard*	*Classification/value*
International		
International Agency for Research on Cancer (IARC)	Carcinogenicity	Group 1[a]
World Health Organization (WHO)	Air quality unit risk factor for lifetime exposure to a concentration of 1 μg m^{-3}	1.5×10^3
	Air quality guideline: lifetime risk level (1:100.000)	0.0066 μg m^{-3}
	Drinking water	0.01 mg l^{-1}
Australia	Drinking water	0.007 mg l^{-1}
Argentina	Drinking water	0.05 mg l^{-1}
Bangladesh		
Chile		
China		
Croatia		
Ecuador		
Ghana		
India		
Nepal		
Thailand		
Vietnam		
Canada	Drinking water	0.01 mg l^{-1}
European Union		
Japan		
Taiwan		
Australia/New Zealand	Air quality guideline	0.0055 μg m^{-3}
	Food	1 mg kg^{-1}
	Salt	0.5 mg kg^{-1}
Canada	Ambient Air Quality Criteria (AAQC)	0.3 μg m^{-3}/24-h
	Foods	0.1–3.5 ppm
	Veterinary drugs	0.5–2 ppm
European Union	Air quality	6 ng m^{-3}
German – MAK[b]	Carcinogenicity	Class 1[c]
The United States		
American Conference of Industrial Hygienists (ACGIH)	TLV[d]	0.01 mg m^{-3}
	Carcinogenicity	TLV-A1[e]
National Institute for Occupational Safety and Health (NIOSH)	REL[f]	0.002 mg m^3
	IDLH[g]	5 mg m^3
	Carcinogenicity	Ca[h]
Occupational Safety and Health Administration (OSHA)	PEL[i] organic As	0.5 mg m^{-3}
	PEL inorganic As	10 μg m^3
	PEL arsine (AsH_3)	0.2 mg m^{-3}
	PEL[i] organic As: industry + shipyards	0.5 mg m^{-3}
	Carcinogenicity	Ca[j]
	Air quality guideline: inhalation unit risk	4.3×10^3 μg m^{-3}

EPA	Risk-specific concentration (RsC) [1:100.000]	0.002 $\mu g\ m^{-3}$
	Drinking Water Equivalent Level (DWEL)	0.01 $mg\ l^{-1}$
	Drinking water unit risk	$5 \times 10^5\ \mu g\ l^{-1}$
	Water: Maximum Contaminant Level (MCL)	0.01 $mg\ l^{-1}$
	Residue tolerance: DMA	2.8 ppm
	Residue tolerance: MMA	0.35–0.9 ppm
	Residue tolerance: arsanilic acid	2 ppm
	Carcinogenicity	Group A[k]
	RfC[l]	No data
	RfD[m]	3×10^4 $mg\ kg^{-1}$ per day
FDA	Drinking and bottled water	0.01 $mg\ l^{-1}$
	Food: poultry meat and eggs[n]	0.5 $mg\ kg^{-1}$
	Food: swine	0.5–2 $mg\ kg^{-1}$
	Food additives: as in titanium dioxide	1 $mg\ kg^{-1}$
	Food additives: synthetic iron oxides	3 $mg\ kg^{-1}$
	Pesticide/herbicide As_2O_3 residue in fruits and vegetables	0.35 $mg\ kg^{-1}$
National Toxicology Program (NTP)	Carcinogenicity	K[o]
US Department of Agriculture (USDA)	Prohibited in organic crop production	Arsenic

[a]Carcinogenic to humans.
[b]Maximale Arbeitsplatz-Konzentration (MAK) (Germany).
[c]Substances that cause cancer in man.
[d]Threshold limit value.
[e]Confirmed human carcinogen.
[f]Recommended exposure limit.
[g]Immediately dangerous to life or health.
[h]Potential occupational carcinogen.
[i]Permissible exposure limit.
[j]Carcinogen.
[k]Human carcinogen.
[l]Reference concentration (RfC) – an estimate of a continuous inhalation exposure concentration to people (including sensitive subgroups) that is likely to be without risk of deleterious effects during a lifetime.
[m]Reference dose (RfD) – an estimate of a continuous oral exposure level to people (including sensitive subgroups) that is likely to be without risk of deleterious effects during a lifetime.
[n]Chickens are not to be fed with arsenic supplements within 5 days of slaughter.
[o]Known human carcinogen.

arsenate, lead arsenate, and sodium arsenite. The use of many inorganic arsenic compounds in agriculture was voluntarily terminated by the mid-1960s. However, lead arsenate was not officially banned by the EPA until 1988. While uses on food crops and the use of arsenic acid as a defoliant on cotton plants were voluntarily terminated in 1993, EPA officially canceled the last agricultural use of arsenic acid in the United States on 3 November 1999. All arsenic-containing pesticides were officially restricted in the United States in 2013 with only MSMA remaining on the market as of the date of writing of this article. In 1987, USEPA terminated the use of inorganic arsenic for nonwood pesticides. Effective 31 December 2003, treatment of wood with CCA for residential uses was completely phased out by both EPA and the EU. However, CCA is still used in some far eastern Pacific Rim countries as of the date of writing of this article. Arsenic is no longer produced in the United States; all of the arsenic used in the United States is imported.

The EPA's Integrated Risk Information System (IRIS) has derived a chronic oral reference dose (RfD) of 0.0003 mg As/kg/day for inorganic arsenic. This value was based on a no observed adverse effect level (NOAEL) of 0.0008 mg As/kg/day for dermal effects, and possible vascular complications of individuals exposed to arsenic in well water using an uncertainty factor of 3 (to account for the lack of reproductive data and uncertainty in whether the NOAEL accounts for all sensitive subpopulations of individuals) were applied. No reference concentration (RfC) for chronic inhalation exposures to arsenic was reported. EPA has been assessing the IRIS document on inorganic arsenic since 2003. As of late 2014, this revision/reassessment is still in progress.

In 2008, FDA set 23 ppb as the 'level of concern' for inorganic arsenic in apple and pear juices based on noncarcinogenic effects. In 2011, the 'Dr. Oz' television show broadcast a program revealing results of its own testing of apple juice samples revealing 36 ppb arsenic in apple juice, but their studies neglected to separate inorganic from organic arsenic. Later that year, Consumer Reports magazine tested 88 samples of apple and grape juices and found that 25% of the samples exceeded federal drinking water standards of 10 ppb. Consumer Reports recommended that the arsenic limit for apple juice be set at 3 ppb; however, based upon these data and public pressure, in July 2013, FDA revisited the 23 ppm value and determined that it should be the same as the preexisting arsenic limit for bottled drinking water – 10 ppb. The January 2015 issue of Consumer Reports reviewed the FDA data on rice and grains and conducted tests on 697 samples of its own. They developed and proposed a point system for managing rice exposure since the inorganic arsenic content of rice varies greatly depending on the type of rice and where it was grown. Based upon the test data, they have identified better choices with much lower levels of inorganic arsenic exposure, including white basmati rice from India, Pakistan, or California and sushi rice grown in the United States. Additionally, they call upon FDA to set standards for arsenic in rice-based foods.

IARC indicates that there is sufficient evidence of a relationship between exposures to arsenic and inorganic arsenic compounds and human cancer to classify arsenic as a group 1 carcinogen (carcinogenic to humans). EPA has determined that arsenic is a human carcinogen by both the inhalation and oral routes and is classified as a group A carcinogen. EPA has calculated an oral cancer slope factor of 1.5 mg kg^{-1} per day and a drinking water unit risk of 5×10^5 μg l^{-1} for inorganic arsenic based upon human dose–response data. The inhalation unit risk for cancer is calculated to be 0.0043 μg m^{-3}.

Advisory values are generated by ATSDR, ACGIH, IARC NIOSH, and WHO targeting public health professionals involved in preventing health risks of environmental exposures, as well as specialists and authorities involved in the regulation of chemicals. While they provide a scientific basis for legally enforceable standards, the values generated by these organizations alone are not enforceable. The EPA RfDs are not enforceable standards. However, EPA uses RfDs as risk assessment benchmarks to generate regulations to maintain levels not to exceed the RfD. The more common global arsenic guidelines and regulations are presented in **Table 2**.

See also: Arsenic: Properties and Determination; Carcinogenic: Carcinogenic Substances in Food; Food Fraud; Rice: Types and Composition; Toxins in Food: Naturally Occurring; Trace Minerals and Trace Elements.

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Ascorbic Acid: Physiology and Health Effects

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Introduction

Vitamin C also known as L-ascorbic acid is an essential dietary nutrient required for numerous biological processes of the body. Human and several other animal species including primates and guinea pig are unable to synthesize it due to a deficiency of L-gulonolactone oxidase, a terminal enzyme in the biosynthetic pathway of ascorbic acid, because of mutation in the gene encoding for the enzyme. Therefore, vitamin C must be solely obtained from the diet to maintain a normal metabolic functioning of the body. Many fruits and vegetables including citrus fruits, guava, tomatoes, red and green peppers, kiwifruit, and broccoli are the predominant sources of vitamin C supplying more than 80% of the daily needs. Thus, consumption of adequate serving of fresh fruits and vegetables daily will ensure vitamin C adequacy. Diet insufficient in vitamin C (defined as plasma vitamin C levels of $<11\ \mu mol\ l^{-1}$) leads to a lethal condition known as scurvy, characterized by gingival changes, pain in the extremities, and hemorrhagic manifestation leading to death. On the other hand, marginal and suboptimal plasma and vitamin C levels have been associated with increased risk of death from all cause of mortality and cancer.

Over the years, vitamin C has received a greater scrutiny beyond its antioxidant function. Research trends in vitamin C have moved forward from preventing deficiency to investigating its therapeutic value. In conjunction with this, the recommendation for vitamin C intake is being reviewed in light of new evidences pertaining to the role of vitamin C in health and diseases. In fact, this issue remains a debatable topic, as there is no common ground on the amount needed to maintain optimum health. Vitamin C has been controversially used to prevent or delay the occurrence of chronic diseases and to treat certain illnesses. The aim of this article is to discuss briefly the established aspects of vitamin C physiology and its relation to health and disease conditions. Current evidences on the therapeutic role of vitamin C in several chronic illnesses including cardiovascular disease (CVD), cancer, and respiratory illnesses will be discussed.

Physiology of Vitamin C

Ascorbic acid is present in all parts of the body at varying concentration in the plasma, bone cells, and cellular immune system. Body stores of vitamin C can be affected by dietary intake, excretion rate in renal tubule, fecal losses, and metabolic losses. Most of the physiological functions of vitamin C are related to its oxido-reduction properties. It acts as a cofactor for hydroxylases and monooxygenase enzymes involved in the synthesis of collagen, carnitine, and neurotransmitters. Ascorbic acid maintains the metal ions' active center in reduced form to keep hydroxylase and oxygenase in their optimal activity and thereby enhance hydroxylation functions. These will be described briefly in the following subsections.

Absorption, Metabolism, Storage, and Excretion

Upon ingestion of vitamin C, the intestinal absorption that takes place is based on two known pathways (Figure 1): (1) the facilitated diffusion mediated by the facilitative glucose transporter (GLUT) and (2) a saturable-substrate transport mechanism via ascorbate-specific transporter (sodium vitamin C transporter (SVCT)). Oral vitamin C is absorbed for approximately 70–90% at moderate intake (30–180 mg day^{-1}), but absorption rate falls below 50% at intake above 1000 mg day^{-1}. Pharmacokinetics studies revealed that consumption of at least 200 mg day^{-1} of vitamin C in healthy young adults leads to plasma concentration of more than 50 μM, which is a nearly complete oral bioavailability, indicated by leukocyte saturation and minimum urinary excretion. This suggests that ingestion of vitamin C orally produces plasma concentrations that are tightly controlled.

Absorbed vitamin C is transported in the plasma as free anion ascorbate and is distributed to all tissues. At cell levels, particularly in the osteoblast, muscle, and retinal cells, an oxidized form of vitamin C known as dehydroascorbic acid (DHA) is taken up by GLUT and then reduced internally to ascorbic acid. Because DHA shares the same transporter as glucose, this leads to competitive inhibition particularly during altered serum glucose levels such as hyperglycemia secondary to diabetes. On the other hand, active transportation of ascorbate via SVCT is subjected to substrate feedback inhibition indicating a regulatory role in maintaining ascorbate concentration in the cells.

In terms of body store, a total body content of vitamin C in healthy adults ranges from 300 mg to 2 g with higher concentrations maintained in the leukocytes, eyes, adrenal glands, pituitary gland, and brain. Excessive vitamin C intake that is not metabolized is excreted mainly in the urine, although some can also be found in the feces. Maximum metabolic losses of vitamin C in healthy nonsmoking men range from 40 to 50 mg day^{-1}. Smokers are found to have 50% higher metabolic losses than nonsmokers.

In human, the main route of removal of ascorbic acid is through urinary excretion. For this purpose, ascorbic acid is oxidized to dehydroascorbic acid, which is subsequently hydrolyzed to diketogulonic acid. The catabolism of ascorbic acid is completed by decomposition of diketogulonic acid to various compounds such as oxalic and threonic acids (Figure 2).

Safety Levels and Toxicity

Generally, vitamin C is relatively safe even at doses up to several grams per day. This is due to the fact that the

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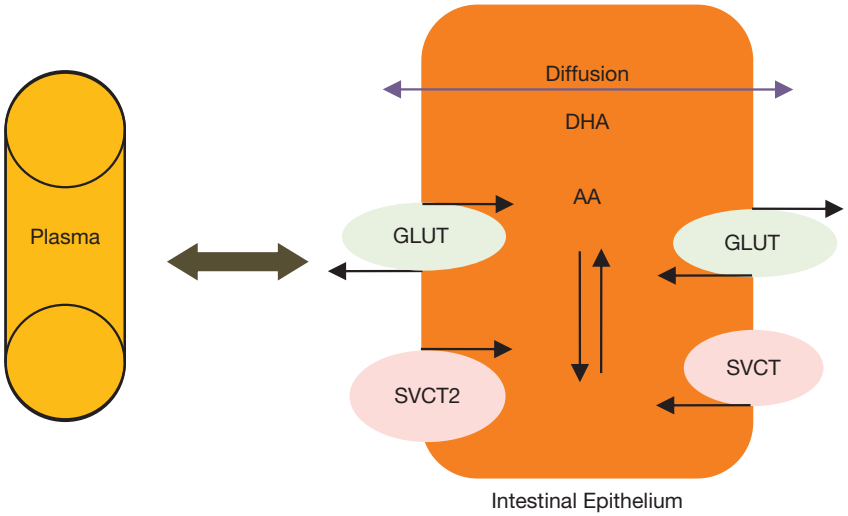

Figure 1 Vitamin C absorption across the intestinal epithelium. GLUT, glucose transporter; SVCT, sodium vitamin C transporter; AA, ascorbic acid; DHA, dehydroascorbic acid.

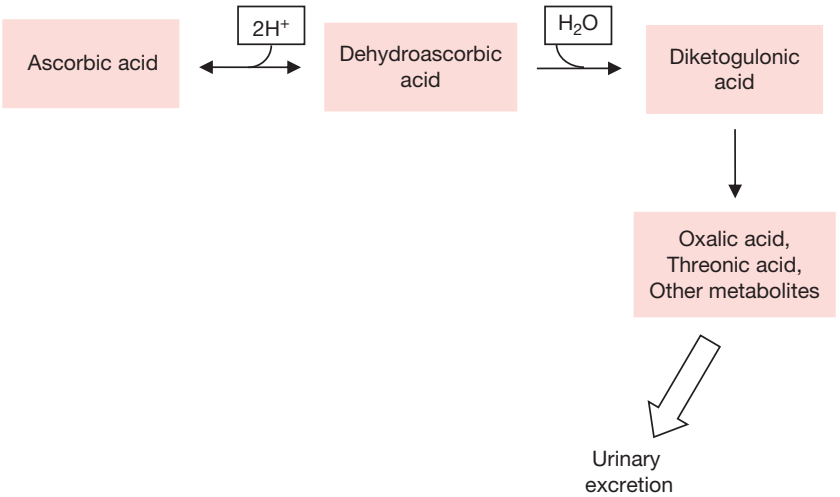

Figure 2 Catabolic pathway of vitamin C.

physiological concentration of vitamin C in the body is tightly regulated by alteration in the intestinal absorption, kidney excretion, and cellular transport. The upper tolerable level, the highest level of daily nutrient intake at which it is likely to pose no risk of adverse effects for most individuals, of vitamin C for adults is set at 2 g day^{-1}. Vitamin C intake at dose beyond 3 g day^{-1} is associated with gastrointestinal disturbance due to the unabsorbed ascorbate. However, participants that were administered intravenous vitamin C up to 100 g per infusion within a few hours in a pharmacokinetics study did not report any adverse effects, demonstrating the safety of this vitamin at megadose.

Nevertheless, high doses of vitamin C are contraindicated in special population including chronic kidney disease patients and hyperoxaluria due to inability to excrete excessive oxalate from metabolic conversion of vitamin C. Meanwhile, in patients with glucose-6-phosphate dehydrogenase deficiency, high doses of vitamin C could induce acute hemolysis. Moreover, high doses of vitamin C could hamper copper absorption and thus inhibit copper-containing superoxide dismutase, an important enzyme in antioxidant defense. On the other hand, very high doses of vitamin C could enhance intestinal iron absorption to a dangerous level among individuals with hereditary diseases such as hemochromatosis and thalassemia. It has been shown in some studies that excessive vitamin C intake (>1 g day^{-1}) may induce severe urine acidification, leading to impaired excretion of weak acids and bases, resulting in deposition of cystinate and urate in the urinary tract and formation of kidney stones.

Mechanism of Action of Vitamin C

Vitamin C is a water-soluble vitamin that is mostly known for its antioxidant function, which is defined as "any substance that, when present at low concentration compared to those of an oxidizable substrate, significantly delay or prevent

oxidation of that substrate." This is attributed to two major properties of vitamin C as an ideal antioxidant:

(1) The ability to readily scavenge free radicals including reactive oxygen species and nitrogen species such as superoxide, hydroperoxyl radicals and nitroxide radicals, single oxygen, nitrogen dioxide, and hypochlorite species, thus protecting substrates (e.g., protein, lipids, carbohydrates, and nucleic acid) from oxidative damage
(2) Stability and low reactivity of ascorbyl radical formed from these scavenging activities

When ascorbate quenches free radicals by donating electrons, ascorbyl radical with low reactivity is generated. Ascorbyl radical reacts with another radical to form DHA. The ascorbyl radical and DHA can be returned to reduced form (ascorbate) by electron donors like glutathione and NADPH.

Vitamin C acts as an electron donor to several enzymes – some participate in collagen hydroxylation and some other in carnitine and catecholamine biosynthesis. Vitamin C acts as cosubstrate to reduce the active center of metal ion of various mono- and dioxygenases and maintain them in a reduced state. Moreover, vitamin C has also been shown to spare other intercellular antioxidants such as glutathione by maintaining them at reduced state. Vitamin C can also regenerate α-tocopherol from the α-tocopheroxyl radical; thereby, it acts as an important co-antioxidant in the absence of which vitamin E can act as a pro-oxidant. Similarly, vitamin C could also regenerate one-electron oxidation products of various antioxidants including urate, glutathione, and β-carotene (Figure 3). However, *in vitro* experiments demonstrate that vitamin C could also act as pro-oxidant. Its pro-oxidant activity is agreed to be dependent on the concentrations used and on the presence of transition metal ions like copper and iron. It has been reported that high doses of exogenous iron (200 mg) and ascorbic acid (75 mg) release iron from iron-binding proteins and promote *in vitro* serum lipid peroxidation of guinea pigs. Ascorbate reduces these metals, which may react with H_2O_2 and subsequently •OH production; this reaction is known as Fenton chemistry. Reduced metals may also react with lipid hydroperoxides that results in production of lipid alkoxyl radicals; subsequently, these lipid alkoxyl radicals can initiate and promote lipid peroxidation. However, such effect is preventable in the presence of sufficient antioxidants (like glutathione). In addition, since metal ions have the capacity to transfer one electron, in biological systems, they bind to proteins; thus, these metal ions become less effective than free-radical catalysts. In line with this, no convincing data are available to demonstrate that this actually occurs in human.

Despite a wealth of knowledge on the antioxidant function of vitamin C from cultured cells and experimental animals, data in human are relatively scarce. This is partly attributed to the fact that human studies may be cofounded by various factors including the bioavailability, presence of illnesses, genetic variations especially with regard to vitamin C transporter, erroneous in dietary estimation, and lack of specific biomarkers. The most conclusive evidences to demonstrate antioxidant effects of vitamin C may be coming from lipids, DNA, and protein oxidation biomarkers. Several lines of evidence indicate that supplementation with vitamin C significantly reduced lipid peroxidation biomarkers (e.g., F_2-isoprostanes) and DNA damage (e.g., lymphocyte DNA damage – comet assay). Nevertheless, this effect is dependent on baseline vitamin C concentration. Supplementation with vitamin C cannot further reduce the oxidative damage if tissues levels of vitamin C are already saturated.

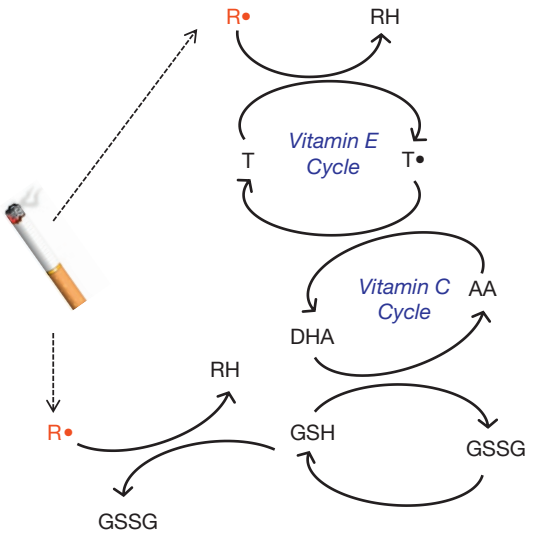

Figure 3 Role of vitamin C in neutralizing free radicals and its interrelationship with other antioxidants. Abbreviations: R•, free radicals; T, tocopherol (vitamin E); T•, tocopheroxyl radicals; AA, ascorbic acid; DHA, dehydroascorbic acid; GSH, glutathione; GSSG, glutathione disulfide (oxidized glutathione).

Dietary Intake and Recommendation

Various factors have been identified to affect vitamin C requirement. These include bioavailability, nutrient-to-nutrient interactions, and gender. There are various dietary guidelines available from diverse professional bodies pertaining to vitamin C intake recommendation. One of the commonly referred to is dietary references intake that was developed and maintained by the Institute of Medicine of the National Academy (see 'Further Reading' section). Based on this guideline, adult male requires 90 mg day^{-1} of vitamin C, while nonpregnant and lactating women require 75 mg day^{-1}. Deutschland-Austria-Confoederatio Helvetica (D-A-CH, 2013) and the European Food Safety Authority (EFSA, 2013) recommend higher intake (D-A-CH: 100 mg day^{-1} for adults regardless of gender; EFSA: 110 mg day^{-1} for men and 95 mg day^{-1} for women >18 years old) to outweigh the benefit of higher intake on the reduction in the risk of chronic diseases. Generally, vitamin C requirement is increased in special population including pregnant and lactating women as well as smokers when compared to their age group counterpart (Table 1). This is to reflect the different physiological requirements in these groups. In pregnant women, higher requirement is needed to account for expansion of blood volume and active transfer to the fetus, while for lactating women, to account for vitamin C transfer to breast milk. For heavy smokers (i.e., smoking more than 20 cigarettes per day), higher intake is needed to account

Table 1 Dietary reference intake for vitamin C

	Gender	
Age group	*Male (mg day^{-1})*	*Female (mg day^{-1})*
0–6 months[a]	40	40
7–12 months[a]	50	50
1–3 years	15	15
4–8 years	25	25
9–13 years	45	45
14–18 years	75	65
≥19 years	90	75
Pregnancy/lactation		
14–18 years	–	80/115
≥19 years	–	85/120
Smokers	Additional 35 mg day^{-1}	

[a]For age group of 0–12 months, the DRI was calculated based on adequate intake (AI), while the rest of the age group was based on recommended dietary allowance (RDA). Source: Institute of Medicine. (2000). *Vitamin C. DRI Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*, pp. 95–185. Washington, DC: National Academy Press.

for reduced absorption and increased daily turnover pertaining to higher oxidative stress in this population.

Vitamin C intake in various nutrition surveys in the United States and Europe indicated adequacy levels. For example, based on the National Health and Nutrition Examination Survey of the United States (NHANES 2009–10), average levels of vitamin C intake among males and females over 20 years old are 95.6 and 82.7 mg day^{-1}, respectively. Similarly, average vitamin C intake (excluding supplements) among adult males and females in European countries ranges from 69 to 139 mg day^{-1} and 65 to 138 mg day^{-1}, respectively. However, vitamin C intake in some Asian countries may not reach the adequacy levels. This has been demonstrated in a publication from India that showed that the available supply of vitamin C is 43 mg per capita per day, which ranges from 27 to 66 mg day^{-1} depending on locality. This is supported by the fact that plasma levels of vitamin C among native South Asian populations are relatively much lower when compared to South Asian living abroad. It is to acknowledge that differences in methodologies may have an impact on the accuracy of this country-to-country comparison.

Role of Vitamin C in Human Health and Diseases

There is substantial amount of literature from *in vitro* animal model and human studies that investigate the role of vitamin C in health and chronic diseases. For the purpose of this article, only studies involving human will be included for discussion. This is based on the fact that ascorbic acid is not an essential nutrient for most animals – thus, studying genetically variant strain incapable of synthesizing ascorbic acid may not fully recapitulate vitamin C transport functions and imitating disease condition associated with vitamin C depletion and supplementation as in human. The role of vitamin C in human health and disease with its potential mechanism of action is summarized in Table 2.

Collagen Biosynthesis and Wound Healing

Collagen is the main structural protein in various connective tissues, including those found in tendons, ligaments, skin, cornea, cartilage, and blood vessels. Different types of collagens that are the main components of the extracellular matrix comprise ≈30% of total protein mass. The main structural protein of the interstitial extracellular matrix is type I collagen, whereas type IV collagen is found predominantly in the basement membrane. In the extracellular matrix, collagens behave as chief constituents of the physical barrier against invasion of cancer cells. In human skin fibroblasts, ascorbate has been suggested to stimulate the generation of types I and III collagen. Vitamin C plays an essential role during collagen biosynthesis, acting as a specific electron donor for proline hydroxylase, lysine hydroxylase, and procollagen-proline 2-oxoglutarate 3-dioxygenase, all of which take part in the posttranslational hydroxylation of peptide-bound proline and lysine residues of collagen, imperative for stable collagen helices formation. Procollagen is a precursor molecule to the protein collagen that contains unusual amino acid sequence: hydroxyproline and hydroxylysine, converted from proline and lysine after the procollagen molecule has been synthesized. The enzyme involved in this process of hydroxylation, prolyl hydroxylase, requires ascorbic acid, iron, and α-ketoglutarate to catalyze the formation of hydroxyproline. Throughout the process of hydroxylation, the enzyme-bound iron is oxidized (Fe^{3+}) and then ascorbic acid reduces the iron to ferrous state (Fe^{2+}), thus reactivating the enzyme. In a comparable reaction, ascorbic acid acts as a cofactor for a copper-dependent lysyl hydroxylase that contributes in the hydroxylation of lysine to hydroxylysine. Therefore, deficiency in vitamin C results in impaired collagen biosynthesis.

Wound healing process also heavily relies on dietary sufficiency, especially of vitamin C. Ascorbic acid plays an important role in all phases of wound healing. During 'inflammatory phase,' (i.e., the body's natural responses to injury), vitamin C is required for neutrophil apoptosis and clearance as well as to neutralize free radical formed as part of scavenging process. As the wound progresses to 'proliferation phase' (i.e., the wound is rebuilt with new granulation tissues comprising collagen and extracellular matrix), vitamin C is needed as a cofactor for synthesis of collagen tissues. While during 'maturation phase' (i.e., remodeling of collagen from type III to I, occurring after the wound has closed), vitamin C is needed to maintain integrity of collagen production and scar formation. Such role has been demonstrated in a randomized, double-blind, placebo-controlled trial involving 20 trauma patients with disorders in wound healing. Supplementation with antioxidant (including vitamin C) has been shown to reduce time to wound closure. Even though the exact role of vitamin C cannot be ruled out due to the mixture with some other antioxidants in the supplementation, vitamin C may act synergistically with the other antioxidant nutrients to promote wound healing. In addition to collagen, ascorbic acid plays a role in the synthesis of other connective tissue constituents, like fibronectin, elastin, proteoglycans, bone matrix, and elastin-associated fibrillin.

Table 2 Role of vitamin C in health and diseases and its potential mechanism of action

Health and disease condition	*Vitamin C role*	*Possible mechanism of action*
Cancer	Pro-oxidant (high dosage) for adjunct cancer treatment	AA needs to be administered intravenously in order to achieve effective concentration. High dosage of vitamin C has been shown to – create pro-oxidant environment and accelerate irreversible damage of the tumor cells, – stimulate apoptotic pathway of the tumor cells, – result in intermediate formation of H_2O_2 via ascorbate radicals, which in turn causes breaks in DNA and mitochondria – prevent cancer spread by increasing collagen synthesis, inhibiting hyaluronidase, increasing extracellular matrix, and walling within the tumor cells
	Antioxidant for cancer prevention (anticarcinogenic effect)	Normal to high physiological levels of plasma AA to neutralize mutagenic free radicals, reduce oxidative stress, and prevent DNA damage
Cardiovascular diseases	Antioxidant/anti-inflammatory properties	AA reduced oxidizability of LDL. LDL oxidation is one of the key steps in atherosclerosis. AA decreases adherence of monocytes to endothelium, inhibits proinflammatory cytokines, and improves production of the endothelium-dependent nitric oxide
	Antihypertensive effect	AA enhances production of nitric oxide, a potent vasodilator, by increasing intracellular concentration of tetrahydrobiopterin, a cofactor for nitric oxide synthase. AA also improves nitric oxide bioavailability
	Lipid-lowering effect	Suboptimal AA intake affects the activity of two cholesterol-regulating enzymes, namely, ACAT and CETP. Increase in ACAT leads to higher LDL-C, while increase in CETP will result in lower HDL-C. AA as an antioxidant may protect VLDL and promote its removal from plasma. Moreover, AA may increase fatty acid utilization in hepatocytes by enhancing carnitine synthesis. Taken together, increased VLDL removal and β-oxidation of fatty acids lead to lower TAG
Cataract and ocular diseases	Antioxidant	Lens is prone to opacification due to its high protein content. Maintaining adequate blood levels of AA may reduce the risk of cortical, nuclear, and PSC cataract by providing protective effect against oxidative stress-related etiology
Collagen biosynthesis and wound healing	Acts as electron donor to specific enzymes of collagen biosynthesis	AA is involved in the synthesis and cross-linkage of collagen and has impact on vascular integrity and capillary bed strength
Iron absorption	Potent enhancer/ physiological role	AA enhances intestinal absorption of nonheme iron by chelating/maintaining iron in reduced form. AA may interact and reduce dietary inhibitors of iron including phytates
Osteoporosis and fractures	Acts as electron donor to specific enzymes of collagen biosynthesis	Collagen is an essential component of bone tissue. AA mediates osteoclastogenesis and osteoblastogenesis. AA is also involved in bone collagen synthesis, by acting as a cofactor of hydroxylation reaction within collagen fiber
Sepsis	Antioxidant	Low levels of AA in critically ill patients are correlated with multiple organ failure. AA inhibits apoptosis of endothelial cells, stimulates proliferation, and prevents the loss of barrier function in sepsis condition

Note: AA, ascorbic acid; DNA, deoxyribonucleic acid; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; TAG, triacylglyceride; ACAT, acyl-CoA:cholesterol acyltransferase; CETP, cholesterylester transfer protein.

Synthesis of Neurotransmitter

Vitamin C is considered as a cosubstrate for copper-containing dopamine-β-monooxygenase that converts neurotransmitter dopamine to norepinephrine by hydroxylation of the dopamine side chain. In addition, ascorbic acid seems to play a role in the hydroxylation of tryptophan that results in the formation of serotonin in the brain. It has been shown that glutamatergic and dopaminergic neuron activity has been closely associated with changes in concentrations of extracellular ascorbic acid in the brain. Results of animal model and cell culture experiments suggested that ascorbic acid plays an important role in the developing nervous system, mainly for the growth and maturation of glial cells and myelin.

Vitamin C and Iron Absorption

Dietary vitamin C is known to enhance the intestinal absorption of nonheme iron by reducing intraluminal iron to a more absorbable ferrous form. In addition, ascorbic acid can interact with dietary inhibitors of iron absorption. Increase in iron absorption with vitamin C may not cause adverse consequences in healthy people. However, such enhancement of iron absorption following consumption of high doses of vitamin C can be of concern in case of iron overload, like β-thalassemia and homozygous hemochromatosis, as vitamin C can worsen iron overload and cause tissue damage.

In addition, while vitamin C increases iron absorption, it can interact with iron and promote oxidative damage. This is an additional concern about high supplemental intakes of

vitamin C that worsen iron overload and, subsequently, its related pathology. Although high dose of iron intake may not be an important factor in overloaded iron, iron-ascorbate couple has been reported to have a strong pro-oxidant nature, and elevated levels of serum ascorbic acid have been shown to restrain the ferroxidase activity of ceruloplasmin; as a result, oxidative damage will be enhanced by amplified ferrous iron.

Cardiovascular Health

CVD including coronary heart disease (CHD), strokes, cardiomyopathy, and other heart diseases remain to be the leading causes of morbidity and mortality worldwide. Although CVDs have been previously described as a 'wealthy nation disease,' it is projected that by 2020, CVD will be the major cause of death in most developing nations. Major risk factors associated with CVD include increasing age, male gender, dyslipidemia, hypertension, cigarette smoking, family history, obesity, and physical inactivity.

Oxidative damage of biomolecules such as lipids, DNA, and proteins has become a working hypothesis that is widely accepted in terms of its relation with initiation and development of CVD. Several lines of evidence indicate that oxidation of low-density lipoprotein (LDL) is the key factor contributing to atherogenesis; thus, reduction in LDL oxidizability has become one of the therapeutic targets of vitamin C. Moreover, vitamin C contributes to cardiovascular health through its antioxidant properties, decreasing adherence of monocytes to the endothelium and improving the production of endothelium-dependent nitric oxide (NO). However, studies investigating the relationship between vitamin C consumption and CVD show inconsistent findings. Observational studies present a link between high vitamin C intake and reduced risk of CVD, yet, randomized controlled trials (RCT) on the effects of vitamin C supplementation on endothelial function show conflicting results; some studies indicated improvement in endothelial function, whereas others presented no effect of vitamin C supplementation. Therefore, for the ease of discussion, the evidence will be grouped into three main components:

(1) Relationship and effects of vitamin C on overall mortality and morbidity associated with cardiovascular health
(2) Effects of vitamin C on blood pressure
(3) Effects of vitamin C on plasma lipids

Relationship and effects of vitamin C on overall mortality and morbidity associated with cardiovascular health

Several epidemiological studies have provided quite encouraging results yet inconclusive regarding the relationship of vitamin C intake and cardiovascular health. In a large European prospective cohort study involving more than 20 000 participants, the relationship of plasma vitamin C and incidence of heart failure for a mean follow-up of 12.8 years was assessed. The results showed an inverse association between plasma vitamin C and risk of heart failure after adjustment of cofounders such as age, sex, smoking, systolic blood pressure, and physical activity; every 20 $\mu mol\ l^{-1}$ increase in plasma vitamin C was associated with 9% relative reduction risk of heart failure. This however may reflect overall intake of fruits and vegetables; thus, specific constituents of the diet leading to reduced heart failure risk cannot be ruled out. A meta-analysis of prospective studies to investigate the relationship of vitamin C intake, plasma level, and risk of stroke was published. The analysis included 12 prospective studies on vitamin C intake and six prospective studies on circulating vitamin C (plasma/serum levels). Results of this analysis suggest that both vitamin C intake and circulating vitamin C are significantly inversely associated with the risk of stroke.

From the clinical trial perspective, a 6-year antioxidant supplementation (vitamin C and E) among 520 subjects with the end point of common carotid artery intima-media thickness. The supplementation was associated with significant regression of atherosclerotic lesions in participants with low baseline plasma vitamin C concentration. However, most data from recent RCTs that evaluate role of vitamin C for prevention of CHD or stroke are not as consistent as the population studies and thus failed to produce convincing evidence to advocate supplementation. Some of the limitations of RCTs and the reason for disparate finding between epidemiological studies have been discussed elsewhere. These include limitation in dietary recalls to estimate vitamin C intake and the failure of the intervention study to exclude subject who already achieved saturation in circulating vitamin C as supplementation may not provide any additional benefit. Furthermore, duration of supplementation and sample size are relatively small in many RCTs.

Effects of vitamin C on blood pressure

Vitamin C has also been hypothesized to confer antihypertensive effects. This is basically derived from the fact that vitamin C enhanced endothelial functions via nitric oxide production. Furthermore, epidemiological study has also shown inverse correlation between plasma levels of vitamin C and blood pressure. A meta-analysis of RCTs to examine the effects of vitamin C supplementation on blood pressure was conducted. A total of 29 trials were included in the analysis with a median vitamin C supplementation of 500 $mg\ day^{-1}$ for a median duration of 8 weeks. It was found that vitamin C modestly reduced blood pressure, with the pooled changes in SBP and DBP being – 3.84 mm Hg and – 1.84 mm HD, respectively. Nevertheless, this analysis was cofounded by heterogeneity of the data, relatively small sample size, and, more importantly, variable control of antihypertensive medications for each of the trials. In short, further evidence is needed to outweigh the benefit of vitamin C supplementation on blood pressure.

Effects of vitamin C on plasma lipids

In line with epidemiological evidence that vitamin C has an inverse association with the development of atherosclerosis, it has also been hypothesized that this effect could be mediated by lipid-modifying effects. In a meta-analysis involving 13 RCTs, it was found that supplementation with at least 500 $mg\ day^{-1}$ vitamin C among hypercholesterolemic subjects led to significant reduction of LDL-C ($-7.9\ mg\ dl^{-1}$) and triglycerides ($-20.1\ mg\ dl^{-1}$). Nevertheless, the magnitude of changes is considered modest, thus the need for further evaluation on the cost-effectiveness of the supplementation.

Cancer

Carcinogenesis, or development of malignant cells, is a multistage process. It is generally accepted that free radicals developed by carcinogens are implicated in the carcinogenesis process. Vitamin C has received a great scrutiny in terms of its role in the prevention and treatment of malignancy. However, the effect of vitamin C on treatment and prevention of cancer is very inconsistent despite a vast number of studies in this area. In a recent meta-analysis based on the epidemiological studies involving more than 8000 lung cancer cases, it was found that vitamin C decreased risk for lung cancer in a dose–response manner; lung cancer risk decreased by 7% for every 100 mg day^{-1} increased in the intake of vitamin C. Similarly, another meta-analysis of cohort studies that investigated the relationship of plasma antioxidant levels (α-tocopherol, retinol, and vitamin C) with the risk for breast cancer revealed that plasma level of vitamin C was significantly lower in breast cancer subjects when compared to control. However, when these data were controlled for menopausal status, continent, and study design, the significant relationship between plasma vitamin C and breast cancer was only seen in case–control type of study. Vitamin C may not be effective in treatment of active cancers when used alone; its supplementation has been suggested to enhance life quality and longevity of cancer patients; thus, it is being studied as an adjuvant in cancer therapy. Some of the proposed mechanisms include vitamin C role in protecting cells from oxidative DNA damage, thus blocking initiation of carcinogenesis. This is supported by several studies that showed that plasma vitamin C at normal to high physiological level (60–100 μmol l^{-1}) concentration neutralized mutagenic free radicals, reduced oxidative stress, and thus prevented DNA damage. The evidence from population studies however does not translate into similar results in randomized controlled trials. A recent meta-analysis of RCTs and also a prospective cohort study reported no survival advantages and no effects of oral vitamin C supplementation on the incidence and mortality from prostate cancer. On the other hand, vitamin C has also been used in the treatment of malignancy by taking advantage of its pro-oxidant capacity. Extracellular ascorbate can destroy cancer selectively, with no effects on normal cells possibly through production of hydrogen peroxide. Pharmacological doses of ascorbate can be attained only by intravenous (IV) administration, due to the fact that orally administered vitamin C is hampered by limited absorptive capacity of the intestinal tract. Thus, this hypothesis provides a plausible explanation for the failure of many orally supplemented studies discussed earlier. In a systematic review that covered 39 articles from RCTs, phase I and II, and observational study, the authors concluded that high-quality studies on IV vitamin C are limited; however, current studies provide some basis for pharmacological benefits of IV vitamin C, thus justifying needs for a larger clinical trials. Some of the positive effects of IV vitamin C observed in these studies include a decrease in chemotherapy-related side effects such as nausea, insomnia, and constipation and improved time to relapse and quality of life of the patients.

In a pilot RCT, administration of high doses of IV vitamin C (75 g or 100 g per infusion) twice a week for 6 months during chemotherapy and an additional 6 months after chemotherapy among stage III/IV ovarian cancer patients leads to fewer side effects/toxicities related to chemotherapy and less adverse event when compared to the control group. Some of the proposed mechanisms include that IV vitamin C acted as pro-oxidant to induce DNA damage and depleted cellular energy (adenosine triphosphate (ATP)). Moreover, pro-oxidant ascorbate stimulates ATM/AMPK pathway leading to mTOR inhibition and death of ovarian cancer cells. Another proposed mechanism for IV vitamin C includes intermediating formation of extracellular hydrogen peroxide (H_2O_2). H_2O_2 diffusion into tumor cells causes DNA and mitochondria to break leading to cell death. Moreover, IV vitamin C therapy has also been shown to work synergistically with chemotherapy drug, gemcitabine, by sensitizing cancer cells to chemotherapy, leading to a synergistic cytotoxic response.

Despite some promising outcomes in many of the uncontrolled trials and case–control studies related to IV vitamin C, these data are subjected to criticism. Risk of bias in these studies is high due to heterogeneity of the study design, small sample size, nonstandardized dosage, and incomplete information on the pharmacokinetics and pharmacodynamics of IV vitamin C.

To summarize, the evidences available to date provide an interesting finding; however, they are not conclusive to suggest any clinical benefits of vitamin C in the prevention and treatment of malignancy.

Cataract

Cataract is characterized by increase in lens opacity or cloudiness leading to decrease in vision and eventually blindness. The World Health Organization revealed that cataract remains the leading cause of visual impairments worldwide, responsible for 51% of all causes of blindness worldwide. During the aging process, the lens undergoes biochemical, physiological, and functional changes especially on its protein constituent as a result of exposure to various insults including free radicals.

Vitamin C is naturally present in the lens at 50-fold of the concentration found in the plasma. Several lines of evidence indicate that aging process is associated with lower ascorbate content of the lens that may compromise lens functions. Many of the epidemiological studies demonstrated that higher vitamin C levels are associated with diminished risk of cataract. Interestingly, based on a systematic search and meta-analysis of the observational studies, it was found that plasma ascorbate is inversely associated with age-related cataract in the Asian population but not in the Western population.

Data from RCTs, however, are not as encouraging. In a meta-analysis that included nine randomized control trials involving more than 117 000 individuals, supplementation with one or more antioxidants (i.e., vitamin C, β-carotene, and vitamin E) for the purpose of prevention of cataract yielded negative results. The study concluded that there is no evidence to recommend antioxidant supplementation as means to prevent or slow the progression of age-related cataracts. Data from the Swedish Mammography Cohort, involving more than 24 000 women that were followed for 8.2 years, revealed even alarming results. In this study, it was found that vitamin C supplement users among women aged ≥ 65 had actually increased risk of cataract by 38%.

Asthma and Respiratory Illnesses

Asthma is a chronic inflammatory airway disease characterized by variable, reversible airway obstruction and episodic symptoms of wheezing, chest tightness, and/or cough. Asthma can develop at any stage in life but often starts during early childhood. According to the Global Asthma Report (2014), it is estimated that more than 300 million people suffer from asthma and 14% of the world's children were likely to have had asthmatic symptoms last year. Asthma is often triggered by environmental insults, including common colds caused by respiratory viruses.

Vitamin C is one of the major antioxidants present in lung tissue and lining fluid. This is to reflect its role in providing antioxidant protection against exposure to oxygen, as well as air pollutant including ozone and nitrogen oxides. Similarly, vitamin C may also implicate in inflammatory response during asthmatic attack by providing protection against oxidative damage. A cross-sectional study indicates that ascorbate concentration in the lung of subjects with mild asthma is reduced. In a population study involving more than 2000 patients, vitamin C intake was associated with enhanced pulmonary function. A systematic review on intervention with vitamin C to reduce common cold-induced asthma was done. In this article, the author described three intervention studies involving a total of 79 subjects. Vitamin C doses provided in all of the three studies were ranged from 1 to 5 g day^{-1}, so as the outcome measures (one study on asthma exacerbation and the two studies on bronchial sensitivity to histamine). These studies provide a positive indication in which vitamin C supplementation is found to reduce the occurrence of infection-induced asthma attacks by 78% and decrease bronchial hypersensitivity. However, further study with larger sample size is needed to confirm these observations. Similarly, in another meta-analysis, it was found that vitamin C is able to alleviate exercise-induced asthma.

Another common respiratory illness is 'common cold.' The term 'common cold' basically refers to viral infection of the upper respiratory tract, characterized by a group of symptoms including nasal discharge and obstruction, sore throat, cough, lethargy, and fever. Although common cold is non-life-threatening, however, it is the leading cause of doctor visits and absenteeism from work/school. Vitamin C has traditionally been claimed to be useful in the prevention and treatment of the common cold. Despite a large number of publications on this aspect, the evidence to support the use of vitamin C supplements to reduce the incidence and duration of common cold is relatively weak. In a recent meta-analysis involving 29 trials that include more that 11 000 participants, it is found that regular supplementation of vitamin C (200 mg day^{-1} or more) had no effect in reducing common cold incidence but had modest effect in reducing the duration of common cold symptoms. However, the authors recommend further evaluation in special population including runners, skiers, swimmers, and soldiers working in subarctic conditions given some positive data on this population.

Osteoporosis and Fractures

Osteoporosis is a progressive deterioration of bone mass and bone tissues leading to bone fragility and increased risk for fracture. Globally, osteoporosis is responsible for more than 8.9 million fracture cases annually. It has been estimated that 1 in 3 women and 1 in 5 men over the age of 50 will experience osteoporotic fracture. Vitamin C plays an important role in maintaining bone integrity by several mechanisms including mediating osteoclast differentiation and accentuating osteoblastogenesis and bone collagen synthesis in osteoblasts, an important process in bones remodeling, maintenance, and repair. *In vitro* and *in vivo* studies demonstrated that vitamin C deficiency leads to decrease in bone matrix stability and weakening bone structure. Quite a handful population-based cohorts demonstrated positive association of vitamin C intake and higher bone mineral density. However, data in human intervention studies are scarce. An RCT involving 90 subjects was conducted. The studied subjects were supplemented with either placebo ($n=30$), or 500 mg vitamin C + 400 IU alpha-tocopherol ($n=30$), or 1000 mg vitamin C + 400 IU alpha-tocopherol for 12 months ($n=30$). The study showed that the intervention with 1000 mg vitamin C and 400 IU of alpha tocopherols led to significantly lower hip bone loss compared to control group. The extent of vitamin C role in preventing bone density loss is questionable due the presence of alpha-tocopherol. In short, more high-quality human intervention trials are needed to rule out the therapeutic role of vitamin C in preventing or delaying bone loss.

Conclusion and Recommendation for Future Study

Vitamin C has undoubtedly been proved to confer significant antioxidant effects in *in vitro* and animal studies. Similarly, several lines of evidence indicate the potential of vitamin C in counteracting with inflammation and oxidative stress, an underlying process of many chronic and acute diseases. Many of the epidemiological studies have generally shown potential therapeutic roles of vitamin C in the prevention and treatment of chronic diseases. However, this has not been translated, at least conclusively, in many RCTs. This is attributed by the fact that our understanding of vitamin C effects at the molecular level in human physiological system is incomplete. Nevertheless, future research in vitamin C should be streamlined by considering several issues including characteristics of study population that may benefit from vitamin C intervention and assessment of baseline vitamin C status, as patient with saturated levels of plasma vitamin C may unlikely benefit from additional oral supplementation. With emergence of 'omics' technology, it is hoped that this will address some of the limitations and controversies observed in animal and human studies by designing a high-quality mechanism-based intervention study.

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Antioxidants: Characterization and Analysis; Ascorbic Acid: Properties, Determination and Uses; Bioavailability of Nutrients; Citrus Fruits; Dietary References: US; Iron: Biosynthesis and Significance of Heme; Iron: Physiology of Iron; Oxidation of Food Components; Storage Stability: Mechanisms of Degradation; Vitamins: Overview.

Further Reading

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Ascorbic Acid: Properties, Determination and Uses

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Vitamin C

Sources

Vitamin C is widely distributed in plants (80–90% in fruits and vegetables) and animals. Fruits with the most vitamin C include cantaloupe; citrus fruits and juices, such as orange and grapefruit; kiwi fruit; mango; papaya; pineapple; strawberries; raspberries; blueberries; cranberries; guava; and tomatoes and tomato juice. Vegetables with the most vitamin C include broccoli, Brussels sprouts, cauliflower, green and red peppers, spinach, turnip greens, and other leafy greens, sweet and white potatoes (skins), and winter squash. Some cereals and other foods as well as beverages are fortified with vitamin C. Vitamin C is also available as caplets, tablets, capsules, drink mixes, multivitamin and antioxidant formulations (solid and liquid), and stand-alone supplements. Tablet and capsule contents range from 25 to 1500 mg vitamin C.

Chemistry

General properties

L-Ascorbic acid ($C_6H_8O_6$) is the trivial name for vitamin C and is also the biochemical nomenclature name accepted by the International Union of Pure and Applied Chemistry–International Union of the Biochemistry and Molecular Biology. However, its systematic chemical designation is 2,3-enediol-L-gulonic acid-γ-lactone, which was formerly known as *hexuronic acid* (Figure 1). Vitamin C is the generic name for all compounds exhibiting qualitatively the biological activity of ascorbic acid (AA). These include esters of AA (e.g., ascorbyl palmitate) and synthetic forms (e.g., 6-deoxy-L-AA, 33% relative activity) and the primary oxidized form of L-AA, L-dehydroascorbic acid (L-DHAA).

L-AA has chiral centers at carbons 4 and 5 and can exist in four stereoisomeric forms. Enantiomeric pairs are L- and D-AA and L- and D-araboascorbic acid. L-AA and D-araboascorbic acid (more commonly known as D-isoascorbic acid), or erythorbic acid, are epimers differing in the orientation of hydrogen and hydroxyl on carbon 5. Structures of various AA forms are shown in Figure 2. Isoascorbic acid is not present in foods, but is synthesized commercially because of its antioxidant capacity and is used commonly in cured meat products to prevent oxidation and protect color. The stereoisomers have no biological activity other than a small amount from isoascorbic acid (2.5–5% relative to L-AA). Most of the analytic techniques used are unable to differentiate between the epimers. Since isoascorbic acid is used in some processed foods, erroneously high vitamin C content will be determined where more sophisticated techniques are not used.

L-AA and L-DHAA are the primary dietary sources of vitamin C. L-DHAA is considered to have up to 80% bioequivalency with L-AA, but some studies reported it is as low as 10%. If true for the biological activity of L-DHAA in humans, many foods that combine L-AA and L-DHAA composition values for total AA would need to be revised.

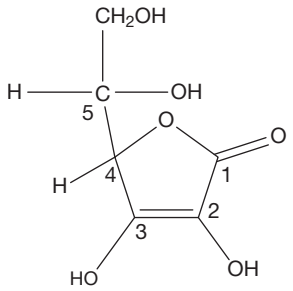
Physical properties of L-AA and its salt and ascorbyl palmitate are shown in Table 1. L-AA is a white to slightly yellow crystalline powder with high water solubility (30 g 100 ml^{-1}) at room temperature. It forms salts, the most important of which are with sodium and calcium salts, which are strongly acidic in aqueous solutions. The salts also have greater water solubility. L-AA complexes with disulfides (e.g., oxidized glutathione), but does not reduce the disulfide bonds. L-DHAA reacts with several amino acids to form brown-colored products (Maillard reaction), which contributes to the spoilage of foods. L-DHAA is not ionized in environments of weakly acidic or neutral pH environments, meaning it is relatively hydrophobic and is better able to penetrate plasma membranes than L-AA.

Spectral properties

The absorption properties of L-AA are dependent on the ionic species present and, therefore, dependent upon the pH of the aqueous media. E1% 1 cm values for L-AA are 695 at pH 2.0 and 940 at pH 6.0. Above pH 5.0, L-AA exists predominantly as the monoanion and the maximal absorption occurs at 265 nm. Associated, at more acidic pH levels, maximal absorption occurs at 244–245 nm. Dissociated, above pH 12.0, maximal absorption occurs at 300 nm. L-AA does not fluoresce, but derivatization with *O*-phenylenediamine (OPD) to form a highly fluorescent product is used advantageously in chemical and liquid chromatography (LC) methods.

Stability

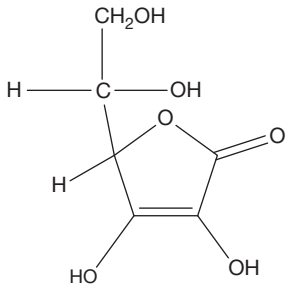
Crystalline L-AA is highly stable in the presence of oxygen when water activity remains low. Due to its strong reducing properties, L-AA is readily oxidized through one- or two-electron transfer. In aqueous solution, L-DHAA is unstable and is hydrolyzed irreversibly to the biologically inactive



L-Ascorbic acid
2-oxo-threo-hexono-1,4-2,3-enediol
Vitamin C

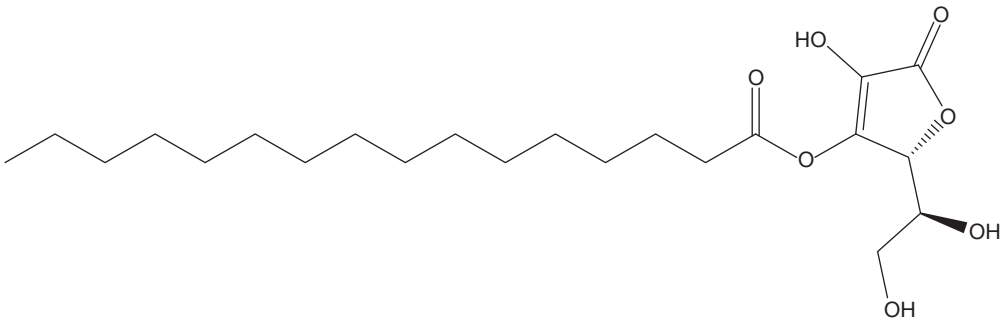
Figure 1 Structure of L-ascorbic acid.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00044-1

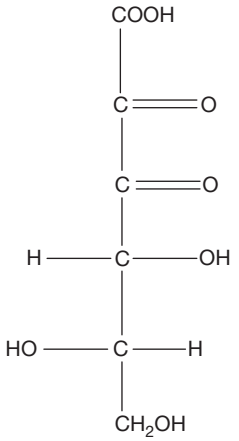


L-Ascorbic acid

L-Dehydroascorbic acid
D-Araboascorbic acid
Isoascorbic acid



Ascorbyl palmitate



Diketogulonic acid
Diketo-L-gulonic acid

Figure 2 Structures of L-ascorbic acid and related compounds.

Table 1 Physical properties of L-ascorbic acid and related compounds

Substance	*Molar mass*	*Solubility*	*Melting point (°C)*	*Crystal form*
L-Ascorbic acid ($C_6H_8O_6$)	176.13	Soluble in water (30 g 100 ml^{-1}) Slightly soluble in alcohol Insoluble in ether, $CHCl_3$, benzene, petroleum, oils, and fats	190–192	Monolithic platelets and needles; white or yellow
Sodium ascorbate ($C_6H_7O_6Na$)	198.11	Soluble in water (90 g 100 g^{-1})		White to slightly yellow powder
Calcium ascorbate $(C_6H_7O_6)_2Ca$	390.31	Soluble in water (5 g 100 g^{-1}) Slightly soluble in alcohol Insoluble in ether		White to slightly yellow crystalline powder
Ascorbyl palmitate ($C_{22}H_{38}O_7$)	414.54	Slightly soluble in oils Soluble in alcohol (22 g 100 ml^{-1})		White to slightly yellow powder

2,3-dioxo-L-gulonic acid. Reducing agents can convert the dehydro form back to L-AA in biological systems. Enzymatic conversion of L-DHAA to L-AA by glutathione dehydrogenase is an important biological defense against oxidative stress.

L-AA can oxidize through one- or two-electron transfer. One-electron reduction utilizes the transition through the L-AA free radical (semi-DHAA). At physiological pH, a bicyclic radical is formed with the loss of a proton, which subsequently becomes a radical anion that is relatively inert, due to its resonance stabilization, but diassociates to L-AA and L-DHAA. The anion radical is an intermediate in the reversible redox system formed by L-AA and L-DHAA. Thus, it is an effective quencher of free radicals, such as singlet oxygen ($^1O_2$).

It reduces ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron (and other metals analogously) and the superoxide radical (O_2^-) to hydrogen peroxide and is oxidized to monodehydroascorbic acid in the process. Reducing agents and glutathione dehydrogenase convert L-DHAA back to L-AA, completing the oxidation-reduction cycle. Classic free-radical termination occurs through reduction of a free radical with L-ascorbate. An electron is transferred to the free radical from L-ascorbate, producing an L-ascorbate radical, which acts as a redox agent. The L-ascorbate radical reacts with itself to form a 1:1 mixture of L-AA and L-DHAA. Two-electron reduction occurs when transition metals catalyze L-AA oxidation. A ternary complex forms between the metal, L-AA, and oxygen, and two p-electrons shift from L-AA to oxygen via the transition metal. The complex then dissociates with the formation of L-DHAA, hydrogen peroxide, and the metal ion. Unless converted back to L-AA, L-DHAA can be quickly hydrolyzes to biologically inactive 2,3-diketo-L-gulonic acid (Figure 2).

Oxygen, temperature, light, transition metal catalysis, pH, the presence of thermal processing conditions, oxidizing lipids, reducing substances, and the possible presence of AA oxidase in biological systems interact to produce a complex set of interactions influencing oxidative stability and the degradation rates of L-AA. Losses during cooking depend on the duration of heating, leaching into the cooking medium, surface area exposed to water and oxygen, pH, presence of transition metals, and other factors that facilitate the oxidation of L-AA and its conversion to inactive forms. The extreme vulnerability of L-AA at or near physiological pH is a primary consideration in most analytic methods. Since routine processing and storage conditions can stimulate the conversion of L-AA to L-DHAA in significant amounts, analytic methods that rely on the oxidation of L-AA to L-DHAA to give a measure of total L-AA are irrelevant. High-performance liquid chromatography (HPLC) detection of L-AA and L-DHAA simultaneously has been reported for biological samples (e.g., plasma stabilized with metaphosphoric acid (MPA)) since early 1990s.

Determinations

L-AA is the only water-soluble vitamin not determined microbiologically. Methodologies have advanced from the bioassays to instrumentally advanced spectrophotometric, fluorometric, electrochemical (EC), and chemiluminescence methods. Chromatographic procedures, especially HPLC, ultraperformance liquid chromatography (UPLC)–mass spectrometry (MS), and capillary electrophoresis (CE), provide an excellent means to resolve L-AA, L-DHAA, and D-isoascorbic acid simultaneously. These separation techniques used with ultraviolet (UV)–visible, fluorescence, or EC detectors are selective and sensitive for quantify L-AA and its isomers from complex biological matrices.

Before a method can be used, it needs to be validated to ensure it is suitable. Guidance documents on method validation, recommended by numerous international reputable organizations, are available such as the Association of Official Analytical Chemists (AOAC), Food and Drug Administration (FDA), Food and Agricultural Organization (FAO), International Conference of Harmonization (ICH), International Union of Pure and Applied Chemistry (IUPAC), and the European Commission. However, there is no single recommended approach for analytic method validation, but there are common elements to validation, for example, selectivity, linearity, stability, accuracy, precision, and the lower limit of quantification (LOQ). Parameters such as limit of detection (LOD), reproducibility, and robustness are also relevant. All these parameters are usually applied for HPLC methodology. For LC–MS, the determination of possible matrix effects (MEs) should be included, especially if MS mode is used in electrospray ionization (ESI).

Extraction and Sample Preparation Procedures

Extraction procedures are designed to stabilize the vitamin before further processing. Careful attention must be given to sample collection and subsequent handling prior to analysis; otherwise, L-AA may not reflect concentrations in foods as consumed. Extraction solutions should maintain an acidic environment, chelate metals, inactivate AA oxidase, and limit soluble oxygen as well as precipitate starch and proteins. The choice depends on the sample matrix and the analytic procedures. Reagents that usually limit L-AA destruction to $<5\%$ include 3–6% MPA containing acetic or sulfuric acid or 0.005 mol 1–1 ethylenediaminetetraacetic acid (EDTA).

MPA, while not compatible with some LC methods, is used most commonly in other methods. MPA inhibits L-AA oxidase and metal catalysis and precipitates proteins that aid in extract clarification. MPA is often combined with other acids and/or organic modifiers (acetic acid, methanol, acetonitrile, trichloroacetic acid (TCA), and citric acid) and stabilizers ((EDTA) and monosodium glutamate) to prevent degradation. Adding ethanol or acetone to the MPA extract precipitates solubilized starch from vegetables, such as potatoes, legumes, and maize for spectroscopic methods. Acetone is also useful to remove metabisulfide and sulfur dioxide from dried fruit products and fruit juices. EDTA is added as a metal chelator in vitamin C extractants to sequestrate copper in MPA and TCA but is ineffective for oxalic acid.

All extraction procedures should be completed rapidly in subdued or yellow light (570–590 nm) to limit oxidation. In general, fresh rather than frozen samples are better for L-AA analysis, since L-AA degrades during freezing or freeze-drying. Homogenization of samples should avoid heating. Samples should be in liquid nitrogen during storage. Freeze-drying is not recommended for sample concentration or storage since vitamin C stability decreases in the porous matrix. It has been reported that L-AA content is higher when food samples are prepared and analyzed on the same day rather than analyzed

from frozen samples (losses of up to half). It has also been reported that L-AA losses during the entire freezing process range between 10% and 80% (average *c*.50%). When samples with high moisture content are blended, the stabilizing solution should be added before blending. Stability of total AA (L-AA + L-DHAA) in serum can be extended with the addition of dithiothreitol or MPA (50 g l^{-1}), which effectively stabilizes the vitamin at −70 °C.

It has been shown that L-AA degrades significantly in autosampler vials during HPLC analysis. This is because the internal surface of the glassware contains materials, such as metal, which promote degradation. Sample tubes for collection, processing, and storage are also subject to this problem. Procedures such as soaking the vials in 0.5 mol 1-1 NaOH or 1 mol 1-1 HCl (30 min) before rinsing with distilled deionized water and soaking in and rinsing with distilled deionized water have been recommended to prevent degradation of L-AA. Similarly, handling and storage have been shown to impact stability of L-AA prior to analysis. For example, vitamin C is higher in pears prepared and sampled with minimal cutting, to reduce induction of ascorbate oxidase and other oxidases that catalyze L-AA oxidation, freezing each sample in liquid nitrogen and storage at −80 °C. Thus, it is necessary to undertake a validation study, to determine stability for embarking on analysis of a food. More research is also necessary to compare the stability of L-AA under ideal conditions compared with those typically used.

Traditional Methods

Oxidation–reduction methods

2,6-Dichloroindophenol (DCIP) titration is an established method to determine L-AA content. DCIP works on the principle of L-AA reduction to a colorless solution from the deep blue color of the oxidized dye. Subsequently, L-AA is oxidized to L-DHAA and any excess dye is pink in the acidic solution, forming a visual end point for the titration. The end point can be determined visually (518 nm). There are a few drawbacks to this method, the most important being titration is limited to quantitation of L-AA only. L-DHAA cannot be measured, unless it is first reduced to L-AA. The titration is also unable to differentiate between L-AA and isoascorbic acid, meaning this method cannot be used with processed and cured meats containing isoascorbic acid. DCIP titration is suitable for fresh juices and multivitamin supplements that do not contain significant quantities of copper or iron. However, highly colored extracts from fruits and vegetables, for example, can mask color changes at the end point. In this respect, solid-phase extraction (SPE) can extend DCIP titration to highly colored samples such as multivitamins, soft drinks, fruits, and vegetables, because cleanup removes copper, iron, sulfite, and other interfering reducing substances, such as cysteine and glutathione. The method can be adapted for L-DHAA by reducing it to L-AA with cysteine before cleanup. This relatively simple approach increases the sensitivity of DCIP, and inclusion of SPE would decrease limitations of the established method.

AOAC Official Method 967.21, Ascorbic Acid in Vitamin Preparations and Juices, 2,6-Dichloroindophenol Titrimetric Method, AOAC Official Methods of Analysis 45.1.14 (AOAC Method 967.21) has been recommended for the analysis of L-AA in beverages and juices for the purpose of nutrition labeling. The AOAC Official Method 985.33, Vitamin C ((Reduced AA) in Ready-to-Feed Milk-Based Infant Formula) (Chapter 50.1.09), is also based on the DCIP titration. The method differs from Method 967.21 at the extraction stage of the method. AOAC International has updated the change of method for vitamin C determination in infant formula and adult/pediatric nutritional formula (AOAC SMPR 2012.012) in its newest edition (19th edn. 2012).

Derivatization methods

There are two types of derivatization methods, *o*-phenylenediamine (OPD) condensation and 2,4-dinitrophenylhydrazine (DNPH). OPD condensation with L-DHAA is one of the most useful derivatization reactions to determine total vitamin C and gives a highly fluorescent quinoxaline product (Ex $\lambda = 350$ and Em $\lambda = 430$). AOAC Official Method 967.22, Vitamin C (Total) in Vitamin Preparations, Microfluorometric Method – The AOAC Task Force on Methods for Nutrition Labeling recommended that Method 967.22 be used for most food matrices. The manual microfluorometric method has been modified to semiautomated analysis with DCIP and *N*-bromosuccinimide oxidation replacing Norit oxidation and the direct addition of Norit slurry in MPA to the food sample to oxidize L-AA and L-DHAA during extraction (AOAC International Method 984.26, Vitamin C in Food, Semiautomated Fluorometric Method (Chapter 45.1.16)). The modified method is rapid (40 assays per hour) with the same sensitivity and specificity as the AOAC International Method 967.22.

DNPH reacts with ketone groups of L-DHAA under acidic conditions to form a red osazone derivative. DNPH is useful for the analysis of total AA if sugars are not present. Norit and DCIP oxidize L-AA to L-DHAA. Derivatization is completed with the addition of DNPH and the color produced on acidification with sulfuric acid. Maximal absorption occurs between 500 and 550 nm. Most methods measure the DNPH derivative at 520 nm. DNPH has not been used commonly compared with OPD derivatization. This method does not compare in specificity and simplicity to the microfluorometric method for total AA. Hence, a rapid DNPH-based microtiter plate assay for the determination of AA in plasma and leukocyte has been developed. The method is capable of high sample throughput and small sample volumes and requires smaller amounts of reagents than traditional DNPH methods.

Enzymatic methods

Enzymatic conversion of L-AA to L-DHAA coupled to a determinative step (e.g., spectrophotometry), OPD and other derivatizations, and EC determinations of oxygen uptake have been used to assay L-AA in biological samples. Ascorbate oxidase and ascorbate peroxidase activities, represented by the following equations, convert L-AA to L-DHAA. Total vitamin C and isoascorbic acid can be quantified at levels as low as 0.2 μg g^{-1}. L-DHAA can be quantified by omitting enzymatic oxidation.

Ascorbate oxidase

$$\text{L-AA} + {}^{1}/_{2}\,O_2 \rightarrow \text{L-DHAA} + H_2O$$

Ascorbate peroxidase

$$\text{L-AA} + H_2O_2 \rightarrow \text{L-DHAA} + 2H_2O$$

Spectroscopic and optical methods combined with flow injection and sequential injection analysis

L-AA can be assessed spectrophotometrically, based on the redox properties of L-AA on reaction with hexacyanoferrate (III) and oxidation using the Cu (II)–neocuproine complex or determination of iodine in reaction with AA. The reduction of Fe^{3+} to Fe^{2+} and Cu^{2+} to Cu^{+} is frequently incorporated in newer methods. Metal ion reduction is associated with the creation of a reduced metal ion complex with various dyes, which exhibit concomitantly a color change. Such color changes, while easily measured spectrophotometrically, can also be used for more simple visual tests or test strips for the present/not present determination and/or approximation of vitamin C content. Dyes that have been used include 2,2′-dipyridyl, pyridine-2,6-dicarboxylic acid, *p*-carboxyphenyl fluorone, 4-(2-pyridylazo) resorcinol, 1,10-phenanthroline, 2,4,6-tri(2-pyridyl)-1,3,5-triazine and ferrozine, and many others.

Other optical methods for vitamin C include chemiluminescence. Some researchers have determined the L-AA content of samples using flow injection analysis and the more advanced sequential injection analysis (SIA), as well as combined approaches. Spectrophotometric and electrochemical detection (ECD) methods are most commonly used with both analyses.

Advances in Vitamin C Analysis

Capillary electrophoresis

CE has been used extensively for the analysis of fat- and water-soluble vitamins, including vitamin C in pharmaceuticals, and fruit or vegetable products. Two basic techniques that have been used most for vitamin C analyses are capillary zone electrophoresis (CZE) and micellar/microemulsion electrokinetic capillary chromatography (MECC). These techniques are highly versatile, faster, more efficient, and more cost-effective than alternatives, but they are also less sensitive than HPLC. CZE is very useful for higher-concentration matrices, dietary supplements, and fruits and vegetables. It is normally applied with water-soluble samples while MECC is able to resolve neutral samples on the basis of partitioning between the aqueous electrolyte and a pseudostationary phase (PSP) of charged molecules. Hence, MECC is more adaptable to complex food matrices. However, hydrophobic analytes (e.g., fat-soluble vitamins) can precipitate out during electrophoresis due to their low solubility in the aqueous MECC buffers.

More recently, adaptations to MECC (MEECC) have been developed to overcome its inherent weakness with hydrophobic analytes. Microemulsions are stable, isotropically clear, systems containing oil (e.g., octane) and water, stabilized by a surfactant and cosurfactant. The adapted method (MEECC) uses micelles as the PSP in CE. The separation mechanism is based on electrophoresis coupled with chromatography, which makes it possible to separate neutral and charged analytes simultaneously. For instance, a novel microemulsion system consisting of sodium dodecyl sulfate (SDS), Brij 35, 1-butanol, and heptane has been developed for 10 fat- and water-soluble vitamins (including vitamin C), which can be separated in 35 min. In addition, using an SDS microemulsion, modified with 21% 1-butanol and 18% acetonitrile as the running buffer, allowed 13 water- and fat-soluble vitamins to be separated, simultaneously, in 30 min.

LC for vitamin C determination

LC methods, especially HPLC, are the preferred method for the analysis of L-AA, L-DHAA, and isoascorbic acid from natural sources. Previous reports have compared the efficacies of traditional methods with HPLC where they demonstrate similar results for only a limited number of samples. HPLC methods, which demonstrate high selectivity and sensitivity, are essential for the analysis of L-AA in many foods because of their complexity. Furthermore, both L-AA and L-DHAA can be quantified using appropriate HPLC methods.

Since L-AA is nonvolatile and hydrophilic in nature, reversed-phase HPLC is most commonly used, although ion-exchange, ion-exclusion, and hydrophilic interaction LC have also been used (Table 2). The validation parameters for different L-AA HPLC methods are shown in Table 2 (published between 2010 and 2014). There are a set of widely accepted key elements for the validation of HPLC methods: selectivity, linearity, stability, accuracy, precision, and the lower LOQ. Additional parameters that may be relevant include LOD, reproducibility, robustness, and ruggedness.

For the analysis of liquid samples, direct injection into the HPLC system, after dilution with the mobile phase, is a common procedure. For solid samples, solid–liquid extraction is usual. In LC-based methods, MPA (with and without glacial acetic acid) has been used most commonly for extraction. Samples are homogenized thoroughly with the solvent, centrifuged or filtered, and injected in the HPLC, again following dilution with the mobile phase. UPLC has also been applied to the analysis of L-AA in foods, with the main advantages of shorter analysis times and much less solvent compared with conventional HPLC. Mobile phase pH is usually adjusted to below the L-AA pK_a (4.17) to prevent degradation. An HPLC chromatogram for L-AA in a standard solution and in grapefruit is shown in Figure 3(a).

L-AA presents a strong absorption in the UV region (245–270 nm), making UV absorbance the most popular detection technique. In addition, fluorescence detection (FD) and ECD have also been used as well as MS after LC separation. MS is the most sensitive analytic technique for the identification of the molecular masses of unknown compounds in various modern research disciplines, including food science and nutrition. The mass spectrum of L-AA is shown in Figure 3(b). The LOD ranged between 0.02 and 0.16 $\mu g\,ml^{-1}$ for ECD, 1.2×10^{-3} and 7.2 $\mu g\,ml^{-1}$ for UV, and $\sim$0.27 $\mu g\,ml^{-1}$ for FD (Table 2). Usually, ECD is more sensitive than UV, although the wide LOD ranges make it difficult to perform an accurate comparison. Fluorescence is also more sensitive than UV, even though derivatization makes it more time-consuming than UV. The low levels of L-DHAA in most samples make quantitative analysis with any HPLC detector difficult. Hence, L-DHAA is often reduced to L-AA before chromatographic separation, and it is measured as total AA, that is, the sum of L-AA and L-DHAA. This procedure requires two separate

Table 2 An overview of validated HPLC methods for vitamin C determination in various food samples

Analyte	*Sample*	*Sample preparation*	*HPLC conditions*	*Method validation*	*Reference*
L-AA and gallic acid	Indian gooseberry juice	Dilution with MeOH/water (70:30, v/v)	Zorbax SB RP-C18 (250 × 4.6 mm, 5 μm) A: 0.1% (v/v) acetic acid B: MeOH DAD (278 nm)	Selectivity: t_R and UV spectra comparison R^2: 0.9998; 30–90 μg ml^{-1} (ES) LOD: 1.42 μg ml^{-1}; LOQ: 4.73 μg ml^{-1} (*S/N*) Repeatability: 1.04%; int. precision: 1.48% Accuracy: 99.37% (recovery tests)	Sawant et al.
L-AA	Fruit juices	Filtration, centrifugation, and dilution with mobile phase	Hypersil GOLD (250 × 4.6 mm, 5 μm) KH_2PO_4 buffer (pH 2.8) UV (254 nm)	Selectivity: t_R and UV spectra comparison R^2: 0.9990; 1–300 μg ml^{-1} (ES) LOD: 0.5 g ml^{-1} (*S/N*) Repeatability: $<$2%; Accuracy: 95.8–102.1% (recovery tests)	Nour et al.
L-AA, iso-AA	(a) Fruit juices (b) Chestnut, ham	(a) Dilution with 6.25% MPA, 2.5 mM TCEP, 2.5 mM EDTA (b) SLE with 5% MPA-2 mM TCEP-2 mM EDTA; centrifugation	TSK gel Amide-80 (4.6 × 100 mm, 5 μm) ACN: 0.1% TFA (90:10) UV (244 nm)	Selectivity: t_R and UV spectra comparison $R^2 > 0.9989$; 0.5–25 μg ml^{-1} LOQ: 1.5 μg ml^{-1}; 3.7 μg ml^{-1} (*S/N*) Repeatability: 1.43–1.92% (L-AA); 2.59–3.01% (iso-AA) (Horwitz criteria) Accuracy: 96.41–103.49% (recovery tests) Robustness: % organic in mobile phase; HPLC flow rate; column temperature Stability: concentration, temperature	Barros et al.
L-AA, L-DHAA	Fruits and vegetables	SLE with 0.05% EDTA; centrifugation; cleanup with C18 cartridges	ProntoSIL C18 (250 × 3 mm, 3 μm) 0.2% (v/v) formic acid ESI-MS	Selectivity: t_R and UV spectra comparison $R^2 \geq 0.9970$ LOD: 0.013 μg ml^{-1} L-AA; 0.011 μg ml^{-1} L-DHAA (*S/N*) LOQ: 0.044 μg ml^{-1} L-AA; 0.038 μg ml^{-1} L-DHAA (*S/N*) Repeatability: 1.6–2.8% L-AA; 1.1–2.7% L-DHAA Accuracy: 81–109% (recovery tests) matrix effect	Fenoll et al.
L-AA	Health drinks	Dilution with 0.56% MPA; centrifugation and filtration	Symmetry C18 (250 × 4.6 mm, 0.5 μm) Acetic acid in water/methanol (95:5) (v/v) DAD (245 nm); ESI	R^2: 0.9979; 0.1-0.6 μg ml^{-1} LOD: 0.01 μg ml^{-1}: LOQ: 0.1 μg ml^{-1} (visual approach) Repeatability: 0.75–1.34%; int. precision: 0.78–1.25% Accuracy: 97.5–98.6% (recovery tests) Robustness: % MeOH in mobile phase Stability: temperature	Konda et al.
TAA (reduction with TCEP)	Fruits, vegetables, fruit juices, and dried spices	LLE or USLE with 5% MPA-1 mM EDTA-5 mM TCEP; centrifugation and filtration	C18 Synergi Hydro-RP (250 × 4.6 mm, 3 μm) A: 0.05% (w/v) formic acid B: 0.02% (w/v) *o*-phosphoric acid UV (254 nm)	Selectivity: CRMs ES (0.01–50 μg ml^{-1}) LOD: 0.6–0.9 μg g^{-1}; LOQ: 2 μg g^{-1} (*S/N*) Repeatability: 0.8–3.6%; int. precision: 1.1–4.8% (CRMs) Accuracy: 97–103% (recovery tests)	Tarrago-Trani et al.
L-AA, L-DHAA, TAA, other organic acids	Vegetables	SLE with 4.5% MPA	Sphereclone ODS (250 × 4.6 mm, 5 μm) 1.8 mM H_2SO_4	R^2: 0.9980; 100–2000 μg g^{-1} (ES) LOD: 0.8 μg g^{-1} (*S/N*) Precision: 5.3% (AOAC)	Sánchez et al.

Table 2 (Continued)

Analyte	*Sample*	*Sample preparation*	*HPLC conditions*	*Method validation*	*Reference*
			(pH 2.6) UV (245 nm)	Accuracy: 90.1–94.6% (recovery tests)	
L-AA, L-DHAA, TA	Fruits and vegetables	SLE with MPA (3 g per 100 ml); centrifugation	Spherisorb C18 (150 × 4.6 mm, 3 μm) 0.01 M dihydrogen ammonium phosphate (pH 2.6) PDA (254 nm); ESI-MS	Selectivity: t_R and UV spectra comparison R^2: 0.9995 (1.9–62.5 μg ml^{-1}) ES (statistical analysis) Precision: 0.003% Accuracy: 89.9–113.1% (recovery tests) Stability: reducing agents, pH, temperature	Chebrolu et al.
L-AA	Beverages	MEPS with methanol/water solution (10:90, v/v)	LiChrospher 100 RP-18e (250 × 4 mm, 5 μm) A: water acidified with acetic acid (pH 2.94); B: MeOH (80:20) UV: 265 nm	R^2: 0.9994; 40–1000 μg ml^{-1} (ES) LOD: 7.2 μg ml^{-1}; LOQ: 24 μg ml^{-1} (*S*/*N*) Repeatability: 10% Accuracy: 97.46–106.88% (recovery tests) Stability: light, temperature, pH	Adam et al.
L-AA, L-DHAA, TAA	Fruits and vegetables	SLE with 3% MPA-8% acetic acid – 1 mM EDTA; centrifugation	Acquity HSS (100 × 2.1 mm, 1.8 μm) 0.1% formic acid in water (v/v) PDA (245 nm)	Selectivity: t_R and UV spectra comparison R^2: 0.9999; 0.05–2 μg ml^{-1} (ES) LOD: 0.022 μg ml^{-1}; LOQ: 0.067 μg ml^{-1} Repeatability: 0.9–3.9%; Accuracy: 87–103.7% (recovery tests)	Spínola et al.
L-AA, TAA	Strawberries	USLE with 3% MPA-8% acetic acid; centrifugation	Phenomenex Gemini C18 (250 × 3 mm, 5 μm) 0.03 M sodium acetate/acetic acid buffer, 5% MeOH UV (251 nm)	R^2: 0.9960 L-AA; 0.9980 TAA; 0.004–0.020 μg ml^{-1} (ES, statistics) LOD: 0.0012 μg ml^{-1} L-AA; 8.8×10^{-4} μg ml^{-1} TAA (*S*/*N*) LOQ: 0.0034 μg ml^{-1} L-AA; 0.0025 μg ml^{-1} TAA (*S*/*N*) Repeatability: 1.5% L-AA; 1.8%; TAA Accuracy: 92.3–100.6% L-AA; 94.3–104.8% TAA (recovery tests) Robustness: % MeOH, pH, and flow rate of mobile phase	Van de Velde et al.
L-AA, TAA	CRMs (milk, nutritional formula, cereals)	USLE with 40% MPA; centrifugation	YMC C18 Pro (250 × 4.6 mm, 5 μm) A: 0.02 M KH_2PO_4 buffer (pH 3.1) B: ACN (gradient elution) UV (243 nm)	Selectivity: CRMs R^2: 0.9986; 1000–4000 μg g^{-1} (IS) LOD: 0.05 μg g^{-1} (*S*/*N*); LOQ: 0.7 μg g^{-1} (*S*/*N*) Repeatability: 2.7–6.5%; int. precision: 3.8–4.5% (CRMs) Accuracy: 93.7–106.4% (CRMs)	Thomas et al.
L-AA	Grapes	USLE with 96% acetic acid; USLE with 2% MPA; centrifugation	Kromasil C18 (100 × 2.1 mm, 3.5 μm) 0.1% (v/v) acetic acid + MeOH; UV (245 nm) ESI-MS	Selectivity: t_R and UV spectra comparison R^2: 0.9990; 0.5–15 μg ml^{-1} (ES) LOD: 0.32 μg ml^{-1} (*S*/*N*) Repeatability: 2.3–4.2% Accuracy: 96.9–102.4% (recovery tests)	Matei et al. (2013)
L-AA	Peppers	USLE with 3% MPA–EtOH (2:8); centrifugation	C18 Gemini (250 × 4.6 mm, 5 μm) A: 0.03 M phosphoric acid; B: MeOH (gradient elution) UV (254 nm)	Selectivity: t_R and UV spectra comparison R^2: 0.9981; 3.91–62.5 μg ml^{-1} (ES) LOD: 0.26 μg ml^{-1} (*S*/*N*) Repeatability: 0.86% (t_R); int. precision: 2.93% (t_R) Accuracy: 97.1–98.8% (recovery tests)	Bae et al.

(*Continued*)

Table 2 (Continued)

Analyte	*Sample*	*Sample preparation*	*HPLC conditions*	*Method validation*	*Reference*
L-AA, TAA	Exotic fruits, juices, and fruits' pulp	Dilution with 10% PCA–1% MPA + TCEP; filtration	Synergi Hydro-RP (150 × 4.6 mm, 4 μm) 20 mM $NH_4H_2PO_4$ (pH 3.5) + 0.015% (w/v) MPA PDA (246 nm)	Selectivity: t_R and UV spectra comparison R^2: 0.9995; 1–100 μg ml^{-1} (ES, statistics) LOD: 0.035 μg ml^{-1}; LOQ: 0.09 μg ml^{-1} (S/N); Repeatability: 0.43–0.7%; int. precision: 3.67% Reproducibility < 2 Accuracy: 96.6–97.3% (recovery tests); ME Robustness: pH and flow of mobile phase, temperature Stability: temperature	Valente et al.

Abbreviations: ACN, acetonitrile; CRMs, certified reference materials; DAD, diode-array detector; ESI–MS, electrospray ionization–mass spectrometry; EtOH, ethanol; LLE, liquid–liquid extraction; SLE, solid–liquid extraction; USLE, ultrasound-assisted solid–liquid extraction; LOD, limit of detection; LOQ defined as the lowest concentration of L-AA where RSD 10%; int. precision, intermediate precision; MeOH, methanol; ME, matrix effect; *S/N*, signal-to-noise ratio; TAA, total ascorbic acid; TCEP HCL, Tris-[2-carboxyethyl] phosphate hydrochloride; precision, includes both repeatability and intermediate precision; all PCA solutions are expressed in % (v/v); all MPA solutions are expressed in % (w/v); they are all prepared in water. RSD = authors do not mention which kind of precision is studied.

Source: Modified from Spínola, V., Llorent-Martínez, E. J., and Castilho, P. C. (2014). Determination of vitamin C in foods: current state of method validation. *Journal of Chromatography A*, 1369, 2–17. Copyright (2014), with permission from Elsevier.

chromatographic runs, and L-DHAA is calculated by the subtraction of L-AA from total AA.

External standard calibration is usual for vitamin C quantification (Table 2) due to its simplicity and use of the same calibration curve for different matrices. Internal standards (IS) have also been used, but these must be selected carefully so characteristics and physical/chemical behavior are as close as possible to L-AA without causing interference. The best IS is, therefore, stable isotope labeled L-AA.

EC determination of vitamin C

EC techniques for L-AA have received considerable interest because of their sensitivity, rapid response, simple operation, and low costs. Since minimum requirements are needed for sample pretreatment (extraction), these techniques are often preferred compared with laborious instrumental methods for L-AA determination where results can be obtained in complex media. EC determination of L-AA is due to electrooxidation of L-AA to L-DHAA, which involves two electrons.

Among the EC methods applied for L-AA determination, researchers have used polarography and voltammetry. Polarography is not widely used because it requires removal of interfering compounds using separation techniques such as extraction, ion exchange, and chromatography. Voltammetric/amperometric analysis using different electrodes (e.g., modified metal or carbonaceous electrodes and nanoparticle and ceramic composites with suitable chemical and biochemical sensors) has been developed more recently. For example, glassy carbon and carbon-paste electrodes, platinum electrode, and hanging drop mercury electrode have been used in L-AA determination. Since L-AA is one of the most electroactive biomolecule, it is difficult to determine its concentration at unmodified carbon or bare metal electrodes, due to the occurrence of surface reactions associated with its previously described EC behavior.

Chronopotentiometry is another EC technique based on oxidation or reduction of analytes on the electrode surface in steady solutions with a constant current. Oxidation (transition) time represents quantitative characteristic, while oxidation potential is a qualitative characteristic of the analyte. It has been found that L-AA content of fermented milk products does not differ significantly when determined using chronopotentiometry or HPLC. Indeed, for some applications, chronopotentiometry is more sensitive, reliable, and rapid.

Recent studies have reported various chemically modified electrodes, such as gold nanoparticles self-assembled on a L-cysteine-modified glassy carbon electrode (GCE), tetraoctylammonium bromide-stabilized 1,6-hexanedithiol-modified gold electrode, multiwalled carbon nanotubes (MWCNTs) with methylene blue-composite film-modified electrode, 3-mercaptopropyl-functionalized silica network gold nanoparticle-modified electrode, and carbon-paste/cobalt Schiff base composite electrodes, with suitable chemical and biochemical sensors, not only decrease excess voltage and enhance electrocatalytic peak current but also solve a common problem encountered at unmodified surfaces, such as the peak overlapping in the determination of L-AA.

MWCNTs are a new type of porous nanostructure in carbon materials, possessing properties such as high electrical conductivity, larger surface-active groups-to-volume ratio, chemical stability, and significant mechanical strength. Modified electrodes based on MWCNTs have been used widely for the study of L-AA, including a carbon nanotube–ionic liquid gel-modified electrode, layer-by-layer assembled functionalized carbon nanotube and polyaniline multilayer film-modified electrode, cytochrome c immobilization on a poly-3-methylthiophene/MWCNTs hybrid-modified electrode, silver hexacyanoferrate nanoparticles/-carbon nanotube-modified electrode, and a chloro-[3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropanoato (2-)]iron (III)/MWCNTs (Fe(III)P/MWCNTs).

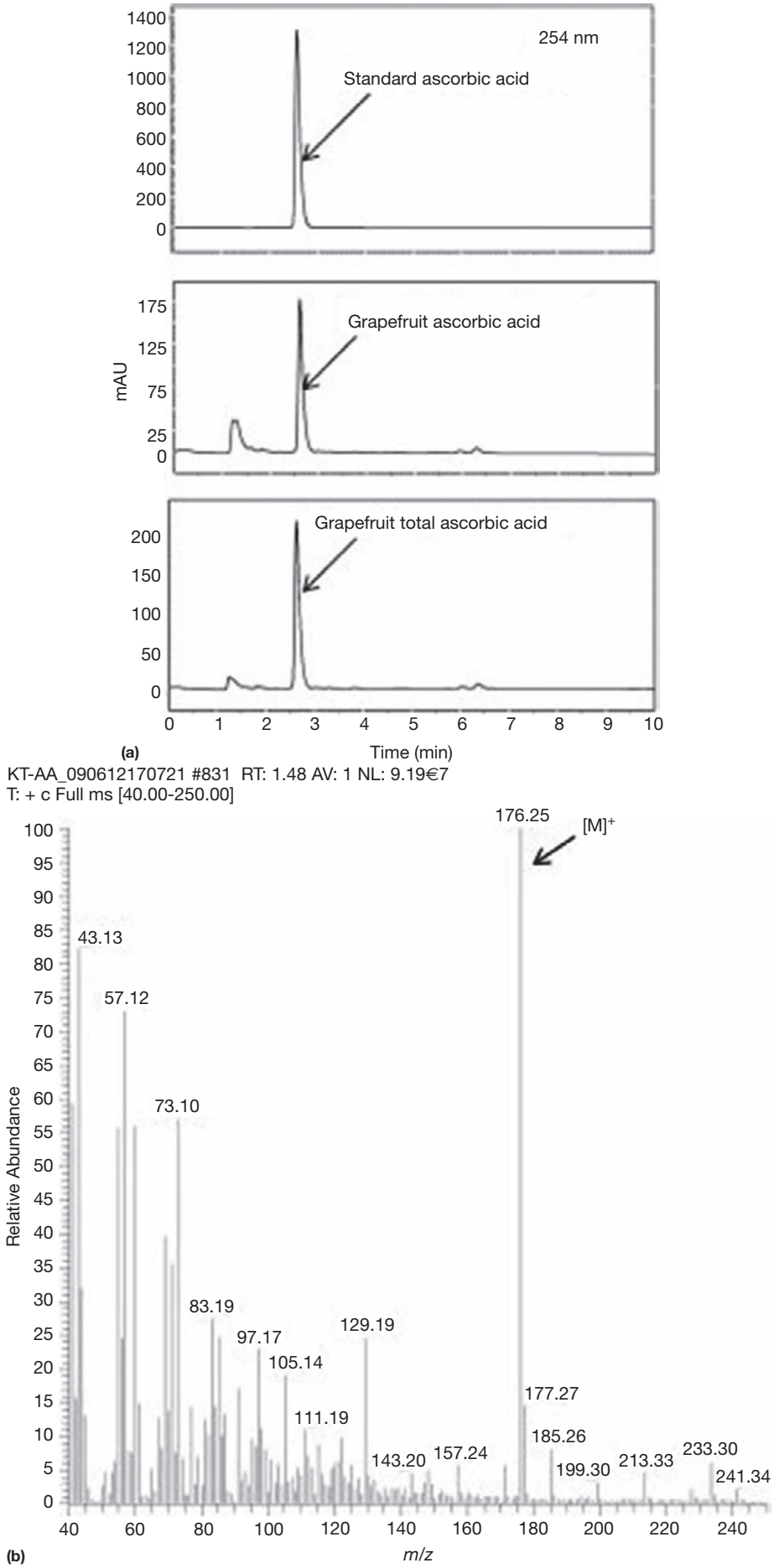


Figure 3 (a) Chromatograms for L-AA in standard solution and in grapefruit extract. (b) Mass spectrum of L-AA, $[M]^+$ (m/z) = 176.25. Reprinted from Chebrolu, K. K., Jayaprakasha, G. K., Yoo, K. S., Jifon, J. L., and Patil, B. S. (2012). An improved sample preparation method for quantification of ascorbic acid and dehydroascorbic acid by HPLC. *LWT-Food Science and Technology* **47**(2), 443–449. Copyright (2012), with permission from Elsevier.

A recent report demonstrated functionalized poly(3,4-ethylenedioxythiophene) (PEDOT) films, prepared by the incorporation of two electrospecies (e.g., ferrocenecarboxylic acid [Fc-] and ferricyanide $[Fe(CN)_6]^{4-}$) as doping anions during the electropolymerization of PEDOT at GCEs, from an aqueous solution to prepare composites [PEDOT/Fc- and PEDOT/$Fe(CN)_6]^{4-}$, which could be used as EC sensors. These nanostructured films combine the advantages of PEDOT (high conductivity and stability) with electroactive species (good EC activity) and have been used as EC sensors for the determination of vitamin C in food samples. These sensors have been shown to possess high selectivity with no interference from other potential competing species. This modified electrode was applied successfully for the determination of analytes, such as vitamin C, in urine and serum samples using the standard addition method with satisfactory results.

Uses

L-AA is used widely as a food additive with many functional roles, based mainly on its oxidation–reduction properties. Its functional roles include uses as a nutritional food additive, antioxidant, browning inhibitor, reducing agent, flavor stabilizer, modifier and enhancer, color stabilizer, dough modifier, and many other roles. Ascorbyl palmitate was developed to provide a form with greater lipid solubility for cooking purposes and antioxidant preparations.

See also: Acids: Natural Acids and Acidulants; Antioxidants: Characterization and Analysis; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: High-Performance Liquid Chromatography; Mass Spectrometry: Applications; Oxidation of Food Components; Spectroscopy: Types; Storage Stability: Mechanisms of Degradation; Vitamins: Overview.

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Authenticity of Food

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Abbreviations

APCI	Atmospheric pressure chemical ionization
ATR	Attenuated total reflectance
CE	Capillary electrophoresis
COI	Cytochrome-*c* oxidase subunit I
CP-MAS	Cross polarization-magic angle spinning
DART	Direct analysis in real time
DESI	Desorption electrospray ionization
DRIFTS	Diffuse reflectance infrared Fourier transform spectroscopy
EASI	Easy ambient sonic-spray ionization
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
FT-ICR	Fourier transform ion cyclotron resonance mass spectrometer
FT-MIR	Fourier transform mid-infrared
FWHM	Full width at half maximum
GC	Gas chromatography
HCA	Hierarchical cluster analysis
HPLC	High-performance liquid chromatography
HR	High resolution
HRM	High-resolution melting
HR-MAS	High-resolution magic angle spinning
HRMS	High-resolution mass spectrometer
HSI	Hyperspectral imaging
ICP-MS	Inductively coupled plasma mass spectrometry
IR	Infrared
IRMS	Isotope-ratio mass spectrometry
IT	Ion trap
LC	Liquid chromatography
LDA	Linear discriminant analysis
LF	Low field
LOD	Limit of detection
LOQ	Limit of quantification
LTQ-FT-ICR	Linear trap quadrupole-Fourier transform ion cyclotron resonance mass spectrometer
LTQ-Orbitrap	Linear trap quadrupole-Orbitrap mass spectrometer
LTQ-TOF	Linear trap quadrupole-time of flight mass spectrometer
MALDI-TOF-MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MAS	Magic angle spinning
MIR	Mid-infrared
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS^3	Triple-stage mass spectrometry
MS^n	Multistage mass spectrometry
MSI	Multispectral imaging
MS/MS	Tandem mass spectrometry
NIR	Near-infrared
NMR	Nuclear magnetic resonance spectroscopy
NMRI	Nuclear magnetic resonance imaging
PAGE	Polyacrylamide gel electrophoresis
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphism
PTR-MS	Proton transfer reaction-mass spectrometry
Q-Orbitrap	Quadrupole-Orbitrap mass spectrometer
QqLIT	Quadrupole linear ion trap
QqQ	Triple quadrupole
QqTOF	Quadrupole time-of-flight mass spectrometer
SERRS	Surface-enhanced resonance Raman scattering
SERS	Surface-enhanced Raman scattering
SNIF	Site-specific natural isotope
SRM	Selected reaction monitoring
TOF-MS	Time-of-flight mass spectrometer
TRS	Transmission Raman spectroscopy
Vis	Visible

Introduction

Food authenticity is of great importance for consumers, food authorities, and food industry because incorrect labeling of foods could be related to various types of fraudulent practices. Diseases related to foodstuff consumption raised the awareness around aspects like geographic origin and agricultural practices. Food authentication became an important issue and has been faced with different analytical approaches like DNA-based technologies, infrared spectroscopy, isotope analysis, separation techniques, mass spectrometry, and NMR. Several 'omics' definitions appeared for the holistic understanding of

different molecular aspects like function, physiology, and pathophysiology of plants and mammals. One above all, namely, 'metabolomics' offers information on the global metabolite composition (metabolome) of a given biological system and its dynamic response towards stimuli. Most suited analytical techniques appears to be NMR, mass, and hyphenated chromatographic techniques (LC–MS, GC–MS, LC-DAD-NMR/MS, etc.), and the high-throughput qualitative screening of a biological sample is defined with the widely accepted term 'metabolic profiling.' The large amount of data obtained by these analytical techniques requires the use of multivariate statistical analysis for sample comparison and discrimination analysis. In this article, selected applications in food authenticity assessment are presented.

Mass Spectrometry

Mass spectrometry (MS) holds a central position in food analysis, and its applications expand in food authenticity-related studies (i.e., traceability or determination of geographic origin and adulteration). MS methods currently yield higher sensitivity when compared to other spectroscopic techniques such as NMR, which normally detects medium- to high-abundance metabolites. MS-based analysis may be targeted or nontargeted. Targeted analysis is a conventional analysis where the method is developed based on standards. Nontargeted analysis theoretically allows the detection and characterization of any compound present in the sample. Two different analytical approaches are followed in nontargeted analysis:

- 'Metabolic profiling' refers to the analysis of a class of chemically related compounds or is associated with a particular metabolic pathway.
- 'Metabolic fingerprinting' refers to the analysis of the total set of metabolites, avoiding focus against certain classes of compounds. When individual metabolites are not identified, 'metabolite fingerprinting' is used for a rapid classification of samples.

In targeted analysis or in metabolic profiling study, selective extractions are needed, and usually, mass spectrometry is coupled to a separation technique such as LC for semipolar compounds; GC for thermally stable, volatile, and semivolatile compounds; or CE for ionic, weakly ionic, and polar metabolites. Data from MS coupled to a separation technique contain the extra information of retention/migration time. MS/MS has been the cornerstone technique for targeted analysis. Several publications on the applications of MS/MS in the detection of contaminants for food quality and safety purposes are present. The QqQ and the IT mass spectrometers give nominal masses and perform MS/MS. The QqQ-MS/MS is one of the most popular instruments for the analysis of complex food samples because of its high sensitivity, selectivity, and specificity for identification and for its quantitation capabilities when operated in MRM mode. The use of new-generation fast-switching QqQ and the hybrid fast-switching QqLIT mass spectrometers significantly enlarges the number of analytes that can be detected in a single MS/MS run up to 200 compounds with 2 SRM transitions. In addition, the IT can be operated in enhanced product ion mode and can perform MS^3 for the characterization of unknowns. To this direction, UPLC-Q-LIT-MS was applied for nontargeted screening and structure identification of the banned azo dye Allura Red AC and its photodegradation products in a beverage. Initially, a nontargeted screening with enhanced MS (EMS) in full scan mode was carried out as a survey scan. When the signal of a detected compound exceeded a defined threshold, the acquisition of both enhanced resolution (ER) and enhanced product ion spectrum (EPI) was automatically activated. In EMS mode, the third quadrupole works as an ion trap, accumulating the ions of interest, while in the ER mode, the isotope ratio is confirmed, and in the EPI mode, the ion trap is used to provide higher abundance of product ions through an enhanced MS/MS scan. The chemical structures of the unknown species formed in the photodegradation process were proposed on the basis of the molecular mass.

HRMSs operating in full scan mode like TOF-MS, Orbitrap-MS, and FT-ICR-MS provide high selectivity and sensitivity and through accurate mass measurements can provide possible identification of unknown compounds and are therefore suitable for nontargeted analysis. High-speed GC-TOF-MS was applied in nontargeted analysis and in particular the fingerprinting of volatile compounds. More than 100 compounds could be identified in coffee in only 7.9 min, and through PCA statistical evaluation, the geographic origin of samples could be determined. Identification was performed by the comparison between experimental and reference/library mass spectra and the comparison between experimental and literature retention indexes. Recently introduced hybrid mass spectrometers such as QqTOF, Q-Orbitrap, and TOF–TOF combine the features of tandem mass spectrometry and accurate mass measurement of fragment ions as an additional tool for confirmation. Additionally, with the hybrids LTQ-TOF, LTQ-Orbitrap, and LTQ-FT-ICR mass spectrometers, multistage MS^n can be used for structural elucidation. LTQ-Orbitrap was applied for the determination of the polyphenolic profiles of unifloral honeys where a total of 43 compounds were detected in less than 5 min and samples were classified according to their botanical origin. Although the chromatographic profiles obtained were similar for all samples, there was a significant difference in the content of some polyphenolic compounds. Floral markers were suggested and honeys derived from perennial plants and from annual plants could be discriminated. Some phenolics were identified and quantified using the available standards, while in the absence of standards, the identification of the corresponding compounds was based on the search for the $[M_H]^-$ deprotonated molecule together with the interpretation of its fragmentations with the help of an Internet database of accurate mass spectrometry data as a reference library. The identification of four different phenolics with almost identical masses was able through the high-sensitivity accurate mass scan. This example points out the main advantages of hybrid mass spectrometry compared to triple quadrupole MS to the screening and identification of unknown compounds, the possibility of using exact mass to calculate the most favorable elemental composition, and the determination of the accurate mass of the product ions in the MS^n data-dependent scan. When the objective is metabolic fingerprinting, the extraction protocol should be nonselective or direct analysis without sample preparation should be followed through direct infusion or measurements on the surface of samples. To this direction, ambient

ionization MS techniques, such as DART, DESI, EASI, and PTR-MS, broke through in the past few years, apart from ESI and APCI sources commonly used with LC and GC, and their applications in foodomics are still being explored. DART-TOF-MS was successfully applied to recognize the origin of beers without any sample preparation and no prior separation procedures. By the same technique, the different olive oil categories could be differentiated, and in addition, olive oil adulteration with hazelnut oil could be revealed. The polar and triacylglycerol profiles were acquired, and additions of 6% and 15% (v/v) hazelnut oil could be detected, respectively. EASI was used to estimate the quality of nut oils through their triacylglycerol profiles. The method allowed the identification of authentic oils and adulterated oils with 5% soybean oil in a few minutes and without sample preparation. PTR-MS allows monitoring of volatile organic compounds (VOCs) through the soft chemical ionization technique used and, although a rather new technique, its capabilities in the field of geographic origin have already been explored in wine, truffles, and Grana cheese. When applied in olive oils, the country of origin could be by 86% classified correctly, 74% of samples were successfully classified according to region of origin, and the district of origin yielded a success rate of only 52%. MALDI-TOF-MS is a widely used technique for the analysis of protein and peptide biomarkers (proteomics) primarily separated through electrophoresis but has become a popular method for direct analysis applied to small molecules. MALDI-TOF-MS has been used to detect adulteration in bovine milk powder with 10% ($m\,m^{-1}$) of non-milk fats and oils through their triacylglycerol profiles and also to determine both qualitatively and quantitatively melamine and its derivatives. Melamine could be determined with an LOQ down to 0.5 $\mu g\,g^{-1}$ and with the limited dried-droplet sample preparation. Significant limitations of direct MS analysis is the ionization suppression and its effect on sensitivity caused by the presence of multiple chemical species and other matrix components that have considerable impact on the ionization of metabolites and also the overlapping of MS signals of isobaric molecules such as structural isomers or enantiomers.

Finally, ICP-MS can also be used to perform food authentication through the elemental composition of food. The fact that isotopic composition of the constituents of agricultural products depends on geographic origin and on production-related factors has made IRMS another promising approach used for assessing traceability. Both techniques have been successfully applied to wines to examine the influences of geographic origin, year of vintage, grape cultivar, and meteorologic conditions.

In any case, the amount of data provided mainly from fingerprinting strategies is of great complexity, and the use of specific software for data treatment is of utmost importance. Usual data-processing steps involve peak detection, integration, data alignment, and normalization before scaling and multivariate statistical analysis.

NMR Spectroscopy

Nuclear magnetic resonance (NMR) is a spectroscopic technique that allows the analysis of samples in all physical states, providing detailed information at molecular level. A single experiment led to analyze several classes of chemical compounds in a noninvasive, highly reproducible way and in a pretty low experimental time. Materials, like foods as well, could be in different aggregation states: crystalline, amorphous, liquid-like, or liquid. Depending on these states, different NMR techniques could be applied, starting from solid-state NMR, going through NMR imaging, and finishing with liquid state. Technology allowed a big drop in the field strength of the magnets: the field strength could vary from very few fraction of tesla up to latest 1.2 gT. Different NMR techniques and instrument requirements result in different data: high resolution (HR), low resolution or low field (LF), high-resolution magic angle spinning (HR-MAS), imaging (NMRI), and site-specific natural isotope fractionation (SNIF) NMR. HR allows both qualitative and quantitative analyses of samples as well as molecular structure determinations in solution; data obtained from complex matrices like foods enable great potentiality in food metabolomics as a tool for monitoring product quality and authenticity. Several studies that appeared in the literature have focused on the combined use of NMR spectroscopy and chemometrics, dealing with geographic origin or quality determination of foodstuffs, olive oil and wine being the major targets. From the NMR point of view, the ^{1}H NMR spectrum of olive oil is dominated by fatty acid signals. For a deeper evaluation of the minor components, such as aldehydes, terpenes, and sterols, accurate spectra recording conditions need to be applied. The first article concerning the geographic characterization of extra-virgin olive oil (EVOO) samples investigated different olive varieties coming from four Italian regions. PCA and HCA performed on selected ^{1}H NMR resonances due to minor components such as sterols, *n*-alkanals, *trans*-2-alkenals, and other compounds led to a very good sample classification according to the origin. Many other studies followed investigating virgin or extra-virgin olive oils of different harvests and cultivars, from different Mediterranean areas, obtaining a good discrimination by applying different statistical protocols on ^{1}H, ^{13}C, ^{31}P NMR, and/or isotopic ratios from ^{13}C and ^{2}H data. The high prices of EVOOs led to adulteration practices with cheaper oils. In this context, a methodology to detect the presence of a percentage of at least 10% of sunflower and red palm oils in EVOOs was proposed, by employing a low-field (0.25 T) unilateral NMR method, performing a non-destructive analysis through sealed bottles. Many other studies focused on the chemometric analysis of NMR metabolite fingerprinting data of wine to investigate provenance, vintage, aging, or grape cultivar. NMR spectroscopy has been employed to analyze other food matrices to assess authenticity, quality, and geographic origin, to detect adulteration, and to assess safety, among them being beer, fruit juice, balsamic and traditional balsamic vinegar of Modena, tea, dairy products, meat, fish, cereals, vegetables, tomato paste, honey, and coffee (Figures 1 and 2). The role of HR NMR in liquid state applied to food chemistry is growing progressively during these last years even though, as far as we know, NMR has never been accomplished as an official analytical methodology, with the only exception of olive oil quality determination for Lazio region in Italy, obtained with a regional law in late 2001.

NMRI is mainly applied to investigate texture or fluid motions/distribution in foods. The relatively low spectral resolution attainable from these studies is largely compensated by

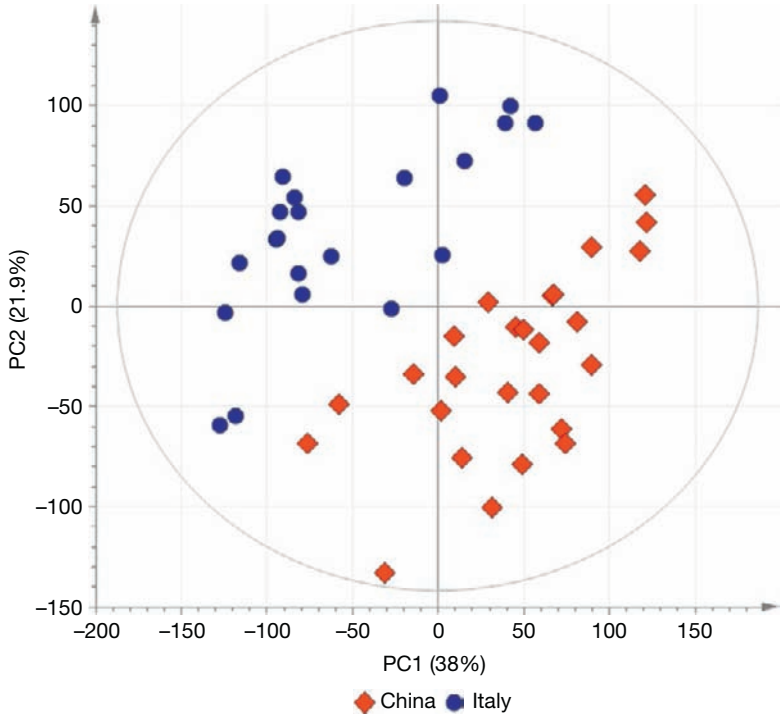

Figure 1 Geographic discrimination between 21 Italian (blue dots) and 26 Chinese (red diamonds) triple-concentrated tomato paste samples by performing PCA analysis on ^{1}H NMR data. Reproduced from Consonni, R. and Cagliani, L. R. (2010). Nuclear Magnetic Resonance and chemometrics to assess geographical origin and quality of traditional food products. *Advances in Food & Nutrition Research* **59**, 87–165.

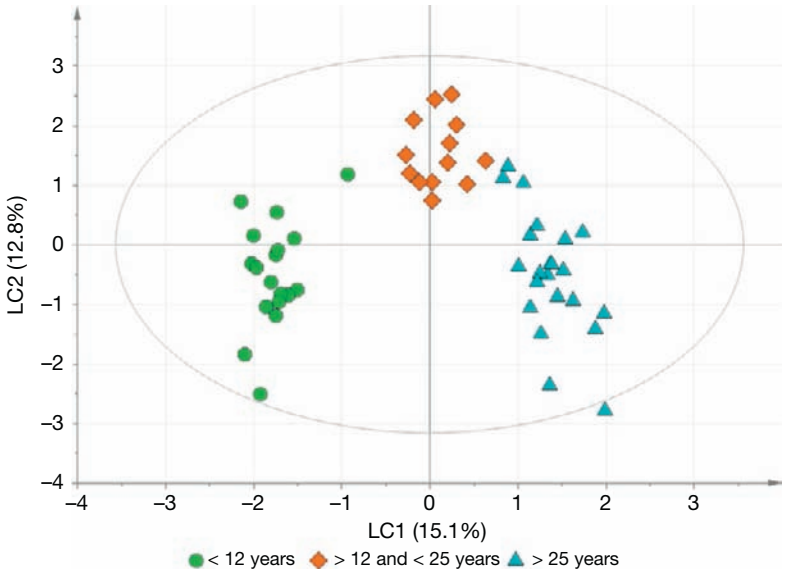

Figure 2 Score plot of hierarchical PLS-DA analysis performed on ^{1}H NMR data of 53 balsamic and traditional balsamic vinegar of Modena: 18 balsamic (green dots), 13 traditional <12 and >25 years (orange diamonds) and 22 traditional >25 years (blue triangles).

3-D images acquired on the basis of relaxation parameters or chemical shift selective values. Water/oil distribution investigations can be easily performed by NMRI, thus allowing maturation studies as well as microstructural studies and compounds/water distribution in intact foods. In the last years, solid-state NMR became a routine spectroscopy in food science, overcoming several technical aspects present in the early stage of this spectroscopy, going through magic angle spinning (MAS), ^{13}C cross polarization (CP-MAS), and ^{1}H high-resolution magic angle spinning (HR-MAS). This NMR spectroscopy was formerly applied to synthetic polymers, in the solid state, to approach crystalline organization. Flour and polysaccharides polymorphisms were first investigated in food analysis, but then, other solid foods were investigated as well.

The last mention is for SNIF-NMR, a relatively old technique, firstly introduced by Prof. G.J. Marten at the University of Nantes more than 20 years ago (patented in 1981) as the most powerful stable isotope techniques in beverage authentication and now the official method of the International Organization of Vine and Wine (OIV) and AOAC International for detecting sugar addition to fruit juice and the natural origin of vanillin. This technique is based on the detection of nonrandom distribution of deuterium in organic molecules.

Vibrational Spectroscopy

Vibrational spectroscopic techniques, both infrared (IR) absorption and Raman scattering, are based on the vibrational transitions of the molecules contained in a sample. In IR spectroscopy, the energy of these transitions is provided by radiation in the IR regions of the electromagnetic spectrum, between the visible and the microwave wavelengths, with mid-infrared (MIR) and near-infrared (NIR) being among the most useful in food authentication studies, as revealed by a large number of related reports. The MIR region lies between 2500 and 25 000 nm (4000–400 cm^{-1}), while the range of wavelengths for NIR is from 780 to 2500 nm (approx. 12 820 to 4000 cm^{-1}). For a particular vibration to be IR active, it must exhibit a change in dipole moment during the vibration. Different chemical bonds absorb at different wavelengths depending on the atoms connected, the surrounding molecules, and the type of vibration occurring. MIR may be used to study fundamental (i.e., stretching and bending) vibrations and associated rotational–vibrational structure, while the higher-energy NIR can excite overtones or combination bands mainly due to the fundamental bonds involving hydrogen atoms. Even though the spectrum obtained by NIR contains more complex structural information comprising broad, overlapped peaks, it may be a characteristic of a sample and may act as a 'fingerprint.' MIR spectra, apart from the peaks associated with characteristic vibrations of the functional groups, contain also the molecular fingerprint region (1500–500 cm^{-1}) with well-defined bands that could provide spectral features useful to identify a sample. The spectral peaks observed in case of MIR are narrow, often sharper, and better resolved than NIR. Sample presentation may take a variety of forms depending on the physical state of sample, including reflectance, transmittance, transflectance, or fiber optics with respect to NIR. In MIR, sampling techniques include transmission-based methods using transmission cells (e.g., KBr disks and ZnSe windows) and methods in reflectance mode, such as attenuated total reflectance (ATR) and diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). The introduction of Fourier transform technique in vibrational spectroscopy has increased its use in food analysis, as there is no scan speed limitation compared to the original dispersive instruments. FT-IR is a powerful tool for food authentication due to its capability to obtain information about many constituents simultaneously in a single spectral analysis. The combined use of FT-IR with multivariate analysis has been applied to distinguish extra-virgin olive oils from four different European countries. A similar approach has been used for the discrimination of 250 saffron samples from Greece ($n = 40$), Iran ($n = 87$),

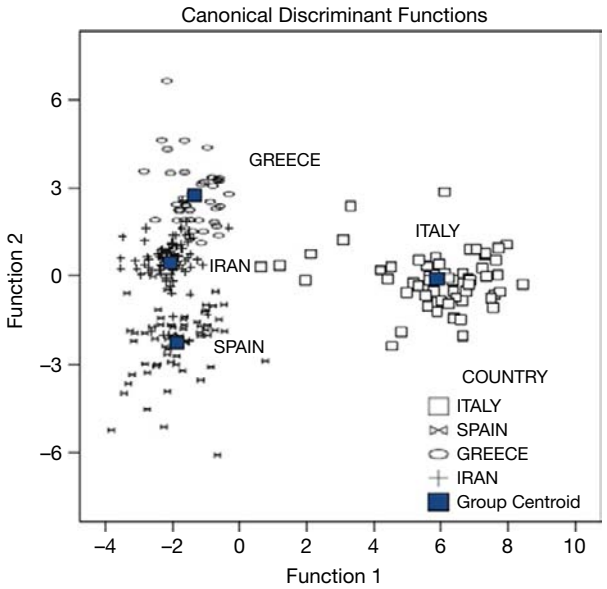

Figure 3 Plot obtained by stepwise canonical discriminant analysis of 250 saffron samples from four different countries, that is, Greece, Iran, Italy, and Spain, based on FT-IR analysis of their volatile extracts. Reproduced with permission from Anastasaki et al. (2010). *European Food Research and Technology* **230**, 71–577.

Italy ($n = 60$), and Spain ($n = 63$). The mid-IR spectra of saffron filament samples and their nonpolar volatile extracts were collected and 93.6% of the samples were correctly classified. Overall, the correct classification rates for samples from Greece, Iran, Italy, and Spain were 90.0, 89.5, 96.7, and 98.4%, respectively (Figure 3).

NIR has allowed the identification of maturity, origin, and variety of white wine grapes. Two varieties, Chardonnay and Viura, were tested and 97.2% of grapes were correctly classified according to their variety, while the correct classification of Chardonnay grapes according to their origin was 79.2%. The application of NIR spectroscopy in combination with Vis spectroscopy and chemometrics has been investigated for the geographic classification of wine. Different multivariate methods were used to classify Australian and Spanish Tempranillo red wines according to their geographic origin, and the best models reported correctly classified 100 and 84.7% of the Australian and Spanish wine samples, respectively.

As regards Raman spectroscopy, the samples are excited with a source of monochromatic radiation that may be in the visible (Vis) or NIR regions of the electromagnetic spectrum. While IR spectroscopy detects vibrations during which the electrical dipole moment changes, Raman spectroscopy is based on the detection of vibrations during which the polarizability changes. Bonds that connect two similar parts of a molecule (e.g., C=C group) tend to be more active in Raman than in IR spectroscopy, as the symmetrical stretches are more intense in Raman spectra. On the other hand, groups such as C=O, N–H, and O–H, with strong mid-IR absorption bands, present very weak vibrations in Raman spectroscopy, since the bonds are only weakly polarizable. Thus, water is practically 'invisible' in the Raman spectroscopy, which is quite useful for

water-based foods. However, dispersive Raman spectroscopy suffers from interference by fluorescence. The use of NIR laser sources can prove beneficial, while FT-Raman instrumentation with an excitation source at 1064 nm results in significantly lower fluorescence and photochemical degradation of samples. Complementary information on fundamental vibrational modes can be obtained from Raman and MIR spectra, since some vibrations are detected primarily by MIR and others primarily by Raman scattering. Raman spectroscopy is becoming increasingly important in food analysis. Significant work has been undertaken to evaluate authenticity and possible adulteration of olive oil. FT-Raman spectroscopy coupled with multivariate calibration has been applied for determining adulteration of Greek extra-virgin olive oils (EVOOs) with sunflower oil at levels lower than 5% by weight. By employing Raman spectroscopy and PCA, pure EVOOs can be distinguished from EVOOs adulterated with hazelnut oil. In addition, the combination of Raman spectroscopy with chemometrics has been evaluated for authenticating the PDO labels of six French virgin olive oils (VOOs) and for determining the fatty acid and triacylglycerol (TAGs) compositions of VOOs. According to the classification model built, 92.3% of French PDOs and 100% of PDO samples made with only one principal cultivar were correctly classified. Raman spectroscopy has been similarly investigated to confirm botanical and geographic origin of European honey, indicating that the major differences among the honey samples were mainly due to their botanical origin. Among the more advanced Raman techniques, surface-enhanced Raman scattering (SERS) and surface-enhanced resonance Raman scattering (SERRS) present many advantages for the development of selective and sensitive analytical procedures. SERS is a very sensitive technique, employing roughened metal substrates (e.g., gold and silver nanoparticles or surfaces) to largely enhance the Raman scattering signal (typically 10^{3-6} over conventional Raman scattering). Another advantage is that fluorescence is quenched from adsorbed molecules, and also, positive identification of an analyte or an analyte mixture may be provided with high selectivity. As a key example, SERS combined with multivariate statistical approaches has been used for the reliable quantification of the banned food dye Sudan I in chili powder over the range of 10^{-3} to 10^{-4} mol l^{-1}. Other modern techniques include transmission Raman spectroscopy (TRS), confocal Raman microscopy, and Raman sensing using fiber optics. Both Raman and IR spectral profiles are currently used for hyperspectral (HIS) or multispectral imaging (MSI). IR and Raman are also emerging techniques in 2-D correlation spectroscopic approach, which employs MIR, NIR, and Raman spectroscopic probes.

During the last two decades, the number of reports on the use of NIR, Raman, and mainly MIR spectroscopy for food authenticity issues (i.e., adulteration, geographic origin, production process, and traceability) has increased significantly. The range of applications includes various foods and beverages such as cereal and cereal products, coffee, dairy products, edible oils and fats, fish, fruits and vegetables, fruit juices, honey, meat and meat products, spices, and wine. By integrating vibrational spectroscopy with multivariate data analysis (chemometrics) strategies, potential analytical tools are developed for industries and regulatory agencies. Such methods are time-saving and nondestructive, require minimal or no sample pretreatment, and enable the cost-effective characterization of samples during the development, processing, quality control, and inspection of foodstuffs. Handheld, portable FT-IR and Raman instruments are also becoming more widely available to bring the benefits of these techniques out of the laboratory. Vibrational spectroscopy may not eliminate the need for more sophisticated analyses, but compared to classical reference methods that typically rely on 'wet chemistry,' it can provide greener analytical methods for screening samples prior to further examination.

Separation Techniques

Among the separation techniques, liquid chromatography, gas chromatography, and capillary electrophoresis make feasible separation and identification of almost any kind of compounds present in a food sample, thanks to continuous advances in both separation and detection capabilities. Their wide applicability in quality control laboratories due to such an efficiency and the affordable capital cost made them candidates to examine food conformity with their label, namely, the origin (geographic and varietal species) and/or the production practice (organic vs conventional). Groups of compounds (targeted analysis), or even the whole spectra/electropherogram serving as a 'fingerprint' containing the maximum information (nontargeted analysis), are examined usually with pattern recognition methods (supervised or unsupervised) to identify possible marker(s) for food authenticity. Nonetheless, the validity of the findings depends on the experimental design of sampling (number, number of harvests, sampling areas, etc.).

The geographic origin and the cultivar employed for virgin olive oil production are often related to its health benefits and the particular sensory properties. Thus, finding markers that could address these two issues became of high importance to protect both the producers and the consumers. This is highlighted by the fact that even for products officially registered as, for example, protected designation of origin (PDO), the availability of a certain analytical method to verify the claim in the label is not necessarily available. The profile of triacylglycerols (TAGs) and that of fatty acids (FA) have been used since many decades for geographic origin discrimination between countries or regions within the same country. Characteristic example is the discrimination of 10 main producing areas in Greece based on the FA content of 1293 samples belonging to 24 harvests after treatment with nonlinear discriminant analysis. The data from both profiles have been even combined to increase classification efficiency. The composition of FA and TAGs is genetically determined; however, pedoclimatic conditions and agronomical practices may have a certain influence justifying their usefulness. Sterols, hydrocarbons, aliphatic alcohols, and sesquiterpenes have also been used, and lately, polar phenolics have also been proposed. All these markers can be detected by GC and HPLC techniques following in most cases official protocols but coupling data to chemometric treatment (Figure 4).

Olive oils produced in different countries are usually facile to be distinguished even based solely on FAs. Focusing on studies regarding PDOs in the same country, the findings are rather controversial. Sesquiterpenes, namely, α-muurolene and α-farnesene contents except for discriminating Apulian from foreign oils, were found suitable to differentiate subbrands of Terra di Bari PDO oils produced in the neighboring areas of the

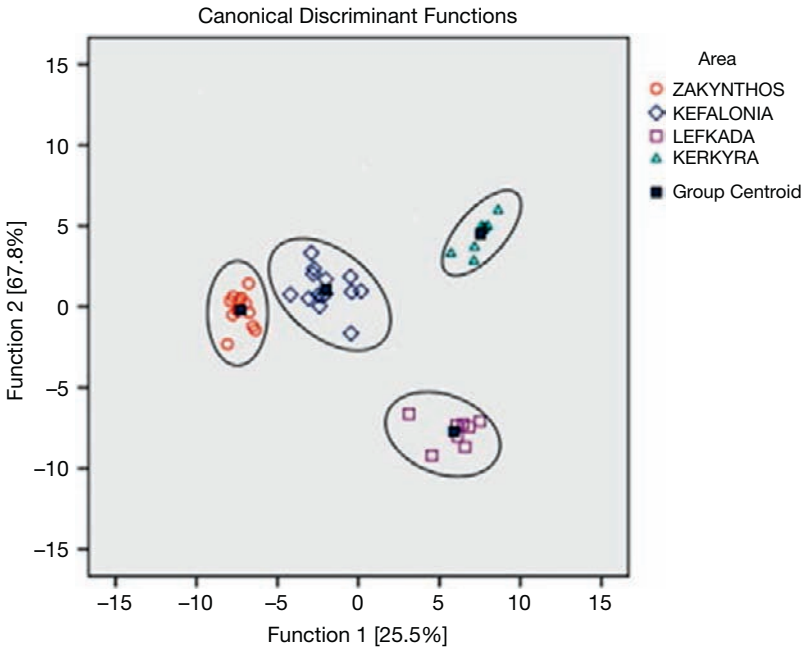

Figure 4 Classification of olive oil samples from western Greece according to geographic origin using selected volatile compositional data. Reproduced with permission from Poularekou et al. (2011). *Journal of Chromatography A* **1218**, 7534–7542.

Apulian region. However, such a finding derived from the analyses of 21 samples and data was considered preliminary. In a study with a larger amount of samples (914 samples and three production seasons), the discrimination of the Ligurian PDOs ($n = 210$) from oils of other regions in Italy as well as from other countries ($n = 704$) based on volatile profile obtained with solid-phase microextraction/GC-ion trap MS resulted in properly recognized and predicted with 90.1% and 81.1%, respectively. These findings were achieved using artificial neural network as a tool. Other studies point out that discrimination may be a difficult task and overlapping can take place in cases where PDOs are produced in close geographic regions and derive from the same cultivar or from a mixture of cultivars presenting compositional similarities (coupage). For example, studies carried out over a 6-year harvest period for five French PDOs showed that Aix-en-Provence and Vallée des Baux derived via blending of same cultivars even at different portions could not be clearly distinguished with the combined information of TAGs and FAs. Such a difficulty was also stressed regarding the analysis of Spanish PDOs for two consecutive harvest years. The oils produced in the various provinces of Andalusia presented overlapping even if 64 compounds obtained by GC and HPLC (fatty acids, sterols, alcohols, and hydrocarbons) were treated with LDA. In the same study, however, the importance of sophisticated statistical approach combined with a multidisciplinary strategy was demonstrated. Thus, employing artificial neural network improved significantly the classification (>90%). Even so, the findings for PDOs presented the highest error of prediction in comparison to those related to sample discrimination from other countries, regions, and provinces.

Similarly, regarding the botanical origin (cultivar), a single marker is also difficult to be proposed due to the complexity of the oil matrix and the variety of factors affecting its composition. The markers with discrimination power are rather comparable to those used for the geographic origin and consequently determined with the same separation methods. It has been stressed however that though FAs and TAGs provide basic information for the cultivars, minor components such as sitosterol and avenasterol, tyrosol and hydroxyltyrosol, (E)-hex-2-enal, lutein, and β-carotene can be more informative for the botanical origin differentiation.

Consistent markers capable of differentiating oils on the basis of cultivation practice are not yet available. However, due to the growing interest in organic olive oil by the consumers, further studies are under way.

Concerning wine authenticity studies, phenolic compounds, biogenic amines, and volatiles have been proposed as useful markers, and within phenolic compounds, both flavonoids and nonflavonoids have been identified. Significant is the contribution of anthocyanin as markers to differentiate European from South American wines. These compounds are determined with LC. Usually, studies regard different countries; nevertheless, there are some concerning different zones. For example, using the content of 13 phenolic compounds and chemometrics, the discrimination of wines from three different Spanish appellations, namely, Penedes, Rioja, and Ribera del Duero, was feasible with a classification rate higher than 96%. As evidenced, characteristic markers were, for example, gallic acid for Penedes, *trans*-coumaroyl tartaric and *trans*-caffeoyl tartaric acids for Rioja, and the flavonol myricetin for Ribera del Duero wine samples. The multivariate curve resolution (MCR) technique allows the resolution of the chromatographic peaks obtained assisting more accurate quantification of phenolics and increasing the information via revealing the presence of coeluting peaks. The application of sub-2 μ particle columns that allows the separation of several isomers seems to be important. With the latter material and LC–MS/MS analysis of phenolic compounds, several wines

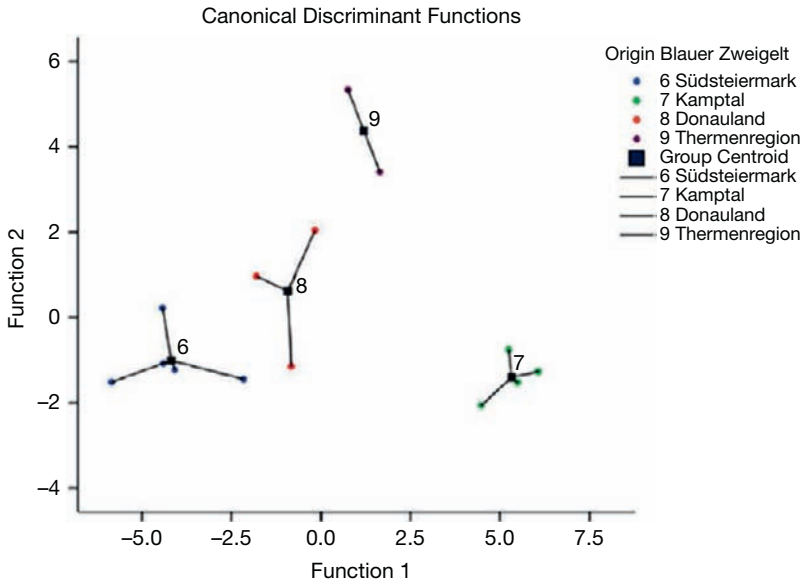

Figure 5 Geographic origin-based discriminant analysis of the grape variety Blauer Zweigelt originating from the region Südsteiermark, Kamptal, Donauland, and Thermenregion (Austria) based on LC–MS/MS phenol analysis. Reproduced with permission from Jaitz et al. (2010). *Food Chemistry* **122**, 366–372.

from 11 Austrian regions could be classified employing multivariate statistics (Figure 5).

Nonetheless, there are also various studies where phenolic compounds are combined with other compositional data (ethanol, calcium, etc.) within the frame of multistrategic approaches for efficient classifications as exemplified for a series of rose Spanish PDO wines, namely, Ribera del Duero, Rioja, Valdepeñas, and La Mancha. The combination with data regarding the levels of biogenic amines made feasible differentiation of wines from different regions of Italy such as Basilicata (cis-resveratrol, total polyphenols, spermidine, and tryptamine), Calabria and Campania (agmatine and trans-resveratrol), and Puglia (cadaverine, ethanolamine, histamine, putrescine, and tyramine) regions. Wine volatiles have been shown adequate for the geographic origin of monovarietal wines from the Azores, Canary Islands, and Madeira islands. The volatile pattern was obtained with solid-phase extraction/GC–MS and treated with chemometrics.

Similar markers have been proposed for the discrimination of grape wine varieties, though among the most recommended are the volatiles. A useful approach to discriminate wines from white grape varieties is based on the determination of shikimate content and the protein profile analysis, whereas for red wines, the shikimate content combined with anthocyanin profile. Shikimate can be determined with the combination of C18 and a cation exchange column according to the method suggested by the International Organization of Vine and Wine, whereas proteins and anthocyanins with CE and high-performance reverse-phase chromatography, respectively.

Information about possible discrimination between organic and conventional wines is rather scarce. Biogenic amines have been reported to be lower in organic wines, whereas phenolic compounds and other compositional parameters were not able to make a clear discrimination despite some quantitative differences. Further studies are on the way to obtain more consistent results.

DNA-Based Technology

A growing consciousness of the food composition led to an increased DNA-based investigations for food authentication also due to the bovine spongiform encephalopathy (BSE) crisis. The incorrect labeling of foods could represent a commercial fraud, and moreover, the nondeclared potential allergens content could cause health problems for consumers. The majority of works was focused on DNA analysis employing polymerase chain reaction (PCR) to amplify specific areas of DNA that could be analyzed with different methods such as the commonly spread electrophoresis techniques.

The application of PCR in authentication of food involves mostly analysis of meat-based foods, fish, and seafood products, which are highly priced foodstuff often susceptible to adulteration. Several studies concerned the detection of different kinds of not declared meat in foods; a species-specific PCR assay targeting mitochondrial D-loop region for the identification of pork in raw, heat-treated samples and in adulterated ones up to 0.1% has been developed. Furthermore, by PCR amplification of the COI gene and detection of species-specific sequences by hybridization, a multidetection test for the identification of different meat species like pork, beef, lamb, horse, cat, dog, and mouse has been achieved resulting in a suitable method for routine inspections. The identification of fish species is usually based on morphological analysis, but this approach resulted however very difficult and not suited to investigate fishes available in the market. As a matter of fact, fishes are often sold in pieces or have been subjected to processes altering the natural aspect. In this context, several DNA-based methods, mainly based on the amplification of

mitochondrial DNA, have been developed, allowing the detection and even the differentiation of closely related fish species. PCR-RFLP technique followed by polyacrylamide gel electrophoresis (PAGE) was proposed for the identification and the discrimination of ten salmon species based on the amplification of a region of the cytochrome *b* mitochondrial gene. Furthermore, the differentiation of closely related species of flatfish such as the sole and Greenland halibut was achieved by species-specific PCR targeting a nuclear gene and by PCR-RFLP of a mitochondrial gene. Moreover, DNA-based methods have been applied for authenticity assessment of dairy products as well, allowing the identification of milks from different biological sources such as the identification of bovine milk in buffalo dairy products. This was achieved by using a high-resolution melting (HRM) as post-PCR method allowing the detection and the quantification of bovine milk in buffalo mozzarella, butter, cream, yogurt, and other buffalo products. The same approach, resulting in a very cost-efficient high-throughput method, has been employed successfully to check the authenticity of Leguminosae species, grapevine cultivars, and PDO sweet cherry cultivar (Tragana Edessis) and to differentiate *Citrus* species and hybrids and bilberry from other berry species. DNA-based methods are also used to detect genetically modified organisms (GMOs) or food allergens; for example, rice, maize, and soybean were investigated and the presence of potential food allergens in, for example, almond and hazelnut was searched in foods. In this context, a tetraplex real-time PCR method was used very recently to quantify simultaneously traces of four allergens such as soy bean, celery, and white and brown mustard in commercially available foods. This latter approach resulted in a powerful method for routine analysis being time-saving with respect to singleplex real-time PCR systems. Finally, DNA analysis is furthermore employed for detecting gluten, normally evaluated with protein analysis, in declared 'gluten free' foods, resulting in most of the cases comparable to ELISA test. In conclusion, the latest results obtained with the most adopted analytical techniques have been presented, highlighting their great potentiality in food authenticity issues. These studies pave the way for possible future investigations aimed to increase the knowledge in food science.

See also: Chemometrics; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Consumer Protection Legislation; Food Fraud; Infrared Spectroscopy: Applications; Mass Spectrometry: Applications; Mass Spectrometry: Principles and Instrumentation; Spectroscopy: Types.

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http://www.moniqa.eu/authenticity – MoniQA Network, Food Authenticity Working Group.

Avocado

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Avocado Fruit Production

History, Cultivation, and Fruit Growth

Avocado originated in Central America, and the fruit has been consumed as part of the diet by the indigenous peoples of that region for more than 5000 years. The word 'avocado' derives from a corruption of the Spanish words 'ahuacate' or 'aguacate,' which are adaptations of the Aztec 'ahuacatl.' The fruit of the first vegetatively propagated cultivar 'Fuerte' is pear-shaped, and this characteristic probably resulted in the inappropriate colloquialism, 'avocado pear.' However, the horticulturally and agriculturally correct term for the fruit is simply avocado.

The botanical name for avocado is *Persea americana* Mill., and three ecological or horticultural races (in some of the literature erroneously referred to as botanical varieties or subspecies) are recognized. The Mexican race has been referred to as *Persea americana* var. *drymifolia*, the Guatemalan race as *Persea americana* var. *guatemalensis*, and the West Indian race as *Persea americana* var. *americana*. However, some researchers have concluded that the validity of these 'botanical varieties' is questionable and requires further study. We suggest the use of ecotype, or horticultural race, which is embedded in the avocado literature. Fruits of the tropical lowland West Indian ecotype are usually large with a thick peel and have a low flesh oil content (<8%) and higher water and sugar content. Trees of the Mexican ecotype yield fruits with high oil content (up to 30%). Those of the Guatemalan ecotype are recognized as having the most horticulturally desirable traits, have intermediate oil content, and are characterized by a 'nutty' flavor. Among the most important commercial cultivars of the 'subtropical' avocado are 'Hass', 'Fuerte', 'Ettinger', and 'Pinkerton', which were all selected from chance seedlings with superior fruit quality. Several countries have extensive selection and breeding programs, which are starting to produce fruit of improved quality. 'Hass' is overwhelmingly the most sought-after subtropical cultivar, having eclipsed 'Fuerte' in Europe and the United States, and forms the basis of the subtropical avocado export industry to Europe and the United States. Several new selections of 'Hass'-like cultivars with improved quality have recently been commercialized.

Resequencing studies to resolve avocado genetic diversity using wild accessions from Mesoamerica and Central America have shown that although avocado is a subtropical tree crop and a predominantly outcrossing plant, genetic variation is very low in comparison with temperate species and much lower than in annuals. The Mexican and Guatemalan ecotypes, whose progeny today form the basis of the technologically advanced 'subtropical' avocado industries, are indigenous to subtropical and tropical highland climates. The so-called 'West Indian' avocados are lowland tropical in origin. Mexico (by far the largest producer), California, Israel, Spain, Chile, South Africa, Peru, Columbia, and Australasia *inter alia* all have industries based on Guatemalan and Guatemalan × Mexican hybrid cultivars. However, virtually all subtropical countries grow avocados to some extent. Large crops of 'West Indian' and 'West Indian' × Guatemalan hybrid avocados are also produced in tropical countries, from less technologically advanced industries. Tropical avocado industries, with the conspicuous exception of Florida, the United States, are usually based on seedling (not grafted) trees. The fruits, although making a substantial contribution to the diets of mostly poor, tropical lowland peoples, are horticulturally and nutritionally inferior to the selected subtropical cultivars.

The avocado tree is evergreen and flowers in early spring, and fruitlets are usually visible by late spring/early summer. Typically, careful and correct orchard management practice is required to ensure the production of quality fruit. Among the most important ecophysiological factors that affect fruit production are irradiance, temperature, water stress, and salinity. Botanically, the fruit of the avocado is described as a berry. Fruit growth continues for anything from 20 to 60 weeks or more, depending on the cultivar, environment, and cultural practice. The fruit is harvested when horticulturally mature as it does not ripen on the tree. Harvested fruit are washed, graded, occasionally waxed, and packed in cartons typically holding 4–6 kg of uniform fruit. European markets prefer the individual fruit weight to be in the range of 200–350 g, for which higher prices are usually paid. It is now known that the final fruit size of avocado is the result of the number of cell divisions during fruit set and fruit growth. Cell division is dependent on *inter alia* isoprenoid biosynthesis, carbohydrate metabolism, maintenance of plant hormone homeostasis, and mineral nutrition. Nitrogen is used as a 'manipulator element' to control tree vigor and must be managed appropriately as excessive tree vigor is counterproductive to yield and good fruit quality. The production of quality fruit in high summer rainfall areas typically requires the application of zinc, boron, potassium, and calcium, the latter from liming. Indeed, quality is associated with relatively high calcium and low nitrogen in the fruit flesh.

Today, there is an increasing global concern about the potential negative effects of commercial agriculture-derived greenhouse gas (GHG) emissions and, in particular, those from intensive production systems like avocado. The energy balances and GHG emissions from the production of export avocados in Mexico (the largest producer and supplier) show no significant difference between 'organic' production and conventional production. Energy consumption and GHG emissions of these two cultivation methods have been quantified at 55 and 56 GJ ha^{-1} and 3.30 and 3.57 ton CO_2 equiv ha^{-1}, respectively. Organic production systems consume three times more renewable energy than conventional production, and dependence on fossil fuel inputs, machinery, and synthetic/organic N-fertilizers negates any difference. Even

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so, experience has shown that it is extremely difficult to control the devastating *Phytophthora cinnamomi* root rot pathogen in 'organic' orchards, where tree injections or foliar sprays of phosphonate chemicals are prohibited.

Fruit Maturity and Ripening

Oil content and/or dry matter of the fleshy pulp, comprising the mesocarp and with a thin layer of endocarp, which surrounds the single large seed, is used by most producers to determine harvestable maturity and consumer acceptability. For the cultivar 'Fuerte', a minimum oil content of 8% (dry mass) was applied in California for many years, but today, this has been replaced by a minimum dry matter standard of ~21%, depending on the cultivar and region. This is based on the percentage moisture (determined using a microwave oven), which is an indirect measure of oil content based on the cultivar-specific constant of the sum of percentage moisture and percentage oil. By 1983, percentage flesh dry matter (or its reciprocal, percentage moisture) had become standard for the determination of avocado fruit maturity and is now used worldwide. Persistence of a living seed coat (not a testa) until horticultural maturity and full fruit size, and then seed coat drying and browning, is an additional maturity criterion. Mature fruits do not shrivel postharvest and will have acceptable eating quality.

Avocado fruits are classified as climacteric (i.e., fruits that can be harvested mature, but hard and unripe, and then undergo normal softening and ripening). An increase in ethylene biosynthesis is typical of climacteric fruits. Maximum ethylene production usually coincides with maximum respiration rate (amount of carbon dioxide released). Cold storage delays and reduces this climacteric rise in respiration.

Although detachment of the fruit is not a prerequisite for ripening of most climacteric fruit, avocado fruits do not ripen while firmly attached to the tree. Postharvest, avocado fruits typically ripen within 5–20 days at temperatures between 15 and 24 °C. However, 'Hass' displays large heterogeneity and is unpredictable in the time taken to reach edible ripeness. Detailed analysis of ripening of 'Hass' fruit has revealed five distinct clusters based on ripening speed, viz., fastest ripening, ~9 days; cluster-2, ~13 days; cluster-3, ~17 days; cluster-4 ~20 days; and slowest ripening, >22 days. Whether a similar ripening pattern exists for other commercial avocado cultivars is currently unknown.

Ripening and fruit softening are both delayed by precooling of the fruit immediately after harvest to 5–6 °C. This strategy is used by most producers to maintain fruit quality during transport/export. A return to ambient temperature after a period of cold storage sees the acceleration of the ripening process. Ripening in avocado can also be stimulated by postharvest exposure to the plant hormone, ethylene, and inhibited by ethylene antagonists such as 1-methylcyclopropene (1-MCP).

During ripening, the fruit undergoes marked changes in a variety of biochemical processes. These include an increase in respiration (i.e., consumption of oxygen and release of carbon dioxide), an increase in ethylene production, changes in texture (i.e., fruit softening), and the production of volatiles such as β-caryophyllene (28% of total volatiles), α-copaene (11%), α,β-cubebene (8%), α-farnesene (6%), decanal (6%), and heptanal (3%), which impart flavor.

The substrate for respiration in avocado fruit appears to be carbohydrate and not oil. However, some degradation of oils does take place. Indeed, metabolomic profiling has revealed that accumulation of linoleic acid (C 18:0) occurs more slowly and to reduced levels in slowest ripening fruit.

Sugar import into developing fruit is accompanied by a transition from symplastic (i.e., cell-to-cell) to apoplastic (i.e., across the cell wall/plasma membrane) transport. The early symplastic route in developing fruit leads to the accumulation of starch, whereas the later apoplastic route in more mature fruit supports the accumulation of soluble sugars. In 'Hass' avocado trees, D-*manno*-heptulose, a seven-carbon sugar, and perseitol, a seven-carbon-containing polyol, are the dominant soluble carbohydrates. By monitoring changes in fruit flesh, sugar content, and composition over the course of development until harvestable maturity, the fate of tree-derived sugars could be deduced. Fructose is at high concentration in young fruits and declines from 22% to about 4% of the total soluble sugars at harvestable maturity. Similarly, glucose declines from about 19% to <1% of total soluble sugars. The concentration of perseitol remains fairly constant throughout the avocado fruit growth at about 25%, whereas D-*manno*-heptulose increases from 15% in immature fruit flesh to 53% of the total soluble sugars in fruit near harvestable maturity. Interestingly, D-*manno*-heptulose is abundant in early season 'Hass' fruit flesh but almost absent in late season fruit. After harvest and during ripening, sucrose, glucose, fructose, and D-*manno*-heptulose contents decline as these sugars are consumed during the respiratory climacteric. The inhibition of ripening with 1-MCP and the relatively new palladium-promoted ethylene scavenger (e+™ Ethylene Remover) result in sustained levels of D-*manno*-heptulose and perseitol. So, the inhibition of ethylene action and removal of ethylene affect avocado fruit ripening similarly.

In addition to changes in sugar metabolism, the respiratory climacteric occurs coincident with upregulation of gene expression including polygalacturonase (*PamPG*), putative NAD^+-dependent sorbitol dehydrogenase (*PamSD*), three acyl-CoA synthetases (*PamACoAS1*, *2*, *3*), and accumulation of triglycerides.

Fruit softening is a consequence of changes in cell wall metabolism resulting from alterations in cellulase (EC 3.2.1.4), polygalacturonase (EC 3.2.1.15), pectinesterase (EC 3.1.1.11), and β-galactosidase (EC 3.2.1.23) activities. In some cultivars (e.g., 'Hass'), peel color changes from green to purple/black owing to the accumulation of anthocyanin (e.g., cyanidin 3-*O*-glucoside) pigments. Other changes include a slight increase in the concentration of glucose and fructose in the fruit flesh.

Processing of Avocado

Avocado is usually consumed fresh in salads, as a savory dish, as a sandwich filling, as guacamole, or as a dessert when sweetened. It was the extraction of oils from avocado pulp and the production of guacamole that really started avocado processing. Apparently, the production of guacamole on a commercial scale was started as long ago as 1964 in California,

and by 1981, 6000 tons of fresh avocado was being processed annually. Guacamole is a highly regarded component of Mexican cuisine.

For processing, the preferred cultivar is 'Hass' because of its superior flavor, flesh (pulp) color, 'keeping' quality, and year-round availability. Fruits with a 25% dry matter content (or 13% oil) are considered ideal for processing. Avocado flesh is also marketed frozen, processed into a sauce, dehydrated to a powder, and extracted for its oil.

Guacamole, Avocado Sauce, and Oil

Before processing can take place, fruits are uniformly ripened at 22 °C in the presence of ethylene gas for 2–5 days. When the fruits are soft, they are cooled to 5 °C and surface-sterilized in 200 mg of hypochlorite per liter. After removal of the fruit stalks (pedicels) and seed, the pulp (mainly mesocarp) is mechanically separated from the peel. The pulp is then mixed with the ingredients (usually includes lime or lemon juice, salt, and salsa or picante) to yield a uniform textured guacamole. The guacamole is then placed in containers and stored frozen.

The process for the production of avocado sauce is similar to that used for the preparation of guacamole up to and including pulp separation. For sauce, the pulp is sheared in a high-speed blender with water, emulsifiers, and spices. Sauces are usually packaged in polyethylene containers and then stored frozen.

Dehydration of avocado is by either spray or drum drying in much the same way as for the preparation of milk powder, but the product is less attractive than that of sugar-storing fruits.

Oil is extracted from avocado with organic solvents, hydraulically (pressing), or by centrifugation. The last method is more desirable as the product is free from solvent residue. Nevertheless, solvent extraction allows for the recovery of various fractions, some of which are used in the pharmaceutical industry. For example, avocado unsaponifiables (including hydrocarbons, tocopherols, triterpenes, sterols, and other unidentified compounds) seem to be beneficial in the treatment of periodontal and osteoarticular diseases. They function by stimulating the deposition of repair material in affected areas by enhancing transforming growth factor beta (TGF-β) in articular cartilage. Avocado oil is also used in cosmetic preparations such as skin moisturizers and body lotions. It has cooking properties very similar to olive oil but is more expensive.

Nutritional Status

The Spanish word 'aguacate' (literally 'testicle tree') refers to the widely held belief among native Central Americans in the aphrodisiac properties of avocado fruits. Early exporters developing the European market cunningly exploited this belief to gain acceptance for the then little-known avocado fruit with an 'acquired taste.' Nowadays, avocado is known to be a highly nutritious fruit and contains higher quantities of soluble and insoluble fiber and protein than many other fleshy fruits. Avocado is also a rich source of potassium and the vitamins E and C and β-carotene (provitamin A). Although low, a vitamin A value of 150 mg RE (retinol equivalents) per kilogram has been reported. Furthermore, the monounsaturated fatty acids in avocado effectively reduce blood levels of the undesirable low-density lipoprotein (LDL) cholesterol while increasing the levels of the beneficial high-density lipoprotein (HDL).

Nutrient Composition/Density

Unlike starch staple foods that are described as providing 'empty calories,' the avocado is a nutrition-rich 'protective' fruit. The energy content per 100 g serving has been estimated at 800 kJ, depending on the cultivar and growing conditions. A detailed analysis of the composition of avocado pulp, mainly mesocarp (the edible portion of the fruit) of a typical subtropical cultivar, has revealed a nutritional status summarized in Figure 1.

Avocado pulp contains approximately 2.3% protein on a fresh-weight basis, which is between two and ten times that of most other fleshy fruits and vegetables analyzed. Although avocado contains all of the essential amino acids (i.e., it is 'complete'), it is not generally regarded as nutrient-dense for protein, certainly not in comparison to meat or eggs, for example. A mixture of soluble and insoluble fiber is ideal in the diet, and avocado contains appreciable quantities of both (2.1% and 2.7%, respectively).

In avocado flesh, the sum of percentage water plus percentage oil is constant in the range 88–91%, and for each gram of water lost, there is a 1 g increase in oil, in fruit containing between 8% and 22% oil. Changes in oil content are also associated with changes in fiber and protein. Therefore, the energy value of avocado is almost entirely due to its oil content, and any variation is due solely to changes in the percentage oil. It is probably this observation that led to the mistaken idea that consuming avocado increases body mass. On the contrary, the addition of avocado to the diet has been shown to cause a small but significant average weight loss. One possible explanation is that avocado speeds up the basal metabolic rate in humans. Another hypothesis relates to the type of oil, and avocado is rich in the beneficial monounsaturated fatty acids.

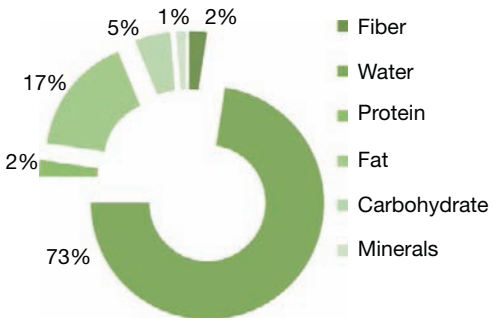


Figure 1 Nutrient content and composition of subtropical avocado fruit flesh. Data are the average between the minimum and maximum values reported by Ahmed, E. M. and Barmore, C. D. (1980). Avocado. In: Nagy, S. and Shaw, P. E. (eds.) *Tropical and subtropical fruits: composition, properties and uses*, pp. 121–156. Westport, CT: AVI; Seymour, G. B. and Tucker, G. A. (1993). Avocado. In: Seymour, G. B., Taylor, J. E. and Tucker, G. A. (eds.) *Biochemistry of fruit ripening*, pp. 53–81. London: Chapman & Hall; Slater, G. G., Shankman, S., Shepherd, J. S. and Alfin-Slater, R. B. (1975). Seasonal variation in the composition of Californian avocado. *Journal of Agricultural and Food Chemistry* **23**, 468–474, and references cited therein.

The fatty acid content and composition of avocado have been iterated in most texts on the physiology and biochemistry of fruit growth and ripening. Avocado lipids can be divided into the following fractions: (1) neutral lipids (tri-, di-, and monoacylglycerols), (2) phospholipids, (3) glycolipids, and (4) free fatty acids. The neutral lipid fraction constitutes 96% of the total lipid content of avocado, and the majority of these are triacylglycerols. The major triacylglycerols identified in avocado are dioleyl palmitin, triolein, dioleypalmitolein, linoleyl oleyl palmitin, and linoleyl diolein. Within each of these triacylglycerols, C18:1, C18:2, C16:0, and C16:1 are the major fatty acids present. The relative concentration (percentage of total lipid) of each is in the range 59–81% (C18:1), 7–14% (C18:2), 7–22% (C16:0), and 3–11% (C16:1). These values are comparable with those for olive oil and make avocado, which is easily digested, an ideal substitute for olive oil in cooking and in the preparation of salad dressings.

The phospholipid composition of the edible portion of avocado fruit is surprisingly much less documented. Nevertheless, by means of high-performance liquid chromatography–electrospray ionization tandem mass spectrometry, phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidic acid (PA), and *lyso*-phosphatidylcholine (LPC) have been unequivocally characterized. PE and PI contain the less unsaturated C16:0/C18:1 fatty acid moieties while PC and PA show a prevalence of C18:1/C18:1, and in LPC, C18:1 prevails.

Unsaturated fats are either mono- or polyunsaturated, and the ratio of polyunsaturated fatty acids to saturated fatty acids (P/S), which is used as an indicator of nutritional value by nutritionists, is about 0.74 for avocado. A diet high in polyunsaturated fatty acids reduces the level of the undesirable LDL, whereas a high dietary level of monounsaturated fatty acids also maintains the levels of the desirable HDL. Thus, it should not surprise that results from trials in which the monounsaturated-rich avocado was used as a dietary component revealed a significant decline in total cholesterol with preservation of the HDL level. Likewise, substituting avocado for butter, margarine, and cheese significantly reduced blood cholesterol levels and increased HDL up to 16%. Accordingly, avocados have gained acceptance by the Heart Foundations of several countries and can even be prescribed for convalescent heart patients.

Fat- and Water-Soluble Vitamins and Antioxidants

The vitamin content and composition of avocado are shown in Table 1. Although vitamin D has been identified in avocado, no values appear to have been published. It has been reported, however, that the vitamin D content of avocado is higher than that of butter and eggs. Vitamins C and E and provitamin A (β-carotene) are believed to function as antioxidants and protect against damage from oxygen 'free radicals.' Although oxygen is essential to life processes, it is also damaging if converted to reactive oxygen species (e.g., the superoxide anion or the hydroxyl radical). These can cause cell mutation and contribute to aging, cancers, arthritis, and heart disease. The three antioxidant vitamins are effective at disarming these reactive oxygen species, and it has been stated that for each of the three and for any given daily kJ proportion, the avocado provides about twice the proportion of this nutrient.

Table 1 Vitamin content and composition of avocado fruit flesh

Component (per 100 g fresh weight)	*Concentration range*[a]
β-Carotene (provitamin A) (IU)	370–750
α-Tocopherol (vitamin E) (IU)	1.6–2.4
Ascorbic acid (vitamin C) (mg)	1.6–30.0
Biotin (mg)	3.2–10.0
Choline (mg)	17–22
Folacin (mg)	30–62
Niacin (mg)	1.4–3.5
Pantothenic acid (mg)	0.25–1.14
Pyridoxine (vitamin B_6) (mg)	0.22–0.62
Riboflavin (vitamin B_2) (mg)	95–230
Thiamine–HCl (vitamin B_1) (mg)	60–240
Phytyl menaquinone (vitamin K) (mg)	0–8
Calciferols (vitamin D)	Unknown

[a]Concentration is dependent on the cultivar and stage of ripening.

Source: Values were derived from data published by Ahmed, E. M. and Barmore, C. D. (1980). Avocado. In: Nagy, S. and Shaw, P. E. (eds.) *Tropical and subtropical fruits: composition, properties and uses*, pp. 121–156. Westport, CT: AVI; Seymour, G. B. and Tucker, G. A. (1993). Avocado. In: Seymour, G. B., Taylor, J. E. and Tucker, G. A. (eds.) *Biochemistry of fruit ripening*, pp. 53–81. London: Chapman & Hall; Slater, G. G., Shankman, S., Shepherd, J. S. and Alfin-Slater, R. B. (1975). Seasonal variation in the composition of Californian avocado. *Journal of Agricultural and Food Chemistry* **23**, 468–474, and references cited therein

Extracts prepared from 'Hass' fruit pulp, containing carotenoids and vitamin E, inhibit growth of both androgen-dependent and androgen-independent prostate cancer cell lines. A putative mode of action appears to be cell cycle arrest at the G_2/M transition and is accompanied by an increase in the expression of the cyclin-dependent kinase inhibitor protein, p27. In addition to the antioxidant vitamins, 2(*R*)-(12*Z*,15*Z*)-2-hydroxy-4-oxoheneicosa-12,15-dien-1-yl acetate and persenones A and B have been isolated from avocado leaves and fruit pulp, respectively, as inhibitors of superoxide and nitric oxide generation. These compounds do not scavenge reactive oxygen species (like the vitamins) but rather suppress free-radical generation and may therefore be effective chemopreventive agents in inflammation-associated carcinogenesis. Indeed, a 4-pyridinyl derivative of 2(*R*)-(12*Z*,15*Z*)-2-hydroxy-4-oxoheneicosa-12,15-dien-1-yl acetate, a polyketide isolated from the leaves due to growth inhibition of silkworm (*Bombyx mori*) larvae, shows cytostatic and proapoptotic activity in human breast cancer cells. Thus, the consumption of avocado may be one way of ingesting more of the antioxidants that help to protect against cancers, heart disease, and aging. Indeed, antioxidant activity in early harvested fruit after storage for 35 days is much higher than that in late harvested fruit after storage for 21 days. Therefore, avocado can be harvested earlier for economic benefits according to the market and can keep high nutritional value for human health benefits.

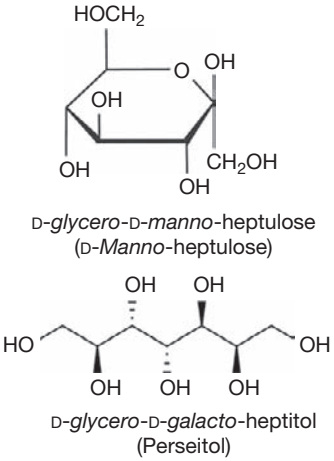
Mineral Composition

The mineral content and composition of avocado are presented in Table 2. As shown, avocado is a rich source of potassium, which is purported to protect against the risk of strokes in humans and reduce the incidence of strokes by up to

Table 2 Mineral content and composition of avocado fruit flesh

Mineral (mg per 100 g fresh weight)	*Concentration range*[a]
Potassium	340–723
Magnesium	40–60
Phosphorus	20–80
Calcium	10–15
Sodium	5–15
Iron	0.5–2
Boron	1–3

[a]Concentration is dependent on the soil type, cultivar, and cultural practice.
Source: Values were derived from data published by Ahmed, E. M. and Barmore, C. D. (1980). Avocado. In: Nagy, S. and Shaw, P. E. (eds.) *Tropical and subtropical fruits: composition, properties and uses*. pp. 121–156. Westport, CT: AVI; Slater, G. G., Shankman, S., Shepherd, J. S. and Alfin-Slater, R. B. (1975). Seasonal variation in the composition of Californian avocado. *Journal of Agricultural and Food Chemistry* **23**, 468–474, and references cited therein



D-*glycero*-D-*manno*-heptulose
(D-*Manno*-heptulose)

D-*glycero*-D-*galacto*-heptitol
(Perseitol)

Figure 2 Structural formulas of D-*manno*-heptulose and its related acyclic heptitol, perseitol.

40%. One of the confounding factors in the cause of strokes is increased blood pressure, which has been associated with a high intake of sodium. The avocado is as low in sodium as it is high in potassium.

Phosphorus, calcium, and magnesium are abundant minerals in avocado. Most other minerals, including iron, occur in amounts of less than 1 mg per gram fresh weight of edible fruit.

Carbohydrates

During avocado fruit ripening, there is an increase in the flesh (pulp) concentration of glucose and fructose. In addition to these monosaccharides, avocado also contains several unusual sugars including the seven-carbon sugar alcohol, perseitol, and its reduced form, D-*manno*-heptulose. The structures of these are shown in Figure 2. 'Hass' avocado fruit pulp sugar content and composition is given in Table 3.

Studies in the 1940s indicated that ingestion of avocado could lead to the presence of sugar in urine. It was later discovered that D-*manno*-heptulose, the major reducing sugar in avocado, could induce hyperglycemia when administered to humans and other animals orally. Even so, there is no unequivocal information linking the consumption of avocado with diabetic symptomatology. In fact, the D-*manno*-heptulose content of the edible portion of avocado declines by up to 80% as the fruit ripens. Thus, any amounts of D-*manno*-heptulose ingested are likely to be very low, and some cultivars appear to have none in harvested fruit. However, toxicity to avocado fruit reported for some birds and animals may be linked to consumption of hard, unripe flesh.

Table 3 Sugar content and composition of ripe avocado fruit flesh

Sugar (mg per 100 g fresh weight)	*Concentration range*[a]
Glucose	0.14–1.28
Fructose	0.08–0.65
D-*Manno*-heptulose	0–3.82
Perseitol	0–2.06

[a]Concentration is dependent on the cultivar and stage of ripening.
Source: Values were derived from data published by Liu, X., Robinson, P. W., Madore, M. A., Witney, G. A. and Arpaia, M. L. (1999). 'Hass' avocado carbohydrate fluctuations. II. Fruit growth and ripening. *Journal of the American Society of Horticultural Science* **124**, 676–681; Seymour, G. B. and Tucker, G. A. (1993). Avocado. In: Seymour, G. B., Taylor, J. E. and Tucker, G. A. (eds.) *Biochemistry of fruit ripening*, pp. 53–81. London: Chapman & Hall, and references cited therein

Interestingly, D-*manno*-heptulose is regarded as an inhibitor of hexokinase (EC 2.7.1.1) activity with a $K_i = 0.5$ mM. Hexokinase phosphorylates hexose/hexulose during metabolism to give the corresponding 6-phosphate derivatives, which are then metabolized in the glycolytic pathway to provide energy and organic substrates. Whether this biochemical pathway is affected by a diet rich in avocado (and therefore some D-*manno*-heptulose) in humans is unknown.

Nutritional Benefits and Costs

Nutrient Density

The extraordinary nutrient density, at least of the fruits of subtropical avocado cultivars that form the basis of world trade and of consumption in the affluent first world, has been noted. In fact, the subtropical avocado has been described as the most nutritious of all widely grown fleshy fruits. Consumption of avocado is therefore associated with improved overall diet quality, nutrient intake, and reduced risk of metabolic syndrome. Thus, comparatively small amounts of avocado are extremely helpful in upgrading the quality of the diet in an increasingly health-conscious world. Furthermore, the relatively small amounts consumed per serving (80–100 g) are no cause for concern where affluence has contributed to overweight or even obesity. The problem in poor, mostly tropical, third-world countries is very different, and here, the energy and nutrient density of the fruit, even of less nutritious tropical cultivars, can make a substantial contribution to guard against malnutrition.

Protection from Coronary Heart Disease

Coronary artery disease results from the buildup of cholesterol and other lipids in coronary arteries. Cholesterol is carried by

lipoproteins, and LDL causes the most damage. HDL, however, protects vessel walls from atherosclerosis.

Monounsaturated fatty acids increase the blood level of HDL. Avocado is high in monounsaturated fats and contains no cholesterol. A diet rich in avocado therefore has a favorable effect on blood fats, decreases cholesterol, and preserves HDL. Trials have revealed that a diet rich in avocado causes a decline in total serum cholesterol of 16% in healthy individuals. In hypercholesterolemic subjects, a decrease in serum cholesterol of 17%, LDL cholesterol of 22%, and triacylglycerols of 22% was observed, while HDL cholesterol increased by 11%. There has, therefore, been a welcome change in attitude toward avocado consumption in people at risk of coronary heart disease.

Hypersensitivity

Hypersensitivity occurs in latex-allergic individuals who react to avocado (and other fruits including banana, melon, and kiwifruit) within 60 min of ingestion. They typically display one or more of the following symptoms: mouth irritation, angioedema, urticaria, asthma, nausea, vomiting, diarrhea, rhinitis, or anaphylaxis. Allergy to avocado is increasing, especially in the United States and Mexico, and has been estimated at around 1% of the general population. Avocado allergy is of particular importance in the 'latex-fruit syndrome' observed in at least 40% of latex-allergic individuals. As mentioned earlier in the text, the allergens elicit diverse immunoglobulin E-mediated reactions in sensitized individuals. Many of the known plant food allergens are in fact proteins produced either following pathogen attack or after induction of the plant stress syndrome. Thus, avocado fruit proteins are of particular importance in food-related allergies.

A recent proteomic analysis of avocado fruit has revealed at least 1012 different proteins including the well-known avocado allergen *Pers a 1* and several proteins suspected of eliciting allergic responses. Included in the latter are polygalacturonase, a thaumatin-like protein, glucanase, and an isoflavone reductase-like protein.

At least two avocado allergens are known: *Pers a 1* and *Pers a 4*. *Pers a 1* is the most widely studied and is considered the major allergen. The *Pers a 1* allergen is a 32 kDa protein that has endochitinase activity and is recognized by 15 out of 20 avocado and/or latex-allergic individuals. This allergen belongs to the PR-3 family of pathogenesis-related proteins and is induced in plants by ethylene treatment, but allergic activity is lost in heating. This probably explains why plant foods containing this allergen that are consumed after cooking (e.g., green beans) are not usually associated with the latex-fruit syndrome.

Antimycobacterial Activity

A patented blend of D-*manno*-heptulose and perseitol termed AV119, which is prepared by extracting fruit pulp in aqueous alcohol and concentrating the solution to obtain a 5% content of the actives, induces human β-defensin 2 production by normal human keratinocytes and satisfactory recovery of the proinflammatory response. More specifically, AV119 modulates the lipopolysaccharide-induced proinflammatory response by blocking the activation of the transcription regulator NF-κB. In addition, AV119 induces aggregation of yeasts, in particular those that are part of the normal human cutaneous commensal flora, and inhibits penetration into keratinocytes.

Other antimycobacterial metabolites from avocado have been isolated from the pulp of unripe fruit and include fatty alcohol derivatives termed avocadenols and avocadin. Both avocadenols A and B, (2*R*,4*R*)-1,2,4-trihydroxynonadecane, and (2*R*,4*R*)-1,2,4-trihydroxy heptadec-16-ene display antimycobacterial activity against *Mycobacterium tuberculosis* with MIC values of 24, 33, 24.9, and 35.7 μg ml^{-1}, respectively.

See also: Allergies: Public health; Antioxidants: Role on Health and Prevention; Cancer: Diet in Cancer Prevention; Carbohydrate: Digestion, Absorption and Metabolism; Carotenoids: Occurrence, Properties and Determination; Enzymes: Functions and Characteristics; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fruits of Tropical Climates: Biodiversity and Dietary Importance; Fruits of Tropical Climates: Dietary Importance and Health Benefits; Malnutrition: Prevention and Management; Phospholipids: Physiology; Phospholipids: Properties and Occurrence; Salad Crops: Dietary Importance; Sugar Alcohols; Tocopherols: Physiology and Health Effects; Trace Minerals and Trace Elements; Triacylglycerols: Structures and Properties; Vegetable Oils: Composition and Analysis; Vegetable Oils: Dietary Importance; Vegetable Oils: Oil Production and Processing; Vegetable Oils: Types and Properties; Vegetarian Diets; Vitamins: Overview.

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B

Bacillus cereus and Other *Bacillus* sp. Causing Foodborne Poisonings, Detection of

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Introduction

Bacteria of *Bacillus* spp. are widely present in natural environments and are common contaminants of foods. They are Gram-positive and aerobic and many species are facultative anaerobes. *Bacillus* sp. form spores (or endospores) in conditions of nutrient depletion and in response to population signals. Spores are in a dormancy state and allow persistence in the environment and in food raw material and resistance to food processing (heat, chemical treatments, high hydrostatic pressure, UV irradiation, etc.) (Figures 1 and 2).

Vegetative cells emerging after spore germination are adapted to growth at a wide range of temperature, pH, and a_w and therefore can easily multiply in food matrices, where they can cause spoilage. The *Bacillus* species *B. cereus* and to a lesser extent *B. subtilis*, *B. licheniformis*, and *B. pumilus* have also been reported as causes of foodborne poisoning. Among those, *B. cereus* represent the most serious concern for public health and food safety because of the number of reported cases and the severity of some of them. *B. cereus* causes two types of syndromes: a diarrheal syndrome following ingestion of *B. cereus* cells and enterotoxin production in the small intestine and an emetic syndrome caused by cereulide ingestion, a heat-stable cyclic peptide. With some exceptions reporting a high incidence in some countries, the relative incidence of *B. cereus* in most developed countries represents a minority (i.e., a few percent) of the outbreaks of foodborne poisonings. However, it is worth noticing that fatal or very severe emetic *B. cereus* outbreaks are increasingly reported since the early 2000s, in particular attributed to liver failure of patients. As a consequence, the assessment of the risk due to *B. cereus* and other *Bacillus* species requires a quantitative evaluation of consumer exposure (prevalence and/or concentrations in foods) and increasingly a qualitative evaluation of the profile of the strains detected in the food. Most detection methods have been developed for *B. cereus*, much more than for other *Bacillus* sp., because of the frequency and the relative severity of *B. cereus* foodborne poisonings as mentioned earlier. Detection of *B. cereus* in foods and other environments meets three objectives:

(i) Evaluation of the population level that should be controlled in foods or to which the consumer could be exposed. Foodborne poisonings are in most instances caused by the ingestion of foods containing at least 10^5 cfu g^{-1}. Evaluation of *B. cereus* concentrations is a key issue for risk management.

(ii) Characterization of the strains present in a food. *B. cereus* strains are highly diverse. A significant effort is dedicated to the determination of the strain potential to cause foodborne poisonings and to the adaptation to the environment in the food chain.

(iii) Detection and quantification of emetic toxin (cereulide) in foods.

Ecological Niches and Routes to Contamination by Foodborne Pathogenic *Bacillus* sp.

Soil is regarded as the natural habitat of spore-forming bacteria. Spores present in soils can be dispersed and can colonize very diverse environmental niches. *B. cereus* and *B. subtilis* spores can be detected in the gastrointestinal tract of vertebrates and vertebrates including mammals. The food production chain can be contaminated by spores through these sources. Molecular tools developed since the early 1990s, such as random amplification of polymorphic DNA (RAPD methods), have allowed in many circumstances a detailed tracing of contamination routes, from soil, to food unprocessed materials, to stored processed dishes. In dairy farms, concentrations of milk with *B. cereus* can also be correlated to concentrations in cow's feces and to dairy cow's feed. Many *Bacillus* spp. are used in different applications exploiting some of their specific properties, which also contribute to dispersion. Contamination of horticultural crops with *B. cereus* could be a consequence of the spreading of *B. thuringiensis*, applied for the insecticidal properties of its parasporal crystal: *B. thuringiensis* is genetically not distinguishable from *B. cereus*. Spores of *B. cereus*, *B. subtilis*, and *B. licheniformis* may also be used for their probiotic properties in humans, for veterinary applications, or in aquaculture.

The Complex Taxonomy of *B. cereus*

B. cereus is part of the genus *Bacillus*. *B. cereus* is phenotypically close to *B. anthracis*, *B. mycoides*, *B. thuringiensis*, *B. weihenstephanensis*, and *B. pseudomycoides*. These genetically very close species form the *B. cereus* group, also known as *Bacillus cereus sensu lato* (Tables 1 and 2). Recent phylogenetic analysis provides a robust description of the genetic structure

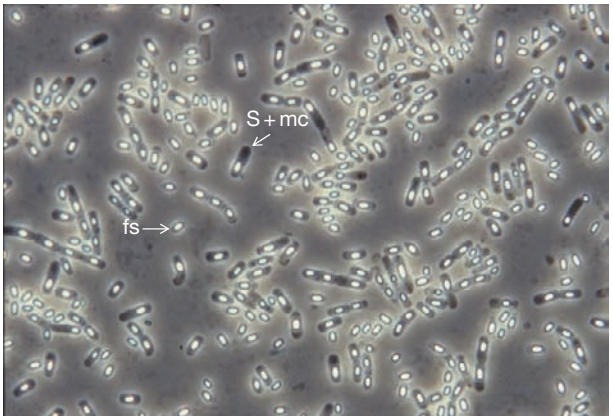

Figure 1 Phase contrast microscopy of *Bacillus* spores under a 1000× magnification. Phase-bright spores (S) are formed within a mother cell (dark phase) (mc) and are released to form free spores (fs). Spore diameter is approximately 1 μm. Picture of Guinebretière MH, INRA PACA.

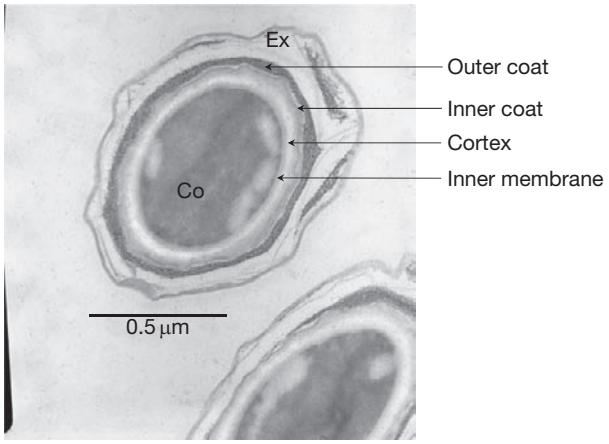

Figure 2 Transmission electron microscopy image of a section of a *Bacillus cereus* spore showing the spore structures. The core (Co) of the spore in dormancy contains DNA protected by small acid-soluble proteins (SASPs). Spore core is highly dehydrated and contains high concentrations of divalent cations (Ca^{2+} and Mg^{2+}) and of dipicolinic acid, specific to bacterial spores. Spore core is surrounded by a membrane similar to that of vegetative cells. The cortex is formed of peptidoglycan, with a structure similar to that of the bacterial cell wall. The cortex is degraded during germination to allow emergence of the vegetative cell and then cell division. Protein coats also contribute to spore protection against some physical and chemical stress. Exosporium (Ex), the outermost structure of spore, has a diverse composition and its role is still poorly known. Exosporium contributes to adhesion of spore to surfaces. Picture of Planchon S and Bornard I, INRA PACA.

of the *B. cereus* group. The correspondence between the phylogenetic groups and the currently defined species is shown in Table 1, together with some phenotypic characterization of the groups and association with outbreaks of foodborne poisonings. The phenotypes of *B. cereus* phylogenetic groups show pronounced variations in the ability to grow at low temperature, low pH, and high salinity and to resist heat. The virulence potential of the phylogenetic groups, evaluated by association (frequency and severity) with foodborne poisonings, shows also marked differences. Some strains of groups III (the one of the emetic strains) and VII have caused the most severe poisonings. In contrast, strains of groups I and VI have almost never been associated with foodborne poisonings. Food authorities do not generally consider this diversity when sampling foods.

Methods for *B. cereus* Enumeration in Foods

Spore Selection and Elimination of Vegetative Cells

B. cereus can contaminate foods as spores or vegetative cells. In some instances, the only enumeration of spores can be recommended, in particular when foods are intended for heat treatment that eliminates vegetative cells. Typically, a few minutes of treatment at approx. 60 °C will inactivate vegetative cells of *B. cereus*, while spores will resist to at least several minutes at 90 °C. Inactivation of vegetative cells prior spore enumeration is therefore achieved with a heat treatment of the samples at 60–80 °C for 10 min or longer. Spores are also unaffected by ethanol in solution unlike vegetative cells. Spore selection and elimination of vegetative cells can also be obtained by exposure of food samples to 95–100% filter sterilized ethanol (ethanol may contain spores) at ambient temperature for 30–60 min at a final concentration of 1:1.

Culture Media and Confirmation Tests

Pure cultures of *B. cereus* can grow on most routine laboratory media such as nutrient or blood agar. Optimal growth is at about 37 °C for most species, except for some psychrotrophic strains (including those identified as *B. weihenstephanensis*), which is more around 30 °C. Selective media for *B. cereus* detection and enumeration have been developed for several decades and are still widely applied worldwide although alternative chromogenic media have been proposed since a few years. Selective PEMBA (polymyxin B–pyruvate–egg yolk–mannitol–bromothymol blue agar) and MYP (mannitol–egg yolk–polymyxin B agar) are based on the resistance of *B. cereus* cells to the antibiotic polymyxin B, which conversely inhibits growth of Gram-negative bacteria and its inability to produce acid from mannitol (no pH reduction diagnosed by no color change of pH indicators such as bromothymol blue or bromocresol purple) and the production of a lecithinase that will precipitate and form a halo surrounding colonies. The addition of trimethoprim has sometimes been suggested for higher selectivity. Colonies are generally large and can coalesce, making plate readings difficult. This problem can be overcome by using large-diameter Petri dishes or reducing the countable colony range to <30. Incubation times and temperature are in the range 30–37 °C for 24–48 h. A method for enumeration of low *B. cereus* counts using MPN (most probable number) determination has been proposed (norm ISO 21871). Cells from colonies presenting the expected *B. cereus* colony aspect should be confirmed as *B. cereus* using confirmation tests. Several have been proposed and differ among national standards. FDA, as published in the Bacteriological Analytical Manual (BAM), proposes identification of those isolates as *B. cereus* that (1) produce large Gram-positive rods with spores that do

Table 1 Phylogenetic groups defined by Guinebretière et al. within *Bacillus cereus sensu lato* and some phenotypic characters

Phylogenetic group	*Current species*	*Association to foodborne poisonings*	*Growth temperature domain*	*Resistance to moist heat*	*Minimal pH, maximal NaCl for growth*
I	*B. pseudomycoides*	N	M	undetermined	4.6, 5%
II	*B. cereus* II *B. thuringiensis* II	M	P	++	<4.3, 8%
III	*B. cereus* III *B. thuringiensis* III *B. anthracis*	H	M	+++	4.6, 10%
IV	*B. cereus* IV *B. thuringiensis* IV	M	M	++	4.6, 10%
V	*B. cereus* V *B. thuringiensis* V	L	MP	++	4.6, 8%
VI	*B. weihenstephanensis* *B. mycoides* *B. thuringiensis* VI	L	P	+	4.6, 6%
VII	*B. cereus* VII or *B. cytotoxicus*	H	MT	+++	<4.3, >10%

Table 2 Main characters distinguishing genetically close species belonging to the *B. cereus* group (*B. cereus sensu lato*)

Species within B. cereus sensu lato	*Character*
B. anthracis	Presence of virulence genes carried by the two plasmids pXO1 and pXO2; nonhemolytic on sheep blood agar. The cause of anthrax in warm-blooded animals
B. thuringiensis	Produces a parasporal crystal consisted of insecticidal proteins. Crystal toxin genes are plasmid-borne
B. mycoides and *pseudomycoides*	Formation of rhizoid colonies
B. weihenstephanensis	Growth at 7 °C and no growth at 43 °C. Presence of certain DNA signatures

not swell the mother cell, (2) produce lecithinase and do not ferment mannitol on MYP agar, (3) grow and produce acid from glucose anaerobically, (4) reduce nitrate to nitrite (a few strains may be negative), (5) produce acetylmethylcarbinol (Voges–Proskauer-positive), (6) decompose L-tyrosine, and (7) grow in the presence of 0.001% lysozyme. The ISO 7932 norm includes aerobic production of acid from glucose, Voges–Proskauer test, and nitrate reduction. The routine French norm NF V08-058 only includes a motility test of presumptive strains and hemolytic activity on blood agar and is easier to perform. MYP or PEMBA is sold ready-to-use by several manufacturers of media and reagents.

Alternative methods are based on the enumeration of colonies on chromogenic medium. These media contain chromogenic substrates that are cleaved by specific enzymatic activities of microorganisms. For example, the chromogenic *Bacillus Cereus* agar (or Brilliance™ *Bacillus cereus* Agar, Oxoid, Basingstoke, UK) contains 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside that is cleaved by β-D-glucosidase and results in white colonies with a blue-green center. Confirmation tests may not be required using this chromogenic medium, as with Bacara™ (AES Chemunex, Bruz, France), and it is usually claimed that these media present a better selectivity that MYP or PEMBA. Some of these chromogenic media Bacara™ and COMPASS *Bacillus cereus* Agar (Biokar Diagnostics, Beauvais, France) have been validated according to ISO 16140 as alternative media for *B. cereus* enumeration in foods. Bacara™ is also recommended by the FDA in the BAM. Enzyme activities implicated in the chromogenic reactions maybe under the regulatory control of regulators showing some polymorphism and suspected to contribute to a typical growth. Some authors therefore suggest that these chromogenic media should still be considered with care.

In conclusion, there is a large panel of methods available for *B. cereus* culture and confirmation. Comparison performed with a common set of strains within the *B. cereus* group shows that each media has its own limitations: for instance, lecithinase activity may not always been detected on MYP or PEMBA, or chromogenic media may not support growth of some *B. cereus* group strains or give a false-negative reaction.

Detection of *B. cereus* Toxins

Several techniques are available for the detection of the cytotoxins Nhe (for nonhemolytic enterotoxin), Hbl (hemolysin BL), and CytK (cytotoxin K) and cereulide, the main virulence factors associated with the *B. cereus* foodborne poisonings. However, it is generally admitted that *B. cereus* diarrheic syndromes are caused by diarrheic toxin production in the small intestine. Diarrheic toxins are proteins sensitive to different proteases such as pronase, pepsin, trypsin, and chymotrypsin and therefore may be degraded by enzymatic activity during the gastrointestinal passage. Their presence in a food is not a reliable indication of a risk and these tests are more useful for characterization of the toxinogenic potential of the strains. Immunological tests specifically targeted to the main virulence factors have progressively replaced biological tests such as ileal rabbit loop or vascular permeability assay, although tissue culture represents alternative for screening: CHO cells, Caco-2 cells, or Vero cells have been used for screening activity of

B. cereus strains. In addition to antibodies developed for laboratory research purpose, commercial immunological tests are proposed for the detection of some of these toxins. The BCET-RPLA test kit (*Bacillus cereus* enterotoxin, reversed passive latex agglutination) (Oxoid, Basingstoke, UK) detects the L_2 component of the three-component toxin HBL and allows a semi-quantitative determination of toxin concentration in supernatants. The TECRA BDE-VIA (*Bacillus* diarrheal enterotoxin visual assay) (TECRA, Roseville, Australia) detects the NheA component of the three-component toxin Nhe. No commercial test is available for CytK detection. A lateral flow assay allows the detection of both HBL (more precisely of its B component) and NHE (the L2 component) with a single test strip (Duopath® *Cereus* enterotoxins assay, Merck KGaA, Darmstadt, Germany). In contrast to diarrheic toxins, the determination of emetic toxin concentration in a food is a good indication of the risk caused by the presence of emetic strains. As with diarrheal toxins, tests of biological activity have been developed such as culture assays of HEp2 cells, on which cereulide causes vacuolization or inhibition of motility of spermatozoids due to the mitochondrial activity of cereulide (sperm boar assay). These methods are not specifically detecting cereulide, but they are appropriate for screening of *B. cereus* isolates. Moreover, sperm boar assay was shown to correlate with liquid chromatography–mass spectrometry methods, which are developed by several laboratories. Although requiring laborious extraction procedures and requiring costly analytical equipment, these methods are getting the reference for cereulide assay, in particular in foods. No pure standard of cereulide is currently available and valinomycin, which shares with cereulide close common structure (both are cyclic dodecapeptide) and potassium transport properties, is generally used as surrogate standard for cereulide.

Molecular Methods of *B. cereus* Detection

B. cereus detection in current routine practices for microbiological analysis of food material is mostly performed with cultivation methods. Nevertheless, alternative methods using PCR amplification of *B. cereus* DNA sequences, increasingly including quantitative real-time PCR amplification, have been proposed and are continuously under evolution. When applied to foods, the sensitivity and reliability of molecular detection methods depend on the quality of the extracted DNA and protocols (of enrichment, separation, cell lysis, analyte concentration, etc.) must be examined with care. Developments for *B. cereus* detection have been favored by thorough genetic investigations and sequencing on strains of the *B. cereus* group. The genetic structure of the toxin operons and the sequencing of toxin genes *nhe*, *hbl*, *cytK*, and *ces* (cereulide synthetase gene) have been achieved by several research groups, and the genome sequence of nearly 200 strains of *B. cereus sensu lato* was available early 2013. These molecular detection methods therefore target a variety of *B. cereus*-specific sequences of varied genes: 16s RNA or *gyrB* genes (reported to have a better phylogenetic resolution among *Bacillus* spp.) present in all bacteria; or genes encoding for *B. cereus* virulence factors, such as hemolysin, phosphatidylcholine-specific phospholipase C gene (*pc-plc*), diarrheal toxins HBL, NHE, and CytK; or the cereulide synthetase gene (*ces*). In particular, they may allow detection of particular type of strains among the *B. cereus* group (for instance, the emetic strains) that may not be identified using plating methods. Worth noting is a high-molecular polymorphism of the enterotoxin genes *nhl*, *hbl*, and *cytK* and possibly also for the emetic strains that need also to be considered for PCR methods development. For instance, *B. cereus* strains may produce two forms of CytK toxin. Strains producing CytK-1 with a high cytotoxic activity and associated with highly pathogenic group VII strains can be discriminated from Cyt-K2 producing strains by a duplex PCR assays amplifying either *cytK1* or *cytK2* genes. In contrast, the prevalence of strains carrying *nhe* or *hbl* can be underestimated, and the characterization of PCR negative strains should be completed with techniques such as Southern hybridization. Because of its stability, detected DNA does not necessarily indicate living cells. Detection in samples subjected to an enrichment step generally implies the presence of living populations, which can also be quantified using RNA analysis. The addition of the DNA intercalating dye ethidium monoazide inhibits PCR of DNA from dead bacteria. In addition, the presence of toxin genes does not inform about the actual virulence of strains and complementary tests about actual toxin production or toxic activity needs to be performed. Current studies on *B. cereus* proteome or genome favored by high-throughput sequencing reveal new virulence factors and detail *B. cereus* diversity, which contributes to the identification of biomarkers differentiating between virulent and less hazardous strains. Integration of these biomarkers into new diagnostic tools such as microarrays is the likely future of *B. cereus* detection tools.

B. cereus Prevalence and Concentration in Foods

Quantitative Estimation

B. cereus prevalence and concentrations in foods is well documented and many surveys have been performed worldwide. *B. cereus* has been detected in a very wide range of foods and this is in agreement with the large diversity of foods associated with outbreaks of foodborne poisonings. Concentrations before storage is usually lower than 100 cfu g^{-1}. A noticeable exception is herbs and spices, which may contain concentrations greater than 10^3 cfu g^{-1}. A large survey performed in the United Kingdom showed that 0.08% of nearly 17 000 food samples exceeded 10^5 *B. cereus* cfu g^{-1}. Foods implicated in outbreaks of *B. cereus* food poisonings are usually contaminated at such (or greater) concentrations, which is therefore considered as critical.

Characterization of *B. cereus* Contaminating Foods

Surveys increasingly include some elements of characterization of the isolated strains, which is important for a better characterization of the risk due to food contamination. These include the ability to grow at low temperature, which is particularly relevant for foods intended to be stored at refrigeration temperatures. These surveys may also include information about the virulence profile of the isolated *B. cereus* strains. This is established by the detection of the main toxins (or of their genes). Some examples of such surveys are shown in Table 3.

Table 3 *B. cereus* prevalence and/or counts in foods with a phenotypic characterization of *B. cereus* in relation to its growth behavior and its ability to form toxins

Food (country and source)	*Number of samples*	B. cereus *prevalence and/or concentrations*	*Behavior of isolates at low temperature*	*Toxin and/or toxin genes*
Cooked dishes, fresh vegetables, fresh meat (Belgium, Samapundo et al.)	575	374 Positive samples[a]	2.6% Growing at ≤ 7 °C and 87.9% growing at 10 °C, $n = 380$	0% Harboring the *ces* gene; 52.5% harboring *hblA, hb D, hblC nheA nheB, nheC, and cytK*; $n = 80$
Oil(s) and fat(s), fish, meat, milk, vegetable(s) and their products, flavorings, ready-to-eat foods, pastry (The Netherlands, Wijnands et al.)	33 787	0.24% $> 10^5$ *B. cereus* cfu g^{-1}	4.4% of psychrotrophic strains[b], $n = 796$	97%, 66%, and 50% harboring the genes for NHE, HBL, and CytK; 8.2% positive for cereulide-like production
Fresh foods, heat-treated foods, and combinations (Danemark, Rosenquist et al.)	48 901	0.7% $> 10^3$ *B. cereus* cfu g^{-1}, 0.5% $> 10^5$ *B cereus* cfu g^{-1}		Tests on 40 isolates; 1 emetic strain, all NHE producers; 28 with visible parasporal crystal of *B. thuringiensis*
Dairy foods, cooked dishes, vegetable dishes, spices (The Netherlands, van Netten et al.)	1700	0.4% $> 10^3$ *B. cereus* cfu g^{-1}, 0.1% $> 10^5$ *B cereus* cfu g^{-1}	16% Growing at ≤ 7 °C; $n = 596$	37% of strains growing at 7 °C producing HBL; $n = 51$

[a]At least one presumptive *B. cereus* in 25 g.
[b]Based on the detection 16S ribosomal DNA signature.

The sequence of the *panC* gene allows a rapid affiliation to one of the phylogenetic groups defined in Table 1. An online tool has been developed, which is available at https://www.tools.symprevius.org/Bcereus/english.php. These have enhanced our understanding of the *B. cereus* risk in foods. *nhe* is present in almost any *B. cereus* strains. *hbl* genes are present in a vast majority of strains, but not in emetic strains. Emetic strains are relatively rare (only a few percents of all *B. cereus* isolates) but in much higher proportions in nonrandom food and clinical isolates. Psychrotrophic strains able to grow at 5 °C are also relatively rare, but strains able to grow at 10 °C are quite common. Foodborne poisonings by group III, IV, or VII strains, giving the most severe poisonings, will likely be a consequence of storage at abuse temperature because of the poor ability of these strains to grow at low temperature.

Other *Bacillus* spp.

The presence of *B. subtilis*, *B. licheniformis*, and *B. pumilus* in foods has been documented for a long time but to a much lesser extent than for *B. cereus*. Their incidence, as number and importance of outbreaks of foodborne poisonings, is much lower than the one of *B. cereus*. No selective media have been developed for those species. Their prevalence in the environment and in foods is established from strain isolates on nonselective media. Then strain isolates are identified using a panel of molecular methods, although sequence analysis of several genes predominates. These are 16S rRNA sequence genes, *gyrA*, *gyrB* (encoding gyrase subunits A and B), *cheA* (encoding a histidine kinase), *rpoB* (encoding a subunit of bacterial RNA polymerase), etc., as more than one method is necessary for the differentiation of these species that are genetically very close. Phenotypic tests, available, for instance, in miniaturized API tests (BioMérieux, Marcy-l'Etoile, France), can complement identification. Together, the data strongly suggest that the prevalence and concentrations in foods should be rather similar to that of *B. cereus*. Foods implicated in food poisonings contained at least *B. subtilis*, *B. licheniformis*, and *B. pumilus* 10^5 cfu g^{-1}. Symptoms of food poisonings include vomiting, nausea, diarrhea, and abdominal pain. Poisonings likely result of the ingestion of toxins formed in the foods, similarly to the emetic syndrome due to *B. cereus* emetic strains and cereulide. These toxins have been described as lipopeptides, produced from rare (a few %) toxic isolates. They have been identified by the fractionation of toxic culture extracts revealed by tests on cell culture and then identification using mass spectrometry.

See also: Chilled Foods: Principles; *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; *Clostridium*: Occurrence and Detection of *Clostridium perfringens*; *Clostridium botulinum*; Emerging Foodborne Enteric Bacterial Pathogens; Food Poisoning: Classification; Food Poisoning: Epidemiology; Food Poisoning: Tracing Origins and Testing; Foodborne Pathogens; Heat Treatment: Effect on Microbiological Changes and Shelf Life; Spoilage: Bacterial Spoilage.

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Bacillus: Occurrence

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Introduction

Bacilli are gram-positive, aerobic, spore-forming bacteria that are widely spread in nature. They are present not only in soil, dust, and water but also in plants, animals, and humans. The genus *Bacillus* contains a very large number of species since it has undergone a vast taxonomic expansion with the use of 16SrRNA sequencing. Some *Bacillus* species, namely, *B. cereus*, *B. clausii*, *B. coagulans*, *B. licheniformis*, *B. pumilus*, and *B. subtilis*, have been used as probiotics in commercial nutritional supplements. This has raised some concern because although most *Bacilli* are nonpathogenic, several species can cause disease in animals and humans by the production of various toxins. These pathogenic species will be the focus of the present article.

Seven closely related species of *Bacilli* are grouped under the taxon *Bacillus cereus sensu lato* (*s.l.*), which includes *B. cereus sensu stricto* (*s.s.*), *Bacillus thuringiensis*, *Bacillus anthracis*, *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus weihenstephanensis*, and the recently described *Bacillus cytotoxicus*. These taxa present a high genomic similarity and could have been considered as a single species. The *B. cereus* group members are globally defined by differences in plasmid encoded-features leading to different spectra of toxicity. While *B. anthracis* and *B. cereus s.s.* are pathogenic for humans and/or mammals, *B. thuringiensis* has been used as biopesticide thanks to its virulence toward insect larvae.

Considering the critical speciation of *B. cereus* group members and the variability in their phenotypic features, a new structure for *B. cereus* populations based on ecological differences and growth temperatures has been proposed. Seven major phylogenetic groups were suggested based on a polyphasic approach combining molecular typing (e.g., amplified fragment-length polymorphism), thermal growth spectra, and psychrotolerance. This new classification was structured in two psychrotolerant (II and VI) groups, three mesophilic (I, III, and IV) groups, one intermediate (V) group, and one moderate thermotolerant (VII) group. All groups contain more than one taxon (e.g., group II contains psychrotolerant *B. thuringiensis* and *B. cereus*; group III includes mesophilic *B. thuringiensis*, *B. cereus*, and *B. anthracis*: and group VI comprises psychrotolerant *B. thuringiensis*, *B. mycoides*, and *B. weihenstephanensis*) with the exception of group I (*B. pseudomycoides*) and group VII (CytK-1-producing *B. cereus*). The last group is constituted by highly toxic, moderate thermotolerant strains that are considered as a novel bacterial species and was consequently renamed *B. cytotoxicus*.

Bacillus anthracis

B. anthracis is the etiologic agent of anthrax, which affects all mammals, including humans. It was used as a bioterrorist weapon in 2001 in the United States under the form of bacterial anthrax powder. *B. anthracis* pathogenesis is elicited by the production of anthrax toxins and the elaboration of a protective capsule. *B. anthracis* is persistent in the environment in a state of dormant spores, which are able to survive for long periods in soil, in spite of adverse environmental conditions. If ingested, the spores can generate vegetative forms, which can grow inside the mammalian host and express various virulence factors.

There are three routes of contamination with *B. anthracis* leading to different types of anthrax: cutaneous (through lesion of the skin), gastrointestinal (through ingestion of contaminated food), and respiratory anthrax (through inhalation of contaminated air). All three forms of anthrax may result in a fatal outcome with highly variable mortality rates. The most benign anthrax is the cutaneous form, which is implicated in about 90% of all human cases with a mortality rate below 1%. It is treatable with appropriate antibiotics (e.g., penicillin and amoxicillin) if early diagnosed. Cutaneous anthrax is characterized by the apparition of a small papule that undergoes ulceration and evolves into a typical black eschar. The lesion, where the bacteria remain located, is accompanied by an edema.

The gastrointestinal anthrax is due to the ingestion of spores while eating contaminated meat, followed by the formation of the classical eschar in the gut system, germination of the spores resulting in multiplication of vegetative cells, and expression of both toxins and capsule. The pathological features reflect the actions of the different virulence factors and result in edema, vascular leakage, and bloody diarrhea. The outcome is often fatal due to the difficulty of posing a correct diagnosis.

The most severe and rapid form of anthrax is caused by the inhalation of spores and results in more than 95% of mortality if the disease is not treated early. The spores, 1–2 μm in diameter, are deposited in the alveolar spaces and are then transported into the lymph nodes where vegetative cells can proliferate and spread through the bloodstream. Large amounts of toxins are then produced and provoke acute symptoms. The illness is therefore characterized by two phases: the first prodromal stage during which mild flu-like symptoms appear and suddenly stop after 48 h to progress to the fulminant illness (second stage) exemplified by fever, tachypnea, cyanosis, tachycardia, moist rales, and evidence of pleural effusion that rapidly evolves to coma and death of the patient..

Bacillus cereus sensu stricto

B. cereus s.s. can be found in a wide variety of niches from soil to the intestines of healthy arthropods and healthy humans. The estimated prevalence of *B. cereus* in the intestinal tract of healthy human individuals ranges from 15% to 40% of the population. Nevertheless, soil is probably the main source of contamination as it has been shown to contain 10^5–10^6 of

spores of *B. cereus* per gram. Due to the strong resistance of its spores toward adverse environmental conditions and its ability to live in soil with low nutrient levels, *B. cereus* can contaminate a large variety of agricultural products. Hence, *B. cereus* can be isolated from a wide range of raw and processed foods, including pasta, rice (unhusked and white), dairy and dried milk products, dried foodstuffs, meat, chicken, vegetables, fruits, and seafood. Two major problems for the food industry are associated with *B. cereus*: food spoilage and human illnesses.

B. cereus s.s. is responsible for various types of non-gastrointestinal diseases, including systemic and local infections such as septicemia, bacteremia, endocarditis, pneumonia, pleuritis, periodontitis, osteomyelitis, meningitis, endophthalmitis, and eye infections. Although the toxins implicated in these cases are not precisely known, a large number of potential virulence factors may contribute to the pathogenesis of *B. cereus* such as hemolysins (cereolysin O, hemolysin II, and sphingomyelinase), phospholipases C, and proteases.

B. cereus also poses foodborne health risk to consumers through two types of gastrointestinal illnesses: emesis and diarrhea. The mechanisms involved in these two syndromes are radically different from each other. The scenario is rather simple for emesis since it results from the consumption of dishes contaminated with a single toxin, cereulide. The fact that the toxin is preformed in food leads to a very short incubation time of 1–5 h. On the opposite, the situation is still unclear for the diarrheal syndrome notably regarding the potential toxins responsible for the disease. It has been postulated that the disease is caused by the ingestion of *B. cereus* cells that grow and generate enterotoxins inside the human intestinal tract, which explains the longer incubation time of between 8 and 16 h. The infective doses of *B. cereus*, typically 10^3–10^5 of colony-forming units per gram, are difficult to assess because they depend on various factors including, for instance, the amounts of toxins produced or the susceptibility of target population. As for numerous other toxi-infections, it is recognized that the more severe forms of the illness are associated with young people and elderly people.

The emetic form of food poisoning was first identified in the 1970s and was associated with the consumption of fried rice. It was only in 1995 that the structure of the causative toxin was identified and named cereulide. The diarrheal type of *B. cereus* food poisoning was first described by Steinar Hauge in 1955, after an outbreak of gastroenteritis that occurred in a hospital and that was related to vanilla pudding contaminated with *B. cereus*.

Different enterotoxins are considered as potential candidates for causing the diarrheal syndrome: the enterotoxin hemolysin BL (HBL), the nonhemolytic enterotoxin (NHE), the necrotic cytotoxin K (CytK), and the enterotoxins FM (entFM) and T (BceT). However, the latter two have never been demonstrated to be directly involved in diarrheal food poisoning. The CytK enterotoxin has been discovered last, following a serious outbreak of gastroenteritis that caused the death of three people in France. Two different forms of CytK, CytK-1 and CytK-2, were subsequently described and CytK-1 displays a greater cytotoxic activity based on cellular tests performed on human intestinal cells. It should be noted that due to the lack of appropriate animal model, the actual contribution of these putative enterotoxins (alone or in combination) in the etiology of *B. cereus* diarrhea should be taken with great care. Further research is clearly needed to get more insights into this particular pathogenesis.

Bacillus weihenstephanensis

B. cereus s.l. strains, which are able to grow below 7 °C and up to 43 °C, are considered as psychrotolerant. They are mostly clustered within the *B. weihenstephanensis* group, notably based on their 16S and 23S rRNA and the presence of a particular variant of the cold shock protein A (*cspA*) gene. However, psychrotolerant strains other than *B. weihenstephanensis* are also present in the *B. cereus* group. Furthermore, some intermediate forms, considering the genetic and phenotypic features between the mesophilic strains and the psychrotolerant strains, may exist.

Both enteropathogenic and emetic *B. weihenstephanensis* have been reported, and these discoveries draw attention to a potential risk of food poisoning from psychrotolerant strains in cooked chilled foods. However, up to now, *B. weihenstephanensis* has not been implicated in food poisoning in spite of the presence of its virulence factors.

Bacillus cytotoxicus

Although several strains of *B. cytotoxicus* were isolated from purees associated with food poisonings, its natural habitat is still unknown. This new species shares high genetic proximity with the *B. cereus s.l.* group but displays distinct phenotypic features, notably its thermotolerant ability (minimal and maximal growth temperatures at 20 and 50 °C, respectively), its inability to hydrolyze starch, and its auxotrophy for tryptophan. As already indicated, the first thermotolerant *B. cereus*-like strain that was isolated (later designated as *B. cytotoxicus*) was related to a lethal outbreak in which three people died of necrotic enteritis. The CytK enterotoxin implicated in this outbreak was further characterized to be a CytK-1, which is a marker for *B. cytotoxicus*. At first, only five strains were clustered in this new genomic species, and three of them originated from France, one from Germany, and one from Norway. However, a recent study performed on the occurrence of *B. cytotoxicus* in potato products taken from retail outlets and from catering showed that the frequency of this thermotolerant species is not negligible and certainly higher than previously thought.

Bacillus thuringiensis

Thanks to its entomopathogenic virulence, *B. thuringiensis* has been extensively used in agriculture as a biopesticide to control insect pests and insect vectors of major human and animal diseases such as dengue, malaria, and yellow fever. The use of *B. thuringiensis* insecticidal toxins in transgenic crops was successful and beneficial, leading to higher yields and to significant reduction in the use of chemical insecticides, which were associated with environmental pollution and chronic human health issues. The crystal polypeptides, or δ-endotoxins,

produced by *B. thuringiensis* possess a highly specific activity against various species of insects belonging to six different taxonomic orders: *Lepidoptera*, *Diptera*, *Coleoptera*, *Hymenoptera*, *Hemiptera*, *and Blattaria*. Certain noninsect organisms such as nematodes, mites, and protozoa are also sensitive to *B. thuringiensis* entomotoxins.

The *B. thuringiensis* entomotoxins are considered as inoffensive for humans, vertebrates, and plants because (i) they are specifically activated in the intestinal tract of target insect larvae (alkaline pH and sensitivity to specific proteases) and (ii) they specifically bind to receptors only present in the intestines of insects. However, one needs to remain cautious since some *B. thuringiensis* strains have been involved in food poisonings and were detected in ready-to-eat foods and in fresh fruits and vegetables. Interestingly, some strains of *B. thuringiensis* are capable of producing potential diarrheal enterotoxins similar to those produced by *B. cereus s.s*. Although the number of food poisonings due to *B. thuringiensis* is extremely limited, the critical distinction between *B. cereus* group members with classical culture confirmation procedures may lead to underreporting.

Occurrence of *B. cereus* in the Environment and in the Food Chain

B. cereus is a ubiquitous soil microorganism and is commonly found in raw, dried, and processed foods. Due to the resistance of *B. cereus* spores to heat, radiation, disinfectants, and desiccation (see preceding texts) and their strong ability to adhere to surfaces, *B. cereus* is also a common contaminant of food production equipment. These characteristics enable the bacterium to contaminate all kind of foods.

Food safety is based on random food testing in order to confirm that the food meets certain control criteria. These criteria should be scientifically determined, for example, by a risk analysis approach, which includes several steps from hazard identification to risk characterization. Considering microbiological risk assessment, the *hazard* is the causative agent present in the food and the *risk* is the probability of adverse health conditions, as stated by the *Codex Alimentarius* Commission and the World Organisation for Animal Health. Total elimination of *B. cereus* contamination from food and food production is unfortunately impossible. Given that normal cooking procedures will make spores germinate and that a subsequent multiplication of vegetative cells and toxin production may occur under improper storage conditions, concern should be raised about practices of food storage in households and restaurants. Furthermore, the vegetative growth may occur over a broad range of temperatures, including refrigerator temperature. This raises concern about the safety of prepacked, ready-to-eat food with regard to *B. cereus*.

Foodborne Outbreaks

The total number of outbreaks caused by *Bacillus* spp. toxins within the EU has increased by 41.9% since 2006. Although not negligible, the accurate number of food poisonings caused by *B. cereus* is not known since it is not a reportable disease and it is not always diagnosed. Sporadic cases are more common than general outbreaks.

Diarrheic Syndrome: Prevalence

Reports on foodborne zoonotic diseases are annually published by the European Union in order to evaluate the impact of zoonosis in the food chain across Europe. Information on foodborne outbreaks is collected from EU member states and helps to compare the prevalence of zoonotic agents between countries although the diversity of conditions regarding the notification of outbreaks impairs this comparison. In these reports, bacterial toxins hold a significant place in the list of reported foodborne outbreaks although no distinction between toxins produced by species from the *Bacillus*, *Clostridium*, and *Staphylococcus* genera is made. In 2012, bacterial toxins were responsible for 14.5% of the total reported outbreaks and *B. cereus* alone involved 2022 human cases, 126 hospitalizations, and three fatalities. Unfortunately, no distinction between emetic and diarrheal types has been made in the reported outbreaks. Yet, the diarrheal type of food poisonings seems to be more frequently reported than the emetic type. Types of foods that are involved in diarrheal food poisoning are also more diverse (e.g., vegetables, meat, desserts, spices, and dairy products).

In Belgium, for instance, *B. cereus* outbreaks are reported every year. The majority of these outbreaks have been associated with the diarrheal syndrome (up to eight outbreaks in 2011), while only one to three emetic cases were reported on a yearly basis. In 2011, *B. cereus* was even the most frequently detected pathogen in reported foodborne outbreaks.

Emetic Syndrome: Cereulide Prevalence

Emetic strains of *B. cereus* have been shown to be very rare in the environment and several studies have determined this low frequency to be around 1–2%. Yet, several cases of emetic intoxications associated with the consumption of rice or pasta dishes have been described worldwide.

Cooked rice and its improper storage represent one of the major risk factors in emetic food poisoning. Indeed, rice is implicated in 95% of the emetic food poisoning cases. Furthermore, two prevalence studies performed in Asian restaurants revealed that *B. cereus* is frequently present (37.5% and 56%, respectively) in the served fried rice dishes. By directly detecting the toxin in rice dishes served in restaurants, a higher prevalence of cereulide was found than previously studied by targeting cereulide-producing strains, even though the toxin concentrations were relatively low (1–4 ng g^{-1} food), as to provoke acute symptoms.

A recent study showed a remarkable high prevalence of emetic strains in sunsik samples (powdered food prepared from grains, fruits, and vegetables) although only a low *B. cereus* contamination level of 10–200 CFU g^{-1} was found. A large-scale prevalence study performed on food samples from general food monitoring programs in Bavaria showed that the incidence of emetic strains is clearly underestimated. This study also indicated that the risk of emetic syndrome is not restricted to starchy foods. These new findings tend to reinforce the

relevance of the hypothesis that emetic *B. cereus* may be more prevalent, and possibly diverse, than previously thought.

Lethal and nonlethal outbreaks due to emetic *B. cereus* have been reported. Severe outcomes are generally observed for children, while adults who had consumed the same meal usually recovered. General outbreaks did also occur and affected up to 116 people. The toxin concentrations range implicated in food poisonings is wide, probably because the victims ate different portions sizes and/or because some victims were more subject to intoxications (e.g., children and elderly people). The total amount of toxin ingested ($\mu g\ kg^{-1}$ body weight) is an essential value to take into account in order to estimate the risk of intoxication since the emesis-inducing dose in humans is currently not known.

Fatal Cases

Although the diarrheal and the emetic syndromes caused by *B. cereus s.s.* are generally mild, some serious outbreaks leading to fulminant death of healthy people have recently put *B. cereus* at the forefront of lethal foodborne pathogens.

Five emetic outbreaks leading to fatal outcome have occurred worldwide and most of them were related to the consumption of pasta or rice. The victims were young (below 20 years old) and the death occurred within hours. Outbreaks due to diarrheal syndromes are more frequently described in the literature but fatal cases are very rare. One fatal outbreak occurred in 1998 in a nursing home for elderly people in France. Forty-four people became ill following the ingestion of contaminated vegetable puree, which led to the death of three people. In Belgium, a fatal case occurred in 2002 in a nursing home. Seventeen people became ill after the ingestion of contaminated minced meat, eleven of them were hospitalized, and two of them died.

Concern about *B. cereus* foodborne outbreak is still largely underestimated despite several fatal cases in recent times. The implication of cereulide in lethal case involving the death of a healthy 20-year-old man has proven that emetic food poisoning could be very brutal and that it does affect other target groups than only children or elderly people.

Conclusion

The genus *Bacillus* contains a large variety of spore-forming species that are widely distributed in nature. With the advance in molecular sequencing, the genus has undergone a profound reclassification and several groups have been defined. Although the majority of *Bacillus* members are nonpathogenic, some of them can cause diseases in humans and animals. Within the *B. cereus* group, species such as *B. anthracis* and pathotypes of *B. cereus* are well known as being pathogenic for humans.

In view of the widespread prevalence of *B. cereus* spp., the presence of its spores in food is inevitable. The high resistance of these spores to adverse conditions ensures that the spores can survive food processing and generate new vegetative cells when returned to favorable conditions.

Besides sporulation, another major issue posed by the pathotypes of *B. cereus* for food safety is their ability to produce toxins. Dietary exposure to toxin-producing *B. cereus* strains leads to gastrointestinal illnesses in humans. The toxin production may occur in food matrices (preformed toxin) or in the human gut (*in vivo* produced toxins) leading to different types of diseases.

Although underestimated, the number of foodborne outbreaks due to this organism is not negligible. Moreover, several lethal cases have been clearly attributed to *B. cereus*. Next to gastrointestinal diseases, *B. cereus* can also cause various clinical conditions notably in sensitive populations like immunocompromised individuals.

Besides *B. cereus*, which is the most prevalent *Bacillus* food poisoning organism, other *Bacillus* species are also raising concern as regards food poisoning or clinical diseases. In contrast, nonpathogenic strains (e.g., *Bacillus toyonensis* and *Bacillus subtilis* subsp. *subtilis*) are currently used as probiotics underlining the high diversity that exist inside the genus *Bacillus*. However, and more than ever, the toxigenicity of *Bacillus* strains seems a crucial issue that should be thoroughly evaluated before using a strain for commercial purpose (Table 1).

Table 1 Representative *Bacillus* species potentially involved in foodborne toxi-infections

Bacillus *species*	*Potential toxins*	*Comments*
B. cereus	Diarrheic pathotypes: enterotoxins Emetic pathotypes: cereulide	Fatal cases reported Inhibition of boar spermatozoa motility (cereulide activity) Some isolates also involved in local and systemic infections
B. weihenstephanensis (*B. cereus* group)	Emetic strains: cereulide	Toxin production observed No food poisoning case reported yet
B. circulans	Cytotoxins	Toxin production observed, but no food poisoning case reported yet Some isolates also involved in local and systemic infections
B. firmus	Cereulide-like toxins	Toxin production observed, but no food poisoning case reported yet
Lysinibacillus (*formerly Bacillus*) *fusiformis*	Cytotoxins	Toxin production observed, but no food poisoning case reported yet
B. lentus	Cytotoxins	Toxin production observed, but no food poisoning case reported yet
B. licheniformis	Lichenysins	Fatal cases reported (dairy products and industrially produced baby food) Some isolates also involved in local and systemic infections
B. megaterium	Cereulide-like toxins	Toxin production observed, but no food poisoning case reported yet

(*Continued*)

Table 1 (Continued)

Bacillus *species*	*Potential toxins*	*Comments*
B. mojavensis	Surfactin-like components, heat-stable amylolysin	Inhibition of boar spermatozoa motility Toxin production observed, but no food poisoning case reported yet
B. pumilus	Pumilacidins	Implicated in food poisoning Inhibition of boar spermatozoa motility Some isolates involved in local and systemic infections
B. simplex	Cereulide-like toxins	Toxin production observed, but no food poisoning case reported yet
B. subtilis	Surfactin, heat-stable amylolysin, heat-labile substances	Inhibition of boar spermatozoa motility Toxin production observed, but no food poisoning case reported yet

See also: *Bacillus cereus* and Other *Bacillus* sp. Causing Foodborne Poisonings, Detection of.

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Bacteriocins

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Introduction

Bacteriocins are a heterogeneous group of bioactive bacterial peptides or proteins, ribosomally synthesized, displaying antimicrobial activity against other bacteria. Bacteriocins involve peptides or proteins of variable biochemical properties, molecular weight, mechanism of action, spectrum of activity, location, and sequence of amino acids. They manifest antimicrobial activity directed against the same bacterial strains that produced them or against strains of closely related species. Synthesis of bacteriocins takes place under control of genes located in plasmid or chromosomal DNA, which – in parallel – contain genetic determinants of producer's resistance to the produced bacteriocin. Genes that code active protein and genes coding resistance to the protein, genes responsible for export of bacteriocin from the cell, and, occasionally, genes coding for enzymes involved in posttranslational modification of bacteriocins undergo expression in parallel. Bacteriocins are produced both by Gram-positive (*Lactobacillus*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Leuconostoc*, *Pediococcus*, and *Propionibacterium*) and by Gram-negative bacteria (*Escherichia coli*, *Shigella*, *Serratia*, *Klebsiella*, and *Pseudomonas*). Until now, in the open-access database of BACTIBASE, more than 200 bacteriocins were described (January, 2014). Interest in bacteriocins reflects potential application of the metabolites and bacteriocin-forming microbes in medicine and as natural food conserving agents.

Classification of Bacteriocins

Bacteriocins of Gram-Positive Bacteria

Bacteriocins produced by Gram-positive bacteria were classified for the first time by Klaenhammer in 1993. Classification of bacteriocins undergoes continuous alterations, linked to studies on their structure, amino acid sequence, and recognized mechanism of their action. In this article, classification of bacteriocins was presented, taking into account several characteristics including their molecular weight, manifestation of the YGNGVXC motif, the presence of disulfide bridges, activity toward bacteria of *Listeria* genus, and sensitivity to temperature. The proposed scheme of classification of bacteriocins produced by Gram-positive and Gram-negative bacteria is presented in Figure 1.

Class I encompasses lantibiotics or thermostable peptides of molecular weight below 5 kDa. They undergo posttranslational modification. They contain atypical amino acids, such as lanthionine (Lan), methyllanthionine (MeLan), dehydroalanine (Dha), dehydrobutyrine (Dhb), and D-alanine (D-Ala). Lantibiotics were divided into two groups: lantibiotics of type A and of type B, manifesting distinct structural and functional properties. Type A encompasses linear peptides of a positive charge, acting by permeabilization of the cytoplasmic membrane in sensitive cells, while lantibiotics of type B include globular molecules, carrying negative charge or chargeless, of a variable manner of action. The best recognized bacteriocin of class I is nisin, produced by certain strains of *Lactococcus lactis*.

Class II nonlantibiotic bacteriocins, which in their composition contain no lanthionine, represent thermostable bacteriocins of molecular weight below 10 kDa. Both bacteriocins of class I and class II are secreted by ATP-binding cassette transporter proteins. Bacteriocins of class II are produced as prepeptides, which generally contain a leader sequence consisting of 14–30 amino acids, with a conserved processing site, and which do not undergo extensive posttranslational modification. Their spectrum of targets include Gram-positive bacteria with low content of G + C pairs, lactic acid bacteria (LAB), and bacteria of *Listeria*, *Clostridium*, or *Enterococcus* genera. Differences in structure of bacteriocins permitted to distinguish their four subclasses.

The subclass IIa includes pediocin-like bacteriocins with strong activity against bacteria of *Listeria* genus. They carry a hydrophilic cationic region with a conserved YGNGVXC motif and two cysteines linked by a disulfide bridge. They manifest high homology of sequence (38–80% identity of amino acid sequences), particularly in the N-terminal region of their molecules. On the other hand, the C-terminal region is more hydrophobic and differentiated.

The subclass of IIb encompasses dipeptide bacteriocins. They contain a single disulfide bridge, may contain the N-terminal sequence of YGNGVXC, or may be devoid of it. Their example involves lactococcin G from *Lactococcus lactis*, lactacin F from *Lactobacillus johnsonii*, and plantaricin F from *Lactobacillus plantarum*.

The subclass of IIc encompasses cyclic peptides, which possesses the N-terminal and C-terminal regions covalently linked. They contain a single disulfide bridge, but they carry no N-terminal sequence of YGNGVXC. Enterocin AS-48 is the prototype of this group.

The subclass of IId encompasses bacteriocins that are distinct in their structure, secretion mechanism, and manner of action from bacteriocins classified in subgroups of IIa–IIc. Their example involves enterocins L50 (EntL50A and EntL50B), produced by *Enterococcus faecium* L50. The subclass IId contains also bacteriocins activated by thiol groups, such as lactococcin B. In some classifications, subclass IId is rejected.

Class III includes bacteriocins of molecular weight above 30 kDa. They are thermolabile and they are produced mainly by Gram-positive bacteria. These bacteriocins can generally be subdivided into bacteriolysins (e.g., lysostaphin produced by *Staphylococcus aureus* and enterolysin A produced by *Enterococcus faecalis*) and nonlytic antimicrobial proteins (e.g., helveticin J, produced by *Lactobacillus helveticus*).

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00053-2

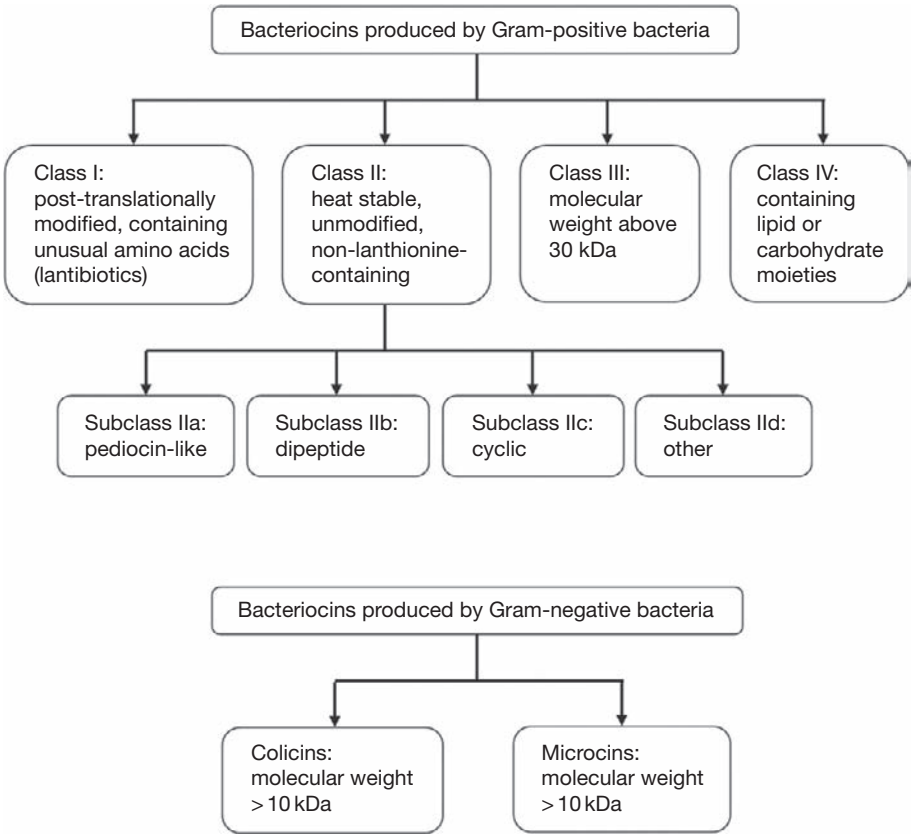

Figure 1 Proposed scheme of classification of bacteriocins produced by Gram-positive and Gram-negative bacteria.

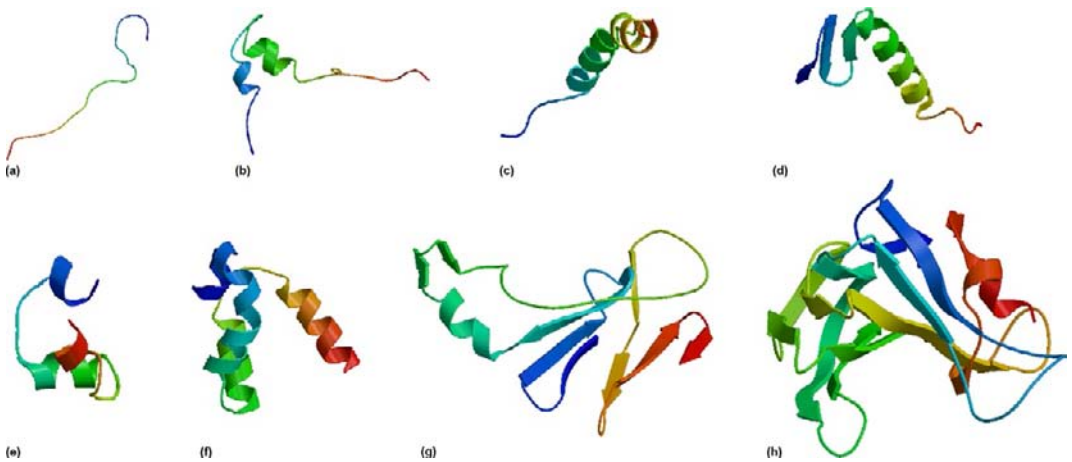

Figure 2 Third-order structures of selected bacteriocins (the models produced using SWISS-MODEL, http://swissmodel.expasy.org, 2014). (a) nisin A; (b) glycocin F; (c) plantaricin F; (d) pediocin PA-1; (e) salivaricin A; (f) enterocin 96; (g) helveticin J; (h) enterolysin A.

Class IV includes bacteriocins, which for full activity require the presence of a lipid or carbohydrate moieties in their molecule.

Third-order structures of selected bacteriocins are presented in Figure 2. Principal characters of selected bacteriocins of Gram-positive bacteria are presented in Table 1.

Bacteriocins of Gram-Negative Bacteria

Bacteriocins produced by Gram-negative bacteria into the environment, which reduce competition from other bacterial strains, are colicins and microcins. Spectrum of activity manifested by bacteriocins of Gram-negative bacteria is more

Table 1 Basic characteristics of selected bacteriocins of Gram-positive and Gram-negative bacteria

Classes of bacteriocins		*Bacteriocin (producer strain)*	*Activity against bacteria*
Gram-positive bacteria	Class I	Nisin A (*Lactococcus lactis subsp. lactis*)	*Enterococcus sp., Lactobacillus sp., Lactococcus sp., Leuconostoc sp., Listeria sp., Staphylococcus sp., Micrococcus sp., Pediococcus sp., Mycobacterium sp., Clostridium sp., Bacillus sp.*
		Mutacin B-Ny266 *(Streptococcus mutans)*	*Gram-positive bacteria*
		Salivaricin A (*Streptococcus salivarius)*	*Gram-positive bacteria*
	Class II Subclass IIa	Enterocin A *(Enterococcus faecium)*	*Enterococcus sp.*, *Lactobacillus sp.*, *Lactococcus sp.*, *Bacillus sp.*, *Listeria sp.*, *Pediococcus sp.*
		Mesentericin Y105 (*Leuconostoc mesenteroides)*	*Lactobacillus sp., Leuconostoc sp., Pediococcus sp., Listeria sp.*
		Pediocin PA-1 *(Pediococcus acidilactici)*	*Pediococcus sp.*, *Lactobacillus sp.*, *Leuconostoc sp.*, *Listeria sp.*, *Bacillus sp.*, *Enterococcus sp.*, *Staphylococcus sp.*
	Class II Subclass IIb	Lactacin F (*Lactobacillus johnsonii)*	*Lactobacillus sp., Enterococcus sp.*
		Lactocin-705 (*Lactobacillus paracasei)*	*Lactobacillus sp.*, *Listeria sp.*, *Streptococcus sp.*
		Plantaricin F (*Lactobacillus plantarum)*	*Lactobacillus sp.*, *Pediococcus sp.*
	Class II Subclass IIc	Carnobacteriocin A *(Carnobacterium piscicola)*	*Carnobacterium sp., Enterococcus sp., Listeria sp., Clostridium sp.*
		Enterocin AS-48 (*Enterococcus faecalis)*	*Bacillus sp.*, *Micrococcus sp.*, *Staphylococcus sp.*, *Enterococcus sp.*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella* Typhimurium, *Shigella sonnei*, *Pseudomonas sp.*
		Enterocin 96 *(Enterococcus faecalis)*	*Enterococcus sp.*, *Lactobacillus sp.*, *Lactococcus sp.*, *Leuconostoc sp.*, *Bacillus sp.*, *Listeria sp.*, *Staphylococcus sp.*, *Salmonella* Typhimurium, *Klebsiella pneumoniae*, *Serratia liquefaciens*, *Proteus vulgaris*, *Enterobacter cloacae*, *Escherichia coli*
	Class II Subclass IId	Enterocin L50A *(Enterococcus faecium)*	*Clostridium sp.*, *Propionibacterium sp.*, *Listeria sp.*, *Lactobacillus sp.*, *Enterococcus sp.*, *Pediococcus sp.*
	Class III	Helveticin J (*Lactobacillus helveticus)*	*Lactobacillus bulgaricus*, *Lactococcus lactis*
		Enterolysin A *(Enterococcus faecalis)*	*Lactobacillus sp.*, *Lactococcus sp.*, *Pediococcus sp.*, *Enterococcus sp.*, *Listeria sp.*, *Bacillus sp.*, *Staphylococcus sp.*, *Propionibacterium sp.*
	Class IV	Glycocin F (*Lactobacillus plantarum)*	*Lactobacillus sp., Streptococcus sp., Enterococcus sp., Bacillus sp.*
Gram-negative bacteria	Colicins	Colicin E2 *(Escherichia coli)*	*Enterobacteriaceae*
		Colicin U (*Shigella boydii)*	
	Microcins	Microcin B17 (*Escherichia coli)*	
		Microcin E492 *(Klebsiella pneumoniae)*	

Jones, E., Salin, V., Williams, G. W. (2005). Nisin and the market for commercial bacteriocins. TAMRC Consumer and Product Research Report No. CP-01-05, July 2005; Franz, C. M. A. P., van Belkum, M. J., Holzapfel, W. H., Abriouel, H., Galvez, A. (2007). Diversity of enterococcal bacteriocins and their grouping in a new classification scheme. *FEMS Microbiology Review* 31, 293–310; Karpiński, T. M., Szkaradkiewicz, A. K. (2013). Characteristic of bacteriocines and their application. *Polish Journal of Microbiology* 62(3), 223–235.

narrow than that of bacteriocins produced by Gram-positive bacteria. Principal characters of selected bacteriocins produced by Gram-negative bacteria are presented in **Table 1**.

The most extensive group is formed by colicins. They are synthesized by over half of *E. coli* strains and also by *Yersinia pestis* (pesticins) and *Serratia marcescens* (marcescins) and by bacteria of *Shigella* genus, *Klebsiella* (klebicins), and *Pseudomonas* (pyocins). Colicins represent large proteins, manifesting molecular weight of 25–80 kDa. As compared to bacteriocins of Gram-positive bacteria, colicins manifest several differences in organization of gene clusters and operons responsible for their synthesis. Also control of their biosynthesis is distinct. All of them are coded by ColE1 plasmids. They exhibit antimicrobial activity targeted at closely related bacterial strains, which carry colicin-binding receptor on cell surface and produce no resistance proteins, capable of inactivating colicins. Their antimicrobial activity involves the formation of ion channels in cell membrane, which results in depolarization of the membrane. Synthesis and export of colicins are lethal for the producer's cell and result in its lysis.

Microcins, the low-molecular-weight peptides, manifesting thermostability and a hydrophobic character, form a separate

class of bacteriocins produced by Gram-negative bacteria. Their activity is mainly directed against bacteria of closely related strains. Microcins are synthesized by *E. coli* bacteria and by a single strain of *Klebsiella pneumoniae*. In view of their mechanism of action and structure and due to genetic criteria, two classes of microcins are distinguished. The first class includes peptides of molecular weight <5 kDa, which undergo posttranslational modifications and which attack mainly intracellular structures. The other class encompasses peptides of molecular weight ranging between 7 and 10 kDa, which do not undergo posttranslational modifications and which lead to damage and destruction of cell membrane of target cells. In contrast to colicins, synthesis of microcins is not lethal for the producer.

Mechanisms of Bacteriocins Action

The mode of action of bacteriocins can be bactericidal or bacteriostatic, determining death or extension of log phase, respectively. At present, at least four types of antibacterial activity of bacteriocins are distinguished, including

- bactericidal activity due to the formation of pores in cell membrane,
- inhibition of cell wall components biosynthesis,
- effect on activity of autolytic enzymes,
- inhibition of bacterial spores' development.

Overview of the mode of action of bacteriocins is presented in Figure 3.

Most of bacteriocins exert a bactericidal effect on sensitive cells frequently inducing the formation of pores in cell membrane. Currently, a few hypothetical models of bacteriocins activity are known. The so-called wedge model is thought to be the most probable, formed on grounds of the sequence and type of interactions between the model lantibiotic, nisin, and the surface of cell membrane of target cells. The model takes into account characteristic properties of type A lantibiotics, first of all positive charge and elasticity of the peptide molecule. The first contact of bacteriocin with a sensitive cell takes place due to electrostatic interactions between a positively charged, hydrophobic bacteriocin molecule and negatively charged phospholipids in cytoplasmic membrane of a sensitive cell. Penetration of peptides in between the double phospholipid layer induces local destruction of the ordered bilayer structure. Nisin enters into reaction together with the membranous peptide glycate precursor, termed lipid II, representing an anchoring molecule and allowing to bind lantibiotics to cell membrane of sensitive bacteria. The mechanism of bacteriocins action involves formation in the cytoplasmic membrane of sensitive bacteria of a transient pore and ion channel complexes. This is accompanied by a passive outflow of small molecules, such as ions of potassium, magnesium, and phosphorus, amino acids, and ATP. In this manner, a disturbance develops in membrane potential and pH gradient and function of the proton pump becomes inhibited. The low level of ATP and ion deficit in the cell results in the inhibition of DNA, RNA, protein, and polysaccharide synthesis. This finally leads to death of the bacterial cell.

Bacteriocins belonging to class II permeabilize the membrane due to pore formation, using the carpet or the barrel stave mechanism. In the carpet model, individual peptides arrange in parallel to the membrane surface and interact with it with no aggregate formation. A temporary derangement of the membrane bilayer structure develops, which results in a local and transient perforation. In the barrel stave model, hydrophilic parts of amphipathic α-helical peptides form pores in the cell membrane. The outer hydrophobic parts of α-helices interact with chains of fatty acids that form phospholipids. In this binding, elements of mannose phosphotransferase (membrane receptors) play a mediating role. Such a type of interaction was demonstrated, that is, in class IIa bacteriocins of LAB. Lethal activity of the bacteriocins reflects a disturbed ionic equilibrium and loss of inorganic phosphates, escaping from the cells through the formed pores. Also, intracellular levels of ATP become drastically reduced, due to increased consumption of ATP, required for maintenance of electric potential, and due to inability to accumulate ATP due to leakage of phosphates from the cell.

In the case of bacteriocins belonging to IIb class, interactions, in line with the carpet model, demonstrate strongly ion-specific properties. Pores induced by lactococcin G are permeable for, that is, cations Na^+, K^+, and choline; those induced by plantaricin E/F are permeable for, that is, Na^+, K^+, H^+, and choline; and those induced by lactacin F are permeable for K^+ and phosphates. Cell death ensues in particular due to loss of potassium ions, accompanied by hydrolysis of ATP by ATPases. The peptides may independently bind to cell membrane, but for pore formation, a synergistic effect is required, resulting from the interaction of two complementary peptides of a dipeptide bacteriocin.

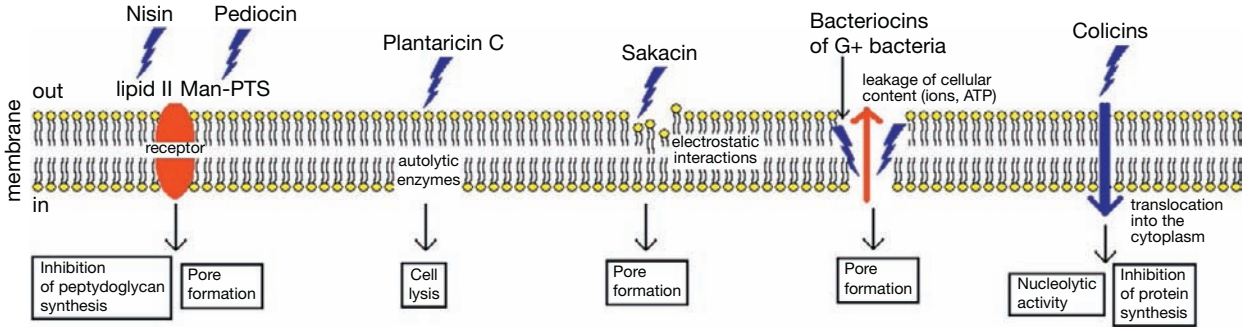

Figure 3 Overview of the mode of action of bacteriocins.

Another mechanism of bacteriocin activity involves their ability to induce lysis of bacterial cells. The process is linked to the interaction of bacteriocins with teichoic, lipoteichoic, and teichuronic acids, representing components of cell membrane. This results in a release and activation of autolytic enzymes, linked to the acids, which results in cell autolysis. Such an action takes place in the case of plantaricin C, produced by *Lactobacillus plantarum* LL441, which induces complete lysis of *L. delbrueckii* subsp. bulgaricus LMG 13551 strain cells. Bacteriocins belonging to lantibiotics class may additionally disturb processes of cell wall biosynthesis. They inhibit synthesis of peptidoglycan at the transglycosylation stage, which is not accompanied by a disturbed biosynthesis of DNA, RNA, or protein. The lantibiotic mersacidin inhibits peptidoglycan synthesis through a specific interaction with the peptidoglycan precursor, lipid II. The sequestering of lipid II prevents its utilization by the transpeptidase and transglycosylase enzymes that install the cross-linked network of the bacterial cell wall. Mersacidin appears to bind to a different portion of lipid II than vancomycin does, indicating that this bacteriocin may prove to have important chemotherapeutic applications.

The mechanism of cell-targeted activity manifested by bacteriocins produced by Gram-negative bacteria includes the depolarization of cell membranes (e.g., colicin E1), damage to DNA (e.g., colicin E2, acting as a nonspecific endonuclease), or inhibition of protein synthesis by inactivation of ribosomal RNA (colicin E3 and cloacin DF13). The bactericidal activity of colicins involves the formation of ion channels in the cytoplasmic membrane of sensitive cells, which leads to depolarization of the membrane also; in the cellular wall of the target bacteria, degradation of peptidoglycan or inhibition of its synthesis may take place. All colicins are coded in Col plasmids. The group of colicin genes consists of a gene coding the toxic protein, resistance-coding gene, and, in most of colicins, the gene coding for a protein facilitating colicin export from the cell and inducing lysis of the cell. Synthesis of colicins may be induced using UV rays or mitomycin C. Colicin E1 is coded by a set of genes representing 'colicin cassette' in ColE1 plasmid. The produced colicin is lethal for the host since its release to the environment results in cell lysis, for which product of *kil* gene is responsible. In normal conditions, the entire system is located in a plasmid in a repressed condition, which results from blocking of the principal promoter (Pcol) by the cellular protein of LexA. Cells, which at a given moment do not produce colicins even if they contain the ColE1 plasmid, are protected from action of exogenous colicin due to a function of *imm* gene product, while the related bacteria containing no such a plasmid become eliminated from the environment. All situations resulting in the destruction of the repressor (LexA protein) and, thus, inducing the cellular SOS system mobilize in parallel colicin production by the entire population of bacteria carrying the plasmid. In standard conditions, synthesis of colicins is switched off in majority of cells and becomes mobilized in stress conditions. In contrast to colicins, synthesis of microcins is not lethal for their producers and is not controlled by the SOS system. Microcins are secreted to medium in the late logarithmic phase of growth, except for microcin Mcc E492, which is produced in the early logarithmic phase.

Medical Application of Bacteriocins

At present, studies are ongoing on the application of bacteriocins in food industry and in medicine. Bacteria of the *Lactobacillus* genus, which produce bacteriocins, are frequently employed in probiotic preparations. The strains of *Lactobacillus* with confirmed probiotic properties belong to the species of *L. bulgaricus*, *L. acidophilus*, *L. casei*, *L. helveticus*, *L. lactis*, *L. salivarius*, and *L. plantarum*. The rods of *Lactobacillus* produce an unfavorable environment for pathogenic bacteria, producing pH-lowering compounds in the alimentary tract and inhibiting growth of the neighboring bacteria. The main duty of probiotic bacteria involves maintenance of microbiological equilibrium in alimentary tract through interactions with pathogenic bacteria. In addition, probiotic preparations manifest an antineoplastic activity.

Inhibiting growth of several pathogenic microbes, probiotic strains reduce incidence of traveler's diarrhea, alleviate the course, and shorten the duration of some bacterial and viral diarrheas (e.g., those induced by *Clostridium difficile*, *Shigella*, *Salmonella*, and enterotoxic strains of *Escherichia coli* or rotaviruses), prevent manifestation, or relieve course of post-antibiotic diarrheas.

To date, studies suggest that selected probiotics exert an antagonistic effect against *Helicobacter pylori*, may lead to their elimination, reduce representation, and/or alleviate inflammatory condition. The experiments indicate differences in antimicrobial effects of *Lactobacillus* genus bacteria. The strain of *L. acidophilus* LB proved to be more active against *H. pylori* than the strain of *L. acidophilus* GG, while the strain of *L. johnsonii* LA1 was found to be more active than the strain of *L. johnsonii* LA10. The bacteriocins with the strongest antibacterial activity against *H. pylori* strains are thought to include lacticins A164 and BH5, produced by *Lactococcus lactis* A164 and *L. lactis* BH5, and bacteriocins produced by *Lactobacillus johnsonii* LA1, *L. casei* YIT 9029, and *L. amylovorus* DCE 471. The studies point also to favorable effects of probiotics in various intestinal inflammatory conditions. It is suggested that just a decreased number of *Lactobacillus* and *Bifidobacterium bacilli* within the alimentary tract may lead to the development of such inflammatory conditions in the intestines.

At present, it is known that bacteria of *Lactobacillus* genus manifest also antagonistic effects toward periodontopathogens, such as *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, and *Porphyromonas gingivalis*. On the other hand, the presence of H_2O_2-producing strains of *Lactobacillus* in periodontal pockets prevents against the development of chronic periodontitis.

Probiotic strains of *Lactobacillus* genus manifest also high ability of adherence to cells of vaginal epithelium and of urinary pathways, forming a layer that exerts a protective effect against colonization by pathogenic microbes. Due to this ability, probiotics and strains of *Lactobacillus rhamnosus*, *L. fermentum*, *L. reuteri* and various species of *Bifidobacterium* in particular proved to be useful in prophylaxis and treatment of infections in urogenital tract.

Mersacidin has been shown to be very effective in treating systemic staphylococcal infections and in eliminating nasal carriage of *Staphylococcus aureus* in a mouse rhinitis model, whereas cinnamycin exerts an inhibitory effect on phospholipase A2,

the enzyme active in synthesis of prostaglandins and leukotrienes in the human immune system. Its action takes place by the sequestration of its substrate, phosphatidylethanolamine. Due to this activity, cinnamycin may prove to have a useful application as an anti-inflammatory and antiallergic drug.

Microcins produced by various *E. coli* strains play a significant role in preventing chicken infections with *Salmonella* bacteria since microcins are capable of inhibiting growth of pathogenic *Salmonella* strains. Colicins and microcins are also used in infections with *E. coli* O157:H7. Microcins and colicins manifest their activity against strains producing shiga toxin and also against other *E. coli* strains of serotype O. The use of colicin- and microcin-producing bacteria as probiotics may markedly reduce pathogen levels in cattle alimentary tract and in this way prevent against infection with pathogenic strains. Potential medical application of selected bacteriocins is presented in Table 2.

Application of Bacteriocins in Food Preservation

In recent years, high interest has been shown regarding the potential for extending food durability using compounds produced by microbes, including bacteriocins. The substances exhibit a number of attractive properties: They are tasteless, they manifest no odor or color, and they easily penetrate into structure of food products. In contrast to chemical preserving agents, they are fully safe for human body. However, before bacteriocins can play a role of biopreserving agents used at an industrial scale, detailed investigations have to be completed on the compounds and they have to be legally accepted as food supplements.

Three typical applications of bacteriocins for the biopreservation of food include

(1) the addition of purified bacteriocins to food products;
(2) the inoculation of a food product with LAB, which will manufacture bacteriocin in the product itself;
(3) the use of an ingredient in food processing that has been previously fermented with a bacteriocin-producing bacterial strains.

Since bacteriocins are isolated from foods such as meat and dairy products, which normally contain LAB, they have unknowingly been consumed for centuries. LAB include Gram-positive cocci and rods belonging to genera of *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Streptococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Carnobacterium*, *Enterococcus*, *Tetragenococcus*, *Vagococcus*, *and Weissella*. LAB play a defining role in the preservation and microbial safety of fermented foods, thus promoting the microbial stability of the final products of fermentation. Protection of foods is due to the production of organic acids (e.g., lactic acid and acetic acid), carbon dioxide, ethanol, hydrogen peroxide, and diacetyl; antifungal compounds such as fatty acids and phenyllactic acid; bacteriocins; antibiotics such as reutericyclin; and aroma compounds. LAB are broadly used for the production of fermented food and

Table 2 Medical application of selected bacteriocins

Disease/infection:	*Bacteriocin (producer strain)*
Bovine mastitis	Nisin A (*Lactococcus lactis* subsp. lactis)
	Uberolysin (*Streptococcus uberis* 42)
Human mastitis	Nisin A (*Lactococcus lactis* subsp. lactis)
Multidrug-resistant strains of bacteria	Nisin F (*Lactococcus lactis* subsp. lactis)
	Mutacin B-Ny266 (*Streptococcus mutans* ATCC 202022)
	Lacticin 3147 (*Lactococcus lactis* DPC 3147)
	Pumilicin 4 (*Bacillus pumilus* WAPB4)
	Warnericin NK (*Staphylococcus warneri* NK)
	Bacillocin B602 (*Paenibacillus polymyxa* NRRL B-30509)
	E50–52 (*Enterococcus faecium* NRRL B-30746)
Cutaneous diseases	Nisin F (*Lactococcus lactis* subsp. lactis)
	Gallidermin (*Staphylococcus gallinarum* Tu3928)
	Epidermicin N101 (*Staphylococcus epidermidis* 224)
	Enterocin 96 (*Enterococcus faecalis* WHE96)
	Hiracin JM79 (*Enterococcus hirae* DCH5)
	Plantaricin MG (*Lactobacillus plantarum* KLDS1.0391)
Oral caries	Mutacin 1140 (*Streptococcus mutans*)
Periodontal diseases	Subtilosin A (*Bacillus amyloliquefaciens*)
Gastrointestinal diseases	Avicin A (*Enterococcus avium*)
	Piscicolin 126 and Carnobacteriocin BM1 (*Carnobacterium maltaromaticum* UAL307)
	Pediocin PA-1 (*Pediococcus acidilactici* UL5)
	Enterocin CRL35 (*Enterococcus mundtii* CRL35)
	E50–52 (*Enterococcus faecium* NRRL B-30746)
	L-1077 (*Lactobacillus salivarius* NRRL B-50053)
	Microcin C7, colicin 1b, and colicin E1 (*Escherichia coli* H22)
	Microcin J25 (*Escherichia coli*)
Bacterial vaginosis	Lactocin 160 (*Lactobacillus rhamnosus* 160)
	L23 (*Lactobacillus fermentum* L23)

Nishie, M., Nagao, J. -I., Sonomoto, K. (2012). Antibacterial peptides 'bacteriocins': an overview of their diverse characteristics and applications. *Biocontrol Science* 17(1), 1–16; Hammami, R., Fernandez, B., Lacroix, C., Fliss, I. (2013). Anti-infective properties of bacteriocins: an update. *Cellular and Molecular Life Sciences* 70(16), 2947–2967.

dairy products, such as milk, kefirs, yogurts, and cheeses, yielding a specific taste and aroma to the products. Considering the typical association of LAB with food fermentation and also long tradition as food-grade bacteria, LAB have been 'generally recognized as safe.' Due to production of bacteriocins, they protect food, exerting an inhibitory effect on other microorganisms, including pathogenic microbes.

In food preservation, also microcins and colicins can be used. Microcins produced by various strains of *E. coli* play a significant role in the prevention against infections with *Salmonella* in chicken. Moreover, *E. coli* cells are capable of surviving and produce microcins in condition of alimentary product deficits, in acidic environment, and in the presence of bile and proteolytic enzymes. Colicins and microcins can be used also in combating infections with the enterohemorrhagic strain of *E. coli* O157:H7, the reservoir of which includes cattle. The strain produces shiga toxin, particularly dangerous for children. In cases of this strain, antibiotic therapy induces an even more intense secretion of shiga toxin, in this way increasing bacterial virulence. Microcins and colicins manifest their activity against strains producing shiga toxin and against other *E. coli* strains of O serotype, linked with human diseases. Therefore, the addition of colicin- and microcin-producing bacterial cultures as probiotics may significantly reduce levels of pathogens in alimentary tract of the cattle and in this way may prevent against infections with strains pathogenic in humans.

Another significant pathogen that can contaminate food is *Listeria monocytogenes*. Activity against *L. monocytogenes* is manifested by, for example, *L. lactis* DPC4275 strain, producing lactacin 3147, used in the production of a mold cheese. The development of *L. monocytogenes* bacteria is effectively inhibited also by nisin in combination with other antibacterial substances, for example, with pediocin, or in combination with appropriate processing technologies.

Nisin is the most known and commercially used bacteriocin. It is produced by *Lactococcus lactis* and belongs to the group of lantibiotics A, manifesting an elongated shape and affecting sensitive cells by permeabilization of their cytoplasmic membrane. Its activity encompasses a wide range of strains, such as *Lactococcus*, *Enterococcus*, *Staphylococcus*, *Micrococcus*, *Pediococcus*, *Lactobacillus*, *Listeria*, and *Mycobacterium*, as well as spores and vegetative forms of *Bacillus* and *Clostridium*. Ingested nisin is inactivated by trypsin and pancreatin and will have no effect on the gut microflora. In the absence of other preservation methods, nisin does not inhibit Gram-negative bacteria, yeasts, or molds. Consequently, nisin is often used in combination with other synergistic preservation methods (known as hurdle technology), such as low pH and high salt concentrations. Nisin has a double mode of antimicrobial action, binding to lipid II and subsequent inhibition of cell wall synthesis and forming pores in the cytoplasmic membrane. Activity of lysine can be altered under effect of physiological conditions, such as pH, ionic strength, temperature, and growth phase of target cells. Upon exposure to nisin, sensitive cells manifested disturbances in synthesis of DNA, RNA, proteins, and polysaccharides.

Important considerations for the use of nisin as a food preservative are its solubility and stability. Nisin is most stable in acid conditions and is soluble in aqueous environments. A solubility of 12% at pH 2.5 has been reported, decreasing to 4% at pH 5.0. Solubility at neutral pH is very low. It was found to be completely stable on heating to 115.6 °C when the pH value was 2.0 but lost 40% of activity at pH 5.0 and more than 90% at pH 6.8. Large protein molecules, for example, milk proteins, provide a protective effect for nisin when heated. Nisin is insensitive to food pasteurization or tyndallization, which markedly increases sensitivity of bacterial spores to nisin action. As a preservation agent, it is used mainly in products such as the following:

- Maturing cheeses, in which nisin prevents against growth of spores produced by *Clostridium tyrobutyricum*.
- Milk, particularly due to prevention against growth of thermophile bacteria spores, which are able to survive a prolonged pasteurization.
- Canned food, in which lysine kills first of all spores formed by thermophile bacteria of *Bacillus stearothermophilus* and *Clostridium thermosaccharolyticum* species.
- Meat, in the case of which nisin allows to reduce contents of nitrates. In addition, the application of nisin in combination with other antibacterial agents, such as pediocin, or with appropriate processing technologies effectively inhibits the development of bacteria of *Listeria monocytogenes* species. Supplementation with nisin allows also to control processes of alcohol fermentation and to reduce infections with lactic fermentation bacteria, such as *Lactobacillus* and *Pediococcus*. Current application of nisin in food preservation is presented in **Table 3**.

Commercial nisin preparations are made by fermentation of enzymatically digested skimmed milk with added yeast extract by nisin-producing strains of *L. lactis* subsp. lactis. The fermentation is maintained at pH 6–7 by the addition of alkali. Synthesis of nisin starts in the early exponential phase of growth of the bacterium, reaches maximum toward the end of this phase, and ceases when entering the stationary phase. In order to achieve prolonged production, continuous cultures are used. The nisin is concentrated either by a foam extraction or by a membrane filtration process.

Nisin is approved for use in over 40 countries and has been in use as a food preservative for over 50 years. For many years, the most commercially available form of nisin for food preservative uses involved Nisaplin™ (Danisco, DuPont), which contains 2.5% nisin active ingredient, 77.5% NaCl (salt), and nonfat dry milk comprising 12% protein and 6% carbohydrate. Nisin (E 234) is authorized for food preservation in the European Union by Directive 95/2/EC on food additives other than colors and sweeteners. Nisin is currently permitted in semolina and tapioca puddings and similar products (3 mg kg^{-1}), ripened and processed cheese (12.5 mg kg^{-1}), and mascarpone cheese (10 mg kg^{-1}). Specifications for nisin are laid down in Directive 96/77/EC. Studies showed that nisin is safe for human consumption at an acceptable daily intake (ADI) of 2.9 mg/person/day. The panel on food additives, flavorings, processing aids, and materials in contact with food (AFC Panel) issued an opinion on 26 January 2006, on the use of nisin as a food additive, which confirmed the ADI of 0.13 mg kg^{-1} body weight established by the Scientific Committee on Food in 1990. The Joint Expert Committee on Food Additives of the UN Food and Agriculture Organization and the World Health Organization recommended daily intake limits of 60 mg of pure nisin for a 70 kg person. However,

Table 3 Current application of nisin in food preservation

Food type	*Typical target organisms*	*Level of nisin (mg kg^{-1} or mg l^{-1})*
Dairy products (milk and cheese)	*Clostridium* spp. *Bacillus* spp. *Listeria monocytogenes*	0.25–15
Meat (ham, pork, beef, and chicken)	*Salmonella* Typhimurium *Escherichia coli* O157:H7 *Brochothrix thermosphacta* *L. monocytogenes* Lactic acid bacteria	1–25
Seafood (fishes, crabs, and lobsters)	*L. monocytogenes* *C. botulinum*	1–25
Pasteurized soups	*B. cereus* *C. pasteurianum*	2.5–6.25
Canned foods	*C. botulinum* *C. thermosaccharolyticum*	2.5–5
Dipping sauces and salad dressings	Lactic acid bacteria	1.25–6.25
Beer, wine, alcohol	Lactic acid bacteria	0.25–37.5

Cleveland, J., Montville, T. J., Nes, I. F., Chikindas, M. L. (2001). Bacteriocins: safe, natural antimicrobials for food preservation. *International Journal of Food Microbiology* 71, 1–20; Delves-Broughton, J. (2005). Nisin as a food preservative. *Food Australia* 57 (12), 525–527; Jones, E., Salin, V., Williams, G. W. (2005). Nisin and the market for commercial bacteriocins. TAMRC Consumer and Product Research Report No. CP-01-05, July 2005.

there is no maximum limit to the use of nisin in processed cheese in Australia, France, or Great Britain. In the United States, the maximum dose limits for use are 200 mg kg^{-1} in canned and plant protein foods and 500 mg kg^{-1} in dairy and meat products; the more typical dose is 100–200 mg kg^{-1}. In Australia and New Zealand, nisin is allowed in cream products at a maximum of 10 mg kg^{-1}; in crumpets, flapjacks, and pikelets (hot plate flour products) at a maximum of 250 mg kg^{-1}; and in cheese and cheese products, dairy desserts, oil emulsions (<80% oil), tomato products, liquid egg products, beer and related products, dips, sauces, mayonnaises, and salad dressings at levels compliant with good manufacturing practice.

Aside from nisin, technological significance is manifested also by pediocin, bavarian, piscicolin, jensenin, curvaticin, lacticin, and sacacin. It is regarded safe to use in food products of bacteriocins synthesized by strains of lactic fermentation bacteria such as *Lactococcus* sp., *Lactobacillus* sp., *Pediococcus* sp., *Carnobacterium* sp., and *Leuconostoc* sp. An increasingly significant commercial importance is manifested also by another bacteriocin, pediocin PA-1, which can be purchased for use as food additive as 'ALTA 2341.'

Numerous studies are devoted also toward the application of bacteriocins as antibacterial agents in food packs. A nisin-coated low-density polyethylene foil was found to inhibit growth of *Micrococcus luteus* ATTC 1240 in raw and pasteurized milk, while cellulose and polyethylene–polyamide bags containing nisin and lacticin 3147 were found to reduce the development of LAB, *Listeria innocua* and *Staphylococcus aureus* in sliced cheese and ham. The studies involved also food packs based on paper, cardboard, and edible covers.

Conclusions

Bacteriocins as peptides of antibacterial properties manifest high significance for preservation of homeostasis between bacteria. They find application in food industry and in medicine. Bacteriocins produced by LAB have been recognized to be fully safe for humans. Nisin is commercially the most important bacteriocin used in food preservation. At present, bacteriocins and probiotic preparations provide an alternative for antibiotics, used as a supplementation in human food and animal feeding.

See also: Cheese: Chemistry and Microbiology; Fermented Foods: Origins and Applications; Foodborne Pathogens; Lactic Acid Bacteria; *Listeria*: Listeriosis; Meat: Eating Quality and Preservation; Preservation of Foods; Probiotics.

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Bananas and Plantains

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Introduction

Among the tropical fruit crops that are consumed at large, bananas and plantains stand foremost not only because of the quantity produced but also in terms of serving the calorific needs of millions of people especially across Africa and Asia. The term 'bananas' is used to broadly denote the dessert forms and certain cooking types, while 'plantains' represent a group of starchy bananas that are often cooked, fried, or processed. Besides fruits, every other part of the plant has been known to possess commercial or medicinal value, and hence in Sanskrit language, it is described as 'Kalpatharu' meaning 'virtuous plant.' In India, it not only is a food crop but also is completely associated and forms a part in many religious functions and social ceremonies.

Origin and Domestication

Bananas and plantains have originated mainly from Southeast Asian regions with predominant diversity spread across Malaysia, Indonesia, and India and with secondary centers of diversity in West and Central Africa (plantain subgroup) and the highlands (Lujugira subgroup) of East Africa. Archaeological and phytolithic evidences suggest early domestication of wild *Musa* sp. probably for its fibers in Papua New Guinea about 7000 years ago. Over the past several millennia, anthropic migrations, natural mutations, and human selection have favored the banana to be one of the most cultivated and favored fruits across the tropics and subtropics of the world.

Domesticated over thousands of years, most of the present-day seedless (parthenocarpic) cultivated edible forms have arisen because of the contribution from two major wild progenitor diploid seeded species *Musa acuminata* and *Musa balbisiana* that provided the A and B genomes. Cultivar names are often described to denote the ploidy and genomic status. For example, *Musa* 'French plantain' AAB indicates that it is a triploid with a double genomic constitution from *Musa acuminata* and one set of genomic contribution from *M. balbisiana*. Recent cytological techniques, viz., genomic *in situ* hybridization and fluorescence *in situ* hybridization coupled with molecular markers, and quantification of nuclear DNA content using flow cytometry, have paved the way for establishing the ploidy status, parental lineage, and genomic nature of varieties with a high degree of certainty. Recent molecular findings also suggest the contribution of *Musa schizocarpa* (S genome) and *Musa textilis* (T genome) in the evolution of certain varieties.

Botany

Botanically, the bananas belong to the monocotyledonous family Musaceae (Zingiberales) under the revised section *Musa* and with the genus *Musa* consisting 33 species, while some more are believed yet to be discovered from wild forest regions spread across continental Asia. The plants are tall and herbaceous, and the true banana stem is an underground 'corm' from which the leaves are pushed up and the basal leaf sheaths encircle one over the other to form the aboveground 'pseudostem.' The leaves are simple, large, and arranged in circular phyllotaxy. The inflorescence shoots up through the central channel of the pseudostem. The root system is adventitious arising from the corm, and most of the roots are present within a depth of 45 cm beneath the soil. The inflorescence is borne on a long peduncled complex spadix shooting through the pseudostem at the terminal region having an overlapping spathe or bracts in succession, each of which subtends biseriately arranged cymose flower cluster. With the onset of anthesis, the bract subtending the flowers lifts up exposing the flowers. The flowers are zygomorphic, morphologically bisexual, but functionally unisexual, with the basal series functioning as female followed by neuter flowers and the distal ones or terminal portion bearing male forms. Perianth is made of six tepals of which one inner tepal is free while the others connate together. Androecium is made of five fertile stamens and a staminode positioned opposite the free tepal. The gynoecium consists of a single-compound pistil of three carpels, a single style, and an inferior ovary with three locules, with ovules in axile placentation. Abortion of ovules occurs commonly in cultivated varieties during fruit development leading to parthenocarpy. The fruit is a berry, with exocarp (peel) that loosens away from the flesh upon ripening.

Cultivars

Majority of the cultivated varieties are triploids while some are diploids (e.g., 'Ney Poovan' (AB), 'Matti' (AA), and 'Surya Kadali' (AA)). Several synthetically bred tetraploids (e.g., FHIA varieties) are also presently cultivated but in a very limited scale. While there are thousands of varieties available, not all of them are commercially exploited for catering to the larger market or export trade, but are still nurtured in backyards and tribal villages for their distinct appeal, taste, or potential food and nonfood or medicinal uses in many parts of Asia and Africa. Regional preferences and adaptability determine the varieties that are commercially exploited, and hence, 'polyclonal' system of cultivation is prevalent in many parts of Asia, as against the monoculture of Cavendish or plantain cultivars exclusively as plantations in many nations for export trade and domestic consumption. Some of the important cultivars commercially grown are listed in Table 1.

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Table 1 Major cultivars of banana and plantains

Cultivar groups and genomic status	*Usage and regions of cultivation*
'Pisang Mas' ('Sucrier'), 'Pisang Ambon Putih' (AA genome)	Mainly for dessert Malaysia, Indonesia
'Ney Poovan' and 'Kunnan' (AB genome)	Dessert India
'Cavendish' subgroup (AAA genome)	Dessert: 'Giant Cavendish' ('Mons Mori'), 'Dwarf Cavendish,' 'Williams' and 'Grand Naine,' 'Robusta,' 'Poyo,' 'Lacatan' In many countries
Red group (AAA genome)	'Red Banana,' 'Green Red'
East African Highland banana (AAA genome)	Cooking and beer: 'Lujugira' Acidic dessert: 'Yangambi Km 5'
'Gros Michel subgroup' (AAA genome)	Dessert: 'Gros Michel,' 'Highgate,' and 'Cocos' (both mutants of 'Gros Michel') In many countries
'Iholena' subgroup (AAB genome)	Cooking Pacific region
'Maoli-Popoulu' subgroup (AAB genome)	Cooking Pacific
'Mysore' subgroup (AAB genome)	Dessert: 'Mysore' ('Poovan') India, Malaysia, Thailand, Indonesia
Plantain subgroup (AAB genome)	Dessert, cooking, processing: 'French plantain'('Nendran'), 'Horn plantain,' 'False Horn plantain' Predominantly in Africa, in India, and in many other countries
'Pome' subgroup (AAB genome)	Dessert: Hill banana, 'Lady Finger,' 'Prata Ana,' South America, Asia, Australia
Silk subgroup (AAB genome)	Dessert: 'Rasthali,' 'Martaman' India, Bangladesh, South America, Asia, Australia
'Pisang Awak' (ABB genome)	Dessert: 'Karpooravalli' Malaysia, Indonesia, India
'Bluggoe' (ABB genome)	Cooking: 'Monthan', 'Ashy Monthan' Malaysia, Indonesia, India, and other Southeast Asian regions
'Saba' (ABB genome)	Cooking

Table 2 Status of banana production across the world

Geographic region	*Area (ha)*	*Production (tons)*
Africa	1 493 224	15 863 068
America	1 223 055	27 111 707
Asia	2 130 692	57 094 628
Europe	10 465	399 940
Oceania	95 879	1 523 400
Total	4 953 315	101 992 743

Source: FAOSTAT 2014: http://faostat3.fao.org/browse/Q/QC/E

Status of Production

During 2012, according to the estimates of Food and Agriculture Organization, banana cultivation was spread in more than 130 countries across the world in about 4.95 million ha with an overall production of 101.99 million metric tons. More than 50% of the production areas are in India, Brazil, the Philippines, the United Republic of Tanzania, and China. About 60% of African production occurs in Uganda and its neighboring countries, viz., Tanzania, Cameroon, Rwanda, Kenya, Côte d'Ivoire, Congo, and Burundi. The distribution of banana production across the different geographic regions is furnished in Table 2.

Nearly 84% of the global produce is utilized for domestic consumption, and the volume of global gross banana exports is estimated to be 16.5 million tons in 2012. Latin America and the Caribbean countries apart from Ecuador accounted for nearly 79% of the total exports. The contribution from Asia was about 17% with major contribution from the Philippines, while Africa contributed to about 3.66% of the global exports. The major countries that export bananas are Costa Rica, Ecuador, Mexico, Columbia, Honduras, Côte d'Ivoire, Cameroon, and the Philippines. The major importers are the United States (27%), the European Union (27%), Russia (8%), and Japan (7%).

Consumption and Economic Uses

Bananas and plantains are ideally suited for many systems of cultivation, be it large extensive corporate farms covering thousands of hectares or small holder farms with resource poor owners or as a backyard crop in urban settings and are part of many intercropping systems. In many mixed farming systems and multitier system of cultivation, bananas are grown along with coffee, pepper, and oranges in parts of India providing necessary shade to the crops and additional income to the growers.

While dessert bananas are sweet tasting and eaten raw, plantains and cooking banana pulp are boiled, fried, boiled and pounded, roasted, and consumed with cereals, other vegetables, and miscellaneous food complements in many West and Central African countries. Plantains are a major part of their diet for more than 70 million people in West and Central Africa, providing each with at least 200 cal a day. In Uganda, Burundi, and Rwanda, banana per capita

consumption is estimated to be as high as 250–400 kg per year. There is also a specialty group of bananas called as 'beer bananas' from which the juice extracted from the ripe fruits is fermented to produce low-alcoholic drinks in parts of Africa.

Similarly, in parts of Asia, several starchy cooking bananas (ABB) apart from plantains serve as complimenting culinary delight to meals after boiling or roasting. Some of the varieties conserved by tribal groups of Western Ghats of India and northeastern states serve as energetic weaning food. 'Matooke,' an East African Highland cooking banana, is known for staple food preparation by the people in the Great Lakes region especially Uganda. They are harvested from three-quarters to full maturity, peeled, and boiled or steamed in banana leaves for consumption. Banana flour and powder made from green mature bananas are often added as ingredient in bread preparation, in pastries, or as thickening of sauces and soups.

Nutritional Constituents, Bioavailability, and Health Benefits

As an easily available and consumable form, banana fruits are appreciated for their high calorific and mineral contents. The nutritional information from USDA database representing a medium-sized banana is furnished in Table 3.

Carbohydrates and Calorific Value

Bananas and plantains are rich in carbohydrate and poor in fat content and hence widely consumed as low-fat diets. Processed bananas such as chips roasted in edible oil enhance its acceptance and energy value. The large amounts of starch present in the unripe fruits are converted to sugars upon ripening and contribute to the sweetness. The carbohydrates are made of predominantly glucose, fructose, and sucrose. There are also a portion of carbohydrates, which are known as resistant starch and nonstarch polysaccharides, which have low glycemic index or low digestibility.

Table 3 Proximate composition of banana fruit (100 g)

Contents	*Availability*
Water	74.91 g
Energy	89 kcal
Protein	1.09 g
Total fat	0.33 g
Carbohydrate	22.84 g
Total dietary fiber	2.60 g

Vitamins		*Minerals*	
Contents	*Availability*	*Contents*	*Availability*
Vitamin C, total ascorbic acid	8.7 mg	Calcium	5 mg
Thiamin	0.031 mg	Iron	0.26 mg
Riboflavin	0.073 mg	Magnesium (Mg)	27 mg
Niacin	0.665 mg	Phosphorus (P)	22 mg
Pantothenic acid	0.334 mg	Potassium (K)	358 mg
Vitamin B_6	0.367 mg	Sodium (Na)	1 mg
Folate, total	20 μg	Zinc (Zn)	0.15 mg
Carotene, beta	26 μg	Copper (Cu)	0.078 mg
Vitamin A	64 IU	Manganese (Mn)	0.27 mg
Niacin	0.665 mg	Selenium (Se)	1 mg
Vitamin B_6	0.367 mg	Fluoride (F)	2.2 mg

Source: USDA National Nutrient Database for Standard Reference 27 Software v.2.1.5

Proteins and Fats

Bananas are rich neither in proteins nor in fats as evidenced by their very constitutive presence. While there is a slight increase in protein concentration from unripe to ripe stage, the levels of lipids remain almost constant during ripening. The lipid present in the peel is rich in polyunsaturated fatty acids, particularly linoleic acid and linolenic acid. Protein bioavailability is limited in raw 'matooke,' and hence, cooked and extruded matooke flours are suggested to enhance energy efficiency and, for serving with proteinaceous food, to improve the nutritional needs of malnourished children.

Vitamins

The pulp is rich in vitamins A, B, and C. Dopamine and vitamin C contents in bananas act as antioxidants. Among the vitamins, the presence of high amounts of carotenes in Melanesian cultivars is noteworthy (0.20–28 nmol g^{-1} banana). 'Uht en Yap' has been reported to contain 6110 μg of α-carotene/100 g, as compared to 26 μg in Cavendish. Some of the Micronesian varieties having high carotene have the potential to serve as genetic resources for future breeding efforts to improve nutritional qualities of banana. Considerable amounts of the resistant starch present in bananas may limit the bioavailability of carotenoids, but studies have revealed that thermal processing improves the retinol bioefficacy of bananas. The lutein content (carotenoid) levels are more in ripened fruit and lutein helps as an antioxidant. The orange yellow or orange pulp color may serve as an indicator for carotene content.

Minerals

Bananas are known for its high potassium content in the pulp. Besides magnesium, calcium, and phosphorus are also present. Ripe fruits of *Musa* 'Bhimkol' occurring in northeastern India could meet 100% RDA of potassium, zinc, manganese, and selenium and several essential amino acids with 100 g of fresh pulp for a six-month-old infant.

Organic Acids

Citric, malic, and oxalic acids are the major organic acids in banana, and their levels normally increase during ripening. Ascorbic acid, β-carotene, citric acid, and malic acid present in ripe fruits may contribute to the flavor of banana-based fruit juices and other finished products.

Flavonoids and Other Bioactive Compounds

Several bioactive amines such as dopamine, noradrenaline, octopamine, histamine, 2-phenylethylamine, and tyramine have been reported in banana. The pulp of banana also contains many flavonoids. Leucocyanidin present in unripe banana has shown protective effect against aspirin-induced erosions. Gallocatechin, epicatechin, and condensed tannins present in banana pulp albeit in trace quantities may serve as antioxidants. Flavonoids extracted from unripe fruits have been attributed with significant hypolipidemic activities.

Health Benefits of Banana

The information on beneficial aspects on health by bananas and plantains is provided here to briefly narrate the tested and attributed benefits of banana and its products. Bananas are the cheapest and easily consumable source of carbohydrates, minerals, and vitamins and known to boost health in many ways. Bananas can serve the dietary needs substantially at any life stage, be it young active children or toddlers, young adults, adults, pregnant mothers, lactating mothers, middle-aged population, or the very aged people. Almost all of the parts of the banana plant are known to influence health. The potential of bananas as emollient, demulcent, and antiscorbutic has been known for long. The fruits, peel, leaves, roots, and pseudostem of banana have been also described to have antiulcerogenic, antioxidant, antimicrobial, antidiabetic, antihyperglycemic, and hypoglycemic properties.

Reduction on serum glucose levels, improved insulin secretion and glycogen storage, and inhibition of enzyme activity related to glucose absorption have been experimentally proved to corroborate the beneficial effects on the regulation of glucose homeostasis by banana leaves. Flowers are astringent and are used in dysentery, menorrhagia, and diabetes; green bananas are antidiarrheal and used as a curative for intestinal disorders. Root is antibilious and anthelmintic in folk medicines; young leaves are used to cover burns on the skin as cool compress; the astringent ashes of the unripe peel and of the leaves have antidiarrheal and antidysenteric properties. Banana stem is employed as diuretic and helps detoxify the body. It is commonly cooked and eaten to prevent or treat kidney stones possibly by regulating calcium uptake by the presence of potassium. Stem juice of fruited plant is used for treating diarrhea, dysentery, cholera, otalgia, and hemoptysis. The plant is also used in inflammation, pain, and snakebite.

The high calorific value of the fruit and the mineral nutrients make it a favorite fruit among sports personnel especially athletes and tennis players to maintain fluid balance and avoid muscle cramps. The low-salt content and high potassium presence help to avoid high blood pressure and improve the function of the heart muscles. Banana-based energizing drinks and dried banana bars for athletes are now available in the market.

Consumption of fresh banana helps to neutralize excessive acidity in the stomach and is a boon for ulcer patients. Vitamin B_6 present in banana influences the production of neurotransmitters including serotonin and the production of hemoglobin and insulin. Serotonin is implicated with mood elevation and a general sense of well-being. It also helps decrease homocystine (a causative factor in coronary artery disease and stroke episodes) levels within the body. Banana contains small quantities of sitosterol, campesterol, and stigmasterol, which are structurally similar to cholesterol and hence block the absorption of dietary cholesterol and reduce blood cholesterol levels. Further, along with ascorbic acid, it also serves to boost general immunity. The high pectin content in the fruit may help to absorb more water and assist in bowel movement. Apart from apples and oranges, banana fruits have been ascribed to have the potential to protect neurons against oxidative stress-induced neurotoxicity and in combating onset of neurodegenerative Alzheimer's disease.

Banana flour made from green bananas is an attractive alternative to wheat flour especially for those who suffer from celiac disease. Green banana flour is rich in type 2 residual starch, which is not digested in the small intestine but is acted upon by bacteria in the colon resulting in the breakdown of products into short-chain fatty acids and contributing to lowering colon inflammation and factors associated with colon cancer. The dietary fiber present in banana could favorably affect the intestinal health by contributing to increased fecal bulk, shortened colonic transit time, changes in the composition of the gut microbial population, lowered gastrointestinal pH, and changed bile acid profiles. In a recent study taken up at the University of Michigan Medical School, a type of lectin isolated from banana known as BanLec has been reported to be potentially active against HIV by binding naturally to the sugar-rich envelope that encases the HIV virus and blocking its entry into the body.

Allergy to Banana

A very low fraction of the population has been diagnosed with allergic reactions to banana. The allergic symptoms, such as stomach cramps, diarrhea, vomiting, runny nose, watery eyes, headaches, itching, sneezing, and wheezing, are displayed. Individuals allergic to chitinase also are very often reported to be allergic to bananas. The banana allergy is known to be a part of the latex-fruit allergy syndrome. Hypersensitivity to bananas is considered a type 1 allergy with very quick reaction within minutes after exposure to specific allergen. In severe allergic reaction, it may lead to anaphylaxis. Consumption of bananas is also not advisable with alcohol as it may aggravate migraine headache.

Cultivation Aspects

Bananas and plantains are vegetatively propagated from corms with a portion of the stem of the side suckers having sword-shaped narrow leaves. Large-scale production of Cavendish and to a certain extent plantains is now facilitated by tissue culture plants initiated from virus- and disease-free high-yielding mother plants. The plantation density generally varies between 1500 and 2500 plants/ha, and in certain intensive systems, even up to 5000 plants/ha are accommodated. Banana requires more of nitrogen and potassium than phosphorus apart from micronutrients. The dosage rates may vary depending on the cultivar and location. Fertilizer application is carried out in such a way to replenish the soil nutrient levels removed during the growth and to optimally maintain the

physiological health of the plant. Critical leaf nutrient norms for different varieties in different locations are available. The critical content range for leaf N may vary from 1.67% to 3.43%, phosphorus from 0.12% to 0.20%, potassium from 2.20 to 4.40, calcium from 0.40 to 1.40, and magnesium from 0.3% to 0.6%. Irrigation levels are decided by soil water potential and evapotranspiration rates. Drip fertigation systems are advantageously adopted in banana production systems to schedule the nutrient and water requirements depending on the crop stage so as to maximize yield and quality of the produce. Rain-fed production system is in vogue where the rainfall is well distributed throughout the year.

Bananas are also grown in green houses in Israel, Turkey, Morocco, and the Canary Islands. 'Grand Naine' and 'Dwarf Cavendish' are largely grown inside green houses. Periodical leaf removal is necessary to overcome excessive shade in green houses. Removal of side suckers until the plant shoots the inflorescence, periodical removal of dried leaf sheath, and removal of a portion of the inflorescence after last hand opening on the inflorescence are common practices followed. As banana plants are easily prone to wind injury, propping using bamboo or casuarina poles or by providing support with polyester or nylon ropes is important to avoid lodging. Periodical earthing up and weed management during the early phase of the crop until canopy coverage and mulching along the interspaces ensure efficient nutrient and water utilization. Bunch covering using polythene or polypropylene sleeves with 2–4% ventilation is regularly employed to protect the developing banana fingers against cold, bruise injury, and insect damage so as to obtain 'blemishless' fruits.

Under ideal growing and disease-free conditions, banana plantations are ratooned by allowing one of the suckers to follow the main plant for yielding and six to eight crop cycles are possible in such commercial plantings and with spatial arrangements that facilitate mechanization. However, the incidence of fusarium wilt or nematodes may force the grower to plant only a single cycle of crop production. Several pests and diseases affect banana production. Among the diseases, black leaf streak disease or black sigatoka (*Mycosphaerella fijiensis*), yellow sigatoka (*M. musicola*), fusarium wilt (*Fusarium oxysporum* f.sp. *cubense*), bacterial head rot (*Erwinia carotovora*), bacterial wilt (*Xanthomonas campestris*), Moko wilt (*Ralstonia solanacearum*), and viral diseases such as banana bunchy top virus (BBTV), banana streak virus, banana bract mosaic virus, and infectious chlorosis caused by cucumber mosaic virus are important and require timely attention. Among the major pests of banana, corm weevil (*Cosmopolites sordidus*), pseudostem borer (*Odoiporus longicollis*), and banana Aphid (*Pentalonia nigronervosa*) – a known vector for the transmission of the dreaded BBTV – pose threat to banana production. Different nematodes such as burrowing nematode (*Radopholus similis*), root lesion nematode (*Pratylenchus coffeae*), root-knot nematode (*Meloidogyne incognita*), and spiral nematode (*Helicotylenchus multicinctus*) can cause considerable yield loss if not properly managed.

Integrated production practices involving the use of organics; the addition of biofertilizers such as arbuscular mycorrhizae, *Azotobacter*, phosphobacteria, and biocontrol agents such as *Pseudomonas fluorescens*; crop rotations; cover cropping with legumes and sun hemp; and the use of nematode deterrent crops such as marigold and coriander are all currently recommended to maintain soil and plant health status. Organic production of bananas are followed by adopting internationally acceptable compliant norms with due certification from organizations authorized in each country. Bunches are harvested at 70–75% maturity (three-quarters round stage of fingers) for export market and at 85–90% maturity (near-round stage) for local trade. Under proper cultural conditions, a yield level of 80–100 t ha^{-1} from 'Grand Naine' is achievable. Productivity of cooking bananas and plantain may range between 40 and 60 t ha^{-1}.

Banana Processing and Processed Products

Although the majority of the produce is consumed fresh, the scope for processing of bananas and plantains is huge especially to reduce the postharvest losses or to meet the export standards when the produce fails. The extent of postharvest losses is reported to be in the tune of 30–40% in different countries. Several preharvest factors such as nutrient deficiencies and incidence of diseases like black and yellow Sigatoka in the main field (*Mycosphaerella* sp.) and postharvest factors such as improper harvesting methods, poor handling, packaging and transport methods, storage temperature and anthracnose incidence during storage, poor marketing facilities, unorganized marketing structure, and lack of market intelligence contribute to severe postharvest losses. Given the total volume of 101.99 million tons of production across the globe at present, even if a moderate postharvest loss of 15% is considered, then the postharvest losses accounts to nearly 15.3 million tons, which is nearly equal to the volume of bananas produced by the African countries. Processing methods offer the scope for waste reduction and waste utilization.

There are several processed banana products that are being consumed and traded all over the world. Apart from traditional uses for food preparation in parts of Asia and Africa, banana 'figs' produced by sun-drying of ripe bananas, banana powder, banana puree or mashed bananas, banana flakes or chips or crisp fries with edible oils, banana-based alcoholic and non-alcoholic beverages, etc., are some of the food products processed from bananas. There is a growing demand for processed and semiprocessed food, and even the large banana exporters have now diversified into this growing market. The guidelines and procedures used for processing of bananas are outlined here. The quality and outcome of the product so processed depend on the raw produce and varieties involved and may require adjustment of processing parameters.

Banana Chips or Banana Crisps

Banana chips or crisps are processed primarily from plantains. It is also possible to utilize the cooking-type cultivars, viz., 'Bluggoe' ('Saba' and 'Monthan'). In Cameroon, 'Popoulo', 'Pelipita', and plantains, viz., 'French Clair', 'Bâtard', and 'Big Ebanga', are used for the preparation of crisps. Banana chips could play an important role in intervention programs to combat micronutrient deficiencies by virtue of their iron, zinc, and total carotenoid contents.

The process generally includes deep-frying of thin raw banana slices of about 1–2 mm thickness in suitable cooking medium and salting them. Slicing can be done so as to have required shapes such as wholes, quarters, long cut or slivers, and fine

brokens. Blanching of bananas prior to cooking at a temperature range of 65–70 °C for 20–25 min before peeling, slicing, and frying can help to obtain crispier banana chips. The maturity stage is important to obtain properly processed banana crisps as browning occurs if the sugar content is higher in the pulp.

The fruits are harvested at unripe but optimal maturity stage for frying. In many parts of India, coconut oil is the most preferred medium, while in other countries, cottonseed oil or corn oil is used. Palm oil is also used for the preparation of banana crisps, but it has been reported that trans-esterified palm stearin and palm kernel olein (1:1 by weight) blend was highly susceptible to oxidative deterioration during deep-fat frying. Sweetened banana crisps with sugar or honey coatings by osmotic dehydration, spice powder coatings along with mint, and tomato flavorings are other forms of banana crisps that have market value.

Machineries are now available for mechanical slicing of fingers in large volumes for industrial processing. A frying temperature of about 160–170 °C for about 3–3½ min with proper shaking of cooking medium to disperse the heat evenly to the slices is recommended depending on the cultivars. Deep-fat frying at about 180 °C for a shorter time is more effective in preserving carotenoids, ascorbic acid, and potassium content in fruits than extended frying at low temperature. The fried banana chips are then air-dried at room temperature (27–29 °C) or cabinet-dried (60 °C).

Hydrocolloid treatment with 1% pectin has been found to reduce the oil absorption during processing and hence recommended for the preparation of low-fat crisps. High-temperature and high-sugar content in the slice can result in browning by caramelization. The addition of antioxidants like propylene glycol, propyl gallate, and citric acid has been recommended for reducing the rancidity during storage.

The processed crisps can be packed in polythene pouches or aluminum foils. Moisture-resistant laminated aluminum foil packaging is more preferable than polypropylene or polythene packaging as it preserves the crispiness of chips better with low rancid odor over time.

Crisp banana chips can also be produced by using a combination of foaming of banana puree and drying temperature of 80 °C at superficial air velocity of 0.5 m s^{-1}. Foaming agents such as egg albumin and a modified soybean protein and soy protein isolate have been tried.

Banana Flakes

Banana flakes are made by feeding the puree directly to the large chrome-plated drum dryers. As the drum turns, with water evaporation, a film of dried material gets formed on the drum surface. The dry film is then removed as a continuous sheet by employing carefully adjusted knives and transported to an air-conditioned room where it is broken into flakes, sifted, and packaged in bag-in-box containers.

Banana Puree

Banana puree is the processed pulp obtained after peeling and is used in baking, dairy, beverages, mixed fruit smoothie, and baby food products. For industrial processing, after harvesting at optimal maturity, the fruits are graded, are taken to ripening chambers, and are artificially ripened. The fruits are then spray-washed and cleaned, peeled, and fed into a hopper and pump system that forces the fruit pulp through a plate with ¼ inch holes and duplex filter into a vacuum deaerator where removal of air helps prevent discoloration of the pulp. To improve the viscosity of puree, mixtures of pure enzymes such as pectinase, cellulase, and hemicellulase can be added. Acidification of pulp with ascorbic acid or citric acid can reduce enzymatic browning. The puree was then pumped through a series of rotators (scrape heat exchangers) for flash pasteurization (71.5–74 °C for 15–30 s) and immediately cooled. The sterilized puree is then packed aseptically into steam-sterilized can or fiberboard cartons, each lined with a laminated plastic bag under high-pressure steam atmosphere. The processed puree has a storage life of 12–15 months.

Frozen banana pulp can also be obtained by extrusion process after rapid heating the pulp quickly to 120 °C and cooling to 2–3 °C, filling, and quick freezing (−20 °C). Pretreatments of plantain by blanching in hot water, steam, microwave treatment, or calcium chloride modifies the texture and the organoleptic characteristics. Calcium chloride and microwave pretreatments have been found to harden the frozen banana pulp.

Banana Powder

Banana powder is used chiefly in the baking industry for the preparation and fillings for cakes and biscuits. Banana flour, from both green and ripe fruits, enriched with sugar, powdered milk, minerals, and vitamins, is widely used in baby foods. Banana powder contains considerable amounts of resistant starch type 2 (RS2 16–18%), which is not digested by amylase and thereby can help to reduce glucose availability to the bloodstream in diabetics. Besides, the products of the *in vitro* fermentation of RS2 have been known to have the capability to inhibit the initiation and promotion stage in colon carcinogenesis.

For small-scale processing into flour, unripe green bananas (cooking banana or plantain) are peeled, sliced, and chopped into pieces about 5–10 mm thickness and dried by spreading them on a surface of mats or cement floors for drying. The slices can be also placed in a 0.2% solution of sodium metabisulfite (w/v) for about 5 min to prevent browning and then dried in an electrical drier at 60 °C for about 12 h. The dried slices are stored and converted to flour only when needed as the hygroscopic flour tends to absorb moisture and rapidly lose its flavor.

Dark-colored, gelatinized flour resulted when the temperature is raised above 75 °C, as a result of reaction between reducing sugars and amino acids. Changes in particle properties (moisture content and particle size) and storage conditions may influence the flowability of the powder.

Solar driers, industrial-scale cabinet driers, or tunnel are used for processing large volumes. A typical spray dryer can produce 70 kg powder per hour to give yields of 8–11% of the fresh fruit, while drum-drying gives a final yield of about 13% of the fresh fruit. In the latter method, the moisture content is reduced to 8–12% and then further decreased to 2% by drying in a tunnel or cabinet dryer at 60 °C.

Foam mat-drying method is done by foaming fresh banana pulp with the addition of soy protein (10 g/100 g) to induce foaming. A 1% or 2% sodium metabisulfite solution is added to improve the color of the final product. The puree can be

whipped for 12–15 min and the foam mats can be quick-dried using cross flow cabinet driers or by forced air circulation. The dried brittle slices or foam mats are then pounded to produce the banana flour.

Freeze-dried banana powder has been reported to retain maximum 3-methylbutanoic acid 3-methylbutyl ester, 3-methylbutyl acetate, and butanoic acid 3-methylbutyl ester that are responsible for the fruity aroma eugenol and elemicin that give the product its typical mellow aromas, as compared to vacuum-belt and air-drying.

Banana Juice

The pulpy nature of banana does not enable juice production simply by means of pressing the juicy fruits such as oranges. Clear juice (75–80% of pulp weight and a TSS content of 23–26° Brix depending on the variety) can be extracted by mechanical press and through pectolytic enzyme clarification. Pretreatment with cellulase 0.06% and pectinase 0.05% at 45 °C for 2 h resulted in 73% clear juice yield. High-tannin contents, polyphenol, and phenols and peroxidase activities cause browning of juice during processing. The clarified juice could be dispensed into cordials ready-to-serve and blended beverages by adjusting TSS to 18–20° Brix and acidity to 0.3% with sugar, citric acid, and water. After chilling, it forms an excellent thirst-quenching and nutritious drink. Preservatives like sodium benzoate, sodium nitrite, nitrates, sulfur dioxide, carbon dioxide, copper carbonate, and benzoic acid may be needed to extend the shelf life of fresh juice. Processed juice may be packed in aseptic conditions in cans or protective packages.

Banana essence, a clear, colorless liquid that has an agreeable concentrated aroma can be also extracted from ripe fruit for use in desserts, juices, and drinks.

Banana Figs or Dehydrated Banana

Banana figs are dried or dehydrated banana made from fully ripe fruits with sticky fig-like consistency and very sweet taste. Varieties with high total soluble solid content are highly suitable for making figs. The banana slices can be dried after following 60 min immersion in osmotic solution having sugar concentration of 65–70° Brix and heating to a temperature of 60 °C to produce stable dried plantain product. Drying at a temperature range of 60 or 70 °C with an airflow of 1.5 m s enabled rapid drying. Osmotic dehydration of 'Karpuravalli' ('Pisang Awak') banana in 60–70% sugar syrup for 12 h followed by drying in hot air oven at 50 °C gave high-quality figs with excellent color and taste. Wrapping the figs in cellophane paper and packaging in polymeric containers can give an extended storage life of 6 months under ambient conditions.

Canned Slices

Banana slices from early stage of ripening can be stored in syrup (25° Brix) at a pH of about 4.2. These sliced ripe bananas preserved in cans with syrup are used for desserts and fruit salads. Calcium chloride (0.2%) or calcium lactate (0.5%) can act as firming agent. The slices prepared from plantains can be cooked in 40° Brix syrup until the concentration becomes 55–60° Brix. The product may be hermetically packed in cans or in heat-resistant plastic pouches and quick frozen at −23 °C and stored in cold. The cold-stored plantain slices are heated in boiling water for about 15 min before serving.

Fermented Banana Products: Banana Beer, Wine and Ethanol, and Vinegar

In Central and East Africa (Burundi, Kenya, Rwanda, Tanzania, Uganda, and Zaire), the juice from the ripe fruit of varieties known as 'beer bananas' (*Mbidde* clone) is also processed by fermentation to produce a low-alcohol content beverage that is rich in vitamin B. The strained banana juice is fermented using ground roasted sorghum or maize as a source of wild yeast, for a few days after which the 'beer' is consumed within a few days due to poor shelf life. In Uganda, the distilled beer known as 'Waragi' is brewed both commercially and on small-scale home preparation.

Wine can be also made from bananas by mixing water to mashed pulp by 10% and after adjusting the TSS to 26° Brix, followed by sterilization and allowing for fermentation with wine yeast, *Saccharomyces cerevisiae* var. *ellipsoideus* at 24–26 °C for 7 days with intermittent aeration. Pretreatment with pectinase and α-amylase to hydrolyze pectin and starch can help to achieve higher-sugar levels in juice for fermentation. The fermented juice is then filtered and kept under anaerobic condition with water seal for secondary fermentation. After 2 weeks, the secondary fermentation can be stopped and the wine can be racked or centrifuged, bottled, and pasteurized at 50 °C for 20 min. Pasteurization of the banana-based alcoholic beverages increases the ester and alcohol contents. The pasteurized wine upon aging will have a characteristic flavor and aroma.

Banana peel can be also subjected to steaming under pressure coupled with enzymatic action of a cellulase following which fermentation using *Saccharomyces cerevisiae* var. *ellipsoideus*, and distillation can be carried out for the production of alcohol. About 150–200 ml l^{-1} of alcohol can be obtained.

The rejects and overripe banana pulp can be crushed with equal volume of water, and the clear juice obtained after enzymatic softening with 'pectinase' is fermented using a strain of *Saccharomyces cerevisiae* var. *ellipsoideus* and later by acetic acid bacteria for making vinegar containing 5–6% acetic acid.

Banana Jam and Ketchup and Other Food Products

The ripe banana pulp of many varieties can be cooked with equal quantity of sugar along with pectin and acid in the right proportions until it gives a good set to produce jam. Banana pulp can also be mixed with pulp of other fruits to process blended fruit jam. Banana ketchup is made from unripe fruits of cooking varieties. The process involves blanching, peeling, mashing, and cooking with salt, sugar, onion powder, and spices. In the Philippines, banana ketchup serves as substitute for tomato ketchup and has export demand in Asian and European markets too.

The technology for several other value-added products based on banana flour such as biscuits, papads, health drink, baby food, sweet chutney, and cakes has been developed. Banana flour in different proportions have been mixed with

other flours such as cassava flour, wheat flour, and pulses, to produce nutritious foods that can serve to prepare bread, baked products, sweets, savories, and baby foods. Using banana flowers, pickles can be made. The inner core of banana stem can be also steam-cooked and used for the preparation of food.

Bananas as Animal Feed

Green stems, corm, and sun-dried ripe banana peels provide feed for cattle and sheep. Ripe banana rejects, supplemented with protein, vitamins, and minerals, are also used as animal feed. The peels and corm are also directly fed to large animals in several parts of the world.

Nonfood Uses of Banana

Peeled leaf sheaths are used fresh or after drying as packaging material for flowers, betel leaves, fruits, and similar other items in parts of Asia and for lining cooking pits and for wrapping food for cooking or storage in parts of Africa. The leaves of the 'Fehi' banana are used for thatching, packing, and cigarette wrappers.

The pseudostem of banana plant is stripped into shreds, dried, and used for tying packages and making flower garland in southern India. Banana leaves serve as environmentally friendly disposable plates for serving meals in southern parts of India. While some of the banana and plantain varieties have been exploited for fiber extraction from pseudostem besides for fruits, the *Musa* species such as *M. textilis* is exclusively suited for fiber extraction. Banana fiber is extracted from dried petioles and pseudostem of plant is used extensively in the manufacture of papers. The pseudostem fiber has numerous other uses including textile manufacture, making ropes, strings, threads, and for the production of handicraft as baskets, toys, table mats, wall hangings, and lamp shades. Conventionally, in West Africa, fibers from the pseudostem were used in fishing lines.

In the Philippines, it is woven and used in textiles and forms the principal ingredient of special fabric in the Philippines. Efforts are also being made to produce nonwoven fabric from banana fibers. They have several uses like making bags, pots, and vases. Mechanical decorticators for efficient extraction of fiber from banana pseudostem are available now, which are employed by many small-scale entrepreneurs to produce banana fiber.

Future Outlook

Rapid strides being made in science and technology have paved the way to improve delivery of nutritional and health principles through food commodities. Improving postharvest shelf life, improving resistance to pests and diseases, enhancing carotene content in bananas, and the use of bananas as edible vaccines are being targeted through genetic transformation approaches. Newer processing and packaging technologies that are being developed to combine efficiency with preservation of nutrition will help enhance the product diversification and value of processed banana products. Being market-driven globally and managed by large-scale corporate sectors and with ever-persisting demand, banana has a stable future and lucrative market. However, threats due to biological factors such as pests and diseases and abiotic stress factors such as higher-temperature regimes, drought, and salinity that pose severe challenge to this easily available and affordable food crop need to be addressed more urgently. Developing and establishing efficient supply chain management system in developing countries are other important aspects to be focused further.

See also: Berries and Related Fruits; Fruits of Tropical Climates: Dietary Importance and Health Benefits.

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Barley

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Background

Barley, *Hordeum vulgare* L., is an ancient grain with cultivation dating back to 8000 BC in the Fertile Crescent and North Africa in the Middle East. Throughout history, barley has been recognized as a versatile grain that can be cultivated in extreme environments such as the dry lands of North Africa and the high altitude and mountains of Tibet and other areas in Asia. Barley was mainly used as a nourishing human food, but in the nineteenth and twentieth centuries, it evolved largely into an animal feed and malting and brewing grain in most parts of the developed world. These uses drastically reduced barley human consumption, due in part to improved conditions of wheat production, along with the increase use of rice and maize in the human diet. Yet, barley is still considered a staple food in some parts of the world including Asia, the Middle East, North Africa, and Eastern Europe. More importantly, there is increased interest in barley and barley foods as a healthy alternative to refined grains and as a functional food ingredient. This is evident in the recent food health claims from the USFDA (2006), EU EFSA (2011), and Health Canada (2012), linking the consumption of barley with reduced risk of developing coronary heart disease and an ability to reduce the rise in blood glucose after a meal.

Production and Consumption

Barley is an important crop ranking fifth among all crops in dry matter (dm) production in the world. The top five barley producers are the Russian Federation, France, Germany, Ukraine, and Canada. Today, approximately 65% of barley crop is being utilized as animal feed, ≈30% for malting and brewing, and only 2–3% for human consumption. However, trends of breeding selective barley cultivars for human use and food industry are on the rise.

Despite the lack of barley consumption in most developed societies with the exception of Scandinavia and some parts of Eastern Europe, barley is still a stable food in many developing countries.

In Tibet, barley provides 80% of the calories in the diet of rural Tibetans; in Morocco, the average person consumed 68.3 kg of barley a year in 1986. In contrast, the 2009 FAO data show a significant reduction in barley consumption in Morocco to approximately 28 kg/person/year. This is thought to be due to lack of initiatives in barley research and food industry interest in barley. Yet, most individuals in North America and Europe consume on average less than 1 kg/person/year. The development of barley-based food ingredients and products is crucial to boost the consumption of barley.

Barley Cultivars

Barley may be one of the most widely adaptable grains allowing it to be cultivated in contrasting climates and various locations worldwide; it is a genetically diverse grain. This genetic diversity allows barley to be classified as either spring or winter type. Barley is further categorized as either two-row or six-row and hulled or hull-less (naked). Two-row barley has two rows of seeds on each spike, and six-row has six rows of seeds on each spike. Hulled and hull-less barley are distinguished by the presence or absence of a hull tightly wrapping the grain. Any of these types can be further classified into either malting or feed barley depending on the end use of the grain.

Kernels from two-row barley are generally larger and more uniform in size than those from six-row barley due to the crowding of spikelets on the spike in the latter. Hull-less barley is free-threshing or naked grains. According to the grain chemical composition, barley grains are further classified as normal (20–30% amylose), waxy (0–5% amylose), high-amylose starch type (>45% amylose), high-lysine, and high-β-glucan (βG). The mature barley grain also known as kernel or caryopsis is composed of the hulls (for hulled barley), pericarp, seed coat or testa, germ, and starchy endosperm. These parts of the kernel contain both similar and different nutrients in variable amounts. Therefore, when considering barley grains as a functional food or food product, knowledge of these differences would help in using them to add nutritional value and quality to barley-based products.

Chemical Composition

Barley cultivars can vary widely in their chemical composition due to differences in genotype, growing environment, and the interaction between these factors. Normal barley generally consists of approximately 60–70% starch per dm, making starch the most abundant constituent in barley found mostly in the endosperm (Table 1). The next chief constituents are total fiber ranging from 11% to 34% and protein 10–20%; of total fiber, 3–20% is soluble dietary fiber with 5–10% βG depending on the cultivar. Other constituents include 2–3% free lipids and 1.5–2.5% minerals. Barley also contains a myriad of other components including a number of antioxidants, phenolic and bioactive compounds of which preliminary research suggests a significant role for some of these compounds in the health benefits attributed to consuming barley. For example, phenolic acids are linked with barley's ability to inhibit human LDL cholesterol and to scavenge free radicals.

Available Carbohydrates

In general, barley is predominantly composed of glycemic carbohydrates and dietary fiber. There is a small concentration

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Table 1 Water, energy, macronutrients, and fiber contents of barley per 100 g

	Pearl barley, uncooked	*Pearl barley, boiled*
Water (g)	10.6	69.6
Energy (kJ)	1535	510
Energy (kcal)	360	120
Carbohydrates (g)	83.6	27.6
Protein (g)	7.9	2.7
Fat (g)	1.7	0.6
Dietary fiber (g)	5.9	2.0

Source: Price, R.K. and Welch, R.W. (2013). Cereal grains. In: Caballero B. (ed.) *Encyclopedia of human nutrition* (3rd ed.), vol. 1, pp. 307–316. Waltham, MA: Academic Press.

of low-molecular-weight (MW) carbohydrates, which must be included with starch, the predominant constituent, when calculating the composition of glycemic carbohydrates in barley. Barley has a small percentage of simple sugars like glucose, fructose, sucrose, and maltose (range of 0.03–0.83% dB). Starch is the predominant glycemic carbohydrate in barley; in normal barley, starch consists of amylose and amylopectin polymers. Amylose is an essentially linear molecule consisting of glucose monomers joined by α-1–4 bonds. In contrast, amylopectin is a branched α-1–4- and α-1–6-linked molecules. The content of amylose in barley depends on the barley type with normal barley starch consisting of approximately 1:3 ratio of amylose to amylopectin, while waxy barley can range from 0% to 5% amylose of total starch.

Resistant starch (RS) in barley is of the type that is physically inaccessible starch or type I, most seen in whole grains. In cereal grains and their products, the RS proportion of starch is relatively small, typically 0–5% of starch; raw barley contains less than 1% RS. The amount of RS is influenced by factors like the amount of starch present, food processing, cooking, and storage conditions. The concentration of RS in barley can be increased through processing methods such as extrusion cooking and pelleting of barley products.

Fiber

In barley, fiber represents the second major constituent of the grain after starch, but unlike starch, fiber is found throughout the kernel. Fiber can be classified into soluble and insoluble forms. The content of total fiber in barley ranges from 11% to 34%, of which 3–20% is soluble dietary fiber mostly in the form of βG.

β-Glucan

βGs are soluble fiber found in many cereal grains; they are large linear polysaccharides of glucose monomers. Specifically, the mixed linkage (1–3, 1–4)-β-D-glucans are linear homopolymers of D-glucopyranosyl residues. Barley is considered to be the richest source of βGs that account for approximately 75% of the total cell wall polysaccharides in the endosperm cell walls; the rest consists of arabinoxylans, cellulose, glucomannans, and proteins. The recent focus and renewed interest in barley as a human food are largely due to the health benefits attributed to βG. The βG content of barley can range from approximately 2% to 11%, which is generally higher than oats (2.2–7.8%) and wheat (0.4–1.4%).

The health benefits associated with consuming βG-rich foods include lowering blood glucose, insulin, and blood lipids, in particular serum total and LDL cholesterol. Some of these effects have been shown to depend on the capacity of βG to increase the viscosity (defined as a measure of resistance to flow) of intestinal contents, which in turn depends on βG physicochemical characteristics such as MW and solubility.

Physicochemical Characteristics of βG

The physicochemical properties of βG in barley have been suggested to have a key role in affecting postprandial responses in humans. The number of parameters includes the following: MW, solubility, viscosity, microstructure, particle size, chain length, and concentration of βG among other parameters. The physicochemical properties denote an interaction between the physical properties (e.g., structure and MW) and the chemical properties (viscosity and chain length) and their impact on physiological activity *in vivo*.

Data in the literature indicate a strong correlation and interdependence between these factors and glycemic response. In particular, viscosity and MW are thought to have a superior role, and they are mutually associated with the physiological effectiveness of βG. Viscosity is defined as the resistance of a solution to flow, and MW is a measure of the size and weight of the polysaccharides in βG.

Barley Processing and Cooking

The physical and chemical characteristics of barley are important factors to be considered to reinstate barley as a human food. These characteristics are affected by processing, a necessary step in preparing barley for human consumption. The most common barley processing method is pearling, a common commercial process whereby the husk and outer layers of barley grains are removed by friction and abrasion. There has been a general preference, by consumers and food manufacturers alike, for a bright white color of pearled barley and milled barley flours. Yet, in recent years, the increased awareness of whole grains and their products such as whole-grain flour by consumers has diminished the demand for white food products such as white bread and pasta.

Since most barley cultivars are hulled, removing the hulls or dehulling is necessary. Pearling is considered one of the oldest practices used in the processing of barley. This process of abrasion of the barley kernel involves the successive removal of grain tissue starting with the outer layers of barley and working inward. Dehulling by pearling still renders the barley grain a whole grain, because the germ, endosperm, and bran layers are still intact. Depending on the amount of materials removed, the rate of pearling, which is dictated by cultural preferences, can produce barley cultivars labeled as whole grain (only the husk was removed), pot (dehulled and further removal of pericarp), and white-pearled (all the bran and most of the germ and crease removed). Varying the degree of pearling time results in significant alterations in the chemical

and nutritional compositions of barley. These changes include decreasing total fiber but not soluble fiber and increasing concentration of starch and βG; this can be done without significant effects on the endosperm but only up to a pearling degree of 15%. Pearling is considered a beneficial method of creating barley fractions with specific characteristics (i.e., high/low starch and high βG) for different end uses such as the addition of smaller amounts of barley to foods or incorporation of barley as a functional food ingredient.

Barley can also be milled using a roller mill to produce barley flour and bran; this is considered an uncommon practice and needs to be further explored. It is generally assumed that barley bran consists of the testa, pericarp, germ, aleurone, and the subaleurone layers; however, since barley is pearled before milling, the bran and flour composition may differ depending on the degree of pearling. Abrasion milling and sieving is another form of barley milling, which involves the milling of dehulled or hull-less barley by an abrasion mill and sieving the ground material through a series of sieves with an option of different sizes for the openings.

Extrusion cooking is a popular industrial technique for the production of breakfast cereals, breads, pasta, and cooked flour. This process employs simultaneous actions of temperature, pressure, and shear at different levels of intensity. Other forms of cooking include hydrothermal treatments. These processing methods are thought to aim principally on enhancing the nutritional value of barley and creating longer shelf life and convenient barley-based products. Nonetheless, their impact on the metabolic effect of barley in humans is not known.

Processing and cooking cause major changes in the architecture of the grain, mostly in the cell wall matrix. Exposing starch-rich foods to boiled water can result in significant changes to starch properties. Starch can go through transformations that can affect its digestibility such as gelatinization. Gelatinization occurs when starch is heated in water; it is the disruption of molecular structures within the starch granule. This leads to swelling in the granules due to increased water absorption that coincides with leaching of material from the starch granules, mostly amylose. Changes can also occur to the particle size due to either pearling, milling, or cooking, thereby reducing the particle size. This leads to more exposure per surface area to digestive enzymes and consequently accelerates starch hydrolysis and the digestion and absorption processes.

Barley Food Products

Barley product development and improvement in food processing methods received little attention over the last few decades, unlike wheat, for example. Quality standards of barley for food use have not been well established, making it challenging for food industry to select raw materials suitable for barley food product development. Yet, there have been a number of initiatives to breed selective barley cultivars for human consumption and product development. These specialty cultivars, mostly naked cultivars, contain higher βG, indigestible carbohydrate content, and protein quality.

Hull-less or naked barley holds a greater potential for food product development than hulled barley and oats. This is due to superior dietary quality and less processing required. Hull-less barley cultivars require minimal processing leaving the bran layer intact unlike hulled cultivars, which may lose a number of nutritious layers during pearling. There are a number of challenges that face barley food producers such as βG, which has an unfavorable effect on certain baked products leading to low volume and increased water retention. Barley also lacks in the protein gluten, unlike wheat; thus, it does not maintain certain characteristics of bread made from wheat flour. Nonetheless, there are several uses of barley in food products including flat bread, muffins, pastas, noodles, pearled barley, and rice extenders, notwithstanding future novel product development especially those from selectively bred cultivars.

Health Benefits of Barley

The renewed interest in barley comes from its ability to produce favorable effects on two main disease risk factors: postprandial glycemic responses and blood lipids. Barley has also been suggested to help in weight management by increasing satiety due to its low glycemic index and high content of viscous fiber, but the evidence in this area is inconclusive.

Barley and Blood Lipids

Reducing serum LDL cholesterol concentrations has been shown to reduce the risk of coronary artery disease. There is solid evidence in the literature to suggest that whole grains high in viscous soluble fiber such as barley are more effective in lowering blood lipids than other grains such as wheat or rice. The suggested mechanisms of cholesterol lowering after consuming a soluble fiber-rich diet include delayed intestinal absorption of lipids and inhibition of absorption and reabsorption of cholesterol and bile acids alongside an increased excretion of bile acids. These effects are thought to be induced by βG's ability to increase the viscosity of the intestinal contents. Other factors may also be responsible such as the reduced impact of barley on postprandial glucose and inflammatory responses and the fermentation of soluble fiber in the colon, resulting in production of short-chain fatty acids, which inhibit cholesterol biosynthesis. Barley is also a rich source of tocols, such as tocopherols and tocotrienols, which have been shown to have favorable effects on LDL oxidation via their antioxidant action. The ability of barley and βG food products in lowering blood cholesterol is well established, but research investigating other disease end points and metabolic markers such as glycemia, hypertension, inflammation, and satiety is progressing slowly. More importantly, most of the interventions are done in harshly processed barley and barley-enriched food products or in extracted βG with a few using intact whole-grain barley kernels.

Barley and Glycemia

Global data show an unabating upward trajectory in diabetes rates with 366 million people suffering from diabetes in 2011–2012 worldwide. Type 2 diabetes (T2D) is characterized by insulin resistance and reduced insulin secretion. Therefore, food products that decrease plasma glucose and insulin

demands may plausibly reduce the risk of developing T2D. Data also show that elevated blood glucose concentrations produce undesirable consequences on health, an occurrence known as hyperglycemia. Postprandial hyperglycemia is characterized by high blood glucose concentrations post meal, which is a strong predictor for developing T2D. Hyperglycemia and constant fluctuations in blood glucose have been further associated with increasing oxidative stress, protein glycylation, and inflammatory responses, all of which are risk factors for a number of chronic illness that share a common underlying pathophysiological mechanism.

Barley and barley food products have been shown to produce favorable effects on glycemia. The mechanisms responsible for these effects have been suggested to be related to the ability of barley βG in its original state, which possesses a very high MW that exhibits high viscosity at a low concentration. Consuming βG-rich barley can increase the viscosity of the meal bolus in the stomach, reducing the mixing of food with digestive enzymes and delaying gastric emptying. Increasing the viscosity has also been shown to retard the absorption of glucose and slow the rate of starch digestion in *in vitro* digestion model studies. There is solid evidence that the main factor responsible for the low glycemic response to barley foods is related to the viscosity of βG, but other factors such as barley's bioactive compounds may play a significant role.

Other Health Benefits

Barley consumption has been associated with improved bowel function and improved colonic integrity. These benefits in bowel function were evident by improvements in short-chain fatty acid production, especially butyrate and propionate, and in other biomarkers of bowel health such as fecal weight.

Other health benefits include weight management via glucose regulation and increased satiety. Barley consumed as an evening meal was found to regulate postprandial glucose; increase the release of GLP-1, a hormone associated with satiety and glucose homeostasis; reduce later energy intake the following day; and reduce hunger over two subsequent meals, a phenomenon known as second-meal effect. However, evidence from acute studies is not conclusive and other studies did not show similar findings. Research in barley's potential in weight management and/or reduction is still progressing.

See also: Beer: Fermentation; Carbohydrate: Digestion, Absorption and Metabolism; Cooking: Domestic Techniques; Dietary Fiber: Determination; Extrusion Cooking: Chemical and Nutritional Changes; Satiety.

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Beef

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Introduction

The word 'beef' is derived from the Latin terminology '*bos*,' and early English speakers referred to cattle and their meat by the Anglo-Saxon term '*cu*' (a term that later converted to 'cow'). In 1066, Norman French-conquered England showed no interest in the usage of ancestral Anglo-Saxon expressions and introduced the Latin-derived terminologies like '*boeuf*' (commonly referred to as meat of cattle). However, the contrast in terminologies becomes evident in Britain and the etymological journey through several years found to be in the English language for centuries as 'beef.' Generally, beef is the gastronomic name for meat obtained from bovines and can be harvested from cows, bulls, heifers, and/or steers. In general, the beef production system can be categorized based on the source, namely, beef from beef breed, beef from dairy production, or a combination of both. The beef production system also varies depending on the farming structure, resources, feeding system, etc. In terms of beef production, the United States produces nearly about 19% of the world's beef, followed by Brazil (17%), the European Union (13%), China (13%), and India (7%).

Consistent and high-quality beef is one of the most important requirements of the meat industry in order to maintain and expand markets. Like any other meat, beef quality and freshness is often perceived as the most helpful indicator in assessing safety at retail level. The two most important intrinsic beef quality attributes are flavor and tenderness in nearly all beef-consuming countries. Juiciness, color, and texture are the next important, followed by marbling and water holding capacity. Marbling has a favorable effect on juiciness and beef flavor. Both intrinsic (color and leanness) and extrinsic (country of origin and place of purchase) attributes are beneficial for predicting meat quality. The consumer perception of beef eating quality is complex, so does the measurement of quality indexes. The measurement of beef quality attributes is a complex task with high economic impact. There are many tests for quality attributes (tenderness, color, flavor, and water holding capacity) that can be applied to a piece of meat only after it leaves the beef plant. These conventional methods are destructive and time-consuming. Beef processors are constantly looking for alternative noninvasive techniques such as real-time ultrasound, x-ray computed tomography (CT), and magnetic resonance imaging (MRI). The beef eating and technological qualities are affected by several factors including breed, feed system, genotype, preslaughter handling, and slaughter technique employed.

Beef Primary Production

Cattle farming around the world started over 6000 years ago, supplying both meat and milk. Nowadays, cattle rearing for beef production has become more specialized, which varies from country to country. The beef production system can be classified as intensive, extensive, or semi-intensive production system. The intensive system involves cattle confinement with a regular supply of feed; extensive system involves movement of cattle in open area for feed (grazing), whereas semi-intensive involves any combination of both the intensive and extensive system. For example, to produce 1 kg of beef carcass, an intensive indoor dairy bull system would require 16.5 m^2 compared with 42.9 m^2 for an extensive beef breed farming system. Researchers have shown that high-forage systems as opposed to concentrate systems can provide beneficial effects in terms of nutritional profile, meat quality, stability, and sensory characteristics. Moreover, the consumer perceives that the grass-fed beef tastes better, enabling the character of the meat to develop in a natural environment. The environmental impact of three beef production systems, namely, (i) conventional (finished in feedlots with growth-enhancing technology), (ii) natural (finished in feedlots with no growth-enhancing technology), and (iii) grass-fed (forage-fed with no growth-enhancing technology), is shown in Figure 1. A study shows that all beef production systems are potentially sustainable with different environmental impacts; the grass-fed system has the highest carbon footprint per unit beef, followed by the natural and conventional feeding systems.

The beef consumed around the world comes from two main types of enterprises including (i) dairy herds where cattle are milked and meat is a coproduct of milk production and (ii) single-suckler herds where specialized beef breeds are reared for beef production. Significant effort has been made over the past couple of decades to beef breeds for the improved meat production and nutritional profile of meat. The improvement in animal breeding, selection of breeds, and genetic lines within breeds have resulted in an overall leaner beef product. The selection of beef breeds is important for the lipid profile of meat along with genotype, sex, age, nutrition, and management. There are various functional traits that are important for beef production, for example, body size, age at puberty, hot-climate adaptability, fleshing ability, muscle expression, cutability, and marbling. Table 1 shows some common beef breeds of commercial importance with their functional traits.

Beef Secondary Production

Preslaughter Activities

The preslaughter activities of cattle involve a number of critical points that include loading of animals at the farm, transportation from farm to abattoir, unloading of animals at the abattoir, restraining, and handling followed by

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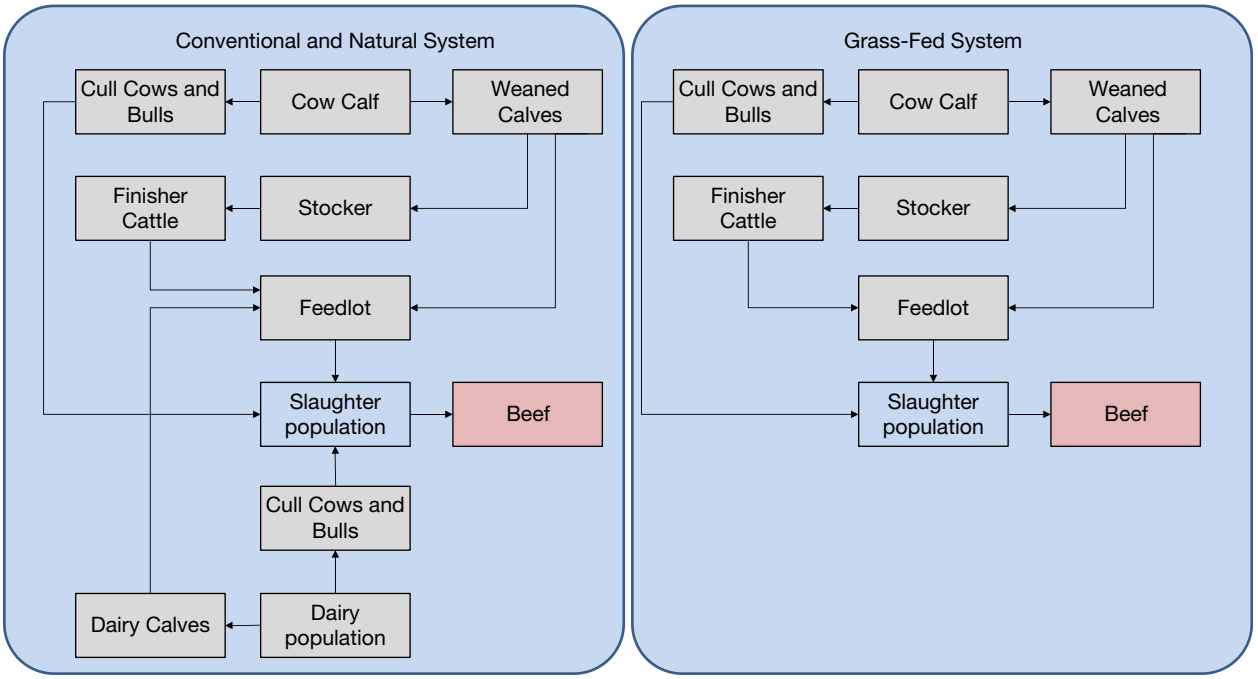

Figure 1 Schematic representation of the beef production systems. Adapted from Capper, J. L. (2012). Is the grass always greener? Comparing the environmental impact of conventional, natural and grass-fed beef production systems. *Animals 2*(2), 127–143.

slaughter. Transportation and loading/unloading are among the main activities that can cause physiological (change in the environment, breakdown of social grouping, and mixing) or physical stress (vibrations, changes in acceleration, confinement, noise, and crowding) in cattle. The transport and handling of cattle can have major implications on animal welfare and meat quality. Feed withdrawal during transportation or fasting during transportation can result in weight loss and also affect the meat quality of the animals that are transported to slaughter.

Recommendations related to the transportation of cattle vary from country to country. For example, in Europe, it is recommended to minimize the duration of transport periods without feed, whereas in New Zealand, livestock should be fasted for 4–6 h prior to a journey to reduce fecal output, facilitating a more comfortable journey. Cattle waiting for slaughter can be stressed and may lead to preslaughter depletion of glycogen in muscles because of many factors, leading to an increase in pH, which is not always ideal for the conversion of muscle to meat. Long-term preslaughter stress including fighting, cold weather, fasting, and transit, which occurs 12–48 h prior to slaughter, depletes muscle glycogen, resulting in meat that has a higher pH and darker color and is drier. The depletion of glycogen in muscles as a result of stress is related to a rapid release of the hormone catecholamines. Short-term acute stress, including excitement or fighting immediately prior to slaughter, produced lactic acid from the breakdown of glycogen. This results in meat that has a lower pH, lighter color, and reduced water binding capacity and is possibly tougher. The management of preslaughter activities is critical in designing effective animal welfare and in improving the quality of meat.

Slaughter

Beef processing begins with the slaughter of the cattle in slaughterhouses or abattoirs, involving several critical processes that lead to the production of fresh meat in the form of quarters. The healthy animal is generally off feed 24 h prior to slaughter with access to water. The age of healthy animals for slaughter varies depending on several factors including breed and feeding regime. The highest quality meat comes from cattle under the age of 36 months, and in some cases, calves are best slaughtered between 3 and 16 weeks of age. The basic commercial processes for live cattle that take place in the abattoir or slaughterhouse are generally uniform across the meat industry. The common processes include stunning, bleeding, hide or skin removal or treatment, evisceration, carcass dressing, and washing, followed by chilling of carcasses as shown in Figure 2. Cattle receiving is the first step with an objective to prepare the animal for slaughtering; the animal's sex, breed, ID number, and live weight are recorded at this stage. Visual screening and antemortem inspections are carried out to identify clinical signs of disease or other abnormalities. Diseased animals that are not fit for human consumption are condemned and are disposed appropriately. Cattle cleared by the inspector for the subsequent production process are presented for stunning. The objective of stunning is to make the animal unconscious before decapitation for animal welfare purposes. Commercially, cattle stunning can be achieved by either mechanical or electrical method as shown in Figure 3. To minimize stress, the animal is restrained using a center track, V-track restrainer, knocking box, or chute. This avoids animal movement and the animal can be positioned for appropriate stunning process. After the stunning process, the animals are shackled to the left hind foot

Table 1 Beef breeds of commercial importance

Breed	*Origin*	*Characteristics*
Angus	The United Kingdom	High growth and size; medium milking potential; early age at puberty; low hot-climate adaptability; high fleshing ability; low cutability[a] and high marbling[b]
Hereford	The United Kingdom	High growth and size; low milking potential; medium age at puberty; low hot-climate adaptability; high fleshing ability; low cutability and medium marbling
Red Angus	The United Kingdom	High growth and size; medium milking potential; early age at puberty; low hot-climate adaptability; high fleshing ability; low cutability and high marbling
Shorthorn	The United Kingdom	High growth and size; medium milking potential; early age at puberty; low hot-climate adaptability; high fleshing ability; low cutability and high marbling
Charolais	France	Very high growth and size; low milking potential; low age at puberty; low hot-climate adaptability; medium fleshing ability; very high cutability and low marbling
Chianina	Italy	Very high growth and size; very low milking potential; low age at puberty; medium hot-climate adaptability; low fleshing ability; very high cutability and low marbling
Limousin	France	High growth and size; very low milking potential; low age at puberty; low hot-climate adaptability; medium fleshing ability; very high cutability and very low marbling
Salers	France	High growth and size; medium milking potential; medium age at puberty; low hot-climate adaptability; medium fleshing ability; high cutability and low marbling
Simmental	Austria	Very high growth and size; high milking potential; medium age at puberty; low hot-climate adaptability; medium fleshing ability; high cutability and low marbling
Brahman	India/the United States	High growth and size; high milking potential; very low age at puberty; very high hot-climate adaptability; high fleshing ability; medium cutability and low marbling
Beefmaster	The United States	High growth and size; medium milking potential; medium age at puberty; high hot-climate adaptability; high fleshing ability; medium cutability and low marbling
Braford	The United States	Medium growth and size; medium milking potential; medium age at puberty; high hot-climate adaptability; high fleshing ability; medium cutability and low marbling
Brangus	The United States	High growth and size; medium milking potential; medium age at puberty; high hot-climate adaptability; high fleshing ability; medium cutability and medium marbling
Red Brangus	The United States	Medium growth and size; medium milking potential; medium age at puberty; high hot-climate adaptability; high fleshing ability; medium cutability and medium marbling
Santa Gertrudis	The United States	High growth and size; medium milking potential; medium age at puberty; high hot-climate adaptability; high fleshing ability; medium cutability and low marbling
Simbrah	The United States	High growth and size; medium milking potential; medium age at puberty; high hot-climate adaptability; medium fleshing ability; medium cutability and low marbling

[a]Cutability or the percentage of lean.
[b]Marbling or intramuscular fat under similar nutrition.

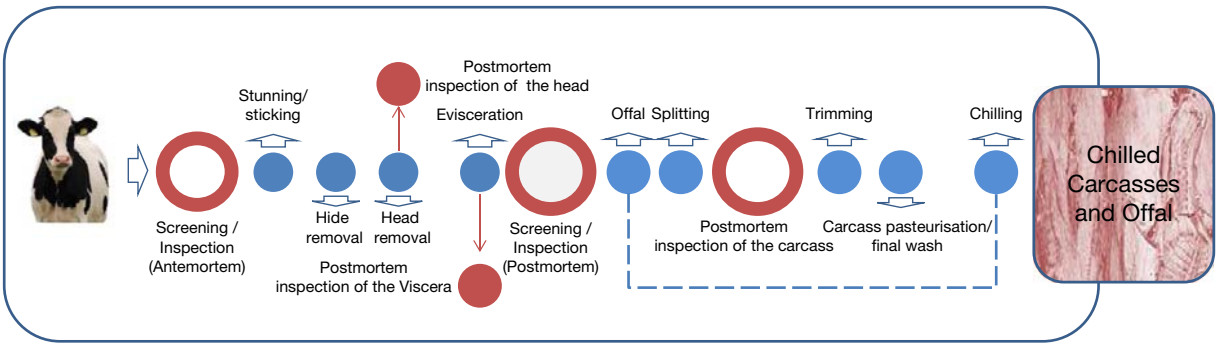

Figure 2 Beef slaughtering process.

when they are in the restrainer. The shackle generally comprises a heavy-duty chain attached to a roller trolley that can move freely on rails. Sticking/bleeding is carried out as quickly as possible after stunning of the animals. The standard method for cattle bleeding is to open the hide at the neck between the brisket and jaw through a longitudinal cut using a clean, sterilized knife, leading to a rapid discharge of blood. Cattle are allowed to bleed for several minutes and blood is collected. Approximately, blood accounts for 3–4% of the animal's live weight. The collected blood may be discarded or processed for various purposes including the use as a human food, feed, or fertilizer.

Hide/fleece washing or dehairing is one of the critical steps in the slaughterhouse immediately after bleeding to reduce the presence of pathogenic microorganisms. Chemical disinfectants can be used to clean hides before hide removal with an

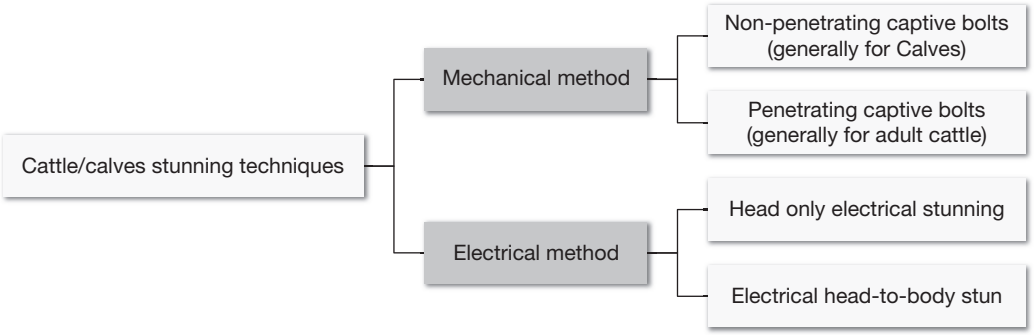

Figure 3 Cattle stunning techniques employed commercially.

aim to reduce microbial load and contaminants. Chemicals including sodium hydroxide, trisodium phosphate, acidified chlorine (sodium hypochlorite with acetic acid), and phosphoric acid have been investigated to date. However, the use of chemicals for hide washing varies from country to country. In the United States, the chemical dehairing process is carried out to remove hair, mud, manure, and other external contaminants from cattle before hides are removed, which should decrease the risk of transferring pathogens, namely, *Escherichia coli* O157:H7 and *Listeria monocytogenes*, to surfaces of beef carcasses. Hide removal, evisceration, carcass washing, and subsequent chilling are critical during beef slaughter and dressing from both meat safety and quality. Manual or mechanical hide removal techniques are generally adopted depending on the size and operational capacity of the slaughterhouse. Extreme care and hygienic conditions are required to avoid carcass contamination and to obtain success, which largely depends on the personnel conducting the activities. Several physical, chemical, or biological interventions have been proposed for carcass washing before the carcass chilling process. The chemicals or disinfectants employed for carcass decontamination include organic acids (acetic acid and lactic acid), sodium hypochlorite, chlorine and chlorine dioxide, trisodium phosphate, hydrogen peroxide, sodium hydroxide, ozone, sodium bisulfate, sodium chloride, acidified sodium chlorite, nisin, potassium sorbate, cetylpyridinium chloride, and activated lactoferrin. However, all of the aforementioned chemical disinfectants are not allowed for carcass washing due to variation in regulations around the world. For example, organic acids such as lactic acid and acetic acid are the most frequently used chemical interventions in commercial plants for beef dressing in countries like the United States, Canada, and Australia. Lactic acid treatment is allowed in the EU, whereas the use of organic acid is not permitted in Japan. Various physical interventions including the use of steam or hot water alone or in combination with organic acid has been proposed for effective removal of pathogenic and spoilage microorganisms. Chemical, physical, or biological disinfectants can be applied at various levels for the treatment of beef hides, carcasses, cuts, and/or trimmings in a variety of applications, namely, (i) spray washing hides prior to hide removal; (ii) spray washing or misting skinned animal preevisceration or postevisceration carcasses, either whole or split prechill; and (iii) misting carcasses, either whole or split, during chilling. Under commercial conditions, the use of multiple sequential interventions at various stages during slaughter should be considered in order to enhance the microbiological safety and shelf life of carcasses. The chilling process of carcasses is the most important step in cold chain for improving the quality, safety, and shelf life of beef meat. Various methods are employed for carcass chilling depending on the capacity and refrigeration facilities available. Refrigeration conditions under which the initial meat chilling takes place have an influence on key quality parameters including color, tenderness, weight loss, and shelf life. For example, studies show that initial chilling of carcasses at 6 °C can decrease in tenderness compared with 8 or 10 °C. Carcass cutting and boning often take place after chilling, since a carcass is easier to handle and cut when it is chilled. However, in some countries, the removal of bones as they come out of the slaughter floor and carcasses at a temperature of more than 20 °C is commonly known as hot boning. Hot boning involves the removal of muscles or cuts before the onset of rigor mortis. In the EU, there are separate requirements for hot-boned and warm-boned meat, whereas there is no distinction in Australia. There are advantages and disadvantages associated with cold boning (removal of bones from chilled carcasses) or hot boning. In general, a higher yield is obtained via hot boning compared with cold boning. This is in part due to elimination of the weight loss that normally occurs during chilling. At various stages of beef processing, diverse edible and inedible by-products are generated, which includes the head, bones, hairs, fat, and other offal. Edible offal for human consumption, including the heart, liver, kidney, tongue, and sweetbread, are either processed at abattoirs or sent to other facilities for subsequent processing. Other by-products are processed for other agri-food applications including pet food or suitably discarded depending on the regulatory regime. In the EU, because of bovine spongiform encephalopathy, the use of dead carcasses for animal feed is prohibited. The cattle slaughtering process and subsequent processing have significant effects on the shelf life, meat quality, and safety profile of carcasses.

Carcass Grading and Evaluation

Beef carcass evaluation is generally the basis for judging the commercial value of the livestock and is consequently one of the most common quality control tests carried out in the meat industry. Carcass quality attributes include tenderness, cut size, fat cover, marbling, meat, and fat color, whereas composition attributes include salable meat yield and proportions of fat,

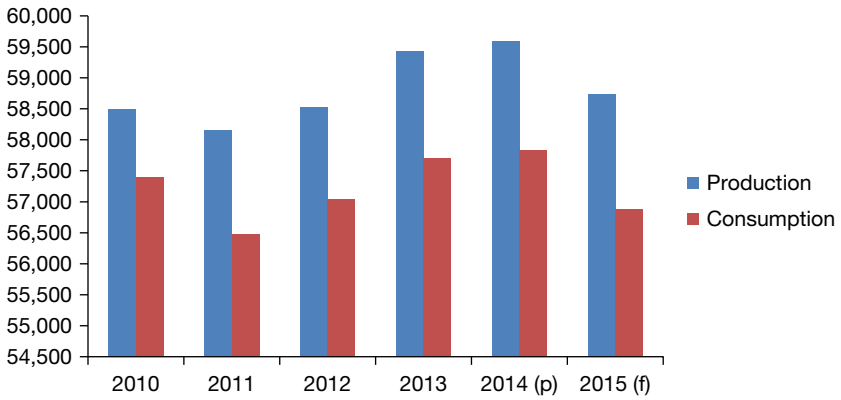

Figure 4 Beef and calf production and consumption trend (1000 tons carcass weight equivalent) (p: projected and f: future). Source: USDA.

lean, and bone. Carcass evaluation is a way to describe the quality of livestock in terms of their suitability and commercial value for various end usage including retail cut and processed meat. A number of approaches are available for the prediction of carcass composition and quality, which may also allow the grading of carcass into various categories. For example, in the United States, the beef carcass is evaluated based on the established Standards for Grades of Slaughter Cattle and Standards for Grades of Carcass Beef. According to this, quality grades are determined by marbling and overall maturity. There are eight quality grade designations: Prime, Choice, Select, Standard, Commercial, Utility, Cutter, and Canner. Prime, Choice, Select, and Standard are classified as young beef (maturity levels A and B) and must be <42 months of age, physiologically. Commercial, Utility, Cutter, and Canner are cow grades from carcasses >42 months of skeletal maturity. Similarly, In the European Union, adult bovine carcasses are classified according to the EUROP grid system, which is based on visual assessment scores according to the defined standards implemented by the European Community Regulations 1208/81 and 1026/91. The EUROP classification scheme includes carcass conformation scores on a 15-point scale with 5 main classes, E (excellent conformation), U, R, O, and P (poor conformation), and 10 subclasses and five main fatness scores (1 (low fatness), 2, 3, 4, and 5 (excessive fatness)) also with 10 subclasses. Mostly, carcass evaluation is done manually by trained graders using photographic references. Various carcass classification schemes adopted worldwide have been criticized due to the subjective nature of the process with a high degree of inconsistencies in such manual grade assessment. With growing concern of qualitative value of carcass and the possibility to improve the consistency of assessment, instrumented carcass evaluation techniques including ultrasound, x-ray CT, nuclear MRI, total body electrical conductivity, and video image analysis (VIA) are gaining importance in the meat industry.

Consumption Pattern

There has been an increasing pressure on the livestock sector including beef to meet the growing demand for high-value animal protein for the ever-increasing population, rising incomes, and urbanization. Beef is the third most widely consumed meat accounting for about 25% of world meat production. Historically, beef consumption is linked to Western culture and is now becoming popular and more affordable in Southeast Asia and Brazil due to an increase in disposable income and changes in society. There is a strong positive relationship between the level of income and the consumption of animal protein. A study observed that beef consumption is associated with an income compared with other meats. Consumers with medium and high income are target for premium beef. Moreover, meat consumption from ruminants has become a status symbol of the growing affluence of the new consumer societies. Cultural and religious factors have also erected in the way of wider diffusion of beef consumption in some countries such as in India. The global beef production and consumption trend is shown in Figure 4. In general, the consumption of beef is heavily and disproportionately concentrated in the industrial countries with an average per capita consumption of beef remained fairly constant in the range of 6.5–6.7 kg per capita. Argentina is the largest consumer of beef accounting for 40 kg per capita, followed by Brazil (25.4 kg per capita) and the United States (23 kg per capita), whereas in the EU (28 countries), the consumption of beef and calves is 10.9 kg per capita based on kilograms of retail weight per capita (carcass weight to retail weight conversion factor is 0.7 for beef and calves). The consumption pattern of beef and calves is projected to remain constant until 2030 years.

Retail Beef Cuts and Products

Various types of fresh beef cuts and beef-based meat products with varying sizes, shapes, tastes, textures, and colors are often visible at butcher shops or retail shops. The full range includes fresh beef, primal or nonprimal cuts, sausages, steaks, burgers, patties, mince, ground beef, charqui, jerky, and other ready-to-eat beef products. The fresh beef cuts may differ from country to country; however, there are eight well-recognized primal beef cuts. Each primal cut is then reduced into subprimal cuts (Figure 5). Individual portions derived from subprimal cuts are referred to as fabricated cuts. The primal cuts of beef are the following:

Chuck: The primal chuck is the animal's shoulder. Since this is a well-used muscle, the chuck contains a high amount of connective tissue and is very tough. It is further cut into subprimal roasts and steaks: blade steak, chuck short ribs, cross-rib pot roast, flat iron steak, ground chuck for hamburgers, and stew meat.

Brisket: The beef brisket is a very tough, course-textured muscle and contains a substantial percentage of fat. It is

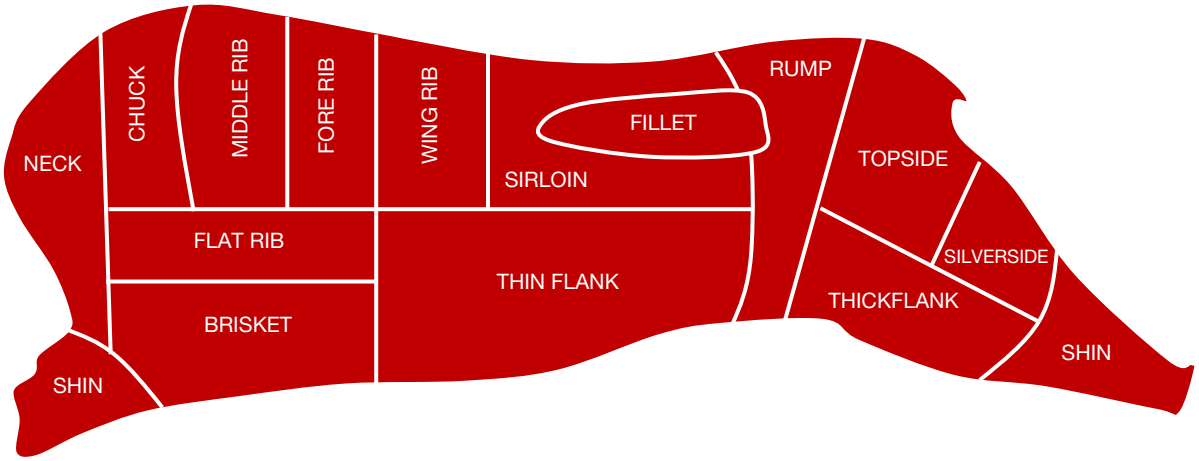

Figure 5 Major cuts of beef.

typically cut into smaller portions that are pickled to produce corned beef brisket or cured to make pastrami.

Shank: Beef foreshank is very flavorful and high in collagen. Typically, it is used in foodservice for making soups and stocks. In retail markets, it is ground for low-fat ground beef.

Rib: It consists of the ribs and a portion of the backbone. The center muscle portion of the rib is quite tender. It also contains large amounts of marbling and produces rich, full-flavored roasts and steaks. It is further cut into subprimal beef short ribs, boneless rib eye roast, rib eye steaks, and roast prime rib of beef.

Short Plate: The short plate contains rib bones and is located directly below the primal rib. It is cut into subprimal short ribs and skirt steaks.

Loin: The loin is located behind the primal rib and produces the most prized cuts of meat. It is further cut into subprimal fillet mignon, porterhouse, T-bone, sirloin butt roast, sirloin steak, and strip steak.

Flank: The flank is located directly beneath the loin. The flank contains no bones, is very tough, but is very flavorful. It is further cut into subprimal flank steak and London broil.

Round: The primal round is the hind leg of the animal and contains the round, shank, and tail bones and aitchbone. It is cut into subprimal round roasts and round steaks.

Nutritional Quality of Beef

Beef, like all meats, is an excellent source of protein. The beef protein is a high-quality, complete protein, meaning it contains all the amino acids necessary to be readily utilized by the body. Beef is a rich source of many minerals including zinc, iron, selenium, phosphorus, magnesium, potassium, and copper. The minerals found in beef are more bioavailable than those from the vegetable sources. Beef is also rich in many vitamins including vitamins B_{12} and B_6, riboflavin, thiamine, and pantothenic acid. Additionally, meat provides a number of saturated and unsaturated fats that are an important source of energy and facilitate the absorption of fat-soluble vitamins including A, D, E, and K. Approximately 50% of beef fat is saturated and the majority of the saturated fatty acids are palmitic acid (16:0) and stearic acid (18:0). The polyunsaturated fatty acid (PUFA) content of beef meat ranges from 11% to 29% of total fatty acids. Table 2 shows the general composition of grass-fed beef. In addition to the traditional essential nutrients,

Table 2 Composition of grass-fed, raw ground beef

Proximate composition	*Units*	*Value per 100 g*
Moisture	g	67.13
Energy	kcal kJ^{-1}	192
Protein	g	19.42
Fat (total lipid)	g	12.73
Carbohydrate (by difference)	g	0
Ash	g	1.71
Minerals		
Calcium (Ca)	mg	12
Iron (Fe)	mg	1.99
Magnesium (Mg)	mg	19
Phosphorus (P)	mg	175
Potassium (K)	mg	289
Sodium (Na)	mg	68
Zinc (Zn)	mg	4.55
Copper (Cu)	mg	0.063
Manganese (Mn)	mg	0.01
Selenium (Se)	µg	14.2
Vitamins		
Total ascorbic acid (vitamin C)	mg	0
Thiamine	mg	0.049
Riboflavin	mg	0.154
Niacin	mg	4.818
Pantothenic acid	mg	0.576
Vitamin B_6	mg	0.355
Folate (DFE)	µg	6
Choline, total	mg	67.4
Vitamin B_{12}	µg	1.97
Vitamin E (alpha-tocopherol)	mg	0.35
Vitamin K (phylloquinone)	µg	1.1
Lipids		
Total SFA	g	5.335
Total MUFA	g	4.8
Total PUFA	g	0.532
Cholesterol	mg	62

Source: USDA nutrient database for standard reference.

beef meat is a potential source of a number of bioactive substances that have been studied for their potential beneficial effects. These meat-based bioactives include taurine, creatine, conjugated linoleic acid (CLA), carnitine, and several endogenous compounds (including ubiquinone, glutathione, lipoic acid, spermine, carnosine, and anserine).

Health Effects

Traditionally, beef is being considered as a highly valued, nutritious food and associated with good health and prosperity. With the growing health awareness and concern, this healthy image for beef meat has gradually been eroded in the last few decades. Health professionals recommend to reduce the overall consumption of fats, and the diet–heart (lipid) hypothesis focused attention on the saturated fat contributed from meat. A number of epidemiological studies have proposed an association of red meat consumption with the development of cardiovascular disease and colon cancer. Therefore, different strategies are being developed, aiming to reduce the intramuscular fat level. These approaches include selective breeding and feeding practices designed to increase the carcass lean-to-fat ratio, improved official carcass classification systems designed to favor leaner production, and modern butchery techniques (seaming out whole muscles and trimming away all intramuscular fat). Research over the past few decades suggests that grass-only diets can significantly alter the fatty acid composition and improve the overall antioxidant content of beef. Grass feeding improves the quality of beef and makes the beef richer in omega-3 fats, vitamin E, beta-carotene, and CLA. There is tremendous potential to enhance the health benefits of beef by the production of high-quality beef from better-bred animals with superior genetics and improved nutritional profile via better feed management.

See also: Meat: Conversion of Muscle into Meat; Meat: Eating Quality and Preservation; Meat: Role in the Diet; Meat: Structure; Pork Meat Quality, Production and Processing on.

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Beer: Fermentation

S Livens, British Beer and Pub Association, London, UK

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Introduction

For much of history, alcoholic beverage production has broadly been the result of 'one-pot cooking', with the final beverage being produced and consumed as a porridge-like 'mash.' Indeed, throughout the world, there are many countries that still produce alcoholic beverages in this way, and photographs of those drinking the fermented liquid through long 'straws' are recognizable from those Egyptian carvings that represent some of our earliest pictographic representations of the brewing process.

There has also been a fair amount of mysticism surrounding the brewing process. In medieval England, the foaming balm at the surface of fermenting vessels was referred to as 'Goddisgoode' and it was widely understood that this foam could be used to restart new fermentations. Words used to describe 'yeast' were based on characteristics that today we associate with the action of yeast during fermentation. Old English words for yeast include *gyst* or *gist*, which share similarities with the Germanic *gischt* meaning 'foam'; even older than this however, the ancient Greek *zestós* and Sanskrit *yásati* are both derived from words that translate as 'boiling.'

Today, our understanding of brewing is far more distinct and simplistically can be separated into four defined stages that each contribute in some way to the necessary characteristics that describe a particular style of beer:

- Brewhouse and wort production
- Fermentation
- Conditioning and maturation
- Packaging

The production of wort, which has already been described within the preceding article, will certainly contribute to the characteristics of a given beer style. However, it is fermentation that establishes the broader platform from which beer will ultimately emerge. Key to alcoholic fermentation is the nutritional composition of wort and the presence of fermentable sugars, which will support the growth and fermentative capabilities of brewing yeast, resulting in the production of alcohol, carbon dioxide, and various flavor-active components that are characteristic of yeast and this stage of production. In addition, yeast will also greatly influence the progress and outcome of the fermentation process. Therefore, for our understanding of fermentation and this stage of beer production, we must consider both yeast and the fermentative process.

Yeast

While yeast is the engine that drives fermentation, it was not until the nineteenth century that we began to fully recognize and even understand yeast as a biological entity. However, while the first classification of yeast as *Saccharomyces*, from the Latin meaning 'sugar fungus,' was applied by the German chemist Meyen, it is the brewing scientist Emil Christian Hansen who made one of the most significant early contributions to our knowledge of brewing yeast.

Hansen studied yeast performance in both ale and lager fermentations while working at the Carlsberg Brewery. His observations noted that whereas a sediment formed at the surface of the warmer, faster ale fermentations, for lager fermentations, which were colder and slower, the same sediment formed instead at the base of the fermentation vessel. Upon further observation, Hansen was able to isolate viable yeast cells from both sets of sediment that, upon repitching, individually demonstrated the same sedimentary characteristics. Hansen had thus successfully demonstrated fundamental differences in the production processes for ale and lager and had independently cultured two separate production yeast strains.

Hansen named the separate isolates *Saccharomyces cerevisiae* (ale yeast) and *Saccharomyces carlsbergensis* (lager yeast), and his work delivered important advantages to the brewing process. The concept of strain purity and an ability to store and propagate yeast as a pure culture introduced improvements to overall beer quality and consistency. However, Hansen's observations further provided new drivers for the manipulation of the fermentation process and, in particular, methods for the recovery of yeast and the importance of temperature in defining fermentation type and for controlling yeast performance.

Yeast Taxonomy

Taxonomy, and particularly in relation to those yeasts responsible for the fermentation of alcoholic beverages, has remained something of a moving feast. Various methods have been employed over the years to separate and define different yeast species. These have been primarily based on simple, observable morphological and physiological differences before becoming based on differences in the fermentation profiles of different carbohydrates and then, finally, in more recent times, based on differences at the molecular genetic level. The kaleidoscopic approaches to taxonomic categorization over the years have led to considerable debate over the identification of microorganisms, including yeast. However, we are now left with a vastly reduced list of strain variants with many previously uniquely defined strains now belonging to the same *S. cerevisiae* group. It is perhaps the almost continual reclassification of lager yeast since the 1970s that has led to the most confusion for brewing scientists. Not least because the ancestry of this 'chimeric' yeast strain, until recently at least, seems to have been well hidden.

Lager Yeast

Early reclassification of the lager yeast *S. carlsbergensis* in the 1970s by Lodder resulted in the new classification of lager production strains as *Saccharomyces uvarum*. Morphologically

and physiologically distinct from *S. cerevisiae*, *S. uvarum* was also reclassified due to its ability to metabolize the sugar melibiose, whereas *S. cerevisiae* cannot. However, in the 1990s, the classification of brewing yeast was once more thrown into confusion when *S. uvarum* was once again reclassified as *S. cerevisiae* before becoming *S. cerevisiae* var. *carlsbergensis* and, then finally, under the name that is today considered to be taxonomically correct – *Saccharomyces pastorianus*.

DNA sequencing has shown that whereas ale yeast is distinct as a species, lager yeast was formed as a hybrid from *S. cerevisiae* and at least one other yeast that has itself only recently been identified. *Saccharomyces eubayanus* was isolated and identified by Liebkind from the forests of Patagonia. Whereas *S. eubayanus* is itself shown to be district from any other yeast strain known today, sequencing of its genome has revealed a 99.5% homology with those areas of the *S. pastorianus* genome that are not related to *S. cerevisiae*. In particular, it is this region that is responsible for the cold tolerance associated with lager yeast and that is also a defining characteristic of *S. eubayanus*.

While admittedly a pun, this simplistic description merely skims the surface of the complexities behind the classification of brewing yeast over the years! It may even be suggested that characterization be based on phenotypic differences given that there is a considerable apparent variation in the flavor profiles of beers produced using the same 'strain' of yeast in addition to differences in yeast performance under different production conditions.

However, notwithstanding these issues and to avoid any further confusion, for the remainder of this article, the terms 'ale yeast' and 'lager yeast' will be used, and on this basis, we can list their principal differences as follows:

Ale yeast	*Lager yeast*
Top fermenting	Bottom fermenting
Optimum fermentation temperature 18–22 °C	Optimum fermentation temperature 7–15 °C
Cannot metabolize melibiose	Can utilize melibiose

Fermentation

There are many different configurations of fermentation vessel and in particular for those used to produce ale. Whether based on construction materials, shape, or indeed the more complex arrangements of vessels and elements as illustrated by the Yorkshire Square or the barrel-based Burton Union system, the common driver for such differing approaches was broadly based on yeast strain.

While changes in vessel shape were undoubtedly more significant following Hansen's characterization of yeast strains, a more contemporary shift towards the requirement for higher-capacity production has also contributed to the basic high- and low-aspect-ratio designs (see succeeding text) most frequently associated with the modern industry.

More recently, however, vessel design is returning to a more ubiquitous format and, in particular, with the realization that some ale yeast strains can show adaptive abilities towards vessel geometry such that those strains once considered as more typical of top fermentation, within reason, can be used in cylindroconical vessels as bottom-fermenting strains.

Certainly, vessel design will impact on the biological and biochemical processes that define fermentation and will therefore have a significant impact on its progress and outcome. Ultimately, all vessels must be 'fit for purpose' and in this way must satisfy a number of fundamental criteria:

- Filling and emptying of the vessel must be achieved as quickly as possible and in such a way as to reduce losses/maximize output.
- The chosen design must ensure efficient and thorough mixing during fermentation.
- Appropriate separation and collection of yeast.
- Efficient and precise control of fermentation conditions.
- Vessel durability, cleanability, and hygiene.

Vessel Construction

There are a number of principal factors that must be considered in the design and construction of fermentation vessels. To begin with, the material used to construct the vessel is important not only for vessel strength and durability but also in terms of issues such as food safety, hygiene, and cleanability.

Cost must not be ignored as a consideration, in terms of both the cost of the base material and the ease with which different materials can be worked on. The construction of early, more 'traditional' fermentation vessels was inevitably based on the availability of local materials. In most cases, this is likely to have been wood or stone; however, a variety of other materials might also be used, including slate and metal.

The final choice of material will also be dictated by the size of the fermentation vessel since the volumes of liquid involved and the extent of CO_2 evolution during active fermentation will exert considerable pressure on the vessel. Therefore, larger vessels will need to be built from more resilient materials, capable of withstanding such rigors, as well to enable sufficient process control and, in particular for lager production, the need for efficient temperature control, which is generally dependent on the thermal conductivity of the material in question.

In some cases, lining of vessels may be necessary to protect the fermenting liquid from toxic or tainting compounds leeching from the construction material. Materials traditionally used for vessel linings are metals such as aluminum or copper; however, glass and composite materials such as epoxy resins and plastics have been used. Vessel linings can protect against microbial contamination and improve the cleanability of the vessel and, in the case of materials that are prone to wear and corrosion, may also increase the life of the vessel. Lining a vessel can also improve rigidity and strength and may offer improved temperature control.

Vessel Geometry

Today, the most common material for construction is undoubtedly stainless steel that, while perhaps not the cheapest of materials, is inert and does not require lining, is extremely durable, and provides excellent thermal conductivity. In this instance, vessel shape becomes a principal concern

for new fermenter design. This will also largely be influenced by the type of beer being produced and in general terms may be described by two distinct geometries.

Low-aspect-ratio design

Vessels used to produce ale are relatively short and either round or square with an open top and a flat bottom. This low-aspect-ratio shape suits the dynamics of top-fermenting yeast, which generally ferment faster than bottom-fermenting lager strains. Ale yeast not only produces considerable amounts of CO_2 during the active fermentation but also is more flocculent. The flocs, or clumps, of yeast entrap rising CO_2 bubbles and are then driven to the surface of the fermenting liquid where the yeast collects and from where it will need to be harvested or 'skimmed' ready for repitching into subsequent fermentations.

High-aspect-ratio design

While lager fermentation vessels may have originally shared a similar design to the 'classic' ale fermenter, today, such vessels are considerably different in both shape and size. Vessel format and design have progressed through various iterations of cylindrical, enclosed, horizontal, and vertical vessel formats. Today's familiar cylindroconical fermentation vessel shape was patented in the 1940s by Nathan who was able to demonstrate considerable improvements in both the time and efficiency of lager fermentations using a high-aspect-ratio design. Nathan reduced overall fermentation time to a period of weeks rather than months based on this new design, which has since become the 'standard' format for fermentation vessel design with the main body of the vessel generally typified by an enclosed, tall, narrow cylinder attached to which is a conical base designed to more efficiently capture and separate yeast from the fermented wort at the end of the process.

Vessel Dynamics

Fermentation dynamics, including yeast performance, are greatly influenced by vessel geometry, which can be evidenced in the principle associated with vessel mixing. Whether in ale or lager fermenters, there is very little, if any, mechanical agitation of the vessel contents. In both cases, vessel mixing is intrinsically linked with the shape of the vessel and the evolution of CO_2 during active fermentation.

While gas evolution is greater in vessels with a low aspect ratio, it is not as vigorous as those with a high aspect ratio. In open, flat-bottom fermenters, CO_2 production will keep yeast suspended within the body of the fermenting wort until flocculation occurs. However, the conical base of cylindroconical vessels creates a concentrated column of rising gas towards the center of the vessel that carries yeast cells up through the body of the fermenting wort. Cooling systems built into the wall of the vessel cause the liquid away from the central rising column to be cooler than the liquid at the center. This changes the density of the wort and provides a region of reduced turbulence. Yeast that has been carried up within the central column is then able to sink back towards the base of the vessel. Finally, as the active fermentation comes to a close, CO_2 production becomes greatly reduced, and combined with vessel cooling, the flocculating yeast will then collect in the vessel cone.

The effects of different vessel geometries on yeast performance are most frequently seen in ale production where there are a number of peculiar formats that have evolved. Fermentation systems such as the Burton Union and Yorkshire Square, which in themselves are something of a rarity today, were designed in particular to accommodate highly flocculent yeast strains that are considerably more difficult to keep suspended during fermentation. Ultimately, this mirrors one of the more important aspects of vessel design that is the removal of yeast at the end of the fermentation process. Unfortunately, within the scope of this article, there is insufficient time to summarize the impact of all of the different possible configurations for vessel geometry on fermentation dynamics. Therefore, we will instead look at the primary differences between high- and low-aspect-ratio vessel geometries and how these impact on the management and recovery of yeast for subsequent fermentations.

Yeast Recovery: Ale Fermentations

One of the important aspects of fermenter design is the ease with which yeast is separated from the fermented wort. Ale yeasts are generally more flocculent than lager strains and, in this way, towards the end of fermentation, are driven to the surface of the fermenting liquid where they will then collect and remain. For this reason, low-aspect-ratio vessels are generally constructed with an open top that enables the easier recovery of yeast. Collection is usually undertaken using a vacuum-based, suction system; however, in its simplest format, this can be achieved using a 'parachute.' This is a metal funnel attached to a pipe that exits through a sealed port beneath the fermentation vessel. The parachute is lowered into the yeast head at the surface of the vessel to allow yeast to be transferred, by gravity, to a sanitized collection vessel situated beneath the fermenter.

Selective cropping is an important concept in brewing and allows for the collection of the yeast that is most optimal in terms of fermentative capacity. Yeast harvested from open fermenters is traditionally of a higher quality in respect of fermentation performance than that collected from lager fermentations. In top fermentations, the first visible yeast 'head' usually occurs quickly and will largely consist of significant amounts of wort solids or 'trub' as well as yeast that is damaged or atypical in terms of anticipated fermentation characteristics. This initial yeast crop is removed and discarded and a second 'head' will then subsequently form. This will contain a much higher concentration of healthier yeast cells and considerably less trub material and will be collected for pitching into subsequent fermentations. In some instances, a final, third crop may also occur. However, this yeast will once again generally be discarded on the principle that it will have flocculated late in the fermentation process and, in addition to exhibiting atypical or undesirable fermentation characteristics, will be less vital in terms of overall health.

The 'open' format of traditional ale fermenters permits easier collection of yeast for serial repitching; however, it also allows for greater risk from microbiological contamination, particularly earlier in the fermentation not only within the cooled or freshly pitched wort but also within the cropped yeast. Additionally, there is an increased likelihood of exposure

of pitching yeast to oxygen, which, if maintained during collection, may result in yeast remaining active during storage and therefore lead to a reduced fermentative capacity on repitching.

Yeast Recovery: Lager Fermentations

When compared with traditional open top fermenters, yeast removal from vessels with a high aspect ratio generally offers fewer options in terms of the actual physical removal of yeast. On this basis, the principle of harvesting yeast is arguably a simpler one and, in some cases, may also offer opportunities for automation, including active separation of the more viable portion of yeast within the cone. With such automated aids, it could be argued that the quality and health of the harvested yeast are at least equal to those taken from open top fermenters. However, there is cost involved in such advances that would favor larger brewers, and indeed, there remain some general caveats.

As for ale fermentations, there are distinct periods of sedimentation for lager yeast. However, in the latter case, the broad lack of opportunities to selectively harvest at different periods during fermentation means that all of the sedimented yeast will collect within the cone and, therefore, harvesting will need to be undertaken with some care.

At the end of fermentation, the yeast within the cone of a cylindroconical vessel can be viewed as being composed of three distinct 'bands.' Much like the first crop of an ale fermentation, the lowest band will be composed of yeast with atypical flocculation characteristics and, as well as containing a high proportion of wort solids, may also carry a greater risk of being contaminated with other microorganisms capable of spoilage. The outer band of yeast, while not carrying a significant amount of 'trub,' will similarly also contain yeast that exhibits atypical brewing characteristics. The central portion of the yeast cone however will generally contain the greatest concentration of healthy yeast cells, which will also exhibit those fermentative characteristics desired by the brewer.

Therefore, while yeast will usually be collected in one instance, harvesting must aim to collect the central portion within the cone and to discard the upper and lower bands. Traditionally, the simplest approach is to collect yeast based on visual prompts, whereby the central portion of the cone would usually present a consistency and 'creamy' color classically associated with healthy yeast. Unsurprisingly, this somewhat objective method is not an exact science and can result in the ongoing and increasing presence of wort solids and 'selecting' for particular undesirable characteristics in pitching yeast.

Today, there are some significant opportunities to improving the efficiency of cropping through the use of automated measurement solutions such as those produced by Aber Instruments Ltd (Aberystwyth, S. Wales). The ABER Yeast Monitor is an in-line device that will directly measure the viability of yeast cells as they are removed from the vessel and that can then specifically select for the healthiest and most viable portion of the crop.

Due to the longer fermentation times associated with lager fermentations, as well as the colder temperatures that are involved, it is more common to 'store' yeast within the cone of the fermentation vessel. In this way, new fermentations are then pitched 'cone to cone.' There are however similar concerns with this method as those associated with the storage of ale yeast, where ongoing fermentation within the cone itself, coupled with longer-term exposure to the greater hydrostatic pressures associated with high-aspect-ratio vessels, can lead to cell stress and damage.

Process of Fermentation

Laying aside the choice of fermenter and the impact of yeast strain on fermentation, the broader biochemistry associated with this process is common to all beer styles and, indeed, to the production of any fermented alcoholic beverage. For beer, this process is defined by the fermentation of wort sugars and the assimilation of other wort constituents with the resulting production of alcohol and CO_2 and a variety of other volatile substances associated with beer flavor. Successful fermentation is therefore highly dependent on the appropriate use of yeast, which must be healthy at pitch and introduced in sufficient quantity.

Yeast Health

In general, pitching yeast will have been harvested and, in particular for ale yeast, stored in special tanks prior to use. Both the extent and progress of previous fermentations and the storage process itself are vitally important for the ongoing health of pitching yeast. Ultimately, and even if the health of yeast is maintained to the greatest degree, there is the likelihood that a new production culture will need to be introduced periodically to ensure consistency of beer flavor and character and the efficiency and predictability of the fermentation process. Here, again, it is vitally important to ensure that the yeast undergoes as little stress as possible through the propagation process to ensure the continuity of the strain characteristics.

Replacing yeast cultures is generally more frequent for lager yeast than for ale. Lager yeast is usually replaced on an eight batch or 'generation' cycle, whereas for ales, this is more likely to be around twelve generations. In some cases, in particular, for the traditional British ale-producing breweries, the production culture may have been in use continually for hundreds of generations or more. While, genetically speaking, ongoing and uninterrupted use will likely lead to a more stable yeast, there will inevitably be some changes to both the flavor and fermentation characteristics associated with that strain. This then becomes a matter for the brewer to determine whether such changes are acceptable and remain within the overall character of the beer in question. However, if catastrophic events would lead to the loss of the production culture, while it is possible to recover or renew the strain, it is unlikely that the essential character of the beer would be preserved.

Maintaining yeast health prior to pitching is therefore a combined effort of ensuring consistent fermentation conditions, providing sufficient wort nutrients, and maintaining appropriate storage conditions over the shortest possible time.

Yeast Storage

At the end of fermentation, due to exhaustion of wort nutrients and sugars, yeast will have developed an internal store of glycogen as an energy reserve. Operationally speaking, it is

important to reduce the potential for yeast to utilize this energy reserve too early. In the case of ale yeast, this necessitates the removal of the culture from the fermented wort as quickly as possible while also looking to minimize contact with beer residue as well as exposure to oxygen or air.

In the case of lager yeast, reducing exposure to oxygen is less of an issue as the culture will generally be held within the cone of the fermentation vessel and that in itself should offer a largely anaerobic environment. However, in all cases, reducing temperature then becomes important in preventing further metabolic activity during storage where ongoing fermentation of residual sugars that are bound up within the yeast can result in significant damage to the health of the cell. Localized hot spots of activity within the bulk-stored yeast can lead to the production of alcohol, CO_2, and heat as a consequence of fermentation and will cause physical damage or weakening of the cells. Metabolically, the energy required for such activity will generally be provided by depletion of glycogen reserves, which are vital to yeast during the very early stages of fermentation.

For yeast held in temperature-controlled storage tanks, low-speed, mechanical agitators can be used to further reduce the likelihood of hot spots of fermentation. However, agitation may increase the potential for exposure to oxygen if this is not effectively excluded from the storage vessel. CO_2 or nitrogen top pressure can be applied; however, whereas this is an effective barrier to oxygen or air, the use of nitrogen in particular can induce a longer lag period in yeast upon repitching.

Yeast Pitching

Pitching of healthy yeast is vital to the progress of fermentation. In particular, at this stage are the quantity of yeast and the introduction of oxygen. Yeast pitching rates will generally fall between 10 million and 25 million yeast cells per milliliter. The final rate will be dependent on many factors including the style of beer, strain of yeast, anticipated patterns of flocculation, and wort strength. Much of this will rely on the experiences of the brewer and knowledge of the performance characteristics of the strain in question. However, over or under pitching can have serious consequences on the progress of the fermentation and will likely result in the inefficient and incomplete utilization of wort carbohydrates.

In particular, over pitching may result in an uncontrolled, 'runaway' fermentation whereby the utilization of sugars will occur at a faster rate than anticipated. The significant production of heat as a consequence of the rapid assimilation of wort sugars, in turn, promotes further rapid and uncontrolled fermentation where the supply of wort nutrients and carbohydrates is quickly exhausted and unable to further support the health of the culture.

Sufficient introduction of oxygen is also important at pitching. However, the quantity of oxygen that is required by yeast will be somewhat dependent on the strain. Brewing yeast cultures range in their demand for oxygen. If the strain in question has a high requirement, then oxygen will need to be delivered as pure oxygen. However, in the case of yeast with a lower demand, this can be achieved using filtered air that has an oxygen concentration just over 20%, providing a maximum of 8ppm oxygen in air-saturated wort.

Fermentation Characteristics

With the exception of time and temperature, the profile of fermentation is the same for ale or for lager and can be described in three distinct phases, borrowed from the classical microbiological model of cell growth:

1. Lag phase
2. Fermentation (growth) phase
3. Stationary phase

Lag phase

The temperature of the wort into which brewing yeast will be introduced will differ depending on yeast strain. For lager yeast, pitching is usually at between 7 and 15 °C, whereas for ales, this temperature is higher, between 18 and 22 °C. Pitching is followed by a period of apparent senescence; however, in reality, yeast is metabolically active as the cells transition through a period of 'acclimatization.'

Wort nutrients, including vitamins, minerals, and metals, are all important to overall yeast health and growth. During this period, dissolved oxygen will be assimilated for the production of sterols and fatty acids required to build and strengthen the cell membrane. Nitrogen is also a key requirement for yeast cell growth and metabolism and in the case of wort will be provided primarily via amino acids. These will be assimilated to build proteins required for cell health and a variety of cellular mechanisms including the production of enzymes needed for the utilization of wort sugars.

Fermentation or growth phase

This stage of the process will see the utilization of the bulk of the fermentable wort carbohydrates with the production of ethanol and CO_2. Many other positive flavor compounds (primarily higher alcohols and esters) derived from yeasts and commonly associated with beer are also produced at this stage.

The utilization of wort sugars will take place in the following order:

1. Sucrose (converted into glucose and fructose before metabolism)
2. Glucose
3. Fructose
4. Maltose
5. Maltotriose

At the start of the fermentation phase, yeast will preferentially utilize glucose before other sugars can be consumed. This period will also involve the production of new cell biomass since the excess of glucose provides sufficient energy for yeast to devote to cell growth and reproduction. However, as glucose is consumed and is no longer present in significant quantity, compared to the amount of yeast, cells switch to a fermentative metabolism whereby the remaining sugars are utilized with greater focus on the production of ethanol and CO_2.

There are a variety of both positive and negative contributors to flavor and aroma produced during the growth phase of fermentation. During cell growth, yeast produces a significant quantity of acetaldehyde, which will then be further converted to alcohol and CO_2. However, the production of compounds referred to as vicinal diketones can be more problematic. These

compounds, primarily diacetyl and 2,3-pentanedione, are a natural metabolite associated with yeast growth that can introduce unwanted flavors to finished beer if they are not removed sufficiently. This forms one of the most important functions of the early, postfermentative, maturation process whereby the concentrations of these compounds, in the presence of yeast, should be reduced to a level that is no longer flavor-active. For this reason, diacetyl reduction is a key indicator of fermentation process efficiency.

Positive flavor attributes associated with fermentation are generally associated with the formation of higher or fusel alcohols and esters. Fusel alcohols are produced initially and are closely associated with the quantity of amino acids in the wort. However, in addition to this, the choice of yeast strain and the temperature of fermentation will have a significant impact on their production. Linked with fusel alcohols is the production of esters. These are the most prevalent positive flavor contributor associated with yeast. There are over 100 different esters in beer with a broad range of characteristics but that typically are described as being fruity, solvent, and banana-like. Generally, esters are produced from the combination of a fusel alcohol and a fatty acid and are influenced by fermentation temperature and yeast strain. However, there are a variety of other effects that can influence the formation of esters, including the composition and strength of the wort, the geometry of the vessel, and the extent of vessel top pressure.

There are a number of factors that can be used to describe or monitor the progress of fermentation. Primarily, an increase in alcohol and CO_2 will be related to the decrease in wort gravity or strength, which simplistically is a measure of wort density and is then related to the concentration of wort carbohydrates. Similarly, yeast cell density within the fermenting wort can be measured as indicator of yeast growth and sedimentation. However, the growth of yeast will also impact on the pH of the wort. As the yeast assimilates wort minerals and nutrients and excretes metabolites such as organic acids, the pH of the wort by the end of fermentation falls to a level of around 4.5. This, along with the depletion of nutrients, presence of hop acids, and the lack of oxygen, is also responsible for the extent of microbiological stability that is associated with beer as a fermented alcoholic beverage.

Stationary phase

The fermentation process comes to an end as the concentration of wort nutrients becomes exhausted, and in particular, those compounds associated with metabolic function and cell membrane health become depleted. At this stage, there will invariably be a residual quantity of fermentable sugar remaining. However, cells will begin to reenter a period of senescence and will start to flocculate. This, along with a reduction in CO_2 production, promotes sedimentation of the yeast within the vessel, which signals the end of the fermentation process.

The end of fermentation is also the point at which those 'creative' processes associated with beer making are completed. In essence, all downstream processes from this stage are concerned with either the modification of flavors or characteristics already produced, typified by maturation or aging, or the preparation of the beer for the appropriate packaging format. There is however one further possible fermentation stage that can be undertaken, and this is associated with cask or bottle 'conditioning.' In this case, a quantity of fermentable extract is required, but rather than a full fermentation, the objective is generally a modest increase in alcohol concentration to enhance the presence of esters and higher alcohols and produce some additional CO_2, which is then used to naturally carbonate the packaged beer.

See also: Alcohol: Properties and Determination; Beer: History and Types; Beer: Raw Materials and Wort Production; Spoilage: Yeast Spoilage of Food and Beverages.

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Beer: History and Types

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Introduction

Beer is a truly international drink, produced and marketed globally; it is the world's most widely consumed alcoholic drink and ranks third overall after water and tea. Alcohol (ethanol) is the most widely used psychoactive agent in the world, and its use is embedded in human culture. With the exception of Oceania and most of North America, tribal peoples from all major parts of the world knew how to make alcoholic drinks, and there have been very few, if any, societies whose people knew about alcohol and yet paid little attention to it.

Ancient people used indigenous plants as a source of fermentable material, and the 'first fermentations' were undoubtedly serendipitous. It is evident that man's early alcoholic drinks often used a mixture of fruit, grain, and honey as a base and were 'mixed' beverages – having affinities with wine, beer, and mead. It was some while before the individual categories of 'beer,' 'wine,' etc. emerged.

'Beer' may be simply described as "an alcoholic drink essentially made by fermenting sugar-rich extracts originating from a variety of plant starches." Today, most beer brewed is derived from cereal grains, which have been partially germinated (malted) – a process that leads to the release of fermentable sugars from starch. Thus, in its broadest sense, the term 'brewing' may be defined as "the combined processes preparing beverages from an infusion of sound grains that have undergone sprouting and the subsequent fermentation of the sugary solution (wort) thus produced by yeast." This results in a proportion of the fermentable carbohydrate being converted to ethanol and carbon dioxide. For reasons of space, most of this article concerns the history of 'European-style' beer, for it is this form that has become truly global.

The transition from nomadic hunter-gathering to a sedentary, crop-growing existence (the 'Neolithic Revolution') was a major step in the history of *Homo sapiens*, and early agriculturalists necessarily used whatever plants available to prepare mind-altering potions and drinks. Several reasons, including population density and climate change, have been forwarded to explain this change of lifestyle, but one school of thought attributes the transformation to the accidental discovery of the physiologically interesting beverages that resulted from fermented moist wheat and barley (i.e., 'beer'). The hunter-gatherers' precursor to beer was a gruel, or porridge, made simply by soaking grains in water.

In theory, any unspoiled grain could be employed provided that the seed had sufficient polysaccharide food reserve (endosperm). Cereal grains are the unique seedlike fruits (caryopses) produced by members of the grass family Poaceae (formerly Graminae). When raw, they present a relatively unattractive foodstuff. A combination of soaking in water or milling and mixing with water renders products that are far more palatable and digestible. Heating the grain/water mixture would have yielded a major improvement in digestibility and nutritional status – resulting from structural changes in starch and protein molecules. In particular, sugars would have been released. These, initially crude, processes have undoubtedly provided the basis for the malting, brewing, and baking industries that we know today.

For a variety of reasons, barley has evolved to become the grain of choice for the brewer, while wheat is preferred by the baker. The histories of beer and bread are intertwined, and some authorities have eloquently made the case for beer being considered as 'liquid bread.' In former times, cereals such as einkorn, emmer, spelt, oats, and rye have been used for brewing in Europe, while outside Europe, rice, millet, maize, and tuberous plants have been/are used.

With the brewing industry now dominated by a few multinational companies, it is difficult to envisage that, for most of its history, brewing was a domestic or, at best, a small-scale commercial enterprise, scarcely removed from its agrarian roots. Enough scientific and archaeological evidence now exists for us to believe that what we know as 'European-style beer' was first produced in the late fourth millennium BC by the Sumerians in southern Babylonia. The Sumerian civilization was located in Lower Mesopotamia – in the alluvial plain between the rivers Tigris and Euphrates – and it was one of the earliest of literate civilizations. Situated in the Fertile Crescent, one of the world's most important centers of cultural development, this region is one of the nuclei of early agriculture (Figure 1). Other centers almost certainly produced their own 'beer,' but we have little or no physical or textural evidence. What we do know is that barley was the ubiquitous cereal staple throughout the archaeological and cultural records of the earliest periods in both Egypt and Mesopotamia.

In brewing terms, the main species of relevance to the evolution of required Near Eastern founder crops are: wild einkorn (*Triticum boeticum*), wild emmer (*T. dicoccoides*) (both wheats), and wild barley (*Hordeum spontaneum*). Assuming that suitable grains evolved, other prerequisites for brewing include the facility to store grain, the ability to control fire, and the development of suitable (heatproof) containers – that is, ceramic technology (which first emerged in East Asia during the Late Pleistocene, ca. 18 000–10 000 BC).

Ancient Near East

The Neolithic Revolution in the lowlands of the Mesopotamian alluvial plain emerged around 7000 BC, but there is no conclusive archaeological evidence for the invention of beer brewing technology as early as this. Agriculture was so successfully organized in Sumer that grain surpluses were not uncommon, and it was to record such events, and subsequent transactions, that writing was developed. Sumerian culture saw the emergence of large cities and the stratification of society into different classes. Beer became one of the surplus

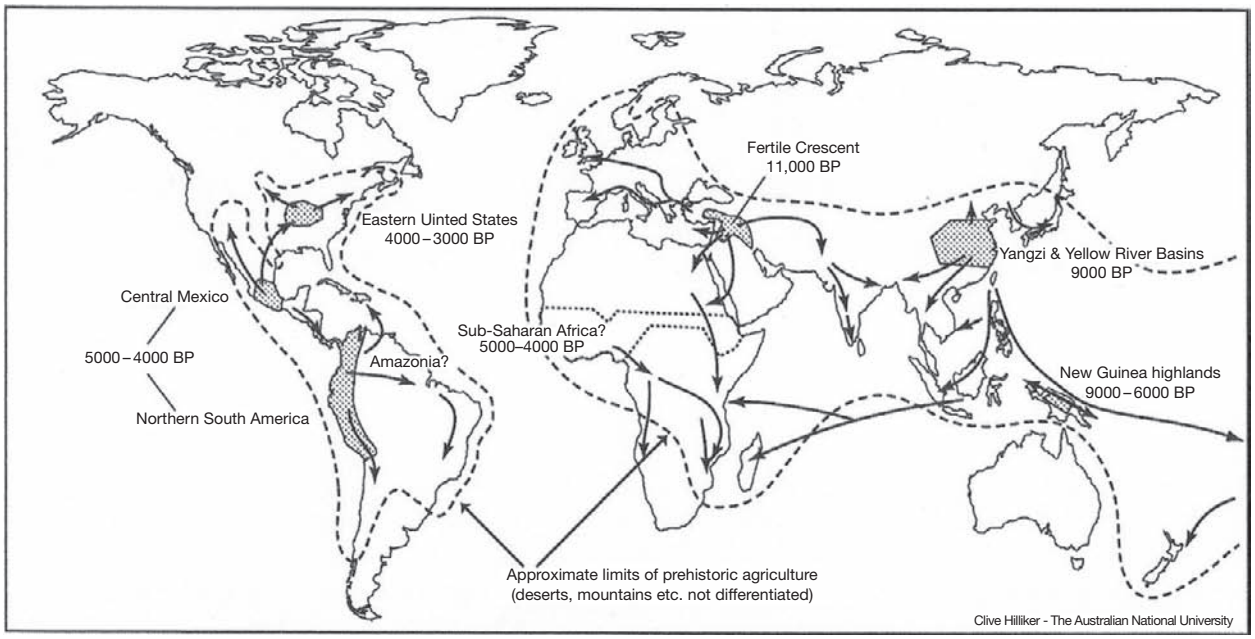

Figure 1 Areas where agriculture originated. Reproduced from Bellwood, P. (2005). *First farmers*: *the origins of agricultural societies*. Oxford: Blackwell, with permission.

products of this new society and brewing came under state control. As a result, the Sumerian bureaucracy of the time provided us with some of the earliest documentations of brewing beer. Proto-cuneiform texts, dating from around 3200–3000 BC, indicate that brewing was no longer a rural pastime, but was a central feature of the economy of Sumerian states. Hundreds of texts describe the administrative activities necessary for the production, distribution, and consumption of beer. Unfortunately, these texts tell us little about brewing technology (this knowledge was assumed!), but they do list raw materials, amounts and types of beer produced, and economic transactions.

Other literary documents yielding information about beer in ancient Mesopotamia can be found in the 'Code of Hammurabi,' which is considered to embody the world's earliest laws. King Hammurabi, who ruled Babylonia from ca. 1792 to 1750 BC, introduced a code of punishments dealing with all aspects of everyday life. Four of these relate to taverns and the distribution and price of beer.

In ancient Mesopotamia, brewers were so important that they were the only profession directly linked to a deity, the beer goddess Ninkasi. A cuneiform text discovered at the Sumerian city of Ur contained the 'Hymn to Ninkasi,' considered to be one of the oldest pieces of literature, and the script contains two Sumerian drinking songs dating from the eighteenth century BC. The first song outlines how Mesopotamian beer might have been brewed, while the second praises Ninkasi for providing beer drinkers with the opportunity to reach a 'blissful mood.'

When it became obvious that air was detrimental to these fermented brews, one saw the development of narrow-necked storage vessels common in archaeological sites in Mesopotamia. It is surmised that such vessels were designed to keep 'bad gasses' (air) out and 'good gasses' (carbon dioxide) in.

Figure 2 Shard of beer jug, ca. 3500–2900 BC from Godin Tepe. The grooves on the inner surface contain traces of 'beer stone.' Reproduced from Michel et al. (1992), with permission.

The first chemical evidence for beer comes from the site at Godin Tepe in the Zagros Mountains of what is now Iran. There is evidence that the neighboring Sumerians exploited this area for some of their essential commodities – and brought their beer-making knowledge with them. Numerous excavated samples of carbonized six-rowed barley have been recovered together with fragments of pottery jars with unique crisscross grooving on the inner surfaces (Figure 2). It is thought that

these grooves were designed to retain the sediment from the beer after storage. Chemical analysis of sediment found in the grooves indicated the presence of calcium oxalate, a major insoluble component of 'beer stone' (a scalelike deposit that accumulates in fermentation vessels and beer storage tanks). Oxalic acid is present in trace amounts in malt and combines during brewing with calcium ions to form the insoluble salt. Ancient jars known to have contained wine, cider, or mead do not show any evidence of calcium oxalate deposits.

Until recently, it has been widely accepted that brewing first commenced in the civilizations of Mesopotamia, but excavations at Göbekli Tepe in southeastern Turkey have indicated that brewing may be much more ancient. At the dawn of the Neolithic (Pre-Pottery Neolithic, i.e., some 6000 years older than Stonehenge, and seven millennia before the Great Pyramid of Giza was built), hunter-gatherers assembled at Göbekli Tepe and created a cultic center. The site ('Tepe' = mound), beset with dozens of massive stone pillars, was not used for habitation, but apparently for executing ancient rituals, many of which involved feasting. Even at this early stage in human history, there is evidence for plant domestication and for beer brewing, and if the latter is confirmed, this would represent the earliest known date for such activity.

From two ancient sites in what is now northern Syria, it is evident that ancient Near Eastern settlements differed in their beer production ethos; sometimes, every household in a settlement brewed, and sometimes, there were specialized (professional?) brewers capable of supplying numerous customers. The former situation is known to have applied to the Late Bronze Age site at Tell Bazi on the eastern bank of the Syrian Euphrates (some 60 Km south of the Turkish border), where seemingly every house contained remains of brewing pots. From excavations carried out at Tell Bazi, it has been possible to reconstruct their malting and brewing methods. Archaeological evidence from the site indicates that two-rowed barley (*Hordeum distichum* L.) was being widely used for malting and brewing, with the possibility that other grains were also used.

Conversely, at Tell Sabi Abyad in the Balikh Valley, 'a brewer' is specifically mentioned in the texts and so beer was centrally brewed and then distributed. A similar situation appertained for bread. Associated with brewing were several types of pottery jars and bowls. Beer brewed here was evidently not sieved or strained, as is suggested by the presence of several bronze filter straws that would have been used to keep large particulate matter out while drinking. Indeed, in a similar vein, the first visual evidence that we have relating to the actual drinking of beer comes from a sealing found at Tepe Gawra, in northern Iraq, dated ca. 4000 BC. The seal depicts two people with bent tubes drinking beer from a large jar (**Figure 3**).

The semimythical Hymn to Ninkasi (of which three copies exist, all written in the Old Babylonian period, ca. 1800 BC) gives us some information about brewing at this time. Most significant is the fact that *bappir* bread (a kind used for storage purposes – only eaten in times of food shortage) was a beer ingredient, as was malt. There is also mention of *bappir* dough being mixed with 'sweet aromatics' using a "big shovel... in a pit." From other sources, it seems as though bappir was always an ingredient of old Sumerian beer.

Ninkasi was head brewer of the great god En-lil and thus of all the gods. It was Ninkasi's responsibility to provide alcohol,

Figure 3 Clay sealing from Tepe Gawra, Zagros Mountains, northern Iraq, showing two people drinking through straws. Dated to 4000 BC. Reproduced from the University of Pennsylvania Museum of Archaeology and Anthropology, with permission.

especially beer, for the temples of the major religious center of Nippur (whose ruins lie ca. 180 km southwest of Baghdad). To the ancient Sumerians, Ninkasi was the personification of beer, and one translation of her name is 'lady who fills the mouth.'

A second seemingly invariable ingredient of beer, although not mentioned in the hymn, appears in protocuneiform documents of the Late Uruk period, and this is *munu* – an entity delivered in sacks, baskets, or vessels. Consensus among scholars is that this translates as malted barley. Emmer, an ancient wheat variety used in ancient Egyptian brewing, is commonly mentioned in ancient Near Eastern documents – but not as a beer ingredient.

A major feature of ancient Near Eastern civilizations was the formation of large conurbations, and it has been argued that beer played no small part in this process. As Alexander Joffe opined: "The appearance of beer has been regarded by some as an indicator of social complexity – the rather prosaic knowledge of brewing being regarded, in a sense following the Sumerian lead, as a sign of civilized behavior."

As urbanization occurred, it was imperative that risks associated with food procurement were minimized, and this was usually achieved through state control. In this respect, beer played an important role in securing the allegiance of the labor force engaged in food production. Also, increased population densities would invariably lead to the contamination of water supplies, and beer, being readily accessible, provided an

obvious alternative drink to water. It was also a cheap source of calories and dietary fiber and was, of course, a stimulant.

The fact that beer was a linchpin of Mesopotamian culture can be gleaned from the *Epic of Gilgamesh* (Gilgamesh was king of Uruk) where the wild, hirsute Enkidu was 'civilized' by a female who taught him to eat bread and drink beer.

Ancient Egypt

Evidence for the production and use of beer in ancient Egypt extends back to the Predynastic era (5500–3100 BC), with early twentieth-century discoveries of beer sediments from jars at Abadiyeh, a Predynastic cemetery on the east bank of the Nile, in Upper Egypt, and at Naqada, one of the largest Predynastic sites in Egypt (situated some 26 km. north of Luxor on the west bank of the Nile). The Predynastic site at Hierakonpolis contained numerous large, fixed vats, and this may represent the earliest known brewery site. Later, it seems as though brewing was mostly a domestic activity and was carried out in smaller, portable, pottery vessels. We also know from Early Dynastic (3100–2686 BC) written records that beer was very important at this time and was a well-established feature of the culture of that period. It is thus highly likely that Egyptian brewing had its antecedents in Predynastic times, and information from the Near East and the Middle East strongly indicates that humans knew how to make bread and brew beer in 6000 BC.

Classical Greek writers (erroneously) credited the Egyptians with having invented beer, and the geographer Strabo (ca. 63 BC to AD 21) commented that "Barley beer is a preparation peculiar to the Egyptians, it is common to many tribes, but the mode of preparing it differs in each." He also noted that it was one of the principal beverages of Alexandria. The Greek historian Diodorus Siculus, in his monumental work *Bibliotheca Historica*, mostly written between 60 and 30 BC, praised the quality of Egyptian barley beer, saying that "They make a drink of barley. . .for smell and sweetness of taste it is not much inferior to wine." Praise indeed from an oenophile. Diodorus Siculus also attributed the invention of beer to the god Dionysus, a god who was rather more associated with wine. To the Greeks, Dionysus was the equivalent of the Egyptian deity, Osiris, who the ancient Egyptians believed had invented beer. Osiris was one of their most important deities, whose principal associations were with fertility, death, and resurrection. Osiris was also credited with spreading beer into countries where the grape was unknown.

In ancient Egypt, beer was king. All sections of the community drank beer, from the pharaoh downward, and it was a product that was inextricably woven into the fabric of daily existence and was a feature of religious festivals and state occasions (when 'special' brews were produced). Beer was drunk daily as a highly refreshing and more reliably potable substitute for water, which in an urban context would become notoriously unhygienic. Beers brewed for everyday drinking would not be highly alcoholic and would have had a very short shelf life; this necessitated daily brewing and immediate consumption. Beer was especially important in regions where the vine would not grow, where it was considered, with bread, to be an indispensable staple. Generally speaking, grapes were much more expensive than grain in ancient Egypt, and so, wine was an expensive commodity.

The barley beer of Egypt was called 'zythos' by the classical writers, a name that refers to its propensity 'to foam.' It was Aristotle's student Theophrastus who first used the term 'zythos' to describe: "Those beverages, which were prepared, like those made of barley and wheat, of rotting fruits." The word has the same Greek derivations as the words 'leaven' and 'yeast.'

Emmer continued (up to the AD fourth century) to be the primary wheat in ancient Egypt long after it became superseded in the Near East. This preference for emmer over free-threshing wheats may have been due to some religious significance. By the New Kingdom period (1550–1069 BC), two types of barley – two-rowed (*H. distichum* L.) and six-rowed (*H. vulgare* L) and emmer (*Triticum dicoccum* Schübl.) – were being used for brewing. Apparently, barley was the predominant brewing cereal during the Old and Middle Kingdoms (2686–1650 BC) but was replaced by emmer by the New Kingdom. Flavorings, where used, consisted of figs, dates, honey, mandrake, lupine, and skirret; hops were not used.

Until the last couple of decades, it was believed that all brewing in ancient Egypt commenced by soaking partially baked barley/emmer bread in water and then allowing the resultant 'gruel' to ferment. This is exactly what happens when the ancient, wheat-based, drink 'bouza' is prepared. Bouza ('boozak' and 'boozeh') is an indigenous drink of Nubia and Sudan, which can now be found in Egypt and other parts of Africa, and is typically drunk by the working classes. It is opaque and can be consumed 'young' or 'old,' according to fermentation time. The latter is obviously more alcoholic (ca. 7% alcohol by volume (ABV)) and has greater nutritive value – with more amino acid and B-group vitamin content. Most bouza is consumed 'fizzy,' for it will still be fermenting. The beer can be partially clarified by passage through a cloth. The alchemist Zosimus of Panopolis gave us AD fourth-century accounts of the preparation of both zythos and bouza.

Work by Delwen Samuel over the turn of this century has shown that, certainly by the New Kingdom, the ancient Egyptians malted grain for brewing. Most work was carried out in the workmen's villages of Amarna and Deir el-Medina, where barley (mainly) and emmer were the brewing grains. A lack of other plant remains (not even dates) suggests that plant flavorings were not used at these sites and that the main body of the beer came from malt.

The importation of wine into Egypt by the Greeks during the Ptolemaic period saw brewing and selling beer become tightly regulated, and later, brewing became a state monopoly, with beer being taxed for the first time. Despite this, the drink was still being consumed during both secular and sacred rituals.

Prehistoric Northern Europe

Barley and wheat are not native to this region, and they were introduced from the Near East/Anatolia. The situation is further complicated by the fact that there is no 'single European Neolithic' per se, and the Mesolithic/Neolithic transition occurred during the sixth and fifth millennia BC. Processing

grains for brewing were vastly different from the Middle and Near East for soaked grains left out to dry merely rotted. Thus, heating (kilning) grain to dryness was a necessity.

Perhaps, the most convincing evidence of germinating and kilning malt comes from the Early Iron Age settlement (fifth to fourth century BC) of Eberdingen-Hochdorf, southwest Germany. This was a Celtic settlement that contained structures seemingly purposely designed for malting and drying grain; the main finds being of hulled barley and spelt (dinkel) (*Triticum spelta* L.).

Artifacts from the Neolithic Orcadian site at Skara Brae indicate that the conversion of barley into malt and ale was an important aspect of life, and evidence from various other Neolithic and Bronze Age sites in Scotland suggests the use of a mixed barley beverage, particularly in association with characteristic ceramic vessels. Most prominent were 'bell beakers' (Figure 4), part of a European culture (the 'Beaker people') that commenced at the end of the Neolithic (ca. 2900 BC) and ended in the early Bronze Age (ca. 1800 BC). Some authorities believe that these beakers were designed for consumption of alcoholic beverages, with beer and mead residues having been identified. The culture, with all its paraphernalia, certainly spread across Europe in the form of a cultural complex and marked a period of unprecedented cultural contact. Brewing knowledge is likely to have spread with the Beaker people.

Classical Antiquity to Medieval Europe

The ancient Greeks are often seen as having been oenophiles in the extreme, even though they were originally beer drinkers. The Minoans, the pre-Greek inhabitants of Crete, almost certainly made barley-based beverages. The Greek prejudice against beer arose from a cultural need to distinguish themselves from 'barbarians' (principally the Celts and the Germanic tribes); to the Greeks, wine was a 'pure, hot, manly drink,' while beer was 'corrupted, cold, and effeminate.' Aristotle declared that men who are drunk on wine fall down face foremost, while those intoxicated with barley beer lie outstretched on their backs! As a result of this attitude, roughly between the eighth century BC and the AD sixth century in Western Europe, wine is documented as the major (only?) alcoholic beverage consumed. This may be partly due to its adoption by 'elites' and/or its increasingly popular use in sacred contexts.

Figure 4 Bell beaker.

Greek prejudices against beer were assimilated by the Romans, but they had a need to meet the needs of their troops in regions of their empire where the grapevine would not flourish. In this context, the earliest written references to beer being brewed in the United Kingdom come from the Vindolanda tablets, dated to the AD first century. Vindolanda was an outpost just south of Hadrian's Wall, from which excavated tablets clearly indicate that beer (*cerveza*) was supplied to Roman soldiers. Also mentioned were 'a brewer' (*cervesarius*), malt, and a brewery. The recovery of malt in Roman contexts from various sites in their northern provinces firmly suggests that the Romans routinely consumed beer, but Roman writers such as Tacitus indicate that beer was the main drink of Celtic and Germanic tribes. Many Iron Age granaries have been found in Britain.

The Celts had been known from Central Europe since around 700 BC and gradually expanded into other parts of Europe. They possessed notable brewing expertise, as noted by Pliny the Elder, who affirmed that "the people of the Western world have also their intoxicating drinks made from corn steeped in water. . .The Spanish provinces have even taught us the fact that these liquors are capable of being kept till they have attained a considerable age." For the Celts, brewing was a domestic activity, typically carried out by women, and this theme was to be continued until brewing became industrialized. Beer at this time was mainly made from barley, although wheat and millet were also commonly used. In certain areas, where barley would not grow, rye and oats were used.

After the fall of the Roman Empire, our information about beer increases because sources increasingly come from northern Europe, where grapevines were more difficult to grow. One legacy left by the Romans was the notion that, in some Celtic tribes, at least, beer was the drink of 'common folk' and wine was for chieftains. Under Roman influence, many Celtic tribes turned to wine, but in regions free from Roman control, such as Ireland, Celtic beer culture survived.

In post-Roman Britain, the Anglo-Saxons were oceanic drinkers of beer (being drunk was considered honorable!), and the drink was certainly widespread during the early Middle Ages in northern Europe. 'Ale' was the most common drink in Wales at this time, but given their propensity for drinking it, there is no recorded evidence of brewing techniques, equipment, or sites from the Anglo-Saxons.

Beer is also widely mentioned widely in Norse literature; Viking beer consumption being sizeable enough for their great god Ódin to warn against drunkenness. They used various names for their beers, including *öl* (which equates to the Old English *ealu*) and *björr* (Old English *beor*), the latter probably containing honey as well. Beer drinking was especially important to Nordic seasonal religious festivals.

After 500 odd years of the Dark Ages in Europe, when there was apparently little innovation in brewing, beer became closely associated with religious establishments. Monasteries were probably the only institutions where beer was regularly brewed on anything like a commercial scale at this time; otherwise, brewing was mostly a household chore. During the Middle Ages (ca. AD 800–AD 1300), people were settling in larger communities, and technology from earlier periods had been fully assimilated, so brewing gradually moved away from the home and became a more commercial venture, certainly for some. The plans of St. Gall monastery in Switzerland, founded in the eighth century, include a kiln, malthouse, mill room, three brewhouses, and a storage facility. Three types of beer, varying in strength, were brewed: *prima melior*, intended for the monks and visiting VIP's; *secunda*, for the lay brothers; and *tertia*, for pilgrims, beggars, etc. These were probably produced by using three consecutive wort runoffs, of decreasing strength, from one mash – a technique that lasted several centuries.

Most importantly, monasteries were responsible for the introduction of hops (*Humulus lupulus* L.) into brewing. Prior to this, innumerable herbs were employed to flavor/stabilize beer; a herb mixture called 'gruit' was widely used. In AD 768, the Abbey of St Denis in Paris mentions 'humlonariae' (hop gardens?), probably the oldest mention of hops, but our first hop documentation in a brewing context arises from the Benedictine monastery in Corbie, near Amiens, where statutes of AD 822 refer to 'the gathering of hops.' A little later, hop cultivation (in 'humularia') is mentioned in documents from the Hochstift monastery, Freising, Bavaria. Early archaeobotanical evidence (ninth to tenth century) for hops was obtained from Haithabu, an important Viking trading station on the Schleswig coast, insinuating that the plant was being traded in Europe. Also notable in that context is the ninth-century 'Graveney boat,' a wooden cargo vessel replete with hops, stranded in the Thames estuary. The exact function of hops in brewing remained a mystery until Abbess Hildegard (1098–1179) of Rupertsberg, near Bingen, avowed: "its bitterness prevents some spoilage in drinks to which it has been added so that they last much longer."

From the early medieval period onward, hops (female flowers) were a common beer additive, especially in regions where sweet gale (*Myrica gale* L.), a major component of 'gruit,' did not grow. Sweet gale seems to have used for brewing in the centuries before and after the birth of Christ in its distribution areas and continued in this role until it was supplanted by *H. lupulus*. As hop usage spread, certain north European towns, such as Bremen and Hamburg, became famous for their beer, much of which was exported to Flanders and the Netherlands. Bremen beer is mentioned in Holland in 1252. Hamburg was to become 'the Hanseatic League's brewhouse,' and in 1369, the town had 457 hop-using breweries and was exporting enormous volumes of beer! All this was only possible because of the incorporation of the hop.

Soon, hop cultivation and use in the brewery spread northward, and Low Country beer arrived in the United Kingdom, being first recorded in Great Yarmouth, Norfolk, 1362/63 import tolls. Hopped beer import and brewing were controlled by mainland Europeans for some time, the English preferring to brew their unhopped 'ale,' and it was not until the early sixteenth century that hops were cultivated in the United Kingdom. In general, the introduction of hops met with resistance – but the agreeable flavor they imparted and their preservative properties meant that they became universally used in brewing. Brewers of unhopped beers relied upon high alcohol content to impart keeping quality, whereas hops also allowed the brewer to produce 'weaker,' stable beers and thus obtain more beer from his grain charge. Gradually, hopped 'beer' became prevalent in conurbations, while unhopped 'ale' survived in rural areas.

To obtain the bitterness from hops, boiling is necessary and this required special (expensive) equipment – the copper or kettle. Such an item would be beyond most domestic brewers, but affordable by commercial brewers – so, the overall pattern of brewing changed. Brewing became more regulated in cities and towns, and guilds emerged to guard the status of the brewer and the quality of beer. An early quality protection measure was the Bavarian 'commandment for purity' (*Reinheitsgebot*) of 1516, which decreed that only malt, hops, and water were to be used in brewing (yeast was added later).

Towards Industrialization

Beer consumption in Europe increased enormously during the fifteenth and sixteenth centuries, and commercial brewing became very profitable; and with profits came taxes. Both beer and its raw materials became taxable, the first beer-related tax in the United Kingdom (on malt) being raised by King James I in 1614. At this time, however, domestic brewing still accounted for around half of beer produced.

By the early 1700s, European cities and towns were expanding, and many urban breweries increased in size to accommodate population increase. The next major step would be brewing on an industrial scale; something spurred by the Industrial Revolution, the first phase of which began in the United Kingdom during the eighteenth century and then spread gradually throughout Europe. Even by the start of the eighteenth century, beer production in London was dominated by 'common brewers,' who supplied various outlets. 'Victualler brewers,' who brewed solely for their own inn or tavern, declined in number, and industrialization of brewing accelerated this decline.

For brewers, the harnessing of steam power was probably the singular most important aspect of the Industrial Revolution, and the first brewery steam engine (from Boulton & Watt) was installed in east London in 1777. By 1801, 14 steam engines were operational in London brewhouses, and the likes of Truman and Whitbread were able to expand dramatically and produce vast amounts of beer. Other factors that enabled brewers to expand were improved iron making and concrete making and improved transport links (canals and then railways). Unhopped ale virtually disappeared from Europe by the seventeenth century.

With industrial-scale brewing came the first 'industrial beer,' London Porter, about which much has been written. Suffice to say the drink probably arose as a response by London brewers to increased malt tax. By comparison to malt, hops were relatively cheap, so by using cheap brown malt and a very high hop rate, a dark, luscious, relatively weak beer with

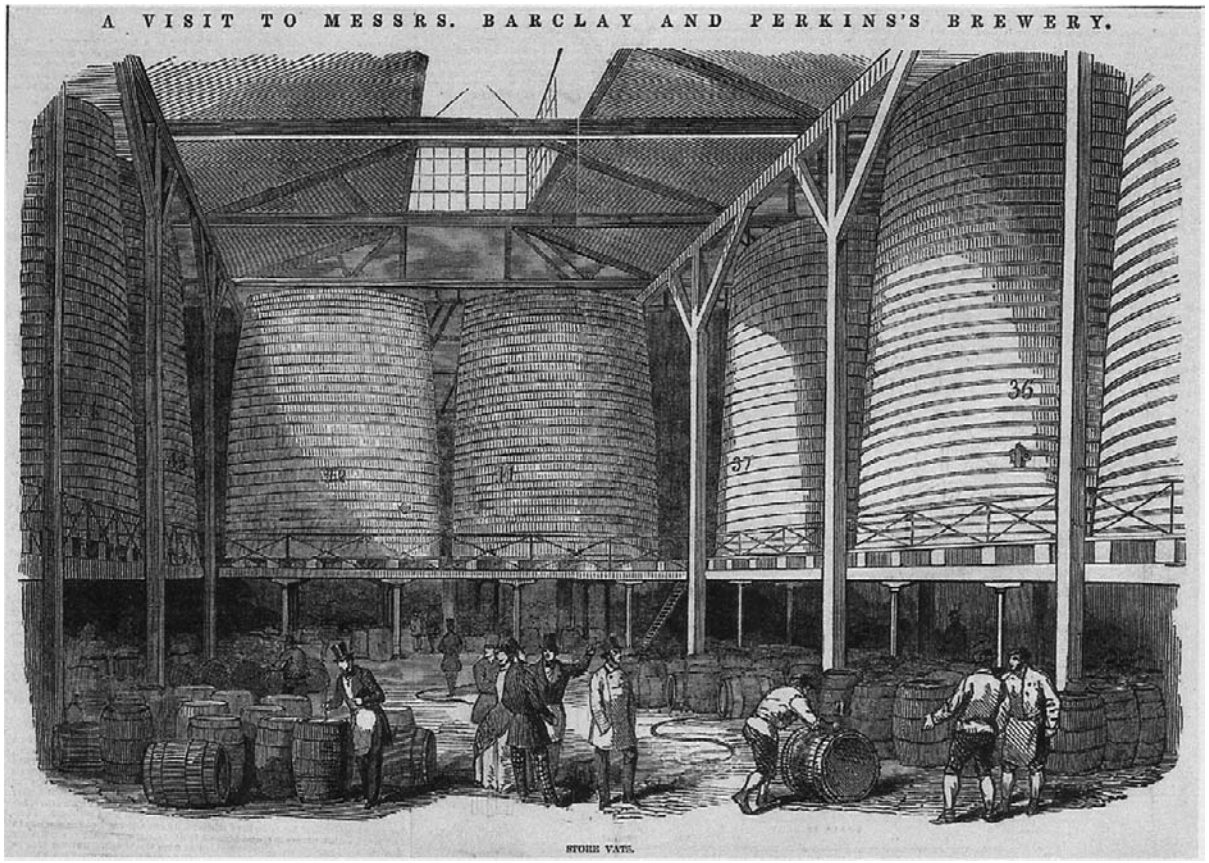

Figure 5 The great porter vats at the Brewery of Barclay, Perkins, and Co., London. Reproduced from 'Illustrated London News,' 6 February 1847.

excellent keeping qualities resulted, which sold to the working class at a competitive price. There was no need for prolonged storage, as was the case with most beers of the time, but its robust nature and low production cost meant that it could be brewed on a huge scale. Enormous wooden vessels (Figure 5) were constructed by some London brewers to accommodate demand – which peaked in the 1820s, London Porter being gradually usurped by mild beers and pale ales.

With large, semimechanized breweries and advancements in science, the late eighteenth century witnessed the first attempts to conduct accurate measurements during brewing. Pioneers were Michael Combrune (thermometry) and John Richardson (saccharometry), who brewed in London and Hull, respectively. Such innovations vastly improved beer quality.

British beers were top-fermented, but in parts of continental Europe, such as Bavaria, bottom fermentation was practiced, probably since the fifteenth century. Brewing here was impossible, even forbidden, during summer months, and beer brewed from October to March was stored for summer consumption. Bavarian monks used nearby caves as cool areas (in the absence of caves, ice (from frozen lakes, etc.) was used in brewery cellars) to store their beer, which proved to be bright, sparkling, and stable. Beer was (necessarily) fermented at low temperature with a low degree of attenuation, and this enabled viable yeast cells to survive after primary fermentation. This yeast continued to ferment slowly during cold storage, and these practices encouraged the cryophilic, bottom-fermenting yeast to evolve. Beers thus produced had a unique flavor profile and were termed 'lagers,' after the German verb 'lagern' ('to store'). These early, bottom-fermented beers bore little resemblance to today's lagers, and the now ubiquitous 'Pilsner' style emerged in 1842 when German brewer, Josef Groll, was appointed head brewer at the Civic Brewery in Plzen. Armed with Bavarian yeast, soft Bohemian water, and local, lightly cured malt, he produced a unique, pale, creamy beer that proved extremely popular and the template for around 95% of today's beer. The style was recreated in Germany, and 'lager yeast' was disseminated all over the globe, Germany becoming a major brewing nation.

Science Plays a Role

Scientific/technological developments at the end of the eighteenth century paved the way for further industrialization of brewing during the following century. Another feature of the nineteenth century in Europe was the gradual spread of bottom-fermented beers at the expense of their top-fermented sisters, the latter only really surviving in Belgium and the United Kingdom. An important innovation was the use of a Carl von Linde mechanical refrigeration machine in Munich's Spaten brewery in 1873. Beer could now be safely brewed all year round and stored with reduced risk of infection. Three years later, Pasteur published his *Études sur la Bière*, which dealt with

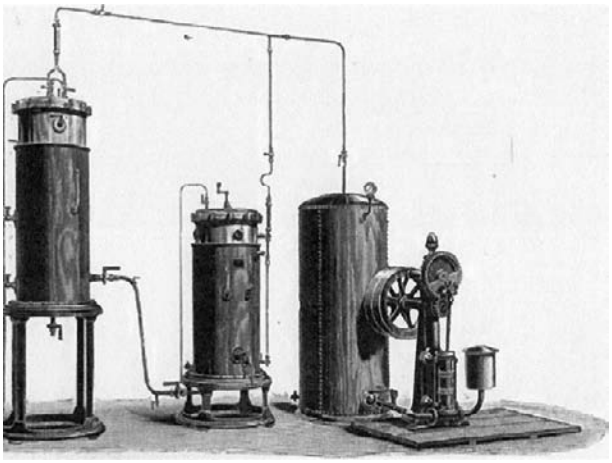

Figure 6 Emil Hansen (1842–1909) – his yeast culture equipment.

beer contamination, having previously proved beyond doubt that the single-celled yeast was the cause of fermentations.

From now on, chemistry and the 'new' science of biology were rigorously applied to brewing, and seminal work was conducted at dedicated laboratories. Most significant was the Carlsberg Laboratory in Copenhagen, where, in 1883, Emil Hansen isolated and propagated the first pure yeast strain (Figure 6). It was bottom fermenting, and he named it *Saccharomyces carlsbergensis*. Hansen's work transformed the way brewers worked and paved the way for our modern fermentation industries. Other fundamental work in the Danish laboratory was carried out by Winge (yeast genetics), Sørensen (pH scale), Kjeldahl (N determination), and Linderstrøm-Lang (protein chemistry).

At the start of the twentieth century, around 69% of all beer was being produced in Germany, the United Kingdom, and the United States, but all three of these countries were soon to see beer volumes fall dramatically, the United States because of prohibition and the other two due to World War I. In particular, the latter changed UK brewing forever – with a fall in beer strength and much more regulation. In Britain, there were 6447 commercial breweries in 1900, but between 1910 and 1920, nearly 1600 breweries disappeared, and, by the start of World War II, only ca. 1400 such enterprises remained. Post-1945, for economic reasons and the takeover mania exhibited by six or seven national brewers (called 'consolidation'!), by 1971, only 170 commercial brewing sites were operational in the United Kingdom, a new low. Inevitably, one of the main casualties of large-scale brewing and a contracting number of brewers was consumer choice.

Not by coincidence, 1971 saw the founding of the Campaign for Real Ale (CAMRA), a UK consumer group determined to improve consumer choice. The number of UK breweries declined slightly after CAMRA's inception, until the nadir was reached in the late 1970s, when some brewers, made redundant by brewery closures, began to start their own small concerns; the microbrewing revolution had started, and from 1980 onward, the brewery count has steadily increased, there now being around 1200. The ethos quickly spread to the United States ('craft beer'), and a similar thing is now happening in Europe and further afield.

Beer Types

There are basically two types of modern beer – 'live' and 'dead' – the difference being whether the product remains in contact with viable yeast or not. If live yeast is removed from beer, by filtration or pasteurization in the brewery, it is described as 'brewery-conditioned,' and no further conditioning will occur once in its container (keg, can, or bottle). In the United Kingdom in particular, beer is often left in contact with live yeast so that a slow secondary fermentation ('conditioning') can take place. This 'live' beer is usually placed in a cask, sometimes a bottle. CAMRA's remit was to save cask-conditioned ('real') beer. With brewery-conditioned beer, once yeast ('life') has been removed, oxygen must be kept at arm's length, and the beer is suffused with CO_2 or a mixture of CO_2/N_2; this enlivens the product.

Since ancient times, man has brewed a variety of beers, as witnessed by the 70 odd types described for Babylonia. The variety of flavoring plants used in brewing over the years coupled with the various sources of extract could have produced an unimaginably large variety of beers. Although today's raw materials are more standardized, it is still possible to brew a vast range of beers, as witnessed at the biennial World Beer Cup held in Denver, Colorado, in April 2014, where there were over 90 style categories for judging! These days, microbrewers tend to lead the way in beer-style innovation. Space permits me to mention only a few examples.

The modern difference between lager and ale is down to fermentation, and in the latter category, the old British distinctions give us 'milds' (light and dark), usually of low strength (<4% ABV) and 'bitters' (light amber–mid brown) (these include the categories 'ordinary' (the so-called 'session' beers, <4% ABV), 'best' (ca. 4.5% ABV), and 'special' (>5% ABV)). Most India Pale Ales (IPAs) fall into the 'ordinary' category – far weaker than their nineteenth-century counterparts. All but the milds are 'hop-driven' beers, and one may broadly categorize other styles as being: 'malt-driven' ('dunkel,' 'Düsseldorf altbier,' 'barley wine,' and 'Scottish ale'), 'roast malt-driven' ('porter' and 'stout'), 'smoky' ('Franconian rauchbier'), and 'fruity and spicy' ('hefeweizen' and 'saison').

Belgium's 'lambic beer' is the oldest surviving commercial brewing style and is a sour wheat (70% malted barley and 30% unmalted wheat) beer brewed in and around Brussels, traditionally between October and April. The essence of such beers is their complex fermentation that involves naturally occurring bacteria and yeasts (i.e., is spontaneous). Only aged (oxidized) hops are used and these are subjected to a lengthy boil. Lambics have unique aromas, and there are several styles: 'young' (ca. 1 year), 'old' (>3 years old), and Faro (sweetened 'old'). Gueuze is a blend of 'young' refermented with 'old.' All are slightly sour (lactic acid) with resinous and cheesy notes, while Kriek is made by steeping Scarbeek cherries in lambic for 6 months. Alcohol contents vary with type.

Another, lesser known, spontaneously fermented beer is 'sourish shchi,' an unhopped, unboiled Russian drink that has a grain bill of malted barley, wheat, rye, and buckwheat. Honey is also used. Yeasts and bacteria for fermentation come from raw materials and equipment. Highly popular in the eighteenth and nineteenth centuries, it survives today (2.0–2.5% ABV) as a sparkling drink, for it is given a secondary

Figure 7 Women chewing maize for 'chicha' production in the town of Arequipa, Peru. Reproduced from Marcoy (1873).

fermentation in champagne bottles. Unlike 'sourish shchi,' the mildly alcoholic (ca. 1.5% ABV), rye-based, Slavic beer 'kvass' is lowly carbonated. Made from malt and soaked rye bread, which is then subjected to alcoholic/lactic fermentation, the drink is healthy and nutritious. Kvass has a strong flavor and sour taste and is usually flavored with fruit and/or herbs.

'Steinbier' is another ancient style, going back to times when the wort boil was raised by repeatedly heating stones in a fire and placing them in the wort. As time progressed, wort would caramelize on the stone surface and impart a sweetish, smoky flavor.

'Weißbier,' the classical wheat beer of Bavaria, also known as 'Hefeweizen' ('yeast wheat') in bottle-condition form, is a turbid (unfiltered) drink made from grist that must contain at least 50% wheat malted wheat. This style probably has similarities with ancient Near Eastern beers. It is normally top-fermented (rare in Bavaria!) and is characterized by phenolic notes, due to the presence of 4-vinyl guaiacol. Special yeast strains are used.

'Kölsch' is a pale, top-fermented beer (ca. 4.5% ABV) brewed only in Köln and Bonn. After the 'ale' fermentation, it is matured at low temperature (3–5 °C). 'Altbier' is similarly brewed and matured; 'Chicha' is a pre-Colombian Andean drink, typically brewed by women and normally made from maize (*Zea mays* L.), although cassava and quinoa can be used. Today, the drink can still be purchased in 'chicharias' throughout Central and South America. The name is derived from *chichal*, which translates as 'with saliva' or 'to spit,' because the original way of breaking down maize starch was to chew the grain and expectorate it for brewing (**Figure 7**). No hops are used, and the beer usually has $<5\%$ ABV.

'Sahti,' a top-fermented, full-bodied, live, turbid Finnish beer (6–8% ABV), is a relic of an ancient farmhouse brewing tradition and is reckoned to be the sole surviving 'primitive' ('fossil') beer style in Western Europe. Grains used are malted barley (and, variously, wheat, oats, and rye), either in malted or unmalted form. ABV is in the 7–12% range. A less potent, partially fermented (sweet) form is also brewed, called 'naisten sahti' ('sahti for women').

In Africa, where the beer market is dominated by Nigeria and South Africa, malted barley and wheat are difficult to cultivate in many areas and are replaced by sorghum, maize, roots, tubers, and plantains. Beer produced from these sources is very different from 'European-style' beers, although sorghum is widely malted for brewing clear, lager-style beers. There is a plethora of African beers, best known being turbid 'kaffir' or 'Bantu' beer, which is known under various tribal names. An artisanal kaffir beer would be expected to contain 2.0–4.0% w/v ethanol, 0.3–0.6% acid (as lactic), and 4–10% solids. Drinking African beer, which is often turbid, was/is often a communal activity and involves drinking through straws (**Figure 8**).

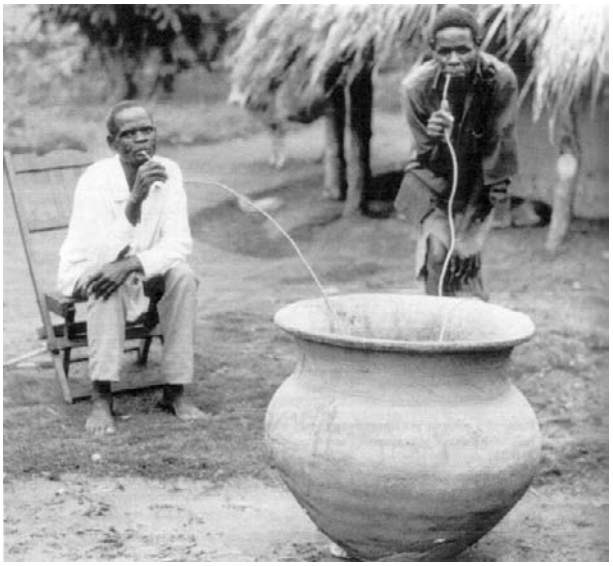

Figure 8 Beer being consumed via straws in Kenya. Reproduced from Dietler and Hayden (2001), with permission.

See also: Alcohol: Metabolism and Health Effects; Alcohol: Properties and Determination; Barley; Beer: Fermentation; Beer: Raw Materials and Wort Production; Bread: Breadmaking Processes; Bread: Chemistry of Baking; Bread: Types of Bread; Cassava: The Nature and Uses; Cereals: Dietary Importance; Cereals: Storage; Cereals: Types and Composition; Enzymes: Analysis and Food Processing; Fermented Foods: Origins and Applications; Fermented Foods: Use of Starter Cultures; Folic acid and Folates: Physiology and Health Effects; Lactic Acid Bacteria; Maize; Millets; Oats; Pasteurization: Effect on Sensory Quality and Nutrient Composition; pH: Principles and Measurement; Quinoa; Rice: Types and Composition; Sorghum: A Novel and Healthy Food; Wheat: The Crop; Yeasts.

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Beer: Raw Materials and Wort Production

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Introduction

The purpose of beer brewing is to hydrolyze the starch from barley malt together with maize (corn), wheat (sometimes malted), rice, sorghum (malted and unmalted), unmalted barley, or sugar/syrups into a sugary nitrogenous, hopped fermentable liquid called wort and convert it into an alcoholic carbonated beverage by yeast. This article will consider the raw materials employed and the production and composition of the resulting wort: the chemistry, biochemistry, and microbiology of wort fermentation.

The wort production process is outlined in Figure 1. Malting and mashing (together with fermentation) are largely enzymatic processes. The principal brewing raw material, malt, contains extractable components (starch, proteins, etc.) and enzymes (amylases, proteases, etc.). However, malt is an expensive raw material. The purpose of malting and mashing is to hydrolyze the starch/protein sources into a sugary nitrogenous fermentable liquid called wort, which will be fermented by yeast into the alcoholic carbonated beverage called beer. Brewing was one of the earliest biological processes to be undertaken on a commercial scale, and it became one of the first processes to develop from a craft into a technology. Beer production is a unit process divided into five distinct, but related, interconnected stages – the first three units will be considered here with the next unit (fermentation). Packaging is a unit process with a distinct and related but separate objective:

- Malting is the germination of barley or other cereal and subsequent drying (or kilning) of it. Malt is produced in malting plants, which are usually these days, but not always, physically separate from breweries. The raw materials are specially selected brewing varieties suitable for malting. The objective of malting from a brewing (and distilling) perspective is to permit the development of enzymes that will hydrolyze proteins and starch during the later stages of germination and during mashing;
- Mashing involves the hydrolysis of proteins/peptides, starch, and other materials from the ground malted barley and unmalted cereals (adjuncts) by a spectrum of enzymes (details later) to produce a water-soluble largely fermentable extract, which can be separated from the insoluble material (called spent grains). This unboiled, unhopped, nonsterile liquid is called sweet wort.
- Sweet wort boiling with the inclusion of hops and/or hop extracts and sometimes sugar and/or syrups to produce the sterile medium called wort.
- Fermentation of oxygenated wort with yeast followed by maturation, dilution (if required) carbonation, and filtration.
- Packaging – used generally to mean kegging, bottling, and canning.

The principal raw materials employed in the brewing process are malted cereals (usually barley (Figure 2), but not always, sometimes wheat and sorghum), unmalted cereals (corn, wheat, rice, sorghum, oats, and barley), and sugar and syrups. Unmalted carbohydrate sources are termed adjuncts (also called a secondary brewing material). Other critical raw materials involved in wort production are hops and the often overlooked primary raw material (considered by some also to be a utility) – water. Some processing aids and additives take part in chemical reactions, for example, carrageen, silica gel, and polyvinylpolypyrrolidone. However, some of these reactions (not all) are more important later in the brewing process during maturation.

Malt and Malting

Wort is a hopped medium that will support yeast growth and fermentation with beer as the end product. It is important that beer is drinkable (beer is not usually supped, it is drunk!) and it exhibits a number of stability characteristics (flavor, physical, foam, and biological). Malt contributes a large number of materials to wort. The principal components of wort are free amino nitrogen (FAN) and fermentable sugars.

The word 'malt' is derived from the Anglo-Saxon 'mealt' and perhaps has the same root as 'melt', referring to grain softening. This occurs during germination or malled (mauled: broken or ground), as malts are milled before being used in brewing (and distilling). Malting is the limited germination of cereal grains – usually (but not always) barley. Sometimes, malt is used 'green' (undried not kilned). The use of the word 'green,' in this context, is obscure, but it may mean cereal color or it may be related to immature or green beer. Although malt made from barley (*Hordeum vulgare*) (Figure 2) is by far the most important, it is also made from wheat, rye, oats, triticale, maize (corn), sorghum, various millets, and rice.

Malting is perhaps the oldest biotechnology. The cultivation of barley and wheat probably began about 10 000 BC and wild grain must have been available earlier. Malting is the controlled germination of cereals, followed by termination of this natural process by the programmed application of heat to dry the grain (kilning). Additional heat is then applied to kiln the grain in order to maintain the enzyme activity and develop the required malt flavor and color. According to the Brewing and Malting Barley Research Institute (BMBRI) based in Winnipeg, Canada, the following barley characteristics are required in order to produce superior malting barley:

- Pure lot of an acceptable variety
- Germination of 96% grains or higher
- No evidence of preharvest germination
- Protein concentration of 11–12.5% on a dry weight basis
- Maximum moisture content of 13%
- Plump kernels of uniform size
- Free from disease, mycotoxins such as deoxynivalenol and chemical residues

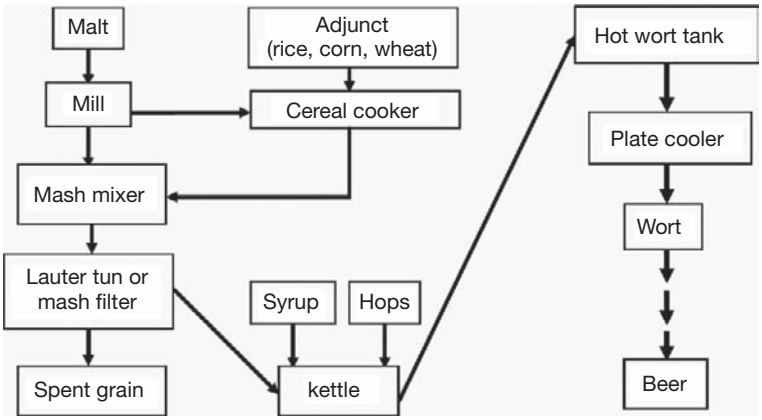


Figure 1 The wort production process.

Figure 2 Barley ears.

- Free from frost damage and weathering
- Less than 5% peeled or broken kernels
- Free of insects, ergot, treated seeds, grit, and odor

The malting process is a blend of pure and applied science involving plant and microbial biochemistry, physiology, chemistry, physics, and engineering. The stages of a typical malting process are depicted in Figure 3. However, there are a number of variations on the basic procedure. The procedure is based on the principle that barley, or another grain, must be converted into malt of the best achievable quality, economically, in the shortest feasible time with the best yield. The choice of malting procedure is guided by these considerations. As malting proceeds, the grain tissues undergo changes. The aleurone cells are partly depleted of their contents but are still metabolically active. The contents of the dead starchy

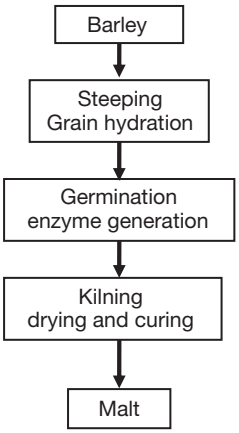


Figure 3 Typical barley malting process.

endosperm are partly degraded and depleted. The embryo metabolizes and grows, chiefly at the expense of hydrolysis products from the starchy endosperm. The hydrolytic and biosynthetic processes proceed simultaneously. There is a net breakdown of polymeric substances, such as starch, and a migration of substances from the aleurone layer and the endosperm to the embryo.

The changes that occur during malting are normally described in terms of physical modification of the grain and an alteration in conventional malt analysis (Table 1), on a biochemical basis. The gross changes are the net result of the degradation of reserve substances. This leads to the interconversion of substances in the living embryo and aleurone layer, the flow of substances to the embryo from the aleurone layer and starchy endosperm, the synthesis of new grain substances, and their incorporation into the raw, growing tissues (the acrospire and rootlets) of the embryo (Figure 4). Allowances must be made for malting losses – the losses of dry matter that occur during the conversion of grain into malt.

The chemical and biochemical changes that take place during malting are complex. They can only be understood by appreciating the range of reactions that occur during the overlapping processes of steeping, germination, and kilning and the effects of deculming and dressing (cleaning) the malt. During steeping, the grain is permitted to imbibe water in

Table 1 Typical barley malt analysis

Moisture (%)	3.8–4.2
Extract (%)	79.9–81.0
Wort color (SRM)	1.4–1.7
Diastatic power (ASBC)	120–145
α-Amylase (DM)	39–49
Malt protein (%)	10.8–12.3
Wort protein (%)	4.9–5.6
FAN (mg/l)	180–220
Wort viscosity (CP)	1.38–1.48
Wort β-glucan (mg l^{-1})	25–150
Friabilimeter value (%)	70–86
Wort fermentability (%)	78–82

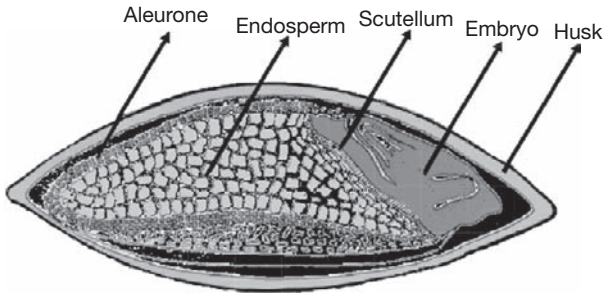


Figure 4 The barley grain.

order to increase the grain moisture from 12–14% to 42–48%. This occurs by immersing the grain in water or by spraying with water and usually a combination of both. The steep water becomes dirty and is replaced at least once in order to maintain the grain fresh. During steeping, the grain swells and softens, and the living tissues resume their metabolism, which had ceased during grain ripening and drying prior to harvesting and during storage awaiting malting. Sometimes, air is blown through the grain–water mixture, aeration, or the grain can be 'air-rested' – the water is drained away and air is sucked downward through the grain. When the grain has achieved the correct moisture content, the water is removed. Usually, this steeped grain is transferred to a germination vessel. In some malting plants, steeping and germination, and occasionally kilning, takes place in one container.

Each batch of grain being malted is referred to as a 'piece.' Following steeping, germination begins and the grain undergoes 'modification.' Modification is an imprecise term that signifies all the desirable changes (biochemical and chemical) that occur when grain is converted to malt. Modification continues during the initial stages of kilning. The three major aspects of modification are

- accumulation of hydrolytic enzymes;
- a variety of chemical reactions that occur in the grain;
- physical changes, which appear as weakening and softening in the grain.

Visible signs in the germination include the initial appearance of a white 'chit' at the end of the grain, followed by a tuft of rootlets or 'culms.' At the same time, the 'acrospire' (coleoptile or shoot) grows. It is covered by the husk in the barley but it grows freely in many other grains. The germinated grain is transferred to the kiln while it is still fresh (called green or undried but it is not really green colored). The moisture content of kilned barley malt is usually 4–4.5%.

Adjuncts

Adjuncts are alternative sources of fermentable extract and are used to replace a proportion of the malt. They may be used usually as less expensive sources of extract. Also, they are used to impart elements of beer product quality such as color, flavor, foam, and drinkability. Alternatively, adjuncts are important if there are taxation considerations that make reduced malt use in brewing financially advantageous, for example, the Japanese legislation that permitted the development of happoshu (containing 25% malt or less) and third category (containing no malt) beers. It should be noted that in Japan, taxation on beer is levied based on the percentage of malt used in the grist.

Although a wide range of brewing raw materials fall within the previously mentioned adjunct definition, attention here will be focussed on the use of adjuncts during three areas of the brewing process: (a) solid unmalted raw materials usually (but not always) processed in the brewhouse, (b) liquid adjuncts (syrups) usually added to the kettle (copper) and some specialty products used for priming beer at a later stage in the process, and (c) malted cereals other than barley, such as wheat and sorghum. Basically, adjuncts are considered to be nonmalted sources of fermentable sugars. Typically (with a few exceptions – discussed later), they contribute only extract, no enzyme activity, and little or no soluble nitrogen and are usually less expensive than malt. It is also considered that adjuncts do not contribute flavor to the finished product. However, this is not always the case. In general, barley tends to give beer a strong, harsh character (particularly stouts), whereas wheat imparts beers with dryness and a stable foam (good head retention). Rice and corn (maize) will give a characteristic light flavor to lager beers.

Starch consists of two types of polymer molecules – the linear amylose and the branched amylopectin. Depending on its source, starch generally contains 20–25% amylose and 75–80% amylopectin. Amylose and amylopectin are inherently incompatible molecules with amylose having a lower molecular weight with a relatively extended shape, whereas amylopectin comprises much larger but more compact molecules. Amylose molecules consist of largely simple unbranched chains with 500–20 000 α-(1→4)-D-glucose units depending on the plant source (very few α-1→6 branches may be found and they will have little influence on the molecule's overall behavior). Amylopectin is formed by nonrandom α-1→6 branching of the amylose-type α-(1→4)-D-glucose structure. The branching is determined by branching enzymes that leave each chain with up to 30 glucose residues. Each amylopectin molecule contains a million or so residues, about 5% of which form the branch points.

Syrups

The major liquid adjuncts used in brewing are glucose syrups, cane sugar syrups, invert sugar syrups (a mixture of glucose and

Table 2 Sugar spectra (%) of brewing worts

	Acid/enzyme syrups	*Enzyme/ enzyme syrups*	*VHMS*[a]	*All-malt wort*
Glucose	65	5	5	8
Maltose	10	55	70	54
Maltotriose	5	20	10	15
Dextrins	20	20	15	23

[a]VHMSs – very high-maltose syrups.

fructose), and syrups with a similar sugar spectrum to wort – details later. Glucose syrups have been available since the mid-1950s. Originally, they were produced by direct acid conversion of starch to a 64–68 DE range. (DE is 'dextrose equivalent' – it is a measure of the reducing power of the solution. For example, starch would have a DE of 0 and pure dextrose (glucose) of 100.) During the mid-1960s, new developments in enzyme technology led to acid/enzyme conversion and production of approximately 64 DE syrups. Table 2 shows the sugar (dextrin) profile of these syrups in comparison to a typical all-malt wort. The only similarity was the content of the higher saccharides or nonfermentables.

The use of liquid adjuncts continued during the 1980s but their use had shortfalls. The high level of glucose became a concern because they induced sluggish and 'hung' fermentations (also called the 'glucose effect'). All brewing raw materials need to be consistent including brewing syrups. However, acid and acid/enzyme syrups depend on the termination of the reaction by chemical or mechanical means when the desired end point is reached, and it is difficult to attain the proper production consistency by operator judgment required due to variables such as temperature, time, pH, and concentration of substrate. In addition, large volume users and breweries located a long distance from the suppliers factory experienced inconsistent syrup color when it is stored for lengthy periods at elevated temperatures. This is the sugar browning reaction particularly with high levels of glucose – this reaction is catalyzed by the presence of metal ions and proteins. This variable quality has forced the syrup manufacture to become more flexible, more competitive, and more adaptive to the wide differences when marketing on an international scale. Also, the use of acid and acid/enzyme syrups had a number of disadvantages such as elevated sulfite concentration, which can cause allergic reactions with some people. In addition, the manufacturing procedure involves exposure to low pH conditions followed by neutralizing with sodium hydroxide or another alkaline base, and as a consequence, elevated syrups and eventually beer sodium levels are frowned upon by the appropriate food and drug authorities. However, although acid/enzyme carbon-treated syrups possess disadvantages, they were acceptable and popular until the late 1980s when there was need for a change. Many changes have occurred in the brewing and wet-milling industries that have led to the development of a novel spectrum of liquid adjuncts. This development has been allied to the increase in adjunct levels in many beers (up to 50% of the wort content) particularly in North American and high-gravity brewing (details later in this article).

With the advent of new technology in enzyme liquefaction and multistage enzyme hydrolysis, production of corn syrups

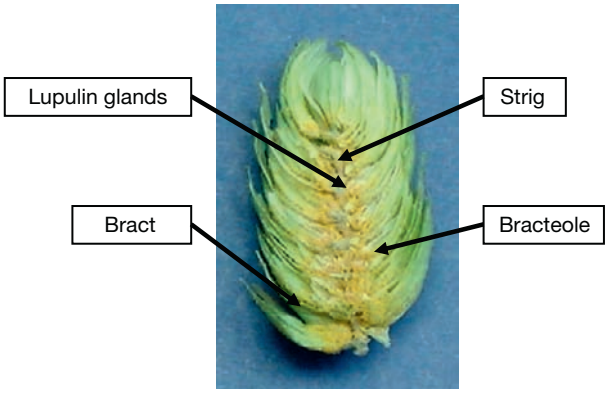


Figure 5 Cross section of a typical hop cone.

with virtually any carbohydrate profile is possible. Table 2 shows the sugar/carbohydrate profile of 'new-generation' very high-maltose syrup (VHMS)/corn (enzyme/enzyme) and its comparison to a typical all-malt wort. The profiles are very similar. This has permitted the brewer to introduce liquid adjuncts into the process without changing the wort sugar profile. Sensory evaluations of beers provided with HMS have revealed no significant differences from beers produced with acid/enzyme syrups. In addition, the sodium levels dropped by as much as 60% in comparison to beers produced with earlier-generation syrups. In the past decade, a further brewing syrup has been prepared. This is VHMS and contains 70% (w/v) maltose and low levels of glucose (Table 2). VHMS is used in high-gravity worts (>18°Plato) as part of a brewing capacity initiative, and the elevated maltose concentration will reduce ester levels in the HG produced beers.

Hops

Hops are the wort ingredient that provides beer with bitterness. It also increases the beer's biological stability, helps stabilize its foam, and greatly influences its taste and aroma. Hops are the flowers or 'cones' (Figure 5) of the plant *Humulus lupulus*. The lupulin glands in the cone contain the important flavoring compounds. The *Humulus* genus belongs to the family of the Cannabaceae, which includes Cannabis (hemp and marijuana) and Celtis (hackberry). Hops are native to the Northern Hemisphere in the temperate zones of Europe, western Asia, and North America and are thought (not proven) to have originated in China. They are now grown commercially in both hemispheres between approximately 30° and 52° latitudes. They are hardy plants that can survive cold winters with temperatures as low as −30 °C. The five recognized taxonomic varieties in the species *Humulus lupulus* are *H. lupulus* var. *lupulus*, European hops; *H. lupulus* var. *cordifolius*, Japanese hops; and *H. lupulus* var. *lupuloides*, *H. lupulus* var. *neomexicanus*, and *H. lupulus* var. *pubescens*, North American natives.

Hops come in two basic market classes, bittering and aroma hops, with a few hops being marketed as dual-purpose varieties. Bittering or kettle hops are added to the sweet wort near the beginning of the boil and aroma hops are added at any time from 30 min before the end of the boil to strike out. They can also be added to the whirlpool, or even later. The addition of

hops to fermented beer is called dry hopping. This practice adds highly volatile essential hop oils to the beer – oils that evaporate into the brew stack during the kettle boil and may even be purged out of the wort during fermentation. The intensity of hoppiness is a matter of beer style and brand. For instance, most American lagers contain minimal hop content and are refreshing to many consumers. At the same time, microbrewers (craft-brewed) add so much hop and produce a beer with almost a 'punishing' hop character. Such beers are growing in popularity, especially in North American bars, and are catching up with other beer cultures around the world but are not for everyone!

Alpha acids, also called humulones, are the sources of most bitterness in beer and make up about 3–4% of the cone's weight in aroma varieties. In superalpha bittering varieties – which are the most recent products of many breeding programs – alpha acids make up more than 20% of the cone's weight. During the wort boil, alpha acids are converted to water-soluble iso-alpha acids or isohumulones, the true bitter compounds in beer. This conversion or isomerization is one of the main objectives of wort boiling and the boiling time must usually be long enough (at least 45 min for most hop varieties) to allow isomerization to take place. The unit of measurement for hop bittering potential is the international bitterness unit (IBU).

Alpha acids are divided into three analogues – compounds of very similar structure – the desirable humulone and adhumulone and the undesirable cohumulone. Cohumulone may make up 15–50% of the hop's total alpha acid content – depending on the hop variety but will also greatly vary depending on variety. It can also vary from one growing year to the next with the same variety. High cohumulone levels in hops tend to result in lower beer foam stability, a harsher, often unpleasant bitterness, and poor aroma profiles. Hops are often marketed, in part, based on their cohumulone content.

Essential oils in hops are responsible for the distinct hop aroma. Some fresh hop varieties smell very 'citrusy' such as Cascade, which has a grapefruit–piney bouquet, whereas others have a more floral bouquet. The main essential oils in hops are humulene, which has a woody, balsamic aroma; caryophyllene, which has a black pepper spicy aroma; myrcene, which has a geranium-like floral aroma; and farnesene, which has a gardenia-like floral aroma. Of these, farnesene either is often completely absent or only occurs in miniscule quantities. Other essential oils, such as linalool with its citrus-like bergamot aroma, although present in only tiny amounts, may have a disproportionately large impact on the overall aroma of certain hop varieties. Approximately 300 compounds are likely contributors to hop aroma profiles. Some of these impart floral, fruity, and spicy notes, whereas others contribute off-aromas and can negatively impart beer aromatics.

There are a number of ways that hops can be introduced into the process:

- Traditionally, leaf hops are added to the boiling wort in the kettle (copper). Bittering hops are added early in the boil in order to extract maximum bitterness into the wort. Aroma hops are added later in the boil to ensure that aroma compounds are not lost through the kettle stack. At the end of boiling, the spent hops (with the bittering and aroma compounds extracted) are removed from the wort with a hop strainer (also called a hop back).
- Dry hopping is the addition of leaf hops to wort during fermentation conditioning or into the barrel in the cellar of a public house or bar. The purpose of dry hopping is to infuse beer with additional fresh hop flavor and aroma. Dry hopping is a cold infusion technique that both intensifies hop aromatics and also adds aromatics that are substantially different from those achieved by late hopping. The alpha acids responsible for hop bitterness are not isomerized and therefore remain insoluble during dry hopping, but testing trials have shown that the bitterness perception of beer can be enhanced by dry hopping, although international bitterness unit values essentially remain unchanged.
- Hop pellets are a form of processed whole hops whereby the dried cones are pulverized using a hammer mill and then extruded through a pelleting die to produce a densely packed pellet. Whereas the hop cone is a flowerlike structure (**Figure 5**) that is both bulky and delicate but not very practical in a brewery, pellets offer brewers a range of advantages over the use of whole hops. For example, whole hops are packed in large bales and require cooled hop storage facilities, whereas pellets are compact and are easy to store. However, while processing hop pellets is likely to break the hop cone's lupulin glands, pellets are much more sensitive to oxygen than whole hops. Consequently, hop pellets must be flushed with an inert gas and vacuum packed but can be stored at room temperature, if required.

Pelletized hops are available in two varieties – Type 90 and Type 45. T-90 pellets are produced from the entire hop flower, whereas T-45 pellets are produced after much of the vegetative matter has been removed from the flower, which concentrates the lupulin content in the pellets. An important brewhouse advantage of pellets compared to baled hops in automated brew systems is the suitability for automated hop dosing equipment. This is because pellets flow more consistently than loose hop flowers (leaf). Because of the overall advantages of pelletized hops, they are used by all sectors of the brewing industry, from the largest brewers to craft brewers including the smallest brewpubs. For example, in North America, pellets are favored over whole hops and hop extracts.

- Hop extracts are one of the many processed products currently available to modern breweries. Currently, over 50% of the hops used by the brewing industry are processed into extracts. Carbon dioxide is the primary solvent employed in the modern hop extraction process. Prior to CO_2, solvents such as ethanol, methanol, methylene chloride, and hexane were used. Ethanol extraction of hops is still practiced on a limited scale particularly by the German brewing industry. Hop extracts possess many advantages. When extracts are used in a brewhouse, it can yield more wort because the hop plant's cellulose has been removed, making wort separation less difficult relative to the use of whole hops or pellets. Also, extracts are concentrated and therefore reduce raw material shipping costs.
- Carbon dioxide hop extracts are often the starting material for a number of enhanced value purified hop extracts such as preisomerized extracts and other modified downstream products such as 'tetra.' These modified extracts have greater utilization properties and therefore higher efficiency when

dosed into beer postfermentation and are very popular with brewers of light (lite) lagers. Indeed, some modified hop extracts provide light stability preventing the light struck (skunky) flavor, and this allows beer to be packaged in clear or green bottles without changes in beer flavor. The use of hop extracts has both its proponents and detractors. Some malt brewers (not all) in the United States and Britain and elsewhere avoid hop extracts entirely, either for philosophical reasons or out of a belief that they are inherently inferior to leaf hops or pellets. Proponents, including some craft brewers, argue that hop extracts allow them to dose bitterness into their beers in a manner that might otherwise be impractical!

Water

Water is the principal ingredient in beer (and distilled beverages). It is both a raw material and a utility. Aside from water, the other major brewing raw materials are malt and unmalted cereals, hops, and caramel (and other coloring materials) together with processing aids and additions. Also, brewery (and distillery) utilities provide the means by which the process can operate. These utilities include the energy source (oil, gas, electricity, and steam) to drive the process, vital services including the supply of water and process gases (CO_2, nitrogen, and oxygen), and facilities for disposal of waste materials.

The ratio of water used as both a raw material and a utility to the volume of beer produced is very important. Over the past 20 years or so, the price of water in many countries has consistently exceeded inflation, and as a consequence, the ratio of water used to the volume of beer produced has decreased. Effluent treatment systems are required to minimize the impact of the process on the environment. With anaerobic treatment systems, it is possible to generate biogas that may be used to replace fossil fuels.

Historically, brewery sites (and distillery sites) were (and still are in many cases) linked to the availability of good water quality, hence the establishment of such brewing centers in Pilsen, Munich, Edinburgh, Burton-on-Trent, St. Louis, Mo., and London, Ontario, and distilling centers in Speyside in the Scottish Highlands. Nowadays, many breweries are situated closer to the consumer and near to the centers of population. However, the criteria for water availability and quality still apply. Water consumption varies markedly from brewery to brewery and is often, but mistakenly, considered as a resource available in limitless quantities at virtually no cost. The actual situation, especially for the more heavily industrial nations, is that water is becoming a limited resource at an ever-increasing price. These costs include the purchase of the water, treating the water en route to the brewery, and treating the resulting effluent.

A brewery has several options for its water supply:

- Groundwaters from wells
- Surface water from rivers, lakes, or streams
- Water from the public supply, which may be derived from either ground or surface sources but treated to comply with international standards

From a consistency point of view, groundwater from wells is preferred, though to keep the supply potable and free of contamination requires appropriate construction and regular maintenance. Surface water is preferred for distilling (especially Scotch whisky). Breweries today make extensive use of water supplied by public utilities or treat the water themselves. Generally, surface water is less microbiologically sound than water obtained from wells or public utilities. In summary, the choice of water supply depends on the availability and on the costs for treatment and quality control. It is often the case that supplies are drawn from multiple sources.

The food and beverage industry is required to use water of 'potable quality' as specified by either the World Health Organization or European Directives. Sometimes, additional quality parameters regarding hardness, alkalinity, and ion content are established by brewers (and distillers) in the interests of the beer (and whisky) character (Table 3).

Breweries often use several qualities of water although in some cases, the brewing, packaging, and general purpose water used is the same (Table 4).

Mashing and Boiling

The purposes of mashing are

- to extract starch, proteins, peptides, lipids, and other components from the malt and adjuncts;
- to render the extract fermentable by ensuring the necessary enzymatic hydrolysis of the previously mentioned components to sugars, amino acid, small peptides, etc.

Table 3 Typical analyses of water used in breweries and distilleries (mg l^{-1})

	Pilsen	*Munich*	*Burton-on-trent*	*Jura*
Dissolved solids	51	536	1226	46
Calcium	7.1	109	268	5
Magnesium	3.4	21	62	6
Bicarbonate	14	171	280	5
Sulfate	4.8	79	638	14
Nitrate	–	53	31	–
Chloride	5.0	36	36	27

Table 4 Water quality during the brewing process

	Quality parameters
Brewing	Potability, hardness, alkalinity, carbon-filtered
Dilution	Potability, carbon-filtered, sterile, hardness, alkalinity, carbonated, reduced dissolved oxygen concentration (50 μg ml^{-1})
Packaging	Potability, hardness, corrosive properties (Langelier index)
General purpose	Potability
Boiler feed	Hardness, pH, silica

Prior to mashing, the malt is milled employing a variety of milling procedures. This is a physical process and beyond the scope of this article. The milled grain is wetted in order to stimulate enzyme activity, which will hydrolyze the starch into fermentable sugars, the proteins into amino acids and small peptides, and the lipids into free fatty acids and sterols. In order to achieve this complex hydrolysis procedure, the mash is subjected to a series of heating and rest periods at set temperatures in order to realize the optimum catalytic conditions with reference to the type of beer being produced. The infusion mashing program is extensively used and is shown in Figure 6. The initial temperature of 52 °C is employed to stimulate both protease and glucanase activities (often called the 'protein rest'), but the rate of activity of the two enzyme systems is unclear. After 15–20 min, the temperature is increased gradually to 65 °C. This temperature is the saccharification temperature and is optimal for both α and β amylase activities. Alpha-amylase is an endoamylase that hydrolyzes 1,4-α-glucosidic linkages in amylose (contains 1α4 linkages) and amylopectin (contains both 1α4 and 1α6 linkages) (Figure 7). This enzyme is virtually absent from mature barley unless it has pregerminated. However, considerable quantities are synthesized *de novo* in the embryo and aleurone layer and large proportions are generated into the starchy endosperm. Beta-amylase is an exoenzyme that catalyzes the hydrolysis of the 1α4 linkages penultimate to the nonreducing chain ends (Figure 7), releasing the disaccharide maltose (G2) and the trisaccharide maltotriose (G3) (Figure 8) and oligosaccharides (also called dextrins) shortened by the removal of two and three glucose residues. Unlike α-amylase, this enzyme is present in unmalted barley. During malting, the level of free β-amylase may initially fall during steeping, and subsequently, during germination, nearly all the β-amylase becomes free, and the bound form disappears. This enzyme will not attack 1α6 or 1α4 linkages immediately adjacent to them.

Another important enzyme during mashing is the debranching enzyme that hydrolyzes1α6 linkages in amylopectin, dextrins, and other oligosaccharides (Figure 7). This enzyme is also known as limit dextrinase and as pullulanase because of its ability to degrade a particular branched bacterial polysaccharide – pullulan. In malt, this enzyme occurs in both freely soluble and bound forms. There exists a heat-stable protein in barley that inhibits malt limit dextrinase. The degree of inhibition increases during malting. However, there is evidence that this enzyme is synthesized *de novo* in the barley aleurone layer during germination.

After approximately 30 min at 65 °C (saccharification rest) (Figure 6), the temperature is raised to 78 °C – mash-off temperature. The principle reason for this mash-off temperature is to inactivate most of the enzymes that were active in the mash. In addition, some final α-amylolysis will occur, whereas at this temperature, β-amylase will be rapidly inactivated. Also, at this temperature, the viscosity of the sweet wort (due to β-glucans and arabinoxylans) will be reduced, and many (not all) microorganisms that are contaminating the malt will be heat inactivated. This sweet wort is separated from the spent grains in a lauter tun or a mash filter.

The sweet wort is boiled in a kettle (also called a copper), usually for 30–60 min with 4–6% evaporation. Boiling is needed to isomerize the hop alpha acids (already discussed). Isomerized hop acids are bitter, whereas nonisomerized hop alpha acids are not. Boiling is also needed to strip out unwanted malt and hop volatiles, to denature proteins and coagulate proteins/polyphenols as hot break, and to establish the wort composition (details later) by terminating all enzymic and microbiological activity surviving the mashing process. As a consequence of boiling and evaporation, there will also be color development (melanoidin reactions) and wort gravity increase that need to be accommodated in order to achieve the finished target for a particular wort. Ideally, a kettle should be fitted with in-line probes in order to follow the isomerization of alpha acids, the disappearance to dimethyl sulfide, the coagulation of colloidal size particles (0.1–10 μm) to form hot break (30–70 μm), and the wort gravity. Currently, this on-line control is not practical, and the brewer has to rely on pragmatic experience to achieve these critical parameters. However, this on-line control cannot be too far in the future!

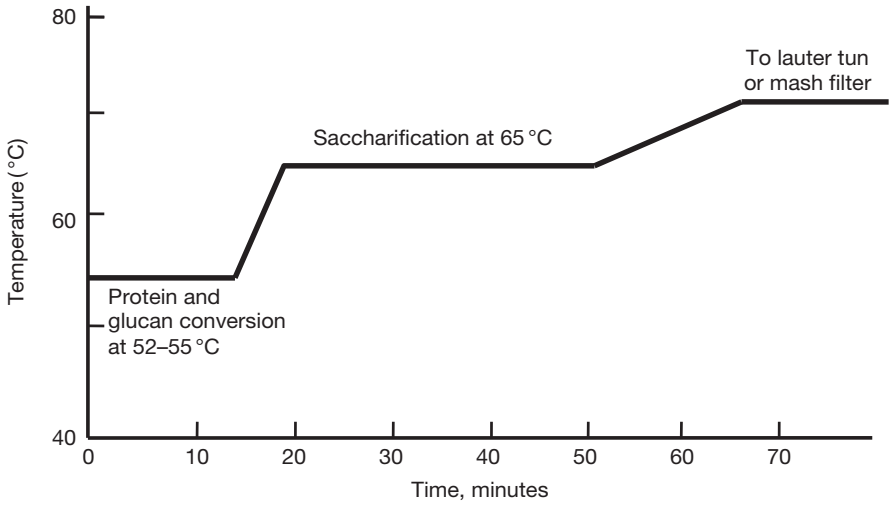


Figure 6 Temperature profile of a typical infusion mashing program.

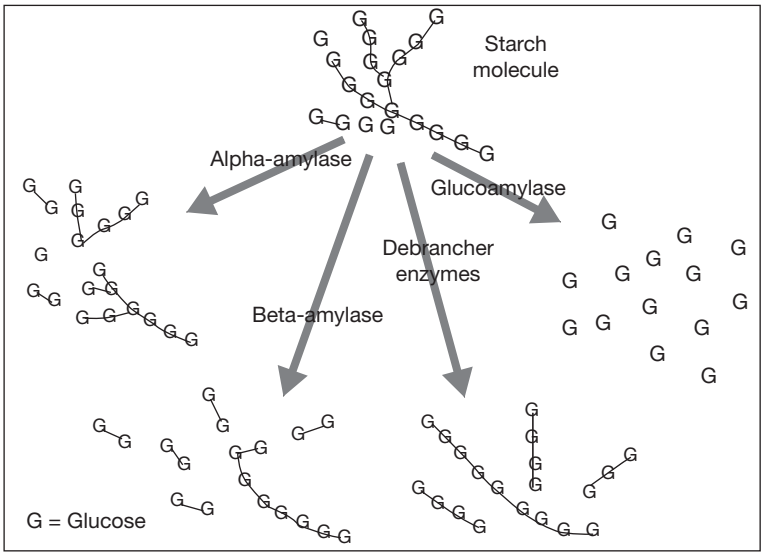
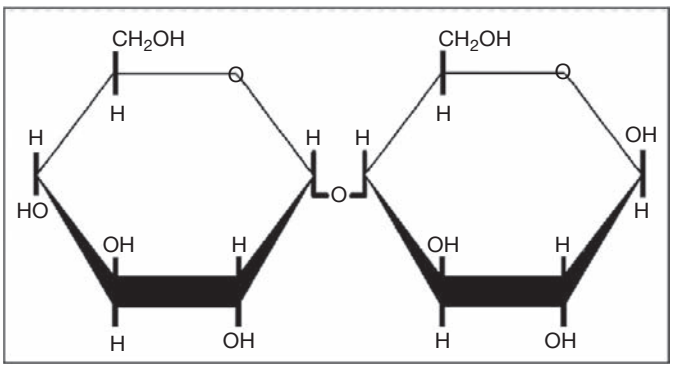
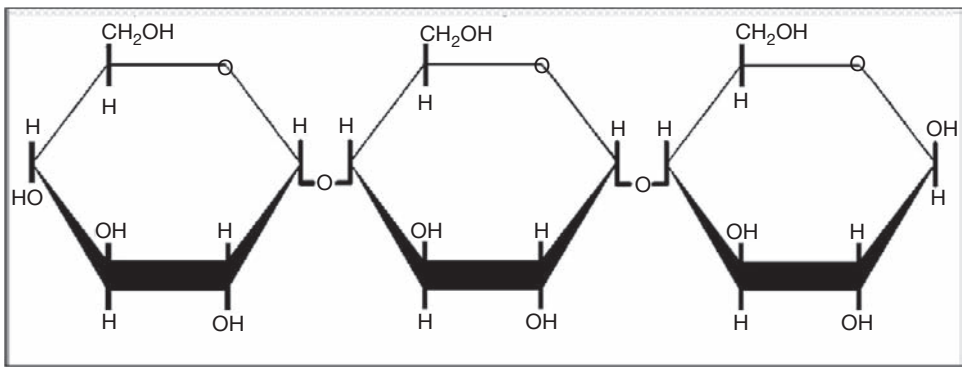


Figure 7 The enzymatic hydrolysis of starch.



Maltose, molecular weight = 342



Maltotriose, molecular weight = 504

Figure 8 Structure of maltose and maltotriose.

Wort Composition

Wort contains the sugars sucrose, fructose, glucose, maltose, and maltotriose, together with dextrin material (Table 2). Also, wort contains a number of amino acids, all of which, with the exception of proline, are metabolized during fermentation. However, it should be reported briefly here that the objectives of wort fermentation are to consistently metabolize wort constituents into ethanol, carbon dioxide, and other fermentation products in order to produce beer with satisfactory quality and stability. Another objective is to produce yeast crops that can be confidently repitched into subsequent brews.

Aside from the actual composition of the wort, its concentration is also important. Traditionally, the wort concentration

(gravity) was 1030–1048 (∝7.5–12°Plato). This produces beers with alcohol concentrates between 3% and 5% (v/v) ethanol. However, beginning in the 1980s, high-gravity brewing (HGB) evolved. This is a procedure that employs wort at higher than normal concentration and, consequently, requires dilution with water (usually deoxygenated), at a later stage in processing. By reducing the amount of water employed in the brewhouse, increased production demands can be met without expanding existing brewing, fermenting, and storage facilities. Dilution with water can occur either entirely or in part at almost any stage in the process, including kettle (copper), strikeout, and prewort cooler; during or after fermentation; during maturation; and pre- and postbeer filter.

HGB has been progressively introduced into breweries around the world for the past half century.

There are a number of advantages and disadvantages to this process. The effects of HG wort on various aspects of fermentation will be discussed later. Other advantages can be summarized as follows:

- Increased brewing capacity – more efficient use of existing plant facilities.
- Reduced energy (heating, refrigeration, etc.), labor, cleaning, and effluent costs
- Improved beer physical and flavor stability.
- More alcohol per unit of fermentable extract because of reduced yeast growth and more of the wort sugars being converted to alcohol.
- HG worts may contain higher adjunct rates and different wort sugar spectra.
- Beer produced from HG are often rated smoother in taste.
- HG brewing offers greater flexibility in product type. From one 'mother' liquid, a number of products can be brewed as a result of dilution and/or use of malt and hop extracts and syrups.

The disadvantages of HG brewing can be summarized as follows:

- Due to the more concentrated mash (increased rate of carbohydrate to water), there is a decreased brewhouse material efficiency and reduced hop utilization.
- Decreased beer foam stability (head retention).
- Difficulties in achieving flavor match compared to equivalent lower-gravity beers.
- High-gravity worts can influence yeast performance with effects on fermentation and flocculation.

See also: Barley; Beer: Fermentation; Beer: History and Types; Fructose and High-Fructose Corn Syrup; Maize; Yeasts.

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Berries and Related Fruits

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Introduction

Botanically, berries are defined as a fleshy fruit that forms from the entire ovary that surrounds the seeds, and true berries include banana, grapes, and blueberries. In this article, the term 'berries' refers to the small, soft, edible, and colored fruits of the plants of the species *Vaccinium*, *Fragaria*, and *Rubus*. Berries have been emerging as important dietary components because of their numerous health benefits. The most popular and commonly consumed berries in North America include blackberries (*Rubus* spp.), red (*Rubus idaeus*) and black raspberries (*Rubus occidentalis*), blueberries (*Vaccinium corymbosum*), cranberries (*V. macrocarpon*), and strawberries (*Fragaria* × *ananassa*). Other berries including bilberries, lingonberries, goji berries (*Lycium barbarum*), and sea buckthorn (SBT; *Hippophae rhamnoides*) are also consumed to a limited extent. Additionally, several exotic berry fruits such as acai berries and honeysuckle (*Lonicera caerulea*) are also gaining importance. This article mainly focuses on the popular berries consumed in North America and those belonging to the genus *Rubus* and *Vaccinium* and some exotic berries including acai berries, honeysuckle, and SBT.

Berries of *Rubus*

Both raspberries and blackberries belong to the genus *Rubus*, which is a member of the Rosaceae family. This genus includes blackberries and raspberries. Blackberries, dewberries, raspberries, and their hybrids are collectively referred to as 'brambles.'

Blackberry (*Rubus* spp.)

Blackberries are grown worldwide, but they prefer regions with mild winters and long moderate summers. North America, Europe, Asia, South America, Central America, and Africa are the main areas of blackberry production. Blackberries are mostly consumed fresh, and also processed into ice cream, jam, marmalade, and other confectionaries. Blackberry fruit is an aggregate fruit consisting of a number of fleshy drupelets, each containing a single seed (pyrene) around the central torus or receptacle. There are three main types of blackberries cultivated based on cane architecture, classified as erect, semierect, and the trailing types. Trailing blackberries bear vigorous primocanes from a single crown that lie on the ground. Trailing blackberries are characterized by flavorful fruit with excellent aroma and with less seeds than other species. Semierect types are characterized by thornless, erect, large, and vigorous canes that grow from a crown and arch to the ground. Semierect and trailing types produce new primocanes only from buds on the crown and are biennial fruiting. Erect blackberries produce primocanes from buds at the crown or from buds on roots. Erect blackberries are characterized by plants that produce stiff, erect canes that are 1–4 m tall. Erect blackberries can be biennial or annual fruiting types. Boysenberry and loganberry are considered as trailing types due to their growth habit. They are highly productive and the berries are similar to the erect type. Blackberries are good sources of vitamins, minerals, and other bioactive compounds such as polyphenols. Glucose, fructose, and sucrose are the principal sugars of blackberries. Malic acid is the predominant organic acid in blackberry. Other acids including ascorbic acid and trace amounts of shikimic, fumaric, and succinic acid have also been detected. Organic acids help in stabilizing anthocyanins and ascorbic acid plays a role in extending the shelf life of berries. Blackberries are rich in polyphenolics such as anthocyanins, ellagitannins, flavonols, flavanols, procyanidins, and phenolic acids. According to various studies, phenolics in blackberries range from 114 to 1056 mg 100 g^{-1} FW.

Raspberry

Raspberries are closely related to blackberries. Raspberry cultivation started in Europe nearly 450 years ago. Russia, Europe, and the pacific coast of North America are the three major raspberry production regions. Raspberries are most productive in areas with mild winters and long, moderate summers. The major production areas of red raspberries in North America are the Pacific Northwest (Oregon, Washington, and British Columbia), California, and the eastern United States (New York, Michigan, Pennsylvania, and Ohio) and also in Mexico and Guatemala. The European red raspberry (*R. idaeus* subsp. *vulgatus* Arrhen.), the North American red raspberry (*R. idaeus* subsp. strigosus Michx.), and the black raspberry (*R. occidentalis* L.) are the most important commercially grown species. There are also yellow raspberries that are mutations of the red genotype and the purple ones are hybrids of the red and black genotypes. Red raspberries are the most widely grown while black raspberries are common only in some areas of the eastern United States. Red raspberries (*R. idaeus*) are widely consumed in fresh, frozen, or processed form, than black raspberries (*R. occidentalis*). Tayberry, loganberry, boysenberry, and youngberry are some important interspecific hybrids of blackberries.

Raspberry canes are biennial with the first year canes called primocanes and the second year canes called floricanes. Commercial red raspberries are either 'summer-bearing' (primocane-fruiting) or 'fall-bearing' (floricane-fruiting) types. Floricane-fruiting cultivars produce canes that are vegetative in the first year, and in the second year, they flower, fruit, and die. The primocane-fruiting raspberries produce canes that are vegetative in the first year, and in the second year, they flower, fruit, die, and are pruned out. Black raspberry cultivars are generally floricane-fruiting. Each raspberry flower has 60–160 ovaries,

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and each ovary contains two ovules, clustered into an aggregate fruit. The fruit ripens about one month after pollination. Raspberry fruit is an aggregate of many individual drupelets. The cohesion of drupelets results from the entanglement of epidermal hairs on the sides and base of the drupelet. Fruit size considerably varies in raspberries ranging from 1 to 5 g. Fruit ripening occurs in three phases and each phase lasts for about 10–12 days. In the first phase, a period of rapid cell division follows pollination. In the later phase, there is a slowdown of cell division while the embryo develops and the seed coat hardens. In the final phase, very rapid growth occurs due to cell enlargement. Ethylene production peaks with ripening, while respiration decreases.

Raspberries are eaten fresh or processed. The major products of processing are individually quick frozen, block-frozen, puree-frozen, canned, juice, concentrate, and preserves. Red raspberries contain an array of health beneficial compounds including essential minerals, vitamins, fatty acids, dietary fiber, and polyphenolic compounds such as flavonoids, phenolic acids, lignans, and tannins. Typically, sugar constitutes 5–6% of ripe raspberry fruit. The main sugars are glucose, fructose, and small amounts of sucrose. Red raspberries are low in energy and a 100 g supplying only 52 kcal. The high fiber content (6.5 g per 100 g) and fructose content regulate blood sugar levels and provide a satiating effect. Soluble solid portion comprises about 9% of the fruit.

Berries of *Vaccinium* (Ericaceae)

Vaccinium is a large complex genus of about 150 species, and it comes under the family Ericaceae. The genus *Vaccinium* includes popular berry fruits such as blueberries, huckleberries, cranberries, lingonberries, and bilberries. The three major cultivated crops of this genus are blueberry, large cranberry, and lingonberry. The genus *Vaccinium* is often divided into several subgenera and sections. The large cranberry (*V. macrocarpon* Ait) belongs to section *Oxycoccus*; and the section *Cyanococcus* (cluster-fruited blueberries) include the highbush blueberry (*V. corymbosum* L.), rabbiteye blueberry (*V. ashei* Reade), and the lowbush blueberry (*V. angustifolium* Ait.). The other species include *V. myrtillus* L. (bilberry and whortleberry) in section Myrtillus, and *V. vitis-idaea* (section Vitis-idaea) are collected mostly from the wild.

Cranberry (*V. macrocarpon* Ait.)

V. macrocarpon Ait. is also known as the cranberry or the American cranberry. Red-fruited species of *Vaccinium* are called as cranberries in North America. Cranberries are commercially cultivated in the United States and Canada. Some production also occurs in Argentina, Chile, and the Netherlands. Cranberry is an evergreen woody perennial mat-forming shrub of about 7 ft with a creeping habit. Pink flowers are borne on young upright shoots. Flower buds form in late summer and open in midsummer of the following year. After pollination, flowers mature late in the autumn. At maturity, berries are bright red with a hard shiny skin. Usually, cranberries are harvested in September through the first part of November. Beds are flooded with 6–8 in of water above the vines, and a harvester is driven through the beds to remove the berries. Cranberries are cleaned, graded, and stored prior to packaging or processing. Cranberries can be stored for several months without any significant quality losses. Shelf life can be extended for several weeks by decreasing the storage temperature to 0 °C.

Cranberry pulp contains lignin, glucose, fructose, and sucrose. In ripe cranberries, acids form 2–3% (w/w), and citric acid forms the primary organic acid. Cranberry also contains several organic acids such as quinic, citric, malic, and benzoic acids. Due to its tartness, most cranberries are processed into products such as juice, sauce, jam, and dried cranberries. Usually, cranberry juice is blended with other fruit juices to reduce tartness. Cranberries are made into a compote or jelly known as cranberry sauce. Cranberries contain about 1.2% pectin. The edible portion of the cranberry is composed of 2.66% glucose, 0.74% fructose, and 0.14% sucrose. Cranberry pulp also contains lignin, glucose, arabinose, and xylose. The overall acid content of ripe cranberries ranges from 1% to 2%.

Blueberry

Blueberries are the most popular berries of the genus *Vaccinium* and subgenus *Cyanococcus*. There are different kinds of blueberries such as the wild-growing lowbush blueberries (*V. angustifolium*) and cultivated highbush blueberries. Cultivated blueberries consist of various species, for example, *V. corymbosum* L., *V. angustifolium* Ait., and *V. virgatum* Ait. The rabbiteye blueberry is *V. virgatum* (*V. ashei)* and also comes under the highbush category. The northern highbush blueberry is *V. corymbosum*. The highbush blueberry is a major crop cultivated in the United States, Canada, Europe, Australia, New Zealand, Chile, and Argentina. The rabbiteye blueberries are commercially produced in southeastern United States. The commercial production of lowbush blueberries is largely confined to Maine (the United States) and Quebec and the Maritime provinces in Canada.

Flowering occurs in early spring, and after pollination, blueberry fruit develops according to a double-sigmoid growth curve. Fruits are borne as racemes or corymbs and berry contains 1–50 seeds embedded in a fleshy and colorless mesocarp. Berries are covered by a waxy cuticle covering the epidermis. Fruit development can be divided into several phases of color development: immature green, translucent greenish white, greenish pink, blue-red, and blue. Fruits ripen in 40–60 days. Blueberries are consumed fresh as dessert fruit and also used for baking. Blueberries are also processed or preserved into jams, pies, syrups, juices, soft drinks, and wines. Most *Vaccinium* fruits for fresh markets are hand-harvested, and for processing, highbush and rabbiteye berries are mechanically harvested.

Blueberries contain 3.5% cellulose and 0.7% soluble pectin. More than 10% of fresh weight of the berry is composed of total sugars. Glucose and fructose constitutes the predominant reducing sugars in blueberries. The acid content of ripe blueberries ranges from 1% to 2% and citric acid forms the primary organic acid (1.2%) in blueberries. Blueberries contain high levels of the amino acid arginine. Blueberries contain 22.1 mg of vitamin C per 100 g FW.

Bilberry

V. myrtillus L. (bilberry, dwarf bilberry, whortleberry, etc.) is similar to the North American lowbush blueberries. The bilberry (*V. myrtillus* L.), a low-growing shrub, is a native of northern and Central Europe and is also found in parts of North America and Asia. It is a small perennial deciduous shrub with slender branches arising from a creeping rhizome. Leaves are bright green and the flowers are globular and waxy with pale green or pinkish petals. Berries are bluish black, globose with purple pigmented flesh and brownish red seeds, and occasionally covered with a gray bloom. Bilberry fruits occur singly in the axils of the lowermost leaves of the vegetative shoot. Bilberries are harvested from the wild in Finland and in other European countries. The berries are used to prepare pies, tarts, syrups, jellies, and wines. Bilberries have a long history of medicinal uses. Glutamic acid and valine are the predominant amino acids in bilberries. Flavonols (quercetin and catechin), ellagitannins, tannins, anthocyanins, and phenolic acids form the phenolic compounds in bilberries. The anthocyanin content in bilberries is relatively higher than the other types of berries including strawberry, cranberry, elderberry, sour cherry, and raspberry. The total anthocyanin content of bilberries ranges from 300 to 700 mg per 100 g FW.

Lingonberry

Lingonberry is popular in Europe and Newfoundland. Lingonberry (*V. vitis-idaea*) is a perennial evergreen shrub that prefers acidic soils and is distributed in circumboreal regions. Lingonberries (*V. vitis-idaea* L.) are predominantly collected from the wild, but this crop has been domesticated recently. They are cold hardy plants and cannot tolerate heat. Lingonberry is commercially cultivated in several locations across Europe, Scandinavia, and also recently in the United States. Major lingonberry-exporting countries are Sweden, Finland, and the Russian Federation. Lingonberries are tart but edible when cooked or processed. It is commonly used in juice, pie fillings, and jam. Lingonberries are abundant in anthocyanin glycosides. The principal amino acids in lingonberries are serine and γ-aminobutyric acid. Lingonberries also have significant levels of benzoic acid. Lingonberries have been used traditionally to treat inflammatory diseases, wounds, gastric disorders, and rheumatism.

Berry Bioactives and Composition

Besides supplying vitamins and minerals, berries are rich sources of several bioactive phytochemicals and antioxidants including phenolic compounds and triterpenoids. Nutritional composition of some selected berries is presented in Table 1. Berries vary greatly in their contents of phenolic compounds. They are crucial sources of phenolic compounds and are represented by flavonoids (flavonols, flavones, flavonones, flavanols, and isoflavonoids), stilbenes, tannins, and phenolic acids (derivatives of benzoic acid and cinnamic acid) in our diet. Studies have shown that varietal differences and differences in the ripeness of the fruit had an effect on the profile and concentrations of phenolic compounds. In the *Vaccinium* genus, the relative amounts of anthocyanins, flavonols, hydroxycinnamic acid derivatives, and proanthocyanidins varied between bilberry, cranberry, and lingonberry. Hydroxycinnamic acid derivatives predominate in blueberries and huckleberries.

Anthocyanins

Anthocyanins are subgroups of flavonoids constituting a large group of water-soluble pigments. They are abundant in berries with red, purple, or blue color. In berries, anthocyanins are composed of aglycones (anthocyanidins) and their glycosides. Anthocyanins function as an antioxidant and a modulator of signal transduction and gene expression. In general, these fruits and their products show strong anti-inflammatory activity. They occur as mono-, di-, or triglycosides, where glycosides are usually substituted at their C3 or less frequently at C5 or C7 positions. Anthocyanins are predominant in *Vaccinium* species, such as bilberry and cranberry, and also in black and red currants and gooseberries. A total of 13 anthocyanins have been detected in cranberry. Among 100 commonly consumed foods in the United States, cranberries are reported as the main source of peonidin. The predominant anthocyanins are the 3-*O*-galactosides and 3-*O*-arabinosides of cyanidin and peonidin. Cranberries have total anthocyanin content ranging from 25 to 100 mg per 100 g fruit.

Anthocyanin profile is quite diverse in blueberry with more than ten anthocyanins, while less diversity is found in chokeberry, strawberry, and raspberry fruits. The total anthocyanin content in blueberry fruit ranges from 85 to 270 mg per 100 g FW. Anthocyanins in blackberries are 3-glycosidic derivatives of cyanidin, delphinidin, malvidin, petunidin, and peonidin. Cyanidin-3-dioxaloylglucoside is unique to blackberries. Raspberries are also rich in cyanidin glycosides. Malvidin-3-galactoside has been found to be the most predominant anthocyanin component in blueberries. The prominent anthocyanins in cranberries are cyanidin-3-monogalactoside, peonidin-3-monogalactoside, cyanidin-3-monoarabinoside, and peonidin-3-monoarabinoside. Cowberries contain high amounts of cyanidin-3-galactoside, and bilberries contain high levels of hydrocinnamic acid and quercetin 3-glucoside, rhamnoside, and arabinoside.

Phenolic Acids

Phenolic acids are major components of berries. Phenolic acids are represented by cinnamic and benzoic acid derivatives. Benzoic acid derivatives include *p*-hydroxybenzoic acid, salicylic acid, gallic acid, and ellagic acid. The common cinnamic acid derivatives include *p*-coumaric acid, caffeic acid, and ferulic acid. Chokeberry is abundant in hydroxycinnamic acid derivatives represented mainly by chlorogenic acid and neochlorogenic acid. Ellagic acid and its conjugates form the majority of phenolic acids in red raspberries. The predominant phenolic acids in strawberries are *p*-hydroxybenzoic acid and *p*-coumaric acid. Among the berries, raspberries and strawberries contain the highest content of ellagitannins. Hydroxybenzoic acid is the most abundant phenolic acid in cranberry followed by hydroxycinnamic acid. Cranberry and blueberry are particularly rich in

Table 1 Nutritional composition of some selected berries (100 g fresh-weight edible portion)

Components	*Unit*	*Blueberry*	*Blackberry*	*Cranberry*	*Raspberry*	*Sea buckthorn*[a]	*Lingonberry*[b]
Water	g	84.2	88.15	87.13	85.75		86.3
Energy	kcal	57	43	46	52	90	52.55
Protein	g	0.74	1.39	0.39	1.20	0.7	0.8
Total lipid (fat)	g	0.33	0.49	0.13	0.65	5.0	1.2
Carbohydrate	g	14.49	9.61	12.20	11.94	6.3	11.5
Fiber, total dietary	g	2.4	5.3	4.6	6.5	6.0	3.7
Sugars	g	9.96	4.88	4.04	4.42	6.3	–
Minerals							
Calcium (Ca)	mg	6	29	8	25	42	20
Iron (Fe)	mg	0.28	0.62	0.25	0.69	0.4	0.4
Magnesium (Mg)	mg	6	20	6	22	30	9
Phosphorus (P)	mg	12	22	13	29	8.6	16
Potassium (K)	mg	77	162	85	151	133	89
Sodium (Na)	mg	1	1	2	1	3.5	2
Zinc (Zn)	mg	0.16	0.53	0.10	0.42	0.0	0.18
Vitamins							
Vitamin C	mg	9.7	21	13.3	26.2	165	11
Thiamin	mg	0.037	0.02	0.012	0.032	0.18	0.05
Riboflavin	mg	0.041	0.026	0.020	0.038	0.07	0.04
Niacin	mg	0.418	0.646	0.101	0.598	0.4	0.5
Vitamin B6	mg	0.052	0.03	0.057	0.055	0.13	0.013
Folate (DFE)	μg	6	25	1	21	10	2
Vitamin B12	μg	0	0	0	0		0
Vitamin A (RAE)	μg	3	11	3	2		1.75
Vitamin A (IU)	IU	54	214	60	33	2.6	–
Vitamin E (α-tocopherol)	mg	0.57	1.17	1.20	0.87	3.0	–
Vitamin D (D2 + D3)	μg	0	0	0	0	0	0
Vitamin D	IU	0	0	0	0	0	0
Vitamin K (phylloquinone)	μg	19.3	19.8	5.1	7.8	11.3	–
Lipids							
Total saturated	g	0.028	0.014	0.011	0.019	0.8	0
Total monounsaturated	g	0.047	0.47	0.018	0.064	1.6	0.1
Polyunsaturated	g	0.146	0.28	0.055	0.375	0.3	0.8
Cholesterol	mg	0	0	0	0	0	0

[a] http://www.fineli.fi/foodlist.php?foodname=A%&lang=en.
[b] http://www.foodcomp.dk/v7/fcdb_details.asp?FoodId=0326.
Source: The USDA National Nutrient Database for Standard Reference; Schauss et al., 2006.

ferulic acid, while bilberry contains *p*-coumaric acid and ferulic acid. Phenolic acids in blackberries are mainly hydroxycinnamic acid and hydroxybenzoic acid and the phenolic acid content range from 7 to 64 mg 100 g^{-1} FW.

Flavonols

Quercetins and kaempferols have been identified in raspberry. In strawberry, kaempferol was detected as the predominant flavonol followed by myricetin and quercetin. Oligomers and polymers represent 85% of the total flavonols and are known as condensed tannins or procyanidins. Flavonol profile of blackberry is quite complex due to the presence of nine quercetin and 3-kaempferol derivatives, as well as other quercetin-derived compounds. Flavonols occur in cranberry as monomers, oligomers, and polymers. The main flavonol compounds in cranberry are glycosides of quercetin, myricetin, and kaempferol. The predominant flavonol is quercetin 3-galactoside. Quercetin exists in many glycosidic forms, and the most abundant form is quercetin galactoside. Myricetin galactoside and arabinoside are also present as well. Cranberries contain substantial amounts of quercetin and its total flavonol content ranges from 20 to 30 mg per 100 g FW. Both blueberries and cranberries are also rich in proanthocyanidins (tannins). A number of studies demonstrate the anticancer potential of quercetin and its derivatives *in vitro* against cell lines derived from the breast, colon, pancreas, and white blood cells (leukemic). Its anticancer actions include the induction of apoptosis, inhibition of epidermal growth factor receptor expression and associated tyrosine kinase activity, reduced expression of ras protein in colon cancer cells, and increased expression of matrix metalloproteinases. Quercetin is a major contributor to cranberry's antitumor property.

Stilbenes

Stilbenes are phenolic compounds that occur in some berries. Resveratrol, pterostilbene, and piceatannol are found in berries including blueberry, cowberry, lingonberry, and acai berry. Resveratrol and its analogs have potent biological properties such as anti-inflammatory, antiaging, antiallergenic, anticarcinogenic, and antimutagenic activities. Studies suggest

that resveratrol can boost apoptosis of cancer cells by enhancing sensitivity to tumor necrosis factor alpha (TNF-α) and reducing NF-κB activation. Studies show that different blueberry varieties of *V. corymbosum*, *V. ashei*, and *V. angustifolium* contain up to 860 ng of resveratrol per gram dry weight of fruit. Cranberry fruit contain about 900 ng of resveratrol per gram dry weight of fruit. Pterostilbene and piceatannol are two analogs of resveratrol, and 100–420 ng per gram fruit was detected in rabbiteye (pterostilbene) and highbush blueberries (piceatannol). Both of these compounds have been shown to possess anticarcinogenic properties to a similar or superior level to resveratrol.

Bioavailability

Only a small portion of the polyphenols (0.5–1%) present in ingested food is actually absorbed. Bioavailability of polyphenolic compounds could be enhanced through biotransformation involving catabolic breakdown, methylation, deglycosylation, etc. According to various *in vitro* studies, phenolic compounds are transported across intestinal epithelium by sugar transporters as glycosides. Following absorption into epithelial cells, these glycosides are converted to aglycones through β-glucosidase-mediated hydrolysis. Aglycones can also be formed in the lumen through the action of membrane-bound lactase–phlorizin hydrolase, and aglycones produced in this way are absorbed passively through the epithelium as compared with conjugation in the ileal epithelium or the liver. Hepatic metabolites (methylated, sulfated, or glucuronidated conjugates) are returned via the enterohepatic circulation (in bile) to the gut lumen. The phenolic compounds that enter the colon consist largely of unabsorbed glycosides and conjugates that have been cycled through ileal and hepatic metabolism. Polyphenols that are associated with food matrix may become unavailable for absorption. These compounds are subjected to metabolism by the gut microbiota after reaching the cecum. For instance, flavonoids undergo ring fission, where the C-ring is degraded resulting in the formation of several phenolic acids.

Berries and Health Benefits

Berries are excellent sources of several antioxidant compounds such as flavonoids, phenolic acids, and vitamins C and E. The hypothesized health benefits related to berry consumption include their role in the prevention of inflammation, oxidative stress, cardiovascular disease (CVD), cancers, type 2 diabetes, obesity, and neurodegeneration.

Antioxidant Activity of Various Berry Compounds

Berries vary in their natural antioxidant capacity, and they owe their antioxidant capability mainly to the various phenolic compounds including tannins, phenolic acids, stilbenes, and flavonoids. The antioxidant compounds in berries exert their action through various mechanisms. Phenolic acids may inhibit the oxidation of vitamin C, carotenoids, and unsaturated fatty acids. Flavonoids can chelate metal, scavenge oxygen radical, and also inhibit lipid oxidation. Anthocyanins could also scavenge free radicals and inhibit the oxidation of human low density lipoproteins (LDL). Carotenoids are also scavengers of active oxygen species preventing the oxidation of LDL. Blueberries and cranberries are ranked among the fruits with high antioxidant capacity owing to the presence of substantial amounts of several phytochemicals such as phenolic compounds. The antioxidant capacity ranges from 13.9 to 45.9 μmol of Trolox equivalents per gram of fresh berry.

Cardiovascular Disease

Evidence from *in vitro* and animal studies suggests that berry bioactive components can influence parameters or factors associated with CVD risks. There is evidence that the addition of berries to the diet could positively affect risk factors for CVD by inhibiting inflammation and improving the plasma lipid profile and free radical scavenging. In a study conducted in middle-aged unmedicated subjects, long-term consumption of moderate amounts of mixed berries resulted in increased high-density lipoprotein cholesterol, decreased blood pressure (BP), and also induced favorable changes in platelet function, indicating that certain constituents of berries alone or in combination play a role in decreasing the CVD risk. Berry and grape consumption has also resulted in increased high density lipoprotein-C (HDL-C) levels and decreased LDL-C levels in healthy, at-risk, and diseased individuals. Some recent study also suggests that berry consumption may also improve insulin sensitivity. Berries have also been associated with the prevention of obesity through interference with lipid digestion and/or through the modulation of lipid metabolism. In dyslipidemic subjects, anthocyanin intake resulted in an increase in plasma HDL cholesterol and a decrease in LDL cholesterol concentrations.

Studies have shown that chokeberry improves blood circulation and can boost the body's immune system. Chokeberry fruit and its products have a protective effect against atherosclerosis, hypertension, and gastrointestinal tract infection. Improvement in vascular function has been reported following intake of pure anthocyanins and cranberry juice (4 weeks) in human subjects. Improvement in endothelium-dependent vasodilation and serum lipid profiles following berry anthocyanin intake was reported in individuals with elevated cholesterol. A decrease in BP has been reported after consumption (6–8 weeks) of mixed berries, anthocyanin-rich tea, and chokeberry and blueberry extracts by individuals with hypertension, myocardial infarction, and markers of metabolic syndrome. Lack of efficacy was noticed in healthy individuals, chronic smokers, dyslipidemic, and obese subjects who consumed blueberry and cranberry juice. Reduction in oxidative stress markers was noticed in overweight and obese children following a short-term blueberry consumption for 8 weeks. Elevated plasma antioxidant capacity was noticed in elderly women and middle-aged men after consumption of strawberry fruit and blueberry extracts. Oxidative DNA damage was significantly decreased in blood cells isolated from human subjects after the consumption of *Aronia*, blueberry, and boysenberry juice for 5 weeks. Bilberry extracts improved circulation in volunteers with a variety of circulatory problems.

Neuroprotective/Neurodegenerative Diseases and Aging

A combination of epidemiological and preclinical studies suggests that the consumption of berries and berry products may

be effective in reversing neurodegenerative symptoms and age-related declines. But a direct association between berry consumption and improvement in neurological health cannot be made at present. A number of observational and human interventional studies have indicated that regular intake of flavonoid-rich plant food and extracts induced improvements in cognitive functions. Evidence from *in vitro* studies also supported the possibility that polyphenolics in berries can beneficially remodel beta-amyloid aggregation *in vitro*. Blueberry consumption and strawberry consumption have been shown to improve memory in older adults.

Diabetes

Polyphenol-rich berry extracts showed α-amylase and α-glucosidase inhibition *in vitro*. Consumption of a berry puree (blueberry, strawberry, black currant, and cranberry) altered the glycemic responses in volunteers. SBT berry induced changes in postprandial glycemic and insulin responses after glucose intake in a human intervention study. Consumption of black currant juice with crowberry powder altered the glycemic and insulin responses of healthy subjects after sucrose-sweetened juice intake. These effects may originate from the inhibition of α-amylase and α-glucosidase activities.

Cancer

The antiproliferative and anti-inflammatory activities of strawberry, raspberry, blueberry, blackberry, and cranberry juices were evaluated against the human stomach, prostate, intestine, and breast cancer cell lines, and the strongest inhibition of cell growth was observed for the raspberry, lowbush blueberry, and cranberry juices. Reduction in the proliferation of the breast cancer (MCF-7) and colon cell lines (HT-29) was noticed on treatment with extracts from fruits and berries including blueberries, black chokeberries, black currant, and raspberries. The raspberry extracts also conferred significant effective protection to DNA damage induced by hydrogen peroxide in the colon cells. A recent study has shown that freeze-dried black raspberry extracts suppressed cell proliferation of human oral carcinoma cells without affecting their viability and also induced apoptosis. The chemoprotective effects of blackberry extracts were demonstrated in studies conducted in a human lung cancer line, A549 with blackberry extracts that inhibited tumor promoter-induced carcinogenesis and associated cell signaling. Cranberry extracts and cranberry press cake showed strong inhibition of the growth of human breast, prostate, skin, and brain cancer cells, which was attributed to its ability to initiate apoptosis and induce G1 phase arrest in the cell cycle. Bilberry extracts induced programmed cell death in human leukemia cells. *In vitro* digested raspberry extracts significantly decreased the population of HT-29 cells in the G1 phase of the cell cycle and showed that raspberry extracts can inhibit key stages in colorectal cancer development, namely, initiation, promotion, and invasiveness.

Different proanthocyanidin fractions from wild and cultivated blueberries also suppressed the proliferation of the androgen-sensitive (LNCaP) and androgen-insensitive prostate cancer cell lines (DU-145). Cranberry flavonoid fractions inhibited the proliferation of various cancer cell lines (androgen-dependent prostate, breast, skin, colon, lung, and brain cell lines) at varying levels. Esters of ursolic acid also inhibited the growth of several types of tumor cells *in vitro* including MCF-7 breast, HT-29 colon, DU-145 prostate, H460 lung, ME180 cervical, and K562 leukemia cell lines. Ursolic acid isolated from cranberry fruit also showed the inhibition of proliferation of HepG2 human liver cancer cells.

Another important anticancer therapeutic property of berries and berry components derives from their ability to inhibit angiogenesis. Studies conducted using extracts from strawberry, bilberry, wild berry, cranberry, elderberry, and raspberry seeds inhibited angiogenesis inhibiting TNF-α-induced vascular endothelial growth factor expression in human keratinocytes. Berries also impaired angiogenesis in human dermal microvascular endothelial cells. Seed flours from berries including black raspberry, red raspberry, blueberry, and cranberry also showed anticancer properties suggesting that berry seed flours have the potential for the development of products for cancer prevention and overall health.

Medicinal Properties of Berry Bioactives

Research has found that bilberry and its products improve the elasticity and permeability of the capillary vessels of the eyeball improving circulation. Cranberry juice has been effectively used for the prevention and treatment of urinary tract infection (UTI). The antibacterial activity of cranberry juice has been known for a long time. It was reported that proanthocyanidins isolated from cranberry fruit exhibited strong bacterial antiadhesion activity offering protection against UTI. Research has found that proanthocyanidins from cranberry are unique and compositionally different from proanthocyanidins from other fruits such as apple and grape. Cranberry juice and cranberry compounds were capable of exerting bacterial antiadhesion activities against *Helicobacter pylori* and oral bacteria including *Streptococcus mutans*. Blueberry compound also exhibited weak antiadhesion activity to *S. mutans*. Ellagitannin fractions from cloudberry and raspberry also demonstrated potent antibacterial activity against *S. aureus*. Recent studies have shown that pure phenolic acids such as hydroxycinnamic acid have bactericidal and bacteriostatic activity against several strains of *Listeria monocytogenes*. *In vitro* studies demonstrated that bilberry and lingonberry extracts containing high amounts of polyphenols exert protective effects against blue LED light-induced retinal photoreceptor cell damage mainly through inhibition of ROS production and activation of proapoptotic proteins.

Sea Buckthorn

SBT (*Hippophae rhamnoides*), a hardy deciduous shrub, belongs to the family Elaeagnaceae. It occurs as a native plant throughout temperate zones including China, Mongolia, Russia, and most parts of northern Europe. It includes several species and *H. rhamnoides* is the most important. In the recent past, it has attracted considerable research attention around the world mainly because of its high nutritional and medicinal value. SBT is a dioecious multibranched and spinescent shrub that grows to 3–4 m in height. Sea buckthorn berries are among the most nutrient-rich fruits in the plant kingdom. The female

plants produce spherical fruit or berries that turn yellow orange or red when ripe. The fruit has a single dark brown glossy ovoid seed surrounded by a soft fleshy outer tissue. Berries are about 3–8 mm in diameter. Berries consist of 68% pulp, seed (23%), and peel (7.75%). Berries are acidic and astringent and unpleasant to eat raw. Sea buckthorn fruit is composed of seed (23%), pulp (68%), and peel (7.75%). Berries are pressed to yield juice (60–85%). Juice is mixed with other sweet juices like apple or grape. Berries are processed into products such as beverages, squash, syrup, wine, beer, preserves, compote, jams, tea (leaves), and jellies. Astringency of fruit juice is minimized by blending with juice or pulp from other fruits in different ratios. Sea buckthorn berries are also included into pies, candies, and liquors. Sea buckthorn oil is also an ingredient in several cosmetic products such as shampoos, lotions, and creams. Juice, oil from pulp and seeds, cream, and pigments are the main commercially utilized sea buckthorn berry products. A yellow pigment can be extracted from sea buckthorn pulp or skin. This pigment contains flavonones, carotene, and vitamin E. Tea is made from the air-dried leaves of sea buckthorn containing many nutrients and bioactive compounds. Pulp oil is another bye product of sea buckthorn processing and is high in carotenoids, tocopherols, and sterols. It also contains the predominant fatty acid, palmitoleic acid.

Sea buckthorn berries are nutrient-dense and are rich in carbohydrates, proteins, fat-soluble vitamins, antioxidants (vitamins C and E, β-carotene, and lycopene), essential fatty acids, phytosterols, and flavonoids, in addition to minerals such as iron and calcium. Berries are exceptionally rich in vitamin C, and the vitamin C content of sea buckthorn has been found to be higher than strawberry, kiwifruit, orange, tomato, carrot and, hawthorn. Sea buckthorn is a rich source of natural flavonoids such as isorhamnetin, quercetin, and aglycones. Studies have shown that sea buckthorn flavonols when ingested with oatmeal porridge had no significant effect on the levels of oxidized LDL, C-reactive protein, homocysteine, and plasma antioxidant potential. Rapid absorption of flavonols was observed by the addition of a small quantity of sea buckthorn oil to the porridge and by doubling the dosage of flavonols in porridge. Extracts of sea buckthorn were also reported to inhibit the cell proliferation of Caco-2 cells and Hep 2 (liver) cancer cell lines *in vitro*. Clinical and human intervention studies investigating the therapeutic potential of sea buckthorn are inadequate. Majority of the medicinal applications of sea buckthorn products in humans are based on anecdotal evidence. SBT seed oil has been clinically used for the treatment of chronic cervicitis, ulcers, and ulcerative stomatitis. In another human intervention study, a significant decrease in waist circumference and vascular cell adhesion was reported in female volunteers fed with SBT berry oil. Further, positive effects of SBT in improving the liver function were noticed in a study conducted in cirrhotic patients indicating that SBT may help in preventing the progression of liver fibrosis.

Honeysuckle

Lonicera caerulea (blueberry honeysuckle) also known as blue honeysuckle, haskap, honeyberry, and edible honeysuckle is a medium-sized shrub with blue edible fruit. This species belonging to the family Caprifoliaceae is a native of the northern hemisphere. This plant species is commercially cultivated in Russia and Japan. Their flavor is described as similar to a combination of blueberries and raspberries. *Lonicera caerulea* plants grow to 0.8–3 m tall and can survive low temperatures without damage. Plants can bear fruits within 1 year of planting. Dark navy blue to purple-colored fruits are oval to long with blue waxy coating. A variety of beneficial compounds such as organic acids, flavonoids, iridoid glycosides, and saponins are present in this plant. Berries contain sugars, lipids, proteins, organic acids, and polyphenols. Honeysuckle fruits contain about 1.52% lipids. Ursolic acid and its derivative pomolic acid were reported in berries. Chlorogenic, caffeic, and ferulic acids form the main phenolic components in *Lonicera caerulea*. The prominent anthocyanins in *Lonicera caerulea* are the glucosides and rutinosides of cyanidin, peonidin, delphinidin, and pelargonidin. In Chinese medicine, the dried bud of this plant has been used for thousands of years for its antipyretic, antidote, and anti-inflammatory properties.

Acai Berries

Acai (*Euterpe oleracea* Mart.) is a palm tree indigenous to South America and grows widely in Brazil, Colombia, Surinam, and in the Amazonian floodplains. Acai palm, also known as the cabbage palm, bears small purple blackberry-like fruit. The state of Para in Brazil is the main producer of acai berry being responsible for 85% of the world production. Acai is a tall, slender multistemmed, and monoecious palm with pinnate leaves that can grow to about 80 ft. A mature tree has about 4–8 well-developed stems. The stem of the palm is smooth and gray in color. Acai palm is propagated by seeds and suckers. It grows well in organic acidic soil and highly warm and highly humid tropical conditions where temperature rarely drops below 10 °C. This palm is adapted to live in waterlogged and flooded areas by developing special root structures known as pneumatophores. Trees start bearing fruit at 3 years and become fully productive 3 years later. Berries can be harvested throughout the year, and higher yields are noticed during August to December. Acai berry is a small drupe and produced in branched panicles of 500–900 fruit. Fruit are spherical and green when young and unripe and turn dark purple on ripening. Fruits of some varieties remain green at maturity and are called white Acai. Fruit are one-seeded and each fruit has a core surrounded by thin stringy fibers covered by a greasy cuticle. The acai berry is about 1–2 cm in diameter and weighs about 0.8–2.3 g. About 80% of the fruit is seed. Seeds are 0.6–1 cm in diameter. The mesocarp of the berry is thin and pulpy surrounding the tough endocarp. The endocarp contains the seed and embryo as well. Depending on the maturity of fruit and the variety, the exocarp is either green or deep purple. The berries are described as having a nutty flavor with metallic taste and an oily texture.

Acai palm is a commercially valuable plant due to its multiple uses. Recently, acai and its products have gained great popularity as a superfood in the United States and North America. Various acai products are now available in the US market including fruit drinks, freeze-dried powder, powdered juice extracts in capsules, and energy bars and snacks. Local inhabitants use acai berry to prepare a thick dark purple juice

by macerating the ripe fruits. The main export product is a mixture of juice mixed with other fruits such as acerola (*Malpighia emarginata*) and guarana (*Paullinia cupana*).

Lipids comprise 50% of pulp and proteins account for about 10% of the dry matter. Extremely high antioxidant capacity was reported in acai pulp with respect to other anthocyanin-rich fruits and vegetables based on the oxygen radical absorbance capacity. The phytochemical profile of acai berries has been characterized. Berries contain a variety of bioactive phytocompounds such as anthocyanins, phenolic acids, proanthocyanidins, and other flavonoids. Cyanidin 3-rutinoside and cyanidin 3-glucoside are the major anthocyanin components. Additionally, lignins have also been identified. Cyanidin 3-glucoside content ranges from 11.1 to 227 mg per 100 g wt. The total anthocyanin content of acai frozen pulp ranges from 282 to 303 mg per 100 g. Low concentrations of resveratrol have also been detected.

See also: Bioavailability of Nutrients; Grapes; Raspberries and Related Fruits; Strawberries.

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Beverage: Health Effects

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We have learned that we are what we drink more than what we eat. Over the past 60 years, the world has undergone a profound transformation, from drinking minimal calories from beverages to the consumption of hundreds of calories per day. More recently, we have learned that we compensate very little in our food intake when we consume additional calories as a beverage. A large literature that encompasses short-term to long-term feeding studies, longitudinal epidemiological studies, intervention studies, and randomized controlled trials has emerged. We know that the form of the beverage does not matter, as there appears to be little variance in compensation when the beverage is fat-, carbohydrate-, or protein-based. In this article, we review our knowledge about the health effects of caloric beverages. This is done in the context of our having drunk water for millennia and developing physiological systems finely attuned to water intake and seasonal hunger. We know a great deal about the relationship between beverage form and energy intake and the subsequent relationship with an array of cardiometabolic problems; however, we do not understand mechanisms underlying this well. The article also discusses additional health problems linked with excessive refined carbohydrate intake and excessive fructose intake. We then present some historical evidence on beverage consumption and discovery and review recent patterns and trends of beverage consumption.

One of the most important aspects of the entire literature of beverages and health is the dearth of research on water and health. We have developed physiological adaptations to protect us against dehydration. Although the bulk of our body is composed of water, we focus far more attention on reducing the intake of all caloric beverages than on understanding the strengths and importance of water.

One of the major dimensions of the sugar and beverages and health debasement reflects the fact that there is an innate human desire for sweetness. If sugar, high-fructose corn syrup (HFCS), and fructose were not sweet, there would be no debate because their consumption would be low. Sweet taste is present in newborns and increases in intensity throughout childhood. It may be that the 'craving' for sweets can even be enhanced by early exposure to intense sweeteners. The food industry has used sugar as a major sweetener for delivery for increasing the amounts of beverages and food over the past half century. The result has been that the consumption of sugar-sweetened beverages (SSBs) rose by a startling 38.5 gallons per person between 1950 and 2000 (from 10.8 gallons per person in 1950 to 49.3 gallons per person in 2000). We have seen small declines since then; however, the industry continues to find new ways to increase liquid sugar consumption by constantly adding new products, be they in fruit juice, energy drinks, vitamin waters, protein waters, sports drinks, and hundreds of new options. Thus, the question 'do current levels of sugar consumption pose a serious health risk to Americans?' seems in crying need of clarity.

Beverage Intake: Effects on Health

Ingestatory Behavior

There is a clear biological basis for reducing intake of sugary beverages, namely, the lack of compensation by reducing food intake when one consumes a caloric beverage. This is particularly true for SSBs but there is an emerging literature that suggests that 100% fruit juice has identical effects to SSBs on our health. There appears to be little reduction in food intake when caloric beverages are substituted for water and other low-nutritive sweetened or 'diet' beverages. This relationship between beverage intake and food intake occurs despite studies showing that individuals who consume caloric beverages feel they are sated. While they may feel more sated, they do not reduce their food intake. Consequently, individuals fed with water or noncaloric or low-calorie sweetened beverages consume reduced total energy intake when compared with those consuming caloric beverages. This relationship appears to hold as food and beverage form changes. That is, whether it is a carbohydrate-, fat-, or protein-based beverage (e.g., SSB, coconut milk, or cow's milk), the relationship holds. A more detailed examination shows that intake of calorically sweetened beverages does not reduce the intake of solid food by a corresponding amount.

The effects of consuming water with meals with various types of caloric and diet beverages (DBs) have been less studied. We undertook a systematic review of English language studies evaluating the impact on energy intake and/or weight status of not drinking water or drinking other beverages compared with water. Relevant clinical trials and epidemiological and intervention studies were collected, and findings across the literature were summarized. Using clinical trials, average differences in total energy intake at test meals (ΔTEI) were calculated across studies for several beverage categories compared to water. The literature for these comparisons is sparse and somewhat inconclusive. One of the most consistent sets of studies compared drinking SSBs with water among adults at a single meal. Total energy intakes were increased by 7.8% (ΔTEI range: −7.5 to 18.9) when SSBs were consumed. Studies comparing noncaloric sweetened beverages with water were also relatively consistent and found no impact on energy intake among adults (ΔTEI = − 1.3, range: −9 to13.8). A set of fewer studies showed that replacing water with milk and juice increased TEI by an estimated 14.9% (range: 10.9 to 23.9).

One-year-long intervention study also promoted water and removed from schools all caloric beverages. This study instituted both educational and environmental interventions to increase water intake in 17 German schools (15 control schools had no intervention). Teachers presented four empirically developed lessons about the body's water needs and the water cycle. Special filtered drinking fountains were installed, water bottles were distributed, and teachers were encouraged to

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organize the filling of water bottles each morning. After 1 year, the children completed 24 h beverage intake recalls in class. Intervention schools had higher water intakes (1.1 glasses per day, $P<0.001$) and lower adjusted risk of overweight (OR = 0.69, 95% CI: 0.48–0.98).

Weight and Children: Trials

Over the past decade, the major addition to our literature on the impact of SSBs has been the effect of SSBs on child weight and risk of obesity. We have summarized research that was reported in detail elsewhere. A total of five studies including 2772 children and adolescents were included in our analysis of SSB trials and body weight. Based on these data, Malik, Pan, Willett, and Hu found a nonsignificant difference in the change in body mass index (BMI) from reducing SSB consumption (weighted mean difference (WMD) −0.17; 95% CI: −0.39, 0.05; $I^2=74.6\%$; P-heterogeneity = 0.003) in the random-effects model (Figure 1). Results from the fixed-effects model were statistically significant (−0.12; 95% CI: −0.22, −0.02). Different statistical models may explain these differences. Metaregressions for intervention modality (education or beverage substitution; $P=0.08$), duration ($P=0.18$), and age ($P=0.84$) were not statistically significant, although power to detect a difference was low with only five studies. When we stratified our analysis by intervention modality, they observed a significant weight reduction among the three studies that provided noncaloric beverages as substitutes for SSB: the summary estimate was (−0.34; 95% CI: −0.50, −0.18; $I^2=0\%$). In contrast, we did not find a significant intervention effect in the two studies that used focussed school-based education to discourage SSB consumption (0.01; 95% CI: −0.19, 0.20; $I^2=59.6\%$).

All studies except for the one by Sichieri et al. showed a beneficial effect or trend of interventions to reduce SSB intake on weight. The study by Sichieri et al. was a school-based intervention that used focussed education to discourage the consumption of carbonated SSBs, but according to the author, the students compensated by increasing their consumption of sugar-added juices and fruit drinks, which may explain the lack of findings. However, in a subgroup analysis, children who were overweight at baseline showed greater BMI reduction in the intervention group, which was statistically significant among girls. Similarly, Ebbeling, Ludwig, and their team, discussed in this same article, found more pronounced benefits of the intervention among adolescents who were overweight at baseline, and Ebbeling et al.'s study, which was conducted exclusively among overweight adolescents, showed the strongest intervention effect among studies included in our analysis. Combining Ebbeling et al. with the subgroup findings from Ebbeling et al., we observed an increased benefit of substituting noncaloric beverages for SSB on weight gain (−0.64; 95% CI: −1.07, −0.21), suggesting that this type of intervention may have greater impact on those who are overweight.

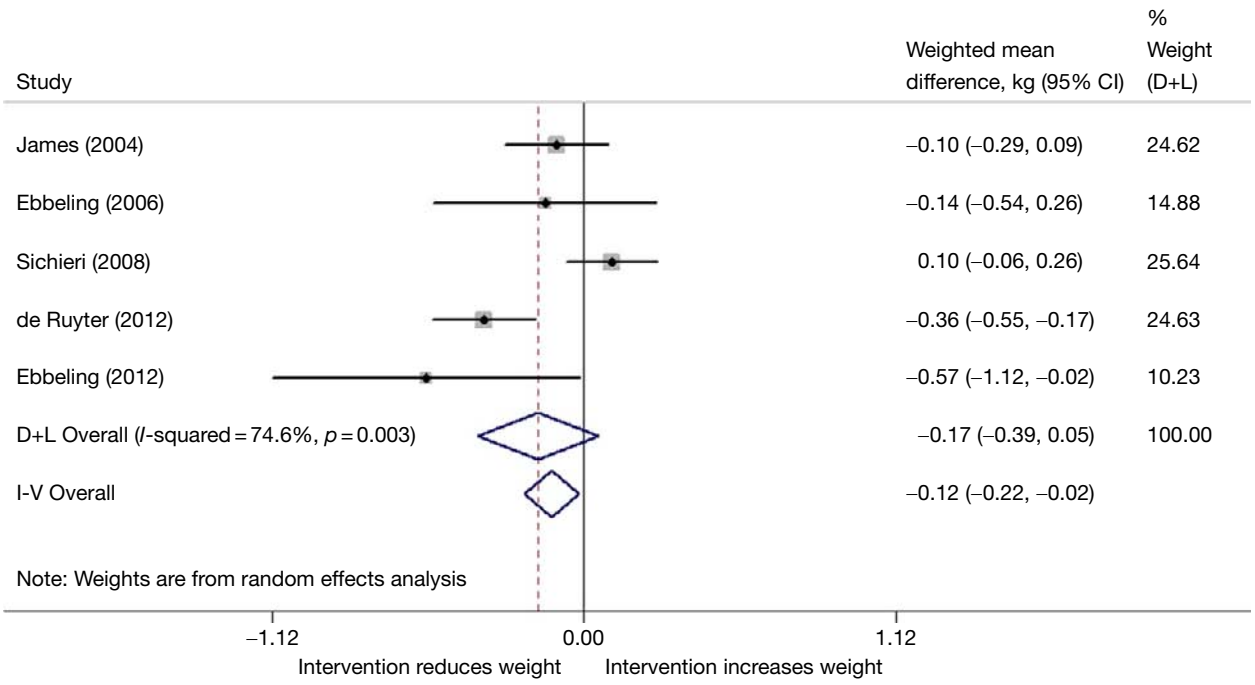

Figure 1 Weighted mean difference in BMI change (95% CI) between intervention and control regimens from RCTs in children. Interventions evaluated the effect of reducing SSB. Horizontal lines denote the 95% CIs; solid diamonds represent the point estimate of each study. The open diamond represents the pooled estimate of the intervention effect and the dashed line denotes the point estimate of the pooled result. Weights are from the random-effects analysis (D + L; DerSimonian and Laird). Pooled estimates from the random-effects analysis (D + L; DerSimonian and Laird) and fixed analysis (I-V; inverse-variance) are shown based on 5 RCTs ($n=2772$). *I*-squared value and *P*-value for heterogeneity are shown. Reproduced from Malik, V. S., Pan, A., Willett, W. C. and Hu, F. B. (2013). Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. *American Journal of Clinical Nutrition* 98, 1084–1102.

There is also extensive epidemiological literature on child weight gain but minimal work on other outcomes. We did not review the epidemiological child weight gain literature here since the major RCTs for children – the two most recent NEJM ones – provide such convincing evidence. Based on the totality of the available evidence from prospective cohort studies, a one serving per day increase in SSB was associated with a 0.06 unit increase in BMI over a 1-year period among children and adolescents and an additional weight gain of 0.12–0.22 kg (~0.25–0.50 lb) over a 1-year period among adults. In children, it is difficult to gauge the impact of our findings, since weight gain in childhood varies as a function of age, maturation, and growth velocity.

Adult Weight

Prospective cohort studies

To our knowledge, the Malik, Pan, Willett, and Hu study noted in the preceding text was the first meta-analysis to evaluate prospective cohort studies of SSB and body weight in adults. This analysis of a 1-year change in weight (kg) in adults was based on seven studies, including eight comparisons and 170 141 men and women. We found that each serving per day increase in SSB was associated with an additional weight gain of 0.22 kg over 1 year (0.22; 95% CI: 0.09, 0.34; $I^2 = 70.2\%$; P-heterogeneity <0.001) from the random-effects model (Figure 2). Metaregressions for age at baseline ($P = 0.32$), duration ($P = 0.37$), use of FFQ to assess diet ($P = 0.26$), sample size ($P = 0.48$), and baseline weight status ($P = 0.10$) were not statistically significant. However, when they stratified the analysis by baseline weight status, they observed greater though nonsignificant weight gain in the two studies conducted among overweight populations (1.22 kg; 95% CI: −0.23, 2.68; $I^2 = 77.5\%$), compared with non-overweight populations (0.15; 95% CI: 0.06, 0.24; $I^2 = 50.3\%$).

Trials

A total of five studies including six comparisons with 292 men and women were included in our analysis of trials in adults. We found a significant difference in change in body weight (kg) between intervention and control regimens (WMD: 0.85; 95% CI: 0.50, 1.20; $I^2 = 0.0\%$; P-heterogeneity = 0.78) from the random-effects model. All studies observed significantly greater weight gain or trends toward greater weight gain in intervention compared to control regimens and there was no evidence of heterogeneity. When stratified by baseline weight status, they observed greater weight gain in intervention

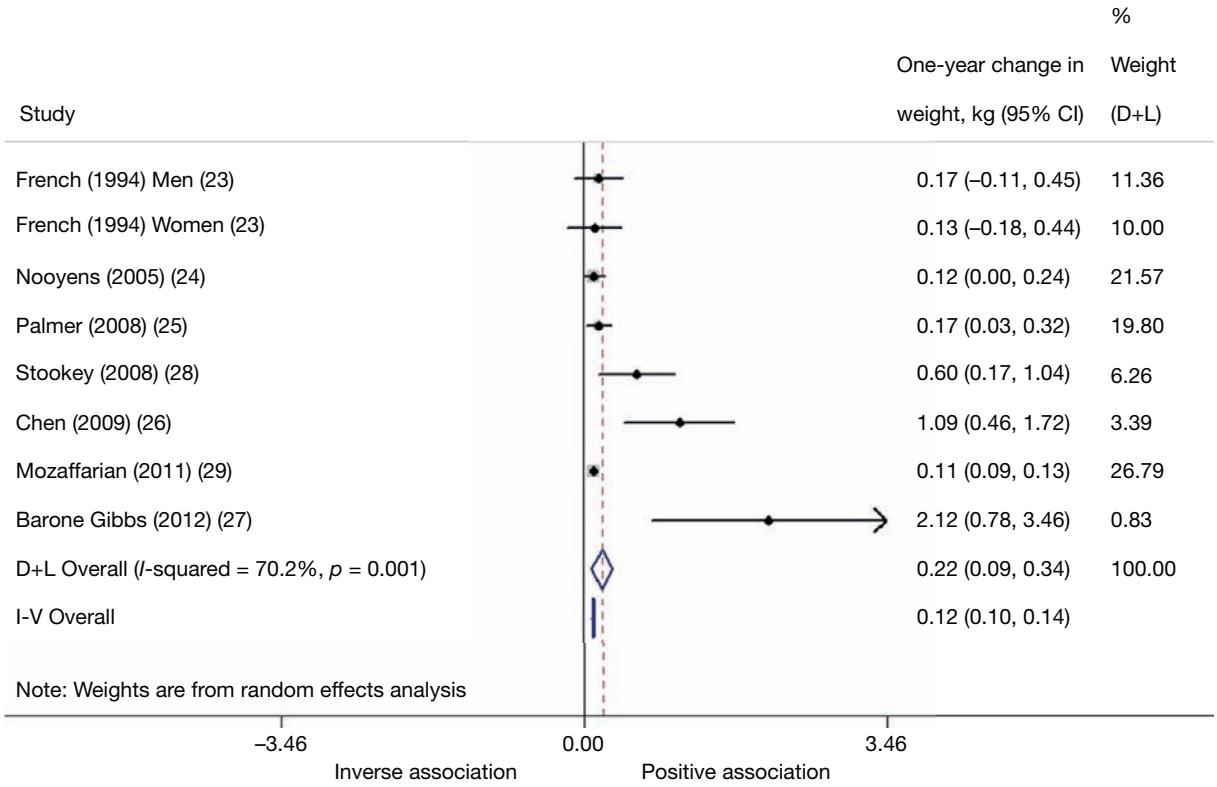

Figure 2 One-year change in weight (kg) per one serving per day increase in SSB, from prospective cohort studies in adults using a change versus change analysis strategy. Horizontal lines denote the 95% CIs; solid diamonds represent the point estimate of each study. The open diamond represents the pooled estimate and the dashed line denotes the point estimate of the pooled result. Weights are from the random-effects analysis (D + L; DerSimonian and Laird). Pooled estimates from the random-effects analysis (D + L; DerSimonian and Laird) and fixed analysis (I-V; inverse-variance) are shown based on seven cohort studies ($n = 174\,252$). *I*-squared value and *P*-value for heterogeneity are shown. Reproduced from Malik, V. S., Pan, A., Willett, W. C. and Hu, F. B. (2013). Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. *American Journal of Clinical Nutrition* 98, 1084–1102.

compared to control regimens among the three studies conducted in non-overweight populations (WMD: 0.89; 95% CI: 0.52, 1.26; $I^2 = 0.0\%$), compared with the two studies conducted in overweight populations (WMD: 0.47; 95% CI: −0.70, 1.63; $I^2 = 0.0\%$). Adding the study by Raben et al. to the analysis, which was excluded because the intervention contained some foods in addition to beverages (~70% beverage and 30% food), increased the overall estimate but introduced some heterogeneity to the studies examined (WMD: 1.06; 95% CI: 0.54, 1.58; $I^2 = 46.3\%$; P-heterogeneity = 0.08).

Adult SSB and Type 2 Diabetes

A growing body of evidence clearly indicates that SSB consumption is associated with increased risk of diabetes through effects on adiposity and independently through other metabolic effects. These results come from our Malik et al. review. We conducted a meta-analysis of eight prospective cohort studies evaluating SSB intake and risk of diabetes. Based on 3,10,819 participants and 15,043 cases, individuals in the highest category of SSB intake (usually 1–2 servings per day) had a 26% (RR 1.26, 95% CI 1.12–1.41) greater risk of developing type 2 diabetes compared with those in the lowest category (none or <1 per month) (Figure 3). A one serving per day increase in SSB was associated with a 15% increased risk for diabetes (RR 1.15, 95% CI 1.11, 1.20). Similar to studies evaluating weight gain, results were generally more consistent among larger and longer studies that did not adjust for potential intermediates in the causal chain such as total energy intake and, in the case of diabetes, BMI (kg m^{-2}). In a study by Schulze et al. in more than 50 000 women with 8 years of follow-up, those that consumed ≥1 SSB per day had an 83% increased risk for diabetes, compared with those consuming <1 per month (p trend <0.001) after adjusting for a number of risk factors. After additional adjustment for BMI, the associated risk decreased to 41% (p trend <0.001), suggesting that BMI accounts for about half of the excess risk. Similar findings were recently reported in the Health Professionals Follow-up Study in over 40 000 men followed for 20 years where SSB was associated with a 24% (p trend <0.01) increased risk of diabetes comparing extreme categories after adjusting for risk factors including pre-enrollment weight change, dieting, total energy intake, and BMI. Although these studies adjusted for various dietary and lifestyle risk factors in their analyses, they are observational and confounding by unmeasured or imperfectly measured factors that may still be present, as SSB may be a marker for a globally unhealthy diet and lifestyle. Randomized controlled trials, on which policies and public health recommendations are often based, are not well suited to evaluate this question because they are greatly affected by intervention intensity and limited by compliance and duration for clinical end points. For these reasons, most interventions have evaluated biological markers of T2D risk or metabolic syndrome.

SSB and Cardiovascular Risk

A small number of prospective cohort studies have evaluated the risk of metabolic syndrome in relation to SSB consumption. Our recent meta-analysis pooled findings from three studies including 19,431 participants and 5803 cases of metabolic syndrome and observed an increased risk of about 20% comparing highest to lowest categories of intake. Two of these studies also looked at SSB consumption in relation to individual components of metabolic syndrome. Dhingra et al. found that individuals that consumed ≥1 SSB per day had a marginal

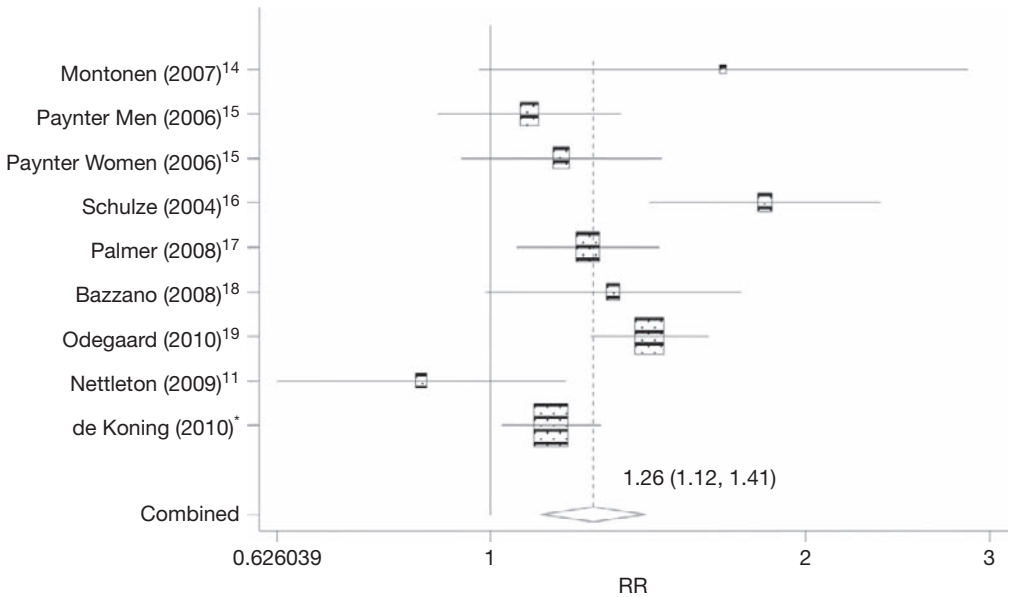

Figure 3 Forrest plot of studies evaluating SSB consumption and risk of type 2 diabetes, comparing extreme quantiles of intake. Random-effects estimate (DerSimonian and Laird method). *Information from personal communication. Reproduced from Malik, V. S., Popkin, B. M., Bray, G. A., Despres, J. P., Willett, W. C. and Hu, F. B. (2010). Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. *Diabetes Care* 33(11), 2477–2483.

18% (RR 1.18, 95% CI 0.96, 1.44) greater risk of developing hypertension compared with nonconsumers after adjusting for baseline hypertension, age, sex, physical activity, smoking, intake of saturated fat, trans fat, fiber, magnesium, total energy, and glycemic index. Results from Nettleton et al. also found a marginal effect of SSBs on incident hypertension (RR 1.10, 95% CI 0.87, 1.39) comparing daily consumers with nonconsumers. These trends are supported by stronger associations in the NHS and NHS II cohorts where women that consumed $\geq$4 SSB per day had a 44% and 28% greater risk of developing hypertension, respectively, compared with infrequent consumers. Studies by Dhingra et al. and Nettleton et al. also found that daily SSB consumers had a marginally increased risk for developing hypertriglyceridemia compared with infrequent consumers after adjusting for risk factors (RR 1.25, 95% (CI 1.04, 1.51) and RR 1.24, 95% CI (0.98, 1.57), respectively). In these studies, daily SSB consumers also had an increased risk for low HDL cholesterol (RR 1.32, 95% CI (1.06, 1.64) and RR 1.24, 95% CI (0.95, 1.61), respectively). In the Coronary Artery Risk Development in Young Adults (CARDIA) study, higher SSB consumption across quartiles was associated with a number of adverse cardiometabolic outcomes: high waist circumference (RR: 1.09; 95% CI: 1.04, 1.14; p for trend, 0.001), high LDL cholesterol (RR: 1.18; 95% CI: 1.02, 1.35; p for trend = 0.018), high triglycerides (RR: 1.06; 95% CI: 1.01, 1.13; p for trend = 0.033), and hypertension (RR: 1.06; 95% CI: 1.01, 1.12; p for trend = 0.023).

Data from short-term trials also provide important evidence linking SSB with cardiovascular risk. Raben et al. found that a sucrose-rich diet consumed for 10 weeks resulted in significant elevations of postprandial glycemia, insulinemia, and lipidemia compared with a diet rich in artificial sweeteners in overweight healthy subjects. A randomized crossover trial among normal-weight healthy men found that after 3 weeks, SSBs consumed in small to moderate quantities (600 ml SSB per day containing 40–80 g of sugar) significantly impaired glucose and lipid metabolism and promoted inflammation. Specifically, LDL particle size was reduced for high-fructose and high-sucrose SSBs and a more atherogenic LDL subclass distribution was seen when fructose- and high-sucrose-containing SSBs were consumed. Fasting glucose and high-sensitivity C-reactive protein increased significantly after fructose, glucose, and sucrose intervention and leptin increased during interventions with SSBs containing glucose. A 10-week intervention comparing the effects of sucrose and artificially sweetened food/beverages on markers of inflammation found that serum levels of haptoglobin, transferrin, and CRP were elevated in the sucrose group compared with the sweetener group.

SSBs may affect the risk of coronary heart disease (CHD) in a relatively short time of just a few years through effects on inflammation that influence atherosclerosis, plaque stability, and thrombosis. Few studies have looked at the association between SSB consumption and clinical CHD. Among over 88 000 women in the NHS followed for 24 years, those that consumed $\geq$2 SSBs per day had a 35% (p trend 0.01) increased risk of CHD compared with infrequent consumers after adjusting for risk factors. Additional adjustment for mediating factors such as BMI, total energy, and incident diabetes attenuated the associations, but they remained statistically significant, suggesting that the effect of SSBs is not entirely mediated by these factors.

A recent meta-analysis of the effect of SSBs on blood pressure was published by Malik, Akram, Shetty, SS Malik, and Njike. All of the final studies reviewed showed a positive relation between SSB intake and hypertension with only 10 of the 12 achieving statistical significance. Their most conservative estimate showed that intake above 12 fl oz of SSB per day would increase the risk of hypertension by at least 6%.

Randomized controlled trials

To date, one well-executed randomized controlled trial has been completed. The CHOICE (Choosing Healthy Options Consciously Everyday) clinical trial focussed on the impact of shifting from normal caloric beverages to either water or DBs among adults involved in active weight loss. The hypothesis was that participants assigned to the beverage substitution groups would achieve greater weight loss at 6 months compared with control participants who made dietary changes of their choosing (DBs were greater than active choices (ACs), and water (WA) greater than AC). Secondary outcomes were to compare the noncaloric beverage groups to the control group on criterion measures of weight loss, waist circumference, blood pressure, glucose, and osmolality as a marker of hydration from 0 to 3 months and 0 to 6 months.

Despite similar or somewhat smaller weight losses, replacement WA showed statistically significant reductions in fasting glucose and improvement in hydration compared with control AC. DB also showed improvements on many of these parameters by 6 months but was not significantly different from AC. In the completer's analysis, the improvements in systolic and diastolic blood pressure in WA compared with AC reached statistical significance. This study showed that approximately a two-serving reduction in caloric beverages resulted in a 2 kg weight loss at 6 months across DB and WA.

In the intent-to-treat analysis, all groups significantly reduced weight and waist circumference and improved systolic blood pressure from 0 to 6 months. Average percentage weight losses ($\pm$SE) at 6 months were DB -2.54% (0.45), WA -2.03% (0.40), and AC -1.76% (0.35); there were no significant differences between groups. DB had greater odds of achieving a 5% weight loss at 6 months compared with AC (OR = 2.29, 95% CI: 1.05, -5.01; $p = 0.04$); WA showed a significant reduction in fasting glucose at 6 months ($p = 0.19$) and improved hydration at 3 ($p = 0.0017$) and 6 ($p = 0.049$) months compared with AC. In a combined analysis, participants assigned to replace beverages were two times more likely to have achieved a 5% weight loss (OR = 2.07, 95% CI: 1.02, -4.22; $p = 0.04$) compared to AC.

The strength of this study is that it is the first randomized controlled trial in adults examining a simple strategy for calorie reduction and weight control, with participants masked to the study purpose, including over 50% racial and ethnic minorities, strong retention rates, 24 h dietary recalls, provision of beverages, an attention control group, and objective weight and physiological outcome measures.

On a population level, the replacement of caloric beverages with noncaloric alternatives could be an important public health message. This strategy also has implications for health care settings, as assessing SSB intake is feasible and the

prescriptive recommendation to replace caloric beverages with noncaloric alternatives is simple and straightforward. Replacing SSB with either DBs or water appears warranted based on these findings.

Does the Type of Caloric Sweetener Matter? Is HFCS the Culprit?

Fructose comes from many sources; however, it first came to fame as a component of HFCS. We now understand that the source of the fructose does not matter and that fructose contained in any sugar (e.g., cane or beet sugar, fruit juice concentrate, honey, and corn syrup) is equally harmful. At one point, there was extensive concern that HFCS might have posed a major impact on health. Bray et al. hypothesized that the large shift toward the use of HFCS coupled with unique metabolic properties of fructose posed a major problem.

Subsequent research has provided clear evidence that all sugars are equal in their impact on cardiometabolic health; nevertheless, the fructose component of sugars such as sucrose and syrup HFCS might lead to additional cardiometabolic risks. These range from gout, linked with the high concentration of uric acid, to many other cardiometabolic complications, particularly related to renal function.

What about Noncaloric Sweeteners?

An increasing component of the beverage market, particularly in high- and middle-income countries, consists of beverages with either noncaloric sweeteners (NCSs) or a combination of caloric sweeteners and NCS. There is minimal evidence that NCS poses any toxicological effect on health. However, there is a large set of epidemiological studies suggesting that the consumption of beverages with NCS is linked with increased cardiometabolic risks including diabetes, metabolic syndrome, and obesity.

Two recent studies question this relationship. A new study by de Koning and colleagues found that an observed association between intake of beverages with NCS and increased risk of T2DM is attenuated and no longer statistically significant following multivariate adjustment for detailed measures of family history, previous weight change, dieting, Healthy Eating Index score, and total energy intake (top bottom quartile of intake: HR (95% CI): 1.09 (0.98, 1.21), p for trend = 0.13). Although de Koning et al. addressed possible confounding by diet more completely than other studies have, their results still could have missed important interactions between dietary pattern and DB consumption.

A second publication went a step further. Using CARDIA longitudinal data, Duffey et al. found that people who consumed DBs tended to be less healthy compared to people who did not consume them. However, there was an important interaction with diet. People who were healthiest tended to be those who ate a 'prudent' or healthy diet (with more fruit, whole grains, nuts, and milk) and did not consume DBs. They had a lower risk of high waist circumference, high triglyceride levels, and metabolic syndrome. But the second healthiest group was those individuals with a prudent diet who also consumed DBs. In contrast, individuals who consumed the 'Western' diet, which had higher amounts of fast food, meat and poultry, pizza, and snacks, had increased risk of heart disease regardless of whether or not they drank DBs.

A recently published study illustrates the complex issues we face related to the use of NCS in beverages and foods. Piernas et al. compared dietary patterns of those in the previously mentioned CHOICE trial. The DB group showed statistically significant decreases in most caloric beverages and specifically reduced more dessert consumption over the study period. The DB group showed statistically significant decreases in most caloric beverages and specifically reduced more on desserts over the study period. This is a small study not powered to examine this topic, but it is suggestive of a potentially important issue.

Mechanisms, History: When Did Caloric Beverages Enter the Human Food System?

We have no clear known mechanisms that explain why when we consume beverages it does not affect our food intake; however, we speculate on this mechanism. It is well known that humans will die within 3–7 days if they do not consume water. Water comprises 75% of the body weight in infants to 55% in the elderly and is essential for cellular homeostasis and life. From the time that primeval species ventured from the oceans to live on land, a major key to survival has been the prevention of dehydration. The critical adaptations cross an array of species, including *Homo sapiens*. To prevent dehydration, reptiles, birds, vertebrates, and all land animals have evolved an exquisitely sensitive network of physiological controls to maintain body water and fluid intake by thirst. Humans may drink for various reasons, particularly for hedonic ones, but most drinking is due to water deficiency, which triggers the so-called regulatory or physiological thirst. We understand from extensive research on the topic that the hydration system of a human is finely tuned to protect us. What is not clear is how this thirst mechanism is different from the hunger or feeding mechanism. We know that humans will die if they do not eat over a 1- to 2-month period depending on their initial weight so there appear to be some evolutionary reasons behind the lack of compensation in food intake when they drink water or caloric beverages.

A consideration of our evolutionary history may help to explain our poor compensatory response to calories from fluids. Elsewhere, we have reviewed the history of eight important beverages: milk, beer, wine, tea, coffee, distilled alcoholic beverages, juice, and soft drinks. Humans may lack a physiological basis for processing carbohydrate or alcoholic calories in beverages because only breast milk and water were available for the vast majority of our evolutionary history. Alternatives to those two beverages appeared in the human diet no more than 11 000 years ago, but *Homo sapiens* evolved over a 100 000- to 200 000-year period. Second, carbohydrate- and alcohol-containing beverages may produce an incomplete satiation sequence that prevents becoming satiated on these beverages.

Current Patterns and Trends of Beverage Intake

Global Aggregate Trends

Across the globe, there is evidence of increasing a shift toward increased consumption of SSBs and more recently also

increased intake of NCS beverages. A recent study examined patterns of carbonated soft drink availability using the two largest and most influential producers of sweetened beverages, The Coca-Cola Company and PepsiCo, who together control 34% of the global soft drink market, examining their product portfolios globally and in three critical markets (the United States, Brazil, and China) from 2000 to 2010. This study used Euromonitor International's definition of 'soft drink,' which includes the aggregation of (i) carbonates/carbonated soft drinks, (ii) fruit/vegetable juice, (iii) bottled water, (iv) functional drinks, (v) concentrates, (vi) ready-to-drink tea, (vii) ready-to-drink coffee, and (viii) Asian specialty drinks. Thus, although the term 'soft drink' may refer specifically to carbonated soft drinks in everyday use, the 34% market share takes into account other beverage categories, with carbonated soft drinks considered a subset of soft drinks overall.

On a global basis, total revenues and energy per capita sold increased from 2000 to 2010, yet the average energy density (kilojoules per 100 ml) sold declined slightly, suggesting a shift to lower-calorie products. Per capita volume sales showed similar worldwide trends during the past decade, with modest increases in sales of carbonated soft drinks alongside marked increases in bottled water, fruit/vegetable juice, and sports and energy drinks.

What is most interesting is the differential trends in the United States versus the developing markets of Brazil and China. Despite the global increase in energy and volume sold per capita by Coca-Cola and PepsiCo from 2000 to 2010, there is a clear decrease in energy and volume sold per capita in the United States over the same time period because of a shift toward reduced kilocalories per milliliter of sales. In contrast, the opposite was true in Brazil and China, with total per capita energy increasing greatly in China and to a lesser extent in Brazil. Daily energy per capita sold by Coca-Cola increased by 41% in Brazil between 2000 and 2010, while daily energy per capita sold by PepsiCo rose by 168%, largely because of increases in energy from carbonated soft drinks. Likewise, in China, daily energy per capita sold by Coca-Cola increased by 215% between 2000 and 2010, while daily energy per capita sold by PepsiCo rose by 147%, also driven by carbonated soft drinks. Energy from carbonated soft drinks alone sold by both Coca-Cola and PepsiCo experienced an even stronger upward growth trend in China between 2000 and 2010. Figure 4 shows the global increases in this one component of soft drink sales from these two companies. The remarkable increases in Brazilian and Chinese sales per capita are presented in Figure 5.

A major objective of the global beverage companies has been to increase sales of water and beverages with NCS and to reduce their total sale of calories. Figure 6 highlights the much lower in average energy density of carbonated soft drinks sold by Coca-Cola and PepsiCo in the United States versus globally and in Brazil and China, as well as the reduction ounce of in average energy density of carbonated soft drinks for both companies. In contrast, the energy density of carbonated soft drinks has not changed in China while there is a slight reduction by PepsiCo in Brazil. Caution is warranted in interpreting Figure 3. First, Coca-Cola and PepsiCo represent only the two largest companies, but in each local market, there are many other companies, few of which have the capacity to promote sales of nonnutritively sweetened beverages like these two companies do. Second, Figures 1 and 2 show overall caloric beverage sales increases represent potentially important current risks to energy imbalance and weight gain.

Discussion and the Future

An expanding literature suggests that caloric beverage intake is linked with obesity and excessive weight gain and increased risk of diabetes and cardiovascular disease. One of the clearest causal linkages between our food supply and excessive weight gain and an array of cardiometabolic problems is the excessive intake of the array of SSBs. Our concern is echoed by experts in the heart, cancer, diabetes, and many other fields as well as in much public debate. Nevertheless, the sugar and beverage sectors represent two of the more powerful of interests in our society, dwarfing the tobacco sector. Country after country is attempting to limit the consumption of SSBs and other caloric beverages, and in many cases, they are succeeding as is seen by the recent 10% SSB tax in Mexico and in equally complex attempts that have yet to succeed in many other countries.

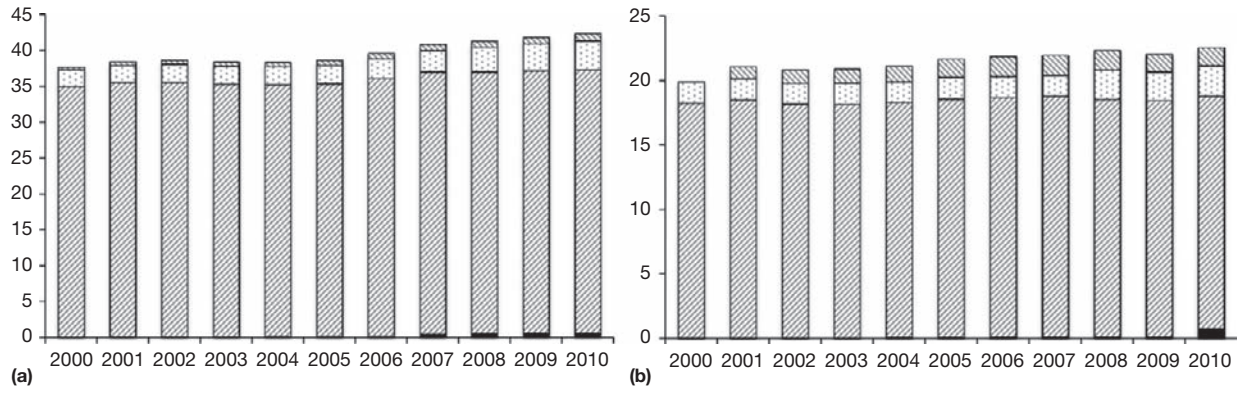

Figure 4 Global trends 2000–10 in daily calories sold. ▨, fruit/vegetable juice; ■, Bottled water; ▧, sports and energy drinks ▨, carbonates. (a) Per capita daily energy sold (kJ) The Coca-Cola Company – world. (b) Per capita daily energy sold (kJ) PepsiCo – world.

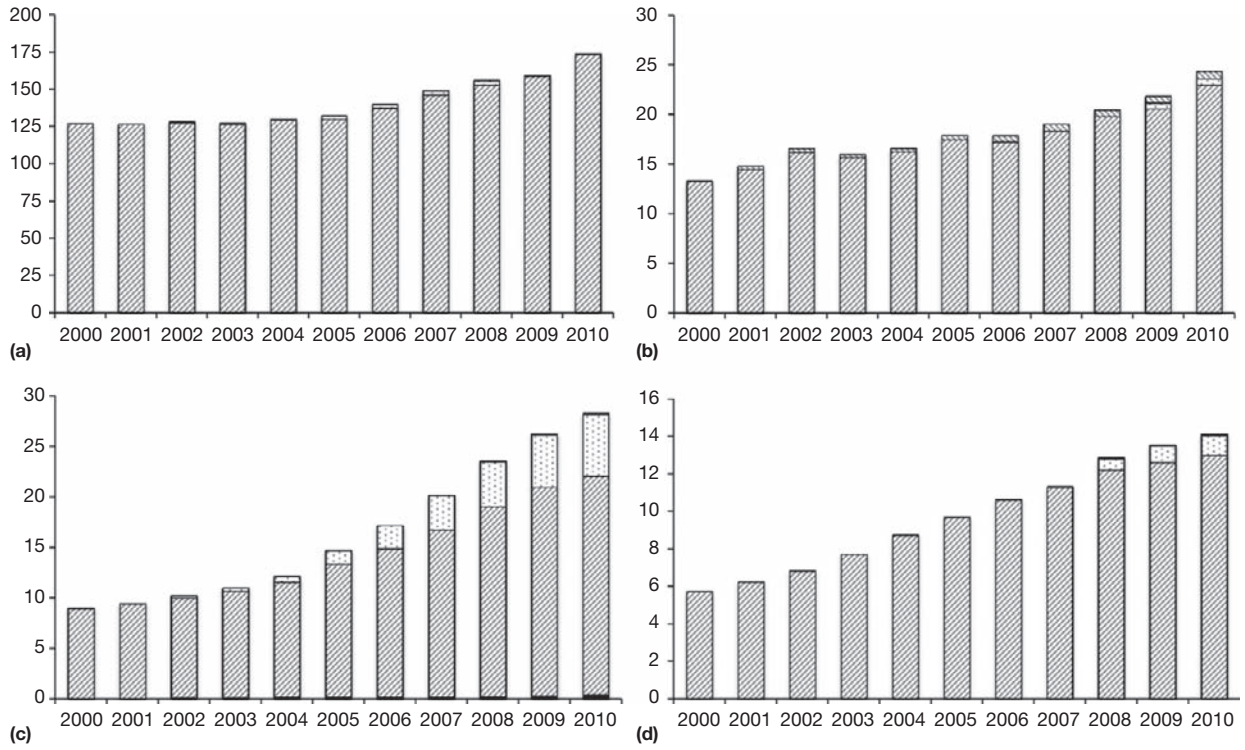

Figure 5 Brazilian and Chinese trends 2000–10 in daily calories sold. ▨, fruit/vegetable juice; ■, Bottled water; ▧, sports and energy drinks. ▨, carbonates. (a) Per capita daily energy sold (kJ) The Coca-Cola Company – Brazil. (b) Per capita daily energy sold (kJ) PepsiCo – Brazil. (c) Per capita daily energy sold (kJ) The Coca-Cola Company – China. (d) Per capita daily energy sold (kJ) PepsiCo – China.

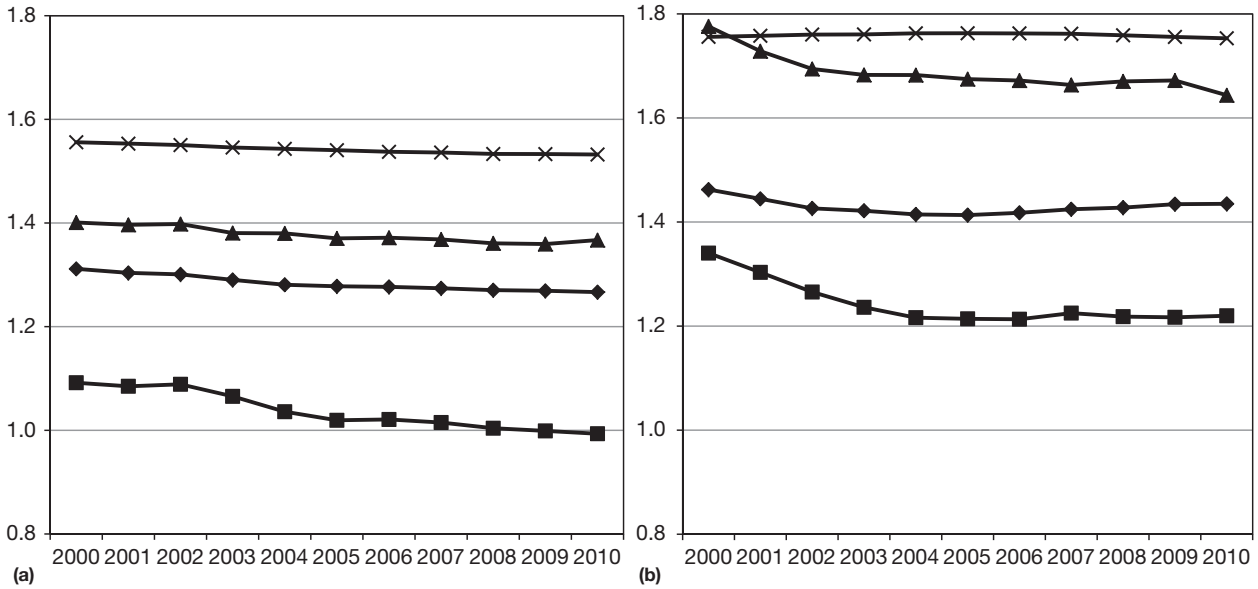

Figure 6 Trends 2000–10 in calories per ounce sold: global, the United States, Brazil, and China. —◆—, World; —■—, the United States; —▲—, Brazil; —×—, China. (a) Kilojoules per 100 ml sold The Coca-Cola Company – carbonates. (b) Kilojoules per 100 ml sold PepsiCo – carbonates.

Acknowledgments

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See also: Beverage: Patterns of Consumption; Nutritional Epidemiology; Obesity: Causes and Prevalence; Obesity: Epidemiology of; Obesity: The Role of Diet.

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Beverage: Patterns of Consumption

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Introduction

Beverages contribute more to hydration than they do to energy intakes. Based on the national food consumption data for the United States, drinking water and caloric and noncaloric beverages accounted for up to 75% of total water intake with the remaining 25% provided by moisture in foods. In contrast, solid foods provide as much as 81.3% of daily calories for people aged over 4 years, whereas caloric beverages provide only 18.7%. Beverages consumed in the United States include drinking water, milk, juices, sodas (carbonated) and fruit-flavored drinks, and coffee and tea.

The nutrient density of beverages has been expressed in nutrients per calorie and nutrients per serving. While drinking water, tap and bottled, contains no calories and no nutrients, other beverages are important dietary sources of vitamin C, potassium, calcium, and other vitamins and minerals. Although sweetened beverages are the biggest source of added sugars, they are not the largest source of dietary calories. Added sugars account for about 13–18% of total daily calories in the American diet, depending on age. On the average, sugar-sweetened beverages (SSBs) account for about 40% of added sugars. Thus, the mean contribution of SSBs to total daily calories in the United States has been estimated at 6–7%.

Consumption patterns of different beverages vary sharply by age. Young children are more likely to drink milk, whereas older adults are more likely to drink coffee. Consumption of both citrus juices and sodas peaks in adolescence before declining in adult life. Consumption patterns can also vary by socioeconomic status (SES). In the United States, consumption of tap water, bottled water, skimmed milk, and diet soft drinks has been linked to higher education and higher incomes. In contrast, the regular consumption of soda and whole milk is linked to lower SES.

Most data on beverage consumption patterns and consumption trends in the United States come from federal agencies. The ongoing National Health and Nutrition Examination Survey (NHANES), conducted by the National Center for Health Statistics, is the primary source of beverage consumption data, based on 1- or 2-day food recall. NHANES data are based on a representative sample of the United States population, spanning different demographics and age groups. The US Department of Agriculture (USDA) maintains national food availability data, which is useful for tracking long-term time trends by beverage category.

Classification of Beverages

All foods and beverages listed as consumed by NHANES participants are included in the USDA Food and Nutrient Database for Dietary Studies (FNDDS) that is available online (http://www.ars.usda.gov/News/docs.htm?docid=12089). The 'What We Eat in America' surveys classify beverages as milk and milk beverages, citrus juices, apple juices (including fruit juice blends), other noncitrus juices, soda (regular and diet), fruit drinks (regular and diet), sports and energy drinks (regular and diet), vegetable juice, water (bottled and tap), flavored and enhanced water, alcoholic beverages, coffee, tea, and meal replacement beverages. This aggregation scheme for beverages is shown in Table 1.

Energy and nutrient characteristics of selected beverages are summarized in Table 2. First, the water content of beverages is high (~90%) and their energy density is low, generally < 50 kcal per 100. Whereas 100% juices and milk contain naturally occurring sugars, sugar is added to SSBs, typically 10 g per 100 g. These amounts correspond to the optimum human pleasure response to sweet solutions. The added sugar can be sucrose (cane or beet sugar) or corn-derived high-fructose corn syrup.

Nutrient density of individual beverages needs to be calculated using a nutrient profiling model. Nutrient profiling is a technique used to rank or rate beverages and foods according to their nutrient composition per reference amount: generally 100 kcal, 100 g or per serving. Profiling models can be based on human nutrient requirements (protein, vitamins, and minerals), on nutrients of public health concern (e.g., added sugars), or a combination of both. Shown in Table 2 are ratings for individual beverages based on the published and validated nutrient-rich food (NRF) index.

The NRF scoring system was designed to be consistent with the Food and Drug Administration (FDA)-regulated Nutrition Facts panel, the principal source of nutrition information for US consumers. Accordingly, reference daily values for each nutrient, based on a 2000 kcal diet, were obtained from FDA sources. The key beneficial components of the positive NRF subscore are protein (50 g), fiber (25 g), vitamin A (5000 IU), vitamin C (60 mg), vitamin E (30 IU), calcium (1000 mg), iron (18 mg), potassium (3500 mg), and magnesium (400 mg). Unlike reference daily values from the Institute of Medicine (IOM), FDA values are not adjusted by age and sex.

Development of the NRF family of indexes closely tracked US federal regulatory standards. The US FDA allows certain foods to carry nutrition and health claims, based on their content of protein, fiber, calcium, iron, and vitamins A and C. Past dietary guidelines identified potassium, magnesium, and vitamin E as shortfall nutrients in the United States diet. In contrast, foods cannot carry health claims if they contain excessive amounts of disqualifying nutrients, typically saturated fat, added sugar, or sodium. The recent definition of 'free sugars' proposed by the Food and Agriculture Organization of the United Nations includes sugars added to beverages during the manufacturing process as well as sugars naturally present in honey and 100% fruit juice. The negative component of NRF was based on saturated fat, added sugar, and sodium. Maximum

Table 1 Types of beverage listed by 'What We Eat in America' participants

Beverage type	*Three most common varieties reported*
Milk and milk beverages	Whole milk, 2% milk, 1% milk
Citrus juices	Orange juice (canned/bottled), orange juice (w/calcium), orange juice (NFS[a])
Apple juices	Apple juice, fruit juice blend, fruit juice (NFS[a])
Other noncitrus juices	Grape juice, pineapple juice, prune juice
Soda (regular and diet)	Regular cola, regular fruit-flavored soft drink (without caffeine), sugar-free cola
Fruit drinks (regular and diet)	Fruit juice drink, fruit-flavored drink (made from powder), fruit juice drink (w/ vitamin C)
Sports and energy drinks (regular and diet)	Gatorade, Powerade, Red Bull (regular)
Vegetable juices	Tomato and vegetable juice (mostly tomato), tomato juice, carrot juice
Water (bottled and tap)	Tap water, bottled water
Flavored and enhanced water	Carbonated water (unsweetened), vitamin water, sweetened carbonated water (e.g., tonic)
Alcoholic beverages	Beer, light beer, red wine
Coffee	Regular coffee (from ground), regular coffee (from instant), decaffeinated coffee (from ground)
Tea	Unsweetened tea, presweetened tea, tea NFS[a] presweetened with sugar
Meal replacement beverages	High-protein meal replacement, ready-to-drink meal supplement/replacement

[a]NFS, not further specified.

Table 2 Energy and nutrient characteristics of selected beverages

Beverage type	*Energy (kcal per 100 g)*	*Water (g per 100 g)*	*Total sugars (g per 100 g)*	*Added sugar (g per 100 g)*	*Nutrient density (NRF9.3/racc)*
Milk, fluid, whole	60	88.3	5.3		38.2
Milk, fluid, 2% fat	50	89.3	5.1		52.7
Milk, fluid, 1% fat	42	89.9	5.2		60.4
Milk, fluid, skim or nonfat, <0.5%	34	90.8	5.1		69.4
Grapefruit juice, carton, bottled	38	90.1	8.9		125.3
Orange juice, cartoon, bottled	42	89.0	8.4		135.8
Fruit juice blend, 100% juice	53	86.3	12.5		123.1
Apple juice	47	87.9	10.9		21.7
Tomato juice	17	93.9	3.6		92.7
Soft drink, cola-type	42	89.1	10.8	10.8	−51.4
Soft drink, fruit-flavored	40	89.5	10.2	10.2	−49.6
Ginger ale	34	91.2	8.7	8.7	−39.3
Fruit drink	48	88.0	11.3	10.1	−42.4
Lemonade	40	89.4	10.0	9.7	−26.6
Orange drink	46	87.4	12.2	11.4	−38.5
Citrus drink with vitamin C added	51	86.7	13.0	11.1	50.6
Orange breakfast drink	44	88.9	10.7	10.7	2.4
Fruit-flavored drink, from powder	35	90.8	8.8	8.8	7.3
Lemonade-flavored drink, from mix	36	90.4	9.4	9.4	−21.0
Fruit-flavored drink, from mix	37	90.2	9.5	9.5	7.1

Nutrient density based on NRF9.3 score per serving.

recommended values per reference amount are 20 g for saturated fat, 125 g for total sugar, 50 g for added sugar, and 2400 mg for sodium.

To calculate NRF values, nutrient composition data for individual foods and beverages were obtained from the USDA FNDDS (composition), linked to the 'What We Eat in America' surveys (consumption). The FNDDS database was supplemented with data on added sugars from other USDA sources and, at the time, did not include data on vitamin D in milk and dairy foods. For each nutrient, content per 100 g of food was converted to percentage daily values (%DV) per reference amount and capped at 100% DV. The positive and negative subscores are calculated as the sum of %DVs for nine beneficial nutrients and three nutrients, respectively. To arrive at the total NRF score for a given beverage, the negative subscore is subtracted from the positive subscore. Scores shown in Table 2 were calculated per beverage serving (~240 ml).

It can be seen that citrus juices (orange and grapefruit), other 100% juices, and tomato juice have the highest NRF scores, largely due to the absence of added sugars and high vitamin C contents. Scores awarded to milk beverages benefited from the presence of protein and calcium; NRF scores increased progressively as the amount of saturated fat declined going from whole to skimmed milk. Beverages with added sugars scored less well, unless they were fortified with high amounts of vitamin C. The NRF scores for sodas and fruit

drinks were less favorable (negative), due to the presence of added sugars and absence of key nutrients. Water (tap or bottled), diet beverages, and unsweetened coffee and tea contain few calories and are not scored for nutrient content.

Beverage Sources

NHANES provides data on diets and health for a nationally representative sample of children and adults. The present analyses of beverage patterns of consumption used NHANES data from three cycles (2005–10), representing both children and adults who completed a 24 h dietary recall. NHANES 1999–2002 collected data using the USDA Automated Multiple-Pass Method administered by trained interviewers. Respondents reported the types and amounts of foods and beverages consumed in the preceding 24 h, from midnight to midnight. The consumption of drinking water was only assessed from 2005 onwards.

Figure 1 shows the contribution of drinking water, caloric and noncaloric beverages, and moisture from foods in total daily water intake. The data are shown in terms of ml water per day (top panel) and as percentages (bottom panel). It can be seen that water and beverages contribute over 70% of dietary water, depending on age, with the remainder provided by moisture in solid foods. Water, tap and bottled, contributed about one-third of total daily water intake and its consumption increased with age. In contrast, the contribution of milk and milk beverages decreased sharply with age; most children consume milk, while many adults do not. The contribution of

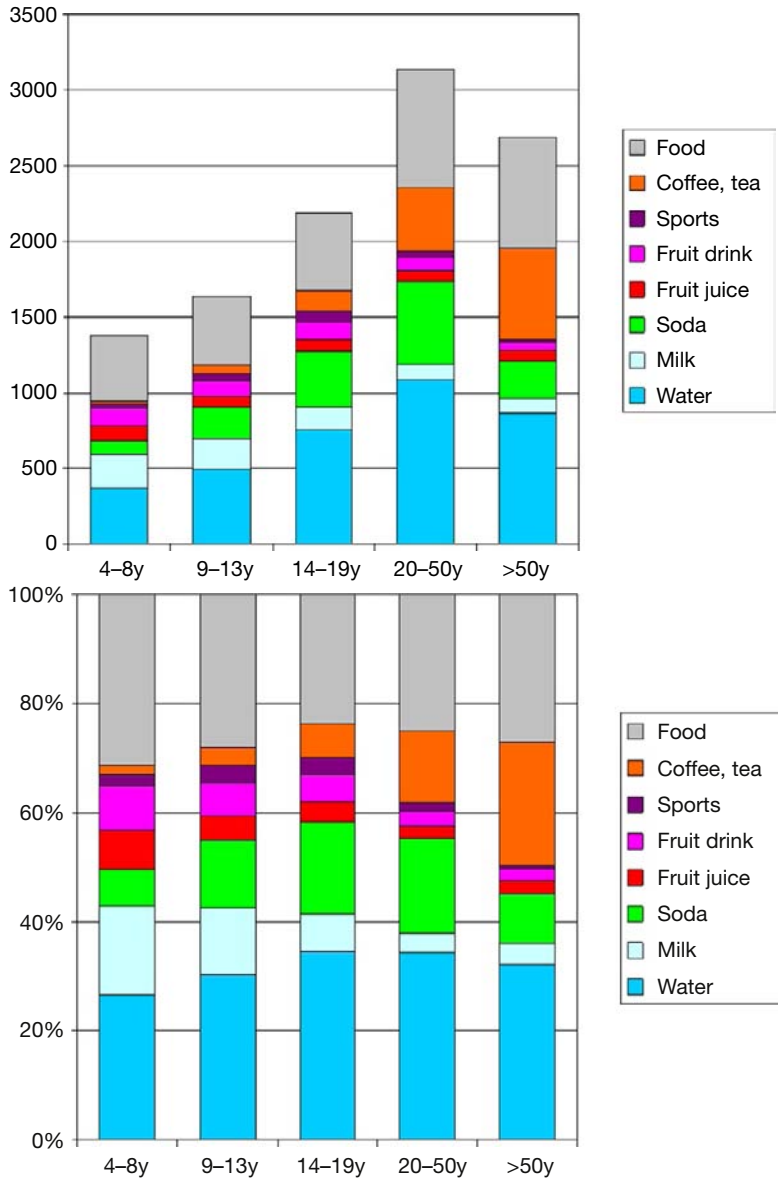

Figure 1 Contribution of water, beverages, and foods to total water intakes by age group. The data are expressed as ml (top panel) and as percentages (bottom panel).

soda to hydration peaked in adolescence and declined in adult life. The amounts of coffee and tea consumed increased with age and were highest among adults aged over 50 years.

Further analyses confirmed that milk was consumed most frequently by younger children (>80%). Soda was consumed by 40–60% of the population, depending on age, with most consumers (60%) in the 20–34-year-old group. The peak consumption of sports and energy drinks was among 14–19-year-olds (11%). Coffee consumption rose dramatically with age: only 1.8% of 4–8-year-olds consumed coffee compared with 68% of adults over 50 years. The age gradient was less dramatic for tea, consumed by 11% of 4–8-year-olds and 33% of over 50s.

Beverages and Hydration

Water requirements to meet hydration needs can be met by plain water, water from caloric and noncaloric beverages, and moisture from foods. As indicated earlier, water and beverages supply much more of the total daily water than food moisture. The contribution of water (tap and bottled) to total water intake has been estimated at 30–37%.

Dietary reference intake (DRI) values for water, established by the IOM in the United States and the European Food Safety Authority in Europe, are based in part on (observed) population intakes of drinking water (tap and bottled), water from other caloric and noncaloric beverages, and moisture from foods. US IOM adequate intakes (AI) for water are shown in Table 3. For children, the recommendations are 1700 ml day^{-1} for boys and girls in the 4–8-year-old group and 2100 ml day^{-1} for girls and 2400 ml day^{-1} for boys in the 9–13-year-old group (IOM 2004).

Table 3 Adequate intake (AI) values from dietary reference values and mean total water intakes (from all sources) by life stage group, NHANES 2005–10

Age	*AI (l day^{-1})*	*Mean (l day^{-1}) (SE)*	*% of sample below AI*
Infants			
0–6 months[a]	0.70	0.91 (0.01)	19.3 (2.7)
7–12 months[a]	0.80	1.16 (0.02)	7.4 (1.9)
Children			
1–3 years[a]	1.30	1.31 (0.01)	55.2 (1.7)
4–8 years	1.70	1.45 (0.02)	74.6 (1.6)
Males			
9–13 years	2.40	1.77 (0.04)	85.2 (1.8)
14–18 years	3.30	2.44 (0.05)	81.9 (2.0)
19–30 years	3.70	3.24 (0.07)	68.4 (2.4)
31–50 years	3.70	3.49 (0.05)	63.6 (1.7)
50–70 years	3.70	3.17 (0.04)	71.7 (1.6)
>70 years	3.70	2.36 (0.03)	91.9 (1.2)
Females			
9–13 years	2.10	1.66 (0.03)	82.7 (1.4)
14–18 years	2.30	1.95 (0.05)	73.4 (2.5)
19–30 years	2.70	2.42 (0.05)	68.2 (2.1)
31–50 years	2.70	2.79 (0.04)	53.1 (1.7)
50–70 years	2.70	2.69 (0.04)	57.5 (1.6)
>70 years	2.70	2.12 (0.03)	80.4 (1.1)

[a]This analysis excludes infants who breast fed. Includes infants consuming infant formula.

Table 3 also shows mean total consumption of water from all sources in relation to IOM guidelines. No group of US children came close to satisfying the DRIs for water. At least 75% of children aged 4–8 years, 87% of girls aged 9–13 years, and 85% of boys aged 9–13 years did not meet DRIs for total water intake. Water volume per 1000 kcal, another criterion of adequate hydration, was 0.85–0.95 l per 1000 kcal, which is again short of desirable levels (1.0–1.5 l per 1000 kcal).

On average, younger adults exceeded or came close to satisfying the DRIs for water. Older men and women failed to meet the IOM AI values, with a daily shortfall of 1218 and 603 ml, respectively. Eighty-three percent of women and 95% of men over 71 years of age failed to meet the IOM AI DRIs for water. However, average water volume per 1000 kcal was 1.2–1.4 l per 1000 kcal for most population subgroups, which is higher than suggested levels of 1.0 l per 1.000 kcal.

Hydration needs are a matter of public health concern. Drinking water is an effective way to assure adequate hydration and may help reduce caloric intake and so improve the dietary nutrient to calorie ratio. However, beverages still contribute fewer calories to the total diet than solid foods.

Beverages and Energy Intakes

Figure 2 shows the contribution of caloric beverages and solid foods, respectively, to total daily energy intakes by age group. These data are from the analyses of the 2005–10 NHANES cycles and are shown as kcal per day (left panel) and as percentages (right panel). It can be seen that total calories, including beverage calories, first increased and then declined with age. Generally, children derive a far greater proportion of their daily calories from liquids than adults.

The principal beverage consumed by young children is milk followed by 100% fruit juice. Soda consumption is low in early childhood, highest in the 14–19-year-old group, and less thereafter. By 50 years of age, only about 10% of total daily energy is provided by beverages.

Added sugars represent a significant proportion of the US diet, supplying 11–17% of total daily energy, depending on age. Soda and sports drinks are the largest food source of added sugars (34.4%) followed by grain desserts (12.7%), fruit drinks (8.0%), candy (6.7%), and dairy desserts (5.6%). Altogether, sweetened beverages contribute about 40% of added sugars to the diet, depending on age.

Sweetened beverages are the largest source of added sugars for every age group. The top sources of added sugars for children 6–11 years are soda and energy and sports drinks (6.6 teaspoons, tsp) (1 tsp ≈ 5 ml) followed by grain desserts (2.5 tsp), fruit drinks (1.5 tsp), candy (1.3 tsp), and dairy desserts (1.1 tsp). Among adolescents, aged 12–19 years, the top sources are soda (10 tsp), fruit drinks (2.5 tsp), grain-based desserts (2.3 tsp), and candy (1.7 tsp). For younger adults, the sources are soda (8.6 tsp), grain-based desserts (2.4 tsp), fruit drinks (1.6 tsp), and candy (1.3 tsp). For older adults, the main sources are soda (3.1 tsp), grain-based desserts (2.5 tsp), dairy desserts (1.2 tsp), and candy (1.0 tsp).

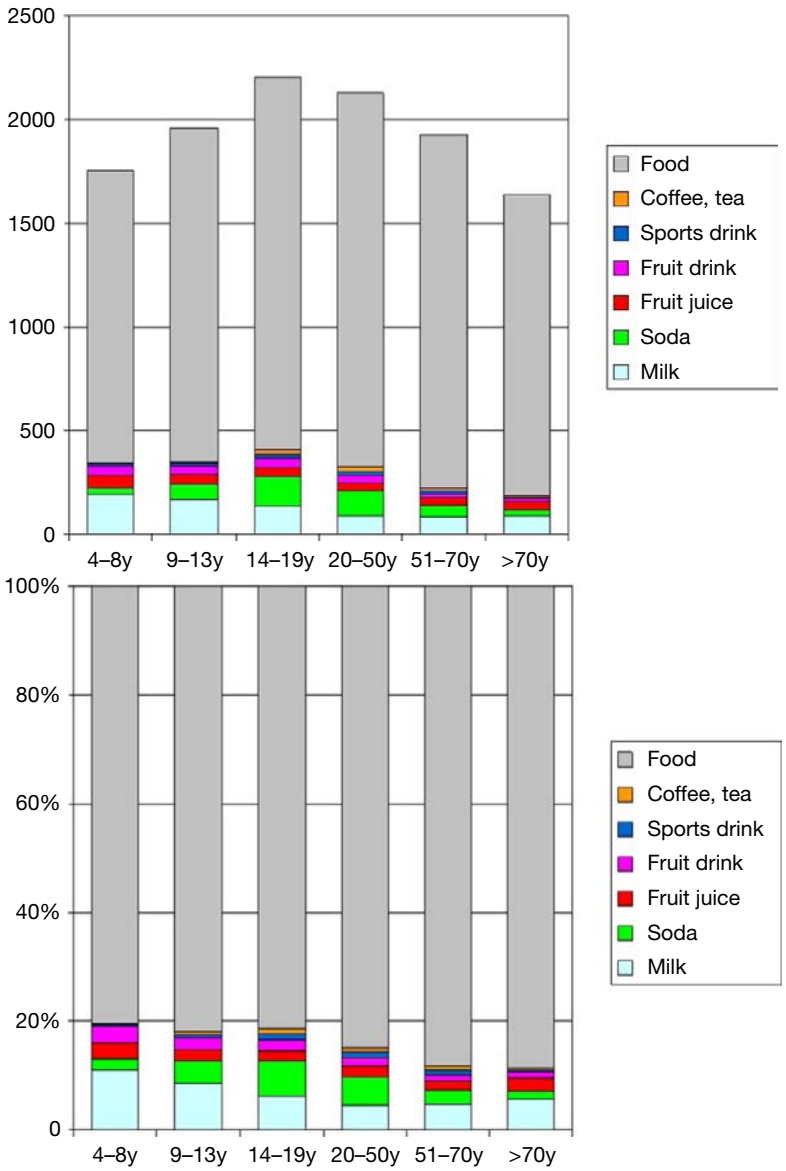

Figure 2 Contribution of water, beverages, and foods to total energy intakes by age group. The data are expressed as kcal per day (top panel) and as percentages (bottom panel).

Whereas soda and energy and sports drinks are clearly the largest single sources of added sugars for every age group, they are not the top sources of calories in the US diet. On average, added sugars from all sources account for ~14% of total dietary energy. Previous estimations by the National Cancer Institute have placed the energy contribution of soda and energy and sports drinks at about 5.5% of daily calories. The present estimate is about 7%, depending on age. As with total calories, most of the added sugars in the US diet are sourced from grocery stores, as opposed to restaurants or schools.

Beverage Consumption Patterns by SES

Few studies have examined beverage consumption patterns by beverage type and SES. In general, lower-calorie beverages are associated with higher incomes. Based on NHANES data, the consumption of diet beverages, sweetened with low-calorie sweeteners, was associated with higher education and higher incomes. Users of diet beverages were also more likely to have better diets, smoke less, and exercise more. Similarly, the consumption of low-calorie skimmed milk was associated with higher SES. Interestingly, the consumption of drinking water (bottled and tap) in the United States was also associated with higher SES. The water effect was significant for adults and marginal for children.

In contrast, the consumption of whole milk and regular SSB has been associated with lower education and lower incomes. The reasons for the observed socioeconomic gradient in beverage consumption are not always clear. Cost provides one explanation. Recent analyses of NHANES data showed a strong SES gradient for the consumption of whole fruit, which was

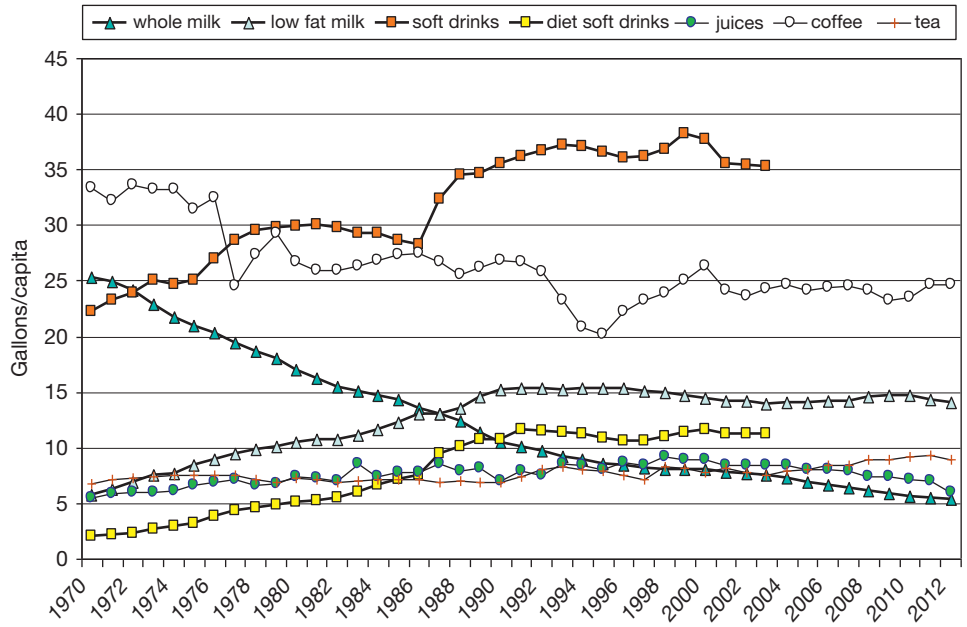

Figure 3 Beverages available for consumption in gallon equivalents per capita per year, USDA trends 1970–2012. Economic Research Service USDA.

attenuated for the lower-cost 100% fruit juice. Whereas whole fruit was more likely to be consumed by higher-income adults with graduate education, 100% fruit juices were more likely to be selected by lower-income teenagers and minority groups. Calls to replace 100% juice with whole fruit in the US diet may result in higher diet costs. On the other hand, there is little cost differential at retail between regular and diet beverages. Why the consumption of tap water should be higher among higher-income group is not entirely clear.

Beverage Consumption Trends

Food balance sheets, prepared by the USDA, represent the annual per capita amounts of food available for human consumption. The figures, expressed in fluid gallon equivalents per capita (for beverages), represent production and imports adjusted for export and stocks.

Figure 3 shows USDA trends data for the period 1970–2012. First, the consumption of whole milk dropped precipitously to be replaced to some extent by low-fat milk. The consumption of soft drinks increased in 1970–2004; subsequent data are not available. The consumption of diet soft drinks has likewise increased in 2004. The consumption of coffee has declined since the 1970s, whereas tea consumption has increase slightly. Bottled waters are missing from the USDA beverage disappearance data. Although no reliable data seem to exist, the consensus is that plain water consumption has increased.

Conclusions

Drinking water and other beverages, caloric and noncaloric, contribute over 70% of total daily water intakes. Even so, most age and gender groups in the United States are not meeting the hydration guidelines issued by the IOM. Drinking water is one way of meeting hydration needs, reducing calories, and so improving nutrient density of the diet. The contribution of beverages to total daily calories needs to be kept in perspective. Caloric beverages contribute 10–20% of daily energy intakes, on the average, depending on age. The bulk of dietary energy for most people is supplied by solid foods. Beverages contribute more to hydration than they do to energy intakes.

See also: Beverage: Health Effects; Coffee: Types and Production; Dairy Products: Dietary and Medical Importance; Fruit Juices; Milk: Role in the Diet; Milk: Sources and Composition; Nutritional Epidemiology; Sports Nutrition; Tea: Health Effects; Tea: Types, Production, and Trade.

Further Reading

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Bifidobacteria in Foods: Health Effects

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Ecology of *Bifidobacterium* spp. in the Human Gut: A First Indicator of Their Biological Functions

Bifidobacteria are indigenous inhabitants of the human intestinal tract, which is one of the most complex microbial ecosystems (microbiota) on the planet, comprising over 100 trillion bacteria in the large intestine. The structure and dynamics of this ecosystem depends on environmental factors as well as on symbiotic interactions with the host that determine their coexistence. After birth, the newborn faces a transition from a sterile-intrauterine life to a foreign environment. The microbial colonization of the newborn's intestine occurs rapidly and constitutes a major exposure to foreign antigens after birth. The newborn's gut is colonized by facultative anaerobes (e.g., *Enterobacteriaceae*, *Enterococcus*, and *Streptococcus*) over the first 24 h and, immediately after, by strict anaerobes (e.g., *Bifidobacterium*, *Bacteroides*, and *Clostridium*), which dominate the ecosystem within a week. The infant gut microbiota shows low diversity and instability within the first 2 years of life and is also highly sensitive to environmental influences, which may partly determine its structure and influence over human health. The ecology of bifidobacteria in the human gut is strongly influenced by the type of milk-feeding at early life stages, when the microbiota constitutes a main factor driving the proper development of the gut anatomy and physiology and the immune system. Epidemiological studies comparing the gut microbiota of breast-fed and formula-fed infants reveal that human milk promotes the dominance of *Bifidobacterium* spp. (representing up to 90% of the total fecal bacteria) in the infant's gut. In contrast, formula-fed infants have a lower abundance of *Bifidobacterium* spp. and an increased abundance of *Clostridium* spp., *Bacteroides* spp., and members of the family *Enterobacteriaceae*. Furthermore, studies show differences in *Bifidobacterium* species composition related to milk-feeding type. Afterward, the progressive introduction of complementary feeding contributes to the establishment of an adultlike microbiota of higher complexity. In adults, *Bifidobacterium* spp. numbers are reduced, representing between 3% and 7% of the microbiota, and tend to decline even more in the elderly.

Bifidobacteria in Human Milk

The effect of breast-feeding on the infant's gut microbiota is mainly attributed to the presence of nondigestible oligosaccharides, which are the third most abundant solid component of human milk, after lactose and lipids, reaching concentrations ranging from 5 to 23 $g\,l^{-1}$ depending on the lactation stage. These are highly diverse glycans that are minimally hydrolyzed by human enzymes and constitute the main energy source for the infant's gut microbiota in the large intestine, exerting a 'bifidogenic' or prebiotic effect. In fact, some species/subspecies of the genus *Bifidobacterium* (*B. longum* subsp. *infantis* and *B. bifidum*) are known to possess genetic and protein machinery (oligosaccharide binding proteins, fucosidases, lacto-N biosidase, etc.) specialized in the utilization of different human milk oligosaccharides. This enables their preferential growth on these substrates and confers a competitive advantage whereby they can outnumber other intestinal bacteria. In addition, other human milk constituents could influence the effect of breast-feeding on bifidobacteria colonization such as relatively lower protein and phosphorus concentrations and lactose content. In recent years, breast milk has also been considered as a potential source of bacteria colonizing the infant's gut. Although the dominant bacterial genera (*Streptococcus* spp. and *Staphylococcus* spp.) in breast milk are not those dominant in the infant's gut, specific strains of *Bifidobacterium* spp. have been identified in the gut microbiota of mother–infant pairs, suggesting their transmission from the mother's breast milk to the infant.

Many epidemiological studies have established associations between breast-feeding and diverse benefits for infant's health, including reduced incidence of infections and to some extent of allergies and obesity, as well as improved bowel function (soften stools and increased stool frequency). This has led to the hypothesis that these beneficial effects may partially result from the role of human milk in promoting the dominance of bifidobacteria. Although direct evidence of the microbiota-mediated effects of breast-feeding is lacking, there are plausible modes of action supporting this hypothesis. The high content of nondigestible oligosaccharides present in human milk promotes colonic fermentation mainly by bifidobacteria, which leads to the generation of organic and short-chain fatty acids and lower stool pH when compared with formula feeding. This presumably contributes to creating a more adverse environment for the growth of pathogenic bacteria, which may also reduce the risk of infections. The reduction in pH and increased short-chain fatty acid concentration might also modulate peristalsis, favoring normal bowel function. Furthermore, increased fermentation is also parallel to increased bacterial mass and osmotic water-binding capacity, contributing to increased stool weight and stool frequency and softer stools in breast-fed babies compared with formula-fed babies. The role of breast milk in immune defenses and allergies could be due to its content in immunologically active compounds (lactoferrin, lysozyme, antimicrobial proteins and peptides, secretory IgA, chemoattractants, cytokines, etc.), which help compensate for the developmental delay of the neonate immune system and gradually stimulate innate immunity and immunologic memory toward of pathogens, while excessive inflammation is avoided, thus preventing immune-mediated disorders. In addition, the primary colonization of the newborn intestine by the microbiota is known to constitute a critical stimulus that accelerates immune system maturation, which could be influenced by the dominance of bifidobacteria

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00065-9

in breast-fed babies. The 'hygiene hypothesis' proposed by Strachan in 1989, and the more recent 'microbiota hypothesis,' suggests that early exposure to pathogens and other microbial antigens helps protect against immune-mediated diseases (Figure 1). This theory is supported by epidemiological studies reporting associations between living in a more 'hygienic' environment and decreased frequency of infections and increased incidence of allergic (e.g., asthma, rhinitis, and atopic dermatitis) and autoimmune disorders. Although this hypothesis does not fully account for the risk of these diseases, it is backed by a plausible mechanistic explanation. In the uterine life, there is a dominance of lymphocyte T-helper 2 (Th2) effector responses to prevent fetal rejection, but persistence of this immune polarization favors the development of a long-lasting atopic phenotype and increases the risk of infections. Nevertheless, appropriate exposure to microbial antigens via infant gut colonization contributes to satisfying the new demands of the neonatal immune system, inducing a change in lymphocyte balance, favoring Th1 and/or Th17 cell responses and T regulatory mechanisms. Accordingly, germ-free mice are deficient in the development of gut-associated lymphoid tissue and antibody production (e.g., IgA) and have fewer and smaller Peyer's patches and mesenteric lymphoid nodes (MLN), isolated lymphoid follicles, CD4+ Th17 cells, and CD4+CCD25+Foxp3+ regulatory cells. Germ-free mice also exhibit epithelial cell immunologic dysfunctions, including reductions in cytokine production, in expression of molecules responsible for interactions with lymphocytes (e.g., MHC) and in antimicrobial peptides (e.g., defensins and REG3γ), in the number of CD8+ T cells with cytotoxic capacity, and in expression (or localization) of innate immune receptors such as Toll-like receptors (TLR). By contrast, these effects can be totally or partially reversed by intentional colonization of the germ-free intestine with commensal microbiota or specific bacteria or bacterial products (e.g., TLR agonists). TLRs are expressed by epithelial cells and antigen-presenting cells (dendritic cells (DCs) and macrophages) and are responsible for the initial recognition of specific microbial components (e.g., RNA, DNA

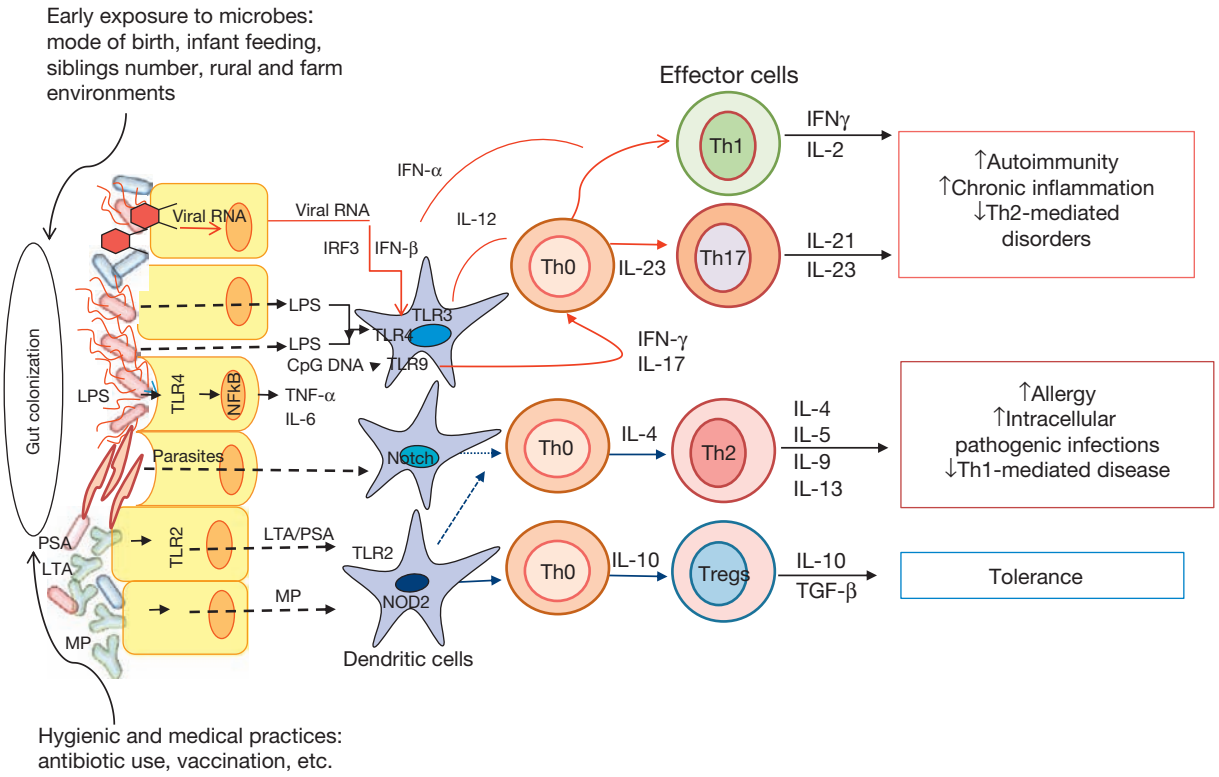

Figure 1 'Hygienic and microbiota hypothesis' according to which early exposure to microbes, partly via gut colonization of the newborn intestine, influences innate immunity and T-cell polarization and determines the risk of developing specific diseases or tolerance. TLR3- and TLR4-activated epithelial and DCs by viral RNA and LPS from Gram-negative bacteria, respectively, may promote the differentiation of T *naive* cells (Th0) into Th1 cells, which increases the risk of chronic inflammatory and autoimmune disorders and reduces the risk of infections by intracellular pathogens and inhibits Th2 responses. TLR9-activated epithelial and DCs by CpG DNA from bacteria enhance Th1- and Th17-type cytokine production (IFN-γ and IL-17, respectively) and increase the risk of autoimmunity and reduce the risk of allergy and infection by intestinal parasites. TLR2-activated DCs by lipoteichoic acids (LTA) from Gram-positive bacteria and some parasites and NOD2-activated DCs by muramyl dipeptide (MP) from Gram-positive bacteria may induce the differentiation of Th0 cells into Th2 cells, which increases the risk of allergy and reduces the risk of chronic inflammatory and autoimmune disorders by inhibiting Th1/Th17 immune responses and contributing to release of anti-inflammatory cytokines (IL-10). TLR-2 activated DCs by cell-wall polysaccharide (PSA) of *Bacteroides fragilis* and Gram-positive bacteria (e.g., bifidobacteria) may be involved in the generation of regulatory T cells (Tregs) by producing high levels of anti-inflammatory cytokine IL-10. Tregs contribute to establishing systemic tolerance by suppressing inflammatory properties of DC and their ability to induce effector Th1, Th2, and Th17 cells; by promoting a tolerogenic DC phenotype; by acting directly on eosinophils, mast cells, basophils, and resident cells and reducing cell migration to tissues; and by acting directly on B cells, reducing allergen-specific IgE.

motifs, LPS, and other cell-wall components), the discrimination between harmful and harmless antigens, and the development of appropriate innate and acquired immune responses. Ligand binding to TLR promotes interactions with different adaptor proteins, thereby activating three major signaling pathways: nuclear factor (NF)-κB, the mitogen-activated protein kinases (MAPKs), and interferon regulatory factors (IRFs). This also leads to the expression of inflammatory genes, encoding cytokines, cytokine receptors, immunoregulatory proteins, adhesion molecules, stress-associated proteins, and other mediators as well as the recruitment of other immune cells (T cells, DCs, etc.) that together activate an inflammatory response leading to pathogen clearance while avoiding excessive inflammation. Signaling through TLRs also stimulates the maturation of DCs, promoting antigen presentation and allowing them to migrate to draining MLN, where they present antigens to *naive* T and B cells. T-cell differentiation into Th1, Th2, Th17, or regulatory T cells depends on the TLRs involved and the cytokine interacting with *naive* T cells, inducing a characteristic array of cytokine production during differentiation. The balance of these T-cell populations and cytokines in early life is thought to influence the risk of developing certain disorders (Figure 1).

A descriptive study of infants has reported correlations between host gene expression and the gut microbiota structure in breast-fed and formula-fed infants, providing the first evidence for microbiota-mediated effects in humans. The study reports significant enrichment of microbiota virulence-related genes linked to enrichment of immunity and defense gene expression in the host transcriptome of breast-fed babies, which could indicate microbiota-mediated stimulation of host-defense mechanisms and explain reduced incidence of infections.

An observational study conducted in infants and young children also reported a reduced ratio of *Bifidobacterium* to *Clostridium* counts associated with the development of atopic dermatitis instead of atopic disease, suggesting that bifidobacteria play a beneficial role in this disorder. Comparisons of intestinal microbiota composition between children with at least two diabetes-associated autoantibodies and child autoantibody-negative also reported that the low abundance of lactate-producing and butyrate-producing species was associated with β-cell autoimmunity. The same association was established for a lack of two dominant *Bifidobacterium* spp. (*B. adolescentis* and *B. pseudocatenulatum*) and an increased abundance of the genus *Bacteroides*. A number of studies in children with celiac disease (untreated and treated with a gluten-free diet) also reported reduced abundance of *Bifidobacterium* spp. and *B. longum* compared with healthy controls. Similarly, healthy infants at high genetic risk of developing celiac disease showed lower numbers of *Bifidobacterium* spp. and *B. longum* compared with low genetic risk infants. In addition, several studies have reported potential associations between the genus *Bifidobacterium* and obesity, although with less conclusive results. In one study, reductions in *Bifidobacterium* numbers have been shown to precede the development of overweight in children. Two other studies showed positive associations of *Bifidobacterium* spp. with normal weight in adults and with normal weight or normal weight gain in pregnant woman compared with subjects with overweight and excessive weight gain during pregnancy, while two other studies indicate the opposite associations.

In the light of all these epidemiological and ecological studies, associations between alterations in the abundance of *Bifidobacterium* spp. and certain human conditions have been drawn, suggesting a role for this bacterial group in reducing the risk or progression of these disorders. This evidence does not necessarily reflect causality of the underlying disease but has constituted the basis for conducting mechanistic studies mainly in animals and intervention trials in humans to provide direct evidence of the health effects of specific species and strains (generally known as 'probiotics'), as described in the following sections.

Modes of Action of *Bifidobacterium* spp. Shown by *In Vitro* and Animal Studies

More than a hundred years ago, Henry Tissier (Pasteur Institute) isolated microbes, including bifidobacteria, from healthy breast-fed infant feces for the first time, but it is in recent decades that the effectiveness and modes of action of specific bifidobacterial strains have been shown by studies *in vitro* and in animal models (Figure 2). Furthermore, whole-genome sequencing data and functional genomics via the use of next-generation sequencing techniques have made it possible to identify the molecular basis of some of the functional traits and health effects attributed to members of this genus, thus helping to provide plausible mechanisms of action.

Bifidobacteria have been acknowledged for their nutritional functions, contributing to the metabolism of nondigestible oligosaccharides, not only from human milk but also from the diet. Over 9% of annotated genes of bifidobacterial genomes encode enzymes involved in carbohydrate metabolism. This feature could partially contribute to energy recovery and to the generation of fermentation products (e.g., acetate) partly by cross-feeding mechanisms with other intestinal bacteria (e.g., butyric acid) with potential health effects. As described previously, colonic fermentation could improve bowel function and create a harsh environment for pathogen survival. Furthermore, butyrate, which can be generated from acetate directly produced by bifidobacteria, is the main energy source for colonocytes and exerts trophic effects stimulating cell growth and differentiation and production of the glucagon-like peptide-2 (GLP-2), which together strengthen the gut barrier function. Butyrate also stimulates GLP-1 production, which induces satiety and improves insulin sensitivity, and exerts an anti-inflammatory role in the gut and peripheral tissues.

Bifidobacteria can also contribute to supplying essential nutrients such as vitamins. Bifidobacteria are known to be involved in folate biosynthesis, although its production depends on the species/subspecies considered. Whereas *B. bifidum* and *B. longum* subsp. *infantis* are considered high producers of folate, *B. breve*, *B. longum* subsp. *longum*, and *B. adolescentis* are considered low producers. The ability of bifidobacteria to produce folate in animals and humans has also been demonstrated by measuring the fecal levels of folate *in vivo*, although the extent to which it is utilized by the host remains unclear. Bifidobacteria could also contribute to

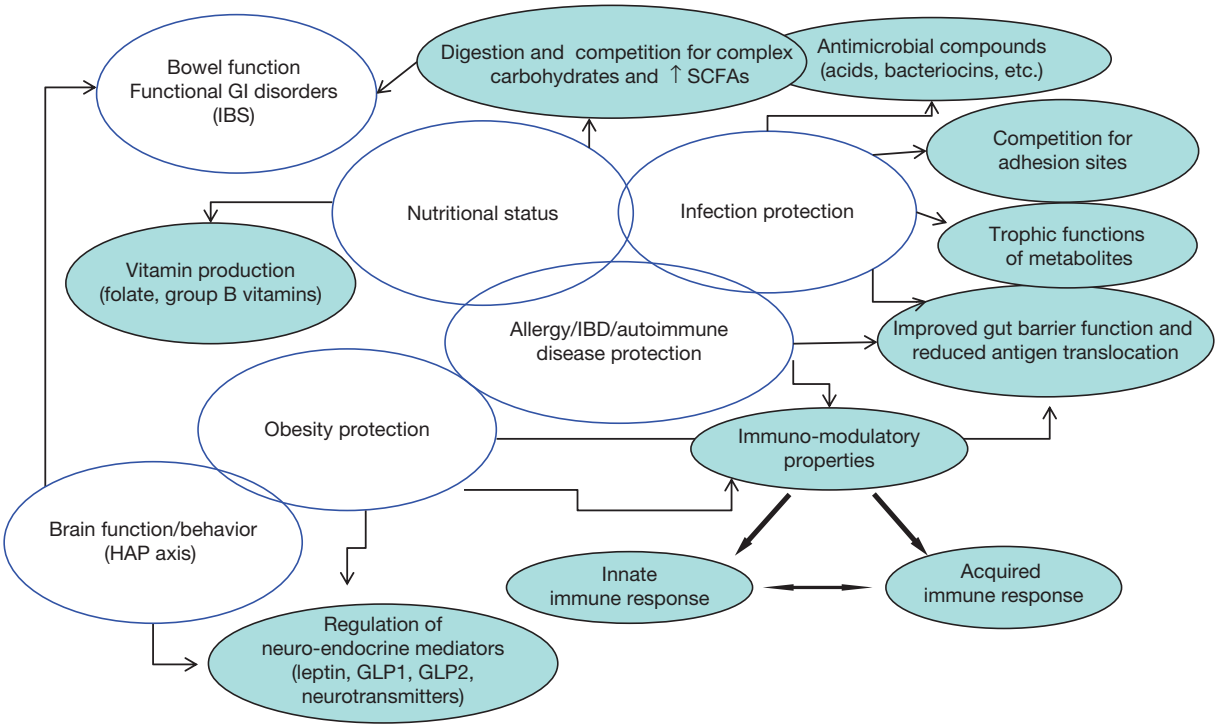

Figure 2 Nutritional and health effects of *Bifidobacterium* strains demonstrated by *in vitro* and animal studies.

synthesizing other B vitamins since transcriptomic analysis revealed that bifidobacterial genes predicted to be involved in biosynthesizing several B vitamins and folate are strongly expressed in fecal samples of adult subjects. Also, the soy fermentation process by *B. longum* R0175 has been reported to lead to thiamine and pyridoxine generation. Although most of the biosynthetic pathways of vitamins are incomplete in the bifidobacteria genomes characterized to date, it is likely that other bacteria complement these pathways since the whole human fecal metagenome is enriched in genes that specify biosynthetic enzymes for biotin, riboflavin, pantothenate, ascorbate, thiamine, and folate production.

Multiple mechanisms have been proposed to play a role in bifidobacterial protection against bacterial pathogens and virus. Such mechanisms include modification of environmental conditions and production of antimicrobial compounds (acids and antimicrobial peptides like bacteriocins), competition for nutrients and adhesion sites, and modulation of the host immune defense mechanisms. Preclinical trials in models of infection have shown that some bifidobacterial strains enhance resistance to microbial pathogens (bacteria and virus) through activating nonspecific cell phagocytosis, increasing cytokine levels, increasing natural killer-cell activity, and/or increasing levels of immunoglobulins. In addition, some bifidobacteria (e.g., *B. infantis* 35624) exert protective effects in models of infection through downregulating the NF-κB pathway, triggered by TLR4 signalling, and inducing T regulatory cells (Tregs) and anti-inflammatory cytokines (IL-10), thereby preventing excessive inflammation.

Bifidobacteria have also been evaluated *in vitro* and in animal models with a view to being used to treat and prevent chronic inflammatory and autoimmune disorders. In animal models, some *Bifidobacterium* strains have been demonstrated to be effective against the deregulated immune response characteristic of allergy, which involves Th2 cell activation with IL-4, IL-5, IL-9, and IL-13 cytokine production; high levels of allergen-specific IgE production and eosinophilia; and recruitment of inflammatory cells to inflamed tissues. Bifidobacteria are thought to help ameliorate or prevent allergy by diminishing Th2 lymphocyte polarization and/or enhancing Th1 lymphocyte polarization and inducing Treg cells and anti-inflammatory cytokines. This has been demonstrated in models of food allergy induced with ovalbumin (e.g., *B. longum* AH1206) and models of atopic dermatitis induced by dermal treatment with house dust mite extract and dinitrochlorobenzene (e.g., IRT5 probiotic product, comprising a combination of bifidobacteria and other lactic acid bacteria).

The role of bifidobacteria in chronic inflammatory bowel disease (IBDs), apparently caused by loss of tolerance to the host's own microbiota, has also been evaluated in animal models of colitis. In this mouse model, the protective action of the product VSL#3, comprising a blend of bifidobacteria and other lactic acid bacterial strains (*Lactobacillus casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *B. longum*, *B. breve*, *B. infantis*, and *Streptococcus salivarius* subsp. *thermophilus*), was related to IL-10 induction and TGF-β-expressing T cells.

Animal studies have also evaluated the potential role of bifidobacteria against autoimmune diseases, resulting from the loss of self-tolerance and abnormal reactivity against the host's own antigens. Diabetes type 1 is a T cell-mediated autoimmune disease characterized by a response in which pancreatic insulin-producing B cells are destroyed. Studies using animal models of nonobese diabetic mice have shown that the probiotic mixture VSL#3, containing bifidobacteria and

other lactic acid bacteria, prevented diabetes by diminishing the destruction of insulin-producing beta cells, presumably mediated by increased infiltration of positive IL-10 mononuclear cells in the pancreas and upregulation of IL-10 mRNA expression, as well as mononuclear cells producing IL-10 in Peyer's patches and spleen. Celiac disease, caused by a dysfunctional immune response to cereal gluten proteins of autoimmune nature, has also been considered a target for bifidobacteria. An animal model of gliadin-induced enteropathy has been used to reveal that *B. longum* ES1 administration reduces inflammatory cytokine production (TNF-α) in the small intestine, increases anti-inflammatory cytokine (IL-10) production, and reduces the CD4+ T-cell population in the peripheral blood.

The effects of bifidobacteria and some prebiotics inducing dominance of bifidobacteria in murine models of obesity have also been evaluated in recent years. These studies generally reveal that a prebiotic-induced rise in total bifidobacteria and administration of specific strains (e.g., *B. pseudocatenulatum* CECT 7765) exert beneficial effects by reducing metabolic dysfunction (e.g., increased serum lipids and glucose levels, reduced tolerance to glucose, and reduced hepatic steatosis) and chronic low-grade inflammation associated with obesity. These effects are presumably mediated by the ability of some bifidobacteria to restore alterations in the gut barrier function, reducing the translocation of inflammatory molecules from the gut to the periphery (e.g., LPS from Gram-negative bacteria), modulating the neuroendocrine system function (e.g., stimulating GLP-1 and GLP-2 production and reducing leptin production), and reducing inflammatory cytokine production and the infiltration of macrophages and effector T cells in the gut and peripheral tissues, which leads to metabolic impairment.

Gut microbiota is also thought to influence brain development and function, as well as behavior, according to recent evidence from murine models. In this context, bifidobacteria have been studied for their potential role in regulating the stress response, which involves the hypothalamic–pituitary–adrenal axis (HPA) and is regulated by both psychological and physical stressors (e.g., infections). The exaggerated response of germ-free mice to mild restraint stress, reflected in an increased release of corticosterone and ACTH, has been shown to be reversed by monocolonization with a strain of *B. infantis*. In a maternal separation model of psychological stress, a *B. infantis* strain also reduced corticosterone levels. The modes of action by which bifidobacteria may regulate the HPA axis and stress responsiveness include reduction of proinflammatory cytokine production and/or modulation of neurotransmitters (e.g., serotonin and catecholamines) and neurotrophic factors (e.g., brain-derived neurotrophic factor (BDNF)). In this regard, *B. infantis* 35624 was shown to elevate plasma tryptophan levels, a precursor of serotonin, by modulating the expression of enzymes (e.g., indoleamine-2,3-dioxygenase) involved in the tryptophan degradation pathway in Sprague–Dawley rats. A strain of *B. longum* has also been shown to reverse infection-induced behavioral changes associated with decreased hippocampal BDNF mRNA expression, without affecting cytokine or tryptophan metabolism and independently of vagus nerve activation. Furthermore, administering a blend of *L. helveticus* R0052 and *B. longum* R0175 reduced anxiety in rats. Overall, the ability of bifidobacteria to regulate the stress response demonstrated in animal studies to date may be a mechanism ameliorating stress-related gastrointestinal disorders, such as irritable bowel syndrome (IBS), as well as metabolic disease related to alterations in the HPA axis. However, such possible applications are still speculative.

Health Effects of *Bifidobacterium* spp. Evidenced by Human Intervention Studies

The Probiotic Concept and the Regulation of Health Claims

In 2001, probiotics were defined as "live microorganisms that when administered in adequate amounts confer a health benefit to the host," which implies that microorganisms must be alive and administered in effective doses. Strains of the genus *Bifidobacterium* are one of the main probiotics sold for human consumption because of their safety record in foods and evidence for potential relationships with health from epidemiological, *in vitro*, animal, and some human studies. The probiotic definition supposed an advance in the harmonization of criteria a microorganism must fulfill to be qualified as a probiotic, and this concept has been generally accepted by the scientific community. This term has also been used generically on labels and publicity to communicate a health effect for consumers. However, communicating the health effects of foods and food supplements, such as bifidobacteria, is now regulated in most countries. Regulation stipulates how the benefits of specific bacterial strains should be substantiated and transmitted to consumers. According to the health claim legislation in the European Union (Regulation EC No. 1924/2006), health claims for foods should be substantiated by providing evidence for their efficacy in high-quality human intervention studies and, particularly, in randomized placebo-controlled trials (RCTs) conducted in the target population (healthy general population or subgroups) for which the claim is made. Within this regulatory framework, two types of health claims can be made: (i) claims related the role of a food on maintenance/improvement of normal physiological functions and (ii) claims related to the role of a food in reducing a risk factor for disease. Moreover, the microorganism for which claims are made must be identified at genus, species, and strain levels. In this scenario, provided in the succeeding text is a summary of the existing evidence on beneficial health effects of specific bifidobacterial strains based on RCT in humans.

Evidence of Health Effects of *Bifidobacterium* spp. on Infants and Young Children

Bifidobacterium strains, added to infant or follow-up formulas or as food supplements, have been evaluated in RCT conducted in infants and young children. Such studies have contemplated diverse physiological and health-related effects including reductions in incidence or severity of gastrointestinal infections, antibiotic-associated diarrhea (AAD), respiratory tract infections, colic and irritability, and allergic manifestations (atopic eczema, allergen sensitization, and wheeze/asthma); contribution to normal bowel function (stool

frequency and consistency); and effects on growth related parameters, mainly as part of safety evaluations.

The role of bifidobacteria in reducing the risk of gastrointestinal infections is one of the main outcomes evaluated in infants and children. The results reported in RCTs conducted with *B. lactis* Bb12 alone (also named *B. animalis* subsp. *lactis* CNCMI-3446 and *B. bifidum* in literature) are contradictory. However, several studies evaluating infant formulas supplemented with *B. lactis* Bb12 combined with *S. thermophilus* or with both *S. thermophilus* and *Lactobacillus helveticus* point for a role of the experimental formulas in the reduction of the risk of nonspecific infections compared with control formulas, using diarrhea for diagnosis and as main outcome.

A recent meta-analysis evaluated the efficacy of probiotic in preventing AAD in a pediatric population, showing some probiotics to be effective, particularly at high doses (*L. rhamnosus* LGG or *Saccharomyces boulardii*) but not of the bifidobacteria evaluated (strains of *B. lactis*) to date.

Evidence for the role of bifidobacteria in reducing the risk of respiratory tract infections is much more limited than evidence for gastrointestinal infections. One RCT reported no significant difference in the rate of bronchopneumonia between infants fed with chemically acidified formula with added *B. lactis* Bb12 and infants fed with standard formula. A second RCT also reported no significant difference in the number of days with respiratory illness or in the number of respiratory illness episodes between the *B. lactis* Bb12-supplemented formula group and the control formula group.

The effects of infant formulas supplemented with either *B. lactis* Bb12 or *B. lactis* Bb12 and *S. thermophilus* on colic, crying, and irritability have not been conclusive either, since one study did not report effects on crying, while another reported lower frequency of colic or irritability but failed to define colic and irritability.

Bifidobacterium strains have also been evaluated for their possible role in reducing the risk of atopic disorders (atopic dermatitis, atopic sensitization) and asthma/wheeze in children, based on their immunoregulatory effects evidenced *in vitro* and in animal models. Of the high-quality RCT included in the most recent systematic reviews and meta-analysis reporting these outcomes, only one evaluated a *Bifidobacterium* strain alone, while most of trials evaluated combinations of strains (bifidobacteria plus lactobacilli) or *Lactobacillus* alone. A single study evaluated whether *B. animalis* subsp. *lactis* HN019 administered as a food supplement to mothers and infants prenatally and postnatally could prevent the development of atopic dermatitis (eczema), atopic sensitization (skin prick tests), and parent-reported symptoms of asthma and rhinoconjunctivitis in early life in a high-risk birth cohort of infants. However, infants receiving *B. animalis* subsp. *lactis* did not show reduced prevalence of any of the health disorders after 2 or 4 years of follow-up. Studies using bacterial strains other than bifidobacteria or combined with bifidobacteria (e.g., *B. lactis* B12,*B. lactis* AD011, *B. longum* BL999, *B. bifidum* BGN4, *B. bifidum* W23, *B. lactis* W52, or *B. breve* Bb99) conclude that prenatal probiotic administration to mothers and/or early-life probiotic administration to infants may reduce the risk of atopic sensitization, total IgE levels, incidence of atopic dermatitis, and IgE-associated atopic dermatitis for some probiotic combinations and intervention protocols, but not the risk of asthma/wheeze. The reported effects seemed to be dependent on the duration of the follow-up period, the study population group (general or at risk population), and the probiotic strains evaluated. Results clearly indicated that the effects of pooled data are only indicative and cannot be attributed to specific bifidobacteria but only to the combinations of bacteria tested and to the specific dose and administration pattern applied.

Some studies evaluating the effects of infant formulas supplemented with *B. lactis* Bb12 or *B. longum* BL999 combined with *L. rhamnosus* LPR on bowel function have not reported effects, in either stool frequency or consistency.

Studies evaluating the possible role of *B. lactis* Bb12 alone or combined with other lactic acid bacteria in the growth of healthy infants in early infancy (less than 4–6 months) or beyond have generally reported adequate growth comparable to control groups. Exceptionally, one study showed a significantly greater growth rate toward the end of the intervention period for infants fed probiotics than the control group, but with questionable physiological significance, considering the magnitude of the difference (equal to 0.5 SD). Similar studies on healthy infants of HIV-positive mothers only reported either faster head growth or increased *z*-scores, but parallel differences were not detected in weight for age, length for age, or head circumference between groups, reflecting inconsistency or lack of real significance in the differences detected. One RCT conducted with *B. longum* BL999 alone, as well as combined with *L. rhamnosus* LPR, reported no effects on weight gain compared with the control groups. Studies conducted with other bifidobacterial strains and combinations as food supplements have not reported adverse effects on growth, mainly evaluated as a safety indicator. Although there is very limited scientific evidence on the effects of probiotics on this outcome, it is generally considered that probiotics do not influence growth in healthy infants either positively or adversely. Major flaws in studies conducted to date are the small sample sizes, the short study duration, and the inclusion of wide age ranges with large differences in developmental processes and growth rates.

Therefore, there is modest evidence that a bifidobacterial strain (*B. animalis* Bb12) in formula acidified with lactic acid bacteria could help to reduce the risk of nonspecific acute gastrointestinal infections. Other strain combinations including bifidobacteria together with lactic acid bacteria may also reduce the risk of some atopic disorders. However, studies with strain combinations do not enable us to draw definitive conclusions for a specific role of bifidobacteria.

Evidence for Health Effects of *Bifidobacterium* spp. on Adults

The potential benefits of bifidobacteria on the adult population evaluated to date concern mainly bowel function improvement and reduction in the risk of AAD. The potential therapeutic effects of bifidobacteria on diverse conditions such as IBD, including Crohn's disease and ulcerative colitis, and IBS have also been evaluated in adults but with a view to their use in clinical practice. Despite the relatively large number of RCT conducted with probiotics in adults, most studies evaluating bifidobacteria used combinations with strains belonging to other bacterial genera and, therefore, cannot be used to draw conclusions on the effectiveness of bifidobacteria as such.

A recent systematic review and meta-analysis of RCT related to effects on bowel function of different probiotic strains supplied as foods or food supplements in adults reported that *B. lactis* DN-173 010 and *B. lactis* HN019 reduce intestinal transit in IBS subjects with constipation and in subjects with functional gastrointestinal disorders, respectively. The analysis of pooled data from studies conducted with different strains also suggests that the effect of probiotics on intestinal transit is greater on subjects with constipation compared with controls and in older compared with younger subjects. Nevertheless, these general conclusions are of limited value since effects could be strain-specific and comparisons between different population groups should be performed with the same strain or combination. In addition, the studies pooled had important limitations, including the fact that they were small and of short duration, some populations groups were underrepresented (young middle-aged adults), and some studies administered the probiotics in food matrixes with other ingredients (e.g., prebiotics) influencing intestinal transit.

Although recent meta-analysis combining studies conducted with different strains and strain combinations suggests that probiotics may reduce the risk of AAD in adults, most studies have been conducted with strains belonging to other than the *Bifidobacterium* genus or with bifidobacteria (e.g., *B. lactis* Bb12, *B. breve* Bb99, *B. longum* PL03, and others not well identified) combined with strains belonging to other genera, and therefore, conclusions cannot be drawn on the effectiveness of bifidobacteria alone. A study on the effect of yogurt containing *Bifidobacterium* DN-173-010 in preventing the side effects of *Helicobacter pylori* eradication therapy did not report reduction of diarrhea.

Well-designed RCTs supporting bifidobacteria administration in IBD management are very limited. Two RCTs performed with a bifidobacteria-fermented milk containing *B. breve* strain Yakult, *B. bifidum*, and *L. acidophilus* and pooled data from three RCTs conducted with the product VSL#3 indicated that these bacterial combinations contribute to inducing remission in patients with active UC.

Two studies have demonstrated the efficacy of *B. infantis* 35624 for ameliorating pain and providing global relief, as well as for reducing bloating and flatulence/distension in IBS patients irrespective of bowel habit subtype. In a large-scale study of IBS patients with constipation, *B. animalis* DN-173 010 was reported to improve bloating, particularly when live bacteria were administered. However, this outcome was not beneficially influenced in another trial on women with minor gastrointestinal symptoms but without any specific disorder. A single RCT also reported that a blend of *L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* 99, and *Propionibacterium freudenreichii* ssp. *shermanii* JS reduced the total symptom score, which included abdominal pain, distension, flatulence, and borborygmi compared with a placebo, but there was no difference in the individual symptoms except for borborygmi. There are also uncertainties regarding the effects of the probiotic combination VSL#3 on IBS because results are inconsistent across studies.

Therefore, there is evidence that *B. lactis* DN-173-010 and *B. lactis* HN019 may modulate intestinal transit particularly in subjects with functional gastrointestinal disorders and *B. infantis* 35624 may ameliorate IBS symptoms. Strains combinations including bifidobacteria can reduce the risk of AAD and induce remission of UC from a clinical perspective, but the effects of bifidobacteria *per se* cannot be inferred.

In summary, there is a lack of well-designed RCT examining effects of bifidobacteria alone to draw conclusions on their health benefits and to be able to support cause–effect relationships in adults or infants and young children. There is a need to establish effective doses and conditions of use for specific strains or strain combinations and administration patterns. This should make it possible to provide consumers with reliable claims on foods and food supplements that could contribute to healthier lifestyles and reduction of disease risk.

See also: Allergies: Public health; Bacteriocins; Chilled Foods: Principles; Colon: Diseases and Disorders; Dairy Products: Dietary and Medical Importance; Energy Metabolism; Fermented Foods: Fermented Milks; Folic acid and Folates: Physiology and Health Effects; Food Allergies: Occurrence and Analysis; Obesity: Causes and Prevalence; Obesity Management; Probiotics; Yogurt: Yogurt Based Products.

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Bioactive Peptides in Foods

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Introduction

Bioactive peptides are food-derived peptides that exert a positive effect beyond that of basic nutrition following consumption in humans. Bioactive peptides are small and usually contain between 3 and 20 amino acid residues, and their bioactivities are based on their amino acid composition and location within the sequence of amino acids that make up the peptide. They are inactive in the sequences of their parent proteins but may be released by enzymatic hydrolysis by proteolytic enzymes during gastrointestinal (GI) digestion, during fermentations with generally recognized as safe bacteria such as lactobacilli, or during food processing. In order to exert a positive health effect, bioactive peptides must cross the GI barrier and survive enzyme degradation. Bioactive peptides are often multifunctional and can exert several beneficial physiological effects at different target sites once liberated in the human body. They play different roles in preventing diseases and modulating the physiological systems, being involved in the GI system such as the antiobesity and satiety peptides; the cardiovascular system such as antihypertensive, antithrombotic, antioxidant, and hypocholesterolemic peptides; the immune system such as antimicrobial, cytomodulatory, and immunomodulatory peptides; and the nervous system, such as opioid peptides.

Numerous bioactive peptides have been reported in recent years as naturally present or generated from food proteins of different origins like milk, eggs, soya, fish, and meat. In this sense, the most extensively studied bioactivity during the last decade has been the antihypertensive activity through the measurement of angiotensin I-converting enzyme (ACE) inhibitory activity. The reason for this interest is mainly because high blood pressure is one of the major, independent risk factors for cardiovascular diseases and the main reason of death in developed countries. Peptides with this type of activity are generally termed as bioactive peptides even though other activities such as antioxidant, antimicrobial, opioid, antithrombotic, and antidiabetic also fit into the general bioactive peptide term.

Bioactive Peptides from Food Proteins

Bioactive peptides may already be present in the food as part of its composition like carnosine and anserine, but they can also be generated by spontaneous endogenous hydrolysis of food proteins caused by endogenous enzymes, by controlled hydrolysis with commercial proteolytic enzymes, or by release from parent proteins in foods once ingested and during its GI digestion. All these pathways of generation are briefly summarized.

Bioactive Peptides Naturally Generated from Food Proteins

Proteins in foods contain sequences that can exert a physiological effect when consumed. These sequences are inactive when present in the parent proteins but can exert its bioactive effect if the respective peptide is released. The generation of peptides in food systems can occur:

(i) During aging or storage by the action of endogenous muscle peptidases such as cathepsins, peptidyl peptidases, aminopeptidases, and carboxypeptidases. As an example, meat and fish proteins are frequently hydrolyzed during aging and/or refrigerated storage.

(ii) During food processing such as fermentation or curing processes. In this sense, fermented meat and fish proteins have been described to be hydrolyzed by both endogenous muscle and microbial peptidases, which contribute to the bioactive peptide release during fermentation and ripening processes. A similar situation is reported in dairy products like cheese and yogurt where bioactive peptides are generated by proteolytic activities of starter microorganisms. Some lactic acid bacteria have been reported to be very active from this point of view.

(iii) The generation during long periods of ripening. In this case, endogenous muscle peptidases may act for several months releasing a large number of bioactive peptides in the final product (e.g., dry-cured ham). In ripening processes, the chance to obtain a wide variety of small peptides such as di- and tripeptides is very high due to the prolonged and unspecific action of the muscular enzymes, resulting in a very rich source of bioactive peptides.

Bioactive Peptides Generated Through Hydrolysis of Food Proteins with Commercial Proteolytic Enzymes

The use of commercial proteolytic enzymes for the production of bioactive peptides is another interesting area extensively developed in recent years. Peptidases from animal, plant, and microbial sources, or combinations of these, have been utilized for various processes in the food industry including the digestion of food proteins for the generation of bioactive peptides. Proteolytic enzymes, like papain, thermolysin, Alcalase®, Neutrase®, Flavourzyme®, and Actinase E®, have been used for the production of enzymatic hydrolysates of meat or fish proteins.

The main objective of these studies is focused on getting the maximum use of industry by-products, giving them an added value, and reducing the environmental impact. Thus, in most cases, proteins from food by-products constitute the substrate for obtaining enriched bioactive peptides fractions. Examples of this have been described in studies regarding the use of trimming, bones (or backbones), blood, and skin obtained from meat and fish industries.

A typical industrial production of bioactive peptides from food by-products by using proteolytic enzymes under controlled conditions is described in Figure 1.

Bioactive Peptides Generated Through GI Digestion of Ingested Food Proteins

The ingestion of food proteins can result in the liberation of bioactive peptides that were encrypted in the protein structure.

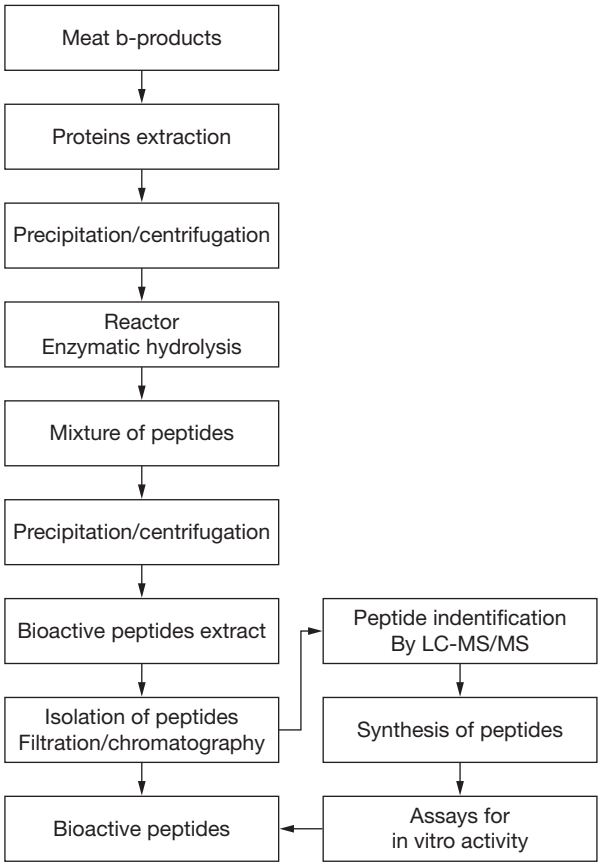

Figure 1 Flow diagram for the generation of bioactive peptides through the enzymatic hydrolysis of edible meat by-products. Reproduced with permission from Mora, L., Reig, M. and Toldrá, F. (2014). Bioactive peptides generated from meat industry by-products. *Food Research International* **65**, 344–349.

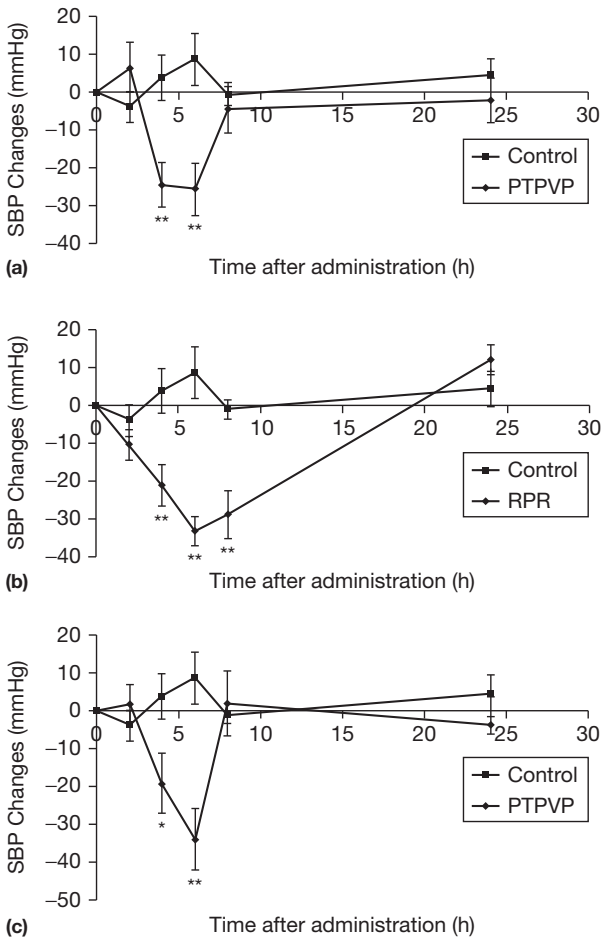

Figure 2 Antihypertensive effect of single oral administration of ACE inhibitory peptides PTPVP, RPR, and KAPVA. Each point indicates the mean of systolic blood pressure of eight SHRs, and the vertical bars represent the standard error. Treatment in each case was control (distilled water) and (a) peptide PTPVP, (b) peptide RPR, and (c) peptide KAPVA, dose 1 mg peptide/kg BW. Significant difference from control at each time: $*P < 0.05$ and $**P < 0.01$. Reproduced with permission from Escudero, E., Toldrá, F., Sentandreu, M. A., Nishimura, H. and Arihara, K. (2012). Antihypertensive activity of peptides identified in the *in vitro* gastrointestinal digest of pork meat. *Meat Science* **91**, 382–384.

Thus, bioactive peptides can be generated when food is consumed during GI digestion by enzymes involved in this digestion such as pepsin, trypsin, and chymotrypsin. The routes of generation have been confirmed by performing *in vitro* studies, using proteins like myosin, actin, tropomyosin, and troponin, as well as by simulated digestion by using trypsin and/or chymotrypsin reproducing the real digestion. These studies are usually followed with *in vivo* studies to verify the bioactivity of the generated peptides. In fact, some of the peptides generated from nebulin (RPR sequence) and titin (PTPVP and KAPVA sequences) proteins after simulated human GI digestion of pork meat were synthesized and tested for their antihypertensive activity in spontaneously hypertensive rats (SHRs) showing an important decrease of the systolic blood pressure in comparison to the control, as shown in Figure 2.

Functional Activity of Bioactive Peptides

As have been previously described, bioactive peptides present in foods can exert a physiological effect when they are consumed. Once peptides have been orally ingested, they are susceptible to be attacked by enzymes involved in the GI digestion. Some small peptides can also be digested by brush border membrane peptidases and intracellular peptidases or even peptidases from the microbial flora present in the intestine. The released peptides must be able to cross the intestinal membrane and be transported intact into the bloodstream where they will reach their target sites and then exert their respective bioactivity. In fact, it is crucial that no degradation of the peptides takes place during their transport in the intestine in order to be allowed to cross the gut barrier with no modifications on their amino acid sequence.

Different types of activity have been reported in the literature, and the most relevant are summarized in the succeeding text.

ACE Inhibitory Activity Peptides

The most extensively studied bioactive peptides generated from food proteins are ACE inhibitory peptides that have the ability

to prevent hypertension and have been used for pharmaceutical and physiological purposes. These peptides reduce blood pressure through inhibition of ACE in the body and usually exert its antihypertensive effects a few hours after oral administration. ACE is the most distributed dipeptidyl carboxypeptidase in mammalian bodies, usually membrane-bound in vascular endothelial cells. ACE plays a critical role in the regulation of blood pressure in the renin–angiotensin system and converts the inactive decapeptide angiotensin I into the potent vasoconstricting octapeptide angiotensin II, resulting in an increase in blood pressure (Figure 3). ACE also inactivates the antihypertensive vasodilator bradykinin. Therefore, by inhibiting the catalytic action of ACE, the elevation of blood pressure can be reduced or even suppressed in the body. Thus, ACE inhibitory peptides interact with noncatalytic binding sites in the ACE enzyme resulting in its inhibition and in lowering the blood pressure. The concentration of an ACE inhibitory peptide needed to inhibit 50% of ACE activity is defined as the IC_{50} value. However, such IC_{50} values do not always correlate with their real antihypertensive physiological effect in the body. In fact, it has been reported in the literature that some peptides with a powerful ACE inhibitory activity *in vitro* are inactive by oral administration. The reason could be that some substrate-type peptides might be hydrolyzed by ACEI enzyme resulting in smaller peptides showing weaker activity.

Originally, ACE inhibitory peptides were obtained from gelatin hydrolysates. Currently, there are hundreds of ACE inhibitory peptides identified in many protein hydrolysates from many types of foods like milk, fish, meat, eggs, soybean, corn, wheat, and seaweed. Recently, ACE inhibitory peptides are also being assayed *in vivo* to test their antihypertensive properties. These studies are developed by using SHRs that receive oral administration of such peptides, and its antihypertensive effects are followed after several hours of ingestion. As an example, peptide AAATP was identified in ACEI inhibitory extract of dry-cured ham, and its activity was tested *in vitro* by using the synthesized peptide obtaining an IC_{50} of 100 μM. The antihypertensive effect of AAATP peptide was clearly established when it was administered to spontaneously hypertensive rats, showing a decrease of systolic blood pressure by 25.6 ± 4.5 mmHg after 8 h of oral administration, as shown in Figure 4.

Opioid Peptides

Endogenous opioid peptides are thought to be involved in the response to stress in animals. Opioid receptors exist in the nervous, endocrine, and immune systems of mammals, and there are three types (μ, κ, and δ). They are known to regulate various endocrine systems, including the hypothalamic–pituitary–adrenocortical axis, and underlie the phenomenon of stress-induced analgesia. Although the first endogenous opioid peptides were identified in the late 1970s, opioid receptor ligands generated from foods not made in mammals were used thousands of years before the detection of endogenous opioids. In this sense, the drug opium was used as analgesic or antidiarrheal agent, whereas the major representative, morphine, was isolated by Serturner around 1800. Opioid peptides derived from foods have some advantages in comparison with endogenous opioid peptides as they are considered more stable and show fewer side effects, with addiction and high dependency caused by synthetic and endogenous opioid peptides being the main concern when they are prescribed.

In foods, opioid peptides are described as peptides that have an affinity for an opioid receptor and are able to exert opiate-like effects by affecting the nerve system and GI functions. Opioid peptides have been reported in hydrolysates from milk casein, wheat gluten, and blood hemoglobin. A tyrosine residue is usually found at the N-terminal end, and an aromatic residue is located in the third or fourth position. The first opioid peptides derived from food proteins were described in milk; β-lactorphin with sequence YGLF showed a decrease in the systolic blood pressure of 23 ± 4 mmHg after oral administration to SHRs. β-Casomorphins are casein-derived peptides very well characterized in literature that are

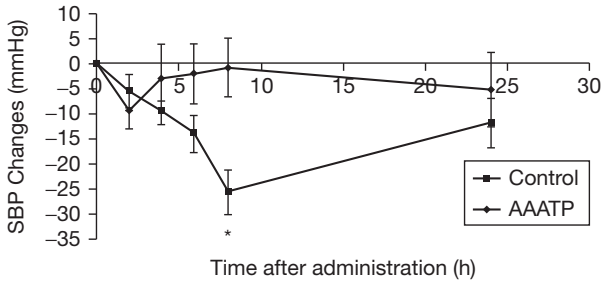

Figure 4 Antihypertensive effect of single oral administration of peptide AAATP. Each point indicates the mean of systolic blood pressure of six SHRs, and the vertical bars represent the standard error. Treatment was control (distilled water) and peptide AAATP. Significant difference from control at each time: $^*P < 0.05$. Reproduced with permission from Escudero, E., Mora, L., Fraser, P. D., Aristoy, M. C., Arihara, K. and Toldrá, F. (2013). Purification and Identification of antihypertensive peptides in Spanish dry-cured ham. *Journal of Proteomics* **78**, 499–507.

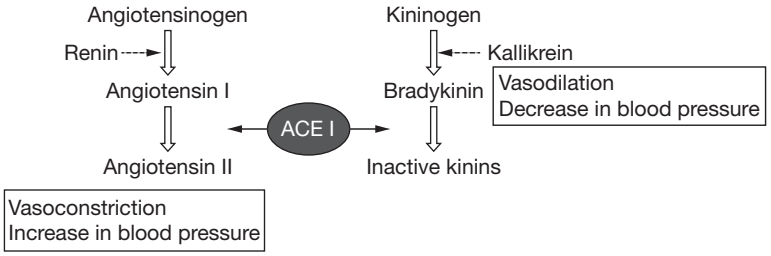

Figure 3 Brief description of the renin–angiotensin system, the main blood pressure, and water balance system in the human body.

involved in the regulation of gut functions, acting as antidiarrheal agents.

Antioxidant Peptides

The intake of antioxidants may decrease the risk of cardiovascular disease and certain types of cancer. Meat and fish contain some endogenous antioxidant peptides in muscle such as glutathione, carnosine, and anserine, which have some relevant physiological roles, especially related to oxidative stress. However, many different commercial enzymes and combinations of enzymes can be used to generate hydrolysates from different meat and fish muscles resulting in the generation of antioxidant peptides. These antioxidant peptides can act by preventing the formation of free radicals or introducing substances that compete for the existing radicals. Its antioxidant properties are highly dependent on the amino acid composition of the sequence of the peptide, so histidine, tyrosine, methionine, lysine, and tryptophan are well-known amino acids present in antioxidant peptides. Several antioxidant peptides have been identified from the hydrolysis of soybean, milk, eggs, fish, and meat proteins.

On the other hand, during the processing of some foods such as dry-cured ham, proteins are hydrolyzed by endogenous enzymes generating a high number of peptides showing bioactive properties. As an example, several peptides identified

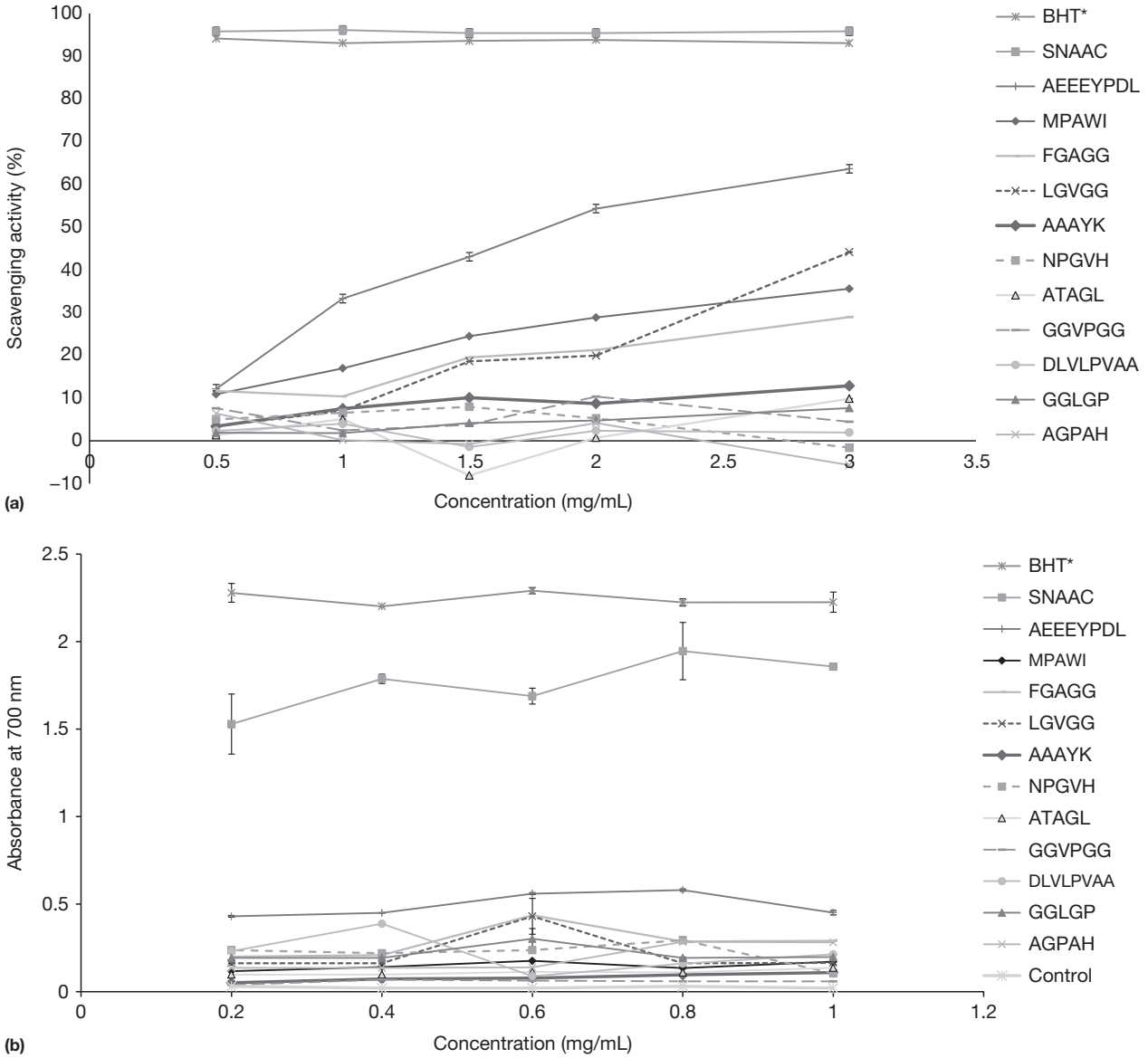

Figure 5 (a) DPPH radical-scavenging activity at different concentrations of the synthesized peptides. (b) Reducing power of different concentrations of synthesized peptides. Values represent means of three independent replicates ($n = 3$). *The synthetic compound 2,6-di-tert-butyl-4-methylphenol (BHT) was used as positive control. Reproduced with permission from Mora, L., Escudero, E., Fraser, P. D., Aristoy, M. C. and Toldrá, F. (2014). Proteomic identification of antioxidant peptides from 400 to 2500 Da generated in Spanish dry-cured ham contained in a size-exclusion chromatography fraction. *Food Research International* **56**, 68–76.

from a size-exclusion chromatography fraction in Spanish dry-cured ham that showed antioxidant activity in the analysis of DPPH radical-scavenging and ferric-reducing power have been synthesized and tested *in vitro* at different concentrations as shown in Figure 5.

The most active peptide was SNAAC, showing an IC_{50} of 75.2 μM in DPPH radical-scavenging and 205 μM in ferric-reducing antioxidant power analysis as shown in Figure 6.

Antidiabetic Peptides

Obesity is a major recognized risk factor for type-2 diabetes and constitutes a relevant public health problem. Main strategies that are being recommended to reduce type-2 diabetes incidence are exercise and a healthy diet.

Dipeptidyl peptidase IV is a serine protease able to cleave preferentially X-proline or X-alanine dipeptides from the N-terminus of different substrates. This enzyme is able to act against glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic peptide. A therapeutic strategy against type-2 diabetes could consist of the reduced degradation of GLP-1 by inhibition of dipeptidyl peptidase IV. Thus, some drugs developed for the control of diabetes are based on the inhibition of the enzyme dipeptidyl dipeptidase IV (DPPIV), but they may have secondary effects in human body. This is the reason why natural bioactive peptides with such activity would be very interesting.

In this respect, peptides like KA and AAATP have been reported as strong inhibitors of this enzyme with IC_{50} values of 6.27 and 6.47 mM, respectively. Also, encrypted peptides in canary seed have showed inhibitory activity against DPPIV and ACE, targets for diabetes and hypertension treatments. These seeds are traditionally used in diabetes and hypertension treatment.

Immunomodulating Peptides

Peptides exerting its action on the immune system are very interesting and attractive in clinical medicine, as they affect both the immune system and cell proliferation responses. In fact, immunomodulatory peptides can regulate lymphocyte proliferation in humans, modulate certain cytokines

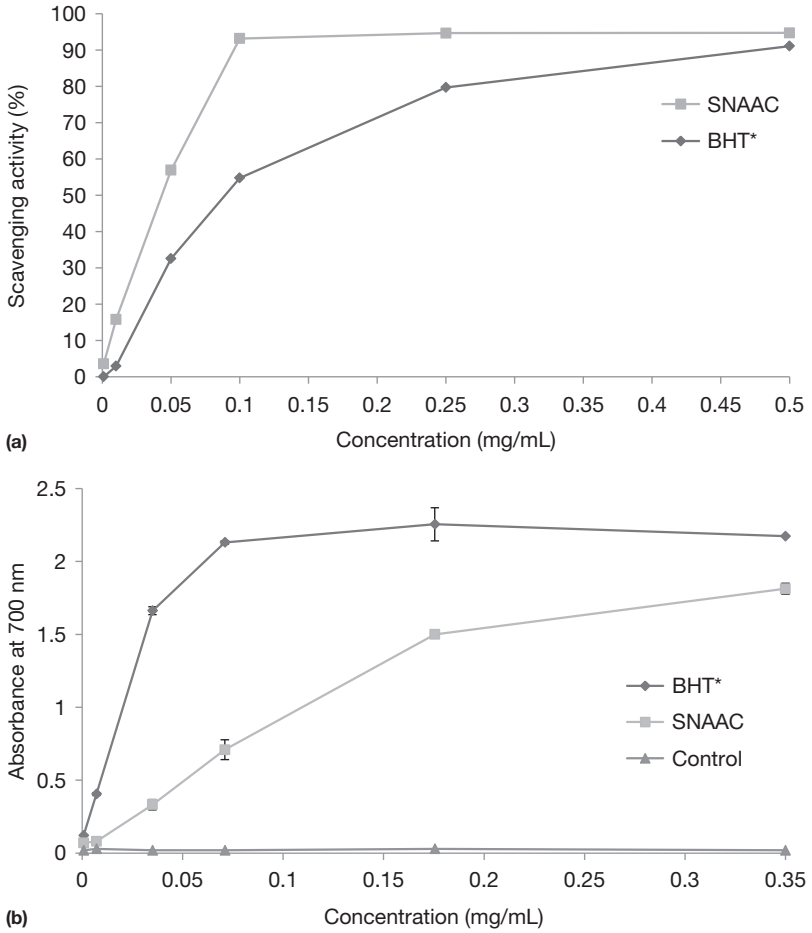

Figure 6 (a) DPPH radical-scavenging activity at different concentrations of the synthesized peptide SNAAC. (b) Reducing power of different concentrations of synthesized peptide SNAAC. Values represent means of three independent replicates ($n = 3$). *The synthetic compound 2,6-di-tert-butyl-4-methylphenol (BHT) was used as positive control. Reproduced with permission from Mora, L., Escudero, E., Fraser, P. D., Aristoy, M. C. and Toldrá, F. (2014). Proteomic identification of antioxidant peptides from 400 to 2500 Da generated in Spanish dry-cured ham contained in a size-exclusion chromatography fraction. *Food Research International* **56**, 68–76.

production, and stimulate macrophage activity. Main immunomodulatory peptides described in literature are released from milk proteins, probably due to the high interest in the regulation of the immune system development in newborn infants.

For instance, some milk casein hydrolysates stimulate the immune system, while other peptides generated by pancreatin or trypsin may inhibit the proliferative responses of murine splenic lymphocytes and Peyer's patch cells. Also, whey proteins have been described to contain many immunomodulatory peptides forming part of whey proteins that can be released by GI digestion after consumption or produced *in vitro* after enzymatic hydrolysis. However, in order to get therapeutic amounts of immunomodulatory peptides, it is necessary to obtain them from controlled hydrolysis as bioactive peptides derived from GI digestion are probably scarce for any significant effects on human body due to their low concentration when arriving to the bloodstream. In fact, three immunomodulating peptides were obtained from Alaska pollack through trypsin hydrolysis. The amino acid sequences were Asn-Gly-Met-Thr-Tyr, Asn-Gly-Leu-Ala-Pro, and Trp-Thr, with lymphocyte proliferation rates of 35.9%, 32.9%, and 31.3% in the presence of 20 μg ml^{-1} purified peptides, respectively.

Antimicrobial Peptides

Antimicrobial peptides have been described to be present in humans, plants, and animals, but as most bioactive peptides, they can also be generated through the hydrolysis of food proteins. These peptides constitute a potential source allowing to increase the natural defense of the organism against invading pathogens.

Antimicrobial peptides have been mainly isolated from milk and egg. In fact, lactoferrin and lysozyme bactericidal domains have been widely studied as sources of antimicrobial peptides. The best investigated antimicrobial peptide is the fragment 17–41 of lactoferrin, known as lactoferricin. Ovotransferrin, α-lactalbumin, and β-lactoglobulin are further examples of food proteins that have been described as sources of antimicrobial peptides.

Antimicrobial peptides are effective against different bacteria such as *Staphylococcus aureus* and *Escherichia coli*, among others, and against yeast. The mode of action depends on the type of peptide, but its effects are by forming pores on bacterial membranes, causing thinner membranes interacting with other components in the cell or acting in a detergent-like manner.

Mineral-Binding Peptides

Mineral-binding peptides help solubilizing minerals such as calcium and are considered beneficial in the prevention of dental caries, osteoporosis, hypertension, and anemia. Thus, these peptides are responsible for the remineralization and the increase in absorption of calcium and other minerals in the intestine. Several milk protein-derived peptides generated by enzymatic hydrolysis have shown good ability to trap mineral and trace elements constituting a strategy for the improved absorption of minerals. In this sense, casein-derived phosphopeptides show mineral-binding properties as their phosphorylated serine residues can form salts with some minerals such as calcium. On the other hand, some peptides derived from hoki and Alaska pollack frame proteins have been known due to their calcium-binding properties, showing similar effects to those observed in casein-derived peptides.

See also: Ham: Dry-cured Ham; Histidine-containing Dipeptides: Properties and Occurrence in Foods; Pork Meat Quality, Production and Processing on; Proteins: Chemistry, Characterization, and Quality.

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http://crdd.osdd.net/raghava/ahtpdb/cond.php – Database on antihypertensive peptides.

http://www.uwm.edu.pl/biochemia/index.php/pl/biopep – Biopep database. This gives a databse on bioactive peptides.

Bioavailability of Nutrients

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Background

Correct assessment of the adequacy of nutrient intakes from the diet requires not only knowledge about nutrient content of ingested foods but also the extent to which the nutrient is available for absorption and utilization in the human body.

Bioavailability is the technical term used to convey the fact that not all of the nutrients ingested will be absorbed, irrespective of whether consumed in the form of food or supplements. Bioavailability aims to describe the effects of a sequence of metabolic events, including digestion, solubilization, absorption, uptake and release, enzymatic transformation, secretion, and excretion, on nutrient utilization. Thus, the supply of nutrients – and increasingly nonnutrient bioactive compounds – to the human body depends not only on the amount in a food but also on its bioavailability. Understanding bioavailability helps to optimize diets and set appropriate recommendations.

The bioavailability of macronutrients, that is, carbohydrates, proteins, and fats, is usually high with more than 90% of the amount ingested being absorbed and utilized in the body. On the other hand, for micronutrients, such as vitamins and minerals, and bioactive phytochemicals such as flavonoids and carotenoids, absorption and utilization can vary widely after ingestion.

Defining Bioavailability

Until a nutrient passes from the digestive system into the bloodstream, it has little or no value. Bioavailability can be explained as the amount of a nutrient absorbed from the gut that becomes available for normal physiological functions or storage.

The Variability of Nutrient Bioavailability

The bioavailability of nutrients is highly variable and can be influenced by numerous factors, including physiochemical properties, such as chemical binding form; the matrix in which the nutrient is incorporated; the presence or absence of other food components that enhance or inhibit absorption; metabolization after absorption; host-related factors (including state of health, genetic factors, age, and lifestyle); and other individual factors.

Enhancers and Inhibitors

Nutrients can interact with one another and with other dietary components at the site of absorption, affecting a change in bioavailability or – if enhancers and inhibitors cancel each other out – a nil effect. Enhancers can act in different ways, such as keeping a nutrient soluble or protecting it from interaction with inhibitors. For example, because carotenoids are fat-soluble, adding small quantities of fat or oil to a meal (3–5 g) improves their bioavailability. Similarly, meat, fish, and poultry, while containing highly bioavailable iron, are also known to enhance the absorption of iron from other foods ingested at the same time. Although this *meat factor* has yet to be identified, it has been suggested that muscle protein may exert an influence.

Inhibitors, on the other hand, may reduce bioavailability by binding the nutrient in a form that is not recognized by uptake systems on the surface of intestinal cells, rendering the nutrient insoluble and, thus, unavailable for absorption, or by competing for the same uptake system. For example, phytic acid is abundant in certain plant foods (e.g., pulses, wholegrain cereals, seeds, and nuts) and strongly binds minerals, such as calcium, iron, and zinc in soluble or insoluble complexes, which are unavailable for absorption. Ways to reduce phytic acid content of foods include fermentation (e.g., extensive leavening of whole-wheat bread dough) or the soaking and germination of pulses.

The inhibitory effect of food constituents can also be used advantageously, as in the case of phytosterols. These compounds can be extracted from certain plant foods and added in higher doses (about 2 g per portion) to a variety of other foods (e.g., spreads and fermented milk drinks) to reduce the absorption of cholesterol, be it from dietary sources or produced in the human body.

Bioavailability of Specific Nutrients

Protein–energy malnutrition, vitamin A, and iron deficiencies are some of the most common forms of malnutrition experienced globally and often coexist. For effective intervention and dietary guidelines, the bioavailability of these nutrients, as supplied from different food sources, is particularly important. The bioavailability of these nutrients is distinct and influenced by different factors, providing good examples of the complexity of nutrient bioavailability.

Protein and Amino Acids

Although protein is a macronutrient that is easily absorbed by the human body, its bioavailability is directly linked to digestibility. To be most bioavailable, a meal needs to supply all the required essential amino acids in the correct proportions.

Amino acids are the central units in protein metabolism. They are incorporated into various proteins and converted to metabolically essential compounds, such as nucleic acids, creatine, and porphyrins. Of the 20 amino acids in human proteins, 12 can be made by the body and are known as nonessential amino acids. The remaining eight (8) (isoleucine,

leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) must be obtained from the diet and, thus, are termed essential or indispensable amino acids. Sufficient intakes of essential amino acids and adequate amounts of nitrogen for the body to produce the nonessential amino acids are important for protein metabolism.

Protein quality

The nutritional quality of food proteins varies, depending on the essential amino acid composition. Foods that contain essential amino acids at levels that facilitate tissue growth and repair are known as complete protein foods, supplying high-quality proteins. Amino acids containing sulfur (including methionine and cysteine) most commonly limit the nutritional value (quality) of proteins in the human diet. Concentrations of these sulfur-containing amino acids are, generally, considered lower in legumes and fruits than in food of animal origin. The roles of these amino acids in the human body are crucial as methionine is the amino acid initiating the synthesis of almost all eukaryotic proteins and cysteine (due to its ability to form sulfur bonds) has an important role in protein structure. Other essential amino acids, lysine and tryptophan, are also consistently found at lower concentrations in plant-based rather than animal-based foods; for example, tryptophan and lysine are limiting in corn; lysine in wheat, sorghum, and other cereals; and methionine in soybeans and other legumes. For further reading on the global protein quality debate, refer to the 2011 report of the FAO Expert Consultation on dietary protein quality evaluation in human nutrition (FAO, 2011).

In addition to protein quality, digestibility (absorption), chemical integrity, and inhibitors are three key properties of foods that can influence the bioavailability of amino acids.

Amino acid digestibility

Amino acid digestibility explains the proportion of amino acids consumed that is absorbed. It is not a fixed value, but reflects the interaction between food and the host and, therefore, may be subject to individual variation. Although the digestibility (absorption) of macronutrients, including protein, is relatively high, protein utilization is influenced by total dietary energy intake and the quality of protein in terms of meeting metabolic demand.

Chemical integrity

Chemical integrity describes the proportion of amino acids absorbed in a form that can be utilized. Some amino acids present in foods may be structurally unavailable (i.e., absorbed in a form that cannot be utilized). This is most likely to be encountered in foods that are heat-treated, oxidized, or subjected to other processes that limit amino acid bioavailability. Heat treatment leads to the formation of Maillard compounds and reduced lysine availability. Oxidization of sulfur-containing amino acids reduced the bioavailability of tryptophan and threonine. High pH induces racemization of L-amino acid residues to D-isomers and formation of cross-linked amino acids, such as lysinoalanine, which also reduces bioavailability.

Inhibitors

Many foods contain bioactive (protein or nonprotein) substances that may inhibit amino acid bioavailability by affecting either digestibility or utilization postabsorption. These inhibitors may be naturally occurring (e.g., tannins, phytates, trypsin inhibitors, glucosinolates, and isothiocyanates), formed during processing (e.g., D-amino acids and lysinoalanine), or formed during genetic modification of crops (e.g., lectins in lentils) (lectins suppress growth at low levels and are toxic at high levels).

Vitamin A

Vitamin A is a generic term used for a group of structurally related chemical compounds known as retinoids, which may be naturally occurring or synthetic compounds with or without vitamin A biological activity. Figure 1 shows the chemical structures of some retinoids.

Vitamin A is often used as a general term for all compounds that exhibit the biological activity of retinol. Vitamin A activity in the diet derives from two sources: preformed vitamin A, such as retinyl esters or retinoids, and provitamin A carotenoids, such as β-carotene, α-carotene, and β-cryptoxanthin. Although an essential nutrient, vitamin A is needed only in small amounts and is necessary for normal functioning of the visual system, growth and development, and maintenance of epithelial cellular integrity, immune function, and reproduction.

Retinol (preformed vitamin A)

Vitamin A *in vivo* is, generally, found in the free alcohol form (retinol) or esterified with a fatty acid (retinyl ester). Available in a pure form by chemical synthesis or as vitamin A palmitate or acetate, it is a pale yellow solid, which dissolves freely in oils and fats, but is insoluble in water. When vitamin A intake is adequate, more than 90% of the total body vitamin A is located in the liver, which releases the nutrient into the circulation as needed. The major dietary forms of vitamin A are long-chain fatty acid esters of retinol, commonly found in foods of animal origin, such as glandular meats, liver and fish liver oils (especially), egg yolk, and whole milk and dairy products.

Preformed vitamin A is absorbed in the small intestine. Generally, the bioavailability of retinol is high, ranging from 70% to 90%. Factors such as dietary fat and intestinal infections can affect the absorption of vitamin A. Products of fat digestion (e.g., fatty acids, monoglycerides, cholesterol, and phospholipids) and secretions in bile (e.g., bile salts and hydrolytic enzymes) are essential for the efficient solubilization of retinol. The absorption of retinol appears to be reduced in individuals with diarrhea and intestinal infections or infestations.

Carotenoids

Carotenoids are lipid-soluble plant pigments found in photosynthetic plants and animal tissues. About 600 carotenoids have been isolated and characterized in nature, and about 10% of these can be metabolized to vitamin A in a variety of animal species, including humans. Both provitamin A carotenoids, such as α- and β-carotenes and cryptoxanthins, and non-provitamin A carotenoids, such as lutein, zeaxanthin, and

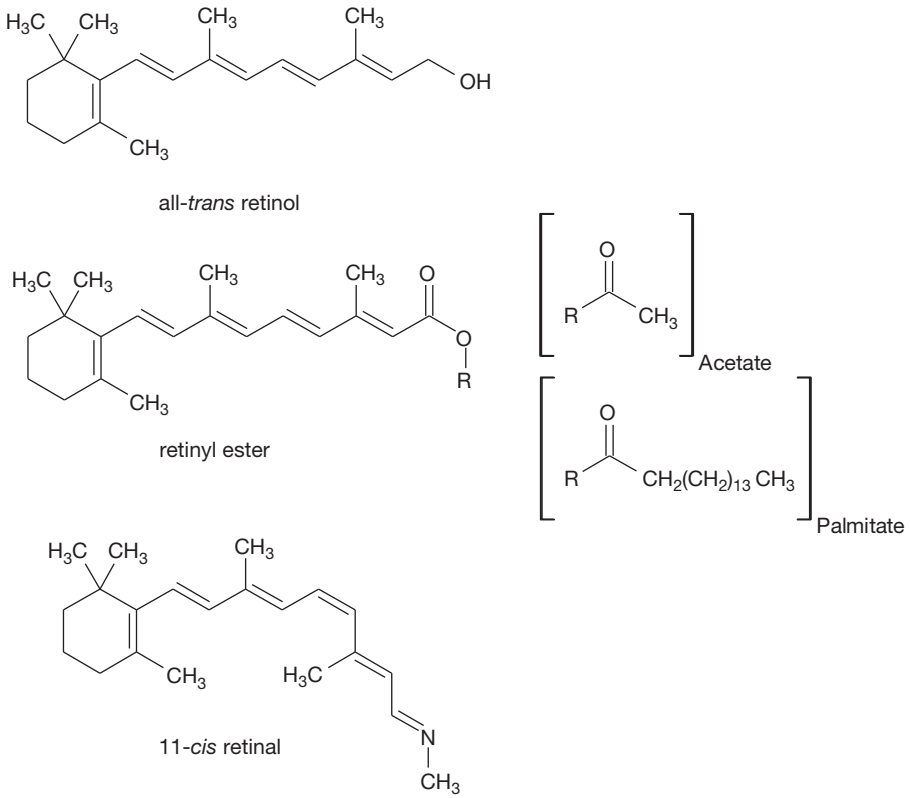

Figure 1 Chemical structures of different retinoids. All-trans retinol is by definition vitamin A, and 1 mg of all-trans retinol is equal to 1 retinol equivalent (RE). When a fatty acyl group is esterified to the hydroxyl terminus of all-trans retinol, retinyl ester is formed for storage. The most abundant retinyl esters are those of palmitic, oleic, stearic, and linoleic acids. Retinyl acetate and palmitate are often used as dietary supplements, but do not occur naturally. Retinol can be reversibly oxidized to retinal, the 11-cis isomer, which is essential for the visual cycle. Reproduced from Packer, L., Kraemer, K., Obermüller-Jevic, U. and Sies, H. (eds.) (2005). Carotenoids and Retinoids: Molecular aspects and health issues, Illinois: AOCS Press.

lycopene, are present in the human blood and tissues and have a variety of functions. The structures of these carotenoids are shown in **Figure 2**. Provitamin A carotenoids are an important source of dietary vitamin A that are found primarily in dark-green leafy vegetables, such as spinach, and in orange and yellow vegetables and fruit, such as carrots, mango, and papaya, although their bioavailability is significantly more variable than that of preformed vitamin A (retinol).

The bioavailability of carotenoids is affected by various factors. Different carotenoids have different levels of vitamin A activity depending upon the *efficiency* of their absorption and the *rate* of their conversion to vitamin A. Recent research has shown that the bioavailability of traditional dietary sources of β-carotene is considerably lower (around half to a quarter) than was previously assumed. Conversion factors for estimating vitamin A obtained from plant foods were revised from 6:1 to 12:1 (μg β-carotene–retinol activity equivalent (RAE)) and 24:1 for other provitamin A carotenoids in a mixed diet. There is also a wide variation in vitamin A equivalency ratios, which can be affected by food- and diet-related factors and health, nutritional, and genetic characteristics among human populations.

Various diet- and host-related factors affect the bioavailability of carotenoids. These factors have been evaluated and extensively reported by Castenmiller and West (1998), De Pee et al. (1998), Van Het Hof et al. (2000), and Yeum and Russell (2002).

Bioavailability of carotenoids

The main diet-related factors influencing carotenoid bioavailability are the food matrix, amounts ingested, and habitual diet type. The nutritional status, health, and genetic characteristics of human populations can also affect the absorption and bioavailability of carotenoids.

Release of the carotenoids from the food matrix is important in the absorption process. The rupture of the plant cell walls by processing (e.g., heating or pureeing) promotes the release of β-carotene from the cells before and during digestion and, therefore, facilitates solubilization and absorption. Generally, the bioavailability of β-carotene from fruits is higher than for vegetables, as the cell wall structure in fruits is usually weaker than in most vegetables and leaves. Furthermore, inhibitors of carotenoid absorption present in fruit are also less than in leafy vegetables.

The composition of the diet (due to nutrient-to-nutrient interactions) affects, to a large extent, the absorption of carotenoids. The second step in absorption, which may affect bioavailability, involves the incorporation of carotenoids into mixed micelles. Among other factors, the formation of these micelles is dependent on the presence of fat in the intestine. Therefore, ingestion of fat, along with carotenoids, is thought to be crucial although only a small amount of fat is necessary to enhance carotenoid absorption. As expected, fat-soluble compounds that cannot be absorbed, such as sucrose polyester (a fat replacer), reduce carotenoid absorption. Also, as dietary

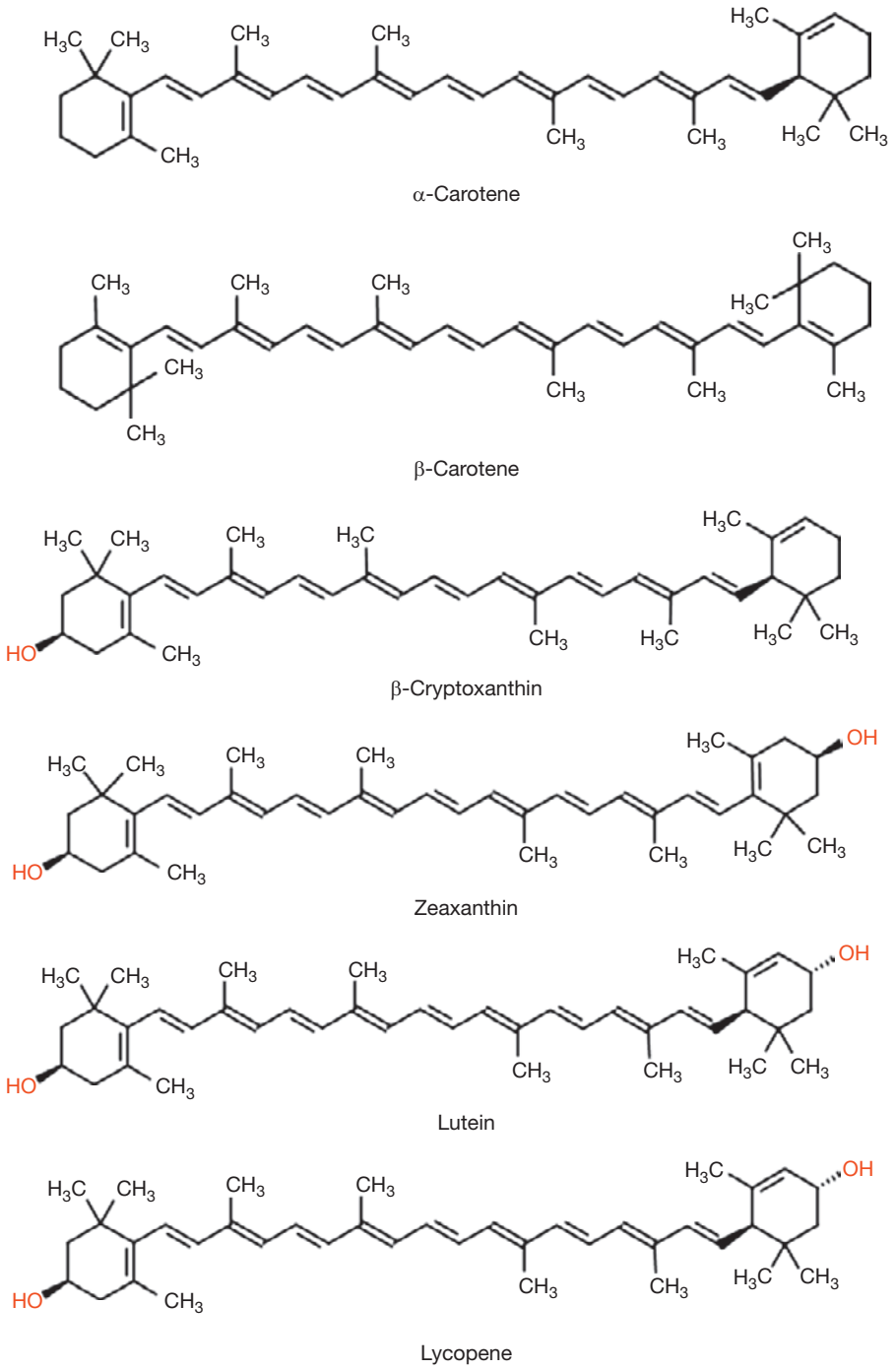

Figure 2 Chemical structures of major provitamin A carotenoids (α-carotene, β-carotene, and β-cryptoxanthin) and non-provitamin carotenoids (lutein, zeaxanthin, and lycopene) found in food.

fiber content increases, the absorption of β-carotene decreases. Dietary fiber reacts with bile acids and, thereby, decreases the absorption of fat and fat-soluble nutrients. The presence of dietary fiber in vegetables and fruits may explain in part the reduced bioavailability of carotenoids from plant foods.

Simultaneous ingestion of carotenoids may also reduce absorption of any of the carotenoids due to interactions at the intestinal level. Studies on simultaneous ingestion of carotenoids indicate that lutein may interfere with the absorption of β-carotene resulting in reduced bioavailability. It has also been found that with pharmaceutical doses of β-carotene, conversion of β-carotene to vitamin A decreases as the oral dose of β-carotene increases. Further research is required to identify the mechanisms behind these interactions.

Furthermore, the absorption of carotenoids is highly likely to be dependent on vitamin A status of the host. Consuming β-carotene-rich foods leads to an increase in serum retinol levels only when these are initially low. The serum response

to β-carotene is higher in women than in men; however, this could in part be attributed to differences in body weight and composition. Intestinal helminthic infections are associated with malnutrition, which may be mediated through impaired fat absorption and reduced vitamin absorption, particularly of vitamin A.

Iron

Minerals (and other nutrients) exist in different chemical forms in food, which can influence bioavailability. Iron is a classic example: there are two primary forms of dietary iron, namely, heme and nonheme. The former is only found in animal products, such as red meat, fish, and poultry. The heme iron content of animal sourced foods is estimated at 40% of the total iron, but data suggest that a portion of red meat provides considerably more heme iron than a portion of white fish, for example. Heme iron is a component from hemoglobin and myoglobin (see Figure 3), which explains why it is only found in animal tissue. Nonheme iron is found mostly in plant-based foods and makes up the remaining estimated 60% of iron found in animal products.

The types of iron (heme or nonheme) notably influence bioavailability. Approximately 90% of dietary iron is consumed in the nonheme form. However, due to low bioavailability, it constitutes only approximately half the iron absorbed by the body. The absorption of nonheme iron is usually much lower than heme iron. In general, the rate of nonheme iron absorption is related to its solubility in the upper part of the small intestine. The presence of soluble enhancers, such as ascorbic acid, and inhibitors, such as phytates, polyphenols, and calcium, consumed in the same meal will have a notable effect on the amount of nonheme iron absorbed. Heme iron is much less affected by other dietary factors and contributes more significantly to absorbable iron.

Inhibitors and enhancers of iron bioavailability

Nonheme iron that enters the common iron pool in the digestive tract is absorbed to the same extent, depending on the balance between inhibitors and enhancers and the iron status of the individuals.

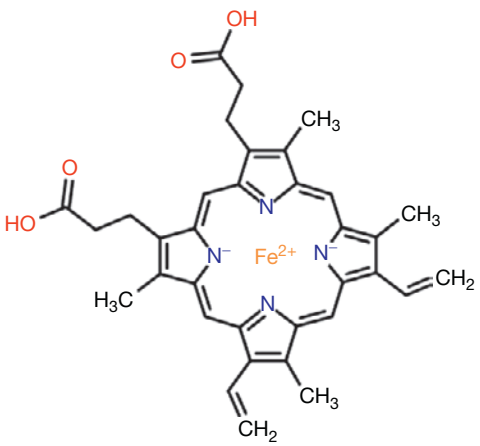

Figure 3 Chemical structure of heme iron as found in food from animal sources.

Phytate and polyphenols in plant-based diets are the main inhibitors of nonheme iron absorption. The negative effect of phytate on iron bioavailability is dose-dependent, and any food processing and preparation methods, such as milling, heating, soaking, germination, and fermentation, which degrade phytate to varying extents, will have a positive effect on iron absorption. Controversies exist on the inhibitory effect of oxalic acid in spinach and cabbage and nondigestible carbohydrates in beans on iron absorption, as these foods are also good sources of ascorbic acid, which enhances iron absorption.

Calcium and dairy products have also been shown to have a negative effect on nonheme iron absorption, but what separates these from other inhibitors is the ability to also inhibit heme iron absorption. Single-meal studies show a negative effect of calcium on iron absorption, but multimeal studies, with a variety of other inhibitors and enhancers, indicate calcium has only limited impact on iron absorption. In a recent study, the two major milk protein fraction (casein and whey) and egg protein (albumin) were reported to have a negative effect on iron absorption in humans. Although phytate has been shown to be the major inhibitor in soy, even after complete phytate digestion in soy protein isolates, significant inhibition of iron absorption can be observed. Thus, in soy, it was concluded that both phytate and a protein fraction inhibit iron absorption.

Ascorbic acid has been shown convincingly to enhance iron bioavailability in a dose-dependent manner. This effect is largely due to its ability to reduce ferric to ferrous iron. Ascorbic acid has also been shown, at least partially, to counteract the inhibitory effects of both phytate and polyphenols on nonheme iron absorption.

Small amounts of meat are recognized to enhance the absorption of nonheme iron from plant foods, although the mechanism(s) is unknown. Studies also support the enhancing effect of cysteine-containing peptides following the proteolysis of meat muscle.

Vitamin A and β-carotene can enhance nonheme iron absorption and increase hemoglobin levels, although several studies suggest this is only observed in iron-deficient individuals. Host-related factors that influence the absorption of heme and nonheme iron include mainly iron status, other nutritional deficiencies, infection, genetic disorders, and physiological state.

As with inhibitors and enhancers, the iron status of an individual mainly influences the absorption of nonheme iron, while heme iron absorption is less affected. There is an inverse relationship between iron status and iron absorption. Protein–energy malnutrition, riboflavin, and vitamin A deficiencies have also been shown to impair iron metabolism and absorption; correction of these nutritional deficiencies will improve iron absorption.

Iron deficiency often coexists in a burden of disease in seemingly well-fed, overweight populations, due in part to iron absorption being reduced by the peptide, hepcidin. Hepcidin is a regulatory hormone secreted by the liver, which inhibits iron absorption. It secretion is increased in chronic inflammation and obesity.

Achlorhydria might also be a substantial cause of iron deficiency, mainly in elderly people, among whom atrophic gastritis is common and gastric acid secretion is low. Gastric acid is needed to maintain ferric iron in solution and make it bioavailable. However, heme iron does not appear to be

affected by the lack of acid and is normally absorbed in individuals with atrophic gastritis.

Other common causes of reduced iron absorption and/or iron deficiency are mucosal atrophy in celiac disease and, possibly, *Helicobacter pylori* infection, although no consensus has been reached. For further reading on iron bioavailability, refer to Heath and Fairweather-Tait (2002), Zimmermann and Hurrell (2007), and Hurrell and Egli (2010).

Conclusions

Nutrients react differently once ingested and absorption can be influenced by a variety of factors, including quality of the food source and the matrix in which it is consumed; the composition of the whole meal, inhibitors, and enhancers; and the status of the host. Although bioavailability is only a partial measure of the benefit from a nutrient, this factor quantifies the amount entering the bloodstream. Once in the bloodstream, the nutrient must cross cell membranes before it can nourish cells. In addition to nutrient content of foods, nutrient bioavailability should also be taken into consideration when nutrition-sensitive policies, nutrition interventions, and dietary guidelines are developed. However, it should be noted that bioavailability is not a constant value and needs to be considered with caution in the context of multiple factors, both intrinsic and extrinsic, which can affect the bioavailability from food- and nonfood sources of nutrients.

See also: Amino Acids: Metabolism; Ascorbic Acid: Physiology and Health Effects; Carotenoids: Occurrence, Properties and Determination; Carotenoids: Physiology; Iron: Biosynthesis and Significance of Heme; Protein: Digestion, Absorption and Metabolism; Retinol: Physiology; Retinol: Properties and Determination.

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Biofilms

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Introduction

Bacteria predominantly exist in nature as complex microbial communities that are attached to surfaces and held together by a matrix of extracellular polymeric substances (EPS). These microbial communities are also known as biofilms, of which the community members are exposed to microbial competition, communication, and collaboration. Bacteria within the biofilm are known to employ physiological cooperation and spatial organization to increase both their metabolic efficiency and their resistance to fluctuations in the local environment. As a consequence, the biofilms have an enhanced metabolic capacity and ability to withstand environmental stresses and host immune responses compared with their planktonic counterparts. The developmental life cycle of the biofilm can be categorized into five stages (Figure 1): (i) initial attachment, where microbial cells adhere to a substratum through weak, reversible van der Waals forces and where the adsorption of inorganic salts and organic compounds to the surface, known as surface conditioning, is important for the initial attachment of microbial cells to abiotic surfaces; (ii) irreversible attachment, where the cells anchor themselves more permanently using EPS components and cell adhesion structures such as surface pili; (iii) maturation I, where a thin biofilm is formed from layered cells and small clusters, during which surface-attached cells can migrate to top of clusters, and increased cell division and aggregation lead to (ii); (iv) maturation II, where clusters develop into large microcolonies and many cells have displaced from the substratum to form channels and voids; and (v) dispersion, where parts of the biofilm undergo cell death and detachment and a subpopulation of cells regain their motility to leave the microcolony to colonize new surfaces. Understanding the biofilm formation mechanisms will greatly facilitate the development of strategies for biofilm control.

Biofilms play an essential role in environmental sustainability and human health. In natural environments, they contribute to bioremediation of toxic compounds released from human activities. In waste treatment, biofilms are used to remove unwanted organic compounds to supply clean water to society. Biofilms formed by commensal bacteria that live on the surface of our skin and intestinal tract behave as a 'virtual organ' and modulate our immune function and facilitate nutrient utilization. However, biofilms formed by bacterial pathogens are also found in the human body and are the root of many persistent and chronic bacterial infections. Examples of biofilm-associated infections include the following: (1) periodontal disease, where dental plaque, a multispecies biofilm, causes dental caries, gingivitis, and periodontitis; (2) cystic fibrosis lung infections, where bacterial biofilms resist antimicrobial treatment and phagocytosis, eventually leading to lung failure; and (3) bacterial infections from medical implants, as many pathogenic bacteria are able to form biofilms on the materials used for catheters, artificial joints, mechanical heart valves, and other devices. Biofilms also cause a significant portion of acute infections, particularly in the food industry. Biofilms formed in food preparation and water distribution systems can result in contamination of food products, causing acute foodborne illnesses. Such biofilms are a burden to public health and industry, in particular when notorious bacterial pathogens form biofilms in the food processing environment. Such pathogens include *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Vibrio* spp., *Campylobacter jejuni*, *Clostridium* spp., and *Listeria monocytogenes*.

Methods to Study Biofilms

Microscopy

Microscopy has been one of the major tools to provide information on biofilm morphology, phylogeny, and architecture. Epifluorescence microscopy and confocal laser scanning microscopy (CLSM), which are based on the excitation and detection of fluorescence emission, are the most commonly used microscopy techniques. By using microbial strains that have been tagged with fluorescent proteins, biofilm morphology and structure can be easily observed. Furthermore, the transcriptional fusions of specific gene promoters to the fluorescent proteins can be used to observe the regulation of specific genes within biofilms via fluorescence. Live–dead stains such as SYTO 62 and propidium iodide are useful for cell viability assays. In epifluorescence microscopy, fluorescent objects below and above the focal plane are also excited and emission-detected, resulting in a less sharp image. In contrast, CLSM is able to filter fluorescence emitting from other planes via a pinhole and thus collects fluorescence from the selected focal plane only. Thus, CLSM is preferred for high-quality 3-D micrographs in thick samples (such as biofilms). Figures 2(a) and 3 give a 2-D and 3-D visualization of a mixed-species biofilm by epifluorescence microscopy and CLSM, respectively.

Scanning electron microscopy (SEM) images specimens at subnanometer resolution by scanning it with a focussed beam of electrons. Electrons interact and are scattered by sample atoms to generate signals that contain information about the sample's surface topography and composition. Conventional SEM techniques require that specimens be conductive, and thus, biological samples were usually coated with conductive material such as gold. In addition, specimens were dry as imaging had to be done under high-vacuum conditions. However, samples can be now imaged uncoated in low-vacuum and moist conditions in an alternative low-voltage mode of SEM operation called environmental SEM. Figure 2(b) shows SEM micrographs of biofilms.

Atomic force microscopy (AFM) images specimens at nanometer to subnanometer resolution by scanning the sample surface with a silicon nitride or silicon tip attached to a

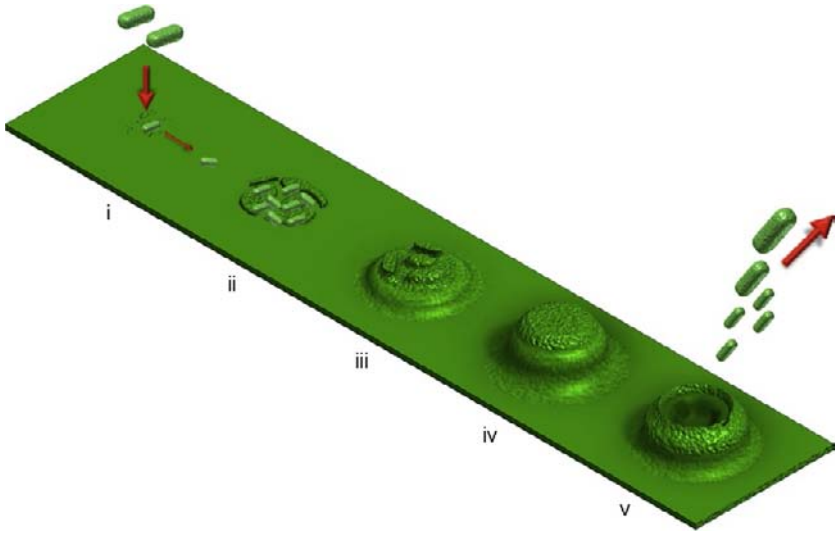

Figure 1 Different stages of the biofilm life cycle: (i) initial attachment, where cells attach via nonspecific forces; (ii) irreversible attachment, via EPS components such as pili and polysaccharide secretion; (iii) maturation I, with formation of cell layers and clusters; (iv) maturation II, where microcolonies may develop and properties specific to the biofilm lifestyle such as increased resistance emerge; and (v) dispersal, which leads to the colonization of new surfaces.

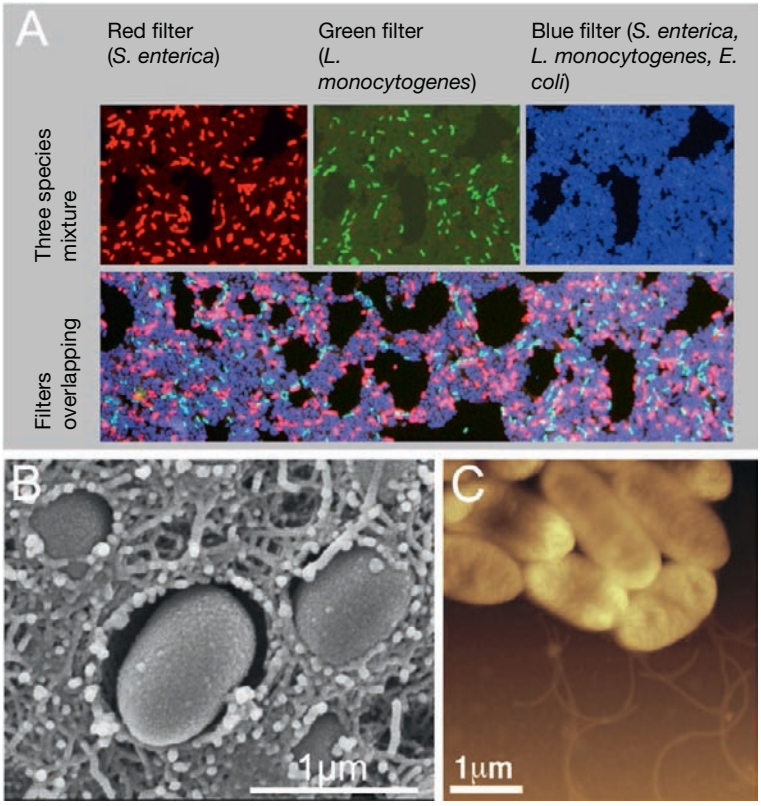

Figure 2 (a) Visualization of a three-species biofilm of *Salmonella enterica*, *L. monocytogenes*, and *E. coli* using epifluorescent microscopy and peptide nucleic acid fluorescence in situ hybridization staining. Adapted from Almeida, C., Azevedo, N. F., Santos, S., Keevil, C. W. and Vieira, M. J. (2011). Discriminating multi-species populations in biofilms with peptide nucleic acid fluorescence in situ hybridization (PNA FISH). *PLoS One* **6**(3). http://dx.doi.org/10.1371/journal.pone.0014786, with permission. (b) Visualization of *E. coli* biofilm by scanning electron microscopy. Adapted from Serra, D. O., Richter, A. M., Klauck, G., Mika, F. and Hengge, R. (2013). Microanatomy at cellular resolution and spatial order of physiological differentiation in a bacterial biofilm. *mBio* **4**(2). http://dx.doi.org/10.1128/mBio.00103-13, with permission. (c) Visualization of *Pseudomonas aeruginosa* biofilm by atomic force microscopy. Adapted from Yang, L., Hu, Y., Liu, Y., Zhang, J., Ulstrup, J. and Molin, S. (2011). Distinct roles of extracellular polymeric substances in *Pseudomonas aeruginosa* biofilm development. *Environmental Microbiology* **13**(7), 1705–1717. http://dx.doi.org/10.1111/j.1462-2920.2011.02503.x, with permission.

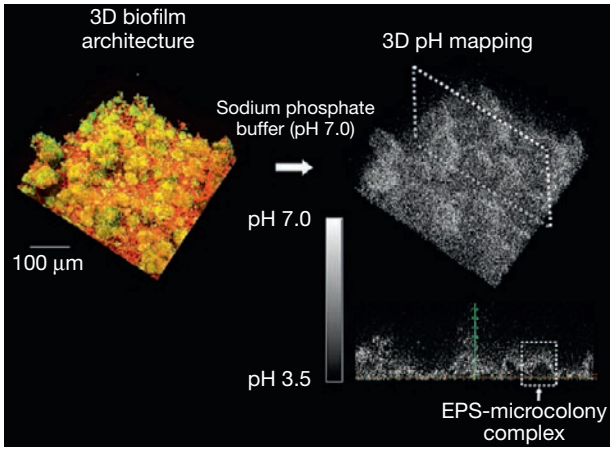

Figure 3 Confocal images of exopolysaccharide (red) and bacteria cell (green) components in mixed-species oral biofilms. Exopolysaccharides create spatial heterogeneities through localized cell-to-matrix interactions. Acidic pockets are only found in the interiors of microcolonies that are protected by exopolysaccharides, which impede rapid neutralization by sodium phosphate. Adapted from Xiao, J., Klein, M. I., Falsetta, M. L., et al. (2012). The exopolysaccharide matrix modulates the interaction between 3D architecture and virulence of a mixed-species oral biofilm. *PLoS Pathogens* **8**(4), e1002623. http://dx.doi.org/10.1371/journal.ppat.1002623, with permission.

cantilever. Interactive forces between the tip and specimen surface deflect the cantilever. Tip-sample interaction forces plotted against the tip-sample distance give the AFM force–distance curve. In this manner, AFM is able to quantify aspects of cell adhesion forces and biofilm/substratum interactions. The gradient of the force–distance curve, as the tip is driven into the sample surface, is a measure of sample compliance or stiffness. Figure 2(c) shows AFM micrographs of biofilms.

Fluorescent In Situ Hybridization

A staining technique of particular importance for the study of microbial biofilms is fluorescent in situ hybridization (FISH). FISH is used to assess the phylogenetic identity, morphology, spatial distribution, and quantity of species in polymicrobial communities. In FISH analysis, fluorescently labeled oligonucleotide probes are complementary to and hybridize to target phylogenetic markers at 16S or 23S rRNA in permeabilized microbial cells. Probes can be designed to target narrow or broad phylogenetic groups. After incubation and hybridization with the target, unspecific and unbound probes are washed away. The degree of homology between the probe and target, called the stringency of the hybridization, is adjusted during incubation and washing steps. The biofilm is then observed using fluorescent microscopy such as the CLSM and the images are analyzed by software such as 'daime' (http://www.microbial-ecology.net/daime/). Figure 2(a) shows biofilms analyzed by FISH.

Sequencing Technologies

The rapid progression of the DNA sequencing technology has greatly facilitated the current understanding of the complex biofilm communities. A large percentage of the microbial species from natural biofilm communities is difficult to culture and thus cannot be studied via routine culture-based approaches. Metagenomics and metatranscriptomics are now widely used for characterizing the microbial species composition and their physiology within biofilms. In metagenomic analysis, the entire genetic material is extracted directly from biofilm samples and then subjected to DNA sequencing by either 16S rRNA amplicon pyrosequencing approach or high-throughput short-read sequencing approach. The microbial taxonomy, genome content, and metabolic capacity can be elucidated via metagenomic analysis. To further analyze the activity of different microorganisms within the complex biofilm communities, metatranscriptomic analysis, whereby the total RNA is extracted directly from biofilm samples, converted to cDNA, and then subjected to DNA sequencing by high-throughput short-read sequencing approach, can be employed.

Biofilm Matrix

Up to 50–90% of the total organic matter in the biofilms consists of EPS that are actively secreted by the microbial cells. The EPS mainly consists of exopolysaccharides, proteins, extracellular DNA (eDNA), and humid acids and lipids and creates the heterogeneous biofilm architecture.

Exopolysaccharides

The exopolysaccharides, which often constitute the major portion of the EPS in biofilms, are long-chained in nature and function as a scaffold of biofilms and cross-link the bacterial cells together. A singular bacterial species can synthesize multiple types of exopolysaccharides with distinct roles from each other and in different stages of biofilm formation. Many of the exopolysaccharides are highly charged, which aids in absorption of water and ions, such as calcium and magnesium cations from the environment. This helps protect biofilm cells from desiccation and buffer against pH changes. Exopolysaccharides are also known to protect microbial cells from harmful conditions such as treatment by disinfectants and UV irradiation. Figure 3 shows how exopolysaccharides contribute to these properties in a mixed-species oral biofilm.

Proteins

Secreted proteins are abundant in the EPS and play important roles on structure and physiology. Extracellular carbohydrate-binding proteins such as lectins can link the bacterial cell to the exopolysaccharides and thus stabilize the EPS network. Another group of high-molecular-mass surface-associated proteins belonging to the Bap (biofilm-associated protein) family functions as adhesins for primary attachment of cells to abiotic surfaces and intercellular adhesion. Thus, they are important for biofilm formation and are highly conserved among various bacterial species. The Bap family proteins have also been shown to mediate interactions between bacterial cells and host cells, which facilitate the pathogen colonization and the establishment of persistent infections. In addition to these structural proteins, there are many enzymatic proteins that

are anchored within the EPS that can alter the local microenvironment of biofilms (see section 'Alteration of Microenvironment').

Extracellular DNA

Besides exopolysaccharides and proteins, eDNA is now recognized as a common EPS component, which can constitute a substantial amount of the biofilm EPS. eDNA was initially found to mediate early-stage biofilm formation of *Pseudomonas aeruginosa* and could interact with bacterial cells through surface pilus structures and mediated interspecies interactions. The eDNA is released from bacterial cells by active excretion or cell death. It is able to bind to cationic antimicrobials, such as aminoglycosides and antimicrobial peptides, and stop them from reaching the bacterial cells within the biofilm.

Biofilm Regulation

Cell-to-Cell Communication (Quorum Sensing)

The high cell density and thick EPS in biofilms can retain high concentrations of bacterial secreted signaling molecules and thus enhance the bacterial cell-to-cell communication (quorum sensing). Quorum sensing is an intercellular signaling mechanism widely distributed among different microorganisms that regulate gene expression in response to small diffusible signaling molecules. The three most common signal molecules used by different bacterial groups are the oligopeptides, *N*-acyl homoserine lactones (AHLs), and autoinducer-2 (AI-2) (Figure 4). Quorum sensing regulates the expression of hundreds of genes and many of these genes, which are involved in motility control, biosurfactant synthesis, and EPS synthesis, are required for biofilm formation and effective stress response to harmful environmental conditions. Foodborne pathogens such as *Salmonella*, *Vibrio cholerae*, and *Serratia liquefaciens* all produce quorum sensing molecules to regulate their motility and biofilm development.

The AHL-mediated quorum sensing is well characterized in Gram-negative bacteria. Figure 5 illustrates the LuxI–LuxR quorum sensing system from *V. fischeri*. The *luxI* gene expresses the LuxI protein, which synthesizes the AHL molecule, *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL), which is also known as autoinducer-1 (AI-1). OHHL is secreted to the external environment. The concentration of OHHL is low in the early phase of growth and increases as the bacterial population increases (especially in biofilms). When the OHHL reaches a critical threshold level, it reenters the bacterial cell to bind to the LuxR protein receptor and activate the LuxR receptor. The LuxR–OHHL complex then recognizes its target genes by a *lux* box (20-base-pair inverted repeat in the promoter region) and activates or represses transcription of these genes. The LuxI–LuxR system is a classic type of quorum sensing system and its homologues are found in many Gram-negative bacteria, including several foodborne pathogens such as *Aeromonas hydrophila* and members of the genus *Vibrio*. The formation of mature biofilms on stainless steel coupons by *A. hydrophila* requires the synthesis of *N*-butyryl-L-homoserine lactone (C4-HSL), and its AHL synthase mutant could not form a mature biofilm. Biofilm formation in *V. cholerae* is controlled by multiple quorum sensing systems that simultaneously regulate the expression of exopolysaccharide biosynthesis genes.

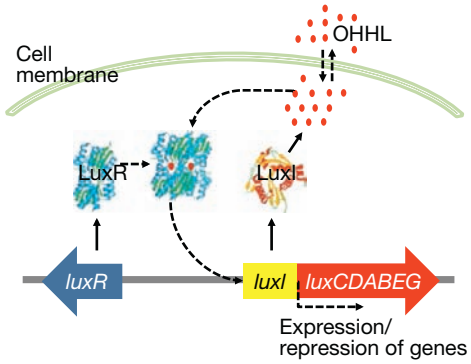

Figure 5 LuxI–LuxR quorum sensing system in *Vibrio fischeri*.

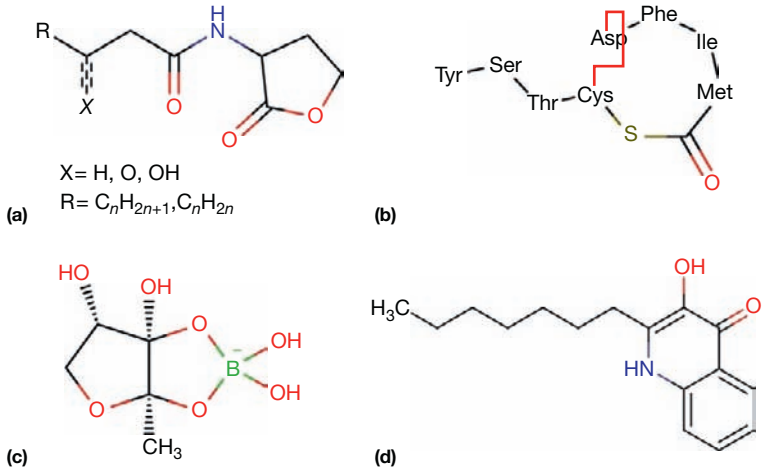

Figure 4 Examples of signaling molecules used in quorum sensing among bacteria. (a) *N*-Acyl homoserine lactones (AHLs), (b) autoinducing peptide-1 (AIP-1) in *Staphylococcus aureus*, (c) autoinducer-2 (AI-2) in *V. harveyi*, and (d) pseudomonas quinolone signal (PQS) in *Pseudomonas aeruginosa*.

Gram-positive bacteria employ oligopeptides as signaling molecules in their quorum-sensing regulation. Unlike the AHL, the oligopeptides bind to their two-component sensor kinase-based receptors on the cell surface, rather than intracellular receptors. The binding of oligopeptides to the receptors can initialize a series of phosphorylation–dephosphorylation reactions and lead to the phosphorylation of the response regulators. The activated response regulators eventually regulate the expression of quorum sensing-controlled genes and biofilm formation. The accessory gene regulator (agr) quorum sensing system was originally identified in *Staphylococcus* spp. and is the best-characterized oligopeptide-based quorum sensing system to date. agr quorum sensing positively controls virulence but negatively regulates the biofilm formation of *Staphylococcus* spp.

The autoinducer-2 (AI-2)-mediated quorum sensing is found in both Gram-negative and Gram-positive bacteria. The LuxS autoinducer synthase produces 4,5-dihydroxy-2,3-pentanedione (DPD), which is the precursor to a set of AI-2s. DPD can be actively transported into cells via the LuxS-regulated (Lsr) transporter. The internalized DPD is then phosphorylated to AI-2 by LsrK family kinase. AI-2 then binds with its specific receptor (LsrR family protein) and subsequently turns on/off the transcription of quorum sensing-regulated genes. The AI-2 quorum sensing is required for biofilm formation on surfaces used in animal production watering systems by the foodborne pathogen *C. jejuni*. Mutants that lack the AI-2 quorum sensing form much less biofilm than wild-type strain. AI-2 quorum sensing positively controls biofilm formation by *E. coli* but negatively controls biofilm formation by *Bacillus cereus* and *Staphylococcus aureus*.

c-di-GMP Signaling

A distinct physiological marker for biofilm cells is the high level of intracellular content of cyclic di-GMP (c-di-GMP). c-di-GMP is a secondary messenger that plays an essential role in determining the lifestyles of a wide range of bacteria. Intracellular c-di-GMP is synthesized by multiple diguanylate cyclases (DGCs) and degraded by phosphodiesterases (PDEs) (Figure 6). The presence of multiple DGCs and PDEs offers bacteria a great degree of flexibility to modulate their intracellular c-di-GMP content under different environmental conditions. c-di-GMP regulates gene expression via c-di-GMP effector proteins (e.g., PilZ domain protein) and c-di-GMP-I riboswitches, which change conformation drastically upon binding to c-di-GMP. High intracellular content of c-di-GMP downregulates bacterial motility and upregulates the synthesis of EPS. In contrast, lowering the intracellular c-di-GMP content facilitates bacterial motility and causes biofilm dispersal. In *Salmonella*, the expression of curli fimbriae and the major exopolysaccharide cellulose is enhanced by high intracellular c-di-GMP. The thin aggregative fimbriae and cellulose were shown to enhance the resistance of *Salmonella* to desiccation and prolong the survival of *Salmonella* colonies on plastic surfaces for several months even without the supply of exogenous nutrients. c-di-GMP positively regulates the production of exopolysaccharide by *V. vulnificus*, which contributes to its biofilm formation.

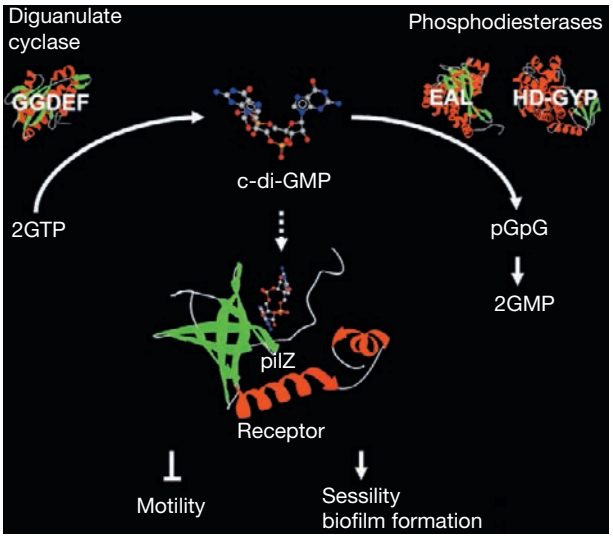

Figure 6 Schematic of c-di-GMP-mediated signaling in bacteria.

Microbial Ecology of Biofilms

Differentiation in Biofilms

Physiological heterogeneity within the biofilm is a main distinguishing factor between the metabolism of biofilm cells and planktonic cells. The EPS fixes bacterial cells in their respective positions, and through their metabolic activities and diffusional processes within the EPS, steep concentration gradients of nutrients, signaling compounds, and bacterial waste are generated within the biofilm. The biofilm cells then differentiate via a spatially patterned gene expression as a response to these gradients, resulting in a wide range of physiological states. For example, cells located at the surface of biofilms are generally fast-growing and behave more closely to planktonic cells, as they have first access to nutrients and oxygen in the medium. In contrast, cells deep within biofilms exhibit slow growth rates and anaerobic respiration due to lack of nutrients. This self-generated diversity acts as a kind of insurance to survival upon attacks that differ mechanistically and increase the overall robustness of the biofilm.

Biofilm growth also drives the production and evolution of genetic variants within bacterial strains. For example, strain variation in colony morphology; swimming and swarming; production of pigments, surfactants, and exopolysaccharides; nutritional requirements; and other phenotypes are commonly found in biofilms but not in planktonic cultures. Endogenous oxidative stress that specifically occurs in biofilms damages cellular DNA and causes double-stranded DNA breaks. These breaks are then repaired by a mutagenic mechanism involving recombinatorial DNA repair genes, including the RecA protein, to generate the genetic variants in biofilms. The emergence of such variants is increasingly being recognized as an important strategy for niche specialization and stress tolerance (see section '**Development of Persister Cells**').

Interspecies Interactions in Biofilms

Biofilms in the natural environment are complex and often consist of multiple microbial species. Microbial cells in the

mixed biofilms interact with each other extensively through metabolite cross-feeding, cross talk of signaling molecules, cross-linking of matrix components, and horizontal gene transfer (Figure 7). These interactions further shape the structures and functions of microbial communities.

Cross-linking of different matrix components is important for coaggregation, a process where different bacterial species attach to one another via specific EPS molecules and receptors. Coaggregation can greatly accelerate the formation of multispecies biofilms and is most well studied in the dental plaque of the human oral cavity using the sequential colonization model. In this model, the primary colonizers, such as *Streptococcus gordonii*, colonize the pellicles on the tooth surface within a short period. The major periodontal pathogen *Porphyromonas gingivalis*, which is a secondary colonizer, can coaggregate with the primary colonizer *Streptococcus gordonii* by binding the streptococcal SspB protein via its fimbrial FimA protein and Mfa1 protein. The secondary colonizer then coaggregates with *Fusobacterium nucleatum*, an essential bridging organism that can coaggregate with many other oral organisms and integrate them into the dental plaque.

It is well known that microorganisms not only communicate with members of the same species but also respond to signal molecules produced by other species to modulate their behavior. The colocalization of different microbial species within multispecies biofilms makes it easy for signaling molecules produced from one bacterial species to diffuse into another bacterial species. Some of these signals are recognized and generate a response, known as cross talk, further shaping the structure and functions of the biofilm community. For example, long-chain AHLs can antagonize the C4-HSL-dependent quorum sensing system in *A. hydrophila*, a Gram-negative, aquatic bacterium that not only mainly affects fish but also can cause gastroenteritis in humans. Interference with quorum-sensing regulation during biofilm formation by foodborne pathogens might open new research avenues toward the current efforts to eliminate these food processing-associated biofilms.

The formation of multispecies biofilms might have important ecological impacts on food safety and human health. For example, the mixed-species biofilms formed by Shiga toxin-producing *E. coli* and *Salmonella enterica* serovar Typhimurium were found to be more resistant to sanitization due to sharing of EPS components. Additionally, environmental bacterial species isolated from fresh produce processing facilities were found to incorporate *E. coli* O157:H7 into their biofilms and increase the survival potential of this bacterium. Mixed-species biofilms of *L. monocytogenes* and *Pseudomonas putida* have greater resistance *to* the disinfectant benzalkonium chloride than *Pseudomonas putida* biofilms alone. Understanding of the interspecies interactions during multispecies biofilm formation and how to prevent them might present a novel antibiofilm strategy for the food industry.

Resistance Mechanisms of Biofilms

Reduction of Penetration by EPS

The protection of microbial cells by EPS is one of the most evident causes of enhanced resistance of biofilms to antimicrobial agents. The high density of the EPS together with its binding properties to toxic compounds results in an effective barrier that is able to dramatically reduce the penetration of antimicrobial agents in deeper parts of the biofilm such as the microcolonies (Figure 8). Thus, the remaining microbial cells can thrive once the antimicrobial treatment is ceased. For example, alginate is able to block the diffusion of gentamicin and tobramycin antibiotics into *Pseudomonas aeruginosa* biofilms, and the diffusion efficiency can be improved after treating the biofilms with alginate lyase. Disruption of eDNA in the matrix, which is able to bind to cationic antimicrobials, was shown via the addition of DNase to the biofilm to enhance the efficiency of antimicrobial treatments.

Alteration of Microenvironment

The thick EPS not only reduces the penetration of antimicrobial agents but also generates antimicrobial-tolerant microenvironment within the biofilms together with microbial cells. Microbial cells are able to secrete enzymes to the surrounding environment under stress conditions, and the EPS can serve as a structure scaffold to hold these enzymes. Typical extracellular enzymes found in the biofilm EPS include catalase, which protect biofilm cells against reactive oxygen species, and β-lactamases, which protect biofilm cells against β-lactam class antibiotics.

Induction of Stress Responses

Biofilm cells have different physiology compared with free-living cells. The slow nutrition penetration and accumulation of waste products within the biofilm matrix can lead to physiological differentiation of biofilm cells and induction of stress response. For example, the sigma factor RpoS, which is normally expressed in stationary-phase cells, was found to be required for biofilm formation by *E. coli*. RpoS was reported to play a role in conferring peroxide resistance in *E. coli* serotype O157:H7 biofilms. Endogenous oxidative stress is able to cause double-stranded DNA breaks for cells in some part of biofilms, and recombinatorial DNA repair can lead to population diversity in biofilms. *L. monocytogenes* biofilms have been shown to accumulate superoxide and hydroxyl radicals, resulting in DNA damage and induction of the DNA repair system and SOS response via the RecA protein for the generation of genetic variants (see section '**Differentiation in Biofilms**').

Development of Persister Cells

Antibiotic stress can lead to the generation of dormant variants that are highly tolerant to antibiotics, known as persisters. Exposure of *E. coli* to DNA-damaging antimicrobial agents can trigger the expression of the *tisB* gene that encodes a small membrane-acting peptide. The synthesis of the TisB peptide can decrease proton-motive force and ATP levels, thus shutting down cell metabolism and inducing the cell to enter a dormant, nondividing state. Antibiotics that are able to kill nongrowing, stationary cells are rare, and none are effective against persisters. After antibiotic stress has been removed, persisters regain their active, dividing state and repopulate the biofilm. It has been estimated that persister cells are present at

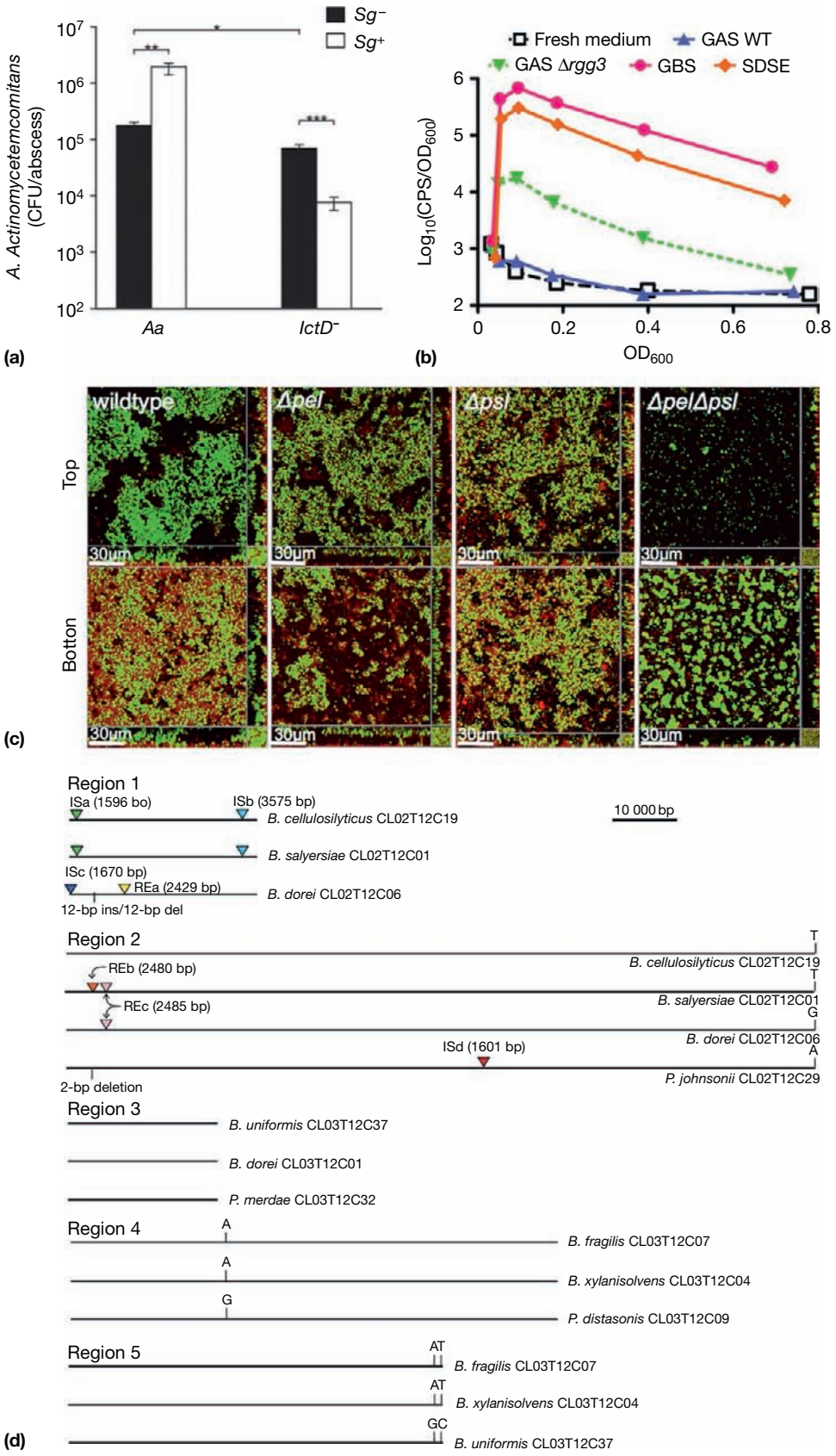

Figure 7 *(Continued)*

0.1–1.0% in the biofilms of *Pseudomonas aeruginosa*, *E. coli*, and *Staphylococcus aureus* (Figure 8).

Biofilm Control Strategies

Conventional Approaches for Biofilm Eradication

A lot of research effort has been focussed on the control and eradication of biofilms that grow in unwanted places and are detrimental to industrial and public health systems. The most efficient approach for biofilm control is to prevent the attachment of bacterial cells to surfaces. Various strategies have been developed for inhibiting microbial attachment. Engineered surfaces with certain physical properties such as special micropatterned topography and surface roughness were shown to reduce microbial attachment. Moreover, chemically modified surfaces with microbicidal capacity and antimicrobial drug-releasing capacity were shown to maintain their antimicrobial attachment properties for long periods of time.

Conventional physical and chemical approaches using cleaning and disinfection procedures have been extensively used over the years to remove biofilm-contaminated surfaces.

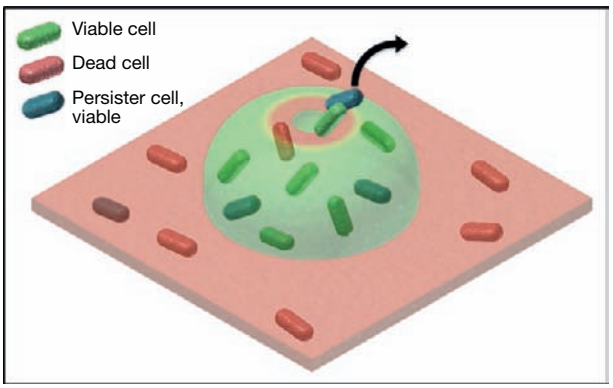

Figure 8 EPS reduce access of antimicrobial compounds and host responses into the biofilm, and cells deep within the microcolonies remain viable. Red shading indicates areas with increased cell death and green shading indicates areas with increased cell viability. Stress from antimicrobials can induce dispersal in biofilms, causing the remaining live and persister cells to leave the microcolony and colonize other surfaces. Persister cells in the thin undifferentiated areas of the biofilm remain viable.

The cleaning procedures are able to remove the thick biofilm EPS via chemical reactions through alkali-based and acid-based agents in combination with mechanical forces (such as flushing and scrubbing). Proper cleaning can significantly disrupt the biofilm EPS and lead to more efficient penetration of disinfectants into the biofilm and increased killing of microbial cells. Conventional disinfectants include ethanol, chlorine dioxide, hydrogen peroxide, and antibiotics. While these chemicals are able to control biofilms to a certain extent, they leave out the possibility of chemical contamination and development of resistant populations. Thus, there is a need for the development of the next generation of biofilm control strategies.

Next-Generation Approaches for Biofilm Eradication

Targeting the microbial quorum sensing and c-di-GMP signaling systems are two promising approaches for biofilm control. These two strategies do not pose a strong selective pressure to raise resistant mutants since they are not based on direct microbial cell killing. Quorum sensing regulates the secretion of antibiotic-inactivating enzymes into the biofilm matrix and cell migration. Small molecules that can interfere quorum sensing have been found from natural and synthetic libraries and were termed as quorum sensing inhibitors. Quorum sensing inhibitors have been shown to be able to greatly impair the biofilm structure stability and resistance. Compounds that activate PDEs and degrade intracellular c-di-GMP have been reported. These compounds are able to cause dispersal of mature biofilms. In addition to quorum sensing inhibitors and c-di-GMP-reducing compounds, phages were also shown to be able to eradicate biofilms due to the fact that they can produce polysaccharide depolymerase enzymes that can specifically degrade the exopolysaccharide of biofilms and cause cell lysis.

Conclusion

Biofilm formation is an important adaptation and survival strategy commonly employed by bacteria. Bacteria in the biofilm are protected from adverse environmental factors and immune response by the EPS. Chemical gradients generated throughout the biofilm enable bacteria to exist in a wide range of physiological states, thereby providing insurance effects in

Figure 7 **(Continued)** (a) Example of metabolite cross-feeding. Oral pathogen *Actinomycetemcomitans* needs to catabolize L-lactate produced by oral commensal *Streptococcus gordonii* in order to establish coculture. Bacterial colony-forming units per abscess in mouse thigh. *Aggregatibacter actinomycetemcomitans* monoculture strains are black bars and cocultured strains with *Streptococcus gordonii* are white bars. Adapted from Ramsey, M. M., Rumbaugh, K. P. and Whiteley, M. (2011). Metabolite cross-feeding enhances virulence in a model polymicrobial infection. *Plos Pathogens* **7**(3), e1002012. http://dx.doi.org/10.1371/journal.ppat.1002012, with permission. (b) Example of cross talk of signaling molecules. Group A streptococcus (GAS) responds to signaling molecules produced by Group B streptococcus (GBS) and *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) in spent supernatants. GAS response is measured via induction of luminescence expression in a P_{shp2}–lux reporter. Adapted from Cook, L. C., LaSarre, B. and Federle, M. J. (2013). Interspecies communication among commensal and pathogenic streptococci. *mbio* **4**(4). http://dx.doi.org/10.1128/mBio.00382-13, with permission. (c) Example of cross-linking of matrix components in different species. Exopolysaccharides in *Pseudomonas aeruginosa* (green) are required for cross-linking and close association of *Staphylococcus aureus* (red), and the differential expression of exopolysaccharides greatly affects the spatial organization of the species in the mixed biofilm. Adapted from Chew, S. C., Kundukad, B., Seviour, T., et al. (2014). Dynamic remodeling of microbial biofilms by functionally distinct exopolysaccharides. *mBio* **5**(4). http://dx.doi.org/10.1128/mBio01536-14, with permission. (d) Strong evidence of horizontal gene transfer. Five large chromosomal regions, each present in a minimum of three of the coresident intestinal *Bacteroidales* strains at near 100% DNA identity. Adapted from Coyne, M. J., Zitomersky, N. L., McGuire, A. M., Earl, A. M. and Comstock, L. E. Evidence of extensive DNA transfer between bacteroidales species within the human gut. *mBio* **5**(3). http://dx.doi.org/10.1128/mBio.01305-14, with permission.

changing environments. Biofilms can be composed of multiple species that interact with each other.

Microscopic techniques such as CLSM in combination with fluorescent tagging and staining are commonly employed in biofilm research for the visualization and understanding of biofilm physiology. In mixed-species biofilms, FISH and sequencing technologies such as metagenomics and metatranscriptomics are used to study phylogenetic groupings, synergy, and competition among members of the biofilm.

Biofilm formation is regulated by intercellular quorum sensing signaling and intracellular c-di-GMP signaling. New methods for biofilm control are being developed based on interfering these two signaling mechanisms. The employment of novel biofilm control methods is believed to improve the effects of antibiotics and disinfectants, which are often ineffective against biofilm and may also generate drug resistance in bacteria.

See also: Campylobacter: Health Effects and Toxicity; *Clostridium botulinum*; *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; Foodborne Pathogens; *Listeria*: Listeriosis; *Salmonella*: Salmonellosis; Shigella; *Staphylococcus*: Food Poisoning; *Yersinia enterocolitica*: Detection and Treatment.

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http://biofilmcourse.ku.dk – Biofilm Online Course – University of Copenhagen.

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http://www.scelse.sg – Singapore Centre on Environmental Life Sciences Engineering (SCELSE).

Biogenic Amines

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Introduction

Biogenic amines (BAs) are naturally occurring organic compounds, formed and degraded through the normal or physiological metabolism of microorganisms, plants, and animals, which possess biological activity. Their molecular weight is close to or less than 200 Da. They are basic nitrogenous heat stable compounds, primarily formed by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones. All BAs are invested with some specific physiological roles in live organisms, but their excessive production or intake can induce adverse reactions. The name of most BAs is assigned depending on the name of the precursor amino acid. Based on the number of amine groups, BAs can be classified into monoamines, diamines, and polyamines, and according to the chemical structure into aliphatic, aromatic, and heterocyclic amines (Table 1). Traditionally, the term BAs includes amines that can arise from direct decarboxylation of amino acids such as histamine, tyramine, tryptamine, agmatine, cadaverine, putrescine (which can also derive from agmatine by hydrolase activity), and phenylethylamine. Other BAs such as spermine, spermidine, serotonin, octopamine, dopamine, and norepinephrine require for their generation some condensation and/or hydroxylation reactions, while methylamine and ethylamine can come from direct amino acid decarboxylation but are primarily formed as metabolic and degradative products.

In foods and beverages, BAs can be generated by the enzymatic activity on proteins and amino acids of raw animal or vegetal tissues and by the amination of aldehydes and ketones, but their formation primarily occurs through microbial activity and decarboxylation of free amino acids. BAs most commonly found in foods are histamine, tyramine, tryptamine, cadaverine, putrescine, phenylethylamine, spermine, and spermidine. Other BAs have been reported, such as agmatine in fish, seafood, fermented meats, and fermented drinks; octopamine in meat and fish products; dopamine and serotonin in fish, meat, and fruits; norepinephrine in meat and fruits; and methylamine and ethylamine in wine and fish.

BAs in Organisms

BAs are compounds with biological activity that play specific physiological roles in prokaryotic and eukaryotic organisms, including bacteria, fungi, plants, and animals. They are also nitrogen sources and precursors for the synthesis of proteins, nucleic acids, alkaloids, and hormones.

In microorganisms, BAs are involved in the supply of energy through the generation of proton motive force, protection from acid, osmotic and oxidative stress, and DNA regulation. In some bacteria, the presence of decarboxylases responsible for BA generation has been considered a virulence factor. Bacteria able to produce histamine can cause tissue damage, facilitating the colonization and invasion of the host, while those able to produce tyramine such as *Escherichia coli* O157:H7 can enhance their adhesion to mucosa and colonization. They can act as essential precursors for siderophore biosynthesis in *Bordetella* sp and *Vibrio anguillarum*. In plants, BAs are implicated in physiological processes such as cell division and differentiation, flowering, fruit development, synthesis of nucleic acids and proteins, membrane stability, and response to stress and senescence, and may also play a defense role against insects and herbivores.

Histamine is generated from histidine by the enzyme histidine decarboxylase. In animals, it is produced and stored primarily in mast cells, basophils, eosinophils, enterochromoaffin-like gastric cells, and histaminergic neurons. Histamine is found in the brain, lungs, stomach, intestines, uterus, ureters, and muscles, and exerts its effects by binding to H-receptors (H1–H4). It acts as a neurotransmitter and local hormone, modulating gastric secretion, heart contractibility, cell growth, contraction of smooth muscle, circadian rhythm, body temperature, food intake, memory and cognition, immune response, and allergic and inflammatory reactions.

Tyramine derives from the enzymatic decarboxylation of tyrosine. In humans, it primarily arises from the diet and has been found in the brain, spinal cord, heart, kidney, liver, lungs, and spleen. It acts as a neurotransmitter and as a catecholamine-releasing agent, modulating peripheral vasoconstriction and cardiac output and increasing blood levels of glucose.

Tryptamine comes from enzymatic decarboxylation of tryptophan and has been primarily found in the brain, but also in the lungs, heart, intestines, liver, kidneys, and urine. It acts as a neurotransmitter and as a serotonin-releasing agent, modulating behavior and food intake.

Cadaverine and putrescine derive from the enzymatic decarboxylation of lysine and ornithine, respectively. Putrescine can additionally result from agmatine by ureohydrolase or iminohydrolase activities. They have been found at low levels in all the tissues and organs, including brain and gut and secretions such as urine and sperm, regulating gene expression, membrane stabilization, cell growth and differentiation, gut development, and maturation of newborns, while high levels are usually found in ulcerated, necrotic, and tumoral tissues.

Phenylethylamine comes from enzymatic decarboxylation of L-phenylalanine in nervous tissue and neurons. It has been found in the brain and spinal cord, and acts as a neurotransmitter and stimulant in the central nervous system, inducing the release of norepinephrine, dopamine, and serotonin and modulating cognition, behavior, and perception.

Spermine derives from spermidine, which comes from putrescine, by spermine synthase and spermidine synthase, respectively. They were first described in sperm, but are present in all the tissues of mammals. At low levels, they act as modulators of gene expression, cellular growth and differentiation, embryonic development, and cytokine-mediated inflammatory response. High levels have been found in tumoral tissues.

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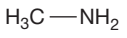
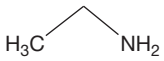
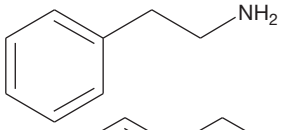
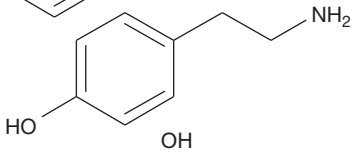
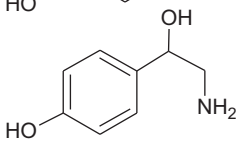
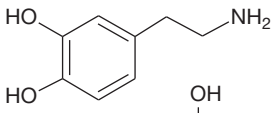
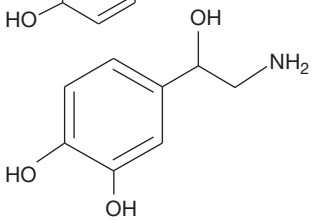
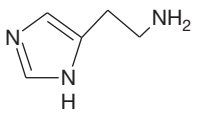
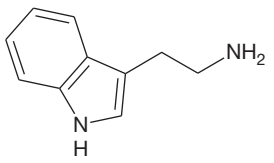
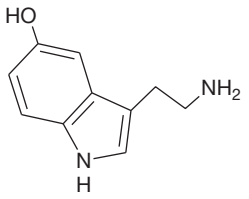
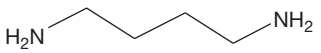
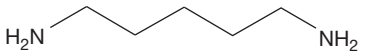
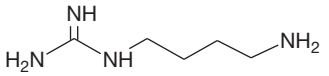
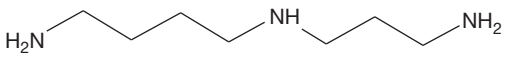
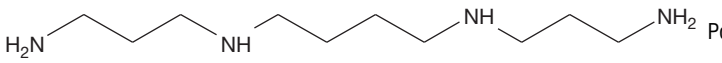
Table 1 Characteristics of biogenic amines found in foods, animals, plants, and microorganisms

Name (MW g mol^{-1})	*Molecular formula*	*Structural formula*	*Classification*	*Precursor amino acid*
Methylamine (31.06)	CH_5N		Monoamine aliphatic	Glycine
Ethylamine (45.08)	C_2H_7N		Monoamine aliphatic	Alanine
Phenylethylamine (121.18)	$C_8H_{11}N$		Monoamine aromatic	Phenylalanine
Tyramine (137.18)	$C_8H_{11}NO$		Monoamine aromatic	Tyrosine
Octopamine (153.18)	$C_8H_{11}NO_2$		Monoamine aromatic	Tyrosine
Dopamine (153.18)	$C_8H_{11}NO_2$		Monoamine aromatic	Tyrosine
Norepinephrine (169.18)	$C_8H_{11}NO_3$		Monoamine aromatic	Tyrosine
Histamine (111.15)	$C_5H_9N_3$		Monoamine heterocyclic	Histidine
Tryptamine (160.22)	$C_{10}H_{12}N_2O$		Monoamine heterocyclic	Tryptophane
Serotonin (176.22)	$C_{10}H_{12}N_2O$		Monoamine heterocyclic	Hydroxytryptophane
Putrescine (88.15)	$C_4H_{12}N_2$		Diamine aliphatic	Ornithine
Cadaverine (102.18)	$C_5H_{14}N_2$		Diamine aliphatic	Lysine
Agmatine (130.19)	$C_5H_{14}N_4$		Polyamine aliphatic	Arginine
Spermidine (145.25)	$C_7H_{19}N_3$		Polyamine aliphatic	Arginine Ornithine
Spermine (202.34)	$C_{10}H_{26}N_4$		Polyamine aliphatic	Arginine Ornithine

Agmatine comes from enzymatic decarboxylation of arginine. It has been found primarily in the gut but also in the spleen, lungs, and adrenal glands, and at lower levels in plasma, the heart, liver, kidneys, muscles, and the brain. It acts as a neurotransmitter and as a neuromodulator in mental disorders and stress, and exerts hypoglycemic and antinociceptive effects.

Serotonin comes from enzymatic decarboxylation of hydroxytriptophan. It is primarily found in the gut, especially in enterochromaffin cells, but also in the central nervous system and platelets. It acts as a neurotransmitter and modulates circadian rhythm, food intake, cognition, and behavior.

Dopamine derives from decarboxylation of L-DOPA, which comes from tyrosine or phenylalanine by hydroxylase activity in neuron medulla cells and adrenal glands, but it is also found in cardiovascular tissue, the pancreas, and the gut. It acts, by interaction with D-receptors (D1–D5) and other endogenous receptors, as a neurotransmitter and as a hormone, modulating cognitive and motor functions, memory, nausea, and vomiting, inhibiting prolactin secretion, and decreasing food intake. It is the precursor of epinephrine and norepinephrine.

Norepinephrine (or noradrenaline) comes from dopamine by dopamine-β-hydroxylase activity. It is produced and stored in the secretory granules of chromaffin cells of the adrenal medulla and postganglionic neurons, and can be liberated into the blood system and distributed to all tissues in response to alert or stress situations. It acts as a neurotransmitter and as a hormone, and interacts with adrenergic receptors, exerting a sympathomimetic effect inducing vasoconstriction and hyperglycemia and enhancing attention and concentration.

Octopamine comes from degradation and hydroxylation of tyramine. In invertebrates it is equivalent to dopamine, while in vertebrates it is found in nervous tissue and the brain, exerting stimulant effects, mobilizing fat from adipose deposits, and increasing blood pressure.

Methylamine and ethylamine are hydrosoluble volatile BAs, which primarily arise from the metabolism and degradation of products. In animals, they are rapidly eliminated by urine and excretions.

BAs in Foods

As described, BAs are bioactive endogenous compounds naturally present at low levels in raw vegetal or animal tissues. They can be generated by the endogenous enzymatic activity on proteins and amino acids of vegetal and animal tissues, and by chemical amination of aldehydes and ketones in foods, but they are usually associated with microbial decarboxylation of free amino acids. In foods, this process requires the presence of decarboxylase-positive microorganisms, the availability of amino acids, and the existence of conditions (pH, water activity, nutrients, temperature, and redox potential) that allow microbial growth and decarboxylase activity. Practically all foods contain proteins or amino acids and therefore may contain BAs. Their presence has been reported in a wide range of products, including meat, fish, vegetables, fruits, milk and its derivatives, nuts, chocolate, and fermented beverages, some of them at high concentrations (Table 2). The different BA levels greatly depend on the foodstuff, the manufacturing and storage conditions, and even the size and part of the product since BAs are heterogeneously distributed in the food matrix.

In raw-fresh products BAs are usually present at low levels and come from endogenous origin, while in processed and/or stored products they are generated by microbial decarboxylation of amino acids during fermentation and/or spoilage processes. In nonfermented foods BAs are produced during growth of spoilage bacteria, and their presence above certain levels is considered indicative of alteration. However, the concentration of BAs in food does not always necessarily correlate with the counts of spoilage organisms, because they are not all decarboxylase-positive. Moreover, a food product can contain high levels of BAs without showing evident signs of alteration. In fermented foods, the generation of BAs is associated with the growth and metabolism of lactic acid bacteria during the fermentation and/or ripening stages characteristic of these products.

The presence of microbial strains able to decarboxylate free amino acids is essential for the formation of BAs in foods. This capacity has been described in many different genera, species, and strains of Gram-positive and Gram-negative bacteria. Microbial capability of BA production seems to be strain dependent rather than genus- or species-specific. Different genes encoding for decarboxylating enzymes have been identified. These genes may be located on the bacterial chromosome or on plasmids, and can be transferred not only vertically but also horizontally between bacteria.

Foods represent an exogenous source of BAs, which are involved in many physiological functions in animals. But the intake of foods with high levels of BAs can induce toxic or adverse effects such as pseudoallergic reactions and gastrointestinal and vascular or hemodynamic disorders. Even neurological disturbances have been described for tyramine, phenylethylamine, serotonin, dopamine, and norepinephrine, while carcinogenic effects have been associated with cadaverine and putrescine, and cytotoxic effects with spermine and spermidine. Besides this, some BAs such as cadaverine, putrescine, spermine, and spermidine can react with nitrites during processing, storage, and cooking of foods, yielding N-nitrosamines that can act as mutagenic and carcinogenic agents.

Fish and Seafood

Fish and seafood products are among the foods with the highest BA concentrations, usually histamine. Indeed, histamine poisoning or histaminosis associated with fish consumption is also known as scombrotoxicosis. Fish such as tuna, bonito, sardine, anchovies, swordfish, and mackerel, belonging to the *Scombridae*, *Scomberesocidae*, *Clupeidae*, *Engraulidae*, *Coryphaenidae*, and *Pomatomidae* families, are the species most commonly associated with incidents of histamine intoxication. Other nonscombroid species including salmon, amberjack, and cape yellowtail can be also implicated. This is probably due to their high content of endogenous histidine, primarily in dark muscle, and to the capability of marine bacteria, in particular Gram-negative species, of exerting their decarboxylase activity even at low temperatures. The main microorganisms associated with the generation of histamine and other BAs in fish and seafood products are spoilage Gram-negative bacteria belonging to the genera *Citrobacter*, *Klebsiella*, *Escherichia*, *Proteus*, *Salmonella*,

Table 2 Maximum reported levels (mg kg^{-1} or mg l^{-1} of product) of the major biogenic amines in some foods and beverages

Product	*HI*	*TY*	*TR*	*CAD*	*PUT*	*SPD*	*SPM*	*PEA*	*AGM*
Fish and seafood									
Fresh tuna	30	99	na	6	5	12	37	3	13
Canned tuna	20 000	5	na	4470	2000	10	35	na	na
Fresh mackerel	1270	189	89	2860	560	24	50	38	na
Smoked mackerel	17 880	na	na	2520	490	4	8	na	na
Cephalopods	9	24	4	164	190	6	13	14	na
Shellfish	49	28	28	151	174	92	149	44	na
Dairy products									
Feta cheese	846	246	na	828	193	na	na	5	na
Ripened raw milk cheese	510	454	na	328	176	43	22	41	69
Ripened pasteurized milk cheese	65	301	na	na	175	40	19	na	13
Blue raw milk cheese	1041	1051	na	757	876	72	19	27	na
Blue pasteurized milk cheese	127	527	na	89	238	29	2	na	na
Meat products									
Fresh meat	5	38	na	13	8	20	70	na	3
Cooked products	11	108	1	12	139	9	36	2	27
Dry-fermented sausages	515	743	91	790	505	91	119	52	43
Dry-cured products	128	295	na	64	331	16	62	19	8
Fermented beverages									
White wine	3	5	na	1	10	2	1	2	7
Red wine	27	19	2	14	108	5	na	16	22
Sherry wine	3	3	na	7	25	na	na	1	na
Beer	22	68	10	51	31	7	15	8	47
Cider	7	4	na	na	12	na	na	na	na
Vegetable products									
Spinach	27	8	7	4	24	23	6	1	na
Cucumber	na	2	na	na	29	4	0.1	1	na
Tomato sauce	5	14	15	12	43	17	4	3	na
Ketchup	9	34	22	31	53	33	12	na	na
Sauerkraut	200	900	na	300	550	50	2	2	7
Fermented soy	4620	35 680	930	6340	12 340	62	69	59	5508
Eggs and derivatives									
Boiled egg	na	na	na	na	0.4	0.2	0.6	na	na
Liquid pasteurized egg	na	9	na	3	17	na	na	na	na

HI, histamine; TY, tyramine; TR, tryptamine; CAD, cadaverine; PUT, putrescine; SPD, spermidine; SPM, spermine; PEA, phenylethylamine; AGM, agmatine; na, data not available. Data from Shalaby. (1996). *Food Res. Int*. 29:675–690; Kalač, Švecová & Pelikánová (2002). *Food Chem*. 77:349–351; Ruiz-Capillas & Jiménez-Colmenero (2004). Crit. Rev. *Food Sci. Nutr*. 44:489–499; Moret, Smela, Populin & Conte (2005). *Food Chem*. 89:355–361; Moreno-Arribas & Polo (2008). *Food Microbiol*. 25:875–881; Kim, Mah, & Hwang (2009). *Food Chem*. 116:87–95; Atiya Ali, Poortvliet, Strömberg & Yngve (2011). *Food Nutr. Res*. 55:5572, pp. 1–8; Konakovsky, Focke, Hoffmann-Sommergruber et al. (2011). *Food Add. Contam. Part A* 28:408–416; Linares, Martín, Ladero, Alvarez, & Fernández (2011). *Crit. Rev. Food Sci. Nutr*. 51:691–703; Prester (2011). *Food Add. Contam. Part A* 28:1547–1560; Galgano, Caruso, Condelli, & Favati (2012). *Front. Microbiol*. 3:199, pp. 1–7; Latorre-Moratalla, Bover-Cid, Veciana-Nogués & Vidal-Carou (2012). *Front. Microbiol*. 3:169, pp. 1–9; Rêgo, Menezes, Figueiredo et al. (2014). *Poultry Sci*. 93:1018–1022.

Shigella, *Vibrio*, *Morganella*, *Hafnia*, *Serratia*, *Enterobacter*, *Aeromonas*, *Pseudomonas*, and *Photobacterium*. Storage under inadequate conditions, due to temperature abuse and cold chain break, is considered the major cause of scombroid poisoning.

Besides histamine, other major BAs such as putrescine, cadaverine, tyramine, tryptamine, spermine, spermidine, and agmatine have been detected in fish and seafood products. The amine index $[(\text{putrescine}+\text{cadaverine}+\text{histamine})/(\text{putrescine}+\text{cadaverine}+\text{histamine}+\text{tyramine}+\text{tryptamine}+\text{methylamine}+\text{spermine}+\text{spermidine})]\times 100$ has been proposed as a quality indicator that must be lower than 25. For canned tuna, the chemical index $[(\text{putrescine}+\text{cadaverine}+\text{histamine})/(1+\text{spermidine}+\text{spermine})]$ has been proposed as a quality indicator that must be lower than 1. Minor BAs in fish and fish products include octopamine, dopamine, noradrenalin, serotonin, ethylamine, and methylamine, for which respective levels up to 130, 201, 131, 12, 339, and 195 mg kg^{-1} have been reported. Levels of other amines such as dimethylamine and trimethylamine have been proposed as indicators of frozen storage deterioration and fish freshness, respectively.

In shellfish, cephalopods, crustaceans, and bivalves, BAs different from those described for fish have been found, usually at lower levels due to the fact that these products are more prone to spoilage and evident alterations, which result in rejection by the consumer.

Milk and Dairy Products

In milk, low levels of some BAs such as spermine, spermidine, and putrescine, probably from endogenous origin, are usually found. In cheese and other fermented or ripened dairy products such as kefir or kumis, high levels of BAs can be found, while buttermilk and yogurt do not usually contain significant

levels. The microorganisms associated with the generation of BAs in cheese are Gram-positive bacteria, in particular lactic acid bacteria such as *Lactobacillus*, *Enterococcus*, *Carnobacterium*, *Pediococcus*, *Lactoccus*, *Leuconostoc*, and *Oenococcus*, but also *Clostridium* and *Bacillus*. Yeasts such as *Debaryomyces*, *Candida*, *Yarrowia*, and *Pichia*, and molds such as *Geotrichum* and *Penicillium*, are also able to produce BAs.

After fish, cheese is the product most commonly implicated in outbreaks of histamine poisoning. Cheese represents an ideal matrix for BA generation because of its diverse microbiota and the intense casein degradation that occurs in many varieties. High levels of tyramine, above 1000 mg kg^{-1}, and histamine can be reached, especially in aged cheeses made from raw milk. Tyramine poisoning associated with cheese consumption is known as the 'cheese reaction,' with symptoms such as headache and hypertensive crisis. It was first described in a patient treated with monoamine oxidase (MAO) inhibitors for depression.

Other BAs such as putrescine, cadaverine, phenylethylamine, and tryptamine, and to a lesser extent spermine, spermidine, and agmatine, have also been found in cheese. Their levels greatly differ between cheese varieties, manufacturing processes, and even sections of the cheese. Milk pasteurization and hygiene tend to lower the accumulation of BAs in cheese. On the contrary, the addition of proteolytic enzymes to accelerate cheese ripening may enhance BA formation. The type of starter culture used for cheese manufacture also influences the generation of BAs in cheese.

Meat and Meat Products

Fresh meat usually contains some endogenous levels of spermine, spermidine, putrescine, cadaverine, tyramine, histamine, agmatine, and noradrenaline. During processing and storage, high concentrations of tyramine, cadaverine, putrescine, and histamine can be formed due to fresh meat spoilage bacteria. The sum of cadaverine and putrescine levels has been proposed as an index of freshness or acceptability of the product, which must be under 20 mg kg^{-1}. Microorganisms associated with BA generation in meat and noncured meat products are mostly Gram-positive bacteria of the genera *Lactobacillus*, *Carnobacterium*, *Enterococcus*, and *Brochothrix*, but also Gram-negative bacteria of the genera *Pseudomonas*, *Serratia*, *Enterobacter*, *Citrobacter*, *Hafnia*, *Proteus*, and *Rahnella*.

In fermented or ripened meat products, large amounts of BAs, especially tyramine, cadaverine, and putrescine, can be formed due to microbial activity. The microorganisms associated with BA generation in these products have been reported as lactic acid bacteria including *Lactobacillus*, *Carnobacterium*, *Pediococcus*, *Lactoccus*, *Leuconostoc*, and *Oenococcus*; other Gram-positive bacteria such as *Clostridium and Bacillus;* and Gram-negative bacteria such as *Pseudomonas*, *Citrobacter*, *Enterobacter*, *Serratia*, *Morganella*, *Escherichia*, and *Klebsiella*. Yeasts including *Debaryomyces*, *Candida*, *Yarrowia*, and *Pichia*, and molds such as *Geotrichum* and *Penicillium* are also able to produce BAs. In cured-brined meat products, strains of *Micrococcus* and *Staphylococcus* may also be implicated. The sum of tyramine, histamine, tryptamine, and phenylethylamine has been proposed as an indicator of hygienic conditions and good manufacturing practices in the production of fermented-ripened sausages, in which it should not exceed 200 mg kg^{-1}. In fermented dry-cured products, the sum of putrescine, cadaverine, histamine, and tyramine has been suggested as a quality index that must be lower than 500 mg kg^{-1}.

Other BAs have been described in meat and its derivatives, including agmatine, at levels up to 43 mg kg^{-1}, and octopamine, serotonin, and dopamine, which usually appear at very low concentrations, close to 1 mg kg^{-1}.

Fermented Drinks

Fermented alcoholic beverages, including wine, beer, and cider, can contain significant amounts of BAs, although generally at lower levels than in fish, cheese, and meat products. However, because ethanol is an inhibitor of MAO and interferes with BA detoxification, they may represent a considerable risk for consumer health.

The most abundant BAs in these products are histamine, tyramine, and putrescine, and to a lesser extent phenylethylamine and cadaverine, but also agmatine, methylamine, and ethylamine. These compounds are considered to arise from lactic acid bacteria metabolism, especially during malolactic fermentation, while yeasts (*Debaryomyces*, *Candida*, *Yarrowia*, *Saccharomyces*, *Kloeckera*, *Metschnikowia*, *Brettanomyces*, and *Pichia*) and molds (*Geotrichum* and *Penicillium*) have a lesser influence, although some BAs have been found in grapes and malt.

BA levels can greatly increase during aging and storage of fermented alcoholic beverages. Higher levels have been generally found in red wine, sherry-type wine, and beer than in white wine, rosé wine, cider, or fruit-based wines such as apple, cherry, plum, peach, or pear wines. In aged wines, putrescine levels have been suggested to indicate poor hygiene or inadequate storage conditions. In rice- or cereal-based wines, amounts of BAs higher than 100 mg l^{-1} have been detected, probably due to the intense proteolytic and fermentative steps during their manufacture.

Vinegar is obtained from alcoholic beverages through the conversion of ethanol into acetic acid by bacteria, and therefore it can also contain BAs, although usually at lower levels than in wines. The common BAs in vinegar are histamine and putrescine, and to a lesser extent spermidine, spermine, and agmatine, followed by tyramine. Higher levels of BAs have been reported for balsamic and sherry vinegars than for red wine, white wine, or apple wine vinegars.

Fruits and Vegetables

Low concentrations of different BAs are naturally present in fruits and vegetables as endogenous compounds, metabolites, and intermediates. During processing and storage of fruits and vegetables, BAs can be generated from the enzymatic activity of raw tissues and from microbial activity. The common microorganisms associated with BA generation in fruit and vegetables are Gram-negative spoilage genera such as *Pseudomonas*, *Aeromonas*, *Stenotrophomonas*, *Enterobacter*, *Hafnia*, *Salmonella*, *Escherichia*, *Klebsiella*, *Serratia*, *Morganella*, *Rahnella*, and *Pantoea*. Overall, the main BAs in these products are phenylethylamine, tyramine, tryptamine, putrescine, and cadaverine. Other BAs have been found at high levels, including histamine in tomatoes

and spinach or noradrenaline in plums and orange juice. High amounts of noradrenaline and dopamine have also been reported in bananas. Phenylethylamine may be present at high levels in mushrooms, cocoa, and derivatives such as chocolate. Serotonin concentrations up to 400 mg kg^{-1} have been found in butternuts and up to 36 and 170 mg kg^{-1} in banana pulp and peel, respectively, and high levels have also been reported in roasted coffee grains. Pyrrolidine, a cyclic secondary amine, may accumulate in pepper and soya, while some algae have been found to contain high amounts of histamine, cadaverine, and dopamine.

Fermented vegetable products including sauerkraut, fermented soybean, and kimchee can contain putrescine, histamine, tyramine, and cadaverine at high levels due to the growth and metabolism of their typical microbiota during the fermentation stage of manufacture. Microorganisms associated with the generation of BAs in these products include lactic acid bacteria such as *Lactobacillus*, *Enterococcus*, *Carnobacterium*, *Pediococcus*, *Lactoccus*, *Leuconostoc*, and *Oenococcus*, and some genera of yeasts and molds.

Eggs and Derivatives

Tyramine, cadaverine, and putrescine have been reported in eggs and their derivatives, but generally at low levels. The primary BA formers in eggs are *Enterobacteriaceae* such as *Escherichia*, *Salmonella*, *Shigella*, *Enterobacter*, and *Proteus*, but strains of *Achromobacter*, *Lactobacillus*, *Leuconostoc*, *Pseudomonas*, *Pediococcus*, *Streptococcus*, *Propionibacterium*, and *Clostridium* can also be implicated. Eggs and derivatives are prone to spoilage, which usually results in rejection by the consumer before high amounts of BAs can be formed. These products are more frequently involved in foodborne diseases related to the presence of pathogens such as *Salmonella* than in adverse reactions or intoxications due to BAs.

Legal Limits of BAs in Foods

The incidence of BAs in virtually all foods has been reported worldwide. In spite of these compounds being present at high levels in foodstuffs and beverages (Table 2), specific legislation concerning the maximum allowed concentration of BAs in the different products is still lacking.

According to the European Food Safety Authority (EFSA), by taking into consideration not only the potential BA levels in foods but also the average daily intake of the different products, the highest exposure values for histamine correspond to fish, followed by cheese and fermented sausages; for tyramine to beer and cheese, followed by fermented fish and fermented meat products; for putrescine to fermented vegetable products, fruits, and juices, followed by fermented sausages, meat products, and cheese; and for cadaverine to cheese, followed by fish and fish products. For phenylethylamine the highest exposure values correspond to fermented sausages and cheese; for tryptamine to cheese, fish, and fermented sausages; for agmatine to fermented foods and beverages; and for spermine and spermidine to meat products, followed by fish, cheese, and vegetables.

The most frequent events of BA intoxication have been reported in Japan, the United States, and the United Kingdom, but very likely many cases in other countries have occurred without being detected or officially reported. Foodborne outbreaks associated with BAs are generally underreported because of the usually mild nature of disturbances, confusion with allergic reactions, and misdiagnoses. On this basis, it is very difficult to establish safety levels or legal limits.

The most studied BA in foods and the only one with established legal limits for human consumption is histamine, and to a lesser extent tyramine. Histamine poisoning associated with fish consumption has been frequently reported in the United States, Canada, Japan, the United Kingdom, and other countries, while histamine poisoning associated with cheese consumption has been widely reported in the Netherlands, the United States, France, and Canada. The European Union established legal limits for histamine below 100 mg kg^{-1} for raw fish and below 200 mg kg^{-1} for cured or brined fish belonging to the *Scombridae* and *Clupeidae* families, while a more stringent level of 50 mg kg^{-1} was adopted in the United States. In wine, the European Union established legal limits for histamine, which range from 2 to 10 mg l^{-1} depending on the regulations of the different countries. In meat products, a recommended upper limit of 100–200 mg kg^{-1} for histamine was proposed in the Netherlands and the Czech Republic, and an upper limit of 100 mg kg^{-1} for total BAs in some other countries.

Overall, histamine levels above 500 mg kg^{-1} or tyramine levels above 1000 mg kg^{-1} are considered toxic and dangerous for human health. In susceptible or unhealthy adults and in children, these levels can be considerably lower. Although upper limits of 100–800 mg kg^{-1} for tyramine and 30 mg kg^{-1} for phenylethylamine have been proposed, no legal limits for most foods have been adopted. Information about human toxicity and safety levels for the rest of BAs remain scant.

Analytical Methods for BAs in Foods

In the United States, the official method for analyzing histamine in foods is the Association of Official Agricultural Chemists (AOAC) procedure, which requires homogenization of the food sample in methanol, filtration, separation by anion exchange chromatography, derivatization with *o*-phthalaldehyde, and spectrophotometric determination. In the European Union, the high-performance liquid chromatography (HPLC) technique is the official method for the quantification of BAs.

The main problem for the determination of BAs in foods is the presence of potentially interfering compounds due to the complexity of sample matrices. Samples usually require extraction with solvents or reagents or solid-phase methods, followed by derivatization steps. BAs are of many different chemical structures, and their concentrations in foods greatly vary. Analytical procedures for the separation, identification, and quantification of BAs in foodstuffs include

- HPLC after derivatization of the sample, and determination by fluorescence, ultraviolet (UV), or electrochemical detectors – this analytical technique is the most widely used for BA quantification

- Gas chromatography (GC) coupled to electrical conductivity, flame ionization, or electron capture detectors
- Capillary electrophoresis or capillary isotachophoresis systems, coupled to a UV detector or a mass spectrometry (MS) system
- Thin-layer chromatography (TLC) and visualization under UV light
- Fluorometric methods based on the fluorescence of BAs at certain pH and/or on their interaction with reagents yielding fluorescence derivatives, such as *o*-phthalaldehyde and β-naphthol
- Enzymatic methods including radioimmunoassays, enzyme-linked immunosorbent assay system (ELISA), and biosensors based on enzymatic reactions between enzymes such as MAO and BAs, followed by detection by electrochemical or spectrophotometric devices

Indirect methods, based on the detection of the producer microorganisms instead of the direct determination of BAs themselves, include

- Detection of producer microorganisms, by growth and screening for decarboxylase-positive strains on differential agars
- Detection of microbial genes encoding for decarboxylating enzymes, by conventional PCR (polymerase chain reaction) techniques or real-time quantitative PCR (q-PCR) techniques

Methods for the Control of BAs in Foods

As described, the generation and accumulation of BAs in foods require the presence of decarboxylase-positive microbiota that can exert their activity on free amino acids or protein substrates. In this context, the simplest methods for the control of BA production in foods are based on the reduction or inhibition of microbial growth during processing and storage, which can be achieved by regulating the environmental conditions that affect microbial growth and metabolism such as temperature, pH, and salt concentration, together with the selection of high hygienic quality raw materials and the use of good manufacturing practices. The addition of antimicrobial compounds or preservatives including sulphites, nitrites, sorbates, phosphates, and organic acids such as citric, malic, and succinic acid, can also reduce microbial growth and retard BA accumulation. Some spices and natural substances have been described to inhibit BA formation. Examples are curcumin, capsaicin, piperine, thymol, ginger, garlic, green onion, red pepper, clove, and cinnamon, and some easily fermentable carbohydrates such as glucose and sucrose at concentrations above 3%.

The control of redox potential in foods can be achieved by packaging technologies including vacuum packaging or modified atmosphere packaging (MAP), which inhibit or reduce microbial growth. The use of active packaging, which in addition to providing the food with a protective physical barrier releases substances with antimicrobial properties (chitosan, polypeptides, essential oils, and bacteriocins), is gaining ground for controlling microbial growth and metabolism, and subsequently for preventing the accumulation of BAs. Edible active packaging materials have been designed for specific foodstuffs and purposes. Intelligent packaging systems that provide the user with information on the storage conditions of the food contribute to controlling the risks associated with the accumulation of BAs.

In fermented foods, the typical lactic acid bacteria are primarily responsible for BA generation. Addition of either starter cultures selected on the basis of their decarboxylase-negative traits or strains from which the decarboxylase activity potential has been eliminated constitutes an advantageous straightforward procedure for the manufacture of safe fermented products. Decarboxylase-negative strains of *Lactobacillus sakei*, *Staphylococcus xylosus*, *Staphylococcus carnosus*, and *Pediococcus pentosaceous* allow the fermentation and ripening of meat and vegetable products without generating BAs.

Another interesting approach is the inoculation of foods or beverages with microorganisms able to degrade BAs. Some strains of *Brevibacterium linens*, *Micrococcus varians*, *Virgibacillus* sp, *Lactobacillus curvatus*, *Staphylococcus xylosus*, and *Arthrobacter crystallopoietes* possess enzymes capable of oxidizing and degrading BAs. Further research is still required for their application in foods and beverages with satisfactory results.

Conventional thermal methods such as pasteurization reduce the numbers of pathogenic and spoilage microorganisms, extending the shelf life of the product and retarding the generation of BAs. However, some foods and beverages cannot be submitted to thermal treatments due to the nutritional, sensory, and textural modifications induced by high temperatures. As an alternative, emerging technologies for food preservation are being investigated for the control of BAs in foodstuffs. Ionizing radiation, such as gamma rays, electron beams, and X-rays, achieve microbial inactivation in foods, reduction of BA production, and, potentially, they could also induce the radiolytic degradation of BAs in a dose-dependent manner. Irradiation at 5, 10, and 15 kGy has been shown to breakdown putrescine, spermidine, and histamine, respectively, in distilled water. However, other reports indicate that the irradiation of foods such as pepperoni and ham could increase the formation of BAs such as phenylethylamine, spermidine, cadaverine, and tryptamine, probably due to the generation of free radicals and the alteration of proteins. High pressure processing (HPP) allows the inactivation of microorganisms by alteration of microbial cell morphology, structure, genetic material, and physiological functions, thus extending the shelf life of the product without impairing its quality attributes. Hydrostatic pressures above certain levels also inactivate enzymes, including microbial decarboxylases. The effects of HPP greatly depend on pressure level, time of treatment, moment of application, and type of food. Treatments at 200 MPa for 10 min reduced putrescine and cadaverine levels in meat batter; 350 MPa for 15 min reduced cadaverine, putrescine, and tyramine in fermented sausages; 400 MPa for 10 min reduced tyramine, putrescine, and cadaverine in frankfurters; and 600 MPa for 5 min reduced tyramine, putrescine, tryptamine, histamine, phenylethylamine, and cadaverine in raw ewe milk cheese and tyramine, putrescine, histamine, and cadaverine in raw cow milk cheese. In contrast, treatments at 50 MPa for 72 h greatly increased tyramine levels in goat milk cheese; 300 and 400 MPa for 15 min increased

tyramine levels in squid; and 600 MPa for 5 min increased putrescine, tryptamine, and phenylethylamine in blue-veined cheese, probably by inducing cell lysis and releasing non-inactivated decarboxylases into the medium.

See also: Allergies: Public health; Amino Acids: Metabolism; Biogenic Amines: Toxicology and Health Effect; Cheese: Chemistry and Microbiology; Cheese: Composition and Health Effects; Consumer Protection Legislation; Cured Foods: Health Effects; Fermented Foods: Composition and Health Effects; Fish: Dietary Importance and Health Effects; Food Allergies; Food Poisoning: Classification; Sausages and Comminuted Products: Dry Fermented Products; Wines: Wine and Health.

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Biogenic Amines: Toxicology and Health Effect

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Introduction

Biogenic amines (BAs) (histamine, tyramine, putrescine, cadaverine, agmatine, spermidine, and spermine) are organic, basic, nitrogenous compounds of low molecular weight, present in plant, microbial, and animal cells and can be detected in raw and in fermented foods. On the basis of their chemical structure, they can be divided into three groups: aliphatic (putrescine, cadaverine, spermine, and spermidine), aromatic (tyramine and phenylethylamine), and heterocyclic (histamine and tryptamine). According to the number of amine groups, they can be classified as monoamines (tyramine and phenylethylamine) and diamines (histamine, putrescine, and cadaverine). Generally, exogenous BAs are produced through decarboxylation of amino acids by bacterial activity during food or beverage fermentation or spoilage. However, their production is a strain-specific characteristic, more widely distributed among certain genera or species, suggesting that horizontal gene transfer may account for their dissemination between strains. During food processing, BA formation is possible only under specific conditions: the availability of free amino acids and presence of aminoacid decarboxylating microorganisms and of an environment that is favorable for enzyme activity and bacterial growth. In fact, the amount and type of BAs formed in foods are strongly influenced by both intrinsic food characteristics (pH, water activity, and microbiota) and extrinsic parameters (storage time and temperature). Fish and fishery products, dairy products, meat and meat products, fermented vegetables, soy products, and alcoholic beverages (wine and beer) are rich in BAs with histamine, tyramine, putrescine, and cadaverine being the most common. These compounds show both physiological and toxicological effects for human health (Table 1). They are precursors for the synthesis of hormones, alkaloids, nucleic acids, and proteins and have an important role as neurotransmitters or are needed for critical biological functions. For this reason, in eukaryotic cells, their biosynthesis is essential. They are involved in natural biological processes such as synaptic transmission, blood pressure and body temperature control, gastric acid secretion, allergic response, and cell growth and differentiation. However, if their level reaches a critical threshold, they can be hazardous to human health causing adverse reactions such as nausea, respiratory distress, hot flush, sweating, heart palpitations, headache, bright red rash, burning sensations in the mouth, and alterations in blood pressure.

BAs: Physiological Effects

BAs are involved in the modulation of several pathways related to the physiology and development of eukaryotic cells. For instance, histamine can act as local hormone and neurotransmitter and is present in mast cells, blood cells, and neurons. It shows different effects in different mammalian and invertebrate organisms by binding to its four receptors (H1, H2, H3, and H4) on and/or in the cellular membrane, resulting in multiple biological responses. It causes smooth muscle cell contraction, vasodilatation, increased vascular permeability and mucus secretion, tachycardia, alterations of blood pressure, arrhythmias, gastric acid secretion, and nociception in nerve fibers. These receptors are localized in different sites. H1 receptors are found in the brain and peripheral tissues and are implicated in the circadian rhythm control, attention and cognition, and vascular and bronchial muscle responses to histamine in allergic processes. H2 receptors are widely distributed in body tissues and play a specific role in the regulation of gastric acid secretion and contraction of intestinal smooth muscle. H3 receptors control histamine synthesis and release, allergic hypersensitivity. Its binding to this receptor induces smooth muscle cell contraction, blood vessel dilation, and, therefore, an efflux of blood serum into the surrounding tissues, initiating the inflammatory process. H4 receptors have been found on hematopoietic cells, highlighting their importance in inflammatory conditions.

Serotonin, derived from tryptophan decarboxylation, is a vasoconstrictor and acts on the regulatory system of emotions. Agmatine has an antidepressant action and is involved in pain regulation.

Tyramine and β-phenylethylamine belong to the so-called 'trace amine' family and show strong structural similarities to classical monoamine neurotransmitters and maintain the basal neuronal within defined physiological limits. Trace amines are also involved in amplification/reinforcement mechanisms. Intuitively, such a function could have important implications in higher cognitive functions and memory formation. These compounds also seem to play an important role in Parkinson's disease. This disease is characterized by the loss of dopaminergic neurons. Trace amines potentiate dopaminergic responses; this apparent reciprocal relationship between trace amine levels and dopaminergic tone makes the trace amines a prime candidate for playing a role in the compensation mechanisms against the loss of dopaminergic neurons. Recently, new G protein-coupled receptors activated by trace amines have been discovered. This specificity applies a new nomenclature for these receptors, that of trace amine-associated receptors. They are mainly located in the blood vessels, explaining the effect of tyramine on blood pressure.

Also, dietary polyamines show important physiological roles supporting a normal metabolism and maintaining optimal health and regulating the intracellular polyamine synthesis. They are a group of polycationic amines ubiquitously distributed in microbial, plant, and animal cells. Putrescine (1,4-diaminobutane), spermidine (*N*-(3-aminopropyl)-1,4-diaminobutane), and spermine (*N*,*N*-bis-(3-aminopropyl)-1,4-diaminobutane) are the main polyamines. They originate from decarboxylation of arginine and ornithine by putrefactive bacteria. This explains why polyamines are present in

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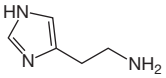
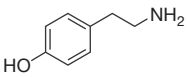
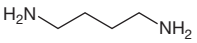
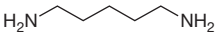
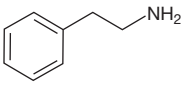
Table 1 Main biogenic amines present in foods and their toxicological effects

Biogenic amine	*Chemical structure*	*Precursor*	*Food class*	*Physiological effects*	*Toxicological effects*
Histamine		Histidine	Vegetables, fish, fermented drinks and foods, dairy products	Local hormone and neurotransmitter, gastric acid secretion, cell growth and differentiation, circadian rhythm, attention and cognition, onset of allergic reactions	Headache, nasal secretion, bronchospasm, tachycardia, extrasystoles, hypotension, edema (eyelids), urticaria, pruritus, flushing, and asthma
Tyramine		Tyrosine		Peripheral vasoconstriction, increased cardiac output, increased respiration, elevated blood glucose, release of norepinephrine	Headaches, migraine, neurological disorders, nausea, vomiting, respiratory disorders, hypertension
Putrescine		Ornithine		Regulation of gene expression, maturation of intestine, cell growth and differentiation	Increased cardiac output, tachycardia, hypotension, carcinogenic effects
Cadaverine		Lysine		Regulation of gene expression, maturation of intestine, cell growth and differentiation	Hypotension, bradycardia, lockjaw, and paresis of the extremities
β-Phenylethylamine		Phenylalanine	Vegetables, fermented drinks and foods, dairy products	Cognitive functions and memory formation, dopaminergic responses	Increased blood pressure, migraine

high amounts in fermented foods. The estimated values for the daily polyamine intake in different studies vary between 250 and 550 mmol. Mediterranean regions show a higher intake of total polyamines (700 mmol day^{-1}) than the United Kingdom and northern Europe (350–500 mmol day^{-1}). These compounds are absorbed from the intestinal lumen and distributed to all organs and tissues in the body and their levels depend on biosynthetic and catabolic enzymes such as spermidine/spermine acetyltransferase, flavin-containing polyamine oxidase, copper-containing diamine oxidase (DAO), and probably other amino oxidases and on the production by microorganisms residing in the intestinal tract and also contribution from the diet. Polyamines exist in cells in both free and conjugated forms, which regulate the cellular pool of free polyamines. Conjugated forms are bound covalently to a partner molecule and can be released by hydrolysis with a strong acid. On the basis of their chemical structure, under physiological conditions, putrescine, spermine, and spermidine are 2, 3, and 4 positive charges, respectively; therefore, they can interact with negatively charged structures of cells such as cellular membrane constituents and nucleic acids. Generally, polyamine–DNA interactions can modulate DNA–protein interactions: polyamines enhance the binding of gene-regulatory proteins to the respective response elements. For example, spermine facilitated the binding of estrogen receptor to its response element, while Oct-1 binding to DNA was inhibited in the presence of high concentrations of polyamines.

Polyamines are involved in cellular growth, maturation of the intestinal tract, and proliferation. Moreover, they regulate the differentiation of immune cells and the inflammatory reactions, and they inhibit pulmonary immunologic and intestinal immunoallergic responses: in children, high polyamine intake during the first year has been significantly correlated to food allergy prevention, which is important for the maintenance of normal growth and maturation of the intestinal tract. Generally, their concentration decreases with age in the brain, kidney, spleen, and pancreas suggesting that maintenance of polyamine level from the diet is important to keep the functioning of these organs in the elderly. Recently, a possible implication of spermine and spermidine in diabetes has been postulated since they show an antiglycation effect at physiological concentration. Moreover, these two polyamines exert an anti-inflammatory effect through the inhibition of proinflammatory cytokine synthesis and the reduction of leukocyte function-associated antigen-1 expression, which is involved in immune cell activation and inflammation.

BAs: Toxicological Effects

Several studies have demonstrated that BAs have toxicological effects for humans even at low concentrations. Therefore, their occurrence in foods is becoming an economic problem directly linked to the influence of these compounds on health. In healthy persons, dietary BAs can be rapidly detoxified by

amine oxidases, whereas persons with low amine oxidase activity are at risk of their toxicity.

Histamine is physiologically the most important BA. Humans can tolerate up to 180 mg of pure histamine orally without having noticeable effects. Endogenous histamine is generated by the enzyme histidine decarboxylase in mast cells, basophils, enterochromaffin-like cells in the gastric mucosa, histaminergic neurons, and some epidermal cells. It is only synthesized as necessary and is degraded immediately.

Histaminosis symptoms, due to ingestion of histamine-rich food or of alcohol or drugs that release histamine or block DAO that converts histamine into imidazole acetic acid, occur up to few hours after the poisoning and resemble an allergic reaction. The main clinical manifestations are hypotension, flushing, and headache, while the increased capillary permeability causes urticaria, hemoconcentration, and eyelid edema. Histamine also acts on the gastrointestinal system causing the contraction of smooth muscles leading to abdominal cramps, diarrhea, nausea, and vomiting. Moreover, it exerts a stimulatory action on the heart by increasing its contractility and exhibiting palpitations and tachycardia, while it is a potent stimulant of sensory and motor neurons producing pain and itching associated with the rash. Symptoms can be reduced by a histamine-free diet or be eliminated by antihistamines. However, because of the multifaceted nature of the symptoms, the existence of histamine intolerance has been underestimated and $\sim$1% of the population has histamine intolerance.

It is difficult to establish a toxic level of histamine, because this depends on the individual detoxifying activities. In fact, BA catabolic enzymes can be inhibited by different drugs, antidepressant drugs, and alcohol. Intoxication is characterized by an incubation period ranging from a few minutes to hours, with symptoms that are usually noticeable for a few hours only. Results from the limited number of studies suggested a potential no observed adverse effect level of 50 mg histamine for symptoms of headache and flushing, but this was based on a limited number of individuals. The amount ingested leading to acute effects is often unknown and several other factors such as potentiation of acute effects by other BAs, alcohol, or medication could not be excluded. The limited published information available suggested a potential acute reference dose of 50 mg of histamine per healthy person. Fish has been incriminated in the majority of histamine poisoning (scombroid fish poisoning or histamine fish poisoning) with histamine levels >500 mg kg^{-1} or less. Some responses mediated by histamine and its receptors (vasodilatation, smooth muscle cell contraction, alterations of blood pressure, stimulation of nociceptive nerve fibers, tachycardia, and arrhythmias) are related with the main symptoms of scombroid poisoning. Given the role of histamine in this syndrome, alteration in the action of histamine catabolic enzymes can have negative consequences. Four main mechanisms have been described to explain scombroid poisoning, and they are (i) impairment of DAO activity due to either genetic predisposition, gastrointestinal diseases, or medication with DAO inhibitors; (ii) mast-cell degranulation to release endogenous histamine in the human body; (iii) potentiation of histamine toxicity by other compounds present in toxic fish; and (iv) undiscovered histamine receptor agonists. The European Union Commission (EU, 2005) specifies fish species associated with a high amount of histidine and establishes its critical levels: For those matured in brine products, the critical concentration is 200 mg kg^{-1}, while for simple fish products, the concentration is 100 mg kg^{-1}, based on the average of nine samples. Of the nine samples, no two can be higher than 100 mg kg^{-1} (and 200 mg kg^{-1}) levels but none can be higher than 200 mg kg^{-1} (or 400 mg kg^{-1} for enzyme-matured products). In the United States, a level of 50 mg kg^{-1} is used.

After fish, cheese is the next most commonly implicated food item associated with histamine poisoning. The histamine concentrations in cheeses that were implicated in outbreaks ranged between 850 and 1870 mg kg^{-1}. Other foods such as sausages, sauerkraut, wine, or other fermented foods can contain high concentrations of histamine and its potentiators, but these are rarely reported to be implicated in histamine poisoning.

According to Rapid Alert System for Food and Feed (RASFF), about 100 histamine intoxication outbreaks have been registered between 2005 and 2010. The estimation of the dose/exposure level causing histamine intoxication is based on the detection of the BAs in the suspected food or on the patient reports. Moreover, it should be considered that histamine toxic effects are enhanced in the presence of other BAs such as putrescine and cadaverine.

Tyramine, phenylethylamine, and tryptamine are related to neuromodulating functions and could have a role in human disorders such as schizophrenia, depression, attention deficit disorder, and Parkinson's disease. In particular, tyramine promotes the efflux of catecholamines from the sympathetic nervous system and the adrenal medulla increasing arterial blood pressure and heart rate by peripheral vasoconstriction, resulting in hypertensive crisis. Tyramine also dilates the pupils and the palpebral tissues, causes lacrimation and salivation, accelerates respiration, and increases the blood sugar content.

Tyramine is also associated to the 'cheese reaction,' a pathological condition that is commonly associated to adverse interaction between monoamine oxidase inhibitors (MAOIs), a class of antidepressant drugs, and high amounts of dietary tyramine causing hypertensive crisis (Figure 1). Increased amounts of tyramine in the blood can cause the release of excess noradrenaline and blood pressure increase. So clinicians proposed the so-called MAOI diet in which tyramine intake is controlled by the reduction of tyramine-rich foodstuffs in the diet, in particular aged cheese, fermented meat, sauerkraut, soy sauce, etc. In healthy people, from 600 mg to 2000 mg of tyramine administrated in a meal would be needed to cause a minimal systolic blood pressure increase, whereas in individuals medicated with MAOI drugs, much less dietary tyramine is needed to produce negative effects.

Also, polyamines are related to some negative effects on human health. The metabolic requirement for polyamines is particularly high in rapidly growing tissues as it happens in tumors. Polyamines are involved in the proliferation of neoplasms in the gastrointestinal tract, and there is increasing evidence that putrescine and spermidine have a role in promoting the malignant transformation of cells. They are involved in cell growth and proliferation. In particular, putrescine is involved in colorectal cancer development and in gastric carcinomas caused by *Helicobacter pylori*. In fact, putrescine can interact with some pathogenic microorganisms since it is an essential component of their outer structure and has been related to virulence factors in many Gram-positive and Gram-negative pathogens.

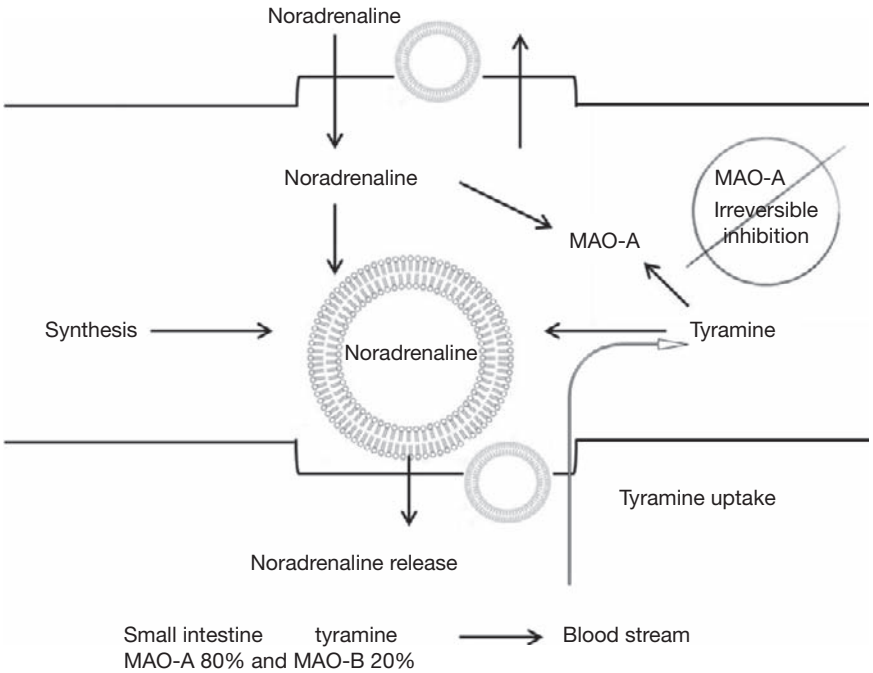


Figure 1 When intestinal MAOs are inhibited, tyramine can induce noradrenaline release from peripheral adrenergic neurons causing hypertensive crisis. Modified from Youdim, M. B. H. and Weinstock, M. (2004). Therapeutic applications of selective and non-selective inhibitors of monoamine oxidase A and B that do not cause significant tyramine potentiation. *Neurotoxicology* **25**, 243–250.

The involvement of polyamines in cancer allowed the development of drugs interfering with polyamine biosynthesis or their biological role. Some examples are ornithine decarboxylase inhibitors and polyamine structural analogs and derivatives. However, tumor cells can uptake extracellular polyamines deriving from the diet or produced by gastrointestinal bacteria, bypassing the effect of these therapeutic agents. Therefore, another approach based on the inhibition of polyamine synthesis in tumor cells and the reduction of the main exogenous sources (e.g., foods rich in polyamines) has been proposed. It gave interesting results also because polyamine deprivation stimulates the antitumoral immune response. However, it has not yet been experimentally proved that altering of the dietary polyamine intake can help cancer patients.

Polyamines are also related to oral acute and subacute toxicity in Wistar rats. The levels for acute toxicity were 2000, 600, and 600 mg kg^{-1} body weight for putrescine, spermidine, and spermine, respectively, while no effects were observed with concentrations of 180, 83, and 19 mg kg^{-1} body weight for putrescine, spermidine, and spermine, respectively.

Apart from a direct effect in promoting the transformation of cells, polyamines subjected to heat can generate secondary amines that can generate nitrosamines with well-known carcinogenic, mutagenic, and teratogenic activities. This is of particular importance in some fermented meat products to which nitrates and nitrites are added as additives.

Detoxification System

The catabolic pathway of BAs is generally regulated by oxidases classified as MAO and DAO that catalyze the deamination of BAs producing hydrogen peroxide and by specific amine methyltransferases (MT) involved in histamine and tryptamine removal. The gastrointestinal tract is the most important source of exogenous BAs. Indeed, the higher activity of BA detoxification system was measured in the gut lumen and liver. In normal physiological conditions, the enzymatic barrier, localized in the intestinal epithelial cells, is considered to play a protective role against the resorption of dietary BAs (Figure 2).

MAO (EC 1.4.3.4) was first discovered as tyramine oxidase by Hare in 1928, since it catalyzed the oxidative deamination of tyramine. Only, in 1968, Johnston discovered that two MAO isozymes existed: MAO-A is primarily present in the stomach, intestine, and placenta with noradrenalin and octopamine as preferred substrates and defects have been linked to depression and abnormally aggressive behavior. MAO-B is predominantly found in the brain and selectively deaminates nonpolar aromatic amines (as phenylethylamine and dopamine). MAO-B expression in the brain increases during aging and may be linked to disorders such as Alzheimer's disease, while a particular allele of the MAO-B gene has been linked to Parkinson's disease. While Parkinson's disease is often treated with L-DOPA, the addition of a MAO-B inhibitor such as deprenyl dramatically increases its neuroprotective effect. They are FAD-dependent enzymes sharing a high sequence identity (~70%) even if they have different substrate specificities, and mammalian MAOs are bound to the outer mitochondrial membrane through a C-terminal transmembrane polypeptide. MAO-A and MAO-B genes probably derive from the duplication of a common ancestral gene as suggested by their identical exon–intron organization. Interestingly, MAO-A and MAO-B amino acid sequences are conserved (>87% and 88.3% identity, respectively) in humans, rats, and bovines underlining the

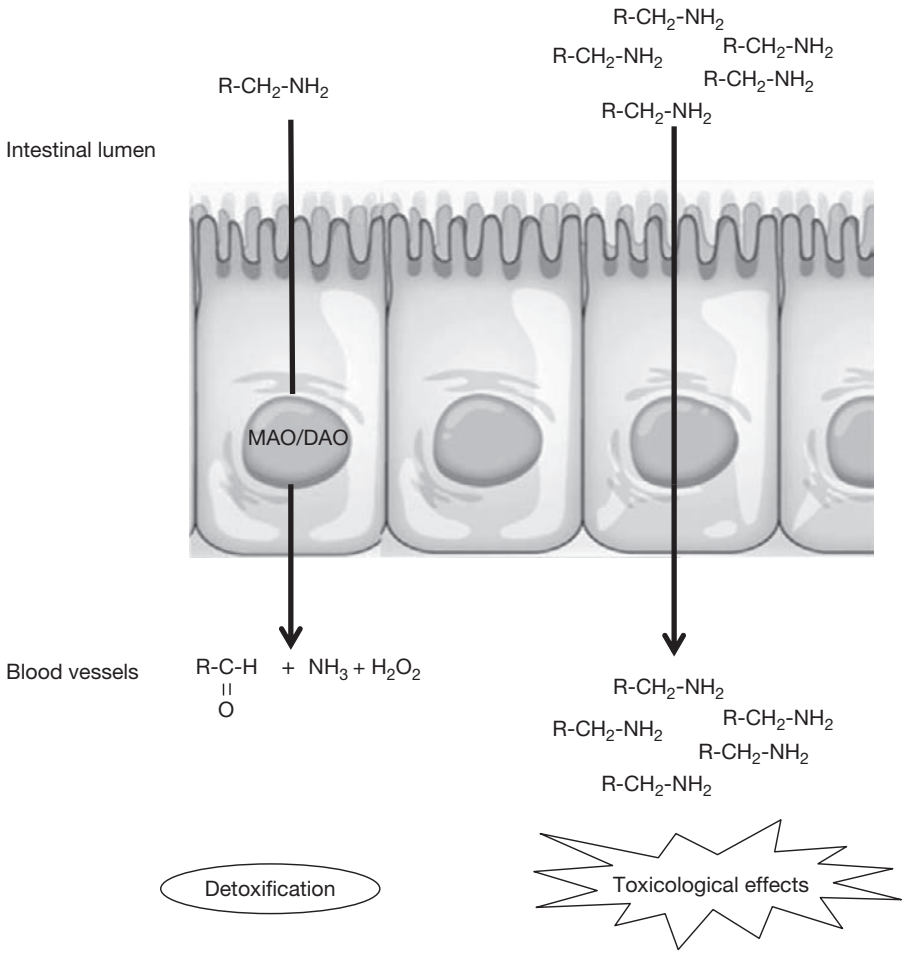


Figure 2 Scheme for the overall oxidative deamination reaction catalyzed by MAOs at the intestinal level. The intake of foods containing high BA amount inhibits detoxification system.

effect of the evolutionary pressure to maintain the physiological function of these enzymes among mammalian species. Tyramine is a substrate for either form of MAO; MAO-A is responsible for tyramine intestinal metabolism preventing its systemic absorption. Tyramine and phenylethylamine are also substrates for *N*-MT; their *N*-methylation generates noradrenaline. Tyramine can be further converted into octopamine.

The main enzyme for the metabolism of ingested histamine is DAO that converts histamine into imidazole acetic acid, which can be conjugated with ribose before excretion. Histamine *N*-methyltransferase (HMT), the other important enzyme inactivating histamine, is a cytosolic protein that can convert histamine only in the intracellular space of the cells. HMT converts histamine into methylhistamine, which is then converted by MAO into *N*-methylimidazoleacetic acid. The ultimate end products of histamine metabolism are excreted in the urine. An impaired histamine degradation based on reduced DAO activity and the resulting histamine excess may cause numerous symptoms mimicking an allergic reaction.

The activity of all of these detoxifying enzymes can be negatively influenced by food components, such as other amines, alcohol and its metabolite acetaldehyde, and phenols, acting as potentiators.

In particular, alcohol and acetaldehyde enhance BA toxicity increasing the permeability of the intestinal wall to these compounds.

Moreover, some drugs can act as inhibitors of MAO and DAO. Some of them are drugs used for the treatment of stress, depression, Alzheimer's disease, and Parkinson's disease and are painkillers, antihypertensive drugs, antibiotics, and agents reducing gut motility. A recent study reported that tobacco smoke reduces MAO levels by up to 40% and several cigarette smoke compounds can also inhibit MAO enzyme activities.

Conclusion

BAs play essential roles in the normal development, metabolism, and physiological functions of humans. However, they are very frequently involved in human pathologies causing neurological disorders, gastrointestinal diseases, abnormal immune responses, cancer, etc. Further studies are needed to evaluate the factors influencing BAs formation to understand how these compounds could affect consumers. In addition, there are large gaps in the establishment of dose–effect relationship. The role of various substances that enhance the

toxicity of BAs and the existence of synergic effects has been demonstrated, and therefore, the determination of the amine concentrations in each case is not enough to assess their toxic potential.

See also: Biogenic Amines; Cancer: Diet in Cancer Prevention; Fish: Dietary Importance and Health Effects; Food Allergies.

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Biosensors

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Introduction

Food safety has become an emerging issue since bovine spongiform encephalopathy and dioxins scandals that occurred in the late 1990s. In the European Community, public confidence in healthy food reached the minimum level, and policy makers spent many efforts to improve the healthiness of food products and to enhance the level of consumer's health protection. Different control strategies have been adopted by international, national, and regional authorities in order to reassess consumer confidence and trust in food, food industries, and government. Stringent food safety and quality analysis represent the keystone of the plan of action in order to protect public health. It is a global issue that requires the concerted efforts of all the actors of the entire supply chain, research organism, control authorities, and politicians.

Within the European Community, the hazard analysis of critical control point system has been officially effective since 1995, setting guidelines and musts concerning hygiene in food production and becoming a legal obligation on 1 January 2006. The food sector is imposed to assess and maintain a system for hazard analysis testing to identify critical points relevant to food safety regardless of the process stage, guaranteeing the healthiness of products at each point of the supply chain. The producers have the penal responsibility of food safety. Food plants are heavily constrained to maintain a rigorous monitoring system for food analysis to ensure compliancy with legislation and rules, as laid out by governments and regulatory authorities.

In this context, it is natural to accept the importance of detection systems that are accurate, sensitive, inexpensive, and preferably portable for on-site testing. Biosensors represent rapid screening tools and their use as detection platforms is expected to increase continually, while the conventional methods will be abandoned.

A biosensor is defined by the International Union of Pure and Applied Chemistry (IUPAC) as a "device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signal." It is composed of three main components: the biological recognition element, the transducer, and the signal readout system. Once the analyte interacts with the bioreceptor, the transducer translates the recognition event into a measurable signal that is then converted into an appropriate output.

The first biosensor was realized in 1960 by Dr Leland C Clark. He developed the first prototype glycemia sensor, an 'enzyme electrode' for the measurement of glucose levels in the blood. The device relies on a thin layer of glucose oxidase entrapped onto an electrode via a semipermeable membrane and monitors the oxygen consumed by the enzyme-catalyzed reaction:

$$\text{Glucose} + \text{oxygen} \rightarrow \text{gluconic acid} + \text{hydrogen peroxide}$$

The enzyme is able to convert electroinactive substrates into electroactive products: when monitoring the signal for oxygen, Dr. Leland Clark found that it would decrease in proportion to the concentration of glucose in the solution. Alternatively, H_2O_2 can be monitored as the result of the oxidation of glucose.

Biosensors are considered powerful tools for the analysis of biomolecular interactions in clinical, biochemical, and environmental analyses. Applications of biosensors are developed majorly for environmental and bioprocess monitoring, quality control of food, agriculture, antibioterrorism, and medical systems. Those innovative devices offer advantages over current analytic methods. They are selective, inexpensive, portable, and easy to use. For these reasons, they represent a valid alternative to conventional methods used in the food industry for hazardous food contaminants detection such as mycotoxins, heavy metals, pesticides, antibiotic residues, and pathogenic microorganisms.

Major advantages of biosensors over traditional techniques are reported in Table 1.

Nanotechnology is playing a crucial role in the development of biosensors. The combined use of micro- and nanofabrication techniques leads to the creation of materials and devices typically with dimensions smaller than 100 nm. The advantages of this smaller-scale approach are supported by the possibility to

- reduce unit costs through mass production,
- reduce sample volumes into the range of microliters or less,
- reduce reagent costs,
- reduce analysis time and sample manipulation exploiting microfluidics,
- reduce power consumption,
- realize portable devices,
- increase biosensors sensitivity.

Biorecognition Elements

Biosensors can be classified by their biorecognition elements or their transduction principles.

The bioreceptor is a molecular species that binds the analyte through a biochemical reaction. It is a fundamental part of the device because it influences the overall biosensor performance. It can belong to one of the five major categories: antibodies, enzymes, whole cells, nucleic acids, and phages.

Affinity-based recognition elements specifically bind to the target and are characterized by high sensitivity, selectivity, and versatility because they can be easily generated for a wide range of different targets. Antibodies are the most important affinity-based recognition elements. They are proteins produced by the immune system to bind specific antigens by noncovalent interactions. The antigen binding site has a particular fold that provides a sort of 'lock and key' fit for a specific antigen.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00072-6

Table 1 Comparison between traditional analytic techniques and biosensors

Traditional analytic techniques	*Biosensors*
Expensive	Cost effective
Time-consuming	Real-time detection
Well-trained personnel	Simple use
Heavy sample manipulation	Limited sample preparation
High-tech equipment	Simple and portable devices

These biological compounds are sensitive and selective but have some limitations in food and environmental analysis. Three types of antibodies are present: polyclonal, monoclonal, and recombinant antibodies. Polyclonal antibodies are produced by immunizing animals and extracting and purifying the sera. They are derived from multiple plasma cells so they can recognize target compounds from different molecular regions. Although polyclonal antibodies can be produced at relatively low cost, they can bind compounds nonspecifically leading to false-positive responses. Moreover, besides the ethical question for the use of animals, toxic molecules or molecules with very low molecular weight are not immunogenic so they cannot elicit immune responses in the host.

Monoclonal antibodies are derived from a single clonal hybridoma, so they have the same structure and recognize only one epitope of the target molecule leading to a higher specificity. However, the production process is very expensive and time-consuming, and like the polyclonal antibodies, they are not highly stable molecules and lose their binding properties under unfavorable environmental conditions. Recombinant antibodies are the product of genetic manipulation of antibody genes for high-affinity antibody production.

Innovative affinity-based recognition elements are represented by phages, nucleic acids, and molecularly imprinted polymers (MIPS). They have different characteristics that will be discussed later, but generally, they show good affinity and specificity and high stability and their production is cheap, fast, and reproducible, avoiding batch-to-batch variations. Since the involvement of animal immunologic systems is not necessary for their creation, it is possible to realize a proper recognition element for the detection of poorly immunogenic or toxic compounds too.

Enzymes are proteins that have an active site for the binding of substrate in their catalytic activity with features similar to the antigen binding sites of antibodies. They are not usually used as biorecognition agent but as a component of a multiple biorecognition system where the enzyme is included for the amplification of the signal. However, biorecognition elements have been reported in literature for the detection of certain organic phosphate pesticides.

Whole bacterial cells can be used in biosensor devices as recognition and processing systems for the analyte as using bacteria induces production of enzymes to detect the desired molecule.

Bacteriophages are viruses that infect bacteria and exploit microbial cell structure to duplicate their DNA and replicate themselves. These viruses display peptides or proteins on their surface that can strongly bind different targets such as proteins, small molecules, and whole cells. Phages can be selected on the basis of target affinity and used as a specific recognition element of a biosensor. In bacteria detection, the phage itself specifically recognizes its particular host strain by specific receptor molecules outside the bacterial cell, and the binding can be so specific that only certain strain could be revealed advantages in using phages are the following: Their production by infecting bacteria is cheap and fast, which takes only a few hours; they are very stable in harsh environmental conditions. For example, they are active in a wide range of pH values (from 3 to 11) and at high temperatures. Moreover, they show high organic solvent resistance, retaining their infectivity of 99% in acetonitrile, 80% in methanol, and 50% in ethanol. Besides phages, the surface peptides or proteins identified as good binders can be chemically synthesized or produced by recombinant expression in bacterial cells and directly used as recognition elements.

Nucleic acids are used to detect specific genes or specific DNA sequences exploiting natural affinity between single-stranded DNA used as the probe and its complementary sequence belonging to the sample. When a specific sequence of DNA is near the probe, the two strands bind and form the classical double-helix DNA structure and the hybridization process is revealed by the transducer. Double-stranded DNA can be used as the probe too, for detection of intercalating agents, which get inserted into the helical conformation of the double-stranded oligonucleotide. Nucleic acids recognition elements are very stable and easily synthesized and stored and can be chemically modified during synthesis, in order to enhance stability, affinity, and specificity and facilitate the functionalization of surfaces. Moreover, they can be easily labeled with fluorophores or enzymes allowing flexibility in assay development.

Aptamers are short strands of nucleic acids properly designed to specifically bind to a target molecule and are considered the next-generation element for molecular recognition. This technology exploits the capability of a single strand of DNA or RNA to fold into three-dimensional structures thanks to self-annealing properties and to recognize the analyte primarily by the shape of the binding site and not by their nucleotide sequences. Aptamers are derived from the Latin word *aptus*, which means to fit. They are isolated from oligonucleotides library using the *in vitro* selection known as SELEX (systematic evolution of ligands by exponential enrichment) technique. A library of oligonucleotides containing a portion of randomized sequence is incubated with the target. Only nucleic acids strongly linked to the target molecule are retained and amplified by polymerase chain reaction, while others are removed by washing steps. Actually, the technology is still in its infancy so various aptamers have been selected for small molecules, supramolecular structures, and whole cells as targets but they have not been tested with biosensing platforms yet. The small size of these nucleic acids ligands allows the formation of a high-density monolayer anchored to the biosensor surface, and thanks to their ability to renature, aptamers can undergo several cycles of denaturation and renaturation. In this way, an aptasensor can be recyclable by adding a chaotroping agent to break the target–aptamer complex, setting the receptor free for next sensing analysis.

The last class of affinity-based recognition elements are MIPs. MIPs are synthetic cross-linked materials with artificially generated recognition sites. Starting from a solution of polymerizable

Table 2 Comparison between different biorecognition elements

Biorecognition element	*Affinity*	*Versatility*	*Specificity*	*Stability*	*Cost*
Polyclonal antibody	+++	++	++	++	++
Monoclonal antibody	+++	++	+++	++	+
Enzymes	++	+	+	++	++
Whole cells	++	+	++	+++	+++
Nucleic acids	+++	++	+++	+++	++
MIPs	++	+++	+	+++	+++

monomers, cross-linkers, and target molecules, the binding sites are formed by polymerizing monomers arranged around the target and removing the template molecule by extensive washing steps. The resulting polymer holds recognition sites complementary to the target in size, shape, and position of the functional groups.

A summary of the characteristics of different recognition elements used in biosensor devices is reported in Table 2.

Immobilization Methods

For better performance of biosensors, biological and physical components must be kept in close proximity to each other. The analyte must have free access to the biocomponent and the integrity of the interaction must be retained. The immobilization step is crucial for biosensing procedure. The functionality of the receptor must be preserved, the recognition sites will be sterically free, and care must be taken to avoid chemical inactivation during the immobilization stages. There is not a universal immobilization method suitable for every application. In order to choose the correct immobilization method, some factors have to be taken into account such as the type of transducing element, the physical properties of the analyte, and the nature of biocomponent to be immobilized.

The biological probe can be anchored on carrier matrices by adsorption, by covalent binding, or by physical retention.

Adsorption of biomolecules on insoluble support relies on nonspecific interaction such as hydrophobic effects: It is a very simple method with wide applicability and it is capable of high biomolecule loading. Physical adsorption mainly relies on van der Waals forces and occasionally on hydrogen bonds so the linkage is weak.

Binding molecules covalently to the support is the most commonly used approach. The covalent binding is very strong, and there is no or very little chance of leaving of biorecognition element from the support.

Biorecognition elements can be bound to the surface through a bifunctional molecule that acts as cross-linker. The most commonly used are glutaraldehyde, cyanogen bromide, and ethyl chloroformate.

Concerning immobilization by physical retention, two different techniques are available: matrix entrapment and membrane enclosure. Entrapping biomolecules in gels or fibers can be useful in analysis involving small substrates and products. Semipermeability of membranes allows both biomolecules confinements and free passage for substrate and reaction products.

Transduction Mechanism

Based upon the transduction method, biosensors are typically classified into optical, mechanical, and electrochemical sensor platforms.

Optical Biosensors

Optical biosensors allow the detection of analytes in complex matrices with minimal sample manipulation; they require a low reagent volume and have a low signal-to-noise ratio. For these reasons, they are particularly appealing in food safety and food quality evaluation. In particular, different optical sensors for rapid detection of pathogenic bacteria, toxins, and contaminants in food have been recently proposed.

Transduction principle is mainly based on changes of surface characteristics of the device when the analyte binds to the sensing layer of the sensors by sorption or complex formation of the affinity binding agent and target molecule.

Target detection and quantification are determined by measuring the refractive index, absorbance, and fluorescence properties of analyte molecules or chemo-optical transducing medium. The working principle of optical biosensors involves measuring changes in the amplitude, phase, frequency, or polarization of light. These devices are composed of a light source, components to generate light with specific characteristics, a modulating agent, a sensing area, and a photodetector. The advantages of optical biosensors are the ease of use, speed of the assay, and the possibility to perform multiplex analysis: samples can be investigated with many wavelengths simultaneously without any interference. Recently, various optical sensors for rapid detection of pathogens, toxins, and contaminants in food have been developed. For example, *Escherichia coli* detection and quantification have been performed in only 15–20 min.

This class of biosensors comprises optical fibers, planar wave guides, resonant mirrors, ellipsometry, total internal reflection fluorescence, surface plasmon resonance (SPR), fluorescence and ultraviolet/visible (UV/vis) spectroscopy, and microarray. Among this group of devices, the most promising are optical fibers and SPR sensors.

Fiber-optic biosensors

The working principle of these devices is quite simple: a light beam goes into the sample through one fiber, interacts with the media, and is reflected and collected from a second fiber to be transferred to a photodetector.

Table 3 Examples of some applications of fiber-optic biosensors

Target analyte	*Matrix*	*LOD*
C. botulinum toxin A	Buffer	5 ng ml^{-1}
Staphylococcal enterotoxin B	Buffer	0.5 ng ml^{-1}
Escherichia coli O157:H7	Ground beef samples	1 CFU ml^{-1}
Listeria monocytogenes	Buffer	10^3 CFU ml^{-1}
Listeria monocytogenes	Hotdog samples	10^7 CFU ml^{-1}
Salmonella	Hotdog samples	10^4 CFU ml^{-1}

The optical fibers can be divided into intrinsic or extrinsic sensors. In the first case, the binding between the probe and analytes occurs within an element of the device, while in the extrinsic sensors type, the optical fiber is exploited in order to couple light to and from the region where the light beam is influenced by the target recognition. Usually, the antibodies are immobilized on the distal end of the fiber, and the incident light introduced at the proximal end travels through the fiber causing excitation of the fluorophores attached to either the antigen or an associated antibody depending on the assay configuration.

There is a rapid development of various fiber-optic biosensors for the detection of botulinum toxin, staphylococcal enterotoxin, *E. coli* O157:H7, *Listeria*, and *Salmonella* spp. Table 3 reports some literature examples of calculated limit of detection (LOD) and tested matrices.

SPR biosensors

SPR is a charge density oscillation that exists at the interface of any two materials with opposite dielectric constants, which can be induced by both electrons and photons.

Plasmons are the collective vibrations of an electron gas or plasma surrounding the atomic lattice sites of a metal. At a specific resonance wavelength of light, the momentums of the photon and the plasmon are matched and the energy of the photons can be transferred to free electrons at the interface. The excited surface plasmons can be considered as an electromagnetic surface wave that propagates along the interface and decays exponentially with distance normal to the interface. SPR determines a dip in reflectance at the specific wavelength, caused by the absorption of optical energy in the metal. This phenomenon facilitates the study of interactions at the metal surface as the evanescent wave propagates to a depth of approximately one wavelength. SPR instruments containing an antibody layer detect minute changes in the local refractive index on binding of the antigen. When target molecules bind to the antibodies immobilized on the metal surface, the resonance shifts to longer wavelengths and amount of shift accordingly reflects the concentration of bound analytes. The most widely used SPR platforms employ the Kretschmann configuration (Figure 1). They are based on prism coupling and angle-dependent detection and show a higher sensitivity and resolution with respect to the devices that operate by diffraction grids. In the case of total internal reflection, the light leaks an evanescent wave field across the interface from the glass to the sample solution characterized by a lower refractive index. In the metal layer, plasmons can be excited by the incident light only at the proper combination of energy (wavelength) and incident angle, and a characteristic absorption of energy via the

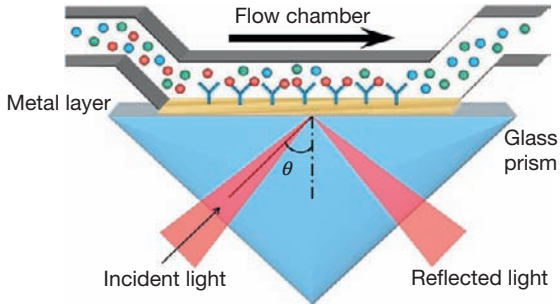

Figure 1 Schematic representation of SPR biosensor in Kretschmann configuration.

evanescent wave field occurs. The SPR is seen as a dip in the intensity of the reflected light at the SPR angle. The refractive index of the medium near the sensor surface affects the SPR angle and changes when biomolecules attach to the surface leading to a shift in the SPR angle.

Considering that antibodies are about 10 nm in diameter, the binding events perturb the evanescent field and hence the SPR, which therefore alters the angle of the light at which the SPR occurs proportionally to the amount of target binding. Since the light does not travel through the sample, it is possible to analyze also nontransparent samples such as serum or food extracts.

SPR biosensors can detect molecules even at femtomolar concentration and were used for detection of whole bacterial cells as *E. coli* O157:H7, *Salmonella*, and *Listeria* at traceable concentrations or *Staphylococcus aureus* enterotoxin B and botulinum toxins.

Quantum dots

Quantum dots are semiconductor nanoparticles that fluoresce when excited by a light source. They are characterized by unique optical properties thanks to their very small sub-wavelength size, which can be exploited for biosensing.

Quantum dots are considered as a promising alternative to traditional fluorescent dyes. Electronic energy levels are governed by the size of the nanoparticles; therefore, emission bands are tunable by changing the size of quantum dots. In contrast to the fluorescent dyes, the emission bands are very narrow due to the quantum electron confinement, so they can be used to detect multiple quantum dots labels without overlap of spectral emission, a feature that has facilitated multiplexed detection.

In contrast to organic dyes, quantum dots are very stable and are not susceptible to photobleaching. Moreover, these nanoparticles are very efficient fluorophores, making them bright labels. A single quantum dot provides signal equivalent to 20 rhodamine molecules, allowing more sensitive assays than those employing organic dyes.

Quantum dots can incorporate a zinc sulfide shell around the core, readily functionalized using amine chemistry methods, which allows water solubility and conjugation to antibodies and oligonucleotide probes. Nonspecific interactions are avoided by a polyethylene glycol modification of the ZnS shell.

Antibody-modified quantum dots have been used for the immunostaining of whole *Listeria monocytogenes* cells, to

visualize *E. coli* and to quantify marine iridoviruses. *E. coli* has been labeled and visualized in samples containing a mixture of bacteria using quantum dots functionalized with specific bacteriophages.

Surface-enhanced Raman spectroscopy

Raman spectroscopy is a vibrational analytic technique able to give detailed structural information on proteins, nucleic acids, and whole pathogens.

Nevertheless, this method shows a low sensitivity, and pathogens can be identified on the basis of their inherent spectra only at very high concentrations. In surface-enhanced Raman spectroscopy (SERS), the signal is enhanced by a factor of 10^4–10^9, simply using receptors to bind or adsorb pathogens to specially designed metallic surface with nanostructured roughness. Two formats are available for SERS-based biosensor devices: intrinsic configuration and extrinsic configuration. In the extrinsic format, a reporter molecule characterized by a simple but strong spectrum is used to generate a signal for detection. The molecule is bound to a metallic nanoparticle functionalized with a specific antibody for the target analyte, and this complex acts as a labeling reagent similar to a fluorophore. The narrow SERS bands are more suitable with respect to broad fluorescent bands and permit multiplexed detection and enhance the sensitivity. Extrinsic SERS detection has been successfully carried out for proteins, viruses, pathogen bacteria, and DNA sequences specific to HIV genes via hybridization assays.

In the intrinsic format, the signal is generated directly by the target molecule. Antibody or oligonucleotide probes on the sensing area anchor target molecule to the nanostructured surface, and the inherent Raman spectrum or molecular fingerprinting is measured. This format is particularly advantageous because it is reagentless and label-free and discriminates specific from nonspecific binding.

Thanks to optimized SERS substrates, it is possible to identify pathogen bacteria. These substrates allow the differentiation of bacterial or viral species, as well as pathogens having minor changes, such as gene deletions, and permit accurate classification of bacteria at the strain level.

Mechanical Biosensors

Generally, mechanical sensors are mass-sensitive sensors and can be considered as balances that react to small mass changes producing a measurable electrical signal. They are based on the piezoelectric effect: Certain solid materials accumulate electric charges in response to mechanical stresses. The phenomenon is reversible so the mechanical distortion can be generated by applying an electrical field to some faces of the crystal.

The main advantage of the piezoelectric transduction approach includes the ability to perform label-free measurements of the binding process, including real-time analysis of binding kinetics.

Quartz crystal microbalance

Quartz crystal microbalance (QCM)-based biosensors are an alternative to conventional analytic methods, due to the sensitive mass detection capabilities and the possibility to monitor binding events in real time. Quartz crystal sensors are coated by a thin layer of a metal serving as an electrode on both sides of the crystal. Applying an alternate current between the two electrodes, an acoustic wave propagates across the devices and the sensor oscillates at a specific base frequency. The resonance frequency depends on the crystal physical properties and those of the medium. The change in the resonance frequency is related to the mass accumulated on the crystal by the Sauerbrey equation:

$$\triangle f = -\frac{2f_0^2}{A\sqrt{\rho_q \mu_q}} \triangle m$$

where $\triangle f$is the change in resonance frequency, f_0 is the resonant frequency of the crystal, A is the active area of the sensors between the electrodes,ρ_q is the density of quartz, and μ_q is the shear modulus of the quartz.

The working principle is the decrease in resonance frequency after the binding of the target organism. The resonance frequency shift is proportional to the mass bound to the vibrating crystal. Bacterial species that have been detected with QCM include *Salmonella*, *Pseudomonas*, and *E. coli*. Concerning the detection of bacterial cells characterized by soft mass, QCM with dissipation monitoring is able to give insight into the viscoelastic properties of the adsorbed mass. The bacterial cells do not fully couple to the crystal oscillations and thus will dampen it and will be underestimated. The main drawback of this type of biosensor is a low sensitivity, because a relatively large mass must be bound to produce a measurable change in signal, so it is not capable of small molecule detection directly, but some sort of signal amplification is required.

An alternative to bulk wave sensing is represented by surface acoustic wave devices. The electrodes are located on the same side of the crystal and the wave transmits along the single side of the crystal, increasing the sensitivity.

Micro and nanomechanical cantilevers

Cantilevers are one-end clamped beams (Figure 2) and represent one of the major promising label-free biosensing platforms.

They can operate in static or dynamic mode. In static mode, the cantilevers are functionalized with a specific receptor only at

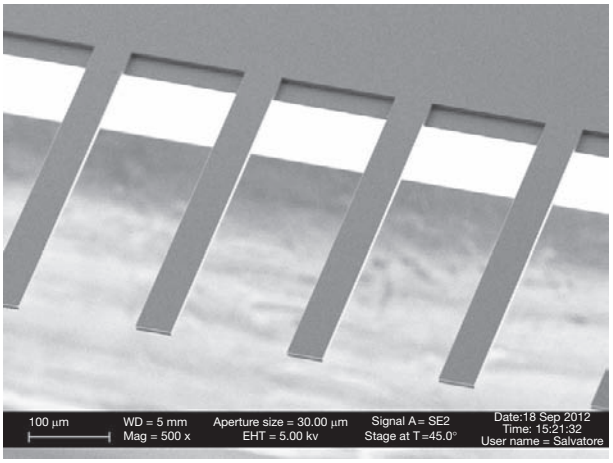

Figure 2 Scanning electron microscopy (SEM) image of a microcantilever array.

the free end of the beam, and when the target binds to the surface, a deflection of the cantilever free end occurs due to the surface stress variation. The static approach is subjected to important restriction as stabilization problems occur due to thermal drift, low sensitivity, and difficulty in comparing deflection with the amount of the added mass. For quantitative analysis, a dynamic approach is recommended. The working principle is rather simple and similar to QCM: cantilever resonates at a specific frequency that can be easily monitored. If target molecules bind to the cantilever-functionalized surface, the mass added to the sensor leads to a shift in the resonance frequency linearly proportional to the total mass bound to the probes. Using the following equation, it is possible to easily link the shift of the resonant frequency $\triangle f$ to the added mass $\triangle m$:

$$\triangle m = -2\frac{\triangle f}{f_0} m$$

where f_0 and m are the resonant frequency and the effective mass before the binding event, respectively. The mass resolution is typically in the nanogram range, if vibrational curves are monitored directly in liquid samples, while in vacuum conditions, the mass sensitivity can be increased, enabling the detection of masses at the zeptogram level. The technique can be successfully integrable as a diagnostic tool on a lab-on-chip platform, reducing assay time and sample manipulation, thus providing portable analytic devices. Simply varying the molecular recognition strategies, these biosensing platforms can be used to detect different targets such as microorganisms, biomolecules, and nucleic acids. In the literature, examples of detection of small immunogenic molecules such as toxins, pesticides, hormones, and antibiotics have been reported.

Electrochemical Biosensors

A chemical sensor is a device that transforms chemical information like concentration of a specific sample component or total composition into an analytic signal, thanks to a chemical reaction. Electrochemical biosensors are a subclass of chemical sensors and specifically react with the target producing electrical signal proportional to the concentration of the analyte. These kinds of molecular sensing devices intimately couple a biological recognition element to a solid electrode surface or electrode arrays, which respond to applied electrical impulses and convert biological recognition process into a useful electrical signal. The major advantage of electrochemical transduction is the possibility to operate in complex and turbid media and to construct inexpensive and miniaturizable devices.

Electrochemical sensors are classified into three groups: amperometric, potentiometric, and conductometric biosensors.

Amperometry is the most common transduction method used in biosensor development thanks to the high sensitivity, wide linear range, and quick response.

In amperometric configuration, a constant potential is applied between the reference and working electrode, measuring continuously the output current associated with the reduction or oxidation of an electroactive species involved in the recognition process: the current generated is linearly related to the target concentration. Amperometry is widely used in enzymatic essays in which hydrogen peroxide is produced in reaction process. Hydrogen peroxide is oxidized at a constant potential to form oxygen molecules, and the electrons result in a measurable current directly proportional to the H_2O_2 concentration. This kind of biosensor is able to detect *E. coli* in 30 min at a concentration of 100 cells ml^{-1}. One of the major drawbacks of these sensors is the interference on the presence of different electroactive compounds within the sample, which can interfere with the signal. In this case, the use of selective membranes can be useful to control the access of compounds to the electrodes.

In potentiometric devices, charge potential accumulation in the working electrode is monitored in comparison with the reference electrode, when no current flows between them; *E. coli* was detected in 1.5 h at 10 cells ml^{-1} concentration, monitoring pH variation caused by NH_3 produced by the bacteria. Potentiometric biosensors can be used in continuous, on-line, and in real-time monitoring, they are portable and cheap devices, but poorly selective.

Conductometric biosensors measure variation in conductance/resistance due to the charges produced during enzymatic conversion; impedimetric-based biosensing platforms detect variations in electrical properties arising from biorecognition events at the surface of modified electrodes. Microbial metabolic activity induces an increase of conductance, while impedance decreases: so these biosensors can be used for pathogen detection. Since 1992, impedance technique is accepted as screening technique for *Salmonella*, *Enterobacteriaceae*, *Listeria* spp., and coliforms that can be successfully detected even if the sensitivity is lower with respect to other sensors. *E. coli* can be quantified in a range of 10^4–10^7 CFU ml^{-1}. *Bacillus cereus* has been detected through antigen–antibody interaction in only 6 min with a sensitivity of 10^1–10^2 CFU ml^{-1}.

The miniaturization of electrodes to micrometric scale allows the packing of numerous transducers elements onto a small biochip device and hence the development of portable high-density arrays, satisfying current requirements for multianalysis. Generally, microelectrodes have great advantages over conventional ones such as low resistance, rapid attainment of the steady state, use of small solution volumes, and high signal-to-noise ratio.

One of the major drawbacks of electrochemical sensors is the inadequate sensitivity. Transport rate on the redox couple to the electrode surface, that is electron transfer kinetics, and diffusivity of electroactive species are very important parameters to consider in order to improve device performances. Moreover, the rate of electron transfer at charged transducer surface is influenced by solvation of redox probes and other ionic species in solution. Other obstacles in adapting electrochemistry to biosensors are the immobilization of the probes, which usually leads to a partial loss of antibody binding capacity, and regeneration, generally incomplete or impossible due to lability of antibodies. However, regeneration is necessary for sensor calibration.

Nanotechnology is a promising way for development of ultrasensitive electrochemical biosensors. Nanomaterials have favorable electronic properties, electrocatalytic activity, high surface area able to enhance the signal, and specific physicochemical bioassays, due to the nanometric size. They have been exploited to promote electrochemical reactions, to achieve direct wiring of enzymes to the electrode surface, and to amplify the signal of biorecognition events.

Impedance biosensors have been investigated for bacteria detection exploiting electrical properties of bacteria cells when they are attached to or associated with electrodes. Magnetic nanoparticles functionalized with antibodies are useful to separate cells from a mixture of bacteria or food matrices, concentrating a target of small volume, applying a magnetic field. In this way, the sensitivity is improved. Recently, the pathogenic bacteria *E. coli* O157:H7 have been successfully detected by impedance biosensors in ground beef samples in 35 min at a concentration of 8.0×10^5 CFU ml^{-1}.

In 2007, Sadik and collaborators described the integration of a fully automated electrochemical sensor with pattern recognition techniques, for the detection and classification of bacteria kingdom at subspecies and strain level. A high-throughput 96-electrode dissolved oxygen sensor measures the difference in the oxygen consumed by different bacteria classes and strains over time, and the oxygen reduction was monitored at a fixed potential.

Biosensors represent a challenging emerging field that has not by any means reached its full potential.

Unfortunately, most biosensors are still prototypes and their introduction in analytic laboratory as routinary commercial devices is not realistic yet. It is not even clear which biorecognition elements or transduction principle will be most productive, so the biosensing research field will continue to evolve in different directions. Generally, robustness of the biorecognition system is the major concern for many applications.

The future of biosensing applications is represented by development of array biosensors, biochips, and lab-on-a-chip for multiplex analysis. They will integrate all processing steps into a microanalytical system, allowing the assay of a large number of samples in few minutes and reducing the use of a high volume of reagents. Actually, microfluidic systems, sample preparation modules, sensitive detection modules, and robust assay methodologies still require further researching efforts prior to develop these kinds of biosensing platforms.

See also: Emerging Foodborne Enteric Bacterial Pathogens; Foodborne Pathogens; Food Poisoning: Classification; Food Poisoning: Epidemiology; Food Poisoning: Tracing Origins and Testing; Heavy Metal Toxicology; Immunoassays: Principles; Mycotoxins: Toxicology; Nucleic Acids.

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Biscuits, Cookies, and Crackers: Chemistry and Manufacture

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Introduction

Biscuit and biscuit-like products have been prepared and consumed by humans for hundreds of years. The term *biscuit* is derived from the Latin word *biscoctus*, which means twice cooked/baked. Its origins date back to Roman times, when certain foods needed to be completely dried so that they could be stored for long periods of time. The term *biscuit* is widely used for biscuits, cookies, and crackers alike in different parts of the world. In the United States, for instance, it is known as a 'chemically leavened bread type product.' It is also called 'scone' in New Zealand, 'biscuit' in the United Kingdom and 'cookie and cracker' in the United States. Different names are given to different products depending on their textural differences and extent of hardness. Second classification for biscuits, cookies, and crackers is usually done on the forming method used during their manufacturing and are therefore classified as fermented, developed, laminated, cut, molded, extruded, deposited, wire-cut, coextruded, and so on. The biscuits and biscuit-like products can be further classified by type and extent of value addition of products like chocolate chips, dried fruits, cream, jams, jellies, and others and also on the secondary processing applied during their manufacturing, including sandwiching, chocolate enrobing, cream filling, center filling, and others.

Dough Ingredients and Their Role

Wheat flour is the major ingredient used in the manufacture of biscuits. Flour is considered a toughener and provides texture, shape, and hardness to the biscuits and biscuit-like products. Apart from wheat flour, some nongluten flours (rice, maize, barley, millets) are also used to produce gluten-free biscuits (Table 1).

The principle proteins of wheat flour are gliadins and glutenins, which produce gluten on addition of water and in return provide structure to products. Soft wheat flour containing up to 9% protein is preferred for manufacturing most of the biscuits and biscuit-like products, whereas for production of crackers medium-strength flour with a protein content of 10.5% or more is preferred. Sweeteners are the second major ingredient after flour used during biscuit production, and they impart sweetness, texture, and color to the product and also increase shelf life. Usually during the manufacturing of biscuits a combination of sucrose and sugar syrup is used, as the former gives texture by forming amorphous glass during baking, and the latter is responsible for color production of the final baked product by enhancing the browning reactions.

Oils and fats are added during the manufacturing of biscuits, as they lubricate the structure of the product and also contribute to texture. Sources of fat and oil range from animal fat (butter, butter oil) to vegetable oil (palm oil, peanut oil, etc.) and are selected by regional preference. The function of fat during manufacturing of biscuits is to interrupt the formation of the gluten network by surrounding the flour particles and making the product softer or imparting soft and smooth texture. Other than that, fats are also used in secondary processing like preparation of cream for cream fillings, surface spraying, and coatings. Water has various functions to perform in biscuit manufacturing like gluten formation, dissolving minor and major ingredients, and controlling dough temperature.

Surfactants/emulsifiers increase the dispersibility and spread the fat more uniformly over other main ingredients. Mostly lecithin, mono- and diglycerides, diacetyltartaric acid esters of fatty acids, polysorbate 60, and others are used as emulsifiers. Surfactants modify the behaviors of liquids and do form a complex with the protein–starch structure, thereby strengthening the fat film and delaying dough setting during baking. Antioxidants, which retard the oxidative rancidity in fats and increase product shelf life, are also added during biscuit manufacturing, for example, butylated hydroxyanisole, butylated hydroxytoluene and tert-butyl hydroxytoluene. Food additives are added either with the purpose to increase shelf life, ease processing, or enhance sensory properties; different additives, such as leavening agents, colors, acids, flavorings, preservatives, and stabilizers are added. Salt is added as a flavor carrier to make gluten tougher and to slow down the fermentation rate. The common leavening agents utilized during the manufacturing of biscuits are sodium bicarbonate and ammonium bicarbonate, which produce gases/CO_2 and ammonia during dough baking and make them porous and light. Different artificial or natural flavoring compounds are used in biscuits to enhance flavor, taste, and smell. Usually spices, herbs, essential oils, and synthetic flavors are used. Biscuit color is an important indicator of quality, as it makes the product more attractive. Plant pigments, caramel color, or artificial coloring agents are the most commonly used coloring agents. Other minor ingredients like milk and milk products or eggs contribute toward nutrition, color, flavor, and texture of the final product (Table 2).

Manufacturing of Biscuits, Cookies, and Crackers

This section describes the methods, processes, and equipment involved in the manufacturing of biscuits, cookies, and crackers. Differences between these products can be made on formulation, usage level of fat and sugar, method of forming of the dough, and the textural properties of the naked products. The manufacturing process generally involves mixing, shaping, and baking steps, which are common for all the products and are discussed as follows:

Table 1 Functions of ingredients in biscuit, cookie, and cracker manufacturing

Ingredients	*Role*
Flour	Forms a viscoelastic network Holds all the ingredients uniformly in the dough Provides texture, hardness, and shape
Sweeteners	Provide sweetness, color, and flavor Provide texture
Shortenings	Interrupt the gluten network of flour Shorten the dough (reduce hardness) Provide texture and improve the eating quality of biscuit
Water	Form gluten in dough Dissolves ingredients and controls dough temperature
Emulsifiers	Maintain uniform dispersion of immiscible substances Spread the fat more uniformly into dough
Yeast and enzymes	Responsible for fermentation (crackers) Modify the gluten proteins and partially break down pentosans in flour
Antioxidants	Retard the oxidative rancidity in fats Increase storage life of fats and increase biscuit shelf life
Leavening agents	Produce gas Increase volume and improve texture
Salt	Toughens gluten Enhances flavor Slows down the rate of fermentation
Milk products	Add nutrients, flavor, and texture Also responsible for Maillard reaction
Egg	Provides nutritional value, structure, and color
Flavor	Enhances flavor, taste, and smell
Colors	Provide better look Make the product attractive

Premixing

Weighing of ingredients is done according to formulation and considering the batch size before the mixing operation starts. Weighing of ingredients is mostly manually done when the batch size is small, but for continuous operations automatic weighing systems are used. Ingredients like flour and sugar are conveyed pneumatically, while liquid shortening and water can be pumped into the mixer. Minor ingredients are usually mixed with water and then added into the overhead mixer hopper. Ingredients like skim milk powder have a tendency to form lumps, so a proper sequence of adding minor ingredients must be followed.

Mixing

The primary purpose of mixing is to bring about a complete and uniform dispersion of ingredients to form a homogeneous dough within a decided time period. In the case of biscuits, cookies, and crackers, mixing mainly involves blending, dispersing, dissolving a solid ingredient into a liquid medium like shortening or water, kneading, developing of dough, and discharging of dough into trolleys or on conveyor belts for further processing. The mixing time usually ranges from 15 to 25 min and depends on factors like flour characteristics, formulation, and temperature of the dough during mixing. Biscuits and cookies generally differ from other baked products such as bread due to a lower moisture content in the final product, and therefore rheological characteristics of dough are different depending on the formulation. Mixers typically have programmable variable-speed drives, which give bakers the opportunity to gently fold in large inclusions without breaking them apart. The two principal type of mixers used for mixing the dough for biscuits, cookies, and crackers can be categorized into batch and continuous mixers, and the latter ones can be further classified into various types.

Batch mixers

The batch mixers are comprised of high-speed horizontal mixers and vertical mixers with detachable bowl.

High-speed horizontal mixers

High-speed multipurpose batch mixers can handle every type of dough for biscuit, cookie, and cracker manufacturing. They offer high levels of automation, ease of use and cleaning, and outstanding reliability to guarantee low-cost maintenance. Unique blade design ensures good dispersion and rapid dough development. They usually consist of a horizontally mounted bowl that encloses the drive motor and are double jacketed, which helps to control the dough temperature during mixing. Usually cold/hot water is circulated in the jacket as a coolant/heating medium. The bowl of the mixer is fixed and has a door at the bottom, which opens and allows the discharge of the dough. The rate of discharge can be increased by rotating the beaters, which can rotate on a horizontal axis.

The beaters are driven horizontally within the bowl and may be fixed to one or two shafts. The action whereby the dough is cut and sheared depends on the exact shape and speed of the blades, but in many mixers the stator is fixed in the bowl, which provides an additional means for cutting the dough. The production rate of mixers is closely tied to the ingredient feed arrangements, but it can range from 500 to 1200 kg h^{-1} for hard dough and up to 650–1300 kg h^{-1} for short dough. The blades of high-speed horizontal mixers are of several types, including high-speed angled wing, parallel bar, and sprag, depending on shapes, action, and product. The frame is fitted over a base plate to retain the load of the mixing chamber. The Z and sigma type are the most commonly used mixing blades: they are fitted inside the mixing chamber and can rotate at different speeds to facilitate mixing of different dough types. To unload the dough from the mixer, a programmable logic controller (PLC)/electrically operated tilting mechanism is also provided.

The advantages of high-speed horizontal mixers are that they are fitted with a high-speed motor with variable speed options to enhance mixing control. The shaftless blades allow the rapid dispersion of ingredients and efficient blending compared to vertical mixers. The PLC and supervisory control and data acquisition (SCADA) control allow easy operations. It can tilt up to 150°, allowing complete discharge of the dough, which can be transported via conveyor/trolleys into the hopper of depositor/rotary molder or sheeting line. To facilitate easy cleaning, they have a 90° reverse tilt option. For loading the dry ingredients, it has pneumatic slide valves fitted overhead and can be operated at 15–80 rpm. It provides more flexibility to the production line.

Table 2 Formulations of biscuits

	Short dough formulations		*Hard dough formulations*					
			Cream cracker		*Soda cracker*			
Ingredients	*Milk biscuit (%)*	*Butter cookie (%)*	*Sponge (%)*	*Dough (%)*	*Sponge (%)*	*Dough (%)*	*Deposited butter cookie (%)*	*Gluten free cookies (%)*
Flour (soft)	100	100		66.7 + Sponge of cream cracker		36.9 + Sponge of soda cracker	100	
Flour (hard)			33.3		63.1			
Corn flour								97.08
Pregelatinized starch								2.92
Granulated sugar		16.67					35	36.96
Powdered sugar	32.38	15.15						
Cane syrup 80%								1.95
Invert syrup 70%	2.86						4	
Malt extract 80%				1.34		0.95		
Dough fat	17.86			28				11.67
Butter		49.70			12.6		60	11.67
Fresh egg		3.79					3.00	5.84
Lecithin	0.10				0.24			
SMP	0.24						5	9.73
Lactalbumin								1.52
Caseinate								12.65
Fresh yeast			0.5		0.63			
ABC	0.17	0.45						2.04
Soda	0.76	0.17		0.67		0.49	0.2	
ACP	0.10							0.51
Salt	0.29	0.76	0.02	1.68		1.89	1	
Liquid flavor	0.10						0.1	0.1
Protease enzyme						0.005		
Recycle biscuit	7.17						10	
Water	23	0	13	40	23.7	5	7	18

Disadvantages of using a horizontal mixer are that most of the ingredients are fed manually except flour, sugar, and oil through the feeding chute, which is located on top of the mixer, and on several occasions unhygienic conditions can prevail due to spillage of ingredients outside the chute. Due to fully enclosed systems, the mixing process cannot be manually observed. Only a single operation can be performed at an instance like charging, mixing, or discharging. Due to high speed, huge weight vibrations are caused during mixing of dough. The discharge of the dough is not always rapid, and it also prevents free movement of dough, as the blades are centrally located to the shafts.

Vertical mixers

Vertical mixers were used extensively before the introduction of horizontal mixers. These types of mixers are used extensively by small bakeries and also during production of crackers. Vertical mixers are also known as spiral or detachable bowl mixers and usually have a wheeled manger or tub with round ends and flat bottom. Beaters are connected with an overhead frame and mounted vertically with horizontal shafts and arms. It may contain one to three beaters and can operate at fixed positions. Bowl and beater position is adjustable and can move in either planetary or stationary direction, which allows gentle rolling and cutting actions. Capacity of mixers can vary from two to three dough batches per hour, and beaters usually rotate at a speed of 20–25 rpm.

The advantage of vertical mixers is they are suitable for almost all types of dough and are available in different capacities. The ingredients can be loaded manually in the tub from the top of the mixer. The dough requiring fermentation/resting time can be manufactured with this mixer. Different types of dough and batters can be mixed by changing beaters/blades assemblies. The mixing process can be visually monitored, and further mixing of any ingredient can be performed. The sequence of ingredient adding can be controlled, and over- or

undermixing of the dough/batter can be avoided. Cleaning is much easier as compared to horizontal mixers, as the bowl and the blades assembly can be detached from the main frame for cleaning.

Vertical mixers usually operate at a very low speed and therefore require more mixing time as compared to horizontal mixers. In vertical mixers the dough is subjected to different mixing action at the top and bottom of the tub, which results in the formation of an uneven dough. The tub and dough handling equipment are generally very heavy and require special equipment like hydraulic-operated trolleys to move them, which is quite laborious. The jacket and temperature sensors are disconnected and reconnected every time while mixing and moving the bowl of the mixer for loading/discharge of dough (Figure 1).

Continuous mixers

Continuous mixers offer a uniform and consistent dough stream for the production lines, which guarantees a consistent product throughout production. These mixers run on continuous feeding systems and can be operated with a precreaming, dosing section in which ingredients can be fed at start or at successive intervals along the length of the screw/rotor of the mixer. Different arms and stators can be attached in the continuous mixer along the length of the barrel, and the mixing action can be altered depending on the consistency of the dough. The orifice at the end of the rotor/screw provides the desired shape to dough pieces. Dough temperature is controlled by water jackets, and mixing time can be varied by adjusting the barrel length. Continuous mixers can provide complete automation as they have a compact system.

Continuous mixers can operate with minimum manpower, lower supervision, and minimum wastage, and uniformity in dough consistency is required for running the equipment. Continuous mixers are favorable for manufacturing products that require no standing time for the dough. Compared to horizontal/vertical mixers, continuous mixers are only suited for manufacturing a single product, as the changeover is not easy, being difficult to optimize the procedure. The cost and complexity is higher in case of any problem as starting and stopping a continuous mixer is much more difficult.

Figure 1 High-speed horizontal mixer.

The Forming Process

In the manufacture of biscuits, cookies, and crackers, the mixing and baking facilities may be used in common, but the forming process is specific for each type of product. Before starting the forming process the dough may be rested for some time to allow fermentation or gluten development in products like crackers and hard dough biscuits. The standing time and the dough temperature are critical in controlling the final quality of the product. The dough is fed into the hopper usually by a trolley tipping device or manually from the trolley. The forming process is specific for every product and can be generally classified into three types: (1) sheeting, laminating, gauging, and cutting; (2) rotary molding; and (3) extrusion of dough through dies. Dough with a fully developed gluten network is generally sheeted and strong, inextensible dough is laminated. Rotary molding is applicable to dough with less-developed gluten, and the soft dough is generally extruded.

Sheeting, Gauging, and Cutting

Sheeting and cutting is the most preferred method for the manufacturing of products like cracker and cookie dough pieces. In this method the fully developed dough is fed by an automatic dough feeder in different configurations onto the hopper of a three- or four-roll sheeter. The rolls are configured to create a compression chamber, which makes a homogeneous dough. This unit allows constant dough feeding and scrap integration to form a consolidated dough sheet of even volume prior to the first gauge roll section of the downstream forming equipment. These sheets are then converted into a uniform sheet of desired thickness (4–5 mm). Three-roll arrangements are generally used for the sheeting process, but on many occasions two-/four-roll arrangements are also deployed. The three rolls are arranged in the form of an inverted triangle below the hopper. In the three-roll arrangement, the two top rollers are known as forcing rolls, and one of the top rollers along with the third roll forms a gauge and hence is called gauge roll. One of the forcing rolls has a rough/grooved surface so that it can facilitate the drawing of the dough from the hopper into the sheeter, and the rest of the two rolls are smoothed. The thickness of the outcoming dough sheet is adjusted by varying the gap between the forcing and the gauging rolls. After the sheet is prepared, it is discharged onto the conveyor belt, and it can be either a front or a back discharge (Figures 2 and 3).

The series of heavy steel rolls called rollers is used to reduce the thickness of the dough sheet to a desired thickness and smoothness. The diameter of the gauge rolls varies from 150 to 400 mm; they have a smooth surface and are mounted vertically one above the other. The last gauge roll acts as a calibration unit, and it has larger diameter rolls. They can be completed by a height scan unit and skinning air ventilators, whereas all conveyors are provided with automated belt tensioning and tracking systems. For most of the products, two to

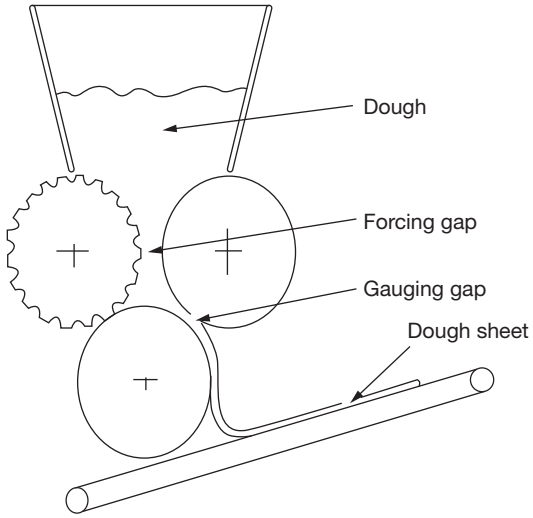

Figure 2 Front discharge sheeter. Sheeting process. See text for explanation. Reproduced from Duncan, M. (eds.) (2011). *Sheeting, gauging and cutting in biscuit manufacture*, *Manley's Technology of Biscuits, Crackers and cookies* (4th ed.). Cambridge: Woodhead Publishing Limited.

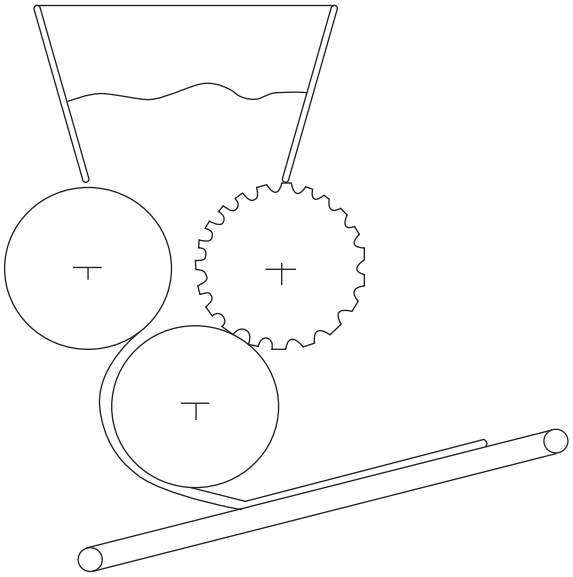

Figure 3 Back discharge sheeter. Sheeting process. See text for explanation. Reproduced from Duncan, M. (eds.) (2011). *Sheeting, gauging and cutting in biscuit manufacture*, *Manley's Technology of Biscuits, Crackers and cookies* (4th ed.). Cambridge: Woodhead Publishing Limited.

three pairs of rollers are used, and the gap between the two rollers can be adjusted up to a clearance of 0.1 mm by moving the rollers in an upward or downward direction. Care has to be taken while adjusting the gap between the consecutive pair of rollers, and the thickness of the sheet must not be reduced more than 50% at any rolling operation. As the rollers are smooth, the chances of dough sticking to the roller are increased, so to avoid this, a scraper is fitted that helps to scrap the sheet and transfer the same onto the conveyors.

The laminator is used in the production of laminated products such as crackers, hard-sweet biscuits, and baked snacks that have a unique property of a light and crisp texture. Laminators are available in two forms, that is, vertical and horizontal, the former of which is often used by cracker manufacturers. Laminating is done for several reasons: (1) it helps to repair the sheet that was formed using a simple pair of rolls; (2) uniform stress distribution can be achieved by turning the folded dough through 90°; (3) consecutive and repetitive cycles of rolling and folding cause more working of dough and develop a delicate structure in baked products; and (4) flaky structure can be obtained in products by spreading fat between two layers. Before the use of automatic laminators the process was performed by hand. Cut-sheet laminators are also used, which are servo motor driven and can operate at high production rates, where the dough sheets are precisely cut into sheets and layered before passing on to the forming and cutting equipment. The number of layers formed during the laminating process affects the final quality of the product, and therefore the takeaway conveyor and the relative rate of the laminator must be controlled for obtaining an equal number of layers.

After the dough sheet is sheeted out from the final gauge roller, a relaxing web is placed between the final gauge roll and the cutting assembly. It is desirable to relax the dough more often in puff or other laminated types to facilitate shrinkage. Flutes are formed on the relaxing web as the speed of the relaxing web is less as compared to the conveyor after the final gauge roll. After the relaxing web, the sheet is again straightened by increasing the speed of the conveyor carrying the sheet to the cutter. The cutting operation can be carried out either to only cut the sheet with a die or to create different designs on the dough piece. Reciprocating and rotary cutters are the two types of cutting processes used by the biscuit and cracker manufacturers. Reciprocating cutters consist of heavy block cutters that stamp out one or more pieces at a time. For maintaining a perfect size and shape, it is necessary that the dough sheet travels at constant speed under the cutter, which drops over the dough sheet, moves along with the dough, and comes to the original position before dropping again. The speed of reciprocating cutters usually ranges from 100 to 200 cuts per minute. Rotary cutters consist of a rotary metal cylinder and may be available either as single or double rotary cutters. A pair of engraved rolls embosses and then cut the dough pieces from a continuous sheet. The rotary cutting rolls are generally made up of bronze or gunmetal and are fitted with molded plastic cups that are engraved. The number of cups on each roll depends on the diameter and length of the roll. After the cutting, the remaining dough, called scrap, can be reused either by mixing it with a fresh batch of dough in the sheeter with the help of the 'scrap return,' or it is added in the horizontal mixer while kneading a new batch.

Rotary Molding

Rotary molding is of great use for handling short dough type biscuits: a single machine produces dough pieces, whereas a huge line is involved in sheeting and cutting. Rotary molding is used to manufacture short dough products like biscuits and sandwich cookies because it is possible to control biscuit

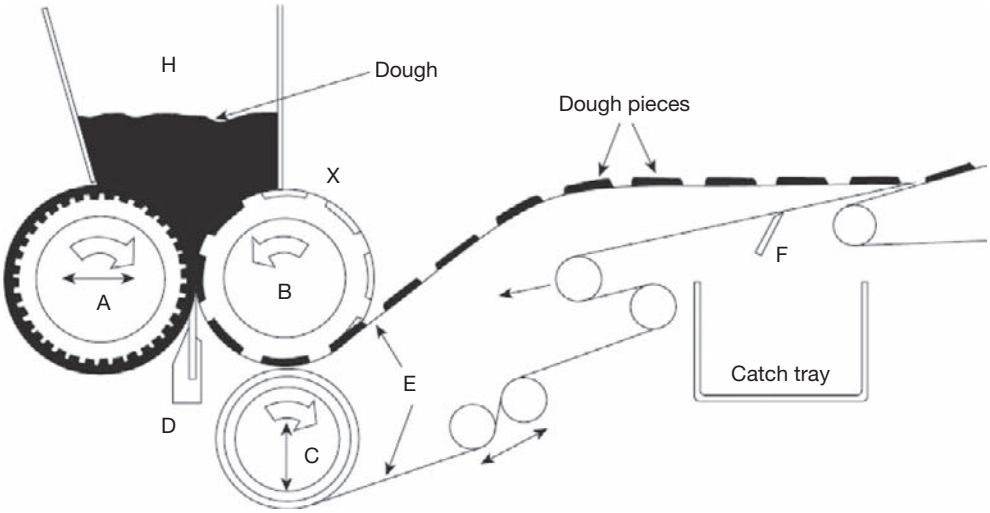

Figure 4 Rotary molding process. See text for explanation. Reproduced from Duncan, M. (eds.) (2011) *Laminating in biscuit manufacture*, *Manley's Technology of Biscuits*, *Crackers and Cookies* (4th ed.). Cambridge: Woodhead Publishing Limited.

weight and texture. The process involved in rotary molding is illustrated in Figure 4.

The general arrangement of a rotary molder consists of a set of three rolls arranged in triangular fashion and placed below the dough hopper. The forcing roller (a) made of steel, holds onto a blanket of dough as it has grooves of various patterns and then forces the dough into the engraved roller. The mounting roller (b) is engraved over a smooth surface, which forms the dough pieces, and the molds are made of plastic or bronze. The molds are engraved with the name and the design pattern, which creates impressions on the dough piece when it is extracted with the help of the extraction web and the extraction roll (c). Excess dough from the forcing roller is scrapped away by a scrapper (d), which is made of steel, and the tip is located below the center of the forcing and the mounting rollers. The extraction roller is made up of steel with rubber coating, and an extraction web (e) passes around it, which is pressed against the forcing roller to extract the dough pieces. The extraction web is usually made from mainly polyurethane or cotton and is cleaned for excess dough/broken dough pieces by a scraper knife (f), and the scrap is collected in the catchment tray. The dough pieces are than passed on to a web, which then transfers the dough pieces onto the baking belt.

Extrusion

Extrusion is one of the simplest ways of making dough pieces and is done by forcing soft short dough through orifices by means of a pump or rollers. Batter like dough is easy to extrude rather than mold or sheet. Extrusion is of great advantage while handling sticky dough and dough containing coarse particles such as nuts, flakes, or chocolate chips. The extrusion process involves wirecut machines and rout presses, which may be further subdivided into angled overhead wirecut and dualtex rout press. Operating speed of wirecut can be as high as 300 rows per minute with a minimum wastage of dough (1–2%) and up to 3800 kg for dualtex root presses.

Dough extrusion machines consist of a hopper placed above a set of two rolls, which force the dough into a balancing/pressure chamber underneath. The rolls can run continuously or intermittently, with a reverse motion for a short period, which causes a suck back action at the nozzle or dies located at the base of the pressure chamber. The extruder is generally positioned directly over the oven band or on a moving conveyor with continuous feeding of trays. Rout types and wirecut extruder are positioned over a normal canvas conveyor. The dies of the extruding machine are placed approximately 70 mm above the takeaway conveyor, or oven band, and its positioning can be adjusted to meet specific product requirements. The orifice size of the dies determines the size of the dough piece, whereas the extrusion rate can be adjusted by the speed of the forcing rolls. The extrusion rate is affected by dough consistency, head of the dough inside the hopper (it is advocated to maintain the dough within a fairly close limits and it is usually found that low levels of dough tend to give less weight variation than high), and pressure in the chamber.

Baking

Baking is the most important manufacturing process, and the final product quality and shelf life rely on the effectiveness of the oven to bake the product. During baking, dough pieces experience changes in density, the structure becomes porous, moisture is reduced, and the surface becomes colored. A traveling or band oven is extensively used for industrial baking processes, whereas small bakeries rely on simple ovens or static ovens, which usually have a heated box with a door and different trays and can be heated by means of electricity, gas, or wood.

The traveling or band oven is a tunnel that is enclosed, is insulated, and bears different sections/zones. Oven length ranges from 30 to 150 m, with an average length of about 60 m and a band width of 1–2 m. The oven consists usually of 3–7 zones with different temperature and air profiles, which

are controlled separately as for the baking profile of the product. In a continuous oven, temperature and heat transfer conditions can be controlled throughout the oven during the baking process. Industrial ovens usually run on fuels such as petroleum gas, oil, or electricity, which heat the atmosphere around the product either directly or indirectly via heat exchangers. The band or trays serve as the baking surface inside the oven. The selection of the oven band is product specific as it affects the quality of the finished product by altering the heat transfer at the bottom of the product itself. The band can be a continuous sheet of steel, which may or may not be perforated and is also available in the form of a wired mesh type. A terminal drum is provided at each end of the oven, and the drive drum, which drives the continuous band and also returns it to the panning end, is located at the oven exit. The feed end is equipped to adjust the tension of the band to hold it tight and prevent any damage. The oven band is supported by metal or graphite skids to prevent sagging. The speed of the band usually decides the baking time of the product, for example, short dough biscuits as compared to hard dough biscuits require a shorter baking period.

Ovens for industrial baking purpose are classified into direct fired, indirect fired, and hybrids. The fuel combustion provides heat to the oven, and usually oil and gas-fired ovens are used. The most common type of oven is the direct gas-fired (DGF) oven, as it offers great flexibility for baking products with different characteristics. In DGF ovens process conditions can be varied for different products including temperature profile, turbulence, and humidity conditions. Ribbon burners burn the fuel and transfer the heat on the band from above and below by an air circulation (turbulence) system. DGF ovens can handle a wide range of products such as hard crackers to very soft cookies. Other direct-fired ovens include the forced convection direct fired and the convector-radiant type. The indirect-fired oven is also known as the convective oven in which hot gases are passed evenly above and below the band and across the full width of the oven. Indirect-fired ovens are provided with separate burners for each zone and usually the fuel is burned outside the baking chamber. Indirect-fired ovens are further classified into indirect-fired forced convection and indirectly fired cyclotherm. In the latter type, parallel tubes run above and below the oven throughout the length of oven in which hot air is supplied through fans to heat the product and circulates back the outgoing cold air to burners. The indirect-fired forced convection oven is similar to the forced convection direct-fired oven, but the difference is that zone burners heats the air through heat exchangers that pass through the plenum chamber. Hybrid ovens are a combination of DGF, direct, and indirect ovens and have varying heating, thermal efficiency, heat transfer, and airflow characteristics that affect product quality. Hybrid ovens respond to specific needs for any kind of biscuit, cookie, and cracker. Most hybrid ovens feature a DGF section for the first part of the baking when the air movement is often undesirable as it dries the outer layers and prevents proper flow and lift. During the drying and coloring processes in the later stages of baking, the air movement is beneficial so a convection section is used.

The baking profile is different for almost all types of biscuits, cookies, and crackers and depends on the formulation and the desired textural product characteristics. Baking time varies from 3 to 12 min and depends on the product. The shortest baking time is usually for crackers and the highest one for cookies. Temperature also varies from 140 to 240 °C, varies from zone to zone, and is product specific.

Cooling

Freshly baked products leaving the oven must be cooled before packaging or secondary processing. The product leaving the oven has a temperature of about 100 °C. Cooling is important because warm biscuits or cookies might not able to withstand the packaging process if too soft, and also the packaging material may shrink and the product quality may deteriorate due to condensation of water vapor inside the packed product. In the industrial production, the cooling process starts at the oven exit: the biscuits are transferred from the oven band to an open conveyor using a doctor's knife and then transferred on the cooling web, which is made of cotton canvas, and carried through the cooling conveyor to cool naturally in the ambient air. The cooling conveyor is either a single- or a two-tier arrangement, depending on the factory layout. A two-tier system is often used when it is desirable or necessary to turn biscuits over during cooling. This avoids uneven moisture distribution, which leads to biscuit cracking. The turning of biscuits also helps in quality control by identifying the faults on the bottom of the biscuits/cookies like burned particles. The length of the cooling conveyor varies from 1.5 to 2 times the length of the oven. Usually the product is cooled up to 40–45 °C, and those who require secondary processing like cream filling are cooled to 18–26 °C. To achieve greater control over the biscuit temperature, forced air cooling is also employed at the end of the cooling process.

Secondary Processing

Typical secondary processing involves the deposition of cream, jam, or marshmallow on the biscuit or the enrobing with chocolate or coating with icing, which gives the product a different appearance, texture, and taste. Cream sandwiching is a process in which two or three biscuits are sandwiched with cream filling between them. The filling generally contains sweet cream with 30–40% fat and 60–70% sugar with added color and flavor. Sandwich machines usually contain 2–6 lanes and can produce 400–800 sandwich biscuits/lane/minute. Apart from sandwiching, icing, jellying, marshmallowing and chocolate coating can also be done.

Packaging

Biscuits require immediate protection as they are highly hygroscopic in nature and tend to gain moisture from the atmosphere, which leads to spoilage. The overall biscuit packaging involves primary, secondary, and tertiary packaging that have different functions and requirements. The primary package is generally in the form of flow wraps, slugs, sachets, displays, tubes, and shrink wrappings, which are usually laminates made up of polypropylene, plastic-coated papers, and

metal boxes. The secondary packaging is usually carried out for packing of wholesale/bulk packets. The tertiary packaging generally utilizes corrugated boxes.

See also: Barley; Biscuits, Cookies and Crackers: Nature of the Products; Bread: Chemistry of Baking; Bread: Dough Mixing and Testing Operations; Browning: Non-enzymatic browning; Cereals: Types and Composition; Colors: Properties and Determination of Natural Pigments; Extrusion Cooking: Principles and Practice; Fats: Classification and Analysis; Fatty Acids: *Trans* Fatty Acids; Food Additives: Classification, Uses and Regulation; Fructose and High-Fructose Corn Syrup; Gums: Properties and Uses; Milk Powder; Vegetable Oils: Types and Properties; Whey and Whey Powders: Production and Uses.

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Biscuits, Cookies and Crackers: Nature of the Products

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Introduction

There are hundreds of varieties of biscuits, cookies, and crackers found across the globe. Diverse terminology in different parts of the world causes some confusion regarding the distinction between biscuits and cookies. In North America, the term biscuit refers to baking powder biscuits or buttermilk biscuits, which are savory quick breads made with flour, shortening, milk, salt, baking powder, and occasionally baking soda. High levels of shortening in the formula produce a tender and flaky texture. They are similar to British scones but are prepared with a leaner formula, which does not contain egg and sugar. In the United Kingdom and most English-speaking countries outside of North America, the term biscuit refers to small, chemically leavened, cake-like products, which have high sugar, high shortening, and low moisture contents. These products are called cookies in the United States and Canada. In this text, biscuits are the type found in the United Kingdom that is synonymous with cookies.

Biscuits and Cookies

In general, biscuits and cookies are small, flat, cereal-based, baked products containing shortening, sugar, and chemical leavening. While soft wheat is the most common, other cereal grains such as oats, rye, corn, and barley are sometimes utilized. Most biscuits and cookies have a low moisture content of less than 5%. They vary widely in size, shape, formulation, preparation method, and flavor. The texture varies from crisp and hard to soft and chewy. Some undergo secondary processing to create sandwiched, iced, coated, filled, and multiple other types of final products. Biscuits and cookies have a relatively low risk of microbial spoilage due to the high shortening, high sugar, and low water contents. They also do not stale like bread and other higher-moisture baked products. The most common cause of loss of eating quality is due to moisture migration. Moisture uptake by crisp, hard products causes them to become undesirably soft and soggy, while moisture loss from soft, chewy products renders them dry and hard.

Biscuits and cookies are often broadly characterized based on their dough properties and then further distinguished by the technique used to shape and place the dough onto the oven band for baking (Figure 1). The two broad categories of biscuit and cookie doughs are hard doughs or short doughs. Wafers are a third category. Although wafers are not technically biscuits or cookies, they are often included in the category because they are manufactured by biscuit and cookie manufacturers.

Hard Dough

Hard doughs contain higher water, lower shortening, and lower sugar than short doughs. The most common mixing method is a single-stage mix in which all of the ingredients are mixed together in one step. The gluten is developed during mixing in hard doughs. The formulation and developed gluten network result in a tight, stiff dough that can be sheeted and then cut or stamped into shapes.

One problem with hard doughs is that if the gluten is too strong and elastic, the dough has a tendency to tear during sheeting and the cut pieces shrink back and distort prior to entering the oven. In order to manufacture hard dough biscuits and cookies on a continuous production line, some formulas contain sodium metabisulfite or sodium sulfite. These are reducing agents that break some of the disulfide bonds in the gluten. As a result, mixing time is shortened, resting time between mixing and sheeting is eliminated, and the dough becomes less extensible so it does not tear or shrink back after cutting. Hard dough biscuits and cookies do not spread (become larger in diameter) during baking. However, they do rise in the oven then shrink or collapse during cooling to produce a thicker final product.

Short Dough

The most common type of biscuits and cookies found around the world is made from short doughs. Short dough biscuits and cookies are also the most diverse, varying greatly in ingredients, size, shape, and flavor. Short doughs are typically made using weak soft wheat flours that contain 9% or lower protein content, and the formulas contain low levels of water and high levels of sugar and shortening. Most short doughs are prepared using a multistage mixing process that starts with creaming the sugar, shortening, and liquid. The liquid may be added as water, or it may be a component of wet ingredients such as fresh eggs, milk or other milk products, and liquid flavorings. The flour and dry ingredients are added to the creamed mixture in a separate mixing step. Short doughs can also be made using a single-stage mix procedure in which all of the ingredients are mixed into a dough in one mixing step. Biscuits and cookies made using a single-step method are inferior to those made with the multistage process. The structure of the cream plays an important role in the properties of the dough and of the final products. The gluten in short doughs is not developed during mixing for several reasons: the high levels of sugar and shortening delay gluten development; the water level is not high enough to completely hydrate the gluten, which prevents it from developing; and the water is encapsulated in the shortening cream where it is not available to hydrate the gluten. In contrast to hard doughs, which are extensible and elastic, short doughs are cohesive and plastic. Short dough consistency is thick enough to almost be pourable but not as thick as bread dough. Some short doughs can be sheeted and then cut or stamped into the desired shapes, while other short doughs are extruded through nozzles or dies. Most

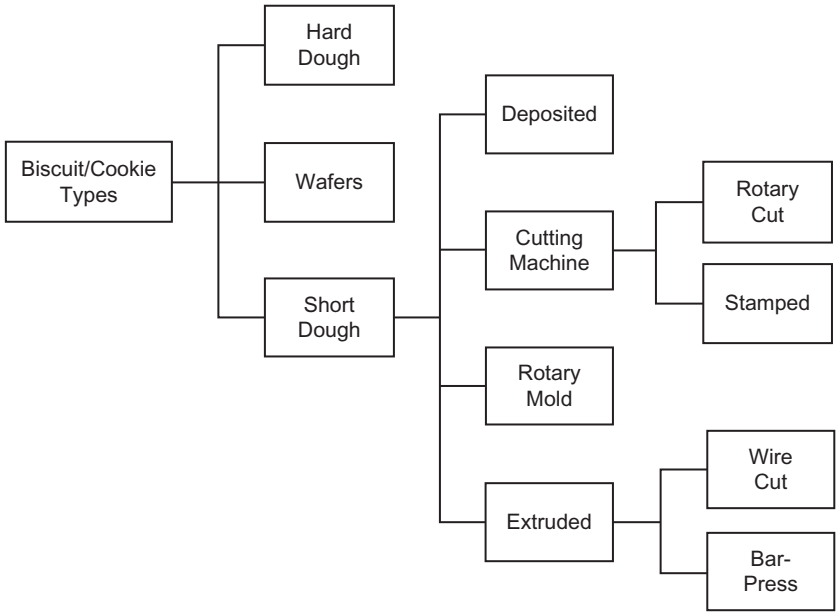

Figure 1 Types of biscuits and cookies.

short dough products retain their shape until they go into the oven and then spread or flow during baking, becoming thinner and larger in diameter. Some types of short doughs, however, do not spread at all and maintain their shape and any designs that are embossed on them.

There are several processes for shaping short doughs, which are classified by how the biscuits and cookies are placed on the baking band (Figure 1). They include deposit, rotary molding, cutting machine, and extruded processes. The cutting machine process includes both rotary cut and stamped products, while extruded products are subdivided into wire cut and bar-press (also known as rout-press) processes.

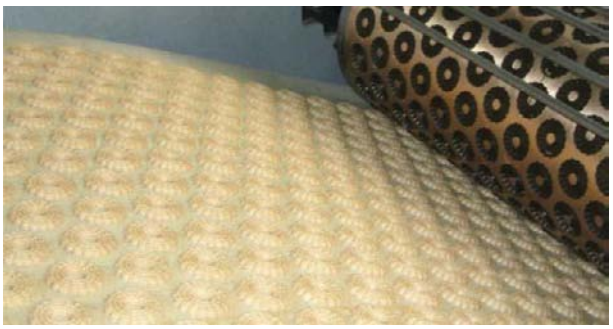

Figure 2 Rotary mold process. Photo courtesy of Reading Bakery Systems (Robesonia, PA).

Rotary Mold Process

Rotary mold is one of the most common production processes for short doughs. The formula is high in sugar, high in shortening, and low in water (less than 20% including the moisture in the flour). The resulting dough not only is crumbly, lumpy, and stiff but also is cohesive and pliable due to the high level of shortening. The dough is forced into engraved molds on a rotating roll for shaping and embossing the top surface. As the roll turns, the shaped dough pieces are extracted and fall onto a canvas belt where they travel into the oven and are baked (Figure 2). Rotary mold biscuits and cookies do not rise or spread in the oven and retain the designs that were embossed on the surface. This process only works with dry, crumbly doughs. Rotary mold biscuits and cookies are economical to produce because there is no scrap dough to recycle, the labor requirement is low, and there is little water to drive off in baking, which keeps energy costs low. The most famous example of a rotary mold cookie is the Oreo®.

Extruded Process

A wide variety of biscuits and cookies are produced using the extrusion process. These are made from soft doughs that rise and spread during baking. Extruded short dough products fall into two categories: wire cut and bar-press (also known as rout-press).

Wire cut process

Wire cut is another widely used production process for short dough products. In the wire cut process, soft, short dough is extruded through an orifice or die and cut with a reciprocating wire (Figure 3). The size of the orifice and speed of the wire control the size of the dough pieces. The dough hopper is positioned directly above the oven band so the cut biscuits or cookies fall directly onto the band. The dough must be cohesive enough to hold together but short enough to be cut cleanly by the wire. If dough consistency is not correct, the pieces are distorted during cutting and are not consistently or correctly positioned on the baking band. The wire cut process works

Figure 3 Wire cut process. Photo courtesy of Reading Bakery Systems (Robesonia, PA).

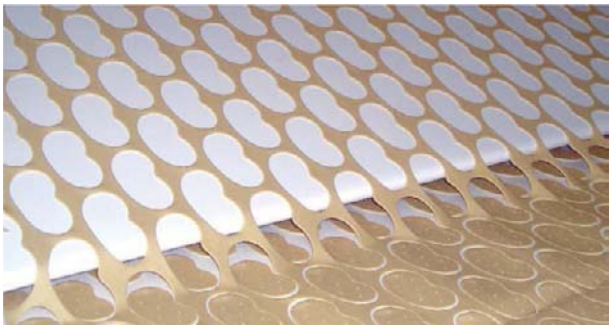

Figure 4 Rotary cut process. Photo courtesy of Reading Bakery Systems (Robesonia, PA).

well for biscuits and cookies with inclusions such as flavored baking chips, dried fruit pieces, and nuts. Chocolate chip and oatmeal raisin are examples of wire cut biscuits or cookies. The wire cut process can also be used to make coextruded products. This is done by extruding two different doughs or a dough and a filling from separate hoppers through a single orifice to combine them into a single product.

Bar-press process

The bar-press or rout-press production process is similar to the wire cut procedure except that the base of the dough chamber contains a die plate with nozzles. The nozzles are shaped to form a design. Some nozzles can rotate while the dough is extruding to produce twists, swirls, or other fancy designs. Inclusions are not added to the formula to prevent clogging of the nozzle. The dough is continuously extruded in short strips, which are then cut into individual pieces and baked. The formula contains high levels of shortening and sugar. Proper dough consistency is critical to ensure the dough is short enough to cut cleanly yet cohesive enough to hold the shape and design. The resulting biscuits and cookies have a soft, delicate texture and are quite fragile. They are difficult to package due to their fragility and irregular shapes. Biscuits or cookies made by this process include Danish butter cookies, Viennese whirls, and spritz. Multiple depositors can be used to combine doughs of different flavors or colors (coextrude) into a single product or to make filled products such as fig bars.

Deposit Process

Biscuit and cookie doughs that are deposited contain the highest level of shortening and sugar in the short dough category. They are also called soft doughs because their soft, semifluid consistency is more similar to batter than dough. The dough is extruded through a nozzle and deposited directly onto the oven band. The pieces are formed by cutting off the flow of the dough at the appropriate interval to get the desired size. Biscuits and cookies made with this method exhibit high spread during baking. Some products increase in diameter as much as 80%. Nilla Wafers® are produced using the deposit process.

Cutting Machine Process (Rotary Cut or Stamped)

In the production of biscuits and cookies by the cutting machine process, shapes are cut from a sheet of dough. The cutting is done by a rotary cutter or a stamper. Historically, the shapes were cut using a stamper that is a heavy block with the desired shapes cut into it that stamps down into the dough. This technique has been widely replaced with the rotary cutter (Figure 4), which is a rotating metal cylinder with the desired shapes cut into it that rolls continuously over the dough sheet.

In the process, the thickness of the dough sheet is gradually reduced by passing it through a series of rollers. The sheeting line typically contains two or three pairs of rolls. The thickness of the dough is reduced less than 50% in each subsequent pass through the rolls. This method produces a fair (20%) to large (60%) amount of scrap (pieces of dough between the cut shapes) depending on the shape being cut. The scrap is collected and either returned to the mixer or, more commonly, reincorporated into the dough during the sheeting stage.

Cutting machine short dough formulas contain more water than rotary mold formulas but are lower in sugar compared with many other types of biscuits and cookies. The high water, low sugar, and sheeting process develop the gluten in the flour. This keeps the shapes from spreading and distorting during baking. Animal cookies and gingerbread people are produced using the cutting machine process.

Wafers

Wafers do not fit the definition of biscuits and cookies but are categorized with them because consumers view them in the category, and they are manufactured and sold by biscuit and cookie manufacturers. Wafers are thin and extremely crisp. They are available in many diverse shapes including flat sheets, hollow sheets, cups, cones, and fancy shapes. They are typically not consumed as is but are components of biscuits, cookies, and candy bars and serve as edible containers for ice cream and other desserts.

The wafer formula contains no or low sugar, no shortening, and high water. The flour used is typically soft white wheat flour of short extraction, so it is very low in protein with high purity (low ash). The resulting batter is very thin and smooth. The gluten is not developed in the batter. Gluten formation in the batter is detrimental and leads to processing issues. For this

reason, batters are mixed in small batches to avoid overmixing and to minimize holding time between mixing and baking. The batter is deposited into heated molds that close and lock, which sets the thickness. Baking temperature is high and baking time is short, between 1.5 and 2.5 min. Although the formula contains sodium bicarbonate, it is added to adjust the pH rather than as a leavening agent. The leavening action comes from steam produced during baking. After baking, the wafer sheet is flexible enough to form into desired shapes before it cools. Some products are formed from the baked sheets, and others are produced by baking the batter in shaped molds. After baking and forming, the wafers are extremely dry, which gives them an undesirably soft texture. For this reason, wafer products go through a conditioning process in which the moisture content is increased to 5–6%. This increases the hardness and crispness of the wafers and makes the final products more stable. Wafer products, especially cones and bowls, are extremely fragile; thus, there is a high level of product loss due to breakage during processing.

Crackers

Crackers are regarded by some as being savory cookies, while others consider them to be unsweetened, salty, crisp biscuits. Crackers are typically consumed as a snack or as a bread substitute. The wheat flours used for cracker production typically contain higher protein content and are stronger than flours used for biscuits and cookies. Saltine crackers and cream cracker formulas contain both hard and soft wheat flours. In general, crackers contain low shortening, low sugar, and low moisture. Their low moisture content makes them resistant to microbial spoilage and gives them a long shelf life. Depending on the type, the leavening is by yeast or chemical leavening. Some types are also leavened by steam during baking.

Crackers are made from hard doughs that are laminated. Laminated doughs are thin sheets of dough, which are alternately layered with shortening. Puffing occurs between the layers, producing a light, crisp product. The stronger flour allows the dough to retain the layers formed during laminating.

The three types of crackers are saltine crackers (soda crackers), cream crackers, and snack crackers (Figure 5). The formulation and production processes vary widely for the three types.

Saltine Crackers

Saltine crackers (also known as soda crackers) are the best known fermented crackers consumed in the United States. They are made using a sponge and dough process. First, a sponge containing strong hard wheat flour, yeast, water, and an inoculum (also called a buffer or old sponge) is prepared and given a long fermentation time of 16–24 h. This long fermentation is critical to develop the proper flavor and texture in the final crackers. During fermentation, flavor compounds are produced by the action of the baker's yeast (*Saccharomyces cerevisiae*) and the *Lactobacillus* bacteria, which were introduced into the sponge in the inoculum. The quality of the saltine

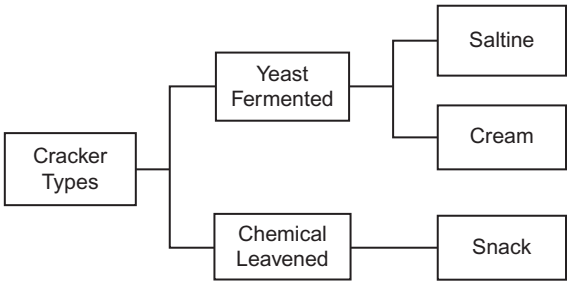

Figure 5 Types of crackers.

crackers is greatly impacted by the activity and addition level of the inoculum. The yeast and bacteria compete for the fermentable carbohydrates in the sponge. When the activity and level of the yeast is lower than that of the *Lactobacillus*, the bacteria dominates and produces enough acid to lower the pH from 6 to 4. This is the desired situation. The drop in dough pH is the most important reason for the lengthy fermentation. Wheat flour contains a native protease enzyme that becomes active at pH 4.1. This protease is thought to be important in creating the correct texture of the saltine crackers. On the other hand, if the yeast level is too high or the activity of the *Lactobacillus* is too low, the yeast dominates and saltine crackers have a completely different flavor and texture profile. The action of the native protease enzyme is also the reason why saltine crackers produced with short fermentation are of poor quality with a bland flavor and hard texture.

After the sponge fermentation is complete, weak soft wheat flour, shortening, salt, and sodium bicarbonate (baking soda) are added and mixed into a fully developed dough. The dough is then allowed to ferment for an additional 4–6 h after which additional sodium bicarbonate is added to raise the dough pH into the 7–8 range. This step is important for several reasons. The raise in pH stops the action of the native protease, so the gluten is not too degraded, which would result in a dough that is too weak to be sheeted and to maintain the distinctive layers. The yeast also becomes active again and dominates fermentation at the higher pH to produce more flavor compounds and strengthen the dough to help produce the correct texture. Additionally, the sodium bicarbonate gives the crackers their characteristic taste and is the reason they are also known as soda crackers.

When the dough has been properly fermented, it is sheeted to about 0.3 mm thick and laminated into six or eight layers. Laminating is folding (lapping) the dough back upon itself in the same direction to create layers. During the process, the dough is also turned 90° so that it is sheeted in both directions (cross sheeted). Cross sheeting aligns the gluten in both directions so that it will expand properly during baking to prevent misshapen crackers. The laminated dough is then passed through several sets of sheeting rollers, which gradually reduce the dough thickness from 2.5 cm to 0.3 mm. A rotary cutter perforates the dough into 50 mm squares. The dough is then docked using a nine-pin pattern consisting of three rows of three docking holes. The docking pins pass through the entire sheet and seal the top and bottom layers together. Salt is sprinkled on top of the dough if desired and the crackers are

baked. The dough sheet is baked intact and then mechanically broken into individual crackers after cooling.

Saltine crackers are baked on mesh bands in tunnel ovens that are 100 m long at very high temperatures of 250–300 °C in a very short time of 2.5–3 min. At these high temperatures, the water in the dough flashes off as steam and causes the layers to puff. The docking holes seal together the layers of dough, so the puffing is controlled and occurs only between the holes. The mesh band allows the moisture to be removed from under the crackers so they do not curl. The thickness of the cracker increases from 0.3 to 4 mm during baking.

The crackers have a low moisture content of 2–2.5% when they exit the oven. They must be cooled slowly to prevent checking or cracking. The puffing between the layers and the low final moisture content give saltine crackers a light, flaky, layered texture. Proper storage is critical to maintain the crisp texture. If the crackers take up even a small amount of moisture, the texture will become undesirably soft and soggy.

Cream Crackers

Cream crackers are popular fermented crackers consumed in the United Kingdom. Cream crackers differ significantly in size, appearance, flavor, and texture compared with saltine crackers. Cream crackers can be made by a sponge and dough process or using a single-stage procedure. The sponge and dough process used to make cream crackers is similar to that used for saltine crackers, but the formula is quite different as shown in Table 1. Compared with saltine crackers, the cream cracker sponge contains much less flour and half the level of yeast. An inoculum is not added into the cream cracker sponge, so the pH of the sponge does not drop appreciably from its original value of around 6. After the sponge fermentation is complete, weak soft wheat flour, shortening, salt, and sodium bicarbonate are added. The majority of the flour is added in the dough stage rather than into the sponge. Additionally, the level of shortening is significantly higher, and the level of sodium bicarbonate is significantly lower than the levels used in saltine cracker production. In cream cracker production, the sponge is fermented for 12–16 h, and the dough is fermented for an additional 1–3 h.

Cream crackers can also successfully be made using a single-stage process in which all of the ingredients are mixed into a dough in a single mixing step. The formula typically contains a blend of 50% strong flour and 50% weak flour, shortening, yeast, salt, sugar, sodium bicarbonate, and water. The yeast level is higher than that used in the sponge and dough system and is set at a level such that the dough will double in size between completion of mixing and end of fermentation. The dough is fermented for 4–16 h.

Table 1 Basic formulas for saltine and cream crackers

	Flour weight basis (%)	
Ingredient	*Saltine crackers*	*Cream crackers*
Sponge		
Flour (strong)	70.0	30.0
Compressed yeast	0.4	0.2
Water	33.0	30.0
Dough		
Flour (weak)	30.0	70.0
Shortening	11.0	20.0
Salt	1.5	1.0
Sodium bicarbonate	1.0	0.2

The makeup procedure is the same for doughs made by both the sponge and dough and the single-stage methods. After fermentation is complete, the dough is sheeted and laminated. Laminating is folding (lapping) the dough back upon itself in the same direction to create layers. Cracker dust (a mixture of 100 parts flour, 33 parts shortening, and 1 part salt) is sprinkled onto the dough between the layers during the lamination procedure. The application rate is 9–18% based on the dough weight. The cracker dust keeps the dough layers separated and provides some lift (rise) during baking to produce a cracker with a flaky structure. During the lamination process, the dough is also turned 90° so that it is sheeted in both directions (cross sheeted). Cross sheeting aligns the gluten in both directions so that it will expand properly during baking and prevent misshapen crackers. The laminated dough is then passed through several sets of sheeting rollers, which gradually reduce the dough thickness to the desired level. Individual crackers measuring 65×74 mm are cut from the dough and docked to seal the layers. The scrap (dough between the cut pieces) is recycled back into the dough at the dough mixing step. The crackers are baked on a mesh band in a very hot oven (210–250 °C) in a short time (4.5–5 min). During baking, the layers expand between the docking holes. The final thickness of a typical cream cracker is 6.5 mm. Cream crackers have a higher moisture content and higher shortening content than saltine crackers, which gives them a softer texture and a richer flavor. The high shortening level makes oxidative rancidity a problem during storage. As with all dry products, moisture uptake during storage can degrade product quality by making the crackers soft and soggy.

Snack Crackers

Snack crackers are also known as savory crackers, cocktail crackers, or cheese crackers. They come in many different flavors, shapes, and sizes. Some are topped with items such as seeds, herbs, cheese, and salt. The most common shape is round. Round and unusually shaped products yield a large quantity of scrap after cutting that is reincorporated back into the mixer or into the dough during sheeting. Compared with saltine crackers and cream crackers, snack crackers contain sugar and more shortening. A few types are yeast-leavened and fermented, but most are chemically leavened. Some contain flavoring compounds. In addition to imparting flavor, many flavoring compounds have a weakening effect on the dough, which may help with sheeting and laminating by making the dough more extensible. If the dough is too strong, proteolytic enzymes or sulfite (sodium metabisulfite or sodium sulfite) is often added to break down some of the gluten to make the dough more extensible during sheeting and lamination, so the cracker pieces do not shrink or distort after cutting.

Snack crackers are prepared using a single-stage mixing process in which all of the ingredients are mixed together at once to make dough, which then may or may not be rested. The dough is then sheeted, laminated, cut, docked, and baked.

Most snack crackers are sprayed with oil as they leave the oven. The oil gives the cracker a shiny appearance, imparts flavor, and helps any applied toppings adhere to the surface.

See also: Leavening Agents; Oxidation of Food ComponentsWater in Foods: Water Content, Water Activity and Their Effects on Food StabilityWheat: Grain Structure of Wheat and Wheat-based Products.

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Relevant Website

www.thebcma.org – Biscuit Cookie and Cracker Manufacturers' Association (B&CMA).

Boron

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Sources and Patterns of Consumption

Boron is a ubiquitous mineral element that is present naturally combined with oxygen in seawater, freshwater, rocks, and soil. Boron concentrations in surface water range widely from 0.001 to 150 mg l^{-1}. Most freshwater concentrations of boron, usually in the form of boric acid, are below 0.4 mg l^{-1} and are not lowered by the treatment for drinking water. Boron is recognized as an essential nutrient for plants; thus, all plant material contains boron. The amount of boron in plant tissues and species varies greatly. Monocotyledons are lower in boron content (usually in the range of 2–6 mg kg^{-1} dry weight) than dicotyledons (usually in the range of 20–60 mg kg^{-1} dry weight). The amount of bioavailable boron in soil can affect the boron content of plants. Foods from plants grown on soil low in boron will have lower boron content than from the same plants grown on soil high in boron because boron accumulates in terrestrial plants. However, boron does not magnify through the food chain. Boron is distributed throughout animal soft tissues and fluids in concentrations mostly between 0.015 and 2.0 μg g^{-1} fresh weight. Bone, fingernails, and teeth can contain several times these concentrations. For the general population, diet and occasionally drinking water are the major sources of boron. As shown in Table 1, the richest sources of boron are fruits, vegetables, pulses, and nuts. Wine, cider, and beer are also high in boron. Dairy products, fish, meats, and most grains are poor sources of boron. A typical daily intake of boron through the diet ranges from 0.75 to 1.35 mg. However, the consumption of specific foods with high boron content will increase its intake significantly. For example, one serving of wine or avocado provides 0.42 and 1.11 mg of boron, respectively. Diets high in meat and processed grains often will provide less than 0.75 mg day^{-1}.

Availability, Absorption, and Metabolism

Boron biochemistry is essentially that of boric acid, which is readily soluble in water. Borates are converted into boric acid when dissolved in water. Dilute aqueous boric acid solutions are composed of $B(OH)_3$ and $B(OH)_4^-$ at the pH of blood (7.4). Because the pK_a of boric acid is 9.15, the abundance of these two species at pH7.4 is 98.4% and 1.6%, respectively. Boric acid forms ester complexes with hydroxyl groups of organic compounds when they are adjacent and *cis*. The importance of the *cis* arrangement of the hydroxyl groups is shown by the lack of reaction of boric acid with polysaccharides such as glucose, glucuronic acid, and xylose because of the lack of appropriately paired hydroxyl groups. Among the many substances of biological interest with which boron forms complexes are pyridoxine, riboflavin, dehydroascorbic acid, and ribose. Through its formation of a diester with the ribose moiety in adenosine, boron also forms complexes with biomolecules such as diadenosine polyphosphates, cyclic ADP ribose, *S*-adenosylmethionine, and oxidized nicotinamide adenine dinucleotide (NAD). The formation of boroesters most likely is a determinant of the response to nutritional and toxic intakes of boron. Several naturally occurring organoboron compounds have been identified. These compounds include antibiotics produced by microorganisms, the plant cell component rhamnogalacturonan-II, and the bacterial extracellular signaling molecule autoinducer-2. All these compounds are boroesters.

About 85–90% of ingested boron is rapidly absorbed; it is excreted mostly in the urine shortly after ingestion. Because there is no usable radioisotope of boron, the study of its metabolism has been difficult. It is believed that most ingested boron is converted into boric acid, the dominant species of boron compounds hydrolyzed at the pH of the gastrointestinal tract, and then absorbed and excreted in that form. During transport in the body, boric acid most likely is weakly attached to organic molecules containing *cis*-hydroxyl groups.

Health Effects

Early Benefits Discoveries and Their Fate

Recognition that there are benefits and detriments to boron in defined quantities in food apparently began in the 1870s. At that time, it was determined that borax and boric acid could be used to preserve foods. For the next 50 years, borates were considered one of the best methods for preserving or extending the palatability of foods such as fish, shellfish, meat, sausages, bacon, ham, cream, butter, and margarine. Boron probably was more beneficial to humankind at this time than most people realize; it had a vital role as a preservative in preventing food crises during World War I. However, as early as 1902, German and American scientists began to question whether large amounts of borates in foods were innocuous. In 1904, a report summarized a study performed for the US Department of Agriculture (USDA) in which human volunteers were fed borax or boric acid in small doses for extended periods or large doses for short periods of time. Boric acid in doses greater than 500 mg (77 mg boron) per day for 50 days resulted in disturbances in appetite, digestion, and health. It was concluded that boric acid at 4000 mg (699 mg boron) per day was a limit beyond which harm to humans would occur. Subsequent to this report, the opinion that boron posed a risk to health grew. By the mid-1920s, many countries of the world began legislating against the addition of borates to food. The study performed for the USDA apparently is the only one in which humans were exposed to regular controlled, relatively high dosages of boron. However, that study combined with a few cases of misuse of boric acid in hospitals, such as using boric acid as a bactericide on massive burns and open wounds, which do not have skin to prevent entry into the body, and mistakenly feeding babies a boric acid solution instead of a

Table 1 Examples of boron content in foods in various food groups

Food	*Boron ($\mu g\ 100\ g^{-1}$)*	*Food*	*Boron ($\mu g\ 100\ g^{-1}$)*
Fruits		*Vegetables*	
Apple, raw, with skin	187 ± 14	Squash, winter, baked	161 ± 10
Orange, raw	107 ± 4	Lettuce, iceberg, raw	46 ± 4
Peach, raw	171 ± 7	Broccoli, cooked	105 ± 7
Pear, raw	227 ± 11	Tomato, raw	63 ± 2
Plums	218 ± 27	Beans, snap, cooked	109 ± 10
Grapes, raw	140 ± 1	Carrots, raw	130 ± 7
Cherries, sweet, raw	228 ± 3	Potato, baked	67 ± 9
Avocado, raw	1430 ± 42	Sweet potato, baked	90 ± 1
Nuts/pulses/grains		*Animal products*	
Peanuts, roasted	583 ± 58	Beef, ground, cooked	9 ± 2
Pecans, roasted	264 ± 13	Pork, ham, cooked	9 ± 2
Beans, navy, cooked	155 ± 12	Turkey breast, cooked	20 ± 1
Beans, pinto, cooked	205 ± 16	Egg, cooked	10 ± 1
Farina, wheat, cooked	10 ± 0	Milk, whole	27 ± 3
Corn grits	10 ± 0	Yogurt, plain	32 ± 2
Rice, white	9 ± 0	Cheese, American	30 ± 1

Values obtained from Hunt, C. D. and Meacham, S. L. (2001). Aluminum, boron, calcium, copper, iron, magnesium, manganese, molybdenum, phosphorus, potassium sodium, and zinc: concentrations in common Western foods and estimated daily intakes by infants; toddlers; and male and female adolescents, adults, and seniors in the United States. *Journal of the American Dietetic Association* **101**, 1058–1060.

sugar solution, created the general belief that boron was nothing more than a poison for humans. Only during World War II were the restrictions against boron eased; food shortages were making food preservation a major concern in many countries. After the war, restrictions were gradually reimposed, and by the middle 1950s, boron as a food preservative was essentially forbidden throughout the world. Joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives stated in 1962 and 1967 that boric acid and borates should not be used as a food preservative. The restrictions and statements occurred in spite of the fact that no case of boron intoxication was ever reported when boron was properly used as a preservative in food.

In 1910, findings were reported indicating that boron is essential for plants. However, conclusive evidence and acceptance of the essentiality of boron for plants are usually considered as coming from reports in the 1920s. Boron deficiency in plants has multiple and diverse physiological consequences including abnormalities in sugar transport, respiration, free radical generation and detoxification, and in carbohydrate, indole acetic acid, RNA, ascorbate, and phenol metabolism. Interestingly, although over 50 years have elapsed since boron was discovered essential, the biochemical reason for many of these deficiency signs has not been definitively identified. Finding boron essential for plants apparently was a stimulus for the efforts to show boron as an essential element for animals in the 1930s and 1940s. These attempts were unsuccessful, most likely because of the use of unsuitable diets and the determination of variables, such as growth, that were not sensitive to boron deprivation. As a result, students were taught that boron was a unique element because it was essential for plants, but not for animals.

The dogma about boron being a poison and being only essential for plants apparently inhibited the examination of whether nutritional intakes of boron were beneficial for higher animals and humans until 1981 when seminal reports about boron being beneficial for bone health appeared. Since then, similar to plants, boron has been found to have diverse beneficial effects in animals and humans including in bone growth and maintenance, brain function, and perhaps cancer, cardiovascular disease, and diabetes risk reduction. In addition, boron was found essential for frogs and zebra fish to complete their life cycle. Boron deprivation in male frogs resulted in atrophied testes, decreased sperm counts, and sperm dysmorphology, and, in female frogs, atrophied ovaries and impaired oocyte maturation. Boron deprivation also resulted in necrotic eggs and high mortality in embryos. Boron deficiency also resulted in high mortality in zebra fish embryos. Although there are some suggestive findings indicating that boron might impair embryonic development in mice, the critical experiment demonstrating boron is essential for mammals is lacking.

Bone Health

Early findings indicating that boron was beneficial for bone health included boron deprivation decreasing the maturation of the bone growth plate in chicks and inducing limb teratogenesis in frogs. Since then, much evidence from animal and cell culture experiments supports the limited human findings suggesting that nutritional amounts of boron are beneficial for bone growth and maintenance.

Animal experiments indicate that the beneficial effect of boron on bone is especially noticeable in trabecular and alveolar bone growth and maintenance. Boron deprivation of rats from conception to age 21 weeks was found to detrimentally affect trabecular bone characteristics. In lumbar vertebra, bone volume fraction and trabecular thickness were decreased, and trabecular separation and structural model index were increased. An increase in the structural model

index indicates less of a more desirable platelike structure. Boron deprivation in rats also has been shown to decrease alveolar bone (primary support structure for teeth) repair that is initiated immediately after tooth extraction. Boron deprivation decreased osteoblast surface and increased quiescent bone-forming surface in the alveolus such that bone volume fraction was depressed 14 days after tooth extraction. Boron deprivation for 9 weeks also impaired alveolar bone formation without tooth extraction in mice. The deprivation decreased osteoblast surface and increased quiescent bone-forming surface in both the lingual side and the buccal side of periodontal alveolar bone.

The changes in trabecular bone induced by boron deprivation may affect bone strength and thus might increase the risk for osteoporosis. Boron deprivation has been found to decrease bone strength variables in femurs of pigs and rats. Boron supplementation also was found to increase mean trabecular density and thickness, trabecular bone volume, and cortical bone volume of femurs from rats with retinoic-induced osteoporosis. One human study examining the possibility that boron may reduce the risk for osteoporosis found that 6 months of a daily supplement of 226 mg (6 mg boron) of a sugar–borate ester commonly found in fruits and vegetables (calcium fructoborate) incorporated into margarine improved bone density in 66 of 100 patients with osteoporosis. This finding resulted in the suggestion boron in the form fructoborate may be a good adjuvant in the treatment of osteoporosis.

Other human studies involving boron also support the suggestion that boron is beneficial for bone maintenance and thus reduces the risk for osteoporosis. Proinflammatory cytokines stimulate osteoclastic bone resorption, which apparently is the basis for inflammatory stress being associated with osteoporosis. Such inflammatory stress also is associated with arthritis, which has been found to be affected by nutritional intakes of boron. A 6 mg day^{-1} boron supplement in the form of calcium fructoborate alleviated subjective measures of mild, moderate, or severe osteoarthritis in 20 subjects. After 8 weeks of supplementation, 80% of patients with mild or moderate osteoarthritis reported reduced or eliminated use of painkillers. In addition, joint rigidity essentially disappeared, and mobility was markedly increased. Patients with severe osteoarthritis, who were supplemented with 12 mg day^{-1} of boron as calcium fructoborate, had a more subdued improvement in mobility and rigidity but did report a significant reduction in painkiller use. The findings in this study, however, are weakened by the nonblinding to treatment and the lack of placebo controls. Subsequently, a double-blind, placebo-controlled pilot study with middle-aged subjects with primary knee osteoarthritis found that, when compared to a placebo, boron supplemented at 3, 6, or 12 mg day^{-1} as calcium fructoborate for 15 days significantly improved inflammatory markers serum C-reactive protein, plasma fibrinogen, and erythrocyte sedimentation rate. In another short-term double-blind, placebo-controlled study of subjects with knee osteoarthritis, ingestion of 108 mg (2.9 mg boron) calcium fructoborate twice daily resulted in decreased C-reactive protein at days 7 and 14 compared to day 1 baseline. In addition to these osteoarthritis studies, a cross-sectional study that enrolled 106 subjects with rheumatoid arthritis and 214 controls found significantly lower serum boron concentrations in rheumatoid arthritis subjects, and rheumatoid factor titer was a significant predictor of low serum boron concentrations.

In addition through modulating inflammatory stress, boron may be beneficial through enhancing the utilization of hormones involved in bone health. In postmenopausal women, the increases in serum 17β-estradiol and plasma copper induced by estrogen therapy were significantly higher when boron at 3.25 mg day^{-1} instead of about 0.25 mg day^{-1} was consumed. The higher boron intake also enhanced the effect of estrogen therapy on serum triglyceride and immunoreactive ceruloplasmin concentrations. The combination of estrogen therapy with the higher boron intake was most effective in increasing serum 25-hydroxy vitamin D concentration. The human findings are consistent with a report that boron increases the efficacy of estrogen supplementation in rats. In ovariectomized rats fed with an AIN-76 diet containing 0.4 mg kg^{-1} boron, a 5 mg kg^{-1} boron supplement significantly increased the beneficial effect of 17β-estradiol supplementation on trabecular bone volume fraction, bone growth plate density, and trabecular separation. The combination of boron and 17β-estradiol, versus either of these alone, also markedly improved the apparent absorption of calcium, phosphorus, and magnesium and the retention of calcium and phosphorus.

Other findings supporting the suggestion that nutritional amounts of boron have beneficial effects on bone health include cell culture studies showing that boron supplementation at 1 or 10 ng ml^{-1} compared to supplementation at 0 and 0.1 ng ml^{-1} increased mineralized nodule formation and mineralized tissue-associated mRNA expressions of type 1 collagen, osteopontin, bone sialoprotein, osteocalcin, and RunX by cultured osteoblasts (MC3T3-E1). In addition, the boron supplementation increased bone morphogenetic protein 4, 6, and 7 levels.

High circulating homocysteine and depleted *S*-adenosylmethionine also have been associated with the incidence of osteoporosis and arthritis. It has been reported that plasma homocysteine increased and liver *S*-adenosylmethionine decreased in rats fed diets containing 0.05–0.15 mg kg^{-1} boron compared to rats fed diets supplemented with 3 mg kg^{-1} boron.

Recent studies involving bioactive glasses, which are used for bone tissue engineering and in situ bone tissue regeneration, provide supporting evidence that boron is beneficial for bone formation. Animal and cell culture studies indicate that bone formation is enhanced when bioactive glasses are modified to contain boron. Some of this enhancement might be the result of inducing angiogenesis, which is critical for wound repair and tissue engineering. Borosilicate bioactive glass ionic dissolution products were found to increase angiogenesis in quail embryos.

To summarize, animal studies have shown that boron deprivation detrimentally affects the structure and makeup of bone. Human, animal, and cell culture studies have shown that nutritional amounts of boron are positively associated with factors involved in bone formation and maintenance and have identified several potential mechanisms through which boron might affect bone health. The findings described strongly support the contention that nutritional intakes of boron are beneficial for bone formation and maintenance.

Central Nervous System Function

Findings showing that nutritional intakes of boron have beneficial effects on central nervous system function are more limited than those with bone. However, they are among the most supportive in demonstrating that boron is a beneficial bioactive food component for humans. Under well-controlled dietary conditions, boron supplementation (3 mg day^{-1}) to older men and women consuming diets providing boron at about 0.25 mg day^{-1} for 63 days altered electroencephalograms (EEGs) such that there was a shift toward more activity in the low frequencies and less activity in the high dominant frequencies of the EEG spectrum. Increased low-frequency activity is typical of states of reduced behavioral activation and has been associated with impaired memory performance. Subjects supplemented with boron after deprivation exhibited improved psychomotor skills of motor speed and dexterity and cognitive processes of attention and short-term memory. Studies with rats gave findings consistent with those from the human studies. Boron deprivation in rats affected brain electrical activity in a manner similar to nonspecific malnutrition and heavy-metal toxicity. Since the 1990s when the human findings were obtained, further studies involving the effect of boron intake on central nervous system function in humans apparently have not been reported. Recently, however, it was found that boron-deprived rats were less active than rats fed diets supplemented with 3 mg kg^{-1} boron. Boron deprivation reduced the number, distance, and time of horizontal movements, front entries, margin distance, and vertical breaks and jumps in a spontaneous activity evaluation. Feeding fish oil (has anti-inflammatory effects) instead of safflower oil in the diet attenuated the response to boron deprivation.

Additional human studies are needed to confirm that boron deprivation has a negative effect on central nervous system function. However, based on findings indicating that boron has positive effects on vitamin D metabolism or utilization, circulating homocysteine levels, and *S*-adenosylmethionine, which affect brain function, it seems reasonable to suggest that nutritional intakes of boron have a positive effect on the central nervous system.

Cancer Risk Reduction

One of the most recently suggested beneficial effects of boron is a reduced risk for some types of cancer. This suggested benefit was initiated by an epidemiological study that found an inverse association between dietary boron and prostate cancer. Since then, several studies have shown that boron inhibits the growth of some types of cultured prostate and breast cancer cells and human prostate adenocarcinoma tumors in nude mice. Boron also has been inversely associated with cervical and lung cancers. A study of cervical smears from 472 women with a mean boron intake of 8.41 mg day^{-1} and 587 with a mean intake of 1.26 mg day^{-1} found 15 cases of cytopathological indications of cervical cancer in the low-boron-intake women and none in the high-boron-intake women. In a study of 763 women with lung cancer and 838 matched healthy controls, boron intake was inversely associated with the incidence of lung cancer. The odds increased substantially if the women were not on hormone replacement therapy. This finding is consistent with that of nutritional intakes of boron enhancing estrogen therapy in human and animals described earlier.

The beneficial effect of boron in cancer risk might be through affecting the immune response. Pigs fed with a low-boron-diet (1–2 mg kg^{-1}) for 95 days exhibited a significantly higher skinfold thickness response to an intradermal injection of phytohemagglutinin than pigs supplemented with boron (5 mg kg^{-1} diet). A nutritional amount (3 mg kg^{-1}) supplemented to a low-boron diet (0.2 mg kg^{-1}) more than doubled the serum antibody concentrations to injected antigen (human typhoid vaccine) in rats. Boron status also was found to affect populations of blood cells involved in the immune or inflammatory response in humans. Perimenopausal women with average boron excretions of 1.1 and 3.0 mg day^{-1} during placebo and boron supplementation periods, respectively, had an increased white blood cell numbers, an increased percentage of polymorphonuclear neutrophils, and a decreased percentage of lymphocytes during the boron supplementation period.

Other Possible Health Effects

In addition to animal and human studies showing that nutritional intakes of boron affect circulating homocysteine and C-reactive protein, boron in concentrations similar to those in blood was found to decrease Ca^{2+} release from ryanodine receptor-sensitive stores. Thus, it has been hypothesized that boron is bioactive through binding NAD^{+} and cyclic ADP ribose, which induce the release of Ca^{2+} from the endoplasmic reticulum. This binding might result in the inhibition of the release of Ca^{2+}, which is a signal ion for many processes including insulin release and the inflammatory response.

Biochemical findings suggest that boron might be beneficial through reducing the risk for the metabolic syndrome and diabetes. Chronic inflammatory stress is a risk factor for these pathological conditions. Some limited evidence has been reported supporting the suggestion that boron facilitates the action or release of insulin. In rats fed a diet containing 0.2 mg kg^{-1} boron, a boron supplement of 2 mg kg^{-1} diet reduced plasma insulin but did not change plasma glucose concentrations. Another finding was that peak insulin release from the isolated perfused pancreas of boron-deprived chicks was almost 75% higher than from the pancreas of boron-supplemented chicks. The difference was especially noticeable when the perfusate was supplemented with glucose. Boron deprivation also has been reported to induce a modest but significant increase in fasting serum glucose concentrations in older men and women fed with a low-magnesium, marginal copper diet.

Both high circulating homocysteine and chronic inflammatory stress indicated by high serum C-reactive protein are considered risk factors for cardiovascular disease. Because both animal and human findings indicate that nutritional intakes of boron may alleviate these risk factors, boron might have a positive effect on heart health. However, these biochemical changes are the only findings that indicate such an effect. Perhaps, an effect on cardiovascular health had an influence on the finding in northern France that the mortality rate was significantly lower when drinking water contained greater than 0.3 mg l^{-1} than when it contained less than 0.3 mg l^{-1}. The

biochemical and drinking water findings suggest that further studies should be considered for the determination of whether there is an association between boron intake and cardiovascular health.

Intakes Affecting Health

Nutritional

Both animals and humans deprived of boron exhibit positive health effects when provided with intakes of boron that may be achieved through consuming foods of plant origin. In human depletion–repletion experiments, participants responded to a 3 mg day^{-1} boron supplement after consuming a diet supplying boron at only 0.2–0.4 mg day^{-1} for 63 days. Extrapolations from animal experiments indicate that to achieve optimal health effects of boron, intakes greater than 0.5 mg day^{-1} are needed and that boron supplementation is unlikely to elicit a response in individuals consuming at least 1 mg day^{-1}. These data were used by the World Health Organization to suggest that an acceptable safe range of population mean intakes of boron for adults could be 1–13 mg day^{-1}. This indicates that usual intakes of boron above 1 mg day^{-1} promote human health.

In the United States, a survey conducted between 1994 and 1996 indicated that boron intakes ranged from a low of 0.35 mg to a high of 3.25 mg day^{-1} for adults. The median intakes for various age groups of adults ranged from 0.87 to 1.13 mg day^{-1}. Findings from a study involving 43 postmenopausal women in eastern North Dakota found that average urinary excretion of boron, which is a good indicator of dietary intake, was less than 0.5 mg day^{-1} for two women and between 0.5 and 1.0 mg day^{-1} for 14 women. These findings suggest that a significant number of people could benefit through an increased intake of boron through diets high in fruits, vegetables, nuts, and pulses.

Safe intakes

The US Institute of Medicine Food and Nutrition Board has not set an adequate intake level for boron. However, they set a tolerable upper intake level of 20 mg day^{-1}. As indicated earlier, the World Health Organization first suggested that 13 mg day^{-1} would be a safe upper intake level, but this was later increased to 0.4 mg kg^{-1} body weight or about 28 mg day^{-1} for a 70 kg person. The European Union established an upper intake level for total boron intake based on body weight that results in about 10 mg day^{-1} for adults. These safe upper intake levels indicate that people living in areas where boron is high in food and water are unlikely to be adversely affected. Support for this statement is the finding that no adverse effects on health were found in 66 men (mean age, 39 years) residing in a high-boron area for 36 years who had a calculated daily boron excretion of 6.77 mg l^{-1}. The drinking water in the area where they resided had boron concentrations that ranged from 2.05 to 29.00 mg l^{-1}, with a mean of 10.2 mg l^{-1}.

Conclusion

Substantial evidence exists indicating that boron in nutritional amounts and by plausible mechanism of actions has health effects that may impact the risks for or severity of arthritis, osteoporosis, cancer, and impaired central nervous system function. Limited evidence suggests that boron intake also might affect the risk for diabetes and cardiovascular disease. Consideration should be given for providing dietary guidance for boron. Such guidance could be to consume foods resulting in a diet that provides about 1 mg of boron per day.

See also: Dietary Practices; Dietary References: US; Functional Foods; Trace Minerals and Trace Elements.

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Brandy and Cognac: Consumption, Sensory and Health Effects

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Brandy in a Global Spirit Context

Market Trends

According to Euromonitor, the global spirit market in 2014 was estimated to be around 21 billion liters. Brandy and Cognac sales contributed to 8% of the global spirit sales at approximately 1.6 billion liters, making it the third largest category, after vodka and whisky that sold around 4 billion and 3 billion liters, respectively. The IWSR reported that the retail value of brandy and Cognac sales in 2013 amounted to 31.4 billion USD. The top markets for brandy are Brazil, Germany, Russia, India, and the Philippines, while substantial volumes are also sold in the United States, South Africa, China, and the Ukraine. In comparison, the main markets for Cognac, according to the Bureau National Interprofessionel du Cognac (BNIC), are the United States, Singapore, China, the United Kingdom, Germany, Hong Kong, France, the Netherlands, Norway, and Finland. Interestingly, sales in the Western markets are driven by the more affordable VS category while the Eastern market sales are driven by superior quality Cognacs. Over recent months, numerous Cognac producers have announced a decline in their profits, largely attributed to the Chinese government's crackdown on luxury spending and gifting, which has resulted in a slowdown in the country's Cognac and high-end Scotch market. Nevertheless, the region remains a priority export market.

Consumer Trends

Volume and sales trends indicate that there is a growing consumer penchant for expensive luxury spirits. Yet, according to Euromonitor's trend forecast for 2014 and beyond, Cognac will be seen less as a traditional drink that can be given as a gift and will feature more prominently in the mixology arena. Younger Cognac and Armagnac products in the VS category are increasingly being consumed in tall drinks and cocktails. Brandies that contain larger portions of more neutral column distilled spirits, such as South African blended brandies, can be also served very successfully in long mixed drinks and is often consumed mixed with cola soft drinks. Modern mixology trends move toward using ingredients that complement the fruity, sweet, and spicy attributes of brandy. The wide variety of combinations, featuring botanicals, fruit juices, confectionary ingredients, and spices being used in brandy cocktails, demonstrates the versatility of the product category.

In contrast, premium pot distilled brandies and older Cognac and Armagnac products are generally consumed neat or diluted with water or on ice to preserve the flavor complexity. Brandy, Cognac, and Armagnac have also become part of the fine dining experience as food pairing partners due to its smoothness and rich flavors.

Production of Brandy

Grape Varieties Used for Brandy Production

A wide variety of grape cultivars are used to produce Cognac, Armagnac, and brandy. Cognac is made mainly from the cultivars 'Ugni Blanc' and 'Folle Blanche' and Armagnac with 'Folle Blanche', 'Ugni Blanc', 'Baco', and 'Colombard'. Brandy produced in other countries is made not only from the previously mentioned cultivars but also from 'Chenin Blanc', 'Arien', 'Flame Tokay', and many other cultivars. The unique character of the individual cultivars contributes to the distinctive taste profiles of the eventual brandies.

Base Wine Production

Base wine, or the wine made specifically for the production of brandy, Cognac, and Armagnac, differs significantly from table wine. First, the grapes are picked early to ensure high acid concentrations and lower sugar levels (18–20° Balling). The addition of sulfur dioxide is common practice with the production of table wine. However, the addition of sulfur during the production of base wine for brandy is uncommon. For example, the level of total sulfur in the base wine must be lower than 20 mg l^{-1}. Otherwise, a reaction between sulfur and copper of the pot stills results in the undesirable formation of copper sulfate. In other countries, such as Spain, the base wine is transported over long distances from the point of winemaking to the point of distillation. Consequently, sulfur is used to protect the base wine from oxidation. The levels of volatile acidity and total phenolic content must be below 0.7 g l^{-1} and 250 mg l^{-1}, respectively, depending on the regulations per country. The yeast sediment that forms during the fermentation of the base wine is either included or not in the distillation process, depending on the style of brandy produced.

Distillation Process

The base wine is now distilled, and during this process, the alcohol concentration is increased from 9–11% to 72% alcohol by volume (abv) for Cognac and brandies from South Africa and other countries or 53–60% abv for Armagnac. In this process, depending on the method used or style that is needed, the wine is heated in a still until it separates into its components, which evaporate at various points on the temperature scale. The more volatile the component, the lower the temperature at which it evaporates, leaving behind the impurities and heavier compounds.

For Cognac and some brandies, distillation is performed in copper pot stills in a two-phase process that creates a complex spirit. In the first phase, the base wine is distilled into low wine. The alcohol concentration of the low wine is between 28% and 30% abv. This is essentially a concentration process resulting in

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the removal of a large proportion of water and soluble solids from the wine. The second stage of distillation involves distilling the low wine into brandy. Three fractions of the liquid are drawn in sequence. These fractions are known as the heads, hearts, and tails, respectively. The heart fraction is the most important, as it is rich in desirable aromas and flavor compounds and it is this fraction that is retained for maturation.

Although the main apparatus used for the production of brandy is the pot still, distillation can also be carried out using a column still (continuous distillation). Armagnac from France, Spanish brandy, and several brandies from the United States are produced with column stills. This apparatus is composed mainly of stainless steel and not copper, although some stills are modified to consist both materials. Distillation takes place continuously and not in batches as with the case of pot still distillation, and the distillate obtained has an alcohol concentration ranging from 50% to 90% abv. Distillates having a high (80–90%) alcohol concentration is less aromatic than that obtained from pot still distillation. The decision made as to what apparatus should be used for the production of brandy is dependent on the style of brandy desired.

Maturation and Blending

After the distillation process, the distillate is placed in oak barrels to mature. These barrels vary with regard to their size, type of wood, and the length of the maturation period that is determined by the laws and regulations that govern each country. After maturation, the brandy is blended with distilled water to decrease the alcohol concentration, and additives such as caramel, sugar, and oak infusion may in certain instances be added. Again, these additions are dependent on the legal classifications of each country.

Sensory Perception and Flavor Chemistry

The sensory perceptions that form part of the brandy, Cognac, and Armagnac consumers' product experience include appearance, aroma, and mouthfeel. These perceptions are the response to a complex chemical structure that is influenced throughout the entire brandy production process as described earlier in the text.

Color and Appearance

Brandy color hues range from greenish tints to straw yellow, golden, and topaz. Brandy can also differ in terms of color intensity, from lightly colored to dark, but never hazy or opaque. Brandy color is influenced by barrel maturation and color rectification with flavorless caramel. The color of brandy has a significant impact on how the aromas and mouthfeel of the product are perceived. Both Cognac and Armagnac color hues tend to darken with aging; young products are typically described as golden or honey in color, while older products are described as amber or mahogany.

Brandy Aroma: Origin and Perception

The compounds responsible for brandy aroma can be traced back to those originating from the grapes and formed during alcoholic fermentation, which are subsequently concentrated into the heart fraction during distillation. Cognac literature also describes the formation of notes such as cocoa and grilled nuts, derived from the cooking process that occurs as the base wine is heated for distillation. Compounds that are extracted, formed, or modified during wood maturation also have a significant contribution to the flavor profile of brandy, Cognac, and Armagnac.

Aroma compounds that originate from grapes include six-carbon alcohols, terpenes, aldehydes, and products of carotenoid degradation such as α-ionone. These compounds typically impart fruity, floral, and green aromas. The presence of grape-derived compounds is influenced by the grape cultivar, terroir, climate, and viticulture and harvesting practices.

Esters are one of the major fermentation-derived flavor compounds contributing to brandy aroma and are known to impart fruity aromas. The final ester content of brandy is influenced by several factors: the ester composition of the base wine, the occurrence of malolactic fermentation during base wine production, the presence of yeast lees during distillation, and the point at which the heart fraction is cut during distillation. Other by-products of alcoholic fermentation that contribute to brandy aroma, through synergistic and masking effects, are higher alcohols, volatile medium- and long-chain fatty acids, and aldehydes.

During Cognac distillation, the heating process can result in ester and terpene hydrolysis. Maillard reactions and Strecker degradation processes can also take place as a result of the heating process, leading to the formation of furans, pyridines, pyrazines, aldehydes, and acetals, all of which can contribute to Cognac aroma.

The most important aromatic compounds that can affect brandy aroma, as a result of wood maturation, are oak lactones, phenolic aldehydes, and furanic aldehydes. The quantities at which these compounds can be found in brandy depend not only on the wood species used but also on the seasoning and toasting practices during barrel manufacturing. Wood maturation affects brandy aroma through flavor compounds present in the wood that are directly extracted from wood lignins into the spirit, where they can undergo further conversions. Alcohol in the spirit can also react with wood lignins to form new flavor compounds, as well as modifications of spirit congeners carried over from distillation.

The wood species used during barrel maturation have been found to impact significantly vanilla, woody, caramel, burned/toasted, green, tails, and rubbery aromas. Oak wood tends to have more intense woody notes due to the presence of *cis*- and *trans*-β-methyl-γ-octalactones, which do not occur in other wood species. Wood toasting has been associated with an increase in vanilla, woody, spicy, caramel, burned, toasted, dried fruits, and smoke notes. However, this is not a linear effect, as degradation of oak lactones and furanic aldehydes at very high toasting temperatures can result in a decrease in woody and vanilla characters. Wood toasting has also been found to have a decreasing effect on fruity, green, tails, and glue-like aromas in brandy. These characters are further decreased during the maturation process. As the maturation period lengthens, the vanilla, woody, rancid and spicy, toasted, smoke, and caramel notes become more intense. Reports have indicated that most of the flavor evolution that takes place during brandy maturation occurs within the first 3 years of maturation.

In Cognac production, the presence of 'rancio' notes is an indication of the age and refinement of the product. This term refers to a complex set of aroma notes that develop during the

maturation process and is the result of a mixture of aromatic methyl ketones and compounds resulting from the oxidative degradation of oak components after at least 10 years of maturation.

Evaluation of Brandy Aroma

Due to the volatility of the flavor compounds at the alcohol level of brandy, it is recommended not to swirl the product prior to nosing as is commonly done during wine evaluation. Instead, brandy aroma can be best appreciated by starting to hold the glass a few centimeters away from the nose. Here, the delicate fruity and floral notes can be observed without being overpowered by ethanol. Gradually, the product can be brought closer to the nose to introduce first the sweet and nutty flavors and finally the heavier woody flavors. Aroma wheels have been developed for South African brandies and Cognac and Armagnac as aroma recognition aids during product evaluation.

Brandy aroma wheel

The South African brandy aroma wheel categorizes the types of aromas perceivable in brandy into a two-tiered system shown in Figure 1. Although the aroma wheel was developed for South African brandies, the wheel is representative of the characteristics that can be perceived in brandies from other countries. The fruity, muscat, floral, woody, toasted, nutty, sweet, and spicy characters indicated on the aroma wheel are considered as positive and, therefore, adding to quality. In contrast, characters such as tails, green, rubbery, and solvent-like detract from quality.

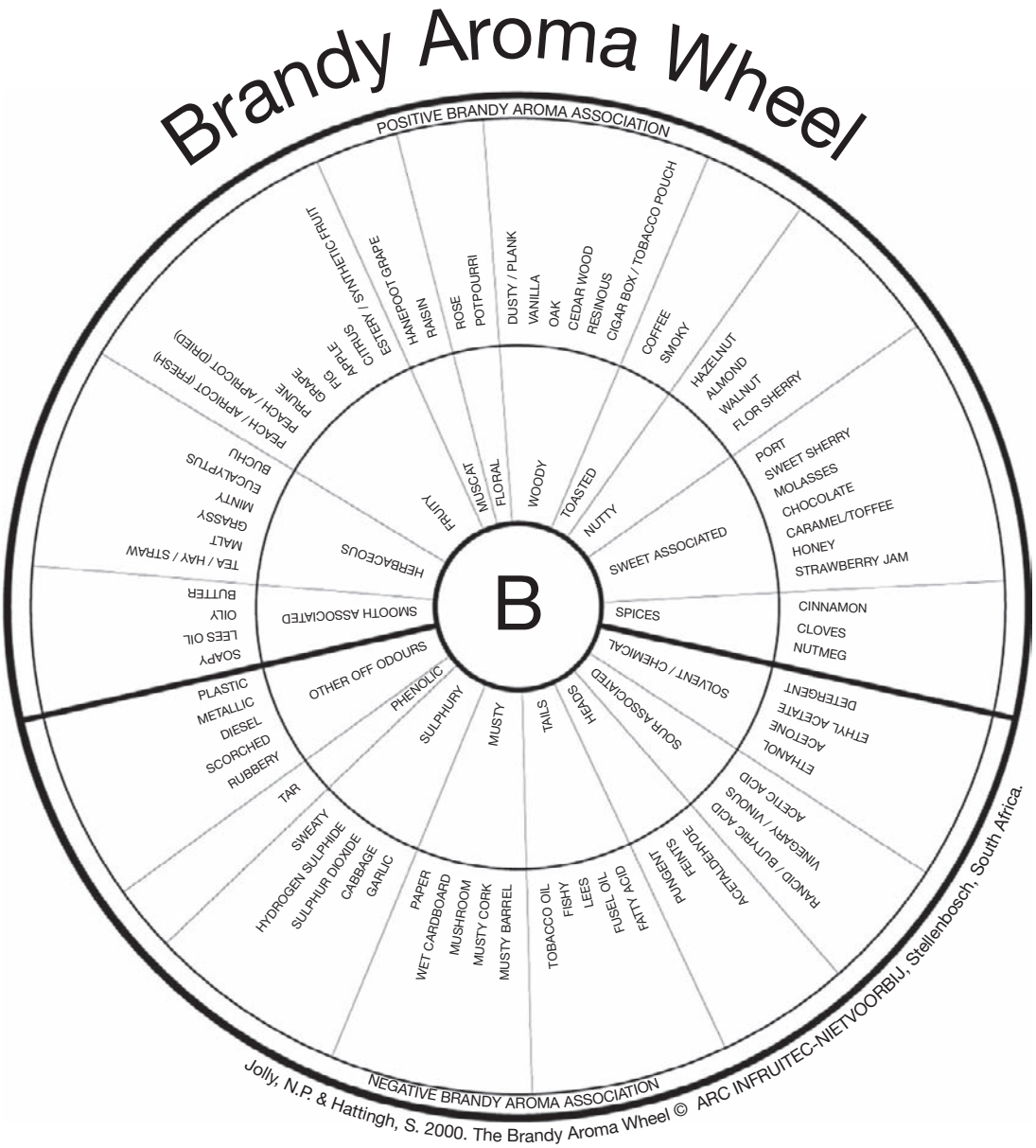

Figure 1 Aroma wheel developed for South African brandies. Reproduced from Jolly, N. P. and Hattingh, S. (2001). A brandy aroma wheel or South African brandy. *South African Journal of Enology and Viticulture* **22**, 16–21.

Cognac aroma wheel

The Cognac aroma wheel was developed by the BNIC and includes over 60 aromatic notes. Unlike the brandy aroma wheel, where the tiers are organized into aromatic families that contribute positively and negatively to brandy quality, the Cognac notes were categorized into four families corresponding to the four seasons: spring, summer, autumn, and winter (Figure 2). The reasoning behind this unique format was to encourage consumer engagement in Cognac appreciation. Prior to the development of the seasonal aroma wheel, the flavor developments that occur during Cognac maturation have been modeled for each of the four main aroma families: fruity, floral, woody, and spicy.

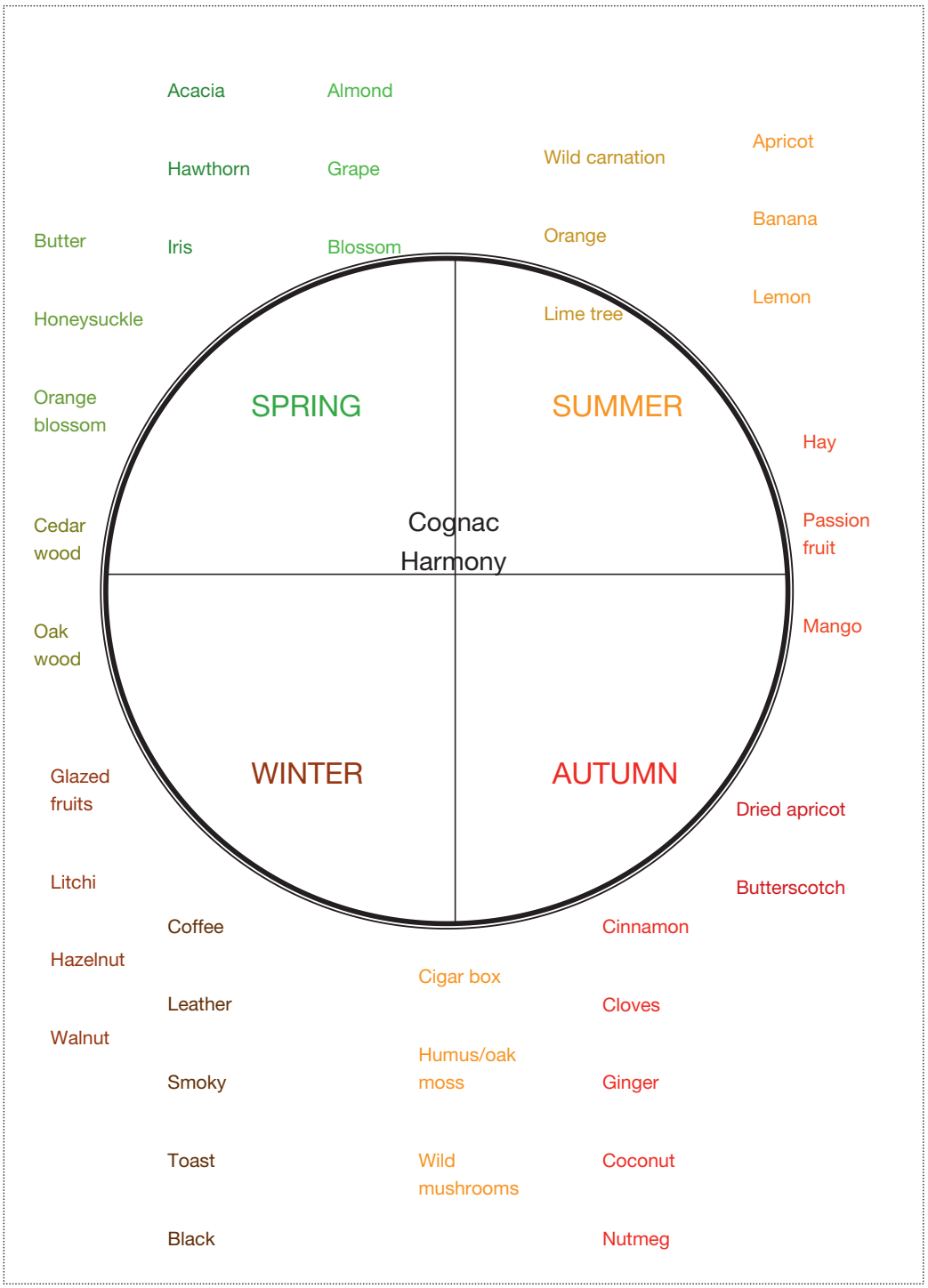

Figure 2 The cognac aroma wheel (Lenoir J (2009) *Cognac Aroma Wheel*. Second International Cognac Summit. Bureau Interprofesionel du Cognac).

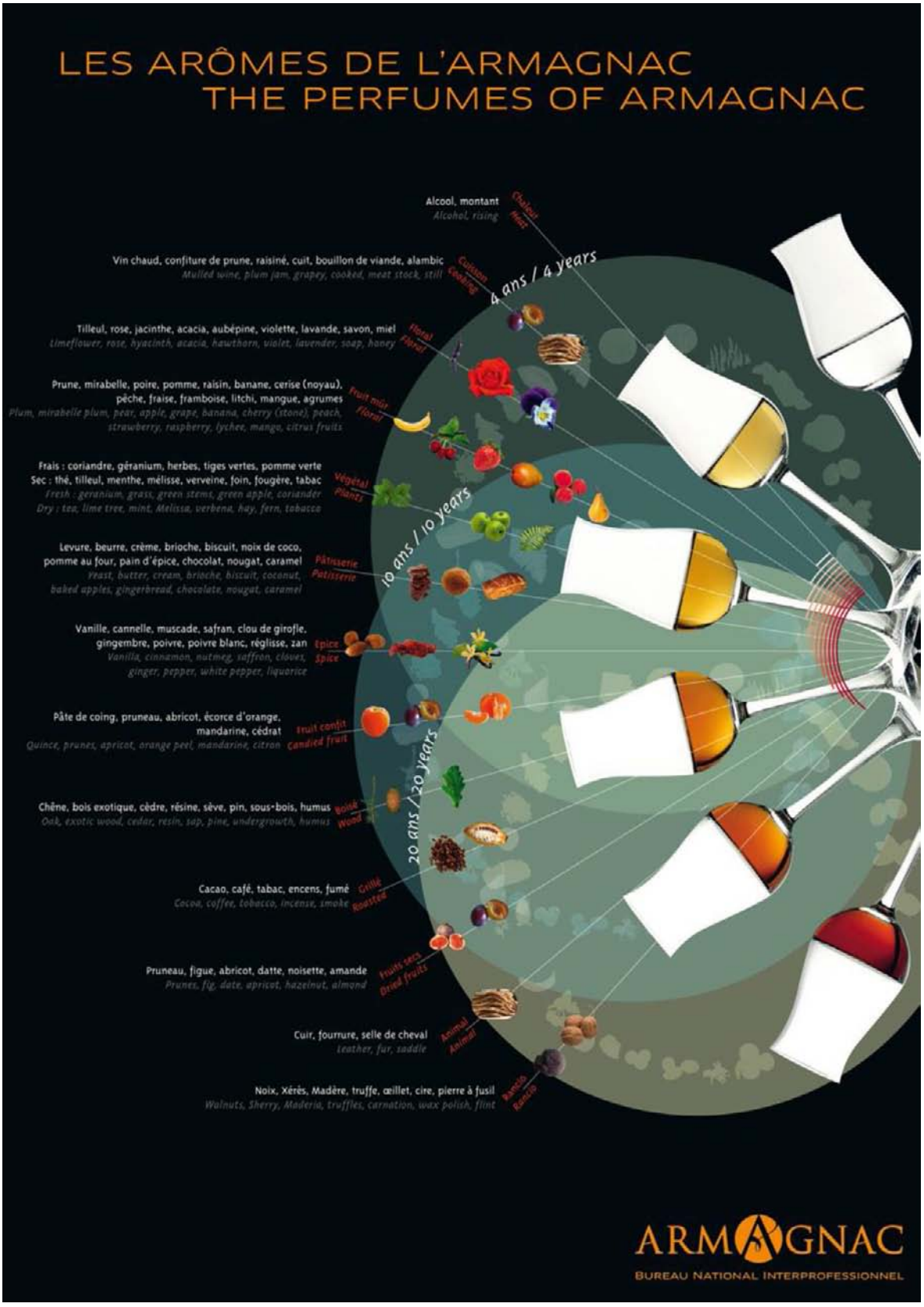

Figure 3 Aroma wheel developed for Armagnac (Bureau National Interprofessionel de l'Armagnac).

Armagnac aroma wheel

Similarly, the Bureau National Interprofessionel de l'Armagnac (BNIA) developed an aroma wheel for Armagnac evaluation. The Armagnac wheel is organized into three overlapping circles that illustrate the flavor evolution that takes place during Armagnac aging (Figure 3). The first circle shows the notes associated with Armagnac products aged for up to 4 years, followed by those associated with 10-year-old products, and finally 20-year-old products. The wheel also includes a color chart showing the variety of color hues associated with Armagnac, ranging from pale to amber.

Brandy Mouthfeel Perception

Brandy mouthfeel develops during wood maturation as low-molecular-weight and hydrolyzable tannins are extracted from oak. These compounds impact the perception of smoothness, burning, astringency, bitterness, and body. As brandy matures, its body and flavor complexity increases, whereas astringency and alcohol burn decrease.

A Comparison of the Cardioprotective Effects of Brandy and Wine

It is well-known that moderate alcohol consumption has a beneficial influence in decreasing the incidence of coronary heart disease. In fact, several epidemiological, case–control, and cohort studies suggest that there might be a causal relationship. Recent reports also indicate an association between light to moderate consumption of red wine and a decreased incidence of diabetes and reduced risk of cardiovascular disease and consequential mortality.

The health benefits of wine are thought to be linked to interactions between the antioxidant, polyphenol, and ethyl alcohol contents that are especially prevalent in red wine. These compounds are known to have anti-inflammatory, anti-atherogenic, and vasorelaxant properties and can also contribute to preventing lipid toxicity, decreasing aggregation of platelets, which leads to blood clotting, and improved blood viscosity.

A South African study recently compared the health effects of the moderate consumption of brandy, which contains more ethyl alcohol and less health-promoting polyphenols than wine, to those of moderate consumption of red wine in healthy adults. The consumption of both wine and brandy resulted in a significant increase in high-density lipoprotein (HDL) cholesterol, which favors heart health.

However, evidence suggests that not everyone may benefit from the health benefits of moderate alcohol consumption; gene–environment interactions may play a role in whether consuming moderate amounts of alcohol has a positive or negative effect on an individual's cardiovascular health. For instance, it has been found that moderate consumption of both red wine and brandy resulted in a significant increase in triglyceride levels in individuals carrying risk-associated, low-penetrance mutations of the HFE gene, previously shown to underlie hereditary hemochromatosis.

Genotyping performed as part of personalized risk assessment may identify a high-risk genetic subgroup of individuals likely to derive less benefit from alcohol consumption. Nutrition intervention may be more effective to lower cardiovascular risk when the genetic background is taken into consideration to identify of gene–environment interactions. This type of assessment may be particularly relevant to patients expressing the metabolic syndrome: a constellation of cardiometabolic risk factors including central obesity, hypertension, impaired glucose tolerance, and dyslipidemia (high triglyceride levels and/or low HDL levels). Further investigations into the role of genes and environment, in assessing mechanisms underlying the benefits of alcohol use and cardiovascular disease risk, may lead to the development of standardized limits for safe alcohol consumption, guided partly by individual genotype.

See also: Alcohol: Metabolism and Health Effects; Alcohol: Properties and Determination; Brandy and Cognac: Manufacture and Chemical Composition; Gin; Rhum–Ron–Rum: Technology and Tradition; Vodka; Whisky, Whiskey and Bourbon: Composition and Analysis of Whisky; Whisky, Whiskey and Bourbon: Products and Manufacture; Wines: Champagne and Sparkling Wines – Production and Effervescence; Wines: Madeira, Port and Sherry Fortified Wines – The *Sui Generis* and Notable Peculiarities. Major Differences and Chemical Patterns; Wines: Types of Table Wines; Wines: Wine and Health; Wines: Wine Production; Wines: Wine Tasting.

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Brandy and Cognac: Manufacture and Chemical Composition

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Introduction

Various spirit drinks (alcoholic beverages) are produced globally today under the name brandy. This term includes alcoholic beverages obtained exclusively from wine spirits or from a mixture of wine spirits and wine distillate. Wine spirit is produced by batch distillation, in small pot stills (alembic), or by continuous distillation, in small column-still distillation. On the other hand, wine distillate is produced by continuous distillation in tower column stills. For the production of most brandies, a maturation period in wooden barrels is required. Expanding the definition even more, in some countries, brandy includes spirits from other fruits like plum and apricot, molasses, or even pure alcohol. In this article, we will consider only the grape brandy.

The term cognac, according to European legislation, falls within the category of 'wine spirits,' and not in that of brandies. It is produced solely in the homonymous Cognac region of France. But while the word brandy is considered a generic term, cognac is referred to as a variety. Juxtaposition of the words brandy and cognac, even in the title of this article, can create various kinds of misunderstandings. Confusion also results from the fact that 'wine spirits' in official European Union documents is used as for broad category of alcoholic beverages, to which cognac belongs to, and simultaneously serves as raw material for the production of both cognac and brandy. Brandy, according to European Union legislation, can only be produced from wine spirit (distillate at $<86\%$ (v/v)) or by mixing wine distillate (distillate at $<94.8\%$ (v/v)) provided that wine distillate does not exceed a maximum of 50% of the alcoholic strength of the final product. Besides the difference in the alcoholic strength, there is no other legal distinction between wine spirits and wine distillates. In practice, wine spirits contains a higher amount of volatile aromatic compounds (congeners) than wine distillate. Wine distillate may be similar in composition to wine spirits, or more often, it is almost identical to pure ethyl alcohol. Pure ethyl alcohol 96.1% (v/v) is used for the production of other alcoholic beverages and is almost completely free of volatile aromatic compounds. Some brandies are made using similar processes as cognac, that is, without the addition of wine distillate, but for the production of 'wine spirits,' redistilled wine distillate can also be used as starting material. Moreover, always in accordance with EU legislation, 'wine spirits' may be released without employing a maturation step, while for brandy, this step is obligatory. US legislation considers cognac as a geographically designated type of brandy. It is thus obvious that the definition of brandy is quite complex since it reflects not only actual differences in the production process but also economic interests. Even more complicated is the case of labeling. There is a gap of legal EU definitions regarding sweetening and maturation indications on the label. On the contrary, cognac maturation period and the corresponding indications are strictly defined by French legislation. It should also to be noted that spirit drinks originated from grape fermentation fall in the category of fruit spirits. Grape marc is another category of spirit drink.

Regarding chemical composition, wine spirits (such as cognac) are characterized by a higher content of aromatic components compared to brandies made by wine distillates. Sensory characteristics of both categories are very closely related to the region of origin. Cognac is considered a superior-quality product and as such it enjoys higher prices. Sensory evaluation of wine spirits is similar to that of wine tasting since it includes assessment of color, odor, mouth feel, flavor, and aftertaste. The primary concern of a producer is the lack of features that render a product defective. Such defects are the perceptions of sulfur; oxidation odors; unripe, rotten, sour taste; and burning sensation. Nose examination involves typicality and elegance, a complex of aromatic richness, and complexity. Mouth evaluation focuses on intensity, harmony, and persistence. It is difficult to define precisely the sensory characteristics and correlate them with the corresponding chemical composition. The simultaneous existence of more than one attributes along with their interactions defines elegance and hence quality. Several olfactory attributes are characteristic. The most significant are the aromas of grape and fermentation origin, such as the flower and fruity odors and the maturation aromas of wood origin, like smoke, vanilla, wood, and spice. The characteristic mouth feel sensation is the low content of acids and a burning sensation due to the high ethanol content. The world production of spirit drinks is about 20 billion liters, 1.2 of this is brandy, while only 0.1 is cognac.

Grape Production

Viticulture and Grape Varieties

The soil in which the vines are grown is an important factor that determines wine quality. The composition and structure of the soil affect the productivity of the vineyard and consequently the quality of the distillates. High production yields are undesirable, since they result in excessive dilution of the aromatic ingredients. Grapes should undergo a progressive maturation, in order to reach the best possible aromatic quality. Plantation and cultivation of a vineyard should follow the best viticultural practices to produce high-quality grapes, wines, and distillates. Abundant rainfall during maturation period may lead to the development of diseases and to the presence of putrid odors in the distillates. Low temperatures could lead to insufficient ripening and immature flavors, while high temperatures can accelerate the oxidation of many

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odorant compounds. The condensation that takes place during the distillation process results in a significant increase in the concentration of the compounds responsible for the brandy's intense and strong flavor. For this reason, the selection of white wines with relatively neutral flavor is preferred for brandy and wine spirit production. Aromatic grape varieties with strong and persistent flavor may be appropriate for the production of high-quality wines. However, they are unsuitable for brandy production since they result to distillates with too intense an aromatic character.

An acre, depending the region and year, can produce 5000–50 000 kg of grapes and potential strength in ethanol (after fermentation) 7–14% (v/v). For example, one acre producing 15 000 kg of grapes can produce 10 000 l of wine for distillation at 12% (v/v) ethanol at 20 °C, corresponding to 1200 l of pure ethanol. From this wine is produced approximately 2500 l of spirit drink of 40% (v/v). A small part of ethanol is lost during distillation and maturation.

Grape Berries

Grapes constitute the raw material for winemaking. Sometimes, in order to enhance vine protection, pesticide application is required. Wine spirits, wine distillates, and consequently the grapes should be as free as possible from pesticides used against insect, fungal, or viral pests. Although pesticides may have an influence on the fermentation process, they do not affect the sensory quality of the wine and distillate. In contrast, the addition of sulfur during vine spraying or dusting can produce undesirable reduction odors, often attributed to H_2S. Carbohydrates (mainly glucose and fructose) are the most abundant constituents of must after water. Sugar concentration in normally ripened grapes varies from 130 to 260 g l^{-1}, resulting, after fermentation, in wines with alcohol strengths ranging from 8% to 14% (v/v), respectively. Regulation of must acidity (mainly due to tartaric, malic, and citric acids) is not essential for distillate quality, since these acids are not volatile. Moreover, wine phenolic compounds, like tannins and anthocyanins, are not volatile and thus do not affect distillate quality. This is the reason why fresh distillates are always colorless.

Vinification

Must Extraction from Grape Berries

The harvest should not necessarily coincide with maximal sugar concentration, but with aromatic ripening. Grapes must be transported as quickly as possible to the winery for must extraction. Putrid grapes should be removed with careful screening. Must extraction and vilification is similar to white wine making. The stalks are removed from the grapes, which are then crushed and pressed. Continuous presses have the disadvantage of increasing the presence of solid particulates from the grapes and releasing undesirable compounds with herbaceous aromatic character. Only the must that is produced by the application of low pressure (extraction of about 80% of the total must) is used. Must that is extracted using high pressure is usually fermented separately and it is distilled in continuous tower column stills to produce pure alcohol.

In general, sulfur dioxide is not added to musts and wines, since it could be transferred into the distillate and consequently decrease its quality by neutralizing the aromatic perception. In certain cases, however, where the hygienic condition of the grapes is not adequate, 0.01 g $l^{-1}$of sulfur dioxide may be added to the must. The time that elapses between pressing and the start of fermentation should be the minimum possible, due to the absence of sulfur dioxide that protects must and wine from oxidation and microbial spoilage.

Methanol (methyl alcohol) is not produced by alcoholic fermentation. It is formed exclusively from the enzymatic hydrolysis of the methoxyl groups of pectins during fermentation. It is always present in very small quantities in wine. However, in wine spirits, it is found in higher concentrations ranging 0.30–0.70 g l^{-1} of pure alcohol (ethanol). Its smell and taste are similar to ethanol, and since it is present in low concentrations, it does not affect the sensory quality of the spirit. It affects, however, spirit safety since its toxicity is well known. EU legislation requires a limit lower than 2.0 g l^{-1} of pure alcohol. Unripe grapes and continuous presses may induce herbaceous character by liberating compounds, such as hexanols (1-hexanol and 2-hexanol) and hexenols (*cis*-3-hexene-1-ol, *trans*-2-hexen-1-ol, *cis*-2-hexen-1-ol). 1-Octen-3-ol is characterized by a mushroom odor and it is found in grapes infected by *Botrytis cinerea*. β-Damascenone is an isoprenoid ketone that is naturally present in grapes, and it is a highly odoriferous compound with a powerful and pleasant fragrance providing a fruity-flowery and honey-like character.

Fermentation, from Must to Wine

Wine designed for wine spirits and brandy production is produced from must by yeast fermentation. Alcoholic fermentation of must occurs spontaneously or by addition of dry industrial yeasts. During the period between fermentation and distillation, several chemical reactions take place and the wine composition alters. Care should be taken to keep this period as short as possible. Distillation can immediately take place after the end of fermentation.

Volatile Substances, from Wine to Distillate

Ethanol (ethyl alcohol) is the most abundant compound in wine after water. It is produced by the alcoholic fermentation of glucose and fructose and ranges at levels 8–14% (v/v). During alcoholic fermentation, various aromatic compounds (congeners) are produced besides ethyl alcohol. It is obvious that the aromatic content of the distillate is much higher than that of the corresponding wine. The content of congeners in distillate is expressed in grams per liter of pure alcohol (g l^{-1} of pure alcohol), in order to be independent of distillation and dilution with water. The ethyl alcohol content of both wine and spirit drinks is expressed as volume per 100 cubic centimeters of finished product at 20 °C and is denoted by % (v/v).

Alcohols possessing more than two carbon atoms are known as higher alcohols or fusel oils. They are synthesized by yeasts during fermentation. Wine higher alcohol content remains

almost unaffected before distillation. Quantitatively, the most important higher alcohols are the straight-chain alcohols 1-propanol, isobutyl (methyl-2-propanol-1), and amyl (a mixture of 2-methyl-1-butanol and 3-methyl-1-butanol) alcohols. Most straight-chain alcohols and their esters have a strong pungent smell. At low concentrations, they contribute to the aromatic complexity but, at higher levels, are characterized by penetrating odors, which mask the aromatic finesse. They reach concentrations in the order of 2.5–5.0 g l^{-1} of pure alcohol in distillates.

Acetic acid is the main volatile acid that has a significant contribution to volatile acidity. It has a vinegar-like intense odor. Although it commonly occurs in wine, it typically occurs at detectable levels only in wines spoiled by acetic acid bacteria. In distillates, its concentration ranges from 0.20 to 1.0 g l^{-1} of pure alcohol. Other carboxylic acids, such as propionic acid and butyric acids, may also be present and they are also associated with bacterial activity before distillation. Butyric acid is characterized by unpleasant buttery and cheesy aromas. Hexanoic, octanoic, decanoic, dodecanoic, myristic (14 carbon atoms), palmitic (16 carbon atoms), and stearic (18 carbon atoms) acids are mainly formed by yeasts.

Ethyl esters are produced by yeasts. They can also be present in grapes, but their amount and sensory importance are often negligible. Esters are present in fresh brandies, and since they have fruity aspects, they have an important contribution to the development of their aroma. Over 160 esters have been identified in wines and most of them are also present in brandies.

Of the monocarboxylic acid esters, the most important are those based on ethanol and saturated carboxylic acids, such as hexanoic (caproic), octanoic (caprylic), and decanoic (capric) acids. Preserving wines before distillation in the presence of lees has been connected with increased content of ethyl esters.

Distillates of wines that have remained in contact with lees contain greater amounts of ethyl decanoate and ethyl dodecanoate. Wine and distillates also contain ethyl esters of long-chain fatty acids (14–18 carbon atoms). The presence of the fatty acid ethyl esters in quantities higher than 5 mg l^{-1} may deteriorate the quality of the bottled spirit, depending on the alcohol content of the product and the storage temperature.

The most prevalent ester in wine is ethyl acetate. A small quantity is produced by yeasts during fermentation, but it is mainly formed by the activity of the aerobic acetic acid bacteria. Wine distillates and brandies contain about 0.4–0.8 g l^{-1} of pure alcohol.

Esters of acetic acid and higher alcohols are also significant since they may provide a fruity character. For example, isoamyl acetate, which has a characteristic banana odor, influences positively wine distillate and brandy aroma. Low fermentation temperatures favor synthesis of fruity esters, such as isoamyl, isobutyl, and hexyl acetates, while higher temperatures favor the production of higher-molecular-weight esters. Juice clarification favors ester synthesis and retention.

Acetaldehyde (ethanal) is the major aldehyde found in wine. It is one of the early metabolic by-products of yeast fermentation. More acetaldehyde is produced through autoxidation of ethanol. An important quantity is bound to sulfur dioxide in cases where it is has been added to the base wine. In distillates and brandies, it is found in concentrations ranging from 0.20 to 0.25 g l^{-1} of pure alcohol.

Other aldehydes that may be found in brandies are formaldehyde, 5-hydroxymethylfurfural, acrolein, propionaldehyde, butyraldehyde, benzaldehyde, isovaleraldehyde, and *n*-valeraldehyde.

Isobutanal at concentrations higher than 25 mg l^{-1} may provide a herbaceous character to the wine distillate and brandy. *trans*-Nonenal is characterized by a paperlike sense, while octanal contributes to the aroma complexity by adding an orange flavor.

Diacetyl (2,3-dioxobutane) is a ketone produced during wine fermentation through oxidation of acetoin, a degradation product of citric acid. It has an important influence on sensory evaluation since its odor is characterized as sweet, buttery, or butterscotch-like.

Distillates may contain extremely low concentrations of different unpleasant (rotten eggs and garlic) volatile sulfur compounds, like hydrogen sulfide, carbonyl sulfide, sulfur dioxide, thiols, sulfides, polysulfides, and thiosterols.

Most aldehydes and acetals are more volatile compared to other aroma-related compounds, and hence, they are distilled early giving a pungent odor in the heads. The volatile acids are distilled throughout the distillation process. Higher alcohols and ethyl acetate are found in higher proportions in the first distillate fractions, and gradually, their presence decreases, while the opposite is a trend observed for ethyl lactate.

Finally, distillates derived from alembic batch distillation contain 99% of initial content of ethanol, 50–60% of methanol, 90–95% of higher alcohols, 3–5% of the 2-phenyl ethanol, 70–75% of esters, and in particular 40–60% of ethyl acetate.

Wine with higher alcohol and polyol content is not influenced by the time that elapses after the end of fermentation in contrast with its ester content, which can decrease significantly. The most affected esters are those that possess the aromatic properties of interest (such as isoamyl, hexyl and phenylethyl acetate, ethyl caproate, ethyl caprylate, ethyl caprate, and ethyl laurate). An undesirable increase in ethyl acetate, ethyl lactate, diethyl succinate, acetaldehyde (ethanal), and acetic acid of the base wine content is usually observed simultaneously.

Distillation

During the distillation process, volatile components are extracted from wine and are partially concentrated into the distillate. The aim of the distillation is to remove the largest amount of water and recover the maximum amount of ethanol and the positive characteristic aromas while minimizing the off-flavors.

A peculiarity of the production of spirits is that the base wine is distilled while it is in contact with the lees. The lees contain dead yeast cells, whose autolysis enriches wine with aromatic components present in the cell walls. It should be noted, however, that the lees must be as free as possible from grape material, since they may provide herbaceous odors that will be transferred into the distillate. In continuous distillation, wine lees are removed just before distillation since their presence could block the distillation equipment and delay the whole process. Thus, in order to modify the spirit

composition, the distillers should control two crucial parameters: wine flow rate and the temperature of distillation and distillate.

Small Pot-Still Batch Distillation

Apparatus

Small pot stills (Figure 1) for batch distillations are usually made of copper. Copper is an excellent conductor of heat and is the most common material used for distillation apparatus. Moreover, it is considered advantageous as it is easily manipulated and reacts with undesirable components of the distillate, such as fatty acids and sulfur compounds, creating insoluble sediments. In this way, components with unpleasant odors are partially removed during distillation.

The pot still can be heated indirectly by steam circulating through copper heating exchangers located a few centimeters above the bottom of the apparatus. The heating may also be achieved by passing steam through a jacket located at the bottom. Habitually, wine is heated directly by flame. For this purpose, a stove fed by wood or gas is required. The type of heating system should be carefully designed to perfectly adjust the shape and the distance of the flame from the boiler, in order to avoid local overheating, which may result in burned odors.

The pot still consists of four consecutive sections. Above the boiler, a cover (hat) is mounted that ends in a pipe, which leads vapors to the fourth part, the cooling coil. The cover prevents overflow during boiling and acts as a reflux condenser. A further part that the pot still may have is the preheater. It can save energy (fuel), time, and cooling water by increasing the temperature of the wine just before distillation. The disadvantage of using an alcohol preheater, however, is the loss of ethyl alcohol.

The condenser comprises a serpentine or many flutes. The temperature of the cooling water must not be too low or exceed 20 °C, since aromatic substances might prematurely condense or ethyl alcohol may evaporate, respectively. The distillate must be collected in stainless steel containers. The sampler is an essential component of the machinery that provides sample shots when needed. An alcoholmeter permits the simultaneous measurement of the alcoholic strength of the distillate during the distillation process.

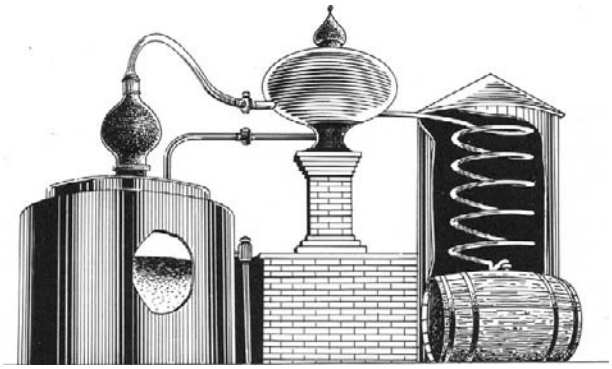

Figure 1 Small pot still (alembic), for batch distillation.

First Distillation

Distillation is accomplished at two successive times (steps). The first step is to produce a distillate called 'low wine' and the second one gives the 'heart,' the wine spirit. Both steps are necessary since after the first, a distillate of approximately 30% (v/v) is obtained, while the second step raises the alcohol content up to 70% (v/v).

When the temperature reaches the boiling point, vapors pass through the preheater, the cover, and the pipe and enter the condenser where condensation takes place. And the distillate is collected in an appropriate container. Since ethyl alcohol is more volatile than water, vapors are initially very rich in ethyl alcohol, while a gradual reduction takes place with time, increasing the boiling point. Thus, during the distillation process, the composition of the distillate changes continuously and the liquid becomes gradually poorer in ethyl alcohol. Finally, the residual consisting mainly of water and nonvolatile wine components remains in the boiler. Such ingredients are organic acids, salts, tannins, and minerals. The average alcoholic strength of 'low wine' is between 24% and 32% (v/v), depending on the alcohol content of the wine distilled and the distillation rate.

In some cases, separate heads and tails are isolated, even after the first distillation step, in order to result in a distillate with less congeners.

Second Distillation

After the first distillation, the boiler is emptied of the distillation residue. The second distillation is called the final distillation. Usually, the whole quantity of wine is distilled before proceeding to the second distillation, in order to avoid possible spoilage.

The second distillation requires special attention and expertise, since the distillate must be separated into three fractions, the 'heads,' 'heart,' and 'tails.' The 'heart' is the wine distillate, the part of the distillate that contains the desirable volatile components necessary for maturation. The distillation rate is adjusted taking into account that after the end of the process, the alcohol content of this fraction should be higher than 70% (v/v). The separation is achieved through monitoring of the temperature and volume of the distillate, the indication of the alcoholmeter, and the results of the sensory evaluation. The separation between the heart and tails is performed at 54% (v/v). The distillation technique employed depends on the wine and the desired final product.

Examples of Distillation

For example, 2500 l of wine, at 12% (v/v), can produce about 1000 l of 'low wine' (28% (v/v)) after 12 h of distillation. Distillation is ended when the indication of the alcoholmeter, placed at the exit of the distillate, is about 2% (v/v). A small portion of the ethanol content is lost during distillation. 2500 l of 'low wine' 28% (v/v) will yield about 40 l of 'heads,' 900 l of 'heart' (70% (v/v)), and 500 l of 'tails' after 14 h of distillation.

According to another technique, three fractions are obtained in the first distillation and four fractions are obtained after the second distillation. This greater fractionation allows

better separation. For example, the first distillation results in 1% heads, 28% heart, and 5% tails. The second distillation results in 1% heads, 28% heart, 28% second, and 5% tails. Heads and tails of the first and second distillations are mixed with the wine or the first distillate and redistilled or are distilled separately to produce a distillate of inferior quality.

Volatile Compounds Formed During Distillation

During the distillation process, a number of chemical reactions take place together with the evaporation of the volatile compounds. These reactions, which involve esterifications, acetalizations, Maillard reactions, and Strecker degradations, can greatly affect spirit quality.

An aldehyde having a sensory impact of 'baked' in distillates is furfural (ranging 0.5–82.5 mg l^{-1} of pure alcohol). Its synthesis involves sugar oxidation and is activated by heat. It is mainly produced during distillation from the remaining pentose content of the lees, and consequently, it is highly influenced by the distillation system employed. For this reason, the concentration of wine distillate and brandy in furfural varies largely. Furfural and its derivatives may also derive from both the wooden cask and the possible addition of caramel. Double distillation enhances the amount of all furanic species.

Ethyl carbamate is a potential carcinogenic compound and its presence is strictly monitored in wines and spirits. Yeasts may be involved in its synthesis through the production of carbamyl phosphate and by the synthesis and degradation of urea.

Minerals Found in the Distillate

Although wine contains different metals, these metals are not volatile and, therefore, are not found in the distillates. Distillates acquire their metallic content by contact with different metals, like aluminum and cadmium. Copper is a very important trace element, due to its heat-conducting and chemical properties. Distillates always contain a small amount of copper. This is not due to the copper content of the base wine, but rather to the copper parts of the distillation apparatus. During distillation, copper combines with other compounds such as butyric, caproic, caprylic, capric, and lauric acids resulting in precipitates, improving the quality of the distillates. Most of the copper found in wines originates from sprays based on the disinfectant properties of copper sulfate, used to treat vines for mildew. Copper could combine with caprylic, caproic, and lauric fatty acids, whose odor resembles that of cheese, as well as with long-chain fatty acids, which could produce insoluble soaps. As far as distillation is concerned, its presence is connected with higher-quality distillates. Distillation in stainless steel distillers results in poorer-quality brandies. The process can be improved by the addition of exogenous copper or copper sulfate.

Small Column-Still Continuous Distillation

Small column-still continuous distillation apparatuses (Figure 2) comprise a boiler with 5–15 plates (disks), a limited number of perforated trays, and a cooler with two chambers. In the upper chamber, wine for distillation is preheated by vapors of distilled wine, and in the lower chamber, the vapors are condensed by cool water. The wine to be distilled initially enters in the cooling chamber, and then, it enters into the second plate. The liquid passes progressively though the plates until it reaches the lower part of the boiler where it is simultaneously heated and evaporated. The vapors of ethanol, being more volatile, reach the upper part of the apparatus, passing progressively through the plates. Condensation occurs when vapors come in contact with the liquid, which is simultaneously heated. Parts of the vapors reach the upper part of the boiler, where they are cooled in the preheater chamber. Such distillation equipments operate continuously, with a constant supply of wine to be distilled. The distillate produced, depending on the number of disks used, has an alcohol content of approximately 70% (v/v). This process is used for the production of Armagnac, a wine distillate produced in the homonymous region of France.

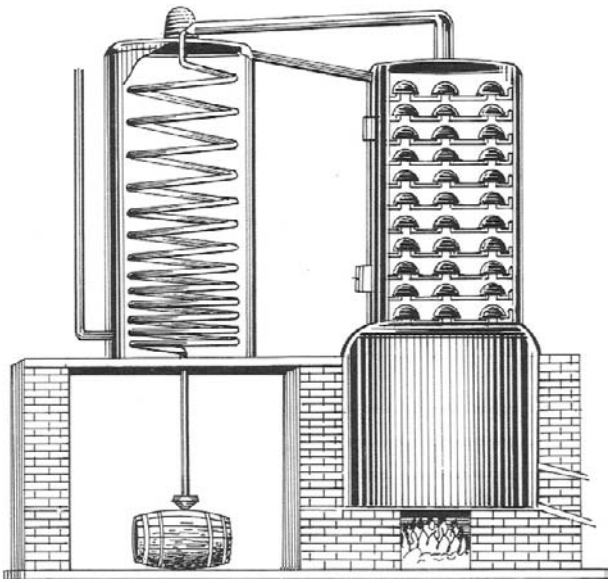

Figure 2 Small pot still, for continuous distillation.

Continuous Tower Distillation

In order to produce pure ethanol, continuous tower distillation columns (Figure 3) are employed. The liquid to be distilled is constantly supplied into the apparatus, which may consist of 2–6 towers. In this way, water is removed together with part or whole of the volatile compounds (flavor components and congeners). A very common continuous distillation apparatus used for the production of pure alcohol or wine distillates consists of three columns. The wine to be distilled enters the first column, where steam sequentially passes through the disks flowing from the lower to the upper part of the column. The column material is bronze or stainless steel and the disks contain 'bells' (Figure 4). In each bell, the steam comes into contact with the descending liquid, resulting in the gradual depletion of ethanol. The liquid reaching the lower part of the tower is thus free of ethanol, while the vapors rich in

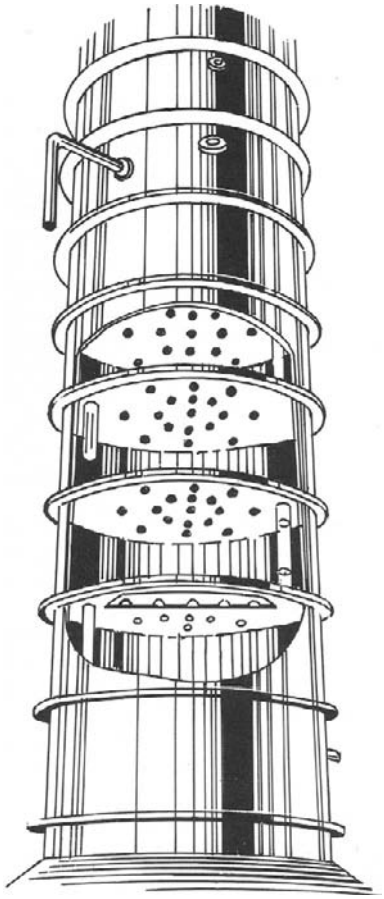

Figure 3 Tower columns still for continuous distillation. Every tower column still contains the appropriate number of discs.

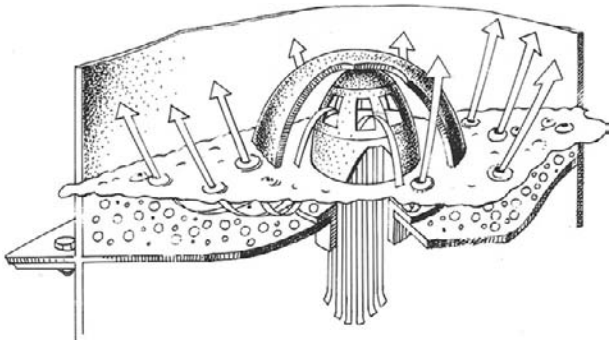

Figure 4 Every disc contains a number of 'bells.' In each 'bell' steam comes into contact with the descending liquid causing the gradual depletion of ethanol.

ethanol (50–96% (v/v)) enter the converter. Part of the steam is condensed and refluxes in the column. The nonvolatile components and most of the water are removed from the lower part of the first column as 'washy' (vinasses). The undesired components are further removed from the second and third columns. Ethyl alcohol is isolated from the third or fourth disk of the third column. Fusel oils (mainly higher alcohols) are also collected from the lower part of this column from different disks. Depending on their volatility, they are grouped in low and high fusel oils. The term 'oil' is due to their insolubility in water. Part of the water is deposited on the bottom of the column as 'washy phlegm.' The wine distillate is collected from the first, second, or third column depending on the desired amount of congeners.

Maturation in Wooden Casks

Maturation may be defined as a period of oxidative and/or nonoxidative aging. Maturation of the distillates ('wine distillate' or 'wine spirit') takes place in wooden, mostly oak, casks. This process may last from several months to several years. Relatively young distillates are placed in casks with a maximum capacity of 225–1000 l, while distillates aged for longer time periods can be stored in 5000 l casks. During this period, there is a loss of 2–3.5% of the distillate volume per year due to evaporation.

During the aging process, the distillate acquires a very characteristic flavor, distinctly different from that of the fresh distillate. The sensory attributes of the distillates such as color, flavor, and mouth feel are greatly influenced by the botanic species of the wood, the different heat treatments applied to barrels, the time that the wooden barrel has been used, and the aging time. The aroma complexity is enhanced due to the extraction of certain wood compounds (volatile and nonvolatile) into the distillate. For instance, tannins, which are polymeric phenolic substances, are extracted from the wood and have a significant contribution to brandy and cognac flavor. Furthermore, during barrel aging, chemical reactions may also occur between the components of wood and distillates, which can also add complexity to the sensory character of the final product.

Oak wood consists of 40–45% cellulose, 20–25% hemicellulose, 25–30% lignin, and 8–15% tannins. The natural color of brandy and cognac is due to the presence of tannins. During its first use, the wood contributes mainly to the toasty aroma of spirits and, while during the second and third uses, donates more vanilla flavor.

The first year of aging usually takes place in new oak casks, whereas subsequently, they may be placed in used casks. Sherry brandies (Brandy de Jerez) are aged in casks of American oak (*Quercus alba*) that previously contained sherry wine. Brandy de Jerez ages according to the traditional dynamic system known as *Soleras y Criaderas*. The phenolic and furanic content of Brandy de Jerez increases considerably during aging.

In spite of their wide acceptance, the use of wooden barrels is both time-consuming and expensive. Therefore, recently, the use of wood fragments (staves and tablets) in order to promote accelerated aging process has become an interesting economic alternative.

Volatile Compounds from Casks

The different volatile compounds extracted from wood, mainly furanic and phenolic compounds, are positively correlated with overall brandy and cognac quality. Concentration of furanic compounds, such as furfuryl ethyl ether, furfural,

2-acetylfuran, and 5-methylfurfural, varies according to the type of cask and aging time. Furanic aldehydes derive from the thermal degradation of polysaccharides derived from wooden casks.

Eugenol, cis-β-methyl-*c*-octalactone, furfural, 4-hydroxy-2-butenoic acid lactone, hexanoic acid, and guaiacol are considered important compounds and are derived from wood. Toasting, a typical process in barrel construction, can strongly modify the volatile composition of the wood, particularly the levels of furanic aldehydes (furfural, 5-methylfurfural, with toasted almond aromas, and 5-HMF), volatile phenols (syringol and 4-allyl-syringol), propanoic acid, 4-hydroxy-2-butenoic acid lactone, and vanillin. When the wood is not toasted, extraction during aging is limited.

Nonvolatile Compounds from Casks

The aging of spirits in oak barrels is a complex process. Direct extraction of wood components, degradation products of wood macromolecules, and reactions between the components of the distillate itself and those originating from the wood (polymerizations, esterifications, acetylizations, and hydrolysis) may occur, in addition to major oxidation processes. Apart from ellagitannins, oak releases a certain number of other compounds into brandies, mainly lignins. Depending on conditions, oak may also release polysaccharides, mainly consisting of hemicelluloses that contribute to spirit flavor.

Total acidity in brandies is initially due to the presence of volatile acids, such as acetic acid. Acetic acid content increases during aging by oxidation of ethyl alcohol. In addition, total acidity progressively increases due to the extraction of phenols from oak casks. Phenols are weak acids that gradually contribute to fixed acidity. New brandies have pH values between 4 and 5, while during maturation, pH falls to 3.5.

Change of Volatiles during Maturation

The ethyl ester content increases during aging, as a consequence of the slow esterification of different organic acids with ethanol. As the distillate matures, ethyl esters become less flavor-active, due to an increase in their solubility in aqueous ethanol by the wood-extracted materials.

Methanol is reduced during aging in casks. Ester synthesis and hydrolytic breakdown continue nonenzymatically during aging, based on the distillate chemical composition and storage conditions.

Isobutanal provides a herbaceous character to the brandy. During maturation, its content declines due to acetylization and selective evaporation.

Blending and Bottling

In order to obtain the final product, various wine distillates of different ages, from different casks, are blended. Pure water is added to reduce the alcoholic strength to 40% (v/v). In most cases, caramel is also added, since it contributes to the development of color. Caramel addition is quite common in the production of aged spirit beverages because it provides an amber coloration attractive to consumers. The chemical composition of caramel is complex, owing to the large number of substances produced, as a result of the pyrolysis of carbohydrates, such as sucrose, glucose, and starch.

Cold stabilization usually follows to ensure the removal of the excess quantity of ingredients, such as tannins, which could develop turbidity and thus affect the quality of the final product. Brandy and cognac are stabilized after two days at −4 °C. Finally, filtration and bottling follow.

After the Sale

Spirits are microbiologically more stable than wines and beer, due to the high alcohol content. In addition, brandy and cognac are not suitable for bottle aging. After opening, spirit drinks gradually become oxidized and their quality deteriorates.

See also: Alcohol: Properties and Determination; Brandy and Cognac: Consumption, Sensory and Health Effects; Rhum–Ron–Rum: Technology and Tradition; Tannins; Tequila: Raw Material, Classification, Process, and Quality Parameters; Vodka; Whisky, Whiskey and Bourbon: Composition and Analysis of Whisky; Wines: Types of Table Wines; Wines: Wine Production; Wines: Wine Tasting.

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Brassica: Characteristics and Properties

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Background

The brassicas comprise a large and diverse group of widely consumed vegetables. *Brassica* is the Latin name of a genus that is taxonomically placed within the Brassicaceae (Cruciferae), which is one of the ten most economically important plant families in the world. The genus *Brassica* includes, but is not limited to, the following vegetables: bok choy, broccoli (calabrese and sprouting broccoli), Brussels sprouts, cabbage, cauliflower, Chinese cabbage, kale, kohlrabi, mizuna, swede (rutabaga), and turnips. Rapeseed or canola, one of the world's most widely grown oilseed crops, is also a nonvegetable *Brassica* species and is discussed elsewhere. Other closely related vegetables within the Brassicaceae family (also known as brassica or cruciferous vegetables, crucifers, cole crops, or mustards) include radish (*Raphanus sativus*), watercress (*Nasturtium officinale*), arugula (*Eruca sativa*), horseradish (*Armoracia rusticana*), maca (*Lepidium meyenii*), mashua (*Tropaeolum tuberosum*), wasabi (*Wasabia japonica*), and cress (*Lepidium sativum*). One of the common characteristics of brassica vegetables is that they all contain glucosinolates, sulfur-containing compounds that are hydrolyzed to produce the so-called mustard oils, which impart characteristic tastes and odors to these vegetables.

Vegetables are considered to be an essential part of a balanced diet. The brassica vegetables in particular add considerable visual or aesthetic appeal to a meal, since many of them are leafy and green. They are rich sources of dietary fiber, and many are good sources of calcium, carotenoids (provitamin A), vitamin C, and certain beneficial phytochemicals. They also have distinctive flavors and textures, which enhance palatability in the eyes of many consumers, though they evoke quite violent negative emotions in others.

Historical Origins of Modern Brassica Vegetables

Most scholars place the wild cabbage at the head of the family tree that has led to the development of the 400 or so varieties of *Brassica oleracea*. Although there is still considerable disagreement among scientists as to precisely how all of the modern brassica vegetables arose, there are widespread references to these vegetables in writings dating back many centuries. Their taxonomy, too, is a subject of considerable debate and should be regarded as being in a state of flux. Genus, species, variety, subvariety, subspecies, botanical group, and cultivar designations are frequently interchanged in the descriptive literature. Commonly recognized taxonomic designations are used herein, wherever possible.

It is widely agreed that there are six major vegetable *Brassica* species. Three are monogenomic, and three are amphidiploid (being diploid for two genomes, each originally contributed by a different species). These major categories are thus commonly designated as having A, B, or C genomic compositions, and their relationships are shown in **Table 1**.

The ancestral wild cabbage was almost certainly a seaside plant of northern European or Mediterranean origin. All of the wild brassicas today occur in cliffs and rocky islets in fairly isolated places. Wild *B. oleracea* varieties still grow as perennials along the coasts of northern Spain, western France, and southern and southwestern Britain. Over time, some 400 varieties have been created, which include cabbages, kohlrabi, oilseed rape, Brussels sprouts, cauliflower, and broccoli. Cabbages were not eaten by the Hebrews or the Egyptians. None of the crucifers are mentioned in the Old Testament, and the only crucifer mentioned in the New Testament (Mark 4:30–32) is mustard seed. The ancient Romans and Greeks, however, were quite familiar with cabbages and cauliflower and believed that eating cabbage during a banquet would prevent one from becoming drunk. Dietary cabbage intake was discussed in the writings of Pythagoras, Diogenes, and Cato, to name a few. Cato, for example, recommended cabbage in the diet to prevent disease and prolong life and claimed to owe his procreative prolificacy (he had 28 sons) to cabbage. In the Middle Ages, cabbage plasters were used for medicinal purposes, and cabbage was used for cough syrup and wound dressings. In the 1700s, they were used aboard ships for their vitamin C content and were used as dressings to combat gangrene.

Broccoli (from the Italian 'brocco,' arm or branch) is widely presumed to have developed from the wild cabbage that was native to coastal Europe and spread through the Near East to the Orient between 2000 and 2500 years ago. Some authorities consider that sprouting broccoli (something resembling modern-day broccoli) was first domesticated and cultivated in Italy during ancient Roman times. A vegetable that was probably broccoli was described by the Roman botanist Pliny (first century CE). There is no consensus, however, on the translation of the early name 'cyma,' and there is concern that the writings of early botanists may have confused 'broccoli' and 'cauliflower' as we know them today. Others maintain that early selection and domestication of broccoli may have been made in Asia Minor, with a cultivated form being brought to Italy by early traders in the 1500s. What is certain, however, is that broccoli (also known at that time as Italian asparagus or sprouting broccoli) was introduced to England around the 1720s. The following passage from Stephen Switzer's *The Practical Kitchen Gardiner* (1727) illustrates the point:

> As for the broccoli, there are three kinds of it, one of which yields sprouts button'd at their points, or headed like small collyflowers; another sort with curl'd leaves, which produce sprouts button'd on the points like asparagus; and a third with curl'd leaves of a pale green colour, which yield sprouts like the red kind; the two are to be had at several places about London; but the first is very rare to be had, but from some few gentlemen that have them yearly from Italy. . .

Table 1 Haploid chromosome number and genomic compositions of the six major *Brassica* species

Species	*Haploid chromosome number*	*Genome*
B. rapa	10	A
B. nigra	8	B
B. oleracea	9	C
B. juncea	18	AB
B. napus	19	AC
B. carinata	17	BC

By the late 1700s, broccoli was introduced to the American colonies, where it was grown by Italian immigrants on the East Coast of the United States but was otherwise little known until the 1920s. In 1912, the Stokes Seeds company brought broccoli seed into the United States and started selling to growers in 1918. In 1923, the D'Arrigo Brothers Company initiated field trials in California and, by 1925, was shipping ice-pack freight car loads of broccoli back to the east coast.

Commonly Cultivated Brassica Vegetables

Broccoli, Including Broccoli Sprouts (*B. oleracea* var. *italica*)

This is also known as calabrese or sprouting broccoli. The most valued portions of broccoli plants are the heads, which are inflorescences consisting of immature fully differentiated flower buds and tender upper stems. Both primary and secondary inflorescences are eaten, and lower stems are eaten too, but are not as prized due to their tough outer 'rind.' Broccoli is available commercially as fresh or frozen florets and is used raw in salads or as vegetable crudités. It is also frequently cooked and served by itself as well as being a component of many cooked and stir-fried dishes. There are over 100 commercial hybrid cultivars of broccoli, derived from a limited number of landraces or open pollinated cultivars that include purple sprouting, purple cape, purple Sicilian, white sprouting, and calabrese or green sprouting broccoli. Cut broccoli shoots or florets are very perishable and must thus be cooled (e.g., vacuum cooling) very soon after picking. Crushed ice or an ice slurry is typically blown into cartons of broccoli within a few hours of their being picked. California and Mexico in North America and Italy, France, and Spain in Europe are the major production areas. Consumption of broccoli in the United States has been steadily climbing since about 1970. In 1970, the total per-capita consumption was about 0.7 kg and is presently (2012 figures) about 3.6 kg. The development of hybrids in the late 1970s and their subsequent marketing by the vegetable seed companies were responsible for much of the increased consumption in the 1970s and 1980s. Impetus for the dramatic increase in consumption over the past 20 or so years has come from the health aura, which broccoli has recently enjoyed. Broccoli and kale are regularly identified as the vegetables eaten most often for health reasons, including cancer prevention and high-fiber, vitamin C, folate, and calcium content. Broccoli sprouts (seeds germinated in water, without soil, and grown as green sprouts for a few days) have added further evidence to the literature on broccoli's health benefits and are currently being evaluated in clinical trials for their ability to mitigate or prevent a wide spectrum of chronic diseases.

Broccoli Raab (*B. rapa* var. *rapa*)

Broccoli raab is also known as broccoli rabe, broccoli babe, rapini, broccoletti di rape, broccoletto, turnip broccoli, cima di rapa, Italian turnip, sparachetti, or taitcat. Broccoli raab has also been classified botanically as *B. campestris* or *B. ruvo*. It is a bitter-tasting vegetable similar in appearance to Chinese kale but non-heading and with thinner stems and smaller flowers than broccoli. It is long been considered a delicacy in Italy, typically lightly sautéed with garlic. It has recently gained interest in the United States and is considered a 'new' vegetable by some.

Brussels Sprouts (*B. oleracea* var. *gemmifera*)

Brussels sprouts are a relatively young member of the brassica family. They originated in Belgium in the 1500s and by the 1700s were appearing on tables around the world. Brussels sprouts grow as a tall ($\sim$1 m) single-stem biennial from which the axillary buds, resembling miniature cabbage heads, are harvested. They are generally eaten after cooking (steaming or boiling) and are available commercially either fresh or frozen. Brussels sprouts require a long growing season, and vegetable quality is adversely affected by warm weather.

Cabbage (*B. oleracea* var. *capitata*)

Cabbage has been cultivated, and even revered, as a vegetable by the ancient Greeks as far back as 2600 years ago, and it has also had a long history of medicinal use. There are scores of references to its use for such diverse purposes as the prevention of drunkenness, headache, stomach ailments, and even cancer. Cabbage leaves have long been used as poultices for application to tumors, and even in modern times, they have been under investigation as a means for preventing or treating breast engorgement in nursing mothers.

All cabbages have heads formed from tightly packed leaves. There are many distinct head types, the most common being Wakefield (with small, early, white, pointed heads; for fresh market), red (leaf surfaces are pigmented and they have medium very firm, round heads; for fresh market and storage), Danish Ballhead (round, very firm heads and light green leaves; for fresh market and storage), and Savoy (some authorities designate this *B. oleracea* var. *sabauda*) (round, loose heads with crinkled or blistered leaves; for fresh market and storage). Cabbage is typically eaten fresh, processed into a salad-like dish called coleslaw, and boiled or eaten as a fermented and pickled product called sauerkraut. Cabbages are important as a fresh market crop as well as a processing crop in most parts of the world and rank in the top ten vegetables in both sales and volume in North America and much of Europe.

Cauliflower (*B. oleracea* var. *botrytis*)

The edible portion or head of cauliflower is composed of tightly clumped, undifferentiated shoot apices on top of

hypertrophied, highly branched fleshy stem tissue commonly referred to as curds. Referred to by Mark Twain as "nothing but a cabbage with a college education," cauliflower curds, unlike broccoli, are actually degenerate shoot tips that are most frequently white in color (lacking chlorophyll), although purple, green, and orange cultivars now exist. Cauliflower is not as cold-tolerant as many other brassica vegetables. It is grown commercially in France, Italy, the United Kingdom, and North America, and it is eaten in the same manner as broccoli as well as being pickled. It is available commercially as fresh or frozen curds.

Charlock (*B. kaber*, also *B. arvensis*, or *Sinapis arvensis*)

Charlock is also known as kaber. The seeds of this plant, likely of Mediterranean origin, are used as a condiment, and the leaves are eaten as a potherb. The seeds are not as pungent as those of other mustards.

Chinese Cabbage (*B. rapa* var. *pekinensis*)

Chinese cabbage is also known as napa, napa cabbage, pe-tsai, wongbok, or chihli. This is a vegetable of major importance in China (over 300 000 ha grown), Korea, Taiwan, and Japan. Grown as an annual crop, most cultivars are biennial and produce tight, compact, cylindrical heads. This vegetable has been cultivated in China for over 1600 years and accounts for a major fraction of the total vegetable consumption in certain (northern) areas of the country. In Korea, it is fermented to produce (preserved) kimchi, which is thus a yearlong, ubiquitous commodity in that country.

Chinese Kale (*B. oleracea* var. *alboglabra*)

This is also known as gai lan, Chinese broccoli, gai lon, gai larn, kai laan, white-flowered broccoli, or fat-shan. Compared to broccoli, Chinese kale has more, slender, dark green leaves, longer stems, and very few florets, which are similar to those of broccoli. Flower buds, flower stalks, and young leaves are consumed, primarily in salads and stir fries. Chinese kale is relatively new to Japan, western Europe, and US cuisines but extensively grown in Taiwan, Southeast Asia, and China. It is relatively fast growing and heat-tolerant.

Chinese Mustards (*B. rapa* ssp. *chinensis*)

These include bok choy, pak choi, choy sum, and Shantung cabbage and are also known as Chinese white cabbage and celery mustard. Pak choi and bok choy are sometimes errantly referred to as Chinese cabbages. *B. rapa* ssp. *parachinensis*, also known as mock pak choi, choy sum, cai tai, or saishin, has been cultivated since the fifth century CE in Asia and continues to be very important vegetables, especially in China. Pak choi is the more leafy cultivar, and bok choy is notable for its massive leaf midribs, which are white and fleshy. This subspecies is a biennial, which is grown as an annual for its edible leaves. Plants can reach 0.6 m tall and can weigh over 2 kg. Leaves are usually consumed fresh but are also dried after blanching, for use through periods when fresh vegetables are not plentiful.

Collards (*B. oleracea* var. *sabellica*)

Available commercially as fresh, canned, or frozen leaves, collard greens are popular in the southern United States, where they are grown along the eastern portion of the country. As such, it is a much more heat-tolerant crop than its close relative, kale. The plants are nonheading and up to 1.25 m tall, and the broad, flat, or slightly furrowed leaves form as a rosette on a minimal stem.

Colza (*B. napus* var. *napus*)

Colza is also known as vegetable rape, xi yang you cal, and chou navet. Colza oil (expressed from seeds) is commonly consumed in India and China. Foliage and sprouted seeds are eaten in salads or as a potherb; inflorescences are prepared like broccoli.

Kale (*B. oleracea* var. *acephala*)

There are many 'kales,' some of which are classified taxonomically into other *Brassica* species or varieties. Kale includes kitchen kale, green kale, dwarf Siberian kale, marrow stem kale, tronchuda kale, curly leaf kale, Scotch kale, tree kale, and borecole. Although highly variable, kale is characterized by a nonheading rosette-like whorl of foliage; a short, erect stem; and large, upright, curly leaves. They are used mainly for their edible foliage and are generally eaten cooked, but are sold fresh, canned, and frozen, or used as garnish. Among the commonly grown kales are the following:

- Branching bush kales (sometimes classified as *B. oleracea* var. *fruticosa*): also known as cow kale or borecole (sometimes classified as *B. oleracea* var. *selenesia*), these were often cultivated in the past for their edible foliage and have been used extensively for animal fodder.
- Thousand-headed kale (*B. oleracea* var. *ramosa* or *B. oleracea* var. *millecapitata*).
- Inflorescence kales (a term used by some to describe cauliflower, broccoli, and related brassica vegetables).
- Galega kale (also known as couve galega): a traditional and widely grown Portuguese nonheading kale with long petioles, large midribbed leaves, and an elongated stem that can reach 3 m; leaves are picked one by one and used for traditional soups or for animal feed.
- Marrow stem kale (*B. oleracea* var. *medullosa*): a particularly prolific type of kale used exclusively for animal feed.
- Siberian kale (also classified as *B. napus* var. *pabularia*): also known as Hanover salad, a leafy vegetable, similar to collards, which is used fresh in salads and cooked as a potherb.

Kohlrabi (*B. oleracea* var. *gongylodes*)

This is also known as knol khol or turnip cabbage. The edible portion of kohlrabi is the fleshy, swollen, tuber-like enlargement of the short, unbranched stem, which may be white, green, or purple and develops just above the surface of the soil. This vegetable developed in northern Europe about five centuries ago. It resembles turnip and rutabaga in flavor and texture but becomes highly fibrous if not harvested at peak maturity.

Mizuna (*B. rapa* ssp. *japonica*)

Also known as mibuna, curled mustard, or Japanese greens, mizuna is a cool-tolerant relative of the leafy turnips that has recently been introduced to the West.

Mustard (Various Latin Binomials)

Plants from this diverse group have been used worldwide for centuries as a condiment (mustard seed; also known as black mustard, brown mustard, and *B. nigra*) and as a vegetable (mustard greens; *B. juncea*). Most well known among the mustards are

- white or yellow mustard (*B. hirta*, *B. alba*, or *Sinapis alba*),
- Chinese mustard (*B. japonica*),
- black mustard (*B. nigra* or *Sinapis nigra*) and field mustard (*B. campestris*),
- Ethiopian mustard and Abyssinian mustard (*B. carinata*).

Many of the so-called brown mustards (*B. juncea*) have been assigned unique variety names. Common English and Chinese names are as follows:

- *B. juncea* var. *capitata* (capitata mustard, jie qiu jie)
- *B. juncea* var. *crassicaulis* (bamboo shoot mustard, sun zi jie)
- *B. juncea* var. *crispifolia* (cut-leaf mustard and curled mustard, mi tuo jie)
- *B. juncea* var. *foliosa* (small-leaf mustard, xiao ye jie)
- *B. juncea* var. *gemmifera* (gemmiferous mustard, bao zi jie)
- *B. juncea* var. *involuta* (involute mustard, juan xin jie)
- *B. juncea* var. *latipa* (wide petiole mustard, kuan bing jie)
- *B. juncea* var. *leucanthus* (white-flowered mustard, bao hua jie)
- *B. juncea* var. *linearifolia* (line mustard, feng wei jie)
- *B. juncea* var. *longepetiolata* (long petiole mustard, chang bing jie)
- *B. juncea* var. *megarrhiza* (tuberous-rooted mustard, dal tou jie)
- *B. juncea* var. *multiceps* (tillered mustard, fen nie jie)
- *B. juncea* var. *multisecta* (flowerlike leaf mustard, hua ye jie)
- *B. juncea* var. *rugosa* (large-leaf mustard, brown mustard, Indian mustard, and mustard greens; rai and dai ye jie)
- *B. juncea* var. *strumata* (strumous mustard, tsatsai and zha cai)
- *B. juncea* var. *tumida* (swollen stem mustard, jing liu jie)
- *B. juncea* var. *utilis* (peduncled mustard, tai jie)
- *B. rapa* var. *narinosa* (broad-beaked mustard, wu ta cai and taasai)

Swede (*B. napus* var. *napobrassica*)

Also known as rutabaga in the United States, swede is considered a 'root crop,' though, technically, this is not accurate. It is an annual crop grown as animal fodder and consumed by human beings after cooking or pickling. It has been grown for about three centuries, originating in Sweden and spreading throughout Europe. The flesh is white or orange and similar in flavor and texture to turnips with equivalent, excellent storage characteristics but low commercial value. Swede not only is much more hardy than the turnip but also takes much longer to mature. The leaves are used as a potherb.

Tendergreen (*B. rapa* ssp. *perviridis*)

Also known as spinach mustard, mustard spinach, or komatsuna, this leafy relative of the turnip is reasonably cold-tolerant, surviving temperatures as low as $-15\,^{\circ}C$. It has large, dark green, mildflavored foliage, which is eaten fresh and pickled, primarily in Korea, Taiwan, and Japan.

Texsel Greens (*B. carinata*)

Of Ethiopian origin, the early growth of this plant is valued for its high protein and vitamin C content and is eaten raw in salads or lightly boiled as a spinach substitute. It has a milder flavor than collards or mustard greens.

Tronchuda Cabbage (*B. oleracea* var. *costata*)

Also known as Portuguese cabbage, couve tronchuda, Galician cabbage, braganza, or sea-kale cabbage, these are loose-headed cabbages that have large leaves with succulent midribs. It is believed that the many landraces of this vegetable arose from an initial hybridization of cabbage and kale.

Turnip (*B. rapa* var. *rapifera*)

Turnip is very similar to rutabaga in that it is a 'root crop' (technically incorrect) that produces high amounts of biomass per hectare, is high in starch content, and has very favorable storage characteristics. It appears to have been around for about 4000 years, originating in eastern Europe and Siberia and gradually spreading across Europe. As with swedes, turnips are generally eaten after cooking and can also be processed for use in pickled or mixed vegetables. Turnip greens are eaten in season as a fresh leafy green vegetable.

Turnip Rape (*B. campestris* var. *oleifera*)

The seeds of this plant produce an oil that is sometimes used in cooking, and it has relatively high levels of unsaturated lipids. It is becoming more popular as an oilseed but is distinct from the very widely grown oilseed rape (*B. napus* var. *oleifera*). The foliage of the plant is used as a potherb and garnish.

'New' Brassica Vegetables

A number of 'new' brassica vegetables have been produced under trade names, primarily by cross hybridization between existing taxa. These include the following:

- Broccolini: a cross between Chinese kale and broccoli trademarked by Mann Packing Co. (California, the United States)
- Asparation: a cross between Chinese kale and broccoli trademarked by Sakata Seed Inc. (California, the United States)
- Broccoflower (*B. oleracea* var. *botrytis*): a bright green cauliflower originating in Holland and trademarked by T & A (Tanimura & Antle, California, the United States) about two decades ago

Regional Preferences

Brassica vegetables are a large group of primarily herbaceous plants that includes a number of the world's most commonly cultivated vegetables. Though the progenitor species likely originated in the Mediterranean region, the cultivated brassica vegetables are of cosmopolitan distribution. Cultivars have been adapted for worldwide production, from the tropics to the Arctic Circle. The largest cabbages in the world have, in fact, been grown near Fairbanks, Alaska, the United States.

Brassica vegetables include a large number of taxonomically closely related, but morphologically and organoleptically diverse, plants. These species have been cultivated for many centuries and have been extensively crossed and hybridized. Many cultivars have been developed in microenvironments or very small geographic regions where they have remained essentially isolated for decades, or even centuries. For example, certain small villages or regions in Italy have their own very distinctive broccoli cultivars. Since these vegetable gene pools have remained isolated for hundreds of generations of selection, there has been considerable development of phenotypes that diverge from a common ancestor. The relationships of some of the more common brassicas are detailed in Figure 1.

Dietary and Commercial Importance

From a nutritional standpoint, these vegetables are perhaps best recognized as excellent sources of vitamin C, fiber, calcium, and certain phytochemicals such as carotenoids (provitamin A) and glucosinolates (which have been the source of considerable recent scientific research as cancer protective agents). The contents of vitamins, minerals, and phytochemicals are, however, highly dependent on both genetic and environmental variables. Plant cultivar or variety; the environment in which the plant is grown (e.g., amount of sunlight, drought stress, and temperature); the conditions under which it is harvested, stored, and transported to market; and the way in which it is prepared for the table and consumed all play key

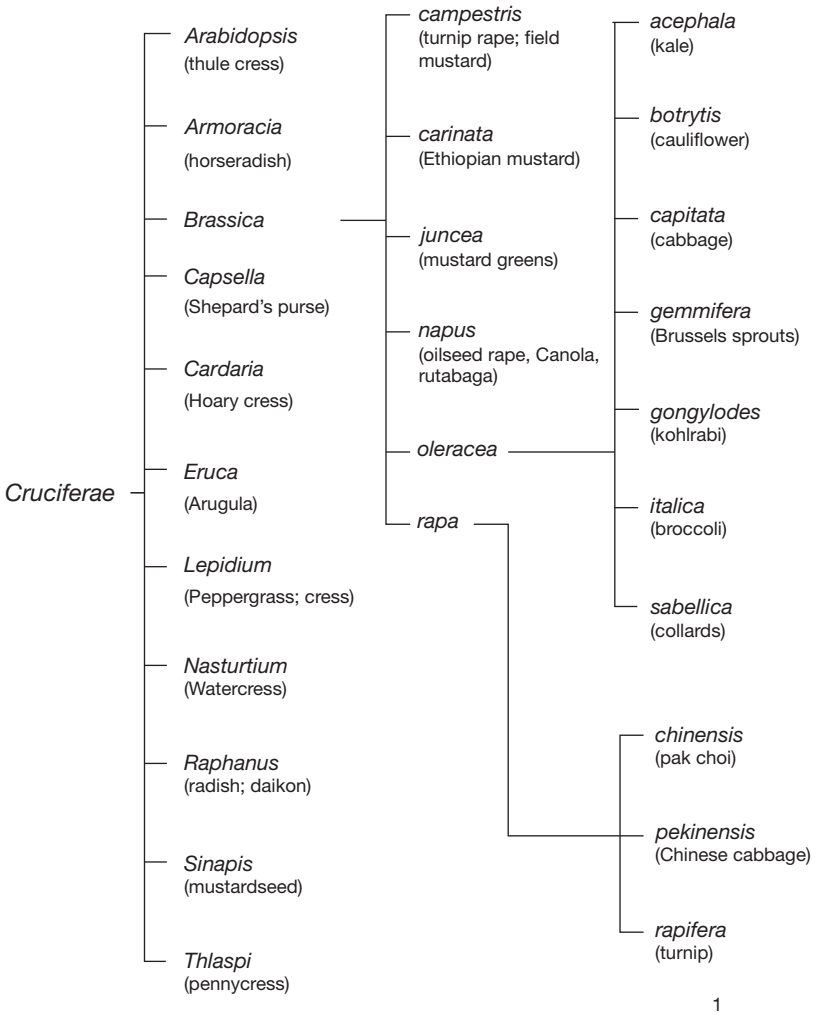

Figure 1 The world's most commonly known brassicas. Note that there are many hundreds of edible brassica species. Three of the well-known species are not commonly eaten, *Arabidopsis* (a genetic 'model organism'), *Capsella*, and *Cardaria*, which actually contains a pervasive and invasive weed in western US rangeland in which the phytochemical sulforaphane was first identified.

roles in the ultimate nutritional value of that vegetable. Water-soluble components such as vitamin C and glucosinolates (see succeeding text) are easily leached out in the pot liquor during cooking. These and other beneficial chemicals can exhibit a tremendous gradient from one portion of the plant to another, and it is often quite difficult to assess these differences without performing sophisticated chemical analyses. Nonetheless, the brassica vegetables remain among the best sources of the dietary components mentioned earlier and should be consumed regularly as part of a diet rich in fruits and vegetables.

In the West, broccoli, Brussels sprouts, cauliflower, cabbage, and kale are the most significant brassica components of the diet. In the East, the so-called oriental brassicas (Chinese cabbage, tendergreen (spinach mustard), bok choy, pak choi, mizuna, celery mustard, and Chinese mustard) as well as spinach mustard and mizuna greens, Chinese kale (Chinese broccoli), and daikon (Japanese radish – a cruciferous root crop) are the main brassica vegetables of commercial and dietary importance.

Cultivation and Postharvest

The brassica vegetables are hardy, cool-season vegetables that grow best in temperatures in the range of 15–20 °C and have similar cultural requirements. Cabbage plants that have been hardened off can tolerate temperatures as low as −4 °C for short bursts, and broccoli and cauliflower plants thrive in light frosts. Almost all cole crops decline in quality when temperatures are in excess of 26–27 °C. Under irrigation (guaranteed water supply), broccoli crops can be harvested in 10–15 weeks from direct seeding, cabbage crops in 13–17 weeks, and cauliflower crops in about 13–16 weeks, depending on the temperature and climate. Brassica vegetables can be grown on a wide range of soil types, and in addition to direct seeding, many crops are now grown from glasshouse transplants. All brassica vegetables are more or less susceptible to the same diseases and insect pests. More specific agronomic advice is beyond the scope of this article.

Although some brassica crops do not need cooling after harvest, broccoli and cauliflower require immediate prechilling to 4 °C. These crops are typically hydrocooled or immersed in an ice slurry. They must be refrigerated for transport and storage and have a storage life of about 2 weeks (broccoli), to 4 weeks (cauliflower), to as much as 6 months for some cabbage varieties grown in cooler climates.

Nutritional Value and Chemical Composition

Table 2 summarizes the nutritional value and chemical composition of broccoli, Brussels sprouts, cabbage, red cabbage, Savoy cabbage, Chinese cabbage, cauliflower, collards, cress, kale, kohlrabi, mustard greens, tendergreen, and swede.

Vitamin A, Vitamin C, Selenium, Calcium, and Fiber

The brassica vegetables are excellent sources of calcium, fiber, vitamin A (in the form of β-carotene) or provitamin A, and vitamin C (especially broccoli, kale, and tendergreen). The element selenium can be incorporated into the tissues of brassica vegetables (in particular broccoli), where it can reach rather high levels, and the vegetable may be of therapeutic value as a source of this antioxidant element due to its special availability from such tissues.

Phytochemical Attributes (e.g., Glucosinolates/Isothiocyanates, Carotenoids, and Flavonoids)

All of the brassica vegetables contain glucosinolates, at concentrations of up to 3% by weight in the seeds of some plants. These sulfur-containing compounds and their breakdown products have long been known for their fungicidal, bactericidal, nematicidal, and allelopathic properties, as well as for the goitrogenic or antinutritional glucosinolates in the protein-rich, defatted meal from widely grown oilseed crops (e.g., rapeseed) and in some domesticated brassica vegetables (e.g., Brussels sprouts). When used as animal feed, rapeseed meal can have pronounced deleterious health consequences on livestock due to ingestion of excessive quantities of 'progoitrin' (e.g., when livestock are fed a meal produced from defatted rapeseed containing the progoitrin), which may interfere with thyroxine production, drastically reducing iodine supply to the thyroid gland and resulting in the development of goiter and other associated problems. Recently, however, other members of this large group of compounds (e.g., glucoraphanin, sulforaphane, phenethyl isothiocyanate, benzyl isothiocyanate, crambene, and indole-3-carbinol) have attracted intense research interest because of their cancer chemoprotective and antioxidant attributes. Certain compounds (e.g., sulforaphane) have been shown to be potent inducers of mammalian detoxication enzymes, which facilitate the deactivation and excretion of many carcinogens from the body. The use of a number of these compounds in a dietary strategy for cancer prevention is now being investigated in clinical trials worldwide, particularly as very young plants (e.g., broccoli sprouts).

The brassica vegetables are generally very rich sources of the antioxidant and provitamin A carotenoids (e.g., lutein, zeaxanthin, and β-carotene). These compounds are long-chain, fat-soluble substituted hydrocarbons, which are the light-gathering accessory pigments typically found in the leaves, stems, and inflorescences of most plants. Since these are typically the plant organs that are eaten, they can be regarded as typical 'dark green leafy vegetables' and good sources of such compounds (Table 2). Certain *Brassica* vegetables, such as kale and broccoli, are particularly rich in these compounds, and the edible head of cauliflower is devoid of them. A mutant, orange cauliflower plant was found growing in a Canadian field about 40 years ago, however, which is currently being investigated as a very potent source of β-carotene and a very useful system for scientists to unravel some of the biochemical and molecular mysteries still surrounding carotenoid production in plants. Though an orange cauliflower is now being marketed, the application of this research may result ultimately in the introduction of higher-carotenoid varieties of some of the world's staple crops and a reduced incidence of vitamin A deficiency (which can be reduced by the ingestion of β-carotene-rich foods).

The brassica vegetables, though not unique in this respect, are also good sources of various flavonoids and their

Table 2 Nutrient composition per 100 g serving of selected raw brassica vegetables[a]

Nutrients	*Broccoli*	*Brussels sprouts*	*Cabbage*	*Red cabbage*	*Savoy cabbage*	*Pak choi*	*Pe-tsai*	*Cauliflower*	*Collards*	*Cress*	*Kale*	*Kohlrabi*	*Mustard greens*	*Tendergreens*	*Swede*
Gross composition															
Water (g)	86.00	86.00	92.18	90.39	91.00	95.32	94.39	92.07	89.62	89.4	84.04	91.00	90.70	92.20	89.43
Energy (kcal)	34	43	25	31	27	13	16	25	32	32	49	27	27	22	37
Protein ($N \times 5.95$) (g)	2.82	3.38	1.28	1.43	2.00	1.50	1.20	1.92	3.02	2.60	4.28	1.70	2.86	2.20	1.08
Total lipid (g)	0.37	0.30	0.1	0.16	0.10	0.20	0.20	0.28	0.61	0.70	0.93	0.10	0.42	0.30	0.16
Carbohydrate (g)	6.64	8.95	5.80	7.37	6.10	2.18	3.23	4.97	5.42	5.50	8.75	6.20	4.67	3.90	8.62
Fiber, total dietary (g)	2.6	3.8	2.5	2.1	3.1	1.0	1.2	2.0	4.0	1.1	3.6	3.6	3.2	2.8	2.3
Ash (g)	0.87	1.37	0.64	0.64	0.80	0.80	0.98	0.76	1.32	1.80	2.01	1.00	1.36	1.40	0.71
Sugars, total (g)	1.7	2.2	3.2	3.83	2.27	1.18	1.41	1.91	0.46	4.4	2.26	2.6	1.32	–[b]	4.46
Minerals															
Calcium (mg)	47	42	40	45	35	105	77	22	232	81	150	24	115	210	43
Iron (mg)	0.73	1.4	0.47	0.8	0.40	0.80	0.31	0.42	0.47	1.30	1.47	0.40	1.64	1.50	0.44
Magnesium (mg)	21	23	12	6	28	19	13	15	27	38	47	19	32	11	20
Phosphorus (mg)	66	69	26	30	42	37	29	44	25	76	92	46	58	28	53
Potassium (mg)	316	389	170	243	230	252	238	299	213	606	490	350	384	449	305
Sodium (mg)	33	25	18	27	28	65	9	30	17	14	38	20	20	21	12
Zinc (mg)	0.44	0.42	0.18	0.22	0.27	0.19	0.23	0.27	0.21	0.23	0.56	0.03	0.25	0.17	0.24
Copper (mg)	0.049	0.070	0.19	0.017	0.062	0.021	0.036	0.039	0.046	0.170	0.499	0.129	0.165	0.075	0.032
Manganese (mg)	0.21	0.337	0.16	0.243	0.180	0.159	0.19	0.155	0.658	0.553	0.659	0.139	–	0.407	0.131
Selenium (μg)	2.5	1.6	0.3	0.6	0.9	0.5	0.6	0.6	1.3	0.9	0.9	0.7	0.9	0.8	0.7
Vitamins															
Vit C (mg)	89.2	85.0	36.6	57.0	31.0	45	27	48.2	35.3	69	120	62	70.0	130	25
Vit B_1 (mg)	0.071	0.139	0.061	0.064	0.070	0.040	0.040	0.05	0.054	0.080	0.110	0.050	0.080	0.068	0.090
Vit B_2 (mg)	0.117	0.090	0.040	0.069	0.030	0.070	0.050	0.060	0.130	0.260	0.130	0.020	0.110	0.093	0.040
Niacin (mg)	0.639	0.75	0.234	0.418	0.300	0.500	0.400	0.507	0.742	1.000	1.00	0.400	0.800	0.678	0.700
Pantothenic acid (mg)	0.573	0.309	0.212	0.147	0.187	0.088	0.105	0.667	0.267	0.242	0.091	0.165	0.210	0.178	0.160
Vit B_6 (mg)	0.175	0.219	0.124	0.209	0.190	0.194	0.232	0.184	0.165	0.247	0.271	0.150	0.180	0.153	0.100
Folate (μg)	63	61	43	18	80	66	79	57	129	80	141	16	12	159	21
Vit B_{12} (μg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vit A (IU)	623	754	98	1116	1000	4468	318	0	5019	6917	9900	36	3024	9900	2
Vit A, RE (μg)	31	38	5	56	56	223	16	0	251	346	500	2	151	495	0
Vit D (IU)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vit E, α-TE (mg)	0.78	0.88	0.15	0.11	0.17	0.09	0.12	0.08	2.26	0.70	1.54	0.48	2.01	1.704	0.30
Vit K (μg)	101.6	177	76	38.2	68.8	45.5	42.9	15.5	437.1	541.9	704.8	0.1	257.5	–	0.3
Lipids															
Saturated, total (g)	0.039	0.062	0.034	0.021	0.013	0.027	0.043	0.013	0.055	0.023	0.091	0.013	0.010	0.015	0.027
Monounsaturated (g)	0.011	0.023	0.017	0.012	0.007	0.015	0.023	0.034	0.030	0.239	0.052	0.007	0.092	0.138	0.025
Polyunsaturated (g)	0.038	0.153	0.017	0.08	0.09	0.096	0.072	0.031	0.201	0.228	0.338	0.048	0.038	0.057	0.088

(*Continued*)

Table 2 (Continued)

Nutrients	*Broccoli*	*Brussels sprouts*	*Cabbage*	*Red cabbage*	*Savoy cabbage*	*Pak choi*	*Pe-tsai*	*Cauliflower*	*Collards*	*Cress*	*Kale*	*Kohlrabi*	*Mustard greens*	*Tendergreens*	*Swede*
Cholesterol (mg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–
Pigments (*carotenoids*)															
β-Carotene (μg)	361	450	42	670	600	2681	190	0	2991	4150	5927	22	1790	–	1
Lutein/zeaxanthin (μg)	1403	1590	30	329	77	40	48	1	4323	12500	8198	0	3730	–	19
Lycopene (μg)	0	0	0	20	0	0	0	0	0	0	0	0	0	–	14
Refuse[c] (*% of total*)	39	10	20	20	20	12	7	61	43	29	28	54	7	7	15

[a]Data from USDA Nutrient Database for Standard Reference, Food Group 11, Release SR27 (2014; http://www.ars.usda.gov/Services/docs.htm?docid=8964).

[b]No data provided.

[c]Refuse is some combination of the total biomass, which is typically not used in food preparation, for example, stems; crowns; spoiled, damaged croute leaves; leaf stalks; cores; trimmings; or root base.

conjugates to which significant antioxidant activity has been ascribed. For example, the prevention of lipid peroxidation for which these compounds appear to be reasonably well suited may be an important mechanism for reducing the severity of age-related degenerative diseases such as arthritis, cardiovascular disease, and cancer.

See also: Antioxidants: Role on Health and Prevention; Cancer: Diet in Cancer Prevention; Food-Herbal Medicine Interface; Functional Foods; Glucosinolates from the Brassica Vegetables and Their Health Effects; Mustard; Papayas; Pesticides and Herbicides; Pesticides and Herbicides: Residue Determination; Pesticides and Herbicides: Types of Pesticide; Pesticides and Herbicides: Types, Uses, and Determination of Herbicides; Rapeseed Oil/Canola; Salad Crops: Root, Bulb, and Tuber Crops; Vegetarian Diets.

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Relevant Websites

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http://clinicaltrials.gov/ – A registry and results database of publicly and privately supported clinical studies of human participants conducted around the world. It is a service of the US National Institutes of Health.

http://www.ers.usda.gov/topics/food-choices-health/food-consumption-demand.aspx – The USDA's Economic Research Service.

Bread: Breadmaking Processes

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Breadmaking Processes

Different methods that allow the conversion of flour and other ingredients into bread have evolved with time. In practice, many of the variations in the common breadmaking processes are small and usually consist of variations about a central 'standard' process, so that we are able to group them into a small number of generic processes in order to consider the changes that occur within them and their contribution to final product quality.

The different processes have a single, common aim, namely, to convert wheat flour into an aerated and palatable food. In achieving this conversion, there are a number of largely common steps that are used; they may be described as follows:

- The blending of wheat flour and water, together with yeast and salt and other specified ingredients in appropriate ratios
- The hydration of the gluten-forming proteins and development of the gluten structure through the application of energy during mixing (sometimes referred to as 'kneading')
- The incorporation of air bubbles within the dough during mixing
- The continued 'development' of the gluten structure and modification of the rheological properties of the dough so as to improve its ability to expand when gas pressures increase with the generation of carbon dioxide gas during fermentation (often referred to as 'ripening' or 'maturing')
- The creation or modification of flavor compounds in the dough
- The subdivision of the dough mass into unit pieces
- A preliminary modification of the shape of the pieces
- A short delay in processing to modify further the rheological properties of the dough pieces
- The final shaping of the dough pieces
- The fermentation (proof) and expansion of the pieces
- Final expansion of dough pieces and fixation of the final bread structure during baking

The differences between breadmaking processes are mainly associated with mixing and kneading, air incorporation, and the creation and development of the gluten structure, in summary all of those operations that in practice deal with the formation of a large dough bulk. The dividing and shaping processes make small contributions to product quality, and the processes of proving and baking are common to all breadmaking processes. Since it is mixing stages, which determine most of the bread quality, this section will concentrate on the main features of the different types of breadmaking process.

The Nature of Dough Development and Its Contribution to Bread Quality

Dough development is a poorly defined term covering complex changes in bread ingredients, which are set in motion when the ingredients first become mixed. The changes are associated with first the formation of gluten, which requires both the hydration of the proteins in the flour and the application of energy through the process of kneading. In the process of developing bread, dough changes are brought about to the physical properties of the dough and in particular its ability to retain the carbon dioxide gas, which will later be generated by yeast fermentation. This improvement in gas retention ability is particularly important when the dough pieces reach the oven. The modification of gluten structure can be achieved by a number of different physical and chemical processes, and various combinations of these form the basis of the different groups of breadmaking processes, which are in common use.

It is important to distinguish between gas production and gas retention in fermented doughs. Gas production refers to the generation of carbon dioxide gas as a consequence of yeast fermentation. Not all of the gas generated during processing, proof, and baking will be retained within the dough before it finally sets in the oven. The proportion that will be retained depends on the development of a suitable gluten matrix within which the expanding gas can be held. Gas retention in dough is therefore closely linked with the degree of dough development that occurs and as such will be affected by a large number of ingredients and processing parameters, which are not necessarily independent of one another.

The production of a defined cellular structure in the baked bread depends entirely on the creation and retention of gas bubbles in the dough during mixing. During mixing, these are a mixture of air (oxygen and nitrogen) and later the carbon dioxide gas coming from fermentation. The numbers and size of gas bubbles, which are created in the dough during mixing, have the greatest impact on bread quality. There is some modification of the gas bubble size populations in the dough during processing, but by the time that the dough leaves the mixer, the final cell structure has largely been decided.

The Major Breadmaking Process Groups

The methods by which dough development is achieved in the bakery may be fitted into five described as follows:

- *Straight dough bulk fermentation*, where resting periods (floor time) for the dough in bulk after mixing and before dividing are used
- *Sponge and dough*, where a part of the dough formulation receives a prolonged fermentation period before being mixed with the remainder of the ingredients to form the final dough, which is then processed without further delay
- *Rapid processing*, where the dough is fermented in bulk for a limited period or not at all before dividing
- *Mechanical dough development*, where significant and measured quantities of energy facilitate dough development and the dough moves without delay from mixer to divider for further processing

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- *Sourdough methods* based on the spontaneous fermentation from naturally occurring wild yeast and other microorganisms

Straight Dough Bulk Fermentation

Variations in this group are based on different periods of bulk fermentation time and fermentation control by temperature, yeast level, or both. The essential features can be summarized as follows:

- Mixing of the ingredients to form a homogeneous dough usually at low speeds and for extended time periods (20–30 min) with a temperature at the end of mixing in the region of 21–27 °C.
- Resting of the mixed dough in bulk for a prescribed time (sometimes called floor time), commonly based on flour quality, yeast level, dough temperature, and the bread variety being manufactured.
- Partway through the bulk fermentation period, there may be a remixing of the dough, commonly referred to as 'knock-back' or 'punching down.'

Typical straight dough formulations only contain a few ingredients as shown in Table 1. Usually, recipe yeast levels are higher with shorter bulk fermentation times. Since the mechanism for dough development in bulk fermentation depends to a significant degree on yeast activity, dough temperature plays a major role in determining the time at which full development is achieved for a recipe with a given yeast level. For a given flour, bread volume improves with increasing bulk dough standing time (Figure 1). However, the protein content and qualities of flours used in bulk-fermented doughs are closely linked with the length of the bulk fermentation period, and in general, the stronger the flour, the longer the fermentation period. Typical white flour protein contents would be 12–13% (14% moisture basis) or greater.

While the only essential ingredients required are those given in Table 1, other ingredients are sometimes added for making bread by bulk fermentation. The typical rates of addition for these optional ingredients and the properties they confer to the dough and the bread are given in Table 2. 'Improvers' consisting of a low level of oxidizing agent or enzyme-active material may be added to bulk-fermented

Table 1 Recipes for bulk-fermented doughs

	3 h (%)	*1 h (%)*
Flour	100	100
Yeast	1	2
Salt	2	2
Water	57	58

Table 2 Optional ingredients in bulk fermentation

	Percentage of flour weight	*Improvement*
Fat	1.0–2.0	Gas retention Crumb softness
Emulsifiers	0.1–0.3	Gas retention Crumb softness
Enzyme-active malt flour	0.1–0.2	Gas production Gas retention Crust color
Enzyme-active soya flour	0.2–0.5	Crumb whiteness
Skimmed milk powders	Up to 2.0	Crust color Flavor

Figure 1 Effect of bulk fermentation time on bread quality.

doughs. 'Knocking back,' 'punching down,' or remixing of the bulk dough may occur partway through the fermentation time. Advantages that are claimed for these operations include equilibration of dough temperatures throughout its bulk and the incorporation of more air into the dough to improve yeast activity.

Sponge and Dough

The elements of sponge and dough processes are similar to those for bulk fermentation in that a prolonged period of fermentation is required to effect physical and chemical changes in the dough. In sponge and dough, this is achieved by the thorough fermentation of part of the ingredients rather than all of them. The key features of sponge and dough processes are the following:

- A first stage in which parts of the total quantity of flour, water, and other ingredients from the formulation are mixed to form a homogeneous soft dough with a final temperature around 20 °C.
- A bulk resting period for the sponge so formed under controlled conditions.
- Mixing the sponge with the remainder of the ingredients to form the dough with a final temperature of 20–27 °C.
- Immediate dividing and processing of the final dough.
- The sponge may be replaced with a flour brew based on a higher proportion of liquid.

The preparation of the sponge may be carried out with low- or high-speed mixers. Key roles for the sponge are to modify bread flavor and contribute to the development of the final dough through the modification of its rheological properties. To maintain the right flavor profile in the finished product, the sponge fermentation conditions should be closely controlled. During sponge fermentation, there is a decrease in sponge pH. This low pH makes a unique contribution to the rheological character in the final dough and has the effect of producing a softer and more extensible gluten network after the second mixing.

Table 3 Examples of sponge and dough formulations (ingredient proportions expressed as percentage of total flour weight)

	Sponge	*Dough*
UK 16 h sponge		
Flour	25.0	75.0
Yeast	0.18	1.75
Salt	0.25	1.75
Water	14.0	43.0
Fat	0.0	1.0
North American 4 h sponge		
Flour	65.0	35.0
Yeast	2.4	0.0
Salt	0.0	2.3
Water	40.0	25.0
Improver	0.1	0.0
Milk solids	0.0	3.0
Sugar	0.0	6.0
Fat	0.0	3.0

Examples of sponge and dough formulations are given in Table 3, one of which is for a typical 16 h (overnight) sponge in the United Kingdom and the other a 4 h example from North America. Additions of improvers are not essential to the production of bread by sponge and dough methods since a contribution towards dough development is made directly by the sponge. However, as shown by the example of a North American recipe in Table 3, improver additions are common. There will be different potential effects from the different oxidizing agents present if the improver is added to the sponge side of the process. Late-acting oxidizing agents have little or no effect until the dough reaches the prover (proofer) while faster-acting oxidizers, such as ascorbic acid and azodicarbonamide, will act in the sponge-mixing stage. In the case of ascorbic acid, oxygen is required for oxidation of the dough proteins to occur. Flour protein contents are usually not $<12\%$ (14% moisture), and Hagberg falling numbers are high.

Rapid Processing

Some short-time dough processes based on no bulk fermentation time are included under this heading. They have the common element that they include improvers to assist in dough development and the reduction of any bulk fermentation period.

Activated Dough Development

This process was developed during the early 1960s and became popular in smaller bakeries. Its essential features are

- the addition of a reducing agent, usually L-cysteine hydrochloride,
- the addition of oxidizing agents,
- the addition of a fat or an emulsifier,
- extra water in the dough to compensate for the lack of natural softening,
- extra yeast to maintain normal proving times.

When first introduced, potassium bromate, ascorbic acid, and L-cysteine hydrochloride were common components in the improver, but in many parts of the world, potassium bromate is no longer used. Since the dough development process in activated dough development was mostly chemically induced, low- or medium-speed (spiral) mixers can be used. A short period of bulk fermentation (typically <30 min) before dividing may follow mixing, and final dough temperatures are in the region of 25–27 °C.

The Dutch Green Dough Process

This process was developed in the Netherlands, and while the mixed dough passes without delay to dividing, significant periods of resting are involved in the total process. The name 'green' refers to the fact that after the mixing, the dough is considered to be underdeveloped or 'green' in classic bakery parlance. Dough development continues not in bulk but by resting the divided dough pieces between successive (two or three) processes. The essential features are as follows:

- Mixing in a spiral-type mixer or extra mixing in a speeded-up conventional low-speed mixer to a final dough temperature of 25–27 °C.
- The dough is divided immediately after mixing.
- The divided dough pieces are rounded and rested for 35–40 min.
- The pieces are rerounded and again rested before final molding.

Improvers are often added to assist with dough development in the absence of bulk fermentation time. The most common ingredients of the improvers are ascorbic acid, enzyme-active materials, and emulsifiers. Flour protein contents are around 12% (14% moisture basis). There is no appreciable softening of the dough from fermentation before dividing, and so water additions will be higher than in bulk fermentation.

Mechanical Dough Development

With mechanical dough development, there is no bulk fermentation period, and dough development is achieved in a short space of time in the mixer with the addition of improvers and extra yeast. The most common process considered under this heading is the Chorleywood bread process (CBP), which was developed by the British Baking Industries Research Association based at Chorleywood, the United Kingdom, and launched in 1961. The essential features of the CBP are

- mixing and dough development in a single operation lasting between 2 and 5 min at a fixed energy input carried out with a high-speed mixer;
- the addition of low levels of an improver (0.3–1.0% flour weight), commonly containing ascorbic acid, a high melting point fat, emulsifier or fat, and emulsifier combination and process enzymes;
- the addition of extra water to adjust dough consistency to be comparable with that from bulk fermentation;
- the addition of extra yeast to maintain final proof times comparable with those obtained with bulk fermentation;
- the control of mixer headspace atmosphere to achieve given bread cell structures.

The aim of the CBP is the same as all other breadmaking processes, which is to modify the protein structure in the dough to improve its ability to stretch and retain gas from yeast fermentation in the prover; in the case of the CBP, this is achieved within 5 min of starting the mixing process. Compared to bulk fermentation, the CBP offers a number of advantages including

- a reduction in processing time,
- space savings from the elimination of the bowl of dough at different stages of bulk fermentation,
- improved process control and reduced wastage in the event of plant breakdowns,
- more consistent product quality,
- financial savings from higher dough yield through the addition of extra water and retention of flour solids, which are normally fermented away.

The role that the delivery of energy to the dough mixing plays in optimizing bread quality with the CBP can be seen in Figure 2. As the level of energy per kilogram of dough in the mixer increases, so bread volume increases, and with this comes a reduction in cell size and increased cell uniformity. Typical work inputs lie in the range of 11–13 Wh kg^{-1}, though some strong flours may require higher work input so as to fully develop their breadmaking potential. Consequently, flours used in the CBP are commonly based on wheat blends, which deliver flours requiring close to 11 Wh kg^{-1} dough. The CBP makes more effective use of flour protein than some other breadmaking processes, and so in some circumstances, it is possible to reduce the overall level of flour protein with compromising bread quality. The role of that energy input during mixing has yet to be fully explained, and while it is

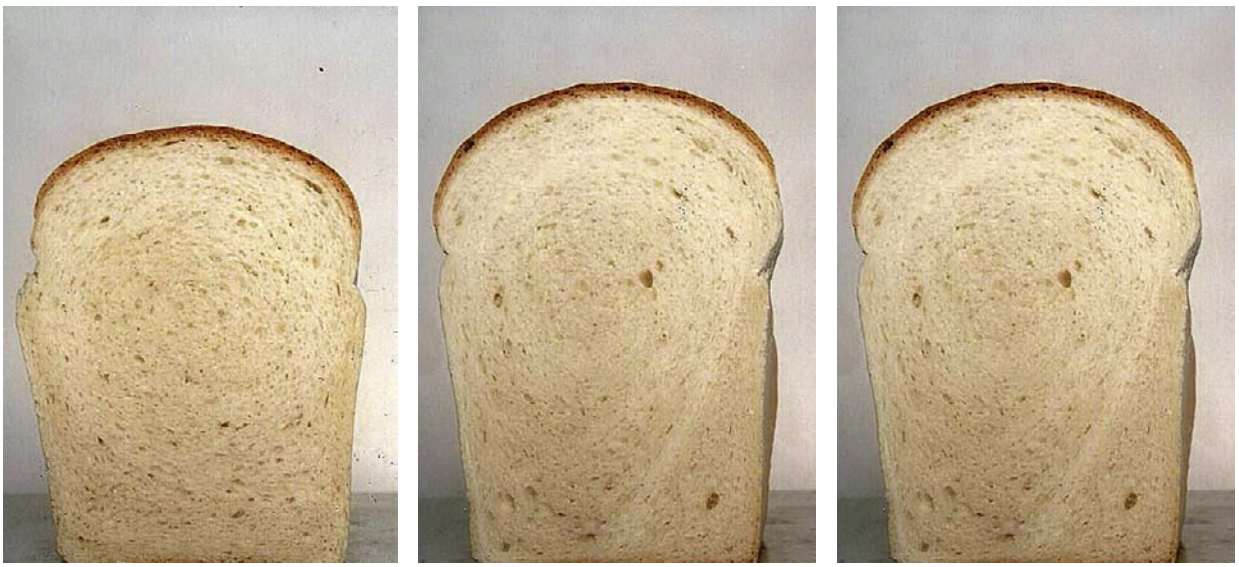

Figure 2 Effect of energy input during CBP dough mixing; left to right, 5, 8, 11 Wh kg^{-1} dough in the mixer.

very likely that the high energy inputs are capable of mechanically breaking the disulfide bonds holding the original protein configurations together, this is not the only reactions that are involved. No doubt, the breaking of weaker hydrogen bonds is also involved.

The input of energy during mixing with all breadmaking processes causes the final dough temperature to rise above that that can be calculated from the weighted average of the dough ingredients. Temperature rises in the CBP are higher than many other mixing actions, and some bakers may see this as a disadvantage in trying to control yeast activity. Control of final dough temperature is delivered using chilled water, the addition of flaked ice, or from the use of chilling jackets around the mixing bowl itself. Commonly with the very short processing times, which are used after mixing, it is possible to run final dough temperatures up to 30 °C that offers the advantage that chemical reactions are enhanced, which can lead to reductions in the level of additives needed in the improver.

In contrast to the situation in other breadmaking systems, many CBP-compatible mixers offer the advantage that the cell structure of the final product can be manipulated by changing the atmospheric conditions in the mixer. When first launched, the main control in the CBP was achieved by the application of a partial vacuum during mixing. Changes in permitted ingredients and the greater reliance on ascorbic acid as the oxidizing agent led to the development of the so-called 'pressure–vacuum' mixer, which could be used with above and below atmospheric pressures sequentially to deliver a wide range of product cell structures. The requirement to add extra water in the CBP arises because dough mixed under partial vacuum has a drier, firmer feel, and the requirement is for soft easily machined dough in most plant bakeries.

Sourdough Processes

The manufacture of sourdough bread has a long history and is based on the spontaneous fermentation of flour through the symbiotic relationship between bacteria and wild yeasts. Variations in the microflora, fermentation conditions, types, and ratios of raw materials are responsible for differences in the functionality of the sour and subsequent flavor in the bread. Sourdough technology is commonly based on wheat or rye flours or a mixture of both. Such breads have distinctive acid flavors largely arising from the ratio of acetic to lactic acid flavor notes, and the manufactured breads are denser with a less aerated structure than many other wheat breads. The preparation of a mother dough cannot proceed without a continuing food source for the microbial activity, and so its reproduction requires a 'top-up' with more flour (source of starch) on a regular basis. The symbiotic relationship between bacteria and yeast is important in sustaining fermentation with bacteria fermenting the more complex (larger molecular weight) sugars and yeasts metabolizing the by-products of the bacterial fermentation. For bakers who do not wish to manufacture and maintain their own starter, pre-prepared, dried sours are commercially available.

Some of the common sours may be described as follows:

- *Levain* based on 'wild yeasts' including *Saccharomyces* (S.) and *Candida* (*C.*) families and the presence of *Lactobacilli* (*L.*)
- The San Francisco sourdough associated with *L. sanfranciscensis* because it was in San Francisco, the United States, that the microorganism concerned was first isolated and identified from dough
- The *Poolish* (Polish-style sponge) that is a relatively liquid system comprising equal parts of flour and water
- The *Biga* commonly used in Italy with the addition of baker's yeast and fermented overnight (12–16 h)
- Rye flour sours

Processing the Dough to Bread

Each of the breadmaking processes has particular advantages and disadvantages, but most types of bread and fermented goods can be made with each of them. Much of the difference between the different processes revolves around the mixing and processing equipment; there may be some variations, but essentially, the processes of proving, baking, and cooling are common to all of the earlier processes.

After the bulk dough has been mixed, it will be cut into smaller unit pieces for processing to the required product. The weights of the individual pieces vary according to local custom and practices, but commonly, there will be some legislative control on the final bread weight that impacts the size of the dough piece, which will be used for processing.

Commonly, the dough pieces are subjected to two shaping processes separated by a short resting period. For many processes, the common first shaping is to a round or ball shape. The resting periods will vary from a few to 20 min or so with the longer periods encouraging gas production by the yeast and the creation of more open cell structures in the final product. The second molding stage fixes the final shape of the product whether it is destined to be baked in a pan or the oven hearth. Care is usually taken not to damage the gas bubble structure in this stage. Many final shaping operations are based on elongating the ball shape, rolling it up like a Swiss roll and then adjusting the shape of the cylinder that has been formed.

Once the final shape has been achieved, the individual pieces in pans or on trays are transferred to the prover for the last of the fermentation stages. In the prover, controlled conditions of temperature encourage gas production by the yeast, and the dough expands in volume. Humidity is also controlled to avoid drying out of the dough piece surface, which would otherwise restrict dough expansion.

The final stage of the breadmaking process is the conversion of the foam in the dough (the closed bubble structure) into an open spongelike structure in the bread. This foam-to-sponge conversion is achieved by heating the dough at temperatures above 200 °C and is accompanied by further expansion (oven spring) and the loss of water, especially from the crust. The complex changes require control of many different molecular, chemical, and physical changes to the dough components and are further complicated by the fact that the poor thermal conductivity of dough means that the changes occur at different parts of the product at different times. In essence, the foam-to-sponge conversion happens on a thermal front moving from the crust to the center of the product, and it is progressive change that is responsible for much of the character that we see in the final product.

However, the key to achieving a particular product character lies with choices made at the mixing stage; in essence, the nature of the baked products is set up by creating a stable foam in the mixer (the dough), expanding the foam in the prover, and converting the foam to a sponge (bread) in the oven.

See also: Bread: Chemistry of Baking; Bread: Dough Mixing and Testing Operations; Emulsifiers: Types and Uses; Enzymes: Functions and Characteristics; Wheat: Grain Structure of Wheat and Wheat-based Products; Wheat: The Crop; Yeasts.

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Bread: Chemistry of Baking

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Introduction

The breadmaking process is a dynamic process in which flour constituents are subjected to numerous physicochemical changes. Physical changes have been considered in a previous article; thus, only chemical variations in flour until bread will be considered in this article. Breadmaking is a dynamic process with continuous physicochemical, microbiological, and biochemical changes induced by the mechanical–thermal action and the activity of the yeast and lactic acid bacteria (LAB) together with the activity of the endogenous enzymes. Mixing involves mechanically and hydration-induced alterations, whereas during proofing, enzymes are mainly implicated and changes related to temperature increase occur during baking. The two main flour biopolymers, starch and proteins, undergo the most dramatic changes during the breadmaking process. The gluten proteins are largely responsible for the rheology of wheat flour dough, structural formation during mixing, and gas holding, whereas the role of starch is mainly implicated in final textural properties and product stability after baking.

Nevertheless, it must be also taken into account that breadmaking has experienced numerous changes in the way of processing and raw materials used. Commercial bakeries have understood very soon the changes in consumer lifestyles and shift their production processes, products, and even distribution channels to meet the new society requirements. Different alternatives have been developed for adapting breadmaking to the consumer demands and for facilitating the baker's work. Breadmaking stages have been extended including mixing the ingredients, dough resting, dividing and shaping, proofing, and baking, with great variation in the intermediate stages depending on the type of product. Low-temperature technology has been initially applied to bakery products to solve the economic losses associated with the bread staling problem that produces a decrease of consumer acceptance. Nowadays, the technology of frozen dough, par-baked bread, and frozen bread is being incorporated as routine processes. The partial baking consists in baking the bread dough until the structure is fixed, giving a product with structured crumb and without a crunchy crust that only requires a very short baking time in the retail bakery. Therefore, these alternative breadmaking processes promote additional chemical changes that were not considered in conventional breadmaking.

Additionally, the nature and extent of the chemical alterations produced during breadmaking are greatly dependent on the specific characteristics of each cereal flour. Since wheat flour is the most common flour used in making bread specialties, this article will be focussed on changes occurring in wheat flour during breadmaking.

Chemical Changes During Mixing

In breadmaking, mixing is one of the key steps that determine the mechanical properties of the dough, which have a direct consequence on the quality of the end product. Mixing allows hydration of flour constituents and it supplies the necessary mechanical energy for developing the protein network. Protein particles are disrupted during mixing and aligned yielding a three-dimensional viscoelastic structure with gas-retaining properties. Part of the protein phase (gliadin and glutenin) of flour has the ability to form gluten, a continuous macromolecular viscoelastic network, when sufficient water is available for the hydration of constituents and mechanical energy input is supplied for distributing flour components. Gluten consists of two main subfractions: glutenins that confer strength and elasticity and gliadins that impart viscosity to dough. In particular, proteins mainly involved in the viscoelastic properties of the dough are the high-molecular-weight glutenin subunits (HMWGS), which affect dough viscoelasticity in a similar and remarkable way than the water content. Gluten proteins contain a predominance of hydrophobic amino acids, particularly glutamine that has a strong tendency to form hydrogen bonds between protein strands. Moreover, the glutenin protein chains of the subunits contain amino acid cysteine with thiol groups, which form disulfide bridges that embrace the glutenin macropolymer and the gluten complex together. The more complex glutenins comprise high- and low-molecular-weight glutenin subunits that are linked together by disulfide bonds. Nevertheless, noncovalent interactions of mesoscopic glutenin aggregates are also involved in the development of the viscoelastic network. The proportion and size distribution of those proteins are critical for breadmaking and influence the mixing, kneading, and baking properties of dough. The properties of this network are governed by the quaternary structures resulting from disulfide-linked polymer proteins and hydrogen bonding aggregates. In this structure, also, tyrosine cross-links contribute to dough elasticity, suggesting that a radical mechanism involving endogenous peroxidases might catalyze the dityrosine formation during breadmaking.

During mixing, dough is exposed to large uni- and biaxial deformations, and a continuous protein network is formed, which is stabilized by disulfide bonds and modified thiol/disulfide interchange reactions. Depolymerization and repolymerization or cleaving and reforming of the sodium dodecyl sulfate (SDS)-unextractable polymers occur by the repeated breaking and reforming of disulfide bonds within and between gluten proteins, where glutenin subunits are released in nonrandom order, indicating a hierarchical structure. These exchange reactions require the participation of oxidizing–reducing systems, whose components occur naturally in flour. Mixing studies confirmed glutenin aggregation changes at constant temperature during dough processing and handling. Mixing induces an increase in the amount of total unextractable polymeric protein and large unextractable monomeric proteins; particularly, the amount of HMWGS increases with a parallel decrease in the amount of polymeric proteins from SDS-extractable proteins. Monomeric proteins and medium-molecular-weight proteins

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do not change in quantity during breadmaking. It seems that some type of rearrangement takes place during the breadmaking process to release proteins of smaller molecular weight. A direct relationship between polymeric glutenin in flours and loaf volume has been found with different wheat genotypes.

Gluten is a nonpure protein system with a main contribution to the unique properties of wheat dough properties, but nonprotein components also have significant effects on dough rheological properties. Mixing also promotes the solubilization of arabinoxylans due to mechanical forces, and this solubilization continues during resting due to the endoxylanase, xylosidase, and arabinofuranosidase activities.

The other large biopolymer that plays an important role in the breadmaking process is starch. Amylose and amylopectin are the constituents of the starch granule. This biopolymer provides fermentable sugars to yeast and has a significant contribution to dough rheology, especially during the baking process.

Protein–lipid interactions are also crucial in the breadmaking process. Polar lipids or the free fatty acid component of the nonstarch lipids has a positive effect on dough formation and bread volume. Conversely, nonpolar lipids have a detrimental effect on the bread volume. During mixing, free lipids in flour are majorly associated with gluten proteins, and nonpolar lipids are retained within the gluten network through hydrophobic forces, leading to the physical entrapment of lipids within the proteins. In addition, glycolipids are associated with glutenins through hydrophobic interactions and hydrogen bonds, and phospholipids interact with either the gliadins or lipid-binding proteins.

Fermentation of Bread Dough

Bacteria, yeasts, and fungi are naturally present in cereal flours at levels around 10^4–10^6 CFU g^{-1}. Flour endogenous microflora together with baker's yeast and sourdough is responsible of the main biochemical changes during fermentation. *S. cerevisiae* is present in bakery's dough due to its intentional addition to speed up proofing. Sourdough comprises yeast and LAB, generally at a ratio of 1:100. The main genera of yeast include *Saccharomyces* and *Candida*, whereas most common LAB are *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Weissella*. Nevertheless, the dominant LAB in sourdoughs depend on flour type (refined or whole wheat), environmental and processing conditions, and recipe. In this scenario, it can also be taken into account the role of endogenous and added enzymes that are commonly used as processing aids in bakeries.

Flour, yeasts, and LAB contain different enzymes that modify dough characteristics and fresh bread quality. Proteolytic enzymes from both flour and microorganisms also act on proteins releasing short-chain peptides and amino acids that will contribute to the organoleptic and nutritional quality of bread. The specific metabolic activities of microorganisms are responsible for the dynamics in nitrogen compounds, showing different metabolic rates for acidic, basic, aliphatic, and aromatic amino acids.

Yeast Action During Fermentation

During proofing or fermentation, the yeast metabolism results in carbon dioxide release and growth of air bubbles previously incorporated during mixing, leading to the expansion of the dough, which inflates to larger volumes and thinner cell walls before collapsing.

The yeast breaks down carbohydrates (starch and sugars) into carbon dioxide and alcohol during the alcoholic fermentation. A significant reduction in the level of reducing sugars occurs along fermentation. The carbon dioxide causes the dough to rise (ferment or proof), and the alcohol produced mostly evaporates from the dough during the baking process. Amino acids are absorbed by yeast and LAB and metabolized as a nitrogen source for growth producing an increase in the amount of gas released.

During fermentation, thousands of tiny bubbles surrounded by a thin film of gluten grow as fermentation proceeds. The endoenzyme α-amylase facilitates the breakdown of hydrated starch granules to shorter chained, unbranched molecules known as dextrins. Subsequently, the enzyme β-amylase, an exoenzyme abundant in flour, hydrolyzes available glucose chains (oligosaccharides) or damaged starch to maltose.

Yeast metabolism during fermentation is responsible for bread aroma. Aroma development in bread crumb has been found to be dependent not only on yeast concentration and fermentation time but also on the mixing stage. Major aroma compounds are alcohols, aldehydes, 2,3-butanedione (diacetyl), 3-hydroxy-2-butanone (acetoin), and esters. Aldehydes and their respective alcohols are produced inside the yeast cell from the degradation of flour amino acids via the Ehrlich pathway. The esters are produced in the yeast cell by an enzymatic reaction between acetyltransferases, acetyl coenzyme A, and various alcohols, whereas the ketones are formed from acetohydroxy acids leaked from the yeast cell. In addition, oxidation compounds from flour lipids greatly contribute to the aroma profile of bread crumb. The formation of the aroma compounds increases with yeast concentration, but the formation of volatile compounds derived from the lipid oxidation compounds is independent of yeast concentration. Fermentation temperature affects the concentration of products of lipid oxidation. Specifically, increasing fermentation temperature favors the formation of 1-heptanol, hexanal, heptanal, octanal, decanal, and 2-pentylfuran, whereas low fermentation temperature favors the formation of ethyl acetate, ethyl hexanoate, and ethyl octanoate in bread.

The type of baker' yeast used during fermentation affects the bread aroma profiles because they lead to different volatile compounds. The fermentation compounds 2,3-butanedione and 1-propanol are the predominant aroma compounds followed by 3-methylbutanal, 2-methyl-1-propanol, and ethyl acetate.

Long yeast fermentation could result in excessive degradation of the protein network leading to the flattening of bread rolls and/or the production of monochloropropanediol isomers, which are considered potential genotoxic carcinogens. Changes in the total or individual content of amino acids and peptides along the different steps of breadmaking modify the organoleptic characteristics of bread. The contribution of low-molecular-weight proteins to the taste and flavor of bread depends on the content of peptides rich in basic and hydrophobic amino acids released during fermentation and baking, the proportion of hydrophilic peptides in unfermented bread, and the balance of endo- and exoproteases activities. The

amino acid profile during breadmaking reveals that the total amino acid content (particularly for ornithine and threonine) increases by 64% during mixing and undergoes a decrease of 55% during baking, being the most reactive amino acids glutamine leucine, ornithine, arginine, lysine, and histidine.

Since initial studies with *S. cerevisiae*, great advances have been reached using specific strains to improve nutritional quality of breads. γ-Aminobutyric acid (GABA) is consumed by yeast during fermentation. To prevent the loss of GABA, mutants defective in the assimilation of GABA have been isolated from a baker's yeast strain. These mutants could be applied to breadmaking fermentation in order to maintain GABA at a high level in dough.

When bread is enriched in inulin-type fructans, the added fructans can be degraded up to 75% by yeast during dough fermentation. The extent of this hydrolysis strongly depends upon the average degree of polymerization of the fructan.

Biochemical Changes Promoted by Sourdough

Sourdough greatly contributes to the flavor and functional properties of the final product. LAB play a major role in sourdough fermentations. Sourdoughs are usually grouped into three types. Type I includes the sourdough that uses part of the previous fermentation. Type II is a semifluid preparation that contains dough-souring supplements and type III refers to dried preparations: both these two require the addition of baker's yeast as leavening agent.

Facultative heterofermentative LAB are important for the production of sourdough bread with porous crumb and contribute to the sensory quality, while obligately heterofermentative LAB, with their metabolic products, influence the flavor and promote the leavening. LAB action results in the production of short-chain fatty acids that decrease the dough pH (Figure 1), which in turn induces the activation of cereal proteases involved in the proteolysis of gluten. The released peptides are then hydrolyzed to amino acids by intracellular LAB peptidases, which release into the media peptides and amino acids, and the former might be flavor precursor compounds. Therefore, the sourdough fermentation leads to an increase in the amount of amino acids, in opposition to yeasted dough where only a decrease in the amino acid content is observed due to the microorganism metabolism. In general, wheat doughs started with LAB show a gradual increase of valine, leucine, lysine, and also proline. Additionally, the action of LAB proteases and peptidases on soluble polypeptides and proteins leads to an increase of short-chain peptides that contribute to dough plasticity and gluten elasticity. Nevertheless, the amino acid dynamics during breadmaking are greatly dependent on the breadmaking process; for instance, in the production of steamed bread, alanine undergoes the highest loss (17.1%), followed by tyrosine (12.5%), whereas leucine was the least affected amino acid.

Lately, a careful sourdough selection has been presented as an alternative for reducing the allergenicity of gluten containing baked goods, thanks to its action on the proline-rich

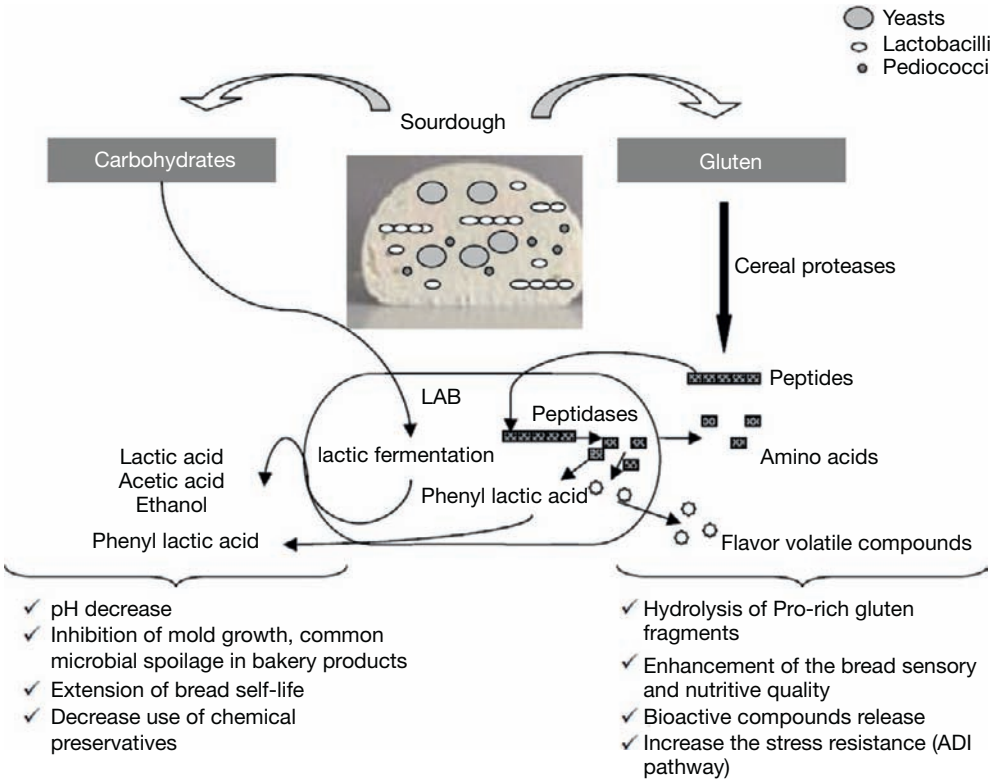

Figure 1 Action of lactic acid bacteria in sourdough fermentation. Reproduced from Rollán, G., Gerez, C. L., Dallagnol, A. M., Torino, M. I. and Font, G. (2010). Update in bread fermentation by lactic acid bacteria. In: Mendez-Vilas, A. (ed.) *Current research, technology and education topics in applied microbiology and microbial biotechnology*. Badajoz, Spain: Formatex, with permission.

peptide fragments. Sourdough LAB, after a careful selection, have been used as sources of proteolytic enzymes to decrease the concentration of gluten during breadmaking with the objective to reach complete degradation of gluten to get gluten-free breads (with less than 10 ppm gluten content).

LAB action increases the shelf life of bakery products due to the production of antifungal compounds. Lactic and acetic acids, together with other bioactive compounds like phenyllactic acid from phenylalanine metabolism and cyclic dipeptides (L-Leu-L-Pro and L-Phe-*trans*-4-OH-L-Pro), are very effective antifungal compounds against *Aspergillus*, *Fusarium*, and *Penicillium*, the main contaminants in bread. Selected LAB like *Lactobacillus plantarum* VTT E-78076 and *Pediococcus pentosaceus* VTT E-90390 or *Lactobacillus brevis* are able to inhibit the growth of rope-forming *Bacillus* strains (*Bacillus subtilis* and *Bacillus licheniformis*). Moreover, the acidification induced by LAB effectively delays the starch retrogradation associated with bread staling. In fact, the current trend for green label products drives to look for propionate-producing strains that could replace preservatives.

Different sourdoughs have been used as a source of microbial phytases resulting in a more efficient reduction of the phytate content in wheat sourdough breads than in yeast-fermented breads. Most LAB produce phosphatase activity with low levels of activity against phytate. The use of selected LAB with specific activities like phytate-degrading enzymes as starters for breadmaking could be a good alternative for obtaining whole wheat bread with low phytate content and in consequence with increased nutritional value regarding mineral bioavailability. Two lactobacilli L-M15 and L-ID15 have shown high phytate-degrading activity hydrolyzing phytates and leading different low-phosphate complex compounds.

Enzymes Contribution During Fermentation

Different processing aids, namely, enzymes, are also used in breadmaking to improve the quality of the baked products by reinforcing the role of gluten, providing fermentable sugars, and/or contributing to stabilize the hydrophobic–hydrophilic interactions. Enzymes have been extensively used in the production of cereal-based products with different purposes. In addition to the traditional starch-hydrolyzing enzymes – amylolytic/dextrinizing, saccharifying, and debranching enzymes – incorporation of nonstarch polysaccharide-degrading enzymes and lipid- and gluten-modifying enzymes has proved to be effective as dough conditioners and strengtheners, initial crumb softeners, enhancers of the activities of yeast and endogenous flour enzymes, bread flavor enhancers, and antistaling principles. α-Amylases are the enzymes most frequently used in baking. The polysaccharides obtained from the hydrolytic activity also participate in the Maillard reactions that take place during baking. Enzyme effects are already apparent immediately after mixing and continue during resting, significantly changing the viscoelasticity of doughs and the biochemical protein pattern.

Three different enzymes activities (transglutaminase, glucose oxidase, and laccase) have been used with the aim to increase gluten strength and consequently to improve dough functionality for breadmaking. Commercial lipases developed for breadmaking are able to reach and act on the low amount of triglycerides of the dough, even though in a limited free water environment. Lipases show a synergistic effect with pentosanases and/or amylases, reducing or avoiding their softening effect on doughs.

Whole wheat flour is rich in fiber, minerals, vitamins, and many phytochemicals (phenolic compounds, sterols, tocopherols, tochotrienols, phytic acid, etc.), which may partly account for its beneficial effect on human health. However, phytic acid (myo-inositol hexakisphosphate) or phytate is considered an antinutrient due to its complexing action on minerals decreasing their bioavailability. A reduction of the phytate content can be achieved by adding exogenous phytic acid-degrading enzymes that release lower myo-inositol phosphates decreasing or eliminating their antinutritional effect.

Chemical Changes During Baking

When dough gets into the oven, a progressive increase in the temperature is produced along baking. Dough constituents and microorganisms are affected by thermal constraints, although the extent of the effect is dependent on their thermal stability. As the temperature raises, the rate of fermentation and production of gas cells increases and this process continues until the temperature of yeast inactivation is reached (around 45 °C). In the case of yeast and LAB, they are acting and generating carbon dioxide and alcohol until their thermal death. The yeast metabolism is even sped up as a consequence of the increased amylolytic activity due to the starch gelatinization. The action of amylase on starch is approximately two-fold every 10 °C rise. Endogenous enzymes present in the dough are inactivated at different temperatures during baking. β-Amylase denatures at lower temperature (57–71 °C) as compared to alpha-amylase, which denatures at temperatures ranging from 65 to 95 °C.

A decrease in extractable protein is produced with time of baking. That insolubility is due to the protein cross-linking derived from the formation of disulfide bonds during baking. As temperature increases, a progressive reaggregation of disulfide-linked SDS-insoluble proteins is produced. Heat-induced reaggregation of glutenin macropolymer starts at around 36 °C. Glutenin apparently starts aggregating into SDS-insoluble structures between 35 and 45 °C. It is rather difficult to detect a variation in the amount of free thiol groups between 30 and 50 °C, but a significant decrease occurs at temperatures higher than 50 °C. Those thermally induced aggregations result in a decrease of the storage modulus (G') of the gluten proteins that has been associated with protein unfolding. In general, gluten proteins show a minimum value of G' at 57 °C, reflecting the thermal transition derived from the protein cross-linking involving SH/SS interchange, oxidation, and hydrophobic interactions. When proteins are denatured, the gluten strands surrounding the individual gas cells release water molecules with a simultaneous transformation into the semirigid structure that will yield the bread crumb.

In parallel, starch granules absorb the water available in the medium and swell, although the degree of swelling is restricted by limiting availability of water. Starch gelatinization begins at around 55 °C (depending on the type of starch), and it is associated with absorption of water, while gluten denaturation

is associated with its removal. Therefore, a major change during baking is the redistribution of water from the gluten phase to the starch phase. After starch swelling, the amylose chains leach out into the aqueous intergranular phase promoting the increase in the viscosity that continues until the temperature constraint leads to the physical breakdown of the granules, which is associated with a reduction in viscosity. Pasting performance of wheat flours during cooking and cooling involves many processes such as swelling, deformation, fragmentation, disintegration, solubilization, and reaggregation that take place in very complex media primarily governed by starch granule behavior. During cooling of the loaf, the gelation process of the starch takes place, in which the amylose chains leached outside the starch granules during heating are prompted to recrystalize. The reassociation between the starch molecules, especially amylose, results in the reordering of the starch molecules leading to a gel structure.

The sugars and breakdown products of proteins released from the enzyme activity are then available to sweeten the breadcrumb and to participate in the Maillard or nonenzymatic browning reactions, responsible for the attractive brown color of the crust. When sugar is heated to around 170 °C, which only occurs on the bread surface, the caramelization reaction where the molecules polymerize to form colored substances proceeds. The browning reaction occurs at high temperatures and low water activity, consisting of the reaction between reducing sugars and either protein- or other nitrogen-containing substances, and it produces colored compounds, named melanoidins. These reactions impart color and flavor to bread. Free amino acids play an important role in the generation of bread flavor precursors, through the formation of the Maillard compounds during baking. In fact, leucine, proline, isoleucine, and serine reacting with sugars form typical flavors and aromas described as toasty and bread-like, while excessive amounts of leucine in fermenting doughs lead to bread with an unappetizing flavor.

However, besides the beneficial chemical reactions, during baking, some other undesirable chemical interactions also occur. Alarm bells sounded some years ago due to the levels of acrylamide present in bakery products. The asparagine is the precursor of acrylamide formation in cereal baked products, especially bread. Other precursors of acrylamide include gluten, but their role is only important when low levels of asparagine are found in dough. In this context, glycine acts hindering the formation of acrylamide from asparagine, and the more asparagine is in the dough, the stronger is the inhibitory effect of glycine. The content of reducing sugars also affects the formation of acrylamide. Because of that, the selection of LAB excreting lower amylolytic activity in the medium and with higher proteolytic activity is recommended to reduce the formation of acrylamide. The addition of glycine but not asparagine caused an increased browning reaction during baking. The addition of glycine also increases the intensity of the browning reaction during baking. Different ways for controlling the production of acrylamide have been proposed, like monitoring the content of asparagine in the raw materials, selecting ingredients, adding the enzyme asparaginase for reducing the content of asparagine during breadmaking, and even applying glycine on the surface of the fermented dough.

Vitamin content is also affected during the breadmaking process. The yeasted breadmaking process leads to a 48% loss of thiamine and 47% of pyridoxine in white bread. Native or endogenous folates show good stability to the baking process and in some breadmaking processes even an increase in endogenous folate content in dough and bread has been observed.

Alternatives to the Common Breadmaking Process: Low-Temperature Technologies

In the last decades, breadmaking processes have been adapted to the new consumer demands, and subzero and low temperatures have been included in the flow diagrams for interrupting the processes before or after fermentation, or when partial baking was completed, for obtaining partially baked breads. These new technologies have facilitated the launching of a great number of fresh baked goods available at any time of the day.

Freezing affects the baking performance of frozen bread dough due to its effect on yeast and gluten network. The freezing rate and the frozen storage conditions have strong influence on yeast activity. A slow freezing rate is usually recommended to preserve its activity; for instance, low-freezing-rate conditions (air velocity of 1 m s^{-1}, −20 °C) result in the highest yeast activity, and in opposition, high-freezing-rate conditions (air velocity of 4 m s^{-1}, −40 °C) result in the lowest gassing power. During freezing and frozen storage, the number of viable yeast cells decreases and, as a consequence, a reducing compound (glutathione) is released, which can break down the disulfide bonds among proteins leading to a weakening effect on the gluten. Besides, hydrophobic interactions become weak when temperature decreases, which can partially explain the steady deterioration of the gluten network during frozen storage. Therefore, the reduction in the dough resistance induced by freezing and thawing operations has been partially related to certain compounds released from dead yeast cells after freezing and thawing.

The gluten network can also be disrupted due to the presence of ice crystals formed during freezing; as a consequence, the gluten network weakens and decreases its water retention ability, affecting its ability to retain CO_2. Frozen hydrated gluten forms a continuous, homogeneous, and not fibrous network that can be deteriorated due to the growth of ice crystals confined into dough matrix capillaries and the growth of bulk ice. Water transport from the gluten to the starch occurs during frozen storage, although the extent depends on the storage temperature. At −15 °C, the water content in the gluten phase decreases by approximately 1% over the first period of storage, and then, it reaches an apparent steady state. In opposition, at −25 °C, the amount of ice does not change. In general, the amount of freezable water in frozen doughs increases with frozen storage, confirming the water migration and ice crystallization.

The lipid fraction is removed from the gluten protein due to the decrease in water in the continuous protein phase promoting the fusion of lipid droplets and an increase in their size. Final effect will be baked breads with a flat surface, harder and coarser crumbs, and uneven distribution of big air cells. After prolonged frozen storage, the proportion of alcohol-soluble

protein increases with a simultaneous decrease in the HMWGS, which suggests depolymerization of the protein matrix during frozen storage, and some starch granules exhibit internal damage and become separated from the gluten matrix.

The technology of bake-off or partially baked bread is another type of breadmaking where low temperatures are applied. The market of partially baked bread has rapidly grown owing to the product being already sized, shaped, and partially baked: thus, no skilled personnel are needed at the retails for finishing the product. Nevertheless, a careful control of proofing, partial baking, chilling, and freezing conditions is necessary because of their great impact on the fresh bread quality. The critical time and temperature control required for the two-step baking is a major problem. Full baking has a superior quality in comparison with its frozen and thawed full-baked counterpart. Optimal time for the initial partial baking lies within the range from 74% to 86% of the time needed for the full baking in conventional breadmaking. Par-baked bread can be stored under frozen or low-temperature conditions. Par-baked loaves stored at low temperatures show progressive crumb hardening and rapid crystallization of the amylopectin chains, but the heat applied during the full-baking process can reverse those processes and the extent of that improvement is directly related to the duration of par-baked bread storage. When par-baked loaves are stored under subzero temperatures, no retrogradation of amylopectin is detected during storage, but some structural changes are produced on the starch as indicated by the increase in amylopectin enthalpy observed during aging of the full-baked breads. Nevertheless, the Milton-Keynes process has been designed to stabilize the par-baked breads by using a vacuum cooler to stabilize the crusty par-baked structure without producing wrinkling or shriveling of the loaf during the storage at ambient temperature.

Breadmaking is a very complex process, in which diverse sources of alterations are compiled in a food system. Mechanical stress besides the biochemical action of microbial and flour endogenous enzymes and finally thermal constraints are responsible for unaccountable changes in all the flour constituents. The understanding of the ingredients (flours, yeast, sourdough, and enzymes) and the process conditions allows the modulation of the biochemical changes associated with breadmaking.

See also: Bread: Breadmaking Processes; Bread: Dough Mixing and Testing Operations; Bread: Types of Bread; Fermented Foods: Use of Starter Cultures.

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Bread: Dough Mixing and Testing Operations

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Introduction

The fundamental basis of utilizing wheat flour as one of the most important food source around the world is its unique property of forming dough and developing gluten when it is mixed with water. Wheat gluten is a protein–lipid–carbohydrate complex formed as a result of specific covalent and noncovalent interactions from flour components during dough making as the components are hydrated and energy from mechanical input from the mixing process is provided.

Wheat varieties at the same protein level were found to differ in their bread-making quality, giving the first indication of protein quality. Protein content and its composition are important determinants of good bread-baking quality. Gluten-forming proteins contribute 80–85% of the total wheat protein and are the major storage proteins of wheat. They belong to the prolamin class of seed storage proteins. Gluten proteins are largely insoluble in water or dilute salt solutions. Two functionally distinct groups of gluten proteins can be distinguished: monomeric gliadins and polymeric (extractable and unextractable) glutenins. Gliadins and glutenins are usually found in more or less equal amounts in wheat. Large level of polymorphism of wheat prolamins results in a special effect in relation to the overall functional properties of wheat dough. During dough formation when prolamin proteins are hydrated and form the gluten network, the numerous structurally similar but slightly different proteins produce a mass in which several characteristics (such as size, polarity, charge distribution, solubility, and viscosity) show a continuous distribution in a relatively large interval. This structural feature provides a unique characteristic of gluten proteins among any other protein systems.

Phases of Bread-Making Process

The bread-making process has several functions, accomplished at different stages in the preparation and baking of dough: (a) mixing of flour and water, together with yeast, salt, and other ingredients in specified ratios to form the dough; (b) developing the gluten structure of hydrated proteins through application of energy during mixing (a stage often termed 'kneading'); (c) incorporating air bubbles within the dough during mixing; (d) continuing the development of the gluten structure after kneading to improve its ability to expand when gas pressures increase (a stage termed 'ripening' or 'maturing'); (e) creating or modifying flavor compounds in the dough; (f) subdividing the dough mass into unit pieces; (g) modifying the shape of the divided dough pieces; (h) resting to allow further modification of the dough pieces' physical and rheological properties; (i) shaping to achieve required configuration; (j) proofing (fermenting and expanding) the dough; and (k) expanding and fixing the dough into its final shape by baking.

Mixing and Dough Making

Dough mixing is a very important stage in the bread-making process. The extent of mixing has a critical impact on final bread quality. The mixing process promotes different physical, chemical, and physicochemical modifications that contribute to the dough development.

Chemical, Physical, and Physicochemical Alterations during Dough Mixing Stages

Dough chemistry involves a series of interactions between carbohydrates, lipids, and proteins. The principal physical science involved with dough making is rheology (see in the succeeding text). Good baking quality depends on several rheological properties such as extensibility exceeding a minimum level, viscosity, strain hardening, and optimal resistance to deformation. Dough is viscoelastic, combining properties of a Hookean solid with those of a non-Newtonian viscous fluid. Dough is essentially foam, which becomes sponge after baking. The transformation from the closed cell structure of a foam to the open cells of a sponge is one of the many changes that occur during dough processing.

Individual dough-property parameters describe only certain essential elements of dough properties. Depending on the final product, different levels of these attributes are required to get superior processing quality. For example, the balance of dough strength and extensibility is believed to be the most important factor governing the suitability of a flour to make good bread. However, for different types of breads and even for different types of processing technologies, a diversity of dough strength and extensibility values may provide the optimum balances needed in each case.

The complexity of relating protein composition to quality derives from the fact that the question can (and has to) be investigated on different levels of protein composition, namely, protein content, the ratio of polymeric proteins to monomeric proteins, the ratio of high-molecular-weight (HMW) glutenin subunits to low-molecular-weight glutenin subunits, and the proportions of x-type and y-type HMW glutenin subunits. These various parameters can be determined for a specific flour sample to see if there is a 'good balance' between the various components in the sample, thereby to satisfy quality-related criteria. The polymeric glutenin is mostly responsible for the elasticity of the dough, whereas the monomeric gliadins are the extensibility-related characters in the system. Thus, the ratio of polymeric proteins to monomeric proteins (the glutenin-to-gliadin ratio) can be directly related to the balance of dough strength and extensibility of the sample.

Two preconditions must be met for the production of dough with the right properties: (a) appropriate proportioning

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of the individual ingredients as established in a well-balanced dough formulation and (b) homogenous distribution of these ingredients throughout the dough mass. In its essentials, dough mixing involves the combining and blending of the formula ingredients and then applying sufficient physical work to the mixture to transform it into a cohesive mass with the requisite viscoelastic properties. In large-scale commercial bakery production, the major ingredients (flour and dry sweeteners) are normally weighed by automatic scales that feed directly into the mixers, while water, liquid shortener, and liquid sweeteners are piped into the mixer through meters that can be preset to deliver specified volumes.

In addition to achieving a thorough dispersion of the ingredients into a homogeneous mixture, the dough mixing process in bread making has the further important objective of physically developing the gluten proteins into a coherent three-dimensional structure that will impart to the dough the desired degree of plasticity, elasticity, and viscosity. The initial mixing phase must physically hydrate the flour particles and incorporate air to nucleate gas cells responsible for leavening. In other words, mixing has three functions: (a) creating a homogenous mass from ingredients of differing characteristics, (b) developing (kneading) the dough sufficiently to ready for subsequent processing, and (c) occluding air into the mass to form the cell structure necessary for finished crumb quality.

At the beginning of the mixing, blending action leads to an even distribution of the dough ingredients and ensures hydration and swelling of flour particles. Wheat flour dough or batter may appear to be uniform and well mixed, but actually, it is multiphasic: starch, gluten proteins, lipids, and water representing the principal phases. Furthermore, the form of these phases changes during periods of mixing that prepare them for separation or food uses. Microscopic changes begin with the instant formation of protein fibrils at first contact of water and flour particles. Slow mechanical development induces these fibrils to coalesce into fibrous bands or tendons and segregates the starch into clusters. When flooded with a displacing fluid, this open, spongelike structure readily releases the entrained starch. Additional development disbands the protein into relatively fine, uniformly distributed, and networked or webbed filaments that entrap the starch and gas bubbles formed when the dough is fermented and baked. The physical properties of hydrated wheat proteins are the result of covalent and noncovalent interactions of wheat gluten proteins. These interactions are altered by the repeated extension, tearing, and compression during mixing or development. Specific chemical effects include (1) disulfide bond disruption, (2) chain disentanglement and rupture, (3) disulfide–sulfhydryl interchange, (4) formation of dityrosine cross-links, (5) formation of new disulfide cross-links, (6) free radical interactions, and especially (6) reorientation leading to enhanced hydrogen bonding.

Mixing produces a homogeneous gluten film regularly distributed around the starch granules. The dough must be mixed for a specific time (referred to as optimum dough development) to ensure optimal loaf volume and bread texture. Stopping mixing before the optimal point results in undermixed dough that gives bread of inferior volume and crumb quality. The optimal mixing requirement is a specific characteristic of each wheat flour. Beyond optimum dough development time, overmixing induces dough stickiness, decreases dough consistency (due to degradation by shearing effects), and negatively affects bread quality.

Stability of the gluten–starch matrix – the primary stabilizing factor for expanding gas cells against disproportionateness and coalescence – depends on its tendency to strain harden. The phenomenon of strain hardening appears to depend on the balance between strength and extensibility of the entangled network of polymeric proteins of wheat flour. Extensibility ensures slippage of the maximum number of statistical segments between entanglements, whereas strength prevents disruption of the entangled network of polymeric proteins. Thus, to ensure stability of gas cells, the dough needs to be not only sufficiently extensible to respond to gas pressure but also strong enough to resist collapse.

A good gas-holding capacity of dough is necessary for producing a loaf of bread with light and even crumb. The strain hardening properties of gluten are vital to avoid early rupture of gas 'cell wall' during proofing. The gas phase of bread, which makes up more than 70% of the final volume of a loaf, has a major influence on its textural and sensory attributes. Controlling the gas phase volume is a major challenge as during proving and early stages of baking gas must be captured within bread dough, only being released at the end of baking. The main factors, important in determining the gas cell structure, include (1) the formation of the initial foam structure during mixing and (2) stabilization of the foam structure, including those factors governing bubble disproportionateness and coalescence. There is particular focus on the role that the thin film lining the bubbles may play in stabilizing the foam structure of a risen dough. The surface properties of components have been suggested to be the important factor to the stability of gas cells. Recently, proteomic methods have been used to identify foam-forming soluble proteins from dough that may play an important role in stabilizing gas bubbles in dough and hence influence the crumb structure of bread. Proteins from a soluble fraction of dough (dough liquor) or dough liquor foam have been separated and identified. Major polypeptide components included β-amylase, tricitin, and serpins, with members of the α-amylase/trypsin inhibitor family being particularly abundant. Neither prolamin seed storage proteins nor the surface-active protein puroindoline was found.

Differences in gluten quality can significantly affect the bread-making potential. Strength is conferred by the fraction of polymeric proteins having molecular weight greater or equivalent to a critical size, MT, (250 000 kDa), and the fraction of gluten protein smaller than MT may counter the strength by acting as diluents. The optimum balance seems to exist when the relative proportions of polymeric proteins greater and smaller than MT are roughly 60:40. Shift in the balance to either side will decrease loaf volume. Increase in smaller proteins (less than MT) may decrease stability of the gluten–starch matrix due to a lesser number of entanglements per chain. On the other hand, increase in strength conferring proteins may prevent sufficient expansion of the gluten–starch matrix required to increase loaf volume due to reduced slippage of gluten polymers through entanglement nodes as a result of increase in number of entanglements per chain. The secondary stabilizing mechanism involves thin liquid lamellae stabilized by adsorbed surface-active compounds (lipids and

proteins) at the gas–liquid interface. Liquid lamellae prevent coalescence and disproportionateness of gas cells when they come in close contact with each other during the late proving and early baking stages of bread making, that is, when discontinuities begin to appear in the gluten–starch matrix. Flour lipids at their natural levels do not influence rheological properties of the gluten–starch matrix surrounding the gas cells, as measured by the dough inflation system. Nevertheless, the small amounts in which these lipids are naturally present are sufficient to influence surface properties.

This short and simplified description of well-accepted observation-based hypothesis of dough behavior underlines the essential importance of size distribution of gluten proteins in relation to their role in determining dough properties. As a consequence of this, a combination of reducing agents, oxidizers, and proteolytic enzymes is frequently used in the baking process to alter dough properties through their effects on the disulfide bonds in the gluten structure. Functional additives play a big role in modern bread making. Among the improvers, ascorbic acid is the most important in modern bread formulation to oxidize flour proteins to improve gluten strength. Other such ingredients (and their functions) include azodicarbonamide (oxidizing agent), cysteine (reducing agent), mono- and diglycerides (emulsifiers and antistaling agents), calcium propionate (mold inhibitor), stearoyl lactylate (dough strengthener), soy flour (crumb whitener), dextrose (fermentable sugar source for the yeast), ammonium chloride (nitrogen source for yeast), enzymes (starch and protein adjustment), and, occasionally, gluten (strengthener).

Dough Preparation Systems

No single standard method for mixing ingredients to create dough is followed by all bakers; instead, more than a half-dozen different procedures can be used. The detailed characterization of these methods is shown in Table 1. Baker preference, product type, and plant practice determine the choice of method. Preparation of the dough can be done in batches or continuously, and fermentation times vary from none to several hours.

Today, most commercial bakers prepare dough as separate batches, sized sufficiently to permit an uninterrupted production schedule but not too large to risk overaging the dough as it waits in the divider hopper. The continuous mix method was developed during the 1950s to automate dough preparation. At one time, it was used by the majority of bakeries producing white pan bread, but this method fell out of favor when consumer demand for variety bread increased starting in the late 1970s. Technologies that grew up around continuous mix such as water brews and liquid sponge, however, remain in wide use for lines dedicated to baking long runs of fast-food buns and similar products.

Table 1 Overview of the most generally used dough preparation systems

Dough system	*Bread and related product produces*	*Advantages*	*Disadvantages*
Straight dough	Lean formula hearth bread, pita bread, 100% whole wheat	Good flavor Medium process time Good mixing tolerance	Difficult dough handling Long mixing time Poor fermentation tolerance
Sponge and dough	Sponge and dough bread and rolls	Good fermentation tolerance Superior product score Good dough handling Longer product shelf life	Poor mixing tolerance Long process time High-cost equipment Larger space equipment
Liquid sponge	Sponge and dough bread and rolls	Uniformity of product Medium process time Good flavor with high amount of flour in the sponge	High-cost equipment Limited to 50–60% of flour in sponge Lack of flavor and shelf life with low flour in sponge
Continuous mix	White pan bread, hamburger/hotdog buns	Same advantages as liquid sponge if fermented Less equipment, labor, and space used	Limited to 50–60% of flour in sponge Lack of crumb strength Lack of flavor and shelf life with less fermentation
No-time dough	Frozen dough, bagels, hard rolls, pizza crusts, dinner rolls, variety pan bread, English muffins	Short production time Greater flexibility Less equipment and space Superior yeast survival in freezing	Lack of flavor Lack of shelf life Higher ingredient costs Problem with floor time
Chorleywood process	Hamburger/hotdog buns, variety pan bread, rye bread	Tolerant to low-protein flours Short production time Greater flexibility No floor time problems	High equipment cost High energy cost Lack of flavor Lack of product shelf life
Authentic sourdough process	Authentic sourdough breads and buns	Sourdough flavor Increased shelf life 'Blistered' appearance Chewy, resilient texture	Very long process times Nurturing of sponge Less consistency Increased space requirement

Source: O'Donell (1996).

Among mixing methods being practiced commercially, the most prevalent is the sponge-and-dough process that involves two mixing stages, namely, one of the sponge and the other of the dough. The sponge mixing stage aims at homogeneous ingredient dispersion and flour hydration and is normally of relatively brief duration, whereas the more critical phase of dough development is reserved for the more extensive mixing of the final dough. In the straight-dough method, as well as in those systems that employ various forms of liquid preferment, there is but one mixing stage in which complete dough development must be achieved.

White pan bread is highly standardized, has well-recognized quality characteristics, and represents the main product style in many parts of the world. Some countries, notably France, take the baguette as the standard product. Table 1 shows the advantages and limitations of the most important methodologies based on the excellent review of Mihalos.

Testing Operations

Introduction

Dough testing methodologies are essential tools through the whole wheat chain from basic research, prebreeding, selection for quality attributes during breeding, characterizing source material, quality control, process, and product development in the wheat-based food industry.

Testing operations can be classified as direct dough testing methods and indirect methods where dough properties are estimated from chemical, physicochemical (spectral), or physical (sedimentation) characteristics. Dough properties, directly related to the bread-making quality of the sample, describe certain viscosimetric or rheological properties of the dough, so the dough testing investigations apply viscosimetric and rheological principles. In general, viscosimetric methods show strong relationships to the starch composition of the samples, while the rheological properties are directly related to both qualitative and quantitative aspects of gluten protein composition.

Some basic information on the direct dough testing methods is given here, with special emphases on the small-scale and microscale testing methods, which revolutionized our understanding about structure–function relationships in wheat dough in the last 30 years. Only some key references are given about the principles, solutions, and achievements of indirect methods in the paragraphs summarizing future trends in the area.

Basic and Empirical Rheology

Rheology is the science of the deformation and flow of materials as a response to physical stresses. The deformation can be classified as elastic or inelastic, while the flow properties of a material can be described as plastic or viscous behavior. Ideal elastic bodies undergo reversible elastic strain when anisotropic forces are applied. In this case, the applied energy is partly stored. In case of ideal viscous body, irreversible changes can be observed, where the exerted energy is transformed. Viscous fluids generally exhibit viscosity, while solids exhibit elasticity.

The aim of fundamental rheology is trying to describe and model the physical behavior of materials by studying the relationships between molecular composition and the observed deformation. Widely applicable instruments (viscometers, rheometers, etc.) and/or specific, often purpose-built methods are used for this purpose. The resulting information, however – despite its scientific merit – does not satisfy the demands dictated by the practice: Fundamental rheology methods often do not differentiate enough among samples and, most importantly, they are not suitable for high-throughput, reasonably cheap routine application in selecting for quality in plant breeding or in case of quality control in the food industry.

During the first quarter of the last century, several empirical rheological equipments and methodologies have been developed, and the last 100 years proved that these standardized methodologies can be applied fruitfully for the comparison/rating of samples derived from the breeding or industrial operations. The collected/archived data derived from these analyses form an invaluable knowledge base based on which the new wheat cultivars and new wheat products of the future can be developed.

Traditional empiric rheological methods and instruments

As it was mentioned earlier, when wheat flour, water, and other related ingredients are mixed, the whole system undergoes a number of chemical reactions and physicochemical and physical changes during dough formation. The type and the rate of these changes highly depend on the composition of wheat flour, on the ingredients, and on the parameters of dough mixing like length mixing, energy input, and temperature. Molecular processes related to the aforementioned changes can be monitored by the continuous measurement of physical (rheological) properties of dough from the starting stage of homogenization through the formation of protein–carbohydrate–lipid–water complex (gluten) until its certain break caused by overmixing.

Wheat dough has both elastic and flowing properties; therefore, it shows a complex viscoelastic behavior. Therefore, the main challenge in the development of empiric rheological methods and instruments has been how one can apply adequate external forces on the dough to measure both elastic strain and viscous flow in one system.

Two principally different empiric rheological methodologies have been developed: *mixing methods* to monitor dough formation and *stretching* methods for the determination of dough strength and extensibility. In case of the latter process, dough is mixed to its optimum consistency in a separate process followed by a relaxation step before stretching. So, the two methods together mimic the industrial bread-making technology with one important exception: instead of a full formulation of the dough, including yeast, dough is mixed here with either water or salt solution.

Mixing methods: The traditional simple solution for detecting the physical changes in a dough is the utilization of standardized (laboratory) mixers with the recording of torque on the mixing arm(s) and/or bowl. The first recording laboratory mixers – regarded as the forerunners of the first commercial machines developed later on, the Swanson–Working

mixograph and Brabender farinograph – have been developed by the Hungarian inventor Jenő Hankóczy.

The working principle of the farinograph is to monitor the torque (energy requirement) during the continuous but relatively gentle mixing of wheat flour – added water system at constant speed and temperature (at 30 °C). The sigmoid (or Z-type) shape of the mixing blades is very unique, which is able to knead and extend the dough, periodically. The torque that arises from dough resistance against mixing was originally measured using a special balance system and replaced later with electronic recording systems. Basically, dough resistance, detected in this equipment, is determined by the rheological properties of dough, particularly viscosity, but the surface properties of the dough, sticking to the bowl walls and blades, also contribute to the measured values. The comparability of different dough behaviors (or flour quality) is ensured with the standardization of maximum dough resistance.

Beyond the mixing parameters such as mixing speed and resistance measured with this system, there is the function of the flour behavior and the amount of water added to the flour. The huge success of the farinograph spreading all around the wheat chain as early as the 1930s derived from the idea of using this direct relationship between the amount of water in the system and resistance for the determination of the most important quality attribute of the flour, *water absorption*, which is the amount of water needed to be added to the flour to reach the constant consistence (500 or 600 farinograph or Brabender units, BU) of dough.

The routine investigation on the farinograph is a two-step process: water absorption of the flour is determined through a 'titration-like' process, followed by the main mixing experiment using this amount of water to characterize the rheological properties of the dough such as dough development time, stability, and degree of softening (**Figure 1**). The detailed description of the farinograph method including the evaluation of curves is summarized in different standard methods (ICC, AACCI, ISO, etc.).

The second traditional type of recording dough mixer is the mixograph. The main difference between these two mixers is the mixing action. The mixograph equipped with pin mixers, where a pull–fold–repull type of movement is applied causing much greater mechanical stress on dough than that in the farinograph or other Z-arm-type mixers.

In case of mixing with mixograph, there is no predetermined optimum consistency of dough; therefore, other methods have to be used for measuring the optimal water absorption. Two methods are used in practice: (a) Samples are compared with a uniform amount of water added and

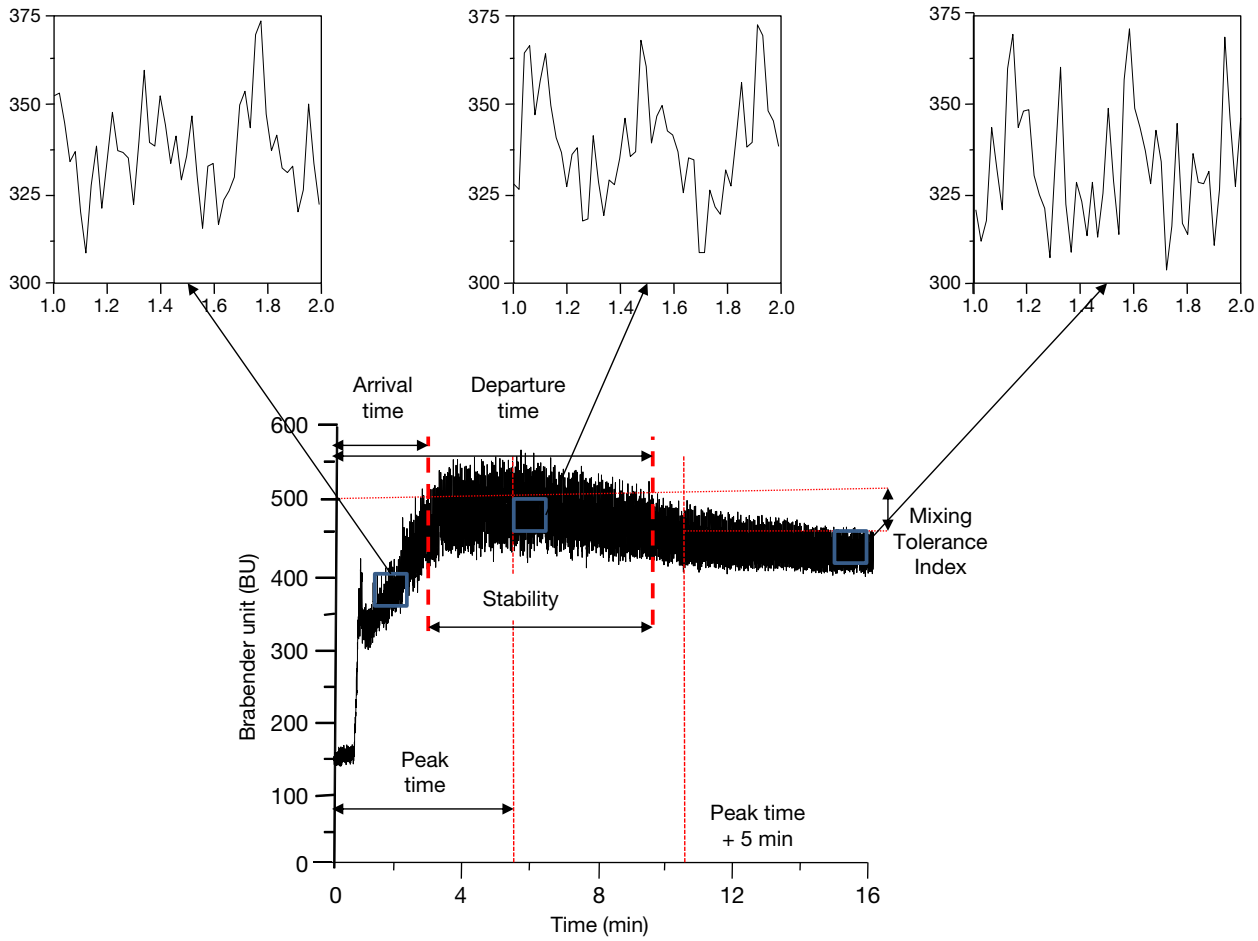

Figure 1 Important farinograph parameters.

(b) the water used in the mixing experiments is calculated based on the protein content of the investigated flours. The parameters generally determined by the evaluation of the recorded mixogram (Figure 2) are as follows: peak time (similarly to the dough development time), maximum peak height, the height of the curve at a specified time after peak (characterizing the tolerance against overmixing, similarly to the farinographic stability), the angle between the ascending and descending portions of the curve (tolerance angle, T), the weakening angle (W), and the area under the curve are defined.

High-speed recordings obtained with a 35 g mixograph equipped with a strain gage allowed the high-resolution monitoring of the mixing action. These recordings provided data essential for developing a mathematical model of dough mixing: dough mixing on pin mixers can be interpreted as a complex, periodic series of pushing and stretching the dough around the pins. Each individual peak represents one of these circles, and so, their size and shape are characteristic to the stage of dough development, and they can be used to determine dough strength and elasticity of the dough (details (a), (b), and (c) of Figure 2 illustrate three regions of the high-resolution mixing curve ((a), (b), and (c)), illustrating the hydration, dough development, and overmixing phases of the mixing). Bandwidth parameters (BWPR, BWBD, and TMBW), directly related to elasticity, in the mathematical model are also shown.

Some other instruments developed by different producers (valorigraph, doughLAB, etc.) work on the same or similar principle as described earlier, with different sizes of mixing bowls and arms for mixing 10–300 g of flour.

Stretching methods: Elasticity is the most unique property of wheat dough, and it mostly depends on the protein–wheat gluten composition and quality. Extensibility of wheat dough is responsible for the extent of expansion during leavening; therefore, it basically determines the baking performance and the quality of final products. In all stretching tests, to determine extensibility, the dough produced by one of the standardized mixing methods is then submitted to large deformations until rupture occurs and the resistance against stretching strain is recorded.

Two types of extension methods are used: in uniaxial extension test, the dough is stretched in one direction, while in case of biaxial method, the dough is extended in two opposing direction. The most traditional and commonly used equipment is the Brabender Extensograph, introduced in 1936. The operation of this instrument is based on the principle of mechanical stretching in simple tension. The investigated dough samples are prepared in the farinograph mixer with optimum water absorption, and then, aliquot pieces of the dough are molded with special tools. During the measurement, the resistance of formed dough pieces to stretching and the distance the dough stretches before breaking are recorded on the extensogram (Figure 3). The following parameters are determined/calculated: the maximum resistance (R_{max}), the resistance at a constant extension (generally at 50 mm, Rx), extensibility (the maximum length of extension before rupture, E), the ratio of maximum resistance to extension (as an indicator of the balance between elastic and viscous behaviors, R_{max}/E), and the area under the curve (as extensional work, A). In some cases, the applied methods can differ in some parameters, like constant extension and resting time of dough before measurement. The desired quality of dough means a good combination of dough resistance and extensibility.

The first device for measuring the biaxial extension character of wheat dough was also developed by Hankóczy, while the principle of dough inflation test was developed by Marcel

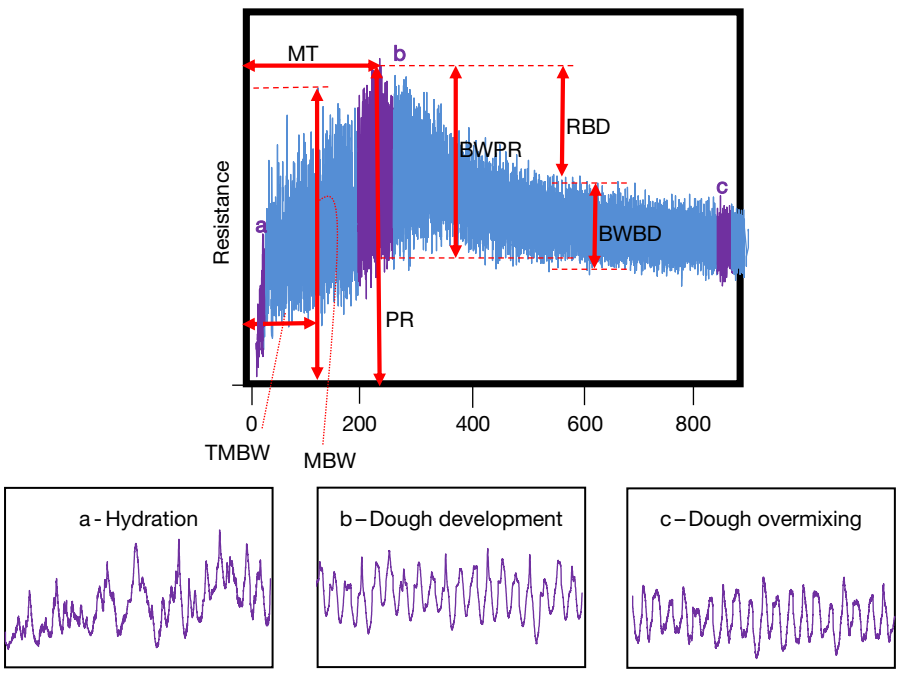

Figure 2 The most important parameters determined from the mixograph curve. MT, mixing time; PR, peak resistance; RBD, resistance breakdown; BWPR, bandwidth at peak; MBW, maximum bandwidth; TMBW, time to maximum bandwidth. High-resolution data recording of regions a, b, and c show the stages of hydration, dough development, and overmixing, respectively.

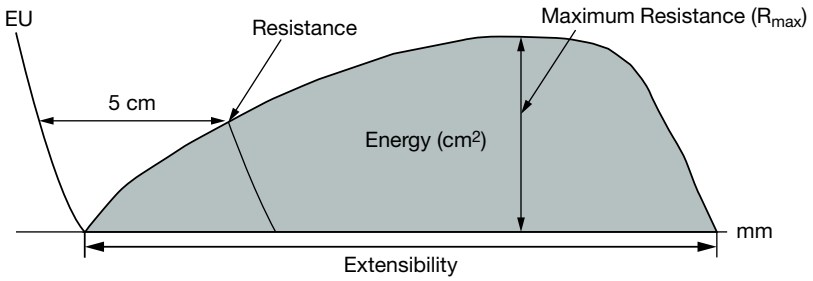

Figure 3 The determination of dough strength (R_{max}) and extensibility from the extensograph curve (extensogram).

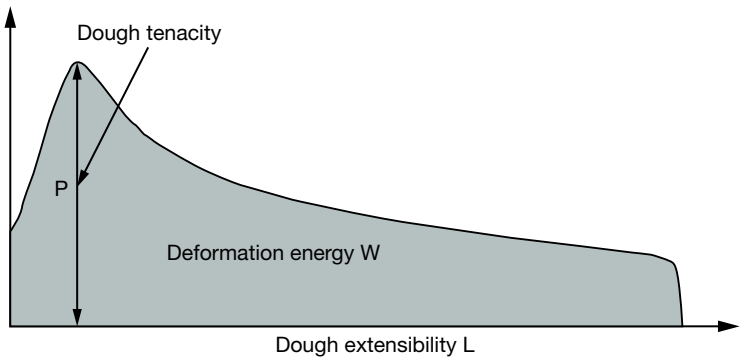

Figure 4 The determination of dough tenacity (P), configuration ratio(P/L), and deformation energy (W) and from the alveograph curve (alveogram).

Chopin in 1927. Today, the most widely known and standardized biaxial extension test is the alveograph method, which is based on the principle of dough inflation or bubble expansion technique. This procedure mimics the microprocesses occurring in dough during fermentation in macroscale, namely, the formation of thin membranes around the CO_2 bubbles. The Chopin Alveograph consists of a special thermostated, one-screw mixer for mixing and extrusion of dough, a bubble blowing apparatus, and the recording manometer. During the measuring procedure, dough disks are prepared, rested, and then inflated by constant air flow. The pressure inside the dough bubble until rupture is measured and recorded on the alveogram (Figure 4). The most generally read or calculated parameters are the maximum overpressure (an indicator of the dough tenacity, P), the average abscissa at rupture (characterizes the extensibility, L), configuration ratio (P/L), and the area under the curve (as deformation energy, W).

The extension tests are also used for investigating the effects of natural or artificial modifying agents, like bugs, enzymes, oxidants or reducing components, and lubricants. The results, recorded curves, and determined parameters of the two methods are very similar. However, because of the different mixing procedures and measuring principle, the comparability of the results is limited and depends also on the type and variety of the samples.

Viscometry as a tool for investigation of the hot phase of the bread-making process

The conventional dough rheology is mainly connected to the protein-dependent dough properties in the first, not heated phase of bread making. Starch as the main component of wheat flour also affects the quality-related properties, even the rheological properties of the dough, mostly as diluent of the proteins and as a consequence of altered hydration during gelatinization in the oven.

Additionally, starch is exposed to enzymatic breakdown, depending on the α-amylase activity of the flour and physical braking during the milling process resulting in damaged starch. An optimal level of enzymatic activity and amount of damaged starch are necessary for the optimal fermentation processes in baking. However, high enzyme activity or a high ratio of hydrolyzed starch results in a weaker water-holding capacity, resulting in serious drop in the end-product quality. Therefore, starch-related viscosity-based characterization of samples is an essential part of source material quality control in the baking industry, and the balanced amylolytic activity is part of the selection criteria during breeding.

Starch properties are usually studied at high temperatures – similar to conditions of the baking process. Generally, the starch characterization is performed by different viscometers, carrying out measurements on temperatures appearing in the technology. The most frequently used standardized method for investigation of α-amylase activity of the grain/flour is the determination of falling number on a special falling viscometer.

While the falling number is a one-point measurement, rotational viscometers are suitable for continuous measures and therefore for more complex characterization of pasting properties of cereal flours and also isolated and modified starch products. The viscometers are heated with constant heating rate, or protocols with optional heating programs are applied depending on the sample types and the goals of measurement. In the first case, Brabender Amylograph® or similar apparatuses are used, and at the beginning of gelatinization (°C), maximum viscosity value and gelatinization temperature (°C) are determined from the registered viscosity curves

according to international standards. In case of instruments working with programmable heating rates (e.g., Rapid Visco Analyser® (RVA), by Perten Instruments, or Micro Visco-Amylo-Graph® by Brabender GmbH), the pasting properties are followed continuously during heat increasing, constant heating, and heat decreasing periods. Next to the already mentioned parameters, viscosity breakdown during cooling, holding strength, and final viscosity are determined. The interpretation of the measured parameters is shown in Figure 5. These partly standardized methods allow to characterize the effects of enzymes and the hydration and viscosity development of hydrocolloids, predicting the quality of starch–protein matrices after cooling (i.e., bread crumb quality), and are applied on much wider areas than the characterization of wheat quality. The applied conditions model better the bread-baking processes in hot phases, but in all cases, the pasting properties are measured in dilute flow-water suspension. Therefore, the adaptation of the measured parameters to the real dough/bread system is not unambiguous.

Investigation of dough fermentation and real baking process

Simplified dough rheometers serve very useful parameters, but their applicability for prediction of baking quality is limited mainly because the fermentation processes, the presence and distribution of the gas (CO_2) phase, and the heating effects significantly modify the rheological behavior of the dough system.

The frequently used laboratory test for overall characterization of baking quality of wheat flour is the baking test. It is 'the ultimate' method, being the real baking process, simulating the industrial conditions in laboratory environment. However, these trials are time-consuming and labor-intensive, and the interpretation of measured parameters (volume, sensory, and texture properties) is partly subjective. Because of some critical phases of fermented dough processing (like proofing and heating), continuous and better reproducible measurements are required. The Rheofermentometer (Chopin Technologies) is suitable for measuring the dough development and tolerance, the intensity of gas production, and the rate of gas retention. The similar but more complex Maturograph® combined with Oven Rise Recorder (Brabender GmbH) is able to measure the proving and baking quality of dough, including the changes of elasticity during the whole process.

Small-scale and microscale testing

The development of very small-scale dough testing equipment and the associated automated interpretation of the resulting mixing curves has provided better reproducibility and removed operator bias, resulting in more objective assessment of the experimental variables. Several small-scale mixographs have been developed and used in different laboratories requiring 2 and 10 g of flour. The 2 g mixograph test procedure was originally designed to mimic the traditional scale methods: development of equipment and procedures included validation against the large-scale standard methods. A 10 g mixing bowl farinograph has been available since the early 1980s, while its 4 g analogous machine has been developed and its commercially available version, the micro-doughLAB, recently produced by Perten. Similar scaling-down processes have happened also in relation to the extension measurements, developing a prototype of microextension tester or the Kiefer-rig and the microdough inflation system for the TA-XT2 Texture Analyzer (Stable Micro Systems). The microextension tester has proved practical to use dough from the 2 g mixograph with micro baking facilities scaled to employ 2.4 g of dough per loaf. The traditional and small-scale dough testing methods have been found to be highly related. Essential member of the microscale machinery is the METEFEM Laboratory micro mill for supplying flour for the micro methodology. This mill is able to make flour even from one single grain and provides milling yield results from 20 g of grain, comparable to those from traditional milling tests.

Beyond applying the microscale and small-scale methodology in breeding for selection to quality in much earlier stages, these developments have facilitated a wide range of research in which either only limited amounts of test material have been available or the more objective, precise assessment of data offered extra benefits.

The spin-off of the developments of small-scale dough testing methodology has been that parallel with the development of small-scale machinery, the electronic data handling

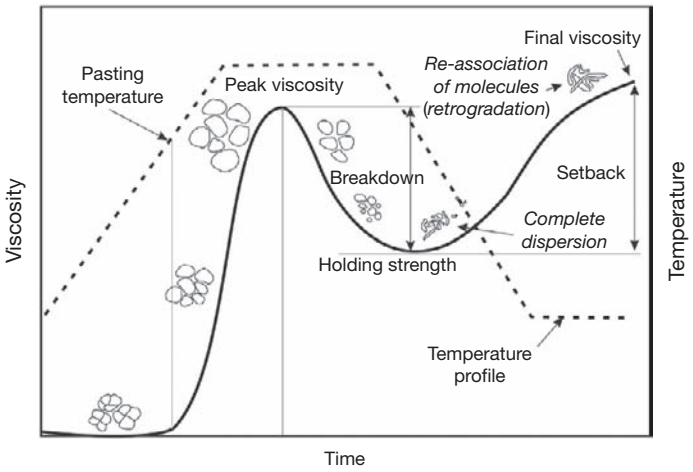

Figure 5 Characterization of the states of starch with rotational viscometers.

and processing and the specific software for calculating the mixing parameters and/or their analogous versions have been adapted for the traditional instruments; even, 'mobile' PC-based versions have been made to attach them onto industrial mixers.

New Developments

Modern bakeries employ high-energy and low-temperature mixing in the production of raw and frozen dough products. However, batch variation in mixed dough quality remains a problem. Traditional instruments used to study the mixing characteristics of doughs were unable to mimic this low-temperature mixing process. The doughLAB and the Mixolab equipments have the capabilities to alter thermal and mechanical energy inputs during mixing.

As it was mentioned earlier, different mixing procedures (straight, continuous, high-speed mixing, etc.) are applied in the baking industry. The amount and the intensity of energy input also affect the rheological properties of dough and so the final quality of baking products. In the case of the mentioned methods and instruments, constant mixing speeds are used. The recently developed doughLAB® (Perten Instruments) is a flexible recording rheometer, which can be used with both conventional z-arm and high-speed mixing actions. The latest one is able to emulate the high rates of mechanical energy input, applied in modern rapid baking systems.

A newly developed small-scale and rapid instrument is the GlutoPeak (Brabender GmbH), where a high-speed mixing action is applied in a thermostated flour–water slurry. The gluten proteins are separated and aggregated by the high-speed sharing effects; the gluten network is formed resulting to an increase in the measured torque. Further intensive mixing destroys the gluten structure; therefore, the resistance of the dough against mixing is decreasing. The time to maximum resistance and the value of peak maximum are the two most informative parameters, determined from the recorded Gluto-Peak diagrams.

The Mixolab System (Chopin Technologies) monitors the resistance of a dough during the dough formation phase and then through a heating/cooling/heating process (Figure 6) in a spiral mixer, mimicking the whole bread-making process. Phase 1 of the curve is equivalent to that of the farinograph, while information derived from phases 2–5 is similar to those of RVA. So, the Mixolab System enables the determination of the contribution of both protein and starch components of the dough in its rheological properties in a single test. Therefore, it is able to perform continuous measurement throughout a simulated baking process, which means that one can use the same instrument for several applications.

Trends and Future

Recent achievements in fundamental rheology to develop new rheological tests applying the knowledge base of modern polymer rheology principles such as the measurement of extensional strain hardening provide the basis to future developments of novel, practical equipments and methodology, suitable for routine evaluation of wheat-based end products.

Cumulative demand of the consumer for healthier, more nutritive bread is a challenge in the whole wheat chain: new quality attributes have to be considered and monitored. The best example for this trend is the effects of applying whole wheat meal and/or ingredients with higher fiber content as source materials. Besides their direct involvement in the development of protein–carbohydrate–lipid complex, altered fiber content alters drastically the water intake of the flour, changing

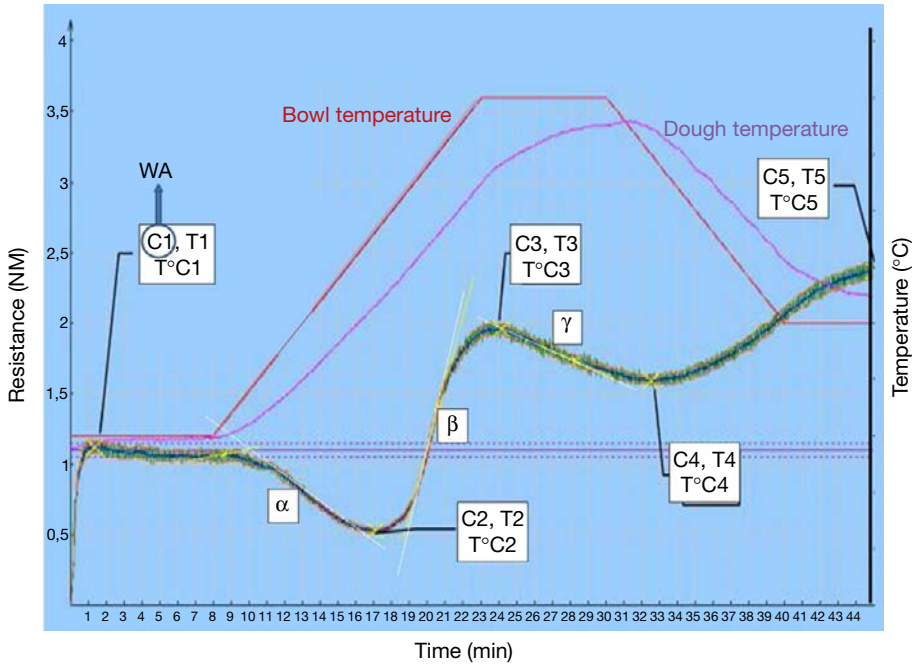

Figure 6 Mixolab parameters to characterize the mixing and heating/cooling related attributes of the dough.

the usual hydration process and therefore altering the physical and physicochemical conditions during dough mixing.

With the appearance of wheats with much more variation in starch composition (waxy wheat and high-amylase wheat), starch-related quality evaluation, largely using viscosimetric techniques, will get significantly more emphases in quality evaluation.

Similarly to milling, where the application of NIR technique to monitor the composition of source material and end products is widespread, there is a strengthening trend to utilize the advantages of spectroscopic analytic and monitoring techniques also in the baking industry, especially continuously monitoring the process of dough development in the mixer. The NIR technology has the potential to provide an invasive or noninvasive mean of probing chemical changes that occur during dough development because the absorbances in the spectra can be directly related to the chemical dough components (water, starch, protein, and fat).

Further spread of the application of indirect, high-throughput relatively cheap methods is expected in the future in prebreeding and breeding, applying small and micro methods such as the Micro Zeleny Tester, NIR, and Raman spectrophotometric techniques to estimate quality attributes and methods predicting important dough properties such as dough strength and extensibility or water absorption based on the genetic and chemical composition of the flour.

In the breeding process to select for quality, the application of traditional dough testing methodologies (farinograph and extensograph) will be the key process also in the future, because of the archived, invaluable data of previous generations of breeding material, determined on these equipments for decades. It is therefore essential to fully understand and characterize the relationships between quality attributes determined by means of traditional methods and those derived from new generation test equipments applied by the industry for process and product development and quality control.

Breeding and industrial objectives are aimed to be achieved through monitoring end-product quality rather than a set of dough parameters – a trend, appearing in the last decade that will fundamentally alter the quality control in the future. A recently developed equipment extensively used already in both basic research and developmental activities is the C-cell digital image analysis for the objective investigation of crumb structure of bread loaves, providing incomparably more insight about bread-making quality than traditional baking test determining loaf volume.

See also: Bread: Breadmaking Processes; Bread: Chemistry of Baking; Bread: Types of Bread; Cakes: Types of Cakes; Cereals: Types and Composition; Food Fraud; Pasta: Manufacture and Composition; Rheological Properties of Food Materials; Starch: Structure, Property, and Determination; Wheat: Grain Structure of Wheat and Wheat-based Products; Wheat: The Crop.

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Bread: Types of Bread

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Introduction

Cereals are basic, popular, and healthy raw materials, providing excellent vectors for nutrition, diversity, and innovation. Bread, made basically not only from wheat but also from other grains, is a staple food widely consumed all over the world since ancient times (10 000 BC) providing approximately half of the consumed carbohydrates. The nearly ubiquitous consumption of bread places it in a position of global importance in international nutrition. Bread was initially baked in the home using simple home-ground whole-wheat flours, and styles of bread, as well as the methods used to make them, evolved to satisfy local tastes and eating habits. Flour milling and subsequent breadmaking on a commercial scale emerged as vital components of local societies, and wheat-based foods evolved into the current diversity.

Bread baking is based on mixing flour and other ingredients with water to make dough, leavening with yeast that produces carbon dioxide, and baking to stabilize the solid protein foam formed, resulting in an elastic porous network. Bread is an excellent carrier of macro- and micronutrients and bioactive components that fulfills an increasing number of nutritional and health claims. Over the last and current decades, bread is being revisited as a key cereal-based baked goods addressed to specific targeted groups of population (low calorie density for obesity prevention and control, gluten-free for celiac patients, and high-fiber goods to alleviate the low current intake of dietary fiber), and a wide array of tailor-made bread is increasingly available.

In this article, the production and consumption of bread types across the world are presented, the role of wheat bread and nonwheat bread in nutrition and health is emphasized and updated, and the value-added bread addressed to specific groups of population is described.

Production and Consumption of Bread

A study by the European Commission in 2010 found that the European bread market was around 32 million tonnes in 27 EU countries. Across the whole of the European countries, the market share of the industrial bakers versus the craft bakers was approximately 50/50, but there were great differences in different countries. One area of continued growth throughout Europe is the market for frozen dough and par-baked products, which has transformed the market so that cooperatives and industrial baking companies are flourishing at the expense of the craft sector. In-store bakeries continue to be a growing sector. In the United Kingdom, supermarket in-store bakeries produce around 13% of the bread, with craft bakeries 7% and the remaining 80% produced by industrial bakers. Bread production is relatively stable in most countries, but there are some countries that are still showing a long-term trend of a slow decline, 1–2% per year, including the United Kingdom and Germany. Bread consumption patterns differ widely within the EU, but most countries have an average consumption of 50 kg of bread per person per year. The market structure varies throughout Europe: in the United Kingdom, the industrial sector represents 80% of production; it is 40% in Germany, 35% in France, about 81% in the Netherlands, and 19% in Spain. Germans and Austrians eat the most bread, around 80 kg per year, while the United Kingdom, Spain, and Ireland are at the bottom of the list with an annual consumption of less than 50 kg. White bread is the most commonly consumed bread (around 60%) in the UK population, although the average amount consumed as a proportion of the total bread intake was lower than those levels reported 10 years ago in the 2000–01 National Diet and Nutrition Survey (NDNS). As of 2000, the country with the largest *per capita* consumption of bread was Turkey with 199.6 kg per person. Turkish people eat more than three times their own body weight in bread annually. Turkey is followed in bread consumption by Serbia and Montenegro with 135 kg and Bulgaria with 133.1 kg. There continues to be increased demand for greater variety of bread than ever with ethnic bread becoming more popular in Europe and greater varieties of whole-wheat breads with oats, bran, seeds, etc. There is also a growing trend for increased production of sliced and wrapped bread in many countries across Europe, including Germany and France. There will be continued growth in morning goods and speciality bread with many opportunities for innovation.

With respect to product innovation and development, health trends will continue with whole grain, fiber, and omega-3, all being important contributors. There will be a continued decrease in bread consumption as alternative foods and bakery-type products are increasingly available. In North America, according to the Economic Research Service, USDA, *per capita* grain availability, adjusted for losses, increased from 43.2 to 61.7 kg per year between 1970 and 2009, which corresponds to an increase in energy availability from 432 to 619 kcal per day during this interval. *Per capita* availability of flour and cereal products varied from 199.5 to 194.7 kg per year between 2000 and 2010. The breakdown of products between 2000 and 2012 was wheat flour (146.3–134.4 kg), rye flour (0.5–0.5 kg), rice (19.2–20.4 kg in 2010), corn products (28.4–33.9 kg), oat products (4.4–5.2 kg), and barley products (0.7–0.6 kg). Consumers are interested in natural, convenience, and indulgence and growing out-of-home consumption, meaning less time spent on home food preparation and consumption. In South America, wheat-bread consumption is high only in Chile and Argentina. In other South American countries, the staples are maize and rice. In Argentina, wheat is the major source of calories and the second source of proteins after beef. Products derived from wheat supply 31% of the average Argentine's daily energy intake of some 12 000 kJ.

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Per capita bread consumption is 96 kg per year in Chile, 74 kg per year in Argentina (70 kg for craft bread and 4 kg for industrial bread), 28 kg per year in Peru, 27 kg per year in Brazil, and 24 kg per year in Colombia. After Germany, Chile is the country with the highest per capita bread consumption in the world.

Bread Worldwide

Ethnic cereal-based foods are generally defined as products that are unique to a particular geographic region or products that are produced from grains that are indigenous to a particular region. Western ethnic foods are defined as those wheat flour-based foods that initially became staples in western Europe and, with European colonization, were transported around the world.

Western Ethnic Bread

The evolution and spread of western ethnic foods can be traced back to the ages, and it is possible to identify a number of key phases in baked goods development (Figure 1). *Pita* is the oldest of known bread types indicating a range of potential countries of origin surrounding the Mediterranean Sea. Pita bread is a slightly leavened wheat bread, which is rolled flat and is either round or oval in shape and variable in size. It is commonly used as a wrap for a variety of filled pocket or rolled sandwiches and has spread throughout the world as a popular convenience food. *Sourdough* is a term used to describe bread produced where the leavening agent is a naturally occurring wild yeast or bacterium. The bread has a characteristic 'sour' flavor and is as old as the history of leavened bread itself. Natural leavens or 'starter' cultures consist of wild/natural yeast and *Lactobacilli* maintained over time and used repeatedly. They produce bread with unique flavor, texture, and keeping qualities and have been around for thousands of years. *Hearth bread* is the term used to describe the traditional baking method where a fermented dough piece is baked on the floor of an often wood-fired oven. It is still widely used in small bakeries around the world, with an almost limitless range of local shapes and styles of bread. Hearth bread should have a distinctive appearance, taste, and aroma with an open and coarse texture. *Baguette* (French bread and Vienna bread) is a hard crusty style of loaf associated with France, but developed in Vienna in the mid-nineteenth century. Baguette is whiter, lighter, and sweeter than the traditional sourdough bread that it was destined to replace. The golden-brown baguette, with its crisp crust, sweet taste, characteristic external appearance with surface cuts, and white crumb with large irregular holes, is one of the most famous of all of the world's bread styles. Many regional adaptations of the baguette have developed across the world with the pandesal in the Philippines and the popular hearth-type bread produced widely in Vietnam as examples. *Pan bread* is the term used to describe bread produced in a baking pan. It is a relatively recent innovation (eighteenth century) that originated in England and Holland, which are the only European countries that routinely produce bread on a large scale in pans. The regular shape, size, and weight of the finished loaf led to the development of the sliced and wrapped loaf. Pan bread is produced by a range of methods, varying in mixing intensity, the use of rapid-acting maturing agents, and the length of bulk fermentation. The *straight dough* method is the traditional method of breadmaking with a bulk fermentation stage of between 2 and 6 h, depending on yeast levels, dough properties, and the preferences of local consumers. This method is still widely used in small bakeries in many parts of the world. The *rapid dough method* relies on full dough development during mixing, with no need for normal bulk fermentation as in the straight dough process. Doughs are divided, molded, and deposited direct into baking pans for proofing and baking. Bread is produced in 2 h from start to finish, using medium-strength and medium-protein flour. This is the popular method of bread manufacture in Australia, and it has spread to other countries that use Australian wheat. The *sponge and dough baking* method is popular in the United States, and successful marketing programs have seen it gain popularity in many Asian countries. The finished baked product has fine, even texture and excellent flavor that has contributed greatly to its popularity. The first stage or 'sponge' is made by mixing a portion of the total flour and water together with the yeast and improver. The sponge is allowed to ferment up to 4 h before the balance of the flour, and other ingredients are added (including salt, which, if added sooner, would inhibit fermentation) and remixed to form the complete dough. The dough is then allowed to relax and then divided, molded, and baked in a conventional manner to produce bread of high specific volume. The basic recipe of *focaccia* is thought to have originated from the Etruscans or ancient Greeks. It is likely to be the early precursor of pizza and, while popular in Italy, has spread all over the world. It comprises a yeasted bread dough, often mixed or spread with oil, herbs, onion, garlic, sage, rosemary, or oregano, and cooked quickly at high temperatures. A *bagel* is a bread product, traditionally shaped by hand into the form of a ring from yeasted wheat dough, which is initially boiled for a short time in water and then baked. The result is a dense, chewy, doughy interior with a browned and sometimes crisp exterior. Bagels are often topped with seeds baked on the outer crust, with the traditional ones being poppy or sesame seeds.

Figure 1 Assorted Western-style breads.

Some may have salt sprinkled on their surface, and there are also a number of different dough types such as whole grain or rye.

Ethnic Breads

The first bread produced was probably the cooked version of a grain paste, made from ground cereal grains and water. Descendants of this early bread are still commonly made from various grains worldwide, including *lavash* (Iran and Turkey), *sangak* (Iran), *tortilla* (Mexico), *chapatti* and *roti* (India), and *pita* (Middle East). Flatbreads are a simple form of bread made from flattened dough (Figure 2). They may be leavened or unleavened, and they are still a very important staple food among the people of the Middle East and India. Their use is spreading throughout the Western world due to their versatility. Flatbreads are staples in northeast Africa (Ethiopia, Eritrea, and Sudan). They are made from a variety of different cereals, especially sorghum, finger millet, and teff, and resemble pancakes. Unlike pancakes served in the West, many of these products have a leavened texture and an acidic flavor. These characteristics are a result of a mixed lactic acid bacteria and yeast fermentation. Probably the two most well-known flatbreads are *injera* and *kisra*. *Injera* is a large (some 50 cm in diameter), spongy-textured pancake about 5 mm thick from Ethiopia and Eritrea. Its surface has a honeycomb-like appearance, very similar to a natural sponge, which is created by the escaping carbon dioxide during the steaming process. Teff is the preferred cereal for making *injera* in Ethiopia. This is due to the superior keeping qualities of teff *injera* when compared with *injera* made from other cereals such as sorghum. *Kisra*, from Sudan, is much thinner (1–1.5 mm thick) and smaller (~30 cm in diameter) than *injera*. It is more like a flexible thin wafer. These flatbreads remain almost exclusively homemade products despite the fact that there are flourishing commercial bakeries in countries such as Ethiopia and Sudan. In Kenya, wheat flour chapatis are a very popular home-baked food in all communities. It is probable that this type of flatbread was introduced by Indians who settled in the country in the late nineteenth and early twentieth centuries.

Steamed bread and buns are consumed throughout Southeast Asia but mostly in China, Japan, and Korea. The characteristics of steamed breads and buns vary from country to country and region to region. Steamed buns can be filled (*bao*) or not filled (*mantou*). *Mantou*, which is unfilled, is the only product called steamed bread. *Char siu bao*, steamed buns with barbecue pork filling, has white skin and splits open on steaming. This splitting is a positive quality characteristic. Char siu bao is very soft and cake-like and the highest quality is high in sugar (up to 30%) although low in fat. Low-sugar *char siu bao* with a low-fat filling could contribute to a healthy diet. Hong Kong-style *char siu bao* is made with low-protein soft wheat and is usually sold fresh. The buns are usually chemically leavened with ammonium hydroxide, which is released during baking when fresh. Singapore-style *char siu bao* is firmer than Hong Kong-style and uses higher-protein flour.

Figure 2 Flatbreads worldwide (clockwise from the left): *kitta*, *kisra*, *injera*, *roti* (chapati), *tortilla*, and *lavash*.

Value-Added Bread

It raises a great deal of recent interest that minor cereals, ancient crops, and pseudocereals, besides wheat, constitute highly nutritional grains with potential breadmaking applications. The concept of using South American, African, and Asian traditional raw materials and fermented foods as a template for wheat-based, wheat-free, and gluten-free foods in Europe and North America is in good accordance with the interest in westernized countries for ethnic foods with revisited value addition. Indigenous foods from different cultures and civilizations with ethnic eating habits are moving in a globalized world with strong immigration movements, encompassing the use of traditional raw materials as ingredients in novel foods. The general growing demand for novel, tasty, and healthy foods together with the increasing number of people suffering from celiac disease has given birth to a new market consisting of cereal products made from gluten-free grains. In this challenging market, oat, rice, corn, sorghum, millet, quinoa, amaranth, and buckwheat have gained a special position, as basic ingredients used singly and/or in associated blends to make gluten-free, wheat-free, and wheat composite breads with variable sensory acceptability and nutritional value (Table 1).

Moreover, the interest in leguminous flours as ingredients for bread production is growing. High-legume wheat blends appear as an efficient strategy to obtain sensorially accepted and nutritionally enhanced bread (Table 2) with no dramatic technological impairment when structuring agents (gluten/hydrocolloids) are incorporated.

Chickpea and green pea incorporation into bread formula decreased and delayed the starch hydrolysis and concomitantly reduced the expected glycemic index. The use of defatted soybean flour promoted a boost of bread antiradical activity. Structuring agents helped restore dough viscoelasticity in highly legume-replaced wheat matrices and apparently obstructed starch-degrading enzyme accessibility causing a slowdown of starch hydrolysis kinetics.

Nutritional and Health-Related Aspects of Bread

Grains are sophisticated reservoirs of macronutrients, cell wall polysaccharides (dietary fiber), and many biologically active

Table 1 Chemical and nutritional information of single and blended cereal bread

Nutrient	*Oat (O)*	*Khorassan*	*Spelt*	*Rye (R)*	*Buckwheat (BK)*	*Wheat (WT)*	*O:R:BK:WT, 20:20:20:40*
	Per 100 g bread, as is						
Moisture (g)	33.6	31.6	29.7	30.2	29.6	28.1	38.9
Fat (g)	3.26	0.33	0.47	0.34	0.80	0.33	1.01
Protein (g)	13.57	11.26	10.36	6.99	12.59	9.60	9.43
Ash (g)	1.73	1.40	1.79	1.26	1.78	1.10	1.40
Digestible carbohydrates (g)	36	49	53	50	46	59	42
Total dietary fiber (g)	11.91	6.23	5.02	11.30	9.65	1.88	7.29
Soluble dietary fiber (g)	2.92	0.28	1.04	3.56	1.70	0.85	1.96
Insoluble dietary fiber (g)	8.99	5.95	3.98	7.74	7.95	1.03	5.33
Resistant starch (g)	4.28	1.11	1.08	4.31	4.77	0.94	3.15
β-Glucans (g)	2.30	0.17	0.42	0.92	0.62	0.10	0.81
Minerals (mg)	444	377	542	388	438	336	396
Ca (mg)	10.6	10.0	17.2	26.9	20.1	24.7	21.4
Cu (mg)	0.2	0.2	0.2	0.1	0.2	0.1	0.2
Fe (mg)	2.2	1.8	1.4	1.9	1.7	0.7	1.4
Mg (mg)	78.3	52.4	61.2	30.5	20.9	24.9	35.9
Mn (mg)	1.9	1.3	1.6	1.2	0.8	0.3	0.9
K (mg)	199.9	147.4	201.2	155.4	197.4	62.8	136
Na (mg)	149.2	162.6	258.2	170.8	195.6	241.9	200
Zn (mg)	1.8	1.4	1.5	0.9	1.1	0.5	0.97
Total phenolics (mg)	280	219	188	209	209	238	745
Phenolic bioavailability (%)	9	16	17	16	17	17	74
Energy (kcal)	225	242	267	228	237	278	292

minor constituents. As 30–70% of daily energy according to the country income is derived from cereal-based foods, their role in nutrition is important. Cereal-based foods are an important source of carbohydrates, protein, dietary fiber, especially B vitamins, and minerals. The starchy endosperm gathered most of the scientific and technological interest for food processing until the end of the previous century. The recognition of the importance of the outer grain layers for health maintenance created the interest to reveal the types, amounts, and potential physiological significance of various vitamins, minerals, and phytochemicals in the whole grain and food made thereof. It has also been recognized that food structure at different levels, to a large extent modified in food processing, is important in controlling the rate and extent of digestion of nutrients and the bioavailability of phytochemicals. In the European Prospective Investigation into Cancer and Nutrition (EPIC) study populations, 27% of total carbohydrate intake was from bread. The majority of bread, as well as other baked goods, is made of refined white wheat flour, free of the outer grain layers rich in cell wall material and associated phenolic compounds, minerals, and vitamins. Epidemiological studies constantly show that the intake of whole grain and cereal dietary fiber protects against the rapidly increasing chronic diseases related to a sedentary lifestyle, such as cardiovascular disease and type 2 diabetes.

Energy and Macronutrients

With an energy content of around 2.2 kcal (9.2 kJ) per gram, bread is considered a 'medium'-calorie food from an energy density perspective. Most of the energy in bread comes from starch; therefore, bread is generally classified as a 'starchy food.' A medium slice of white bread typically provides 86 kcal (361 kJ). Continental breads (such as focaccia) contain oils (usually olive oil, rich in monounsaturated fatty acids) that increase the calorie content, with a 50 g serving of *focaccia* containing, on average, 180 kcal (756 kJ). An average slice of *ciabatta* (around 45 g) contains, on average, 116 kcal (487 kJ). Whole-wheat (2.2 g/100 g) and brown flours (2 g/100 g) therefore contain slightly more fat than white flour (1.2 g/100 g).

Although white flour and brown flour provide a similar profile of saturated (e.g., palmitic acid), monounsaturated (e.g., oleic acid), and polyunsaturated fatty acids (e.g., linoleic acid), the specific flours differ in their fatty acid concentration, with whole-wheat flour providing the greatest proportion of fatty acids. The amount of fat/100 g of bread is small. However, the addition of fat during the breadmaking process or in meal preparation can increase the fat content. Bread also contains considerable amounts of protein and carbohydrate.

The amount of fiber in the bread depends on the flour used to make it. A slice of 40 g of white wheat bread will deliver about 1 g of dietary fiber, and a similar slice of whole-wheat bread would deliver 3–4.5 g. During the day, the choice of bread has a large effect on the intake of dietary fiber, and six portions of whole-wheat bread would provide close to the recommended intake of dietary fiber of 25–35 g per day. A range of minerals, vitamins, and phytochemicals have been detected to be concentrated in the same grain parts as most of the dietary fiber polymers (Table 3), and there is increasing evidence about their biological activity and possible interference with various disease pathologies.

Micronutrients

Bread provides various micronutrients, including calcium, iron, zinc, copper, magnesium, manganese, selenium, and some B vitamins, such as folate (Table 3). Any food that

Table 2 Proximate chemical and nutritional composition of flours (per 100 g flour, d.b.) and blended breads (per 100 g fresh blended bread)

Sample code	Protein (g)	Total dietary fiber (g)	Insoluble dietary fiber (g)	Soluble dietary fiber (g)	Fat (g)	Ash (g)	Digestible carbohydrates (g)	Energy (kcal)	Moisture (g)
Flours									
Wheat (WT)	14.13	2.19	1.20	0.99	1.56	0.63	82	–	14.32
Commercial barley (CB)	11.27	15.2	10.05	5.15	1.69	1.52	58		12.8
High β-glucan barley (HBGB)	17.74	32.1	18.5	13.71	5.38	1.83	35		8.3
Yellow maize (YM)	5.68	3.55	0.99	2.55	2.16	0.66	88		12.99
White maize (WM)	5.25	4.31	0.77	3.54	1.56	0.56	88		13.54
Teff (T)	13.05	12.19	7.40	4.80	5.06	2.21	67	–	11.90
Chickpea (CP)	16.56	22.2			6.16	2.60	43		9.9
Soybean (SB)	49.68	13.6			3.24	5.81	17		10.5
Green pea (GP)	25.12	14.56	8.50	6.05	1.27	2.58	57	–	8.17
Buckwheat (BW)	19.71	13.52	6.58	6.93	3.44	2.05	61	–	11.70
Breads									
T:GP:BK:WT, 7.5:7.5:7.5:77.5	11.6	2.9	1.63	1.24	3.6	–	50	283	32.3
T:GP:BK:WT, 15:15:15:55	12.2	4.3	2.42	1.88	3.8	–	48	283	31.9
T:GP:BK:WT, 15:7.5:15:62.5	11.7	3.8	2.13	1.67	3.8	–	51	295	29.3
T:GP:BK:WT, 15:15:7.5:62.5	12.2	3.8	2.21	1.63	3.7	–	48	282	32.2
CP:GP:SB:WT:CMC: GL, 20:20:2:48:5:5	11.58	8.18	3.46	4.72	1.28	0.80	31	201	46.5
CP:GP:SB:WT:CMC: GL, 20:14:8:52:3:3	12.33	7.52	3.78	3.74	1.46	0.99	34	217	42.9
CP:GP:SB:WT:CMC: GL, 20:8:14:48:5:5	14.42	8.79	3.84	4.95	1.52	1.12	30	216	42.3
CP:GP:SB:WT:CMC: GL, 14:14:8:58:3:3	12.93	7,24	3.43	3.81	1.36	0.98	42	249	34.8
WT:CB, 60:40	7.31	4.01	2.77	1.24	0.56	0.56	39	198	38.6
WT:HBGB, 60:40	8.1	11.91	8.22	3.69	1,11	0.96	25	166	40.5
WT, 100%	11.1	1.4	0.83	0.59	3.4	–	51	283	32.9

provides 15% or 30% of the recommended dietary allowance (RDA) for a specific vitamin or mineral, per 100 g, is considered a 'source of' or 'high in,' respectively, the named vitamin or mineral. As Table 3 outlines, bread can be considered a 'source of' and/or 'high in' many micronutrients.

Phytochemicals

The highest amounts of phytochemicals are found in the outer layers of grains and include phenolic compounds, phytosterols, and tocols (tocopherols and tocotrienols). These plant substances have been substantiated to have antioxidant properties *in vivo*. A study examining the total antioxidant capacity of various foodstuffs, including cereals (such as wheat) and 18 cereal products, found that whole wheat, buckwheat, and wheat bran had the greatest total antioxidant capacity value, while white flour showed the lowest total antioxidant capacity value, indicating that flour containing the outer layers of the wheat grain and germ has a greater potential antioxidant activity than white flour. In a recent study, the polyphenol qualitative and quantitative profile and antiradical properties of enzyme-digested extracts mimicking human gastrointestinal conditions, from single oat, rye, buckwheat, and wheat and multigrain quaternary bread at different levels of wheat flour replacement, have been investigated, and the relationships between total and individual polyphenols and kinetics of reduction of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) have been established. The total phenol content among the single bread extracts was significantly higher in buckwheat (808 mg gallic acid equivalents (GAE) per kg) and decreased in the following order: buckwheat > wheat > oat > rye. Mixed bread exhibited increased polyphenol content (up to 1135 mg GAE per kg) and higher polyphenol bioaccessibility (from 55% to 80%) with a higher degree of wheat replacement. Similar trends were found for antioxidant power. DPPH reduction followed two-step kinetics in all cases, where a considerable amount of phenolic acids and flavonoids corresponds to high antiradical activity and to slow kinetic rates (protocatechuic acid and ferulic acid), particularly for mixed grain bread extracts.

Role of Cereal Foods in Diet: Nutrition and Health Claims

Numerous epidemiological studies during the past 15 years have shown the protective role of whole grain foods against chronic diseases, such as cardiovascular disease, type 2

Table 3 Vitamins and minerals of wheat and rye bread

		Wheat						*Rye*	
		Brown		*Whole grain*		*White*		*Whole*	
Per 100 g bread			*% RDA*		*% RDA*		*% RDA*		*% RDA*
Vitamins									
Vitamin K	μg	7.8	6	2.2	3	3.1	4	1.2	1
Thiamine	mg	0.4	25	0.4	27	0.5	30	0.4	29
Riboflavin	mg	0.2	18	0.3	20	0.3	19	0.3	20
Niacin	mg	4.7	26	4.4	22	4.4	22	3.8	19
Vitamin B6	mg	0.2	6	0.3	17	0.1	4	0.1	4
Folate	μg	50.0	21	118	29	111	28	110	27
Pantothenic acid	mg	0.7	8	0.5	5	0.2	2	0.4	4
Choline	mg	23.9		~		14.6		~	
Betaine	mg	180		~		102		~	
Minerals									
Ca	mg	107	14	91.0	9	151	15	73.0	7
Fe	mg	2.4	19	3.5	19	3.7	21	2.8	16
Mg	mg	82.0	12	53.0	13	23.0	6	40.0	10
P	mg	202	15	176	18	99.0	10	125	12
K	mg	248	5	204	6	100	3	166	5
Na	mg	472	22	487	20	681	28	660	27
Zn	mg	1.8	8	1.3	8	0.7	5	1.1	8
Cu	mg	0.4	8	0.3	13	0.3	13	0.2	9
Mn	mg	2.1	56	1.5	74	0.5	24	0.8	41
Se	μg	40.3	41	29.5	42	17.3	25	30.9	44

RDA, recommended dietary allowance (percent daily values for adults or children aged 4 or older and based on a 2000 calorie reference diet) of different breads.
Source: www.nutritiondata.com; US Department of Agriculture, Agricultural Research Service (2012). USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov; Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Elements. Food and Nutrition Board, Institute of Medicine, National Academies, http://www.nap.edu.

diabetes, and colon cancer. Although it has been calculated that consuming up to 50% of all grain foods as refined grain foods, when they do not contain high levels of added fat, sugar, or sodium, is not associated with increased disease risk, whole grain foods are considered to be protective for health. Cereal dietary fiber is considered to be the main contributor to the health protective effects of whole grain foods. In addition, the potential role of phenolic and other minor grain constituents in health maintenance has been emphasized.

Following reports of epidemiological studies showing the importance of whole grain, numerous actions have been taken to promote the use of cereal foods containing whole grain and dietary fiber. In 2003, the WHO/FAO expert consultation recommended that whole grain cereals, fruits, and vegetables are the preferred sources of nonstarch polysaccharides, the intake of which should be 20 g per day. Dietary recommendations in the United States (http://www.foodpyramid.com/mypyramid/) are to consume at least half of cereal foods as whole grain, and the Nordic dietary recommendations also recommend eating cereal foods as whole grain products (http://www.norden.org/fi/julkaisut/9). In Denmark, whole grain foods have been promoted by a campaign and logo (http://www.fuldkorn.dk/english/) with the aim of reaching the recommended daily intake of 75 g whole grain, and in the United States, by a stamp and communication by the Whole Grains Council (http://wholegrainscouncil.org/whole-grain-stamp) helping consumers to reach a daily intake of whole grain of at least 48 g.

In 1999, the US Food and Drug Administration (www.fda.gov) accepted a health claim for whole grain foods: "Diets rich in whole grain foods and other plant foods and low in total fat, saturated fat, and cholesterol may reduce the risk of heart disease and some cancers." The European Food Safety Authority (EFSA) has not accepted a health claim concerning whole grain foods, but some health claims can be used in connection with cereal ingredients, such as wheat and oat bran, rye fiber, and oat β-glucan. Rye fiber contributes to normal bowel function for food, which fulfills the high fiber claim with this fiber. Barley grain fiber, oat grain fiber, and wheat bran fiber contribute to an increase in fecal bulk for food, which fulfills the high fiber claim with every fiber. Wheat bran fiber contributes to an acceleration of intestinal transit for food, which fulfills the high fiber claim with this fiber with a daily intake of ≥ 10 g of wheat bran. The consumption of arabinoxylan (AX) from wheat endosperm as part of a meal contributes to a reduction of the blood glucose rise after that meal for food that contains at least 8 g AX-rich fiber per 100 g of available carbohydrates in a quantified portion. Consumption of β-glucans from oat or barley as part of a meal contributes to the reduction of the blood glucose rise after the meal for food that contains ≥ 4 g of β-glucans from oats or barley for each 30 g of available carbohydrates in a quantified portion. β-Glucans contribute to the maintenance of normal blood cholesterol levels for food that contains ≥ 1 g of β-glucans from oats, oat bran, barley, and barley bran or from their mixtures per quantified portion, 3 g needed per day. Replacing digestible starches with resistant

Table 4 Nutritional composition of refined wheat flour breads with added dietary fiber

	Sandwich bread		*White pan bread*		*Pan bread*	
Nutrition facts, per 100 g bread	*Reference*	*Low-calorie high-fiber bread*[a]	*Reference*	*High-fiber bread*[b]	*Reference*	*High-fiber light bread*[c]
Moisture (g)					36.1	43.3
Energy (kcal)	270	198	241	232	238	132
Protein (g)	7.5	8.1	7.6	7.1	13.90	13.51
Digestible carbohydrates (g)	50	38.6	47.9	45.1	42.33	16.66
Starch (g)		36.7				
Sugars (g)		1.9	1.0	1.0		
Fat (g)	4.1	0.94	2.0	2.0	1.45	1.21
Saturated (g)	2.1	0.12	0.6	0.6		
Total dietary fiber (g)	2.8	8.5	2.0	6.1	4.53	23.24
Soluble (g)			0	4.3	1.28	2.79
Insoluble (g)					2.84	19.37
Sodium (g)	0.5	0.47	0.6	0.6		
Ash (g)					1.69	2.08

[a]Includes wheat fiber VITACEL WF 101 from Rettenmaier & Söhne GmbH.
[b]Includes polydextrose Litesse from Danisco Sweeteners Ltd.
[c]Includes a mix of soluble and insoluble fibers. Patent No 200601668. CSIC. 2006.
Source: Collar, C. (2008). Novel high fibre and whole grain breads. In: Hamaker, B. (ed.) *Technology of functional cereal products*, pp 184–214, Cambridge: Woodhead Publishing Limited.

starch in a meal contributes to a reduction in the blood glucose rise after that meal for food with the final content of resistant starch is at least 14% of total starch.

In Europe, the nutrition and health claims directive also provides a list of allowed nutrition claims, many of which are also relevant to cereal foods, such as 'source of fiber' for products containing at least 3 g DF/100 g product and 'rich in fiber' for those containing 6 g DF/100 g product (**Table 4**).

Protein, vitamins, and omega-3 and polyunsaturated fatty acids are also among those food components where nutrition claims may be used to show a high content. In addition to AX, β-glucans and cellulose, resistant starch, lignin, and other phenolics associated with carbohydrate polymers are part of the cereal dietary fiber complex. Despite the known health effects, recommendations, labeling, and communication campaigns, the majority of cereal foods consumed are made of refined flour and contain less dietary fiber and other health-promoting compounds than are present in the whole grain raw material.

See also: Amaranth; Cereals: Dietary Importance; Ethnic Foods; Quinoa.

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Browning: Enzymatic Browning

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Introduction

Browning that occurs widely in many fruits and vegetables, and some seafood products, is initiated by the enzymatic oxidation of phenolic compounds, resulting in the formation of brown-colored substances. Polyphenol oxidase (PPO) is a group of copper proteins that catalyzes the oxidation of phenolics to quinones, which produce a series of brown pigments. These reactions are thought to be the major cause of the enzymatic browning of many fruits and vegetables during handling, storage, and processing. The initial products of oxidation are quinones, which rapidly condense to produce relatively insoluble brown polymers (melanins). This enzymatic browning problem is of considerable importance to the food industry as it greatly influences the nutritional quality and appearance, reduces the consumer's acceptability, and, therefore, results in significant economic impact to both food producers and the food-processing industry. It is estimated that over 50% of loss in fruits and vegetables occurs as a result of enzymatic browning, and in particular, tropical and subtropical fruits and vegetables are the most susceptible to these reactions. Moreover, highly prized and economically valuable seafood is extremely vulnerable to deteriorative enzymatic browning. The millions of pounds of seafood, specifically shrimp, caught or imported into processing facilities must be treated with chemical preservatives to control and/or eliminate this discoloration process.

Owing to its tremendous economic impact to the food industry, inhibition of enzymatic browning caused by PPO in food products has been widely investigated. The most important factors that determine the rate and degree of enzymatic browning of fruits and vegetables involve the concentrations of both active PPO and phenolic compounds, pH, temperature, compartmentalization between enzymes and substrates, and oxygen availability of the tissue. Understanding the enzymatic browning process is necessary in order to control it effectively and to obtain high-quality product that is acceptable to consumers. This article reviews the browning-related enzymes, browning substrates, browning reaction properties, and control of the enzymatic browning, with an emphasis on fresh fruits and vegetables after harvest.

Polyphenol Oxidase

PPO (E.C. 1.10.3.1), a Cu-containing enzyme, is also referred to as catechol oxidase, tyrosinase, phenolase, catecholase, diphenol oxidase, or *o*-diphenolase. PPO is found in almost all higher plants, including fruits and vegetables. It is known that PPO is synthesized as preproteins and contains putative plastid transit peptides at the N-terminal region, which target the enzyme into chloroplast and thylakoid lumen in plants. In addition to its existence in higher plants, PPO is also found in some seafood products, such as shrimp and lobster. Its role in browning reactions causes deterioration in food quality by changing the nutritional and organoleptic properties and hence consumer acceptability.

The action mechanism of PPO is based on its capacity to oxidize phenolic compounds. When the plant tissue is damaged, resulting in the rupture of plastids, the cellular compartment where PPO is located leads to the enzyme contact with the phenolic compounds released by rupture of the vacuole, the major storage organelle of these compounds. It is considered that the active site of PPO contains two copper atoms and the enzyme catalyzes two different reactions in the presence of molecular oxygen: the hydroxylation of monophenols (monophenolase activity) and the oxidation of *o*-diphenols to *o*-quinones (diphenolase activity) (Figure 1). This reaction is generally followed by nonenzymatic polymerization of the quinones giving rise to the formation of melanins, pigments of high molecular mass, and dark color. Yoruk and Marshall reviewed the sequence of biochemical reactions leading to the formation of melanin from the oxidation of phenolic amino acid tyrosine. The rate of enzymatic browning of fruit and vegetables depends largely on specific activity of PPO and concentrations of phenolic compounds, the pH, and temperature. Table 1 gives some parameters of PPO from different fruit and vegetable sources with an emphasis on the optimum pH and temperature.

It is interesting to note that another phenol-oxidizing enzyme, laccase (*p*-diphenol: oxygen oxidoreductase, E.C. 1.10.3.2), appears in some higher plants. Laccase is active on both *o*- and *p*-diphenol substrates and is clearly differentiated from other PPOs by its unique ability to catalyze the oxidation of the latter. In litchi pericarp, the oxidative product of 4-methylcatechol catalyzed by PPO *in vitro* can accelerate anthocyanin degradation, resulting in the formation of brown-colored substances. Furthermore, the anthocyanase (identified now as a laccase) from litchi pericarp catalyzed the hydrolysis of sugar moieties from anthocyanin to yield anthocyanidin. Thus, it might be suggested that laccase contributes to litchi pericarp browning by rendering major phenolic constituents (anthocyanins) accessible to PPO. Properties of the laccase involved in anthocyanin degradation in combination with the oxidation of other phenolics caused by PPO require detailed elucidation. Moreover, the different contribution of laccase to enzymatic browning in some fruits and vegetables requires also clarification.

In higher plants, PPO is believed to be membrane-bound. PPO exists in soluble and membrane-bound forms. Limited activity of PPO in some fruits and vegetables such as apple, apricot, banana, pear, cucumber, eggplant, litchi, potato tubers, sunflower, and various *Prunus* fruits may be attributed to its tight binding to membranes; however, latency may not be

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00090-8

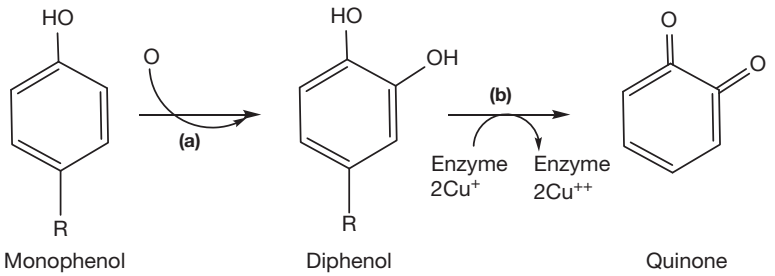

Figure 1 Reactions of (a) hydroxylation and (b) oxidation catalyzed by polyphenol oxidase.

Table 1 Parameters of PPO extracted from different fruit and vegetable sources

Source	*Substrates with higher affinity*	*Km (mM)*	*Optimum pH*	*Optimum temperature (°C)*	*References*
Apple (cv. Amasya)	4-Methylcatechol Catechol	3.1 34.0	7	15	Oktay et al. (1995)
Apricot	4-Methylcatechol		5–5.5		Fraignier et al. (1995)
Artichoke	4-Methylcatechol Catechol	10.2 12.4	6	25	Aydemir (2004)
Avocado	4-Hydroxyanisole		5		Espín et al. (1997)
Banana (cv. Anamur)	Catechol	8.5	7	30	Ünal (2007)
Cherry	4-Methylcatechol		4.5		Fraignier et al. (1995)
Cucumber	Catechol		7		Miller et al. (1990)
Eggplant	4-Methylcatechol		5–6.5		Perez-Gilabert and Carmona (2000)
Field bean	Catechol		4		Paul and Gowda (2000)
Grape (cv. Victoria)	Chlorogenic acid Catechin		5.0	40	Rapeanu et al. (2006)
Kiwifruit	Catechol		7.3		Park and Luh (1985)
Lettuce	Chlorogenic acid Epicatechin	0.67 0.91	5–8	25–35	Heimdal et al. (1994) Fujita et al. (1991)
Litchi	4-Methylcatechol	10	7.4	70	Jiang et al. (1997)
Longan	4-Methylcatechol		6.5	35	Jiang (1999)
Loquat	Chlorogenic acid	1.0	6.5	30	Sélles-Marchart et al. (2006)
Mango (cv. Tainong)	Catechol Pyrogallol		7	30	Wang et al. (2007)
Medlar	Epicatechin L-Dopa	4.0 4.7	6.5	35	Dincer et al. (2002)
Mulberry	Pyrogallol	1.2	7.5	20	Arslan et al. (2004)
Olive	4-Methylcatechol		5.5–7.5		Ben-Shalom et al. (1977)
Peach	4-Methylcatechol		5		Fraignier et al (1995)
Peppermint	Catechol		7	30	Kavrayan and Aydemir (2001)
Pineapple	Catechol		6–7		Das et al. (1997)
Plum	4-Methylcatechol		4–5.5		Fraignier et al(1995)
Potato	Chlorogenic acid		4.5–5 and 6–6.5	40	Cho and Ahn (1999); Sanchez-Ferrer et al.(1993)
Spinach	Dopamine		8		Sheptovitsky and Brudvig (1996)
Strawberry (cv. Elsanta)	Catechol	5.9	5	25	Dalmadi et al. (2006)
Persimmon	Catechol	12.4	7.5	20–40	Özen et al. (2004)
Sunflower	Gallic acid	1.11	7.9	45	Raymond et al. (1993)
Yacón root	Caffeic acid Chlorogenic acid	0.2 1.1	6.6	30	Neves and Silva (2007)

dependent just on membrane integrity because it persists even after release from the plastid and requires activation in broad bean, spinach, grape, mango, peach, pear, and sago palm. Activation of PPO can be induced by frost and aging, fatty acids, alcohols, denaturants, detergents, acid and alkali, protease treatment, sonication, and mild heat treatment. Removal of PPO-bound inhibitors may also result in activation of latent enzyme. It is mostly believed that endogenous proteases are involved in activation of latent PPO by cleaving a certain region. Therefore, protease inhibitors are usually used

to avoid further PPO activation by endogenous proteases during isolation. In addition, it is suggested that PPO activity may be regulated *in vivo* by oxygen concentration and pH value as the levels of both change in the chloroplast of the intact tissue. In some cases, latent PPO could be activated by pathogen attack, signifying the possible involvement of an activation process in a response to infection, which accounts for the rapid appearance of black spots when fruits and vegetables decay.

In higher plants, PPO has been described as a multiple gene family. Seven PPO genes (PPOs A, A′, B, C, D, E, and F) were identified from tomato and the PPO gene is encoded in the nucleus and translated in the cytoplasm and then the pro-PPO formed is transported to the chloroplast where it is cleaved by a protease, producing the active form. So far, PPO genes have been cloned from some fruits and vegetables, such as pear, sweet potato, grape, and apple, with their expression properties analyzed in relation to browning potential. In higher plants, predicted molecular weights range from 57 to 62 kDa. Mushroom PPO is generally thought to contain four subunits with a total molecular weight of 128 kDa. It can be expected that molecular biology techniques will help our understanding of the property of PPO and how to control enzymatic browning.

PPO exhibits a wide range of substrates in higher plants (Figure 2). Significant variations in the activities of PPOs exist from different fruit and vegetable sources. Types and relative concentrations of natural phenols vary largely for different fruit and vegetable sources (Table 2). For example, in the 'DeChaunac' grape, caffeic acid as a PPO substrate is oxidized at much faster rates than other structurally related substances, while in the 'Koshu' grape, chlorogenic acid is most rapidly oxidized, followed by caffeic acid and catechin. In addition, PPO isoforms in different fruit and vegetable tissues may also exhibit differential substrate specificities and variations in their relative activities toward monophenols and *o*-diphenols. For instance, two isoforms isolated in banana bud showed the maximum relative activity toward dopamine, but these enzymes exhibited obvious differences in their activities toward chlorogenic acid, L-dopa, and (+)-catechin. So far, all of the PPOs discovered have the ability to convert *o*-dihydroxyphenols to *o*-benzoquinones using O_2 as the second substrate, but not all PPOs hydroxylate monophenols.

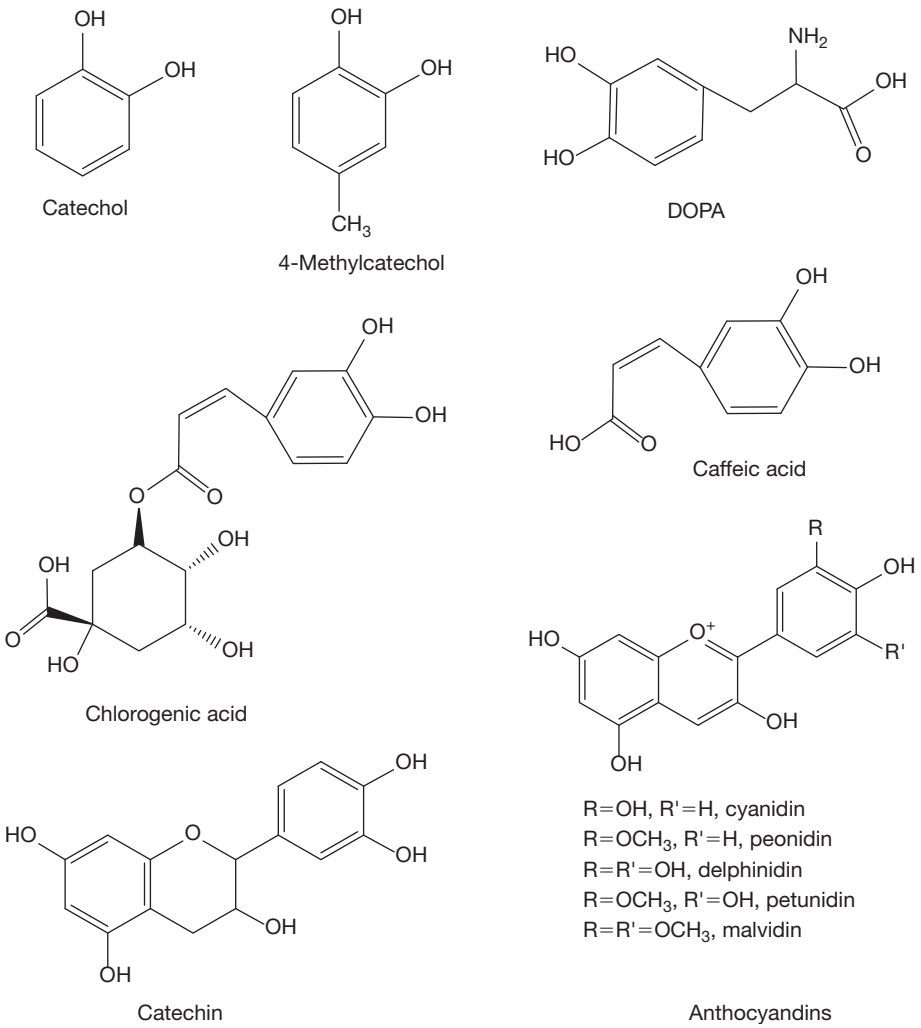

Figure 2 Structures of some natural substrates of polyphenol oxidase.

Table 2 Relative specificity of PPO substrates

Substrates	*Apple*	*Peach*	*Strawberry*	*Field bean*	*Grape*	*Litchi*	*Longan*
Di- or triphenols							
Catechol	100	100	9	100	5.9	167	91
4-Methylcatechol	181	103	80	140	74	100	100
Chlorogenic acid	102		11	0	51	0	0
L-Dopa		23		22.6	5.4		
Catechin	54	539	100	0	21		
Caffeic acid		7	13	0	100		
Gallic acid		5		0	0		
Pyrogallol	38	182		62	24	3273	281
Monophenols							
Tyrosine	3	0	0	0	0	0	0
p-Cresol		0		0	0	0	0
p-Coumaric acid		0	0				

Peroxidase

The relative significance of PPO activity is obscured somewhat by the presence of peroxidase (POD, E.C. 1.11.1.7), a similar oxidative enzyme. It is relatively difficult to ascribe a significant role to POD in enzymatic browning when one of its substrates, hydrogen peroxide (H_2O_2), is generally present at very low concentrations in plant cells. Recent evidence collected indicates clearly that POD could enhance browning reactions in the presence of ongoing PPO-mediated browning reactions. While the mechanism of this PPO-coupled browning is not well understood, it is possible that the PPO-mediated generation of quinones can lead to H_2O_2 accumulation, providing a higher concentration of this free radical species, thus enabling the occurrence of the POD-mediated oxidation of polyphenols. The profile and concentration of polyphenol substrates in plant tissues will also influence the potential for the POD-mediated polyphenol browning. Furthermore, the POD-related browning can be distinguished by the addition of an H_2O_2 quenching agent such as catalase, which will prevent browning caused by the POD-mediated reactions. Further work should be conducted with PPO inhibitors such as tropolone to determine whether the POD-mediated browning can occur without the existence of the PPO-associated browning. It would be premature to argue that the POD-mediated polyphenol browning is a consistently significant component in enzymatic browning of fresh fruits and vegetables after harvest, although there are sufficient questions raised by the current literature to encourage further work in this area.

Control of Enzymatic Browning

Table 3 gives the latest information about the conventional and alternative methods used currently to control enzymatic browning. The principle of the enzymatic browning inhibition involves the inactivation or inhibition of PPO activity, inhibition of the oxidation of phenolic compounds, low O_2 or O_2 accessibility, and delayed senescence in relation to compartmentalization between enzymes and substrates. Technologies to control enzymatic browning include heat treatment, PPO inhibitors, chelating agents, acidulants, reducing compounds, antiaging or signal substances, edible coatings, modified atmosphere packaging, controlled atmosphere, and cold storage.

Heat treatment is the most used method for stabilizing foods because of its capacity to destroy microorganisms and to inactivate enzymes. As mentioned earlier, PPO has a temperature range where it exhibits activity. In general, exposure of PPO to temperatures of 50–90 °C can destroy the catalytic activity, but the time required to inactivate the enzyme depends largely on the product. Thus, heat inactivation of PPO is feasible by applying temperatures of >50 °C for a short time. Temperatures of >60 °C for 3 min are sometimes used to treat red grapes before vinification, while heat shock in chlorinated water at 50 °C for 2 min inhibits effectively the surface browning of fresh-cut lettuce. However, the heat treatment may produce undesirable color and/or flavor as well as undesirable changes in texture. In general, the heat inactivation of PPO is more suitable for fresh fruits and vegetables after harvest to control enzymatic browning before they are dried or processed.

Many inhibitors of PPO have been applied for the control of enzymatic browning of fresh fruits and vegetables after harvest. The major PPO inhibitors include aromatic carboxylic acids such as benzoic and cinnamic acids and their derivatives. Strength and inhibition types follow cinnamic acid (noncompetitive) > 4-hydroxycinnamic acid (competitive) > 4-methoxycinnamic acid (noncompetitive). 2-Hydroxycinnamic acid has no inhibitory effect on the diphenolase activity, possibly due to a region different from the active site, and hinders the binding of substrate to the enzyme through steric hindrance or by changing the protein conformation. Diamine derivatives of coumarin and 4-hexylresorcinol are effective inhibitors of black spot formation in shrimp and 4-hexylresorcinol can also inhibit mushroom PPO, but they are not good inhibitors of grape PPO. Furthermore, application of 0.002% or 0.005% 4-hexylresorcinol does not inhibit enzymatic browning of fresh-cut artichokes. It is also noted that citric acid may function as a PPO inhibitor through its chelating action and ascorbic acid through its site-directed specificity toward histidine residues on the PPO protein. Application of citric acid can inhibit effectively the skin

Table 3 Treatments to inhibit enzymatic browning of fresh fruits and vegetables after harvest

Fruits and vegetables	*Treatment*	*References*
Apple	10 μl l^{-1} NO gas and 10 mg l^{-1} NO donor compound 2,2′-(hydroxynitrosohydrazino)-bisethanamine	Huque et al. (2013) and Wills et al. (2007)
Avocado	300 nl l^{-1} 1-methylcyclopropene	Hershkovitz et al. (2005)
Bamboo shoot	1.0 mM salicylic acid	Luo et al. (2012)
	0.5 mM sodium nitroprusside (a nitric oxide donor)	Yang et al. (2010)
Cauliflower	Application of an antifog shrink film	DeEll et al. (2003)
Celery	Humidified controlled atmosphere (5 kPa O_2 plus 5, 15 or 25 kPa CO_2)	Gomez and Artes (2004)
Cherimoya	0.5% L-cysteine dip	Campos-Vargas et al. (2008)
Eggplant	1 μl l^{-1} 1-methylcyclopropene for 6 h	Massolo et al. (2011)
	Exposure to 5 ml kg^{-1} ethanol vapor in a sealed container for 5 h at 20 °C	Hu et al. (2010)
Grape	0.5% burdock fructooligosaccharide	Sun et al. (2013)
	0.5% or 1% chitosan coating	Shiri et al. (2013) and Choi (2007)
	2 and 4 mM salicylic acid dip	Ranjbaran et al. (2011)
Lettuce	Heat shock in chlorinated water for 3 min at 50 °C	Roura et al. (2008)
Litchi	0.5% salicylic acid and 1% isoascorbic	Kumar et al. (2013)
	1 mM pyrogallol	Jing et al. (2013)
	2 M HCl	Salomao et al. (2012)
	60 g l^{-1} sodium metabisulfite	Liang et al. (2012)
	0.1% (v/v) *n*-butanol aqueous solution	Sun et al. (2011)
	25 μg l^{-1} methyl jasmonate	Yang et al. (2011)
	40 mM ascorbic acid and 1.0% (w/v) chitosan	Sun et al. (2010)
	0.25–2% chitosan dissolved 6% citric acid	Ducamp-Collin et al. (2008)
	Exposure to pure N_2 gas for 6 h	Liu et al. (2007)
	Plastic bag packaging	Sivakumar and Korsten (2006)
	Citric acid or tartaric acid solution at pH 0.8, 1.0, or 1.3 plus 1% (w/w) chitosan	Joas et al. (2005)
Longan	2% chitosan/30% nanosilica hybrid film	Shi et al. (2013)
	7.5% sodium metabisulfite	Hai et al. (2011)
Loquat	625 nl l^{-1} 1-methylcyclopropene followed by modified atmosphere packing	Oz and Ulukanli (2011)
	10 μM methyl jasmonate	Cao et al. (2008)
Lotus (fresh-cut root)	1.2% chitosan coating, followed by micro-perforated polyethylene packing (30 μm thickness)	Xing et al. (2010)
Mushroom	2% O_2 + 10% CO_2	Ye et al. (2012)
	An ethanolic extract from licorice roots (*Glycyrrhiza glabra*) or 1 μg ml^{-1} 3-(2,4-dihydroxyphenyl propionic acid)	Nerya et al. (2006)
Peach	80% N_2O	Nah et al. (2012)
	0.2% ascorbic acid or/and 5 M nitric oxide	Zhu et al. (2009)
Pear	0.5–1 μl l^{-1} 1-methylcyclopropene	Moon et al. (2008)
	4% calcium chloride	Mahajan and Dhatt (2004)
	0.2% diphenylamine or 0.3% ethoxyquin	Feng et al. (2004)
Persimmon	0.5% calcium nitrate or calcium chloride	Ferri et al. (2008)
Pineapple	38 °C for 60 min	Weerahewa and Adikaram (2005)
	0.1 μl l^{-1} 1-methylcyclopropene for 18 h	Selvarajah et al. (2001)
	2% $CaCl_2$ solution immersion	Goncalves et al. (2000)
Plum	0.5% ascorbic acid by vacuum infiltration	Shao et al. (2011)
Pomegranate	0.1, 0.5, or 1.0 mM acetyl salicylic acid	Sayyari et al. (2011)
Olive	3% sodium metabisulfite solution at pH 3.0	Segovia-Bravo et al. (2012)
Strawberry	60, 75, and 90 °C for 3 or 5 min	Holzwarth et al. (2012)
Sweet cherry	*Aloe vera* diluted 1:3 with distilled water and then immersed for 5 min	Martinez-Romero et al. (2006)

browning of litchi fruit and surface browning of fresh-cut Chinese water chestnut during storage. Kojic acid is found in fermented Japanese foods, and the use of kojic acid at concentrations ranging from 1 to 4 mM exhibits a significantly antibrowning effect on apple juice. This agent inhibits PPO activity and also chemically reduces brown pigments on colorless compounds.

Reducing agents are extensively used in the food industry. The application of reducing compounds is, to date, the most effective control method for enzymatic browning caused by PPO. Studies have revealed that ascorbic acid, pyrogallol, cysteine, bisulfites, and thiol compounds have a direct inactivating effect on PPO, in addition to their ability to reduce benzoquinones to *o*-dihydroxyphenols; the reducing compounds are oxidized in the process. The most widespread reducing agents used for enzymatic browning control are sulfiting agents. The reducing compound sulfite is generally used by the industry in controlled-atmosphere chambers with burning sulfur. However, in recent years, there has been increasing concern over the sulfur residue present in fresh fruits and

vegetables, particularly as some consumers are sensitive to sulfites. In the United States, sulfur is only registered as a postharvest treatment on grapes. Therefore, studies have been made to find efficient inhibitors without toxic effects. In these studies, cysteine has been shown to be an effective compound to prevent browning. It reacts with *o*-quinone intermediates to produce stable and colorless products. However, even at a low concentration, cysteine produces an undesirable odor, limiting its use in food processing. Thus, development of sulfur-free treatments to control enzymatic browning is imperative.

The use of acidulants is another approach widely used in food processing to reduce enzymatic browning. Acids naturally present in some edible food products, such as ascorbic acid, citric acid, and malic acid, shift the food pH to 3 or lower. In general, PPO present in fruits and vegetables is more active in the pH range of 4.0–8.0 (Table 1); and thus, the enzyme activity drastically decreases at a higher acid environment. Acidity may reduce the strong binding of the enzyme to its active site of copper. In fact, acidulants only partially prevent enzymatic browning of fresh fruits and vegetables compared with bisulfite; in combination with edible coatings, such as chitosan, enhanced inhibition of enzymatic browning may result.

Oxygen concentration or O_2 accessibility influences greatly enzymatic browning. A high percentage of molecular O_2 can be replaced with either N_2 or CO_2 to slow down or prevent enzymatic browning. As oxygen is required by PPO to initiate the browning reaction at the surface wound, the use of O_2-impermeable packaging, edible films, antifog shrink films, modified atmosphere packing, or controlled atmosphere may be beneficial in preventing the onset of enzymatic browning. In recent years, chitosan coatings have been extensively investigated for the control of enzymatic browning in fresh fruits and vegetables after harvest and show a potential for commercial application (Table 3). In addition, prevention of mechanical bruising during the shipping of fresh fruit and vegetables after harvest is important to avoid O_2 accessibility, while compression and vibration can be prevented by the use of soft packaging.

An alternative approach for the control of enzymatic browning involves delaying the senescence of fresh fruits and vegetables after harvest. It is well known that membrane deterioration is an early and characteristic senescence feature of fresh fruits and vegetables during storage. Although plant cells possess both enzymatic and nonenzymatic antioxidant systems to alleviate membrane lipid peroxidation and protect cellular membranes from radical oxygen species (ROS), irreversible senescence occurs, due to oxidative membrane damage induced by the imbalance between ROS and ROS-scavenging systems.

An accumulation of ROS may induce a loss of membrane integrity. Accordingly, a gradual loss of compartmentalization between enzymes and substrates could lead to the enzymatic oxidation of phenolic compounds to brown-colored polymers. As a result, application of some antiaging agents or signal substances such as calcium nitrate, calcium chloride, ethanol vapor, NO, N_2O, salicylic acid, methyl jasmonate, and 1-methylcyclopropene effectively inhibits the enzymatic browning of apple, avocado, grape, litchi, loquat, peach, pear, persimmon, pineapple, pomegranate, eggplant, or bamboo shoot after harvest by delaying senescence (Table 3). Among these applications, 1-methylcyclopropene is used commercially.

New Approaches for the Control of Enzymatic Browning

Current research on genetic engineering methods such as antisense RNA and gene silencing will help increase our understanding of the enzymatic browning caused by PPO and how to control it to improve crop quality. Molecular biology techniques have helped to explain the confusion regarding the multiple forms of PPO isolated from many fruits and vegetables. The recent work to reduce browning capacity of plants and, in turn, to improve crop quality is the control of PPO levels *in vivo* by means of antisense RNA strategy. In light of the molecular and genetic data available, it is believed that manipulation or regulation of PPO gene expression in order to diminish adverse effects of PPO on quality of fruit and vegetable crops without application of additives will be a future challenge. Antisense downregulation of PPO, for instance, in potato and mushroom has already been performed. Genetic transformation has also been conducted, and a transgenic apple (*Malus* x *domestica*) shoot with reduced PPO expression had lower browning potential than a control shoot. Furthermore, PPO activity of 'Russet Burbank' potato was inhibited by sense and antisense PPO RNAs from a tomato PPO cDNA under the control of the 35S promoter from the cauliflower mosaic virus, and tubers from five lines exhibited reduced browning correlated with low PPO activity. It appears that inhibited expression of PPO genes will be applied to prevent enzymatic browning in a wide variety of food crops without the application of various additives.

Conclusion

PPO is the key enzyme considered to be responsible for food browning. Despite the fact that the involvement of PPO in enzymatic browning has been studied for more than a century, many questions still remain about the enzyme itself as well as the enzymatic browning mechanism. The biochemical properties involved in the action of PPO in numerous fruits and vegetables have been investigated widely. Some models explaining PPO activity have provided additional insight to our understanding of the molecular structure of PPO reactions. Genes encoding PPO have been isolated and characterized from some fruits and vegetables, and all these studies verify the plastidic location of the unclearly coded protein. Current approaches to the understanding and control of enzymatic browning caused by PPO will make it possible to obtain crops of improved quality for marketing and storage. However, revealing all the complexity of PPO gene regulation to control enzymatic browning remains a prime challenge. In view of consumer concerns, products of such genetic engineering technology are not likely to be practical in the short term. Additionally, the food industry still faces the problem of how to

prevent enzymatic browning while considering food safety, regulations, marketability, and cost.

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See also: Antioxidants: Characterization and Analysis; Browning: Non-enzymatic browning; Colors: Properties and Determination of Natural Pigments; Enzymes: Functions and Characteristics; Food Additives: Classification, Uses and Regulation.

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Browning: Non-enzymatic browning

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Introduction

Heat treatment is very common during the processing and storage of foods. Thus, thermal processing allows the improvement of food value attributes – such as organoleptic properties – and health attributes, getting healthier and more nutritious foods. Sterilization treatments, frying, roasting, baking, etc., reaching temperatures up to 220 °C, induce a series of food transformations leading to the formation of new compounds that affect, in general, the acceptability of the product by consumers. Some of these transformations are collectively known as nonenzymatic browning (NEB), responsible for many of the flavors and colors in foods that have undergone thermal processing.

NEB is a set of complex reactions produced in thermally treated foods giving rise to the formation of brown colors without the intervention of enzymes. This effect is desirable in many foods such as bread, breakfast cereals, candies, coffee, and chocolate, where the toasted aroma and color are expected. However, NEB produces undesirable effects during the processing and storage of different liquids or dehydrated foods – for example, milk, fruit juices, eggs, and fruits – due to the formation of undesirable aroma and/or colors and loss of nutritive value (degradation of vitamin C or essential amino acids).

NEB can be divided in three different reactions: ascorbic acid (AA) degradation, caramelization (degradation of sugars), and the Maillard reaction (MR) (sugar–amino acid reaction), although part of the compounds formed during AA degradation or caramelization can take part in the MR. The conditions where such reactions take place are reviewed in Table 1.

AA Degradation

L-Ascorbic acid (AA) or vitamin C is a highly water-soluble and strongly reducing substance with acidic properties. This chemical behavior is related to its 2,3-enediol structure conjugated with a carbonyl group (a lactone), which makes this molecule very sensitive to different forms of degradation. The decomposition of AA occurs mainly at slightly acidic pH, medium/high water activity, and moderate temperature in fruits, vegetables, and meat products, giving rise to the reversible production of dehydroascorbic acid (DHAA) and the irreversible hydrolysis to 2,3-diketogulonic acid (DKGA) (Figure 1). There are two different pathways, one oxidative and another non-oxidative. One of the main differences between them is the higher production of furfural in the non-oxidative pathway. The selection of the oxidative or the non-oxidative pathways depends on the presence of metallic catalysts such as Cu^{2+} and Fe^{3+}.

Oxidative Degradation

During the oxidative degradation under aerobic conditions, the AA molecule is oxidized to DHAA, which is the main precursor of the degradation products. DHAA is hydrolyzed to DKGA, which suffers a subsequent decarboxylation, dehydration, and/or polymerization to produce different byproducts such as brown polymers. As stated earlier in the text, the primary factors that influence the oxidative degradation of AA are the pH, oxygen partial pressure, and the presence of metal ions.

Effect of pH

The food's pH influences the oxidative degradation of AA in a nonlinear manner because of the different oxidation sensitivities of its ionic species (Figure 2): the ascorbate dianion (A^{2-}) is much more sensitive than the monoanion (AH^-), which is more sensitive than the protonated AA. In foods, at acidic pH, the predominant species are the protonated and AH^- species, due to the pK_{a1} of the C3 hydroxyl group ($pK_{a1} = 4.04$). However, at basic pH, AA is much more sensitive to aerobic degradation because the pK_{a2} of the C2 hydroxyl group is 11.4. Therefore, at a pH higher than 8.00, there is a high portion of A^{2-}.

Effect of metallic ions

The oxidation of AA can develop either catalyzed by metallic ions or not catalyzed. In the first studies on the oxidation of AA catalyzed by metals, the rate constant (first order) was thought to be relatively high ($5.87 \times 10^{-4}\ s^{-1}$). However, recent experiments under noncatalyzed conditions show that this constant is rather low ($6.00 \times 10^{-7}\ s^{-1}$) at pH = 7.0. Therefore, the noncatalyzed reaction is not spontaneous. In addition, when metals are present at concentrations of parts per million in foods, the rate constants are higher than those obtained in the absence of metals. The principle of metal catalysis is shown in Figure 3.

The oxidation speed in the presence of metals is proportional to the oxygen partial pressure (0.40–1.00 atm) under aerobic conditions. However, under low oxygen partial pressure (<0.20), the oxidation speed is independent of oxygen concentration and depends on the amount of metal catalysts. The chemical nature of the metal, its oxidative state, and the presence of chelators play an important role in AA oxidation. For example, Cu^{2+} is 80 times more reactive than Fe^{3+} while the ethylenediaminetetraacetic acid (EDTA)–Fe^{3+} chelate is 4 times more reactive than Fe^{3+}. However, in the case of copper, its catalyzing activity is almost lost when chelated with EDTA.

The chemistry behind AA oxidation

In foods and solutions, AA is in equilibrium with AH^-, which can be oxidized to the semidehydroscorbate (AH·) radical and then to DHAA by a one-electron oxidation. However, the main

Table 1 Key points of nonenzymatic browning reactions

Mechanism	*Ascorbic acid degradation*	*Caramelization*	*Maillard reaction*
Oxygen	Yes/no	No	No
Amino groups	No	No	Yes
Optimum pH	Slightly acid	Basic/acid	Basic
Heat	Mild	Strong	Mild
Water activity	Medium/high	Low	Low/medium
Nutritional relevance	Yes	No	Yes
Toxicological relevance	No	Yes	Yes

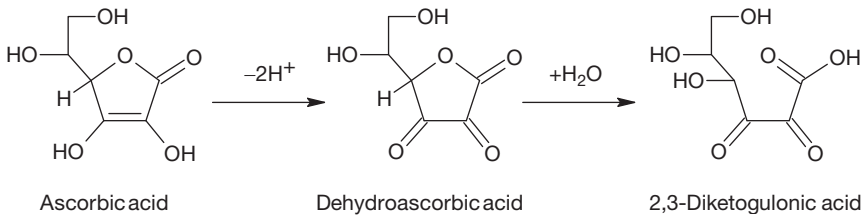


Figure 1 Oxidation of ascorbic acid (AA) to DHAA and DKGA.

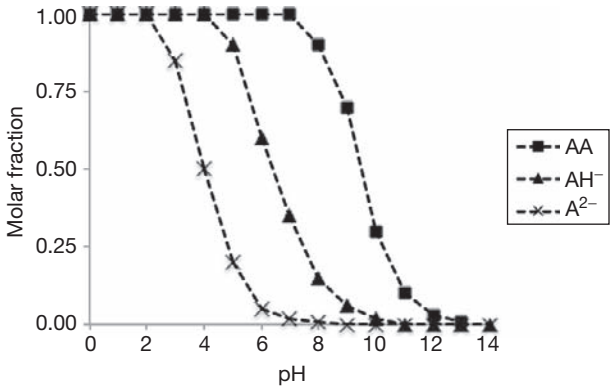


Figure 2 Effect of pH over the distribution of ascorbic acid species.

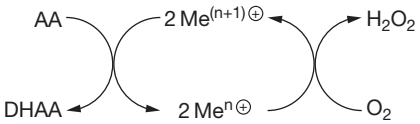


Figure 3 Principle of metal catalysis of aerobic AA oxidation.

pathway for the oxidative degradation of AA starts when a metal species, oxygen, and AH^- are linked in a ternary complex that generates DHAA. The main steps of the metal-catalyzed oxidation of AA are depicted in Figure 4. The ascorbate monoanion is oxidized to AH· due to the presence of a metal catalyst as seen. This reaction gives rise to the formation of superoxide, duplicating the reaction speed. AH· will end the radical reaction by the formation of DHAA, which will be hydrolyzed irreversibly to DKGA in the next step.

Non-oxidative Degradation

In an acidic medium under anaerobic conditions, such as canned foods like vegetables and tomato juices, the

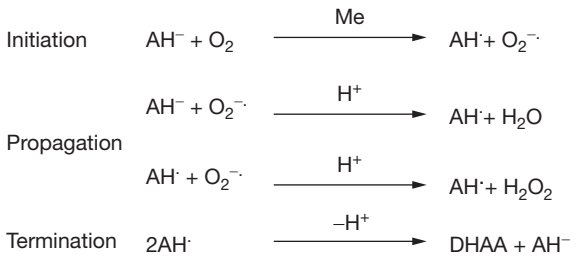
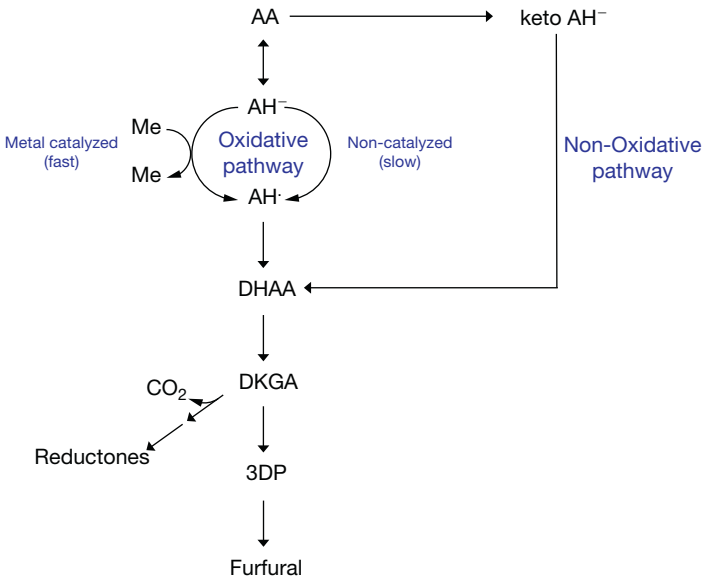


Figure 4 Metal-catalyzed oxidation of AA.

non-oxidative degradation of AA starts with the formation of the keto form of HA^- followed by the irreversible hydrolysis of the lactone group to DKGA, as in the case of the aerobic oxidation. This anaerobic degradation is negligible in fresh food, but in canned ones, it is accelerated by the presence of metal catalysts, especially copper. In addition, the non-oxidative pathway is also accelerated at acidic pH (3.00–4.00) probably by favoring the direct hydrolysis of the 1,4-lactone. The scheme of the reaction is shown in Figure 5. The next steps are shared by the oxidative and the non-oxidative pathways due to the common precursor DKGA. This chemical compound reacts by means of different chemical reactions, for example, decarboxylation, dehydration, and conjugation with other chemical species, giving rise to the formation of colored and flavored compounds. In addition, some other compounds like ethylglyoxal, 3-deoxypentosone, reductones, or furfural formed from DKGA breakdown may participate also in the MR.

Caramelization

Caramelization is another kind of NEB, obtained when sugars are heated over their fusion temperature, giving rise to an enol intermediate and final dehydration products. This is a useful method to produce color and flavor enrichment during cooking of sugar-rich foods, such as bread baking or coffee roasting.



AA: ascorbic acid. AH^-: ascorbic acid monoanion. AH^+: semidehydroascorbate radical. Me: Metal catalyzer. DHAA: dehydroascorbic acid. DKGA: diketogulonic acid. 3DP: 3-deoxypentosone.

Figure 5 AA degradation pathway.

Table 2 Main flavor compounds derived from caramelization

Heterocyclic compounds			*Carbocyclic compounds*
Furans	Furanones	Pyrones	
Furfural	Hydroxymethylfuranone	5,6-Dihydromaltol	2-Hydroxy-3-methyl-cyclopentenone
2-Hydroxyacetylfurane	Dihydroxymethylfuranone	5-Hydroxy-5,6-dihydromaltol	3-Methyl-2-cyclopentenone
5-Hydroxymethylfurfural			2,3-Dimethyl-2-cyclopenten-1-one

These thermal treatments increase the temperature at the food surface to above 120 °C. In addition, a pH range from 3.00 to 9.00 increases the development of caramelization, as well as compounds such as ammonia, sulfite, or oxidizing agents like Cu^{2+}. Therefore, the fine-tuning of these parameters allows the obtention of foods with caramellike aroma or caramellike color. For example, sucrose syrup can be heated in an acidic-buffered solution to obtain a flavored caramel, whereas the same solution when heated in the presence of sulfuric acid and ammonia gives rise to the formation of a colored caramel. Some of the compounds responsible for the caramellike aroma are included in Table 2. They produce flavor ranging from mild-sweet caramel aroma to burning bitter.

Monosaccharide Reactions

The first step of caramelization in monosaccharides comprises an intramolecular rearrangement (enolization) followed by β-elimination of water (dehydration). This is the key reaction because it initiates subsequent reactions (dicarboxylic cleaving, retro-aldol reaction, aldol condensation, and radical reaction) that give rise to aliphatic sugar degradation products, which react further to produce oxygen heterocyclic and carbocyclic compounds. These compounds, in addition to their aroma properties, possess conjugated double bonds, which in turn absorb light and produce the caramellike color. A scheme of these reactions is shown in Figure 6. At acidic pH, the enolization and dehydration allow the maintenance of the carbon chain length. However, at basic pH, there is a predominance of fragmentation by retro-aldolization reactions and further reaction of the fragments by aldol additions.

Reactivity in acidic conditions

As stated in Figure 5, during the heating of acidic foods – for example, fruit juices – enolization and subsequent dehydration are the predominant reactions. The first step involves the slow enolization to produce enediol compounds, which are important chemical species in caramelization. As shown in Figure 7, glucose can enolize to fructose, giving rise to the formation of two different enediol compounds (1,2-enediol and 2,3-enediol). They can continue the reactions by subsequent dehydrations producing different deoxyosones, which can undergo further dehydrations and cyclization, giving rise to the formation of heterocyclic compounds like 5-hydroxymethylfurfural

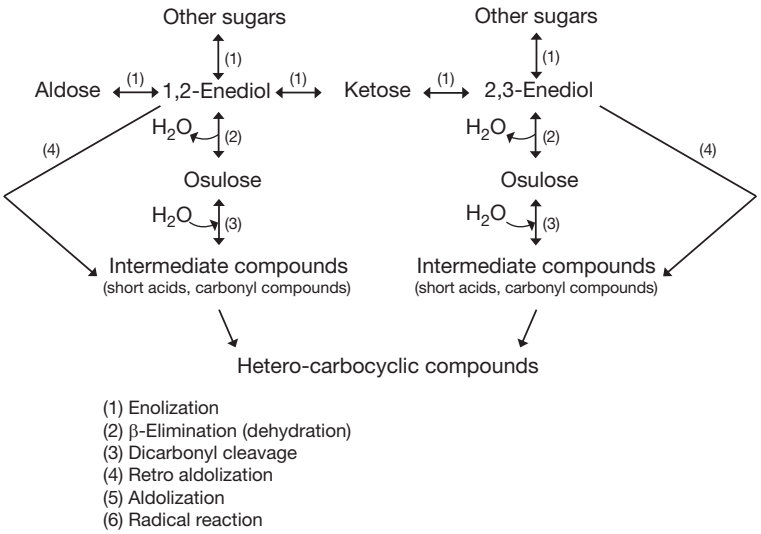


Figure 6 Main pathways of monosaccharides caramelization.

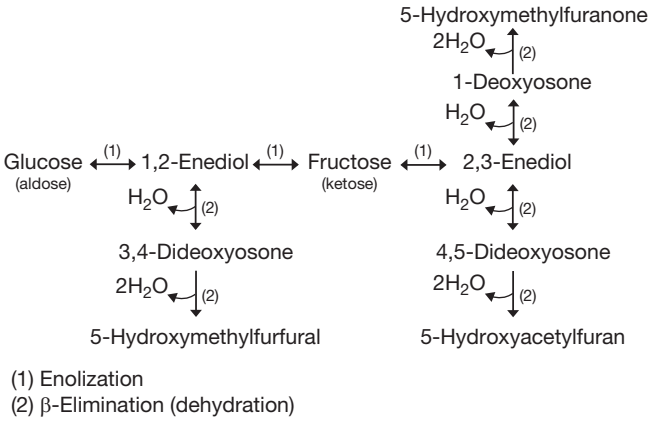


Figure 7 Caramelization of glucose–fructose.

(HMF), 5-(hydroxymethyl) furan, furfuryl alcohol, and/or 5-(hydroxymethyl)furanone. Pentoses give rise to the formation of furfural as the main degradation product, whereas hexoses produce 5-HMF. Dehydration reactions are very important, yielding a large amount of water released to the medium (up to 40% by weight). In addition, the release of short-chain fatty acids, such as formic or acetic acid, produces a drop in the food's pH. Finally, intramolecular glycosidic bonds can be formed during caramelization. For example, the heat treatment of glucose syrup above 100 °C gives rise to the generation of 1,6-anhydroglucopyranose while the heating of sucrose caramel produces 1,6-anhydroglucopyranose, 1,6-anhydroglucofuranose, and difructosedianhydride.

Reactivity in basic conditions

In alkaline foods, such as some baked goods, the enolization reactions stated in the previous sections are favored. In addition to this, there is also a degradation of the carbon skeleton. The enolization steps give rise to the generation of other sugars like mannose or psicose by the Lobry de Bruyn–van Ekenstein rearrangement. The enolization can be also favored by oxidizing reagents such as Cu^{2+}, which produces carboxylic acids and hydroxy acids. In addition, the dicarbonyl cleavage and retro-aldolization reactions produce hydroxy aldehydes and hydroxy ketones like glyceraldehyde and dihydroxyacetone. Further reactions (retro-aldolization and β-dicarbonyl cleavage) give rise to the generation of large amounts or aromatic compounds (as stated in Table 2).

Oligosaccharide Reactions

When a mixture of oligosaccharides–polysaccharides is heated over their fusion point, the first reaction taking place in acidic medium is the glycoside hydrolysis, releasing different amounts of mono- and disaccharides, which react faster than the oligosaccharides. In addition to glycosidic bond hydrolysis, the subsequent formation of glycosides – that is, transglycosidation and formation of oligomers – is also possible in an acidic medium. For example, during glucose caramelization, different amounts of the disaccharides trehalose, maltose, isomaltose, and gentobiose are found. In the case of sucrose syrup, more oligomers of fructose with a degree of

polymerization (DP) up to 25 units are also generated. So, caramelization is not only a degradative reaction. In the case of basic foods, disaccharides can suffer the Lobry de Bruyn–van Ekenstein rearrangement like monosaccharides. For example, lactose can be transformed into lactulose by the catalytic activity of sodium aluminate.

Apart from these reactions, enolization and dehydration with further aldol-like reactions also occur, to produce low-molecular-weight compounds such as HMF, like in the case of monosaccharides. However, the formation of HMF (and others) is favored in oligosaccharides because of the cleaving of the glycosidic bond between the monosaccharide residues is more efficient than water elimination by glucose. Therefore, the 4-osulose formed at the reducing end by 1,2-enolization gives rise to HMF and an oligosaccharide with one sugar less (Figure 8).

The desirable effect of caramelization development is the production of caramellike color and flavor. The caramelization of both monosaccharides and oligosaccharides generates colored compounds. Most of them are low-molecular-weight compounds such as dihydrofuranones, cyclopentenolones, cyclohexenolones, and pyrones. In addition, high-molecular-weight polymers are also produced by the fusion of reactive intermediary compounds like osuloses. These reddish-brownish colloidal polymers range from slightly acidic to basic in nature, although their exact chemical composition is not known.

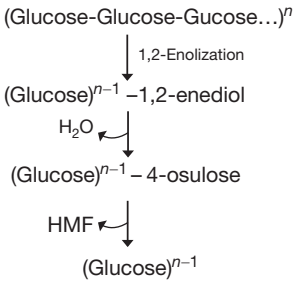


Figure 8 Fragmentation of oligosaccharides by caramelization.

Maillard Reaction

The MR is a set of chain chemical reactions that give rise to the formation of brown pigments with modifications in color, odor, and taste of thermally treated foods. It occurs most readily at low-intermediate water activity and basic pH. The general scheme of the reaction can be seen in Figure 9.

(A) The first step of the MR consists of the condensation of a carbonyl group with an amino group, and after dehydration, an unstable Schiff base is formed, which is transformed rapidly into an *N*-substituted glycosylamine. This reaction is reversible because in a strong acidic medium, the sugar and the amino acid can be regenerated. The amino group can be a free amino acid, the side chain of an amino acid (like lysine) incorporated in a protein, or the amino group of the last amino acid in each protein. In the case of the carbonyl groups, they are usually reducing sugars, although they can be also carbonyl compounds from the intermediate stages of the MR and/or lipid oxidation.

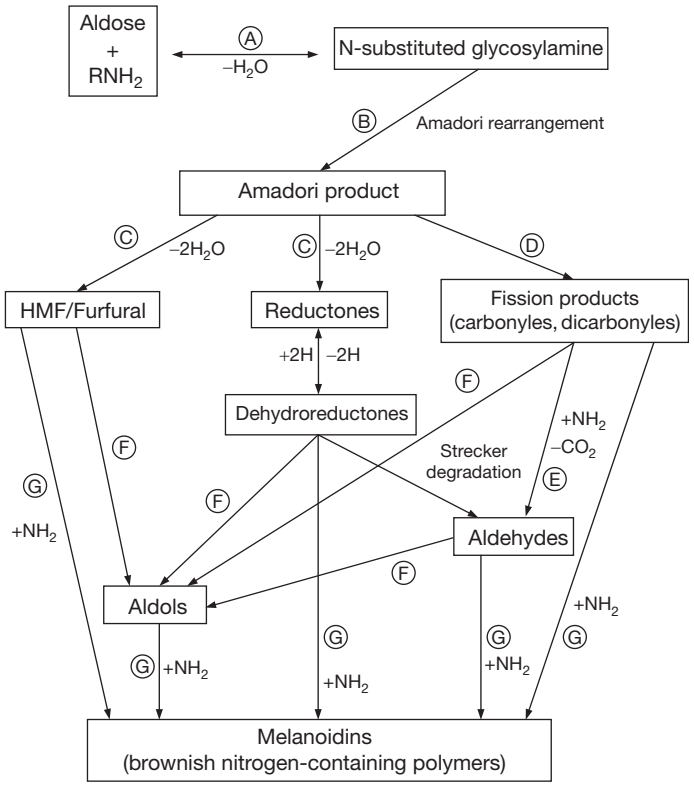


Figure 9 The Maillard reaction (MR).

(B) The next step consists of the irreversible rearrangement of the *N*-substituted glycosylamine. When the molecule is an *N*-substituted aldosylamine, by means of the Amadori rearrangement, the 1-amino-1-deoxy-2-ketose is formed. However, when the starting product is an *N*-substituted ketosylamine, a 2-amino-2-deoxy-2-ketose is formed by means of the Heyns rearrangement. The Amadori and Heyns products are decomposed, depending on the pH and temperature of the medium, giving rise to the formation of different intermediate compounds. These comprise the intermediate steps of the MR.

(C) The Amadori products can decompose by a retro-Michael reaction, removing the amino group and further dehydration. Different osuloses – named 1-, 3-, or 4-deoxyosone or reductones – are formed. At low pH, a 1,2-enolization occurs, giving rise to the final formation of HMF and furfural. A 2,3-enolization takes place at basic pH. The reductones so formed can be dehydrated again to form dehydroreductones, which form polymers by reacting with amino groups at the advanced stages of the MR.

(D) The Amadori compounds can be split into different fission products, such as acetaldehyde.

(E) The interaction of amino acids with dicarbonyl compounds, like dehydroreductones or fission products, is known as the Strecker degradation and implies the loss of amino acids in foods. As a result of this degradation pathway, new aldehydes with one carbon atom less – lost as CO_2 – are formed.

The next steps ((F) and (G)) are known as the advanced steps of the MR, where two different classes of compounds are formed: melanoidins and volatile aromatic compounds.

(F) Volatile aromatic compounds are formed directly from the Amadori compounds and do not need the mediation of free amino groups. However, 1% of the total volatile compounds produced are formed by the reaction of 2-deoxyglucose with amino acids. As in the case of the Strecker degradation, many different chemical odorants are formed, like pyrazines, pyrroles, thiazoles, and thiophenes. For example, alquilpyrazines are the main flavors of roasted meat, while boiled meat gives rise to other products like 2,4,5-trimethyl-3-thiazoline.

(G) *Melanoidins* are brown polymeric anionic compounds produced by means of the condensation of aminated products of the intermediate stages of the MR such as *N*-substituted pyrroles, *N*-substituted 2-formylpyrroles, and 2-furaldehyde. Melanoidins have a wide molecular weight and different absorbance spectra with maxima in the ultraviolet (280 nm) and visible (420 nm) ranges.

Chemical Links between AA Degradation, Caramelization, and the MR

In the previous sections, the different chemical pathways of AA degradation, caramelization, and the MR have been described. Although each set of reactions depends on a different series of variables, all of them share some chemical species that can take part in another kind of NEB. Therefore, the development of one of these pathways may lead to a higher development of a different pathway. The complete set of chemical links is shown in Figure 10.

The degradation of AA and caramelization share similarities, such as the pH influence and the formation of some chemical

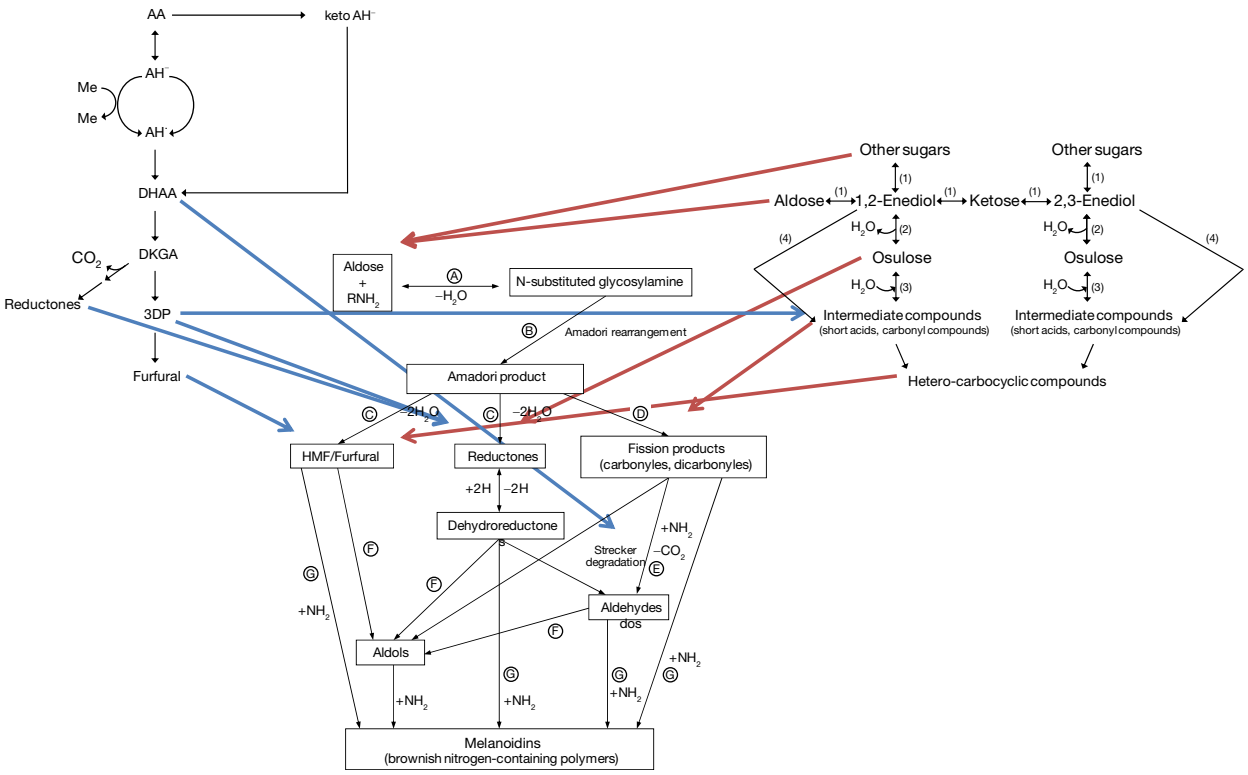


Figure 10 Chemical links among AA degradation, caramelization, and the MR.

species like furfural or reductones. These carbonyl compounds take part in the Strecker degradation and DHAA. The DHAA reacts as an amino acid to generate scorbamic acid, which gives rise to the formation of reddish–yellowish oligomers. In addition, the subsequent polymerization of these compounds and other carbonyls produces melanoidins (nitrogen-containing polymers) or nonnitrogenous polymers (caramel-like).

In the case of caramelization, the most direct link with the MR comes from the reaction of monosaccharides with amino compounds. Therefore, the enolization reactions can produce more aldoses to increase the development of the MR. Furthermore, the hydrolysis of oligosaccharides to produce monosaccharides also increases the speed of the MR. Other highly reactive species formed during caramelization – osuloses – can also take part in the MR through the formation of dehydroreductones or other carbonyl compounds. In addition, the heterocyclic compounds – such as HMF – generated during caramelization reactions can be also included in the structure of melanoidins. Caramelization can be also favored by the formation of 3 DP during AA degradation. This chemical species can react with further caramelization intermediate compounds to produce hetero- and carbocyclic compounds.

When foods are heated, the first reactions taking place are AA degradation and MR. Because of the relative low amount of AA, large amounts of furfural or reductones cannot be produced, but DHAA can readily come into the MR through the Strecker degradation. When temperature exceeds 100 °C, the surface of foods dehydrates, increasing the rate of caramelization. Furthermore, the reductones generated during the intermediate-advanced steps of the MR speed up the formation of hetero- and/or carbocyclic compounds of caramelization. Furthermore, the formation of short-chain carboxylic acids during the MR and caramelization decreases the food's pH, increasing again the development of brownish polymers from the MR and caramelization.

A good example of a MR–caramelization collaboration could be biscuit baking. The biscuit surface is covered with beaten egg and the dough goes into the oven, where the temperature increases slowly. As temperature reaches 50 °C in the inner part of the biscuit, amino compounds and sugars start to react through the MR. The inner part will never exceed 100 °C, so caramelization will never develop. At the same time, in the outer part, the MR will develop due to the presence of glucose and egg proteins. This will create a large pool of intermediate compounds that will take part in caramelization when the temperature reaches 120 °C. At this time, an extensive brownish color and flavor will develop at the surface by the combined action of the MR and caramelization.

Conclusions

The Western diet includes different heat treatments, which influence both the organoleptic and health attributes of foods. Depending on their composition and the conditions of the heat treatment, that is, temperature, time, humidity, pH, and the presence of metals, different chemical reactions take part. The degradation of AA is developed in foods like fruits or vegetables submitted to heat treatment and preservation, that is, canned vegetables or jams, while caramelization is produced mainly in foods that undergo a severe heat treatment, such as coffee or cocoa roasting. In the case on the MR, those foods rich in proteins and carbohydrates, like milk, are prone to this kind of NEB. These three reactions usually take part at the same time during heat treatment, and due to their interconnected chemical pathways, they generate a plethora of by-products responsible for the special color and aroma of heat-treated foods. Furthermore, the MR and lipid oxidation share other pathways, reinforcing the idea that food processing and storage produce alterations by many different ways. Therefore, under a technological point of view, it is complex to stimulate any of these reactions trying to keep the others stable. However, the knowledge of their shared chemical pathways allows to food producers the development of healthier and appetizing foods.

See also: Acrylamide; Antioxidants: Characterization and Analysis; Ascorbic Acid: Properties, Determination and Uses; Biscuits, Cookies, and Crackers: Chemistry and Manufacture; Bread: Chemistry of Baking; Caramel: Methods of Manufacture; Coffee: Types and Production; Cooking: Domestic Techniques; Drying: Effect on Nutrients, Composition and Health; Extrusion Cooking: Chemical and Nutritional Changes; Maillard Reaction; Milk: Processing of Milk; Oxidation of Food Components; Pasteurization: Effect on Sensory Quality and Nutrient Composition; Polycyclic Aromatic Hydrocarbons; Proteins: Chemistry, Characterization, and Quality.

Further Reading

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Relevant Websites

www.imars.org/online/ – International Maillard reaction Society.

Buffalo Milk

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SD Kalyankar, Government College of Dairy Technology, Udgir, India
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Introduction

The world has two main types of buffaloes: (1) riverine buffaloes (*Bubalus bubalis*) and (2) swamp buffaloes (*B. carabanesis*), whereas the third type known as Mediterranean buffaloes evolved from these two major types. The riverine buffalo is found in South Asia and Southwest Asia, whereas the swamp buffalo is present in East Asia and Southeast Asia. The Mediterranean buffaloes are found in Italy, the Balkan states, Turkey, and in some parts of Russia. The best buffaloes of the world are found in the Indian subcontinent, and India is the leading buffalo country, which produces 96 million tons of milk annually. Pakistan produces 27 million tons annually. Buffalo milk (BM) is ranked second after cow milk (CM) in the world as the BM produced is more than 12% of the world's milk production. About 70% of the total BM is produced by India. World milk production has doubled in the last decade, with BM production ranking second after bovine milk.

Buffalo breeds of India are known for their high production potential and high efficiency for utilization of low-quality roughages and sustaining poor-quality husbandry practices than cattle. The major population of buffalo is from rural India where farmers keep 1–4 milk animals as a subsidiary occupation for sustainable rural livelihood and nutritional security. The most important traits contributing largely toward profit are high milk-producing ability and low maintenance cost. Various compositional and functional properties render the BM eminently suitable for manufacture of dairy products such as ultra-high temperature (UHT) cream, dried ice cream mixes, dairy whiteners, edible casein, and caseinates. However, from a technological point of view, BM is often not considered an ideal fluid for manufacture of several types of cheeses, milk powders, evaporated and condensed milk, and infant formulas.

Due to several biochemical differences between BM and CM, conventional processing technologies are often unsuitable and cannot be applied directly for processing BM. Emerging R&D trends in BM processing suggest that there is an ample scope for tailoring the technology, particularly in the developing world where buffaloes enjoy a preeminent position in milk production. Several region-specific traditional milk products owe their unique characteristics to BM. The suitability of buffalo as a milk producer is now distinctly gaining importance throughout the world. A major bottleneck in the enhancement in production potential of buffaloes has been the inadequate research and paucity of information about it. It is often falsely presumed that the scientific information generated on cattle can be extrapolated to buffaloes.

Nutritional Significance of Milk

Milk is termed as the almost complete food for human diet. It is the first food of the newly born human being and other mammals. It is a food that contains all the nutrients required for the newly born baby, pregnant mothers, patients, and the old age people. There is no doubt that milk and milk products have played a key role throughout the development of human civilization and supply most of the essential nutrients in significant amounts than any other single food. It is very essential for the growth and development of a newly born child. Milk contains all the essential nutrients like protein, fat, lactose, vitamins, and mineral matter for normal growth and performing different functions for the body systems. Not only is it the most important food during early childhood, but also, in one form or another, it continues to be used for normal diet throughout the life span. It is also the most versatile of all the animal-desired food commodities, and it is a component of the diets of many physical forms like cheese, yogurt, ice cream, ghee, milk powders, and many other forms of fluid milk. The calcium content is higher in BM than in milk from cow, and it contains more colloidal calcium and phosphorus. Buffaloes are the second largest source of milk supply in the world. In India, nearly half of the milk processed by the organized dairies comes from buffaloes. The BM is richer in fat than milk from cattle. Generally, it has also higher levels of proteins, lactose, and ash, although these differences are not as high in fat. The absence of β-carotene in BM, which is present in CM, is another notable characteristic. The swamp buffalo has traditionally been regarded primarily as working animals especially in China and other rice-growing countries of the Far East. A website depicting worldwide buffalo distribution is given at the end of 'Further Reading' section of this article. It is, however, milked in many countries and yields a product rich in butter fat and similar in all respect to that of milch river breeds. It is probable that the animal has a considerable potential for milk production if managed and bred to that end.

Milk Proteins

BM proteins are complete proteins of high quality, that is, they contain all the essential amino acids in the proportions required by the body. The energy value of BM proteins is 17.2 J g^{-1}. The BM is much preferred by the consumer for its rich nutrition and is drunk or transformed into valuable products such as indigenous traditional dairy products, cheese, yogurt, and ice cream. The composition of major and minor milk constituents in BM and CM is compared in Tables 1 and 2.

BM has a higher content of fat, lactose, casein, whey proteins, and minerals than CM. All of the casein in BM is present in the micellar form, while in the CM, only 90–95% of the casein is in the micellar state and the rest is present in serum phase. The proportion of bovine casein, which is micellar, depends on the temperature range and gravitational force used to sediment casein micelles. The size of casein micelles is

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00093-3

Table 1 Compositional difference in major constituents of BM and CM

		Composition						
Type of milk	*Country*	*Water*	*Total solids*	*Fat*	*Solids not fat*	*Protein*	*Lactose*	*Ash*
Buffalo	Italy	83.14	16.86	7.22	9.64	3.95	4.88	0.81
Buffalo	Egypt	83.60	16.40	6.37	10.03	3.87	5.00	0.79
Buffalo	USSR	82.00	18.00	8.00	10.00	4.32	4.96	0.84
Buffalo	India	82.98	17.02	7.06	9.96	3.90	5.28	0.78
Cow	India	86.07	13.93	4.90	9.43	3.42	4.91	0.70
Cow	The United States	86.61	13.39	4.14	9.25	3.58	4.96	0.71

Note: Casein content of buffalo milk is higher (3%) than that of cow milk (2.65%).

Table 2 Compositional difference in minor constituents in BM and CM

	Concentration (%)	
Constituents	*Buffalo milk*	*Cow milk*
Total ash	0.80	0.73
Calcium	0.18	0.12
Magnesium	0.02	0.01
Sodium	0.05	0.05
Potassium	0.11	0.15
Phosphorus	0.10	0.10
Citrate	0.18	0.18
Chloride	0.07	0.1
Ca/P ratio	1.8	1.2

Source: Sindhu, J. S. (1999). Physico-chemical properties of cow and buffalo milk in relation to milk processing. In: *Advances in processing and preservation of milk*: *A compendium of short term course notes*. Karnal, Haryana: National Dairy Research Institute

bigger (110–170 nm) in BM compared to CM (range 50–500 nm, mean 120–150 nm). The voluminosity of casein micelles in BM is 2.68–3.72 ml g^{-1} compared with that of CM casein that is 4.18 ml g^{-1}. Similarly, the solvation (hydration of casein micelles) of BM is lower (2.60–2.90 g water/g casein compared to 3.48 g water/g of casein from CM). The casein micelles scatter the light, which accounts for the opacity of milk. The opacity of BM casein micelles is three times higher than that of CM casein. It is determined by spectrophotometry. The BM casein micelles contain higher levels of calcium and magnesium but lower levels of sialic acid and hexose. Lactoferrin content of BM is 32 mg/10 ml compared with that of CM that is 15 mg/100 ml. No genetic polymorphism is exhibited by either the caseins or whey proteins in BM. The presence of immunoglobulin, lactoferrin, lactoperoxidase, and lysozymes in BM makes it a good dietary and health food.

The BM and colostrum, like that from cows, also contain minor bioactive components such as peptides, hormones, and growth factors, which had advantageous intestinal effects in rat trials. The BM has slightly higher concentrations of β-lactoglobulin than CM, and it is also the major whey protein, while human milk contains no β-lactoglobulin, which contains essential and branched chain amino acids, and a retinol-binding protein, which has the potential to modulate lymphatic responses. It can yield antibacterial peptides, which are released after proteolytic digestion with trypsin and are active against Gram-positive bacteria.

Milk Fat

BM fat contains higher proportions of high-melting triglycerides (9–12%) than CM (5–6%), and as a result, it is thicker. Similarly, the proportion of butyric acid-containing triglycerides is higher (50%) in BM than in CM (37%). As a consequence of the higher proportion of butyric acid-containing triglycerides, the emulsifying capacity of BM fat is superior than that of CM fat. Fat globules are bigger in BM (4.15–4.6 μm) than in CM (3.36–4.15 μm). These are rendered chargeless at much higher pH (4.5–4.6) in BM compared with that of CM (pH 4.3).

The BM fat contains less (0.22%) free fatty acids than CM fat (0.33%). The concentration of unsaponifiable matter (392–398 mg/100 ml) is also lower in BM than in CM (414–450 mg/100 ml). Similarly, phospholipid content of BM is also less (21 mg/100 ml) compared with that of CM (37.37 mg/100 ml). The total and free cholesterol contents are 275 mg and 210 mg, respectively, per 100 g of BM ghee, which is much less than the corresponding values of 330 mg and 280 mg/100 g in CM ghee. On the other hand, esterified cholesterol of BM (64 mg/100 g) is much higher than that of CM ghee (48 mg/100 g).

The nutritive interest in BM products is also higher than that in CM because of its derived products, which could be a good source of conjugated linoleic acid (CLA) for humans, like other food products from ruminants. The CLA refers to a group of polyunsaturated fatty acids (PUFAs) that exist as positional and stereoisomers of conjugated dienoic acid (18:2). The predominant isomer in foods is the cis-9,trans-11 CLA also called rumenic acid and the trans-10,cis-12 CLA found primarily in foods containing beef or dairy products. Synthetic mixtures of CLA can also be readily purchased as nutritional supplements and are composed primarily of the cis-9,trans-11 CLA and trans-10,cis-12 CLA isomers.

Numerous potential physiological effects have been attributed to CLA including those related to its potential antiadipogenic, antidiabetogenic, anticarcinogenic, and antiatherosclerotic properties. The CLA content is much higher in foods derived from ruminants than those from nonruminants and with milk having higher content than meat, because of the ability of ruminants to biohydrogenate dietary unsaturated fatty acids with the help of bacteria present in the rumen.

In dairy products from BM, the CLA concentrations typically range from 2.90 to 8.92 mg CLA/g fat, and the cis-9,trans-11 CLA isomer makes up between 73% and 93% of the total CLA. The CLA content of cheeses typically ranges from 3.59 to 7.96 mg CLA/g fat. CLA content of cows' milk ranges from 3.38 to 6.39 mg CLA/g fat. The amount of CLA found in dairy and beef is a direct reflection of the diet the animals are fed with. It was found that CLA concentration increases linearly when animals were pasture-fed and decreases when grass intake declines.

The CLA content of BM fat can be influenced by manipulating the type of dietary supplement fed to dairy animals. Supplementing the diet with polyunsaturated oils that contain either corn oil or sunflower oil increases CLA content of milk fat substantially. The BM has better emulsifying power than that of CM because of its higher percentage of butyric acid (50%) having triglycerides as compared to CM, which has only 37%. Due to this factor, more butter and ghee is obtained from BM. The BM is also less prone to hydrolytic rancidity than CM.

Milk Salts

The concentration of calcium and magnesium is about 1.5 times higher in BM than in CM. On the other hand, the concentration of sodium, potassium, and chloride is lower in BM than in CM. The content of colloidal calcium and magnesium (160 mg/100 ml and 9 mg/100 ml, respectively) in BM is much higher than the levels 80 mg/100 ml and 3 mg/100 ml, respectively, of CM. Only about 20% of calcium and 55% of magnesium in BM are present in dissolved state compared with 33% and 75%, respectively, in CM. The Ca/P ratio in BM is much higher (1.8) than in CM (1.2).

Vitamins in BM

The BM is a rich source of most water-soluble and fat-soluble vitamins. The average concentration of vitamins in milk of buffalo, Indian cow, and Western cow is summarized in Table 3. The vitamin A content is higher in BM (340 IU kg^{-1}) than in CM (230 IU kg^{-1}). However, due to the absence of carotenoids and the high fat content in BM, its total vitamin A potency per unit weight of fat is less than that of CM. Similarly, the tocopherol (vitamin E) content of BM is slightly higher (334 μg kg^{-1}) than that of CM (312 μg kg^{-1}). However, due to higher fat content of BM, its fat is poor in tocopherol (26 μg g^{-1} fat) compared with that in CM (35 μg g^{-1}).

Pigments in BM

Biliverdin IX alpha, a latent blue-green pigment, occurs in fresh BM. This pigment is absent in CM and is considered an important characteristic of BM. The average concentration of biliverdin in skim milk of Murrah and Surati buffaloes is 51.8 and 65 μg/100 ml, respectively. The concentration of this pigment in BM varies significantly at different stages of lactation and lactation number. Biliverdin was primarily associated with α, β, and γ caseins and the proteose-peptone fraction of BM. Biliverdin is converted to bilirubin during storage and souring of BM. This pigment binds lipids and imparts the characteristic greenish-yellow appearance to BM fat and butter prepared by traditional fermentation process.

Other Constituents

The BM is rich in taurine (6 mol l^{-1}), compared with that in CM (4.0 mol l^{-1}). On the contrary, the concentration of urea in BM, 17–22 mg/100 ml, is much lower than the level, 37–40 mg/100 ml, in CM. The levels of lipase and alkaline phosphatase are lower in BM than in CM. However, the free amino acids are present at a higher concentration (0.44%) in BM than in CM (0.15%).

Factors Causing Variation in the Composition of BM

Similar to the differences in CM, changes in BM composition are due to breed, feed, stage of lactation, season, lactation number, and genetic polymorphism. These variations would strongly affect the manufacturing conditions, sensory quality, and nutritional properties of the dairy products.

Breed

The breed of buffalo has a notable effect on the milk composition and yield traits. A comparison of the composition of milk from four different breeds of Indian buffaloes revealed that Murrah was the best-performing breed for fat, total

Table 3 Average concentration of vitamins in different types of milks

	Concentration in milk		
		Zebu	
Vitamins	*Buffalo (*Bubalus bubalis*)*	*Bos indicus*	*Bos taurus*
Vitamin A (IU ml^{-1})	340	230	136–157
Thiamine (μg ml^{-1})	0.2–0.5	0.2	0.2–0.8
Riboflavin (μg ml^{-1})	1.59	2.33	1.7
Pyridoxine (μg ml^{-1})	3.25	2.6–3	–
Ascorbic acid (mg/100 g)	6.72	1.94	1.65–2.75
Tocopherol (μg g^{-1})	334.2	312.2	–

Source: Sahai, D. (1996). Compositional profile of buffalo milk. In: *Buffalo milk*: *Chemistry and processing technology*. Karnal, Haryana: SI Publishers

protein, and casein contents. The Mehsana breed was better for solids-not-fat (SNF) and Bhadawari for total solids (TSs). Apart from the wide differences in fat content of buffalo breeds, differences in other milk constituents were not reported.

Feeding

The quality, quantity, and composition of the diet, especially the quantity and quality of proteins, have been reported to affect the composition of BM. Feeding buffaloes on a diet containing added fats increased milk yield and fat content and, in particular, with dietary tallow. A positive correlation has been reported between the energy content of the diet and fat, milk protein, and lactose contents of BM.

Age of Animal

It is a well-documented fact that the TS, SNF, lactose, and ash content increased with the increase in the number of lactations while the fat and total protein contents were not affected.

Stage of Lactation

The fat and TSs content increased, lactose decreased, and protein and ash initially decreased before reincreasing with advanced stage of lactation.

Season

Variations in composition of BM during various seasons are reported by several workers. These variations in major milk constituents have been reported. The percentages of fat, solids, and SNF were the highest during summer. Also, the percentages of Ca, P, K, Na, Cu, Mn, and Fe were the highest in summer and the lowest in winter.

Genetic Polymorphism of Milk Proteins

The genotypes of α-s1 and α-s2 caseins had no significant correlation with milk composition except for a weak ($P \leq 0.1$) correlation being reported between α-s2 casein and TS.

Differences in Physicochemical Properties of BM and CM

Precise and in-depth information on the physicochemical and functional attributes of milk is an essential prerequisite for its automated industrial processing. Most of the prevalent processing technologies apparently originated in the Western world, where CM and milk products predominate. Therefore, processing strategies were essentially based on the knowledge of the chemistry and functionality of CM. Species-related differences in milk composition have become the subject of topical interest, as processing of milk from species other than cow is being adopted by the dairy industry in several countries of the world. With the emergence of buffaloes as a predominant milk species particularly in countries of Asia, Africa, and Latin America, coupled with rapid dairy development, efforts have been made to develop and adopt appropriate technologies for BM processing. It becomes essential to understand the unique physicochemical and functional properties of BM to overcome the various challenges encountered during its processing. BM has higher specific gravity, viscosity, curd tension (CT), pH, oxidation–reduction potential (Eh), thermal conductivity (TC), and thermal expansion than CM, but its heat capacity and rennet stability are lower than that of CM. In the fluid state, BM is as stable as CM due to various physicochemical factors. On the contrary, the heat stability of concentrated milk product is significantly lower than that of BM. Owing to differences in physical properties, milk from the two species behaves differently when processed for manufacturing of the products.

Physical Properties of BM

Specific Gravity

BM is characterized by its higher specific gravity than CM. Colostrum had higher specific gravity (1.061) than normal BM (1.037). It is also confirmed that the specific gravity of colostrum and normal BM and that of mastitic BM had a lower specific gravity of 1.014 and 1.028 in clinical and subclinical cases, respectively.

Viscosity

The viscosity of BM is generally higher than that of CM. However, the viscosity of milk from both species is largely dependent on fat content. Skim, standardized (3% fat), and whole BM (6.1% fat) showed viscosities of 1.33, 1.70, and 2.02 cP, while skim, standardized (3% fat), and whole CM had viscosities of 1.17, 1.44, and 1.66 cP, respectively. This may explain the variations in the viscosity of BM cited in different studies. A rapid decrease in the viscosity of postpartum BM from 6.80 cP for the first milking to reach the 1.64 cP on the sixth day (normal milk) was also recorded. The incidence of mastitis increased the viscosity of BM to 2.79 and 2.43 cP for milk from animals with clinical and subclinical mastitis, respectively. At pH 8.6 and 10.8, the viscosities of BM were twice as high as those of CM, which was attributed to induced changes in the interactions between water and casein micelles.

Freezing Point

The cryoscopic index of milk is related to its soluble constituents (i.e., lactose and soluble salts) and is usually used to detect water added to milk. The freezing point of BM (−0.518 °C to −0.590 °C) is less than that of CM. The FP of BM is affected by season (−0.528 °C and −0.531 °C in warm and cold weathers, respectively) and farm size (−0.532 °C and −0.519 °C in small and large farms, respectively) and between organic and conventional farming methods (-0.526 ± 0.01 and -0.537 ± 0.01, respectively). The FP of BM in Germany ranged from −0.5509 °C to −0.5146 °C.

Thermal Conductivity

Knowledge of the thermal properties of milk is essential to the design of heat exchanger, condensers, and evaporators

commonly used in dairy plants. The average TC of whole BM has been reported to be 0.5689 ± 0.00734 at 42–43 °C. Normal variations in the ranges of fat and SNF had no significant effect on the TC of BM. However, the TC of BM was higher than that reported for CM, which was attributed to the differences in the fat and fat/SNF ratio of the two milks.

Electrical Conductivity

Electrical conductivity (EC), like other milk properties, is related to the milk composition, particularly the ionized constituents. BM has a lower EC (average 9.17 ± 1.51 mmhos) in comparison to CM (11.12 ± 1.56 mmhos).

Buffering Capacity

The pH of BM decreased more slowly than that of CM during acidification due to the higher buffering capacity (BC) of BM resulting from the high casein and inorganic phosphate contents of BM. The p*Ka* of BM (pH 5.32) was higher than that of CM (pH 4.90). However, both milks showed a higher BC at the acid side than the alkali side of the titration curve.

Technologically Important Characteristics of BM

Curd Tension

Early studies have shown that the CT of rennet-coagulated BM is nearly 1.5-fold that of CM mainly due to its high casein and calcium contents. It is reported that the CT for CM and BM is 27.90 and 32.25 g, respectively. A higher CT value of 55.70 g has been reported for BM, which was attributed to differences in animal breeds and methods used for the determination of CT. However, the CT of BM was greatly reduced by heating to 85 °C, boiling, and homogenization. The addition of phosphate and citrate to heated milk further decreased the CT of BM. Lowering the pH increases the CT of rennet-coagulated BM. However, for milk containing NaCl, the CT increased when the pH was lowered to 6.0, but further lowering of the pH caused the CT to decrease.

Heat Stability

Wide variations have been reported in values for the heat stability of BM due to the heating temperature as well as in the methods used to measure the heat stability. However, most studies agreed that BM was less heat-stable than CM, which is attributed to the high fat and Ca contents, and high negative correlations have been reported between fat and Ca contents, respectively, and the heat stability of BM.

pH

Alteration in pH caused considerable changes in the heat stability of BM and exhibited type A milk with a maximum (~pH 6.7) and minimum (~pH 6.9) stability.

Acid Gelation

The acid-induced gelation of BM using glucono-delta-lactone (GDL) was monitored using thromboelastography that can separate gelation into two phases, the onset gelation time and the time to get it firm. The pH at GT ranged from 5.5 to 5.9, which was higher than that reported for CM (pH 5.1–5). The pH at GT of BM increases with increase in protein content, which may explain the higher pH at GT of BM as compared with CM. Also, the pH of BM at K_{20} was 5.40–5.65, which was higher than that of CM. Linear relations were found between GT, K20 of BM, and GDL concentration and gelation temperature.

Urea Level

The low level of urea in BM (17.5 mg/100 ml) as compared with CM (40 mg/100 ml) was considered to be a factor responsible for the low heat stability of BM. The addition of small amounts of urea has been reported to improve the heat stability of BM.

TS Content

To produce 1 kg of cheese, only 5 kg of BM is required compared with 8 kg of CM required to produce the same quantity of cheese. Similarly, 10 kg of BM is required as compared with 14 kg of CM for the production of 1 kg of butter. The BM is preferred by dairies because it is best for making Mozzarella cheese. Cheddar cheese from BM is also superior to CM cheese because of its better nutritional value and acceptability. Better calcium and phosphorus ratio and less sodium and potassium in BM than in CM make it a better nutrition supplement for infants. This very fact attracts 30–35% higher price to BM as compared to CM. Its average fat globule size is high (2.04 μm) as compared to CM, that is, 1.86 μm. Similarly, its calcium level is high and cholesterol level is low ($0.65\ mg\ g^{-1}$) as compared to CM ($3.14\ mg\ g^{-1}$). The BM has 11.42% more protein than CM. The BM also has a high level of natural antioxidant named tocopherol peroxidase. The patients allergic to CM can benefit from consuming BM.

Rennet Coagulation Time

BM is characterized by a faster coagulation than CM. Also, the rennet coagulation time (RCT) of BM was almost unchanged when the milk was diluted with an equal volume of water while a similar treatment markedly increased the RCT of CM. A close correlation was found between the RCT and the levels of colloidal calcium in diluted milk from different species. RCT increased sharply at a colloidal calcium level of <50 mg/100 ml. The RCT of BM was affected differently than the RCT of CM due to the type of milk-clotting enzyme. For example, using *Endothia parasitica* protease, both BM and CM showed similar RCT, while BM coagulated faster with the use of calf rennet. The RCT of BM is more sensitive to the addition of NaCl, H_2O_2, and Na_2CO_3 than that of CM. Storing of BM at 7 °C for up to 24 h had a slight effect on its RCT while the RCT of CM increased under the same conditions. The increase in the RCT of BM by heat treatments is less pronounced than that

of CM. These differences can be attributed to the differences in the colloidal phase of the two milks and explain the differences in the behavior of buffalo's and cow's milks during cheesemaking.

Comparison of Quality of Dairy Products from BM and CM

Edible Casein and Caseinates

Due to the higher content of casein in the form of larger micelles and presence of all of the casein in the micellar state, it is easier to manufacture edible casein and caseinates from BM. The yield of these products is also higher from BM due to lower losses in the whey because of bigger size and low hydration of the micelles.

Coffee and Tea Whiteners

Due to higher protein, fat, and calcium content in BM, the yield of whitener from BM is higher. The product is superior due to higher whitening capacity when made from BM. The bigger size and greater opacity of casein micelles from BM may be responsible for better quality product. The higher emulsifying capacity of BM fat may also be responsible for the better dispersion of whitener used in coffee or tea.

Yogurt

BM is better suited for the manufacture of yogurt, as its manufacture is easier and there is no need for prior concentration or addition of dried milk due to higher TS in fat.

Condensed Milks (Sweetened and Unsweetened)

BM behaves quite differently from CM during production and storage of condensed and evaporated milk. This is due to (1) differences in micelle composition of milk proteins, that is, casein; (2) higher levels of milk proteins (both casein and serum proteins), milk fat and lactose; and (3) higher calcium content and lower heat stability of milk.

Ice Cream

BM is considered as a better source of fat for ice cream due to higher emulsifying capacity. Further, BM ingredients produce better body and texture in ice cream.

Infant and Health Foods

Better absorption of fat due to higher emulsifying capacity; better absorption of calcium due to higher concentration of calcium, magnesium, lactoferrin, free amino acids, esterified cholesterol, and taurine; and lower concentration of sodium, potassium, chloride, urea, and free and total cholesterol in BM compared to CM are beneficial for human nutrition. These attributes make BM superior than CM as an ingredient for infant and health foods, provided its CT is reduced to improve the digestibility.

Problems in the Manufacture of Some of the Cheeses from BM

Cheddar cheese is largely manufactured mainly from CM in major cheese-producing countries. However, in India, the major share of milk production is from buffaloes. The adaptation of well-known technology for the production of various products from BM posed many problems primarily because of its qualitative and quantitative differences. CM, in general, is considered to be the most suitable raw material for cheese. The main problems encountered in the manufacture of hard-type cheese from BM have been faster renneting time, lower retention of moisture, slower lipolysis and proteolysis, and poor flavor, body, and texture development. The BM cheese is criticized for its higher fat content and hard, rubbery, and dry body and texture. The defects in BM cheddar cheese are attributed mainly to the physicochemical and compositional characteristics of milk. The high BC of BM due to its higher calcium, phosphate, and casein content is the cause of slower development of acidity. Faster renneting time may be attributed to its higher colloidal content (about 160 mg/100 ml) compared to only 8 mg/100 ml in CM. The lower retention of moisture in the curd may be the result of low solvation (hydration) of its calcium compared to CM casein. Hard, rubbery, and dry body may be due to the high CT, which, in turn, is the result of higher content of casein with bigger size of the micelle, high content of calcium and magnesium more so in the colloidal state, large proportion of solid fat with bigger size of the globules, and low voluminosity and solvation of its casein micelles compared to those of CM. The slower rate of proteolysis and lipolysis is the cause of higher CT of BM. Mozzarella cheese manufactured from BM is the most highly valued pasta filata cheese in Italy and the United States. The BM cheeses in general are becoming increasingly popular throughout the world, and its demand is rising at a rate that is among the highest for any food product. The high demand in specialty dairy products from BM is due to its high sensory quality along with the high adaptability of the animals. BM is reputed for its richness and creaminess. Certain varieties of cheese like Mozzarella and white pickled Domiati cheese are superior in quality when made from BM. The manufacture of Domiati cheese from BM is easier, as the handling of curd is easier due to more firm curd and the yield is higher.

Technology for Manufacture Cheese from BM

In view of the earlier-mentioned problems associated with the manufacture of BM cheese, research was initiated in the area of cheese in early 1960s at the National Dairy Research Institute, Karnal, India. Since a major share of milk production is from buffaloes and BM is known to be unsuitable for cheese production, attention was given to this raw material. Cheddar cheese, the most common variety made in India, does not develop proper flavor, body, and texture when it is made from BM. The main problem is the faster rate of syneresis, which results in lower moisture content in finished cheese. This, in turn, affects adversely the three most important biochemical reactions, that is, glycolysis, proteolysis, and lipolysis,

which constitute the major activity in cheese, flavor development. In order to overcome this problem, attempt should be made to develop a manufacturing technique, which would ensure greater retention of moisture and accelerated rate of glycolysis, proteolysis, and lipolysis. A presalting method was developed at this institute, which envisages the addition of 1% salt to the cheese milk that resulted in the best product. However, the addition of salt to the milk makes the whey unsuitable for utilization in food products.

See also: Condensed Milk; Cheese: Processing and Sensory Properties; Cheese: Types of Cheeses – Hard; Cheese: Types of Cheeses – Soft; Dairy Products: Dietary and Medical Importance; Food Allergies; Ice Cream: Uses and Method of Manufacture; Milk Powder; Protein: Food Sources; Yogurt: Dietary Importance.

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Butter: Manufacture

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Introduction

Butter is a dairy product made by churning fresh or fermented cream or milk. Conversion of milk fat into butter is a very old way of preserving milk fat. Butter accounts for a major portion of the nutritive value of milk. Butter is generally used as a spread and a condiment, as well as in cooking applications, such as baking, sauce making, and pan frying. Butter consists of butterfat, water, and milk proteins. Most frequently made from cow milk, butter can also be manufactured from the milk of other mammals, including sheep, goats, buffalo, camels, and yaks. The most dominant source for production of butter today is bovine milk. Throughout the centuries, butter was manufactured at farms in small quantities with considerable variation in quality. In the nineteenth century, industrial production of the butter started through centralization and mechanization, which resulted in substantial improvement in the quality of butter. The largest butter-producing countries are the United States, Germany, France, New Zealand, and Russia. According to the Codex Alimentarius Commission under the joint FAO/WHO Food Standards Programme, butter is a fatty product derived exclusively from milk. A 100 g portion of butter must contain a minimum of 80 g fat and a maximum of 16 g water and nonfat milk solids.

According to the USDA, one tablespoon of butter (14 g/0.5 oz) produces 420 kJ (100 kcal), all from fat, 11 g (0.4 oz) of which 7 g (0.25 oz) are saturated fats and 30 mg (0.46 g) are cholesterol. In other words, butter consists mostly of saturated fat and is a significant source of dietary cholesterol. For these reasons, butter has been generally considered to be a contributor to health problems, especially heart disease. For many years, vegetable margarine was recommended as a substitute, because it is higher in unsaturated fat and contains little or no cholesterol. In recent decades, though, it has become accepted that the trans fatty acids contained in partially hydrogenated oils used in typical margarines significantly raise undesirable low-density-lipoprotein (LDL) cholesterol levels as well. Trans-fat free margarines have since been developed. Proponents of the consumption of organic butter, such as the nutritionist Mary Enig, state that, because butter is nutritious and 'is rich in short and medium chain fatty acids,' this can have a positive effect on health and prevent disease. Butter contains only traces of lactose, so moderate consumption of butter is not a problem for lactose intolerant people. People with milk allergies need to avoid butter, which contains enough of the allergy-causing proteins to cause reactions. Butter can play a useful role in dieting by providing satiety. A small amount added to low fat foods such as vegetables may stave off feelings of hunger.

Historical Background

The art of butter making has a long history. In India, ghee has been a symbol of purity and an offering to the Gods – especially Agni, the Hindu God of fire – for more than 3000 years. References to ghee's sacred nature appear numerous times in the Rig Veda, circa 1500–1200 BCE. The tale of the Lord Krishna during his childhood stealing butter remains a popular children's story in India today. Since India's prehistory, ghee made from butter has been both a staple food and used for ceremonial purposes such as fueling holy lamps and during funeral prayer.

Manufacture of creamery butter has been confined to the 'colder' regions of the world, where gravity creaming has been successful. References to butter are found in the Old Testament. In the past, butter was an article of commerce and a sign of wealth. Up to the middle of the nineteenth century, factory butter making was unknown. Most of the butter was made on the farm from cream obtained by gravity creaming. The cream was decanted into a wooden churn and subjected to shear and mild aeration with the help of a stirrer or by rotating the vessel. Once the fat formed clumps, butter milk was removed and the fatty mass gathered and excess moisture removed. This process hardly met modern hygiene standards. In most cases, cream gets soured before converted into butter. The wooden churns were extremely difficult to keep clean. Lack of refrigeration would lead to swift growth and proliferation of putrefactive organisms. Addition of common salt to the butter grains prior to working was the only preservation methods available in those days. The presence of significant quantities of lactic acid from the sour cream would have contributed to the subsequent preservation of the butter. Butter has also been stored in containers immersed in peat swamps, taking advantage of the lower temperature and virtually anaerobic conditions.

An ancient method of butter making, still used today in parts of Africa and the Near East, involves a goat skin half filled with milk, and inflated with air before being sealed. The skin is then hung with ropes on a tripod of sticks, and rocked until the movement leads to the formation of butter. The late nineteenth century witnessed the inventions of mechanical cream separators and mechanical refrigeration. The advantages of heat treatment to improve the keeping quality of dairy products were soon realized. This led to the establishment of creameries, where milk was separated, and the availability of larger quantities of cream led to the mechanization of butter making. Initially, the churns were of wooden construction, essentially a scale-up of the barrels used for hand production, but then were slowly replaced by aluminum and then stainless steel until the technology was overtaken in the second half of the twentieth century by the development of continuous butter making processes. By the beginning of the twenty-first century, batch churning had been replaced in dairies by continuous churning processes.

Classification of Butter

Many types of butter are found in the market. These differ with the type of cream from which they are made and with variations in

the manufacturing process. Unless specifically mentioned, the different kinds of butter may or may not have been salted. A brief description of several kinds of butter is as follows:

- *Pasteurized cream butter*: Usually made from pasteurized sweet cream. Such butter has a milder flavor than that made from similar cream not pasteurized.
- *Ripened cream butter*: Made from cream in which a pleasant delicate aroma has been developed before churning by ripening (i.e., inoculating the cream with a lactic culture and holding it at a desired temperature). Properly made, ripened cream butter has a delicate flavor.
- *Unripened cream butter*: Made from unripened cream. The flavor of such butter is usually mild.
- *Salted butter*: Butter to which salt has been added.
- *Unsalted butter*: Contains no added salt.
- *Sweet cream butter*: In this case, the acidity of the churned cream does not exceed 0.20%.
- *Sour cream butter*: Made from cream which has more than 0.20% acidity.
- *Fresh butter*: Such butter has not undergone cold storage. (Usually, fresh butter is not kept for more than 3 weeks.)
- *Cold storage butter*: This butter has been stored at a temperature of about −18 °C (0 °F) for some time. (Generally cold storage butter is from 1 to 6 months old when offered for retail trade.)
- *Dairy butter* (USA): This is butter made on a farm. It is usually made from unpasteurized sour cream, which has not been standardized for acidity. This butter generally has a sour flavor due to the high acid content of the cream.
- *Creamery butter*: This is butter made in a creamery or dairy factory. It is more uniform in quality than 'dairy butter.'

Industrial Butter Making

There are two completely different methods for manufacturing butter. These are the churning method and the emulsification method. In the churning method, crystallization of the fat takes place in cream, followed by a phase inversion in which the oil-in-water emulsion of the cream is turned into a water-in-oil emulsion by strong mechanical treatment. The fat content is then concentrated by draining off the buttermilk. The butter is finally plasticized by mechanical working.

In the emulsification method, the aforesaid first three subprocesses are carried out in reverse order. First, the fat emulsion is concentrated to a fat content corresponding to the composition of the final product, then a phase inversion is carried out followed by crystallization, and finally a coherent fat mass is formed and plasticized (Figure 1).

Churning Method of Butter Manufacture

The basic principle of the churning method is that air is mixed into cream where it forms foam. Simultaneously, some of the fat globule membranes are disrupted leading to liquid fat being squeezed out of the damaged fat globules and spread at the interface of the foam making fat globules stick to the lamella of the foam. By further agitation, the foam collapses, and the fat globules are forced so closely together that they coalesce into small lumps. These lumps are further pressed into small butter granules.

A batch-type butter churn may vary in capacity from a few liters to a maximum of about 45 000 l. These churns were originally made of wood but later were replaced with stainless steel. After cleaning and disinfecting the churn, it must be specially prepared to prevent the butter from sticking to the surface. With wooden churns, this is achieved by scalding with boiling water and immediately cooling with chilled water. This treatment leaves a film of water on the surface of the wood and prevents the butter from adhering to it. All wooden equipment must be kept wet until used.

Batch butter churns may be barrel or cone shaped with fixed or rotating internal 'workers.' As the churn is rotated, the combined actions of rotating and beating cause the cream to break, forming the butter grains (fatty phase) and buttermilk (aqueous phase). During the first few turns, gases (e.g., carbon dioxide from heterofermentative fermentation) may be liberated from the cream. In order to maintain an even pressure within the churn, it is necessary to release these gases. This is done by depressing a small valve in the lid of the churn. Each churn has an indicator glass to see what is happening inside the churn. When hand churning, the cream feels heavier when it begins to thicken. This takes about 15–20 min from the beginning of churning. The cream breaks and forms small grains of butter which are clearly seen on the indicator glass. The actual size of the butter grains varies according to the type and size of the churn. For hand churning, the grains should be kept small, approximately 3 mm in diameter – traditionally stated as the size of wheat grains.

Chilled water at approximately 5 °C is used to harden and control the size of these grains, as well as to remove the traces of buttermilk. Washing reduces the yield and is not necessary if the cream is of good quality and all the necessary hygienic precautions have been observed. Traditionally, well washed butter will have a longer shelf life than unwashed and overworked butter. Salt may be added dry or in the form of brine as a final wash. The addition of brine (10% solution) to butter grains has been used to reduce the need for chilled water. This can be important during warm weather when there is a lack of chilled water. It prevents streakiness due to uneven mixing of the salt. The butter grains are 'worked' to expel excess moisture, create an even, fine distribution of water droplets, and produce a close textured, evenly colored product. During the period of working, drainage, and addition of dry salt, samples are tested to determine the salt and moisture contents. The operator determines the 'end-point' of working when the moisture content is between 15.5% and 16% and by visual assessment of the butter. At this stage, the butter is removed from the churn in readiness for packing. The moisture content of butter must not exceed the legal maximum limit of 16%. Manufacturers attempt to be as near that limit as possible to ensure the maximum yield.

Pretreatment of the Cream Prior to Churning

It is necessary to concentrate the emulsion in milk to a fat content of about 35–42 g/100 g or even higher in a centrifugal separator. The cream is heated to 85–110 °C for 10–30 s in a plate heat exchanger in order to kill any pathogens and to reduce the load

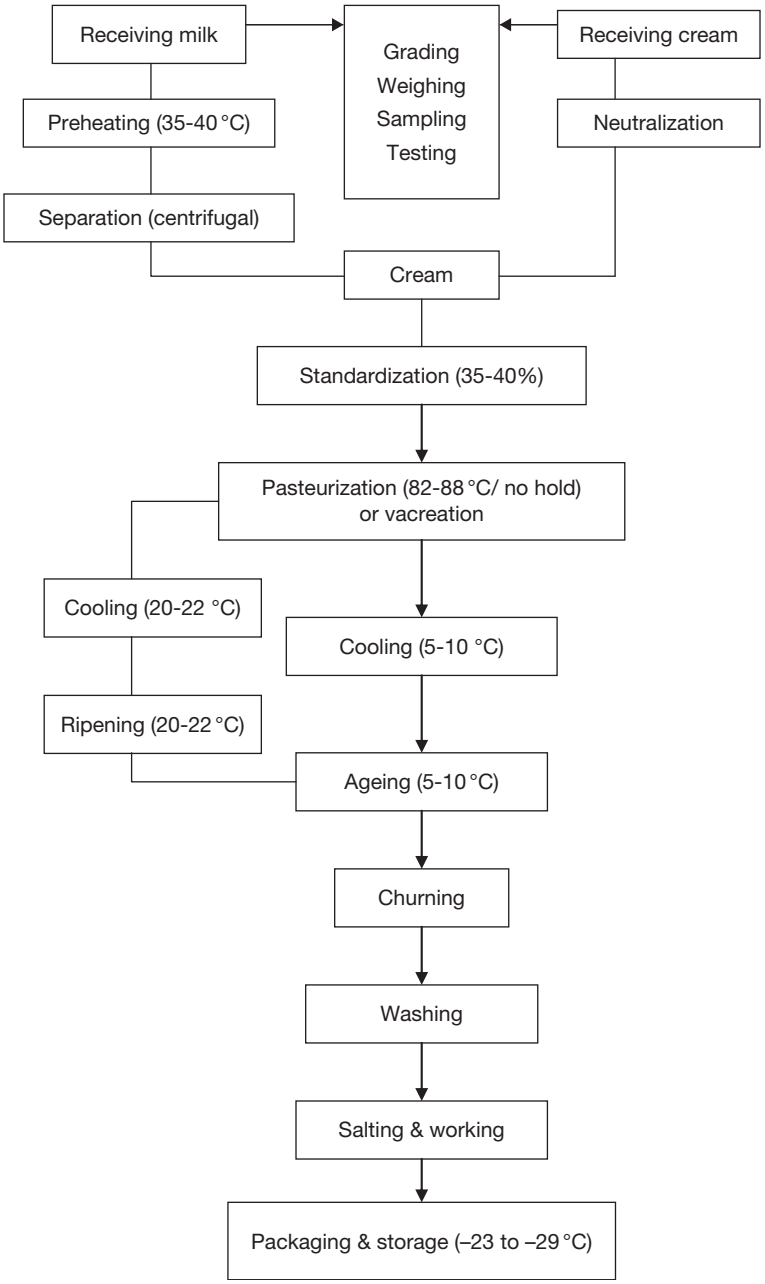

Figure 1 Process flow diagram for butter.

of spoilage-type microorganisms. It is possible to combine high-temperature-short-time (HTST) treatment with vacuum deodorization, which is termed as vacreation. A vacuum chamber could be inserted after the heating unit in the machine. Such treatment might have a fine effect on the flavor of the butter, for instance, if flavors originating from feeding of the cows occur. The system is mainly used in countries where dairy cows fed on pasture with strong tasting weeds, which cause off-flavors in the milk. The cream is cooled immediately after heat treatment as churning is impossible unless the milk fat is solidified. In one cooling procedure, the cream is cooled directly to a low temperature (4–5 °C), kept overnight, and then churned. This treatment results in formation of mixed fat crystals, also called corn crystals, in which a considerable part of the low melting triacylglycerols, due to the fast cooling, is trapped in a crystal lattice formed by high-melting triacylglycerols. Butter churned from such cream will have a lower fat content and, therefore, a very firm consistency and rather poor spreadability. When milk is converted to butter, four basic main changes – concentration, crystallization, phase inversion, and plasticizing – are necessary.

Manufacturing Process

The cream treatment has a strong effect on both the butter-making performance and the quality of the butter. It is

performed in four main stages, namely pasteurization, vacreation, cooling, and microbial and/or physical cream ripening.

Pasteurization

Cream is separated from milk by centrifugation. Normally, the raw milk is preheated to above 40 °C to ensure that all of the fat is in a liquid state so that the milk-fat globules are less susceptible to shear damage. The optimum temperature for separation is 63 °C, higher temperatures causing denaturation of whey proteins which, though not critical for butter making, may adversely affect the properties of the skimmed milk. For batch churning the cream may be separated at 35 or up to 40 g fat 100 g^{-1}, while for continuous butter makers the fat content is normally 40–48 g fat 100 g^{-1}, depending on the particular machine. In order to kill pathogens and technologically harmful microorganisms, as well as to inactivate lipolytic and proteolytic enzymes, the cream is heated to 85–110 °C for 10–30 s.

A few very small manufacturers may batch pasteurize the cream at 63–66 °C for a minimum of 30 min. The minimum treatment is at 72 °C for 15 s, though most use a slightly more severe treatment, such as at 74–76 °C for 15 s as a common practice when using the HTST treatment. Specially designed plate heat exchangers may be used to minimize physical damage to the fat globules. Severe heat treatments should be avoided to minimize the generation of a cooked flavor and to minimize the uptake of copper onto the fat globule membrane from the serum.

Vacreation

This process is applied when there are problems with taints in the milk, whether from pasture weeds consumed by the cattle or as a result of storage problems, and further treatment is needed. Undesirable flavors arising from microbial action, from high-temperature pasteurization, from the feed of the cows, or from unpleasant aromas in the milking shed, are removed. A vacreator is used for multistage vacuum treatment of cream. This equipment has now been replaced by spinning cone evaporators in which the volatile compounds are removed from a thin film under vacuum. Where less severe flavor problems may occur, the cream is heated to ~90 °C, then flash cooled by spraying into a chamber where a pressure of ~20 kPa is maintained. The loss of water on cooling is accompanied by reduction in any other volatile component. However, this treatment also has drawbacks regarding butter yield and basal moisture content.

Cooling

Following the pasteurization/vacreation stage, the cream is shock-cooled to 6–8 °C. However, if cultured cream butter from a very 'soft' cream (pasture feeding) is to be produced, cooling occurs at about 20 °C. Then, the cream undergoes physical and/or microbial ripening.

Butter from Sweet Cream

Sweet cream for butter making is potentially the simplest to prepare. The hot cream should be cooled as quickly as possible, usually within the plate heat exchanger used for pasteurization.

Milk fat contains a very wide range of fatty acids, and hence triglycerides, crystallizing as a mixture of predominantly α- and β-crystals. The continuing crystallization releases more heat, mainly within 2 h from cooling, and causes the cream to warm by about 2 °C. The extent of the crystallization will depend on the temperature and on the composition of the fat. Ideally, the cream should be cooled from 4 to 5 °C immediately after pasteurization, so that even with the release of the remaining latent heat the temperature should remain below 7 °C. When this is not possible, additional cooling should be provided, either by cooling pads on the tank wall or by circulation through an external heat exchanger. The cooled cream should be held for at least 4 h before butter making to permit adequate crystallization – at least 50% of the milk fat should be crystalline. Overnight aging is the preferred approach when butter making is carried out on a single shift.

Ripened Cream Butter

The mechanization of butter making, particularly the introduction of pasteurization and adequate refrigeration, prevented the development of acidity and associated fermented flavors in the cream. In many markets, these flavors are highly desired and steps were taken to reintroduce an appropriate microflora and carry out a controlled fermentation. Pasteurization conditions were usually more severe than for sweet cream; for example, 90–95 °C for 15 s or 105–110 °C with no hold. The increased protein denaturation reduces the redox potential, aiding growth of the culture. Cooling is limited to about 20 °C, with a typical fermentation time of 12–18 h depending on the activity of the starter.

The culture normally consists of a mixture of mesophilic lactic acid bacterial strains of *Lactococcus lactis* subsp. *lactis* and *Lac. lactis* subsp. *cremoris* providing lactic acid while citrate positive strains of *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* produce flavor compounds, predominantly diacetyl and its precursor, acetoin. The inclusion of *Leuconostoc mesenteroides* subsp. *cremoris* or *Leu. mesenteroides* subsp. *citrovorum* increases diacetyl production while avoiding a yogurt-like flavor by reducing acetaldehyde to ethanol. The fermentation must progress to a pH below 5.3 for the generation of diacetyl.

The physical ripening of the cream was originally developed in Scandinavia (Alnarp method) and it helps to optimize the consistency of butter. This process lasts several hours and includes a sequence of hot/cold levels (e.g., 'cold–warm–cold' or 'warm–cold–cold'). Without this procedure, the butter would often be too firm or sometimes too soft and tend to oil off, according to the largely varying milk fat composition. The basal moisture content and the fat losses in the buttermilk can also be influenced by physical cream ripening.

Continuous Butter Making

Continuous butter making machines began to be widely used in the 1960s. These were so successful that, within a decade, most of the batch churns used in creamery manufacture had been superseded. The important advantages of these machines are better hygiene, and control of quality and process efficiency was constantly improved. In the past 55 years, the Fritz method

of continuous butter making has become the leading technology in Western Europe and many other countries. This method is based on similar steps to that of the traditional batch method, but converts relatively small quantities (at any point of time) at a much higher rate, creating the potential for greater production capacity and process control. This method gives an hourly output of 5 tonnes and a production of more than 10 tonnes per hour is even possible.

Cream Feed to the Continuous Butter Making Machine

So as to operate optimally with a minimum of corrective action, the continuous butter making machine must be supplied with a consistent feed. It requires cream of consistent composition (fat, pH), physical characteristics (viscosity, degree of fat crystallization), temperature, and feed rate throughout the working schedule. These requirements can be met by bulking together the cream for a day's production schedule in a single silo. The cream can then be supplied within ±0.5% to the machine by a programmable-logic-control (PLC)-controlled, variable speed pump. The pipelines should be designed and constructed to ensure that the pump is neither starved nor receives excessive shear exerted on the cream during transfer. Flow rates of 0.2–0.4 m s^{-1} are satisfactory for sweet cream with slightly lower rates needed for cultured creams, depending on viscosity.

The most appropriate aging temperature is often lower than the optimum temperature for destabilization of the cream in the machine. It is expensive to introduce that energy by mechanical action. This drawback can be corrected by passing the cream through a preheater using warm water as the heating medium. Small temperature differentials of 1–2 °C should be employed to minimize the risk of overheating. The cream outlet temperature should be controlled within ±0.25 °C of the target temperature. This temperature will vary with the fatty acid profile in the cream. Higher temperatures are needed in the winter to compensate for the greater proportion of saturated fat, so that the temperature will approach that needed for 50% of the fat in the globules to be in the liquid state.

There is also a tendency for the cream feed temperature to be lowered with increasing fat content. As a general rule, all handling of cream prior to butter making should avoid damage to the milk-fat globules, because damaged fat globules will tend to agglomerate and may block the pipelines. However, controlled destabilization has been used in the past as a pretreatment immediately before the machine to increase its production capacity.

Fritz Process of Continuous Butter Making

The GEA Ahlborn (Germany), the Continab (Simon Freres, France), the Pasilac (Denmark), and the Westfalia (Germany) are the major manufacturers of continuous butter making machines. Although the design features vary with some minor differences, the basic principles remain the same. The butter maker consists of: (1) the beating section, (2) churning section, and (3) the working section. The basic sequence of operations in the Fritz process starts with the feeding of the cream into the churning cylinder. Due to the rapidly rotating beaters (about 1000 rpm) in the cylinder, this process only lasts a few seconds. Buttermilk and granules drop into the subsequent separating section. This consists of a rotating cylinder (35–42 rpm) in which after-churning takes place first, that is, the granules are built up in size. Most of the buttermilk is drained off at that stage. Chilled water circulates in the jackets of the churning sections to minimize the temperature rise in the butter. The butter granules then drop through a slide into the first working section from where they are moved with parallel contra rotating augers and start to form a continuous mass, which is forced through a series of plates with orifices.

On the downstream side of the plates, cruciform beaters contribute to the working and flow of the butter mass. There are flutes in the auger sections to assist draining of the buttermilk. The degree of working is controlled by the speed drive and the pitch angle of the beater. At the end of the first working section, salt is added, if required, as a slurry of 40–60% salt through 1–3 injection points close to the final orifice plate. If indirectly cultured butter is to be made, lactic starter cultures, acid, and flavor concentrates are injected at the same points. In this case, the remaining moisture content at these points (the basal moisture content) must not be higher than 13.5%; otherwise, the common maximum permitted water level of 16% could be exceeded when adding the culture concentrate. For this reason, the first kneader operating at low shear rates presses as much of the residual buttermilk out as possible. Before entering the second working section the butter mass is 'evacuated,' that is, exposed to a reduced pressure of 25–60 kPa, whereby its air content falls from approximately 4–7% (v/v) to approximately 0.1–0.5%. This helps avoid laminations in bulk butter and confers a smooth though firmer texture to it, which is said to be appreciated by the consumers.

Processing Variables

Butter yield and its properties, such as consistency, moisture content, and oiling-off, are affected by numerous interrelated process variables. These include the following.

Machine Variables

Machine variables include the beater speed, first kneader speed, second kneader speed, and reduced pressure at vacreation, as summarized here.

There is an optimum beater speed at which the moisture content is minimum. Higher speeds result in 'overchurning' and speeds below the optimum result in the butter moisture content causing 'underchurning.' Both overchurning and underchurning soften the butter mass, which in turn affects the working efficiency.

As the speed of the kneaders is decreased, the time for draining the buttermilk from the butter mass is extended and the butter moisture falls. In order to have the option of canceling this effect for the second kneader, a drain cock at its bottom side can be closed. Because it is generally easier to achieve low moisture content in a hard than in a soft fat, the temperature of the first kneader is reduced by injecting cooled water or buttermilk. The kneader configuration influences the amount of working given to the butter, which in turn affects the sizes of water droplets. A significant fraction of too large moisture

droplets (diameter >10 μm) allows microbial growth and affects the storage quality of the butter.

Cream Variables

Cream variables include fat content, fat composition, the cooling regime, and the salt content in cultured cream.

High cream fat contents are desirable because of higher butter yields (about 0.2% fat losses in the buttermilk vs. 0.05% in the skim milk) and the lower incidence of off-flavors. On the other hand, achievement of correct moisture content (which also depends on the process variables) relates to the fat in the cream, often at about 40–42%. At lower fat levels, the energy demand may exceed the motor capacity, and the butter tends to be underchurned. If the fat content is higher, it is difficult to reduce power to the level required, and the butter tends to be overchurned.

Proper destabilization and agglomeration of the fat occur at an optimum solid-to-liquid fat ratio. At too high or too low values of this ratio, higher beater speeds must be used. More moisture is beaten into the butter, and more fat is lost in the buttermilk. Hence, there is also an optimum cream temperature in the range of 8–14 °C yielding both a minimum basal moisture content and minimum fat losses. However, oxidative (e.g., fishy) flavors arising at higher temperatures must also be considered.

The optimum cream temperature, in its turn, is influenced by the way it has been attained, that is, by the previous temperature treatment. Because the solid to liquid fat ratio at a given temperature depends on fat composition, numerous machine and cream parameters have to be adjusted according to the fat concerned.

Vacreation tends to increase the range of globule sizes. Small fat globules are harder to disrupt than large ones, hence the fat losses in the buttermilk are higher with smaller ones. Very large fat globules, on the other hand, are easily damaged under vacreation.

The presence of salt in cultured cream butter accelerates the autoxidation, the inverse effect occurring with salted sweet cream butter. Overall, butter making depends on numerous interrelated factors which have to be carefully adjusted against each other to keep the process performance and quality parameters within their optimum ranges.

Packaging of Butter

Butter packing may be accomplished in bulk or retail packs. Because butter is relatively stable and the profitability is lower than for many other dairy products, it has been used as a balancing wheel for surplus milk fat. As such, the production has commonly been out of balance with market needs; this is particularly so in those countries where the dairy industry is geared for export rather than for supply to the domestic market. The butter is placed in bulk packs – in older and smaller butter plants, the butter, possibly batch-produced, is packed into 25-kg cartons. Originally, a loose parchment lining was used, but this has been replaced by blue-pigmented polyethylene bags, as this gives better protection.

Nowadays, freshly churned butter is collected first in a butter silo, with an auger to help feed the butter to the pump, providing a break in the product flow so that any interruption in the packing does not cause interruption. The bulk butter packing uses two-stage filling to ensure accuracy and minimum giveaway. Smaller bulk packs can be produced to comply with manual handling restrictions. The shelf-life of the bulk butter may be extended considerably by storing it in frozen conditions at below −18 °C.

Most retail butter is packed in either parchment or a parchment-aluminum foil laminate. Parchment is cheaper but is permeable to moisture vapor and ultraviolet rays so that the surface of the butter can suffer from both surface drying and oxidative rancidity, the latter being reduced by the application of pigments such as titanium dioxide to the outer surface of the parchment. Foil laminate protects the butter from ultraviolet rays and only permits moisture vapor and gas interchange at the seams, thus aiding in a longer shelf-life.

Some specialist butters may use transparent films. Though the film gives good protection against moisture loss, the risk of oxidative rancidity on the surface is higher than for parchment. Preformed plastic containers, often polypropylene, are more expensive and tend to be used for soft butters and hybrid products that would be too easily damaged in film wraps. Butter portions, typically less than 20 g in weight, for catering and institutional use, are filled either into foil laminates, where the consistency on filling can be critical, or into plastic trays with a foil or aluminum film cover, in a form-filled seal operation.

See also: Buffalo Milk; Butter: Properties and Analysis; Cream: Types of Cream; Dahi; Dairy Products: Dietary and Medical Importance; Fatty Acids: Essential Fatty Acids; Fatty Acids: Metabolism; Fatty Acids: *Trans* Fatty Acids; Fermented Foods: Fermented Milks.

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Butter: Properties and Analysis

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Butter Manufacturing and Butter Regulation

The use of milk fat goes back to several millennia ago, since animals started to be domesticated. However, the first written reference is around 2000 BC. Butter has been used not only as food but also for cosmetic purposes, for medical purposes, and for religious ceremonies. Butter manufacturing was first based on the separation of the cream by gravity settling, followed by a manual churning in wooden churns. The obtained butter grains were separated from the buttermilk and then manually kneaded. The continuous churning process used nowadays for the production of butter replaced most of the batch process former used. Different manufacturing technologies exist for continuous butter making; however, the most typical is the Fritz process. The Fritz process involves concentration of the cream via centrifugation, to 40% fat approximately, and then a thermal treatment of the cream is performed. The purpose of the thermal treatment is to crystallize part of the milk triglycerides, which will initiate partial coalescence, hence butter grain aggregation during the churning step. During churning, the butter grains are separated from the water phase, the buttermilk, which is subsequently removed. The butter grains are then processed in the working section; here, the excess of water is removed, and the water droplets are homogeneously distributed in the continuous fat phase. Therefore, butter is referred as a water-in-oil emulsion. The final consolidation of butter occurs during storage, in the first 2 weeks from manufacturing. Margarine manufacturing differs from butter manufacturing on the mixing and emulsification steps. During margarine production, milk fat, vegetable oils, water phase, and emulsifier are mixed and then emulsified. Cooling and an intensive mechanical working are followed.

The increase in consumers' expectations and the innovation in production technologies led to an extensive variety of spreadable-fat products available on the market. In general, spreadable-fat products are differentiated by their origin, animal or vegetable fat, and by the amount of fat. Standards and guidelines for butter are internationally designated by the Codex Committee on Milk and Milk Products, which belongs to the Codex Alimentarius Commission. At European Union level, the Council of the European Union established a classification, with annexed nomenclature, for the producers. This is laid under the Council Regulation (EC) No 445/2007. Generally, under the nomenclature of spreadable fats fall all the products with fat content between 10% and 90% (w/w), which are solid at 20 °C. However, the most known spreadable-fat products are butter, margarine, and butter blends. Butter, as quoted in the Council Regulation (EC) No 2991/94, is obtained by exclusively milk fat in which content must be between 80% and 90% (w/w), with a water content not higher than 16% (w/w) and dry nonfat milk material below 2% (w/w). Under the same condition for the amount of fat, but when vegetable and/or animal fats are used instead of milk fat for manufacturing, the obtained product is referred to as margarine. Dairy blends are referred to products obtained by combining milk fat with vegetable fats in which the amount of fat is between 80% and 90% (w/w). Spreadable-fat products that are labeled as 'reduced fat' have a fat content between 41% and 62% (w/w). For butter and blends with a fat content lower than 41%, the nomenclature of 'low fat' or 'light' is used. 'Three-quarter fat' and 'half fat' can also be used to refer to products with a fat content between 62–60% and 41–39%, respectively. For 'reduced fat' and 'low fat' or 'light' butter and blends, the member states can choose to use the term in their own language to reflect the same product. In the case of spreadable-fat products imported from non-Community countries, the same requirements as those produced in the European Union must been fulfilled. Butter production is also regulated by national legislation, brand regulation, and company specification.

In addition to conventional butter, other milk fat products are available in the market, for example, whipped butter, flavored butter, confectionary butter, anhydrous milk fat, butter oil, butter powder, and ghee. These are mainly used as ingredients in bakery and confectionary industries.

World Consumption and Distribution

In the last decade, production of butter has been increasing from 4.660 million tons to 5.202 million tons. By 2023, a 24.05% increase in butter production is expected. Europe leads in world butter production, with 66% calculated as share average in 2000–2012, followed by the Americas, Oceania, Asia, and Africa, with 16.7%, 8.3%, 7.1%, and 2%, respectively. The top five world producers, calculated as average in 2000–2012, are the United States, Germany, France, New Zealand, and Russian Federation, with 0.67 million tons, 0.44 million tons, 0.43 million tons, 0.41 million tons, and 0.26 million tons, respectively. New Zealand is the first world exporter of butter with 0.35 million tons, followed by the European Union countries. France, Germany, and Luxembourg account for the highest kilogram per capita butter consumption in the world, which is equal to 7.4, 6.2, and 6.10 kg per capita per year, respectively. Conversely, the lowest per capita consumption, indicated as below 1 kg per capita per year, is reported in Mexico, Brazil, Colombia, Greece, Spain, Iran, Israel, Turkey, Egypt, South Africa, China, Japan, Mongolia, and South Korea. This is mainly due to the higher temperature reported in most of the aforementioned countries and/or to an alternative source of fat more accessible than bovine milk fat, for example, olive oil or buffalo milk fat. The reported data illustrate the current scenario for butter manufactured from bovine milk; however, buffalo, goat, sheep, and camel milk

are also used for butter manufacturing. Buffalo butter manufacturing has been increasing in the last decade, from 0.41 to 0.83 million tons. Asia and Africa, which are characterized by the lowest consumption and production share for bovine butter, head up in the production of buffalo butter with 81.6% and 18.4%, respectively, calculated as average in 2000–2012. India is the first producer of buffalo butter, with 0.6 million tons, followed by Egypt, China, and Iraq, with 83, 8.77, and 1.45 thousand tons, respectively. At present, no data are reported on the production and trade of butter from other sources.

Milk Fat Composition and Structure

The origin of the textural and functional properties of butter must be sought on milk fat composition and structure. Milk fat composition and the stereolocation of fatty acids (FAs) in the triacylglycerol (TAG) molecules influence the crystallization behavior, which in turn affect the microstructure, hence the macrostructure and textural properties of butter. Among natural fats, milk fat is one of the most complexes, as both structure and chemical compositions. Bovine milk fats are shaped as spherical globules, the size of which is between 2 and 8 μm, and their concentration is $\sim 10^{10}$ globules ml^{-1}. A complex milk fat globule membrane, composed of lipids and proteins, the thickness of which lies in the range of 10–20 nm, surrounds the TAG core of milk fat. The primary role of the membrane is to physically stabilize the milk fat, and to protect them from enzymatic oxidation and lipolysis. The core of the milk fat globule accounts for 98.3% (w/w) TAGs of the total composition; the remaining 1.7% includes phospholipids, diacylglycerols, sterols, free FAs, and monoacylglycerols stated in decreasing order. The amount and composition of milk FAs vary considerably among breeds of cow, feeding regimes, seasons, stages of lactation, and genetic variations. Approximately 400 FAs have been identified in milk fat; among these, only 14 are present in amounts higher than 1%. The presence of short-chain FAs makes bovine milk fat a distinct fat, both in flavor and in functional properties, when compared to other natural fats used for the manufacturing of spreadable-fat products, as rapeseed, sunflower, or olive oils. Saturated FAs, with a carbon chain length varying from 4 to 18, account for 70–75% of total milk fat. Among those, palmitic acid (C16:0), stearic acid (C18:0), and myristic acid (C14:0) are predominant with 22–35% (w/w), 9–14% (w/w), and 8–14% (w/w) average range, respectively. Conversely, among the unsaturated FAs, oleic acid (C18:1 *cis*9) and palmitoleic acid (C16:1 *cis*9) account for 20–30% (w/w) and 1–3% (w/w) average range, respectively.

Textural Properties of Butter: Macro- and Microstructure

The optimal consistency of butter is smooth, slightly firm, and plastic. In addition, butter should have good resistance to cutting, being fairly spreadable, and it should be easy to melt in the mouth without leaving a greasy sensation. To study and therefore to be able to modify the mechanical and functional properties of butter, hence its macrostructure, a clear understanding of its microstructure is needed.

Microstructure

The microstructure of butter is the utmost attribute for its textural and functional properties. The microstructure of butter consists of a tridimensional fat-crystal network interrupted by intact and partially disrupted milk fat globules, water, and air droplets, which are all dispersed in a liquid fat phase. Accordingly, butter is often referred as water-in-oil emulsion. Figure 1 shows the microstructure of butter, both schematically (a) and as observed by confocal laser scanning microscope (CLSM) (b). The crystals in the network are held together by two principal types of bonds, primary and secondary bonds. Primary bonds are the cardinal bonds of the entire crystal network, due to their strength and irreversibility. On the contrary, secondary bonds that hold crystals together by van der Waals forces are weaker and irreversible, underlying the thixotropic character of butter. Sintering, which refers to the formation of strong solid bridges between crystals and crystal clusters, might also occur. In addition, stearic and electrostatic forces might be involved in the system, as milk fat globules and proteins are also present. All the elements of the microstructure, including their characteristics as size and shape, and the interactions between them, such as the forces involved between elements, contribute to the mechanical and functional properties of butter. Principal focus is given to the effect that the solid-to-liquid ratio, the crystal polymorphisms, the presence of intact fat globules, and the continuity of the crystal network, as a number of contact points between crystals, have on the microstructure of butter, hence on its textural and functional properties.

Macrostructure Textural Properties of Butter

Butter is a pseudoplastic fat, or viscoelastic product, upon application and consequently removal of stress; it is able to regain some of the original shape. Different analytic and sensorial measurements have been used to describe textural properties of butter, and different descriptors have been correlated with different measurements. The most common descriptors for butter, evaluated by sensorial and rheological measurements, are hardness, spreadability, brittleness, stiffness, elasticity, and stickiness.

How Microstructure Elements Affect the Textural Properties of Butter

Crystal Network and Milk Fat Globules

The amount of solid fat in which the optimal range at 5 °C is 30–40% has often been considered one of the main factors responsible for the textural properties of butter, in particular for hardness and stiffness. However, the size of the crystals (1–20 μm) and crystal clusters (20–100 μm), thus the amount and type of bonds between them, and the amount of fat globules contribute to the to the microstructure properties more than the solid fat content (SFC). Generally, formation of small crystals lead to a finer crystal network with more contact points,

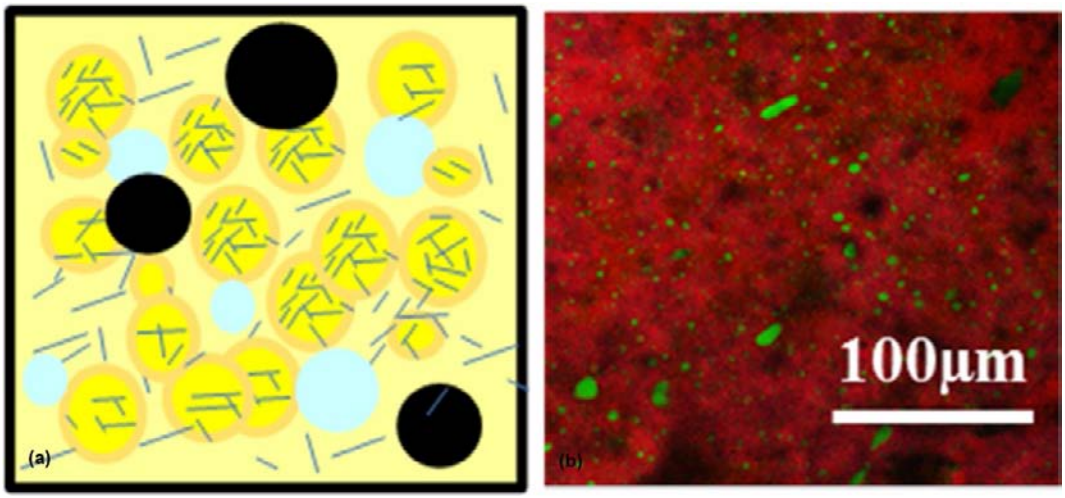

Figure 1 Microstructure of butter. (a) Schematic representation of butter as water-in-oil emulsion. The light blue area represents the water phase, the yellow area is liquid oil, the yellow circle is the fat globule containing liquid oil and crystals (petroleum bars), and the black circle is air. Not to scale. (b) CLSM images of butter after 28 days of storage. The black shadow on the image background represents the crystal network. The red area is the liquid fat of the system, and the green areas are protein/water phases. The scale bar indicates 100 μm. Adapted from Buldo, P. (2013). Crystallization of fat in and outside milk fat globules. Effect of processing and storage conditions. PhD thesis. Denmark: Aarhus University. ISBN: 978-87-92936-45-5.

which results in a firmer butter. On the contrary, larger crystals form a crystal network with few contact points that is more prone to fracture; hence, a softer butter is obtained. Yet, by increasing the amount of intact fat globules, the crystal network will be interrupted and the crystallization process will be shifted in favor of milk fat globules. The crystal network present within the milk fat globules does not contribute to the hardness and stiffness of butter. Therefore, butter with a high amount of intact fat globules has a weak structure and good spreadability. The latter is a consequence of the high melting faction of the TAGs that are inside the milk fat globules. The crystal network presents outside the milk fat globules, hence in the continuous liquid fat phase, leads to a stronger and firmer structure; therefore, it is the main element contributing to textural properties of butter. This highlights that the amount of SFC is not important as much as the ratio of solid fat present in and outside the milk fat globules for the textural properties of butter. The amount of solid fat present outside the milk fat globules is also responsible for the brittleness of butter. By increasing the volume fraction of fat crystals in the continuous phase, brittleness increases correspondingly. On the contrary, increasing the amount of intact milk fat globules will result in an increased structure deformation, hence elasticity. In conclusion, by increasing the amount of intact fat globules and thus by decreasing the amount of solid fat in the continuous phase, a more elastic and spreadable, thus less hard and brittle, butter is obtained.

Polymorphism

The polymorphism of milk fat is another element contributing to the textural properties of butter. In the majority of spreadable-fat products, including butter, β′-crystals with traces of β are found. The β′-crystals is the most desirable form in butter as it gives a smooth mouth sensation, due to its needle shape (<5 μm) and to its stability through the storage conditions. However, allowing the thermal treatment of the cream to a low cooling rate and/or the product to a prolonged storage time at refrigerator temperature, an irreversible polymorphism transition to a more stable crystal form, β, can sometimes be achieved, but only to a small extent. β-Crystals result in a sandy and coarse mouthfeel and in a poor crystal network and brittle structure, as a consequence of their large conformation (>20 μm) and platelet-like shape; therefore, they are undesirable in butter. α-Crystals are not likely to be present in spreadable-fat products, due to their metainstability. The crystal polymorphism is not a challenging factor on the microstructure and textural properties of butter, as it is fairly constant on the desired form; therefore, it is considered as a negligible element.

Water and Air Droplets

Despite their number concentration, 10^{10} ml^{-1}, water droplets do not have a significant contribution to the microstructure of butter, thus to its textural properties. This is due to their homogeneous distribution and small size (1–125 μm). However, they are relevant for microbiological safety, organoleptic properties, and chemical stability of butter. By increasing droplet size, for example, by coalescence, or if inhomogeneous distribution appears, microbiological growth, chemical instability (e.g., fat oxidation), and alteration of the organoleptic properties will occur.

Air droplets are the element that is present in the lowest share, below 5% (v/v). The size of air droplets is variable, as also is their number; however, the majority of them have a diameter of 20 μm or greater. Generally, by reducing the amount of air a harder butter is achieved, and vice versa by increasing the amount of air. Negligible consideration is given to them, as they are fairly stable through the shelf life of butter.

Alteration of Textural Properties of Butter During Storage and Handling

Butter leaves the producers at its best textural and functional properties; however, during transportation and storage, mainly during handling of butter at the consumers' place, textural changes might occur to the product. During handling, intended as temperature fluctuation, for example, from the refrigerator (5 °C) to the dinner table (25 °C) and vice versa, butter results in an increase in hardness, stiffness, brittleness, and elasticity. The changes on textural properties after temperature fluctuations, thus after melting and recrystallization of milk fat, are caused by the formation of a denser and stronger crystal network in the continuous fat phase, as a consequence of fat globule flocculation.

Butter Flavor and Color

The characteristic flavor of cultured butter is associated to the diacetyl and δ-decalactone compounds. Although sweet cream butter is associated to mild and 'nutty' flavor, this is mainly due to the presence of short-chain free FAs, aldehydes, methyl ketones, and lactones, which are depending on the feeding regime of the cows. In addition, aromatic characteristics associated to salty, cooked, grassy, and stale can also depict butter flavor. A typical rancid butter off-flavor can be caused by increased concentration of butyric and caproic acid. A conspicuous amount of volatile compounds is present in butter; however, their contribution to the final flavor is still undefined. Some aromatic compounds of butter are located in the water droplets.

Butter color varies from light to dark yellow. The color of butter is influenced by the amount of β-carotene present in the feed of the animals, hence in the milk. Generally, pasture has higher content of β-carotene than hay. The breed of the cow also influences the color of butter, for instance, milk from Jersey and Guernsey has a higher content of β-carotene than milk from other breeds.

How to Modify Butter Properties

Several approaches can be used to modify the textural and functional properties of butter. These can be classified as alteration of the composition and modification of the technological process parameters. The FAs composition can be changed in favor to the FAs more desirable in the final product. This can be achieved either by feeding regime or by fat fractionation. Increasing the amount of unsaturated FAs, for example, increasing fresh pasture or feeding encapsulated unsaturated fats, results in a softer butter. A softer butter can be obtained also by increasing the amount of low melting milk fraction through fat fractionation. However, butter with a higher melting fat fraction results in a reduced oiling off and moisture migration. To better address this topic, further knowledge is needed on the effect that TAG composition has on the textural properties of butter. The addition of minor components to the cream, for example, phospholipids, can increase the hardness of spreadable-fat products by increasing the crystal size and by promoting polymorphism transition toward a more stable form. In addition, phospholipids reduce graininess and tendency to oil off in butter.

Available Methods to Study Butter Properties

In order to fully characterize butter properties, a variety of analyses need to be performed. Table 1 shows the available methods to study butter properties.

Microscopy Techniques

As mentioned earlier, the microstructure of butter is the starting point to study the textural and functional properties of butter. Therefore, a proper visualization of the microstructure would give a first insight of the butter properties. CLSM is so far the best technique available to visualize the microstructure of butter. Images obtained by CLSM clearly identify the crystal network, the liquid fat, the fat globules, and the water and air droplets (Figure 1(b)). Yet, the resolution is low to allow a clear visualization of the single crystal or crystal cluster. The latter could be overcome by using a polarized light microscope (PLM); however, the microstructure complexity of butter makes it not suitable for this scope.

Rheology Techniques

Rheology techniques are commonly used to characterize the structure and texture of butter. In small deformation rheology, the applied stress or shear strain, at a given frequency and within the linear viscoelastic region (LVR), oscillates the microstructure without breaking it. Consequently, the time-dependent stress or strain is measured. On the other hand, by applying large deformations, a nonlinear response, which implies breakdown of the microstructure, is achieved. Outside the LVR, by applying large deformations, strength of primary bonds is monitored, whereas within the LVR, the strength of the secondary bonds is characterized. Common techniques among large deformation rheology are sectility, extrusion, compression, and penetration tests. A combination of oscillatory shear tests and compression or penetration tests characterizes the micro- and macrostructure, resulting in an accurate description of the textural properties of butter. Yet, the right technique, including test parameters and geometry used, should be carefully evaluated based on the purpose of the measurement and considering their effect on the outcoming data.

Oscillatory Rheology

In oscillatory shear tests, several geometries have been used to study the textural behavior of butter during crystallization and on the final product. Among these, parallel plate–plate, corrugated plate–plate, cone–plate, bob cup, starch cell, and vane cup, all available in different sizes and materials, have been tested. The most suitable geometry to study the microstructure of butter is the parallel plate–plate or the corrugated plate–plate, as change in structure caused by sample loading is minimized; in addition, these geometries are not destructive

Table 1 Summary of some of the methods available to study crystallization, microstructure, and textural properties of butter

Methods	*Principle*	*Applications*	*Advantage*	*Disadvantage*
Confocal laser scanning microscopy (CLSM)	Specimen is excited by a laser that is scanned in a focal plane. Fluorescence light is emitted and it is focussed as a confocal point	Observation of complex structure in a defined depth; particle size determination; fractal analysis	Detection of several component phases; 3-D images; high contrast, definition, and signal-to-noise ratio; easy and relatively fast sample preparation; emulsion stability	Low resolution
Polarized light microscope (PLM)	A polarized light is used to illuminate the sample. Due to the birefringence of crystals, they will appear bright, whereas liquid and amorphous regions of the specimen will appear dark	Observation of crystallization process and crystal structure; particle size determination	Inexpensive technique; contrast-enhancing technique	Not suitable for complex system such as butter or multiphase emulsions; limited resolution to the order of 1 μm
Oscillation rheology	Applying a constant stress or strain to the material	Measures the variation in strain as a function of the applied stress and vice versa	Determination of several parameters (G'; G'', G^*; $\tan\delta$)	Difficult sample handling and not highly reproducible with hard fat
Uniaxial compression	A sample between two parallel plates is compressed at constant load and velocity	Determination of the force needed to induce a change in a material	Easy and fast; several textural descriptor can be estimated from the data	No highly reproducible
Penetration test	A geometry is penetrated into the sample either at constant load or at constant speed	Determination of the hardness	Easy and fast, widely used	Results are dependent on parameters and geometries
Differential scanning calorimetry (DSC)	Temperatures and heat flows are associated with transitions in materials as a function of time and temperature	Determination of thermal behavior, solid fat content, and crystal polymorphism	Fast and simple; measurements performed at isothermal and nonisothermal temperature	Shape of the thermogram changes based on the cooling rate used; difficult sampling with plastic fats; shear cannot be applied
Dilatometry	Measures the change in sample volume	Determination of solid fat content	Reproducible	Laborious, time-consuming and inaccurate; measurements at constant temperature
Pulsar nuclear magnetic resonance (p-NMR) – direct method	Measures the signals from the hydrogen nuclei. Calculates the ratio between the hydrogen in the solid phase and the total number of hydrogen (solid and liquid phases)	Determination of solid fat content and crystal polymorphism	Fast, simple, and highly precise	Not accurate; measurements at constant temperature
p-NMR – indirect method	Measures the liquid part of the samples and refers it to the fully melted samples	Determination of solid fat content	Accurate	Complex sample preparation and time-consuming
x-Ray diffraction	Bragg's law is used to determine the distance between the reflections formed by the x-ray scattered from the electrons of the samples	Determination of polymorphism and lamella stacking of TAGs	Accurate	Expensive instruments, often not available

Source: Buldo, P. (2013). Crystallization of fat in and outside milk fat globules. Effect of processing and storage conditions. PhD thesis. Denmark: Aarhus University. ISBN: 978-87-92936-45-5.

on the sample during measurements. Parallel plate–plate geometry has often been implicated of causing wall slippage; this might be the consequence of free fat on the surface of spreadable-fat products or adhesive failure of the sample, which, under normal conditions, is not the case for butter. On the other hand, special attention should be paid when using the corrugated plate–plate with plastic fat, because the sample can stick between the open spaces of the plate leading to artifact measurements. The cone–plate geometry is also not recommended for butter, due to the leakage of oil from the samples as the required gap is achieved. The geometries mounted with a cup or a cell, thus with vane, are not suitable to study the textural properties of spreadable-fat products, due to the changes in structural conformation caused by the vane; as a consequence, the measurements will be linked to a structure artifact. Crystallization behavior of milk fats during butter

manufacturing can be studied by a bob cup, since no continuous crystal network is present until phase inversion occurs. Measurements from oscillatory tests display the viscoelastic behavior of butter, with the elastic modulus (G′) always greater than the viscous modulus (G″), within the LVR and before the yield point. The elastic modulus is used to quantify the crystal network, whereas the complex modulus (G*) gives an overview of the total structure characteristics.

Large Deformation Rheology

Among large deformation rheology tests, sectility or extrusion test is often used. Briefly, during the sectility test, a cutting steel wire moves at constant speed into the butter, whereas during extrusion test, a piston with a constant speed extrudes the sample from an orifice. In both tests, the outcoming force or stress is used as firmness measurement. Penetration and compression tests are also used frequently. A wide variety of geometries are available, for example, cone, needle, cylinder, sphere, and plate, all in different sizes and materials. The most used geometry is the cone for penetration test and the plate for compression. During penetration test, the geometry penetrates into the sample either at a constant speed or with a constant load, and the response of the material, as the force needed to penetrate the material or the distance traveled by the geometry, are recorded as a function of time. The American Oil Chemists' Society official method, which uses a cone with 20° angle, is based on the distance traveled by the cone under the force of gravity. The outcoming data are often referred to product hardness or firmness. Compression test is performed in the presence of geometries larger than sample, for example, plate geometry; therefore, a uniaxial compression occurs. The outcoming data provide additional information to a penetration test, for example, brittleness. Despite that, at the moment, the cone remains the most used geometry for the study of butter texture. The interpretation of the obtained data is relative to the parameters and geometries selected. For instance, the selected speed for compression is correlated to the response of the material, as higher speed corresponds to a higher force. In addition, the recorded forces are influenced by the size and shape of the geometry and by the dimension of the sample. Consequently, the choice of the most appropriate technique and parameters and the interpretation and comparison of results might be challenging despite a simple sample preparation and performance of the measurements. For the aforementioned tests, the obtained firmness measurements have often been related to the spreadability parameter evaluated by sensorial test.

Crystallization Study: Differential Scanning Calorimeter, p-NMR, and x-Ray Diffraction

Additional knowledge for the characterization of the textural properties of butter is obtained by the study of the crystallization occurring during manufacturing and storage of butter. Crystallization in butter is often studied by differential scanning calorimeter (DSC), pulsed nuclear magnetic resonance (p-NMR), and x-ray diffraction. DSC measures the heat flow, as a function of time and temperature in a controlled atmosphere, to identify the phase transitions of the material. DSC has been widely used to study the thermal behavior of butter. The obtained thermogram is mainly used to gain information on the melting and/or crystallization temperature of butter. Milk fats have a melting/crystallization temperature's range from −40 to +40 °C. Solid fat content and crystal polymorphism of butter can also be obtained by DSC; however, literature is scarce on this topic. So far, the best available methods to measure the level of SFC are dilatometry and direct and indirect p-NMR. The dilatometry is based on the change of volume of the sample, whereas p-NMR measures the signals from the hydrogen nuclei, which are distinguished by the free induction decay. The direct NMR method calculates the ratio of the number of hydrogen in the solid phase to the total number of hydrogen. The indirect method measures only the liquid part of the sample and refers it to a calibration performed on the fully melted sample. Crystal polymorphism and lamella stacking of TAGs molecules present in butter are determined by wide- and small-angle x-ray diffraction, respectively. The distance between the intensities of the scattered light, as a function of the scattered angle and the original wavelength, is calculated by Bragg's law.

Conclusions

Butter is considered an exclusive product for its unique flavor and nutritional value; as a result, it is used nearly in all world countries. Textural and functional properties of butter are closely related to the microstructure elements and to their distribution and interactions in the system. A combination of different analyses is needed to characterize the functional and textural properties of butter. Rheological tests reflect the behavior of the microstructure elements. Oscillation rheology tests performed with parallel plate–plate geometry, together with CLSM images, contribute to microstructure characterization. Penetration and compression test are linked to butter macrostructure, thus to its textural properties. The main microstructure element affecting butter texture is the fat network outside the milk fat globules. A continuous crystal network consisting of small crystals outside the milk fat globules leads to a harder and more brittle butter than a microstructure characterized by a higher number of milk fat globules and/or by large crystals, hence with a solid phase present within the globules rather than in the continuous liquid phase.

See also: Butter: Manufacture; Codex Alimentarius; Cream: Types of Cream; Dairy Products: Dietary and Medical Importance; Fats: Classification and Analysis; Fats: Production and Uses of Animal Fats; Fatty Acids: Determination and Requirements; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fatty Acids: Metabolism; Fatty Acids: *Trans* Fatty Acids; Food and Agriculture Organization of the United Nations; Food Classification and Description; Ghee; Margarine: Composition and Analysis; Milk: Processing of Milk; Milk: Role in the Diet; Milk: Sources and Composition; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Oxidation of Food Components; Phospholipids: Properties and Occurrence; Rheological Properties of Food Materials; Triacylglycerols: Characterization and Determination; Triacylglycerols: Structures and Properties.

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Cadmium: Properties and Determination

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Abbreviations

AAS	Atomic absorption spectroscopy
AOAC	Association of Official Analytical Chemists
CEN	European Committee for Standardization
CONTAM	Scientific Panel on Contaminants in the Food Chain, European Food Safety Authority
EFSA	European Food Safety Authority
EN	European standard
ESI-MS	Electrospray mass spectrometry
FAAS	Flame atomic absorption spectroscopy
GFAAS	Flame graphite furnace atomic absorption spectroscopy
HPLC	High-performance liquid chromatography
HSSR-BGC	High-speed self-reversal background correction
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
IEC	Interelement correction
ISO	International Organization for Standardization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
ML	Maximum level
MSF	Multicomponent spectral fitting
PC	Phytochelatins
PTWI	Provisional tolerable weekly intake
TDS	Total dissolved solids
UNEP	The United Nations Environment Programme

Cadmium (Cd) is a nonessential element with chemical and physical characteristics that convert it into a toxic element for the environment and living creatures. It was discovered by a German chemist, Friedrich Stromeyer, in 1817. The name comes from the Latin word *cadmia*, meaning calamine (zinc carbonate, $ZnCO_3$), or from the Greek word *kadmeia*, which has the same meaning. Toxic trace elements, including Cd, are currently a cause of concern for the committees and agencies responsible for food safety and health. Consequently, opinions have been expressed about cadmium in recent years, providing important information about various aspects concerning this element.

Physical and Chemical Properties

Cd is a soft, malleable, blue-white metal that tarnishes in air and is soluble in acids and insoluble in alkalis. Boiling cadmium gives off a yellow-colored vapor that is extremely toxic. Its melting point is 321 °C (610 °F) and its boiling point is 765 °C (1410 °F). The density of Cd is 8.65 g cm^{-3}. It is a transition metal, belonging to group 12 in the periodic table, together with zinc and mercury. The element has an atomic number of 48, an atomic mass of 112, and one main oxidation state (+2), although a few compounds have been reported in which it is +1. It has eight naturally occurring isotopes: ^{106}Cd, ^{108}Cd, ^{110}Cd, ^{111}Cd, ^{112}Cd, ^{113}Cd, ^{114}Cd, and ^{116}Cd. Of these isotopes, ^{114}Cd and ^{112}Cd are the most common, with an abundance of 29% and 24%, respectively. It is not usually present in the environment as a pure metal, but rather combined with other elements such as oxygen, chlorine, or sulfur to form Cd oxide, Cd chloride, or Cd sulfate, respectively. Cd sulfate and Cd chloride are soluble in water, whereas elemental Cd, Cd oxide, and Cd sulfide are almost insoluble. Cd is easily complexed with some organic compounds, although these compounds have not been found in the general environment because they decompose quickly. There is some evidence that in certain foods, cadmium is bound to metallothionein-like proteins.

Cd metal has specific properties that make it suitable for a wide variety of industrial applications. These include excellent corrosion resistance, low melting temperature, high ductility, and high thermal and electrical conductivity. An interesting property of Cd is its effect in alloys. In combination with certain metals, it lowers the melting point: Lichtenberg's metal, Abel's metal, Lipowitz's metal, and Newton's metal.

Occurrence of Cadmium

The abundance of Cd in the Earth's crust is estimated to be about 0.1–0.2 ppm. It is considered the 65th most abundant element. The only important ore of Cd is greenockite, or Cd sulfide (CdS). This ore does not have a sufficient concentration of Cd to be mined profitably. Most Cd is obtained as a

by-product from the smelting of zinc (Zn), lead (Pb), or copper (Cu) ores.

Cd is released to the environment from natural and anthropogenic sources. Natural sources include volcanic activity, weathering of rocks, burning of vegetation, sea-salt spray, and mobilization of Cd deposited in soils, sediments, and landfills. Anthropogenic sources include mining and smelting of Zn ores, combustion of fossil fuels for electricity and heating, incineration of municipal waste, industrial and agricultural wastes, and manufacture of phosphate fertilizers. Figure 1 shows the changes in the emission of Cd to the environment in the years 1990–2010 in member countries of the European Environment Agency (EEA). Although emissions to the environment have decreased in recent years, they are still a cause for concern for regulatory organizations.

Once it is in the environment, Cd moves easily through soil and water and can be taken up by certain crops and aquatic organisms. In fact, for the nonsmoking population, food is the main source of exposure to this element. The latest report on Cd issued by the contaminants panel of the European Food Safety Authority (EFSA) shows that the main dietary sources of this element are cereals and cereal products, vegetables, nuts and pulses, starchy roots or potatoes, and meat and meat products. However, the highest concentrations are found in seaweed, fish and seafood, chocolate, fungi, oilseeds, and edible offal.

Maximum limits (MLs) of Cd have been established for some food groups: European Commission (Regulation (EC) No. 1881/2006), China (GB2762-2012), and Australia/New Zealand (Standard 1.4.1). The food groups considered in these standards are the ones that present the greatest problems: vegetables, cereals, pulses, meat, and seafood. MLs vary within a given food group: cereals, 0.1–0.2 mg kg^{-1}; vegetables, 0.05–0.2 mg kg^{-1}; fruit, 0.05 mg kg^{-1}; seafood and derived products, 0.1–0.3 mg kg^{-1}; crustaceans, 0.5 mg kg^{-1}; bivalves and cephalopods (without viscera), 1 mg kg^{-1}; meat, 0.05–0.2 mg kg^{-1}; liver, 0.5 mg kg^{-1}; and kidney, 1 mg kg^{-1}. For viscera, the Australia/New Zealand standard allows higher MLs: liver, 1.25 mg kg^{-1} and kidney, 2.25 mg kg^{-1}. This standard also establishes an ML for chocolate and cocoa powder (0.5 mg kg^{-1}).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a provisional tolerable weekly intake (PTWI) of 7 μg kg^{-1} of body weight. The health risk related to Cd exposure has recently been reevaluated by EFSA's Scientific Panel on Contaminants in the Food Chain (CONTAM), which has established a tolerable weekly intake (TWI) of 2.5 μg kg^{-1} body weight. Table 1 shows the intake reported for various

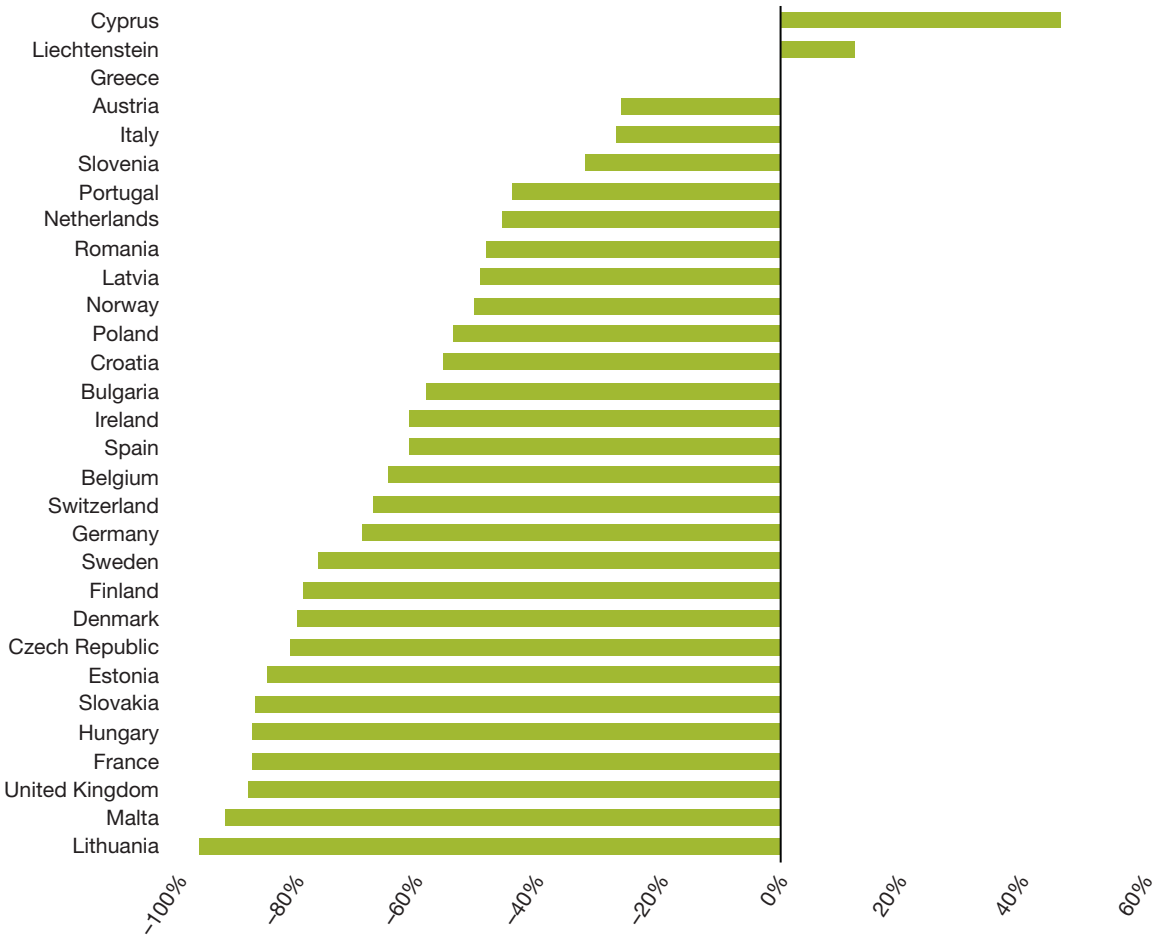

Figure 1 Change (%) in cadmium emissions 1990–2010 (EEA member countries). Reproduced from European Environment Agency (EEA) website (http://www.eea.europa.eu/data-and-maps/daviz/change-in-cadmium-emissions#tab-chart_1), with permission of European Environment Agency.

Table 1 Weekly cadmium intake in various countries

Country	*Intake ($\mu g\ kg^{-1}$ body weight/week)*	*Main food contributors*	*Year*
China	3.12 (average)	Rice, meat, and vegetables	2013
Japan	2.91–3.15 (range)	Rice	2004
Hong Kong	2.49 (average) 5.71 (high consumers)	Vegetables, fish and seafood products, cereals	2000
The United States	0.98–1.26 (average, lower to upper bound)	Vegetables, grains, and mixtures	2003
Spain (Catalonia)	1.83 (average)	Cereals, fish and shellfish, tubers	2003
The Netherlands	1.26 (average)	Wheat, potato, spinach, and carrot	1999–2002
Germany	1.4 (average) 2.35 (high consumers)	Vegetables and cereals	2014
Cyprus	4.7 (mean) 2.1–12.9 (range)	Meat and offal, cereals, vegetables	1997–2000
Sweden	0.81–3.15 (range)	Meat, potatoes, and cereals	2004–2007
France	1.12 (mean) 1.89 (95th percentile)	Bread and dried bread products, potatoes and potato products	2006–2007
Ireland	2.38–3.08 (average, lower to upper bound) 5.39–6.09 (95th percentile, lower to upper bound)	Vegetables (especially potatoes), cereals, and bread	2001
The United Kingdom	1.63 (mean) 2.8 (97.5th percentile)	Potatoes, bread, and miscellaneous cereals	1997

countries. The review that EFSA carried out indicates that the adult European population's exposure to Cd is near or above the TWI and points to vegetarians and children as populations at risk that may double the TWI. The CONTAM concludes that current exposure to Cd at the population level should be reduced.

Determination of Cadmium in Foodstuffs

When analyzing any trace element – and Cd is no exception – one must take a series of precautions to avoid mistakes and misinterpretation of the data obtained. It is necessary to work with material previously treated with diluted acid (5–10% HNO_3) to eliminate possible contamination and with ultrapure water with an electrical resistivity of 18.2 mΩ. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. A more detailed description of the measures to be taken to determine trace elements in general and Cd in particular is given in the European Committee for Standardization (CEN) standard EN 13804:2002.

There are various methods for determining Cd in food, proposed by international organizations – CEN, AOAC (Association of Official Analytical Chemists), and ISO (International Organization of Standardization) – or by national organizations. Table 2 shows the methods proposed by the international organizations, which differ with regard to sample preparation and the method employed for detection.

Sample Digestion Methods

Foods are complex samples that require a preliminary mineralization treatment to decompose the matrix and dissolve the element. This mineralization is the most decisive step for obtaining quantitative determination of the element. Various methods for sample mineralization are commonly reported in the literature for Cd determination: dry ashing in a muffle furnace, pressure digestion using concentrated acid, and acid digestion by heating at atmospheric pressure.

Dry ashing

Dry ashing is done in crucibles that are first dried and then subjected to a temperature ramp in a muffle furnace. Typical ashing temperatures are 450 to 550 °C at atmospheric pressure. This step is common to all the Cd determination methods that use dry ashing; however, the method for dissolving the ash varies and may be complex. Some methods are limited to dissolving the ash in 6 M HCl and evaporating to dryness. The residue is then dissolved with 0.1 M HNO_3 for subsequent detection. This is the principle of AOAC method 999.11 and of standard EN 14082:2003 for the analysis of cadmium in foodstuffs.

Dry ashing has a series of advantages; it is possible to preconcentrate the analyte, the sample does not need much handling, and it is a method that uses a smaller volume of reagents than other forms of digestion. The main disadvantage of this method has to do with losses resulting from the formation of volatile compounds. It has been shown that losses in foods can be caused by the formation of $CdCl_2$, which depends on the matrix and the digestion temperature. A study conducted on tomato leaves showed small Cd losses (up to 7%) in dry ashing at temperatures not exceeding 500 °C. However, the losses increased to 30% when the final ashing temperature was raised to 900 °C. These losses caused by volatilization can be reduced by the use of ashing aids, which form complexes or molecules with the elements and prevent their volatilization. Another drawback of dry ashing is the time required for complete mineralization of the sample (12–24 h). Analytic instruments have been developed recently to dry ash samples using microwave heating. These devices can be programmed to remove most of the moisture initially (using relatively low heat) and then convert the sample to ash (using relatively high heat). Microwave instruments greatly reduce the time required to carry out an analysis, with the analysis time often

Table 2 Official methods for analyzing cadmium in food products

Reference	*Method*	*Year*
EN 14082	Determination of lead, cadmium, zinc, copper, iron, and chromium by atomic absorption spectrometry (AAS) after dry ashing	2003
EN 14083	Determination of lead, cadmium, chromium, and molybdenum by graphite furnace atomic absorption spectrometry (GFAAS) after pressure digestion	2003
EN 14084	Determination of lead, cadmium, zinc, copper, and iron by atomic absorption spectrometry (AAS) after microwave digestion	2003
EN 15763	Determination of arsenic, cadmium, mercury, and lead in foodstuffs by inductively coupled plasma mass spectrometry (ICP-MS) after pressure digestion	2009
AOAC Official Method 973.34	Determination of cadmium in foods by atomic absorption spectrophotometric method	1974 (final action)
AOAC Official Method 986.15	Arsenic, cadmium, lead, selenium, and zinc in human and pet foods by atomic absorption spectrometry (AAS) and anodic stripping voltammetry (ASV)	
AOAC Official Method 945.58	Determination of cadmium in foods by dithizone method	1993 (final action)
AOAC Official Method 999.10	Determination of lead, cadmium, zinc, copper, and iron in foods by atomic absorption spectrophotometry after microwave digestion	1999 (first action)
AOAC Official Method 999.11	Determination of lead, cadmium, copper, iron, and zinc in foods by atomic absorption spectrophotometry after dry ashing	1999 (final action)
ISO 15774	Animal and vegetable fats and oils – determination of cadmium content by direct graphite furnace atomic absorption spectrometry	2000
ISO 6561-1	Fruits, vegetables, and derived products – determination of cadmium content. Part 1: Method using graphite furnace atomic absorption spectrometry	2005
ISO 6561-2	Fruits, vegetables, and derived products – determination of cadmium content. Part 2: Method using flame atomic absorption spectrometry	2005

being less than an hour. The major disadvantage of the microwave heating method is that it is not possible to analyze as many samples simultaneously as in a muffle furnace.

Wet digestion

Wet digestion is performed by using combinations of oxidizing acids (HNO_3, conc. $HClO_4$, and conc. H_2SO_4), nonoxidizing acids (HCl, HF, H_3PO_4, diluted $HClO_4$, and diluted H_2SO_4), and H_2O_2. Most of the methods currently employed use HNO_3 as the primary oxidizing agent in combination with H_2O_2. Closed systems working under pressure are generally used, but systems open to atmospheric pressure (open digestion) may also be employed.

Digestion in open systems has traditionally been done in open vessels or tubes on a hot plate or in an aluminum or graphite heating block. In addition to conventional heating, it is also possible to use microwave irradiation. The most notable advantage of this technique is its low cost, especially if one is working with digestion blocks. One of the main disadvantages of working with an open system is that the temperatures are limited by the acid solution's boiling point. The boiling point of HNO_3 is 122 °C, very low for complete digestion of foodstuffs with a high concentration of fat or protein. In these cases, H_2SO_4 is added (boiling point, 338 °C), which raises the digestion temperature, although the presence of sulfates may affect determination by spectroscopic methods. Furthermore, a high temperature in an open system may bring about losses by volatilization, which can be avoided partially by working under reflux conditions. There is now instrumentation for performing open digestion, which incorporates reflux condensers and is automated. These new systems are an alternative to pressure systems, permitting digestion of a larger quantity of sample without causing overpressure problems. Although the open digestion has been used for decades, there are no official methods for the determination of Cd in foodstuffs based on acid digestion by heating at atmospheric pressure.

Pressure digestion systems can typically achieve temperatures in the 200–260 °C range. This results in a dramatic acceleration of the reaction kinetics, allowing digestion reactions to be carried out in a matter of hours (conventional heating) or in less than an hour (microwave assisted). The European Standard (EN) 13805 specifies a method for the pressure digestion of foodstuffs intended for the determination of trace elements. The mineralization method most often applied for determining Cd in foodstuffs is microwave-assisted digestion, using nitric acid as the oxidizing agent and H_2O_2. This approach is the basis for various official methods: EN 14084:2003, AOAC Official Method 999.10, and EPA Method 3051. There are also methods that leave the way in which the closed system is heated open (microwaves or pressure digestion apparatus with conventional heating): EN 14083:2003 and AOAC Official Method 2013.06.

Cadmium Quantification Methods

The most common analytic procedures for measuring cadmium concentrations in food samples use atomic absorption spectroscopy (AAS) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) or mass spectrometry (ICP-MS). These three analytic methods are compatible with most of the digestion procedures described in the previous section.

Various forms of AAS have been used for the determination of Cd. Possibly the one most commonly used at present is GF-AAS (graphite furnace atomic absorption spectroscopy). There are various official methods that use GF-AAS as a detection technique for determining Cd in foods (EN 14083:2003,

AOAC 999.10, AOAC 999.11, ISO 6561-1:2005, ISO 15774:2000, and China national standard GB/T 5009. 15-2003). Most of these methods require prior preparation of the sample by dry ashing or pressure digestion, except ISO 15774:2000, developed for the determination of cadmium in animal and vegetable fats and oils, in which the sample is placed directly in the graphite furnace without prior mineralization treatment.

The principle of GF-AAS is the incineration and atomization of the samples (after digestion) in a graphite tube furnace with platform connected to an atomic absorption spectrometer. The measurement of Cd content is obtained from the absorption observed at a wavelength of 228.8 nm. Various developments have helped to improve this methodology in recent years (improved electrodeless discharge and hollow cathode lamps for increased light output). The limits of this methodology (0.03–0.08 $\mu g\,l^{-1}$) permit determination of most food samples, especially those that may be considered problematic because of their high cadmium content.

Some studies show that the determination of Cd in GF-AAS suffers from background and spectral interferences. Zn(II), Co (II), and Ag(I) ions can cause positive interferences, while Ge (IV), Cu(II), Pb(II), and Sb(III) can produce negative interferences. There have also been reports of background interference caused by the presence of iron (Fe). In order to prevent these interferences and also to avoid possible losses of Cd during the thermal treatment, it is necessary to add a matrix modifier, palladium (Pd), nickel (Ni), ammonium phosphate, and a mixture of Pd and Mg being the ones most used. There have also been improvements in the correction systems (Zeeman background correction versus D_2 correction) and the introduction of samples (use of L'vov platforms). In recent years, the high-speed self-reversal background correction (HSSR-BGC) system has been presented as a powerful background corrector to avoid background and spectral interferences in Cd determination (Figure 2).

Flame atomic absorption spectroscopy (FAAS) is a technique that has been very widely used for the determination of Cd for many years. There are some official methods that leave the choice of FAAS or GF-AAS as the detection method open (AOAC 999.10, AOAC 999.11, and ISO 6561-2:2005). FAAS requires a liquid sample to be aspirated, aerosolized, and mixed with combustible gases, such as acetylene and air or acetylene and nitrous oxide. The mixture is ignited in a flame whose temperature ranges from 2100 to 2800 °C. During combustion, atoms of the element are reduced to free, unexcited ground state atoms, which absorb light at characteristic wavelengths.

FAAS is a simple, fast determination method that costs less than other techniques, and it is very selective because atomic lines are sharp. However, its sensitivity is less than that of other detection systems used for the determination of Cd in food. The reported detection limits of this method in food are various orders of magnitude higher (4–10 $\mu g\,l^{-1}$). For this reason, the analyte is generally preconcentrated and separated prior to determination by FAAS. There are various preconcentration methods, including coprecipitation, liquid-liquid extraction, cloud-point extraction, solid-phase extraction, and dispersive liquid-liquid microextraction. After preconcentration, detection limits closer to those of other techniques used for determining Cd in food have been reported (0.60–5 $\mu g\,l^{-1}$).

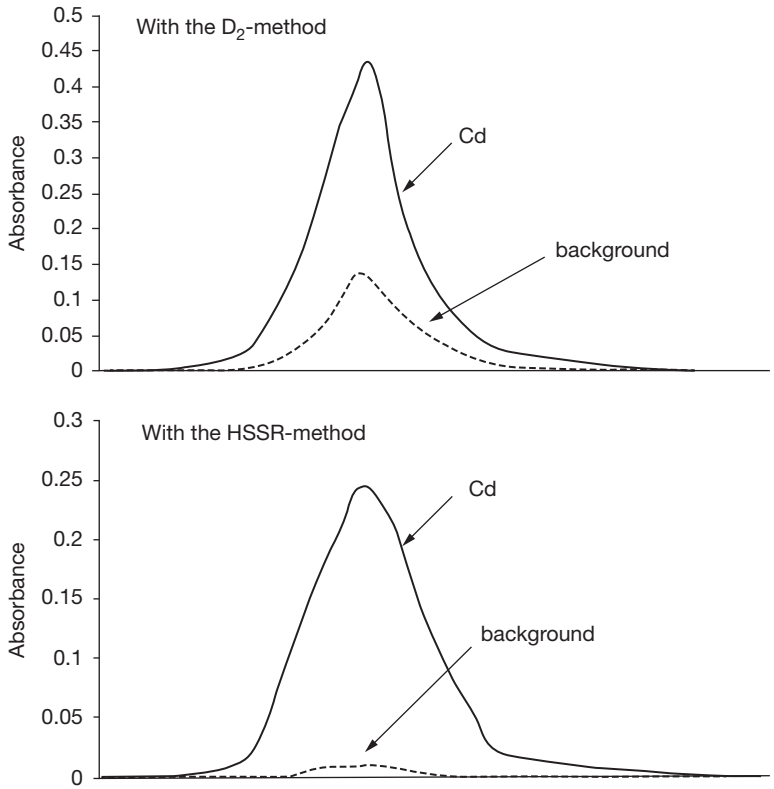

Figure 2 Background correction with D_2 and HSSR method in the determination of cadmium by GF-AAS. Reproduced from Waterlot, C. and Douay, F. (2013). *Measurement* **46**(8), 2348–2358, with permission of Elsevier.

ICP-AES is based on the generation of plasma by ionization of argon gas using the electromagnetic field created by high-frequency electric current. Initially, the liquid sample (previously digested) is converted into a stream of fine droplets (aerosol). In the spray chamber, separation of the aerosol occurs; the large droplets go to the drain and the fine droplets are carried to the plasma, which generates temperatures around 10 000°K. At this temperature, all elements become excited and emit light at their respective wavelengths. The radiation emitted from the plasma is then collected by wavelength and amplified to yield intensity measurements. The official methods established by the various organizations for the determination of Cd in food do not use ICP-AES as the detection method. Only one CEN method uses ICP-AES for analysis of animal feeding stuffs (EN 15510:2007).

This analytic technique has the same disadvantage as FAAS: its detection limit (0.1–1 $\mu g\, l^{-1}$) is approximately 100 times greater than that of GF-AAS and 1000–10 000 times greater than that of ICP-MS. In some cases, analyte preconcentration processes similar to those described for FAAS have been used. The advantages of this method are the possibility of determining a large number of elements simultaneously and the fact that the cost of acquisition and maintenance is less than that of ICP-MS. Furthermore, among all the commonly used analytic atomic spectrometry techniques, ICP-based techniques are probably the ones with the fewest interferences; however, nebulizer, chemical, and spectral interferences are all present. The chemical interferences are generally of less importance because the high temperature and the inert atmosphere of the Ar plasma help to reduce them; nebulizer interferences in ICP-AES (also known as matrix effects) can arise from physical and chemical differences between reference standards and samples or between samples, such as the inconsistent presence of matrix salts and organic compounds, or different viscosities and surface tensions of the liquid. In these cases, one can try to work by diluting the sample or using the method of additions and employing internal standards.

The most severe interferences in ICP-AES are spectral, due mainly to the excitation and subsequent emission of spectral lines for every element in the sample as well as the Ar added to facilitate plasma generation. Emission at 228.80 nm is the strongest line for Cd. If arsenic (As) is present in the sample, it can interfere with Cd measurements owing to spectral overlap from the nearby As line at 228.812 nm. Similarly, for the 214.439 nm Cd line, there is also an Fe line only 6 pm apart that can lead to severe interferences in the presence of high amounts of Fe. Spectral overlaps may be avoided by using an alternate wavelength, or high resolution (if available), or can be compensated for by the use of interelement correction (IEC) equations or multicomponent spectral fitting (MSF) (Figure 3).

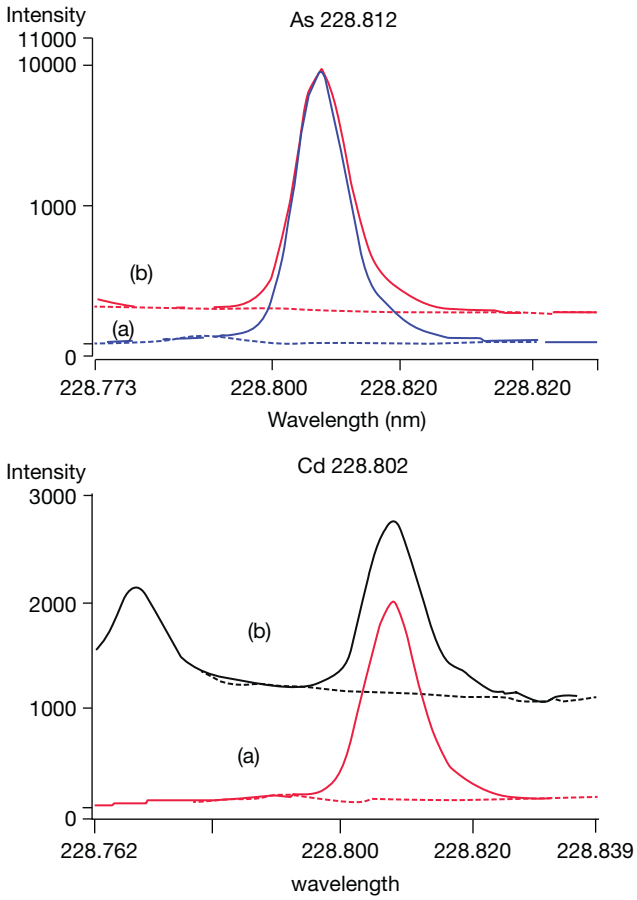

Figure 3 Line overlap of As and Cd at 228.8 nm in ICP-OES. Reproduced from Asfaw, A. and Wibetoe, G. (2009). *Spectrochimica Acta Part B* **64**, 363–368, with permission of Elsevier.

Table 3 Spectral interferences in Cd detection by ICP-MS

Isotope	*Abundance*	*Interference*
^{110}Cd	12.5	$^{39}K_2^{16}O^+$
^{111}Cd	12.8	$^{95}Mo^{16}O^+$, $^{94}Zr^{16}O^1H^+$, $^{39}K_2^{16}O_2^1H^+$
^{112}Cd	24.1	$^{40}Ca_2^{16}O_2$, $^{40}Ar_2^{16}O_2$, $^{96}Ru^{16}O^+$
^{113}Cd	12.22	$^{96}Zr^{16}O^1H^+$, $^{40}Ca_2^{16}O_2^1H^+$, $^{40}Ar_2^{16}O_2^1H^+$, $^{96}Ru^{17}O^+$
^{114}Cd	28.7	$^{98}Mo^{16}O^+$, $^{98}Ru^{16}O^+$, ^{114}Sn
^{116}Cd	7.49	$^{100}Ru^{16}O^+$

Source: May, T. W. and Wiedmeyer, R. H. (1998). *Atomic Spectroscopy* **19**(5), 150–155, with permission of PerkinElmer Corporation.

In ICP-MS, the argon plasma is also used as the excitation source; however, in contrast to ICP-AES, the plasma is used to generate ions that are introduced into the mass analyzer. The ions are separated and collected in relation to their mass-to-charge ratio. The main advantage of this method is its sensitivity, with detection limits (0.00001–0.001 $\mu g\, l^{-1}$) much lower than those of any other technique used for the determination of Cd. One disadvantage in comparison with ICP-AES and FAAS has to do with the concentration of total dissolved solids (TDS). In ICP-AES, with the proper equipment, up to 20% TDS can be run. However, in ICP-MS, TDS concentrations only range up to 0.2%, unless specialized nebulizers and torches are used. Moreover, it is an expensive technology to acquire and maintain, and it requires highly qualified operators. Currently, a considerable number of the studies that report Cd concentrations in foods use this methodology. This technique is also the basis of standard EN 15763:2010.

In ICP-MS, the determination of certain elements may be affected by spectral and matrix interferences. The spectral interferences may be due to isotopes of other elements (isobaric interferences) or to the formation of molecules or combinations of atoms among the elements present in the matrix and those that come from the air and the argon plasma (polyatomic or molecular interferences). A series of spectral interferences have been identified in the determination of Cd by ICP-MS (Table 3). The most sensitive isotope for Cd is at mass 114; however, there is also a minor isotope of tin (Sn) at this mass. This means that if there is Sn in the sample, quantitation using ^{114}Cd is difficult. Another element that can cause spectral interference is molybdenum (Mo). The formation of different species with oxygen creates interferences in the determination of all the isotopes of Cd except ^{106}Cd. This latter isotope, however, is low in abundance (1.2%) and is insufficiently sensitive to yield sub-ppb detection limits.

Various approaches can be used to try to eliminate these interferences, such as mathematical correction (a useful method for correcting interference caused by Sn) or the use of collision and reaction cells that use ion–molecule collisions and reactions to cleanse the ion beam of polyatomic and molecular interferences before entering the analyzer (used for eliminating MoO interferences). However, the best and probably most efficient way to remove spectral overlaps is the use of a high-resolution mass spectrometer.

The determination of total concentrations of Cd in foodstuffs is important because it is a way of keeping watch on food safety. In recent years, however, attempts have been made to take a further step, to identify the interactions between this element and the food matrix. The possible bonds with more complex molecules may finally be a conditioning factor affecting its subsequent intestinal absorption and arrival in the systemic circulation. In plants, there are reports of the formation of complexes with phytochelatins (PCs) as a cellular response to this toxic element. Different methods have been used in recent years to determine Cd-PC complexes: high-performance liquid chromatography (HPLC)-ICP-MS and HPLC-electrospray mass spectrometry (ESI-MS). This kind of approach is currently one of the most novel lines of research in the analysis of Cd in foods.

See also: Cadmium: Toxicology; Heavy Metal Toxicology; Infrared Spectroscopy: Applications; Spectroscopy: Types.

Further Reading

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Relevant websites

http://www.efsa.europa.eu/en/efsajournal/pub/980.htm – European Food Safety Authority (EFSA).

http://www.epa.gov/ttn/atw/hlthef/cadmium.html – US Environmental Protection Agency (USEPA).

http://www.icco.org/sites/sps/documents/Cadmium%20Workshop/EU%20Commission%20-%20DG%20Sanco.pdf – Directorate-General for Health & Consumers. European Commission.

http://www.who.int/ipcs/assessment/public_health/cadmium/en/ – World Health Organization (WHO).

Cadmium: Toxicology

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Sources and Production

Cadmium is a naturally occurring soft metal in the Earth's crust. It has an average crustal abundance of 0.1–0.5 parts per million and is ranked the 64th most abundant natural element on Earth.

Cadmium can be released from the rocks through natural processes such as weathering and erosion and then transported to rivers and oceans. In marine sediments, the cadmium concentration is typically higher than that on the land, with an average abundance of 1 part per million. Other natural processes resulting in cadmium emissions include volcano eruptions and forest fires.

Cadmium can also be released to the environment through various human activities. A large part of anthropogenic emission occurs during the extraction, smelting, and refining of metals, especially zinc, lead, and copper, from ores that also contain cadmium. The burning of fossil fuels such as coal, oil, and gas and the use of cadmium-containing phosphate fertilizers also contribute to the total emission. Starting from the 1930s, cadmium as the by-product of the metal processes has been found useful in many industrial products, mainly in the areas of metal coating, plastic stabilization, color pigments, and nickel–cadmium batteries. With its wide application, cadmium enters the environment during the manufacture, use, and disposal of these industrial products. High levels of cadmium have been found in the soil close to hazardous waste sites because of spills and leaks. Since cadmium does not break down in the environment, it stays in the water and soil for long periods of time and can be taken up by plants, fish, and animals.

The anthropogenic emission of cadmium used to be about ten times the amount of natural emission. However, due to the increasing global awareness of cadmium-related health and environmental issues, the traditional application of cadmium has diminished in many countries during the last 20–30 years. Today, cadmium is primarily used in the manufacture of nickel–cadmium batteries, and anthropogenic emissions have dropped significantly.

Patterns of Consumption

Route of Exposure

Humans can be exposed to cadmium from food, water, and air. Food is the major source of cadmium exposure for nonsmokers in a non-occupational environment. In unpolluted areas, the exposure from drinking water is usually less important compared with the exposure from the food because of the relatively lower levels. Though the burning of fossil fuels and cadmium-containing household waste can release cadmium into the air, the exposure from the air for non-cadmium workers is usually negligible. Cigarette smoking can be an important source of cadmium, because the tobacco plant characteristically accumulates relatively high concentrations of cadmium in its leaves. In heavy smokers, the contribution of cadmium exposure from smoking can be comparable with that from the diet. In this article, only dietary exposures will be discussed.

Levels in Food

The presence of cadmium in food results from the contamination of soil and water. Cadmium can be taken up by certain plants and aquatic organisms and accumulate in the food chain. In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) conducted a reevaluation of cadmium in food. In response to the JECFA's call, cadmium occurrence data from a total of over 155 000 food samples were submitted for review. The majority of these data were submitted by the European Food Safety Authority (EFSA), covering 19 European Union member countries. Besides, ten other countries (China, Japan, Singapore, Vietnam, Brazil, Chile, Canada, the United States, Australia, and Ghana) also submitted cadmium occurrence data. The food industry submitted cadmium occurrence data from food products distributed and used worldwide. A description of these data is given in Table 1.

For most food categories, the national mean cadmium concentrations range between nondetected (ND) and 0.04 mg kg^{-1}. Shellfish/mollusks, meat and poultry offal, nuts and oilseeds, coffee, tea and cocoa, vegetables (especially dried), and spices are among the top food categories with the highest cadmium levels (0.1–4.8 mg kg^{-1}). The food categories containing relatively low cadmium include eggs (0.0001–0.007), dairy products (ND–0.004), animal and vegetable fats (ND–0.006 mg kg^{-1}), meat muscle (0.001–0.003 mg kg^{-1}), fruits (0.001–0.007 mg kg^{-1}), and finfish (ND–0.008 mg kg^{-1}). The cadmium levels in grains usually contain medium levels of cadmium (wheat, 0.009–0.04 mg kg^{-1}; rice, 0.004–0.02 mg kg^{-1}; and oats, 0.003–0.02 mg kg^{-1}).

Dietary Exposure

The mean total dietary exposure is calculated as the sum of the cadmium exposure of all food categories; each is the product of the mean cadmium occurrences and the average consumption for the total population. Different countries and regions may apply their own methodology to assess the food consumption, normally from either a retrospective dietary history recall or a prospective dietary record. These surveys can capture an individual's food consumption from 1 day to 7 days, depending on the length of the survey. The results can be used to estimate a daily or weekly exposure. At its 73rd meeting in 2010, the JECFA committee recommended that these exposure estimates be extrapolated to a monthly basis due to cadmium's exceptionally long half-life. A daily or weekly exposure estimate can be translated into a monthly exposure estimate by multiplying 30 or 4, respectively, and expressed in μg cadmium per kilogram body weight per month (μg kg bw^{-1} $month^{-1}$).

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00097-0

Table 1 A summary of cadmium levels in foods

Region	*Country*	*No. of samples*	*Top food categories with the highest cadmium concentrations (mg kg^{-1})*	
Asia	China	1491	Mollusks	0.599
			Fish and seafood	0.077
			Meat and poultry	0.042
			Vegetables	0.019
			Pulses/legumes	0.016
	Japan	67	Shellfish/mollusks	0.346
	Singapore	482	Dried vegetables	0.986
			Shellfish/mollusks	0.288
			Coffee, tea, and cocoa	0.149
			Nuts and oilseeds	0.086
			Spices	0.024
	Vietnam	317	Mollusks	0.487
Europe	19 EU countries	131167	Seafood and products	0.215
			Edible offal/products	0.206
			Coffee, tea, and cocoa	0.074
			Vegetables/nuts/pulses	0.067
			Spices	0.062
Latin America	Brazil	2241	Meat kidney	0.025
			Poultry kidney	0.012
			Meat/poultry muscle	0.003
	Chile	9	Shellfish/mollusks	0.949
North America	Canada	706	Shellfish/mollusks	4.820
	The United States	7411	Meat/poultry kidney	0.498/0.464
			Shellfish	0.429
			Spices	0.228
			Poultry liver	0.176
			Nuts and oilseeds	0.131
Pacific region	Australia	532	Roots and tubers	0.033
			Nuts and oilseeds	0.019
			Shellfish/mollusks	0.014
			Cereals/grains (exclude wheat and rice)	0.013
			Baked goods	0.012
Africa	Ghana	144	Finfish	0.00002
Worldwide	Industry-used ingredients	10929	Mollusks	4.213
			Coffee, tea, and cocoa	1.750
			Shellfish	0.648
			Dried vegetables	0.330
			Spices	0.106

JECFA (2010). Cadmium, Tables 5 and 6. If data from multiple food categories are reported, only the top 5 food categories with the highest cadmium occurrence are listed.

Several countries and regions have conducted their own total diet study (TDS) to estimate the national dietary cadmium exposure. Some countries have separate survey systems to collect the occurrence data and the food consumption data. In the United States, for instance, the occurrence data come from the TDS, and the consumption data come from the National Health and Nutrition Examination Survey (NHANES). The estimated mean dietary cadmium exposures for countries with available data are listed in Table 2.

The national mean cadmium exposures in adults are estimated to range from 2.1 to 12.0 μg kg bw^{-1} month^{-1} in these countries and regions. For children 0.5–12 years old, the exposures reported by Australia and the United States range from 3.9 to 20.6 μg kg bw^{-1} month^{-1}. Dietary exposure for vegetarians, as reported by the EFSA, can reach as high as 23.2 μg kg bw^{-1} month^{-1}.

A provisional tolerable weekly intake (PTWI) for cadmium of 7 μg·kg bw^{-1} was established by the JECFA in 1988 and has been widely accepted. At its 73rd meeting in 2010, the JECFA reevaluated cadmium. Based on its adverse renal effects, the committee established a new provisional tolerable monthly intake (PTMI) of 25 μg·kg bw^{-1}·month^{-1}.

The Contribution of Food Categories to the Total Dietary Exposure

The cadmium exposure from a certain food category is calculated as the product of the cadmium concentration and the level of consumption for that food category. Therefore, the food categories with the highest cadmium concentrations may not be the categories that contribute the most to the total cadmium exposure. For instance, though the cadmium concentration in grains is not the highest among all food categories, cadmium exposure from grains may still be the main source of total dietary exposure in certain populations due to the large consumption of the staple foods. On the other hand, though certain spices can contain high levels of cadmium, the exposure from spices may not be comparable with other food sources due to the fairly low consumption.

Table 2 Dietary cadmium exposure levels in different countries

Country	Source of occurrence data	Source of consumption data	Mean cadmium exposure in adults ($\mu g\ kg\ bw^{-1}\ month^{-1}$)
Australia	TDS 2000–01	1995 Australian National Nutrition Survey	2.4,[a] 7.2[b] (males) 2.1,[a] 6.6[b] (females)
China	TDS 2007	TDS 2007	9.9[c] (range 0.5–36.5, varied by province)
Europe	EFSA 2009	EFSA 2008	9.1[c] (range 7.6–11.8, varied by country)
The United States	TDS 2004–08	NHANES 2003–06	4.6[a] (range 4.1–5.5, varied by age)
Chile	TDS 2001–02		9[d]
Japan			12[d]
Lebanon	TDS 2004		5.2[c]
Republic of Korea			7.7[b]

[a]ND = 0.
[b]ND = LOD.
[c]ND = LOD/2.
[d]ND: not specified.
JECFA (2010). Cadmium. Tables 10, 12, and 13.

The contribution of each food category to the overall dietary cadmium exposure varies by country and geographic region, primarily due to the differences in the regional cadmium occurrences and dietary habits. For example, the main dietary sources of cadmium in Japan are rice, vegetables, seaweed, and seafood, while the main sources in some European countries are cereals and grains, animal offal, and vegetables. In European vegetarians, the main contributors can be vegetables, pulses, and nuts.

The cadmium contributions of different food categories also depend on the geologic location. Taking China as an example, in Sichuan province, cereals accounted for 85% of the total exposure, and the cadmium levels in the cereals are about six times the national average. In Shanxi and Shanghai provinces, vegetables account for 62% and 69% of the total cadmium exposure, respectively. Meat is the major source of cadmium in Heilongjiang and Hubei provinces, while seafood contributes 88% of the total dietary cadmium in Liaoning province.

The Biomarkers for Cadmium Exposure

Many biomarkers have been used to reflect the level of cadmium exposure, including cadmium levels in the blood, urine, feces, liver, kidney, hair, toenail, etc. The most commonly used exposure biomarkers are blood cadmium and urinary cadmium. It has been generally accepted that urinary cadmium indicates the total body burden, while blood cadmium only reflects recent exposures. Many human studies have shown that the level of urinary cadmium is positively correlated with daily dietary cadmium exposure.

High Exposures

Foods grown in cadmium-contaminated areas can have high cadmium levels, leading to high cadmium exposures in people who consume these foods. An example is the epidemic of itai–itai disease in Japan around the 1930s, resulting from the consumption of rice grown in cadmium-contaminated fields. In some highly mineralized geographic areas, such as central Jamaica, high levels of cadmium naturally occur in the soil, resulting in high levels of cadmium in local vegetables and foods. High urinary cadmium and the incidence of kidney diseases have been reported in the local population.

Availability, Absorption, and Metabolism

In humans, the average absorption of ingested cadmium is about 5% in adults. The absorption of cadmium in newborns and infants can be higher. The factors affecting the gastrointestinal absorption of cadmium include age, gastric pH, diet type, and nutrition status. There has been evidence that zinc and iron compete with cadmium for absorption, probably through a common transporter. As a result, the bioavailability of cadmium is lower in zinc and iron-rich foods, such as oysters, and people with low body iron store can have higher cadmium absorption. It has been suggested that the generally lower body iron store in women accounts for their generally higher body burden of cadmium.

In the liver, cadmium binds with metallothionein (MT), a cysteine-rich protein that can bind metals through the thiol groups on its cysteine residues. This process reduces the amount of free cadmium, preventing free cadmium from exerting its toxic effects. However, some cadmium–MT complex is transported to the kidneys and accumulates in the renal proximal tubule by receptor-mediated endocytosis. In the kidneys, the cadmium–MT complexes are degraded by lysosomes, resulting in the release of free cadmium. Though the newly released free cadmium can induce more MT in the kidney, renal tubular damage starts to occur when free cadmium exceeds a certain level, at which the newly synthesized MT is not enough to neutralize it. This cadmium level is called the 'critical value,' commonly quoted as 200 $\mu g\ g^{-1}$ renal cortex.

Cadmium is widely distributed in the body, with the highest concentrations found in the liver and the kidneys and lower concentrations found in the brain, bone, and fat. In humans with non-occupational exposure, the majority of cadmium body burden is found in the liver, the kidneys, and muscle. Long-term exposure to cadmium leads to selective accumulation in the liver and renal cortex, such that up to 75% of the total body burden is found in these organs.

Cadmium does not readily cross the fully developed placenta or blood–brain barrier in humans. Evidence has shown that cadmium can induce MT in the placenta. The placenta retains most cadmium after low exposures, so the concentrations of cadmium in the fetuses and neonates are much lower than the corresponding concentrations in pregnant women.

Once cadmium has entered the body, it is eliminated from the body at an extremely slow rate. The most important route of elimination of absorbed cadmium, urinary excretion, normally gets rid of only less than 0.01% of the total body burden

per day. The daily urinary excretion rate is usually proportional to the level of body burden and slowly increases with age. The amount of cadmium eliminated from fecal excretion is sometimes comparable with that from urine. Other routes of elimination, such as from hair and breast milk, are insignificant. Due to the extremely slow rate of elimination, the biological half-life of cadmium in humans is very long, approximately 10–30 years.

Health Effects

Cadmium has no known biological function in animals and humans. Occupational exposure to cadmium, mainly through inhalation, can result in metal fume fever, chemical pneumonitis, pulmonary edema, and lung cancer. In this article, only health effects related to oral exposure are discussed.

The acute oral toxicity of cadmium is rarely seen in humans. Exposures to high concentrations of cadmium from heavily contaminated food or beverages can result in GI symptoms including nausea, vomiting, and abdominal pain. The no-observed-adverse-effect level (NOAEL) of a single oral dose is estimated to be 3 mg elemental cadmium per person, and the reported lethal doses range from 350 to 8900 mg.

Toxic effects from chronic oral exposure are a much greater concern than from acute exposures, because they require lower exposure levels and occur more frequently. Chronic exposures to cadmium from contaminated foods have been associated with damages of multiple organs and systems, including the kidneys, bones, and cardiovascular system. Adverse health effects in the endocrine and the neural systems and several types of cancer have also been reported.

Renal Effect

The kidneys are the major target of cadmium toxicity. Limited autopsy examinations in patients with long-term, heavy oral cadmium exposure found kidneys with granular surface, extensive tubular atrophy, and interstitial fibrosis. The molecular mechanisms of cadmium-induced nephrotoxicity are not yet clearly defined, but evidence has shown that mitochondria could be one of the earliest target organelles. Three mechanisms have been proposed: the generation of reactive oxygen species that results in apoptosis or autophagic cell death of renal tubular cells, inhibition of the Na–K–ATPase transport system, and the stimulation of calcium release from the endoplasmic reticulum, causing damage to the mitochondrial membrane.

Renal tubular damage

One of the earliest manifestations of cadmium nephropathy is the damage of the proximal renal tubule, resulting in an elevated excretion of low-molecular-weight proteins (LMWPs) due to the reduced reabsorption. This type of damage is measured by the urinary levels of LMWPs, commonly beta-2 microglobulin (β2MG), retinol-binding protein (RBP), or the activity of N-acetyl-beta-glucosaminidase (NAG). Numerous epidemiological studies have shown that the level of urinary LMWPs is positively associated with cadmium exposure, with a nonlinear dose–response curve. The overall data suggest a two-phasic response, with a rapid increase of LMWPs occurring after the exposure reaches a certain point. The JECFA estimated this transition point as U–Cd at 5.24 μg g creatinine^{-1}. Other estimates for this transition point include U–Cd at 10 μg g-creatinine^{-1} and urinary β2MG at 1000–1500 μg g creatinine^{-1}.

Urinary LMWP biomarkers serve as sensitive screening end points for early kidney damage due to cadmium exposure. In clinical labs, urinary β2MG levels that are less than 300 μg g creatinine^{-1} are usually considered 'normal,' and patients with higher levels are generally recommended for further examination. Moderate tubular proteinuria (β2MG or RBP levels in the range of 300–1000 μg g creatinine^{-1}) is indicative of early or minor tubular damage. However, due to a large functional reservoir of the kidneys, this level of exposure does not lead to clinical symptoms in most cases. In addition, tubular damage at this level is usually reversible after the exposure has ceased. Severe tubular proteinuria, that is, tubular dysfunction, occurs when β2MG or RBP levels are higher than 1000 μg g creatinine^{-1}. At this stage, more generalized biochemical abnormalities can be present, as seen in the Fanconi syndrome. Also, the tubular damage seems to be irreversible. Impairment of tubular function would be progressive even if cadmium exposure is discontinued.

Glomerular damage

Cadmium-induced glomerular damage was evident in a number of *in vivo* and *in vitro* animal studies. The generation of reactive oxygen species has been suggested as the molecular mechanism. Several animal studies and case reports have indicated that cadmium-induced renal damage could eventually progress to end-stage chronic kidney disease (CKD), which requires kidney transplant.

Cadmium-induced glomerular damage has been reported in Japanese patients with *itai–itai* disease. The association between cadmium exposure and decreased glomerular filtration rate (GFR) has been observed in polluted areas in Japan, Poland, and Thailand, but not in Belgium. In the general population, significantly reduced GFR was observed in a Swedish population of women at a mean urinary cadmium level as low as 0.6 μg Cd l^{-1} (comparable with 0.8 μg g creatinine^{-1}). Evidence from the US NHANES and the Korean NHANES has suggested an association between cadmium exposure and reduced GFR or increased albuminuria. These studies provide strong support for the role of cadmium as a CKD risk factor.

Kidney stone

Kidney stone formation has been reported as a long-term health effect among cadmium workers with tubular proteinuria. It has been suggested that calcium metabolism and citric acid uptake could be interrupted by cadmium, leading to urolithiasis. The limitation of these occupational studies is the potential confounding factors resulting from coexposure to other toxic elements, such as lead and arsenic. In the general population, relevant data are extremely limited and the effect remains inconclusive.

Diabetic nephropathy

In experimental animals, cadmium treatment caused pathological changes in pancreatic tissues and elevated blood glucose. Human studies, on the other hand, have given inconsistent

results to support a causal association between cadmium and diabetes. However, a synergistic or potentiating effect of cadmium has been observed in laboratory animals with spontaneous or drug-induced diabetes. Two European cross-sectional studies and one study in China found that diabetic-related renal tubular damage was more severe in those with high cadmium exposure than those with lower cadmium exposure.

Bone effects

Cadmium-related bone disorders were first reported among individuals with long-term, high exposure from contaminated food. In Japan, the endemic of *itai-itai* disease in the 1940s raised health concerns of rice crops grown in cadmium-polluted areas. *Itai-itai* disease is one of the most severe forms of chronic cadmium intoxication. It is characterized by severe bone pains due to osteomalacia, followed by increasingly waddling gait due to bone deformities, and progresses into more severe clinical symptoms so the patients lose the ability to walk, eventually leading to death.

Cadmium may exert its bone effects through interference with calcium homeostasis and vitamin D metabolism. In several animal and human studies, cadmium stimulated the formation of osteoclasts, leading to enhanced bone resorption. Changes in bone metabolism, bone mechanical properties, and bone mineral density (BMD) have been shown in experimental animals following long-term cadmium treatment. In humans, epidemiological studies conducted in Belgium, Sweden, China, the United Kingdom, and the United States consistently showed a negative correlation between cadmium exposure and BMD, suggesting cadmium as a risk factor for osteoporosis.

Since osteoporosis is the main cause of fractures in postmenopausal women, it is scientifically plausible that cadmium is also a risk factor for bone fracture. This hypothesis can be supported by a study in female rats, in which low-level chronic exposure to cadmium enhanced the risk of long-bone fractures. In humans, there have been very few epidemiological studies designed to investigate the relationship between cadmium exposure and the risk for fracture, and they are often different in the selection of study population, the type of fractures recorded, and the adjustment of confounding factors. The dose–response information is lacking, and a threshold dose for bone effects has not been established.

Cardiovascular effects

The cardiovascular effects of cadmium have been observed in animals with the absence of significant renal disease, primarily reported as increased systolic blood pressure. The pathological and biochemical changes have included oxidative damage, endothelial cell apoptosis, lipid accumulation, and interaction with calcium channels.

There have been a great number of epidemiological studies designed to investigate the association between cadmium exposure and hypertension in humans. Among population studies with a considerable large sample size, the results vary from significant positive relationships (in Thailand and Korea), to a weak association (in the United States), to no association (in Belgium), to a negative association (in Japan). Most studies used a cross-sectional design, making it difficult to support a causal association. It has to be noted that different cadmium exposure biomarkers (urinary cadmium and blood cadmium) were used in these studies, and different confounding factors were controlled. Examples of these factors include age, alcohol consumption, cigarette smoking, coexposure to lead, diabetes, reduced renal function, and the use of hypertension treatment medication.

The association between cadmium exposure and the risk of atherosclerosis, peripheral arterial diseases, ischemic heart disease, and stroke has been investigated in a limited number of ecological or cross-sectional studies. Because of the complicity of the etiology and diagnostic criteria for these cardiovascular conditions, the results are often inconsistent, and the overall evidence is not strong enough to support a causal association.

Cancer

Cadmium and cadmium compounds have been classified as known human carcinogens by the International Agency for Research on Cancer (IARC) and the National Toxicology Program (NTP). Most of the evidence is based on the occupational exposure through inhalation, which has been most clearly associated with lung cancer.

Cadmium does not bind directly to DNA, yet it may induce oxidative stress and act as a DNA-damaging agent. In a rodent *in vivo* micronucleus study, cadmium chloride induced micronuclei formation in bone marrow and peripheral blood. In addition, cadmium has also been found to compromise the repair of DNA adducts in an *in vitro* system. Chemical carcinogenesis has not been shown in animals except for one rat study, in which a zinc-dependent increase in prostatic proliferation was observed.

In humans, only a limited number of epidemiological studies have demonstrated an association between chronic cadmium exposure and cancers of the breast, endometrium, kidney, pancreas, and urinary bladder. Further studies are needed to confirm these results.

Other Effects

The reproductive and developmental effects of cadmium have been reported in several animal studies. In pregnant rats, oral exposure to cadmium induced the level of metallothionein in the placenta. Elevated levels of cadmium were found in dam blood, the placenta, and fetal blood and fetal brain. Other developmental effects observed in animals include reduced birth weight and skeleton malformation. In humans, the level of cadmium in maternal blood is not highly correlated with that in fetal blood, but is correlated with the level in the cord blood and the placenta.

Some neurotoxicity and neurobehavioral effects have been observed in rodents, including changes in the levels of certain neurotransmitters and abnormal behavior in offspring. In humans, the blood–brain barrier can limit cadmium's access to the central nervous system. However, infants and children may be more susceptible because the blood–brain barrier has not fully developed in the early life stages.

See also: Cadmium: Properties and Determination; Dietary Exposure Assessment; Dietary Surveys: National Food Intake; Heavy Metal Toxicology; Renal Function and Disorders.

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Caffeine: Characterization and Properties

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Introduction

In the minds of most Europeans caffeine is strongly associated with coffee. Indeed, this compound was first isolated in coffee 200 years ago, and this isolation led to its naming. Caffeine occurs in plants of different families, with traditional styles of human consumption. It has a mild stimulating effect on the central nervous system. Caffeine-containing coffee and tea plants are important contributors to the global economy. Since the seventies of the last century, the biosynthesis of caffeine, its in-plant transfer, and degradation have been thoroughly investigated.

Studies on caffeine's mechanism of action, stimulation, and health effects are ongoing. New fields of use have provoked increasing research. The next article in this encyclopedia is dedicated to caffeine consumption and related health effects.

Discovery and Naming

In 1819, the German poet Goethe presented some coffee beans to the chemist F.F. Runge in Jena for investigation. Runge was successful in this task. The aqueous extraction of the beans, both green and roasted, the precipitation of coffee acids, and separation eventually allowed the crystallization of the substance he called 'Kaffeebase,' the base of coffee, identifying its activity on salamanders. He published his methodology and product description in early 1820. Much later, in 1866, Runge recounted the start-up story, with his introduction to Goethe.

Concurrent research on coffee was done using similar procedures at some other centers in Europe. Within a short interval of time, multiple investigators had identified the same substance. Later in 1820, F. von Giese published his findings on coffee in present-day Estonia, calling the substance 'Kaffeestoff.' In 1821, the French senior pharmacist P. J. Robiquet verbally presented his results to the pharmaceutical society in Paris, and he used the word 'caféine,' which he had adopted from an unconvincing attribution by R. Chenevix, some years earlier. This name was accepted by scientific consensus, and in 1822, it was accepted for a short entry in a dictionary of medicine by P.J. Pelletier. The following year, the term caffeine came out at the Royal Academy of Science in Paris, in a report on alkaloids by Pelletier, in which he mentioned Robiquet's earlier note. Pelletier and his companion B. Caventou had ceased their own research on coffee in favor of Robiquet. In 1823, the latter published the details of his extraction as part of another dictionary's article on coffee, and his procedure became the standard methodology of its time, being discussed in depth in the French pharmaceutical journal (*Journal de Pharmacie et des Sciences Accessoires*) in 1826.

The community researching caffeine in Paris was open to guest scientists from all over Europe, and members communicated with corresponding societies via the exchange of letters and printed proceedings, posted per stagecoach.

Reporting to the Swedish academy about the research in France and the related notes of 1826, with all his authority, J.J. Berzelius acknowledged the seniority of Runge on the isolation of caffeine, thus disclosing his significance to the European scientific community.

In the following years, the search for the active mechanisms of other stimulating plants of human interest and use resulted in several discoveries of substances that were later identified to be identical to caffeine. In 1826, guaranine was detected in guaraná seeds by T. Martius. In 1827, V. Oudry identified theine in tea leaves, and its equivalence to caffeine was clarified by Martius in 1840 and C. Jobst in 1838. In 1836, an alkaloid was found in maté leaves by B. Trommsdorf, and this substance was identified as caffeine by J. Stenhouse in 1843. In 1865, caffeine was detected in the kola nut by W.F. Daniell, and in cocoa beans by E. Schmidt in 1883. All these researchers thoroughly described and characterized the compound, based on its physical and chemical properties, as crystalline needles, slightly bitter tasting, and soluble in hot water.

Caffeine's molecular formula, $C_8H_{10}N_4O_2$, was determined by C. H. Pfaff and J. Liebig in 1832. In the century's last quarter, the eventual structural formula of caffeine was fixed (Figure 1), first proposed by L. Medicus of Wuerzburg, Germany, based on cleavage reactions in 1875, and later confirmed via synthesis from well-known educts by E. Fischer and L. Ach in Berlin in 1898. The total synthetic pathway is shown in Figure 2.

Dimethylurea reacts with malonic acid to form the pyrimidine ring with properly placed methyl and keto groups; nitrosylation with nitrous acid, with subsequent reduction on platinum, introduces the amino group in position 5, where reaction with potassium cyanate provides the missing atoms for the imidazole part, followed by acidic ring closure to form dimethyl uric acid. Via chlorination with phosphorus pentachloride in position 8, as well as reduction with fuming hydroiodic acid, the dimethyl xanthine theophylline is produced. The last step is methylation with methyl iodide in position 7 to yield caffeine. The synthesized caffeine was checked against naturally occurring caffeine, using mixed melting point methods.

Nomenclature

In the nomenclature of the twenty-first century, the molecule caffeine is referred to as 1,3,7,trimethyl-2,6-dihydro-purin-2,6-

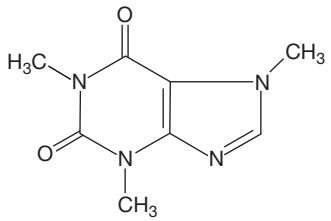


Figure 1 Caffeine – structural formula.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00098-2

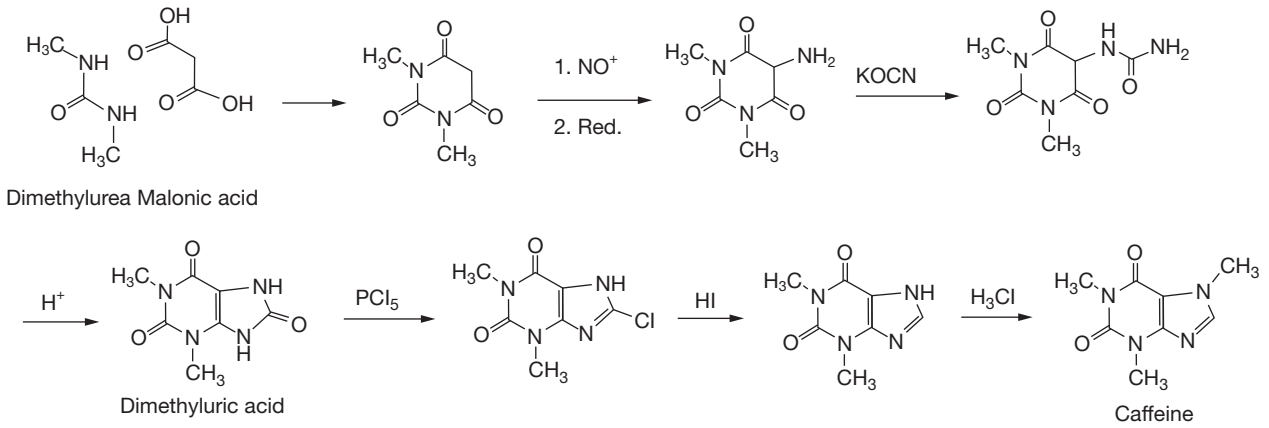


Figure 2 Reaction scheme of Fischer's synthesis of caffeine 1898 (edited from Kunz, H., (2002). Emil Fischer – unequalled classicist, master of organic chemistry research, and inspired trailblazer of biological chemistry. *Angewandte Chemie, International Edition*, 41, 4447).

dione, or by the shortened form 1,3,7-trimethylpurine-2,6-dione. Caffeine is accepted as synonymous, however. The parent skeleton is purine, a bicyclic system, which consists of two fused heterocycles, the five- and six-membered imidazole and pyrimidine, respectively. The multiplicative prefix trimethyl marks the substituents, and the suffix dione indicates the functional keto groups in caffeine. Purine is a constituent of nucleic acids.

The name purine was created by Emil Fischer in 1884. In 1897, he proposed its ring numbering, as shown in Figure 3. This customary naming and numbering was used in the standard reference works of that time, the German *Beilstein Handbook of Organic Chemistry* and in the American *Chemical Abstracts* since their first editions. In 1957, the parent name purine was declared to be a 'retained trivial name' in the rules for the nomenclature of organic chemistry established by the International Union of Pure and Applied Chemistry (IUPAC), which was based in Oxford, United Kingdom, at the time, and in the same paper Fischer's customary numbering system for purine was accepted as an exception to the systematic numbering. These naming conventions were then confirmed repeatedly. In December 2013, with a new approach in organic nomenclature, purine was upgraded to a 'preferred IUPAC name' (PIN), a decision published in the IUPAC's actual *Blue Book*. The IUPAC's naming principles continue to "allow alternatives, in order to preserve the diversity and adaptability of the nomenclature to daily activities in chemistry and in science in general," as stated in the *Blue Book*, that is Favre and Powell.

There is no doubt that this modus operandi would matter to purine (PIN) and caffeine. 'Caffeine' is allowed, without explicit IUPAC mentioning, as an alternative for the trimethylated purine dione. The more comprehensive name is 3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione. Another trivial name widely used for the unmethylated purine dione is xanthine; its subsequent methylation forms part of the well-studied metabolism involved in caffeine biosynthesis and degradation. Caffeine may also be named 1,3,7-trimethyl-xanthine. Figure 4 shows the covalent structure of caffeine – the customary skeleton numbering is marked at the locants. Classic mesomeric zwitterionic structures using the valence bond model (depicted in Figure 4(a)) have stepped down in favor of the molecular orbital view;

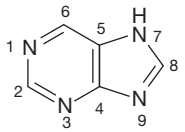


Figure 3 Purine – structural formula with customary numbering.

computational methods with software package allow calculation of partial charges (marked in Figure 4(b) and visualization of molecules.

Physical and Chemical Property and Safety Data

The chemical and physical data for caffeine are well known. It is a solid, odorless, slightly bitter-tasting, sublimating substance that crystallizes into white needles. In solution, it absorbs in the ultraviolet region, and solubility differences aid analytical separation and purification. Caffeine is both hydrophilic and lipophilic, thus easily passing through biological membranes and barriers. Its bitterness is perceived throughout the oral cavity; it is described as long lasting, and it is used as a standard for bitter-tasting calibrations. When crystallized from aqueous solutions, caffeine forms a nonstoichiometric stable hydrate. Caffeine salts, like citrate, are part of pharmaceutical compounds. Anhydrous caffeine occurs in two modifications. The low-temperature-stable form is the β modification, and at 141 °C, it is transformed to the α modification, which has a higher rate of dissolution. On cooling, the α phase converts very slowly back to the β phase, which is important for pharmaceutical preparations.

Several spectroscopic methods are available for caffeine analysis and authenticity control. The basic spectra are referenced in the analytical section.

Caffeine in solutions undergoes self-assembly: When a critical aggregation concentration is exceeded, caffeine molecules in solution cluster predominantly to dimers, with coplanar positions of the caffeine moieties, staggered at about 90°, with a distance of 3.5 Å.

The association of caffeine with the different phenolic compounds of caffeine plants follows the same principles. The

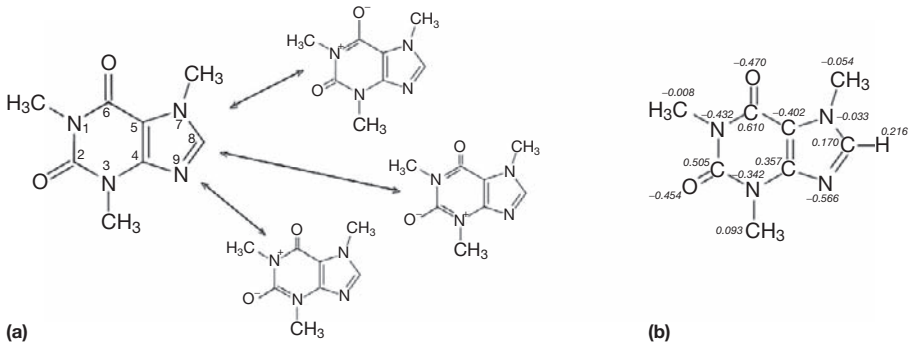


Figure 4 Caffeine – covalent structure with customary numbering, mesomeric structures (a) and atomic partial charges (b), the latter with data from Tavagnacco, L., Schnupf, U, Mason, P. E., et al., (2011). Molecular dynamics simulation studies of caffeine aggregation in aqueous solution. *Journal of Physical Chemistry B*. **115**, 10957–10966.

complexes are investigated since the fifties of the last century, inter alia by using ultraviolet (UV), infrared (IR), crystallographic, and nuclear magnetic resonance (NMR) data. With the latter method, the associates are observed by changes in the NMR chemical shift of caffeine's methyl protons, studied both in model solutions and in coffee brew.

Table 1 shows the property figures of caffeine, outlined in the *Chemical Abstracts Register*, which contains references offering additional information.

In regard to occupational health effects, the vendors of chemicals are obliged to provide a material safety data sheet for the substances in their portfolio, with property and safety data as well. For caffeine, the acute toxicity estimates on the possible route of intake reported are low, and the risk of accidental ingestion, contact, or inhalation at the worksite is minor. Thus, in the hazard classes under scrutiny, caffeine is assigned to the least hard categories. The respective labeling is at the lowest warning level. For transport, no dangerous goods marking is necessary.

Attempts to manage this information led to a compilation of International Chemical Safety Cards, a system in which caffeine is included with a peer review status, as of 1998, with partial updates in 2005. To enter the system, the substance name is requested, or its number in the registry of the Chemical Abstracts Service, CAS, in the Registry of Toxic Effects of Chemical Substances, RTECS, or the number in the Hazardous materials description of the United Nations (UN number).

Health-related property data for caffeine, with a focus on carcinogenicity, were covered by a monograph of the International Agency for Research on Cancer of the World Health Organization (IARC) in 1991. Caffeine is in Group 3, not classifiable as to its carcinogenicity to humans.

In 1958, the American Food and Drug Administration (FDA) declared caffeine as 'generally recognized as safe,' when added to cola-like beverages up to 200 mg caffeine per liter of beverage — the famous status 'GRAS,' confirmed in 1978 with a report. Since then, newly designed compositions came to the market. In November 2010, FDA sent a warning letter to producers of caffeinated alcoholic beverages, informing them, that caffeine co-consumption with alcohol would not be covered by the GRAS definition of cola-like drinks.

The scientific opinion of the European Food Safety Authority on the safety of caffeine, issued in May 2015, evaluates the published studies on the topic. The conclusion was, that single doses up to 200 mg caffeine and habitual intakes up to 400 mg per day from all sources (3 or 5.7 mg per kg bodyweight, respectively) do not give rise to safety concerns for adults. For pregnant women, the habitual consumption should be halved. For children and adolescents, a first approach is based on the bodyweight frame, until specific data for this age group will become available.

Caffeine Sources and Uptake

Caffeine is said to be the most widely consumed psychoactive substance. It is used as a stimulant in both food and pharmaceuticals, and its consumption and effects are considered in the next article in this encyclopedia.

Sources of caffeine include plants and direct chemical synthesis.

Industrial Sources

For pure caffeine, data on industrial production are rare. The Organization for Economic Cooperation and Development (OECD) in Paris has submitted summaries in a 2002 "Screening Information Data Set" (SIDS) of high-volume production chemicals. The data are repeatedly cited: "1999 the estimated world production amounted to 10.000–15.000 tons including 3.000–4.000 tons of natural caffeine" from decaffeination. Since then, a moderate increase in production has occurred. In 2011, China was the main supplier of synthetic caffeine, and the country's leading manufacturer reports a production volume of 8000 tons.

In the early 1900s, industrial decaffeination from caffeine plants was developed in Europe, with a focus on the production of decaf coffee. At about the same time, caffeine production via the decaffeination of imported tea wastes started in the United States, in response to soft drink demand. This process is still in use, as recently reported from Turkey and India, which were number 6 and number 2 in 2013 world tea production. The decaffeination of coffee pulps (i.e., the coffee waste in coffee-producing countries) is also being developed.

The changed priorities for coffee decaffeination are best illustrated by the title of a scientific article on decaf in 2012 "Which is the by-product: caffeine or decaf coffee?" In beverage

Table 1 Properties of caffeine, from the CAS Registry (if not otherwise marked); website source www.cas.org accessed February 2015, identifying the references therein

Property	*Value*		
Molecular formula	$C_8H_{10}N_4O_2$		
Molecular mass	194.19 Da		
Status at ambient conditions	Solid, dimorphic		
Appearance, taste and smell	Colorless ('white'!), bitter tasting, odorless		
Crystalline forms[a]	Hydrate 0.8 H_2O, stable up to 51.5 °C, dehydration to stable β phase		
	Anhydrous β phase		
	Anhydrous α phase (stable up to sublimation/melting)		
	Metastable α phase; on cooling down from stable α phase, back-transformation to the β phase is kinetically restrained		
Dimers in aqueous solution[b]	Self-associated caffeine dimers, coplanar, staggered, at concentrations above the solubility limit[c]		
Stability point of hydrate 0.8 H_2O	51.5° (quadrupol point)[d]		
Transformation point $\beta \rightarrow \alpha$	141 °C[a]		
Sublimation point	178 °C		
Melting point (in fused capillary)	236 °C		
Density [g cm^{-3}]	1.45 at 25 °C/α phase[e]		
	1.23 at 18.7 °C[f]		
Ultraviolet absorption maximum λ_{max} (nm)	274 (aqu.)		
Apparent molar absorption coefficient[h] ε_a (l mol^{-1} cm^{-1})	9.750 ± 75 (aqu., $c = 0001\%$), lit. average[g]		
Dissociation constant	pKa 10.4 at 40 °C (acid–base constant)		
Mass solubility aqueous (mass ratio, %)	Concentr (%)	Temp (°C)	Remarks
	2.2	20.0	pH 6.9
	46.8	83.9	
	81.0	100.3	Extrapol. value
Mass solubility, other solvents	Ethanol: slightly		
	Ether: insoluble		
	Chloroform: very good		
Dipole moment, *D*	3.7 (benzene)[i]		
Octanol/water partition ratio	−0.091 at 23 °C (log Pow)		

Property data taken from the CAS Registry online (February 15th, 2015) and the following:

[a]Bothe, H., Cammenga, H. K. (1979). Phase transitions and thermodynamic properties of anhydrous caffeine. *Journal of Thermal Analysis* **16**, 267—275.

[b]Iza, N., Gil, M., Montero, J. L., and Morcillo, J. (1988). Self-association of caffeinein aqueous solution. Study of dilute solutions by normal and second derivative UV absorption spectroscopy. *Journal of Molecular Structure*, **175**, 25–30.

[c]Sanjeewa, R. and Weerasinghe, S. (2011). Study of aggregate formation of caffeine in water by molecular dynamics simulation. *Computational and Theoretical Chemistry* **966**, 140–148.

[d]Epple, M., Cammenga, H. K., Sarge, S. M., Diedrich, R., and Balek, V. (1995). The phase transformation of caffeine: investigation by dynamic X-ray diffraction and emanation thermal analysis. *Thermochimica Acta* **250**, 29–39.

[e]Bothe, H. and Cammenga, H. K. (1980). Composition, properties, stability and thermal dehydration of crystalline caffeine hydrate. *Thermochimica Acta* **40**, 29–39.

[f]Pfaff, C. H. and Liebig, J. (1832). Ueber die Zusammensetzung des Caffeins. *Annalen der Pharmacie* **1**, 17–20.

[g]Eisenbrand, J. and Pfeil, B. (1956). Beitrag zur Bestimmung des Coffeins in verschiedenen Lebensmitteln. *Z. anal. Chemie* **151**, 241–258.

[h]Mejri, M., BenSouissi, A., Aroulmoji, V., and Rogé, B. (2009). Hydration and self-association of caffeine molecules in aqueous solution: comparative effects of sucrose and β-cyclodextrin. *Spectrochimica Acta Part A* **73**, 6–10.

[i]Weiler-Feilchenfeld, H. and Bergmann, E. D. (1968). *Israel Journal of Chemistry* **6**, 823.

manufacture, the eventual claim 'natural' might be a driving force, to use added caffeine obtained from a plant product.

Milestones in the development of industrially feasible caffeine synthesis include the Traube synthesis of 1900 (too many steps with low yields, but a backbone of further improvements), the Bredereck synthesis of 1950 (developed during World War II, facing the constraints of limited accessibility of educts by starting with xanthosine from yeast), and the novel Zajac synthesis of 2003, starting with inexpensive uracil. For the latter, a reaction scheme is given in Figure 5. Uracil is double-methylated with methyliodide, the 1,3 dimethyl-uracil is nitrated with a mixture of nitric and sulfuric acid, and it is then reduced with iron or hydrochloric acid in tetrahydrofuran to yield the respective amino-uracil. The latter is acylated with formic acid and nitrated again at the remaining 4-position of uracil; heating with iron in acetic acid allows simultaneous reduction and intramolecular heterocyclization to produce theophylline, which is N-methylated to yield caffeine. For almost a century, the fully synthetic caffeine could not compete economically with caffeine from decaffeination, however.

Agricultural Production

Volumes of agricultural products are published by FAO, the United Nation's food and agriculture organization in Rome, on a regular basis. Caffeine-containing plants deliver globally traded commodities of high overall economic value. For the most important caffeine products, Table 2 shows the ranking

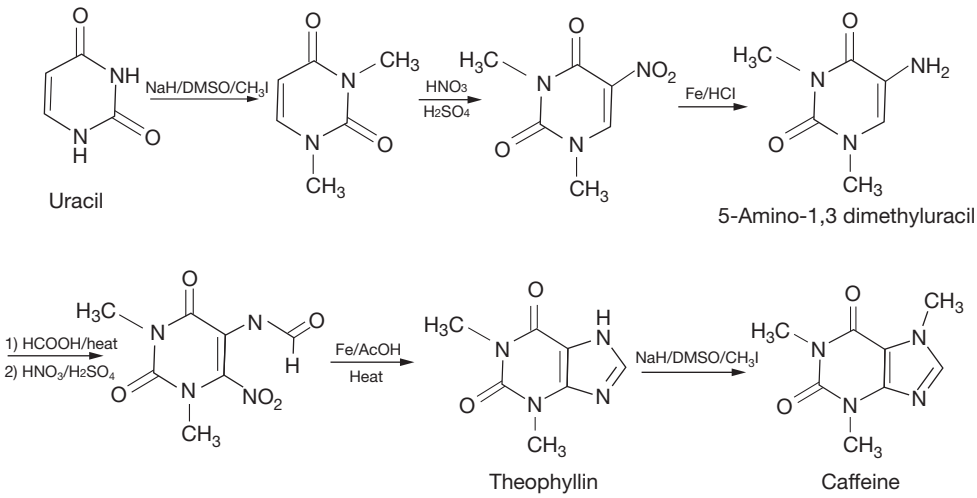


Figure 5 Reaction scheme for Zajac's novel method of caffeine synthesis 2003 (edited from Zajac, M.A., Zakrzewski, A.G., Kowal, M.G. and Narayan, S. (2003). A novel method of caffeine synthesis from uracil. *Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry*, **33**, 3293.)

Table 2 Caffeine sources from plants, with plant botanical naming, caffeine contents, and average production volumes of the caffeine products

Plant source	*Botanical species*	*Caffeine content (% d.b.)*	*Production average (tonnes per year)*	*Market forms, remarks*	*Use*
Guaraná seeds	*Paullinia cupana* Kunth	2.5–5%	3,900	Seeds, dried, powdered or pounded into a stick and roasted	Beverage/masticatory (caffeine supply for other products)
Tea leaves	*Camellia sinensis* (L.) Kuntze	2–4.5%	4,800.000	Black, oolong, green, and white tea from different postharvest treatments	Beverage (dust and stalks used to gain caffeine)
Coffee seeds	*Coffea canephora* Pierre ex A.Froehner	1.5–2.6%	8,600,000	Roast coffee, roast and ground coffee, instant coffee, convenience preparations	Beverage (caffeine from decaffeination used for other products)
	Coffea arabica L	0.9–1.9%			
Coffee husks	*Coffea arabica*			Arab specialty 'Qishr'	Beverage
Kola nuts	*Cola nitida* (Vent.) Schott & Endl.	1.5%	290,000	Dried	Masticatory/beverage
Maté leaves	*Ilex paraguariensis* A.St.-Hil.	0.5–1.5%	790,000	Dried, ground, and slightly roasted	Beverage
Cacao beans	*Theobroma cacao* L.	0.2%	4,500,000	Cacao beans, cocoa mass, cocoa powder	Beverage, chocolate bars

Plant source: customary name.

Botanical species: *The Plant List* (2013). Version 1.1. Publication on the Internet; http://www.theplantlist.org/ (accessed January 1st, 2015) Caffeine content: Mass percent on dry basis.

Production average: 2009-2013 data from FAOstat (web sourceww.faostat3.fao.org accessed February 2015); for Guaraná from IBGE (weweb sourcew.ibge.gov.br/home/estatistica/indicadores/agropecuaria/lspa/ accessed February 2015).

in caffeine content and the volumes of agricultural production as 5-year averages.

The data suggest that caffeine plants, roughly calculated, can provide about 300 000 tons of the biomolecule for human uptake per year. That is an order of magnitude above the amount of the chemical as derived from industrial synthetic production.

In the FAO definition of agricultural corps, coffee, tea, and maté are stimulant crops because they contain caffeine, but cacao, due to its additional nutritional value, is both a stimulant and food crop; kola nuts are separately categorized as edible nuts. Guaraná seeds, which are the highest in caffeine content, are not included in the FAO statistics, because their agriculture is negligible in volume. For the purposes of this article, guaraná data are taken from publications of the Brazilian Institute of Geography and Statistics (IBGE). Table 2 also mentions Qishr, husks of the coffee seed, traditionally used in Arabian countries to prepare a tea-like beverage; coffee husks are mentioned in the FAO commodity list, but without reported quantities; it is not a primary crop.

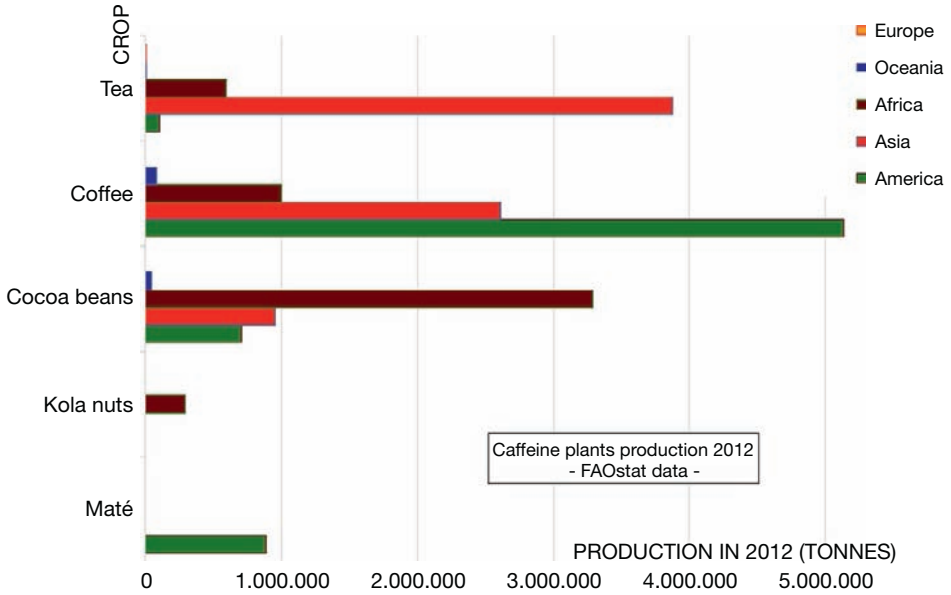


Figure 6 Agricultural production of caffeine containing plant commodities per crop and continent, 2012 volumes in tonnes, data from FAOstat (web sourcewww.faostat3.fao.org accessed January 2015).

Natural Occurrence and Volumes

In all nature, more than 60 plant species are known to contain caffeine, and coffee and tea are almost ubiquitously present to consumers. Surprisingly, up to the end of the fourteenth century, Europe was one of the few regions of the world where caffeine-containing drinks were entirely unknown.

For an outline, the 2012 crop volumes are summarized per continent in Figure 6. Tea is predominantly grown in Asia. Cocoa cultivation is centered on the African continent. Coffee production volumes are more balanced around the globe's tropic belt. Kola nut and maté are produced exclusively in Africa and America, respectively. According to the FAO, 'Oceania' comprises Australia, New Zealand, and the Pacific islands, which are fine but very small origins of some caffeine plants.

Political and economic factors influence the long-term production pattern: the FAO archives maintained since 1961 reveal an explosive coffee increase in Asia since 1995—Vietnam!—a starting coffee volume in China, and a decline of tea from Europe. The teas from the territories of the former Soviet Union are gone.

Uptake

The ways of human uptake of caffeine vary widely.

It is swallowed in beverages produced from caffeine-containing plants, such as coffee, tea, maté, and chocolate (these beverages mark the main uptake in volume), and in water-based flavored drinks, so called soft and energy drinks, to which caffeine is added. Caffeine is eaten in products such as chocolate bars, and it has been traditionally chewed, as with kola nuts. New routes of uptake arise. To facilitate on-the-go ingestion, energy drink preparations are offered as gels to swallow; some energy preparations for accelerated uptake also include chewing gums, patches with gels or microemulsions for skin penetration, and vapor sticks to imitate the idea of e-cigarettes.

Caffeine is used in pharmaceutical preparations in great volumes, primarily as an adjuvant in analgesic combinations, both in over-the counter and in prescription medicine, and in minimal volumes in orphan drugs for rare diseases such as neonate apnea. Caffeine is also part of some cosmetic formulations with transdermal uptake.

Ingested caffeine is directly absorbed in the mouth or via the stomach and intestine within 45 min, and it readily spreads into all body tissues. Caffeine clearance happens via catabolism in the liver, with successive demethylations, oxidation to uric acid, cleavage of the imidazole ring with remaining uracil, and finally the break down to ammonia and exhaled carbon dioxide. Only a little caffeine is excreted unchanged.

Dispersal

Caffeine's primary use by humans, either as a food or drink preparation or via pharmaceutical or cosmetics production, causes the chemical to enter waste waters and landfill leachates. The biodegradation of caffeine into constituent compounds requires some 3–6 weeks. Due to its biodegradability, bio- and geoaccumulation are not expected. Nevertheless, increasing dispersal in coastal waters is observed, and caffeine is found in marine fauna of coral reefs.

Caffeine-Growing

Caffeine is located in different tissues and organs within the plants that produce it; in these locations, it experiences synthesis, storage, transfer, metabolism, and degradation.

Biosynthesis

Caffeine biosynthesis in plants is well established during the last 40 years, with comprehensive overviews since 2000, notably by Ashihara. It follows a general pathway, starting with the DNA's purine base xanthosine. This is methylated at N-7 using xanthosine-methyltransferase, which depends on *S*-adenosyl-L-methionine (SAM) as a methyl donor, itself turning to S-adensoyl-homocystein (SAH). Next, the ribosyl unit is removed with the help of *N*-methylnucleosidase. The resulting 7-methylxanthine is methylated at N-3, catalyzed by another enzyme, 7-methylxanthine transferase (theobromine synthase), again with SAM as methyl donor, to produce theobromine. Further methylation with 3,7-dimethylxanthine transferase (caffeine synthase) at N-1 of the xanthine eventually leads to caffeine. The first methylation at the xanthosine level is the rate-determining stage.

The structures of the involved enzymes are known, their encoding genes have been consolidated in a multicentered work on the coffee genome in 2014 ('provides insight into the convergent evolution of caffeine biosynthesis', says the title). The main biosynthetic pathway is shown in Figure 7, which marks the involved enzymes together with their enzyme code numbers. Additionally, minor routes via theophylline or paraxanthine exist. Methylxanthines from the side pathways of biosynthesis may accompany caffeine in the composition of caffeine-plant products. They form plant-specific methylxanthine patterns, providing a useful tool for plant-source identification.

In-plant Localization

In the plant cells, caffeine is synthesized in the cytoplasm, translocated, and stored in intracellular membrane-bound compartments, the central vacuoles. It is in tight complexation with phenolic molecules of the respective plant.

These phenolics are chlorogenic acids for coffee and mate; catechins for cocoa, kola nut, and guaraná; and tannins for tea. In the respective complexes, different interactions take place. Equilibrium constants are determined. The caffeine chlorogenate complex is described as an 1:1 type, π-molecular complex stabilized by hydrophobic interactions.

The release of caffeine from its complex in plant products was an analytical challenge of the early encounters with caffeine in coffee: Runge achieved the separate preparation of Kaffeesäure (coffee acid) and Kaffeebase (caffeine) by breaking the complex via the precipitation of the acid with lead acetate and the isolation of caffeine from the filtrate. Robiquet achieved the same result with magnesium acetate.

Basic research was done, comprising the localization of xanthines in the tissues and organs of the plant, and their ways and distribution during plant development. The key aspects are similar in all caffeine plants.

Caffeine as a Secondary Metabolite

For plant biology, caffeine is classified as a secondary metabolite, that is, it is not primarily essential and active for growth and reproduction, but it is useful for adaptation to the environment. Caffeine may serve as a chemical defense against predators, or it might contribute to the allelopathic prevention of competing up-growth, or it might help attract supporters – a fashionable keyword is 'bioecology.'

As the plant accumulates caffeine in direct proportion to the risk of predation, it is synthesized in the outer parts of young leaves and fruits, and on their epicuticular waxes. On the ground surrounding the individual plant, the phytochemical is released by the seed coat of dropped fruits or from fallen leaves, which may prevent the germination of competing seeds on the same ground. On the other hand, the caffeine content of the blossom's pollen and nectar acts as an incentive to pollinators attracted by the scent; and crosspollination by bees may help to maintain the genetic diversity of caffeine-containing plants. The experimental confirmation of the presumed intention-and-effect chains requires a sophisticated study design, however. With the turn of the century, the general

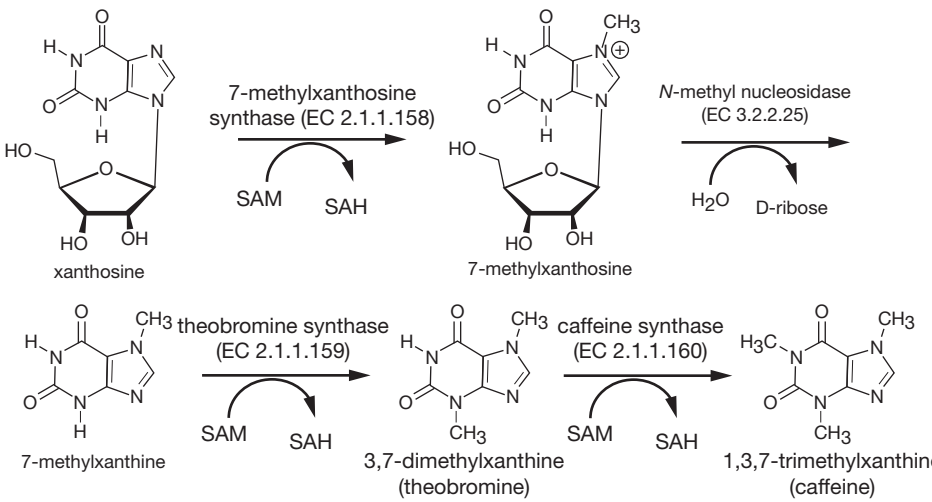


Figure 7 Main pathway of caffeine biosynthesis in plants compiled from different sources: (1) Ashihara, H., Kato, M. and Crozier, A. (2011) Distribution, biosynthesis and catabolism of methylxanthines in plants. In Fredholm, B.B. (ed.) *Methylxanthines*. p.17. Berlin: Springer. 2) Kanehisa, M., (2013). KEGG database, Pathway map Caffeine metabolism. Kyoto, Japan (web source http://www.genome.jp/kegg/ accessed June 2015).

emphasis of research changed to field studies in order to convince the producers to adopt the ideas of bioecology and moderate agroforestry. Follow-up crop management is covered in the articles on coffee, tea, and cocoa.

Caffeine-Containing Plants

The most important caffeine-containing plants are named in Table 2. Of these, coffee, tea, and cocoa are itemized in individual articles of this encyclopedia: two articles are dedicated to cocoa, four deal with coffee, and three articles describe tea.

Maté, kola nut, and guaraná are discussed in more detail in this article. They are the next most commonly produced caffeine-containing plants. In the years 2011–13, their use as herbal medicine in the European Union was assessed, with a focus on the use of maté, kola nut, and guaraná as powdered leaf or seed, all of which appear to be nontoxic preparations for oral use.

Other caffeine synthesis happens in some citrus plants, where nectar and pollen may contain caffeine.

Caffeine plants grow in the tropic zone north and south the equator. Further climatic requirements limit their cultivation to distinct environments, altitudes, and geographic regions. Weather conditions in the crop year are connected to strong up- and downturns in the outcomes of sensitive countries, with big economic impacts.

Figure 8(a)–8(d) shows the 2013 worldwide distribution of the six main caffeine crops, using the basic data from the FAO, aggregated by country, as well as Brazilian statistics.

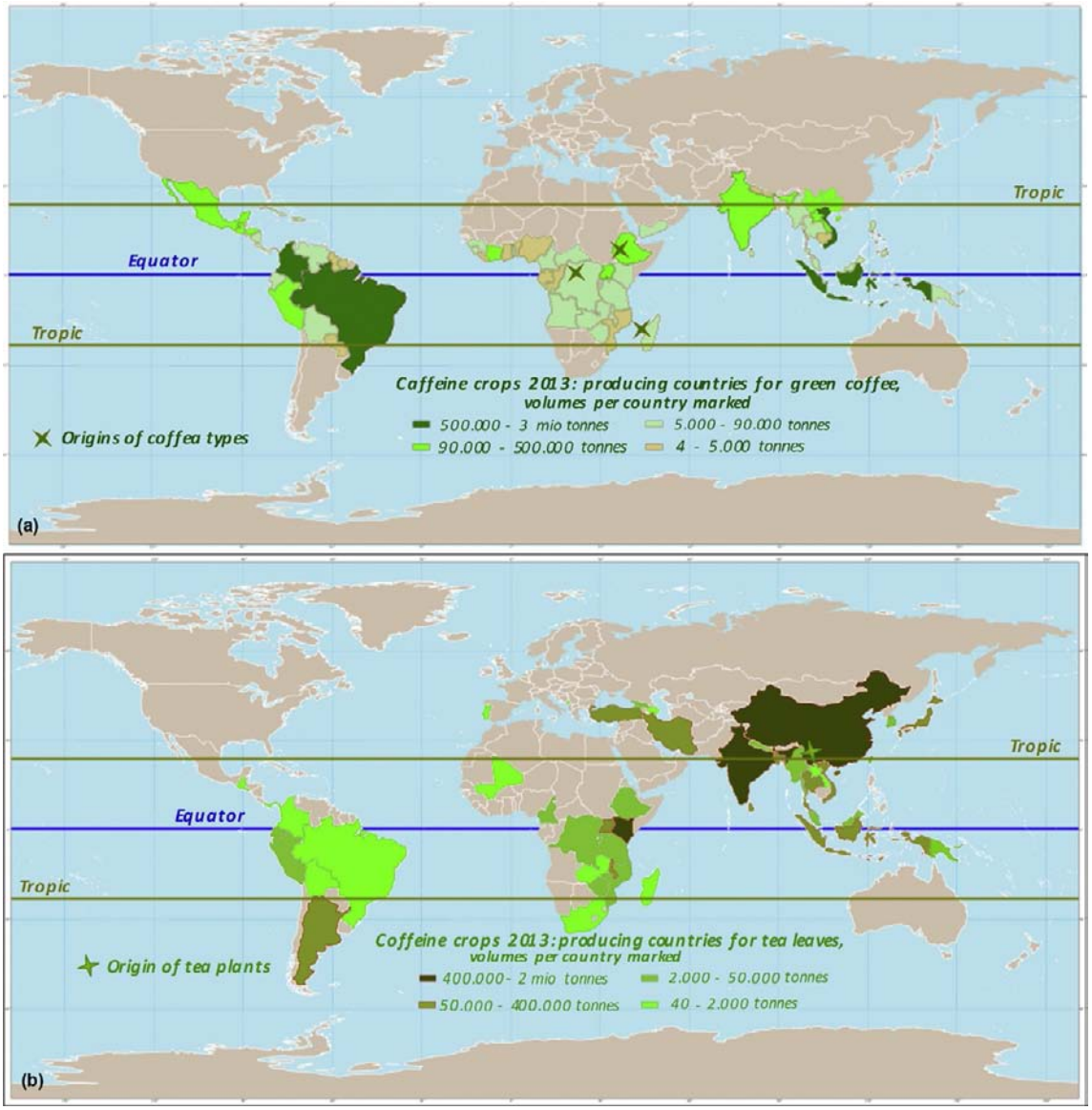


Figure 8 (a–d) Agriculture on caffeine products 2013, crop- and country wise marked on world maps, subtitles within the figures data from FAO and (for Guaraná) Brazil statistics. Web-sourcesFAOstat, http://faostat3.fao.org/download/Q/QC/E (accessed 11 Jan 2015 and 6 March 2015), and IBGE, http://www.ibge.gov.br 'Levantamento Sistemático da produção Agrícola', LSPA 2014 (accessed 6 March 2015).

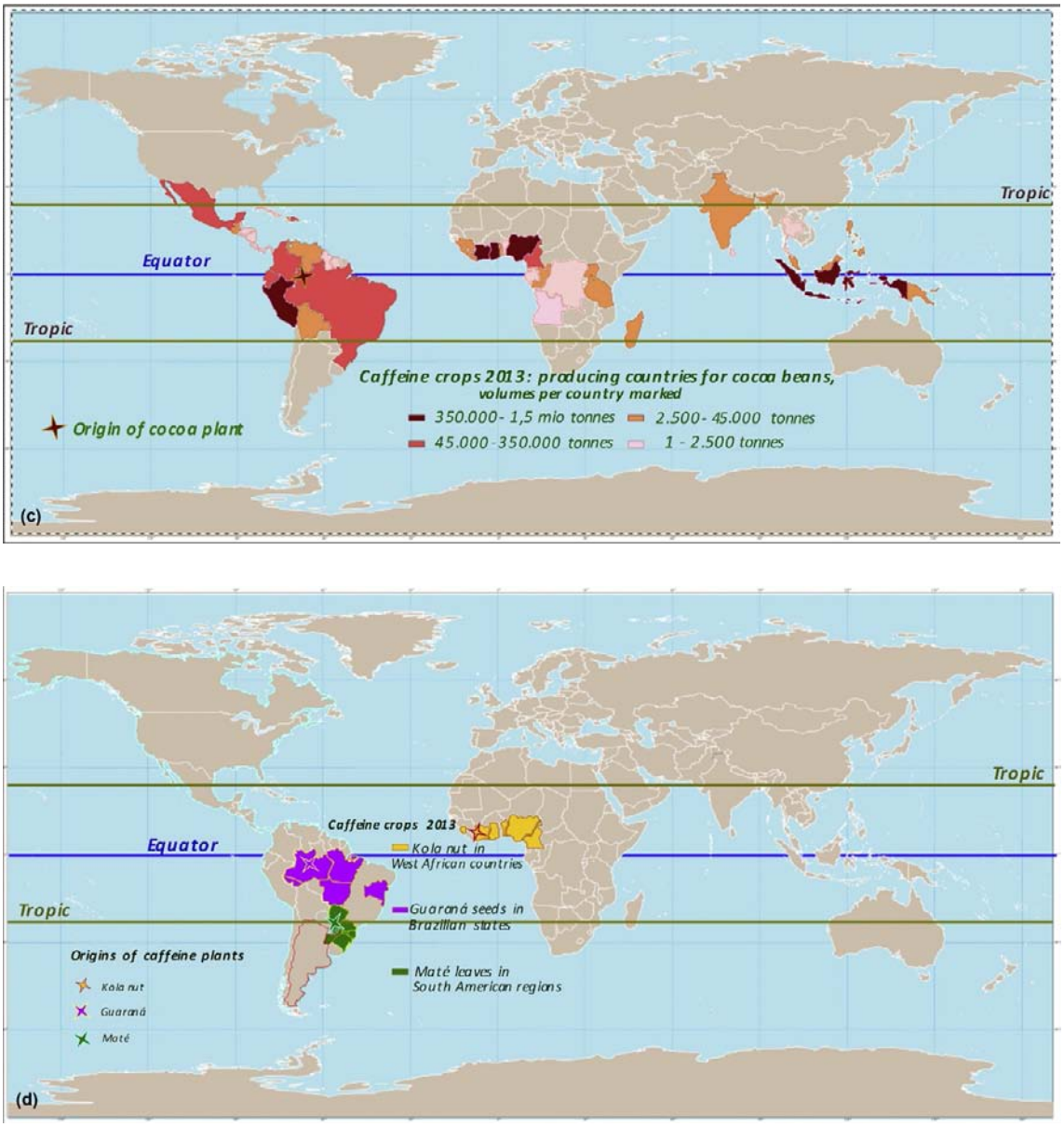


Figure 8—cont'd

The figures visualize the respective cultivation belts around the globe in four crop dedicated maps. The basic grids are world maps with political borders, with mark-ups on the surface of the countries that cultivate the respective caffeine-containing plants; the differences in production volumes are discretely implied by coloration. The place of the plants' genetic origin is indicated, as established by history and sophisticated phylogeographic analysis, lastly by Anthony for coffee in 2010.

Figure 8(a) shows the 80 countries that maintain coffee crops; Brazil is by far the biggest producer; Ethiopia, the cradle of Arabica coffee, is no. 7; on the Northern rim of the tropics, China is emerging with her Southern regions (only those are marked).

Figure 8(b): Fifty countries cultivate tea, outreaching the tropic belt to both sides; China is the top producer.

Figure 8(c): Sixty countries have cacao agriculture, with an eye-catching concentration very near the equator, in West Africa (Ivory coast!) and Indonesia.

Figure 8(d) the production sites of kola nut, mate and guaraná do not overlap, thus they are visualized together in Figure 8(d). The productions are retained at the genetic origin of the plants. Kola nut is grown in some coastal countries of West Africa. Mate is harvested in an even smaller region in the triangle of Argentina, Brazil, and Paraguay, where only the really producing districts are marked. Guarana has spread from its Amazonas origin to neighboring Brazilian states; only those are marked.

The following descriptions are in order of the product's caffeine content, as given in Table 2.

Guaraná

The seeds of the guaraná plant, *Paullinia cupana*, of South America are traditionally consumed as a stimulant beverage. Guaraná belongs to the family *Sapindaceae*, genus *Paullinia* L.; the species is *Paullinia cupana* Kunth; and the chemotaxonomy was accepted for *The Plant List* in 2012. Another *Paullinia* species has caffeine in its bark, *Paullinia yoco* R. E. Schult. & Killip, found in the frontier region of Colombia, Peru, and Ecuador. Guarana's caffeine is most concentrated in the seeds, in complexes with the phenolics catechine and epicatechine.

Guaraná is native to the tropical rainforest of the broad Amazonian basin, where it has been gathered and used since pre-Columbian times. In 1669, a Portuguese missionary described the plant and its history, and named the plant. Linné installed the genus *Paullinia* in 1753. Humboldt collected his specimen of *Paullinia cupana* further northward in present-day Venezuela in 1821, stating, "crescit in ripa obumbrata fluminis Orinoci, prope S. Fernando de Atabapo; floret Majo" or "is growing on the umbrageous banks of the river Orinoco, near San Fernando de Atabapo; it is flowering in May," cited from Bonpland and Humboldt.

In its original form, guaraná was a woody liana, as shown in Figure 9, reaching the forest canopy, at heights up to 12 m.

Figure 9 Guarana vine with fruits, scandent on a rainforest tree in East Ecuador, September 2010 (courtesy of the photographer Geoffree R. Gallice, University of Florida, Gainsvillle, USA, under collective common license by Geoff Gallice from Gainesville, FL, USA (Guaraná) [CC BY 2.0 (http://creativecommons.org/licenses/by/2.0)], via Wikimedia Commons; accessed March 2015).

Over generations of crop growing, it was domesticated to a scandent shrub, up to 2 m tall and 4 m in diameter. In their Amazonas indigenous territory, the Sateré Mawés grow these shrubs in orchards around their homes; they recruit new plants by gathering seedlings from the rainforest. Colonists in the same region cultivate the plants in plantation-style arrangements, however, propagating them by cuttings.

The guaraná plant blossoms with white corollas in clusters directly on the branches. It is monoecious with separate male and female flowering periods even on the same branch, and pollination is done by insects. The plant develops bunches of eye-catching fruits, each with a red mantle, white mesocarp flesh, and a dark seed, so that the fruit resembles an open eyeball.

The Satare Mawes have strong traditions that guide the processing of the fruit: they pick the bunches when the outer skin of the fruit bursts, and the eyes open. Immediate post-harvest treatments include roasting, and the seeds are unharnessed, separated from residual testae, and pounded with mortar and pestle. The grist is thoroughly kneaded and rolled into a cylinder. After some weeks in the smokehouse, the guaraná achieves its reliable storage form, the 'baston' or guaraná stick. For beverage preparation, the powder is obtained by grating the hard stick by means of the bony tongue of a special fish or a slab of basalt.

At the beginning of the twentieth century, guaraná was introduced as flavoring in bottled soft drinks named 'Guaraná,' with several fabrication sites and nationwide acceptance. As the use and production of Guaraná increased, an extension of the agriculture was required, as was a simplification of the many laborious stages of traditional processing, and an adaption to industrial procedures. For soft drink manufacture, guaraná was utilized as a syrup prepared via the alcoholic extraction of seeds after simple roasting and grinding.

Over the last 70 years, international demand for guaraná has grown, given its use as a natural source of caffeine. Cultivation was expanded to regions outside the Amazon, southward to Bahía and Mato Grosso, with plants provided by research institutes. Climatologic and edaphic conditions in these states are similar to the Amazon Basin, and plantations in Bahia account for some 70% of national guaraná production (Brazilian official data acquisition for 2013), with nearly 90% national use. The discussion on the ethnobotanic heritage of the crop is ongoing (see the 2013 article titled 'Who owns Guaraná?').

Tea

Tea-leaf beverages have been consumed in Asia for thousands of years, and tea is the world's second largest agricultural commodity with caffeine content by volume. The tea plant belongs to the family *Theaceae*, genus *Camellia*, and species *Camellia sinensis* (L.) O. Kuntze, with two main varieties, *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica*, as well as some minors. In 1753, Linné had installed this genus, calling it *thea* with one species, *Thea sinensis*; a separate genus was the decoratively flowering *Camellia japonica*. Later, *Thea sinensis* and *camellia japonica* were reorganized with the common genus name *Camellia*; this section deals with the species C. *sinensis*, the tea plant.

Tea was first cultivated in China, followed by Japan, some thousand years ago, with India and Indonesia starting to cultivate it in the nineteenth century. On plantations, tea is an evergreen shrub. The primary crop consists of the tender leaves. Their postharvest treatment up to the final product is done in the countries of origin.

The tradable commodities are the tea leaves; they are brewed for beverages, esteemed as stimulants for their caffeine content. The series of phenolics accompanying the caffeine in tea provokes a specific profile of caffeine release from the drink. The caffeine metabolism follows the general pathways.

Tea is explicitly treated in articles 685–687 of this encyclopedia.

Coffee

Coffee beans are the most important primary product of the caffeine-containing plants, and the second internationally traded commodity in value, following mineral oil. The coffee plant's evolutionary source is Africa and Madagascar, with west central Africa for the robusta coffee, and Ethiopia for arabica coffee, the latter being used in beverages since about one thousand years ago.

The coffee plant belongs to the family *Rubiaceae*, genus *Coffea* L., with the economically important species being *C. arabica* L., *C. canephora* Pierre ex Fröhner (the arabicas and the robustas), and some minors. In the context of the project 'World Checklist of Rubiaceae', its chemotaxonomy was elucidated and consensually accepted, cornerstones are publications by Davis 2006 and Bremer 2009.

Since the seventeenth century, coffee cultivation has expanded from its Afro-Arabian homelands into all climatically suitable regions in the tropics. Occasionally, it succeeded other tropical crops, which experienced an economic decay, including sugar in Indonesia or Hawaii. Coffee plantations are often run by smallholders. The intensity of crop management ranges from monoculture (i.e., no shade, high inputs) to polyculture (shade overstory retained, fewer inputs, agroforestry).

Coffee is a tropical shrub with cherry-like fruits. They are processed after harvest to yield the 'naked' dried beans, the trade commodity green coffee, as defined in the International Coffee Agreement between producer and consumer states. Final treatment prior to consumption is the green beans' roasting and grinding; it takes place in the consumer country. The last step is the preparation of the beverage.

The different aspects of coffee are covered in the coffee articles of this encyclopedia.

Kola Nut

The kola nut has long been known as a source of caffeine stimulation. It is native to the West African coast, very near the equator. Cultivation centers include Sierra Leone/Liberia, Nigeria/Cameroon, and Gabon. It has been domesticated in West Africa, and it has likely been cultivated in the forests of Sierra Leone since the fourteenth century. The tree is constituent of the lowland forest, requiring a hot, humid climate and capable of withstanding 3 months of dry season. Kola may be cultivated even in drier areas wherever ground water is available.

The kola plant belongs to the cacao family. The classification is in a state of flux. In 2003, it changed its place from *Sterculiaceae* to *Malvaceae*, following the angiosperm phylogeny group. The genus remains *Cola* (Vent.) Schott & Endl., with the species are *C. nitida* (Vent.) Schott & Endl., Gbanja kola, cultivated around Ghana and Nigeria, *C. acuminata* (P. Beauy,) Schott & Endl., Abata kola, which is distributed from Togo to Angola and cultivated in the tropical forests of West Africa, and some minor ones.

The phenolics, accompanying the kola nut's caffeine, are tannic acid, catechins, and, to a lesser extent, chlorogenic and quinic acids. The nuts have a bitter, astringent taste, getting milder on drying. Chewing is common in the local population, used against fatigue and hunger, and a beverage is prepared by boiling the powdered seeds in water. Exported to Europe and America, the kola nut is used as an ingredient in the production of beverages in those areas.

When grown from seed, kola needs about 3 months to germinate, but propagation from cuttings is also possible. After 4 years, when the tree has grown to a height of 2–3 m, the first fruits appear. Full maturity is reached in 10 years, with harvests up to the age of 100 years. Yields of 300 nuts per tree are considered good. Old kola trees may serve as shade trees for cocoa plantations.

The plant is an evergreen, up to 20 m high, with a rather short stem and a dense crown. It has large leaves, variable in shape and size, and its flowers are male or hermaphrodite, with wind or insect pollination. Fruits are ripe after 5–6 weeks. Botanically, they are no nuts but comprise of a set of voluminous warty follicles gathered into a star, with each follicle containing 5–10 ellipsoid seeds, about 2.5 cm in diameter and colored red or white depending on the variety. Ripe fruits are dispersed by bats, birds, and squirrels.

The kola fruits are harvested before full maturity, using knives mounted on long poles. The fruits are immediately opened, and the seeds recovered and left in heaps for fermentative decay and sun drying. After some days, the seeds are washed and put in baskets on fresh leaves to be stored under surveillance.

Kola nuts are habitually used in West Africa for social and cermenonial purposes. Without kola seeds, in Nigeria and many West African countries, traditional hospitality, cultural, and social ceremonies are considered to be incomplete.

In a 1995 document on edible nuts as non-wood forest products, the potential of kola nuts is discussed: "Considering how much cola nuts are appreciated in West Africa while being virtually unknown elsewhere, expectations for an expanding market are reasonable." (Wickens, 1995).

Maté

Maté is native in South America, in the woodlands of the Paraná River in eastern Paraguay. The leaves are used to make a tea-like beverage, and they have been collected since pre-Hispanic times. Throughout the twentieth century, maté was once again cultivated in the mountainous southern parts of Brazil, northern Argentina, and Paraguay, reestablishing the abandoned plantations that Jesuit monks had run in eighteenth and nineteenth centuries. The traditional 'extractive'

production is practiced in parallel, with leaves gathered from 'wild' forest trees, before regular postharvest treatment.

In plant taxonomy, maté belongs to the family *Aquifoliaceae*, genus *Ilex* L., species *I. paraguariensis* A. St.-H. (called yerba maté). Linné named the genus in 1753, and Auguste Saint-Hilaire gave the maté a description in 1822. Other species are *I. vomitoria* Ait. (the yaupon holly of the southern United States, archaeobotanically identified as 'black drink' from 1000 years ago) and *I. guayusa* Loes., cultivated in Ecuador, with evidence of its use for 1500 years. The decorative Christmas holly known in Europe, *I. aquifolium*, is a relative without caffeine. Maté leaves contain 0.5–1.5% caffeine, and the accompanying phenolics are the chlorogenic acids.

The maté tree is 8–15 m high, evergreen, and dioecious, flowering from October to November, fruiting from March to June, and pollinated by insects. The perennial leaves are about 8 cm long, olive-green, and leathery, with slightly crenate dentate margins. The plant needs constant, moderate rainfall and average temperatures of about 22 °C, although it can tolerate extremes down to −6 °C. The conditions are met in its region.

For harvest, older leaves and tender branches are taken. Soon after, the crop is flash-dried through direct heat for 1 min at 300 °C (blanching, zapecado). In subsequent processing stages, the leaves are dried with hot air (secado) and coarsely ground. A seasoning storage for several months can be added (aging, maturation), followed by final grinding and sieving. Modifications are developing, both with economic and ecologic aims. They have influence on the products' appearance and composition.

In 2010, an Argentine study on the bioactive compounds variation during maté processing steps revealed that these steps lead to an increased caffeine content in the product, as compared to the green leaves after harvest. The marked augmentation was observed after the zapecado step, from 0.9 to 1.5% in caffeine, with parallel increase of chlorogenic acid (1.8 to 2.1% for the mono-CQA and similar for di-CQA's). As possible reason, a degradation of nucleic acids is discussed, with release of purins for biosynthesis of caffeine.

In Latin America, the beverage is prepared in a special gourd with hot or cold water. Maté is often drunk as part of the consumption ritual in a social setting, using a metal straw called a 'bombilla'; the straw ends in a closed bulb of teaspoon size with perforations, to avoid the aspiration of fines from powdered maté leaves when the infusion is sucked up through it. The drawing in **Figure 10** incorporates maté leaves, blossoms, fruit, and the classical drinking equipment. Maté is predominantly consumed in South America, and export is preferentially done in final consumer packaging, such as individual tea bags (1–2 g). For use as an ingredient in the food or dietary supplement industries, it is also supplied as maté tea concentrate.

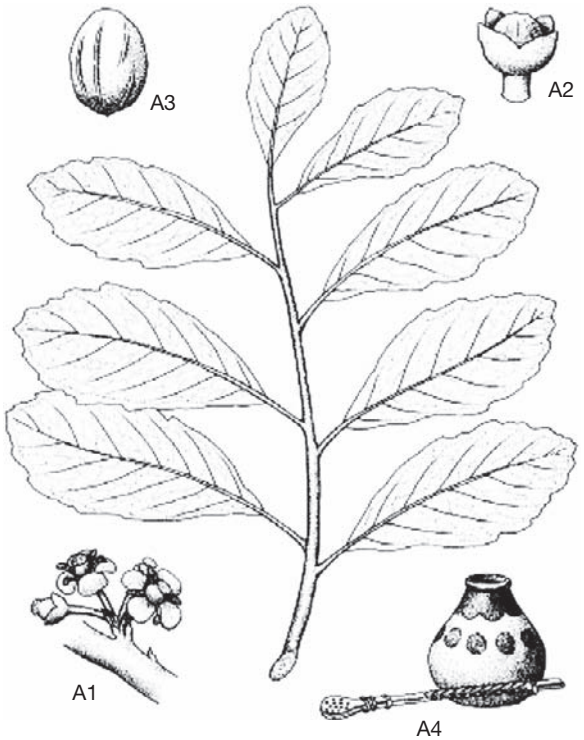


Figure 10 Maté, ensemble of twig with leaves, inflorescence, flower, fruit and consumption utilities. (Source: Hernández Bermejo, J.E. and León, J. (1994). *Neglected crops: 1492 from a different perspective*, p.247. Rome: FAO Publications Division. Reproduced with permission of the Food and Agriculture Organization of the United Nations.)

Cocoa

Cacao beans were the first caffeine-containing plant products that Columbus encountered in 1502. At that time, they were cultivated by the Maya in Central America. Cacao is a neotropic plant, possibly originating from the lowland rainforests of the Amazon Basin. Today, it is spread within a narrow zone 10 degrees of latitude south and north of the equator around the globe. Cacao belongs to the family *Malvaceae*, genus *Theobroma*, species *Theobroma cacao* L. As with the kola nut, the classification of cacao was merged, in 2003, with *Sterculiaceae*. The reorganization was carried out by the Angiosperm Phylogenetic Group, and it is on the way to be accepted. The caffeine content of cacao in the chocolate mass (ground beans) averages 0.2%, the lowest in the series we look at here, and it is clearly overshadowed by cacao's theobromine content of 1.2%.

Cacao is an understory tree, propagated by seed or cuttings and reaching maturity with 4–5 years of age; it grows up to 10–12 m in height, with leathery leaves and small, pinkish flowers directly on the trunk and branches. Here, the fruits or 'pods' develop, each yielding 20–50 cacao beans, which equate to 20–30 pods per harvest. The beans are ground and processed to make chocolate. Pulp and pods are also used for food and forage. Cocoa has its own articles in this encyclopedia.

Analytics

Since the early seventies of the last century, the analytical characterization of caffeine is preferentially done via chromatographic separation and spectroscopic identification. This preference represents a return to the roots of caffeine research: the first to observe chromatographic separations was Runge, the caffeine man, in 1850.

As coffee and tea are globally traded commodities, their characteristics are important to international trade. They are an item governed by the International Standard Organization (ISO), with emphasis on caffeine as a main constituent. For ISO coffee, the determination of caffeine in coffee was one of the first standards, published 1983, following an American AOAC procedure of 1979, which relied on liquid chromatographic separation and ultraviolet spectrometric quantification of caffeine via UV absorption at 276 nm. The method was explicitly described in a European directive of that time, even with an illustration of the chromatographic columns.

When liquid chromatographic procedures developed toward high-performance liquid chromatography (HPLC), caffeine determination was soon affected. The standard technique is now HPLC on reversed phase columns, with quantification again via UV absorption, as suggested with Figure 11, where the UV spectrum is an insert to the HPLC chromatogram. Actually, ISO provides HPLC/UV methods both for tea and coffee, with each one applicable to the specific products on the market. Chromatograms allow separation of the other methylxanthines accompanying caffeine in the same run. The specific distribution patterns of the different caffeine plants may serve as an analytical tool for authenticity proof, and "although several detection modes have been recently proposed, UV detection is still the first choice in the liquid chromatography analysis of these analytes due to its simplicity and reliability," as emphasized in a Venezuelan research article on methylxanthines in cocoa beans in 2007. The HPLC chromatogram for this section originates there.

Other classic spectroscopic figures transport further constitutive information:

Caffeine's infrared spectrum, shown in Figure 12, provides the two C=O valence bands of C-2 and C-6, well separated at 1700 and 1650 cm^{-1}, in 2013 with molecular calculations attributed to in-phase and out-of-phase stretching vibrations of the C=O bonds respectively, and in the fingerprint region, the methyl C–H separated from the ring C–H vibrations.

The proton nuclear magnetic resonance spectrum, in Figure 13, shows the signals of the methyl proton groups, with

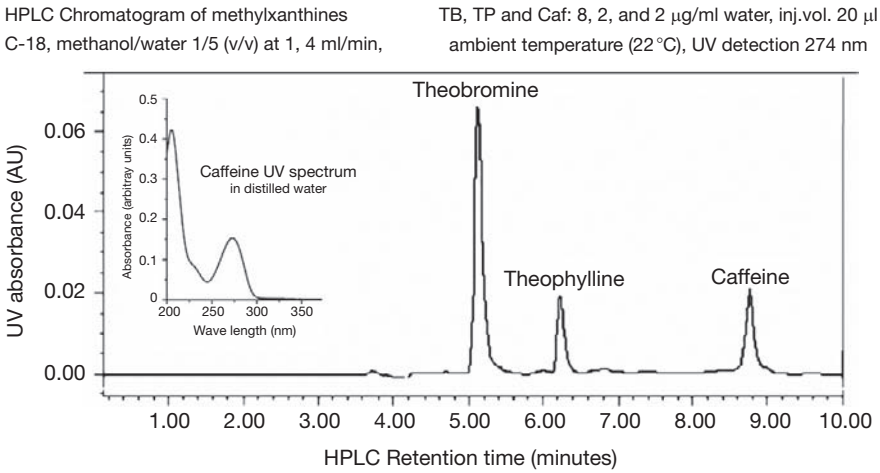
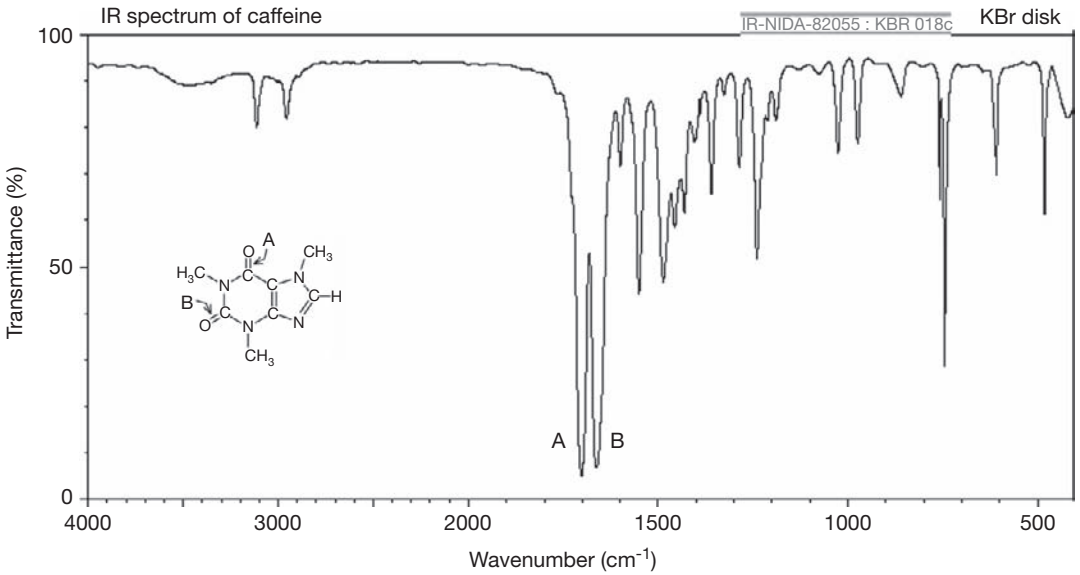
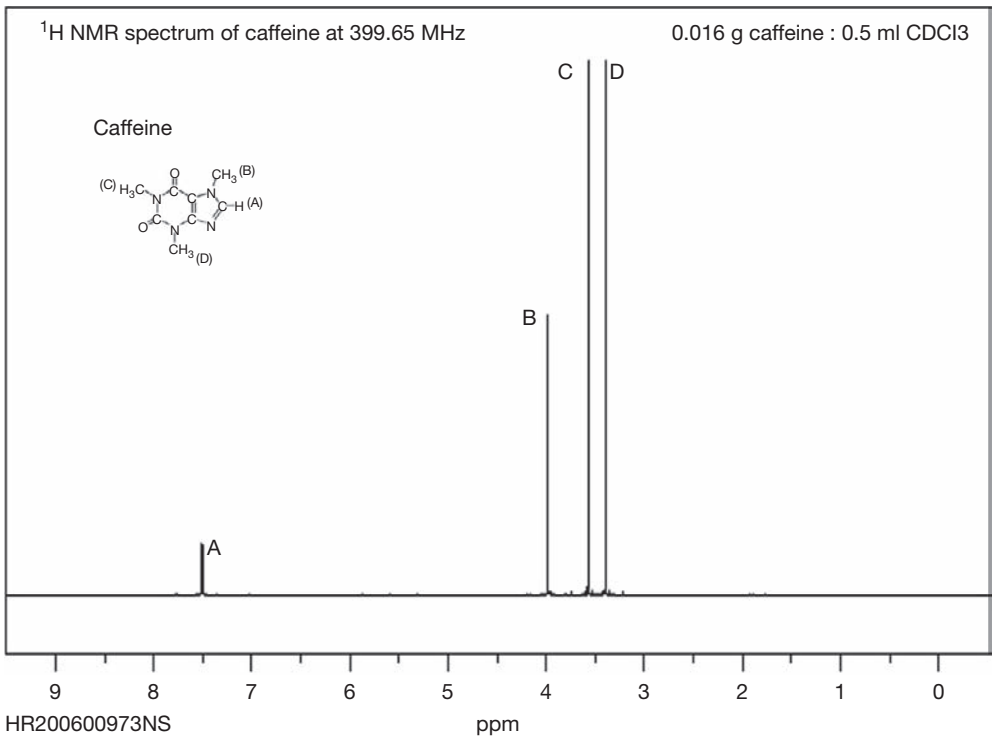


Figure 11 HPLC chromatogram of methylxanthines caffeine, theophylline, and theobromine in standard solution,[A] with UV spectrum of caffeine[B] as insert edited from several sources: [A]Brunetto, M.R., Gutiérrez, L., Delgado, Y. et al. (2007). Determination of theobromine, theophylline and caffeine in cocoa samples by a high-performance liquid chromatographic method. Food Chemistry 100, 463, [B]Belay, A. (2010). Measurement of integrated absorption cross-section, oscillator strength and number density of caffeine in coffee beans by integrated absorption coefficient technique. Food Chemistry 121, 587.



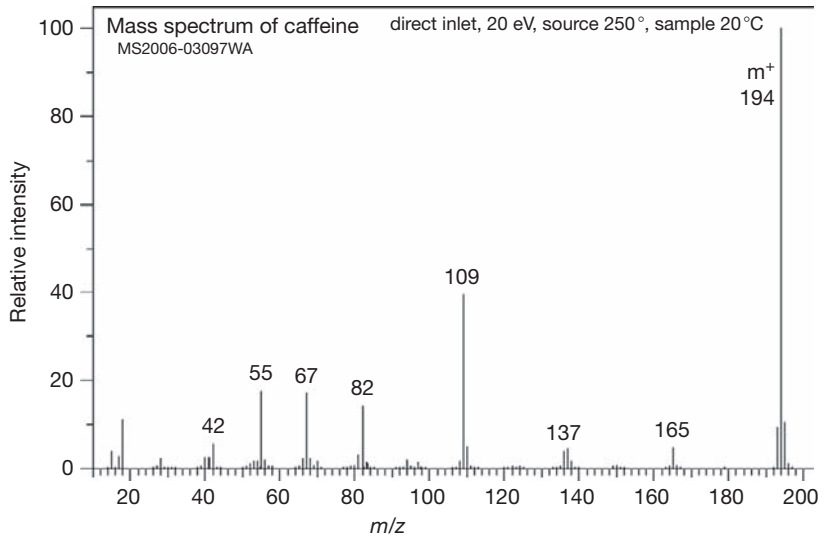
from SDBS, Spectral Database for Organic Compounds, by courtesy of National Institute of Advanced Industrial Science and Technology (AIST), Japan, published online at SDBSWeb: http://sdbs.db.aist.go.jp (accessed Jan. 25th, 2015)

Figure 12 Infrared spectrum of caffeine in KBr disc, adapted from AIST spectra base, Japan; caffeine formula inserted, relation to the C-O-bands marked.



SDBS, Spectrum Database for Organic Compounds, by courtesy of National Institute of Advanced Industrial Science and Technology (AIST), Japan, published at SDBSWeb: http://sdbs.db.aist.go.jp (accessed Jan. 25th, 2015)

Figure 13 Nuclear magnetic resonance spectrum of caffeine in $CDCl_3$ solution, adapted from AIST spectra base, Japan; caffeine formula inserted, relation to the proton chemical shifts marked.



from SDBS, Spectral Database for Organic Compounds, by courtesy of National Institute of Advanced Industrial Science and Technology, (AIST), Japan, published online SDBSWeb: http://sdbs.db.aist.go.jp (access ed Jan. 25th, 2015)

Figure 14 Mass spectrum of caffeine, adapted from AIST spectra base, Japan; main fragments marked.

individual chemical shifts, magnetically shielded from each other. The single-ring proton, with its characteristic aromatic chemical shift, has a weak spin–spin-coupling to the protons of the adjacent 7-methyl group, reducing their signal height by splitting into a doublet, and splitting itself into a quartet.

In addition to ^{1}H-NMR, caffeine can be analyzed by ^{13}C and ^{15}N nuclear magnetic resonance, and, further on, with two-dimensional resonance techniques such as heteronuclear multiple bond coherence (HMBC) and heteronuclear single quantum coherence (HSQC), to investigate interactions between protons and distant carbons or nitrogens, including ^{13}C and ^{15}N.

In the mass spectrum, Figure 14, the last of the basic spectra, the molecular ion peak, dominates as the base signal, with only a few fragmentations. Remarkably, no methyl fragments are present: the MS-fragmentation starts with a ring cleavage at the pyrimidine part, with a cut off of methyl isocyanate (m/z 55), leading to the fragment m/z 137 and, after CO elimination, to the dominant fragment ion m/z 109. The rest of the MS are the broken bits.

Mass spectrometry is commonly used following the chromatographic separation of the analyte mixture, with GC/MS or HPLC/MS/MS as well established combinations. The mass spectrum may serve as a proof of authenticity for the caffeine origin in caffeinated soft drinks and energy drinks. A 2012 research article was entitled "Caffeine in your drink: natural or synthetic?" The isotopic composition of carbon reveals whether the caffeine in the drink is truly natural, as might be claimed, or synthetic with a carbon isotope pattern of fossil times.

Caffeine in Registries, Inventories, and Databases: Identifiers and Registry Codes

Caffeine is defined in several scientific and administrative registries. These registries have their roots in individual or corporate private initiatives or in regulatory intentions, and they are administered by either private or governmental services. The actually most accessed registers are listed in Table 3, together with the individual numerical identifiers for caffeine they provide.

By far, the most widely used is the Chemical Abstracts Service (CAS) registry, with caffeine having CAS registry number 58-08-2. The low numbers of this registry ID indicates the very early uptake of caffeine into the registry. Two later codes had to be deleted. The CAS number may often serve as an entrance to online work with elaborate regulatory listings.

The CAS has been run by the American Chemical Society since 1907. Caffeine is placed in the abstracts section "Biomolecules and their synthetic analogs." The first CAS entries of caffeine referred to German abstracts on the early caffeine synthesis by Fischer and Traube. In 1965, the service with registry numbers started. With modern tools, it may easily be accessed for pooling substance-related data and literature. The caffeine property data in Table 1 are generated from this source on-line.

Another important source, the *Beilstein Handbook of Organic Chemistry*, was started in Germany in 1881, and it incorporated papers from 1771 onward. It organized the scientific literature according to distinct chemical substances. In systematic order, caffeine had its place in Volume 26, within the nitrogen-containing heterocycles. The print version continued until the fifth multivolume supplement, covering the literature until 1979. The tradition was continued with the powerful Beilstein online database, which was taken over by Elsevier and reorganized in Elsevier's Reaxys, which integrated the older data almost completely. The Beilstein registry number is now called the Reaxys registry number, for caffeine numerically the same.

In 2011, the International Union of Pure and Applied Chemistry (IUPAC) introduced the International Chemical Identifier code (InChI). It provides structural information on chemical

Table 3 Inventories containing caffeine with registry codes

Inventory/register and reference	*Administered by*	*Short name*	*Code for caffeine*
Chemical Abstracts Register, online services since 1980	Chemical Abstracts Service CAS Columbus, OH, USA	CAS RN	58-08-2
Reaxys, continuing the Beilstein Online Database	Reed Elsevier London	Reaxys Registry Number	17705
IUPAC International Chemical Identifier	InChI Trust, Cambridge, UK[a]	InChI code	InChI = 1S/C8H10N4O2/c1-10-4-9-6-5(10)7(13)12(3)8(14)11(6)2/h4H,1-3H3
InChI key.	IUPAC,Research Triangle Park, NC, USA	InChI key	RYYVLZVUVIJVGH-UHFFFAOYSA-N
Inventory on classification, labeling, and packaging of dangerous substances (CLP)[b].	European Commission, Brussels	EINECS	200-362-1
Adapted by follow-up regulations[c].	European Chemicals Agency (ECHA), Helsinki	EC No.	200-362-1
Index of harmonized classification and labeling[d]	ECHA, Helsinki	Index No.	613-086-00-5
Registry of Toxic Effects of Chemical Substances, originally based in the USA	BIOVIA Foundation,San Diego, CA, USA[e]	RTECS	EV6475000
Hazardous materials description of the United Nations[f]	United Nations Committee of Experts on the Transport of Dangerous Goods	UN No.	1544[g]
Harmonized Commodity Description and Coding System (HS)[h]	World Customs Organization WCO, Brussels[i]	HS Code	2939.30

[a]With members IUPAC, NIST, CAS, Elsevier, FIZ, and others
[b]Tracing back to the respective European directive of 1967
[c]The last (EC) 1272/2008, Annex VI
[d]Annex VI of Reg. (EC) 1272/2008
[e]With licenses to CAS, Can. NIOSH, Elsevier and others
[f]With hazard classes for safe transportation of hazardous chemicals, based on the United Nations Recommendations on the Transport of Dangerous Goods – Model Regulations since 1957
[g]The UN number 1544 is entitled 'solid alkaloids, not otherwise specified', and 'alkaloid salts, not otherwise specified'; caffeine is dealt with in these items, but not explicitly mentioned.
[h]With common nomenclature and classification of goods (customs tariffs) since 1968 (tracing back to the principles of the former General Agreement on Tariffs and Trade GATT); actually in 2015 laid down in in Council Regulation (EEC) No 2658/87.
[i]With regular amendments; the next HS 2017 Edition was finalized in January 2015.

substances, using a string of ciphers and letters: a textual code fit for search engines. Developed in cooperation with the US National Institute of Standards (NIST), it is now held by the InChI Trust, which incorporates other relevant media organizations. The InChI code is increasingly used, replacing the earlier, less powerful SMILES code. Additionally, the shorter InChI key of IUPAC works like a registry number, providing identification and basic data for the substance requested.

The European Inventory of Existing Commercial Chemical Substances (EINECS) number follows a regulatory approach, with an inventory focused on any handling of the substances concerned. The cutoff date for 'existing' was 1981, and caffeine is included in it. The inventory was expanded step-by-step to other groups of chemicals, with an increasing volume of code numbers, and thus, a new name for the same identifier came up: EC number.

A similar approach in the United States led to the Registry of Toxic Effects of Chemical Substances (RTECS) number, established in 1971 by the National Institute for Occupational Safety and Health (NIOSH), and caffeine is included in the RTECS as well. After several transfers and mergers since 2001, RTECS was shifted from Accelrys, in 2014, to BIOVIA, which is owned by the French commercial group Dassault Systèmes. Dassault gives license other registry organizations, such as the CAS. Even though a series of other nation's analoga to EINECS or RTECS exist, these registry codes have gained remarkably broad acceptance.

Meanwhile, the EC number serves as a software key to the Europe-wide registration of chemical substances required by law. For caffeine, industry has submitted a dossier to the European Chemicals Agency (ECHA), in Helsinki, to notify it about caffeine's property and safety data, with a focus on the manufacturing workplace. In the EU, the comprehensive 'chemical index number' is included in the same regulation as the European Communities (EC) Number, again including caffeine.

The United Nations have a register with recommended coding for substances and articles dangerous to transport. Caffeine is considered part of 'solid alkaloids n.o.s. or alkaloids salts n.o.s. poisonous,' positioned in Class 6.1 Toxic, with a common four-digit code. The abbreviation 'n.o.s.' stands for 'not otherwise specified.' For caffeine, an outside marking on transport containers is not required.

In the Harmonized System (HS) of commodity description and coding, maintained by the World Trade and World Customs Organizations, 'Caffeine and its salts' have an HS

Code number identifying it as an organic chemical in the subgroup 'vegetable alkaloids' (From 2017 onwards, the subgroup will be identified as 'alkaloids.'). This classification is independent of caffeine's origin, whether from chemical synthesis or from decaffeination. The caffeine plant crops, as important internationally traded commodities under customs, are placed in the HS chapters for these vegetable products.

Table 3 is a rendering of registry codes for caffeine in several databases.

The EC Number of **Table 3** need not be confused with the Enzyme Commission's EC number, which identifies the enzymes catalyzing specific reactions. The number has been systematically designed and attributed by the International Union of Biochemistry and Molecular Biology since 1955. Enzyme numbers for the enzymes involved in the biosynthesis of are indicated in **Figure 5**.

Other mighty registers, with a history from medieval times, are the national pharmacopeias. They identify substances for pharmaceutical use and provide specifications and safety information. Caffeine is included in them. Its generic name 'caffeine' serves as pharmaceutical International Nonproprietary Name (INN) for the World Health Organization (WHO). Since 1965, the European pharmacopoeia has incorporated more and more the national texts, as laid down in the European Pharmacopoeia Convention. Under the WHO umbrella, harmonized pharmacopoeial texts are exchanged with the United States and Japanese pharmacopoeias (USP and JP, respectively).

Literature on the medical and physiological aspects of caffeine can easily be found in the Medline databases of the US National Institutes of Health and the National Library of Medicine via the search engine Pubmed. The search on 'caffeine' yields some 30 000 results.

A broad list of identification numbers is presented by the web-based information service of the Wikipedia foundation, which is authored by voluntary contributors, and has gained an increasing reputation during the last decade. Within the different language versions, the English article on caffeine dates back to 2001. Articles compile actual data, which are cross-linked intensively.

Legislative aspects and requirements belong to the part on consumption, and will be covered by the next article.

See also: Caffeine: Consumption and Health Effects; Cocoa: Composition and Health Effects; Cocoa: Production, Chemistry, and Use; Coffee: Analysis and Composition; Coffee: Decaffeination; Coffee: Health Effects; Coffee: Types and Production; Food Colloids and Emulsions; Tea: Analysis and Tasting; Tea: Chemistry and Processing; Tea: Health Effects.

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Caffeine: Consumption and Health Effects

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Introduction

Caffeine is probably the most utilized pharmacologically active substance in the world. As such, due to the caffeine exposure of the majority of the population through different foods and drinks, this substance has engaged the interest of the scientific community. The effects of caffeine are dependent on multiple factors, such as the dose, contribution sources, individual responses, and body weight.

Caffeine belongs to the alkaloid family of chemicals. Prominent in this family are the methylxanthines, such as caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylxanthine), and theobromine (3,7-dimethylxanthine). All of them are derived from purine (the xanthine group is 2,6-dioxopurine); theophylline and theobromine have two methyl groups, while caffeine has three.

Caffeine's molecular formula is $C_8H_{10}N_4O_2$, and its molecular weight is 194.19. The chemical structure of caffeine is shown in Figure 1.

Consumption of Caffeine

Reference Levels for Caffeine Exposure

There are no European guidelines for caffeine intake for the general population. However, a conclusion that there was not an apparent justification for worries about caffeine's potential carcinogenic and mutagenic effects in humans when ingested at normal doses was taken in the eighties of the last century. In humans, moderate caffeine doses between 200 and 300 mg are frequently associated with well-being effects, such as memory improvement, increased energy, and alertness.

For non-alcoholic beverages, the maximum content of caffeine as a flavoring is 150 mg kg^{-1}. In 2012, the European Commission adopted two regulations that established a new list of authorized flavorings in the EU. The European Commission requested that the EFSA (European Food Safety Authority) deliver a risk assessment and scientific opinion about caffeine as a flavoring. However, it was not possible – through the usual procedures – to assess caffeine as a flavoring substance by the Scientific Panel on Food Additives, Flavorings, Processing Aids, and Materials in Contact with Food because data on normal and maximum levels of usage were not available. The referred panel was aware that new toxicological data on caffeine had been published in 2003, and that this data should be considered.

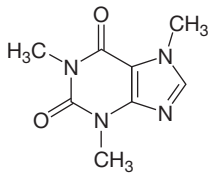

Figure 1 Chemical structure of caffeine.

Recently, the EFSA Panel on Dietetic Products, Nutrition and Allergies deliver a scientific opinion on the safety of caffeine providing advice on caffeine intakes that do not give rise to concerns about adverse health effects for the general healthy population.

Nevertheless, the main sources that currently contribute to caffeine exposure can be summarized as follows:

Tea

According to Chinese tradition, new leaves from the plant *Camellia sinensis* from the southern China mountains have been used to make tea since 2737 BC. It is believed that the Portuguese were the first Europeans to drink tea during their sixteenth-century expeditions to the Far East. In the seventeenth century, its consumption was first reported in the Netherlands and France. It was also in the seventeenth century that tea reached England; however, its generalized consumption only became established in the eighteenth century when the first teahouses appeared. Currently, tea is consumed all over the world, but mainly cultivated in China and India. *Camellia sinensis* is the scientific name of the plant. All varieties of tea are derived from this plant; the differences result from the way the leaves are processed (oxidation or fermentation). There are five main groups of tea: green tea, paocong, oolong, pu-erh (red tea), and black tea. The main difference between these products is the degree of oxidation of tea flavonoids, which are low in green tea.

The smaller the leave particles are, the greater the amount of caffeine extracted during tea preparation is. This probably depends on the bigger surface of particles in comparison to their volume. Studies show that caffeine extraction increases with the duration of fermentation. The temperature of the water used to brew the tea also affects its caffeine content: the hotter the water is, the amount of caffeine extracted increases. Another factor of the caffeine content of tea is related to the duration of the infusion. Thus, instructions about water temperature and infusion duration are usually provided on the package label by the producer.

Benefits of tea consumption are believed to be due to flavonoids that have an antioxidant effect. However, considering these positive effects, there are still many aspects related to tea consumption that need to be better understood; especially about the chemical compounds and their mechanisms of action.

It is important to highlight the difference between tea and herbal infusions made from herbs, spices, or fruits, and not from the plant *Camellia sinensis*.

Caffeine content of the leaves or commercial teabags is on average 30 mg. According to the Food Standards Agency (UK), caffeine levels in tea vary from 1 to 90 mg per dose, the average being 40 mg. However, in general, it could be considered that the caffeine content varies from 12.5 mg l^{-1} (non-caffeinated tea) to 282.5 mg l^{-1} (regular tea). Caffeine levels were tested in white, green, and black tea for different extraction time

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00099-4

periods. The caffeine contents ranged between 14 and 61 mg per cup. However, it was observed that infusion duration affects caffeine concentration in tea.

Coffee

Coffee is one of the most consumed beverages in the world. It is an infusion of roasted coffee bean grounds from plants of the *Coffea* genus. Coffee seeds are contained in berries that, once ripe, are processed (roasted) and dried. The history of coffee began in Ethiopia, and coffee has been attributed 'energizing' properties since the fourteenth century. There are two coffee species with commercial value, *Coffea arabica* and *Coffea robusta*. Coffee beans of these two species have different chemical compositions. In coffee, there are more than 1000 different compounds, many of them formed during the roasting process, which gives them their unique flavors and aromas. There are three groups of substances in coffee that stand out by their importance: caffeine, diterpenes (cafestol and kaheol), and other polyphenols. *Arabica* beans have more lipids, trigonelline, and saccharose, while *robusta* beans have more caffeine and chlorogenic acid in their products. During the decaffeination and filtration processes, some components are removed, such as caffeine and lipid fraction.

Caffeine levels in coffee vary widely. The amount of caffeine per cup is influenced by the preparation method (boiled, filtered, or espresso method). The average concentration of caffeine in ground coffee is approximately 105 mg per portion/dose with variations between samples ranging from 15 to 254 mg (espresso, filtered, and cappuccino coffee samples). The average caffeine amount per cup (150 ml) of ground roasted coffee is approximately 85 mg, but could be of approximately 60 mg in instant coffee, or only 3 mg in decaffeinated coffee. The average levels of caffeine in espresso coffee samples is 106 mg per cup, ranging from 25 to 214 mg.

Soft drinks

The expression 'carbonated water' first appeared in 1978. In the 1830s, the first attempts were made to add other ingredients, such as barks and leaves to increase carbonated water's potential benefits. As a result the first flavored carbonated drinks appeared.

Consumers are constantly searching for new flavors and formulations of soft drinks, innovation being the key to the success of the food industry. Concerns about health and lifestyle modifications have been influencing changes in soft drink consumption, primarily resulting in the increase of low calorie, or no-sugar-added varieties of the most common soft drinks. To satisfy consumer expectations, new formulations containing plant extracts, such as guarana and ginseng, have been put on the market. However, there are older formulations containing caffeine extracts. These formulations are the colas.

Colas and iced teas are the most popular soft drinks made from plant extracts. Cola is extracted from the nuts of the African tree *Cola acuminata* and *Cola nitida*. In the original formula, cola syrup also contained small amounts of cocaine. This mix became famous in 1886 in Atlanta (USA). Today, the drink is manufactured without cocaine. Its main ingredients are: water, sugar or sweeteners, caramel color, natural flavors, phosphoric acid, carbon dioxide and, of course, caffeine.

The United States had an important role in the history of tea through the invention of iced tea in 1904. Although these soft drinks are still produced using tea extracts, particularly green and black tea, they have other ingredients that separate them from plain iced tea, including high levels of added sugars. The most common iced tea ingredients are: water, sugar or sweeteners, tea extracts, flavors, juices, acidity regulators, and antioxidants.

In recent years, it was suggested that consumption of soft drinks with added sugar increases the risk of type 2 diabetes, heart disease, and other chronic diseases.

Caffeine levels in soft drinks differ by brand. However, in Europe, there is a legal limit for caffeine levels as a flavor of 150 mg kg^{-1}, or per l.

Caffeine levels in a cup of a soft drink (200 ml) vary between 20 and 60 mg. Caffeine levels range from 88 to 171 mg l^{-1} in regular cola soft drinks, and 81 to 124 mg l^{-1} in diet cola soft drinks.

Energy drinks

Energy drinks first made their appearance in Europe and Asia in 1960. In 1987, a well-known brand of energy drinks was launched in the Austrian market. This brand was launched in the United States in 1997, which contributed to the trend of high caffeine content energy drink consumption, which was enhanced by aggressive industry marketing. This kind of product usually has the word 'energy' in the product name, and contains high levels of caffeine and other additional ingredients not usually found in soft drinks. It is important to distinguish energy drinks from sports drinks. Sports drinks may have in their composition: carbohydrates, minerals, electrolytes, and flavorings. Their customary name suggests they aid in restoring water and electrolytes lost to sweating during physical activity. However, energy drinks refer to different types of beverages containing non-nutritive substances, such as caffeine, guarana, taurine, ginseng, L-carnitine, creatinine, glucuronolactone, alleged to enhance physical performance, and also high levels of sugar (glucose, dextrose, and sucrose) and small amounts of vitamins and minerals. The high variability and availability of energy drinks, often associated with being attractive, to younger generations increases the concerns about potential caffeine effects on children, and adolescents' physiology and behavior.

Plant extract soft drinks, such as cola or iced tea, usually contain flavorings, sugars and/or sweeteners, and, frequently, caffeine. However, energy drinks have more caffeine than soft drinks, and are heavily marketed to adolescents. Caffeine, as a component of energy drinks, is associated with impulsivity and the search for a new sensation in the young, promoting sexual activity, smoking, drug and alcohol abuse, and the increase of risky behavior while driving. These behaviors can be intensified with the combined consumption of energy drinks and alcohol.

Energy drink consumption is a potential health risk for the general population, but is especially alarming in younger people because of the high caffeine levels and other substances not usually found in the food chain. Therefore, it is suggested that energy drinks should not be present in the diets of children and adolescents because of the stimulant effects.

Energy drinks may contain high levels of caffeine, approximately 80 mg per 250 ml per can, equivalent to three cans of cola soft drinks, or to a cup of instant coffee.

Caffeine Consumption in Different Countries

Data on energy drink consumption in specific consumer groups in the EU was collected from the following 16 EU

member states: Austria, Belgium, Cyprus, Czech Republic, Germany, Greece, Finland, France, Hungary, Italy, Poland, Romania, Spain, Sweden, the Netherlands, and the United Kingdom. For adolescent consumers (10–18 years old), the mean exposure to caffeine from energy drink consumption is 23.51 mg day^{-1} and 0.38 mg kg^{-1} body weight (bw) per day. The mean daily exposure to caffeine from all sources (energy drinks, coffee, tea, caffeine-containing soft drinks, chocolate, and cocoa) is 184.92 mg day^{-1}, and 3.01 mg kg^{-1} bw per day for energy drink consumers. For total exposure to caffeine, the mean daily exposure from all sources is 149.20 mg day^{-1}, and 2.45 mg kg^{-1} bw per day.

In the United States the mean caffeine consumption is approximately 1 mg kg^{-1} bw per day in children and 3 mg kg^{-1} bw per day in adults. Other data from the United States, with the objective of estimating caffeine exposure from caffeine-containing drinks and cocoa and chocolate snacks, demonstrated a mean daily exposure to caffeine of 109 mg day^{-1}.

In New Zealand it is estimated that a mean exposure to caffeine in different subgroups of the population, namely, adolescents from 13 to 19 years old, is 82 mg day^{-1} and 1.2 mg kg^{-1} bw per day.

In Argentina, the mean daily exposure to caffeine by adolescents and young adults from 11 to 15 years old and 16 to 20 years old is 2.3 mg kg^{-1} bw per day and 4.1 mg kg^{-1} bw per day, respectively. Mate (an infusion of mate leaves, of which consumption is a tradition in Argentina) was the main source of caffeine.

The mean daily exposure to caffeine in France is 19.3 mg day^{-1} for the 11–14 year old age group and 33.5 mg day^{-1} for the 15–17 year old age group. This exposure is the result of the following sources of caffeine: soft drinks, energy drinks, coffee, tea, and cocoa/chocolate.

Soft drinks were identified as the main source of daily caffeine exposure for adolescents in northern countries (Finland, Norway, Denmark, Iceland, and Sweden). Daily exposure to caffeine from cola soft drinks, coffee, and tea for the 10–13 year old age group was 50.6 mg day^{-1}; and for the 14–18 year old age group the exposure was 66.3 mg day^{-1}. In Finland, 5% of all the 14–15 year old adolescents studied had a total daily exposure to caffeine from energy drinks and/or coffee greater than 2.5 mg kg^{-1} bw per day. 13-year-old Norwegian adolescents had a daily exposure to caffeine of 44.15 mg day^{-1} from the following caffeine sources: soft drinks, coffee, and tea. In Icelandic adolescents, between 15 and 19 years old, daily exposure to caffeine form soft drinks, energy drinks, coffee, and tea was 62.8 mg day^{-1}. Approximately 70% of this exposure was from the consumption of cola soft drinks.

Data from Portugal demonstrates that approximately 42% of adolescents consume energy drinks. Approximately 17% consume energy drinks combined with alcohol. The major contributors to caffeine exposure are coffee and energy drink. The total daily mean exposure to caffeine is 0.47 mg kg^{-1} bw per day. However, 3.30% of individuals exceed the daily exposure to caffeine of 2.5 mg kg^{-1} bw per day.

Effects of Caffeine on Human Health

Caffeine is a neurological stimulant, producing biological effects on a majority of body organs. Caffeine's primary cellular action is to block adenosine receptors. Blockage of these receptors by caffeine and related substances stimulates tissue and organ activity, including of the central nervous system.

Pharmacokinetics of Caffeine

After ingestion, caffeine is readily absorbed in the gastrointestinal tract and enters the blood stream. The maximal concentration of caffeine on blood is reached within 1 h to 1 h 30 min after ingestion.

The main metabolic pathway in humans (70–80%) is *N*-3-demethylation to paraxanthine, also known as 1,7-dimethylxanthine. This reaction is achieved by the liver enzyme CYP1A2, which is responsible for over 95% of primary caffeine metabolism. Beyond paraxanthine, caffeine's main metabolites are 1-methylxanthine, 1-methyluric acid, 5-acethylamine-6-formylamine-3-methyluracil, and 1,7-dimethyluric acid (17U). These metabolites are formed by secondary metabolism from paraxanthine by CYP1A2, CYP2A6, N2-acetyltransferase, and xanthine dehydrogenase. Four CYP isoforms are involved in caffeine metabolism at a concentration of 3 mmol l^{-1}, but for concentrations below 1 mmol l^{-1}, CYP1A2 and CYP1A1 are the most important isoenzymes.

After distribution, caffeine is metabolized in the liver and eliminated in urine. Caffeine's half-life in organisms varies depending on age, physiological, and pathological status, usually taking 4–5 h in human adults. However, it can be longer, up to 100 h in patients with liver disease, children, newborns, and pregnant women. Smoking enhances caffeine removal from the blood stream because of its actions on CYP1A2.

Caffeine is an adenosine receptor antagonist. Adenosine has the capacity to condition some of the effects on the central and peripheral nervous system. Caffeine has a similar structure to adenosine and acts as a competitive antagonist to adenosine receptors (A1 e A2A). It has the ability to produce effects opposite to adenosine effects, namely on the central nervous system, and on vasoconstriction. Caffeine intake causes the release of norepinephrine, dopamine, and serotonin in the brain, increasing catecholamine circulation, according to the inhibitory effects of adenosine.

The wide interindividual variability of CYP1A2 activity on its substrates, such as caffeine, is due to factors such as gender, ethnicity, genetic polymorphisms, and exposure to inducers. The metabolism of caffeine is affected by genetic determinants, age, pregnancy, diet, or life style (i.e., smoking, environmental factors, medication, including oral contraceptives, and disease types).

Pharmacokinetics of caffeine on dogs is consistent with the slow elimination of caffeine in newborns when compared with adults. The elimination of caffeine in newborns is reduced due to an immature liver enzymatic system.

Before 8–9 months of age, children have a reduced ability to metabolize caffeine. Approximately 85% of the consumed caffeine is excreted, unchanged, in their urine. In adults, this rate is only 1–5%. Caffeine's half-life is 20–30% lower in women than in men. The half-life in newborns is between 50 and 100 h, but gradually approaches the half-life of adults at approximately 6 months old. During pregnancy, the half-life of caffeine increases by 4 h through the first trimester of pregnancy, and 18 h during the third trimester of pregnancy.

General Effects of Caffeine

Caffeine, at low doses, stimulates the central nervous system and causes diuresis, smooth muscle relaxation, cardiac/heart muscle stimulation, and increased gastric secretion in adults. The consumption of caffeine is recognized for having positive effects on human health, namely in the prevention of type 2 diabetes mellitus, cardiovascular disease, cancer, and Parkinson disease. Nevertheless, this mechanism is not entirely known, and benefits are not believed to be related to caffeine because decaffeinated coffee has similar effects. However, epidemiological studies have been performed to relate caffeine consumption and the risk of bone fracture, but their results have been inconclusive.

High doses of caffeine seem to produce anxiety, nausea and nervousness. Children and women of childbearing age are risk groups that need specific recommendations about caffeine intake. Studies demonstrate that women of childbearing age should not consume more than 300 mg caffeine per day, equivalent to 4.6 mg kg^{-1} bw per day for a 65 kg individual; while children should restrict caffeine consumption to $\leq$2.5 mg kg^{-1} bw per day.

However, a maximum daily caffeine limit for children of 95 mg day^{-1} was suggested.

The northern countries have performed a caffeine risk assessment on children and adolescents to assess toxicological food risks. The following limits for tolerance and abstinence symptoms to caffeine and anxiety syndrome were defined: a LOEL (lowest observed effect level) of 1.0–1.3 mg kg^{-1} bw per day, and a LOAEL (lowest observed adverse effect level) of 2.5 mg kg^{-1} bw per day.

Caffeine tolerance syndrome is defined as the necessity to intake progressively higher doses to reach the desired objective. Currently, there is no reference value established for caffeine exposure, such as an acceptable daily intake. An exposure of 2.5 mg kg^{-1} bw per day has been suggested as a conservative upper toxicological limit, utilized as a base for risk assessment for children and adolescents, with limited evidence. For instance, Health Canada did not establish a final upper limit for caffeine intake for adolescents of age 13 or older because of insufficient data. Health Canada suggests that daily caffeine intake for this population should not exceed 2.5 mg kg^{-1} bw per day. This limit was defined for adolescents because the upper limit for adults may not be appropriate for younger adolescents with a lower body weight that are still developing. This is based on a general precautionary principle.

The structure of caffeine protein receptors is similar in children and adults. Thus, caffeine affinity to adenosine receptor is age independent. Nevertheless, differences in caffeine excretion are one of the main differences of caffeine susceptibility because the main enzyme responsible for its metabolization, CYP1A2, has different activity depending on age. Caffeine elimination is very low in newborns under 1 year old (approximately) but increases until puberty. At puberty, elimination decreases, though always with interindividual variations.

Differences in the sensitivity to caffeine among men and women were observed, which found more pronounced sleep disorders in women than in men; this difference associated with the stimulant effects of caffeine consumption.

Adolescents are a risk group for the toxicity originated by excessive caffeine intake. Caffeine metabolism decreases during adolescence because of the high natural progression of growth hormones, increasing the risk of toxic caffeine effects on this population.

Death caused by excessive caffeine intake is uncommon and only a few cases were reported. An acute lethal dose was estimated at approximately 10 g per person for adults.

Caffeine DL_{50} oral (median lethal dose) is approximately 200 mg kg^{-1}, approximately 12 g. For an adult, this would be the equivalent of rapidly consuming approximately 50 cups of coffee.

The excessive, chronic, or acute use of caffeine can result in 'caffeinism,' defined as caffeine dependency. Symptoms include restlessness, insomnia, muscle twitching, tachycardia, gastrointestinal disturbances, and sometimes the exacerbation of preexisting anxiety or panic states, depression, or schizophrenia. One of the adverse effects of its excessive intake is caffeine intoxication, classified in the WHO international list of diseases: F15.0 – mental and behavioral disorders due to the use of other stimulants, including caffeine.

Energy drinks, coffee, and soft drinks that contain caffeine can increase the risk of caffeine intoxication in abstinent individuals or in usual consumers. The potential of acute toxicity due to the consumption of energy drinks can be higher than it is from other sources of caffeine for several reasons: lack of appropriate labeling, as consumers are not entirely informed of the amount of caffeine that they will consume; advertising, because several energy drinks have 'health claims' such as increased performance, endurance and concentration; and consumer profile, by the absence of sales restriction of energy drinks to children and adolescents (which are a risk group for caffeine intoxication).

Caffeine and pregnancy

A low caffeine intake, less than 150 mg day^{-1}, during pregnancy will not affect the fetus. However, high doses, higher than 300 mg day^{-1}, equivalent to more than three cups of tea per day, should be avoided during pregnancy because they are associated with an increased incidence of congenital defects.

Caffeine intake involving pregnant women after 20 weeks of pregnancy showed a higher risk of miscarriage for women with a caffeine intake of 200 mg day^{-1} when compared with women without caffeine intake. For daily caffeine intake above 200 mg, the risk increased significantly when compared with women without caffeine intake. The fact that a high consumption of caffeine can cause adverse effects on fertility should be a concern, as well as a recommendation to women of childbearing age that an upper limit of 300 mg day^{-1} should also be avoided. Thus, moderation when consuming caffeine from any source during pregnancy should be taking into consideration.

Caffeine and cardiovascular effects

The association between coffee intake and cardiovascular risk factors was investigated. There is evidence that caffeine raises blood pressure and catecholamine levels, but reduces heart rate after acute intake.

In hypertensive patients, caffeine intake produces an acute increase of blood pressure for 3 h or more. However, recent evidence does not support an association between long-term coffee intake and the increase of blood pressure, or regular intake of coffee and cardiovascular diseases risk in hypertensive patients.

Caffeine has a minimal effect on electrocardiograms. Nevertheless, the results are inconsistent with susceptibility for arrhythmia.

Available data suggest that moderate consumption of caffeine, ≤ 400 mg day^{-1}, does not negatively affect cardiovascular health. There is not enough epidemiological data to conclude about coronary heart disease risk or death associated with an intake ≥ 1000 mg day^{-1}.

Medical recommendations advise moderation of caffeine consumption for patients with cardiovascular disorders, such as tachycardia, palpitations, and arrhythmias.

In regards to coffee, there is no clear evidence of association with hypertension risk, myocardial infarction, or other cardiovascular diseases.

Regular and moderate coffee consumption is not a hazard for health, and can even be associated with positive effects on cardiovascular health. Coffee is a complex drink that contains hundreds of biological compounds associated with beneficial health effects. For the cardiovascular system, coffee consumption may reduce the risk of hypertension, coronary heart disease, congestive heart failure, arrhythmias, and spills. However, it can negatively affect lipid profiles, depending on how drinks are prepared. In addition, possible advantages of the regular consumption of coffee should be evaluated in relation to potential risks due to high levels of caffeine.

Caffeine and bone and calcium imbalance

A minor depressor effect of caffeine on the calcium intestinal absorption and no effect on total calcium excretion in a 24-h time period was suggested. Epidemiological studies show that this negative outcome can be partially justified by the inverse relation between milk and the consumption of caffeine-containing beverages; a high caffeine intake is frequently a marker for a low calcium intake. The consumption of beverages containing caffeine is, therefore, a risk factor for osteoporosis. However, there is no evidence of a harmful effect of caffeine on the bone system for individuals who ingest the recommended daily doses of calcium.

A direct action of caffeine is increased calcium urinary excretion, because of the antagonistic mechanism of adenosine receptors. Acute caffeine intake increases urinary calcium excretion. In general, there is evidence that, in younger individuals, calcium absorption can increase to compensate for urinary losses, while elderly individuals are less adaptable.

Contrary to its predominately vasodilator effect, adenosine acts in the kidney as a vasoconstrictor.

Caffeine and human behavior

The effects of caffeine's impact on cognitive function, including alert state, surveillance, memory, and mood are not consistent. Inconsistencies may be due to the different methodologies, individual personalities, hour of the day when studies were conducted, or other uncontrolled and confusing factors, such as alcohol and smoking. In general, caffeine increases the alert state and decreases fatigue, improving the performance of surveillance and simple tasks.

However, extremely high caffeine doses may increase anxiety; but these consequences are rare with normal caffeine consumption doses. In adults with preexisting anxiety disorders, caffeine can have an exacerbating effect. Caffeine abstinence seems to be associated with negative effects; however, the studies are inconclusive.

Association between caffeine and sleep demonstrate an unwanted drowsiness reduction when the individual is working during the night, or with sleep deprivation. However, consumption patterns suggest that individuals control caffeine intake to not interfere with sleep. Thus, moderate caffeine consumption by healthy adults could not be associated with adverse effects on behavior.

Caffeine and the renal system

Caffeine is known as a diuretic substance. However, long-term studies show a decrease of the diuretic effect, leading to hypokalemia. The proposed mechanism of action includes the activation of β_2 adrenergic receptors and diuresis.

Caffeine and carcinogenicity

According to the IARC (International Agency for Research on Cancer), there is not enough evidence on caffeine to conclude about its carcinogenicity in humans. Therefore, caffeine cannot be classified with regard to carcinogenicity in humans.

Effects of caffeine on children and adolescents

In the 1990s, approximately 75–90% of children consumed caffeine regularly. Its consumption continued to grow, both in frequency and in quantity. Beneficial effects of caffeine consumption by children and adolescents are limited and restricted to athletic and short-term sports performance. However, several adverse effects have been reported in children and adolescents relating to caffeine intake. Caffeine intake has been associated with behavioral changes, hypertension, and hypercholesterolemia. However, no consistent scientific evidence is available.

Increased risk of adverse effects related to energy drink consumption can affect, in particular, children with cardiovascular, renal and hepatic diseases, diabetes, behavior and mood disorders, hypothyroidism, and specific pharmacological therapy. Physiological responses to caffeine in children and adolescents, namely increased blood pressure, were dose-dependent. Nevertheless, children and adolescents should not be considered as miniature adults because the effects of caffeine can be different from the effects attributed to adults. Additionally, child and adolescent brains are growing; thus, a healthy diet and an adequate number of sleeping hours are essential for proper child development.

Caffeine intake can compromise adolescent sleep. Moderate or high caffeine consumers have more sleep interruptions and disorders, and feel more tired in the morning. Caffeine can improve attention, but can also raise blood pressure and cause sleep disorders in children.

The relationship between caffeine consumption and drug abuse in adolescents demonstrates that consuming four or more drinks per day containing caffeine was associated with aggressive behavior, bad manners, and an increase in smoking habits.

Caffeine is frequently utilized in children and adolescents to improve school and sports performance; however, the results are not consistent. Caffeine is believed to improve sports performance by enhancing muscle contraction and decreasing effort and fatigue.

Conclusion/Final Remarks

The importance of caffeine is evident. Since antiquity, people have sought to perfect the form of their caffeine drinks. However, energy drinks currently constitute a concern because it is not possible to find a consensus in the literature and/or among regulators about the 'definition' of this type of beverage. The difficulty of distinguishing between energy and sports drinks raises concerns. In addition, there are many incorrect social perceptions, especially among young people, about the benefits and harmful effects of energy drinks. Moreover, energy drinks are easily accessible in the market; there are no age restrictions for their purchase, and costs are similar to those of common refrigerated beverages.

As such, it becomes necessary to sensitize the entities responsible for risk communication to the at-risk population, namely children and adolescents. Also, for this reason, there is strong need for regulatory entities to establish maximum caffeine content limits, and also labels with warnings about potential health risks. Moreover, European legislation determines that all beverages having $\geq$150 mg caffeine per liter must be labeled with the statement "High caffeine content. Not recommended for children or pregnant or breastfeeding women" in the same field of vision as the name of the beverage, followed by a reference in brackets to the caffeine content, expressed in mg per 100 ml.

These health warnings should be extended to advertising on television, and to the heavy marketing that the energy drink industry utilizes in the various channels of communication, sponsorship, and advertising gifts distribution.

Finally, the combination of the consumption of energy drinks and alcoholic beverages, or energy drinks and exercise should be avoided. Also, energy drink consumption by sensitive populations (individuals with cardiovascular disease, hypertension, insomnia, and anxiety, etc.) should be avoided. Moreover, at-risk population groups that are more sensitive to caffeine's adverse or toxic effects, such as children, adolescents, pregnant women and breastfeeding mothers should avoid consumption of energy drinks. Thus, the entities responsible for risk communication should provide adequate information about the risks associated with excessive caffeine intake.

See also: Adolescent Nutrition; Alcohol: Metabolism and Health Effects; Caffeine: Characterization and Properties; Carcinogenic: Carcinogenic Substances in Food; Cocoa: Composition and Health Effects; Coffee: Analysis and Composition; Coffee: Decaffeination; Coffee: Health Effects; Coffee: Types and Production; Hypertension and Diet; Pregnancy: Dietary Guidance for Pregnancy; Renal Function and Disorders; Risk Assessment of Foods and Chemicals in Foods; Sports Nutrition; Tea: Analysis and Tasting; Tea: Chemistry and Processing; Tea: Health Effects; Tea: Types, Production, and Trade.

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Relevant Websites

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https://www.food.gov.uk/ – FSA – Food Standards Agency.

Cakes: Types of Cakes

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Introduction

Cake products are found around the world and vary widely. Good quality cakes have a large volume and a crumb grain that is uniform with many small, fine cells and no large holes or tunnels. The definition of cake also varies in different parts of the world. In general, cakes are sweet baked products, which contain high levels of sugar, fat, eggs, and water. Most cakes are made using soft wheat flour with a low protein content of 7–9%. The soft wheat flour is often pin-milled to produce a very fine (small) particle size. Smaller flour particles have more surface area and are able to absorb more liquid and produce better quality cakes.

Cakes are classified by the methods used to produce them. Common classifications include layer cakes, foam cakes, and pound cakes. Layer cakes are further divided into high-ratio and low-ratio cakes and are also categorized based on the method used to prepare the batter, which includes multistage mix, single-stage mix, or continuous mix. Foam cakes can be further classified by the level and source of fat in the formula. Examples are angel food (no fat), sponge (egg yolk only), and chiffon (egg yolk and added fat). Pound cakes are typically made using the multistage (creaming) method. Pound cakes were named based on the original formula, which contained 1 lb of flour, 1 lb of butter, 1 lb of sugar, and 1 lb of eggs.

Layer Cakes

Layer cakes are quite common across the globe. They typically consist of multiple sheets of cake stacked together with some type of filling (frosting, jam, preserves, etc.) between the layers. A range of flavors, ingredients, toppings, shapes, and sizes vary enormously. At the basic level, layer cakes are produced using a batter system, which is high in sugar, water, and fat. The first step in layer cake production is making the batter. Although the procedure varies for the different methods, the objectives of mixing are the same: (1) to combine all of the formula ingredients into a smooth, uniform batter, (2) to form a stable emulsion of fat in water, and (3) to incorporate air cells. Both high-ratio and low-ratio layer cakes can be prepared using three different mixing procedures: multistage mix, single-stage mix, and mechanical mix. The three methods vary by how air is incorporated into the batter, which affects how stable the batter is during bench time (time between mixing and baking) and the texture of the final baked cake.

Multistage Mix Process

Cake batters made using the multistage mix process have excellent stability and produce cakes with a uniform, fine crumb grain (large number of small-sized air cells). In the first mixing stage, solid fat and sugar are creamed together. The wet ingredients and flour are then incorporated in two or three subsequent mixing steps. The purpose of creaming is to incorporate air into the fat. The sugar crystals help cut the fat to increase air incorporation. Because the air cells are trapped in the shortening, they are very stable and will not coalesce in the batter during bench time (holding or processing time between mixing and placement into the oven). When the shortening melts during baking, the air cells are released into the aqueous phase where the leavening gases produced by the chemical leavening reaction and water vaporization can diffuse into them to leaven (raise) the cake.

Single-Stage Mix Process

Commercial cake mixes made by home consumers and institutions are made using a single-stage mixing process. The cake mix contains the dry ingredients and fat, which are processed through a cake finisher that acts like a grinder to bind the fat onto the surface of the flour particles. This ensures that there is no free fat in the system, which would destabilize the foam generated during mixing. To prepare the cake, the consumer simply adds the liquid ingredients and mixes the batter for a few minutes. Air is trapped in the aqueous phase during mixing. This is possible because the formula contains surfactants such as propylene glycol monostearate to assist in air incorporation and stabilization.

Cakes made by the single-stage mix are simple to prepare but have poor stability and will not withstand bench time. During standing, the air cells in the batter quickly coalesce (go together) to form large bubbles, which can rise out of the batter, resulting in poor volume and poor crumb grain that is not uniform with coarse cells, large holes, and tunnels. The high level of surfactants also makes the cake delicate and tender, which is good for eating but not desirable for commercial cakes, which may be stacked and/or transported.

Continuous Mix Process

Specialized mixers are used for the production of cakes by the continuous mix process. In this method, all of the ingredients are made into a slurry, which is fed into the mixing head where the batter is mixed at high speed, while air is injected into it. Air is incorporated into the aqueous phase due to the high energy input. This method is the most expensive of the three methods. Batters made using this process are very stable and produce cakes with a firm texture. For these reasons, this production method is used for most commercial cakes.

High Versus Low Ratio

Layer cakes can be made using a high-ratio or low-ratio formula (Table 1). The main difference is the level of sugar relative to the level of flour. High-ratio layer cakes are typically

Table 1 Typical high- and low-ratio layer cake formulas

	High ratio (% flour weight)	*Low ratio (% flour weight)*
Flour	100	100
Sugar	140	100
Shortening	55	45
Milk	95	68
Whole egg	76	66
Baking powder	1.3	1.3
Salt	0.7	0.7

consumed in the United States, while many European countries prefer low-ratio layer cakes.

During baking, the high water content in the batter allows the starch to gelatinize during baking, which helps set the structure of the cake during baking and results in a uniform, aerated crumb grain and a cake with a tender texture. The ratio of sugar to flour dramatically impacts the properties of the batter during baking and affects the volume and structure of the baked cake. High-ratio cakes contain higher levels of sugar than flour. The sugar level generally ranges from 125% to 140% based on the weight of the flour. Chocolate cakes can tolerate even higher levels of sugar and may contain as much as 180% sugar on a flour weight basis. Examples of high-ratio cakes include angel food, sponge, chiffon, and layer cakes consumed in the United States. The sugar level controls the temperature at which the cake structure sets (transforms from a fluid batter to solid foam) by its effect on the temperature at which the starch gelatinizes and the egg protein denatures. In high-ratio layer cakes, the high level of sugar increases the denaturation temperature of the egg and the gelatinization temperature of the starch. The gelatinization temperature of the starch is increased to a temperature higher than the boiling point of water. During baking, the internal temperature of the cake does not exceed the boiling point of water; thus, the starch does not fully gelatinize in the interior of a cake baked with a high-ratio formula. As a result, the cake collapses during cooling. For this reason, flour used for high-ratio layer cake production is typically chlorinated. The chlorine gas reacts with the protein, lipids, starch, and other components of the flour, thereby changing their functionality. The reaction with the protein produces hydrochloric acid (HCl), which alters the pH of the flour. The change in pH does not impact the baking properties but does provide a way to measure the degree of chlorination that the flour received. Untreated flour has a pH of approximately 6.1, while highly chlorinated flour needed for production of high-ratio layer cakes typically has a pH ranging between 4.7 and 4.9. It is the oxidation of the starch that is the mechanism by which chlorine gas improves the baking performance of the flour. Oxidized starch is able to absorb water more quickly than native starch and will swell at a faster rate after gelatinization. This allows the viscosity of the batter to increase at a faster rate in the oven. As a result, the starch is completely gelatinized and the structure of the cake is fully set by the end of baking so it does not collapse upon cooling.

In low-ratio cake formulas, the sugar level is equal to or lower than the level of the flour. In low-ratio cakes, the sugar level relative to the flour level is not high enough to significantly raise the starch gelatinization temperature. Thus, it is not necessary to use chlorinated flour for the production of low-ratio layer cakes. Examples of low-ratio cakes include pound cakes and layer cakes consumed in Europe.

The viscosity of the batter is critical in order to produce a cake of high quality. Proper batter viscosity ensures the cake will have large volume and a uniform, fine crumb grain. The batter viscosity must be high enough to keep the starch and the air cells suspended. If the batter is too thin (low viscosity), the starch granules can settle to the bottom of the cake during baking. This results in a tough, rubbery, dense, gummy-appearing layer at the bottom and a fluffy, foamy-appearing layer at the top of the cake. The air bubbles will also coalesce (go together), creating large bubbles, which can become buoyant enough to rise to the surface of the batter and be lost, resulting in a cake with low volume. Large air bubbles that do not rise out of the cake will produce an open, irregular crumb grain containing large holes or tunnels.

Layer cakes are leavened by chemical leavening and steam. It is important to select the correct leavening system for the desired color, texture, volume, and crumb grain characteristics. Some varieties of cakes are leavened by steam alone and others by steam in addition to chemical leavening agents, such as baking soda (sodium bicarbonate) and baking powder. The baking powder most often used is double-acting, which contains sodium bicarbonate and two leavening acids. One leavening acid reacts at room temperature (fast-acting) to help nucleate the batter during mixing, while the other reacts in the oven (slow-acting) to expand the air cells during baking. When using baking powder, it is important to select one with the correct slow-acting leavening acid that will react at the proper time during baking. If the leavening acid reacts too early before the batter viscosity is high enough, the air cells will diffuse out of the batter, resulting in low volume and a coarse crumb grain. On the other hand, if the leaving acid reacts too late after the structure is set, the cake cannot expand, resulting in low volume, and the buildup of pressure inside the cake can destroy the crumb grain and may cause the top surface to crack.

Foam Cakes

Foam cakes are characterized by their extremely light, fluffy, and spongy texture. Eggs are the most important ingredient in the formula and play a critical role in leavening and the structure of the cake. In the production of foam cakes, the eggs are whipped into foam. The stability of the foam is critical to the final volume and texture of the cake. Fat inhibits foam formation and creates softer, less stable foams. For this reason, the stiffness and stability of foams generated using egg whites alone are the highest followed by whole egg. Egg yolk alone does not form into foam. In formulas containing whole egg, it is common to whip the egg whites into foam and then gently fold them into a separately prepared batter that contains the egg yolk later in the process. In the preparation of an egg white foam, all mixing bowls, utensils, and baking pans should be

free from grease. For this reason, it is common for egg white foams to be prepared in metal rather than plastic bowls, which can retain enough residual fat from other products to destroy the foam. Foam cakes are also commonly baked in pans, which are not greased, even if the formula contains whole egg. Foam cakes can be classified by the level and source of fat in the formula and include (1) no fat, (2) fat from egg yolk only, and (3) fat from egg yolk plus fat added in the form of butter, shortening, or oil. Examples are angel food (no fat), sponge (egg yolk only), and chiffon (egg yolk and added fat).

Angel Food Cake

Angel food cake, also known as angel cake, contains only egg whites and is an example of a no fat foam cake. Angel food cakes have a light, airy structure and a spongy or chewy texture due to their high sugar content and the absence of fat. A typical formula is shown in Table 2. The batter is prepared by whipping the egg whites with sugar and cream of tartar to produce stiff foam. The sugar helps stabilize the foam. Although cream of tartar is a leavening acid (tartaric acid), it is not added as a leavening agent but rather to reduce the pH of the egg white proteins in order to improve their whipping ability. The air whipped into the foam is the leavening agent. Once stiff foam is produced, flour and flavorings are very gently folded in. Extreme care must be taken to not stir too vigorously and break the foam.

The flour used in angel food cakes is weak soft wheat flour that has a very low protein content of about 4%. Often, wheat starch is added to dilute the flour. The function of the flour in angel food cake is completely different than in almost every other bakery good. In most baked products, the gluten protein is the most important component of the flour and responsible for the structure and quality of the baked product. In angel food cake, the starch is the most important component of the flour. When the starch gelatinizes during baking, it absorbs water, which dries the foam and causes the structure to set. Angel food cakes are typically baked in tube pans, which are tall, round metal pans that have a hollow metal tub in the center (Figure 1). The center tube helps the center of the cake bake completely. After baking, the tube pan is inverted while cooling to prevent the cake from collapsing.

Table 2 Typical foam cake formulas

	Angel food (% flour weight)	*Sponge (% flour weight)*	*Chiffon (% flour weight)*
Flour	100	100	100
Sugar	215	143	108
Egg white	277		
Whole egg		143	143
Cream of tartar	4	1	1
Oil			40
Water			80
Baking powder		4	4
Salt	1	1	2

Sponge Cake

Sponge cakes contain a small amount of fat, which comes from the use of whole eggs (egg yolk). These cakes are richer and more flavorful than angel food cakes. In general, sponge cakes are prepared using a combination of a batter and foam. The batter is prepared by beating the flour, egg yolks, and half of the sugar. Separately, the egg whites and remaining half of the sugar are whipped into foam, which is carefully folded into the egg yolk batter. In some cakes, the whole egg is whipped instead of the egg whites being whipped separately. Sponge cakes are baked in a variety of differently shaped pans. The spongy structure of the cake lends itself well to rolling; thus, sponge cake is often used to produce rolled and filled cake desserts.

Chiffon Cake

Chiffon cakes are the richest and most flavorful of the foam cakes. The texture is light and airy but denser than angel food and sponge cakes. The formula contains whole egg and added fat in the form of butter, shortening, or oil (Table 2). The fat impedes the foaming ability of the eggs, so these cakes also contain chemical leavening in order to produce expanded cakes with light and airy textures. In general, chiffon cakes are prepared using a combination of a batter and foam. The batter is prepared by beating the flour, egg yolks, fat, water, and flavorings and some of the sugar. Separately, the egg whites and remaining sugar are whipped into foam, which is carefully folded into the egg yolk mixture. The batter has a thinner consistency (lower viscosity) than the other types of foam cakes. Chiffon cakes are traditionally baked in tube pans (Figure 1).

Pound Cake

Pound cakes are rich and have a dense but tender texture. Originally, pound cakes were made using 1 lb of flour, 1 lb of butter, 1 lb of sugar, and 1 lb of eggs. Modern formulas still

Figure 1 Tube cake pan.

Table 3 Typical pound cake formula

	Percent (flour weight)
Flour	100
Sugar	100
Shortening	50
Milk	50
Whole egg	50

contain those basic ingredients but the proportions have changed and chemical leaveners have been added. A typical formula is given in Table 3. Pound cakes are typically made using the multistage (creaming) method. The cakes are usually baked in loaf or Bundt pans. Sponge cake batter lends itself well to the addition of ingredients such as cream cheese, sour cream, coconut, nuts, raisins, and other dried fruits and a multitude of flavoring agents.

See also: Acids: Natural Acids and Acidulants; Aerated Foods; Emulsifiers: Types and Uses; Gums: Properties and Uses; Leavening Agents.

Further Reading

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Calcium: Physiology

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Introduction

Calcium is the most abundant mineral in the human body. Approximately 99% of calcium in the body is found in the skeleton as hydroxyapatite, a crystalline complex of calcium and phosphate. The remaining 1% of calcium found in the body is found in the blood, extracellular fluid, and soft tissues. Calcium is a nutritionally essential mineral that plays a critical role in a number of processes. It provides the skeleton with rigidity, hardness, and strength to bear a variety of intrinsic (e.g., muscle contraction) and extrinsic (e.g., gravity, trauma) forces. It also plays an important metabolic role in facilitating muscle contraction, blood coagulation, enzyme activity, nerve synaptic function, secondary messengers, hormone release, and membrane permeability. Calcium is so vital in supporting physiological processes of the body that its concentration in the serum and extracellular fluid is maintained within a very narrow range. If dietary intake of calcium is low, the body mobilizes calcium from skeletal tissue, a dynamic calcium reservoir, to maintain serum calcium levels. Chronic insufficiency of calcium ultimately leads to decreased bone mass, the development of osteoporosis, and increased risk for sustaining fragility fractures.

Dietary Sources of Calcium

Calcium is naturally found in a plethora of foods; however, its richest source is dairy. A cup of milk or yogurt provides approximately 300 mg of calcium, while 50 g of cheese including Swiss, goat, low-fat cheddar, mozzarella, Gruyère, and some processed cheese slices contains 250–500 mg of calcium. Several other natural sources of calcium include green leafy vegetables, nuts, seeds, and beans (Table 1). Vegetables contain much lower levels of calcium per serving compared to dairy products. Thus, it is easier and more practical to consume dairy products to meet daily calcium requirements. Calcium-fortified foods are becoming increasingly more common in the North American market as an alternative for achieving adequate dietary calcium intake. Calcium-fortified foods include fruit and juice beverages, soy and almond beverages, rice beverages, breakfast cereals and bars, granola bars, breads, and tofu. These calcium-fortified foods may contain up to 300 mg of calcium per serving.

Patterns of Consumption

From 2007 to 2012, the consumption of dairy and, more specifically, milk has decreased among Canadians and Americans. This pattern of decreased milk intake can also be seen globally among several European countries (e.g., Belgium, France, Iceland, Italy, the Netherlands, Spain, Sweden, and Switzerland) as well as in Iran and New Zealand. In North America, calcium intake remains a concern for all age groups. According to the Canadian Community Health Survey, a federal cross-sectional survey on the health status, health-care utilization, and health determinants for the Canadian population, 65% of men and 72% of women aged 31–50 years do not consume the minimum recommended daily servings of dairy. Similarly, 83% of girls and 61% of boys aged 10–16 years do not meet the recommended 3–4 servings of dairy per day. In the United States, 85% of the population does not meet the recommendations for dairy intake. Because it is easier to meet daily calcium needs through dairy intake, strategies such as the Get Enough campaign in Canada and Milk, a Force of Nature campaign in Europe have been launched by various lobbying organizations to increase daily calcium intake through dairy and other foods. For those who cannot meet their calcium requirements with food, the medical community promotes the use of calcium supplements to achieve recommended intake levels.

Recommended Intakes Across the Life Span

The recommended intakes of calcium and vitamin D for both the United States and Canada were reassessed in 2010 by the Committee to Review Dietary Reference Intakes for Vitamin D and Calcium established by the Institute of Medicine. Dietary reference intakes (DRIs) were updated to replace adequate intakes (AIs) for all age groups, except for infants, with estimated average requirements (EARs) and recommended dietary allowances (RDAs), due to currently available data on bone health outcomes. These current data include large-scale randomized trials and calcium balance studies. Data were not sufficient to establish an EAR for infants; thus, the AI is used to reflect an intake level based on estimates that are assumed to be adequate. However, current data have facilitated the development of an upper level (UL) for infants, which was not previously specified. Table 2 lists the DRIs for calcium by life stage group in Canada and in the United States.

Bioavailability, Absorption, Regulation, and Distribution in the Blood

Bioavailability

There are several natural and fortified dietary sources of calcium as well as numerous calcium supplement products. The degree to which the body can absorb calcium can vary substantially between these sources (Table 1). Calcium is considerably bioavailable from the consumption of dairy foods. Some calcium-fortified foods and beverages also have calcium bioavailabilities that are comparable to that in dairy, while

Table 1 Calcium content and estimated absorption from various food products

Food	*Serving size*	*Calcium content (mg)*[a]	*Fractional absorption (%)*	*Estimated calcium absorbed (mg)*	*Servings required to equal 250 ml of milk*
Milk products					
Milk	250 ml	312	32.1	100	1.0
Cheddar cheese	42 g	303	32.1	97	1.0
Vegetables					
Kale	65 g	47	58.8	28	3.6
Broccoli	71 g	35	52.6	18	5.6
Spinach	90 g	122	5.1	6	16.7
Brussels sprouts	78	19	63.8	12	8.3
Nuts and seeds					
Almonds	28 g	80	21.2	17	5.9
Sesame seeds, no hull	28 g	37	20.8	8	12.5
Legumes					
Beans, white	110 g	113	17.0	19	5.3
Beans, pinto	86 g	45	17.0	8	12.5
Beans, red	172 g	41	17.0	7	14.3
Fortified foods					
Orange juice (fortified with calcium citrate malate)	250 ml	300	36.3	109	0.9
Tofu, calcium set	126 g	258	31.0	80	1.2
Soy beverage (fortified with tricalcium phosphate)	250 ml	300	24.0	72	1.4
Soy beverage (fortified with calcium carbonate)	250 ml	300	21.1	63	1.6

[a]Based on a half-cup serving size unless otherwise noted.

Selected data from Weaver, C. M. and Plawecki, K. L. (1994). Dietary calcium: adequacy of a vegetarian diet. *American Journal of Clinical Nutrition* **59**, 1238S–1241S; 'Dairy Nutrition' www.dairynutrition.ca/.

others do not. This may be explained by the chemical nature of the calcium compound used to fortify the food or beverage product and the food or beverage matrices to which the calcium is added. The bioavailability of calcium from the consumption of some vegetables (e.g., cauliflower and broccoli) is even higher than that from dairy and calcium-fortified foods. However, vegetables contain much lower levels of calcium per serving compared to dairy products and calcium-fortified foods (Table 1). Another consideration is that some vegetables and plant foods contain other components (i.e., oxalates and phytates) that form salt complexes with calcium that render them unabsorbable by the body. Indeed, it is the amount of calcium along with its bioavailability that determines how much calcium a particular food source will provide per serving. Thus, dairy foods, and more specifically, milk, continue to serve as the major food source of calcium in Western diets. In North America, 75% of dietary intake of calcium comes from dairy. With regard to supplemental calcium, these may also vary in bioavailability. Some research suggests that differences in bioavailability among calcium supplements may be linked to the form in which the supplement is found (i.e., tablet, powder, or liquid) or the acid to which it is bound; however, these findings are mixed. Interestingly, supplemental calcium shows a comparable absorption efficiency as dietary calcium.

Absorption and Regulation

Approximately 90% of calcium absorption occurs in the small intestine with smaller amounts absorbed through the colon. Calcium absorption occurs by two major pathways, an active transcellular pathway that primarily occurs in the duodenum and a passive paracellular pathway that occurs through specialized membrane domains (i.e., tight junctions) throughout the small intestine but predominately in the more distal regions, particularly in the ileum. In active calcium transport, calcium moves across the brush border of the microvilli of the small intestine and into the enterocyte through calcium-selective channels called transient receptor potential vanilloid type 5 (TRPV5) and type 6 (TRPV6) (Figure 1). Calcium is then transported across the enterocyte to its basolateral side by a transport protein (calbindin-D) where it then gets extruded into interstitial space (lamina propria) by transport proteins (basolateral plasma membrane calcium pump (PMCA) and the sodium calcium exchanger) before making its way into the circulation. In paracellular calcium transport, specialized membrane domains (tight junctions) located between the apical and basolateral membranes of the enterocyte form a barrier to the movement of ions, proteins, and other macromolecules across the intestines. These tight junctions are driven by the luminal electrochemical gradient for calcium. Thus, when calcium levels inside the lumen of the small intestine are high, passive calcium entry into the enterocytes is stimulated by the paracellular pathway (Figure 1).

Transcellular calcium transport is almost exclusively regulated by 1,25-dihydroxyvitamin D (calcitriol), the hormonally active metabolite of vitamin D, while paracellular calcium transport is driven by a downhill concentration gradient of calcium between the luminal space and the interstitial space.

Table 2 Dietary reference intakes of calcium (mg day^{-1})[a]

Age	*AI (mg)*	*EAR (mg)*	*RDA (mg)*	*UL (mg)*
Infants				
0–6 months	200	–	–	1000
6–12 months	260	–	–	1500
Children				
1–3 years	–	500	700	2500
4–8 years	–	800	1000	2500
Males				
9–13 years	–	1100	1300	3000
14–18 years	–	1100	1300	3000
19–30 y	–	800	1000	2500
31–50 years	–	800	1000	2500
51–70 years	–	800	1000	2000
>70 years	–	1000	1200	2000
Females				
9–13 years	–	1100	1300	3000
14–18 years	–	1100	1300	3000
19–30 years	–	800	1000	2500
31–50 years	–	800	1000	2500
51–70 years	–	800	1000	2000
>70 years	–	1000	1200	2000
Pregnancy				
14–18 years	–	1100	1300	3000
19–30 years	–	800	1000	2500
31–50 years	–	800	1000	2500
Lactation				
14–18 years	–	1100	1300	3000
19–30 years	–	800	1000	2500
31–50 years	–	800	1000	2500

AI, adequate intake; EAR, estimated average requirement; RDA, recommended dietary allowance; UL, tolerable upper intake level.

[a]As set out by the Institute of Medicine (2011). *Dietary reference intakes for calcium and vitamin D*. Washington, DC: National Academy Press.

Even though the rate of calcium absorption through the transcellular pathway is approximately three times greater than that through the paracellular pathway, the greatest proportion of calcium absorption when dietary intake is high occurs in the ileum through the paracellular pathway. This is because the sojourn time of chyme in the lower half of the small intestine (hours) is substantially higher than that in the duodenum (minutes). Thus, when dietary intake of calcium is normal or high, the major factor that determines the amount of calcium absorbed is the quantity ingested. When dietary intake of calcium is low, a high proportion of calcium that is absorbed occurs through the transcellular pathway in the duodenum. Thus, in addition to 1,25-dihydroxyviamin D, low dietary intake of calcium also regulates the efficiency of the transcellular pathway. Other factors that affect the transcellular transport of calcium include estrogen status and age.

Distribution in the Blood

Approximately 99% of calcium in the body is stored in the skeleton as hydroxyapatite, while the remaining 1% of calcium in the body is located in the blood, extracellular fluid, and soft tissues. Calcium in the blood is found in three forms: (1) ionized or free calcium, which accounts for approximately 50% of total calcium concentration; (2) protein-bound calcium (bound mainly to albumin), which accounts for approximately 30–40% of the total serum calcium concentration; and (3) complexed or chelated calcium, which accounts for approximately 10% of the total blood calcium concentration. Ionized calcium is the physiologically active form of calcium that plays an important metabolic role in maintaining bone homeostasis, facilitating muscle contraction and relaxation, nerve synaptic function, blood coagulation, enzyme activity, secondary messengers, and hormone release. Protein-bound calcium is not usable by tissues due to its inability to diffuse through membranes. Chelates serve to bind calcium for maintaining homeostatic cytosolic concentrations.

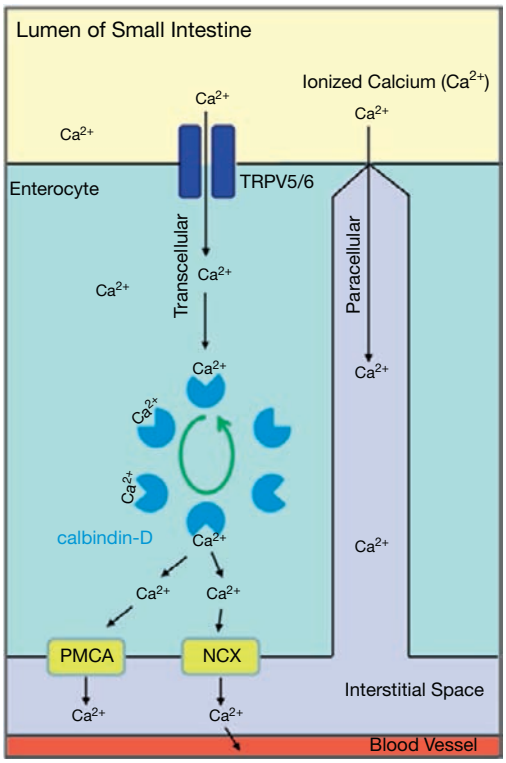

Figure 1 Active and passive calcium transport across the intestines. In active calcium transport, calcium moves across the brush border of the microvilli of the small intestine and into the enterocyte through calcium-selective channels (i.e., transient receptor potential vanilloid type 5 (TRPV5) and 6 (TRPV6)) and then is transported across the enterocyte to its basolateral side by calbindin-D. Calcium is then extruded into interstitial space via the basolateral plasma membrane calcium pump (PMCA) and the sodium calcium exchanger (NCX) before making its way into the circulation. In paracellular passive calcium transport, tight junctions located between the apical and basolateral membranes of the enterocyte form a barrier to the movement of ions, proteins, and other macromolecules across the intestines. Transport across these tight junctions is driven by the luminal electrochemical gradient for calcium. Modified from Hoenderop, J. G. J., Nilius, B. and Bindels, R. J. M. (2005). Calcium absorption across epithelia. *Physiological Reviews* **85**, 373–422.

Calcium Balance

Calcium balance is determined by its dietary intake, absorption, and excretion and is tightly regulated by the

complex interplay between the intestines, bones, and kidneys. Calcium balance is maintained when the net absorbed calcium through the intestines and kidneys (reabsorption) matches the net excreted calcium through the feces, urine, and the skin. Thus, if an adult ingests 1 g of calcium, it is estimated that approximately 0.35 g will be absorbed in the small intestine. This occurs under the tight regulation of the calciotropic hormones. Calcium balance will be achieved if the kidney excretes the same amount of calcium (0.35 g) that the small intestine absorbed. This is accomplished by a combination of filtration of calcium across the glomeruli and subsequent reabsorption of the filtered calcium along the renal tubules. Adaptations of the intestines, bones, and kidneys to various stimuli occur to compensate for changes throughout the life span (e.g., growth, hormones, injury, and disease).

Hormonal Regulation

There are three major hormones involved in controlling calcium balance. These are parathyroid hormone (PTH), 1,25-dihydroxyvitamin D, and calcitonin. Other hormones, such as estrogens, stanniocalcin, thyroid hormones, insulin, and adrenal corticosteroids, contribute to the maintenance of calcium homeostasis.

Parathyroid hormone

PTH is secreted into the circulation by the parathyroid glands in response to low blood calcium levels and acts primarily on the kidneys and bones to increase blood calcium levels. PTH acts on the kidneys by directly stimulating renal tubular calcium reabsorption, which increases circulating levels of calcium. PTH also directly stimulates the breakdown of bone (bone resorption) to increase circulating levels of calcium. The absorption of calcium through the small intestine is indirectly regulated by PTH due to the action of PTH on increasing the activity of 1-α-hydroxylase, the enzyme that catalyzes the hydroxylation of 25-hydroxyvitamin D (calcifediol) into the active hormone, 1,25-dihydroxyvitamin D (calcitriol).

1,25-Dihydroxyvitamin D

1,25-Dihydroxyvitamin D enhances active transcellular transport of calcium through the small intestine in response to decreased calcium intake. It also mobilizes calcium and phosphate from the bone and increases renal reabsorption of calcium by increasing transport proteins (i.e., TRPV5, TRPV6, calbindin-D, and PMCA). The action of 1,25-dihydroxyvitamin D in increasing blood levels of calcium occurs when adequate substrate (i.e., vitamin D) is provided, mainly through sun exposure, diet, or supplemental intake.

Calcitonin

Calcitonin is synthesized and secreted into the circulation by parafollicular cells of the thyroid gland in response to high circulating levels of calcium. Calcitonin acts to reduce circulating levels of calcium by decreasing bone resorption through the inactivation of bone-resorbing osteoclasts. It also lowers blood calcium levels by reducing calcium absorption through the small intestine and renal tubular calcium reabsorption.

Dietary Control

Vitamin D

Vitamin D must be hydroxylated in two subsequent reactions by two separate hydroxylase enzymes in order to produce the active hormone metabolite 1,25-dihydroxyvitamin D that plays a major role in increasing blood levels of calcium (see section '**Hormonal Regulation**'). Thus, through the action of its hormone metabolite, vitamin D is critical for supporting calcium homeostasis. However, vitamin D is not found naturally in most foods that are commonly consumed. Fleshy fish including salmon, mackerel, and herring are naturally rich in vitamin D (219–392 IU per 2.5 ounce serving). Other good food sources of vitamin D include egg yolk and milk. In Canada and in many other countries, vitamin D is added to foods such as milk, yogurt, soy beverages, and margarine in an effort to achieve adequate daily intakes of vitamin D. Moreover, supplementation with vitamin D is becoming increasingly more common as recommendations for vitamin D have increased substantially over the past decade.

The other major source of vitamin D for humans is synthesis by skin exposed to ultraviolet B (UVB) photons from sunlight. When UVB photons reach the surface of the skin, they are absorbed by 7-dehydrocholesterol in the plasma membrane of the epidermis, transformed to previtamin D, and then rapidly converted to vitamin D. Vitamin D is then hydroxylated into 25-hydroxyvitamin D in the liver, which is then hydroxylated in the kidneys to form the active hormone 1,25-dihydroxyvitamin D. Sun exposure can result in adequate production of vitamin D in humans. However, there are several factors that can limit the number of UVB photons that reach the surface of the skin and that contribute to vitamin D deficiency in many countries including Canada and the United States. These include the zenith angle of the sun, melanin, use of sunscreen products, and pollution in the air.

Vitamin D plays a critical role in maintaining calcium balance because balance is almost exclusively regulated by 1,25-dihydroxyviamin D, the hormonally active metabolite of vitamin D (see section '**Hormonal Regulation**'). 25-Hydroxyvitamin D concentration in the serum is the functional indicator of vitamin D status. Some research suggests that optimal calcium absorption is achieved when serum levels of 25-hydroxyvitamin D are at a minimum of 50 nmol l^{-1}. Others suggest that optimal calcium absorption is achieved when serum levels of 25-hydroxyvitamin D are above 75 nmol l^{-1} and even 80 nmol l^{-1} in certain populations such as postmenopausal women.

Phosphorus

Phosphorus is a nutrient that is easily obtained in the Western diet and is naturally found bound to oxygen as phosphate. Phosphorus is naturally found in appreciable amounts in milk, milk products, meat, beans, lentils, nuts, and grains. Phosphorus is increasing in the food supply due to the increasing addition of phosphate additives that are sources of inorganic phosphate to processed food products. Inorganic phosphorus is commonly added to processed food products to improve their taste, shelf life, and speed of preparation. In the United States, the greatest contributors of phosphorus in the diet are milk and dairy, followed by grain-based dishes, breads, poultry, pizza, vegetables, meats, and processed food

products. The absorption rate of naturally occurring phosphorus is between 40% and 60%, whereas the absorption rate of inorganic phosphorus ranges between 80% and 100%. Inorganic phosphorus has a much greater absorption rate and efficiency compared to organic phosphorus because it does not require enzymatic digestion in the stomach.

Studies have shown that the effects of phosphorus on various physiological processes are in part determined by the accompanying levels of dietary calcium. However, if dietary intake of calcium is inadequate, this balancing relationship is nullified and neither phosphorus nor calcium will support normal physiological function. In one trial, a one-time consumption of 1500 mg of phosphorus, which is over twice the RDA, showed decreases in serum calcium levels in healthy premenopausal women. Other researches reported high circulating levels of PTH in premenopausal women that consumed phosphorus at 1319 mg day^{-1} but consumed low levels of calcium (<800 mg day^{-1}). However, recent data have shown that calcium absorption and phosphorus absorption through the intestines and kidneys are independent of each other. In addition, others have shown that a 150% increase in dietary phosphorus does not alter calcium balance when calcium intakes are low, medium, or high. Indeed, phosphorus is a constituent of hydroxyapatite that makes up bone tissue. Thus, phosphorus is essential for bone accretion. Whether and under which circumstances phosphorus modulates calcium balance continues to be a subject of research interest and controversy.

Protein

The role of high-protein diets on calcium balance is complex. Dietary protein, primarily from animal sources, generates endogenous acids through the oxidation of sulfur amino acids and phosphoproteins. These acids are suspected of inducing a negative calcium balance and subsequent demineralization of the bone. Indeed, controlled feeding studies have demonstrated that an increase in protein intake by 40–60 g day^{-1} results in a 0.3–0.8 unit reduction in urinary pH. However, further studies demonstrate that other factors such as ammonia excretion and hormones (e.g., insulin and glucocorticoids) may be more involved in urinary calcium excretion from high intakes of dietary protein. Controlled feeding studies have shown that high intakes of purified proteins such as casein, dried egg whites, and wheat gluten increase urinary calcium excretion by 0.7–2.2 mg g^{-1} of supplemental ingested protein. This effect occurs from an increase in glomerular filtration rate and a decrease in fractional tubular reabsorption rate when dietary protein intake increases one- to threefold. Other studies have demonstrated no changes in urinary calcium excretion when comparing diets high in meat consumption versus those with low meat consumption. Some foods such as meat and dairy products contain components other than protein that may lower urinary calcium excretion when adequate levels of calcium intake are achieved. Thus, the effect of diets high in protein on urinary calcium excretion depends on the nature of the dietary protein.

While high intakes of dietary protein are speculated to result in a negative calcium balance, studies examining this relationship have reported mixed findings. Some studies observed negative calcium balance with high-protein diets compared to low-protein diets, while others observed no changes in calcium excretion and calcium balance. These conflicting findings may be explained by a number of factors including the difficulties in measuring whole-body calcium balance, the nature of the ingested protein, and other factors such as level of dietary intakes of calcium and phosphorus. It is possible that increased urinary calcium excretion from high intakes of dietary protein is a result of increased intestinal absorption; however, more study is required in this area. Interestingly, when adequate levels of calcium intake are achieved, increases in protein intake are positively correlated with bone mass in all age groups. Protein stimulates the production and activity of insulin-like growth factor-1 that stimulates bone formation and the regulation of bone metabolism.

Sodium

The average dietary intake of sodium in the United States and Canada is approximately 3400 mg day^{-1}, which is more than twice the AI (1500 mg day^{-1}) and significantly higher than the UL (2300 mg day^{-1}), as set out by the Institute of Medicine. Sodium and calcium compete for reabsorption in the kidneys. Thus, high dietary intakes of sodium increase urinary calcium excretion. However, adding potassium to a high-sodium diet may help decrease calcium excretion. Evidence demonstrates that for every 2290 mg of sodium ingested, an average of 40 mg of calcium is excreted, without any adaptive compensatory mechanisms. Thus, it is reasonable to expect a daily loss of approximately 60 mg of calcium among Canadians and Americans who consume approximately 3400 mg of sodium per day.

Potassium

According to the 2010 Dietary Guidelines for Americans Advisory Committee, potassium is considered to be one of the four major shortfall nutrients in the American diet; similar shortfalls are seen in the dietary intakes of Canadians. The average intake of potassium in the United States is just over half of the dietary requirements. A variety of foods are rich in potassium including bananas, papaya, dark leafy greens, avocado, milk, yogurt, various dried beans, fish, nuts, and seeds.

Studies have shown that intake of potassium improves calcium balance. In adult and elderly men and women, supplementation with potassium citrate at 0, 60, or 90 mmol day^{-1} for 6 months resulted in improved calcium balance. Net renal acid excretion and urinary calcium excretion both decreased compared to placebo, while no changes in fractional calcium absorption were observed. Other studies have also shown that supplemental intake of potassium regardless of its form (i.e., potassium bicarbonate or potassium citrate) reduces urinary calcium. The mechanism whereby potassium reduces urinary calcium excretion is unclear, but appears to be independent of net renal acid excretion.

Caffeine

Research indicates that caffeine does not affect calcium balance when dietary levels of calcium are adequate. Studies demonstrating that caffeine from coffee and tea increases urinary calcium excretion may be explained by the acute diuretic action of caffeine that does not persist past 24 h. While caffeine intake decreases calcium absorption, this effect is small and may be

counterbalanced by adding one to two tablespoons of milk to one cup of caffeine-containing coffee.

Health Effects: Bone and Cardiovascular Complications

Composition and Remodeling of Bone

The skeleton is composed by weight of approximately 60% inorganic matter, 30–32% organic matter, and 8–10% water. The inorganic phase consists of primarily hydroxyapatite, a crystalline complex of calcium and phosphate, while the organic phase is composed of primarily type I collagen and various noncollagenous proteins. The mineral complex in the bone serves to strengthen the collagen composite, providing more mechanical resistance to the tissue. It also serves as a source of calcium, phosphate, and magnesium ions for mineral homeostasis.

The bone is a dynamic tissue whereby continuous remodeling serves to maintain structural and metabolic homeostasis in the body. When the coupling action of the bone-forming osteoblasts and the bone-resorbing osteoclasts is balanced, old bone is replaced by an equal amount of new bone; thus, there is minimal net change in bone mass. Unbalanced remodeling occurs when one of the two processes (bone formation or bone resorption) is quantitatively unequal, often resulting in the loss of too much bone. This form of unbalanced bone remodeling is common in postmenopausal women resulting in an increased risk for the development of osteoporosis and fragility fractures.

Osteoporosis

Osteoporosis is a skeletal disease characterized by compromised bone strength, predisposing an individual to an increased risk of fractures. Approximately 10 million Americans and 2 million Canadians are affected by this disease. The most common sites of osteoporosis-related fragility fractures are at the wrist, vertebrae, and hip. The consequences of fragility fractures include chronic pain, decreased quality of life, loss of independence, fear of falling, development of restrictive lung disease, and increased mortality. Along with its significant consequences to morbidity and mortality, osteoporosis has an annual cost of 20 billion dollars to the American and Canadian health-care systems combined, and it is expected that this economic burden will rise due to rapidly aging populations.

Osteoporosis is considered to be a pediatric disease with geriatric consequences because the bone mass (bone mineral density, BMD) that is accrued during growth and development determines bone health later in life. BMD in later life is a function of peak bone mass and the rate of subsequent bone loss during aging. Adequate calcium intake throughout growth and development is necessary to ensure normal mineralization of the bone. Other important factors that determine bone mass include age, weight, physical activity, menopausal status, smoking, alcohol intake, and use of corticosteroid hormones. Assessment of BMD is commonly performed using dual-energy x-ray absorptiometry and provides a measure of the amount of mineral within a selected area of the bone. In humans, BMD is a predictor of fracture risk and is routinely determined to diagnose osteoporosis. However, bone weakness results from a loss in both bone quantity (BMD) and bone quality. Bone quality is determined by a number of factors such as bone microstructure, bone turnover, and material properties. In addition, there are several known risk factors for sustaining a fragility fracture that are independent of BMD. Thus, other fracture assessment tools such as the WHO Fracture Risk Assessment Tool (FRAX) and the Canadian Association of Radiologist and Osteoporosis Canada (CAROC) risk assessment systems have been developed to predict 10-year fracture risk by assessing BMD and clinical risk factors such as body weight, height, smoking status, alcohol intake, history of fracture, and use of glucocorticoids.

Relationship Between Calcium and Bone Health

Despite evidence demonstrating that calcium intake consistently reduces bone turnover by up to 20%, there are surprisingly insufficient data to draw clear conclusions about the relationship between calcium and protection against osteoporosis when examining BMD and fracture risk. This is partly due to an absence of trials that have investigated a dose–response relationship between calcium and bone health. In addition, very few studies have investigated the action of calcium alone on bone health. Rather, the combined effect of calcium and vitamin D is often included in bone trials. Other reasons for insufficient data on the relationship between calcium and markers of bone health include an absence of information on background diets, vitamin D intake, circulating levels of 25-hydroxyvitamin D, which is the functional indicator of vitamin D status, and other characteristics of study subjects such as hormonal status and physical activity levels that affect calcium balance.

Adequate calcium intake is important to achieve normal bone accretion during growth and development as it plays a role in building peak bone mass. During the first year of life, the average accretion rate of calcium is approximately 100 mg day^{-1}. This rate increases in older healthy children to about 140 mg day^{-1}. Between the ages 9 and 18 years, the accretion rate of calcium further increases, particularly in boys. BMD also increases significantly with age until approximately 17.5 years in boys and 15.8 years in girls. Thus, it is logical that AIs of calcium should be met to support normal bone accretion. Studies demonstrate that daily intake of calcium levels that are higher than needed to support normal bone accretion does not confer extra benefits to bone mineral content (BMC) or BMD. In addition, it is unclear whether bone mass gained through dairy intake or calcium supplementation is maintained post-intervention.

During adulthood, achieving adequate calcium intake is a target for supporting calcium balance and the maintenance of bone. However, little information exists on the level of calcium needed to achieve calcium balance or the maintenance of bone mass in the adult population. One study reported higher ultrasound bone mass measurements in physically active premenopausal women with higher calcium intake compared to those with lower calcium intakes, regardless of physical activity levels. Another study reported less trochanteric BMC loss in premenopausal women with high calcium intake compared to those with lower calcium intake. In men, studies have reported

that calcium intake was not related to BMD at the calcaneus, lumbar vertebrae, or femur.

During pregnancy, calcium requirements of the fetus are high particularly during the third trimester when the skeleton is undergoing mineralization. To adapt to fetal calcium requirements, intestinal calcium absorption of the mother increases early in pregnancy to result in a net positive calcium balance. During the third trimester, calcium transfer from the mother to the fetus increases substantially resulting in a maternal calcium balance that is zero or slightly negative by the end of pregnancy. Little evidence exists on the effects of calcium supplementation during pregnancy on maternal BMD. One study demonstrated that calcium supplementation during low calcium intake (600 mg day^{-1}) results in improved bone health in infants.

As mentioned earlier, calcium intake consistently reduces bone turnover by up to 20%, and this is associated with a reduction in bone loss during the postmenopause. While some trials conducted in younger postmenopausal women have not demonstrated a clear benefit to the bone in response to increases in calcium intake, others have demonstrated that calcium intake results in small increases in BMD and is associated with a lower fracture risk. This benefit has also been observed in older postmenopausal women when fracture risk is more prevalent. Given that most studies have provided calcium supplementation in combination with vitamin D, more data on the effects of calcium alone on BMD and fracture risk during the postmenopause and aging are needed. It is generally accepted that calcium may play a role in mitigating bone loss rather than protecting against osteoporotic fractures during aging.

Calcium Intake and Cardiovascular Disease Risk

As mentioned earlier (see section '**Patterns of Consumption**'), calcium intake remains a concern in the United States, Canada, and several other countries. To help achieve daily calcium requirements, calcium supplements are commonly consumed, especially by middle-aged and elderly women. However, recent evidence has suggested that calcium supplement intake, alone or in combination with vitamin D, increases adverse cardiovascular events. Studies and meta-analyses have reported opposing conclusions, highlighting the current lack of consensus for calcium supplementation recommendations for supporting bone health during aging. Inconsistencies in evidence surrounding calcium intake and cardiovascular complications may be explained by the lack of randomized controlled trials specifically designed to investigate the effect of calcium supplements on cardiovascular disease. Some inconsistencies between studies may also be explained in part by reduced power from subgroup analyses and the inclusion of both men and women in some trials. The mechanism of adverse action of calcium on cardiovascular risk is unknown. National and international osteoporosis foundations (e.g., Osteoporosis Canada) recommend reaching daily calcium requirements from all sources (food and supplements). Nevertheless, in light of the recent controversy surrounding calcium supplements and cardiovascular complications, calcium supplement use in the United States has decreased from 22% in 2011 to 17% in 2012.

See also: Bioavailability of Nutrients; Caffeine: Consumption and Health Effects; Calcium: Properties and Determination; Dairy Products: Dietary and Medical Importance; Milk: Role in the Diet; Osteoporosis; Pituitary Gland: Pituitary Hormones; Potassium: Physiology; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health.

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Calcium: Properties and Determination

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Calcium

Calcium is an essential mineral nutrient for both plants and animals. It is a soft alkaline earth metal with a shiny silver surface, which gradually reacts with the atmosphere to form a dull gray-white coating of calcium oxide and calcium nitride. Discovered by Sir Humphry Davy in 1808, calcium is the fifth most abundant element by mass in both the Earth's crust (3.5%) and the human body (1.5%). This article focuses on the basics of calcium chemistry, dietary intake and the influence of food composition, food processing and bioavailability, and methods of calcium analysis in foods and biological samples.

Chemistry of Calcium

The chemical element calcium, symbol Ca, atomic number 20, is a group IIA metal (alkaline earth metal), which does not exist as a pure element in nature. Calcium has melting and boiling points of 840 and 1484 °C, respectively, and readily reacts with oxygen and the halogens, including fluorine, chlorine, bromine, and iodine. The electron configuration of calcium is $1s^2 2s^2 2p^6 3s^2 3p^6 4s^2$, and it has two valence electrons and commonly occurs as a divalent cation (Ca^{2+}). Its reactivity results in calcium existing in the form of common mineral compounds including carbonates (e.g., limestone, marble, and chalk), sulfates (e.g., gypsum and alabaster), fluorides (e.g., fluorite), phosphates, and silicates. The name calcium is derived from 'calx,' Latin for lime (calcium oxide), which was used by the Romans in mortar for building in the first century. However, excavations at a site in Eastern Turkey have identified the use of lime as a construction material as long ago as 7000–14 000 BC.

Calcium has an atomic weight of 40.08 and a total of 24 isotopes, of which five are stable, with Ca-40 predominating at over 96% relative abundance (Table 1). In addition, Ca-48 has such a long half-life that it is also generally considered stable. The remainder are radioisotopes, with several being available for use in research, including Ca-41, Ca-45, and Ca-47. For practical reasons, Ca-45 is the most commonly used calcium radionuclide for biological investigations. The least stable radionuclide is Ca-34 with a half-life shorter than 35 ns.

Calcium as an Essential Nutrient

Calcium is an essential nutrient for all living organisms, occurring in both plants and animals in the form of solid mineral salts and also dissolved in solution. These forms allow calcium to facilitate a range of biological roles including key structural and metabolic processes such as muscle contraction, digestion, blood clotting, and cell signaling. Calcium plays key structural roles in bones, teeth, and plant cell walls and membranes. An adult contains approximately 1.2 kg of calcium, 99% of which is found in the skeleton and teeth in the form of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], an inorganic crystalline structure consisting of calcium and phosphorus. Calcium is involved in the maintenance of the mineral composition of teeth, which undergo constant mineralization and demineralization in response to pH and dietary exposure in the mouth. In man, the structural integrity and permeability of biological membranes are maintained by calcium binding to proteins and phospholipids. In plants, calcium forms salt bridges between pectin molecules in the middle lamella, stabilizing adjacent cell walls. This stabilization is essential for cell walls to retain their structure and hold their contents, which is particularly important in developing fruits. Some plants also accumulate calcium in their tissues, thus increasing rigidity.

Many cellular processes in plants and animals rely on signaling, which involves the movement of the calcium ion, Ca^{2+}, into and out of the cytoplasm. An increase in cytosolic calcium is responsible for triggering a range of biological processes. Consequently, cellular calcium concentrations need to be tightly controlled within a narrow range in order to maintain appropriate functionality.

Overview of Occurrence in Foods

In the majority of industrialized countries, milk and dairy products, including cheese and yogurt, provide the greatest contribution to calcium dietary intake in adults. Approximately 70% of the calcium present in the food supply in the United States is in the form of milk and dairy products, with about 10% in fruits and vegetables, 5% in grain products, and the remainder in other foods. The relative importance of these food sources to an individual's calcium intake depends on the amounts consumed. However, as the main food sources of calcium for most adults consuming omnivorous diets in developed countries are milk and dairy products, this food group typically accounts for the bulk of total calcium intake at around 35–50%. Although the concentration of calcium in cereal products and vegetables is generally much lower than dairy products, the amount of these foods eaten means that foods of plant origin do make a significant contribution to total calcium intakes for most people (20–30% in the United States and the United Kingdom, with approximately 14% from bread due to calcium fortification of white flour).

In Europe, Australasia, and North America, adult dietary intakes of calcium are generally around 800–1000 mg day^{-1} compared with some areas of the developing world, such as rural Gambia, which has calcium intakes in women and children of approximately 300 mg day^{-1}. This difference reflects the lower consumption of milk and milk products in the developing world. Vegans and others who do not consume milk and dairy products may need to take particular care to ensure adequate calcium intake from their diet or may require

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Table 1 Stable isotopes and selected radioactive nuclides of calcium

Stable isotopes		*Selected radioactive nuclides*				
Isotope	*Relative abundance (%)*	*Nuclide*	*Decay*	*Half-life*	*Energy (MeV)*	*Intensity (%)*
Ca-40	96.941	Ca-41	Electron capture	1.02×10^5 years	0.42	100
Ca-42	0.641	Ca-45	β^- (no γ emitted)	162.7 days	0.26	100
Ca-43	0.135	Ca-47	β^-	4.536 days	1.98	16
Ca-44	2.086				0.68	84
Ca-46	0.004		γ		1.30	75
Ca-48	0.187					

a supplemental source of calcium, particularly during periods of rapid growth. Lacto-vegetarians, who do not avoid milk and dairy products, generally have little difficulty in attaining adequate calcium intakes.

Other foods that contain considerable amounts of calcium include tinned fish such as sardines or salmon (largely due to the calcium in bones and skin), soymilk substitutes, tofu (especially if it has been processed with calcium), and certain seeds and nuts, but typical eating habits indicate that these do not contribute much to total calcium intake for most people. Leafy green vegetables are also good sources of calcium, but absorption from them is generally low.

Factors Influencing Calcium Bioavailability

Bioavailability is the degree to which a nutrient is absorbed and utilized by the body for normal metabolic functions. In the case of calcium, this generally means the amount that is absorbed and incorporated into bone. There are several physiological and dietary factors that impact calcium bioavailability. Physiological factors include calcium and vitamin D status, age, rate of growth, disease, pregnancy, and lactation. The impact of dietary factors on calcium bioavailability is often difficult to delineate from physiological factors. Bioavailability of calcium is strongly affected by the physical and chemical forms in which it is consumed. The relative solubility of calcium compounds and the degree of ionization caused by gastric acid modulate calcium absorption from the gastrointestinal tract. Calcium is also more bioavailable when consumed with food than on an empty stomach, partly due to the longer transit time through the gastrointestinal tract.

There are a range of foods, nutrients, and other compounds that either enhance or inhibit calcium absorption when consumed simultaneously. Phytate, uronic acid, and oxalate are well-established plant-based inhibitors of calcium uptake, acting through the formation of insoluble complexes in the gut.

Serum vitamin D (as calcitriol, the active form of vitamin D) concentration is positively associated with calcium absorption and accounts for approximately 25% of its variability. Intestinal calcium absorption declines with age; but treatment with 25-hydroxyvitamin D_3 (the precursor of calcitriol) has been demonstrated to enhance absorption in elderly individuals. With the exception of vitamin D, potential enhancers of dietary calcium absorption are generally less well defined than inhibitors and may have only limited influence on the intestinal absorption of calcium ingested with a meal.

Absorption from the gastrointestinal tract is not the only consideration in bioavailability; dietary sodium, fat, and protein can influence the amount of calcium excreted in urine and consequently not available for essential functions in the body.

Fat significantly influences calcium absorption in cases of fat malabsorption, such as steatorrhea. Fatty acids (particularly saturated fatty acids) bind to calcium to form insoluble soap complexes in the intestine resulting in increased loss of calcium in the feces. The effect of protein on calcium absorption is the subject of debate; some research suggests a positive correlation between protein intake and calcium absorption, which in turn leads to an increase in urinary calcium excretion. However, the impact of increased calcium turnover on bone in response to dietary calcium needs to be fully determined. Other dietary components influencing calcium absorption and excretion include caffeine and alcohol. Caffeine has a slight negative impact on calcium absorption, with no significant effect on urinary calcium excretion, while the exact mechanisms underpinning the increased risk of osteoporosis and reduced bone mass in chronic alcohol consumers require further research.

Sources of Calcium Intake

Calcium is unevenly distributed in the human diet. Some foods are rich sources of calcium, while others are relatively poor. As a result of developments in food processing and the introduction of fortification policies and practices, the increasing availability of calcium-fortified foods and dietary supplements containing calcium salts is leading to a wider range of rich dietary sources of calcium.

Foods of Animal Origin

The calcium content of cow's milk is relatively unaffected by the animal's diet, stage of lactation, or climate. Approximately two-thirds of calcium in milk is bound to the milk protein, casein, and to a much lesser extent other proteins, phosphorus, and citrate, with the remainder as unbound calcium. Removal of the fat fraction from milk leads to slight increases in calcium concentration when comparing skimmed, partly skimmed, and whole milk (Table 2). Despite processing, dried milk powder and yogurts retain virtually all their calcium, whereas hard cheeses and butter suffer approximately 20% and 80% losses, respectively. Soft cheeses and cream cheese generally contain less calcium on a wet-weight basis due to their higher

Table 2 Calcium content of selected animal source foods

Food name	*Serving size*	*Weight (g)*	*Calcium (mg)*	*Concentration (mg g^{-1} as is)*
Milk and dairy products				
Milk, whole, 3.3% milk fat	250 ml	258	291	1.13
Milk, partly skimmed, 2% milk fat	250 ml	258	302	1.17
Milk, skim	250 ml	259	324	1.25
Milk, partly skimmed, 2% milk fat with added milk solids	250 ml	260	372	1.43
Milk, condensed, sweetened, canned (Eagle Brand™)	15 ml	19	55	2.89
Milk, evaporated, partly skimmed, canned, undiluted, 2% milk fat	15 ml	16	44	2.75
Yogurt, vanilla or fruit, 1–2% milk fat	175 ml	185	227	1.23
Cheese, cheddar	50 g	50	361	7.22
Cheese, mozzarella, 22.5% milk fat	50 g	50	269	5.38
Cheese, cottage, 1% milk fat	125 ml	119	73	0.61
Meat, fish, and egg				
Egg, poached	1 large	50	27	0.54
Beef, ground, lean, crumbled, pan-fried	75 g	75	11	0.15
Beef, mechanically deboned, raw	90 g	90	436	4.84
Pork chop, center cut, lean, bone in, pan-fried	1 chop	69	16	0.23
Chicken, broiler, breast, meat, fried	75 g	75	12	0.16
Salmon, Chum (keta), baked or broiled	75 g	75	11	0.15
Salmon, Chum (keta), canned, drained with bone (unsalted)	75 g	75	187	2.49
Sardine, Atlantic, canned in oil, drained with bone	1 can	106	405	3.82

Data from Health Canada (2008). Nutrient value of some common foods. Ottawa: Health Products and Food Branch, Health Canada, Government of Canada Publications, except for meat data, which are from the Canadian Nutrient File, Health Canada, version 2010.

water content. The calcium content of cheeses also depends on the process used in preparation and whether the calcium precipitates with the milk solids or remains in the whey. Cheeses precipitated with lactic acid, such as cottage or cream cheese, contain lower calcium levels in the curd, as most of the calcium remains soluble in the acid whey. Rennin coagulation that is used in the production of cheddar or mozzarella yields cheeses with a higher calcium content as the curd is formed before significant acidification takes place.

Meat and fish are not typically rich sources of calcium (Table 2). Mechanically deboned meats can contain much higher levels of calcium due to the abrasion of bone during the deboning process. Tinned salmon provides higher levels of calcium than fresh salmon filet (Table 2), because the bones and skin tend to be consumed along with the flesh. The latter also applies to small fish consumed whole, such as sardines and anchovies. Calcium is also generally higher in crustaceans than in fin fish.

Foods of Plant Origin

Foods of plant origin are generally not particularly rich sources of calcium, and some contain significant levels of inhibitors to calcium absorption such as phytate or oxalate. However, due to the large amounts consumed, plant-based foods generally make a significant contribution to total calcium intake in developed countries. Whole grains and seeds generally provide more calcium than most fruits or vegetables (Table 3). Fortification of refined flours and breakfast cereals can significantly increase the contribution of these foods to total calcium intake. Similarly, the high calcium content of baking powder and the variety of calcium-containing food additives used (e.g., as dough conditioners or yeast nutrients – see the succeeding text) add to the amount of calcium in baked goods.

While spinach appears to contain a reasonable amount of calcium (129 mg per 95 g serving; see Table 3), its high oxalate content renders a large proportion of the calcium insoluble and therefore much less bioavailable. As a result, it has been estimated that over 15 servings of spinach would be required to obtain the same amount of absorbable calcium as one glass of milk (Table 4). Turnip greens contain a similar level of calcium to that found in spinach, but without the oxalate, consequently two servings of turnip greens provide a similar level of absorbable calcium to one glass of milk (Table 4). However, these types of calcium absorption measurements are often determined under laboratory conditions by feeding only the single food, rather than a whole diet. Calcium bioavailability from a given food source can be substantially modified by other foods eaten at the same time. Calcium absorption from milk has been shown to decrease from 33% to 27% when consumed with spinach, whereas calcium absorption from spinach increased from 3% when fed alone to 11% when consumed with milk. In a Western mixed diet, the overall calcium absorption by adults typically averages about 25–30%.

Processing Effects on Food Calcium

There are numerous food additive uses of calcium salts that may add appreciable amounts of calcium to some foods. The Food Chemicals Codex, ninth edition (2014), lists over 30 calcium compounds used as food additives or processing aids. Some of the common functions of calcium-containing food additives (and examples of the calcium compounds used) include dough conditioners such as calcium carbonate, calcium iodate, calcium lactate, calcium oxide, calcium phosphate, and calcium

Table 3 Calcium content of selected plant-based foods

Food name	*Serving size*	*Weight (g)*	*Calcium (mg)*	*Concentration (mg g^{-1} as is)*
Vegetables and fruit				
Beans, snap (green, yellow, Italian), fresh or frozen, boiled, drained	125 ml	71	33	0.46
Carrots, raw	1 medium	61	20	0.33
Peas, green, frozen, boiled, drained	125 ml	85	20	0.24
Spinach, boiled, drained	125 ml	95	129	1.36
Apple, with skin (7 cm diameter)	1 apple	138	8	0.06
Banana, raw	1 medium	118	6	0.05
Oranges, raw	1 fruit	131	52	0.40
Grain and cereal products, nuts, and seeds				
Wheat flour, all purpose	125 ml	66	10	0.15
Bread, white, commercial	1 slice	35	53	1.51
Corn meal, dry	125 ml	73	4	0.05
Flax seeds, whole and ground	15 ml	11	36	3.27
Soy flour	125 ml	53	127	2.40
Tofu, regular, soft or firm prepared with magnesium chloride (nigari)	150 g	150	122	0.81
Tofu, regular, medium firm or firm prepared with calcium sulfate	150 g	150	347	2.31
Tofu, fried, prepared with calcium sulfate	150 g	150	1442	9.61
Almonds, roasted, salted	60 ml	35 g	93	2.66
Peanuts, all types, shelled, roasted	60 ml	37 g	20	0.54
Hazelnuts or filberts, dried	60 ml	34 g	39	1.15

Data from Health Canada (2008). Nutrient value of some common foods. Ottawa: Health Products and Food Branch, Health Canada, Government of Canada Publications, except for tofu data, which are from the Canadian Nutrient File, Health Canada, version 2010.

Table 4 Bioavailability of calcium from various food sources

Food	*Serving size (g)*	*Ca content (mg)*	*Fractional absorption (%)*	*Servings equivalent to 240 ml milk*
Milk	240	300	32.1	1
Soy milk (unfortified)	120	5	31.0	60.4
Almonds, dry roasted	28	80	21.2	5.7
Sesame seeds, no hulls	28	37	20.8	12.2
Broccoli	71	35	52.6	5.0
Brussels sprouts	78	19	63.8	8.0
Spinach	90	122	5.1	15.5
Turnip greens	72	99	51.6	1.9
Tofu, calcium set	126	258	31.0	1.2
Pinto beans	86	44.7	26.7	8.1
Kale	85	61	49.3	3.2
Rhubarb	120	174	8.54	9.5
Sweet potatoes	164	44	22.2	9.8

Selected data from Weaver, C. M. and Plawecki, K. L. (1994). Dietary calcium: Adequacy of a vegetarian diet. *American Journal of Clinical Nutrition* 59 (suppl.), 1238S–1241S, except for pinto beans, kale, rhubarb and sweet potatoes, which are taken from Weaver, C. M., Proulx, W. R. and Heaney, R. (1999). Choices for achieving adequate dietary calcium with a vegetarian diet. *American Journal of Clinical Nutrition* 70 (suppl.): 543S–548S.

sulfate; pH adjusters such as calcium hydroxide and calcium phosphate; antioxidants such as calcium ascorbate; preservatives such as calcium propionate, calcium disodium EDTA, and calcium sorbate; anticaking agents such as calcium stearate, calcium phosphate, and calcium silicate; and thickeners such as calcium gluconate and calcium alginate. The percentage composition of calcium in these compounds ranges considerably; for example, calcium gluconate is 9% calcium, calcium carbonate is 40% calcium, and calcium oxide is 71% calcium by weight. Solubility also differs considerably between compounds, with some such as calcium carbonate being relatively insoluble at neutral pH, whereas others such as calcium acetate or calcium chloride are highly soluble. Tofu set with calcium sulfate contains considerably more calcium than tofu set with magnesium chloride (Table 3). Firm tofu contains a higher concentration of calcium than regular tofu due to the lower water content (and consequently higher solids). Fermentation (including leavening of bread) or germination of selected plant foodstuffs can increase the availability of calcium due to breakdown of complexing compounds such as phytic acid. The calcium content of meat can be increased by processing or cooking in acidic solutions due to the dissolution of calcium from bone. For example, pork spare

ribs or chicken cooked with vinegar contain more calcium than the uncooked flesh.

Tap water typically contains calcium and many other elements and may make some contribution to total calcium intake particularly in hard water areas. In some parts of Europe, it is estimated that tap water contributes up to 4% of daily calcium intake. Water hardness is measured as milligrams of calcium carbonate equivalents per liter, and hard water may be over 300 mg of calcium carbonate per liter. The World Health Organization recommends water containing 80–100 mg calcium carbonate per liter as ideal. Calcium carbonate is 40% elemental calcium by weight; therefore, a 250 ml glass of water may provide 30 mg of elemental calcium in some areas. The calcium content of bottled waters varies widely from less than 10 mg l^{-1} to around 200 mg l^{-1} and up to 600 mg l^{-1} with calcium fortification. The hardness of water added during processing may also influence the calcium content of foods to some extent. However, losses through soaking and/or boiling may amount to 5–25% of the calcium content of a variety of vegetables that are commonly prepared in this way.

Foods may have nutrients added for a variety of reasons including restoration of processing losses or to provide similar nutrient levels to dairy products where they are used as a substitute, such as soy 'milk,' 'yogurt,' and other plant-based beverages, which may be fortified with calcium. Foods used for special dietary purposes such as meal replacements, formulated liquid diets, and slimming foods are generally fortified with calcium to prevent nutritional deficiency. Fortification, which in this context refers to the addition of calcium with the intent of raising the calcium content of the food for the consumer, is often undertaken in response to legislation. However, regulations and practices concerning food fortification may vary considerably between countries.

Supplements

Nutritional supplements and antacid medications can make a significant contribution to calcium intakes for some individuals. Over 40% of the US population (including almost 70% of older women) uses dietary supplements containing calcium. Calcium carbonate is found in many over-the-counter antacid preparations, which can provide up to 400 mg of calcium per day. However, calcium citrate, which is absorbed similarly when taken with or without food, is beneficial for people with achlorhydria, inflammatory bowel disease, or absorption disorders. Other forms of calcium in supplements or fortified foods include gluconate, lactate, and phosphate. Multivitamin and multimineral supplements may also contain calcium, though usually in smaller amounts. Natural source supplements include oyster shell and dolomite, although concerns have been raised concerning the potential for lead contamination in both forms, along with aluminum, arsenic, mercury, and nickel in the latter.

Analysis of Calcium in Foods and Biological Samples

The assessment of nutritional calcium status is difficult, and there are no reliable measures of status. Circulating calcium concentrations are tightly regulated via the endocrine system, varying little in response to widely differing dietary calcium intakes (see Calcium: Physiology). Consequently, deviations of calcium concentration from this narrow range are medically significant, and in this setting, the measurement of total plasma or serum calcium or specific measurement of the ionized calcium fraction is important. About half of the calcium in serum is ionized, not bound to proteins or low-molecular-weight ligands, and considered to be the active form; therefore, it is a better measure of functional levels. However, the marker is influenced by other factors such as pH and age.

Urinary calcium has been used as a marker of calcium status, but the influence of other dietary confounders (such as protein, sodium, and caffeine) on urinary calcium excretion makes it unsuitable for many applications. Urinary levels are relatively well regulated and therefore generally not sensitive to small changes in status. Total calcium in plasma or urine can be measured by atomic absorption spectrophotometry, either directly on the diluted sample or following sample mineralization ('ashing' – see the succeeding text) or by colorimetric assay (e.g., using arsenazo III or orthocresolphthalein complexone) on a clinical autoanalyzer. Ionized calcium in plasma can be assessed using an appropriate ion-sensitive electrode. Assessment of intracellular calcium levels in the research laboratory can be undertaken using fluorescent probes such as FURA 2 or QUIN.

Neutron activation measurements are considered the gold standard in assessing total body calcium content. However, its application for research is limited, and it tends to be used as a means of calibrating other methods. The method involves exposure to radiation, thus limiting its uses. It measures the proportion of Ca-49 in the body using gamma-ray counts. Calcium nutritional status is more often assessed indirectly through balance studies or by measurement of bone mineral concentration or bone mineral density, reflecting the major structural role played by this mineral nutrient. There are a variety of techniques that can be employed to measure the mineral content of bone. One of the most commonly used is dual-energy x-ray absorptiometry (DXA), which uses x-ray technology to measure either the whole body or specific bone regions. This technique can be used in all population groups (except pregnant women) but may be less accurate as a measure of calcium status in the elderly when bone degeneration occurs naturally. Measurements, however, can be influenced by fat mass and distribution and even by the methodology and machine used. Computerized tomography (CT) offers a three-dimensional assessment of bone content, but the equipment is less portable and the radiation dose significantly greater than for the DXA method. A third method of assessment is quantitative ultrasound, which is normally limited to measurement of the peripheral skeleton. Although this technique offers portable equipment with no radiation exposure, the precision of the method is questionable as results do not correlate well with DXA measurements.

Other putative measures of calcium status such as bone remodeling markers and functional enzyme measures are not specific to the mineral and therefore have limited use. A number of bone remodeling markers (such as breakdown products and enzymes) can be measured to assess calcium status in either serum, including osteocalcin and alkaline phosphatase, or

urine such as hydroxyproline, pyridinoline, deoxypyridinoline, N-telopeptide, and C-telopeptide. However, concentrations of each of these proposed markers can be influenced by a number of other factors including life stage, particularly menopause.

Sample Preparation

Most foods and biological tissue samples can be prepared for the analysis of total calcium content using either dry ashing (in a muffle furnace) or wet ashing (acid digestion) techniques or a combination of the two. Dry ashing typically involves combustion at elevated temperature (e.g., 500–550 °C) until organic matter is fully destroyed, followed by dissolution of the ash in a suitable acid for subsequent analysis. Wet ashing techniques typically involve the destruction of organic matter by heating in concentrated nitric acid until a pale straw color is attained and may feature additional oxidation steps with perchloric acid or hydrogen peroxide to clarify the sample solution. In wet ashing procedures, particularly for samples with a high calcium content such as bone meal, sulfuric acid is generally to be avoided due to the ready precipitation of calcium from solution by sulfate, forming plaster of Paris (calcium sulfate hemihydrate) or gypsum (calcium sulfate dihydrate).

Determination of Calcium Content of Foods and Tissues

Determination of the calcium content of foods and tissues is routinely undertaken using atomic absorption spectrophotometry, although a number of alternative techniques exist including titrimetric methods using EDTA or $KMnO_4$, neutron activation analysis, or inductively coupled plasma emission spectrometry. Details of sample preparation and analytic procedures for a variety of food sample types are published by the Association of Official Analytical Chemists.

Atomic Absorption Spectrophotometry

For atomic absorption spectrometric analysis of calcium, 0.1–1.0% (w/v) lanthanum is included in the analytic working solution as a matrix modifier to reduce anion interferences due to phosphate or sulfate, which otherwise can form refractory complexes and depress the absorption of light by atomic calcium.

Absorbance is measured at the 422.7 nm calcium spectral line, following atomization in a reducing air–acetylene flame, and compared with certified analytic standard calibration solutions. A reducing flame gives a higher sensitivity, though an oxidizing flame may give a higher precision where this is critical. Typical analytic working ranges are obtained up to 5 mg l^{-1} in the analytic working solution when using a standard nebulizer assembly and may be approximately doubled with the use of a high-sensitivity nebulizer (see Spectroscopy: Atomic Emission and Absorption).

$KMnO_4$ Titrimetric Method for Calcium in Wheat Flour (Method from the Association of Official Analytical Chemists)

After dry ashing and dilution of the ash to a suitable volume with demineralized water, bromocresol green indicator is added along with enough 20% sodium acetate solution to change the pH to 4.8–5.0 (blue). The sample solution is covered and heated to boiling. The calcium is precipitated by slow addition (1 drop every 3–5 s) of 3% oxalic acid solution (w/v) until pH is 4.4–4.6, as indicated by a distinct green shade. The solution is then boiled for 1–2 min and allowed to settle overnight. The supernate is filtered through quantitative paper, Gooch, or fine fritted glass filter, and the beaker and precipitate are washed with small portions of ammonium hydroxide (1 + 50). A mixture of 125 ml of water and 5 ml of sulfuric acid is added to the precipitate with heating to 80–90 °C. The solution is finally titrated at 70–90 °C with 0.01 M $KMnO_4$ to a slight pink end point, 1 ml of permanganate solution equating to 1 mg of calcium.

See also: Calcium: Physiology; Spectroscopy: Types.

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Campylobacter: Health Effects and Toxicity

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Introduction

The name *Campylobacter* was coined from two Greek words: καμπύλος (*kampylos)* meaning curved and βακτηρῖα (*bakteria*) meaning rod. The genus *Campylobacter* was initiated in 1963 by Sebald and Véron upon observing a *Vibrio*-like bacterial strain that had been isolated from abortion cases in ewes and cattle could not ferment sugar and had a different G+C content to that of *Vibrio cholerae*. Chronologically, McFadyean and Stockman who had concluded that a *Vibrio*-like bacterium was responsible for abortions in Devon longwoolled ewes in the United Kingdom reported the first case in 1906. This was closely followed by a similar report in 1918 by Smith and Taylor who had observed that a *Vibrio*-like bacterium was responsible for abortions in cattle in the United States of America. Due to the similarity between these two bacteria, Smith and Taylor had classified them into the genus *Vibrio* and collectively named *Vibrio fetus*. This bacterium was renamed as *Campylobacter fetus*, and to avoid such errors in taxonomy and nomenclature of *Vibrio*-like bacteria that were being isolated, Sebald and Véron initiated and defined the genus *Campylobacter* as comprising Gram-negative, slender, and curved bacteria, which are (a) motile by means of polar flagella (Figure 1), (b) microaerophilic with a strictly respiratory metabolism, and (c) not producing acid in carbohydrate-containing media and which have a DNA G+C content of 29–36%. This contrasted the genus *Vibrio*, which had been previously defined as comprising bacteria that ferment glucose and have a DNA G+C content between 40% and 53%. As a result of improved taxonomy methods, particularly genotypic methods, the genus *Campylobacter* presently has 25 species and 9 subspecies (see Table 1).

Unlike in sheep and cattle, the isolation of *Campylobacter* spp. from human feces before 1968 was not possible because an isolation technique had not been developed. All the *Vibrio*-like bacteria that had been isolated in humans before originated from blood samples. The first undisputable *Campylobacter* human infection was reported in 1947 by Vinzent and coworkers who identified *V. fetus* (presently *C. fetus*) to have caused abortion in two women who had been admitted to the hospital for four weeks due to fever. In 1957, Elizabeth King isolated two *Vibrio*-like bacteria from the blood of patients with diarrhea but failed to isolate any bacteria from the feces of the patients. In spite of failure to isolate bacteria from the feces of the patients, King concluded that *Campylobacter* caused the enteric infections. Dekeyser and Butzler confirmed King's conclusion in 1968. They isolated *V. jejuni* (presently *C. jejuni*) from feces and blood of a 20-year-old female who was admitted in July 1968 in St. Peter University Hospital in Brussels with severe diarrhea and fever using a filtration technique. Since then, better techniques for isolating and identifying *Campylobacter* from feces have been developed. These techniques have revealed that *C. jejuni* and *C. coli* are one major cause of human gastroenteritis. Interestingly, epidemiologic studies have constantly shown that *C. jejuni* is the leading cause of bacterial gastroenteritis worldwide and *C. coli* is responsible for a significant lower percentage of cases. Therefore, in this chapter we discuss (i) general characteristics of *C. jejuni*, (ii) epidemiology of *C. jejuni* infections, (iii) *C. jejuni* enteritis and associated postinfectious manifestations, (iv) pathogenesis of *C. jejuni*, and (v) treatment of campylobacteriosis.

Characteristics of *C. jejuni*

C. jejuni are Gram-negative, nonspore-forming, microaerophilic (5% oxygen), spiral-shaped rods measuring 1.5–3.5 μm in length and 0.2–0.4 μm in width. They possess a single circular chromosome of 1.64–1.7 million base pairs (30.6% G+C), which is predicted to encode about 1650 proteins including 52 ribosomal proteins. The bacterial cells have a unique corkscrew motility propelled by flagella located at the poles of the bacterial cell. The growth temperature ranges from 30 to 45 °C with an optimum of 42 °C. *C. jejuni* does not multiply at temperatures below 30 °C but remains viable for many months at these temperatures. Some studies have shown that *C. jejuni* cells remain viable at 4 °C for up to 7 months. They grow in a pH range of 5.0–9.0 with an optimum of 6.5–7.5. *C. jejuni* is sensitive to desiccation and heat (it can be easily destroyed above 48 °C) and is unable to grow in NaCl ≥ 2%. In contrast to many other bacteria, amino and carbon acids are the major carbon sources because they do not utilize glucose or other hexose sugars as carbon source. But particular strains are able to metabolize L-fucose. *C. jejuni* survives and thrives in a wide range of hosts.

Epidemiology

C. jejuni is the most prevalent cause of human gastroenteritis in both developing and developed countries. In developing countries, it mainly affects children below 5 years, while in developed countries it mainly infects adults. In addition, in developing countries, incidences of campylobacteriosis are not season-specific but in developed countries most cases are reported during summer.

In spite of these differences, *C. jejuni* are commensal organisms in the intestines of wild birds and farm and domestic animals including goats, sheep, cattle, swine, poultry, dogs, and cats. Also, they are found in untreated water and water bodies such as lakes, dams, and rivers. Consequently, contact with farm and domesticated animals, raw milk, milk products, untreated water, undercooked poultry, contaminated meat products, barbecue meat, minced meat, both processed and unprocessed sea foods, international travel, and restaurant eating are major risk factors. Epidemiological and

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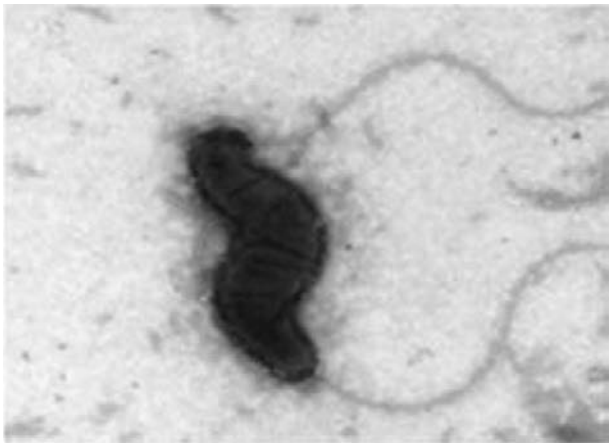

Figure 1 Electron micrograph of *C. jejuni* strain B2. The bacterial cell has at both poles a flagellum, which ensures its cellular motility. Thanks to Michael Hoppert, Department of General Microbiology, University of Göttingen for the picture.

microbiological studies have shown that poultry remains to be the major source of human infection.

According to the European Food Safety Authority (EFSA), 214 000 cases of infection were reported in the year 2012 in the European Union. It is assumed that the actual number of cases of human campylobacteriosis is at approximately nine million per year in the EU. The costs incurred by campylobacteriosis for the public health systems and due to loss of productivity in the EU are estimated by the EFSA to approximately EUR 2.4 billion per year. The disease burden of *Campylobacter* enteritis and its postinfectious sequelae is approximately 0.35 million disability-adjusted life years (DALYs) per year. Contaminated broiler meat is assumed to account for 20–30% of the cases, while 50–80% may be attributed to the chicken reservoir as a whole (laying hens in addition to broilers).

The Foodborne Diseases Active Surveillance Network, a collaboration among the Centers for Disease Control and Prevention (CDC) and 10 US state health departments, estimates the incidence per 100 000 population in 2012 to 14.30. Among these *Campylobacter* isolates with species information, 90% were *C. jejuni*, 8% were *C. coli*, and the remaining 2% belonged to other *Campylobacter species*.

Table 1 The bacterial species included in the genus *Campylobacter* (in alphabetical order)

Species/subspecies/biovar	*Host/habitat*	*References*
C. avium	Chicken, turkey	Rossi et al. (2009)
C. canadensis	Whooping crane	Inglis et al. (2007)
C. coli	Swine, poultry, sheep, dogs	Skirrow (1977)
C. concisus	Humans	Love et al. (1984)
C. curvus	Swine, humans	Vandamme et al. (1991)
C. cuniculorum	Rabbits	Zanoni et al. (2009)
C. fetus ssp. *fetus*	Sheep, goats, cattle	van der Graaf-van Bloois et al. (2014)
C. fetus ssp. *venerealis* bv. *intermedius*	Cattle	
C. fetus ssp. *venerealis*	Cattle	
C. gracilis	Dogs, humans	Han et al. (1991)
C. helveticus	Cats, dogs, humans	Stanley et al. (1992)
C. hominis	Humans	Lawson et al. (1998)
C. hyointestinalis ssp. *hyointestinalis*	Swine	Gebhart et al. (1983)
C. hyointestinalis ssp. *lawsonii*	Swine	On et al. (1995)
C. insulaenigrae	Seals, sea lions, elephant seals,	Foster et al. (2004)
C. jejuni ssp. *doylei*	Humans	Steele and Owen (1988)
C. jejuni ssp. *jejuni*	Cattle, chicken, sheep, swine, turkey, humans, wild birds, etc.	Skirrow (1977)
C. lanienae	Swine, cattle	Logan et al. (2000)
C. lari ssp. *concheus*	Shellfish	Debruyne et al. (2009)
C. lari ssp. *lari*	Shellfish	
C. laridis	Humans	Benjamin et al. (1983)
C. mucosalis	Swine	Lawson et al. (1975)
C. peloridis	Shellfish	Debruyne et al. (2009)
C. rectus	Humans	Vandamme et al. (1991)
C. showae	Humans	Etoh et al. (1993)
C. sputorum bv. *faecalis*	Cattle, humans, sheep, swine	Vandamme and On (2001)
C. sputorum bv. *paraureolyticus*	Cattle, humans, sheep, swine	
C. sputorum bv. *sputorum*	Cattle, humans, sheep, swine	
C. subantarcticus	Gray-headed albatrosses, black-browed albatrosses, gentoo penguins	Debruyne et al. (2010a,b);
C. ureolyticus	Cats, swine, canines, humans	Han et al. (1991)
C. upsaliensis	Dogs, cats, poultry, humans	Goossens et al. (1990)
C. volucris	Black-headed gulls	Debruyne et al. (2010a,b)

C. jejuni Enteritis and Associated Post-infectious Manifestations

C. jejuni affects human health by causing an infection that is known as *Campylobacter* enteritis or human campylobacteriosis. This infection is a foodborne disease that is characterized by the following clinical symptoms: fever, abdominal pain, watery or sometimes bloody diarrhea, headache, dizziness, nausea, and vomiting. These symptoms usually start 2–4 days after ingestion of *C. jejuni* and may clear after 5–7 days. The *C. jejuni* minimal infectious dose is above 500 viable bacteria. Although this disease is self-limiting, post-infectious manifestations, namely, Guillain–Barré syndrome (GBS), reactive arthritis (ReA), and inflammatory bowel disease (IBD), can arise after recovery. Below is a brief description of each post-campylobacteriosis manifestation:

(a) *GBS*: GBS is an autoimmune disorder in which the body's immune system 'mistakenly' attacks human GM gangliosides – lipids found in the central nervous system – leading to acute neuromuscular paralysis and consecutive muscle weakness. There are four types of GBS, namely, Miller Fisher syndrome (MFS), acute motor axonal neuropathy (AMAN), acute inflammatory demyelinating polyradiculoneuropathy (AIDP), and acute motor and sensory axonal neuropathy (AMSAN). *C. jejuni* is the leading cause of GBS and has been linked to triggering MFS, AMAN, and AMSAN, but not AIDP. It triggers these autoimmune disorders because its cell wall surface contains lipooligosaccharides (LOSs) that resemble ganglioside structures on the surface of the Schwann cells and the coat of the nerve axons. As a consequence of this molecular mimicry, the immune system releases autoantibodies leading to neuritis and axonal degeneration. GBS usually develops 3 weeks after recovery from *Campylobacter* enteritis and is characterized by slow recovery and severe residual disability.

(b) *ReA*: ReA is a spondyloarthropathic syndrome characterized by inflammation of the joints and tendons occurring after gastrointestinal infections with *C. jejuni* as well as *Shigella dysenteriae*, *Salmonella enterica*, *Yersinia enterocolitica*, and *Yersinia pseudotuberculosis* or genitourinary infections especially with *Chlamydia trachomatis*. The mechanism of ReA is poorly understood. However, interleukin IL-1, IL-6, and TNF-α have been implicated in *C. jejuni*-associated ReA. In some cases, ReA occurs together with an inflammation of the eye's conjunctiva or uvea, as well as of the urethra (and rarely additionally of the uterine cervix in women). This inflammatory triad is called Reiter's syndrome.

(c) *IBD*: IBD is established when a gastrointestinal tract immunologic disorder leads to chronic recurrent inflammation of the colon and small intestine. Gut host cells and gut commensal microflora continuously communicate creating a sustained normal gut homeostasis. The diversity and load of the human normal gut microbiota are influenced by the individual's genetics and diet. It mostly comprises *Bacteroides* spp. and *Prevotella* spp. A disruption of the normal gut homeostasis by factors including smoking, diet, drugs, geography, social stress, and psychological elements leads to changes in the diversity of the gut microflora. This in turn promotes intestinal epithelial invasion by *C. jejuni* and *S. enterica* and gut commensals, which are recognized by nucleotide-binding oligomerization domain (NOD) proteins and Toll-like receptors. Sensing of these receptors stimulates the mucosa-associated immune system leading to intestinal inflammation.

(d) *Other possible C. jejuni-associated diseases*: Besides the above-mentioned clinical manifestations, campylobacteriosis was also associated with the postinfectious irritable bowel syndrome, celiac disease, and potentially with the so-called immunoproliferative small intestinal disease.

Pathogenesis of *C. jejuni*

The clinical features of campylobacteriosis are a result of the interaction between *C. jejuni* bacterial cells and the enterocytes of the host. This section looks at the *Campylobacter* virulence-associated factors and counteracting enterocytal factors: *C. jejuni* interaction with both the host microbiome and the innate immune system.

Campylobacter Virulence-Associated Factors

The environment in the lower intestinal tract is hostile for colonization with *C. jejuni*. In the process of adjusting its metabolic systems in response to this unfavorable environment, *C. jejuni* ends up secreting proteins, which lead to the disruption of the intestinal epithelium. This epithelial irritation results in clinical symptoms such as diarrhea. Also, certain *C. jejuni* surface structures interact with enterocytes contributing to their disruption. These secreted proteins and surface structures are collectively termed *Campylobacter* virulence-associated factors. They include the following:

(a) *Flagella, motility, and chemotaxis*: Motility helps bacteria to effectively colonize their environment. *C. jejuni*'s flagella and 11 different types of chemoreceptors allow it to migrate within the mucosa in search for a suitable environment. Interestingly, *C. jejuni* migrates to certain areas of the mucosa that have energy sources, like carboxylic acids, amino acids, and L-fucose. In addition to motility, the flagellin A (*flaA*) and flagellin B (*flaB*) subunits undergo phase variation, hence playing a role in evasion of the host immune response.

(b) *Toxin*: *C. jejuni* produces a toxin known as cytolethal distending toxin (CDT). This toxin is made up of three subunits: CdtA, CdtB, and CdtC. The CdtB subunit has a DNase 1-like activity; hence, it randomly cuts DNA of enterocytes to release 5′-phosphorylated di-, tri-, and oligonucleotide fragments leading to enterocyte cell cycle arrest, disrupting cell protecting structures such as the cell wall and eventually death of enterocytes. The subunits CdtA and CdtC are binding proteins for delivering CdtB into the cytoplasm of enterocytes. *C. jejuni* CDT is essential for invasion and mucosal inflammation.

(c) *Adhesion factors*: Attachment of *C. jejuni* to the epithelial cell surface is an important step in invasion of the mucosa and in some situations for evasion of the immune response. Unlike similar Gram-negative enteric pathogens

such as *E. coli* and *S. enterica*, adherence of *C. jejuni* to enterocytes is not mediated by pili or fimbriae. It is mediated by structures that are found on the cell surface of *C. jejuni*. These structures include the following: (i) CadF binds to fibronectin bound to the surface of enterocytes and is required for invasion of the epithelium. (ii) The fibronectin domain-containing lipoprotein FlpA (Cj1279c) also binds to fibronectin. (iii) The surface-exposed lipoprotein JlpA binds to heat shock protein 90 (Hsp90). (iv) Further examinations have shown that lipoprotein Cj0091; periplasmic adhesion protein Cj0268c; fibronectin/fibrinogen-binding protein Cj1349; the autotransporter proteins CapA and CapB; flagellin A (FlaA); hemolysin TlyA/Cj0588; the major antigenic peptides PEB1, PEB3, and PEB4; and p95 adhesin play a role in *C. jejuni* adherence. Their binding sites on enterocytes and the outcome of their binding to enterocytes are under investigation.

(d) *Invasion factors*: The process of *C. jejuni* internalization into enterocytes and associated factors has been a focus of research over the last 20 years. To date, it has been established that (i) internalization of *C. jejuni* into enterocytes is an energy-dependent process and (ii) the bile salt, deoxycholate, present in the lower intestinal tract stimulates *C. jejuni* to synthesize and secrete a set of proteins termed as *Campylobacter* invasion antigens (Cia) which play a role in internalization. *C. jejuni* synthesizes and excretes at least eight Cia proteins, but presently, only four, namely, CiaB, CiaC, CiaD, and CiaI, have been functionally characterized. In addition, some other *C. jejuni* proteins and host cell factors like actin have been shown to play a role in *C. jejuni* internalization.

(e) *Survival strategies within host cells*: Upon internalization, *C. jejuni* survives and multiplies within enterocytes in membrane-bound compartments that are commonly referred to as *Campylobacter*-containing vacuoles (CCV). Poor nutrition, low pH, and antibacterial molecules including superoxide, hydrogen peroxide, halogenated oxygen molecules, and lysosomal enzymes characterize the CCV. Therefore, *C. jejuni* must have strategies to survive and multiply in this hostile microenvironment. Currently, three survival strategies have been identified. Firstly, *C. jejuni* synthesizes Cia proteins, which enable it to survive within enterocytes. For example, CiaI deviates CCV from the canonical endocytic pathway, thus avoiding union of CCV with lysosomes. Secondly, within enterocytes, *C. jejuni* undergoes a significant metabolic downshift leading to the downregulation of several metabolic pathways. Thus, amino acid biosynthesis and biosynthesis pathways associated with prosthetic groups, fatty acids, lipids, pentose phosphate, purine, and pyrimidine are downregulated, as well as catabolic pathways associated with degradation and utilization of amino acids and C1 compounds and pathways involved in the transport of nutrients, metal ions, and amino acids. Thirdly, within eukaryotic cells, *C. jejuni* changes its mode of respiration from a mode that uses many terminal electron acceptors including oxygen, nitrate, fumarate, nitrite, trimethylamine *N*-oxide (TMAO), and dimethylsulfoxide (DMSO) to a mode that uses only the readily available fumarate as the terminal electron acceptor (fumarate respiration). However, its survival strategy against toxic molecules remains unclear because unlike other intracellular pathogens such as *Salmonella typhimurium*, catalase does not make a contribution to its survival within epithelial cells.

(f) *Glycolipids*: Glycolipids are lipids with covalently attached carbohydrates whose function is to provide energy and serve as markers for cellular recognition. *C. jejuni* has two kinds of glycoproteins or lipid-bound polysaccharides: lipooligosaccharides (LOSs) and capsular polysaccharides. LOSs form a major part of the *C. jejuni* outer membrane. They contain a terminal structure similar to the human gangliosides GM1a and GM2, and in response to the microenvironment in the enterocytes, LOSs undergo phase variation with the terminal β-1,3-linked galactose residue (Gla) being switched on or off creating diverse strains in a population. LOSs play the following roles in pathogenicity: (i) LOS phase variation promotes *C. jejuni's* ability to adapt to the enterocyte's hostile environment, (ii) LOSs mediate adherence and invasion, (iii) structural similarity to human gangliosides GM1a and GM2 and phase variation help in evasion of host immune response, and, (iv) as explained in the section on *C. jejuni* enteritis and postinfectious manifestations, LOS's similarity to human gangliosides GM1a and GM2 plays a role in the pathogenesis of GBS.

The polysaccharide capsule is an external structure of *C. jejuni*. Like LOSs, it undergoes phase variation, hence playing a role in adherence, invasion, and evasion of the host immune response. Also, it is important for the survival of *C. jejuni* outside its natural host.

(g) *Glycoproteins*: Glycoproteins are proteins with carbohydrate residues that are covalently attached to the hydroxyl (—OH) group of the R group of serine or threonine or the amino group ($—NH_2$) in the R group of asparagines in a process called glycosylation. The former is known as *O*-linked while the latter is known as *N*-linked. Proteins may undergo glycosylation during or after translation. *C. jejuni* has both glycosylation systems. The *O*-linked system is diverse and adds pseudaminic acid and related sugars to the immunodominant flagellin. As a result of its association with the flagella, it is essential for adaptation to changing host environment and evasion of host immune response. The *N*-linked system is conserved and adds the bacillosamine-containing heptasaccharide to over 30 proteins. It plays a role in adherence, invasion, and enhancement of the stability of virulence-associated proteins.

Host Defense

The human gastrointestinal tract poses two defenses against *Campylobacter* invasion. These are the following:

(a) *Microbiota barrier*: The human gastrointestinal tract harbors a diverse group of genetically different commensal microbial species. This group of microorganisms is collectively referred to as microbiota. The dominant species in microbiota of a healthy individual are from the following genera: *Lactobacillus*, *Veillonella*, *Bacteroides*, *Peptococcus*, *Escherichia*, *Peptostreptococcus*, *Bifidobacterium*, *Eubacterium*,

Fusobacterium, and *Clostridium*. However, these species vary from one healthy individual to another due to age, genetics, diet, and environment. These microorganisms live in the lumen, the outer mucus layer, within intestinal crypts and on the surface of the mucosal epithelial cells. Microbiota benefit the host in the following ways: breaking down complex nonabsorbable compounds into simple absorbable molecules, maturation of the immune system, and maturation of the intestinal mucosa. In addition, the microbiota plays an important role of defending the host against invasion by all invading pathogens including *C. jejuni* using the following strategies: first, competing for niches and nutrients and, second, secreting bactericidal metabolic by-products including reactive oxygen species, short-chain fatty acids, and bacteriocins. This phenomenon is known as colonization resistance or microbiota barrier.

(b) *Immune barrier*: The human gastrointestinal tract is protected with both the adaptive and innate immune systems, which complement each other. The adaptive immune system is triggered when the presence of *C. jejuni* stimulates the synthesis of interleukin 8 (IL-8). Consequently, IL-8 activates the maturation of macrophages, T-cells, and B-cells, which clear the invading *C. jejuni*. The innate immune responses are initiated when *C. jejuni*'s cell wall structures are detected by nucleotide-binding oligomerization domain-containing protein 1 (NOD1) or Toll-like receptors (TLRs). The interaction of *C. jejuni* cell wall structures and NOD/TLR activates nuclear factor kappa B (NF-κB), which stimulates the production of various cytokines that mediate maturation of dendritic cells (DCs) into antigen-presenting cells (APCs). The APCs capture *C. jejuni* and present them to the mature T-cells for elimination.

Current Model of Disease Pathogenesis

The process of campylobacteriosis establishment starts when *C. jejuni* gets an opportunity to adhere onto enterocytes upon the disruption of the microbiota barrier by diet or chemicals such as antibiotics. For instance, studies that have been carried out in humanized gnotobiotic mice to determine which commensal microorganisms promote *C. jejuni* invasion have shown that eating a typical Western diet such as pizza, cheese, French fries, and burgers elevates the dominance of *Escherichia coli*, *Clostridium innocuum*, *Eubacterium dolichum*, *Catenibacterium mitsuokai*, and *Enterococcus* spp. and reduces the levels of *Bacteroides* spp. and *Prevotella* spp. leading to campylobacteriosis. Once *C. jejuni* adheres to enterocytes, the flagellar type-III-homologue secretion system releases Cia proteins that facilitate *C. jejuni* internalization through M cells. The internalization process is unique because it shares the characteristics of both trigger and zipper mechanisms. Once internalized, *C. jejuni* survives and multiplies in vacuoles avoiding clearance by lysosomes. In response to the vacuolar environment, *C. jejuni* synthesizes several proteins that damage the host cells. During internalization, *C. jejuni* releases CDT, which damages the DNA of the host cell leading to cell death and activates the adaptive immune response. At the same time, NOD and TLR recognize *C. jejuni* and activate the innate immune response. The activities of both the adaptive and innate immune responses lead to local inflammation. While the activities of Cia damage tight junctions leading to diarrhea.

Treatment

Usually, campylobacteriosis is a self-limiting disease that requires no interventional treatment. In most cases, campylobacteriosis patients are treated with fluid and electrolyte substitution (especially potassium). However, in severe cases especially immunosuppression or HIV infection, patients should be treated with antibiotics immediately if laboratory results indicate a *C. jejuni* or *C. coli* infection. *C. jejuni* is usually sensitive to macrolides, tetracyclines, aminoglycosides, carbapenems, and chloramphenicol but increasingly resistant to cotrimoxazole and fluoroquinolones. In spite of a wide range of antibiotics available for the treatment of campylobacteriosis, the macrolide erythromycin remains the antibiotic of choice.

See also: Campylobacter: Properties and Occurrence; Campylobacter: Species Detection; Diarrheal Diseases; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; *Escherichia coli* and Other *Enterobacteriaceae*: Occurrence and Detection; *Salmonella*: Detection; *Salmonella*: Properties and Occurrence; *Salmonella*: Salmonellosis; *Yersinia enterocolitica*: Properties and Occurrence; *Yersinia enterocolitica*: Detection and Treatment; Zoonoses.

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Campylobacter: Properties and Occurrence

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Introduction

The genus *Campylobacter* was established in 1963 and comprised species that had been referred to for some time as 'anaerobic *Vibrio* species.' At this time, the main interest in campylobacters was in their role as causes of reproductive diseases in cattle and sheep. Interest in the group greatly intensified as a result of fundamental studies published by Butzler and colleagues in 1973 and Skirrow in 1977 demonstrating their involvement with diarrhea in humans. At the present time, it is widely recognized that *Campylobacter* is a taxonomically and ecologically diverse genus, with taxa found in mammalian, avian, molluscan, and reptilian hosts. Species are well established as frequent causes of gastrointestinal disease in humans, abortion, and infertility in animals and have been associated with periodontal disease too. Some are regarded as commensal organisms. This article outlines their taxonomy, distribution, associations, and core phenotypic characteristics.

Campylobacter: A Brief Taxonomic Overview

Detailed taxonomic studies of microorganisms were significantly hampered prior to the use of DNA-based methods, and the use of the G+C ratio was crucial in the initial description of *Campylobacter* in 1963 that included *Campylobacter fetus* and '*C. bubulus*.' A subsequent and more thorough study in 1973 considered additional misclassified 'anaerobic *Vibrio*' species, thereby adding *C. jejuni*, *C. coli*, and *C. sputorum*. In contemporaneous developments, seminal studies in the 1970s by Butzler and colleagues and Skirrow clearly demonstrated the relevance of *C. jejuni* as an important cause of diarrhea in humans. With *C. fetus* long established (since 1919) as an animal pathogen involved with spontaneous abortion in cattle and sheep (predominantly *C. fetus* subsp. *fetus*) and infectious infertility (*C. fetus* subsp. *venerealis*), interest in the prevalence, distribution, and significance of these organisms increased markedly. As a consequence, many new taxa were discovered and described in association with enteric, septic, gastric, reproductive, and oral diseases of humans and animals and even in the environment ('*C. nitrofigilis*,' since transferred to the genus *Arcobacter*). There are presently (November 2014) 26 species that are validly described in accordance with the Bacteriological code and one ('*C. troglodytis*') published but awaiting validation through publication in the *International Journal of Systematic and Evolutionary Microbiology*.

It is important to recognize that advances in taxonomic methods, notably those that have been applied to establish genetic and phylogenetic relationships, have had a major impact on the taxonomic structure of *Campylobacter*. Arguably, that with the greatest influence to date has been the widespread application of 16S rRNA gene sequence analysis to reveal broad evolutionary patterns. As a consequence, several species first described as *Campylobacter* species were reassigned to related, but distinct genera including *Helicobacter*, *Arcobacter*, and *Sulfurospirillum*. The differentiation of species in these genera is not trivial; although *Arcobacter* species have an ability to grow at lower temperatures and in an aerobic atmosphere, and gastric *Helicobacter* species possess a sheath around their flagellae (characteristics not found among campylobacters), many species may be found in the same habitat, making identification with all but molecular methods taxing. Notably, *C. coli* and *H. pullorum* are indistinguishable using commonly available biochemical methods alone, and both have been found in human diarrhea and poultry.

Figure 1 summarizes the phylogenetic relationships among *Campylobacter* species and related bacteria in the wider Epsilonproteobacteria class that includes *Arcobacter* and *Helicobacter*.

Campylobacter: Distribution and Pathogenicity

Table 1 summarizes the current taxonomic composition of the genus, alongside clinical significance (if any) in humans, host distribution, and foodborne vector potential of member taxa.

C. avium was described in 2009 as a novel species isolated from chickens and a turkey. Given that it shares these ecological niches with the established pathogen *C. jejuni*, it is not unreasonable to surmise that *C. avium* could be a cause of foodborne illness in humans. Indeed, given that the trait of hippuricase activity is also shared by these species (that remains the most frequently used phenotypic marker for *C. jejuni*), the potential for *C. avium* infections to be misidentified as those caused by *C. jejuni* seems credible. Nonetheless, there has been no report of human disease associated with *C. avium* thus far, even where suitable molecular culture-independent methods have been used.

C. canadensis was described in 2007, having been isolated from captive whooping cranes in Canada. There have been no descriptions of the organism in human illness as yet, and since wild birds are regarded as a low risk of human campylobacteriosis, this is unlikely to change if the host range of *C. canadensis* does not extend beyond the current domain.

C. coli is generally regarded as the second most frequent cause of classical campylobacteriosis in humans after *C. jejuni*, its closest known genetic relative. In 2003, of campylobacteriosis cases in the United Kingdom were attributed to *C. coli* infections, with a substantive (£4 million) societal cost. The host distribution of *C. coli* closely resembles that of *C. jejuni*, including many common food vehicles such as chicken, pork, and beef, with its frequency in pigs especially noteworthy. Interestingly, molecular epidemiological studies identify poultry and ruminants as the most important reservoirs of human infection. A case-to-case study in the United Kingdom conducted in 2002 suggested *C. coli* infections were more likely to be associated with the consumption of halal meats, meat pies, offal, pâté, and bottled water. The bottled water

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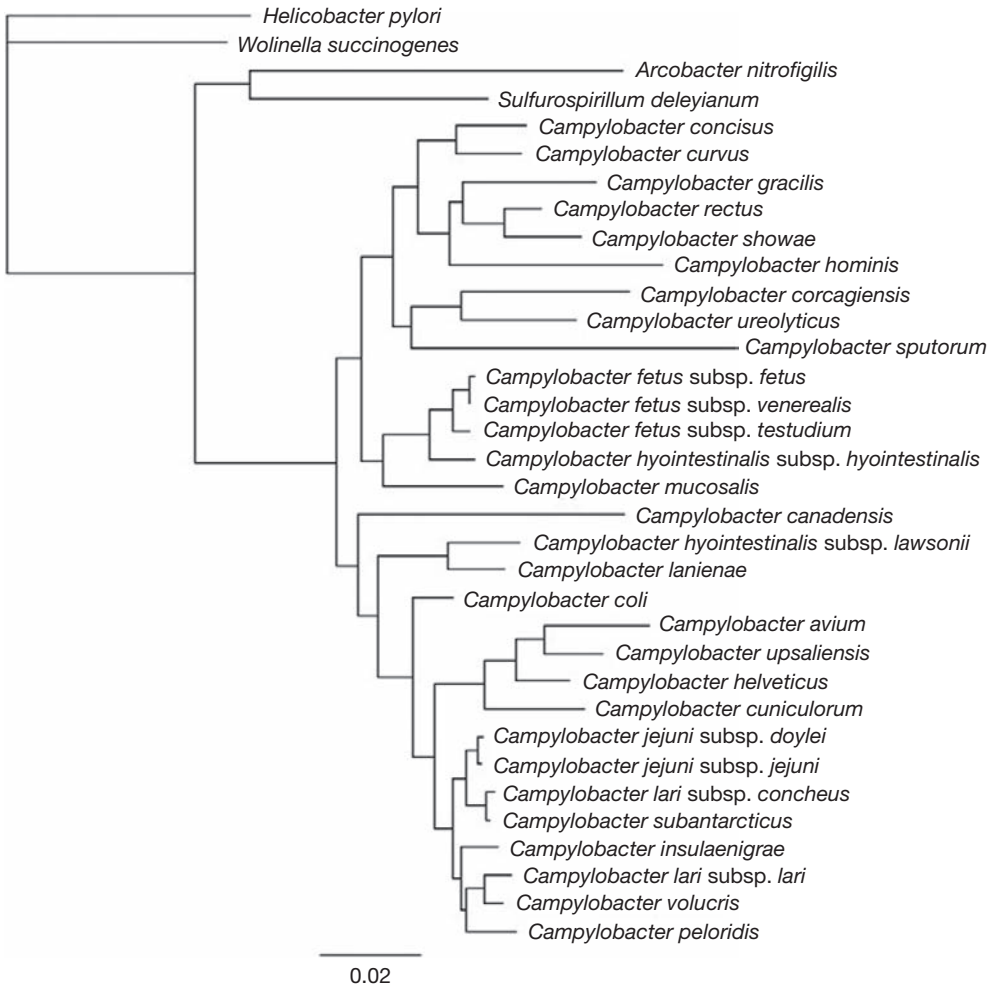

Figure 1 Phylogenetic tree for the type strains of the validly described taxa within the *Campylobacter* genus based on nucleotide sequence similarity of the 16S rRNA gene. The type species of closely related genera were also included and *Helicobacter pylori* was used as the out-group. The tree was constructed in Geneious 6.1.7 (Biomatters, available at www.geneious.com) using the Tamura–Nei genetic distance model and neighbor joining with a 70% similarity cost matrix, a gap open penalty of 12, a gap extension penalty of 3, and global alignment with free end gaps as the alignment type.

association is intriguing, given that while reported outbreaks are rare, two of the three we are currently aware of are waterborne.

C. concisus was originally described from the human oral cavity, but since this time, there have been many subsequent descriptions of its presence in human diarrheal cases. It is well known to be a genetically heterogeneous species, to the extent where analyses such as quantitative DNA–DNA hybridization and amplified fragment length polymorphism analysis indicate that at least two, and possibly up to six, genomically distinct taxa may be identified that potentially represent distinct species. However, the current lack of phenotypic markers to distinguish such taxa has led to the usage of the term 'genomospecies' to distinguish these genetically distinct but phenotypically indistinguishable groups. Two genomospecies appear dominant and a simple polymerase chain reaction (PCR) test may be used to differentiate these. It has been hypothesized that the different genomospecies have different pathogenic potentials too, yet the current evidence is conflicting. What is indisputable is that, where appropriate isolation or detection methods are employed, *C. concisus* can be found frequently not only in cases of human diarrhea and cases of inflammatory bowel disease (IBD) but also in stools from evidently healthy persons. Comparative genomic analysis of six strains revealed a range of genes associated with metabolism, adherence, and invasion that may explain the differences in patient outcomes if infected with *C. concisus*. Recent studies have identified *C. concisus* in domestic pets and various food animals including cattle, pigs, and chicken, highlighting the need to better understand the organism's pathogenic potential.

C. corcagiensis was described in 2014 resembling, but distinct from, *C. ureolyticus* and isolated from the feces of captive lion-tailed macaques. The pathogenicity of the species is as yet undetermined, as is its wider distribution. However, since we are unaware of any reports of *C. ureolyticus* in foods, and lion-tailed macaques surely being a rare exposure route, *C. corcagiensis* is an unlikely foodborne pathogen.

C. cuniculorum was first isolated from rabbit ceca in 2009 and was shown in a 2013 study to be widespread among wild rabbits in Italy. The pathogenicity of the species is as yet undetermined, but if the frequency of the organism's detection

Table 1 Distribution and human clinical significance of *Campylobacter* species

Taxon	*Human clinical associations*	*Host species*	*Foodborne illness association*
C. avium	None at present	Poultry	None reported
C. canadensis	None at present	Whooping cranes	None reported
C. coli	Gastroenteritis	Poultry, pigs, sheep, dogs, ostriches	Yes
C. concisus	Gastroenteritis, inflammatory bowel disease	Humans, dogs, cats, pigs, chicken	Potentially
C. corcagiensis	None at present	Lion-tailed macaques	Unlikely
C. cuniculorum	None at present	Rabbits	None reported
C. curvus	Uncertain	Humans, pigs	None reported
C. fetus subsp. *fetus*	Septicemia, gastroenteritis, abortion	Cattle, sheep	Potentially
C. fetus subsp. *venerealis*	Septicemia	Cattle, sheep	Unlikely
C. fetus subsp. *testudinum*	Gastroenteritis, septicemia	Turtle, skink, snake species	Potentially
C. gracilis	Uncertain	Humans	No
C. helveticus	None at present	Cats, dogs, pigs	None reported
C. hominis	None at present	Humans	None reported
C. hyointestinalis subsp. *hyointestinalis*	Gastroenteritis	Pigs, cattle, deer, sheep, hamsters, monkeys, elephants	Potentially
C. hyointestinalis subsp. *lawsonii*	Gastroenteritis	Pigs	Potentially
C. insulaenigrae	Gastroenteritis, septicemia	Seals, sea lions	Potentially seafood
C. jejuni subsp. *jejuni*	Gastroenteritis, septicemia, polyneuropathies	Poultry, cattle, pigs, sheep, dogs, cats	Yes
C. jejuni subsp. *doylei*	Gastroenteritis, septicemia, gastritis	Humans, chicken[a]	Unlikely
C. lanienae	None at present	Pigs, cattle	None reported
C. lari subsp. *lari*	Gastroenteritis, septicemia	Wild birds, chickens, horses, dogs	Potentially
C. lari subsp. *concheus*	None at present[b]	Humans, shellfish, seagulls	None reported
C. mucosalis	None at present	Pigs	None reported
C. peloridis	None at present[b]	Humans, shellfish	None reported
C. rectus	Periodontal disease	Humans, dogs[d]	None reported
C. showae	Periodontal disease	Humans, dogs[d]	None reported
C. sputorum[c]	Abscesses, gastroenteritis	Humans, sheep, cattle, pigs	Potentially
C. subantarcticus	None at present	Wild birds	None reported
C. troglodytis	Gastroenteritis	Chimpanzees	None reported
C. upsaliensis	Gastroenteritis	Cats, dogs	None reported
C. ureolyticus	Wound infections, urethritis, gastroenteritis	Humans, cattle	None reported
C. volucris	None at present	Black-headed gulls	None reported

[a]The identification of strains from chicken samples is controversial. See text.
[b]Some strains have been isolated from human feces but no pathological association was described.
[c]Comprises three biovars differentiated by catalase and urease production: bv. sputorum, bv. faecalis, and bv. paraureolyticus.
[d]May represent as-yet undescribed novel, but closely related species.

in rabbits in Italy is echoed in other countries, *C. cuniculorum* may be of interest to study in more detail, in nations that have a known appetite for the consumption of rabbit meat.

C. curvus was originally described as '*Wolinella curva*' and recovered from the oral cavity of humans. An extensive polyphasic taxonomic study reclassified the organism into the genus *Campylobacter* at which point the specific epithet was also emended. *C. curvus* is not regarded as a cause of periodontitis, but there are rare reports of its involvement with hepatic abscesses, premature birth, and pediatric IBD. The role of *C. curvus* in these conditions has not been established. The use of a sensitive culture method recovered *C. curvus* from both bovine and porcine sources, indicating the potential for foodborne transmission.

C. fetus was one of the two taxa (the other was '*V. bubulus*,' since reclassified as *C. sputorum*) that formed the genus *Campylobacter* in the original taxonomic proposal. It remains the type species of the genus. Subsequent to inception, *C. fetus* has been divided into three subspecies. *C. fetus* subsp. *fetus* may be found in the intestine of cattle and sheep and may also cause abortion in these animals if the reproductive tract is contaminated. In humans, *C. fetus* subsp. *fetus* may cause septicemia, abortion, and gastroenteritis. To date, there has not been a definitive link of human illness to the consumption of contaminated food, but the organism has been recovered from meat and liver samples from sheep, cattle, and pigs; chicken meat; turkey carcasses; vegetables; and milk. Thus, human consumption of contaminated foods is a risk for *C. fetus* infection, as is direct animal contact.

In contrast to *C. fetus* subsp. *fetus*, *C. fetus* subsp. *venerealis* appears highly adapted for colonization of the bovine genital tract; such infections may lead to abortion, but more importantly infertility, where the venereal nature of infection may be associated with substantial economic losses to breeding

programs. Reports of human infections are extremely rare and limited to cases of vaginosis and septicemia.

C. fetus subsp. *testudinum* is the most recently described subspecies and its natural hosts are a range of reptiles including turtle, skink, and snake species; it has also been recovered from clinical cases of septicemia, diarrhea, and pulmonary edema. Several human cases described consumption of Asian speciality dishes, some of which contained ingredients including turtle, frog, and eel. These data imply *C. fetus* subsp. *testudinum* infections in humans may be foodborne, where reptiles and perhaps eels are used as ingredients. It should be noted that the evidence to definitively link infections to foodborne consumption is presently circumstantial and that eel species are not a proven host for *C. fetus* subsp. *testudinum*.

C. gracilis was first described as '*Bacteroides gracilis*' and in association with human periodontitis. A polyphasic taxonomic study formally assigned the organism to the *Campylobacter* genus. The role of *C. gracilis* in periodontal disease is unclear. The organism has been detected in stool samples from both healthy and diarrheic dogs and humans using culture-independent methods. To our knowledge, detections in food animals have not been reported. Current evidence indicates *C. gracilis* is not a foodborne cause of gastroenteritis.

The principal hosts of *C. helveticus* are domestic pets (cats and dogs), from which this species was first described. Strains have been identified in porcine cecal and carcass swab samples, but not on pork meat. There are as yet no reports of *C. helveticus* infections in humans, but it should be noted that the close phenotypic and genetic similarities between this species and *C. upsaliensis* (a known human pathogen) do not preclude the possibility that historical cases of human *C. upsaliensis* disease may have been misidentified episodes of *C. helveticus* infections. Nonetheless, there is currently no evidence to support that hypothesis.

C. hominis was first detected in human feces using a PCR approach in 1998 and subsequently isolated in 2001 using an anaerobic growth protocol, suggested by the close phylogenetic association with *Campylobacter* species for which culture conditions of anaerobiosis appear to be preferred. There are presently no reports of *C. hominis* in animals, or with diseases in humans, and it is generally believed that this bacterium has a commensal role.

C. hyointestinalis is divided into two closely related but distinct subspecies with substantive phenotypic, genotypic, and ecological differences. *C. hyointestinalis* subsp. *hyointestinalis* has been found in fecal and intestinal samples of pigs, cattle, deer, sheep, hamsters, monkeys, and elephants; in many of these animals, there has been an association with diarrhea. Cases of gastroenteritis in humans have also been reported, including an outbreak for which the implicated (but not proven) source was raw milk. The relatively wide spread of host animal species in which *C. hyointestinalis* subsp. *hyointestinalis* is found and its presence in human gastroenteritis are indicative of foodborne pathogenic potential. The other subspecies, *C. hyointestinalis* subsp. *lawsonii*, was first isolated in 1995 from the porcine stomach. Since then, a 2002 report linked isolates from a case of human diarrhea and pigs in the same household with genetic typing has been published. Furthermore, *C. hyointestinalis* subsp. *lawsonii* has been detected in 2014 in 26.7%, 55.6%, and 65.4% of infant stool samples from Bangladesh, Peru, and Tanzania, respectively. Other studies published in 2011 and undertaken in Ireland and Canada have detected *C. hyointestinalis* in human diarrhea, but were unable to identify to subspecies level. The data thus far suggest each *C. hyointestinalis* subspecies may be an agent of foodborne non-*jejuni*, non-*coli* illness, with prevalence rates varying markedly between countries. However, further work is needed to substantiate this and the role that *C. hyointestinalis* plays in human gastroenteritis.

C. insulaenigrae has been recovered predominantly from marine mammalian species including sea lions, a porpoise, and several seal species. To date, there is one formally published report of human infection, an Australian case of enteritis and septicemia in a 60-year-old female with end-stage renal failure. While this patient was clearly compromised, there was no known prior contact with marine mammals and the route of infection must remain speculative. A second report was presented at the 2014 New Zealand Microbiology Society conference, a case of bacteremia and enteritis, which *may* have been associated with the consumption of mussels (T. Blackmore, personal communication). It should be noted that seals are native to New Zealand. These cases suggest both direct contact and exposure to or consumption of contaminated water or food may be transmission routes for *C. insulaenigrae* to humans, where marine mammal species are extant.

C. jejuni comprises two subspecies, of which *C. jejuni* subsp. *jejuni* is by far the better studied, most widespread, and most important from a foodborne illness perspective. This is substantiated from a review of outbreaks worldwide where, when species-level identification was performed, *C. jejuni* subsp. *jejuni* was far more frequently found to be the cause compared with the closely related species *C. coli*. The organism is extensively distributed among production animals and domestic pets, with consumption of contaminated food, water, and milk and direct animal contact all establishing transmission pathways. The infectious dose appears comparatively small, the incubation period ranges from 18 h to 8 days, and post-infectious complications can include polyneuropathic disorders including Guillain–Barré and Miller Fisher syndromes and reactive arthritis. It has been suggested that the pathogenic potential of individual strains may vary. Suggestions of host specificity or at least host preference of certain strain types have been made from genotyping studies, and differential survival rates between strains has also been used to argue the 'successful clone' hypothesis, first proposed to the best of our knowledge in 2006. Equally, it has been suggested that the absence of host-associated phenotypic or genetic markers among multilocus sequence type 21 strains from different sources may argue for generalist rather than specialist adaptation. Strain NCTC 11168 was the first *Campylobacter* spp. genome to be sequenced and publicly released; while there are a further 15 *C. jejuni* subsp. *jejuni* strain genomes completed and published as of 12 November 2014, decreasing costs and increased access to genome sequencing infrastructure are resulting in many more being made available for study.

In contrast, *C. jejuni* subsp. *doylei* is reported only rarely in human disease and has been found in cases of gastritis, diarrhea, and septicemia. Its scarcity may in part be related to a key phenotypic difference between the two subspecies; *C. jejuni* subsp. *doylei* is unable to grow at 42 °C, a common component

in methods for the isolation of enteric campylobacter. This trait would also explain the absence of *C. jejuni* subsp. *doylei* in poultry, given that this is also the core temperature of the host birds. The report of *C. jejuni* subsp. *doylei* in 19 of 31 chickens examined by Kaakoush and colleagues in 2014 is questionable from this ecological perspective as well as the use of the 16S rRNA gene sequence for speciation, since this does not possess sufficient resolution to distinguish such closely related taxa. Aside from this single report in chickens, no animal host has been identified for *C. jejuni* subsp. *doylei*. Additional distinctions between *C. jejuni* subsp. *doylei* and *C. jejuni* subsp. *jejuni* include the inability of the former to reduce nitrate and produce cytolethal distending toxin, both of which have been shown to be the result of gene deletion.

C. lanienae was first recovered from fecal samples of asymptomatic abattoir workers, with subsequent studies demonstrating its presence in pigs, cattle, sheep, wild boar, and red deer. Although the presence in pigs and cattle and detection in humans suggest zoonotic transmission from direct contact or consumption of contaminated meats, the absence as yet of any reported disease symptoms in humans currently indicates *C. lanienae* may be of limited concern, although clearly, it deserves close monitoring.

C. lari is another species known to be genetically and taxonomically diverse and is currently divided into two subspecies. *C. lari* subsp. *lari* was first described in 1983 as '*C. laridis*' with the species epithet corrected to *C. lari* later. Strains have been described from diverse sources, including seagulls, ducks, cows, shellfish, dogs, and horses, and reports of human infection have included gastroenteritis, abdominal pain, septicemia, and infections associated with surgical implants. Outbreaks associated with contaminated water have been described. *C. lari* subsp. *concheus* is more recently described (2009) with strains thus far recovered from human feces and shellfish. Its pathogenicity has yet to be determined but, given the sources from which it has been recovered, should be regarded as a potential enteropathogen that can be transmitted from the consumption of contaminated food; shellfish are as yet the only verified animal host. The diversity of *C. lari* extends to a third, genetically diverse group referred to as urease-positive thermophilic campylobacters on the basis of their most discerning phenotypic trait, urease production. Strains have been recovered from human diarrhea, marine and river waters, and shellfish and on this basis should be regarded as potential human pathogens that may be transmitted by contaminated water or food, notably shellfish.

First described in 1975 as a subspecies of *C. sputorum* but elevated to species status in a later polyphasic taxonomic study, *C. mucosalis* was initially recovered from pigs in association with lesions of porcine intestinal adenomatosis, necrotic enteritis, regional ileitis, and proliferative hemorrhagic enteropathy. The role of *C. mucosalis* in such diseases is questionable given the identification in 1995 of the intracellular *Lawsonia intracellularis* as the predominant pathogen. Two cases of enteritis in humans described in 1993 attributed to *C. mucosalis* were subsequently shown to be misidentified *C. concisus* strains in 1994. More recently, the use of sensitive isolation methods has recovered *C. mucosalis* from beef and pork products. Genetic methods have detected strains in canine feces and at low frequency in healthy and diarrheic human stools. Current evidence casts considerable doubt on *C. mucosalis* being an important foodborne pathogen.

C. peloridis was described in 2009 following a taxonomic study of bacteria resembling *C. lari*. This study refined the taxonomic structure of *C. lari* (see earlier) and also determined that some strains from human stools and shellfish were distinct enough to warrant recognition as a distinct species, namely, *C. peloridis*. Its pathogenic potential has yet to be determined, but its close resemblance to the established enteropathogen *C. lari* and occurrence in shellfish suggest it would be prudent to maintain an awareness of the organism.

C. rectus was first described (as '*Wolinella recta*') in 1981 in association with human periodontal disease, where it remains as one of several bacterial species that may be involved with this condition. There have been several reports (most recently using culture-independent molecular tools) of the organism in human diarrhea and in dogs. While the role of *C. rectus* in periodontal disease is substantiated, its role as a potential zoonotically transmitted cause of gastroenteritis is far less clear.

C. showae is another species that was first identified in 1993 to play a role in periodontal disease. As with *C. rectus*, *C. showae* has also been detected in cases of human gastrointestinal disease, and in dogs, and the pathogenic and zoonotic potential of these species may be considered to be equivalent at this time.

Along with *C. fetus*, *C. sputorum* was one of the first 'anaerobic *Vibrio* spp.' to be reclassified as *Campylobacter*, although here it was referred to as '*C. bubulus*' and was subsequently reclassified as a biovar of *C. sputorum*, which was later rescinded in 1998 since the defining phenotypic tests were found to be irreproducible and invalid. *C. sputorum* is divided into three biovars based on their ability to produce catalase and urease, with bv. sputorum negative in both traits, bv. faecalis catalase-positive only, and bv. paraureolyticus urease-positive. *C. sputorum* can be found in cattle, pigs, and sheep as host species and has been reported in humans as an oral commensal, in diarrhea, and in abscesses. Its pathogenic potential is largely undetermined, but the body of evidence thus far suggests it to be a possible risk to immunocompromised persons, with foodborne transmission being a credible, if presently unsubstantiated, pathway.

C. subantarcticus has so far only been recovered from wild birds in the subantarctic. While this species is closely related to the pathogenic *C. lari*, there are currently no reports of human infections, and wild birds are generally regarded as a low-risk exposure route for human campylobacteriosis.

'*C. troglodytis*' was first described in 2011 from fecal samples of wild chimpanzees in national parks located in Tanzania. A 2014 study of *Campylobacter* spp. in infant diarrheal samples in developing countries including Tanzania, and also Peru and Bangladesh, revealed '*C. troglodytis*' in 18 of 53 representative samples examined for emerging campylobacters. These limited studies suggest (but do not prove) pathogenic and zoonotic potential, or contamination via a common source.

C. upsaliensis was first isolated in 1983 from canine feces and described as the 'catalase-negative or weak campylobacter' in light of its (at that time) quite distinctive phenotype. Domestic cats and dogs remain the host species that are most commonly associated with *C. upsaliensis*; recent exemplar studies have found it in 37% of dogs and 44.6% of cats. In addition, three of 31 samples (9.7%) in commercial broiler

chickens in a 2014 Australian study harbored *C. upsaliensis*; a separate 2013 Swedish study in which 2% of rodents were positive suggests that vectors other than domestic pets potentially pose an exposure pathway and, with rodents especially, a possible contamination route for animal husbandry. In humans, *C. upsaliensis* is considered to be pathogenic, with diarrhea and septicemia being recognized outcomes. *C. upsaliensis* is less frequently reported from human disease compared with other enteropathogenic species such as *C. jejuni* and *C. coli*, although its more pronounced sensitivity to antibiotics commonly used in routine isolation protocols may contribute to underdetection.

The taxonomic status of *C. ureolyticus* had remained unclear for some time, given its original (1978) classification as a *Bacteroides* species was questioned with the advent of phylogenetic analyses; a more detailed polyphasic taxonomic examination in 2010 reassigned the organism to the genus *Campylobacter*. The first reports of clinical associations encompassed abscesses, non-gonococcal urethritis, and soft tissue infections, with more recent studies implicating the species as a cause of gastroenteritis, ulcerative colitis, and Crohn's disease. The status of *C. ureolyticus* as a primary pathogen is, however, unproven and controversial, given that it can be detected in healthy and diarrheal patients. Infraspecific differences in virulence properties have been proposed to explain this. Recent studies suggest cattle may be a reservoir for *C. ureolyticus*; thus, contaminated meat or milk may represent foodborne infectious sources of note, if subsequent studies prove organismal pathogenicity.

C. volucris is a *C. lari*-like species isolated from black-headed gulls. The pathogenic potential of the species is undetermined, and thus far, no association with human illness has been reported.

Campylobacter: General Properties

The genus *Campylobacter* represents a distinct phylogenetic group within the class Epsilonproteobacteria that includes *Arcobacter*, *Sulfurospirillum*, *Helicobacter*, and *Wolinella* (**Figure 1**). Nonetheless, species within the genus exhibit some remarkably diverse properties. Cells may be spiral (e.g., *C. fetus* and *C. jejuni*), curved (e.g., *C. concisus*), or rod-shaped (e.g., *C. gracilis* and *C. hominis*) and either aflagellate (*C. gracilis*, *C. hominis*, or *C. ureolyticus*) or with one or more polar flagella (other species). They have a preference for nutritionally complex media, but many species can be cultured on blood-free media and some species will grow on a minimal medium. One important unifying feature is their particular atmospheric gaseous requirements. Historically, taxa have been considered to require microaerobic (3–5% O_2 content) conditions (e.g., *C. jejuni* and *C. fetus*); microaerobic conditions, but with a H_2 requirement (e.g., *C. concisus*); or anaerobic conditions (e.g., *C. rectus* and *C. ureolyticus*). However, recent studies in 2011 have indicated that all species can be cultured in a single specified microaerobic atmosphere comprising 3% O_2, 7% H_2, 10% CO_2, and 88% N_2. This atmosphere, combined with the use of blood agar media and 0.60–0.65μM filtration, has successfully recovered a wide range of species from food products. This approach was derived from 'the Cape Town protocol' used in Professor Lastovica's laboratory for many years to examine clinical samples, with a remarkable recovery rate of an extensive range of *Campylobacter*, *Arcobacter*, and *Helicobacter* species.

Campylobacter: Incidence and Significance

Campylobacter species are widely regarded as the most frequent bacterial cause of acute gastroenteritis worldwide; reports of campylobacteriosis often do not differentiate the various enteropathogenic species (*C. jejuni* subsp. *jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*) that together have been reported at rates per 100 000 population varying from 42.6 across the 27 European Union (EU) member states in 2013, to 44 in the United States, to 383.5 in New Zealand. The rate of campylobacteriosis in New Zealand has since dropped to 156.8 following a range of interventions in poultry production. It is widely recognized that these reported rates represent only a fraction of the actual disease burden as a consequence of underdiagnosis and underreporting; for example, the true incidence of campylobacteriosis across EU states has been estimated at 1860 cases per 100 000 population in 2013, representing an average underreporting rate of 43.6. In low-to-middle income countries, campylobacter infections are estimated to cause 8.4% of the total burden of diarrheal disease.

As high as these figures are, it should be noted that they represent reports of species such as *C. jejuni* for which isolation methods have been predominantly designed. With some studies demonstrating essentially equivalent numbers of taxa such as *C. concisus*, the burden of disease may be even higher and perhaps accounting for some of the majority of acute gastroenteritis cases for which routine analyses fail to identify a pathogen. Although much progress has been made in understanding *Campylobacter*, there is much that remains to be understood.

See also: Campylobacter: Health Effects and Toxicity; Campylobacter: Species Detection.

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Relevant Websites

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www.gold.jgi-psf.org – Genomes online database.

Campylobacter: Species Detection

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Introduction

The genus *Campylobacter* comprises Gram-negative, non-spore-forming, microaerophilic microorganisms. Cells are slender, spirally curved rods often motile by means of a single polar flagellum at one or both ends. It encompasses microorganisms of human and veterinary medicine interest; most species are pathogenic for humans and/or animals, and they are commonly found in the reproductive organs, intestinal tract, and oral cavity of humans and animals. A phylogenetically related genus that is an important human pathogen as well is *Arcobacter*. In general, campylobacters can be differentiated from arcobacters by the higher optimal growth temperatures (25–30 °C for arcobacters compared with 30–42 °C for campylobacters) and the aerotolerance of arcobacters. Taxonomy of the *Campylobacter* genus has evolved in the last 30 years and presently contains 16 species. Five of them, namely, *C. upsaliensis*, *C. helveticus*, *C. lari*, *C. jejuni*, and *C. coli*, are established or possible enteropathogens for humans, are found in food and water, and are thermotolerant.

The two subspecies of *C. jejuni*, *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*, are currently recognized as the first causative agent of foodborne infection in developed and developing countries. In contrast to the well-known foodborne pathogen *Salmonella*, which is also the second causative agent of foodborne disease in developed countries, *Campylobacter* is responsible for sporadic cases rather than structured disease outbreaks. *C. jejuni* asymptomatically colonizes chickens and other avian species, and chicken meat is recognized as the most common food vehicle that causes intestinal disease in humans. *C. jejuni* infection in humans is symptomatic as the microorganism invades the intestinal epithelial layer resulting in inflammation and diarrhea. The basis for the different outcomes of infection in humans versus chicken is not well understood. Human campylobacteriosis is a self-limiting disease. However, a correlation exists between human *C. jejuni* infection and the development of Guillain–Barré syndrome (GBS), an autoimmune disorder of the peripheral nervous system. It is now recognized that GBS is a severe, potential sequel of *Campylobacter* infection.

The role of *Campylobacter* in human foodborne infection has only recently been recognized, and this is partly due to the difficulties of detection of this microorganism in foods. Such difficulty hinders proper epidemiological studies to assess the burden of disease caused by *Campylobacter* spp. Additionally, the lack of harmonized sampling and analysis procedures limits the efforts directed toward determining *Campylobacter* prevalence in foods and eventually proposing preventive measures to control foodborne infections. This article describes the currently available *Campylobacter* detection methods, with particular emphasis on the *C. jejuni* species, due to its significance for the food safety.

Detection Methods Based on Culture Media

Culture media for the detection of *Campylobacter* were initially developed for microbiological analysis of feces (medical samples) and are based on the use of a basal medium, supplemented with antibiotics. Furthermore, increased temperature of incubation (42–43 °C) facilitates the detection and isolation of thermotolerant campylobacters. The use of these culture media subsequently has been extended to the detection in food and water (food samples). As for the detection of other foodborne pathogens, an enrichment step is commonly performed for *Campylobacter*. Furthermore, a pre-enrichment is often employed in order to recover sublethally damaged cells of *Campylobacter*, common in samples as a consequence of food processing/preservation technologies employed. The microaerophilic nature of *Campylobacter* is taken into consideration in culture media development. In particular, ingredients need to be added in media, which neutralize the toxic effects of substances that are formed in the presence of oxygen and light. Neutralization can be achieved by the addition of ferrous sulfate, sodium metabisulfite, and sodium pyruvate, in some cases also combined with blood, or alternatively by the addition of charcoal. It should be highlighted that the addition of blood in the media used for enrichment can greatly influence downstream molecular biology applications since it is a renowned inhibitor of PCR amplification. Furthermore, the atmosphere of incubation greatly influences the recovery of *Campylobacter*; therefore, it needs to be adjusted accordingly. Usually, conditions of 5–7% O_2, 10% CO_2, and 80% of N_2 and/or H_2 are employed.

Apart from the increased temperature, antibiotics are the main selective agents employed in enrichment and plating media. Common antibiotics used are polymyxin, trimethoprim, and colistin, active against Gram-negative bacteria; vancomycin, rifampicin, cephalosporin, and bacitracin, active against Gram-positive bacteria; and cycloheximide, amphotericin B, and nystatin, which are employed as inhibitors of yeasts and molds.

Preston and Bolton broths are used for enrichment purposes, while Preston agar and mCCD (modified charcoal, cefoperazone, deoxycholate) agar are the most common plating media. On such media, the typical appearance of *Campylobacter* colonies is flat, glossy, and moist, with a tendency to spread.

Figure 1 presents an outline of the EN ISO 10272-1 method for the detection of *Campylobacter* spp. in food and feedstuff. Common tests performed on suspected colonies for confirmation are Gram staining and cell morphology, motility, and microaerobic growth tests at 25 °C (negative for *Campylobacter* spp.), aerobic growth test at 41.5 °C (negative for *Campylobacter* spp.), and oxidase test (positive for *Campylobacter* spp.). The identification of confirmed colonies as *C. jejuni* is mainly based on hydrolysis of hippurate; among *Campylobacter* spp., only *C. jejuni* is positive for this test. Furthermore, identification can

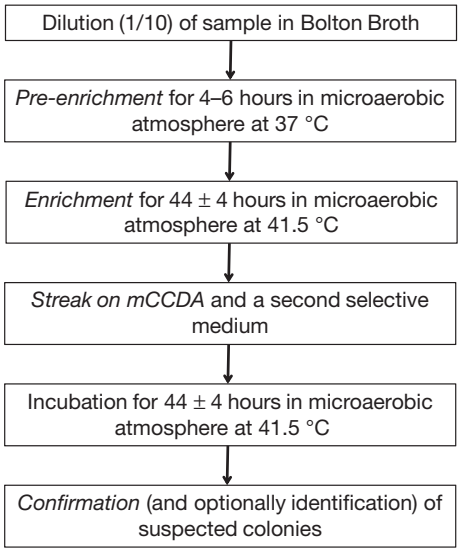

Figure 1 Outline of the ISO 10272-1 method for detection of *Campylobacter* spp. in food and feed.

be performed by commercially available latex agglutination assays with polyclonal antibodies raised against antigens of main *Campylobacter* species. Alternatively, identification can be performed by molecular methods, as described in the succeeding text.

It is widely recognized that recovery of *Campylobacter* from food samples (especially chicken meat) is highly dependent on the choice of enrichment broth and selective plating medium. This is due to (i) varying sensitivities, within the *Campylobacter* spp., to the antibiotics added in the media, (ii) difficulties in recognizing *Campylobacter* spp. on the plating media because often they form atypical colonies, (iii) the presence of sublethally injured cells in the samples, and (iv) outgrowth of *Campylobacter* during enrichment, especially when present at low concentrations, by competing bacteria. Under certain conditions, direct plating is preferable to plating after enrichment. Such an approach, however, is inadequate for samples with a low number of *Campylobacter* or when cells are sublethally injured and there is a need for resuscitation prior to plating. The difficulty in recovering *Campylobacter* from a food sample increases the risk of obtaining false-negative results. Despite significant efforts in recent years, monitoring of *Campylobacter* in foods and risk assessment still suffer from the lack of reliable and standardized culture-dependent protocols.

Species Identification by PCR

As reported in the preceding text, *Campylobacter* species identification may be performed by phenotypic tests. Such an approach presents certain limitations. First, it should be underlined that all members of the *Campylobacter* genus can be considered fastidious microorganisms; therefore, any test that is based on growth in culture media displays inherent disadvantages. Second, discrimination of the closely related members of the genus can be problematic due to the increased phenotypic relatedness or atypical results by some representative members. Alternative methods for identification have been developed that are based on PCR. These methods have mainly focused on human pathogenic campylobacters and are proposed as single species-specific PCR or multiplex PCR (mPCR) for simultaneous identification of different species in one reaction. Various genes have been used as targets, and **Table 1** summarizes the wealth of PCR protocols developed for the identification of *Campylobacter* species of food safety interest.

Typing

Typing refers to the process of identifying isolates of one species to the strain level. It allows the classification of clonally, and epidemiologically, related isolates and their differentiation from unrelated isolates. Typing of an isolate usually follows the identification of the species level, and it is indispensable for the epidemiological investigation of a foodborne outbreak or for tracking foodborne pathogens through the food chain. Ultimately, typing may allow better control and prevention–intervention measures to limit the prevalence of pathogens in food. Clearly, typing of *Campylobacter* isolates is an important task in microbiological analysis of foods, and it can be based on phenotypic or genotypic methods. Among phenotypic methods, serotyping (Penner scheme serotyping of heat-stable antigens detected by passive hemagglutination and to a lesser extent Lior scheme serotyping of heat-labile antigens detected by bacterial agglutination) is widely accepted and has been broadly applied for typing. With the advent of application of molecular biology methods in food and medical microbiology, different genotypic methods have been proposed and are discussed in the succeeding text.

Genotypic Methods

(i) Sequence polymorphism of the genes encoding for flagellin proteins in *Campylobacter* has been exploited for typing purposes. The *flaA*, or both *flaA* and *flaB* genes, can be amplified by PCR and subsequently subjected to restriction fragment length polymorphism. Alternatively, a *flaA* PCR product can be analyzed by denaturing gradient gel electrophoresis. Typing based on the *fla* gene (s) represents a simple and quick method, and the results correlate with heat-labile serotyping results (at least for some serotypes).

(ii) Pulsed field gel electrophoresis (PFGE) is considered the method of choice for typing of different pathogens, particularly during investigation of foodborne outbreaks. It should be highlighted that an international network of laboratories (PulseNet International) detects and defines foodborne outbreaks by applying PFGE as a fingerprinting method. Standardized protocols and a well-structured database of electrophoretic profiles for different foodborne pathogens (including *C. jejuni*) allow comparison of results between laboratories and facilitate the detection of outbreaks. The main disadvantage of the PFGE method is the lengthy and labor-intensive preparation of the samples for electrophoresis. This aspect renders the method impracticable in routine analysis or if a large number of isolates need to be typed.

(iii) Ribotyping essentially consists in digestion of genomic DNA, separation in agarose gel electrophoresis, and southern blot hybridization with probes targeting the rRNA-encoding genes. This method may be used for

Table 1 Summary of published PCR protocols for the identification of various species of *Campylobacter*

Method developed	*Species identified*	*Gene target*	*Additional features*	*Reference*
Single PCR	*C. jejuni*	*ceuE*, encoding a siderophore transport protein		Gonzalez et al., *Journal of Clinical Microbiology* **35**, 759–763
Single PCR	*C. coli*	*ceuE*, encoding a siderophore transport protein		
Single PCR	*C. jejuni*	*omp50*, encoding a porin	A scheme based on *omp50* amplification and hippurate hydrolysis is proposed for the identification of *C. coli* and *C. lari*	Dedieu et al., *Journal of Clinical Microbiology* **42**, 2301–2305
Multiplex PCR	*C. jejuni* *C. coli*	*mapA*, encoding a membrane lipoprotein *ceuE*, encoding a siderophore transport protein	*Campylobacter* genus identification by 16S rRNA gene primers	Denis et al., *Letters in Applied Microbiology* **29**, 406–410
Single PCR	*C. jejuni*	*hip*, encoding a hippuricase	A set of primers targeting the 16S rRNA gene was proposed for the differentiation of *C. jejuni* and *C. coli* from all other *Campylobacter* species	Linton et al., *Journal of Clinical Microbiology* **35**, 2568–2572
Single PCR	*C. coli*	Primers targeting partial aspartokinase coding gene and a downstream ORF		
Multiplex PCR	*C. jejuni* *C. coli* *C. lari* *C. upsaliensis*	*lpxA*, gene involved in lipid A biosynthesis		Klena et al., *Journal of Clinical Microbiology* **42**, 5549–5557
Multiplex PCR	*C. jejuni* *C. coli*	Gene encoding a putative oxidoreductase subunit *ceuE*, encoding a siderophore transport protein	*Campylobacter* genus identification by primers *cadF* gene (encoding an outer membrane protein) primers	Nayak et al., *Molecular and Cellular Probes* **19**, 187–193

typing of different bacterial species and it has also been applied for *C. jejuni* and to a lesser extent for *C. coli*, *C. upsaliensis*, and *C. lari*. Ribotyping for *Campylobacter* showed a low discriminatory power (i.e., ability of the method to discriminate two strains that are unrelated). It can be considered a complicated method with low throughput capacity, and therefore, an automated protocol (i.e., riboprinting) has also been proposed.

(iv) Random amplified polymorphic DNA is basically a PCR performed with short primers in low-stringency conditions. The resulting electrophoretic band patterns are used as fingerprints of the isolates. This method has been applied for *Campylobacter* sp. typing. It is a method that is easy to apply and fast but has the major disadvantage of low reproducibility.

(v) Amplified fragment length polymorphisms (AFLPs) are differences in restriction fragment lengths that occur within a genome and are the consequence of the presence/absence of restriction enzyme recognition sites. AFLP analysis is a typing method that is based on selective amplification of restriction fragments from total genomic DNA digested with restriction enzymes. It has been used as a typing method for *C. jejuni* with good reproducibility results. It is a rather complicated and laborious method but offers the advantage of analyzing a random portion of the whole genome of a microorganism without the need for prior sequence knowledge.

(vi) Multilocus sequence typing (MLST) consists in PCR amplification and sequencing of a number (usually 6–8) of housekeeping genes and comparison of the sequences obtained from different isolates. It is a very powerful typing method extensively used for strain identification of foodborne pathogens, including different members of the *Campylobacter* genus. Typing by MLST has proved useful, and its application has expanded with the availability of high-throughput sequencing platforms. The method is reproducible and has a high discriminatory power. Furthermore, it offers an additional advantage compared to the methods described in the preceding text: since typing is based on DNA sequences (and not some form of electrophoretic profile), direct comparisons between laboratories, as well as unambiguous database construction, are facilitated. MLST may be considered currently the 'gold standard' for typing of different *Campylobacter* spp., including *C. jejuni*.

It should be noted that *C. jejuni* and *C. coli* show significant intraspecies biodiversity and are characterized by genetic instability. This is partly due to the fact that both microorganisms are naturally competent and, therefore, prone to horizontal genetic exchange. Consequently, an essential characteristic of the typing method to apply for *Campylobacter* is the stability of the marker. MLST is not affected by genetic instability since it is based on the study of variation that slowly accumulates within a population and it has allowed the description of *Campylobacter* population structure. It is expected that whole-genome sequencing of multiple isolates will further contribute to the understanding of the diversity of *Campylobacter*.

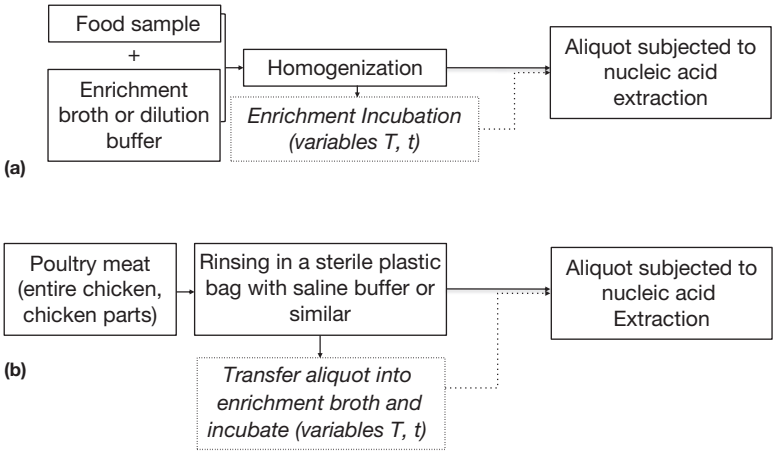

Figure 2 Work flow of microbiological analysis by a culture-independent approach. (a) The first step in the analysis involves a homogenization of the sample; after this step, it is possible to extract nucleic acids directly or after enrichment. (b) For poultry samples rather than homogenization, rinsing can be performed, and the subsequent analysis can be performed on the rinse, either directly or after enrichment. Abbreviations: *T*: temperature, *t*: time.

Direct Detection and Quantification

As evidenced in the preceding text, detection of *Campylobacter* spp. by the use of culture media is problematic. Currently, no universal enrichment and/or plating medium exists. This can be related not only to the fastidious nature of the microorganism but also to the physiological state of the cells in the various food matrices. Apart from stressed or sublethally injured cells, the viable but nonculturable (VBNC) state has been described for *Campylobacter* spp. Cells in the VBNC state by definition do not grow on culture media. Entry into the VBNC state seems to be a survival mechanism when cells encounter unfavorable conditions. It has yet to be determined if VBNC cells are/may become virulent and eventually cause disease to humans. Independently of the fate of VBNC cells, a method should be able to detect them in order to accurately describe the prevalence of *Campylobacter* in foods. Another nonnegligible inadequacy of detection based on culture media is the long time to result, considering the various steps of microbiological analysis, that is, pre-enrichment, enrichment, and selective media incubation. To overcome these specific limitations of culture-dependent microbiological analyses, that is, incomplete and possibly biased recovery and determination of *Campylobacter* and length of analysis that does not coincide with timely corrective actions, food microbiologists are increasingly investigating alternative approaches that are based on molecular biology methods. Application of molecular methods has the advantage of bypassing at least some of the culturing steps, thereby circumventing these limitations. Figure 2 summarizes the work flow of a microbiological sampling and analysis by means of molecular methods applied in a culture-independent approach. As shown, for poultry samples, it is possible to perform the analysis on homogenates or on rinses of whole carcass or parts. The most commonly employed culture-independent approach involves extraction of nucleic acids (usually DNA) and quantitative PCR (qPCR) amplification.

qPCR can be considered the best alternative to traditional culture plating for the detection (and if possible quantification) of pathogens in foods. It is currently recognized as the molecular method of choice when a culture-independent approach is employed for foodborne pathogen detection. Important parameters of the developed qPCR protocols that need to be taken into consideration are specificity, limit of detection, and, in case where the protocol is intended for quantification, the linearity range. Specificity is determined by testing the qPCR protocol with a large number of nontarget species, and no amplification should be obtained. This is a parameter that strictly depends on the choice of the target gene and subsequently on the primer/probe design. The limit of detection and the linearity range are strongly dependent on the quality of nucleic acid that can be extracted from a given food sample. Calibration curves can be constructed in order to determine these parameters. Calibration curves correlate cell concentration (usually expressed as log CFU (colony-forming units) per gram or milliliter of food) to threshold cycle (C_t), determined as the cycle number of the PCR when amplification is detected (the output of qPCR). Such calibration curves are commonly constructed by artificially inoculating serial dilutions of the target microorganism in the food sample of interest and subsequently extracting and amplifying the DNA by qPCR to recover the C_t value for each cellular concentration. Each calibration curve is characterized by a linear regression equation, a coefficient of linearity that indirectly indicates the linearity range, and a detection limit. The equation can be used for the quantification of the target microorganism in a naturally contaminated food sample.

As mentioned in the preceding text, DNA is commonly employed in qPCR. This choice is based on the easiness of extraction and manipulation of this nucleic acid as compared to RNA. It should be underlined that DNA is a stable molecule and can persist long after cell death. For this reason, the use of DNA in qPCR has been criticized as it can result in positive amplification from dead cells. Alternatively, RNA can be used as a target since it is an unstable molecule and generally is considered to have a short half-life. This characteristic of the RNA renders it a good indicator of vitality; that is, the RNA of dead cells is rapidly degraded and is not being detected. The choice of the RNA molecule to target in a qPCR is also important. It is recognized that rRNA is more stable with respect to mRNA and differences in stability have also been observed among mRNA molecules. For this reason, the suitability of an RNA molecule to serve as an indicator of vitality needs to be verified.

So far, no suitable RNA vitality target has been proposed for *Campylobacter*. When RNA is used as a target, alternatively to qPCR, the nucleic acid sequence-based amplification (NASBA) can be used. This is an amplification method that is performed at isothermal conditions with RNA as a target. NASBA protocols for detection of viable *Campylobacter* spp. have been proposed that employ both rRNA and mRNA as targets.

As shown in Figure 2, an enrichment step before nucleic acid extraction is sometimes required. An enrichment step is deemed necessary when the detection and/or quantification limit of the method is not compatible with the purpose or the needs of the microbiological analysis. For foodborne pathogens, it is essential to determine the presence or absence in a certain quantity of sample. If this detection limit cannot be achieved, an enrichment step is performed. Such enrichment step can be considered a double-edged sword. On the one hand, after enrichment, it is no more possible to quantify the initial population present in the sample; on the other hand, DNA amplified after enrichment can be attributed to live cells.

qPCR protocols have been optimized for detection and/or quantification of *Campylobacter* in food. Protocols mainly address thermophilic *Campylobacter* detection, and several target genes have been used. These include the *cadF* gene (encoding a membrane protein involved in adhesion), part of the 16S rRNA-encoding gene, the *hip* gene (encoding the hippuricase), and the *rpoB* gene (encoding the β-subunit of the RNA polymerase). Performance of the developed qPCR protocols has been tested with artificially contaminated samples. Such protocols represent potentially valuable tools for the determination of the prevalence of *Campylobacter* in foods. So far, there has been limited application of the qPCR directly in food samples for the detection and quantification of *Campylobacter*. In the studies conducted so far, two important observations have been made. First, an underestimation of the *Campylobacter* prevalence in the food samples can result from the sole application of culture-dependent methods; that is, when both culture-dependent and culture-independent methods are applied in parallel, there are a higher percentage of samples that result positive by the latter as compared to the former. Second, a higher prevalence is determined in the samples without enrichment; this observation has been made also by the culture-dependent methods and can be due to the presence of stressed or injured cells that are not able to propagate in the selective medium used for the enrichment. Further application of the qPCR in food and environmental samples that will provide quantitative data of prevalence of *Campylobacter* and that can be used for quantitative risk assessment is urgently needed. In order to obtain robust quantitative data regarding *Campylobacter* prevalence in foods, standardized and validated procedures have to be developed and adopted. The lack of standardized procedures regarding type of sample, employment of enrichment broth and relative details (type of broth and time and temperature of enrichment), DNA (and/or RNA) extraction and qPCR amplification, is currently hindering efforts of determining the true prevalence of *Campylobacter* spp. in food samples, particularly in poultry.

Conclusions

In order to perform quantitative risk assessment, tools must be available that can accurately determine the *Campylobacter* prevalence in food and determine the contamination level. At present, *Campylobacter* is the leading cause of human gastroenteritis in developed countries. The advancement achieved in just a few years regarding molecular identification and typing of *Campylobacter* has been important in determining the role of poultry meat in the transmission of disease to humans. Although it is widely recognized that the main risk factor that leads to *Campylobacter* infection is manipulation and consumption of poultry meat products, available microbiological data regarding prevalence and contamination load do not allow for a valid risk assessment. Improved and standardized application of culture-independent methods will permit accurate quantification of *Campylobacter* in foods and will allow a quantitative estimation of *Campylobacter* risk related to consumption of various foodstuffs. Such estimation can also be used for proposing preventive measures in the food chain.

See also: Campylobacter: Health Effects and Toxicity; Campylobacter: Properties and Occurrence; Emerging Foodborne Enteric Bacterial Pathogens; Foodborne Pathogens; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Zoonoses.

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Cancer: Diet in Cancer Prevention

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Introduction

Cancer remains the second most common cause of mortality and accounts for about 20% of all deaths in industrialized countries. Carcinogenesis, the process from initiation with a mutagen through promotion and tumor formation, usually occurs over a long period of time. Patterns of cancer rates in various countries point to environmental influences including diet as important contributors to both initiation and prevention of cancer. The unifying characteristics among cancer cells include their ability for uncontrolled growth and replication, the avoidance of apoptotic signals, and their capacity to increase invasiveness and angiogenesis. Nutrients that may interfere with any one or more of these aspects are viewed as potentially anticarcinogenic or chemopreventive. Given the complex nature of the collection of diseases we call cancer, it is unlikely that a single compound will be identified as the ultimate chemopreventive agent. Rather, bioactive nutrients may interact synergistically or antagonistically with other food components and influence the expression or activity of more than one gene product. Thus, a very complex nutrient–gene environment is created, potentially leading to an effective chemopreventive strategy. Most of the supporting data for chemoprevention with dietary nutrients are derived from preclinical cell culture (*in vitro*) or animal model (*in vivo*) experiments, studying a single nutrient in relation to selected gene products in carcinogenesis. Epidemiological human studies are usually case–control or prospective cohort studies, frequently relying on food frequency questionnaires and extrapolation of nutrient content from databases. Randomized clinical trials with single nutrients or single foods have mostly shown conflicting results. This article principally deals with the role of diet in the prevention of cancer rather than dietary components implicated in contributing to tumorigenesis (e.g., aflatoxin and alcohol) or treatment of established cancers. It also should be noted that often several dietary factors are associated with effects on carcinogenesis at more than one organ site. Finally, the importance of a balanced diet in health and cancer prevention needs to be stressed. Supplementation with supranutritional doses of any given nutrient may lead to potentially harmful effects, especially in individuals who are already nutrient-replete or who are oxidatively stressed (e.g., smokers). To this day, neither the American Institute for Cancer Research (AICR) nor the National Institutes of Health (NIH) recommends dietary supplements in supranutritional levels for the general population as a cancer-preventive strategy.

Maintaining a Healthy Weight

Body fatness, or obesity, and a progressively sedentary lifestyle appear to be an ever-increasing problem in many developed countries. Rates of overweight and obesity are now more prevalent than they have ever been. Correlation with hundreds of cohort and case–control studies has provided convincing evidence that body fatness is associated with a host of diseases and increases risk for developing esophageal, pancreatic, colorectal, breast, endometrial, and renal cancers. Frequently, the correlation between caloric restriction and cancer prevention relates to decreased inflammation – a prime contributor to tumor promotion. Therefore, maintenance of a healthy weight throughout a person's life is regarded as one of the most important ways to protect against cancer. Several phytochemicals, including the polyphenols curcumin, daidzein, epigallocatechin gallate, genistein, and resveratrol, have been reported to exhibit potential effects against adiposity and thus obesity and against obesity-related cancers. Their cancer-preventive effects are further described in the succeeding text.

Dietary Fiber

The definition of fiber appears to vary depending on the organization and manufacturer. Both naturally derived and synthetically manufactured dietary fibers are now ubiquitously present in the food market and mostly present a collective term to describe a spectrum of nondigestible, unrefined carbohydrates, including nonstarch polysaccharides, oligosaccharides, lignin, and analogous polysaccharides. Legumes, minimally processed cereals, and various fruits and vegetables are considered to be good sources of natural fiber, and a vast number of dietary supplements have flooded the market with soluble/insoluble fiber products.

Experimental animal studies consistently have suggested that dietary fiber plays an important role in cancer prevention. In epidemiological studies, the protective benefits of fiber intake in terms of cancer prevention remain somewhat controversial. For colorectal cancers, the evidence appears stronger. A recent meta-analysis of case–control and cohort studies, which included 20 trials involving 10 948 subjects with colorectal adenoma, supported the hypothesis that high dietary fiber intake is associated inversely with colorectal adenoma risk. Furthermore, in the recent 11-year follow-up analyses of the European Prospective Investigation into Cancer and Nutrition (EPIC) study, inverse associations of total dietary fiber intake with colorectal cancer regardless of age, sex, or anthropometric and lifestyle variables were described. The beneficial role of dietary fiber in the reduction of colon cancer risk may relate to the ability of fiber to reduce the contact time of carcinogens within the intestinal lumen and to promote healthy intestinal microbiota, thus enhancing bile acid deconjugation and production of short-chain fatty acids and modulating inflammatory bioactive substances. Intestinal bacteria ferment fiber into short-chain fatty acids such as butyrate, which has been shown to function as a histone deacetylase inhibitor, thus

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epigenetically regulating gene expression to inhibit cell proliferation, induce apoptosis, and therefore suppress tumorigenesis. For other types of cancer, the evidence remains controversial. However, during the 11.6-year follow-up of the 825 men newly diagnosed with prostate cancer in a population-based prospective study in 43 435 Japanese men, total fiber and insoluble fiber intake were associated with a decreased risk of advanced cancers. These results suggested that consumption of dietary fiber was associated with a decreased risk of prostate cancer.

Inorganic Nutrients

Among the micronutrients, trace minerals are inorganic substances that are frequently essential components of enzymes and other proteins. Specifically, the trace mineral selenium has been implicated in cancer prevention. Selenium, which is found in the active site of selenocysteine, the twenty-first amino acid, is incorporated into selenium-containing proteins (selenoproteins). Selenoproteins appear to be the primary responsible connection in selenium's role as a cancer chemopreventive agent. Whereas animal models convincingly demonstrated beneficial effects of selenium supplementation, the evidence from human clinical cancer prevention trials remains controversial. Early trials in populations with relatively low levels of serum selenium demonstrated decreased incidence of prostate, colon, and lung cancers with dietary selenium supplementation. However, the 2008 Selenium and Vitamin E Cancer Prevention Trial (SELECT), which investigated prostate cancer incidence in a selenium-replete population of over 35 000 men, found no positive health benefits. Considering the recent evidence that certain selenoproteins not only prevent but also can promote cancer, it may not be surprising that the 2014 follow-up analyses of SELECT found that those men who started the trial with a high selenium baseline, compared with those with low levels, increased their risk of developing high-grade prostate cancer twofold. The AICR and the NIH currently do not recommend dietary selenium supplementation for cancer prevention.

Phytochemicals

Phytochemicals include tens of thousands of nutrients and secondary plant metabolites, and many are involved in the plant's defense against aggression by pathogens or ultraviolet radiation; some serve as pigments. Upon consumption of vegetables, herbs, seeds, nuts, and fruits, a diverse range of these nutritive and nonnutritive and potentially pharmacologically active phytochemicals are ingested. Mechanistically, phytochemicals may interfere with the cancer process at various stages and through diverse modes of action. Additionally, many of these phytochemicals lack toxicity, making them potential agents for cancer prevention.

Phytochemicals may help protect against lipid peroxidation through their antioxidative abilities, where free radicals are prevented from removing electrons from membrane lipids, thus averting cellular damage. More intriguingly, *in vitro* and *in vivo* studies have shown many phytochemicals to prevent environmental mutagens, to which we are principally exposed through the food and water we consume, from binding to DNA, thus inhibiting cancer initiation. This may be accomplished by suppression of either expression or catalytic activity of carcinogen-activating enzymes, also known as phase I enzymes, such as the cytochrome P450 (CYP) enzymes and epoxide hydrolases. Without endogenous activation, the ingested exogenous carcinogen is impeded from binding to DNA, thus preventing potential mutations. The chemopreventive process via the reduction of phase I enzyme activity is aided by stimulus-induced activation or increased expression of phase II enzymes, which include various isoforms of glutathione *S*-transferases (GST), uridine 5′-diphosphoglucuronosyltransferase (UDP-glucuronosyltransferase, UGT), NAD(P)H dehydrogenase (quinone)-1 (NQO1), sulfotransferases (SULT), *N*-acetyltransferases (NAT), and methyltransferases (e.g., *S*-methyl transferase and catechol *O*-methyl transferase, COMT). These phase II enzymes attach polar groups to the CYP-activated carcinogen to enable efflux and subsequent excretion of the potentially harmful molecule, and risk of cancer initiation will have been reduced. It should be noted that human genetic polymorphisms exist for several phase II enzymes, in which case phytochemicals may have increased or decreased effects on the expression or activity of these enzymes.

Several phytochemicals also have been shown to interfere with tumor promotion, inhibiting the survival and expansion of established cancer cells. The suppression of cell proliferation and induction of apoptosis has been shown for a variety of dietary compounds. Induction of cell cycle arrest through upregulation of the tumor suppressor P53 or inhibition of signaling pathways involving the oncogenes Akt and Ras may result in decreased cell proliferation, thus restricting tumor growth. Tumor progression and invasiveness may be inhibited by phytochemical-induced inhibition of enzymes involved in the regulation of inflammation, such as cyclooxygenase-2 (COX-2), or those that regulate angiogenesis, the process by which new blood vessels are formed to provide nutrients to the growing tumor, such as the vascular endothelial growth factor (VEGF). Lastly, phytochemicals and other exogenous compounds have been shown to elicit epigenetic alterations, including DNA (de)methylation and histone (de)acetylation, impacting microRNA expression, all of which influences gene expression. The epigenetic mechanisms of many dietary components are not well understood and are the subject of intense investigations.

Vitamins

Dietary vitamins are important to consider in cancer prevention because of their ability to stop reactive oxygen species (ROS) and reactive nitrogen species (RNS) by accepting electrons from free radicals thus neutralizing their damaging effects on polyunsaturated fatty acids, proteins, carbohydrates, and DNA. Therefore, dietary antioxidative vitamins have the capability of counteracting harmful effects of otherwise carcinogenic chemicals. Case–control studies have frequently demonstrated that increased consumption of foods correlating

with increased vitamin consumption results in decreased cancer incidence.

Vitamin A and Carotenoids

Vitamin A is fat-soluble and is found in preformed and provitamin forms. Preformed vitamin A, which includes retinol and related compounds, is acquired from animal sources, whereas provitamin A, such as β-carotene and related carotenoids, is acquired from plant sources; both forms of vitamin A have antioxidant properties and thus may contribute to free radical scavenging. Because vitamin A is important for cell-mediated immunity and humoral immunity, a vitamin A deficiency may contribute to cancer susceptibility. Retinoids and other vitamin A species have been shown to have cancer-preventive properties *in vitro*, because of their influence on epithelial proliferation and differentiation. However, among the various human clinical trials, greater exposure to retinol was not associated with decreased prostate cancer risk and in some cases appeared to modestly increase the risk of cancer.

Carotenoids' main function in plants appears to be as accessory photopigments, thus providing fruits and vegetables their red and yellow colors. Observational studies in humans previously found an inverse correlation with carotenoid intake and cancer risk. Of the many carotenoids, β-carotene and lycopene have received the most research interest. A 2001 study examining serum β-carotene levels and breast cancer risk in women found an inverse association. However, clinical cancer prevention trials have been unable to support these observations. The Physicians' Health Study found no effect of β-carotene on lung cancer, and in two other major clinical trials, high doses of β-carotene showed a significant increase in risk of developing lung cancer in study participants, who were current or former smokers. While the relationship between vitamin A and cancer prevention in nonsmokers remains unclear, the lack of consistency among the various cancer prevention trials with β-carotene led the AICR and NIH currently to not recommend β-carotene supplementation to those who are current or former smokers or those who have been exposed to asbestos.

Lycopene, a carotenoid found in diets rich in tomatoes and tomato products, is a very powerful antioxidant, with about twice the antioxidant activity of β-carotene. In various cell-based and animal studies, lycopene targeted antioxidant/detoxification enzymes and the Ras signaling pathway. Among the various observational studies in humans, some reported that consumption of tomato products appeared to lower risk of certain types of cancers, including prostate cancer, and lycopene preferentially accumulates in the prostate tissue. Because tomato products also contain a variety of minerals, vitamins, and other phytochemicals, it was unclear whether the beneficial effects were due to lycopene itself. This was further put into question when experimental studies demonstrated that rats that consumed tomato powder had a much lower cancer risk, compared with rats receiving lycopene supplements. Several human case–control studies using lycopene supplements gave conflicting results: whereas some studies indicated a protective effect against prostate cancer, other studies found little to no effects. The association between lycopene and prostate cancer is rather complex, and a 2006 study suggested that the association between lycopene and prostate cancer may be modified not only by other dietary antioxidants but also quite possibly by the gene encoding for the DNA repair protein XRCC1. Even though there appears to be insufficient evidence to support lycopene as a (prostate) cancer-preventive agent, increased consumption of tomato products as part of a healthy diet may be beneficial.

Vitamin B Complex

The B vitamins are a group of eight water-soluble vitamins, which are important as parts of coenzymes. As such, they are essential for cell and organismal growth and development and other physiological functions. Vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_3 (niacin), vitamin B_5 (pantothenic acid), vitamin B_6 (pyridoxine), and vitamin B_7 (biotin) have not been shown to act as cancer-preventive agents. Vitamin B_{12} (cobalamin) is required for proper red blood cell formation, neurological function, and also DNA synthesis. Vitamin B_{12} has been shown to help increase genetic stability and DNA repair and, like vitamin B_6, is involved in homocysteine metabolism. In terms of cancer prevention, very few randomized clinical prevention trials have been completed with individual B vitamins, and there appeared insufficient scientific evidence to support supplementation with B vitamins. However, in the recently (2013) analyzed data from the Women's Health Initiative Observational Study, vitamins B_2 and B_6 intakes from diet and supplements were associated with a decreased risk of colorectal cancer in postmenopausal women. New cross-sectional case–control and intervention trials with vitamin B_6 in colorectal and other cancers are currently underway.

Folate (vitamin B_9) is the generic term utilized for both the naturally occurring folate in food and folic acid, which describes the oxidized monoglutamate form that is used in dietary supplements and fortified foods. Much of the supplementation has focussed on folic acid's hitherto unexplained ability to reduce the risk of fetal neural tube defects. Potentially important for cancer prevention, the conversions of homocysteine to methionine in the synthesis of the methyl donor *S*-adenosyl methionine and the methylation of deoxyuridylate to thymidylate in DNA synthesis are folate-dependent. Thus, epigenetic regulation of gene expression through DNA methylation using folate supplementation has been proposed. A number of epidemiological studies suggested that folate might inhibit development of cancers of the urogenital system and of the gastrointestinal tract. A number of clinical trials examined the effects of folic acid supplementation on colorectal cancer risk. However, the results were controversial and did not support the early findings. In at least one study, the risk of adenoma development increased with folic acid supplementation and secondary analyses demonstrated an increased risk of prostate cancer. A prospective study in 2011, however, found an inverse association between folate intake and risk of the early pre-adenoma stages of colorectal cancer. Thus, folate, like some other nutrients (e.g., selenium), may play a dual role in cancer, whereas dosage and timing of exposure may determine whether this nutrient will prevent or promote cancer. Furthermore, common ethnic differences in genetic variations (polymorphisms) in the gene encoding for the enzyme methylene tetrahydrofolate reductase exist,

influencing its enzymatic activity and subsequent individual dietary folate needs. For those reasons, the NIH strongly caution against consumption of high doses of folic acid.

Vitamin C

Vitamin C, L-ascorbic acid, is an essential nutrient for humans and other primates and, beyond its role in collagen synthesis, is a very strong, water-soluble antioxidant. The molecule donates electrons to intra- and extracellular free radicals, therefore being oxidized to dehydroascorbic acid. Dehydroascorbic acid is then converted back into ascorbic acid, and the process can be repeated. Epidemiological studies have suggested that consumption of foods with high vitamin C content is inversely correlated with most types of cancers. However, even though vitamin C is known to limit formation of carcinogens, evidence from human clinical trials and prospective cohort studies has been inconsistent. Most clinical prevention trials have failed to establish a link between vitamin C, alone or in combination with other micronutrients, and cancer incidence or mortality. While it remains unclear whether vitamin C may be cancer-preventive, the tight regulation of plasma and tissue vitamin C levels in humans may not allow an increase above saturation levels, even with supplementation.

Vitamin D

Vitamin D is produced endogenously when skin epithelial cells synthesize this lipophilic vitamin in response to ultraviolet rays from sun exposure. Additional, albeit small, quantities may be consumed through dietary sources such as oily fish, meat, egg yolks, and fortified products such as milk or margarine. Because of moderately successful sunscreen/protection strategies to reduce UV-related skin cancer incidence, vitamin D synthesis may be declining. Calcitriol is the physiologically active vitamin D metabolite (1,25-dihydroxyvitamin D). Vitamin D promotes calcium absorption, thus enabling normal bone mineralization, and plays important roles in the reduction of inflammation and modulation of immune function. Epidemiological and many preclinical studies indicated that vitamin D played a role in the prevention of colorectal, prostate, and breast cancers. *In vitro* evidence suggested that vitamin D inhibits genes involved in apoptosis. Conversely, vitamin D inadequacy was thought to increase cancer risk. However, clinical prevention studies remain inconclusive, partially because vitamin D intake and serum 1,25-dihydroxyvitamin D concentrations may not correlate. Therefore, while vitamin D is an essential nutrient and important for general health, studies do not support excessive vitamin D intake for the purpose of cancer prevention.

Vitamin E

There are eight naturally occurring forms of vitamin E found in plants: α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol; α-tocopherol (vitamin E) is the biologically active form. Vitamin E has distinctive antioxidative properties and plays an important role in the protection of lipoproteins from free radical damage. Furthermore, vitamin E enhances the immune system and plays a role in anti-inflammatory processes. Several clinical trials have investigated whether vitamin E intake or supplementation may benefit in the prevention of colorectal, prostate, or other forms of cancer. Some early studies found promising results, indicating that vitamin E supplementation was associated with decreased risk of population-specific cancer mortality. Many subsequent studies, however, showed no association between vitamin E intake and prostate or colorectal cancer risk, and one study in 2004 pointed to vitamin E plus β-carotene supplementation increasing mortality. Recently, the study SELECT, which investigated the supplementation of selenium and/or vitamin E in men, found a statistically significant twofold increase in high-grade prostate cancer incidence in men who had low baseline levels of selenium and took the vitamin E supplement. Thus, insufficient data exist to support vitamin E supplementation as a cancer-preventive agent, and the NIH caution against daily use of large doses of vitamin E (400 IU), because of a potential increased risk of cancer.

Vitamin K

Vitamin K describes a group of related, essential nutrients: vitamins K1, K2, and K3. Acute vitamin K deficiency is life-threatening due to the liver's requirement of vitamin K for synthesis of coagulation factors to promote blood clotting and to prevent abnormal bleeding. Vitamin K can be obtained through consumption of foods, including green leafy vegetables, okra, asparagus, prunes, and avocado. Importantly, humans also obtain vitamin K from their intestinal microbiota. Preclinical investigations suggested that vitamin K inhibited proliferation and induced apoptosis in liver cancer cells. Since then, some small human clinical prevention trials indicated that vitamin K supplementation lowered the risk of developing liver cancer among Japanese women with hepatitis C-induced cirrhosis. However, the results were not statistically significant. Another clinical trial in 2006 suggested that a subtype of the vitamin K2 group might reduce recurrence of liver cancer after surgery. Even though follow-up studies failed to show an effect, vitamin K is now being tested along with other drugs after liver cancer surgery. Available scientific evidence thus far does not support the use of vitamin K supplements for primary cancer prevention.

Glucosinolates

Glucosinolates, which groups the bioactive isothiocyanates, thiocyanates, and indoles, are found in the plant family Brassicaceae, which includes vegetables such as broccoli, cabbage, and mustard. Epidemiological, preclinical, and clinical studies have demonstrated that dietary glucosinolates in amounts achievable in a normal healthy diet help in slowing down carcinogenesis or risk of cancer without toxic effects. The most intensely studied glucosinolate in cancer prevention, by far, is sulforaphane, the bioactive metabolite of glucoraphanin, a compound found in high concentrations in broccoli sprouts. Sulforaphane is a very potent inducer of the transcription factor nuclear factor (erythroid-derived 2)-like-2 (NFE2L2 or Nrf2) and thus increases expression of the phase II enzyme NQO1. Administration of high glucosinolate

concentration containing beverages has very eloquently been demonstrated to decrease the amount of carcinogen-DNA-adducts, and the cancer preventive and other health effects of glucosinolates are covered in great detail elsewhere in this encyclopedia.

Lectins

The plant secondary metabolites chlorophyll, phthalides, and phytic acid (including inositol phosphates) make up the lectins, which are commonly found in legumes and grain products. Phytic acid, in its bioavailable inositol form, has been shown to be a beneficial cancer-preventive agent in cell and animal studies, especially in breast, prostate, and colorectal cancers. Several human clinical intervention trials with inositol are currently ongoing or have recently been completed and are awaiting analysis. In animal models, chlorophyll inhibited carcinogen uptake and tumorigenesis. Few randomized, clinical trials with lectins have been conducted. Even though chlorophyllin (commercially available mixture of synthetic chlorophyll derivatives) has been shown to inhibit aflatoxin–DNA adduct formation in Chinese, to date, there is not enough evidence to suggest effectiveness in cancer prevention with chlorophyll.

Omega-3 Fatty Acids

In epidemiological studies, consumption of fish and fish products, including fish oil supplements, has been associated with decreased cardiovascular risk and cancer incidence. The health benefits of omega-3 versus omega-6 fatty acids were based on the evidence that omega-6 fatty acids, which predominate in the typical US diet, may stimulate tumor growth through prostaglandin-E2 synthesis, whereas omega-3 fatty acids, particularly eicosapentaenoic acid and docosahexaenoic acid, may suppress tumor formation. However, studies in humans have provided conflicting results. Even though strong evidence exists that omega-3 fatty acids may be helpful in cardiovascular disease, a 2006 meta-analysis of 38 studies conducted over the past 40 years on the effects of omega-3 fatty acids on cancer found no effect overall.

Organosulfurs

The thiol-containing organosulfur compounds of garlic, onion, and some other bulbous plants include diallyl sulfide, diallyl disulfide, and diallyl trisulfide, as well as the dithiole thiones found in cruciferous vegetables such as the cabbages. Among the various beneficial health effects attributed to the organosulfur compounds, antibacterial and anticancer activities have received much attention. Diallyl sulfide increased apoptosis in cancer cell lines and in animal models. Diallyl disulfide and diallyl trisulfide increased expression of phase II enzymes and inhibition of cancer initiation in preclinical models. Only few human clinical trials have been conducted with organosulfur compounds, and a 2006 randomized trial on long-term garlic supplementation showed no beneficial effects on the prevalence of precancerous gastric lesions or on gastric cancer incidence in a Chinese population.

Phytosterols

Plant seeds, nuts, grains, and unrefined vegetable oils and margarine are good sources of phytosterols. Because of structural similarity, plant phytosterols can inhibit uptake of dietary cholesterol. Observational data in humans suggested that consumption of dietary phytosterols correlates with a reduction in colon, breast, and prostate cancer risk. Phytosterols appear to interfere with tumor promotion and progression through influencing hormone-dependent growth of endocrine tumors, slowing of cell cycle progression, and boosting of immune recognition of cancer cells. For example, β-sitosterol aided in cell cycle arrest and prevented cell proliferation. However, even though some epidemiological studies have inferred that higher intakes of plant foods containing phytosterols were associated with a decreased risk of stomach and breast cancers, it remains to be elucidated whether phytosterols were the active components and whether these potential cancer-preventive activities can be replicated in humans.

Polyphenols

Polyphenols, which are found in abundance in spices, herbs, seeds, nuts, vegetables, berries, many beverages, and fruits, are the most abundant antioxidants in the human diet. Structurally, they are defined by the presence of large multiples of phenol rings, many of which contain various active groups, such as hydroxyl or meth(ox)yl groups. Much like the endogenous radical scavengers (e.g., glutathione), polyphenols donate one of their electrons to the ROS, leading to neutralization and efflux in a nontoxic form. In addition to being direct antioxidants, polyphenols have also been shown to be indirect antioxidants and to induce antioxidant enzymes, including glutathione peroxidase, catalase, and superoxide dismutase. Current experimental evidence strongly supports a contribution of polyphenols to the prevention of cancers, and polyphenols reverse epigenetic alterations and inflammation. However, because doses much higher than achievable in the human diet are usually utilized in these models, uncertainty persists regarding applicability to cancer prevention in humans. Within the structurally diverse polyphenols, molecules are grouped into classes primarily based on the number of phenol rings.

Flavonoids

Flavonoids are benzo-*c*-pyrone derivatives and thus share the common structure consisting of two aromatic benzene rings, which are separated by an oxygen-containing pyran ring. Based on the degree of oxidation of the pyran ring and their position of functional groups, the over 5000 naturally occurring flavonoids and their glucosides are divided into six subclasses: flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols. A seventh subclass, the proanthocyanidins, which consists of polymers of flavonol units, is often added.

Flavonoids are found in a variety of plants. In cell and animal models, many individual flavonoids have been shown to affect a variety of biological activities, many of which may interfere with carcinogenesis. Several epidemiological studies have supported evidence for an inverse relationship between flavonoid intake and cancer risk, but human studies remain difficult to interpret. This may be partially explained by the low oral bioavailability of many flavonoids and the significant inter-individual variability in metabolic enzyme expression and activity as a result of genetic and environmental factors.

Flavones and their 3-hydroxy derivatives (flavonols) comprise the largest subgroup among all polyphenols. Their many functional groups, including glycosides, methoxides, and other acylated products, can attach to their three rings. The most common flavonol aglycones are quercetin and kaempferol, and they both have an estimated 279 and 347 different glycosidic combinations, respectively. In human cancer cells, quercetin, kaempferol, and many methoxylated flavones have been shown to induce apoptosis and inhibit cell proliferation and/or angiogenesis. The flavones chrysin and galangin inhibit the activity of the enzyme aromatase, thus resulting in anti-estrogenic effects, important in hormone-related cancers. Luteolin, a flavonoid found in parsley, inhibited COX-2 and VEGF expression, thus preventing tumor progression and angiogenesis in preclinical models. However, a 2009 human prospective cohort study with over 38 000 women, which assessed intakes of quercetin, kaempferol, myricetin, apigenin, and luteolin with food frequency questionnaires, has not supported a major role of these flavonoids in cancer prevention.

Isoflavones are mostly found in the leguminous (beans) family of plants. Genistein and daidzin are almost exclusively found in soybeans, and high consumption of soy products is associated with decreased breast cancer risk in Asian populations. Isoflavones are able to bind to the mammalian estrogen receptors and thus are often referred to as phytoestrogens, even though human estrogen still has a 1000-fold higher effect. Similar to some flavones, genistein inhibits aromatase activity. Genistein can also aid in the induction of phase II enzymes, thus preventing cancer initiation. Whereas animal studies provided convincing evidence for a role of genistein and daidzin in cancer prevention, human studies have been unable to replicate these findings. This may be partially explained by the differential isoflavone metabolism in rodents and humans, but not enough human studies have been conducted to verify.

The polymers of proanthocyanidins are responsible for the astringent characteristic of many fruits and beverages. Anthocyanidins, primarily responsible for the red, blue, and purple pigments of most flowers, fruits, and vegetables, mainly exist in glycosidic forms, which are commonly referred to as anthocyanins. Even though anthocyanidins and anthocyanins have been effective in preclinical cancer prevention studies, human trials have not correlated intake of specific anthocyanidins and anthocyanins with decreased cancer incidence.

Lignans

Our intestinal microbiota metabolize plant-derived lignan precursors into enterolignans, enterodiol, and enterolactone. The latter two are classified as phytoestrogens because of their ability to mimic some of the effects of estrogens. Therefore, research on lignans in cancer prevention has primarily focussed on hormone-associated cancers, such as breast, endometrial, ovarian, and prostate cancers. Studies have reported conflicting results. Several prospective cohort studies found no association between lignan intake and breast or other cancers. In contrast, a 2009 meta-analysis limited to postmenopausal women showed a 15% reduction in risk of breast cancer with high lignan intake. In a 2013 data analysis of a multisite phase II randomized controlled clinical trial, 147 patients with prostate cancer who participated in a presurgical trial of flaxseed supplementation were investigated. Significant inverse correlations between total flaxseed-derived urinary lignan metabolites and the nuclear cellular proliferation marker, Ki67, in tumor tissue were observed. Furthermore, enterolignans hindered cancer cell proliferation via VEGF-associated pathways. Analyses of the EPIC study in 2014 showed significant inverse associations with lignans and bladder cancer risk. However, at present, it is not clear whether high intakes of plant lignans offered significant protective effects against any cancers or if other phytochemicals present in the diet were involved.

Stilbenes

Many stilbenes, including resveratrol, are found in red wine and other dark berries and possess anticancer activity in animal and *in vitro* models. Resveratrol has been shown to inhibit the proliferation of many different human cancer cell lines. Although absorbed by enterocytes, resveratrol has limited bioavailability and is quickly metabolized. Thus, many of the early studies investigating resveratrol in the diet failed to find any correlation to cancer risk. Recently, smaller human studies with very large daily doses of supplemented resveratrol have found inverse correlations to breast and colon cancer incidence, whereas resveratrol levels achieved with a normal Western diet have not been associated with cancer prevention.

Other Important Polyphenols

Curcumin, a strong antioxidant from turmeric, prevented weight gain (obesity) and cancer in animal models. The molecular mechanism in cancer prevention remains to be elucidated but likely involves induction of apoptosis in cancer cells and inhibition of cell proliferation through upregulation of the tumor suppressor P53. Because of the poor intestinal absorption, new delivery strategies are being developed to improve curcumin's bioavailability.

Ellagic acid and its derivatives, which are found in berries and nuts, promoted apoptosis and cell cycle arrest in bladder, breast, cervical, pancreatic, and prostate cancer cell lines, as well as those of the aerodigestive tract. This polyphenol also exerts antiangiogenesis effects in breast cancer cells. Ellagic acid appears to accumulate in epithelial cells and has been shown to irreversibly bind to cellular DNA and protein. In animal models, ellagic acid was shown to inhibit the incidence of chemically induced small intestinal adenocarcinomas and to reduce intestinal inflammation. While ellagic acid has been suggested as support in chemotherapy-induced toxicity, clinical trials with the single compound have not been conducted thus far.

Rosmarinic acid, which is a dimer of caffeic acid, showed anti-inflammatory, antioxidant, and antimutagenic characteristics. It inhibited angiogenesis through inhibition of VEGF and interleukin-8 *in vitro* and significantly reduced the levels of ROS in endothelial cells. Whereas studies with rosmarinic acid have been successful for allergy studies, the effect of this compound in cancer prevention in humans remains to be investigated.

Capsaicinoids, polyphenolic amides found in chili peppers, induced apoptosis and inhibited CYP 3A4 and 3A5 in cell and animal models. Information regarding the metabolism and bioavailability of capsaicin in different tissues and organs and its potential cancer-preventive effects in humans is limited. A prospective study investigating capsaicin as a chemopreventive agent for prostate cancer is currently planned.

Future Directions

There is little doubt that diet influences carcinogenesis and can be employed as a cancer-preventive strategy. Investigations into individual genotypes of nutrient- and carcinogen-metabolizing genes will become essential in order to understand and measure outcomes. The complex gene–nutrient interactions will need to be further investigated before individuals or diseases can be identified that will benefit from intervention with specific phytochemicals in cancer prevention. Still, a pill will unlikely be able to offset an unhealthy lifestyle or replace the established benefits of a healthful lifestyle with a primarily plant-based diet.

See also: Aflatoxin: A Global Public Health Problem; Alcohol: Metabolism and Health Effects; Glucosinolates from the Brassica Vegetables and Their Health Effects; Selenium: Properties and Determination; Trace Minerals and Trace Elements.

Further Reading

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Candies and Sweets: Sugar and Chocolate Confectionery

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Candies and Sweets: Sugar and Chocolate Confectionery

Candies and sweets, collectively known as confections, are defined as foods whose main characteristic is sweetness. It is typically understood that a candy is a rather small, defined food item. Confectionery products are divided into two categories:

Sugar confectionery: Sugar is the main ingredient.
Chocolate confectionery: Chocolate is the characterizing ingredient, either as entirely made up of chocolate or as a coating or inclusion.

Patterns of Consumption

Globally, sugar confectionery accounts for about 39% of candy consumption and chocolate confectionery about 61%. This ratio can vary widely among countries. Confectionery consumption is increasing in countries with a growing middle class, such as Brazil and India, and in countries with traditionally low sugar consumption, such as China and Japan. As populations become more prosperous, chocolate consumption tends to increase. In more developed countries, confectionery consumption shows little growth from year to year and has declined in some nations.

Table 1 shows candy consumption for a few countries on a yearly and daily basis. Northern and Western European countries are the highest consumers of chocolate confectionery in the world.

In 2013, the global confectionery market was estimated to be worth $171 billion, with chocolate representing $110 billion. US candy sales for the same period were $33.9 billion.

In Asia and Latin America, sugar confectionery tends to predominate. In parts of Asia, less sweet confections are preferred. In Japan, candy must be aesthetically pleasing. Kit Kat bars are wildly popular in Japan, and there is a tradition of constantly introducing new and unusual flavors, such as wasabi, green tea, soy sauce, miso, and sweet potato. Throughout Asia, gummy candies with fruit flavors are preferred.

The Nordic countries are among the top global consumers of confectionery. Sweden has a high consumption of sugar confectionery and Switzerland has the highest chocolate consumption.

In a number of countries (India, China, and Mexico), candies are thought of as mainly for gift-giving occasions or for children. With cultural changes caused by globalization, candy consumption begins to be considered for everyday snacking. Chocolate was traditionally considered an expensive luxury, but with smaller sizes and lower prices, consumption has increased rapidly in Asia and Latin America.

Large spikes in confectionery consumption occur during Easter, Halloween, and Valentine's Day, when confectionary is traditionally gifted. Each holiday has its own set of traditional treats.

Ingredients

Sugar and Other Nutritive Sweeteners

The single most important ingredient in confectionery is sugar. Along with sweetness, sugar provides structure, bulk, and preservative properties in candies. Sugar confectionery is made from a mixture of sucrose and glucose syrup in various proportions. Chocolate production uses only sucrose. Molasses, brown sugar, and honey are used for specific flavor effects in various confections. Demerara sugar is a special type of golden-brown sugar made from cane juice used in some high-cocoa-content chocolate bars.

Sugar, chemically known as sucrose, is a disaccharide made up of one molecule of glucose and one of fructose. Unlike most other monosaccharides and disaccharides, sucrose is a non-reducing sugar, which confers a high degree of stability, so it can be cooked to a high temperature without breaking down or undergoing browning reactions. Under temperate storage conditions, sucrose does not pick up moisture and can remain stable for years. Sucrose sets the standard for sweetness and functionality in candy making, and all other sweeteners are compared with it.

The two commercial sources of sucrose are cane sugar and beet sugar, with cane sugar representing 78–80% of world sugar production. Production methods differ between cane sugar and beet sugar, but the sucrose from each is chemically identical.

Sugar refineries produce several types of sugars:

- Granulated white sugars, with a range of defined crystal sizes obtained by screening
- Confectioners' sugars of various particle sizes and cornstarch content
- Brown sugars of various grades – light, medium, dark, agglomerated, and liquid
- Liquid sugars of various concentrations of dissolved sucrose that may contain invert sugar
- Specialty sugars, which have added ingredients or may be colored

Within these categories is a wide range of products. Since there are no standard definitions, the names companies use for the same product can be different. Screened sugars have been sieved to give specific crystal size ranges. The smaller crystals dissolve more readily and are preferred for confectionery. Coarser crystals, called sanding sugar or decorating sugar, can be used for dusting the surface of jellies and other confections to give surface sparkle and a mild crunch. Sugar used in confectionery must be highly refined and of the highest quality.

Confectioners' sugar is produced by pulverizing white sugar and then screening it to 75 or 45 μm size. To prevent caking, 3% cornstarch is added. Some is also available without cornstarch. Confectioners' sugar is recommended for fondant production.

Table 1 Sugar and chocolate confectionery consumption per capita per year and per capita per day

	kg per capita per year			*g per capita per day*		
Country	*Sugar*	*Chocolate*	*Total*	*Sugar*	*Chocolate*	*Total*
Japan	1.73	2.23	3.96	4.74	6.11	10.85
Brazil	1.83	2.16	3.99	5.01	5.92	10.93
France	3.50	6.50	10.00	9.59	17.81	27.40
The United Kingdom	5.26	10.29	15.55	14.41	28.19	42.60
Germany	5.95	11.60	17.55	16.27	31.78	48.05
The United States	6.15	5.46	11.61	16.85	14.96	31.81
Denmark	8.64	7.65	16.29	23.67	20.96	44.63

Invert sugar is a mixture of glucose and fructose produced by acid or enzymatic hydrolysis of liquid sucrose.

Glucose syrup is the other essential ingredient in sugar confectionery production. Its most important function is to control or prevent crystallization in hard candy production; it also provides humectancy, helps maintain texture, and stabilizes the product. Glucose syrups are about 40–50% as sweet as sucrose.

Glucose syrups are produced from cornstarch (the United States) or wheat starch (Europe) by acid or enzymatic hydrolysis, which produces different grades. Standard glucose syrup, also called confectionery syrup, is 42 DE syrup. DE stands for 'dextrose equivalent' and is a measure of the degree of hydrolysis and the amount of reducing sugar present. The two types of 42 DE syrup used in confectionery are glucose syrup and high-maltose syrup.

Labeling sugars

On labels in the United States, the quantity, in grams, of all carbohydrates is listed and then broken out as dietary fiber and 'sugars.' Sugars include all nutritive sweeteners, including corn syrups, sugar, lactose, and brown sugar. The different types of sweeteners are listed separately in the ingredients list.

Sugar-Free Candies: Sugar Alcohols

Candies labeled 'sugar-free' contain sugar alcohols or artificial sweeteners or a combination of both. Sugar alcohols, also known as polyols, are carbohydrates, but they are not sugars. Sugar alcohols include erythritol, xylitol, glycerol (glycerin), hydrogenated starch hydrolysates (HSH; polyglycitol), isomalt (isomaltitol), lactitol, maltitol, mannitol, and sorbitol. Xylitol is about as sweet as sucrose and maltitol about 90% as sweet. The other sugar alcohols are 40–60% as sweet as sucrose. Some sugar alcohols – xylitol, erythritol, and mannitol – have a pronounced cooling effect, similar to mint. Isomalt and maltitol have a less cooling effect. Sugar alcohols are noncariogenic (do not promote tooth decay). They contain 2–3 calories per gram. Erythritol has only 0.2 cal g^{-1}. They do not raise blood glucose, so they are suitable for diabetics. However, they have a laxative effect if consumed in excess.

Other Ingredients

Acidulants provide the tart flavors in sour candies, enhance fruit flavors, and provide preservative properties. Common acidulants include citric acid, potassium citrate, malic acid, fumaric acid, lactic acid, calcium lactate, sodium citrate, tartaric acid, and ascorbic acid. Different acids can be blended to obtain a desired level of sourness and taste duration.

Confectionery fats contribute to the tenderness, structure, and texture in candy. Cocoa butter, the fat in chocolate, is considered the 'gold standard' of confectionery fats. Its sharp melting curve, close to body temperature, allows it to melt in the mouth, and the proper alignment of its fat crystals provides the desirable snap of chocolate when it is broken. Because of its expense, cocoa butter is not used in other types of confectionery; other fats are used instead, including butter and vegetable fats, the most important of which are coconut oil and palm kernel oil, usually in a partially hydrogenated form, designed to have similar properties to cocoa butter. Other fats include partially hydrogenated soybean and cottonseed oils and fully hydrogenated palm kernel oil and coconut oil. The FDA has recently proposed revoking the GRAS (generally recognized as safe) status of partially hydrogenated oils, which presents a challenge to confectioners to find alternatives that provide the same functions in candies. Many hard candies and jelly/gummy candies do not contain any fat.

Dairy: A wide range of dairy products are used in confectionery – fluid whole milk, skim milk, cream, evaporated milk, sweetened condensed milk, evaporated milk, milk protein concentrates, butter, anhydrous milk fat, whey, a range of milk powders, and lactose (milk sugar). Milk is a significant ingredient in milk chocolate, fudge, caramel, toffee, and pralines. Milk used in chocolate must be in dry form, as water will prevent chocolate from flowing properly and ruins its texture.

Emulsifiers have many important functions in confectionery: viscosity reduction, lubrication, control of sugar crystallization, aid in dispersion of ingredients, stabilization of structure, and prevention of sticking. The most widely used emulsifier is lecithin, derived from soy. Commercial lecithin is a mixture of phosphatides and sterols. Other emulsifiers include polyglycerol polyricinoleate, sorbitan esters, polysorbates, mono- and diglycerides of lactic and tartaric acids, sucrose esters, and propylene glycol monoesters. In chocolate confectionery, emulsifiers prevent the separation of cocoa butter from cocoa solids and slow the development of bloom.

Flavors: Sometimes, an ingredient's flavor profile is very complex, with many different chemicals contributing to the odor, such as coffee and chocolate flavors. Sometimes, one chemical characterizes a flavor, for example, benzaldehyde in almonds, eugenol in cloves, and vanillin in vanilla. If only the

characterizing chemical is used, as in less expensive candies, the flavor profile may be one-dimensional; other flavor compounds present, for example, in vanilla extract, round out and add complexity to the flavor.

Some flavors are developed during cooking or roasting. Sugar heated with proteins or bicarbonate will produce an array of pleasant flavors that are described as 'browned,' brown sugar, or caramelized, in a reaction known as the Maillard reaction.

Dairy ingredients provide milky, creamy, and buttery flavors; heating produces cooked cream, browned butter, and butterscotch flavors.

The complex flavor of chocolate is developed through a series of processes, from fermentation of the cocoa beans to roasting the nibs and conching.

Flavors used in confectionery can be artificial or natural or a combination of both and must be able to withstand the candy making process. The FDA maintains a list of flavor compounds that are GRAS. Over 3000 compounds have been listed to date.

Food coloring: The most common colorants used in confectionery are artificial colors that are either oil- or water-soluble. There is a growing trend toward using natural colorants, but these are more expensive and tend to be heat-sensitive and fade.

Fruits are used as inclusions in many candies, the most popular being raisins. Other fruit products used in confectionery include dried fruits, pastes, juice, and juice concentrates.

Glazes, coatings, and polishing agents; *antistick and release agents*: Coatings and glazes improve the appearance and stability of candies, providing gloss, increased shelf life, adhesion of sugar crystals to encapsulate acidulants on the surface of jelly candies, fat barrier, moisture resistance, and antistick properties. The common confectionery glazes are carnauba wax, shellac, beeswax, and gum arabic. Shellac is extracted from the *Laccifer lacca* insect. Carnauba wax is extracted from the leaves of a Brazilian tree, *Copernicia prunifera*. Beeswax is secreted by bees. Gum arabic is used to give chocolates a brilliant shine. A less common coating is made from a corn protein, known as zein, which provides a vegetable-based glaze that competes with shellac.

Gelling agents provide body, texture, and structure to chewy, jelly-type candies. The gelling agents used in candy making are gelatin, pectin, modified starches, and egg albumin (egg whites). Gelatin, derived from cows or pigs, is widely used in gummy candies, fruit jellies, and gumdrops. Egg whites produce a structured foam and are used as a gelling or whipping agent to provide a characteristic flavor and an airy, open texture in marshmallows, nougat, and divinity. Pectin and modified starches are used as vegetable substitutes for gelatin and egg whites. Each gelling agent confers different properties to a candy, and they are often used in combination to achieve desired effects.

Nuts, seeds, and coconut: The tree nuts used in candy making are almonds, pecans, walnuts, hazelnuts, and pistachios, available whole, broken, and finely chopped or as meal, flour, butter, or paste. Peanuts and peanut butter are among the most popular candy ingredients. While peanuts function like tree nuts, they are a legume. Nuts are roasted or pasteurized before use in confectionery for both flavor development and microbiological safety. Seeds used in confectionery include sesame and sunflower. Spice seeds such as fennel, caraway, and anise are used sparingly for flavor. Halva, an ancient confection popular in the Middle East, is made from sesame seed paste. Marzipan is made from almond paste.

Botanically, coconuts (*Cocos nucifera*) are classified as a fruit. The coconut is made up of a fibrous outer husk and a hard inner shell. When immature, coconut water fills the shell; as the coconut matures, the coconut kernel or 'meat' is formed as a pure white layer on the inner surface of the shell. In mature coconuts, the water is absorbed and the meat is about one-half inch thick. This meat is the source of coconut oil. The meat (full fat or partially defatted) is dried and shredded or powdered for use in candies. Coconut candy is very popular in Latin America, where it is known as *cocada*.

Preservatives are used to prevent rancidity in fats and oils. The most common preservative is tert-butylhydroquinone. Safe usage has been set to an upper limit of 0.02% of the fat and oil contents by the FDA and the European Food Safety Authority. Another common preservative is citric acid. Not all commercial confectionery products contain preservatives.

Salt is used to enhance sweet flavor and to round out and blend complex flavors. Sodium levels are required on labels and are in the range of 10–45 mg per serving (equivalent to 25–114 mg of sodium chloride). Salt flavor in confectionery is popular in chocolate and caramel. Salt sprinkled on the surface of chocolate and caramel confections enhances flavor and provides a subtle crunch. Sea salt is often used because it adds a certain cachet to the confection and is perceived to have a different taste than regular salt.

Asian ingredients not found in Western-style confectionery are red bean paste, glutinous rice flour, and rice malt syrup. Red bean paste is made from cooked, mashed, and sweetened adzuki beans. Rice malt is considered to be healthy.

Methods of Production

The production of candy depends on boiling sugar and corn syrup in various proportions in water or milk to specific temperatures, sugar concentrations, and moisture contents and controlling the crystallization of the sugar. Within these constraints, a wide range of confections are produced. The two general categories of sugar confectionery include soft-boiled and hard-boiled (hard candy) production. Soft-boiled confections have a higher moisture content and a creamy texture. Hard candies can be hard and dense, brittle, or crispy and crunchy. The process of crystallizing sugar in soft-boiled confections, such as fudge and pralines, is known as graining. Sugar cooked and solidified to the point where crystals are no longer present, as in brittles and hard candy, is considered to be in a glassy state. Water content in sugar confectionery is typically low, ranging from 1.5% to 6.5%. Hard candies have the lowest moisture content. Candies with higher water content include jellies, marshmallows, fondants, and creams.

In both commercial and home cooking, a series of cooking stages are used as a shorthand way to determine when the sugar mixture has reached the proper temperature and sugar concentration to produce the desired texture. For home cooking, a small dollop of the cooked syrup is dropped into cool water, and the form the quickly cooled syrup takes is an indicator of

Table 2 Temperature ranges for boiling sugar confections

Type of confection	*Cooking stage*	*Degrees C*	*Degrees F*	*Sugar conc %*
Syrup	Thread	101–112	215–233	80
Fudge, pralines, fondant	Soft ball	112–116	234–240	85
Caramels	Firm ball	117–120	242–248	87
Nougat, gummies, divinity, marshmallows	Hard ball	121–131	250–268	92
Taffy, butterscotch	Soft crack	132–143	270–290	95
Lollipops, toffee, brittles, hard candy	Hard crack	149–154	300–310	99

the cooking stage. It is both a visual and tactile test. Table 2 shows the various cooking stages for different types of candies.

Fondant and Frappé

Fondants and frappés form the base of many confectionery recipes. Fondant is prepared from a mixture of sucrose and corn syrup concentrated with cooking to about 86–90% solids and 10–14% moisture and worked until smooth and pliable. It is a soft to firm white mass consisting of microscopic sugar crystals ($\leq$20 μm) dispersed in a saturated sugar solution. The corn syrup helps to keep the fondant hydrated and prevents the crystals from growing larger. Fondant is a confection in its own right such as filling in chocolate candies with or without added flavors and colors, as well as an ingredient in other confections, to add grain to caramels, fudge, and nougats and to make creams.

Frappé is a mixture of egg whites or dry egg albumin and sugar whipped into boiled corn syrup. Frappé is an aerating ingredient added to fondants and creams to lighten their texture and to make nougats. Nougat can be hard or soft. Soft nougat is a common filling in many popular candy bars, such as Three Musketeers, Baby Ruth, and Milky Way.

Panning

Panning is the process in which a confectionery center is coated with a sugar or chocolate shell. The centers are placed in a round, tilted pan, called a dragée pan, with an opening for adding ingredients and air for drying. The pan is filled with syrup and rotated until the centers are evenly coated and the syrup dries. The process can be repeated several times depending on the nature of the desired shell. Sugar is added to aid drying. Soft panning refers to shells that are soft, such as for jelly beans. Hard panning refers to shells that are hard; M&M's and Jordan Almonds are examples. Examples of chocolate panning include malted milk balls, nuts, and raisins.

Another process for coating confectionery with chocolate is called enrobing, which is akin to dipping the center into molten chocolate to cover it. Today, enrobing machines carry out the process.

Aeration of Hard Candies

Aeration is the process of injecting air into a hard-boiled candy mass. It is an important process for lightening the texture of hard candies and providing crispiness. Aeration is done in one of four ways.

Pulling is the most common method. The candy mass is repeatedly stretched and folded to incorporate air bubbles, producing a silky, fibrous appearance. Taffy is an example. Adding bicarbonate releases carbon dioxide, which puffs up the mass, resulting in a porous, low-density, crispy candy. Vacuum expansion produces a low-density honeycomb structure. In the vacuum process, pulled candy pieces are placed under vacuum causing the incorporated air bubbles to expand rapidly. Lastly, there is continuous cooking with air injection, which produces a consistent product.

Production of Chocolate and Chocolate Confectionery

Chocolate production is a complex and lengthy process. After harvesting ripe cocoa pods, the beans and surrounding pulp are removed and fermented, during which time the beans turn brown, the pulp disappears, and flavor precursors develop. The beans are dried, often in the sun but sometimes in kilns, and transported to the factory where chocolate is produced. The dry beans are cleaned, roasted, and winnowed. Roasting continues the development of the chocolate flavor. Winnowing causes the roasted shells to crack, and the nib (kernel) inside is removed. The nibs are ground and liquefied to produce chocolate liquor, which is composed of cocoa solids and cocoa butter.

Cocoa solids and cocoa butter are separated and blended in the required proportions with sugar and milk powder (for milk chocolate). The chocolate mixture is put into a machine called a conche. During the conching process, the chocolate mass is continually ground and scraped from the sides to create a smooth consistent texture and to reduce particles to a size that can no longer be sensed on the tongue. Conching produces frictional heat that helps to develop, blend, and mellow the chocolate flavor.

After conching, chocolate is tempered by repeated, controlled heating and cooling cycles to produce the desired crystal structure of the cocoa butter fat. The fat molecules in cocoa butter can crystallize into six different polymorphic crystal forms: I, II, III, IV, V, and VI. Each form has a different melting point, stability, gloss, and hardness. Only one form, known as V crystal, gives the desired texture, shiny appearance, melt in the mouth sensation, smoothness, snap when broken, and keeping qualities. When cocoa butter is allowed to cool naturally, it produces a mixture of crystal forms and lacks the desired qualities. Proper tempering allows the melted chocolate to cool very slowly, producing the highest proportion of V crystal. Crystal forms I–IV have lower melting points, so a continued process of melting these without melting V crystals, with subsequent slow cooling, eventually produces a majority

of V crystal. Crystal form VI has an even higher melting point; it forms from V crystal after several months of storage at room temperature, which then causes a drop-off in quality and the phenomenon known as fat bloom.

There are many types of chocolate ranging from dark, bittersweet, semisweet, and milk. The United States and other countries have standards of identity for chocolate types. The typical milk chocolate bar contains 25–30% cocoa, while a sweet dark chocolate bar contains 30–40%. In recent years, chocolate bars with up to 85% cocoa content have come on the market due to perceived healthy qualities of dark chocolate.

Ganache is a mixture of heavy cream and semisweet chocolate used as the basis of the truffle center. Couverture chocolate is a very high-quality chocolate with added cocoa butter that is used by professionals for dipping, coating, and molding.

Compound coating or confectionery coating (compound chocolate) is made from a combination of cocoa, vegetable fat, and sweeteners and is used to enrobe some candy bars. Since it contains no cocoa butter, it does not need to be tempered. It is a lower-cost alternative to chocolate coatings. According to FDA regulations, if a product contains no cocoa butter, it cannot be called 'chocolate' on the label but may be referred to as 'chocolate-flavored.'

White chocolate contains no chocolate solids other than cocoa butter. According to the FDA, it must contain at least 20% cocoa butter, at least 14% total milk solids, and no more than 55% nutritive carbohydrate sweeteners. White confectionery chips that are sometimes erroneously referred to as 'white chocolate' have substituted vegetable fats for cocoa butter and are considerably less expensive.

Shelf Life of Confectionery

Shelf life refers to the amount of time a food product will retain its quality prior to consumption. 'Best before' dates on labels are intended to inform the consumer of the shelf life of a product. This label does not indicate that the candy is no longer safe to eat after that date, just that the quality may not be up to standard. Candy quality can deteriorate in several ways. The sugar in a hard-grained confection, such as toffee, can crystallize, making it grainy. Moisture can migrate out of a candy to the surface, making it sticky. In layered candies, there can be bleed-through from one layer to the other, affecting texture, taste, and color.

Chocolate can develop white spots on the surface, known as bloom or fat bloom, which looks like mold but is actually an indication that the cocoa butter crystals are converting to less stable forms and leaching onto the surface of the chocolate. It could mean that the chocolate was not properly tempered, but this also happens when chocolate is stored for a long time. Formation of bloom is speeded up if the chocolate is subjected to fluctuating temperatures. The texture of the chocolate can be adversely affected, becoming dry and crumbly. These are all quality issues; the candy is still safe to eat.

Health Effects

The main health concerns about consuming confectionery are contributing too much added sugar and calories to the diet and the risk of dental caries. Consuming a small amount of candy in a well-balanced diet is acceptable. However, diabetics must watch their sugar intake. For some individuals, there are concerns about allergens and gluten content. Sugar is known to lead to dental caries/cavities when dental hygiene is not optimal.

Candy manufacturers have responded to concerns about obesity by decreasing serving sizes and developing smaller bars and packages, often called 'fun size' or 'snack size.' Miniaturized 'bite size' candy bars are also coming to market. The National Confectioners Association recommends a daily consumption of candy not exceeding 50–100 calories a day. The data in Table 1 show that Americans' average daily consumption is less than 32 g, but this amount exceeds 100 calories.

In attempts to have a more favorable label (known as a 'clean label') and a healthier product, confectioners are exploring ways to reduce or substitute fat, salt, and sugar. In 2012, Nestlé announced it would remove all artificial colors, flavors, and preservatives in all their confectionery products sold in the United Kingdom.

Functional Confectionery and Healthy Ingredients in Nuts and Chocolate

A functional ingredient is one that confers a health benefit when added to a food. Examples include vitamins, probiotics, omega-3, resveratrol, taurine (for energy), and fiber. A small but growing confectionery niche has added functional ingredients, sometimes blurring the boundary between food and food supplements. Consumers are eager to find foods that will solve their health problems.

Nuts and chocolate have been shown to possess numerous healthy constituents. A growing body of research shows that chocolate has many benefits for cardiovascular and cognitive health, due to the presence of antioxidant compounds, such as flavanols, polyphenols, and proanthocyanidins. While polyphenols constitute 12–18% of the dry weight of whole cocoa beans, it has been shown that the various chocolate processing states, such as fermentation, roasting, and alkalization, contribute to some loss of these compounds. Research is still needed on which specific ingredients are the most important and what the effective dose is to achieve a benefit. Nuts are rich in antioxidants and good fats.

Health Effects of Selected Ingredients

Allergens. According to the FDA, major food allergens include milk, eggs, tree nuts, wheat, peanuts, and soybeans, all common ingredients in candies. Allergens must be listed on the label.

Artificial dyes. A recent study from Purdue University found that some candies had 29–33 mg of artificial dyes, which is of concern because levels in this range can affect behavior in a small percentage of children.

Black licorice. Glycyrrhizin, the sweet agent in licorice, is alleged to have many health benefits but is also known to cause high blood pressure and arrhythmia if consumed in large quantities and can cause potassium levels to fall. The FDA has warned against heavy consumption of black licorice but has not set a daily limit and lists it as GRAS, with usage

Figure 1 Sugar confectionery in Seville, Spain.

amounts for certain foods: up to 16% glycyrrhizin in hard candy and 3.1% in soft candy.

Caffeine. Caffeine, as much as 50 mg, is added to a few candies, such as some jelly beans, marshmallows, and chocolate bars, for its energy-imparting qualities. In May 2013, the FDA announced it will investigate the safety of caffeine being added to food products, especially its effect on children.

Gluten. Gluten will be present in a candy if the starch used as a gelling agent comes from wheat. Candies with barley malt and any confectionery with wafers or cookies included will also contain gluten. In August 2014, the FDA issued a final rule to define the term 'gluten-free' for voluntary use in the labeling of foods. For a food to be labeled gluten-free, it must contain 20 ppm of gluten or less. The Hershey Company provides a list of all their gluten-free confectionery on their website.

Zein. Zein, a prolamin protein derived from corn gluten meal, is gluten-free and safe for those with celiac disease or gluten intolerance. The term 'corn gluten' causes confusion, and many people think that zein is a gluten protein. There is no gluten in corn, and, according to Wikipedia, the term arose colloquially. Zein is preferred by vegans and others who object to the use of shellac, an insect product, in confectionery glazes, but the consumer has little knowledge or control over this.

Kidney-friendly candy. People with chronic kidney disease or on dialysis need to restrict their intake of phosphorus, potassium, and salt, which are found in some confections. Phosphorous and potassium are not required on labels. The DaVita company has a list of permissible candies on their website.

Metallic dragées. Dragées are small hard spheres of sugar, about 4 mm diameter, coated with a colored or metallic glaze, used for decorating cakes and cookies. There is concern about the metallic content, especially of the silver ones, and although claimed to be safe by the manufacturers, the state of California banned them in 2003. The FDA declares that dragées are nonedible and require jars to carry labels saying 'for decoration only.'

Salt. Most commercial candies contain salt, and the amount of sodium per serving is required on the label. Confectionery sodium levels are in the range of 10–45 mg per serving, with some confections as high as 100–145 per serving. This amount of sodium constitutes from 0% to 6% of the % daily value. Not all sodium in commercial candies comes from sodium chloride (table salt). Other sources of sodium include sodium salts of acidulants.

Sugar alcohols. Sugar alcohols can cause a laxative effect if too much is consumed, and foods are required to have a warning label to that effect. However, excessive consumption of sugar alcohols can cause a laxative effect, and the maximum safe daily amounts are in the range of 20–50 g depending on the sugar alcohol. The FDA mandates that foods with 20 g or more of mannitol and 50 g or more of sorbitol must carry the warning label 'Excess consumption may have a laxative effect.'

Vegan considerations. Many candies are not vegan because of the presence of milk, egg, or gelatin. Confectioner's glaze made from shellac is an animal product from insects. Beeswax may also be considered nonvegan.

Perspective on Confectionery in Food and Health

By mixing sugar with a few other ingredients, an almost infinite variety of candies and confections can be created. They are colorful, pretty, and tasty. Candy and confections are small packets of food, meant to provide pleasure and to be eaten in moderation. Behavioral studies suggest that pleasurable foods can help to achieve and sustain a healthy diet. Chocolate, in particular, is associated with producing a sense of well-being, which is attributed to the many natural compounds it possesses. However, even though many ingredients in candies are healthy or have healthy ingredients, the amount present in a piece of candy or chocolate is far too small to have an effect, and moderate consumption is key to maintaining a healthy diet (**Figure 1**).

See also: Aerated Foods; Antioxidants: Role on Health and Prevention; Caramel: Properties and Analysis; Cocoa: Composition and Health Effects; Cocoa: Production, Chemistry, and Use; Food Allergies:

Occurrence and Analysis; Functional Foods; Glucose: Properties and Analysis; Maillard Reaction; Nuts: Health Effects; Phenolic Compounds: Bioavailability and Health Effects; Sugar Alcohols.

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Canning: Process of Canning

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Introduction

Canning process was introduced about two centuries ago, and for long time, it has been one of the main means of food preservation, together with chilling and freezing. The food canning history began in the late eighteenth century in France when Nicholas Appert discovered that the application of heat to food in a sealed glass container prevented food spoilage. Later, Peter Durand developed a method of sealing food in tin containers; this idea was improved by Bryan Dorkin and John Hall who installed the first commercial cannery in England. Some years later, L. Pasteur gave a reasonable explanation for canning's effectiveness when he demonstrated that microorganisms were responsible of food spoilage. Gradually, the production of canned foods became mechanized and the developments associated to food canning continue today.

Conventional canning is a method of food preservation in which a food is placed in hermetically sealed containers and heated to destroy microorganisms. The main objective of heat application is to destroy pathogenic and spoilage microorganisms, and at the same time, the hermetic container prevents contamination by new microorganisms. Although the use of metal containers is common, there are other alternatives as glass jars, plastic cans, and retort pouches. The level of heat applied to a food depends on several factors: acidity of processed food, density, composition, resistance to heat transfer of food, heat resistance of microorganisms of interest in food, initial load of microorganisms, container, heating medium, equipment, processes, etc. After heating, canned foods are cooled and then handled at room temperature maintaining container integrity and preventing recontamination of product.

Some Concepts Related to Canning

In order to evaluate the suitable amount of heat for the canning process, several concepts must be studied. Among these concepts, the following are considered:

pH

pH is one of the most relevant factors when designing canned foods; the pH of the food plays a key role in determining the extent of heat processing needed to insure a safe and stable final product. The pH value of a food represents the molar concentration of hydrogen ions (in other words, the acidity level of a product); this concentration decreases as the pH value of the food increases. So a low pH value means a higher concentration of hydrogen ions. The range of pH values is between zero and 14. A pH of 7 is neutral (corresponds to pure water), while values less than 7 are acidic and those greater than 7 are basic or alkaline.

In the canning industry, a low-acid food is defined as a food having a pH higher than 4.6, while an acid food is defined as a food with a pH value equal or lower than 4.6. This simple classification of foods considers a reference pH value of 4.6. This value is based on *Clostridium botulinum* spores. *Clostridium botulinum* spores are very resistant to several conditions, including heating; if these spores survive, they have a chance to germinate and grow. However, *Clostridium botulinum* spores will not grow if the pH of a food is 4.6 or less. These spores in low-acid foods must be killed by heating during the canning process, and low-acid foods must receive a severe thermal process, so they are pressure-cooked at high temperatures (around 120 °C) during long periods of time. Examples of low-acid foods include fish, meat, poultry, and most vegetables and their products. On the other hand, acid foods are processed with less heating because *Clostridium botulinum* spores are unable to germinate and grow at pH 4.6 or less. In canning processes for this kind of foods, no pressure cooking is required and pasteurization temperatures are used (temperatures under boiling point of water). Pasteurization temperatures are suitable to inhibit most microorganisms, including vegetative cells of *Clostridium botulinum*. Bacterial spores can survive these processes; however, these spores will not grow at low pH, and the food will remain stable at room temperature (commercially sterile). Examples of acid foods include most fruits and their products.

In addition to low-acid and acid foods, another category of foods is used: acidified foods. Acidified foods are containing a significant amount (over 10%) of naturally low-acid ingredients. The pH of the low-acid ingredients is lowered by the addition of acid in the formulated food. This acid may be added directly or by the use of naturally acid ingredients. No matter how the acidification is achieved, all the low-acid components must take up enough acid to drop their pH below 4.6 within 24 h. Acidified foods are thermal processed at moderate temperatures, similar to those of acid foods.

Commercial Sterility and Microorganisms

The condition of commercial sterility is reached when a product that has been processed will neither represent a danger to consumer's health nor spoil. In the case of conventionally canned low-acid foods, the processes are designed to destroy spores of *Clostridium botulinum* and reduce chances of survival of spores of spoilage microorganism. Bacterial spores are more resistant to heat than vegetative cells. The concept of commercial sterility implies to render foods free of microorganisms capable of growing in the product at room temperature at which the finished product is intended to be held during storage, distribution, and trading. The thermal destruction of microorganisms generally obeys a first-order reaction kinetics. That is, the destruction rate of microorganisms is dependent on the concentration of microorganisms. Mathematically, the first-order kinetics of microbial destruction can be expressed as follows:

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$$-\frac{dN}{dt} = kN \quad [1]$$

where $-\frac{dN}{dt}$ is the rate at which microbial concentration decreases, N is the microbial concentration, and k is the first-order reaction rate constant.

Integrating eqn [1] between limits, N_0 at $t_0 = 0$, and N at time t results

$$-\int_{N_0}^{N} \frac{dN}{N} = k\int_0^t dt \quad [2]$$

$$-\ln N + \ln N_0 = k(t-0) \quad [3]$$

$$\log N = \log N_0 - \frac{kt}{2.303}. \quad [4]$$

The expression of eqn [4] describes a straight line with slope $-\frac{k}{2.303}$ and y-intercept $\log N_0$. Also, D-value is defined as $D = \frac{2.303}{k}$. The D-value is the time to destroy 90% of a microbial population when it is heated at a lethal temperature. D-value is a measure of microbial thermal resistance; it is specific for each microorganism under given conditions. At a given temperature, greater D-values are related to higher thermal resistance of a microorganism.

On the other hand, the minimum sterilization level for *Clostridium botulinum* during canning process when the concept of commercial sterility is used is expressed as 12 log cycles or decimal reductions (12-D). It means that if hypothetically a container would have a microbial load of 1×10^{12} cells of *Clostridium botulinum*, after this thermal process, only one cell would survive. If a particular food cannot support the growth of *Clostridium botulinum*, another microorganism of interest can be used to monitor the condition of commercial sterility.

Clostridium botulinum has been chosen as the reference microorganism in the canning industry because it has several useful characteristics that make it suitable for this task. It is a rod-shaped anaerobic bacterium that forms very resistant spores; it can grow in foods with a pH value greater than 4.6. This microorganism is responsible for botulism, a mortal disease caused by the ingestion of a neurotoxin generated by the vegetative form of *Clostridium botulinum*. This neurotoxin is one of the most potent poisons in nature. The vegetative form of *C. botulinum* is not resistant; however, its spore is highly resistant to heating. These spores can be found in several raw foods; under definite conditions, they can germinate, grow, and generate the toxin. Among the suitable conditions to germinate the spore, pH above 4.6, anaerobic environment, and room temperature are found. Potentially, these conditions can be found in low-acid canned foods. So destruction by heating is required in these products.

Although other microorganism may be present at raw materials used to prepare canned foods, they are less important than *C. botulinum* because they show less resistance to heat or they do not endanger the consumer's health.

On the other hand, *Clostridium sporogenes* (PA 3679) is significantly more resistant to heat than *C. botulinum*; it is a nontoxic obligate anaerobic sporeformer mesophilic microorganism, and it is used to determine safe thermal processes for low-acid foods. For acid foods, thermal processes are based on facultative anaerobes, such as *Bacillus coagulans*, *Bacillus macerans*, and *Bacillus polymyxa*; these microorganisms are more sensible to heat.

Preparation of Foods for Canning

In order to obtain wholesome canned foods, good manufacturing practices must be used during storage, handling, and preparation of raw foods and ingredients. Some problems can appear because of unsuitable handling of foods previous to thermal process. It is important to minimize the microbial contamination of food in each operation prior to heat treatment; otherwise, the microbial population increases and designed thermal process could be insufficient to reach the condition of thermal sterility.

Depending on the characteristics of foods, different preparative actions are taken to process a product.

Fruits and vegetables are usually peeled, pitted, destemmed, and seeded. Some leafy vegetables (like spinach) are heated (blanching) before canning to remove air and facilitate the packing of leaves in containers; in other cases, the heating inactivates enzymes to prevent some changes in product, or it can increase the initial temperature of food. High-acid fruits or vegetables can be thermally processed before they are placed in containers.

Usually, meats are precooked, boned, and compacted before thermal processing.

In the case of seafood, most fish and shellfish are boned or shelled before packing; however, some smaller fish, like sardines, are packed with their bones, considering that they will soften during the thermal process.

Thermal Process

Essentially, there are two categories of thermal processes in the canning industry. One is based on the use of retorts (or autoclaves: conventional canning), and the other is the aseptic processing of foods. In processes using retorts, containers are filled with food, followed by sealing them and then heated using steam under pressure until both container and food become sterilized together. In aseptic processing, a liquid food is sterilized outside the container by means of heat exchangers, and then it is cooled in the same equipment. The cooled sterile food is then filled and sealed in a container that has been previously sterilized; these operations are carried out in a sterile environment at room temperature. Summarizing, the retort processing is an 'in-container' sterilization, which can be applied to all types of foods, while aseptic processing is an 'out-of-container' sterilization used for liquid foods. Additionally, a third category of thermal processes can be considered; it is the atmospheric processing or pasteurization, applied to acid and acidified foods that require only a mild heat treatment.

The thermal process must be specific for each product, container type and size, and type of sterilization equipment. The thermal process is obtained by combining information about thermal destruction of microorganisms and product heating data in the proposed sterilization equipment. The amount of heat needed to destroy an expected number of microorganisms can be determined according to information on thermal resistance of microorganisms of interest in the product and the level of microbial destruction planned with the thermal process. On the other hand, the product heating

data usually are obtained from heat penetration tests that measure the temperature/time profile in containers during the thermal process. It is a good practice to obtain heat penetration data under the 'worst-case' conditions likely to be encountered during the thermal process to be sure that a suitable process is achieved. This is the reason why the tests are made with temperature sensors located at the slowest heating point inside the container. In some cases, the use of inoculated packs with microorganisms of known heat resistance can be helpful to design a thermal process. Product heating data are affected by intrinsic (product characteristics) and extrinsic (related to heating equipment) factors. Several mathematical methods have been developed to use the aforementioned information to design or analyze a thermal process.

Equipment

Sterilizers of different types are used on conventional canning. Both batch and continuous systems are available. In batch retorts, cans are loaded in crates and introduced into the retort; they are heat-treated and then unloaded. Examples of batch retorts are the vertical and horizontal steam (or water) still retorts; in this equipment, crates of containers are loaded into the retort, closing the vessel, and heating the containers; then the cooling is carried out by cutting off the steam and adding cool water. In addition, agitating batch retorts are also available. On the other hand, in continuous retorts, filled sealed containers are automatically and continuously moved from atmospheric conditions to a pressurized steam environment, held during the process time, and then moved again into an atmospheric condition for further handling. Examples of continuous retorts are the continuous rotary cooker and the hydrostatic sterilizer. It is common to use steam or hot water as the heating medium or, less usually, a mixture of steam and air. Food containers may be held stationary or rotated.

Most aseptic systems consist of heaters, a holding tube, and coolers. Equipment used for heating during aseptic processing of foods includes several types of heat exchangers; among these are the scrapped surface, the plate, and the tubular heat exchangers; these are indirect heat exchangers because heating medium does not mix with food. Also, there is equipment using direct steam injection in the food.

Pasteurization is normally carried out at temperatures under 100 °C, and it can use hot water baths. At the end of the thermal process, cold water is used for cooling. During the cooling stage of metal and glass containers, it is advisable to remove them at temperatures around 38 °C, so surface water evaporates, preventing corrosion problems. Pasteurization of bulk liquids can be accomplished in heat exchangers; they use as heating medium steam or hot water.

Each type and size of heating equipment (sterilizer) has its particular characteristics and must be known to establish a specific thermal process. With this information, the limits of accuracy of both time and temperature given to the food container can be known.

Postprocess Handling of Canned Foods

Achievement and maintenance of container integrity are essential for microbiological safety and to minimize spoilage. It is important to take appropriate measures to prevent leaker contamination. Among factors involved in control of container leaks are inspection of seam formation, care in seam abuse during passage of containers throughout the handling equipment, disinfection of water used to cool the containers, avoiding wet handling of containers, etc.

On the other hand, canned foods are commonly intended for long storage periods at ambient temperature, so containers must be kept dry. Also, during distribution, the use of sharp knives to open shrink-wrapping and cardboard cases in store may be a problem.

Nutritional and Health Concerns of Canned Foods

The main objective of thermal processes for canned foods is to achieve the condition of commercial sterility. However, as a consequence of thermal treatment, several components in the food can be affected, changing its characteristics, including sensorial and nutritional attributes. So thermal processes must be optimized in order to guarantee that stable and safe products are obtained and that they are quality foods with the suitable retention of sensorial and nutritional characteristics.

Nutrients

In canned foods, the nutrient retention varies depending upon the process, the product, and the nutrient, among other conditions. For a long time, there were canned products in which a significant amount of vitamins was lost during heating, but other nutrients remained in high levels, like protein and calcium. During storage, nutrient losses were less evident. According to new trends in thermal processing, canned foods provide at least the same nutritional value as fresh produce and their frozen counterparts when prepared for a table dish; inclusive, in some cases, canned products can provide higher nutrient levels than fresh produce. This is because fresh produce are exposed to several detrimental conditions (intrinsic enzymes, environmental factors, microorganisms, deleterious reactions, etc.).

Principles that can be applied to describe the effect of heating on microbial destruction also can be applied to other changes occurring in foods, that is, nutrients, quality factors, and enzymes.

Hazards

Most operations in canning processes are oriented to ensure the safety of canned foods. Frequently, the canning industry uses the principles of a system called hazard analysis and critical control points (HACCP) to achieve this goal. Among others, HACCP specifies control points (operations) during food processing to prevent health risks with canned foods. The following are some health concerns associated with canned foods that are commented:

In the case of troubles of microbial origin, it is important to consider that naturally acid foods and acidified products will not support the growth of food pathogen microorganisms, so mild thermal treatments (like pasteurization) are enough to destroy microorganism in the food. Food processors are most

concerned about low-acid foods, like meats, fish, mushrooms, and vegetables; in these products, *Clostridium botulinum*, which causes botulism, must be destroyed by thermal processes, which also destroy other microorganisms that may poison or spoil the food. In addition to thermal process, a suitable and sanitary handling of food before and after heating to prevent the manufacture of unsafe foods is important. There are reports involving other microorganisms different from *C. botulinum* that had the opportunity of growing before thermal process or recontaminating the heated product yielding a dangerous product. This is why systems like HACCP are helpful to obtain safe products.

Another point of interest related to health involves potentially hazardous or toxic substances in container materials that may migrate into the food inside the container. One example of such substances is lead, which was used to solder the metal parts of a can. In the three-piece cans, the lead-soldered side seam has been replaced by a welded side seam. The welding process uses electrodes that apply pressure and electric current to form the side seam. In the welded seam, the problem of lead leaching into the canned food is eliminated. At present, practically lead-soldered cans are not in use. Another example of hazardous substance in container materials is bisphenol A (BPA). This substance is used as an ingredient in the manufacture of internal can coatings, which are used to prevent or retard the interaction between metal cans and their contained food substances. Currently, there are dispute statements that suggest potential risk from BPA exposure; some chemical companies have begun developing BPA substitutes, but on the other hand, more information about BPA impact on consumers' health is required.

Containers for Canned Foods

Historically, the first canning processes used glass containers, with the inconvenience that they were bulky, brittle, and expensive. However, glass containers are still widely used in different sizes and shapes, because they present several desirable properties (glass is inert, transparent, resistant, etc.).

Immediately, the tin cans appeared. Despite the use of metal cans for almost two centuries, innovations in can manufacture have been made up to present days. For example, currently, metal cans are made of recyclable metals (steel and aluminum); additionally, the inside of cans is coated to prevent interaction between metal and food components. The use of the three-piece can is common, but the use of the two-piece can is continuously increasing, and this is justified because the manufacture of two-piece cans (drawing and redrawing (DRD) and drawing and ironing (D&I) methods) is more efficient and economical in comparison with the conventional production method of welding three-piece cans. Also, there are savings with the continuous gauge reduction in manufacture of metal cans. Other innovations in metal cans are the use of ring pulls to open a can and the system of easy-open ends.

On the other hand, the concept of a can ('a container that can store and protect foodstuffs for long periods at room temperature') must be reevaluated since this concept has led to a number of interesting and important developments in other materials and formats. The following are some examples:

(1) Aseptic carton, like the one used by Tetra Pak for milk and other liquid foods is subjected to aseptic processing. This special carton is made with several laminated materials including plastic, aluminum foil, and cardboard.
(2) Cardboard bottles made from recycled paper and low-density polyethylene liner; it has been proposed for milk packing.
(3) Paperboard cans combining paperboard and plastics.
(4) Aluminum foil lamination pouches that can show some advantages compared to traditional metal cans.
(5) Barrier plastics combined with structural polyolefins in can or can-like shapes represent a type of container that could provide all the food protection properties required.

In addition to the traditional cylindrical shape of a can, there are other shapes in use, like trays, cups, pouches, bowls, and bricks.

Future Trends

Traditionally, the analysis and design of thermal processes to achieve commercial sterility are based on mathematical models that assume that inactivation of bacterial spores and vegetative cells, including those of *Clostridium botulinum*, follows first-order kinetics; with this information, thermal resistance parameters (D and z) are evaluated. This approach has provoked that thermal processes evaluated by these means yield safe foods from the microbial point of view; however, these products are probably overprocessed. At the present time, there is evidence that microbial inactivation by heat has a different behavior. In addition, today, better mathematical and computational resources are available to analyze and evaluate thermal processes and generate improved mathematical models, which have been validated experimentally for specific microbial populations. According to this information, theories of thermal processing must be reexamined to guarantee not only safe foods but also quality products.

Related to the last point, food processors are concerned about optimization of thermal processes looking not only for safe and stable foods but also for a reduction in costs and energy in the operation. On the other hand, consumers are demanding nutritional value, chemical safety, sensorial attributes, and, in general, overall quality in their products. To reach these objectives, the canning processes must be reexamined and optimized taking into account the availability of new materials, equipment, and tools to analyze the processes.

Another point of interest is the availability of new sterilization technologies. In canning, sterilization is accomplished by heat and in some cases by a combination of heat and acid. However, nowadays, alternative processes, such as microwave heating, or nonthermal processes such as ultrahigh pressure or UV radiation, are available. These late processes have been used in combination with heat to generate canned foods. Foods processed with these alternative technologies are reported to have minimal quality and nutritional loss as compared to those obtained by conventional thermal processing methods.

See also: *Clostridium botulinum*; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; Heat

Treatment: Effect on Microbiological Changes and Shelf Life; Heat Treatment: Principles and Techniques; Pasteurization: Effect on Sensory Quality and Nutrient Composition; Pasteurization: Principles and Applications; pH: Principles and Measurement; Pickling; Preservation of Foods; Sterilization of Foods.

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Relevant Website

http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=113 – Regulatory information from the U.S. FDA related to thermally processed low-acid foods packaged in hermetically sealed containers.

Caramel: Methods of Manufacture

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Introduction

The term caramel has three meanings, that is, (i) solid or semisolid mass for making candies and decoration of cakes pastries and other desserts; (ii) syrup for flavoring sauces, gravies, and some beverages; and (iii) liquid colorant for food and selected pharmaceuticals. All of them are available by burning sugars, and the properties and applications of resulting products depend on the temperature and duration of the burning.

Solid or Semisolid Caramel

Solid or semisolid caramel is a beige to brown color mass resulting basically from burning sugar at temperature depending on the source applied. For fructose, galactose, glucose, sucrose, and maltose, it is 110, 160, 160, 160, and 180 °C, respectively. The process proceeds stepwise. When it begins from aqueous solution of sugar, first, evaporation of water has to take place at 100 °C. Otherwise, the solid source can be thermally liquefied. When sucrose is taken as a source, the material for semisolid (soft) candies is formed at 129 °C. Making hard candies takes heating to 165–168 °C. Light, medium, and dark caramels are formed at 180, 180–188, and 188–204 °C, respectively. The so-called black Jack caramel with burnt odor results from burning sugar at 210 °C.

That kind of caramel is standardized on the level of particular manufacturers who use their own compositions of ingredients. There are several more or less complex methods of making caramel. They are based on caramelization of sugars with some additives. One of them is making traditional Middle East sweet kaimak from sugar and milk fat or cream at elevated temperature. Milk provides a softness of the product, which distinguishes the caramel from hard candies. Corn or maple syrup is added to increase the sweetness and prevent aggregation into grains usually resulting from too high level of sugar in the product. Fat is frequently added to provide a taste. Among fats, butter is superior but it is also the most expensive. Therefore, commonly, a blend of various fats is in use, and in good-quality products, butter is an indispensable component. Other ingredients being in use are whey, calcium hydrogen carbonate, salt, flavor, molasses, and corn starch.

There is a patent for producing caramel of a high content of fructose oligosaccharides. Thus, either mono-, oligo-, or polysaccharides, eventually some sugar alcohols together with sugar syrups, wheat gluten, eventually deaminated, an oil, and lecithin are initially treated in a ball mill, followed by 5–15 min caramelization at 130–160 °C. In other patents, the same procedure is carried out with the addition of an organic acid, for instance, citric acid.

Caramel is hygroscopic. Thus, it should be stored in tightly closed nonmetal containers. The hygroscopicity of the caramel decreases with a content of the noncaramelized saccharide.

Caramel Syrups

Caramel syrups are usually homemade although they are also commercially available. House ladies burn sugar, chiefly sucrose, and use resulting syrup *in situ*. The process begins from melting sugar, and then, due to dehydration, initial bubbling increases, producing massive foams. Simultaneously, decomposition reactions generate brown color and acrid smoke. Usually, at this stage, the process is stopped. Temperature of the process at this stage provided formation of furan-2-aldehyde from pentoses or 5-hydroxymethyl furyl-2-aldehyde from hexoses. Under conditions of burning, their low-degree polymerization occurs. The products being low-molecular-weight furan derivatives do not dispose with any extended chromophore system. Simultaneously, some part of monomers still resides in the syrup, making it suitable for flavoring rather than for coloring.

Caramel syrups can be stored in closed glass containers for 2–3 weeks at room temperature and for 2–3 months in refrigerator. The shelf life of caramel syrups even at room temperature can be significantly extended by blending on five hot volumes of a sugar base holding syrup containing granulated sugar, water, corn syrup, and glucose with three volumes of a burnt sugar syrup. The caramel syrups are used for flavoring some dishes, particularly sauces, but since they are alcohol-soluble, they are applicable for flavoring and coloring liquors. They have specific bitter taste and slightly burnt odor.

Caramel Colors

Sources

Mono- and disaccharides are common sources for manufacturing caramel colors. The source has some impact to the course of the caramelization and properties of the final product. Reduced sugars caramelize more readily than nonreducing sugars. Since some residual sugar is left in the product, the caramels have slightly different organoleptic properties and different stability. Caramel colors manufactured from molasses have poor quality, and they are rich in potassium. Fruit juices and extracts have also been considered as a stock for caramelization.

For economical reasons and availability of mono- and disaccharides, in some parts of the world, oligo- and polysaccharides and even starch waste have paid attention as the source for the caramel manufacture. They were first subjected to either acid-, base-, or enzyme-catalyzed hydrolysis into starch hydrolyzates containing 70–85% reducing sugars (DE

30–58). Caramels from the stock prepared by enzymatic hydrolysis have a greater tendency to crystallize. They are more hygroscopic and less viscous as they contain more low-molecular-weight dextrins. Also, malt and soybean carbohydrates can yield caramel.

Caramel Classes

Caramels are the colloidal species, and retaining this property when applied as a colorant is a necessary condition for their effectiveness. As every micellar system, they discharge and precipitate as brown floccules at an isoelectric point. For that sake, there is no one universal caramel color for all types of food. The caramel colors spread into four classes as shown in Table 1 taking color intensity and binding to diethylaminoethyl cellulose (DEAE cellulose) and phosphoryl cellulose as criteria.

Catalysts and Additives

Several additives have been used to stimulate the caramelization and add some flavor and aroma. The use of α-hydroxy carboxylic and proteogenic α-amino acids and their salts could be rationalized in terms of the formation of secondary food aromas on the thermal reactions of those acids with saccharides. Usually, caramels of the III and IV classes are contaminated with neurotoxic 4(5)-methylimidazole (MeI) up to the level of ~200 mg kg^{-1}. The decrease in the content of MeI was an additional argument for the use of such catalysts. In order to provide the stability of the color of the caramels, some inhibitors such as magnesium sulfate or chloride, potassium metabisulfite, and sodium sulfite, sulfate, or polysulfate may be added.

The additives and catalysts in use are subjected to some limitations according to local regulations. The US federal law accepts the following acid additives: acetic, citric, phosphoric, sulfuric, and sulfurous acids. Ammonium, calcium, potassium, and sodium hydroxides are the accepted alkaline additives. Ammonium, sodium, or potassium carbonates, bicarbonates, phosphates (including dibasic phosphates and monobasic phosphates), sulfates, and sulfites are accepted salts. The use of polyglycerol esters of fatty acids as antifoaming agents in amounts not greater than that required to produce the intended effect is also legal.

Table 1 Classes and types of caramel colors [44]

Class	*Sort*	*Color intensity (ε_{max} at 560 nm)*[a]	*Characteristics*
I	Plain caramel E 150a	0.01–0.02	Not more than 50% of the color is bound by DEAE cellulose and not more than 50% of the color is bound by phosphoryl cellulose
II	Sulfite caramel E 150b	0.06–0.10	More than 50% of the color is bound by DEAE cellulose and it exhibits an absorbance ratio of more than 50
III	Ammonia caramel E 150c	0.08–0.36	Not more than 50% of the color is bound by DEAE cellulose and more than 50% of the color is bound by phosphoryl cellulose
IV	Sulfite ammonia caramel E 150d	0.10–0.60	More than 50% of the color is bound by DEAE cellulose and it exhibits an absorbance ratio of not more than 50

Each class of caramel is prepared using a different catalyst suitable for manufacturing caramel of different isoelectric point, thus suitable for coloring products of different acidities (all caramel colors are acidic). Typically, all (i) ammonium, sodium, and potassium carbonates/bicarbonates, (ii) sulfites, (iii) ammonia, and (iv) ammonia/sulfite combination are used to prepare caramel of I, II, III, and IV type, respectively.
[a]The parameter is expressed in terms of a product having a color intensity of 0.10 absorbance units taken at 560 nm in 10 mm quartz cell.

Caramel Manufacture

The whole process proceeds usually batchwise in entirely stainless steel, preferably 316 stainless steel, installations that include either open or pressure kettles, lines, agitators, fillers, and storage tanks. In the case of pressure kettles, at up to 160 °C, the typical gauge pressure is 483 kPa (70 psi, 5 atm). The selection between processes involving either the open or pressure kettles depends on the type of caramel required. There are some contradicting opinions on the role of atmosphere under which caramelization takes place. The caramelization in the open but under oxygen-free atmosphere provided products of higher tinctorial strength.

The process requires thorough maintaining the reaction parameters. They are adjusted accordingly to the caramelized source and the desired product and then strictly controlled throughout the process. Modeling approaches to the caramelization are available. Composition of resulting caramel depends on reaction temperature and time and the concentration of the reagents. On batchwise production, in-process controls are crucial for making uniform product. The tinctorial strength of the product is proportional to the time of heating. The initial setting-up isoelectric point is essential. Its corrections throughout the process are complicated and sometimes impossible. It is selected depending on the required class of caramel. Then, the course of the process is monitored through regular checking the tinctorial strength and viscosity of the samples. The latter is a function of the rate of dehydration of the source. That rate influences the properties of the final product. It can be corrected by manipulating with temperature and mutual contact of reagents. The final stage of the processing involves killing heat. It is also very important. It involves decreasing temperature of the reaction mixture to ~30 °C. Spraying with water through nozzles is not recommended as such caramel is fairly unstable. Instead, spraying caramel in large volume of 4:1 mixture of ethanol and diethyl ether is proposed as providing stable product of high tinctorial strength. There are also other methods for killing heat. Perhaps, employing heat exchangers is now the most common approach.

Caramelization can be performed without any catalyst. This process takes heating the substrate to 190–250 °C under atmospheric, reduced, or elevated pressure. The latter method

was recommended for caramelization of starch syrups to initiate reaction. Caramels from the process run above 200 °C dispose with low tinctorial strength and they are acrid.

Catalysts provide decreasing temperature of caramelization. For technological reasons, among all catalysts in use, ammonia is superior as it provides the lowest temperature (130 °C) of caramelization at the shortest time of the process and the caramel of the highest tinctorial strength. However, the level of MeI in resulting caramel is the highest. Considerable amount of MeI is also formed in the caustic sulfite-/ammonia-catalyzed process. Caramels of both those classes containing MeI as low as 25 mg kg^{-1} are available in modified processes. The manufacture of ammonia caramel in continuous process involves pumping a heated stream of corn syrup through a reaction zone under pressure to which preheated ammonia is injected. It accelerated the formation of the caramel, whereas the rate of the formation of MeI is lower. In such manner, the concentration of MeI in caramel decreases. Class IV caramel of reduced MeI content is manufactured in close vessel applying the ammonium bisulfate catalyst blended with acid to afford $pH < 5$.

The use of mineral acids and alkali also offers manufacture of caramels at lower temperature. Compared to ammonia and caustic sulfite/ammonia caramels, they have a higher content of 2-furaldehyde from pentoses and 5-(hydroxymethyl)-2-furaldehyde from hexoses and higher content of components from dehydration and condensation. On selection of the catalyst and the burning parameters, resulting Linner hue index (H_L) describing the caramel redness should be taken into account. H_L is defined as $10 \log(A_{510}/A_{610})$ where A denotes UV–vis absorbance taken at 510 and 610 nm, respectively.

The tinctorial strength of caramels can additionally be enhanced applying some physicochemical methods. They involve ultrafiltration. This method helps to remove some MeI. Another approach combines size exclusion chromatography with centrifugation (10–2000 *g*).

Solid caramels are also available. To meet the goal, viscous caramel at 120 °C is treated with ammonium carbonate, sucrose and orthophosphoric acid added, cooling to 100 °C and finally treating the blend with citric acid and sodium bicarbonate. In another solution, caramel is thickened with cereals such as rye flour and conditioned at 80–85 °C and pH 3.5–5.5. Alternatively, a blend of starch with dextrins can be used as a thickener. Ajinomoto has patented caramelizing extrusion of mono- and disaccharides at 150–300 °C. In contrast to liquid caramel colors, solid caramels may have $pH > 7$.

The caramelization can be stimulated by UV light and γ-radiation. Sonication ruins caramel micelles and causes flocculation.

Caramel Stability and Storage

Caramel colors are unstable, and on storage, the caramelization progresses. It is manifested by gradual increase in its tinctorial strength. Cooling slows down but does not eliminate this process. After prolonged storage, caramels irreversibly resinify yielding amorphous gel. When stored at low temperature in plastic-lined drums or barrels, it can withstand the storage for up to 5 years. When stored at ambient temperature, their shelf life reaches hardly 2 years.

See also: Caramel: Properties and Analysis.

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Caramel: Properties and Analysis

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General Introduction

Watching white crystalline sugar turning brown into caramel, producing an enticing and appetizing aroma, must be one of the first childhood exposures to chemistry for most humans. Caramel constitutes one of mankind's oldest and most important dietary materials. Despite the high profile of this dietary material, due to its familiarity and due to its economic importance, with around one-third of the total 150 Mt of sugar produced annually processed by heat treatment to form caramel and related products, an understanding of the chemistry of caramelization remains in its infancy.

Caramel refers to a material obtained from carbohydrates, in particular mono- and disaccharides, at elevated temperatures. The products of this thermal transformation are referred to as caramelization products. The term caramel is synonymously employed as well for caramel coloring used as a food additive in many food products and beverages. This will be described separately later in this article. Caramel is furthermore used as a synonym for certain kinds of candies with caramel as the main ingredient, which is not described further here.

The process of caramelization has been known since the early days of cooking, when sugar-rich products were heated. Indeed, whenever carbohydrate-rich food raw materials are heated, products of caramelization will be formed. Carbohydrates have been treated thermally by humans since ancient times. Mankind began cultivating wheat around 12 000–10 000 BC, with archaeological evidence from central Turkey (e.g., Göbekli Tepe or Çatalhöyük sites), when a genetic mutation in wheat resulted in a nonshattering rachis suitable for harvesting. The most definite archaeological evidence of early thermal treatment of carbohydrates originates from an illustration in the ancient Egyptian tomb of Ramses III displaying a royal bakery. The earliest records of a material we now would consider as caramel date back to recipes from the Arabian Peninsula using honey as the raw material for caramelization around AD 900. In the eighteenth century, recipes using caramelization became more widespread due to the wider availability of sucrose obtained from sugarcane (cross-reference sugar/sucrose). In the nineteenth century, caramel, in particular caramel color, obtained commercial significance once again due to a wider availability of sucrose with sugar beet cultivation starting in Europe and the rise of industrial food production.

Chemistry

The onset of the caramelization process varies with the type of mono- or disaccharide used. Sucrose **3**, for example, starts melting at 135 °C without color change. Development of color and caramel aroma starts around 143–160 °C. On cooling, a brittle glass-like material results. E150a, a standard caramel color, is obtained at temperatures above 160 °C resulting in a dark brown to blackish color accompanied by a bitter aroma. Other monosaccharides start the caramelization process at lower temperatures, such as fructose **2** at 110 °C, glucose **1** and galactose at 160 °C, and maltose at temperatures above 180 °C (for chemical structures, see Figure 1).

Analytic Methods

Purity

A series of analytic procedures exist for defining purity standards of caramel. Just to give one example, the US Pharmacopeia requires a specific gravity lower than 1.30 g cm^{-3}, an ash content below 8%, and a simple purity check involving the absence of a precipitate upon the addition of 0.5 ml phosphoric acid to a 20 ml sample. Furthermore, threshold levels for arsenic, lead, and mercury have also been defined.

Color Index by Colorimetry

Color is the most important property of caramel. The color strength of caramel is typically defined as its tinctorial power, K_{560}. This is the absorbance of a 0.1% weight/volume solution measured through a 1 cm light path at a wavelength of 560 nm (nm) using a spectrophotometer. The higher the value of the absorbance, and consequently, the tinctorial power, K_{560}, the darker the caramel color.

The color tone of the caramel color is also important. This is defined by the hue index, which is the measure of the color hue or red characteristics. It is a function of the absorbance at 510 and 610 nm. Generally, the higher the tinctorial power, K_{560}, the lower the hue index and the lower the red tones. Various other indices are in use around the world.

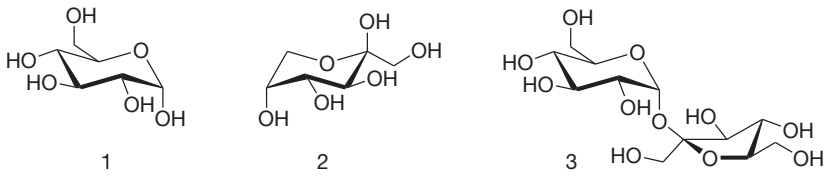


Figure 1 Chemical structures of typical saccharides used in caramel production.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00112-4

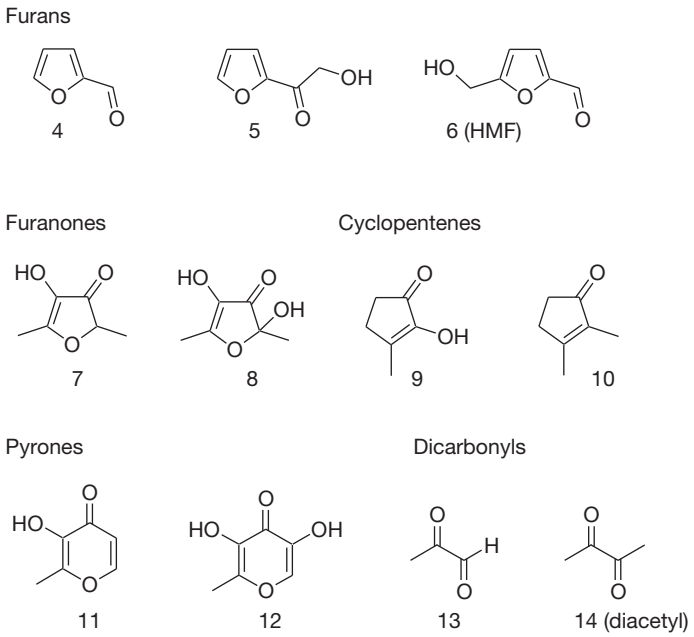


Figure 2 Chemical structure of typical volatile compounds from caramel.

Gas Chromatography

For analysis and characterization of volatiles in caramel, gas chromatography (GC) methods have been traditionally employed for separation. As detectors, GC-FID, GC-nose, and GC-MS (gas chromatography–mass spectrometry) are used. For the majority of caramel volatiles (for structures, please see Figure 2), GC-MS data are contained in commercial spectral libraries such as NIST and allow straightforward identification of caramel volatiles from GC-MS data. It should be noted that other aroma volatiles not formed in caramelization also possess an aroma classified as caramel-like in sensory evaluation panel testing.

Liquid Chromatography–Mass Spectrometry

In the last years, high-resolution MS has evolved as a powerful technique to study complex mixtures. Due to its unsurpassed resolving power, combined with tandem MS methods for structure elucidation and innovative data interpretation strategies developed recently, a comprehensive characterization of the chemical composition of caramel was possible.

Reaction Mechanisms

In caramelization chemistry, it is advisable to separate caramelization reactions, which occur under simple heat treatment in pure mono- and disaccharides from Maillard reaction products, which require the presence of an amine base, most frequently amino acids.

The following reaction types dominate caramelization chemistry summarized by Krohn and Golon et al.:

- Enolization
- Dehydration
- Dicarbonyl cleavage
- Retro-aldol reactions
- Aldol reactions
- Glycosidic bond formation
- Hydration

Volatile Compounds

Within the volatile fraction of caramel, a series of heterocyclic and carbocyclic compounds are formed including furans **4–6**, furanones **7** and **8**, cyclopentenes **9** and **10**, and pyrones **11** and **12** (see Figure 2). Retro-aldol reactions of saccharides yield dicarbonyl compounds such as **13** and **14**. Diacetyl **14**, associated with a butterscotch-type flavor, is one of the best-characterized aroma compounds in caramel, which is accompanied by several hundreds of other identified flavor compounds. The mechanism of formation has been discussed in detail by Blank and Krohn.

Nonvolatile Fraction

Within the nonvolatile fraction, caramelization leads to a significant reduction of the starting material saccharides (90% and more) and yields several ill-defined products as a complex mixture. The scientific investigation of caramel started around 1860 with a seminal paper by French chemist A. Gelis, who noted that upon heating of sucrose, dramatic chemical changes occurred. He named the products originating from heated sucrose as caramelans and caramelins depending on their molecular weight. In 1967, Kitaoka classified the reaction products of caramelization into three distinct classes: caramelans, tetramers of hexoses ($C_{24}H_{36}O_{18}$); caramelens, hexamers of glucose ($C_{36}H_{50}O_{25}$); and caramelins, polymers of glucose ($C_{125}H_{188}O_{80}$). Although this classification is reported on many websites and even in food chemistry

textbooks, with an added hint that caramelization is accompanied by loss of water from carbohydrates, this early classification has never attracted the attention of other research groups or has ever been further substantiated, probably due to a lack of analytic methods able to unravel the complexity of the product mixture formed in caramelization.

Recent work by Golon and Kuhnert using liquid chromatography in conjunction with high-resolution and tandem MS could first of all show that in a typical caramelization reaction, around 1000 analytes with different m/z ratios can be detected. The number of actual reaction products is due to the presence of isomerism in carbohydrate chemistry and is impossible to estimate. The observed reaction products could be classified according to reaction types encountered in their formation.

Using a domino tandem MS approach, the products formed in caramelization of the most important dietary carbohydrates including monosaccharides, disaccharides, and polymeric carbohydrates, such as starch and cellulose, were investigated by Golon and Kuhnert.

In typical high-resolution MS spectra of caramel, around 300 intense signals could be observed on average (see Figures 3 and 4). The total number of signals resolved in an MS experiment is around 1000. A combination of van Krevelen and Kendrick analysis (see Figures 3 and 4) revealed that the products formed fall into four distinct compound class categories: (1) oligomers of sugars (up to dodecamers); (2) dehydration products of sugars mainly of oligomeric nature; (3) polyaromatic heterocycles; and (4) minor lipid-like redox products.

Kendrick analysis with normalization of water addition revealed that several homologous series of dehydration products were formed with a loss of up to eight water molecules starting from a given oligomer (shown in Figure 4).

Targeted tandem-LC-MS measurements showed that all compounds formed existed as multiple isomers, and in some cases, close to the theoretical number of isomers were resolved chromatographically. Tandem MS could confirm the structure of carbohydrate oligomers in most cases, although all issues of stereo- and regiochemistry remain open due to a lack of reference materials. For dehydrated products, tandem MS data revealed that dehydration always starts at the reducing end of the oligosaccharide leading to, after loss of the first three water

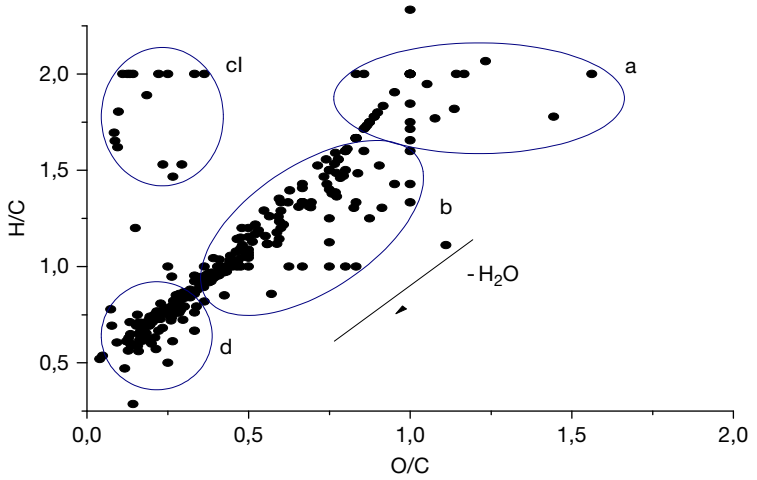


Figure 3 Van Krevelen diagram of high-resolution ESI-MS data in the negative-ion mode of thermally treated mannose (a) carbohydrates, (b) dehydration products, (c) lipid-like compounds, (d) condensed aromatic heterocycles. H/C, hydrogen/carbon ratio; O/C, oxygen/carbon ratio.

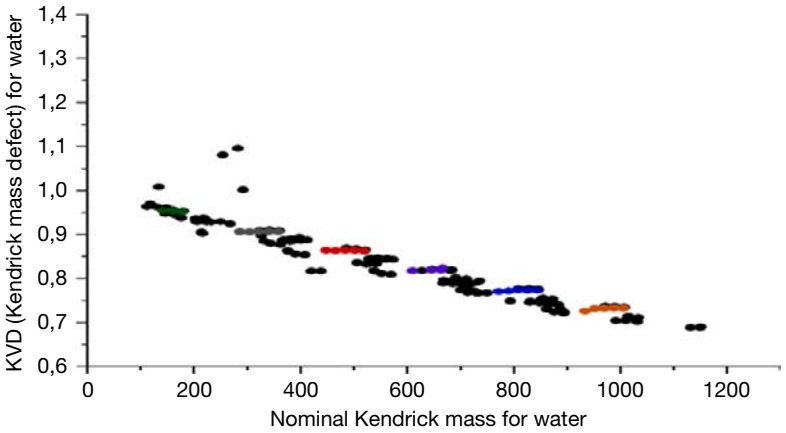


Figure 4 Kendrick diagram from high-resolution ESI-MS data in the negative-ion mode of thermally treated glucose normalized to loss of water. Colored parallel lines to x-axis indicate homologous series of up to eight losses of water.

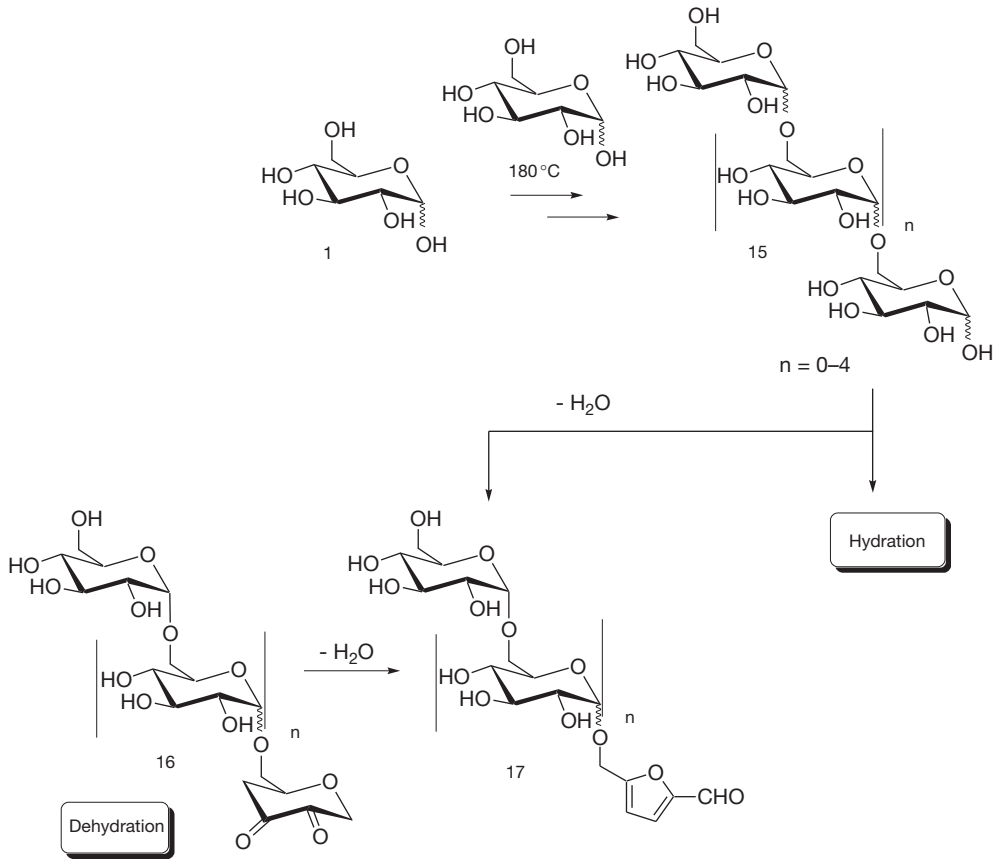


Figure 5 Reaction scheme of typical products formed in caramelization of monosaccharides.

molecules, a furanoid moiety at the reducing end (either 5-hydroxymethylfurfural or acetylfuran derivatives). Selected tentative structures **15–17** are shown in Figure 5.

When comparing the reactivity of various carbohydrates, Golon and Kuhnert observed that galactose moieties are most reactive, probably due to their axial 4-OH group, which allows the formation of reactive bicyclic hemiacetal intermediates.

Caramel Coloring

Caramel color is produced by thermal treatment of carbohydrates, in pure form or after addition of selected reagents. A wide range of raw materials are used as carbohydrate source, including fructose, glucose, invert sugar, sucrose, malt syrup, molasses, starch hydrolysates, and fractions thereof. Acids used include sulfuric, sulfurous, phosphoric, acetic, and citric acid; alkaline reagents include ammonium, sodium, potassium, and calcium hydroxide; and the salts used include ammonium, sodium, and potassium carbonate, bicarbonate, phosphate, sulfate, and bisulfite. Antifoaming agents, such as polyglycerol fatty acid esters, are added as processing aids during manufacture. Its color ranges from pale yellow to amber to dark brown. The resulting caramel color bodies are found to be either anionic or cationic in nature depending upon the reactants used in their manufacturing.

Table 1 Classification and application of industrial caramel colors

Class	*E number*	*Description/synonyms*	*Application*
Class I	E150a	Plain caramel, caustic caramel, and spirit caramel	Whiskey and other high-proof alcohols
Class II	E150b	Caustic sulfite caramel	Vegetable extracts, cognac, sherry, and vinegars
Class III	E150c	Ammonia caramel, baker's caramel, confectioner's caramel, and beer caramel	Beer, sauces, gravies, baking goods, and confectionery
Class IV	E150d	Sulfite ammonia caramel, acid-proof caramel, and soft drink caramel	Soft drinks

Source: Wikipedia: http://en.wikipedia.org/wiki/Caramel_color.

Internationally, the United Nations Joint Food and Agriculture Organization recognizes four classes of caramel color, differing by the reactants used in their manufacture, each with its own INS or E number, shown in Table 1.

Caramel coloring is developed as a result of heating several commercially available carbohydrates, including glucose, fructose, sucrose, and starch, in the presence of acidic (e.g., sulfuric

acid, phosphoric acid, acetic acid, and citric acid) or alkaline reagents (e.g., hydroxides, carbonates, and bicarbonates of ammonium, sodium, or potassium), which lead to caramel color compounds that are cationic or anionic in nature. In some case, phosphate, sulfate, and bisulfite salts can also be used. In general, four groups of caramel colorings, identified by their E number, have been internationally recognized and are presented in Table 1. The choice of caramel color depends on the requirements of a given application and therefore on the color intensity required. Additionally, stability factors for the food matrix used, including stability in aqueous ethanol, stability in the presence of salts (adjustment of the net charge of color bodies plays a role here), stability at pH 3 or 2, and stability in the presence of tannins, also need to be considered.

It should be noted that slight variation of manufacturing process parameters (starting material, temperature, and time) allows the production of a large range of different qualities of product within each caramel category and also results in differences in chemical composition and physical properties. This is further evidenced by variation in 4-methylimidazole (4-MEI) **18** and 2-acetyl-4-tetrahydroxybutylimidazole (THI) **19** concentrations in E150c and E 150d, respectively (see section 'Toxicology'). Variations in chemical composition in industrial caramel color have not been systematically studied, and no published material is available on the composition of the nonvolatile caramelization products other than for E150a caramel. It must, however, be assumed that the presence of ammonia compounds in E150c and E150d caramels will lead to Maillard chemistry and hence a chemical composition completely different if compared to E150a caramel.

Dietary Burden

The EFSA panel on food additives and nutrient sources added to food provided figures for the average intake of caramel from different classes. These data mainly originated from food questionnaires from the UK population and are divided into intake from adult and children population. Table 2 provides data on the average daily intake of E 150a–d, whereas Table 3 provides a summary of the different classes of foods that contribute to the intake of E 150 a–d.

From the data, in particular from the children population, it becomes obvious that minimum and maximum daily intakes vary dramatically by a factor of 5–10. A total intake for an average person of 70 kg bodyweight can be calculated from these data resulting in an intake of ~35 g day^{-1}. The maximum figures clearly exceed the recommended daily intakes. Interestingly, the types of food contributing to caramel intake vary dramatically if adults and children are compared. In the adult population, alcoholic drinks are the main contributors, whereas in the children population, sweet goods such as confectionary, fine bakery goods, and desserts are the main contributor.

Table 2 Estimated dietary intake of caramels in UK population

	Children population		*Adult population*
Class	*Minimum intake (mg kg^{-1} body weight per day)*	*Maximum intake (mg kg^{-1} body weight per day)*	*Average intake (mg kg^{-1} body weight per day)*
E150a	76.9	427.2	136.6
E150b	8.7	34.6	21.7
E150c	21.7	302.4	295.0
E150d	23.2	506.2	89.4

Source: EFSA panel on food additive report.

Toxicology

Generally in caramel, polycyclic carbocycles and heteroaromatics potentially form in thermal treatment and heterocycles 4-MEI **18** and THI **19** are under scrutiny for their adverse health effects. All four classes of caramel colors have been previously evaluated by the EU Scientific Committee for Food (SCF), by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and by the Nordic Council of Ministers (TemaNord). Both JECFA and SCF concluded that an acceptable daily intake (ADI) figure was not required E150 a, considering that it contains no chemical reagent used in manufacturing and therefore resembles caramel formed in normal cooking processes. For E150b, an ADI of 0–160 mg kg^{-1} body weight per day and, for E150b and E 150d, an ADI of 200 mg kg^{-1} body weight per day were established. These threshold values are based on information indicating that its chemical composition was similar to and intermediate between E150a plain caramel and E150d caramel. For E150c caramel, an ADI of 200 mg kg^{-1} body weight per day with the stipulation that THI **19** concentration should not exceed 10 mg/kg color on a color intensity basis was set. In 2010, the International Programme on Chemical Safety (IPCS) concluded similarly that commercially produced caramel color has the same toxicological properties as caramel produced by cooking or heating sucrose, except for those prepared using ammonium (E150c and E150d) (Figure 6).

The US Food and Drug Administration classifies and regulates caramel color in Title 21 CFR § 73.85 as a generally recognized as safe color additive exempt from certification. The IPCS has concluded that caramel color does not exhibit carcinogenicity or mutagenicity, referring to the available literature.

Data on the toxicokinetics of the caramel colors are very limited and show little uptake of the high-molecular-weight fraction of the color bodies from the gastrointestinal tract, with the bulk of the material being excreted in the feces. Animal studies on E150c ammonia caramel have shown evidence of lymphocyte depression and other evidence of immunotoxicity, which are considered to be due to the presence of THI, a potent immunosuppressant, in this caramel.

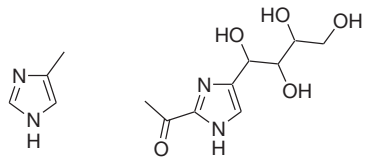
Caramel colors have been extensively tested for genotoxic potential in a variety of assays *in vitro* and *in vivo*. The results in *in vitro* systems were generally negative, with a few marginally positive findings, and no positive findings have been reported in *in vivo* assays.

Long-term toxicity studies carried out on E150c/d caramel were similar to and did not reveal any pattern of toxicity in addition to available 90-day oral toxicity studies. No evidence of carcinogenicity was seen in 2-year studies in rats on E150c/d.

Table 3 Estimated dietary intake of caramels according to food type in UK population

	Children population		*Adult population*	
Class	*(%)*	*Food type*	*(%)*	*Food type*
E150a	12–55	Nonalcoholic drinks	30	Nonalcoholic drinks
	15–32	Fine bakery products	27	Beer and cider
	11–48	Deserts	16	Soups
	11–32	Sauces and seasonings	10	Sauces, seasonings, and pickles
	12–56	Pickles		
	11–32	Soups		
	16–49	Malt bread		
E150b	12–53	Fine bakery products	50	Beer and cider
	11–41	Deserts and milk products	20	Soups
	11–22	Ice creams		
	12–45	Sauces, seasonings, and pickles		
	18–54	Soups		
	19–55	Malt bread		
E150c	13–45	Fine bakery products	48	Beer and cider
	12–45	Deserts	22	Sauces, seasonings, and pickles
	12–79	Sauces, seasonings, and pickles		
	12–45	Vinegar		
E150 d	13–51	Nonalcoholic drinks	65	Confectionary
	20–81	Confectionary	23	Nonalcoholic drinks
	10–29	Bakery goods		
	10–24	Sauces, seasonings, and pickles		
	10–34	Malt bread		

Source: EFSA panel on food additive report.



18 19

4-methyl-imidazole THI (2-acetyl)-4- tetrahydroxybutyl imidazole

Figure 6 Chemical structures of imidazoles 4-MEI (**18**) and THI (**19**) from E150c and E150d.

In a complementary study in mice, there was as well no evidence for a carcinogenic potential of E150d. In 2007, the National Toxicology Program issued a report summarizing the results of toxicological testing conducted on 4-MEI **18** in rats and mice. A 2-year study in rats was inconclusive regarding carcinogenicity, however, a mouse study of equal length revealed an increased incidence of certain lung tumors. These studies were conducted in rodents at levels of 4-MEI that far exceed current estimates of human exposure to 4-MEI from the consumption of E150c/d in food products and beverages. The latter level is higher than the current maximum level for THI required in the specifications for E150c.

According to the Food Chemicals Codex, 4-MEI in caramel color is allowed up to 250 ppm on a color-adjusted basis, which means 250 ppm maximum for every 0.100 color absorbance of a 0.10% solution at 610 nm.

In conclusion, the use of caramel colors can at the current stage be considered as safe if ADI values and maximum concentrations for 4-methylimidazole and THI are met.

See also: Browning: Non-enzymatic browning; Carbohydrate: Digestion, Absorption and Metabolism; Chromatography: Focus on Multidimensional GC; Colors: Properties and Determination of Natural Pigments; Fatty Acids: Fatty Acids; Flavor Enhancers: Characteristics and Uses; Fructose: Sources, Metabolism, and Health; Glucose: Properties and Analysis; Maillard Reaction; Sucrose: Properties and Determination.

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Carbohydrate: Digestion, Absorption and Metabolism

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Digestion

Mouth and Stomach

Digestion of polysaccharides, namely, starch, begins in the mouth. Salivary α-amylase, also known as ptyalin, hydrolyzes the α-(1-4) glycosidic bonds in linear glucose polymers. This enzyme is unable to hydrolyze α-(1,6) linkages in branched polymers, terminal α-(1-4) linkages, and α-(1-4) linkages near branch points; thus, the primary end products of amylase digestion are oligosaccharides, maltose, maltotriose, and α-limit dextrins (small and branched glucose polymers). Digestion by salivary α-amylase is brief and incomplete as the enzyme is inactivated by the acidic gastric juices in the stomach.

Smaller carbohydrates, such as trisaccharides and disaccharides, are not enzymatically digested until they reach the small intestine.

Small Intestine

As food passes from the stomach to the small intestine, bicarbonate (HCO^{3-}) is released to neutralize gastric juices and pancreatic α-amylase is released to resume the enzymatic digestion of starch that began in the mouth. Similar to salivary α-amylase, this enzyme is only able to hydrolyze linear portions of glucose polymers. The end products of maltose, maltotriose, and α-dextrin are then further hydrolyzed by enzymes contained in the microvilli of the small intestine, often referred to as brush border enzymes. These glycosidases are also responsible for the digestion of disaccharides such as sucrose and lactose.

The major brush border enzyme complexes responsible for carbohydrate digestion are glucoamylase, sucrase–isomaltase, and β-glycosidase (lactase). Table 1 describes the enzyme activity for these different complexes and their primary location in the small intestine. Glucoamylase continues to hydrolyze the larger end products of starch digestion, such as maltotriose and α-limit dextrins. Unlike α-amylases, which are endoglucosidases (can only hydrolyze α-(1,4) bonds within a linear glucose polymer), glucoamylase is an exoglucosidase that can begin hydrolyzing α-(1,4) linkages at the nonreducing end of a glucose polymer to release individual glucose moieties. However, this enzyme is still unable to hydrolyze α-(1,6) glycosidic bonds so the end products of digestion from glucoamylase are primarily glucose and isomaltose (a disaccharide of glucose bound by an α-(1,6) linkage).

Sucrase–isomaltase is a single brush border enzyme with two catalytic subunits that have different substrate specificity. The sucrase–maltase subunit is responsible for the digestion of sucrose to glucose and fructose and maltose to two glucose moieties. The isomaltase–maltase subunit is responsible for the digestion of isomaltose and maltose into two glucose moieties. This enzyme complex is critical as it accounts for 100% of sucrose digestion and almost all of the small intestine's ability to hydrolyze an α-(1,6) glycosidic linkage.

The β-glycosidase enzyme complex is also made up of two subunits, thus the reason this complex is often called lactase–phlorizin hydrolase. The lactase subunit hydrolyzes the β-(1,4) bond of lactose to release glucose and galactose. Glycolipids are the predominant substrate for the phlorizin hydrolase subunit, resulting in the release of a monosaccharide (glucose or galactose) and ceramide.

Large Intestine

The end products of carbohydrate digestion are monosaccharides, which are quickly absorbed in the small intestine. However, any carbohydrates that escape digestion and absorption in the small intestine, such as dietary fiber, pass into the large intestine where they are then fermented by microbes residing in the intestine. For more information on dietary fiber and the role of large intestine microbes on health, see elsewhere in this Encyclopedia.

While the presence of polysaccharides, such as dietary fiber, may be beneficial for the health of the large intestine, the presence of small carbohydrates, such as oligosaccharides, disaccharides, and monosaccharides, in the large intestine can lead to intestinal discomfort, such as bloating, gas, and diarrhea. Small carbohydrates are generally highly osmotic and will bring a large amount of water into the large intestine, often resulting in diarrhea. Smaller carbohydrates polymers are also rapidly fermented by the microbes in the intestine, resulting in the formation of gas and possibly feelings of bloating and flatulence.

Sugar alcohols, or polyols, are small monosaccharides and disaccharides that generally escape digestion and absorption in the small intestine. While some sugar alcohols occur naturally in fruits and vegetables, they are also frequently used in the food industry as noncaloric sweeteners for sugar-free and reduced-sugar foods. Since most of these polyols escape digestion and absorption, they pass into the large intestine and are fermented by the microbiota. These polyols are also highly osmotic so overconsumption can cause symptoms such as diarrhea, gas, and bloating. In fact, in the United States, some foods containing polyols that may be consumed in amounts that would cause discomfort carry a warning label that 'excess consumption may have a laxative effect.' For more information on sugar alcohols, see elsewhere in this Encyclopedia.

Absorption and Transport

Intestinal Absorption

Once carbohydrates are digested, the resulting monosaccharides are quickly absorbed by the cells of the small intestine (enterocytes). Because monosaccharides are polar molecules,

Table 1 Primary enzymes involved in carbohydrate digestion

Enzyme/enzyme complex	*Enzyme action*	*Location*	*Substrates*	*End products*
Salivary α-amylase	Hydrolyzes internal α-(1-4) glycosidic bonds in linear glucose polymers	Mouth	Starch (amylose, amylopectin) Glycogen	Oligosaccharides Maltose Maltotriose α-Limit dextrins
Pancreatic α-amylase	Same as salivary α-amylase	Small Intestine Secreted by the pancreas into the duodenum	Starch (amylose, amylopectin) Glycogen	Oligosaccharides Maltose Maltotriose α-Limit dextrins
Glucoamylase	Hydrolyzes α-(1-4) glycosidic bonds at the nonreducing end of linear glucose polymers	Small intestine Activity extends entire small intestine with highest activity in the ileum	Oligosaccharides Maltose Maltotriose α-Limit dextrins	Glucose Isomaltose
Sucrase–isomaltase	Sucrase–maltase subunit hydrolyzes (α-1) → (β-2) glycosidic bond in sucrose and the α-(1-4) glycosidic bond in maltose Isomaltase–maltase subunit hydrolyzes the α-(1,6) glycosidic bond in isomaltose and the α-(1-4) glycosidic bond maltose	Small intestine Activity highest in the jejunum	Sucrose Maltose Isomaltose	Glucose Fructose
β-Glycosidase	Lactase subunit hydrolyzes the β-(1,4) bond of lactose Phlorizin hydrolase subunit hydrolyzes the bond between monosaccharides and ceramides	Small intestine Activity highest in the jejunum	Lactose Glycolipids	Glucose Galactose Ceramide

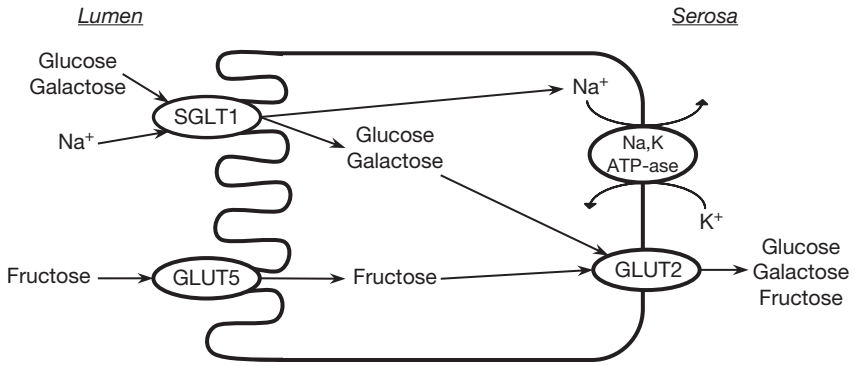

Figure 1 Absorption of monosaccharides.

transporters are required to carry them across the cell membrane. The specific transport mechanisms differ for the different monosaccharides.

Glucose and galactose are absorbed via the sodium-dependent transporter, SGLT1, located on the luminal side of the small intestinal cells. The monosaccharides are transported from the lumen to the enterocyte against a concentration gradient while the sodium is cotransported into the cell down a concentration gradient (Figure 1). In order to maintain the sodium gradient between the lumen and the enterocyte, sodium is pumped out of the enterocyte by a sodium–potassium ATPase in the basolateral membrane. Fructose transport into the enterocyte is facilitated by the GLUT5 transporter that is not sodium-dependent. This transporter can also facilitate the transport of glucose across the membrane, but the affinity for fructose is much greater. Once inside, the enterocyte monosaccharides are transported through the serosal side of the cell by GLUT2 transporters.

Transport into the Tissues

After being absorbed, monosaccharides are carried through the bloodstream to the tissues of the body. Virtually every cell in the body contains one or more types of GLUT transporters, signifying the importance of carbohydrates, particularly glucose, as a major fuel source for the body. As many as 14 different isoforms of GLUT transporters have been discovered; however, GLUT1–5 transporters have been the most thoroughly studied. Table 2 describes the functions of the different GLUT transporters and where they are found throughout the

avoidance of specific carbohydrate triggers or minimizing or eliminating starch from the diet.

Congenital disorders in carbohydrate absorption are also quite rare, and treatment can be more difficult. In glucose–galactose malabsorption, a mutation in the gene for SGLT1 leads to the inability to absorb glucose or galactose in the diet. Symptoms include diarrhea, dehydration, bloating, and abdominal pain and can begin as early as the first day of life. The primary treatment is to remove most carbohydrates from the diet with the exception of fructose. In some cases, individuals with this condition improve as they age and develop greater glucose tolerance. A mutation in the gene for the GLUT2 transporter leads to a condition called Fanconi–Bickel syndrome. As discussed previously, the GLUT2 transporter is the primary monosaccharide transporter in the intestine, but it is also expressed in the liver, kidney, and pancreas. Therefore, in addition to carbohydrate malabsorption, individuals with this condition will also have systemic effects, such as renal nephropathy and hepatomegaly.

Food Factors

There are several properties of foods that can impact digestion and absorption of carbohydrates, including physical structure, processing, and the presence of other macronutrients in the food. There is considerable research interest in this area as controlling the rate of digestion and absorption of glucose may have an important impact on health conditions, such as type 2 diabetes.

Alteration in the physical structure of carbohydrates, particularly starch, within foods has been shown to change the digestibility of the carbohydrates. For example, ground rice has been shown to be digested and absorbed more rapidly than whole, unground rice. Similarly, carbohydrates from pureed beans are more rapidly absorbed than those from whole beans. It is likely that the process of grinding or pureeing breaks down the semicrystalline structure of starch, thereby increasing the available surface area of the carbohydrate to digestive enzymes and thus the rate of digestion and absorption.

Other types of processing can also impact carbohydrate digestibility, particularly starch. Most of the starch contained in cooked foods is gelatinized. Starch gelatinization is the hydration of starch during cooking and is an essential process to make baked goods and pasta. During gelatinization, the starch granule swells and bursts, making the starch much more accessible to digestive enzymes. In foods where there is incomplete gelatinization of the starch, such as in pumpernickel bread, that often include intact grain kernels, digestion and absorption of the starch carbohydrates tend to be slower. Also, as gelatinized starch cools, it can undergo the process of retrogradation where the starch molecules attempt to recrystallize. Often, this process can result in the formation of resistant starch. As the name suggests, these recrystallized starch molecules are generally more resistant to digestive enzymes and either escape digestion or are more slowly digested and absorbed.

The presence of other macronutrients and food components, such as protein, lipid, fiber, and antinutrients, can also change the digestion and absorption rate of carbohydrates. When carbohydrates are consumed along with protein and/or lipids, the digestion and absorption rate appears to be slower, as evidenced by a lower glycemic response compared to consuming the carbohydrate alone. This may be due in part to the formation of a protein matrix around starch granules, as is often seen in grains. However, there may also be interactions between starch and protein that occur after ingestion as the addition of protein-based foods, such as fish and cheese, to breads or pasta can change the digestion and absorption of carbohydrates. Complexes can also form between starch chains and fatty acids that can slow the digestion and absorption of carbohydrates. The complexes may be more resistant to digestive enzymes, but starch–lipid complexes can also change the solubility and gelatinization temperature for starch, which could also impact digestibility. Dietary fiber has probably been the most-studied food component for impacting carbohydrate digestibility. The fibers that have shown the greatest impact on carbohydrate digestion and absorption are viscous dietary fibers because of their ability to slow transit in the intestine and interfere with enzyme accessibility to carbohydrates. Antinutrients, such as phytic acid and α-amylase inhibitors, have also been shown to interfere with carbohydrate digestion. Inhibitors of α-amylase can occur naturally in foods such as beans and grains. The impact of these inhibitors as they exist in the food seems fairly small; however, when isolated and concentrated, these inhibitors can have a significant impact on blood glucose levels.

Metabolism of Carbohydrates

Catabolism for Energy

The primary fate of absorbed carbohydrates is to be catabolized for cellular energy in the form of ATP. The energy value of carbohydrates is typically estimated to be 4 kcal g^{-1}. This value was determined by Atwater's calculation of the heat of combustion of various food carbohydrates. However, the true caloric value of carbohydrates can vary significantly. Some insoluble fibers, such as cellulose, have practically no caloric value since they are minimally digested and absorbed. Some soluble fibers, as well as sugar alcohols, can be partially digested and easily fermented, which makes their caloric value, on average, around 2 kcal g^{-1}. Most mono- and disaccharides have a caloric value ranging from 3.75 to 3.95 kcal g^{-1}, while highly digestible starches can yield as much as 4.2 kcal g^{-1}.

Glycolysis

Once taken into the cells, glucose is phosphorylated by a hexokinase to glucose 6-phosphate. This helps retain glucose within the cell and maintain a concentration gradient for continued entry of glucose into the cell. Glucose 6-phosphate is the precursor for several metabolic pathways, including glycolysis, pentose phosphate pathway, and glycogen synthesis. Hexokinase activity can differ in tissues with liver cells having a lower affinity (higher K_m) than hexokinases in other tissues. This ensures that active tissues get the glucose they need for metabolism, and when glucose levels rise, more is taken up by the liver for storage. Hexokinase in the liver (known as glucokinase) also has no feedback inhibition from its product,

glucose 6-phosphate. This is essential for the liver to be able to control blood glucose levels and generate glucose 6-phosphate for glycogen storage when excess glucose is present. In other tissues, excess glucose 6-phosphate can inhibit hexokinase, which then allows the concentration of free glucose in the cell to rise, eliminating the concentration gradient and stopping further transport of glucose into the cell.

To enter the glycolytic pathway (Figure 2), glucose 6-phosphate is isomerized to fructose 6-phosphate. Fructose 6-phosphate is then phosphorylated by phosphofructokinase-1 (PFK-1) to form fructose 1,6-bisphosphate. This is the committed step into glycolysis; thus, PFK-1 is highly regulated by the energy status of the cell with high ATP/AMP levels inhibiting the enzyme and low ATP/AMP levels activating the enzyme. Fructose 1,6-bisphosphate is cleaved by aldolase to form two 3-carbon units: dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. Dihydroxyacetone phosphate is isomerized to glyceraldehyde 3-phosphate, and these two molecules are again phosphorylated to form 1,3-bisphosphoglycerate. This step also generates NADH, which can be utilized to produce energy in other aerobic metabolic pathways. Through a series of additional steps, both three-carbon units are eventually converted to two pyruvate molecules and all four phosphate groups are removed to yield four ATPs. However, since two ATPs were utilized by hexokinase and PFK-1, the net energy yield of glycolysis is two ATPs.

Fate of pyruvate and NADH from glycolysis

The fate of pyruvate and NADH generated during glycolysis will depend on the presence of oxygen in the cellular environment and the presence of mitochondria. If oxygen is available, both NADH and pyruvate can be further oxidized to produce more ATP. However, if there is a lack of oxygen in the tissues, such as skeletal muscle during anaerobic exercise, or in tissues lacking mitochondria, such as erythrocytes, pyruvate and NADH cannot be used to generate more ATP.

In aerobic conditions, pyruvate can enter the mitochondria where it is oxidized to acetyl-CoA and enters the TCA cycle. The TCA cycle metabolizes pyruvate completely to CO_2 and in the process donates electrons to NAD^+ and FAD. These electrons are then passed through the electron transport chain in the mitochondrial membrane to generate ATP. The NADH molecules generated by glycolysis cannot enter the mitochondria, but under aerobic conditions, shuttle systems between the cytosol and the mitochondria can ensure the electrons from NADH get inside the mitochondria and into the electron transport system to generate ATP. These two shuttle systems are the glycerol 3-phosphate shuttle and the malate–aspartate shuttle.

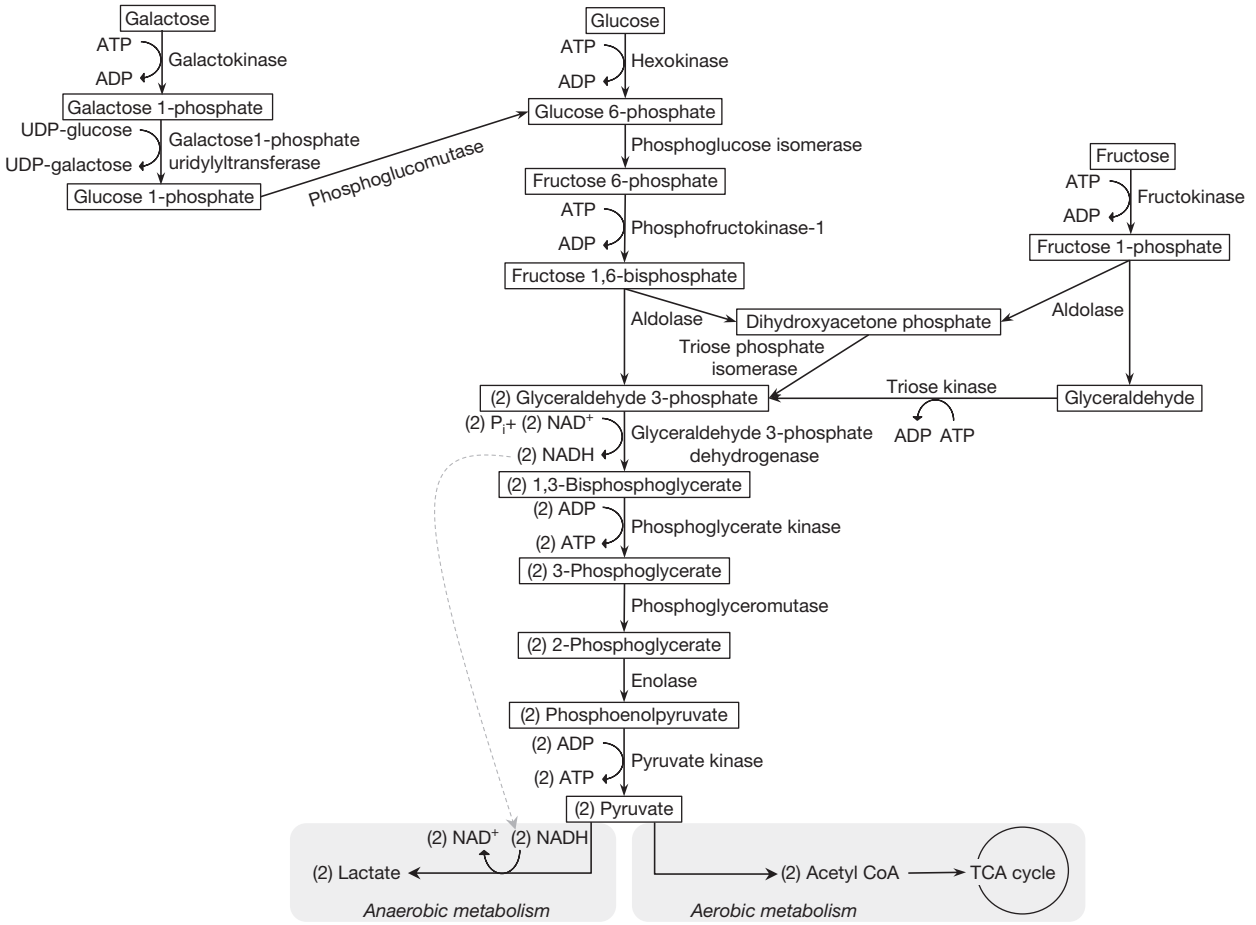

Figure 2 Aerobic and anaerobic glycolysis and entry points for fructose and galactose.

The glycerol 3-phosphate shuttle transfers electrons from NADH to dihydroxyacetone phosphate to form glycerol 3-phosphate, which can pass through the mitochondrial membrane. Similarly, the malate–aspartate shuttle transfers electrons from NADH to oxaloacetate to form malate, which can then enter the mitochondria.

In anaerobic conditions, or when there is a lack of mitochondria, the fate of pyruvate and NADH is closely linked. Pyruvate is converted to lactate by lactate dehydrogenase, a process that requires NADH. The conversion of pyruvate to lactate ensures that NAD^+ is regenerated for glycolysis to continue and prevents a buildup of pyruvate in the cell. Lactate can be released by cells into the bloodstream and taken up by other tissues as a fuel source or converted back to glucose by the liver in a process called the Cori cycle.

Fructose and galactose metabolism

The preferred carbohydrate source for cellular metabolism is glucose. Therefore, fructose and galactose are converted to intermediates in glycolysis. Fructose is metabolized in the liver to form glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, both intermediates in glycolysis. Galactose is also metabolized primarily in the liver to form glucose 6-phosphate, which can enter glycolysis. Figure 2 summarizes fructose and galactose metabolism and the entry of the metabolites into glycolysis. As we will discuss more in the succeeding text, since these monosaccharides can contribute intermediates in glycolysis, they may also contribute to the production of glucose through gluconeogenesis.

Maintenance of Glucose Homeostasis

Because of the importance of glucose as an energy source for the body, it is critical that tissues always have an available source of glucose, even during times of fasting. Gluconeogenesis, which occurs primarily in the liver, is the process by which glucose is generated. Most of the steps of glycolysis are reversible, and this is the primary means by which the liver will synthesize glucose.

Gluconeogenesis

Gluconeogenesis (Figure 3) is essentially a reversal of glycolysis, and the primary substrates for gluconeogenesis are pyruvate, lactate, glycerol, and amino acids. Each of these substrates can be converted to intermediates in the gluconeogenic pathway. Lactate can be oxidized to pyruvate to enter the gluconeogenic pathway. Glycerol is an intermediate of lipid metabolism and can be converted to dihydroxyacetone phosphate, while alanine is converted to pyruvate through alanine aminotransferase.

Since cellular energetics favor glycolysis, ATP is required to drive gluconeogenesis, and there are four key enzymes that enable the reversal of glycolysis to favor the production of glucose. The conversion of pyruvate to phosphoenolpyruvate requires two enzymes in gluconeogenesis even though the reverse reaction in glycolysis required only one. The enzyme involved is pyruvate carboxylase, which requires ATP and converts pyruvate to oxaloacetate. Oxaloacetate is then converted to phosphoenolpyruvate. The next several steps are a reversal of glycolytic enzymes and result in the conversion of phosphoenolpyruvate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, which then condense to form fructose 1,6-bisphosphate. To reverse phosphofructokinase-1 to return fructose 1,6-bisphosphate to fructose 6-phosphate requires another unique enzyme for gluconeogenesis, fructose 1,6-bisphosphatase. Likewise, the removal of a phosphate to convert glucose 6-phosphate back to glucose requires a unique enzyme, glucose 6-phosphatase. Once glucose is formed, it can be released by the liver to provide fuel to cells.

Similar to the enzymes in glycolysis, the enzymes in gluconeogenesis are also regulated to ensure a steady supply of glucose. Because of the similarity of the glycolytic and gluconeogenic pathways, generally, the activation of an enzyme in one pathway results in the inhibition of the 'paired' enzyme in the reverse pathway. For example, AMP is an activator of phosphofructokinase-1 to favor glycolysis and the generation of ATP. Conversely, AMP is an inhibitor of fructose 1,6-bisphosphatase, which favors gluconeogenesis rather than glycolysis. The enzymes in gluconeogenesis and glycolysis are also subject to hormonal regulation. During the fed state, insulin is released, which inhibits gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase. Alternatively, during fasting, glucagon levels rise, which induces gluconeogenic enzymes.

Storage of Glucose

The importance of glucose for energy is again demonstrated in the ability of the body to store glucose in the readily available form of glycogen. Most of the glycogen is stored in the liver and skeletal muscle with the purpose of maintaining blood glucose and providing a quick energy source for active cells, respectively. For greater depth into glycogen, see elsewhere in this Encyclopedia.

Glycogenesis

Glycogen is a glucose polysaccharide composed of α-1,4 linked glucosyl units with occasional branching created by α-1,6 linkages. This branching allows for more rapid breakdown when glucose is needed since there are many chains for enzymes to begin degrading. Glycogen synthesis begins with glucose 6-phosphate, the result of the phosphorylation of glucose by hexokinase (the first step in glucose metabolism). Phosphoglucomutase then catalyzes the conversion of glucose 6-phosphate to glucose 1-phosphate. UTP is then utilized to form UDP-glucose, which is necessary for glucose to be added to the glycogen chains. Glycogen synthase then transfers UDP-glucose molecules to the glycogen chains.

Glycogen synthase is the key regulatory enzyme of glycogenesis and is regulated by blood glucose levels as well as insulin and glucagon levels. As expected, elevated blood glucose and insulin will trigger glycogenesis, while fasting and elevations in glucagon will inhibit this pathway. Since skeletal muscle glycogen is primarily for rapid energy needs rather than the maintenance of blood glucose, skeletal muscle glycogenesis is also regulated by the energy state of the cell, with AMP inhibiting glycogenesis and triggering glycogenolysis.

Glycogenolysis

Different enzymes control glycogen degradation and glycogen synthesis. The main enzyme involved in glycogen degradation is glycogen phosphorylase, which catalyzes the removal of glucose 1-phosphate from the terminal end of a glycogen chain. A debranching enzyme is also required as glycogen phosphorylase cannot cleave glucose molecules near a branch

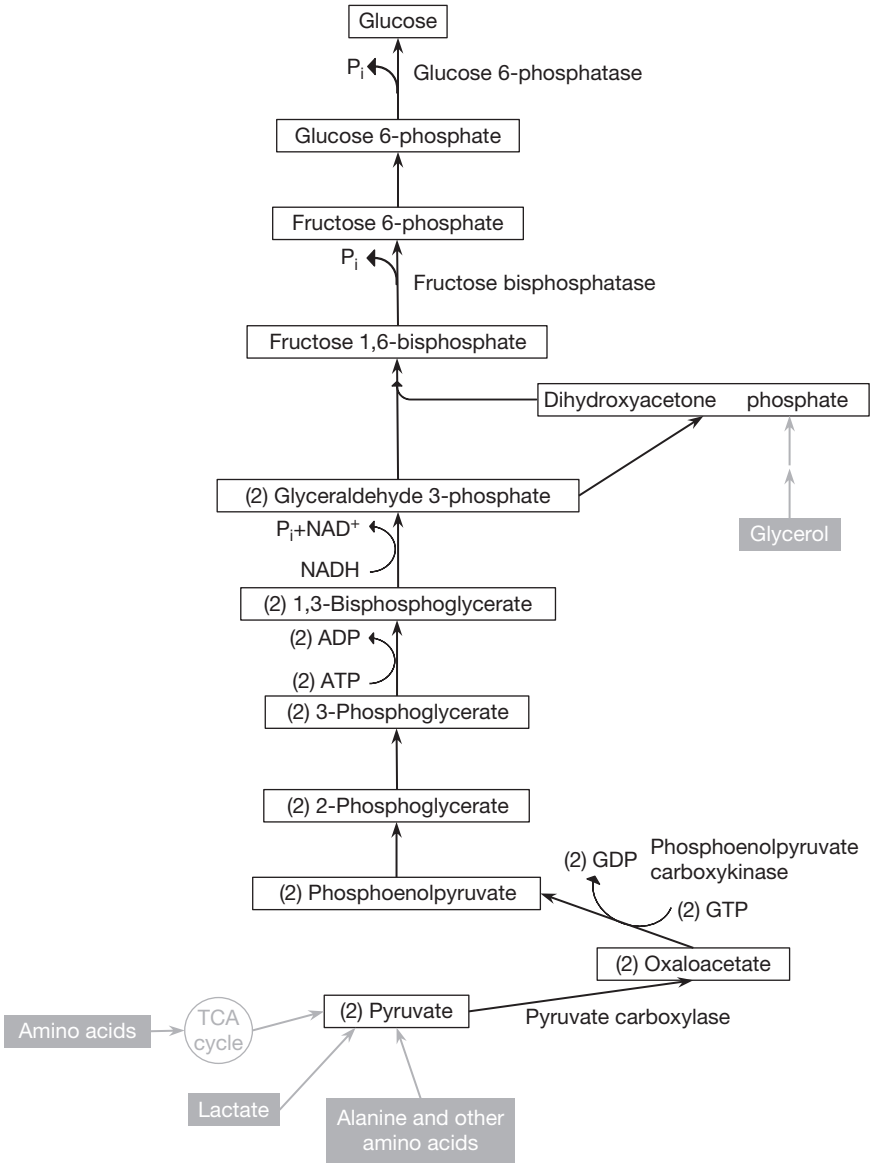

Figure 3 Gluconeogenesis, relationship to glycolysis, and entry points for noncarbohydrate substrates.

point. In the process of removing the branch point, a small amount of free glucose is released. Glucose 1-phosphate is converted back to glucose 6-phosphate. In the skeletal muscle, this will likely immediately proceed into glycolysis to provide the cell with needed energy. However, in the liver, glucose 6-phosphatase removes the phosphate from glucose 6-phosphate to generate free glucose that can then be used to maintain blood glucose levels. These enzymes are regulated by the same hormonal mechanisms as glycogenic enzymes.

Other Metabolic Pathways for Carbohydrates

Glucose metabolism can also be used to generate precursors for biosynthetic pathways, such as amino acid and lipid formation. Pyruvate and intermediates in the TCA cycle can be used for the carbon skeleton of amino acids, such as alanine and glutamate. Acetyl Co-A and glycerol 3-phosphate can contribute to the formation of fatty acids and glycerol backbones, respectively, in lipid formation. The pentose phosphate pathway is an alternative pathway for anaerobic glucose oxidation and can yield NADPH for biosynthetic pathways and as a defense against cellular oxidative damage, as well as ribose 5-phosphate, a precursor for nucleic acid synthesis. UDP-glucose, the precursor for glycogen synthesis, can also be used as a precursor for the formation of lactose in the mammary glands as well as the formation of glycoproteins and glycolipids.

Physiological and Genetic Factors Impacting Carbohydrate Metabolism

As there are a number of pathways involved in carbohydrate metabolism, this also means there are a number of physiological and genetic conditions that can impact carbohydrate metabolism. Fortunately, genetic conditions are fairly rare. However, disorders of carbohydrate metabolism that are

acquired over time, such as type 2 diabetes, are becoming more common. In this section, we will give a brief overview of these conditions that impact carbohydrate metabolism, and for greater depth, see elsewhere in this Encyclopedia.

One of the most common disorders of carbohydrate metabolism is diabetes. There are multiple forms of diabetes (type 1, type 2, and gestational), but all have in common elevated blood glucose levels resulting from the inability of glucose to enter cellular tissues to be oxidized for energy. In type 1 diabetes, this is due to the inability of the pancreas to make insulin, but in type 2 diabetes and gestational diabetes, insulin production may be normal, but the tissues are resistant to the action of insulin. See elsewhere in this Encyclopedia for more information on these conditions.

Inborn errors of carbohydrate metabolism typically result from defects or deficiencies in certain enzymes involved in carbohydrate metabolism. In fructose metabolism, a deficiency in fructokinase can lead to elevated levels of fructose in the urine, which is relatively benign. However, a deficiency in aldolase B (hereditary fructose intolerance), which cleaves fructose 1-phosphate into precursors of glycolysis, can lead to hypoglycemia as an accumulation of fructose 1-phosphate inhibits gluconeogenesis and glycogenolysis. In galactose metabolism, classical galactosemia results from a deficiency in galactosyl uridylyltransferase, which causes a buildup of galactose 1-phosphate, which can lead to systemic problems, such as hepatomegaly, cataracts, and brain damage. While some of these deficiencies can lead to serious physiological issues and potentially death if not treated, most individuals are able to successfully manage their condition by avoiding the offending sugar in their diet.

Deficiencies or defects in enzymes involved in the direct metabolism of glucose have much more serious effects and, because of the reliance on glucose for energy, are difficult to treat with diet. For example, disorders in the metabolism of pyruvate due to deficiencies in pyruvate dehydrogenase or pyruvate carboxylase, while rare, typically lead to neurological issues, lactic acidosis, seizures, developmental delay, and failure to thrive. In the most severe cases, infants with these conditions will not survive. There are a number of enzyme deficiency diseases that have been grouped together in a family of glycogen storage diseases. As many as 11 different diseases have been characterized, each involving a different enzyme in glucose metabolism and varying in their severity and occurrence. For example, glycogen storage disease type V (McArdle's disease) is a deficiency in muscle glycogen phosphorylase with fairly minor effects, such as exercise-induced muscle pain and myoglobinuria. However, glycogen storage disease type IV (Andersen disease), which results from a deficiency in the glycogen branching enzyme, leads to failure to thrive and is usually fatal. While several types of glycogen storage diseases exist, the overall incidence of glycogen storage diseases is 1 in 20 000–25 000. While the frequency of each type is not completely known, it appears that glycogen storage disease types I, II, III, and VI are the most common, while types IV, V, and VII are very rare. While all types must be closely monitored, with treatment, the prognosis is better for those with types I, III, V, and VI. Type IV, type 0, and sometimes type II can result in early death.

See also: Food Intolerance: Lactose Intolerance; Fructose: Sources, Metabolism, and Health; Glucose: Glucose Intolerance; Glucose: Metabolism and Regulation; Lactose; Prebiotics; Starch: Structure, Property, and Determination; Starch; Sucrose: Dietary Importance.

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Carcinogenic: Carcinogenic Substances in Food

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Introduction

Substances that are known or suspected to be carcinogenic to experimental animals and/or humans are widespread throughout the environment. They occur naturally in the physical environment and are found in a very large number of higher plants, fungi, and microorganisms, many of which are part of the human diet. Some carcinogens have also been introduced into the human diet as a result of traditional cooking and preserving practices. Although carcinogens act through a wide variety of mechanisms, a substantial number have a common mechanism of action in that they react with the genetic material of the body, DNA. These so-called genotoxic carcinogens generally require metabolic activation by the 'host' animal to express their carcinogenicity. Although substantial efforts are being made to develop short-term, nonanimal tests to predict the carcinogenicity of chemicals, animal bioassays remain the only reliable method for establishing the potential of a chemical to be a carcinogen and form the basis of current approaches for the control of potentially carcinogenic chemicals in the human diet.

Naturally Occurring Carcinogens

It has been estimated that the total number of known chemicals exceeds 7 million and that the great majority are naturally occurring. Although only a very small proportion (perhaps less than 0.01%) of these chemicals have been tested for carcinogenic potential in laboratory studies, a high proportion (as high as 50% in some evaluations) have been found to be positive. Therefore, even allowing for the imperfect selection and testing process, it is likely that there are a very large number of naturally occurring carcinogenic chemicals in the universe of chemicals and therefore in the food we eat.

Naturally occurring substances identified as carcinogens in animals and/or humans by the range of approaches available for this purpose include inorganic compounds, organometallic compounds, and both simple and complex organic chemicals (see Table 1). These materials are present in the environment either as naturally occurring minerals or as a result of natural processes acting in the environment such as combustion, radioactive decay, or biodegradation of plant materials to oils. They are also widespread throughout the plant kingdom in both edible and nonedible plants and in many fungi and in unicellular organisms.

Inorganic Chemical Carcinogens

Many metallic elements are present as contaminants in food, being derived from a range of sources including the water used in food processing, soil residues, packaging, and cooking equipment. A number of metals and some of their salts have been shown to be carcinogenic in animals and/or humans, particularly to the lungs. These include arsenic, beryllium, cadmium, chromium, and nickel. Little is known about the mechanism by which metals cause cancer, although evidence is emerging that some metal ions affect the fidelity of an enzyme involved in the biosynthesis of DNA resulting in abnormal DNA being produced. A number of naturally occurring radioactive elements are also carcinogenic, particularly to the lungs. These include uranium and radium (metals) and radon (a gas), and these may act by damaging DNA directly or by increasing oxidative damage as a result of an increase in reactive radical species. In addition, some naturally occurring minerals such as asbestos, silica, and talc are known to be carcinogenic to animals and humans under some circumstances, probably acting by activating macrophages to generate damaging active oxygen species.

Organic Chemicals: Complex Natural Mixtures

The earliest association made between the development of cancer in humans and exposure to an essentially natural rather than man-made chemical was that between scrotal (skin) cancer and soot by Percival Pott in 1775. However, the specific chemical(s) responsible (polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene and 7,12-dimethylbenzanthracene) were not identified until more than a century later. Since then, a number of other naturally occurring materials have been shown to be carcinogenic. These have included mineral oils, shale oils, and wood dust/shavings, the oils being carcinogenic to the skin, and wood dust to the nasal cavity. Inadvertent ingestion of small amounts of such materials with food may be difficult to avoid.

Organic Chemicals in Higher Plants

Although the acute toxicity of many plant species has been known since written records first appeared, only comparatively recently has the carcinogenicity of plant-derived products been recognized. The list of confirmed animal carcinogens present in plants is still relatively small, and few, if any, are confirmed or suspected human carcinogens. However, developments in analytic chemistry will allow an increasingly detailed inventory to be made of chemicals in plants, which will undoubtedly result in the discovery of many more carcinogens in our foodstuffs. The identification of over 1000 chemicals in coffee beans and the observation that whereas only 3% of the chemicals had been tested for carcinogenicity, nearly 70% of these tested positive are clear pointers to future directions. Although it can be argued that the majority of these compounds are present at very low levels in plants and so the risk to man from any individual compound may be small, reliable methods for assessing both hazard and risk of low-level

exposure are not well developed. In addition, methods for assessing the hazard from complex mixtures of chemicals are also poorly developed, resulting in additional uncertainty in evaluating the risk-posed by natural materials (see the succeeding text). The identified chemical carcinogens in plants tend to be secondary metabolites, often present as part of the plants natural defense mechanism against predation (i.e., natural pesticides), and as such are widespread in fruit, vegetables, herbs, and spices (see Table 2).

One of the first classes of toxic compounds in plants to be identified was the pyrrolizidine alkaloids from the genus *Senecio*. Subsequently, more than 200 related compounds have been isolated from numerous genera and species, many of which are potent liver toxins and liver carcinogens. Other classes of alkaloids found in the plant kingdom include derivatives of the nicotine alkaloids, such as *N*-nitrosonornicotine, which are present in tobacco leaves and are known to be carcinogenic to animals. Tobacco leaves also contain a range of compounds that have been shown to potentiate the carcinogenic effect of the alkaloids present.

Many other classes of carcinogenic plant products have been identified. These include glycosides of azoxy alcohols such as cycasin, an animal carcinogen at various sites including the liver and also a possible human carcinogen. Arecoline, an alkaloid from betel (areca) nuts, is believed to cause mouth cancer in humans, while isoprene glycosides, such as ptaquiloside found in bracken, are liver carcinogens, and phenolic alkylbenzenes such as safrole present in many herbs and vegetables are also principally liver carcinogens. Other phenolic compounds including flavonoids, such as quercetin, rutin, and kaempferol, and tannins, such as trapain and brevifolin, are potent mutagens, but evidence for their carcinogenicity is lacking. In fact, many of these compounds have been shown to exert anticarcinogenic effects.

Table 1 Examples of naturally occurring substances that are believed to be carcinogenic in experimental animals and/or humans

Inorganic chemicals
Arsenic; beryllium; chromium; cobalt; cadmium; lead; manganese; nickel
Polonium; radium; uranium; radon (gas)
Asbestos; silica (glass fiber); talc
Organic chemicals – complex mixtures
Mineral oils; shale oil; soot; wood shaving/dust
Organic chemicals in higher plants
Cycasin (cycads) arecoline (betel nuts); safrole (*Sassafras*); pyrrolizidine alkaloids (Boraginaceae and Compositae); ptaquilosides (bracken [*Pteridium*]); nitrosoalkaloids (tobacco [*Nicotiana*])
Organic chemicals in lower-order plants and microorganisms
Agaratine (mushrooms); aflatoxin, ochratoxin, sterigmatocystin (*Aspergillus* spp. and others); mitomycin, streptozotocin, daunomycin, actinomycin (*Streptomyces* spp.)

Organic Chemical Carcinogens in Other Edible Plants and in Microorganisms

Chemical carcinogens are also found in a wide range of lower plants, such as fungi, and in microorganisms. Simple and complex hydrazines are found in many species of mushroom and have been shown to produce tumors in many tissues in experimental animals. Mycotoxins such as aflatoxin B_1 and the related polynuclear compounds produced by *Aspergillus* species are some of the most potent carcinogens known, being active at dose levels in the nanogram per kilogram range. Human exposure to such compounds occurs when cereal crops or nuts are stored in humid conditions, as they are in many parts of equatorial Africa and China. Aflatoxin B_1 is one of the few established human carcinogens found in the plant kingdom. Other carcinogenic compounds produced as natural products include the antibiotics adriamycin and daunomycin and the antineoplastic agent streptozotocin isolated from microorganisms of the genus *Streptomyces*.

Carcinogens Produced by Food Processing

Despite the widespread occurrence of potentially carcinogenic chemicals in the plant kingdom, most foodstuffs contain only low levels of these chemicals. However, it has now been recognized that a number of processes used in food preparation/processing can introduce significant amounts of carcinogens into the food or the local environment. The most widely studied of these processes is preservation of meats and fish by salting or smoking, grilling or broiling, and cooking in vegetable oils.

Traditional methods for preserving meat and fish involve either salting or smoking. Epidemiological evidence has been found for an association between an increased incidence of

Table 2 Some naturally occurring carcinogenic plant pesticides (a) and their sources (b)

(a)	
Chemical class	*Examples*
Aldehyde	Crotonaldehyde; benzaldehyde; hexanal
Hydrazine/hydrazone	*N*-methyl-*N*-formylhydrazine; methylhydrazine; pentanal methylformylhydrazone
Alcohol	Methylbenzyl alcohol; catechol
Ester	Ethyl acrylate; benzyl acetate
Simple heterocycles	Coumarin; hydroquinone; safrole; sesamol; 8-methoxypsoralen
Polyphenols	Quercetin
(b)	
Generic source	*Examples*
Fruit	Apple; apricot; cherry; grapefruit; lemon; melon; peach; pear; pineapple
Root vegetables	Carrot; onion; parsnip; radish; turnip
Brassica	Broccoli; Brussels sprout; cabbage
Herbs	Coriander; dill; fennel; mint; sage; tarragon
Spices	Allspice; caraway; cardamom; nutmeg; paprika; turmeric

cancer of the mouth and pharynx and intake of salted meat and fish. It seems likely that a reaction between sodium nitrate and/or nitrite used for preserving the meat and alkylamides present in the meat results in the formation of *N*-nitrosamines and nitrosamides. These compounds have been shown to be potent carcinogens in animal experiments to the mouth, pharynx, and other sites. Levels of nitrosamines in cured meats and fish can be as high as 100–200 ppb (parts per billion) for the simple alkylnitrosamines and between 10 and 100 ppb for volatile heterocyclic nitrosamines. Although dose levels required to induce tumor formation in animal studies are substantially higher than those likely to be ingested by man, there is a concern that the presence of nitrosamines in food presents a significant hazard to man.

Preservation of meats and fish by smoking has also been shown to introduce chemicals known to be carcinogenic to animals, particularly PAHs, although direct evidence for an association between an increased incidence of human cancers and consumption of smoked meat and fish is lacking.

The frying or grilling of meats and fish has been found to generate significant quantities of heterocyclic nitrogenous compounds derived from amino acids present in foods. These so-called cooked food mutagens include 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (methyl-IQ$_x$), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), and 2-aminodipyrido[1,2-a:3′,2-d]imidazole (Glu-P-2). They are some of the most potent bacterial mutagens known and have been shown to induce a wide range of tumors in animals. Levels as high as 500 ppb have been found in grilled chicken, and it has been suggested that they may be implicated in the induction of colon and breast cancers in humans. PAHs can also be generated by the grilling of meat and fish and both carcinogenic and noncarcinogenic compounds have been identified. Levels of one particular PAH in foods, the carcinogen benzo(a)pyrene, have been reported to vary from <1 ppb in grain to more than 30 ppb in singed meat.

Heating oils in order to cook foods has also been found to generate a range of carcinogenic chemicals, including PAHs. However, many of the compounds produced are volatile and may therefore represent more of a hazard to the cook than to the food consumer. Thus, cooking with unrefined rapeseed or soya bean oil, which contain significant levels of the polyunsaturated fatty acid linolenic acid, has been shown to result in the release of aldehydes including formaldehyde, acetaldehyde, and acrolein, hydrocarbons including 1,3-butadiene and benzene, and other chemicals. Many of these compounds are mutagenic to bacteria and carcinogenic in animals, and in areas of the world where such cooking practices are common (e.g., China), the incidence of lung cancer in the exposed population is high. The International Agency for Research on Cancer (IARC) considers that emissions from high-temperature frying are 'probably carcinogenic to humans.'

Mechanisms of Carcinogenicity

It is well established that cancer is a multistep process and that chemical carcinogens can induce neoplasia by a wide range of mechanisms involving either interaction with the hereditary material of the organism or interference with one of the many cellular control systems. The former compounds, known as genotoxic carcinogens, interact directly with DNA, resulting in a permanent heritable change to a cell following replication (i.e., an altered genotype). In contrast, nongenotoxic (the so-called epigenetic) carcinogens do not interact directly with DNA but cause cancer by other mechanisms.

Chemicals that react with DNA are invariably electrophiles (i.e., they possess one or more electron-deficient centers in the molecule) that target the nucleophilic (electron-rich) sites in the DNA. The electrophilic center may be present in the molecule itself (activation-independent) as in β-propiolactone, dimethyl sulfate, and α,β-unsaturated aldehydes or be generated following metabolism (activation-dependent) in the target species.

Examples of classes of compounds that are converted to reactive electrophiles by oxidative metabolism include nitrosamines, chlorinated alkanes, hydrazines, and PAHs. Because of the inherent reactivity of these species, they react not only with DNA but also with other cellular macromolecules such as RNA and proteins. These reactions protect the cell against the carcinogenicity of the chemical by reducing the amount of electrophile available to react with DNA, but may lead to other forms of damage and ultimately cell death.

The enzyme system considered to be mainly involved in the activation of chemicals to carcinogenic species is the so-called mixed function oxidase system. This enzyme complex is centered on cytochrome P450 and is present in most, if not all, of the organs of the body. The enzyme system consists of a very large family of related isoenzymes of differing substrate specificity and has a widespread distribution in the animal kingdom. Early work with this enzyme system suggested that only certain isoenzymes were responsible for the activation of carcinogens, although it is now clear that different isoenzymes may activate the same compound in different species.

Most chemical carcinogens appear to be substrates of one particular isoenzyme called CYP1A1. Molecular modeling has shown that only relatively flat (planar) molecules are oxygenated by this cytochrome. Common carcinogens activated by this isoenzyme include PAHs, aflatoxins, and 9-hydroxyellipticine, whereas the related isoenzyme CYP1A2 activates arylamines and amides such as 2-acetylaminofluorene and the cooked food mutagens. Other subfamilies of cytochromes involved in activating carcinogens include CYP2E1, which is known to act on a wide range of small molecules such as dialkylnitrosamines, urethane, vinyl monomers and haloalkanes, and CYP3A, which also activates PAHs, aflatoxins, and cooked food mutagens.

The chemistry of the activation process varies with the type of carcinogen. The oxidation of aflatoxin B_1, for example, results in the formation of the 8,9-epoxide in a single step, whereas the activation of PAHs, such as benzo(a)pyrene, is a multistep process involving an epoxide that is converted to a diol by epoxide hydrolase, which is then converted to the proximate carcinogenic species, a diol epoxide. Activation of arylamines and amides to DNA reactive species, in contrast, frequently involves an initial oxidation step to an *N*-hydroxy derivative, which is then further metabolized to a highly reactive *N*-*O*-ester. This latter reaction is catalyzed by a transferase

enzyme, usually sulfotransferase or acetyltransferase for arylamines and glucuronotransferase for arylamides. Other oxidative reactions result in the formation of unstable compounds that decompose spontaneously to the ultimate carcinogenic species. Thus, simple nitrosamines are oxidized by CYP2E1 to an α-hydroxy intermediate that breaks down to the electrophilic alkyldiazonium ion.

Enzyme systems other than the mixed function oxidase system may also be involved in the metabolic activation of carcinogens. Thus, for aflatoxins, there is evidence that prostaglandin H synthetase can activate this group of compounds, and for arylamines, oxidation may be carried out by prostaglandin peroxidase, by myeloperoxidase, or by flavin-containing monooxygenases.

The direct metabolic activation of compounds to carcinogenic species by phase II metabolism, a process normally associated with detoxification, can also occur. Thus, safrole and related compounds are converted to their sulfate esters, the ultimate carcinogenic species by the phase II enzyme, sulfotransferase.

Metabolic Activation of Epigenetic Carcinogens

Since there is no common mechanism describing the action of epigenetic carcinogens, making predictions as to the likely carcinogenic potential of these chemicals is extremely challenging, and generalizations concerning the effect of metabolism on the activity of chemicals acting by a nongenotoxic mechanism are not possible. The activity of a number of epigenetic carcinogens is reduced as a result of metabolic activation, although in the case of one group of epigenetic carcinogens that produce renal tumors in the rat by binding to and preventing the degradation of a specific kidney protein, alpha-2-microglobulin, metabolic activation is required for carcinogenic activity. Compounds acting by this mechanism include isophorone and isolimonene, which are present naturally in many fruits. Similarly, a wide range of structurally diverse chemicals induce liver tumors in rodents due to their ability to induce the proliferation of hepatic peroxisomes. Food contaminants such as phthalate diesters, which leach out of packaging materials, fall into this category, although no naturally occurring food chemical has yet been found to be a peroxisome proliferator. Some examples of nongenotoxic mechanisms of carcinogenesis are shown in Table 3.

Carcinogenicity Tests

Animal Bioassays

As the mechanism of carcinogenesis in both humans and animals is not well understood, the only acceptable procedure for determining whether a chemical is likely to be a carcinogen is the examination of experimental animals exposed to the suspect material under carefully controlled conditions. This procedure relies on the assumption that animals will behave in essentially the same way as humans to carcinogen exposure; that is, the mechanism of tumor induction will be similar in both experimental animals and humans. Mechanistically based, short-term tests for carcinogenicity prediction not involving experimental animals are still a distant and elusive goal.

The basic approach for carcinogenicity testing involves administering the test material to two suitable animal species for a considerable proportion of their natural life span. Because of their small size and relatively short life expectancy, the rat and mouse are the species of choice, although the hamster is occasionally used. In the United States, inbred strains of animals are widely used (the F344 rat and the $B6C3F_1$ hybrid mouse), although outbred strains are commonly used in Europe. To examine the carcinogenic potential of food components, the test substance is usually given in the diet, although in some circumstances, administration may be in the drinking water or by gavage. The study continues until a certain proportion in one or other of the treatment groups has died or has been killed in a moribund state. As a minimum, 50 animals are allocated at random to each of the experimental groups, allowing a statistically significant carcinogenic effect to be detected if five animals in a test group develop tumors and no animals in the control group do. During the study, the animal's clinical state is regularly monitored, and at the end of the study, a complete necropsy is performed on all surviving animals. Tumors are assessed by a histopathologist, and an attempt is made to determine whether any tumors seen were the cause of the (early) death of the animal (fatal tumors) or were unrelated to the death (incidental tumors). The procedures of these bioassays are conducted under rigorous conditions defined by the Code of Good Laboratory Practice.

Tests are essentially of two types: The first, used widely under the National Toxicology Program in the United States, is designed to examine the ability of the test material to induce cancer in the species used; the second is aimed at determining the cancer incidence in respect of dose – a classical dose–response study. The former requires a few treatment groups, including a relatively high-dose group in order to maximize

Table 3 Some examples of nongenotoxic mechanisms of carcinogenesis

Mechanism	*Examples of chemical classes*
Promotion	Phorbol esters; barbiturates; chlorinated hydrocarbons
Receptor-mediated (e.g., peroxisome proliferation)	Phthalate diesters; hypolipidemic drugs; chlorinated herbicides
Endocrine modulation	Androgens and estrogens (e.g., 17β-estradiol); antithyroid agents
Immunosuppression	Cyclosporine
Tissue specific toxicity	Metals (e.g., arsenic and beryllium)
Cytotoxicity	Metal chelators; branched chain hydrocarbons

the chance of detecting a carcinogenic effect, whereas the latter requires a wide range of dose groups to define accurately the dose–response relationship.

The analysis of a carcinogenicity bioassay is aimed at determining whether the administration of the test chemical has resulted in an increase in the incidence of tumors at one or more sites compared with the normal background level. In order to accomplish this analysis, two major confounding factors may have to be taken into consideration. The first is the effect of differences in mortality rates between the control and treated groups and the second is the effect of differences in food intake and its consequence on body weight. Both factors can substantially alter the tumor pattern observed in different groups. Early deaths may prevent the animals from reaching tumor-bearing age, and reduced food intake, due perhaps to unpalatability of the diet with the test material admixed and the associated reduction in body weight, may result in a considerable reduction in tumor incidence.

The interpretation of the results of a bioassay is complex, but most authorities work to the 'weight of evidence' principle. This evidence is taken in the light of the adequacy of the bioassay, which is dependent on some of the factors previously discussed. Strong evidence for the compound being a genotoxic carcinogen would be increased malignant tumor incidence in two species, with tumors at multiple sites showing a clear dose–response relationship. Rare or unusual tumors at a site would be given added weight. Equivocal evidence may result from a statistically marginal result or only an increase in commonly occurring benign tumors. Tumor development in only one species and in association with species-specific toxicity is characteristic of nongenotoxic (or epigenetic) carcinogens. Sometimes, problems associated with such findings may be clarified by further mechanistic studies or by reference to historical data. When the data from bioassays are considered in human risk assessment, other factors must clearly also be taken into consideration. These may include evidence of genotoxicity in short-term tests and data on metabolism and potential human exposure. Furthermore, a measure of risk at doses substantially below the bioassay dose may be needed. This may require extrapolation using mathematical models. As yet, no general agreement has been reached as to the most appropriate method, and so, the calculated risk given by different methods may vary considerably. Thus, the final assessment may be made on quite pragmatic grounds, in which the experience and expertise of a number of individuals are drawn on to reach a consensus opinion.

Short-Term Predictive Tests

A large number of test systems have been developed to detect damage to the genetic material of cells in an attempt to predict carcinogenic potential and thereby reduce the reliance on animal tests. *In vitro* assays for detecting genotoxicity (i.e., damage to the cells genome that may be directly or indirectly heritable) include tests to detect gene mutation using bacterial or mammalian cells and the so-called indicator tests that detect mechanistic changes associated with the formation of mutations, such as the binding of foreign molecules to the DNA bases. *In vitro* tests are backed up by short-term *in vivo* tests to confirm that the effects seen *in vitro* are realized in the whole animal. These tests are usually undertaken in rats, mice (ordinary and transgenic), and/or hamsters but can also be done in fruit flies (see Table 4). Since many of the cell systems used are unable to activate metabolically the majority of test chemicals, an exogenous mammalian metabolizing system, the so-called S-9 mix, is incorporated into the assay. Chromosome damage seen in such tests includes chromosome and chromatid gaps and breaks, rings, fragments, dicentrics, translocations, and inversions. A short-term *in vivo* assay measuring unscheduled DNA synthesis in rat liver or gut is recommended by most regulatory authorities if there are a positive response in any *in vitro* assay and a negative response in an *in vivo* cytogenetics assay. Other test methods and end points are under consideration by regulatory authorities as indicators of genotoxic potential including the COMET assay for assessing DNA damage and aneuploidy, the change in chromosome number resulting from damage to the cellular architecture (spindle) controlling chromosome replication.

The last two decades has seen extensive efforts to determine whether short-term tests are suitable for predicting carcinogenic potential. The early validation studies suggested good predictability, with correct identification of over 90% of carcinogens (high sensitivity) and over 90% of noncarcinogens (high specificity). In later evaluations, a much lower figure (60%) was obtained. However, when carcinogens known to

Table 4 Short-term test systems for predicting carcinogenic potential

Test system	*Cell used*	*End point*
Bacterial mutation	*Salmonella typhimurium* TA strains *Escherichia coli* WP2	Reversion to histidine independence
Mammalian gene mutation	Chinese hamster lung (V79) Chinese hamster ovary (CHO) Mouse lymphoma (L5178Y) Human transformed lymphoblastoid (TK6)	Loss of HPRT, TK, or Na^+/K^+ ATPase expression
Chromosome aberration *in vitro*	Chinese hamster fibroblast (CHL) Chinese hamster ovary (CHO) Human peripheral blood lymphocytes(PBL)	Chromosome/chromatid aberration (gaps, breaks, and deletions)
Chromosome damage *in vivo*	Bone marrow erythrocytes (mouse)	Micronuclei induction
Heritable damage *in vivo*	Rodent germ cells	Dominant/lethal mutations; heritable translocations, etc.

HPRT, hypoxanthine phosphoribosyl transferase; TK, thymidine kinase.

react by nongenotoxic mechanisms (e.g., hormones or peroxisome proliferators) were excluded, the predictability was improved suggesting that short-term tests are suitable for detecting those carcinogens that act by a genotoxic mechanism.

Although many regulatory authorities have guidelines for carcinogenicity evaluation, which include short-term tests, they all still require animal studies as the ultimate test for carcinogenicity. However, the use made of short-term tests varies. In the United States, the Food and Drugs Administration recommends a battery of short-term tests for all additives for which cumulative dietary intake is expected to exceed 1.5 μg per person per day in order to assist in the interpretation of animal feeding studies. Some bodies, such as the IARC, use short-term tests as an adjunct to animal carcinogenicity studies in their evaluation processes, giving added weighting in their assessment of likely human risk to an animal carcinogen that is also positive in short-term tests.

However, until a consensus can be reached as to what a positive or negative result in an animal feeding study means in terms of whether the compound may or may not be a human carcinogen, the further development of better (faster/cheaper) short-term tests may be a futile exercise.

Monitoring and Control of Hazards and Risks

The complex mixture of chemicals that constitute food, together with the uncertainty of the specific role of the various components in the diet, has made the control of potential carcinogens in food difficult. In particular, the realization that animal carcinogens, as identified by standard animal bioassays, are widely distributed in the general environment, including food, has made control by total elimination impossible. Control of toxic agents in food particularly contaminants and additives has been achieved by examining their hazard in animal studies. Thus, the establishment of a no observable adverse effect level (NOAEL) is followed by the setting of an acceptable daily intake (ADI) through extrapolation based on the relative sensitivity of animals and humans to toxic events. This extrapolation may also take into consideration other properties of the chemical concerned, such as genotoxic potential. For genotoxic carcinogens, however, it is generally considered that there is no NOAEL, and therefore, acceptable intake is based on estimation of likely risk. A maximum risk of between 10^{-5} and 10^{-6} cancers in a lifetime is considered as an acceptable risk by most authorities, particularly those in the United States (in California, 10^{-5} is called the no significant risk level), and acceptable exposure estimates are determined by extrapolation from animal data. In California, the extrapolation (scaling) factor used to estimate human potency from rat potency is 5.5. For nongenotoxic carcinogens (and for some genotoxic carcinogens, particularly those that act as aneugens), it is considered that the NOAEL approach is acceptable, since the carcinogenic response is the result of a prior toxic event for which a no observed effect level (NOEL) can be determined. In most circumstances, an uncertainty (safety) factor of 100 is applied to the NOAEL, to allow for interspecies variation ($\times 10$) and interindividual variability ($\times 10$). In the case of carcinogens where a NOAEL approach is considered acceptable, sometimes, an extra safety factor is required on the NOEL for the tumors (not necessarily the same as the overall NOAEL for the study). If that is the case, the NOEL for the tumors ÷ high safety factor is calculated and the overall NOAEL for the study ÷ the normal safety factor is calculated and the lower resultant quotient is used to set the ADI. More recently, the two factors of 10 have each been subdivided into pharmacokinetic and pharmacodynamic components to reflect increased understanding of the mechanisms underlying the development of toxicity and to allow for factors associated with special groups such as infants and children. It must be said that the scientific basis to support either the acceptable risk or the NOAEL approach is quite limited as even for the best documented cases, the mechanism of the carcinogenic effect is poorly understood, and the relative sensitivity of laboratory rodents and humans is rarely known.

The unequivocal identification of human carcinogens is difficult since direct experimental approaches are precluded. Thus, epidemiological studies involving both prospective and retrospective studies and both cohort and case control studies may have to be employed. These techniques have limited applications to diet-associated carcinogenesis and have proved most useful in identifying specific carcinogens in the workplace or those used as therapeutic agents, where it is easier to quantitate exposure. The specific problem in identifying dietary carcinogens relates to the complexity of diet, the difficulty in identifying specific components, and the sensitivity of the epidemiological methods themselves. It would seem likely that epidemiological data will only be able to link specific chemical carcinogens in food with a carcinogenic effect in a few favorable circumstances, since such chemicals are likely to be present at low levels and induce only a small increase in tumor incidence over background levels. One such example was the identification of a carcinogenic hydrazone in the mushroom *Gyromitra esculenta*, as a result of an epidemiological study in Finland. Such methods have also indicated the relative importance of lifestyle factors in carcinogenesis: in particular, associations have been made between lack of dietary fiber and colon cancer, between a low intake of fresh fruit and vegetables and stomach cancer, and between excess dietary fat and colon and breast cancers, although the specific chemicals responsible have not been identified with any certainty.

Most of the activity aimed at controlling carcinogens in food has been directed at preventing addition of potentially carcinogenic substances to the existing background level of natural carcinogens. This has been tackled through the application of laws governing the adulteration of food, the first of which were enacted in the mid-nineteenth century in the United Kingdom. The relevant UK legislation was the 1990 Food Safety Act, governing the nature and quality of food and its nutritive value. This act, like its forerunner, the 1955 Food and Drug Act, requires that the constituents of food should not be injurious to health and is still in force. There is separate legislation for particular groups of compounds in food, such as pesticides, veterinary medicines, food additives, and food contact materials. Many aspects of food safety including carcinogens in food are now governed under European Union regulations, with risk assessments undertaken by the European Food Safety Authority in Parma, Italy.

The position in the United States up to 1958 was similar to that in the United Kingdom. Food was considered adulterated if injury could arise from its use. Legislation was based on

traditional food, added substances, and unavoidable added substances (contaminants). For added substances, listed in an inventory of over 3000 chemicals and often referred to as 'Everything Added to Food in the United States' (EAFUS), the food was considered adulterated if the added substance could render the food injurious to health; for unavoidably added substances, a balance was applied between the essential nature of the food material and the degree of contamination. These strictures applied to both carcinogenic and noncarcinogenic toxicants. In 1958, a change in emphasis was introduced through the Food Additives Amendment. This established a licensing scheme for substances deliberately added to foods or for substances that could migrate into food, but excluded materials that were generally, through usage, regarded as safe (GRAS). For licensing purposes, the material has to be shown to be 'safe' for its intended use, although in theory at least, the GRAS substance could be a carcinogen.

In 1958, the Delaney Clause was enacted; this required that if there was evidence of carcinogenicity in any test system, the material should be prohibited from food usage. Improved analytic techniques have shown that many foods contain both unintentionally added and natural carcinogens, such as polynuclear aromatic hydrocarbons, nitrosamines, mycotoxins, and arylamines, and no form of regulation could control these materials. Furthermore, bulk components of food may themselves play an important role in the development of carcinogenesis. The recognition that the exclusion of all potentially carcinogenic additives (under the Delaney Clause) is a practical impossibility has given way to the concept of safe tolerance and that safe levels may be set by appropriate, conservative risk assessment in which an insignificant lifetime risk of developing tumors of, for example, 10^{-6} is considered acceptable.

Mixtures of Substances

One criticism of present methods of risk assessment is that assessment is carried out singly; that is, it ignores possible combined action of multiple components in the diet and multiple routes of exposure. The enactment of the Food Quality Protection Act (FQPA) of 1996 in the United States changed this, as the act mandated consideration of all pathways of exposure (aggregate risk assessment) and of exposure to more than one pesticide at a time (cumulative risk assessment). There are a number of methodological problems with the latter, but considerable progress has been made in the United States. However, the most dramatic progress has been with regulated substances particularly pesticides and with end points other than carcinogenicity. The UK Department of Health published a report on risk assessment of mixtures, and the European Union, as a whole, has made further progress.

See also: Carcinogens: Identification of Carcinogens; Mutagens; Mycotoxins: Classification; Mycotoxins: Occurrence and Determination; Mycotoxins: Toxicology.

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Carcinogens: Identification of Carcinogens

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Introduction

According to Globocan's estimates, 14.1 million new cancer cases and 8.2 million cancer deaths occurred worldwide in 2012, and the burden of cancer is expected to increase over the coming decades because of continuing global demographic and epidemiological transitions, particularly in low- and middle-income countries. More than 20 million new annual cancer cases have been forecast for 2025.

Identifying carcinogens and limiting human exposure are key aspects of cancer prevention. The International Agency for Research on Cancer (IARC) Monographs Programme is an authoritative source for the identification of carcinogenic hazards in the environment. These hazards include chemicals, complex mixtures, occupational exposures, physical agents, biological agents, and lifestyle factors. In order to identify such hazards, the IARC Monographs employ international working groups of independent scientists who evaluate human exposure, epidemiological evidence, evidence from experiments in animals, and from mechanistic studies. Working groups make scientific, qualitative judgments on the evidence for or against carcinogenicity based on the available data.

Each *IARC Monograph* includes information on the production and use, occurrence, sources, and routes of human occupational and environmental exposure. All pertinent epidemiological studies are considered in order to asses the risk of developing cancer in different population groups. Several criteria are used to asses causality and temporality, the precision of estimates of effect, biological plausibility, and the coherence of the overall available literature. From an experimental point of view, an agent is considered to be carcinogenic when its administration to experimental animals induces a statistically significant rise in the incidence of one or more histological types of neoplasia, reduces latency, or increases the severity or multiplicity of cancers, as compared to results in animals not exposed to the substance. The IARC Monographs consider studies that follow the guidelines for conducting long-term carcinogenicity experiments, such as those developed by the Organisation for Economic Cooperation and Development (OECD). Finally, the evaluation of mechanistic data may provide evidence of carcinogenicity and also help in assessing the relevance and importance of findings of cancer in animals and in humans. The nature of these data depends on the biological activity of the agent being considered. Relevant topics may include toxicokinetics, mechanisms of carcinogenesis, susceptible individuals, populations and life-stages, and other adverse effects.

Since 1971, more than 900 agents have been evaluated by the *IARC Monographs* of which more than 400 have been identified as carcinogenic to humans (IARC Group 1), probably carcinogenic to humans (IARC Group 2A), or possibly carcinogenic to humans (IARC Group 2B). These categories only refer to the strength of the evidence that an exposure is carcinogenic, as opposed to the extent of its carcinogenic activity (potency). Classifications may change as new information becomes available. The IARC classifies an agent as *carcinogenic* when sufficient evidence in humans supports a causal relationship between exposure to the agent and an increased risk of cancer in target organs (the identification of a specific target organ or tissue does not preclude the possibility that the agent might also cause cancer at other sites). When the evidence in humans is less than sufficient but evidence in experimental animals and on relevant mechanisms of carcinogenicity in exposed humans are strong, the agent may be classified as carcinogenic as well.

At present, the IARC Monographs have classified the following agents in food and drink as carcinogenic to humans: aflatoxins, ethanol in alcoholic beverages, arsenic in drinking water, and Chinese-style salted fish. This article reviews the listed agents with regard to the source of exposure, patterns of consumption, and evidence of carcinogenicity in humans. The article also discusses several other food components identified as probable or possible carcinogens, as well as food agents recommended by the IARC Monographs for future assessment.

Sources and Production

Aflatoxins

Aflatoxins are natural carcinogens produced primarily by the common fungus *Aspergillus flavus* and the closely related species *Aspergillus parasiticus*. Aflatoxin B1 is the most common and potent of this large group of mycotoxins. It develops when the temperatures are between 24 and 35 °C and the moisture content exceeds 7% (e.g., at latitudes between 40° N and 40° S of the equator). Contamination may occur before harvest, resulting in potentially high levels of aflatoxins in dietary staples, such as maize, peanuts, and rice, and continuing difficulty in eliminating aflatoxins from these products. Various spices sometimes contain aflatoxins, but tree nuts and a wide range of other foods are contaminated less frequently.

With other crops, the prevention of aflatoxin formation mainly relies on the avoidance of contamination after harvest through the use of rapid drying and good storage practices.

Alcoholic Beverages

The predominant commercially produced alcoholic beverages are beer, wine, and spirits. They may be consumed in combination to increase the alcoholic strength of the beverage (e.g., wine may be fortified with spirits). In many developing countries, other types of alcoholic beverage, such as sorghum beer, palm wine, or sugarcane spirits, are produced at home or locally through the fermentation of seeds, grains, fruit, vegetables, or parts of palm trees, using a fairly simple production process.

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The basic ingredients for beer are malted barley, water, hops, yeast, and sometimes wheat. Beer is available in nearly all countries from transnational producers, their subsidiaries, joint ventures, or local partners. Different beer products may vary widely in ethanol content, from low or no-alcohol beers (pure alcohol, 0.5–2.5%), to stronger stouts, ales, and other malt-based products containing up to 14% pure alcohol.

Nearly all wine is produced from grapes, but wine is also made from other fruits and berries. Wine usually ranges in strength from 10% to 14% pure alcohol. Products with higher alcohol content, such as sherries, ports, cognacs, and brandies, are generally classified as fortified wines, and their alcohol content may be as high as 20–22%.

Spirits are frequently produced from cereals (e.g., corn, wheat), beets, molasses, grapes, cane sugar, potatoes, and other fruits. Distilled spirits vary widely in strength because the distillation process may produce liquids with very high alcoholic content. Approximately 54% of distilled spirits worldwide are so-called traditional or local products. Some previously local products have become global commodities, such as Scotch whisky and tequila, which are now industrially produced and traded internationally. Other spirits continue to be made using low-level traditional technology, which in some cases (e.g., the *kachasu*, or kill-me-quick, of Southern Africa) may involve the use of any available ethanol-strengthening agents, including battery acid. Other regional or national products include the *aguardiente* or cane spirits from parts of Latin America, the Republic of Korea's *shochu*, Mexico's *pulque*, India's *arrack*, and products specifically consumed by local ethnic or tribal groups. Local products are often produced for home use or trade in the informal sectors of developing economies.

Arsenic in Drinking-Water

Arsenic is a naturally occurring metalloid, and it is mainly transported through the environment by water. Arsenic is a component of more than 245 minerals, especially sulfide-bearing mineral deposits, and it has a strong affinity for pyrite, which is one of the more common minerals in the earth's crust. The weathering of rocks converts arsenic sulfides into arsenic trioxide, which enters the arsenic cycle as dust or by dissolution in rain, rivers, or groundwater, thus entering the food chain (e.g., arsenic may be abundant in certain seafoods). The form and concentration of arsenic depend on several factors, including whether the water is oxygenated (e.g., arsenites predominate under reducing conditions, such as those found in deep well-water), the degree of biological activity associated with the conversion of inorganic arsenic to methylated arsenic acids, the type of water source (e.g., water from the open ocean vs. surface freshwater or groundwater), and the proximity of the water source to arsenic-rich geological formations and other anthropogenic sources. The natural and anthropogenic occurrence of arsenic in drinking water has been recognized as a major public health issue in several regions of the world, where high concentrations in fruit, vegetables, grain, and meat have been reported.

Chinese-Style Salted Fish

In southern China, about 20 different fish, such as red snapper, threadfin, Spanish mackerel, croaker, and Japanese mackerel, are prepared in salted form. Salted fish are prepared by salting, brining, dry-salting, pickle curing, or a combination of these treatments. In brining, fish are placed in a solution of crude salt and water until the fish tissue has absorbed the required amount of salt. For dry-salting, fish are mixed with dry salt, and the resultant brine, produced by the dissolution of the salt in the water content of the fish, is allowed to drain away. During pickling or pickle-curing, the fish is mixed with salt and stored under the brine (pickle), which forms when the salt dissolves in the water extracted from the fish. The fish are then dried under the sun for 1–7 days, depending on the size of the fish and the weather. Salted fish prepared in this way are called tough- or hard-meat-salted fish. Softening the fish by decomposition prior to salting produces soft-meat-salted fish that are usually stored for up to 5 months before consumption.

During the drying of salted fish, infestation by insects can be a serious problem, especially in damp weather. In southern China, the high average annual temperature and humidity are favorable to the growth of bacteria such as *Staphylococci*.

Human Exposure

Aflatoxins

Ample evidence suggests that a large proportion of sub-Saharan Africa, south-east Asia, and Latin America experience high-level chronic dietary exposure to foodborne mycotoxins, particularly aflatoxins. The growth of aflatoxin-producing molds is greatly favored by the high temperature and relative humidity, in combination with inadequate processing facilities, storage, or transportation. Aflatoxins have been found in a variety of agricultural commodities, but the most pronounced contamination has been identified in maize, peanuts, cottonseed, and tree nuts. Children are chronically exposed to high levels of aflatoxins in areas where food contamination is endemic and this pattern of exposure continues throughout life. Acute poisoning following exposure to high doses of aflatoxin (aflatoxicosis), is a major public health problem. However, evaluating the extent and severity of aflatoxin's exposure in developing countries can be difficult since diseases in these regions often go unreported, and only major outbreaks are known to investigators. A precise characterization of the exposure might be derived from direct assessments (quantitation of biomarkers such as urinary aflatoxins and aflatoxin-albumin adduct, or the 249*TP53* mutation), in combination with reports of contamination in foods sampled from markets and trade shipments, reports of acute poisoning incidences, and the measurement of organ toxin levels in postmortem reports.

Alcoholic Beverages

Nearly 2 billion adults regularly consume alcoholic beverages, with an average daily consumption of 10–12 g of ethanol (about one drink). Alcoholic beverages have been an integral part of many cultures for thousands of years, and the use of fermented alcoholic beverages persists in all tribal and village societies, except those in Australia, Oceania, and North America. In general, men consume substantially more alcoholic drinks than women, but also age and socioeconomic status are key factors

in determining the levels of alcohol consumption. Within almost all populations, consumption varies widely, usually as a function of availability, price, culture, religion, and dependency. Worldwide, the average consumption is nearly four times higher in high-income countries than it is in low-income countries, and alcohol consumption tends to be highest in Europe, North America, and Oceania. The most recent data from the OECD show that adult individuals (aged ≥ 15 years) drink an average of 9.4 l of pure alcohol each year. Consumption varies within countries, however. Many people do not consume alcoholic drinks, and alcoholic beverages are illegal in Islamic countries. Some people drink occasionally, and others consume at least 15–25% of their dietary energy as alcohol. Consumption also varies with regard to the different types of beverage. Beer is the most widely consumed alcoholic drink worldwide, and it is particularly popular in Europe, North America, and Oceania. Wine is mainly consumed in Europe, Australasia, and the Americas, with highest levels of consumption in western and southern Europe. The data on average consumption of spirits or liquors is scarce. An increasing number of young people report excessive alcohol consumption, especially in high-income countries. Excessive alcohol consumption is also referred to as binge drinking, and it is defined as the consumption of ≥ 60 g of pure alcohol on the same occasion on at least one day in the last month.

Records do not yet track the sales of alcoholic beverages that are not taxed in the country where they are consumed. As a consequence, the average consumption of alcoholic drinks, internationally and nationally, is underestimated. This unrecorded drinking includes the consumption of homemade or informally produced alcohol (legal or illegal), smuggled alcohol, alcohol intended for industrial or medical uses, and alcohol obtained through cross-border shopping.

Arsenic in Drinking Water

Nonoccupational exposure to arsenic occurs mainly through food, except in areas with high levels of arsenic in drinking water. High concentrations of arsenic have been measured in drinking water in large areas of Bangladesh, China, West Bengal (India), and smaller areas of Argentina, Australia, Chile, Mexico, Taiwan (China), the United States, and Vietnam. In some areas of Japan, Mexico, Thailand, Brazil, Australia, and the United States, mining, smelting, and other industrial activities have contributed to elevated concentrations of arsenic in local water sources. Several other regions have reported drinking water that is highly contaminated with arsenic. In most of these regions, the drinking-water source is groundwater, which is naturally contaminated from arsenic-rich geological formations. The epidemiological evidence from drinking-water exposure indicates that carcinogenicity is related to exposure to As^{III} and As^{V}, which are the predominant oxidation states of arsenic under reducing and oxygenated conditions, respectively.

Chinese-Style Salted Fish

Salted fish is popular among coastal populations in southern China and in Southeast Asian countries, where it is often used as an accompaniment to other dishes or rice. Although the amount consumed at any one time is small (not more than 10 g), the dish may appear at every meal. Salted fish mixed with rice has also been used as a traditional weaning food, as well as a food for infants. The consumption of salted fish in Chinese populations has been declining since the second half of the twentieth century, and consumption during weaning and early childhood is now rare. Both cultural changes and other methods of preserving food may be responsible for this decrease.

Evidence of Carcinogenicity

Aflatoxins

The adverse toxicological consequences of aflatoxins in populations are quite varied, owing to a wide range of exposures that lead to a panel of acute effects, including rapid death, impaired growth, immune suppression, and chronic outcomes, such as hepatocellular carcinoma (HCC). The mold-produced aflatoxins were first considered by the IARC Monographs in 1971 and identified as carcinogens in animals before they were shown to be human liver carcinogens (IARC Group 1) in 2009. The relationship between aflatoxin exposure and liver cancer risk is one of the most extensively documented examples of a widely disseminated environmental carcinogen. Several key steps in the development of aflatoxin-induced carcinogenesis are now well accepted by the research community, and provide strong evidence that the mechanism of action involves activation to a genotoxic metabolite, formation of DNA adducts, and induction of a specific mutation in codon 249 of the tumor suppressor *TP53* gene. Geographically distinct cohort studies have independently found that aflatoxin exposure significantly increases the risk of developing HCC. In addition, several case–control studies have confirmed that aflatoxins act synergistically with hepatitis B virus (HBV) to increase the risk of liver cancer 12-fold.

Alcoholic Beverages

As evaluated by the IARC Monographs, the consumption of alcoholic beverages causes cancers of the oral cavity, pharynx, larynx, esophagus, colorectum, liver (HCC), and female breast. An association has also been observed with pancreatic cancer. For the majority of the above-cited cancer sites, risk estimates emphasize a dose-dependent relationship that does not vary by beverage type and does not show a threshold of intake, since the adverse effect is observed even at consumption of less than 1 alcoholic drink per day. For cancers of the upper digestive tract a synergistic effect with tobacco smoking is observed. Drinking patterns play an important role in modulating this relationship. Alcohol consumption results in exposure to acetaldehyde, which is derived from the beverage itself and formed endogenously. Both acetaldehyde and ethanol associated with the consumption of alcoholic beverages are carcinogenic to humans (IARC Group 1). Acetaldehyde is detoxified by aldehyde dehydrogenases (ALDH). The ALDH2*2 variant allele, which encodes an inactive enzyme, is prevalent (up to 30%) in East Asian populations. Heterozygous carriers, who have about 10% enzyme activity, accumulate acetaldehyde and have higher relative risks of

alcohol-related esophageal, head and neck cancers, as compared to individuals with the common alleles.

Among nonsmokers, reported relative risks for esophageal squamous cell carcinoma and laryngeal cancer range from 0.74 (95% CI: 0.47–1.16) for light- ($\leq$1 drink corresponding to 10–12 g of ethanol) to 3.09 (95% CI: 1.75–5.46) for high- ($\geq$4 drinks corresponding to 50 g of ethanol) alcohol daily intakes. Summary effect estimates for colorectal cancer are 1.11 (95% CI: 0.90–1.38) at 1 drink per day and 1.41 (95% CI: 1.16–1.72) at more than 4–5 drinks per day. Associations between alcohol consumption and HCC are generally found at high intakes, with significant increased risk of about 30–40% occurring at consumption levels >40 g day^{-1}. The risk of breast cancer at 10 g day^{-1} ethanol intake appears to be increased by 8% for postmenopausal women and 9% for premenopausal women. Future research should aim to determine if and to what extent these relative risk functions differ by cancer incidence and mortality.

The overall risk of cancer in men who consume more than two alcoholic drinks per day and in women who consume more than one alcoholic drink per day is 6% higher than it is in people with lower alcohol consumptions. Based solely on the discussed evidence on cancer, even small amounts of alcoholic drinks should be avoided. In addition, excessive alcohol drinking is associated with many other acute consequences, such as alcohol poisoning, injury, and violence.

Arsenic in Drinking water

Epidemiological studies indicate that exposure to arsenic through inhalation or drinking water causes cancer of the lung, skin, and urinary bladder. In northern Chile, a case–control study reported significantly increased risk of lung cancer with increased exposure to arsenic from drinking water, with an odds ratio increasing to 7.1 (95% CI: 3.4–14.8). Evidence also points to a dose–response relationship between drinking arsenic-contaminated water and bladder cancer within exposed populations. The highest risks were seen for people who had experienced over 40 years of exposure, with an odds ratio of 4.1 ($P<0.01$). Dose–response relationships are also suggested for skin cancer (squamous cell carcinoma of the skin) within the exposed populations. A retrospective cohort study reported a standardized mortality ratio of 28 (95% CI: 11–59) for skin cancer deaths, based on seven observed deaths.

The available evidence also indicates an association between exposure to arsenic in drinking water and the development of tumors at several other sites, including the kidney, liver, and prostate. However, various factors prevent one from drawing a solid conclusion, and the possibility of chance or bias cannot be ruled out.

The organic arsenicals monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are the active ingredients of some herbicides, and they are metabolites of inorganic arsenic. Given the sufficient evidence that DMA caused cancer in experimental animals, and because MMA is extensively metabolized into DMA, both compounds are classified as possibly carcinogenic to humans (IARC Group 2B).

Chinese-Style Salted Fish

The IARC Monographs reviewed uncooked salted fish in different countries and concluded that Chinese-style salted fish causes cancer of the nasopharynx and possibly of the stomach. Two possible mechanisms for the observed cancer risk are (1) the formation of *N*-nitroso compounds, including *N*-nitrosamines, during the preparation of salted fish and (2) the activation of the oncogenic Epstein–Barr virus (EBV).

The effect of salted fish on the risk of nasopharyngeal cancer seems to be most pronounced for consumption in early childhood and during weaning, while the association with adult consumption appears weaker. The association remains after adjustment for EBV status. The effect observed on the risk of stomach cancer is modest, and adjustment for important confounding risk factors (including smoking, alcohol, and *Helicobacter pylori* infection) is missing in several studies. Also, studies lack an investigation of the association between salted fish and EBV-positive gastric carcinomas.

Other Food Carcinogens and Future Perspectives

In addition to the previously discussed factors, the IARC Monographs have assessed the evidence for the carcinogenicity of several other food components. The dried leaves of the *Yerba mate* plant are mainly consumed in Argentina, Brazil, Paraguay, Bolivia, Chile, Ecuador, and Uruguay. Most often, the leaves are prepared in a hot infusion beverage drunk from a gourd or other container through a straw, which places very hot fluid at the oropharynx and esophagus. Hot mate drinking has been classified as probably carcinogenic to humans (IARC Group 2A). Several epidemiological studies support the role of hot mate in increasing the risk of upper gastrointestinal tract cancers. A population-based survey conducted in Brazil showed that the mean temperature just before consumption was 69.5 °C. Thus, temperature could act by directly damaging the mucosa or accelerating metabolic reactions, including those with carcinogenic substances in tobacco and alcohol. At present, it is unclear whether the potential carcinogenic effect of mate is due to the components of the plant, to the temperature at which it is consumed, or both, and the possibility of residual confounding by alcohol drinking and tobacco smoking cannot be excluded. The IARC Monographs will re-evaluate mate drinking, in light of new evidence in support of an association between mate consumption and esophageal squamous cell carcinoma.

Processed meat, such as ham, bacon, sausage, and hot dogs, contains high concentrations of preformed nitroso compounds, some of which are potential carcinogens (IARC Group 2A). Red meat contains heme iron, which promotes carcinogenesis through its catalytic activity on the formation of nitroso compounds and lipid oxidation end-products such as 4-hydroxynonenal. In some processed red meats, heme iron is nitrosylated, because curing salt contains nitrate or nitrite. Also, polycyclic aromatic hydrocarbons, heterocyclic aromatic amines, and aldehydes are formed in meat when amino acids and creatine react at cooking temperatures greater than 180 °C. Numerous studies have shown an association between high intakes of processed meat and colorectal cancer. Consumption of red and processed meat has also been found to be associated with a risk of stomach and pancreatic cancers. In addition to the carcinogenicity of nitroso compounds, the relationship between processed meat and stomach cancer could be due to the high salt content of processed meats. In light of the

cumulating evidence, the IARC Monographs will evaluate the carcinogenic effects of consuming red meat and processed meat.

The consumption of sugar-sweetened beverages may promote carcinogenesis by increasing insulin and glucose levels, and appears to be associated with obesity and diabetes. In turn, overweight and obesity are associated with the onset of cancer at several sites including colon, breast (postmenopausal), endometrium, kidney, oesophagus, and possibly pancreas and ovary. The IARC Monographs will evaluate the potential carcinogenic effects of widely used artificial sweeteners such as aspartame and sucralose.

Several other chemicals in food constituents, contaminants, and flavorings have also been previously evaluated as possibly carcinogenic to humans (IARC Group 2B). These substances include coconut oil, 2,4-hexadienal, methyleugenol, methyl isobutyl ketone, 1,3-dichloro-2-propanol, 3-monochloro-1,2-propanediol, and 4-methylimidazole.

In addition to the agents mentioned above, the IARC Monographs have identified for future evaluation: acrylamide, furan, and 5-(hydroxymethyl) furfura, which are food agents commonly found in cooked foods; coffee; dimethylformamide, which is a widespread contaminant of water; and iron in food and supplements. The evaluation of these substances is considered a high priority for public health, owing to the accumulating evidence on adverse effects.

See also: Aflatoxin: A Global Public Health Problem; Alcohol: Metabolism and Health Effects; Alcohol: Properties and Determination; Beverage: Health Effects; Carcinogenic: Carcinogenic Substances in Food; Nitrites and Nitrates; Polycyclic Aromatic Hydrocarbons.

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http://www.who.int/dietphysicalactivity/en/ – WHO Global Strategy on Diet, Physical Activity and Health.

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Carotenoids: Occurrence, Properties and Determination

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Occurrence

Carotenoids are red, orange, and yellow isoprenoid polyene pigments produced by bacteria, algae, and plants. In nature, more than 700 carotenoids exist, and an annual bioproduction of 100 million tonnes makes carotenoids among the most widespread and largest groups of pigments in nature. Carotenoids are found throughout the plant kingdom and are the major endogenous pigments on flowers, fruits, and vegetables and exogenous pigments in insects, birds, and fish. Carotenoids are also present in green vegetables and leaves, but their colors are masked by chlorophyll. In autumn, however, degradation of chlorophyll means carotenoids are responsible for the typical red, orange, and yellow colors of autumn leaves.

Carotenoids are classified into two main subclasses: carotenes and xanthophylls where the oxygenated xanthophylls are dominant. In addition to the wide variety of carotenoids in nature, carotenoids occur in different stereoisomers (*E*/*Z*) and optical isomers (*R*/*S*) (Figure 1).

The ability to produce carotenoids is mainly reserved to some bacteria, algae, and the higher plants, while higher-order animals are dependent on diet for their carotenoids. However, subsequent transformations of carotenoids obtained from the diet may lead to animal-specific carotenoids that are not normally found in organisms incapable of synthesizing carotenoids *de novo*.

In general, carotenoids in bacteria and algae provide a wider variety of structural types than those found in higher plants. The hydroxyl carotenoids often occur in the form of derivatives, that is, xanthophylls in fruits as fatty acid esters in contrast to the xanthophylls found in leaves. In fungi, some tertiary alcohols and pigments also occur as fatty acid esters. Carotenoid–protein interactions arise where a stoichiometric combination between a protein and astaxanthin-related carotenoids is present. The best-known example is astaxanthin, which was believed to bind weakly to hydrophobic sites of actomyosin in salmon flesh. However, recent research has indicated astaxanthin is associated with other muscle proteins as well as actomyosin, and the major astaxanthin-binding protein in salmon muscle is α-actinin. Other carotenoids, such as β-carotene and lutein, appear in association with proteins without specific interactions. In plants, it is assumed that carotenoids are located in the grana of the chloroplasts in the form of chromoproteins. In addition to these interactions between proteins and carotenoids, a more specific link between proteins and carotenoids also exists; one example is the blue carotenoprotein, crustacyanin, in lobster shell, which typically adopts the color of the carotenoid when cooking denatures the protein–carotenoid complex.

Distribution of Carotenoids in Food

Among the wide variety of carotenoids, only 50–60 of them are present in human diets. Of these, ~35 including nine metabolites are present in human plasma and tissues.

The main sources of natural occurring carotenoids are fruits and vegetables and salmonid fish. In addition, eight natural groups of carotenoids are used as food colorants; these include α-, β-, and γ-carotene (E160a); bixin, norbixin, and annatto (E160b); capsanthin, capsorubin, and paprika (E160c); lycopene (E160d); β-apo-8′carotenal (E160e); ethyl ester of β-apo-8′carotenal (E160f); and lutein. For human health, E160a, E160d, and E161 are probably the most important because they are powerful antioxidants. Those carotenoids presented in fruits and vegetables can, due to their distribution, be categorized as belonging to three different subgroups: (1) green fruits and vegetables, (2) yellow-red fruits and vegetables, and (3) yellow-orange fruits and vegetables.

Green fruits and vegetables: Among green fruits and vegetables, green beans (*Phaseolus vulgaris*), lima or butter beans (*Phaseolus lunatus*), calabrese broccoli, Brussels sprouts, cabbage, kale, kiwi, lettuce, honeydew muskmelon, green peas, spinach, and green-harvested wheat are important sources of carotenoid in human diet. Green fruits and vegetables are rich in chlorophylls and several carotenoids, including epoxycarotenoids, lutein, zeaxanthin, α-carotene, β-carotene, and traces of α- and β-cryptoxanthin. Except for epoxycarotenoids, all these carotenoids are found in human plasma.

Yellow-red fruits and vegetables: Among yellow fruits and vegetables, apricots, cantaloupes, carrots, pumpkin, and sweet potatoes predominate as carotenoid sources in human diets. Common carotenoids are α-carotene, β-carotene, γ-carotene, ξ-carotene, phytofluene, and phytoene. Among red fruits and vegetables, pink grapefruit, tomatoes, and watermelon are typical sources of carotenoids such as lycopene, ξ-carotene, β-carotene, phytofluene, and phytoene. All carotenoids occurring in yellow-red fruits and vegetables give rise to high concentrations of carotenoids in human plasma following dietary intake.

Yellow-orange fruits and vegetables: Among yellow-orange fruits and vegetables, maize, mango, papaya, peaches, prunes, squash (acorn – *Cucurbita pepo* var. *turbinata*), squash (winter – *Cucurbita* spp.), oranges, and citrus fruits dominate as carotenoid sources in human diet. Typical carotenoids include epoxycarotenoids (not observed in human plasma), lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, ξ-carotene, phytofluene, and phytoene.

Carotenoid distribution in *Salmonidae* spp. is dependent on the carotenoids in the fish diet. The reddish color typically associated with farmed Atlantic salmon is due mainly to the synthetic but nature-identical carotenoid, astaxanthin, which is commonly used in salmonid fish feed for muscle pigmentation. This carotenoid also dominates in wild salmonid consuming krill and other shellfish. Canthaxanthin is also allowed as an additive in salmonid feed. However, the use is more limited due to the potential for the formation of crystalline deposits in human retina despite the relevant European Food Safety Authority (EFSA) panel concluding that total anticipated combined exposure to canthaxanthin, for adults and children, from food

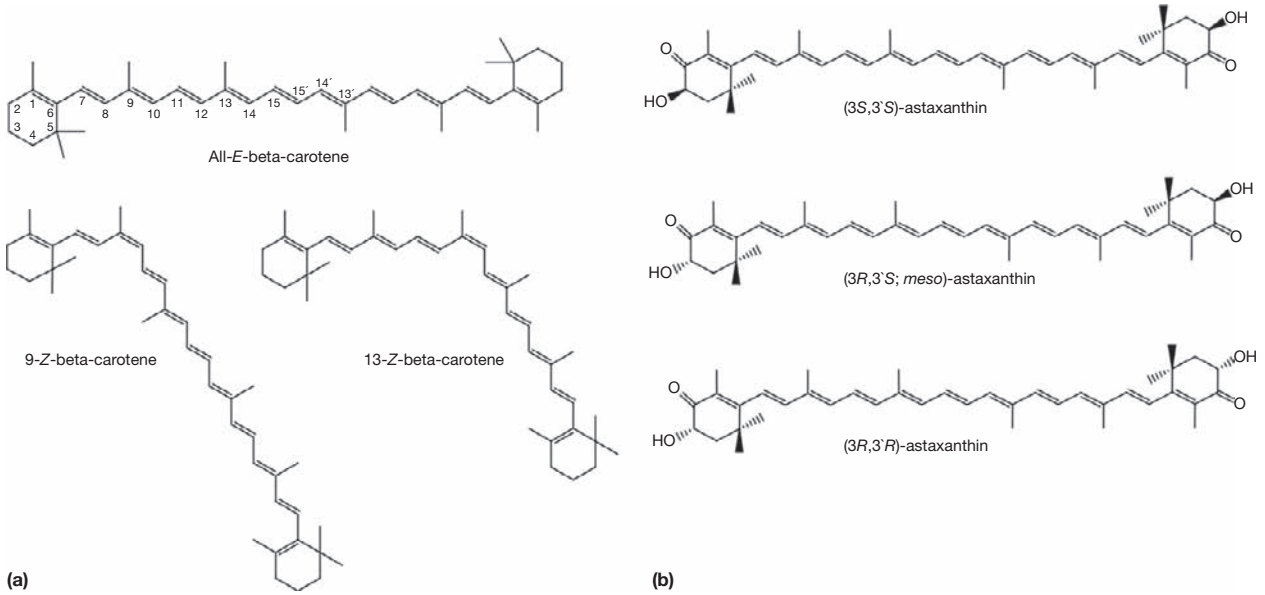

Figure 1 (a) Examples of geometric isomers of β-carotene. (b) Optical isomers of astaxanthin.

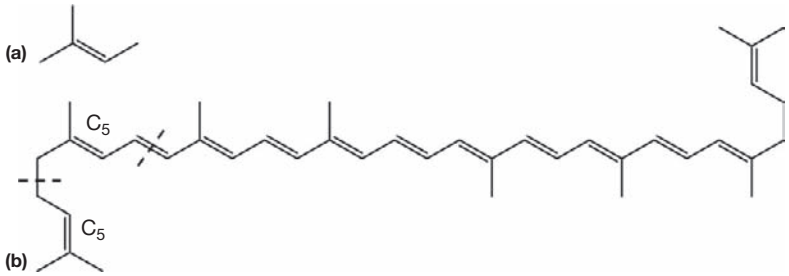

Figure 2 The basic C_5-isoprene units (a) and lycopene (b).

and feed additives is unlikely to exceed acceptable daily intake (0.30 mg kg bw^{-1} day^{-1}). The introduction of vegetable oils and alternative microbiological sources of carotenoids in the feed has, over recent years, introduced traces of other carotenoids such as lutein, zeaxanthin, adonirubin, and β-carotene in the salmon flesh. In production of organic salmon, natural sources of astaxanthin are required, which include the yeast *Xanthophyllomyces dendrorhous* (previously *Phaffia rhodozyma*) and the bacteria *Paracoccus carotinifaciens* (Panaferd-AX).

Properties

The basic carotenoid structure is lycopene, a C_{40} conjugated polyene chain built up of eight C_5-isoprene units, as shown in Figure 2.

This polyene chain represents a chromophore responsible for the characteristic colors, going from colorless (phytoene, three conjugated double bounds), to light yellow (ζ-carotene, seven conjugated double bounds), to yellow (4,4′-diaponeurosporene, nine conjugated double bounds), to red (paprika pigment capsanthin, 10 conjugated double bounds), to orange (β-carotene, 11 conjugated double bounds), to pink (bacterioruberin, 13 conjugated double bounds), to blue with an increasing numbers of conjugated double bounds. When associated with proteins, as carotenoproteins, a dark blue color is obtained such as crustacyanin in lobster shell.

In nature, carotenoids have several functions such as attracting pollinating insects in flowers, indicating maturity in fruits, recognizing and attracting the bird mating process, and signaling molecules in several animal species. Carotenoids are also capable to both transfer and acceptance of photon energy (carotenoid + light energy → ^{1}carotenoid). In photosynthesis, carotenoids absorb visible light where chlorophylls have only weak absorbance. The energy is effectively transferred to the chlorophyll, resulting in singlet-excited chlorophyll (^{1}carotenoid + chlorophyll → carotenoid + ^{1}chlorophyll). Carotenoids are also an important defense against light damage of cells in green plants, algae, and photosynthetic bacteria.

Carotenoids are labile compounds and may decompose when exposed to light, oxygen, high pressure, or potentially other chemical components. In addition, carotenoid stability is affected by esterification and carotenoid–protein interactions. For example, astaxanthin stability in salmon muscle is related to the strength of protein–astaxanthin binding. Several studies have shown decomposition of carotenoids during food processing, where a distinct effect of process parameters can be observed. Some factors affecting carotenoid stability during

processing are increased temperature, prolonged processing time, high concentrations of oxygen and/or sodium chloride, prooxidants, rancidity, and high pressure.

In recent decades, the presence of carotenoids in our food supply, and their role in human health, has been of unprecedented interest. Some carotenoids are vitamin A precursors, and about 35 carotenoids are found in human plasma, depending on dietary composition. Interest in the relationship between carotenoids and human health goes back to the 1930s, but the breakthrough that placed carotenoids among the most important food components occurred in 1981. In a *Nature* publication, the question 'Can dietary β-carotene materially reduce human cancer rate?' was raised. The answer was yes, and 3 years later, a new keystone paper dealing with β-carotene as a newly recognized antioxidant stimulated many carotenoid researchers to search for new functions of carotenoids related to human health. Even after more than 30 years of intensive research, however, it is still not clear if carotenoids have an important role as antioxidants *in vivo*, although their consumption is associated with reduced risk of a range of age-related diseases including cancer.

Antioxidant Properties

Several papers have reviewed antioxidant actions of carotenoids. The role of carotenoids as antioxidants is complex; under some circumstances, carotenoids show a prooxidant effect although this remains controversial and may not distract from their health benefits.

Reactive oxygen species (ROS) are generated constantly in the body by metabolism, and the mitochondria seem to be the subcellular center for ROS production. In addition to ROS generated during respiration and metabolism (e.g., $^1O_2$, $OH^{\bullet}$, $O_2^{\bullet-}$ $ROO^{\bullet}$, and H_2O_2), humans are also exposed to exogenous sources of free radicals (e.g., radiation, tobacco smoke, and pesticides). Free radicals and singlet oxygen can be beneficial, because they kill pathogenic organisms that invade the body, but oxidative stress occurs if there is an overproduction of these extremely reactive components. When oxidative stress arises, ROS/reactive nitrogen species and free radicals react with fatty acids in cell membranes, enzymes, nucleic acids, and endothelial cells causing damage, which may lead to lyses, mutation, and/or inflammation correlated with aging and chronic diseases. Studies show that diets containing carotenoids are related to reduced risk of these pathological conditions.

Carotenoids are known to affect many different cellular pathways. The antioxidant properties of carotenoids are due mainly to excellent physical quenching of singlet oxygen ($^1O_2$) where the energy absorbed to produce triplet oxygen ($^3O_2$) is converted to rotary and vibratory energy by the carotenoid chromophore system. The quenching rate of carotenoids increases with increasing numbers of double bounds and varies with functional groups and chain structure. The role of carotenoids as radical scavengers is more limited. However, several studies have shown that carotenoids also effectively trap free radicals but via a different mechanism compared with more usual chain branching antioxidants (initiation, propagation, and termination). One suggested mechanism is an addition reaction between the carotenoid molecule and peroxyl radicals, which produces a resonance stabilized carbon-centered radical. Several studies (*in vitro*) have verified β-carotene to be highly reactive with peroxyl radicals but only at low oxygen tension (lower than 150 mmHg/20 kPa (atmospheric pressure is 101 kPa)). This correlates well with oxygen pressures found in most tissue under physiological conditions, which supports β-carotene as an antioxidant *in vivo*. At high oxygen pressure, β-carotene behaves as a prooxidant.

Antioxidant actions of carotenoids in living organisms are complex. Several studies have shown that carotenoids interact synergistically with several other antioxidants. The best-known example is probably between β-carotene and α-tocopherol (vitamin E) in protection of lipid peroxidation *in vivo*. Another example, however, is zeaxanthin in combination with ascorbic acid (vitamin C) and vitamin E, which together protect human retinal pigment cells against oxidation induced by photoreactions. To explain the synergism between different antioxidants, it is important to consider the different properties of the molecules involved; some of them are hydrophilic and others hydrophobic. This means they are located in different surroundings throughout membrane systems and subcellular regions. Vitamin C is water-soluble and traps radicals in plasma and cytosol, whereas the carotenoids and vitamin E are fat-soluble and are presented in the lipid phase of membranes. In addition, vitamin C enhances vitamin E activity by reducing the tocopheroxyl radical, while the carotenoid cation radical is repaired by vitamin C.

Provitamin A Carotenoids

Provitamin A activity of carotenoids is well studied, and for humans, the activity is limited to carotenoids that have at least one unsubstituted β-end group (Figure 3). Typical examples of provitamin A carotenoids in humans are α-carotene, β-carotene, and β-cryptoxanthin. On the other hand, species such as the salmonids can convert astaxanthin and other xanthophylls to vitamin A when the dietary supply is insufficient. In humans, the conversion of β-carotene to vitamin A takes place in the intestine and other tissues. Thus, after an oral dose, both intact β-carotene and its metabolite retinol can be found in the circulation. In mammals, the conversion of β-carotene into vitamin A is supposed to happen via two potential pathways: The central cleavage pathway includes the cleavage of the 15,15′ C-double bond producing two molecules of retinal. Alternatively, one molecule of retinal can be produced after stepwise oxidation of β-carotene beginning at any of the double bonds in the conjugated polyene chain.

Vitamin A (retinol), and its chemically related forms retinal and retinoic acid, is essential for development, growth, health, and survival of most living organisms. The role of retinal in

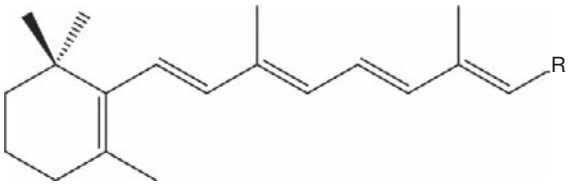

Figure 3 Unsubstituted β-end group.

chromophore pigments, such as rhodopsin, is well understood and vitamin A deficiency is linked directly to reversible night blindness, which may be followed by irreversible loss of sight. Vitamin A deficiency is also linked to impaired immune function, resulting in increased incidence of respiratory and gastrointestinal infections, and measles. In Western societies, where sources of vitamin A are unlimited (e.g., egg, meat, and fish), the conversion of provitamin A carotenoids is limited (<30% of daily intake). In developing countries, where vitamin A deficiency still is a problem, both supplementary programs and food-based interventions are used to increase the vitamin A intake among the population. *Golden Rice* is an example of a fortified food developed for use in areas where their dietary supply of vitamin A is poor. Genetically engineered, this rice produces β-carotene in the edible grain. Details of Golden Rice were published in *Science* in 2000. In 2005, a new variety – Golden Rice 2 – was announced producing 23 times more β-carotene than the original variety.

Carotenoids and the Human Eye

Vision is probably the most important human sensory input, and the largest part of the brain is, therefore, dedicated to processing signals from both eyes. Good vision can be explained as clear imaging and is depending on eye(s) operating across a wide range of light levels. For humans, good vision includes properties like contrast activity, motion perception, and color description. Zeaxanthin and lutein accumulate in the human eye and are known to be important in good vision and reduced risk of age-related macular degeneration. A diet rich in lutein and zeaxanthin, or taken as a food supplement, is known to improve significantly the contrast activity of the eye, which is especially important in dim light. The mechanism is complex and includes both types of photoreceptor cells (rods and cones). Rods are responsible for good night vision, whereas cones function best in daylight. In twilight, signals from both rods and cones are mixed together to mediate vision. Rod cells respond well to bluish light, but blue light is blurred and has many other disadvantages. Both lutein and zeaxanthin filter out blue light and are, therefore, important components in rods to achieve good vision during twilight. The largest concentration of lutein and zeaxanthin is found in a small region of the retina called the yellow spot. This crucial area of the retina gives us the best visual performance, and lutein and zeaxanthin have an important role in extending the usefulness of cone-mediated vision in midrange light levels.

Carotenoids in Skin Photoprotection

Sunlight consists of both UVB (290–320 nm) and UVA (320–400 nm) radiation. UVB is absorbed mainly in the epidermal skin. Activation of proinflammatory cytokines, via photodamage induced by UVB radiation, can lead to sunburn, and through direct interactions with DNA, UVB can cause DNA mutations and skin cancer. UVA may also cause sunburn, but it has a significant role in photoaging. Moreover, UVA can penetrate deeper into dermal skin and induce generation of ROS. ROS-induced mutations of mitochondrial DNA may lead to reduced activity of enzymes involved in oxidative phosphorylation, affecting normal energy metabolism. Carotenoids are involved in the protection of skin against sun-induced damage, be it singlet oxygen ($^1O_2$) quenching, molecules that increase optical density of the skin, or as a source of vitamin A. Provitamin A molecules can be converted into retinoic acid, which is recognized to be a therapeutic agent against photodermatoses. The major carotenoid found in human skin is β-carotene, which is a provitamin A carotenoid. β-carotene is normally found in the epidermal skin, increasing reflection and scattering of light. Several other carotenoids, such as lutein, zeaxanthin, cryptoxanthin, and β-cryptoxanthin, may also accumulate in human skin. The absorption maximum of carotenoids ranging from ~400 to 500 nm is, however, not optical in the absorption of UVA/UVB. Together with carotenoids, eumelanin (a dermal skin pigment that absorbs light across a broad range of the visible spectra) has an important function, as an energy-absorbing pigment, in photoprotection against skin damage.

Carotenoids and Oxidative Stress-Related Diseases

Numerous epidemical, interventional, and clinical studies exploring the role of carotenoids in human health have been performed, and some have found positive effects of different carotenoids on cancer and other diseases associated with oxidative stress. Oxidative stress-related diseases include inflammatory bowel diseases, retinal ischemia, cardiovascular disease and restenosis, AIDS, acute respiratory distress syndrome, and neurodegenerative diseases, such as stroke, Parkinson's disease, and Alzheimer's disease. Treatment with antioxidants including the carotenoids is possible because oxidative injury is present in such diseases. Carotenoids often operate together with other components, including other antioxidants and/or trace elements. For example, selenium is essential for human health, specifically the immune system, but selenium compounds together with carotenoids have also been shown to inhibit carcinogenesis in rodents and humans. Another example is the inhibition of stomach cancer using the combined application of β-carotene, vitamin E, and selenium.

One of the clearest findings in nutritional epidemiology is the relationship between high intakes of fruits and vegetables and reduced rates of cardiovascular diseases including coronary heart disease and stroke. Reductions in coronary disease risk factors and reduced cardiovascular mortality are particularly important. The mechanism for these benefits is not clear, but a lot of attention has been focussed on protection against chronic diseases and the importance of micronutrients with antioxidant properties (e.g., carotenoids). Carotenoids are stored in the liver or in the adipose tissue and are incorporated into plasma lipoproteins during transport. Carotenoids are, therefore, of major importance as a dietary factor against increased risk of chronic diseases, such as heart and vascular diseases, cancer, and diabetes. Most studies dealing with carotenoids, as agents protecting against heart and vascular diseases, have focussed on those carotenoids occurring most commonly in the diet, namely, α- and β-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin. Several other carotenoids may also protect against age-related diseases due to the similarities in their chemical structures. In addition to the established mechanisms, including quenching of $^1O_2$ and scavenging of free radicals, associations between carotenoids and

inflammatory markers, such as C-reactive protein or other biomarkers of vascular disease, are likely.

Carotenoids and the Human Immune System

The human immune system is complex, but carotenoids seem to have an important role in protecting cells against injury induced by ROS. White cells are very active and generate large amounts of ROS during normal activity. For example, the mitochondrial electron transport system generates ATP using oxygen consumed by the cell and ROS, which can damage important functions of the mitochondria. Similarly, phagocytes use ROS to kill host and invading cells. Carotenoids protect subcellular organelles against oxidative injury and are, therefore, important components enhancing cell-mediated immune responses.

In human immunodeficiency virus (HIV)-positive patients, for example, carotenoid deficiencies are more common compared with HIV-negative individuals. HIV infects, specifically, CD4+ cells, which are important components of the immune system. Several studies have shown that supplementation with carotenoids increases CD4+ cells, leukocytes count, and the CD4+/CD8+ ratio in HIV-infected individuals, improving survival rates.

Determination

Isolation, characterization, and determination of carotenoids are performed differently depending on the information available about the sample of interest. Initial steps include extraction with an organic solvent; tetrahydrofuran is used widely because of the excellent solubility of all known carotenoids, but other solvents like hexane (C_6H_{14}), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), methanol (CH_3OH), and ethyl acetate (EtOAc) are also used. After extraction, partition into an organic solvent (EtOAc, *tert*-butyl methyl ether (TBME), or CH_2Cl_2) is common. For tissue containing chlorophylls and/or carotenoid esters, saponification to remove chlorophylls or fats from certain foods may be necessary. In some cases, however, saponification has to be performed under a modified atmosphere (e.g., N_2) to avoid oxidation of xanthophylls (e.g., saponification of astaxanthin esters together with oxygen results in the formation of astacene). Separation of the carotenoids in an extract using HPLC–UV/Vis should, thereafter, be optimized on a reversed-phase and/or normal-phase column, either of which may be suitable for specific carotenoids of interest. To identify unknown carotenoids, it is sometimes necessary to purify the extract by column chromatography, and/or preparative HPLC, for isolation and characterization of (*E*/*Z*) and (*R*/*S*) carotenoids. Purified carotenoids can, potentially, be identified using several analytic techniques, such as UV–Vis spectroscopy, mass spectroscopy (MS), nuclear magnetic resonance, and circular dichroism spectroscopy. Some carotenoids can be identified in comparison with HPLC–UV/Vis-MS profiles for synthetic or isolates. Confirmation of structures using MS can be performed for definitive identification.

Due to poor stability of free carotenoids, extraction from biological tissue can be challenging, especially those containing large amounts of unsaturated lipids. Carotenoids can easily decompose or be isomerized when exposed to high temperatures, light, and a range of chemicals or be oxidized when exposed to oxygen and acids. To improve the stability of carotenoids during extraction, it is possible to add antioxidants or solid CO_2 to the sample. Extracted sample stability can also increased with the addition of butylated hydroxytoluene and/or pretreatment using a solid-phase extraction column. In addition, to obtain good results, it is important to protect samples from heat and light during processing.

Chromatographic analyses of carotenoids are similarly difficult, and the choice of column and mobile phases relevant for analysis depends on type of material or tissue being used, the variety of carotenoids, carotenoid concentrations, and analytic equipment available. Since carotenoids are hydrophobic, normal-phase chromatography is used commonly. However, several reversed-phase systems have also been developed with good repeatability.

Detection of carotenoids after HPLC separation is based mainly on UV–Vis detectors due to the excellent UV–Vis properties of these chromophores where maximum absorption (λ_{max}) increases with increasing numbers of conjugated double bonds. Thus, the yellow acyclic ζ-carotene with seven conjugated double bonds (λ_{max} at 378, 400, and 425 nm in acetonitrile/ethyl acetate/methanol (85:10:5)) absorbs light at much shorter wavelengths than the red acyclic carotenoid lycopene (λ_{max} at 444, 470, and 502 nm in petroleum ether). Both the shape of the spectrum (spectral fine structure) and λ_{max} are characteristics of the chromophore (Figure 4). Most carotenoids absorb at one to, at most, three wavelengths, resulting in a three-peak spectrum. The percentage ratio between absorption peaks III and II is often calculated (absorption peaks III/II × 100%) as an indicator of fine structure. However, this information is not sufficient to identify carotenoids. In addition to the main peaks at λ_{max}, *Z* isomers show an extra peak at shorter wavelengths (~320–350 nm).

Several chromatographic systems have been developed to analyze carotenoids from fruits and vegetables as well as leaves. Carotenoids from green fruits and vegetables are often analyzed on a C18 reversed-phase column using a gradient system of mixed mobile phases; mixture A contains acetonitrile (CH_3CN) and CH_3OH (90:10) and mixture B consists of hexane, CH_2Cl_2, and CH_3OH (45:45:10). Another typical HPLC column used is a normal-phase silica-based, nitrile-bonded column also using a gradient system of mixed mobile phases, where mixture A contains hexane, CH_2Cl_2, and CH_3OH (75:25:0.3) and mixture B hexane, CH_2Cl_2, and CH_3OH (75:25:1). This system is well suited to analyze carotenoid epoxides, *E*/*Z* dihydroxycarotenoids, and mono- and diketocarotenoids. For analyses of *E*/*Z* isomers of hydrocarbon carotenoids (carotenes), columns with low carbon loading or large pore sizes are preferable. This can be a C30 reversed-phase column with a gradient system consisting of different mixtures of TBME, CH_3OH, and H_2O. Another excellent system is a C18 reversed-phase column with low carbon loading. In this system, a mixture of CH_3CN, CH_3OH, hexane, and CH_2Cl_2 (85:10:2.5:2.5) is normally used for the mobile phase.

In fish and marine species, astaxanthin is often analyzed on an H_3PO_4-modified silica gel column (e.g., Lichrosorb SI60-5, 125 × 4.0 mm, 5 μm, Hichrom, Reading, the United Kingdom)

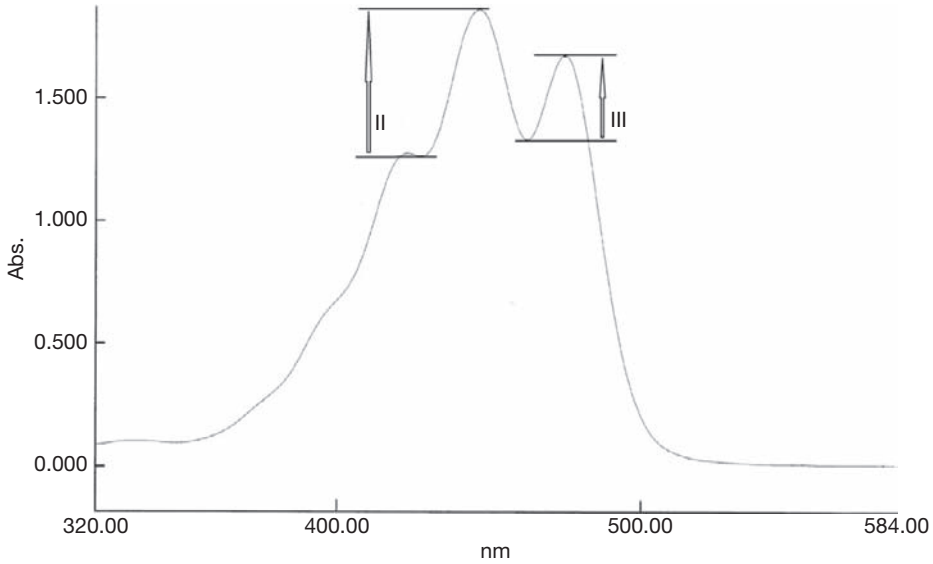

Figure 4 UV–Vis spectrum of lutein showing a typical three-peak spectrum. Calculation of % ratio between absorption peaks III and II (absorption peaks III/II $\times$ 100%) is often used as an indicator of specific fine structures of carotenoids.

Table 1 Relevant HPLC columns for the separation of carotenoids in extracts from natural products and biological samples

HPLC column	*Type of carotenoids*
C18 reversed-phase[a]	Monohydroxycarotenoids and carotenes
C18 reversed-phase (low carbon bonding)[b]	*E*/*Z* isomers of carotenes
C30 reversed-phase[c]	*E*/*Z* isomers of carotenes
Silica-based, nitrile-bonded column[d]	Carotenoid epoxide, *E*/*Z* dihydroxy-, mono-, and diketocarotenoids
Tris-(3,5-dimethylphenylcarbamate) chiral column[e]	*R*/*S* isomers of hydroxycarotenoids
$H_3PO_4^-$-modified silica gel (SI60-5) column[f]	Astaxanthin

[a]Khachik et al. (1986). *Journal of Agricultural and Food Chemistry* **34**, 603.
[b]Khachik et al. (1989). *Journal of Agricultural and Food Chemistry* **37**, 1465.
[c]Sander and Wise (1993). *Journal of Chromatography* **656**, 335.
[d]Humphries and Khachik (2003). *Journal of Agricultural and Food Chemistry* **51**, 1322.
[e]Khachik et al. (2002). *Investigative ophthalmology and visual science* **43**, 3383.
[f]Vecchi et al. (1987). *Journal of High Resolution Chromatography & Chromatography Communications* **10**, 348.

and absorption detected at 470 nm with hexane and acetone (86:14) as the mobile phase (isocratic, flow 1.0 ml min^{-1}). This system separates *E*/*Z* isomers of astaxanthin and other actual carotenoids, such as canthaxanthin, zeaxanthin, and lutein. A new method for homogenizing, extracting, and quantifying astaxanthin, canthaxanthin, lutein, and zeaxanthin from fish flesh was developed in 2010 by a group at the Norwegian Seafood Federation, FHL. This method was developed to standardize pigment analyses in salmon tissue to avoid conflicting information arising from variable results obtained using different analytic methods.

Normally, a standard HPLC column is not capable of separating carotenoids' optical isomers (*R*/*S*). To do that, specific chiral columns are used where an amylose *tris*-(3,5-dimethylphenylcarbamate) chiral column together with a mobile phase containing hexane and propan-2-ol is an option. This system is well suited to separate *R*/*S* stereoisomers of hydroxycarotenoids.

To summarize, HPLC separation of carotenoids in extract from foods and human plasma and tissues is best accomplished by employing a combination of reversed- and normal-phase chromatography and chiral chromatography. A summary of relevant HPLC methods is presented in **Table 1**.

See also: Antioxidants: Role on Health and Prevention; Ascorbic Acid: Physiology and Health Effects; Berries and Related Fruits; Cancer: Diet in Cancer Prevention; Carotenoids: Physiology; Chromatography: High-Performance Liquid Chromatography; Food Additives: Classification, Uses and Regulation; Fruit Juices; Genetically Modified Microorganisms; HIV Disease and Nutrition; Infrared Spectroscopy: Applications; Retinol: Physiology; Rheological Properties of Food Materials; Selenium: Properties and Determination; Solanaceous Fruits Including Tomato, Eggplant, and Peppers; Spectroscopy: Types; Tocopherols: Physiology and Health Effects; Vegetable Oils: Composition and Analysis.

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Carotenoids: Physiology

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Introduction

Carotenoid Discovery

Carotenoids are a group of red, orange, and yellow pigmented molecules that are synthesized by photosynthetic plants, algae, bacteria, and fungi but not animals. The discovery of carotenoids can be attributed to Heinrich Wilhelm Ferdinand Wackenroder who, in his 1831 publication, described a method where he pressed carrots, diluted the juice with water, and extracted the liquid with ether. After the liquid evaporated, he was left with 'a yellow fatty oil and carotin.' Since the discovery of 'carotin,' over 600 naturally occurring carotenoids have been identified. Although hundreds of carotenoids have been documented, alpha- and beta-carotene, beta-cryptoxanthin, lutein, lycopene, and zeaxanthin account for 90% of carotenoids found in the human diet and body.

Physical Properties of Carotenoids

Carotenoids typically have a 40-carbon chain backbone composed of eight isoprene molecules. Carotenoids are differentiated and produce different pigments, via modifications to the isoprenoid backbone through cyclization of end groups and oxidation. The amount of pigmentation depends not only on the accumulation of carotenoids but also on the regulation of genes involved in carotenoid synthesis, degradation, and storage. There are two major subgroups of carotenoids found in nature: carotenes, which are made up of carbon and hydrogen molecules, and xanthophylls, which are oxygenated carotenes. The composition of alternating double bonds, which is common to all carotenoids, allows them to absorb light in the visual range of the spectrum.

Figure 1 contains chemical structures of the six most prevalent carotenoids in the human diet.

Overview of Carotenoid Function

Plants, specifically fruits and vegetables, produce the majority of carotenoids in the human diet. Research conducted across many species has shown that the core carotenoid biosynthetic pathway is conserved in most plants. More information regarding the carotenoid biosynthetic pathway can be found in the 'Further Reading' section of this article. In plants, carotenoids have a critical role in photoprotection, light capture, abscisic acid (ABA) and strigolactone production, and attracting pollinators. Carotenoids are utilized by humans as an important source of vitamin A and have been shown to have a significant role in maintaining eye health, immune function, and disease prevention.

Carotenoid Physiology in Plants

Photoprotection and light capture

Carotenoids have a fundamental role in harnessing energy during photosynthesis as the key components of light-harvesting complexes in photosynthetic organisms. Additionally, carotenoids protect photosynthetic machinery in the presence of excess light. During the assembly of photosystems, which carry out the primary photochemistry of photosynthesis, carotenoids bind to light-harvesting complexes where they best absorb sunlight in the blue-green visible range ($\lambda = 450–550$ nm). This complements the nearby chlorophyll molecules that absorb light in the red range ($\lambda = 800–850$ nm). Carotenoids then transfer this excitation energy to adjacent chlorophylls to contribute significantly to photosynthesis. Moreover, carotenoids quench triplet chlorophyll, scavenge reactive oxygen species, which can damage cell membranes and proteins, and dissipate excess energy via xanthophyll-mediated nonphotochemical quenching. The utility of carotenoids in photoprotection can be demonstrated with carotenoid-deficient mutants that display bleaching, delayed greening, or even lethal phenotypes.

Abscisic acid and strigolactone production

Carotenoids are also the precursors of key signaling molecules and hormones, such as ABA and strigolactones. The β-xanthophylls, violaxanthin, and neoxanthin are cleaved via members of the 9-*cis*-epoxycarotenoid dioxygenase gene family to produce the hormone abscisic acid. Abscisic acid mediates response to environmental stresses, usually through the control of stomatal aperture and transpiration during high salinity, temperature, or light exposure as well as drought, and may induce many stress-related gene products. Additionally, abscisic acid promotes several developmental processes, including seed dormancy, germination, and maturation and the transition between vegetative growth and reproductive growth.

Strigolactones are carotenoid-derived terpenoid lactones that inhibit shoot branching. Strigolactones were initially characterized for their ability to trigger germination of parasitic plant seeds, such as *Striga*, when present in the root exudates of their host. More recently, however, strigolactones have been identified to play a crucial role in the mutualistic symbiotic interaction with arbuscular mycorrhizal fungi, which improves nutrient uptake of more than 80% of land plants. Strigolactone production is dependent upon the action of carotenoid cleavage dioxygenases, which are responsible for cleaving numerous carotenoids.

Utility as attractants

Carotenoids result in brightly pigmented plant organs that attract birds and insects, assisting in the dispersal of pollen and seeds and, thereby, aiding in plant pollination and reproduction. In addition to providing visual cues, carotenoid

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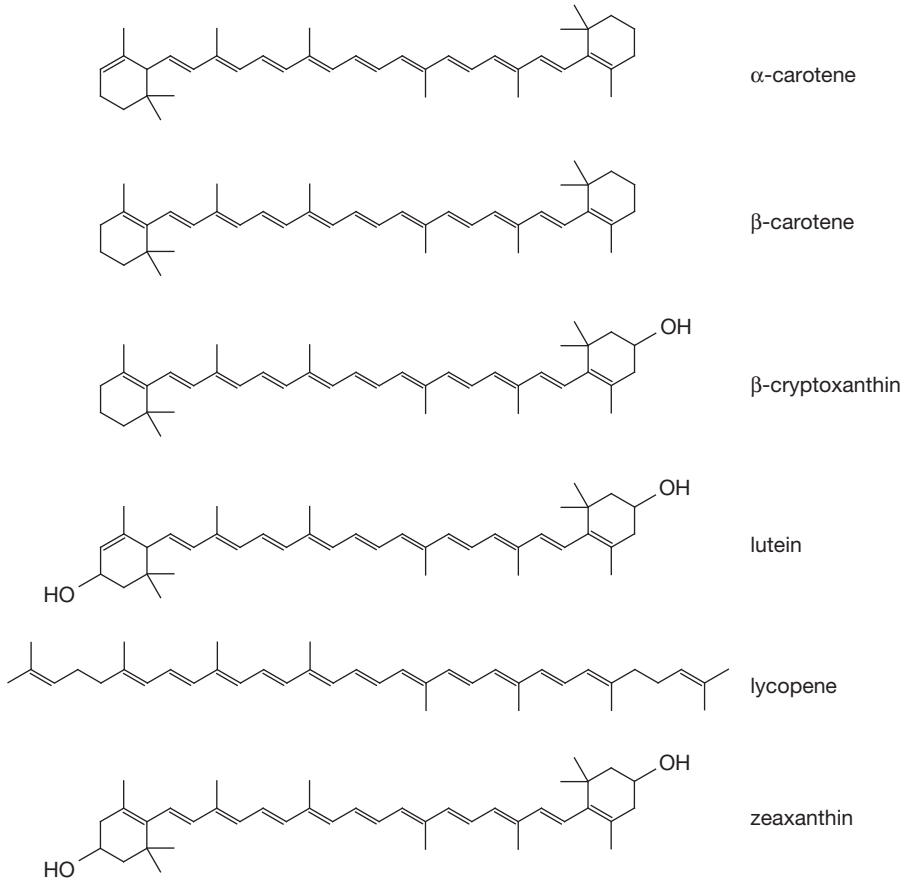

Figure 1 Chemical structures of the carotenoids found most often in the human diet.

derivatives serve as substrates in the biosynthesis of plant volatile compounds that further attract insects and animals for pollination and seed dispersal. Furthermore, carotenoid pigments may serve as visual cues that signal undesirable plant flavors or even antinutritional or lethal compounds. Consumers deciding to purchase carotenoid-based products also use pigmentation intensity and uniformity as an acceptability criterion.

Carotenoid Physiology in Animals

Utility in reproduction, predation, and consumer appeal

Dietary carotenoids can accumulate in animals, particularly birds and fish, where they are important for sexual behavior, reproduction, or avoidance from predation. Accumulation of dietary carotenoids can boost immunity and advertise health, leading to preferential selection by sexual partners.

The pea aphid (*Acyrthosiphon pisum*) was the first animal known to have acquired the carotenoid biosynthetic machinery necessary to produce carotenoids, which provides the reddish pigmentation distinguishing them from green forms. Phylogenetic analysis indicates the acquisition of carotenoid genes was likely the result of a horizontal gene transfer from the fungi to the aphid genome. The red-green color polymorphism appears to be maintained by frequency-dependent selection imposed by natural predators, which search for prey using different visual cues, resulting in differential susceptibility of the red and green individuals.

Animal-based food products consumed by poultry, fish, and mammals are frequently supplemented with carotenoids to provide a dietary vitamin A source as well as to color meat and animal products, making them more appealing to consumers. For the same reason, medicines and cosmetic products are often colored with carotenoids. The nutraceutical industry synthetically manufactures several carotenoids on an industrial scale, including beta-carotene, lycopene, and zeaxanthin, for a wide range of food products and cosmetics, such as vitamin supplements and other health products as well as animal feed additives.

Prevalence, uptake, detection, and recommended dose

Prevalence

Carotenoids have a critical role in human nutrition because they are an essential source of provitamin A – particularly in developing countries – and carotenoid-rich foods provide many other phytonutrients, plant-based compounds that have a potentially beneficial role in the prevention and treatment of human disease. Provitamin A is metabolized in the body into retinal and retinoic acid, the active forms of vitamin A, which support a plethora of physiological processes

including vision, reproduction, embryonic growth and development, immunity, and cellular maintenance. As humans are unable to synthesize carotenoids, they must ingest plant products or animal products that have been enriched with carotenoids to meet daily health recommendations.

The six most commonly ingested carotenoids in the human diet (see Figure 1) can be separated into two groups: (1) provitamin A carotenoids (alpha-carotene, beta-carotene, and beta-cryptoxanthin) and (2) non-provitamin A carotenoids (lutein, lycopene, and zeaxanthin). Most provitamin A comes from orange and yellow fruits and vegetables as well as some leafy green vegetables (see Table 1). Preformed vitamin A, retinol and retinyl ester, is found in animal products, such as liver and fish oil, meat, poultry, dairy, eggs, and fortified cereals. Carotenoid and vitamin A supplements are also available to improve low dietary intake.

High concentrations of the non-provitamin A carotenoids (e.g., lycopene) can be found in red and pink fruits and vegetables (see Table 2). Tomatoes and tomato-based foods account for over 85% of the dietary intake of lycopene. The remaining non-provitamin A carotenoids (lutein and zeaxanthin) are often difficult to separate so are typically reported together. Rich sources of lutein and zeaxanthin include dark leafy greens, egg yolks, and corn products (see Table 3). Non-provitamin A carotenoids have high levels of antioxidant activity and have been implicated in the prevention of vision impairment, cancer, and cardiovascular disease.

Uptake

The bioavailability of carotenoids is dependent on the degree of food processing, the nature of the food matrix, amounts of dietary fat and fiber coingested, and factors related to the individual. Generally, food processing (e.g., chopping, pureeing, and cooking) will break down plant tissue releasing carotenoids and increasing absorption. The presence of dietary fat in the meal will aid solubilization of free carotenoids, as they are lipophilic compounds. For example, egg yolks may be a better source of lutein and zeaxanthin, compared with some fruits and vegetables, because of their high fat content. In contrast, the presence of dietary fiber will have a negative effect on carotenoid absorption. Finally, human-based factors, including current vitamin A status, malnutrition, intestinal inflammation, and genetic profile, will determine the overall bioefficiency after ingestion and processing.

Table 2 Fruit and vegetable products containing high concentrations of lycopene

Description	*Lycopene (g/100 g)*
Grapefruit, raw, pink, and red	1419
Guava sauce, cooked	3909
Guavas, common, raw	5204
Papayas, raw	1828
Tomato juice, canned	9037
Tomato products, canned, paste	28 764
Tomato sauce, canned	13 895
Tomatoes, crushed, canned	5106
Tomatoes, red, ripe, canned	2537
Tomatoes, red, ripe, raw	2573
Tomatoes, sun-dried	45 902
Watermelon, raw	4532

Nutrient data were sourced from the USDA National Nutrient Database SR27, 2014.

Table 1 Fruit and vegetable products containing high concentrations of provitamin A carotenoids

Description	*β-Carotene (g/100 g)*	*α-Carotene (g/100 g)*	*β-Cryptoxanthin (g/100 g)*
Carrots, canned	5331	2743	0
Carrots, frozen, cooked, boiled	8199	3716	199
Carrots, raw	8285	3477	0
Collards, frozen, chopped, cooked, boiled	6818	127	28
Kale, frozen, cooked, boiled	8823	0	–
Kale, raw	5925	56	81
Melons, cantaloupe, raw	2020	16	1
Orange juice, frozen concentrate	53	19	191
Papayas, raw	274	2	589
Peppers, sweet, red, cooked, boiled	1525	18	460
Peppers, sweet, red, raw	1624	20	490
Persimmons, Japanese, raw	253	–	1447
Pumpkin, canned	6940	4795	0
Pumpkin, raw	3100	4016	0
Spinach, canned	5881	0	–
Spinach, cooked, boiled	6288	0	–
Spinach, frozen, chopped, or leaf	7035	0	–
Squash, winter, butternut, cooked, baked	2793	1130	3116
Squash, winter, butternut, raw	4226	834	3471
Sweet potato, canned, mashed	5219	0	–
Sweet potato, raw	8509	0	7
Tangerines (mandarin oranges), canned	193	133	503
Tangerines (mandarin oranges), raw	155	101	407
Turnip greens, frozen, cooked, boiled	6459	0	–

Nutrient data were sourced from the USDA National Nutrient Database SR27, 2014.

Table 3 Food products containing high concentrations of lutein and zeaxanthin

Description	Lutein + zeaxanthin (g/100 g)
Chard, Swiss, cooked, boiled	11 015
Collards, cooked, boiled	6197
Collards, frozen, chopped, cooked, boiled	10 898
Corn grain, yellow	1355
Cress, garden, cooked, boiled	8402
Egg, whole, cooked, hard-boiled	353
Kale, cooked, boiled	18 246
Mustard greens, frozen, cooked, boiled	6672
Noodles, egg, spinach, cooked, enriched	2232
Spinach, canned	10 575
Spinach, cooked, boiled	11 308
Spinach, frozen, chopped or leaf, cooked, boiled	15 691
Spinach, frozen, chopped or leaf	12 651
Turnip greens and turnips, frozen, cooked, boiled	9532
Turnip greens, frozen, cooked, boiled	11 915

Nutrient data were sourced from the USDA National Nutrient Database SR27, 2014.

Once ingested, a fraction of carotenoids are solubilized into micelles, which are molecular aggregates transporting fat-soluble materials. Solubility depends on homogenization, heat treatment, and the presence of dietary fat and fiber. Carotenoids are absorbed via passive diffusion into the gastrointestinal mucosal cells, which act as a selective barrier. Subsequently, they are packaged into chylomicrons and released into the lymphatic system where they are taken up by the liver and stored or reexcreted into the bloodstream. Vitamin A liver stores are in the form of retinyl esters. Carotenoids are differentially absorbed by the tissues and are typically targeted to the eye macula, liver, lungs, adipose tissue, brain, prostate, and skin. Typically, about 10% of ingested carotenoids are assimilated.

Vitamin A bioefficiency can be reduced in the presence of certain medications. Cholesterol-lowering drugs cholestyramine (Questran) and colestipol (Colestid), antiobesity drug orlistat (Xenical), or the coingestion of mineral oil may reduce the absorption of fat-soluble compounds including carotenoids. Manufacturers of these medications recommend patients take dietary supplements to increase their vitamin A and carotenoid intake.

Detection

Carotenoid levels are usually assessed using blood plasma, in particular plasma retinol levels. These levels are useful for accessing vitamin A inadequacy, but not effective for detecting marginal levels. Relative dose–response tests analyze plasma retinol levels before and after administration of small quantities of food or supplements. Supplementation studies analyze plasma retinol levels over a longer period of time, usually days or weeks. While these studies are very useful, they lack detailed analysis of food matrix composition and other absorption factors. Several digestion and assimilation assays *in vitro* have been used to analyze these factors instead.

Recommended intake

Provitamin A carotenoids are the only carotenoids recognized as essential for humans. The recommended dietary allowance (RDA) for vitamin A is given in terms of micrograms (μg) of retinol. A provitamin A RDA of 900 μg day^{-1} for males and 700 μg day^{-1} for females (14 years and older) is sufficient to support normal gene expression, immune function, and vision. The RDA for women who are pregnant or breastfeeding can be as high as 1300 μg day^{-1}, although supplements containing vitamin A are not recommended for pregnant women; no risks are associated with beta-carotene intake during pregnancy. Twelve micrograms of dietary beta-carotene provide the human body with 1 μg of retinol, which gives beta-carotene a retinol activity equivalent (RAE) ratio of 12:1. Alpha-carotene and beta-cryptoxanthin each have an RAE ratio of 24:1 while beta-carotene in oil, as a supplement, has a ratio of 2:1. Finally, preformed vitamin A, consumed as an animal product, has an RAE ratio of 1. Although non-provitamin A carotenoids are not recognized as essential nutrients, they are often linked with combating oxidative stress, eye health, and prevention of chronic disease. With this in mind, recommendations of 5000–7000 μg day^{-1} for lycopene and lutein plus zeaxanthin have been suggested, with higher intake possibly increasing prevention of certain diseases.

Carotenoid deficiencies and toxicities

Deficiencies

Plasma retinol levels lower than 0.7 μmol l^{-1} or 200 μg l^{-1} indicate vitamin A deficiency. While not common in industrialized countries, vitamin A deficiencies are more common in developing countries where individuals have limited access to preformed vitamin A and/or supplements as well as diets consisting almost exclusively of staple starch-based crops, which are typically low in provitamin A. The World Health Organization estimates that over 190 million preschool-age children and over 19 million pregnant women around the world have serum retinol concentrations below 0.7 μmol l^{-1}. Further, it is estimated that 650 000 children under the age of 5 die from vitamin A deficiencies each year.

Groups most at risk for vitamin A inadequacy are premature infants, young children, pregnant or breastfeeding women, and individuals with cystic fibrosis. Premature infants do not have adequate liver stores of vitamin A at birth and their serum retinol concentrations remain low for the first year of life. Infants and young children who are breastfed exclusively will have insufficient levels of vitamin A if the mother's breast milk volume and vitamin A content are suboptimal. Pregnant and breastfeeding women require additional vitamin A stores for fetal growth and development as well as their needs. Finally, those with cystic fibrosis typically have pancreatic insufficiency, making it difficult to break down and absorb nutrients from their diet. An estimated 15–40% of cystic fibrosis patients have a vitamin A deficiency.

To date, no known deficiency symptoms have been identified in individuals consuming low-carotenoid diets as long as they still consume adequate provitamin A, preformed vitamin A, or vitamin A supplements. However, the National Cancer Institute, American Cancer Society, and the American Heart Association suggest consuming a variety of fruits and vegetables daily, partly to increase intake of carotenoids from the human diet.

Toxicity

Excess consumption of beta-carotene- and lycopene-rich food sources results in the benign conditions carotenodermia and lycopenemia, respectively, which cause a deep yellow and an orange discoloration of the skin, respectively. These conditions can be reversed by reduced consumption of carotene- and lycopene-rich foods.

Vitamin A toxicity (hypervitaminosis A) is caused by overconsumption of preformed vitamin A. Large doses of beta-carotene have never been associated with vitamin A toxicity. However, severe cases of hypervitaminosis A may result in liver damage, hemorrhage, and coma. Toxicity is associated with prolonged intake of preformed vitamin A in excess of ten times the RDA. Most multivitamin A supplements contain RDA information to prevent cases of toxicity.

Disease prevention

Vision

The first sign of vitamin A deficiency is often night blindness or the inability to see in low light and darkness. More advance deficiencies lead to xerophthalmia, where the conjunctiva becomes dry, thick, and wrinkled and, ultimately, if untreated, can cause blindness as a result of corneal damage.

Lutein and zeaxanthin are present at high concentrations in the macula lutea, the central part of the retina, which is yellow in color, and are known to provide eye photoprotection. The chemical structures of lutein and zeaxanthin allow them to absorb efficiently relatively high-energy short-wavelength light, reducing the amount of blue or UV light that reaches critical eye structures. Filtering protects the underlying cell layers from light-induced oxidative damage and improves visual perception.

Higher concentrations of lutein and zeaxanthin in the body have been associated with lower risk of developing age-related macular degeneration (AMD) and cataracts. The World Health Organization reports that cataracts and AMD comprise 51% and 5% of the major causes of blindness, respectively. While cataracts are the principal causes of blindness in aging adults from developing countries, AMD is the leading cause of blindness in those over 65 years of age in industrial countries. As the world's population continues to age, the prevalence of AMD and cataracts is estimated to increase sharply in the next 10–15 years. Aging appears to be the most likely factor contributing to the onset of AMD and cataracts. AMD contribution factors include increased exposure to UV and blue light, excess oxidative stress, environmental factors, and diets high in polyunsaturated fatty acids. External factors influencing the development of cataracts include poverty, poor nutrition, and strict vegetarian diets that lack important antioxidants and phytonutrients. A multicenter eye disease study found that higher dietary intakes of carotenoids, specifically lutein and zeaxanthin, are associated with reduced AMD risk. Other studies have found that those consuming diets rich in lutein and zeaxanthin are significantly less likely to require cataract extraction or develop cataracts. Further, experts have recommended that the consumption of at least 6000 μg day^{-1} of dietary lutein and zeaxanthin from fruits and vegetables may decrease the risk of AMD.

Immune function

Vitamin A is an immune-stimulant, which is essential for the human immune system to function normally. Retinol and its related metabolites maintain the integrity and function of the skin and mucosal cells and support the development and differentiation of white blood cells. Vitamin A deficiencies increase the risk of infections, particularly diarrhea and measles, and reduce resistance to infectious diseases. In regions of the world where vitamin A deficiency is common, over 1% of those with measles die and the World Health Organization recommends giving vitamin A supplements to children with measles.

Cancer

Several studies have suggested that the non-provitamin A carotenoid, lycopene, and lycopene-rich diets and increased serum levels of lycopene significantly reduce the risk of prostate cancer, particularly aggressive types. Exploratory studies have also found that men with enriched lycopene intakes from cooked tomatoes and tomato products are less likely to develop prostate cancer than men with lower intakes. There is also growing evidence that lycopene may play a protective role in other cancers including breast, lung, gastrointestinal, cervical, ovarian, and pancreatic.

Carotenoids have also been implicated in the prevention of skin cancer by contributing lifelong photoprotection against exposure to the harmful effects of the sun. Additional research has documented the significant role that natural and synthetic retinoids may have in the reduction of carcinogenesis in the skin, breast, liver, colon, and prostate. While diets high in carotenoid-rich foods are associated with reduced risk of some cancers, high-dose beta-carotene supplements have been linked with increased risk of lung cancer in smokers and former asbestos workers. Most experts feel the risks of taking high-dose beta-carotene supplements outweigh any potential cancer prevention benefits, particularly in smokers or other high-risk groups.

Antioxidant activity

Carotenoids provide antioxidant properties by absorbing high-energy short-wavelength light and scavenging reactive oxygen species. While studies invariably show that non-provitamin A carotenoids provide protection from DNA damage, provitamin A carotenoids show mixed effects. Studies that were carried out using low (dietary) concentrations of provitamin A carotenoids found protective effects, while those using higher concentrations (in excess of high-dietary intakes) were associated with an increase in DNA damage. These findings may be caused by provitamin A carotenoids acting as a prooxidants rather than antioxidants in high concentrations.

Osteoporosis

The most prevalent metabolic bone disease, osteoporosis, may be caused by oxidative stress to the skeletal system. Natural and synthetic antioxidants, such as non-provitamin A carotenoids, may counteract the damaging effects of oxidative stress that are produced during intensive exercise and among smokers.

Cellular communication and differentiation

Independent of the antioxidant activities, carotenoids have been shown to stimulate intercellular communication by increasing or inhibiting the expression of specific genes. Through the regulation of gene expression, vitamin A metabolites have a major role in cellular differentiation and specialization. Vitamin A metabolites, such retinoic acid, are essential for fetal development, especially the limbs, heart, eyes, and ears.

Summary

A large body of research has contributed significantly to our understanding of carotenoids since their discovery almost 200 years ago. It is quite clear that carotenoids orchestrate several essential roles in both plant and animal physiologies. In plants, carotenoids help harness additional energy by capturing light at wavelengths outside the range of chlorophyll activity and also provide photoprotection from reactive oxygen species. Furthermore, the accumulation of carotenoid pigmentation, in both plants and animals, helps with reproductive success and advertises health. In animals, especially humans, provitamin A carotenoids are converted to vitamin A, which is critical for maintaining healthy vision, immune response, and cellular communication and differentiation. The RDA for vitamin A in healthy male and female adults (age 14 +) is 900 and 700 μg day^{-1}, respectively. Diets rich in vibrant orange fruits and vegetables, such carrots, squash, sweet potatoes, and cantaloupe, provide high concentrations of provitamin A. While there is no current RDA for non-provitamin A carotenoids, these compounds have been shown to have antioxidant activity and may help prevent AMD, cataracts, and some cancers. High concentrations of the non-provitamin A carotenoid, lycopene, can be found in red and pink fruits and vegetable products, such as tomatoes, tomato products, and watermelon. The non-provitamin A carotenoids lutein and zeaxanthin are most prevalent in dark leafy greens, egg yolks, and corn products. Bioaccessibility of carotenoids from the diet is increased when foods are cooked and the carotenoids coingested with fat. While the importance of carotenoids in plant and animal physiology is clear, there are still many facets to be explored. This article focussed on the six most common carotenoids in the human diet, but there are still over 600 other carotenoids that need further investigation.

See also: Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Bioavailability of Nutrients; Carotenoids: Occurrence, Properties and Determination; Colors: Health Effects; Colors: Properties and Determination of Natural Pigments; Colors: Properties and Determination of Synthetic Pigments; Hypovitaminosis A; Retinol: Physiology; Retinol: Properties and Determination.

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Casein and Caseinate: Methods of Manufacture

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Introduction

Casein is the most important protein component in milk, both quantitatively and nutritionally, accounting for about 80% of milk's total nitrogen. It was used in industries producing paper, textiles, paint, leather, fiber, and other materials. Edible casein and caseinates are also long established dairy byproducts with uses in many foods. Casein is a very rich source of essential amino acids, with the only possible exception of cysteine. It is a phosphorylated and glycosilated complex synthesized by mammary glands. It is constituted of three different polypeptide chains (αs_1, αs_2, and ß) held together by noncovalent interactions. Casein fractions are organized in micellar aggregates that also contain bivalent cations (calcium and smaller amounts of magnesium), ranging 20–300 nm in diameter. This structure allows highly stable dispersion of hydrophobic fractions in a colloidal state by the action of hydrophilic bonds.

The amount of casein in whole milk varies according to the animal breed and stage of lactation. It is generally in the range 24–29 $g\,l^{-1}$. Casein contains 0.7–0.9% phosphorus, covalently bound to the protein by a serine ester linkage. Consequently, casein is known as a phosphoprotein. All the amino acids that are essential for humans are present in casein in high proportions, with the possible exception of cysteine. Thus, casein is considered to be a highly nutritious protein. It exists in milk in complex groups of molecules called as micelles. The micelles consist of casein molecules, calcium, inorganic phosphate, and citrate ions, and they have a typical molecular weight of several hundred million daltons.

In terms of physical chemistry, the casein micelles exist in milk as a very stable colloidal dispersion. As a protein, casein is made up of hundreds of individual amino acids, each of which may have a positive or a negative charge, depending on the pH of the milk system. At some pH value, all the positive charges and all the negative charges on the casein remain in balance (i.e., the net charge on the protein is zero); this pH value is known as the isoelectric point (IEP), which is 4.6 for casein. The IEP is the pH at which the protein is least soluble. Milk has a pH value of about 6.6, at which the casein micelles have a net negative charge and are quite stable. Casein consists of several individual casein components (αs1-, αs2-, β-, and κ-casein), each having slightly different properties.

Casein is precipitated from skim milk by acidifying it to produce acid casein, or the milk is treated with rennet to produce rennet casein. The precipitated casein curd is separated from the whey, washed, and dried. The water-soluble derivatives of acid caseins, produced by reaction with alkalis, are called caseinates. Edible casein is a long-established dairy byproduct used as an ingredient in many food products, including dairy products. The general development of food technologies and their applications has increased the production of and demand for casein. Its manufacture differs from that of nonedible casein (also called industrial casein) in that casein for food is produced under sanitary conditions. Further, during its manufacture, food-grade chemicals are used and sufficiently heat-treated to make the casein safe for human consumption. The intensive investigation into manufacturing technologies over the years and the introduction of efficient plant designs have immensely improved the technology for edible casein production.

Production of Caseins: A Scenario

The world production of caseins and caseinates is hard to define due to lack of a significant data. However, it could be 430 000–460 000 tons. The largest producers of caseins are New Zealand (~150 000 tons), Netherlands (~85 000–10 000 tons), and Germany (25 000–40 000 tons). The world market for casein or caseinates used in the food industry fluctuates between 200 000 and 2 500 000 tons. The United States is the biggest importer of caseins. The demand for food-grade casein in the US is about 25 000 tons per annum, and the corresponding demand for caseinates is around 30 000 tons. About 20% of this demand is for nutraceutical applications. Casein is also utilized for the manufacture of imitation cheeses. Japan is the second largest importer of casein.

The production of edible casein is only economically feasible when the whey is efficiently and economically utilized. This has been one of the main reasons why edible casein is not produced on a large scale. Most of the requirements of this byproduct, even for industrial uses, were met through import. During 1999–2014, a few new large, automatic, and continuous manufacturing plants have begun to produce edible casein, lactose, and whey protein concentrates. The production of caseinates has not picked up in many dairying countries, including India, however, because of its high drying cost, low bulk density, and high packaging, storage, and transportation costs.

Casein Manufacturing Processes

Processes for the manufacture of edible casein from milk are well known all over the world (Figure 1). The efficient separation of fat from milk is essential. For this, filtered and warmed milk (40–45 °C) should be separated in a hermetic cream

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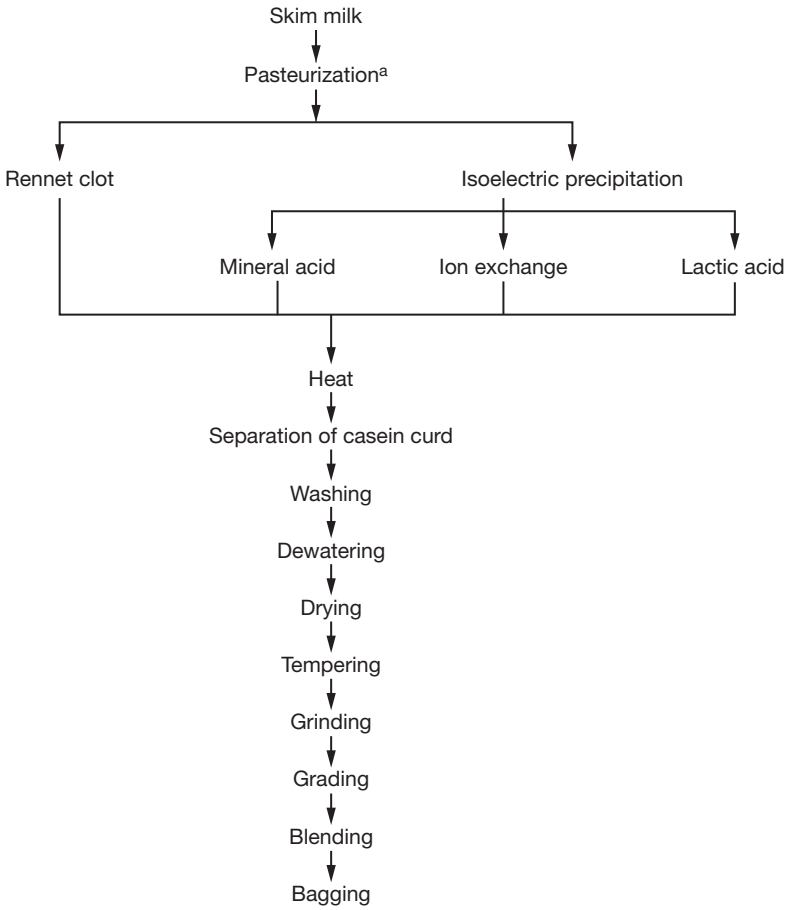

Figure 1 Manufacture of the various types of caseins. [a]Milk for the manufacture of rennet casein for nonfood use is not pasteurized.

separator so that the fat in the skim milk is reduced to less than 0.05%. Achieving the microbiological standards for edible casein also requires the pasteurization of either or both the milk and the curd. Heat treatment tends to produce a higher yield of casein. Some researchers hold that the heat treatment of milk for casein manufacture causes slight insolubility and other defects.

Separation

To extract the casein from milk, it is first separated by means of centrifuges to produce cream and skim milk. Skim milk can thus be considered to be the raw material from which casein products are made.

Precipitation

An important use of surplus skim milk is in the production of casein. Casein exists in milk as a calcium caseinate–calcium-phosphate complex. When an acid is added to the milk, this complex is dissociated. As the pH of the milk is lowered, the calcium is displaced from the casein molecules by hydronium ions, H_30^+, and the calcium phosphate associated with the complex is converted into soluble Ca^{2+} ions and $H_2PO_4^-$ ions. At about 5.3 pH, the casein begins to precipitate out of solution, and at the IEP of casein (about pH 4.6), maximum precipitation occurs. At this pH all the calcium is solubilized. Not only is the calcium from the caseinate molecule removed, but the calcium phosphate is also liberated in its soluble form. This allows the plant to wash the soluble salts from the curd in order to achieve low ash content in the final product.

Ideally, all the casein in a sample of milk would be precipitated simply by adding enough acid to bring the pH value to ~4.6. However, the reaction between the acid and the caseinate complex is not instantaneous, and the pH tends to rise slowly with time. Therefore, ample time should be allowed for achieving equilibrium conditions. When casein is precipitated from skim milk by the direct addition of acid, the temperature and pH of precipitation and the mechanical handling of the curd during its formation are very important in determining the subsequent properties of the curd.

Enzymatic coagulation

In the case of the enzymatic coagulation of casein, the pH of the milk does not change. Instead, the coagulation depends on the addition of a specific enzyme, chymosin/rennin, which cleaves a highly charged portion from the κ-casein, called glycomacropeptide. This action causes the remainder of the κ-casein (now called para-κ-casein) to lose its considerable

power in stabilizing the micelles in milk, and the result is the formation of a three-dimensional gel network or coagulum of casein in the presence of calcium ions. This reaction is essential for the manufacture of virtually all cheeses and in the production of rennet casein.

Acid coagulation

Casein precipitated by acid usually includes the name of the acid in its description, as with hydrochloric acid casein and lactic acid casein. Any of the acid precipitation processes can be used to produce edible casein. The choice of the method for reducing the pH of skim milk to precipitate casein is governed by the cost of acid. The lactic fermentation process is attractive in terms of its cost effectiveness. For lactic acid casein, the pasteurized skim milk is cooled to 22–26 °C and inoculated with a 0.5% starter of mixed lactic starters and incubated for 14–16 h, during which time the pH reduces to 4.6, producing a coagulum. The coagulum is cooked to 50–55 °C to create a curd firm enough for subsequent processing. The acid and heat help in the syneresis of whey.

The use of mineral acids has the advantage of completely continuous operation with no holding time for coagulation. Hydrochloric acid is a superior coagulating agent. When sulfuric acid or hydrochloric acid is used to precipitate curd, it should be diluted before being added to the skim milk; otherwise, the local action of the acid may injure the curd, even though the agitation is rapid. Within reasonable limits, the more dilute the acid the better the quality of the casein produced.

Temperature of precipitation

The kind of curd formed is sensitive to heat. Curd precipitated at temperatures below 35 °C is very soft and fine, and consequently, it is slow to settle and difficult to wash without loss. Precipitated at temperatures between 35 and 38 °C, the curd is coarse, provided stirring is not too fast. Stirring is necessary to distribute the acid uniformly, but rapid stirring at temperatures below 38 °C produces a curd so fine that it settles very slowly during drainage and washing, and it may be lost to some extent in the whey and washings. The curd can be made firm either by heating to a temperature above 38 °C or lowering the pH to 4.1. Curd precipitated at about 43 °C has a texture resembling chewing gum, being stringy, lumpy, and coarse, containing practically no fine particles, and separating cleanly from the whey.

High-grade casein, which is low in ash and readily soluble, is made by the grain-curd process in which the pH and temperature are closely controlled. The best product is made by the use of hydrochloric acid, but lactic and sulfuric acids may be used successfully. The temperature of the skim milk should be held close to 35 °C for hydrochloric acid curd. The pH 4.1 is adjusted by adding dilute acid slowly with continuous stirring. It produces a granular curd that is easy to drain and wash.

Draining of Whey

After precipitation, curd gets settled. Whey should be removed from contact with the curd as soon as possible. The longer the curd remains in contact with the whey, the more difficult it is to wash out acids, salts, whey protein, and lactose, as the freshly broken curd tends to anneal itself, thereby enclosing these constituents within a protein film.

Washing of Casein Curd

The washing of casein curd is one of the most important steps in casein manufacture as most quality improvement in casein is achieved through efficient washing. Large portions of lactose, minerals, and acids are trapped within the curd, which prevents their ready removal during washing of the curd. It is necessary to allow sufficient holding time during each washing stage in order to permit the diffusion of these whey components. The diffusion rate depends on the size and permeability of the curd particles and the purity, amount, and rate of movement of the wash water. Smaller size and better permeability of the curd particles are important for efficient washing. Three separate washes of casein curd are required with contact times of 15–20 min each. As soon as the whey is removed the wash water should be added in equal quantity to the whey that has drained off. The curd should be well stirred in the wash water, either by rakes or by mechanical agitators, but care should be taken not to break the curd into fine particles. Firm and friable curd particles are required to avoid creation of excessive fines. Rubbery and plastic curds cannot be washed effectively. Efficient washing can be achieved through the removal of as much whey as possible at the whey off stage. The temperature and pH of wash water are important factors affecting quality of casein.

Temperature of wash water

Casein curd acts like a sponge in the water, contracting to expel water when heat is applied (termed as syneresis) and relaxing when the water temperature is lowered. Heating leads to hard and rubbery curd, while cold water softens it and causes the curd to be fragile and readily broken. The temperature of the first wash should be the same as the precipitation temperature in order to produce good curd shrinkage. With lactic casein, higher temperatures (70 °C or above) are necessary at some stage of washing to reduce the bacteria, which multiply during the incubation of milk with starter. The temperature of the final wash water should be adjusted to 32–40 °C for better expulsion of water during subsequent pressing.

pH of wash water

The pH of the water should be around 4.6 for the first two washings to avoid the formation of a gelatinous layer over the curd particles in excessively acid water, as well as the softening and dispersion of the curd in alkaline waters. The gelatinous layer, if formed over the curd particles, inhibits the drainage of salts and lactose from the curd. Making the pH of the wash water the same as that of casein helps maintain the equilibrium. Dilute sulfuric acid is preferred for this purpose because casein is much less soluble in this acid than it is in hydrochloric acid. The third wash should be given with neutral water.

Pressing of Casein Curd

The efficient pressing of washed casein curd is important for minimizing the energy required for the removal of the remaining water by drying. Inadequate pressing leads to the formation of lumps of curd on subsequent grinding. It also produces a hard, impervious surface that resists the escape of moisture from the inside, a condition known as case hardening. The mechanical removal of water is cheaper than thermal vaporization.

The batch pressing operation is usually an overnight operation (not less than 12–15 h) with 34 kg cm^{-2} pressure. The proportion of water in washed curd and its ease of removal depend on the type of curd made. The appropriate pH and a temperature of 41 °C produce a firm, friable curd that drains and presses well. The final moisture content is usually 55–60%.

Milling and Drying of Casein

Pressed curd is prone to microbial attack and, therefore, should be shredded and dried as promptly as practicable. Pressing produces particles of uniform size and surface for drying. Uneven drying leads to large particles or lumps that dry on the outside, forming a hard, impervious outer surface that prevents the diffusion of the remaining moisture from the interior of the particles.

The ground curd is spread on trays. It should be spread evenly, and no more than 0.9–1.1 kg of curd should be placed on a tray of 75 × 75 cm. The bottom tray on each truck should have a finer mesh than the others, or it should be covered with a cloth to catch fine particles that may sift through the other trays. The proper control of the temperature and humidity of the air coming in contact with the curd are essential for the efficient drying of casein. The inlet air temperature of 52–57 °C is suitable for any type of curd. Once started, drying should not be interrupted until the moisture content is about 8%. Properly dried casein has the same fine, granular characteristics as the properly ground curd from which it is made.

Tempering, Grinding, Sieving, and Bagging of Casein

Tempering: It is the holding of casein for a period of 24 h to allow efficient cooling, hardening, and even distribution of moisture throughout the batch. Casein shows variation in moisture content during a day's run, as it comes from the drier. The most efficient tempering consists of recirculating the dried casein by pneumatic conveyers. It has the advantage that the air used for the transport of the casein assists in cooling the curd.

Grinding: The casein must be cooled before grinding because warm casein is plastic and causes 'burn on' of the rollers. Grinding and sieving are necessary to produce the highest proportion of the product in the size range desired by the buyer. The grinding is completed by roller mills, pin mills, or hammer mills. For the production of 60- and 80-mesh casein, pin mills are much more efficient than hammer mills.

Sieving: The grinding operation is followed by sieving into various mesh sizes, and then bagging. Common mesh sizes are 30–40 mesh casein, 60-mesh casein, and 90-mesh casein.

Bagging: The casein is packed in sacks or bags of 100 or 200 lb capacity, as prescribed by the grade classification of the casein. Burlap sacks lined with closely woven cloth or heavy papers, or three-ply paper bags may be used for bagging of caseins.

Continuous Casein Manufacture

Due to the advancement of technology and automation, continuous casein-manufacturing plants have replaced batch processes for large-scale production. These plants reduced the manufacturing costs for improving the value of milk proteins, related to that of dried milk, and also elevated the status of casein for both industrial and food uses. These plants are also highly labor-saving, because a large casein plant, with continuous hydrochloric acid precipitation and a capacity of 14 000 l h^{-1}, requires only one person to operate it. The systems are designed to accurately measure pumping rates in order to ensure a constant flow of milk and acid, mixing acid with skim milk at controlled temperatures of 25 °C or lower. This ensures equilibrium conditions before coagulation begins, automatic regulation of steam injection to achieve the coagulation temperature, and a holding tube capable of obtaining complete coagulation and a well-settled curd, all of which lead to less than 1% losses of casein in whey.

After precipitation, casein curd is concentrated by passage over stationary, inclined and fine mesh screens, which remove between 70% and 90% of the whey. Several dairy companies have installed and are successfully operating roller presses and lately decanters for removing the whey. Hydrocyclones may be employed to recover fine particles from whey and wash water. For continuous washing of casein curd, the most common procedure now adopted is a counter flow which reduces both the volume of water needed and the loss of casein fines; the technique involves as many as five washing stages. These storage tanks are of sufficient capacity to permit an average holding time of 20–30 min. Continuous curd pressing is done in mechanically driven roller presses, by belt, or by passing the material through decanters, where water is sufficiently expelled for subsequent economical drying.

There are a number of types of equipment for the drying of casein. The most widely used in recent years is a vibratory type of drier. The curd passes through a mill to reduce it to even-sized particles, which then travel by means of a vibratory action over trays of perforated stainless steel, transferring to successively lower trays. The heated air flows through the beds of curd from the bottom to the top, thus encountering layers of curd of increasing water content and providing improved efficiency of heat utilization.

Method of Manufacture of Caseinates

The caseinate is prepared from freshly precipitated acid casein curd or from dry acid casein by reaction with dilute alkali solutions (Figure 2). Sodium caseinate is the most commonly used form of casein, and it is used in wide range of processed food products as a source of protein, and for their physicochemical, nutritional, and functional properties. Next to sodium caseinate, calcium caseinate is common and finds its use in both pharmaceutical preparations and as a food ingredient. It functions as supplier of both calcium and protein. The specifications for this product vary with its end use, but they frequently include a limitation of calcium content to within the range of 1.0–1.5%.

Manufacturing Process

The fresh acid casein curd is preferred over dried casein as a raw material because the former yields caseinates with blander

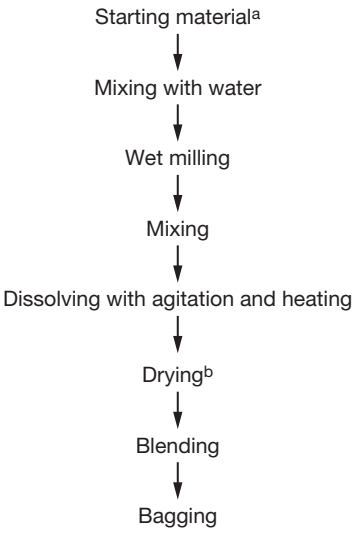

Figure 2 Conventional method for the manufacture of caseinates. [a]Either fresh, acid casein curd, or dried casein; [b]usually spray- or roller-dried.

flavor than does the latter. Caseinates prepared from dry casein will also incur the additional manufacturing costs associated with the drying, dry processing, bagging, and storage of the casein prior to its conversion to sodium caseinate. However, in countries that import casein, buyers may still prefer to purchase casein and produce their own sodium caseinate. Casein should have a low calcium content (0.15% dry basis) in order to produce a caseinate solution with a low viscosity, and a low lactose content (0.2% dry basis), with the goal being sodium caseinate with the best color, flavor, and nutritional value. Control of the curd characteristics is also important to ensure rapid dissolution.

Sodium Caseinate

The manufacture of sodium caseinate consists of the formation of a casein suspension, solubilization of casein using sodium hydroxide, and drying the sodium caseinate produced.

Casein Suspension and Solubilization

The main difficulties experienced in the conversion of acid casein to sodium caseinate are as follows:

(a) The very high viscosity of sodium caseinate solution of moderate concentration limit the solids content for spray drying to 20%.
(b) The formation of a relatively impervious, jelly-like, viscous coating on the surface of casein micelles impedes their dissolution on the addition of alkali. To overcome the former difficulty, it is essential that the pH and temperature are controlled during conversion, because these influence viscosity. The latter challenge can be overcome by reducing the particle size by passing the casein and water mixture through a colloid mill prior to the addition of alkali.

After the final casein wash, the curd is dewatered to about 45% solids and then mixed with water (to 25–30% solids) prior to entering the colloid mill. The temperature of the emerging slurry, which has a pasty consistency, should be below 45 °C, because it has been observed that milled curd can reagglomerate at higher temperatures.

Addition of Alkali and pH Control

The common alkali used in the production of sodium caseinate is sodium hydroxide in the form of 2.5 M solution. The quantity of alkali required is generally 1.7–2.2% by weight of the casein solids. Other alkalis such as sodium bicarbonate or sodium phosphates may be used, but the amounts required and their costs are both greater than those of sodium hydroxide. Hence, they are used only for specific purposes, such as the manufacture of citrated caseinate. The addition of the dilute alkali, preferably by dosing into the recirculating line just prior to the pump, must be carefully controlled with the aim of reaching a final caseinate pH of 6.6–7.0.

Dissolving

The viscosity of sodium caseinate solutions can be expressed as a logarithmic function of the total solid concentration. Each dissolving vat, therefore, must be equipped with a powerful agitator and a high speed recirculating pump. In addition to concentration, temperature, and pH, the calcium content of the curd, the type of alkali used, and seasonal and genetic factors also affect the viscosity of the product. Once the alkali has been added to the casein, it is important to raise the temperature as quickly as possible to 60–70 °C, in order to reduce the viscosity. During the dissolving operation, the incorporation of air should be kept to a minimum, because caseinate solutions form very stable foams.

Drying

The homogeneous sodium caseinate solution is usually spray-dried in a stream of hot air. In order to ensure efficient atomization of the sodium caseinate solution, the solution must have a constant viscosity as it is fed to the drier. It is common practice to minimize the viscosity by preheating the solution to a temperature of 90–95 °C just prior to spray drying. Care should be taken to minimize the time for which the caseinate solution is at high temperature.

Other Caseinates

The manufacture of potassium and ammonium caseinates is very similar to that of sodium caseinate, although, in the case of ammonium caseinate, a lot of the ammonia is evaporated from the solution during the drying process. A solution of sodium caseinate, like those of potassium and ammonium caseinates, has a straw-like color, and it is completely different in appearance from milk. Solutions of calcium caseinate, on the other hand, are very white and opaque (even whiter than milk), and they are less viscous than solutions of the other caseinates. Calcium caseinate solutions are produced by

adding a slurry of lime (calcium hydroxide) in water to a casein curd–water mixture, and the combined slurries react at a relatively low temperature (<45 °C) until the neutralization is completed. Use of higher temperatures before neutralization is likely to result in the precipitation or coagulation of the partly reacted calcium caseinate, with the probable dumping of the contents of the reaction vessel. All caseinate powders have a white appearance.

Composition of Casein and Caseinates

The typical composition of casein and caseinates is shown in Table 1. The caseins produced from lactic, sulfuric, or hydrochloric acid precipitation are almost indistinguishable from one another. The rennet casein differs from acid casein particularly in ash content and pH. During the acidification process, the calcium and inorganic phosphate, which are associated with the casein micelles in milk, are dissolved and leached from the curd leaving only the organic phosphorus and a small residue of calcium. Rennet casein contains about 3% calcium and approximately 1.4% phosphorus. Sodium and calcium caseinates are spray-dried products, their moisture content is much lower than that of the caseins, and their protein content is higher. With a pH in the range 6.5–7.0, sodium caseinate usually contains 1.2–1.4% sodium, whereas the calcium content of calcium caseinate is in the range 1.3–1.6% (Table 2).

Properties of Casein and Caseinates

Solubility

Acid and rennet casein are insoluble in water. Virtually all applications of casein products require them to be dissolved first. Consequently, before use, acid casein must be dissolved using an alkali to produce a solution with a pH of 6.5 or higher. For nonfood, technical applications, acid casein may be dissolved in other alkalis, such as borax or ammonia, usually to a somewhat higher pH (7.5–9.5, or higher) than that used for edible applications.

Table 1 Composition of casein products

Parameters	*Acid casein*	*Rennet casein*	*Sodium caseinate*	*Calcium caseinate*
Moisture (%)	12.0	12.0	3.8	3.8
Protein (%) (Nx6.38)	90.0	84.0	91.4	91.2
Ash (%)	2.5	7.5	3.8	3.6
Lactose (%)	1.0	1.0	0.1	0.1
Fat (%)	2.0	2.0	1.1	1.1
pH	–	–	6.5–6.9	6.8–7.0

Table 2 Mineral content of caseinates

Minerals	*Sodium caseinate*	*Calcium caseinate*
Sodium (%)	1.2–1.4	0.1
Calcium (%)	0.1	1.3–1.6
Iron (mg kg^{-1})	3–20	10–40
Copper (mg kg^{-1})	1–2	1–2
Lead (mg kg^{-1})	<1	<1

Water Absorption and Viscosity

Casein products can absorb substantial amounts of water, and, as a result, they can modify the texture of dough or baked products, serve as the matrix former in cheese-type products, produce specialized plastic materials, or increase the consistency of solutions such as soups. They are good film-formers and find use in whipping and foaming applications, and in emulsions of fats or oils in water.

Melting Properties

Casein exhibits melting properties that are unique among proteins. Following limited proteolysis, casein becomes thermoplastic and flows when heated. A similar affect can be achieved by chelating of some of the calcium ions present. These phenomena are the basis for the melting of natural cheeses and the production of process or imitation processed cheeses. A structure must exist before a substance can be said to melt. With caseins, this structure may be obtained by precipitation with calcium, acid, or the addition of rennin. Casein does not form thermal gels and has little functionality in applications that require temperature set.

High heat stability and the ability to melt are the two properties of caseinates that make them difficult to replace in many food applications. The demand for casein for use in products such as cheese analogs (processed cheese, mozzarella cheese) depends on the formation of a protein matrix from calcium caseinate, which undergoes thermomelting similar to its processed cheese counterpart.

Whipping/Foaming Ability

Caseinates generally produce higher foam overruns, but they also produce less stable foams than egg white or whey protein concentrates. The excellent surfactant property of the amphiphilic casein is also responsible for its use in whipped toppings, cake mixes, and ice cream.

Nutritional Properties

The nutritional quality of a protein is primarily determined by its essential amino acid content. For adult man, eight amino acids are essential. These are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine; the infant requires histidine as well. In comparison with an ideal reference protein composition that was developed by the FAO in 1973, casein contains an adequate amount of all the essential amino acids, with the possible exception of the sulfur-containing amino acids methionine and cysteine.

See also: Bioactive Peptides in Foods; Coffee: Analysis and Composition; Milk: Sources and Composition; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health.

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Cashew Nuts

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Sources and Production

Cashew nut is made up of a fruit in which the kernel is embedded. The real fruit of the cashew is commonly a nut. It is a kidney- or heart-shaped achene, in any normal variety. Its color varies from bottle green to grayish brown (dried fruit). It is attached to the end of a fleshy footstalk or peduncle, which is in fact the receptacle of the flower, that is, broadened and swollen, and forms the false fruit. The nut is composed of kernel and pericarp or shell. The kernel is slightly curved back on itself and forms two cotyledons, representing about 20–25% of the nuts weight. It is wrapped in a thin, difficult to remove peel (testa), reddish-brown membrane, which in turn approximates to 5% of the whole nut. Figure 1 shows the different cashew nut parts. Cashew is of considerable economic importance because its components have numerous uses. The annual production of cashew nuts (with shells) is the highest of all tree nuts, with a value of more than 3.5 million tons. Cashew nut, native to Brazil, was introduced about two centuries ago to the Goa region of India, which became one of the major producers of cashew nuts, accounting for almost 50% of the total world export. Operations involved in the processing of cashew kernels are basically cooking; drying; cutting; decortication; peeling; classification; frying, in the case of roasted almonds; and packaging. The cashew kernel is of high food value with about 40–57% oil and 21% protein contents. It is an important delicacy, which is mainly used in confectionery and as a desert nut. Cashew is globally one of the most popular tree nuts and is eaten as a snack or incorporated as an ingredient in a variety of foods. Cashew ranks third in the international tree nut trade with over 20% of the market. The kernel can be roasted and consumed; it can also be used as an adjunct in chocolate and chicken feeds. Powdered milk used in the standard milk chocolate recipe could be replaced with 25% roasted cashew kernel. In view of the increasing production of cashew globally, there is a need for an increased utilization of the cashew nut, especially the nutritious cashew kernel.

Patterns of Consumption

Lipids and proteins in edible nut seeds account for the major portion, typically 50–90%, of seed weight and are well known to significantly influence seed properties. In recent years, nut seed lipids have received significant attention due to not only their importance in sensory properties but also their possible role in human health and weight management. In a study, the cashew nut diet was more efficient than the other experimental diets in promoting weight gain in rats, but all these diets were less efficient than the casein diets. The protein quality of edible seeds and nuts studied varied significantly, with protein digestibility-corrected amino acid score values ranging from 57% for *baru* almond to 90% for cashew nut. Thus, the cashew nut has the best-quality protein among these foods, mainly because of its essential amino acid profile (AAS = 103%). The inclusion of these seeds and nuts grown in Brazil in healthy diets has been recommended, especially in vegetable-based diets, since a portion (20 g) of cashew nuts provides around 10% of the protein Dietary Reference Intake for adults. It has also been reported that the amino acid score (AAS) values of the seeds and nuts studied (Table 1) were higher than those reported in the literature for the cashew nut (AAS = 103% vs. 95%).

Several studies showed the inclusion of cashew nut bran at levels of 7.5 and 15% in animal broiler diets, decreasing the levels of cholesterol, palmitic (C16:0) acid, and linoleic (C18:2) acid and increasing oleic acid (C18:1) content of abdominal fat. It was demonstrated that 13% cashew nut bran in the diet did not contribute to increase oleic acid (C18:1) in goat meat.

Availability, Absorption, and Metabolism

Nutritionally, cashew nut provides a lot of energy, providing a reasonable balance of carbohydrates and lipids and proteins; its high energy and protein content makes cashew nut an ideal supplement for feeding children, pregnant women, nursing mothers, and convalescents. The almond flavor appreciated by much of the population allows its incorporation in various types of dishes and culinary delights, increasing the nutritional value of the diet. Table 2 shows the approximate composition of almond cashew, according to various authors.

The most abundant of the minerals present in the cashew nut was potassium (38.5 ± 0.0) followed by magnesium and calcium with values of 36.4 ± 0.2 and 21.4 ± 0.1, respectively. The least abundant were copper, iron, and zinc. Anacardein, the predominant soluble globulin in cashew, also known as CMP, is a multimeric globulin (13S) and constitutes about 40–45% of the total cashew soluble seed proteins. Tree nut allergies affect about 1% of the population and are one of the leading causes of fatal and near-fatal food-induced allergic reactions. Patients with a severe allergy to tree nuts rarely become tolerant of these foods, and these allergies can persist throughout life. Cashew proteins seem to be very potent allergens. Six of 30 (20%) patients with type I hypersensitivity to cashew nut were reported to experience an anaphylactic reaction in one study. Ingestion of chocolate candy containing cashew nut has also been reported to cause an anaphylactic reaction. In the United Kingdom, recent wide availability of cashew nut butter-type spread has increased the potential for a higher rate of exposure. It has been reported that 0.08% of British 4-year-olds are allergic to cashew, whereas 40% of 142 French patients allergic to peanuts were found to be sensitized to cashew. On the other hand, only Brazil nuts (24.5%), cashews (20.9%), macadamia nuts (17.1%), and pistachios

Figure 1 The cashew nut parts.

Table 1 Essential amino acid composition of the cashew nut and amino acid score (AAS) according to the WHO/FAO/UNU requirement pattern*

Amino acid (mg of amino acid/g protein)	*WHO/FAO/UNU requirement pattern*	*Edible seed (cashew nut)*
His	16.0	28.4
Ile	31.0	31.2
Leu	61.0	69.5
Lys	48.0	49.6
Met+Cys	24.0	30.0
Phe+Tyr	41.0	72.1
Thr	25.0	37.1
Trp	6.6	16.9
Val	40.0	40.2
Total	292.6	375.0
AAS (%)	100	103

*Data are mean of two replicates.

Source: Freitas, J. B., Fernandes, D. C., Czeder, L. P., Lima, J. C. R., Sousa, A. G. O. and Naves, M. M. V. (2012). Edible seeds and nuts grown in Brazil as sources of protein for human nutrition. *Food and Nutrition Sciences* **3**, 857–862.

Table 2 Proximate composition of cashew kernel according to different authors

Components (g/100 g)	*Maia et al. (1971)*	*Melo et al. (1998)*	*Akinhanmi et al. (2008)*	*USDA (2009)*	*Vincent et al. (2009)*	*Robbins et al. (2011)*
Moisture	7.65	5.05	7.20	5.20	5.52	–
Ash	2.43	2.40	2.80	2.54	4.41	–
Oil	45.06	46.28	49.10	43.85	34.95	44.1
Protein	21.29	22.1	36.30	18.22	27.31	–
Carbohydrate	22.51	7.93	1.40	30.19	25.39	–
Crude fiber	–	–	3.20	3.30	1.42	–

Table 3 Fatty acid composition of cashew

Fatty acids	*% of total lipid*
14:0	n.d.[a]
16:0	11.14 ± 0.29
16:1 ω7	n.d.
18:0	9.08 ± 0.08
18:1 ω9	56.87 ± 0.83
18:2 ω6	22.22 ± 0.61
18:3 ω3	n.d.
20:0	0.68 ± 0.01
20:1 ω9 – n.d.	n.d.
20:2 ω6 – n.d.	n.d.
22:0	n.d.

[a]n.d., not detected.

Source: Robbins, K. S., Shin, E. C., Shewfelt, R. L., Eitenmiller, R. R. and Pegg, R. B. (2011). Update on the healthful lipid constituents of commercially important tree nuts. *Journal of Agricultural and Food Chemistry* **59**, 12083–12092.

(13.3%) were higher in saturated fatty acids. This is one reason that Brazil nuts, cashews, and macadamia nuts were excluded from the USDA qualified health claim for tree nuts. In the same study, it was observed that cashew was the only nut type possessing all of the minor sterols elucidated with the exception of Δ7-stigmastenol. Table 3 shows the composition of fatty acids in cashews.

Cashew nut skins are a natural source of phenolic compounds; the levels of (+) catechin (5.7 g kg^{-1} DM) and (−) epicatechin (4.46 g kg^{-1} DM) in the testa of cashew nuts are higher than those reported for green tea and chocolate. The testa-containing cashew nuts possess significantly higher amounts of carotenoids and tocopherols, but a lower level of thiamine when compared to testa-free kernels.

Health Effects

Nuts constitute a good source of certain vital bioactive compounds that could elicit many health benefits in human beings. Cashew nut contains the best-quality protein for humans. Results of several epidemiological studies suggested that there may be a connection between frequent nut consumption and reduced incidence of several chronic diseases. Long-term consumption of nuts has been associated with a lower risk of body weight gain and obesity. The consumption of nuts as a part of the healthy diet has a positive influence on the fatty acid profile of persons with type 2 diabetes. Analysis of the effects of the inclusion of cashew nut in the diet on the antioxidant status of human subjects with metabolic syndrome resulted in an increased antioxidant capacity. Nut consumption seems to apply a cholesterol-lowering effect and reduce the risk of lipoprotein-mediated cardiovascular disease, and recent emerging scientific findings have demonstrated that the bioactive constituents of whole nuts have cardioprotective, antiobesity, anticancer, and antioxidant effects by a number of different mechanisms. Cashew nut represents one of the cheapest major sources of nonisoprenoid phenolic lipids, which have a variety of biological properties and medicinal applications and have demonstrated a potential antioxidant activity. Quantitative determination of the major phenolic lipids in cashew apple, kernels, and shells of cashew nut at various stages of development suggested the possibility of fatty acid-type biogenesis of these phenolic lipids. The presence of unsaturated fatty acids, tocopherols, squalenes, and phytosterols and the antioxidant activities of various bioactive compounds such as phenolic, flavonoids, phospholipids, sphingolipids, sterols, and tocopherols were reported in cashew nut samples. Furthermore, the ethanolic extract of cashew nut testa has exhibited significant antioxidant activity, and the polyphenolic compounds present in the testa appear to contribute to the antioxidant activity. Concerning the functional properties of cashew nuts, recently, the isolation of protein from defatted cashew nut shell (CNS), with the crude protein product containing 91.07% protein, was reported. Under its natural conditions, the solubility of this protein isolate is comparable (74.02%) to that of mustard green meal protein. The solubility of the protein isolate decreases with decreasing pH, with the minimum solubility observed at its isoelectric point (pH 3). The water-holding capacity, oil-holding capacity, foaming capacity, foam stability, emulsifying capacity, and emulsion stability were found to be 2.56 cm^3 H_2O/g protein, 4.28 cm^3 oil/g protein, 76.88%, 70.98%, 62.0%, and 79.0%, respectively. The profiles of these functional properties were determined with varying pH values and NaCl concentrations, and improved properties were observed in the alkaline pH range and in the presence of NaCl. Electrophoretic analysis showed that the high-molecular-weight protein globulin was the major protein in the protein isolate. The cashew nuts have been associated with two distinct hypersensitivity reactions. The first, contact or systemic dermatitis, has been linked to cardol and anacardic acid found in the CNS oil, both of which are related to poison ivy urushiol. The second type of hypersensitivity reported from North America and Europe is IgE-mediated food allergy; reactions can range from atopic dermatitis to fatal systemic allergic reactions. Recent studies on cashew nut

allergy suggest that the prevalence of cashew nut allergy is increasing. Cashew nut consumption by allergic patients can cause severe reactions, including anaphylaxis. The major cashew allergens are Ana o 1, Ana o 2, and Ana o 3. Ana o 1 is a 50 kDa vicilin-like protein resistant to heat and proteolysis. The other two known allergens are Ana o 2,a 33 kDa legume-like protein, and Ana o 3, a 13 kDa 2S albumin. All three allergens were classified as seed storage proteins. Patients allergic to cashew nut (50% (10of 20 sera) are sensitized to recombinant Ana o 1, 62% (13 of 21 sera) to recombinant Ana o 2, and 81% (21 of 26 sera) to recombinant Ana o 3 determined by Western immunoblotting allergens from these families of seed storage proteins) are known to be allergic to other tree nuts and legumes and seeds.

See also: Allergies: Public health; Amino Acids: Determination; Amino Acids: Metabolism; Antioxidants: Role on Health and Prevention; Food Allergies: Occurrence and Analysis; Food Allergies; Functional Foods; Nuts: Health Effects; Phenolic Compounds: Bioavailability and Health Effects; Selenium: Properties and Determination; Tocopherols: Physiology and Health Effects; Trace Minerals and Trace Elements; Triacylglycerols: Characterization and Determination; Triacylglycerols: Structures and Properties.

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Cassava: The Nature and Uses

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Introduction

Cassava (*Manihot esculenta* Crantz) is a woody shrub that belongs to the spurge family (Euphorbiaceae). Cassava, an annual crop native to South America, is also called manioc or yucca. It is now extensively cultivated throughout tropical and subtropical regions, mainly for its edible tubers as a source of carbohydrates. In some communities, its green leaves are also consumed as a vegetable, although leaves are rich in cyanogenic glycosides and require careful processing. Starch is made from cassava root and used in many culinary and industrial applications (Figures 1 and 2).

The domestication of cassava took place over 10 000 years ago in west central Brazil, and it became a staple food among pre-Columbian Americans. It was introduced to Africa by Portuguese in the sixteenth century, where it replaced native African crops. Along with maize, another introduction from South America, it is now one of the most important staple crops in Africa. Currently, Africa accounts for over half of the world's cassava production. The popularity of cassava in Africa originates from the fact that African slaves who migrated to South America brought back with them the knowledge and technology of cassava cultivation, processing, and cooking to Africa when they returned home.

Cassava originated in tropical rainfed areas, and therefore, the yield is not optimal under dry climatic conditions. Nonetheless, cassava is still considered one of the most drought-tolerant crops, and its importance for food security in drought-prone areas is widely recognized. However, it contains cyanogenic antinutrients that can cause serious health effects when improperly processed cassava is consumed. During drought, cassava is often the only crop that is able to survive the low moisture, and the cyanide content in cassava is elevated during such times as a normal stress response of the plant. Another constraint is that cassava is low in protein, and an exclusive dependence on cassava-based diet can cause serious health consequences.

Cassava has been a crop consumed mainly where it is produced and has not been considered for intra- or international trade due to factors that prevent long-term storage and lengthy transportation. However, with modern technology, this picture is now changing, and cassava products are becoming an important export in some countries, notably Thailand.

With these in mind, this article discusses the production, patterns of consumption, nutritional profile, and health effects of cassava. This article also describes projects aiming at improving the agronomic and nutritional qualities of cassava.

Sources and Production

Leading Countries in Cassava Production

A phylogenetic study by Olsen and colleagues indicates that the origin of cultivated varieties of cassava, *Manihot esculenta* subsp. *flabellifolia*, occurred in regions around west central Brazil, and its cultivation started at least 10 000 years ago.

Cassava was introduced to other parts of the world, mainly by Portuguese colonists, and it is now cultivated in most tropical and subtropical countries. Over half of the cassava production takes place in Africa, followed by Asia and South America. According to an FAO report published in 2008, top five countries in terms of cassava production were Nigeria, Brazil, Thailand, Indonesia, and the Republic of Congo (in order of the amount produced). These five countries account for 57.0% of the world production. Of note, since 1980s, Nigeria has dramatically increased its output and became the top country in cassava production.

Advantages and Disadvantages of Cassava

Several traits of cassava make it an ideal crop in dry areas and during drought. First, cassava plants can survive drought conditions and resume growth when water becomes available, either from rain or irrigation. It can grow in marginal soils without fertilizers, where other crops cannot grow; however, the yield can be compromised. Second, it can be harvested in varied times after planting, ranging approximately from 6 months to 3 years, unlike grain crops such as rice and millet, which must be harvested during a narrow window of time. Third, per area yield is higher than most other crops. Studies show that cassava can produce 250×10^3 cal ha^{-1} day^{-1} under excellent growing conditions and when supplied with organic and chemical fertilizers. This compares favorably with 200×10^3 for maize, 176×10^3 for rice, 114×10^3 for sorghum, and 110×10^3 for wheat. Last, but probably as important as other factors, cassava cultivation is generally easy requiring little care such as weeding. Besides, cassava root is less prone to insect and rodent damages, as it grows underground.

Despite these excellent traits that made cassava popular throughout the tropics, it is nonetheless slow-growing and may take 10–12 months to harvest roots. This is significantly longer than grain crops such as rice and millet. A number of pests and diseases are known for cassava, and they are often devastating, causing famines.

Cassava Cultivation

Cassava is propagated vegetatively using stem cuttings, although it flowers and produces seeds. The stem cuttings for planting, 10–15 cm long, are obtained from disease-free stakes of 8–15-month-old cassava plants. Planting should take place when there is sufficient rainfall to promote good sprouting. Seeds are not used as planting materials for cassava production. However, they are used for breeding purposes.

Three different ways are known to plant cassava cuttings, and the best way is chosen depending on the soil moisture, pest occurrence, and how the tubers are harvested. Vertical planting, with two-thirds of the cutting in the soil, produces tubers deep in the soil, and therefore, they are relatively difficult to pull out, but it is suited in the area with low rainfall. This

Figure 1 Cassava plants in a smallholder farm in Honiara, Solomon Islands.

Figure 2 Cassava roots sold at a market in Honiara, Solomon Islands.

method produces roots with good soil anchorage. Angled planting, with two-thirds in the soil, produces tubers that are easy to harvest and suitable in loam soil. In horizontal planting, cuttings are placed horizontally, approximately 10 cm deep in the soil. This method produces multiple stems but the tuber size may be small. Horizontal planting is used in mechanized planting.

The first and the best harvest takes place 9–12 months after planting. Tubers can grow in the soil up to 3 years. However, under extensive rainy and waterlogged conditions, starch in the tuber can get hydrolyzed into sugars, and the tubers become difficult to cook as the starch content decreases. As well, older tubers tend to become fibrous and woody, thus often unsuitable for consumption. Thus, harvesting of the tubers should be planned based on weather conditions.

Cassava is mainly grown to harvest its roots, but cassava leaves are also edible after extensive cooking, as the raw leaves are rich in cyanogenic glycosides. Cassava leaves are an excellent source of nutrients that lack in its roots, such as protein, vitamins, and minerals. Older leaves tend to have undesirable levels of cyanogens, and therefore, young leaves are preferred. In some African countries, especially Cameroon, Congo, Malawi, and Tanzania, cassava leaves are popular and used in local recipes.

Pests and Diseases

As most other vegetatively propagated crops, it tends to accumulate viruses over the period of cultivation. Among a number of viruses infecting cassava, cassava mosaic virus is one of the most important viruses that cause diseases. It is vectored by whiteflies. A new mutated strain of this virus was found in Uganda in 1980s, and it is spreading throughout Central Africa. Another important virus infecting cassava is cassava brown streak virus that can cause total crop failure. The use of virus-free planting material is the key to prevent the diseases. Quarantine procedures must be in place when cassava germplasm is exchanged internationally. New virus-tolerant/virus-resistant cassava strains are available that are produced by breeding.

Insect pests include cassava mealybug (*Phenacoccus manihoti*) and cassava green mite (*Mononychellus tanajoa*). These pests can be biologically controlled using natural enemies introduced from South America, the cassava's center of origin. Nematode damages may not be evident from casual observation of the field because the pathogen's main entry is through the roots. However, it is a major concern, and control measures can potentially increase the yield significantly.

Currently, climate change appears to affect many parts of the world in various ways. For example, Pacific countries are experiencing droughts more often in recent years than before, causing food shortages. Most popular energy sources in the Pacific are root crops, including taro and sweet potato. Compared with grain crops, root crops are susceptible to postharvest degradation and pest damages. Therefore, cassava is gaining popularity as a food security crop because it can survive in the soil even in dry times and the harvesting time is flexible. Climate change also alters the occurrence and epidemiology of pests and diseases. Timely monitoring is essential to minimize the damages.

Cyanogenic Glycosides

Despite many of cassava's desirable traits, it requires careful processing, as it contains cyanogenic antinutrients, making it potentially poisonous and occasionally fatal. Depending on the amount of cyanogenic glycosides, cassava varieties are classified into two types, 'bitter' and 'sweet.' Bitter varieties contain toxic levels of cyanogenic glycosides and therefore not usually suitable for human consumption without extensive processing. Sweet varieties contain relatively low levels of cyanides and are edible. However, they tend to be less tolerant to pests and diseases. Historically, the two types of cassava were classified into two separate species, *Manihot esculenta* (the bitter cassava) and *Manihot palmata* (the sweet cassava). However, molecular phylogenetics indicated that the two types should be classified as a single species. In fact, the bitterness (cyanogenic glycoside content) depends on factors such as soil, climate, and physiological status and not on a genotypic character.

Postharvest and Transport

Cassava is difficult to store once it is harvested because of a process termed postharvest physiological deterioration. In this process, a natural healing response to physical damages that

involves coumaric acids fails to switch off in harvested cassava tubers. The tubers become completely oxidized in 2–3 days after harvest, making them worthless.

Another hindrance comes from the fact that ~70% of fresh weight of cassava is water. Therefore, it is heavy and difficult to transport a large amount of fresh cassava, especially in Africa where road conditions are not ideal and transportation options are limited. For this reason, unprocessed cassava is generally consumed locally. Cassava can be dipped in wax after harvest, and this enables long-range shipping and storage. Cassava is also stored frozen when it can be stored for many months or dried in sunlight after chopping into pieces.

Patterns of Consumption

Preparation of Cassava

Cassava is grown mainly for its starchy roots. It is a major staple crop in many tropical and subtropical countries. However, in some areas, especially in Central Africa, cassava leaves are also consumed as a vegetable. Cassava is an important source of nutrients in these areas, and unique local recipes were developed to prepare cassava cooked with accompanying ingredients that provide proteins, vitamins, and minerals that are not abundantly present in roots.

Most tissues of cassava contain cyanogenic glycosides, in both sweet and bitter varieties. Cyanogenic glycosides found in cassava are linamarin and lotaustralin. However, linamarin is more abundant than lotaustralin and accounts for over 90% of the total cyanogenic glycosides.

Bitter varieties of cassava contain a larger amount of cyanogenic glycosides than sweet varieties, and these are the varieties that are preferentially cultivated in many countries because they can resist pests and diseases and deter thieves. Therefore, it must be processed before it is consumed safely. Five ways to minimize the toxic effects are practiced to safely consume cassava. First, cassava varieties with low toxicity can be selected. However, by doing so, other favorable traits may be compromised. Second, cyanogenic glycosides can be removed by soaking in water. Third, maceration of cassava tissues will release endogenous enzymes to decompose cyanogenic glycosides. Fourth, enzymes of microbial origin can be utilized for the decomposition. Lastly, cyanogenic glycosides can be reduced by heating.

Cassava roots can be simply eaten boiled or fried to accompany greens, meat, or fish dishes. Cassava is also a versatile crop that can be used in numerous different recipes all over the world. Some examples are described here to illustrate its versatility.

Cassava Recipes

Cassava meals

Fufu: Cassava root is boiled and then pounded into a dough to make this popular staple food in African and Caribbean countries. It is often mixed with yam and plantain banana. Fufu can be made with a flour of cassava and other plants. Similar food made with maize flour is consumed in East Africa and called ugari. Fufu is usually eaten with a soup, which is often scooped with fufu made into a spoonlike shape.

Gari: Gari is a granular flour made from fermented cassava roots. Its color is off-white, and it is popular in Nigeria and other Western African countries. To make gari, freshly harvested cassava root is peeled and grated, and then, it is fermented for 3–7 days. The fermentation process removes the toxic cyanide and also contributes to make the desirable flavor. Then, it is sieved and heated for preservation. Heating serves three purposes: to destroy the enzymes and microorganisms, to remove cyanide, and to dry the gari. It has a shelf life of up to 6 months if stored properly. To serve, gari is soaked in water with roasted peanuts, dry fish, sugar, etc.

Farinha de mandioca (Brazil): Farinha de mandioca is grated and dried cassava roots used as condiments or a side dish. It is commercially available in varying degrees of coarseness. To make farinha de mandioca, cassava roots are first detoxified by soaking in water. Then, the detoxified roots are ground and squeezed to remove most of the liquid content. The dried pulp is subsequently dried on a saucepan. Western Amazon is the major area for the production of farinha de mandioca.

Breads, cakes, fries, and noodles

Cassava bread: Cassava flour is mixed with wheat flour to make bread in some African countries such as Ghana, Cameroun, Congo, Malawi, and Nigeria. The cassava flour used for this purpose must be of high quality.

Cassava cake (the Philippines): Grated cassava root is mixed with coconut milk, eggs, and butter. It is one of the most popular sweets in the Philippines.

Cassava cake (Pacific): Similar to the cassava cake in the Philippines, grated cassava root is mixed with coconut milk in many Pacific countries such as Papua New Guinea, Solomon Islands, and Vanuatu. Meat and other ingredients are often sandwiched between the layers of cassava cake. It is typically sold wrapped in banana leaves (**Figure 3**).

Cassava pone (Caribbean countries): Cassava pone is a dessert originated from Trinidad and Tobago, but it became popular also in other Caribbean countries such as Guyana. To make cassava pone, cassava is grated and mixed with grated coconut, coconut milk, sugar, and spices and then baked.

Brazilian tapioca: A crepe made with cassava powder, 'Brazilian tapioca' is topped with shredded coconut, chocolate, or fruit jam and eaten for breakfast or dessert.

Figure 3 Cassava cake wrapped in banana leaf sold at Arawa Market, Bougainville, Papua New Guinea.

Cassava fries: Cassava can be a substitute for French fries. Cassava fries are popular in Central and South American countries and Malaysia.

Cassava derivatives

Cassareep (Guyana): Cassareep is a juice from bitter cassava, boiled to form a thick, black syrup, with added spices and condiments (e.g., cayenne pepper, cinnamon, cloves, salt, and sugar). It is a base for a variety of sauces prepared in South America. Cassareep gives food distinctive bittersweet flavor and is also used as a preservative. Cassareep is produced and exported from Guyana.

Cassava leaves

Mixed vegetables: Cassava leaves can substitute any green leafy vegetable. Leaves are not widely consumed, compared with the roots, but in many communities, they are part of local diet. For example, in coastal regions of Kenya, cassava leaves are washed, pounded, and boiled in salt water for an hour and added to other vegetables such as onions and tomatoes that are prefried. Curry powder and coconut cream are used to season such mixed vegetables. The dish accompanies starchy staples.

Cassava leaves: Boiled cassava leaves are eaten with sambal (shrimp paste) or tempoyak (fermented durian) in Sarawak.

Saka saka (Congo): 'Saka saka' is made by crushing the greens in a mortar and pestle and then boiling in water with other ingredients (palm oil, onion, garlic, capsicum, eggplant, okra, etc.), until it is cooked to a pulp. It is commonly served with fish, meat, or chicken.

Tapioca recipes

Tapioca pearls: Tapioca is shaped into small balls, typically with a diameter of 2–8 mm, depending on their use. In Asian countries, they are used in desserts. Tapioca pearls are often referred to as 'sago' pearls, because they are similar to those made from starch derived from sago, a palm species. Due to its lower price, tapioca pearls can be used as a substitute for sago pearls.

Sagu: 'Sagu' is a cold dessert popular in Brazil made with tapioca pearls, cinnamon, and cloves cooked in red wine.

Tapioca tea (Taiwan and other Asian countries): Also known as bubble tea, this is a cold milk tea served with tapioca pearls. It is served in a cup and drunk with a large straw. Originally developed in Taiwan in the 1980s, it is now enjoyed throughout Asia (Figure 4).

Udon (Japan): Tapioca is mixed with wheat flour to improve the texture of frozen udon noodles in Japan. The frozen cassava-wheat udon is considered to be of superior culinary quality.

As a food additive: Tapioca is used in food industry to improve the taste and texture and to give consistency or stickiness to many products, including confectionary, yogurt, and noodles.

Alcoholic beverages

Kasiri (cassava beer): Alcoholic beverages are produced from cassava in many countries. Amerindians in Suriname and Guyana produce a cassava beer called kasiri from grated cassava roots by pressing to extract its juice and then fermentation of the juice.

Figure 4 Tapioca tea served at a tea stall in Taipei. Take note of the black tapioca pearls at the bottom of the tea.

Cassava beer (South Africa): An industrial scale production of cassava beer has started by SABMiller. The beer, named Impala, uses traditional home brew recipe from villages.

Cassava starch in food industry

Besides the aforementioned recipes, cassava and its derivatives such as tapioca are widely used for food additives and as an ingredient of processed foods, often to enhance the texture and other qualities of the products. Some applications include the use as thickeners for soups, sauces, and baby foods; as binders for sausages and other processed meats; and as stabilizers in ice cream.

Nutrition, Availability, Absorption, and Metabolism

Nutrition Profile of Cassava

Cassava roots are essentially a source of carbohydrates and very poor in protein and fats, although it is a good source of calcium and vitamin C. The main amino acids in the protein are arginine, histidine, isoleucine, leucine, and lysine. In the tropics, cassava is the third most important carbohydrate source, only after rice and maize, and in arid areas and in times of drought, it is often the only source of nutrition (Table 1).

Cassava is the third largest source of food carbohydrates in the tropics, after rice and maize.

Protein content in cassava can be increased by biofortification through genetic engineering and breeding. An alternative approach is the use of solid-state fermentation. Solid-state fermentation is a century-old technology that utilizes fungal

Table 1 Nutrition information on cassava (raw)[a] (mg/100 g)

Proximates		
Nutrient		
Water	g	59.68
Energy	kJ	667
Protein	g	1.36
Total lipid (fat)	g	0.28
Ash	g	0.62
Carbohydrate, by difference	g	38.06
Fiber, total dietary	g	1.8
Minerals		
Calcium	mg	16
Iron	mg	0.27
Magnesium	mg	21
Phosphorus	mg	27
Potassium	mg	271
Sodium	mg	14
Zinc	mg	0.34
Copper	mg	0.100
Manganese	mg	0.384
Selenium	μg	0.7
Vitamins		
Vitamin C, total ascorbic acid	mg	20.6
Thiamin	mg	0.087
Riboflavin	mg	0.048
Niacin	mg	0.854
Pantothenic acid	mg	0.107
Vitamin B6	mg	0.088
Folate, total	μg	27
Folic acid	μg	0
Folate, food	μg	27
Folate, DFE	μg	27
Choline, total	μg	23.7
Betaine	mg	0.4
Vitamin B12	μg	0.00
Vitamin B12, added	μg	0.00
Vitamin A, RAE	μg	1
Retinol	μg	0
Carotene, beta	μg	8
Carotene, alpha	μg	0
Cryptoxanthin, beta	μg	0
Vitamin A	IU	13
Lycopene	μg	0
Lutein + zeaxanthin	μg	0
Vitamin E (alpha-tocopherol)	mg	0.19
Vitamin D (D2 + D3)	μg	0.0
Vitamin D	IU	0
Vitamin K (phylloquinone)	μg	1.9
Lipids		
Fatty acids, total saturated	g	0.074
Fatty acids, total monounsaturated	g	0.075
Fatty acids, total polyunsaturated	g	0.048

[a]According to USDA National Nutrient Database for Standard Reference (2014) United States Department of Agriculture web site available from http://www.nal.usda.gov/fnic/foodcomp/search/. Accessed in September, 2014.

species and has been used for manufacturing soy sauce and other products in Asia and particularly in Japan. Smith and colleagues reported an increase in the protein content of cassava roots using solid-state fermentation with the fungus *Sporotrichum pulverulentum*.

Cassava is generally considered a poor source of carotenoids. However, carotenoid content in cassava roots can range widely from 1.02 to 10.40 μg g^{-1}, and its retention depends on how the cassava roots are processed. Therefore, it may be possible to improve cassava for the carotenoid content by conventional means using breeding and improved processing technologies. Cassava is also a moderate source of folate, thiamine, pyridoxine, riboflavin, and pantothenic acid.

Cassava root contains minerals such as zinc, iron, magnesium, copper, and manganese and is an important source of these minerals for poor communities. Besides, it is a decent source of potassium.

Young cassava leaves are a good source of vitamins and protein. Of note, it is rich in vitamin K. Vitamin K plays a role in the treatment of Alzheimer's disease and also is involved in bone mass building by osteotropic activity in the bones.

The low protein status of cassava can be an advantage for kidney patients who require diet with low protein content.

As mentioned earlier, cassava is a poor source of certain essential nutrients. Nonetheless, many sub-Saharan African communities depend on cassava for their dietary needs. The result is, according to the World Health Organization, the malnutrition that often leads to blindness, disability, or death. Estimated 250 000–500 000 children die each year in these countries. Enhancing nutritional properties of cassava could, in theory, correct this and save many lives. Donald Danforth Plant Science Center in St. Louis, Missouri, the United States, is working with African scientists to develop two cassava varieties, one for West Africa and one for East Africa. For West Africa, a popular cassava variety is improved in beta-carotene and iron in the roots. For East Africa, a variety resistant to cassava mosaic virus is fortified with beta-carotene, and resistance to cassava brown streak virus is added. This initiative is funded by Bill & Melinda Gates Foundation.

Cyanogen as an Antinutrient

Antinutrients are compounds typically found in crop plants that interfere with nutrient absorption by the human body. Cyanogenic glycosides are the most important antinutrient in cassava. They have an important role for plants to deter herbivory and resist pests and diseases. In modern cultivars, high antinutrient traits have been selected out. However, in the case of cassava, cyanogenic property is still retained in all varieties, perhaps, due to their benefits and also difficulty to breed cyanogen-free varieties.

Cyanogenic glycosides are found in a number of food crops. They are, in their original forms, relatively nontoxic. However, when plant material is macerated upon chewing, hydrogen cyanide (HCN) is released by the action of endogenous enzymes (Table 2).

According to a report by Santana and colleagues, cyanide is produced in cassava as the result of the hydrolysis of linamarin by linamarase, forming an acetone cyanohydrin, which is subsequently transformed to release HCN, either spontaneously or enzymatically.

Recently, an Ohio State University team, led by Richard Sayre, developed a cassava line that is nearly free of cyanide by blocking the expression of the genes that are responsible for linamarin synthesis. The plant contains reduced amount of cyanogens in leaves (by 60–94%) and in roots (by 99%), compared with unmodified cassava. Whether this approach is

Table 2 Food sources of cyanogenic glycosides and amount of hydrogen cyanide (HCN) produced[a]

Plant	*HCN (mg/100 g)*	*Glucoside*
Bitter almonds	250	Amygdalin
Cassava root	53	Linamarin
Whole sorghum	250	Dhurrin
Lima bean	10–312	Linamarin

[a]According to Shibamoto, T. and Bjeldanes, L. F. (1993). *Introduction to food toxicology*. California: Academic Press, San Diego.

practical requires careful greenhouse and field testing as linamarin may play important physiological roles.

Health Effects

Effects of Cyanogens

Despite its high yield and low water requirement for growth, cassava consumption has major health problems. As stated earlier, cassava contains cyanogenic glucosides that liberate HCN by endogenous enzyme termed linamarase. Therefore, proper processing is necessary to reduce the toxic compounds to a safe level for consumption. However, long-term effects of the low levels of cyanide are also a concern.

The symptoms of cyanide poisoning appear several hours after the ingestion of improperly processed cassava or cassava derivatives. They include vomiting, vertigo, collapse, and sometimes death. Sulfur can convert cyanide to thiocyanate in human body. Therefore, the poisoning is treatable with an injection of thiosulfate.

The conversion of cyanide to thiocyanate requires a reserve of sulfur-containing amino acids that are also building blocks for other proteins essential for normal physiological functions. Therefore, detoxification depletes the sulfur reserve of the body that leads to various protein deficiency symptoms.

However, this thiocyanate is a compound known to affect the function of thyroid gland to store and process iodine and cause goiter.

In many tropical and subtropical communities, cassava is the only crop that survives during famines. At such times, the normal and often lengthy process of cyanide detoxification is cut short, resulting in incomplete removal of the toxins. Besides, during the drought, cyanogenic glycoside content increases in cassava as a physiological response to the stress, elevating the chance of cyanide poisoning epidemics. Such epidemics often occur in poverty-stricken communities in Africa.

The processing of cassava, by soaking, by heating, or by fermentation, can remove most of the cyanogens, but the processed flour and other products can still contain toxic levels of cyanide. To improve the safety, Howard Bradbury of Australian National University recently developed a simple method to remove the cyanide in cassava flour. The flour is mixed with water to make a thick paste and spread into a thin layer over a basket and kept in the shade for 5 h. During this process, an endogenous enzyme breaks down the cyanide, which escapes into the air. The flour is then safe for consumption.

Besides ingestion of cyanide, poisoning can occur during the detoxification process from the vapors.

Disorders Due to Chronic Toxicity of Cyanogen

Konzo

Konzo is a neurological disorder typically characterized by a sudden onset of an irreversible (but nonprogressive) and symmetrical spastic paralysis/tetraparesis. Konzo is associated with the consumption of cyanogenic glucoside from improperly processed bitter cassava, combined with low-protein diet deficient in sulfur-containing amino acids. Epidemics of this disorder are reported from many cassava-consuming areas in Africa.

This disorder affects women and children under the age of three. In the communities affected by konzo, the position of women in the hierarchy may be lower than that of men, who have better access to nutritious foods that are low in cyanogens and high in protein. Women may be responsible for the processing cassava and exposed to toxic vapor from the preparation. Breast-fed children, typically under the age of three, are not likely to suffer from konzo, as the toxin is not passed from mother to breast milk.

As described earlier, the production of cassava is on the increase, and the likelihood of drought is rising globally in the face of global warming. Therefore, it follows that the cases of konzo will also increase and healthcare workers should monitor any outbreak of this disease.

Tropical ataxic neuropathy

This is a disease in cassava-eating countries in the tropics. Its symptoms include paresthesia (tingling) of the sole of the feet, weakened arms, blurring or loss of vision, ataxic/or spastic gait, deafness, and weakness and thinning of legs. It is considered to be a result of chronic ingestion of subtoxic levels of cyanide. It is suspected to be linked to thiamine deficiency. Most tropical ataxic neuropathy patients are adults, while konzo affects primarily children and women of childbearing age.

Goiter

A goiter is a disease with symptoms of swollen neck or larynx due to the enlargement of the thyroid gland. Iodine deficiency accounts for more than 90% of the goiter cases worldwide. When the body breaks down cyanide in cassava, it generates thiocyanate, which limits the ability of thyroid gland to store iodine, causing iodine deficiency. The function of thyroid is severely compromised without iodine. Iodine deficiency can be corrected by using iodized table salt.

Other Disorders from Cassava Consumption

Malnutrition

As cassava is naturally low in protein and other essential nutrients, diet based solely on cassava can lead to malnutrition.

Kwashiorkor

Extreme dependence on cassava for dietary requirements can lead to a condition known as kwashiorkor, caused by protein deficiency. Lack of protein causes an osmotic imbalance in the digestive system that results in the swollen gut due to the

retention of water (edema). Edema is often mistaken by uninformed parents as a sign of good nourishment, while in fact, it is a form of malnutrition. Getting more protein and calories is the best treatment for this condition.

Allergy to cassava

People who are sensitive to latex can be allergic to cassava. A few cases have been reported that patients with latex allergy suffered from anaphylaxis after eating cassava. It is attributable to a chitinase that cross-reacts with prohevein in latex, because the N-terminal domain of the class I chitinases shows homology to prohevein. These chitinases are also found in avocado, banana, and chestnut.

Medicinal and Other Uses

Cassava has been claimed to be effective for treating prostate cancer. However, the American Cancer Society discounts this claim, as there is no convincing evidence from controlled studies.

A derivative of cassava juice, cassareep, exhibits antiseptic property and is used as an ointment and for the treatment of eye diseases.

Cassava is free of gluten that is present in wheat flour. Therefore, it is a good substitute for the people who are sensitive to gluten.

Conclusion

Cassava is a crop of primary importance in many parts of the world, especially in poverty-stricken, arid areas, as it is often the only crop that can survive the drought and poor soils. Recent research found many food and industrial uses of this traditionally subsistence crop, and the production is on the increase. Many cassava projects to improve the nutritional status of the people who depend on this crop are being implemented and must be continued. Research on the diseases associated with cassava diet should be continued to improve the welfare of the cassava-dependent communities.

See also: Antinutritional Factors in Legume Seeds: Characteristics and Determination; Bread: Types of Bread; Famine, Hunger, and Undernourishment; Malnutrition: Concept, Classification and Magnitude; Malnutrition: Prevention and Management; Protein: Requirements; Toxins in Food: Naturally Occurring.

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Cellulose

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Cellulose Origin

Cellulose is an almost inexhaustible polymeric raw material with fascinating structure and properties. Cellulose and its derivatives are used in countless commercial products ranging from paper and textiles to pharmaceuticals and foods. In nature, cellulose is the main structural component of the plant cell wall (e.g., cottons and woods) and also exists in some lower plants, such as algae and mosses. In addition, cellulose can also be produced by certain bacteria and fungi. Regardless of the different sources, the chemical structure of cellulose remains the same.

Cellulose Chemical Structure

Cellulose is a linear polymer composed of repeating units of glucose rings. Figure 1 shows the molecular structure of cellulose, which is composed of repeating β-D-glucopyranosyl building blocks that are covalently linked by β-1,4-glycosidic bonds between the hydroxyl groups of C4 and the C1 carbons. The beta linkage between these building blocks makes an extended and rigid conformation with each glucose ring 180° from its neighbor. Although the cellulose molecule is a simple polymer composed of thousands of identical glucose units, lots of cellulose chains are aggregated together in parallel by hydrogen bonds to form a highly compact, fully extended cellulose sheet structure. These sheets assemble by van der Waals interactions. Each cellulose chain is composed of one reducing end terminated with C1–OH group, which is in equilibrium with the aldehyde structure. The other end is a nonreducing end with a C4–OH group. This highly packed, linear-chain homopolymer is responsible for the stable property of cellulose and its aqueous insolubility.

Such molecular structure imparts cellulose with its characteristic properties of hydrophilicity, crystallinity, and chemical modifying variability due to the abundant presence of hydroxyl groups. These hydroxyl groups are basis for extensive hydrogen bond network, which gives rise to a highly rigid and ordered cellulose molecular structure. These hydroxyl groups also provide sites for etherification to make cellulose ethers.

Cellulose Biosynthesis

The majority of cellulose is produced from higher plants, such as cotton, trees, and ramie. In very few cases, cellulose is present in an almost pure state, for example, in cotton seeds. In most cases, however, cellulose exists in wood embedded in the matrix of hemicellulose, lignin, and other cell wall components. These matrices play roles in hindering the degradation and utilization of cellulose biomass. Cellulose can also be generated from certain bacteria (e.g., *Gluconacetobacter xylinus*, previously called *Acetobacter xylinum*), fungi, algae, and animals (tunicates). Bacterial cellulose is typically pure cellulose with high crystallinity and long fiber structure. In contrast to plant cellulose, the absence of lignin and hemicellulose allows easier extraction and purification of cellulose for various applications.

Cellulose Microfibril Organization

The biosynthesis of cellulose has been investigated in details over the past decades. In nature, cellulose exists as a composite of many glucan chains, called microfibrils. It is proposed that cellulose is initially synthesized as individual linear glucan chains, and a number of glucan chains are packed together by hydrogen bonds to form cellulose crystalline sheets, and these sheets are further aggregated together by van der Waals' interaction to form elementary fibrils. Elementary fibrils are then assembled into larger units, forming microfibrils, bundles, which are in turn packed into fibers.

Cellulose microfibrils have varied sizes depending on their origins (Figure 2). Small microfibrils from higher plants (also called elementary fibrils) usually contain approximately 36 glucan chains or less, while large microfibrils from algae contain more than 1000 chains. Microfibrils adopt different shapes and aspect ratios depending on their origin.

Cellulose Crystal Structure

Cellulose has varied crystal forms: cellulose I, II, III, and IV. Cellulose I, also called native cellulose, is the most abundant form occurring mainly in plant cell walls. Cellulose I exists in two distinct allomorphs (Iα and Iß). It has been revealed that cellulose Iα has a triclinic unit cell, while Iß has a monoclinic unit cell. Cellulose produced by bacteria and algae is enriched in Iα, while the cellulose synthesized from higher plants is predominantly Iß, and cellulose from tunicate is considered to be almost pure Iß. Cellulose I can be irreversibly transitioned to a more stable crystalline form, cellulose II. Cellulose II is always found in mercerized (alkali-treated) cotton and in regenerated cellulose. Cellulose III is prepared by the treatment of native cellulose with anhydrous ethylamine or liquid ammonia. Cellulose IV is produced with certain treatments at high temperature.

The crystal structure of cellulose has been extensively studied by several structure-analysis methods, such as x-ray diffraction (XRD), 13C solid-state nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, and neutron diffraction analysis. The crystallinity index of cellulose crystal allomorphs is shown in Figure 3, in which different XRD patterns are observed for different allomorphs. The crystal structure and crystallinity contribute to the high insoluble property of

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00127-6

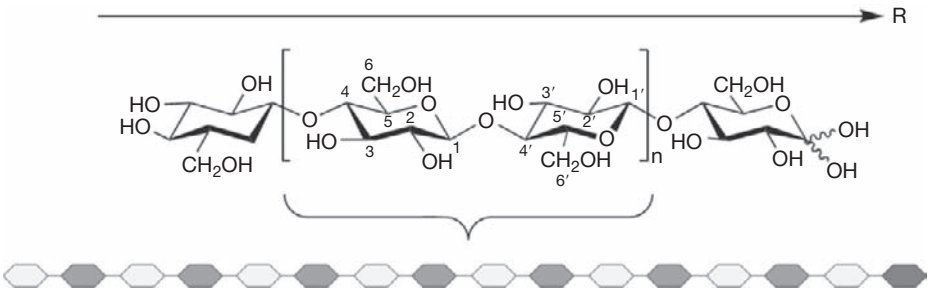

Figure 1 Molecular structure of a typical cellulose chain. The repeating units of cellobiose are denoted in gray. The reducing end (R) of cellulose chain is indicated in dark gray. Taken from Perez et al. (2010).

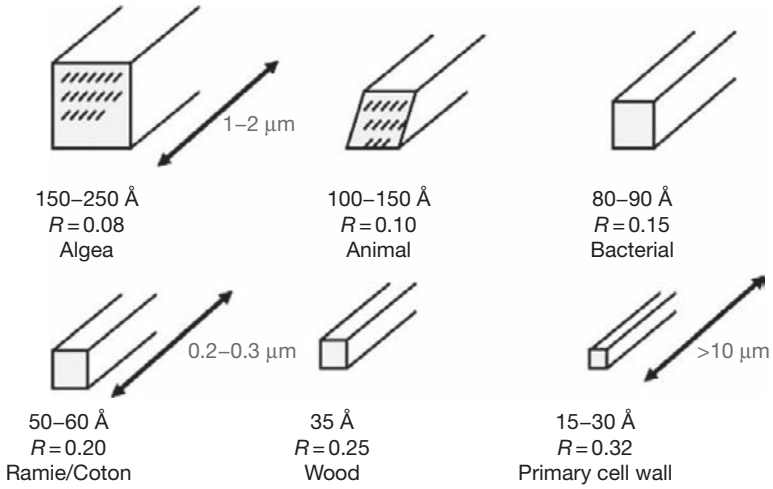

Figure 2 Schematic representations of the cross sections of typical cellulose microfibrils from different sources. R represents the ratio of the number of surface chains to the total number of cellulose chains. Taken from Perez (2010).

cellulose. This highly ordered crystalline structure needs to be destroyed in order to make cellulose material soluble or more accessible to solvents.

Cellulose Molecular Weight and Degree of Polymerization

The chain length of cellulose is described as the degree of polymerization (DP), which varies with the source and treatment of the raw material. Cotton and higher plant fibers usually have DP in the range of 800–10 000; bacterial cellulose has similar DP. In the case of wood fibers, the DP values are 300–1700. Regenerated cellulose contains 250–500 repeating units. As cellulose is an unbranched polymer, high DP is associated with high molecular weight (M_W). In soluble cellulose derivatives, M_w and DP are critical in determining the rheological properties, for example, viscosity and flow behavior. Generally, M_w and DP are positively correlated with solution viscosity.

Production of Cellulose Derivatives

The presence of many hydroxyl groups (three hydroxyls per cellulose building block, anhydroglucose unit (AGU)) makes it possible for etherification of those hydroxyl groups to produce various cellulose ethers. This occurs via the following reaction:

$$\mathrm{ROH} + \mathrm{R'Cl} \rightarrow \mathrm{ROR'} + \mathrm{HCl}$$

R′ can be methyl, ethyl, etc. ROH represents one of the three hydroxyl groups in an AGU.

Cellulose ethers are widely used for coatings, films, membranes, drilling, pharmaceuticals, and foods. Some modified celluloses are generally approved as food additives, such as methylcellulose (MC) E461, hydroxypropyl cellulose (HPC) E463, hydroxypropyl methylcellulose (HPMC) E464, methyl ethyl cellulose (MEC) E465, sodium carboxymethylcellulose (CMC) E466 (also called cellulose gum), and ethyl cellulose (EC) E462 (Figure 4). Those modified celluloses (hydrocolloids) are derived from cellulose raw materials by various chemical reactions.

Generally, modified celluloses are made through a solubilization step in alkali solution to first form alkali cellulose (eqn [1]) and then with an etherification reaction to incorporate side groups onto the main polymer chain. MC is made by treating alkali cellulose with methyl chloride (eqn [2]). HPMC is made by treating alkali cellulose with propylene oxide under methyl chloride (eqn [3]). EC is made by treating alkali cellulose with ethyl chloride (eqn [4]).

$$\mathrm{R\text{--}OH + NaOH} \longrightarrow \mathrm{R\text{--}ONa + H_2O} \qquad [1]$$

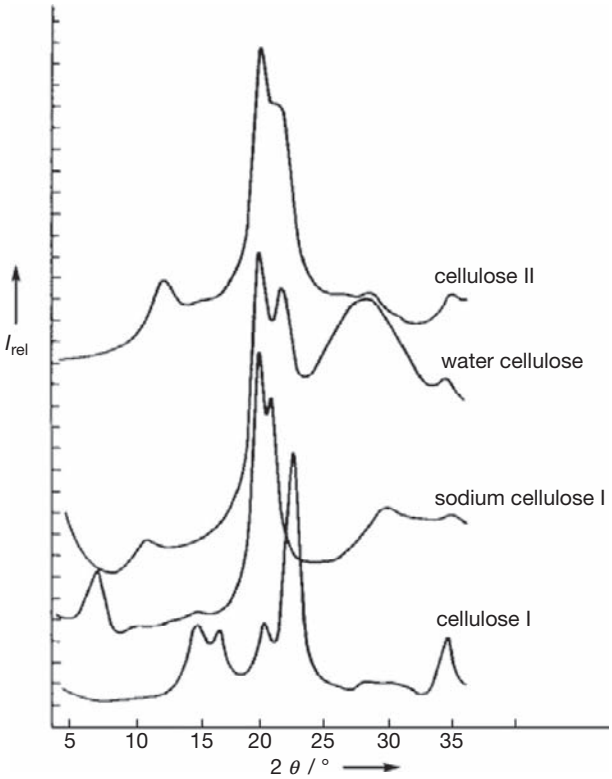

Figure 3 XRD (x-ray diffraction) patterns of cellulose in different allomorphs. Taken from Klemm et al. (2005).

$$\text{R--ONa} + \text{CH}_3\text{Cl} \longrightarrow \text{R--OCH}_3 + \text{NaCl} \quad [2]$$

$$\text{R--ONa} + \text{CH}_3\text{Cl} + \text{H}_3\text{C}\text{—}\underset{\text{O}}{\overset{\text{H}}{\text{C}}}\text{—}\text{CH}_2 \longrightarrow \text{R--OCH}_2\text{CHOCH}_3 + \text{NaCl} \quad [3]$$

(CH_3 substituent on the CHOCH$_3$ group)

$$\text{R--ONa} + \text{CH}_3\text{CH}_2\text{Cl} \longrightarrow \text{R--OCH}_2\text{CH}_3 + \text{NaCl} \quad [4]$$

Figure 5 shows a simplified block flow diagram of the process to produce MC and HPMC. This process typically includes the production of alkali cellulose and reaction of alkali cellulose with other reagents, followed by purification, drying, and packaging. These two products are manufactured in a similar process except propylene oxide that is used in the reactor step for HPMC. Due to the high reactivity of epoxy, alkali cellulose first reacts with propylene oxide to form sodium alcoholate, which subsequently reacts with methyl chloride to produce HPMC.

Cellulose Derivatives in Food Applications

MC and HPMC

MC and HPMC show similar properties and are used to achieve similar functionalities in food applications. They hydrate in cold water and gel upon heating. Schematic description of dispersion, hydration, and gelation of MC and HPMC as a function of temperature is shown in Figure 6. As seen, the polymer powder is dispersed into water above its solubility temperature, hydrates when the temperature drops below the solubility temperature, and gels when the temperature increases above the gelation temperature. Since the gelation process is reversible, the gel reverts back to liquid state upon cooling. The solubility and gelation temperatures of different chemistries of HPMC and MC are provided in Table 1.

The other properties of MC and HPMC are water binding, surface activity, and adhesion. Based on these properties, MC and HPMC grades have been used to provide boil-out control, binding, moisture retention, volume improvement, and texture enhancement in various food applications including bakery fillings; bakery; frozen desserts; meat analogs; and processed meat applications.

While incorporating MC and HPMC into the food formulations, two main considerations must be kept in mind: First is to avoid agglomeration of MC/HPMC when incorporating into water-based system and second is to solubilize MC/HPMC to benefit fully from its functionality. There are three main methods that can be used to incorporate MC/HPMC into food formulations:

- *Preparation of MC and HPMC solution*: Desired amount of MC/HPMC is dispersed into one-third of required water that is above the solubility temperature of the particular MC/HPMC material. The remaining two-third of the water is added cold while stirring. The mixture then is cooled to hydration temperature of the MC/HPMC and stirred for at least 2 h.
- *Blending with dry ingredients*: Desired amount of HPMC/MC grades is blended with other dry ingredients to avoid agglomeration or lumps. Water is added at correct temperature to hydrate while stirring.
- *Dispersion in oil*: Desired amount of HPMC/MC grades is dispersed in organic solvents/oil. The dispersion is then added to the water at correct temperature to hydrate.

Example of Food Applications for MC and HPMC

Meat analogs

Meat analogs are food products that are designed to mimic the appearance, flavor, and texture of meat products. Their consumption has recently been increasing for various reasons including personal beliefs, health concerns, and social causes. Soybean proteins, wheat gluten, cottonseed proteins, and other plant proteins are used to formulate meat analogs. MC is used in the formulation as a binder to help the product maintain its shape and have a firm texture. Thermal gelation holds the ingredients together and reduces moisture loss during heat treatment while providing a structure to help achieve desired meat-like textural properties at the consumption temperature. Addition of MC also emulsifies the fat and helps prevent oil separation.

The ingredient list to formulate soy patties is provided in Table 2. Cold water is placed in a mixer with an attached wire whip. Gluten and MC are dry-mixed together and added into the water while mixing at medium speed until slurry is formed. Beef flavoring, dextrose, and salt are blended into the slurry while mixing for 1 min at high speed. Texturized soy protein

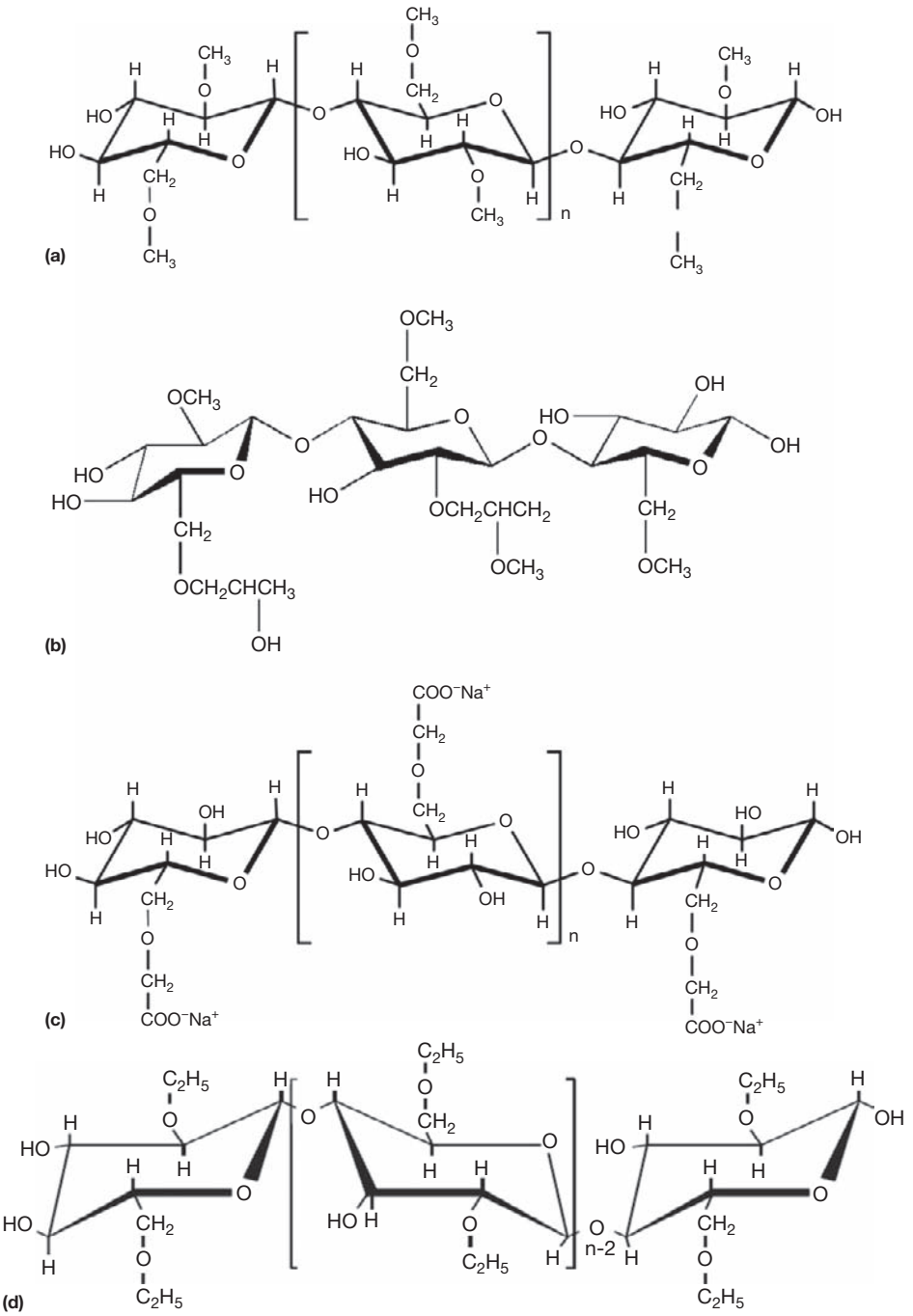

Figure 4 Structure of chemically modified cellulose: (a) methylcellulose (MC); (b) hydroxypropyl methylcellulose (HPMC); (c) carboxymethylcellulose (CMC); (e) ethyl cellulose (EC).

is added next and mixing continues for 5 min. Then the soy protein concentrate is added to the bowl and mixed for five more minutes. The resulting mixture is refrigerated or frozen prior to forming soy patties to promote hydration and binding of MC.

Bake-stable fillings

MC and HPMC are commonly used in both salty and sweet bakery filling formulations to provide stability during baking. During the baking or microwaving process, the viscosity of the filling decreases, causing the filling to boil and run out of the dough product. This phenomenon is known as boil-out. With the addition of MC/HPMC, the filling gels during baking and prevents boil-out.

A formulation for bake-stable chocolate filling for pastry applications is provided in Table 3. Cold water is placed in a mixer with an attached paddle. The dry ingredients are blended together and added into the cold water at low speed and mixed for about 3 min until the mixture is homogeneous. Then, the oil is added into the mixture while blending at high speed.

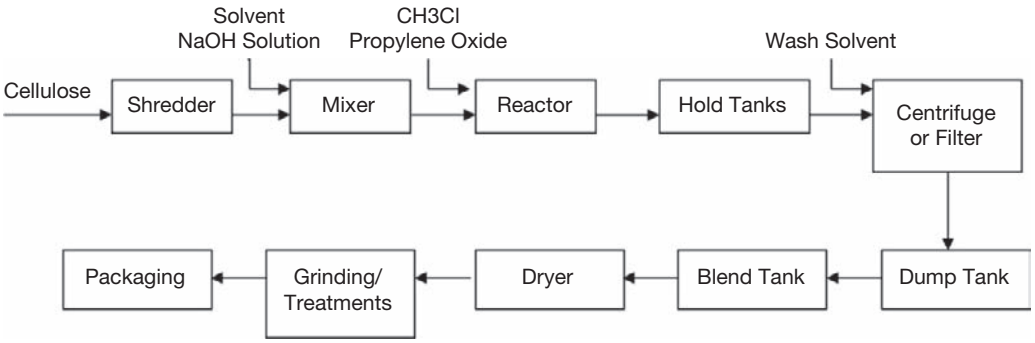

Figure 5 Manufacturing process of MC and HPMC.

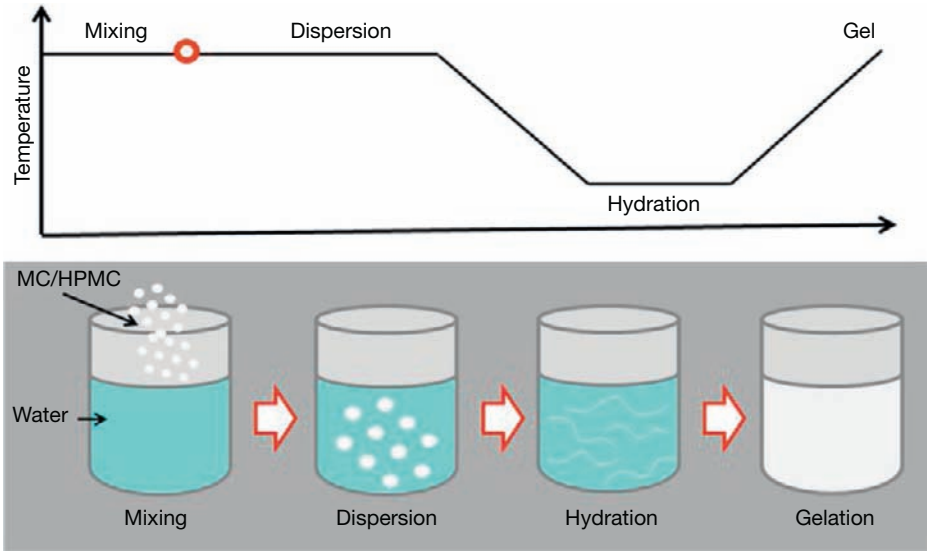

Figure 6 Schematic description of dispersion, hydration, and gelation of methylcellulose and hydroxypropyl methylcellulose as a function of temperature.

Table 1 Hydration and gelation temperatures of methylcellulose and hydroxypropyl methylcellulose as a function of their chemistries

		Hydration temperature		*Gel temperature*	
Type	*Chemistry*	*F*	*°C*	*F*	*°C*
MC	SG A	54	12	110–114	38–44
MC	A	68	20	122–131	50–55
HPMC	E	90	32	136–147	58–64
HPMC	F	90	32	143–154	62–68
HPMC	K	120	50	158–194	70–90

SG A, E, F, and K chemistries are differentiated by the degree of substitution by methyl and hydroxypropyl groups.

Table 2 The ingredient list for soy patties

Ingredients	*%Weight*
Cold water	63.50
Texturized soy protein	19.50
Flaked soy protein concentrate	3.20
Modified gluten	4.50
Oil	3.20
Beef flavoring	4.40
Methylcellulose[a]	1.00
Dextrose	0.45
Salt	0.25

[a]Methylcellulose: METHOCEL™ SGA 16 M FG.

Mixing continues for two more minutes at high speed, and the resulting chocolate filling is refrigerated or frozen prior to usage to ensure complete hydration of MC. While baking, MC containing filling will gel and prevent boil-out. As a result, the filling will remain within the pastry, and visual and sensory attributes of the end product will be preserved.

Carboxymethylcellulose

Carboxymethylcellulose (CMC) is an anionic, water-soluble cellulose derivative. Solubility of CMC depends on the DP as well as the degree of substitution and the uniformity of the substitution distribution. Water solubility of CMC would increase with decreased DP and increased carboxymethyl

Table 3 The ingredient list for bake-stable chocolate filling for pastry

Ingredients	*%Weight*
Cold water	33.50
Oil	30.00
Sugar	19.25
Cocoa powder	10.00
Skimmed milk powder	5.00
Cook-up freeze–thaw-stable starch	1.75
Hydroxypropyl methylcellulose[a]	0.50

[a]Hydroxypropyl methylcellulose: METHOCEL K4M FG.

Table 4 The ingredient list for cocoa drinks

Ingredients	*%Weight*
Milk	90.46
Sugar	6.5
Cocoa powder	2
Carboxymethylcellulose[a]	1
K-carrageenan	0.04

[a]Carboxymethylcellulose: WALOCEL CRT 100 PA.
Source: Trademark of The Dow Chemical Company.

substitution and substitution uniformity. The viscosity of the solution increases with increasing DP and increasing concentration.

CMC is soluble in water at any temperature. Because of its highly hygroscopic nature, CMC hydrates rapidly. Rapid hydration may cause agglomeration and lump formation when the CMC powder is introduced into water. Lump creation can be eliminated by applying high agitation while the powder is added into the water or preblending the CMC powder with other dry ingredients such as sugar before adding into water.

Due to its high solubility and clarity of its solutions, CMC is commonly used in beverages and beverage dry mixes to provide rich mouthfeel. It is also used in acidified protein drinks to stabilize protein and prevent it from precipitating. CMC is also added to syrup and sauce formulations to increase viscosity. Bakery is another application where CMC is commonly used to improve the quality and the consistency of the end product. In tortilla breads, for example, it is used to improve the process ability of the dough and the textural properties of the end product, including foldability and rollability.

Example Food Applications for CMC

Cocoa drinks

CMC is used in cocoa drink formulations to control viscosity, improve body and mouthfeel, stabilize solid particles, and prevent syneresis and sedimentation at hot and cold temperatures. The ingredient list to formulate cocoa drinks is provided in Table 4.

Ice cream

Blends of hydrocolloids and emulsifiers are used in ice cream formulation to contribute body, provide creamy mouthfeel, increase overrun and the amount of air incorporated into ice cream mix, and prevent ice crystal growth during freeze–thaw cycle. CMC is included in the blends to improve freeze–thaw stability of ice cream. Medium-viscosity CMC at a level of 0.02–0.5% (weight) is used in ice cream formulations to eliminate crystal growth and prevent undesired sandy mouthfeel.

Availability, Absorption, and Metabolism

Fermentability and Fiber

Dietary fibers impact all aspects of gut physiology and are a vital part of a healthy diet. There is a need for the development of novel fiber-rich foods that both are acceptable to the consumer and have proved health benefits. However, many fibers (particularly those that are rapidly fermented) can result in gas production, bloating, and diarrhea.

Several cellulose ethers are recognized as fibers. For example, HPMC is a nonfermentable soluble dietary fiber, which has been used in the manufacturing of many foods for several decades. It has a long safety record and is generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA) with intake concentration of up to 20 g day^{-1}. Cellulose ethers have unique physical properties apart from most dietary fibers. Because of this, the Association of Official Analytical Chemists (AOAC) published a special test method (AOAC 2006.08) to analyze the soluble fiber content provided by HPMC, MC, and CMC.

Health Effects

Cellulose ethers have been used in foods for several decades as functional additives. During this time, most of the uses focused on the ability of these polymers to modify the rheology of the food. However, within the last decade, a new focus has emerged due to their ability to provide broad health benefits when included in the diet. Cellulose ethers can be used as fibers, to substitute allergenic food ingredients, to shift fat content to healthier oils, to influence satiety, and to blunt postprandial insulin levels.

Replacement of Gluten in Bakery Products

Celiac disease (CD), an immune-mediated enteropathy, is one of the most common lifelong disorders on a worldwide basis. At present, the only available treatment for CD is strict adherence to a gluten-free diet, which means a permanent withdrawal of gluten from daily food. The majority of leavened cereal-based products are made of wheat flour or other cereal flours containing gluten. Gluten is an essential structure-building component in bread and other bakery products. Its removal impairs the dough's capacity to properly develop during kneading, leavening, and baking. This results in baked goods with very low volume and a dense pore structure. Hydrocolloids and mixtures of hydrocolloids can mimic the structuring role of gluten due to their physical properties. The usage of HPMC or the combination of HPMC and CMC (WELLENCE™) as a gluten replacement in baked goods has been in practice since at least 1985. The pictures in Figure 7

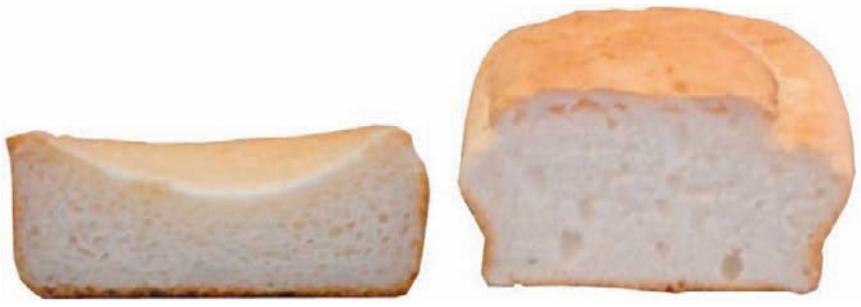

Figure 7 Visual images of gluten-free bread with and without METHOCEL™ addition.

demonstrate the ability of HPMC to build a structural network inside the product ensuring that cakes and breads retain the desired shape and volume.

Reduction of Fat Update in Foods

Deep-fried food is globally popular due to its distinct texture (crispy crust and tender and moist inside) and taste. However, with the increasing global obesity rate, according to the World Health Organization (WHO), an increasing number of health-conscious shoppers look for convenient, healthy, and satisfying fried food for themselves and their families. Technologies have been developed in recent years to make healthier fried foods with reduced/low-fat products. An interesting approach to reduce the health risks of consumer foods is to reduce the overall fat content of fried food by a coating with cellulose derivatives. Cellulose derivatives such as CMC, MC, and HPMC are used in the batter or breadcrumbs to reduce oil absorption during frying, for example, in doughnuts, fried dough products, and structured, extruded, and coated products. MC and HPMC in batters as viscosifying agent make a uniform and consistent batter for continuous batter coating manufacturing. MC and HPMC also improve the adhesion property of coating onto the food matrix and help to control batter pickup and the adhesion of seasoning and flavoring agents onto food surface. Most importantly for fat reduction, the thermal gelation and film-forming properties help to form a protective film barrier, which hinders the heat and mass transfer during deep frying, thus reducing the amount of oil absorption/ penetration into foods.

'Healthier' Fats

Although *trans* and saturated fats have beneficial attributes from the standpoint of food formulation, including firmness, reduction of oil migration, and leakage, they have also been linked to detrimental health effects. As a result, the WHO recommends that fat consumption should be shifted towards unsaturated fatty acids as opposed to saturated and *trans* fats. However, fat sources composed of mostly unsaturated fatty acids are in liquid form and lack structure at room temperature. As a consequence, they create significant challenges during food processing and adversely affect the product quality when used as a direct substitute for solid fats. An emerging strategy is to structure oil without the presence of *trans* or saturated fats. Cellulose ether, EC, is known to solubilize in liquid oil at elevated temperature and gel upon cooling. The resulting EC-oil gel has the desired structure that has potential to replace *trans* and saturated fats. The incorporation of edible EC-oil gels into food systems is now an active area of research, and applications currently under investigation include finely comminuted meat products, cream fillings, and bakery applications including puff pastry.

Figure 8 An image of layer formation in a puff pastry product that has 91% saturated reduced fat compared to puff pastry formulated with butter.

Puff pastry is a light and flaky type of pastry that contains several layers of thin rolled dough, which are formed by repeatedly rolling and folding the pastry dough. The roll-in is part of the puff pastry formulation that is composed of more than 90% fat and it is placed between the dough to assure separate layer formation during the rolling and folding. The fat used in the formulation of the roll-in needs to be highly saturated so that it is in the semisolid state at room temperature, to be spreadable onto the dough, and to help create the layers. However, due to its high saturated fat content, laminated pastry is undesirable for a healthy diet. Therefore, reducing saturated fat content of puff pastry without compromising the sensory properties is desirable. Figure 8 shows an image of a puff pastry that was prepared with a roll-in containing EC-oil gels. The pastry has 91% less saturated fat compared to a puff pastry formulated with butter.

As the figure shows, the resulting pastry had desired layer structure and a light and flaky texture.

Reduction of Blood Cholesterol

Elevated levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) are associated with an increased risk for coronary heart diseases. In several human clinical trials, HPMC was found to reduce TC and LDL-C while having only a minor effect on high-density lipoprotein cholesterol (HDL-C), thus improving the HDL/LDL ratio. For example, it was found that HPMC with varying dose and viscosity combinations showed LDL-C reductions ranging from 6.1% to 13.3% compared to a nonsignificant reduction (1.9%) in the control group. Changes in total and non-HDL-C paralleled those for LDL-C. Concentrations of HDL-C were not altered significantly. A recent study demonstrates that HPMC is an effective adjunct to statin therapy for further lowering atherogenic lipids and lipoproteins in humans with primary hypercholesterolemia. In the European Union (EU), the Commission Regulation No. 432/2012 allows the following health claim: "HPMC contributes to the maintenance of normal blood cholesterol levels."

In a diet-induced obesity mouse model, a significant decrease in the concentrations of plasma cholesterol was seen when the mice were fed with cationic hydroxyethyl cellulose (cHEC). Plasma TC was 16.7%, 19.7%, and 25.3% lower in mice that were fed with the 2% or 4% cHEC-supplemented diet.

Glycemic Response

Diabetes is associated with numerous adverse health outcomes, including cardiovascular diseases. Dietary fibers produce viscous solutions in the digestive system, blunt postprandial glucose, and insulin excursions. The degree of viscosity appears to be inversely related to glycemic response, with the more viscous dietary fibers producing greater effects. The fibers form viscous solutions when mixed with the gastrointestinal (GI) tract contents, slowing gastric emptying and thickening small intestine contents. This may reduce contact between food and digestive enzymes and interfere with diffusion of nutrients to absorptive surfaces, thus slowing the rate at which glucose molecules become available for absorption at the small intestine brush border. It has been suggested that lowering dietary glycemic load may be advantageous for individuals at risk for type 2 diabetes, coronary heart disease, and obesity.

Several studies have suggested that consumption of HPMC has potential therapeutic values in the management of risk factors for type 2 diabetes and cardiovascular disease. Consumption of high-viscosity (HV; 1% 5 Pa·s) and ultrahigh-viscosity (UHV 1% 7.5 Pa·s) HPMC with a meal significantly blunted postprandial insulin excursions and was well tolerated in overweight and obese men and women. However, only UHV HPMC blunted the peak glucose level. Further studies in subjects with and without type 2 diabetes mellitus have demonstrated that inclusion of HV-HPMC in a meal significantly blunts the postprandial glucose response. In a hamster model, it was demonstrated that after 8 weeks of feeding, 8% HPMC supplementation had better efficacy in glucose reduction compared to natural fibers such as pectin.

In the EU, the Commission Regulation No. 432/2012 allows the following health claim: "Consumption of HPMC with a meal contributes to a reduction in the blood glucose rise after that meal (4 g of HPMC per portion)."

Effects on Lipid Metabolism

HPMC modulates plasma lipoprotein profiles and hepatic lipid levels. HPMC is not absorbed by the body, but its presence in the intestinal lumen increases fecal fat, sterol, and bile acid excretion and as a result changes hepatic lipid metabolism. It has been suggested that HPMC may be facilitating fat excretion in a biased manner with preferential fecal excretion of both *trans* and saturated fats in hamsters fed with fast-food diets.

In preliminary studies, maturing hamsters on a high-fat diet put on significantly less body weight when they were supplemented with HPMC than the control animals, due primarily to the reduced deposition of abdominal fat tissue and fat accumulations in their livers and skeletal muscles. In obese mice, 4% and 8% HPMC supplementation in a high-fat diet led to significant weight loss. Also reductions in plasma cholesterol, glucose, and insulin levels were seen, which are strongly correlated with reduced leptin concentrations. Moreover, an increase in the fecal secretion of total bile acids, sterols, and fats indicated altered fat absorption when HPMC is incorporated in the diet. The data indicate that HPMC not only reduces body weight but also normalizes the metabolic abnormalities associated with obesity. The data suggest as well that the effect of HPMC on glucose and lipid homeostasis in mice is mediated through improvement in leptin sensitivity resulting from reduced fat absorption. HEMC was shown to be similarly effective in improving the lipid metabolism under high-fat diet condition.

Satiety

Overweight and obesity, as well as consequent cardiovascular disease, are primarily driven by overavailability of food and an increasingly sedentary lifestyle. One approach to treatment is to manipulate appetite and reduce food intake through control of satiety (inhibition of hunger as a result of having eaten). Dietary fibers are thought to impact satiety, by a viscosity effect. Viscous soluble fibers may be useful because they prolong the intestinal phase of nutrient digestion and absorption. Materials incorporated into the diet that form a gel mass in the stomach, such as alginate and pectin, have been shown to enhance satiety by distending the stomach wall. Novel food-grade MC (SATISFIT^{TM}-LTG cellulose) was developed to gel at temperatures below the body temperature and is not influenced by pH. It was shown *in vivo* by magnetic resonance imaging trials that SATISFIT^{TM}-LTG forms a gel mass that persists for at least 2 h. In contrast, the conventional MC that does not gel at body temperature clears the stomach rapidly. An initial clinical trial with healthy human volunteers demonstrated clear perception of greater satiety. Analysis of results from the visual analog scale indicates that appetite recovery is slower with novel

gelling MC than with control products. A significant reduction in the energy intake observed in the human trial might be explained by the gelation of the MC in the stomach. The satiety effect has been shown to last for at least 2 h after ingestion of the product. Further, human trials have to be performed to validate impact on satiety and on compensation effects.

See also: Fructose: Sources, Metabolism, and Health; Proteins: Chemistry, Characterization, and Quality; Sucrose: Properties and Determination.

Further Reading

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Cereals: Dietary Importance

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Introduction

Cereal-based foods are by far the major source of food, energy, protein, B vitamins, and minerals for the current world population estimated in more than 7.3 billion people (Figure 1). Basically, the population growth experienced during the past two centuries has been closely associated with the production of cereal grains. In most countries, diets have a single cereal as the primary staple. The most widely used are rice, wheat, and maize, which provide 93% of the total cereal calories (Figure 1). These grains constitute the main staple for Asians, Europeans, and Americans, respectively. In Africa and India, sorghum and millets are widely grown and consumed.

According to the Food and Agriculture Organization, the amount and proportion of food energy and protein provided by cereals in human diets in 2011 were 1296 kcal, nearly 50% of the average per capita caloric intake. Likewise, cereals provided 31.9 g protein of the total estimated daily intake of 73.9 g protein (Figure 1). Cereal grains are considered primarily as caloric or starchy foods and, more recently, as an important source of dietary fiber. However, their protein quality, especially for infants, is marginal. In developing countries, cereals are usually consumed with legumes and pulses, thus significantly increasing protein intake and, more importantly, enhancing protein quality by the improvement of the essential amino acid profile. Protein and/or calorie malnutrition (*kwashiorkor and marasmus*) is prevalent among groups of people who have inadequate food intake or rely solely upon cereals and/or starchy roots or tubers as their source of protein.

Plant breeders have developed maize, sorghum, and barley genotypes with high lysine, which results in an improved protein quality and nutritional value. A high-lysine and high-tryptophan maize, called quality protein maize, with satisfactory grain yield and structure is being commercially grown in different places around the world (Brazil, China, Ghana, and other African countries).

Carbohydrates

Starch is the most abundant carbohydrate in cereals and the main contributor of calories (Table 1). It is composed of molecules of branched amylopectin and linear amylose. Most cereals contain 70–75% amylopectin. Waxy maize, rice, and sorghum contain more than 95% amylopectin. Most food processes partially or totally gelatinize the starch granules, making the molecules more prone to amylases.

Starch is highly digestible by the human digestive system. Practically, all starch disappears in the gastrointestinal tract. A small portion of the starch (1–5%) resists enzymatic hydrolysis when cereal foods are thermally abused. This residual or resistant starch can be quantified in the soluble dietary fiber residue and is highly susceptible to fermentation in the hindgut.

Other factors that can decrease starch digestibility are excessive amounts of fiber and enzyme inhibitors. The digestible energy of cereals is negatively related to the grain fiber content and positively related to the grain lipid content. Refined grains contain more digestible energy than whole counterparts (Table 1).

Cereals contain small quantities of soluble carbohydrates such as glucose, fructose, maltose, and sucrose and therefore are suitable for diabetics. Highly refined cereals have a higher glycemic index compared with whole grain products.

Whole cereal grains are considered a rich source of dietary fiber (Table 1). However, foods from grains have marked differences in the amount and type of fiber. Fibrous components are concentrated in the pericarp or bran. The dietary fiber content in cereal-based foods varies greatly depending on the extent of milling. Refined flours lose most of the fiber during milling. All cereals are considered a rich source of insoluble dietary fiber constituted by cellulose, insoluble hemicellulose, and lignin, which speeds up intestinal transit and binds carcinogens. Epidemiological evidence has related a high-fiber diet to a lower incidence of diabetes, obesity, and diverticular disease. A high dietary fiber consumption benefits diabetics because of the reduced diffusion of glucose in the intestinal mucosa and the diminished insulin secretion rate. Oats, barley, and rye are considered good sources of soluble dietary fiber constituted by arabinoxylans, β-glucans, and soluble hemicelluloses, which increase the excretion of bile salts and dietary cholesterol, thus lowering blood cholesterol levels.

Protein

Cereal grains provide 43% of our total protein intake in 2011 (Figure 1). Genotype, environment, and growing conditions affect the amount of protein in the kernel. In general, brown rice and oats contain the lowest and highest protein content, respectively (Table 1). Protein quality is mostly dictated by the amino acid profile and digestibility (Table 2). The apparent protein digestibilities in cereals range from 80% to 90%. Sorghum (especially kernels with condensed tannins), whole barley, rye, and oats are consistently lower in digestibility than other cereals. Milling, decortication, fermentation, and germination all increase protein digestibilities because of the removal of fiber components.

The storage protein in cereals (prolamins) contains small amounts of essential amino acids, especially lysine. The albumins and globulins, mainly located in the germ, contain the best essential amino acid profile. High-lysine cultivars of maize, sorghum, and barley contain lower amounts of prolamins and higher amounts of the other protein fractions. Thus, they have a more balanced protein.

For all cereals, the most limiting amino acid is lysine. Oats, rice, rye, barley, and the high-lysine cultivars contain a more

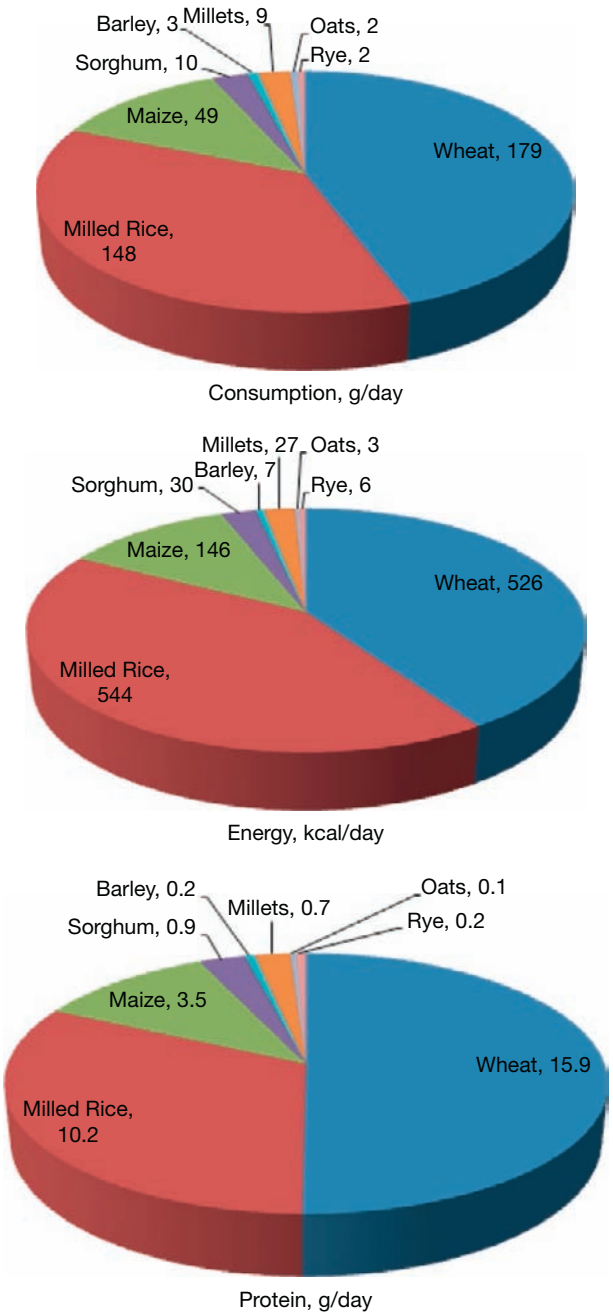

Figure 1 Consumption, energy, and protein daily intake of different cereals in the year 2011. Data taken from FAO (2014). Electronic page: http://apps.fao.org/. Rome: Food and Agriculture Organization.

favorable essential amino acid composition than the rest of the cereals (Table 2). Lysine deficiency is observed particularly in postweaned infants who suffer *kwashiorkor* and rely on cereals and/or starchy tubers as the only food source. The supplementation of cereal-based foods with small quantities of legumes or animal foods improves protein intake and protein quality. The next most limiting amino acids for maize and the rest of the cereals are tryptophan and threonine, respectively.

Decortication or milling partially or totally removes the pericarp and germ tissues, therefore improving the digestibility and reducing the lysine level. Malting improves protein quality due to enzymatic degradation of proteins. Fermentation improves protein quality via improved digestibility and *de novo* synthesis of lysine by the fermenting biomass.

Lipids

Lipids are relatively minor constituents in cereal grains (Table 1). However, cereal lipids are rich in the essential fatty acid, linoleic acid (18:2 Δ 9,12; 30–60% of total fatty acids) and practically devoid of saturated fatty acids. Cereals contain trace quantities of phytosterols; they do not have any cholesterol. Cereals with yellow endosperm (i.e., yellow corn, yellow sorghum, and durum wheat) contain some provitamin A activity imparted by β-carotenes. Various types of tocopherols are responsible for the vitamin E activity of cereal grains. Degermination of cereal grains greatly reduces the content of lipids and tocopherols.

Minerals and Vitamins

The pericarp, germ, and aleurone layer are rich in vitamins and minerals. Refined cereal products therefore lose part of these important nutrients. The enrichment of cereal-based foods is aimed toward the replacement of minerals (Fe and recently Zn) and vitamins (thiamine, riboflavin, niacin, and folic acid) lost during milling. In many countries, the fortification of cereal-based foods is required by law.

Phosphorus is the mineral found in greatest amounts (Table 3). Unfortunately, its availability is low because most is associated with phytic acid. The phytic acid has the property of binding strongly with other cations, thus decreasing their bioavailability. Phytic acid decreases significantly during sprouting and fermentation due to activation of phytases. These processes considerably improve the bioavailability of minerals (Table 4).

In general, cereals are poor source of calcium, except for finger millet and teff (Table 3). Some food processes, such as nixtamalization of maize for tortilla production, greatly increase calcium. The bioavailability of this mineral in lime-cooked tortillas is high.

Cereals are considered a good source of potassium and are practically devoid of sodium. Whole grains provide a significant source of magnesium, iron, zinc, and copper, which are reduced by degermination, decortication, and milling.

Cereals are also considered an important source of B vitamins (except B_{12} or cobalamin), but dried matured grains do not contain vitamin C. B vitamins are concentrated in the aleurone layer. Beriberi (a thiamine deficiency disease), endemic in Eastern and Southern Asia, is prevalent among people who consume milled rice. Milled rice contains about 10% of the thiamine of brown rice (Table 3).

Niacin is found in a free and bound form and can be synthesized from tryptophan. The alkali treatment of maize for tortilla production considerably improves niacin bioavailability because the glycosidic bond that renders it unavailable is alkali-labile. Niacin deficiency produces pellagra, which causes dermatitis, diarrhea, and dementia, and has been prevalent in regions of Southern Africa where people rely on maize as the main food source.

Table 1 Proximate and nutrient composition of cereal grains[a]

Proximate composition									*Dietary fiber*	
							Digestible energy			
Cereal	*Protein (%)*	*Ether extract (%)*	*Crude fiber (%)*	*Ash (%)*	*NFE (%)*	*Starch (%)*	*kcal kg^{-1}*	*kJ kg^{-1}*	*Total (%)*	*Soluble (%)*
Wheat										
Hard	14.4	2.3	2.9	1.9	78.5	64	3865	16181	12.1	1.7
	11.5–17	1.8–2.8	2.8–3	1.8–2	75.2–82.1					
Soft	9.9	2.8	2.7	1.7	82.9	69	3865	16181	12.1	1.7
	8–12	2.6–2.9	2.5–2.8	1.8–1.9	80.4–85.1					
Durum	13.2	2.8	2.8	2	79.2	70.2	4056	16981	12.1	1.7
	12–15.6	1.8–3.8	2.4–3.1	1.8–2.1	75.4–82					
Rice										
Paddy	7.5	2.4	10.2	4.7	75.2	–	2821	11810	–	–
	6.7–9	1.7–2.7	8.4–12.1	3.4–6	70.2–79.8					
Brown	9.2	2.5	0.9	1.5	85.9	77.2	4321	18091	3.7	0.9
	8.3–10.1	1.8–3.3	0.7–1.2	1.2–1.8	83.6–88					
Milled	7.8	0.5	0.4	0.6	90.7	90.2	3938	16488	1.3	0.4
	7.3–8.3	0.3–0.6	0.2–0.6	0.3–0.9	89.6–91.9					
Maize										
Dent	9.1	4.4	3	1.7	81.8	71.8	4056	16982	12.8	1.1
	8.1–11.5	3.9–5.8	2.4–3.5	1.4–2	77.2–84.2					
Flint	11.1	4.9	2.2	1.7	80.1	–	4056	16982	–	–
	9.5–12.8	4–5.8	1.6–2.8	1.4–2	76.6–83.5					
Popcorn	12.1	4.6	2.3	1.8	79.2	62.3	–		13.1	0.4
	11–13.2	4–5.3	2.2–2.4	1.6–1.9	77.2–81.2					
Sweet	13.2	4.6	2.7	2.3	77	54.1	4145	17354	9.4	1.2
	12.1–14.2	3.7–9	2.2–3.2	2–2.7	70.9–80.1					
Barley	11.5	2.2	5.6	2.9	77.8	58.5	3543	14833	15.4	3.9
	7.5–15.6	1.8–2.6	5.3–5.9	2.6–3.1	72.8–82.8					
Rye	13.4	1.8	2.1	2	80.7	68.3	3794	15885	16.1	3.8
	12.6–14.5	1.6–2.2	1.6–2.6	1.7–2.2	78.5–82.5					
Oats										
Whole	17.1	6.4	11.3	3.2	62	52.8	3058	12803	–	–
	12.4–24.4	4.5–10.3	10.4–14.3	2.9–3.4	47.6–69.8					
Groats	16.9	7.4	1.6	2.1	72	65	4316	18070	12.5	6.6
	13.8–22.5	5.9–8.4	1–3.3	1.9–2.4	65.2–77.4					
Sorghum	11	3.2	2.7	1.8	81.3	73.8	3880	16245	11.8	1
	7.3–15.6	0.5–5.2	1.2–6.6	1.1–4.5	68.1–89.9					
Millets										
Pearl	14.5	5.1	2	2	76.4	60.5	2822	–	7.0	0.6
	8.6–19.4	1.5–6.8	1.4–7.3	1.6–3.6	62.9–86.9					
Finger	8	1.5	3	3	84.5	59	–	–	–	–
	6–10.9	1–4.6	2–6.8	2.3–3.9	73.8–88.7					
Italian	11.7	3.9	7	3	74.2	59.1	3395	–	–	–
	6–14	1.2–5.2	2.6–8.6	1.5–3.6	68.3–88.7					
Proso	11	3.5	9	3.6	72.9	56.1	3636	–	–	–
	6.4–12.8	2.9–4.9	4.6–12	1.4–5	65.3–84.7					
Japanese	11.8	4.9	14.3	4.9	64.1	62	–	–	–	–
	11.2–12.7	2.5–6.3	13.9–14.7	4.7–5	61.3–68					
Fonio	8.7	2.8	8	3.8	76.6	–	–	–	–	–
	5.1–10.4	2.1–5.2	4.6–11.3	1.8–6	67.1–86.4					
Kodo	10.4	3.7	9.7	3.6	72.6	72	–	–	–	–
	6.2–13.1	3.2–4.9	8.4–11	3–4.1	66.9–79.2					
Teff	10.9	2.4	2.4	2.2	82.1	–	–	–	–	–
	7.9–12.6	2.3–2.5								

[a]All values are expressed on a dry matter basis. Protein conversion factors: for wheat, 5.7; rice, 5.95; other cereals, 6.25; NFE = nitrogen-free extract.

Source: Serna-Saldivar, S. O. (2003). Cereals: dietary importance. In: Caballero, B., Trugo, L. and Finglas, P. (eds.) *Encyclopedia of food sciences and nutrition* (2nd ed.), pp. 1027–1033. London: Academic Press; Serna-Saldivar, S. O. (2010). *Cereal grains: properties, processing and nutritional attributes*. Boca Raton, FL: CRC Press (Taylor & Francis Group).

Table 2 Amino acid composition of cereal grains[a]

	Wheat		*Maize*		*Rice*		*Sorghum*		
Amino acid (g per 100 g of protein)	*Hard*	*Durum*	*Normal*	*High-lysine*	*Brown*	*Milled*	*Normal*	*High-lysine*	*Barley*
Essential									
Phenylalanine	4.6	4.1	4.8	4.3	5.2	5.2	5.1	4.9	5.2
Histidine	2	1.9	2.9	3.8	2.5	2.5	2.1	2.3	2.1
Isoleucine	3	3.6	3.6	3.4	4.1	4.5	4.1	3.9	3.6
Leucine	6.3	7	12.4	9	8.6	8.1	14.2	12.3	6.6
Lysine	2.3	2.2	2.7	4.3	4.1	3.9	2.1	3	3.5
Methionine	1.2	0.9	1.9	2.1	2.4	1.7	1	1.6	2.2
Threonine	2.4	2.9	3.5	3.9	4	3.7	3.3	3.3	3.2
Tryptophan	1.5	–	0.5	0.9	1.4	1.3	1	0.9	1.5
Valine	3.6	4.6	4.9	5.6	5.8	6.7	5.4	5.1	5
Nonessential									
Aspartic acid	4.7	4.7	6.4	7.7	9.3	9.8	6.4	7.5	6
Glutamic	30.3	32.3	19.2	17.1	17.3	19.3	20.6	20.1	25.5
Alanine	3.1	4.8	7.7	6.3	5.8	5.8	8.6	8.4	2.1
Arginine	4	3.5	4.8	6.9	9.5	8.8	3.5	4.5	4.6
Cysteine	2.8	–	1.4	–	2.3	2.2	1.6	1.5	1.8
Glycine	3.8	6.5	3.8	5	4.8	4.8	2.9	3.5	3.9
Proline	10.1	13.4	8.2	9.1	5	4	7.9	7.6	11.6
Serine	4.2	5.7	4.6	4.7	5.3	4.3	4.1	4.2	3.8
Tyrosine	2.7	2	4.2	3.5	4.2	5	3.2	4.2	2.8
Amino acid score (%)	42.3	40.4	49.6	79	75.4	71.7	38.6	55.1	64.3
Protein digestibility	89.8	87.9	88.2	88.9	88.5	89	85.9	88.9	84.9

				Millets						
Amino acid (g per 100 g of protein)	*Rye*	*Oats*	*Groats*	*Pearl*	*Finger*	*Proso*	*Japanese*	*Italian*	*Kodo*	*Teff*
Essential										
Phenylalanine	5	5.4	4.2	5.2	5.2	5.2	5.9	5.5	5.8	5.7
Histidine	2.4	2.4	2.2	2.2	2.2	2.2	1.9	2.9	1.8	3.2
Isoleucine	3.7	4.2	3.9	4.4	4.4	4.6	4.5	5.9	3.1	4
Leucine	6.4	7.5	7.4	11	9.5	12.9	11.5	14.1	8.6	8.5
Lysine	3.5	4.2	4.2	2.9	2.9	2.2	1.7	2.2	3.2	3.5
Methionine	1.6	2.3	2.5	2	3.1	2	1.8	2.6	1.7	4.1
Threonine	3.1	3.3	3.3	3.9	4.2	3.3	2.7	4.3	2.9	4.3
Tryptophan	0.8	–	1.3	2.3	1.5	0.9	1	1.4	0.8	1.4
Valine	4.9	5.8	5.3	5.7	6.6	5.1	6.1	5.1	4.2	5.5
Nonessential										
Aspartic acid	6.7	9.2	8.9	8.6	6.5	5.7	6.1	6.9	6.3	6.6
Glutamic acid	24.7	21.6	23.9	20.7	20.3	20.4	23.9	18.8	23.1	24.8
Alanine	2.4	5.1	5	8.5	6.2	10.7	9.3	8.9	5.5	5.7
Arginine	5.9	6.4	6.9	5.3	4.5	3.2	3.6	2.8	3.6	5
Cysteine	2	1.7	1.6	2.1	2.6	1.6	2.7	1.4	1	0.9
Glycine	4	5.1	4.9	3.3	4	2.2	2.3	2.9	3.8	3.8
Proline	9.1	5.7	4.7	6.6	7	7.2	10.1	10.6	7.2	5.5
Serine	4.1	4	4.2	4.9	5.1	6.3	5.6	5.8	4.1	5.2
Tyrosine	2.6	2.6	3.1	3.2	3.6	2.4	2.4	2.6	3.8	3.9
Amino acid score (%)	64.3	77.2	77.2	53.3	53.3	40.4	31.2	40.4	58.8	64.3
Protein digestibility	86.7	86.2	90.6	89	–	89.9	–	89.8	–	–

[a]Essential amino acid requirements for infants (g per 100 g of protein): lysine, 5.44; methionine and cysteine, 3.52; threonine, 4; isoleucine, 4; leucine, 7.04; phenylalanine and tyrosine, 6.08; histamine, 1.4; tryptophan, 0.96; tyrosine and cysteine are not essential amino acids but they can spare the requirements for phenylalanine and methionine, respectively. Source: Serna-Saldivar, S. O. (2003). Cereals: dietary importance. In: Caballero, B., Trugo, L. and Finglas, P. (eds.) *Encyclopedia of food sciences and nutrition* (2nd ed.), pp. 1027–1033. London: Academic Press; Serna-Saldivar, S. O. (2010). *Cereal grains: properties, processing and nutritional attributes*. Boca Raton, FL: CRC Press (Taylor & Francis Group).

Table 3 Mineral and vitamin compositions of cereal grains

Nutrients	Wheat		Maize	Rice		Sorghum	Barley
	Hard	Durum		Brown	Milled		
Minerals							
Ca (%)	0.03	0.04	0.03	0.03	0.02	0.04	0.04
P (%)	0.35	0.51	0.29	0.25	0.12	0.35	0.56
Phytic acid (%)	0.97	–	0.71	0.56	–	0.77	1.06
K (%)	0.36	0.49	0.37	0.17	0.10	0.38	0.50
Na (%)	0.04	–	0.03	0.03	0.00	0.05	0.02
Mg (%)	0.14	0.17	0.14	0.19	0.03	0.19	0.14
Fe (ppm)	40.1	47.8	30	28	19	50	36.7
Co (ppm)	0.05	–	0.1	0.07	0.01	3.1	0.04
Cu (ppm)	4.9	5.6	4	4.2	2	10.8	15.1
Mn (ppm)	40.0	33.5	5	24	12	16.3	18.9
Zn (ppm)	30.9	41	20	18	10	15.4	23.6
Vitamins							
Thiamine (mg per 100 g)	0.57	0.67	0.38	0.34	0.07	0.46	0.44
Riboflavin (mg per 100 g)	0.12	0.11	0.14	0.09	0.03	0.15	0.15
Nicotinic acid (mg per 100 g)	7.40	11.10	2.80	4.62	1.60	4.84	7.20
Pyridoxine (mg per 100 g)	0.35	0.43	0.53	0.92	0.45	0.59	0.44
Pantothenic acid (mg per 100 g)	1.36	–	0.66	1.35	0.75	1.25	0.57
Biotin (mg per 100 g)	0.01	–	0.01	0.01	–	0.02	0.01
Folacin (mg per 100 g)	0.04	0.04	0.03	0.02	0.02	0.02	0.04
Carotenes (mg kg^{-1})	0.2	0.2	29.5	–	–	15.7	1
Vitamin E (mg kg^{-1})	12.8	28	24	1.7	0.14	13.8	24.8

Nutrients	Rye	Oats	Groats	Millets						
				Pearl	Finger	Proso	Italian	Kodo	Teff	Fonio
Minerals										
Ca (%)	0.05	0.11	0.08	0.01	0.33	0.01	0.01	0.01	0.17	0.03
P (%)	0.36	0.38	0.51	0.35	0.24	0.15	0.31	0.32	0.45	0.18
Phytic acid (%)	0.97	1.80	–	–	–	0.32	–	–	–	–
K (%)	0.47	0.47	0.44	0.44	0.43	0.21	0.27	0.17	0.31	0.16
Na (%)	0.01	0.02	–	0.01	0.02	0.01	0.01	0.01	0.02	0.02
Mg (%)	0.11	0.13	0.14	0.13	0.11	0.12	0.13	0.13	0.18	0.40
Fe (ppm)	38	62	47.2	74.9	46	33.1	32.6	7	14.9	36
Co (ppm)	–	0.05	–	0.50	0.10	–	–	–	0.06	3.30
Cu (ppm)	9	4.7	4.8	6.2	0.3	8.3	9.2	–	4.4	15
Mn (ppm)	58.4	45	46	18	7.5	18.1	21.9	–	2.5	30
Zn (ppm)	32.2	37	35.8	29.5	15	17.2	21.4	–	6.7	30
Vitamins										
Thiamine (mg per 100 g)	0.69	0.77	0.72	0.38	0.48	0.63	0.48	0.32	0.45	0.30
Riboflavin (mg per 100 g)	0.26	0.14	0.16	0.22	0.12	0.22	0.12	0.05	0.10	0.10
Nicotinic acid (mg per 100 g)	1.52	0.97	0.91	2.70	1.30	1.32	3.70	0.70	2	3
Pyridoxine (mg per 100 g)	0.34	0.12	0.21		–	–	–	–	–	–
Pantothenic acid (mg per 100 g)	0.73	1.36	1.23	1.09	–	1.10	0.82	–	–	–
Biotin (mg per 100 g)	0.01	0.02	–	–	–	–	–	–	–	–
Folacin (mg per 100 g)	0.05	0.06	–	–	–	–	0.02	–	–	–
Carotenes (mg kg^{-1})	–	–	–	5.4	–	–	–	–	–	–
Vitamin E (mg kg^{-1})	16.6	16.7	17	19	22	–	31	–	–	–

Source: Serna-Saldivar, S. O. (2003). Cereals: dietary importance. In: Caballero, B., Trugo, L. and Finglas, P. (eds.) *Encyclopedia of food sciences and nutrition* (2nd ed.), pp. 1027–1033. London: Academic Press; Serna-Saldivar, S. O. (2010). *Cereal grains: properties, processing and nutritional attributes*. Boca Raton, FL: CRC Press (Taylor & Francis Group).

Table 4 Effects of cereal processing on the nutritional value

Processing method	*Effects on nutritional value*
Dry milling, decortication, degermination	The removal of the pericarp and germ tissues considerably modifies the chemical composition. Refined products are practically fiber-free and contain lower amounts of oil, minerals, vitamins, and even essential amino acids. In addition to physical losses, thermal treatments usually lower the bioavailability of important vitamins. The protein quality of refined products is inferior compared with whole grains because the proteins of the germ contain a more balanced essential amino acid composition
Germination or sprouting	Germination or sprouting has been an effective way to improve the nutritional value of cereal-based products. The high enzymatic activity of malted cereals improves nutrient digestibility, mineral bioavailability, and the bioactivity of some nutraceuticals
Fermentation	Fermentation causes similar benefits as germination. Fermentation is known to improve protein quality due to higher nitrogen digestibility and *de novo* production of some amino acids such as lysine and tryptophan. In addition, these processes lower the presence of antinutritional factors. Fermented breads usually contain more protein and better protein quality due to yeast
Cooking	Water, alkali, or acid cooking slightly reduces protein digestibility due to the insolubilization of prolamins and formation of disulfide bonds. Maize and sorghum are frequently alkali-cooked to produce traditional foods such as tortillas and Tô. Cooking in the presence of alkali leachates (i.e., wood ashes, lime, or potassium hydroxide) slightly lowers protein quality and lysine bioavailability. Lime cooking of maize solubilizes albumins, globulins, and prolamins with the consequent increase in residual or nonextractable proteins. These changes slightly reduce protein digestibilities. Lime cooking of maize increases calcium content, which is highly bioavailable. It is also known that lime cooking enhances niacin bioavailability. This is very relevant or important in countries where pellagra is still endemic among some groups
Parboiling	Parboiling of rice and other cereals such as sorghum and millets produces important changes in the nutritional value. The thermal treatment gelatinizes starch and hardens the endosperm, reducing the kernel susceptibility to break during milling. Parboiled rice has higher quantities of vitamins and minerals because the nutrients located in the aleurone diffuse into the inner endosperm during hydration and parboiling

Source: Serna-Saldivar, S. O. (2010). *Cereal grains: properties, processing and nutritional attributes*. Boca Raton, FL: CRC Press (Taylor & Francis Group).

Effect of Processing on Nutrient Composition

Humans usually consume processed cereal products instead of whole grains. Most processed foods are manufactured from refined milled products such as decorticated grains, flours, meals, semolina, and grits. Commonly, cereals are milled and then cooked in order to produce an array of foods. Milling and cooking have a profound effect on the nutritional value of prepared foods. Table 4 summarizes the main effects of processing on the nutritional value of cereal-based foods.

Phytochemicals and Nutraceutical Properties of Cereal Grains

Besides the dietary fiber, cereals are considered as a good source of important antioxidants and nutraceuticals, which exert proved positive effects in human health because they combat oxidative stress, chronic diseases, and cancer. Table 5 summarizes the main phytochemicals associated with cereals with their main effects on human health.

Among the cereals, sorghum and maize have higher antioxidant activity compared with wheat, oats, and rice.

Phenolic compounds are divided into three major categories: simple phenolics, flavonoids/anthocyanins, and tannins. All cereals contain simple phenolics and only brown sorghum condensed tannins. The most relevant simple phenolic is ferulic acid in its free, conjugated, and bound forms. It is considered as a potent antioxidant and a nutraceutical that prevents inflammation, cancer, LDL oxidation, and neuron degeneration (Table 5). Cereals may contain significant amounts of flavonoids such as flavanols, flavanones, flavones, and anthocyanins. Red-, blue-, and purple-pigmented corn kernels are rich in anthocyanins, and several reports indicate that these are similar to the ones found in red wine. The main anthocyanins are cyanidin and peonidin glycosides, which diminish the incidence of various chronic and degenerative diseases. Their health benefits have been related to their high-antioxidant and antiradical activities. Sorghum is the most promising cereal in terms of flavonoids and nutraceutical potential. For instance, sorghum may contain flavan-3-ols, flavan-4-ols, and anthocyanins such as apigeninidin and luteolinidin. Among flavanols, the flavan-4-ols have particular therapeutic interest because of their antitumor activity and enhancement of immune response (Table 5).

Cereal lipids can be subdivided into nonpolar, polar, and nonsaponifiable. By much, the most abundant type is the nonpolar fraction consisting of triglycerides. In all cereal grains, except brown rice and oats, the major fatty acid component is linoleic acid followed by palmitic acid (16:0). In brown rice and groats, oleic acid (18:1 $\triangle 9$) is the major unsaturated fatty acid.

The lipid fraction of cereals contains significant amounts of phospholipids such as phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, and phosphatidylserine. These polar lipids are considered nutraceuticals because they form part of cell membranes and keep their integrity. Phosphatidylcholine helps to keep the proper functioning of the liver and to transport lipids, and its deficiency is related to increased susceptibility to hepatic cancer. Lecithin and choline lower the risk of

Table 5 Major nutraceuticals associated with cereal grains and their health benefits

Nutraceutical compound			
Family	*Class*	*Anatomical part/main cereals*	*Preventive or therapeutic effect*
Phenolics	Simple phenolics such as ferulic acid	Mainly associated with the pericarp. Present in all cereal grains	Prevent oxidative stress, cancer, cholesterolemia, atherosclerosis, and aging
	Avenanthramide	Associated with the outer layers of oats (pericarp, aleurone)	There are 35–40 phenolic alkaloids that occur as amides of substituted anthranilic acids. They are anti-inflammatory and anti-irritant and prevent chronic inflammatory disease, cardiovascular disease, cancer, diabetes, Alzheimer's disease, and schizophrenia
Flavonoids	Anthocyanidins Flavonols Flavan-3-ols Flavanones	Mainly associated with the aleurone of blue- and red-colored maize. Flavanones (eriodictyol and naringenin) are found in lemon-yellow sorghums	Prevent oxidative stress, cancer, cholesterolemia, atherosclerosis, and aging
	Condensed tannins	Mainly associated with *testa* of high-tannin or brown sorghums	Prevent oxidative stress, cancer, cholesterolemia, atherosclerosis, stomach ulcers, and aging
Carotenoids	Carotenes	Mainly associated with starchy endosperm in yellow endosperm cereals	β-Carotenes are converted to vitamin A or retinol. Prevent cancer and cardiovascular disease and strengthen the immune system
	Xanthophylls *Lutein* *Zeaxanthin* *Cryptoxanthin*	Mainly associated with yellow endosperm maize, wheat, and sorghum	Prevent age-related macular degeneration and cataracts (opacity of the crystalline lens of the eye). Slow down symptoms of retinitis pigmentosa. Prevent cardiovascular disease and cancer
Phytosterols	Sitosterol Stigmasterol Campesterol Avenasterol	Mainly associated with the germ, pericarp, and aleurone of most cereals	Compete with cholesterol for absorption and therefore is hypocholesterolemic. Prevent cardiovascular diseases
Fibers	Soluble	Mainly associated with oats and rice bran	Improve the function of the gastrointestinal tract, increase viscosity, and lower glycemic index. Reduce risk of diabetes and hypercholesterolemia. Most soluble fibers are probiotic because they readily ferment in the hindgut yielding short-chain fatty acids that inhibit hepatic HMG-CoA reductase
	Insoluble	Mainly found in the pericarp associated with cell walls of all cereal grains	Improve gastrointestinal functionality. Increase bile acid binding and fecal bulk and reduce constipation, hemorrhoids, diverticulitis, and cancer of the large bowel
Phytic acid Inositol	Inositol hexakisphosphate	Mainly associated with the aleurone and pericarp of all cereal grains	Antioxidant, antineoplastic properties in breast, colon, liver, prostate, and skin cancers, leukemia, and sarcomas
Polyunsaturated fatty acids	Linoleic acid (18:2 ω6) Linolenic acid (18:3 ω3)	Mainly associated with the germ or *scutellum* of all cereals	Help to reduce cholesterol and hyperlipidemia. Linoleic and linolenic acids are transformed into prostaglandins and nutraceutical fatty acids EPA and DHA
Lecithin and choline	Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine	Mainly associated with the germ	Phosphatidylcholine (lecithin), phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine are essential for proper function of the cell membranes and brain. Slow down the process of cell aging and prevent high cholesterol. Choline is used for the synthesis of acetylcholine, which is the main neurotransmitter

(*Continued*)

Table 5 (Continued)

Nutraceutical compound			
Family	*Class*	*Anatomical part/main cereals*	*Preventive or therapeutic effect*
Vitamins	Tocopherols	Mainly associated with the germ of all cereals	Tocopherols are important antioxidants. α-Tocopherol or vitamin E is considered as the second line of defense against oxidative stress. Prevent cardiovascular disease and high cholesterol and improve mental health and brain functionality and are considered antimutagenic and anticancer
	Folic acid	Mainly associated with the aleurone of all cereal grains	Prevents neural tube defects in newborn babies and miscarriages and helps for proper brain development. Lowers homocysteine and therefore helps to prevent cardiovascular diseases
Policosanols	Long-chained alcohols (waxes): Octacosanol Triacontanol Hexacosanol Dotriacontanol	Mainly associated with the outer part of the pericarp and germ Maize and sorghum contain significant quantities	Have beneficial physiological activities such as reducing blood lipid levels and platelet aggregation

Source: Serna-Saldivar, S. O. (2010). *Cereal grains: properties, processing and nutritional attributes*. Boca Raton, FL: CRC Press (Taylor & Francis Group).

cardiovascular disease and positively affect brain and mental development of both the fetus and infants, and their chronic inadequacy may be related to Alzheimer's disease. Phosphatidylinositol and phosphatidylserine are lipotropic, reduce blood triglycerides and fatty liver, and prevent bipolar disorders and Alzheimer's disease.

Phytosterols such as β-sitosterol, campesterol, avenasterol, and stigmasterol inhibit the absorption of cholesterol from the small intestine, thus effectively lowering both serum total cholesterol and LDL.

Other important groups of antioxidants are the carotenoids and xanthophylls, which are minor constituents of cereal grains. They are most abundant in yellow maize, yellow sorghum, and durum wheat. The consumption of yellow endosperm cereal grains can potentially benefit at least 1 million children that die every year due to weakness and deficiency of vitamin A and 350 000 more that become completely blind. The main nutraceutical role of carotenoids to humans is the molecular protection against free radicals. From the nutritional viewpoint, the most important carotenoid is β-carotene because one molecule is converted into two of the active forms of vitamin A or retinol in a normal human system. In addition, β-carotene can regenerate the activity of vitamin E and possibly other oxidized antioxidants. β-Carotene also acts as an antioxidant that scavenges free radicals deep in human LDL and HDL and in cell membranes. Lutein, zeaxanthin, and cryptoxanthin are xanthophylls that have received special attention because they prevent macular degeneration highly associated with blindness in geriatric patients.

Tocopherols and tocotrienols are responsible for the vitamin E activity of plant tissues. Various combinations of all eight tocols are found among cereals. The most predominant forms are α-tocopherol, γ-tocopherol, and α-tocotrienol. Among the structural parts of cereal grains, tocol derivatives are most abundant in the germ. These compounds are potent antioxidants that block the formation of free radicals in cell membranes. Thus, they prevent cardiovascular diseases and LDL oxidation and strengthen the immune system.

See also: Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Barley; Carotenoids: Occurrence, Properties and Determination; Cellulose; Cereals: Types and Composition; Dietary Fiber: Determination; Energy: Intake and Energy Requirements; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fermented Foods: Origins and Applications; Functional Foods; Maize; Millets; Niacin; Phenolic Compounds: Bioavailability and Health Effects; Phenolic Compounds: Occurrence, Classes, and Analysis; Phospholipids: Properties and Occurrence; Phytic Acid: Properties, Uses, and Determination; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Proteins: Chemistry, Characterization, and Quality; Pulsed Electric Fields; Retinol: Properties and Determination; Rice: Role in Diet; Sorghum: A Novel and Healthy Food; Starch: Sources and Processing; Starch; Tannins; Tocopherols: Properties and Determination; Tortillas; Wheat: Grain Structure of Wheat and Wheat-based Products.

Further Reading

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Relevant Websites

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www.fao.org – Food and Agriculture Organization of the United Nations.
www.grainmilling.org – Grain Milling & Processing: Cereal Foods.
www.usda.gov – U.S. Department of Agriculture.
www.worldbank.org – The World Bank.

Cereals: Storage

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Introduction

Storage of cereal grains is critical in terms of food security for the more than 7.3 billion people inhabiting the world. The main purpose of grain storage is to equilibrate supply and demand, and this postharvest operation is a key step in the complex logistics of moving grain from producers to processors and grain products from processors to consumers.

Most cereal grains have to be stored on farms or commercial grain elevators because they are harvested in specific seasons of the year and are gradually utilized by the various industry segments. Generally, imported grains are also stored for significant periods of time because they are usually acquired in large quantities in order to keep low costs and large inventories. The main objective of storage is to provide wholesome cereals, free of insects, insect fragments and rodent filth, mold damage, mycotoxins, and pesticides.

The final target is to manage stored grain wisely with insignificant losses while maintaining its nutritional and functional qualities.

The FAO has estimated that grain storage losses in most developing countries, mainly located in tropical and subtropical areas of the globe, are up to 35% higher compared with developed counterparts due to lower investments in proper infrastructures. Therefore, there is a great opportunity to upgrade storage systems in order to diminish losses and assure food quality especially for people with scarce economic resources. If new and better storage facilities are built, the world can save at least 15% of the total cereal production estimated in 2013 in more than 2.78 billion tons. Worldwide storage facilities have been calculated in less than 65% of all production, which indicates a lack of infrastructure especially in developing countries. An ever-growing population and the food crisis demand for new storage facilities at collection points close to the harvest areas, especially in regions where industrialization is not completed yet. Up to 70% of world's food reserve crops are still stored outdoors in bags piled on raised earthen platforms or stored bagged in godowns. Only about 20% is stored in modern silos.

Cereal grains deteriorate due to intrinsic and/or extrinsic reasons. The intrinsic deterioration is due to respiration, whereas the extrinsic deterioration is caused by biotic agents such as insects, molds, and rodents. Regardless of the sort of losses, grains lose dry matter, quality, and nutritional and economic values as raw materials and feedstocks. From the health viewpoint, the consumption of mold-infested grains could lead to animal or human mycotoxicosis. The toxins of greatest concern are aflatoxins because of their known carcinogenesis and fumonisins, ochratoxins, T-2, and zearalenone.

Grain Deterioration

The key to maintain cereal grains under optimum conditions is the control of their moisture. When grain moisture exceeds the permissible level, increase of the grain respiration rate is activated. Stored cereals have latency, that is, limited respiration, when maintained under their critical moisture content (considered 14%). Below this critical moisture, pests have more problems to reproduce and survive. The temperature and air relative humidity are the most important environmental factors affecting grain deterioration, insect infestation, and mold growth. High grain moisture, environmental temperature, and air relative humidity increase the grain metabolic activity and enhance the growth and development of insects and molds.

Intrinsic Deterioration

This grain deterioration is caused by the metabolic activity resulting from respiration. The grain generates energy or heat, carbon dioxide, and water. The activation of the grain releases important enzymes that break down lipids, proteins, and starch generating carbon dioxide as the end-respiration metabolite and hydrolyzed compounds, which are detrimental for functionality and quality of processed or finished products.

In general terms, the intrinsic grain deterioration favors the extrinsic because most pests require water as one of the most relevant substrates. The air relative humidity plays an important role in the susceptibility of the grain to deterioration. The grain can surpass its critical moisture content when it is exposed at a high air relative humidity (more than 70%). When the cereal grain surpasses its critical moisture content (14%), it activates and generates heat that catalyzes the respiration process. This is the most common way how adequate stored grains lose latency and progressively deteriorate. More importantly, the respiration process that generates heat and water attracts insects and molds, which damage kernels that contain more than 1.5 and 3.5% moisture above the critical.

Grains tend to equilibrate with the environmental moisture and are hygroscopic when exposed at high relative humidities. Commonly, storage facilities situated in tropical areas are the most difficult to manage because of the high temperatures and air relative humidities.

Extrinsic Deterioration

The extrinsic deterioration is the most important in terms of grain losses. It is mainly caused by insects, followed by molds. Insects can proliferate at relatively lower grain moisture or water activities than molds. Rodents and birds also play an important role in extrinsic grain deterioration especially in open storage facilities. All these biotic agents cause direct and indirect losses. The indirect damage is due to insect fragments and feces, rodent hair and feces, and bird droppings that can contaminate a given lot of grain with pathogenic bacteria. The presence of these contaminants is highly penalized by regulatory agencies.

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Management of Stored Grains

Grains are preferably stored in weather- and pest-proof storage structures so that their viability, food energy, nutritional quality, and marketability can be assured. Grain elevators are centers where the grain is concentrated with the aim of preserving and improving the grain grade. Incoming lots of grains are in most cases sampled; analyzed in order to assign class, grade, and other quality attributes; weighed; unloaded; cleaned; and stored. The most critical operation is the one related to testing grain quality because it is related to the economic value of the grain and will determine other important management decisions such as the need for drying, cleaning, commingling, and end use.

Grain Analysis

After sampling, most grain lots are inspected and graded by licensed inspectors or by experienced employees. The main grain quality factors are moisture, test weight, dockage, heat damage, insect damage, and mycotoxins. Moisture is generally determined with the electronic moisture meter or a near-infrared apparatus. The first determines moisture via electric conductivity, whereas the second, by scanning whole or ground kernels in the infrared spectrum.

Test weight is a measure of the apparent grain density and is the most widely used parameter, related to grain condition and therefore to its commercial value. It is negatively affected by insect and mold damage. The most common way to perform a measurement of it is using the universal dockage test meter equipped with different sets of sieves for each specific type of cereal. Heat-damaged, insect-damaged, moldy, and/or sprouted kernels are visually identified and manually removed and quantified.

One of the main concerns in grain elevators is the acquisition and merchandising of mycotoxin-contaminated kernels. This is because domestic and export markets have strong federal regulations regarding the maximum allowable amounts of these metabolites. In most countries, the maximum aflatoxin permitted level for direct human and animal use is 20 and 200–300 ppb, respectively. Needless to say, when grains exceed 20 ppb and contain less than 250 ppb, they have to be sold as animal feed with a discount price. Grain elevators routinely test for mycotoxins, especially aflatoxins. Suspected grains are usually first observed under ultraviolet light to see their fluorescence. This practical test does not determine the type of mold nor the mycotoxin level; however, it is used as a screen test to decide if the sample must be further tested with a quantitative assay. The most common assay to determine mycotoxins is based on a quick solvent extraction (methanol–water, ethanol, chloroform, etc.) of the mycotoxin, followed by filtration and quantification via ELISA (enzyme-linked immunosorbent assay) columns.

Weighing and Grain Unloading

Grain shipments are usually weighed on platform scales and then dumped into underground bins via gravity. Most grain elevators are equipped with a hydraulic platform that positions the locked truck in an angle so to speed up the unloading process and to assure the complete removal of the grain. A similar system is used to unload trainloads. The train wagon is positioned into the unloading zone, and then, the gate or bottom door is opened to discharge the grain directly into an underground bin. Depending on the grain characteristics, the discharged lot is dehydrated, precleaned, or simply conducted to storage facilities. Grains are elevated or conveyed using screw conveyors or buckets.

Grain Dehydration

Some cereal grains such as paddy rice are usually harvested at high moisture contents and therefore need artificial drying before storage. The aim of this operation is to lower the grain moisture content to levels adequate for storage to maintain grain viability and to keep to minimum grain physical damages such as stress cracks. This is especially relevant for paddy rice, which is commonly harvested at moistures above 22%. Drying temperatures of up to 45 °C are generally safe although higher temperatures may be used in cereals used for feed.

Artificial drying is the most common practice to lower the moisture content of cereal grains. There are batch or continuous dryers differencing in the air flow: cross, concurrent, or counterflow. Regardless of the dehydration technique, the principal factors to regulate are air temperature and relative humidity, airflow, depth of the grain, and the desired rate of dehydration. The heated air is injected at a flow rate of approximately 1 m^3 per ton.

Grain Cleaning

Grain cleaning is a common operation before storage especially when the grain is going to be directly channeled to the food industry. Cleaning improves grade, increases uniformity of grain lots, and decreases amounts of damaged grains. The grain with lower foreign material will be more stable during storage because the extraneous matter contains high amounts of insect eggs and mold spores and serves as physical protection to biotic agents. The cleaning system typically consists of air aspirators and sifters equipped with magnets to trap metals. Air aspiration removes most of the light contaminants such as plant material, glumes, and empty kernels, whereas milling separators are designed to remove larger and smaller contaminants than the grain. Milling separators generally consist of two or more sieves positioned one on top of the other on an oscillating or vibrating frame. Other cleaning apparatuses seldom used by grain elevators but frequently used by milling industries include gravity and disk separators and color sorters.

Grain Rotation

Turning is the process of moving bulk stored grains with conveyors within the storage facilities. The operation is practiced in order to break hot spots in storage, facilitate insect control, and make more efficient the aeration or ventilation operation. Insecticides can be effectively applied during rotation, and if fumigation is necessary, tablets of aluminum phosphide can be distributed throughout the grain mass. The main disadvantage of grain rotation is that it increases kernel damage and the incidence of broken kernels.

Grain Aeration

Aeration is considered as the cheapest preventive measure for the preservation of grains. It is defined as the movement of air through a bed of stored grain. Its main advantages are that the quality of grain is maintained without moving the grain, the significant reduction of the wear and tear on both the grain and handling machinery, and the suitability and effectiveness in applying fumigants. The aim of this operation is first to equalize stored grain temperature to prevent moisture migration, remove sour and off-odors caused by molding, and cool the grain to prevent or minimize hot spots and insect and mold growth. Most aeration systems consist of perforated air ducts positioned in X or Y configurations on the floor before the facility is filled. The ducts are fed with outside air by fans placed on the external walls of the bins.

Types of Storage Facilities

There are many designs of grain storage facilities. The facilities should be designed to protect cereals from weather, insects, molds, rats, and birds. Facilities range from a simple pile of unprotected grain on the ground to expensive storage bins or elevators that contain equipments for sorting, cleaning, drying, fumigating, and transferring grain lots to trucks, railcars, barges, and ships.

Storage on the Ground

This is the simplest method and the one that requires the lowest economic inputs. It is widely used as a short-term or transitional storage especially after harvesting. However, since the grain is exposed to the air, it is susceptible to weather conditions and biotic infestations. The grain is simply unloaded and conveyed into the prepared ground getting the typical hill configuration. The slope and form of the grain hill is critically important to minimize losses due to rain. The slope of the grain hill should minimize the penetration of the rainwater, and the ground floor of the pile should also help to absorb water. When the grain pile is exposed to rainfall, a 5 cm deep external grain layer forms, protecting the rest of the grain. The ground systems can be improved when a circular contention wall of approximately 1 m tall is built with grain bags, by placing aeration ducts on the ground and by covering the surface or external part of the stored grain with plastic covers.

Underground Storage

Underground storage is considered as one of the oldest practices to store grains. The method protects kernels from adverse environmental conditions and inhibits pests due to the low oxygen and high carbon dioxide concentrations. The underground facility should be hermetically sealed in order to preserve the grain. The grain will gradually utilize the oxygen in the headspace producing carbon dioxide that will decrease the respiration rate and create an adverse environment for insects and molds.

Grain Elevators

Grain elevators are the most widespread facilities to store cereal grains. They are called elevators because the unloaded grain is conveyed with augers or bucket elevators into the top of the storage bins and discharged by gravity. There are many different types of grain elevators. The most widely distributed throughout the globe are the flat bins and silos built from concrete or steel. Flat or horizontal facilities are usually built wider and lower than silos to reduce cost and side pressures. There are round steel or concrete bins designed to resist the maximum volume of grain to store. They are usually built with concrete floors; the roof tends to follow the slope of the pile of grain, and they are equipped with fixed loading and unloading mechanical systems.

Upright silos are round, hexagonal, or even square-shaped concrete or steel bins usually constructed in rows, so one straight conveyor can service a whole series of bins. The most common form is round because it is the most resistant and is built in such design to take advantage of the interstitial spaces to increase storage capacity. Silos are often built on two or more rows of cylindrical bins with diameters ranging from 2 to 10 m. The grain is usually elevated by a leg consisting of an endless vertical belt equipped with attached buckets. The inferior part of the silo is usually conical to aid in the grain unloading.

Controlled Atmosphere Storage

The objective of controlled atmosphere storage is increasing the concentration of carbon dioxide in airtight facilities to reduce grain respiration and growth of insects and molds. Most insect species of stored grains will perish when the oxygen concentration is less than 2%. Fungi can still grow at lower oxygen concentrations (0.2%), but infestations will occur only if the lot is stored at moisture contents above 16%. Low-oxygen conditions can be created by the use of carbon dioxide or nitrogen gases. Carbon dioxide is more effective than nitrogen for killing insects. The gases are injected from cylinders or by addition of dry ice (solid carbon dioxide) to a silo before sealing. The major advantage of using controlled atmosphere storage is the reduction of pesticides for controlling insects.

Insects

There are between 20 and 30 insect species that attack cereal grains during storage. The warm temperatures of tropical and subtropical areas are the preferred habitat for these insects, whereas the cold weather inhibits insect activity. Insects are the main agents responsible for grain losses in the world. Besides the direct losses, insects contaminate grains and processed products with fecal material, uric acid, weblike material, and body fragments. Insects are classified as primary or secondary according to their habits and characteristics (Table 1). Primary insects are more harmful because they have the ability to damage sound kernels. They usually perforate the grain for feeding and reproductive purposes. These insects mainly consume the endosperm and germ tissues and use the grain as site for oviposition and larvae development. Secondary insects are

Table 1 Biology and habits of the most common insects that infest cereal grains and their products

Common (scientific name)	*Biology, habits, and type of damage*
Lepidoptera	
Angoumois grain moth (*Sitotroga cerealella*)	Adults measure 7.6 mm long and 12.7 mm wide (extended wings). The front and back wings are yellow and gray. The hind wings have a characteristic pointed tip with posterior hairs. The female lays 40–300 eggs over the grain surface. The larvae tunnel into the kernels where they complete their life cycle of 5 weeks. The 5 mm long larvae are white caterpillars with three pairs of legs. Before pupating, the larvae prepare a thin escape hole through which the moths emerge. During development, the larvae consume up to 50% of the grain. Larvae develop in a brown pupa after to 2–3 weeks. The infestation is characterized by the extensive webbing over the lot of grain
Indian mealmoth (*Plodia interpunctella*)	Cosmopolitan insect that in the adult stage measures 5–10 mm long. The basal and distal halves of the front and hind wings are light coppery- and dark coppery-colored. The 13 mm long larvae differ in color from white to green. Adult moths lay clusters of 12–30 eggs on the grain surface totalizing 60–300 eggs during the life cycle. Eggs hatch into larvae, which is the destructive stage. The larvae usually come to the outside of the kernels to spin cocoons and pupate. The adult emerges from the pupa and under optimum conditions has a 4–8-week cycle. The infested grain usually has the typical webbing
Mediterranean flour moth (*Anagasta kuehniella*)	The moth is light gray-colored measuring 6–12.5 mm long. The wings are distinctively marked with two black lines in zigzag. The female lays on flours and crevices eggs that hatch 3–6 days later into measure worms. The larvae pupate in silk cocoons for 8–12 days. The adult emerges from the pupa and have a life cycle of 9–10 weeks during warm weather
Coleoptera	
Maize weevil (*Sitophilus zeamais*) Rice weevil (*Sitophilus oryzae*) Granary weevil (*Sitophilus granarius*)	These primary insects are the most destructive. The head has a pair of antennae and a prolonged snout. The granary weevil is brown or black and does not have functional hind wings. The maize and rice weevils are reddish brown to black and have two light spots on each front wing. The hind wings function as flight wings. The 5 mm long adults perforate the grain to lay one egg. Each female may lay from 300 to 400 eggs that hatch 5–15 days later. The larva develops inside the grain and gradually consumes the grain during 15–40 days. The life cycle under optimum conditions lasts 4–5 weeks
Lesser grain borer (*Rhyzopertha dominica*)	Primary insect that in the adult stage measures 2.5 mm long. The brown or black beetle has a cylindrical shape and is capable of flying. Both the larvae and adults are destructive. Females lay 2–30 eggs outside the grain. The cream-colored larva with a dark head and three pairs of legs perforates the grain and develops inside in approximately 60 days
Larger grain borer (*Prostephanus truncatus*)	The larger grain borer is a small, dark-brown, elongated, cylindrical beetle about 4.2 mm long. It is similar in appearance to the lesser grain borer. It is considered one of the most harmful tropical pests especially in maize granaries
Confused flour beetle (*Tribolium confusum*) Red flour weevil (*Tribolium castaneum*)	These secondary insects are 3.6 mm long and brown/reddish and are considered the most destructive in flours and processed grain products. The antennal segments of the confused flour beetle gradually increase in size toward the tip, whereas the antennae of the red beetle end with three abruptly enlarged segments. The female lays 400 or more sticky eggs (6–12 per day) on sacks, cracks, or grain products. The eggs hatch into a white-colored 1.5 mm long worm. These insects leave feces and dead bodies, cast skins and exoskeletons, and excrete quinones that produce off-odors and undesirable color changes in flour
Sawtoothed grain beetle (*Oryzaephilus surinamensis*) Merchant grain beetle (*Oryzaephilus mercator*)	The reddish-brown saw-toothed and merchant beetles are relatively small insects (2.5 mm long) with six toothlike projections on each side of the thorax. Generally, these beetles feed on damaged grains, flour, and processed foods
Yellow mealworm (*Tenebrio molitor*) Dark mealworm (*Tenebrio obscurus*)	These cosmopolitan mealworms are one of the largest secondary pests that infest stored products

Source: Serna-Saldivar, S. O. (2010). *Cereal grains: properties, processing and nutritional attributes*. Boca Raton, FL: CRC Press (Taylor & Francis Group).

opportunistic because they attack grains that have already been damaged by primary counterparts or processed products such as flours or processed foods.

Table 1 summarizes the habits, characteristics, and biology of the principal insects that attack cereal grains during storage. Five primary pests cause most of the insect damage. These are the granary, rice, and maize weevils; the larger and lesser grain borers; and the Angoumois grain moth. The large grain borer is of economic importance in tropical areas around the globe. Temperature is the most important environmental factor affecting insect development and reproduction. Most insects do not reproduce at temperatures lower than 12 or higher than 34 °C. The optimum temperature for reproduction is about 26 °C.

Molds

After insects, grain molds are the most important biotic agents negatively affecting grain viability and quality. Molds have potent lipolytic, amylolytic, and proteolytic enzymes that degrade stored nutrients; moreover, they can produce mycotoxins that have the potential to cause serious diseases and deaths in humans and domestic animals. The most relevant genus of storage molds are *Fusarium*, *Aspergillus*, and *Penicillium* (Table 2). These molds usually infest cereal grains when they contain moisture in the range of 16–20% and the air relative humidity and temperatures are 85% and 25–30 °C, respectively. The main harmful effects of storage fungi are lower

seed viability, grain discoloration, nutrient degradation, mycotoxin production, grain heating, and generation of musty off-odors.

Diseases related to the consumption of mycotoxins have been known for hundreds of years. Cases of ergotism in the seventeenth century and ochratoxicosis during the Middle Ages are well documented. Aflatoxins are now recognized among the most potent naturally occurring carcinogens in nature. During the past decade, fumonisins have received special attention because of their potent harmful health effects on equines and humans. Table 2 summarizes the main types of mycotoxins found in stored cereal grains and their harmful effects in humans, domestic animals, and poultry. Undoubtedly, maize is the most susceptible cereal to mold infestation and mycotoxins. This is because the cob is covered with husks creating an ideal and protective environment for molds.

Rodents

Rodents are considered the most destructive vertebrates in the planet. The main reasons why these mammals cause huge economic losses are their wide range of adaptation, high reproduction rate, and the complexity for their control.

The most common rodents present in grain storage facilities are the Norway and roof rats and the house mice. Table 3 summarizes the features of these species that destruct grains in the field and throughout storage.

Table 2 Characteristics and toxicological effects of the main mycotoxins that occur in cereal grains and their products

Mold	*Mycotoxin*	*Toxic effects on humans and domestic animals*
Aspergillus flavus, *Aspergillus parasiticus*	Aflatoxins	There are several types of aflatoxins; the most relevant and toxic in cereals is B_1. Other important metabolites are B_2, G_1, G_2, M_1, and M_2. These are of great importance because they cause toxicity at very low concentrations (10 ppb). Aflatoxins produce acute hepatitis, widespread hemorrhages, and poor immunologic response and are potent carcinogens and mutagenic agents
Aspergillus ochraceus, *Penicillium verrucosum*	Ochratoxin	Ochratoxins are isocoumarin derivatives bound to phenylalanine. The toxicosis known for several centuries involves progressive renal failure and atrophia, anemia, polyuria, anorexia, headaches, and uremia. In most instances, the disease is fatal
Fusarium moniliforme, *Fusarium verticillioides*, *Fusarium proliferatum*	Fumonisin	They are the group of toxins that have received more recently attention. They are highly toxic to livestock, mainly horses. In humans, fumonisins have been related to esophageal cancer and interference with folic acid metabolism. Therefore, they can exacerbate fetal malformation such as neural tube defects
Fusarium graminearum	Zearalenone	Chemically is an acid lactone with a phenol–resorcinol configuration. Swine are the most affected animals because it causes the 'estrogenic syndrome' or animal feminization, characterized by vulvovaginitis, vaginal prolapse, and infertility in sows and testicular atrophy, infertility, and swelling of the mammary glands in boars
Fusarium	Trichothecenes (T-2 toxin)	About 180 trichothecenes are known. High-moisture cereals are frequently contaminated with these toxins considered as potent protein synthesis inhibitors and immune suppressors. Trichothecenes can produce the fatal syndrome named 'alimentary toxemia'
Fusarium graminearum	Deoxynivalenol (DON) Vomitoxin	Vomitoxin is a deoxynivalenol derivative. The toxin causes feed refusal, vomiting, and lower feed efficiency. The beer industry is especially concerned about these toxins because they may contaminate beer when infested barley or malt is used

Source: Serna-Saldivar, S. O. (2010). *Cereal grains: properties, processing and nutritional attributes*. Boca Raton, FL: CRC Press (Taylor & Francis Group).

Table 3 Biology and features of rodents commonly present in grain storage facilities

Type of rodent	*Biology and features*
Norway rat (*Rattus norvegicus*)	They grow in temperate and urban areas. The robust adults weigh 300 g and have small eyes and ears and short and blunt noses and their hair coats are usually dark. The most distinctive feature is that the tail is shorter than the body. The adults are aggressive and dominant and daily consume an average of 28 g food. The dam has the capacity of producing up to 7 litters per year of 8–12 pups each. This rat excretes large (1.9 cm long) fecal pellets with blunt ends
Roof rat (*Rattus rattus*)	This rat is comparatively smaller compared with the Norway counterpart, but it adapts better to tropical regions and its color varies from black to grayish white. Two distinctive characteristics are that the ventral or abdominal part is lighter than the rest of the body and that the tail is longer than the body length. In addition, the rat has larger eyes and ears and a pointed nose. These rats can climb and live in overhead areas especially when they coexist with Nordic rats. The roof rat excretes fecal pellets of 1.2 cm long with pointed ends
House mouse (*Mus musculus*)	House mice are small cosmopolitan rodents common to human dwellings that only weigh 15 g. They have a longer tail compared with the body, a light-brown or gray hair color, a pointed nose, small eyes, and long ears. Females give birth up to 8 litters per year of 9–12 pups each. They specialize in consuming cereal grains, and the control program should consider that these animals are excellent climbers. The house mouse droppings average 0.6 cm long with pointed edges

Source: Serna-Saldivar, S. O. (2010). *Cereal grains: properties, processing and nutritional attributes*. Boca Raton, FL: CRC Press (Taylor & Francis Group).

See also: Aflatoxin: A Global Public Health Problem; Controlled Atmosphere Storage: Applications for Bulk Storage of Foodstuffs; Drying: Principles and Types; Enzymes: Functions and Characteristics; Food and Agriculture Organization of the United Nations; Insect Pests; Maize; Mycotoxins: Classification; Mycotoxins: Occurrence and Determination; Mycotoxins: Toxicology; Pesticides and Herbicides: Types of Pesticide.

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Cereals: Types and Composition

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Introduction

Cereals belong to the *Gramineae* family commonly known as grasses. Most of these plants are perennial; however, all commercial cereal grains are annual. It is believed that prehistoric men selected plants that yielded large kernels in a relatively short period of time. Since then, mankind has manipulated cereals in order to increase their yield through a better adaptation to different ecosystems, climates, and soils. This continuous plant breeding effort has sustained the dramatic increase of the world population experienced since 1900 (from approximately 1.7 billion in 1900 to more than 7.3 billion in year 2014).

Cereals have a wide array of assets and advantages. They yield high amounts of food per surface area and mature grains that are not perishable when properly stored. Furthermore, these grains concentrate calories and other nutrients in a relatively small package that has sustained mankind since they became sedentary. Among the major food groups, cereals are undoubtedly the largest supplier of calories and proteins for the average human being.

According to the Food Agriculture Organization, cereals are by far the most important foods with an annual production exceeding 2.78 billion tons in year 2013. Cereals are the staple for the world inhabitants and the major source of feed for animals that supply meats, eggs, and dairy products.

Among cereals, three contrasting grains, rice, wheat, and maize, yield approximately 89% of the total production. Rice and wheat are almost exclusively channeled to human foods, whereas maize is widely used as feedstock for animals and fuel ethanol. Maize is the cereal with the highest production and yield followed closely by rough rice and wheat. The United States harvests 35% of the world production due to the use of high-yielding hybrids including an increasing number of genetically modified organisms (GMOs). Wheat is still considered the icon of cereals because of its versatility to produce a wide array of leavened foods, which is possible due to its unique gluten properties. Wheat is the cereal that contributes the most in terms of human caloric intake. About 45.5% of the wheat is produced in China, India, the United States, and Russia.

In 2014, more than 75% of the paddy rice was harvested in China, India, Indonesia, Bangladesh, Vietnam, and Myanmar. The per capita consumption of rice in developing Asian countries averages 80 kg $year^{-1}$, with the people from Myanmar, Vietnam, Bangladesh, Cambodia, and Indonesia being the most dependent on this cereal grain with yearly consumptions of 195,167, 160, 147, and 140 kg per capita, respectively.

Barley is currently the fourth cereal grain in terms of production with a global production of 144.7 million tons in year 2013. Rye, barley, and oats are mainly planted in Europe, Russia, and the United States. These winter small grains are also planted for forage or as dual-purpose crops. About 65% of the rye is harvested in Eastern Europe due to its adaptation to harsh winters and poor soils. In these regions, rye flour is usually mixed with wheat flour for the production of many traditional breads and other bakery goods. Likewise, most of the barley and oats are planted in United States, Canada, and Europe for food and feed. Barley is used for malting, brewing, and distilled alcohol spirits, whereas milled oats or groats are used for the production of composite breads and other bakery items.

Sorghum is currently the fifth cereal grain in terms of production with a global production of 61.4 million tons in year 2013. It is directly used to produce a wide array of traditional foods in Africa and India and almost exclusively used for animal feed in the United States, Mexico, and other Latin American countries. Sorghum as feed competes strongly with maize although is considered to have a slightly lower nutritional value.

The millets are grown as subsistence crops because of their hardiness and droughts resistance. These cereals are mainly produced in developing countries of Africa and Asia. The main producers are India, Nigeria, Niger, and China. The African countries located south and west of the Saharan desert (Mali, Burkina Faso, Niger, and Nigeria) have traditionally depended on millets and sorghum as the main source of nutrients.

The most relevant features of cereal plants are stems with nodes where buds and leaves originate, alternate spear-shaped leaves, and an inflorescence that could be a spike or panicle. These bear multiple flowers enclosed in glumes that upon fertilization and maturation yield monocotyledon starchy fruits composed of pericarp, germ, and endosperm. The cotyledon is located in the germ's *scutellum* and is considered as the first reserve tissue, whereas the endosperm is the largest part where protein and starch are stored as secondary reserve tissues. Cereals are divided according to the photosynthesis pathway into two groups: C_3 plants that form three-carbon compounds via the Calvin-Benson metabolic cycle where rice wheat, barley, rye, triticale, and oats are included and C_4 plants such as maize, sorghum, and millets that form four-carbon compounds via the acidic Crassulacean metabolic pathway. C4 cereals generally grow in hot climates with a high light intensity and are more efficient in terms of CO_2 utilization and water and nutrient uptake.

The inflorescences of wheat, barley, rye, and triticale are spikes, whereas rice, sorghum, oats, and all millets produce panicles. In the specific case of maize, the cob is classified as a fused panicle or central axis. Maize, rye, sorghum, and pearl millet cross-pollinate, whereas wheat, oats, barley, and rice self-pollinate.

The cereal fruit is botanically named caryopsis and consists of three major anatomical parts: pericarp, endosperm, and germ. There are some species that loose the glumes or husks during harvesting and others that tightly retain these protective coats. Maize, sorghum, wheat, rye, triticale, and pearl millet are examples of naked caryopses, whereas rice, oats, and barley are covered caryopses.

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The pericarp or bran is the outer layer that covers the kernel and contains most of the fiber, whereas the endosperm is the main anatomical part in terms of quantity and food utilization. It could constitute up to 75% of the total grain weight and is mainly composed of starch and protein and is practically devoid of fiber. The starch is stored in granules that are classified as simple or compound. Among cereals, only rice and oats synthesize compound starch granules. The germ, which encloses the *scutellum* and embryo, contains most of the oil and is also rich in protein and vitamins. The *scutellum* or cotyledon and endosperm are considered as the first and second reserve tissues, respectively. These anatomical parts store nutrients necessary for germination.

Adaptation and Cytogenetic Origin

The various cereal genus and species are adapted to produce in most of the ecosystems around the globe.

Maize (*Zea mays* L.)

Maize is the most produced cereal crop in the world and the most adapted to different ecosystems. Ancient Mesoamerican farmers started to select and manipulate teosinte (*Zea mexicana*) and in a couple of centuries transformed this native grass into several pre-Columbian maize races. Today, plant breeders recognize more than 300 races that evolved from these prehistoric ethnic groups. The Mesoamericans created maize by transforming a tiny two-rowed ear of teosinte of about 3 cm long into the first small maize ears with four ranks of paired female spikelets. This transformation took perhaps only 100–200 years. The maize crop produces under irrigation and in dryland conditions; however, the plant is highly susceptible to frosts. Among commercial cereals, maize is the only one that has the male and female flowers separated. The staminate or male inflorescence is borne in the tassel and the pistillate or female inflorescence on the ears. These are located in the top and middle parts of the plant, respectively. Maize is classified according to color into white or yellow and kernel configuration into dent or corneous or flinty. The main specialty types are popcorn, waxy, high-amylose or amylomaize, sweet, blue, Cuzco, and quality protein.

Rice (*Oryza sativa*)

Rice is considered as a sacred plant in Asia and is still the most important food for more than 50% of the world population. Archaeological evidence has proved that rice was planted and used at least 4000 years BC. In developing Asian countries, the yearly per capita intake of rice is higher than 80 kg. Production data indicate that about 90% of the world rice production is harvested in Asia. Rice is planted in tropical and subtropical regions of the world close or neighboring the equatorial line where there is high relative humidity and rainfalls. Rice is also traditionally grown in some European countries such as Italy, Spain, and France. The agronomics of rice is different from other cereals because this plant is generally planted on flooded soils and by transplanting seedlings previously grown in nurseries. Three major groups of rice are widely recognized: Japonica, Indica, and Javanica. The first two are the most relevant. Japonicas are usually high yielding and produce short caryopses that upon cooking yield sticky or glutinous rice. On the other hand, Indicas usually yield less compared to Japonica counterparts and produce long caryopses that upon cooking yield drier and firmer rice. Commercially, rice is classified as long, medium, and short. The main specialty rices are glutinous, black or purple, red, Basmati, and Risotto.

Wheat (*Triticum* sp.)

Wheat was one of the first cultivated plants and is considered as the most important cereal in terms of energy supply for humans. It is planted in temperate regions and considered as a winter crop. In the early part of the 1900s, taxonomists recognized three species that possessed different chromosome numbers: diploid, tetraploid, and hexaploids, which contain 14, 28, and 42 chromosomes, respectively. Commercially, the wheats planted nowadays are hexaploids and tetraploids. The hexaploids are the most widely grown throughout the planet and provide the hard and soft kernels suited for the production of yeast-leavened breads and cookies/cakes and other related chemically leavened bakery items, respectively. The tetraploid durum (*Triticum durum*) is one of the most cultivated today to fulfill the pasta market. Wheat is the only cereal that possesses functional gluten. The flour upon hydration and the mechanical work of mixing forms a cohesive and elastic dough suited for the production of leavened bakery items and pasta products. Wheat is classified according to kernel hardness into hard, soft, or durum, color into red or white, and growth habit into winter and spring. The main specialty types are waxy and the ancient types known as spelt, einkorn, and emmer.

Barley (*Hordeum vulgare*)

Barley was one of the first crops cultivated by men since the origins of farming. Historically, barley preceded wheat as a food grain in ancient Egypt and since then it has been consumed in many cultures. Barley ranks fourth among cereals and significantly contributes to the world's food supply as human food, malt products, and livestock feed. Approximately 64% of the world barley is channeled to the feed industry. Cultivated barley is one of the 31 *Hordeum* species. Most are diploids ($2n = 14$ chromosomes) with about the other half being tetraploids or hexaploids. Barley is a cool season crop cultivated in the spring and summer at temperate latitudes. It is cold-tolerant and considered the most drought-resistant, alkali, and salt-tolerant among the small grains. Barley is classified into malting or feed and into two- or six-rowed types. The main specialty types are hull-less or naked, waxy, high-amylose and hyproly, or high lysine.

Sorghum (*Sorghum bicolor*)

Sorghum originated in equatorial Africa where it has been grown for more than 2000 years. It is one of the cereals with the highest genetic variability because more than 30 000 selections are preserved in the world collection bank located in India. Sorghum

continues to be a traditional crop in Africa and India and constitute a major source of calories and protein for millions of people. About half of the world production is processed into a wide array of traditional foods. In developed countries, sorghum has gained popularity because of its drought resistance and high productivity especially when planted under good agronomic practices and inputs. Sorghum is the preferred cereal in semidesert areas of the world or in areas where maize strives due to high temperatures, poor soils, or lack of rainfall. Sorghum is classified as high- and low-tannin types. Brown, bird-resistant or high-tannin sorghums have a reduced nutritional value and are grown because of their agronomic advantages including bird resistance, decreased weathering, mold infestation, and sprouting. Most cultivated sorghums do not contain condensed tannins and have similar food and feed nutritional value when thermal treated compared to maize. White sorghums are currently viewed as an excellent source of gluten-free flours or meals suited for the production of snacks, cookies, breads, and beer. Good-quality sorghums can substitute maize in most of industrial applications including bioethanol. The main specialty types of sorghum are waxy, popping, black or Shawaya, high-lysine, and lemon.

Rye (*Secale cereale*)

Rye has a better resistance to harsh winters, cold weather, and lack of water compared to wheat and is generally planted in less fertile soils under dryland conditions. It is primarily a winter crop that is sown in the fall, becomes dormant during the winter, and is harvested early in the spring. Occasionally, rye is sown in the spring and harvested during the summer. Most rye is harvested in Europe and Russia and is dry-milled and processed similarly to wheat. The grain has been traditionally used for the production of flat breads and yeast-leavened breads, which varies in crumb color from practically white to dark-brown and in taste from a mildly sour to a strong distinctive acidic. Rye flour is preferred for the production of sour breads and is also used for the manufacture of crackers, cookies, and breakfast cereals. Compared with wheat flours, rye flours deteriorate faster but contain higher levels of protein, pentosans, and minerals.

Oats (*Avena sativa*)

The origin of oats can be traced back to approximately 2000 BC in the Middle East or surrounding Mediterranean areas. *Avena sativa* and *Avena byzantine* are the two most widely grown. Oats are a cool season crop and ranks sixth in world cereal production. The demand for oats for human consumption has steadily increased due to its nutritional health implications, the best protein quality among cereals, and especially due to the unique properties of its dietary fiber. In the processing of oats for human foods, the hulls or glumes are removed and the naked caryopsis, named groats, is generally consumed as whole grain. The main drawback of whole oats and their milled fractions is the high fat content prone to oxidation. That is the main reason the dry milling of oats is aimed toward the deactivation of lipolytic enzymes via thermal treatments. Flaked and milled oats are mainly used for the production of breakfast cereals, nutrition bars, cookies, and composite breads.

Triticale (*Triticum secale*)

Triticale is the only plant species created by man who crossed wheat and rye. It was first deliberately produced in 1876 although the first varieties emerged in the 1930s in Russia, but it was until the 1960s when it was commercially planted. Triticale is a hybrid resulting of the cross of wheat (mother) and rye (father) with the aim of obtaining kernels with the best features of these cereals (gluten functional and better agronomic performance or hardiness and resistance to pests). Triticale has regained popularity during the past 20 years. World production in 2013 (14.6 million tons) was approximately three times greater than in 1993. However, most of the triticale currently grown does not meet gluten expectations and therefore is mainly used as forage or feed. In 2013, the triticale planted area was approximately 3.85 million hectare.

Millets

The grasses known collectively as millets are a set of highly variable small-seeded cereal species indigenous to different areas of the world. In some African countries such as Niger, Mali, Burkina Faso, and Gambia, the yearly consumption of millets amounts to 160, 71, 55, and 52 kg, respectively. In 2012, about 29.9 million tons were directly used for the production of many African and Asian traditional foods. Similar to sorghum, millets are mainly adapted to semidesert, tropical, and subtropical areas of the world, but they are usually planted on barren and low-moisture soils and under hot environmental conditions. These cereals are of special value in semiarid regions because of their short growing cycle. Most millets are viewed as either subsistence or cash crops in developing and developed countries, respectively. Average yields of millets seldom exceeds 1 ton Ha^{-1}.

The most relevant millet is pearl (*Pennisetum americanum*), which originated in the corridor from Western Sudan to Senegal. It is believed to be one of the earliest domesticated millets because kernels have been found in West African sites inhabited 2000 BC. Pearl is the most productive millet and is used instead of sorghum with the advantage that it possesses a better nutritional value. Pearl millet produces a very unique cylindrical panicle containing hundreds of oblong–round-shaped kernels.

After pearl millet, the most relevant are foxtail (*Setaria italica*), proso (*Panicum miliaceum*), and finger (*Eleusine coracana*). Foxtail millet is especially important in China, Japan, and India. Proso or common millet (*Panicum miliaceum*) originated in Manchuria and first appeared as a crop in Transcaucasia and China 5000 BC. It is considered as one of the most drought-resistant millets. The kernels are small (2–3 mm) and can be cream, yellow, orange-red, or brown in color. Kernels are usually traditionally milled into flours for preparation of a wide array of traditional foods or used for birdseed. Finger or ragi millet originated in Ethiopia and reached India between 3000 and 4000 years ago. Its name is due to the digitally arranged panicle. Finger is among one of the most productive millets with average yields of about 1.8 tons Ha^{-1}. The two main races are the African highland, widely grown in the cooler higher altitudes regions of East Africa and Asia, and the Afro-Asiatic lowland. Finger millet is generally dry-milled into flour for the production of flatbreads, dosa and rotis.

Kodo (*Paspalum scrobiculatum*) and barnyard (*Echinochloa frumentacea*) millets are indigenous to the Indian subcontinent. Kodo kernels are enclosed in hard horny persistent husks that are difficult to remove. Barnyard is considered as one of the fastest growing among all millets.

Teff (*Eragrostis tef*) originated 4000 BC in northern Ethiopia. Maximum production is achieved at high altitudes (1800–2000 m), with rainfall of 450–500 mm and a temperature range of 10–27 °C. Today, teff accounts for about one quarter of the total cereal production in Ethiopia where it is mainly processed into the traditional fermented pancake known as injera. Among millets, teff produces the smallest kernels (< 1 mm in diameter), which are rich in dietary fiber and iron and contain a better protein quality and calcium compared to other cereals.

Fonio (*Digitaria exilis*) is an important subsistence crop in the savannas of West Africa where it has been planted because of its short growing cycle (2 months) and resistance to arid conditions, scarce rainfalls, and poor soils. Fonio also yields very small kernels that are milled into flours, which are mainly used for preparation of porridges, couscous, traditional breads, and opaque beers.

Physical and Chemical Properties

The grain physical properties of cereal grains are depicted in Table 1 and the chemical composition in Table 2. Cereal grains are composed of carbohydrates, protein, lipids, vitamins, and minerals.

All cereals are classified as starchy grains because they contain at least 60% of this carbohydrate. The starch is a polymer of glucose units joined by α 1–4 and 1–6 glycosidic bonds that after digestion provide most of the energy consumed by humans. The starch is an excellent source of energy because in practical terms it is completely digested and utilized by a normal human being. The starch has contrasting properties if it is in its native, gelatinized, or retrograded stages. Most foods processed are aimed toward the partial or total starch gelatinization because the thermal-treated starch turns into molecules with high affinity for water that yield viscous doughs, gels, or slurries. The starch molecules are stored in granules, which are packed with amylose and amylopectin molecules. The amylose contains about 1500 glucose molecules in a linear chain joined by α 1–4 glycosidic bonds. The amylopectin or branched starch is composed of glucose molecules not only linked by α 1–4 glycosidic bonds but also containing branches that occur when α 1–6 bonds form. Only about 4–5% of the total glycosidic bonds are α 1-6. These glucose polymers have a molecular weight of 108×10^6 g mol^{-1} (600 000 glucose units/amylopectin molecule) and structurally are divided in type A, B, and C chains. The starch of most cereals contains 75% amylopectin and 25% amylose. However, some cereals such as maize, wheat, rice, barley, and sorghum can contain from 95% up to 100% amylopectin and therefore are almost amylose-free. These cereals are named waxy because their endosperm

Table 1 Physical properties of cereal grains

Cereal	Test weight (kg hl^{-1})	1000 kernel weight (g)	Dimensions				Density (g cm^{-3})
			Length (mm)	Width (mm)	Thickness (mm)	Length: width ratio	
Maize							
Dent	68.5–78.0	240–370	8.0–17.0	5.0–9.8	–	1.1	1.20–1.36
Popcorn	82.0–83.0	130–151	8.0–8.6	5.3–6.0	4.0–4.4	1.4–1.5	1.37–1.39
Paddy rice							
Long	56.0	21–24	8.9–9.6	2.3–2.5	1.8–1.9	3.8:1–3.9:1	–
Medium	58.5	23–25	7.9–8.2	3.0–3.2	1.9–2.1	2.5:1–2.6:1	–
Short	60.0	26–30	7.4–7.5	3.1–3.6	2.1–2.3	2.1:1–2.4:1	–
Wheat	–	–	4.0–10.0	2.5–4.5	–	2.0	–
Hard	80.9	20–32	–	–	–	–	–
Soft	77.8	30–40	–	–	–	–	–
Durum	74.0–80.0	20–60	–	–	–	–	–
Barley							
Hulled	46.0–71.0	17–57	8.0–14.0	2.0–4.5	–	3.4	–
Sorghum	68.5–77.3	23–35	3.0–5.0	2.5	1.6	1.6	1.20–1.35
Rye	62.5–73.5	16–32	4.5–10.0	1.5–3.5	–	2.9	–
Oats							
Hulled	41.3–52.9	24.7–35.8	9.3–11.1	2.9–3.0	–	3.5	–
Groats	75.9–80.1	16.5–26.0	5.3–6.5	2.1–2.3	–	2.7	–
Triticale	77–79	28–45	–	–	–	–	1.44
Millets							
Pearl	76.0–80.0	4–15	3.0–5.5	1.5–3.0	1.2–2.4	2.4	1.25–1.30
Foxtail	–	5	2.0	2.0	–	1.0	–
Finger		1.8–3.8	1.0–1.8	1.0–1.5	1.0–1.5	1.0	1.24
Proso	72.7	–	–	–	–	–	–
Fonio	–	0.5	–	–	–	–	–
Tef	–	0.13–0.4	1.0–1.5	0.8–1.0	–	1.4	–

Source: Serna Saldivar, S.O. (2010). *Cereal grains: properties, processing and nutritional attributes*. Boca Raton, FL: CRC Press (Taylor & Francis Group).

Table 2 Chemical composition of cereals grains

Cereal	*Protein (%)*	*Fat (%)*	*Crude fiber (%)*	*Minerals (%)*	*NFE (%)*[a]
Maize					
Dent	9.1	4.4	3.0	1.7	81.8
	8.1–11.5	3.9–5.8	2.4–3.5	1.4–2.0	77.2–84.2
Popcorn	12.1	5.2	2.3	1.8	78.6
	11.0–13.2	4.6–5.8	1.8–2.6	1.4–1.9	76.5–81.2
Rice					
Paddy-Rough	7.5	2.4	10.2	4.7	75.2
	6.7–9.0	1.7–2.7	8.4–12.1	3.4–6.0	70.2–79.8
Brown	9.2	2.5	0.9, 1.5	85.9	83.6–88.0
	8.3–10.1	1.8–3.3	0.7–1.2	1.2–1.8	
White	7.8	0.5	0.4	0.6	90.7
	7.3–8.3	0.3–0.6	0.2–0.6	0.3–0.9	89.6–91.9
Wheat					
Hard	14.4	2.3	2.9	1.9	78.5
	11.5–17.0	1.8–2.8	2.8–3.0	1.8–2.0	75.2–82.1
Soft	9.9	2.8	2.7	1.7	82.9
	8.0–12.0	2.6–2.9	2.5–2.8	1.8–1.9	80.4–85.1
Durum	13.2	2.8	2.8	2.0	79.2
	12.0–15.6	1.8–3.8	2.4–3.1	1.8–2.1	75.4–82.0
Barley	11.5	2.2	5.6	2.9	77.8
	7.5–15.6	1.8–2.6	5.3–5.9	2.6–3.1	72.8–82.8
Sorghum	11.0	3.2	2.7	1.8	81.3
	7.3–15.6	0.5–5.2	1.2–6.6	1.1–4.5	68.1–89.9
Rye	13.4	1.8	2.1	2.0	80.7
	12.6–14.5	1.6–2.2	1.6–2.6	1.7–2.2	78.5–82.5
Oats					
Oats	17.1	6.4	11.3	3.2	62.0
	12.4–24.4	4.5–10.3	10.4–14.3	2.9–3.4	47.6–69.8
Groats	16.9	7.4	1.6	2.1	72.0
	13.8–22.5	5.9–8.4	1.0–3.3	1.9–2.4	63.4–77.4
Triticale	15.2	1.9	2.2	1.9	78.6
	12.6–17.2	1.6–2.2	2.0–2.5	1.8–2.1	77.4–80.8
Millets					
Pearl	14.5	5.1	2.0	2.0	76.4
	8.6–19.4	1.5–6.8	1.4–7.3	1.6–3.6	62.9–86.9
Foxtail	11.7	3.9	7.0	3.2	74.2
	6.0–14.0	1.2–5.2	2.6–8.9	1.5–3.6	68.3–88.7
Finger	8.0	1.5	3.0	3.0	75.0
	6.0–10.9	1.0–4.6	2.0–6.8	2.3–3.9	73.8–88.7
Proso	11.0	3.5	9.0	3.6	72.9
	6.4–12.8	2.9–4.9	4.6–12.0	1.4–5.0	65.3–84.7
Barnyard	11.8	4.9	14.3	4.9	64.1
	11.2–12.7	2.5–6.3	13.9–14.7	4.7–5.0	61.3–68.0
Kodo	10.4	3.7	9.7	3.6	72.6
	6.2–13.1	3.2–4.9	8.4–11.0	3.0–4.1	66.9–79.2
Teff	10.9	2.4	2.7	2.6	81.4
	7.9–12.6	2.3–2.5	2.4–3.0	2.2–2.9	79.0–85.4
Fonio	8.7	2.8	8.5	3.8	73.6
	5.1–10.4	2.1–5.2	4.6–11.3	1.8–6.0	62.7–80.0

[a]Nitrogen-free extract (NFE) that gives an indication of nonfibrous carbohydrates (starch and sugars).

Source: Serna Saldivar, S.O. (2010). *Cereal grains: properties, processing and nutritional attributes*. Boca Raton, FL: CRC Press (Taylor & Francis Group).

acquires this appearance when viewed with the naked eye. These waxy cereals and their starches have special industrial uses.

The second most abundant chemical component is the various types of protein fractions distributed in the different anatomical parts of the cereal kernel. Those associated with the endosperm are commonly denominated gluten proteins, whereas those associated with the germ are the albumins and globulins. The range of protein of cereals is from 8% to 16% (Table 2). Proteins from cereals have good rate of digestibility but lack of an adequate essential amino acid balance or composition especially for preschool infants and children. The most limiting amino acid is lysine followed by tryptophan in maize and threonine in most other cereals.

Cereals are considered as a good source of dietary fiber. From the health viewpoint, the importance of dietary fiber

and consumption of whole grains has greatly increased during the past years. The dietary fiber is viewed as therapeutic for people with diabetes, high cholesterol, and gastrointestinal problems. Both insoluble and soluble dietary fibers have positive health benefits. The insoluble fiber increases the peristaltic movement, increases the fecal bulk, and prevents constipation, hemorrhoids, diverticulosis, and colon cancer. On the other hand, the soluble fiber reduces blood cholesterol and lowers the sudden increase in blood glucose levels. Both types of fibers have the ability to bind bile acids decreasing cholesterol. The insoluble fiber is mainly composed of cellulose and lignin, whereas the soluble counterpart is composed of hemicellulose, arabinoxylans, and β-glucans.

Lipids are relatively minor constituents of cereal grains. Cereal lipids are divided into polar and nonpolar and are mainly located in the germ. In most cereals, the nonpolar triglycerides constitute more than 95% of the total fraction. Maize, sorghum, and pearl millet are the cereals that contain the highest amount of these compounds, whereas oat is considered the only cereal that contains significant quantities of lipids in the starchy endosperm. The ash or mineral fraction material is mostly associated with the glumes, pericarp, and aleurone layer. The most abundant mineral is phosphorus that is generally bound to phytates. Unfortunately, cereals are low in essential minerals iron and zinc.

Nutritional Value

Cereals grains and their products provide most of the total food intake and nutrients that sustain mankind. According to the FAO in 2011, an average human being daily consumed more than 403 g of total cereals and obtained approximately 1296 kcal and 31.9 g protein.

Most of the calories are derived from the starch, which is almost completely digested and utilized in a normal human system. Another advantage of starch is that it releases the glucose at a slower rate to the blood stream and therefore is suited for diabetics. The consumption of whole cereals rich in dietary fiber is even better because of their lower glycemic index. The consumption of whole grain foods lowers energy density, blood cholesterol, and glucose and reduces the incidence of several cancers, mainly colon. Oat-based products exert positive health effects because they contain significant quantities of both insoluble and soluble dietary fibers. The rest of the cereals have a higher ratio of insoluble to soluble fiber and therefore do not exert the same positive effects as the oat's dietary fiber.

The major drawback of cereals is their low protein quality. Protein quality is affected by the digestibility rate and mainly by the essential amino acid balance. Cereals usually contain from 8% to 12% protein (**Table 2**), have a good rate of protein digestibility (80–90%), but unfortunately lack lysine, the most important essential amino acid in practical human nutrition. The malnutrition problems still widely distributed throughout the world are almost always observed in infants living in places where cereals provide most of the daily food intake. The problem worsens when diets lack of legumes and/or animal-based products.

Cereals are considered a good source of all B vitamins except B_{12}, but they are low in fat-soluble vitamins and devoid of vitamin C. It is common to observe vitamin A deficiencies in populations that depend on cereals. Scientists are trying to develop high β-carotene varieties using molecular biology with the aim of reducing vitamin A deficiency and night and permanent blindness endemic to some regions of Asia and other parts of the world.

See also: Barley; Biscuits, Cookies, and Crackers: Chemistry and Manufacture; Bread: Types of Bread; Cereals: Dietary Importance; Dietary Fiber: Determination; Maize; Millets; Oats; Pasta: Manufacture and Composition; Protein: Food Sources; Rice: Role in Diet; Rice: Types and Composition; Sorghum: A Novel and Healthy Food; Starch: Sources and Processing; Starch: Structure, Property, and Determination; Wheat: Grain Structure of Wheat and Wheat-based Products; Wheat: The Crop.

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Chapatis and Related Products

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Introduction

Chapati is the oldest in wheat-based food category that is consumed by a majority of the population in the Indian subcontinent and also widely consumed in the United Kingdom, Middle East, and other countries with an Asian ethnic community. Also known as Roti, it is used as a staple diet by a major segment of the population of the Indian subcontinent. It is a flat and unleavened baked product made from whole wheat flour. Chapati constitutes an important source of dietary proteins, calories, some vitamins, and minerals for the poorer section of the Indian population. In India, about 75% of the wheat grown within the national boundaries is used for the preparation of chapati.

There are some other similar flat bread products like chapati (leavened or unleavened) that are prepared from wheat flour and are popular worldwide, that is, tandoori roti, naan, parotha, phulka, kulcha, poori, litti, missi roti, tortilla, matzo, pita, yufka, sangak, balady, barbari, taftoon, lavash, ciabatta, baati, bafla, and gyro bread. Whole wheat flour is the main ingredient for chapatis and related other products, but sometimes yeast and fat are also included in the formulation to improve the dough handling, mixing, and textural properties. Good-quality chapatti should be pliable, soft in texture, light creamish brown in color, and slightly chewy and should have a baked wheat aroma.

In this article various types of chapati and related flat bread products, their traditional preparation methods and the advancement made in this direction are discussed.

History of Chapati

Flat breads have been known to humans for over the last 6000 years. Babylon is the oldest to have a baker's oven in the world in 4000 BC. In the old kingdom of Egypt, as long ago as 2500 BC, hot ashes or heated stone slabs were used to bake flat bread. These were the Egyptians who passed their bread-making skills, including fermentation, to the Greeks and Romans. Bread occupied an important place in Roman culture and religion; therefore, large commercial bakeries were developed in the second century BC to meet the demands of increasing bread consumption. The possibility of mashing edible grains into a paste and then baking into a flat bread was discovered by the Sumerians in ancient Mesopotamia (modern-day Iraq).

Flat breads in Scandinavian tradition were stored for lean months and tough weather. Flat breads, made in large batches and sometimes in the form of flat rounds with holes in the center, were strung together and stored hanging. Tandoor originated in Persia (Iran) and was brought to India via Afghanistan by Arabs way back in 3000 BC. Small mud-plastered ovens have been found in Harappa and Mohenjo-Daro. Abu'l-Fazl ibn Mubarak, vizier of Mughal Emperor Akbar, has also mentioned chapati in his book 'Ain-i-Akbari,' in the sixteenth century.

Classification of Flat Breads

Flat breads can be classified in two different categories: single layered and double layered. Single-layered bread is again classified in two subgroups as leavened/fermented (risen by a process of yeast fermentation) and unleavened. According to this classification barbari, gomme, lavash, tandýr, and pide are classified as leavened single-layered flat bread, whereas yufka and parotha are classified as unleavened single-layered flat bread, and pita (Arabic flat bread) and baladi are double-layered flat bread.

Double-layered flat bread is widely used in Middle Eastern and North African countries and is gaining popularity in Western countries. High extraction flour (flour having a good amount of fiber) is generally used for double-layered flat bread, making it acceptable as a high dietary fiber food.

Some Popular Flat Breads Worldwide and Their Preparation

The flat breads are usually produced from a simple recipe consisting of flour, salt, and water in varying proportions; however, sometimes the manufacturer also uses optional ingredients like yeast fat, skim milk powder, and certain additives for quality improvement and shelf life enhancement. In addition to the numbers of emulsifiers, hydrocolloids and enzyme preparations have been added to the recipe to modify the process conditions to offer the consumer fresh bread at any time.

In different regions of world different flat breads are produced and consumed.

China

Green Onion Pancakes

Green onion pancakes are made with all-purpose flour, oil, and minced scallions (green onions).

Sanchuisanda

Sanchuisanda are baked in ashes.

Europe

Blintz/Blini (Russia)

Blintz is a yeast fermented thin pancake, somewhat similar to a crepe, although yeast is not used in crepe preparation.

Ciabatta (Italy)

Ciabatta is an Italian white bread made from wheat flour and yeast. The loaf is somewhat elongated, broad, and flat like a slipper and somewhat collapsed in the middle. It has become

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popular across Europe and the United States and has been widely used as sandwich bread since the late 1990s.

Crepes (France)

Crepes are a very thin pancake made of wheat flour. The word *crepes* is derived from the Latin word crispa, meaning 'curled.' Crepes are considered a national dish. They are now also popular in North America and South America.

Flat Bread (Norway)

Flat bread of Norway is made with barley flour, salt, and water.

Focaccia (Italy)

Focaccia is a traditional Italian bread somewhat similar to pizza. It has various flavors and shapes such as the 'focaccia al rosmarino,' 'schiacciata' in Tuscany. Each region of Italy has its own local recipe for making focaccia.

Lefse (Norway)

Lefse is a traditional soft, Norwegian flat bread made by using potato, milk or cream (sometimes lard), and flour and cooked on a griddle.

Pizza (Italy)

Pizza is a traditional Italian thin flat bread baked on a very hot stone-oven at temperature about 900 °F/450 °C. Traditional topping is done with tomato sauce, mozzarella cheese, and basil.

Rye Flat Bread

Rye flat bread is produced in the Scandinavian countries. For preparation, mixing of barley, oat, and rye flours with water is done; the dough is rolled to a thin layer; and it is baked on a hot plate. Cohesive dough is produced and allowed to ferment for 45 min. Dough pieces weighing 400 g are rounded and sheeted to 0.7–1.0 cm thickness. A round central portion of the sheeted dough (5.0 cm diameter) is cut and removed. It is then again fermented for 30–45 min and baked at 230 °C for 30 min.

Indian Peninsula

Wheat-Based Products

Bhatura

Bhatura, a deep-fried Indian bread, is soft and fluffy and is often enjoyed with chole (chickpea curry), making the dish 'chole bhature.' The ingredients required are maida, yogurt, ghee or oil, and yeast. The lactic acid in the yogurt reacts with baking soda to make dough light and rise (6 h). The dough, once kneaded well, is left to increase in volume. Small balls of this dough are then sheeted either by hand or using a rolling pin. Then they are deep fried (175 °C) for about 40 s until they turn into puffed, light brown, elastic, chewy, and soft fluffy bread.

Chapati

Chapati is a single-layered, unleavened Indian traditional flat bread. Usually whole wheat flour is used for chapati preparation, but sometimes yeast and fat are also included in the formulation to improve the dough handling, mixing, and textural properties. It is usually consumed immediately after preparation. Wheat flour, salt, oil, and an appropriate amount of water are the basic requirements in the formulation thereof. Chapati is prepared by mixing flour with water and other ingredients for development of dough, sheeted, and baked for a short time on a hot pan. Chapatis are prepared generally in a circular shape of about 15 cm diameter and 0.2 cm thickness.

Khakhra

Khakhras, generally served as breakfast, are thin crackers made from mat bean, wheat flour, and oil. Khakhras are hand-made and roasted snacks. They are delicious, crunchy, and healthy and can be enjoyed with pickles and chutneys.

Kulcha

Kulcha is a typical North Indian leavened bread prepared with refined wheat flour. Various ingredients such as wheat flour dough, mashed potatoes, onion, and lots of spices are rolled into a flat round bread and then baked in a tandoor until it turns golden brown. After baking, it is smeared with butter and then enjoyed with spicy chole. Dough is prepared by adding salt, sugar, oil, curds, and yeast to a flour and mixed using hot water. Kneaded dough is covered by a damp cloth and incubated in a warm place for 2 h. Then the dough ball is rolled into flat circular kulchas and baked on a pan with small quantity of butter/oil on its surface.

Litti/baati

Litti/baati is a traditional staple food of the Bihar state of India. Litti is a variation of baati and is stuffed with sattu, flour made of roasted Bengal gram dal. Being high in nutrients and fiber, sattu litti is an ideal power brunch food for field workers and is served with chokha made of boiled potatoes, roasted brinjal, and ghee.

Sattu flour is known for its cooling effects, is considered a must-have food during summers, and is relished in form of littis, parantha, laddoos, and drink. Another peculiar feature of this platter is the extensive use of mustard oil. The aroma and the slightly pungent taste provided by mustard oil gives an interesting flavor to the dish. Litti is traditionally baked over cow dung cakes in villages, but can be baked in gas tandoor or electric ovens as well.

Naan

Naan is a traditional Indian leavened flat bread, made from maida, enriched with yogurt, and baked in a tandoor. A variety of naan is available in the market, for example, Peshwari naan (filled with nuts and raisins), Kema naan (stuffed with minced meat), or Kashmiri naan. It is relatively more nutritious than chapati and roti, due to milk, curd (yogurt), and egg in its formulation.

Parotha

Parotha is a typical Indian flat bread. The main ingredients are wheat flour, water, oil, and salt, whereas sugar and egg are optional ingredients. Two types of Parotha are generally prepared in India: South Indian Parotha and North Indian Parotha, differing in extraction rate of flour. South Indian parotha is multilayered, circular-shaped, creamish white-colored flat bread made from refined wheat flour (maida). In south India, parotha is one of the staple food items. It is also called Kerala parotha, Ceylon parotha, or simply parotha. It possesses light brown spots on the surface. Soft pliable handfeel, soft and slightly chewy texture with distinct layers, optimum oiliness, easy breakdown in the mouth, and pleasant taste and aroma are the characteristics of a good-quality parotha. In north India various types of parotha are prepared by stuffing with potato, cauliflower, and so on. A special type of parotha, that is, Lachcha Parotha is very famous in north India.

Poori

Poori is close to chapati in terms of consumption. It is an unleavened flat bread prepared in many of the countries in South Asia including India, Pakistan, and Bangladesh. It is consumed for breakfast or as a snack or light meal. It is an unleavened traditional deep-fried product (oil content 28–30%) of India, prepared from whole wheat flour dough. Wheat flour dough is sheeted to a circular shape of about 10–12 cm diameter and 0.1–0.3 cm thickness and then deep-fried in vegetable oil to a soft pliable texture with typical aroma. Due to its high oil content it has limited use for calorie conscious people.

As poori cannot be made from any cereal/pulse flours other than wheat, in many regions of the world it is prepared from blends of imported wheat flour and locally grown maize (*Zea mays* L.), jowar (*Sorghum vulgare* Pers.), and Bengal gram (*Cicerarietinum* L.). The possibilities of using composite flours for poori making have been studied. Incorporation of maize, Bengal gram, and jowar flours to whole wheat flour (atta) at 10%, 15%, and 20% levels, respectively, yielded fully puffed pooris with a very good rating. Texture of pooris containing up to 20% of Bengal gram flour had better texture than those containing maize or jowar flour. Pooris of good overall acceptability could be obtained from composite flours based on atta incorporated with maize/jowar flours at the 15% level and Bengal gram flour at the 20% level.

Tandoori roti

Tandoori roti is also an unleavened single-layered flat bread similar to chapati. Its preparation is also similar to chapati. The flour is mixed with water, shortening, salt, sourdough, or yeast and then sheeted and baked in a tandoor. It is creamish brown to brown in color. The tandoor is an oval in-ground oven, the walls of which are plastered with clay. Wood or natural gas is burned to heat the tandoor. With the help of a cloth pad, the sheeted dough is placed on the heated walls of the tandoor. The roti takes 60–90 s for baking depending upon the heat in the tandoor.

Non-Wheat-Based Products

Bhakri

Bhakri is made primarily with sorghum flour, water, and oil. It is sometimes made with suitable blend of wheat flour. This is also a flat and unleavened bread.

Dosa or dosai

Dosa is a crispy fermented crepe or savory pancake originated from South India. It is made from rice, black gram, and salt. A mixture of rice and black gram that has been soaked in water is ground finely to form a batter. The proportion of rice to black gram is basically 4:1 or 5:1. The batter is allowed to ferment overnight. A thin layer of the batter is then spread evenly onto a hot greased tava (griddle). A crisp pancake is formed. A dosai is served hot, either folded in half or rolled like a wrap, usually with aloo curry, chutney, or sambar.

Pappad

Pappad is a thin and crispy Indian cuisine sometimes described as a cracker or flat bread. It is served as an accompaniment to a meal in India. Pappad is also used as an appetizer or a snack and can be enjoyed with various toppings, such as chopped onions, chutney, or other dips and condiments.

Mediterranean/Middle East

Aish Mehahra

Aish Mehahra is a product of Egypt. It is made with ground fenugreek seeds (5–10%) and maize.

Baladi

Baladi is also a product of Egyptian origin. It is a pocket bread and is used as kebab wrap, sandwich bread, or as a spoon. Wheat flour and whole wheat flour are used to prepare traditional Egyptian pita bread Aash Makamar and Aash Baladi, respectively.

Barbari Bread

Barbari is an Iranian/Persian flat bread. Hazara migrants, referred to by Iranians as 'Barbars' brought it to Iran in the nineteenth century. This is most commonly baked in Afghanistan.

Bazlama

Bazlama is a product of Turkey and Middle East countries. It is a single-layered leavened flat bread having a creamy yellow color and circular shape with an average thickness of 3 cm and diameters ranging from 10 to 20 cm.

Lavash

Lavash is a fermented single-layered flat bread of Armenia. It is formed in an elliptical shape, 20–30 cm long, 10–20 cm wide, and 0.3–0.5 cm thick. The ingredients required are flour, salt, water, and baker's yeast. All the ingredients are mixed and kneaded into a fully developed dough and allowed to ferment for 30–60 min at 30 °C. The dough is then weighed, divided, rounded, and final proofed for 15–20 min at 30 °C and then sheeted. Baking is done at a temperature of 320 °C for 15–40 min in an oven.

Matzo

Matzo is a very thin bread of Israel having a cracker-like texture.

Pide

Pide, mostly consumed in the holy month of Ramadan, is a circular flat bread made of leavened dough of slight viscosity. It has a thickness of 1.5–2 cm and a diameter of 20–25 cm. The ingredients required for Pide production are flour, salt, water, shortening, sugar, baker's yeast, and some additives. Pide resembles Iranian barbari and Indian tandoori naan bread. The ingredients are mixed and kneaded for 15–20 min to obtain homogenous dough. The dough is fermented for 40–50 min at 30 °C, then divided in an appropriate weight and rounded. The dough is sheeted, and final proofing is done for 30–40 min at 30 °C. Baking is done at a temperature of 320 °C for 18 min in the oven.

Pita

Pita, also Pitta, breads, also called Arabic bread, balady, shamy, Syrian bread, and pocket bread, are circular, leavened double-layered flat breads that originated in the Middle East. It is prepared with flour, water, baker's yeast, and salt. All the ingredients are mixed into a fully developed dough with a temperature of 24.5–25.5 °C and fermented for about 1 h. The dough is then rounded and intermediately proofed for 15–20 min. Sheeting is done, and then final proofing is given for 30 min at 30 °C and 95% RH. Then baking is done in a hot hearth oven at 370–500 °C. Top and bottom crusts are formed instantaneously as the loaves are exposed to such a high temperature. Subsequently heat penetrates the interior of the loaf and transforms the interior moisture into steam within some 30–45 s. Steam expands the loaf into a puffed-up form. Thus the baking creates the voids in the interior upon cooling.

Sangak

Sangak is single-layered leavened flat bread of Iranian origin. Sangak is usually prepared as 70–80 cm long, 40–50 cm wide, and 0.3–0.5 cm thick. There are indented blisters on the bottom crust. The top crust contains small blisters and is sprinkled with sesame or poppy seeds. The ingredients required for preparation are flour, water, sourdough, and salt that are mixed and fermented for 2 h. A portion of the dough (500 g) is sheeted, docked, and transferred onto the hot pebbles of the oven. The temperature of pebbles is kept between 350 and 500 °C, and the baking time required is 2–4 min.

Taftoon

Taftoon, also known as Tanoor bread, is a leavened single-layered and docked flat bread. Docking avoids pocket formation during baking. It is popular in the southwestern part of Iran. The ingredients required are flour, active dry yeast, salt, and water. Taftoon preparation involves mixing, dividing, proofing, sheeting, docking, baking, and packaging. Crust color becomes reddish brown. High-quality tanoor is of uniform thickness with an even distribution of small blisters on the top crust.

Yufka

Yufka is a Turkish unleavened single-layered flat bread. It is circular, cream-colored, 1–2 mm in thickness, 4–5 cm in diameter. The flour is mixed with water, salt, a little vinegar or lemon juice, and very little olive oil and then kneaded. The dough is divided, rounded, and then baked on both sides on a hot plate for a shorter time about 15–30 s.

North America

Pancake

A pancake is a thin, flat cake often round prepared from a batter and cooked on a hot griddle or frying pan. Some use a yeast-raised or fermented batter to make pancakes. Most pancakes are cooked one side on a pan and flipped to cook the other side.

Southeast Asia

Khanombuang

Khanombuang is a product of Thailand made with rice flour. Khanombuang are usually first topped or filled with coconut cream, followed by sweet or savory toppings such as shredded coconut, or egg yolks, or chopped scallions. They are thin and crispy with a juicy interior.

Roti Canai

Roti canai is circular flat bread prepared in Malaysia. It is known as roti prata in Southern Malaysia and Singapore and is similar to the Indian Kerala porotta.

Various chapati products are depicted from Figures 1–3.

Ingredients Required for Preparation of Flat Breads

Quality of used raw materials as well as bread making methods affect the quality of bread. The desired quality of bread is a good digestibility, a delicious and fine aroma, and no easily crumbling or staling characteristics. The basic ingredients are flour, water, salt (sodium chloride), and naturally fermented starter dough with either baking powder or baker's yeast. In addition, sugar, butter, vegetable shortening, or nonfat dry milk may be added to enhance taste and aroma.

Flour

Flour is the basic ingredient in a flat bread recipe as it affects the texture and sensory properties. The quality characteristics of chapati depend on the quality of wheat used and the processing conditions given to convert it into whole-flour. Flours of different extraction rates give different bread types with colors varying from creamy white to pale brown. Total protein content is the most important character of wheat flour that affects the bread quality. Flat breads such as lavash, taftoon, barbari, and sangak are generally produced from soft white wheat flours. It has been observed that the quality of flat bread is

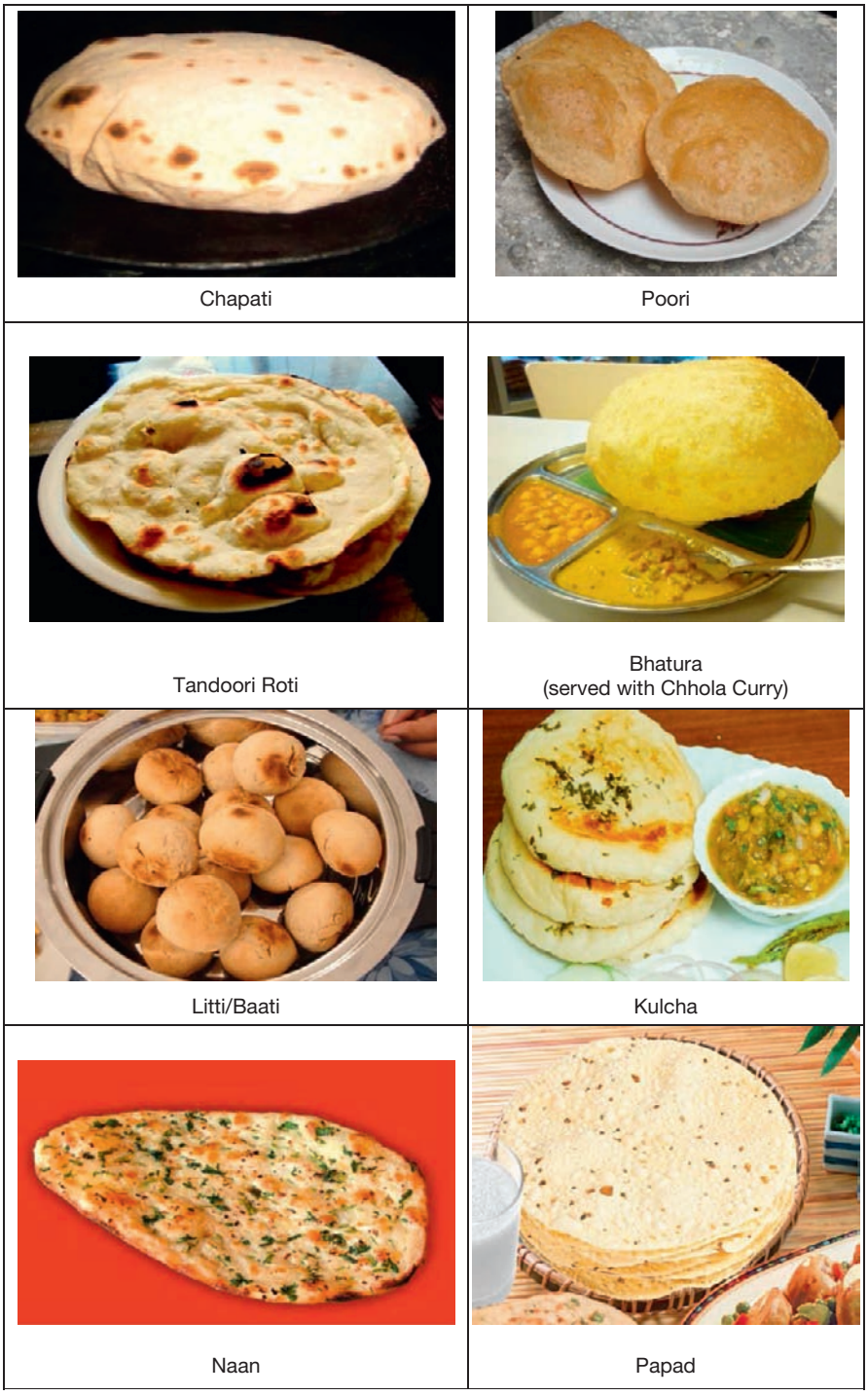

Figure 1 Various flat breads of Indian subcontinent.

(Continued)

superior when made with hard wheat flour associated with medium gluten strength, high starch damage, higher water absorption capacity, and good mixing and sheeting properties. The addition of durum wheat flour and Indian wheat flour raises the quality in chapati production. Wheat varieties with higher levels of lipids give chapatis with a softer texture.

Water

Water is a basic component that helps to get a homogeneous mixture of other ingredients and provides a desired viscoelastic structure to dough. Water helps dissolving hydrophilic components such as salt, sugar, and insoluble proteins and forms

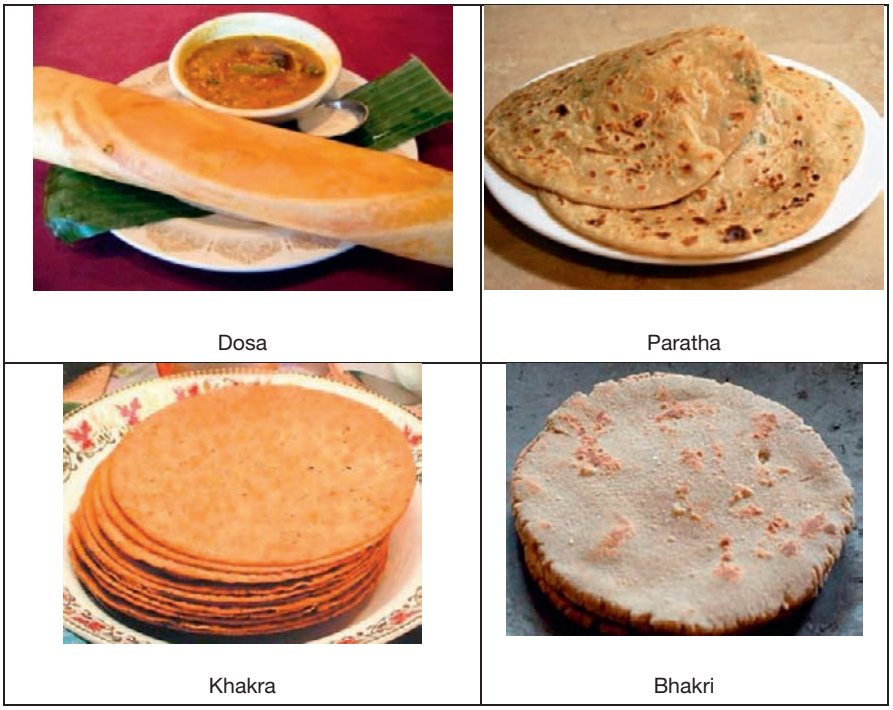

Figure 1 (Continued)

gluten by hydrating insoluble proteins in water. The amount of water used during flat bread production depends on the type of flour and bread. Approximately 50% water results in finely textured, light flat bread. Some artisan bread formulas contain from 60% to 75%. In yeast-leavened flat breads, the higher water absorption results in more carbon dioxide bubbles and a coarse bread structure. The addition of bran to the flour influences its water-absorbing capacity as it contains pentosans that have a high ability to absorb water.

Salt

Salt is an important ingredient in bread making. Use of salt in appropriate quantity is required to get a high bread quality. It should have a high solubility level in water and should be physically clean, bright, and white. Salt is mainly used for flavor and taste enhancement in flat bread production. The normal range of salt addition is about 0.5–3% of flour weight. Without salt, the weak flours make wet doughs, therefore the addition of salt improves the strength of proteins and gives good texture and better loaf volume and controls the action of yeast for optimum leavening of dough.

Baker's Yeast or Sourdough

Flat breads may be leavened or unleavened. The fermentation or rising of dough is due to leavening agents like yeast, sourdough, and baking soda. The leavening agent generates gas (CO_2) within the liquid phase that leavens the dough. Baker's yeast helps forming aroma, maturing dough by fermentation of some fermentable carbohydrates available in dough, and rising dough by formation of carbon dioxide.

Sourdough is also used for flat bread fermentation. Sourdough is a piece of dough saved from the previous batch that is then mixed with flour, salt, and water to produce bread. Sourdough is a complex biological system having a dynamic interaction among endogenous lactic acid bacteria and yeasts. Sourdough fermentation improves texture and palatability of whole grain, fiber-rich or gluten-free products, stabilizes or increases levels of various bioactive compounds, retard starch bioavalibility, and improves mineral bioavailability.

Baking powder is the most widely used leavening agent in flat bread production due to its low cost. Utilization of baking soda acts as a leaving agent and can change the color and flavor of bread.

Utilization of Composite Flours in Chapati/Flat Bread Making

Cereal flours other than wheat or starchy materials can also be used for chapati making if wheat flour is not available in sufficient amount or is an expensive imported material. Sweet potato has been used in Kenya for chapati making partially replacing wheat flour Protein content and nutritive value of wheat flour products can be improved by using flours from other cereals or legumes. For example, soy flour has been incorporated in chapatis to improve nutritional quality. Barley flour and germinated wheat flour have been incorporated in wheat flour to yield acceptable chapatis. Researchers have studied organoleptic acceptability of chapatis made from a combination of durum and aestivum wheat supplemented at different levels with Bengal gram flour and soy protein concentrate. The nutritional quality of chapati has been improved by the addition of soy flour or rice bean flour to wheat flour. Triticale could be blended with wheat at a level of 50% to make chapatis. Various oilseed flours (cotton seed, mustard, and

Baladi

Aish Mehahra

Barbari

Lavash

Pita

Matzo

Pide

Sangak

Yufka

Bazlama

Figure 2 Various flat breads from Middle East.

(Continued)

Taftoon

Figure 2 (Continued)

sunflower seed flours) can be/are also added with whole wheat flour for chapati making. Oilseed flours, because of their high contents of protein and essential amino acids, especially lysine, significantly improve the quality of wheat flour. Flaxseed and linseed flours have been incorporated in wheat flour to prepare omega-3 rich chapatis. Rice bran addition in wheat flour up to 20% is acceptable for increasing the dietary fiber content of chapati.

Addition of Green Leafy Vegetables in Chapati to Fortify Micronutrients

Wheat flour is a staple food for half of the world's population, it nevertheless does not guarantee a balanced diet, as it lacks micronutrients. Balanced diets are not available to a large population of the world, particularly those in developing countries, where multiple micronutrient deficiencies are more common than single deficiencies due to poor consumption and poor bioavailability of micronutrients. To tackle this issue, traditional flat breads like chapati/parotha are prepared by incorporating green leafy vegetables to enhance the nutritive value. Green leafy vegetables have been used as valuable additives for different products as they are inexpensive, easily available, and quickly cookable products. Hence, they can be used to overcome the deficiencies prevalent in vulnerable groups of socioeconomic classes. Some studies report that when chapatis are added with spinach, their shelf-life is extended up to 12 months by using an antimycotic agent like sorbic acid, in pack-sterilization, and storing them in PFP packaging materials. Curry leaves can also be incorporated in chapatis and up to a 10% addition of curry leaf powder is found acceptable. Moreover, when chapatis is fortified with curry leaf powder, calcium and carotene content increases. Less-utilized leaves of beet root (*Beta vulgaris* L.), carrot (*Daucuscarota* L.), cauliflower (*Brassica oleracea* L.), and turnip (*Brassica rapa* L.) can also be used in flat breads. Leaf mixture addition is accepted up to 10% in missi roti and dosa, and it significantly increases calcium and iron content in the products. Dried leaves of bathua (*Chenopodium album* L.) are added in paratha, and the results show that the carotene and iron content increases in the product.

Changes in Chapati During Storage and Their Prevention

The desired sensory quality characteristics of chapati are greater pliability, soft texture, light creamish brown color, slight chewiness, and baked wheat aroma. Freshly baked flat breads are soft and elastic. When kept at room temperature, chapati becomes stale within a few hours, and the texture becomes hard and tough. The shelf-life of freshly baked chapaties is 24–36 h, and due to mold growth, ropiness, and texture deterioration depending upon storage conditions, it becomes unsuitable for consumption. Various attempts have been made to preserve chapatis for more than 6 months with the use of antimycotic agents like propionic acid, sorbic acid, and other ingredients. However, during storage the chapatis develop a slight bitter taste due to preservatives. Recently, long shelf-life chapatis were developed by lowering the concentration of sorbic acid in combination with the biopreservative nisin. Highly acceptable no-preservative chapatis can be prepared through a thermal process at a F_0 value of 3.0. The product remained stable and highly acceptable even after a storage period of 12 months under ambient temperature (14–35 °C) conditions. Development of a retort pouch has enabled the preservation of traditional products like chapattis for long-term storage and increased their commercial scope and viability.

Automation in Chapati Making

Chapati making is not only a household practice. It is being done at a large scale in hotels, hostels, *Gurudwaras*, and so on. Hence there is a need of an automated chapati making plant. A chapati making machine developed at the Central Food Technological Research Institute, Mysore, for defense canteens and hotels is a fine example. It comprises two units: (a) chapati sheeting and (b) chapati baking. This machine is useful to prepare chapati on a large scale (at a commercial level). A traditional chapati maker, made up of iron and marble (Figure 4), is also available in the market. Automated baking and sheeting machines are also available in the market, and they reduce the chapati making time (Figures 5 and 6).

Flat Bread of Norway

Blitz-Blini

Chinese Pancake

Tortillas

Focaccia

Arepa

Ciabatta

French Crepes

Roti Kanai

Rye Flat Bread

Figure 3 Other flat breads from different parts of the world.

Scientific Interventions in Chapati Making

Various researchers have given their contribution in the field of flat bread making. Some important findings are as follows:

1. Partially baked chapati shows better retention of quality as compared to fully baked ones during storage at ambient and frozen conditions.
2. Flour extraction rates, fermentation times, and baking conditions directly affect the bioavailability of magnesium in flat breads.
3. Shortening and sodium stearoyl-2-lactylate (SSL) are of great help in retarding the staling of flat breads.
4. A molder was designed to produce flat breads with a variation of 0.01 mm to get consistent thickness flat bread, being thickness a major bread quality.
5. High alpha amylase flours are better in continuous production of flat breads by direct extrusion cooking.
6. Flour with 11–13% protein and 85% extraction was best suited for the production of tandoor bread.
7. Storage quality of flat breads made with different types of wheat can be improved by adding SSL (0.5%) and calcium propionate (0.5%). Shelf life can be extended up to 8 days at room temperature.
8. Durum is not a suitable variety for making good-quality flat breads or chapatis.
9. Guar gum increases the softness, extensibility, and other sensory attributes in fresh bread. Though extensibility and softness reduce significantly during storage at room temperature, refrigerated chapati containing guar gum shows less loss in extensibility up to a period of 2 days.
10. Proteinase and xylanase improve the quality of parotha by modifying rheological properties of the dough, glucose oxidase reduces the spread ratio, fungal alpha amylase produces a fragile bread lacking typical chewiness.
11. High-protein flours with wet gluten, high sedimentation value, and increased particle size give better-quality Iranian breads.
12. Gluten and oxidizing chemicals like ascorbic acid or potassium bromated increase extensibility and viscosity, compressive hardness and cohesiveness while reducing agents decrease them, and increase dough adhesiveness.
13. Moisture content, *in vitro* enzyme digestibility, and water-soluble starch decrease steadily during the storage at room temperature and at refrigerated temperature. Refrigerated temperature slows the rate of staling, but the texture of the chapatis becomes progressively harder at both room and refrigerated temperatures.

Figure 4 Roti maker.

Figure 5 Continuous baking machine.

Conclusion

Chapati and other related flat breads are used as staple diet of a major segment of population globally. It is a source of macro- as well as micronutrients. The preparation and formulation of flat bread varies with country and community. Various other cereals as well as oil seed and legume flours can be added to the main ingredient of chapati, that is, wheat flour to enhance its

Figure 6 Continuous sheeting machine.

nutritional value. Automation has also being done in chapati and related flat bread product preparation.

See also: Barley; Bread: Breadmaking Processes; Bread: Chemistry of Baking; Bread: Dough Mixing and Testing Operations; Bread: Types of Bread; Millets; Rice: Role in Diet; Rice: Types and Composition.

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Introduction

Milk has an average composition that varies depending on the species (human, cow, goat, and buffalo; see Table 1), the breed, the animal's feed, and the stage of lactation. In cheesemaking, the curds obtained after coagulation of milk will usually undergo significant modifications (Figure 1), depending on the type of cheese. Said modifications are caused by the specific technological procedures, which influence the activity of the microbial flora present throughout the cheese processing, and the enzymes native or added to milk. All together, these factors lead to the final product – cheese – that has a chemical composition quite different from milk (Table 2).

However, for simplification, those transformations may be considered essentially caused by three chemical reactions – proteolysis, lipolysis, and glycolysis – acting on milk major components: proteins, fats (lipids), and sugar (lactose).

Protein and Proteolysis in Cheese

Proteins are chains of amino acid molecules connected by peptide bonds (Figure 2). There are many types of proteins, and each has its own amino acid sequence (typically containing hundreds of amino acids). There are 22 different amino acids of which nine are essential (they cannot be made by the human body), and they can combine to form protein chains.

The amino acids within protein chains can bond across the chain and fold to form three-dimensional structures. Proteins can be relatively straight or form tightly compacted globules or be somewhere in between. The term 'denatured' is used when proteins unfold from their native chain or globular shape, due to a physical (heat), chemical (acids), or enzymatic (rennet) action.

On average, cow's milk contains 3.3% total protein. Milk proteins contain all nine essential amino acids required by humans. The milk proteins can be divided into two main groups: the casein family that contains phosphorus and will coagulate or precipitate at pH 4.6 and the serum (whey) proteins that do not contain phosphorus and remain in solution in milk at pH 4.6. The principle of coagulation, or curd formation, at reduced pH is the basis for cheese curd formation. In cow's milk, approximately 82% of milk protein is casein and the remaining 18% is serum or whey protein.

The casein family of protein consists of several types of caseins (α, β, κ), and each has its own amino acid composition, genetic variations, and functional properties. The caseins are suspended in milk in a complex called a micelle (see Figure 3). The high phosphate content of the casein family allows it to associate with calcium and form calcium phosphate salts. In cheese manufacture, casein is cleaved between certain amino acids, and this results in a fragmented protein, which will precipitate.

The behavior of the different types of caseins (α, β, κ) in milk when treated with heat, different pH (acidity), and different salt concentrations provides the characteristics of cheeses. The serum (whey) protein family consists of approximately 50% ß-lactoglobulin, 20% α-lactalbumin, blood serum albumin, immunoglobulins, lactoferrin, transferrin, and many minor proteins and enzymes. Like the other major milk components, each whey protein has its own characteristic composition and variations.

Degradation of proteins in cheesemaking occurs essentially by means of enzymes (proteases) coming from several sources: the native milk, airborne bacterial contamination, enzymes or bacteria that are added intentionally, and somatic cells present in milk. Casein proteolysis in cheesemaking starts when rennet (mainly chymosin) is added to milk and the enzyme acts on casein causing the aggregation of casein micelle, which will precipitate (coagulate). Because this enzyme is acidic (its activity is optimized in acidic environment), a starter culture is previously added to milk so to cause a slight, but important, pH lowering. Afterward and during cheese ripening, proteolysis occurs by the action of enzymes native to milk (plasmin) or released by lactic acid bacteria from the starter culture, leading to the formation of smaller fractions such as peptides or even amino acids (Figure 4).

The degree of breakdown of proteins in a cheese can be followed via the analytic method called electrophoresis as shown in Figure 5. The bands in each column represent proteins, polypeptides, or peptides, whose size is more or less inversely proportional to their distance of migration, from top to the bottom of the column. Peptides released during cheese proteolysis have a key role in the development of cheese taste, and a few may also exert specific bioactive or physiological effects such as opioid, antihypertensive, immunomodulatory, and antimicrobial.

Lipids and Lipolysis in Cheese

Fats are made from individual fatty acid molecules attached to glycerol, a three-carbon backbone. The most common type of fat is called a triglyceride, which contains three fatty acids attached to the backbone. Because there are many different fatty acids that can be attached to the backbone (see Figure 6), there are many different types of triglycerides or fats. Fatty acids may be saturated, which means that each carbon has a single bond to another carbon and two hydrogen atoms, or fatty acids may be unsaturated, which means that a carbon has two bonds to the adjacent carbon, called a double bond, and a single bond to another carbon and a hydrogen atom.

Other fatty compounds include phospholipids, which make up approximately 1% of the fat in milk.

Milk contains approximately 3.4% total fat, and cheeses will have an average ten times more fat than milk. Milk fat contains approximately 65% saturated, 30% monounsaturated, and 5% polyunsaturated fatty acids; thus, cheese is a high-caloric food also containing high levels of saturated fatty acids (butyric, myristic, palmitic, and stearic acids), which,

Table 1 Average chemical composition of milk of different species (for 100 g of fresh milk)

Species	*Water (%)*	*Proteins (%)*	*Fat (%)*	*Lactose (%)*	*Mineral salts (%)*
Buffalo	82.2	4.8	7.5	4.7	0.8
Goat	86.5	3.9	4.3	5.8	0.8
Ewe	80.9	6	7.5	5.4	1.1
Cow	87.5	3.2	3.7	4.6	1

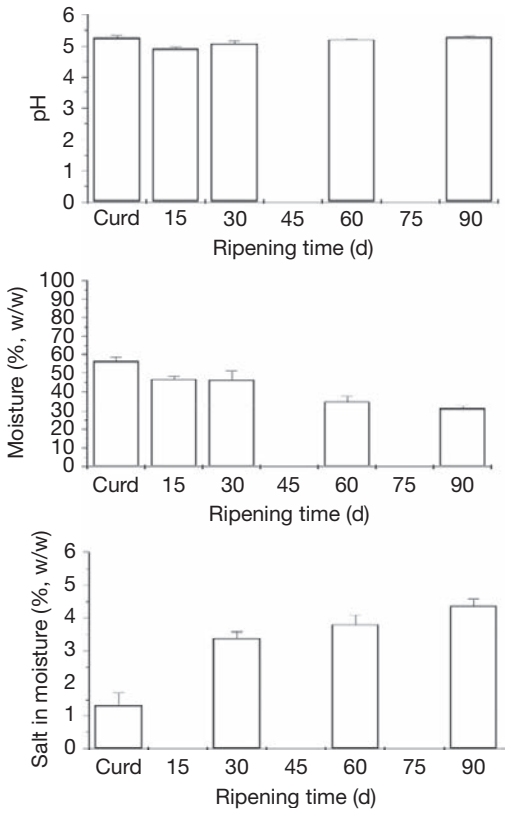

Figure 1 Evolution of physicochemical parameters of an experimental cheese (average ± standard deviation) throughout ripening.

Table 2 Composition (average ± standard deviation, $n = 4$) of São Jorge cheese by 90, 120, and 210 days of ripening

Ripening time (days)	*Parameters*		
	Moisture (%)	*Fat content (%)*	*pH*
90	36.19 ± 0.93^a	34.5 ± 0.4^a	5.27 ± 0.09^a
120	36.78 ± 0.66^a	32.5 ± 0.3^b	5.43 ± 0.04^b
210	36.63 ± 1.29^a	32.0 ± 0.6^b	5.36 ± 0.03^b

Different letters represent means with statistical differences for $p < 0.05$.

when consumed in excess may contribute to onset of high blood cholesterol and associated diseases. The conjugated linoleic acid (CLA) is a *trans*-fatty acid present in milk and cheese that is beneficial to humans in many ways. The biological properties of dietary CLA are attracting considerable interest because studies suggest that it may have a powerful anticarcinogenic, immunomodulating, growth-promoting, lean body mass-enhancing, and antidiabetic properties.

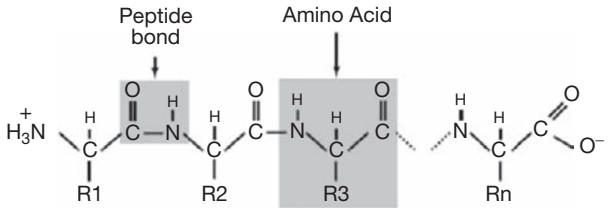

Figure 2 Structure of proteins (R1, R2, etc. are radicals specific to each amino acid. The number of amino acids in the caseins of milk varies from 199 to 209).

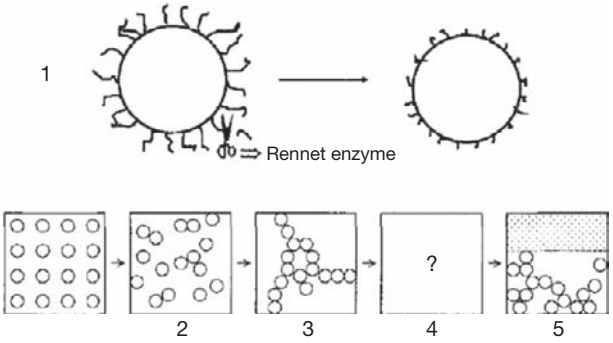

Figure 3 Model sequence of casein micelles changes during gelation by action of rennet leading to precipitation (coagulation) of casein.

The contents of CLA in cow's raw milk may vary from 0.2% to 3.7% of total milk fat, and it is established that dairy products derived from ruminants fed predominately on pasture are richer in CLA. Cheeses are thus considered important sources of CLA, with typical content values varying between 8 and 18 mg g^{-1} of fat.

Lipolysis (degradation of fats by enzymes called lipases) is crucial in the development of cheese aroma and taste. Throughout ripening, lipases transform the milk fat into short-chain fatty acids that may be volatile. The type of the starter culture, the length of the ripening period, and the conditions prevailing are important in defining the rate and type of lipolysis.

Sugar and Fermentation (Glycolysis)

The principal carbohydrate in milk is lactose (Figure 7). The concentration of lactose in cow's milk is relatively constant and averages about 5%.

Glycolysis or fermentation is the chemical reaction by which milk sugar (lactose) is transformed by the microflora into lactic acid and energy and other components.

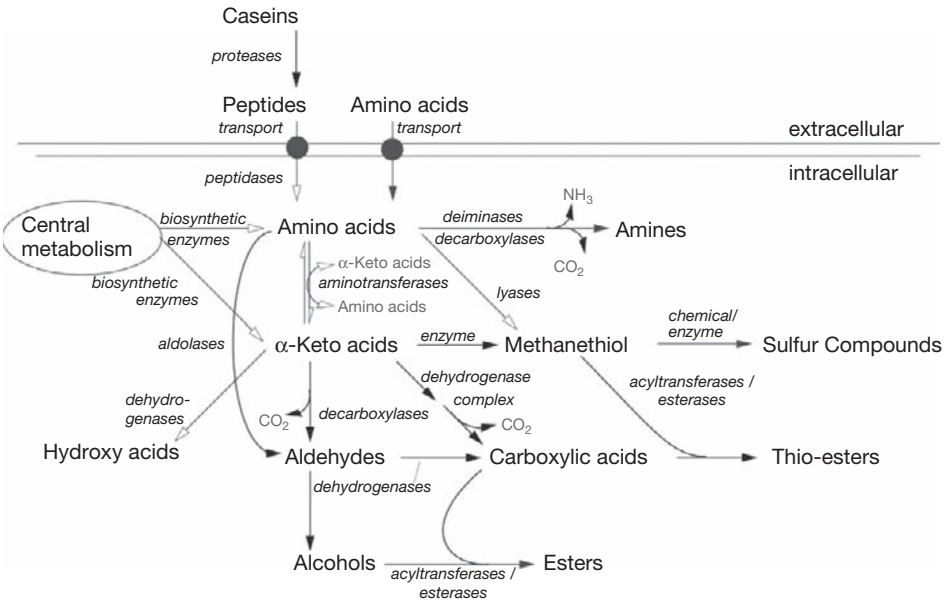

Figure 4 General pathways leading to intracellular metabolites and their degradation routes to potential flavor compounds. More specifically, pathways from methionine to flavor compounds (methanethiol, thioesters, and sulfur compounds) are shown. Adapted from Kranenburg, R., Kleerebezem, M., Vlieg, J. H., et al. (2002). Flavour formation from amino acids by lactic acid bacteria: predictions from genome sequence analysis. *International Dairy Journal* 12, 111–121.

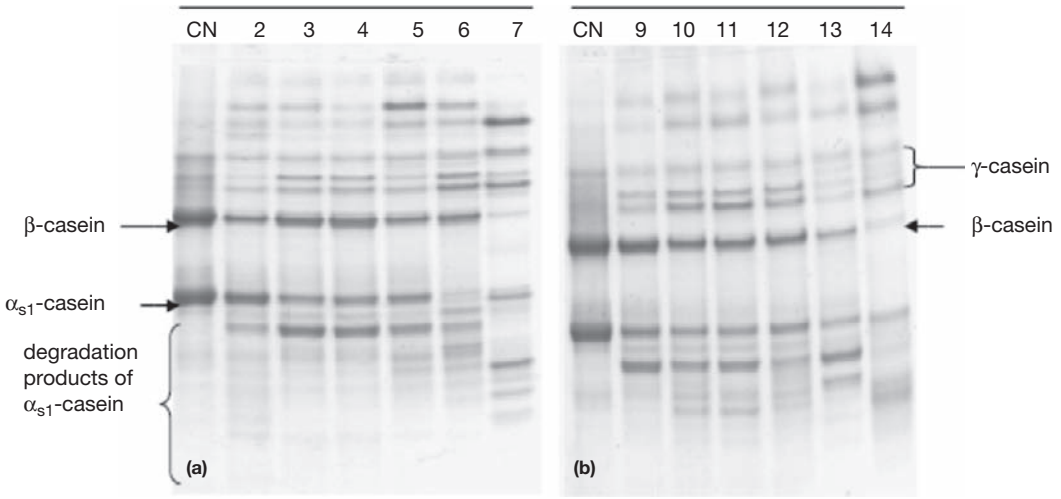

Figure 5 Evolution of proteolysis via urea–polyacrylamide gel electrophoresis in São Jorge cheeses from dairies A and B, by 1, 15, 30, 60, 90, or 130 days of ripening. Lanes 1, 8, and 15: Na-caseinate; lanes 2–6: cheese A; lanes 9–14: cheese B. Reproduced from Kongo, J. M., Gomes, A. M., Malcata, F. X. and McSweeney, P. L. H. (2009). Microbiological, biochemical and compositional changes during ripening of São Jorge – a raw milk cheese from the Azores (Portugal). *Journal Food Chemistry* 112, 131–138.

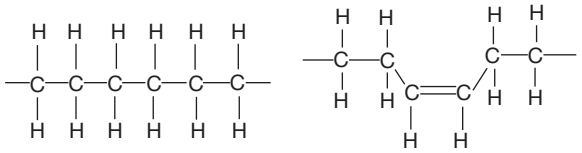

Figure 6 Chemical structure of a saturated (left) fatty acid and unsaturated (right) fatty acid.

The accumulation of lactic acid leads to the lowering of the pH of the curd, thus making it inhospitable for many unwanted bacteria and helping in syneresis (whey release). The amount of lactic acid released depends on the starter culture type and on the technological specificities used in the cheesemaking. Long-ripened cheeses may be depleted of lactose making them suitable for consumption by lactose-intolerant consumers.

Cheese Microbiology

Cheesemaking is based on the application of LAB in the form of defined or undefined starter cultures that are expected to cause a rapid acidification of milk through the production of lactic acid, with the consequent decrease in pH, thus affecting a

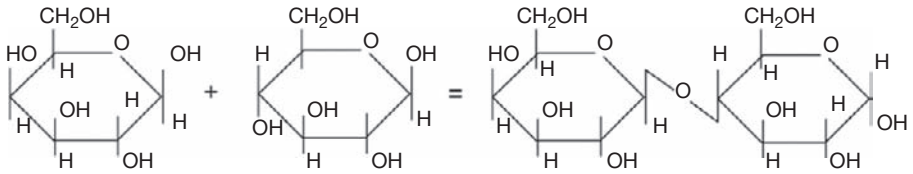

Figure 7 Lactose structure made of one molecule of glucose and one molecule of galactose.

number of aspects of the cheese manufacturing process and ultimately cheese composition and quality.

The LAB or non-LAB microflora of cheese may originate from three main sources: strains present in milk, those of the starter culture added, and those from adventitious 'in-house' contamination. While in industrial cheeses, bacteria from the starter culture will dominate from early manufacture to late ripening, traditional cheeses usually owe their typical flavors to the presence of microorganisms from (raw) milk and from 'in-house' contamination; in this case, cheeses obtained from distinct milk batches and farms will likely exhibit different viable numbers of several species. Although several species have been identified in various research works, the groups described in the succeeding text are those most frequently found in traditional cheesemaking.

Lactic Acid Bacteria

LAB are the most important group of microorganisms in milk and most dairy products. Species identified in cheese may be homofermentative, such as lactococci, or heterofermentative, such as lactobacilli. Distinct groups of LAB show different degrees of proteolytic activity in cheese, but their main role apparently is the formation of aminic and ammoniacal nitrogen, by degrading peptides and metabolizing amino acids – hence playing a complementary role to rennet in milk protein degradation throughout the whole manufacture process of cheese. The main bacteria associated to cheese and other dairy products are shown in Table 3.

The earliest productions of cheeses were based on the spontaneous fermentation, resulting from the development of the microflora naturally present in the raw milk and its environment. The quality of the end product was a reflex of the microbial load and spectrum of the raw material. Spontaneous fermentation was later optimized through backslopping, that is, inoculation of the raw material with a small quantity of whey from a previously performed successful fermentation, and the resulting product characteristics depended on the best-adapted strain dominance. Today, backslopping is still used to produce many artisanal raw milk cheeses, that is, those bearing the PDO (Protected Designation of Origin) status. The starter culture applied in this so-called natural fermentation is usually a poorly known microflora mix that, although having a predominance of LAB, may also contain non-LAB microorganisms, and its microbial diversity and load are usually variable over time.

Nonstarter Lactic Acid Bacteria, Yeasts, and Molds

As previously stated, the microbiology of a cheese, namely, those made from raw milk, is complex. Most cheeses will often be contaminate by LAB bacteria from the dairy environment (nonstarter lactic acid bacteria), which may also play a role in the development of the final cheese aroma, and by other groups such as enterococci, Enterobacteriaceae, micrococci, and molds and yeasts, the so-called in-house contaminants.

The wide variation in the dynamics of the species in association with the prevalent physical and chemical peculiarities of each cheese creates the diversity of aroma and taste among

Table 3 Main bacteria associated with cheeses or other fermented products

Species/subspecies	*Main uses/other comments*
Lactococcus	
L. lactis subsp. *lactis*	Mesophilic starter used for many cheese types
L. lactis subsp. *lactis* biovar diacetylactis	Used in Gouda, Edam, sour cream, and lactic butter
L. lactis subsp. *cremoris*	Mesophilic starter used for many cheese types
Streptococcus	
S. thermophilus	Thermophilic starter used for yogurt and many cheese types particularly hard and semihard high-cook cheeses
Lactobacillus	
L. acidophilus	Probiotic adjunct culture used in cheese and yogurt
L. delbrueckii subsp. *bulgaricus*	Thermophilic starter for yogurt and many cheese types, particularly hard and semihard high-cook cheeses
L. delbrueckii subsp. *lactis*	Used in fermented milks and high-cook cheese
L. helveticus	Thermophilic starter for fermented milks and many cheese types particularly hard and semihard high-cook cheeses
L. casei	Cheese ripening adjunct culture
L. plantarum	Cheese ripening adjunct culture
L. rhamnosus	Cheese ripening adjunct culture
Leuconostoc	
L. mesenteroides subsp. *cremoris*	Mesophilic culture used for Edam, Gouda, fresh cheese, lactic butter, and sour cream
Brevibacterium	
B. linens	Used in smear surface-ripened cheeses, Camembert, Stilton, and Limburger and as a cheese-ripening adjunct culture
Propionibacterium	
P. acidipropionici	Used in Gruyère and Emmental cheeses
P. freudenreichii subsp. *shermanii*	Used in Gruyère and Emmental cheeses

Source: Broome, M. C., Powel, I. B. and Limsowtin, G. K. Y. (2003). Starter cultures: specific properties. In: Regisnki, H., Fuquay, J. W. and Fox, P. F. (eds.) *Encyclopedia of dairy sciences* (vol. I), pp.269–275. London: Academic Press.

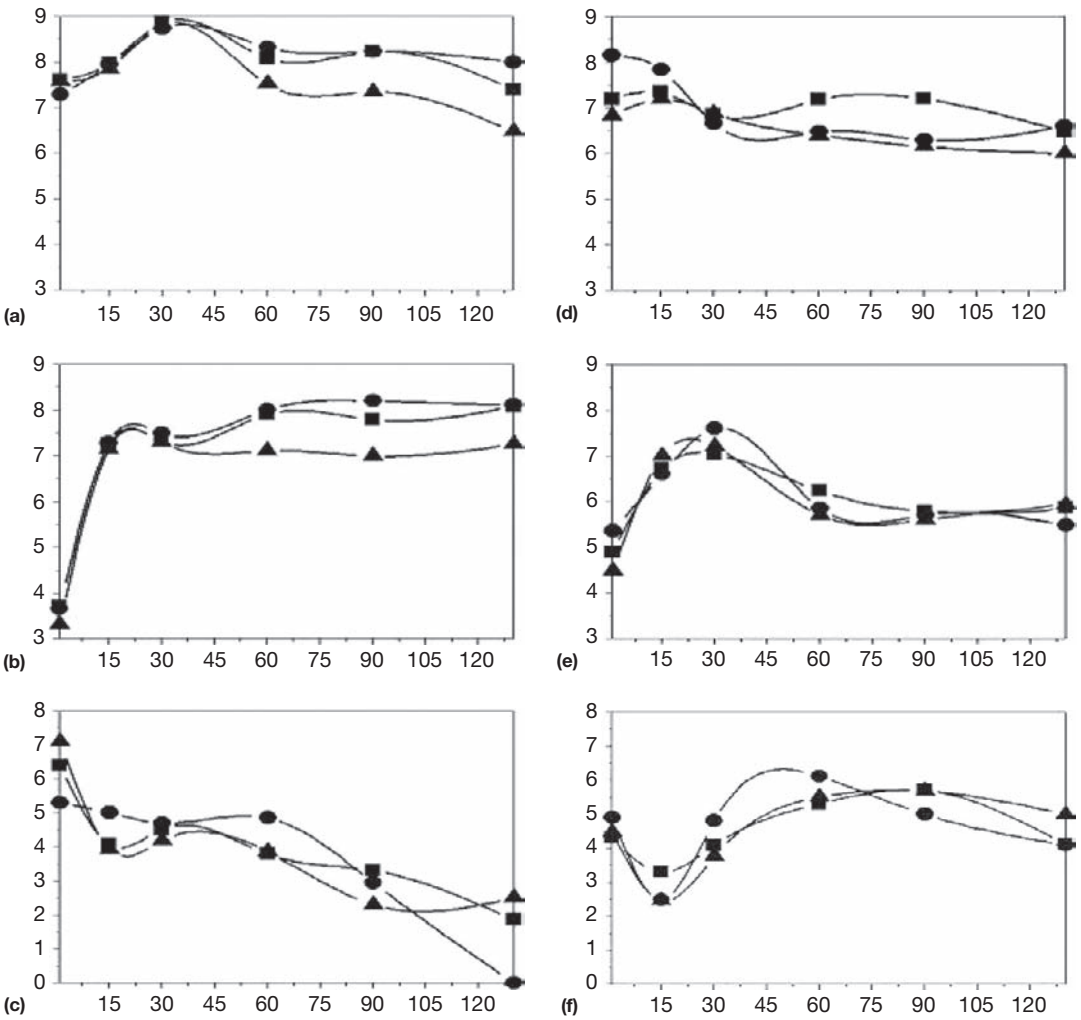

Figure 8 Evolution of viable numbers of the microflora in São Jorge cheeses from dairies A (■), B (●), and C (▲) by 1, 15, 30, 60, 90, and 130 days of ripening: (a) total mesophiles, (b) lactobacilli, (c) Enterobacteriaceae, (d) lactococci, (e) enterococci, and (f) yeasts and molds.

cheeses. Many strains isolated from raw milk cheeses were associated with the formation of more complex volatile profiles and higher scores for some sensory attributes. This, associated with a greater typical microflora biodiversity, makes raw milk cheeses more prone to developing more intense flavor than cheeses made from pasteurized milk.

Molds and yeasts are usually present in raw milk, and they do not survive pasteurization. Thus, unless the cheese is made from raw milk, its contamination with yeasts and molds is essentially caused by reinfection during manufacturing by yeasts and molds present in the environment of cheese factories, like walls and shelves of ripening rooms, air, equipment, water, milk, and brine. Yeasts can metabolize lactic acid in the cheese, causing the rise in pH and leading to the formation of the characteristic undesirable yeasty or fruity flavor and gas in cheeses. However, due the production of metabolites, for example, short-chain fatty acids and other compounds, some specific strains contribute to maturation and aroma formation.

The characteristic feature of some mold-ripened cheese types is extensive proteolysis and lipolysis, releasing peptides and fatty acids that contribute to the development of distinctive flavor and aroma, as in the case of Camembert cheese. Wild types of molds such as *Aspergillus flavus* may influence the organoleptic characteristics of cheeses or even produce mycotoxins that represent a potential health risk to the consumer. The most common molds found in raw milk cheeses belong to the genera *Geotrichum*, *Aspergillus*, *Mucor*, *Fusarium*, and *Penicillium*.

Finally, recall also that poor hygienic practices, inadequate pasteurization, or poor handling during processing may allow for accidental presence of pathogens such as *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* O157:H7, *Campylobacter jejuni*, and *Staphylococcus aureus* in cheeses.

Figure 8 shows the microflora dynamics throughout ripening of São Jorge raw milk cheese.

See also: Cheese: Composition and Health Effects; Cheese: Processing and Sensory Properties; Cheese: Types of Cheese – Medium; Cheese: Types of Cheeses – Hard; Fermented Foods: Fermented Milks; Lactic Acid Bacteria.

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Cheese: Composition and Health Effects

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Introduction

Cheese is a dairy product strongly appreciated by consumers at large, not only due to the unusual complexity and variety of its sensory attributes but also owing to its outstanding nutritional value. Cheese constitutes an important dietary source of essential nutrients and health-promoting compounds, such as proteins, amino acids, bioactive peptides, lipids (e.g., polyunsaturated fatty acids (PUFAs), and conjugated linoleic acid (CLA) isomers), minerals, vitamins, and polyphenolic compounds. However, cheeses are also characterized by high contents of fat, saturated fatty acids (SFAs), *trans*-fatty acids (TFAs), cholesterol, and salt, features that have contributed to its negative health image, arising from the association between intake of these components and an increased risk of cardiovascular diseases. Due to this association, nutritional recommendations promote the reduction of cheese consumption, ignoring that such product may also be a good dietary source of beneficial nutrients. In fact, several studies suggest no or inverse relationship between cheese intake and the risk of cardiovascular disease. Moreover, dairy products including cheese potentially may have many other beneficial physiological properties, such as anticariogenic, antihypertensive, and anticarcinogenic effects, as well as beneficial effect on bone health.

Cheese nutritional composition is determined by several factors, such as characteristics of milk used (which depend on numerous factors linked to the animal source, including species, breed, stage of lactation, physiological status, and diet) and the cheesemaking and the ripening conditions, resulting in a wide diversity of cheese types worldwide – each one with distinct and unique sensory and nutritional properties. Although the chemical composition of cheeses varies considerably, overall, they are composed mainly of fat, protein, vitamins, and minerals, which are retained in the curd during manufacture, and of relatively small amounts of water-soluble constituents (whey proteins, lactose, and water-soluble vitamins and minerals), once these milk components are lost in the whey. The proximate composition of some cheeses is shown in Table 1.

Fat and Cholesterol

Consumers are more aware of the relationship between diet and health, and the great consumer concern regarding to cheese intake is not only due to high levels of fat in some cheese types but also due to its fatty acid composition. The fat content of cheese is very variable, with some cheeses with very low levels of fat (e.g., the cottage cheese has <4 g per 100 g of fat), but most cheeses have between 20 and 40 g per 100 g of fat, which may even represent about 50% of the cheese weight (e.g., cream cheese) (Table 1). Fat constitutes an important source of energy and essential fatty acids in human diet, but on the other hand, its consumption has been claimed to increase the risk of cardiovascular diseases – particularly in the case of cheese fats rich in SFAs and TFAs.

Milk and dairy products are characterized by high contents of SFAs and TFAs and low levels of PUFAs, as the result of the extensive microbial metabolization of dietary lipid in the rumen, where the lipids are hydrolyzed and the released unsaturated fatty acids are biohydrogenated with the production of high level of SFAs, as well as TFAs. Cheese fat contains about 60–70% of SFAs, palmitic acid (16:0) being the most abundant SFA, followed by myristic (14:0) and stearic acids (18:0) (Table 2). Monounsaturated fatty acids (MUFAs) represent 20–30% of total fatty acids in cheese, while only 4–6% of total fatty acids are PUFAs (Table 2). TFAs may represent about 5% of total fatty acids in cheeses (Table 2), being composed mainly of 18:1 *trans*-isomers. Vaccenic acid (18:1 *trans*-11) is the major 18:1 *trans*-isomer in ruminant-derived food products; however, under feeding systems based on the utilization of high levels of the concentrates, 18:1 *trans*-10 may become the major 18:1 *trans*-isomer.

Cheese constitutes an important source of fat in human diet, contributing significantly to SFA and TFA intake. Results on TFAs in foods in Europe – TRANSFAIR study – showed that in western Europe countries, cheese contributes between 3.0% and 14.0% of total fat intake, providing between 5.9% and 24% of total SFA intake and between 5.4% and 33.8% of total TFA intake. Consumption of SFAs and TFAs has been associated with deleterious effects on human health, such as increase of the cardiovascular disease risk. So, the present nutritional guidelines from WHO/FAO recommended that intake of SFAs and TFAs should not exceed 10% and 1% of total energy intake, respectively. However, individual SFAs and TFAs seem to have different biological effects that are not considered in nutritional recommendations. Overall, SFAs are known to increase the total and low-density lipoprotein (LDL) cholesterol; however, only lauric (12:0), myristic (14:0), and palmitic (16:0) acids seem to have a cholesterol-raising effect, whereas stearic acid (18:0) appears to have neutral cholesterolemic effect. Moreover, hypercholesterolemic SFAs seem to have distinct cholesterolemic effects, but the comparative effect among such SFAs is still inconsistent.

Trans-fatty acids are provided in human food by several sources but mainly by ruminant-derived foods and partially hydrogenated vegetable oils. The content and profile of the TFAs from each source are distinct, depending on the mechanism of their production. Contrary to what happens in ruminant fat that has as the main TFA vaccenic acid (18:1 *trans*-11), in partially hydrogenated vegetable oils, the TFA profile is characterized by higher diversity of the main TFAs. The results

Table 1 Proximate composition of selected cheeses (per 100 g)

Cheese type	*Moisture (g)*	*Fat (g)*	*Protein (g)*	*Carbohydrates (g)*	*Cholesterol (mg)*	*Energy (kcal)*	*Reference*
Caerphilly	41.8	31.3	23.2	0.1	90	375	[1]
Cabrales	36.5	34.6	23.5	0.2	–	407	[2]
Camembert	50.7	23.7	20.9	Tr	75	297	[1]
Cebreiro	59.4	22.2	14.9	1.8	–	266	[2]
Cheddar	36.0	34.4	25.5	0.1	100	412	[1]
Cream cheese	45.5	47.4	3.1	Tr	95	439	[1]
Cottage	79.1	3.9	13.8	2.1	13	98	[1]
Edam	43.8	25.4	26.0	Tr	80	333	[1]
Emmentaler	35.7	29.7	28.7	Tr	90	382	[1]
Feta	56.5	20.2	15.6	1.5	70	250	[1]
Gouda	40.1	31.0	24.0	Tr	100	375	[1]
Idiazabal	30.9	38.8	25.7	0.6	–	455	[2]
La Serena	42.2	26.3	24.3	2.0	–	342	[2]
Majorero	38.4	28.7	25.7	2.2	–	370	[2]
Manchego	31.6	38.0	24.7	1.3	–	446	[2]
Mozzarella	49.8	21.0	25.1	Tr	65	289	[1]
Parmesan	18.4	32.7	39.4	Tr	100	452	[1]
Pecorino	44.1	27.4	25.5	–	–	–	[3]
Roncal	30.0	38.2	26.2	1.3	–	454	[2]
Roquefort	41.3	32.9	19.7	Tr	90	375	[1]
São Jorge	37.0–38.0	30.0–34.0	23.2–25.8	–	–	–	[4]
Serpa	35.7–50.4	19.2–35.5	18.0–28.2	–	–	–	[5]
Serra da Estrela	45.4	25.2	22.1	–	–	–	[6]
Terrincho	52.3–54.7	23.3–25.1	18.3–24.2	–	–	–	[7]

Tr, trace.

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on biological effects of TFAs from ruminant and industrial sources are still not completely clear. However, some studies suggest that the current levels of TFAs from ruminant origin consumed in diets do not contribute significantly to cardiovascular disease risk. Moreover, 18:1 *trans*-11 is considered as a beneficial TFA, once it is the precursor of endogenous synthesis of CLA in animals and humans.

The putative deleterious effect of milk and dairy products on health, particularly on cardiovascular diseases, has been the target of extensive researches, and recent epidemiological studies have shown that higher intake of milk and dairy products may not be detrimental to cardiovascular health and even contribute to cardiovascular disease risk reduction. Most of these evidences are related to milk, and only few studies evaluated the association between cheese consumption and cardiovascular disease risk. However, overall, these few studies suggest no association or inverse relationship between cheese intake and the risk of cardiovascular disease. The potential beneficial effects of cheese consumption on cardiovascular diseases may be due to several healthy nutrients present in cheeses, such as vitamins, minerals, bioactive peptides, and lipids, such as PUFAs and CLAs.

In recent years, CLAs have received much attention due to their potential beneficial properties to human health. CLA refers to a mixture of positional and geometric isomers of linoleic acid with a conjugated double-bond system. Several studies realized in animal models and cell cultures have shown that CLAs exhibit numerous beneficial physiological activities, including anticarcinogenic, antiobesitic, antiatherogenic, antidiabetogenic, immunomodulatory, and osteosynthetic effects. Ruminant fat is naturally rich in CLA, particularly in rumenic acid. However, the cheese content in CLA varies considerably, reflecting the CLA levels in the original milk, with CLA levels between 0.5 and 1.71 g per 100 g of total fatty acids (**Table 2**). Cheese constitutes an important dietary source of CLA in human diets, and in some countries, it is the major source of CLA, such as Portugal where it is estimated that cheese contributes to 40% of total CLA intake.

To meet the consumers' demand for healthy foods has led to the development of reduced- and low-fat and even fat-free cheeses. Nevertheless, fat is essential for the development and perception of the correct sensory characteristics of cheeses; it has been reported that fat removal leads to defective cheeses in terms of flavor, texture, and appearance. So, the uncharacteristic sensory properties of low-fat cheeses when compared with full-fat cheeses may compromise the popularity of such cheese type, since the consumers cannot be willing to sacrifice the

Table 2 Fatty acid composition of selected cheeses (g fatty acid 100 g^{-1} of total fatty acids)

	SFAs					*MUFAs*		*PUFAs*			
Cheese type	*12:0*	*14:0*	*16:0*	*18:0*	*Total*	*18:1* cis-*9*	*Total*	*CLA*	*Total*	*Total TFAs*	*Reference*
Abondance	4.06	12.2	28.1	8.25	70.1	17.8	24.7	0.69[a]	4.03	1.65[b]	[1]
Azeitão	–	–	–	–	70.9–73.8	–	17.5–18.9	0.90–0.98	4.11–4.47	4.57–4.77	[2]
Caerphilly	3.29	10.6	29.6	9.16	69.5	20.3	27.2	0.52	3.29	2.50	[3]
Cantalet and Salers	4.06	12.1	27.5	8.35	70.3	17.8	24.8	0.67[a]	3.99	1.59[b]	[1]
Cows' cheese	3.94	12.0	35.1	7.26	71.4	19.9	25.0	0.85[a]	3.65	–	[4]
Évora	–	–	–	–	71.2–73.2	–	18.7–19.4	0.88–0.93	4.16–4.35	4.68–4.82	[2]
Ewes' cheese	3.87	9.79	23.9	10.8	68.2	16.1	26.1	1.61	5.66	–	[5]
Mozzarella	4.01	12.6	26.4	9.81	67.3	–	26.8	0.85	6.02	4.69	[6]
Manchego	4.07	9.23	25.2	10.2	68.8	19.6	26.5	0.89	4.69	1.68[c]	[7]
Nisa	–	–	–	–	67.9–69.2	–	21.0–22.1	1.1–1.2	4.49–4.60	5.11–5.18	[2]
Pecorino	2.94	8.53	20.5	10.5	62.5	17.7	22.6	0.96	4.53	3.44	[8]
Rocamadour	4.05	11.5	27.7	9.45	70.5	17.5	24.3	0.67[a]	3.99	1.77[b]	[1]
Soft goats' cheese	4.27	8.82	23.9	11.1	65.4	19.8	26.4	0.76	5.31	3.97	[9]
Tomme de Savoie	4.06	12.1	27.5	8.35	70.3	17.8	24.8	0.66[a]	3.99	1.59[a]	[1]

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; TFAs, *trans*-fatty acids.

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[a] 18:2 *cis*-9, *trans*-11.

[b] 18:1 *trans*-11 + 18:1 *trans*-10.

[c] 18:1 *trans*-11.

typical sensory perceptions associated with a particular cheese variety for a plain reduction of fat intake.

Changes of the cheese fatty acid profile, in order to reduce its saturation and increase the levels of beneficial unsaturated fatty acids, particularly $n-3$ PUFAs and CLAs, also have been extensively explored. The final content of fatty acids in cheese is mainly dependent on the fatty acid levels of the unprocessed milk, and the utilization of milk with improved fatty acid profile appears to be a good approach to manufacture cheeses with enhanced nutritional value. Milk fatty acid composition is determined by several animal intrinsic factors, like species, breed, genotype, lactation and pregnancy stages. In addition, the milk fat composition is largely dependent on the diet supplied to animals. Several studies show that there is opportunity to improve naturally the nutritional value of milk fat through changes of animal diet and nutritional strategies to increase the concentration of specific beneficial fatty acids in milk fat, which have been a major target in ruminant milk research. Utilization of diets based on fresh pasture and supplementation of diets with lipid sources rich in PUFAs has shown to be effective to improve the fatty acid profile of milk and dairy products, decreasing its content in SFAs and increasing beneficial fatty acids, including CLAs and $n-3$ PUFAs. Although the strategies for improving the milk fatty acid composition have been the goal of numerous studies, the effect of dairy products with modified fatty acid composition through manipulation of the animal diet on cardiovascular disease is little known. The current evidence is still insufficient, but a few studies show that intake of milk and dairy products with improved fatty acids composition via modulation of animal diets may have beneficial effect on cardiovascular risk in both healthy and hypercholesterolemic individuals.

Enrichment in $n-3$ PUFAs also has been experimentally attained by incorporation of lipid sources rich in $n-3$ PUFAs, such as fish and flaxseed oils, at different stages of cheesemaking. Moreover, *in vitro* studies have shown that several strains of food-grade microorganisms are able to convert linoleic acid (18:2 $n-6$) into CLA, and the enrichment of cheese with CLA by utilization of microbial cultures has been explored. Nevertheless, the utilization of CLA-producing microorganisms only has allowed a marginal CLA enrichment in cheese. Unlike fat removal, the enrichment of cheese with CLAs and $n-3$ PUFAs by animals' diet manipulation or by cheese fortification with low levels of lipids has minor effect on sensory characteristics of cheeses, resulting in cheeses with good acceptability by

consumers, demonstrating the feasibility of producing these cheeses with high nutritional value.

The cheese cholesterol content is consumers' concern regarding the consumption of cheese, due to generic association of dietary cholesterol to increase the total cholesterol and LDL cholesterol. However, epidemiological and clinical studies indicate that for the general population, the dietary cholesterol constitutes a minor risk for the development of cardiovascular diseases, once the dietary cholesterol has little effect on the plasma LDL/high-density lipoprotein cholesterol ratio. The cholesterol content of cheese varies among cheese types, with values of cholesterol < 100 mg per 100 g of cheese (Table 1), not constituting the cheese intake, due to the supply of cholesterol a significant risk for development of cardiovascular disease.

Protein

Cheese has high biological value proteins. The protein content of cheese varies considerably among cheese types, ranging between about 3 g per 100 g of protein (e.g., cream cheese) and 40 g per 100 g of protein (e.g., parmesan cheese) (Table 1). The protein of cheese is composed mainly of caseins (α_{s1}-CN, α_{s2}-CN, -CN, and κ-caseins). Caseins constitute a rich source of essential amino acids, calcium, and inorganic phosphate. Throughout cheese ripening, the casein molecules are hydrolyzed, producing a great diversity of peptides and eventually free amino acids. Several studies have shown that many of the peptides formed during cheese ripening have physiological activities, such as angiotensin-converting enzyme (ACE)-inhibiting peptides, phosphopeptides, immunopeptides, and casomorphins and antimicrobial and antioxidant peptides. In Table 3 are several cheeses types where bioactive peptides were identified.

The ACE-inhibiting peptides constitute the majority of the bioactive peptides detected and investigated in cheese. ACE plays an important role in blood pressure regulation – converting the angiotensin I into a potent vasoconstrictor, the angiotensin II, and inactivating a vasodilator, the bradykinin, resulting in an increase in blood pressure. Several studies showed that many cheeses have a potential to lower blood pressure. For example, *in vitro* studies have reported high ACE-inhibitory activity ($> 70\%$ of inhibition) in water-soluble extract obtained from Italian cheeses, as Gorgonzola and Italico, and Spanish cheeses, as Idiazabal, Manchego, Roncal, Mahon, goat cheese, and Cabrales. It is known that the ACE-inhibitory peptides derived from dairy products are less potent than the drug commonly used to control high blood pressure in hypertensive individuals. However, the cheese bioactive peptides may constitute a natural dietary approach with the potential to control hypertension.

In some cheeses also, phosphopeptides have been identified (Table 3). Such peptides may interfere with mineral absorption, due to their ability to bind and solubilize the minerals, aiding the mineral absorption in the intestine. Moreover, it is well documented that phosphopeptides exert anticariogenic effect, inhibiting the caries lesion by promoting the recalcification of the dental enamel and by inhibiting the adhesion of plaque forming bacteria. Moreover, due to their ability to bind and solubilize the minerals, phosphopeptides have been associated with other beneficial effects, as prevention of osteoporosis, hypertension, and anemia.

The proteolytic phenomena throughout cheesemaking depend on numerous factors like the chemical and microbiological characteristics of feedstock milk, the specific steps along cheesemaking, and the environmental patterns prevailing during ripening. The pattern and extent of casein degradation during ripening influence strongly on the sensory properties of the final cheese, as well as its nutritional value; therefore, the great diversity in cheese manufacture with regard to the milk characteristics and cheesemaking conditions contributes to a great variety of cheeses, each one showing distinct and unique sensory and nutritional properties. Moreover, the profile of bioactive peptides in ripened cheeses is dependent on the equilibrium between their formation and degradation. So, the cheese bioactivity seems to depend not only on the manufacturing conditions but also on the ripening stage.

Carbohydrates

Most cheeses show residual levels of carbohydrates (Table 1). Lactose constitutes the main carbohydrate in milk, but during cheese manufacture, it is lost in whey. Moreover, the residual lactose that is retained in cheese curd is fermented to lactic acid during ripening process. A considerable percentage of the world population is lactose-intolerant. Due to the lack of enzyme lactase needed for hydrolysis of lactose into monosaccharides, the lactose absorption is compromised in lactose-intolerant individuals. In this population, the lactose consumption leads to the development of several symptoms, such as diarrhea, abdominal discomfort, and flatulence. However, the ripened cheeses are free of lactose, thus constituting a dairy product adequate to lactose-intolerant people.

Minerals

Cheese is a good dietary source of several minerals, such as calcium, phosphorus, and magnesium. The beneficial effect linked to cheese consumption has also been related to its mineral composition, mainly with its high levels of calcium, which show positive effects on various disorders, namely, controlling hypertension, osteoporosis, obesity, and dental caries.

High levels of sodium also are found in cheeses, as result of the cheese salting step. Salting constitutes the major source of sodium in cheese and, depending the cheese variety, may occur in different ways – salt can be added directly to the cheese curd (e.g., cheddar and cottage cheeses) and rubbed into the surface of the molded cheese (e.g., some blue-type cheeses), or the molded cheese is immersed in brine solution (e.g., Edam, Feta, and Gouda cheeses). Salting is an important step in the manufacture of the majority of cheeses, playing a relevant role in cheese preservation and development of its adequate sensory properties. Moreover, salt addition to cheese provides a source of sodium and chloride ions, which play an essential role in a number of life processes, such as in nutrient absorption and transport, in the regulation of blood pressure, and in the

Table 3 Bioactivity of peptides identified in several cheese types

Cheese type	*Bioactivity of peptides*	*Reference*
Cheddar	ACE-inhibitory activity Phosphopeptides	[1; 2]
Emmentaler	Immunostimulatory Antimicrobial Phosphopeptides ACE-inhibitory activity	[3; 4]
Gouda	ACE-inhibitory activity	[4]
Blue	ACE-inhibitory activity	[4]
Camembert	ACE-inhibitory activity	[4]
Edam	ACE-inhibitory activity	[4]
Havarti	ACE-inhibitory activity	[4]
Comté	Phosphopeptides	[5]
Herrgard	Phosphopeptides	[6]
Roquefort	ACE-inhibitory activity	[7]
Extra hard, hard, semihard, and soft cheeses of Swiss origin	ACE-inhibitory activity	[7; 8]
Gorgonzola	ACE-inhibitory activity	[9]
Mozzarella	ACE-inhibitory activity	[9]
Italico	ACE-inhibitory activity	[9]
Crescenza	ACE-inhibitory activity	[9]
Parmigiano-Reggiano	Phosphopeptides Precursor of opioid peptide	[10]
Grana Padano	Phosphopeptides	[11]
Manchego	ACE-inhibitory activity	[12]
Idiazabal	ACE-inhibitory activity	[12]
Rocal	ACE-inhibitory activity	[12]
Goats' cheese	ACE-inhibitory activity	[12]
Cabrales	ACE-inhibitory activity	[12]
Festivo	ACE-inhibitory activity	[13]
Cheese-like system of ovine milk	ACE-inhibitory activity Antioxidant activity	[14]

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transmission of nerve cell impulse. However, the high levels of salt in cheese have favored the negative health image of cheese, due to association of high salt intake with an increased risk of development of several chronic diseases, as hypertension, stroke, osteoporosis, kidney stones, or cardiovascular diseases.

The amount of salt is variable among cheese types, with values between 0.7 g per 100 g in Emmental cheese and 6 g per 100 g of NaCl in Domiati cheese (Table 4). Overall, cheese contributes relatively small amounts to sodium daily intake; for example, in France, where the cheese consumption is high, cheese contributes only to 9.2% of total sodium intake. Nevertheless, to satisfy the consumer concerns over high levels of sodium intake, various types of cheese have been developed with reduced sodium content. Different approaches for reduction of the sodium content in cheese have been explored, such as decrease of the NaCl amounts added; partial or total replacement of NaCl by other salts like KCl, $MgCl_2$, and $CaCl_2$; and addition of flavor enhancers. Overall, these approaches have led to positive results, producing cheeses with reduced sodium content well accepted by consumers. However, great reductions of salt addition lead to the development of cheeses with undesirable sensory properties. Moreover, it is reported that

Table 4 Salt content of selected cheeses (g 100 g^{-1})

Cheese type	*Salt*	*Reference*
Cabrales	2.56	[1]
Camembert	2.5	[2]
Cheddar	1.5	[2]
Edam	2.0	[2]
Emmentaler	0.7	[2]
Feta	3.0	[2]
Gouda	2.0	[2]
Gruyére	1.1	[2]
Idiazabal	2.77	[1]
Manchego	2.40	[1]
Pecorino	1.2	[3]
Roncal	2.26	[1]
Roquefort	3.5	[2]
São Jorge	4.9–5.1	[4]
Serpa	0.83–3.0	[5]
Serra da Estrela	3.1	[6]
Terrincho	0.94–2.9	[7]

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sometimes, the substitution of NaCl by other salts results in cheeses with unsuitable flavor.

On the other hand, cheese has a low content of iron and zinc. In order to enhance cheese nutritional value and its contribution to iron and zinc intake, cheese fortification with these minerals has been explored. Several cheese types have been successfully fortified with these minerals without relevant effects on their sensory properties; however, higher fortification levels lead to changes in sensory properties of cheese, which may limit the consumer acceptability by the product.

Vitamins

Vitamins are widely recognized for its importance in human health, contributing to multiple and different vital functions in the organism. Cheeses constitute an excellent source of liposoluble vitamins, as retinol, carotene, vitamin D, and tocopherols. Although the majority of water-soluble vitamins of milk are lost in the whey during cheesemaking, some water-soluble vitamins, such as vitamin B_{12}, riboflavin, niacin, and folate, are present in sufficient levels in cheese. Therefore, cheese consumption could play a significant role in vitamin supply, contributing to adequate vitamin intake.

Some vitamins are widely distributed in food and human deficiency in such vitamins is improbable. However, for other vitamins, very few natural sources are available, as the case of vitamin D, which may result in insufficient consumption. Fortification of foods already normally consumed is a good strategy to achieve an adequate intake of these nutrients. The fortification of cheeses with vitamins, like vitamins A and C and particularly vitamin D, has been explored. Overall, the results have shown that fortification of cheeses with vitamins can be successful by enhancing vitamin content in these cheeses without compromising their sensory properties.

Polyphenolic Compounds

In recent years, the polyphenolic compounds have received much attention due to their potential beneficial properties to human health. Dietary polyphenolic compounds exhibit a variety of biological activities, including protection against oxidative stress and several degenerative diseases. So, owing to the beneficial health effects associated with the consumption of polyphenols, foods enriched in these compounds, including cheeses, are demanded. The polyphenols in cheeses may result of several circumstances, including: transference from feed and from animal metabolism to the milk, arising of the amino acid catabolism or the enzymatic activity in product, as well as from direct incorporation of polyphenolic compounds to milk or during cheesemaking processing, and finally through contamination from the environment.

The polyphenols are secondary metabolites produced by plants and therefore widespread throughout the plant kingdom. Several studies showed that utilization of dietary sources rich in polyphenols in ruminant nutrition – such as forage, shrubs, and industrial by-products – besides being able to change the chemical composition of milk, including its fatty acid profile, can also lead to transference of various polyphenolic compounds of the feed to milk and dairy products. On the other hand, the addition of single phenolic compounds or extracts rich in polyphenolic compounds in unprocessed milk or during cheesemaking in order to manufacture cheese with increased levels of polyphenolic compounds also has been explored. Both approaches seem to be feasible to improve the nutritional value of cheeses. However, independent of their origin, the polyphenolic compounds have a strong impact on sensory characteristics of cheese and at high levels may lead to undesirable changes in flavor and color of cheeses.

See also: Cheese: Chemistry and Microbiology; Dairy Products: Dietary and Medical Importance.

Further Reading

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Cheese: Processing and Sensory Properties

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Introduction

Cheesemaking implies the elimination of whey (the watery portion of milk) after coagulation of milk, thus resulting in the concentration and preservation of the most important milk nutrients – protein, fat, and minerals. For many cheeses, namely, those undergoing the process of ripening, the optimization of coagulation and whey elimination (syneresis) are obtained by first adding a lactic acid bacteria (LAB) starter culture to milk. At this initial phase, the starter culture is expected to cause a slight and rapid acidification of milk through the production of lactic acid, with the consequent decrease in milk pH. Next, rennet (an acidic enzyme) is added to coagulate the milk, and this, with the contribution of the subsequent cheesemaking steps, leads to formation of the initial texture and the almost insipid flavor of the cheese. Ripening, a slow process that may last from 1 to 24 months depending on the cheese variety, is the final step that imparts the final and most significant changes of cheese flavor. Thus, the final sensorial features of a cheese are more or less a consequence of the combined actions of all steps involved in its processing. Consequently, understanding and controlling the effect of each of the processing steps during cheesemaking is crucial toward directing the process to deliver the final product in mind. Also, due to the key role the sensorial factors play in the cheese consumer's choice, attempts to understand and control the dynamics of the development of texture and flavor have been an important part of the activity in the field of food science.

Cheese Processing and Starter Cultures

The basic principles involved in cheese manufacture have remained the same for over 1000 years. Basically, milk must be first heat treated to kill the harmful bacteria that it may harbor. Starter cultures, usually LAB, are then added to the milk and their growth 'ripens' the milk and later helps to develop the desired flavors and aromas in the cheese. For raw milk cheeses, in case a starter culture is added, it can be of a pure or mixed undefined (whey from previous day) type. The starter culture produces lactic acid, a natural preservative that, besides acting on cheese taste, will cause the pH of milk to drop, thus creating optimal conditions for the activity of rennet that is usually added afterward. This will cause the coagulation of the main protein present in milk - casein - by lowering its solubility, leading to its precipitation and formation of a gel that will separate into curds and whey. LAB may later have a different number of metabolic activities, namely metabolizing citric acid, acting on the breakdown of the protein (in conjunction with the rennet and enzymes from milk), as well as on the breakdown of the diglycerides formed from the milk triglycerides, and in some cheeses, on the breakdown of hippuric acid to benzoic acid. The formed curds are then heated according to the type of cheese, and salt is added as a preservative, flavor enhancer, and syneresis promoter, reducing also the *aw* of the curd. Finally, the pressed curds are put to the ripening process so that the required biochemical reactions that lead to specific flavors will occur.

Fresh, soft, or hard cheeses are made essentially by the same process with slight differences in one or more of the steps described (see Figure 1). Thus, while fresh cheeses are ready for use as soon as the manufacturing process is complete, hard cheeses are allowed to mature in temperature- and humidity-controlled rooms from 1 to 24 or more months depending on the type of cheese.

From coagulation, cutting, and pressing, the pH of the curds will usually change markedly (see Figure 2). Later, during ripening, the pH will usually keep dropping in most cheese varieties as shown in Table 1, and the pH of the final cheese will usually vary between 4.8 and 5.4 as a result of the continuous acidification by the starter culture, which also contributes to the release of volatile components in the ripening curd.

Cheese Texture

Texture can be defined as the sensory manifestation of structure of the food and the manner in which this structure reacts to applied forces. Texture affects the immediate perception of a consumer to the quality of a product, through vision, kinesthesia, and hearing, making it a key flavor indicator. The final

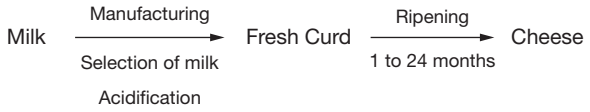

Figure 1 Essential steps involved in most cheese processing.

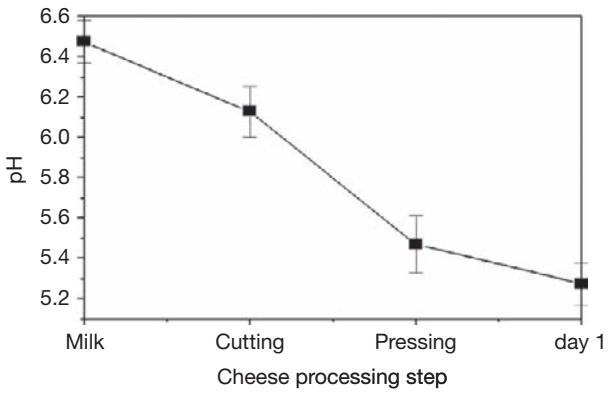

Figure 2 Evolution of pH (average + standard deviation) in an experimental cheese.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00136-7

Table 1 Typical pH versus time profiles for several cheese varieties (time is in minutes unless otherwise noted)

	Swiss-type		*Gouda*		*Cheddar*		*Feta*		*Cottage*	
Operations	*Time*	*pH*	*Time*	*pH*	*Time*	*pH*	*Time*	*pH*	*Time*	*pH*
Add starter	0	6.60	0	6.60	0	6.60	0	6.60	0	6.60
Add rennet	15	6.60	35	6.55	30	6.55	75	6.50	60	6.50
Cut	45	6.55	70	6.50	75	6.50	115	6.4	300	4.80
Drain or dip into forms	150	6.35	100	6.45	195	6.3	130	NA	360	5.0
Milling	NA	NA	NA	NA	315	5.45	NA	NA	NA	NA
Pressing	165	6.35	130		390	5.40	NA	NA	NA	NA
Demolding	16 h	5.30	8 h	5.40	10 h	5.20	24 h	4.6	NA	NA
Minimum pH	1 week	5.20	1 week	5.20	1 week	5.10	1 week	4.4	NA	NA
Retail	6 months	5.6	6 months	5.6	4 months	5.3	6 weeks	4.4	2–14 days	5.2

texture of a cheese is developed throughout ripening, a slow process involving the concerted series of biochemical, microbiological, and chemical reactions, which are predetermined by the manufacturing processes. Generally, a cheese may be classified as having a typical soft, semisoft (medium), or hard texture, and the said classification is related to the cheese water and fat contents, to which many factors will contribute.

As previously stated, processing conditions – temperature of coagulation, cutting and cooking the curds, pressing, salting, and ripening – will affect directly the cheese texture or hardness. The pH at drain determines the retention of rennet (which participates in hydrolysis of α_{s1}-casein) and the amount of plasmin (a native milk proteinase that is responsible for much of casein breakdown in the curd), while the salt-to-moisture ratio affects the amount of intact casein. Finally, the changes in body and texture that transform the rubbery, elastic mass of curd to a cheese with a firm close texture are essentially the results of the three primary biochemical processes – glycolysis, lipolysis, and proteolysis – occurring mainly during ripening. With aging, the cheese texture will also change as a result of the evaporation of available water on the surface, leading to a 'drier,' harder, and less deformable cheese. Finally, the storage temperature (or ripening temperature) impacts the rate of proteolysis, namely, proteolysis of α_{s1}-casein hydrolysis.

In general, cheese texture will change markedly in the first 1–2 weeks of ripening as the hydrolysis of a small fraction of α_{s1}-casein by the rennet to the peptide α_{s1}-I results in a general weakening of the casein network. The following and usually relatively slow (occurs over a period of months) change in texture is often associated with the rate of proteolysis, which in turn is controlled largely by the proportion of residual rennet, plasmin, microflora peptidases, salt-to-moisture ratio, and storage temperature.

Cheese composition also affects the final texture, as both dissolved calcium in the cheese serum and calcium bound to the protein network have been shown to affect the rate of proteolysis, and cheeses having a higher fat content are less firm and more elastic.

Sodium chloride affects both the matrix and the serum phases of the cheese, which, in turn, affects the overall texture. It is known that brine-immersed cheeses show dramatic changes in texture during the early stages of aging. Many soft cheeses are obtained by a combination of a low degree of syneresis and the use of specific types of vegetable curds (such as cardosin) or starter cultures (viz., specific fungi of the group *Penicillium*), which are highly proteolytic and thus will contribute to loosening the matrix formed by casein to peptides and amino acids, involving high fat content and water.

Table 2 Definitions of textural properties attributed to cheeses and other dairy products

Textural properties	*Definition*
Adhesiveness	Work necessary to overcome the forces between dissimilar materials
Chewiness	Energy required for masticating a solid food material until it is ready for swallowing
Cohesiveness	The strength of the internal bonds making up the body of the product or degree to which the sample deforms before rupture
Gumminess	Energy required to disintegrate a semisolid food material to a state ready for swallowing
Hardness	Force necessary to attain a given deformation
Resilience	How well a product fights to regain its original position
Springiness	Degree or rate at which the sample returns to its original shape/size after partial compression between tongue and palate

In conclusion, the texture of a cheese is a reflection of its microstructure, formed essentially by the casein matrix entrapping fat globules and bounded to water, forming a structure that is affected by the biochemical activities occurring during ripening, which change the amount, type, and physicochemical relation of the said components.

A cheese may have several important textural properties as listed in Table 2.

Flavor in Cheese

While the perception of a cheese texture also contributes to its general flavor, texture may be seen as a simpler contact by means of the sense of touch, while flavor usually requires a more profound use of our senses of vision, taste, and smell with the food product.

As previously stated, the initial fresh curds from any cheese type taste more or less the same. It is only during ripening that the biochemical processes occurring by the action of LAB and

milk native enzymes present in the curd will cause the main contribution to cheese flavor development. Those primary changes are followed and overlapped by a host of secondary catabolic changes, including deamination, decarboxylation, and desulfurylation of amino acids and β-oxidation and esterification of fatty acids. While the primary reactions are mainly responsible for the basic textural changes of the curd and for the basic flavor of cheese, the secondary transformations are responsible for the finer aspects of cheese flavor and also modify the texture. A number of metabolites, important contributors to a cheese's flavor, are formed from the three main milk components during ripening, as shown in Tables 3 and 4.

It can thus be stated that a cheese flavor is composed of a complex large number of dissolved volatiles of low-molecular-weight compounds and ions, which act synergistically in the perception of flavor, and their sensory contribution may not be directly dependent on their comparative concentration. If in excess, any of these metabolic products may also result in an off-flavor appearance in cheeses.

Many of the enzymes involved in the said processes may be dependent on cooperation between strains, making the metabolic processes by LAB important in flavor formation.

Biochemical Reactions and Cheese Flavor Development

Cheese flavor is a direct result of three biochemical reactions generally known as glycolysis, lipolysis, and proteolysis.

Table 3 Flavor compounds formed from main milk components during ripening

Casein	*Milk fat*	*Lactose and citrate*
Peptides	Fatty acids	Lactate
Amino acids	Keto acids	Pyruvate
Acetic acid	Methyl ketones	CO_2
Ammonia	Lactones	Diacetyl
Pyruvate		Acetoin
Aldehydes		2,3-Butanediol
Alcohols		Acetaldehyde
Carboxylic acid		Acetic acid
Sulfur compounds		Ethanol

Glycolysis

The fermentation of lactose is one the first LAB activities that contribute to flavor in cheese. The general simplified pathway of lactose metabolism (glycolysis) by LAB (starter and nonstarter LAB (NSLAB)) in cheese is shown in Figure 3.

Lactose can be metabolized to lactate and then to acetate and diacetyl by strains of *Lactococcus lactis diacetylactis* and *Leuconostoc* spp. and CO_2, which is responsible for the characteristic eyes of many cheeses, and to citrate that contribute to flavor.

In the case of many traditional cheeses, a mixed culture of unknown composition is used in the so-called *back slop* method.

Lipolysis

Lipolysis in cheese is important as a process leading to the formation of many flavor metabolites due to the activity of

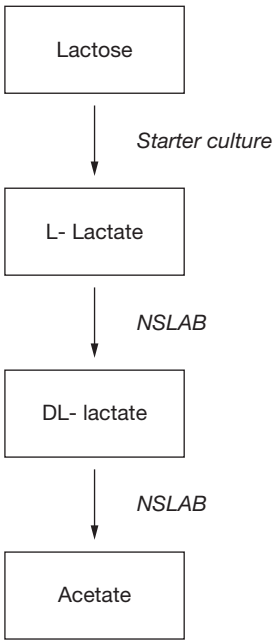

Figure 3 Simplified pathway of lactose metabolism in LAB.

Table 4 Some key flavor components in known variety of cheeses

	Cheese variety			
Metabolism of	*Gouda*	*Cheddar*	*Camembert*	*Swiss-type*
Peptides/amino acids	3-Methylbutanal Methanethiol Dimethyl sulfide Methylpropanol	3-Methylbutanal Isovaleric acid Methanethiol	3-Methylbutyrate Methanethiol Benzaldehyde	Methional 3-Methylbutanal Skatole
Sugar	Diacetyl	Propanoic acid Diacetyl	2,3-Butanedione	Propanoic acid Diacetyl
Fat	Butyric acid Butanone Hexanal	Butyric acid Acetic acid Butanone	Butyric acid 1-Octen-3-ol 2-Undecalactone	Ethyl butyrate Ethyl hexanoate Ethyl-3-methylbutanoate

Source: Smit G, Smit BA, and Engels WJM (2005). Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheeses products. *FEMS Microbiology Reviews* 29: 591–610.

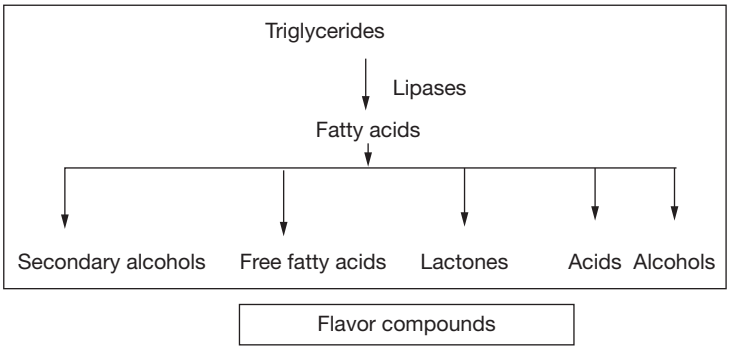

Figure 4 A simplified pathway of milk triglyceride and fatty acid flavor compound formation during cheese ripening.

such enzymes as lipases and esterases. The hydrolysis of triglycerides, which constitutes more than 98% of cheese fat, is the main biochemical transformation of fat occurring during ripening and causes the production of short-chain free fatty acids (FFAs) that contribute to the aroma of cheese, depending on the amount of the aqueous phase and the pH of a cheese. LAB play an important role in this process that leads to the formation of flavor. The presence of specific FFA can give the perception of flavor such as rancid, sharp, goaty, soapy, and coconut-like. FFA can be further hydrolyzed to methyl ketones, which are responsible for the characteristic aroma of blue cheeses. Lactones have also been identified as possessing strong aroma that may be important in overall cheese flavor. A simplified pathway of milk fat metabolism is shown in Figure 4.

Proteolysis

Proteolysis is probably the most important biochemical event in cheese ripening accounting for the development of a number of organoleptic features, encompassing both flavor and texture.

Thus, early-stage hydrolysis (primary proteolysis), that leads to the formation of large specific peptides, and the later-stage proteolysis (secondary proteolysis) will occur when the said peptides are digested into smaller ones and even free amino acids by enzymes from starter or nonstarter microorganisms, leading to changes in texture and taste of the cheese. Generally, the rate of breakdown of α_{s1}-casein is greater at lower storage pH than the rate of breakdown of β-casein, thus, changes in pH during storage affect the rate of proteolysis and consequently texture.

Proteolysis in cheese is defined as changes in β-, γ-, and s-casein peptides and other minor proteins that lead to the formation of large water-insoluble peptides and smaller water-soluble peptides from the action of rennet (chymosin), milk native proteases, and peptidase enzymes from starter and NSLAB. The hydrolysis of casein to high-molecular-weight peptides is thought to be primarily the result of chymosin and plasmin, while the subsequent hydrolysis of high-molecular-weight peptides is primarily the result of proteolytic enzymes from LAB. In general, many proteolytic compounds contribute to the typical aroma of a cheese. They are a result of different reactions including deamination, transamination, decarboxylation, and cleavage of the amino acid side chain by the microflora. Products such as keto acids, aldehydes or carboxylic acids, ketones, lactones, esters, alcohols, aldehydes, pyrazines, sulfurous compounds, carbonyl compounds, FFAs, free amino acids, and salts have been detected and reported to contribute to cheese aroma. In general, different nitrogen catabolisms will result from different starter cultures, and such products as 3-methylbutanal, an important volatile compound formed during the ripening of Parmesan cheese, or methyl alcohols and methyl aldehydes derived from the branched-chain amino acids, leucine, isoleucine, and valine, may be formed. A general overview of proteolysis and the pathway of formation of methylbutanal are shown in Figures 5 and 6.

As consequence of the dynamic of activity of LAB throughout ripening, cheese taste and texture will obviously change with aging, as more biochemical reactions occur and because moisture and salt content and pH also change throughout ripening.

Cheese Microflora and Flavor Development

Fermentation with LAB is a cheap and effective food preservation method that can be applied even in more rural/remote places and leads to improvement in texture, flavor, and nutritional value of many food products. LAB have a long and safe history of application and consumption, namely, in cheese processing, thus, being generally recognized as safe.

Cheesemaking is based on the application of LAB in the form of defined or undefined starter cultures that are expected to cause a rapid acidification of milk through the production of lactic acid, with the consequent decrease in pH, thus, affecting a number of aspects of the cheese manufacturing process and ultimately cheese composition and quality.

Traditionally, mixed or undefined strain starter cultures composed of a number of strains of *Lactococcus* spp., *Lactobacillus*, and even *Enterococcus* spp. were used.

The earliest productions of cheeses were based on spontaneous fermentation, resulting from the development of the microflora naturally present in the raw milk and its environment. The quality of the end product was a reflex of the microbial load and spectrum of the raw material. Spontaneous fermentation was later optimized through backslopping, that is, inoculation of the raw material with a small quantity of whey from a previously performed successful

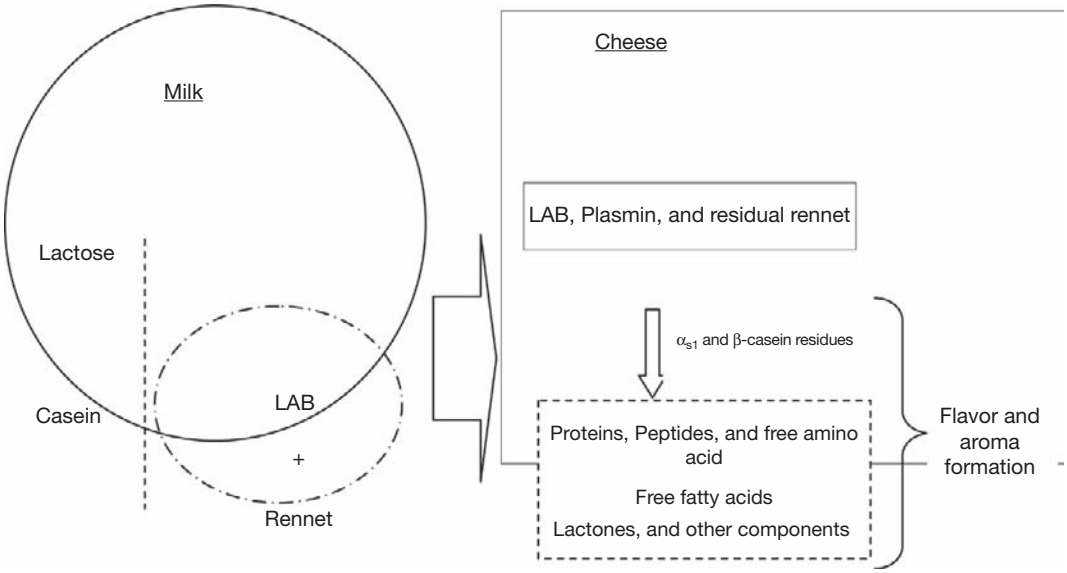

Figure 5 General view of proteolysis during cheese processing.

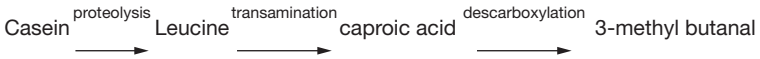

Figure 6 3-Methylbutanal formation by LAB during ripening of cheese.

fermentation, and the resulting product characteristics depended on the best-adapted strains dominance. Today, backslopping is still used to produce many artisanal raw milk cheeses, namely, those bearing the PDO (Protected Designation of Origin) status, which are considered to be an important source of LAB genetic diversity. The starter culture applied in this, so-called natural fermentation, is usually a poorly known microflora mix that, although having a predominance of LAB, may also contain non-LAB microorganisms, and its microbial diversity and load are usually variable over time.

Moreover, traditional cheeses also obtain their flavor intensity from the NSLAB, which are not part of the normal starter flora but develop in the product, particularly during maturation, as a secondary flora.

In the high-output cheese industry, defined cultures are rather used, optimizing the process, namely, the lactic acid formation. Bacteria existing in the processing environment – the so-called nonstarter lactic acid bacteria – often contaminate the cheeses, thus, having also a role in flavor development. For specific varieties of cheese, such as 'blue cheeses,' a starter culture made up of specific molds such as the *Penicillium* spp. is also used. Due to a lower diversity of the microflora in industrial cheeses, they are commonly recognized as developing weaker flavors.

The starter culture in cheesemaking is thus very important for two obvious reasons of producing lactic acid via metabolism of lactose: lowering the pH of the curd (recall that at lower pH values, undesirable bacteria have more difficulties to grow and therefore the pH history during processing of the cheese is a good indicator of the actual product safety) and producing the distinctive flavors and textures via metabolism of fat, lactose, and protein.

Undesirable flavors, namely, bitterness, may develop in cheeses, often as consequence of combined excessive activity of rennet and mesophilic starter cultures. Bitterness is considered a result of excessive accumulation of bitter peptides (usually of 2–23-amino-acid residues), predominantly containing hydrophobic amino acids. Excessive use of certain types of rennet and starter cultures with high proteolytic activity can lead to formation of bitterness and it has been shown that this defect often occurs in cheeses made without NaCl and in low-fat cheeses. Also, it seems that fast acid producers, heat-tolerant strains grown under uncontrolled pH conditions, are more prone to producing high levels of bitter components. Off-flavors such as rancidity may develop due to excessive or unbalanced lipolysis caused by lipases/esterases from starter or NSLAB, from enzymes produced by psychrotrophic bacteria, or from indigenous milk lipoprotein lipase. Finally, late gas blowing and other off-flavors in certain hard cheeses result from the metabolism of lactate (or glucose) by *Clostridium* sp. to butyric acid and H_2.

Texture and Flavor Analysis

While texture (mouthfeel) of a food product may be subjective, it plays a key role in consumer acceptance and market value of a food product, namely, cheeses. The sensory measurements of a cheese may be taken by consumers (say, in shopping malls, supermarkets, or any other area having a large number of consumers) or by a trained panel. The purpose of a consumer taste test is usually for a general evaluation (acceptance/rejection) of a dairy product, while the trained panels are

trained to detect a specific food characteristic (bitterness, sandiness, and crumbliness) with a high degree of reproducibility.

However, attempts have been made to standardize measurements of texture and flavor. In fact, food texture characterization is rooted on human sensory evaluation; however, many instrumental methods have been developed to analyze some physical and chemical properties in finished products, allowing for corrections and control of the manufacturing and storage processes in order to consistently deliver the expected final product.

The evolution of a cheese microstructure can be followed by microscopy techniques, generally known by such acronyms as TEM, SEM, and CLSM, imaging techniques, and infrared spectroscopy, while mechanical properties such as firmness and hardness (texture profile analysis (TPA)) may be evaluated by mechanical methods using a testing machine such as the Instron model (Figure 7), a penetrometer, or a rheometer. Figures 8 and 9 are results of a study of texture characterization of samples of São Jorge cheeses at different ripening periods.

Due to good separation efficiency and versatility, gas chromatography methods have found increasing acceptance and application in food science and in technology for separation and identification of a large variety of compounds. These techniques are thus helping to elucidate and understand the mechanisms and chemistry of flavor formation in cheeses.

A cheese map developed for cheddar cheeses is shown in Figure 10. Different cheddar cheeses from around the world can be placed in the map due to their specific flavors.

In conclusion, cheese flavor and texture are complex and slow processes resulting from the extent of the biochemical pathways of glycolysis, lipolysis, and proteolysis, which are

Figure 7 Texture testing machine Instron model 4501, series H3279.

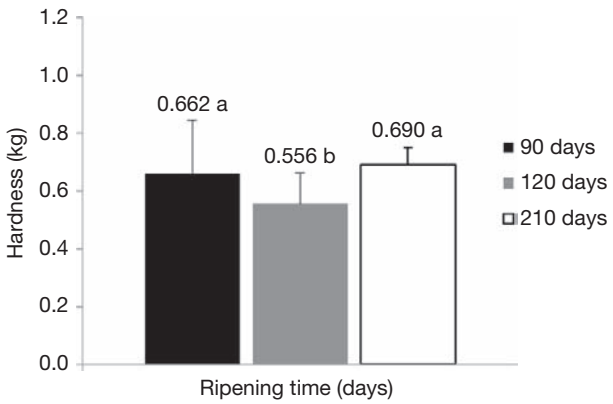

Figure 8 Hardness of São Jorge cheese (average ± standard deviation, $n = 154$) by 90, 120, and 210 days of ripening. Different superscript letters represent mean values that are statistically different for $p < 0.05$.

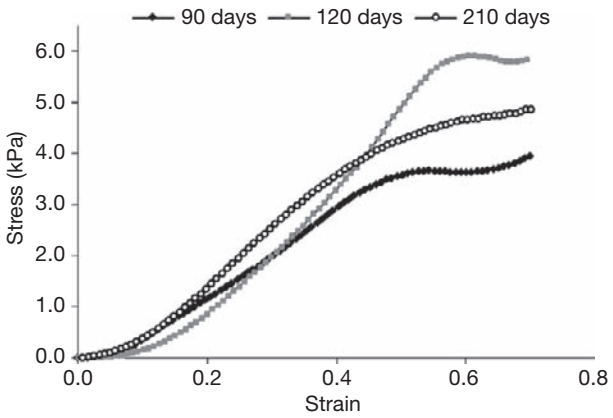

Figure 9 Stress–strain curves of São Jorge cheese with 90, 120, and 210 days of ripening.

Figure 10 Cheddar cheese flavor map.

affected by the cheesemaking specificities and the microflora (starter and NSLAB) present in the curd and by the native enzymes of milk and rennet. Studies and attempts to control cheese flavor are important activities in food science due to the key role flavor and texture play in the consumer's choice of a cheese.

See also: Cheese: Chemistry and Microbiology; Cheese: Composition and Health Effects; Cheese: Types of Cheese – Medium; Cheese: Types of Cheeses – Hard; Cheese: Types of Cheeses – Soft; Fermented Foods: Fermented Milks; Lactic Acid Bacteria.

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Cheese: Types of Cheese – Medium

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Introduction

Semihard cheeses are probably the largest group of known cheeses. Production is usually by rennet coagulation, after slight acidification of milk with lactic acid from added or adventitious starter culture. The amount of moisture removed from the curd depends on the water temperature/time used in the cooking and wash of the curd. Higher temperatures during cooking or washing cause the curd to contract and expel more moisture. Typically, these cheeses mature for 15 days to 3 months. Roquefort, mozzarella, Stilton, manchego, Gorgonzola, provolone, Gouda, Edam, and São Jorge cheese are part of the long list of medium cheeses. The texture and aroma of medium cheeses change during ripening due to solubilization of calcium phosphate, proteolysis, lipolysis, and loss of moisture, which are all dependent on the manufacturing technology such as the starter used, temperatures used for cooking the curds, degree of syneresis, and salting, as these will determine the pH, moisture content, degree of proteolysis, and calcium and fat content of the cheese.

PDO Cheeses

Many traditional PDO (protected designation of origin) cheeses are medium cheese types, and in Europe, they are perceived as being an important part of the cultural heritage in places where they are made.

Consumers in Europe have defined traditional food product as 'a product frequently consumed or associated with specific celebrations and/or seasons, normally transmitted from one generation to another, made accurately in a specific way according to the gastronomic heritage, with little or no processing/manipulation, distinguished and known because of its sensorial properties and associated with a certain local area, region or country.' The PDO status (Figure 1) or 'appéllation d'origine contrôlée' is a designation applied to foodstuffs (cheeses) that are produced, processed, and prepared in a given geographic area using a recognized and unique technology, determined by human and natural factors dependent on the area where it is produced, such as the raw milk used in the cheesemaking. This is usually seen as a way of protecting and increasing the market for foodstuffs with typical characteristics. About 8% of the cheese produced in the EU (i.e., 3–4% of the world's production) is protected by a PDO registration, and these includes names such as Grana Padano, Comté, queso manchego, Serra da Estrela, São Jorge, Feta, and many more.

Because these cheeses are in general produced in small factories and represent an important source of income for local population, their protection may fulfill an important socioeconomic role in creating local jobs and in maintaining the agricultural population in areas, which could otherwise be abandoned. In fact, the World Intellectual Property Organization indicates that the PDO system was developed because of the perceived necessity of providing solutions against fraudulent commercial practices, related to the origin of agricultural products.

PDO cheeses are often made from raw milk; thus, as a way of establishing the required improvements toward increasing their safety, many scientific projects have been set in Europe toward characterization and improvement in processing of traditional cheeses.

Semi-hard cheeses also encompass the so-called "industrial" cheeses, which, in contrast to PDO cheeses, are produced in high output factory plants. The increasing scientific knowledge, started with discoveries by Pasteur and the technological development in the dairy field, allowed cheeses (even if initially they were of the artisanal type) to be manufactured in a more controlled and directed processing in large plants. These cheeses may often be considered as having a weaker taste, as compared to PDO ones, however, they are the most available, and thus, most consumed especially for large urban areas around the world. In fact the high-output dairy industry is one of the largest food industries in the world.

For medium cheeses in general, the starter culture – LAB species intentionally added to milk – plays a crucial and complex role in the development of final aroma and taste. Their central function is the fermentation of the milk sugar (lactose) to lactic acid and the degradation of milk protein and fat. During processing, after the protein (casein) has been coagulated, the resulting decrease in pH due to the lactic acid released by LAB will result in moisture expulsion (syneresis) and curd shrinkage, influencing also the final cheese texture. The starter culture is an active contributor to the shelf life and safety of cheese, gives a sharp, fresh flavor to the curd, and is essential in typical taste and aroma development throughout ripening.

The changes in body and texture that transform the rubbery, elastic mass of curd to a cheese with a firm close texture are the results of protein and fat degradation. The release of volatile components from the curd gives the aroma to cheese and associated flavors, and the pH of the final cheese will usually vary between 4.8 and 5.4 (Figure 2) as a result of the continuous acidification by the starter culture.

Mozzarella

Mozzarella (Figure 3) is one the most famous cheeses due to its association to pizza food. Mozzarella is a low-moisture medium cheese with an unusually broad compositional range in terms of moisture (45–52%) and fat (about 30–50% fat in dry matter), and it is a rindless cheese that can be eaten fresh. The essential quality attributes of mozzarella (pizza cheeses) are its functional properties such as flowability, stretchability, browning, free oil formation, and shredability. Mozzarella is a rennet-coagulated

cheese, and thermophilic starter cultures are commonly used in its processing allowing for a fast rate of acidification that, in combination with a short time processing, results in a less syneresis and thus higher moisture in the final cheese. In the final stage of processing, the curds are stretched while heating with hot water. Its processing requires a unique step, at the later phase of its processing, at which the curds are stretched at a high temperature. While traditionally it was made from buffalo milk coagulated with a starter derived from the whey of a previously successful batch (backslopping), the world's high demand requires that most of the mozzarella cheeses available now are made from cow's milk.

Figure 1 Labeling seal for a PDO product.

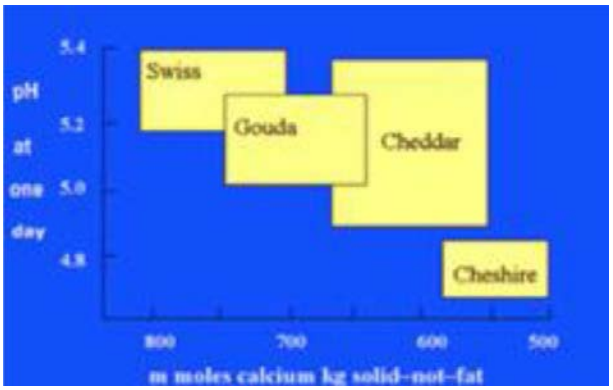


Figure 2 Average pH variation in different types of cheeses. Reproduced from Lawrence, R. C., Gilles, J. and Creamer, L. K. (1983). The relationship between cheese texture and flavour. *New Zealand Journal of Dairy Science and Technology 18*, 175.

Roquefort

Roquefort (Figure 4) is a popular French cheese, reported to be called in France the 'cheese of kings and popes.' This cheese is protected by AOC (PDO) guidelines. Roquefort cheese is moist and breaks into little pieces easily. Genuine Roquefort is made from sheep's milk, and after aging for 3–5 months, the cheese is creamy with a sharp, tangy, salty flavor. Roquefort belongs to the group of the so-called blue cheeses due to the blue-colored veins it develops from the growth of *Penicillium roqueforti*, which is added to the curd or introduced through holes poked in the rind.

Gouda

Gouda (Figure 5) is a Dutch cheese named after the city of Gouda in the Netherlands and one of the most popular cheeses in the world. It is a semihard cheese with a unique flavor and smooth texture. Gouda is typically made from pasteurized cow's milk although some artisan may use raw milk, sheep's or goat's milk, to produce cheeses that will go for longer time, and Gouda cheeses of different age are commonly sold. To enhance the flavor of the cheese, herbs, seasonings, and nuts may be blended, and at the final stage, it may have a fat content of c.31 g/100 g of cheese.

Manchego

The manchego (Figure 6) is produced in La Mancha region of Spain, home to Don Quixote. It may be made from unpasteurized sheep's milk under the PDO guidelines or in the industrial version from pasteurized milk.

The rind is inedible with a distinctive, traditional herringbone basket weave pattern, pressed on it. It may be sold as semicurado (young manchego aged around 3 months), curado (manchego cheese aged for 6 months), or viejo (manchego cheese aged for a year becomes crumbly in texture while the interior of the cheese acquires a butterscotch color, having a fat content of c.57%).

São Jorge

São Jorge (Figures 7 and 8) is a PDO Portuguese traditional cheese made from cow's raw milk on the island of the same name. For its processing, raw milk from grass-fed cows is heated to 31 °C followed by the addition of a small amount of whey natural starter culture from a previous processing

Figure 3 (Left) Curds of mozzarella being stretched and (right) blocks of mozzarella cheeses ready for consumption.

Figure 4 A slice of Roquefort cheese showing the typical paste with spots or veins of the mold *Penicillium* sp.

Figure 5 Wheels of stacked Gouda cheeses ripening on wood shelves.

batch. After coagulation, the curds are cut, washed, salted, molded (10–15 kg size), and ripened under controlled temperature and humidity for a minimum of 3–7 months. After this period, members of the Brotherhood of St. George Cheese perform the required tasting tests, before the cheese is allowed to formally enter the market bearing the PDO seal.

Patterns of Cheese Consumption

The consumption of cheese has in general improved significantly over the years across the world, making the cheese industry a lucrative business. Cheese production and markets have thus emerged as important elements of the dairy industry over the past three decades. Industrially or traditionally (deli)

Figure 6 Manchego cheeses ripening on wood shelves. Notice the patterns of herringbone basket weave pattern on the rind.

produced, and mostly prepared from milk of cow, sheep, or goat, cheese continues to be a popular addition to everyday diet especially in the Western world.

Factors Affecting the Growth of Cheese Consumption

In general, the increasing of population and income, together with the growing popularity of dairy products, particularly among developing country consumers is a key factor behind strong demand in the medium term. Demand continues to be encouraged by the growing influence of retail chains and multinational companies in these countries, which is facilitating improved consumer access to dairy products. The demand for dairy products is expected to remain particularly strong not only in important developing dairy markets such as North Africa, the Middle East, and East Asia but also in more mature markets such as those in the European Union, the United States, and Russia.

Dairy product consumption in developed countries may increase only modestly, with the exception of cheese, for which growth may be 16% by 2020 as compared to the 2008–10 base period. New packaging technology, more convenience, and possible substitutability with meats help boost cheese consumption. In developing regions, the consumption of all products increases vigorously at around 30% from the base period, driven by increasing population and income levels.

The rate of growth and per capita consumption of milk and milk products remain significantly different among regions (Figure 9). Cheese from cow's milk represents 95–96% of the total cheese production, and the European countries registered the main production increase, with a similar tendency observed in the United States in the last decades.

LDC countries consume less than 50 kg per person per year on average, compared with 100 kg per person for developing countries, while the developed regions of North America and Europe consume well in excess of 200 kg per person (in milk equivalent). Such a per capita consumption disparity represents an investment potential and future opportunities for both the domestic and global dairy sectors.

Figure 7 São Jorge cheeses at an early ripening phase (left) and a wheel showing a casein seal, which is affixed in all cheese wheels.

Figure 8 A wheel of São Jorge cheeses at market (Toronto, Canada) and 4- and 3-month, respectively, cheeses at the final phase before going to market.

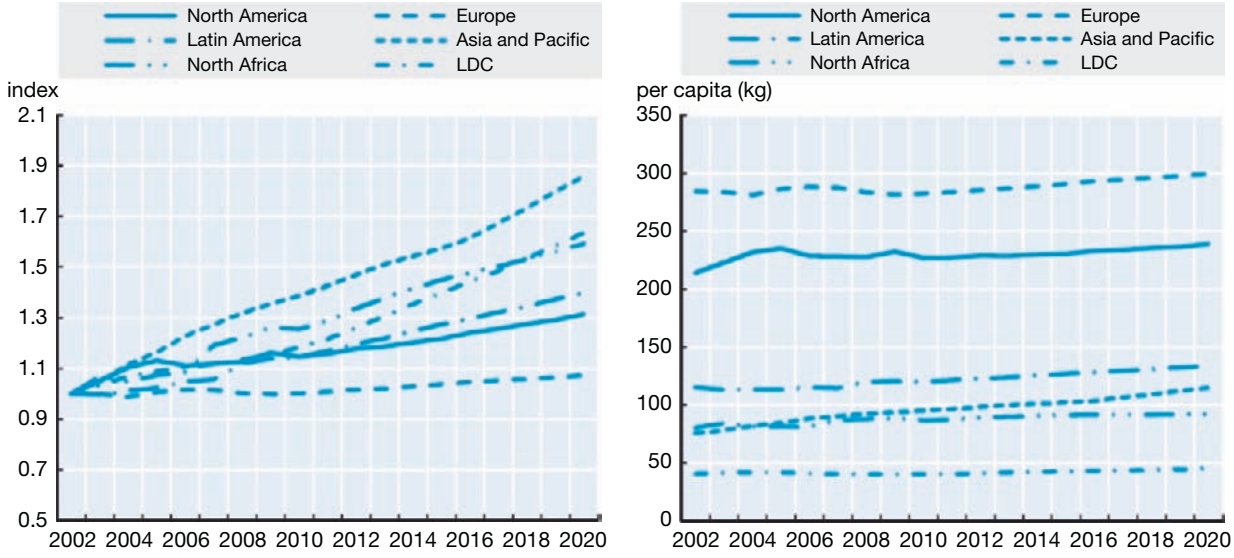
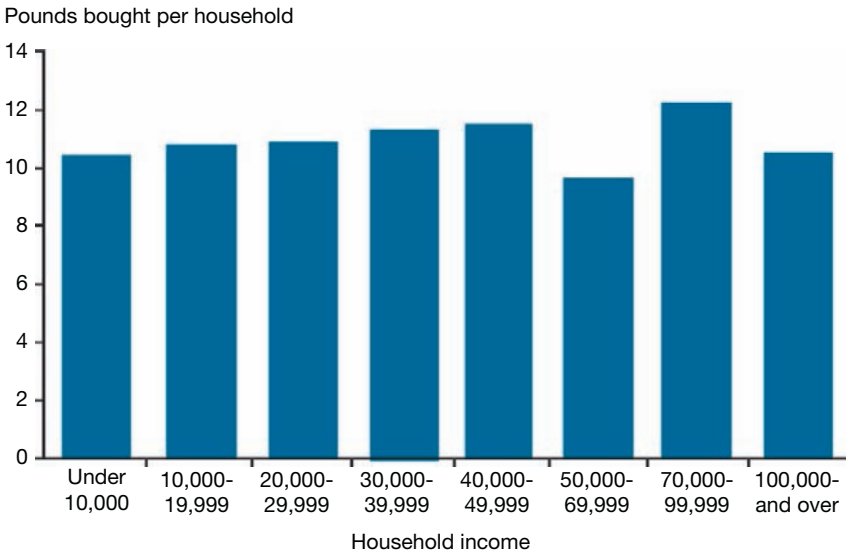
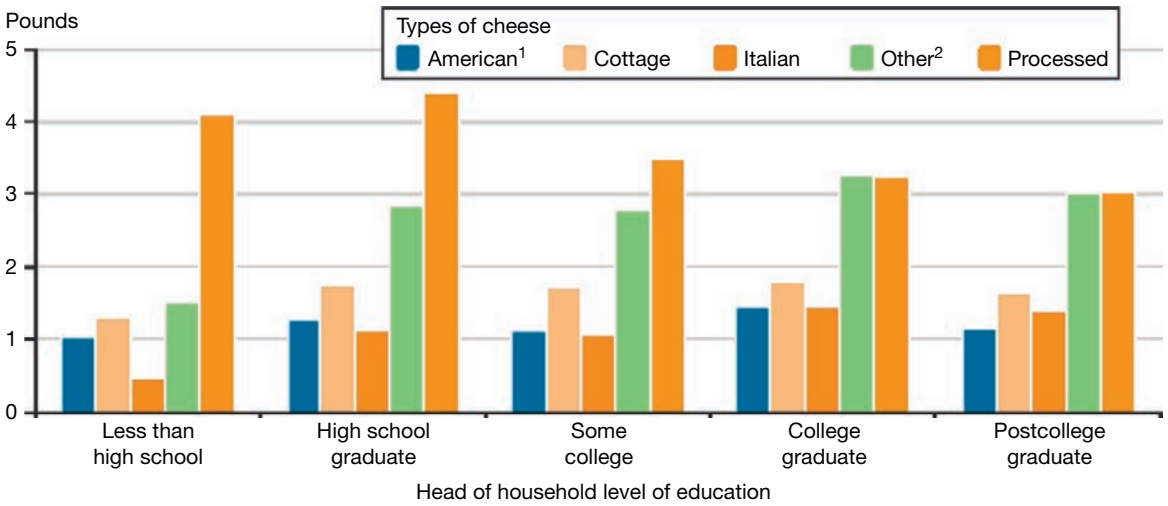


Figure 9 World dairy consumption levels and growth projections (source OECD-FAO Agricultural Outlook 2011–20). Left panel: Index of milk and dairy product consumption growth (in milk equivalent, 2002 = 1). Right panel: Levels of milk and dairy products per capita consumption growth (in milk equivalent). LDC, least developed countries.



All cheese types = American, Italian, processed, cottage, and other as defined by Nielsen. Other cheeses, as defined by Nielsen Homescan, are cheeses that do not fall into the American, Italian, cottage, or processed categories.

Figure 10 Per capita cheese purchases of all cheese types, by household income in the United States in 2005. Reproduced from Davis, C. G., Blayney, D. P., Dong, D., Stefanova, S. and Johnson, A. (2010). Long-term growth in U.S. Cheese Consumption may slow. A report from the economic research service, www.ers.usda.gov, accessed on 15/05/2014.



[1]American cheeses are Cheddar, Colby, Monterey, and Jack.

[2]Other cheeses, as defined by Nielsen Homescan, are cheeses that do not fall into the American, Italian, cottage, or processed categories.

Figure 11 Per capita cheese purchases by female heads of households. Reproduced from Davis, C. G., Blayney, D. P., Dong, D., Stefanova, S. and Johnson, A. (2010). Long-term growth in U.S. Cheese Consumption may slow. A report from the economic research service. www.ers.usda.gov, accessed on 15/05/2014.

In fact, cheese consumption in developed economies will be fraught by challenges, such as a matured market profile, limited growth in population, and most importantly the fast aging population, which account for lesser per capita consumption than the younger generation. Therefore, increase in cheese consumption within these markets is likely to be marginal and mainly associated with changes in form and type of dairy products consumed.

In the United States, reports on consumption using selected demographic and economic factors indicate that income, age, racial/ethnic factors, location, gender, and education influence cheese consumption in different, but significant, ways (see Figures 10 and 11).

Food preferences and consumer attitudes are progressively gaining importance as a significant determinant of the demand for milk and dairy products, particularly in high-

income countries as consumers become more affluent and more educated.

Health and nutritional issues have become important factors in determining the overall demand and the compositional demand for milk and dairy products. Demand is progressively becoming orientated toward more natural products with a reduction in the demand for products that are perceived to be 'unhealthy' (e.g., fats). Research shows that in the United Kingdom, for example, health consciousness increases with income and age and women tend to be more health conscious than men.

Food safety has emerged as an important global issue with international trade and public health implications. In recent years, food safety incidents have seriously affected consumer attitudes toward food, which in turn has led to significant changes in food consumption and purchasing patterns. For example, following the EU BSE outbreak in 2000, the consumption of cheese as a protein substitute to beef and veal increased in many member states.

Quality (including factors such as taste, freshness, branding, and packaging) is an important factor driving purchasing decisions for milk and dairy products. The drive toward quality products has tended to result in a shift in demand from commodity-type products to value-added products.

Production ethics are receiving more attention from consumers when making their purchasing decisions, particularly in higher-income countries. Concern about the environment and animal welfare has grown considerably, resulting in an increasing demand for milk and dairy products that are perceived by consumers to be more 'environmentally' and 'animal-friendly.' These include organic, welfare-friendly, and locally produced milk and dairy products. Research has found that gender, age, educational level, and socioeconomic status are important factors, and young, educated women of a higher socioeconomic status tend to be more concerned about production ethics.

Strategies and the Future of the Cheese Industry

In the present context where purchasing decisions are being increasingly guided by price (Figure 12), cheaper yet healthy and wholesome foods are surfacing back into the spotlight, and with *small is beautiful* attitude, consumers are shifting their preferences from imported cheese brands to locally produced cheeses.

In general, consumption levels and cheese markets in developed countries will tend to show nearing saturation; thus, the focus of the global cheese industry now shifts toward emerging markets. Such markets (Asia, Latin America, Middle East, and Africa) are projected to display superior growth rates in the near future. Large population and rising incomes in these nations will prove to be the major driving factors for exceptional growth in dairy, namely, cheese consumption. Growth in this market will primarily be driven by improving income levels, strengthening consumer sentiment/confidence, waxing propensity to spend, and increase in the adoption of Western-styled food.

The EU cheese market is in general changing, forcing cheese suppliers to rethink their business models and explore new opportunities for growth. The main drivers behind these changes are falling growth rates in the EU, more pressure on margins because of higher revenues in powder and butter products, and volatility in commodity cheese markets.

The actual economic recession has certainly slowed cheese consumption pattern especially in the developed countries; however, the future outlook for global cheese market may still be bright. In the postrecession period, innovation and product diversification will be prominent market strategies for manufacturers and suppliers, satisfying new consumer demand with a diversity of cheese such as organic cheeses.

Looking at the new markets, new cheeses that suit the demand in developing dairy markets like China and India may be important.

Also, the growing demand for dairy products that meet consumers' changing diet and nutritional needs may result into strong growth for innovative and healthier cheese products, such as lactose-free goat cheese products and half-fat, reduced fat, and low-salt cheeses. The maturation process in the making of hard and semihard cheeses adds further complexity and risk in the market. Because the maturation process takes from a few weeks to over a year for cheeses like Gouda,

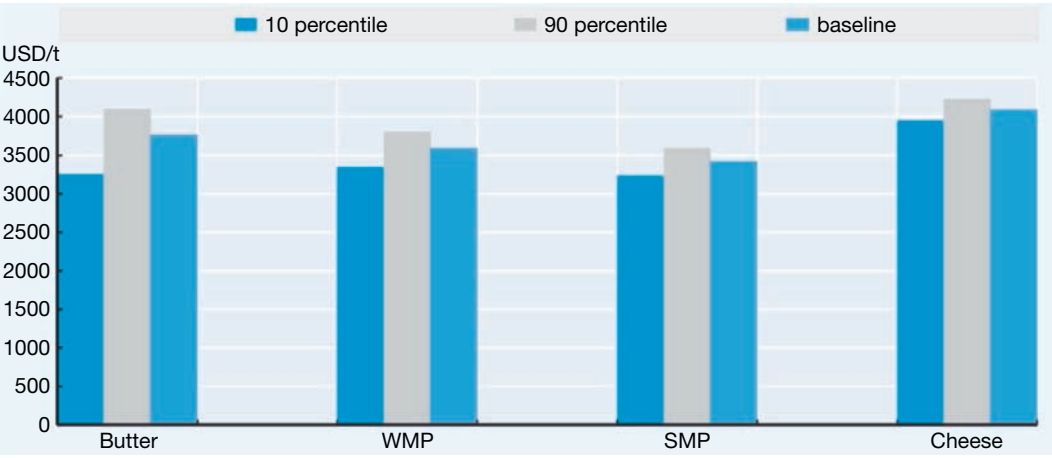


Figure 12 Projections of world dairy product prices in 2020. OECD-FAO Agricultural Outlook 2011–20.

Edam, and Emmental, it creates a time lag that makes a market-driven approach very difficult for these cheese types. Another area to possibly look at may be the production linked to the regions where they are produced such as products with protected geographical indication (PGI) or protected designations of origin (PDOs) for artisanal, organic, or other specialty cheeses.

Consumers are willing to pay more for regionally produced specialty cheeses when they combine good quality and taste with a strong marketing story (see Figure 13). Consumers have a growing interest in cheeses with a sense of history and tradition – and that is turning them away from processed cheeses and toward products unique to a single region or even a single farmstead. Natural, organic, artisan, and local cheeses, as well as cheeses made from blends of milk, are all part of this trend, and specialty cheese stores use these cheeses to distinguish their range from the large retailers.

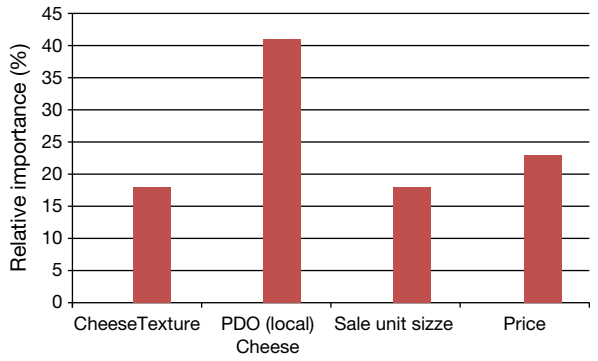


Figure 13 Finding consumer segments based on their preferences for four cheese attributes.

Ethnic Cheeses

In the US market, Italian cheeses are the most popular of ethnic cheeses – so popular that US production of Italian cheeses surpassed that of American natural cheeses for the first time in 2006. Italian cheeses accounted for almost 4 billion of the 9.5 billion lbs. of cheese produced in 2006. A large share of that volume was consumed in restaurants and other food service establishments. In fact, Latin American and Spanish cheeses are no longer a niche market, as an increasing number of non-Hispanic consumers incorporate them into their cooking. Half of the top 10 fastest-growing specialty cheeses in the United States at retail are Hispanic varieties (see Figure 14).

In Europe, specialty cheeses – PDO and PGI cheeses – represent a considerable market (Figure 15). As previously explained, PDO covers agricultural products and foodstuffs that are produced, processed, and prepared in a given geographic area using recognized know-how, while protected geographical indication covers agricultural products and foodstuffs closely linked to the geographic area. At least one of the stages of production, processing or preparation, takes place in the area.

Nutritional and Health Effects

Similar to soft cheeses, medium cheeses are nutritious foods rich in protein, fat, fat-soluble vitamins, and calcium. Many of the cheeses in this group are the so-called Gourmet cheeses making them highly priced cheeses in the market. As in the case of hard cheeses, medium cheeses may as well be suited for lactose-intolerant consumers as in many varieties, lactose is mostly depleted due to its use by the microflora present throughout processing and ripening, also having in general

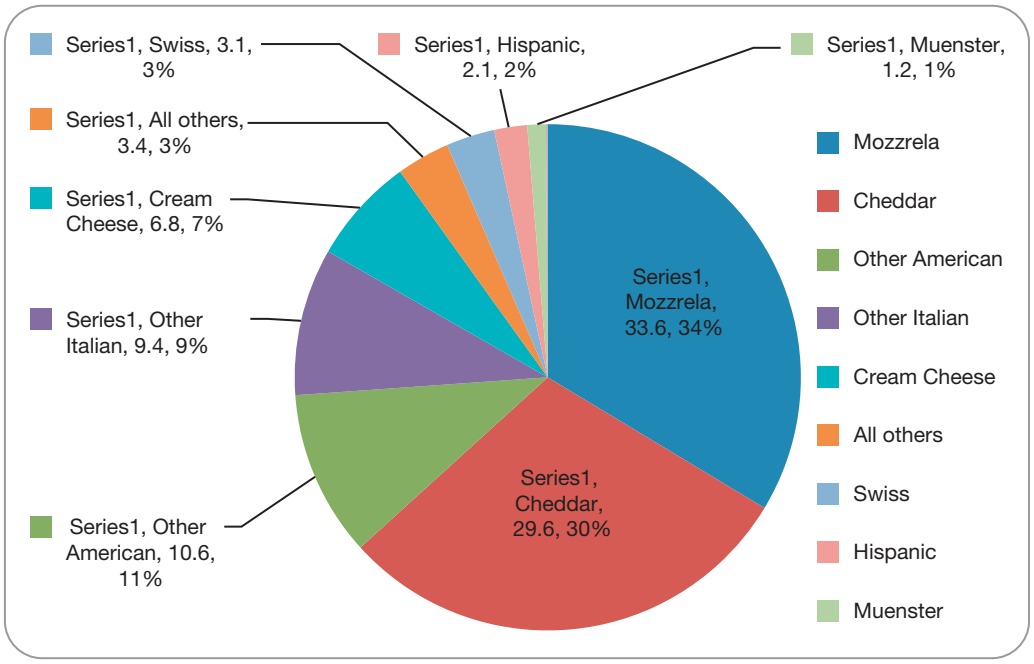


Figure 14 US cheese production by variety. USDA Dairy Products Annual Summary 2011.

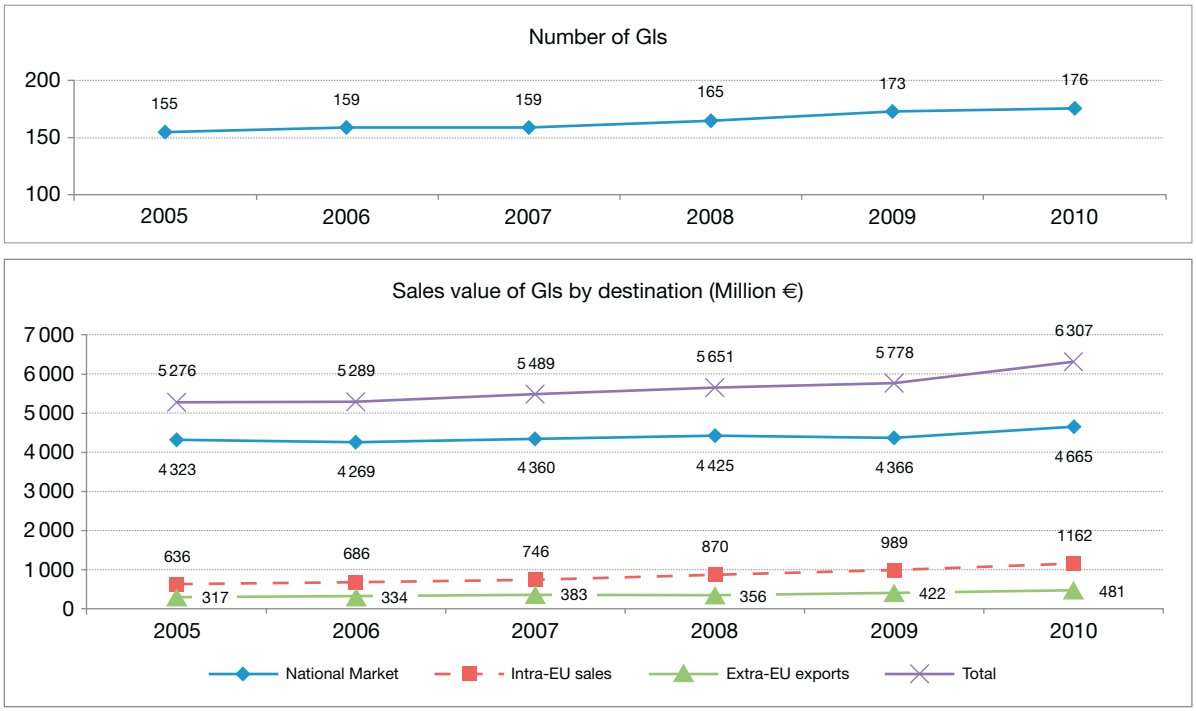


Figure 15 PDO and PGI cheese products and foodstuff sales in Europe. European Commission; Agriculture and Rural Development: public statistics, survey and estimates, 2008.

higher contents of short chain fatty acids as compared with nonripened soft cheeses.

See also: Cheese: Chemistry and Microbiology; Cheese: Composition and Health Effects; Cheese: Processing and Sensory Properties; Cheese: Types of Cheeses – Hard; Fermented Foods: Fermented Milks; Lactic Acid Bacteria.

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Cheese: Types of Cheeses – Hard

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Introduction

Hard cheeses have lower moisture contents than other types of cheeses, and their processing in general requires cooking the curds to increase the release of whey. Due to their extensive ripening period, hard cheeses develop very distinct strong taste and aroma, and the starter culture, which must withstand the harsh conditions in the cheese to exert their proteolytic, lipolytic, and acidification activity, plays a key role in the processing of said cheeses. Nutritionally, hard cheeses are high in protein and fat, and there are some research evidences showing that they may have higher contents of CLA (conjugated linoleic acid) than their softer counterparts. Finally, lactose is in general absent or at very low levels in hard cheeses, which is an advantage for lactose-intolerant consumers.

Varieties

Cheddar

Cheddar cheese is probably the most widely purchased and eaten cheese in the world, especially in Anglo-Saxon countries. It is a hard cheese that matures over a period of time between 9 and 36 months. Its name is associated with the processing technique known as 'cheddaring,' which is a step used during cheesemaking to give cheese a dense, layered texture. It consists in cutting up the curds into smaller pieces to expel whey, and the more they are, the more liquid will drain from them and the harder the resulting cheese will be. While this step is common in most medium and hard cheeses, it is taken one step further for Cheddar cheese, as the curds are cut up and then pressed together into slabs and then the slabs of curds are stacked on top of each other. The weight of stacking the slabs of curds on top of one another presses out even more moisture. Then, the slabs of curds are cut up again, pressed into slabs again, and stacked again. The process continues until so much whey is expelled that after aging, the cheese will have a crumbly, layered, dense texture.

Idiazabal

Idiazabal (Figure 1) is a traditional, farmhouse, hard cheese made from raw milk of sheep in the Basque and Navarra regions of northern Spain. Named after the village of Idiazabal, the cheese received a protected designation of origin (PDO) distinction in 1987. Traditionally, the cheeses are made at early summer in higher zones of pastures and left in the rafters to mature. By the end of summer, the cheeses are ready for sale. Idiazabal is produced in the shape of a cylinder, with a smooth and hard natural rind that is pale yellow to amber in color.

Emmental

Emmental is a PDO cheese produced in the central cantons of Switzerland. Traditionally, it is made from unpasteurized cow's milk. The aroma is sweet, the flavor is fruity and acidic, and it is sold with a rind covered by paper with the producer's name on it. Inside, Emmental has the typical image of a Swiss cheese showing walnut-sized holes, which result from entrapped air produced by the starter culture *P. freudenreichii* that at later phase of the cheese production consumes the lactic acid and releases carbon dioxide gas, which slowly forms the bubbles that make holes.

Gruyere

Gruyere cheese is named after a Swiss village, and it is traditionally made form unpasteurized cows' milk, with a rusty brown, hard, and dry rind pitted with tiny holes. The cheese is darker yellow than Emmental but the texture is more dense and compact. After coagulation, cutting the curds and molding and pressing, the cheese is salted in brine for 8 days and curing from 3 to 10 months.

Parmesan

The Parmesan cheese is among the top cheeses chosen by cheese connoisseurs. The PDO designation states that for a cheese to be called as Parmesan, it has to be produced from cows grazing on fresh grass and hay. Parmesan cheese has a hard, gritty texture and is fruity and nutty in taste, and it is mostly consumed grated over pastas or used in soups and risottos although it can also be eaten on its own as a snack. Total processing time may last up to 2–4 years.

São Miguel

São Miguel cheese (Figure 2) is made from pasteurized milk from cows essentially grass-fed all year round on the island of the same name in Azores. At earlier ripening phase, the cheeses may be sold under the brand Famoso, while São Miguel requires 7–9 month of ripening. The cheese has a unique and distinctive rind colored black.

Source and Production

Cheese is a bacterial ecosystem that changes during ripening due to a series of biochemical transformations. When these changes are balanced, they result in a large variety of flavor compounds and thus the highly desirable aromas and flavors of different cheeses. To obtain their typical more intense taste and aroma, hard cheeses in general require extensive ripening periods.

Figure 1 A wheel of ripen Idiazabal cheese.

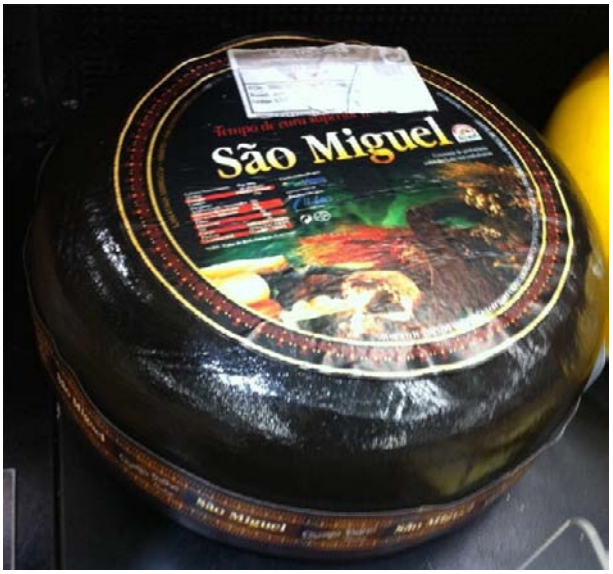

Figure 2 São Miguel cheese wheels showing typical rind color and paste texture.

While the general four processing steps are common for most varieties (see Figure 3), some specific details will differ and contribute to the peculiarities of each cheese. Such differences may be associated to the characteristics of the milk, starter culture type, time and temperature of coagulation, cooking and procedures for whey drainage, salting type, and ripening conditions.

Chemical Quality of Milk

Factors such as the type of the animal, its feed, and season may influence the quantity of calcium, casein, and whey proteins present in milk and consequently influence the quality and rate of milk coagulation during cheese processing. Recall that curd formation in the cheese vat is important for controlling the structure, moisture content, and rheological properties of the cheese. Industrially, corrections to the milk composition can be made by adding calcium chloride or powder (protein) milk, thus increasing the quality and consistency of the curds.

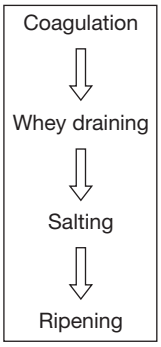

Figure 3 General cheeses processing steps.

Microbiological Quality of Milk

Hard and semihard cheeses manufacture requires that the fresh curd may be matured for several months or even years, which enables even slow-growing microorganisms to manifest themselves. In general, the microbial diversity underlying the benefits of raw milk cheese depends on both the milk microbiota and traditional practices, including inoculation practices. Thus, traditional or local know-how from farming to cheese processing may help in maintaining both the richness of the microbiota in individual cheeses and the diversity between cheeses throughout processing.

The microbiota in raw milk may be highly complex, and especially in raw milk cheeses, this will be reflected in the cheese taste and aroma. In fact, it has been shown that flavor is more intense and rich in raw milk cheeses than in processed ones and that many strains isolated from raw milk cheeses were associated with the formation of more complex volatile profiles and higher scores for some sensorial attributes. This abundant native microbiota can express in raw milk cheeses, which is not the case in cheeses made from pasteurized or microfiltered milk. The wide variation in the dynamics of the species in association with the prevalent physic chemical peculiarities of each cheese creates the diversity of aroma and taste among cheeses. Throughout ripening, the microflora biodiversity will usually decrease in cheese cores and only a small number of LAB species resist to the harsh conditions of hard cheeses. The living bacteria, as well as the enzymes from the bacteria dying off throughout the ripening process, play a key role in the final taste and aroma development of hard cheeses.

Starter Culture and Rennet

Cheese ripening is characterized by a number of microbiological changes and many biochemical changes that are caused by enzymes from the rennet, the milk, and the microorganisms associated with the cheese. The starter culture added to milk during cheese processing is expected to lower the pH of milk thus increasing the rate of syneresis and contribute to the development of the organoleptic characteristics of the cheese (less

important in fresh cheeses). Thus, using the right type and amount of starter culture is crucial to bring about more or less consistently the biochemical activities (proteolysis, lipolysis, and glycolysis) that are important for the development of aroma in cheeses.

The hydrolysis of the casein by rennet in cow's milk transforms the caseins from a stable colloidal system to an unstable one forming a gel that eventually can expel liquid by syneresis. The rate of the enzymatic hydrolysis of casein in milk is proportional to the amount of rennet added, and in this way, the rennet concentration indirectly affects aggregation.

While the starter bacteria generally die off after manufacture, they continue to contribute to ripening since their cells burst open after death releasing their enzymes into the cheese. In most long ripened cheeses, wild nonstarter lactic acid bacteria (NSLAB) grow slowly during ripening and may eventually reach high numbers.

In certain varieties, very obvious microbiological changes occur. The distinctive eyes (holes) in Swiss cheese are caused by the growth of a secondary bacterial flora, which converts the lactic acid produced by the starter bacteria to other products, including carbon dioxide gas, some of which is trapped in the cheese to form bubbles.

pH

Changing the pH of the milk strongly affects the structure of the micelles and the renneting reaction, as the rate of enzymatic breakdown of casein is very dependent on pH. Rennet (i.e., chymosin) is an acidic enzyme, with an optimum activity in milk around pH 6.0, thus, decreasing the milk natural pH increases the rate of proteolysis considerably. Lowering the pH of the milk leads to a decrease in coagulation time, the main effect probably being the increase in enzyme activity; aggregation is also affected. All together, these changes have an effect in whey expulsion and consequently in the inner conditions that will prevail throughout the long ripening process of hard cheeses. Different cheese varieties usually have different pH profiles throughout all the processing and ripening phases.

Temperature

In general, milk will not clot when the temperature is below 15 °C. This is an effect of the inefficiency of the aggregation reaction since the enzymatic hydrolysis of casein still proceeds at low temperature; heating will then lead to almost immediate coagulation, a phenomenon termed cold renneting. Coagulation and syneresis are optimized at a typical temperature for each hard cheese.

Manufacturing of Some Hard Cheeses

Cheddaring is a unique step in the making of Cheddar cheese. It is a multistep process that reduces whey content, adjusts acidity, adds characteristic flavor, and results in a denser and sometimes crumbly texture. Cheddaring implies that cooked curds are cut and then left to set forming loaves. The loaves are then cut, turned over, and stacked repeatedly. This process is complete when the acidity of the whey is between 5.1 and 5.3. The loaves now are milled, salted and put into molds, pressed, and put to ripening for a year or more, depending on the variety of Cheddar being processed.

In the case of Parmesan cheese, its traditional processing begins with the evening milk being kept overnight in metal trays, to allow the cream to rise to the top, skimmed, and combined with the whole milk from the morning milking. The milk is warmed in large cauldrons and naturally fermented whey from the previous day's production is added as a starter. Natural calf's rennet is then added to coagulate the milk and curds form after about 20 min.

Using a balloon whisk called a spino, the cheesemaker whisks the curds obtaining curds the size of a grain of wheat separated from the whey. All the mixture is cooked at a specific temperature and left to stand and allow the curds to sink to the bottom of the cauldron where they knit together forming a spongy mass. Using specific equipment and procedures, the cheesemaker lifts the curd mass and, dividing it in half, wraps each in a muslin. The cheeses are hung on poles to shed excess liquid, while the whey drains out, some of which will be used as starter culture in the next day cheese processing.

The curds are inserted in molds and, after pressing, become salty where they stay for about 24 days. The cheese wheels are then transferred to the curing rooms, where they remain usually for 1 year, being turned regularly, wiped, and brushed. Finally, through a series of tests, independent inspectors determine whether the cheese meets the standards of the Consorzio del Formaggio Parmigiano Reggiano.

An important and the longest part of the production of the Le Gruyere is the 'affinage,' that is, maturation. After the curds are put into molds, they must be ripened in caves with humidity near 94%. If the humidity is lower, the cheese dries out. If the humidity is too high, the cheese does not mature and becomes smeary and gluey. The temperature of the caves should be between 13 and 14 °C. Different combinations of temperature and humidity will result in cheeses of different characteristics such as harder or more crumbly.

The uniqueness of Emmental cheeses resides in the combination of a thermophilic starter culture with a culture of propionic bacteria that typically releases carbon dioxide gas, which slowly forms the bubbles that make eyes or holes typically present in Emmental cheeses. Emmental is somewhat a difficult cheese to produce because of the complicated eye-forming fermentation that requires specific conditions for the propionic bacteria to produce the gas in the required and not excessive amount.

Cheese Problems

The microbiological and biochemical changes that occur during ripening, and hence the flavor and aroma of the finished product, are largely predetermined by the manufacturing process. While the extensive ripening period of hard cheeses contributes to the development of their taste and aroma, it may also increase the chances of occurrence of a number of potential defects in the product.

A defect so-called 'early blowing' may appear some time after the cheese has been manufactured. This is usually a consequence of an unbalanced presence and activity of bacteria of

the group Enterbacteriaceae, which may be present in raw milk or introduced into pasteurized milk or curds by improper hygienic practices. High hygienic standards and pasteurization in general decrease substantially these type of problems.

Another problem common in hard cheeses is the so-called late blowing (**Figure 4**), associated with the presence of resistant spores from such bacteria as *Clostridium tyrobutyricum*, which when in vegetative form produces gases causing unwanted discontinuity of the cheese paste after 2–3 months after the cheeses have been manufactured. *C. tyrobutyricum* are commonly present in milk from cows fed with hay contaminated with the spores. Milk centrifugation (bactofugation), to eliminate spores, high-quality hay or addition to milk of preservatives such as nitrite, or lysosima are the common ways to lower the chances of appearance of the late blowing problem.

While an optimum activity of the starter culture is desirable, the excessive activity of the starter culture and of the rennet may lead to formation of undesirable substances and to defects such as bitterness, a consequence attributed to excessive proteolysis and formation of short hydrophobic peptides. The inhibition of citrate-fermenting starter cultures by bacteriophages (or for that matter, antibiotics present in milk) may also permit residual levels of citric acid to remain in the curd. In fact, inhibition of starter cultures is said to be a cause of explosion of entire wheels weighing more than 24 kg. Heterofermentative NSLAB are capable of fermenting citrate with the production of carbon dioxide. As a result, gassing may occur, with splits and fissures developing in Parmesan production.

The involvement of yeasts in the maturation and spoilage of many cheese types is complex and in many cases difficult to differentiate; however, they may also contribute to flavor development within and on the surface of Gorgonzola and Camembert.

Finally, concerns about safety of raw milk cheeses are the basis for legislation requiring that raw milk cheeses must have a minimum maturation time of 60 days, forming an integral part of the HACCP (hazard analysis critical control point) plan so that pathogens such as *Salmonella* spp., at levels that might

Figure 4 Intense gas production in cheeses contaminate by *Clostridium tyrobutyricum*.

Table 1 Some nutritional facts of hard cheeses as compared to fresh cheeses

		Cheese variety		
Nutrient	*Unit per 100 g*	*Cheddar*	*Parmesan*	*Fresh cheese*
Water	g	37	29	51
Energy	Kcal	406	392	299
Total lipids	g	34	26	24
Saturated fats	g	20	16	13
Protein	g	24	36	18
Total sugars	g	0.28	0.8	2.4
Minerals				
Calcium	mg	675	1184	566
Magnesium	mg	27	44	24
Phosphorus	mg	473	694	385
Potassium	mg	76	92	129
Sodium	mg	644	1376	751
Vitamins				
Riboflavin	mg	0.43	0.33	0.2
Vitamin A	IU	994	781	806
Vitamin E	mg	0.78	0.22	0.4
Vitamin D	IU	24	19	110
Cholesterol	mg	102	68	69

Source: USDA, National Nutrient Database for Standard Reference Release 27 www.usda.gov (accessed on 15/01/2015).

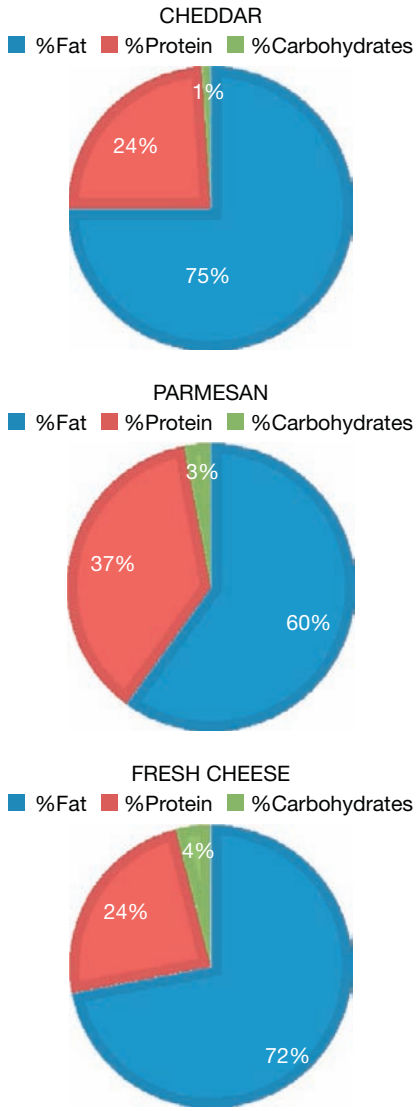

Figure 5 Caloric ratio for the three varieties of cheeses.

be encountered naturally, will be presumed to have died before the cheese is consumed.

Microbiological problems associated with cheese manufacture can be summarized in three categories: (a) failure to make the product as intended, (b) defects resulting in spoilage or loss of quality of the finished product, and (c) contamination that may result in an unsafe product. It has also been emphasized that these problems are likely to be highly variable, depending on the type of cheese, source of milk, processing, season of the year, geographic location and storage, distribution, and consumption.

Nutritional Considerations

Hard cheeses can be considered as highly concentrated sources of protein and fat, having low carbohydrate (lactose) contents (see **Table 1** and **Figure 5**). The protein content in cheese, especially hard cheeses, is of high biological availability, in the form of peptides and amino acids resulting from degradation of casein. The fat contents in hard cheeses (may be above 52%, mainly in the form of saturated fatty acids) make them to be high-caloric food products. Hard cheeses are also good sources of calcium and fat-soluble vitamins such as vitamins A and D as well as of CLA.

See also: Cheese: Chemistry and Microbiology; Cheese: Composition and Health Effects; Cheese: Processing and Sensory Properties; Cheese: Types of Cheese – Medium; Fermented Foods: Fermented Milks; Lactic Acid Bacteria.

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Cheese: Types of Cheeses – Soft

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Introduction

Cheese can be defined as a consolidated curd of milk solids in which milk fat is entrapped by coagulated protein (casein).

It is believed that accidentally, man found that milk coagulate and sour, separating into curds and whey if exposed to natural heat in specific containers, and this was found to be a good way of preserving the valuable nutrients present in milk, making cheese an important component of human diet since then. Athletes in the Roman Empire had cheese as a preferred food choice, which was described then as 'a luxurious specialty for the palate of the kings.'

Today, cheesemaking is a major industry worldwide, as advances in food science and technology have led to large-scale industrialization, allowing for mass production with a focus on guaranteeing products with microbial safety and textural consistency. However, much is still practiced on a relatively small-scale accounting for a rich diversity of cheeses still available. At a small scale, cheesemaking may still be seen as an art, namely, in what concerns the processing of the so-called traditional cheeses, where the cheesemaker experience and empirical knowledge are key factors in obtaining cheeses of unique taste and flavor.

The removal of water during cheese processing increases the concentration of most nutrients (proteins, fat, mineral salts, and vitamins with the exception of lactose) present in milk, making cheese a nutrient rich food. Cheeses can be classified based on a number of criteria; however, classifications based on the source of milk, method of coagulation, or texture (which is largely determined by moisture and fat contents) are the most common. The latter allows the classification of cheeses as: 'soft,' 'semisoft' (medium), or 'hard.' Texture is an important quality parameter that determines the identity of a cheese and greatly affects its consumer preference and acceptance, and although it may mean different things to different people, cheese texture and general appearance such as mouthfeel are the primary quality attribute of any dairy food. Many instrumental methods do exist today to evaluate the rheology and structure of a product (texture); however, humans have been the cornerstone of food texture characterization.

Source and Production

There are more than 200 types of cheeses and their making involves a complex number of sequence procedures associated with specific biochemical transformation, which, as a whole, influences the development of cheese flavor, as well as the yield and composition, and thus the overall quality of the final cheese.

However, for convenience, all processes can be broken down into a number of relatively simple unit operations (Figure 1), and slight variations of these and the use of different milks (Table 1) combine to generate the huge range of cheeses available today.

At high output industry, due to the high volumes of milk being processed (Figure 2), cheesemaking is highly automatized and thus expected to be less prone to variations among batches. Although not compulsory (as for typical raw milk cheeses), a heat treatment equivalent to pasteurization is usually applied at the start of processing. The milk is then cooled to the fermentation temperature, which will differ from cheese to cheese but usually in the range of 30–37 °C, followed by the addition of the starter culture. This helps to ensure a safe product, and for cheeses undergoing the ripening phase, it leads also to a reliable fermentation, keeping variations in the course of industrial manufacture of cheese to a minimum. The salting of the curd is common to all cheeses giving it more taste and increasing the microbial safety of the curd due to the preservation properties of salt.

However, cheeses can be processed via more basic technology as happens in artisanal cheesemaking (Figures 3 and 4). These cheeses, also called traditional or specialty cheeses, are essentially handmade and when made from raw milk are usually considered to be of more interest in terms of taste and aroma (see Figure 5) than their industrial counterparts. Thus, artisanal cheesemaking is still an important part of the cheese market in the world. In fact, artisanal cheeses are often seen as alternative products that may actively contribute for consumer's health, although some concerns exist related to their safety. In particular, raw milk for processing raw milk cheeses should be of a standard microbial quality ($<10^4$ cfu ml^{-1}), which is a key factor in obtaining products of highest quality.

Whatever the process (industrial or artisanal), the resulting cheeses may be classified based on their moisture and fat contents as 'soft' (fresh), 'semisoft' (medium), or 'hard' cheeses; see Table 2.

Thus, there are hundreds of different types of cheese, but each is made using similar principles of coagulating the proteins in milk to form curds and then separating the curds from the liquid whey. Depending on the type of cheese, milk proteins may be coagulated in different ways, of which the most common include: (i) adding rennet (or 'chymosin') – an enzyme extracted from calves' stomachs or produced by micro-organisms, or from plant rennet extracts, (ii) via fermentation that releases lactic acid, and (iii) via addition of such acids as lemon juice, lime juice, or vinegar or by combination of above methods. The different cheese flavors and textures arise from a combination of such factors as the type of milk, the amount of fat in the milk, the bacteria culture used to ferment the milk, and specially from the processing conditions applied during cheese-making and ripening. Cheeses classified as soft-unripe, usually have a very short shelf-life as such.

Soft cheeses have water content in the range of 50–80% and, depending on the variety, may not undergo the ripening

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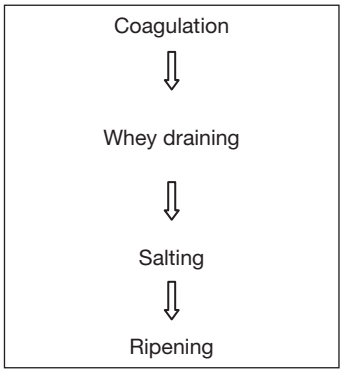

Figure 1 General cheesemaking steps.

phase, namely, in the case of the so-called fresh cheeses; thus, their processing may not require the addition of a starter culture. Soft cheeses are easier to make than hard cheeses and are traditional foods that are popular in many countries. In some varieties, the whey is drained to leave the cheese, which is pressed into blocks and stored in brine until it is sold. Coagulation may be achieved using rennet, or organic acids such as lactic acid, lemon juice or vinegar. These cheeses are soft, moist, and creamy and include curd cheese, paneer, queijo fresco, cottage cheese, Brie, Serra da Estrela cheeses, and others.

Table 1 Average chemical composition of milk of different species (for 100 g of fresh milk)

Species	*Water (%)*	*Proteins (%)*	*Fat (%)*	*Lactose (%)*	*Mineral salts (%)*
Buffalo	82.2	4.8	7.5	4.7	0.8
Goat	86.5	3.9	4.3	5.8	0.8
Ewe	80.9	6	7.5	5.4	1.1
Cow	87.5	3.2	3.7	4.6	1

Note: In cheese, these nutrients will appear on average ten times more concentrated.

Figure 2 High-output cheeses industry layout: (left) a closed cheese vat of high capacity (10 000–20 000 l) and (right) brine salting of cheeses in a large-scale plant processing 20 tons of cheese a day.

Figure 3 A small-scale artisanal cheese plant unit processing 50 kg per day of a traditional PDO (protected designation of origin) cheese.

Early coagulation phase with cheesemaker tasting the consistency of the gel

Cutting the curd after renneting

Scalding

Draining the whey and milling the curd

More detailed view of curd being moulded

Figure 4 Cheese processing steps at small-scale unit (the last image shows the cheeses after being pressed and ripening for 10 days).

Soft Unripened Cheeses

Queijo Fresco

The most simple known fresh cheese is queijo fresco (fresh cheese, queso blanco). Queijo fresco (see Figure 6) is a common type of cheeses in Southern Europe and Central and South America. Its processing simple requires the heating of milk followed by the addition of rennet or a food-grade organic acid (acetic, citric, or lactic acid or even lemon juice). After coagulation and whey drainage, the curds are salted, hooped, and packaged. The texture and mouthfeel of queijo fresco depend on the severity of heat treatment given to milk, the acid type used, and mode of clotting. If the curds are pressed, the cheese will have a more rubbery texture especially if it is made from low-fat milk. After packaging, queijo fresco is ready to eat and has on average 5–7 days of shelf life if kept in refrigeration.

Cottage

Cottage cheese (see Figure 7) is a common type of cheeses in Anglo-Saxon countries. It may be available in low-fat and non-fat forms; while regular cottage cheese only has 4% of fat by weight, low-fat cottage cheese has only 2%. Cottage cheese has long been considered an ideal source of protein for those on weight loss diets because it can be made in a variety of low-fat forms, it is inexpensive, and it can be used in a multitude of recipes. Billions of pounds of cottage cheese are consumed each year in the United States, probably as a result of a public's perception of cottage cheese as a health food.

Cottage cheese manufacturing typically begins with pasteurized skim milk, which is then inoculated with active lactic starter to raise the acid content and rennet to speed coagulation. Rennet is added for a rapid coagulation of milk in the cheesemaking process. Depending on the type of cottage cheese being made and the amount of active lactic starter used, the inoculated milk is held for 5–16 h at a temperature of 32° C (89.6 F). This allows the lactic starter to reproduce and acidify the milk, and the mixture to coagulate properly. The resulting coagulated milk is then cut into cubes that will form the curds that are seen in cottage cheese. Then, the mixture is cut, increasing whey draining, and rinsed with worm and cold water. Most other forms of cheese are then pressed, but cottage cheese is not, thus retaining significantly more moisture and does not form a solid block. The addition of cream, depending

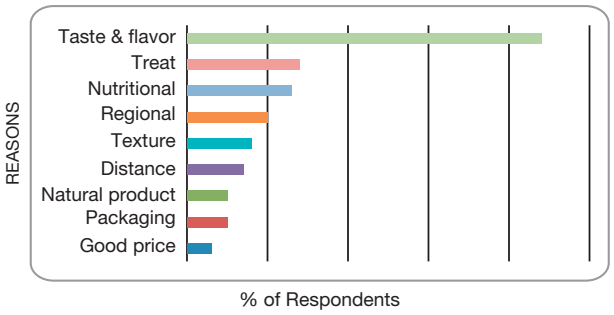

Figure 5 Consumer reason for purchasing Irish farmhouse cheese.

Figure 6 Portuguese queijo fresco is a soft cheese type with red pepper sauce on top as is usually consumed.

Figure 7 Cottage cheese, a fresh cheese type.

Table 2 Most representative groups of cheeses and their texture classification

Type	*Moisture content*	*Fat content*	*Name*	
			Unripened	*Ripened*
Soft	50–80%	<40%	Cottage, quark, cream, queijo fresco (queso blanco)	Camembert, Brie, Neuchâtel, queijo Serra da Estrela
Semisoft	40–50%	<35%	Mozzarella, Roquefort, Stilton, Gorgonzola, São Jorge, Provolone, Gouda, Edam	
Hard	30–40%	<30%	Cheddar, Idiazabal, Gruyère, Parmesan, Emmental, São Miguel	

Table 2 GLUT transporters: location and importance

Transporter	*Location*	*Importance*
GLUT1	Erythrocytes Brain endothelial cells Blood–placenta barrier	High-affinity transporter in cells that rely almost exclusively on glucose for energy Critical for fetal development
GLUT2	Intestine Liver Kidney Pancreas (β-cells)	Low-affinity transporter that becomes more active when glucose levels are high. Important for the absorption of monosaccharides and for ensuring uptake of excess glucose in the liver. May also be used by the pancreas as a trigger for insulin release. Critical for glucose reabsorption by the kidney
GLUT3	Brain neurons	High-affinity transporter in a tissue that relies almost exclusively on glucose for energy
GLUT4	Skeletal muscle Cardiac muscle Adipose tissue	High-affinity transporters induced by insulin to increase glucose uptake into cells Failure of these transporters to respond to insulin leads to insulin resistance
GLUT5	Primarily intestine Testes Liver Skeletal muscle Adipose tissue	The predominant fructose transporter in tissues Critical in testes as fructose is an important energy source for sperm

body. Most of the GLUT proteins that transport glucose can also transport galactose and sometimes fructose; however, fructose is most frequently transported across tissues by GLUT5. Fructose and galactose levels in the blood are magnitudes lower than glucose due to the proportion of these monosaccharides in the diet compared to glucose and because galactose and fructose are rapidly taken up and metabolized by the liver.

The role of the tissue in carbohydrate metabolism is reflected by the type of GLUT transporter present in the cells. Tissues and cells that absolutely depend on glucose for fuel, such as erythrocytes (red blood cells) and brain endothelial cells, have higher concentrations of GLUT1 transporters. The GLUT1 transporter has a high affinity (low K_m) for glucose, thus ensuring that even at low blood glucose concentrations, the cells will be able to obtain glucose for energy. GLUT1 transporters are most concentrated in the erythrocyte (as much as 10% of the membrane protein), to not only ensure these glucose-dependent cells receive glucose but, possibly, also provide greater capacity for the bloodstream to carry glucose. GLUT1 also mediates glucose transport to the placenta and is critical throughout fetal development. Mice *in utero* that do not express GLUT1 do not survive gestation, and humans that have the genetic disease of GLUT1 deficiency syndrome experience developmental delays, seizures, and neurobehavioral issues from infancy.

Tissues that are involved in the absorption and regulation of blood glucose, such as the liver, kidney, pancreas, and intestine, express more of the GLUT2 transporter, which has a lower affinity (high K_m) for glucose. Thus, when glucose concentrations are high, these transporters become more active. In the liver, GLUT2 transporters begin to remove glucose from the bloodstream as glucose levels rise, thereby helping to regulate blood glucose levels. The liver is capable of converting and storing excess glucose for release at a later time when glucose levels are lower. In the pancreas, GLUT2 likely plays a role in glucose sensing that triggers the release of insulin as blood glucose levels rise. The expression of GLUT2 in the renal tubules of the kidney is critical for the reabsorption of glucose into the bloodstream and prevention of glucosuria.

Skeletal and cardiac muscle and adipose tissue predominantly express the insulin-dependent glucose transporter GLUT4. This transporter is fairly specific for glucose with little to no uptake of galactose or fructose. As insulin levels rise in response to an elevation in blood glucose, GLUT4 transporters are recruited from the intracellular compartment to the cell membrane. Most of the glucose in the bloodstream is taken up by skeletal muscle cells through this mechanism, making GLUT4 an important transporter for regulating blood glucose levels. In fact, disruption of the translocation of GLUT4 to the cell membrane is commonly known as insulin resistance and contributes to the development of type 2 diabetes. To learn more about the role of carbohydrates in health conditions, such as type 2 diabetes, see elsewhere in this Encyclopedia.

Glycemic Response

The change in the levels of glucose in the bloodstream after absorption of carbohydrate is considered the 'glycemic response.' Many physiological and food-related mechanisms influence the glycemic response to food, such as the type of carbohydrate consumed, the clearance of glucose from the bloodstream, and other components within the food (e.g., fat and protein). These factors are further discussed in the section in the succeeding text. For more information on the regulation of glucose in the bloodstream, see elsewhere in this Encyclopedia.

Factors Impacting Digestion and Absorption of Carbohydrates

Physiological and Genetic Factors

There are several congenital disorders that can lead to impaired digestion and absorption of carbohydrates. Disorders impacting digestion are typically defects in brush border enzymes, such as sucrase–isomaltase and lactase. Malabsorption disorders that are not a result of inadequate digestion are usually a result of genetic disorders in transporters or secondary to other digestive disorders, such as celiac disease.

Congenital deficiencies in digestive enzymes, such as sucrase–isomaltase, lactase–phlorizin hydrolase, and glucoamylase, are rare conditions but have been identified and characterized in various populations. The symptoms of these conditions are often similar: diarrhea, bloating, abdominal discomfort, vomiting, and dehydration; therefore, diagnosing these conditions often requires identifying the specific dietary carbohydrate trigger (e.g., sucrose or lactose) and sometimes intestinal biopsies. Treatment generally involves

on the type of cottage cheese being made, and of salt may follow.

Soft Ripened Cheeses

There are a number of soft cheeses that undergo a ripening period that usually may go up to 1 month. Examples of these are Brie, Feta, Camembert, Serra da Estrela cheese, and others.

Feta

Feta (Figure 8) is a protected designation of origin (PDO) cheese and may be the most famous Greek cheese occupying more or less 70% of the Greek cheese market.

Traditionally, the cheese is made by mixing 30% goat's milk with sheep's milk, although nowadays, goat's and cow's milk are also made. The coagulation requires the use a starter culture (a mixed culture that may contain *Lactococcus lactis*, *Lactobacillus bulgaricus*, *L. helveticus*, and *Streptococcus thermophilus*) and afterward the addition of rennet, draining the way followed by brine salting. Feta texture is creamy or crumbly dry, varying according to age, local environment, animal breeds, and starter cultures. Feta aged for 4–6 weeks is sold in blocks, and because it is a high-salt cheese sometimes, it is washed underwater to remove the extra saltiness.

Brie

Brie (Figure 9) and Camembert are the types of so-called mold ripened cheeses and are produced from whole or semi-skimmed cow's milk to which rennet is added. After coagulation and after curds are firm, they are injected with a mold infusion of *Penicillium candidum* or *P. camemberti*. The cheese is then cast as several layers of cheese into molds and then kept for around 18 h. After this, the cheese is salted and aged for minimum of 4 weeks. *P. candidum* or *P. camemberti* have a high proteolytic and lipolytic activity; thus, they are important contributors to development of the typical taste of Brie. There are several varieties of Brie cheese including plain, herb, and others with combinations of milk products. The microbial dynamics during Brie as well as Camembert ripening may be complex, implying succession of different types of microorganisms such as yeasts, molds, and lactic acid bacteria, each one prevailing at pH values and different ripening phases.

Camembert

Camembert (Figure 10) is a soft high fat cheese, also made from cow's milk. Camembert is ripened as a small round cheese and sold as such, so it is fully covered by the rind. Good Camembert cheese is bland, hard, and crumbly in texture when fresh. As the cheese matures, it forms a smooth, runny interior and a white bloomy rind that is typical to Camembert cheese. It has a rich, buttery flavor. The rind is bloomy white caused by a white fungus of the *Penicillium* group.

Figure 8 Blocks of Feta cheeses.

Figure 9 Brie cheese with its characteristic white rind.

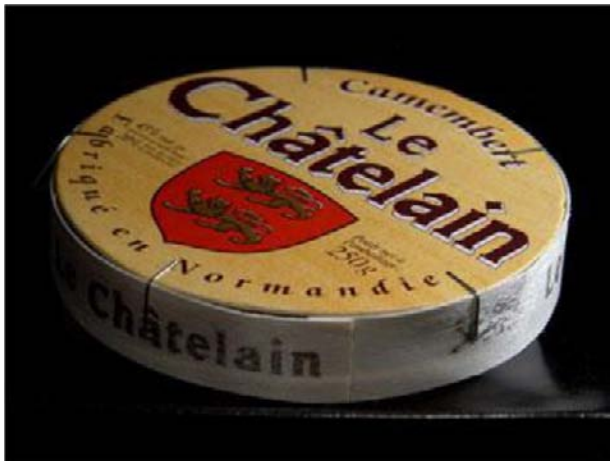

Figure 10 An elegant package of Camembert cheeses.

Figure 11 (Left) Serra da Estrela cheese with typical texture and rind covered by a cloth; (right) the thistle flower of cardo plant (*Cynara cardunculus*).

Serra da Estrela

Serra da Estrela is a PDO (Protected Designation of Origin) (Figure 11) Portuguese cheese and has been made for centuries by shepherds in the mountains of Serra da Estrela in the Beira region from sheep's milk. The cheese is entirely handmade, with the curds being broken up by hand. Serra da Estrela is so soft that it is almost spreadable. The affinage takes 30–40 days. Interestingly, Serra da Estrela among other Portuguese traditional cheeses is coagulated with a thistle rennet from a plant named cardo. The high proteolytic activity of the vegetal coagulant associated with the high fat content of ewe's milk gives the soft spreadable texture typical of Serra da Estrela cheese.

Nutritional and Health Effects

In general, cheeses are highly nutritious foods reach in protein, fat, fat soluble vitamins, and calcium. Due to their content in fat, cheese is a high caloric food; thus, efforts have been undertaken toward making low-fat cheeses. As reduction in fat results in an unpleasant rubbery texture, low-fat cheeses are usually of fresh (soft) type. Fresh unripened cheeses may still have considerable amount of lactose, and this, associated with a high moisture content, makes them more prone to spoilage by bacteria, thus having a short shelf life.

See also: Cheese: Types of Cheese – Medium; Cheese: Composition and Health Effects; Cheese: Processing and Sensory Properties; Cheese: Types of Cheeses – Hard; Fermented Foods: Fermented Milks; Lactic Acid Bacteria.

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EDITED BY Benjamin Caballero, Paul M. Finglas and Fidel Toldrá

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ENCYCLOPEDIA OF
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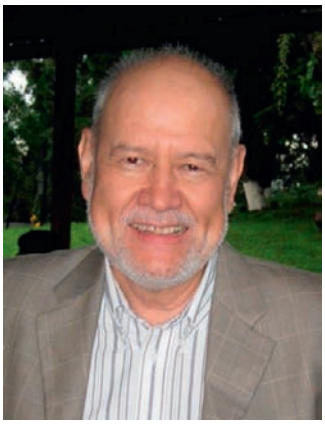


Benjamin Caballero is professor of International Health and of Maternal and Child Health (Bloomberg School of Public Health), and professor of pediatrics (School of Medicine) at Johns Hopkins University.

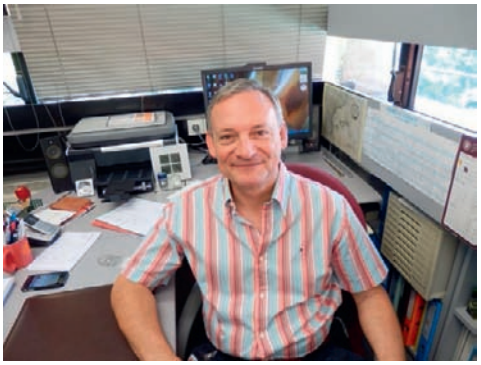
He obtained his MD from the University of Buenos Aires, his MSc in biochemistry from the University of San Carlos, and his PhD in neuroendocrine regulation from MIT, in Cambridge, MA. He started his academic career as assistant professor of pediatrics at Harvard Medical School and director of the Nutrition Unit of Boston Children's Hospital, and subsequently became the founding director of the Center for Human Nutrition at Johns Hopkins University, in Baltimore.

Prof. Caballero has focused his research on child nutrition and health in developing countries. In particular, he has explored the combination of undernutrition and overweight that has become increasingly prevalent in low- and middle-income countries. He was a member of the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences, USA, and of a number of expert panels created by the Institute, including the Dietary Reference Intakes (DRI) Committee, the Expert Panel on Macronutrient Requirements, and the Childhood Obesity Task Force. He was also a member of the Dietary Guidelines for Americans Advisory Committee, of the Scientific Advisory Board of the Food and Drug Administration (FDA), and of a number of advisory committees of the National Institutes of Health (USA).

He is the editor-in-chief of the *Encyclopedia of Food Sciences and Nutrition*, a 10-volume work on food production, consumption and biological effects. He is also editor-in-chief of the *Encyclopedia of Human Nutrition*, which received the Book of the Year Award from the British Medical Association. His *Guide to Dietary Supplements* summarizes the current scientific basis for the use of mineral and vitamin supplements. His book *The Nutrition Transition: Diet and Disease in the Developing World* explored the impact of demographic and economic development on diet- and lifestyle-related diseases in developing countries. His book *Obesity in China* summarizes research conducted in rural and urban China to track the impact of socioeconomic development on health outcomes. He is also coeditor of a widely used textbook on human nutrition, *Modern Nutrition in Health and Disease*.

He is a member of the Spanish Academy of Nutritional Sciences, and a Fellow of the American Society for Nutrition and of the Royal Society of Medicine (UK). Recent awards include the Donald Medearis Lectureship from the Massachusetts General Hospital/Harvard Medical School, the Mataix Prize for lifetime achievements in nutrition science from the Spanish Academy of Nutritional Sciences, the Ancel Keys Prize for achievements in international public health, and the Thompson–Beaudette Lectureship from Rutgers University.

Paul Finglas joined the Institute of Food Research in 1981 and is currently head of the Food Databanks National Platform and Research Leader in Food and Health at the Institute (http://www.ifr.ac.uk/science/platform/FD/default.html). He has, for most of his science career, been involved in a wide range of research in food composition and analysis, and the nutritional effects of micronutrients in food and health research. Paul has considerable experience of co-coordinating both national and international projects (e.g., EuroFIR, TDS-EXPOSURE, Bacchus and QualiFY (all EU FP7), and is currently of the spin-out EuroFIR AISBL, a non-profit international association based in Belgium, from one of these projects. Paul has a broad range of experience in science publishing and is currently editor of the journals *Food Chemistry* and *Trends in Food Science and Technology*, and was one of the coeditors for the *Encyclopedia of Food Science and Nutrition* (2nd Ed.). Paul has a degree in chemistry from Aston University in Birmingham and has published over 150 publications on a wide range of topics in food science and nutrition.



Fidel Toldrá holds a BSc in chemistry (1980), high degree on food technology (1981) and PhD in chemistry from the University of Valencia (1984). Professor Toldrá was a Fulbright postdoctoral scholar at Purdue University in West Lafayette (US, 1985–86) and visiting scientist at the University of Wisconsin-Madison (1991 and 1995), and the Institute of Food Research-Bristol (UK, 1987). Currently, he is research professor at the Instituto de Agroquímica y Tecnología de Alimentos (CSIC), in Paterna, Valencia (Spain). He is also associate professor of food technology at the Polytechnical University of Valencia.

Prof. Toldrá has focused his research on food biochemistry and its relationship with nutrition, quality and safety. He has filed 12 patents, directed 22 PhD thesis and published over 245 manuscripts in recognized scientific journals and more than 115 chapters of books. His h-index is 41. Prof. Toldrá has authored two books and edited/co-edited more than 30 books for major publishers like CRC Press, Wiley-Blackwell, Elsevier and Springer.

Prof. Toldrá is the European editor of *Trends in Food Science and Technology* (2005–) and associate editor of *Meat Science* (2014–); he was the editor-in-chief of *Current Nutrition & Food Science* (2005–2012), section editor of the *Journal of Muscle Foods* (2009–2010) and guest editor of 12 special journals issues. He is a member of the editorial boards of *Food Chemistry*, *Food Analytical Methods*, *Journal of Food Engineering*, *Journal of Food and Nutrition Research*, *The Open Nutrition Journal*, *The Open Enzyme Inhibition Journal*, *Recent Patents in Agriculture*, *Food and Nutrition*, *Food Science & Nutrition* and *Current Opinion in Food Science*.

He has been a member of the Scientific Panel on food additives, flavorings, processing aids and materials in contact with foods (periods 2003–2008) and the Scientific Panel on flavorings, enzymes, processing aids and materials in contact with foods (2008–2015) of the European Food Safety Authority (EFSA) acting as Chairman of the Working groups on Irradiation (2009–2010), Processing Aids (2011–2014) and Enzymes (2010–2015). He was a member of FAO/WHO group of experts to evaluate chlorine-based disinfectants in the processing of foods (2008–2009). He was a member of the Executive Committee of the European Federation of Food Science and Technology (EFFOST, 2002–2009). He is a Fellow of the International Academy of Food Science and Technology (IAFOST, 2008) and of the Institute of Food Technologists (IFT, 2009–). He received the Iber Award on Food and Cardiovascular Diseases (1992), the Institute Danone award in Food, Nutrition and Health (2001), the International Prize for Meat Science and Technology from the International Meat Secretariat (2002), GEA award on R+D activity from the Valencian Community (2002), and the Distinguished Research Award (2010) and Meat Processing Award (2014), both from the American Meat Science Association.

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Siân Astley has worked extensively with individuals and organizations throughout Europe from a variety of disciplines including research, food and biotech industries, and the media. She is the author of more than 300 popular science articles for magazines and trade publications as well as 25 peer-reviewed papers, and she was awarded her Diploma in Science Communication in 2009 (Birkbeck University of London). After 14 years as a bench-scientist, Siân became communications manager for NuGO, one of the first FP6 networks of excellence, and was the European communications manager for the Institute of Food Research in Norwich (UK) until April 2012. Currently, she is the training and communications manager for the European Food Information Resource (EuroFIR AISBL) supporting training within EU-funded research projects and networks, and communication of research activities.

David J. Baer is a supervisory research physiologist with the US Department of Agriculture's Beltsville Human Nutrition Research Center located in Beltsville, Maryland. He serves as the research leader for the Center's Food Components and Health Laboratory and also serves as the director of the Center's Human Studies Facility.

Dr. Baer conducts controlled dietary intervention studies to investigate the relationship between diet and the risk for chronic degenerative diseases, especially cardiovascular disease, cancer, and diabetes in people. His research also includes studies on the health impacts of weight gain and determining the calorie content of foods. Some of the dietary interventions he has investigated include the effects of different types of protein, fats and fatty acids, fiber, margarine, butter, plant sterols, salad dressings, nuts, whole grains, berries, alcohol, and tea on overall nutrition and health. In addition to dietary intervention studies, Dr. Baer is involved in research studies to validate food survey methodologies and in developing new methods for dietary assessment.

Dr. Baer earned his bachelor's degree from the University of Illinois and his master's and doctorate in nutrition from Michigan State University.

Marina Carcea was awarded a master degree in agricultural sciences at the University of Pisa, Italy "cum laude" in 1980, and a PhD in food science also "cum laude."

She is currently a senior researcher in the Research Center on Food and Nutrition of the Council for research in agriculture and analysis of agricultural economy (CRA-NUT formerly INRAN National Research Institute on Food and Nutrition) and she was the director of the Cereals Research Programme in INRAN. CRA-NUT is a primary research institute in Italy under the egis of the Ministry of Agriculture. Dr. Carcea joined INRAN in 1989 after having worked in Italian and English universities (Queen Elizabeth College, King's College, and University of London) and after a two-year contract with the Food and Agriculture Organization (FAO) of the United Nations (UN), Rome.

She has a vast experience in the field of research on foods, cereals in particular. In recent years, her main research interests have been: chemical characterization and study of the functional properties of cereal components; study of the interactions between components and of the interrelationships between the biochemical properties of components and the technological properties of the raw material and derived products; development of new, cereal-based products; development of methods to assess technological parameters of the raw material; nutritional value of cereals; and developments of protocols for quality assurance of cereals, food authenticity.

She has taken part and/or co-ordinated several research projects within national or international programs (European Commission, FAO) involving several institutions. She is the author of more than 160 scientific publications, mostly in international journals, eight book chapters, and two scientific books. She delivered lectures on her research activity at about 150 national and international congresses and she seats in several national and international committees (Italian Ministry of Agriculture, Codex Alimentarius, and European Commission) regarding food and nutrition topics. She is also a member of the editorial board of scientific journals.

From 1994 to 2006, she has also been a lecturer of food science and technology at the University of Tor Vergata, Rome, Italy.

She is a founding member of AISTEC, the Italian Association of Cereal Science and Technology. Since 1996, she is an elected member in the Executive Committee of the same association and since 2009, president of the association.

Since 2000, she is the Italian National Delegate of the International Association for Cereal Science and Technology (ICC) and she was also the president of the same association for 2011–2012.

In 2004, she was the first woman to be awarded the International Harald Perten Prize for her excellent research achievements in the field of cereal science and technology.

She is also a member of the Georgofili Academy in Florence, Italy.

Lawrence J. Cheskin graduated from Dartmouth Medical School and completed a fellowship in gastroenterology at Yale–New Haven Hospital. He is an associate professor of health, behavior, and society at the Johns Hopkins Bloomberg School of Public Health, with a joint appointment in International Health–Human Nutrition, and in medicine (GI) at the Johns Hopkins University School of Medicine. Dr. Cheskin is also a founder and director of the Johns Hopkins Weight Management Center, a comprehensive treatment program for obesity.

In his research, Dr. Cheskin has studied the effects of medications on body weight, the gastrointestinal effects of olestra, how cigarette smoking relates to dieting and body weight, and the effectiveness of lifestyle and dietary changes in weight loss and weight maintenance.

He is also the author of four books: *Losing Weight for Good*, *New Hope for People with Weight Problems*, *Better Homes and Gardens' 3 Steps to Weight Loss*, and *Healing Heartburn*. Dr. Cheskin has appeared on television news programs and lectured to both professional and lay audiences on the topics of obesity and weight control.

Nigel Cook is a graduate of the University of Dundee. After postdoctoral research in the Universities of Aberdeen and Leicester, he moved to the Central Science Laboratory (now the Food and Environment Research Agency (FERA)) at the Food Science Laboratory, Torry, Aberdeen in September 1994, before relocating to new facilities in York. At FERA, he studies the transmission of pathogens, particularly enteric viruses, through foods and the environment. He has a visiting professorship at the Katholieke Universiteit Leuven in Belgium. He is a councilor of the International Association for Food and Environmental Virology. He is a project leader within the standardization working group ISO TC34 SC9 WG6, currently developing a standard for detection of *Cryptosporidium* and *Giardia* on berry fruits and leafy green vegetables. He was a coordinator of the European Framework 7 project "Integrated monitoring and control of foodborne viruses in European food supply chains (VITAL)," and a chair of COST Action 929 "A European Network for Environmental and Food Virology" from 2006 to 2010. Between 2009 and 2014, he was a member of various European Food Safety Authority's Working Groups preparing opinions on the risk of foodborne viruses, and represented the European Communities on the Codex Committee on Food Hygiene Working Group developing *Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food*. He was a member of the UK Advisory Committee on the Microbiological Safety of Food's Viral Infections Subgroup. He was the founding editor of the journal *Food and Environmental Virology*, published by Springer.

Luca Simone Cocolin graduated in 1994 in food science with a grade of 110/110 and remark followed by food biotechnology PhD studies from 1995 to 1998. In February 1999, he defended his thesis acquiring the title of PhD in food biotechnology. From 1998 to 2001, he received a scholarship from the Friuli Venezia Giulia region (Italy). From November 1, 2001, he was an assistant professor at the University of Udine, Faculty of Agriculture, Food Science Department, Italy, and in October 1, 2006, he became an associate professor at the University of Torino, Italy. In January 2014, he had the habilitation for full professor and from June 2015, he is the full professor in food microbiology at the University of Torino.

From September 2008, he is an executive board member of the International Committee on Food Microbiology and Hygiene (ICFM) part of the International Union of Microbiological Societies (IUMS) (http://www.icfmh.org/). From January 2008, he is the editor-in-chief of the *International Journal of Food Microbiology* and he is a member of the editorial board of *Applied and Environmental Microbiology*, *Food Analytical Methods*, *Frontiers in Food Microbiology*, and *Frontiers in Nutrition and Food Science Technology*. He regularly reviews paper for *Food Microbiology*, *Meat Science*, *Journal of Applied Microbiology*, and *Letters in Applied Microbiology*. He is a co-author of more than 180 papers on national and international journals and he attended national and international congresses with oral and poster presentations. On Scopus (www.scopus.com, consulted on March 2015) he has 172 documents reviewed, which were cited 3520 times, resulting in an h index of 33.

His scientific interests comprise:

development, optimization, and application of molecular methods for the detection, quantification, and characterization of foodborne pathogens;
study of the microbial ecology of fermented foods (mainly sausage, cheese, and wine) by using culture independent and dependent methods;
bioprotection: molecular characterization of bacteriocin production and its study *in vitro* and *in situ*;
selection of new putative probiotics from artisanal fermented foods; and
study of the human microbiome and its influence on human health.

Christopher Duggan, for the past 25 years, has been performing clinical trials in the fields of pediatric nutrition, gastroenterology, and global health. His early work centered on the management of diarrheal diseases in children, including trials that demonstrated the feasibility and efficacy of oral rehydration solutions (ORS) for diarrhea management in the United States and globally. In collaboration with colleagues at Harvard TS Chan School of Public Health and Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania, Dr. Duggan and colleagues are evaluating the efficacy of micronutrient supplementation in infants and young children born to women with or at risk of HIV infection. Recent studies include the development of new biomarkers of environmental enteric dysfunction as well as the evaluation of nutritional status on neurodevelopment. With colleagues at St John's Research Institute in Bangalore, India, he is evaluating the efficacy of maternal vitamin B12 supplementation on biochemical and clinical parameters during pregnancy and infancy. He is a course co-director of the Bangalore, Boston Nutrition Collaborative (http://bbnc.globalhealth.harvard.edu). Past and present research support has come from the National Institutes of Health, the Gates Foundation, and the World Health Organization.

In addition to his global health research interests, he is a pediatric gastroenterologist and a nutrition physician at Boston Children's Hospital where he directs the Center for Nutrition (http://www.childrenshospital.org/nutrition). He is a medical director of the Center for Advanced Intestinal Rehabilitation, one of the largest centers in the United States for the care of children with intestinal failure/chronic diarrhea syndromes (http://www.childrenshospital.org/cair). He is also the course co-director of an inaugural Harvard College course "Nutrition and Global Health" and mentors undergraduate, graduate, and postdoctoral students at HMS and HSPH (http://www.hsph.harvard.edu/nutrition-and-global-health/).

He is a professor of pediatrics at Harvard Medical School and a professor in the Department of Nutrition at the Harvard TS Chan School of Public Health (http://www.hsph.harvard.edu/faculty/christopher-duggan/).

Jed William Fahey's current research concerns elucidating the mechanisms of how plants protect themselves against unfavorable and stressful conditions, and how this understanding can be translated to chemoprotection of eukaryotic mammalian systems. This work draws on elements of natural product chemistry, enzymology, nutritional epidemiology, and clinical research in order with isothiocyanates (e.g., sulforaphane) and glucosinolates. His work led to the discovery that broccoli sprouts are an exceptionally rich and consistent source of phytochemicals that induce the detoxification of carcinogens, and to the development of methods for their detection and for assessing their metabolism in humans. He discovered that one of the inducers, sulforaphane, has potent antibiotic activity against *Helicobacter pylori*, a causative agent of peptic ulcer and stomach cancer, and followed up with trials in animals and in *H. pylori*-infected humans. Ongoing collaborations examine the effects of broccoli, Moringa, and the other plants and their phytochemicals against a range of chronic diseases. Dr. Fahey has for years taught courses in chronic disease prevention and nutrition at both medical and public health schools.

Manuel Franco is an associate professor at University of Alcalá in Madrid (Spain) where he leads the social and cardiovascular epidemiology research group (http://www3.uah.es/cardiosocialepi/). He is also an adjunct professor at Johns Hopkins University (Baltimore).

Prof. Franco's work focuses on the social determinants of cardiovascular diseases and its major risk factors as diet. His methodological interests include the measurement of the urban environment and large social and economic changes in relation to cardiovascular health. He is the lead investigator of the Heart Healthy Hoods, study funded by the European Research Council, that will study the urban environment in relation to cardiovascular health in Madrid (http://hhhproject.eu/). This longitudinal study will be collecting neighborhood level data (via audits, Google Street view, photovoice, and qualitative methods) and linking them to clinical outcomes collected from patients enrolled at the City of Madrid primary healthcare clinics. Prof. Franco trained in Spain and Germany to obtain his MD and obtained his PhD from Johns Hopkins Bloomberg School of Public Health working with Dr. Ana Diez-Roux in the MESA study on food environment and dietary patterns. He has published over 30 international high impact articles and collaborates with universities in the United States, Europe, and Latin America.

Maria Glibetic is a research director of Centre of Research Excellence in nutrition research, Institute for Medical Research in Belgrade, University of Belgrade, Serbia, and member of executive board of directors for food data association EuroFIR AISBL. She is an experienced basic and nutritional scientist with over 250 scientific publications and presentations. Maria has considerable experience in leading national and international projects and since 2006, she participated in nine EU funded projects including EuroFIR, EURRECA, BaseFOOD, CHANCE, BACCHUS, and ODIN. Maria and her team are responsible for the creation of the first online national food database, for designing food data management system, and for the development of different nutritional tools for intake analysis. She was a principal leader of many nutrition intervention studies evaluating the plant bioactive component effects on human cardiovascular health. She leads postgraduate department for integrated nutritional sciences at University of Belgrade, where she teaches two courses.

Linda Harvey obtained her PhD from the University of East Anglia, UK. She is currently the head of the Human Nutrition Unit at the Institute of Food Research, Norwich, UK. Her research interests include micronutrient requirements, bioavailability, and metabolism.

Ronald Jackson received his bachelor's and master's degrees from Queen's University and doctorate from the University of Toronto. His time in Vineland, Ontario, and subsequent sabbatical at Cornell University, redirected his interest in *Botrytis* toward viticulture and enology. As part of his teaching duties at Brandon University, he developed the first wine technology course in Canada. For many years he was a technical advisor to the Manitoba Liquor Control Commission, developing sensory tests to assess candidates of its sensory panel, and was a member of its external tasting panel. He is the author of *Wine Science: Principles and Applications, 4th edition* (2014), *Wine Tasting: A Professional Handbook, 2nd edition* (2009), *Conserve Water, Drink Wine*, and chapters and technical reviews in other multiple books and encyclopedia. He is retired in Bronte, Ontario, but remains active writing, cycling, doing yoga, and traveling, as well as being a fellow in the Cool Climate Viticulture and Oenology Institute, Brock University, St. Catharines, Ontario, Canada.

Joe P. Kerry is a senior college lecturer and the head of the food packaging research group in the School of Food and Nutritional Sciences at University College Cork (UCC). He received his doctorate in microbiology at University College Galway in 1995. Prof. Kerry is also a qualified member of the Institute of Packaging. He is very involved in national and international research projects both at fundamental and applied levels. Primary research interests address various aspects of food packaging, shelf-life stability, food composition, and numerous aspects of food quality, particularly in relation to muscle foods. He also has very strong links with industry and his research team assists companies in relation to many aspects of new food product development. He has over 220 publications in peer-reviewed international journals, over 300 presentations at major international conferences, along with several other significant publications. His expertise includes use and manipulation of modified atmosphere packaging systems for use with foods, use of extrusion technology for the manufacture of food products/packaging materials, and applications and sensor/new technology developments within the area of food packaging, especially in the area of smart packaging.

Frédéric Leroy, after studying bio-engineering at Ghent University, obtained a PhD in applied biological sciences at the Vrije Universiteit Brussel in 2002, where he continued his academic career at the research group of Industrial Microbiology and Food Biotechnology (faculty of sciences and bio-engineering sciences). As associate professor, his lecturing activities include courses in food science and technology (i.e., "Nutrition," "Technology of animal products," "Food microbiology and ecology," and "Quantitative and predictive microbiology"). Dr. Leroy's research primarily deals with the ecology and functional roles of bacterial communities in (fermented) foods, in particular with respect to the generation of quality, safety, and/or nutritional and health advantages. Focus is mostly on meat products, but other food systems are also being studied, including fermented milks and sourdough breads. In addition, his research interests relate to elements of tradition and innovation in foods, both from a technological and societal point of view.

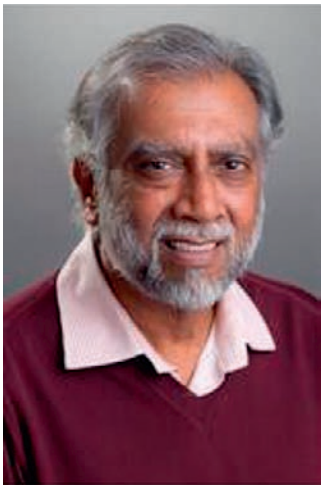
Catherine M. Logue completed her undergraduate and postgraduate degrees in Ireland and earned a PhD in meat microbiology from the University of Ulster, UK in 1996. Dr. Logue was a faculty member at North Dakota State University from 1999 to 2011 rising through the ranks of assistant to associate and full professor. In 2011, she re-located to Iowa State University's College of Veterinary Medicine and is a professor of veterinary microbiology and preventive medicine. Dr. Logue is also the director of faculty and staff advancement and equity for the college. Her research interests focus on foodborne pathogens of food animals and the contamination of meat and meat products destined for human consumption. Her research studies the detection, isolation, and characterization of a range of foodborne pathogens such as *Salmonella*, *Campylobacter*, *Listeria*, *Escherichia coli*, and methicillin-resistant *Staphylococcus aureus* (MRSA) in poultry, bovine, and swine. She also focuses her research on antimicrobial resistance in commensals and pathogens of production animals. She has been an author and a co-author on more than 90 peer-reviewed papers and book chapters as well as more than 150 abstracts and presentations at national and international meetings.

F. Xavier Malcata graduated in chemical engineering in 1986 from the University of Porto (Portugal), received a PhD in chemical engineering/food science from University of Wisconsin, Madison (USA) in 1991, and his habilitation in food science and engineering by Portuguese Catholic University in 2002. He was the dean of College of Biotechnology of Portuguese Catholic University, the chairman of Portuguese Society of Biotechnology, Portuguese representative at VI and VII European Union Framework Programs of research and development, expert for European Food Safety Agency, and a co-ordinator of Portuguese Engineering Accreditation Board in chemical engineering for Northern Region. He is currently a full professor at University of Porto.

His major research interests have focused on technological improvement of traditional Portuguese foods and upgrade of byproducts thereof, development of nutraceutical ingredients and functional foods, design and optimization of enzymatic reactors for edible oil processing, characterization of plant proteases toward cheese and whey cheese manufacture, production of starter and nonstarter cultures from adventitious microflora, and optimized application of unit operations to food processing.

With an academic career of independent research and teaching for more than two decades, Prof. Malcata published more than 450 papers in refereed journals worldwide, wrote 11 books, and prepared more than 45 chapters for edited books. Among many international distinctions, he was

recipient of Ralph H. Potts Memorial Award in 1991 by American Oil Chemists' Society (AOCS, USA), Foundation Scholar Award – Dairy Foods in 1998 by American Dairy Science Association (ADSA, USA), Young Scientist Research Award in 2001 by AOCS, Canadian/International Constituency Investigator Award in physical sciences and engineering in 2002 and 2004 by Sigma Xi (USA), Danisco International Dairy Science Award in 2007 by ADSA, Scientist of the Year Award in 2007 by European Federation of Food Science and Technology (the Netherlands), Samuel C. Prescott Award in 2008 by Institute of Food Technologists (IFT, USA), International Leadership Award in 2008 by International Association for Food Protection (IAFP, USA), Elmer Marth Educator Award in 2011 by IAFP, Distinguished Service Award in 2012 by ADSA, and William V. Cruess Award in 2014 by IFT. He has been elected for the honor societies of food science (Phi Tau Sigma, USA), scientific research (Sigma Xi, USA), and engineering (Tau Beta Pi, USA). He was also elected for fellow of IFT, ADSA, AOCS, and International Academy of Food Science and Technology.



Gopinadhan Paliyath is a professor at the Department of Plant Agriculture, University of Guelph, and the research program director for "Food for Health," under the UG/OMAFRA partnership. Dr. Paliyath is a biochemist and has an interest in various aspects of fruits and vegetables, specifically the nutraceutical components and their mechanism of action. He obtained his BSc Ed degree (botany and chemistry) from the University of Mysore, MSc degree (botany) from the University of Calicut, and PhD degree (biochemistry) from the Indian Institute of Science, Bangalore. Subsequently, he did postdoctoral work at Washington State University, University of Waterloo, and University of Guelph. Dr. Paliyath's research is focused on the biochemistry of plant senescence, specifically pertaining to postharvest biology and technology of fruits and vegetables. Investigations on the role of phospholipase D (PLD) in membrane homeostasis and signal transduction have led to advances in the understanding of the mechanism of membrane deterioration that occur during stress and senescence. Another aspect of his research is focused on understanding the mechanism of action of food components in disease prevention. The efficacy, bio-accessibility, bioavailability, and molecular mechanisms of action of nutraceuticals in fruits and processed products in relation to their cancer-preventive and anti-inflammatory actions are being investigated using mammalian cell lines, and animal model systems.

Dr. Paliyath has developed technologies and products for enhancing the shelf life and quality of fruits and vegetables based on PLD inhibition. R&D activities relevant to the industry sector include: (1) optimization of an enhanced freshness formulation for application to various fruits, vegetables, and flowers; (2) developing methods for nutraceutical carriers that would enhance the functional food quality and delivery (e.g., stabilizing lycopene in tomato juice, sauce, etc., for health beneficial effects); and (3) developing novel technologies to enhance the cancer-preventive ingredients in fruit products, etc. Patents awarded include: (1) # 6,514,914 (US) and 2,298,249 (Canada); (2) #7,198,811 (USA), 4141387-1 (Japan), 260738 (Mexico), 1469736 (Turkey), 028 284763 (China), and 223077 (India). The patents describe the use of nanoformulations based on hexanal and other generally regarded as safe (GRAS) ingredients for enhancing the shelf life and quality of fruits, vegetables, and flowers by pre or postharvest treatments. These technologies are currently being evaluated for extending shelf life and quality of mango in India and Sri Lanka with the assistance of the Canadian International Food Security Research fund. The collaboration involves researchers from Canada, India (Tamil Nadu Agricultural University), and Sri Lanka (Industrial Technology Institute).

Dr. Paliyath is also the research program director for the food and health theme-related activities under the OMAF/MRA/University of Guelph research partnership. He serves on the editorial board of several journals. He is a member of American Chemical Society and Canadian Society of Plant Biologists.

(Total-refereed publications in journals – 92; patents and intellectual properties – 2; disclosures – 4; chapters in books – 27; nonrefereed contributions – 10; research reports – 28; conference proceedings – 88; edited books – 9; book reviews – 6 (Google Scholar: h index – 31, i10 index – 68, citations – 4332; RG score – 35.63)).

Yolanda Picó is a full professor of nutrition and food science at the University of Valencia since 1998. She is currently the head of the research group on food and environmental safety of the University of Valencia. Her research interests are the development of new analytical methods to determine organic contaminants in food and the environment, identification of unknown compounds by liquid chromatography–mass spectrometry, micro-extraction separations, and environmental and food safety. To the date, she is the author of nearly 200 peer-reviewed papers, 170 scientific papers in journals of SCI, 25 book chapters, and editor of four books on food and environmental safety.

Vieno Piironen is a professor of food chemistry at the Department of Food and Environmental Sciences, University of Helsinki, Finland. She received her PhD in food chemistry at the University of Helsinki in 1987 and has approximately 35 years of experience in food research and education on bachelor, master, and PhD levels. She has participated actively in international research and education projects and networks. Her research has focused especially on chemical and nutritional properties, reactions and analysis of lipids, vitamins, and other bioactive compounds. Research on vitamins has been active from the beginning of 1980s. She has studied both lipid- and water-soluble vitamins; their chemical and nutritional properties and importance in foods and diets as well as factors influencing vitamin levels. In addition, development of analytical methods for different vitamers as well as validation and harmonization of the methods through international collaboration have been among the priorities. Recent collaboration projects have focused on enhancing vitamin contents in cereal-based foods by plant breeding, utilization of vitamin-rich grain fractions, and bioprocessing. Currently, the research focus lies on investigating microbial *in situ* synthesis of folate, vitamin B12, and other B vitamins in cereal and legume matrices as a means to improve nutritional quality of foods and to develop new food applications. In lipid research, different lipid classes and their chemical and enzymatic reactions in food matrices are studied. Diverse methods are used to study proceeding of oxidation from primary products to monomeric oxides, volatiles, and polymerization products and to study possibilities to control oxidation. Controlling enzymatic reactions leading to off-flavors in cereal and legume matrices is also among the interests. Phytosterols and their conjugates have been studied as natural food components belonging to the dietary fiber complex. On the other hand, questions related to sterol enrichment such as oxidation susceptibility and mechanisms as well as factors affecting oxidation reactions have been of interest. She has also studied nutrients and anti-nutrients in legumes and more recently started research on utilization of high value components in microalgae. She has approximately 160 papers in international journals and a number of other publications.

David Rodríguez-Lázaro is a doctor in veterinary medicine (DVM), specialized in food science (BSc and MSc) and molecular microbiology (PhD). He is a senior scientist at ITACyL and an assistant professor of microbiology at the University of Burgos. He has performed research stays in the Danish Institute for Food and Veterinary Research (Denmark), the University of Prague (Czech Republic), the Food and Environmental Research Agency (UK), and the University of Bristol (UK). He was a Leverhulme visiting professor in the Institute of Advanced Sciences in the University of Bristol during the years 2004 and 2005 and Marie Curie research fellow in the faculty of medical and veterinary sciences in the University of Bristol (UK) until 2007.

His research interest is focused on the establishment of reliable, quantitative molecular strategies for detection of important food-borne pathogens from environmental sources and various types of foodstuffs, the characterization of the prevalence of the main foodborne pathogens in food and food-related environments, and the development of emergent food preservation processes and their effects in the microbial virulence. He has participated in a number of coordinated EU-funded projects such as PROMISE, BASELINE, VITAL, FOOD-PCR, SACROHN, and MONI-QA, having established active links with the leading European research groups working in food safety.

He has published more than 100 international scientific papers and book or book chapters regarding food safety. He is currently a member of the editorial board of *Applied and Environmental Microbiology*, *International Journal of Food Microbiology*, *Food and Environmental Virology*, and *International Journal of Food Contamination* and the editor-in-chief of the journal *Food Analytical Methods*. He was awarded with the XV Jaime Ferrán Award in 2013 by the Spanish Society for Microbiology for his promising scientific career in microbiology.

Turid Rustad is a professor and the head of the food science group at the Department of Biotechnology, Norwegian University of Science and Technology. The main research focuses on the biochemistry of marine raw materials, the relationship between biochemistry and quality, and changes in raw material properties during processing. Studies of enzymatic activities in different raw materials have been linked to studies of changes in the biochemistry of these raw materials. She has worked with characterization of composition and enzymatic processes in a wide range of different raw materials, such as fish, fish by-products, and zooplankton in relation to different storage and processing methods such as chilling, heating, superchilling, and frozen storage.

Noel W. Solomons has lived and worked in Guatemala for 40 years. He was born and educated in Massachusetts in the United States. As a young child, he became an amateur naturalist and was a nature counselor at various summer camps; this would guide him to a career in science. In his young adulthood, he would participate in the civil rights and anti-war movements, only to become disillusioned by the intractable nature of the injustice elements in the fabric of American society. As a physician by graduate training, he performed his university studies at Harvard College and Harvard Medical School; it was during overseas electives in his medical training that he visited Peru and Colombia and committed to an expatriate life trajectory outside of his homeland. Clinical training included a residency in internal medicine and infectious diseases at the Hospital of the University of Pennsylvania and specialization in gastroenterology and clinical nutrition at the University of Chicago. He became a resident of Guatemala in 1974 as an affiliated investigator at the Institute of Nutrition of Central America and Panama. He would later commute for eight years to a faculty position in the Department of Nutrition and Food Science of the Massachusetts Institute of Technology. Assuming a full-time Guatemala commitment in 1985, he co-founded the Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM) where he remains its scientific director. Over 40 local university theses have been completed by Central American students in that institution as well as an equal number of master's degree research projects from international students from Europe, and North and South America. He has supervised doctoral dissertations for 12 PhD candidates from the United States, Canada, Germany, Spain, and the Netherlands through CeSSIAM.

Dr. Solomons has 332 publications indexed on Medline. In addition, he has edited two books and contributed over 100 articles, reviews, editorials, and commentaries in nonindexed venues and over 50 book chapters. These are dedicated to the scientific and academic interests of his career including: clinical nutrition; human growth and body composition; lactose maldigestion; dietary intake, nutritional status, intestinal absorption, and food fortification related to various micronutrients (vitamins, trace elements, and essential fatty acids); complementary feeding; nutrition in aging and chronic disease; and the interaction of malnutrition and infection.

Among the honors bestowed upon Dr. Solomons are the International Nutrition Prize of the International Union of Nutritional Sciences and the Kellogg Prize of the Society for International Nutrition Research. He is a fellow of the American Society of Nutrition. He is an academic member of the Guatemalan Academy of Medical, Physical and Natural Sciences and the Spanish Academy of Nutrition and Food Science. He was the awardee of the 2010 National Medal for Science and Technology for Guatemala.

He has been a visiting professor in university courses in Mexico, Peru, Brazil, Indonesia, and Spain. He currently holds adjunct professorial appointments at the Boston University School of Public Health, and the Friedman School of Nutrition Science and Policy and the Department of Community Medicine and Public Health, both at Tufts University. He is a founding board of directors, member of the Hildegard Grunow Foundation in Munich and the Essential Nutrient Foundation of Singapore. Finally, Dr. Solomons is a coordinator for Central America of the Nevin Scrimshaw International Nutrition Foundation in Boston, and an associate editor for the Foundation's *Food and Nutrition Bulletin*. He serves on editorial boards for ten scientific journals.

Maria Tsimidou is a professor of food chemistry and the head of the Laboratory of Chemistry and Technology in the School of Chemistry at the Aristotle University of Thessaloniki (AUTh), Greece. Her teaching is food chemistry, analysis, quality control, and food legislation. Research interests are related to virgin olive oil chemistry, quality and authenticity, saffron chemistry, authenticity and quality, antioxidant activity of plant extracts and constituents, new sources of targeted bioactive compounds (squalene, carotenoids, and phenols), and analytical procedures for their determination. She has published many research papers, review articles, and contributions to scientific books and encyclopedias on the above-mentioned topics. Currently, she is an associate editor in the *European Journal of Lipid Science and Technology* and chairs the COST ACTION FA1101 "Saffronomics."

Jorge Welti-Chanes earned his degree in biochemical engineering (1976) and master of science in food engineering (1978) at Tecnológico de Monterrey (ITESM, Mexico), later he moved to Spain to perform his doctoral studies in chemistry, in the area of food technology, obtaining his degree at the University of Valencia. He is currently the national director of graduate studies at School of Engineering and Sciences at Tecnológico de Monterrey also is professor and researcher in the areas of biotechnology and food at the same institution. He started his academic activity in 1976 as a university professor of ITESM, has additionally been a full professor at the National Polytechnic Institute (IPN, Mexico) and the University of the Americas, Puebla, Mexico (UDLA). He has an experience of 37 years as a teacher and university researcher, 20 of which were spent in combination with the development of administration work in education, science and technology. In the UDLA, he was teaching in the Departments of Chemistry and Biology and Chemical Engineering and Food, in the latter was responsible for the leadership for a period of a year and subsequently became dean of the School of Engineering (1986–1988). From January 1989 to June 2002, he was an academic vice chancellor at UDLA. He has published 14 books and has over 200 scientific publications in refereed journals and books, has given more than 250 presentations at international conferences. He is an associate editor of the journals *Food Engineering Reviews* and *Journal of Food Science* and participates as a member of the editorial boards of *Journal of Food Engineering* and *Current Opinion in Food Science*. In May 2011, he received the Life Achievement Award by the International Association for Engineering and Food (IAEF), for his career as a researcher and academic worldwide, and in January 2014, the Romulo Garza Award from the Tecnológico de Monterrey for the impact of their research work and as recognition for being one of the most productive researchers in the life of Tecnológico de Monterrey. He has been the president of ISOPOW and IAEF and is the currently president of the International Society of Food Engineering (ISFE).

Peter J. Wilde graduated in biophysics at the University of East Anglia in 1985 and has been researching the colloidal and interfacial properties of food systems at the Institute of Food Research (IFR) for over 25 years. IFR is the only publicly funded UK research institute that focuses on the underlying science of food and health to address the global challenges of food security, diet, and health, healthy aging, and food waste. IFR is the one of eight institutes that receives strategic funding from the Biotechnology and Biological Sciences Research Council (BBSRC). It also receives funding from government agencies and departments, the EU, charities, and industry, from the UK and overseas.

Pete's research expertise is the interfacial behavior of proteins and other surface active components in food relevant systems. The aim is to determine how the molecular and interfacial processes control the functionality of foams and emulsions. Currently, the functional aspects of his research have focused on improving the dietary impact of emulsified foods. These include fundamental studies on how interfacial layers control emulsion rheology to develop novel fat reduction strategies; the design of interfacial structures to control lipid digestion to promote satiety or the delivery of fat-soluble nutrients and drugs; and to determine the physico-chemical role played by the salivary film in perceiving fat content in emulsions. The impact of this research will be to aid the rational design of foods with enhanced nutritional benefits to address the global challenges of obesity, type 2 diabetes, and other major diet-related conditions.

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HOW TO USE THE ENCYCLOPEDIA

All articles in the encyclopedia are arranged alphabetically as a series of entries.

1. Contents

Your first point of reference will likely be the contents. The complete contents list appears at the front of each volume providing volume and page numbers of the entry. We also display the article title in the running headers on each page so you are able to identify your location and browse the work in this manner.

2. Cross-references

The majority of articles within the encyclopedia have an extensive list of cross-references that appear at the end of each article, for example:

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Iron: Biosynthesis and Significance of Heme; Iron: Physiology of Iron.

3. Index

The index provides the volume and page number for where the material is located, and the index entries differentiate between material that is a whole article; is part of an article, part of a table, or in a figure.

4. Contributors

A full list of contributors appears at the end of volume 5.

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INTRODUCTION

Until a few decades ago, virtually all known health effects of foods were related to their content of essential nutrients. The clinical description of most diet-related illnesses mirrored the signs of essential nutrient deficiencies, such as pellagra, beriberi, and others. Consequently, the key public health concern regarding diet was ensuring that everyone consumed enough food. It was only in the past 50 years that large-scale epidemiological observations began to associate chronic diseases like diabetes and cardiovascular disease with nonessential diet constituents such as saturated fat, fiber, and cholesterol. Taking advantage of the emergence of digital informatics, these studies were able to manipulate increasingly large sets of data and provide, for the first time, a picture of the secular changes in the health of large populations and its association with what they ate regularly. These findings progressively shifted the concern from eating enough to avoiding excessive consumption of certain foods. Eating enough was replaced by eating well.

But it turned out that defining how to eat well is far more complex than defining minimum needs of essential nutrients. First, there is no single paradigm to study those relationships, given the wide variety of biological mechanisms and the long exposures involved. Second, many of the experimental models used to define essential nutrient needs are not applicable to the study of long-term effects of diets in free-living populations. And it is now clear that experiments with isolated dietary compounds do not reflect the actual effects of the complex food matrix we consume daily. Finally, while the discovery of essential nutrients and their role in health was the domain of a few specialties speaking a common language (primarily biochemists and physiologists), the study of the long-term effects of whole diets in humans must of necessity involve epidemiologists, social and behavioral scientists, food scientists, clinicians, policy experts, etc., making far more difficult the development of consensus and foundational concepts.

It is thus not surprising that today we have still not achieved a stable consensus on how to eat 'well.' Furthermore, while few nonscientists would care about the minimum requirement of a vitamin to sustain life, there are plenty of opinions among nonscientists on how to eat 'well.'

Our goal in preparing this encyclopedia has been to contribute to the understanding of that complex diet–health relationship by providing a multidisciplinary, integrative and accurate source of information. We aim to serve the needs not only of established and in-training scientists, but also of the increasingly important group of professionals who are key to disseminate and sustain the practice of science: journalists, science writers, science administrators, fund raisers, donors, and policymakers. In preparing this work, we had the enormous advantage of working with one of the publishers with the most extensive expertise in major reference works, Elsevier. This first edition builds on the impressive breadth of knowledge of over 922 authors and on the tireless work of our editorial advisory board. We are very grateful to all of them.

Benjamin Caballero
Paul Finglas
Fidel Toldrá

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Chemometrics

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Introduction

Chemometrics can be defined as the chemical discipline, which makes use of mathematical, statistical, and logical methods to extract meaningful information from experimental data and to optimize processes and/or products. It is evident from this definition that it accompanies all stages of the chemical measurement process, from sampling to interpretation passing through the definition of the optimal experimental conditions and data collection and processing. Even if it finds its roots in analytical chemistry, chemometrics is highly interdisciplinary and its domain of application is becoming wider and wider. However, since the very beginning, food-related issues have constituted an important field of application of chemometric techniques. Indeed, foodstuffs are rather complex matrices and their authentication often relies on a holistic characterization through the measurement of different chemical indexes or through the recording of whole instrumental fingerprints. The construction of traceability models for the verification of labeling compliance of products with a designated origin, the monitoring and control of food production processes, the correlation of volatile compound profiles with the sensory evaluation of a trained panel, and the indirect quantification of one or multiple analytes based on cheap and rapid spectroscopic techniques are only a few emblematic examples of application where the use of chemometrics is not only advisable but also necessary.

Chemical Data: Types and Representation

As anticipated, the main goal of chemometrics is to extract useful and meaningful information from chemical data. Therefore, prior to illustrating the chemometric toolbox with all its plethora of methods, it is essential to understand the data, which constitute the basis for modeling. A chemical system is usually characterized and described by a set of measured or calculated variables, which can be either quantitative or qualitative in nature. In the former case, variables are defined on a numerical (interval or ratio) scale, can undergo any kind of algebraic operation, and can assume an infinite (continuous) set of values; examples of these types of indices are pH, concentration, and temperature. On the other hand, qualitative variables may assume only a discrete set of values, which are often categorical or expressed in the form of attributes: the presence or absence of a constituent, genuine/adulterated, and sensory panel evaluation on a 1–5 scale are all examples of such kind. Usually, one variable only is not enough to characterize a chemical system, so that multiple descriptors are recorded on the same sample: these may come from different instruments or analytic procedures (e.g., total acidity, peroxide number, and concentration of iron or calcium) or be acquired with the same platform (e.g., absorbances at different wavelengths or detector intensities at different retention times, in the case of spectroscopic or chromatographic profiles, respectively). The resulting data are then *multivariate* and each sample may be described by a numerical row vector $\mathbf{x}_i$, collecting the results of all the measurements performed:

$$\mathbf{x}_i = \left[x_{i1}x_{i2}x_{i3}\ldots x_{ip}\right] \qquad [1]$$

p being the total number of variables. Accordingly, when more than a single sample is analyzed, the data can be arranged into a matrix $\mathbf{X}$, having as many rows as the number of samples (n) and, as already stated in eqn [1], as many columns as the number of variables (p):

$$\mathbf{X} = \begin{pmatrix} x_{11} & \ldots & x_{1p} \\ \vdots & \ddots & \vdots \\ x_{n1} & \ldots & x_{np} \end{pmatrix} \qquad [2]$$

Each row of the matrix $\mathbf{X}$, then, contains the results of the experimental measurement performed on a particular sample, while each column is made of the values of a single variable along all the samples.

One of the advantages of the matrix representation in eqn [2] is that it has a geometric counterpart: Each variable can be thought as an axis in a (hyper-)space having p dimensions so that the value of the matrix element x_{ij} represents the coordinate of the ith object along the jth direction. Accordingly, each sample can be described as a point in the p-dimensional space (see Figure 1).

Experimental Design

Although this aspect is often still neglected by many chemists, chemometrics enters the analytical process long before the data processing and interpretation stage, on one hand as it is necessary to sketch the most appropriate sampling strategies in order to have representativeness and to meet specific requirements (e.g., a target accuracy or precision) and, on the other, as the quality of the data obtained and the possibility of retrieving the information sought strongly rely on a careful experimental planning. Indeed, as already pointed out by Fisher, "Statistical procedure and experimental design are only two different aspects of the same whole, and that whole comprises all the logical requirements of the complete process of adding to natural knowledge by experimentation."

By the term experimental design, one indicates the family of techniques whose aim is to identify a parsimonious set of experimental conditions. The aim of experimental design techniques is (a) to understand the effect of controlled variables (factors) on one or more responses (often with the final intent of optimization) and (b) to define an empirical model (response surface) for the relation between the responses (dependent variables) and the factors (independent variables), which may be used either to support the optimization process

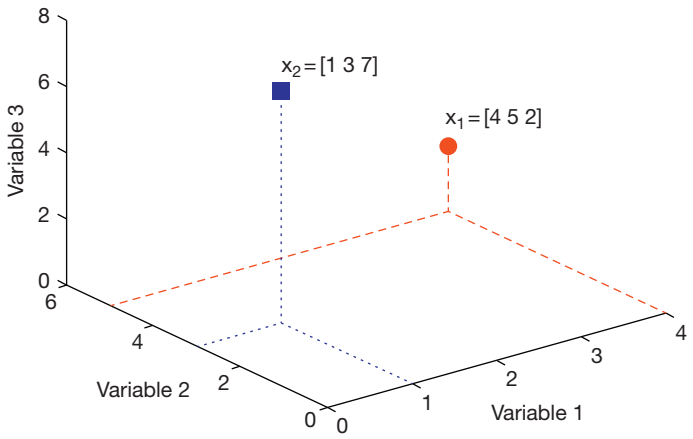

Figure 1 Geometric representation of data vectors as points in the multidimensional space of the variables.

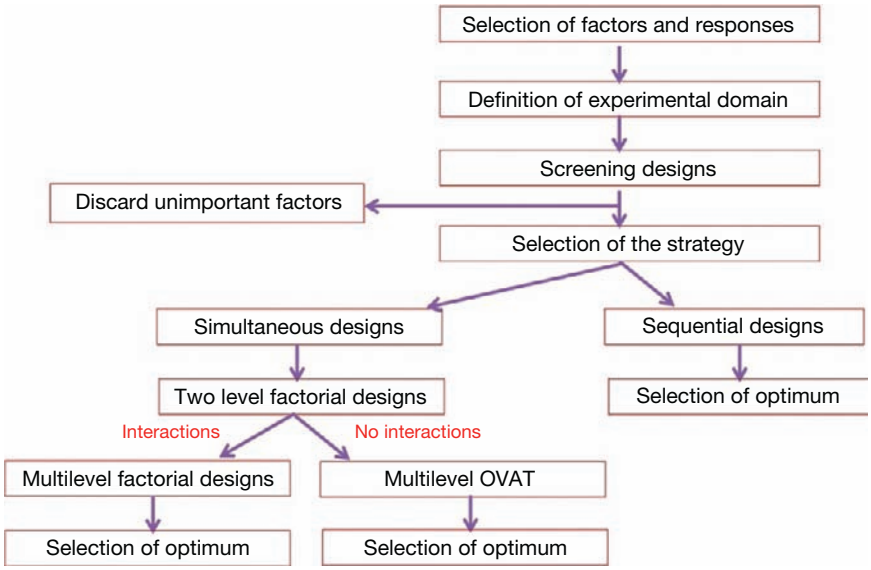

Figure 2 Flowchart of experimental design.

defined in point (a) or for prediction purposes. In both cases, a systematic and efficient mapping of the experimental domain, at the same time looking for the minimum number of experiments to be performed, is sought.

Given the scopes summarized earlier, the family of experimental designs encompasses a wide range of techniques of increasing complexity, as evidenced by the pipeline shown in Figure 2.

Screening designs are the most parsimonious ones, as they include only the experiments needed to estimate in a semiquantitative fashion the main effect of the investigated factors, not taking into account the possible interactions among variables. A linear relation is assumed between the response(s) y and each of the f controlled factors:

$$y = b_0 + b_1x_1 + b_2x_2 + \ldots + b_fx_f \qquad [3]$$

where b_0 is an offset term, corresponding to the predicted response in the center of the experimental domain, while $b_1 \ldots b_f$ are estimates of the effect of the factors $1 \ldots f$ on y. A direct consequence of the assumption of linearity is that only two levels are investigated for each factor and the resulting strategies are on one hand the so-called saturated or supersaturated factorial and on the other the Plackett–Burman designs. Screening designs are normally used to have information on which of possible factors may be discarded as very likely unaffecting the response. After screening, it is necessary to choose the strategy that may be more appropriate for the specific problem: Indeed, there are two options available, the factorial approach and the sequential approach. The sequential strategy is most suited when there are only a few factors, the goal is optimization of (possibly) a single response, and there is no interest in calculating the response surface. It operates by selecting a small number of initial experiments and then iteratively choosing the coordinates for the successive experiment based on the coordinates and the responses of the previous ones, up to when a stopping/optimality criterion is met. On the other hand, in the factorial approach, the whole set of

experiments to be conducted is defined a priori, before any of those is actually carried out: This approach is in general to be preferred, but it is for sure the one to be adopted when the effect of controlled factors on multiple responses has to be investigated and also when modeling is the purpose. In this context, two level factorial designs, that is, factorial designs in which each factor is controlled only at two values, allow to assess the main effect of the factors and at the same time to verify the presence of interactions. Indeed, they assume a linear model with mixed terms, which, in the case of two factors, takes the form

$$y = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 \quad [4]$$

where b_{12} is the term that accounts for the interaction between factor 1 and factor 2, while all other terms have the same meaning as in eqn [3]. Although such mathematical assumption is often too approximate to lead to reliable estimation of the main effects and interaction terms or to accurate predictions of the response values, still, two level factorial designs allow to have a semiquantitative understanding of the relation between dependent and independent variables and to assess the presence or absence of interactions.

When the linearity assumption is released, so that nonlinear (almost always second- or third-order polynomial) relationships are modeled, factors cannot be controlled at two levels only anymore, so that multilevel designs are needed. The use of multilevel designs, the most famous of which are the central composite, the Box-Behnken, and the Doehlert ones, together with the family of optimal designs (D-optimal, A-optimal, and so on), allows to better approximate the response surface, which, in the case of three factors, usually takes the form

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{23}x_2x_3 + b_{13}x_1x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad [5]$$

where b_{11}, b_{22}, and b_{33} account for the effect of the squared factors and all the other terms have the same meaning as in eqns [2] and [3]. Specific designs may also be used when the factors to be investigated are the constituents of a mixture or when they are qualitative variables.

Exploratory Analysis

Exploratory analysis is a strategy/philosophy that makes use of a variety of techniques to summarize the main characteristics of a data matrix in an easy-to-understand form, often with visual graphs, without using a statistical model or having formulated a hypothesis. Its main purposes are to maximize insight into a data set, to uncover underlying structure, to extract important variables, to detect outliers and anomalies, to test underlying assumptions, and to develop parsimonious models. All these characteristics suggest that exploration is and should always be the first step in data analysis, even when the final goal is some kind of predictive modeling.

According to the definitions reported earlier, exploratory data analysis is normally carried out by means of two families of techniques: projection methods and clustering algorithms. The need for the projection method is evident when thinking that, in the situations when many variables are measured on the samples, which is what normally occurs with chemical systems, graphical inspection of the data in the original p-dimensional space described in the section '**Chemical Data: Types and Representation**' may result difficult or even unfeasible. Indeed, as the name suggests, projection methods operate by projecting the data on a relevant subspace, which can be used to summarize the data conveniently and explore the data set using plots and figures, in order, for instance, to find patterns, relation, differences, and similarities between objects and variables. The most famous and used multivariate projection method is for sure principal component analysis (PCA), whose aim is to find the best low-rank approximation of the original data in a least squares sense. This is equivalent to state that PCA identifies those directions in the multivariate space, which in turn account for the maximum explained variance and which are mutually orthogonal, and projects the original data onto this reduced subspace. Mathematically, the PCA model can be expressed by the following equation:

$$\underset{n\times F}{\mathbf{T}} = \underset{n\times p}{\mathbf{X}}\ \underset{p\times F}{\mathbf{P}} \quad [6]$$

where $\mathbf{T}$ is the matrix collecting the coordinates (*scores*) of the sample onto the new reduced (F usually $\ll p$) set of variables (called principal components), while the matrix $\mathbf{P}$ contains the coefficients of the projection (*loadings*). The same relation can be rearranged as in eqn [7], to show the so-called *bilinear* nature of the PCA decomposition:

$$\mathbf{X} = \hat{\mathbf{X}} + \mathbf{E} = \mathbf{TP}^{\mathrm{T}} + \mathbf{E} \quad [7]$$

where the matrix $\mathbf{E}$ (*residuals*) accounts for the portion of the variability originally present in the data, which is not explained by the PCA model and the hat ($\hat{}$) indicates that $\mathbf{X}$ is approximated by the model. Indeed, bilinear modeling, that is, the approach that assumes that a data matrix $\mathbf{X}$ may be approximated by the product of two low-rank matrices, one representing the coordinates of the samples onto a reduced subspace and the other expressing the variable contribution to the definition of the subspace, is one of the core elements of chemometrics. Due to its characteristics, PCA represents the optimal tool to perform exploratory data analysis, as it can provide information about the relationship among samples and among variables, together with the possibility of noise filtering and outlier detection. Graphical representation of the samples in the principal component space (*scores plot*) allows to identify the presence of clusters or groups in the data or to evidence some trends; on the other hand, correlation between the experimental variables or irrelevant descriptors may be assessed by visually inspecting the elements of the loadings matrix (*loadings plot*). An example of the application of PCA in the context of food analysis may be observed in **Figure 3**, where the scores and the loadings plots for a problem involving the authentication of honey samples are shown. In particular, the data set was made on the results of 15 chemical analyses on 73 honey samples coming from different botanical origin (sulla, heather, eucalyptus, chestnut, honeydew, and wildflower).

Inspection of the scores plot in **Figure 3(a)** evidences the presence of different clusters in the data: If no information about the origin of samples were available, one would have stated that there were seven different groups of honey; however, integrating the scores plot with the information available,

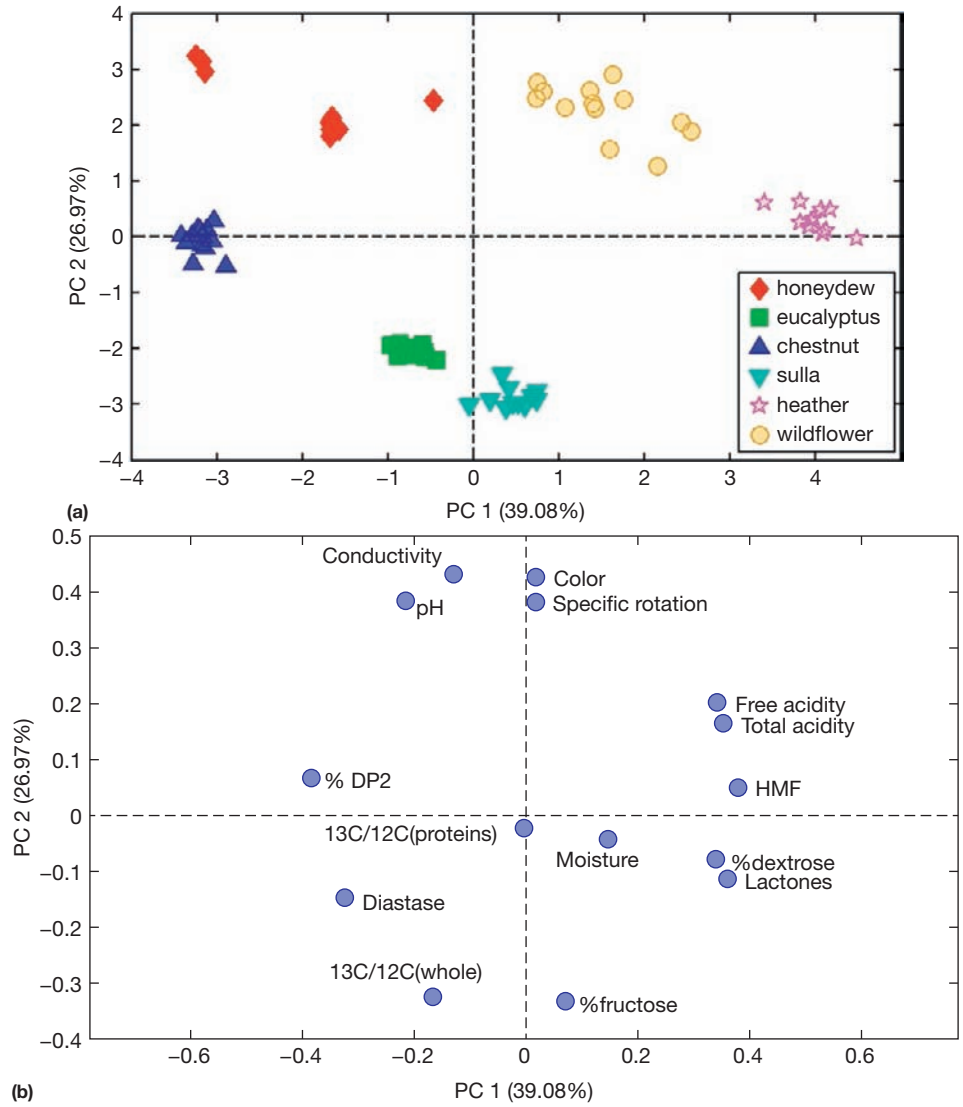

Figure 3 Principal component analysis of honey data set. (a) Scores and (b) loadings plots.

it is possible to affirm that there are six clusters, one for each of the different floral origin of the samples and that the group corresponding to honeydew is further split in two subclusters. Moreover, in general, it is also possible to see how the clusters corresponding to multifloral origin (honeydew and wildflower), which are separated from the unifloral ones along PC2, are less homogeneous, as it could be expected. Information about the variables can, instead, be obtained by looking at the loadings plot reported in Figure 3(b): At first, one can observe that dextrose and lactones are correlated, as well as color and specific rotation or free and total acidity, as they fall very close to one another in the PC space; on the other hand, the carbon isotope ratio in the proteic fraction does not seem to contribute much to the model as its loadings are almost zero. Lastly, comparison of the scores and the loadings plot allows to interpret the differences observed among samples in terms of the original variables. As stated, unifloral honeys are separated from multifloral ones along the second principal component: looking at the loadings plot, this means that the former are characterized by a higher fructose content and carbon isotope ratio, at the same time showing a lower pH, conductivity, color, and specific rotation. On the other hand, wildflower is separated from honeydew along PC1, and the same component differentiates also among the various unifloral honeys: this means that in going from honeydew to wildflower or from chestnut to heather, there are a systematic increase of dextrose, lactones, free and total acidity, and hydroxymethylfurfural (HMF) and a corresponding decrease of diastase and DP2.

The second family of techniques normally used for exploratory data analysis are clustering algorithms. As the name suggests, the aim of clustering techniques is to look for the presence of groups (usually of samples, but it is possible to apply the same concepts to variables or to both) in the data, based on the calculation of some kind of similarity or dissimilarity index. Indeed, the underlying idea is that object belonging to the same group will be similar to one another and dissimilar to the object belonging to other clusters, so that

the definition of a proper measure to quantify (dis-)similarity plays a key role for this family of methods. In many applications, dissimilarity is expressed in terms of a distance measure in the multivariate space, but other indexes may also be used. Once the proper way of quantifying (dis-)similarity is chosen, clustering may be carried out according to two different approaches: hierarchical and partitional. In hierarchical clustering, as the name suggests, objects are ranked from the most similar to the most dissimilar in a hierarchical fashion, and the main result is a graphical representation called a *dendrogram*. In particular, at the beginning of the hierarchical clustering procedure, each sample is considered a cluster per se; at each step, the two most similar clusters are merged together and the procedure is continued until all objects are joined into a single group. This approach is called agglomerative (bottom up) and it is the most widely used: hierarchical clustering may also be carried out in a divisive way (top down), by starting from a single cluster and iteratively arriving to as many groups as the number of objects, but this approach is more computationally intensive and, therefore, it is rarely adopted. Here, it must be stressed that, whatever the approach, hierarchical clustering does not provide a unique grouping: partitioning of object into clusters may be achieved only by cutting the dendrogram at a specific level, which usually corresponds to a large distance among the merged groups. As an example, the dendrogram corresponding to agglomerative hierarchical clustering for the honey data set already presented in the case of PCA is reported in Figure 4.

The dendrogram in Figure 4 shows very clearly the presence of clusters among the data, and also, as already evidenced by PCA, the groups of honeydew and wildflower samples are less homogenous than the other ones (their final merging distance is higher). Moreover, it can be also seen how the identified number of clusters would vary, depending on the level at which the dendrogram be cut: If one chooses a threshold distance of 8 (dashed line), then honeydew samples would be seen as two different groups and a total number of seven clusters would be identified; instead, if a threshold distance of 10 (continuous line) be adopted, then the number of detected groups would be six. On the other hand, partitional clustering attempts to directly decompose the data into a predefined number of groups by minimizing some criterion, which should reflect the local structure. The most famous member of this family of algorithm is k-means where the criterion function is the sum of the squared distances of each sample to its nearest cluster centroid:

$$E = \sum_{i=1}^{N} ||\mathbf{x}_i - \mathbf{m}_{C(\mathbf{x}_i)}||^2 \quad [8]$$

where $\mathbf{m}_{C(\mathbf{x}_i)}$ is the vector collecting the coordinates of the centroid of the cluster closer to $\mathbf{x}_i$.

Calibration

When dealing with food analysis or characterization, exploratory data analysis, although necessary, may not be sufficient, as many problems may require the prediction of one or more quantitative or qualitative properties of the samples. Calibration is the use of empirical data and prior knowledge for determining how to predict quantitative information $\mathbf{Y}$ from available measurement $\mathbf{X}$ via some mathematical transfer function f:

$$\mathbf{Y} = \hat{\mathbf{Y}} + \mathbf{E}_Y = f(\mathbf{X}) + \mathbf{E}_Y \quad [9]$$

where the matrix of residuals $\mathbf{E}_Y$ collects the variance in $\mathbf{Y}$ not accounted for by the regression model, that is, the difference between the actual values of $\mathbf{Y}$ and those predicted by the

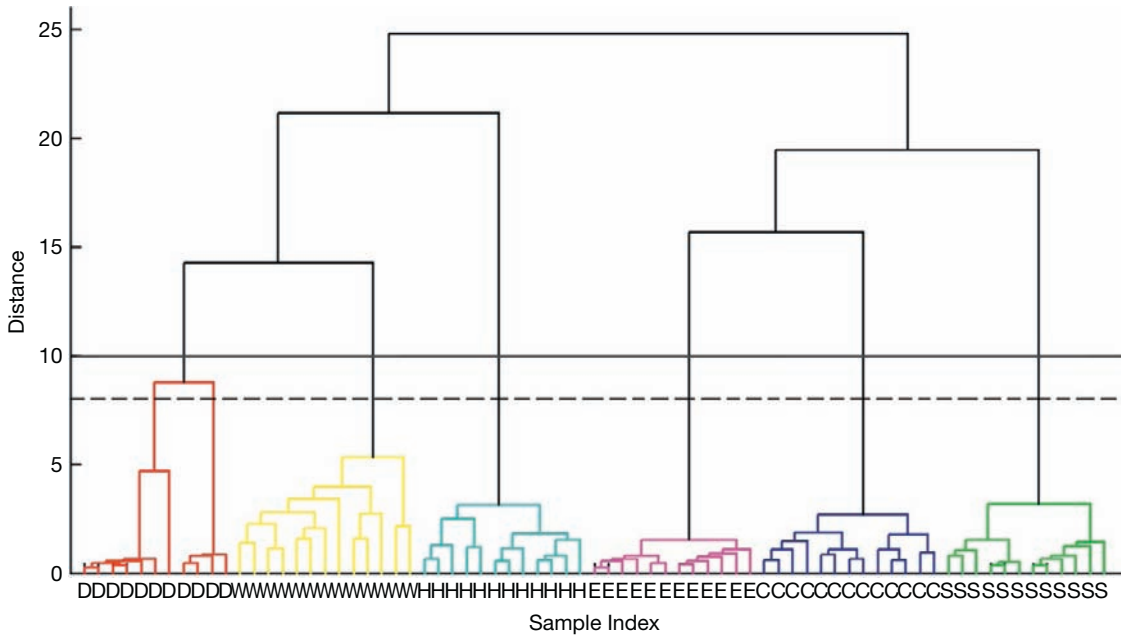

Figure 4 Dendrogram resulting from hierarchical clustering of honey data. Dashed and dotted lines (corresponding to distance values of 8 and 10, respectively) indicate two possible threshold values, which may be used to cut the dendrogram in order to define sample grouping.

model ($\hat{\mathbf{Y}}$). In food analysis, calibration plays a key role as quite often, the direct measurements of the properties of interest may not be feasible or it may be too expensive, time-consuming, or inaccurate, so that quantification is usually based on secondary measures (e.g., chromatographic peak area, absorption or emission intensity, current, and voltage). In this context, the simplest relation between the experimental measurements collected in **X** and the properties to be predicted is a linear one:

$$\hat{\mathbf{Y}} = \mathbf{XB} \qquad [10]$$

where **B** is a matrix of the so-called regression coefficients, which constitute the model parameters and uniquely define the mathematical relation. In particular, each column of the regression coefficient matrix collects the weights associated with the independent variables for the prediction of the corresponding column of the **Y** matrix, and this information can be used also for the sake of interpretation. Indeed, in principle, the larger the absolute value of the coefficient b_{ij} (the ith row and jth column element of **B**), the higher the contribution of the ith X-variable to the prediction of the jth response. However, particular care should be taken when inspecting the values of **B**, as their magnitude directly reflects the scales of both **X** and **Y**. Moreover, when dealing with instrumental fingerprints, interpretation can be further hindered by the presence of overlapping signals: As an example, one may consider the case when a spectroscopic measurement is used to predict the concentration of an analyte in a solution. If no interferent is present, the regression coefficients would resemble the spectrum of the pure analyte; however, if the solution contains also an interferent, whose spectrum partially overlaps with that of the analyte, the regression vector no longer looks like the pure spectrum because negative parts and shifts in position of peak maximum are introduced. Accordingly, there may be cases when a negative regression coefficient is correctly obtained for a variable that is positively correlated with the response. Operationally, the most straightforward way of estimating the regression coefficients in eqn [10] is provided by multiple linear regression (MLR). MLR is the multivariate generalization of the univariate least squares method; the values of the coefficient are calculated as the ones, which minimize the sum of squared residuals:

$$\min_{\mathbf{B}} \left(||\mathbf{E}_Y||^2\right) = \min_{\mathbf{B}} \left(||\mathbf{Y} - \hat{\mathbf{Y}}||^2\right) \qquad [11]$$

Accordingly, the matrix **B** is estimated as

$$\mathbf{B}_{MLR} = \left(\mathbf{X}^T\mathbf{X}\right)^{-1}\mathbf{X}^T\mathbf{Y} \qquad [12]$$

Unfortunately, even if MLR is the simplest method for linear regression, it is quite often inapplicable to the matrices resulting from food analysis and characterization. Indeed, in order for the term $(\mathbf{X}^T\mathbf{X})^{-1}$ in eqn [12] to be estimated accurately, the matrix **X** should meet some mathematical requirements, which are rarely fulfilled in problems involving instrumental fingerprinting of real-world samples: the number of samples should be lower than the number of predictors and the variables should be as uncorrelated as possible from one another. In this context, the bilinear approach introduced in the section '**Exploratory Analysis**' for PCA, by involving the projection of the samples onto a low-dimensional space of orthogonal variables, represents a way of overcoming the previously mentioned limitations. In particular, principal component regression (PCR) is a calibration method that originated by directly combining a preliminary dimensionality reduction step by means of PCA with the calculation of a MLR model on the resulting scores. In mathematical terms, at first, the matrix **X** is decomposed into the product of scores and loadings according to eqn [7], and successively, the scores are used as predictors to build the MLR model:

$$\hat{\mathbf{Y}} = \mathbf{TV} \Rightarrow \mathbf{V} = \left(\mathbf{T}^T\mathbf{T}\right)^{-1}\mathbf{T}^T\mathbf{Y} \qquad [13]$$

V being the matrix of regression coefficients relating **Y** to **T**. By combining eqns [13] and [7], it is possible to obtain the model coefficients directly in terms of the original independent matrix **X**:

$$\hat{\mathbf{Y}} = \mathbf{TV} = \mathbf{XPV} = \mathbf{XB}_{PCR} \Rightarrow \mathbf{B}_{PCR} = \mathbf{PV} \qquad [14]$$

Due to the nature of PCA decomposition, PCR provides a reliable answer to the drawback discussed earlier for MLR, even if it may not provide the best low-rank representation of the data to be used for calibration purposes. Indeed, principal components are calculated as those directions in space that account for the maximum variance in the data set; however, when multiple sources of spurious (irrelevant) variation are present in the data set, the directions of maximum variance may not account for the correlation with the responses to be predicted. On the other hand, partial least squares regression (PLS) makes active use of the information in **Y** to define the low-rank subspace onto which the data should be projected. In PLS, both the **X**- and the **Y**-blocks are decomposed in a bilinear fashion, and the axes of the low-dimensional subspace (called latent vectors) are chosen so that the scores of **Y** (**U**) have maximum covariance with those of **X** (**T**) and are linearly dependent, through what is called the *inner relation* (last one of the following equations):

$$\begin{aligned} \mathbf{T} &= \mathbf{XR} \\ \mathbf{Y} &= \mathbf{UQ}^T + \mathbf{E}_Y \\ \mathbf{U} &= \mathbf{TC} \end{aligned} \qquad [15]$$

where **R** is a matrix of weights, governing the projection of the **X**-block, **Q** are the loadings for the **Y**-block, and **C** is a diagonal matrix of coefficients. Also, in the case of PLS, it is possible to combine the equations in eqn [15] to obtain a matrix of regression coefficients directly relating **Y** to **X**:

$$\begin{aligned} &\mathbf{Y} = \mathbf{UQ}^T + \mathbf{E}_Y = \mathbf{TCQ}^T + \mathbf{E}_Y = \mathbf{XRCQ}^T + \mathbf{E}_Y = \mathbf{XB}_{PLS} + \mathbf{E}_Y \\ &\mathbf{B}_{PLS} = \mathbf{RCQ}^T \end{aligned} \qquad [16]$$

However, inspection of regression coefficients is not the only tool for interpretation, when dealing with PLS: the bilinear nature of the relations reported in eqn [15] suggests that scores and loadings plots also constitute a valid support to model understanding and diagnostics.

Although, for all the methods described so far, a linear relationship between the property (or properties) to be predicted and the secondary measurements is assumed, this need not always be the case: the important is to have a defined calibration equation in order to be able to make predictions on future samples, so that several nonlinear algorithms have

been proposed in the literature to tackle with more complex functional dependencies. In this framework, since a detailed discussion would be far beyond the scope of the present article, it is just worth mentioning, among the various possibilities, kernel- or dissimilarity-based approaches, neural networks, or locally weighted regression.

Classification

Food-related issues may not always call for a quantitative prediction, and rather, problems such as the authentication of a good, its quality control, and traceability (just to cite a few) involve the assessment of one or more qualitative properties. For instance, one may be interested in assessing whether a product is organically grown or not, or if a wine was produced in Italy, Spain, South Africa, or Chile, or, again, if a food will be good, acceptable, or bad according to consumer preferences. From a chemometric standpoint, all those methods, which deal with the possibility of predicting one or more qualitative responses on a set of samples, belong to the family of classification tools. Indeed, classification techniques aim at building models, which, based on the values of the measured variables, assign a sample to a *category* or *class*, the latter being a group of objects sharing similar characteristics. Accordingly, in the language of classification techniques, the wine authentication problem, cited earlier as an example, would involve four categories ('Spain,' 'Italy,' 'South Africa,' and 'Chile'), while the three classes 'good,' 'bad,' and 'acceptable' would be considered for the food preference one. Since samples can be represented as points in the multivariate space of the variables, classification may be seen under a geometric perspective as the search for surfaces identifying regions of space where it is more likely to find objects belonging to a particular category. In this context, it is particularly useful to operate a distinction between two possible approaches: discrimination and class modeling. Discriminant techniques partition the space in as many regions as the number of categories in the data set, so that if an object falls in the region corresponding to a particular class, it is univocally assigned to it; as a consequence, each sample is predicted to belong to one and only one of the categories postulated by the problem. On the other hand, modeling techniques, as the name suggest, try to model each category independently on the others and operate by identifying a region of the multivariate space where it is likely to find samples from that particular class (the model space): If a sample falls within that region, it is accepted by the class model; otherwise, it is rejected. Accordingly, when more than one category is modeled, a sample can be accepted by only one class (and then be univocally assigned to it), by more than one (i.e., confused), or by none (and be considered an outlier). In the remainder of the section, discriminant and modeling approaches will be further discussed through the illustration of two widely used methods, respectively, partial least squares discriminant analysis (PLS-DA) and soft independent modeling of class analogies (SIMCA).

PLS-DA is a discriminant classification technique based on the PLS algorithm already described in the section 'Calibration,' and it was introduced to overcome the limitations suffered by traditional methods such as linear (LDA) and quadratic (QDA) discriminant analysis, in the presence of ill-conditioned $\mathbf{X}$ matrices. Indeed, the same kind of problems (high number of highly correlated variables), which make MLR unsuitable to build regression models, hinders the applicability of LDA and QDA for classification. Accordingly, after finding a suitable coding that allows to transform a classification problem into a regression one, the use of the PLS algorithm represents a solution to these drawbacks. In particular, PLS-DA is based on coding the information about class belonging into a binary dummy matrix $\mathbf{Y}$ having as many rows as the number of samples and as many columns as the number of classes: the matrix element y_{ij} will be equal to 1 if the ith sample belongs to class j or to zero if it does not. A PLS model is then built between the experimental matrix $\mathbf{X}$ and the dummy matrix $\mathbf{Y}$, and classification is achieved on the basis of the predicted values $\hat{\mathbf{Y}}$, usually via the introduction of a suitable threshold. An example of application is reported in Figure 5, where the use of PLS-DA on chromatographic data to discriminate olive oils from the PDO Sabina from other extra virgin oils is shown. In this case, since there are only two categories, due to the symmetry of the problem, the dummy matrix $\mathbf{Y}$ boils down to a vector $\mathbf{y}$ in which 1 indicates 'Sabina' and 0 'other oils.' Classification is then achieved by setting a threshold of 0.5 to the predictions: all the samples for which the predicted response is higher than the threshold are classified as Sabina oils, while all the others are recognized as from other origins.

Differently than what happens for discriminant methods, modeling techniques focus on capturing the similarity among samples belonging to the same category, rather than the differences between individuals from competing classes. Under many respects, they can be considered as outlier detection techniques, as their aim is to verify whether a sample fits the model of a particular category or not. In particular, SIMCA operates by describing the class-related variability in the experimental fingerprint using a PCA model of appropriate dimensionality:

$$\mathbf{X}_G = \mathbf{T}_G\mathbf{P}_G^T + \mathbf{E}_G \quad [17]$$

where the subscript G indicates that only the samples from category G are used to define the projection. Then, for each sample, an overall distance to the class model, measuring the extent of outlyingness, is defined as the combination of the distance to the model space (which is a function of the residuals) and the distance within the model space (which accounts for the distance of the sample scores to the origin of the PC space). Accordingly, if the distance to the model is below a prespecified threshold, the sample is accepted by the category; otherwise, it is rejected. When more than a single category is modeled, a straightforward way of representing the results of SIMCA is the so-called Coomans plot, which is shown in Figure 6 for the same data set used to exemplify PLS-DA.

The axes of the Coomans plot represent the sample distances to the two investigated categories, and the thresholds used to define acceptance/rejection by the class models divide the plot in four different regions: Objects falling in the uppermost left region of the plot are univocally accepted by the class 'Sabina,' while those mapped onto the rightmost lower part are uniquely accepted by 'other oils'; the samples falling in the lowermost left part of the plot are confused between the two categories, while those in the uppermost right regions are considered as outliers by both classes.

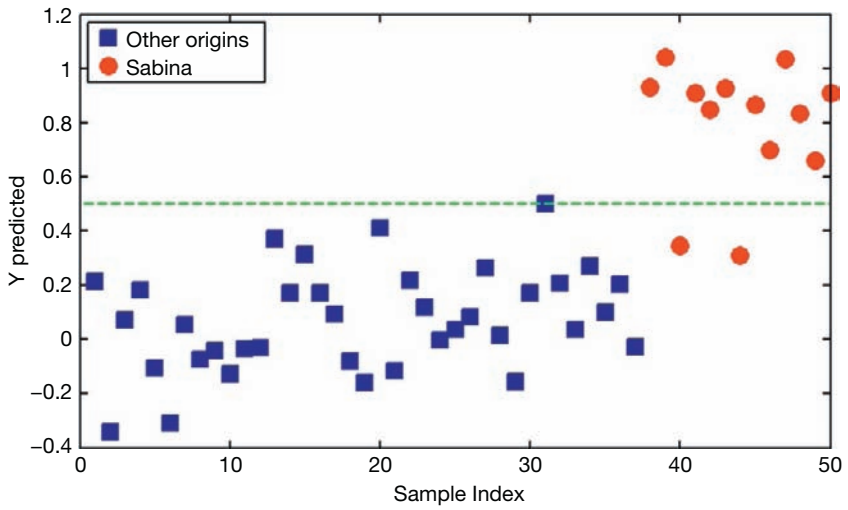

Figure 5 Illustration of how classification is accomplished in PLS-DA: samples are assigned to one or the other class based on the predicted *y* values and the green dashed line indicates the classification threshold. Accordingly, all samples except the two Sabina oils that fall below the threshold are correctly classified.

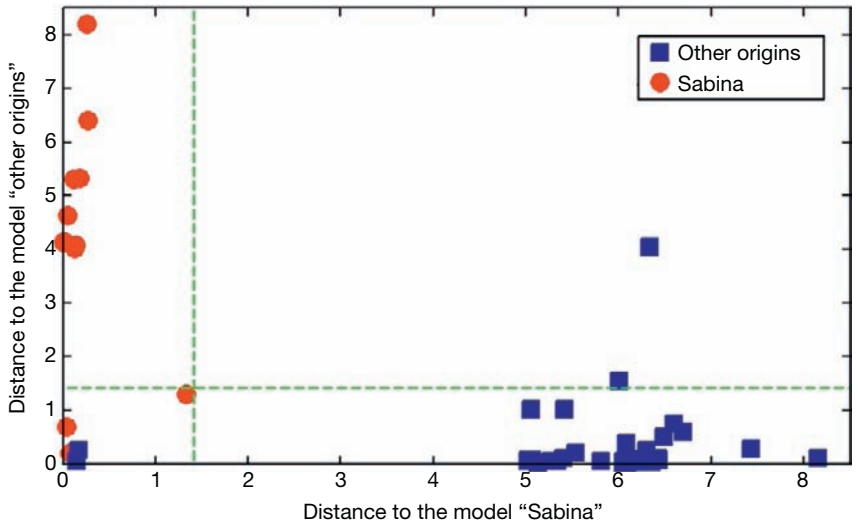

Figure 6 SIMCA modeling of the olive oil data set: Coomans plot. The dashed lines indicate the acceptance thresholds used by the two class models.

A Continually Increasing Toolbox

Although the topics presented in the previous sections cover most of the fields of application of chemometrics to food analysis and characterization, the chemometric toolbox is continuously evolving to match the increase in the complexity of the problems to be tackled and, at the same time, in the availability of high-throughput instrumentation. For instance, multiway and multiset resolution techniques may be used to extract chemically relevant profiles from data collected by means of hyphenated techniques or, in general, resulting from experiments where signals are recorded as a function of different sources of variability. On the other hand, hyperspectral image analysis techniques allow to extract both spatial information (e.g., texture and homogeneity) and spectral information from the samples, while data fusion approaches combine information from multiple sources into a holistic characterization of the sample for both exploratory and predictive purposes.

In general, one may affirm that chemometric techniques constitute an essential and valid tool for all of those who are involved at different levels in the characterization and analysis of foodstuff.

See also: Authenticity of Food; Food Fraud; Infrared Spectroscopy: Applications.

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Cherries (*Prunus* spp.): The Fruit and Its Importance

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Introduction: Cherry Taxonomy and Types

Commercially, the two most important cherry species are sweet cherry (*Prunus avium*, L.) and tart cherry (*Prunus cerasus* L.), both tree fruits native to Southeastern Europe and Western Asia. They are closely related and graft-compatible and will hybridize to form interspecific (Duke) cultivars. Sweet cherry (diploid, with a base chromosome number of 8 and a somatic number of 16) probably originated between the Black Sea and the Caspian Sea, but it spread into Europe in ancient times. Tart cherry (tetraploid, with a base chromosome number of 16 and somatic chromosome number of 32) is native to the same areas as sweet cherry, and there is good evidence that crosses between *Prunus avium* and the ground cherry (*Prunus fruticosa* Pall) gave rise to tart cherry. There are other cherry species, but most, for example, Nanking cherry (*Prunus tomentosa*), have limited commercial value as fruits.

Sweet cherries can be divided into two major types based on fruit characteristics. Heart-type cherries are ovoid or heart-shaped with relatively soft flesh, often ripening early. Most of the commercially important cultivars, however, are of the Bigarreau type with firmer, crisp-fleshed fruit, ripening mid to late season. Fruit flesh may be red or yellow, and the skin may be dark (red to nearly black) or light (yellow-red to yellow-white).

Many sweet cherry cultivars grown throughout the world originated in Europe, but a number of important ones were selected or bred in local cherry districts. European cultivars grown in the United States are Napoleon (Royal Ann), Black Tartarian, Eagle, Early Purple, Early Rivers, Elkhorn, Hedelfingen, Knight's Early Black, Lyon, and Schmidt. The cultivars Windsor, Van, Sam, Vista, Victor, Sue, Vega, Summit, and Stella were developed in Canada. Chinook and Rainier were developed in Washington. Bing, Lambert, Black Republican, Corum, and Hoskins were selected and developed in Oregon. Chapman, Burbank, Bush Tartarian, and the new cultivars Mona, Larian, Jubilee, Berryessa, and Bada originated in California. Recent introductions include Ulster and Hudson from New York and Angela from Utah.

The most important sweet cherry cultivars in the Western United States, where over 80% of the US crop is produced, have been dark-fruited, crisp-fleshed cultivars: Bing (the leading cultivar in North America), Van, and Lambert. But others may be available because of their use as pollenizers or as the result of recent fresh market demand for large, light-colored, and crisp-fleshed fruits from cultivars like Rainier. Firmness, size, color, and soluble solids are all important market considerations, and growers in regions where summer rains are prevalent, for example, the eastern United States and Eastern Europe, are at a disadvantage because the main cultivars are often the softer-fleshed, rain cracking-resistant types, for example, Emperor Francis, Hedelfingen, and Schmidt. In these regions, light-fleshed cultivars, Rainier, Napoleon (Royal Ann), Corum, and Emperor Francis, are best for making into maraschino cherries (because pigment is undesirable), but a few are nonetheless grown for the fresh market. Napoleon is also used for canning. Bing is mainly a fresh market cultivar, and Lambert is used both for canning and fresh market. Black Republican and other very firm, dark cherries are good for freezing.

Tart cherry fruit are generally soft, juicy, and depressed-globose in shape, but colors may range from the Morello types with red to dark red flesh and juice to the Amarelle types with nearly colorless juice and flesh.

Although new cultivars are being tested, there are only a few tart cherry cultivars commonly grown in North America, ranging from the light red Early Richmond, to the medium red-skinned Montmorency, to the late dark red English Morello, but Montmorency is still the standard. In Western Europe, Schattenmorelle and Sternsbaer are common, but many others are grown in Russia, Slovenia, Romania, and Hungary. Most, unlike sweet cherry, are more or less self-fertile and generally do not require pollenizers. Almost all of those grown in the United States and Western Europe are harvested mechanically and sold for processing, primarily as a frozen or canned ingredient for use in manufactured food products such as pies, but more recently as a dried fruit product, and in Europe and other areas, there have been uses developing for juice, liqueur, and marmalade production and combinations with yogurt.

Production Areas

Turkey, the United States, Iran, and Russia are large producers of both sweet and tart cherries (FAO Statistics, Table 1). In some areas of northern Europe, tart cherries are, after apples, the second most important fruit grown. Otherwise, within Europe, tart cherry production is concentrated in Eastern Europe, Slovenia, Hungary, and Romania, while sweet cherry production is more common in Western Europe, Italy, Switzerland, France, and Spain. Sweet cherry production is increasing in the Southern Hemisphere, New Zealand, Australia, and Chile, for fresh shipments to northern markets in their winter season. In the United States, sweet cherry cultivation has also been increasing, with production mostly in the west, not only in Washington but also in Oregon and California. Most US tart cherry production occurs near the Great Lakes, primarily in Michigan with 70–75% of the US crop, which with New York, Wisconsin, and Pennsylvania totals 90–95% of the US crop.

Average world production values for sweet and tart cherries are now approximately 1 200 000 and 2 200 000 metric tons, respectively. Wide annual supply fluctuations, especially regionally, in both sweet and tart cherries characterize production and create high risks in product availability and price change for producers, processors, and marketers. Annual US

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Table 1 Average values and yields of tart (sour) and sweet cherries (top 20 producers) over the years 2010–12

Country	*Sour cherries average annual yield (MT)*	*Sour cherries value ($1000)*	*Sweet cherries average annual yield (MT)*	*Sweet cherries value ($1000)*	*Total average yield (MT)*	*Total average value ($1000)*
Turkey	188388	115033	445734	566649	634122	681682
The United States	179667	109708	324057	411964	503723	521672
Iran	166733	101811	200864	255353	367598	357163
Italy	165893	101297	111006	141118	276898	242415
Spain	102744	62737	95179	120998	197923	183735
Chile	77159	47114	78716	100069	155875	147183
Uzbekistan	76687	46826	80333	102125	157021	148951
Syria	55677	33997	67540	85861	123217	119858
Ukraine	32267	19702	72800	92548	105067	112250
Russia	29575	18059	71567	90980	101142	109039
Romania	17833	10889	74225	94359	92058	105249
Greece	16333	9973	47567	60470	63900	70443
Poland	13821	8439	39727	50504	53548	58943
Austria	12238	7472	53954	68590	66192	76063
China	9096	5554	32000	40680	41096	46234
France	7308	4462	41135	52293	48443	56755
Germany	6778	4138	30290	38507	37069	42645
Lebanon	6616	4039	22833	29027	29449	33066
Serbia	6494	3965	24322	30919	30816	34884
Bulgaria	5338	3259	24842	31581	30180	34840

Source: United Nations FAO statistics.

tart cherry production ranged, for example, from 38000 to 133000 metric tons in the last several years, but there has been a gradual downward trend in average production since the mid-1960s to about 120000 tons (in 2014), with a farm value of about $50 million, but the processed value is at least thrice that. Sweet cherry production, however, has been increasing, especially recently as markets develop in Japan and the Far East of the Pacific Rim for fresh cherries grown in the Western United States and elsewhere.

Between 2010 and 2012, the world's cherry acreage increased by 4.2%, reaching over 400000 ha, according to the United Nations Food and Agriculture Organization (FAO). Turkey has the largest cherry acreage worldwide (increasing its share from 11% to 12% in 2012), with the United States being the second with 8.7%. Italy is third with Syria (at 7.4%) and then Iran and Spain, with shares of 7.2% and 6%, respectively. Chile's share increased (from 3.4% to 3.8%). Turkey also increased its share in the world's production, with 21.3%. The United States was second with 17%, while Iran, Syria, and Italy reduced their shares to 8.9%, 4.6%, and 3.6%, respectively. Chile ranked sixth in world production, with a 4% share of the total in 2012, a sharp increase compared with the 2.8% in 2010.

Growth and Management

Flowering and fruit set – Sweet cherry flowers are in clusters of two to four usually borne laterally on short spurs on 2-year-old twigs or near the base of longer 1-year-old shoots. Floral initiation takes place in July, after the crop is harvested, and only on buds where the subtending leaves opened relatively early in summer. Flower buds are of the unmixed type and do not give rise to a lateral or bourse shoot. As a result, flowering spurs, unlike those on apples and pears, do not remain productive. Flowers generally have a single pistil but in very hot summers may form two pistils that result in undesirable double fruits. With few exceptions, for example, 'Stella' (and its progeny) and the new 'Lapins' and 'Sweetheart' cultivars, commercial sweet cherries are self-sterile (self-incompatible) and therefore require another cultivar for pollination. There are, however, intrasterile groups, where none of the group will cross-pollinate any other member of the group. Bing, Lambert, and Napoleon are one such group.

Tart cherry flowers develop much like sweet cherry, with buds of two to four flowers on either spurs or lateral buds. Tart cherry cultivars range from compatible to self-incompatible. Montmorency, for example, is only partially compatible but is always grown without a pollinator. Fruit set, however, clearly limits yield on the fully incompatible cultivars, but overcropping may occur in other cultivars with excessive flowering or fruit set resulting in too few leaves or leaf buds to develop fruit of adequate size and quality.

In both sweet and tart cherries, flower development and fruit set may frequently be harmed by late frosts, although tart cherry is hardier and generally blooms later than sweet cherry. Wide annual supply fluctuations are consequently common in major growing areas for both species due to spring frosts or to low midwinter temperatures where lack of wood hardiness is a contributing factor. Sweet cherries are less hardy than apples, but some tart cherry cultivars may be as hardy as the apple cultivars McIntosh or Northern Spy. In addition, the best-quality sweet cherry cultivars tend to be more susceptible to rain cracking and do best in regions with dry summer growing conditions. Fruit also develops good quality in regions often too cool for peaches or apricots. Climate also dictates that

cherries be grown where winter chilling temperatures (about 1000 h for most sweet cherries, longer for tart cherry) are adequate to break rest; thus, cherry culture is generally limited to cooler temperate regions.

Tree size and rootstocks – Tree size plays a central role in production of quality fruit. Dwarf trees have many advantages: Light penetrates better, favoring photosynthesis; the tree produces more and better fruit; spraying can be done more efficiently, usually with reduced use of chemicals; and dwarf trees are easier to harvest. Cherries are no exception, but dwarfing rootstocks have not until recently been available for either sweet or tart cherry. The common rootstocks, 'Mazzard' and 'Mahaleb,' only slightly affect tree size, if at all. 'Colt' is similar and may be somewhat drought- and cold-susceptible. Recently, however, dwarfing rootstocks have been developed in several breeding programs, and is being tested. For example, of 17 cherry rootstocks developed in Giessen, Germany, most produce relatively large trees, but two of these rootstocks give trees about 25% of the standard, and several rootstocks developed in Belgium may also be promising.

Harvesting and handling – Sweet cherries are almost all hand-harvested, particularly those intended for the fresh market. Avoiding pitting and bruising throughout harvest, sorting, and packing is a major problem in delivering high-quality fruit to the fresh market. Bruise susceptibility of some white- or yellow-fleshed cultivars may even require field packing to minimize loss. Mechanical harvesting of tart cherries for processing, however, has been a major technological development, which substantially reduces grower costs. A grower and his family plus some high school students (a crew of 6–8) can mechanically harvest as much as 200–300 hand pickers formerly did. The results include huge savings in direct labor costs, large reductions in housing costs, and substantial savings in labor fringe costs. The US tart cherry industry used essentially completely mechanical harvesting during the 1970s, although there have since been additional improvements in equipment and techniques.

Some aspects of cherry processing have also substantially changed, which has led to greater efficiencies and product quality in the cherry industry. Almost all tart cherry processors have adopted electric-eye sorting equipment, which substantially reduces in-plant sorting labor, and destemming equipment efficiently removes the stems from mechanically harvested cherries. Although picking of sweet cherries for the fresh market is still by hand, subsequent handling has been improved dramatically very recently with the substitution of hydraulic flumes for conveyor belts to reduce bruising and pitting throughout sorting and packing.

Quality Factors

Soluble solids (primarily hexose sugars and sorbitol) and fruit color (depending on the type) are the best indicators of quality for both sweet and tart cherries, although fruit acid level may be important in tart cherry. Except for soluble carbohydrates, vitamins A and C, and certain flavonoids that may be important as antioxidants in some cultivars, cherries are relatively low in nutrients, but calcium, iron, magnesium, phosphorus, and copper contents are high compared to apple, peach, grape, and strawberry. High-quality tart cherry fruit typically has at least 15% soluble solids, while sweet cherries should have nearly 20% (or higher). Standards for harvesting and marketing may, however, often be lower. Optimum conditions also often vary with use. To facilitate brining (bleaching in sulfur dioxide solutions for maraschino cherries), fruit may be picked prematurely before color and soluble solids are adequate for the fresh market. Stem fruit removal force is carefully monitored for tart cherries that will be mechanically harvested, and abscission may be brought on by treatment with ethephon, which releases ethylene, expediting abscission and fruit drop in response to mechanical shaking.

Disorders, Diseases, and Pests

Disorders – In processing brined sweet and tart cherries, the solution pocket problem involves subepidermal splits in the flesh, which fill with brine solution and with ruptured cell contents. Time of harvest, degree of turgidity at brining, temperature, or any procedures that reduce either the sugar or water content of the fruit will tend to decrease the problem.

Rain cracking (swelling followed by rupture of the epidermis) of sweet cherries occurs mostly during the harvest period when the fruit is mature or nearly so and has been wet with rain for some time. Primary cause is absorption of water directly through the skin of the fruit and not through the root system. Cultivar cracking susceptibility has been tested extensively. In testing, Bing, one of the best-quality cultivars, was worst, followed by Napoleon, Lambert, Emperor Francis, Giant, Schmidt, Yellow Spanish, and Montmorency, which did not crack. In another ranking, Bing invariably cracked very badly and was followed by Lambert, Giant, Gil Peck, and Hedelfingen. In still another test, Van, Merton Glory, Vega, and Vista were very susceptible, while Emperor Francis, Schmidt, and Sam were less, and Sue, Kristin, Ulster, and Early Rivers were the least susceptible. From the long-range viewpoint, breeding programs under way ultimately may produce desirable crack-resistant cherries. Cracking may be reduced by some chemical treatments, for example, rain-activated calcium sprayers, but results with hormone (auxin (NAA) and gibberellin (GA_3)) applications are equivocal. In some parts of the world, for example, Europe, covering trees with plastic film has been widely used to avoid cracking. Elsewhere, this approach or a high tunnel method has also been used to promote earlier flowering and fruiting, thus capturing high early market returns that justify the extra costs.

Pitting of sweet cherry is a condition in which areas near the surface of the fruit become sunken, forming dimples or pits, and may occur before or after harvest, and there are at least three different sources: usually from bruising during handling, from feeding by sucking insects such as the soldier bug, and perhaps from physiological injuries, for example, adverse low-temperature stress during postharvest cooling or growing conditions.

Diseases – Bacterial canker, one of the most important sweet and tart cherry pathogens, is caused by two different pathogens, *Pseudomonas syringae* and *Pseudomonas morsprunorum*, and is characterized by oozing of gum (gummosis) at infection sites. Disease development is most prevalent during the

cool, wet periods of early spring. Crown gall, caused by *Agrobacterium tumefaciens*, can affect sweet and tart cherry rootstocks and is characterized by galls forming usually near infection sites caused by wounds, sometimes man-made, for example, cultivation injuries, or due to damage from subterranean chewing insects or rodents. Some rootstocks, however, are only moderately susceptible, and some hybrids may be tolerant. Brown rot, caused by the fungi *Monilinia fructicola* or *M. laxa*, affects both sweet and tart cherries and reduces yield in infected and decaying blossoms, twigs, and fruit. Fruit decay after harvest is also a problem. The fungi persist in mummified fruit on the tree and the ground and infection continues from these inocula the following spring. Growing regions with cooler and drier summers provide some relief. Cherry leaf spot, *Blumeriella jaapii* (*Coccomyces hiemalis*), is the most serious disease affecting tart cherry and most ground cherries. Infection occurs in the spring on expanding leaves and continues throughout the season under favorable conditions, for example, high humidity. Severely infected leaves become chlorotic and abscise, and if defoliation is severe, fruit may not ripen properly and tree vigor and hardiness are reduced. Powdery mildew (*Podosphaera oxyacanthae*) is similar. Other fungal pathogens may sometimes be important, causing blights, crown or root rots, and replant (orchard reestablishment) problems. Cherry dieback is thought to be a complex of several disorders, one of which may be mycoplasma disease. X-disease, leafhopper-transmitted and often devastating, is also due to mycoplasma.

Several viruses cause poor vegetative growth, reduce yields, and may even result in tree death, but others are symptomless. Among the most severe is prunus necrotic ringspot virus, which is pollen-transmitted and present in all cherry-growing areas of the world. Little cherry (prune dwarf) disease is another pollen-transmitted virus and very destructive. *Prunus* stem pitting disease is caused by the tomato ringspot virus and is spread by nematodes.

Pests – Bird damage can be very serious, and some areas may require protective netting to reduce predation. The cherry fruit fly passes the winter in the soil as a pupa, adult flies emerge in late spring, and females feed on surfaces of leaves and fruit and lay eggs in the nearly ripe fruit. On hatching, the larvae (maggots) feed on the fruit flesh. The larvae are easily killed by holding fruit near 0 °C, but fumigation, until recently most often with methyl bromide, may be required to meet quarantine restrictions for shipping overseas. Other pests include black cherry aphid, plum curculio, European red mite, peach tree borer, and two-spotted mite.

Economic Problems and Future Developments

Although the tart cherry industry is facing a serious problem of excessive productive capacity in some years and persistent oversupplies, this industry has adopted new technologies and practices in the last 10 years, which substantially improve its cost efficiency and productivity. Much of the newly planted acreage uses efficient trickle irrigation and closely planted orchard systems that also involve hedging techniques. These recent new techniques, especially in combination, provide large increases in yields per hectare and hence substantial reductions in costs.

Considerable genetic diversity still exists in Eastern Europe and Russia, the center of origin for both tart and sweet cherries. Although breeding programs have been limited, exploiting that diversity should do much to overcome the growing, handling, and processing problems that face growers using the industry standards, the sweet 'Bing' and the tart 'Montmorency' in the United States. Sweet cherry growers especially need dwarfing rootstocks and spur types for growth control, and all growers need cultivars with disease and pest resistance, less self-sterility, and a range of maturities so that there are longer seasons for fresh markets. Sweet cherry growers also need cultivars with rain-cracking resistance and, for postharvest fresh market quality, resistance to bruising. Tart cherry growers need new cultivars for diversifying and strengthening marketing options, for example, fresh and frozen juice products, dyes for cosmetics and the food processing industry, and dry stem scars and small freestone pits to facilitate handling and processing. A combination of new marketing strategies and products for tart cherries and advances in breeding of both sweet and tart cherries would clearly benefit the economic potential of the entire cherry industry.

See also: Apples; Berries and Related Fruits; Drying: Effect on Nutrients, Composition and Health; Fruit Juices; Peaches and Nectarines; Plums and Related Fruits; Strawberries.

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Chilled Foods: Effects on Shelf-life and Sensory Quality

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Introduction

The commercial cold chain has been evolved together with the progress on *Food Science and Technology*. In the past, chilled food was a term used for those items that need to be kept under refrigeration because of quick microbial spoilage. Sometimes, these products were seafood and fish caught in remote areas of the world and needed to be transported to specific markets. However, nowadays, the chilled food chain also includes some novel products that must be kept under refrigerated conditions; temperature abuse is not an option because of the microbial risk inherent in the product. These novel products are called cooked-chilled foods or ready-to-eat meals.

During the storage of chilled foods, temperature must be kept at specific values and recorded to ensure the microbial safety of the product. Consumers must be aware of the importance of controlling the temperature of these products and make sure their responsibility to handle the product from the store to the final consumption. Even if the product is kept with ice on a boat or kept on a supermarket on the fridge, temperature must be carefully monitored to ensure that spoilage microorganisms are growing slowly or the microbial growth is delayed.

Several microbial species have been identified in specific products and these are the ones that should be monitored during the storage; microorganisms such as *Bacillus* spp. are frequently found in chilled goods and it is recognized as one of the foodborne organisms, and high counts of this sporeformer microorganism can promote gastrointestinal diseases. Pathogens such as *Listeria monocytogenes*, or even spores of *Clostridium botulinum*, can be identified in some chilled foods, because of a poor pasteurization process, lack of hygienic measures, cross contamination, or underprocessing of the product.

Furthermore, sensory quality of chilled foods can be drastically compromised because of some chemical reactions taking place during storage, such as rancidity, changes in color and flavor, or changes in texture. Refrigerated temperatures delay some of these chemical reactions but do not eliminate them completely. Even if the reaction rate is slow, the chemical processes are taking place, and if the product is stored for several weeks, noticeable changes on sensory properties could be observed.

This article presents a brief discussion about some microbial and sensory changes in chilled foods. The specific microbial groups of representative food products are included as well as some of the novel approaches to minimize microbial risks and to improve the current technologies used to preserve chilled foods. Sensory changes in some refrigerated products and some novel strategies used by food technologists are also included.

Chilled Foods

The term chilled foods includes a number of products: some of them are ready-to-eat, others require some quick preparation by the consumer, and another category includes chilled products that will be used as ingredients for other products. In general, chilled foods must be kept at a temperature $\leq 5\ ^{\circ}C$ to ensure the microbial quality of the product through the chilled chain until they reach the consumer. Chilled foods include entrées, pasta, vegetables, fresh soups, salad dressing, desserts, deli products, dips, ready-to-eat meals, different kinds of meat, fish and seafood, and poultry products. All of them, regardless of the product, must follow the food safety regulations in each country for processing, handling, transportation, storage, and final consumption. Often, a hazard analysis and critical control point (HACCP) program is followed to process, preserve, handle, prepare, transport, and package chilled foods.

Some of the main concerns of chilled foods are related with chemical, sensory, and microbial changes of the product by the end of the shelf life when the consumer is eating the product. Chemical changes on the product can seriously compromise the nutritional quality of the product leading to a food item without the original nutrient content. Sensory changes are related more with the acceptability of the product by the consumer; even though these changes do not put at risk the health of the user, they might affect the perception and consumption of the product, making it unsuitable for eating. Finally, one of the most important characteristics of the chilled foods is the microbial quality; even though the growth of the microorganisms is delayed during storage of these products because of low temperature, there are some species such as psychrophilic microorganisms that can grow and represent a risk for the consumption. Besides, some of the emerging pathogens might stay alive on the product and grow if the temperature is not controlled, producing a high risk of foodborne illnesses.

Shelf Life

The shelf life of the product depends on several factors such as initial microbial counts, the quality of the raw ingredients, the processing technology, the addition of antimicrobials, and the use of preservation factors such as decrease of pH or a_w and storage temperature. The chemical composition of the product will also be an important factor to consider during the shelf life studies, as the richer in nutrients the product is, the faster its microbial growth. Furthermore, a_w is an important fact to consider during the shelf life, especially for chilled foods that have high moisture content. Water is important not only for microbial growth but also for the promotion of chemical reactions on the product.

Regardless of the chemical composition of the product and the initial microbial population, an additional aspect to consider during the shelf life is the packaging material and the packaging conditions of the product. Some packaging materials represent a strong barrier against moisture, oxygen, and temperature, which together will extend considerably the shelf

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life of the product. However, on the chilled food chain, some products are not packaged because they are sold 'fresh and raw' such as fish or produce or some products have a very weak barrier with very basic packaging materials. Some products might be packaged using some specific conditions such as vacuum and modified or controlled atmospheres, which also contribute to the extension of the shelf life. Also, several preservation factors have been tested together with low temperatures during food processing to extend the shelf life of different products; physical and chemical hurdles have been used as shown in Table 1.

Microbial Shelf Life

Fish and seafood

Several marine products such as seafood and fish have a short shelf life because of the chemical reactions taking place together with the postmortem changes. All of these chemical changes can promote faster microbial growth of the product. Spoilage microorganisms on fish include aerobic and proteolytic bacteria, coliforms, and lactic acid species. Pathogens on fish include *Vibrio cholera*, *L. monocytogenes*, *Escherichia coli*, and *Salmonella* spp., among others (Table 2). Numerous studies have been conducted to incorporate some compounds on the ice that keeps the temperature of chilled foods low. Some of the compounds have antimicrobial properties to delay the microbial growth and extend the shelf life of the product. Studies have been done with plants and herbal extracts, for example, thyme, oregano, clove, basil, and rosemary, among others. Other compounds that have been tested together with ice include some organic acids, alone and in combination. Some examples are citric, ascorbic, and lactic acids. The food products tested include marine species such as seafood (anchovies) and different kinds of fish (sardines and mackerel, among others). In most of the cases, the shelf life of the product has been considerably extended when comparing with control samples kept only on ice; microbial loads reported for muscles are considerably lower for those treated products compared with control samples. Counts of aerobes, anaerobes, psychrotrophs, Enterobacteriaceae, and lipolytic and proteolytic microorganisms are affected because of the presence of acids and free radicals.

This traditional method to preserve fish, using ice, is commonly used for transportation from the origin to the final market of the fish. Boxes with ~30% of ice are used to keep the fish with low microbial growth and minimize the chemical reactions during transportation.

In the last few years, a new approach has been studied to reduce the amount of ice required for transportation of fish. Superchilled fish has 10–15% of ice since the temperature of the product is reduced to about 1–2 °C below its freezing point. The ice is surrounding the product, protecting it from microbial spoilage and enzymatic reactions, creating an ice shell within the product. Other studies on keeping chilling temperatures on fish include the use of ice with different shapes such as the traditional flake ice or the use of small spherical ice crystals known as slurry, flow, or fluid ice. The latest provides an extended shelf life of the fish rather than the flake ice (up to two times longer) because of the direct contact of the crystals within a bigger superficial area of the fish delaying microbial growth and preserving the texture of the product.

Other approaches that have been researched to extend the shelf life of fish include the use of chilling slurry, edible films,

Table 1 Examples of preservation factors used together with refrigerated conditions to extend the shelf life of chilled foods

Food item	*Physical factors*	*Chemical factors*
Fish and seafood		Herbal extracts
Ready-to-eat meals (meats and vegetables)	Irradiation	
Ready-to-eat meals (beef)	High hydrostatic pressure	
Fish		Essential oils
Shrimp	Vacuum packing	
Seafood		Gas flushing
Fish		Organic acids
Fish	Vacuum packing	Modified atmosphere packaging (MAP)
Ready-to-cook meats and fish		Chitosan
Fish		Bacteriocins (nisin)
Fish	Different shapes of ice crystals	
Sausages		Olive oil
Fish	Super chilling (partly freezing)	

Table 2 List of microorganisms found in chilled food according to the product

Product	*Microorganisms*	*Product*	*Microorganisms*
Fish and seafood	Lactic acid bacteria (LAB)	Fresh produce	Coliforms
	Vibrio spp.		Yeast and molds
	Listeria monocytogenes		Lactic acid bacteria (LAB)
	Escherichia coli		*Salmonella*[a]
	Pseudomonas spp.		*E. coli* O157:H7[a]
	H_2S-producing bacteria		*L. monocytogenes*[a]
Rabbit meat	*Pseudomonas* spp.	Lamb	*E. coli*
	Lactic acid bacteria (LAB)		Lactic acid bacteria (LAB)
	Brochothrix thermosphacta		*Pseudomonas* spp.
	Enterobacteria		*Yersinia enterocolitica*
Cooked-chilled foods[a]	*L. monocytogenes*		
	Clostridium botulinum		
	Bacillus cereus		
	Clostridium perfringens		

[a]No common microorganisms but they might be present if the food has not been properly handled.

and modified and/or controlled atmosphere. The use of chilling slurry has been tested in cod, basically using seawater slurry (−2 °C) that can reduce the temperature of the product faster than regular ice. During the shelf life, chilling slurry has shown better results on the fish because of the low microbial growth and the freshness of the product. However, some of the problems associated with the use of chilling slurry are associated with the weight gain and salt intake of the fish, both considered drawbacks for the product.

Meat products

One of the meat that are highly valued is lamb; some of the most important markets are away from the highest consumers, for example, the lamb from New Zealand needs to travel to foreign markets that sometimes takes several weeks to reach its final destination. The vacuum-packed lamb needs to keep a temperature below −1.5 °C to ensure a shelf life between 60 and 70 days. Some of the bacteria that can be found in lamb meat include *E. coli*, *Pseudomonas* spp., lactic acid bacteria, and even *Yersinia enterocolitica* (Table 2). It is essential to keep the product under strict temperature conditions to delay the microbial growth; besides, such as in other meat products, cross contamination can occur during the handling of the animal.

Miscellaneous foods

As part of this category, there is a group of very complex foods that include all the cooked-chilled foods that have meat, poultry, fish, and vegetables as part of the list of ingredients. Sales on these products have been drastically increased in the last few years because of the variety of products, innovative concepts, and developed products that fit several lifestyles. The average shelf life of these products is about 5 days when the temperature has been ≤3 °C. However, if the temperature is not well controlled, the products might represent a risk for consumption because of the kind of bacteria that can grow on them. These products are generally pasteurized and packaged such as mashed potatoes, pork chops, ratatouille, minced fish, spaghetti, and mac and cheese. A comprehensive list of these products is presented in Table 3. However, one of the main risks of these products is associated with the presence of some pathogenic microorganisms such as *L. monocytogenes* and spores of *C. botulinum* that can survive the pasteurization and be latent on the product. These cooked-chilled foods are also known as refrigerated processed foods of extended durability (REPFED) ready-to-eat meals.

Depending on the thermal treatment applied to this kind of products, there are three categories of REPFEDs:

(a) Mild thermal treatment at 70 °C for 2 min. Basically, this treatment is applied to inactivate *L. monocytogenes* in at least 6 log reduction. These products are pasteurized in the package used to sell them. However, this mild thermal treatment cannot inactivate spores in the product and represents a risk for the consumer if the food is not adequately handled by the consumer until the final consumption.
(b) Medium thermal treatment at 90 °C for 10 min. This treatment has the goal to inactivate the strains of nonproteolytic vegetative cells of *C. botulinum*, *Bacillus cereus*, and *L. monocytogenes*. The product is pasteurized in the package used to sell it, and because the thermal treatment is not really strong, some spores can resist the process and survive. However, the temperature used for this process can produce some thermal damage on the spores reducing the possibility of producing a foodborne problem.
(c) Repackaged chilled foods. This kind of products is pasteurized in some packaging material and after is repackaged in the intended final package. Sometimes, these products are pasteurized in opened containers and after packaged to be sold. The big risks in these products are related with cross contamination after the pasteurization with pathogenic strains.

Cold storage is one of the hurdles used on these products to extend the shelf life and delay the microbial growth; however, most of the time, a previous thermal treatment and some other preservation factors (such as reduction of pH, a_w, and antimicrobials) are used together.

One of the microorganisms that have been also associated with chilled foods is *B. cereus*. The spores of this microorganism can survive high temperature during conventional pasteurization but it can also survive refrigeration temperatures and grow during the storage of food, representing a microbiological concern. *B. cereus* is frequently associated with foodborne gastroenteritis. Several foodborne outbreaks have been reported in the food industry in the last decades because of the presence of spore-forming bacteria, mainly from the genera *Bacillus*. The vehicle of these microorganisms has been mainly associated with the vegetables that are part of most of these cooked-chilled foods.

Chemical Shelf Life

One of the main chemical reactions taking place on fish is oxidation of the lipids; these reactions known as rancidity are responsible of the generation of off-flavors and odors on the product. Marine products have a characteristic odor that can be easily noticeable. However, when there is lipid oxidation

Table 3 Examples of cooked-chilled foods (REPFEDs) available on the market; classification is made based on the main ingredient

Meat	*Poultry*	*Fish*	*Vegetable*	*Pasta*
Pork chops	Chicken and rice	Salmon and rice	Mashed potatoes	Spaghetti
Meatballs with tomato paste	Curry chicken	Fish in sauce	Spinach mash	Cannelloni
Veal stew	Chicken with vegetables	Minced fish	Ratatouille	Penne with vegetables
Beef burgers	Grilled chicken	Crab cakes	Carrots	Mac and cheese
Sliced ham	Turkey meat	Lobster cakes	Leak and potato mash	Lasagna
Roast beef	Poultry sausages	Surimi	Rice with vegetables	Fresh pasta salad

because of the changes in pH during the shelf life, this odor changes to a very unpleasant and putrid smell because of the several biochemical reactions taking place, in addition to microbial growth. Also, there are chemical changes associated with proteins and microbial growth together with enzymatic activity, releasing some nitrogen that is associated with fish deterioration. Some metabolites coming from the microbial activity such as amino acids are related with the protein hydrolysis taking place on fish and are also responsible for changes on the texture of the product.

On the other side, in some meat products, the chemical reactions taking place during the chilled storage are part of the natural process of tenderization of the product. Changes in pH, water-soluble compounds, and lipids on meat muscle during the storage will promote some desirable chemical reactions that provide the product with the characteristic flavor during the cooking process. For example, on beef, the biochemical changes during the postmortem period involving the adenosine triphosphate (ATP) degradation will produce specific flavor precursors on the product once it is cooked. These changes involve the reaction between sugars and amino acids producing specific volatile compounds. Nevertheless, the degree of production of these compounds is also influenced by other factors such as the diet and race of the animal, the season, the animal age, and the conditions of slaughtering, among others.

Because the quality of the product depends on the control on the chemical reactions taking place during the storage, several efforts focus on how to optimize these chemical changes. Some examples are the reduction of temperature keeping the product in special chambers, the use of special packaging materials and conditions, the incorporation of some chemicals on the ice, and the use of antioxidants and radical scavengers, among others. The use of antioxidants has been extensively documented; several compounds have been tested in different food products to delay microbial growth, to inhibit enzymes or reduce the chemical reactions catalyzed by them, and to scavenge the free radicals in those products having rancidity problems during storage.

Sensory Quality

The basic sensory evaluation of food products includes the assessment of odor, flavor, taste, texture, and appearance. During chilling of foods, some physicochemical changes take place; in some cases, these changes are part of the conditioning process of the product, for example, the postmortem changes mentioned earlier (such as ATP degradation) on fish, beef, pork, and poultry products. The flavor and aroma compounds in different kinds of meat are present on the water-soluble and lipid fractions of the product. Furthermore, these biochemical changes will impact the texture, general acceptability, and overall sensory quality of the product. The chemical reactions associated with the sensory characteristics of the food involve sugars, lipids, and amino acids.

Several studies have shown that the conditioning process of beef and pork meat provides better products in terms of sensory characteristics when the product is kept at chilled conditions as long as 21 days. Important and significant changes are observed in aroma, flavor, taste, and texture characteristics such as tenderness, juiciness, and chewiness when the meat is allowed for a long conditioning process. Another example is the use of CO_2 snow and brine chilled ($-2\ ^\circ C$) in ground beef; both techniques are able to delay the microbial growth on the meat up to 21 days. The use of CO_2 snow produced also a better texture on the product having a more tender meat; meanwhile, the chilled option showed cooking losses on the product.

Some specific products from beef, such as the heart and liver, which are used as by-products for other industries, are preserved as chilled foods. However, when the product is removed from the animal and immediately packaged under vacuum conditions, the meat is better preserved in terms of weight loss, off-flavor generation, and minimum microbial growth. Then, it is highly recommended for this kind of products to package the product under vacuum just as the postmortem period starts.

The use of herbs has also been used in the meat industry to improve the sensory quality of some products such as chilled lamb. Some extracts from rosemary have antioxidant effects, and once applied to chilled lamb, the product can have a longer shelf life not only with minimum lipid oxidation but also with excellent sensory properties in terms of flavor, texture, color, and aroma.

The main sensory issues observed on fish during the shelf life are related to the loss of freshness observed on the flesh, the changes on pigmentation, the presence of off-flavors, and the appearance of the skin. Regarding some of the novel applications in chilled foods, some of the previously mentioned studies using herbal extracts to extend the shelf life of fish have shown positive results regarding sensory characteristics. When the ice contains some herbs such as oregano, thyme, and rosemary, the product acquires a similar taste and flavor, making it more appealing to the consumer.

Furthermore, when fish is stored under specific preservation factors, the shelf life can be extended and the quality improved. For example, studying the use of superchilled ($-2\ ^\circ C$) and chilled ($4\ ^\circ C$) salmon and having the same fish under modified atmosphere, it is possible to extend the shelf life considerably. Products under superchilled conditions are able to show good quality and lower microbial counts (<1000 cfu g^{-1}) after 24 days of storage (total aerobic plate, H_2S-producing bacteria, and psychrotrophic counts). The superchilled salmon shows also acceptable sensory properties after 21 days of storage. The salmon kept under modified atmosphere and chilled was spoiled after 10 and 7 days, respectively.

Fruits and Vegetables

In this group, there are not only some products highly consumed such as the ready-to-eat salad blends because of the practical approach for the consumer but also some other products with lower demand such as some fruit cuts sold as chilled food or just regular fresh produce. Chilled salads represent a very good example of vegetables that have a reduced shelf life because of the natural characteristics of the ingredients. Some brands are sold using modified atmospheres to provide additional hurdles for the microorganisms. In most of the cases, the

microbial growth is delayed long enough to provide a safe product for the consumer but all the biochemical reactions affect considerably the sensory characteristics of the product, mainly the texture. Although chilled fruits and vegetables can be considered a safe product, it is well known that some foodborne outbreaks come from these products such as the salad blends involving pathogens such as *Salmonella*. Also, some consumers prefer to purchase fresh produce and disinfect and prepare them at home rather than use commercial chilled vegetables.

Some common products kept under chilling conditions and sold by piece include leafy greens, mushrooms, cucumbers, green and red peppers, carrots, herbs, squash, green beans, turnips, and celery, among others. These products are kept under chilling conditions to preserve their freshness and delay the microbial growth. However, when these products remain on cold storage for longer periods of time, the known chilling injury is observed. Some of these products kept at 4–5 °C and 95% of relative humidity can start to show some signs of injury such as tissue electrolyte leakage, stress because of ethylene production, and changes observed on appearance.

Conclusions

The chilled food chain represents a high percentage of the food production around the world. Hundreds of food items are currently sold as final products or ingredients and hundreds are new in the market each year. However, one of the main concerns related with these goods is still the microbial quality because of the constant foodborne outbreaks and the possibility of microbial growth during storage. It is really important to monitor the storage temperature of chilled foods throughout the production chain until the product reaches the consumer. Research has been done and is ongoing in the food technology field trying to identify new preservation factors to be used together with low temperatures not only to extend the shelf life of the product but also to ensure that pathogenic microorganisms are completely eliminated from the product after processing. The main challenge is to find these preservation factors strong against microorganisms but gentle with the nutritional and sensory quality of the product.

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Chilled Foods: Modified Atmosphere Packaging

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Rationale

Preservation of food products, by quality optimization and shelf life extension, is an important challenge of the food industry that allows the valorization of the final food product. Because food products are very sensitive to temperature, the reduction and maintenance of low temperatures are a crucial technology for food preservation. The technological development of chilling equipment in the last century allowed a wider range of food items in the market and enlarged the number of consumers. With the benefits of preservation by chilling, foods do not need to be so severely processed, such as in the traditional operations of canning, drying, smoking, and salting, and could be presented in the fresh state, minimally processed, or mildly processed as in pasteurization. Therefore, chilled foods, submitted to low-impact processing, result in higher quality and nutritional retention, however, typically with a relative short shelf life that reduces product marketability. Thus, this type of products may take advantage of the hurdle concept, associating chilling with the modified atmosphere packaging (MAP) technology in order to enlarge product shelf life from 50% to 400%. One success example of this hurdle technology concept is the application of MAP in chilling-sensitive produce, allowing to overcome the impact of low-temperature injury.

Modified Atmosphere Packaging

MAP technology relies on modification of the atmosphere inside the package and surrounding the food product that leads to an increased product shelf life, by acting at the microbiological, metabolic, and physicochemical levels in the product.

The modified atmosphere inside the package is maintained over storage time due to the control of gas transport through the packaging material that should have different barrier properties depending on whether it is nonrespiring or respiring products. Packaging of nonrespiring products needs a high-barrier material for gases in order to maintain the atmosphere injected, since no relevant metabolic activity exists in the product. Packaging of respiring products, such as fresh fruits and vegetables, is more complex than nonrespiring products because it involves the interplay between the natural respiration process of the fresh products and the gas exchange through the package containing the product, to generate and maintain the adequate atmospheric composition to product preservation. Thus, the packaging material needs a selective permeability to the gases involved.

MAP consists in the generation of an atmosphere that is different than normal atmospheric air. The typical composition of atmospheric dry air is 78.08% (v/v) N_2 (nitrogen), 20.96% O_2 (oxygen), 0.93% Ar (argon), and 0.04% CO_2 (carbon dioxide) and other trace gases. Normally, nitrogen, oxygen, and carbon dioxide are the three most common gases used in MAP. In very limited cases, it is also used carbon monoxide, ozone, ethylene oxide, nitrous oxide, helium, neon, argon, propylene oxide, ethanol vapor, hydrogen, sulfur dioxide, and chlorine. The presence of O_2 inhibits the growth of anaerobic microorganisms but promotes the growth of aerobic ones and some undesired reactions of oxidation and vitamin loss. The increased level of CO_2, compared with air, leads to a bacteriostatic and fungistatic effect, in part due to the acidic effect of dissolved CO_2 in the product, and also contributes to respiration rate reduction of many respiring products. The lower level of O_2, compared with air, has a positive effect on respiration rate reduction. Superatmospheric O_2 (30–80%) may have an identical effect on reducing the metabolic processes. The inert and tasteless N_2 gas, due to its low solubility in food products and inhibition of aerobic spoilage microorganisms growth, is normally used as filling gas to maintain the atmospheric pressure and avoid package collapse.

Even though vacuum packaging (VP) may be interpreted as a modification of the original package atmosphere and in that sense could be considered a type of MAP, normally, it is not regarded in literature as a MAP system due to the large differences between both packaging systems; in fact, VP has no atmosphere inside the package.

The term scientifically used as modified atmosphere is commercially substituted by protective atmosphere in food labeling, in order to be easily understood and not raise suspicions from consumers. Another term close, which sometimes is wrongly applied as synonymous, is controlled atmosphere that should not be confused with modified atmosphere. In controlled atmosphere, the gas composition surrounding the product is continually monitored and regulated to maintain the desired gas concentrations, normally applied not in a package but in a container or a storage room. Thus, the control of the atmosphere in modified atmosphere is less precise than in controlled atmosphere.

MAP is a technology that despite its wide commercial application in Europe dates back to the 1970–80s of last century meets the demands of today's consumer and market trends for safer and healthier products and convenience products, products already sliced and ready to eat or to prepare. This preservation technology, combined with temperature control, does not need to use chemical preservatives and based on the concept of barriers ('hurdles') allows the reduction of salt and additives in the product, maintaining its nutritional value. Moreover, the increase in the shelf life of the product has the effect of reducing product losses, thus reducing distribution costs and widening the market for such products. Thus, with the use of chilling and MAP technologies for preservation, many food products may be available out of season and/or shipped to distant consumer markets with a high standard

quality. However, the following limitations should also be pointed out: (i) increased product cost, (ii) loss of benefits once the pack is opened or leaked, (iii) restricted temperature control, (iv) specific recommended atmosphere for each type of product, and (v) consequently development of a MAP solution for each type of product.

The two most common types of retail MA packages are ellipsoid–cylindrical polymeric bags with sealed ends and parallelepipedic trays heat-sealed across the top with polymeric films. Polymeric films are the most popular among available barriers to create modified atmospheres. These materials present different properties depending on the chemical structure, production process, and additives. The plastic material may be composed of only a film (monolayer) or a set of layers of different polymers (multilayer). Since there is no monolayer plastic material having properties that make it suitable for all applications in the food industry, it is necessary to combine the properties of various plastics in a multilayer system suitable for each case.

The main desired factors to consider in the selection of the polymeric material for MAP are

- type of packaging (e.g., flexible or rigid package or tray with a semirigid cover);
- permeability properties of CO_2 and O_2;
- permeability to water vapor;
- physical properties (e.g., strength, transparency, and durability);
- effectiveness and strength of heat sealing;
- resistance to degradation by chemicals;
- nontoxic and chemically inert;
- easy printing on the outer surface.

The most common plastic polymers used for packaging foods are EVOH (ethylene vinyl alcohol), PVC (polyvinyl chloride), PVDC (polyvinylidene chloride), PET (polyethylene terephthalate), PP (polypropylene), PE (polyethylene), amorphous polyester, and nylon. Typically, these polymers are coated on the inside face with a chemical agent for dispersing droplets of condensed water during storage, ensuring good visibility of the product. LDPE (low-density polyethylene) and PVC have permeability characteristics that make them most suitable for the packaging of respiring products and PVDC and polyester in the case of products with low respiration rates.

MAP for Nonrespiring Chilled Foods

Definition

MAP of nonrespiring products consists of complete removal of the original atmosphere inside the package, replacing it with the desired atmosphere by injection at the time of closing or sealing of the package, without additional manipulation of the atmosphere inside the package. Therefore, the package has a crucial role in maintaining the initial atmosphere throughout storage time of the product.

The main reason of food preservation in nonrespiring products is the control of microbial growth, and the most common atmospheres used are the ones rich in CO_2 and poor or absent in O_2. There are many studies in literature studying different atmospheres in specific products to conclude the most appropriate atmosphere for the preservation of that specific product.

Design

The successful application of MAP technology is dependent on a correct design of the packaging system, taking in attention of the gas transfer phenomena between outside and inside packaging material and between inside atmosphere and product itself. If the desired atmosphere composition is initially injected, the purpose of the packaging material will be to maintain over time, as close as possible, that initial atmosphere. The packaging material should therefore be

- CO_2 barrier (after the initial injection of CO_2 into the atmosphere within the packaging, the packaging is intended to prevent the outflow of this gas over time since CO_2 has a beneficial antimicrobial activity),
- O_2 barrier (after the initial removal of O_2 from the atmosphere within the packaging, the packaging is intended to prevent the entrance of O_2 over time that would lead to color changes and reduce product acceptability by the consumer, with the exception of red color fresh meat that is favorable in the presence of O_2),
- barrier to aromatic compounds (in order to prevent loss of natural flavor of the product and prevent entry of off-flavors to the product),
- barrier to water vapor (normally, food products have a higher water content that facilitates its loss; thus, the packaging should prevent the exit of water that decreases product sensorial quality and commercial value).

However, temperature fluctuations in the distribution chain occur frequently, and the presence of condensed water inside the package is depreciated; water condensed could be a good medium for microbial growth and consequently limits the shelf life of the product. Therefore, it would be desirable that the packaging would be capable of absorbing the water released from the product.

The volume of gas should usually be between two and three times the volume of the food product due to the high solubility of CO_2 in the product.

Examples of MAP Applications

The main MAP applications in nonrespiring products are in cheeses, fresh meat, cured and smoked meat-based products, snacks (potato chips), fresh fish, fresh pasta, coffee, bakery products, and ready-to-eat foods.

For food products where the main spoilage parameter is oxidative rancidity, the gas mixture should be O_2-free. In opposition, the presence of O_2 in the MA composition is used in fresh red meat in order to avoid the red color loss. Usually, a mixture of around 20–60% CO_2 and 40–80% N_2 is adequate if the main objective is the microbial control. The use of concentrations of 20–30% (v/v) CO_2 and nonuse of O_2 for cooked and smoked meat-based products are common nowadays.

MAP for Respiring Chilled Foods

Definition

MAP of fresh produce is an atmospheric modification that relies on the interplay between the natural process of product respiration and gas exchange through the package. The main goal is the control of the metabolic activity, using low-O_2 and high-CO_2 atmospheric compositions that interfere in many metabolic processes. The potential effects of low levels of O_2 and high levels of CO_2 are related with reduction of (i) respiration rate, (ii) ethylene production and sensitivity to ethylene action, (iii) developmental alterations, (iv) incidence and severity of certain physiological disorders, and (v) susceptibility to decay, with the resultant benefit of retarding senescence and extending the shelf life of the fresh produce. Each different product and in some cases different cultivars have different responses to low O_2 and high CO_2. Exposure of fresh fruits and vegetables to O_2 levels below their tolerance limits or to CO_2 levels above their tolerance limits may hazard the product and decrease their storage life. The beneficial and injurious effects of low O_2 and high CO_2 specifically for fresh fruits, fresh vegetables, and fresh-cut fruits and vegetables were thoroughly studied and presented in scientific literature. The normal gas mixtures are 1–5% O_2 and 0–20% CO_2.

Types of MAP

The modified atmosphere inside a package can be achieved by either passive or active procedures. In passive procedure, the package is closed with the atmospheric air, and due to product respiration process, there are increase in carbon dioxide (CO_2) and a depletion of oxygen (O_2) concentration, changes that are regulated by the gas exchange through the package so that, at equilibrium, adequate low O_2 and high CO_2 concentrations are reached. The O_2 concentration decreases and the CO_2 concentration increases until the product respiration rate equals the gas exchange rate through the package and steady state values are attained. At steady state, the O_2 flow entering the package is equal to the O_2 consumed by respiration, and the CO_2 flow leaving the package is equal to the CO_2 produced by respiration. Throughout the transient period, the O_2 and CO_2 concentrations are not the most adequate to product preservation. In order to achieve the desired atmosphere more rapidly, the modification of the atmosphere in the package can be accelerated by the active procedure, although an increasing cost is inconvenient. In the active one, the procedure is similar with the nonrespiring products: removal and replacement of the atmosphere before closing the package. In this procedure, there is no lag time to achieve the optimal gas concentrations, and the interplay between the product respiration and the gas exchange through the package will allow to maintain the desired gas concentrations over product shelf life.

Design

An MAP system not properly designed may be ineffective or even shorten the storage life of a product. If respiration is much slower than the gas exchange through the package, the initial concentrations will almost not be altered. In opposition, if respiration is much faster than the gas exchange, O_2 concentrations will rapidly deplete and anaerobic respiration will be induced, thus the importance of a correct design of the packaging system. MAP design can be improved by the development of mathematical models used to predict the gas concentrations inside a package instead of the 'trial and error' approach. Modeling a MAP for a particular food product requires the analysis of different components of the system package–environment–commodity: (i) the gas exchange through package, (ii) the recommended gas concentrations, and (iii) the respiration rate for a specific product and storage conditions. Several models have been developed to predict the gas concentrations inside an MAP system with different levels of mathematical complexity.

Examples

MAP has a special interest in high added value and highly perishable products, achieving an increase in shelf life of up to 800%. For example, the export of highly perishable products such as strawberries and other red fruits needs an increased product shelf life. The recommended storage conditions under MAP for strawberry are 4–10% (v/v) O_2 and 15–20% (v/v) CO_2, at 0–5 °C combined with high relative humidity (90–95%). The use of MAP to extend the shelf life of mushrooms has been extensively reported, and the recommended MAP conditions for mushrooms at optimum storage temperature (0–2 °C) combined with high relative humidity (95%) are 3–5% (v/v) O_2 and $<12\%$ (v/v) CO_2, depending on each mushroom species.

Another example of high added value and highly perishable products are the fresh-cut ones. Fresh-cut products have shorter shelf life than intact products, owing to cell damage. Thus, chilling and MAP technologies for extending fresh-cut product shelf life may have a major impact on the fresh-cut market. The higher respiration rates of fresh-cut products, as well as their higher tolerance to CO_2 in general, require the use of packaging materials with a high O_2 transfer rate. Recent advances in MAP have been driven by the requirements of minimally processed vegetables. An important commercial application of MAP is cut lettuce. The success is attributed to retardation of browning, coupled with maintaining a fresh appearance. The recommended MAP conditions for cut lettuce at optimum storage temperature (0–5 °C) combined with high relative humidity (90–95%) are 0.5–3% (v/v) O_2 and 5–10% (v/v) CO_2. Other common fresh-cut products taking advantage of MAP are shredded carrots (2–5% (v/v) O_2 and 15–20% (v/v) CO_2 at 0–5 °C).

See also: Apples; Brassica: Characteristics and Properties; Cheese: Processing and Sensory Properties; Chilled Foods: Packaging Under Vacuum; Chilled Foods: Principles; Controlled Atmosphere Storage: Applications for Bulk Storage of Foodstuffs; Controlled Atmosphere Storage: Effect on Fruit and Vegetables; Convenience Food; Cured Foods: Health Effects; Meat: Eating Quality and Preservation; Pasteurization: Principles and Applications; Preservation of Foods; Storage Stability: Mechanisms of Degradation.

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Chilled Foods: Packaging Under Vacuum

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The Role of Oxygen in Food Spoilage

The presence of oxygen is one of the major factors of spoilage of foods and causes the following:

(1) Oxidation reactions, damaging vitamins, fatty substances, pigments, and flavoring substances that are often catalyzed by enzymes
(2) Growth and activity of aerobic microorganisms (aerobic bacteria, yeasts, and molds)

It is therefore essential, in order to prolong the freshness of foodstuffs, to eliminate the presence of oxygen in contact with the foodstuff itself, and to prevent further access of oxygen during storage. Vacuum packaging is one of the simplest and most widely used systems to achieve this objective. This type of packaging has enjoyed increasing success since the late 1950s, in parallel with the development of the technology of plastics owing also to the changes in the distribution chain of perishable foods requiring increased hygiene and storage life.

This article outlines the principles of vacuum-packaging technology and the features of the packages utilized and describes the main fields of application of vacuum packaging to chilled foods.

Vacuum Packaging: A Definition

'Vacuum packaging' is a term improperly but commonly used to define a packaging system that implies the reduction of the partial pressure of atmospheric gases (oxygen being 20% of them) inside a package. A vacuum inside a package can be obtained mainly through two systems:

- Steam flushing of the headspace
- Sucking of air from the package headspace by means of equipment (vacuum chamber and nozzle) based on a vacuum pump

The former system is mainly utilized in hot packaging of shelf-stable products, which are generally packaged in rigid containers. The elimination of air is achieved by means of a steam flush that replaces atmospheric gases inside the package; steam condensation in the package headspace after chilling reduces the inner gaseous pressure. The latter system is commonly utilized for packaging of perishable foodstuffs that have to be stored in chilled conditions. In this case, packaging equipment based on a vacuum pump is generally utilized in combination with flexible packages, which, after evacuation, are closed hermetically (by means of a seal or sometimes a tight clip) and collapse on the packaged product once the package is exposed to atmospheric pressure. Depending on the type of equipment employed, a residual pressure of atmospheric gases as low as 500 Pa can be obtained in the package.

Figure 1 illustrates the main systems utilized for evacuating flexible packages:

(1) Nozzle (Figure 1)
(2) Single vacuum chamber (Figure 1)
(3) Divided vacuum chamber (Figure 1)

Packaging Materials Utilized for Vacuum Packaging

Plastics are the main raw material utilized for manufacturing the flexible materials employed in vacuum packaging of chilled foods. These materials must have the following properties:

- Flexibility
- Mechanical resistance to various forms of abuse (abrasion, puncture, and flex cracking)
- Gas-barrier properties adequate to the application requirements (generally expressed as gas permeation rates)
- Thermosealability or clippability
- Good optics (haze and gloss)
- Printability
- Suitable dimensional behavior (dimensional stability to heat, formability after heating, and shrinkability after heating)

To combine and balance to the required level of the earlier mentioned properties, it is often necessary to mix different types of individual materials. Different resins can be mixed together (resin blends), resin additives (such as plasticizers, pigments, stabilizers, and slip agents) can be used, and, more commonly, a multilayer material is produced, each layer being composed of a distinct individual material that imparts its properties to the overall structure.

Individual components most commonly used in manufacturing flexible materials utilized for vacuum packaging are listed in the succeeding text, together with their abbreviations and main properties:

- Polyethylene (PE): sealability, formability, moisture barrier, and low cost
- Polypropylene (PP): moisture barrier, thermal resistance, and dimensional stability
- Ethylene–vinyl acetate (EVA) copolymer: easy sealability and thermal shrink properties
- Ionomers: mechanical strength, easy sealability, grease resistance, and formability
- Polyamides (PA): mechanical strength, gas barrier, and formability
- Polyesters (PET): mechanical resistance, heat resistance, and gas barrier
- Ethylene–vinyl alcohol (EVOH) copolymer: gas barrier and easy processability in coextrusion
- Polyvinylidene chloride (PVDC): gas barrier and grease barrier

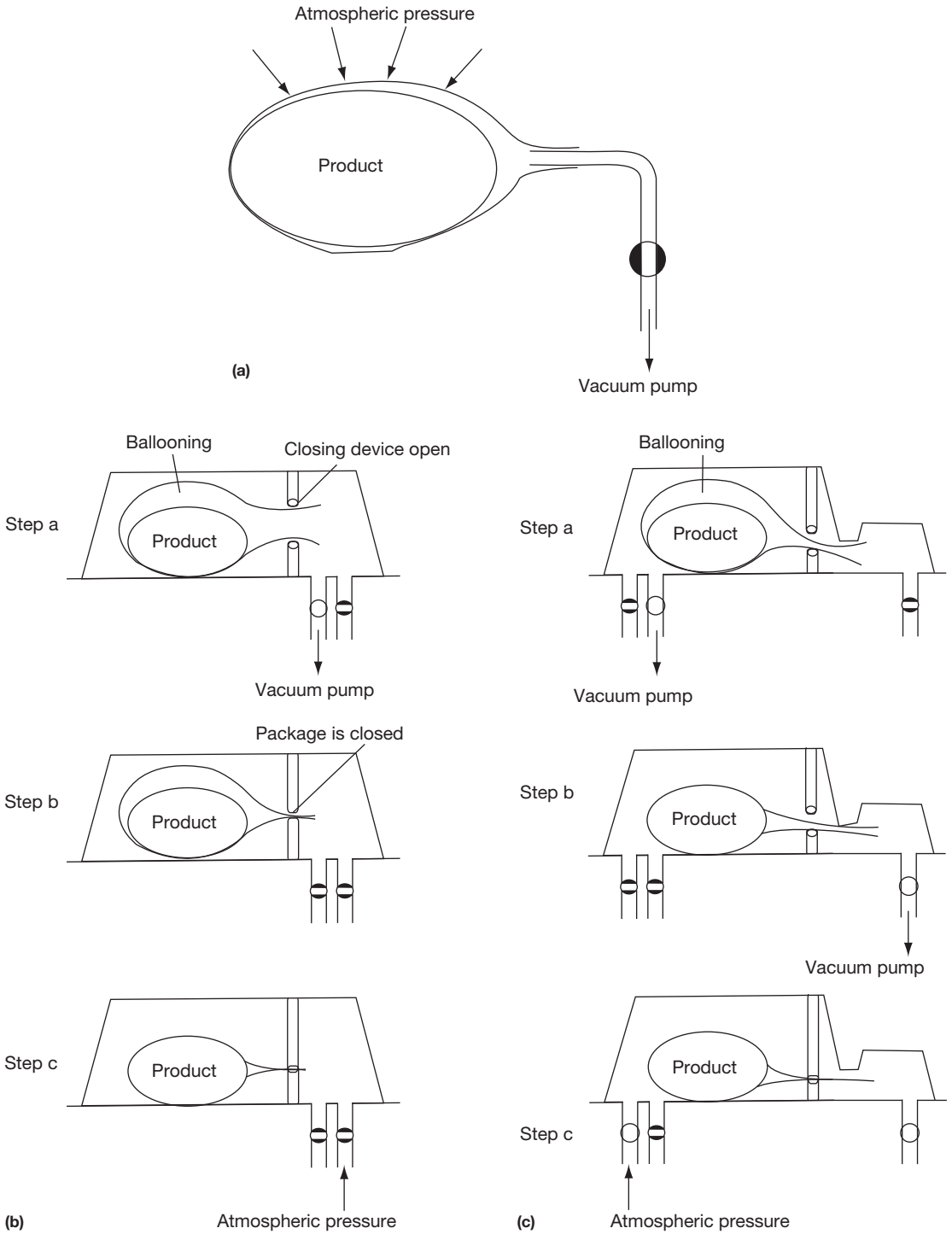

Figure 1 Main systems utilized for obtaining vacuum packages. (a) Nozzle system. Air is extracted from the package (a bag or a pouch) through a nozzle, and then, the package is closed. This is the simplest way of extracting the air, but it does not allow high levels of vacuum in the package. (b) Most common system utilized for evacuating bags, pouches, and thermoformed packages. (c) Divided vacuum chamber. This allows a better evacuation of the package headspace by using two separate chambers where a vacuum is applied sequentially. Reproduced from Chilled storage: packaging under vacuum. Macrae, R., Robinson, R. K. and Sadler, M. J. (eds.) (1993). *Encyclopedia of food science, food technology and nutrition*. Academic Press.

PE, PP, EVA, and ionomers are classified in the wide family of resins called polyolefins. Aluminum foils and vacuum-metallized plastic films are also utilized because of the excellent gas-barrier properties of aluminum.

Multilayer materials are manufactured using various techniques:

(1) Coextrusion: the molten resins are combined in the final structure by extruding them through a round or flat extrusion die, which keeps them separate in discrete layers.
(2) Lamination: previously extruded plastic films and, sometimes, aluminum foil are joined together by means of glues (glue lamination) or with resins that have adhesive properties (extrusion lamination).
(3) Coating: preextruded films are coated with a layer of molten or dissolved resin (a latex).

Package Configurations Used for Vacuum Packaging of Chilled Foods

These can be classified into four main categories: shrink bags such as Cryovac, pouches, thermoformed packages, and skin packages such as Darfresh (Cryovac and Darfresh are registered trademarks of Cryovac Inc., a subsidiary of Sealed Air Corporation).

Shrink Bags

Shrink bags are available in the form of premade bags that can be prepared in different packages (e.g., taped bags) to allow their utilization on automatic equipment. The main feature of these bags is their ability to shrink when exposed for a short time to heat (for instance, a few seconds at 90 °C). This behavior is the consequence of treatment imparted to the packaging material during its production (oriented polymeric chains that retain a built-in tension, making them able to shrink when relaxed by heating).

Shrinking increases the packaging material thickness (which varies between 40 and 120 μm), improving mechanical resistance and gas-barrier properties; determines a tighter package around the product, limiting dripping out of juices in moist products such as meat; and improves the appearance by eliminating excess of packaging material around the product. Shrink bags, at the onset of their introduction on the market in the 1950s, were monolayer PVDC materials, but subsequent technological evolution gave rise to complex coextruded multilayer structures having polyolefins as the main components and gas-barrier layers made of PVDC or EVOH.

Some materials are electronically cross-linked to improve mechanical properties.

Shrink bags are used for vacuum packaging of industrial units of fresh meat, processed meat, and cheese and for consumer units of processed meat and cheese.

Pouches

Pouches can be utilized in two forms:

- Premade plastic envelopes of different sizes that are loaded with the product, then evacuated, and sealed in vacuum chambers
- In-line prepared pouches from machines using rollstock material (horizontal form fill seal and vertical form fill seal machines)

The earlier mentioned machines, starting from a flat web, produce a film tubing that can be either horizontal or vertical (hence the different definitions), which is filled with the product, sealed transversely, and cut into final packages.

The machines can be equipped with a vacuum chamber to evacuate the pouch before final sealing. Form fill seal machines are widely employed in food packaging; however, their utilization for vacuum packaging of perishable foods is rather limited as thermoforming is preferred whenever a high packaging output and automation of packaging operation are required.

Premade pouches are commonly utilized for vacuum packaging of industrial units of fresh red meat such as whole primal cuts, processed meat (ham, bacon, salami, and bologna), and cheese. Typical structures of materials for pouches, obtained by means of glue or extrusion lamination, are based on bioriented PA6 or PET (biorientation plus heat setting enhances dimensional stability and mechanical resistance), which can be coated with PVDC to increase the gas-barrier properties and are laminated to suitable sealing layers (PE, EVA, or PP and ionomers when heat resistance for pasteurization or cooking in the package is requested). For long storage-life applications (pasteurized cooked ham or sausages) where maximum gas-barrier properties are needed, aluminum foil is also used.

The total thickness of the materials mentioned earlier varies between 70 and 250 μm, the higher thickness being employed for heavy and hard products.

Thermoformed Packages

These are obtained on continuous thermoforming machines, which use rollstock materials. Two rolls are used, one for the bottom web, which is unwound, heated by a warm plate, and formed into cavities where the product to be packaged is subsequently loaded, and one for the top web, which is sealed onto the bottom web inside a vacuum chamber from which the air has been removed. Thermoforming is widely applied to the packaging of perishable foodstuffs because of the flexibility of the process, the packaging speed, and the ease of automation.

A wide variety of packaging materials are utilized. Bottom webs are generally based on PA laminated or coextruded with all types of polyolefins. PVDC and EVOH are used when high gas-barrier properties are needed. Some bottom webs are also heat-shrinkable after thermoforming.

Top webs are generally laminated structures similar to those used for pouches, the thickness of which seldom exceeds 100 μm. Metallized materials are often used in the top web formulation.

Thermoforming is utilized for packaging many kinds of perishable products, both consumer and industrial units, including whole ham and fresh meat primal cuts, with the exception of the biggest units of cheese and processed meat.

Skin Packages

Skin packaging represents an evolution of thermoforming where the top web is softened by means of heat and subsequently formed onto the packaged product.

The bottom web can be either flexible or rigid and can be preformed into a tray or a product-sized cavity into which the product to be packaged is loaded.

In skin packaging, the top film conforms tightly to the product shape with advantages in terms of appearance, limited product crushing due to vacuum, and the possibility of contour sealing around the product, limiting the exudation of liquids from the product. Because of its appealing appearance and the high cost of the packaging materials, skin-packaging utilization is limited to consumer or family units of meat (both fresh and processed), fish, prepared meals, and cheese.

Top webs are usually based on ionomeric resins or on coextruded structures of polyolefins. PVDC or EVOH provides the necessary gas barrier.

Bottom webs are generally either laminated or coextruded structures similar to those used for thermoforming.

When a rigid tray is required, polyvinyl chloride (PVC), polystyrene (PS), or PET is employed.

Influence of Vacuum Packaging on the Storage Behavior of Chilled Foods

Fresh Meats and Poultry

Meats contain an abundance of nutrients necessary for the growth of microorganisms; they are particularly rich in soluble organic substances such as carbohydrates, amino acids, and nucleotides. Therefore, spoilage of fresh meat and poultry during chilled storage is mainly due to the growth of aerobic psychrophilic bacteria belonging to the Pseudomonadaceae family and to the *Moraxella–Acinetobacter* group. The growth of these bacteria results in development of off-odors (due to hydrogen sulfide, ammonia, amines, and indole) and bacterial slimes, which contribute to meat discoloration.

Vacuum packaging in oxygen-impermeable materials limits oxygen supply to the typical aerobic spoilage microflora, providing conditions suitable only for the slower-growing lactic acid bacteria, which, in chilled conditions, require several weeks to produce off-odors.

In addition, vacuum packaging has an impact on meat color, which is mainly due to the presence in the muscle tissue of myoglobin, a conjugated protein where the protein moiety (globin) is bonded to a heme group.

The iron atom of the hematin nucleus can form complexes with different ligands and can be in either the ferrous (Fe^{2+}) or ferric (Fe^{3+}) oxidation state. The globin can be in either the native or the denatured state. Among the different complexes of heme, globin, and ligands, three are important in fresh meat:

- Oxymyoglobin, with oxygen as the ligand and iron in the ferrous state, characterized by a bright red color
- Myoglobin, with water as the ligand and iron in the ferrous state, characterized by a purplish-red color
- Metmyoglobin, with water as the ligand and iron in the ferric state, characterized by a brown color

Oxymyoglobin is the pigment normally present on the surface of meat exposed to air and gives the meat its bright and attractive color.

During storage, as a consequence of the growth of aerobic bacteria that reduce the availability of oxygen on the meat surface and of the reduction in the capability of the meat to reduce its own metmyoglobin level, metmyoglobin tends to predominate, imparting its brown color to the meat surface, which contributes to consumer rejection of the product.

In vacuum-packaged meat, as a consequence of preventing oxygen access to the meat surface, the pigment is of the myoglobin color, and the meat appears darker than a sample exposed to air or packaged in modified atmospheres. Displaying of vacuum-packaged consumer units of fresh meat can create problems of consumer acceptance because of the purplish color of the meat surface. In this case, proper consumer warning has to be given to explain the origin of the color and the advantages of vacuum packaging in terms of prolonged storage life.

The storage life at 0–2 °C of fresh primal meat vacuum-packaged in bags or pouches is 4–8 weeks, allowing full aging of meat types such as beef that require maturation. Consumer units in the form of meat slices have a storage life of 2–3 weeks.

Processed Meats

It is useful to classify the many existing types of processed meats into two main categories of products:

- Products having high water activity, the production processes of which often imply cooking (frankfurters, patties, bologna, fresh sausages, cooked ham, and luncheon meats)
- Cured products with low water activity (raw ham, salami, and bacon)

All the products mentioned earlier can benefit from vacuum packaging, which avoids surface drying and mold growth and greatly slows down the oxidation of fat and pigments. Pasteurizable packages are also commonly used for microbial stabilization of some products (cooked ham, patties, and frankfurters). Furthermore, cook-in-the-package technology has been developed for the production of cooked ham, allowing optimization of the production process, hygienic quality, and yield.

The chilled storage life of vacuum-packaged processed meats is very variable, depending primarily upon product composition and packaging material characteristics, and can vary from a couple of weeks (certain types of fresh sausages) to several months (cured products and pasteurized or cook-in-the-package products).

Cheese

Vacuum packaging is mainly applied to hard and semihard types of cheese, for both industrial and consumer units, and its effect in storage-life extension is due mainly to

- elimination of surface drying,
- slowing down of fungal growth,
- limitation of oxidation of fatty substances.

An interesting application of vacuum packaging is the curing in the package technology that is utilized for certain types of cheeses (Emmentaler, Gouda, and Edam). The cheese is vacuum-packaged at an early curing stage and cured inside

the package. This technique improves yield (limiting rind formation and water loss) and allows a certain product standardization. Curing in the package is particularly demanding in terms of gas transmission properties of the packaging material; a good compromise is necessary between a sufficiently high carbon dioxide permeability to allow the escape of gas formed inside the cheese during curing and a sufficiently low oxygen transmission rate to avoid mold growth. The gas transmission rate requirements can vary from cheese type to cheese type and also depend upon production conditions, which are often variable among different dairies.

Fish

Wet fish is probably the most perishable type of foodstuff because of the high content of soluble substances in the flesh, many of which contain nitrogen and triglycerides characterized by polyunsaturated fatty acids (in fatty types of fish such as clupeids, salmonids, and scombroids). In addition, fish flesh is characterized by a high level of enzymatic activity, which plays a major role in the early stages of spoilage.

Vacuum packaging retards the growth of aerobic spoilage bacteria and limits the oxidation of fat. However, vacuum-packaged fish is very perishable and has to be carefully stored at temperatures below 2 °C, the normal storage life being 5–7 days. Prepackaged consumer units of wet fish, utilizing skin packages, have recently been introduced at a commercial level due to the need for a distribution system capable of handling in a relatively easy way a product characterized by bad smells, dripping out, and hygienic and preparation problems at the consumer level. For processed fish (smoked, salted, and pickled), vacuum packaging is commonly utilized because it limits the spoilage factors of this kind of product (mainly fat oxidation and mold growth). The storage life of vacuum-packaged processed fish depends upon the water activity level, ranging between 2 and 3 weeks for slightly salted fish and a few months for highly salted fish.

Prepared Foods

Vacuum packaging is also utilized for a wide range of prepared foods (delicatessen products, cooked meats and poultry, prepared meals, and salads).

The spoilage mechanism and perishability of these products are related to their chemical composition (pH and additives) and heat treatment (pasteurization), and therefore, their storage life is very variable, ranging from 2 weeks to several months.

An interesting application of vacuum packaging to prepared foods is the cook-in-the-package technique, which implies packaging under vacuum of raw or partially cooked foods that are cooked inside the package, pasteurized (when required), chilled and stored, and reheated and unpackaged at the time of consumption. This technology allows the rationalization of meal preparation in central units (e.g., in institutional kitchens and restaurant chains) because of the storage life of the prepared meal (1 week or more) and it has also been introduced to food-processing plants to prepare meals to be distributed chilled at the retail level.

See also: Controlled Atmosphere Storage: Applications for Bulk Storage of Foodstuffs; Lactic Acid Bacteria; Meat: Structure; Oxidation of Food Components; Spoilage: Bacterial Spoilage.

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Chilled Foods: Principles

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Introduction

Nowadays, the population exodus to urban areas has required some improvements in food preservation techniques and greater distribution services of perishable products. Due to modern consumer preferences of quality, the most important method of preservation and distribution to the main world market is by chilling and freezing. Today's lifestyles have also changed consumer behavior, product preferences, and eating trends resulting in new requirements for food producers.

Chilling is one of the preservation techniques that is most applied with food products, having been established in the nineteen century. Because of the complexity of the refrigeration process, it needed more time to be developed than the heating process. People learned to produce heat about 500 000 years ago, but the chilling process started about 150 years ago—although some reports from the second century (BC) established the use of 4° of coldness for medical purposes. By definition, the refrigeration process can be described as a simple reduction of the ambient temperature (i.e., if the ambient temperature is around 40 °C the product transport can operate with a refrigeration system to reduce the temperature to 30 °C), even though some authors have considered the refrigeration temperatures to only be in the range of 0–10 °C, based on the preservation principle involved.

The refrigeration principle consists of a reduction in the product temperature which reduces the kinetic energy, ultimately minimizing the rate of motion of the molecules inside the product. This rate determines the speed at which many important reactions take place. In the case of food products, many spoiling reactions can be controlled by temperature, such as: microbial growth, physiological processes (ripening of fruits and vegetables), and chemical and enzymatic reactions (enzymatic and non-enzymatic browning, vitamin degradation, lipid oxidation, and biocompound degradation).

Basically, all food products can be preserved by refrigeration for some time, depending on the final temperature reached and the chilling rate. However, the final storage temperature and the amount of time that the product is subjected to refrigeration have different effects that can compromise the product quality. In this sense, in rapidly cooled peaches, a serious defect can be observed, known as 'wooly texture,' in which a lack of juiciness without variation of the tissue water content is shown. Another typical case of the effect of the refrigeration process on food products is the phenomenon called 'cold shortening,' which is an irreversible reduction of the muscle tissue – that can be detected in beef, lamb, and pork meat – when the temperature is reduce rapidly.

This contribution establishes the principles related to the chilled process, equations related, new techniques, and also, improvements in the refrigerant compounds employed in food processing.

Chilling

The chilling process can be described as the method to remove heat from a product. It is the most benign process applied to food, presenting only a few changes in flavor, texture, and nutritious value. In general, refrigeration temperatures for food product preservation are below 15 °C and above the freezing point. Although human beings have been using cold storage since ancient times – by placing products in ice or at low temperatures outside – the refrigeration process, or mechanical refrigeration, has no more 150 years of application.

Due to their nature, food products are vulnerable to spoiling, reducing their commercial life by three mechanisms:

- The growth of live organisms can contaminate and deteriorate food products. Microorganisms, such as bacteria, parasites, and molds can be in the air and soil. Insects and rodent can contaminate food products with their own microbial fauna.
- Chemical reactions, such as browning or oxidation, can produce different compounds causing changes in flavor or texture of the product.
- Biochemical reactions, like respiration, ripening, and browning (in vegetables and animal meat after slaughter) are basically carried out by enzymes within the product. Also, these reactions may reduce the product quality and other physical phenomena such as gain or loss of moisture that reflects on moisturize or dehydration.

On the other hand, the term 'shelf life' refers to the timeframe that the product (stored under the recommended conditions) will:

- remain safe for consumption;
- be certain to retain desired sensory, chemical, physical, and microbiological characteristics; and
- fulfill any label declaration of nutritional information.

This definition of shelf life implies the storing conditions, which, when referring to refrigerated conditions, may vary enormously due to temperature changes during the transportation and in-home handling before their consumption.

The main effect of chilling is the decrease of storage temperatures, which also implies a reduction in the rate of the reactions mentioned above, presenting an increase in the shelf life of the product. The deterioration rate can lessen as the temperature decreases to the minimum in the range of the refrigeration temperatures. Although, even below freezing temperatures, some reactions still take place in some products.

The most important effect of the chilling process on food products is the reduction of the microbial growth, which increases the shelf life of the product. The most common temperature for microorganism growth is around 36 °C; but microbial growth has been detected in extremely low (−34 °C), and extremely high (100 °C) temperatures as well.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00143-4

The microbial grow division in this large range of temperature refers to three main groups. The first group of microorganisms that grow well at or below 7 °C, and have their optimum between 20 and 30 °C, are referred to as *psychrotrophs*. Further, the group that can grow well between 20 and 45 °C, with their optimum range between 30 and 40 °C, are referred to as *mesophiles*. While the group that grows well at and above 45 °C, with their optimum range between 55 and 65 °C, are referred to as *thermophiles*.

Psychrotroph microorganisms that can grow under refrigerated conditions are: a. bacteria *Shewanella*, *Alcaligenes*, *Flavobacterium*, *Corynebacterium*, *Lactobacillus*, *Micrococcus*, *Pseudomonas*, *Psychrobacter*, *Brochothrix Enterococcus*, and others, being *Pseudomonas* and *Enterococcus* the most commonly detected and c. molds c strains of *Aspergillus*, *Cladosporium*, and *Thamnidium*. Under refrigerated conditions, the growth of some yeast has been detected, but in less numbers than bacteria and molds.

Although shelf life is enhanced by chilling, it only increases by a small amount of time compared to other preserving techniques, such as heat treatment, freezing, or drying.

Further, it is important to point out that chilling can be applied not only to enhance the product shelf life, but to control the growth reaction rate of specific microorganisms desirable in food products. This is the case of the chilled ripened process in some cheeses, meats, and wines.

It can be assumed that the lower the storage temperature, the higher the shelf life; but this is not always true. Some products, mainly fruits and vegetables, may suffer when they are stored at temperatures close to 0 °C. For instance, peaches can suffer undesirable internal and core browning (in a process called woolliness) if they are stored at temperatures close to 0 °C. In the case of potatoes, when they are stored below 3 °C quality deterioration occurs as a consequence of changes in the starch–sugar system that produces the accumulation of sugars. Table 1 presents the most common deterioration problems related to cold storage. Further, much research has been carried out to find the best temperature storage conditions for different food products, with the intention to reduce undesirable reactions and optimize their shelf life. Table 2 presents the optimum storage conditions for some food products.

Moreover, another parameter that must be considered during food refrigeration storage is the relative humidity. If the water activity in the product is higher than the relative humidity inside the refrigeration room, there will be loss of moisture in the product. On the contrary, if the relative humidity in the refrigeration room is higher than the water activity, water condensation may occur, which, in many cases, produces microbial growth and spoilage of the product. In this sense, high relative humidity conditions (between 90% and 95%) are preferred for fruit and vegetable products—based on their high moisture content. On the contrary, products with low moisture content, such as cheese or nuts, should be stored under reduced relative humidity conditions ($<85\%$). To prevent microbial growth in the refrigeration chamber with high relative humidity, it is necessary to avoid water condensation on the chamber walls.

The Refrigeration Process

Refrigerants

Refrigerants are chemical substances that can take the heat out from the product and release it outside the refrigeration system. The substances employed in vapor compression systems, such as NH_3, CO_2, and R134 are named primary refrigerants

Table 1 Deterioration of fruits and vegetables under refrigeration storage conditions

Product	*Critical storage temperature (°C)*	*Type of deterioration between the critical and freezing temperature*
Mango	10–13	Gray discoloration of peel Unequal ripeness
Melon	7–10	Spot, peel putrefaction No ripeness
Apple	2–3	Internal and core browning, scald, moist breaking
Orange	3	Spot, brown marks
Sweet potatoes	13	Internal discoloration, spot Rotting
Eggplant	7	Scald on surface Rotting
Olive	7	Internal browning
Avocado	4–13	Brown discoloration in pulp
Squash	10	Rotting
Green beans	7	Spot and redness
Lime	7–9	Spot
Lemmon	14	Spot, red mark
Papaya	7	Spot, rotting No ripeness
Cucumber	7	Spot, rotting
Pineapple	7–10	Green color upon ripening
Banana	12–13	Opaque color upon ripening
Watermelon	4	Spot, unpleasant odor
Tomato (green)	13	Light color upon ripening, rotting
Tomato (ripe)	7–10	Rotting, softening, soaked water

Table 2 Optimum storage conditions different foods

Food product	*Storage temperature (°C)*	*Relative humidity (%)*	*Storage life (days)*
Banana	11–15.5	85–95	7–10
Apricot	−0.5 to 0	90	7–14
Bean (snap)	7	90–95	7–10
Broccoli	0	95	10–14
Carrot	0	98–100	28–42
Celery	0	95	30–60
Cherry	−1	90–95	14–20
Chicken	Just above the freezing point	85–90	20–30
Cucumber	10–15	90–95	10–14
Eggplant	7–10	90–95	7–10
Fish	Just above the freezing point	90–95	7–10
Lemon	10–14	85–90	30–180
Lime	9–10	85–90	40–140
Lettuce	0–1	95–100	14–20
Meat	Just above the freezing point	85–90	10–15
Milk (pasteurized)	Just above the freezing point	<85	10–15
Mushroom	0	90	3–4
Peach	−0.5 to 0	90	14–30
Plum	−1 to 0	90–95	14–30
Potato	3–10	90–95	150–240
Spinach	0	95	10–14
Strawberry	−0.5 to 0	90–95	5–7
Tomato	4–10	85–90	4–7
Watermelon	4–10	80–90	14–20

Table 3 Application of different refrigerant compounds used in the industry

Refrigerant						
Formula	*Number*	*Latent heat (kJ kg^{-1})*	*Boiling point 100 kPa (°C)*	*Environmental classification*	*Toxicity*	*Flammability*
CCl_3F	R-11	194.20	23.8	CFC	Low	Low
CCl_2F_2	R-12	163.54	−29.8	CFC	Low	Low
$CHCl_2F$	R-21	254.20	−44.5	HCFC	Low	Low
$CHClF_2$	R-22	220.94	−40.8	HCFC	Low	Low
	R-123	170.47	27.83	HCFC	Low	Low
$C_2H_2F_4$	R-134	217.28	−26.05	HFC	Low	Low
NH_3	R-717	1328.48	−33.3		High	High
CO_2	R-744	352.00	−78.5		Low	Low

and are used to chill the product. Substances employed for the transportation of the chilled product from one cooled place to another are named secondary refrigerants. In this sense, solutions with a freezing point below 0 °C are used as secondary refrigerants; the most commonly employed of these solutions being ethylene glycol, propylene glycol, and calcium chloride. Their properties are similar; however, propylene glycol has the advantage of being safe if it comes into contact with food.

The first refrigerant applied in the industrial vapor compression systems was ethyl ether, around 1850. After, other refrigerant products were employed, such as carbon dioxide, methyl chloride, butane, ammonia, ethane, and gasoline—among others. During the decade of the 1920s, after many factory workers were injured and or killed due to leaks of these first refrigerants, their use was limited and finally prohibited for use in the industry.

Later, during the first part of the twentieth century, the company DuPont developed the refrigerant R-21; the first member of the chlorofluorocarbon family. Sometime later, the company developed R-12 (Freon), naming it as the most adequate refrigerant for commercial use. However, during the 1970s, it was discovered that Freon had a negative impact on two worldwide problems: the thinning of the protective ozone layer at the stratosphere (protecting the ground from ultraviolet radiation from the sun); and the increase in global warming by the greenhouse effect on the atmosphere. For these reasons, the refrigerants R-12 and R-22 have been replaced by other kinds of refrigerants, such as R-134a and R-123. Table 3 presents the main characteristics of the primary refrigerants used in the industry and in domestic equipment. From this, the most important characteristics are the vaporization heat and low boiling point, followed by the toxicity and

flammability. Even though the ammonia (R-717) has good thermodynamic properties, the high toxicity and flammability make it extremely difficult to use in chilling industry.

Refrigerants can be classified by the halocarbon numbering system. This system was first adopted by the DuPont Company. But also, it has been adopted by some professional associations, such as the American Association American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE). The general model to name a refrigerant is R-*xyz*C, where R stands for refrigerant, x stands for the number of carbon atoms, y stands for the number of hydrogen atoms and z stands for the number of fluorine, chlorine atoms. Table 4 presents the classification convention for this system.

Further, there is a classification systems presented by the International Union of Pure and Applied Chemistry (IUPAC). In this case, the refrigerants are classified by chemical composition, being:

- CFC. Chlorine-Fluor-Carbon compounds. They are presently banned because of their ozone depletion potential (ODP). Production stopped world-wide in 1995 and usage in 2000 (e.g., R12, R13, R500).
- HCFC. Hydro-Chlorine-Fluor-Carbon. The use of these refrigerants is not allowed because of their zero ODP, their production is going to stop in 2015, and usage in 2030, worldwide, but in the European Union, in 2001 for new equipment, in 2010 for old equipment, and in 2015 for any use (e.g., R22, R409).
- HFC. Hydro-Fluor-Carbons. Their use is allowed because of their zero ODP, but they contribute to the global warming potential (GWP). Fluorocarbons, where all hydrogen atoms are replaced by fluorine atoms, are often called perfluorocarbons (PFC) (e.g., R134a, R410A, R404A, and R407C).
- HFO. Hydro-Fluor-Olefin. It almost has equal thermal behavior to that of R132a, but with a much smaller GWP. The most common R1234yf (2,3,3,3-Tetrafluoropropene, $C_3H_2F_4$) is being used as a replacement for R134a in automobile air conditioners.
- Natural refrigerants (no ODP or GWP): such as air, water, ammonia (R717, NH_3), carbon dioxide (R744), and propane (R290, C_3H_8), are flammable, or toxic, or have very large vapor pressures.
- Halons. Containing bromine which replaces some chlorine compounds. This refrigerants were developed in the 1960s, and were employed more as a fire-fighting agents. However, because of the ODP, their production stopped in the 1990s.

There are signs used to detect a leakage of the refrigerants, such as odor in natural (ammonia) and artificial (hydrocarbons) refrigerants, some physical effects (bubbling), chemical effects (catalytic reaction in platinum wires) and fluorescence excitation with ultra-violet lighting to detect CFC, HCFC, and HFC refrigerants.

Cryogenic chilling is the term applied to refrigerants that change their phase by absorbing latent heat to cool the food. There are only three kinds of cryogen refrigerants: solid carbon dioxide, liquid carbon dioxide, and liquid nitrogen.

The phase change mechanism in solid carbon dioxide is by sublimation (solid to gas), removing latent heat of around 352 kJ kg^{-1} at −78 °C. Liquid cryogens remove latent heat by vaporization, obtaining around 358 kJ kg^{-1} at −196 °C for liquid nitrogen. In the case of liquid carbon dioxide, the latent heat is similar to the solid. Further, the gas obtained also absorbs sensible heat as it warms from −196 °C (liquid nitrogen) or from −78 °C (CO_2) to give a total refrigerant effect (the total amount of heat that can be removed by the refrigerant) of

Table 4 Refrigerants nomenclature by halocarbon numbering system

Code	*Significance*	*Examples*
R*xyz*	Halocarbons x = number of carbon atoms −1 y = number of hydrogen atoms +1 z = number of fluor atoms	R12: (R012) 1C → x = 0, 0H → y = 1, 2F → z = 2, plus 2Cl, that is, dichloro-difluoro-methane, CCl_2F_2 The number of Cl is calculated by diminishing the number of flourine R22: (R022) 1C → x = 0, 1H → y = 2, 2F → z = 2, R134a: 2C → x = 1, 2H → y = 3, 4F → z = 4, $C_2H_2F_4$ Plain hydrocarbons were also included: R50 = CH_4, R170 = C_2H_6, R290 = C_3H_8
R4xx	Zeotropic mixtures xx = chronological order A,B, for different compositions, as they become accepted	R404A: R125 + R143a + R134a, 44/52/4 in %wt R410A: R32 (CH_2F_2) + R125 (C_2HF_5), 50/50 in %wt* R407C: R32 + R125 + R134a, 23/25/52 in %wt
R5xx	Azeotropic mixtures xx = chronological order	R500: 50% R125 + 50% R134a wt was the first on the market R502: 48.8% R22 + 51.2% R115 was the third on the market R507: R125 + R143a, 50/50 in %wt
R-7xx	Inorganic compounds xx = molar mass (rounded) a,b,. . . for different isomers, as they become accepted	R717: ammonia (MNH_3 = 17 g mol^{-1}) R718: water (MH_2O = 18 g mol^{-1}) R744: carbon dioxide (MCO_2 = 44 g mol^{-1}) R764: sulfur dioxide (MSO_2 = 64 g mol^{-1})
RxyzBt	halons xyz as for CFCs and t = bromine	R12B1: 1C, 0H, 2F, 1Br, 1Cl
Halons	Halons	Halon 1211 = CF_2ClBr, bromochlorodifluoromethane, liquid
Xyzt	xyzt for C, F, Cl, and Br atoms	Halon 1301 = CF_3Br, bromotrifluoromethane, gas Halon 2402 = $C_2F_4Br_2$, dibromotetrafluoroethane, liquid

690 and 565 kJ kg^{-1}, respectively. This effect can be used to chill diced meat or vegetables at up to 3 ton h^{-1}, making the plant operation and storage processes faster.

The main disadvantages of these substances are their cost (because most of the time the refrigerant is not reusable), and their ability to cause asphyxia, which is higher for carbon dioxide, and to a lesser extent with nitrogen. Regulations are set for a maximum safe limit for operators of 0.5% CO_2 by volume, and that excess carbon dioxide must be removed from the processing area by an exhaust system to ensure operator safety, which also incurs additional setup costs. Other hazards associated with liquefied gasses include cold burns, frostbite, and hypothermia after exposure to intense cold.

A refrigerant's safety classification is based on the combination of toxicity and flammability. According to the ANSI/ASHRAE Standard 34-1997, safety groups are classified as follows:

- A1 lower toxicity and no flame propagation
- A2 lower toxicity and lower flammability
- A3 lower toxicity and higher flammability
- B1 higher toxicity and no flame propagation
- B2 higher toxicity and lower flammability
- B3 higher toxicity and higher flammability

The Refrigeration Cycle by Vapor Compression

The refrigeration process taking place in household and warehouse fridges includes a mechanism that removes energy from a place with low temperature and transfers it to a place with high temperature. In the first place, the second law of the thermodynamics indicates that heat flows from a place with higher temperature to a place with lower temperature to equilibrate the temperature in the system. However, according to the Clausius statement, heat can flow from a place with low temperature to a place with higher temperature by applying some effort. In the refrigeration process, the objective is to transfer heat from a place with low temperature to a place with higher temperature. To achieve this, the refrigerant uses the transformation enthalpies presented when it changes from the liquid to vapor. A scheme of this process is presented in Figure 1. The refrigeration process starts in point 1, called the compression point, in which the fluid in a vapor state receives energy from the compressor and circulates to the point 2. At point 2, the fluid increases its pressure, temperature, and enthalpy. Then, the refrigerant enters into the condenser, where the fluid liberates heat to the system surroundings—due to the temperature differences between them. As a consequence of this heat transfer, the refrigerant decreases its temperature and condenses, presenting a phase change from the vapor to the liquid phase at constant pressure (point 3). Afterward, the refrigerant flows through an expansion valve, with a decrease in pressure in an isoentropic process, to obtain a vapor–liquid mixture (point 4). Finally, the mixture enters into the evaporator (which corresponds to the inside part of domestic fridges) where the refrigerant receives heat from the food products, until it reaches the conditions presented in point 1 to restart the cycle.

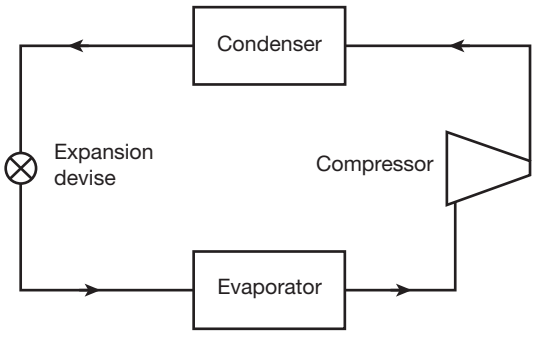

Figure 1 Chilling cycle system (Qi is the heat remove from the food product; Qo is the heat released to the environment).

Further, the refrigeration cycle can be described in a pressure-enthalpy diagram (Figure 2). First, the refrigerant increases the pressure during the compression process (points 1–2). Later, there is a decrease in the enthalpy during the condensation that takes place at constant pressures (point 2–3). After, the fluid reduces its pressure in the expansion valve at isoenthalpic conditions (points 3–4). Finally, the refrigerant increases its enthalpy (by the increase in the temperature) at isobaric conditions (points 4–1) to restart the process.

In this diagram, the amount of energy released by the refrigerant in the condenser can be determined by the enthalpies in points 2 and 3. In this sense, the heat flow released by the refrigerant is:

$$\dot{Q}_s = w(\hat{H}_3 - \hat{H}_2) \quad [1]$$

In the eqn [1], w represents the flow mass of the refrigerant and H represents the enthalpy per unit mass of refrigerant. In this case, the heat value obtained is negative because the amount of energy in point 2 that represents the enthalpy of the refrigerant when the fluid leaves the compressor is higher than in point 3, when the refrigerant leaves the condenser.

To know the amount of energy removed by the refrigeration system, it is necessary to apply the eqn [2]. In this equation, w represents the flow mass of the refrigerant, H_1 represents the enthalpy in point 1 after the evaporation process in the chilling chamber, and H_4 (point 4) represents the energy of the refrigerant before flowing to the evaporator.

$$\dot{Q}_E = w(\hat{H}_1 - \hat{H}_4) \quad [2]$$

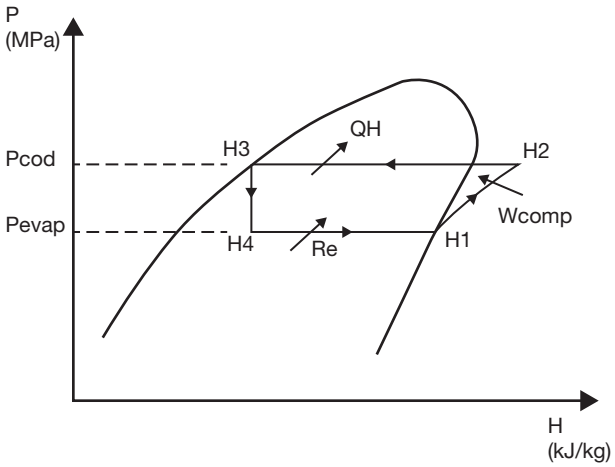

Figure 2 Chilling cycle system in an enthalpy–pressure diagram.

The value of Q_E represents the most important part of the refrigeration system, a parameter known as refrigerant capacity. Another important parameter in the refrigeration process is the coefficient of performance (COP), which is defined as the quotient of the refrigerant capacity and the work externally provided to the system (in the compression operation) (eqn [3]).

$$\mathrm{COP} = \frac{(\hat{H}_1 - \hat{H}_4)}{\hat{H}_2 - \hat{H}_1} \quad [3]$$

Further, the refrigeration facilities used for these applications must be designed to overcome heat gain through the walls of the system, and also to perform the descent of the temperature. To calculate the energy required for chilling food products, the following equations must be applied (eqn [4]).

$$Q = mCp(T_i - T_f) \quad [4]$$

- Q is the total energy removed from the food product
- m: mass of the product being chilled (kg)
- Cp: Specific Heat value (kJ kg^{-1} K) of the food product
- $T_i - T_f$: Difference between the initial and final chilling temperature (K)

There is abundant information regarding the Cp of different food products above the freezing point and below the freezing point. However, the Siebel equation for above freezing products ($T_k < 274$ K) can be applied to predict the Cp in kJ kg^{-1} K; in which, 'a' is the mass fraction of water in the food product (eqn [5]).

$$Cp = 3.3494a + 0.8374 \quad [5]$$

Moreover, the size of the refrigeration plant and the processing time required to chill different products are calculated using unsteady-state heat transfer methods. Many assumptions are made to simplify the estimation, that is, the initial temperature of a food is constant and uniform throughout the product and the temperature of the cooling medium, respiratory activity, and all thermal properties of the food are constant during cooling.

Total heat load = Heat from respiration
+ Sensible heat of containers
+ Heat evolved from operators and lights
+ Heat loss through roofs and walls
+ Heat loss through floor

A *British thermal unit* is defined as the amount of heat energy required to raise the temperature of one pound of water 1 °F from 59 to 60 °F; and 1 Btu h^{-1} = 0.293 watt (W).

Another unit of refrigeration widely used in the industry of ventilation and air conditioned is ton of refrigeration, or simply ton, being 1 ton = 12 000 BTU h^{-1} of heat removed. This equals the heat absorbed by 1 ton (2000 lb) of ice melting at a temperature of 32 °F over 24 h, and because the heat of fusion of ice at 32 °F is 144 Btu lb^{-1} (eqn [6]).

$$1\,\mathrm{ton} = \frac{1 \times 2000 \times 144}{24} = 12000\,\mathrm{BTU\,h^{-1}} \quad [6]$$

The conversion factor of 1 ton in the SI system is 1 ton = 3.516 kW

Refrigeration System Derivations

With the intention of reducing the power needed in the compression stage or achieving higher evaporation values than in the regular vapor compression cycle, many derivations of this arrangement have been developed, and they are presented in the following sections.

Chilling system with gas separation (flash economizer)

In systems with a gas separation arrangement, the fraction of vapor is separated from the saturated liquid in a manner that, conditions of a saturated liquid that, when expanded produces a mixture (point 3), which can be separated into a liquid (point 4) and a vapor (point 6). This vapor is send into the compressor, while the liquid is fed into an expansion valve to the evaporator; for this reason, the system needs two compressors. The power savings in the compressors by employing this system can be up to 16% (Figure 3).

Systems with two compressors and one evaporator

A useful option to achieve an important reduction in the power needed to compress the refrigerant is the application of a two-stage compression systems. In this system, a two-stage compression and intermediate refrigeration section are employed. This system requires higher initial investments, but the savings in power in the long term, and the low temperature achieved in the evaporator section justify its use.

Systems with two compressors and two evaporators

Sometimes different refrigeration temperatures are required in diverse processes in a food industry in the operation plants. Then it is required to develop a chilling system with more than one evaporator. Evaporators operating at two different temperatures can work in an efficient way in a two-stage system, employing an intermediate refrigerator and vapor separator. In this system, the point 3 corresponds to the saturation temperature $T3$ of the second evaporator, and is set by this temperature (Figure 4).

To evaluate this system, it is necessary to carry out a mass and energy balance in the different sections of the arrangement.

The next assumption is taken within the system, where w is the flow mass in the system (eqn [7]):

$$w_1 = w_2 = w_7 = w_8 \quad [7]$$

Balances in the evaporators:

$$Q_{E1} = w_1(\hat{H}_1 - \hat{H}_7) \quad [8]$$

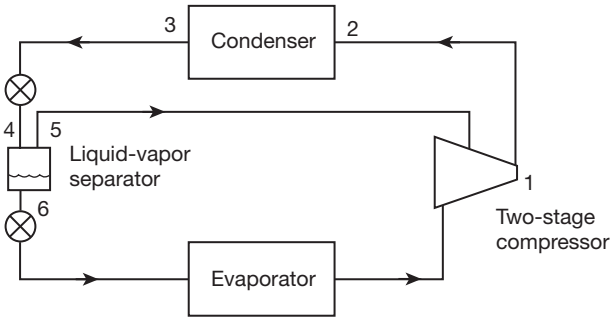

Figure 3 Expansion process with gas separation.

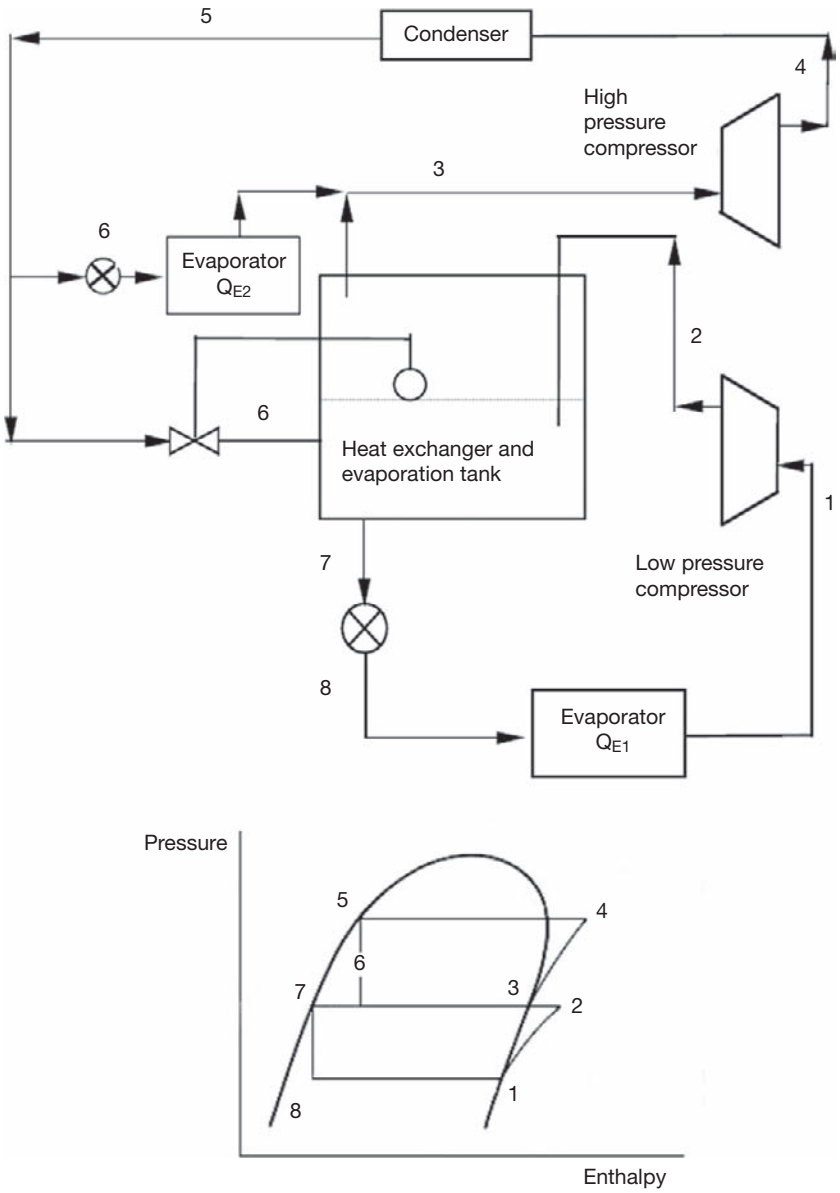

Figure 4 Operation with two compressors and two evaporators.

$$Q_{E2} = w_6\left(\hat{H}_3 - \hat{H}_6\right) \quad [9]$$

Balances in the second evaporator and intermediate refrigerator:

$$w_2\hat{H}_2 + w_5\hat{H}_5 + Q_{E2} = w_3\hat{H}_3 + w_7\hat{H}_7 \quad [10]$$

Also, in this subsystem, the flow mass can be determine by the eqn [11].

$$w_2 + w_5 = w_3 + w_7 \quad [11]$$

Since $w_2 = w_7$, it is also true that $w_3 = w_5$; therefore:

$$w_3\left(\hat{H}_3 - \hat{H}_5\right) = w_2\left(\hat{H}_2 - \hat{H}_7\right) + Q_{E2} \quad [12]$$

Power of the compressors:

$$\text{First compressor}: (Pow)_1 = w_1\hat{W}_1 = w_1\left(\hat{H}_2 - \hat{H}_1\right) \quad [13]$$

$$\text{Second compressor}: (Pow)_2 = w_3\hat{W}_2 = w_3\left(\hat{H}_4 - \hat{H}_3\right) \quad [14]$$

Chilling system with heat exchanger

In this arrangement, the liquid refrigerant, before passing through the expansion valve, is sent to a heat exchanger with the intention of increasing the heat in the system (and it facilitates the state change to vapor). The vapor flowing after the evaporator, before entering the compressor, is providing the heat in the heat exchanger (Figure 5).

Magnetic refrigeration

As an emerging technology, magnetic refrigeration has the potential to achieve high energy efficiency using environmentally friendly refrigerants. These devices use the magnetocaloric effect (MCE), defined as the temperature change that most of the magnetic materials present when

they are subjected to a changing magnetic field. Since the MCE in the best magnetocaloric materials available presents a maximum temperature change of 4 K in a magnetic field of 1 T, the magnetic refrigeration device must use a regenerative process to obtain a temperature span high enough to be applied for refrigeration purposes. The only disadvantage of this technology is the high price of the magnets.

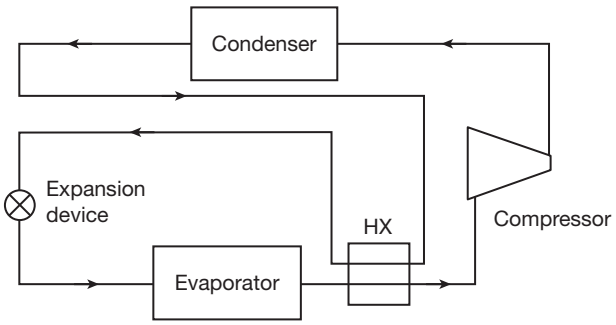

Figure 5 Chilling system with heat exchanger.

One of the most recent devices with interesting results is the one developed with a rotating mechanism in which the magnet rotates with magnetocaloric material. The magnet's (which has a high price, one of the disadvantages of this technology) design consists of a complex arrangement of permanent magnets and soft magnetic materials, which are assembled in the shape of an inner rotor. This device seems to be the prototype with better characteristics for being successfully applied in refrigeration systems. It also can help to obtain the higher temperature span in the system.

Refrigerated transport

The refrigerated transport of chilled foods must be seen as a global operation, taking into account the movement of chilled products from one cold storage area to another without variations in the temperature. For this, the actual movement of food products is carried out by way of refrigerated vehicles, ships, and aircrafts, the latter being the shipment mode least used. In the movement of products, temperature preservation is crucial to delivering products of the highest quality. Use of the finest

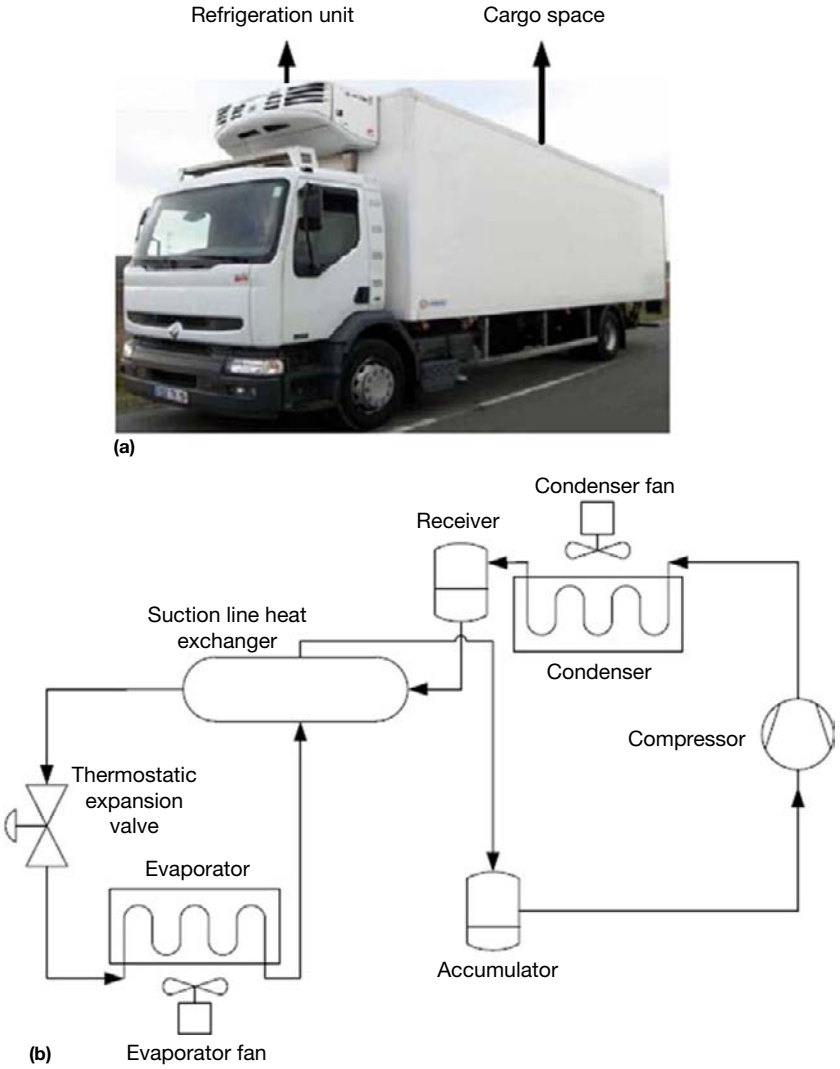

Figure 6 (a) A refrigerated truck system and (b) schematic of the refrigeration unit.

facilities cannot compensate bad manufacturing or handling practices, such as mistakes in the loading, packaging, or fluctuations in the storage temperatures. Furthermore, it is important that the temperature of the refrigeration equipment be adequate; but it is even more important to make sure that the food product is stored at the correct temperature.

The amount of refrigerated containers around the world is getting bigger every day. In 2002, at least a million refrigerated road vehicles, and about 400 000 refrigerated containers were reported in use all over the world. The temperature range of the refrigeration equipment is wide, from an insulated box containing ice, to a complex container equipped with computer systems to maintain the temperature, depending on the product being transported, within a range of −25 to 30 °C.

A typical refrigeration transport system is presented in Figure 6. In this, the refrigeration unit interacts with the truck cargo space to regulate the space to a certain temperature. Figure 6 shows the scheme of the refrigeration unit, in which all the components are interconnected, forming a complete vapor compression system. As in the common chilling system, the system employed in the vehicle uses energy to extract heat from the refrigeration chamber and transfer it to the external environment to maintain the product temperature.

See also: Chilled Foods: Effects on Shelf-life and Sensory Quality; Chilled Foods: Modified Atmosphere Packaging; Chilled Foods: Packaging Under Vacuum; Freezing Theory.

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Chlorophyll

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Sources and Production

Chlorophyll is a natural green pigment found in photosynthetic organisms such as plants and algaes. In higher plants and algaes, except the blue-greens, chlorophyll is located in chloroplasts. Chlorophyll takes an important role in human diet as it is consumed as a part of vegetables and fruits. To date, there have been no standardized and certain information about the chlorophyll content of plants in literature. The change in chlorophyll contents depends on cultivar, harvest time, ripening stage, and parts of the plant. Storage conditions and processing of plants may also play an important role. Besides, various extraction and quantification methods cause incompatible chlorophyll contents. Chlorophyll contents of some raw vegetables, obtained from different studies, are given in Table 1.

Due to having green color and widespread occurrence in many plants, chlorophyll might be thought of as a natural coloring agent in foods. However, it is not a suitable coloring agent due to its unstable nature against processing conditions. Therefore, to have stable green coloring agents, metal-substituted chlorophylls are commercially produced by chemical modification of natural chlorophylls. The most popular and industrially used metal-substituted chlorophyll is water-soluble copper chlorophyllin. It is produced from crude chlorophyll extract as a result of hydrolysis of phytyl and methyl esters, removal of cyclopentanone ring, and replacement of magnesium by copper. It is generally present as its sodium or potassium salt.

Chemical and Physical Characterization of Chlorophyll

The basic chemical structure of chlorophyll is a cyclic tetrapyrrole. Tetrapyrrole is not only found in the structure of chlorophyll but also in some other biologically important molecules such as heme and vitamin B_{12}. It has four pyrrole rings linked together at their carbon atoms via a one-carbon bridge. It is generally present bound with a metal ion, which provides different biological functions to the molecule. In chlorophyll, four pyrrole rings are linked with methine (—C═) bridges, and the four nitrogen atoms are coordinated with a central magnesium atom (Figure 1). Beside four pyrrole rings (A, B, C, and D), chlorophyll contains an additional fifth isocyclic ring (ring E). A monounsaturated isoprenoid alcohol (phytol) esterified to propionic acid located at C-17 gives chlorophyll a hydrophobic character.

There are five types of well-known chlorophylls named as chlorophyll a, b, c, d, and e. Apart from them, chlorophyll f has been identified in recent years. Chlorophyll a and chlorophyll b are the most abundant types in higher plants. They are generally present in an average ratio of 3–1. However, it has been stated that this ratio varies depending on genetic and environmental factors. Chlorophyll a and chlorophyll b differ from each other due to a chemical compound on the second pyrrole ring (ring B) at the C-7 position. Chlorophyll a has a methyl group ($-CH_3$) at the C-7 position, whereas chlorophyll b has a formyl group ($-CHO$). Chlorophyll d has a very similar structure with chlorophyll a, except that it contains formyl group instead of vinyl group ($—HC═CH_2$) at the C-3 position. Although chlorophyll a, b, and d are formed from the dihydroporphrin macrocycle named as chlorin, chlorophyll c has a fully unsaturated porphyrin macrocycle. It also has a propenoic acid (a *trans* acrylic acid) at the C-17 position instead of propionic acid. This propenoic acid is not esterified with a long-chain alcohol like phytol as in the other chlorophylls. Molecular structures of major chlorophylls are shown in Figure 1.

Chlorophylls have many conjugated double bonds with the ability to absorb visible light. However, all chlorophylls have specific wavelength absorptions due to their differences in structures causing different green hues among plants. The absorbance spectrums of chlorophylls show two dominant bands as Q-band and Soret band. Chlorophyll a has characteristic bands in the 432 and 669 nm regions that correspond to the Soret band and Q-band, respectively. The corresponding bands for chlorophyll b are located at 455 and 644 nm, respectively. Chlorophyll c has characteristic bands between 578 and 630 nm and between 443 and 450 nm. Due to being structural similarity, absorbance spectrum of chlorophyll d is very close to that of chlorophyll a.

In chloroplasts, chlorophylls are found bound to carotenoids, lipids, and lipoproteins via noncovalent bonds. For that reason, chlorophylls are easily extracted with organic solvents, especially polar solvents such as acetone, methanol, and ethanol.

Effect of Processing and Storage Conditions on Chlorophyll

Stability of chlorophyll depends on storage and processing conditions such as pH, temperature, oxygen, metals, and enzymes. Depending on the conditions, there could be changes in molecular structure of chlorophyll causing discoloration that is an indicator of low quality. Thus, several studies have been carried out to prevent chlorophyll degradation.

pH

Chlorophyll is susceptible to low pH conditions. Chlorophyll degradation with the effect of acidic conditions is the result of degradation of chlorophyll to pheophytin (Figure 2). This reaction is called pheophytinization, and two hydrogen ions replace the magnesium ion found in the center of the porphyrin ring. As a result, the bright green color of chlorophyll turns to olive-brown, which causes negative consumer perception. In

the presence of chlorophyllase enzyme, pheophytin losses its phytyl group, resulting in pheophorbide formation. Pheophorbide also has an olive-brown color like pheophytin.

Although acidity is not enough for the formation of pheophytin at the beginning of the process, pH may decrease during the process. For instance, fermentation of vegetables lowers the pH in brine causing chlorophyll degradation. Besides, higher process temperatures trigger the formation of pheophytin in processed vegetables. Natural organic acids found in food matrix and fatty acids arising from lipid hydrolysis might be released as a result of heat treatment.

To prevent pheophytin formation and accordingly discoloration, alkalizing agents such as magnesium carbonate might be used. However, alkalizing agents cannot maintain alkaline conditions permanently. It is especially important during long period of storage that results in color losses.

Table 1 Chlorophyll content of some vegetables

	Chlorophyll content (mg kg^{-1} fresh weight)
Spinach	791[a], 1083[b], 1270[c]
Green beans	71–133[d], 75[a]
Brussels sprouts	32[e], 60[c]
Broccoli	21[a], 79[c], 128[b]
Parsley	632[a], 995[f]
Cucumbers	36[a]
Green peas	50[a]
Leeks	87[a]
Green paprika	38[a]
Zucchini	68[a]
Celery	23[a], 34[g]

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Heating

Heat treatment is widely applied in food processing, especially for pasteurization, sterilization, blanching, and drying purposes. Chlorophyll is susceptible to heat treatments, which causes some structural changes. Mild heat treatments result with the formation of chlorophyll isomers (Chlorophyll a′ and Chlorophyll b′). They differ from chlorophyll

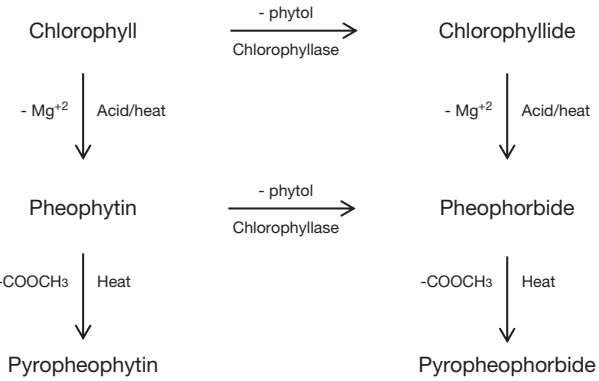

Figure 2 Formation of chlorophyll derivatives and factors affecting.

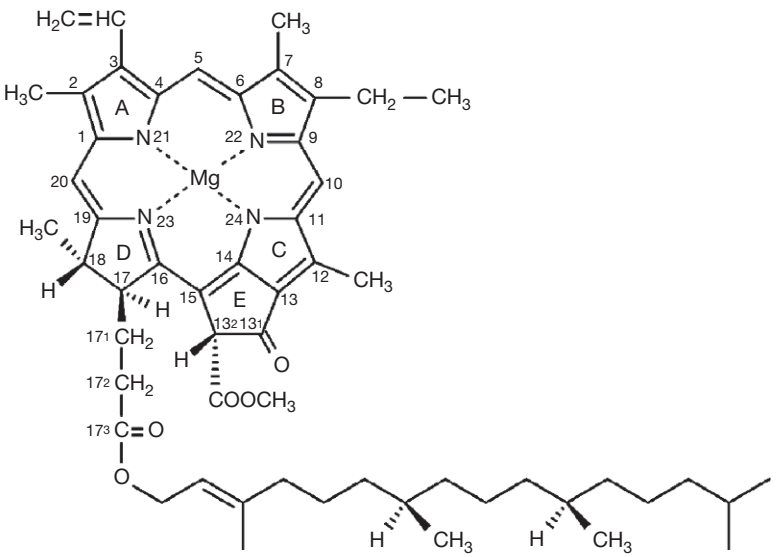

Figure 1 Molecular structure of chlorophylls.

with carbomethoxy group ($-COOCH_3$) at the C-13^2 position. Heat treatments may also cause loss of Mg^{2+} ion and formation of pheophytin. Chlorophyll a leads to formation of pheophytin more rapidly than chlorophyll b, as its heat stability is lower. Increased temperatures or long periods of heat treatments result in the formation of pyroderivatives of chlorophylls (pyropheophytin and pyropheoporbide); those have no carbometoxy group at the C-13^2 position (Figure 2).

Blanching is a heat treatment implemented prior to freezing or canning of vegetables to inactivate enzymes. This treatment provides protection of nutritional and sensorial quality. However, conditions that require inactivating enzymes are different from each other. It has been reported that blanching conditions required to inactivate lipoxygenase (LOX) causes more chlorophyll loss than peroxidase (POD). Thus, it could be said that blanching conditions required to inactivate LOX are not proper for protection of chlorophyll.

To preserve the green color of fruits and vegetables, control of the thermal treatment is important. It has been shown that high temperature in a short time might be an effective approach to preserve chlorophyll.

Enzymes

Enzymes such as chlorophyllase, LOX, POD, and polyphenol oxidase induce loss of quality in fruits and vegetables. Chlorophyllase, a thylakoid membrane glycoprotein, is found in green plants and causes the destruction of chlorophyll. Its activity continues during the processing and storage period of fruits and vegetables. Chlorophyllase cause the conversion of chlorophyll to chlorophyllide by catalyzing cleavage of the phytol group. Chlorophyllide is known as a green chlorophyll derivative. Formation of chlorophyllide is desirable to preserve the green color in canned vegetables. However, when acid is present in the medium, chlorophyllide losses its magnesium ion and causes pheophorbide formation. Olive fermentation could be a good example for formation of chlorophyllide and then pheophorbide. Besides, chlorophyllase hydrolyzes pheophytins, which gives rise to the formation of pheophorbide (Figure 2).

The formation of chlorophyllide depends on temperature conditions as chlorophyllase is activated in a temperature range (60–82.2 °C) and degraded at higher temperatures (>100 °C). Studies have shown that degradation of chlorophylls to pheophytins and degradation of chlorophyllides to pheophorbides follow first-order kinetics.

Besides chlorophyllase, POD and LOX enzymes in fruits and vegetables play a crucial role in chlorophyll degradation. LOX oxidizes fatty acids containing *cis*,*cis*-1,4-pentadiene structure. LOX is responsible for formation of hydroperoxides and free radicals that cause khaki/yellow or colorless products. Oxidative degradation of chlorophyll is named 'chlorophyll bleaching.' LOX could be inactivated by heat treatment. Therefore, blanching is generally performed for canned or frozen vegetables to inactivate LOX and protect color.

POD is one of the oxidoreductase enzymes and is found in many cell organelles including chloroplast. Although POD plays a significant role in ripening of fruits and vegetables, it is also responsible for chlorophyll degradation. In the degradation mechanism of chlorophyll via POD, phenolic compounds are oxidized with hydrogen peroxide. As a result, formed phenoxy radicals oxidize chlorophyll to colorless compounds.

Metals

Chlorophyll forms complex with some metal ions, which causes some changes in color. In canned vegetable foods, the magnesium ion at the center of chlorophyll might be replaced with iron. This replacement results in an undesirable brown-gray color. If zinc and copper salts are present in the medium during processing, some greener areas on the surface of vegetables could appear. This color change is called as 'regreening.' From this point of view, green vegetables are blanched in water containing certain amounts of Zn^{2+} and Cu^{2+} salts, and they become greener than their natural forms. Formation of complexes between chlorophyll and Zn^{2+} and Cu^{2+} are named the 'Veri-Green process.' These metal complexes are more stable than natural chlorophyll. The complex formation rate could change depending on types of chlorophylls, metals, and environmental conditions such as pH. Although complexes provide stable green color, their usage might cause detrimental health problems. Zn-chlorophyll complexes are preferably used in the industry, as Cu-chlorophyll complexes are not innocent.

In canned foods, after pheophytin is formed with the effect of heat, it is complexed with zinc and resulted with formation of zinc-pheophytin. Decarboxymethylation of zinc pheophytin causes the formation of zinc pyropheophytin. Another suggested pathway for zinc pyropheophytin formation is direct complexation of zinc with pyropheophytin.

Surface-active anionic compounds affect the formation of metal complexes. These compounds adsorb onto the chloroplast membranes, which results in increasing the negative surface charge and thereby increasing complex formation.

Oxidation

Chlorophyll is found in many vegetable oils such as virgin olive oil and rapeseed oil. During bleaching of edible oils, chlorophyll and its derivatives are generally removed or their concentration is reduced as much as possible. Due to being sensitizers, chlorophyll and its derivatives might cause photosensitized oxidation in edible oils. Photosensitized oxidation should be taken into account, as it is as important as autoxidation by means of oxidation products. Low-molecular-weight off-flavor compounds, degradation products of fatty acids, and oxidized polymers are some of the products that cause unacceptable consumer perceptions.

Photosensitizers such as chlorophyll, riboflavin, and myoglobin absorb light energy and turn into an excited single-state sensitizer. Following that, an excited triplet-state sensitizer is generated from the excited single-state sensitizer via intersystem crossing. An excited triplet-state sensitizer might follow two pathways. In the type I pathway, sensitizers play a role as free-radical initiators. An excited triplet sensitizer reacts with a substrate such as linoleic acid by donating an

electron or accepting hydrogen. Accordingly, free radicals are generated, and they react with triplet oxygen via a free radical oxidation mechanism. In type II pathways, an excited triplet sensitizer reacts with triplet oxygen, which results in formation of a singlet sensitizer and singlet oxygen. After that, the singlet oxygen interacts with unsaturated fatty acids, and hydroperoxides are produced. The amount of hydroperoxides depends on the number of double bonds of unsaturated fatty acids.

When chlorophyll is dissolved in alcoholic solutions or exposed to air, oxidation at the C-13^2 position might occur. Oxygen or oxygen-containing compound are located in the C-13^2 position of the chlorophyll.

Free radical scavengers such as carotenoids and tocopherols prevent chlorophyll-sensitized oxidation of fatty acids. β-carotene, a singlet oxygen quencher, prevents oxidation in edible oils. Moreover, storage in a controlled atmosphere might be used for the same purpose.

Refining

Chlorophyll and pheophytins are critical compounds and impart a greenish color to edible oils. Chlorophyll is especially responsible for color of olive oil that depends on the types and ripeness degrees of olives. However, excess chlorophyll causes an unacceptable consumer perception. Chlorophyll and its derivatives could be partly removed by decolorization step of refining process. However, removing chlorophyll completely is a difficult and not economical process.

Bioavailability and Health Effects

Knowledge about potential health-related benefits of chlorophyll has drawn attention in recent years. Therefore, many studies about the bioavailability of chlorophyll have been carried out to expose the effectiveness of chlorophyll on human health. However, investigations about the bioavailability of chlorophyll generally focus on *in vitro* studies.

Absorption of natural chlorophylls might change depending on such factors as type, solubility, digestive stability, and food matrix. During digestion, natural chlorophylls are converted to pheophytins because of acidity in the stomach. As a result, Mg-free derivatives might be predominantly found in human feces. According to studies, the absorption of Mg-free chlorophyll derivatives by intestinal cells is easier than that of natural chlorophyll. It has been also found that specific metallo-chlorophyll derivatives like Zn-pheophytin are stable in the gastric conditions and available for absorption.

Commercial copper chlorophyllin is a complex mixture containing different chlorin-based components such as copper chlorin e^6 and copper chlorin e^4. These chlorin-based components are absorbed at different rates. The absorption rate of copper chlorophyllin components might be changeable depending on the food matrix that may protect them from ingestion conditions.

Although chlorophyll causes photosensitized oxidation, it might show an antioxidative effect based on some conditions. If chlorophyll is exposed to light, the prooxidant effect of chlorophyll increases. However, it plays an important role as an antioxidant in dark medium. Change of chlorophyll structure also affects antioxidant activity. It has been reported that the antiradical capacity of metallo-derivatives such as Mg-chlorophylls and Zn-pheophytins is much more than that of metal-free derivatives such as pheophytins and pyropheophytins. In addition to natural chlorophyll derivatives, it has been remarked that copper chlorophyllin displays antioxidant activity against oxygen species.

Chlorophyll and its derivatives have antimutagenic and anticarcinogenic effects. These effects are against many mutagens and carcinogens such as polycyclic aromatic hydrocarbons, heterocyclic amines, and aflatoxin B_1. According to a common opinion, the protective mechanism of chlorophyll is formation of complex between porphyrin ring of chlorophyll and carcinogens–mutagens. However, the chemical structure of mutagens–carcinogens is an effective factor for formation of a complex. As a result, DNA-adduct formation is prevented and bioavailability of dietary carcinogens or mutagens is reduced. It has been shown that green vegetables might decrease the risk of colon cancer in humans. Moreover, it has been stated that chlorophyll and its derivatives prevent skin cancer when experimental animals are used. Studies have also shown that chlorophyllin has a significant antimutagenic effect against antitumor drugs that might have detrimental effects on healthy cells.

The amount of chlorophyll in the diet is significant to determine contribution of chlorophyll consumption to human health. Because of its high concentration in vegetable-rich diets, health benefits of chlorophyll might increase. As can be seen from **Table 1**, the average chlorophyll content of green beans (90 mg chlorophyll per kg vegetable) is less than that of parsley (800 mg chlorophyll per kg vegetable). Therefore, the health effect of chlorophyll in green bean could be provided by a lower amount of parsley.

See also: Carotenoids: Occurrence, Properties and Determination; Carotenoids: Physiology; Colors: Health Effects; Colors: Properties and Determination of Natural Pigments; Colors: Properties and Determination of Synthetic Pigments; Cooking: Domestic Techniques; Storage Stability: Shelf Life Testing.

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Cholecalciferol: Properties and Determination

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Background

Cholecalciferol (vitamin D_3) is the common form of vitamin D synthesized in animals. Ergocalciferol (vitamin D_2) is the form that is synthesized mainly in plant sources such as mushrooms. The term vitamin D usually covers both forms, and it plays a crucial role in the regulation of intestinal absorption and metabolism of calcium and phosphate, both of which are vital for the healthy homeostasis of bones. Despite being known as a vitamin, vitamin D is a prohormone that is converted to its hormonal active form (1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$ or $1,25(OH)_2D_2$)). Vitamin D was discovered in the early twentieth century from research on the treatment of rickets, a bone disorder caused by vitamin D deficiency in children. The original vitamin D, which was named D_1, was a mixture of compounds that was prepared by ultraviolet (UV) radiation of ergosterol that was found in plants and fungi. In 1931, vitamin D_1 was purified by Askew and the compound ergocalciferol or vitamin D_2 was isolated. At that time, synthesis of vitamin D in human was not understood, as ergosterol could not be found in human tissues. Few years later, Windaus synthesized 7-dehydrocholesterol (ergosterol equivalent in human skin) from cholesterol, and its UV-radiated product was named cholecalciferol or vitamin D_3.

Physical and Chemical Properties

Cholecalciferol is a white crystalline powder with the chemical name 9,10-seco(5Z,7E)-5,7,10(19)-cholestatriene-3β-ol. As shown in Figure 1, its chemical structure differs from that of steroids by the bond cleavage at 9–10 followed by ring opening, which is responsible for antirachitic activity. This ring opening reaction occurs in human skin on exposure to UV-B radiation (290–315 nm) of 7-dehydrocholesterol (provitamin D_3). As shown in Figure 1, the ring opening is followed by the formation of a double bond to produce previtamin D_3. Subsequent temperature-dependent rearrangement of this double bond produces cholecalciferol with a characteristic conjugated triene structure. Once formed, it is carried into circulation by vitamin D-binding protein. Cholecalciferol itself is biologically inactive, and its conversion to function biologically comprises two enzymatic hydroxylation reactions, producing 25-hydroxyvitamin D_3($25(OH)D_3$) followed by $1,25(OH)_2D_3$. Ergocalciferol, which is ingested through plant food sources, also undergoes similar process to produce the biologically active form $1,25(OH)_2D_2$. The structures of the vitamin D analogues commonly found in human plasma are shown in Figure 2, and these and many other analogues found in plasma and other biological fluids are collectively called vitamin D.

Although vitamin D is traditionally implicated in bone health, its deficiency was found to have effects on many other diseases such as hypertension, diabetes mellitus, cancer, and autoimmune and cardiovascular diseases. As a result, there has been a proliferation of research on vitamin D in recent times. Some research indicates that different forms of the vitamin D may be responsible for this variety of physiological effects. In general, the activities of vitamins are expressed in international units (IU). Expression in IU is useful in expressing the total activity when several forms of the vitamin with different biopotencies are present in one food source. With vitamin D, both cholecalciferol and ergocalciferol are considered to have a similar potency: 1 IU = 0.025 μg. However, the potencies of other forms of vitamin D are not available in IU; therefore, this concept is not as widely used as with other vitamins such as vitamin A. Evaluation of potencies of different forms of vitamin D is made more challenging due to the fact that each form may have different physiological effects. As shown in Figure 2, in terms of chemical structure, cholecalciferol differs from ergocalciferol at the C17 side chain where ergocalciferol has an additional methyl group at C24 and an extra double bond between C22 and C23. It is worth mentioning that cholecalciferol and ergocalciferol possess similar physical and chemical properties. Table 1 lists the physical and chemical properties of cholecalciferol and ergocalciferol. While their potencies in humans are considered equivalent by pharmacopoeias, some studies have demonstrated that cholecalciferol is more potent than ergocalciferol and better absorbed by the intestine. It has also been found that its major circulating metabolite $25(OH)D_3$ has higher affinity than that of ergocalciferol (25-hydroxyvitamin D_2 ($25(OH)D_2$)) to vitamin D-binding protein, resulting in a longer half-life.

Cholecalciferol is the common form that is consumed as vitamin D. It has been extracted from fish liver oil, but nowadays, it is mostly synthesized for use in nutritional supplements and in fortified foods. Despite its insolubility in water due to high lipophilicity, cholecalciferol is readily soluble in a wide range of organic solvents including alcohols and both chlorinated and nonchlorinated hydrocarbons such as chloroform and hexane. Cholecalciferol undergoes a reversible thermal isomerization to previtamin D_3 and/or degradation when stored in alcoholic solutions and exposed to oxygen and light. However, it is stable for long periods when stored in absolute ethanol without the presence of oxygen and light at low temperature (−20 °C). It is also stable in oils, fats, and fortified food, when stored in dark at low temperatures. It has been found that cholecalciferol is protected against degradation in food matrices and once released from them can be vulnerable to degradation by light and oxygen. Cholecalciferol is stable in alkaline solutions but not in acidic conditions. In acidic conditions, it undergoes isomerization to inactive forms such as isotachysterol and 5,6 trans isomers.

Occurrence and Forms in Food

It is well known that cholecalciferol is primarily obtained via exposure of the skin to sunlight. Some critical factors including skin pigmentation, time of day, sunscreen use, altitude, skin

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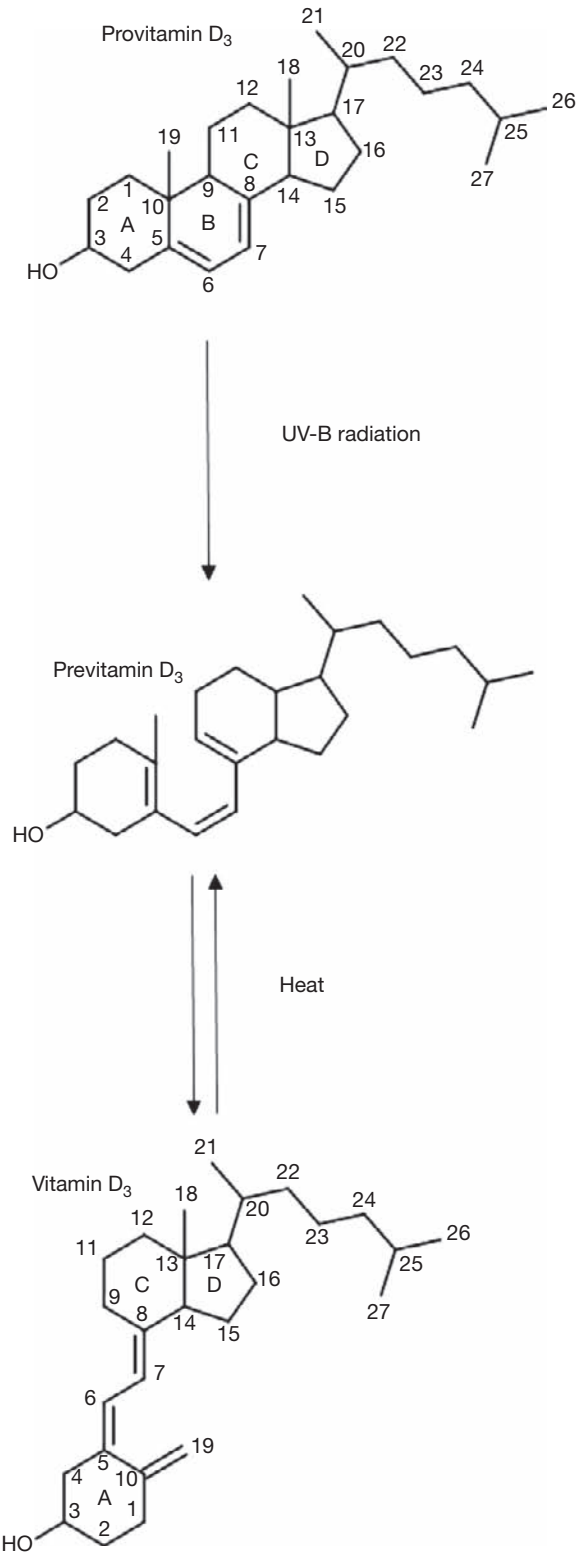

Figure 1 Photochemical conversion of provitamin D_3 to vitamin D_3.

type, age, obesity, latitude, clothing, and season directly influence the vitamin D intake from the sun. Due to concerns of the depleted ozone layer and skin cancer, the significance of sunlight in vitamin D production has decreased in recent years; hence, dietary intake has become the main source as vitamin D deficiency has become common.

Vitamin D can be naturally found in a limited number of foods. Cholecalciferol is usually present in animal foods, while ergocalciferol can be found in fungi (mushrooms) and plants. Provitamins (7-dehydrocholesterol and ergosterol) are present in animals and plants (including the ones used as food sources by humans); therefore, exposure to sunlight produces vitamin D in them. Mushrooms dried in sunlight are considered a good plant source of vitamin D_2. Fish liver oils are regarded as the best source of cholecalciferol. Fish, such as tuna and salmon, are generally accepted as relatively good sources. Both cholecalciferol and ergocalciferol can be found in milk, cholecalciferol being the predominant form. Seasonal variations can affect the concentration of cholecalciferol in cow's milk due to changes in sunlight exposure of its skin. Although alimentary forms are mainly composed of ergocalciferol and cholecalciferol, small portions of calcidiol and calcitriol can also be found in some dietary products. Table 2 provides a list of nonfortified foods containing vitamin D with their respective concentrations. It should be noted that the analytical methods available for measuring vitamin D in food were usually developed to measure only the two calciferols; therefore, the reported values may not reflect the total amounts that include the contributions by other forms.

Fortification of Foods

The serious concern of the adverse outcomes (wrinkling and skin cancer) that can occur with high exposure to sunlight has decreased obtaining vitamin D through sunlight exposure. In addition, the general trend of low-fat diets causes vitamin D deficiency because this fat-soluble vitamin is removed along with fat during the production of low-fat food. Although an optimal cholecalciferol daily intake level has not yet been established, current recommended daily intake is in the range 400–600 IU. This is difficult to obtain through dietary sources alone without exposure to sunlight. As a result, vitamin D supplementation is commonly prescribed. In order to address the vitamin D deficiency in a large fraction of the population, a common practice used in many countries is to fortify certain foods with vitamin D. However, fortification of food with vitamin D must be monitored because of toxicological concerns such as hypercalcemia with fatal consequences at very high levels. Either cholecalciferol or ergocalciferol is used in the fortification of food, although cholecalciferol is the preferred form. There is no consensus about the extent to which food fortification should be performed and the prevalent action varies according to the legislation of each country.

Common vehicles of vitamin D fortification are milk, milk-based beverages, milk powder, margarine, and cereal products. Cholecalciferol is usually added to the milk in oily form due to its easier solubilization in oil-based foods. Milk powder is fortified by mixing cholecalciferol powder with milk powder into the bulk. In this process, it is important to make sure particle sizes of milk powder and vitamin powder are compatible in order to avoid separation of the components, leading to a heterogeneous mix. It must be stored in a cool and dry space to ensure minimal cholecalciferol loss. In the case of

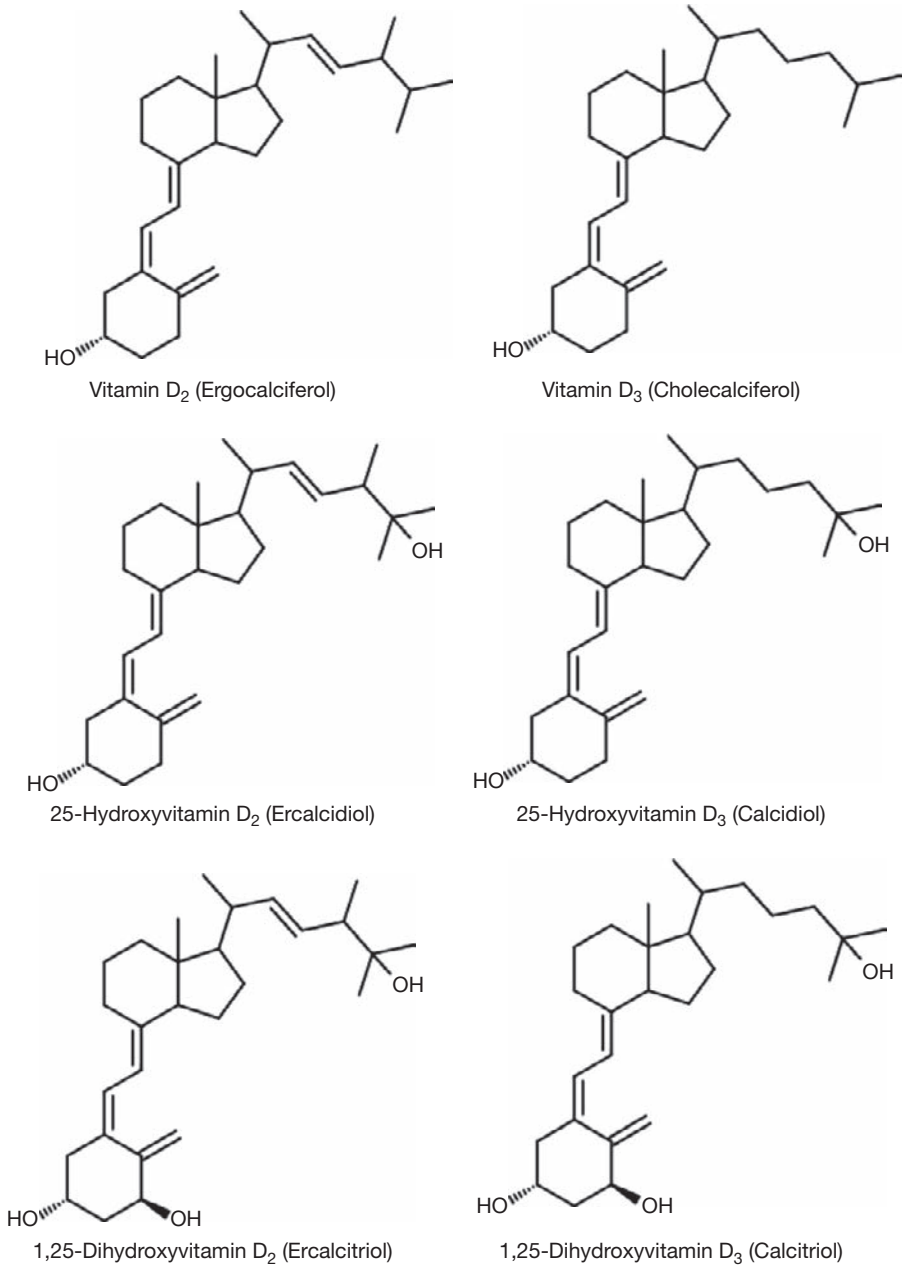

Figure 2 Chemical structures of ergocalciferol, ercalcidiol, ercalcitriol, cholecalciferol, calcidiol, and calcitriol.

margarine, the fortification is performed by mixing oily cholecalciferol with a fraction of warmed oil, followed by homogenization with the fat blend prior to the emulsification procedure. In addition to fortified foods, cholecalciferol can be obtained from vitamin D supplements that generally contain 1000 IU in each tablet.

Analytical Methods

Unlike with photosynthesized vitamin from skin, with dietary intake, vitamin D levels can rise to over 400 ng ml^{-1} (about 2 mg or 80 000 IU in total), leading to vitamin D toxicity. The natural levels of vitamin D in common foods are very low and the fortified levels are also relatively low unlike with most other vitamins. The determination of cholecalciferol content in foods is a challenging analytical task as, in natural foods and even in fortified foods, only small amounts are present and a wide range of compounds are extracted along with cholecalciferol that cause difficulties in sample preparation and instrumental analysis.

Vitamin D molecules in foodstuffs and biological fluids are bound to lipids and/or proteins; therefore, chemical bonds need to be broken to release the vitamin before analysis. In general, saponification is used to release vitamin D and to remove other lipids from foodstuff, and solvent-based protein

Table 1 Properties of ergocalciferol and cholecalciferol

Properties	*Ergocalciferol (vitamin D_2)*	*Cholecalciferol (vitamin D_3)*
Molecular formula	$C_{28}H_{44}O$	$C_{27}H_{44}O$
Molar mass (g mol^{-1})	396.63	384.62
Molar volume	406.9 cm^3 mol^{-1}	396.9 cm^3 mol^{-1}
Melting point (°C)	115–118	84–85
p*K*a	14.74	14.74
log *P*	9.148	9.085
λ_{max} (nm)	264.5	264.5
Extinction coefficient ($E^{1\%}_{cm}$ (hexane))	459	485
Extinction coefficient ($E^{1\%}_{cm}$(ethanol))	462	485
Optical rotation (chloroform)	+52°	+52°
Mass solubility (water)	(Sparingly soluble (5.9E − 5 g l^{-1}))	(Sparingly soluble (6.5E − 5 g l^{-1}))
Molar solubility (water)	(Sparingly soluble (1.5E − 7 mol l^{-1}))	(Sparingly soluble (1.7E − 7 mol l^{-1}))

Table 2 Dietary sources of vitamin D

Food source	*Vitamin D content (IU/100 g)*
Beef (meat)	13
Beef (liver)	8–40
Pork (meat)	84
Pork (liver)	40
Chicken	50–65
Poultry	80
Poultry skin	900
Butter	35
Eggs	28
Cheese	12
Cream	50
Cabbage	0.2
Spinach	0.2
Corn oil	9
Cod liver oil	10 000
Cod	85
Shrimp	150
Salmon	220–440
Sardines	1500
Mackerel	120
Herring liver oil	140 000
Herring	330

precipitation is used to release it from biological fluids. In saponification, vitamin D compounds are released from lipoprotein complexes. In protein precipitation, vitamin is released from the proteins by denaturing the protein. Commonly used saponification procedure consists of mixing the sample with ethanolic potassium hydroxide and heating to approximately 80 °C for about 1 h. The unsaponifiable portion containing the vitamin is then extracted using a nonpolar solvent such as hexane. Subsequently, the organic phase is removed by evaporation and the residue reconstituted in a suitable solvent depending on the analytical method used. Commonly used protein precipitation method consists of mixing the sample with acetonitrile to precipitate out the proteins by denaturation. The precipitated proteins are removed after centrifugation, the supernatant is dried under nitrogen or by evaporation, and the residue is reconstituted in a suitable solvent depending on the analytical method used. The extracted vitamin is subsequently detected and quantified using techniques such as ELISA, radio immunoassay, or high-performance liquid chromatography (HPLC) with UV spectrophotometric or mass spectrometric detection (HPLC-UV or HPLC–mass spectrometry (MS)). HPLC–MS is the most common chromatographic technique used nowadays due to its superior selectivity and sensitivity relative to HPLC-UV. However, vitamin D compounds produce weak signals in MS detector because of their poor ionizability. Recently reported methods have overcome this problem by derivatization with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) to produce Diels–Alder adducts that have better ionization efficiency in MS detection.

Chromatographic separation is usually carried out on a reversed-phase analytical column, and the detection wavelength commonly used with UV detection is 265 or 280 nm. Electrospray ionization and atmospheric pressure chemical ionization are the most frequently used mass spectrometric ionization modes, and most methods used tandem mass spectrometric detection (HPLC–MS/MS) rather than HPLC–MS to achieve better specificity and sensitivity.

There are many different immunoassay kits commercially available for vitamin D assay. With immunoassays, the vitamin extract is subjected to an antibody-specific vitamin D assay. The detection step of immunoassays is based on different spectroscopic techniques such as UV–visible, chemiluminescence, Raman scattering, and radioactivity measurements. Two major limitations of immunoassays for vitamin D analysis are matrix effects and the inability to distinguish between 25(OH)D_3 and 25(OH)D_2. Raman spectroscopy for immunoassay has shown high sensitivity, enabling the detection of vitamin D metabolites at very low concentrations; however, this method still cannot distinguish between the different vitamin D metabolites.

Despite being the best method for vitamin D analysis, one of the major drawbacks of HPLC–MS or HPLC–MS/MS is that the MS detection is susceptible to matrix effects. Matrix effects in MS occur when the compounds that coelute with the analyte interfere with the ionization process causing ionization suppression or enhancement. As there is no way to ensure complete elimination of the interfering compounds that coelute with the analyte, the only way to obtain accurate data is to remove the contribution from the interferences at the quantification step. The inclusion of stable isotope-labeled (SIL) internal standards

(IS) in both the samples and standards followed by quantification using internal standard calibration has become the most common method used to correct for matrix effects in MS detection. As the chemical properties and ionization process of SIL-IS are almost identical to those of the vitamin, and as it elutes at the same retention time as the vitamin and experiences the same extent of matrix effects, it provides the best option to correct for matrix effects when IS calibration method is used. As a separate SIL-IS is needed for each form of the vitamin quantified, and some of them are not commercially available, many published methods for vitamin D had no corrections applied for matrix effects of MS detection. Some methods used a single SIL-IS for all vitamin forms although this does not remove matrix effects from all vitamin D analogues. Quantification with HPLC-UV methods has used either IS calibration using noncoeluting compounds such as laurophenone or retinyl acetate or external standard calibration.

Eleven analytical methods for the analysis of vitamin D in foodstuffs have been validated by the Association of Official Analytical Chemists International (AOACI); however, only four of them have been used in laboratories. In summary, these methods are similar, in which saponification is used to extract the vitamin D content and HPLC-UV is used for quantification. The scope of these methodologies is only vitamin D_3, limiting their applications to food matrices containing other forms of vitamin D. In addition, these approaches were validated for a limited number of food matrices, containing high levels of fat, which make them inappropriate to the application in matrices with low-fat content such as cereals and juices.

See also: Calcium: Physiology; Calcium: Properties and Determination; Cereals: Dietary Importance; Chromatography: High-Performance Liquid Chromatography; Dairy Products: Dietary and Medical Importance; Fish: Fish in the Human Diet; Fish Oils: Composition and Health Effects; Fish Oils: Production and Properties; Immunoassays: Principles; Milk: Role in the Diet; Milk Powder.

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Cholesterol: Absorption, Function and Metabolism

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Introduction

Cholesterol plays an essential role in maintaining membrane integrity and proper fluidity. It is an abundant component of the plasma membranes of eukaryotic cells, which makes up around 25–30% of the total lipids present in cell membranes. The cholesterol contents of cell membranes are tightly regulated and have an impact on cell permeability. The large hydrophobic domain of cholesterol fits into spaces between phospholipids, thereby preventing diffusion of water-soluble molecules through the membrane. Furthermore, cholesterol reduces the fluidity of cell membranes with its rigid structure and stabilizes membranes under different conditions. Alterations in membrane fluidity further influence membrane transport processes, including Na^+/K^+-ATPase. Although around 70% of plasma cholesterol is esterified, cholesterol in cellular membranes is in free form.

In addition to the role in the stability and architecture of the plasma membrane, cholesterol is a precursor for the biosynthesis of bile acids, vitamin D, and steroid hormones in mammals. Fluctuations of circulating levels of cholesterol under physiological conditions are controlled by a series of mechanisms that balance between *de novo* synthesis, absorption of dietary cholesterol, and removal from peripheral tissues. Therefore, cholesterol levels in circulation depend not only on the amount of cholesterol supplied from the diet (exogenous) and synthesized in the body (endogenous) but also on the removal, uptake from cells, and the conversion of cholesterol into other molecules (bile acids and steroid hormones). Concentration of intracellular cholesterol is coordinated by the same mechanisms, in line with the phases in the cell cycle. All these processes are tightly regulated and maintain concentration of cholesterol within a normal range. However, many chronic noncommunicable diseases are associated with alterations in these mechanisms, including cardiovascular diseases and cancer.

Cholesterol Biosynthesis

Most animal cells are able to produce cholesterol, but the production rates vary depending on the cell types and organ function. Cholesterol *de novo* synthesis is a highly regulated, multistep process known as the mevalonate pathway. Primary place of cholesterol production is the liver, which contributes 20–25% of total cholesterol synthesis, while cholesterol is also synthesized in the intestines, adrenal glands, and reproductive organs. The only precursors for the biosynthesis of cholesterol are two-carbon metabolite of acetate, acetyl coenzyme A (acetyl CoA). Two acetyl-CoA molecules form acetoacetyl CoA, which is combined with another acetyl CoA and then hydrated to 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) in the cytoplasm. This reaction is catalyzed by the enzyme HMG-CoA synthase. The next step is the reduction of HMG-CoA to mevalonate (the key intermediate in cholesterol biosynthesis) by the enzyme HMG-CoA reductase in microsomes, which requires NADPH. This is an irreversible and rate-limiting step in the cholesterol biosynthesis, which is commonly used as therapeutic target in hyperlipidemic disorders using statin drugs (HMG-CoA reductase-competitive inhibitors).

HMG-CoA reductase is placed in the membrane of the endoplasmic reticulum (ER). This enzyme contains multiple transmembrane domains and a catalytic domain that converts HMG-CoA into mevalonate. The catalytic domain (C-terminal domain) faces the cytosol, while the membrane domains play an important regulatory role. Namely, these domains are sterol-sensitive, and they change the conformation of the enzyme when interact with sterols. Hence, when cholesterol level in the cell is high, conformation changes downregulate the activity of HMG-CoA reductase, and less mevalonate is produced. Lower synthesis is compensated by increased rate of uptake of cholesterol from blood to cells, thereby lowering the level of cholesterol in the circulation. On the contrary, when levels of cholesterol in cells decline, they respond by increasing the gene expression of not only proteins included in cholesterol biosynthesis, in particular HMG-CoA reductase, but also proteins responsible for the uptake of cholesterol from the external environment, such as low-density lipoprotein (LDL) receptor. In this way, cholesterol concentration in cells increases.

Further, conversion of mevalonate to 3-isopentenyl pyrophosphate includes three reactions, which all require ATP. These reactions, as well as those that follow, are carried out in the ER. The next steps involve the synthesis of farnesyl pyrophosphate, which is used for the synthesis of squalene. Squalene is converted to lanosterol through series of cyclization and migrations of hydrogen and methyl groups. Finally, cholesterol is synthesized from lanosterol in several reactions including oxidation, reduction, and demethylation. The whole process consists of 37 reactions.

Cholesterol Transport

Although cholesterol in synthesized in the ER, its concentration in the ER is very low. This is because the largest part of synthesized cholesterol is transported to other sites. Most of the cholesterol is carried to the plasma membrane, where it modulates membrane fluidity and maintains barrier between cell and environment. However, cholesterol is also sent to other organelles. Some of them are a part of the secretory pathway (e.g., the Golgi complex and lysosomes), and they receive cholesterol by vesicular transport. For the other intracellular destinations, such as the mitochondria, lipid transport proteins deliver cholesterol. These proteins interact with the ER membrane, bind cholesterol, then diffuse through the cytoplasm to target organelles, and release cholesterol into their membranes.

Furthermore, cholesterol is delivered to circulation. Given that cholesterol is a highly insoluble molecule, it is transported

through circulation by endogenous transporters called lipoproteins. The five major classes of lipoproteins are chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL, and high-density lipoprotein (HDL), and their characteristics are presented in Table 1. Lipoproteins have a common structural configuration, although the relative lipid and protein composition of each lipoprotein varies. All of them contain phospholipid monolayer with anchored proteins, which protect inner hydrophobic core of neutral lipids, for example, cholesterol esters and triglycerides. On the surface of lipoproteins, there is some free cholesterol as well. Based on this architecture, lipoproteins are ideal carriers for lipophilic compounds. In addition to other lipids, lipoproteins not only transport and deliver dietary cholesterol to peripheral tissues but also remove the excess cholesterol from peripheral tissues to the liver, thereby maintaining the homeostatic balance. However, each class of lipoproteins has different structures, specific functions, and thus different effects on health. From the clinical point of view, LDL cholesterol and HDL cholesterol are particularly attractive, even though other lipoproteins have clinical significance as well.

Cholesterol Absorption

Absorption of dietary cholesterol is closely related to plasma concentrations of cholesterol. In human diet, the major sources of cholesterol are egg yolk, meat, and dairy products. A variable proportion of dietary cholesterol is esterified to fatty acids, while the rest remains free. However, only free cholesterol appears to be absorbed, so cholesterol esters have to be hydrolyzed prior to absorption, mostly by the action of pancreatic enzymes. Additional and often overlooked source of cholesterol originates from the bile, and all biliary cholesterol is nonesterified and therefore suitable for absorption. Moreover, among 60–80% of cholesterol that is absorbed from the diet and the bile, greater part originates from the bile since it is already prepared to be absorbed.

The main sites of cholesterol absorption are the duodenum and proximal jejunum. The process occurs through the intestinal mucosal cells on the surface of the intestinal villi. Digestion of lipids starts in the stomach when the food is mixed with gastric and lingual enzymes, forming a crude emulsion. This emulsion is mixed in the duodenum with pancreatic enzymes and bile salts. Pancreatic enzymes, lipase, cholesterol esterase, and phospholipase A all have hydrolytic functions and liberate cholesterol from the esters. Since many products of pancreatic enzymes are poorly soluble, the role of bile salts is to emulsify lipids, acting as a surfactant. The obtained mixture of lipids, triglycerides, and cholesterol is readily soluble and is able to pass through the thin water layer around luminal cells. In this way, the mixture comes up to absorptive enterocytes. Although it has been thought for years that lipids passively diffuse through the plasma membrane of enterocytes, recent studies have shown that several proteins are involved in intestinal transport of cholesterol. The cholesterol carriers that are identified so far are NPC1L1, SR-B1, CD13, CD36, P-glycoprotein, and annexin 2–caveolin 1 complex. Some of them affect intracellular concentration of cholesterol and are controlled by transcription factors. However, the list of protein mediators of intestinal cholesterol transport is likely still incomplete.

Inside the intestinal cell, cholesterol is shuttled to the ER, where it is packaged into chylomicrons. Chylomicrons are large particles (around 1 um), with lipid-containing core (similar to the other lipoproteins) and the surface with anchored apoproteins, mostly apoB-48, apoE, A-1, A-2, and A-4. Amphipathic nature of apoproteins makes chylomicrons soluble in blood and lymph fluid. Because of the size of chylomicrons, they cannot pass through the blood vessels, but instead enter the lymph. This secretion into the lymph fluid requires apoB-48 on the surface of chylomicrons. From the lymph, chylomicrons enter the bloodstream via the thoracic duct and the right subclavian vein. Thence, they are sent to the adipose and muscle tissue that utilize a part of the triglycerides in chylomicrons, before arriving at the liver. The triglycerides from chylomicrons are extracted by the enzyme lipoprotein lipase (LPL) while passing through the capillaries of the muscle and adipose tissue and used by muscles or stored in adipocytes. The remaining particle is called chylomicron remnant. The remnant is denser than chylomicron; it contains less triglycerides and thus is relatively rich in esterified cholesterol. It is rapidly removed from the circulation by the liver, via LDL receptors or the LDL receptor-like protein (LRP). Chylomicron receptor LRP is placed on the surface of hepatocytes, interacts with apoE in the remnants, and thus facilitates the uptake to the liver.

In the liver, lipids are repackaged into VLDL particles, which necessarily contain apoB-100, synthesized mainly in hepatocytes. The amounts of apoB-100 are controlled by the amount of lipids: When more lipids are available, the

Table 1 Composition and physical properties of lipoprotein classes

Lipoprotein	Chylomicrons	VLDL	IDL	LDL	HDL
Source	Intestine	Liver, intestine	VLDL	VLDL	Liver, intestine, VLDL, chylomicrons
Diameter (nm)	90–1000	30–90	25–35	20–25	5–25
Density (g ml^{-1})	<0.95	0.95–1.006	1.006–1.019	1.019–1.063	1.063–1.210
Total lipids (%)	98–99	90–93	88–90	78–80	43–67
Triglyceride	85–88	50–55	25–30	10–15	3–15
Free cholesterol	1	8–10	8–10	8–10	2–10
Cholesteryl esters	3	12–15	32–35	37–48	15–30
Phospholipids	8	18–20	25–27	20–28	26–46
Protein (%)	1–2	7–10	10–12	20–22	33–57
Apoprotein	A-I, A-II, B-48, C-I, C-II, C-III	B-100, C-I, C-II, C-III, E	B-100, C-I, C-II, C-III, E	B-100	A-I, A-II, C-I, C-II, C-III, D, E

proteolysis of apoB-100 is reduced and the production of VLDL increases. In contrast, when the concentration of lipids falls down, more apoB-100 is proteolyzed and the generation of VLDL decreases as well. Therefore, the concentration of VLDL is regulated by the proteolysis of apoB-100. VLDL are secreted from the liver, and they are the main carriers of endogenously produced triglycerides. As with chylomicrons, VLDL is also subjected to LPL-mediated hydrolysis of triglycerides in capillary walls, and then, surface proteins (with the exception of apoB-100) are transferred to HDL. One important protein involved in the transfer of cholesteryl esters and triglycerides between VLDL, LDL, and HDL is facilitated by cholesterol ester transfer protein (CETP), which is also an important target for cholesterol-lowering drugs. Therefore, both chylomicrons and VLDL deliver triglycerides to tissues for storage or as a source of energy. Although a part of the remnants of VLDL is removed by the liver, most are converted to LDL via IDL. In addition, there is another pathway for lipid transfer by chylomicrons and VLDL, heparan sulfate proteoglycan-mediated pathway.

After hydrolysis of triglycerides from VLDL, IDL particles are produced. Some of IDL are taken up by the liver, through the binding of apoE to the LDL receptors. Nevertheless, the majority of IDL is modified by hepatic lipase forming LDL.

LDL Cholesterol

In LDL particles, cholesterol makes ~50% of the weight, while around 25% are proteins. The crucial protein component is apolipoprotein B-100 (apoB-100), along with 80–100 additional ancillary proteins that increase LDL solubility. Core of the LDL particles is highly hydrophobic and contains mostly triglycerides and cholesterol esterified by various fatty acids. The basic function of LDL is delivering cholesterol to cells for storage or further biogenesis, by receptor-mediated endocytosis involving the uptake of the whole lipoprotein particle. Especially during periods of rapid growth and development, LDL particles provide cholesterol to most peripheral tissues via the LDL receptor. In spite of this important function, high levels of LDL cholesterol in serum strongly correlate with increased risk of cardiovascular events. Thus, LDL cholesterol is widely called 'bad cholesterol.' Furthermore, recent evidence showed possible relationship between oxidized form of LDL and atherosclerosis. The oxidation of LDL is a complex process, which affects both the protein and the lipids: they undergo oxidative changes and form complex products. Primarily, nonenzymatic oxidative changes in amino acids result in extensive alteration in the apoB-100 composition and structure. In addition, lipid peroxidation causes generation of aldehydes and ketones that covalently modify amino acid side-chain residues of apoB-100 and the other proteins. Modified LDL is then scavenged and degraded by macrophages. They bind oxidized LDL by the scavenger receptor, which is expressed primarily on macrophages. The receptor–LDL complex is taken up and then targeted to the lysosome for degradation. If macrophages phagocyte a high amount of LDL, cholesterol accumulates in their cytoplasm, which becomes filled with lipid droplets. The lipid droplets in the cytoplasm give the macrophages a foamy appearance, because these cells are named foam cells. They are not harmful as such, but they can accumulate in the wall of large blood vessels, leading to the formation of an atheroma, which are known as precursors for atherosclerosis. The unrestricted generation of foam cells and its ability to promote inflammatory responses and differentiation of monocytes in the arterial wall imply that oxidized LDL is a key factor in the development of atherosclerosis.

In order to be packaged into lipoproteins, a large amount of cholesterol is esterified with long-chain fatty acids. In this way, the problem of transport of insoluble cholesterol through circulation is solved. However, cholesterol esters cannot pass through membrane and delivery is enabled by lipoprotein receptors. A prototype of these receptors is LDL receptor, which mediates the uptake and lysosomal degradation of plasma LDL, thereby providing cholesterol to cells. This is a cell surface transmembrane protein that binds LDL on apoprotein sites and carries it into the cell by receptor-mediated endocytosis. After binding LDL, the receptor–LDL complex is taken up, clustered, and then endocytosed by clathrin. Then, the vesicle is fused with an acidic endosome. The decrease in pH induces a conformational change in the LDL receptor, which releases the LDL cholesterol, and the receptors either are then destroyed or can be sorted into vesicles and returned to the surface of the cell. The remaining endosome is delivered to the lysosome, which is more acidic than endosome. Lysosomal enzymes degrade the protein component and hydrolyze the cholesterol esters. The liberated cholesterol can be used by the cell for the synthesis of plasma membranes, vitamin D, and steroid hormones or stored in the cytoplasm in the form of cholesterol ester droplets.

This mechanism is crucial for efficiently removing the excess LDL and maintaining desirable levels of LDL in blood. Therefore, it is important that cells express an adequate amount of LDL receptors on their surfaces and that their function is appropriate. Several genetic mutations in the LDL receptor diminish its function or reduce the number of receptors on the cell surface, leading to increased levels of serum LDL and hypercholesterolemia.

LDL Receptor Mutations

Mutations in the LDL receptor gene cause inherited disorder characterized by high serum cholesterol level, called familial hypercholesterolemia (FH). FH is principally an autosomal dominant disorder. LDL receptor is located on chromosome 19 at 19p13.1–p13.3, and more than 1000 mutations have been identified in this gene so far. In addition, mutations in two other genes also produce the FH phenotype: the apolipoprotein B-100 gene that codes the most important protein component of LDL and proprotein convertase subtilisin/kexin type 9 (PCSK9). Nevertheless, mutations in LDL receptor gene are more common and affect about 1 in 500 individuals. These genetic changes are classified in five broad classes according to the effect on LDL receptor function. Class 1 mutations affect the synthesis of the receptor in the ER, and these mutations are alternatively referred to as 'receptor-negative' mutations. In class 2 mutations, the LDL receptor is not transported from the ER to the Golgi that leads to the degradation of the receptor. In class 3 mutations, the binding of LDL to the receptor is improper. In class 4 mutations, the internalization of the receptor–ligand complex is inhibited, and in class 5 mutations, the internalized receptors cannot recycle properly. The classes

2–3 are often termed 'receptor-defective.' Class 5 mutations cause relatively mild phenotype of FH, since LDL receptors are still present on the cell surface, although they have to be newly synthesized. Classes 2 and 3 are identified as the most common.

Most people with FH are heterozygotes, having one normal copy of the LDL receptor gene and one mutated copy of the gene. These people have an increased risk of cardiovascular disease that typically begins in their forties or fifties. However, homozygotes have two mutated genes for the LDL receptor, inherited from both affected parents. In these people, clinical picture is more severe and their FH clinical onset of symptoms usually appears in childhood or adolescence.

HDL Cholesterol and Reverse Cholesterol Transport

Unlike LDL, HDL cholesterol is widely known as 'good cholesterol' because of its strong inverse association with the risk of cardiovascular disease. HDL particles play the largest role in removing cholesterol from the peripheral tissues to the liver, suppressing cholesterol accumulation in the peripheral tissues. The essential function of HDL cholesterol is the initiation of a multistep process, reverse cholesterol transport, via plasma to the liver, which processes the excretion of excess cholesterol from the body. Furthermore, many other atheroprotective activities have also been attributed to HDL, such as antioxidant, anti-inflammatory, hemostasis, and endothelial cell maintenance functions. It prevents oxidative modification of LDL, thereby reducing the generation of macrophage foam cells and atheroma plaque formation within the arteries. HDL also decreases the activity of macrophage chemotactic factor 1 that participates in low-grade systemic inflammation and signals the infiltration of monocytes to arterial walls. In addition, HDL acts opposite to LDL inhibiting platelet aggregation and enabling activity of nitric oxide synthase, which is also inhibited by oxidized LDL. Nevertheless, the most important function of HDL is the promotion of reverse cholesterol transport, especially removing cholesterol from atherosclerotic plaques. A number of epidemiological studies have suggested that low level of this lipoprotein induces the development of coronary artery diseases. On the contrary, high HDL cholesterol level is associated with the reduction of incidence of atherosclerosis and cardiovascular disease.

HDL constitutes a heterogeneous group of lipoproteins that differ in density, size, lipid composition, and apolipoprotein content, as well as electrophoretic mobility. Based on the electromobility determined by electrophoresis, HDL particles could be separated into two major subfractions, HDL2 and HDL3. Although the proteomics of HDL is very complex, the majority of HDL particles contain apolipoprotein A-I (apoA-I), which is the most abundant apolipoprotein in human plasma in healthy population. The second most abundant protein in HDL is apoA-II, which is also included in many HDL particles. In addition, over 85 other proteins have been identified so far, showing proteomic complexity and diversity of HDL that is related to numerous functions of HDL.

ApoA-I synthesis in the liver and intestine is the first step in HDL metabolism. The next step is the interaction of ApoA-I with cells expressing ABCA1, a member of the ABC transporter superfamily (ATP-binding cassette transporters) that transports the specific substrates across cell membranes utilizing the energy of ATP binding and hydrolysis. ABCA1 is situated at the plasma membrane and intracellular organelles, where it can facilitate transport of various molecules across extra- and intracellular membranes. Although studies in animal models of tissue-specific ABCA1 deficiency confirmed that hepatic ABCA1 was crucial for HDL biogenesis, nonhepatic tissues are also very important. The ABCA1 pathway not only is involved in lipid efflux from both peripheral and hepatic tissues but also is important for the formation of nascent HDL and maintenance of plasma HDL levels. At first, lipid-poor apoA-I removes free cholesterol from peripheral cells through ABCA1 transporter to generate nascent pre-βHDL. This particle, consisting of a phospholipid bilayer surrounded by a protein coat, has a great affinity for free cholesterol and collects cholesterol, which is then stored in the lipid bilayer. This process is enhanced by the activation of the lecithin–cholesterol acyltransferase enzyme, which esterifies free cholesterol stored in the bilayer with fatty acyl groups from lecithin (phosphatidylcholine), forming hydrophobic cholesterol esters. The obtained esters are moved to the hydrophobic core, which is growing up. During circulation, nascent HDL increases binding more cholesterol and phospholipids. The shape of HDL is then changing from the disk to a sphere in a complex process mediated by ABCG1, hepatic lipase, endothelial lipase, CETP, and phospholipid transfer protein. At this step, further cholesterol collection is ceased; mature spherical HDL particle is formed and recognized by the liver. Mature HDL interacts with other apoB-containing lipoproteins, such as IDL and VLDL. Thus, ABCA1 and its transporter are important for HDL metabolism, and the defective ABCA1 transporter due to mutations leads to severe reduction of HDL, a rare inherited disorder called the Tangier disease.

In humans, HDL cholesteryl esters are cleared from plasma via two major pathways. In the first one, CETP facilitates an exchange for triglyceride in HDL cholesteryl ester and transfer to LDL, which is returned to the liver through the LDL receptor-mediated pathway. The other way is nonendocytic hepatic uptake of HDL by scavenger receptor B1, multiligand receptor that binds not only HDL but also VLDL and LDL. The final step for the elimination of cholesterol from the liver is secretion into bile, either directly or after conversion to bile salts. Several transporters are involved in biliary cholesterol secretion, such as ABC transporters (ABCB11, ABCB4, and ABCG5/G8), the Niemann–Pick C1-like protein 1, the phosphatidylserine flippase, ATPase class I type 8B member 1, and the HDL cholesterol uptake receptor – SR.

Cholesterol-Lowering Drugs

Regarding the established role of the total and LDL cholesterol in atherosclerosis and CVD, the American College of Cardiology (ACC) and the American Heart Association (AHA) proposed in 2013 the guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults. Atherosclerotic cardiovascular disease (ASCVD) includes coronary heart disease, stroke, and peripheral arterial disease. Healthy diet, maintenance of healthy weight, and lifestyle modification (regular exercise and avoidance of tobacco) are proposed as crucial

components in ASCVD risk reduction, both prior and with the use of cholesterol-lowering drugs.

The most used cholesterol-lowering drugs are *statins*, a large group of inhibitors of HMG-CoA reductase. A comprehensive meta-analysis from the Cholesterol Treatment Trialists' Collaboration of 27 randomized trials revealed that they reduced the risk of major coronary events by 24% for each 1 mmol l^{-1} reduction of LDL concentration, stroke by 15%, and coronary revascularization by 24%. It has been assumed that fully implemented public policies would result that more than a third of all middle-aged and older adults in the United States and United Kingdom will be recommended for statin therapy.

Statins act on crucial step of cholesterol biosynthesis, the mevalonate synthesis, which is regulated to ensure sufficient production of isoprenoids. The inhibition of cholesterol biosynthesis leads to raised expression of LDL receptors in liver cell membranes, enhancing clearance of the circulating LDL particles from blood, and to raised expression of PCSK9, an enzyme responsible for LDL receptor catabolism. Additional 'pleiotropic' effects of statins (such as reduced vascular and systemic inflammation, including sepsis, and reduced cancer risk) may result from the inhibition of the biosynthesis of isoprenoid intermediates and of small GTP-binding protein, thus influencing the signal transduction in membrane receptors involved in processes of cell proliferation, differentiation, and apoptosis. Statins downregulate antigen presentation and decrease T-cell activation and reduce antigen expression on endothelial cell and the levels of inflammatory cytokines TNF-α, IL-1β, IL-6, IL-8, NF-κB, and C-reactive protein. Furthermore, they reduce the production of reactive oxygen species, inhibit the production of thrombin and thrombomodulin, decrease plasminogen activator inhibitor-1 expression, and increase tissue plasminogen activator levels and tissue factor, thus leading to increased degradation of fibrin. The increased anticoagulation and fibrinolysis prevent endothelial cell disruption.

Statins are strongly recommended as secondary prevention in individuals with clinical ASCVD and as primary prevention in individuals with primary elevation of LDL above 4.9 mmol l^{-1}, in individuals with diabetes, and in those with estimated 10-year ASCVD risk $\geq$7.5% with LDL concentration 1.8–4.9 mmol l^{-1}. High-intensity statin therapy on average lowers LDL by $\geq$50%, moderate-intensity statin therapy lowers LDL by 30–50%, and lower-intensity statin therapy lowers LDL by approximately <30%. In people over 75 years of age, high-intensity statin therapy in secondary prevention is not recommended. These drugs are not routinely recommended for individuals with NYHA class II–IV heart failure or who are receiving maintenance hemodialysis.

Statins are metabolized by cytochrome P450 pathway and thereby occasionally may cause hepatotoxicity (<3% of patients) and myopathy (<0.2% of patients), and in <0.05% of statin-treated patients, muscle injury leads to rhabdomyolysis, myoglobinuria, and renal failure. After over 25 years of wide use of statins, it is proved that benefits of statin therapy are of far greater significance than the risk of adverse effects of statins such as muscle weakness, impairment of hepatic function, the increased incidence of type 2 diabetes, and cataract.

Approved nonstatin agents for LDL reduction include *bile acid-binding resins* (colestipol, cholestyramine, and colesevelam), which have the advantage of not being absorbed systemically and can be used in pregnancy; *fibrates* (fenofibrate, bezafibrate, and gemfibrozil), which mainly decrease triglycerides and increase HDL; *niacin*, which slightly decreases LDL and triglycerides while increasing HDL; *cholesterol absorption inhibitor* ezetimibe, which acts on epithelial Niemann–Pick C1-like protein; and *omega-3 fatty acid* supplements. Although these agents can clearly improve lipid profiles in many patients, contemporary event reduction trials have shown little evidence to support their use either as monotherapy or as an adjunct to statins in general population.

The new agents – mipomersen and lomitapide – were approved in 2013 by the US Food and Drug Administration to treat patients with homozygous FH. *Mipomersen* is an antisense oligonucleotide that binds to a specific 20-base sequence on messenger RNA coding for apolipoprotein B-100. *Lomitapide* is an inhibitor of microsomal triglyceride transport protein assisting in the transfer of triglyceride to apolipoprotein B and also reduces circulating LDL cholesterol by targeting VLDL production.

CETP inhibitors such as anacetrapib and evacetrapib are very effective in raising HDL (>100% in phase 3 trials), but their effects on HDL function are not fully understood, although some data suggest that they have the potential to promote cholesterol efflux capacity. They are also effective in reducing LDL levels (>30%) and concentrations of Lp(a).

The most promising novel agents effective in LDL reduction are *monoclonal antibodies targeting PCKK9*, a protein secreted by hepatocytes that binds to the LDL receptors leading to its cellular internalization and lysosomal degradation. The inhibition of PCSK9 also reduces Lp(a). Three large randomized control human trials on safety and efficacy of alirocumab, bococizumab, and evolocumab are currently ongoing.

See also: Adipose Tissue: Structure and Function of Brown Adipose Tissue; Adipose Tissue: White Adipose Tissue Structure and Function; Cholesterol: Factors Determining Blood Cholesterol Levels; Cholesterol: Properties, Processing Effects, and Determination; Elderly: Nutrition Requirements; Fat Replacer; Fatty Acids: Essential Fatty Acids; Fatty Acids: Metabolism; Fatty Acids: *Trans* Fatty Acids; Hypertension and Diet; Obesity: Causes and Prevalence; Obesity: The Role of Diet; Phospholipids: Physiology; Phospholipids: Properties and Occurrence; Triacylglycerols: Characterization and Determination; Triacylglycerols: Structures and Properties; World Health Organization.

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Cholesterol: Factors Determining Blood Cholesterol Levels

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Introduction

Whole-body cholesterol balance is regulated by the net effects of dietary cholesterol absorption, *de novo* cholesterol biosynthesis, and whole-body cholesterol clearance, mostly by biliary excretion from the liver.

In the intestinal tract, cholesterol originates from two sources: food intake and biliary secretion into the duodenum. Several proteins in the brush border membrane of enterocytes are involved in mediating intestinal cholesterol absorption. Whereas various transporters, including fatty acid translocase/cluster determinant 36, scavenger receptor class B type I (SR-BI), and Niemann-Pick C1-like 1 (NPC1L1), may influence cholesterol uptake, the ATP binding cassette (ABC) transporter family, including several cholesterol carriers (ABCA1, ABCB1, and ABCG5/G8), act as efflux pumps favoring cholesterol export out of absorptive cells into the lumen or basolateral compartment. Among all the cholesterol transporters, the enriched NPC1L1 protein in the apical membrane of enterocytes is considered essential for intestinal cholesterol absorption, and genetic modifications of NPC1L1 in cultured intestinal cells alter cholesterol uptake.

Although all tissues in the body are capable of synthesizing cholesterol from acetyl coenzyme A (CoA), the liver is the main site for *de novo* cholesterol synthesis and stores it as cholesterol ester after esterification by acetyl-CoA acetyltransferase. The major rate-controlling enzyme in hepatic cholesterol synthesis is 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which is used as a pharmacological target of statin treatment.

Cholesterol, as a water-insoluble molecule, needs to be transported in the plasma associated with various lipoprotein particles, such as chylomicrons, very low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs). Approximately 60–80% of cholesterol is transported through the bloodstream in the core of LDL particle. On the other hand, crucial molecules in cholesterol transport are apolipoproteins located on the surface of LDL particles. Each LDL particle contains one molecule of apoB, a lipoprotein responsible for carrying cholesterol to peripheral tissues and binding to LDL receptors. Since more than 90% of the removal of LDL takes place in the liver, the liver determines the rates of LDL clearance from plasma. Hence, the liver is the only organ capable of eliminating excess cholesterol from the body, by either secretion into bile or conversion into bile acids. The movement of excess cholesterol from peripheral tissues to the liver is a result of reverse cholesterol transport (RCT), which is promoted by HDL particles.

The body maintains a stable cholesterol pool by regulating mechanisms of absorption, synthesis, and elimination. The dominant factor that determines cholesterol absorption in the small intestine is intake. Hence, when dietary cholesterol intake is very low, its absorption is upregulated. Conversely, if dietary intake is too high, absorption is decreased and biliary excretion is intensified. The percent of cholesterol absorbed in healthy subjects vary significantly from person to person from 29% to 80%. However, about 25% of the population has exaggerated response to an overload of dietary cholesterol. Additionally, among dietary and genetic factors, many physiological and pathological conditions can influence plasma cholesterol levels.

Aging

Numerous studies have demonstrated that regardless of physical activity levels and nutritional status, levels of plasma total cholesterol rise progressively with age. Plasma LDL cholesterol (LDL-C) levels increase progressively from young adulthood to approximately age 60 in men and to age 70 in women. Prolonged intestinal transit time may be a factor for increasing cholesterol absorption with aging. Slow intestinal transit is associated with an increased rate of bacterial biotransformation of bile acids, with enhanced enterohepatic recirculation of deoxycholic acid. At the same time, diet rich in cholesterol and saturated fatty acids (SFA), usually presented in elderly subjects, can be the reason for increased cholesterol absorption. Another possible explanation could be a gradual reduction in the rate of LDL clearance from the circulation, presumably as the result of a reduced activity of LDL receptors, diminished number of functioning hepatic LDL receptors, and prolonged turnover time ('downregulation') of the recirculating LDL receptors. In response to cholestyramine, a bile acid-binding agent, the elderly can reach the same values of LDL clearance as younger individuals, which explains that they still have the capacity for 'upregulation' of hepatic LDL receptors. Another explanation for reduced LDL receptor expression may be a consequence of a reduced hepatic demand for cholesterol, due to a reduced bile acid synthesis occurring with age. In mice, aging increases biliary cholesterol secretion and reduces level of hepatic bile acid synthesis, which hence increases the susceptibility of developing cholesterol gallstones in elderly animals. This finding may be explained with the decreased activity of hepatic 7-α hydroxylase, an enzyme that metabolizes cholesterol to biliary salts. Some of the rise in LDL-C levels with age could be related to the sedentary lifestyle and increase of body weight, which is typical in older population. In addition, various concomitant diseases (diabetes mellitus, hypothyroidism, and nephropathy) and commonly used drugs more frequently present in the elderly population are associated with hypercholesterolemia. Also, it has been shown that growth hormone (GH) has key roles in cholesterol metabolism

and that its secretion is reduced with aging. Experiments performed in rodents have demonstrated that the administration of GH is able to completely reverse age-dependent increase in plasma cholesterol and the reduced levels of bile acid synthesis to the same level as seen in young animals.

Gender

Compared with men, women have lower levels of LDL-C and VLDL cholesterol and higher HDL cholesterol (HDL-C) levels. There is no difference in cholesterol absorption fraction between men and women. Lower concentrations of LDL-C are associated with accelerated LDL production and enhanced LDL clearance, which may be explained with higher levels of estrogens in women. Studies in rats have shown that estrogen treatment is followed by an increase of hepatic LDL receptors and a faster clearance of LDL particles. The sex difference in HDL concentrations is associated with greater synthesis rate of apolipoproteins A-I and A-II, major proteins of HDL particle responsible for fat efflux from peripheral tissues to the liver. It has been shown that postmenopausal women have higher plasma cholesterol levels than premenopausal women of the same age. In postmenopausal women, the decrease in plasma estrogen levels after menopause may play a significant role in the reduction of the clearance of LDL particles and subsequent increase of LDL-C. Estrogen replacement treatment has been shown to markedly decrease LDL-C in dyslipidemic postmenopausal women. Sexual dimorphism in cholesterol metabolism cannot be explained only by the different levels of sex hormones. There are studies that showed that surgically induced menopause without hormone replacement therapy has no effect on plasma cholesterol concentrations when compared with surgical control group (hysterectomy with conservation of the ovaries). There are seemingly many factors that need to be explored in the future, and one of them is certainly the difference in insulin action between men and women, with higher rate of circulating insulin and therefore greater suppression of lipolysis in women. It is unlikely that differences in body composition are responsible for this phenomenon, because sex differences in cholesterol metabolism exist even they are matched for percentage of body fat. Androgen action is most likely not responsible for the sex differences in plasma LDL-C concentration. Testosterone administration is associated with only modest reduction of LDL-C concentration when given to hypogonadal men in replacement doses and has no effect on LDL-C concentration in eugonadal men.

Genetic Factors

Serum lipid levels are associated with genetic factors. It has been estimated that genotype participates with around 40–60% in the serum total cholesterol variability. There is growing evidence about association between 95 genetic loci and LDL-C, HDL-C, and triglycerides, discovered in genome-wide association studies. In the Framingham Heart Study (FHS), offspring and third-generation cohorts (3110 participants) of middle-aged to elderly adults with available genomic DNA were genotyped for lipid single-nucleotide polymorphisms (SNPs), and genetic risk scores (GRS) were calculated for each individual and lipid fraction. The results showed modest association between lipid GRS and corresponding lipid, which was the strongest with the long-term average lipid measure, and between HDL-C GRS and TG GRS. Lu et al. investigated whether common genetic variants in genes involved in cholesterol metabolism could predict the plasma cholesterol levels. They found that out of 361 SNPs in 243 genes, 23 SNPs were associated with plasma total cholesterol levels. The results of the study with the multiple gene approach reported that 10 out of 17 candidate genes were associated with lipid levels in Caribbean Hispanic subjects, where the genetic variants on three genes, APOA5, APOB, and CYP7A1, accounted for the largest proportion of lipids variation. The longitudinal cohort of black and white siblings, enrolled in the Bogalusa Heart Study, showed association of long-term levels and trends of LDL-C with chromosomes 1 and 19. There are several evidences of strong connections between ten common variants in the genes for LPL, CEPT, and APO and plasma lipid concentrations in children according to the Gene–Diet Attica Investigation on childhood obesity (GENDAI). Significantly higher total cholesterol and LDL-C were observed in APOE $\epsilon 4$ carriers compared to $\epsilon 3/\epsilon 3$ homozygotes and $\epsilon 2$ carriers. The association of APOE genotype with total cholesterol/HDL-C ratio was further modulated by body mass index (BMI). Carriers of the cholesteryl ester transfer protein (CETP) TaqIB B2 allele had significantly higher HDL-C and lower total cholesterol/HDL-C ratio compared to B1/B1 individuals. Suggested potential prediction of lifelong exposure to an adverse lipid profile in children is very important for applying precautionary principle and preventive measures.

Dietary Factors

Modern diet is characterized by the high intake of SFA, refined starches and sugars, both known to have adverse effect on serum lipids levels. In addition, the human diet contains a large portion of oxidized fatty acids and oxidized cholesterol because of the food processing (e.g., frying and heating).

Lipid Intake

It is clear that plasma cholesterol levels correlate to the quality and quantity of dietary lipid intake. Tarahumara and Guatemalan Indians who are consuming a diet low in fat exhibit low serum total cholesterol and LDL-C. However, when these people are placed on typical Western diet, their total cholesterol and LDL-C increase and synthesis of VLDL increased, mainly due to increased flow of free fatty acids (FFA) to the liver. In subjects with increased BMI, it has been demonstrated that excess body weight correlates with increased synthesis and turnover of cholesterol in the adipose tissue.

SFA and Cholesterol

Effects of dietary fats and cholesterol on circulating cholesterol levels, among others, depend on the type of fats and individual characteristics. A high intake of dietary cholesterol increased fasting LDL levels by $\sim$10% in a dose-dependent manner, while 12% reduction of fasting LDL levels reduced coronary

risk by 19%. It was estimated that each 100 mg increase in dietary cholesterol intake resulted in an increase of 0.05 mmol l^{-1} in serum LDL-C concentration. On the other hand, very-low-fat diets caused dyslipidemia (high triglycerides and low HDL-C concentrations). In the 'Great Fat Debate,' scientists emphasized that the adequate replacement of SFA in diet is crucial in terms of reduction of LDL-C and cardiovascular risk. The general recommendation is to minimize dietary saturated fat by displacing it with unsaturated fat, primarily polyunsaturated. However, in practice, people usually replace it with refined carbohydrates, which also increase cardiovascular risk.

Polyunsaturated and Monounsaturated Fatty Acids

The beneficial effect of unsaturated fatty acids on lipid metabolism in man is well documented. Supplementation of diet by linoleic acid ($n-6$) leads to a decrease in total cholesterol, LDL-C, and HDL-C levels and in LDL/HDL ratio. Lowering effect on serum total cholesterol and LDL-C could come from polyunsaturated and monounsaturated fatty acids from the corn oil. Favorable fat content from the corn oil positively influence HDL-C, rising its level and also increasing ratio of HDL-C to total cholesterol and decreasing ratio LDL-C to HDL-C. The ($n-3$) fatty acids have an effect different from that of the ($n-6$) fatty acids, in that they enhance HDL-C levels. The effects of $n-3$ PUFA on circulating levels of plasma lipoproteins have been shown to be variable. No consistent changes have been observed in LDL-C or HDL-C concentrations with $n-3$ PUFA consumption. Greater part of this variability is attributed to a lack of control of confounding factors such as dietary cholesterol, fat level, and fatty acid composition. The most comprehensive study evaluating benefits of fish oil (i.e., EPA + DHA) supplementation represents meta-analysis comprising 47 placebo-controlled randomized trials, which found clinically significant reduction of triglycerides (by 0.34 mmol l^{-1}), no change in total cholesterol, and slight increases in HDL-C and LDL-C (by 0.01 and 0.06 mmol l^{-1}, respectively) in hyperlipidemic subjects. Another meta-analysis of 21 trials evaluating lipid outcomes after fish oil consumption found net change in triglycerides (0.30 mmol l^{-1}), HDL-C (0.04 mmol l^{-1}), and LDL-C (0.15 mmol l^{-1}) and no effect on total cholesterol. According to previous reports, the mechanisms by which dietary $n-3$ PUFA may induce changes in plasma LDL-C levels by decreasing absolute rates of LDL synthesis and catabolism without any effect on the fractional catabolic rate. Studies using rats have shown that consumption of fish oils alters hepatic LDL receptor expression. Now, it is known that the effect of EPA/DHA on blood lipids (including cholesterol) is based on the action of these $n-3$ PUFAs as ligands of the various PPAR isoforms and on the modulation of the signaling pathway of the transcription factor SREBP. Principal transcription factor that binds the promoter region of the genes coding for proteins controlling cholesterol homeostasis is SREBP-2. However, the fact that since SREBP-2 is not directly ligated by DHA (EPA), a relationship between PPARa ligation and SREBP-2 activation is presumed and is still not unequivocally explained. The last relationship is possibly species-dependent. Furthermore, the more efficient absorption of dietary cholesterol by rodents compared to humans may account for the differences in the extent of observed increases in LDL-C. The limited but rather consistent increases reported in LDL-C in humans consuming fish oils may be due to the relatively small proportion of dietary cholesterol being delivered to the liver compared to the amount delivered to the livers of hamsters. Improvement of HDL-C together with insulin resistance index HOMA (homeostasis model assessment) was noted in hemodialyzed patients, after EPA + DHA supplementation. That was explained by probably activation of PPAR isoforms and consequently improving insulin signaling on postreceptor level. It was shown that consumption of fatty seafood can modulate fasting insulin, ghrelin, and leptin during an 8-week intervention in obese young adults. Effects were partly gender-specific, and the most consistent effect on circulating hormones was mediated by weight loss. PPARα agonists are the center of a regulatory hub impacting fatty acid uptake, fatty acid activation, intracellular fatty acid binding, mitochondrial and peroxisomal fatty acid oxidation, ketogenesis, triglyceride turnover, lipid droplet biology, gluconeogenesis, and bile synthesis/secretion.

Therefore, it is important to understand the effects of dietary $n-3$ PUFA on cholesterol metabolism and circulating lipid levels and their interrelation with other dietary components, so that the beneficial effects of $n-3$ PUFA are not compromised.

As to the influence of oleic acid ($n-9$), controversial results have been published. Some authors did not see modification of HDL-C levels, but others showed an increase of HDL-C especially after an olive oil regime. Significant increase of HDL-C and apo A1 levels among walnut consumers without modified regular diet was established. The increase of HDL-C may be due to the fatty acid composition of walnuts, as it provides mostly linoleic acid ($n-6$), alpha-linolenic acid ($n-3$), and oleic acid ($n-9$), or might be related to the nature of the protein amino acids.

Dietary Fiber, Soy Protein, and Plant Sterols

The serum levels of total cholesterol, LDL-C, Apo A1, and Apo B were significantly lower in one Chinese ethnic group, who consume diet primarily based on corn and then on rice, soy, buckwheat, sweet potato, and pumpkin products, compared with another Chinese ethnic group, who primary consume rice and then corn, broomcorn, potato, and taro products, which are more harmful for lipid profile. Corn-based diet of the first group is rich with dietary fiber that reduces serum total cholesterol level in healthy and hyperlipidemic subjects and with plant high-quality protein that might raise serum HDL-C levels and promote the transportation and excretion of free cholesterol.

Consumption of soy protein significantly decreases concentrations of total cholesterol, LDL-C, and triglycerides in circulation. It was shown that after 24 weeks of treatment based on soybean, patients with combined hyperlipidemia, isolated hypercholesterolemia, and isolated hypertriglyceridemia have decreased LDL-C level by 38%, 32%, and 8%, respectively. Results of the meta-analysis showed association of soy protein with significant decreases in serum total cholesterol by 77%, LDL-C by 5.25%, and triglycerides by 7.27% after short initial period and significant increases in serum HDL-C by 3.03% after more than 12 weeks of consumption. A better effect on the lipid profile was found after intake >80 mg. Dietary fiber from fruits, vegetables, and whole-grain products lowers levels of total

cholesterol and LDL-C possibly through the bile acid metabolism and alteration in serum sex hormone concentrations.

Plant sterols (around 2 g day^{-1}) have been shown to block intestinal absorption of cholesterol and lower total plasma LDL-C.

In nutritional epidemiology, examining the relation between diet and its effect should not be focussed on the intake of single nutrients, food items, or food groups, but should be focussed on the overall diet and food preparation methods and eating patterns. If we want to achieve healthy serum lipid levels and prevent chronic diseases, we should follow general diet recommendations to consume great amount of fruits, vegetables, nuts, and fish; moderate amount of dairy and vegetable oils; and whole-grain foods in place of refined starches and sugars and to avoid sugar-sweetened beverages, processed meats, and foods that contain partially hydrogenated vegetable oils.

Alcohol Consumption

Serum lipid level is associated with alcohol consumption. Effects depend in part on the amount of consumed alcohol, so that moderate intake protects individuals against cardiovascular diseases. Alcohol consumption was the independent negative risk factor for insulin resistance and improved lipid profiles that are known to be worsened by insulin resistance. The increase in HDL-C and decrease in LDL-C in drinkers result from the inhibition of CETP that promotes transfer of cholesteryl ester from HDL to VLDL (a precursor of LDL) and LDL. Inconsistent results among earlier studies may partly result from variability in the prevalence of obesity among subgroups and according to the amount or frequency of alcohol consumption. Harmful effect of heavy alcohol consumption may be attributable to increased triglyceride synthesis.

Coffee Consumption

Coffee is consumed as a beverage worldwide; however, its effect as a cardiovascular risk factor is still controversial. Roasted coffee contains naturally present antioxidants and others that are formed during the roasting process. Chlorogenic acids and caffeine may play a role in the inhibition of lipid peroxidation, free radical scavenging, and anti-inflammatory activity. However, coffee also contains diterpenes, cafestol, and kahweol. High consumption of these compounds can raise serum levels of total cholesterol and LDL-C. Most of them are retained by the paper filter, which substantially reduces the cholesterol-raising effects. It should be mentioned that instant coffee was associated with lower serum concentrations of total cholesterol and higher serum LDL-C level. Average change in total cholesterol for each cup ranged from 0.007 to 0.026 mmol l^{-1}. When examining the effects of coffee beverage on serum lipoprotein levels, we should take into account coffee preparation such as the use of milk, sugar, ice cream, and alcohol.

Physical Activity

The sedentary lifestyle is one of the principal risk factors of highly prevalent illnesses such as type 2 diabetes, cardiovascular disease, osteoporosis, and some cancers. Association between sedentary lifestyle and the current pandemic of obesity and metabolic syndrome is clear. Attempt to exactly measure the effect of sedentary lifestyle on metabolic syndrome and other cardiovascular risk factors, based on duration of leisure-time physical activity, have shown that even 25 min day^{-1} produces benefits, better HDL-C function or higher level of paraoxonase 1 activity. The difference for glycemia, total cholesterol, and HDL-C level disappears after adjustment for age, gender, and cigarette smoking. However, low physical fitness among young adults has been shown to longitudinally predict hypercholesterolemia and, among middle-aged adults, hypertension. This association was attenuated when adjusted for obesity.

Seasonal variation in physical activity has been reported to coincide with seasonal changes in blood lipid levels, particularly total cholesterol. Environmental changes in ambient temperature, daylight, and monthly precipitation are thought to induce seasonal changes in physical activity, particularly by extreme environmental factors (e.g., hot or cold temperatures). Significant increase in nonoccupational activity due to yard work and exercise or recreational activities was noted during the warmer months. Estimates of the amplitude of seasonal variation in activity energy expenditure in this report were consistent with those in the Framingham Offspring Study. The FHS is a population-based prospective family study that began in Framingham, MA, in 1948 with the recruitment of the Original Cohort. In 1971, children of the Original Cohort, called the Offspring Cohort, were enrolled, and finally, in 2002, the grandchildren of the Original Cohort were enrolled making the FHS the longest-running family-based study in history. For the past 62 years, investigators at the FHS have collected data related to CVD and its risk factors. Recent public health recommendations have noted the importance of environmental factors as potential barriers to regular participation in healthful levels of such activity.

Environmental Contaminants

There are increasing evidences of the role of environmental contaminants (e.g., heavy metals and persistent pollutants) in serum lipid level variation. Nonoccupational exposure to various chemicals can occur through contaminated drinking water, foods, air, and cigarette smoking, and even low-level exposure may be harmful to health. Hereafter, examples of the influences of the most common contaminants on lipid metabolism will be described.

Arsenic is widely distributed in the environment and usually contaminates drinking water sources. Experimental studies on rats have shown an increase in total cholesterol level after 10–20 weeks of exposure to arsenic, added as arsenite or arsenate in drinking water. This effect becomes more significant under high-cholesterol diet and when the exposure occurs earlier in life. Mechanism by which arsenic modifies cholesterol metabolism has not been yet elucidated. Possible way is modulation of RCT that transfers cholesterol from the periphery back to the liver by modifying the densities of cholesterols.

Altered lipid metabolism (low HDL-C, hypertriglyceridemia, and high total cholesterol and LDL-C level) may be a

consequence of chronic *cadmium* exposure, possibly due to decreased plasma lipoprotein lipase activity and increased activity of HMG-CoA reductase.

The results of relationship between blood *lead* level and serum cholesterol and lipoprotein levels are conflicting. Occupational exposure to lead was positively associated with levels of total cholesterol and HDL-C. Lead-exposed patients had decreased total cholesterol and LDL-C and increased HDL-C levels. Recently, an association between blood lead and total cholesterol based on an age-adjusted model has been confirmed.

Fat-soluble chlorinated *organics*, such as dioxins, furans, polychlorinated biphenyls (PCBs), and chlorinated pesticides, were related with unfavorable serum lipid levels. Correlation between serum PCBs levels and plasma lipid levels was firstly showed in occupational studies. The strong relationship between PCBs levels and cholesterol and triglycerides was reported with average value for the sum of PCB congeners of 4.2 μg. The proposed mechanisms by which PCBs affect serum lipids are the activation of certain cytochrome P450 enzymes and increased synthesis of lipids.

Perfluorooctanesulfonate (PFOS) used as surfactant in a wide variety of commercial products has been recently recognized to disrupt serum lipid level. Positive association between plasma concentration of PFOS and serum concentration of HDL-C and negative association with total cholesterol/HDL-C ratio and triglycerides level were reported. The possible pathway is peroxisome proliferation, leading in hepatotoxicity and alteration of lipids homeostasis.

It was postulated in the systematic association study about broad environmental correlation to lipid levels. There was favorable association of HDL-C (3–4% higher HDL-C) with iron and mercury exposure and unfavorable association with PCBs, dibenzofurans, organochlorine pesticides, and all persistent organic pollutants. Multiple environmental factor analysis enabled better understanding of their relationship with characteristics in the general population.

Cigarette Smoking

Cigarette smoking is a well-known risk factor for atherosclerosis and may influence serum lipid levels. Significant increase in serum total cholesterol, triglycerides, VLDL, and LDL-C and decrease in protective HDL were reported among chronic smokers, hypertensives, and chronic smokers with high blood pressure. However, confounding factors such as diet, BMI, stress, alcohol consumption, and physical activity have not been controlled in some studies. The effect of smoking on the serum lipid profiles may be influenced by age, gender, and different smoking statuses. It was found that smoking was associated with lower total cholesterol and LDL-C levels in elderly men and in middle-aged women and also with decreased HDL-C levels in 65–74-year-old men and 55–64-year-old women when compared with nonsmokers. The data from meta-analysis were in contrast due to differences in study populations and their dietary habits, physical activities, lifestyle, or public health awareness. Positive association between current smoking and dyslipidemia was reported in women but not in men. Further, current female smokers in the age groups between 20 and 34 years and of 50 years or older have had the highest risk of dyslipidemia. The possible reason for gender difference in the association between cigarette smoking and unfavorable serum lipid levels could be an interaction of some hormonal factors with components of the inhaled smoke. The nicotine provokes the secretion of catecholamines and other hormones (e.g., cortisol and GH) leading to an increased serum concentration of FFA, which stimulates hepatic secretion of VLDL and triglycerides. Cigarette smoke has great oxidative potential and can promote oxidative modifications in LDL and other biomolecules and may contribute to additional endogenous oxidant formation, through its effects on the inflammatory-immune response. Some natural antioxidants such as dietary polyunsaturated fats and vitamin E are connecting with lower risk for atherosclerosis, although they may contribute to lipid oxidation in smokers. Further studies are needed to explain the mechanism by which cigarette smoke changes serum lipid levels and what are the most responsible substances for these changes, since cigarette smoke besides nicotine contains multiple toxic compounds.

Stress

Serum cholesterol levels are changing under emotionally stressful situations. Numerous studies have demonstrated that acute and chronic stressors are related in alterations in cholesterol concentrations. Investigators have established a negative link between cholesterol and psychological and physical aggressions. However, there is a positive correlation between cholesterol and psychological stress. This correlation can be explained with increased peripheral fat tissue lipolysis caused by heightened sympathetic nervous system activity and increased levels of catecholamines, glucocorticoids, and glucagon. The final result of these processes is increased release of fatty acids into the circulation. In contrary, deficit of serotonin and lower cholesterol levels have been implicated with physical aggression and increased incidence of accident, suicide, and homicide. The possible explanation can be that in primitive man, cholesterol served as a sentinel compound for survival. Hence, when primitive man was experiencing lack of food, low blood cholesterol was leading and preparing him for food seeking and increased risk in hunting. Chronic stress induces both functional and structural adaptations within the hypothalamo–pituitary–adrenal axis that are suggestive of long-term alterations in neuroendocrine reactivity to subsequent stressors. Experimental evidence of chronic stress-induced hyperlipidemia in animal models has been documented by significant increases of blood total cholesterol, LDLs, and triglycerides and decrease in HDLs concomitant with increased oxidative stress.

Diseases

Obesity

It is hard to interpret the influence of obesity itself on cholesterol metabolism, due to the confounding of metabolic disorders accompanied with this condition. Abnormalities, such as hypertriglyceridemia, hyperglycemia, and insulin resistance,

commonly seen in obese population, may affect the distribution and size of lipoprotein particles independently of adiposity. The typical dyslipidemia observed in obesity does not include total cholesterol level disturbances. Obesity is characterized by the higher development of small dense, more atherogenic LDL particles. Viscerally, obese men were found to have significantly reduced LDL receptor binding of lipoproteins compared with lean healthy controls. Although hypercholesterolemia is not a concomitant feature of insulin resistance and obesity, both of them are characterized by decreased expression of hepatic LDL receptors due to higher rates of hepatic cholesterol synthesis and diminished cholesterol absorption.

Many studies show that total cholesterol and LDL-C concentrations respond more weakly to diets low in saturated fat and cholesterol in obese than in lean subjects, due to large amount of cholesterol in the enterohepatic pool in obese people. Additional amount taken in with the diet would not be recognized as small to activate hepatic LDL receptors, and hepatic LDL receptors are most probably suppressed by this large stream of endogenous cholesterol from enterohepatic circulation. Therefore, the most effective way for the obese to normalize their blood cholesterol is to lose weight. This shows us that cholesterol metabolism is tightly regulated so that if cholesterol synthesis is upregulated, cholesterol absorption is diminished and vice versa.

Diabetes Mellitus and Metabolic Syndrome

Considerable attention has been focussed on dyslipidemia accompanying diabetes and metabolic syndrome. Metabolic syndrome is generally characterized by abdominal obesity, insulin resistance, hypertension, and blood lipid disorders including high TG and low HDL-C, high apoB, and small dense LDL-C. Metabolic syndrome is associated with increased endogenous cholesterol synthesis and reduced intestinal cholesterol absorption predominantly as a consequence of visceral obesity, independently of overall obesity. It has been proposed that hyperinsulinemia in insulin resistance may upregulate the expression of SREBP-1c, a transcription factor that stimulates the synthesis of fatty acids and the production of VLDL particles. However, SREBP-2, another transcription factor that upregulates *de novo* cholesterol synthesis, does not appear to be affected by hyperinsulinemia or hyperglycemia. Besides elevated liver VLDL production, decreased postprandial triglyceride metabolism represents a major pathway of the hypertriglyceridemia that characterizes diabetic condition. Increased cholesterol absorption has also been reported in type 2 diabetes with slightly increased concentrations of total cholesterol and LDL-C. It has been shown that changing carbohydrate content of a mixed meal altered the postprandial accumulation of chylomicrons. Furthermore, a high glucose level alters the genetic expression of various genes involved in HDL metabolism in HepG2 cells, including human ABCA1, SR-BI, and hepatic lipase.

Cushing Syndrome

It is well known that chronic overt hypercortisolism, as in Cushing syndrome, is characterized by systemic alterations leading to increased risk of metabolic and cardiovascular diseases. Cortisol excess could inhibit insulin secretion, glucose uptake, and glycogen synthesis; worsen insulin sensitivity; and increase gluconeogenesis. However, it was hypothesized that adrenal incidentaloma (the presence of adrenal mass and patient has no signs of hormonal excess or obvious underlying malignancy) may be itself an unrecognized manifestation of the metabolic syndrome. The pattern of dyslipidemia in Cushing syndrome is the same as in metabolic syndrome or in type 2 diabetes.

Hypothyroidism

Hypothyroidism is associated with increased TC and LDL-C due to limited synthesis of the LDL hepatic receptors. Also, the activity of HMG-CoA reductase is significantly lowered, which may explain the poor response to hypolipemic treatment.

Acromegaly

Patients with acromegaly have a relative risk to present glucose alterations, with a 2.6 times and 2.1 times higher risk of impaired glucose tolerance and diabetes, respectively, than the general population, and show a higher prevalence of hypertriglyceridemia and low HDL-C. Active acromegaly in women is strongly associated with higher visceral adiposity dysfunction, insulin resistance, and the features of MetS; therefore, more careful metabolic management is suggested in acromegalic women.

Chronic Renal Failure

The typical dyslipidemia in *chronic renal failure* is characterized by hypertriglyceridemia and low levels of HDL-C, as the result from decreased lipoprotein lipase activity. The decreased level of hepatic lipase observed in renal failure may account for the presence of IDLs and the high HDL2 subfraction. The pattern of dyslipidemia depends on the degree of proteinuria and the kind of terminal renal failure treatment. Total and LDL-C can also be increased, with a predominance of the small dense LDL in the event of proteinuria or peritoneal dialysis.

Drugs

Many drugs, besides lipid-lowering drugs, can affect serum cholesterol levels. It has been reported that thiazide diuretics increase total and LDL-C levels by 5–10% in a dose-dependent way. These side effects are short term and not contraindication for their use. Loop diuretics have a similar effect. In contrary, the use of potassium-sparing diuretics and indapamide shows no changes in cholesterol levels. The effects of beta-blockers on total cholesterol and LDL-C are negligible. Furthermore, they could decrease HDL-C by ~5–20%. However, Celiprolol, a selective beta1 blocker with weak beta2 sympathomimetic activity even improves the lipid pattern. The only antihypertensive agents that lower total and LDL-C levels are alpha1-blocking agents.

Oral estrogen preparations given to postmenopausal women, premenopausal women, women with polycystic ovary syndrome, men with prostatic carcinoma, and male-to-female transsexuals reduce total and LDL-C and increase HDL-C levels.

Combined (estrogen/progestogen) hormone replacement therapy has similar effect. Tamoxifen, a selective receptor estrogen modulator, beneficially affects both total cholesterol and LDL-C, by decreasing them, due to its partial estrogenic activity. Danazol, a drug that is used in treatment of endometriosis, induces an increase of LDL and decrease of HDL-C level. Isotretinoin treatment increases LDL and total cholesterol levels by ~15% compared to pretreatment levels. Patients treated with immunosuppressive therapy (cyclosporine, azathioprine, and corticosteroids) show increased level of total cholesterol, LDL-C, and HDL-C in spite of the combination of drugs and gender of the patient. The largest effect on cholesterol metabolism has cyclosporine in female population, and it is associated with small dense, more atherogenic LDL particles. Tacrolimus does not affect total cholesterol and LDL-C levels.

Protease inhibitors, especially ritonavir, increases total cholesterol level by 30–40%.

Considering anticonvulsive therapy, carbamazepine seems to have most effect on TC levels, which are more pronounced in women and independent of the dosage. Contrary, valproic acid is the only anticonvulsant with a more favorable effect on the lipid profile.

Conclusions

In this article, we try to summarize all factors that determine cholesterol balance in the body, from absorption, to synthesis, to clearance. Some of them, as age, gender, and unbalanced food with more saturated fats and refined carbohydrates, are well known. However, there is growing amount of evidence about the benefit effect of diet, which contains more fatty seafood, corn, soy protein, olive oil, and dietary fiber from fruits, vegetables, and whole grain. Maybe, it is possible to improve, by epigenetic impact with regular diet, known specific genetic variants of genes involved in cholesterol metabolism in childhood. We also emphasize that improvement of low physical fitness could reduce hypercholesterolemia. In the future, we have to pay attention to environmental contaminants (e.g., heavy metals and persistent pollutants) that are able to influence lipid metabolism.

See also: Cereals: Dietary Importance; Cholesterol: Absorption, Function and Metabolism; Fish: Dietary Importance and Health Effects; Soy Beans: Dietary Importance.

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Cholesterol: Properties, Processing Effects, and Determination

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Properties

Introduction

Cholesterol is the most highly regarded small molecule in biology because of the great research effort by multidisciplinary scientists and the number of highly decorated research awards, especially the 13 Nobel Prizes, bestowed upon those scientists dedicating their work to study the structure, biosynthesis, biological functions, absorption and metabolism, and quantification of this fascinating molecule. Cholesterol, a complex four-ring molecule with essential biological functions, is surprisingly synthesized from the most basic two-carbon substrate, acetate (Figure 1). Although being an essential cellular component of animal tissues and the sole precursor of many steroid hormones, cholesterol creates various health complications by being oxidized or accumulating elsewhere excessively, such as in the artery wall, which are preconditions to the formation of atherosclerosis. Cholesterol was first discovered in 1815 as a component of human gallstones by the French chemist M.E. Chevreul. The structure of cholesterol was correctly identified in 1932; however, the process amazingly had begun decades before without modern technologies such as nuclear magnetic resonance. From then on, using isotopic tracers, the discovery of various biosynthetic and metabolic pathways of cholesterol began.

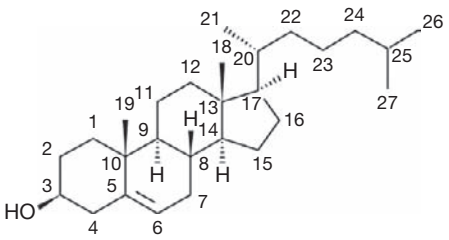

Figure 1 Cholesterol: the most regarded small molecule in biology. ACD/ChemSketch Freeware 2012, Advanced Chemistry Development, Inc., Ontario, Canada.

Chemical Properties

Naturally occurring sterols, including cholesterol, have 1,2-cyclopentano-phenan-threne skeleton with 27–30 carbons, a hydroxyl group at C-3, and a minimal 7-carbon side chain at C-17 of the ring structure (Figure 2). The variation in the structures of the ring skeleton and the side chain differentiates mammalian and plant sterols. Cholesterol is the most abundant sterol in animals and the vital structural component of animal plasma membranes and is essentially absent in plants.

Cholesterol can be found free or esterified to long-chained fatty acids in animal tissues. Hepatocytes need cholesterol to synthesize lipoproteins and bile acids, whereas other cell types incorporate cholesterol into their cell membranes. Cholesterol required for cellular functionality can be taken exogenously through low-density lipoprotein (LDL) from the bloodstream or synthesized endogenously from acyl-coenzyme A (CoA). Cholesterol is hydrophobic, which is enhanced by the loss of the hydroxyl group through esterification. Cholesterol is transported in the bloodstream by lipoproteins, primarily in the form of cholesterol esters. Because of their hydrophobicity, cholesterol esters are more suitable for cellular storage, whereas free cholesterol acquired by cells through hydrolysis of cholesterol esters accumulates in the cell membranes as a structural component.

Uptake and Use

Isotopic labeling techniques confirmed that a two-carbon metabolite of acetate in acetyl-CoA was the sole building block for the biosynthesis of cholesterol in the endoplasmic reticulum. In addition to endogenous biosynthesis, cholesterol is also available to enterocytes from dietary sources, bile, and turnover of intestinal mucosal epithelium. Cholesterol enters the intestine in the free-form by the action of pancreatic cholesterol esterase. It is packed into more hydrophilic micelles containing conjugated bile acids, monoglycerides, and lysolecithin to approach the absorptive cells. Approximately 60–80% of dietary and biliary cholesterol is absorbed; however, biliary cholesterol is to a greater extent absorbed because it is already in the form of micelles. Cholesterol is transported in the circulatory system and to the body tissues by plasma lipoproteins, which share a common structure of a neutral lipid core consisting of triglycerides and cholesterol esters, surrounded by a monolayer of phospholipids, free cholesterol, and alipoproteins. Chylomicrons (synthesized in the intestine) and very low-density lipoproteins (VLDL, largely synthesized in the liver) deliver triglycerides to tissues for storage or energy purposes, and they are converted to LDL in the process. The LDL can also be synthesized in the liver. The LDL primarily transports cholesterol to tissues and glands for storage or further synthesis. Both endogenously synthesized and exogenously absorbed cholesterol must be returned to the liver for excretion or degradation. The removal of cholesterol at the extrahepatic tissues and in the circulatory system is facilitated by the high-density lipoprotein (HDL). The HDL is removed by the liver or steroidogenic tissues through a selective uptake process of cholesterol and other lipid components.

Cholesterol, if not stored as cholesterol esters in the cytoplasm, is used for structural purposes in the cell membrane or the synthesis of bile acids, steroid hormones, and vitamin D. Cholesterol contributes up to 30% of lipid mass in the cell membrane and is vital to the less fluid and more structured lipid phase. The oxidation of cholesterol in tissues, governed by enzymatic pathways, yields important steroid precursors; however, the oxidation caused by food processing through

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nonenzymatic pathways usually results in biologically toxic oxysterols.

Cholesterol Content in Foods

Cholesterol in the human diet primarily comes from meat, poultry, eggs, dairy products, and seafood (Tables 1–4). Therefore, it is expected that, of all the food groups presented in the USDA National Nutrient Database for Standard Reference (SR), only those without the previously mentioned protein sources such as vegetables, spices and herbs, cereals, and vegetable cooking oils have an undetectable amount of cholesterol. The database provides the cholesterol content of various types of protein sources in various forms of raw, cooked, and further processed products, which makes it possible to extrapolate the approximate cholesterol content of similar foods. Cholesterol is an integral component of the animal cell membrane, and it is also deposited in fat droplets; therefore, cholesterol content of fresh ingredients does not change much unless there is a remarkable change in the structure and metabolism of muscle or fat cells. Factors influencing cholesterol content across protein sources are species, diet, muscle oxidative pattern, muscle fiber type and structure, and fat content.

In general, raw and cooked meat and poultry have approximately 40–100 mg of cholesterol per 100 g of meat (Table 1). Few exceptions, such as nonenhanced, cooked chicken dark meat, can contain up to 150 mg/100 g. Chicken skin, both raw and cooked, also has more than 100 mg/100 g. Mechanically deboned meat and poultry have an approximately 10–20% greater amount of cholesterol because of bone marrow contamination. Variety meats have much greater cholesterol content, from 300 mg/100 g of raw liver to 3000 mg/100 g of braised brain. Egg and dairy products are other sources of cholesterol in the human diet (Table 2). With an exception of more than 200 mg/100 g in few salted butter products, cholesterol content

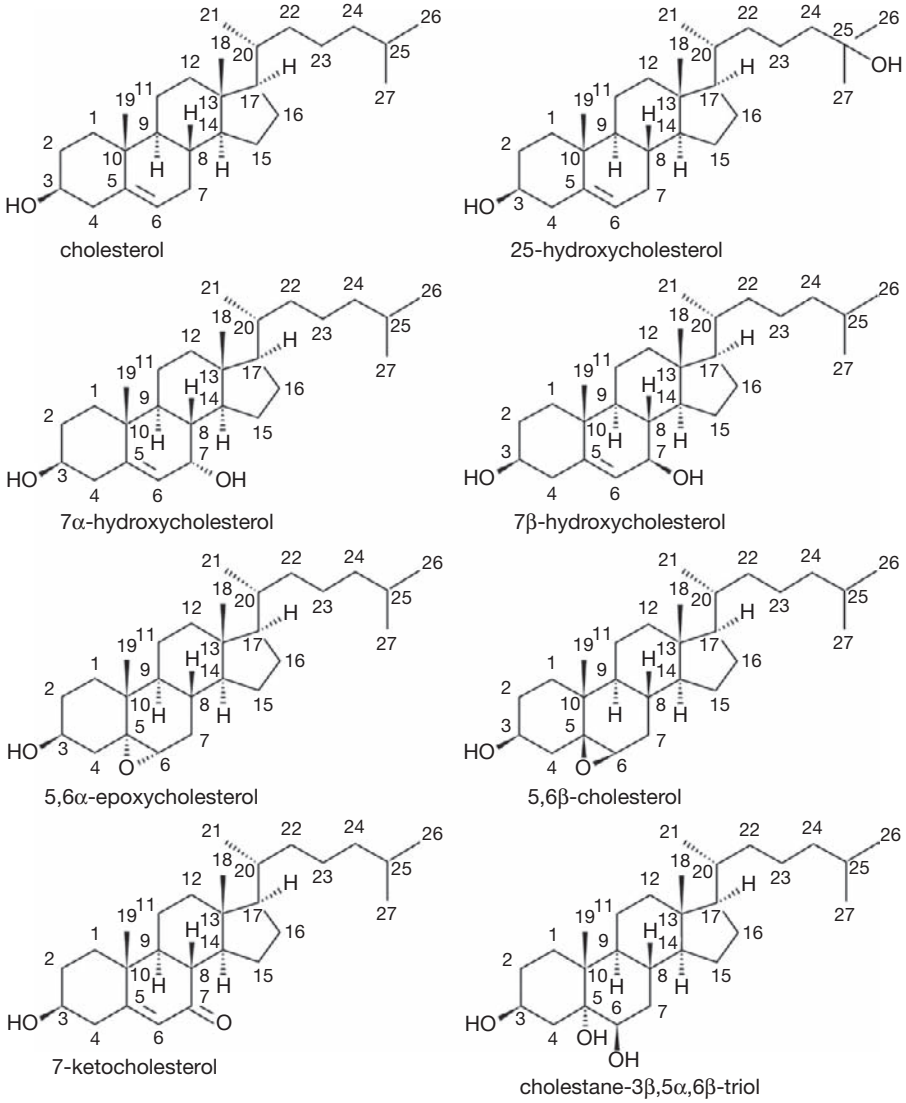

Figure 2 Structures of cholesterol and the most commonly found cholesterol oxides. ACD/ChemSketch Freeware 2012, Advanced Chemistry Development, Inc., Ontario, Canada.

Table 1 Cholesterol content of meat and poultry

Meat and poultry	*Cholesterol content, mg/100 g*
Chicken	
Chicken, broiler or fryers, breast, skinless, boneless, meat only, raw	73
Chicken, broilers or fryers, breast, meat only, cooked, roasted	85
Chicken, broilers or fryers, dark meat, drumstick, meat only, raw	89
Chicken, broilers or fryers, drumstick, meat and skin, cooked, roasted	130
Chicken, broilers or fryers, skin only, cooked, roasted	83
Chicken, broilers or fryers, skin only, raw	109
Pork	
Pork, fresh, leg (ham), whole, separable lean only, cooked, roasted	94
Pork, fresh, leg (ham), whole, separable lean only, raw	68
Pork, fresh, loin, whole, separable lean and fat, raw	63
Pork, fresh, loin, whole, separable lean only, cooked, roasted	81
Pork, fresh, loin, whole, separable lean only, raw	59
Pork, fresh, spareribs, separable lean and fat, cooked, braised	121
Pork, fresh, spareribs, separable lean and fat, raw	80
Beef	
Beef, grass-fed, strip steaks, lean only, raw	55
Beef, ground, 70% lean meat/30% fat, patty, cooked, broiled	82
Beef, ground, 70% lean meat/30% fat, raw	78
Beef, rib, whole (ribs 6–12), separable lean and fat, trimmed to 1/8" fat, all grades, cooked, broiled	82
Beef, rib, whole (ribs 6–12), separable lean and fat, trimmed to 1/8" fat, all grades, raw	70
Beef, round, top round, separable lean and fat, trimmed to 1/8" fat, all grades, cooked, braised	90
Beef, round, top round, steak, separable lean and fat, trimmed to 1/8" fat, all grades, raw	69
Beef, short loin, porterhouse steak, separable lean only, trimmed to 1/8" fat, all grades, cooked, grilled	82
Beef, short loin, porterhouse steak, separable lean only, trimmed to 1/8" fat, all grades, raw	58
Lamb	
Lamb, domestic, foreshank, separable lean and fat, trimmed to 1/4" fat, choice, cooked, braised	106
Lamb, domestic, foreshank, separable lean and fat, trimmed to 1/4" fat, choice, raw	72
Lamb, domestic, loin, separable lean only, trimmed to 1/4" fat, choice, cooked, broiled	95
Lamb, domestic, loin, separable lean only, trimmed to 1/4" fat, choice, raw	66
Veal	
Veal, leg (top round), separable lean and fat, cooked, braised	134
Veal, leg (top round), separable lean and fat, raw	78
Veal, loin, separable lean and fat, cooked, braised	118
Veal, loin, separable lean and fat, raw	79
Variety Meats	
Beef, variety meats and by-products, brain, cooked, pan-fried	1995
Beef, variety meats and by-products, brain, cooked, simmered	3100
Beef, variety meats and by-products, brain, raw	3010
Beef, variety meats and by-products, liver, cooked, braised	396
Beef, variety meats and by-products, liver, raw	275
Chicken, gizzard, all classes, cooked, simmered	370
Chicken, gizzard, all classes, raw	240
Chicken, liver, all classes, cooked, simmered	563
Chicken, liver, all classes, raw	345
Pork, fresh, variety meats and by-products, brain, cooked, braised	2552
Pork, fresh, variety meats and by-products, brain, raw	2195
Pork, fresh, variety meats and by-products, heart, cooked, braised	221
Pork, fresh, variety meats and by-products, heart, raw	131

Source: US Department of Agriculture, Agricultural Research Service. (2014). USDA National Nutrient Database for Standard Reference, Release 27; http://www.ars.usda.gov/ba/bhnrc/ndl.

of dairy products is less than 100 mg/100 g. Cholesterol in dairy products is accumulated for storage purposes in the form of cholesterol esters within the fat globules; therefore, more concentrated and more fatty dairy products have more cholesterol, for example, most milk products with approximately 4 mg/100 g, regular cream cheese with 110 mg/100 g, and fat-free cream cheese with 12 mg/100 g. Most cottage cheeses are low in cholesterol, around 4–10 mg/100 g. Similarly, egg products containing more yolk have much more cholesterol than those containing more egg white because majority of the lipid components of eggs are located in egg yolk. Whole chicken eggs have approximately 372 mg cholesterol/100 g, whereas cholesterol in most egg white powders is undetectable. Moreover, whole eggs from turkey or goose can have as much as 900 mg/100 g. Most cholesterol in eggs occurs in free-form, whereas much of cholesterol in dairy products is esterified.

Fish generally has similar or lower cholesterol content compared with meat and poultry, for example, raw and cooked tuna with 38–49 mg/100 g. Some fish such as salmon or trout can have up to 60 mg/100 g of raw meat and up to 100 mg/100 g of cooked meat. Shellfish and crustaceans have much greater cholesterol content than meat, poultry, and fish. Crustaceans such as shrimps, crabs, and lobsters have lower cholesterol content than mollusks, in the range of 50–70 mg/100 g of raw and 90–120 mg/100 g of cooked meat. Some canned and breaded shrimp products of mixed species can have up to 138–252 mg/100 g of meat. Squid has been reported to have the greatest cholesterol content among seafood, in the range of 200–300 mg/100 g for both raw and cooked products. Mollusks with shell such as oysters have similar cholesterol content to that of crustaceans (**Table 3**).

Processed meat products and other processed foods formulated with cholesterol-containing protein sources vary greatly in cholesterol content, from a few milligrams in 100 g of soups

Table 2 Cholesterol content of dairy foods and eggs

Dairy foods and eggs	*Cholesterol content, mg/100 g*
Butter, salted	215
Butter, whipped, with salt	219
Cheese, cottage, creamed, large or small curd	17
Cheese, cottage, low-fat, 1% milk fat	4
Cheese, cream	110
Cheese, cream, fat-free	12
Cheese, gouda	114
Cheese, low-fat, cheddar, or colby	21
Cheese, mozzarella, part skim milk	64
Cheese, mozzarella, whole milk	79
Cheese, pasteurized process, American, fortified with vitamin D	100
Egg, duck, whole, fresh, raw	884
Egg, goose, whole, fresh, raw	852
Egg, quail, whole, fresh, raw	844
Egg, turkey, whole, fresh, raw	933
Egg, white, raw, fresh	0
Egg, whole, raw, fresh	372
Egg, yolk, raw, fresh	1085
Milk, fluid, 1% fat, without added vitamin A and vitamin D	5
Milk, low-fat, fluid, 1% milk fat, with added vitamin A and vitamin D	5
Milk, nonfat, fluid, with added vitamin A and vitamin D (fat-free or skim)	2
Milk, nonfat, fluid, without added vitamin A and vitamin D (fat-free or skim)	2
Milk, reduced fat, fluid, 2% milk fat, with added nonfat milk solids, without added vitamin A	8
Milk, reduced fat, fluid, 2% milk fat, with added vitamin A and vitamin D	8
Milk, reduced fat, fluid, 2% milk fat, without added vitamin A and vitamin D	8
Milk, whole, 3.25% milk fat, with added vitamin D	10

Source: US Department of Agriculture, Agricultural Research Service. (2014). USDA National Nutrient Database for Standard Reference, Release 27; http://www.ars.usda.gov/ba/bhnrc/ndl.

Table 3 Cholesterol contents of seafood

Seafood	*Cholesterol content, mg/100 g*
Crustaceans, crab, Alaska king, cooked, moist heat	53
Crustaceans, crab, Alaska king, raw	42
Crustaceans, shrimp, mixed species, canned	252
Crustaceans, shrimp, mixed species, raw	126
Fish oil, cod liver	570
Fish, salmon, Atlantic, farmed, cooked, dry heat	63
Fish, salmon, Atlantic, farmed, raw	55
Fish, salmon, pink, canned, drained solids	83
Fish, seatrout, mixed species, cooked, dry heat	106
Fish, trout, rainbow, farmed, raw	59
Fish, tuna, fresh, bluefin, cooked, dry heat	49
Fish, tuna, fresh, bluefin, raw	38
Mollusks, cuttlefish, mixed species, raw	112
Mollusks, octopus, common, raw	48
Mollusks, oyster, eastern, cooked, breaded and fried	71
Mollusks, oyster, eastern, farmed, cooked, dry heat	38
Mollusks, oyster, eastern, farmed, raw	25
Mollusks, oyster, eastern, wild, cooked, moist heat	79
Mollusks, oyster, eastern, wild, raw	40
Mollusks, squid, mixed species, cooked, fried	260
Mollusks, squid, mixed species, raw	233

Source: US Department of Agriculture, Agricultural Research Service. (2014). USDA National Nutrient Database for Standard Reference, Release 27; http://www.ars.usda.gov/ba/bhnrc/ndl.

to more than 100 mg in 100 g of ready-to-eat sausages (Table 4). The variation is a direct result of diverse formulations, ingredients, cooking processes, storage conditions, and the oxidation of cholesterol during processing. Cholesterol content of meat, poultry, eggs, dairy products, and seafood provided by the SR and their labeled proportions can be used to calculate the cholesterol content of processed foods. Many restaurant foods are also available in the database. It is expected that restaurant foods containing large amounts of animal fat and ingredients such as chicken dark meat, bacon, and shrimp are abundant in cholesterol.

Dietary Recommendations

Despite the fact that cholesterol is a necessary physiological and structural component, the body synthesizes adequate levels of cholesterol to meet physiological needs. Evidence exists that in certain individuals, excessive consumption of dietary cholesterol may raise serum LDL cholesterol, thereby increasing the risk of cardiovascular disease. As such, in the *Guidelines for Americans 2010*, the US Department of Agriculture and the US Department of Health and Human Services recommend that Americans consume less than 300 mg of cholesterol per day to help maintain normal levels of serum cholesterol as well as a healthy ratio of HDL/LDL cholesterol.

Processing Effects

Effects on Cholesterol Content

Most value-adding processes cause little chemical effect on cholesterol content of foods. Cholesterol is primarily diluted or concentrated through food processing because of changes in protein, lipid, and moisture contents. Therefore, cholesterol-reducing strategies for foods normally involve protein and fat replacement by those from plant sources and the development of further processes that compensate for the loss of texture and flavor, such as extrusion or restructuring of protein, lipid hydrogenation, and flavor addition (natural or synthetic). It is also important to note that the replacement by plant sources will dramatically increase the content of phytosterols, many of which are estrogen analogs and have potentials to cause physiological effects on human. An important aspect of food processing that changes cholesterol concentration is the loss of moisture. Cooking or any heat application usually increases cholesterol content because moisture is lost, whereas cholesterol is retained. However, some cholesterol can be lost during

Table 4 Cholesterol content of processed foods

Processed foods	*Cholesterol content, mg/100 g*
Beef, bologna, reduced sodium	56
Blood sausage	120
Bockwurst, pork, veal, raw	93
Cooking oils, vegetable oils	0
Fast foods, biscuit, with egg and bacon	235
Fast foods, biscuit, with egg and ham	156
Fast foods, cheeseburger; double, large patty, with condiments and vegetables	55
Fast foods, cheeseburger; single, regular patty; plain	43
Fast foods, cookies, animal crackers	16
Fast foods, cookies, chocolate chip	21
Fast foods, croissant, with egg and cheese	170
Fast foods, croissant, with egg, cheese, and bacon	167
Fast foods, croissant, with egg, cheese, and ham	140
Fast foods, croissant, with egg, cheese, and sausage	123
Fast foods, English muffin, with egg, cheese, and Canadian bacon	168
Fast foods, hotdog, plain	45
Fast foods, onion rings, breaded and fried	0
Frankfurter, beef and pork	50
Frankfurter, chicken	96
Liver cheese, pork	174
Liver sausage, liverwurst, pork	158
Pork, oriental style, dehydrated	67
Salad dressing, Italian dressing, commercial, regular, without salt	67
Salad dressing, Italian dressing, reduced fat, without salt	6
Salad dressing, ranch dressing, commercial, regular	26
Salad dressing, ranch dressing, reduced fat	16
Salami, cooked, beef and pork	89
Salami, cooked, turkey	76
Sausage, chicken and beef, smoked	70
Sausage, turkey and pork, fresh, bulk, patty or link, cooked	84
Snacks, potato chips, cheese flavor	4
Snacks, potato chips, reduced fat	0
Soup, bean with bacon, condensed, single brand	3
Soup, bean with frankfurters, canned, condensed	9
Soup, bean with ham, canned, chunky, ready-to-serve	9
Soup, bean with pork, canned, condensed	2
Soup, beef noodle, canned, condensed	4
Soup, beef with vegetables and barley, canned, condensed, single brand	6
Soup, cheese, canned, condensed	3
Soup, chicken with star-shaped pasta, canned, condensed, single brand	4
Soup, cream of asparagus, canned, condensed	4
Soup, cream of celery, canned, condensed	11
Soup, cream of chicken, canned, condensed, single brand	7
Spices	0

Source: US Department of Agriculture, Agricultural Research Service. (2014). USDA National Nutrient Database for Standard Reference, Release 27; http://www.ars.usda.gov/ba/bhnrc/ndl.

cooking because of oxidation or fat drip. Oxidation occurs more easily to free cholesterol, for example, cholesterol in eggs or lean tissues, whereas cholesterol esters in fat droplet are more likely to be lost through cooking, for example, chicken skin or fat tissues. Many studies have documented an increase in cholesterol content in cooked meat and poultry compared with raw and have explained the phenomenon, at least in part, through the migration of cholesterol from adipose tissues or poultry skin to cooked lean tissues. The USDA Nutrient Data Laboratory, responsible for quality control of data published in the SR, routinely uses dry matter basis (based on solid content) to evaluate data. Occasionally, depending on cooking methods, some chicken products cooked with skin on showed an increase in solid percentage, more than what was expected through moisture loss, and an increase in dry matter-based concentration of cholesterol, which could only be explained by the migration of lipid from the skin, which has more cholesterol than cooked lean tissue. Interestingly, such a phenomenon was not observed in chicken cooked with skin side down or with added water because of either fat drip or water dilution, respectively.

Oxidation of Cholesterol

Cholesterol can be oxidized during cooking, heat applications such as extrusion or pasteurization, irradiation, and prolonged processes such as fermentation or storage. The oxidation of cholesterol has a similar mechanism to that of unsaturated fatty acids. With a double bond between C5 and C6, the single bonds C4–C5 and C6–C7 are most prone to the attack by free radicals from lipid oxidation and reactive oxygen species. Cholesterol esters, however, are more susceptible at C7. Cholesterol oxidation products (COPs), cholesterol oxides or oxysterols, have similar carbon backbone to that of cholesterol but possess more oxygen-containing functional groups such as hydroxyl, ketone, or hydroperoxide (**Figure 2**). Most of the added functional groups are located at C7 and sometimes at C5 and C6. Rarely does the oxidation happen on the side chain, such as at C25 (25-hydroxycholesterol). To initiate the oxidation, a free radical can subtract a hydrogen at the C7 next to the C5=C6 double bond, creating a free radical in the cholesterol structural rings. The migration of this free radical and subsequent formation of hydroperoxide, hydroxyl, or ketone derivative preferably occur at C7 rather than C4 because of the existing hydroxyl group at C3. Reactive oxygen species such as triplet oxygen may attack C5=C6 double bond in an addition mode to form 5,6-epoxycholesterol. This epoxy can be hydrated to form a very toxic triol (cholestane-3β,5α,6β-triol). The degradation of hydroperoxide at C7, however, forms hydroxyl radical and 7-alkoxyl radical, which becomes 7-hydroxycholesterol through hydrogen abstraction or 7-ketocholesterol through reaction with 7-peroxyl. In addition, further dehydration of cholesterol and intermediate products by prolonged heating can promote the formation of conjugated cholesta-3,5-dien, 7-ketocholesterol, cholesta-3, 5-dien-7-one, and cholesta-3, 5, 7-trien. Cholesterol 7-hydroperoxide and intermediate free radicals such as 7-alkoxyl, 7-peroxyl, or hydroxyl propagate new free radicals from other cholesterol or unsaturated fatty acid molecules to start the chain reaction (**Figure 3**). Factors influencing cholesterol oxidation are similar to those affecting fatty acid oxidation, such as pH and the availability of free radicals, reactive oxygen species, light, antioxidants, and metal catalysts. The physical state of cholesterol, which influences the exposure and the arrangement of the ring structure, the side chain, the double bond, and the hydroxyl group, is also very

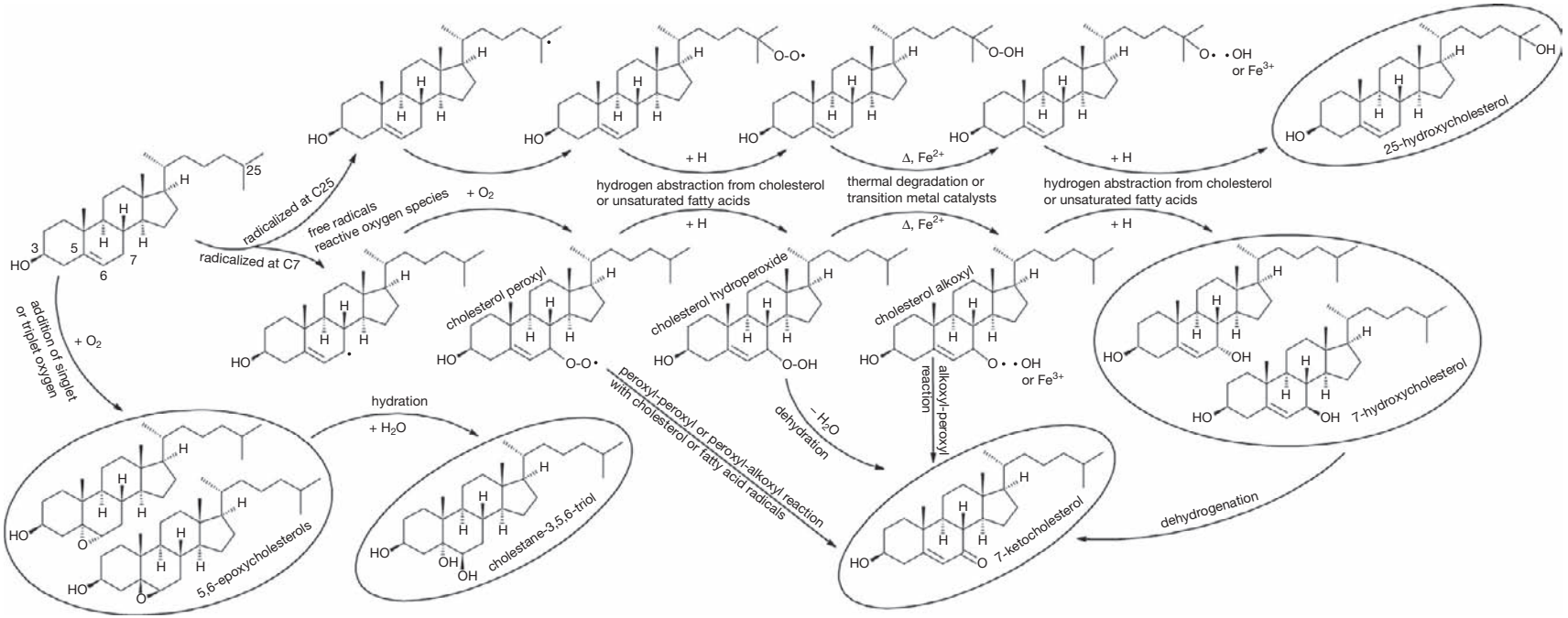

Figure 3 Primary pathways of cholesterol oxidation by free radicals. ACD/ChemSketch Freeware 2012, Advanced Chemistry Development, Inc., Ontario, Canada.

important to the oxidative degree and the production of oxysterols. Cholesterol esters are less susceptible to oxidation than free cholesterol, as evidenced by a much greater percentage of cholesterol lost in dried egg powder (primarily containing free cholesterol) than that in cooked separable fats (primarily containing cholesterol esters) found in the SR and various studies. In addition to heat applications, irradiation has been reported to accelerate the autoxidation of lipids including cholesterol. However, refrigeration, freezing, and vacuum packaging provide a protective effect, as expected. Moreover, it is important to recognize the photosensitized oxidation of cholesterol during retail display under fluorescent light. Muscle foods are especially susceptible because myoglobin, muscle pigment, is a photosensitizer and significantly increases the generation of the excited singlet oxygen. Singlet oxygen can be added directly to the double bond of cholesterol molecule. Initial products of photosensitized oxidation are primarily 5-hydroperoxycholesterol and 6-hydroperoxycholesterol, which differ from those generated through free radical-mediated pathways. As previously discussed, stable oxidation products are epoxycholesterols and cholestanetriol. Most commonly found oxysterols in foods are 7-OH, 7-keto, 5,6-epoxy, 3,5,6-triol, and the side-chain derivative 25-OH (Figures 2 and 3).

Cholesterol Oxides in Foods and Health Implications

Similar to cholesterol, cholesterol oxides are limited to foods of animal origin and seafood. Irradiation, direct heating, and processing methods with high heat or high degree of dehydration for a prolonged period have been reported to produce more oxysterols than the milder processing methods. The 7-hydroxycholesterol derivative was most commonly found in egg products at an average concentration of 0.2 to less than 50 ppm. Spray-dried egg yolk, however, was reported to have more than 100 ppm of cholesterol oxides, most were epoxycholesterol. More cholesterol oxide derivatives, as previously mentioned, have been found in meat and poultry products. Using beef tallow for prolonged deep-frying was reported to oxidize 25% cholesterol, most of which was converted to 7-hydroxycholesterol and conjugated dien and trien derivatives. Moreover, prolonged storage of dried pork for 3 years could oxidize more than 30% cholesterol, the products of which also included 5,6-epoxycholesterol. Cholesterol oxides have not been found in milk regardless of fat content even after prolonged heating. However, dehydration, fermentation, and prolonged storage seem to be the primary causes of cholesterol oxidation in dairy products. Examples of cholesterol oxides found skim milk powder are especially troubling, including up to 2.5 ppm of toxic triol and 25 ppm of 7-ketocholesterol. A level of up to 17 ppm of 5,6-epoxycholesterol, 7-hydroxycholesterol, and other side-chain oxidation derivatives has also been reported in butter and cheeses. Grated cheeses have much more cholesterol oxides, possibly because of greater surface area that promotes oxidation. Seafood has a low concentration of cholesterol oxides, approximately 5–9 ppm, which is primarily in products subject to smoking and prolonged storage. Dry-heat cooking methods seem to increase cholesterol oxides in fish more than moist-heat cookery.

The structures of cholesterol oxides resemble that of cholesterol; therefore, they can be delivered to various tissues in similar pathways and interfere with the biological functions of cholesterol. Cholesterol oxides have an increased polarity compared with cholesterol, which allows them to be absorbed more efficiently into the bloodstream. Macrophages also absorb LDL with oxidized cholesterol at a much greater rate. COPs have been reported to have greater atherogenic and cytotoxic effects than cholesterol. The atherogenic effects of cholesterol oxides were found in animal models at prolonged ingestion rates of 1–40 mg kg^{-1} body weight. Although most oxidation derivatives cause cytotoxicity in culture of various cell types, only 25-hydroxycholesterol and cholestanetriol have been reported to cause severe necrosis *in vivo*, for example, aortic muscles and endothelium of rabbits. Radioactive labeling indicates that VLDL and LDL, the preferred cholesterol transporter to human vascular cells, are also the preferred mode of transportation for cholesterol oxides. Therefore, oxidized VLDL and LDL are suspected to cause damage to the endothelial integrity and death of the vascular muscle cells. In addition, cholestanetriol, 7-ketocholesterol, and 25-hydroxycholesterol interfere with cholesterol synthesis, membrane formation and integrity, and cell growth of various tissues *in vitro* and *in vivo*. Recently, evidence indicates that cholesterol oxides, primarily epoxycholesterols, can also be mutagenic and even carcinogenic. They were reported to cause mutation in the lungs of hamsters and tumors in skin of mice. Some cholesterol oxides, for example, 7α-hydroxycholesterol, are physiological metabolites and have not been found to exhibit adverse effects on human health.

Quantification

Cholesterol and cholesterol esters are usually determined together as total cholesterol, although cholesterol esters are usually hydrolyzed to the free-form. Cholesterol must be extracted and separated from other interfering lipid compounds, especially fatty acids before it can be quantified. Although various means can be used to measure cholesterol, gas chromatography (GC) with flame ionization detection (FID) and high-pressure liquid chromatography (HPLC) with ultraviolet (UV) detection or mass spectrometry (MS) have become the predominant techniques. The majority of the recent cholesterol data in foods, however, have been generated by GC-FID, including those in the SR.

Extraction

Cholesterol is traditionally analyzed together with fatty acid composition; therefore, lipid extraction has been the first step of most cholesterol determination procedures found in the literature. The lipid extraction employs mixtures of midpolar and nonpolar solvents, among which the 2:1 (v/v) chloroform/methanol is most popular. The two most commonly used methods of this mixture were proposed by Folch and coworkers in 1957 and Bligh and Dyer in 1959, which have been modified by many others. Other solvent mixtures of hexane, diethyl ether, isopropanol, and butanol have also been examined; however, none comes close to the recovery of the chloroform/methanol mixture. The polarity of the extraction mixture is very important, especially in a two-step procedure (a polar solvent followed by a

nonpolar one), because cholesterol exists in a slightly polar free-form (with hydroxyl group) and a much less polar esterified form. Cholesterol, as a structural component of the cell membrane, is also bound by other polar compounds such as proteins and phospholipids. After chloroform/methanol extraction, the organic layer is separated from the aqueous layer. It is important to maintain a chloroform/methanol/water ratio of 8:4:3 to prevent the selective loss of lipid compounds into the aqueous phase, including cholesterol.

Following lipid extraction, lipids are saponified primarily using concentrated ethanolic or aqueous potassium hydroxide (KOH) solutions to liberate cholesterol from the esterified form with fatty acids. With cholesterol becoming more and more important because of its health implications, analysts have explored shortcuts to simplify the analytic procedures, the most important of which has been the use of direct saponification when cholesterol is the only lipid compound of interest. Direct saponification yields either similar or better recovery compared with the traditional two-step extraction/saponification procedure. In some cases of complex matrices, especially those with high emulsifying capability such as eggs or emulsion-type meat products, direct saponification is much more accurate and precise. Samples of various natures are directly hydrolyzed in either ethanolic or aqueous KOH solution, which is followed by an extraction of the unsaponifiable compounds by a nonpolar solvent, similar to that of lipid extraction. Toluene or a mixture of hexane and a miscible alcohol is most commonly used. Hexane is sometimes used alone; however, at least three extractions are needed to obtain the exhausted recovery and maintain the ratio of cholesterol and an internal standard. Toluene so far has been the best solvent for cholesterol extraction, accommodating a wide range of polarity with a single extraction. However, the use of toluene is prone to the formation of an emulsion, especially with the direct saponification because soap, an emulsification agent, is formed during saponification. Extraction using toluene, therefore, is followed by a careful cleanup process. A few cautions are needed for direct saponification technique such as the strength of the KOH solution, saponification time, and type of KOH solution (i.e., alcoholic or aqueous).

Derivatization

Cholesterol is usually converted to the suitable derivatives for various means of measurement. GC requires volatility and thermal stability, whereas liquid chromatography requires sensitivity and specificity, that is, enhanced UV absorption or more informative ion fragments if cholesterol is quantified by UV absorption or MS, respectively. Recently, cholesterol has been determined without derivatization because of dramatic improvements in columns and detectors.

Trimethylsilyl (TMS) ether is the most common cholesterol derivative. The ether provides great volatility and improves peak shape because the hydroxyl group is converted to a much less polar ether group, preventing the interaction with the polar sites of the GC columns. Among various derivatizing reagents and conditions, N,O-bis(TMS)trifluoroacetamide (BSTFA) in 1% trimethylchlorosilane is recommended because of the maximum ether conversion and the ability to produce hydrofluoric acid, which volatizes silicon dioxide and helps maintain the sensitivity of the FID detector. In addition, the use of butyrate and acetate cholesterol esters has been explored; however, the resultant conversion rates and peak shapes were compared less favorably than those of the TMS ether. The ether, on the other hand, is susceptible to moisture; therefore, moisture-free environment and moisture trap for the GC system are required.

Free cholesterol has been analyzed by HPLC-UV, HPLC-photodiode array (PDA), and HPLC-MS. Liquid chromatography provides great resolution between cholesterol and other interferences; however, the UV detection is much less sensitive than the FID. MS is typically used for definitive purposes rather than for routine analysis. Cholesterol, if not derivatized, does not yield informative mass fragments because it has only one functional group. Recently, the development of high-temperature, low-bleed GC columns with fused silica and bonded or cross-linked stationary phase has allowed for the determination of free cholesterol by GC-FID. The temperature program can be isothermal or gradient, depending on the interferences that need to be separated from cholesterol. Free cholesterol often interacts with silanol groups on the surface of the fused silica, which causes the tailing of the cholesterol peak. This phenomenon can affect the linearity of the calibration curve if a wide range of standard concentrations and a less polar internal standard such as cholestane are used. A column with a nonpolar stationary phase, for example, (5%-phenyl)-methylpolysiloxane, and a thick stationary film, that is, more than 0.25 μm and preferably 1 μm, can be used to improve peak shape and linearity. However, a nonpolar column may not provide the best resolution for a mixture of unsaponifiable compounds, the majority of which are slightly more polar than the saponified fatty acids. A thick stationary phase can increase column bleeding and hinder the sensitivity; therefore, high-temperature limits of the columns are important. The column technology nowadays seems to provide adequate thermal stability through cross-linked and bonded phases. Longer conditioning time during column installation can be helpful.

Separation

Chromatographic separation of cholesterol depends primarily on columns; and in the case of HPLC, it also depends on the separation mode, that is, normal-phase or reversed-phase. Cholesterol has been most commonly determined by GC; therefore, many GC columns are available. Packed columns have been mostly replaced by capillary columns because of much better resolution and reproducibility. Most capillary columns used in cholesterol analysis have typically 10 000–30 000 theoretical plates, providing a much better resolution than the 3000–5000 theoretical plates of the packed columns. Cholesterol is classified as a nonpolar compound with a slight polarity provided by one hydroxyl group; therefore, both nonpolar and polar stationary phases have been used. A nonpolar phase (100% dimethylpolysiloxane) such as HP-1, DB-1, SE-30, CP-Sil 5CB, or SPB-1; a slightly polar phase (5%-phenyl-methylpolysiloxane) such as HP-5, DB-5, SPB-5, RTX-5, or ZB-5; and a midpolar phase (50%-phenyl-methylpolysiloxane) such as DB-17, DB-1701, NB-17, or CP-Sil24CB are the most commonly used GC stationary phases for cholesterol determination. The low-polarity phase with 5% phenyl group has been used much more than other two

phases in analyzing both free cholesterol and derivatized cholesterol. Cholesterol derivatives have been separated on a thin-film column, that is, less than 0.25-μm film thickness, whereas a thicker film has been used for free cholesterol. A thickness of more than 0.5 μm will improve peak shape of free cholesterol as previously discussed but will significantly prolong the retention of cholesterol in the GC column, sometimes unnecessarily.

Most HPLC methods for cholesterol determination were not developed for routine analysis. The advantage of HPLC is that it can be used to separate cholesterol, cholesterol esters, triglycerides, diglycerides, tocopherols, and other sterols without derivatization, and sometimes in a single run. It has been reported that the GC does not provide an adequate separation of free cholesterol from phytosterols and tocopherols, whereas such a separation can be accomplished by manipulating the HPLC mobile phase polarity in both normal-phase and reversed-phase modes. Normal-phase HPLC mostly employs highly pure silica microparticles (5 μm) such as μPorasil, Inert-Sil, or Spherisorb and a 1–3% isopropanol/hexane mobile phase. Other polar columns of alcohol-bonded silica and cyanopropylsilica have also been used. Reversed-phase mode employing a nonpolar column offers better selectivity because it allows for more manipulation of mobile phase polarity. Most compounds coexisting with cholesterol in a post-preparation mixture are also retained better on a reversed-phase column. Reversed stationary phase commonly used for cholesterol determination consists of an octyl (C8) or octadecyl (C18) being bonded to a highly pure silica microparticle (5 μm). The C18 columns provide excellent retention and require mobile phases with lower polarity. Common organic modifiers for reversed-phase HPLC are acetonitrile, ethanol, methanol, and isopropanol. Recently, ultrahigh-pressure liquid chromatography (UPLC) has been used more for cholesterol determination because it uses much less solvent and decreases the retention time significantly to approximately 5 min. UPLC can also increase the resolution substantially through using submicron particle sizes.

Detection

Cholesterol derivatives have been quantified by FID or MS although the latter is primarily used for research or definitive purposes. A flame ionization detector provides great sensitivity and linearity over a wide range of concentrations. MS, however, is the method of choice when cholesterol is analyzed in a more complex mixture and expected to coelute with other unsaponifiable compounds, such as phytosterols or tocopherols. MS is better used for cholesterol derivatives than free cholesterol because cholesterol derivatives respond better to the ionization and produce more informative ion fragments for identification and quantification. Free cholesterol, like many sterols with very few functional groups, does not respond well to atmospheric pressure ionization such as electrospray; therefore, electron impact ionization in a vacuum chamber is usually employed. The detection limit for FID and MS is in the range 1 ng on-column. The MS can be more sensitive, depending on the ion-monitoring mode.

In addition, cholesterol and cholesterol derivatives have been successfully determined by UV, fluorescence (FD), evaporative light-scattering, and electrochemical (ECD) detections, which are usually coupled with an HPLC system. UV detection is the most commonly used technique, which is probably a matter of convenience more than technical considerations because of the issues with specificity or sensitivity. Cholesterol has been detected in the range of 202–210 nm with the maximum absorption at 205 nm. A simple scan of spiked cholesterol standard may be needed to confirm λ_{max} in a specific solution. A PDA detector improves specificity of UV detection because of multiwavelength monitoring. Fluorescence-generating or fluorescence-tagging reagents such as 3,4-dihydro-6,7-dimethoxy-4-methyl-3-oxoquinoxaline-2-carbonyl azide and naproxen acyl chloride can be used to convert cholesterol and other sterols to fluorescing derivatives, which will substantially increase specificity and sensitivity. The limit of detection (LOD) for the FD is within few picograms on-column. UV absorption can be enhanced to a LOD of 0.1 ng at a much more favorable λ_{max} of 250 nm by converting cholesterol to cholest-4-en-3,6-dione in the Jones oxidation reactions. Such a LOD is very unusual for UV detection. Electrochemically active derivatives of cholesterol such as carbamate ester can be quantified by ECD at very sensitive levels of picograms on-column. Cholesterol can also be determined on the ECD without precolumn derivatization in an oxidation mode, however, with much less sensitivity at nanogram levels. An evaporative light-scattering detector is mostly suitable for cholesterol determination in simple biological fluids and is a generic technique, having a similar LOD but lower precision compared with those of UV detection. Electrochemical and fluorescence detectors are most specific and sensitive for the HPLC technique but may require cumbersome precolumn preparation.

Determination of COPs

Similar to the determination of cholesterol, that of COPs follows a series of extraction (direct or postextraction saponification), derivatization, separation, and detection processes. The separation and detection can be achieved by both GC and HPLC with various detection techniques, also similar to those used for cholesterol. Cholesterol oxides, however, normally occur at very low concentrations, approximately 1% of cholesterol content, and in various forms, which makes the extraction process extremely important. Direct saponification has been recommended to separate COPs from the interferences because it eliminates selective losses of COPs during lipid extraction. Hot alkali conditions, however, can generate more cholesterol oxides or alter the ketone derivatives. Some researchers have suggested that the selection of saponification temperature depends on the COPs of interest and the nature of food matrices and that the cold saponification may be more suitable for the ketone derivatives. Antioxidants such as butylated hydroxytoluene can be added to prevent further oxidation of COPs. Preparative chromatography has been used to isolate COPs directly from lipid extract or to remove cholesterol from the unsaponifiable materials and, in some cases, is the most important step to improve selectivity and sensitivity. Cholesterol oxides, similar to cholesterol, can be determined in the free-form or derivatized form. However, COPs are much more susceptible to further degradation. Therefore, the GC determination usually requires derivatization, primarily to TMS ethers, to improve the thermal

stability. Although GC, coupled with FID or MS, offers relatively quick and sufficient separation of major COPs, the use of temperature programs hinders the ability of this technique to analyze thermally sensitive COPs. As a result, HPLC is preferred for COP determination. Most COPs are more polar than cholesterol; therefore, normal-phase HPLC has been used more than reversed-phase HPLC. However, reversed-phase HPLC methods, especially those using C18 columns, are becoming more and more popular for the same reason previously discussed. Although the use of a UV detector has been the detection method of choice, some COPs do not possess UV chromophores. In addition, a large number of COPs occur in foods, similar to the case of flavor volatiles, which makes an absolute separation and identification almost impossible. Hence, HPLC-MS will probably be the primary technique to study COPs in foods in the future because MS is a much more specific technique and some COPs have been suggested to contribute to Alzheimer's disease, Parkinson's disease, multiple sclerosis, and inflammation. Recently, the isotope dilution MS has also been employed to study the roles of COPs in various pathological pathways of these diseases.

See also: Adipose Tissue: Structure and Function of Brown Adipose Tissue; Adipose Tissue: White Adipose Tissue Structure and Function; Beef; Cholesterol: Absorption, Function and Metabolism; Cholesterol: Factors Determining Blood Cholesterol Levels; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Dairy Products: Dietary and Medical Importance; Fats: Classification and Analysis; Fatty Acids: Determination and Requirements; Fermented Foods: Fermented Meat Products; Fish: Dietary Importance and Health Effects; Fish: Fish in the Human Diet; Lipoproteins; Meat: Role in the Diet; Meat: Structure; Milk: Role in the Diet; Milk: Sources and Composition; Phospholipids: Physiology; Phospholipids: Properties and Occurrence; Phytic Acid: Properties, Uses, and Determination; Pickling; Pine Kernels; Pineapple; Pituitary Gland: Pituitary Hormones; Plums and Related Fruits; Polycyclic Aromatic Hydrocarbons; Pork Meat Quality, Production and Processing on; Poultry: Processing; Shellfish: Role in the diet.

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Choline: Physiology

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Metabolism of Choline

There have been several comprehensive reviews of the metabolism and functions of choline that describe its role in the synthesis of the phospholipids in cell membranes, methyl metabolism, cholinergic neurotransmission, transmembrane signaling, and lipid–cholesterol transport and metabolism. Choline can be acetylated, phosphorylated, or oxidized.

Choline, methionine, and folate metabolisms interact at the point that homocysteine is converted to methionine (Figure 1). Betaine–homocysteine methyltransferase catalyzes the methylation of homocysteine using the choline metabolite betaine as the methyl donor. Elevated plasma homocysteine is an independent risk factor for cardiovascular disease and stroke in humans. Treatment with betaine or choline also lowers elevated plasma homocysteine in humans. In an alternative pathway, 5-methyltetrahydrofolate–homocysteine methyltransferase regenerates methionine. In addition, tetrahydrofolate is needed to scavenge one-carbon groups when betaine is metabolized. Perturbing metabolism of one of the methyl donors results in compensatory changes in the other methyl donors, owing to the intermingling of these metabolic pathways. Rats ingesting a choline-deficient diet have diminished tissue concentrations of methionine and *S*-adenosylmethionine, doubled plasma homocysteine concentrations, and diminished hepatic methylfolate content. These effects are reversible by refeeding choline. Rats fed with diets deficient in both methionine and choline for 5 weeks have hepatic folate concentrations that are half of those present in controls. Rats treated with the antifolate, methotrexate, have diminished pools of choline metabolites in the liver. Severe folate deficiency induced in rats, by feeding an amino acid-defined diet containing no folate and succinylsulfathiazole for 4 weeks, has resulted in hepatic choline and phosphocholine concentrations that were 65% and 80% lower, respectively, than in controls.

Dietary Requirements

Though there is a pathway (in all tissues, but most active in the liver) for the *de novo* biosynthesis of the choline moiety via the sequential methylation of phosphatidylethanolamine using *S*-adenosylmethionine as the methyl donor (Figure 1), only some of the demand for choline can be met by using methyl groups derived from one-carbon metabolism. Animals and humans fed with a choline-deficient diet deplete choline stores and develop liver dysfunction. Healthy male humans with normal folate and vitamin B_{12} statuses fed with a choline-deficient diet have diminished plasma choline and phosphatidylcholine concentrations and develop liver damage (elevated plasma alanine aminotransferase). Liver cell death occurs in choline deficiency, because hepatocytes initiate programmed cell death (apoptosis) when deprived of choline. Because methyl supplementation with betaine, methionine, folate, or vitamin B_{12} does not prevent apoptotic death induced by choline deficiency in hepatocytes, it must be that depletion of intracellular choline moieties, rather than depletion of methyl groups, is the critical parameter involved in the induction of apoptosis. Some humans (male and female) fed with total parenteral nutrition solutions devoid of choline, but adequate in methionine and folate, develop fatty liver and liver damage that resolve when a source of dietary choline is provided. Fatty liver occurs because choline is required to make the phosphatidylcholine portion of the very-low-density lipoprotein particle. Animals fed with a choline-deficient diet may also develop growth retardation, renal dysfunction and hemorrhage, or bone abnormalities. A metabolite of choline, betaine, is used in the kidney glomerulus as an osmolyte, and for this reason, choline-deficient animals have trouble excreting concentrated urine.

Human studies of choline requirements in women, children, or infants have not been conducted. Thus, we do not know whether choline is needed in the diet of these groups. Female rats are less sensitive to choline deficiency than are male rats perhaps because estrogen enhances females' capacity to form the choline moiety *de novo* from *S*-adenosylmethionine. Pregnancy may be a time when dietary supplies of choline are especially limiting. Though female rats are resistant to choline deficiency, pregnant rats are as vulnerable to deficiency as males are. During pregnancy, large amounts of choline are delivered to the fetus across the placenta, and this depletes maternal stores of the various forms of this nutrient. At birth, humans and other mammals have plasma choline concentrations that are much higher than those in adults. Also, the need for choline is increased during lactation, because so much must be secreted into milk; lactating rats are more sensitive to choline deficiency than are nonlactating rats.

The Institute of Medicine (IOM) recently made recommendations for choline intake in the diet. There were insufficient data with which to derive an estimated average requirement for choline, and so, only an adequate intake can be estimated. The IOM report cautioned, "this amount will be influenced by the availability of methionine and methyl-folate in the diet. It may be influenced by gender, and it may be influenced by pregnancy, lactation, and stage of development. Although AIs are set for choline, it may be that the choline requirement can be met by endogenous synthesis at some of these stages." The IOM recommendations are shown in Table 1.

The recent report by Jacob that folate deficiency in humans exacerbates choline deficiency highlights why studies of choline–folate–homocysteine interrelationships in humans are important for the future refinement of these recommendations.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00155-0

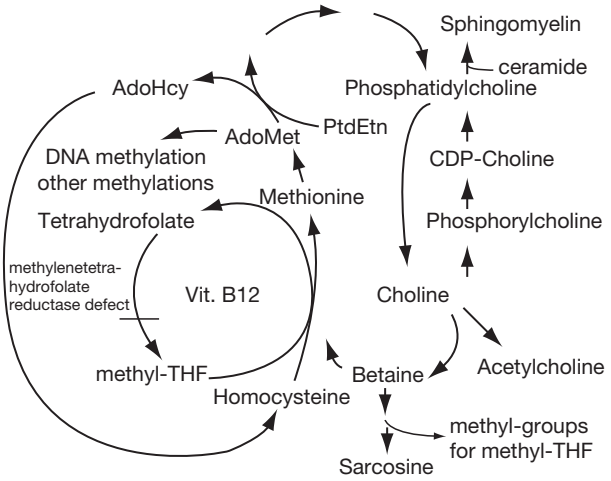

Figure 1 Choline, folate, and homocysteine metabolisms are closely interrelated. The pathways for the metabolism of these three nutrients intersect at the formation of methionine from homocysteine. PtdEtn, phosphatidylethanolamine; AdoHcy, *S*-adenosylhomocysteine; and AdoMet, *S*-adenosylmethionine.

Table 1 Institute of Medicine recommendations for choline intake in the diet

AI for infants	0–6 months	125 mg per day, 18 mg kg^{-1}
	6–12 months	150 mg per day
AI for children	1–3 years	200 mg per day
	4–8 years	250 mg per day
	9–13 years	375 mg per day
AI for males	14–18 years	550 mg per day
	19 years and older	550 mg per day
AI for females	14–18 years	400 mg per day
	19 years and older	425 mg per day
AI for pregnant women	All ages	450 mg per day
AI for lactating women	All ages	550 mg per day

AI, adequate intake level.

The plasma choline concentration varies in response to diet and can rise as much as twofold after a two-egg meal. Fasting plasma choline concentrations vary from 7 to 20 μM, with most subjects having concentrations of 10 μM. Individuals that have starved for up to 7 days have diminished plasma choline, but levels never drop below 50% of the normal. The plasma phosphatidylcholine concentration also decreases in choline deficiency, but these values are also influenced by factors that change plasma lipoprotein levels. The fasting plasma phosphatidylcholine concentrations are ~1–1.5 mM.

Choline and Brain Development

Choline availability during embryogenesis and perinatal development may be especially important. There is a sensitive period in rodent brain development during which treatment with choline (about three times the dietary intake) produces long-lasting enhancement of spatial memory that is lifelong. This period occurs during embryonic days 12–17 in the rat (rats give birth on day 21; mice give birth one day earlier and probably have a slightly different period of susceptibility). Choline supplementation during these critical days elicits a major improvement in memory performance at all stages of training on a 12-arm radial maze. Choline deficiency during the critical time frame reduces memory performance. The choline-induced spatial memory facilitation correlates with changes in the birth, death, and migration of cells in the hippocampus during fetal brain development, with altered distribution and morphology of neurons, biochemical changes, and electrophysiological changes in the hippocampus. Human and rat brains mature at different rates; the rat brain is comparatively more mature at birth than the human brain, but in humans, hippocampal development may continue for months or years after birth.

Choline and Cancer

Dietary deficiency of choline in rodents causes the development of hepatocarcinomas in the absence of any known carcinogen. Choline is the only single nutrient for which this is true. It is interesting that choline-deficient rats not only have a higher incidence of spontaneous hepatocarcinoma but also are markedly sensitized to the effects of administered carcinogens. Several mechanisms are suggested for the cancer-promoting effect of a choline-devoid diet. A progressive increase in cell proliferation that is related to regeneration after parenchymal cell death occurs in the choline-deficient liver. Cell proliferation and its associated increased rate of DNA synthesis could be the cause of the heightened sensitivity to chemical carcinogens. Methylation of DNA is essential to the regulation of expression of genetic information, and the undermethylation of DNA observed during choline deficiency (despite adequate dietary methionine) may be responsible for carcinogenesis. Choline-deficient rats experience increased lipid peroxidation in the liver. Lipid peroxides in the nucleus are a possible source of free radicals that could modify DNA and cause carcinogenesis. Choline deficiency activates protein kinase C signaling, usually involved in growth factor signaling in hepatocytes. Finally, a defect in cell suicide (apoptosis) mechanisms may contribute to the carcinogenesis of choline deficiency.

Summary

Choline in the diet is important for many reasons. As our understanding of the importance of folate and homocysteine nutrition increases, there should be increased interest in how choline interacts with folate and homocysteine metabolisms. Recent findings about choline in brain development should stimulate comparable studies in humans. During the next few years, it is likely that food composition data will be available for choline, and this will make it possible to examine interactions between choline, folate, and methionine when considering epidemiological data. In addition, we should learn more about choline requirements in women. For these reasons, interest in choline as a nutrient for humans should be sustained.

See also: Cancer: Diet in Cancer Prevention; Phospholipids: Properties and Occurrence; Riboflavin: Properties and Determination; Vitamins: Overview.

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Choline: Properties and Determination

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Introduction

Choline is an essential nutrient that is required for normal cell function, with involvement in lipid synthesis and transport, cellular methylation reactions, neural tube development, stem cell proliferation and apoptosis, and cholinergic neurotransmission. Choline is a precursor for phosphatidylcholine, which comprises over half of the phospholipid content in mammalian cell membranes, and is critical in the transport of excess triglycerides from the liver. As a result, choline deficiency has been associated with the development of hepatosteatosis, or fatty liver, and is the only nutritional deficiency found to cause cancer in the absence of known carcinogens. One important cellular methylation reaction involving choline is in the biosynthesis of methionine, one pathway for which in mammals involves the choline metabolite betaine. The alternative pathway involves folate metabolism, linking the intakes of these nutrients through a sensitive metabolic equilibrium. This shared pathway, through methyl group donation, is believed to be responsible for neural tube closure *in utero*, explaining a fourfold increase in risk for neural tube defects in the children of pregnant women with choline deficiency. Rodent studies have similarly indicated that choline supplementation during pregnancy altered the brain structure and long-term potentiation of offspring through increased stem cell proliferation and decreased stem cell apoptosis; the reverse was observed for mothers with choline deficiency. Choline is also the precursor for acetylcholine, one of the most common neurotransmitters responsible for influencing the function of the brain, heart, and numerous other organs and organ systems (Figure 1).

Estimation of Choline Intake

Choline is produced in the body as a by-product of the biosynthesis of phosphatidylcholine. In this process, *S*-adenosylmethionine is converted to phosphatidylcholine by the enzyme phosphatidylethanolamine-*N*-methyltransferase, and a new choline molecule is formed. The production of choline via this pathway, however, is not sufficient to support proper function, and thus, dietary supplementation of choline is necessary. The one exception may be in women of childbearing age; high levels of circulating estrogen may be linked to upregulated biosynthesis of choline. Adequate intakes (AIs) for choline have been established by the US Institute of Medicine at 425 mg day^{-1} for women and 550 mg day^{-1} for men. The AI increases to 450 or 550 mg day^{-1} for women who are pregnant or lactating, respectively. In a monitored dietary intake study, the average daily choline consumption was determined to be 630 mg day^{-1} for men and 440 mg day^{-1} for women, levels comparable with the AIs. Tolerable upper limits were determined as the lowest level causing adverse effects (hypotension) in humans to be 1000 mg day^{-1} in children 1–8 years of age, 2000 mg day^{-1} from 9 to 13 years of age, 3000 mg day^{-1} from 14 to 18 years of age, and 3500 mg day^{-1} over age 19. In addition to hypotension, other adverse effects from high choline intake include fishy body odor (from triethylamine production) and cholinergic side effects such as sweating, vomiting, and diarrhea.

Choline intakes can be attributed to a number of choline-related compounds, including free choline and betaine, and a number of esterified forms such as phosphocholine (PCho), phosphatidylcholine (PtdCho), glycerophosphocholine (GPCho), and sphingomyelin (SM) (Figure 2). Choline is a small quaternary amine containing two carbons and a hydroxyl group. Betaine, also known as glycine betaine or trimethylglycine, is formed from choline by oxidation of the alcohol group to a carboxylic acid. Because the oxidation of choline to betaine is irreversible, however, the intake of betaine is not typically included in the considerations for choline intake. In the phosphorylated forms of choline, the hydroxyl group is replaced with a phosphate (PCho and SM) or glycerophosphate group (GPCho and PtdCho). In the polar PCho and GPCho molecules, the phosphate and glycerophosphate groups are the respective molecular termini. In PtdCho, the phosphate group joins the choline molecule to a glycerol moiety esterified with two fatty acids, forming a phospholipid. A large number of structures make up the group of phospholipids classified as PtdCho, with fatty acids that vary from 16 to 22 carbons with varying degrees of saturation. In SM, the phosphate group connects the choline molecule with a ceramide, which contains a sphingosine (an unsaturated 18-carbon amino alcohol) and a fatty acid with 16–24 carbons and up to one degree of saturation. These structural differences result in a group of compounds with significantly different chemical characteristics.

In 2004, the US Department of Agriculture (USDA) released a database outlining the choline content of 630 common food items, and a selection of the entries from this database have been summarized in Table 1 to represent foods high in choline from a variety of categories. The USDA database includes individual values for betaine, free choline, PCho, PtdCho, GPCho, and SM and a summed total choline content determined in each food product. Foods highest in total choline content include egg yolks, liver (from beef, veal, chicken, and turkey), and wheat germ. The total choline content, however, is a mathematical sum of free choline, PCho, PtdCho, GPCho, and SM and may not represent the bioavailable choline content upon consumption. Betaine content is not included in the calculation of total choline because, as mentioned previously, the conversion from choline to betaine is irreversible, and therefore, the consumption of betaine does not directly translate to choline intake. Studies indicate that in a rat model, the bioavailabilities of choline, PCho, and GPCho are similar, but studies have not been conducted to translate these findings to human subjects; no data are available to elucidate the

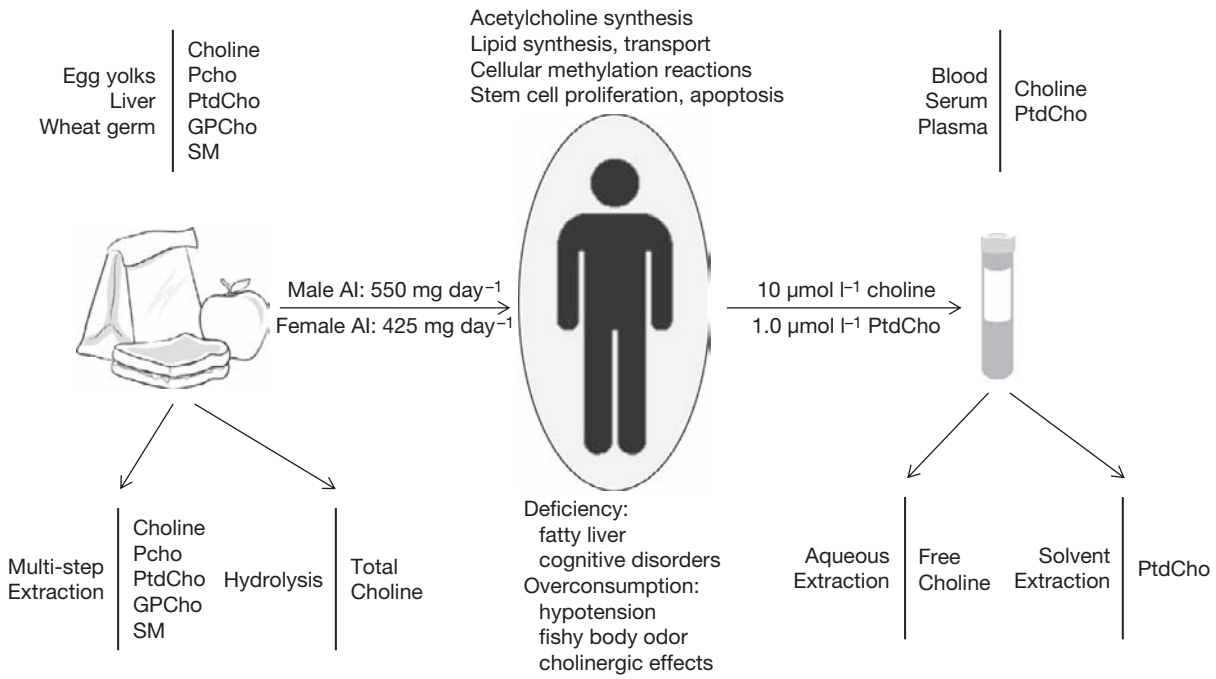

Figure 1 The interrelationship and analytical determination of choline-containing compounds in dietary intake, metabolism, and health status.

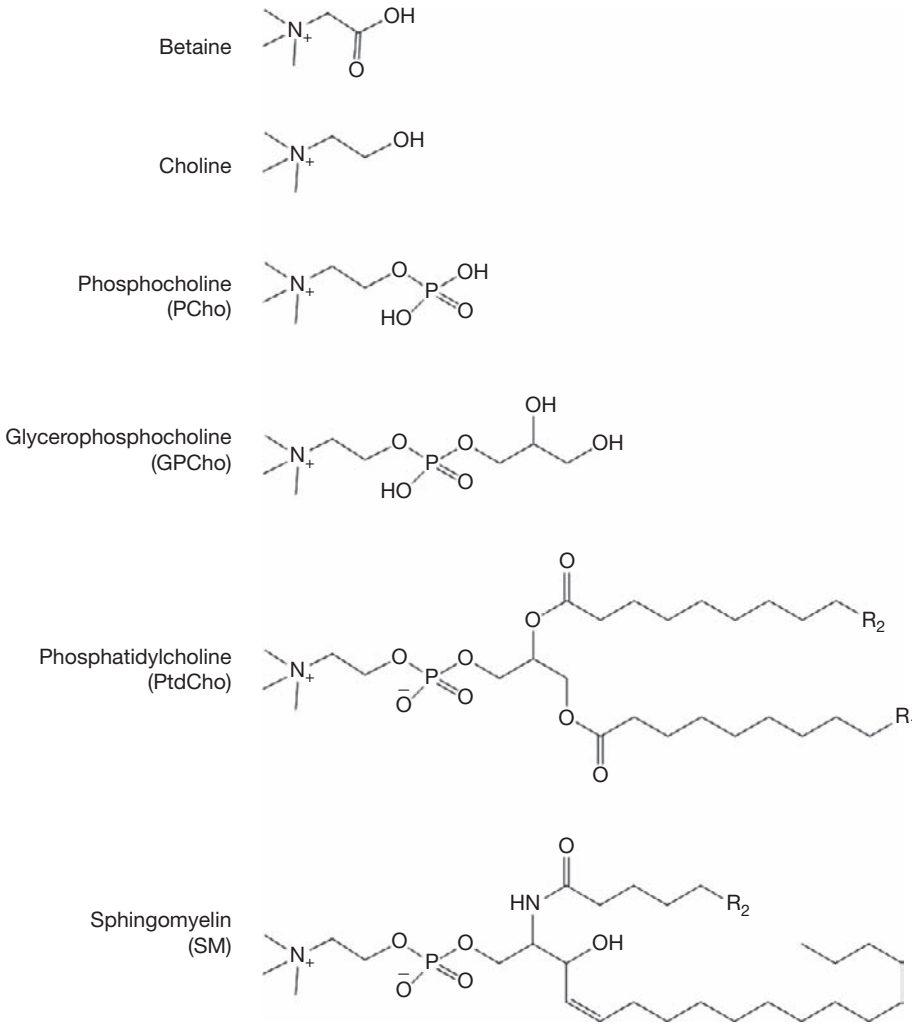

Figure 2 Chemical structures of choline-related compounds.

Table 1 Foods high in choline content

Food description	*Category*	*Free choline (mg/100 g food)*	*Total choline*[a] *(mg/100 g food)*	*Major form*
Egg, yolk, raw, fresh	Dairy, eggs	1.3	682.4	PtdCho
Beef, variety meats and by-products, liver, cooked, braised	Beef	61.8	426.1	PtdCho
Beef, variety meats and by-products, liver, cooked, pan-fried	Beef	56.7	418.3	PtdCho
Veal, variety meats and by-products, liver, cooked, pan-fried	Lamb, veal, game	92.9	411.0	PtdCho
Veal, variety meats and by-products, liver, cooked, braised	Lamb, veal, game	88.6	398.8	PtdCho
Beef, variety meats and by-products, liver, raw	Beef	56.2	333.2	PtdCho
Veal, variety meats and by-products, liver, raw	Lamb, veal, game	85.3	309.9	PtdCho
Chicken, liver, all classes, cooked, pan-fried	Chicken, turkey	69.1	308.5	PtdCho
Chicken, liver, all classes, cooked, simmered	Chicken, turkey	47.9	290.1	PtdCho
Egg, whole, cooked, fried	Dairy, eggs	0.7	272.6	PtdCho
Egg, whole, raw, fresh	Dairy, eggs	0.6	251.0	PtdCho
Egg, whole, cooked, hard-boiled	Dairy, eggs	0.6	225.2	PtdCho
Turkey, liver, all classes, raw	Chicken, turkey	63.8	221.9	PtdCho
Turkey, liver, all classes, cooked, simmered	Chicken, turkey	9.7	220.2	PtdCho
Chicken, liver, all classes, raw	Chicken, turkey	49.2	194.5	PtdCho
Turkey, heart, all classes, cooked, simmered	Chicken, turkey	3.9	172.5	PtdCho
Cereals ready-to-eat, wheat germ, toasted, plain	Breakfast cereals	69.2	152.1	Free choline
Pork, cured, bacon, cooked, pan-fried	Pork	12.3	130.8	PtdCho
Cake, chocolate, dry mix, regular, prepared without frosting	Baked products	5.4	128.4	PtdCho
Turkey, heart, all classes, raw	Chicken, turkey	24.6	126.8	PtdCho
Pork, cured, bacon, cooked, microwaved	Pork	11.6	124.7	PtdCho
Spices, mustard seed, yellow	Spices, herbs	46.2	122.6	PtdCho
Pork, cured, bacon, cooked, baked	Pork	12.3	119.3	PtdCho
Pork, fresh, loin, center loin (chops), bone-in, separable lean only, cooked, broiled	Pork	2.2	102.8	PtdCho
Coffee, instant, decaffeinated, powder	Beverages	93.7	101.9	Free choline
Mutton, roasted from mutton sandwich	Lamb, veal, game	1.7	100.6	PtdCho
Beans, navy, mature seeds, raw	Legumes	49.9	87.4	Free choline
Fish, cod, Atlantic, cooked, dry heat	Finfish, shellfish	17.7	83.7	PtdCho
Noodles, egg, dry, enriched	Cereal grains, pastas	50.4	78.7	Free choline
Nuts, pistachio nuts, dry roasted, with salt added	Nuts, seeds	10.7	71.5	PtdCho
Candies, milk chocolate	Sugars, sweets	9.1	46.1	GPCho
Salad dressing, mayonnaise, soybean oil, with salt	Fats, oils	0.2	46.0	PtdCho
Brussels sprouts, cooked, boiled, drained, without salt	Vegetables	23.4	40.1	Free choline
Orange juice, frozen concentrate, unsweetened, undiluted	Fruits	8.1	19.9	Free choline

[a]Total choline includes the sum of free choline, glycerophosphocholine (GPCho), phosphocholine (PCho), phosphatidylcholine (PtdCho), and sphingomyelin (SM).

Source: Howe, J. C., Williams, J. R., Holden, J. M., Zeisel, S. H. and Mar, M.-H. (2004). *USDA database for the choline content of common foods*. Beltsville, MD: US Department of Agriculture.

differences in the human absorption of the various forms of choline. The water- and lipid-soluble forms of choline are known to have different absorption mechanisms in the human body, which translates to differences in both the method and rate of distribution to tissues. It is also interesting to note that in animal products (meats, dairy, and eggs), the major choline-containing compound identified is PtdCho, while in plant products (fruits, vegetables, and grains), PtdCho is not the major choline compound. In addition to endogenous choline and choline-containing compounds, choline can be added to foods and supplements as choline chloride, choline bitartrate, or lecithin. The PtdCho content of lecithin, a natural emulsifier of plant or animal origin, is roughly 25% by mass, corresponding to approximately 3–4% choline by mass.

In order to establish whether AIs are met, accurate and precise analytical methods must be utilized for determination

of choline and choline-related compounds in foods and food products. In general, two approaches have been taken in the characterization of foods for total choline content: extraction-based methods in which free choline and each choline ester are determined independently and hydrolysis-based methods in which all choline esters are hydrolyzed to free choline and the total free choline content is subsequently determined. The extraction approach, while complicated and time-consuming, is valuable because the concentration of each choline-related compound can be determined independently, and therefore, the bioavailability can be considered with respect to evaluation of total choline intake. This approach requires multiple steps to isolate the hydrophilic (free choline, betaine, PCho, and GPCho) and hydrophobic (PtdCho and SM) components and separate quantitative analyses of the two groups. Conversely, hydrolysis approaches utilize acid, base, and/or enzymes to cleave the esters from the choline moiety, allowing a single analysis to be performed to determine the total concentration of hydrolyzed free choline. The hydrolysis approach is appealing because the overall methodology is often more simple and rapid, but unfortunately, the efficacy of the chemical hydrolysis of the choline esters does not reflect the bioavailability of the various chemical forms.

Once choline intakes are established, appropriate methods are needed to assess the choline status in the population through measurement of choline or choline biomarkers in plasma and/or serum. Experts believe that choline health status is most accurately reflected in the concentration of choline and/or PtdCho in plasma. Fasting choline concentrations in human plasma have been reported in the range of 7–20 $\mu mol\,l^{-1}$, with a median concentration of 10 $\mu mol\,l^{-1}$. Concentrations of PtdCho in human plasma have been reported in the range of 1.0–1.5 $\mu mol\,l^{-1}$, representing mostly the PtdCho that constitutes plasma lipoproteins. The methods for determination of choline and PtdCho in plasma and serum face the same challenges as those for determination in foods, as the extraction and sample cleanup steps are different for the hydrophilic choline and hydrophobic PtdCho. In determination of biomarkers, however, the concentrations of choline and PtdCho are both measured; hydrolysis-based approaches for determination of total choline content are not routinely utilized.

Extraction-Based Approaches for Total Choline Determination in Foods

Extraction-based approaches that separately quantify individual choline-containing compounds in foods and food products are desirable from a nutritional standpoint, as the bioavailabilities of the various choline esters may not be equivalent, leading to misrepresentation of total choline from a simple summation. These approaches typically include both aqueous and organic extraction steps (either simultaneously or in parallel) followed by direct analysis to determine the concentration of each choline-containing compound in the sample. Most approaches rely on a classic liquid–liquid extraction approach in which the sample is diluted to a level of approximately 80% water and combined 1:3 with a mixture of chloroform and methanol (2:1). After homogenizing, an additional one part chloroform is added and one part water in distinct steps separated by additional mixing. The mixture is then allowed to settle; the upper water/methanol layer contains the hydrophilic components, including free choline, betaine, PCho, and GPCho, while the lower chloroform layer contains the hydrophobic PtdCho and SM. This method has been demonstrated to remove 94% of the lipid content into the chloroform layer, with no significant cross extraction between the two layers. Reprocessing of any remaining solid material with an additional aliquot of chloroform has been found to remove the remaining lipid-soluble components, and by combination of the separate chloroform layers, quantitative extraction can be achieved. Other approaches for extraction of choline-containing compounds from food and food products have been described using a parallel approach in which the sample is split, hydrophilic compounds are isolated using dilute acid, and hydrophobic compounds are isolated using a combination of chloroform and methanol.

Once choline-containing compounds have been isolated from a food or food product, they must be isolated from one another for accurate quantitation. Some approaches separate the various compounds using preparative techniques such as liquid chromatography (LC) and thin-layer chromatography (TLC); isolated compounds are then hydrolyzed individually to release choline, and the free choline is measured by a technique such as gas chromatography-mass spectrometry (GC-MS). Performing separate analyses for each compound is time- and resource-intensive, however, which has led to improvements in the analytical approach. Newer methods have converted the preparatory approach described in the preceding text into the overall analytical approach, removing the need for compound collection and separate analysis. In these approaches, both the aqueous and organic extracts can be analyzed using the same LC approach with different mobile phase compositions, leading to a fivefold reduction in the analysis time. This approach has been coupled with mass spectrometry (MS) using stable isotope-labeled internal standards to assign the values reported in the USDA database (Table 1). In addition, the use of an isotope dilution approach to quantitation greatly improves the accuracy and precision of an LC-MS method by reducing errors from sample handling and variability in sample injection and ionization within the mass spectrometer.

Improvements in column technology and better understanding of fundamental chromatographic processes have also led to improvements in the simultaneous determination of choline-containing compounds. Utilization of hydrophilic interaction liquid chromatography (HILIC) may be the future of total choline analysis, as both hydrophilic and hydrophobic components can be analyzed simultaneously, again reducing the analysis time by a factor of 2. Coupled with isotope dilution-tandem mass spectrometry (ID-MS/MS), this type of analysis can even provide information about the distribution of various fatty acid moieties of the choline esters PtdCho and SM. Although still in its infancy, this approach may offer the full suite of information needed to truly understand choline intake from foods in a more straightforward and high-throughput technique.

Hydrolysis-Based Approaches for Total Choline Determination in Foods

Despite concerns about equivalence in bioavailability of different choline-containing compounds in foodstuffs, a majority of

historical approaches for determination of total choline rely on hydrolysis by acid, base, and/or enzymes, which removes all information about the distribution of choline esters. The benefit of hydrolysis, although the sample preparation can be labor- and time-intensive, is that the subsequent determination of the total liberated choline becomes significantly more straightforward than the analysis of individual choline esters.

Classically, colorimetric approaches have been widely used for choline determination. Following the release of choline-containing compounds from foods using three cycles of hot methanol extraction, liberated choline can be reacted to form a Reinecke salt, which is easily detected by absorbance at 520 nm. While this method is simple and straightforward, losses have been observed during the preparation of the precipitate, and the method lacks specificity as the reagent forms the colored product with any amine. The AOAC Official Method of Analysis for choline in infant formula and milk (999.14) is also a colorimetric reaction with choline liberation by acid and enzyme hydrolyses. The reaction mixture includes choline oxidase to release hydrogen peroxide, which will react with peroxidase, 4-aminoantipyrine, and phenol to form a quinone imine dye with absorbance at 505 nm. Modifications to this approach have replaced the phenol and 4-aminoantipyrine with dichlorofluorescein, added a basic hydrolysis step to release PtdCho from lecithin, and added acid-washed decolorizing carbon (Norit) to the sample to remove interference from vitamin C, making the approach more broadly applicable to foods and dietary supplements other than infant formula and milk. Methods based on choline oxidase are more specific for choline, but as demonstrated by the number of necessary modifications, interferences are still possible in the formation of the detectable colored compound.

To increase specificity for choline and remove interferences from other sample components that might be simultaneously extracted, separation-based approaches have been developed. Some methods utilized base hydrolysis to generate triethylamine, which can be determined by GC with flame ionization detector (FID) or MS. Like the colorimetric methods described previously, however, these methods are specific only for quaternary amines and may result in a positive bias from other compounds in the food matrix. Other methods utilized acid hydrolysis or a combined acid and enzyme hydrolysis followed by LC-IDMS or ion chromatography with conductivity or ion-exchange membrane detection. Approaches that did not utilize an enzymatic step with the acid hydrolysis yielded results up to 15% lower than those using a combined approach, because PCho is not completely hydrolyzed under acidic conditions. However, in one modification that conducted the acid hydrolysis in a microwave digestion chamber, an additional enzyme hydrolysis step was not necessary to completely extract total choline from food products. Another approach has also been reported utilizing base hydrolysis, followed by capillary electrophoresis with indirect absorbance detection in the presence of 1-methylimidazole as a carrier electrolyte.

Other less common methods for quantitation of choline in foods and food products have utilized the reaction of choline oxidase with choline from sample extracts by monitoring the consumption of molecular oxygen or the generation of hydrogen peroxide. More specifically, molybdenum-catalyzed oxidation of iodide by hydrogen peroxide has been detected potentiometrically for quantitation of total choline in foods. Biosensors have also been utilized for the detection of O_2 decrease or H_2O_2 increase upon introduction of a choline-containing sample to a reactor containing choline oxidase, in either a standalone or flow-through design. One potential benefit of the biosensor approach lies in the ability to determine choline and choline esters using the same biosensor, by analyzing the same sample pre- and post-hydrolysis. These methods have not been widely adopted, however, as LC and LC-MS equipment are more common in food laboratories, and the use of biosensors for routine analysis has not yet been demonstrated as robust.

Determination of Choline for Health Marker Status

For evaluation of choline health status, the most commonly measured biomarkers are free choline and PtdCho in the serum and/or plasma. Free choline and PtdCho are often separated from the serum and/or plasma matrix by liquid–liquid extraction as described previously for foods; free choline can also be isolated from serum or plasma using ion-exchange resins. Early methods for determination of free choline and PtdCho in plasma and serum involved a radioenzymatic assay in which free choline is extracted into the aqueous layer by liquid–liquid extraction (as described previously) and converted to ^{32}PCho using ATP-γ-^{32}P in the presence of a choline kinase catalyst or ^{32}P-labeled acetyl-CoA in the presence of choline acetyltransferase. Following the separation from the labeled precursor via electrophoresis or ion-exchange chromatography, the radioactive decay of ^{32}PCho is measured by scintillation counting. The organic phase can be analyzed separately for PtdCho by isolation with preparative LC or TLC, hydrolysis to release choline, and repetition of the free choline determination procedure by radioenzymatic assay. PtdCho can also be measured via total phosphorus content by colorimetric assay. These assays are simple, fast, reproducible, and specific for choline and PtdCho and have been demonstrated to have little interference from other choline esters; the only significant drawback is the sensitivity of the assay to the source of the enzymes used in the various hydrolysis and reaction steps.

Several other traditional chemical methods have been utilized as well for determination of choline and PtdCho in biological fluids. Chemiluminescence approaches have been explored, utilizing the reaction of choline with choline oxidase to form peroxide and the subsequent oxidation of acridinium-9-carboxamide or luminol catalyzed by peroxidase. These methods also described the option for hydrolysis of PtdCho by phospholipase D to form free choline, allowing both biomarkers to be determined by the same approach. Another approach utilized the decay of the fluorescence of choline oxidase (native or derivatized) as the reaction with free choline progresses. While these traditional methods have the benefit of simplicity and low instrumentation demand, developing technologies demand demonstration of specificity and increased accuracy and precision for true understanding of clinical health.

Instrumental approaches have been explored more recently to give a more accurate profile of choline and choline esters in plasma and serum. Some of the methods used for food analysis (described previously) have also been applied to clinical analyses, separating the various choline-containing compounds

using preparative chromatography such as LC or TLC. Isolated compounds are then hydrolyzed individually to release choline, and the free choline is measured by a technique such as GC-FID or GC-MS. GC can also be coupled to radiometric assays described previously, by using GC to separate the radiolabeled compounds of interest and monitoring the radioactivity at the column exit. To utilize any GC-based technique, the choline must first be volatilized. Although one pyrolysis–GC-MS technique has been described, the most common approach to volatilization is via chemical derivatization, which can add lengthy sample preparation time and potential for the introduction of error or bias. To reduce error or bias that may result from sample preparation, techniques using stable isotope-labeled compounds as internal standards have been described. By spiking ^{2}H-, ^{13}C-, or ^{15}N-labeled variants of each compound of interest into samples at the start of processing, inefficiencies in extraction, sample losses, and variability in molecular ionization can also be mitigated.

Other approaches have utilized LC with electrochemical detection following postcolumn enzymatic reaction with choline oxidase to generate and electrochemically detect hydrogen peroxide. Free choline has also been derivatized with 1-naphthyl isocyanate prior to LC to utilize fluorescence detection or with 3,5-dinitrobenzoyl chloride prior to LC to utilize absorbance detection. Because choline is a relatively small, hydrophilic molecule, early approaches to LC often included the addition of an ion-pairing agent, which led to challenges in maintaining a robust, reproducible separation. As described for GC-based techniques, the utilization of an internal standard is critical in LC determination of choline as well. The numerous sample preparation and analysis steps allow the possibility for various sources of bias and error in the final analytical determination of choline, and the proper use of an internal standard can reduce the impact.

Taking advantage of the benefits of an internal standard approach, most recent methods for choline determination in serum and plasma have utilized LC with MS or MS/MS detection and isotope dilution for quantitation. As described previously for food analysis, emerging technologies such as HILIC allow simultaneous analysis of choline and PtdCho. Many current approaches utilizing isotope dilution describe the addition of an acetonitrile or methanol solution containing the internal standards to force protein precipitation from serum or plasma. Following centrifugation, these samples can then be directly analyzed by HILIC LC-MS/MS. Because similar methods have been demonstrated in foods for the profiling of numerous choline-containing compounds, it stands to reason that an HILIC LC-MS/MS method could be used to screen serum or plasma samples if a new choline biomarker is discovered.

Conclusions

Choline is a small quaternary ammonium compound that plays an important role in numerous metabolic processes and can be present in numerous forms, each having distinct chemical and nutritive properties. To understand the role of choline in health and function, foods (intake) and biological fluids (output) must be accurately monitored for choline content. As a result, understanding and interpretation of the role of choline in human health rely heavily on the availability of appropriate analytical methodologies.

See also: Choline: Physiology; Eggs: Composition and Health Effects; Folic acid and Folates: Physiology and Health Effects; Food Composition Databases; Human Milk: Composition and Nutritional Value; Infants: Nutritional Requirements; Phospholipids: Physiology; Phospholipids: Properties and Occurrence; Pregnancy: Dietary Guidance for Pregnancy; Sports Nutrition.

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Chromatography: Combined Chromatography and Mass Spectrometry

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Introduction

The advanced analytic technologies are revealing a complex profile regarding the food and health research. Most foodstuffs are produced from living organisms and tissues, thus reflecting the complexity of the biological systems they are coming from. Generally, two types of opposite food components, namely, beneficial and hazardous ones, gain the emerging attention. These components play a key role in food nutritional, functional, or hazardous properties. Among the toolkits of techniques developed to investigate food at wide scale including small molecules, peptide, and proteome, chromatography combined with MS has gained popularity especially because of its ability to handle complex food matrices. The chromatography–MS methods demonstrate high resolution ratio, specificity, speed, and reliability of the analytic response in a high-throughput mode. For these reasons, chromatography–MS methods have been extensively employed in food analysis.

In this article, applications of chromatography–MS to food safety and quality is discussed in detail, including detection, identification, and quantification. In this way, the identification and determination of food could be addressed, especially for the certification and traceability of foodstuffs. Moreover, the relationship between structure and function in food systems could be clarified.

Combined Chromatography to MS Strategies

MS method is based on the production of ions, which are subsequently separated or filtered according to their mass-to-charge (m/z) ratio and then detected. The resulting mass spectrum is a plot of the (relative) abundance of the generated ions as a function of the m/z. It provides excellent selectivity, which is of utmost importance in quantitative trace analysis. The mass spectrometer is a highly sophisticated and computerized instrument, which basically consists of ion source, a mass analyzer, and a detector. In principle, liquid chromatography (LC), gas chromatography (GC), and capillary electrophoresis (CE) are used as the separation technique, while the mass spectrometers are employed as a detector. The different physical and chemical properties between analytes and their relative affinity for the stationary/mobile phases promote their separation. The molecules are retained in the column and then elute (come off) from the column at different times (retention time) that depend on the analyte affinity for the mobile phase. This allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately, by breaking each molecule into ionized fragments and detecting these fragments using their m/z. However, online chromatography–MS systems offer additional value, especially in terms of selectivity that allows sensitive and specific analysis in food for various purposes.

Mass Spectrometer

A mass spectrometer contains three major components, namely, an ion source, a mass analyzer, and a detector. The analyte previously separated from other analytes and the matrix bulk eluted to the ion interface, where a portion of the sample is converted into ions. The ions that meet certain characteristics are trajected through the mass analyzer and onto the detector. The mass analyzer is used to sort the ions (according to their m/z values) and to calculate their abundances.

With the ion source, the target of interest is ionized and then transported by magnetic or electric fields to the mass analyzer. For gases and vapors, electron ionization (EI) and chemical ionization (CI) can be applied. For liquid and solid biological samples, electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) are the most commonly used ionization sources. Inductively coupled plasma (ICP) sources can carry out cation analysis, although it appears seldom in food analysis. In the combination of HPLC/GC to MS, the ionization sources are classified according to hard and soft ionization of the molecule. Hard ionization types, such as EI, provide a high degree of fragmentation and important information for structure of unknown compounds. However, the EI source is not the most appropriate for HPLC–MS, because of the short EI shelf life at atmospheric pressure. On the country, EI source can be used in GC–MS due to its high vacuum. Soft ionization indicates that ions are formed using lower residual energy, such as fast atom bombardment (FAB), chemical ionization (CI), atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI), and MALDI. These sources provide mostly the molecular ions (or protonated or deprotonated molecules and few fragments).

Mass analyzers separate the ions according to their m/z ratio by using an electric and/or magnetic field. There are different types of mass analyzers. Time-of-flight (TOF) analyzer records the pass-through time by using an electric field to accelerate the ions with the same potential, thus allowing the most rapid reach of lighter ions. Nowadays, TOFs provide accurate m/z. Quadrupole mass filter can chose ions in a certain range of m/z by using oscillating electrical fields and can filter m/z as the quadrupole ion trap. Three-dimensional quadrupole ion trap can trap and eject ions sequentially. A linear quadrupole ion trap can trap ions in a two-dimensional quadrupole field, instead of a three-dimensional quadrupole field as in a 3-D quadrupole ion trap. In the Orbitrap method, ions are electrostatically trapped in an orbit around a central, spindle-shaped electrode.

The final element of the mass spectrometer is the detector. The detector records the charge induced or the current produced when an ion passes by or hits a surface. The electron multiplier, Faraday cups, or ion-to-photon detectors are commonly used.

GC–MS

GC–MS method combines the features of GC and MS to identify different substances within single food sample. The use of a mass spectrometer as detector in GC was developed during the 1950s after being initially assembled by James and Martin in 1952. GC involves an oven and inside of it capillary column with proper dimensions (length, diameter, and film thickness) and optimized phase properties that helps to separate the targets of interested by volatilization using a temperature gradient of the oven. The mobile phase is an inert carrier gas (as helium or nitrogen), while the stationary phase is a microscopic layer of liquid or polymer on an inert solid support (silica). In principle, the targets of interest are volatilized and then interact with the GC stationary phase coated in the column walls. Each compound elutes at a different time (retention time) depending on their boiling point and affinity by the stationary phase.

The typical components of GC instruments are carrier gas, flow controller, sample injector, column oven, column, and detector (any type of mass analyzer). In the GC separation process, a certain volume of gaseous or liquid sample is injected into the column head by using, usually, a microsyringe (solid-phase microextraction fibers can also be used). The carrier gas sweeps the targets through the GC column and, then, the analyte elute of the column when the oven temperature raises its boiling point. Different adsorption strengths between the targets and stationary-phase materials allow also variations in retention time, in favor of the identification and determination by MS.

Recently, comprehensive two-dimensional (2-D) GC, or GC × GC – originally developed in 1991 by Professor Phillips – was employed to separate targets that are difficult to separate by conventional GC. The two GC columns are connected sequentially, the 1-D column is a conventional one and the 2-D column is a short fast one. Between the 2-D columns, a modulator is employed to collect small fractions of the effluent from 1-D, focus them as a narrow pulse, and transfer them to the 2-D. This so-called modulation cycle is repeated throughout the 2-D GC run. For example, a typical modulation is the thermal modulation. The liquid nitrogen is used to immobilize all the components eluting from 1-D. A hot stream pulse mobilizes a part of the compounds through the second column, where the 2-D elution starts again.

GC–MS method offers high sensitivity and resolution power, excellent reproducibility, and extensive and highly reproducible fragmentation, providing excellent identification potential through well-established databases, such as the NIST library. Other advantages are the easy use of the technique and its low cost. The major disadvantage is that GC–MS is by its nature limited to the analysis of small volatile molecules and molecules that can be made volatile. The problem of by-product formation and degradation needs consideration.

The GC-MS methods allow much finer degree of substance identification than either of the unit used separately. It is difficult to make an accurate identification by single GC or MS methods. The MS process normally requires a pure sample, while GC using a traditional detector (e.g., flame ionization detector) cannot differentiate between molecules that coeluted in the same peak. GC–MS method reduces the possibility of error, because two different molecules that behave in the same manner in GC and MS are very rare. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC–MS analysis, it typically increases certainty that the analyte of interest is in the sample. Two different molecules can also have a similar pattern of ionization in a mass spectrometer (isobaric interferences). However, accurate mass instruments reduce or eliminate the possibility of these interferences.

LC–MS

LC–MS is a powerful technique that has very high sensitivity and selectivity and so is useful in many applications such as food safety. This separation principle is similar to GC (different affinities of the analytes for the stationary/mobile phases). However, in GC, the analyte separation is between the liquid stationary phase and the gas mobile phase, while, in HPLC, the analyte separation is between the solid stationary phase and the liquid mobile phase. Then, analytes are separated according to their polarity independently of whether they are volatile or not. In HPLC, the separation is forced by a liquid at high pressure (as the mobile phase) through a column. This HPLC column is previously packed with a stationary phase generally composed of irregularly or spherically shaped particles. Usually, octadecylsilyl (C_{18}) is used as stationary phase with pure or pH-adjusted water–organic mixtures (as water–acetonitrile or water–methanol), which is termed reversed-phase liquid chromatography (RP-LC). Silica gel is also can be applied as stationary phase with neat or mixed organic mixtures, which is called normal-phase liquid chromatography (NP-LC). RP-LC is most often used because of its superior separation capabilities.

LC–MS is currently (probably) the most widely used mass spectrometry technology, due to its ability to separate and detect a wide range of molecules. The method allows for the collection of both qualitative and quantitative data and can achieve pgL^{-1}level of detection.

The combination of the separating potential of liquid chromatography and the analyzing power of mass spectrometry makes LC–MS a highly useful tool for analytical chemists. Due to its high selectivity and sensitivity, it is finding increasing use in the analysis of a wide range of substances in complex mixtures. The major challenge in coupling of LC with MS is posed by the fact that gas-phase ions must be produced in order to obtain a mass spectrum.

There are many types of LC–MS interface, such as ESI, APCI, atmospheric pressure photoionization (APPI), and MALDI. Currently, there are mainly two types of API interfaces. One is ESI, which is best suited to ionic compounds with high polarity and the other APCIs. As already mentioned, ESI is a soft ionization technique, producing little fragmentation. For better structural information, ESI interface can be coupled with tandem MS (ESI-MS/MS).

CE–MS Method

CE includes a group of electrokinetic separation methods carried out in submillimeter capillaries and in micro- and nanofluidic channels. In these methods, analytes migrate through electrolyte solutions under the influence of an electric field.

Analytes can be separated according to ionic mobility; additionally, they may be concentrated by means of gradients in conductivity and pH. The identity of sample components can be established by direct coupling of the capillary electrophoresis with mass spectrometers. Commonly, to connect both instruments, the capillary outlet is introduced into an ESI source modified to introduce a sheath liquid that closes the electrical circuit. CE-MS offers a powerful tool for the targeted analysis of polar metabolites, such as amino acids, organics acids, and nucleotides with superior resolution and sensitivity.

Application in Food Analysis

Food products can be regarded as complex mixtures and consist of beneficial and hazardous compounds that naturally occur in food or are introduced during food processing. Beneficial food components include lipid, carbohydrate, polyphenols, carotenoid, amino acids, peptides, and proteins, while the hazardous ones consist of mycotoxin, marine toxin, plant toxins, pesticide, and veterinary drugs. As example of natural beneficial food compounds, lipids such as fats and sterols are able to store energy and act as structural components of cell membranes. The positive health benefits associated with the consumption of omega-3 fatty acids on infant development, cancer, cardiovascular diseases, and various mental illnesses, such as depression, attention-deficit hyperactivity disorder, and dementia, should also be kept in mind. On the contrary, toxic substances generally originating from crop protection treatments against pest, agrochemical treatments, or packaging materials have been frequently found in foodstuffs. Pesticide residues can cause acute and chronic health effects in human and livestock, ranging from simple irritation of the skin and eyes to the destruction of the nervous system, reproductive problems, and even cancer. All these compounds, toxic or beneficial, can be determined using chromatography combined with mass spectrometry. In the next section, the determination of both, beneficial and hazardous compounds, in food is discussed.

Analyzing Beneficial Food Components via Chromatography–MS

Lipid

Lipids are macronutrients that contribute significantly to the nutritional and sensory value of food. Lipids can be found in various forms, such as free fatty acids, acylglycerols, phospholipids, sphingomyelins, glycosphingolipids, steroids, and bile acids. Some lipids have simple structure (as fatty acids), but many others have complex and variable structures. The structures can be ascertained and completely resolved by HPLC–MS or GC–MS methods, helping to establish the structure/function relationship. HPLC–MS method is one of the most powerful tools for the analysis of lipid components in food. Lipid content from various foodstuffs has been extensive investigated. To simplify sample preparation, HPLC–MS method has been an attractive alternative. The main trend of diverse lipids' analysis is the employment of HPLC–MS methods. Triacylglycerols, fatty acids, carotenoids, and phospholipids found in various foodstuffs have been studied in detail via HPLC–MS method. In addition, the use of tandem mass spectrometry (MS/MS) enhances dramatically their structural elucidation, for example, individual triacylglycerols. Besides, GC–MS is also used to analyze free fatty acids and triacylglycerols, although it required derivatization procedures (e.g., transformation of free fatty acids in the methyl esters).

Carbohydrate

Carbohydrates, the most abundant natural products, can be classified according to their degree of polymerization (DP). They can be divided initially into three principal groups, namely, simple sugars (DP 1–2), oligosaccharides (DP 3–9), and polysaccharides (DP > 9). They are one of the most important components for nutrition and health, because they have important physiological role. They can serve as structural elements in plants (as cellulose), important sources of dietary fiber, and major sources of energy (as starch or glucose) in food. For the reasons mentioned earlier, carbohydrates are specially targeted via HPLC–MS or GC–MS methods in food. Monosaccharides and small oligosaccharides have been traditionally analyzed via GC–MS. However, the use of derivative reaction in GC–MS method hampered its application to larger oligosaccharides and molecular conjugates. Due to the high polarity and low volatility of carbohydrates, ESI ionization is usually preferred over APCI ionization. Carbohydrates can be hardly observed in negative ionization, due to its lower sensitivity, while they can be sensitive in the positive ionization mode. For example, the addition of Li salts with low concentration to the HPLC eluents produces $[M+Li]^+$ ions and can increase the sensitivity of carbohydrate compounds in food.

Carotenoids

Carotenoids are lipid-soluble pigments responsible for the color of a wide variety of fruits and vegetables. Some of them are provitamin A carotenoids, subsequently transformed into vitamin A, which can prevent serious eye diseases, such as night blindness; susceptibility to infection; rough, scaly skin; and retarded tooth and bone development. There are about 700 carotenoids in nature, but only about 50 have provitamin activities. Of those 50 compounds, the three most important precursors of vitamin A in humans are α-carotene, β-cryptoxanthin, and β-carotene.

A range of LC-based techniques have been used to analyze carotenoids, most of them coupled to a PDA or UV–vis detector. Although LC separation coupled to UV–vis instruments has been the most common analytic method for determining carotenoids qualitatively and quantitatively, the spectra of many carotenoids are very similar, so many researchers have complemented the identification of carotenoids using other detectors, such as MS. With UV and PDA systems, it is impossible to provide molecular structure information for identification, especially for unknown carotenoids in complex sample matrices. The MS instruments are used to overcome spectral interferences in UV–vis and, therefore, to achieve high sensitivity in complex mixtures and to obtain molecular structure information on the basis of the molecular mass and fragmentation pattern under tandem MS (MS/MS and MS/MS/MS).

For HPLC–MS, usually ESI, sometimes APCI ionization, is used. There are many examples of analysis and quantitation of vitamins in food via HPLC–MS. Most carotenoids are apolar compounds, so APCI (possibly also APPI) ionization may be more advantageous than the more commonly used ESI.

HPLC–MS/MS has also been used to distinguish between structurally related molecules and their epoxidized forms, products of carotenoid oxidation, that are potential oxidative stress markers and difficult to profile due to their small amounts and the difficulty in separating them from hydroxyl carotenoids.

Amino Acids, Peptides, and Proteins

There is currently a general trend in food science towards the consideration of food as an affordable way to prevent diseases. In this sense, one of the main challenges is to improve our limited understanding on the interaction of food compounds with genes and their subsequent effect on proteins and metabolites; this knowledge should allow a rational design of strategies to manipulate cell functions through diet, which is expected to have an extraordinary impact on our health in the nondistant future.

Proteomics is the large-scale analysis of a proteome that includes all the expressed proteins in a particular biological system at a given time, whereas peptidomics is the analysis of all peptide content within an organism, tissue, or cell (peptidome) including not only the peptide present in the system but also the transient products of protein degradation. In general, the major difficulty in the analysis of protein and peptides comes from the different physicochemical properties of proteins, the high number of peptidic sequences that can become available, and the huge dynamic concentration range of both families of compounds in real samples. Proteomics and peptidomics offer multiple applications in food science including food processing, food quality, food safety, and characterization of healthy food ingredients.

LC–MS has been extensively used in the identification and determination of amino acids, peptides, and proteins. In earliest studies, for example, conventional RP-HPLC with chiral derivatization agents was used for the determination of amino acids. However, these methods were not applicable in real food samples because of the insufficient sensitivity and the interference from the numerous coexisting amino acids. For the determination of peptides and proteins, HPLC–MS plays a critical role. By using RP-HPLC, ion exchange, hydrophobic interaction chromatography, etc., peptides and proteins can be separated via the differences in surface hydrophobicity or surface charge. These methods provide the practical technologies to separate complex food matrices, and the affinity chromatography allows the purification of targeted peptides and proteins. After HPLC separation, most peptides and proteins in the low concentration range can be identified by MS techniques.

CE-MS has been mainly applied for proteomics, peptidomics, and metabolomics studies. Capillary electrophoresis techniques coupled to MS are ideal analytic techniques for different 'omics' approaches such as metabolomics, proteomics, and peptidomics mainly due to the particular characteristics of this separation technique. CE provides fast and highly efficient separations, low reagent and sample consumptions (CE is particularly well suited for samples that are volume-limited), and high versatility considering the different CE modes available. The main drawback of CE is its poor sensitivity, but it can be improved by combining CE with MS detection, while the use of preconcentration strategies can give further sensitivity gain. Besides, the use of MS as detector provides additional selectivity and structural information of the detected compounds.

Polyphenols

Flavonoids are polyphenolic compounds, usually found in food and beverage. The motivation of flavonoids analysis using HPLC–MS or GC–MS methods arises from their potential beneficial effects on human health. Flavonoids are usually present in low amounts in a complex matrix of plant extracts and thus generally are difficult to isolate in higher quantities. This, combined with their good sensitivity in electrospray, makes HPLC–MS the method of choice for flavonoid analysis. To increase selectivity (often a critical aspect) and high mass resolution and to increase structural information, tandem mass spectrometry is often used in HPLC–MS analysis of flavonoids.

In the other side, several CE-ESI-MS methods have been developed for the analysis of flavonoids, using high-pH running buffers containing ammonium acetate and MS detection in the negative-ion mode. Other natural compounds bearing phenol structures have been analyzed by CE-MS. Thus, a comparison between HPLC-ESI-MS and CE-ESI-MS for the analysis of phenolic compounds from red wines showed that in spite of CE providing much higher separation efficiency than HPLC, 24 compounds could be identified by HPLC–MS vs. 13 compounds identified by CE-MS in the mentioned red wine extracts.

Food additives

Food additives are strictly limited; only additives explicitly authorized may be used in food. Monitoring foodstuffs for additives is an area of increasing concern and importance. Due to its excellent figures of merit, HPLC–MS is often a method of choice. Food additives are groups of substances commonly classified according to their application and not to their chemical structure. Some of these are small molecules (like benzoic acid used for conservation), some are macromolecules (like the 'infamous' guar gum, causing concern a few years ago), some are synthetic products, while others are natural extracts. For these reasons, it is difficult to generalize the 'proper' analytic method to be used for their characterization. Some methods are compound-specific (and do not estimate total amount of a given class of compounds); some characterize groups of substances. Often, both identification and quantification are required. Generic procedures for the simultaneous extraction of various classes of food additives and residues in various matrices are in use.

Analyzing Hazardous Food Components via Chromatography–MS

Mycotoxin

Mycotoxins are toxic secondary metabolites of fungi (usually molds) that readily colonize crops or foodstuffs during storage. Most often, these are present in cereals and oil seeds. A

database reported 474 mycotoxins and fungal metabolites, while previously unknown metabolites have recently been created. These toxins are dangerous even in very small amounts. Therefore, for some mycotoxins, maximum residue level of aflatoxins B_1, for example, as low as $0.1\mu gkg^{-1}$ has been established by the EU for baby foods and processed cereal-based foods for infants and young children (please see EC No 1881/2006). For mycotoxin analysis, two different strategies can be followed to develop and validate analytic methods. One is to set up a screening method to detect and roughly quantify as many metabolites as possible, reducing the selectivity in extraction and cleanup steps. The other approach is to develop a high-performance method to accurately quantitate a few selected mycotoxins in selected cases. For both approaches, a number of methods have been developed and validated by diverse research groups. The combination of HPLC with MS/MS has proved to be a powerful tool for the simultaneous determination of different classes of mycotoxins.

Besides mycotoxins, also their metabolites often formed in plants and animals might be hazardous for man. Such conjugated or masked mycotoxins can also be analyzed with LC–MS/MS methods, very well suited for high sample throughput, and should be included into multimycotoxin analysis for the direct detection and characterization of metabolites in complex biological and food matrices.

Mycotoxin analysis uses a variety of mass spectrometric methods. Electrospray (in both positive-ion mode and negative-ion mode), APCI, and APPI ionizations are all frequently used. Triple-quadrupole instruments are often used for targeted analysis, while high-resolution analysis is used to look for unexpected derivatives. When quantitation is needed, using isotope-labeled standards, whenever available, is highly advantageous for accurate and precise analysis.

Pesticide residues

Pesticide residues in food are a growing concern, both for the consumers and for legislation. Widespread use of pesticides necessitates their trace analysis in vegetables and various food products. There are several hundred active ingredients and thousands of formulations currently in use. Like in the case of mycotoxins, a large number of compounds need to be screened and, if found, accurately quantified. Simultaneous analysis of pesticides requires development of efficient high-throughput methods.

A significant fraction of pesticide trace analysis is based on HPLC–MS. In most cases, tandem mass spectrometry is utilized, as it allows simplification of sample treatment prior to the analysis and achieves multiresidue analysis in a single chromatographic run. When quantitation is needed, using isotope-labeled standards, whenever available, is highly advantageous for accurate and precise analysis.

For chromatography, often, UHPLC (ultrahigh-performance liquid chromatography) is used. UHPLC allows fast and efficient separation and analysis is usually performed in less than 15 min chromatograms. For analysis, most often, a C18 column with small particle size (1.7 m) is used. The most often used mass spectrometer is the triple quadrupole (QqQ) that achieves tandem mass spectrometry. The QqQ literally has three quadrupole mass analyzers in the same instrument. The first analyzer is set to transmit the precursor (usually the molecular) ion; in the central quadrupole, collision-induced dissociation (CID) takes place, while the second analyzer transmits the expected product ion, which is detected. In this instrument, several precursor ion → product ion transitions are selected (selected reaction monitoring (SRM)) to increase sensitivity.

Increasing selectivity is of prime importance for analyzing trace components in complex materials. For this reason, conventional selected ion monitoring (SIM) in a single quadrupole is usually not considered sufficiently selective to identify pesticide traces in food. Besides tandem mass spectrometry, analysis using high mass resolution (HRMS) is another direction to increase selectivity. This can be carried out using time-of-flight (TOF), Orbitrap, or Fourier transform (FT) mass spectrometers.

A further option to increase accuracy and reliability of pesticide analysis is the use of isotope dilution (that means to use the isotopically labeled analyte as internal standard). This enhances accuracy of analysis and at the same time may allow a simplified sample preparation process. However, it increases the costs of analysis significantly, so for this reason, it is rarely used for routine pesticide analysis in the agrifood sector. Pesticide analysis is one of the most important applications in the agrifood sector. A large variety of different foodstuffs are routinely analyzed, including baby food, vegetables, vegetable oil, honey, fruit juice, wine, and milk.

Conclusion

In the past years, there has been an emerging investigation for the food safety and food quality analysis using chromatography–MS methods, including HPLC, GC, and CE. These combined methods allow the rapid, reliable, and accurate analysis, identification, and quantification in food matrices. State-of-the-art MS instruments enhanced analytic sensitivities for the identification of trace components of food and provide a powerful tool for analyzing various foods to meet the requirements of food legislation or the concerns about toxicity. Combined MS (LC–MS, GC–MS, etc.) and MS/MS methods can reduce the time and labor-consuming sample preparation processes and improve the selectivity for both qualitative and quantitative analyses of food samples present in complex matrices. The advances of simple, robust, and reliable portable chromatography–MS method can promise an on-site, rapid, accurate identification and quantification of unknown compounds in food.

See also: Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Food Poisoning: Tracing Origins and Testing; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto.

Further Reading

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Chromatography: Focus on Multidimensional GC

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Introduction

Over the last decades, consumers' preferences have been addressed to healthier and flavorsome food with higher nutritional value: the driving force has been food quality. Primary condition for quality is safety that is closely related to the compliance with legal standards on human health risks, the environment, animal welfare, protection of natural resources, and ethical requirements. On the other hand, the sensory impact due to flavor, smell, and appearance has assumed an equally important role. In this perspective, food end products, semifinished products, and raw food matrices require control analyses to establish quality requisites and to verify compliance with legal standards.

Since its introduction in the early 1950s, gas chromatography (GC) demonstrated to be an effective, flexible, and sensitive technique for food analysis and has been successfully adopted as the 'core' of the analytic process for

- flavor and aroma characterization (*sensomics*);
- studies of composition and of origin authentication;
- characterization of the volatile compounds from vegetable matrices, essential oils, and differently produced extracts;
- fat analysis and characterization (short-chain fatty acids, fatty acids methyl esters (FAMEs), triglycerides, sterols, etc.);
- residue and contaminant determination (pesticides, veterinary drugs, endocrine-disrupting chemicals (ECDs), food packaging migration products, and environmental contaminants such as polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs).

However, one-dimensional GC not always provides adequate efficiency and selectivity to obtain the required results in terms of analyte separation (and as a consequence identification and quantitation), in particular when complex samples have to be analyzed. On the other hand, although the new generation of mass spectrometric detectors thanks to improved performances (new ionization sources/interfaces and analyzers) enables reliable identity confirmation and quantitation even without a separative step, chromatographic separation provides additional information on analyte physicochemical properties that in some cases are necessary for unknown identification (i.e., isomers).

Multidimensional chromatography (MDC) has therefore emerged as a valuable alternative thanks to the peak capacity enhancement as well as the possibility to combine orthogonal discrimination principles (including those provided at the detection level) to investigate sample composition and to process results to obtain higher and further levels of information.

MDC combines two, or more, independent (or nearly independent) separation steps, increasing significantly the separation power of the resulting system. In the early 1980s, the comprehensive definitions of MDC already distinguished those system configurations providing a simultaneous zone displacement from those adopting coupled columns that provide sequential displacement of analytes.

More recently, because of modern instrumental innovations and MDC developed applications, a more general definition of MDC as '*n*-dimensional analysis that generates n-dimensional displacement information' was proposed.

When gas-phase separations are considered, the most intuitive and descriptive definition was given by Marriott, who has defined multidimensional gas chromatography (MDGC) as "the process of selecting a (limited) region or zone of eluted compounds issuing from the end of one GC column, and subsequently subjecting the zone to a further GC displacement."

A typical MDGC separation focuses on a fixed number of fractions eluting from the first dimension (^{1}D), the so-called heart-cuts (H/C) (the approach is referred to as heart-cut multidimensional gas chromatography (H/C MDGC)) that are subsequently analyzed in the second dimension (^{2}D), with the aim to improve separation by combining different discrimination principles exploiting 2-D-system selectivity. Alternatively, when a fast and continuous heart-cutting (named modulation) is implemented by applying sampling periods compatible with the ^{1}D peak width, the resulting MDGC approach is named comprehensive 2-D GC or GC × GC.

Figure 1 shows a schematic description of monodimensional and different multidimensional GC instrumental configurations; Figure 1(a) reports a conventional monodimensional GC system, and Figure 1(b) a typical H/C MDGC including a switching system (or valve V) located at the junction between the two columns. The system also includes an auxiliary gas line from an electronic pressure controller (aux EPC) that provides flow for the switching valve and/or a makeup flow for the ^{2}D column that is located in a separate oven. The system is also equipped with a primary detector (Det 1) and with a monitor detector (Det 2), optional. Figure 1(c) shows a comprehensive two-dimensional system configuration that includes a modulator (M) that interfaces the two columns (^{1}D and ^{2}D) coupled in series. The detection is placed at the end of the ^{2}D column.

The next sections present basic operative principles, instrumental configurations, and applications of H/C MDGC and comprehensive two-dimensional gas chromatography (GC × GC).

Heart-Cut Multidimensional Gas Chromatography

Basic Operative Principles and Instrumental Configurations

A classical H/C MDGC configuration basically consists of two conventional capillary columns (e.g., 30 m × 0.25 mm ID × 0.25 μm film thickness – d_f) coated with stationary phases differing in selectivity, connected in series. Columns can be located in a single or in two separate ovens, and a suitable interface is located between the two dimensions enabling to

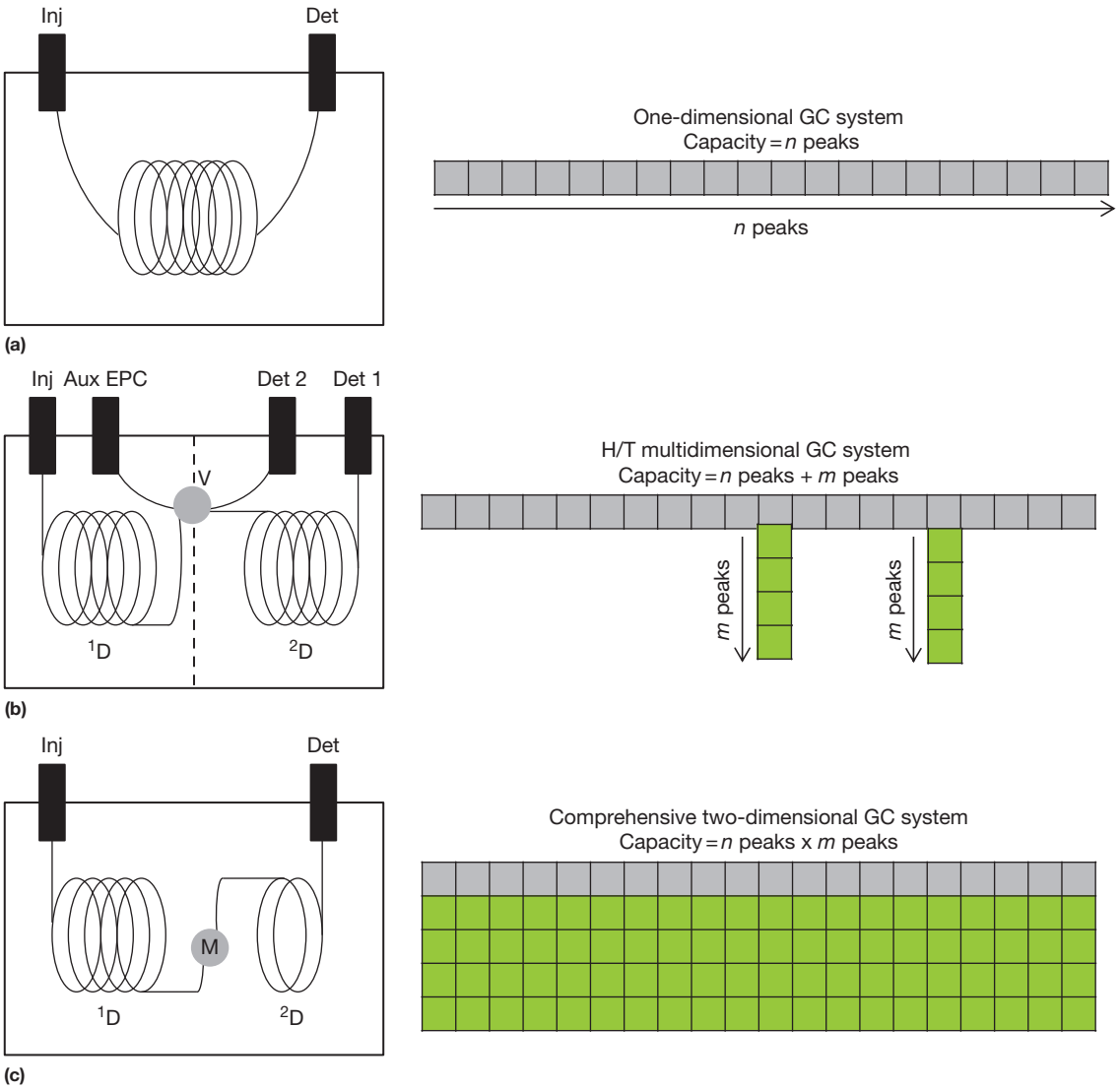

Figure 1 Schematic visualization of mono- and multidimensional GC instrumental configurations and their actual peak capacity: (a) shows a typical one-dimensional system, (b) shows an H/C MDGC, while (c) shows a comprehensive two-dimensional system. Inj, injector; aux EPC, auxiliary gas electron pressure controller; Det 1, primary detector; Det 2, monitor detector; V, switching interface; M, modulator; ^{1}D, first dimension column; ^{2}D, second dimension column; n^{1}D, peak capacity; m^{2}D, peak capacity.

transfer chromatographic bands from the ^{1}D to the ^{2}D column (see Figure 1(a)). The use of a cryotrapping device, between the two capillaries, position V in Figure 1(a), makes the system more efficient, affording a better band focussing and reconcentration that results in an improved ^{2}D peak capacity and higher system sensitivity for trace analytes.

The transfer devices developed since the beginnings of the technique can be classified in three groups: in-line and out-line valves, the most popular systems, and valveless systems.

Valve-based systems, although straightforward in principle, are affected by several limitations due to efficiency and inertness. Band dispersion caused by dead volumes and retention time fluctuations may occur. The modern SilTite™ technology enabled to overcome valves surface activity and improved mechanical robustness when installed in a hot oven.

Six-port–two-position W-type valves from Valco, live-coupling/switching pieces, and Deans-switch interfaces utilizing Valco microvolume T-piece are nowadays the most popular devices for valve-based systems in MDGC.

However, thanks to the evolution electronic pressure control systems and their integration into the GC, most of the MDGC systems adopt nonintrusive, valveless flow-modulated devices (based on pneumatic flow switching). Some examples of H/C MDGC systems available on the market are the multi-Deans-switching technology from Shimadzu, the moving capillary stream switching (MCSS) from Brechbühler, the capillary flow technology (CFT) from Agilent, the Swafer™ microchannel system from PerkinElmer, and the SilFlow™ microchannel device (MCD) from SGE. Effluent-switching devices by Agilent, PerkinElmer, and SGE are based on microchips that connect individual switch components into a single, small deactivated microchip plate.

The Swafer™ system has laser-fabricated microchannels on circular metal disks present in the device body and is available

with different configurations allowing flow switching and splitting between two or four inputs and two or up to four outputs. With the SilFlow™ technology, the MCD has options for three-port or four-port GC splitters. Advantages of microfabricated devices rely on the possibility to overcome and eliminate chromatographic distortions (peak tailing, peak broadening, and/or analyte loss) thanks to surface inertness, low thermal mass, and low dead volumes.

From an operative point of view, they are based on a microfluidic Deans-switch technology; a description of the principle of operation is reported in section '**Thermal and Pneumatic Modulators**.'

H/C MDGC Applications in Food Analysis

One of the most remarkable fields of the application of multidimensional gas chromatographic techniques is the analysis of medium-to-high complex mixtures of volatiles and semivolatiles that include aroma-active compounds, botanical tracers, and origin authentication markers. Thanks to MDGC technology, food flavor analysis undoubtedly experienced advances and improvements, in terms of results' accuracy and reliability. Key features in flavor analysis include the possibility to obtain better separations, after ^{1}D prefractionation of complex samples and improved sensitivity for target analyte detection due to higher system loadability that is not affected by column or detector overloading; in addition, when separation is followed by olphactometric detection to screen for sensory-active compounds, GC effluent can be diverted to a suitable sniffing port and contemporarily subjected to further separation and/or detection.

Another field where MDGC is very popular is chiral recognition of chiral food flavors; the adoption of chiral selectors as stationary phases for capillary columns in the ^{2}D provided a further approach for flavor authentication.

Figure 2 shows an interesting application of ES-MDGC-MS for the characterization of wine aroma and in particular in the identification of the chiral flavor analytes responsible of the rose note of a Riesling wine.

A further advancement in flavor authentication is represented by MDGC coupled with isotopic ratio mass spectrometry (IRMS). MDGC–IRMS affords to define the origin of diagnostic flavor compounds in consideration of the natural variation in isotope distribution. Constant-flow MDGC–combustion/pyrolysis–IRMS was successfully applied to the authenticity assessment of (*E*)-α(β)-ionone from raspberry cultivars.

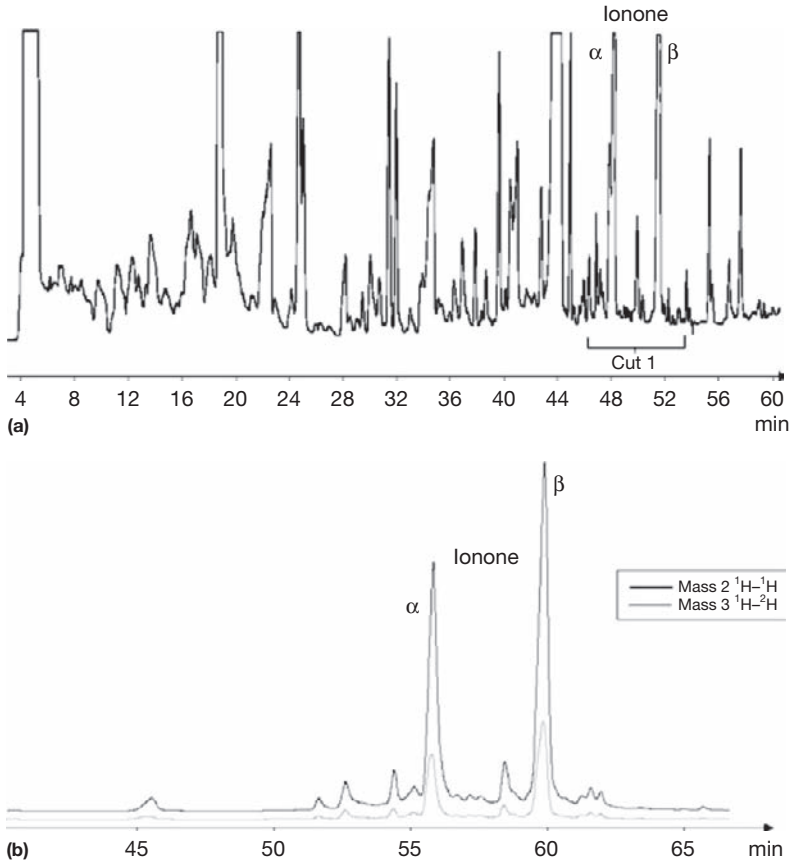

Figure 2 Isolation and characterization of aroma-active compounds in Riesling wine. (a) One-dimensional GC-FID/O on polar stationary phase (Carbowax 20M) enables to identify an aroma-active zone with rose notes. The heart-cut window (inset) shows the same region separated on an apolar (5% phenyl–95% polydimethylsiloxane) ^{2}D column. (b) Combining MS and olphactometric detection, analytes responsible for the rose aroma are univocally identified. (c) Separation of rose oxide enantiomers from a standard racemic mixture by ES-MDGC-MS on a 6TBDMS-DiAc-β-CD together with their odor threshold (OT) values. Lower trace reports the enantiomeric distribution of rose oxides found in a Riesling wine. Reproduced from Marriott, P. J., Chin, S. T., Maikhunthod, B., Schmarr, H. G. and Bieri, S. (2012). Multidimensional gas chromatography. *Trends in Analytical Chemistry* **34**, 1–21.

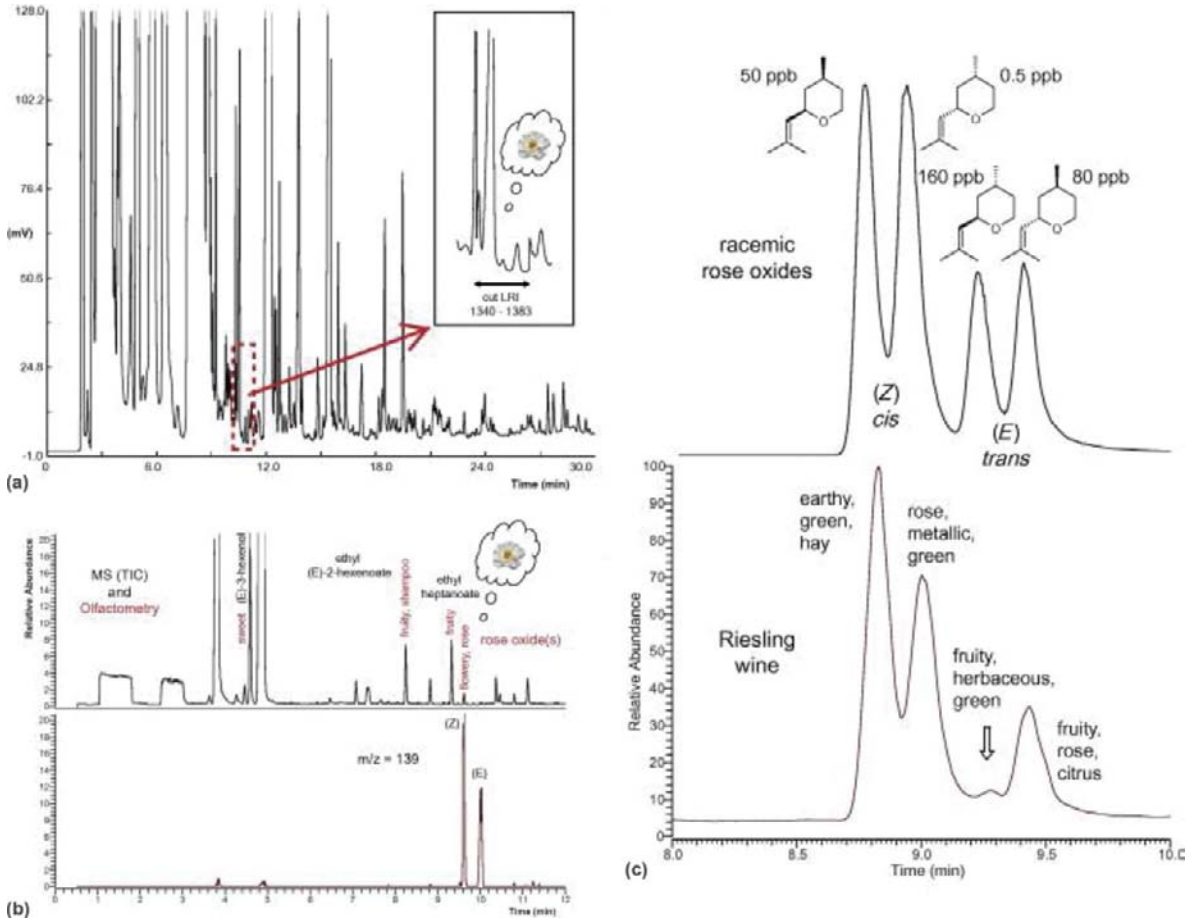

Figure 3 One-dimensional chromatogram (a) with FID detection and H/C MDGC – pyrolysis – isotope ratio mass spectrometry (H/C MDGC–P–IRMS) separation of the ionones eluting region (b) with MS selected ion monitoring (SIM) detection of a raspberry extract. $^{2}H/^{1}H$ isotope ratios were measured by H/C MDGC–P–IRMS run on a twin-oven system from HP model 6890 GC with autosampler A200S (CTC Analytics, Zwingen, Switzerland); multicolumn switching system MCS2 (Gerstel, Mülheim, Germany) coupled to an isotope ratio mass spectrometer (DeltaplusXL) via a pyrolysis reactor (ceramic tube (Al2O3), length = 320 mm, 0.5 mm i.d., and reactor temperature = 1450 °C); and an open split (Thermo Electron, Bremen, Germany). The ^{1}D was equipped with an Rtx-1701 column (30 m × 0.25 mm ID, 1 μm d_f) (Restek, Bad Homburg, Germany) and ^{2}D column with a ZB 5 (30 m × 0.25 mm ID, 0.50 μm d_f) (Zebron, Phenomenex, Aschaffenburg, Germany). The following conditions were employed: splitless injection (injector temperature, 220 °C); ^{1}D temperature program, starting from 100 °C, isothermal for 2 min, and increasing by 2–220 °C min^{-1}, isothermal for 40 min; transfer line temperature, 220 °C; and cut, 46–54 min. The ^{1}D retention times of the ionones were determined by a monitor detector, FID, temperature = 250 °C. The ^{2}D column temperature program started from 60 °C, isothermal for 2 min; increased by 6–180 °C min^{-1}, isothermal for 10 min; and increased by 1.5–220 °C min^{-1}, isothermal for 10 min; carrier gas flow (helium) was 0.8 ml min^{-1}, constant flow. Reproduced from Sewenig, S., Bullinger, D., Hener, U. and Mosandl, A. (2005). Comprehensive authentication of (*E*)-α(β)-ionone from raspberries, using constant flow MDGC-C/P-IRMS and enantio-MDGC-MS. *Journal of Agricultural and Food Chemistry* **53**, 838–844.

Figure 3 reports the total chromatogram (a) and the resulting H/C MDGC chromatogram (b) of a raspberry extract measured by MDGC–IRMS. For accurate and precise isotopic measurement of (*E*)-α-ionone and (*E*)-β-ionone, it is necessary to cut exclusively the ^{1}D band eluting in correspondence to (*E*)-α(β)-ionone. The chromatographic system of this example includes a ^{1}D Rtx-1701 column (30 m × 0.25 mm ID × 1 μm d_f) and a ^{2}D equipped with a ZB 5 column (30 m × 0.25 mm ID × 0.5 μm d_f).

H/C MDGC has demonstrated to be a valuable tool to solve targeted analytic problems: quantitation of informative analytes and identification and abundance assessment of authenticity markers. However, when comprehensive investigation has to be run, through untargeted approaches, multidimensionality has to be extended to the whole analytic run.

Two-Dimensional Comprehensive Gas Chromatography (GC × GC)

Basic Operative Principles

In H/C MDGC, dedicated time-programmable interfaces transfer automatically, and online, fractions consisting of a few components (peaks) eluting from the ^{1}D column for a limited time fraction of the whole chromatographic run to the ^{2}D column; conversely, in GC × GC, each component eluting from the first column is online and automatically trapped, refocussed, and reinjected into the second column. This operation is run by a modulator, thermal or valve-based focussing interface, within a fixed time (4–8 s), which is also the time allowed for the analysis in the ^{2}D.

Comprehensive MDGC (i.e., GC × GC) was firstly introduced by Phillips in 1996, and today, it is widely accepted. This technique has superior separation performance than H/C MDGC due to the comprehensive application of multidimensional separation to the entire sample in every single analytic run. Figure 1 shows how H/C MDGC (Figure 1(b)) and GC × GC (Figure 1(c)) effectively exploit system separation power and the resulting theoretical peak capacity compared to one-dimensional separations (Figure 1(a)).

The core of a GC × GC system is the modulator that accumulates (or traps), refocuses, and rapidly releases fractions eluting from the ^{1}D to the ^{2}D column. These operations are run within a fixed time frame, named modulation period (PM), and repeated across the entire chromatographic run. Modulation has to be sufficiently rapid to preserve the original ^{1}D separation, while ^{2}D separation has to be fast enough to be finished before the injection of the subsequent fraction from ^{1}D effluent.

Appropriate selection of columns' dimensions, combination of stationary phases, PM, and timing enables the full exploitation of system potentials, optimized peak capacity, and high reproducibility in terms of 2-D peaks' distribution over the chromatographic plane and accurate mass transfer (accuracy in quantitative determinations).

Thermal and Pneumatic Modulators

Modulators can be essentially divided into two main categories on the basis of the operative principle: thermal and pneumatic modulators. Thermal modulators use positive and/or negative temperature difference, with respect to the GC oven, to transfer fractions from the ^{1}D to the ^{2}D. Common feature of these systems is the sensitivity enhancement, obtained by the band refocussing promoted by temperature difference, being their duty cycle equal to 1, meaning that the mass transfer is complete without leaks.

Conversely, pneumatic interfaces adopt valves connected either in-line or out-line with the column set, for band transfers.

Until 1998, when a hybrid cryogenic/pneumatic interface was firstly implemented, all modulators were thermal and basically inspired to or developed on Phillips models.

Above all, and excluding prototypes and/or innovative thermal interfaces still close in research and development laboratories, commercial instrumentation is nowadays equipped with different design two-stage thermal modulators.

Briefly, fundamental steps include the following:

I step: The narrow band eluting from the ^{1}D (generally a 25 m × 0.25 mm ID × 0.25 μm d_f) is focussed under a cold jet stream (CO_2, liquid N_2, or compressed air for high-molecular-weight analytes). Focussing can be done on a portion of the ^{1}D column, in a deactivated tubing of suitable inner diameter (generally a narrow bore capillary 0.10 mm ID), or at the head of the ^{2}D column (generally a 1 m × 0.10 mm ID × 0.10 μm d_f).

II and III steps: Reinjection is achieved by heating and volatilizing the condensed fraction; this operation is typically done by switching off the cold jet and letting modulation capillary to be heated by oven temperature (longitudinally modulated cryogenic system – LMCS and dual-jet CO_2 developed by Beens) or by activating an hot jet upstream (loop-type dual jet from Zoex and quad-jet modulators) as depicted in Figure 5. Typically, remobilization has a pulse duration from 10 to a few hundred milliseconds depending on the analyte volatility and on the differential temperature achieved in the cold–hot jet duty cycle.

Analytes are then subjected to a further cycle of focussing and reinjection (*IV step*) to achieve an optimized band width at the entrance of the ^{2}D column. When orthogonal displacement is applied between the two dimensions (by adopting different stationary phases), analytes, not separated in the ^{1}D, can be separated in the ^{2}D.

Despite the superior effectiveness of cryogenic modulation, it is also rather expensive due to the cryogenic fluid consumption and instrumental management. The development of cryogenic-free, low-cost interfaces has therefore been a field of active research in the last few years. Pneumatic modulation has experienced an increasing interest, grown in parallel with the spreading of the technique both in the scientific community and in the industry.

In-line connected pneumatic interfaces adopt multiport diaphragm valves or solenoid valves, some of them also applied in H/C MDGC. The first example of a diaphragm valve-based system was able to sample only very narrow timed fractions from the effluent of the first dimension. With early pneumatic modulators, the band focussing was done by rapid sampling of narrow fractions (50 ms wide, twice a second) from the ^{1}D; being so sharp, they do not need further focussing to enter the ^{2}D column. This interface is not mass-conservative and about 90% of the first column effluent is lost. A step forward was done by the introduction of valve-based systems with flow diversion, which implement the band focussing 'in time' thanks to the adoption of a sampling loop, connected for the 80% of the time in series with the ^{1}D column, and an extra flow of carrier gas toward the ^{2}D column. This system maintains a ^{2}D linear velocity at least 20 times higher than that of the ^{1}D column; the injected band therefore becomes compressed and about 80% of the original mass passes to the second dimension. Alternative configurations may include two parallel ^{2}Ds and consequently two detection systems.

Out-line pneumatic interfaces, located outside the GC oven and not along the sample path, are based on the principles of flow switching implemented in the Deans-switch technology. The device, in its first construction, consisted of a planar metal plate housing a collection chamber connected (via two metal branches) to a three-way solenoid valve. More recently, to attain flexibility and differential flow velocity required by GC × GC, the fixed volume chamber has been replaced by a flow restrictor (a capillary) with selectable inner diameter and length (also define as flexible loop). An electronic pressure-control module supplies the auxiliary gas through a solenoid valve that can be switched. The collection chamber (or the restrictor) is filled periodically with ^{1}D column effluent when the solenoid valve is in the bypass mode. At the end of the collection period, the internal 'loop' is flushed for 100–200 ms by a very high gas flow (typically 20 ml min^{-1}), generated by switching the valve to the inject mode. By setting the first- and second-column flow at a ratio of F_1:F_2 = 1:20, the ^{1}D column band can be compressed (in time) to form a 20-fold narrower

^{2}D column pulse (1.0 s pulse can be injected as a 50 ms pulse). This modulation interface has theoretically no temperature restriction and can be considered truly comprehensive being able to transfer the 100% of the column effluent of the first column into the second column.

Other pneumatic modulators adopting flexible loops and/or multichannel chip-based capillaries, connected via multiport valves to auxiliary gas controllers, have been introduced but to date are not available as commercial products.

Detectors for GC × GC

Narrow peaks of about 50–300 ms base peak width necessitate fast acquisition rates (i.e., at least 50–100 Hz). Flame-ionization detection (FID) with its high maximum frequency of acquisition (50–300 Hz) has been used for years, while element-selective detectors, such as electron capture detector (μECD), nitrogen- and sulfur-chemiluminescence detectors (NCD and SCD), and nitrogen–phosphorous detector (NPD), need careful optimization although in specific field of application they provide useful complementary information for analyte identification. For example, GC × GC with dual detection by NPD and ECD, obtained thanks to microfluidic splitting interfaces, has been successful for multiclass pesticide-residue analysis in vegetable samples.

Mass spectrometry, on the other hand, can be considered the elective detector for GC × GC platforms enabling univocal analyte identification, in combination with retention data, and accurate quantitation. Time-of-flight mass spectrometry (TOF-MS) operating in low-resolution mass detection and fast acquisition is the preferred detector because of its high data-acquisition rate, selectivity, reliable deconvolution of overlapping peaks, and ability to scan for broad mass range. On the other hand, high-resolution TOF-MS, although in principle very straightforward in terms of information potentials related to accurate mass detection, is characterized by slower acquisition speed. The recent introduction of (HR)TOF-MS benchtop instrumentation has opened new perspectives for metabolomics and foodomics applications.

Quadrupole MS (qMS) has experienced in the last years a renewed interest for a number of applications, including qualitative and quantitative. Modern fast quadrupoles (operating with acquisition rate up to 50 Hz for scan and selected ion monitoring (SIM) mode) have in fact demonstrated to be really competitive in terms of quality and reliability of the results provided; in addition, commercial MS databases, prevailing in qMS spectra, can successfully be adopted for unknown identification.

Data Visualization and Elaboration

Although GC × GC is a true two-dimensional separation, the process produces data values that are sequential: chromatographic bands eluting from the ^{1}D are collected, focussed, and reinjected in the ^{2}D where they undergo further separation before detection. In the detector, an analog-to-digital (A/D) converter collects the chromatographic signal at a certain frequency. The digitalized data and related metadata (information about the data) are stored in a file with a specific format for subsequent access. GC × GC systems use an A/D converter to map the intensity of the chromatographic signal to a digital number as a function of time. Single-channel detectors (i.e., FID, ECD, and SCD) produce a single value at each time sample of the chromatogram, while multichannel detectors (such as MSs) produce multiple values (typically over a spectral range) for each time sample.

Data collected are generally stored under proprietary data file format but can be converted to text, as ASCII format comma-separated values (CSV), or to the ASTM format named analytical data interchange (ANDI) that is a standard for chromatography and MS data. Although these standards lack some information specific for GC × GC metadata, as, for example, the modulation cycle, they are commonly used to communicate raw data and to enable elaboration within different software platforms.

Visualization is the next step and enables, at first, a qualitative inspection of chromatographic separation. It can be implemented through *rasterization* by arranging data values acquired during a single modulation cycle as a column of pixels (picture elements), so that the ordinate (*Y*-axis, bottom to top) is the elapsed time for the ^{2}D separation, and then, by arranging these pixel columns so that the abscissa (*X*-axis, left to right) is the elapsed time for the ^{1}D separation. This ordering presents the data in the commonly used right-handed Cartesian coordinate system, with the ^{1}D retention times as the first index into the array.

Data processing extracts higher level of information from raw data enabling peak detection, peak identification, quantitative analysis, and sample comparison, classification, and recognition based on ^{2}D patterns. A detailed description of data processing approaches is beyond the scope of this contribution, but information can be retrieved in suggested further readings. However, an example of advanced fingerprinting is presented in section '**GC × GC Applications in Food Analysis**' for food origin authentication.

GC × GC Applications in Food Analysis

GC plays an important role as an analysis tool for aroma extracts and essential oils, due to the complexity of the matrix and the variable abundance of components from trace (ng/g) to several per cent (g/100 g). Groups of chemically related components (e.g., monoterpenoids and sesquiterpenoids, saturated and unsaturated hydrocarbons, alcohols, carbonyl derivatives, and esters) are common. These compound classes possess similar chromatographic behavior and may have common isobaric molecular ions and/or MS spectra, which makes MS interpretation and analyte identification difficult. The capability to adopt different GC × GC separation mechanisms, as well as producing rationalized ^{2}D patterns, can overcome these problems.

GC × GC in food analysis covers a wide range of applications from the identification and profiling of fats, oils, essential oils, and extracts to food contaminant determinations. Very recently, several authors have demonstrated its potentials in *sensomics* applications by combining advanced sample preparation with multiple detection, including olfactometry in GC (O) × GC-MS platforms.

A typical application of GC × GC in fat and oil characterization is the detailed analysis of FAMEs. FAMEs differ among each other primarily in their chain length, their number of unsaturations, and the *cis*/*trans* configuration of their double

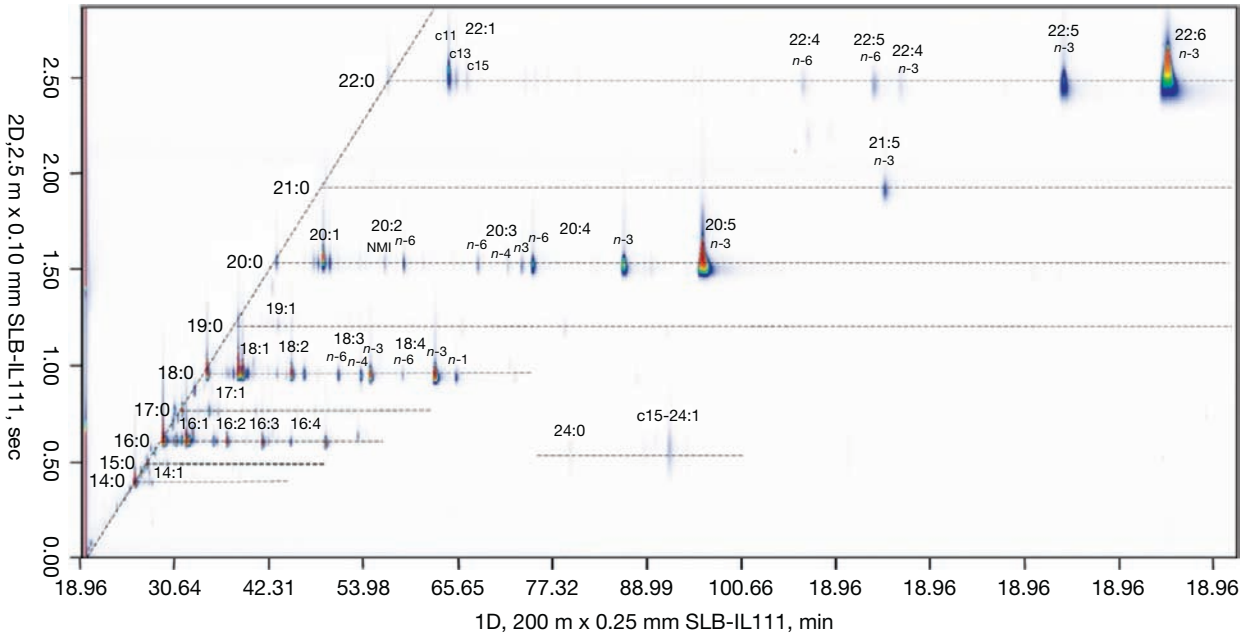

Figure 4 GC × GC separation of FAMEs from a menhaden fish oil. System consisted of an Agilent 7890A GC (Agilent Tech., Wilmington, DE) combined with a Zoex ZX2 dual-stage cryogenic modulator (Houston, TX). The injection port of the gas chromatograph was connected to a 1034 kPa electronic pressure control module, and hydrogen was used as the carrier gas. Supelco SLB-IL111 capillary column (200 m × 0.25 mm ID × 0.2 μm d_f, Bellefonte, PA) was used for ^{1}D separation, and the same capillary column but with different dimensions (2.75 m × 0.10 mm ID × 0.08 μm d_f) was used for the ^{2}D separation. The cryogenic modulator first loop consisted of a 2 m × 0.10 mm deactivated capillary. The 50 cm × 0.15 mm Pd capillary reactor was placed between the ^{1}D column and the modulator. The timing of the modulation was set to 3.5 s; the temperature of the hot jet was set at 350 °C with a pulse of 350 ms. The column oven temperature was set to 170 °C, and the injector port at 300 °C. Analysis was acquired in constant pressure at 690 kPa and the split flow was set to 125.69 ml min^{-1}. Reproduced from Delmonte, P., Fardin-Kia, A. R. and Rader, J. I. (2013) Separation of fatty acid methyl esters by GC-online hydrogenation × GC. *Analytical Chemistry* **85**, 1517–1524.

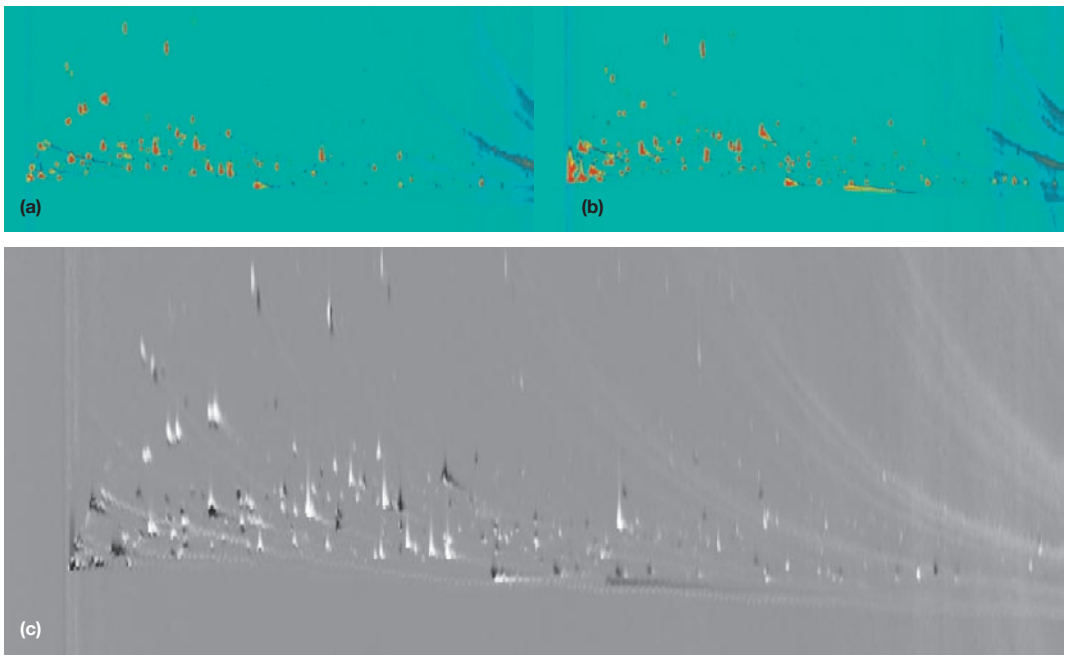

Figure 5 2-D plots of the volatile fraction from an Italian extra virgin olive oil sample and from a defected sample where *muddy* notes where perceived by the official panel (b). Image comparison (c) results, visualized in a grayscale ratio mode (GC-Image™ software), reveal compositional differences by lighter and darker areas. Analysis conditions: HS-SPME sampling, 2 cm StableFlex 50/30 μm DVB–Carboxen–PDMS fiber (Supelco, Bellefonte, the United States); sample amount, 1.000 g; vial volume, 20 ml; sampling time, 40 min; and temperature, 40 °C. GC × GC analyses: GC × GC-MS system: Agilent 6890 GC–Agilent 5975 MSD ionization mode: EI 70 eV (Agilent, Little Falls, DE, USA); transfer line temp., 280 °C; scan range, *m/z* 35–250 in fast scanning mode (10 000 amu s^{-1}). GC × GC interface: KT 2004 loop modulator (Zoex Corporation, Houston, TX, USA); modulation time, 4 s. Column set: ^{1}D, CW20M column (30 m × 0.25 mm ID, 0.25 μm d_f); ^{2}D OV1701 column (1 m × 0.10 mm ID, 0.10 μm d_f) (MEGA – Legnano (Milan), Italy). Analysis conditions: injection mode, split; ratio, 1/20; temp., 250 °C; carrier gas, helium; T. program, 40 °C (1 min)/ 2.5 °C/min/260 °C (5 min).

bonds. Recently, a straightforward investigation approach was proposed. This approach uses identical highly polar separation columns based on ionic liquids (i.e., SLB IL-111 from Supelco) with an online chemical derivatization made on a Pd tubing in the presence of hydrogen carrier gas. The hydrogenated derivatives, which are the saturated forms of eluting FAMEs, are successively separated in the ^{2}D on the basis of their chain lengths. Operating under isothermal conditions, saturated FAMEs lie on a straight diagonal line bisecting the separation plane, while FAMEs with the same carbon skeleton but different number, geometric configuration, or position of double bonds lie on lines parallel to the ^{1}D time axis. **Figure 4** reports the pattern of menhaden oil analyzed under isothermal conditions.

^{2}D separation patterns, obtained by comprehensive methods, have an intrinsic potential as unique and highly informative sample fingerprints, being potentially useful for sample characterization, differentiation, discrimination, and classification. However, this improvement in the informative content produces large and complex datasets (consisting of bidimensional retention data, detector responses, and MS spectra) that require suitable data mining: (1) to interpret the higher level of information and (2) to extract useful and consistent data on sample compositional characteristics.

^{2}D fingerprint analysis can be adopted in food authentication as well as in flavor and aroma characterization, to define the so-called product signature in terms of sensory properties (*sensomics*) and botanical/geographic origins and/or to study modifications induced by thermal treatment, through the volatile fraction of the matrix investigated. **Figure 5** reports the results of a pairwise comparison of the volatile fraction of two olive oil samples from Italian productions: an edible extra virgin olive oil and a defected one classified by the official panel as *muddy*. Differences, visualized in the comparative image analysis, correspond to green and red areas of the chromatographic plane where analytes differing for their abundance between samples are present. Black and white regions correspond to analytes present in just one of the two samples.

Brighter areas correspond to ^{2}D peaks with a larger difference between the reference (*muddy* sample) and the analyzed (extra virgin olive oil) patterns and more abundant in the analyzed image; darker areas conversely correspond to ^{2}D peaks whose abundance is higher in the reference (*muddy* sample) image.

See also: Chromatography: Combined Chromatography and Mass Spectrometry; Essential Oils: Isolation, Production and Uses; Essential Oils: Properties, Composition and Health Effects; Fatty Acids: Determination and Requirements; Fatty Acids: *Trans* Fatty Acids; Quality Control in Food Processing.

Further Reading

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Chromatography: High-Performance Liquid Chromatography

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HPLC: The Separation Workhorse

HPLC is a widely applied analytic technique as evidenced by the number of instruments installed, practitioners, analyses performed, and research papers published annually. HPLC is applied in quality control and is the workhorse of the analytic departments within the pharmaceutical and food industries and environmental control. Furthermore, HPLC holds a central place in most research laboratories: HPLC methods are developed to study drug delivery, adsorption and metabolism, genetic expression of proteins, emerging pollutants in the environment, and nutrients in foodstuffs or in the gastrointestinal tract of animal models or humans, to name only a few characteristic examples. Figure 1 depicts the continuous increase in the number of published articles per year based on a literature search on Scopus, using the terms 'liquid chromatography' in combination with one of the terms 'clinical,' 'pharmaceutical,' 'food,' and 'review.' The fact that more than 1000 reviews are published annually on HPLC and its applications is impressive and indicative of the width and significance of the technique. Indeed, the corresponding numbers for atomic spectroscopy or electrochemistry for the year 2013 are approximately five times less.

With the advent of systems biology and the development of the 'omics' fields, for example genomics, proteomics, and metabolomics, HPLC maintains its major position as an indispensable research tool: new high-resolution mass spectrometry (HRMS) instruments such as the time of flight (TOF) and the Orbitrap MS are ideally combined with different modes of HPLC to map the protein or small metabolite content of samples, cells, or organs. As explained later in this article, difficult analytic tasks require extensive method development in both LC and MS and cannot be served by a single technique.

In HPLC, sample constituents are separated as they migrate through a column in different velocities and elute from the system in different times. The analytes take part in a three-mode interaction between the stationary phase, the sample, and the mobile phase. Analytes that are strongly retained on stationary phase elute late, while analytes that do not interact strongly migrate faster and elute sooner through the system. HPLC utilizes a variety of column formats, dimensions, and chemistries, while mobile phases include a wide range of organic solvent and buffer mixtures. Detection of eluted analytes can be achieved by a single detector or multiple detectors connected in series. Figure 2 depicts a schematic of an HPLC system with its components.

Modes of HPLC

HPLC is practiced in discrete modes that employ different separation mechanisms and find application in different areas. The separation mechanism is governed by the selection of the stationary phase. The two major separation mechanisms are the following:

(a) Normal phase (NP), where analytes partition between a polar stationary phase (e.g., silica) and a low-polarity mobile phase (e.g., hexane, chloroform, or dichloromethane). Dipole–dipole and hydrogen bonding interactions are the major contributors; nonpolar analytes elute quickly, while polar analytes interact strongly with the stationary phase eluting later.
(b) Reverse phase (RP), where polarities are reversed, with the stationary phase being less polar than the mobile phase. Polar analytes remain mostly in the mobile phase showing little attraction towards the stationary phase and elute early. Nonpolar analytes are retained by the stationary phase by a combination of interactions, including nonspecific hydrophobic interactions.

Reversed-phase liquid chromatography (RPLC) is by far the widest applied mode largely due to its more robust nature and its compatibility with aqueous samples such as biological, environmental, and food samples and extracts. However, RPLC is restricted to the analysis of medium-polarity and medium-molecular-weight compounds. Polar or high molar mass compounds are usually problematic. The former include some of the most important metabolites in the biochemical pathways: amino acids, nucleotides, organic acids, and amines. These are not retained in RPLC and elute rapidly with the solvent front.

One solution towards the analysis of polar molecules is derivatization, which is used to modify analyte molecules and improve retention and detectability; for example, amino acids are analyzed as derivatives that absorb in UV or exhibit fluorescence. However, derivatization as a reaction of organic molecules imposes a number of drawbacks:

1. Analytes react at different rates.
2. Molecules can behave differently in different environments (different pH or salinity values may yield inconsistent results).
3. An excess of reagents is introduced to the system.
4. Derivatives have a limited lifetime, thus introducing a potential error source.

Rapid advances in MS enabled the development of multianalyte methods that can determine hundreds of analytes in a single run, thus partially overcoming the need for improved analyte detector response. Nevertheless, nonderivatized polar molecules will show poor retention and thus, significant efforts are invested in the development of separation modes appropriate for the analysis of polar analytes and ionic or ionizable species. These include aqueous normal-phase

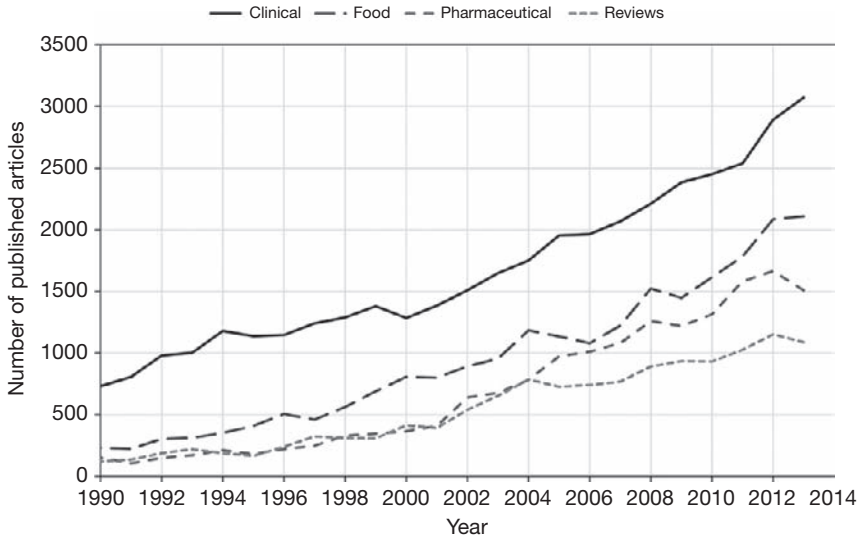

Figure 1 Number of published articles per year, returned when using the terms 'liquid chromatography' and 'clinical,' 'food,' 'pharmaceutical,' or 'review.' Search performed on Scopus on April 27, 2014.

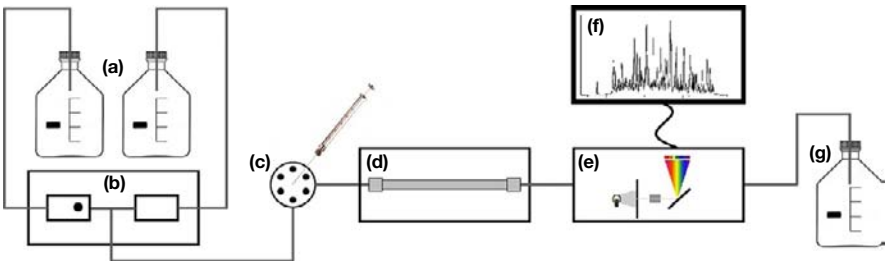

Figure 2 Schematic representation of an HPLC instrument. a: Solvent compartment; b: pump; c: sample injection port or autosampler; d: column compartment; e: detector(s); f: data recording and processing; g: waste.

(ANP) chromatography, ion pair chromatography (IPC), ion exchange chromatography (IEC), and hydrophilic interaction liquid chromatography (HILIC). HILIC can be considered as a reversed reversed-phase chromatography: analytes are separated using principally organic mobile phases and a hydrophilic stationary phase, eluting in order of increasing hydrophilicity. HILIC phases are categorized as neutral (where electrostatic interactions are not evident), charged (where electrostatic forces dominate the separation), and zwitterionic. HILIC is increasingly used in the analysis of polar compounds, providing the elution order reversed to the order provided by RPLC. However, certain polar groups, such as phosphorylated molecules, may remain problematic, so other LC modes are employed: IEC and IPC make use of mobile phases that contain ionic species typically dissolved in the mobile phase (in IEC, ionic species may be immobilized in the stationary phase).

Ionic interactions are stronger and thus harder and slower to disrupt, and as a result, IEC generally provides wider peaks compared with RPLC. IEC is extensively used in the analysis of proteins, taking advantage of ionic interactions between charged proteins and the stationary phase. IEC can be further divided into cation or anion exchange chromatography, while certain stationary phases encompass ampholytes or species that may appear at a neutral or charged state: tertiary amines will acquire a positive charge at low pH but will be neutral at high pH. The strength of the ionic interactions between ligands and analytes is determined by the number and location of the corresponding charges. To facilitate analyte binding, mobile phases of low to medium salinity are initially employed (loading phase). To achieve analyte elution, the salt concentration in the mobile phase is increased, thus eluting analytes in the order of strength of ionic interactions.

On the other hand, IPC uses ion pair reagents to modify the stationary phase: these are dissolved in the mobile phase and interact with the hydrophobic ligand of RP stationary phases. The polar part of the ion pair reagent is exposed to the mobile phase, ready to interact with polar metabolites increasing their retention. IPC when coupled to MS faces severe limitations due to the high background signal generated by many widely used ion pair reagents. The latter contaminate MS systems almost permanently, rendering the MS system practically nonusable for different analyses. IPC has recently found an important application area in the analysis of metabolites involved in central metabolism: intracellular metabolites such as nucleotides, coenzyme A esters, nucleosides, phosphorylated sugars, and metabolites involved in energy metabolism such as AMP, ATP, and NADP.

Additional liquid chromatographic techniques relevant to food and clinical analyses include affinity chromatography

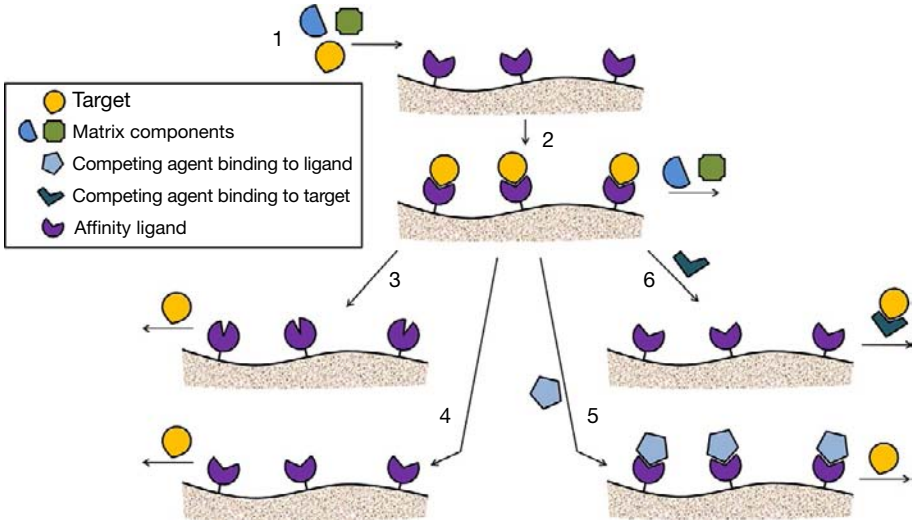

Figure 3 Schematic representation of the binding mechanism and elution protocols applied in affinity chromatography. 1: Sample loading; 2: targeted analytes bound, other matrix components eluted; 3: elution of targeted analyte by change of conditions (pH and ionic strength); 4: isocratic elution; 5: addition of competing agent binding on the affinity ligands; 6: addition of competing agent binding on the targeted compound.

(AC) and size exclusion chromatography (SEC). In AC, separation is based on analyte affinity to ligands or receptors bound on the stationary phase. AC shows potential in the isolation of valuable compounds of biological interest from complex matrices taking advantage of the natural affinity between two counterparts, such as an enzyme for its substrate/inhibitor or an antibody for its antigen. One of the counterparts is immobilized on a chromatographic column, acting as a receiver, binding its specific partner over other compounds. The captured analyte is eluted by changing the conditions in the mobile phase (pH, ionic strength, and competitive analyte) isolated in pure form (Figure 3).

SEC utilizes stationary phases of a controlled porosity that physically block the flow path of the analytes as they migrate through the column. Analytes of molecular volume smaller than the particle pores penetrate deeper and elute slower, compared with relatively large molecules that will be excluded from the pores and elute faster. Using appropriate standards, SEC can be used to determine the molecular weight of synthetic and natural macromolecules, while both AC and SEC are widely used in the separation and isolation of proteins and polysaccharides from complex mixtures.

A recent development is two-dimensional LC (2-D-LC), which is employed to address the need for additional separation power. 2-D-LC is increasingly used in biomarker discovery and identification, in the oil industry, in the analysis of polymers and biopolymers, and wherever additional separation power is necessary. 2-D-LC implements two orthogonal separation mechanisms that run sequentially. The two modes should be based on different complementary separation mechanisms. For example, in protein/peptide analysis, the ion exchange or size exclusion column is coupled online with RP: analytes are separated in the first column and subsequently in the second column (RPLC). The second column is typically smaller in dimension so that analysis is rapid to cope with the continuous flow of analytes eluting from the first column. 2-D-LC requires sophisticated instrument control and data handling in order to synchronize the pumping of different solvents through two columns, movement of the valves to alter the flow path, switch from column to column for loading, and elution and washing. Results are depicted as heat maps or contour plots.

Stationary Phases

The vast majority of HPLC columns utilize porous, spherical octadecyl (C_{18})-functionalized silica particles of 3–5 μm diameter, while other chemistries include octyl (C_8), phenyl-hexyl, and amine-modified phases. Fluorinated phases provide alternative selectivity and separation characteristics, exhibiting higher retention for polar compounds.

These phases have typical surface areas up to 400 $m^2\,g^{-1}$ and pore diameters of ~100 Å. Such materials owe their popularity to their relative ease of manufacturing in large quantities, simplicity of surface modification, chemical and mechanical stability, excellent batch-to-batch reproducibility, and extensive documentation on their utilization as separation media. However, the growing demand for analysis of increasingly complex samples and higher sample throughput has driven the development of new stationary phases, with numerous new materials introduced every year. Perhaps, the most important recent development was the introduction of ultra-high-pressure LC (UHPLC) systems in the early 2000s. UHPLC employs sub-2 μm particles and accommodates operating pressures higher than 1000 bar. This development necessitated major advances in column technology and instrumentation. Apart from the obvious advantages of higher separation power that lead to higher specificity (analytes are less likely to coelute with interferences) and sensitivity (lower background noise), UHPLC can increase the analytic throughput: an HPLC method of 10 min per sample may be reduced to 3–4 min in UHPLC. The major advantages and operating parameters of UHPLC are presented in Table 1.

Table 1 Characteristics and typical operating parameters of UHPLC

Characteristics of UHPLC	*Comments*
Operating parameters	Pressure: $\leq$20 000 psi ($\sim$1400 bar) Flow rate: $\leq$5 ml min^{-1} Particle size: sub-2 μm particles Column dimensions: $\geq$to 2 mm i.d. Detection rate: $\geq$40 points s^{-1}
High throughput	$\sim$3–10-fold increase in throughput vs. HPLC, without loss of resolution
Rapid method development	Ideal for rapid column and mobile phase screening and method optimization
High resolution	Up to $\sim$3-fold increase compared with HPLC
Reduced solvent consumption	Typical 5–15-fold reduction vs. HPLC due to shorter analysis times and smaller i.d. columns
High sensitivity	$\sim$3–10-fold increase of mass sensitivity
Higher precision	$\sim$2–3-fold increase in retention time and peak area precision
Compatibility	Compatible with high temperatures, 2-D-LC and core–shell columns

Despite its clear advantages, UHPLC is associated with significant equipment costs that hinder global transition from HPLC. To this end, core–shell or superficially porous particles have recently attracted significant interest. These have a solid, impenetrable core that supports a thin, porous layer, typically from 0.2 to 0.6 μm (Figure 4). In totally porous particles, diffusion paths within the pores are longer, increasing peak broadening and decreasing column efficiency; superficially porous particles exhibit superior mass transfer properties, reducing peak broadening and enhancing column performance. In fact, the efficiency for columns of 2.7 μm core–shell particles rivals that for sub-2 μm totally porous particles, while the operating back pressure is halved, eliminating the need for UHPLC instruments. An example of the performance of 5 μm core–shell vs. fully porous particles is shown in Figure 5.

Monolithic columns were introduced mainly aiming to overcome limitations of mass transfer described as the C term in the Van Deemter equation. Compared with particulate materials, monoliths exhibit very low C values that do not vary significantly within the flow rate. Monoliths comprise a polymer or silica network where pores are interconnected generating an unobstructed flow path that in contrast to particulate columns does not have closed pores. Mass transfer is enhanced enabling higher flow rates. Monoliths have found application mostly in the analysis of larger molecules (e.g., proteins and polynucleotides) and in large-scale biotechnology applications, for example, in the form of convective media conveniently used for the purification of target proteins produced in bioreactors.

Furthermore, nano-LC and chip-based LC are increasingly used especially in the analysis of biomolecules such as proteins. Nanoflow enhances analyte sensitivity and at the same time is well suited to handle reduced sample volumes. Recent developments bring HPLC in the core of the MS tip (e.g., in the form of ionKey/MS^{TM}). While developments such as the latter appear attractive, especially for use by less-trained personnel, their adaptation requires significant capital investment costs.

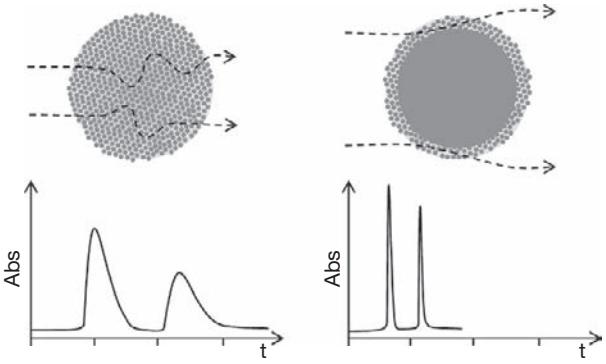

Figure 4 Schematic depicting the cross sections of fully porous (left) and core—shell (right) silica particles with the corresponding chromatographic traces showing sharper peaks on the core—shell material.

Column chromatography will most likely continue to dominate as it provides unparalleled versatility and peak and loading capacity. Chip-based LC and other such formats that have been presented in recent years are still in the development stages and, although some promise has been shown, are still a long way from claiming the HPLC market.

Instrumentation

Analytical instrumentation of (U)HPLC shows continuous development since the introduction of the method in mid-1970s. Instrument manufacturers have invested in the development of higher quality materials, instruments, and software, and by the end of the twentieth century, HPLC technology had overcome most reproducibility and stability issues faced in its first decades. In the early days, HPLC suffered from issues such as the use of additives, the blockage of tubes and columns, the introduction of unnecessary peak broadening, and poor quality of frits, ferules, connectors, etc. Such issues have now been addressed with the development of advanced quality or fit-for-purpose media: Metal-free systems can be used for the analysis of proteins; sample preparation removes particulate matter before clogging the system; developments in the production phase allow high column batch-to-batch reproducibility: change to a new column of the same material will not result in difference in method performance. As a result, analysis throughput has increased dramatically, while method transfer and interlaboratory repeatability are greatly enhanced.

Detection and Hyphenation

Detection in HPLC typically utilizes optical, electrochemical, and spectroscopic detectors. These should provide fast data acquisition to cope with the flow dynamics of LC. Given that ten or more data points are required for optimum peak quantitation, fast data acquisition becomes of primary importance for accurate quantitative analysis in HPLC and UHPLC in particular, where peaks may have widths of less than 5 s.

In practical terms, a spectrophotometric detector is an integral part of the modern (U)HPLC instrument and thus hyphenation means coupling LC to a mass spectrometer. UHPLC–MS dominates the field of biopharmaceutical, food,

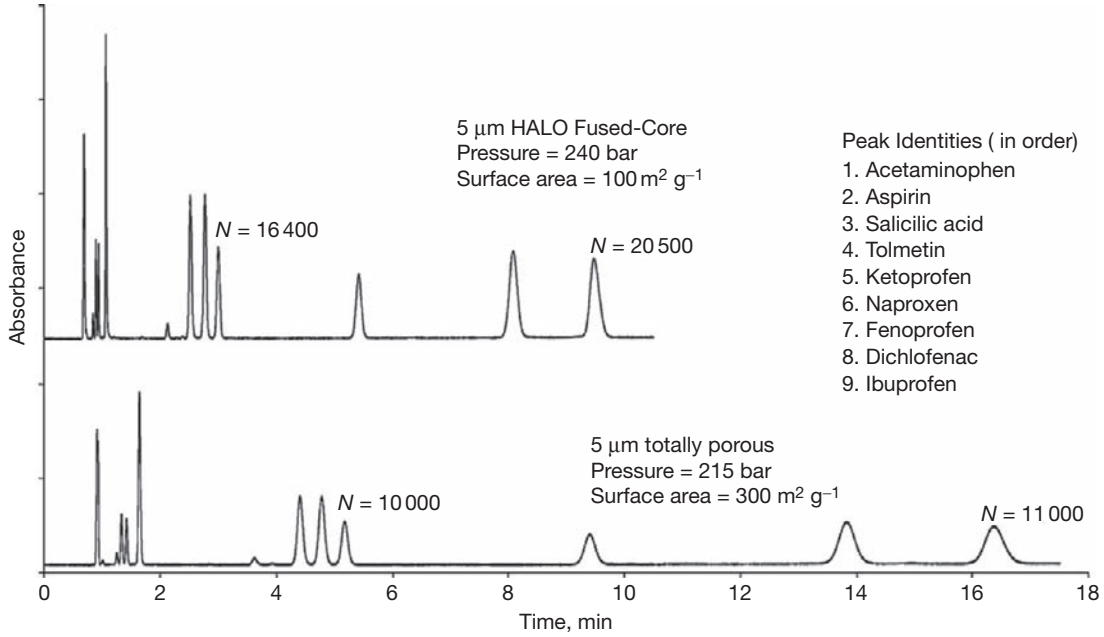

Figure 5 Comparative separations with 5 μm particles: fused-core vs. totally porous. Column dimensions: 4.6 mm × 150 mm; temperature: 35 °C; flow rate: 2.0 ml min^{-1}. Reprinted with permission from Elsevier from DeStefano, J. J., Schuster, S. A., Lawhorn, J. M. and Kirkland, J. J. (2012). Performance characteristics of new superficially porous particles. *Journal of Chromatography A* **1258**, 76–83.

and environmental analyses. MS spectroscopists may consider HPLC as merely an introduction step to the MS system, while chromatographers argue that MS is another type of LC detector; however, the truth is that separation is necessary prior to MS for a number of reasons, such as the need to separate isomeric/isobaric substances and the drive to avoid ion suppression effects. No matter the sophistication and the cost of the MS analyzer, such incidents would lead to poor or no analyte detection even for analytes of high concentrations. Implementation of HPLC, and UHPLC in particular, reduces this threat, albeit it cannot eliminate it, by adding an orthogonal element of separation: molecules are first separated according to physicochemical properties and are next introduced to MS.

Applications

This section aims to illustrate selected examples of HPLC applications in the analysis of biological and food samples. LC–MS has become the gold standard in a number of application fields and this has been recognized by regulatory authorities, which require its application for the majority of analyses of primary importance such as contaminants in foods, prohibited substances (e.g., steroids in foods or biological samples), narcotics, pharmaceuticals, and endogenous metabolites. Adaptation of the technology is enforced in order to ensure that ion suppression effects are minimized in the analysis of unknown samples and that analyte identification by a combination of retention time and MS signal is unambiguous: identification is verified by retention time, precursor and product ion mass, and product ion ratios and is corroborated against reference standards analysis using explicitly set tolerance levels.

Application of HPLC in Food Analysis

Our food supply includes a variety of increasingly processed materials from various sources. Substances such as fertilizers, pesticides, veterinary drugs, growth promoters, colorants, antioxidants, natural and artificial sweeteners and flavors, natural and synthetic vitamins, and carbohydrates are used to improve productivity and thus increase competitiveness and profit margins of the food industry. To ensure food safety and nutritional quality, regulations have been established for the assessment of food quality, the assessment of nutritional intake, and traceability especially for foods and products with designation of origin and so forth. Regulation agencies often deal with the detection of prohibited substances and substances with maximum permitted levels such as chemical additives, residues, and contaminants in food products explicitly specifying methodological considerations to harmonize methods and ensure interchangeability of results across different laboratories and states.

Increasingly, food analysis methods are built around HPLC, which has proved to be an optimal technology for detecting and/or quantifying the vast majority of food analytes. In recent years, the demands for high sample throughput, high efficiency, and high resolving power have significantly boosted (U)HPLC technology in areas of analytic research. Several multianalyte methods have been developed offering simplicity and reduction in cost. The key requirement for developing such multimethods is the application of generic extraction procedures with a careful examination of matrix effects. Recently, methods covering more than 250 veterinary drugs, pesticides, mycotoxins, and other potential chemical contaminants have been developed and are applied in routine analysis.

Fused-core columns have been applied in the analysis of bisphenols in canned food and soft drinks, phenolic

compounds in beverages, and banned substances in egg, honey, and milk. HILIC has proved to be complementary to RPLC in the analysis of foods: meat, milk and its products, eggs, packaged food, natural products, honey, and cereals. Fluorinated phases found use in the separation of tocopherols, alkaloids in natural products, and photoinitiators in packaged food. Higher temperatures (i.e., >60 °C) may expedite analysis due to the reduction of solvent viscosity and back pressure. Using elevated temperatures, 12 triazine herbicides were separated in 2.5 min. Other applications showed separation improvements (increase in resolution) at very low temperatures (5 °C) for the analysis of photoinitiators in packaged food.

Sample preparation is still one of the most important steps of an analytical method. Effective sample preparation is important to reduce matrix interferences and achieve reliable analytic results. A sample preparation method should be fast, simple, accurate and facilitate the analysis of a large number of samples. Modern sample preparation techniques such as on-line solid-phase extraction (SPE), supercritical fluid extraction (SFE), turbulent flow chromatography, and molecularly imprinted polymers (MIPs) can permit high-sensitivity HPLC analysis in the parts per trillion (ppt) range. Utilization of advanced techniques in the determination of target analyte groups such as antibiotics and growth promoters in a variety of food products has improved sensitivity and selectivity, cleaning up target analytes and removing matrix-interfering compounds.

Current rapid development of UHPLC has led to its increasing adoption as the approach of first choice for confirmatory analysis for multiple-target analysis in a variety of food products. Despite technological challenges, such as narrower peaks, and the impact of sample matrix effects, the overall injection cycle times have shortened, detection limits have improved, and chromatographic resolution and the number of compounds in multianalyte methods continue to increase. We expect the speed of analysis to increase further by using even higher temperatures and pressures, allowing the separation of a much larger number of analytes in the field of food analysis.

Application of HPLC in Health-Related Analyses

Contemporary bioanalytical laboratories are equipped with HPLC or UHPLC systems used in the analysis of small molecules or macromolecules in trace or semipreparative quantities. A major advantage is that basic instrumentation may be conveniently modified for a variety of purposes. Hence, a single LC pump can deliver the mobile phase or can be combined with additional pumps in 2-D-LC systems for, for example, proteomics or for purification and isolation of analytes of interest for characterization purposes.

Two decades ago, pharmaceutical industry laboratories relied on HPLC and immunoassays for drug discovery and development: HPLC assays accounted for more than 80% of pharmacokinetics/pharmacodynamics (PK/PD) needs and toxicity studies. The landscape is now totally dominated by LC–MS/MS where RPLC is a key element of method development and validation. Regulatory bodies such as the FDA and EMEA publish explicit guidelines for the validation of LC-based methods.

The development of UHPLC facilitates high-throughput screening (HTS), a powerful tool that enables the performance of routine analysis in, for example, food quality control or the environmental monitoring sector. The pharmaceutical industry utilizes HTS to promote products to the next development step at a faster rate: from combinatorial synthesis to drug target finding and lead finding, structure–activity evaluation, *in vitro* and *in vivo* application, and PK/PD assessment. As a drug candidate moves through the different sections of a pharmaceutical industry, assays are developed, and for all these purposes, the advantages offered by (U)HPLC remain unparalleled.

HPLC and LC–MS have achieved significant penetration in clinical laboratories, substituting other types of assays, such as ELISA or photometric assays. The major drivers behind this development are the benefits offered by HPLC and LC–MS with regard to detection specificity compared with cross-reactivity often observed in enzymatic assays or immunoassays. HPLC and LC–MS in particular can offer unambiguous detection and wide dynamic ranges for quantitation even when multianalyte methods are applied. HPLC is now used in parallel to GC–MS, and both methods cover the majority of potential target analytes in the clinical environment. GC–MS remains indispensable in the analysis of small volatile molecules, organic acids, and other key analytes in clinical and forensic toxicology; however, HPLC–MS/MS offers higher sensitivity while reducing the time and labor required for sample preparation (e.g., for derivatization necessary prior to introduction in GC). A major limitation however, is the lack of commercial mass spectral libraries, which necessitates the generation of local databases (lab-built libraries). At present, HPLC is practiced in virtually all hospitals in the developed world, while most of the major hospital laboratories are equipped with a variety of LC–MS and LC–MS/MS systems. Analytic tasks are distributed to specialized laboratories that are national reference centers for the analysis of groups of key analytes (e.g., amino acids, endocrine disruptors, and antibiotics). HPLC–MS/MS systems are widely used in clinical chemistry laboratories and pharmacology, toxicology, and forensic facilities. These laboratories are tasked with the analysis of toxins and drugs of abuse, therapeutic drug monitoring (TDM; such as the quantification of pharmaceuticals with a narrow window of therapeutic level concentration, e.g., certain groups of antibiotics and the majority of immunosuppressants and antipsychotic drugs), clinical trials of new pharmaceuticals, and the emerging field of bioequivalence of generic drugs. Emerging fields such as the assessment of occupational exposure and workplace drug testing represent new application areas.

Conclusions

HPLC offers an indispensable analytic tool in modern science, with thousands of new methods and protocols published every year. Instrument and column manufacturers continue to improve the specifications, performance, and quality characteristics of their products making the technology more robust, reliable, and capable to address emerging analytic problems resulting from modern human activities. As HPLC represents a

key element in the majority of bioanalytical and biotechnology fields, the central position of the technology seems secure for many years to come. A general advice for the practitioners, especially those coming from other scientific fields and using HPLC only as a tool for a certain purpose, is to remember that LC represents a dynamic process; hence, results can be influenced by different physicochemical factors. The sophistication of current state of the art is based on sound scientific principles and decades of dedicated work from thousands of scientists from the manufacturer and the research sectors. This striking science can be recognized every day in the separations achieved in thousands of LC systems globally. Separations undreamed of some years ago can now be achieved conveniently and offered as kits or standard operating protocols (SOPs). The continuing investments from the academia and the manufacturers will maintain the drive toward lower detection limits, higher resolution power, and higher quality separations.

See also: Antibiotics and Drugs: Residue Determination; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Mass Spectrometry: Principles and Instrumentation.

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Chromatography: Supercritical Fluid Chromatography

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Introduction

Supercritical fluid chromatography (SFC) is a variant of high-performance liquid chromatography (HPLC) in which a supercritical fluid is used to replace the liquid mobile phase. HPLC, in particular reversed-phase liquid chromatography, has long established itself as a pertinent analytical technique in the food and health areas. Decades of application and research have led to this established technique being the mainstay of analysis in all steps of food analysis and health analysis.

SFC is considered as a valuable alternative over HPLC because the mobile phase properties differ significantly. A supercritical fluid has properties intermediate between those of a liquid and a gas. The temperature and pressure of a component in its supercritical state should be higher than the critical values, T_c and P_c, respectively, as seen in Figure 1. When a component is in its supercritical state, it cannot be liquefied by increasing the pressure. Supercritical carbon dioxide (CO_2) is most commonly used in SFC, mostly because it is cheap, readily available, and environmentally friendly. Moreover, it is very safe to be used in the food industry since it is easily removed from samples by simple expansion and evaporation.

SFC was from its early years recognized as a hybrid of HPLC and gas chromatography (GC). SFC was acknowledged as a technique that enables rapid separations, comparable to HPLC, and allows separation of substances with high boiling points, which is also possible in GC. The properties of supercritical CO_2 differ from those of liquids used in HPLC, in the sense that CO_2 is less viscous and has a higher diffusivity than liquids. This leads to rapid mass transfer, therefore resulting in a higher sample throughput, faster equilibration, and shorter cycle times. Since solvent consumption is low, waste generation is also negligible.

Supercritical fluids are used for extraction and for chromatography. Food products analyzed or produced by the utilization of supercritical fluids are seen in everyday life. Some of these products are highlighted in Figure 2.

The main difference between an HPLC and SFC system is that in SFC, the pumping system needs to be adapted to allow the pumping of a compressible fluid (Figure 3). The second difference is that there is a pressure regulation system, which restricts the column outlet flow and creates a system back pressure, keeping CO_2 in its super-/subcritical state. It is known that the solvent strength of the supercritical mobile phase is strongly related to its density (being controlled by temperature and pressure). It is also well known that the polarity of the nonpolar carbon dioxide can be increased by the addition of polar organic solvents, such as methanol. Hence, the partition between the mobile phase and stationary phase, of analytes with varying polarities, can be changed and their retention altered.

SFC can be coupled with a number of other techniques, for example, mass spectrometry (MS), ultraviolet (UV)–Vis spectroscopy, flame ionization detection (FID), LC, and supercritical fluid extraction (SFE). These numerous advantages are quite appealing and are possibly the reasons why there has been an increase in the utilization of this technique in both the food and health sectors. This is reflected by the number of publications in the area, which has shown a steady increase in the last years.

SFC is a versatile technique and can be used to analyze a large number of compounds. However, it has the unique ability of analyzing thermally labile and nonvolatile substances. Substances with such properties are also found in the food and health industries, for example, thermolabile pesticides, fatty acids, and numerous medicinal drugs. Moreover, SFC is a powerful technique in the separation and analysis of chiral substances. A chiral compound is one that consists of stereoisomer pairs referred to as enantiomers that have nonsuperimposable mirror images. Chirality may lead to one enantiomer being responsible for the biological activity, while the other might be much less potent as in the case of adrenaline. Antagonistic, toxic, and no effects are also possible with the other enantiomer. Twenty-five percent of pesticides used in the food industry are also chiral. The importance of chirality was highly recognized after the thalidomide (Softenon®) disaster in the early 1960s. Thalidomide was being used by pregnant women to treat morning sickness. However, it was later shown that one enantiomer was responsible for the teratogenic effect, affecting fetuses during their development, resulting in babies with missing extremities. This article will focus on the applications of SFC in food, health, and drug analysis. The studies presented are not exhaustive, but they will give the reader a good understanding of the applicability of SFC in different domains.

SFC in Food Analysis

Food analysis is a sector of vital importance in analytical chemistry, which may sometimes prove to be a challenge due to the number and wide variety of compounds that must be analyzed. These compounds range from nutrients and compounds with bioactivities to pesticides, contaminants, or illicit substances that are considered harmful. Food analysis with SFC focussed mostly on lipids and fat-soluble vitamins, probably due to the high solubility of these analytes in CO_2. However, the trend is moving to use SFC also for the analysis of more polar compounds, for example, amino acids and carbohydrates, and the analysis of compounds with low volatility or UV absorbance such as triterpenoid compounds. This section presents an overview of different applications of SFC in food analysis. Table 1 shows a summary of SFC applications in food analysis, which will be discussed shortly.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00160-4

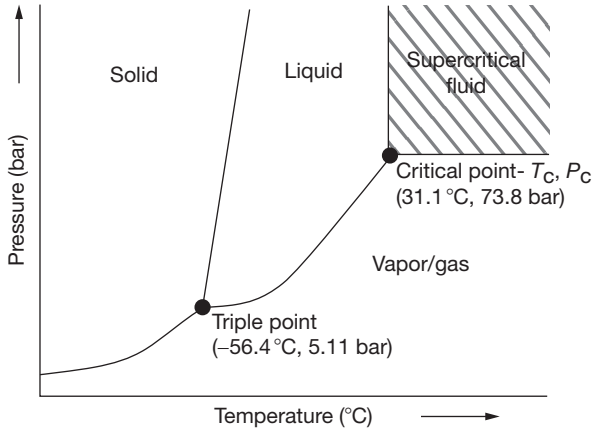

Figure 1 Phase diagram for carbon dioxide.

Analysis of Lipids

Lipids play a vital role in cellular signaling and energy storage, while phospholipids are the main cell membrane constituent. Lipids are hydrophobic compounds, but they vary greatly in their polarity due to the diverse moieties that can form part of lipids. Some include hydrophobic acyl chains; others, hydrophilic phosphoric acid or sugar moieties. Therefore, analyzing lipids in a mixture is not easy. The study of lipid classes in their natural environment is referred to as lipidomics.

An SFC–MS method that allows the simultaneous analysis of lipids with diverse structures and polarities has been established by Bamba et al. SFC–MS was used to analyze a complex mixture of 14 lipids, including phospholipids, glycolipids, neutral lipids, and sphingolipids, extracted from the leaf of *Catharanthus roseus*. All lipids were successfully separated within 15 min. This system is suitable for studies on lipidomics because it is useful as a fingerprinting method for the screening of diverse lipids and also for the profiling of the specific constituents.

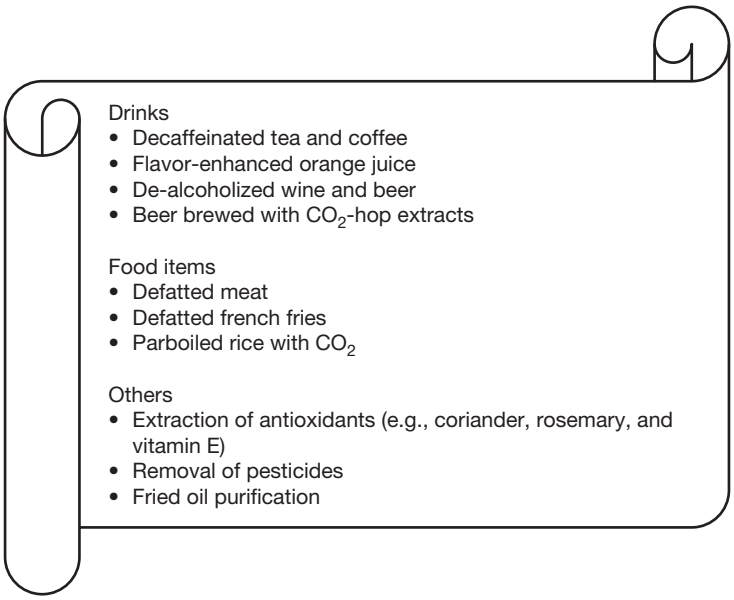

Figure 2 The application of supercritical fluids to daily food production.

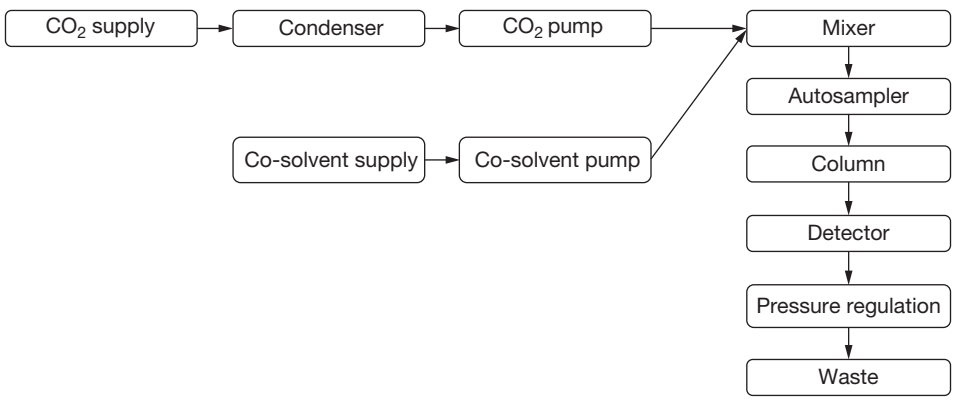

Figure 3 Supercritical fluid chromatography process diagram.

Table 1 Summary of some SFC applications in food analysis (abbreviations are explained in the text)

Analyte	*Sample*	*Detector*	*Stationary phase*	*Mobile phase*	*References*
Lipid analysis					
DHA and EPA concentrates	Transesterified tuna oil	UV	Octadecyl silica	Pure CO_2	[1]
EPA-EE	Fish oil mixtures	Not specified	Silica	Pure CO_2	[2]
Phospholipids	Soybean flakes	Not specified	Alumina (preparative)	CO_2 modified with a 5–30% ethanol/water 9:1(v/v) eluant	[3]
Fat-soluble vitamins					
β-carotene	Standard mixture	DAD	ODS and C30	CO_2 and varying percentages of methanol	[4]
Tocopherols	Olive by-products	MS	Capillary column pack with silica particles and 10% polyethylene glycol	Pure CO_2	[5]
Tocopherols	Standard mixtures	UV	Silica	CO_2 and varying percentages of ethanol	[6]
Carotenes, vitamin E, sterols, and squalene	Palm oil	UV	Silica	CO_2 with 4% ethanol	[7]
Carotenoids and their epoxidized products	Human serum and LDL samples	MS	Silica, bonded phenyl, carotenoid, and ODS	CO_2 and varying gradients of methanol with 0.1% ammonium formate as additive	[8]
Nutraceuticals					
Phenol diterpenes	Rosemary	UV	Specially designed column packed with silica particles coated with nonpolar SE-54 (5% phenyl silicone, 95% methyl silicone)	CO_2 and varying percentages of ethanol	[9]
Carnosic acid and carnosol	Rosemary	FID	Specially designed column packed with silica particles coated with nonpolar SE-54	Pure CO_2	[10]
Davanone	Natural davana oil	UV	2-Ethylpyridine	CO_2 and a methanol gradient (1–20%)	[11]
Polyphenols	Grape seed extract	UV	2 Diol columns coupled in series	CO_2 and a methanol gradient (17–45%) with different additives	[12]
Glucobrassicin	White cabbage	UV	Silica	CO_2 and varying gradients of methanol	[13]
Sinalbin	Mustard	UV	Silica	CO_2 and a methanol gradient (4–15%) with trifluoroacetic acid as additive	[14]
Monitoring of pesticides					
Triazole pesticides	Standard preparations	UV	Chiralpak AD	CO_2 and varying percentages of methanol, ethanol, or 2-propanol and with triethylamine and trifluoroacetic acid as additives	[15]
Multiple pesticide residues	Canned foods, fruit, and vegetables	UV	Silica	CO_2 and varying percentages of methanol	[16]
Other compounds					
Underivatized amino acids	Standard mixtures	ELSD	Diol bonded to silica	CO_2 and varying gradients of methanol and water, triethylamine and trifluoroacetic acid as additives	[17]
Caffeine, fructose, glucose,	Standard mixture	ELSD	Ethylpyridine bonded to silica	CO_2 and a methanol gradient (5–50%)	[18]

(*Continued*)

Table 1 (Continued)

Analyte	*Sample*	*Detector*	*Stationary phase*	*Mobile phase*	*References*
sucrose, and NHDC					
Triterpenoid compounds	Apple pomace extracts	ELSD	Ethylpyridine bonded to silica and phenyloxy propyl	CO_2 and varying percentages of methanol	[19]

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The following three studies concerning lipids were developed at analytical scale, optimized, and then upscaled to the preparative level. The first study ([1] – refer to Table 1) focuses on the isolation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) ethyl ester concentrates from transesterified tuna oil using SFC. The interest in these compounds is coming from the fact that EPA and DHA polyunsaturated fatty acids (PUFAs) are found in fish oils in ranges between 6% and 26% weight of the total fatty acids. These fatty acids have shown clinical benefits in cardiology, neurology, and mental health, among others. They continue to be studied due to their market in food additives and supplements and also in pharmaceutical products.

Pettinello et al. ([2] – refer to Table 1) investigated the attainment of enriched fractions of eicosapentaenoic acid ethyl ester (EPA-EE). Different operating conditions were investigated at bench scale. The final conditions (Table 1), which gave a purity of EPA-EE of 90% and a recovery of 49%, were used as a starting point for pilot plant experiments. This study continues confirming the versatility of SFC, in that it can be used for a number of purposes, starting from separation and analysis to large-scale production.

A subgroup of lipids is the phospholipids. These are components of living cell membranes and play an important part in enzyme activation – thus, phospholipids are important in nutrition. SFC was used to fractionate phospholipids in a multiunit process after being extracted from soybean flakes ([3] – refer to Table 1). This process produced purities above 75% for the individual phospholipids, phosphatidylcholine, and phosphatidylethanolamine.

Analysis of Fat-Soluble Vitamins, Including Carotenoids

Fat-soluble vitamins (A, D, E, and K) play a major role in anabolism and catabolism. Deficiency of these vitamins can lead to serious diseases, such as night blindness (vitamin A), rickets (vitamin D), rupturing of blood cells and cancer (vitamin E), and blood coagulation disorders (vitamin K). Methods for the extraction and enrichment of these vitamins from natural sources like plants and seeds are constantly sought after, to be able to produce food supplements. SFC has been widely used for this group of compounds as can be seen from the following examples.

Tocopherols (α, β, and γ) are components of vitamin E and are particularly interesting for the food industry because of their antioxidant and nutraceutical activities. SFE and SFC were used to isolate tocopherols from olive by-products ([5] – refer to Table 1). The residue obtained from olive pomace was subjected to SFE with subsequent fractionation by two successive depressurizations. Tocopherols were separated and quantified using SFC subsequently. This study thus resulted in the isolation of tocopherols in an environmentally friendly way.

In a study by Jiang et al. ([6] – refer to Table 1), the effects of temperature, pressure, and modifier concentration in the mobile phase on the retention factor and resolution of tocopherols were studied. It was concluded that the separation of

α-tocopherol from γ- and δ-tocopherols was best at 18 MPa and 80 °C and with 5% ethanol in the mobile phase. This study also showed that the resolution of tocopherols increases when the temperature increases and the pressure decreases.

Another study ([7] – refer to Table 1) investigated the application of SFC to quantitatively analyze minor components found in palm oil. Carotenes, five isomers of vitamin E, sterols, and squalene were separated in <20 min. Results obtained from this study were comparable with those from an established analytical technique, such as GC. However, the SFC method showed several advantages. The conventional analysis of palm oil components requires the use of several techniques, such as GC, HPLC, and UV–Vis spectroscopy since no technique is able to identify all components. Moreover, the different techniques require different sample preparations. Using SFC, labor and time were saved for the sample preparation and separation of all components ($\approx$25 min) from the palm oil. This study also showed that there was a significant difference in the concentrations of vitamin E isomers analyzed by SFC and HPLC. Prolonged exposure of vitamin E isomers to organic solvents in HPLC resulted in destruction of the compounds, leading to lower levels found than in the SFC analysis.

Carotenoids are naturally occurring fat-soluble pigments, of which some act as provitamin A. Their potent antioxidant properties have led to increased investigations as potential anticancer, antiaging, and cardiovascular drugs. Carotenoids are known to be sensitive to temperature; therefore, low temperatures have to be employed in their analysis. This means that analysis takes place in subcritical conditions. Lesellier's research group ([4] – refer to Table 1) worked on the method development and improvement of the separation of β-carotene *cis*/*trans* isomers. The factors that affected retention differences were the dielectric constant of the modifier, solubility parameters, type of mobile phase, and stationary phase conformation.

Oxidation of carotenoids leads to the formation of epoxycarotenoids, which may be better markers for low-density lipoprotein (LDL) oxidation, an important process in atherosclerosis. A decrease in the carotenoid levels is seen before the increase in lipid peroxide levels during the oxidation of LDL. However, the low levels of carotenoids in the blood cannot be directly correlated to oxidative stress levels, because of individual differences in dietary intake. A method employing SFC–MS was used to identify epoxycarotenoids proving the sensitivity of the technique and therefore its applicability to biomarker screening ([8] – refer to Table 1).

Nutraceuticals

A number of natural products, including plants, contain constituents that show biological activities. A nutraceutical is defined as a food that possesses medicinal or nutritional properties, including the prevention and/or cure of an illness. Rosemary, davana, and grape seed extract are examples of such harvests, from which beneficial products can be separated and isolated by SFC as discussed in the succeeding text. Processes can then be scaled up to ease their productivity.

Rosemary is well known for its antioxidant properties. The most active compounds are the phenol diterpenes, primarily carnosic acid and also carnosol, rosmanol, and epi- and isorosmanol. Ramirez et al. ([10], [11] – refer to Table 1) worked on the separation and isolation of compounds with antioxidant and antimicrobial properties. Initially, the group focussed on the optimization of the separation of carnosic acid from other antioxidants in rosemary extracts obtained by SFE. Then, semipreparative SFC was used to obtain fractions with enhanced functional activities. The biologically active fractions were then shown to have improved antioxidant and antimicrobial properties. The optimized method resulted in two very active fractions. The CO_2-based mobile phase with a low percentage of modifiers has advantages for upscaling, since the lower the percentage, the lower the costs and residues incurred and the easier the evaporation of the mobile phase.

Davana is an aromatic herb from India. The oil of davana was initially only recognized for its importance in the perfume industry. However, davanone, a sesquiterpene, is a principal component of this oil. Davanone exhibits antifungal and antispasmodic properties. This compound was separated from the bulk matrix or natural davana oil by SFC ([11] – refer to Table 1). The final sample was nearly 100% optically pure.

Grape seed extract is a popular nutritional supplement with polyphenols that have antioxidant properties. Phenolics vary in their distribution and content depending on the raw material. Kamangerpour et al. ([12] – refer to Table 1) worked on the development of a fast SFC method for the isolation and identification of these polyphenols. The method was then used to separate phenolics in a grape seed extract.

Two other examples of nutraceuticals extracted from plants are glucobrassicin and sinalbin, both glucosinolates, with anticancer properties, coming from the plants of the *Brassicaceae* and the seeds of the *Sinapis alba* L. families, respectively ([13], [14] – refer to Table 1). White cabbage was used as a source of glucosinolates, while mustard was used as a source of sinalbin. The developed SFC methods can be used to separate and isolate the individual compounds of interest.

Monitoring of Pesticides

Pesticide or chemical residues in food, added during planting or processing phases, put the health of consumers at risk of adverse effects. It is therefore vital to monitor, identify, and quantify pesticide levels in food samples. A number of studies have used SFC to separate and analyze pesticides, for instance, contaminants in actual food samples. Toribio et al. ([15] – refer to Table 1) worked on the enantiomeric separation of six triazole fungicides, namely, cyproconazole, propiconazole, diniconazole, hexaconazole, tebuconazole, and tetraconazole. A polysaccharide-based chiral stationary phase was used and the effect of different modifiers studied. It was found that the organic modifier that provided the highest resolutions depended on the analyzed pesticide.

El-Saeid ([16] – refer to Table 1) monitored pesticide residues in canned foods, fruits, and vegetables, obtained from a local market in Houston, Texas. SFE was used to extract the pesticides from the samples, which were then analyzed by SFC. A total of 39% of foods tested were found to contain four pyrethroids, four herbicides, four fungicides, and six carbamates in different food samples. All the tested foods had different levels of pesticide residues ranging from 0.03 ± 0.005 to 0.8 ± 0.12 ppm. This study helped to promote consumer safety by assaying pesticide residues in food items.

Analysis of Other Compounds

Due to the nonpolarity of CO_2, SFC is mainly used in the analysis of hydrophobic or weakly polar compounds. However, by modifying the mobile phase polarity, a wider range of compounds can be analyzed. Figure 4 shows a chromatogram of a separation of amino acids, which are very polar compounds: proline (Pro), threonine (Thr), hydroxyproline (Hyp), serine (Ser), glutamic acid (Glu), and aspartic acid (Asp). Methanol, with water and triethylamine as additives, was used as a modifier to separate mixtures of underivatized amino acids. When a modifier gradient was used, even a higher number of amino acids were resolved in <10 min ([17] – refer to Table 1).

Carbohydrates were also separated with SFC ([18] – refer to Table 1). Mixtures of caffeine, fructose, glucose, sucrose, and neohesperidin dihydrochalcone (NHDC), which is an artificial sweetener, are commonly found in soft drinks. The mixture was separated in <5 min when SFC was used. Another sweetener, sucralose, was also identified and quantified in two sports drinks using SFC. Sucralose is roughly 600 times sweeter than sucrose with only 12% of the calorie count. The beverage marketed as 'low calorie' was indeed found to have a small amount of the sweetener.

Triterpenoids are recognized to possess a variety of biological activities, such as antibacterial, antioxidant, antitumor, antihyperglycemic, anti-HIV, antisclerotic, and cardiovascular activities ([19] – refer to Table 1). These compounds can be found in natural plants and in resinous natural materials. However, these compounds have a low UV absorbance; thus, a UV detector could not be used in this case. An alternative detector, evaporative light scattering detector (ELSD), was used, which gave acceptable peak intensities.

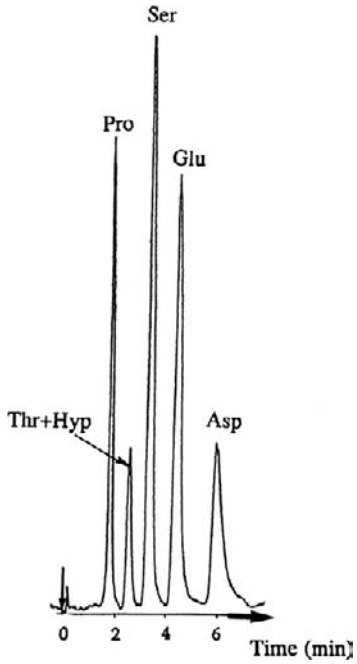

Figure 4 SFC chromatogram of a standard mixture of polar amino acids. Diol stationary phase; flow rate, 5 ml min^{-1}; inlet pressure, 230 bar; temperature, 0 °C; CO_2 modifier (80:20, v/v); modifier, methanol/additives (87.95:12.05, v/v) with water–triethylamine–pyridine (7:0.05:5, v/v) ([17] – refer to Table 1). Reprinted from Packed column subcritical fluid chromatography of underivatized amino acids, Camel, V., Thiebaut, D., Caude, M. and Dreux, M. (1992). Packed column subcritical fluid chromatography of underivatized amino acids. *Journal of Chromatography A* **605**, 95–101. Copyright (1992), with permission from Elsevier.

SFC in Health Analysis

The determination and quantification of drug levels in plasma play an important role in the monitoring and development of modern pharmaceutical products. Data generated from these measurements help in the understanding of toxicological effects and offer insight to a drug's mode of action (pharmacodynamics) and are used to establish pharmacokinetic parameters related to the absorption, distribution, metabolism, and elimination of the drug. Such studies are needed for all drugs in order to obtain regulatory approval. The following are just three examples of the use of SFC for health analysis, which help contribute to the safety and efficacy of medicinal products in patients.

Malaria is still prevalent in many areas of the world, and it continues to be studied to find treatments. Artemisinin, also known as qinghaosu (QHS), mefloquine, and sulfadoxine are all compounds recognized as having antimalarial activity. Developing GC and HPLC methods is challenging because on the one hand, these compounds are thermally labile, while on the other hand, they do not contain a UV, visible of fluorescent chromophore to facilitate their detection. Acidic hydrolysis or basic hydrolysis of QHS was done to produce a UV chromophore, but acidic hydrolysis lacked specificity, while the basic hydrolysis method required a lot of experimental work. An SFC–ECD (electron capture detection) method proved to be sensitive enough to measure QHS levels for therapeutic drug monitoring by Mount et al. This method had a limit of detection of 20 ng ml^{-1}. However, this limit can be lowered further by decreasing the baseline noise of the detector, possibly by using carbon dioxide of a better quality. This SFC–ECD method was also used to quantify mefloquine in blood samples. More work was required for sample preparation when compared with HPLC–UV and GC–ECD methods, but the method proved to be more sensitive and selective than HPLC–UV and more robust than GC–ECD. In another study by Bhoir et al. concerning the method development for the measurement of sulfadoxine levels in human plasma, SFC was found to be sensitive, rapid, and reproducible. The SFC method was found to be superior to an HPLC–UV method in terms of speed and cost.

Ketotifen, an analgesic, is another medicine where pharmacokinetic studies were done by SFC. A method for the determination of (*R*)- and (*S*)-ketotifen in human plasma was developed. Oral ketotifen shows a bioavailability above 92%; however, the maximum plasma concentrations reach low microgram per milliliter levels after a 25 mg dose. SFC was used to plot a plasma drug concentration versus time curve for both enantiomers by Hoke et al. A chiral stationary phase consisting of (*R*)-1-naphthylglycine and 3,5-dinitrobenzoic acid was used. The method was shown to be valuable to

measure ketotifen levels in human plasma, between 0.05 and 2500 ng ml^{-1}, after both topical and oral administration.

A final example concerns the use of the antineoplastic antimetabolite cytarabine, which is used in the treatment of leukemia. To understand better the pharmacodynamics of cytarabine, its plasma concentrations were monitored using an SFC–MS/MS method and an HPLC–MS/MS method by Hsieh et al. The results obtained from both methods displayed equivalent accuracy, and they can thus be used in an alternative manner.

SFC for Preparative Use

Preparative chromatography has had a significant impact on both food and pharmaceutical industries. The advantage of this technique arises from the ease of scalability, robustness of the equipment, and the possibility to separate diverse mixtures. Moreover, this technique can be used routinely for purification, from a few grams to tons per day. When SFC is used for preparative purposes, once again, the technique exhibits advantages over HPLC. In preparative SFC, the mobile phase is recycled online, which is not the case for preparative HPLC. Other advantages of prep-SFC stem from the inherent properties of CO_2 like the higher diffusivity, low viscosity, and retention control by the variation of pressure and temperature. Productivity in preparative chromatography is increased by the use of overlapping or stacked injections. This is a technique where a second injection is made before all the substances from the first injection have eluted from the stationary phase. Restrictions of prep-SFC include samples that are not soluble in methanol or CO_2, high-polarity compounds, and, just like in HPLC, cases where impurities coelute with a particular product.

Prep-SFC is used for the separation of diverse compounds in given mixtures, for example, the production of functional food ingredients. It is commonly utilized for the fractionation and purification of extracts acquired with SFE. Triglycerides, diglycerides, free fatty acids, carotenes, tocopherols, and tocotrienols were successfully separated from crude palm oil. Purified PUFA ethyl esters were obtained from tuna oil, while free sterols and ferulate phytosterol esters were attained from corn bran. The oil that is obtained as a by-product of corn bran dry milling contains cholesterol-lowering phytosterols – which are thus interesting for the nutraceutical market. Prep-SFC was also applied by Li et al. to the separation of polymethoxyflavones (PMFs), mainly nobiletin, tangeretin, 3,5,6,7,3′,4′3-heptamethoxyflavone, and 5,6,7,4′-tetramethoxyflavone, from orange peel extracts. PMFs show anti-inflammatory, anticarcinogenic, and antiatherogenic properties. The botanical species, *Thymus vulgaris* L. is well known for its food flavoring properties. However, thymol, which is a monocyclic terpenoid found in thyme, possesses antiseptic, analgesic, and anti-inflammatory properties. This terpenoid was extracted and isolated by prep-SFC, with a recovery of up to 97% of the total thymol content by Garcia-Risco et al.

Moving to drug analysis, prep-SFC has already been implemented as a routine tool in a number of pharmaceutical laboratories. White has described the integration of prep-SFC for large-scale chiral purification. The technique proved to reduce the overall sample throughput time and running costs while delivering high-purity fractions and acceptable recoveries. In another study by Toribio et al., the semipreparative chiral separation of lansoprazole, pantoprazole, and rabeprazole was presented. The recoveries and production rates obtained for the first eluted enantiomer were lower than those obtained for the second, especially if high-purity levels were aimed at. Several fractions of the first enantiomer were contaminated with the second, decreasing the recovery and production rates. This is possibly due to overloading conditions, where the peaks were distorted in the front.

Other Use of Supercritical Fluids in Food and Health: Extraction

SFE is the most common application for supercritical fluids. The process takes advantage of the properties of supercritical fluids, just like SFC. The major parameters on which the extraction depends are temperature, pressure, and flow rate. Supercritical CO_2 has been used to extract a wide range of analytes, ranging from essential oils to phytochemicals. These extracts can then be used for analytical purposes, for supplementation, and for flavor and fragrance purposes.

In addition to the benefits of using CO_2 in SFC, namely, the higher speed and lack of chemical residue, still, a few more advantages can be seen in SFE. Supercritical CO_2 shows no surface tension. Therefore, it penetrates the pores of heterogeneous matrices rapidly, helping to enhance the extraction efficiency. Moreover, the CO_2 used in an extraction can be recycled and reused. Products obtained from the extraction can then be further separated into different components by SFC. From the analytical point of view, SFE is compatible with SFC since the two techniques can share the mobile phase and some devices, like the CO_2 pump and condenser.

Some constituents extracted by SFE include lycopene from tomato seeds and skins, ginger oleoresin from ginger, oil from coriander seeds, and caffeine, theobromine, and cocoa butter from Brazilian cocoa beans. In the pharmaceutical sector, hyperforin and adhyperforin have been extracted from *Hypericum perforatum* L., commonly known as St. John's wort. Products from St. John's wort are known to have anti-inflammatory, antidepressive, antiviral, and antimicrobial effects.

SFE can also be used to determine the presence of drug abuse in human hair. Amphetamines, such as methylenedioxyethylamphetamine, methylenedioxymethamphetamine, and methylenedioxyethylamphetamine, can be investigated with a fast and reproducible method. The SFC method gives results that are similar to those obtained from established techniques used to extract amphetamines from hair, such as solid phase extraction and liquid–liquid extraction.

Conclusions

This article briefly explained the benefits of using supercritical CO_2 in the food and health sectors, with special reference to the SFC technique. SFC is considered as an environmentally friendly technique since the use of supercritical CO_2 reduces the consumption of organic solvents, CO_2 is obtained as a by-product of fermentation processes, and minimal waste is generated. The high diffusivity and low viscosity of CO_2 enable experiments to be conducted at higher flow rates, therefore achieving a higher analysis throughput without compromising on efficiency. These properties are leading to an expansion of

SFC applications in the food and health industries. The use of SFC continues to expand, and we anticipate it will continue growing in the future.

SFC is maturing and is establishing its position in analytical and preparative analyses. The technique provides highly accurate, precise, and sensitive measurements, especially when coupled with an MS detector. SFC has been conducted on a broad range of food, plant, and biological samples for the analysis of minor components, additives, contaminants, and medicinal products. Moreover, the technique can also be used to characterize compounds, for example, in lipidomics.

The separation mechanism of SFC can be compared with that of normal-phase liquid chromatography (NPLC). Just like in NPLC, in SFC, the polarity of the mobile phase here, supercritical CO_2, can be modified by the addition of polar modifiers. In this way and also by adjusting the density of the CO_2, one can vary the retention of the analytes on the stationary phase. SFC can be seen as a complementary technique to HPLC since many separations that are impractical or impossible to accomplish by HPLC can more easily be achieved by SFC.

Online coupling of SFE with SFC facilitates high throughput analysis as long as the compounds under study are soluble in CO_2. This system is suitable for the analysis of compounds that are easily destroyed during organic solvent extraction, for example, by the high temperature, in a Soxhlet extractor.

The versatility and wide applicability of SFC in the food and health sectors are very promising. More research is needed to discover the full application potential of this technique. In the coming years, more applications of SFC are expected, not only for research purposes but also more in routine agricultural, food, and clinical applications.

See also: Fatty Acids: Determination and Requirements; Herbs: Composition and Dietary Importance; Pesticides and Herbicides: Residue Determination; Pesticides and Herbicides: Types, Uses, and Determination of Herbicides; Supercritical Fluid Extraction; Tocopherols: Properties and Determination.

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Chromium: Physiology

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Introduction

Recently, a paradigm shift has occurred in the understanding of the physiological effects of trivalent chromium, Cr^{3+}. While initially proposed to be an essential trace element for mammals in the late 1950s, the initial studies have been found in hindsight to be methodologically flawed. As a consequence of this and recent data, no unambiguous data for chromium being an essential element exist. The data demonstrating beneficial effects are best interpreted in terms of a pharmacological role for chromium at supranutritional doses. While guidelines from governmental agencies and textbooks have not yet caught up with the recent literature, chromium should be removed from the list of essential trace elements.

Absorption, Transportation, Storage, and Excretion

Chromium Absorption

While aspects of the mechanisms of absorption and transport of Cr^{3+} ions are known, overall, the process is poorly elucidated. Most notably, little is known of the fate of Cr^{3+} taken orally before the Cr reaches the bloodstream. Essentially, no data exist on the forms of Cr^{3+} in food as a result of its very low concentration. The fate of dietary chromium at a molecular level in the digestive tract is also essentially unknown, although >98% of Cr^{3+} passes through without being absorbed. This lack of knowledge is in stark contrast to what has been established for ferric iron (Fe^{3+}), with a similar charge to size ratio as Cr^{3+}. Dietary iron is probably primarily in the ferric form. Absorption of iron takes place in the proximal portion of the duodenum. Unlike Cr^{3+}, whose reduction potential is such that it should not readily be reduced under the conditions in the gastrointestinal tract, ferric ions can be reduced chemically or enzymatically by a brush border ferrireductase of the enterocytes. Subsequently, iron enters the enterocytes as ferrous iron via the transmembrane protein DMT1 (divalent metal transporter 1), which is probably responsible for the entry of a variety of divalent metal ions. The transported iron is then stored or is released from the enterocyte by the basolateral transmembrane protein ferroportin. Iron probably passes through the enterocyte in the ferrous state (Fe^{2+}) and is returned to the ferric state (Fe^{3+}) by ferroxidases for transport in the bloodstream by the protein transferrin (vide infra). The failure of Cr^{3+} ions to be reduced requires a unique absorption system to be present for chromium, compared with other proposed essential metals ions, if chromium is actively absorbed and essential.

However, the preponderance of evidence suggests that chromic ions are passively absorbed, that is, absorbed via simple diffusion. In rats, chromium has been established to be absorbed via passive diffusion. Only a small percentage (<1%) of dietary Cr is absorbed, while the remainder is excreted in the feces. Inorganic chromium salts are generally used to mimic dietary chromium and are absorbed to a similar extent, that is, <1%, over a wide range of doses. (The most commonly used salt is chromium(III) chloride hexahydrate, which is actually the chloride salt of the *trans* isomer of the $[CrCl_2(H_2O)^4]^+$ cation.) Chromium supplementation of the diet results in an increase in urinary chromium loss, and most of the absorbed chromium is rapidly excreted. Recently, rats that were gavage-dosed with $CrCl_3$ were found to exhibit ~0.2% absorption of Cr over a 2000-fold range of doses, consistent with the absorption of simple inorganic chromium(III) salts occurring via diffusion and not active transport. The concentration of Cr in the urine, blood, and tissues is proportional to intake. In fact, Cr content of the liver and kidney for multiple complexes of Cr(III) has been shown to increase linearly with intake. Urinary Cr loss appears to be controlled by intake. When followed by isotopically labeled chromium (^{50}Cr or radioactive ^{51}Cr) in rats or humans with increased urinary Cr loss from diabetes or strenuous exercise, the increased urinary Cr loss has been found to be offset by an increase in Cr absorption. In fact, the increased uptake of water in these conditions may simply result in the increased diffusion of Cr and the subsequent increase in urinary chromium loss.

Additional data come from studies of the absorption of Cr^{3+} from a rat intestinal perfusate with added Cr as $CrCl_3$. A double-perfusion technique has been utilized in which the intestinal vasculature, from the superior mesenteric artery to the portal vein, and the intestinal lumen, from the duodenum to the ileum, were perfused simultaneously. Over a greater than 100-fold range of Cr^{3+} concentrations, Cr absorption was found to be a nonsaturable process, consistent with animal studies. These results led to the conclusion that the Cr^{3+} was absorbed by the nonmediated process of passive diffusion in the small intestine of rats fed with a Cr-adequate diet.

However, the fate of dietary chromium(III) organic complexes could be different to that of the inorganic chromium salts if the complex (or some product thereof) absorbed is different from the form absorbed using inorganic chromium (III) salts. For example, the presence of added amino acids, phyate (high levels), and oxalate in the diet reportedly alters Cr uptake, as does ascorbic acid. Yet, none of these studies disproves that absorption is passive, only that the extent of chromium available for diffusion may be altered by the addition of these potential chelators to the diet and that the potential chelators may alter steps subsequent to absorption. The possibility that complexes of chromium with organic ligands may have altered the absorption properties is part of the impetus behind the various Cr(III) complexes used or proposed as nutritional supplements, although generally, this has not proven to be the case. In fact, studies comparing the blood, urine, and tissue concentration in rats of ^{51}Cr from labeled $CrCl_3$ and the most popular chromium supplements, chromium picolinate and chromium nicotinate, indicate that all are absorbed to a similar extent within experimental error.

Another interesting conclusion that can be drawn from the intestinal perfusate studies is that chromium appears to be

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actively transported out of the intestinal cells, as nearly all of the chromium entering the cells is cleared from the cells (leaving <10% behind to be stored). Yet, no transporter is known for chromium. Cr^{3+} bound to some chelating ligand has been proposed to be transported by monocarboxylate transporters (MCT) as occurs for chelated Al^{3+} (a nonessential metal ion). Chromium complexes with free carboxylates are known; for example, the complex formed between Cr^{3+} and EDTA (ethylenediaminetetraacetate) is the violet [Cr(Hedta)(H_2O)], where one of the carboxylate groups is protonated. More importantly, the common complex of Cr^{3+} and citrate possesses a free carboxylate in the solid state and in solution. This idea is also supported by the perfusate studies. When amino acids were left out of the solutions perfused through the intestinal lumen, less chromium was transported into the vascular perfusate, while additional chromium was retained by the intestinal mucosa. Thus, potential ligands for the chromium may need to be transported into the intestinal cells for chromium to be efficiently transported out of the cells. However, the recent studies showed that monocarboxylate transporters are not involved in Cr^{3+} transport, at least from endosomes in hepatocytes.

Chromium Transport from the Blood to the Tissues

The fate of chromium in the bloodstream is better understood. *In vivo* administration of Cr^{3+} to mammals by injection results in the chromic ions being bound by the iron-transport protein transferrin, while administration of $^{51}CrCl_3$ by stomach tube to rats results in 80% of the chromium immunoprecipitating with transferrin. *In vitro* studies of the addition of chromium sources to the blood or blood plasma also result in the loading of transferrin with Cr(III), although under these conditions, albumin and some degradation products also bind chromium. When chromium is added to the blood *in vitro*, chromium binds to both transferrin and albumin; *in vivo*, only transferrin binds appreciable quantities of chromium. Thus, studies in which Cr sources are added to the whole blood, blood plasma, or serum should be avoided.

Transferrin is an 80 kDa blood serum protein that tightly binds two equivalents of ferric iron at neutral and slightly basic pH values. The protein exhibits amazing selectively for Fe^{3+} in a biological environment because the metal sites are adapted to bind ions with large charge to size ratios and is primarily responsible for the transport of iron through the bloodstream. In humans, transferrin is maintained only on average ~30% loaded with iron; consequently, the protein has been proposed to potentially carry other metal ions, although data to support this under physiological conditions are limited. The similar charge and ionic radii of chromic ions to ferric ions suggest that chromic ions should bind relatively tightly to the protein. *In vitro* studies of the addition of chromic ions to isolated transferrin reveal that Cr^{3+} readily binds to the two-metal-binding sites, resulting in intense changes in the protein's ultraviolet spectrum. Two equivalents of (bi)carbonate are concomitantly bound. Ultraviolet and Raman spectra suggest that each chromic ion binds to two tyrosine residues from the protein, strongly indicating that the chromic ions bind in the two ferric ion binding sites. Visible spectra suggest the d^3 chromic centers are in pseudooctahedral environments. Variable temperature magnetic susceptibility studies confirm that the chromium centers are trivalent (i.e., $S=3/2$). Estimates of the binding constant for chromium and the maintenance of transferrin on average only 30% loaded with ferric ions suggest that the protein appears to be primed to be able to transport chromium through the bloodstream.

Chromium-loaded transferrin has been demonstrated to transport chromium *in vivo*. Injection of ^{51}Cr–transferrin into rats results in incorporation of ^{51}Cr into tissues. Injection of labeled transferrin and insulin results in a severalfold increase in urinary chromium; iron transfer by transferrin had previously been shown to be insulin-sensitive. Thus, transferrin, in an insulin-dependent fashion, can transfer Cr to tissues after which Cr is ultimately excreted in the urine. Cr_2–tranferrin serves as an inhibitor for the binding of Fe_2–transferrin to the surface of reticulocytes, presumably at the site of binding transferrin to transferrin receptor. Cr-loaded human transferrin is a better inhibitor than apotransferrin but not as good as mono- or diferric transferrin.

Chromium Transport from the Tissues to the Bloodstream and Urine

Cr^{3+} appears to be moved from the tissues to the bloodstream and subsequently urine via a peptide named low-molecular-weight chromium-binding substance (LMWCr or chromodulin). The peptide is 10 or 11 amino acids long; is composed of glycine, aspartate, glutamate, and cysteine; and tightly binds four chromic ions. The peptide has been isolated from mammals, chickens, and alligators where all appear to contain the contiguous peptide of sequence Glu-Glu-Glu-Glu-Gly-Asp-Asp. The peptide is rapidly cleared from the body after binding Cr^{3+}. Spectroscopic studies indicate that the chromic ions are bound to the peptide primary via the carboxylate side chain of the aspartate and glutamate residues. The chromic ions appear to be arranged in a multinuclear assembly.

The movement of Cr(III)–LMWCr is stimulated by increases in blood insulin concentration; thus, increased movement of Cr from the bloodstream to the tissues by transferrin results in a subsequent elimination of chromium from the body bound to LMWCr. The peptide appears to be maintained in the tissue in the apo form; levels of the apo form potentially are under homeostatic control. At a minimum, LMWCr functions to rapidly clear Cr from tissues.

The Cr(III)-containing form of LMWCr has been proposed to be the biologically active form of chromium. *In vitro*, it has been found to activate the kinase activity of insulin receptor; this activation was proportional to the Cr content of LMWCr. In a proposed mechanistic scheme, at high intakes of Cr, LMWCr would accumulate to pharmacological levels, bind to the insulin receptor (helping to maintain the receptor in its active confirmation), and result in increased insulin sensitivity. The validity of this proposal requires testing *in vivo*.

Metabolic Functions and Deficiency Symptoms

'Low-Chromium' Diets

Traditionally, evidence for chromium being an essential element for mammals has come primarily from four sources: (i) studies attempting to provide rats with chromium-deficient diets, (ii) studies examining the absorption of chromium as a

function of intake, (iii) studies of patients on total parenteral nutrition (TPN), and (iv) studies looking for an association between insulin action and chromium movement in the body.

The field of Cr nutrition had its beginnings in 1955 when rats fed with a *Torula* yeast-based diet apparently developed impaired glucose tolerance in response to an intravenous glucose load from which a new dietary requirement, coined glucose tolerance factor (GTF), absent from the *Torula* yeast-based diet and responsible for the glucose intolerance was proposed. The active ingredient of GTF was Cr^{3+}. Inorganic compounds containing over 40 different elements (200–500 mg kg^{-1} body mass) could not restore glucose tolerance, while several inorganic Cr(III) complexes (200 mg Cr per kilogram body mass) restored glucose tolerance. Unfortunately, these Cr studies were methodologically flawed. For example, the Cr content of the diet was not reported. The rats were maintained in wire mesh cages, possibly with stainless-steel components, allowing the rats to obtain Cr by chewing on these components. Consequently, the actual Cr intake of the rats in these studies is impossible to gauge, putting into question the suggestion that the rats were Cr-deficient. The use of the large amounts of the metal ions is also of concern; as will be discussed (vide infra), supranutritional doses of Cr pharmacological effects in rats with insulin resistance. These doses are probably about 103 times the typical daily content of a rat's diet.

Details of the 'isolation' of Brewer's yeast GTF were reported in 1977. However, the isolation procedure included harsh procedures such as an 18 h reflux in 5 M HCl, which would hydrolyze any proteins, complex carbohydrates, or nucleic acids. Nicotinic acid was apparently sublimed from the material (although no data or experimental details were presented for the mass spectral, sublimation, or extraction studies). Amino acid analyses indicated the presence of glycine, glutamic acid, cysteine, and other amino acids, although the relative amounts were not reported. The results were interpreted to indicate that GTF was a complex of Cr, nicotinate, glycine, cysteine, and glutamate. In paper chromatography experiments, the material on which the studies in the preceding text were performed gave several bands, only one of which was active in bioassays. Based on this work, GTF has been proposed to be a Cr (III)–glutathione–nicotinate complex as glutathione is a tripeptide of glutamate, glycine, and cysteine. A three-dimensional structure has also been proposed for Brewer's yeast GTF that has two *trans* N-bound nicotinic acid ligands and amino acids occupying the remaining four sites of an octahedral around the chromic center. However, Cr^{3+} has been demonstrated repeatedly to be separable from agents in yeast responsible for *in vitro* stimulation of glucose metabolism in adipocytes. Thus, the component of yeast that is active in the bioassays apparently does not contain Cr. The characterization of a Cr fraction from yeast was performed on a mixture that contained multiple Cr-containing species and other components, and the Cr-containing components of the mixture probably do not reflect what bound Cr initially in the intact yeast. Yet, despite the numerous publications refuting the GTF studies, these studies unfortunately continue to be cited as evidence of the essential role of Cr.

Since the initial GTF studies and prior to 2011, the most notable efforts of work with rats to generate a chromium-deficient diet were studies utilizing high-sugar or high-fat diets. For the high-sugar diet studies, rats in plastic cages (with no access to metal components) were given a purified diet consisting of 55% sucrose and providing 33 ± 14 μg Cr per kilogram diet. A supplemented pool of rats was given with water containing 5 ppm $CrCl_3$. At 12 weeks, Cr-deficient rats had lower fasting plasma insulin concentrations and similar fasting plasma glucose levels compared with supplemented rats; yet, both concentrations were similar after 24 weeks. In intravenous glucose tolerance tests after 24 weeks on the diet, plasma insulin levels tended to be higher in Cr-deficient rats; rates of excess glucose clearance were statistically equivalent. Thus, a high-sucrose diet can lead to hyperinsulinemia that appears to be partially corrected by chromium. This research group also obtained similar results using a high-fat diet that contained 33 mg Cr per kilogram diet. After 16 weeks on the diet alone, rats had higher fasting plasma insulin levels, compared with rats also receiving drinking water containing 5 ppm Cr. Thus, the high-fat diet also appears to induce increased fasting insulin levels, which can be corrected with chromium administration. Some calculations are in order to put this work into perspective. Humans lack signs of Cr deficiency with a daily intake of 30 μg Cr; assuming an average body mass of 60 kg, 30 μg day^{-1} corresponds to 0.5 μg Cr per kilogram body mass per day. Thus, for a rat, this is equivalent to 5 μg Cr per kilogram body mass per day, ten times what a human intakes. Thus, the 'low-Cr' high-sugar and high-fat diets cannot be said to be deficient at all unless rats require more than ten times the chromium that humans do on a per kilogram body mass basis. Consequently, the effects of the diet cannot be attributed to Cr deficiency; the high doses of Cr in the Cr-supplemented rats can only be considered as having a pharmacological role on those rats whose physical condition were impaired by the high-sucrose or high-fat diets and/or the other mineral stresses. In 2011, the effects of a purified diet (AIN-93G without Cr in the mineral supplement, 16 μg Cr per kilogram diet) on male Zucker rats in metal-free cages for 16 weeks were examined. Zucker rats also received the normal AIN-93G diet (i.e., with 1000 μg Cr added per kilogram diet) and the normal diet supplemented with an additional 200 or 1000 μg Cr per kilogram diet. No ill health effects were noted for the rats on the low-chromium diet. Plasma insulin concentrations in glucose tolerance tests decreased as a function of chromium dose indicating a pharmacological effect for the dietary chromium.

Thus, with no added stresses, a purified diet with as little chromium content as possible does not result in symptoms that can be attributed to chromium deficiency, although effects on insulin sensitivity can be observed at supranutritional, that is, pharmacological, doses of chromium. Hence, establishing whether chromium is an essential nutrient is apparently not feasible from traditional nutrition studies, because developing a chromium-deficient but otherwise sufficient diet appears not to be possible. This could be simply because chromium is not an essential element or uniquely because such low amounts of chromium are required that generating a deficiency is not possible. Similarly, no genetic disorder that alters chromium transport or distribution or other function has been identified; the study of such disorders for other metals has pointed to the essential nature of those elements and the biomolecules that utilize the metal. How then can the potential essential nature of chromium (or other elements such as silicon, vanadium, arsenic, and boron that have been proposed to be essential but

in such miniscule amounts that generating a dietary deficiency is impossible) be established? The most obvious method is establishing that a biomolecule containing the element *in vivo* performs an essential biological function at physiological levels of the element. For chromium, this has proved less than straightforward, as several mechanisms for chromium action at a molecular level have been proposed, while none have been adequately established *in vivo*. In fact, major questions shroud each proposal, and open scientific discussions of these issues and the other issues described in the preceding text have proved to be quite difficult given the financial implications of the outcomes.

Total Parenteral Nutrition

Evidence for an essential role of chromium in humans has also been inferred from studies of patients on TPN. Patients on TPN have developed impaired glucose utilization or glucose intolerance and neuropathy or encephalopathy. These symptoms were reversed by chromium infusion while unaltered by other treatments. While limited to about ten individual cases, these studies have been interpreted as providing evidence of clinical symptoms associated with chromium deficiency that can be reversed by supplementation. Curiously, the development of the symptoms that were reversible by chromium supplementation does not correlate with serum chromium levels, indicating either that serum chromium levels are not an indicator of chromium deficiency or that another factor is in operation. Additionally, these incidences of diagnosed potential chromium deficiency have been questioned as they lack consistent relationships between the chromium in the TPN, time on TPN before symptoms appear, serum chromium levels, and symptoms. Yet, subjects, albeit a limited number, with certain conditions not responsive to other treatments appear to have improved glucose control and reduction in insulin needs in response to intravenous chromium.

The level of Cr administered must be examined carefully. In the cases where reversal of symptoms was reported, the TPN solutions provided 2–10 μg Cr per day. All the Cr in the TPN is introduced into the bloodstream, while only ~0.5% of Cr in the regular diet is absorbed into the bloodstream. Thus, 30 μg of Cr in a typical daily diet results in only ~0.15 μg of Cr entering the bloodstream. The TPN solutions utilized in these studies are consequently presenting ~13–67 times the required amount of chromium; thus, based on these data, the TPN solutions cannot be considered Cr-deficient. For the treatments, the subjects received 40–250 μg Cr per day added to the TPN solution to alleviate their conditions, clearly pharmacological doses as the largest dose provided 1.7×10^3 times more chromium than a standard diet when one considers that this administration corresponds to the equivalent of 100% absorption so that the values should be multiplied by 100 for comparison against oral studies. Consequently, the results with the insulin-resistant TPN patients can only be considered as providing evidence for a pharmacological role of chromium. The data are not relevant for examining whether chromium is an essential element.

Not surprisingly, as TPN provides ten or more micrograms of chromium per day, TPN patients accumulate chromium in their tissues. Calls are appearing for the reexamination of the chromium levels in TPN solutions in terms of a need to reduce recommended levels.

Absorption

The absorption of Cr as a function of intake by humans is still widely cited as evidence for the essentiality of chromium, although this is based on a single study. An inverse relationship between dietary chromium intake and degree of absorption was observed, in contrast to rodent studies described in the preceding text. Cr intake was determined from the amount of various food consumed where the Cr content of each food had been previously determined; Cr absorption was estimated from the Cr content of the urine as use of radiolabeled Cr to track the fate of all the administered Cr is not possible in humans. The data suggested that absorption of Cr varies approximately from 0.5% to 2.0% for Cr intakes of ~15–50 μg day^{-1}. This difficult to perform study is far from definitive and desperately requires repeating. For example, a distinct difference is found when the data are separated into male and female subjects. For males, no statistical variation occurs for apparent chromium absorption as a function of intake, while an inverse trend was observed for the female subjects. A thorough statistical treatment including a propagation of error analysis is needed. Additionally, these data are in striking contrast to a similar human study. Chromium absorption was determined to be ~0.4% for free-living individuals; when Cr intake was increased by over fourfold, urinary chromium excretion increased over fourfold while maintaining ~0.4% absorption of chromium for both males and females. The difference between the two studies lies in the range of Cr intakes of ~15–50 μg day^{-1} for the former and ~60–260 μg day^{-1} for the latter, suggesting that an inverse relationship between Cr intake and absorption, if it exists, exists only at the lowest portion of the range of intakes. These results have led to the proposal that Cr homoeostasis is maintained at the level of excretion, not absorption, and that the mechanism of Cr uptake by rats may be different from that in humans. However, the assumption that chromium is in a state of homoeostasis is unproven. In fact, the extent of absorption appears to determine the amount of urinary excretion. As noted in the preceding text, absorption appears to be directly determined by intake – the amount of chromium intake determines the amount of chromium excretion. No evidence for chromium homoeostasis can be derived from these studies (with the exception of the data for female humans).

Thus, in summary, Cr appears to be absorbed by diffusion, not actively transported. The process of chromium absorption could possibly be different in humans (i.e., at least females) from that in rats, but this would seem unlikely and would require more research for this to be firmly established. If chromium intake by humans is active, this transport system would only play a significant role at low chromium intake and would be overwhelmed by diffusion at higher intakes. Clearly, chromium absorption in rodents is not inversely proportional to intake, distinct from that of other reported essential elements. Based on absorption studies, at least in rodents, chromium does not appear to be an essential element; and this probably extends to other classes of mammals.

Chromium Movement

The rates of absorption and transport of Cr are altered in humans with type 2 diabetes and in rodent diabetes models. Increases in blood insulin concentration (such as those following a glucose challenge or similar dietary stress) result in decreases in plasma glucose concentration followed by increases in urinary Cr loss with the amount of increased urinary Cr loss roughly equivalent to the decrease in the amount of blood chromium. Serum chromium levels of diabetic patients are lower than those of healthy subjects, while urinary Cr loss is increased in diabetic subjects. As noted in the preceding text, the increases in chromium loss are accompanied by (if not the result of) increases in chromium absorption. While the association between insulin insensitivity/diabetes and rates of chromium transport is suggestive, it is not proof of an essential role for chromium and is explained in part by the transport of chromium by the iron-transport protein transferrin. A role for transferrin in the detoxification of chromium resulting from increases in chromium absorption associated with insulin insensitivity/diabetes could also explain its phenomenon.

Pharmacological Effects

Supplementation with Cr has been proposed to result in beneficial responses in mammals with demonstrated glucose intolerance or insulin insensitivity, including type 2 diabetes, cardiovascular disease, and related conditions. However, studies in humans tend to be negative or at best ambiguous. The clinical studies, while focused primarily on type 2 diabetic subjects, tend to have small subject pools and not be well designed. One study has dominated attention; 185 adult-onset diabetic Chinese patients participated in the study, in which decreases in the concentration of fasting serum glucose, insulin, hemoglobin A1C, and total cholesterol and decreased glucose and insulin levels in response to glucose challenges were observed as a result of Cr supplementation in a dose-responsive manner. Unfortunately, attempts to reproduce these results with other populations, including Western populations, have been unsuccessful. Two meta-analyses commissioned by agencies of the US government have generated inconclusive results on whether Cr affects symptoms of type 2 diabetes. One in 2002 with funding from the Office of Dietary Supplements of the National Institutes of Health (NIH) identified only four quality studies for analysis and found that the results were inconclusive as the combined results except for those of the Chinese study found no effects. Another in 2007 with funding from the Department of Health and Human Services identified 18 studies. The authors concluded that Cr supplementation might have a modest effect on glucose metabolism in type 2 diabetics but that large heterogeneity combined with the overall poor quality of the studies limited the strength of any conclusions. Unfortunately, the positive effects of the greatest magnitude used in the analysis came from the 12 studies ranked lowest in quality, including the Chinese study. A trend was observed that commercial industry-sponsored studies were more likely to observe beneficial effects. Consequently, the position of the American Diabetes Association (ADA) is that insufficient evidence exists to support the use of chromium to improve glycemic control in people with diabetes.

In contrast, studies with rodent models of diabetes and peripheral tissue insulin resistance generally observe beneficial effects on insulin sensitivity and often also observe beneficial effects of serum cholesterol and triglyceride levels. These rat studies generally provided rats between 80 and 1000 μg (or even more) Cr per kilogram body mass daily for periods of weeks or months. Based only on mass, this would correspond to 5.2–65 mg Cr daily for an average 65 kg human. Even when corrected for the increased metabolic rate of rats compared to humans, this range corresponds to ~1–13 mg Cr daily. Thus, human clinical trials may have only started to approach the dose necessary to see a beneficial effect in humans. The amount of Cr used in clinical trials needs to be increased before determining whether or not chromium supplementation has an effect on individuals with type 2 diabetes, insulin resistance, or related conditions. However, one also cannot rule out that beneficial effects might be unique to rodents and perhaps certain other animals.

Requirements

The basis for the use of chromium as a nutritional supplement stems from chromium being on the list of essential vitamins and minerals under examination by the NRC (National Research Council of the National Academies of Science, the United States) since 1980 when an estimated safe and adequate daily dietary intake (ESADDI) for 50–200 μg was suggested. In 2001, the National Academies of Science established an adequate intake (AI) of chromium of 35 μg day^{-1} for men and 25 μg day^{-1} for women. AI is defined as the recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate. The AI is more conservative than a recommended daily allowance and is expected to cover the needs of more than 97–98% of individuals. Thus, essentially, all Americans are believed to be chromium-sufficient, and little if any need exists for chromium supplementation. The bases for this determination are rather limited. The AI is primarily based on the Cr content of nutritionist-designed diets (on average roughly 35 μg Cr for men and 25 μg Cr per day for women), which turns out to be almost identical to the chromium content of self-selected American diets. Diets in other developed nations appear to be similar in Cr content, if not slightly higher. The National Research Council is currently reviewing dietary requirements of trace elements and vitamins. What decision is made in terms of the status of chromium as an essential element will be interesting.

Dietary Sources and Content in Food

Chromium is ubiquitous in foods but at very low concentrations. However, during processing, particularly in stainless-steel (~10–40% chromium) equipment, the concentration of chromium appears to increase; in fact, most of the Cr in some foods may come from processing. Foods particularly rich in Cr (i.e., >100 ppb) include broccoli and black pepper and certain beers; however, values for vegetables must be considered

carefully because of the variable amount of chromium that comes from soil contamination. The low concentrations of chromium in food, the ease of contamination, and the low suggested dietary requirement for chromium (vide supra) make preparation of a low-chromium (or chromium-deficient) diet difficult if even possible (as this assumes that chromium is actually an essential trace element). Given the amount of Cr in the diet that comes from modern processing, the Cr intake of early humans must have been extremely limited.

Reference Doses

No tolerable upper limit (UL) has been set for Cr^{3+}. No significant health concerns have been found for chromium supplements other than chromium picolinate at current doses. However, a study commissioned by the National Toxicology Program (National Institutes of Health) has found that providing chromium picolinate up to 5% of the diet of male and female rats and mice for 2 years had no conclusive deleterious health effects. This is probably the result of chromium picolinate dissociating in the gastrointestinal tract when provided orally. In contrast, cell culture study and *in vivo* studies suggest that if intact chromium picolinate reaches cells intact that it is toxic and clastogenic, mutagenic, and possibly carcinogenic, hence, the supplement should not be used intravenously, for example, in TPN.

See also: Chromium: Properties and Determination; Cooking: Domestic Techniques; Dietary References: US; Glucose: Glucose Intolerance; Iron: Physiology of Iron; Parenteral Nutrition; Trace Minerals and Trace Elements.

Further Reading

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Relevant Websites

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Chromium: Properties and Determination

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Physical and Chemical Properties

Chromium(6+)

Only two oxidation states of chromium, Cr^{3+} and Cr^{6+}, are generally considered biologically and environmentally relevant and stable; in other words, they are stable in the presence of air and water. Chromium(III) complexes are both kinetically and thermodynamically stable; however, chromium(VI) complexes are kinetically stable but thermodynamically unstable. In the presence of appropriate reducing agents, Cr^{6+} can readily be reduced via Cr^{4+} and/or Cr^{5+} intermediates ultimately to Cr^{3+}. The biochemistries of both Cr^{3+} and Cr^{6+} have controversial histories. The public is generally more familiar with the chemistry of Cr^{6+} (or chromate) because of its toxicity.

Chromium(6+), d^0, is most commonly encountered as the intensely colored chromate, $[CrO_4]^{2-}$, or dichromate, $[Cr_2O_7]^{2-}$, anions. These two species are interconvertible in water. Chromate has a distinctive yellow color and exists at basic pH values. Insoluble $PbCrO_4$ was previously used as the pigment in paint for yellow highway lines. Below pH 6, chromate is in equilibrium with the yellow-orange dichromate anion. Acidic dichromate solutions are potent oxidants. The coordination environment of the chromium centered in both the chromate and dichromate anions is tetrahedral. The intense color of both anions stems from ligand to metal charge transfer bands. Mixed ligand complexes of Cr^{6+} with oxides and halides or oxides and amines are well known, as are Cr(VI) peroxo complexes. The diamagnetic Cr^{6+} center does not give rise to ESR (electron spin resonance) spectra, while NMR (nuclear magnetic resonance) studies of Cr(VI) complexes with oxo, peroxo, and halo ligands are of limited utility.

While Cr(VI) complexes are known to be potentially potent carcinogens and mutagens when inhaled, a serious debate has arisen with regard to the effects of the oral intake of these complexes, as illustrated in recent years by the popular movie *Erin Brockovich*. Chromium(VI) complexes could give rise to these effects through a number of mechanisms, including oxidation of biomolecules by the complexes or by the subsequently generated Cr^{4+} and Cr^{5+} intermediates, reactions of reactive oxygen species generated as by-products of these oxidations, reactions of organic radicals generated in these processes, and the binding of the ultimately generated Cr^{3+} to biomolecules. The relative importance of these mechanisms is far from being deciphered. The nature and significance of the coordination of Cr^{3+} ions to DNA as a result of Cr^{6+} reduction is a current topic of much debate. Chromate can readily enter cell via sulfate (SO_4^{2-}) and phosphate (HPO_4^{2-}) transporters given its similar shape and charge.

Chromium(3+)

Coordination complexes of Cr^{3+} are nearly always octahedral. Consequently, the chromic center has a d^3 electron configuration with three unpaired electrons ($S = 3/2$), one in each of the three t_{2g} orbitals. This configuration is responsible for the kinetic inertness of Cr(III) complexes, where ligand exchange half-times are generally in the range of hours. The hexa-aquo ion of chromium, $[Cr(H_2O)_6]^{3+}$, is purple in aqueous solution. Solutions of the ion are acidic; at neutral and basic pH, the ion readily oligomerizes to give hydroxo-bridged species starting with the $[(H_2O)_5Cr(\mu\text{-}OH)_2Cr(H_2O)_5]^{4+}$ ion. The Cr^{3+} ion has a large charge to size ratio and is considered as a hard Lewis acid, preferring oxygen and nitrogen coordination. With common biomolecules, coordination to anionic oxygen-based ligands such as phosphates and carboxylates would be expected; however, the structure of Cr(III) biomolecules generally is poorly characterized to date.

Common commercially available forms of Cr^{3+} include chromium chloride ($CrCl_3 \cdot 6H_2O$), chromium nitrate ($Cr(NO_3)_3 \cdot 9H_2O$), and chrome alum ($K[Cr(SO_4)_2] \cdot 12H_2O$), which are utilized because of their solubility. The commonly used commercial form of $CrCl_3 \cdot 6H_2O$ is actually *trans*-$[Cr(H_2O)_4Cl_2]Cl_2\ H_2O$. Dissolution of this green solid initially yields green solutions of the $[Cr(H_2O)_4Cl_2]^+$ cation. Chromium chloride, chromium picolinate, and chromium nicotinate are the most popular forms of chromium used in nutritional supplements. Chromium picolinate is the meridional isomer of the tris-chelate complex of Cr^{3+} and the picolinate anion, [Cr(picolinate)$_3$]. Chromium nicotinate is a poorly characterized insoluble polymer of Cr^{3+}, nicotinate, and hydroxide; its composition varies depending on the method of synthesis. The hydrochloride of a complex of Cr^{3+} and methionine ([Cr(L-methionine)$_3$].HCl) and 'chromium propionate' have been used in nutritional supplements for cattle ('chromium propionate' only) and swine in the United States. Trivalent chromium is not approved as a feed additive in the European Union. Although originally characterized as anhydrous chromium propionate, Cr(propionate)$_3$, chromium propionate is actually the trinuclear cation $[Cr(III)_3O(propionate)_6(H_2O)_3]^+$. The structural, spectroscopic, and magnetic properties of this cation and its analogs with other carboxylate ligands have been extensively studied. Limited characterization has been reported on chromium methionine.

The magnetic and spectroscopic properties of chromium (III) complexes do not readily lend themselves to providing much information on the coordination environment of chromic centers in biomolecules. For mononuclear complexes, a magnetic moment close to the spin-only value for an $S = 3/2$ center (3.88 BM) is generally observed. While ^1H and ^{13}C NMR spectra can be obtained on Cr(III) complexes, the spin 3/2 center results in greatly broadened and shifted resonances in NMR spectra. The structure of the complex generally needs to be known in order to interpret the NMR spectra, rather than the reverse. In contrast, Cr(III) complexes can give rise to sharp features in ESR spectra; however, the ESR spectra of biomolecules have often proved to be quite broad, providing limited information. ESR spectroscopy is probably a

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significantly underutilized technique in characterizing chromium in biological systems. Cr^{3+} as an impurity in the Al_2O_3 matrix of emeralds and rubies gives rise to the green and red color of these gems; yet, the electronic spectra of chromium-containing biomolecules are usually very simple. Three spin-allowed $d \rightarrow d$ transitions are expected; two normally occur in the visible region, while the third is expected in the ultraviolet region (where it can be hidden by ligand-based features). No charge transfer transitions generally occur while the visible absorption bands have extinction coefficients of typically less than 100 M^{-1} cm^{-1}. Thus, only relatively concentrated solutions of Cr^{3+} have observable color. Cr(III) complexes are generally stable against oxidation or reduction, although the redox potential can be significantly altered by certain ligands.

Although chromium as the Cr^{3+} ion was proposed to be an essential element about 50 years ago, its status is currently in question, as recent experiments appear to demonstrate that the element can no longer be considered essential. Supplemental nutritional doses of Cr^{3+} have been proposed to result in body mass loss and lean muscle mass development, leading to an appreciable nutraceutical industry being built around chromium. However, these claims have been thoroughly refuted. Chromium has also been suggested to be a conditionally essential element whose supplementation could lead to improvements in carbohydrate and lipid metabolism under certain stress situations, including type 2 diabetes and the effects of shipment of farm animals; this is currently an area of intense and hotly debated research with recent findings suggesting that beneficial effects from Cr^{3+} supplementation are pharmacologically, not nutritionally, relevant. However, while beneficial effects from chromium supplementation have reproducibly been demonstrated in rodent models of diabetes and insulin resistance, well-performed meta-analysis indicates that no clinical effects in humans result from dietary supplementation up to 1 mg chromium per day. Rodent studies use proportionally greater doses based on body mass than the human studies suggesting that clinical trials using larger doses of Cr^{3+} are required to establish if the metal ion has beneficial effects in humans. At the same time, supplementation of the diet with at least certain Cr(III) complexes has been proposed to have potentially deleterious effects, although recent data suggest that oral chromium supplementation is safe.

Determination in Food and Drink

Chromium levels in tissues, body fluids, food components, and other biological samples reported prior to c.1978 are problematic and should be ignored. At this time, improvements in analytic techniques revealed several problems, including appreciable contamination of biological samples (as these samples were often homogenized in a stainless steel blender); in fact, measured Cr levels reflected the levels of contamination not the actual tissue or fluid Cr concentrations, which were extremely small. Another major problem in atomic absorption experiments prior to 1978 was that workers were attempting to measure a tiny signal against a large background; a linear correspondence was actually found to exist between background absorbance and the reported apparent Cr content of samples. Currently, analyses of human blood and urine samples with Cr concentrations above 1 ppb should be considered suspect, unless the subjects are taking chromium supplements; even values above 0.5 ppb may be considered suspect. Consequently, studies prior to 1978 utilizing patients who were believed to be Cr-deficient based on Cr tissue or fluid concentrations and that reported Cr levels in tissues, foods, or fluids of one order to several orders of magnitude too high must be ignored. Thus, with the exception of some ^{51}Cr-labeled tracer studies, the fields of chromium nutrition and biochemistry really began in the late 1970s when chromium uptake and loss could be followed to a reasonable degree of accuracy. At present, chromium levels in tissues and biological fluids are usually determined by graphite furnace atomic absorption spectrometry (GFAAS), although neutron activation analysis (NAA) and inductively coupled plasma-mass spectrometry (ICP-MS) can also be used, with the latter growing in popularity. Inductively coupled plasma-atomic emission spectrometry (ICP-AES) has also seen some use although the detection limit is not suitable for urine and plasma samples so that the technique will not be discussed further. Neutron activation and ICP-MS have also been utilized with stable isotopes of Cr for determining Cr levels in tracer studies, in addition to the continuing use of radioactive ^{51}Cr for such studies. As most researchers have limited access to NAA, the technique will not be discussed further. These techniques are compared in Table 1.

Chromium is ubiquitous in foods but at very low concentrations. However, during processing, particularly in stainless steel (~10–40% chromium) equipment, the concentration of chromium appears to increase; in fact, most of the Cr in some foods may come from processing. Foods particularly rich in Cr (i.e., >100 ppb) include broccoli and black pepper and certain beers; however, values for vegetables must be considered carefully because of the variable amount of chromium that comes from soil contamination. The low concentrations of chromium in food, the ease of contamination, and the low suggested dietary requirement for chromium (30 μg day^{-1}) make

Table 1 Comparison of chromium analysis techniques

	GFAAS	*ICP-AES*	*ICP-MS*	*NAA*
Analysis time	2–3 min for only Cr	2–5 min all elements	2–5 min all elements	days multiple elements
Detection limit	0.1–0.01 ppb	10–1 ppb	1–0.01 ppb	~0.1 ppb
Isotope specific	No	No	Yes	Cr-50
Sample volume	Small	Moderate	Small	Varying
Instrument cost	Moderate	High	Very high	Extremely high
Operating cost	Moderate	High	High	High

preparation of a low-chromium (or chromium-deficient) diet difficult if even possible (as this assumes chromium is actually an essential trace element). Given the amount of Cr in the diet that comes from modern processing, the Cr intake of early humans must have been extremely limited.

Tables of chromium content for various foods are probably of limited utility because of the variability of chromium content of samples based on history (processing, growth conditions, etc.). For example, a 50-fold difference in chromium content has been found for different types of oatmeal. Foods used in nutrition studies need to be analyzed for each study, rather than depending on tabulated values in the literature. The chromium content of commercial rodent chows is surprisingly high and extremely variable, even between lots of the same brand. The amount of chromium in commercial rodent chows is normally about 500 $\mu g\,kg^{-1}$. The purified AIN-93G diet has 1 $mg\,kg^{-1}$ of added Cr^{3+} as potassium chromium sulfate dodecahydrate.

Precautions to Be Taken in Chromium Determination

Chromium concentrations in urine and blood are in the sub part per billion range unless subjects receive chromium supplementation. Hence, the concentration of Cr in these fluids is within about an order of magnitude of the detection limit of current techniques. Thus, contamination is a major concern. Similarly, the chromium concentration of tissues and foods generally range from 1 to 1000 ppb, meaning that contamination can be a major problem as well. For foods, knowing the history of the sample is important as processing can provide more chromium than the food initially contained. Differences in commercial processing can result in large differences in chromium content. Similarly, differences in chromium content of vegetables and other plants in terms of growth conditions are poorly known.

Because of the chromium content of stainless steel, contact with stainless steel should be avoided during sample collection and handling. For example, the use of a stainless steel blender to homogenize samples transfers appreciable amounts of chromium. Needles for venipuncture, thus, can be a challenge. Most of the chromium removed from a stainless steel needle during blood collection appears to be removed by the first 20 ml of blood; consequently, collecting samples after allowing several milliliters of blood to pass through the needle has been proposed. However, contamination, while reduced, still appears to be substantial. Siliconized stainless steel needles seem to be suitable. Plastic cannula or catheters are suitable. For plasma, anticoagulants, for example, ethylenediaminetetraacetic acid, used should also be low in chromium.

Plasticware (such as those made of polyethylene or polypropylene) should be used wherever possible in transferring and storing samples, and all glassware or quartz tubes should be acid-washed. Rubber stoppers are a source of chromium and should be avoided. All water and chemical reagents need to be of ultra high purity. Investigators should wear nonpowdered nitrile or polyvinylchloride gloves. All methods should be verified with the use of a certified chromium standard.

Sample Digestion

Discussion will be limited to samples for GFAAS, for which more data exist. Similar procedures are required for ICP-MS.

Urine

Sample digestion can be avoided for urine samples, although the use of the method of standard addition may be required to remove matrix effects and is recommended. Wet ashing can also be utilized; for example, digestion with HNO_3 and 30% H_2O_2 at subboiling temperatures for 15 h has been utilized. The solid can be dissolved in water for analysis.

Blood

For dry ashing, magnesium nitrate has been added as a matrix modifier to samples in quartz tubes followed by lyophilization using a stainless steel-free lyophilization apparatus. The solid is then ashed in a muffle furnace at 480 °C and then dissolved in HCl for analysis. Following this technique because of the uniformity of samples, the use of the method of standard addition has been reported to not be necessary.

Alternatively, blood plasma has also been enzymatically digested and used directly with the addition of magnesium nitrate as a matrix modifier; the method of standard addition was also utilized.

Tissues and Foods

For such samples, digestion of samples is essential. (Numerous variations of the procedures described have been used.) For dry ashing, magnesium nitrate is added before placing in a muffle furnace; the ash is dissolved in HCl for analysis. For wet ashing, samples can be treated with a mixture of nitric and sulfuric acids and heated to dryness at subboiling temperatures followed by dissolution in water for analysis. A combination of wet and dry ashing has also been used. Samples in borosilicate glass tubes are heated in a muffle furnace at 375 °C for 48 h; subsequently, nitric acid is added to the cooled samples that are then heated in a heating block at 90 °C. Additionally, hydrogen peroxide (50%) is added at time intervals until carbon particles are dissolved. Alternatively, the use of microwave digestion systems has become popular. Nitric acid is added to samples before heating in the microwave digestion system.

Determination Methods

Graphite Furnace Atomic Absorption Spectrometry

In GFAAS, a furnace dissociates a sample into its component atoms. Light from a hollow cathode tube (which is element-specific) is passed through the generated cloud of atoms where the atoms of the element of interest absorb the light. The absorption of light is proportional to the concentration of the element in the sample. The furnace, an electrically heated graphite tube with an opening for sample introduction, replaces the flame in traditional atomic absorption spectrometry. The furnace, in contrast to the flame, generates a higher

Table 2 Furnace program for chromium determination by GFAAS

Mode	Step	Temperature (°C)	Ramp time (s)	Hold time (s)
Dry	1	100	5	20
Dry	2	140	15	15
Char	3	1600	10	20
Atomization	4	2500	0	4
Clean	5	2600	1	3

density of atoms with a longer residence time in the cloud, increasing the sensitivity of the method. The use of modern instruments with their background corrections removes some of the pre-1978 difficulties; however, contamination given the low concentration of chromium in biological samples will always be a potential problem.

Generally, the protocol of Veillon and coworkers or variations thereof are utilized. After being injected into the furnace, samples are subjected to a program of drying (two steps at 100 and 120–140 °C), charring (1150–1350 °C), atomization (2500–2700 °C), and cleaning (2600–2700 °C). A typical program is shown in Table 2.

Inductively Coupled Plasma-Mass Spectrometry

ICP-MS generates the constituent atoms and ions using an ICP. In contrast to GFAAS where the absorption of light is detected, the generated ions are detected directly. The ions are separated based on their charge to mass ratio in the mass spectrometer, generally using a quadrupole analyzer or magnetic sector analyzer. The detection limit of ICP-MS is normally about an order lower than GFAAS based on the number of ions generated in the former compared to the number of atoms excited in the latter. Contamination becomes a greater concern as the level of detection is pushed to lower limits. ICP-MS has the advantage of being isotope-specific, whereas GFAAS is only element-specific. ICP-MS instruments are considerably more expensive than GFAAS, which is why GFAAS is currently more commonly used for Cr analysis. ICP-MS instrument currently also requires more care in operation, although analysis time is shorter than for GFAAS.

Chromium(III) and Chromium(VI) Speciation

Given the different chemistries and toxicities of chromium(III) and chromium(VI) complexes, determining of the concentration of one or the other rather than the total chromium concentration is often desired. However, none of the techniques described earlier can separate complexes of the two oxidation states but rather only measure total chromium content. Techniques, such as colorimetric assays that can specifically test for chromium(IV) species, lack the sensitivity to measure biological concentrations. Consequently, some type of separation technique is required before detection of chromium using one of the determination methods described earlier. This separation must be accomplished without the oxidation states of the chromium changing. The separation also can lead to dilution requiring preconcentration before chromium analysis. The concentration of each ion can be measured, or the concentration of one ion in combination with the total chromium concentration can be measured allowing for the calculation of the concentration of the other ion.

Developing methods to separate these ions at low concentrations without altering the oxidation states is an area of active research. Most research has been focused on water samples that generally lack the complexity of biological samples. Yet, studies have shown that Cr^{3+} and Cr^{6+} concentrations can be determined in urine and plasma samples, although nothing has been adapted for routine use to date. For example, cloud point extraction has been combined with GFAAS to determine the concentrations of Cr^{3+} and Cr^{6+} in human blood serum samples, while ion-pair reversed-phase high-performance liquid chromatography (HPLC) has been coupled with ICP-MS to measure the concentrations of Cr^{3+} and Cr^{6+} in human urine (from chromate workers).

Conclusion

Cr^{3+} and Cr^{6+} ions have distinctly different chemistry. Most notable, Cr(VI) complexes are kinetically stable and thermodynamically unstable, while Cr(III) complexes are both kinetically and thermodynamically stable. In a biological environment, only Cr^{3+} is stable as Cr^{6+} is reduced via Cr^{5+} and/or Cr^{4+} intermediates to Cr^{3+}. This chemistry results in Cr^{6+} being toxic, mutagenic, and carcinogenic, while Cr(III) complexes are generally nontoxic. Chromium concentrations in tissues, body fluids, foods and beverages, and other biological samples are very low. Chromium concentrations in these samples reported prior to c.1978 are problematic (except when radioactive Cr-51 was used as a tracer), and these values must be disregarded as they are often too low by as much as three orders of magnitude. Currently, chromium concentrations in biological samples are generally measured using graphite furnace atomic absorption spectroscopy or ICP-MS, with the former being more popular because of instrument expense and ease of maintenance. Sample preparation must be performed carefully to prevent contamination, particularly from stainless steel. Currently, research in the separation and determination of Cr^{3+} and Cr^{6+} simultaneously is an active area.

See also: Chromium: Physiology; Cooking: Domestic Techniques; Trace Minerals and Trace Elements.

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Cider (Cyder; Hard Cider): The Product and Its Manufacture

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Definition and Origin

Cider is generally defined as an alcoholic beverage obtained by apple juice (apple must) fermentation. Noteworthy, in North America, the term 'cider' is rather associated with a cloudy unfermented and unpasteurized apple juice, whereas the fermented product is called 'hard cider.' In Europe, the fermented product is mainly named 'cider' in the UK, 'cidre' in France, 'sidra' in Spain, and 'apfelwein' in Germany. Another common fruit fermentation is obtained from pears and leads to a product named 'perry' in English or 'poiré' in French. It is worth mentioning that cider can either be a final product ready for consumption or an intermediate product used for apple brandy (e.g., 'Calvados' in Normandy and 'Lambig' in Brittany) production by distillation or cider vinegar via an acetic fermentation. Moreover, Calvados blended with apple must leads to an aperitif-type beverage (i.e., predinner drink) named 'Pommeau' in France.

Like the other major fermented beverages (i.e., wine and beer), cider is one of the oldest alcoholic beverages in the world; Hebrews called it 'sichar,' whereas Romans and Greeks called it 'sicera' and 'sikera,' respectively. From an etymological point of view, the name *cider* would come from 'sicera' (meaning any fermented beverage that is not wine; Cambridge Psalter); this can especially be observed in Normandy, as 'cidre' was originally spelled 'sidre.' Although the name *cider* was not used at the time, during antiquity, a certain number of writings by Pliny the Elder or Palladius refer to alcoholic beverages obtained from apples or pears. During the ninth century, the term 'sicetores,' referring to brewers producing ale but also 'pomacium' from apples, was used by Charlemagne. In France, the first use of the word 'cidre' was found in the Conception de Nostre-Dame (Wace, twelfth century). From this time, and thanks to the invention of the press (thirteenth century), cider production extended to various apple-producing European regions. From the fourteenth to twentieth centuries, technological practices and processes were optimized and led to higher volumes and better-quality products. Nowadays, cider production, although far more limited than wine and beer, can be found on every continent in apple-growing regions worldwide. In Europe, the UK (mainly West Country, West Midlands but also Wales), France (mainly Normandy and Brittany), Spain (mainly Asturias and Basque country), and Germany are the main cider-producing countries, although many others have local productions (e.g., Ireland, Austria, Poland, Sweden, Norway). In North America, hard cider production is done in the United States, Canada, and even Mexico. Interestingly, in Quebec, a new cider type named 'ice cider' (equivalent to icewine in enology and thus using apples with high sugar contents due to natural frost) has recently appeared. In South America, cider is produced in Argentina and Chile. Cider production is also found in Asia (China and Japan), Africa (South Africa), Australia (Tasmania), and New-Zealand.

Although the generic term 'cider' is commonly used, the term 'ciders' is more appropriate to reflect the large variety of fermented apple products. According to production region, cider can be produced from only apples, with the addition of pear or from apple concentrate. Additives such as sugar, acid, or coloring agents can also be used. Sugar content may vary leading to dry, semidry, or sweet ciders. Carbon dioxide (CO_2) may be absent or present and obtained in the final product by carbonation or a secondary fermentation. In this context, beyond the classical definition of the term *cider*, it is important to note that several country regulations exist to define cider and cidermaking. Examples of regulations include the French "Décret n°87–600 du 29 juillet 1987 modifiant le décret n° 53–978 du 30 septembre 1953 relatif à l'orientation de la production cidricole et à la commercialisation des cidres et des poirés," the UK "Customs & Excise Notice 162: cider production" and the Spanish "Orden de 1 de agosto de 1979 por la que se reglamentan las sidras y otras bebidas derivadas de la manzana." In these regulations, authorized products entering the cidermaking process as well as specific analytical parameters (e.g., ethanol, volatile acidity, or SO_2 levels) are defined.

Moreover, to ensure cider product quality, several certifications exist at the national or international (European) level. They are delivered based on certain product specifications and requirements to ensure product authenticity and quality for consumers. Examples of these certifications, which are generally easily recognizable by specific logos on the label, include

- Protected Designation of Origin (PDO) and covers, as defined by the European Commission, "agricultural products and foodstuffs which are produced, processed and prepared in a given geographical area using recognized know-how" (e.g., Cidre Pays d'Auge, Cidre de Cornouaille and Sidra de Asturias).
- Protected Geographical Indication (PGI) and covers, as defined by the European Commission, "agricultural products and foodstuffs closely linked to the geographical area. At least one of the stages of production, processing or preparation takes place in the area" (e.g., Cidre de Bretagne, Cidre de Normandie, Gloucestershire cider, Herefordshire cider, and Worcestershire cider).
- Organic farming corresponds to products that must comply with strict EU requirements covering not only production and processing, but also the control and labeling of organic foods. This certification has gained interest due to strong societal demand but also higher commercial value for the product.
- 'Label Rouge' (red label) is specific to France and attests that the product, examined by a national commission, presents superior overall quality compared to a similar product (e.g., Cidre de Normandie, Cidre Royal Guillevic).

Production

Cider production practices in France (Figure 1) and the UK, although both leading to products called ciders, exhibit major differences that in some instances can even be considered opposite. In the following section, these practices will be used as examples.

Raw Material

Although cider can be produced from any apple cultivar, in Europe, a distinction is traditionally made between dessert, cooking, and cider apples. Cider apples have distinct organoleptic qualities that make them uninteresting for direct consumption linked to their acidity or natural polyphenol content. On the contrary, these traits provide high product qualities in terms of body equilibrium (sweetness, acidity, astringency, and bitterness) that are not obtained using dessert apples. Different cider apple cultivars exist, and more than 1000 have been described, although only a few are used for production. They are classified into six categories based on juice-specific savors:

- *Sweet*: These apple varieties are the blandest of the six categories. They have low acid (<60 mequiv.l^{-1}) and polyphenol (<2 gl^{-1}) contents and up to 15% sugar (cultivars: *Douce Coëtligné, Doux Normandie, Muscadet Petit de l'Orne, Rouge Duret, Sweet Copin, Sweet Alfortd, Nortwood* . . .).
- *Sour*: These apples are mildly sour (60–90 mequiv.l^{-1}) with low polyphenols (<2 gl^{-1}) (cultivars: *Guillevic, Judeline, Rouget de Dol, Locard blanc* . . .).
- *Sharp*: These apples provide sourness and freshness to cider due to their high acidity (>90 and up to 240 mequiv.l^{-1}) and low polyphenol (<2 gl^{-1}) contents (cultivars: *Petit Jaune, Judor, Avrolles, Locard vert, Crimson King, Brown's Apple* . . .).
- *Bittersweet*: This apple type corresponds to intermediate cultivars with low acidity (<60 mequiv.l^{-1}) and high polyphenol (>2 gl^{-1} and <3 gl^{-1}) contents providing bitterness and astringency to cider (cultivars: *Douce Moën, Bedan, Binet Rouge, Bisquet, Ashton Bitter, Dabinett, Yarlington Mill, Tremlett's Bitter* . . .).
- *Bittersharp*: These apples have high acid (>60 mequiv.l^{-1} and up to 240 mequiv.l^{-1}) and total polyphenols (>2 gl^{-1} and up to 6 gl^{-1}) (cultivars: *Cazo jaune, Kingston Black, Foxwhelp* . . .).
- *Bitter*: These apples have low acid (<60 mequiv.l^{-1}) and high total polyphenols (3–9 gl^{-1}) (cultivars: *Marie Ménard, Kermerrien, Petit Amer, Fréquin rouge, Bramtot, Ellis Bitter* . . .).

Traditionally, ciders are not obtained from a single cultivar. Indeed, blending ('pommage') of different apple types allows for the production of balanced ciders with various organoleptic qualities. Also, three maturity stages can be distinguished: early season (beginning to end of September), midseason (beginning of October to mid-November), and late season (mid-November to end of December). After harvest, which is rather mechanically performed nowadays, apples are stored for a maximum of 5 days before processing to reach full maturity and to ensure that all starch has been converted into sugar. This short time period allows good fruit preservation and texture compatible with optimal pressing.

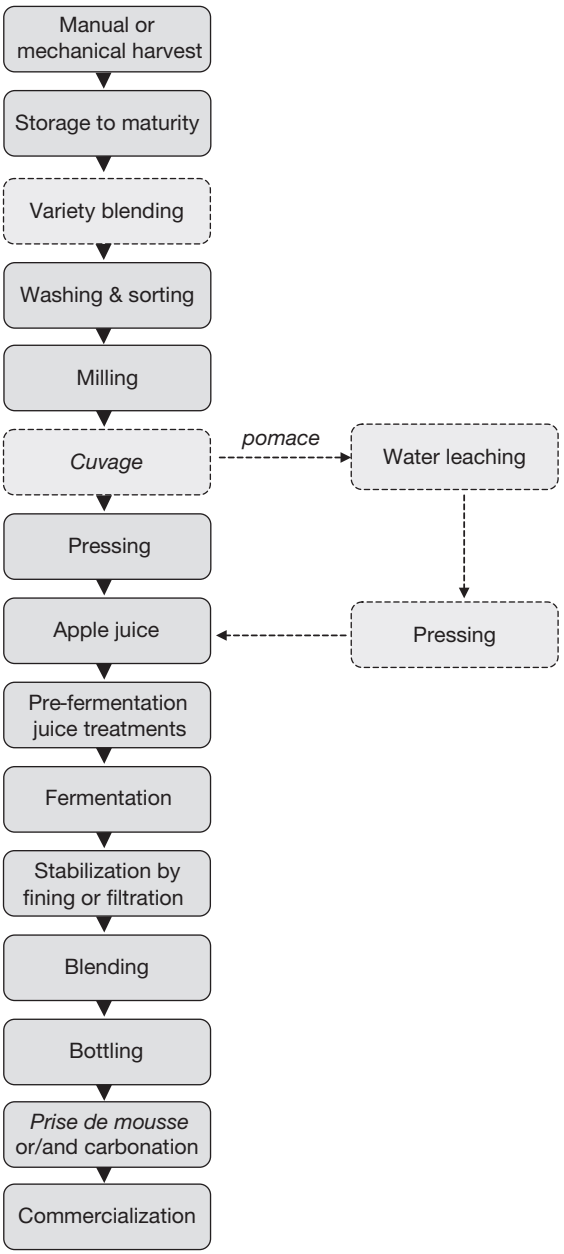

Figure 1 French cidermaking diagram. Steps in lighter gray and discontinued lines are optional.

Milling and Pressing

Before any processing operation, fruit sorting and washing are required to eliminate rotten fruits and potential foreign materials (e.g., wood pieces, grass, soil, and stones) that could impact overall product quality. Rotten fruits are associated with mold contamination and originate either directly from the apple tree itself (*Monilia* spp.) or indirectly after impacts due to falling and being in contact with the ground

(*Fusarium* spp.). All damaged fruit, especially in the case of mechanical harvesting, can also be spoiled by secondary molds belonging to *Botrytis* and *Penicillium* genera. Beyond the rotten aspect of these fruits and potential associated off-flavors, it is worth noting that some molds are also able to produce toxins (mycotoxins) that may have an impact on human health. Milling (*rapâge* in French) is then performed to obtain fruit pulp for more efficient juice (or must) extraction. In general, a high-speed mill corresponds to a wheel bearing graters or coarse knives rotating against a fixed surface; they are calibrated to tear the fruit tissues without smashing the seeds. Fine milling is used for firm apples, whereas rougher milling is used for very ripe apples. In France, an extra step called 'cuvage' (pectin leaching for 2–5 h for better pressing yields) can be performed.

In both the UK and France, the next step involves pressing the pulp by exerting high pressure. Nowadays, technological evolutions have led to the use of semicontinuous, continuous, or even automated presses, although discontinued and more traditional presses can still be encountered in farm and artisanal cider productions. The main types encountered are

- *Horizontal presses*: They correspond to compressible chambers, equipped with several juice ducts, in which the pulp is introduced. Then pressure is applied through the action of a piston or a membrane. Thanks to several cycles of pressure and homogenization, high extraction yields (up to $800\,l\,T^{-1}$) can be reached.
- *Continuous belt presses*: In this case, the pulp is placed between two bands that will go through a series of rollers that apply pressure onto the belt, resulting in a pressing action. This type of press gives yields ranging from 600 to $750\,l\,T^{-1}$.

Pressing leads to two products, on the one hand, a liquid phase corresponding to the juice, also called 'apple must' (*moût pur jus* in French), and on the other hand, a solid phase called pomace (*marc* in French). After coarse screening, the juice is transported through piping systems to stainless steel, HDPE (high-density polyethylene) or fiberglass tanks. Less commonly, wood tanks can still be encountered in more traditional cider productions, even though this material is more difficult to clean and sterilize, thus leading to potential product spoilage.

The obtained pomace (200–400 kg per ton) still contains compounds of interest. A second extraction can therefore be performed by water leaching (countercurrent water percolation of the pomace) or by pressing the watered pomace. These actions lead to diluted juice (*moût de diffusion* or *petit jus* in French) that will be added to the first juice obtained. The extraction step may be facilitated by using enzymatic cocktails (pectinases and hemicellulases, or pectin methylesterase [PME] in the presence of calcium) – by themselves or in combination with the mechanical material.

Prefermentation Juice Treatments

In France, juices are subjected to additional specific steps called *défécation* and *décantation* (keeving and settling in English) to obtain slow and long fermentations. Keeving corresponds to the traditional juice treatment, and about half of French cider juices are still treated by this method.

- Keeving relies on the ability of pectins to form a gel via PME enzyme and calcium action. All compounds necessary for keeving (pectin, PME, and calcium) are naturally present in juice. During PME demethylation, an increase of acidic groups on pectin chains is observed. In the presence of calcium, bonds will form between pectic chains, thus leading to gel formation. After 2–6 days, CO_2 is generated by the onset of the fermentation process, and the bubbling action will force the gel to rise to the top of the tank, carrying along suspension deposits (rich in nitrogen) that form a complex structure called *chapeau brun*. At the same time, complexed materials will also sediment to the bottom of the tank. The clarified juice is situated between the gel and sediment layers. The natural process is slow and not always reliable. Nowadays, in order to increase the reliability of this static system, fungal PME preparations and $CaCl_2$ are added. Recently, a continuous dynamic system, termed *flottation*, has also been used by some cider producers. In this case, the juice is treated for up to 48 h, before being introduced in a specific device with nitrogen bubbling in the presence of $CaCl_2$.
- Settling is obtained by pectin hydrolysis, using a combination of enzymes (PME, endopolygalacturonase and pectinlyase). The clarified juice is obtained by decanting; sometimes this is followed by fining by gelatin addition. This method is widely used by industrial cider producers.

The clarified juice obtained by either method is then transferred to another tank to begin the next production step. Prefermentation clarification generates juices without any suspension deposits and low bacterial counts, thus reducing potential microbial spoilage. Moreover, amino nitrogen content and yeast counts are also reduced, thus leading to a slow fermentation. Polyphenols have also been shown to be affected by these treatments, mainly through a reduction in both flavanol content and average degree of polymerization.

In the UK, keeving is not performed but juice additions are. The goal is to obtain a blend of fermentable sugar sources, including juice but also apple juice concentrates and/or syrups that give a final product of 10–12% alcohol after fermentation. Whereas in the case of French cider production, everything is done to allow for slow fermentation by reducing nutrients, reducing yeast counts, and using cold temperatures. In the UK, the goal is to ferment juices to dryness in as a short time as wine. To achieve this, several nutrients are added to the blend. Although there is obviously no sugar shortage in apple juice, ammonium nitrogen and vitamins (enzymatic cofactors), which are essential for yeast development and thus for fermentation, can be limiting, thereby leading to sluggish or stuck fermentations. This is especially the case for apple juice concentrates and syrups. In this context, ammonium phosphate (250 ppm) will be used to standardize the blend in amino nitrogen content (about 100 mM). Thiamin, biotin, panthoneate, or pyridoxine vitamins can also be added. As performed in France, British cider makers also depectinize the juice using pectinolytic enzyme preparations. This indeed facilitates filtration operations and prevents haze formation.

Finally, sulfur dioxide (SO_2) is an important technological additive that is added during both French and British fermentation processes. It presents two main properties of interest for cider making and is widely used in oenology. First, it exhibits antiseptic activity against bacteria and non-*Saccharomyces* yeast. The multiple SO_2 targets leading to microbial cell death explains why no sulfite resistance has been observed to date. Second, sulfite has antioxidant (leading to sulfate formation in the presence of oxygen) and antioxidative (oxidase inhibition) activities. SO_2 performance is directly linked to pH as only the undissociated form (termed 'molecular SO_2') is active. Therefore, it is recommended to adjust the pH of the juice to pH 3.75 by adding malic acid, the main organic acid found in apples. Moreover, SO_2 interacts with various chemical compounds (especially aldehydes), microorganisms, and solids; therefore only the so-called free SO_2 will be active and participate to the overall juice or blend quality. However, SO_2 can negatively affect cider aroma, in particular due to its action on oxidative flora, and more important is considered to be an allergen (legal limits for SO_2 contents in ciders have been set in Europe). Although no efficient alternative has been found to date, ascorbic acid (antioxidant properties) addition can help reduce SO_2 concentrations.

Fermentation

Fermentation refers to microbial activities that will transform the juice into cider. Like any other technological process, the key factor to obtain the desired end product is control. This is especially true and critical when living organisms are involved. In the context of cidermaking, several technological levers can be used. These levers correspond to biotic factors (microbial inoculation or indigenous flora) and abiotic factors, either intrinsic (pH, amino nitrogen content, sugar content) or extrinsic (temperature, oxygen content). As discussed earlier for amino nitrogen content, the contrast between the French and British processes is also obvious for the fermentation step.

French production relies exclusively on indigenous flora. The typical fermentation temperature is between 5 and 15 °C, due to the fact that cider tanks were traditionally situated outside the buildings and thus directly influenced by seasonal temperatures. This also prevents or inhibits lactic acid bacteria (LAB) and spoilage microorganism growth. The combination of low temperature, low amino nitrogen content, and lower yeast counts (by centrifugation or filtration at strategical time points) yields slow fermentation times, in accordance with the desired organoleptic qualities. In the case of PDO and *Label Rouge* ciders, a 6-week minimum fermentation time before bottling is required. In the cider industry, temperature is regulated between 8 and 10 °C by refrigeration systems to control the fermentation, whereas in traditional ciderhouses, racking, centrifugation or filtration can still be used for control.

French cider production consists of several phases:

- *Oxidative phase*: This phase is named oxidative due to the presence of oxygen in juice and lasts from 5 to 15 days. From a microbiological point of view, high species biodiversity can be observed (Tables 1 and 2) with oxidative or slow fermentative yeasts especially belonging to the *Metschnikowia*, *Hanseniaspora*, and *Candida* genera (Figures 2(a) and 2(c)). During this stage, little alcohol is produced (<1%), but numerous volatile compounds are produced, especially esters that largely contribute to the aromatic qualities of the final product.
- *Alcoholgenic phase (alcoholic fermentation)*: During the transition between the oxidative phase and this phase, microbial diversity largely decreases and the *Saccharomyces uvarum* species becomes dominant (Figures 2(a) and 2(c) and Table 1). Under anaerobic conditions, this species, more adapted to cold temperature than *Saccharomyces cerevisiae* (the main species carrying out the alcoholic fermentation in many fermented beverages), mainly metabolizes sugars into ethanol and CO_2 as well as contribute to the organoleptic qualities of the cider by producing fusel alcohols, acids, and more important, ethyl esters.
- *Maturation phase*: This stage actually corresponds to a slower fermentation period, usually associated with a decrease in *S. uvarum* counts and sometimes with the appearance of other yeast species such as *Lachanceae cidri*, *S. cerevisiae*, *Brettanomyces anomala*, or *B. bruxellensis*. In general, LAB counts can also rise at this time (Figure 2(b)), due to increased fermentation temperatures (climate changes) and their metabolism (especially for the *Oenococcus oeni* species). They induce malolactic fermentation (this transformation sometimes happens concurrent with the alcoholic fermentation). LAB species encountered mainly belong to *Oenococcus*, *Lactobacillus*, *Leuconostoc*, and *Pediococcus* genera (Table 2). Malolactic fermentation results in the decarboxylation of the main apple juice acid (malic acid) to lactate. The loss of a carboxylic (–COOH) function leads not only to the production of CO_2 but also to a reduction in acidity. Although this metabolic step can positively contribute to the overall organoleptic quality of the product, it is not always desired as it may lead to ciders with high pH values (>3.75), compatible with microbial spoilage. Malolactic transformation can be controlled by racking, centrifugation, and filtration operations. The maturation stage can last until bottling.

Except for traditional ciders, British fermentation can be considered as the complete opposite. Indeed, in the UK cider industry, rapid and complete fermentations are performed. To do so, not only are juice compositions adjusted as described earlier, but they are also inoculated with commercial active dried yeasts (*S. uvarum*, *S. bayanus*, or a mix of both). The commercial yeasts usually originate from the wine industry. Notable technological traits of these yeast strains include aroma production, killer activity (favors strain development in the presence of other yeasts), and flocculation (for easy clarification). Inoculation trials, concerning both alcoholic fermentation (even using mixed cultures for ester production), but also malolactic transformation, have been performed in various countries; however, to this date, this method has not yet been widespread.

Usually in the UK cider industry, an aerobic phase is performed to favor yeast sterol production, essential to cope with the final ethanol content. After this step, the inoculated juice is fermented to dryness under anaerobic conditions for one week at temperatures ranging between 15 and 20 °C.

In all cases, alcoholic fermentation is monitored by following density or specific gravity, until the desired residual

Table 1 Yeast biodiversity in cider during the various production steps

Species	*Cider type*	*Dominance*	*Fermentation stages when species can be found*
Arthroascus schoenii[a] *(formerly Endomyces schoenii)*	French cider	+	Cider must, oxidative phase
Cryptococcus sp.[a]	French cider	+	Water for apple transport, cider must, oxidative phase
Candida matritensis	French cider	+	Cider must, oxidative phase
Candida oleophila	French cider, UK cider	+/−	Cider must, oxidative phase
Candida parapsilosis	Spanish cider	+	Cider must, oxidative phase
Candida pomicola	French cider, UK cider	+	Cider must, oxidative phase
Candida sake	French cider	+/−	Cider must, oxidative phase
Candida stellata	French cider	+	Cider must, oxidative phase
Candida tropicalis	French cider	+/−	Cider must, oxidative phase
Dekkera anomala	French cider	++	Maturation phase, bottled cider
Dekkera bruxellensis	French cider	+	Maturation phase, bottled cider
Hanseniaspora osmophila	French cider, UK cider, Spanish cider	+	Cider must, oxidative phase
Hanseniaspora sp.	French cider	+	Alcoholic fermentation phase, maturation phase
Hanseniaspora uvarum	French cider, UK cider, Spanish cider	+++	Cider must, oxidative phase
Hanseniaspora valbyensis	French cider, UK cider, Spanish cider	++	Cider must, oxidative phase or late stages
Issatchenkia occidentalis	French cider	+	Water for apple transport, cider must, oxidative phase
Issatchenkia terricola	French cider	+	Cider must, oxidative phase
Kloeckera sp.	French cider, Spanish cider	+	Cider must, oxidative phase
Kluyveromyces marxianus	French cider	+	Cider must, oxidative phase
Lachanceae cidri (formerly *Zygosaccharomyces cidri*)	French cider	++	Alcoholic fermentation phase, maturation phase, bottled cider
Metschnikowia pulcherrima	French cider, UK cider, Spanish cider	++	Cider must, oxidative phase
Meyerozyma guilliermondii (formerly *Pichia guillermondii*)	French cider, UK cider, Spanish cider	++	Cider must, oxidative phase
Pichia delftensis	French cider	+	Cider must, oxidative phase
Pichia fluxum	French cider, Spanish cider	+	Cider must, oxidative phase
Pichia membranifaciens	French cider	+	Cider must, oxidative phase
Pichia misumaiensis	French cider, UK cider	+	Cider must, oxidative phase
Pichia nakasei	French cider	+	Cider must, oxidative phase
Rhodotorula ingeniosa	French cider	+	Water for apple transport, cider must, oxidative phase
Saccharomyces uvarum	French cider, UK cider, Spanish cider	+++	Alcoholic fermentation phase, maturation phase, bottled cider
Saccharomyces cerevisiae	French cider, Spanish cider, UK cider	++	Alcoholic fermentation phase, maturation phase, bottled cider
Saccharomycetes sp.	French cider	+	Water for apple transport, cider must, oxidative phase
Torulaspora delbrueckii	French cider	+	Cider must, oxidative phase

Dominance based on studies available in the literature and unpublished data (French ciders). +, punctually identified; ++, regularly present; +++, dominant species.
[a]Filamentous fungi.

sugar concentrations are reached (in the UK: dry, in France: $< 28\,g\,l^{-1}$ for 'Brut,' between 28 and $42\,g\,l^{-1}$ for 'Demi-Sec,' and $> 35\,g\,l^{-1}$ for 'Doux' ciders). Malolactic fermentation is monitored by the follow-up of malate and lactate using classical biochemical methods (ex. paper thin layer chromatography).

Postfermentation Treatments

After fermentation, different operations are performed before bottling. In most cases, cider is racked from the lees to clarify and stabilize the product. If not, a limpid and stabilized product is obtained by fining, centrifugation or filtration operations that can be combined or applied individually. Fining consists of adding proteins that interact with polyphenols and form complexes to entrap matter in suspension while settling out. Typical fining agents correspond to gelatin (animal protein) or chitosan (prepared from crab-shell chitin); however, bentonite, a negatively charged absorbent clay, can also be added to accentuate sedimentation. Filtration is performed using either kieselguhr, an unconsolidated form of diatomite, or plate filtration or microfiltration. For traditional ciders, fining and filtration are not performed, thus leading to yeast haze and deposit in the bottle.

Table 2 LAB biodiversity in cider during the various production steps

Species	*Dominance*	*Fermentation stages when species can be found*
Lactobacillus brevis	+	MLF fermentation phase, maturation phase
Lactobacillus buchneri	++	All stages
Lactobacillus casei/paracasei	+	Early and late phases
Lactobacillus collinoides	+++	All stages
Lactobacillus diolivorans	+	Occasionally identified
Lactobacillus mali	+	All stages
Lactobacillus pentosus	+	All stages
Lactobacillus sp.	+	Maturation phase
Lactobacillus fermentum	+	All stages
Lactobacillus plantarum	+	All stages
Leuconostoc mesenteroides	+++	Early, oxidative phase, less frequent in late phases
Oenococcus oeni	+++	Dominant during alcoholic, MLF, and maturation phase, can be present in all stages
Pediococcus ethanodurans	+	Occasionally identified
Pediococcus parvulus	+	All stages
Pediococcus pentosaceus	+	All stages

Dominance based on studies available in the literature and unpublished data (French ciders). +, punctually identified; ++, regularly present; +++, dominant species.

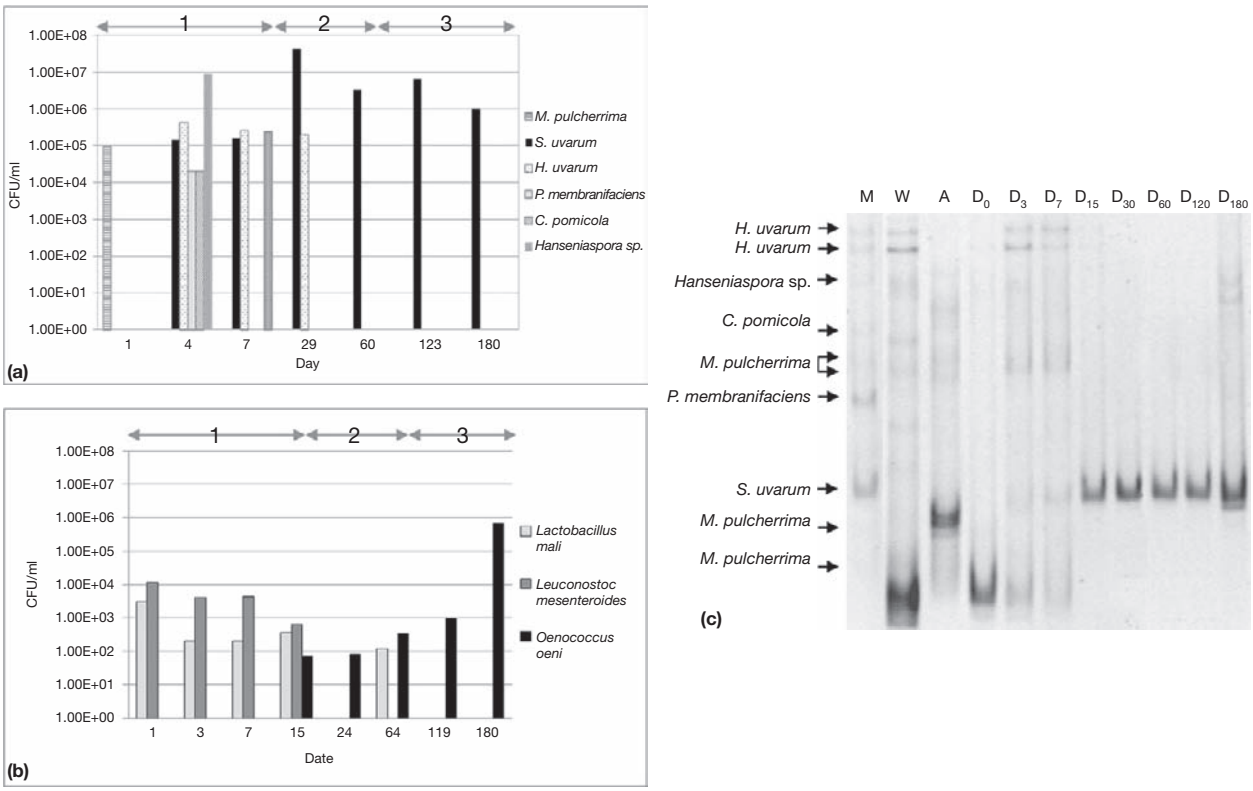

Figure 2 Examples of microbial dynamics during cidermaking observed using culture-dependent (PCR-RFLP for yeast (a) and ARDRA for LAB (b)) and culture–independent (using TGGE for yeast (c)) methods. All methods showed a decrease in biodiversity leading to the dominance of *S. uvarum* for yeast (responsible for alcoholic fermentation) and *O. oeni* for LAB (responsible for malolactic fermentation). 1, 2, 3 correspond to fermentation stages; W, water used to transport apples; and A, apples in silos.

Ciders are then blended based on the experience of the cidermaker. Noteworthy, although limited additions are allowed in most countries (see detailed legislations), in the UK, water is added to reach the desired alcoholic level. Other additives permitted include sweetening agents such as sugar, but also acids, coloring agents, and preservatives to obtain expected organoleptic qualities. Unlike in Germany and Spain, British and French ciders are expected to be effervescent. In industrial or artisanal ciders, carbonation is performed to saturate the liquid with *ca.* 5–6 g l^{-1} CO_2. Natural effervescence is obtained through a supplementary step, called *prise de mousse* in French, and is usually performed during the maturation phase. To do so, indigenous yeast fermentation, or inoculation of active dry yeasts in bottles or in tanks is

performed. The yeasts naturally ferment residual sugars to form CO_2. Temperature, yeast counts, and residual amino nitrogen will be the main factors affecting effervescence in the final product.

Finally, the product is bottled in glass or PET bottles or kegs using an isobarometric filling machine to preserve product effervescence. Pasteurization is possible according to product type and nature of the container (tunnel pasteurization after filling for glass bottles, flash pasteurization and chilling before bottling for PET bottles). Although the temperature and duration applied for microbiological stabilization are not necessarily high, due to pH and alcohol content, this step may still impact the organoleptic characteristics of the final products (typical cooked apples or caramel flavors). The product is then commercialized.

Cider Spoilage

Different types of spoilage can occur during cidermaking and can arise from either the presence of undesirable microorganisms or specific physicochemical parameters. A survey performed in 2004 among French cidermakers showed that microbial spoilage was of greater importance. In terms of frequency and economic impact, the main microbial spoilages are

- *Phenolic off-flavor*: This organoleptic defect is due to volatile phenols usually associated with 'animal,' 'leather,' 'phenolic,' or 'spicy' aromatic notes. This spoilage has been extensively studied in wine and to a lesser extent in cider. It has been shown that *Brettanomyces* spp. yeasts are responsible for the transformation of hydroxycinnamic acids into volatile phenols via vinyl phenol intermediates (these reactions are mediated by hydroxycinnamate decarboxylase, and vinylphenol reductase activities). However, in wine as in cider, hydroxycinnamic acid mainly exists in an esterified form (with tartaric acid in wine and quinic acid in cider) that *Brettanomyces* cannot metabolize. In wine, it was shown that commercial enzymatic preparations, containing a cinnamoyl esterase, were mainly responsible for hydroxycinnamic acid production. In cider, it was recently shown that *Lactobacillus collinoides* could metabolize the main hydroxycinnamic ester, chlorogenic acid (= caffeoylquinic acid), and produce the caffeic acid precursor. This precursur can be further metabolized into ethyl catechol by either *L. collinoides* or *B. anomala* species.
- *Cider-sickness*: This spoilage, known as 'framboisé' in France, is less frequent but can have a severe economic impact. It mainly concerns sweet ciders. It occurs in tanks or in bottles and is characterized by an excessive production of acetaldehyde (often above regulatory limits, see following). This leads to unpleasant flavors (rotten banana, vegetal aromas), haze formation, high pressure in bottles, and excessive foaming. This spoilage is associated with the development of the Gram-negative bacterium, *Zymomonas mobilis* (the subspecies *pomaceae* is associated with British cider-sickness, whereas the subspecies *francensis* is associated with French 'framboisé' ciders). It vigorously ferments sugars into multiple end products including excessive amounts of acetaldehyde and CO_2. Favorable factors are residual sugars, absence of SO_2 addition, pH >3.75, and high amino nitrogen. Acetaldehyde levels are regulated in France (<100–120 mg l^{-1}).
- *Acrolein spoilage*: This spoilage is mainly associated with ciders fermented to dryness and used for apple brandy production. Spoilage is associated with LAB able to degrade glycerol (including *L. collinoides*, although this trait may be strain-dependent). Glycerol is produced (*ca.* 5 g l^{-1}) by yeast during fermentation and provides roundness to the product. Glycerol is degraded via a glycerol dehydratase into 3-hydroxypropionaldehyde (3-HPA). This molecule is a precursor for acrolein and can be nonenzymatically formed via spontaneous dehydration in acidic conditions. It leads to pepper flavors and a strong bitter taste.
- *Lactic acid spoilage*: This is due to the natural metabolism of LAB, associated with their early development before alcoholic fermentation is complete (in the presence of fermentable sugars). It can be easily detected as malolactic transformation only leads to L-lactate while lactic acid spoilage is associated with both D- and L-lactate isomers. Acetic acid is also formed by heterofementative LAB. The economic impact of lactic acid spoilage is rather low.

The following microbial spoilages are less frequent:

- *Ropiness*: This spoilage is characterized by abnormal viscosity associated with the presence of exopolysaccharides (sugar polymers). The type of exopolysaccharides depends on the producing microorganism. Responsible microorganisms in cider have been associated to LAB (e.g., *Pediococcus damnosus*, *Lactobacillus sicerae*), or sporulating bacteria (*Bacillus licheniformis*).
- *Acetic acid spoilage*: This alteration is easily recognizable due to its characteristic acidic taste and vinegar flavor. It is associated with the development of acetic acid bacteria (e.g., *Acetobacter*, *Gluconobacter* spp.) that metabolize ethanol in the presence of oxygen. Acetic acid can then react with ethanol to form ethyl acetate, another off-flavor molecule. Therefore, maintaining the product in anaerobic conditions will prevent this spoilage.

Concerning, physicochemical spoilage ('casse' in French), they are observed when cider is in contact with air and thus, as stated earlier, can be controlled by using anaerobic conditions. In the presence of oxygen, polyphenol oxidation occurs conferring product instability and color change. This reaction is observed in the presence of metals (iron or copper) that contribute to polyphenol complexation. Oxidative spoilage is associated with the polyphenol oxidase enzyme, linked to overripened or moldy fruits. Finally, protein haze, characterized by deposits in bottles, is associated with protein–polyphenol combinations. Noteworthy, protein concentration being low in cider, it is rarely observed.

Cider Composition, Organoleptic Qualities and Health Impact

Composition

Cider composition (Table 3) first depends on apple juice composition and thus not only on the apple varieties but also culture conditions, fruit maturity, and physical (cracks and bruises) or

Table 3 Physicochemical parameters determined for 150 ciders representative of French cider production

		Mass density	Alcohol (%vol)	pH	L-Malate (mg l^{-1})	D-Lactate (mg l^{-1})	L-Lactate (mg l^{-1})	Acidity (mequiv.l^{-1})	Volatile acidity	Sorbitol (mg l^{-1})	Fructose (mg l^{-1})	Glucose (mg l^{-1})	Sucrose (mg l^{-1})	Total sugar (mg l^{-1})
All ciders	From	1000.9	1.4	3.28	0.00	0.04	0.04	1.11	0.03	3.1	8.2	0.1	0.0	8.0
	To	1040.2	7.2	4.22	6.79	1.53	4.35	7.09	1.11	14.1	62.7	20.8	11.8	82.3
	Mean	1017.4	4.1	3.74	1.29	0.24	1.61	2.63	0.42	6.3	34.5	7.1	0.4	39.6
	Median	1016.6	4.2	3.76	0.45	0.16	1.79	2.29	0.43	6.0	34.2	5.8	0.0	37.2
Dry	From	1000.9	2.9	3.43	0.00	0.06	0.04	1.26	0.03	3.7	8.2	0.1	0.0	8.0
	To	1023.4	7.2	4.03	5.05	1.53	3.14	4.37	1.11	14.1	61.4	20.8	2.4	78.1
	Mean	1011.3	4.9	3.75	1.25	0.27	1.48	2.27	0.54	6.5	25.7	4.0	0.1	28.3
	Median	1010.6	4.9	3.75	0.67	0.18	1.71	2.01	0.53	5.8	25.5	3.5	0.0	27.0
Semisweet	From	1010.4	3.0	3.28	0.00	0.05	0.12	1.11	0.04	3.1	22.7	2.3	0.0	24.9
	To	1028.2	5.5	4.22	6.79	0.69	4.35	5.77	0.89	10.5	52.0	15.2	1.1	59.1
	Mean	1018.4	4.2	3.77	1.14	0.19	1.94	2.25	0.44	6.4	37.1	7.2	0.1	42.1
	Median	1018.3	4.2	3.80	0.38	0.14	2.15	2.09	0.50	6.5	36.9	7.1	0.0	42.1
Sweet	From	1014.0	1.4	3.35	0.01	0.04	0.13	1.20	0.04	3.7	30.4	3.7	0.0	11.6
	To	1040.2	5.2	4.06	4.19	0.57	3.45	7.09	0.67	7.3	62.7	18.6	11.8	82.3
	Mean	1025.2	3.1	3.70	1.93	0.18	1.32	3.47	0.24	5.3	45.3	11.7	2.3	53.8
	Median	1025.4	2.8	3.70	2.60	0.14	0.85	3.18	0.15	5.3	46.3	12.4	1.0	54.3

(Source: IFPC)

biological (molds, worm holes) damage. Then, the process and microbial activity during fermentation impact the product. Apple juice is mainly constituted of water (80–90% w/v) but also contains various soluble compounds:

- *Sugars*: They are the principal component of the soluble matter, representing from 75% to 90% (80–170 g l^{-1}). They are encountered in various forms: mono-, oligo-, and polysaccharides (amylose, amylopectin, pectins). However, the main sugar is fructose. It is the only one present in the final fermentation phase, as other fermentable sugars (glucose and the disaccharide: sucrose) are rapidly metabolized into fructose during the first two-thirds of the fermentation. Noteworthy, glucose can still be found in semisweet or sweet ciders. At the end of fermentation, all or part of these sugars are transformed into ethanol (generally 2–8% but up to 12% ABV – alcohol by volume). In ciders, residual sugar concentrations are generally situated between 8 and 67 g l^{-1}. Moreover, sorbitol, a nonfermentable sugar alcohol, ranges from 3 to 14 g l in cider.
- *Acids*: The main acid, as noted earlier, is malic acid. It can occur at concentrations between 1 and 10 g l^{-1}, according to apple variety (*ca.* 85% of total acidity). Citric, acetic, quinic, and citromalic acids can also be detected. Galacturonic acid can be detected when enzymatic preparations have been used due to pectin degradation. In ciders, lactic acid is detected if malolactic transformation has occurred.
- *Polyphenols*: Cider phenolic compounds are (1) flavan-3-ols (composed of the monomers (+)-catechin et (−)-epicatechin and their corresponding polymer: procyanidin; 2–5 g kg^{-1}), (2) hydroxycinnamic acids (caffeic, *p*-coumaric and ferulic acids), and their esters (chlorogenic acid or 5- caffeoylquinic acid being the most represented; 0.3–2.5 g kg^{-1}), (3) dihydrochalcones (phloridzin, phloretin et phloretin-2′-O-xyloglucoside; 0.02–0.10 g kg^{-1}) as well as (4) flavonols (e.g., quercitrin, hyperin, or avicularin) and anthocyanins in smaller quantities.
- *Nitrogenous compounds*: They are only present in small quantities, although their content may vary considerably according to the considered cultivar (40–150 mg l^{-1}). Yeast-assimilable nitrogen substances are of interest as they will condition fermentation speed and achievement (as noted earlier, keeving can be used to control amino nitrogen content in apple juice). They mainly correspond to amino acids, in particular asparagine but also aspartic acid (*ca.* 50% and 5% of total nitrogen, respectively).
- *Vitamins*: Vitamin C is the main vitamin found in apple juice, but is generally lost during the process. Vitamin C may be added in some productions.
- *Minerals*: Iron, potassium, as well as calcium and sodium, are present in both apple juice and cider.
- *Volatile compounds*: These molecules are either varietal (originating from the fruit) or fermentative (originating from microbial metabolism). They belong to various chemical families: alcohols, esters, ketones, aldehydes, phenols, sulfur compounds, and organic acids.

Organoleptic Qualities

Concerning cider taste, the three main components that interact to give the overall perception in the mouth are sugars, acids, and polyphenols. Sugars contribute to sweetness and roundness perception. In this context, fructose, and to a lesser extent sorbitol, largely contributes to the sugary flavor of the final product. Acids contribute to cider flavor and directly influence sweetness perception. A positive acidity perception is associated with product freshness. Finally, polyphenols contribute to several product traits: bitterness, astringency, and color. Concerning aroma, many volatile molecules contribute, according to their sensory perception threshold, to cider aroma. Although some cider aromas are already present in apple juice (apple cultivar aromas such as hexanol, ethyl 2-hydroxycaproate, ethyl lactate, or diacetyl), a large majority are produced during fermentation. The molecules correspond to alcohols like propanol, isobutanol, isopentanol, benzylic alcohols, and 2-phenylethanol (known for its rose aroma). Yeasts also produce esters during the alcoholic fermentation (e.g., ethyl 3-methylbutyrate, 2-phenylethyl

acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate among many others). Terpene derivatives, sulfur-containing compounds, and lactones can also be detected.

All these compounds contribute to the aromatic complexity of the final product and rely on a subtle balance between them (Table 4). As stated earlier, this balance can be affected by several metabolites (e.g., volatile phenol, acetaldehyde, lactic acid, acetic acid) originating from spoilage microorganisms. Moreover, this can also be affected by aroma compounds present at much higher levels than their sensory perception threshold. Noteworthy, the amount of effervescence, bubble size, and persistence will also affect overall product perception.

Health Impact

Like any other food product, a 'benefits and risks' approach should be considered. The first aspect to consider is the nutritional value of the product. As stated earlier, there is not one cider but many ciders; thus a range of nutritional values can be observed according to the considered cider. Examples of values for French dry and sweet ciders are as follows: water 92.70 and 94.1, carbohydrates 2.35 and 3.21, fibers 0.5 in both types, alcohol 3.2 and 1.9 g/100 g, respectively. No fat, starch, or proteins are found.

Potential Benefits

- *Microbial safety*: Due to its intrinsic characteristics (low pH, presence of alcohol and polyphenols – exhibiting bacteriostatic effects – and anaerobic conditions) and, in some cases, the cidermaking process (pasteurization), cider does not contain pathogenic bacteria or permit their growth. Reported cases of *Shiga toxin-producing E. coli* (serotype O157:H7) have been reported in the literature, but are actually associated with the nonfermented and unpasteurized North American nonalcoholic product called cider (by opposition to hard cider).
- *Antioxidant effect*: Cider (like apples and apple juice) is rich in polyphenols that exhibit antioxidant properties. These dietary molecules have been recognized as having health benefits by inactivating free radicals (O_2^- and other reactive oxygen species) naturally produced in the body. In this context, like other polyphenol-rich food and beverages, cider and related apple products may contribute to

Table 4 Organoleptic descriptors associated with cider

Family	*Group*	*Descriptor*
Tastes		Acidic
		Bitter
		Sweet
Aromas	Fresh fruit	Apple
		Pear
		Quince
		Apricot
		Peach
		Grapefruit
		Lemon
		Pineapple
		Passion fruit
		Mango
		Banana
		Raspberry
		Strawberry
		Blackcurrant
		Cherry
	Complex fruit	Crystallized fruits
		Cooked fruit
		Applesauce
		Almond
		Hazelnut
		Chestnut
		Fig
		Prune
	Vegetal	Grass
		Mushroom
		Hay
		Mint
		Moss
		Tobacco
		Yeast
		Tea
Sensation		Sparkling
		Astringent
Aromas	Floral	White flowers
		Honeysuckle
		Violet
		Acacia
		Honey
		Rose
	Spices	Clove
		Cinnamon
		Pepper
		Anis
		Resin
		Cedar
		Pine
	Grilled	Roasting
		Toasted bread
		Sweet bread
		Licorice
		Smokey
	Woody	Undergrowth
		Fresh wood
		Humus
	'Brown'	Butterscotch
		Caramel
		Chocolate
		Vanilla
	Mineral	Flintstone
		Silex
	Milky	Milk
		Butter
	Animal	Leather
		Musk

lowering risks of some chronic medical problems (particularly cardiovascular diseases and cancers).

- *Minerals*: Apple cider is a good source of potassium and iron. It is also low in sodium, making it compatible with a sodium-restricted diet.
- *Low calorie content*: Ciders possess low calorie contents, compared to other alcoholic beverages (especially wine). For example, in France, whereas 79 kcal/100 g were determined for average wine, the following calorie contents have been determined for average cider, dry cider, and sweet cider: 30.2, 32.8, and 27.1 kcal/100 g, respectively. Dry ciders have more calories than sweet ciders due to the fact that, for a same quantity, ethanol represents more calories than sugar (7 vs. 4 kcal g^{-1} for ethanol and sugar, respectively).
- *Gluten-free*: Cider is a gluten-free fermented beverage and thus can be an alternative to beer for gluten-intolerant people. This is especially used as a marketing argument in North America, where a higher percentage of the population is concerned with celiac disease.

Potential Negative Aspects

- *Alcohol*: Although ciders possesses low alcohol contents (generally ranging from 2% to 8%) and observational studies suggest that moderate alcohol consumption is linked with lower risks of coronary heart disease, cider should be consumed in moderation like any other alcoholic beverage. This is especially true for white ciders (7.5 ABV), an almost colorless product made either by processing dessert apples and pomace or by using apple concentrates with glucose or corn syrup addition for alcohol production. This product, although often containing little apple juice, falls under the UK regulation cider definition (Customs Notice 162).
- *Sugar*: According to the amount of residual sugars or sugar added, cider may contain little to high amounts of simple sugars (up to more than 8 g per 100 ml). Therefore, this should be taken into consideration in a balanced diet.
- *SO_2*: As stated earlier, sulfites are added during the production process to control microbial growth. However, SO_2 can also be considered as an allergen to some individuals. In Europe, labeling is compulsory if concentrations of more than 10 mg kg^{-1} or 10 mg l^{-1} (expressed as SO_2) of sulfur dioxide and sulfites are present (Directive 2003/89/EC).
- *Patulin*: This mycotoxin is a secondary metabolite produced by a number of fungal species (*Aspergillus*, *Byssochlamys*, and *Penicillium*) with *Penicillium expansum* being the most frequent. Although patulin can be found in apples and apple juice, studies suggest that it is metabolized by *Saccharomyces* spp. during alcoholic fermentation to form (*E*)- and (*Z*)-ascladiol, apparently less toxic compounds. Although ciders are less at risk, similar to fruit juices, concentrated fruit juices and other fermented drinks from apples or containing apple juice, patulin content is regulated to maximum levels of 50 μg kg^{-1} at the EU level (Commission regulation (EC) N°1881/2006).
- *Biogenic amines*: These molecules result from the metabolism of living organisms (LAB in cider), mainly through the decarboxylation of the corresponding precursor amino acid. Histamine, tyramine, as well as putrescine and cadaverine, can be encountered. They are undesirable due to their physiological activity, especially in sensitive consumers (with natural or drug-related mono-amine oxidase deficiencies). No legislation exists limiting the presence of these metabolites in cider.

See also: Alcohol: Metabolism and Health Effects; Alcohol: Properties and Determination; Apples; Beverage: Health Effects; Beverage: Patterns of Consumption; Lactic Acid Bacteria; Yeasts.

Further Reading

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Cirrhosis

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Malnutrition is a prevalent clinical complication of end-stage liver disease (ESLD) among pediatric and adult populations. The spectrum of malnutrition seems to worsen in relation to the progression of liver dysfunction, further increasing the risk of mortality especially in the case of the patient awaiting transplantation. Optimizing the nutritional status of patients with ESLD is therefore a key component of care.

Underlying Pathways Resulting in Malnutrition

Malnutrition is generally recognized in the setting of nutritional losses, increased nutritional requirements, inadequate ingestion, and/or metabolic disturbances. In patients with ESLD, it is possible that each of these aspects may contribute to malnutrition.

Nutritional Losses and Metabolic Disturbances

Lipids

With regard to malabsorption, hepatic dysfunction with cholestasis results in decreased circulation of bile salts causing nutritional losses of dietary lipids via steatorrhea. Deficiency of the essential fatty acids (EFAs) can result. Since lipids provide a substantial amount of kilocalories (9 Cal g^{-1} of fat), steatorrhea also leads to losses in overall energy intake and poor growth. In an effort to promote increased total fat intake and calorie absorption, medium-chain triglyceride (MCT)-based products are often utilized because of their ability to be absorbed without micelle formation. However, this practice should be weighed carefully for risk–benefit, as this can contribute to a greater degree of displacement of long-chain triglycerides and EFAs (a very high ratio of MCT to total lipid >80% is particularly risky). It is also of note that elongation and desaturation of EFAs are decreased in cirrhosis. The liver of a cirrhotic patient is more likely to depend on fuel from fat stores versus carbohydrates.

Fat-Soluble Vitamins

Steatorrhea often results in deficiencies of fat-soluble vitamins (vitamins A, D, E, and K) in ESLD.

Vitamin A

Vitamin A (retinol) deficiency can lead to poor immune function, growth failure, anorexia, epithelial keratinization, and night blindness. Nearly all of retinol absorption is facilitated by emulsification by bile acids and micelle formation. Vitamin A is available to the tissues primarily via transport by the retinol-binding protein (RBP). When the synthetic function of the liver is affected, the production of RBPs is decreased. However, assessment of true vitamin A status is difficult, since serum retinol levels are not in accordance with actual hepatic stores. Therefore, the use of the retinol/RBP molar ratio can be used to help detect vitamin A deficiency (deficiency defined as a molar ratio <0.8).

Vitamin D

Vitamin D deficiency can lead to rickets and osteomalacia. Micelle emulsification is necessary for vitamin D absorption. It has been estimated that 25–33% of children and adult patients with chronic liver disease will have vitamin D deficiency.

Vitamin E

Vitamin E deficiency can result in peripheral neuropathy, myopathy, and hemolytic anemia. Lipoproteins are required for vitamin E transport. Therefore, in disease states such as cholestasis where lipoprotein production may be altered, it is recommended that vitamin E status is assessed by the ratio of serum vitamin E to total serum lipids (deficiency defined as the ratio <0.6). It has been noted that vitamin E may be more malabsorbed than vitamin D.

Vitamin K

Deficiency of vitamin K can cause bleeding, bruising, and hemorrhage by way of impairing the synthesis of clotting factors, which can be compounded by hepatic failure also. Prothrombin time (PT) is generally used as an indicator of vitamin K status; however, more sensitive measures of vitamin K status (protein induced by vitamin K absence-II (PIVKA-II)) are increasingly available.

Carbohydrates

Cirrhotic patients are also more vulnerable to hypoglycemia, in the setting of decreased hepatic glycogen stores and gluconeogenesis. In this case, ESLD patients, especially infants and young children, are at high risk for catabolism in the fasting state. Insulin resistance also complicates glucose utilization in patients with ESLD.

Protein

The production of several key proteins (including albumins, retinol-binding proteins, protease inhibitors, coagulation factors, iron-binding proteins, and insulin-like growth factors) is decreased when the synthetic function of the liver is impacted by disease. Protein degradation and amino acid oxidation and proteolysis are all increased. Patients with chronic liver disease have altered concentrations of amino acids (AAs), namely, increased concentrations of aromatic AAs and decreased concentrations of branched-chain AAs. Moreover, hyperammonemia may result from decreased function of the enzymes involved in the urea cycle. There is also protein malabsorption due to enteropathy in portal hypertension.

Water-Soluble Vitamins

Pediatric patients may be susceptible to water-soluble vitamin deficiency due to malabsorption and inadequate ingestion. Adult patients, particularly those with alcoholic liver disease, have been found to be deficient in thiamine, folate, vitamin B12, and niacin. Folate and vitamin B12 deficiencies can result in macrocytic anemia, while deficiency of vitamins B6 and B12 and thiamine can cause neuropathy. Thiamine deficiency has been highly associated with alcoholic liver disease, which can ultimately lead to Wernicke's encephalopathy.

Minerals

Iron losses may result from gastrointestinal bleeding, and iron stores may be affected in repeated episodes of bleeding. Zinc and magnesium losses may affect those patients who are prescribed diuretics. Zinc deficiency may also result due to poor intake and decreased absorption. Zinc deficiency may result in altered taste perception and anorexia, which may further contribute to poor intake. Insufficient calcium levels can be found due to steatorrhea and 'hungry bone' phenomenon in vitamin D deficiency. On the other end of the spectrum, copper and manganese may accumulate in the liver to the point of hepatotoxicity, and those patients who may be susceptible should be monitored.

Inadequate Ingestion

Inadequate intake can be a significant causative factor of malnutrition in ESLD. Taste changes, lack of appetite, early satiety, and nausea and vomiting are all possible variables leading to poor intake. Poor appetite and early satiety may be related to increased leptin, tumor necrosis factor, and tryptophan. Poor oral intake may be found in patients who are receiving a salt-restricted diet due to the perceived unpleasant taste or unpalatable nature of the diet. Delayed gastric emptying in the setting of hepatomegaly and ascites can contribute to nausea and discomfort with eating.

Increased Nutritional Requirements

Energy

Hypermetabolism and increased energy requirements are characteristic of adult patients with cirrhosis. Historically, predictive equations have been used to estimate caloric requirements, despite their inaccuracies. A few studies have validated the use of indirect calorimetry to determine the resting energy expenditure (REE) in cirrhotic adult patients. Indirect calorimetry is the gold standard but is not available in all centers. Among the few studies in pediatric patients who underwent measurement of REE, the results generally showed hypermetabolism (upward of 125–140% of estimated needs). When indirect calorimetry is not available, periodic serial assessment of anthropometrics (especially arm circumference and triceps skinfold) can serve as a guide for nutritional provision.

Protein

Patients with ESLD have increased protein needs. For pediatric patients, a minimum of protein intakes of 2 g kg^{-1} day^{-1} has been suggested. However, some studies have shown that intakes of 2 g kg^{-1} day^{-1} do not support appropriate growth. Ranges of 2–4 g kg^{-1} day^{-1} are therefore suggested. Age-based guidelines for children with severe liver disease have not been established. However, it might be reasonable to say that such children might require at least twice the US Dietary Reference Intakes for age. Expressed as g protein/kg/day, these values for healthy children are as follows: 0.5–1 years 1.0 g, 1–3 years 0.87 g, and 4–8 years 0.76 g; males 9–13 years 0.76 g and 14–18 years 0.73 g; and females 9–13 years 0.76 g and 14–18 years 0.71 g. Protein restriction is not recommended in adult or pediatric patients unless they evidence hepatic encephalopathy. Pediatric patients with cholestasis may require more branched-chain amino acids (BCAAs). However, children receiving ready-made commercial formulas, particularly those that contain hydrolyzed protein, generally meet their BCAA requirements. In adults, the ASPEN guidelines recommend the use of standard formulas in critically ill adults with liver disease, with provision of BCAA formulas only in refractory encephalopathy. A 2003 Cochrane review evaluated 11 randomized trials and did not find a benefit to the use of BCAAs in patients with encephalopathy.

Nutritional Assessment in End-Stage Liver Disease

Nutritional assessment in liver disease is challenging yet is the integral basis of nutritional intervention and management. Nutrition assessment should be performed at the initial meeting and routinely for monitoring. Nutrition assessment can be composed of four constituents – anthropometric, biochemical, clinical, and dietary components.

Anthropometry

Anthropometry is a very challenging component of the nutrition assessment in liver disease. Many of the traditional measures of nutritional status, primarily weight, can be affected by fluid status, hepatomegaly, and ascites and therefore may not be a valid indicator of nutritional status. Monitoring of abdominal circumference may be helpful. Length or height may be a better indicator of nutritional status, especially when measured in serial occurrences over a period of time. Growth should be plotted and tracked on the appropriate growth charts (World Health Organization for ages 0–24 months and Centers For Disease Control and Prevention for ages 2–20 years). It can also be helpful to assess weight gain velocity and linear growth velocity (i.e., gains in grams per day or centimeters per month), and these data can be compared with standard reference ranges. Weight-for-length and body mass index (BMI) are also important markers. Monitoring the weight-for-length or BMI is crucial for identifying the underweight and trends in weight changes. Since weight and linear growth can be affected in liver disease, the use of arm anthropometry should ideally be a component of every nutrition assessment. Mid-arm circumference (MAC) and triceps skinfold (TSF) thickness are quick, noninvasive measures of lean body mass and fat reserves, respectively.

Biochemical (See Section on Monitoring)

Clinical

Clinical findings play a large role in the dynamic process of nutrition assessment and monitoring and, in fact, may represent the most important component. Clinical findings such as edema, ascites, hepatomegaly, jaundice, vital signs, and gastrointestinal symptoms can shed light on the nutritional status of patients with ESLD. The skin, hair, and nails of patients should be reviewed or examined. Assessment of muscle wasting, fat reserves, bone fractures, bowed legs, and acanthosis should be considered as well.

Dietary

Lastly, an estimation of dietary and intake history should be performed including administration, route, frequency, use of alternative medicine and/or supplements, behavior, knowledge and beliefs, food security and availability, and physical activity. Information regarding food allergies and tolerance should also be obtained. Careful attention should be paid to assessing formula and enteral and parenteral nutrition intake. Nutritional needs may vary according to the type and severity of liver disease.

Hepatocellular Disorders

Alcoholic Cirrhosis

Excessive alcohol consumption causes a spectrum of liver disease ranging from fatty hepatic infiltration to cirrhosis. Though there is a clear association between alcohol intake and liver disease, only a small proportion of people progress to cirrhosis. It is generally accepted that the risk of the development of cirrhosis increases proportionally with the amount of alcohol ingested. Typically, cirrhosis occurs in patients who ingest more than 30 g of alcohol per day for more than 10 years (standard drinks estimated at 14 g per drink). The highest risk for the development of cirrhosis is associated with ingesting more than 120 g day^{-1} (8.5 standard drinks per day).

Alcoholic cirrhosis poses several nutritional challenges because of its multifactorial nature. Factors that contribute to malnutrition in this group of patients include anorexia, decreased energy intake because of substitution of calories from food for those in alcohol, poor nutrient digestion and absorption, hypermetabolism, and decreased protein synthesis and secretion. In addition, ascites can be present and lead to premature satiety. Also, pancreatic insufficiency may coexist and further lead to malabsorption.

In addition to the typical deficiencies seen in patients with cirrhosis (i.e., fat-soluble vitamin deficiencies and protein–energy malnutrition), individuals with alcoholic cirrhosis are also predisposed to water-soluble vitamin deficiencies. The vitamins and minerals most typically found to be suboptimal in this population include vitamin A, vitamin D, thiamine, folate, pyridoxine, and zinc. Thiamine deficiency (i.e., beriberi and Wernicke–Korsakoff syndrome) may occur in alcoholic cirrhosis due to decreased intake, decreased absorption, and reduced thiamine storage. Thiamine deficiency is so common in adults with chronic alcohol intake that it is generally recommended that these patients receive daily supplementation with thiamine as a preventive measure. In addition, it is important to administer thiamine prior to glucose infusions to prevent Wernicke's encephalopathy (confusion, ataxia, and coma) in these patients. Alcohol consumption can also commonly cause pyridoxine (vitamin B6) deficiency with clinical manifestations of stomatitis, peripheral neuropathy, and confusion. These patients also commonly have folate deficiency, resulting in macrocytic anemia (although liver disease itself can cause macrocytosis). In general, nutritional therapy involves cessation of drinking and providing adequate nutrition of proteins, carbohydrates, and lipids.

Viral Hepatitis

Hepatitis B and hepatitis C can progress to chronic liver disease and ultimately to cirrhosis, though the prevalence of cirrhosis in these two populations is highly variable. Neonates who have acquired hepatitis B infection perinatally have a much higher chance of developing chronic hepatitis than those who acquire it later in life. Hepatitis C, however, has a higher rate of developing into chronic liver disease in adults with approximately 80% of infected individuals developing chronic infection and up to 50% of those individuals developing cirrhosis within 10–20 years. The nutritional recommendations for this patient population are similar to the recommendations for other etiologies of cirrhosis.

Autoimmune liver disease

The two main categories of autoimmune liver disease are autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC). PBC will be further discussed in the hepatobiliary section. Though not all patients with AIH will progress to cirrhosis, the progression may occur. The management of AIH typically involves immunosuppressive medications including azathioprine and prednisone. Since prednisone is often needed for a prolonged period of time, nutritional needs may be required to combat the multiple negative side effects of steroids including glucose intolerance, body composition changes, growth velocity disturbances, and osteoporosis, among others.

Metabolic Disorders

Wilson's Disease

Wilson's disease is an autosomal recessive inherited disease involving improper copper excretion resulting in the accumulation of copper in many organs including the brain, kidneys, liver, and eyes (Kayser–Fleischer rings). In general, nutritional recommendations for this disease involve avoidance of excessive copper in the diet such as found in liver, mushrooms, shellfish, nuts, beans, and chocolate. In addition, providers often use zinc supplementation to help prevent copper absorption from the gastrointestinal tract and chelating agents such as penicillamine and triethylenetetramine (trientine). For cases of severe liver disease, transplantation is sometimes needed despite dietary restrictions and pharmacological interventions.

Hemochromatosis

This is an autosomal recessive disorder that results in increased iron absorption from the gut. Excess iron is deposited in many

organs including the liver and patients typically present in adulthood with lethargy, abnormal liver function tests, hyperpigmented skin, and diabetes. Chronic alcohol use or concomitant viral hepatitis can again exacerbate the progression of liver disease. The treatment for this disease is primarily periodic phlebotomy. In general, patients should avoid supplements containing iron, but diets are generally not restricted from food high in iron, such as red meat, especially in the patients undergoing therapeutic phlebotomy.

Nonalcoholic Fatty Liver Disease (NAFLD)

NAFLD is defined as hepatic fat accumulation without any other known causes for secondary fat accumulation (i.e., excessive alcohol consumption). It describes a range of liver diseases from isolated hepatic steatosis (fatty deposits in the liver without inflammation), to nonalcoholic steatohepatitis (NASH) (which includes steatosis and inflammation), to hepatic fibrosis or cirrhosis and ESLD. The most common risk factors for the development of NAFLD are central obesity, type 2 diabetes mellitus, dyslipidemia, and metabolic syndrome. There is an increasing prevalence of NAFLD over the last few years with it now being considered the most common form of chronic liver disease in the Western world. Nutritional therapy is geared toward weight loss through dietary and lifestyle changes with a reasonable goal of losing 1–2 lbs per week.

Hepatobiliary Disorders (Including Neonatal Cholestasis Disorders)

Neonatal Cholestasis

There are many causes of neonatal cholestasis with the most common being extrahepatic biliary atresia, idiopathic neonatal hepatitis, alpha-1-antitrypsin deficiency, total parenteral nutrition (TPN)-associated liver disease, cystic fibrosis, intrahepatic cholestasis syndromes (i.e., Alagille syndrome), infection, metabolic/genetic disorders (i.e., bile acid synthetic defects and galactosemia), and endocrine disorders (i.e., hypothyroidism). For all of these disorders involving varying degrees of cholestasis, there is an underlying theme involving malabsorption of fat-soluble vitamins and steatorrhea. Typically, nutritional management involves supplementation of vitamins A, D, E, and K. Elemental formulas are preferred because they are rich in MCT and more easily digested. MCTs should account for 50% of the fat intake, and providers should be aware that diets with excessive proportion of total fats from MCTs (>80%) may result in fatty acid deficiency. Overall, these patients often require energy needs estimated at 130–150% of predicted needs for age. Risk factors for poor growth in this population include patients with ESLD, patients awaiting liver transplant, and patients with complications such as bleeding varices and ascites.

Biliary Atresia

Biliary atresia is a pediatric disease of unknown etiology that results in the obliteration of the extrahepatic biliary system. It causes neonatal cholestasis and ultimately progresses to hepatic fibrosis and to liver failure if untreated. It remains the number one cause of pediatric liver transplantation at this time despite surgical intervention attempts with the Kasai hepatoportoenterostomy to restore the drainage of bile. As with many other causes of cholestasis and cirrhosis, severe steatorrhea and malnutrition are common. Extra attention to nutritional status should be paid to children with biliary atresia. Studies have shown that poor nutritional status pretransplant is linked to prolonged hospital stay, increased risk of mortality, and increased cost of medical care. Efforts should be made immediately after the Kasai procedure to improve the nutritional status of this patient population, and clinicians should provide supplemental nutrition via a nasogastric tube if needed and even consider parenteral nutrition if enteral nutrition is inadequate or poorly tolerated.

Primary Biliary Cirrhosis

Primary biliary cirrhosis (PBC) is an autoimmune process that destroys the intralobular bile ducts, eventually progressing to hepatic cirrhosis and ultimately to liver failure. As with other patients with cholestasis, patients with PBC have decreased synthesis of bile acids leading to steatorrhea and decreased absorption of fatty acids. The treatment of PBC is ursodiol, and nutritional efforts should be made to monitor for fat-soluble vitamin deficiencies, osteopenia/osteoporosis, and overall malnutrition. Because PBC often occurs in postmenopausal women, the incidence of osteoporosis is very high in this population.

TPN-Associated Liver Disease

TPN-associated liver disease results from chronic intravenous nutrition, typically in the setting of prolonged enteral fasting. This condition is most frequently observed in pediatric patients with short bowel syndrome who are dependent on intravenous nutrition for extensive periods of time. The cause is multifactorial with a large contributor being enteral starvation. When patients with TPN-associated liver disease have cholestasis, they are at risk for the fat-soluble vitamin deficiencies as mentioned previously. When dependent on intravenous nutrition, providers must be careful to monitor for deficiencies in vitamin E and selenium. Conversely, copper, manganese, and aluminum can accumulate to toxic levels. The treatment of this disease is aimed largely on prevention. Many providers limit the amino acid and lipid components when clinically possible. In addition, though dextrose itself does not promote liver injury, excess dextrose can lead to excess fatty acid synthesis that causes hepatic steatosis.

Nutritional Therapies

In this section, the various types of nutritional support that can be given to a patient with cirrhosis will be reviewed in relationship to outcome. The recent Cochrane review of nutritional support listed in the reference section contains a very detailed summary of randomized clinical trials addressing these questions. The types of support include oral supplements, enteral nutrition, and parenteral nutrition. Outcome has been assessed as nutritional (weight gain, anthropometric measures, serum chemistries such as albumin and bilirubin, and nitrogen

balance), clinical (effects on ascites, gastrointestinal bleeding, and hepatic encephalopathy and rates of infection and postoperative complications), economic (length of stay in the intensive care unit and hospital and readmission rates), and global (mortality). Various means of monitoring will be discussed as will drug–nutrient interactions.

Types of Support

Oral Nutrition

Frequent feeding can ameliorate the preferential early utilization of fat and protein that occurs in patients with cirrhosis. Late evening meals can positively affect nitrogen balance compared with an equicaloric diet without a late evening meal.

Oral Supplements

A multitude of trials have been performed in adults with compensated cirrhosis, malnourished cirrhosis, cirrhosis, and encephalopathy. None of these trials showed an effect on mortality except for one that actually showed increased fatal outcome in the supplemented group. Likewise, there was no difference in the development of encephalopathy, although one randomized controlled trial did show resolution of hepatic encephalopathy in patients receiving supplements with branched-chain amino acids. None showed resolution of ascites although several showed decreased development of ascites. No effect on serum albumin was seen. Several trials examined the effects of oral supplements on gastrointestinal bleeding, and no beneficial effects were observed. Effects of oral supplements on health-related quality of life have been investigated. In general, no benefits were noted with the exception of the effects of branched-chain amino acid-rich supplements on some measures related to hepatic encephalopathy. Likewise, in malnourished patients with cirrhosis undergoing liver transplantation, the use of oral nutritional supplements was not associated with effects on serum bilirubin, ascites, hepatic encephalopathy, postoperative complications, length of stay in the hospital or intensive care unit, mortality rates, or even anthropometrics such as triceps skinfold or mid-arm muscle circumference.

In summary, the administration of oral nutritional supplements to patients with cirrhosis has been associated with few benefits except for decreased development of ascites and improvement of chronic hepatic encephalopathy.

Enteral Nutrition

Most trials failed to show a benefit although one was associated with improved nitrogen balance in medical patients and another showed reduced postoperative complications in surgical patients.

Parenteral Nutrition (PN)

Although most studies of PN have failed to show any specific effect in cirrhotic patients, one showed a reduction in serum bilirubin and improved nitrogen balance. Another showed a reduced incidence of postoperative ascites and yet another showed that PN was associated with a reduction of postoperative infections. However, in general, recipients of PN have better survival versus comparable controls not receiving PN.

Special Nutritional Recommendations

Poor growth secondary to fat malabsorption in cholestasis can be reversed by oral medium-chain triglycerides (but long-chain triglycerides must be administered to avoid essential fatty acid deficiency). Dietary sodium restriction and potassium supplementation should be used to help manage ascites. To prevent muscle wasting and negative nitrogen balance, 1 g kg^{-1} day^{-1} of protein is indicated for adults; 2 g kg^{-1} day^{-1} of protein is recommended for infants and young children. Normalization of zinc status should be done for patients with hepatic encephalopathy (HE). Protein restriction is rarely necessary short term and should never be done in the medium term or long term. Branched-chain amino acid supplements are controversial for acute HE; they do help with chronic HE.

Cholestatic patients require supplementation of fat-soluble vitamins. Supplementation of water-miscible versions of vitamin A is often utilized in clinical practice, but careful monitoring is of paramount importance as toxicity can lead to further hepatic damage. The recommended oral supplements of vitamin A range from 5000 to 25 000 IU day^{-1}. However, shortages of water-miscible vitamin A supplements may limit administration. Vitamin D should be supplemented as vitamin D3 (cholecalciferol). If the serum 25-OH vitamin D is low, amounts of cholecalciferol three to ten times the DRI might be necessary. Vitamin E should be supplemented with the water-miscible form containing D-α-tocopheryl polyethylene glycol succinate, also known as TPGS. Usually, 25 IU kg^{-1} day^{-1} will suffice. Oral supplements of vitamin K 2.5–5 mg two to seven times per week should be given. It has been suggested that all fat-soluble vitamin supplementation be given with TPGS to enhance absorption.

Monitoring

Monitoring the administration of nutrition to a cirrhotic patient includes the assessment of weight, length in a growing child, and anthropometrics, which may be the most reliable noninvasive way to assess nutritional status. Serum metabolites include electrolytes, blood urea nitrogen, creatinine, acid–base status, calcium, magnesium and phosphorus, triglycerides, liver enzymes, complete blood count/differential, platelets, prothrombin time and partial thromboplastin time, iron indexes, trace elements especially zinc and selenium, carnitine, and folate/vitamin B12. Cholestatic cirrhotic patients should be monitored for deficiencies of vitamins A, D, and E. The use of triene/tetraene ratio can be used to assess EFA deficiency, while a lipid panel can be used to assess metabolic syndrome or lipogenic changes. Although albumin and prealbumin should be assessed as indexes of protein malnutrition, the interpretation of values below normal may be difficult as both of these are affected by protein nutrition and hepatic synthesis. Similarly, prolonged prothrombin time may reflect malabsorption of vitamin K, poor hepatic synthesis, or both. Although ammonia is classically measured to monitor the risk of hepatic encephalopathy and as an indication for pharmacological management,

this analyte is notoriously subject to artifact. The ratio of plasma BCAA/aromatic amino acids may be more useful to assess HE.

If nutrients are administered enterally, then gastrointestinal tube placement and nose care should be monitored as needed to assess the correct position of tube placement. If nutrients are administered via an ostomy, then careful site care of the gastrostomy/jejunostomy should be done. If parenteral nutrition is administered via a peripheral or indwelling central venous catheter, then the line site should be assessed frequently for signs of infection.

Given all the limitations of monitoring the nutritional status of cirrhotic patients, it is useful to understand that the subjective global assessment of nutritional status, which has been modified for the cirrhotic patient, is perhaps the most useful means of assessing the nutritional status of patients with this condition. It is simple, reproducible, and validated and includes the history of nutrient intake, physical examination, and anthropometrics that can be easily performed.

Drug–Nutrient Interactions

Some of these interactions of particular importance in a cirrhotic patient are the effects on reduced elimination of potassium by cyclosporine and spironolactone. Cirrhotic patients are generally total body salt-overloaded, so it is particularly important to be aware of how much sodium is being delivered in intravenous lines to support the patient such as saline in arterial lines and/or peripheral intravenous lines for antibiotic delivery. Many parenteral medications are available as sodium salts, further adding to the salt burden of the cirrhotic patient. Antacids and sucralfate can bind phosphorus in the gut and lead to hypophosphatemia. Thiazide diuretics may also lead to phosphaturia and increased excretion of potassium. Hyperglycemia may result from cyclosporin A, tacrolimus, and/or corticosteroids; however, given that marked insulin resistance is characteristic of cirrhosis, the precise cause of hyperglycemia in a cirrhotic patient receiving one or more of these drugs may be difficult to determine. The hepatic metabolism of 25-hydroxyvitamin D is lowered by the anticonvulsants diphenylhydantoin and phenobarbital.

A number of drugs commonly used in cirrhotic patients are incompatible with parenteral nutrition solutions, including amphotericin B, acyclovir, sodium bicarbonate, and ciprofloxacin. Drugs used to treat HE such as lactulose and neomycin may result in nutrient malabsorption.

Overall Recommendations

1. Nutritional assessment by anthropometrics should be done to assess the basis for nutrient recommendations.
2. Given that there is little solid support for specific nutritional interventions in cirrhotic patients, it is reasonable to provide nutrients to meet basic requirements in the most cost-effective manner possible that is easiest for the patient and provides the best quality of life. Thus, the logical order of provision of nutrients is oral nutrition with frequent small meals including a bedtime meal and oral nutritional supplements as necessary to maintain normal vitamin status and manage chronic HE. If this approach does not work, then provision of all or at least partial nutritional requirements via enteral feeding is recommended with PN reserved for those who are unable to meet their nutritional needs via the oral or enteral route. PN should ideally be administered cyclically as opposed to constant infusion and for as short a period of time possible, to avoid metabolic and infectious complications.
3. Monitoring should be done for all cirrhotic patients to assess the safety of nutritional interventions (weekly or more frequently for those receiving parenteral nutrition) and the effect of nutrients on nutritional status (monthly or every other month) for anthropometrics. For selected cirrhotic patients, additional studies could be undertaken to investigate hepatic osteodystrophy (DEXA scan), retinopathy (evoked response electroretinography to assess the functional consequences of vitamin A and E deficiencies), and HE (ammonia and branched chain/aromatic amino acids in plasma and proton magnetic resonance spectroscopy for brain glutamine).

See also: Appetite Control in Humans: A Psychobiological Approach; Cystic Fibrosis, Nutrition in; Enteral Feeding; Malnutrition: Concept, Classification and Magnitude; Malnutrition: Prevention and Management; Parenteral Nutrition; Vitamins: Overview.

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Citrus Fruits

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Citrus Fruits

Citrus fruit is one of the major horticultural crops grown worldwide, and they are the most traded horticultural commodity in the world. The exact place of origin of citrus fruits is still under debate, but it is believed that it originated from Southeast Asia and spread to the other parts of the world. Citrus crop is grown in developed and developing countries as well. Citrus fruits constitute a crucial source of vitamin C. Brazil, the Mediterranean countries, China, and the United States account for about two-thirds of the total citrus production. In the last 30 years, there has been a steady increase in the per capita consumption of citrus fruits worldwide. North America has the highest per capita consumption of citrus fruits in the world followed by South America and Europe. According to FAO, fresh citrus fruit consumption is decreasing in the developed countries while some of the developing countries are showing an increase in consumption. Great variation exists in the types of citrus fruits produced and consumed throughout the various parts of the world. Oranges occupy the major portion of world citrus production followed by mandarins. Oranges form the majority of the citrus crop produced in the United States. About one-third of the citrus fruits produced globally are used for processing.

Members of Citrus Genus

Citrus is the largest genus of the Rutaceae family. The *Citrus* genus is composed of several species and each species has many varieties. Taxonomic identification is very complicated and the precise number of species is still unclear because there are many spontaneous and commercial hybrids. According to the Swingle system, 16 species were recognized and the Tanaka system identifies 156 species in the genus. Citrus fruits can be generally classified into the following categories: sweet oranges (*Citrus sinensis*; blood and acidless oranges), mandarins (such as satsuma (*C. unshi*), tangerines (*C. tangerina*), *C. reticulata*, and clementines (*C. clementina*)), sour/bitter oranges (such as Seville (*C. aurantium*)), lemons (*C. limon*), limes (*C. aurantifolia* and *C. latifolia*), grapefruit (*C. paradisi*) and pomelo (*C. grandis*), hybrids (e.g., tangelos, tangors, and limequats), and citrons (*C. medica*).

Types of Citrus Fruits

Sweet Oranges

Blood, navel, mandarin, and common oranges are the four groups of sweet oranges. Sweet oranges belong to the species *C. sinensis*.

Common Oranges

They are mostly globose but oval or ellipsoid is also common. Common Valencia is oblong to spherical. The base of the fruit is flattened in most varieties. The surface is not only smooth but also finely pitted. Generally, most sweet oranges have yellow to yellow-orange rind and flesh. The fruits in this group are spherical, globose, or ovate. Valencia, Shamouti, and Sathgudi are some sweet orange types.

Blood or Pigmented Oranges

The blood oranges are characterized by pink to red coloration of the flesh, juice, and rind. Coloration is due to the presence of anthocyanin pigments in the rind and flesh of the fruit. Red color develops with warm days and cool nights, which is the main feature of the Mediterranean climate. The common blood oranges are 'Maltese,' 'Moro,' 'Sanguinello Moschato,' etc.

Navel Oranges

This is an important group of fresh fruit orange varieties due to its excellent quality. Navel oranges have a navel-like structure at the stylar end or apex, which is a rudimentary secondary fruit embedded in the primary fruit. Navel orange fruits are ovate to oblong in shape. Most navel oranges, particularly the 'Washington navel' orange variety, the most widely grown navel cultivar in the world, are ellipsoid or obovate in shape. Its fruit's surface is generally smooth and is moderately pitted and pebbled. The fruits are mostly large and seedless. Other common navel oranges are Navelate, Palmer, etc. Orange-colored fruits are juicy and seedless with aromatic flesh. They are excellent for fresh fruit market, but do not keep well on the tree, not adapted to hot arid climate.

Mandarin (*C. reticulata* Blanco)

Most mandarins are deep orange to reddish orange in color when fully mature and are easily peelable. Their fruits are small or large (4–8 cm in diameter) with a low to high collar base and a deeply depressed apex. Their fruits are globose to oblate, 5–9 cm in diameter, with a thin or thick rind with sunken oil glands and a relatively smooth or, sometimes rough, surface. Their fruits are seedless or seeded with nearly 3–7 or more seeds and have 7–17 segments. Most mandarins lose quality and the rind 'puffs' if not picked when internally ripe.

It is an evergreen tree with many spiny branches tolerant to drought and cold. Although mandarins prefer subtropical climate, they can also grow in tropical areas. Mandarin oranges are classified into five groups: Satsuma, King, Willowleaf (Mediterranean), common, and small-fruited. Mandarins are usually eaten fresh or as desserts as they are easily peelable and have a distinctive pleasant flavor.

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Grapefruits (*C. paradisi*)

Grapefruits (*C. paradisi*) are considered to have originated in the West Indies as a hybrid of pomelo or as a mutant. They have many attributes similar to pomelos. They are one of the large citrus fruits (although smaller than pomelos) with diameters of up to 10–15 cm and have commercial possibilities as fresh fruits. They are oblate to spherical in shape, slightly depressed at the stylar end, and flat at the stem end. The rind is medium in thickness, yellow or pink-blushed, and smooth-surfaced. Grapefruits are borne in clusters like grapes and are seeded to seedless. There are red-fleshed and white fleshed cultivars of grapefruits. Red fleshness is due to the accumulation of carotenoid and lycopene pigments. In tropical regions, fruits develop with high juice content, thinner peel, and lower acidity. Grapefruits can stay on the tree from September to July in the northern hemisphere. However, it is not suitable to leave the fruits on the trees for a prolonged time because granulation may develop and seeds may germinate within the fruit. People in North America, Europe, and Japan prefer fresh grapefruit.

Pomelo (*C. grandis* or *C. maxima*)

Pomelo (*C. grandis* or *C. maxima*) originated in southern China and is found growing wildly in China and in many northeastern states of India. Its fruits are the largest among the commercially grown citrus in the world. The fruit diameter commonly ranges from 20 to 30 cm. The fruits are globose to pyriform in shape and are borne singly. Pomelos have a much bigger fruit with thick peels and are less juicy than grapefruits. They also have a firm flesh with crisp carpellary membranes and juice sacs. They are white- or pink-fleshed and are seeded.

Lemon (*C. limon* (L.) Burm.)

Lemons are believed to have originated in India. The lemon tree is a small thorny tree that grows well in subtropics and tropics. The fruit size and shape of the lemon are highly variable from spherical in Baramasi lemon or Eureka to oblong. The fruit color is green to bright yellow at maturity and is with or without a collar at the neck. Thickness and smoothness of rind vary among varieties and lemons are either seedless or seedy. Some varieties have large and prominent nipple, while others have very small, inconspicuous nipple. The flesh of lemon is pale yellow and very acidic. Some varieties of lemon are quite juicy, while others have less juice. The quality of the lemon fruit is excellent in semiarid irrigated areas and coastal areas. In humid tropics, lemon trees produce fruits with coarser peels. The lemon juice is valued for its tart, tangy, and fresh flavor and is used as a common food ingredient in many cuisines. The fruit rind is a source of commercial essential oil and aroma compounds. Lemon trees are cold-sensitive than oranges and grapefruits and the fruit can freeze at low temperatures owing to its low sugar content. Argentina, Spain, California, and Italy are the leading producers of lemon. The lemon fruit structure is similar to other citrus fruits and is composed of the outer peel and the interior edible pulp. The outer peel is composed of the exterior flavedo or the epicarp and the interior white spongy part known as the albedo (mesocarp). The flavedo contains carotenoid pigments, vitamins, and essential oils. The albedo is composed of cellulose and soluble carbohydrates (pectin and protopectin), flavonoids, amino acids, and vitamins.

Acid Lime (*C. aurantifolia*)

Acid lime fruits are high in acid. *C. aurantifolia* grow throughout tropical and warm subtropical regions of the world and the world's leading producers are Mexico, Florida, the West Indies, and Egypt. The most common acid lime is the small-fruited acid lime or *C. aurantifolia* Swingle. It is also known as the 'Mexican' or 'Key' lime in the Florida Keys. The fruits are round or elliptical in shape with a thin or thick rind depending on the variety. The fruits have very small neck, a flat base, and a small nipple at the apex. The highly acidic flesh is juicy with a distinct aroma and flavor. The fruits are mostly seeded but seedless varieties are also commercially available. The fruits of *C. latifolia* Tan. are large (80–100 g) and are oblong in shape. These limes are also known as Tahitian or Persian lime. Lime trees are small shrubby and spiny, and they are cold-sensitive. The rind surface is greenish yellow to yellow at maturity and the tender flesh is juicy.

Sweet Lime (*C. limettioides* Tan.)

Sweet lime is also known as Indian sweet lime or Palestine lime. It is mainly utilized for its medicinal properties. The medium-sized fruit is pale yellow to orange yellow at maturity. The fruit shape is round to oblong and the juice is nonacidic. These limes are not commonly processed.

Citron (*C. medica* L.)

Citrons are grown in India. Citron fruits are long-oval or ellipsoidal to obovate in shape like some of the lemons. Their fruits are quite large with a yellow color and a very thick, rough, and bumpy peel at maturity and are also very seeded.

Calamondin

Calamondin is commonly grown in southern China, Taiwan, Japan, the Philippines, and northern India. It is a small mandarin-like fruit of 3–3.25 cm in diameter, weighing 20–30 g. It has a smooth orange-colored peel that is very thin (1–2 mm). Its fruit has an orange-colored acidic flesh with seeds, but the peel is sweet and edible. It is commonly used for making marmalade and for culinary purposes.

Citrus Fruit Development

Citrus fruits follow a typical sigmoid pattern of growth and development, which is divided into three stages. The initial phase, or phase I, is of slow growth including the period between anthesis and June drop. Phase II is the rapid growth period in which the fruit experiences a huge increase in size by cell enlargement during 4–6 months. In the final phase, or phase III or ripening period, growth is mostly arrested and fruits undergo a nonclimacteric process. Citrus fruits are

nonclimacteric fruits and hence there is no remarkable increase of respiration rate and ethylene production during ripening as in climacteric fruits such as mango or banana. Being nonclimacteric, citrus fruits do not ripen after harvest. Proper handling of citrus fruits is needed during harvesting, storage, transportation, and marketing to minimize wounding, stimulation of respiration, and ethylene production, which can induce physiological disorders and fungal rot. Citrus fruits produce a very low quantity of ethylene after harvest and there is no associated rise in respiration. However, fruits respond to exogenous ethylene supply by an increase in respiration, chlorophyll loss, calyx drying, and abscission. During ripening, citrus fruits change color from green to yellow or orange or orange-red according to the variety. This is called natural degreening, or natural color development. In some citrus fruits, if held on the tree beyond maturity, the yellow-orange color again changes to green, which is called regreening. Regreened fruit, although internally mature, loses marketability. The regreening process can occur on the tree and also after harvest. In most citrus fruits, regreening occurs when the fruit is on the tree, but in pomelo, regreening has been observed in harvested fruit stored in natural light or fluorescent light.

Fruit Morphology

Citrus fruits are classified as a hesperidium. Hesperidium is a modified berry resulting from a single ovary. The fruit consists of 8–16 carpels that form the core of the fruit or segments that contain the seeds and juice. Citrus fruits are characterized by the presence of an outer rind or skin. The rind or peel of citrus fruits is divided into an exocarp or flavedo, which is the outer, colored part, and the mesocarp or albedo, which is the inner colorless (white) or sometimes tinted part. The flavedo consists of the epicarp proper, hypodermis, outer mesocarp, and oil glands. Above the epicarp is a multilayered protective skin or cuticle. The edible pulp of a mature citrus fruit is divided into segments with or without seeds or juice sacs by a thick film or endocarp surrounding the soft central axis. Each segment is surrounded by a continuous endocarp membrane. In the segments, juice is contained in closely packed, club-shaped multicellular sacs, also called juice vesicles, which completely fill the segments. A thin wall called the carpellary septum surrounds the segments. Each juice sac also has a very minute oil gland at the center. The seeds (ovules) are also attached to segment walls by means of axial placentation.

The pomological and organoleptic attributes of citrus fruits are quite diverse. The fruit diameter ranges from a few centimeters in Mandarins to more than 25 cm for some pomelos (*C. grandis*). The fruit's shape also varies: fruits are oblate in tangerines, mandarins, and grapefruits, spherical or oval in sweet oranges, oblong in lemons (*C. limon*) and citrons (*C. medica*), and spherical in limes (*C. aurantifolia*). The fruits usually have 8–16 segments with or without seeds. Albedo forms the major portion of the citron fruits, while it is absent in kumquats and poorly developed in mandarins. The fruit pulp color depends on the presence or absence and abundance of carotenoids and anthocyanins in the pulp, and it can be pale, yellow, orange, or red. The size and shape of the seeds are also variable among species. Navel oranges and Tahiti lime (*C. latifolia*) are seedless, while grapefruit and pomelo have 30–50 seeds. The fruit flavors and essential oils are diverse both qualitatively and quantitatively.

Methods of Consumption

Citrus fruits are consumed fresh or processed into various products. Citrus fruits including oranges, mandarins, tangerines, and grapefruits are peeled and eaten fresh or as desserts. A major portion of citrus fruits produced are utilized for processing. The fresh fruit segments are also added to salads and desserts and on cakes. The most common citrus fruit product is fresh or frozen orange juice concentrate made from freshly squeezed and filtered orange juice. Additionally, citrus fruits are also made into marmalade, jams, jellies, candies, or wine. The dried and pulverized fruits are used for preparing confectionaries and flavoring baked products. Fresh or dried peel of citrus fruits has a variety of uses. The grated outermost layer of the rind, zest, is used in cooking and baking. The pulp, obtained after the extraction of orange juice, is dried and used as an emulsifier and binder in the food and beverage industries. Essential oils extracted from the peel of citrus fruits are used commercially for flavoring drinks, ice cream, pudding, desserts, chewing gum, sweetmeats, ice cream, sherbet, and other products. They are also a used by the pharmaceutical industry for the preparation of drugs, soaps, perfumes, cosmetics, and home cleaning products. Dried orange blossoms and leaves are used to make herbal teas. Fresh juices from limes and lemons are made into refreshing drinks. Lime and lemon juices are also suitable for garnishing and for flavoring dishes and curries. The fruit is also made into pickles, salted preserves, and dried limes and processed into beverages, jams, jellies, and marmalade. Essential oils are also extracted from the peel and are extensively used in flavoring soft drinks, confectionery, chewing gum, sweets, ice cream, sherbet, and other food products.

Biochemical Composition of Fruits

Sugars and Acids

The total soluble solids form 10–20% of the fruit fresh weight and are mainly composed of carbohydrates (70–80%) and small quantities of organic acid, proteins, lipids, and minerals. The insoluble portion mainly contains cell structure polysaccharides. The total solid contents in citrus fruit increase with ripeness due to an increase in sugar content. Sucrose, glucose, and fructose constitute the major fruit carbohydrate components. In mandarins and tangerines, sucrose, glucose, and fructose occur in a general ratio of 2:1:1. Sugars are prominent in tangerine, oranges, and grapefruits, while limes and lemons are rich in citric acid. Additionally, trace amounts of mannose, maltose, heptuloses, and galactose have also been identified in citrus fruits. Sucrose is the major nonreducing sugar in citrus fruits. Sucrose, in very small amounts, is present in limes and lemons. Arabinose, a pentose sugar, has been identified in lime and grapefruit. Starch content in citrus fruits is negligible.

Organic acid contributes to the acidic properties of citric fruits. Citric acid forms the major acid in citrus fruits. Malic acid forms the second most acidic component though present in much lower amounts than citric acid. Other organic acids including succinic, lactic, and oxalic have also been detected in fruits. Organic acids exist in free form or in combined form in salts (such as citrates and malates), esters, or glycosides. The titratable acidity or the free acidity of the citrus fruit juice is mainly due to citric acid contents. The total acidity represents the sum of all acids in free and combined forms. Citric acid is the predominant organic acid in juices, while malic, malonic, oxalic, and quinic acids form the major citrus peel organic acids. Acidity varies with fruit maturity and citrus species. It ranges from 0.5% to 1.5% in orange juice, 0.8% to 2.5% in grapefruit, and 4% to 8% in lemons and limes. In many oranges and grapefruit varieties, acidity declines with fruit ripeness due to a decrease in citric acid content. In lemons, a decrease of pH with an increase in acidity occurs with fruit ripeness. Citrus fruits are very low in fat content and are also not a good source of proteins.

Nutritional Value of Citrus Fruits

Citrus fruits have long been valued as important sources of vitamin C. Besides being deficient in cholesterol, citrus fruits also contain an impressive list of health-benefiting nutrients including essential minerals, vitamins, and dietary fibers. Citrus fruits are principal sources of many bioactive phytochemicals with disease-preventive properties including flavonoids and limonoids.

Minerals

Citrus fruits are good sources of dietary potassium and are relatively low in sodium (3–4 mg per 178 ml of orange juice). The ratio of sodium and potassium plays a role in the maintenance of electrolyte balance. Citric acid in citrus fruit can help in the absorption of calcium and other minerals by acting as a chelator.

Vitamin C

Among vegetables and fruits, citrus fruits are particularly popular as excellent dietary sources of vitamin C and can supply vitamin C content ranging from 23 to 83 mg per 100 g fresh weight on an average. Consumption of citrus fruits in moderate amounts may be the best way to meet the 100% of the RDA for vitamin C. Studies showed that intake of orange juice (500 ml day^{-1}) for two weeks (~250 mg ascorbic acid per day) resulted in increased plasma vitamin C concentrations by 40–64% and reduced oxidative markers in adults. A reduction in plasma lipid peroxidation was observed in healthy adults who consumed orange juice (8 oz or 236 ml) daily (70 mg vitamin C) for 2 weeks.

β-Carotene

The only fat-soluble vitamin occurring in citrus fruits in substantial quantity is vitamin A in the form of provitamin A carotenoids. Carotenes and β-cryptoxanthin form the major vitamin A precursors in citrus fruits. β-Cryptoxanthin is the major vitamin A precursor in tangerines, mandarins, and oranges, while α- and β-carotenes represent a minor portion of carotenoids in some oranges. Among various foods, citrus fruits including oranges and tangerines are the most concentrated dietary sources of β-cryptoxanthin. The content of provitamin A carotenoids varies greatly among different citrus fruits.

Folic Acid

Citrus fruits and their juices are good sources of natural folates, and according to a study in Europe, folate was 100% bioavailable from orange juices and is highly stable. There is strong scientific evidence supporting a positive relationship between dietary folate consumption and the prevention of neural tube defects in infants. Citrus fruits (100 g) can provide up to 10–20% of the RDA for adults and children less than 9 years of age, complementing to the dietary folate requirement. Another study reported that the consumption of orange juice (750 ml) daily for four weeks increased the plasma folate concentrations in adults by 18%.

Dietary Fiber

Cellulose, hemicelluloses, and pectins in citrus fruits are a source of dietary fiber. Pectin is the predominant soluble dietary fiber component of citrus fruits constituting about 65–70% of the total fiber content. Pectin occurs both in the edible portions of fruit and in the inedible residues such as peel, rag, and core. Consumption of fresh citrus fruits such as oranges and grapefruits can contribute significant quantities of pectin to the human diet. Insoluble fiber in citrus fruits is composed of polysaccharides and they play an important role in human diet in preventing digestive disorders because of their water-holding capacity.

Limonoids

Limonoids are one of the most health-benefiting bioactive components of citrus fruits owing to their versatile health-promoting and disease-preventing properties. Citrus limonoids were considered as the culprits causing delayed bitterness of the juices at room temperatures lowering the juice quality. Limonoids are highly oxygenated and modified triterpenes classified as tetranortriterpenoids. The term 'limonoid' is derived from limonin, the first limonoid component identified as the bitter component of citrus seeds. Several other limonoid compounds such as obacunone, nomilin, obacunoic acid, isolimonic acid, deacetylnomilin, ichigan, isoobacunoic, and dictomnolide were also identified. Limonin and nomilin are the predominant limonoids of citrus fruit. Limonoids are grouped into aglycones and glucosides. Aglycones are bitter limonoid compounds in the peels of citrus fruits, while glucosides are tasteless components. Both aglycones and glucosides are present in citrus seeds, but fruit tissue contains only glucosides. More than 50 limonoid aglycones and glucosides have been identified from various *Citrus* species. Evidence from several *in vivo*, *in vitro*, and animal studies suggests that

limonoids have anticancer properties and showed potent cytotoxic activities. In studies conducted using animal models and cell lines, limonoids have been shown to inhibit proliferation of the cancers of the stomach, colon, breast, skin, and pancreas. Limonoids including limonin and nomilin were also found to have antibacterial and antiviral properties. Studies also indicated that the anticancer properties of limonoids can be correlated to the induction of glutathione S-transferase, a major detoxifying enzyme system.

Flavonoids

Citrus fruits are known to be rich sources of polyphenolics such as flavonoids and phenolic acids. More than 60 types of flavonoids have been identified in citrus fruits belonging mostly to 4 different flavonoid groups: flavones, flavanones, flavonols, and anthocyanins. In the genus *Citrus*, flavanones are more abundant in high concentrations than flavones. Flavanones are concentrated in albedo and in the membranes than in the juice sacs. Hesperidin, naringin, and neohesperidin are the major flavonoids in citrus fruits. Citrus flavanones occur in the form of aglycones or glycosides. Hesperidin is the prominent flavonoid in orange and it is not bitter, but less soluble in water. Hesperidin also occurs in mandarins, lemons, and limes. Naringin, the major flavonoid in grapefruits and shaddock, has a bitter taste and it is soluble in water. Seeds and peels of citrus fruits are also rich in phenolic compounds such as phenolic acid and flavonoids. Naringin, neohesperidin, and neoeriocitrin are the main neohesperidoside flavanones present in bergamot, grapefruit, and bitter orange juices. Bergamot, orange, mandarin, and lemon juices contain the rutinoside flavanones: hesperidin, narirutin, and didymin. Caffeic, chlorogenic, ferulic, sinapic, and *p*-coumaric acids are the most abundant phenolic acids present in citruses.

Citrus flavonoids are known for their powerful free radical scavenging and for their anti-inflammatory activities. The anti-inflammatory properties of citrus flavonoids, hesperidin and its flavone analog diosmin, are due to their inhibition of the activities of proinflammatory mediators such as prostaglandins E2 and F2 and thromboxane A2. *In vitro* studies have also shown that citrus flavonoids can inhibit the reactions catalyzed by cyclooxygenase, lipoxygenase, and phospholipase A2. Citrus flavonoids have also been shown to possess platelet anti-adhesive and antiaggregation properties. Epidemiological studies have indicated an inverse relationship between regular consumption of citrus fruits and reduced risk of developing cardiovascular diseases, atherosclerosis, and inflammation. Flavonoids also exhibit antibacterial and antiviral activities. Evidences from a number of studies have demonstrated that citrus flavonoids or the purified single citrus flavonoid components (hesperidin, naringin, naringenin, etc.) possess antiproliferative activities and are capable of inhibiting the proliferation of many kinds of cancer cell lines including melanoma, colon, breast, squamous, leukemia, lung, prostate, colorectal, and hepatomas. Although the exact mechanisms explaining the anticancer properties of citrus flavonoids are still largely unclear, a number of mechanisms have been proposed. Flavonoids have been suggested to exert multiple actions and are involved in the inhibition of metastasis, inhibition of tumor progression, inhibition of angiogenesis in tumor cells, arrest of cell cycle progression, protection of DNA against oxidative damage, inactivation of carcinogens, signal transduction, etc.

See also: Cancer: Diet in Cancer Prevention; Vitamins: Overview.

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Clostridium botulinum

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Introduction

Clostridium botulinum is an anaerobic spore-forming gram-positive bacillus, which is ubiquitous in the environment. *C. botulinum* and in rare cases *C. butyricum* and *C. baratii* can produce neurotoxins that are among the most potent known to man. *C. botulinum* produces eight botulinal neurotoxins (BoNTs), types A–G. Human botulism is usually associated with types A, B, and E and rarely with type F, while animal botulism is usually associated with types C and D. Type G has been reassigned as *C. argentinense*, which has been associated with sudden death but not neuroparalytic illness. Disease from type G is unknown in animals.

C. botulinum can be divided into four subgroups I–IV, differentiated by their biochemical activities. Group I is proteolytic, grows at temperatures between 10 and 45 °C, and can produce toxin types A, B, and F. Group II is nonproteolytic, grows at temperatures between 3 and 45 °C, and can produce toxin types B, E, and F. Those in group II are also termed psychrotrophic because of their ability to grow at temperatures below 5 °C. Groups I and II are the main groups associated with human illness, and those in group I account for almost all reported cases of infant botulism. Group III is nonproteolytic and produces toxin types C and D; and group IV can be weakly proteolytic and produces only toxin type G.

Types of Botulism

There are three main types of botulism, namely, foodborne, infant, and wound. Another two clinical categories are recognized – adult intestinal toxemia and iatrogenic botulism.

Foodborne botulism is the most common form of botulism and is caused from ingestion of foods containing *C. botulinum* toxin. In the case of foodborne botulism, contaminated products may be widely consumed, thus exposing a large number of people to the hazard and may subsequently represent a medical and public health emergency.

Infant botulism affects infants less than 1 year of age and is caused when infants ingest *C. botulinum* spores, which then germinate and produce toxin in the intestine. The spores may come from the environment or from foodstuffs. Honey has been previously implicated in the disease and is no longer recommended for infants less than 12 months. Almost all cases of infant botulism have been due to types A and B.

Wound botulism occurs when *C. botulinum* infects a wound and subsequently produces toxin. It has been associated with major soil contamination through compound fractures or severe traumas/lacerations. The incidence of wound botulism has increased considerably in recent years, particularly among injectors of black tar heroin.

Adult intestinal toxemia is the colonization of the intestine with *C. botulinum* following ingestion of spores and is analogous to infant botulism. Germination, vegetation, and toxin production by *C. botulinum* are not usually supported in the normal adult intestine; however, in a small number of adults who have a bowel abnormality or bowel disease or are undergoing antimicrobial therapy, colonization can occur.

Iatrogenic botulism is the accidental overdose of toxin (e.g., via inhalation by laboratory workers or via injection for therapeutic or cosmetic purposes).

Toxin Action

All of the toxins produced are large single polypeptides (130–150 kDa) of a similar structure. Enzymatic cleavage produces a 100 kDa heavy chain and a 50 kDa light chain linked via a single disulfide bond. In proteolytic strains, the enzymatic cleavage is caused by proteases produced by the cell, while in nonproteolytic strains, an exogenous protease such as trypsin is involved.

The toxin either enters the blood stream directly or is absorbed intact from the gastrointestinal tract. The toxin binds via gangliosides to high-affinity presynaptic receptors. It is then transported into the nerve cell through a receptor-mediated endocytosis process common with dichain toxins.

The toxin acts by blocking the normal release of the neurotransmitter acetylcholine that under normal circumstances triggers contractions of the skeletal muscle. The toxin binds irreversibly, and the recovery of function depends on ultraterminal sprouting of the nerve to form new motor endplates.

Each of the seven toxins (A–F) has different specific toxicities and durations of persistence within the nerve cells.

Symptoms

C. botulinum intoxication in adults is characterized by a descending symmetrical flaccid paralysis. Early symptoms include blurred vision, slurred speech, dry mouth, difficulty in swallowing, and muscle weakness. Botulism, particularly the early symptoms, may be confused with a number of other conditions including poliomyelitis, viral encephalitis and meningitis, myasthenia gravis, Guillain–Barré syndrome, tick paralysis, stroke syndromes, Eaton–Lambert syndrome, alcohol intoxication, drug overdose, antimicrobial-associated paralysis, and atropine, shellfish, or mushroom poisoning.

Early symptoms may vary depending on the route of infection/intoxication. Foodborne botulism has an incubation period ranging from 2 h to 8 days with the majority of cases having an onset 12–72 h postexposure. Nausea, vomiting, abdominal cramps, and diarrhea may precede the neurological symptoms described earlier.

The incubation period for wound botulism is considerably longer than for foodborne botulism and may range from 4 to

18 days. This is due to the organism first causing infection and then the subsequent *in vivo* production of toxin in the wound. Neurological symptoms are as described earlier and may include the presence of a fever.

The incubation period in infant botulism is difficult to ascertain but can be between 3 and 30 days. *C. botulinum* intoxication in infants is characterized by 'floppy baby syndrome.' Infants may be constipated and have feeding difficulties, poor sucking reflex, lethargy, loss of head control, and a feeble cry. In the adult version of intestinal toxemia, clinical manifestations are similar to those observed with foodborne botulism and may include the early gastrointestinal symptoms. In comparison to foodborne botulism, the onset is generally gradual and less dramatic.

In cases of iatrogenic botulism, the incubation period for inhalational exposure is similar to that of foodborne botulism with incubation periods ranging from 24–36 h to several days postexposure. Clinical symptoms are as described earlier while lacking any gastrointestinal symptoms.

In cases of overdose by injection, incubation periods vary depending on dosage. The doses usually recommended for cosmetic purposes are too low to cause disease. However, higher doses injected for treatment of muscle movement disorders have caused systemic botulism like symptoms in patients. Patients who received injections of botulinum toxin at a much higher dose than that recommended for cosmetic purposes have resulted in symptoms of severe botulism and long-term recovery.

In the absence of medical intervention, mortality rates as high as 50–60% have occurred. Mortality rates vary based on the patient age and type of botulism and are generally lower in those aged <20 years. With improvements in critical care management, fatality rates have dropped to *ca.* 16%, and where death does occur, it is often due to delayed diagnosis/treatment. Death is usually via respiratory failure due to paralysis or nosocomial infections (particularly pneumonia) resulting from long-term hospitalization. Mortality rates for foodborne botulism are 5–10%, 15–17% for wound botulism, and $<1\%$ for infant botulism.

Recovery can take weeks to months and full neurological recovery from weakness and fatigue may require as long as 1 year. In 95% of cases, the nerve terminals regenerate and recovery is complete. Extended outpatient rehabilitation is often required.

Complications

Although there are no additional specific complications resulting from botulism intoxication, the potential complications of prolonged paralysis, assisted ventilation, and nutritional support are significant. Nosocomial infections may include pneumonia; urinary tract infections (from indwelling Foley catheters); stress ulcers and sores; thrombophlebitis, cellulitis, and line infections due to peripheral and central intravenous catheters for prolonged periods; fungal infections; and also deep vein thrombosis and pulmonary embolism due to patients being bedridden for weeks to months.

Infectious Dose

The estimated lethal doses for purified crystalline botulinum toxin type A for a 70 kg man are 0.09–0.15 μg when introduced intravenously, 0.7–0.9 μg through inhalation, and 70 μg orally. The precise human toxicities for the remaining BoNTs are uncertain. All seven toxins have been shown to cause inhalational botulism in primates and therefore have the potential to cause human botulism if a significant exposure occurs. It is thought that 1 g of pure toxin dispersed in animal feed would be enough to kill 400 000 adult cows. Type A toxin produces a more severe disease than types B and E because of differences in amount of ingested toxin, absorption, or receptor affinity. Type A intoxication results in respiratory failure occurring more rapidly and more severely than with the other types of botulism. Mortality rates are lower in type B disease than with type A or E, and it has been shown generally that the longer the incubation period, the better the prognosis.

Diagnosis/Confirmation

The early diagnosis of botulinum intoxication is paramount as antitoxin therapy is most effective when administered early. Initial diagnosis is based on clinical history, physical examination, epidemiological history, and electromyography results. Epidemiological histories should include risk factors that may support the diagnosis including black tar heroin injection (wound), laboratory work with botulinum toxins or receiving nonapproved neurotoxin preparations for therapeutic or cosmetic purposes (iatrogenic), and recent consumption of a home-canned product (foodborne).

Diagnosis is confirmed by demonstration of botulinum toxin or the spores in the suspected food or patient specimens (vomit, feces, serum, gastric aspirate, and wound). It has been shown that the toxin may routinely be present in the serum 7–9 days after exposure and in some patients for up to 28 days postexposure.

Infant botulism diagnosis is made on clinical symptoms and confirmed by recovery of the organism or by detection of the toxin in stools.

In cases of inhalational botulism, diagnosis is more difficult as often toxin is not identifiable in the serum/stool.

The definitive diagnosis is made by the mouse bioassay, and positive *in vitro* assays are confirmed using the mouse bioassay.

Treatment

Treatment of botulism required intensive hospital care and may include where necessary mechanical ventilation and administration of antitoxin. The antitoxin is of equine origin and may carry a significant risk of serum sickness, and skin sensitivity testing should always be undertaken before administration. Hypersensitivity is in the region of 10–20%.

Control of the airway and ensuring adequate ventilation are of primary importance, and in some cases, endotracheal intubation may be required.

If exposure is known to have occurred within several hours, then induced vomiting and gastric lavage should be commenced. Even after several days, an emetic agent may be advisable to help eliminate any unabsorbed toxin still present.

Several forms of antitoxin are available and some agencies (e.g., Centers for Disease Control, the United States, and Public Health England, the United Kingdom) recommend the administration of antitoxin based on clinical diagnosis without waiting for laboratory confirmation. The antitoxin neutralizes toxin not yet bound to nerve terminals but does not neutralize toxin already bound.

Additional treatment for wound botulism includes debridement, drainage, and irrigation of the wound. As toxin production may continue until the infection with *C. botulinum* is eliminated, the administration of antibiotics is also likely.

In March 2013, the Food and Drug Administration (FDA), the United States, approved the first botulism antitoxin (BAT) that can neutralize all seven known botulinum nerve toxin serotypes. The BAT heptavalent (A, B, C, D, E, F, and G) (equine) is derived from horse plasma and is now the only drug available for treating adult botulism in the United States. BAT has superseded the use of a licensed bivalent botulinum antitoxin AB and an investigational monovalent botulinum antitoxin E. It is also the only available drug for treating infant botulism that is not caused by nerve toxin type A or B.

In cases of infant botulism, a human-derived botulinum immune globulin (BabyBIG) is available. BabyBIG is obtained from the plasma of immunized adults who have subsequently developed high titers of neutralizing antibodies against BoNTs A and B. BabyBIG is derived of human origin and therefore does not have the high anaphylaxis risk inherent with equine sources or the risk of lifelong equine antigen hypersensitivity. Although supportive intensive care including mechanical ventilation may be required, the use of BabyBIG can significantly reduce the length of hospital stay for infants.

Prevalence in the Environment

C. botulinum is ubiquitous in the environment and can be found in soil; dust; marine and freshwater sediments; intestinal tracts of animals, birds, and fish; vegetables; fruits; leaves; silage; and animal manure.

There is a geographic predilection by *C. botulinum* type with type A spores prevalent in western United States, Brazil, Argentina, and China. Proteolytic type B spores are primarily found in eastern United States and nonproteolytic type B spores in the United Kingdom and continental Europe. Type E spores predominate in aquatic environments and are found in many northern regions such as Alaska, northern Canada, Scandinavia, Russia, and Japan. Types C and D are more associated with the warmer climates of Indonesia, Australia, and South Africa with type C more prevalent in some aquatic environments occupied by waterbirds. Type F, which is found more rarely, has been detected in the soils/sediments of Brazil and Paraguay and in aquatic environments. Type G has been isolated from soil in Argentina and Switzerland.

Prevalence in Foods

Because of the prevalence of *C. botulinum* in the environment, there is potential for a wide range of raw food materials to carry spores. In many cases, the common food vehicles associated with *C. botulinum* are characteristic of the country or culture and the prevalence of the *C. botulinum* type found in that environment. In certain parts of the United States, China, Spain, and Italy, consumption of vegetables contaminated with type A is the most common cause of botulism, while in Alaska, Japan, Canada, and Scandinavia, cases are usually associated with fish/aquatic products contaminated with type E. In central continental Europe, cases are typically from home-cured meats and usually associated with type B spores. In cases of infant botulism, honey has become a recognized source for *C. botulinum* spores.

Group I strains are frequently related to insufficiently processed home-preserved foods such as canned vegetables and cured meats, whereas group II strains are more of a risk in foods processed with mild heat treatments and subject to refrigerated storage (e.g., REPFEDS (refrigerated processed foods of extended durability)).

Commercially prepared foods are rarely implicated in botulinum outbreaks due to stringent controls, but when a failure does occur, mass intoxication may result. In the United Kingdom in 1989, a major outbreak occurred resulting in 27 people affected with one fatality. The outbreak was due to a yogurt containing hazelnut puree, which had been sweetened with aspartame rather than sugar. The processing of the conserve had been inadequate to destroy *C. botulinum* spores. In the United States in 2007, eight people contracted foodborne botulism after eating commercially produced canned food products (including hot dog chili sauce), which had been underprocessed.

The majority of botulism cases today are associated with low-acid home-preserved foods particularly vegetables that have a $pH > 4.6$, for example, peppers, garlic, and carrots. In 2006 in Thailand, 209 people were affected with 42 people developing respiratory failure, neuromuscular failure, and autonomic nervous system failure. The outbreak was due to ingestion of pickled home-canned bamboo shoots.

Global Incidence

In 2010, in 29 of the European Union (EU) and European Economic Area (EEA) countries (all except Liechtenstein), 104 cases of botulism were confirmed. This was a decrease of 21% when compared to 2009 when 132 cases were confirmed. Poland, Romania, Italy, and France accounted for 80% of the confirmed cases. The EU trend has been stable during 2006–10 with the confirmed case rate ranging from 0.02 to 0.03 cases per 100 000 population (European Centre for Disease Prevention and Control, Annual Epidemiological Report 2012). Previous data from the same source indicate similar numbers of cases reported between 2005 and 2010 with 152 cases reported in 2005, 157 cases (109 confirmed) in 2006, 171 cases (129 confirmed) in 2007, and 151 cases (112 confirmed) in 2008. Between 1995 and 2004, there were 2388 reported cases

although not all countries reported for all years. Incidence in Europe (50 countries) is considerably higher than in just the EU/EAA (31 countries).

In the United States, a total of 112 laboratory-confirmed cases of botulism were reported to the CDC in 2010. Of these, foodborne botulism accounted for 9 (8%), infant botulism for 85 (76%), wound botulism for 17 (15%), and botulism of unknown or other etiology for 1 (<1%) cases (Centers for Disease Control and Prevention, Botulism Annual Summary, 2010). Previous data from the same source indicate similar numbers of cases reported between 2005 and 2010 with 145 cases in 2005, 170 cases in 2006, 144 cases in 2007, 153 cases in 2008, and 121 cases in 2009. In general, infant botulism was the predominant cause each year.

Between 1976 and 2007, a total of more than 3000 cases of infant botulism have been globally identified, and as of 2009, infant botulism has been documented in 26 countries representing 4 continents.

In England and Wales, wound botulism accounts for the most cases of botulism with 144 cases between 2000 and 2010. This compares to four cases of foodborne botulism and six cases of infant botulism during the same time period. In the United States between 2005 and 2010, there were 158 cases of wound botulism compared to 573 cases of infant botulism and 101 cases of foodborne botulism. Thirteen cases were of unknown or other etiology.

Survival, Prevention, and Control

In order for foodborne botulism to occur, the food must initially be contaminated with *C. botulinum* spores. The environment provided by the food must be anaerobic and nonacidic with low sugar and salt to enable the germination of the spores into cells capable of growth and toxin production. The food must then be consumed without sufficient prior heating to destroy the toxins (85 °C for >5 min).

To control and prevent botulism, the processing and subsequent storage of the food must be such that it results in either the destruction of the spores or the prevention of their germination, growth, and subsequent toxin production.

C. botulinum produces spores that can survive in a dry state for decades. The spores are highly resistant to heat, desiccation, UV light, and alcohols. They may survive several hours at 100 °C but will not survive exposure to moist heat at 120 °C for 30 min. Spores of the nonproteolytic group II types are more heat-sensitive than the group I types (proteolytic) but can still withstand many of the mild pasteurization techniques currently in use (70–95 °C). In contrast, the toxins are heat-labile and are inactivated by heating at 85 °C for ≥5 min.

Processing/Storage/Shelf Life

The proteolytic group I types cannot grow below temperatures of 10 °C, so are not of concern in cold-distributed foods. These group I types when they grow often cause food spoilage that may indicate to consumers the defective quality of the food. However, the potency of the toxin is such that even tasting a product spoiled by *C. botulinum* may cause intoxication. A case in 2002 occurred where an individual tasted a mouthful of foil-wrapped baked potatoes but did not swallow it due to the spoiled taste. He had however consumed sufficient toxin for botulism to occur and spent more than 6 months in hospital recovering.

The strains of group II are nonproteolytic and can grow at temperatures as low as 3 °C. These strains may show no outward signs of obvious spoilage of the foodstuff and are more of concern in products that are minimally processed and have refrigerated storage, for example, REPFEDS.

The increased consumer demand for fresher less preserved foods has led to the use of milder heat treatments combined with other food preservation hurdles. This includes chilled storage, a controlled shelf life, and occasionally intrinsic preservatives (e.g., reduced pH). Hermetic sealing may be used to create anaerobic conditions that may extend the shelf life, but this may also create conditions favorable for the growth of *C. botulinum* in cases of product abuse. Product abuse (i.e., where there is failure to store a product at a low temperature or to consume within a certain timeframe) may lead to increased consumer risk from group II spores, which can grow and produce toxin at temperatures as low as 3 °C.

C. botulinum spores are resistant to pressure but can be destroyed by combinations of high-pressure and high-temperature treatments. The spores and toxins of all strains are also relatively resistant to the freezing temperatures used for food storage.

Heat Resistance

The proteolytic strains of *C. botulinum* (group I) produce spores that are highly heat-resistant, and these are the principal concern for the safe production of low-acid canned foods. As such, a universal botulinum cook is used in the canning of low-acid foods (pH >4.6). A botulinum cook results in a 12 log reduction of spores at 121 °C for 3 min or an equivalent time/temperature combination. High-acid foods (<4.6) do not support the germination and growth of group I spores, and for these products, the botulinum cook is not required.

The *D*-value or decimal reduction time is the time it takes at a given temperature to reduce a population by 90%. Group I spores are considerably more heat-resistant than group II spores although these still show moderate heat resistance. Group I spores have a $D_{121°C}$ of 0.21 min and a $D_{100°C}$ of 25 min, while group II spores have a $D_{121°C} = < 0.001$ min and a $D_{100°C}$ of <0.1 min. It has been shown that heating group II spores for 5–10 min at 80–85 °C can inactivate their spore germination system due to sublethal injury but that in the presence of lysozyme, germination can still be induced and the heat resistance increased by up to two orders of magnitude.

pH/Sodium Chloride Concentration

The growth of proteolytic *C. botulinum* strains is inhibited at pH <4.6 or by 10% sodium chloride (NaCl), with the minimum water activity to allow growth being 0.96 where NaCl is used as the humectant (Table 1). Growth and toxin production of nonproteolytic *C. botulinum* strains are inhibited at pH <5 or in the presence of >5% NaCl concentration. For these strains, the minimum water activity to allow growth is 0.97 where NaCl is the humectant. It should be noted that water activity

Table 1 Characteristics of *C. botulinum* groups I and II

	C. botulinum *group I*	C. botulinum *group II*
Toxin types	A, B, F	B, E, F
Proteolytic	Yes	No
Minimum temperature	10 °C	3 °C
Optimum temperature	35–40 °C	18–25 °C
Minimum pH	4.3–4.6	5
NaCl	10%	5%
Minimum a_w[a]	0.94	0.97
$D_{121°C}$ spores	0.21 min	<0.001 min
$D_{100°C}$ spores	25 min	<0.1 min

[a]May vary, depending on humectant.

values may vary depending on the humectant used with glycerol permitting growth at lower a_w values. Humectants (salts or sugars) absorb moisture making water less available for microbial growth. The toxins of both groups I and II are stable at low pH.

Disinfectants

C. botulinum spores are readily killed by chlorine-based disinfectants and exposure to formaldehyde. The spores are sensitive to most disinfectants authorized in the food processing sector as long as the correct time/concentration combinations are used.

Detection

A variety of methods exist for the detection of *C. botulinum* toxins and may include ELISA (enzyme-linked immunosorbent assay), PCR (polymerase chain reaction), endopeptidase assays, and lateral flow immunoassays, among others. These methods have been used as screening tools and may provide a rapid result in some matrices. However, the gold standard for the detection of toxin still remains the mouse bioassay. This involves the extraction of the toxin (if required, e.g., from foodstuffs), trypsinization if required (necessary for non-proteolytic strains), and then intraperitoneal inoculation of laboratory mice. Mice are subsequently observed for signs of botulism, which may include ruffled fur, labored breathing, Cheyne–Stokes respiration, and pinching of waist resulting in a 'wasp waist.' The mice are observed for 3 days, but generally, symptoms of botulism will be observed within the first 24 h. The toxin type may be determined by neutralization tests with specific antitoxins. The mouse bioassay is very sensitive and is measured as MLD (minimum lethal dose) with one mouse LD50 corresponding to 5–10 pg and a detection limit of 20–30 pg ml^{-1}. Detection of *C. botulinum* spores usually results from providing a suitable environment, which enables the growth and toxin production of the organism. Detection is then confirmed by the presence of toxin as described earlier.

Therapeutic/Cosmetic Use of Botulinum Toxin

Despite the potency of *C. botulinum* neurotoxins and their involvement in serious disease, their use for therapeutic and cosmetic purposes (particularly of BoNT type A) is increasing. The use of neurotoxins for cosmetic purposes such as reducing the appearance of wrinkles and frown lines is well established but offers only temporary improvement. *C. botulinum* neurotoxin is used in a wide variety of medical conditions associated with muscular hyperactivity, glandular hypersecretions, and pain. These include focal dystonias, spasticity, nondystonic disorders, strabismus, chronic pain and disorders of localized muscle spasms, smooth muscle hyperactive disorders, and sweating and salivary and allergy disorders. The use of neurotoxin does not correct the underlying condition but improves clinical symptoms for a time. The duration and quality of improvement vary depending on the dose, dilution, and method of injection. The effects generally last approximately 3 months after which further injections will be needed. There is a risk of the patient developing resistance, but this has been reduced by giving the lowest effective dose and ensuring long periods between injections.

See also: Canning: Process of Canning; Chilled Foods: Modified Atmosphere Packaging; Chilled Foods: Packaging Under Vacuum; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; Minimally Processed Foods.

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Clostridium: Occurrence and Detection of *Clostridium perfringens*

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Background

Clostridium perfringens was recognized as a potential cause of foodborne illness in the 1950s following the isolation of a large number of the organism from suspect foods involved in foodborne illness. In the United States, it is the second leading cause of bacterial foodborne illness. It was eventually realized that the responsible enterotoxin was produced following the ingestion of a large number of cells from outbreak foods. The cells sporulate in the small intestine and release the enterotoxin. The enterotoxin was subsequently isolated in the early 1970s. Extensive work has been conducted on the mode of action of the toxin using animal models and in cell culture.

C. perfringens is divided into types A–E. Types B–E are typically associated with domestic animals and are responsible for a variety of veterinary illnesses. Type A is associated with foodborne illness and is part of the microflora of soil.

Extensive surveys of the incidence of *C. perfringens* in retail foods were conducted beginning in the mid-twentieth century after realization of its role in foodborne illness. Specialized selective and presumptive media have been developed and modified to take advantage of the special ability of *C. perfringens* for rapid growth at elevated temperature, including in the presence of specific antibiotics.

The Organism

C. perfringens is classified into five types, A–E, depending on their ability to produce four toxins, alpha, beta, epsilon, and iota. Food poisoning is caused by type A, while a more serious gastrointestinal illness, necrotic enteritis, is due to type C but is rare in industrialized countries.

Foodborne illness caused by type A *C. perfringens* is due to the production of an enterotoxin during sporulation in the small intestine following consumption of a large number of vegetative cells in temperature-abused foods. The enterotoxin is encoded by a gene (*cpe*) located on either the chromosome or large plasmids. In the majority of outbreaks, the responsible isolates carry the enterotoxin gene on the chromosome.

C. perfringens is an anaerobe, that is, requires the absence of oxygen for rapid growth. This is readily achieved during cooking procedures in which high temperatures drive off oxygen. In the vegetative (growth) state, the organism possesses the shortest generation time of any bacterium, able to divide in <10 min in protein foods under optimal conditions such as those that may be found following the cooling of large portions of meat, poultry, and gravies.

C. perfringens is a spore-forming organism. Under conditions of limited nutrients, vegetative cells (i.e., those in the growth phase) can enter the spore state, which is a cell from highly resistant to physical insults such as radiation, high temperatures, pasteurization, and sanitizing agents. However, the spores of this organism are inactivated by canning procedures. In the natural environment and in nonoutbreak foods, the organism is often found in the spore state. When placed in conditions of proper nutrients (germinants), such as protein foods, spores can germinate and resume vegetative cell growth.

Occurrence

Reservoirs

The environment

Type A strains have been isolated from most soil samples examined, at levels of up to 10^3–10^4 g. However, soil is not considered a major reservoir as the level of enterotoxin-positive isolates is low.

Feces

C. perfringens is present in the intestinal contents of virtually all animals including most humans examined with great variation in number and between species. Levels may also vary over time. Median counts in healthy individuals range from 10^3 to 10^6 g and are higher in neonates and elderly than adults. There is some uncertainty as to whether humans are reservoirs of foodborne, enterotoxigenic *C. perfringens*.

The widespread presence of *C. perfringens* spores in fecal samples has led to the use of *C. perfringens* as an indicator of fecal pollution of water by certain governmental jurisdictions on several continents. As well, the World Health Organization has also recommended it as a useful indicator of fecal pollution. *C. perfringens* spores were also found to be good indicators of the distribution of sewage sludge on the ocean floor where low temperature and high pressure are less suitable for common indicators of fecal pollution such as coliforms and fecal streptococci.

Food/food animals

Following the identification of *C. perfringens* as an etiological agent of foodborne illness, many surveys of the incidence of the organism in foods were conducted. Early surveys revealed an incidence of approximately 50% (range of 30–80%) of raw or frozen meat and poultry items containing generic (i.e., enterotoxin-positive and enterotoxin-negative) *C. perfringens* (Table 1). Typical ranges in early surveys were at levels up to 10^2 g in meat and poultry, the commodities typically associated with foodborne outbreaks. Certain foods, such as spices, were found to contain the organism at higher levels, even up to 10^3 g. More recent surveys have employed techniques that allow the determination of the presence of the enterotoxin gene. This revealed that enterotoxigenic *C. perfringens* is present at low levels in retail foods (Table 2).

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Table 1 Levels of generic *C. perfringens* in retail foods

	% positive	Levels (per gm or cm^2)
Beef, pork, poultry	52–94	1–100
Pork sausage	39	5–93
Ground beef	50	5–100
Pork carcasses	66	20/100 cm^2
'Raw meat'	70	<3
Raw fruits and vegetables	4	10–100
Spices/herbs	5–100	10–10 000
Frozen foods	3	10–20

Compiled from Guran and Oksuztepe; Juneja et al.; Lindström et al.

Table 2 Levels of enterotoxigenic *C. perfringens* isolates from selected sources

Source	% enterotoxin-positive
Soil	7
Healthy adults	8[a]
Meat, fish, poultry, retail	0.1–1.7
'Raw meat,' retail	8–12
Intestine, food animals	10–40

[a]Compiled from Carmen et al.; Guran and Oksuztepe; Lindström et al.; Li et al.; Miki et al.

Taken together, and in spite of many surveys of retail food, food animals, humans, and the environment, the natural reservoir of foodborne, enterotoxigenic *C. perfringens* remains unknown. It is now generally accepted that <10% of global isolates carry the enterotoxin gene (Table 2).

Entry and Growth in Outbreak Foods

High-protein foods, such as meat and poultry, are typically involved in the outbreaks of foodborne illness. These have been prepared in advance often in large amounts in settings such as cafeterias, restaurants, and caterer preparation sites. Spores of the organism are able to survive the heating steps associated with food preparation. Such spores can then germinate and grow during slow cooling procedures. Because outbreaks of *C. perfringens* typically occur in institutionalized settings, the average outbreak size consists of several dozen cases. However, the relatively mild symptoms of most cases result in the involvement of public health officials when only a large number of people have become ill.

Factors Affecting Growth

The temperature limits for growth of *C. perfringens* are between 15 °C (60 °F) and 50 °C (122 °F) with an optimum at about 45 °C (112 °F). Deficiencies in cooling are involved in virtually all cases of foodborne illness caused by *C. perfringens*. Specifically, slow or improper cooling allows the rapid growth of the organism between 37 and 50 °C. This highlights the need to reduce the size of portions of meat or the volumes of gravies after cooking as spores can survive the internal temperatures. The potentially rapid growth of *C. perfringens* during cooling has led to governmental regulations regarding the cooling of commercially produced, processed meat and poultry. For home and institutional settings, the temperatures recommended by governmental agencies for holding of cooked foods during serving or holding should be followed. These are 5–60 °C (40–140 °F).

Detection

Detection of Viable *C. perfringens*

Methods for detection of *C. perfringens* depend on the level of expected cells. The levels in retail foods are expected to be low and the standard technique involves the most probable number (MPN) method (see Section '**Most Probable Number**'), while the cell number in outbreak foods is much higher and requires the use of specially developed selective and differential media.

Criteria for Outbreak

The typical criteria for implicating the role of *C. perfringens* in outbreak foods involve either (a) detection of enterotoxin-positive spores ($>10^6$ g) in feces of ill individuals, (b) detection of elevated ($>10^5$ g) numbers of vegetative cells in outbreak foods, or (c) detection of enterotoxin in feces of ill individuals.

Most Probable Number

An MPN procedure is used when a low number of *C. perfringens* are expected, for example, in routine surveys of retail foods. For this purpose, iron milk medium containing 2% iron powder is used and is inexpensive and simple to prepare. Selection is based on the appearance of a 'stormy fermentation.' In the reaction, casein is precipitated by acid from the fermented lactose followed by fractionation of the curd into a spongy appearance when the milk is incubated at 45 °C. If results are read after 18 h, confirmatory tests are not necessary.

Examination of Outbreak Foods

Many media for enumeration of *C. perfringens* from outbreak foods have been developed over the years. The currently recommended medium in North America and elsewhere is tryptose-sulfite-cycloserine (TSC) agar. Following anaerobic incubation at 37 °C, *C. perfringens* colonies appear as black due to the reduction of sulfite to sulfide and reaction of the sulfide with iron in the medium to form the black iron sulfite precipitate. Because certain other clostridia may produce black colonies on this medium, confirmation of a selected number of isolates is necessary (see '**Confirmatory Tests**' later).

Confirmatory Tests

Confirmatory tests can rule out the possible (though unlikely) presence of other sulfite-reducing clostridia. The media for this purpose are tubes of motility nitrate (MN) and lactose gelatin (LG). The use of TSC agar together with the LG and MN has been adopted as official first action by AOAC (Association of Official Analytical Chemists) International. Details of the laboratory procedures are described as standard methods by the US Food and Drug Administration and by the International Organization for Standardization. Commercial tests kits for

identification of clostridia are also available. Their use has been described in publications from professional organizations such as the American Society for Microbiology.

Enterotoxin Detection

Detection of *C. perfringens* enterotoxin in feces is a method of confirming the role of *C. perfringens* in foodborne illness. The two available commercial assays are an enzyme-linked immunosorbent assay and a reverse passive latex agglutination. Stools of healthy individuals contain undetectable levels of the enterotoxin. The commercial kits for each technique, respectively, are available from TECHLAB Inc. and Oxoid Inc.

Enterotoxin Gene

As shown in **Table 1** versus **Table 2**, the ability of *C. perfringens* to produce enterotoxin is limited to a small percentage of isolates. To confirm the role of the organism in foodborne outbreaks by the presence of a large number of viable cells or spores, it is necessary to demonstrate that suspect isolates (colonies) possess *cpe*. This is performed using the polymerase chain reaction (PCR) assay. The procedure amplifies *cpe* from lysed cells (i.e., isolates confirmed as *C. perfringens*) allowing the determination of its presence. PCR protocols have been developed to determine the location of *cpe*, that is, whether on the chromosome or on a plasmid, though this distinction is not routinely done or required in investigations of outbreaks.

Possible Medical Applications of *C. perfringens* Enterotoxin

As part of its biological activity in cases of foodborne illness, *C. perfringens* enterotoxin binds to membrane proteins called claudins. Certain solid cancer cell lines often overexpress membrane-associated claudin molecules. This has led to a possible use of *C. perfringens* enterotoxin as an anticancer agent. In particular, tumor necrosis and shrinkage against xenografts of human pancreatic cancer tumors growing on the back of mice have been demonstrated. Current research involves limiting immune responses to the enterotoxin and employing particular enterotoxin fragments. Its potential medical application is an exciting possibility for this toxin.

See also: *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; Food Poisoning: Tracing Origins and Testing; Foodborne Pathogens.

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Clostridium: Food Poisoning by *Clostridium perfringens*

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Introduction

Clostridium perfringens was initially identified as a cause of food poisoning in the 1940s. This bacterium then became known as an important cause of foodborne disease. Many cases of food poisoning due to enterotoxigenic *C. perfringens* are now reported every year, and *C. perfringens* food poisoning ranks among the most common foodborne diseases in industrialized countries. The most important virulence factor is the toxin known as *Clostridium perfringens* enterotoxin (CPE), while the biological properties of this bacterium also contribute to the induction of foodborne disease.

In this article, we described the basic features of *C. perfringens* food poisoning, with a focus on the genetic and biological characteristics of CPE-producing strains, and also introduced novel insights into the molecular mechanism of CPE cytotoxicity.

Characteristics of the Organism

C. perfringens is a Gram-positive, rod-shaped, encapsulated, nonmotile bacterium that causes a broad spectrum of human and veterinary diseases. The virulence of *C. perfringens* mostly attributes its toxin-producing ability; that is, more than 16 different toxins have been identified from human and veterinary disease isolates. Of these toxins, the human gastrointestinal (GI) virulence of *C. perfringens* is known to depend on three toxins, CPE, beta-toxin (the main toxin responsible for necrotizing enteritis in humans), and also possibly beta2-toxin (an additional toxin for human colitis by CPE-producing type A strain). While toxin-producing ability is crucial for human GI diseases, *C. perfringens* food poisoning isolates have several other biological abilities: a rapid doubling time (<10 min for vegetative cells), the ability of vegetative cells to grow in relatively high optimal growth temperatures (43–45 °C), the ability to form environmental stress-resistant spores, and tolerance to air exposure in spite of being classified as an anaerobic bacterium. These properties also play important roles in the induction of *C. perfringens* foodborne illness.

Based on the production of an arsenal of four major toxins (alpha, beta, epsilon, and iota), *C. perfringens* isolates are classified as type A–E. Toxin typing of *C. perfringens* strains was traditionally identified by toxin–antitoxin serum neutralization tests in mice. The recent development of PCR-based schemes permitting toxin genotyping has simplified toxin typing of *C. perfringens* isolates. Food poisoning isolates are most frequently identified as type A, while the most important toxin gene, the *cpe* gene, is rarely found in type C, D, and novel E strains. These type C to E strains have not yet been identified as causative agents of *C. perfringens* food poisoning outbreaks, even though type C to E strains sometimes express an enterotoxin with very similar or almost identical physical, serological, and biological properties to CPE produced by the type A strain. Of these *cpe*-carrying type A–E strains, the CPE-producing type A strain is more widely distributed than the other types of CPE-producing strains. On the other hand, a *cpe*-positive type C isolate was found in some cases of necrotic enteritis (also known as Darmbrand or pigbel), while the *cpe*-positive type C strain may have accidentally been isolated from a patient with type A food poisoning. The *cpe* gene is present on a conjugative transferable large plasmid in most, if not all, type C, D, and E strains. However, genetic organization of the *cpe* region of type C, D, and E strains differs from that of *cpe*-positive type A strains.

Factors Contributing to *C. perfringens* Type A Foodborne Illness

A unique toxin, CPE, plays a major role in *C. perfringens* type A food poisoning. A large number of epidemiological studies confirmed the importance of CPE in GI virulence. First, most type A *C. perfringens* isolates from food poisoning outbreaks produce CPE. Second, a strong positive correlation exists between illness and the presence of CPE, the level of which in a patient's feces is known to cause serious intestinal effects in experimental animals. And third, human volunteers fed highly purified CPE will develop all of symptoms characteristic of *C. perfringens* type A food poisoning.

Animal model experiments revealed that CPE is active on the GI tract; that is, all regions of the small intestine are sensitive to treatment with CPE. In rabbit ileal loops, purified CPE causes the rapid loss of fluid and electrolytes from the GI tract. These effects on rabbit ileal loops can be neutralized with CPE-specific antiserums. Moreover, the ability of CPE-positive *C. perfringens* food poisoning isolates to produce both fluid accumulation and histopathologic damage in rabbit ileal loops is remarkably higher than that of CPE-negative isolates or that of a *cpe* knockout mutant of the food poisoning strain.

The importance of CPE to human GI virulence has been supported by the finding that CPE-producing type A *C. perfringens* strains (most isolates harbor the *cpe* gene on a plasmid) are also isolated from nonfoodborne human GI diseases, including antibiotic-associated diarrhea, sporadic diarrhea, and nosocomial diarrheal outbreak.

The Biochemistry of CPE

CPE is a single polypeptide of 35 317 Da composing 319 amino acids with an isoelectric point of 4.3. This toxin is not a heat-stable enterotoxin; its activity is inactivated by heating for 5 min at 60 °C. It is also sensitive to pH extremes but resistant to some proteases, such as trypsin and chymotrypsin. This toxin is not a secreted toxin because the toxin gene (*cpe*)

does not encode the 5′ signal peptide, which is often associated with secreted toxins.

Native CPE crystal structure analysis revealed CPE to be a three-domain protein with an elongated architecture of beta-sheets. These features of CPE are common among pore-forming toxins, such as *C. perfringens* epsilon-toxin and *Laetiporus sulphureus* hemolytic pore-forming lectin. However, the domain arrangement of CPE is in the opposite order; domain I is composed of the C-terminal region of CPE, whereas domains II and III are formed by the N-terminal region. The C-terminal half of the toxin (domain I) is responsible for specific receptor-binding activity. CPE domain II contains a region that appears to be a transmembrane stem, and this region is crucial for pore formation and cell killing. The CPE region residing in domain II also promotes CPE hexamer formation (~450 kDa SDS (sodium dodecyl sulfate)-resistant large complex, described later). Domain III may undergo structural changes during prepore to pore transition. The N-terminal 37 amino acids of CPE lack a definable structure and are not necessary for toxicity.

Genetics of CPE

Cpe ORF nucleic acid sequences are highly conserved among CPE-positive type A isolates; that is, CPE must have a highly conserved amino acid sequence. Interestingly, the *cpe* gene appears to be harbored on either the chromosome or the large plasmids with a single copy. In the chromosomal *cpe* strain, the *cpe* gene is flanked by insertion sequence (IS) elements (IS*1469* and IS*1470*), which is associated with the putative 6.3-kb *cpe*-containing transposon, Tn*5555*. On the *cpe* region of *cpe*-carrying plasmids, the *cpe* gene is resided by upstream IS*1469* and by downstream IS*1151* or IS*1470*-like elements in almost all type A *C. perfringens* isolates; that is, based on gene arrangement differences in the *cpe* region, most, but not all, CPE-producing type A strains are divided into three *cpe* genotypes: one type of the chromosomal *cpe* strain and two types of the plasmid *cpe* strain. Similar to other toxin genes such as the epsilon-toxin gene, the *cpe* gene is harbored on conjugatively transferable plasmids. Collectively, the *cpe* region in both chromosomal and plasmid *cpe* strains is on a transposon, a mobile genetic element; that is, these IS elements may be related to mobilization or transfer of the *cpe* gene.

Synthesis and Release of CPE

Although CPE-producing *C. perfringens* isolates are divided into three *cpe* genotypes, the amount of CPE expressed by an isolate is not affected by whether that isolate carries the *cpe* gene on the chromosome or a large plasmid. CPE synthesis begins shortly after the induction of sporulation and progressively increases for the next 6–8 h. CPE expression is regulated at the transcriptional level, with significant amounts of *cpe* mRNA being produced during sporulation; however, *cpe* mRNA is not produced during the vegetative growth of *C. perfringens*. This phenomenon is strongly supported by the findings of genetic analyses as follows: expression of the *cpe* gene in type A strains is strictly regulated by sporulation-associated alternative sigma factors, including SigF, SigK, and SigE. SigK and SigE (SigE and SigK are sporulation-associated sigma factors active in mother cells during the sporulation of *Bacillus subtilis*) directly induce the transcription of *cpe* mRNA by binding SigK- or SigE-dependent promoters (P1, P2, and P3) located upstream of the *cpe* ORF. The abundant CPE expression during sporulation also depends on the exceptional stability of *cpe* mRNA (the functional half-life of *cpe* mRNA is approximately 60 min).

Unlike most *C. perfringens* toxins, CPE is not actively secreted; following its synthesis, the CPE protein accumulates in the cytoplasm of the mother cell as a cytoplasmic CPE-containing paracrystalline inclusion body. When sporulation events have been completed, the mother cell lyses with the simultaneous release of CPE into the intestinal lumen, in which then CPE acts as an enterotoxin.

Effects of CPE on the GI Tract

C. perfringens food poisoning is basically a self-limited illness in humans. Therefore, histopathologic studies have seldomly been conducted on humans. Instead, the actions of CPE have been examined using animal models. Using an *in vivo* rat or rabbit ileal loop model, CPE causes the loss of fluid and electrolytes from the GI tract and then produces extensive histopathologic damage to the small intestine; that is, the target organ for CPE is believed to be the small intestine, with the ileum being particularly sensitive to this toxin. CPE-induced histopathologic damage is initially limited to the villus tips of the small intestine; however, the entire small intestinal villus eventually suffers extensive damage over time: desquamation of the villus epithelium, inflammatory cell infiltration (mostly lymphocytes), and slight hyperemia in the mucosa. This CPE-induced tissue damage plays a major role in initiating CPE-induced fluid/electrolyte intestinal transport alterations; that is, the onset of fluid transport changes closely coincides with the development of tissue damage, and CPE levels that produce tissue damage can induce intestinal fluid and electrolyte transport alterations. CPE-induced intestinal damage can develop within 15–30 min of toxin treatment in the rabbit ileum.

The cellular actions of CPE were extensively investigated using a cultured intestinal epithelial cell line. These studies speculated that CPE would act in a multistep process. The current model for the actions of CPE begins with binding to the toxin-specific receptors, claudin-3 and claudin-4, in a temperature-sensitive manner. Claudins are a 24-member family of ~20 to 25 kDa proteins and are the most important proteins in epithelial tight junctions. These tight junction proteins are predicted to consist of four transmembrane domains, two extracellular loops, and cytoplasmic tail-mediating signal cascades. CPE binding to receptor claudin proteins is mediated by the second extracellular loop (at a helix–turn–helix motif) of the tight junction protein. CPE can bind to claudin-3, claudin-4, claudin-6, claudin-7, claudin-8, and claudin-14 (the contribution of claudins-8 and claudin-14 to CPE cytotoxicity is less than that of claudin-4), but not to claudin-1, claudin-2, claudin-5, or claudin-10. CPE–claudin binding rapidly results in the formation of an ~90 kDa small complex, which contains claudin families and claudin receptors along with claudins incapable of binding CPE (nonreceptor claudin(s)).

Within as little as 5 min of CPE binding, six small complexes are then thought to oligomerize into a stable large complex (~450 kDa), which initially assembles as a prepore and rapidly inserts into membranes to form an active pore. The formation of active pores causes the loss of normal plasma membrane permeability properties. These CPE-induced permeability alterations are initially restricted to small molecules of <200 Da, for example, calcium ions and amino acids. As a result of these events, CPE-treated cells die from classical caspase 3-mediated apoptosis at moderate levels of CPE, whereas cells die from oncosis at higher levels of CPE. Thus, large-complex formation is a necessary step in CPE-induced cytotoxicity.

CPE pore formation also leads to morphological damage, which allows the formation of a bigger large complex (~650 kDa). This ~650 kDa large complex contains another ~65 kDa tight junction protein, occludin. This event induces the further disruption of tight junctions.

Collectively, the involvement of tight junction proteins in the actions of CPE causes intestinal paracellular permeability changes. The overall sequence of events on CPE-treated culture cells is believed to lead to CPE's effects on the small intestine both *in vivo* and *in vitro*.

Tolerance of *C. perfringens* to Environmental Stress

Besides its toxin-producing ability, *C. perfringens* type A foodborne illness is commonly the result of inadequate handing of food during cooking, cooling, or holding. The biological properties of *C. perfringens* vegetative cells and spores in these settings contribute to its ability to cause food poisoning by enabling its survival and growth in cooked food; for example, resistance to heat, low temperatures, NaCl, and nitrates (typically used to process and protect foods). Of these tolerant properties to environmental stresses during cooking, extremely high heat tolerance has been a well-established property of *C. perfringens* vegetative cells and spores. The resistance of *C. perfringens* spores to heat is influenced by both environmental and genetic factors. Regarding environmental factors, the spores of *C. perfringens* food poisoning strains can survive boiling in a protective meat-based medium (e.g., cooked-meat medium). Incomplete heating can also induce the germination of *C. perfringens* spores. These resistant properties of spores are genetically based on the products of *ssp* genes, which encode a/b-type small acid-soluble proteins. The three previously identified *ssp* genes (*ssp1*, *ssp2*, and *ssp3*) in *C. perfringens* strains share identical sequences and are also expressed at similar levels in several *C. perfringens* isolates, including strains that produce heat-resistant or less heat-resistant spores. The novel *ssp* gene, *ssp4*, was recently identified. One of the *ssp4* gene products, the Asp Ssp4 variant, is harbored in most chromosomal *cpe* strains, and this variant mostly contributes to the extreme heat resistance of spores made by food poisoning strains with the chromosomal *cpe* gene. This gene product also appears to contribute to sodium nitrate resistance.

Pathogenesis of *C. perfringens* Type A Food Poisoning

Inappropriate cooking and preservation cause the rapid proliferation of the vegetative cells of enterotoxigenic *C. perfringens* in foods. While many ingested *C. perfringens* vegetative cells are likely killed due to the acidity of the stomach, some vegetative cells that survive pass through the stomach and remain viable in a sufficiently contaminated food vehicle (i.e., food containing 10^6–10^7 *C. perfringens* vegetative cells per gram). These surviving vegetative cells multiply and sporulate in the small intestine; hence, this illness is considered to be an infection, not an intoxication. A low-molecular-weight (1000–5000) sporulation factor, produced by both CPE-negative and CPE-positive vegetative cells, may promote *in vivo* sporulation. CPE is expressed during sporulation and then accumulates in the mother cells of *C. perfringens*. After mother cells lyse, CPE is released into the intestinal lumen, in which the CPE quickly binds to intestinal epithelial cells and exerts its action. These events induce fluid and electrolytes losses and also morphological damage to intestinal epithelial cells.

Epidemiology

C. perfringens type A food poisoning annually ranks among the most common foodborne diseases in the United States, Europe, and Japan. Identified outbreaks of *C. perfringens* type A food poisoning commonly involve large outbreaks (the average outbreak size is ~50 to 100 in United States) and often occur in institutions, such as hospitals, school cafeterias, prisons, and nursing homes. This epidemiological feature has largely been attributed to at least four factors. First, large amounts of food are prepared at once in institutions, which is a factor that increases the risk of contamination. Second, the preparation of large amounts of food is associated with insufficient heating, especially the 'core' region of food. Third, large amounts of food are often prepared in advance and held for later serving. These conditions appear to facilitate the growth of heat-tolerant bacterium in prepared food. Fourth, relatively mild and nondistinguishing symptoms develop in most cases of *C. perfringens* type A food poisoning; in other words, *C. perfringens* food poisoning with a small number of cases is not recognized or reported. Therefore, the true prevalence and impact of this foodborne disease appear to be significantly understated.

Molecular Characteristics of Food Poisoning Strains

Isolates from food poisoning outbreaks are mainly classified into three *cpe* genotypes based on the gene arrangement of the *cpe* region, as described earlier. Plasmid *cpe* strains have been identified as the pathogen in human GI diseases such as antibiotic-associated diarrhea, nosocomial diarrhea, and sporadic diarrhea and also as isolates in healthy human feces and environmental samples. Studies using recently developed PCR assays, which allow these '*cpe* genotypes' to be differentiated from each other, revealed that the plasmid *cpe* strain can induce *C. perfringens* type A foodborne outbreaks, similar to the chromosomal *cpe*-positive strains; isolates from approximately two-thirds of outbreak cases harbored the *cpe* gene on the chromosome, and plasmid *cpe* isolates were the causative strain in another one-third of cases. However, as other *cpe*-negative environmental strains, most of these plasmid *cpe* strains produce spores that are less resistant to high and low temperatures, NaCl, and nitrates, than the chromosomal food

poisoning strains. These properties are based on the genetic background of the plasmid *cpe* strains; plasmid *cpe* strains carry the *ssp4* gene, which encodes the less heat-resistant Ssp4 protein the Gly Ssp4 variant.

Source of Enterotoxigenic *C. perfringens* Strains in Food Poisoning Outbreaks

While common food vehicles for *C. perfringens* type A foodborne illness were initially thought to be meat and meat products (notably beef and poultry and gravies) in the United States, various foods have also been identified as a contaminated food in *C. perfringens* type A food poisoning outbreaks. In general, *C. perfringens* is ubiquitous in nature; it is present in soil (at levels of 10^3–10^4 CFU g^{-1}), foods (e.g., approximately 50% of raw or frozen meat contains some level of *C. perfringens*), dust, and the intestinal tract of humans and domestic animals (e.g., human feces typically contain 10^4–10^6 CFU g^{-1}).

In independent surveys investigating various kinds of environmental samples, some surveys identified both types (chromosomal or plasmid-borne) of *cpe*-positive isolates, while other surveys failed to detect *cpe*-positive strains in environmental samples. The reasons for the difficulty in detecting CPE-producing *C. perfringens* in environmental samples are as follows:

(1) Very low number of *C. perfringens* is present in retail food samples, while the *C. perfringens* strain is frequently present in retail food.
(2) Only a small subset of food isolates (less than 5%) can produce CPE, maybe even in putative reservoir(s).
(3) CPE is produced only during sporulation; however, it is sometimes difficult to induce the sporulation of isolates using several kinds of sporulation-specified media (e.g., Duncan–Strong medium).

Results from the limited number of surveys identifying *cpe*-positive strains in investigated samples revealed that *C. perfringens* strains carrying the *cpe* gene on a large plasmid are likely to be the main population in the environment, while the three *cpe* genotypes of the *C. perfringens* strain are broadly distributed. Therefore, foods could be accidentally contaminated with *cpe*-positive strains from the environment in any steps during food processing (Figure 1).

Interestingly, recent studies using molecular assays (multilocus sequence typing and microarray assays) revealed the genetic characteristics of chromosomal and plasmid *cpe* strains. The multilocus sequence typing (MLST) procedure has characterized isolates using the DNA sequences of approximately 450–500 bp internal fragments of multiple housekeeping genes. The different sequences present within isolates of each housekeeping gene have been assigned as distinct alleles, and, for each isolate, the alleles at each of the loci define the allelic profile or sequence type (ST). Using MLST assays, chromosomal *cpe* strains construct a distinct ST from plasmid *cpe* strains, *cpe*-negative strain, and type B to E veterinary strains.

Another molecular assay is a microarray assay, in which the property of nucleic acid sequences specifically pairs with each other between complementary nucleotide base pairs. With a

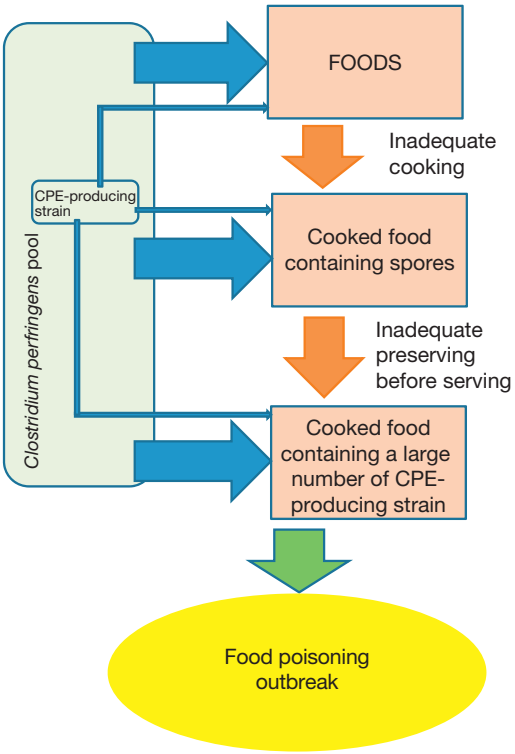

Figure 1 Events leading to *C. perfringens* food poisoning outbreaks, when accidentally contaminated with CPE-producing strain. Foods are more frequently contaminated with non-CPE-producing strains.

completely sequenced *C. perfringens* food poisoning strain genome-based DNA microarray assay, type A chromosomal *cpe* strains have different genetic backgrounds from type A plasmid *cpe* strains and *cpe*-negative type A strains; that is, the gene clusters of myo-inositol, ethanolamine, and biotin synthesis are absent in the variable region of chromosomal *cpe* strains, whereas the gene cluster of cellobiose metabolism is present. Combined with the results of MLST analysis for chromosomal *cpe* strains, chromosomal *cpe* strains and plasmid *cpe* strains are likely to have different habitats in the environment; plasmid *cpe* strains might have adapted to the mammalian intestine environment, while chromosomal *cpe* strain might be present in environments with plant materials.

The principal reservoir(s) of *C. perfringens* type A food poisoning strains has not been examined in detail. The development of a method(s) capable of detecting low numbers of bacterial cells is required to further investigate the source of CPE-producing *C. perfringens* in environmental samples because contaminated samples commonly contain a low number of CPE-producing *C. perfringens* with a small subset of total *C. perfringens* isolates. Unfortunately, the current standard procedures established to diagnose *C. perfringens* food poisoning are only able to detect large numbers of *cpe*-positive strains in samples. Several assays based on the methods of 'microbial source tracking' might be useful for detecting low numbers of vegetative cells and/or spores in environmental samples.

In the future, epidemiological surveys using assays, which can differentiate between the genetic backgrounds of *cpe*-positive and *cpe*-negative strains and are based on microbial

source tracking, help to understand the *cpe*-positive *C. perfringens* habitat in the environment, and based on these epidemiological findings, candidate(s) of native reservoirs of chromosomal and/or plasmid *cpe*-positive *C. perfringens* strains would be identified.

Clinical Features

Food poisoning outbreaks due to type A enterotoxigenic *C. perfringens* typically involve a large number of cases. The primary clinical symptoms associated with food poisoning are moderately severe abdominal cramps, nausea, and watery diarrhea; vomiting and fever are rare. Symptoms of food poisoning by type A *C. perfringens* strains develop 8–24 h after the ingestion of food heavily contaminated with the organism. The illness is generally self-limited but typically lasts 12–24 h. Mild symptoms may last 1 or 2 weeks in some cases.

Fatalities are very rare, occurring in $<0.05\%$ of cases. While everyone is susceptible to *C. perfringens* type A food poisoning, fatalities are commonly caused by dehydration and occur among the very young, very old, debilitated, and chronically ill individuals. Three foodborne outbreaks of CPE-producing strains with fatalities have recently been reported, and isolates in these outbreaks were identified using PCR assays as a type A strain harboring the *cpe* gene on the chromosome. Of these outbreaks, fatalities were attributed to necrotizing colitis, which has features similar to those of necrotizing enteritis caused by the *C. perfringens* type C strain. These fatal cases had nausea and vomiting in addition to abdominal cramps and watery diarrhea.

Diagnosis

A diarrheal disease is difficult to identify as a foodborne illness due to enterotoxigenic *C. perfringens* in a clinical setting. For example, the clinical symptoms and degree of severity of foodborne diseases by *C. perfringens* and *B. cereus* are very similar. Therefore, a laboratory investigation must be performed to identify a diarrheal disease as food poisoning caused by CPE-producing *C. perfringens*.

The most conventional diagnostic criterion to identify *C. perfringens* type A food poisoning outbreaks is the detection of CPE in the feces of patients. However, the usefulness of fecal CPE detection approaches for identifying *C. perfringens* food poisoning outbreaks is limited because fecal samples from suspected cases must be collected soon after the onset of food poisoning symptoms to ensure meaningful results. Serological assays such as the reversed-passive latex agglutination assay and enzyme-linked immunosorbent assay are used to detect CPE in properly collected fecal samples. The detection of CPE in the feces of several patients, who exhibit a common clinical features (common food consumption, typical incubation time, and characteristic symptoms), provides compelling evidence for the occurrence of *C. perfringens* type A food poisoning, while nosocomial type A *C. perfringens* outbreaks rarely occurred via contaminated environments, mainly lavatory equipment.

Further laboratory analyses including bacterial isolation and species identification are reliable approaches to identify *C. perfringens* type A food poisoning. For example, in the United States, bacteriologic criteria used by the Centers for Disease Control and Prevention (CDC) to identify an outbreak include demonstrating the presence of either (i) 10^5 *C. perfringens* organisms per gram of stools from two or more patients or (ii) 10^5 *C. perfringens* organisms per gram of epidemiologically implicated food.

Only demonstrating the presence of *C. perfringens* in suspected food or the feces of patients is insufficient for the unequivocal identification of *C. perfringens* type A food poisoning because *C. perfringens* is ubiquitous and is found in raw food and the GI tracts of healthy individuals. Therefore, to identify food poisoning outbreaks caused by type A *C. perfringens*, isolates from suspected cases should be identified as the type A CPE-producing *C. perfringens* strain. Culture samples using spore-formation medium (such as Duncan–Strong medium) are used to investigate the ability of foodborne disease isolates to produce CPE. However, a definite protocol for CPE expression by food poisoning isolates has not yet been established. Moreover, the *in vitro* sporulation of food poisoning isolates is often difficult to achieve under laboratory conditions.

Instead of investigating CPE-producing ability, molecular assays, for example, simple PCR assays, are available to specifically detect the *cpe* gene. The *cpe* gene must be functional in most, if not all, *cpe*-positive strains because the *cpe* region in the *cpe*-positive *C. perfringens* strains examined, which can produce CPE during *in vitro* sporulation, has almost identical sequences, such as the ORF sequence of the *cpe* gene, upstream sequence of the *cpe* ORF including SigK- and SigE-dependent promoters, and ribosome-binding sites. Developed PCR assays can relatively easily detect the presence of the *cpe* gene carried by *C. perfringens* strains in properly collected contaminated food samples and/or fecal samples from patients with *C. perfringens* type A food poisoning. However, many protocols for the *cpe*-detecting PCR assay also detect the *cpe* gene in type C and type D *C. perfringens* and the *cpe* pseudogene in type E strain; that is, PCR assays detecting the *cpe* gene must be meaningful when combined with PCR assays identifying the *C. perfringens* toxin genotype.

CPE-positive strains must be present, even at very low frequencies, in various foods, healthy human feces, and the environment. Therefore, similarities must be investigated between isolates from feces and suspected food to more strictly diagnose cases and identify contaminated food in foodborne illnesses. To investigate similarities or differences between *cpe*-positive isolates in these samples, identifying the serotype and/or genotype (such as the pulsed-field gel electrophoresis type) of CPE-producing isolates can be an alternative and supplemental approach for diagnosing *C. perfringens* type A food poisoning outbreaks.

Treatment and Prevention

The clinical symptoms of *C. perfringens* type A food poisoning are commonly mild and the clinical course is self-limited. Antibiotic treatment is typically not required. Rehydration

drinks containing important electrolytes are useful for managing the dehydration caused by diarrhea.

CPE-producing *C. perfringens* strains are broadly distributed in the environment, including foods, soil, and kitchen surfaces. Therefore, contamination events to food may occur during the cooking process (food ingredients, cooking, and preserving) (**Figure 1**). For this reason, completely protecting against *C. perfringens* contamination to food(s) is likely to be difficult. Fortunately, a large number of CPE-producing bacterial cells are needed to induce *C. perfringens* food poisoning, which is because many ingested *C. perfringens* vegetative cells are killed when exposed to the acidity of the stomach. Collectively, the most important factors preventing and controlling *C. perfringens* type A foodborne illness are 'careful cooking' and 'careful storage,' which prohibit the vegetative growth of *C. perfringens* in cooked foods. Therefore, reducing the total amount or size of food for heating and cooling (it is difficult to archive high internal temperatures in large amounts of food), appropriate storage at less than 10 °C for cooked foods before serving (growth rates of *C. perfringens* vegetative cells rapidly decrease at temperatures below ~15 °C, with no growth occurring at 6 °C), and the immediate consumption of served foods as soon as possible are the best ways to prevent *C. perfringens* food poisoning.

Biologically, the growth of *C. perfringens* strains is affected by water activity (a_w), reduction potential (E_h), pH (optimal growth at pH 6 to 7), and chemical preservatives (e.g., NaCl). These preservation factors also control the growth of *C. perfringens* vegetative cells and inhibit the outgrowth of germinating *C. perfringens* spores in food.

See also: *Clostridium*: Occurrence and Detection of *Clostridium perfringens*; Diarrheal Diseases; Food Poisoning: Classification; Food Poisoning: Epidemiology; Food Poisoning: Tracing Origins and Testing; Foodborne Pathogens.

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Clostridium: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin

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Introduction

Botulinum neurotoxin (BoNT)-producing clostridia normally called *Clostridium (C.) botulinum* are, from the genetic and physiological points of view, a diverse taxonomic group. The group comprises Gram-positive, anaerobic, rod-shaped, spore-forming bacteria that produce the most toxic biological substance known, botulinum neurotoxin (BoNT). Foodborne botulism is a neuroparalytic disease resulting from neurotoxin-induced inhibition of skeletal and autonomic peripheral cholinergic nerve terminals. It results from consumption of food in which *C. botulinum* has grown and produced botulinum toxin. Without prompt diagnosis and treatment, the resulting cranial neuropathy and symmetrical, descending flaccid paralysis may progress to respiratory failure and death.

BoNTs are 150 kDa proteins produced by *C. botulinum* or neurotoxigenic strains of *C. butyricum* or *C. baratii*. Maximal potency of BoNTs is only obtained after cleavage of the 150 kDa polypeptide into a 100 kDa heavy chain (HC) and a 50 kDa light chain (LC) linked by a single disulfide bond. The HC binds to receptors on neurons and enables the internalization of the LC, a zinc metalloprotease, into presynaptic neurons at the neuromuscular junction. The internalized LC cleaves one of the soluble *N*-ethylmaleimide-sensitive attachment receptor (SNARE) proteins. The LC of BoNT type A cleaves synaptosomal-associated protein 25 (SNAP-25), whereas the LC of BoNT type B cleaves synaptobrevin-2. Cleavage of these proteins prevents the docking of small synaptic vesicles with the presynaptic membrane, preventing the release of acetylcholine into the neuromuscular junction and resulting in flaccid paralysis of the corresponding muscle.

Seven serologically distinct BoNTs (A–G) can be distinguished based on neutralization of toxicity with specific antisera. Recently, a strain of *C. botulinum* producing BoNT type B and another BoNT that is not neutralized by antitoxins to BoNTs A–G has been isolated from a case of infant botulism. It has been proposed that this novel neurotoxin is an eighth serotype – BoNT type H. While sequence data have not yet been made available, genomic studies have indicated that BoNT type H differs substantially from the other seven BoNT serotypes. Human botulism, including foodborne, wound, and infant botulism, is associated with types A, B, E, and, very rarely, F. The majority of cases of animal botulism are caused by type C; cattle and sheep are also particularly susceptible to botulism caused by type D. To date, there is no direct evidence linking type G to disease.

Based on physiological differences, and now supported by whole genome sequencing, *C. botulinum* is divided into four groups: (I) all type A and proteolytic strains of types B and F, (II) all type E and nonproteolytic strains of types B and F, (III) type C and D strains, and (IV) type G strains. Strains producing two toxin types have been reported, as have those producing only one type of toxin but carrying a silent gene for a different toxin type. Groups I and II are commonly referred to as 'proteolytic' and 'nonproteolytic' strains, respectively. Proteolytic strains are relatively resistant to heat and preservatives such as salt, acidulants, and nitrite. They are therefore the most likely forms involved in outbreaks from underprocessed canned foods or from salted, pickled, and otherwise cured products. Exceptions are some dry- and brine-cured hams and pickled fish where *C. botulinum* may develop before the diffusion of the main ingredients conferring safety (e.g., salt and acid) is complete. With few exceptions, infant botulism is caused by proteolytic strains of *C. botulinum*. As a result of their sensitivity to heat and ability to grow at refrigeration temperatures, group II, or nonproteolytic, strains present a risk of botulism from minimally processed packaged foods that have extended shelf lives at refrigeration temperatures. In a few exceptional cases, other clostridia (*C. baratii* producing type F toxin and *C. butyricum* producing type E toxin) have been implicated in foodborne botulism incidents. Type E is the prevailing form of *C. botulinum* in aquatic environments and the most likely form of botulism from preserved fish. Type E is also the main cause of botulism involving the indigenous population of Canada and Alaska. The emphasis here will be on groups I and II since they are involved in human illness. Strains belonging to group III are involved with animal botulism. This article discusses the distribution of botulinum spores in the environment and in foods and detection of the organism and its neurotoxin in foods.

Presence of *C. botulinum* in the Environment

Spores of *C. botulinum* are commonly present in soils and sediments, but their numbers and types vary, depending on the location. The possibility of contamination of food with *C. botulinum* depends on the distribution and incidence of spores in the environment where the food originates.

C. botulinum spores are widely distributed in North America, but the spore load varies considerably, as does the predominating type. Soils in the United States west of the rise of the Rocky Mountains usually contain type A spores, while type B spores predominate in the Eastern United States. Most US type B strains are proteolytic. *C. botulinum* type E is found in damp to wet locations. In the region around the Great Lakes, and particularly around Green Bay of Lake Michigan, high numbers of type E are found in shoreline and sediment samples. Type E is also found in the coastal areas of Washington and Alaska. The distribution of types on the Pacific coast changes with latitude; south of 36N, the prevalent types shift from E to A and B.

Type B predominates in the terrestrial environments of Britain, Ireland, Iceland, Denmark, and Switzerland. It is associated with most botulism outbreaks in Spain, Portugal, Italy, France, Belgium, Germany, and Poland, indicating wide

distribution of this type in the European environment. Type B also predominates in the aquatic environments of the United Kingdom. Most European type B strains are nonproteolytic.

C. botulinum type E predominates in marine and freshwater environments in temperate to subarctic regions. High incidences of *C. botulinum* type E have been recorded from the Nunavik region of Quebec, the northwest coast of Alaska, the Great Lakes, and lakes and rivers of North Japan. High levels of type E spores were found in Scandinavian waters, particularly in the Kattegat, between Denmark and Sweden, and in the Baltic Sea. The presence of high numbers of *C. botulinum* type E in these regions suggests a terrestrial origin of the organism and passive accumulation from land drainage. Type E spores also predominate in the Ukraine and most parts of Russia. High numbers of type E botulism outbreaks associated with fish caught from Lake Baikal suggest a high prevalence of *C. botulinum* type E in the Irkutsk region of Russia. In general, surveys of Asia report lower numbers, with the exceptions of a high incidence of type E spores around the Caspian Sea and a high incidence of all types in the Xinjiang district of China.

Fewer surveys have been carried out in the southern hemisphere. Spores of all types have been detected in South America. In a study of over 2000 Argentine soil samples, 23.5% tested positive for BoNT-producing clostridia, with type A being the most frequently detected. *C. botulinum* type E has been found in fish, oyster, and shrimp samples taken off the Brazilian coast.

As a result of its role in outbreaks of avian botulism across the world, the distribution of *C. botulinum* type C has been widely studied. Based on both surveys and recorded cases of animal botulism, *C. botulinum* type C has been found in several countries including Australia, Japan, Korea, Canada, the United States, Costa Rica, Spain, Sweden, Finland, Germany, Ireland, Italy, Norway, Brazil, South Africa, and Turkey. Genetic recombination between *C. botulinum* type C and type D strains has resulted in *C. botulinum* strains producing a mosaic C/D type toxin. Chimeric type C/D toxin is more lethal to avian species than either type C or type D. Type C botulism has become an emerging and serious problem in poultry flocks in Sweden, causing 13 separate outbreaks in 2008. Recent molecular typing evidence indicates the type C/D strain responsible for avian botulism outbreaks in waterbirds in Spain shares a very high level of genetic similarity with the strains responsible for the Scandinavian outbreaks in poultry flocks. *C. botulinum* type D is the cause of botulism outbreaks in cattle in several parts of the world including parts of the United States, Canada, Australia, Israel, Turkey, Germany, South Africa, and the Netherlands.

In summary, type A spores predominate in soils in the Western United States, China, Brazil, and Argentina, and type B spores in the Eastern United States, the United Kingdom, and much of continental Europe. However, most US type B strains are proteolytic, while most European strains are nonproteolytic. Type E is the predominant type in northern regions and in most temperate aquatic regions and their surroundings. Types C and D are found more frequently in tropical environments. The reasons for this distribution pattern are not well understood. Type A appears to favor neutral to alkaline soils with low organic content, consistent with its virtual absence in the highly cultivated soils of the Eastern United States and Europe. Type B strains predominate in heavily farmed soils in Argentina, Denmark, and the United States. Type E is psychrotolerant, which undoubtedly plays a role in its prevalence in the north and in many aquatic environments. Based on outbreaks in wild and domestic animals, *C. botulinum* types C and D can be found over most continents.

Presence of *C. botulinum* in Foods

Spores of *C. botulinum* are widespread in nature and are found in soils, aquatic sediments, and the gastrointestinal tracts of animals. Contamination of food with *C. botulinum* often occurs during growth or harvesting and is most likely to occur when a product originates in an environment with a high incidence of spores. However, contamination can also occur during or after processing. There have been considerably fewer surveys of foods for contamination with *C. botulinum* than environmental surveys, and they have focussed primarily on fish, meats, and infant foods, particularly honey.

Fish may become contaminated with spores of *C. botulinum* in their environment or during processing and handling. The presence of *C. botulinum*, mostly type E, in fish is readily demonstrated, although the incidence is lower than in environmental surveys. For example, 20% of the fish caught in the Baltic Sea and Finnish freshwaters tested positive for *C. botulinum* type E, while 7% of vacuum-packed hot-smoked products marketed in Finland contained spores of the organism. Other studies have indicated a range from 0% to 100% of fishery products testing positive for *C. botulinum* type E. Most fish-borne botulism outbreaks recorded in Canada, the United States, Russia, Europe, Japan, Egypt, and Iran have been linked to the consumption of smoked, salt-dried, canned, or fermented fish usually eaten without further cooking.

The level of contamination of meats is generally very low, relative to the incidence on fish and fishery products. The few studies indicate that the incidence of *C. botulinum* in meat samples is often less than 10%, and several studies have not been able to detect *C. botulinum* in meats. The concentration of spores in meat is typically <1 spore per kg, with the exception of raw pork and vacuum-packed bacon in the United Kingdom, where some samples had up to 7 spores per kg. Survey results reveal that nearly all toxin types identified from cured meat, raw pork, vacuum-packed bacon, and liver sausage were either type A or type B. *C. botulinum* types A and B are the two serotypes most often associated with botulism outbreaks from meat products. The presence of low numbers of *C. botulinum* in pig and cattle's feces indicates carriage of *C. botulinum* by healthy animals and suggests contamination of carcasses with *C. botulinum* during carcass dressing.

C. botulinum types A or B may be present on vegetables, particularly those harvested from the soil. *C. botulinum* is common in soil and organic fertilizers, usually at low concentrations. However, one survey reported 25 000 spores per kg in soil in China. One product of particular concern is cultivated mushrooms, in which up to 2100 type B spores per kg have been detected. As a result of the relatively frequent occurrence of botulism outbreaks resulting from canned vegetables in California in the early part of the twentieth century, an extensive survey of vegetables from markets and gardens was

conducted. Fruits and vegetables, including asparagus, beans, carrots, celery, corn, lima beans, olives, potatoes, turnips, apricots, cherries, and peaches, all tested positive for *C. botulinum*, primarily type A. Improperly processed or temperature-abused vegetable products, including baked potato, potato salad, carrot juice, olives, mushrooms, bean curd, and onions, have all been implicated in foodborne botulism outbreaks. Home-canned vegetables including beans, carrots, asparagus, and vegetable products stored in oil including garlic in oil and eggplant in oil present an especially high risk of foodborne botulism.

Spores in honey and other infant foods pose a unique hazard because, in some infants, the spores are able to colonize the intestines, produce toxin, and cause infant botulism. By 1984, honey had been implicated as the likely source of botulinum spores in 20 cases of infant botulism in California. Surveys for the presence of *C. botulinum* spores in honey suggest that the incidence ranges from 2% to 26% of samples depending upon the geographic source of honey, with spore levels in random samples of honey in the order of 1–10 spores per kg. However, in honey samples associated with infant botulism, the level is approximately 10^4 spores per kg. The pasteurization process applied to honey is used to inactivate osmophilic yeasts and prevent granulation, but is not sufficient to inactivate spores of *C. botulinum*. Other infant foods have also been examined. While *C. botulinum* has been detected in samples of corn syrup and rice cereal, exposure of infants to botulinum spores via these foods seems to be minimal as the spore levels are low and spores are unlikely to multiply during manufacture and storage. Due to the risk for infant botulism, health authorities in several countries recommend that honey should not be given to infants less than 1 year of age.

Dairy products are rarely vehicles for botulism outbreaks. Six reported outbreaks of botulism caused by cheeses, primarily cottage cheese, occurred in California and New York between 1912 and 1951. One of these outbreaks occurred in Albany, New York, in 1914 and resulted in the death of three persons and was one of the first reported outbreaks caused by *C. botulinum* type B. In October 1993, eight cases resulted after eating a potato stuffed with meat and a commercial cheese sauce. In August 1996, an outbreak of botulism in Italy affected eight people after consumption of mascarpone cheese, either alone or as a component of the desert tiramisu.

Detection of *C. botulinum*

Safety Precautions When Working with *C. botulinum* or BoNT

The extreme toxicity of BoNT requires that specific safety procedures be followed when working with cultures containing *C. botulinum* and BoNT. With the rare exception of wound botulism, *C. botulinum* is noninfectious in healthy adults. As a result of its noninfectious nature, *C. botulinum* is a containment level 2 organism. In Canada, procedures generating significant quantities of toxin, or aerosol formation of toxin, require BSL3 laboratory facilities. All personnel engaged in handling toxic materials must be fully informed about the hazards, and all materials to be autoclaved should be contained in stainless steel boxes with handles. Therapeutic antisera must be available in case of accidental intoxication. Potentially toxic materials should always be contained in unbreakable, leak-proof trays or boxes. This is particularly important in the incubation or manipulation of botulinal cultures, which may contain in excess of 10^5 mouse LD per ml. If toxin should be spilled, it must be inactivated immediately with 0.1 N NaOH.

Traditional Culture Methods

Prevalence rates for *C. botulinum* in foods are not readily available, mainly because of the cost and difficulties of detecting the organism in foods. *C. botulinum* is traditionally detected by enriching food or environmental samples for spores, followed by broth culture and detection of BoNT in the culture supernatant. Common enrichment media for detecting viable *C. botulinum* are cooked meat medium (CMM), CMM glucose, chopped meat glucose starch medium, and tryptone–peptone–glucose–yeast extract (TPGY) broth to which trypsin may be added. Trypsin is necessary to activate the toxin produced by group II organisms and may also inactivate potential inhibitors of *C. botulinum* such as bacteriocins in mixed cultures. While foods may be inoculated directly, the sediments of centrifuged samples are preferred because potential growth inhibitors are removed. At least two tubes of media are inoculated. One is heated at 75–80 or 60 °C, depending on whether the suspected type belongs to group I or II, to select for spores. Alternatively, spores of group II may be selected by holding samples in 50% alcohol for 1 h before inoculation. The other tube is incubated without any heating to allow development of vegetative *C. botulinum* cells in cases where few or no spores are suspected. Adding lysozyme to the medium may increase recovery of heat-injured spores. Group I strains grow optimally at 37 °C, while group II strains have an optimal growth temperature of 30 °C. *C. botulinum* is identified after incubation of the enrichment medium by toxin analysis of the supernatant fluid. Botulinal toxin is detected by injecting serum or extracts from foods and clinical specimens into mice, observing these for lethality, and neutralizing the toxin with specific antisera.

It is not necessary to isolate *C. botulinum* in pure culture from foods in order to demonstrate its presence. If the neurotoxin is present in the nonselective culture after incubation, the toxin-producing organism must have been originally present. If an enrichment culture tests positive for either *C. botulinum* type B or F, the organism must be isolated to determine whether the strains belong to group I or group II. *C. botulinum* can be isolated by streaking toxic enrichment cultures onto *C. botulinum* isolation (CBI) agar. CBI agar is supplemented with cycloserine, sulfamethoxazole, and trimethoprim to inhibit background flora and egg yolk for detection of lipase-positive colonies. Suspect lipase-positive colonies showing spreading and an irregular edge may be picked and restreaked for isolation. Many other common clostridia, such as *C. sporogenes*, produce a lipase reaction on agar plates containing egg yolk. Isolates are grown in TPGY or CMM broth, and toxicity of the culture supernatant confirms identity of the isolate as a neurotoxigenic clostridium. It is important to note that *C. butyricum* is lipase-negative; therefore, *C. butyricum* strains producing type E toxin will appear as lipase-negative colonies on CBI agar. Colony immunoblotting to detect

individual botulinum toxin-producing colonies on an agar plate is a sensitive method for isolation from enrichment cultures with a high background flora.

As an alternative to testing enrichment cultures, or pure cultures, with the mouse assay to detect BoNT, polymerase chain reaction (PCR) can be used to detect the presence of genes encoding BoNT. Multiplex PCR assays (both conventional and real-time-PCR) have been developed to allow simultaneous detection of *botA*, *botB*, *botE*, and *botF* genes in a single reaction. These multiplex PCR reactions allow screening of many samples at once, with the advantages of reduced time to detection without a requirement for use of live animals. Application of multiplex PCR to isolated colonies also allows identification of bot-containing colonies, facilitating isolation of botulinogenic clostridia. As PCR does not detect botulinum toxin, the possibility exists that clostridial strains with silent BoNT genes may be detected, resulting in a false-positive result. PCR has also been used for molecular characterization of *C. botulinum* based on the sequences of the neurotoxin and flagellin genes.

When *C. botulinum* is present in food or environmental samples, it is typically present in low concentrations (<1 to 1000 spores per kg). Quantitative estimates of such low numbers require the use of broth cultures and most probable number (MPN) analysis. Depending on the precision required, three or five tube MPN tests are performed in a similar manner described earlier for the detection of *C. botulinum*. Toxicity testing for the large number of samples involved in MPN analysis is facilitated by using an *in vitro* assay, in place of the standard mouse bioassay.

Detection of BoNT

BoNTs are extraordinarily potent with the parenteral human lethal dose estimated to be 0.1–1 ng kg^{-1} and the oral lethal dose estimated at 1 μg kg^{-1}, thereby requiring an extremely sensitive assay for detection.

Mouse Bioassay

BoNTs are recognized by their lethal action in mice and neutralization with specific antisera. The sample, or an extract prepared by homogenizing it in gelatin phosphate buffer, is clarified by centrifugation and filter sterilized. Trypsin treatment may be required to activate low levels of toxin from nonproteolytic strains. The prepared sample is injected intraperitoneally into mice with and without neutralization with antitoxin. Typical symptoms of botulism are ruffled fur, pinched waist, labored breathing, limb paresis, and general paralysis before death. The time required to onset of symptoms is dependent upon the concentration of toxin in the extract, with symptoms typically occurring within the first 24 h postinjection. Definitive results are obtained if mice injected with untreated sample display symptoms within 72 h, while mice injected with neutralized sample do not display symptoms. The use of an end point earlier than death is encouraged, as ruffled fur, pinched waist, and labored breathing are typical and easily observable signs of botulinum toxin. The mouse bioassay has several advantages including high sensitivity (approximately 10 pg of botulinum toxin) and is sufficiently robust to detect botulinum toxins in a wide range of sample matrices including feces, serum, gastric liquid, a wide range of food samples, and supernatants of bacterial cultures. In addition, the mouse bioassay is the only assay capable of detecting all serotypes, including any possible new serotypes, of BoNTs. In addition to the obvious ethical objection of sacrificing animals, the mouse bioassay has the disadvantages of being slow, expensive, and low throughput.

A refinement of the typical mouse bioassay, nonlethal mouse assays involve subcutaneous injection of mice and observation for flaccid paralysis of muscle near the injection site. Subcutaneous injection at the inguinocrural region in mice has been demonstrated to yield sensitivity results comparable to the typical mouse assay. A nonlethal mouse toe-spread reflex model has been used to detect botulinum toxins in buffer, serum, and milk samples at sensitivities similar to the typical mouse bioassay. Preincubation of samples with specific antitoxins prevents development of paralysis, allowing identification of toxin serotype with these methods. *Ex vivo* assays, using rat or mouse phrenic nerve diaphragm, and the rat intercostal muscle strips assay allow several tests from tissues of a single animal.

Immunologic Assays

The earliest immunologic assays used to detect botulinum toxins included passive agglutination and immunodiffusion assays. Enzyme-linked immunosorbent assays (ELISAs) were first developed for BoNT in the late 1970s and were capable of detecting approximately 400 mouse lethal doses (1 MLD is approximately 10 pg of neurotoxin). Improvements in ELISA technology, such as use of capture antibodies in a sandwich ELISA and generation of specific monoclonal antibodies for capture and detection, have increased the sensitivity of ELISA detection of botulinum toxins to less than one mouse lethal dose per ml of food matrix. Traditional ELISAs used alkaline phosphatase or horseradish peroxidase to cleave a chromogenic substrate. Reporters using signal amplification or chemiluminescence have further increased the sensitivities of immunoassays. Lateral flow assays are rapid and easy to perform with minimum requirements for laboratory equipment or skills. The sensitivity of lateral flow assays is in the range of 5–20 ng, approximately 1000 times less sensitive than the mouse bioassay. Some commercial lateral flow assays have been reported to be limited by matrix effects and have been recommended only for rapid detection of botulinum toxin in bacterial cultures.

Endopeptidase Assays

Development of *in vitro* assays to detect botulinum toxins has accelerated as a result of increased investment to defend against biological threat agents, and also assays are used for potency testing for lot release of therapeutic BoNT (e.g., Botox, Dysport,

or Xeomin). The discovery that botulinum toxin is a specific endopeptidase has led to the development of several assays that rely on detection of the endopeptidase activity of the LC. These assays typically employ a peptide containing a sequence from one of the substrate proteins – SNAP-25, synaptobrevin, or syntaxin. Cleavage of the peptide is measured by several methods including generation of a bioluminescent signal through cleavage of a SNAP-25 luciferase fusion protein, changes in fluorescence emission ratios using fluorescence resonance energy transfer (FRET), and detection of cleavage products using mass spectrometry. Most of the assays relying on endopeptidase activity require an initial step to remove interfering substances and concentration of the neurotoxin. This step often employs immunomagnetic 'pulldown' using magnetic beads coated with antibody to the neurotoxin HC. Endopeptidase assays have the advantage over immunoassays of detecting only botulinum toxins with endopeptidase activity, and not inactive toxins.

Cell-Based Assays

Cell-based assays take detection of biological activity even further by providing a model for BoNT detection that provides information on the activity of the BoNT preparation. This includes cell surface binding, endocytosis, translocation of the LC into the cellular cytosol, and enzymatic activity of the LC on SNARE substrates. This makes cell-based assays ideal for high-throughput screening for botulinum toxin inhibitors. In addition, cell-based assays may present a more accurate indication of the biological activity of therapeutic preparations of BoNTs. The drawbacks of cell-based assays are their limited sensitivity in comparison to the mouse assay and *in vitro* endopeptidase assays, and the difficulty in obtaining quantitative results. Cell-based assays also require the maintenance of cell cultures and take much longer to obtain results, when compared to other *in vitro* assays.

See also: *Clostridium botulinum*; *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Clostridium*: Occurrence and Detection of *Clostridium perfringens*.

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http://www.hc-sc.gc.ca/fn-an/legislation/guide-ld/botulism-botulisme-prof-eng.php – Botulism – Guide for Healthcare Professionals.

http://www.hc-sc.gc.ca/sr-sr/activ/micro/botulism-eng.php – Botulism Reference Service for Canada.

Cobalamin (Vitamin B12): Metabolism and Disorders

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Introduction

Vitamin B12 is the largest and most complex of all the vitamins. The name vitamin B12 is generic for a specific group of cobalt-containing corrinoids, also known as cobalamins, with biological activity in humans. Cobalt gives this water-soluble vitamin its red color. The main cobalamins in humans and animals are hydroxocobalamin, adenosylcobalamin, and methylcobalamin, the last two being the active coenzyme forms.

Although vitamin B12 was isolated almost 60 years ago, its metabolism remains incompletely defined. In practice, cobalamin metabolism is complex and requires many processes and steps, any one of which, if not present, may lead to cobalamin deficiency. Table 1 enumerates the elements establishing the definition of cobalamin deficiency.

This article summarizes the current knowledge on cobalamin metabolism and disorders.

Vitamin B12 Ingestion and Related Disorders

Vitamin B12 is produced exclusively by microbial synthesis in the digestive tract of animals. Therefore, animal protein products are the source of vitamin B12 in the human diet, in particular organ meats (liver and kidney). Other good sources are fish, eggs, and dairy products. In foods, hydroxo-, methyl-, and 5′-deoxyadenosyl cobalamins are the main cobalamins present.

A typical Western diet contributes 3–30 μg of cobalamin per day. The recommended dietary allowance set by the Food and Nutrition Board of the Institute of Medicine (the United States) is 2.4 μg day^{-1} for adults and 2.6–2.8 μg day^{-1} during pregnancy.

Table 2 describes through a synthetic view the different stages of vitamin B12 metabolism used in clinical practice and the corresponding causes of cobalamin deficiency. In clinical practice, vitamin B12 deficiency caused by dietary deficiency is rare.

The dietary causes of deficiency are limited to elderly people who are already malnourished, such as elderly patients living in institutions (they may consume inadequate amounts of vitamin B12-containing foods) or in psychiatric hospitals (strict vegetarians). Studies focussing on elderly people, particularly those who are in institutions or who are sick and malnourished, have suggested a cobalamin deficiency prevalence of 30–40%. The Framingham Study demonstrated a prevalence of 12% among elderly people living in the community. Using a stringent definition, a prevalence of 5% had been reported in a group of patients followed or hospitalized in a tertiary reference hospital.

A dietary cause of cobalamin deficit or deficiency was also found in newborns from strictly vegetarian pregnant women.

Vitamin B12 Digestion and Related Disorders

Dietary vitamin B12, which is bound to proteins in food, is released in the acidic environment of the stomach where it is rapidly complexed to the binding protein and transporter haptocorrin (HC), also referred to as the R-binder or transcobalamin I. About 80% of circulating cobalamin is bound to HC, and serum cobalamin levels have been correlated with serum HC concentrations. Although some unexplained low serum cobalamin concentrations were reported to be caused by mild to severe HC deficiencies, these abnormalities were not accompanied by pernicious anemia and are not thought to cause functional cobalamin deficiency.

Cobalamin continues its route in the gastrointestinal tract and dissociates from HC under the action of pancreatic proteases. Then, cobalamin is associated in the intestine with the intrinsic factor (IF, also known as the S-binder). This complex is essential for the ileal absorption of cobalamin (Table 2).

Digestion disorders related to vitamin B12 deficiency are mainly represented by food-cobalamin malabsorption (FCM) syndrome. This syndrome is characterized by the inability of the body to release cobalamin from food or intestinal transport proteins, particularly in the presence of hypochlorhydria (therefore, the appropriated denomination is 'maldigestion'). The principal characteristics of this syndrome are listed in Table 3.

FCM accounted for at least half of subtle or clinical documented cobalamin deficiency in elderly patients (60–70% in our experience). FCM is caused primarily by atrophic gastritis, related or not to chronic carriage of *Helicobacter pylori*. Other factors that mainly contribute to FCM are long-term ingestion of antacids, such as H2 receptor antagonists and proton pump inhibitors, particularly among patients with Zollinger–Ellison syndrome, and biguanides (metformin). In addition, other FCM inducers include chronic alcoholism, surgery or gastric reconstruction (e.g., bypass surgery for obesity), and partial exocrine pancreatic failure.

It is to note that in the case of FCM, patients can absorb 'unbound' cobalamin through IF or passive diffusion mechanisms. Thus, the recognition of the syndrome permits new developments of oral vitamin B12 therapy.

At this level, it is also important to note that several homozygous nonsense and missense mutations in the gene encoding the gastric IF have been reported to cause hereditary juvenile cobalamin deficiency.

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Vitamin B12 Absorption and Related Disorders

Absorption depends mainly on IF, which is secreted by the gastric mucosa. IF binds cobalamin forming a complex that is absorbed by the terminal ileum (Table 2). This complex is located at the apical side of brush border membranes (BBMs) of polarized epithelia, such as the intestinal apical BBM. It consists of the IF–vitamin B12 receptor named cubilin, a 460 kDa peripheral membrane glycoprotein, encoded by the *CUBN* gene, which was mapped to chromosomal region 10p12.33-p13, and the 48 kDa amnionless protein encoded by the *AMN* gene, a gene essential for mouse gastrulation and localized on human chromosome 14.

The human megalin/gp330/LRP2 receptor, encoded by the LRP2 gene located on chromosome 2q24-q31, is a giant endocytic receptor (600 kDa) of the low-density lipoprotein receptor family that was strongly suggested to play an important role in the stability of the cubilin–AMN complex.

The mechanism of vitamin B12 absorption through the complex IF–cubilin–AMN is Ca^{2+}-dependent.

Mutations in CUBN 7 were reported to cause hereditary megaloblastic anemia 1 (MGA1). MGA1 is a rare autosomal recessive disorder affecting human subjects with neurological symptoms and juvenile MGA.

Two principal mutations were identified in Finnish patients (FM): a mutation named FM1 changing a highly conserved proline to leucine (P1297L) in CUB domain 8, suggesting that this proline is functionally crucial in cubilin, and one point mutation FM2 in the intron interrupting CUB domain 6, which produced a truncated cubilin. Interestingly, a normal-size cubilin protein was identified in urine samples from homozygous FM1 patients, whereas a complete absence of the protein was reported in a patient homozygous for the FM2 mutation. Other mutations were also uncovered but were subsequently identified as polymorphisms after their

Table 1 Definitions of vitamin B12 (cobalamin) deficiency

- Serum cobalamin levels <150 pmol l^{-1} and clinical features and/or hematologic anomalies related to cobalamin deficiency
- Serum cobalamin levels <150 pmol l^{-1} (<200 pg ml^{-1}) on two separate occasions
- Serum cobalamin levels <150 pmol l^{-1} and total serum homocysteine levels >13 μmol l^{-1} or methylmalonic acid levels >0.4 μmol l^{-1} (in the absence of renal failure and folate and vitamin B6 deficiencies)
- Low serum holotranscobalamin levels <35 pmol l^{-1}

Table 2 Stages of vitamin B12 metabolism and corresponding causes of vitamin B12 deficiency

Stages and actors in vitamin B12 metabolism	*Causes of vitamin B12 deficiency*
Intake solely through food	• Strict vegetarianism and patients who are sick in institutions or in psychiatric hospitals
Digestion brings into play the following:	• Gastrectomies
• Haptocorrin	• Pernicious anemia
• Gastric secretions (hydrochloric acid and pepsin)	• Food-cobalamin malabsorption
• Intrinsic factor	
• Pancreatic and biliary secretions	
• Enterohepatic cycle	
Absorption brings into play the following:	• Ileal resections and malabsorption
• Intrinsic factor	• Pernicious anemia
• Cubilin, amnionless	• Food-cobalamin malabsorption
• Calcium and energy	
Transport by transcobalamins	• Congenital deficiency in transcobalamin II
Intracellular metabolism based on various intracellular enzymes	• Congenital deficiency in various intracellular enzymes

Table 3 Main characteristics of the food-cobalamin malabsorption syndrome

Characteristics of food-cobalamin malabsorption	*Associated conditions or agents*
• Low serum vitamin B12 (cobalamin) levels	• Gastric disease: atrophic gastritis, type A atrophic gastritis, gastric disease associated with *Helicobacter pylori* infection, partial gastrectomy, gastric bypass, vagotomy
• Normal results of Schilling test using free cyanocobalamin labeled with cobalt-58 or abnormal results of derived Schilling test[a]	• Pancreatic insufficiency: alcohol abuse
• No anti-intrinsic factor antibodies	• Gastric or intestinal bacterial overgrowth: achlorhydria, tropical sprue, Ogilvie's syndrome, HIV
• No dietary vitamin B12 deficiency	• Drugs: antacids (H2 receptor antagonists and proton pump inhibitors) or biguanides (metformin)
	• Alcohol abuse
	• Aging or idiopathic

[a]Derived Schilling tests use food-bound cobalamin (e.g., egg yolk, chicken, and fish proteins).

detection in normal individuals in the general population. The cubilin P1297L mutation associated with hereditary MGA1 was reported to cause impaired recognition of the cobalamin–IF complex by cubilin.

In addition, a mutation in AMN was reported in recessive hereditary MGA1 and was demonstrated to be essential for a functional cobalamin–IF receptor. This study demonstrated that homozygous mutations affecting exons 1–4 of the human *AMN* gene translated into selective malabsorption of vitamin B12, a phenotype associated with hereditary MGA1. The essential AMN–cubilin interaction was confirmed *in vitro*, thereby explaining the molecular basis of intestinal cobalamin malabsorption syndrome.

In adults, cobalamin deficiency is classically caused by pernicious anemia or Biermer's disease or Addison's disease. It is an autoimmune disease characterized by the destruction of the gastric mucosa, especially the fundus, associated with a primarily cell-mediated autoimmune process and the presence of various antibodies anti-IF and gastric parietal anticell antibodies that target the H+/K+-ATPase α and ß subunits.

Pernicious anemia is the leading cause of cobalamin deficiency and accounted for 40–50% of cases in adults. In the general population, the prevalence of pernicious anemia is 0.1%; in subjects over the age of 60, it reaches 1.9%. The principal characteristics of pernicious anemia have been reported in detail in several reviews and are listed in Table 4. In practice, the diagnosis of pernicious anemia is based on the presence of IF antibodies in serum (specificity, >98%, and sensitivity, around 50%) or biopsy-proved autoimmune atrophic gastritis. The presence of *H. pylori* infection in gastric biopsies is an exclusion factor.

Genetic susceptibility to pernicious anemia appears to be genetically determined, although the mode of inheritance remains unknown. Evidence for the role of genetic factors includes familial co-occurrence of pernicious anemia and its association with other autoimmune diseases. Thus, a certain number of autoimmune diseases occur at a higher frequency in patients with pernicious anemia – around 30% – or among family members of pernicious anemia patients. They can precede the disease or occur after its onset. The association with autoimmune diseases such as type 1 diabetes (insulin-dependent), autoimmune thyroiditis (particularly Hashimoto's thyroiditis), and vitiligo is common. Other associations have also been frequently described, for example, Sjögren's syndrome, celiac disease, and Addison's disease (adrenal insufficiency). Cases of multiple autoimmune syndrome including pernicious anemia have also been documented.

Table 4 Main characteristics of pernicious anemia

Characteristics of pernicious anemia
● Low serum vitamin B12 (cobalamin) levels
● Hematologic abnormalities or neurological manifestations
● Abnormal results of Schilling test using free cyanocobalamin labeled with cobalt-58
● Presence of anti-intrinsic factor antibodies
● Autoimmune gastritis (*Helicobacter pylori*-negative)
● Associated autoimmune diseases (Sjögren's syndrome, Hashimoto's thyroiditis, type 1 diabetes mellitus, etc.)

Since the 1980s, the malabsorption of cobalamin has become rare, owing mainly to the decreasing frequency of gastrectomy and terminal small intestine surgical resection. Several disorders commonly seen in practice might, however, be associated with cobalamin malabsorption. These disorders include exocrine pancreas' function deficiency following chronic pancreatitis (usually alcoholic), lymphomas or tuberculosis (of the intestine), celiac disease, Crohn's disease, Whipple's disease, and uncommon celiac disease.

Vitamin B12 in Blood and Tissues and Related Disorders

After cobalamin is absorbed at the BBM–blood barrier, it dissociates from the IF and reaches the systemic circulation where it associates with transcobalamin II (TCII) (Table 2).

The kidney represents an essential organ where the body's vitamin B12 stores are maintained, and studies demonstrated that the kidney regulates plasma vitamin B12 levels by maintaining a pool of unbound cobalamin that can be released in the case of vitamin B12 deficiency.

The tissular cobalamin–TCII complex uptake is achieved through megalin (LRP2) and transcobalamin II receptor (TCII-R)-mediated endocytosis, which plays a crucial role in cobalamin homeostasis. It is worth mentioning that TCII is responsible for the cellular uptake of vitamin B12 in most tissues and that TC deficiency is associated with severe MGA. Following cobalamin–TCII cellular uptake, TCII undergoes lysosomal digestion, which allows cobalamin separation from TCII and its cytoplasmic transfer.

It has been estimated that there is a delay of ranging from 5 to 10 years between the onset of cobalamin deficiency and the appearance of clinical manifestations, due to important hepatic stores (>1.5 mg) and the enterohepatic cycle. The average vitamin B12 content is ~1.0 mg in healthy adults, with 20–30 µg found in the kidneys, heart, spleen, and brain. Estimates of total vitamin B12 body content for adults range from 0.6 to 3.9 mg with mean values of 2–3 mg. The normal range of vitamin B12 plasma concentrations is 150–750 pg ml^{-1}, with peak levels achieved 8–12 h after ingestion.

Part of the unbound cobalamin serves as a cofactor for methionine synthase-mediated homocysteine catabolism into methionine and methylenetetrahydrofolate reductase (MTHFR)-mediated formation of the vitamin B9 (folate) biologically active form, tetrahydrofolate, which is then involved in the synthesis of purines and pyrimidines. The other part of free vitamin B12 is transferred to the mitochondria where it is transformed into adenosyl-B12, an important cofactor in the methylmalonyl-coenzyme A mutase-mediated formation of succinyl-CoA from methylmalonyl-CoA, the product of odd-chain fatty acid and some amino acid catabolisms.

Biochemically, cobalamin deficiency will cause homocysteine accumulation, increased methylmalonyl-CoA levels, and decreased MTHFR activity. These changes translate into several abnormalities including folate deficiency and subsequent inhibition of purine and pyrimidine formation essential for RNA and DNA syntheses.

The clinical manifestations of these metabolic abnormalities are MGA, neurological defects, malformations of neurological

structures, increased cardiovascular thrombotic risk and renal disease, and methylmalonic acidemia.

Functional cobalamin deficiency can also be caused by defects in the intracellular processing of cobalamin such as abnormal lysosomal digestion of the TCII–cobalamin complex, and subsequent defective lysosomal release of cobalamin, and abnormalities in intracytoplasmic cobalamin metabolism with all the consequences on biochemical reactions in which cobalamin acts as an important cofactor.

Clinical Manifestations of Vitamin B12 Deficiency

Clinical manifestations related to vitamin B12 deficiency are highly polymorphic and of varying severity, ranging from milder conditions, such as fatigue, common sensory neuropathy, atrophic glossitis (Hunter's glossitis), and isolated macrocytosis or neutrophil hypersegmentation, to severe disorders, including combined sclerosis of the spinal cord, hemolytic anemia, and even pancytopenia.

These later years, the use of strict criteria to define vitamin B12 deficiency (see Table 1) has resulted in more recognition of previously unknown or atypical clinical presentations of cobalamin deficiency. The established manifestations of cobalamin deficiency are described in Table 5.

Vitamin B12 Deficiency Therapy

The treatment of established vitamin B12 deficiency is based upon the administration of intramuscular cyanocobalamin. Its efficacy on the resolution of clinical signs and symptoms has been proved.

The classic treatment for vitamin B12 deficiency is based on parenteral administration – in most countries as intramuscular injections – in the form of cyanocobalamin and, more rarely, hydroxo- or methylcobalamin. Hydroxocobalamin may have several advantages due to a better tissue retention and storage. However, the management concerning both the dose and schedule of administration varies considerably between countries.

In the United States and United Kingdom, doses ranging from 100 to 1000 μg per month (or every 2–3 months when hydroxocobalamin is given) are used for the duration of the patient's life. In France, treatment involves the administration of 1000 μg of cyanocobalamin per day for 1 week, followed by 1000 μg per week for 1 month, followed by 1000 μg per month, again, normally for the remainder of the patient's lifetime.

Likewise, oral cyanocobalamin given at treatment doses has been shown to exhibit a similar efficacy to intramuscular cyanocobalamin, resulting in significantly increased levels of serum vitamin B12 and improvements in hematologic parameters. In fact, about 1–5% of free cobalamin (or crystalline cobalamin) is absorbed along the entire intestine by passive diffusion. A systematic review carried out under the auspices of the *Cochrane Metabolic and Endocrine Disorders Review Group* supports the efficacy of oral cobalamin therapy, with a daily dose of 2000 mg initially and then 1000 mg weekly of vitamin B12. However, the efficacy of oral cobalamin in the treatment of severe neurological diseases has not yet been sufficiently documented. Therefore, in these patients, cobalamin must still be administered via the parenteral route.

Our working group has developed an effective oral treatment for FCM and pernicious anemia using *crystalline* cobalamin (cyanocobalamin). Our principal studies of oral cobalamin treatment (open, not randomized studies) are described in Table 6. These other routes of administration have been proposed as a way of avoiding the discomfort, inconvenience, and cost of monthly injections.

Since the 1990s, at least half of these patients were treated with oral cyanocobalamin, with a dose between 125 and 2000 μg day^{-1}. All of the patients who were treated orally corrected their vitamin B12 levels, and at least 80% corrected their hematologic abnormalities. Moreover, half of the patients experienced a clinical improvement on oral treatment. Table 7 shows our '*façon de faire*' (take home recommendations). The following can be proposed: ongoing supplementation is needed until any associated disorders are corrected (e.g., by

Table 5 Main clinical features of vitamin B12 deficiency

Hematologic manifestations	*Neuropsychiatric manifestations*	*Digestive manifestations*	*Other manifestations*
• Frequent: macrocytosis, neutrophil hypersegmentation, regenerative macrocytic anemia, medullary megaloblastosis ('blue spinal cord') • Rare: isolated thrombocytopenia and neutropenia, pancytopenia • Very rare: hemolytic anemia, thrombotic microangiopathy (presence of schistocytes)	• Frequent: polyneuritis (especially sensitive), ataxia, Babinski's phenomenon • Classic: combined sclerosis of the spinal cord • Rare: cerebellar syndromes affecting the cranial nerves including optic neuritis, optic atrophy, urinary and/or fecal incontinence • Under study: changes in the higher functions, dementia, stroke and atherosclerosis (hyperhomocysteinemia), parkinsonian syndromes, depression, multiple sclerosis, autism	• Classic: Hunter's glossitis, jaundice, LDH and bilirubin elevation ('intramedullary destruction') • Debatable: abdominal pain, dyspepsia, nausea, vomiting, diarrhea, disturbances in intestinal functioning • Rare: resistant and recurring mucocutaneous ulcers	• Frequent: Tiredness, loss of appetite • Under study: atrophy of the vaginal mucosa and chronic vaginal and urinary infections (especially mycosis), hypofertility and repeated miscarriages, venous thromboembolic disease, angina (hyperhomocysteinemia)

Table 6 Experience of oral vitamin B12 therapy in the University Hospital of Strasbourg, Strasbourg, France

Study characteristics (number of patients)	*Therapeutic modalities*	*Results*
Open prospective study of well-documented vitamin B12 deficiency related to food-cobalamin malabsorption ($n=10$)	Oral crystalline cyanocobalamin: 650 μg day^{-1}, during at least 3 months	• Normalization of serum vitamin B12 levels in 80% of the patients • Significant increase in hemoglobin (Hb) levels (mean of 1.9 g dl^{-1}) and decrease in mean erythrocyte cell volume (ECV) (mean of 7.8 fl) • Improvement of clinical abnormalities in 20% of the patients • No adverse effect
Open prospective study of low vitamin B12 levels not related to pernicious anemia ($n=20$)	Oral crystalline cyanocobalamin: 1000 μg day^{-1}, for at least 1 week	• Normalization of serum vitamin B12 levels in 85% of the patients • No adverse effect
Open prospective study of well-documented vitamin B12 deficiency related to food-cobalamin malabsorption ($n=30$)	Oral crystalline cyanocobalamin: between 1000 and 250 μg day^{-1}, during 1 month	• Normalization of serum vitamin B12 levels in 87% of the patients • Significant increase in Hb levels (mean of 0.6 g dl^{-1}) and decrease in ECV (mean of 3 fl); normalization of Hb levels and ECV in 54% and 100% of the patients, respectively • Dose effect – effectiveness dose of vitamin B12 $\geq$ 500 μg day^{-1} • No adverse effect
Open prospective study of low vitamin B12 levels not related to pernicious anemia ($n=30$)	Oral crystalline cyanocobalamin: between 1000 and 125 μg day^{-1} during at least 1 week	• Normalization of serum vitamin B12 levels in all patients with at least a dose of vitamin $\geq$250 μg day^{-1} • Dose effect – effectiveness dose of vitamin B12 $\geq$ 500 μg day^{-1} • No adverse effect
Open prospective study of low vitamin B12 levels related to pernicious anemia ($n=10$)	Oral crystalline cyanocobalamin: 1000 μg day^{-1}, during at least 3 months	• Significant increase in serum vitamin B12 levels in 90% of the patients (mean of 117.4 pg ml^{-1}) • Significant increase in Hb levels (mean of 2.45 g dl^{-1}) and decrease in ECV (mean of 10.4 fl) • Improvement of clinical abnormalities in 30% of the patients

Table 7 Expert opinion recommendations for oral vitamin B12 treatment

	Pernicious anemia	*Intake deficiency and food-cobalamin malabsorption*
Parenteral administration (intramuscular)	Cyanocobalamin: • 1000 μg day^{-1} for 1 week • 1000 μg per week for 1 month • 1000 μg per each month, for life (1000–2000 μg day^{-1} for at least 1–3 months in the case of severe neurological manifestations)	Cyanocobalamin: • 1000 μg day^{-1} for 1 week • 1000 μg per week for 1 month • 1000 μg per 1 or 3 months, until the cobalamin deficiency cause is corrected (1000 μg day^{-1} for at least 1–3 months in the case of severe neurological manifestations)
Oral administration	Cyanocobalamin: • 1000 μg day^{-1} for life[a]	Cyanocobalamin: • 1000 μg day^{-1} for 1 month • 125–1000 μg day^{-1}, until the cobalamin deficiency cause is corrected[a]

[a]The effect of oral cobalamin treatment in patients presenting with severe neurological manifestations has not yet been adequately documented.

halting the ingestion of the offending medication or chronic alcoholism or by treating *H. pylori* infection or pancreatic exocrine failure). This may result in lifelong administration or, when applicable, sequential administration.

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See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Bioavailability of Nutrients; Malnutrition: Prevention and Management; Vegetarian Diets; Vitamins: Overview.

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Cobalt: Properties and Determination

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Cobalt Properties

Cobalt is the 27th element of the periodic table (atomic number 27). It was discovered by Swedish chemist Georg Brandt about 1730. Its symbol is Co, and it is included in group 9 (or VIIB) in the periodic table, thus corresponding to the transition metals. It is a brittle, hard, silver-gray metal with magnetic properties similar to those of iron (ferromagnetic). It is alloyed with aluminum and nickel to make particularly powerful magnets. The abundance of cobalt in the Earth's crust is 0.003%. It has a fusion point of 1493 °C, a boiling point of 3100 °C, an atomic weight of 58.93320, and a relative density of 8.86 g cm^{-3}.

Cobalt has an electronic structure of [Ar] $4s^2 3d^7$, and its most common oxidation states are +2 and +3. Nevertheless, some compounds with oxidation states ranging from −3 to +4 are also known. At temperatures below 417 °C, cobalt exhibits a hexagonal close-packed structure (ε-cobalt). Between 417 °C and its melting point of 1493 °C, cobalt has a face-centered cubic structure (α-cobalt).

Cobalt occurs in nature in a fairly widespread but dispersed form, being detectable in trace quantities in many rocks, soils, and manganese-rich marine nodules. The currently exploited sources of cobalt are mainly those where it originates as a by-product of more valuable metals, particularly copper, nickel, zinc, lead, and platinum. It is distributed in minerals such as erythrite or red cobalt [$Co_3(AsO_4)_2 \cdot 8H_2O$], cobaltite (CoAsS), skutterudite [(Co,Ni)As_3], carrollite [$CuCo_2S_4$], linnaeite [Co_3S_4 (+Ni,Cu,Fe)], cattierite [CoS_2], nickel cattierite [(Co, Ni)S_2], heterogenite [$2Co_2O_3CuO \cdot 6H_2O$], or asbolane (mixed manganese–iron oxide with cobalt). In seawater, the metal is present primarily as the cobalt ion and its chloro, sulfate, and carbonate complexes. In shallow waters, up to 98% of the metal can be found in the sediments and in suspended particulate matter. There are several cobalt isotopes with different half-lives among which are ^{56}Co (77.3 days), ^{57}Co (271.8 days), ^{58}Co (70.9 days), ^{59}Co (stable), ^{60}Co (5.3 years), and ^{61}Co (1.7 h).

The main use of cobalt compounds remained as a coloring agent (a rich blue color) to glass, enamels, and porcelain, right up to the twentieth century. It is also employed to make heat-resistant superalloys. Some alloys of cobalt are used in jet turbines and gas turbine generators, where high-temperature strength is important. Cobalt compounds are also important for nonmetallurgical applications, such as catalysts for the petroleum and chemical industries, a drying agent, or in lithium-ion battery electrodes. Cobalt salts of the higher carboxylic acids (cobalt soaps) are used to accelerate drying in oil-based paints, varnishes, and inks. As a ferromagnetic material, it forms permanent magnets and it can be used as an electromagnet. It has also been used in human medicine in the treatment of certain iron-resistant anemia. Finally, radioactive ^{60}Co is used to treat cancer as a medical device for the precise treatment of brain tumors and deformities of blood vessels. Cobalt–chromium alloys are also used as biomaterials because of its high degree of biocompatibility and the low volume of attrition produced at the articulating surfaces.

Cobalt is essential to hematopoiesis by virtue of the fact that it is the metal confined by the corrin ring of vitamin B_{12}. The ring consists of four pyrrole subunits, linked together to form a macrocyclic ring. It is thus like a porphyrin, but with one of the bridging methylene groups removed. The nitrogen of each pyrrole is coordinated to the central cobalt atom. In ruminants, bacteria present in their digestive tracts are able to convert cobalt ions to vitamin B_{12}. It is also associated with glycylglycine dipeptidase enzyme. In the presence of hydrogen peroxide, cobalt ions are able to produce hydroxyl radicals through a Fenton-like mechanism. Good food sources of cobalt include meat muscles, fish, nuts, oats, and green leafy vegetables such as broccoli and spinach. The bioavailability *in vivo* of cobalt ions is relatively limited, because these cations precipitate in the presence of physiological concentrations of phosphates and nonspecifically bind to proteins such as albumin. Dietary factors that seem to have the greatest effect on the ruminal production of vitamin B_{12} are cobalt concentration, roughage content of the diet, and total feed intake.

Cobalt Analysis

Sample Treatment

Most of the methods used for cobalt determination require the availability of a treated sample solution. Previously, the sample must be subjected to a treatment process where the organic matter and other matrix components are destroyed and metal ions for further analysis are released. During this treatment, the risk of sample contamination or analyte losses is high.

A common practice is the incineration of the sample in a muffle furnace using a time–temperature-controlled program. This process is defined as dry mineralization. Process efficiency and analyte retention mainly depend on the food matrix and the time–temperature program applied. The ash residue is usually dissolved with acids (HCl, HNO_3, etc.) after a blanching step.

An alternative to the earlier technique is wet mineralization. This method is based on the oxidizing action of strong acids either alone or in acid mixtures that sometimes include hydrogen peroxide. These reagents have the capacity to release cobalt ions and other trace elements keeping them in solution.

Dry and wet mineralization procedures are often tedious and time-consuming. Another drawback of the earlier mineralization methods lies on the high risk of volatilization of certain mineral elements such as selenium, mercury, or arsenic, thus causing analyte losses. For cobalt determination, this problem is not initially present unless the final objective of the applied technique is oriented to determine these volatile elements simultaneously.

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The microwave digestion procedure could be a faster and more efficient alternative. In this technique, samples are introduced in microwave ovens using pressure vessels. It also allows the use of a smaller volume of reagent. The most significant disadvantage is the risk of explosion due to increase in pressure caused by the destruction of organic matter. The pressure vessels are placed on a turntable to avoid problems arising from lack of homogeneity in the distribution of radiation within the microwave oven.

Despite the discrepancies earlier discussed between these treatment processes, usually, the comparison of dry and wet mineralization and microwave digestion methods did not show statistically significant differences in relation to accuracy and recovery percentage of mineral elements determined.

Preconcentration Methods

In some cases, the cobalt concentration present in food and biological samples could be below the detection limit of the analytic technique used. Another possibility is a strong interfering effect with the food matrix, which impairs the measurement procedure. In these cases, preconcentration and/or separation methods prior to cobalt analysis are needed to improve sensitivity and selectivity. The detection limit of the analyte can be substantially improved if the final volume is reduced. Several procedures, such as solid-phase extraction (SPE), liquid–liquid extraction (LLE), cloud point extraction (CPE), coprecipitation, and membrane filtration, have been proposed and applied for the enrichment and separation of cobalt from a sample matrix. Table 1 summarizes some published papers on cobalt preconcentration techniques in foods.

SPE is an advantageous separation and preconcentration technique for trace metal ions given its simplicity, flexibility, and high enrichment factor. The general procedure is to use a solid phase that separates the analyte of interest from interfering substances of the food matrix. With this purpose, the original sample, previously dissolved or suspended in a liquid phase, is poured on the solid phase on which the analytes are retained. Subsequently, another liquid phase is necessary to elute the retained analytes. Several solid-phase materials have been employed for the preconcentration of cobalt ions. These include chemically modified silica gel with aminothioamidoanthraquinone, cellulose functionalized with 8-hydroxyquinoline, silica gel–polyethylene glycol, β-cyclodextrin cross-linked polymer, Aliquat 336 chloride immobilized in poly(vinyl chloride), glycerol-bonded silica gel, and activated carbon modified by dithiooxamide. Carbon nanotubes have been currently proposed as a novel solid-phase extractant for cobalt determination at trace levels. The hexagonal arrangements of the carbon atoms in graphite sheets, attached to their high surface area, make them a promising solid sorbent for preconcentration procedures. Process parameters such as pH, sample flow rate, and sample volume can influence on the adsorption efficiency as previously studied. Thus, it is shown that the acidity of the solution affects the adsorption process because protons in acid solution can protonate the binding site of the chelating molecules, and hydroxides in basic solution may form complexes causing metal

Table 1 Preconcentration and separation techniques for cobalt analysis

Investigation	*Reference*
Separation of cobalt(II) from nickel(II) by solid-phase extraction (SPE) into Aliquat 336 chloride immobilized in poly(vinyl chloride)	Blitz-Raith, A. H., Paimin, R., Cattrall, R. W. and Kolev, S. D. (2007). *Talanta* **71**, 419–423
Simultaneous coprecipitation of lead, cobalt, copper, cadmium, iron, and nickel in food samples with zirconium(IV) hydroxide prior to their flame atomic absorption spectrometric determination	Citak, D., Tuzen, M. and Soylak, M. (2009). *Food and Chemical Toxicology* **47**, 2302–2307
Acid extraction and cloud point preconcentration as sample preparation strategies for cobalt determination in biological materials by thermospray flame furnace atomic absorption spectrometry	Donati, G. L., Nascentes, C. C., Nogueira, A. R., Arruda, M. A. and Nóbrega, J. S. (2006). *Microchemical Journal* **82**, 189–195
Cloud point extraction for cobalt preconcentration with on-line phase separation in a knotted reactor followed by ETAAS determination in drinking waters	Gil, R. A., Gásquez, J. A., Olsina, R., Martínez, L. D. and Cerutti, S. (2008). *Talanta* **76**, 669–673
Solid phase extraction of Co ions using L-tyrosine immobilized on multiwall carbon nanotubes	Pacheco, P. H., Smichowski, P., Polla, G. and Martínez, L. D. (2009). *Talanta* **79**, 249–253
Miniaturized preconcentration methods based on liquid–liquid extraction and their application in inorganic ultratrace analysis and speciation: A review	Pena-Pereira, F., Lavilla, I. and Bendicho, C. (2009). *Spectrochimica Acta Part B* **64**, 1–15
Silica gel–polyethylene glycol as a new adsorbent for solid phase extraction of cobalt and nickel and determination by flame atomic absorption spectrometry	Pourreza, N., Zolgharnein, J., Kiasat, A. R. and Daystar, T. (2010). *Talanta* **81**, 773–777
Solid phase extraction preconcentration of cobalt and nickel with 5,7-dichloroquinone-8-ol embedded styrene–ethylene glycol dimethacrylate polymer particles and determination by flame atomic absorption spectrometry (FAAS)	Praveen, R. S., Daniel, S. and Prasada Rao, T. (2005). *Talanta* **66**, 513–520
Simultaneous preconcentration of cobalt, nickel and copper in water samples by cloud point extraction method and their determination by flame atomic absorption spectrometry	Xu, H., Zhang, W., Zhang, X., Wang, J. and Wang, J. (2013). *Procedia Environmental Sciences* **18**, 258–263
Simultaneous determination of nickel, cobalt and mercury ions in water samples by solid phase extraction using multiwalled carbon nanotubes as adsorbent after chelating with sodium diethyldithiocarbamate prior to high performance liquid chromatography	Zhou, Q. and Kuifu Zhao, A. X. (2014). *Journal of Chromatography A* **1360**, 76–81

precipitation. On the other hand, a lower flow rate of sample produces a longer contact time with the solid phase, thus increasing the percentage recovery. Finally, low sample volumes result in a higher percentage recovery because the contact time between the metal ions and the active sites of the solid phase increases.

LLE is a preconcentration method in which the metal is distributed between two immiscible liquid phases (usually an aqueous phase and organic phase). Cobalt preconcentration is achieved by adding a chelating agent to the aqueous phase, followed by an extraction of the cobalt complexed into the organic phase. For the analytic measurement of the extracted cobalt ion, a back-extraction step to an aqueous medium, usually acid, is recommended. Nevertheless, LLE has several drawbacks, such as emulsion formation and the use of large and contaminated solvent volumes, which make LLE expensive and environmentally unfriendly. Searching for alternatives to the conventional LLE using negligible volumes of extractant and a minimum number of steps has led to the development of liquid-phase microextraction techniques for cobalt preconcentration.

CPE is related to LLE but has emerged as an alternative due to several reasons: (1) its excellent concentration factors, (2) the smaller required sample size, (3) the use of a small volume of organic solvent that is generally toxic, (4) the use of nontoxic surfactants, (5) the reduction of laboratory residues, and (6) being recognized as a safer, simpler, and cost-effective procedure. CPE is based on the following phenomenon: in much diluted solutions of nonionic surfactant, the monomers are dispersed in the solvent. But above the critical micellar concentration of the surfactant, these monomers associate spontaneously, forming aggregates of colloidal dimensions termed micelles, due to the diminished solubility of the surfactant in water. The surfactant solution becomes turbid because it attains the cloud point. This clouding phenomenon can be induced by changing the temperature, surfactant concentration, or the choice of a compatible pH buffer, resulting in the separation of a single isotropic micellar phase into two phases, a surfactant-rich phase of small volume and an aqueous phase. Choosing an appropriate chelating agent that is able to form with the metal ion a hydrophobic complex, which is entrapped by micellar structures, extraction and subsequent preconcentration of cobalt in the surfactant-rich phase can be achieved. Among the nonionic surfactants commonly used, Triton X-114 (octylphenoxypolyethoxyethanol) is the most feasible for preconcentration cloud point extraction. Regarding chelating agents, 2-(5-bromo-2-pyridylazo)-5-(diethylamino)-phenol (5-Br-PADAP), 1-(2-pyridylazo)-2-naphthol (PAN), ammonium pyrrolidinedithiocarbamate (APDC), and 1-(2-thiazolylazo)-2-naphthol (TAN) have been used for the determination of cobalt.

The separation of dissolved substances by precipitation may occur in two ways. Direct precipitation is produced when a reagent creates an insoluble and very selective precipitate. Alternatively, indirect precipitation is formed when a precipitate of a different compound is produced and the desired analyte is retained by coprecipitation on the surface of the formed precipitate. Further, centrifuging, filtering, or washing separates this precipitate. The coprecipitation technique stands out by its ease and the simultaneous preconcentration and separation of the analytes from the food matrix. Hydroxides of various metal ions as inorganic coprecipitants have been used for the preconcentration of cobalt in food and environmental samples such as magnesium(II), thulium(III), zirconium(IV), tin(IV), and scandium(IV) hydroxide. Other organic coprecipitants such as magnesium-8-quinolinate, iron(III) hexamethylenedithiocarbamate, and bismuth(III) diethyldithiocarbamate have been also employed in cobalt coprecipitation studies, giving very high concentration factors.

Finally, another preconcentration technique is membrane filtration in which cobalt ions are collected in the form of metal chelates over a membrane filter from an aqueous solution by filtration. The filter material has a strong affinity for hydrophobic species in water, which allow retention of these species during filtering. Among the materials used as a filter to cobalt ions are cellulose nitrate or cellulose acetate. APDC, poly(ethyleneimine) or carmine has been used as complexing agents.

Atomic Absorption Spectroscopy

In atomic absorption spectroscopy (AAS), electromagnetic radiation at specific wavelength is passed through a cell containing gaseous free atoms. Atoms are produced in two different ways using AAS: flame atomic absorption spectrometry (FAAS) and electrothermal atomic absorption spectrometry (ETAAS) (graphite furnace and a L'vov platform). Thus, the determination of cobalt using an atomic absorption spectrophotometer requires a light source (wavelength $\lambda = 240.7$ nm) and an atomization source. Standard working conditions for analyzing cobalt by AAS are listed in Table 2.

FAAS uses an air–acetylene or nitrous oxide–acetylene as the most common oxidant–fuel mixtures to produce atomization. Preconcentration methods described in the preceding text permit the use of FAAS for cobalt determination in foodstuffs with detection limits of 0.30–2.4 ng ml^{-1}. However, when conventional FAAS proves impractical because cobalt values are below or near the detection limit of the instrument, it is recommended to use ETAAS in which the atomization is produced by the passage of electrical current through a cylindrical graphite tube (called graphite furnace). This process comprises several steps: preheating, drying, pyrolysis, atomization, and cleaning, which are achieved by a temperature–time interval program (ramp heating). During the drying stage, liquid from the sample is evaporated and driven off. In the pyrolysis stage, some of the concomitants in the sample are removed. During this process, the system is flushed with protective gas (usually argon) to exclude air from the sample compartment and to minimize oxidation of the atomizer material at high

Table 2 Standard conditions for cobalt analysis by AAS

Spectrometer settings	*Cobalt*
Wavelength (nm)	240.7
Lamp current (mA)	5–7
Spectral resolution (nm)	0.1–0.2
Nebulizer	Spoiler
Oxidant	Air
Fuel	C_2H_2
Flame condition	Oxidizing
Deuterium lamp background correction	

Table 3 Experimental conditions proposed for some authors for cobalt determination by ETAAS

Step	*Ramp(s)*	*Hold(s)*	*Furnace temperature (°C)*	*Internal gas flow (l min^{-1})*
Drying 1	1(10)	5	110(200)	1.0
Drying 2	5	15	130(400)	1.0
Pyrolysis	10	30	700(800)	1.0
Atomization	0	5	2200(2400)	0
Cleaning	1	3	2450(2700)	1.0

temperatures. Finally, the temperature is rapidly increased to quickly vaporize and atomize the sample. Table 3 shows operation conditions used for cobalt determination by ETAAS.

ETAAS combined with preconcentration methods has been used for cobalt determination. It can reach detection limits of 1.2–50 ng l^{-1}. In order to reduce matrix effects when ETAAS is used, various matrix modifiers are added.

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

The earlier analytic techniques are sensitive enough to determine cobalt and other trace elements. However, as monoelemental techniques, they could become too slow and tedious when trying to analyze a wide range of trace elements. ICP-OES is a multielemental technique able to determine concentrations of multiple elements in a sample with a single aspiration obtaining a high degree of variability and minimum interferences. The technique is based upon the spontaneous emission of light, with a characteristic wavelength, from atoms and ions previously excited in plasma. The particular wavelength leaving the monochromator is converted to an electrical signal by a photodetector.

Plasma may be defined as a luminous volume of partially ionized gas. It is generated from radiofrequency magnetic fields induced by a copper coil wound around the top of a glass torch. The sample solution is converted to an aerosol and directed into the central channel of the plasma. The plasma maintains a temperature of approximately 10 000 °C in its core where cobalt and other elements are released as free atoms and excited. Therefore, argon plasma is needed to produce atomization and excitation. The nearly complete atomization of the sample minimizes chemical interferences.

For cobalt determined by ICP-OES, a solution detection limit of 5 μg l^{-1} and a sample detection limit of 2.5 μg g^{-1} can be achieved. Selectivity is important to minimize the spectral overlap interferences resulting from rich line emission spectra of cobalt and other elements such as tungsten, niobium, and molybdenum. ICP-OES has been used for cobalt determination in dried fruit, coffee, cocoa, and infant formula. The emission spectral lines used for cobalt analysis are 228.616, 230.786, and 238.346 nm.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS): Cobalt Speciation

ICP can also be coupled with a mass spectrometer leading to inductively coupled plasma mass spectrometry (ICP-MS). The inductively coupled argon plasma is once again used as an excitation source for the elements of interest. However, in contrast to ICP-OES, the plasma in ICP-MS is used to generate ions that are introduced in the mass analyzer through a sampler cone, followed by a skimmer cone. Mass spectrometers are instruments that separate ions according to their mass-to-charge ratio (m/z) and then accurately quantify the resulting ions once they pass through a detector.

There are five types of mass analyzers: quadrupoles, ion traps, time-of-flight, magnetic sectors, and Fourier transform-based ion cyclotrons. Quadrupole mass spectrometry has become increasingly popular in recent years, being currently the most used. Sample introduction can be static, through direct insertion probes, or dynamic. The interconnection with chromatographic equipment is implied in the latter. In fact, as it will be discussed later, the use of high-performance liquid chromatography with the mass spectrometry has recently become a routine technique due to advances achieved in the interconnecting interfaces.

ICP-MS is the method of choice for cobalt and other ultratrace element determinations due to its low detection limit, multielement capabilities, fast routine, and wide linear calibration range. For cobalt, it has been widely applied in clinical analysis, reaching quantification limits as low as 0.20 ng ml^{-1} in oral mucosa cells, 0.01 ng ml^{-1} in human serum, and 0.03 ng ml^{-1} in whole blood.

Regarding interferences, spectroscopic interferences are probably the largest ones in ICP-MS and are caused by atomic or molecular ions that have the same m/z ratio as the analytes of interest. In many cases, it can be avoided by using a different isotope of the element of interest or by measuring another isotope of the interfering element and subtracting from the mass of interest on the basis of the known isotope ratio of the interfering element. Current ICP-MS instrumental software makes a correction for all known atomic 'isobaric interferences'; however, they do not provide corrections for most of the polyatomic interferences. Such interferences are caused by polyatomic ions that are formed from precursors having numerous sources, such as the sample matrix, reagents used for preparation, plasma gases, and entrained atmospheric gases. Table 4 shows the main polyatomic interferences for cobalt determination.

Currently, it is necessary to know not only the precise quantity of trace element that is present in a food or biological sample but also in what form it is present (speciation). Chemical speciation comprises the identification and quantification of different trace elements and the biomolecules to which they are associated, their oxidation states, and the coordination

groups involved. In the last years, a novel scientific discipline was arisen, known as metallomics or chemical speciation. To this sense, cobalt can be present in the environment in different forms. The main compounds of toxicological interest are the metallic form (cobalt metal), the oxides (cobalt oxide and tetroxide), and the salts (cobalt chloride, sulfide, and sulfate). On the other hand, there are other forms of nutritional interest such as a wide variety of cobalamins including cyanocobalamin (CNCbl), hydroxocobalamin (OHCbl), methylcobalamin (MeCbl), and 5′-deoxyadenosylcobalamin (AdoCbl).

To carry out these studies, several methods of high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) coupled with ICP-MS have been reported for cobalt speciation analysis. The separation of cobalt species is based on their retention times. The advantages of using CE as a separation method, in comparison with other chromatographic techniques, are its low cost, applicability to small sample volumes, and high resolving power and low reagent consumption. It has been applied on pharmaceutical preparations of vitamin B_{12} achieving detection limits for the cobalamins ranging from 30 to 70 ng ml^{-1}. Another study using EC-ICP-MS on nutritive supplements and chlorella foods achieved lower detection limits of 0.3, 0.2, and 1.7 ng ml^{-1} for CNCbl, OHCbl, and Co(II) ion, respectively.

In relation to HPLC-IPC-MS, the physical separation capabilities of liquid chromatography with the analytic skill of mass spectrometry are combined yielding high sensitivity and selectivity. It has been used for the analysis of nutritive supplements with detection limits of 0.008, 0.013, and 0.014 ng ml^{-1} for Co(II), OHCbl, and CNCbl, respectively. The following table (Table 5) shows some published studies on cobalt speciation using these hyphenated techniques and on cobalt analysis by ICP-MS.

Table 4 Polyatomic interferences for the determination of cobalt in ICP-MS

Isotope	*Abundance*	*Interference*
^{59}Co	100	$^{43}Ca^{16}O^{+}$, $^{42}Ca^{16}O^{1}H^{+}$, $^{24}Mg^{35}Cl^{+}$, $^{40}Ar^{23}Na^{+}$, $^{40}Ar^{18}O^{1}H^{+}$, $^{40}Ar^{19}F^{+}$

Ion-Selective Electrodes

Ion-selective electrodes are one of the most frequently used potentiometric sensors during laboratory analysis. The main advantage over spectroscopic methods is that most of these spectroscopic techniques are relatively expensive and involve large infrastructure backup and support of expertise. In contrast, ion sensors provide analytic procedures that overcome the earlier drawbacks since they are fast and require minimal sample treatment. Ionophore plays the main role in the sensitivity and selectivity of ion-selective electrodes. A good ionophore shows strong affinity for a particular ionic species than others.

A number of ion-selective electrodes for the determination of cobalt concentration have been reported using materials such as Schiff bases, calixarenes, isothiazoles, and mercapto compounds. However, most of these sensors suffer from disadvantages such as narrow working concentration range, non-Nernstian potential response, high response time, high detection limit, and poor reproducibility.

Recently, PVC membrane electrode (PME) and coated graphite electrode (CGE) have been prepared by using 2-((thiazol-2-ylimino)methyl)phenol as a good ionophore in order to use it as cobalt-selective electrode. The electrodes exhibit a Nernstian slope for cobalt ions with a limit detection of 6.91×10^{-7} mol l^{-1} for PME and 7.94×10^{-8} mol l^{-1} for CGE, which show a proper potentiometric response. Similarly, azines, a condensation product of hydrazine with ketones or aldehydes, can also be used as cobalt-selective electrodes. Thus, palladium(II) dichloro acetylthiophene fenchone azine in a plasticized PVC matrix reveals a good selectivity for cobalt(II) with a detection limit of 8×10^{-7} mol l^{-1}. Finally, the

Table 5 Cobalt speciation and analysis by ICP-MS

Investigation	*Reference*
Determination of cobalamins using capillary electrophoresis (CE) inductively coupled plasma mass spectrometry	Baker, S. A. and Miller-Ihli, N. J. (2009). *Spectrochimica Acta Part B* **55**, 1823–1832
Simple and robust ICP-MS method for simultaneous determination of serum Co and Cr in routine clinical practice	Choi, H. J., Lim, S. J., Park, Y. S. and Lee, S. Y. (2015). *Clinica Chimica Acta* **439**, 91–96
Determination of cobalamin in nutritive supplements and chlorella foods by capillary electrophoresis-inductively coupled plasma mass spectrometry	Chen, J. H. and Jiang, S. J. (2008). *Journal of Agricultural and Food Chemistry* **56**, 1210–1215
Development and validation of an inductively coupled plasma mass spectrometry (ICP-MS) method for the determination of cobalt, chromium, copper and nickel in oral mucosa cells	Martín-Cameán, A., Jos, A., Calleja, A., Gil, F., Iglesias-Linares, A., Solano, E. and Cameán, A. M. (2014). *Microchemical Journal* **114**, 73–79
Simultaneous analysis of 21 elements in foodstuffs by ICP-MS after closed-vessel microwave digestion: method validation	Millour, S., Noël, L., Kadar, A., Chekri, R., Vastel, C. and Guérin, T. (2011). *Journal of Food Composition and Analysis* **24**, 111–120
An ORS-ICP-MS method for monitoring trace levels of cobalt and chromium in whole blood samples from hip arthroplasty patients with metal-on-metal prostheses	Pei, K. L., Kinniburgh, D. W., Butlin, L., Faris, P., Lee, D., et al. (2012). *Clinical Biochemistry* **45**, 806–810
Cobalamin speciation using reversed-phase micro-high-performance liquid chromatography interfaced to inductively coupled plasma mass spectrometry	Yanes, E. G. and Miller-Ihli, N. (2004). *Spectrochimica Acta Part B* **59**, 891–899
Combined use of HPLC-ICP-MS and microwave-assisted extraction for the determination of cobalt compounds in nutritive supplements	Yang, F. Y., Jiang, S. J. and Sahayam, A. C. (2014). *Food Chemistry* **147**, 215–219

use of macrocyclic ligands (such as dipyridine–hexaazacyclotetradecane derivatives) with PME and CGE has also been developed with similar results (detection limits of 6.80×10^{-9} mol l^{-1}).

Colorimetric Methods

These types of techniques are less used for cobalt determination; however, they offer some advantages such as less tedious sample pretreatments and more inexpensive instrumentation. Colorimetric methods were introduced during the 1940s for analyzing cobalt in biological materials and achieved detection limits of 0.005 μg ml^{-1} in blood samples. Recently, a simple naked eye colorimetric method has been developed based on controlling the oxidation level of methylene blue. This redox dye could be tuned from blue to colorless, yellow, and green at different oxidation states. The sensing system is just a simple mixture of methylene blue, 2-aminothiophenol, and copper nitrate, forming a complex that resulted in the reduction of methylene blue from blue to colorless in 3 min. The addition of cobalt ions to this system produces a color change from colorless to brown at low cobalt concentrations and green at high concentrations within 2 min. The sensing of cobalt ions can be achieved by UV–visible spectra or by the naked eye. The authors reported a linear range from 0.1 to 1.1 mM with a correlation coefficient of 0.9982 and a detection limit of 0.04 mM.

Nondestructive Techniques

Neutron activation analysis is not a common technique for cobalt analysis. However, it can be used as a multielement technique when determining a large number of trace elements without destroying the sample. Furthermore, it is a well-established technique for multielement determination at ultra-trace levels with high sensitivities (i.e., a detection limit of 0.6 ng g^{-1} was achieved for cobalt in liquid milk samples). The technique principle is that elements can be radioactive by exposure to neutron irradiation. By monitoring the subsequent decay of these radioisotopes, it is possible to identify and accurately quantify the elements in the sample.

There are three main sources of neutrons for irradiation: nuclear reactors, radioactive neutron sources, and electron–ion accelerators. The former provide the highest neutron fluxes and permit the highest sensitivities for detection and quantification of various elements. Currently, this technique is being used to authenticate the geographic origin of certain foods with a quality mark (e.g., foodstuffs belonging to a protected designation of origin).

x-Ray fluorescence (XRF) is another unusual nondestructive technique for cobalt determination. It is based on bombarding a small area of the sample with x-ray photons, which are sufficiently energetic to cause ionization of inner electrons of the atoms. Because of this unstable atomic configuration, an electron from an outer orbital fills the vacancy of an inner electron, which emits the difference of energy between the two orbitals as a 'secondary' (or fluorescent) x-ray photon. The main difference with ICP-OES is that in XRF, emitted radiation from the ultraviolet–visible region of the spectrum is covered. It has achieved a detection limit for cobalt of 1 μg g^{-1} in fruits and of 0.3 μg g^{-1} in dairy samples.

See also: Authenticity of Food; Cobalt: Toxicology; Infrared Spectroscopy: Applications; Mass Spectrometry: Applications; Mass Spectrometry: Principles and Instrumentation; Potassium: Properties and Determination; Sodium: Properties and Determination; Spectroscopy: Types; Zinc: Properties and Determination.

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Cobalt: Toxicology

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Cobalt Physiology

Cobalt is a trace element widespread in the environment and human exposure is mainly attributed to air breathing and food intake. Skin contact with soil or water containing cobalt may also enhance exposure. This element has both beneficial and harmful effects on human health. From a nutritional point of view, cobalt is part of vitamin B_{12} in which this element is complexed with four nucleic pyrroles joined in a ring called corrin, similar to porphyrins. However, this element may be toxic when human exposure occurs at levels higher than recommended values. The respiratory system is the main target organ as a result of inhalation of high doses of cobalt by metallurgy workers, causing several diseases such as pneumonia, nodular fibrosis, asthma, and lung cancer. It may also cause thyroid damage, high blood pressure, heart effects, vomiting, and diarrhea.

The human body contains an average of 1.1 mg of cobalt, 85% of it being in the form of vitamin B_{12}. Usually, the main source of cobalt in humans is through diet. Its absorption in the digestive tract varies between 5% and 45% in different individuals. This absorption is conditioned by both dietary factors and physiological factors. Cobalt bioavailability is determined by the cobalt intake from diet as well as on its chemical form. Several studies show that cobalt species determine the kinetics of cobalt absorption both through diet and by inhalation routes. Volunteer studies have found that chloride form of cobalt had higher absorption than the oxide form. Moreover, early experiments showed that cobalt urinary excretion is inversely proportional to the dose supply. On the other hand, the digestive absorption of cobalt compounds is also influenced by other concomitants present in the food. The presence of albumin or lactose increases the absorption of the element. Thus, administration of cobalt ions with cow milk may increase the gastrointestinal absorption up to 40%. On the other hand, the bioavailability *in vivo* of cobalt ions is impaired in the presence of physiological concentration of phosphates. Finally, a low iron status promotes cobalt absorption probably by active competition for the same transport mechanism in enterocytes. In relation to physiological factors, gastrointestinal uptake of cobalt differs according to sex, as shown by a research study, where volunteers were supplied with several amounts of soluble cobalt compounds showing significantly higher urinary cobalt levels in women (median, 109.7 nmol $mmol^{-1}$ creatinine) than in men (median, 38.4 - nmol $mmol^{-1}$ creatinine).

Once absorbed, cobalt is mainly present in the organism as cobalt (II) ions and is distributed throughout the body, the liver, kidneys, pancreas, and heart being the main organs where cobalt is accumulated. Most of the cobalt present in serum is bound to albumin, and the free fraction is estimated between 5% and 12%. This latter fraction is in equilibrium with free cobalt present in the interstitial fluid. It has also been identified in serum a histidine-rich glycoprotein that binds cobalt and other elements such as copper, nickel, and zinc. There is not any standard biochemical marker of cobalt accumulation in the human body with age.

Cobalt in the form of vitamin B_{12} is an established treatment for pernicious anemia. It is proclaimed to increase erythropoiesis and physical performance, even in nonanemic subjects. The molecular mechanism by which cobalt stimulates erythropoietin production is not fully understood. It seems that cobalt stabilizes the hypoxia-inducible factors (HIFs), namely, HIF-α-prolyl hydroxylase and HIF-α-asparaginyl hydroxylase, which increase the expression of the erythropoietin gene. Eight non-corrin cobalt-containing enzymes have been identified, which are shown in **Table 1**.

Cobalt may interact with other ion metals. Thus, in human red cells, cobalt shares the same transport mechanism with calcium. The uptake is practically irreversible since cobalt bound in the cytosol is not itself extruded by the Ca pump. Additionally, cobalt *in vitro* can replace iron in the heme group of hemoglobin. Binding to hemoglobin is initially reversible; however, a cobalt fraction starts later to bind strongly probably due to oxidation *in situ* from cobalt (II) to cobalt (III). In acute cyanide poisoning, the capacity of cobalt (II) ions to form stable complexes with cyanide anion may be used to neutralize this toxicant. On the other hand, cobalt can also replace zinc in some enzymes such as alkaline phosphatase and carboxypeptidase.

The main excretion route of cobalt is the urine followed by feces. Studies of whole-body retention of inorganic cobalt in human males after intravenous injection gave an excretion rate after 8 days of 56% in urine and 11% in feces. This cobalt excretion is lower in hemodialysis patients justifying the importance of renal clearance. Urinary cobalt is used in biological monitoring of occupational exposure to cobalt compounds to discriminate occupationally exposed subjects from nonexposed ones and to distinguish subgroups of workers with different degrees of exposure.

Cobalt represents approximately a 4.3% of vitamin B_{12} weight. Considering that daily requirements of vitamin B_{12} are approximately 1 μg and that only 50% of vitamin B_{12} is absorbed by the gastrointestinal tract, the Recommended Daily Allowance (RDA) of vitamin B_{12} is 2.4 μg, which corresponds to 0.10 μg of cobalt.

Exposure Sources

Toxic effects of cobalt may occur as a result of an exposure to high concentrations or through chronic exposure for a long time period at low concentrations (mainly occupational exposure). Among the adverse effects of cobalt toxicity are skin dermatitis and carcinogenic, mutagenic, cardiovascular, and respiratory effects. Although the mechanism of cobalt toxicity

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Table 1 Non-corrin cobalt-containing enzymes and its function in the organisms

Enzyme	*Function*
Aldehyde decarbonylase	It is involved in the conversion of a fatty aldehyde to a hydrocarbon. The only case where the enzyme has been purified is from a green alga and this decarbonylase appeared to have cobalt and porphyrin
Bromoperoxidase	Bromoperoxidase from *Pseudomonas putida* catalyzes the formation of a carbon–bromine bond in the presence of peroxides. It is activated by incubation with cobalt ions but not with other transition metals such as iron, nickel, zinc, and vanadate
Glucose isomerase	It catalyzes the reversible isomerization of D-glucose to D-fructose and is of outmost commercial importance for the industrial production of corn syrup
Lysine 2,3-aminomutase	A bacterial enzyme that also contains cobalt ions that catalyzes the interconversion of L-lysine and L-beta lysine, the first step in lysine degradation
Methionine aminopeptidase	It is a ubiquitous enzyme in both prokaryotes and eukaryotes that catalyzes cotranslational removal of N-terminal methionine from elongating polypeptide chains during protein synthesis. It contains two cobalt (II) ions per active subunit that catalyze hydrolysis
Methylmalonyl-CoA carboxyltransferase	It is a multienzyme complex from prokaryote that couples two carboxylation reactions, transferring a carboxyl group from (*S*)-methylmalonyl-CoA to pyruvate to yield propionyl-CoA and oxaloacetate
Nitrile hydratase	It catalyzes the hydration of nitriles to their corresponding amides. This enzyme is also used as biocatalyst in acrylamide and nicotinamide production, as well as in environmental remediation for the removal of nitriles from waste streams
Proline dipeptidase	Also called prolidase. It is an enzyme that catalyzed the cleavage of bonds between aminoacyl and proline groups

remains unknown, it seems that cobalt ions in the presence of hydrogen peroxide are able to produce hydroxyl radicals in a manner possibly similar to Fenton reaction, causing oxidative stress and oxidative damage to lipids, proteins, and DNA. This would be favored by hyperoxic conditions with oxygen acting synergically with cobalt to promote the formation of free radicals. Cobalt also has a strong affinity for sulfhydryl groups causing displacement of divalent cations in the ion center of metal-activated enzymes and inhibition of vital enzymes such as cytochrome P450, peroxidase, and tyrosine iodinase. This effect has also been demonstrated for DNA repair enzymes (incision and polymerization steps) in which cobalt ions interact with zinc finger domains in the protein.

When the toxicity of cobalt compounds is investigated, it is important to consider various factors. Speciation or chemical form of the cobalt compound influences its solubility and bioavailability. As a result, acute toxicity of these compounds varies from each other (see Table 2). The route and the time of exposure should also be considered for evaluating the risk (single, repeated, short- and long-term exposure, oral, inhalation, etc.). The organ or tissue whose damage gives rise to the critical effect, called critical organ, must be selected. Aiming for a quantitative assessment of risks related to toxicant exposure, risk assessment is applied to environmental and occupational health.

Table 2 Median lethal dose (LD_{50}) of several cobalt compounds in rats

Compound	*LD_{50} (mg kg^{-1})*
Cobalt chloride	80
Cobalt oxide	202
Cobalt chloride hexahydrate	766
Cobalt sulfide	>5000
Cobalt metal	6171

Soil, Water, and Air

Cobalt is present in nature in a fairly widespread but dispersed form, being detectable in trace quantities in many rocks, soils, and manganese-rich marine nodules. Currently, the exploited sources of cobalt are mainly those where it exists as a by-product of more valuable metals, particularly copper, nickel, zinc, lead, and platinum. Cobalt is also present in igneous rocks that are rich in iron. The abundance of cobalt in the Earth's crust is 0.003%. In relation to sediments, cobalt concentration was determined in modern sediments in the Bristol Channel (England), a region that receives a significant input of industrial waste from coal mining, smelting, and other industries. A cobalt concentration of 22.8 mg kg^{-1} in suspended particulates, 16.0 mg kg^{-1} in silts, and 6.4 mg kg^{-1} in sands was found. Similarly, sediments from the Ems (Germany) contained 40 mg kg^{-1} of cobalt and there was a significant contribution from industrial discharges. Cobalt concentration in soils is determined by several factors such as the amount of organic matter, clay mineral content, oxidation–reduction state, pH, and translocation to root and foliage plants. Usually, cobalt concentrations in most soils ranged between 0.1 and 50 μg kg^{-1}.

In seawater, the metal is present primarily as cobalt (II) ion and its chloride, sulfate and carbonate complexes. Cobalt values around 0.1–0.7 μg kg^{-1} are reported. An increase in cobalt concentrations with depth in the oceans is related to organic productivity. The low concentration of cobalt close to the continents probably indicates removal through absorption, coprecipitation, and flocculation of solids. Thus, in shallow waters, up to 98% of the metal can be found in the sediments and in suspended particle matter. Several studies have found an average cobalt concentration in drinking water over 2 μg l^{-1}.

Cobalt concentration in the atmosphere depends on anthropogenic activity and other natural sources that include weathering and local geology, volcanic eruptions, and seawater spray. The average concentration of cobalt in ambient air is situated around 0.4 ng m^{-3}. However, levels up to 0.61 μg m^{-3}

have been detected in urban areas. The residence time of cobalt in the atmosphere depends on the particle size and meteorological factors. Rainwater solubilizes the cobalt species present in the atmosphere. Studies have detected a cobalt concentration in rainwater over 0.3 $\mu g\,l^{-1}$ in rural areas against 1.7 $\mu g\,l^{-1}$ in highly industrial areas.

Dietary Sources

The average daily intake of cobalt from food is estimated to be between 5 and 60 $\mu g\,day^{-1}$ varying between countries. Several studies have reported a mean dietary intake of cobalt of 4–29 $\mu g\,day^{-1}$ in France, 11 $\mu g\,day^{-1}$ in Canada and the United Kingdom, and 60–65 $\mu g\,day^{-1}$ in Turkey. Cobalt-rich food sources include meat muscles, liver, fish, nuts, oats, and green leafy vegetables such as broccoli and spinach. Several studies have analyzed this element using ICP-OES or ICP-MS obtaining an average concentration of 0.25–1.03 $\mu g\,g^{-1}$ in dried fruits, 0.06–0.18 $\mu g\,g^{-1}$ in crucifers, 0.02–0.06 $\mu g\,g^{-1}$ in cereals, and 0.17 $\mu g\,g^{-1}$ in fish liver. As it will be seen later, myocardial effects have been observed in heavy beer consumers. This is because in the early 1960s, some breweries added cobalt salts (chloride or sulfate) to stabilize beer foam, resulting in elevated exposures to cobalt. Some people who consumed excessive amounts of beer (8–25 pints per day) suffered from severe heart problems, causing death.

Cobalt content in human milk depends on feeding mothers and their lifestyles (dietary habits during pregnancy and after the birth, taking medications, smoking, etc.). Cobalt concentration in human milk from Central European women has been found between 0.42 and 2.46 $\mu g\,l^{-1}$. A transfer factor from the mothers' daily intake to milk was reported to be equal to 8.4.

Anthropogenic Sources

Significant exposure to cobalt occurs predominantly at work since environmental concentrations are usually low. Cobalt compounds are used as a coloring agent (to enrich blue color) for glass, enamels, and porcelains. It is also employed to make heat-resistant superalloys. Some alloys of cobalt are used in jet turbines and gas turbine generators, where high-temperature strength is important. Cobalt compounds are also important for nonmetallurgical applications, such as catalysts for petroleum and chemical industries, as a drying agent, or as part of lithium ion battery electrodes. Cobalt salts of the higher carboxylic acids (cobalt soaps) are used to accelerate drying in oil-based paints, varnishes, and inks. As a ferromagnetic material, it forms permanent magnets, thus being used as an electromagnet.

Cobalt levels present in the environment are reported to be relatively high during cobalt powder production in grinding processes, hard-metal industry, and ceramics industry. These high levels of cobalt in the environment result in an increased concentration in blood and urine of the exposed workers. Cobalt exposure has also been detected, although to a lower extent, among diamond polishers and dental technicians. Spain has established a workplace exposure limit of 0.02 $mg\,m^{-3}$ for cobalt.

^{58}Co and ^{60}Co are radioactive isotopes produced from routine operations of nuclear power plants. Although the half-life of these isotopes is relatively short ($t_{½} = 71$ days and $t_{½} = 5.27$ years), small amounts can be released into the environment as cooling water pollutants or radioactive waste. Furthermore, small amounts of cobalt could be released to the atmosphere from incinerator plants that use carbon as fuel or from the vehicle exhaust tubes.

As already mentioned, contaminated soils by highway and airport traffic or other types of industrial pollution could contain high cobalt concentrations. The mobility of cobalt present in the soil depends on the acidity of the medium. Thus, cobalt may be transferred from the plants' edible parts, mainly fruits, grains, and seeds, to the food industries. Although animals eating these plants will accumulate this metal, this fact does not seem to increase cobalt transmission and concentration along the food chain.

Finally, over the last years, the use of cobalt alloys as biomaterial in orthopedic joint replacement has created a new source of cobalt exposure. In dental usage, the term 'steel' became a synonym of cobalt–chromium alloys. On the other hand, the use of metal bearings in hip joint arthroplasty may cause a serious concern because of the wide applicability in susceptible patients.

Allergic Effects

The existence of allergenic reactions to cobalt is well known causing an erythematous and papular dermatitis. Cobalt ions can act as hapten and bind to macromolecular components to produce immunogenic products that can account for allergic reactions. Cobalt allergy occurs as a result of exposure to substances containing this element such as detergents, hair dyes, creams, and fertilizers. Also, the body adornments (jewels) may contain cobalt. A sensitivity of 7.6% has been reported by the North American Contact Dermatitis Group in individuals patch-tested from 1998 to 2000. Similarly, a study carried out in Denmark for 10 years (2003–12) has documented a positive association between cobalt allergy and dermatitis caused by nonoccupational exposure to leather goods.

Photosensibilization of cobalt in cement has been described in bricklayers, itself being manifested by severe and diffuse eczematous dermatitis in the body and limbs. A cross-sectional study developed in the Swedish hard-metal industry showed a prevalence of irritant reaction and hand eczema of 15% and 10%, respectively. Usually, skin cobalt allergy is related to other allergic reactions caused by other metals such as chromium and nickel (cross-reaction).

Dental technology students have an increased risk of developing contact dermatitis during their studies, owing to the use for training purposes of alloys that release high amounts of cobalt (0.0047–820 $\mu g\,cm^{-2}\,week^{-1}$). Dental technicians and students mostly have short and repeated contact with tools and alloys containing this element, which may result in the deposition of large amounts of metal on their skin. A severe, generalized pruritic eczematous rash in an 84-year-old woman with cobalt–chromium prosthetic hip for a fractured femur neck has also been diagnosed in Australia. The replacement of the cobalt–chromium hip prosthesis with a titanium-on-polyethylene one

Table 3 Relevant researches on allergic and carcinogenic effects and genotoxicity of cobalt exposure

Investigation	*Reference*
Association between cobalt allergy and dermatitis caused by leather articles – a questionnaire study	Bregnbak, D., Thyssen, J. P., Zachariae, C., Menné, T. and Johansen, J. D. (2014). *Contact Dermatitis*. doi:10.1111/cod.12319
Cobalt, nickel, and chromium release from dental tools and alloys	Kettelarij, J. A., Lidén, C., Axén, E. and Julander, A. (2014). *Contact Dermatitis* **70**, 3–10
Systemic allergic dermatitis caused by cobalt and cobalt toxicity from a metal-on-metal hip replacement	Wong, C. C. and Nixon, R. L. (2014). *Contact Dermatitis* **71**, 108–128
Cobalt asthma – a case series from a cobalt plant	Sauni, R., Linna, A., Oksa, P., et al. (2010). *Occupational Medicine* **60**, 301–306
Lung cancer risk in hard-metal workers	Moulin, J. J., Wild, P., Romazini, S., et al. (1998). *American Journal of Epidemiology* **148**(*3*), 241–248
Incidence of lung cancer among cobalt-exposed women	Tiichsen, F., Jensen, M. V., Villadsen, E. and Lynge, E. (1996). *Scandinavian Journal of Work, Environment and Health* **22**, 444–450
Lung cancer mortality in a site producing hard metals	Wild, P., Perdrix, A., Romazini, S., Moulin, J. and Pellet, F. (2000). *Occupational Environmental Medicine* **57**, 568–573
Exploring the potential role of tungsten carbide–cobalt (WC–Co) nanoparticle internalization in observed toxicity toward lung epithelial cells *in vitro*	Armstead, A. L., Arena, C. B. and Li, B. (2014). *Toxicology and Applied Pharmacology* **278**, 1–8
The effect of nano- and micron-sized particles of cobalt–chromium alloy on human fibroblasts *in vitro*	Papageorgiou, I., Brown, C., Schins, R., et al. (2007). *Biomaterials* **28**, 2946–2958
Absence of significant genotoxicity in lymphocytes and urine from workers exposed to moderate levels of cobalt-containing dust: a cross-sectional study	De Boeck, M., Lardau, S., Buchet, J. P., Kirsch-Volders, M. and Lison, D. (2000). *Environmental and Molecular Mutagenesis* **36**, 151–160

aided to overcome her dermatitis and the serum cobalt levels decreased from 967 to 232 nmol l^{-1} after 3 months. Table 3 shows several investigations performed over allergic effects of cobalt exposure.

Carcinogenic Effects

Studies on carcinogenic effect of cobalt are inconclusive. Although inhalation of cobalt particles could be related to the increased risk of lung cancer, this carcinogenic effect is not so clear when is contacted by other means. In 1990, the International Agency for Research on Cancer concluded that the epidemiological evidence was inadequate in classifying cobalt or cobalt compounds as human carcinogens and that there was sufficient evidence for the carcinogenicity of cobalt metal powder and cobalt oxide in experimental animals.

Sarcomas in rats have been observed at the site of injection of cobalt salts or cobalt metal powder. However, similar injection studies have provided little evidence of cobalt-induced cancer in mice, hamster, and guinea pigs. The chronic exposure (0–3 mg m^{-3}, 6 h day^{-1}, 5 days $week^{-1}$ during 104 weeks) to aerosols containing cobalt sulfate has shown a major incidence of lung tumors in rats and mice of both sexes. The study has also found a major prevalence of liver hemangiosarcoma in male mice. However, in this case, significant correlations could not be established due also to a higher prevalence of infection by *Helicobacter hepaticus*. In relation to the use of cobalt alloys as biomaterial, several studies have evaluated the effects of implanted Co–Cr alloys on the development of tumors in animals. Four out of six studies reported tumors at the implant site, whereas two studies reported no such increase.

Two epidemiological studies were developed in France to observe the incidence of lung cancer among hard-metal workers. In this case, cobalt exposure is associated with tungsten carbide exposure in the hard metal-manufacturing industry. It was found that these workers had an increased mortality from lung cancer due to the simultaneous exposure to cobalt and tungsten carbide. Although occupational risk was highest among smoking workers, this excess mortality could not be attributed to smoking alone. The toxicity of cobalt–tungsten carbide has been also studied using lung epithelial cells *in vitro*. The toxicity was higher in nanoparticles (80 nm) against microparticles (4 μm), suggesting that internalization may play a role in enhancing toxicity. Another research has estimated the risk of cancer, especially lung cancer, among women workers exposed to cobalt aluminate spinel used in the porcelain factories in Denmark. In that study, a woman group was selected to be exposed to cobalt and further was compared with a woman reference group from cobalt-free department in a factory. The results showed a higher risk of lung cancer in both groups if compared with the whole Danish women population, although the risk was only slightly higher for the exposed group than for the reference group. Moreover, a higher risk of cervical cancer was found in the exposed woman group and of corpus uteri cancer in the reference group. Finally, an increase in mortality from lung cancer was reported among cobalt recovery workers in Soviet plants concerned with nickel extraction. However, there was a simultaneous exposure to nickel and arsenic that could explain this finding.

The use of Co–Cr alloys as hip implants has also been studied in humans for long periods (3–7 years). Most of the studies developed in patients showed that there is no evidence that these metal-on-metal bearing surfaces are associated with an increased risk of any cancer diagnosis. Table 3 shows some relevant studies over carcinogenic effects of cobalt exposure.

Cobalt Genotoxicity

Some *in vitro* studies, with human lymphocytes or mammalian cells, indicate that cobalt (II) ions may affect DNA integrity either directly through a Fenton-like mechanism or indirectly, affecting the repair system of DNA damage. Another study, comparing the effect of fine and nanoparticle alloys of cobalt–chromium on human fibroblast cells, noted that the nanoparticles generated free radicals, cell DNA damage, cytotoxicity and aneuploidy. This genotoxic effect has also been shown with cobalt oxide nanoparticles in human hepatocarcinoma cells through an increase in lipid hydroperoxide and reactive oxygen species generation.

In vivo intraperitoneal administration of cobalt (II) ions (50–100 μmol kg^{-1} as cobalt acetate) in rats produced oxidative DNA damage in renal, hepatic, and pulmonary chromatin. Similarly, chromosomal aberrations have also been found in bone marrow cells of mice after a single oral administration of high doses of cobalt chloride (20–80 mg kg^{-1}). With all the previously mentioned, there is considerable evidence to consider that all soluble cobalt (II) salts have genotoxic effects in animals and *in vitro* models. However, there is no evidence available in humans. Thus, a human study realized by Belgian researchers detected no significant increase of genotoxic effects in workers exposed to cobalt-containing dust, at a mean level corresponding to TLV-TWA (20 μg m^{-3}), as compared to controls. Although several epidemiological studies show that occupational exposure to hard-metal particles is associated with an increased risk of lung cancer and despite the increase of reactive oxygen species tested with *in vitro* models, there is no evidence of a genotoxic effect by cobalt compounds in humans. Therefore, further investigation may be developed in this sense in the future.

Respiratory Effects

Several compounds are thought to cause lung damage through the generation of oxygen-derived free radicals and related reactive compounds. The respiratory system is the most affected organ resulting from cobalt exposure by inhalation. In addition to the possible increase of the incidence of lung cancer, different types of toxic reactions related with respiratory system have been reported, including the upper respiratory tract, the bronchial tree, and the alveolar parenchyma.

Upper Respiratory Tract

Animal studies have shown degeneration of the olfactory epithelium, squamous metaplasia of the respiratory epithelium, and nonneoplastic lesions of the nose and larynx of rats and mice exposed to aerosols containing cobalt sulfate hexahydrate. Furthermore, studies performed on humans showed that inflammation of the nasopharynx seems to be frequent in hard-metal workers. However, it is not clear whether the occurrence of these symptoms is produced by particles containing cobalt or from an immunologically mediated reaction (allergic rhinitis). It has also been observed that subjects working in lignite mines have a high prevalence of atrophic rhinitis, which may be related to an excessive pollution caused by airborne contaminants such as chromium, nickel, cobalt, and lead.

Bronchial Tree

Cobalt has been identified as an occupational agent, which is involved in asthma diseases. Exposure to cobalt salts or cobalt metal released from diamond-polishing activities may cause typical bronchial asthma in a small proportion of workers (generally <5%). Similarly, 7% of workers exposed to cobalt dust at a hard-metal plant of Uppsala (Sweden) reported asthma. Another study showed that chromium and cobalt salts (in the form of cobalt chloride) can be responsible for occupational asthma in workers exposed to higher metalworking fluid aerosols of an aerospace manufacturer of Birmingham (the United Kingdom). A higher urinary excretion of chromium and cobalt was observed in the occupational asthma group than in the asymptomatic control group.

The pathophysiology of cobalt asthma may involve both immunologic and nonimmunologic mechanisms. Japanese researchers showed IgE antibodies to cobalt-conjugated human serum albumin in some of the cobalt asthma patients. For the remaining patients, the mechanism of cobalt-induced asthmatic reaction remains unknown. In order to investigate this mechanism, it could be noted that a positive lymphocyte transformation test with cobalt (II) ions has also been reported in hard-metal asthma, suggesting a role for cellular immunity. On the contrary, a study conducted to analyze all the cases of cobalt asthma encountered in a cobalt plant of Kokkola (Finland) used skin prick tests for cobalt and common environmental allergens. None of these patients had a positive reaction against cobalt in skin prick test, indicating a nonimmunologic mechanism.

Lung Parenchyma

In 1950, severe and fatal pulmonary edema and hemorrhage were firstly described in rats treated intratracheally with cobalt metal powder (500 μg per rat). Similarly, intratracheal instillation of 10–50 mg of cobalt metal produced acute pneumonia with diffuse cellular infiltration and bronchiolitis obliterans. Subchronic response assessed 8–12 months after the acute dose was characterized by the presence of multinucleated cells and a lack of cellular reaction within the alveolar walls. Short-term exposure appears to have fewer adverse consequences. Exposure of rats to aqueous suspension of ultrafine metallic cobalt particles (20 nm) during 4 days at 5 h day^{-1} induced slight and reversible pulmonary injury including focal hypertrophy or proliferation of the epithelium in the lower airways.

Hard-metal pulmonary fibrosis has been known for over 70 years, in subjects exposed to dust produced during mixing, shaping, and grinding of hard metals. Several studies have shown a close relationship between cobalt exposure and lung fibrosis. Thus, parenchymal reaction in some hard-metal workers exposed to dust containing cobalt has been observed. Histological changes varied from intense desquamative alveolitis with giant multinucleated cells to end-stage nonspecific pulmonary fibrosis. A weak association of the disease with a

Table 4 Investigations performed on respiratory effects of cobalt exposure

Investigation	*Reference*
Inhalation toxicity and carcinogenicity studies of cobalt sulfate	Bucher, J. R., Hailey, J. R., Roycroft, J. R., et al. (1999). *Toxicological Sciences* **49**, 56–67
The effect of environmental pollution on the respiratory system of lignite miners: a diachronic study	Sichletidis, L., Tsiotsios, I., Chloros, D., et al. (2004). *Medicina del Lavoro* **95**(*6*), 452–464
Cobalt-induced bronchial asthma in diamond polishers	Geysens, B., Aurwerx, J., Van den Eeckhout, A. and Demedts, E. (1985). *Chest* **88** (*5*), 740–748
Lung function and respiratory symptoms in hard-metal workers exposed to cobalt	Rehfisch, P., Anderson, M., Berg, P., et al. (2012). *Journal Occupational Environmental Medicine* **54**(*4*), 409–413
An outbreak of occupational asthma due to chromium and cobalt	Walters, G. I., Moore, V. C., Robertson, A. S., et al. (2012). *Occupational Medicine* **62**, 533–540
Cobalt asthma – a case series from a cobalt plant	Sauni, R., Linna, A., Oksa, P., Nordman, H., et al. (2010). *Occupational Medicine* **60**, 301–306
Lung function and respiratory symptoms in hard-metal workers exposed to cobalt	Rehfisch, P., Anderson, M., Berg, P., et al. (2012). *Journal of Occupational and Environmental Medicine* **54**(*4*), 409–413
Hard-metal disease: eight workers with interstitial lung fibrosis due to cobalt exposure	Migliori, M., Mosconi, G., Michetti, G., Belotti, L., D´Adda, F. (1994). *Science of the Total Environment* **150**, 187–186
Reversible lung lesions in rats due to short-term exposure to ultrafine cobalt particles	Kyono, H., Kusaka, Y., Kubota, H. and Endo-Ichikawa, Y. (1992). *Industrial Health* **30**(*2*), 103–118

specific human lymphocyte antigen (HLA) polymorphism (glutamate 69 in HLA-DP beta chain) has been reported.

According to the medical literature, bronchial asthma is considered the main etiologic agent inducing hard-metal fibrosis. The essential factor to avoid the progression of the disease is to separate the affected individuals from the work environment. However, in many cases, the disease remains despite the cessation of exposure and not always is managed with steroid treatment. To summarize, Table 4 shows several investigations performed on respiratory effects of cobalt exposure.

Cobalt and Thyroid Function

Animal studies have confirmed that cobalt is a goitrogen (a substance that suppresses the function of the thyroid gland by interfering with iodine uptake). This effect may be related with the capacity of cobalt ions to inhibit tyrosine iodinase enzyme. In a cobalt production plant, a subclinical hypothyroid status has been observed in some workers. Similarly, increased concentrations of total and free serum thyroid hormone have been found in workers exposed to cobalt–zinc sulfate.

Cardiovascular Diseases

Cobalt is considered responsible for a type of myocardiopathy with a specific pathogenesis. As already mentioned, the first cases reported of myocardiopathies associated with the intake of high doses of cobalt were related to beer drinkers. In the second half of the 1960s, in some areas of Canada, the United States, and Belgium, a large number of subjects, all of them great beer drinkers, showed a particular dilatative myocardiopathy associated with other symptoms such as cardiocirculatory insufficiency with dyspnea, hypotension, tachycardia, cyanosis, enlarged heart with reduced cardiac output, and in many cases a large pericardial effusion. The mortality rate of subjects was as high as 50% in some areas. It was observed that these pathologies were attributed to cobalt added as a foaming agent (1 ppm on average) in the form of sulfate or chloride. This meant an estimated daily intake of 6–8 mg of cobalt. Complications in the diagnosis of cobalt myocardiopathy were also related to the possibility that alcohol would also be able to induce such cardiopathy. Thus, cobalt myocardiopathy could be an advanced state or a particular type of alcoholic myocardiopathy. According to some authors, a fulminant form of beriberi due to thiamine deficiency was caused by alcohol and aggravated by cobalt. Finally, other studies claimed the direct toxic role of cobalt.

Several cases of myocardial toxicity have been associated with cobalt industrial exposure. Workers of a cobalt compound plant showed an association between cumulative cobalt exposure and left ventricular isovolumic relaxation time and deceleration time of the velocity of the early rapid filling wave, indicating altered left ventricular diastole. Another study investigated cardiac function of a group of Italian hard-metal workers with or without hard-metal fibrosis. The mean duration of exposure was 10.4 years, ranging from 1 to 40 mg m^{-3}, whereas that level of environmental exposure ranged between 0.009 and 13.6 mg m^{-3} cobalt. An abnormal left ventricular ejection fraction during resting and at exercise was also found, which could be explained by the formation of cobalt deposits in the myocardium after industrial exposure. The subjects also showed ventricular dysfunction and abnormality of the right ventricular function, which could be a manifestation of initial pulmonary artery hypertension or of early occult cor pulmonale.

Several mechanisms about cobalt toxicity on the cardiovascular system have been proposed:

(1) Impairment of antioxidant enzyme activities and myocardial mitochondrial ATP production rate. An experimental study showed a lower activity of manganese superoxide dismutase in rats exposed to a cobalt-supplemented diet for 24 weeks against control group. Activity in the

Table 5 Studies related with cobalt exposure and cardiovascular diseases

Investigation	*Reference*
Quebec beer-drinkers' cardiomyopathy: 48 cases	Morin, Y. L., Foley, A. R., Martineau, G. and Roussel, J. (1967). *Canadian Medical Association* **97**(*15*), 881–883
Cobalt-beer cardiomyopathy: a clinical and pathological study of 28 cases	Alexander, C. S. (1972). *American Journal of Medicine* **53**, 395–417
Cardiac function study in hard-metal workers	D´Ádda, F., Borleri, D., Migliori, M., et al. (1994). *Science of the Total Environment* **150**, 179–186
Chronic cobalt exposure affects antioxidants and ATP production in rat myocardium	Clyne, N., Hofman-Bang, Y., Haga, N., et al. (2001). *Scandinavian Journal of Clinical and Laboratory Investigation* **61**(8), 609–614
The evolution of β-adrenergic dysfunction during the induction of canine cobalt cardiomyopathy	Unverferth, D. V., Fertel, R. H., Thomas, J., et al. (1984). *Cardiovascular Research* **18**, 44–50
Fatal myocardial disease associated with industrial exposure to cobalt	Kennedy, A., Dornan, J. D. and King, R. (1981). *Lancet*, 412–414

respiratory chain enzymes, succinate–cytochrome *c* reductase, NADH–cytochrome *c* reductase, and cytochrome *c* oxidase, was also reduced in the cobalt rats.

(2) Damage of the electromechanical matching of myocardial tissues, probably connected with a decreased concentration of calcium ions in the cell due to damage of the membrane transport pathway induced by cobalt.

(3) Inhibition of the sympathetic tone. A study on cobalt cardiomyopathy in dogs showed changes in the β-adrenergic system.

(4) Finally, an unspecific allergic mechanism was also reported; however, the way that is induced remains unknown.

Table 5 shows some studies related with cobalt exposure and cardiovascular diseases.

See also: Cobalamin (Vitamin B12): Metabolism and Disorders; Cobalt: Properties and Determination.

Further Reading

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Cocoa: Composition and Health Effects

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Description of Commodity

Cocoa is derived from the 'bean' from the cocoa or cacao tree (*Theobroma cacao*), which is cultivated in the equatorial regions, as it requires a hot climate with high levels of precipitation to grow and produce optimal yields. The cocoa 'bean' develops in large and often colorful pods that contain the bitter seeds used in the manufacture of cocoa and, subsequently, chocolate.

Sources and Production

Postharvest, the 'bean' requires a number of processing steps that, ultimately, can influence many of the potential health benefits and organoleptic properties of end products. Processing can not only degrade polyphenolic compounds, reducing bitter flavors, which may be preferable to consumers, but also reduce the availability of compounds that may be responsible for the beneficial bioactive functions.

The initial step, following harvesting and splitting of the pod to remove the 'beans,' is fermentation. The remaining pulp around the 'beans' is removed, which allows the rich flavors associated with cocoa to develop. The exact method varies from country to country; it can be achieved by simply by covering the piles of beans with leaves or with the use of boxes designed especially for this purpose.

Following fermentation, which can take between 5 and 8 days depending on method and country, the 'beans' are dried and winnowed where the 'bean' is cracked to separate the shell from the nib (the part that is used to make cocoa and chocolate). The nibs are roasted to further develop flavor. These roasted nibs are ground before processing to produce cocoa powder and cocoa butter. This has the effect of separating the fat-rich cocoa butter, which is used in chocolate production along with many applications in the cosmetic industry, and the cocoa powder, also often described as fat-free cocoa mass. The distinction of fat-free cocoa mass from cocoa solids is important, as a chocolate high in cocoa solids is not necessarily high in fat-free cocoa mass, which is the cocoa component rich in potentially beneficial polyphenolic compounds. Chocolate, which typically lists cocoa solids as part of the ingredient labeling information, is a composite of cocoa butter and fat-free cocoa mass (cocoa powder).

The separation of the fat-free cocoa mass from cocoa butter is important as it enhances the stability of final products, reducing any separation of fat that can result in unpleasant 'blooming' where a white fatty layer can be seen on the surface of products, as was the case with eighteenth-century cocoa drinks and poorly stored chocolate today. Separation is achieved using methods such as the Dutch process that uses alkali to produce a mellower-tasting product, which may be lower in polyphenols, prior to hydrolytically pressing the powder from the fat or the Broma process, which is a slower process using a combination of sieving and temperature to produce what is known as natural cocoa. Van Houten developed the former method, while the latter was developed in the United States at the Ghirardelli factory. These processes were accompanied by a number of other technological advances in the nineteenth century that helped change cocoa products from a bitter drink to the modern drinking cocoas and chocolates that are enjoyed by millions today. Cocoa and chocolate are products often consumed for pleasure owing largely to their hedonic qualities and luxury image. Initially, this led to research aimed at understanding potential harms, including tachycardia, but this has changed dramatically over the last decade and a half with the investigation of potential health benefits.

The emergence of potential health benefits from compounds found in cocoa is of great interest to both the scientific community and the food industry. Arguably, a disproportionate amount of work is being undertaken using cocoa and chocolate due to commercial interests, which may not exist to fund work with other plant-based foods containing similar compounds. Alongside potential financial interests, a key property of cocoa and chocolate is that they are rich sources of polyphenolic compounds, which are stable over time. Chocolate has been shown to maintain its polyphenol content 2 years after manufacture, with low levels of contaminants and bacteria growth, characteristics not seen in fruit and vegetables. Whereas other commodities, including teas, appear to be less stable, with a shorter shelf life, they are yet to demonstrate similar levels of bioavailability to the polyphenols found in cocoa or chocolate. This, together with good consumer acceptability and desirability, has resulted in over 2000 peer-reviewed papers describing research on cocoa or chocolate in PubMed by August 2014.

A key challenge in the promotion of any potential health benefits derived from the consumption of cocoa and chocolate is the way it is considered as a food by society and the formulations that are most popular. Many products based on cocoa are energy dense, resulting from high levels of fat and sugar. Chocolate as a formulation varies from country to country; often, cocoa and cocoa butter can have added sugar and, potentially, milk and/or other emulsifiers (typically soy lecithin), which in some countries are accompanied by added vegetable fats; in Europe, only illipe, palm oil, sal, shea, kokum gurgi, mango kernels, and copra oil can be used. But this can mean that chocolate contains around 500–530 kcal per 100 g (2050–2250 kJ), 27–32 g of fat of which 16–19 g is saturated, and 45–64 g of added sugar (typically sucrose). When sold as a powder, cocoa contains around 310 kcal per 100 g (1300 kJ), 22 g of fat of which 13 g is saturated, and only a trace of sugar. However, cocoa is consumed rarely in this form, and often, sugar and fat (often from added dairy fat) are added, which can dramatically alter nutritional composition. So, by definition, in some territories, chocolate cannot be considered as a food with a health claim simply because of its high fat and sugar content.

Availability, Absorption, and Metabolism

The mechanism(s) and exact bioactive compounds responsible for the health benefits observed have also been an area of debate. Epicatechin has been proposed as the key biologically active polyphenol, in part not only due to its relatively low molecular weight but also because it has been detected in plasma following ingestion. However, it is equally likely that many of the polyphenolic compounds found in cocoa and chocolate are metabolized in the gastrointestinal tract, degraded and transformed chemically both by stomach acid and by the microflora of the colon prior to absorption. It has been suggested that many of these metabolites could be responsible for the beneficial effects of cocoa and chocolate rather than the compounds native to cocoa after processing.

The complex nature of cocoa, as a source of polyphenols, alongside other bioactive compounds such as theobromine, a bioactive amine with similar effects to caffeine, has been hypothesized to be responsible for many of the health benefits. This is aside from its unique fatty acid and amino acid profiles and the fact that cocoa is a good source of many micronutrients, including magnesium and iron, which also may impact human health. Potentially, this means that it is difficult to identify if the benefits arise from one active compound or a synergistic mix of compounds. In addition, the hedonistic and socially ascribed qualities given to chocolate since the Aztecs could infer benefits purely from its consumption in a Hawthorne-type effect, where individuals improve or modify an aspect of behavior in response to being observed.

Much of the initial work *in vitro* focussed on the antioxidant properties of the phenolic rings in cocoa polyphenols. This is demonstrated by the very high oxygen radical absorbance capacity (ORAC) value for cocoa. However, the effect of these compounds *in vivo* is less related to their antioxidant properties, as their bioavailability is not sufficient to support this mechanism, than the number of pathways targeted by cocoa polyphenols. The most widely studied is perhaps inducible endothelial nitric oxide synthase (eNOS), a key enzyme involved in how blood vessels respond to the sheer stresses caused by changes in blood flow and pressure. Cocoa polyphenols are associated with an increase in nitric oxide, which improves the capacity of the blood vessel to expand and reduces the risk of damage that might lead to the development of atherosclerotic plaques and, ultimately, cardiovascular disease. Other key enzymes acted on by cocoa polyphenols include AMP-activated protein kinase, which can influence insulin resistance and lipid metabolism in a positive way, and the angiotensinogen-converting enzyme pathway, which is key in determining blood pressure.

History of Consumption and Formulation Including Some of the 'Issues'

The consumption of cocoa can be traced back to the civilizations of Central America, especially the Mayans and Aztecs, although it appears to have originated in South America where a wide variety of native cocoa trees can still be found. Cocoa has been cultivated since about 1500 BC. Historically, a number of physiological effects have been linked to cocoa consumption, with its use initially linked to rituals; it was consumed as a bitter drink mixed with chili or even blood by the ruling classes, priests, and warriors. It was suggested by Montezuma II that a soldier could march for a whole day on a single cup of cocoa without getting fatigued. During the time of the conquistadors, Spanish monks recorded in detail the potential health effects of cocoa, including as a treatment for consumption (tuberculosis), digestive problems, and general fatigue. Following these expeditions, cocoa was brought to Europe, but dismissed in many royal courts at the time as moldy almonds, although in Spain, a culture of cocoa drinking did develop. This spread across Europe, despite the fact that cocoa was bitter and the fat component did not form a stable emulsion upon mixing with water. The culture of cocoa drinking was a concern in France, where it was considered a narcotic, most likely as a result of cocoa houses becoming places where intellectuals would meet and, because cocoa provided an alternative to the alcoholic beverages, began to think more radically. However, it was not until the industrial revolution when methods, such as the Dutch process and conching, developed that modern cocoa and chocolate began to be manufactured. Discovered by Rodolphe Lindt, conching physically redistributes fat in cocoa to improve the texture of the end product, mixing cocoa powder for a long time using a paddle that scrapes the surface of the bowl. Air and friction reduce the amounts of volatile fatty acids and oxidize some of the bitter compounds, thus mellowing the taste of the end product. However, this process and others while mellowing flavor have the potential to reduce further levels of bitter bioactive compounds, including epicatechin and theobromine. During this period, Joseph Fry also discovered that, if cocoa butter was added back to cocoa, the product could be molded allowing the first chocolate bars to be produced.

Within much of northern Europe and North America, milk chocolate is more popular than the bitter plain chocolate, probably because of its mellower and sweeter flavor. Milk chocolate was initially developed in Switzerland following the invention of a process to dry milk. Until then, it was only possible to mix milk with cocoa in a drink: Daniel Peter added the dried milk developed by Henri Nestlé to cocoa liquor, creating milk chocolate.

All these developments needed raw ingredients, namely, cocoa and sugar, which came from plantations and colonies of the Dutch, Spanish, and British empires and depended to a large extent on the slave trade up until its abolition. The combination of global trade and industrialization meant that cocoa and chocolate became not just a rare treat for the wealthy, but a product consumed widely by the whole of society. Its high energy content, including saturated fat and sugar, means that chocolate has been implicated as a cause of a range of problems including obesity, poor skin health, and dental caries. However, epidemiological data do not support a link between chocolate consumption and obesity; if anything, the data suggest the opposite. These findings need to be taken with a note of caution, as such studies are prone to a degree of selection and reporting bias, as they rely on participants reporting what and how much chocolate they eat.

Health Effects

Early work studying the Kuna Indians, an indigenous population inhabiting islands near Panama, provided modern insight

into the health effects of cocoa. This ethnic group has little age-related increase in blood pressure or associated hypertension. However, when members of this group moved to the urban environment of Panama City, they display expected levels of hypertension. This could be said to represent the classic 'migration type' study, suggesting a lack of a genetic effect, and the increase in hypertension must be attributed solely to environmental influences. The island-dwelling Kuna consume large quantities of cocoa daily, both as drinks (up to five cups a day) and as an ingredient in cooking. Those living in Panama City, however, consume very little cocoa. Moreover, the island-dwelling Kuna have a threefold higher level of nitric oxide metabolites in their urine compared with those living in an urban environment. These epidemiological data clearly suggested an association between cocoa polyphenols (mainly flavanols) and improved vascular function and support the view that increased nitric oxide, via the induction of nitric oxide synthase, could be the mode of action.

Epidemiological evidence of the effect of chocolate has largely been derived from studies in which data collection was initially undertaken as part of other studies, and the effect of cocoa or chocolate consumption examined as post hoc analysis. The majority of these data have been derived from European prospective study data sets, which are supported by cross-sectional data from the United States and Spain. The cross-sectional natures of European cohort studies lack predictive power because of their design. European studies, however, reported hard end points, including death and myocardial infarction, suggesting that chocolate consumption is associated with a reduced rate of mortality.

Data from the Zutphen Elderly Study reported by Buijsse et al. considered the effects of consuming foods containing cocoa in elderly men over a period of 15 years. The findings suggested no association between sugar confectionary consumption and mortality but did demonstrate a significant inverse association between habitual intake of cocoa-containing foods and cardiovascular mortality rates (as well as all-cause mortality rates). This was in agreement with the cross-sectional data collected at the start of the study, suggesting a significant inverse association between blood pressure and habitual intake of foods containing cocoa. These data were subsequently confirmed by analysis of the Potsdam cohort from the European Prospective Investigation into Cancer and Nutrition reported by Buijsse et al. In this group, chocolate consumption and blood pressure were assessed at baseline and found to be inversely associated in nearly 20 000 individuals aged 35–65 years of age. When followed up, those who consumed the greatest quantity of chocolate had a significantly lower risk of myocardial infarction and stroke than those consuming the lowest amounts (RR = 0.61 (95% confidence interval 0.44–0.87)), with 12% of this effect (95% confidence interval 3–36%) explained by the effect of chocolate on blood pressure after an average follow-up period of 8 years.

Cocoa polyphenolic compounds have been reported to have beneficial effects on cardiovascular risk, including the lowering of blood pressure, in both animal models and clinical trials with human participants. In rats, the consumption of cocoa polyphenols demonstrated not only a reduction in blood pressure but also an increase in the expression of a number of genes associated with endothelial function, which suggests the potential for an epigenetic effect of polyphenols. Upregulated genes associated with reduced blood pressure acted on endothelial function via increased nitric oxide and prostaglandin production.

Many of the clinical studies demonstrating a reduction in blood pressure attributed to the effect of polyphenols have been credited to changes in endothelial function. However, work in individuals with type 2 diabetes has shown an improvement in endothelial function but no change in blood pressure. The effects of the underlying condition and concomitant medication, which may reduce blood pressure but have minimal effect on endothelial function, could explain this apparent contradiction. It has been suggested that the improvement in endothelial function derives from an increase in redox-sensitive activation of the phosphatidylinositol 3-kinase/Akt pathway, which results in the activation of eNOS. This is thought to be via an increase in intracellular free calcium concentration and activation of estrogen receptors. These changes appear to go beyond just nitric oxide with cocoa polyphenols acting on other endothelium factors. The improvements seen in endothelial function have been linked to insulin signaling and, ultimately, the potential to reduce insulin resistance. In individuals with type 2 diabetes, the results are inconsistent. However, in studies with healthy individuals and those with hyperinsulinemia consuming high doses of chocolate (100 g day^{-1}) for 2 weeks, reductions in insulin resistance have been reported consistently. Alongside the potential for better endothelial function and insulin sensitivity, it has been suggested that activation of the AMPK pathway in both the skeletal muscle and liver could explain improvements in insulin sensitivity.

Cocoa and chocolate polyphenols have also been associated with the beneficial effects on dyslipidemia across a range of clinical and nonclinical groups, including individuals with type 2 diabetes. This is in accordance with data that suggest diets rich in plant-derived foods and polyphenols are associated with more favorable lipid and cholesterol profiles and reduced cardiovascular risk. Data from mice models of diabetes suggest that cocoa polyphenols might act via hepatocellular AMP-activated protein kinase (AMPK) along with its downstream target, acetyl-CoA carboxylase. In 2006, Zang et al. reported that the effects of these polyphenols were approximately 200 times more potent than metformin, the most commonly used pharmaceutical agent in type 2 diabetes, on lipid metabolism. As in the case of blood pressure, there are a number of alternate mechanisms by which cocoa polyphenols might act to improve the lipid profile including the modulation of the ATP-cassette binding protein. In data from human studies, improvements in lipid profiles, specifically increased high-density lipoprotein (HDL) cholesterol levels, have been seen in populations with diabetes. Another research suggests that polyphenols may reduce the overall amount and vulnerability of low-density lipoprotein (LDL) cholesterol to oxidative damage. Some of the data from clinical studies are challenging to interpret, as often cardiovascular risk and LDL cholesterol are calculated, rather than measured, creating a potential for bias and/or confounding factors, meaning interpretation needs to be undertaken with care.

Despite dismissing the potential of cocoa and chocolate as having an antioxidant effect *in vivo* because the data from human

studies are largely equivocal, it is still important to consider what is meant by oxidative stress and how it is measured. A number of methods available can be insensitive to changes in response to diet or can be directly influenced by their presence as components of acute food intake, rather than showing any effect upon endogenous redox biology. The relatively poor absorption of cocoa polyphenols resulting in low plasma concentrations compared with other antioxidant compounds, including uric and ascorbic acids, suggests that any effects are likely to go beyond just antioxidant capacity and act via multiple mechanisms. It is logical that the beneficial effects of polyphenols on endothelium function arise from increased synthesis of endothelium-derived factors, such as nitric oxide and prostaglandins, which are also free radical scavengers, resulting in an enhanced pool of potentially beneficial compounds. Along with the effects of cocoa polyphenols that have been demonstrated on superoxide dismutase, this increase in free radical scavengers might explain the reductions in oxidative stress markers including lipid peroxidation and DNA damage.

Cocoa polyphenols may also have positive effects on coagulation and clotting, having been demonstrated to inhibit platelet aggregation, the potential mechanism being via decreased production of superoxide anions and increased nitric oxide synthesis, which in addition would reduce levels of oxidative stress. A further potential effect of polyphenols on health is as 'an antinutrient' or chelating agent. This could be considered as a 'negative effect' with increased risk of micronutrient deficiencies and disease, such as iron-deficiency anemia. However, this chelating effect could potentially reduce chronic disease risk. Both in the gastrointestinal tract and, potentially, in circulation, by binding metal ions that could otherwise be prooxidants, oxidative stress and oxidation of LDL cholesterol and/or DNA damage might be reduced. Currently, these effects are little more than a hypothesis based on mechanistic work *in vitro*. It is clear, however, that beneficial effects of cocoa are likely to be derived from polyphenols and these compounds appear to have multiple modes of action, with the mechanisms described in this article often interacting and overlapping.

Cocoa and chocolate have been proposed to improve cognition, based on mechanisms associated with nitric oxide metabolism. This has been demonstrated in animals, but work in humans has also reported the potential of improved cognition. This concept has been hypothesized as perhaps reducing progression in dementia, but consistent data are not yet available. The effects of cocoa butter as a cosmetic product have been observed for many years; the effect of chocolate on the resistance of skin to ultraviolet light damage has also shown limited promise in clinical studies.

Chocolate and cocoa drinks have been studied as a potential ergogenic aid in sport, for both professional athletes and the general public. Initial studies have suggested that chocolate might delay perception of fatigue and, thus, improve exercise performance. This has been associated with a reduction in glucose oxidation linked to the effects of polyphenols. Data are yet to demonstrate this effect consistently. Other areas of interest in exercise physiology are the potential of cocoa polyphenols to modulate oxidative stress and inflammation associated with exercise; mechanistic work *in vivo* shows promise, but the practical implications are yet to be demonstrated.

Endeavors to explore the health potential of chocolate are less than 20 years old. From the initial work *in vitro* of Waterhouse, Shirley, and Donovan in the mid-1990s to the first human studies reported in 2000, attempts have been made to see if cocoa has the potential to reverse or prevent disease processing pertaining to cardiovascular disease and cognitive decline. The vast majority of these studies have been undertaken in healthy individuals, with a few undertaken in populations with, or at increased risk of, cardiovascular disease. Only five studies have been undertaken in individuals with diabetes, for example, and two with heart failure or following heart surgery. Some of these studies have combined cocoa, typically in the form of chocolate, with other potentially bioactive compounds, such as soy isoflavones and lycopene. It is possible that favorable palatability and acceptability of chocolate mean that it could be used as a carrier or vector for other less palatable plant-derived bioactives in the future.

The key challenge when attempting to ascertain the potential health effects of cocoa and chocolate lies in the way many studies are conducted. There is little consistency in the formulations or even concentrations/doses of polyphenols being consumed by participants. This reflects that cocoa and chocolate manufacturers have, at least, supported many studies financially and, therefore, are unlikely to share commercially sensitive data with respect to recipes and composition. Additionally, many of the studies are limited in duration and often sample size, with very few studies exceeding 100 participants or lasting greater than 12 weeks. The reliability of many studies is also brought into question as, although many studies state that participants were blinded to the cocoa or chocolate they were given, on closer examination, it is apparent that the control product(s) was milk in studies of cocoa beverages or white chocolate in studies of dark chocolate. Under such conditions, it is highly plausible that informed study participants would be fully aware of the arm to which they had been assigned. In a review, Karen Cooper et al. summarized the first 10 years of research on the health effects of chocolate and proposed an 11-point checklist for planning future trials. These included the design of the trials; need to consider the matrix or formulation used; use of chocolate in preference to cocoa; transparency in terms of both independency of the trial from industry and the need to register human trials publically; careful selection of biomarkers, especially those assessing antioxidant effects; and, finally, publication of null or negative results.

Future and Health Claims

Increasingly, in many countries, claims associating food and health are being regulated. In Europe, for example, the European Food Safety Authority (EFSA) has a rigorous assessment process for assessing health claims whether maintaining health or preventing disease, which can be generic or proprietary. The latter provides sole use for a claim for 5 years protecting the investment in intellectual property. To be awarded a health claim, foods need to have an identifiable, well-characterized component with a defined mechanism(s) of action and effect and clinical trial data to support the effect *in vivo*. In 2012, the EFSA gave a positive opinion for a proprietary health claim by Barry Callebaut (BE) for a cocoa product containing flavanols. This was granted under Article 13.5 and, potentially, allows these cocoa and chocolate products to carry the claim that *Cocoa flavanols help maintain endothelium-*

dependent vasodilation, which contributes to normal blood flow. This claim acknowledges the growing body of data from many sources for chocolate and cocoa that have been shown to demonstrate this effect. However, it is perhaps less clear how the general public would interpret this claim and if it would influence purchasing decisions.

Not all health claims linked to cocoa and chocolate have been as successful. In 2012, the USDA withdrew publication of ORAC values from its Nutrient Data Laboratory website (http://www.ars.usda.gov). This decision was based on mounting evidence suggesting that there was no link between the antioxidant capacity of foods and chronic disease risk. Using the PASSCLAIM criteria, which are the basis for a positive opinion for a health claim, it was found that reduction in cardiovascular risk could not be attributed to the antioxidant capacity of polyphenol-containing foods, such as cocoa. This perhaps explains the EFSA judgment in 2010 that found a causal relationship could not be demonstrated. The panel also stated that any beneficial psychological effect (as with physiological effects) could not be linked to the antioxidant activity, content, or properties.

So, although one health claim has reached the EFSA threshold, many have failed due to poor characterization of the bioactive compounds, the absence of clear mechanism(s) of action, or inadequate clinical trial data. This does not mean that chocolate will eventually be labeled with health claims, as nutritional composition and portion size are also important. The Barry Callebaut claim was for 10 g of a specially formulated chocolate, or 2.5 g of cocoa, containing 200 mg cocoa flavanols, more than twice the more typical levels of polyphenols. Otherwise, the energy content and added sugar would make a health claim for chocolate incompatible with established public health messages.

Many of the negative aspects of chocolate and cocoa consumption have not stood up to rigorous examination, with no apparent weight gain seen in studies or when observing populations. However, the shift in focus to consider the beneficial effect of polyphenols and recommendations of higher intakes, especially in an aging population, should be undertaken with caution owing to the largely under studied potential of food–nutrient–drug interactions. It has been suggested that epicatechins and other flavanols can act on cytochrome P450, including CYP3A4, which are responsible for the conjugation of many organic compounds, including a wide range of drugs. Thus, any compounds altering the action of this and similar enzyme systems could impact the circulating concentration of pharmaceuticals and their half-life and, ultimately, increase the risk of side effects or poor control of symptoms. This could lead, in the future, to as yet undefined negative health effects, which need to be studied before recommendations to increase intakes to above that achieved normally, for example, levels seen among the Kuna of Panama.

Conclusion: Cocoa and Chocolate for Health or Pleasure?

It is of note that the history of cocoa and chocolate consumption has been linked with potential health benefits. However, with industrialization, and the increased availability of sugar, a sweeter product has been created, which is associated with unproved negative health effects. This means that chocolate is likely to remain a luxury, nonessential food. However, it should not necessarily be considered as being inherently harmful and has potential for a number of well-defined health benefits. This does not mean that increasing consumption is wise, from both the perspective of sustainability, as ecosystems would need to be changed to grow more cocoa, and health, as eating more chocolate is likely to lead to increased energy intake, weight gain, and, potentially, long-term health consequences.

See also: Antibiotics and Drugs: Drug–Nutrient Interactions; Antioxidants: Role on Health and Prevention; Cholesterol: Factors Determining Blood Cholesterol Levels; Functional Foods; Phenolic Compounds: Bioavailability and Health Effects.

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Cocoa: Production, Chemistry, and Use

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Cocoa Tree and Beans

Cocoa beans represent the seeds of the fruits of cocoa tree (*Theobroma cacao* L., order Sterculiacae), which is a native species of tropical humid forests on the lower eastern equatorial slopes of the Andes in South America. Cocoa was domesticated and consumed for the first time by the Mayas and Aztecs. The growth of the cocoa tree requires warm-humid climates with temperatures between 20 and 30 °C and high and constant humidity, conditions found in areas about 20° latitude north and south of the Equator. Abundant and well-distributed rainfalls are essential because the cocoa tree tolerates a dry season of up to 3 months a year. The cocoa tree is a rather delicate plant that does not tolerate direct strong winds and direct sunlight. In fact, it is generally grown in the shade of other tall trees (banana trees and coconut palms). Trees can reach up to 20 m in height, but are usually kept below 5 m. The fruit is 10–30 cm long and 7–10 cm wide and weighs ~400–1000 g. It is an indehiscent drupe called a pod or 'cabosside.' It can be spherical, cylindrical, pointed or blunt, smooth, or wrinkled. The pericarp has a thickness of 10–15 mm and can be of different colors depending on the ripeness and variety, turning from green to yellow, from red to orange. The optimum ripeness of the fruit varies according to the variety and generally lasts from 4.5 to 7 months. The fruit is harvested twice a year (in February/March and April/July). The summer harvest usually produces fruit of better quality. The seeds, in the number of 20–60 per fruit, are arranged in regular rows and immersed in a mucilaginous acidic pulp containing glucose and fructose and are called beans. Cocoa beans are the only economically valuable part of cocoa tree because they represent the raw material for the production of cocoa-based products. Each cocoa bean consists of two cotyledons (nibs) and a small embryo, all enclosed in a skin (shell). The cotyledons contain two types of cells: storage or parenchyma cells, containing fat globules, protein and starch granules, and pigmented cells, containing polyphenols and methylxanthines.

Cocoa Varieties and Geographical Diffusion

Two major botanical groups of cocoa are currently recognized: Criollo and Forastero. Criollo (native) corresponds to the subspecies 'cacao' and represents the original cocoa, previously consumed by pre-Columbian people. Its diffusion resulted from the dissemination through the Andes toward the lowlands of Venezuela, Colombia, and Ecuador and northward to Central America and Mexico, and to a large number of Caribbean Islands. Today the subspecies Criollo is found in Mexico, Colombia, and Venezuela. It represents only 5% of the world's production due to its great susceptibility to disease; making it difficult to cultivate. Criollo pods are green to red; the seeds are pale, ferment easily, have a pleasant and penetrating aroma, and have an optimum taste. Therefore they are considered an excellent quality cocoa. The Criollo is rarely used alone; most frequently it is used to fortify other mixtures having a weak and not persistent aroma. It can be subdivided into Central American Criollo and Venezuelan Criollo. The Forastero cacao (foreigner) corresponds to the subspecies 'sphaerocarpum' and resulted from dissemination toward the Amazon Valley in Northern Brazil and the Guyanas. In the nineteenth century, Forastero cocoa seeds were taken from South America to the islands of Sao Tomé of West Africa, then to Ghana. From Ghana, Forastero spread to other African countries, the most important of which are the Ivory Coast, Cameroon, and Nigeria. In these countries, there was an immediate increase in cultivated area, and they are now the largest producers of cocoa in the world. Forastero represents about 85% of the world's cocoa production because it is very strong, resistant to disease, and easily cultivated, especially in Africa. The seeds have a strong flavor, not very aromatic and of poor quality, and are generally used in a mixture with other more valuable varieties. Forastero cocoa is now a group very differentiated in several subspecies and hybrids. The best-known Forasteros are the 'Amelonados' with pods resembling melon, which were the predominant types traditionally cultivated in West African countries. The Amazonian subgroups show a wide genetic variability. Some Forastero plants growing in particular areas give cocoa of excellent quality (*cru*) such as Arriba, the national cocoa of Ecuador.

Natural hybridization between Criollo and Forastero led to the origin of a third variety of cocoa named 'Trinitario,' not found in the wild. It has been reported that the Criollo population from Venezuela and the Amelonado-type Forastero from Guyana could have been involved in hybridization leading to the production of Trinitario. It is difficult to specify the characters of Trinitarios as they may have pod and bean characters ranging from those typical of Criollos to those of Forasteros.

Cocoa Beans Harvesting, Fermentation, and Drying

These steps of the process are carried out in the countries of origin. Fresh cocoa seeds undergo fermentation and a drying process to be ready and stable for transport to the countries in which they will be processed to chocolate and related products. Local or regional variations in cocoa plant materials, fermentation procedures, and drying processes lead to a traded good typical of the country of origin; therefore, the composition of the fermented cocoa beans, which is one of the most important factors influencing taste and flavor of the cocoa products, depends not only on the cocoa variety but also on the geographic origin.

Cocoa bean fermentation can be considered the first key stage in cocoa products and chocolate production because

the series of biochemical reactions occurring in the beans is necessary for inducing the pleasant characteristics of cocoa-based products.

The fully ripe cocoa pods are carefully cut from the trees, are gathered into heaps, and can be stored for 2–5 days. This enhances prefermentation activity inside the pods. The harvested pods are broken by hitting against a hard surface, and the seeds are extracted from the fruit together with the surrounding sugary mucilaginous pulp (wet beans) and kept for fermentation immediately. The fresh bean is bitter and is not suitable for manufacture of cocoa products because it does not have any flavor, aroma, or taste of cocoa products. All the standard methods of fermentation essentially involve keeping together in heaps, baskets, boxes, or perforated barrels a mass of a reasonable quantity of wet beans for periods ranging from 4 to 6 days and mixing the mass of beans on alternate days. The natural beans' fermentations are generally carried out according to a traditional process. Among the various methods adopted for fermentation in the different cocoa-producing countries, the heap, tray, and box methods are considered the standard, widely adopted methods. The real substrate of the fermentation is not the bean, but the surrounding pulp that contains about 84.5% water, 10% glucose and fructose, 2.7% pentosan, 0.7% sucrose, 0.6% protein, 0.7% acids, and 0.8% inorganic acids and therefore it is a good substrate where a wide range of microorganisms can develop. Wild yeasts (*Kloeckera* and *Saccharomyces* spp.) and bacteria of genera such as *Lactobacillus*, *Bacillus*, *Pediococcus*, *Acetobacter*, and *Gluconobacter*, are involved in cocoa fermentation, determining alcoholic, lactic acid, and acetic acid fermentations.

The first consequence of fermentation is the loss of most of the pulp around the beans because the pulp substrates are broken down through microbial action; however, more important are the biochemical changes inside the cocoa beans that contribute to a reduction of their bitterness and astringency and improve their color and flavor.

In the first stage of the fermentation, yeasts proliferate and convert sugars to alcohol, then the development of lactic acid bacteria occurs, which contributes to sugar breakdown producing lactic acid. After the pulp has run off, the conditions become more aerobic, and the presence of oxygen allows acetic acid bacteria to take over from the yeasts and convert alcohol to acetic acid. Acids that are synthesized from pulp sugars move into the beans and lower the internal pH. The acetic acid diffusing through the beans causes a breakdown of the polyphenol and lipid membranes of the vacuoles of the cell, and the cell contents become mixed, allowing various enzymatic reactions to take place. One of the most important consequences is the oxidation of polyphenols and their conversion to insoluble forms due to the reactions with proteins; these reactions are responsible not only for the removal of the bitter taste from the beans but also for the strong reduction of total polyphenol content, thus affecting the antioxidant and health properties of cocoa. Oxidation of polyphenols is also responsible for the change of bean color, from violet to brown. During and after fermentation, another fundamental biochemical change occurs: internal beans' autolytic enzymes, in particular carboxypeptidase and aspartic endoprotease, are activated by the low pH; the proteins in the cotyledons undergo hydrolysis, giving rise to amino acids and oligopeptides, essential precursors for the development of cocoa aroma, arising from the reaction of amino acids with sugars (Maillard reactions) during the subsequent step of roasting.

The duration of fermentation ranges from 1.5 to 10 days, depending on the cocoa variety, climate, volume of cocoa mass, and the method adopted. Criollo ferments in a relatively shorter period of 2–3 days, whereas Forastero takes 5–7 days.

For the manufacturer of chocolate or cocoa powders the degree of fermentation of the beans is a major quality criterion. Fully fermented cocoa beans have a brown color. It has been shown that too high contents of nonfermented (slaty color) or partly fermented (violet color) beans result in a lack of cocoa flavor in the end product. The slaty beans cause a very acid and astringent flavor profile, whereas the violet beans cause a bitter and harsh flavor.

The fermented beans have a moisture content of about 55%, too high to permit the storage of the beans. The moisture content has to be lowered to about 6% for safe storage and transportation. Immediately after fermentation, cocoa beans are sun-dried or dried utilizing hot air. Depending on climatic conditions, the beans are exposed to the sun for about 12–20 days. This method generally gives good-quality beans in traditional areas of cocoa production where the weather is sufficiently sunny, as in West Africa. In the areas where the climate remains unsuitable for drying, artificial drying methods became necessary, utilizing hot air with a maximum temperature of about 60 °C. The humidity % after drying is 5–8%, with a weight loss of two-thirds with respect to the fresh bean. The dried beans are packed in jute bags of about 60 kg capacity and traded to transformation countries, where all the following steps (Figure 1) for production of chocolate and cocoa products will take place.

Processing of Fermented Cocoa Beans: Roasting and Production of Cocoa Liquor

A key step of chocolate production is roasting. Roasting is performed in the transformation countries, and flavor is formed during this step from the precursors developed during fermentation and drying of cocoa beans. The aroma precursors in cocoa beans, which include free amino acids, low-molecular-weight peptides, and reducing sugars, develop into the cocoa specific aroma through Maillard reactions during roasting.

Before roasting, cocoa beans are cleaned to remove stones, metals, and other extraneous materials. Beans are cleaned by passing through a series of screens and magnets. In some cases a preroasting process is applied. The roasting can be performed on whole cocoa beans or on cocoa nibs. In most cases the traditional roasting is performed on whole cocoa beans by hot air treatment, and cocoa shells are removed by aspiration (winnowing). Temperatures of roasting are generally lower that 150 °C and for times of from 30 to 120 min. Roasted cocoa beans are then lightly crushed to avoid dust formation, obtaining cocoa nibs.

Roasted cocoa nibs are milled to break down the cell walls and expose the cocoa butter. The resulting product is a homogeneous flowing cocoa paste called cocoa liquor containing

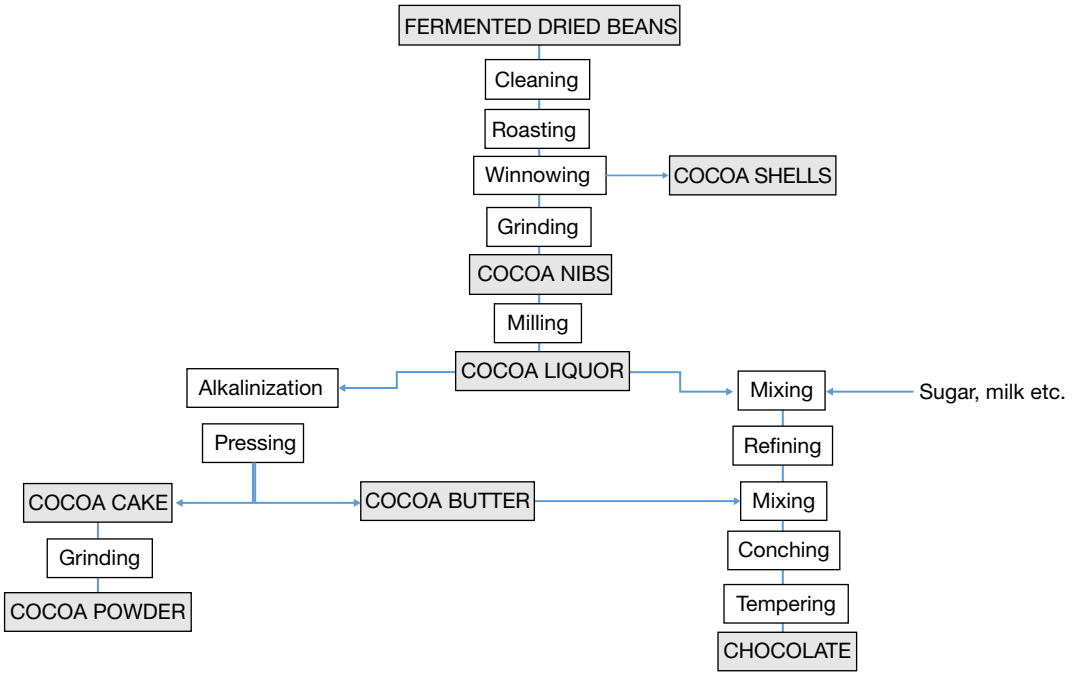

Figure 1 An overview on cocoa bean processing.

about 55% fats. To produce cocoa liquor with improved dispersibility, the cocoa nibs can be subjected to an alkalinization process (Dutch cocoa process): roasted nibs are treated with a diluted alkali solution at 75–100 °C, then neutralized and dried. This treatment causes starch swelling and the formation of porous cell structure of cocoa mass that prevents the formation of sediments in cocoa drinks.

Production of Cocoa Powder and Cocoa Butter

Cocoa powder is widely used in the manufacturing of cocoa-based products as drinks, cake fillings, ice cream, and so on. To convert cocoa liquor (generally alkalinized cocoa liquor) to cocoa powder, a defatting process is performed by pressing liquor in a mechanical or hydraulic press at 400–500 bar and a temperature of 90–100 °C. In this way part of the fat (cocoa butter) is removed, and cocoa cake (compressed cocoa powder) is produced. Cocoa powder is obtained by grinding cocoa cake. Generally the fat content of cocoa powder is 10–24%. Cocoa butter obtained from pressing is separated, filtered, and reused as an ingredient for chocolate and many cocoa-derived products.

Production of Chocolate

Chocolate is obtained from nonalkalinized cocoa liquor mixed with sucrose, cocoa butter, emulsifying agents (lecithins), flavoring compounds (vanillin), and eventually other ingredients (milk, hazelnuts, almonds, etc.). Ingredients are mixed to obtain a homogeneous chocolate paste that is then refined to obtain finer particles of <30–40 μm. The refining step is performed by single or multiple refining rollers. Further key steps in chocolate production are conching and tempering. Refined chocolate paste is powdery and has a harsh and sour flavor, and conching is necessary to obtain fine chocolate of optimal flavor, smoothness, and texture. Conching is performed in round or oblong rotary conche pots in which the chocolate mass is mixed, ground, and kneaded at temperatures of 65–75 °C. Conching times vary from a few hours to many days, depending on the desired final chocolate quality. Generally during conching steps, flavors, emulsifier, and cocoa butter are added. The effect of conching on chocolate paste is a reduction of acidity due to the loss from evaporation of acetic acid and other volatile compounds, loss of moisture, and a more uniform distribution of fats that form a film around each cocoa particle.

Tempering is one of the most critical steps to obtain a product with a stable crystalline form of cocoa butter responsible of the good melting properties and the glossy surface of good-quality chocolate. Tempering is performed by cooling under stirring the cocoa mass derived from conching (from 40–50 to 18–28 °C). Cocoa is maintained at this low temperature for about 10 min, and then is heated to 32 °C. In this way the polymorphic form V of cocoa butter is obtained, with a melting point of 34 °C, close to the human body temperature.

Different chocolate typologies are produced (Table 1) and can be classified according to the different percentages of cocoa liquor and cocoa butter in the final products or according to the addition of ingredients different from cocoa. Today much of the chocolate consumed is in the form of sweet chocolate, with the addition of sugars (sucrose). Milk chocolate is sweet chocolate that in addition contains milk powder. White chocolate contains cocoa butter, sugar, and milk, but no cocoa liquor.

Table 1 Examples of formulations for different kind of chocolate (g/100 g)

Ingredient	*Chocolate (50% cocoa)*	*Chocolate (70% cocoa)*	*Milk chocolate*	*White chocolate*
Cocoa liquor	35	70	12	–
Added cocoa butter	15	–	22	30
Sugar	50	30	51	50
Whole milk powder	–	–	15	20

Chemical Composition

Lipids

Cocoa fat (cocoa butter) represents about 50–58% of the cocoa beans, and its triacylglycerols (97–98% of cocoa butter) mainly consist of palmitic acid (25% of total fatty acids), stearic acid (37%), and oleic acid (34%), with a low amount of linoleic acid (3%). Oleic acid is primarily esterified at the 2-position of glycerol, so the main triacylglycerols are 1,3-dipalmito-2-olein, 1-palmito-3-stearo-2-olein, and 1,3-distearo-2-olein. Cocoa butter is solid at room temperature and melts at temperatures between 30 and 40 °C, depending on the polymorphic form. The amount of cocoa butter in chocolate is 21–35%, depending on the addition of cocoa butter to the cocoa liquor.

Protein, Peptides, Amino Acids, and Amines

Proteins make up 10–15% of the dry weight of cocoa seeds, the second most abundant constituent after cocoa fat. Proteins are the cocoa fraction that undergoes the most intensive modification during fermentation, where microbiological and enzymatic reactions lead to extensive breakdown of cocoa seed proteins, yielding peptides and amino acids that are the important flavor precursors.

Cocoa beans contain four main proteins, albumin, globulin, prolamin, and glutelin. Albumin and globulin are the most important both quantitatively and qualitatively. Globulins are vicilin-like storage proteins consisting of three subunits with molecular masses of 47, 31, and 15 kDa, which are derived from a common 66-kDa precursor. The albumin fraction was identified as a 21-kDa cocoa seed protein having trypsin inhibitory properties. During fermentation, peptide and free amino acids increase and total protein concentration decreases. The globulin protein fraction is the most degraded during fermentation. Cocoa proteins are cleaved to hydrophilic and hydrophobic peptides as well as amino acids through autolysis by two endogenous enzymes, aspartic endoprotease and carboxypeptidase. Fermentation of cocoa beans is fundamental for the activation of these two enzymes by microbial metabolites (such as acetic acid). Changes in the protein composition of cocoa beans have been noted not only during fermentation but also as a consequence of roasting. A decrease in total protein, free amino acids, and albumin are seen during roasting.

Regarding amino acids composition, nonfermented beans contain low levels of free amino acids with a 1:1 ratio between hydrophobic/acidic amino acid, whereas in fermented cocoa seeds this ratio significantly increases to 3:1. The increase in the concentrations of hydrophobic amino acids, such as leucine, alanine, and phenylalanine, is explained by the activity of carboxypeptidase that releases single hydrophobic amino acids and aspartic endoprotease, which hydrolyzes proteins preferentially at the hydrophobic amino acids sites. The free amino acid content recorded in fermented beans ranges from 500 to 1800 mg/100 g, with a prevalence of the hydrophobic amino acids responsible of the formation of Strecker aldehydes and pyrazines, important compounds for cocoa aroma. Among nonprotein amino acids, relevant contents of gamma-aminobutyric acids, ranging from 30 to 100 mg/100 g, were detected in fermented cocoa beans, therefore cocoa can be considered an important natural source of this inhibitory neurotransmitter amino acid.

Low amounts (20 mg kg^{-1}) of biogenic amines deriving from microbial decarboxylation of amino acids occurring during fermentation, as 2-phenylethylamine, tyramine and tryptamine have been found in cocoa. Biogenic amines can be also originated by thermal decarboxylation of amino acids, so their amount is higher in roasted products.

Carbohydrates and Organic Acids

The primary carbohydrates in fermented dried cocoa beans are starch (6%) and cellulose (9%). Soluble carbohydrates include glucose, fructose, sucrose (0.08–1.5%), raffinose, and stachyose. Sucrose is partially hydrolyzed during fermentation, providing reducing sugars precursors of aroma development during roasting. Fiber fraction aside from cellulose contains pentoses (1.5%), galactans, and polymers of galacturonic acid. Fibers are concentrated in cocoa shells.

Organic acids (1.2–1.6%) are primarily formed during cocoa bean fermentation, and the most represented are acetic acid (0.2–0.7%), citric acid (0.4–0.7%), and oxalic acid (0.3–0.5%). Acetic acid is partly lost during cocoa processing, in particular during conching.

Methylxanthines

Cocoa, as coffee and tea, is generally considered a stimulating food, due to the high levels of alkaloids. Theobromine and caffeine in particular are the principal alkaloids found in cocoa. Theobromine (3,7-dimethylxanthine) represents 1.2–2% of cocoa beans, where it is partially bound to tannins in cotyledon cells. During fermentation, the development of acetic acid permits the release of theobromine that migrates from cotyledons to shells. Cocoa shells in fact contain about 1.5% of theobromine, and generally they are reused to extract this alkaloid.

Polyphenols

Cocoa is a rich source of polyphenols: the defatted unfermented cocoa beans contain about 120–180 g kg^{-1} of polyphenolic compounds, representing one of the most concentrated natural sources. The polyphenols in cocoa beans are stored in the pigment cells of the cotyledons. Depending on the amount of anthocyanins, pigment cells are white to deep purple. Three groups of polyphenols can be distinguished: catechins or flavan-3-ols (~37%), anthocyanins (~4%), and proanthocyanidins (~58%). The main catechin is (−)-epicatechin representing up

to 35% of polyphenol content. In smaller amounts, (+)-catechin as well as traces of (+)-gallocatechin and (−)-epigallocatechin have been found. Procyanidins in cocoa consist of oligomers and polymers of catechin and epicatechin. Anthocyanins identified in cocoa include cyanidin-3-galactoside and cyanidin-3-arabinoside. Small quantities of quercetin, quercetin glycosides, naringenin, luteolin, apigenin, clovamide, and phenolic acids such as caffeic, ferulic, gallic, and *p*-coumaric acid have also been found in cocoa products.

The high level of polyphenols in raw cocoa beans is progressively reduced during cocoa processing. During fermentation polyphenols diffuse with cell liquids from their storage cells and undergo oxidation to condensed high-molecular mostly insoluble tannins. These reactions are both nonenzymatic and enzymatic catalyzed by polyphenol oxidase. It is reported that epicatechin and catechin content, respectively, are reduced to ~10–70% during fermentation. Roasting causes a dramatic reduction of some phenolic substances, in particular clovamide, together with an overall decrease of the antiradical and antioxidant properties of cocoa beans/nibs. High temperatures during the cocoa bean roasting process and also the alkali treatments on the cocoa powder induce the epimerization reaction of epicatechin to catechin, reducing its bioavailability. Moreover, some recent works have achieved chiral separations of catechin and epicatechin enantiomers, showing that the prevalent form of catechin in roasted cocoa products is (−)-catechin, a nonnatural in cocoa beans with less bioavailability than (+)-catechin.

Flavor Compounds

Chocolate and cocoa flavors reside in their volatile fraction, which is composed of a complex mixture of up to 500 compounds. The aromatic profile of cocoa beans is very complex and is also dependent on the method and duration of fermentation and drying practices applied. Alcohols, aldehydes, and ketones have been reported as the major groups of compounds found in raw cocoa and at the beginning of the fermentation process (1 or 2 days). Alcohols, esters, and acids (acetic acid mainly) were developed in the middle of fermentation (3–5 days), becoming the most important groups of volatile compounds at the end of fermentation (6–8 days). Alcohols, esters, and pyrazines contents increased during the sun-drying process.

Cocoa fermentation is crucial not only to the formation of significant volatile fractions but also for the development of cocoa–chocolate flavor precursors as amino acids and reducing sugars. *Via* Maillard reactions, cocoa roasting converts flavor precursors formed during fermentation to two main classes of odorant compounds: pyrazines and Strecker aldehydes, and three of these had a strong chocolate flavor: 2-ethylpropanal, 2-methylbutanal, and 3-methylbutanal. Pyrazines were recognized as cocoa/nutty notes: 2,3-dimethylpyrazine, trimethylpyrazine, tetramethylpyrazine, 3(or 2),5-dimethyl-2(or 3)-ethylpyrazine, 3,5(or 6)-diethyl-2-methylpyrazine, and furfurylpyrrole. Conching has an effect on cocoa aroma, although no new key odorant is synthesized during the heating process, and levels of 2-phenyl-5-methyl-2-hexenal, furaneol, and branched pyrazines are significantly increased, whereas most Strecker aldehydes are lost by evaporation.

World Production and Human Consumption

The cocoa-producing countries are all developing countries and localized in Africa, Central America, South America, Asia, and Oceania. World production of cocoa beans is constantly growing: it has increased from 31 000 tons in 1880 to more than 3 000 000 tons in 2002 and about 5 000 000 tons in 2012. Africa is the continent with the highest production (67%). The largest state producer is the Ivory Coast (about 30% of world production). World cocoa production has risen at an average annual growth rate of 3.3% during the period 2002–12.

Between 2002 and 2011/2012, primary cocoa processing growth at an average rate of 2.9% per annum was registered. Europe remained by far the largest cocoa-processing region, followed by the United States. However, with an annual growth rate of 5.6%, the largest regional processing increase occurred in Asia and Oceania. Moreover, in the last 10 years processing in the countries of origin has increased, supported, in some countries, by government policies favoring the export of value-added semifinished products rather than raw cocoa beans.

European regions are also the largest cocoa consumers, accounting for 48% of total world consumption of cocoa followed by the Americas, at 33%. Growing consumption has been observed in Asia and Africa. Consumption of chocolate confectionery products increased by 10% between 2002 and 2010 in selected countries, including the major European countries, the United States, Brazil, Japan, and Australia, corresponding to an annual growth rate of 1.2%. World per capita consumption of cocoa has also seen a similar pattern of growth over the review period, rising from 0.54 kg in 2002 to 0.61 kg in 2010.

Consumer preferences, especially of European and American consumers, has changed over the last 10 years: chocolate aficionados are asking for single-origin premium chocolate and 'high cocoa content' products, with their own distinctive flavors. Cocoa industries are driven to use innovation to appeal to consumers in saturated markets: new flavors, new packaging, and new sizes but also chocolates with health-promoting properties for health-conscious consumers. Sustainable sourcing is also growing in importance for consumers: demand for cocoa grown in a responsible manner is rising, as a consequence of the companies' response to consumer preferences.

See also: Cocoa: Composition and Health Effects.

Further Reading

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Relevant Websites

http://faostat.fao.org/ – Food and Agriculture Organization of the United Nations.
http://www.icco.org/ – International Cocoa Organization.
http://worldcocoafoundation.org – World Cocoa Foundation.

Codex Alimentarius

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About Codex Alimentarius

The Codex Alimentarius, or the food code, is a collection of internationally adopted food standards and related texts developed by the Codex Alimentarius Commission (CAC) and presented in a uniform manner. Codex standards, guidelines, and codes of practice aim at protecting consumers' health and ensuring fair practices in the food trade. Since its beginning, the Codex Alimentarius evolved in the most important international reference point in matters concerning food safety and quality and international food trade.

Codex Alimentarius Commission

The CAC was established by the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO), as an intergovernmental body to implement the Joint FAO/WHO Food Standards Programme, which was established by a FAO Conference resolution in 1961 and a World Health Assembly resolution in 1963. Its principal objective is to protect the health of consumers and to facilitate the trade of food by setting international food standards and other texts that can be recommended to governments for acceptance. The CAC also coordinates all food standards work done by international governmental and nongovernmental organizations.

The CAC is open to the governments of all member nations or associate members of FAO and WHO. It currently has 185 members, and more than 200 intergovernmental and international nongovernmental organizations are accredited as observers. Codex members cover 99% of the world's population. More and more developing countries are taking an active part in the Codex process, in many cases assisted by the Codex Trust Fund, which strives to finance and train participants from such countries to enable efficient participation. Being an active member of the Codex helps countries to compete in sophisticated world markets and to improve food safety for their own population.

The body of Codex Alimentarius consists of CAC, Executive Committee, General Subject Committees, Commodity Committees, ad hoc Intergovernmental Task Forces, and FAO/WHO Coordinating Committees. All Codex Committees, their current status, and host governments are presented in Table 1.

The Procedural Manual of the CAC describes the legal foundations and practical functioning of the CAC and its subsidiary bodies. It sets out the basic rules of procedures for the elaboration of Codex standards and related texts, basic definitions, and guidelines for the operation of Codex committees. It is intended to help member governments participate effectively in the work of the joint FAO/WHO Food Standards Programme.

Member countries communicate with the CAC through the Codex Contact Points that act as the link between the Codex Secretariat and member countries in order to coordinate all relevant Codex activities within their own countries. In order to provide better transparency and to facilitate more efficient participation in Codex activities, member countries are encouraged to establish National Committees for Codex Alimentarius that should include representatives of the relevant ministries, technical experts, decision-makers from the industry, and consumer organizations.

FAO/WHO Trust Fund for Participation in the Codex

FAO and WHO help developing countries to apply Codex standards, to strengthen national food control systems, and to take advantage of international food trade opportunities.

In 2002, FAO and WHO recognized that developing countries and countries with economies in transition were not participating fully in the work of the CAC. In response, the director-generals of WHO and FAO launched the FAO/WHO Project and Fund for Enhanced Participation in Codex (Codex Trust Fund) on 14 February 2003 with the objective of helping developing and transition economy countries enhance their level of effective participation in the Codex activities.

Codex Standards, Codes of Practice, Guidelines, and Recommendations

The Codex Alimentarius includes standards for all the principal foods, whether processed, semiprocessed, or raw, for distribution to the consumers, containing provisions with respect to food hygiene, food additives, residues of pesticides and veterinary drugs, contaminants, labeling and presentation, methods of analysis and sampling, and import and export inspection and certification.

For food safety and nutrition matters, the CAC, as a risk manager, establishes its standards using the principles of risk analysis and bases its work on the scientific advice provided by the independent international risk assessment bodies such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Joint FAO/WHO Meeting on Pesticide Residues (JMPR), Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA), or ad hoc consultations organized by FAO and WHO. Codex standards also address issues related to food quality to ensure fair practices in the food trade. Food standards, guidelines, and recommendations established by the CAC are recognized as reference points for food under the relevant World Trade Organization (WTO) agreements. While being recommendations for voluntary application by members, Codex standards serve in many cases as a basis for national legislation.

Public concerns about food safety issues are often placing Codex at the center of global debates. Biotechnology, pesticides, veterinary drug residues, food additives, and contaminants are some of the issues discussed in Codex meetings. Creating

Table 1 List of all Codex Alimentarius bodies with their status and host countries

Acronym	*Name*	*Status*	*Host*
CAC	Codex Alimentarius Commission	Active	FAO (Rome) and WHO (Geneva)
CCEXEC	Executive Committee of the Codex Alimentarius Commission	Active	FAO (Rome) and WHO (Geneva)
General subject committees			
CCCF	Codex Committee on Contaminants in Foods	Active	The Netherlands
CCFA	Codex Committee on Food Additives	Active	China
CCFAC	Codex Committee on Food Additives and Contaminants	Renamed and reestablished	—
CCFH	Codex Committee on Food Hygiene	Active	The United States
CCFICS	Codex Committee on Food Import and Export Inspection and Certification Systems	Active	Australia
CCFL	Codex Committee on Food Labelling	Active	Canada
CCGP	Codex Committee on General Principles	Active	France
CCMAS	Codex Committee on Methods of Analysis and Sampling	Active	Hungary
CCNFSDU	Codex Committee on Nutrition and Foods for Special Dietary Uses	Active	Germany
CCPR	Codex Committee on Pesticide Residues	Active	China
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Foods	Active	The United States
Commodity committees			
CCCPC	Codex Committee on Cocoa Products and Chocolate	Adjourned sine die	—
CCCPL	Codex Committee on Cereals, Pulses and Legumes	Adjourned sine die	—
CCFFP	Codex Committee on Fish and Fishery Products	Active	Norway
CCFFV	Codex Committee on Fresh Fruits and Vegetables	Active	Mexico
CCFO	Codex Committee on Fats and Oils	Active	Malaysia
CCIE	Codex Committee on Edible Ices	Abolished	—
CCM	Codex Committee on Meat	Abolished	—
CCMMP	Codex Committee on Milk and Milk Products	Adjourned sine die	—
CCMPH	Codex Committee on Meat Hygiene	Adjourned sine die	—
CCNMW	Codex Committee on Natural Mineral Waters	Adjourned sine die	—
CCPFV	Codex Committee on Processed Fruits and Vegetables	Active	The United States
CCPMPP	Codex Committee on Processed Meat and Poultry Products	Abolished	—
CCS	Codex Committee on Sugars	Active	Colombia
CCSB	Codex Committee on Soups and Broths	Abolished	—
CCSCH	Codex Committee on Spices and Culinary Herbs	Active	India
CCVP	Codex Committee on Vegetable Proteins	Adjourned sine die	—
Ad hoc Intergovernmental task forces			
CGECPMMP	Joint FAO/WHO Committee of Government Experts on the Code of Principles Concerning Milk and Milk Products	Renamed and reestablished	—
CXTO	Joint CODEX/IOOC Meeting on the Standardization of Table Olives	Abolished	—
GEFJ	Joint ECE/Codex Alimentarius groups of experts on standardization: Fruit Juices	Abolished	—
GEQFF	Joint ECE/Codex Alimentarius groups of experts on standardization: Quick Frozen Foods	Abolished	—
TFAF	Ad Hoc Intergovernmental Task Force on Animal Feeding	Dissolved	—
TFAMR	Ad hoc Codex Intergovernmental Task Force on Antimicrobial Resistance	Dissolved	—
TFFBT	Ad Hoc Intergovernmental Task Force on Food Derived from Biotechnology	Dissolved	—
TFFJ	Ad Hoc Intergovernmental Task Force on Fruit and Vegetable Juices	Dissolved	—
TFPHQFF	Ad hoc Codex Intergovernmental Task Force on the Processing and Handling of Quick Frozen Foods	Dissolved	—
FAO/WHO coordinating committees			
CCAFRICA	FAO/WHO Coordinating Committee for Africa	Active	Cameroon
CCASIA	FAO/WHO Coordinating Committee for Asia	Active	Japan
CCEURO	FAO/WHO Coordinating Committee for Europe	Active	The Netherlands
CCLAC	FAO/WHO Coordinating Committee for Latin America and the Caribbean	Active	Costa Rica
CCNASWP	FAO/WHO Coordinating Committee for North America and South West Pacific	Active	Papua New Guinea
CCNEA	FAO/WHO Coordinating Committee for Near East	Active	Lebanon

standards that at the same time protect consumers and ensure fair practices in the sale of food is a process that involves specialists in numerous food-related scientific disciplines, together with consumer organizations, production and processing industries, food control administrators, and traders. The finalization of food standards and their compilation into a code that is credible and authoritative require extensive consultation and the collection and evaluation of information, followed by confirmation of final results and sometimes objective compromise to satisfy differing sound, scientifically based views.

There are two main groups of Codex standards: Codex general standards such as the General Standard for Food Additives, General Standard for Contaminants and Toxins in Food and Feed, and General Standard for the Labelling of Prepackaged Foods and specific standards of which the largest number is the group called 'commodity standards.' Representatives of some of the major commodity standards included in the Codex with a year of last modification are presented in the Table 2.

Commodity standards tend to follow a fixed format set out in the Procedural Manual of the CAC. The format consists of the following categories of information:

- *Scope* includes the name of the food to which the standard applies and, in most cases, the purpose for which the commodity will be used.
- *Description* includes a definition of the product or products covered with an indication, where appropriate, of the raw materials from which they are derived.
- *Essential composition* includes information on the composition and identity characteristics of the commodity, as well as any compulsory and optional ingredients.
- *Food additives* includes the names of the additives and the maximum amount permitted to be added to the food. Food additives must be cleared by FAO and WHO for their safety, and the use of food additives must be consistent with the Codex General Standard for Food Additives.
- *Contaminants* contains the limits for contaminants that may occur in the product(s) covered by the standard. These limits are based on the scientific advice of FAO and WHO and must be consistent with the Codex General Standard for Contaminants and Toxins in Food and Feed. Where appropriate, reference is also made to the Codex maximum limits for pesticide residues and for residues of veterinary drugs in foods.
- *Hygiene* makes reference to relevant Codex Codes of Hygienic Practice for the commodity concerned. In almost all cases, it is required that the product shall be free from pathogenic microorganisms or any toxins or other poisonous or deleterious substances in amounts that represent a hazard to health.
- *Weights and measures* contains provisions such as fill of the container and the drained weight of the commodity.
- *Labelling* includes provisions on the name of the food and any special requirements to ensure that the consumer is not deceived or misled about the nature of the food. These provisions must be consistent with the Codex General Standard for the Labelling of Prepackaged Foods. Requirements for the listing of ingredients and date-marking are specified.
- *Methods of analysis and sampling* contains a list of the test methods needed to ensure that the commodity conforms to the requirements of the standard. References are made to internationally recognized test methods that meet the CAS's criteria for accuracy, precision, etc.

The Codex Alimentarius contains more than 200 standards in the prescribed format for individual foods or groups of foods.

Codex codes of practice define the production, processing, manufacturing, transport, and storage practices for individual foods or groups of foods that are considered essential to ensure the safety and suitability of food for consumption. For food hygiene, the basic text is the Codex General Principles of Food Hygiene, which introduces the use of the Hazard Analysis and Critical Control Point (HACCP) food safety management system. A code of practice on the control of the use of veterinary drugs provides general guidance in this area.

Codex guidelines fall into two categories:

- Principles that set out policy in certain key areas
- Guidelines for the interpretation of these principles or for the interpretation of the provisions of the Codex general standards

In the cases of food additives, contaminants, food hygiene, and meat hygiene, the basic principles governing the regulation of

Table 2 Representatives of the Codex commodity standards and the year of last modification

Reference	*Title*	*Last modified*
CODEX STAN 199-1995	Standard for Wheat and Durum Wheat	1995
CODEX STAN 152-1985	Standard for Wheat Flour	1995
CODEX STAN 198-1995	Standard for Rice	1995
CODEX STAN 171-1989	Standard for Certain Pulses	1995
CODEX STAN 175-1989	Standard for Soy Protein Products	1989
CODEX STAN 211/1999	Standard for Named Animal Fats	2013
CODEX STAN 210/1999	Standard for Named Vegetable Oils	2013
CODEX STAN 33-1981	Standard for Olive Oils and Olive Pomace Oils	2013
CODEX STAN 311-2013	Standard for Smoked Fish, Smoke-Flavoured Fish and Smoke-Dried Fish	2013
CODEX STAN 3-1981	Standard for Canned Salmon	2011
CODEX STAN 219-1999	Standard for Grapefruits (*Citrus paradisi*)	2011
CODEX STAN 260-2007	Standard for Pickled Fruits and Vegetables	2007
CODEX STAN 247-2005	General Standard for Fruit Juices and Nectars	2005
CODEX STAN 89-1981	Standard for Luncheon Meat	1991
CODEX STAN 96-1981	Standard for Cooked Cured Ham	1991
CODEX STAN 207-1999	Standard for Milk Powders and Cream Powder	2014
CODEX STAN 275-1973	Standard for Cream Cheese	2010
CODEX STAN 212-1999	Standard for Sugars	2001
CODEX STAN 86-1981	Standard for Cocoa Butter	2001
CODEX STAN 87-1981	Standard for Chocolate	2003

these matters are built into the relevant standards and codes of practice.

There are free-standing *Codex principles* covering the

- addition of essential nutrients to foods,
- food import and export inspection and certification,
- establishment and application of microbiological criteria for foods,
- conduct of microbiological risk assessment (MRA),
- risk analysis of foods derived from modern biotechnology.

Interpretative Codex guidelines include those for food labeling, especially the regulation of claims made on the label. This group includes guidelines for nutrition and health claims; conditions for production, marketing, and labeling of organic foods; and foods claimed to be 'halal.' There are several guidelines that interpret the provisions of the Codex Principles for Food Import and Export Inspection and Certification and guidelines on the conduct of safety assessments of foods from genetically modified plants and microorganisms.

The uniform Codex procedure for the Elaboration of Codex Standards and Related Texts, according to the CAC Procedural Manual, includes eight steps (**Figure 1**).

An alternative accelerated 5-step procedure for the elaboration of Codex standards and related texts, as a subject of an accelerated

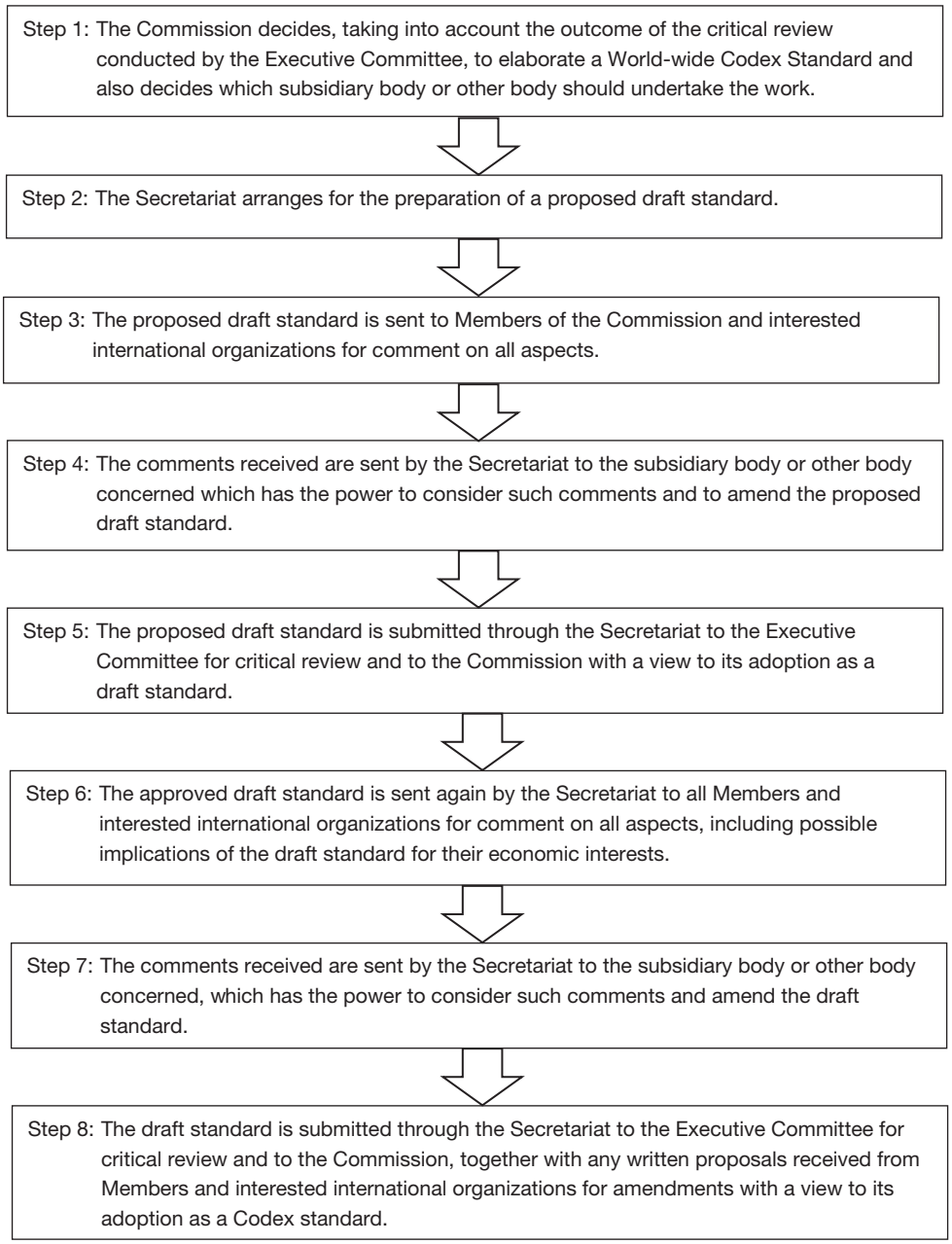

Figure 1 An 8-step procedure for the elaboration of Codex standards.

elaboration process, may also be performed if it is identified by the CAC, or the relevant subsidiary body. The accelerated procedure omits steps 5, 6, and 7 of the regular Codex procedure.

Codex Contact Points are responsible for ensuring that papers are circulated to those concerned within their own country and for ensuring that all necessary action is taken by the date specified.

Risk Analysis

As defined by the CAC, the risk analysis should follow a structured approach comprising the three distinct but closely linked components of risk analysis: risk assessment, risk management, and risk communication, each component being integral to the overall risk analysis. There should be a functional separation of risk assessment and risk management, in order to ensure the scientific integrity of the risk assessment, to avoid confusion over the functions to be performed by risk assessors and risk managers, and to reduce any conflict of interest. Effective communication and consultation with all interested parties should be ensured throughout the risk analysis.

Scientific Basis for Codex Work

Codex committees, when developing standards, apply risk analysis and rely on the independent scientific advice provided by expert bodies organized by FAO and WHO. These bodies also give direct advice to member governments.

From the very beginning, Codex activity is based on the sound science. Experts in a wide range of scientific disciplines have contributed to every aspect of the code to ensure that its standards withstand the most rigorous scientific scrutiny.

The main principles of developing scientific advice are as follows:

- Excellence: Use of internationally recognized expertise, supported by the creation of a platform for global scientific discussions based on best practices in elaborating guidance.
- Independence: Experts contribute in their own capacity and not on behalf of a government or institution. They are required to declare possible conflicts of interest.
- Transparency: Procedures and methods to ensure all interested parties understand the processes for the development of scientific advice and have access to the reports, safety assessments and evaluations, and other basic data.
- Universality: A broad base of scientific data is critical for the elaboration of international standards-setting activities. Therefore, institutions and all interested parties throughout the world are invited to make data available.

Experts responsible for risk assessment should be selected in a transparent manner on the basis of their expertise, experience, and independence with regard to the interests involved.

Risk assessment should incorporate the four steps, hazard identification, hazard characterization, exposure assessment, and risk characterization, and should be based on all available scientific data and on realistic exposure scenarios, with consideration of different situations that include consideration of susceptible and high-risk population groups.

FAO/WHO expert bodies responsible for the scientific advice to CAC and their subsidiary committees are the JECFA, the JMPR, the JEMRA, and other ad hock expert groups conducted by FAO and WHO.

The JECFA is an international expert scientific committee administered by FAO and WHO. It has been meeting since 1956, initially to evaluate the safety of food additives. Its work now also includes the evaluation of contaminants, naturally occurring toxicants, and residues of veterinary drugs in food. JECFA has evaluated more than 2600 food additives, approximately 50 contaminants and naturally occurring toxicants, and residues of approximately 95 veterinary drugs. The committee has also developed principles for safety assessment of chemicals in foods that are consistent with current thinking on risk assessment and take account of developments in toxicology and other relevant sciences.

The JMPR is an expert ad hoc body administered jointly by FAO and WHO in the purpose of harmonizing the requirement and the risk assessment on the pesticide residues. The JMPR has met annually since 1963 to conduct scientific evaluations of pesticide residues in food. It provides advice on the acceptable levels of pesticide residues in food moving in international trade.

The JEMRA began in 2000 in response to requests from the CAC and FAO and WHO member countries and the increasing need for risk-based scientific advice on microbiological food safety issues. JEMRA aims to develop and optimize the utility of MRA as a tool to inform actions and decisions aimed at improving food safety and to make it equally available to both developing and developed countries. JEMRA focuses on the following main areas of work: providing risk assessments for selected pathogens (pathogen–commodity combination risk assessments) to the CAC and to member states, developing guidelines for risk assessment of microbiological hazards in food and water, and providing expert advice on risk management.

FAO and WHO provide expert scientific advice on many aspects of food quality, safety, and nutrition relevant to the work of the CAC. While not officially part of the CAC structure, the FAO/WHO expert consultations provide independent scientific expert advice to the commission and its specialist committees and task forces. The issues being addressed include biotechnology and nanotechnologies.

Codex and International Food Trade

During the last century, a quantity and variety of food traded internationally has grown exponentially. According to FAO statistics, international food trade is more than 500 billion dollars per year market, with billions of tonnes of food produced, transported, and marketed. The increasing global market requires uniform food standards and the Codex international food standards, guidelines, and codes of practice contribute to the safety, quality, and fairness of this international food trade. Consumers can trust the safety and quality of the food they buy and importers and distributers can trust that the quality of food they ordered will be in accordance with their specifications. Reference to the Codex Alimentarius occurs in many bilateral and plurilateral trade agreements.

The WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and the Agreement on Technical Barriers to Trade (TBT Agreement) both encourage the international harmonization of food standards, and

Codex standards have become the benchmarks against which national food measures and regulations are evaluated within the legal parameters of WTO agreements. WTO members that wish to apply stricter food safety measures than those set by Codex may be required to justify these measures scientifically.

See also: Codex Alimentarius Commission: Role in International Food Standards Setting; Food and Agriculture Organization of the United Nations; Food Classification and Description; Food Composition Databases; Food Fraud; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Heavy Metal Toxicology; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling.

Further Reading

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Joint FAO/WHO Food Standards Programme (2013) *Codex Alimentarius Commission procedural manual*, 21st ed. Rome: FAO and WHO.

Relevant Websites

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http://www.fao.org/food/food-safety-quality/scientific-advice/other-scientific-advice/en/ – Other scientific advice at FAO.
ftp://ftp.fao.org/codex – Codex ftp link.
www.standardsfacility.org – Standards and Trade Development Facility.
www.who.int/foodsafety/codex/trustfund/en/ – Codex Trust Fund.
www.who.int – World Health Organization (WHO).
http://www.who.int/foodsafety/chem/jecfa/en/ – JECFA at WHO.
http://www.who.int/foodsafety/chem/jmpr/en/ – JMPR at WHO.
http://www.who.int/foodsafety/micro/jemra/en/ – JEMRA at WHO.
http://www.who.int/foodsafety/en/ – Other scientific advice at WHO.
www.wto.org – World Trade Organization (WTO).

Codex Alimentarius Commission: Role in International Food Standards Setting

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Introduction

Since ancient times, food standards have been laid down by competent authorities or governments for the protection of consumers and the facilitation of trade. Food laws are enacted to protect the consumers against unsafe, adulterated, and misbranded food, and also to facilitate the movement of food across borders. With technological advancements in storage and transportation, the twentieth century has witnessed an exponential increase in food trade. However, the independent development of food standards in different countries led to barriers in trade.

The establishment of the Food and Agriculture Organization (FAO) in 1945 and the World Health Organization (WHO) in 1948 led to an increased focus on food and health and thus began a series of joint expert meetings. In 1950, experts at the first meeting of the Joint FAO/WHO Expert Committee on Nutrition stated, "Food regulations in different countries are often conflicting and contradictory. Legislation governing preservation, nomenclature, and acceptable food standards often varies widely from country to country. New legislations not based on scientific knowledge is often introduced, and little account may be taken of nutritional principles in formulation regulations." The Committee noted that the conflicting nature of regulations may be an obstacle to trade and may therefore affect the distribution of nutritionally valuable food and suggested that FAO and WHO study these problems more closely.

The fourth session of the FAO/WHO Expert Committee on Nutrition stated, "The increasing, and sometimes insufficiently controlled, use of food additives, has become a matter of public and administrative concern." It also noted that the means of solving problems related to food additives may differ from country to country, and this fact "must in itself occasion concern, since the existence of widely differing control measures may well form an undesirable deterrent to international trade." In the same year, 1955, the FAO and WHO convened the first joint FAO/WHO Conference on Food Additives. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) began work and, in its first meeting, articulated the general principles for the use of food additives; a text that still forms the framework for the consideration of food additive use.

At the same time, there were other developments taking place at the international level involving food standards. In 1958, the United Nations Economic Commission for Europe (UNECE) established the Geneva Protocol, in which a harmonized layout for food commodity standards was proposed. The layout still forms the basis of most food commodity standards worldwide, including Codex standards. Similarly, the International Dairy Federation, founded in 1903, had worked on standards and labeling requirements for milk and milk products. This work was taken over by the Joint FAO/WHO Expert Committee of Government Experts on the Code of Principles concerning Milk and Milk Products. The Committee developed formal procedures for the elaboration of its standards, which involved consultation with the governments between the meetings of the Committee itself. After the Second World War, there was a regional effort to harmonize the food standards in Latin America and Europe.

In February, 1961, the Director-General of the FAO, B.R. Sen, actively entered into discussion with the WHO, the UNECE, the Organisation for Economic Cooperation and Development, and the Council of the Codex Alimentarius Europaeus, with proposals that would lead to the establishment of Joint FAO/WHO Food Standards Programme. In November, 1961, the eleventh session of the conference of the FAO passed the resolution by which the Codex Alimentarius Commission (CAC) was established.

The Joint FAO/WHO Food Standards Conference, convened in Geneva in 1962, established the framework for cooperation between the two agencies. The CAC was to be the body responsible for implementing the Joint FAO/WHO Food Standards Programme, and it was also endorsed in May 1963 at the sixteenth World Health Assembly.

The first session of the CAC was held in Rome in October 1963, and it was attended by delegates from 30 countries and 16 international organizations. Currently, the CAC has 186 Codex Members, 185 member countries, and 1 member organization (EU); 220 Codex Observers, 50 international governmental organizations, 154 nongovernmental organizations (NGOs), and 16 UN agencies. The thirty-seventh session of CAC, held in 2014 in Geneva, was attended by delegates from 170 countries; one Member organization; and 28 international governmental, and nongovernmental organizations, including UN agencies. The headquarters of Codex Alimentarius is based in Rome and the Codex Secretariat is also located in the FAO building in Rome. However, the meetings of CAC are held in every alternate year in either Rome or Geneva.

Purpose

The Codex Alimentarius is a collection of internationally adopted food standards and related texts presented in a uniform manner. It develops harmonized international food standards, guidelines, and codes of practice to protect the health of consumers, and to ensure fair practices in the food trade. These food standards and related texts aim at protecting consumers' health and ensuring fair practices in the food trade. The publication of the Codex Alimentarius is intended to guide and to promote the elaboration and establishment of definitions and requirements for foods to assist in their harmonization, and, in doing so, to facilitate international trade. The Commission also promotes the coordination of all food standards work undertaken by international governmental and nongovernmental organizations.

Scope

The Codex Alimentarius includes standards for all the principle foods, whether processed, semiprocessed, or raw, for distribution to the consumer. Materials for further processing into foods should be included to the extent necessary to achieve the purposes of the Codex Alimentarius, as defined. The Codex Alimentarius includes standards/guidelines/codes of practices for food hygiene, food additives, residues of pesticides and veterinary drugs, contaminants, commodities (e.g., milk, meat, fruits and vegetables, and processed food), labeling and presentation, methods of analysis and sampling, and import and export inspection and certification. Thus, it looks at both horizontal and vertical standard setting so far as food is concerned.

Codex standards and related texts are not a substitute for, or an alternative to, national legislation. Every country's laws and administrative procedures contain provisions with which it is essential to comply. Codex standards and related texts contain requirements for food aimed at ensuring for the consumer a safe, wholesome food product free from adulteration, correctly labeled and presented. A Codex standard for any food or foods should be drawn up in accordance with the Format for Codex Commodity Standards and contain, as appropriate, the sections listed therein.

In 1995, the Codex standards, guidelines, and Codes of practice became a reference for food safety in the World Trade Organisation (WTO) Agreement on Sanitary and Phytosanitary measures. The only other organizations mentioned are the World Organization for Animal Health for animal health issues and the International Plant Protection Convention for plant health.

Structure of Codex Alimentarius Commission and Its Subsidiary Bodies

The CAC consists of the following main organizational elements, each of which has a role to play in the achieving the mandate of the Commission (Figure 1):

(a) The Commission
(b) The Executive Committee
(c) The Codex Subsidiary bodies
(d) The Codex Secretariat

Codex Alimentarius Commission

While passing the resolutions at the eleventh session of the FAO Conference in 1961 and the sixteenth World Health Assembly in 1963, which led to the establishment of the Codex Alimentarius Commission, the two bodies also adopted the Statues and Rules of Procedure for the CAC, the main decision making body of the Joint FAO/WHO Food Standards Programme. The legal basis for the Commission's work is provided by the Statutes. The Membership to the Commission is open to all Member Nations and Associate Members of the FAO and/or WHO. The Commission meetings, which are held annually (in July) in Rome or Geneva, are chaired by the Chairperson, and he/she is assisted by the three Vice-Chairpersons.

Executive Committee of the Codex Alimentarius Commission

The Executive Committee consists of the Chairperson and three Vice-Chairpersons of the Commission, as well as the

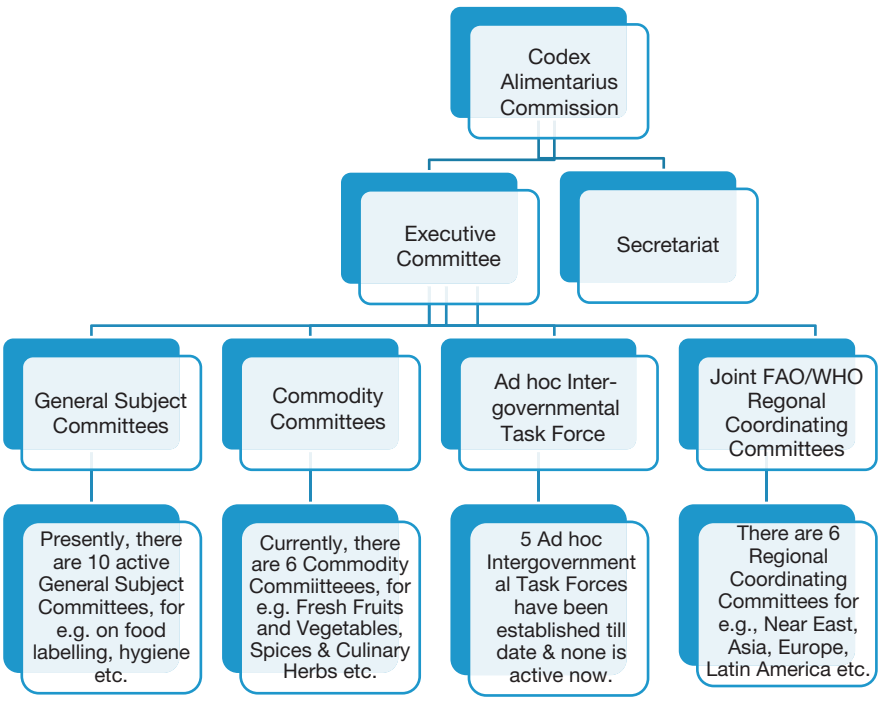

Figure 1 Organizational structure of the Codex Alimentarius Commission.

Regional Coordinators, together with seven further Members elected by the Commission at regular sessions from among the Members of the Commission, one each coming from the following geographic locations: Africa, Asia, Europe, Latin America and the Caribbean, Near East, North America, and South-West Pacific. The Executive Committee acts on behalf of the Commission as its executive organ between sessions of the Commission. In particular, the Executive Committee may make proposals to the Commission regarding general orientation, strategic planning, and programming of the work of the Commission; study special problems; and assists in the management of the Commission's program of standards development, namely by conducting a critical review of proposals to undertake work and by monitoring the progress of standards development.

Subsidiary Bodies Under the CAC

There are two kinds of Subsidiary bodies, the Codex Committees, which prepare draft standards for submission to the Commission and the Coordinating Committees, through which regions or groups of countries coordinate food standards activities in the region. The Commission may also approve a third type of subsidiary body called a Codex ad hoc Intergovernmental Task Force (TF), which is a Codex Committee with very limited terms of reference (TOR), established for a fixed period of time. Section V of the CAC Procedural Manual enlists the table of Committees and their TORs.

The Codex Committees are further divided into the General Subject Committees and the Commodity Committees.

General subject committees

These Committees are so called because their work has relevance for all Commodity Committees, and this work applies across all Commodity Committees. These are sometimes referred to as 'horizontal committees.' Currently, the following ten General Subject Committees are functioning:

- Codex Committee on Contaminants in Foods (CCCF)
- Codex Committee on Food Additives (CCFA)
- Codex Committee on Food Hygiene (CCFH)
- Codex Committee on Food Import and Export Inspection and Certification Systems (CCFICS)
- Codex Committee on Food Labeling (CCFL)
- Codex Committee on General Principles (CCGP)
- Codex Committee on Methods of Analysis and Sampling (CCMAS)
- Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU)
- Codex Committee on Pesticide Residues (CCPR)
- Codex Committee on Residues of Veterinary Drugs in foods (CCRVDF)

Commodity Committees

Commodity Committees are responsible for developing standards for specific foods or classes of foods based on the composition. They are often referred to as the 'vertical standards.' These Committees convene as necessary and go into recess or are abolished when their work is complete. There are currently six active Commodity Committees, as follows:

- Codex Committee on Fish and Fishery Products (CCFFP)
- Codex Committee on Fresh Fruits and Vegetables (CCFFV)
- Codex Committee on Spices and Culinary Herbs (CCSCH)
- Codex Committee on Fats and Oils (CCFO)
- Codex Committee on Processed Fruits and Vegetables (CCPFV)
- Codex Committee on Sugars (CCS)

The following Commodity Committees work through correspondence or are in recess:

- Codex Committee on Cereals, Pulses, and Legumes (CCCPL)
- Codex Committee on Cocoa Products and Chocolate (CCCPC)
- Codex Committee on Meat Hygiene (CCMH)
- Codex Committee on Milk and Milk Products (CCMMP)
- Codex Committee on Natural Mineral Waters (CCNMW)
- Codex Committee on Sugars (CCS)

The following Commodity Committees that have been abolished are as follows:

- Codex Committee on Edible Ices (CCIE)
- Codex Committee on Meat (CCM)
- Codex Committee on Meat and Poultry Products (CCMPP)
- Codex Committee on Soups and Broths (CCSB)

Joint FAO/WHO Regional Coordinating Committees

The Membership is open to all Member Nations and Associate Members of the FAO and/or WHO, which are also members of the CAC within the relevant geographic location. There are six Regional Coordinating Committees, as given below:

- FAO/WHO Coordinating Committee for Africa (CCAFRICA)
- FAO/WHO Coordinating Committee for Asia (CCASIA)
- FAO/WHO Coordinating Committee for Europe (CCEURO)
- FAO/WHO Coordinating Committee for Latin America and the Caribbean (CCLAC)
- FAO/WHO Coordinating Committee for North America and South-West Pacific (CCNASWP)
- FAO/WHO Coordinating Committee for Near East (CCNEA)

Each Committee meets once in a year or once every 18 months or 2 years, depending upon its nature. The Codex Alimentarius Commission has designated a member country of the Commission, which has indicated its willingness to accept financial and other responsibility, such as appointing a Chairperson of the Committee. This country is referred to as the host country. The host country is responsible for appointing the Chairperson of the Committee from among its own nationals. Should this person for any reason be unable to take the chair, the host country shall designate another person to perform the functions of the chairperson for as long as the chairperson is unable to do so.

Ad hoc Intergovernmental Task Force

As per Statute 7 of the CAC, like other subsidiary bodies, the Commission may establish an ad hoc Intergovernmental Task Force (TF) for a specific purpose and specified period.

Presently, there are no TFs that are active. However, the following TFs were established and then abolished on completion of their assigned work as per their TORs:

- Task Force on Animal Feeding (TFAF): 1999–2004 and 2011–2013
- Task Force on Foods Derived from Biotechnology (TFFBT): 1999–2003 and 2004–2008
- Task Force on Fruit and Vegetable Juices (TFFJ): 1999–2005
- Task Force on the Processing and Handling of Quick Frozen Foods (TFPHQFF): 2006–2008
- Task Force on Antimicrobial Resistance (TFAM): 2006–2011

The Codex Secretariat

The Codex Secretariat is located at FAO headquarters in Rome. The Secretariat organizes the meetings of the Commission and the Executive Committee, and it facilitates the work of the Subsidiary bodies in close coordination with the secretariat of the host country.

Scientific Basis for Codex Work

Within Codex, risk analysis is defined as "a process consisting of risk assessment, risk management, and risk communication" (Figure 2). It is a structured, systematic approach that examines the potential adverse health effect consequential to a hazard, or conditions of food, and it develops options for mitigating the risk. Risk analysis should be:

- applied consistently;
- open, transparent, and documented;
- conducted in accordance with both the Statements of Principle Concerning the Role of Science in the Codex Decision-making Process and the extent to which other factors are taken into account and the Statements of Principle Relating to the Role of Food Safety Risk Assessment; and
- evaluated and reviewed as appropriate in the light of newly generated scientific data.

Figure 2 Process of risk analysis.

Risk analysis is an integral part of the decision-making process of the Codex, and the Commission has adopted the definition of risk analysis, as well as the *Working principles for risk analysis for application in the framework of the Codex Alimentarius*. Risk-analysis principles to be applied by the Committees on Food Additives; Contaminants in Food; Residues of Veterinary Drugs in Foods; Pesticide Residues; Food Hygiene and the Nutrition and Food for Special Dietary Uses have been adopted by the Commission.

Risk Assessment

When developing standards, Codex Committees apply risk analysis and rely on the independent scientific advice provided by expert bodies organized by the FAO and WHO, as illustrated in Figure 3. However, scientific advice may also be provided through a series of ad hoc meetings on a given topic or ad hoc expert consultations that are convened only once to address a specific topic.

These bodies also give direct scientific advice to Member Governments. There are four FAO/WHO expert committees, namely: (1) Joint FAO/WHO Expert Committee on Food Additives (JECFA); (2) Joint FAO/WHO Meeting on Pesticide Residues (JMPR); (3) Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA); and (4) Joint FAO/WHO Expert Meeting on Nutrition (JEMNU), with other scientific advice provided by the FAO and WHO. The funds for Codex Scientific Advice are primarily provided by the FAO and WHO, and several countries also support the FAO and WHO in this important work.

A four-step process for risk assessment (Figure 4) is followed by these bodies.

Advice from the JECFA is considered by CCFA, CCCF, and CCRVDF, as it is responsible for providing scientific advice on:

- Food additives
- Contaminants in foods
- Residues of veterinary drugs in foods

Similarly, the JMPR is responsible for evaluating exposure to pesticides and recommending maximum residue levels (MRLs) for the new and periodic review of compounds for dietary intake purposes. There is well-established criteria for the prioritization of compounds for evaluation by the JMPR by the CAC. It is thus responsible for performing the risk assessment upon which the CCPR and, ultimately, the CAC base their risk management decisions.

The JEMRA are commissioned by the CCFH through the FAO and WHO for performing international risk assessments upon which the CCFH and the CAC base microbiological risk management options. The specific request to the FAO or WHO for microbiological risk assessment by the CCFH will include the risk profile document, a clear statement of purpose and the scope of the work to be undertaken, any time constraints facing the committee that could impact the work, and the specific risk management questions to be addressed by the risk assessors.

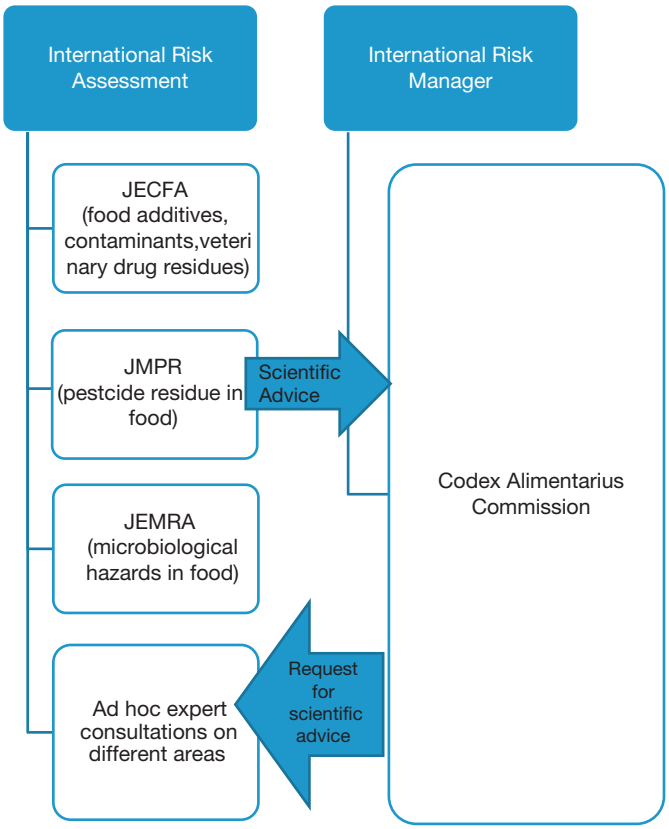

Figure 3 Roles of risk assessor and risk manager.

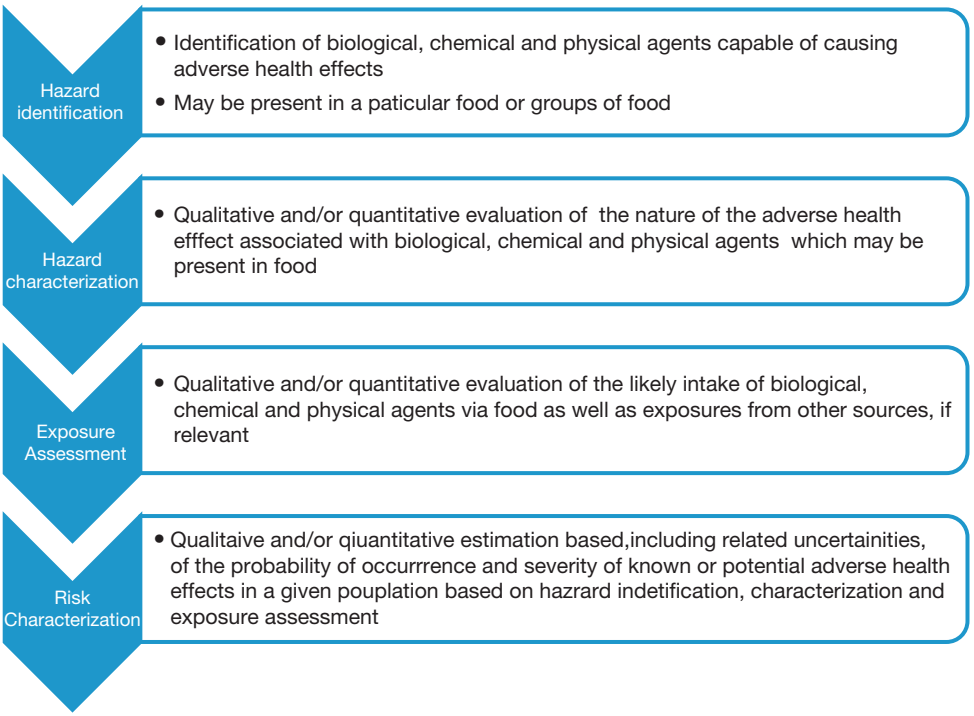

Figure 4 A four-step risk assessment process.

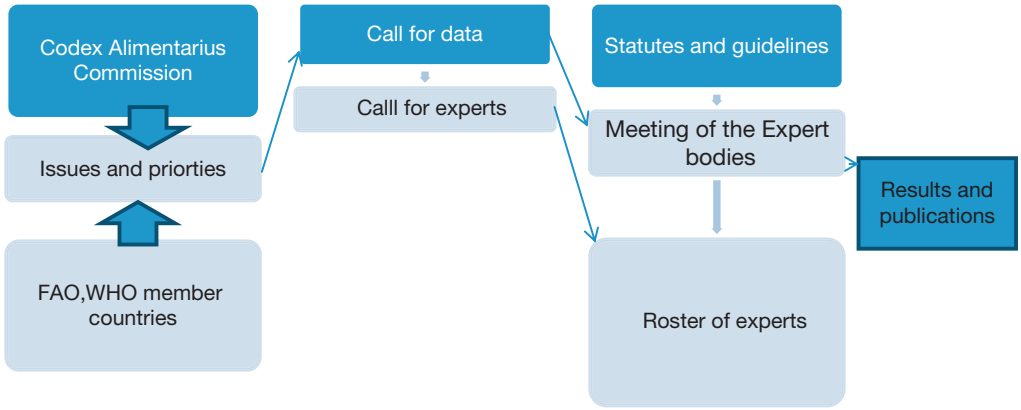

Figure 5 Process for the provision of scientific advice by expert bodies.

So far as the nutritional risk assessment advice to the CCNFSDU and CAC is concerned, the FAO and WHO are acknowledged as the primary sources of this advice.

Procedure for the provision of scientific advice by expert bodies

The steps involved in the provision of the scientific advice are listed in Figure 5.

Identification and prioritization of issues referred by the CAC or by member countries of the FAO and WHO.

- Call for data to facilitate the evaluation of the identified issue: The data can be provided by the industry, wherever applicable, or national data can be provided by the regulatory agencies or scientific institutes.
- The data submitted is examined by a group of experts who are elected in their personal capacity and not as representatives of their country or of the institute where they may be employed. In appointing experts, the FAO and WHO consider the scientific and technical excellence, diversity and complementarity of scientific backgrounds and opinions, and geographical as well as gender balance. The exact composition of the group will depend on the nature of the expert advice required, but it may include representatives of the natural sciences, including chemists, biologists, toxicologists, and public health specialists, as well as experts from other fields.
- Based on the data that has been submitted and the expert group consultations, the outcomes of the risk assessment are published.

Risk Management: Elaboration of Standards by the CAC

When a Codex subsidiary body (i.e., a committee or a task force) proposes to elaborate a standard, code of practice, or related text within its items of reference, it should consider the following:

- Priorities that were established by the Commission in the Strategic Plan of Work
- Any specific relevant strategic project currently being undertaken by the Commission
- Feasibility of completing the work within a reasonable period of time

The CAC has well-established criteria, to be applied in determining priorities for the inclusion of tasks in the program of work of committees and ad hoc task forces. These criteria are generally addressed when a member country makes a submission to a committee for new work or for the review of an existing, or adopted, Codex text. If the proposal falls outside of the committee's terms of reference, the proposal is referred to another committee or reported to the Commission in writing, together with proposals for amendments to the committee's terms of reference.

The initial proposal for a new or ammended Standard or related text comes from a country or group of countries that raises the issue at a Codex committee or an FAO/WHO coordinating committee. A committee proceeds with work on a new standard only once it has been approved by the Commission.

When a committee or task force starts to elaborate a standard whose development has been approved by the Commission, there is a step procedure to be followed. The normal procedure has eight steps, although an accelerated five-step procedure may be used if agreed to by at least two-thirds of the Members of the Commission. Most of the Codex documents are elaborated through this step process. Some Codex documents are developed outside of the step process, such as internal documents to guide the work of a specific Committee.

Project documentation

When a committee or other subsidiary body of the Commission is considering elaborating a standard code of practice, or related text, the committee will prepare project documentation for submission to the Executive Committee and the Commission. This documentation will provide the information required by the Commission to determine whether or not the work should be approved, and will be the basis for the Executive Committee's critical review and monitoring of the progress of the work. This project documentation is not required for individuals maximum residue limits for pesticides

or veterinary drugs, or the maintenance of standards and texts such as the General standard on food additives or international numbering system.

Project documentation should consist of the following:

- Purpose of the proposed standard
- Indication of its relevance to the Codex strategic objectives
- Scope of the proposed standard
- Assessment of the proposed standard against the criteria for the establishment of work priorities
- Proposed time line for completion of the work (including, as a minimum, start date, proposed date for adoption at step 5, and proposed date for final adoption by the Commission)
- Identification of expert advice requirements
- Identification of any issues related to the needs of developing countries.

The preparation of this project documentation is the responsibility of the Member proposing the new work. It should be prepared in sufficient time for the committee to reach consensus on whether or not to recommend the work and subsequent consideration by the Executive Committee and the Commission.

Specific criteria for determining priorities in the work of committees and task forces

The specific criteria that are applied while evaluating a proposal for the elaboration of a standard are different for general and the vertical subject committees as given below:

Criteria applicable to general subject committees

- Contribution to the protection of consumers' health and prevention of fraudulent practices
- Diversification of national legislation and apparent resultant or potential impediments to international trade
- Scope of the work undertaken and the establishment of priorities between the various sections of the work
- Work already undertaken by other international organizations in this field will be considered

Criteria applicable to commodity committees

- Contribution to the protection of consumers' health and prevention of fraudulent practices
- Volume of production and consumption in individual countries and volume and pattern of trade between countries
- Diversification of national legislation and apparent resultant or potential impediments to international trade
- International or regional market potential
- Amenability of the commodity to standardization
- Coverage of the main consumer protection and trade issues by existing or proposed general standards
- Number of commodities that would need separate standards indicating whether raw, semiprocessed, or processed products are to be included in the standard
- Work already undertaken by other international organizations in this field

Procedure for the elaboration of Codex standards

Part 3 and Part 4 of Section II of the *Codex Procedural Manual*, which is updated as and when necessary, lays down the procedures for the elaboration of codex standards and related texts through an eight- or five-step process.

The eight-step uniform procedure for the elaboration is as follows:

Step 1: The Commission decides to elaborate a standard and assigns the work to a committee, after taking into account the outcome of the critical review conducted by the Executive Committee. A decision to elaborate a standard may also be taken by a committee, but it is subject to endorsement by the Commission. In the case of regional standards, the Commission bases its decision on the proposal of the majority of the members belonging to the region or group of countries submitted at the CAC session.

Step 2: The Secretariat arranges the preparation of a proposed draft standard. In the case of MRLs for residues of pesticides or veterinary drugs, as well as MLs in case of the contaminants in food, the Secretariat distributes the recommendations of the JECFA and JMPR and any other information on risk assessment work conducted by the FAO and WHO.

Step 3: The proposed draft standard is sent to members of the commission and interested international organizations for comment on all aspects.

Step 4: The comments received are forwarded by the Secretariat to the concerned subsidiary body or other body concerned, which, as per the TOR, has the mandate to consider the comments and to propose amendments to the draft standard.

Step 5: The proposed draft standard is submitted through the Secretariat to the Executive Committee for critical review and to the Commission for its adoption as a draft standard. The Commission gives due consideration to the outcome of the critical review and the comments that may be submitted by any of its members.

Step 6: The draft standard is sent by the Secretariat to all Members and interested international organizations for comment on all aspects, including possible implications of the draft standard for their economic interests.

Step 7: The comments received are sent by the Secretariat to the concerned subsidiary body or the other body concerned, which has the power to amend the draft standard.

Step 8: The drafts standard is submitted through the Secretariat to the Executive Committee for critical review and to the Commission for adoption as a Codex standard. At this step again, the Commission gives due consideration to the outcome of the critical review and comments submitted by the members, and members may take part in the debate.

The five-step uniform, accelerated procedure for the elaboration

On the basis of a two-thirds majority of votes cast, and taking into account the outcome of the critical review conducted by the Executive Committee, the CAC shall identify those standards that shall be subject to an accelerated elaboration process. While making a decision to use the accelerated elaboration process, relevant consideration could be:

- Matters concerning new scientific information
- New technologies

- Urgent problems relating to trade or public health
- Revision or updating the existing standards

Once the Commission decides to elaborate a standard on the basis of a two-thirds majority of votes cast using the accelerated procedure and assigns the work to a subsidiary body or other body, the Secretariat arranges the preparation of a proposed draft standard, and the proposed draft standard is sent to members of the Commission and the interested international organizations for their comments. During step 2, the recommendations of the risk assessment done by the JECFA, JMPR, and other FAO/WHO consultations are made available. In step 3, the proposed draft standard is sent to the members of the Commission and interested international organizations for their comments, and when standards are subject to the accelerated procedure, members of the Commission and the interested international organizations are notified.

In step 4, the Secretariat forwards comments to the subsidiary body or other body for consideration and amendments to the proposed draft standard, if any. In step 5, the proposed draft standard subject to the accelerated elaborating procedure is sent to the Executive Committee for critical review and then to the Commission through the Secretariat, together with any written proposals from Members and interested international organizations, for adoption as a Codex standard.

Unlike in the eight-step procedure, the proposed draft standard or related text is submitted to the members of the Commission and other international organizations and to the Executive Committee only once.

The final adoption of the Codex standard, either through the eight- or the five-step procedure, can be made only at the plenary session of the CAC.

Measure to facilitate consensus while adopting standards

At the Twenty-sixth Session of the Commission in the year 2003, it was decided that every effort should be made to reach agreement on the adoption or amendment of standards by consensus. All efforts should be made to ensure that the process of standard-setting is open, transparent, inclusive, and science-based.

Risk Communication

After the adoption of the Codex standard by the CAC, it is published and issued to all Member states and Associate Members of the FAO and/or WHO, as well as to international organizations. They are also available on the Codex Alimentarius website (www.codexalimentarius.org).

The documents published by Codex can be classified as:

- Standards and MRLs, which contain mandatory requirements (are identified as CODEX STAN and CAC MRL/ML)
- Codes of practices, which are recommendations in general or for specific manufacturing or handling process (are identified as CODEX RCP)
- Guidelines, directed at the Codex subsidiary bodies and national governments, which are usually advisory texts (are identified as CAC GL)

Methods of analysis are considered as part of the standard or the MRL. Till date, the CAC has adopted the following Codex standards and related texts (Table 1).

Role of Codex Standards in the Framework of International Trade

Following the Marrakesh Agreement in 1994, which led to the creation of the WTO in 1995, the Agreement on the Application of Sanitary and Phytosanitary measures (the SPS agreement) and the Agreement on Technical Barriers to Trade (TBT Agreement) also went into effect. The SPS agreement has recognized and chosen the Codex standards, guidelines, and codes of practices, as well as the recommendations for food additives, veterinary drugs and pesticide residues, contaminants, methods of analysis, and sampling. Similarly, the Codex standards have assumed considerable significance under the Technical Regulations and standards provisions contained in Article 2 of the TBT agreement.

The recognition of the scientifically based Codex standards and the related texts within the framework of the SPS and TBT agreements has given a big flip to the work of Codex and also generated more interest in its Members. There has been increased Member attendance at Codex meetings, particularly from the developing countries.

Conclusion

At its Thirty-sixth Session in 2013, Codex Alimentarius celebrated its fiftieth year of establishment. It is a matter of great satisfaction that the membership has grown from 30 in 1963 to 186. It reinforces the confidence in the organization's work and the extremely important responsibility of laying down the global parameters for the quality and safety of food products for human consumption that the organization is shouldering. However, to maintain relevance in the rapidly changing

Table 1 List of adopted Codex standards and related texts

Type of text	*Number*
1. Codes of practice, both general and for specific production and handling processes	49
2. Guidelines	72
3. Individual food standards	212
4. Miscellaneous	4

The Codex Standards for Food Additives (GSFA, General Standards of Food Additives) list the food additives that have been adopted by the CAC (updated up to 36th session).

Codex MRLs for pesticides (updated up to the 36th session of the CAC), veterinary drugs (updated up to the 35th session of CAC), and residues

Source: www.codexalimentarius.org.

environment, where everyday new scientific developments are taking place, new food safety concerns are emerging, consumer preferences are changing rapidly, and there is increased global trade, the CAC must be more nimble-footed in setting standards. There is a need to make the process of standard-setting more inclusive by making an extra effort to encourage the developing countries to submit the necessary data.

The Codex Strategic Plan for the period 2014–19 that was adopted at the Thirty-sixth Session would need to be followed and monitored to ensure that the twin mandates of the Codex Alimentarius work are followed in letter and spirit.

See also: Food and Agriculture Organization of the United Nations; World Health Organization.

Further Reading

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Relevant Websites

www.codexalimentarius.org – About Codex.
www.wto.org – The WTO and the FAO/WHO Codex Alimentarius.

Coenzymes and Cofactors

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Introduction

Metabolism represents an array of chemical reactions that usually involve the transfer of specific functional groups from which substances are produced, maintained, or destroyed; and energy is made available to produce heat or work. Such transfer reactions often depend on aid from cofactors. Herein, cofactors are described that are involved in (1) bond making and breaking steps, (2) redox reactions, (3) the transfer of functional groups, and (4) changes in cellular energy or chemical potentials. In general, cofactors facilitate the coupling of the spontaneous processes of catabolism to the nonspontaneous processes of anabolism. As biocatalysts, cofactors provide the possibility of differing transition states that lower the activation energy of given reactions. As such, cofactors may increase reaction rates or enable reactions at lower temperatures. Cofactors can also affect the reaction environment or bind to substrates to change physical and chemical properties that make them more susceptible to chemical alteration. When associated with a corresponding apoenzyme, the presence of a cofactor extends the catalytic capacity of the resulting holoenzyme or functional enzyme (i.e., an apoenzyme plus cofactor). Moreover, when linked to specific metabolic processes, a cofactor may provide additional regulatory functions by acting as allosteric modulators.

For clarity and organization, the term cofactor will be used throughout to describe both organic and inorganic compounds that are involved as catalysts in enzymatic or biologically relevant chemical reactions. In this regard, it is also important to note that some terms have very specific meanings. For example, 'coenzyme' will be used to define organic cofactors that work in conjunction with apoenzymes. The term prosthetic group will be used when a given cofactor is nondissociable with respect to its corresponding enzyme; cosubstrate will be used when binding is dissociable. When metals serve as cofactors, they usually fall into two categories, that is, metals important to metalloenzymes or metals important to metal–enzyme complexes. The stability of the interaction defines whether the term metalloenzyme or metal–enzyme complex is used. Metalloenzymes have metal binding constants of 10^8–10^9 or greater. Metal–protein complexes have constants of 10^5 or less. To broaden the discussion, comments regarding biofactors that are responsible for nonenzymatic chemical catalysis, such as the tocopherols, are also included. Throughout the article, a number of examples and descriptions of cofactor function are described; however, our primary descriptive goal is to portray the broad range of mechanistic approaches that are involved in biological conversions important to metabolism in various animal species.

Biochemical Functions

Role of Cofactors in Catalytic Processes

Cofactors aid in creating alternative routes for reactions and, as a consequence, aid in reducing the amounts of energy required to reach the reactions' highest activation transition state. Accordingly, more of a given substrate can proceed to the formation of product. Cofactors improve enzyme–substrate interactions by combinations of the following mechanisms: (1) effecting conformation changes that improve interactions and the binding of a substrate to an enzyme, (2) causing bond strain to align a portion of a substrate so it is better positioned to engage in a reaction, (3) altering the nucleophilic or electrophilic properties of a substrate, (4) altering the environment at the active site of an enzyme (e.g., water structure around the active site), (5) forming transient covalent bonds with residues within the active site to lessen the energy of substrate moiety transfers, and (6) enhancing electron tunneling (increasing movement of electrons to an active site). The active site of enzymes is usually found in a 3-D groove or pocket. Cofactors extend chemical options or improve on entropic considerations by providing an extendable arm to place a substrate or an intermediate closer to the active center of an enzymatic process. Cofactors facilitate transition-state formation, a transition in which the targeted molecule is no longer a substrate, but not yet a product.

As shown in Figure 1, one may describe chemical reactions in terms of the 'activation' or 'free energy' needed to initiate a chemical transition or reaction. In this regard, many reactions proceed with multiple steps and reaction intermediate transition states. Note that the activation energy is lowered, but the initial energy of the starting reactants or their final products is not changed.

Listed in Table 1 are well-recognized cofactors derived from water-soluble vitamins and vitamin K, as well as important nonvitamin-derived cofactors. In addition, examples of essential dietary minerals utilized as cofactors are summarized in Table 2, particularly those involved in redox reactions.

Role in Entasis (Facilitation of Energized States in Proteins and Metalloproteins)

The term entasis comes from the Greek meaning 'to stretch or strain tight.' When metal cofactors are involved, the state of entasis (e.g., conformational strain) is relieved by a loss of an electron from metals that are a part of a protein complex. The electron transfer is more thermodynamically favorable. In many enzymes for which elements, such as calcium, magnesium, zinc, sodium, and potassium, are required or influence

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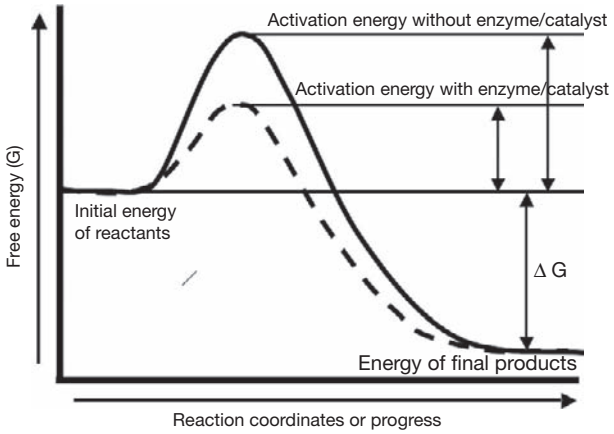


Figure 1 The relationship between reaction progress, activation energy, and the function of enzymes as catalysts. The activation energy (also known as the Gibbs free energy (G) or free enthalpy) is the minimum energy required to start a reaction. With respect to enzyme-catalyzed reactions, simply stated, an enzyme (often with the aid of a cofactor) acts to lower the activation energy needed to start the reaction.

catalytic activity, their roles are often to facilitate entasis-like processes.

In contrast, cofactors derived from organic compounds are less capable of hydrolyzing or changing the chemical character of biological compounds, because of their lower redox potentials. The typically higher reduction potentials of metal-containing complexes and/or the energy associated with entasis circumvent this problem. Metalloenzyme complexes are often several orders of magnitude more proficient as redox catalysts than organic-derived cofactors capable of redox. As it relates to 'entasis,' simple metal complexes tend to equilibrate quickly with substrates and products and other components in the reaction environment. However, when combined in a complex protein structure (i.e., an enzyme), entatic strain caused by the inclusion of metal cofactors makes the initial phases of the reactions much more energetically favorable. The reaction center is also less compromised by nonspecific interactions. Accordingly, an active site of an enzyme can be thought of as existing more in isolation than a component that is in equilibrium with cellular compounds and compounds not directly related to the reaction.

Table 1 Coenzymes and cofactors

Vitamin-derived coenzymes and cofactors

Cofactor name	*Common abbreviations for the cofactor*	*Precursor*	*Modifications leading to cofactor formation*	*Chemical group(s) transferred or types of reactions catalyzed*
Ascorbic acid		Vitamin C	The biosynthesis of ascorbic acid in many animals starts with the formation of UDP glucuronic acid in a pathway important for D-glucuronic acid synthesis. L-gulonolactone, a product of the pathway, reacts with oxygen, catalyzed by the enzyme L-gulonolactone oxidase, to produce ascorbic acid. In humans, L-gulonolactone oxidase is missing; thus, a dietary source of ascorbic acid is required	Ascorbic acid is a reducing agent and antioxidant. It often is utilized as a cofactor for monooxygenase (hydroxylase)-catalyzed reactions
Biotin; coenzyme R		Biotin (vitamin H and vitamin B_7)	Biotin as a cofactor is covalently linked. The enzyme biotinidase catalyzes the cleavage of biotin from biotinyl peptides (the proteolytic degradation products of holo-(biotin-requiring) carboxylases, which allows recycling and reutilization of biotin)	Biotin is essential for carboxylation reactions involving the addition of CO_2, important in the synthesis of fatty acids, isoleucine, and valine and in gluconeogenesis
Cobalamins: Hydroxocobalamin Methylcobalamin Adenosylcobalamin	B_{12}, OHCbl, or B_{12a} MeCbl or MeB12 AdoCbl	Vitamin B_{12}, cyanocobalamin (commercial preparation of B_{12} in which a CN moiety	In humans, the various forms are rapidly interconverted	Together with folic acid, cobalamins are essential for DNA synthesis. Methylcobalamin serves as a source of transferable 'CH_3—' groups.

(*Continued*)

Table 1 (Continued)

Vitamin-derived coenzymes and cofactors

Cofactor name	*Common abbreviations for the cofactor*	*Precursor*	*Modifications leading to cofactor formation*	*Chemical group(s) transferred or types of reactions catalyzed*
		replaces the methyl group)		Adenosylcobalamin serves as a cofactor for isomerization reaction, rearrangements in which a hydrogen atom is directly transferred between two adjacent atoms (e.g., the conversion methylmalonyl-CoA to succinyl-CoA by methylmalonyl-CoA mutase)
Coenzyme A	CoASH	Pantothenic acid (vitamin B_5)	Coenzyme A is synthesized in a multistep process that requires four ATPs, pantothenate, and cysteine	CoASH aids in the transfer of acetyl group and other acyl groups (e.g., TCA-related oxidations and fatty acid metabolism)
Flavin adenine dinucleotide	FAD	Riboflavin (vitamin B_2)	FAD consists of a riboflavin moiety bound to the phosphate group of an ADP molecule	Catalyzes high-energy electron transfer reactions important in oxidative phosphorylation, common cofactor for enzymes with reductase activity
Flavin mononucleotide or riboflavin-5-phosphate	FMN	Riboflavin (vitamin B_2)	Produced from riboflavin by the enzyme riboflavin kinase	Functions as a prosthetic group for various oxidoreductases including NADH dehydrogenase
Nicotinamide adenine dinucleotide or, in older notation, diphosphopyridine nucleotide	NAD or DPN	Niacin (vitamin B_3)	In animal cells, *de novo* tryptophan degradation plus ATP addition or, alternatively, ATP addition to diet-derived niacin	As a cofactor, catabolic oxidative/reduction reactions; as a cosubstrate, polyribosylation reactions
Nicotinamide adenine dinucleotide phosphate or, in older notation, triphosphopyridine nucleotide	NADP or TPN	Niacin (vitamin B_3)	Conversion of NAD into NADP by NAD kinase (requires ATP)	Most often anabolic oxidative/reduction reactions
Pyridoxal-5′-phosphate	PLP	Pyridoxine	Conversion of pyridoxine into PLP by pyridoxal kinase (requires ATP)	Carries out transamination, decarboxylation, and racemization reactions (also see **Table 2**)
Tetrahydrofolic acid	THFA, H_4FA, MTHF	Folic acid (vitamin B_9)	The synthesis of THFA and derivatives (N^5, N^{10}-methylene-THFA, N^5-methyl-THFA, N^5-formimino-THFA, N^{10}-formyl-THFA, N^5, and N^{10}-methenyl-THFA) starts with dihydrofolate reductase (essential to THFA formation from H_2FA). The reactions that lead to derivatives are FA → H_2FA → THFA ↔ methylene-THFA → methyl-THFA	Transfer of methyl, formyl, methylene, and formimino groups and the so-called one-carbon transfers, essential for amino acid, purine, and DNA synthesis
Thiamine pyrophosphate (i.e., thiamine diphosphate)	TPP, TDP, ThDP	Thiamine (vitamin B_1)	Thiamine is converted into TPP by thiamine kinase (requires ATP)	Carries out two-carbon group transfer reactions, α-carbon cleavage (e.g., oxidative decarboxylations), transamination reactions

Table 1 (Continued)

Vitamin-derived coenzymes and cofactors

Cofactor name	*Common abbreviations for the cofactor*	*Precursor*	*Modifications leading to cofactor formation*	*Chemical group(s) transferred or types of reactions catalyzed*
Thiamine triphosphate	TPPP, TTP, ThTP	Thiamine (vitamin B_1)	ThTP is currently suggested to be synthesized by apparent chemiosmotic mechanisms, analogous to ATP synthesis	Nerve cell energy metabolism, sodium gating in nerve cells; in E. coli, TTP is used in metabolic processes induced during amino acid starvation
Vitamin K; 2-methyl-1, 4-naphthoquinone derivatives: phylloquinone, menaquinone	Vitamin K_1 (phylloquinone), vitamin K_2 (menaquinone, also MK-n, where M stands for menaquinone, K stands for vitamin K, and n represents the number of isoprenoid side chain residues)	Vitamin K	Vitamin K_1 is synthesized by plants: the menaquinones are synthesized in the intestinal lumen by bacteria	Vitamin K (in animals) is involved in the carboxylation of certain glutamate residues in proteins to form gamma-carboxyglutamate (Gla) residues. Gla residues facilitate subsequent calcium binding that causes activation of enzymes important to blood coagulation and regulation of calcification in bone and smooth muscle

Nonvitamin-derived coenzymes and cofactors

Cofactor name	*Common abbreviations for the cofactor*	*Chemical modifications leading to cofactor formation*	*Chemical group(s) transferred or types of reactions catalyzed*
Adenosine triphosphate	ATP	ATP is formed by the action of ATP synthase. ATP synthase is a mitochondrial membrane enzyme that uses transmembrane electrochemical proton potential differences facilitate the phosphorylation of adenosine diphosphate (ADP) to ATP	Adenosine triphosphate (ATP) is a coenzyme involved in energy transfer reactions including biosynthetic reactions, motility, and cell division
Adenosine 3,5-cyclic monophosphate	cATP	cAMP is derived from ATP, synthesized by adenylyl cyclase	cATP is important in cell signaling and cell signaling transduction
Cytosine triphosphate	CTP	CTP synthetase catalyzes the last committed step in pyrimidine nucleotide biosynthesis: $ATP + UTP + glutamine \rightarrow ADP + P_i + CTP + glutamate$	CTP is a high-energy molecule similar to ATP but is limited to a smaller subset of metabolic reactions (e.g., synthesis of glycerophospholipids and glycosylation of proteins)
Coenzyme Q, ubiquinone	CoQ, CoQ_{10}	Biosynthesis occurs in 3 major steps: (1) creation of the benzoquinone structure (using phenylalanine or tyrosine), (2) addition of isoprene side chain units, and (3) condensation of the isoprene chain with benzoquinone	CoQ_{10} is found in the membranes of many organelles; the highest concentration is found on the inner membrane of the mitochondria, where it facilitates electron flow. CoQ also has antioxidant potential
Glutathione (GSH)	GSH	GSH is a tripeptide (Gly-Cys-Glu) with a gamma peptide linkage between the carboxyl group of the glutamate side chain and the amine group of cysteine, which is attached by peptide linkage to glycine. Glutathione reduces disulfide bonds formed within cytoplasmic proteins to cysteines by serving as an electron donor. In the process, glutathione is converted to its oxidized form, glutathione disulfide (GSSG). Once oxidized, glutathione can be reduced back by glutathione reductase, using NADPH as an electron donor	Major reducing agent in cells (e.g., is maintained at mM levels). A cosubstrate for glutathione peroxidases and glutathione S-transferase. It is a key component of the glutathione–ascorbate cycle, a system important to maintaining safe hydrogen peroxide levels in cells
Guanosine-5′-triphosphate	GTP	The synthesis and regulation of GTP are complex and involve enzymes, such as inosine-5′-monophosphate dehydrogenase, GMP synthase, GMP kinase, and nucleoside-diphosphate kinase	Analogous to ATP, GTP acts as a substrate for the synthesis of RNA during the transcription process or DNA during DNA replication. It is used as a

(*Continued*)

Table 1 (Continued)

Nonvitamin-derived coenzymes and cofactors

Cofactor name	*Common abbreviations for the cofactor*	*Chemical modifications leading to cofactor formation*	*Chemical group(s) transferred or types of reactions catalyzed*
			source of energy for specific steps in protein synthesis and gluconeogenesis
Guanosine 3,5-cyclic monophosphate	cGMP	cGMP is derived from GTP, synthesized by adenylyl cyclase	cGMP is essential to signal transduction, in particular with G proteins, in second-messenger mechanisms where cGMP is converted to guanosine diphosphate
Heme (various types)	Heme	Heme consists of an Fe ion located at the center of heterocyclic organic ring, called porphyrin. Porphyrins consist of four pyrrolic groups joined together by methine bridges	Hemes are components of hemoglobin and are at the active sites in numerous enzymes and protein important to oxygen metabolism (e.g., myoglobin, cytochromes, catalase, and nitric oxide synthase)
α-Lipoic acid or thioctic acid	ALA	ALA is an organosulfur compound derived from octanoic acid. As a cofactor, ALA is covalently attached by an amide bond to a terminal lysine residue in the lipoyl domain component of the active site of lipoic acid requiring enzymes	Essential for the transfer of acetyl groups in pathways important to energy regulation and production
Molybdopterin, molybdenum cofactor	Mo cofactor	Molybdopterins are synthesized utilizing guanosine triphosphate as a principle substrate	The molybdenum cofactor is used by sulfite oxidase, xanthine oxidoreductase, and aldehyde oxidase. The absence of molybdenum cofactor leads to the accumulation of toxic levels of sulfite
3′-Phosphoadenosine-5′-phosphosulfate	PAPS	PAPS is a derivative of adenosine monophosphate that is phosphorylated at the 3′-position with a sulfate group attached to the 5′ phosphate	PAPS is a coenzyme in sulfotransferase reactions
Pyrroloquinoline quinone	PQQ	Tricyclic quinone derived from the annulation of glutamic acid with tyrosine	PQQ is utilized as a dehydrogenase redox cofactor in many bacteria and recently discovered to serve a similar role in lower eukaryotes. Although it is not currently viewed as a classical cofactor important to enzymatic functions in animals, it is ubiquitous in nature and is present in all tissues. It appears to play a role as a redox agent in cell signaling by interaction with receptors important to Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathways and mitogen-activated protein kinase (MAPK) pathways
S-Adenosylmethionine	SAM-e, SAMe, SAM	SAM is made from ATP and methionine by methionine adenosyltransferase	Serves as a cosubstrate in methyl group transfer reactions. Transmethylation, transsulfuration, and aminopropylation are the metabolic pathways that use SAM as a cosubstrate
Tetrahydrobiopterin	BH_4, THB	Tetrahydrobiopterin is synthesized by reactions involving GTP as a substrate and GTP cyclohydrolase as an important enzyme associated with the synthetic pathway	THB serves as a cofactor for 3 aromatic amino acid hydroxylase enzymes, which are important to the degradation of phenylalanine and synthesis of serotonin, melatonin, norepinephrine, and epinephrine. BHT is also a cofactor for the production of nitric acid by nitric oxide synthases

Table 2 Cofactors involved in redox reactions

Cofactor	*Description*
Metals	
Manganese	Utilized in the mitochondrial antioxidant system (e.g., Mn-dependent superoxide dismutase) and enzymes, such as malic enzyme and pyruvate dehydrogenase
Iron	Utilized in numerous oxidases, mono- and dioxygenases, and reductases, as well as cytochrome P450 enzymes, important in secondary metabolism and mitochondrial function. Iron is also found in heme proteins, such as hemoglobin and myoglobin. Hemoglobin binds oxygen cooperatively via coordination with iron. Active sites containing iron often exist as iron–sulfur clusters containing sulfide-linked di-, tri-, and tetrairon centers in variable oxidation states. For example, both mitochondrial complex I and complex II have multiple Fe–S clusters
Zinc	Utilized in various enzymes such as alkaline phosphatase, alcohol dehydrogenase, matrix metalloproteinase, carboxypeptidase A, and carbonic anhydrase
Cobalt	Utilized in corrin-containing proteins (e.g., as cobalamin) and enzymes, such as methionine aminopeptidase 2 (eukaryotes) and nitrile hydratase (bacteria)
Nickel	Utilized in plant urease, the NiFe hydrogenases in bacteria, and the nickel-tetrapyrrole coenzyme, cofactor F430, which is used by methyl coenzyme M reductase, and minor forms of bacterial superoxide dismutase and glyoxalase
Copper	Utilized in numerous oxidases: superoxide dismutase, mono- and dioxygenases, and reductases. Copper is also used in hemocyanins that transport oxygen in some invertebrate, for example, crustaceans living in environments with low oxygen pressure (e.g., hemocyanins bind oxygen noncooperatively and less efficiently than hemoglobin). Terrestrial arthropods (e.g., spiders and scorpions) also utilize hemocyanins
Molybdenum	Utilized in aldehyde, sulfite, and xanthine oxidases
Nonmetals	
Selenium	Utilized in glutathione peroxidase (reaction: $2\,GSH + H_2O_2 \rightarrow GSSG + 2H_2O$) and Se-dependent deiodinases that convert T_4 to T_3 (the active hormone) by removing an iodine atom from the outer tyrosine ring
Sulfur	Utilized in redox (cysteinyl residues at the active site of proteins). Inorganic sulfur is a part of iron–sulfur clusters in proteins with redox activity (cf. Fe and ferredoxins), which serve as electron shuttles in cells. In bacteria, nitrogenase enzymes contain Fe–Mo–S clusters (important in nitrogen fixation)
H, C, N, O	Utilized in all biologically important organic compounds. Distinguished by being the smallest of the elements that can form stable multiple bonds
Vitamin-derived cofactors	
Ascorbic acid/vitamin C	Utilized in oxidation reactions catalyzed by monooxygenases (hydroxylases), dioxygenases, and oxidases. Such enzymes are often iron- or copper-containing. Ascorbate acts as a reductant to maintain Fe and Cu in their reduced states
Niacin	Utilized as a precursor of the coenzymes: nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Reactions involve the removal of two hydrogen atoms from the reactant in the form of a hydride ion (H:) and a proton (H+). The proton is released into solution, while the reductant RH2 is oxidized and NAD^+ is reduced to NADH by transfer of the hydride to the nicotinamide ring of NAD or NADP. As a general observation, NAD is used mostly in catabolic processes and NADP in synthetic processes
Pyridoxine (vitamin B_6)	Utilized in the hydrolysis of ether linkages in glycogen by glycogen phosphorylase. The phosphate group on the pyridoxal-5′-phosphate donates a proton to an inorganic phosphate molecule, allowing the inorganic phosphate to in turn be deprotonated by the oxygen forming the α-1,4 glycosidic linkages in glycogen
Riboflavin	Utilized as a component of the cofactors FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide) utilized by a variety of oxidases. During the catalytic cycle, a reversible interconversion of the oxidized, semiquinone, and reduced forms of flavin occurs. FMN is a stronger oxidizing agent than NAD and is particularly useful because it can take part in both one- and two-electron transfers; importantly, because oxygen as a reactant prefers one-electron transfers.

Types of Cofactor-Catalyzed Reactions

Many cofactors, particularly those derived from vitamins, are designed for highly specific chemical modifications and reactions. Some of the more important types of reactions are outlined in this section.

Oxidation–reduction reactions

Redox chemical reactions occur with a transfer of electrons (appreciating that some redox reactions occur with no apparent electron net gain or loss in electrons, e.g., reactions involving covalent bonds). When redox reactions occur, the process involves one of the following mechanisms: (1) direct electron transfers, (2) hydrogen atom transfers, (3) hydride ion transfers, and (4) direct combinations with oxygen. Cellular energy release is controlled by means of redox reactions. In this regard, redox proteins and metabolic steps in the regulation of their associated cofactors are often linked or colocated, which provides regulatory continuity, for example, the positioning of mitochondria in cells and the interdependence of mitochondrial and nuclear genes in energy regulation.

Of the vitamin-derived cofactors, flavin adenine dinucleotide (FAD) and riboflavin-5′-phosphate (flavin mononucleotide (FMN)) derived from riboflavin and nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) derived from niacin are perhaps the most common (cf. Tables 1 and 2). However, in animals, more than a dozen metals, nonmetals, nonvitamin-derived cofactors, and ascorbic acid and pyridoxal-5′-phosphate (PLP) play important roles in redox.

Generation of leaving groups

As noted, cofactors function in part by allowing the possibility of differing transition states that lower the activation energy of given reactions. With regard to the generation of leaving groups, this usually implies enriching the nucleophilic or electrophilic character of transition-state intermediates to better engage in nucleophilic or electrophilic substitutions. The vitamin-derived cofactors, thiamine pyrophosphate (TPP) and PLP, serve as excellent examples of such functions.

Thiamine pyrophosphate (TPP) consists of aminopyrimidine and thiazole rings linked by a methylene bridge. The thiazole moiety acts as an ylid (a neutral dipolar molecule containing a negatively charged atom attached to a heteroatom with a formal positive charge). The carbanion character of TPP's thiazole ring is retained in transition-state intermediates. Examples of enzymes in which TPP is used for this purpose are pyruvate, α-ketoglutarate, or branched-chain amino acid dehydrogenase complexes and 2-hydroxyphytanoyl-CoA lyases (see Figure 2). TPP catalyzes the reversible cleavage of a substrate at the carbon–carbon bond connecting a carbonyl group to an adjacent reactive group (usually a carboxylic acid or an alcohol). In the reverse reactions, the TPP–substrate bond is broken, reforming a TPP ylid and substrate carbonyl.

TPP is also a cofactor for transketolases, enzymes associated with the pentose phosphate pathway in all organisms and the Calvin cycle of photosynthesis. In transketolases, TPP accepts a 2-carbon fragment from a 5-carbon ketose (e.g., D-xylulose-5-P) and then transfers the fragment to a 5-carbon aldose (e.g., D-ribose-5-P) to form a 7-carbon ketose (e.g., sedoheptulose-7-P). The second reaction catalyzed by transketolase in the

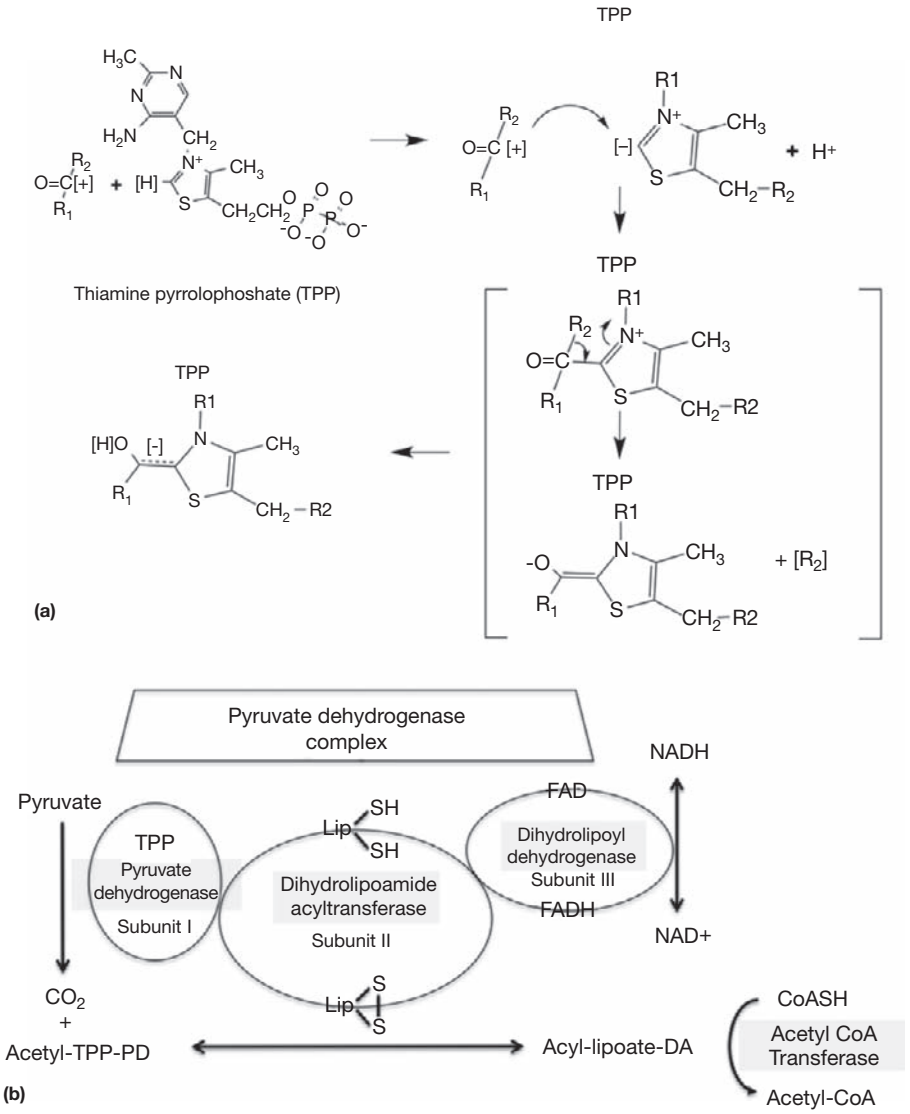


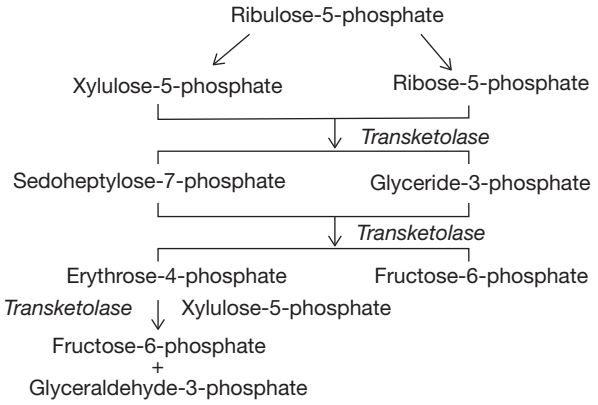
Figure 2 Thiamine. Thiamine (2-[3-[(4-amino-2-methyl-pyrimidin-5-yl) methyl]-4-methyl-thiazol-5-yl] ethanol) as the pyrophosphate derivative, TPP, is exquisitely designed to facilitate the metabolism of carbohydrates. The thiazole moiety of thiamine has carbonium ion character, which is retained in transition-state intermediates. As a consequence, decarboxylation of α-ketoacids derived from glucose may enter energy-generating cycles, such as the tricarboxylic acid (TCA) cycle and citric acid cycle (a). As an example, thiamine pyrophosphate is an essential cofactor for pyruvate dehydrogenase, a component of the pyruvate dehydrogenase complex (b).

pentose phosphate pathway involves the transfer of a 2-carbon fragment from D-xylulose-5-P to erythrose-4-phosphate, which results in fructose 6-phosphate and glyceraldehyde-3-P. This reaction connects the pentose phosphate pathway to glycolysis (Scheme 1).

PLP-dependent enzymes are particularly critical in the metabolism of amino acids. The 4-formyl substituent of PLP condenses with the α-amino group of an amino acid, to form an azomethine (Schiff base) linkage. Next, the conjugated double-bond system extends into PLP's pyridinium nitrogen. This weakens bonding around the α-carbon of the amino acid substrate, which, depending on the enzyme, can lead to transaminations, decarboxylations, or subsequent substitutions at the carbon adjacent to the α-carbon. In PLP-dependent enzymes, the moiety is often attached to a lysyl group within the enzyme. This has the advantage of setting the stage for an initial transimidation with an amino acid, which is a faster reaction than an aldehyde with an amino acid, that is, a classical transamination. Further, the transimidation mechanism allows PLP to be retained in its original state in association with the enzyme at the conclusion of a catalytic cycle. Accordingly, although the binding of PLP to transaminases can be viewed as potentially dissociable, the net result is more covalent in character (Scheme 2).

Acyl activation and transfer reactions

Cofactors involved in the transfer of acyl and acetyl groups include coenzyme A (CoA), lipoic acid, and small molecular weight proteins, acyl carrier proteins (ACPs), that are associated with fatty acid and polyketide synthesis complexes. A phosphopantetheinyl moiety serves as the principle functional component within the structure of CoA and ACP. The phosphopantetheinyl moiety provides a thiol group that reacts with the carboxylic acid group associated with acetate or acyl functions to form thioesters. This step is a prelude to numerous acetyl and acyl group transfers and enolizations. The carbonyl carbon of a thioester is more positively polarized than a corresponding oxy ester, because oxy esters are more stabilized by resonance than thioesters. Activation as a thioester sets the stage for eventual acetyl and acyl transfers. The phosphopantetheinyl moiety also provides a flexible arm to move substrates from one site to the next in complexes, such as those designed for fatty acid and polyketide synthesis (Scheme 3).



Scheme 1 Transketolase reactions.

In an analogous fashion, lipoic acid, α-lipoic (thioctic) acid, provides a functional sulfhydryl group with an extended flexible arm attached via a peptide bond to lysyl residues in subunits usually associated with dehydrogenase enzyme complexes. For example, the role of lipoic acid in pyruvate dehydrogenase is in part outlined in Figure 2. Given that lipoic acid has two adjacent sulfhydryl groups, which are oxidized to form a dithiolane ring, oxidized lipoic acid is sufficiently electronegative to catalyze the transfer of TPP intermediates with carbanion character. The resulting thioester can then be transferred to form CoA intermediates.

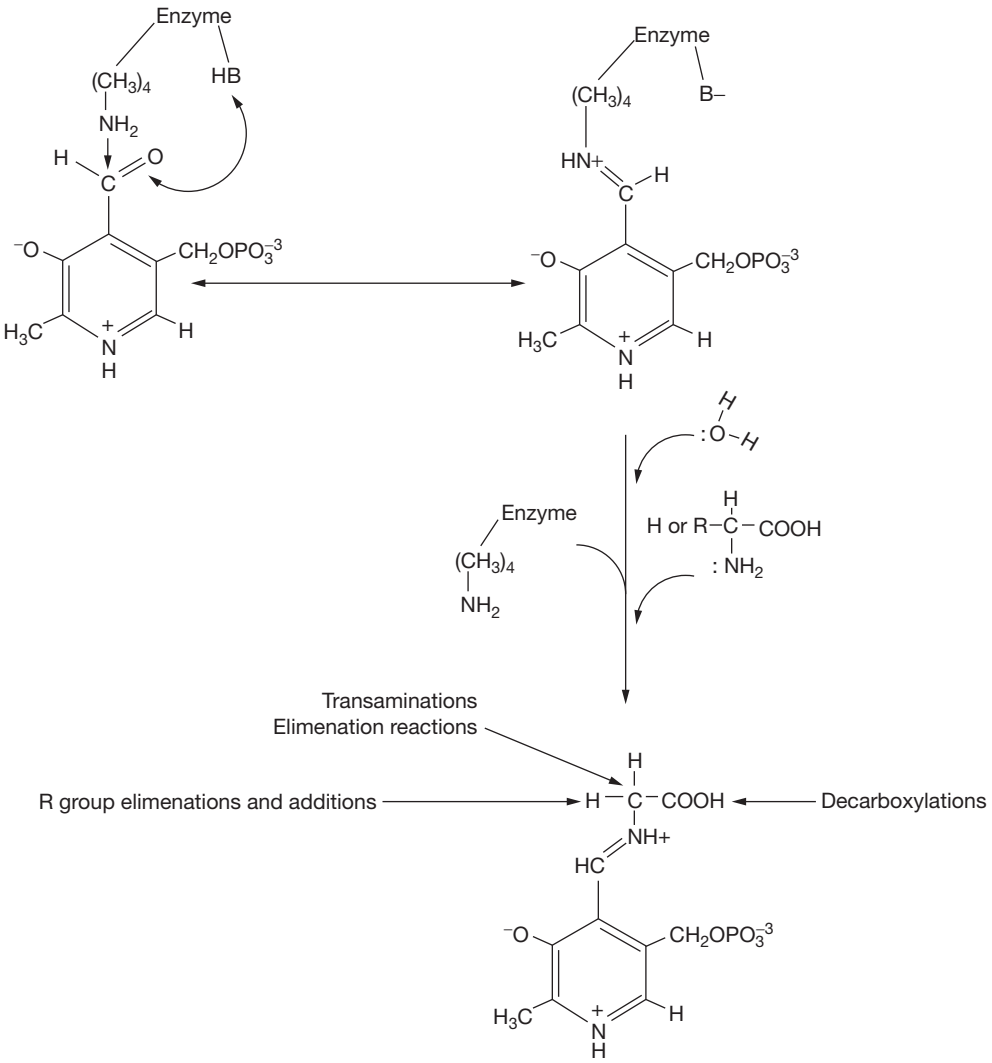
Carboxylations

D-(+)-Biotin is a cofactor designed for the transfer of CO_2 in carboxylase and transcarboxylase enzymes, such as acetyl-CoA carboxylase-α, acetyl-CoA carboxylase-β, and methylcrotonyl-CoA, propionyl-CoA, and pyruvate carboxylases. Biotin is covalently attached to the epsilon-amino group of specific lysine residues at the active site in such enzymes, which are essential to fatty acid synthesis, branched-chain amino acid catabolism, and gluconeogenesis.

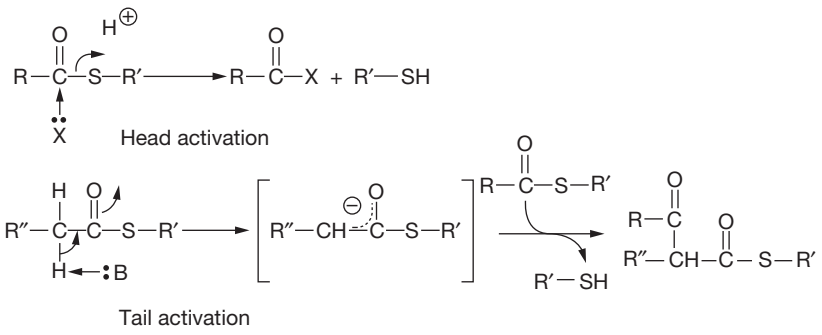
The mechanism involves tautomerization of the ureido nitrogen, a process that enhances its nucleophilicity. Phosphorylation of bicarbonate by ATP represents an independent step to produce carbonyl phosphate, an electrophilic mixed-acid anhydride. The carbonyl phosphate then reacts to generate an active carboxylbiotinyl enzyme. These reactions change the carbon in CO_2 to one with carbanion character, which facilitates subsequent reactions as the conversion of acetate to malonate or pyruvate to oxaloacetate (Scheme 4).

In addition to biotin, the active forms of vitamin K acts as a cofactor that is essential for certain types of carboxylation reactions, particularly those involved for posttranslational modifications of proteins utilized in blood coagulation cascades (e.g., thrombin) and pathways important to the regulation of extracellular matrix (ECM) calcification. Vitamin K-dependent carboxylases catalyze the carboxylation of glutamyl residues to γ-carboxyglutamyl (GLA) residues. The presence of GLA residues facilitates the binding of calcium ions, important to the activation of GLA-containing enzymes (i.e., those involved in blood coagulation) and GLA-containing ECM proteins, such as osteocalcin, important to the control of ECM mineralization. In contrast, in plants and bacteria, vitamin K either functions as an electron acceptor in photosystem I or is important to anaerobic respiration. Compounds with vitamin K activity are derived from 1,4-naphthoquinone derivatives that are combined with varying numbers of isoprenoid units to form the so-called phytyl side chains. Vitamin K includes two natural vitamers designated vitamin K_1 (phylloquinone, synthesized in plants) and vitamin K_2 (menaquinone, present in animal tissues) (Scheme 5).

Vitamin K-dependent carboxylases use vitamin K epoxidation to drive the carboxylation reactions that lead to GLA formation. GLA formation occurs in two independent steps: (1) deprotonation of a targeted glutamic acid residue to form a carbanion intermediate, followed by (2) the addition of CO_2. The mechanism is facilitated by a histidyl residue at the carboxylase active site, which acts as an electrophile that accepts the negative charge on the γ-carbon of the modified glutamyl residue carbanion (Scheme 6).



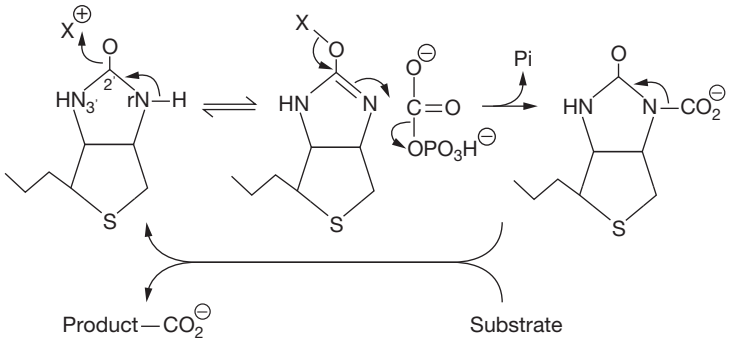
Scheme 2 Pyridoxal-5'-phosphate reactions.



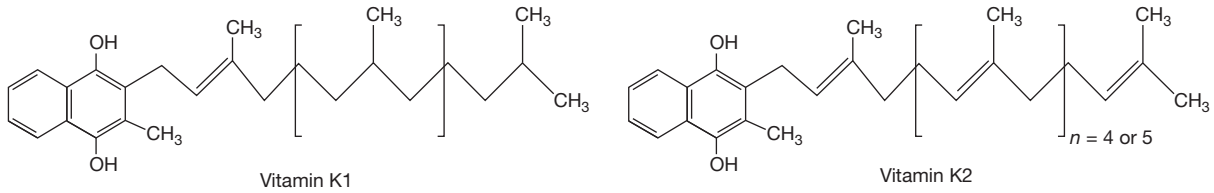
Scheme 3 Thiol-facilitated acyl transfer reactions.

Single-carbon transfer reactions

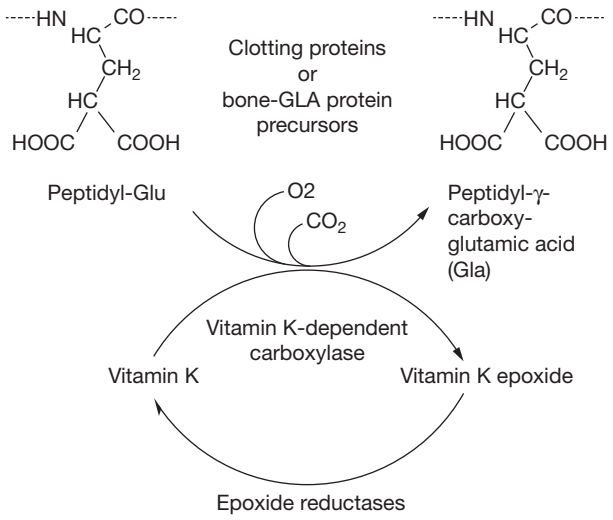
A number of cofactors are involved in single-carbon transfer reactions, in higher organisms, namely, derivatives of folic acid, the cobalamins, and S-adenosylmethionine (SAM). The cofactors derived from folic acid are heterocyclic compounds composed of 4-(pteridin-6-ylmethyl)-aminobenzoic acid that is conjugated with one or more L-glutamate units. The reactions that involve folic acid derivatives include the generation and utilization of formaldehyde, formimino, and methyl groups. For these conversions to



Scheme 4 Biotin activation.



Scheme 5 Forms of vitamin K.



Scheme 6 Vitamin K-dependent carboxylase reaction.

occur, folic acid must be reduced to tetrahydrofolic acid (THFA) (Scheme 7).

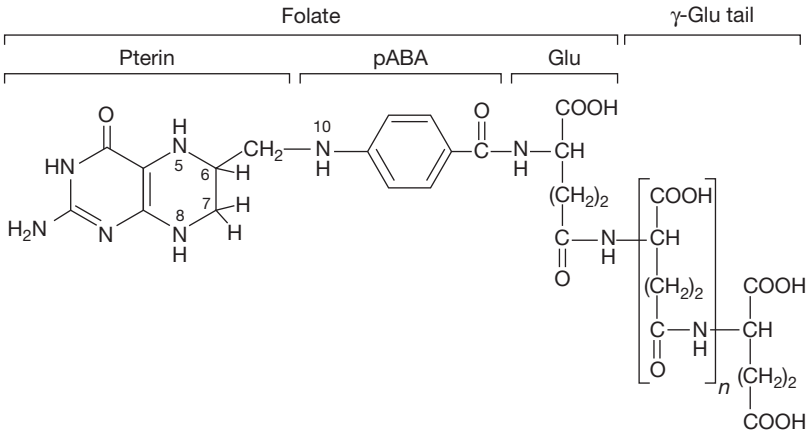
Reduction of the pteridine ring brings the two nitrogens at positions five and ten closer together, which changes their electrochemical properties to facilitate the formation of THFA single-carbon derivatives. The potential derivatives of THFA are N^5-methyl-THFA, N^5-formimino-THFA, N^{10}-formyl-THFA, $N^{5,10}$-methylene-THFA, $N^{5,10}$-methenyl-THFA, and N^5-methyl-THFA. The differing oxidation states of a carbon unit are needed for the range of reactions catalyzed by THFA. For example, the formyl, methanyl, and methylene forms of THFA are utilized for purine and thymidylate (i.e., DNA-related) synthesis. Methylated forms of THFA participate in reactions important to the interconversion and catabolism of amino acids (Scheme 8).

Further, the relative binding of THFA derivatives to given enzymes appears related to the number of the glutamyl residues attached to the 4-(pteridin-6-ylmethyl)-aminobenzoic acid moiety of folic acid. The number of glutamyl residues also dictates specificity and partitioning into given THFA apoenzymes.

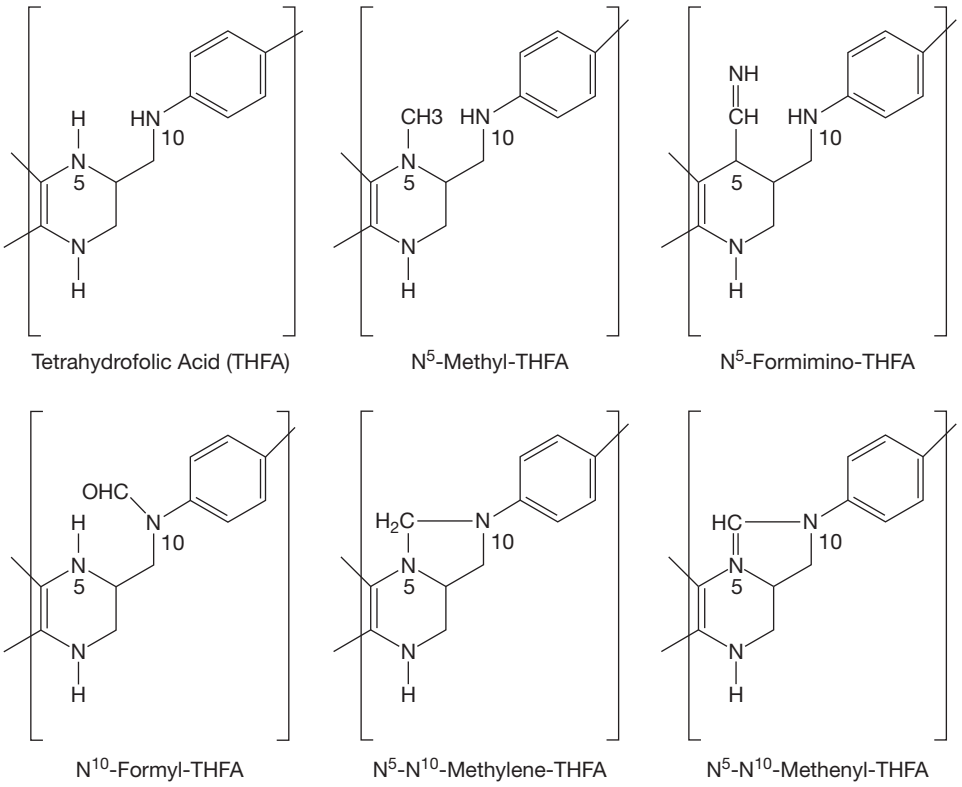
The biochemical basis of the interrelationship between THFA and cobalamin is the maintenance of two functions: (1) nucleic acid synthesis and (2) methylation reactions that are involved in reactions that range from the silencing of genes (DNA methylations) to the synthesis of lipotropic agents and compounds involved as methyl donors, such as choline, betaine, or methylated derivatives of glycine. The most important linkage between folate and vitamin B_{12} occurs at the methionine synthetase step, where 5-methyl-THFA serves as a substrate with homocysteine to form methionine.

There are two forms of vitamin B_{12}. Both forms of vitamin B_{12} consist of a porphyrin-like structure of tetrapyrrole rings containing cobalt at its center. A 5′,6′-dimethylbenzimidazole nucleotide may also linked to the tetrapyrrole rings via a phosphate sugar linkage (see F, Rearrangements on vicinal carbons). Methylated vitamin B_{12} (cobalamin) catalyzes the conversion of homocysteine to methionine by transferring a methyl moiety derived from methyl-THFA to methionine. In effect, vitamin B_{12} in this reaction acts somewhat analogous to a Grignard reagent (e.g., an organometallic complex involved in the transfer of an alkyl group) (Scheme 9).

As noted, for the transfer of methyl groups, SAM also plays a predominate role. SAM is essential to the methylation of phospholipids and production of methylated forms of various amino acids and carbohydrates. SAM serves as the principle methyl source in DNA methylation. The methyl group attached



Scheme 7 Folic acid.



Scheme 8 Derivatives of tetrahydrofolic acid.

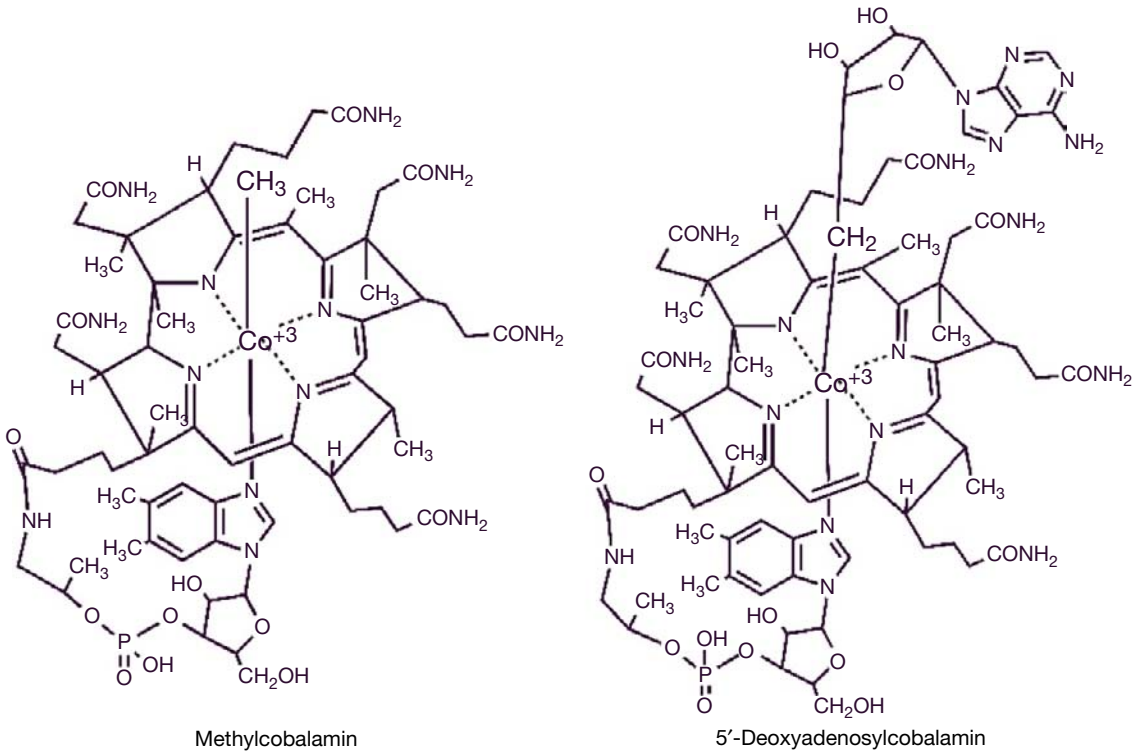
to the methionine sulfur atom in SAM is chemically reactive. This allows donation of this group to an acceptor substrate in transmethylation reactions (Figure 3; Scheme 10).

Rearrangements on vicinal carbons

The rearrangement of vicinal carbon atoms (e.g., an ethenyl group minus one hydrogen atom [$-CH{=}CH_2$] or $R-CH{=}CH_2$ where R can be any other group of atoms) depends on the 5′, 6′-dimethylbenzimidazolyl nucleotide form of vitamin B_{12}. Examples include reactions catalyzed by methylmalonyl-CoA mutase that allows for the oxidation of odd-chain fatty acids and methylation of branched-chain fatty acids, which is important to neural membrane assembly (Figure 4).

Control of reactive oxidants

Reactive oxidant species (ROS) are reactive oxygen ions and peroxides that include superoxide anion, hydrogen peroxide, hydroxyl radicals, and their derivatives (e.g., hypochlorous acid, HOCl). ROS are by-products of the normal metabolism of oxygen, particularly related to mitochondrial oxidations and reactions catalyzed by cytochrome P450 oxidases. High levels of ROS initiate reactions that may alter the structures of important molecules, such as DNA, RNA, proteins, and lipids,



Scheme 9 Derivatives of cobalamin.

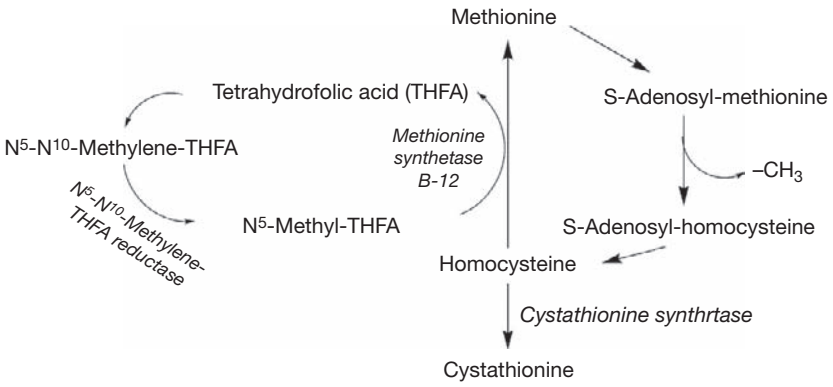


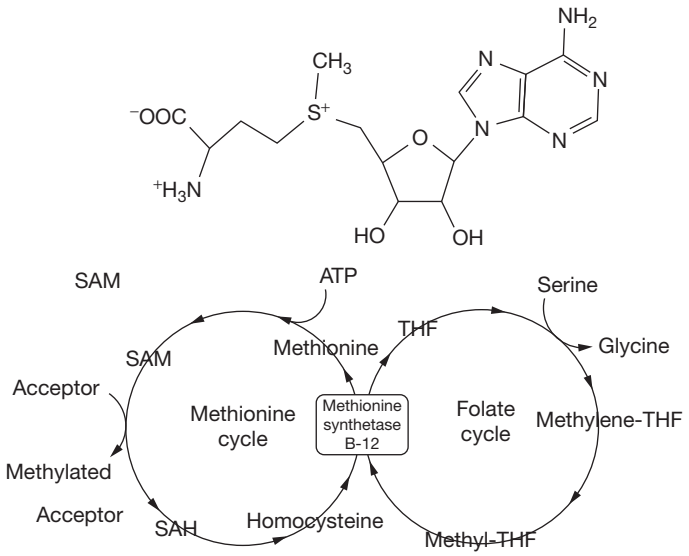
Figure 3 One-carbon metabolism pathway involves the interaction between folic acid, vitamin B_{12}, and S-adenosylmethionine (SAM). Reduced folic acid serves as donors of single carbons in any one of its three forms: 5-methyl-THFA, 5,10 methylene-THFA, and 10-formyl-THFA. The single-carbon donor, 5-methyl-THFA, is used to convert homocysteine into methionine, which can then go to form S-adenosylmethionine, the key substrate in methylation reactions. 5,10-Methylene-THFA is important for the conversion of deoxyuridylate into thymidylate (not shown), and the donor 10-formyl-THFA is used *de novo* purine synthesis (not shown).

particularly lipids comprising cellular membranes. As a consequence of the potentially destructive nature of ROS, a number of systems and compounds have evolved to counteract potentially destructive ROS reactions. A partial list of antioxidant cofactors including some dietary biofactors is given in Table 3.

Of the vitamins and related derivatives, many are apolar and reside mostly in the lipid membranes of cells or cellular organelles. The best example of an essential nutrient that acts as an antioxidant is vitamin E, a group of ten lipid-soluble compounds that includes both tocopherols and tocotrienols. Of the differing forms of vitamin E, γ-tocopherol is the most common (found in many plant oils), and α-tocopherol is considered to be the most biologically active. Compounds with antioxidant activity are often referred to as the first line of defense with respect to cellular membrane protection. Of the other small molecular weight compounds (vitamin-derived cofactors and related metabolites), most appear to act as reductants.

ADP-ribosylation reactions

About half the niacin present in cells as NAD or NADP is used as a substrate for mono- and polyribosylation reactions, that is,



Scheme 10 Relationship between the methionine and folate cycles.

multiple groups of ADP-ribose moieties are transferred to proteins to form long branched chains, in a reaction called poly(ADP-ribosylation). Mono- and polyribosylations are important in the regulation of a broad range of enzymes. In the nuclei of cells, polyribosylation of specific histones is an essential part of DNA repair. ADP-ribosylation is the addition of one or more ADP-ribose moieties to a protein (Scheme 11).

Cofactor Regulation and Generation

Transport and Transporters

The transport of organic cofactors, their intermediates, and metals in and out of cells is an important regulatory mechanism. Integral membrane proteins with specific permeability and/or affinity for cofactors control cellular transport. With regard to metals, given that metal ions are already at their chemically basic level, intracellular metabolism is focused on direct interactions between the metal and its transport pathways. Indeed, in most cases, simple diffusion plays a very minor role. For example, it has been estimated that the 'free' cellular copper and zinc concentrations in cells are no more than a few atoms per cell, because of binding to highly specific intracellular transporters. In general, absorption of metals from intestinal lumen cells into circulation or from circulation into targeted cells occurs through association with protein complexes, such as in the case for iron and transferrin through the transferrin receptor and cobalamin and intrinsic factor through the receptor cubilin, or through a transporter with permeability for the cofactor, as in the case for the SLC39 family of zinc transporters or sodium-ascorbate cotransporters SVCT1 and SVCT2.

For nonmetal-, vitamin-, or nonvitamin-derived cofactors, the processes are also highly regulated and complex. For example, for the vitamin-derived cofactors, disassembly is required for intestinal absorption. Appreciate that vitamins in foods are usually present as cofactors or in highly modified forms. Pancreatic and intestinal cell-derived enzymes (nucleosidases, phosphatases, and peptidases) are key factors in the initial digestive process with the goal of creating smaller or more soluble moieties designed to increase the statistical probability of interacting with luminal receptors for transport out of the intestinal compartment. In circulation, cofactors or their precursors may be specifically bound to transport proteins or loosely associated with proteins, such as albumin and immunoglobulins.

In addition to dietary deficiencies of given precursors (e.g., essential vitamins and minerals), impairments in absorptive mechanisms, through genetic disorders or other pathophysiological conditions, can also lead to cofactor deficiencies. For example, pernicious anemia in the elderly can result from impaired cobalamin absorption in spite of adequate intake due to insufficient gastric intrinsic factor secretion from gastric parietal cells. Vitamin B_{12} absorption is directly dependent on the interaction between intrinsic factor-bound cobalamin and the cubilin receptor, which binds this nutrient–protein complex.

Because various gene products handle the intracellular and whole body metabolism of cofactors, mutations or polymorphisms in such genes can lead to disruptions in cofactor metabolism with significant health consequences. For example, mutations in genes required for intestinal absorption of cofactors can result in a phenotype with close resemblance to symptoms of the corresponding dietary deficiency. Elemental iron absorption is mediated through DMT1, which is encoded by the gene SLC11A2. Mutations in SLC11A2 in both rats and mice lead to microcytic anemia, which is also present in iron deficiency anemia. Likewise, at the cellular level, as would be expected, transporters also play essential and important roles in cofactor regulation. Some examples are listed in Table 4.

Further, mutations in the biosynthesis, recycling, or restoration pathways can resemble the dietary deficiency. For instance, L-gulono-gamma-lactone oxidase is a critical enzyme in the L-ascorbic acid biosynthesis pathway, and mutation of this gene in rats leaves it prone to scurvy. Interestingly, humans and many other primates cannot synthesize their own vitamin

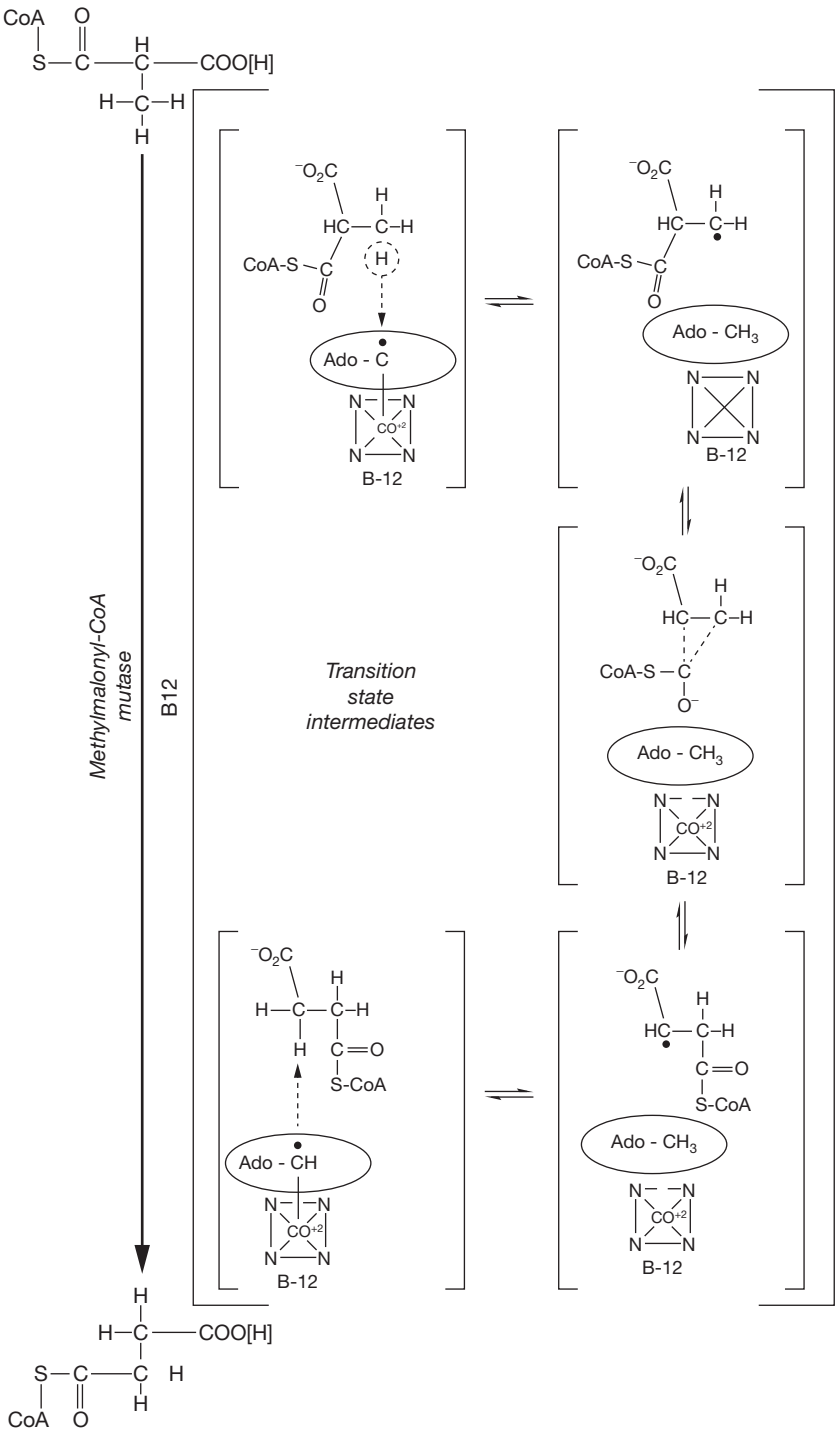
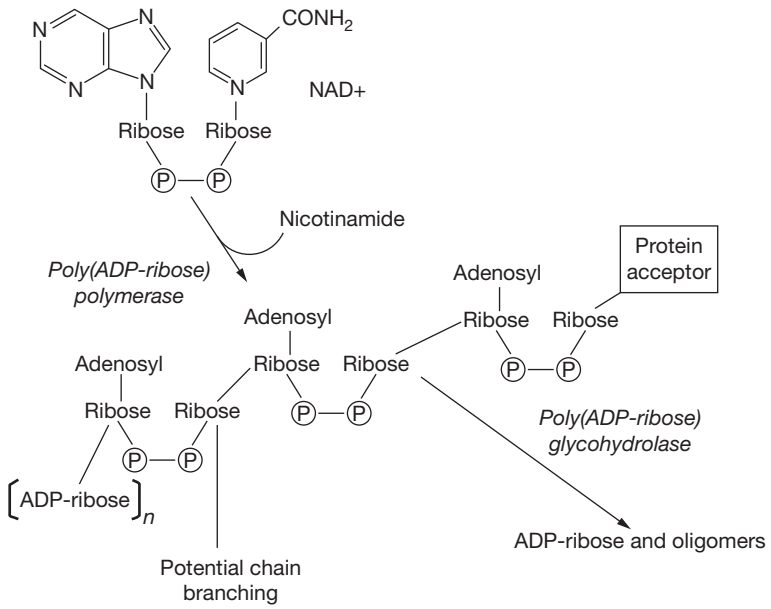


Figure 4 Mutase reactions begin with the homolytic cleavage of the bond between the 5′, 6′-dimethylbenzimidazolyl nucleotide form of B-12 and the Co^{3+}. A free radical is formed; accordingly, coenzyme B-12 in the catalytic process acts as a free radical generator. A free radical is then formed on the vicinal carbon of the substrate, which sets the stage for rearrangements, such as the isomerization of methylmalonyl-CoA to succinyl-CoA.

C because of mutations in the gulonolactone oxidase and as a consequence are sensitive to dietary vitamin C deficiency. Another example of a mutation in cofactor biosynthesis leading to a cofactor deficiency is X-linked sideroblastic anemia, which is caused by a mutation in 5-aminolevulinate synthase gene ALAS2. Mutations in ALAS2 affect the heme biosynthesis pathway, and impairment in 5-aminolevulinate synthase leads to impaired heme synthesis and microcytic anemia concomitant with iron overload due to ineffective use of available iron. It is also important to note that the phenotype because of

Table 3 Cofactors, related enzymes, and selected biofactors with antioxidant activity

Essential compounds (vitamins) and related derivatives	*Function*
α-, β-, γ-, and δ-Tocopherols (vitamin E)	Lipid peroxide initiated chain reaction breakers
β-Carotenes	Singlet oxygen quencher
Lycopene	Singlet oxygen quencher
Ubiquinol (CoQ-10)	Radical scavenger
Ascorbic acid	Reductant
Glutathione	Reductant
Urate	Radical scavenger
Bilirubin	Radical scavenger
NADH, NADPH, $FADH_2$, $FMNH_2$	Sources of reducing potential
Proteins and enzymes	
Superoxide dismutase, depending on the organism, utilizes metals (Cu, Zn, Mn, and Ni) as cofactors	Dismutation of superoxide anions: $[M_{(n+1)+}]\text{-SOD} + O_2^- \rightarrow [M_{n+}]\text{-SOD} + O_2$ $[M_{n+}]\text{-SOD} + O_2^- + 2H^+ \rightarrow [M_{(n+1)+}]\text{-SOD} + H_2O_2$. where M = Cu ($n=1$); Mn ($n=2$); Fe ($n=2$); Ni ($n=2$)
Glutathione peroxidase (utilizes Se as a cofactor and glutathione as a cosubstrate)	Reduces lipid hydroperoxides to their corresponding alcohols and H_2O_2 to water: $2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$
Myeloperoxidase (utilizes Fe as a cofactor)	Controls hypochlorous acid production from hydrogen peroxide and chloride anion
Hydrogen peroxidases (utilizes Fe as a cofactor)	Converts hydrogen peroxide to water
Catalase (utilizes Fe as a cofactor)	Catalyzes the decomposition of hydrogen peroxide to water and oxygen
α-1-Microglobulin (utilizes a critical cysteine residue at its active center to facilitate reactions)	Degrades heme and is a radical scavenger as well as a reductase
Metal binding proteins (e.g., metallothionein, chaperones, and ceruloplasmin)	Sequestration, transport, and regulation of transition metals capable of nonspecific redox when not protein bound
Diet-derived antioxidants/nutraceuticals	
Polyphenol antioxidants (e.g., pyrroloquinoline quinone, hydroxytyrosol, and flavonoids like various flavones; flavanols, such as catechins or epicatechins; flavanones; isoflavone phytoestrogens, such as daidzein and genistein; flavonols such as quercetin (like rutin); and stilbenoids such as resveratrol)	Over 4000 distinct species, many have antioxidant activity; others can effect changes in cell-to-cell signaling, receptor sensitivity, inflammatory enzyme activity, or gene regulation
Phenolic acids (e.g., cinnamic acid, caffeic acid, salicylic acid, and vanillin)	Redox cycling, general antioxidant activity
Carotenoids (e.g., lutein, zeaxanthin, and carotenes)	Over 600 known carotenoids in two classes xanthophylls (which contain oxygen) and carotenes (which are hydrocarbons and contain no oxygen) quench singlet oxygen



Scheme 11 Poly(ADP-ribose) polymerase reactions.

Table 4 Examples of transporters important to the regulation of vitamins and minerals

	Transporter name/symbol	*Solute carrier*	*Genetic disease*
Vitamin examples			
Thiamine (vitamin B_1)	Organic cation transporter 1/OCT1	SLC22A1	Thiamine-responsive megaloblastic anemia
	Thiamine transporter 1	SLC19A2	Lethal encephalopathy, basal ganglia disease
	Thiamine transporter 2	SLC19A3	
Riboflavin (vitamin B_2)	Riboflavin transporter 1/RFVT1	SLC52A1	Brown–Vialetto–Van Laere syndrome, motor neuron disease
	Riboflavin transporter 1/RFVT2	SLC52A2	
	Riboflavin transporter 1/RFVT3	SLC52A3	
Folate	Reduced folate carrier/RFC	SLC19A1	Hereditary folate malabsorption, cerebral folate transport deficiency
	Proton-coupled folate Transporter/PCFT/HCP1[a]	SLC46A1	
	Folate receptor/FOLR1		
Ascorbate (vitamin C)	Sodium-dependent vitamin C transporter 1/SVCT1	SLC23A1	
	Sodium-dependent vitamin C transporter 2/SVCT2	SLC23A2	
Mineral examples			
Iron	Divalent metal transporter 1/DMT1	SLC11A2	Microcytic anemia with hepatic iron overload, hereditary hemochromatosis
	Ferroportin	SLC40A1	
Zinc	Zinc, IRT-like, protein 4/ZIP4	SLC39A4	Acrodermatitis enteropathica; Ehlers–Danlos syndrome (spondylocheiro dysplastic)
	Zinc, IRT-like, protein 13/ZIP13	SLC39A13	
Copper	Copper-transporting ATPase 1/ATP7A	SLC31A1	Menkes disease
	Copper-transporting ATPase 1/ATP7B		Wilson's disease
	High-affinity copper uptake protein 1/CTR1		
Heme	Heme carrier protein 1/HCP1/PCFT*	SLC46A1	Cerebral folate transport deficiency

[a]PCFT/HCP1 can transport both folate and heme.

mutations in cofactor transporter genes can also be lethal. For example, mutation of SLC23A1, which encodes the ascorbic acid transporter SVCT2, in mice leads to perinatal lethality and symptoms that do not resemble scurvy or vitamin C deficiency. The differences (lethal vs. nonlethal) may be due to the developmental timing of the mutations. Mutations that occur very early in development are often more likely to be lethal.

Another consideration is that the some minerals and vitamins may have other biological roles than strictly cofactor functions, in which case the phenotype evident in the mutation of transporter genes may reflect a combination of a lack of cofactor activity and the noncofactor associated effects caused by the reduced transport of the same nutrient. For example, zinc is a cofactor for some enzymes such as carbonic anhydrase and alcohol dehydrogenase, and zinc is also present in many DNA-binding proteins as zinc fingers and can modulate cell signaling.

Cellular Synthesis Pathways and Chemical Modifications Important to Cofactor Cellular Regulation or Modulation of Activity

The regulation of given cofactors occurs at a number of levels. In addition to regulation at the level of transport, many vitamins are subject to chemical modifications that transform them into coenzymes. Thus, the amounts of available cofactors may be controlled by chemical modifications and some cases synthesis. The following sections focus on specific chemical modifications important to organic cofactor activations to highlight the range of cellular strategies used.

Phosphorylation. The addition of a phosphate (PO_4^{3-}) group is often used as a major homeostatic control. In many proteins and enzymes, phosphorylation serves as a type of 'on/off' switch, thereby altering function or activity. Enzymes designated as kinases usually catalyze phosphorylation reactions using ATP as a cosubstrate. Phosphorylation can serve to (1) alter hydrophilic charter and size so that the cofactor is less likely to be transported out of a cell or cellular compartment, (2) improve or alter binding of the cofactor to given enzymes, (3) change a given chemical characteristic of the cofactor, or (4) contribute to overall cellular control because of linkages to the cell's chemical potential, as defined by ADP/ATP concentrations. Some important examples include the following:

- Phosphorylation of ascorbic acid to ascorbate-2-phosphate, which renders the molecule less likely to act as a redox initiator by inhibiting keto–enol tautomerism (e.g., [−COH═COH−] ↔ [−CO−CO−])
- Phosphorylation of vitamin B_6 to PLP, which allows PLP to act as an acid catalyst in glycogen phosphorylase, that is, in addition to its role in transaminases and other PLP-requiring enzymes
- Phosphorylation of NAD to NADP, which results in a cofactor that is more likely to function in a synthetic process (NADP) than catabolic process (NAD)
- Phosphorylation of TPP to thiamine triphosphate, in which neural tissue is involved in ion gating and transport
- Phosphorylation of pantotheine, which facilitates covalent attachment of pantotheine-4′-phosphate to other moieties,

such as intermediates in the coenzyme A synthesis pathway or to ACP subunits

Adenylylations. Examples of adenylylation reactions include conversion of niacin to NAD(P), pantothenic acid to CoA, and vitamin B_{12} to adenosyl B-12 and also the formation of non-vitamin-derived cofactors, such as SAM and 3′-phosphoadenosine-5′-phosphosulfate (an activated form of sulfate). The presence of an adenylyl function can improve binding to targeted protein/enzyme binding domains. Analogous to certain types of phosphorylations, adenylyl transfers represent another way of linking cofactor generation to changes in a cell's chemical potential.

Methylations and Amidations. Examples of methylations include the following:

- Methylation of ascorbic acid to 2-methyl-ascorbic acid. This type of modification can be important in the regulating ascorbic acid's redox activity (inhibits keto–enol tautomerism).
- Methylation of niacin to 1-methylnicotinamide, which facilitates the removal of excess niacin from cells.
- Methylation of aromatic cofactors, which can change the location of reactions on resonating ring structures. For example, the presence of a methyl group can direct electrophiles to attack aromatic molecules at ortho- and/or para-positions relative to the location of the methyl group.

With regard to amidations, the conversion of nicotinic acid to nicotinamide changes the –[COO-] moiety from an acid to a weak base. The hydrogen bonding potential with water and other protic solvents is altered. Similar to methylation, the presence of a $-[CONH_2]$ group is *ortho–para* directing, that is, incoming electrophiles are directed to ortho- or parapositions, which results in the stereospecific transfer of hydride ions from NAD or NADP.

Conjugations utilizing glutamate and isoprene units. As noted, folic acid in cells is a pteroyl derivative containing polyglutamyl residues in γ-linkage. The number of γ-linked glutamyl residues in part determines the binding of folic acid (i.e., pteroylpolyglutamates) to given enzymes. For example, liver thymidylate preferentially binds pteroylpolyglutamates with four glutamyl residues, but derivatives with two to seven glutamyl residues all bind at least 30-fold more tightly than pteroylmonoglutamate. Polyglutamylation can also influence folic acid solubility. For transport in fluids and in and out of cells, the principle form of folic acid is monoglutamylated, which is the most soluble form. In the intestine and cells, conjugated forms of folic acid in foods are converted to the monoglutamyl folic acid by enzymes with γ-linked peptidase activity in order to increase solubility and eventual transport into the body and into cells.

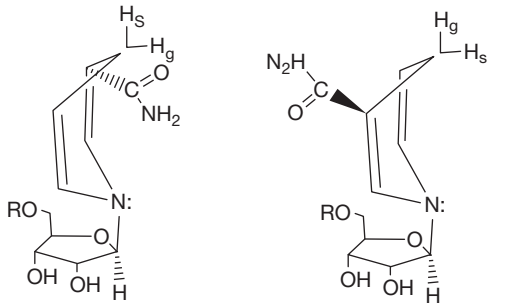
Isoprene conjugation is important to the synthesis of phytyl side chains for numerous biological active biofactors and cofactors (e.g., carotenoids, tocopherols, and phyllo- and menaquinones). As an example of a dietary biofactor, prenylated flavonoids are produced in plants with varying degrees of phytoestrogenic and antioxidant activity. Isoprene units (2-methyl-1,3-butadiene, $CH_2{=}C(CH_3)CH{=}CH_2$) are converted to dimethylallyl diphosphate and isopentenyl diphosphate in pathways designed for synthesis of compounds that have highly divergent functions (e.g., from rubber to biologically actives sterols). For the fat-soluble vitamins, a hydrophobic (phytyl) side chain composed from isoprene units allows such vitamins to partition into lipid membranes of the hydrophobic domains of targeted proteins.

Isomerism. The isomeric form of a cofactor also plays a role in the regulation of given catalytic activities. As an example, dehydrogenases and reductases often show preferences for one or the other of the two isomeric forms of NAD[P]H (the A or pro-R form or B or pro-S form). The two hydrogen atoms of niacin at the 4-position are prochiral. The binding of NAD to dehydrogenases or reductases is such that hydride transfer involves only one side of the nicotinamide moiety. Binding of the nonpreferred form is generally weaker, and the maximum velocity is slower. Examples of dehydrogenase and reductases that prefer the A or pro-R form are alcohol, lactate, and isocitrate dehydrogenases and dihydrofolate reductase, whereas glyceraldehyde-3-phosphate, 3-phosphoglycerate, glutamic acid, and glucose-6-phosphate dehydrogenases and glutathione reductase prefer the B or pro-S form (Scheme 12).

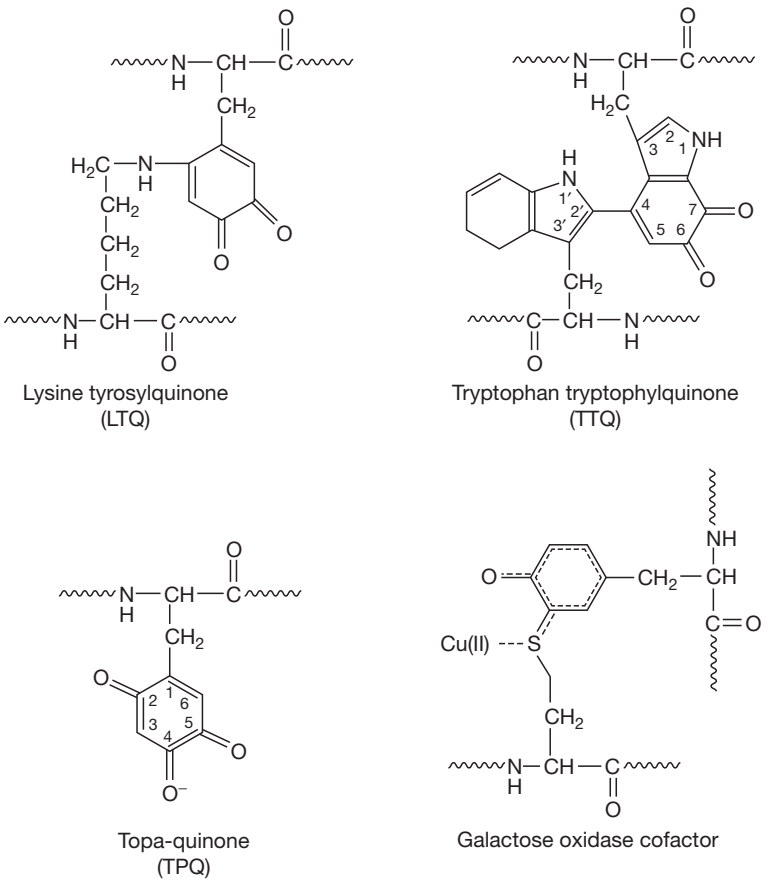
Covalent Attachment and In Situ Cofactor Formation. Many cofactors are covalently attached to the enzymes that they serve. Of the vitamins and vitamin-like compounds, good examples are biotin, lipoic acid, pantothenic acid (as pantotheine), and riboflavin-derived cofactors, FMN and FAD. Indeed, more than 20 flavoenzymes have been described, for which five novel FAD or FMN covalent linkages have been identified (e.g., the covalent linkages to histidyl, tyrosyl, or cysteinyl residues). PLP could also be included, because as noted, imine bonds, although capable of dissociation, are also covalent.

Covalent binding has obvious impact on cofactor metabolism (e.g., metabolic turnover of the cofactor becomes more dependent on protein catabolism before it can be released and potentially reutilized). The thermodynamics constraints of reactions can also be significantly altered (e.g., changes in entropic considerations).

With regard to *in situ* cofactor formation, in the past two decades, the best examples come from the discovery of four novel redox cofactors derived from posttranslational modification of specific amino acids within corresponding enzymes. These include the topa quinone cofactor, found in copper amine oxidases; lysine tyrosyl quinone, found in lysyl oxidase; tryptophan tryptophylquinone, found in bacterial methylamine dehydrogenases; and cysteine-cross-linked tyrosine,



Scheme 12 Isomeric forms of NADH.



Scheme 13 Quinone cofactors.

found in galactose oxidase. The topa quinone cofactor of copper amine oxidase is generated by copper-assisted self-processing of the precursor protein (Scheme 13).

Conclusion

Many of the aforementioned cofactors are found and used in all forms of life. This suggests that they were present in our earliest of ancestors. The recent assignment of quinone derivatives as the main compound class composing 'interstellar' dust grains is important in this regard. For example, one of the quinones that was identified, pyrroloquinoline quinone, is an important redox cofactor used by methylotrophic bacteria and present in all biological tissues examined to date. Further, considerable evidence supports the notion that the formation of RNA was a dominant process in the eventual evolution of life. RNA is capable of self-replication and catalyzing specific biochemical reactions. Accordingly, it is not difficult to envision that cofactors, such as ATP, evolved by a process similar to the formation of RNA (and DNA) as major components of reactions important to sustaining energy relationships.

With regard to the discovery and conceptual understanding of cofactors as catalyst, most of the advances have come in this century. NAD was the first organic cofactor to be identified extending from the early work of Arthur Harden and William Young. They observed that a boiled and filtered yeast extract accelerated alcoholic fermentation in yeast capable of fermentation. As methods important to the study of protein structure and enzyme kinetics improved, the relationships that cofactors play in influencing induced fit and the so-called 'lock and key' mechanisms, as well as entropic parameters, entasis, and quantum tunneling, became more apparent.

Although detailed discussions of specific catalytic mechanisms are beyond the scope of this article, our goal nevertheless was to provide basic descriptions of cofactor functions. Many of which represent the bases for biochemical lesions associated with nutritional deficiency syndromes and conditions. For more detailed discussions of mechanisms, the reader should consult the references listed in section 'Future Readings.' For additional physiological and nutritional descriptions, the articles that are cross-referenced should be consulted, particularly those related to vitamins and minerals that serve as cofactors.

See also: Antioxidants: Role on Health and Prevention; Ascorbic Acid: Physiology and Health Effects; Calcium: Physiology; Cobalamin (Vitamin B12): Metabolism and Disorders; Copper: Physiology; Enzymes: Functions and Characteristics; Folic acid and Folates: Physiology and Health Effects; Hypovitaminosis A; Iodine: Physiology; Iron: Biosynthesis and Significance of Heme; Iron: Physiology of Iron;

Magnesium; Manganese; Retinol: Physiology; Riboflavin: Physiology; Thiamin: Physiology; Tocopherols: Physiology and Health Effects; Trace Minerals and Trace Elements; Vitamin K: Physiology; Vitamins: Overview; Zinc: Physiology and Health Effects.

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Coffee: Analysis and Composition

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Introduction

Coffee is the most traded commodity in the world after oil. Although native to Africa (Ethiopia (Abyssinia)), nowadays, it is cultivated in regions between the Tropic of Cancer and the Tropic of Capricorn.

The generic name *Coffea* covers approximately 70 species, but only two of them are economically important: *Coffea arabica*, commonly arabica coffee, which accounts for three-quarters of world production, and *Coffea canephora* var. *robusta*, commonly robusta coffee. Differences between these two species include ideal growing climate, physical aspects, chemical composition, and characteristics of the brew made with the ground roasted seeds.

Coffee is one of the most consumed beverages in the world and a rich source of antioxidants. The amounts of these antioxidants are influenced by several technological factors. Besides, the antioxidants identified in coffee (chlorogenic acids (CGAs) and volatile and nonvolatile Maillard reaction (MR) products) contribute in different proportions to the overall antioxidant capacity. Several chronic diseases, such as cancer, cardiovascular, inflammatory, and neurodegenerative pathologies, are associated with oxidative stress. The antioxidants present in coffee, together with caffeine, have been proposed as those compounds that mainly contribute to its health properties.

Green Coffee

Composition of Green Coffee

Arabica and robusta green coffees show both qualitative and quantitative differences in their composition (Table 1).

Green coffee composition is dominated by carbohydrates (~60% dry matter), more abundant in arabica coffee than in robusta. Most carbohydrates present in green coffee are insoluble polysaccharides (cellulose and hemicellulose, arabinogalactan, and galactomannan) accounting for ≈50%. The soluble fraction (≈10%) includes disaccharides (sucrose) and monosaccharides (glucose, galactose, arabinose, fructose, mannose, mannitol, xylose, and ribose) in traces.

Proteins, peptides, and free amino acids account for 10–15% of the green coffee dry matter. The main amino acids, both protein-bound and free, are asparagine, glutamic acid, alanine, aspartic acid, and lysine. Several other *N*-compounds are present in coffee, such as caffeine, trigonelline, and nicotinic acid. The caffeine concentration in robusta coffee is approximately double that in arabica.

Lipid contents in green coffee account for 8–18% of its dry matter. The total content in arabica seeds is approximately twofold than that in robusta. Fatty acids in coffee are found primarily in combined forms; most are esterified with glycerol in the triacylglycerol fraction (75%), 20% esterified with diterpenes, and a small proportion esterified in sterol esters (sitosterol (54%), stigmasterol, and campesterol). Most fatty acids in coffee are unsaturated, being linoleic, oleic, and linolenic acids the most abundant. Also, palmitic, stearic, araquidic, lignoceric, and behenic acids and pentacyclic diterpenes (methylcafestol, cafestol, and kahweol) are present.

Regarding minerals, potassium accounts for approximately 40% of the mineral content of ground coffee, and phosphorus, for 4%. The remaining mineral content consists of approximately 30 different elements, including sodium, magnesium, calcium, and sulfur. Trace minerals in coffee include zinc, silicon, manganese, iron, copper, and aluminum, among others.

Green coffee also contains a large amount and variety of polyphenols. The main components of the phenolic fraction of green coffee are CGAs (7–12% dry matter), which are more abundant in robusta coffee. These phenolic acids are formed by the esterification of one molecule of quinic acid and one to three molecules of *trans*-hydroxycinnamic acids in different positions. The main groups of CGA are caffeoylquinic acids (CQAs), dicaffeoylquinic acids (diCQAs), feruloylquinic acids (FQAs), *p*-coumaroylquinic acids (*p*CoQAs), and mixed diesters of caffeoylferuloylquinic acids (CFQAs; Figure 1). CQAs are the most abundant CGAS in coffee, being 5-*O*-CQA the most abundant. More recently, numerous minor CGAs were found in coffee including isomers of dimethoxycinnamoylquinic acids, dimethoxy-hydroxycinnamoylquinic acids, dihydroxy-methoxycinnamoylquinic acids, diferuloylquinic acids, di-*p*CoQAs, and mixed diesters of the earlier described hydroxycinnamic and methoxycinnamic acids. In addition, at least three free cinnamic acids were also identified in green coffee, namely, caffeic acid, ferulic acid, and 3,4-dimethoxycinnamic acid.

Phenolic compounds different from CGAs and related compounds, such as cinnamoyl glycosides, cinnamoyl amino acids, anthocyanidins, and lignans, have also been identified in green coffee in minor amounts.

Roasting Coffee

Roasting Process

The characteristic properties of the coffee beverage, such as flavor and aroma, are developed during the roasting of coffee. The beans are heated to 180–250 °C, from 2 to 25 min, depending on roast technique and desired roasting degree (light, medium, or dark). The initial changes occur at or above 50 °C when the protein in the tissue cells denatures. Three major phases are drying, pyrolysis, and cooling. During the drying phase, most of the free water evaporates keeping the bean temperature at 100 °C. Temperature rise indicates the second phase. At about 150 °C, there is a release of volatile products (water, CO, and CO_2), which results in an increase of 50–80% in bean volume. At 170–200 °C, pyrolytic reactions drastically modify the chemical composition of the bean by the formation of hundreds of substances that give coffee its characteristic aroma and taste. At the

Table 1 Chemical composition of green *Coffea arabica* and *Coffea robusta* seeds

	Concentration[a] (g/100 g)	
Component	*Coffea arabica*	*Coffea robusta*
Soluble carbohydrates	9–12.5	6–11.5
Sucrose	6.0–9.0	3–7
Reducing sugars	0.1	0.4
Polysaccharides	3–4	3–4
Insoluble polysaccharides	46–53	34–44
Hemicelluloses	5–10	3–4
Cellulose, β-1,4-mannan	41–43	32–40
Nitrogenous compounds		
Protein/peptides	10–11	11–15
Caffeine	0.8–1.4	1.7–4.0
Trigonelline	0.6–1.2	0.3–0.9
Lipids		
Triglycerides	15–17	8–12
Diterpenes (free and esterified)	0.5–1.2	0.2–0.8
Minerals	3–4.2	4.4–4.5
Chlorogenic acids	6.7–9.2	7.1–12.1
5-*O*-Caffeoylquinic acid	3.0–5.6	4.4–6.6

Content varies according to cultivar, agricultural practices, climate, soil composition, and methods of analysis.
[a]%Dry matter; water content of raw coffee 7–13%.

end of roasting, the beans then burst due to the increase of internal pressure, and the popping of the beans indicates that roasting must be stopped by cooling (quenching). Despite chemical changes, also, the physical parameters of coffee change considerably. The bean color changes to cinnamon brown, an 11–20% loss in weight occurs, and the specific gravity falls from 1.2 to 0.6 due to volume increase.

Torrefacto is a roasting process in which sugar is added to coffee, usually robusta. This roasting technique is used in several countries of southern Europe and South America where some segments of the population prefer coffees with a dark-brown color, intense aroma, and a strong taste with a tendency to bitterness. This kind of roasting process was initially used to mask negative sensorial attributes in robusta coffees. Nowadays, torrefacto-roasted coffee is usually blended with conventional roasted coffee (robusta) for commercial use.

The degree of roast is reflected on the external color of the beans (from light to dark brown due to pyrolysis of organic compounds). Despite visual evaluation, color analysis is traditionally carried out on ground roasted coffee using techniques based on the reflectance of the light in the visible or in the NIR range of the color spectrum. The use of a standardized tristimulus colorimeter provides lightness (L^*) and chromaticity (a^* and b^*) parameters that can also be applied to calculate the hue angle (H°) and chroma (C^*). The lower the lightness, the darker the roast. Similarly for the Agtron-type spectrophotometer, which uses NIR illumination, other techniques using lasers have been developed to be used directly on coffee beans without grinding.

Influence of Roasting Process in the Composition of Coffee

The roasting process causes several changes to the composition of the coffee beans, because some compounds are degraded or modified, resulting in the development of the characteristic color, aroma, and flavor of coffee. These transformations have been extensively studied, but the composition of roasted coffee is still far from being elucidated. Table 2 provides a rough overview of the main components of roasted arabica and robusta coffee.

Roasting leads to profound changes in the chemical composition of coffee with a simultaneous decrease in the naturally occurring substances in green coffee and the generation of many other compounds derived from the MRs, carbohydrate caramelization, and pyrolysis of organic compounds. Although some compounds formed during roasting might be responsible for the health properties of the coffee brew, potential carcinogenic compounds, such as acrylamide and furan, can also be formed.

Most of the carbohydrates present, such as cellulose and polysaccharides of mannose, galactose, and arabinose, are insoluble. During roasting, some of them are degraded to low-molecular-weight carbohydrates, which are soluble. Sucrose present in raw coffee is decomposed in roasted coffee up to half.

Phenolic compounds suffer great changes during the heating processing of coffee due to their thermal instability. The content of CGAs drops during roasting as shown in Table 3. During the drying phase, when there is still adequate water content, isomerization of CGAs occurs, leading to an increase in 4- and 3-CQAs in roasted coffee. Additionally, some of the CGAs are hydrolyzed to cinnamic acids and quinic acid. The cinnamates may be decarboxylated and transformed into low-molecular-weight compounds. Later in roasting, part of the CGAs lactonize giving rise to several isomers of caffeoylquinic, feruloylquinic, and diCQA lactones (Figure 1). Decomposition of CGAs has a great impact on the quality attributes of roasted coffee and is directly related to flavor development.

The MR involves the condensation of the carbonyl group of the reducing sugars with the amino group of amino acids and proteins. It consists of a complex network of chemical reactions during which a myriad of products of different chemical compositions are formed, commonly known as Maillard reaction products (MRPs). The polymeric brown-colored final products of the MR are called melanoidins and result from the cyclizations, dehydrations, retroaldolizations, rearrangements, isomerizations, and condensations of other MRPs. Melanoidins are one of the major components of roasted coffee, accounting for up to 25% of dry matter. These compounds have different chemical compositions, which are still not fully elucidated, and consequently different properties: nutrient or contaminant binding, antioxidant or prooxidant, and mutagenic or antimutagenic. Because phenolic compounds also participate in the MR, they are at least partly incorporated in coffee melanoidins through noncovalent or covalent bonds. Besides color development, the MR plays a key role in aroma generation. For example, pyrazines, resulting from the degradation of instable MRPs, are potent odorants in roasted coffee. Other main classes of aromatic compounds formed during the MR are aldehydes, ketones, pyridines, oxazoles, thiazoles, and thiophenes.

Furthermore, the addition of sugar at the end of the torrefacto roasting process might intensify the development of MRs and, consequently, increase the antioxidant capacity of coffee.

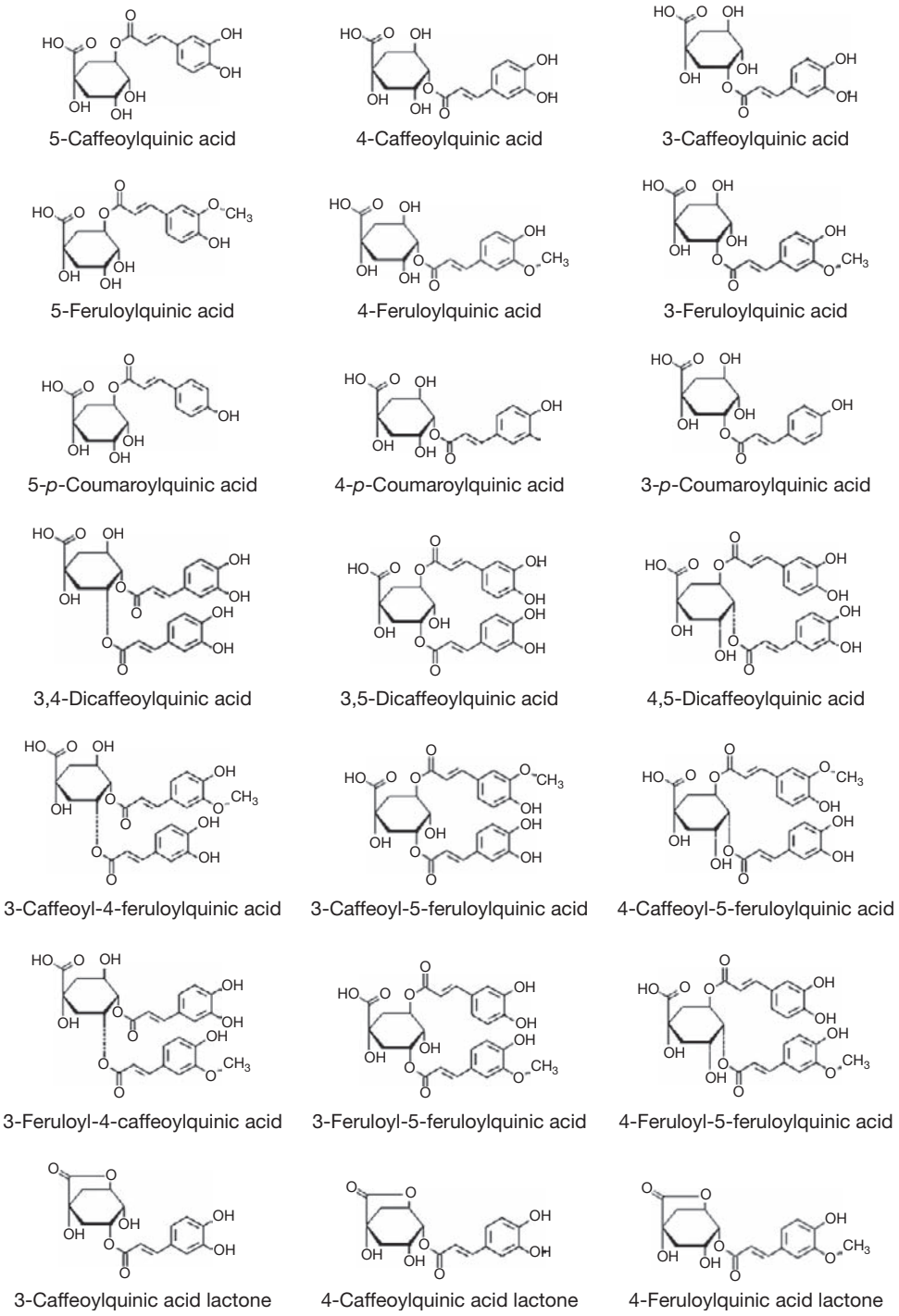

Figure 1 Main chlorogenic acids in coffee.

Protein is subjected to extensive changes when heated in the presence of carbohydrates. There is a shift of the amino acid compositions of coffee before and after bean roasting. The total amino acid content of the hydrolysates drops by about 30% because of considerable degradation. Arginine, aspartic acid, cysteine, histidine, lysine, serine, threonine, and methionine, being especially reactive amino acids, are somewhat decreased in roasted coffee, while the stable amino acids, particularly alanine, glutamic acid, and leucine, are relatively increased. Free amino acids occur only in traces in roasted coffee because they are involved in a series of reactions, for example, the MR, giving rise to potent aroma compounds. Mercaptans, thiophenes, thiazoles, alkylpyrazines, pyrroles, pyridines, and pyrrolizines result from the degradation of sulfur amino acids, hydroxy amino acids, and proline.

The caffeine content of raw arabica coffee is 0.8–1.4%, whereas in the robusta variety, it is 1.7–4.0%. Caffeine is quite thermostable and decreases only slightly during roasting.

Table 2 Chemical composition of roasted *Coffea arabica* and *Coffea robusta*

	Concentration[a] *(g/100 g)*	
Component	*Coffea arabica*	*Coffea robusta*
Carbohydrates/fiber		
Sucrose	Tr – 4.2	Tr – 1.6
Reducing sugars	0.3	0.3
Polysaccharides	31–33	37
Nitrogenous compounds		
Protein/peptides	7.5–10	7.5–10
Caffeine	1.1–1.3	2.4–2.5
Lipids		
Triglycerides	17	11
Diterpenes	0.9	0.2
Melanoidins	25	25

Content varies according to cultivar, agricultural practices, climate, soil composition, and methods of analysis.
[a]%Dry matter; water content of roasted coffee 1–5%.

Table 3 Chlorogenic acid content (%) as a function of the degree of roasting

Raw/degree of roasting	*Arabica*	*Robusta*
Raw	6.9	8.8
Light	2.7	3.5
Medium	2.2	2.1
Dark	0.2	0.2

Source: Belitz, H. D., Grosch, W. and Schieberle, P. (2009). *Food chemistry*. Germany: Springer.

In contrast, trigonelline, which is present in green coffee up to 1.2%, is 50–80% proportionally decomposed to roasting temperature. The main degradation products are nicotinic acid, pyridine, and several alkylpyridines and pyrroles.

The lipid fraction appears to be very stable and survives the roasting process with only minor changes. Linoleic acid is the predominant fatty acid, followed by palmitic acid. The major compounds in roasted coffee beans (coffee oil) are triacylglycerols (78.8%) and diterpene esters (15%). The main diterpenes are cafestol, 16-*O*-methylcafestol, and kahweol. Cafestol and kahweol are partially degraded by the roasting process. Sitosterol and stigmasterol are the major compounds of the sterol fraction.

The potassium is predominant in coffee ash (1.1%), followed by calcium (0.2%) and magnesium (0.2%). The predominant anions are phosphates (0.2%) and sulfates (0.1%). Many other elements are present in trace amounts.

The aroma of roasted coffee is very complex, being composed of a large number of volatiles with different odor qualities, some pleasant, but many others probably below their detection threshold. The concentration, proportion, and synergistic and antagonistic effects among volatile compounds influence the final aroma quality.

The *volatile composition* of roasted coffee depends on many factors, including coffee species/variety, growing and harvesting conditions, storage prior to roasting, degree of roast, and type of roasting machine. The formation of volatile compounds depends on the stability of their precursors and location within the seed. More than 900 compounds with a wide variety of functional groups have been identified until now after roasting in different types of coffee.

The classes of volatile compounds found in roasted coffee are furans, pyrans, pyrazines, pyrroles, aldehydes, ketones, phenolics, hydrocarbons, alcohols, acids, esters, lactones, thiophenes, oxazoles, thiazoles, pyridines, amines, and various sulfur and nitrogen compounds. Not all the volatiles in coffee are odorants, and their contribution to flavor is not usually directly related to their abundance.

The complex aroma is formed during the roasting process by pyrolysis of the water-soluble components, such as sugars, amino acids, and trigonelline. A number of them may be produced by more than one route. The main pathways by which the aroma precursors are degraded are the MR; Strecker degradation and formation of pyrazines and oxazoles; degradation of trigonelline, phenolic acids, lipids, and sugars; breakdown of sulfur and hydroxy amino acids; and proline and hydroxyproline degradation. Generally, carbohydrates produce furans, aldehydes, ketones, and phenols; proteins, peptides, and amino acids produce ketones, pyrroles, and pyrazines; lipids are responsible for only small amounts of aldehydes and ketones given their resistance to changes during the roasting process; CGAs produce phenolic volatile compounds (e.g., catechols, pyrogallol, and phenol); trigonelline produces pyrroles, pyridines, and pyrazines. Almost all thiophenes, oxazoles, and thiazoles are formed during roasting, since they are not usually detected in green coffee.

2-Furfurylthiol is the most important contributor to the aroma of coffee. Its precursors are polysaccharides containing arabinose, for example, arabinogalactans, and cysteine in the free and bound forms. Part of furfurylthiol and the other thiols are present in roasted coffee as disulfide bound to cysteine, SH-peptides, and proteins.

Robusta coffees contain alkylpyrazines and phenols in significantly higher concentrations than arabica, being earthy and smoky/phenolic notes in the aroma profile more intensive. Arabica coffees are usually richer in the odorants of the sweet/caramellike group. In torrefacto coffee, where coffee is roasted with sugar, pyrazines, pyridines, and furans are formed in greater quantity than in conventional roasted coffee due to increases in the Maillard and caramelization reactions.

At least 28 volatile compounds are reported as key odorants of ground and brewed coffees and contribute to the aroma. Most of these odorants have been assigned to sweet/caramel, earthy, sulfurous/roasty, and smoky/phenolic notes. The remaining odorants have a fruity or spicy odor. Several authors suggested that only those compounds which concentrations surpass their odor thresholds are odor-active in food. However, the evaluation of the odor activity values for all of the volatiles in coffee is very laborious because concentration and odor threshold data must be determined by a large number of compounds (Table 4).

One of the first practicable methods to convert the results of instrumental analysis of the volatiles into sensory data was combined hedonic aromatic response measurement analysis and aroma extract dilution analysis. In both procedures, serial dilutions of an extract containing the volatile fraction of a food

Table 4 Volatile compounds in coffee

Class of compound	*Number*
Hydrocarbons	80
Alcohols	24
Aldehydes	37
Ketones	85
Carboxylic acids	28
Esters	33
Pyrazines	86
Pyrroles	66
Pyridines	20
Other bases (e.g., quinoxalines and indoles)	52
Sulfur compounds	100
Furanes	126
Phenols	49
Oxazoles	35
Others	20
Total	841

Source: Nijssen, L. M., Visscher, C. A., Maarse, H., Willemsens, L. C. and Boelens, M.H. (1996). Volatile compounds. In: *Food qualitative and quantitative data* (7th ed.), pp. 72. 1–72.23. Zeist, The Netherlands: TNO Nutrition and Food Research Institute.

are analyzed by gas chromatography–olfactometry (GCO). In this technique, the separated compounds at the effluent from a GC column are evaluated qualitatively one by one by human assessors. An integrated approach involving the joint determination of the volatile compounds by gas chromatography–mass spectrometry (GC–MS) and GCO provides useful information about the most important active contributors to the aroma of different coffees.

One of the key factors in the analysis of the profile of volatile compounds is their extraction. Several methods have been used to study the aroma fraction of roasted coffee and coffee brews. One of the first was the preparation of coffee extracts where volatile compounds were dissolved with organic solvents, as in steam distillation extraction. This method should be performed under mild conditions. The temperature, in particular, is a critical point, due to the instability of, for example, thiols and disulfides. To avoid the formation of artifacts, the temperature during the isolation of the volatiles may not exceed 50 °C for a longer period.

As an alternative to the injection of an organic solvent extract, the vapor phase surrounding the coffee sample (headspace) can be directly analyzed. This method gives the most accurate composition of volatiles. However, under the usual operating conditions, the headspace gas sample is considerably diluted by the carrier gas. This problem can be solved by the injection of the headspace gas directly into the interior of the capillary column (on-column injection), using a purge-and-trap system with an adsorbent or using a static headspace (SH) sampler. Headspace sampling has progressively replaced techniques such as steam distillation or solvent extraction because it is the most suitable for studying high volatile compounds and because its composition represents better the aroma perceived by the consumer. The direct and accurate analysis of volatiles in coffee by SH (SH-GC) requires a careful standardization of instrumental parameters such as sample size, equilibration time and temperatures, and chromatographic conditions required for the separation of volatile compounds. As the injection procedure can be automated using a headspace sampler, errors associated with manual handling are removed.

Solid-phase microextraction has been extensively applied to the study of coffee aroma as a simple, rapid, solvent-free, and inexpensive method. The selection of fibers and optimization of both extraction and desorption parameters are necessary. Polydimethylsiloxane (PDMS)/divinylbenzene (DVB) coating had the highest overall sampling sensitivity, whereas carboxen (CAR)/PDMS was the most effective for small molecules and acids. In coffee, the DVB/CAR/PDMS fiber has been chosen because its three-component composition gives high recoveries for analytes with different structures and polarities.

The separation of volatile compounds is usually achieved by GC. The components eluting from the GC can be detected with an FID, although specific detectors, including the NPD and FPD, are also widely used for coffee volatiles, but MS is nowadays the most common detector used to identify and also to quantify (by total ionic current) coffee volatiles.

The change in the flavor profile from the ground coffee to the brew is caused by a change in the concentrations of these compounds and not by the formation of new odorants. The aroma of coffee is not stable; the fresh note is rapidly lost. Of the highly volatile odorants, methanethiol evaporates the fastest, followed by acetaldehyde.

CGA contributes to body and astringency; sucrose contributes to the color, aroma, bitterness, and sourness; proteins remain perfectly stable, but minor protein components, such as free amino acids, are highly reactive. Trigonelline generates pyridine and may consequently be responsible for some objectionable flavors. Caffeine has no function other than a contribution to the bitterness.

Coffee Brew

The brewing process is essential in brew composition, because the contact of water with roasted coffee grounds is the crucial step for the extraction of coffee compounds. Other factors, such as origin or variety of coffee beans, blending, roasting degree, and grinding, also play a key role in coffee brew composition.

Among the several brewing techniques, filter coffee (drip filter) is the most widely used coffee brew obtained by infusion method, whereas espresso coffee (EC) is the most appreciated coffee brew produced by pressure method. Moreover, in southern European countries such as Italy and Spain, the use of the mocha coffee maker is much extended at domestic level, and the plunger coffee maker is being used more often for coffee aroma lovers. In each case, the technical conditions applied, such as the coffee/water ratio, water temperature, and water pressure, also contribute to the different chemical compositions of coffee brews.

In drip filtration methods, water at 92–96 °C flows through a hardly compressed ground coffee bed, and the extract drips from the brewing chamber into the pot. Turbulence in the brewing chamber prevents water from becoming saturated. This method involves extracting water-soluble compounds, but most of the lipophilic fraction remains in the filter with the solid materials.

In pressure methods, water at approximately 9 bar and 88–92 °C is forced to go through coffee grounds compacted in a small brewing chamber (coffee cake). Rapid brewing time and fine particle size are necessary. In this case, the composition and quality of the brew also depend on the water pressure.

Usually, the extraction of water-soluble components including CGAs, caffeine, nicotinic acid, trigonelline, soluble melanoidins, and hydrophilic volatile compounds is greater at higher temperature and pressure. Galactomannans and arabinogalactans are the predominant polysaccharides that pass into the brew.

Caffeine is the single most frequently determined compound in coffee products, and the methods applied to its analysis have changed dramatically over the last 25 years. Caffeine absorbs strongly in the ultraviolet region (272 nm in water and 276 nm in chloroform), and this provides the basis of countless spectrophotometric methods. At present, chromatographic methods, and mainly reversed-phase HPLC, provide the most reliable way to quantify caffeine in coffee products. A simple aqueous extraction produces very complex extracts, which cannot be directly used. A similar chloroform extract is significantly cleaner but still contains interfering compounds that absorb at 276 nm. The level of these interfering components may be reduced by simple clarification with lead acetate and filtration to remove polymeric materials in samples, solution cleanup with a C18 Sep-Pak cartridge, and the column chromatography. In many cases, the chromatographic conditions allow the simultaneous determination of trigonelline and caffeine. A smaller number of papers have described alternative liquid chromatographic techniques, for example, ion-exchange and even gel-filtration.

The most abundant *phenolic compounds* of coffee are CGAs. The main hydrophilic antioxidant compounds in coffee are 3-, 4-, and 5-monocaffeoylquinic acids and 3,4-, 3,5-, and 4,5-diCQAs. Also, little amounts of caffeic acid, ferulic acid, *p*-coumaric acid, 4-hydroxybenzoic acid, and sinapic acid can be found in coffee. Traditionally, the Folin–Ciocalteu colorimetric method has been used to routinely measure the total phenolic compounds. Although this is the most popular method to evaluate the total phenolic compounds, the Folin–Ciocalteu reagent can be reduced by many electron donors, not only phenolic compounds. Furthermore, to identify and quantify single phenolic compounds is necessary to apply HPLC analysis. Detection of phenolic compounds is usually recorded at 325 nm. Quantification of 5-caffeoylquinic acid (5-CQA) is made by comparing the peak area with that of the standard. Because of the lack of commercial standards, in many cases, quantification of the other caffeoylquinic acids (and other CGAs) is performed using the area of 5-CQA standard combined with the molar extinction coefficients of the respective CQA. Also, in recent years, MS has become the most suitable method to identify and quantify phenolic acids.

The *lipid fraction* (not water-soluble) of coffee is partially extracted due to the high temperature of the water and is present in the brew as an emulsion. However, oil particles are likely to be retained in filters made of paper or similar types of materials. The high pressure used to make espresso and the absence of a filter made of paper, or another lipophilic material to retain the lipids, facilitate their extraction into the brew. Thus, unfiltered coffee and EC brews contain higher amounts of these compounds, including bioactive diterpenes and sterols. The emulsified lipid fraction can be determined by liquid–liquid solvent extraction with, for example, trichloromethane. Total lipids are quantified by weight after evaporation of the solvent. Different amounts have been reported in both espresso and non-EC beverages. For an EC prepared with a 100% arabica coffee, ~5% of the total solids may be considered as typical. Within the unsaponifiable fraction of coffee lipids, two compounds belonging to the diterpene family – cafestol and kahweol – are of relevance to blood cholesterol level; present in roasted arabica beans at levels around 0.6%, they are transferred to the espresso brew in small amounts.

Browned compounds are quantified by measuring the absorbance of the sample at 420 nm after exactly 1 min with a spectrophotometer connected to a thermostatically controlled chamber (25 °C).

Caffeine and the quinic acid lactones are the main bitter substances in the coffee brew. Also, acidity of coffee beverages is an important organoleptic parameter. The presence of acetic, formic, malic, citric, and lactic acids has been detected in the brew, along with quinic acid and CGAs. The content of the last ones is of course smaller when using dark roasted coffee, in which they have largely disappeared. A mineral acid – phosphoric acid – has also been found in brews, deriving from the thermal degradation of phytic acid, the phosphoric acid ester of inositol. Coffee brew acidity cannot be described just by the pH. The measurement of this variable, obtained using electrode-type instruments, reports values ranging from 4.8 to 5.8 (optimum 4.9–5.2), showing an increase with roasting degree and depending on extraction time.

EC is an increasingly popular drink. By definition, EC is a "polyphasic beverage prepared from roast and ground coffee and water alone, constituted by a foam layer of small bubbles with a particular tiger-tail pattern, on top of an emulsion of microscopic oil droplets in an aqueous solution of sugars, acids, protein-like material and caffeine, with dispersed gas bubbles and solids." These characteristics of EC are responsible for their peculiar sensorial properties. A fine EC should have a great amount of persistent, consistent, and hazelnut foam with 'tiger-skin' effect, a bitter/acid balance taste, and a strong body. A good EC cup could be prepared from approximately 7.5 g of finely ground roasted coffee for a volume of 40 ml of water at 9 atm of pressure and 96 °C temperature.

The quality of a good cup of EC is initially evaluated by the quality of its foam. The *foam index* is defined as the ratio, in percentage, of EC foam and liquid volumes measured immediately after the extraction of EC using a 100 ml graduated cylinder. The *persistence of foam* is defined as the time (in minutes) that the liquid phase below the cream layer took to appear during cooling at room temperature. Foamability seems to be influenced by a protein fraction, while foam persistency depends more on a polysaccharide fraction.

Density is little influenced by coffee solids. Differences with respect to pure water are limited to the second decimal digit; this could be explained by the presence of coffee lipids dispersed as an emulsion, which are less dense than water and decrease the overall density. The addition of sugar can raise density to around 1.08 g ml^{-1}. *Viscosity* is influenced by the presence of dispersed phases, related to the amount of lipid droplets in emulsion. Increased viscosity has been associated

with improved body. *Surface tension* is a property of interface, due to the tendency of molecules to diffuse from one material to another: pure water–air interfaces.

The brewing method may influence the hydrolytic degradation of insoluble carbohydrate polymers, adding to the soluble content. Total solids content is the most important characteristic in the chemical composition of EC, often perceived by consumers as 'strength'; a typical figure for soluble carbohydrates is 8 g l^{-1}, corresponding to around 15% of total solids. The *total solids* are determined by oven-drying 40 ml of EC to a constant weight (14 h, 102 ± 3 °C) without filtering. Accordingly, suspended solid materials, emulsified lipids, and solutes are included. The *extraction* is defined as the percentage of total solids with respect to ground roasted coffee dose (7.5 g). The *concentration* is defined as the percentage of total solids with respect to the EC volume (40 ml). The *total solids on filtrate* are determined by oven-drying 40 ml of EC after filtering with Whatman 1 to a constant weight (14 h, 102 ± 3 °C). The true soluble fraction, typically 90% of the total solids, can be determined by oven-drying after filtering the liquid.

Antioxidant capacity. During very intense heat treatments, such as the roasting of coffee, the loss of antioxidant activity of natural antioxidants – mainly represented by polyphenols – by progressive thermal degradation has been found to be minimized by the formation of MRPs. The antioxidant activity of coffee can be measured using different methods. Colorimetric reactions are developed to evaluate the ability of a food compound or product to quench and/or reduce radicals or metals using DPPH, or ABTS radicals, or the FRAP assay, respectively. The antioxidant capacity is usually compared with that of a very well-known antioxidant, such as vitamin E, using Trolox (a water-soluble vitamin E analog). For this reason, these methodologies are usually called Trolox equivalent antioxidant capacity (TEAC) assays. These colorimetric methodologies are currently the most widespread, but all of them are based on similar electron transfer redox reactions and evaluate more than phenolic compounds. Other methodologies like the electron spin resonance spectroscopy have been proposed as much more specific techniques, capable of distinguishing the contribution of phenolic and nonphenolic antioxidants in coffee by means of different stabilized radicals such as Frémy's salt and TEMPO. The basis is the absorption of microwave energy by unpaired electrons, such as those of free radicals, when they are in a magnetic field. Mainly, phenolic compounds can be detected when Frémy's salt is used as the stabilized radical, whereas TEMPO is mainly scavenged by MRPs, such as melanoidins.

ABTS: 2,2´-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt. DPPH: Potassium persulfate, 2,2-diphenyl-1-picrylhydrazyl. Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid. Frémy's salt: Dipotassium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, Frémy's salt (potassium nitrosodisulfonate). TEMPO: 2,2,2,2-Tetramethyl-1-piperidine-1-oxyl.

See also: Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Caffeine: Characterization and Properties; Caffeine: Consumption and Health Effects; Coffee: Decaffeination; Coffee: Health Effects; Coffee: Types and Production; Maillard Reaction; Phenolic Compounds: Bioavailability and Health Effects.

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Coffee: Decaffeination

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Introduction

The term coffee is mostly employed in association with the consumable beverage obtained by extracting roasted coffee with hot water, but it actually comprises a wide range of intermediate products, starting from the freshly harvested fruit (coffee cherries), then to green and roasted coffee beans, to the final product of consumption, the beverage itself. Decaffeinated coffee is defined as 'coffee from which caffeine has been extracted' and, as a commercially available product, corresponds to roasted and ground coffee. The decaffeination process is usually performed on coffee beans prior to roasting (green coffee beans).

Background

Caffeine (1,3,7-trimethylxanthine) is an alkaloid that occurs naturally in coffee, cocoa beans, kola nuts, and tea leaves. It is the most widely consumed and studied psychoactive substance in history, but the general conclusions on its effects on human health are still controversial. Increased alertness and learning capacity and better exercise performance have been cited as some of the positive effects of low to moderate caffeine intake. While some studies have associated caffeine intake with high blood cholesterol, coronary diseases, and cancer, others suggest that its consumption may lower the incidence of suicide and hepatic cirrhosis. Nonetheless, excessive caffeine intake has been associated with a wide variety of health problems, including aggravated heartburn and acid indigestion, anxiety, tachycardia, insomnia, and accelerated osteoporosis. It has also been reported to cause mutation, inhibition of DNA repairs, adrenal stimulation, cardiac arrhythmias, and increased heart output. In light of some deleterious effects and the general controversy around this specific compound, a great deal of effort has been devoted to providing caffeine-free beverages, especially coffee.

Roasted coffee beans contain ~1–2% of caffeine by weight. The decaffeination process removes up to 97% of the caffeine, so that a cup (150 ml) of decaffeinated coffee contains from 1 to 5 mg of caffeine, as opposed to 60–180 mg for the same cup with regular coffee.

It should be kept in mind that coffee is a complex mixture of chemical compounds, and, although earlier studies on the effects of coffee on health have been mostly associated with caffeine consumption, it should be emphasized that this compound is just one of the several bioactive substances that are present in coffee. Unfiltered coffee is a significant source of cafestol and kahweol, and such diterpenes have been implicated in the cholesterol-raising effects of coffee. Some results of epidemiological research indicate that coffee consumption may help prevent several chronic diseases, including type 2 diabetes, Parkinson's disease, and liver disease. Overall, there are little evidence of health risks and some evidence of health benefits for adults consuming moderate amounts of coffee (3–4 cups per day).

Decaffeination Procedures

In the production of coffee, green beans are roasted to generate the coffee soluble solids and volatiles that impart flavor to the brewed beverage. In order to avoid coextraction of aroma components in the decaffeination process, green beans are generally extracted. The conventional methods employed for coffee decaffeination are organic solvent extraction, water decaffeination, and supercritical carbon dioxide extraction. A schematic overview of these procedures is displayed in Figure 1, and a more detailed discussion on each specific procedure is presented as follows.

Solvent Decaffeination

The original processes employed for coffee decaffeination were based on solvent extraction from the green coffee beans. Solvent extraction relies on the solubility of caffeine in various organic solvents including acetone, benzene, ethyl acetate, ethyl alcohol, ethyl ether, and methylene chloride. It can be accomplished either by direct solvent extraction of the beans or indirectly, employing water extraction of the beans, followed by solvent extraction of the caffeine from the water extract.

The first commercial process was developed in Germany (1905), established by the firm Kaffee HAG. Chlorinated compounds were the first caffeine extractants used in this process. Trichloroethylene was commonly employed as the solvent of choice until it became the subject of US Food and Drug Administration investigations and was eliminated in 1977, because of its suspected carcinogenicity, and then replaced by methylene chloride. A wide variety of solvents have been tested over the years, with dichloromethane (DCM) and ethyl acetate being the most employed up to date. DCM has also been recently tested as a caffeine extractor for coffee bean waste, that is, spent coffee grounds. DCM is not selective as other solvents, including methanol and water, although in this specific application, the coffee was roasted as opposed to the green beans used in the traditional processes. Also, caffeine levels will be much lower in this type of waste because most of the caffeine will be transferred to the beverage, given its high solubility in water at elevated temperatures (>90 °C).

The conventional decaffeination process can be divided into four major steps: (1) steaming the beans, (2) extracting caffeine with an organic solvent, (3) driving off the solvent by steam to a tolerable level, and (4) drying the coffee beans. It was early determined that direct organic solvent extraction was either not very effective or rather slow, regardless of the high solubility of pure caffeine in the specific solvent employed. In order to improve extraction and expedite caffeine removal,

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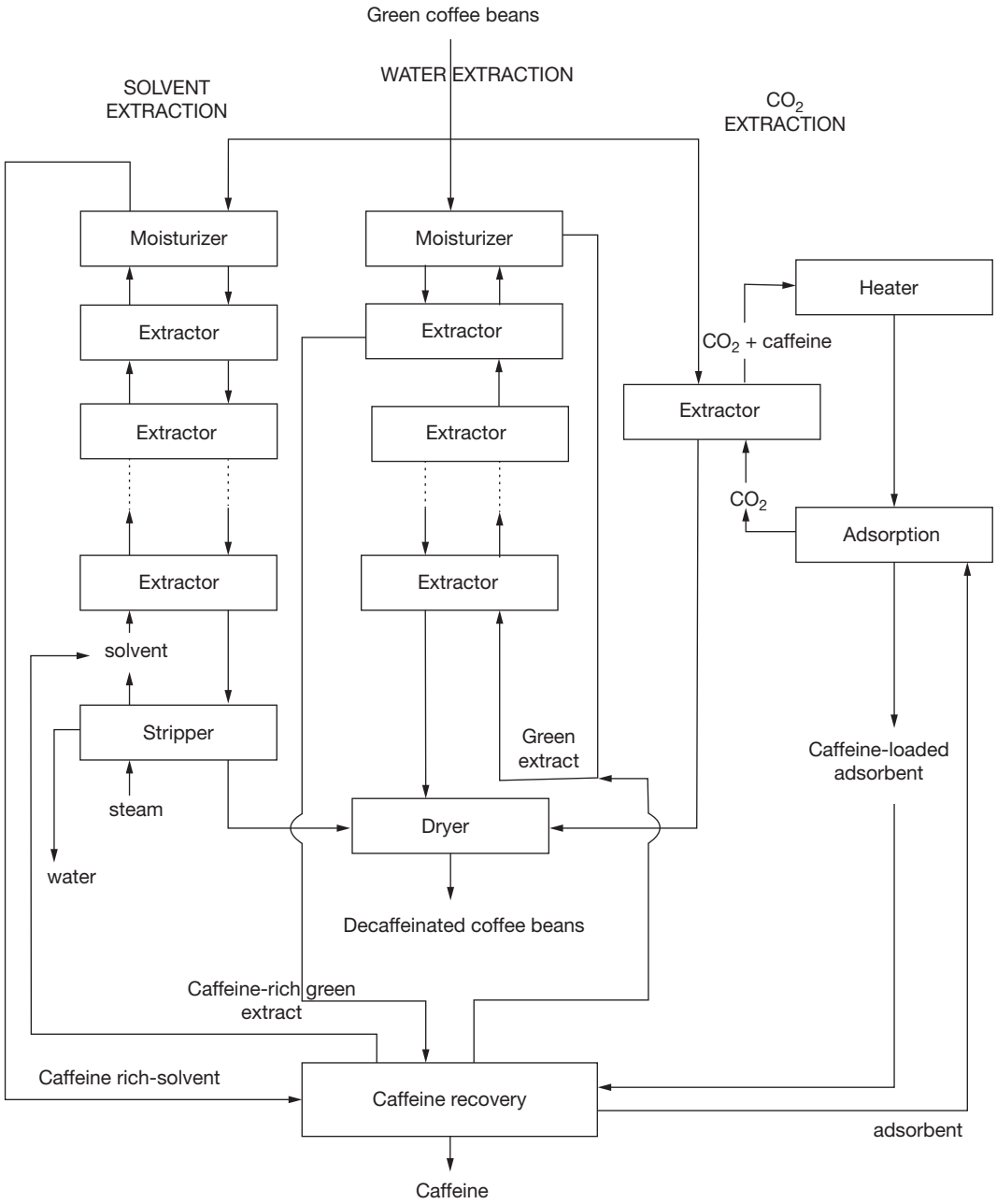

Figure 1 Schematic overview of conventional decaffeination processes.

coffee beans are thus steamed. This will promote swelling of the beans and improve solvent permeability and also break down caffeine complexes. A process patented in 1939 suggested that the coffee beans should be first contacted with water (20 °C) for approximately 1 h prior to steaming. This immersion of the beans is intended to wet the outside surface of the beans and also provides a so-called surface absorption of water, since the water content is raised from 10% to 22%, approximately. Other processes suggested a wetting step after steaming in order to raise the moisture content of the beans up to 40%.

The extraction itself is carried out in a battery of several fixed bed columns with the solvent, at temperatures ranging from 25 to 120 °C, being contacted countercurrently with the bed of beans, for a period of up to 10 h. The column that contains the coffee beans with the lowest caffeine content is separated and solvent-drained. It is then steam-stripped in order to remove all the residual solvent. The decaffeinated coffee beans are then discharged and dried prior to storage. The caffeine-rich solvent that exits the first column of the batch is sent to a caffeine recovery stage, and the resulting caffeine-free solvent is returned to the process. See Figure 1 for a schematic view of the process.

There are some studies introducing modifications over the basic solvent extraction procedure. The extraction time can be reduced down to 3 h by employing a solvent in turbulent flow.

The step of moisturizing the coffee beans can be skipped when dimethyl sulfoxide is employed as a solvent. However, other compounds including sugars are also dissolved, and thus, their concentration must be kept near their saturation point. Some experiments indicate that mixing a suspension of caffeic acid crystals in water with methylene chloride or ethyl acetate provides a significant increase in extraction capacity. A caffeine–caffeic acid complex is rapidly crystallized at room temperature, and the extent of decaffeination is much higher in comparison with the same solvents mixed with water. A patent from 1988 (General Foods Corporation, the United States) proposed that contacting any type of caffeine-containing extract solution with caffeic acid should result in an insoluble caffeine–caffeic acid complex. Several types of extracts/solvents (oil, water, and organic) were tested, employing both green and roasted coffees.

Water Decaffeination

In 1941, a process that aimed at the removal of caffeine from coffee employing the universal 'green' solvent was devised. The so-called water decaffeination (Swiss Water process) consists basically of replacing the commonly employed organic solvents by water, taking advantage of the temperature-dependent solubility of caffeine in such nontoxic solvent. However, there are other water-soluble constituents that should not be extracted in order to maintain flavor qualities. Therefore, in order to prevent their extraction, the extracting water must contain equilibrium quantities of these solubles, with little or no caffeine. Claimed advantages of this process over solvent decaffeination include increased extraction rates, elimination of the solvent stripping system, purer extracted caffeine, and elimination of undesirable compounds extracted by the organic solvents. Also, the pre-steaming and pre-wetting steps are eliminated. The extraction process itself is similar to the one employed for solvent decaffeination, with a battery of columns containing the green beans being contacted countercurrently with the water extract (containing up to 15% green coffee water solubles), for a period of up to 8 h (see Figure 1). The decaffeinated beans are washed with water and air-dried. The wash water is mixed with the caffeine-free water extract in order to maintain the required equilibrium composition of noncaffeine solubles. The caffeine-rich extract is also recycled after caffeine removal. Caffeine recovery is performed by either solvent extraction or adsorption by activated carbon.

CO_2 Decaffeination

The use of supercritical CO_2 extraction for coffee decaffeination started in Germany in 1970. The major advantage of this method is the use of an inert gas as solvent, while the disadvantages are the costly high-pressure equipment and batch processing. The process is usually carried out at pressures ranging from 180 to 300 bars and temperatures of 40–90 °C. Initially, the coffee beans are brought to a moisture content of up to 50% (by wetting, steaming, or both) as in the case of solvent extraction. The beans are then loaded into the extraction vessel, while a solid adsorbent (activated carbon) is loaded into the adsorption vessel. As the CO_2 is circulated through the vessels, caffeine is extracted from the beans and retained by the activated carbon (see Figure 1). The extracted caffeine may also be removed from the supercritical fluid by reducing pressure, thereby decreasing its solubility, or by passing the CO_2 into a washing tower, where the caffeine is absorbed by the water. An alternate of the method represented in Figure 1 consists in placing both the green coffee beans and activated carbon into a pressure vessel, being contacted with CO_2 at 220 bar and 90 °C. The coffee beans are separated from the granular activated carbon by a vibrating sieve. Decaffeinated beans are then dried down to 10% moisture as in the other types of decaffeination procedures.

There are several patents introducing a few modifications and variations over these methods. If a decaffeination rate of over 97% is required, the caffeine/CO_2 mixture can be passed first through a washing tower and then through a column containing the activated carbon. The use of supercritical nitrous oxide has been proposed as a replacement for CO_2, given its higher solvent power under similar pressure and temperature conditions. The use of a CO_2-saturated caffeine-free green coffee extract has also been proposed. After contacting the beans for several hours at high pressures (up to 300 bar) and temperatures (up to 100 °C), there is a rapid drop in pressure, and the beans are washed with the liquid for up to 2 h. The CO_2-saturated caffeine-containing green coffee extract is then decaffeinated with supercritical CO_2 (the caffeine is separated from the CO_2 in a water absorption tower). Other proposed modifications include (i) elimination of the pre-wetting step by employing a varying temperature profile during extraction, (ii) acidification of the beans during the pre-wetting step in order to compensate for acidity losses, and (iii) replacement of the activated carbon by ion exchangers or zeolites. A recent study proposed simultaneous extraction of caffeine and chlorogenic acids from coffee beans by employing both water and supercritical CO_2 flowing countercurrently through the extractor filled with the beans. The nonpolar recovery section height was varied (increased) by adding glass beads to the bottom of the extractor. It was observed that caffeine recovery in the supercritical CO_2 increased with increasing height of the nonpolar recovery section, because it allowed caffeine already dissolved in the water phase to be transferred to the supercritical CO_2 phase.

Although the majority of CO_2 decaffeination processes employ supercritical conditions in order to obtain satisfactory extraction, there is a patent from 1989 (Hermstein & Still, Germany) that proposes the use of liquid CO_2. Moisturized green beans (45–55% moisture) are contacted with water-saturated CO_2 at relatively lower pressures (65–70 bar) in comparison with supercritical extraction. Extraction time is quite high, 60 h, but it is claimed that the flavor quality of the decaffeinated coffee is very good. Caffeine is recovered by decompression of the CO_2–caffeine mixture.

Roasted Coffee Decaffeination

The major drawback of applying the aforementioned methods directly to roasted coffee is the simultaneous extraction of volatiles and aroma components. Nonetheless, a few procedures have been proposed. A patent from 1984 (from Kaffee HAG) described the application of supercritical CO_2 decaffeination to roasted coffee. Extraction takes place in two steps, the

first employing dry CO_2 to extract aroma components and then employing CO_2 to remove the caffeine itself from wetted roasted coffee. Afterward, the water-soluble components are extracted from the decaffeinated coffee and mixed with the aroma-containing CO_2. The resulting extract can then be freeze-dried to produce decaffeinated instant coffee. A few years later, the same company proposed a procedure employing liquid CO_2 to remove caffeine from roasted and ground coffee with minimal extraction of aroma volatiles. Moistened roasted coffee is placed in a pressure vessel connected in a closed cycle with another pressure vessel filled with a strong acid ion exchanger that is selective for adsorption of caffeine. Extraction takes about 2–3 h in a closed system and the CO_2 is kept at low temperatures (15–30 °C). The small amount of roasted coffee components that are absorbed by the CO_2 can be separated by CO_2 evaporation and returned to the roasted coffee. It is claimed that the flavor of the decaffeinated roasted coffee obtained by this procedure is similar to regular roasted coffee.

Other Developments

Water-immiscible oils or fatty materials have also been proposed as extractants for decaffeination processes. Examples include sunflower, soybean, corn, peanut, and coffee oils. The procedure consists basically of the conventional multistage countercurrent extraction, with the coffee beans previously moistened (40–60% water content). Caffeine extraction is carried out at temperatures ranging from 90 to 120 °C. The oil is separated from the green beans by steaming and regenerated by liquid–liquid water extraction. This type of caffeine extractant can also be employed for caffeine removal from the caffeine-rich green extract obtained during water extraction.

Other 'green' solvents have been recently evaluated as alternatives for caffeine removal. A recent study showed the potential of ethyl lactate (ethyl 2-hydroxypropanoate) for such purpose. Accelerated solvent extraction was employed at temperatures ranging from 100 to 150 °C and 10 min extraction time. The recovery of caffeine was in the range of 50–90%. Further studies are mentioned aiming at the use of supercritical CO_2 with ethyl lactate as a cosolvent.

Caffeine Recovery

Large amounts of caffeine are required for addition to beverages and for pharmaceutical uses. Market needs are met either by recovering and refining caffeine from the decaffeination of coffee or via synthetic route. Caffeine removed from coffee decaffeination processes is usually obtained from a caffeine-rich solvent mixture, either an organic solvent or water, or from a caffeine-loaded adsorbent. Caffeine mixed with a volatile solvent can be recovered by solvent evaporation or distillation. If the solvent presents a high boiling point, a countercurrent water liquid–liquid extraction system can be employed. Removal of caffeine from the water–caffeine mixture coming from either this type of process or water decaffeination can be accomplished by (a) liquid–liquid extraction with a volatile solvent (that will be later evaporated) or (b) adsorption by an activated carbon. As previously mentioned, caffeine recovery from processes employing supercritical CO_2 is also based on adsorption.

Recovery of caffeine from the saturated adsorbent can be performed in several ways. The most common procedure is contacting the caffeine-loaded adsorbent with a chemical agent that will provide desorption, usually an acidic solution. Employed acids include acetic, formic, benzoic, citric, dichloroacetic, and lactic, either pure or combined. Other desorption agents include organic solvents such as methyl ethyl ketone, ethyl acetate, and dichloromethane. Caffeine can then be recovered from the resulting solution by employing several methods such as solvent evaporation or distillation, membrane separation, and crystallization. Both water and CO_2-based decaffeination procedures claim the major advantage of employing 'green' solvents. Naturally, such advantage can no longer be claimed if the previously mentioned solvent-based caffeine recovery systems are also employed. Thus, some solvent-free processes have also been devised. In a process developed by Kaffee HAG, in 1985, desorption is accomplished by treating the caffeine-loaded activated carbon with hot pressurized water (300 °C and 90–200 bar) for 1–3 h. The activated carbon is then ready to be reused in the decaffeination process without further treatment. As the adsorption capacity decreases slightly after each cycle of use, the activated carbon must be eventually reactivated at high temperatures. The caffeine is recovered from the aqueous solution by membrane separation or evaporation.

The so-called 'direct caffeine desorption' process, based on two patents from HAG (1986 and 1994), also avoids the use of solvents in caffeine recovery. The caffeine-loaded adsorbent is processed in a three-stage fluidized bed reactor. In the first (upper) stage, caffeine desorption takes place by contacting the adsorbent with an inert gas from combustion (oxygen-depleted) at 360 °C. In the second (middle) stage, nondesorbed caffeine and inorganic impurities are decomposed at a higher temperature. In the third (lower) stage, the activated carbon is regenerated by the gas entering the reactor at 800 °C. The adsorbent is then water-quenched and returned to the decaffeination plant. The caffeine-saturated gas stream is treated in a scrubber system, in which the caffeine is absorbed by water and later crystallized.

Future Trends

There are a few available reports on attempts to produce genetically decaffeinated coffee beans. Transgenic coffee plants with suppressed caffeine synthesis using RNA interference (RNAi) technology have been obtained. Overall, it was concluded that RNAi seemed to confer a global effect on expression of several relevant genes, indicating that reduction of caffeine levels was not due to the suppression of a single enzyme but instead attributed to a disturbance of the whole biosynthetic pathway. It was also concluded that theobromine is the major intermediate in caffeine biosynthesis. Both theobromine and caffeine contents of the plants were reduced by up to 70%, indicating that it should be feasible to produce coffee beans that are intrinsically deficient in caffeine. However, given that caffeine offers protection to young leaves and fruits from predators like larvae and also avoids competition by preventing the growth of neighboring plant species, producing decaffeinated plants through genetic manipulation still presents a few challenges.

Recent innovations in food technologies have led to the use of traditional technologies, such as fermentation and enzyme technology, to produce new health food ingredients, reduce or remove undesirable food components, add specific nutrients or functional ingredients, modify food compositions, mask undesirable flavors, or stabilize ingredients. With that in mind, understanding the mechanisms involved in the degradation of caffeine by either microorganisms or enzymes can help in the development of a bio-based process for caffeine removal.

Bacterial strains found capable of degrading caffeine belonged to *Pseudomonas* and *Serratia* genera, and there is an indication that they can be used for reduction of the caffeine content in bearing plants. A method has been proposed for producing tea leaves with less caffeine content by growing caffeine-degrading bacteria on the surface of the leaf, but no studies on coffee have been reported. Many of the available studies on caffeine degradation by biological action means have focussed on lowering the caffeine content of coffee pulp and husks (residues generated during the wet and dry processing of coffee, respectively) so they can be employed as a supplement for animal feed. A few have focussed on the use of these residues as fermentation substrates for the production of value-added chemicals, and caffeine degradation was merely a consequence of microorganisms' growth. Anaerobic fermentation of coffee pulp resulted in about 13–63% reduction of caffeine in 100 days, whereas aerobic fermentation resulted in 100% degradation of caffeine in 14 days. Bioremediation of coffee pulp and husks to reduce the caffeine content has been mostly studied in fungal systems. Among the microbial communities present in coffee pulp, only a few species like *Aspergillus*, *Penicillium*, and *Rhizopus* were found capable of degrading caffeine. *Aspergillus* and *Penicillium* species degraded caffeine almost with 100% efficiency at 25 °C, whereas the efficiency of degradation decreased to 30% at 30 °C. An effective method has been reported using coffee pulp and husks as substrates for the growth of molds. Caffeine was degraded during the growth of *Lentinus edodes*, whereas caffeine was accumulated in the fruiting bodies of *Pleurotus* sp. Thus, *Pleurotus* sp. could be used to recover caffeine from coffee.

Though the search for a caffeine-degrading microorganism began over 40 years ago, studies in this research area are still needed. The degradation pathway in fungi is still not clearly known. Even in microbial systems, studies have to be performed to ensure that toxic metabolites are not formed by the strain during its growth. More microorganisms that could degrade caffeine need to be isolated. Also, studies employing coffee beans or plants as growth substrates have yet to be conducted. Biological detoxification of coffee pulp by solid-state fermentation using fungi seems promising. However, the initial caffeine concentration and external nitrogen concentration are very crucial for caffeine degradation to be effective. Though the enzymes involved in the degradation of caffeine are known, *in vitro* enzymatic studies for caffeine degradation are not yet available. Also, such enzymes are not very stable, and thus, more studies on enzyme stability and biochemical characterization are required. Such enzymes could be purified and immobilized in a bioreactor, so their caffeine-degrading ability could be studied and optimized. In time, such studies can lead to the development of a biological process for caffeine degradation.

See also: Caffeine: Characterization and Properties; Caffeine: Consumption and Health Effects; Coffee: Health Effects.

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Relevant Websites

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Coffee: Health Effects

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Introduction

Beverages are an important component of our diet. Coffee occupies an important place among beverages, since it holds the second position in consumption after water. On average, people consume 500 billion cups of coffee annually, with a global production of 8 million tons per year. The major consumers are the United States, Brazil, Europe, and Japan.

Coffee (*Coffea* L.) is considered as a valuable and enjoyable beverage, and often, it is consumed due to its stimulatory effects, mainly related to the presence of caffeine (1,3,7-trimethylxanthine): generally, 240 ml of instant coffee contains approximately 100 mg of caffeine. Caffeine content can be, however, influenced by the different technologies used. For instance, green bean dewaxing and wet processing can reduce its content, which remains in the range of 0.65–2.30%.

In addition to caffeine, coffee contains more than a thousand different chemicals, including carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids, and phenolic compounds. Some of them, such as trigonelline, nicotinic acid, chlorogenic acid (5-*O*-caffeoylquinic acid, CQA), melanoidins, and diterpenes (cafestol and kahweol), together with caffeine feature relevant nutritional or functional properties.

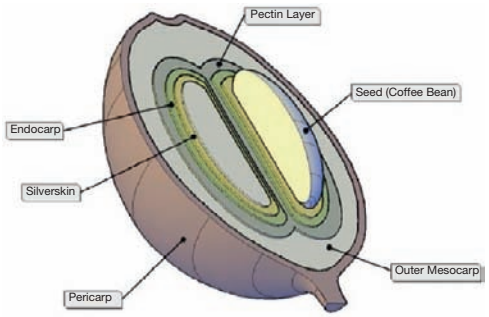
The coffee fruit (also called berry or cherry) is an oval drupe of about 10 mm in size. Coffee beans present an outer skin called pericarp, which is green in unripe and red-violet, deep red, yellow, or orange, depending on the cultivar, in ripe fruits. The pericarp covers the mesocarp (the soft yellowish, fibrous, and sweet pulp), a pectin adhesive layer, which is a highly hydrated layer of mucilage, and the endocarp, called the parchment (Figure 1). Finally, a silverskin covers each hemisphere of the coffee bean (endosperm).

Many factors influence coffee quality, among which the action of microorganisms. Microbial metabolites produced during coffee fermentation can diffuse into the grains and influence the beverage's final quality. There are different kinds of coffee beverages, characterized by different nuances in terms of body, aroma, acidity, and astringency.

Coffee is a complex array of biologically active components that can influence many metabolic processes. The main components are alkaloids (caffeine and trigonelline), phenolic compounds (chlorogenic acids), and diterpenes (cafestol and kahweol) (Table 1).

The main mechanism of action of caffeine is to antagonize adenosine receptors (with its A1 and A2A subtypes); a secondary effect is the inhibition of phosphodiesterases, with the subsequent accumulation of cyclic adenosine monophosphate and an intensification of the effects of catecholamines. Coffee is metabolized in the liver by enzymes known as cytochrome P450 1A2 (CYP1A2). Caffeine effects translate, in most people, in a psychoactive response, which includes increased alertness and attention, through the stimulation of the central nervous system, and in a complex cardiovascular response, mainly consisting of an acute increase in blood pressure. Some benefits related to its consumption include mood enhancement, better exercise performance, and shorter reaction times. Caffeine also produces negative effects, such as sleeplessness, anxiety, restlessness, tension, nervousness, palpitations, tremulousness, and psychomotor agitation. Moreover, the risk of rheumatoid arthritis and osteoporosis increases when coffee is consumed on a regular basis. Some other negative effects can arise strictly related to some special risk groups: for such subjects, a reduced consumption of coffee is recommended (Table 2).

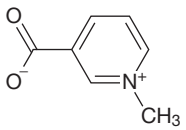
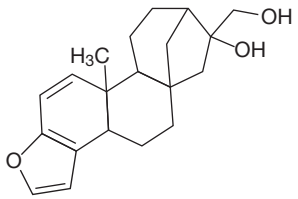
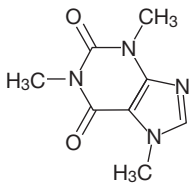
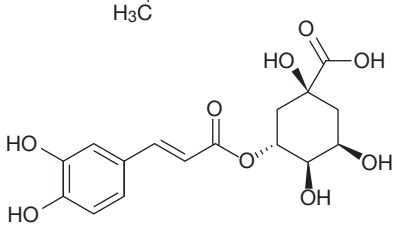
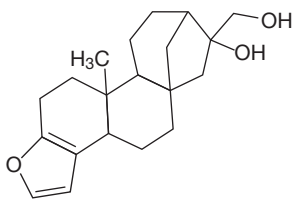
Cafestol and kahweol are also present in coffee. They can increase blood cholesterol and also feature some chemopreventive activity. The way of action of these compounds on lipoprotein metabolism is not completely understood. The only scientific evidence available is that they are absorbed intestinally. The positive association between coffee consumption and serum cholesterol has been observed mainly in Scandinavia and in the United States, where boiled coffee and filtered coffee are very popular. Therefore, the brewing method is probably critical to the cholesterol-raising effect of coffee. Also, French press (cafetière) coffee contains relatively high levels of cafestol and kahweol (6–12 mg/cup).



Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*magnoliopsida*
Order	*Gentianales*
Family	*Rubiaceae*
Genus	*Coffea*
Species	*Arabica; Canephora*

Figure 1 Coffee fruit structure and botanical classification of coffee plant.

Table 1 Main physiological actions of coffee components

Compound	*Molecular formula*	*Amount (mg/100 ml)*	*Physiological action*
Trigonelline		0.4–1.2 nicotinic acid	Antioxidant and antiinflammatory activities
Kahweol		0–0.1 (filtered coffee) 0.7–10 (boiled coffee)	Increased low density lipoprotein cholesterol and total cholesterol Free radical scavenging
Caffeine		30–54	Central nervous system stimulation Elevation of blood pressure Increased alertness Increased metabolic rate Decreased DNA degradation and reduced hydroxyl radicals Antioxidant properties
Chlorogenic acid (CGA)		35–175 CGA 17–87 caffeic acid	Antiinflammatory and antiangiogenic activities Antioxidant properties
Cafestol		0–0.1 (filtered coffee) 0.5–8 (boiled coffee)	Increased low density lipoprotein cholesterol and total cholesterol Free radical scavenging

Chlorogenic acids and caffeic acids are other important healthy components present in coffee. Chlorogenic acids are a family of esters formed between quinic and *trans*-cinnamic acids, which are an important group of dietary phenols. Caffeoylquinic acid (CQA) is the main compound among chlorogenic acids. The content of chlorogenic acids has been reported to range from 70 to 350 mg, which would provide about 35–175 mg of caffeic acid. These compounds show antioxidant activity *in vitro*. Probably, their action *in vivo* is reduced, because the metabolites deriving from their catabolism have lower antioxidant activity than the parent compounds.

Finally, coffee contains some micronutrients, such as magnesium, potassium, niacin, and vitamin E, which may contribute to the health effects of coffee consumption.

Antioxidant and Antibacterial Properties of Coffee

Lifestyle factors play a key role in human health. Traditional diets rich in bioactive components are associated with a lower risk of different illnesses; for example, the consumption of antioxidants or antioxidant-rich foods induces a lower risk of cancer (colorectal, hepatic, renal, ovarian, pancreatic, esophageal, endometrial, and pharyngeal cancers), as demonstrated by several meta-analyses.

Coffee is known as a dietary source of antioxidants and free radical scavengers, such as caffeine, hydroxycinnamic acids (caffeic, chlorogenic, coumaric, ferulic, and sinapic acids), Maillard reaction products, and melanoidins. Compared to other beverages, coffee stands out for its antioxidant potential; some authors reported levels of phenolic compounds in several beverages,

Table 2 Main groups subjected to caffeine's adverse effects and safe limits

Groups	*Risks*	*Limit (mg day^{-1})*
Women of childbearing age	*Conception*: High intakes of coffee or caffeine ranging from 400 to 800 mg day^{-1} are associated with delays in conception *Pregnancy complications*: Levels of at least 300 mg day^{-1} of caffeine increase the risk of spontaneous abortion. This association could be explained by the relationship between nausea and fetal viability *Fetal growth*: Caffeine intakes ranging from 200 to 400 mg day^{-1} induce a decrease in mean birth weight of about 100 g. Mothers of small for gestational age (SGA) infants had higher caffeine intakes in the third trimester of pregnancy than mothers of non-SGA infants *Lactation*: High maternal caffeine intakes cause irritability and poor sleeping patterns in infants	300
Children	Caffeine doses higher than 3 mg kg^{-1} of body weight can result in some behavioral effects (nervousness, anxiety, and sleep disturbances)	45 for 4–6 years old, 62.5 for 7–9 years old, and 85 for 10–12 years old
Older adults	Higher plasma caffeine concentrations could increase the risk of drug interactions and fracture risk, particularly in the presence of calcium and vitamin D insufficiency	400

drinks, and infusions in concentrations ranging from 0.07 to 4.16 mg l^{-1} in the following order: black tea > instant coffee > coke > red wine > violet carrot juice > apricot nectar > Turkish coffee > white wine. Coffee components exhibit some degree of antioxidant activity, so that coffee has the potential of reducing inflammation by decreasing oxidative stress.

Coffee antioxidant potential is associated with the presence of both natural compounds and substances developed during roasting. The temperature and time of the roasting process can impact on total antioxidant activity. Melanoidins, which are high-molecular-weight nitrogenous and brown-colored compounds, are formed during the roasting process. They show antioxidant, antimicrobial, anticariogenic, anti-inflammatory, antihypertensive, and antiglycative activities. Melanoidins from coffee showed higher antioxidant activity than those isolated from other sources, such as beer. All types of coffee preparations (filter, espresso, and Italian style) show antioxidant capacity, since all are effective free radical scavengers. Decaffeinated coffee has lower antioxidant capacity than regular coffee. The antioxidant capacity is about 6–7% after each 200 ml of coffee consumption, resulting in a reduction of inflammation through the reduction of free radicals and other reactive oxygen species.

Roasted coffee extract also features activity against some microorganisms, such as *Staphylococcus aureus* and *Streptococcus mutans* and several species/strains of Enterobacteriaceae (*Serratia marcescens*, *Enterobacter cloacae*, and *Salmonella enterica*). This activity is related to some coffee characteristic components, such as caffeic acid, trigonelline, caffeine, chlorogenic acid, and protocatechuic acid, all melanoidins with well-known natural antimicrobial activity.

Cardiovascular Effects of Coffee

The effects of coffee on the cardiovascular system are largely debated because the underlying mechanisms are complex and because of the considerable variability in individual responses. Many effects of coffee on the cardiovascular system have been attributed to caffeine, although coffee contains hundreds of chemical substances, many of which, such as polyphenols, are pharmacologically active.

Several epidemiological studies have examined the association between coffee consumption and the risk of coronary heart disease, but the findings have been equivocal. Experimental data from short-term and animal studies have suggested detrimental effects of caffeine on blood pressure, insulin resistance, and arrhythmia and have implicated coffee as a potential cardiovascular risk factor. Moreover, cross-sectional studies have shown an association between coffee and plasma cholesterol concentrations. Additionally, evidence from case–control studies has suggested that coffee consumption is associated with a higher risk of cardiovascular disease.

On the other hand, prospective cohort studies generally have not supported the existence of an association between coffee consumption and a higher risk of cardiovascular diseases, indicating that for most healthy people, moderate coffee consumption is unlikely to adversely affect cardiovascular health. Furthermore, several studies have suggested that also for patients with established coronary heart disease, it is safe to continue their habitual coffee consumption. A randomized clinical trial involving patients with acute ST segment elevation myocardial infarction (MI) has evaluated the effects of an acute ingestion of coffee on the autonomic function and cardiovascular health. Coffee ingestion was associated with an increase in parasympathetic tone, and coffee did not increase cardiac arrhythmia. The authors concluded that coffee ingestion is safe and not associated with adverse cardiovascular outcomes in postmyocardial infarction patients.

The relation between coffee consumption and the risk of arrhythmias has also been investigated. Although early animal studies indicated that coffee appeared to cause arrhythmias in a canine model, more recent studies have suggested that coffee does not increase arrhythmias. Actually, long-term coffee drinking might reduce the risk of abnormal cardiac rhythms, including atrial fibrillation.

Controlled interventional studies have shown that in normal adults, even acute high-dose caffeine did not affect cardiac

rhythm and rate and did not cause clinically significant ventricular or supraventricular arrhythmias. Moreover, prospective cohort studies did not find significant association between coffee consumption and the risk of atrial fibrillation. Mechanisms involved in this potential protection against arrhythmias are still largely unknown, but it has been hypothesized that caffeine attenuates negative effects of endogenous adenosine on cardiac electrical conduction.

The cardiovascular risk factor more extensively studied in relation to coffee is blood pressure: an elevated blood pressure is a risk factor for coronary heart disease, congestive heart failure, stroke, kidney disease, and all-cause mortality, and the relation between blood pressure and subsequent outcomes is direct and progressive throughout the usual range of blood pressure, including the nonhypertensive range. Therefore, even a small change in average blood pressure levels may have a major public health effect. Evidence that caffeine is an antagonist of adenosine receptors suggested the biological plausibility that coffee induces vasoconstriction and elevates systolic and diastolic blood pressure acutely. However, whether coffee consumption has chronic effects on blood pressure and cardiovascular disease is far from obvious and remains controversial. Dietary and other lifestyle factors play an important role in hypertension and blood pressure control: excess intake of salt or alcohol, suboptimal dietary pattern, physical inactivity, and excess body weight are here the most important factors. Coffee drinking might add as another dietary factor causing hypertension, but whether coffee intake per se is associated with detrimental long-term blood pressure changes or increases the risk of hypertension remains debated. A recent meta-analysis of 20 randomized, controlled trials and five cohort studies has shown no clinically important effects of long-term coffee consumption on blood pressure or the risk of hypertension in coffee consumers.

Various confounding factors may have influenced the equivocal results of different studies, including differences in the study design, populations examined (age, sex, usual frequency of coffee drinking, and smoking), types of coffee blend, and types of preparations used. Particularly, the design of epidemiological studies may affect findings about the relation between coffee and blood pressure or cardiovascular risk. Observational (cohort) studies usually have a large sample size and can provide insight into the long-term effects of different doses of coffee on blood pressure and possible changes in the effects by gender, age, race, cardiovascular risk profile, and other characteristics. However, observational evidence does not demonstrate causality, because coffee drinking is part of an individual's lifestyle and is related to many other factors, such as alcohol intake, mental stress, and dietary habits, which may also influence blood pressure.

Randomized clinical trials may provide insight into causality, because both the intervention (e.g., the use of coffee or caffeine in tablets) and the control treatment (e.g., decaffeinated coffee or placebo) are randomly assigned to participating subjects, and any confounder that could distort the relation between coffee and blood pressure should be equally distributed in both groups. The limitation of currently available randomized clinical trials on this subject is however that only fixed doses of coffee or caffeine have been studied, and for a relatively short period of time, the number of participating subjects has been limited; and there have often been problems of poor adherence to the assigned treatments. Furthermore, trials may have suffered from unsuccessful randomization or lack of blinding of the participants and/or investigators, which could introduce bias. Therefore, any conclusion on the cardiovascular effects of coffee has to rely on the overall evidence deriving from multiple sources of information.

The variability observed in the results of coffee studies and meta-analyses may be in part explained by the development of tolerance. For example, tolerance to caffeine-induced pressor effects quickly develops in habitual coffee drinkers, and blood pressure may be increased only temporarily in occasional coffee drinkers, while it is usually not elevated in heavy drinkers. It is possible that the complex set of counterregulatory hormones maintaining blood pressure causes tolerance to the humoral and hemodynamic effects of caffeine. In addition, above a certain level of consumption, caffeine's pressor effect might be counterbalanced by other coffee ingredients, including chlorogenic acid, flavonoids, melanoidins, quinide, magnesium, cafestol, and kahweol. Also, potassium, contained in coffee, may lower blood pressure. This may help explain the observed inverse 'J-shape' relation between habitual coffee drinking and the risk of hypertension, which increased up to three cups per day and then slightly decreased at higher amounts of consumption.

Another reason underlying the heterogeneity of responses to coffee drinking is the interaction between coffee components and the individual genetic constitution (nutrigenetics). For example, the enzyme accounting for approximately 95% of caffeine metabolism (the CYP1A2 isoform) has a wide interindividual variability in activity, resulting in rapid or slow metabolism of caffeine. It has been observed that subjects with slow metabolism have an increased risk of MI. Also, blood pressure responses are variable between subjects, with most subjects experiencing an increase in blood pressure, others showing no change, and some even showing blood pressure reductions. An association between gene variants in the adenosine and adrenergic receptors and blood pressure responses to caffeine has been observed. In principle, the exposure to higher blood pressure values as the result of such nutrigenetic interactions in some genetically predisposed individuals may expose such individuals to a higher coffee-related cardiovascular event. Moreover, it has been observed that genetic variants of adenosine receptors may also explain the interindividual variability in the susceptibility to anxiety and sleep changes induced by caffeine.

Besides blood pressure, other cardiovascular risk factors may be affected by coffee consumption. Some coffee compounds other than caffeine also have insulin-sensitizing and anti-inflammatory effects, and recent evidence from prospective cohort studies has suggested an inverse association between coffee and the risk of type 2 diabetes mellitus. This relationship is consistent across age, obesity, and study location (the United States and Europe), independent of potentially confounding dietary and lifestyle factors. Some proposed mechanisms able to explain this association include effects on insulin sensitivity and/or insulin secretion modulated by different minerals, antioxidants, and phytochemical compounds found in coffee. The role of caffeine in increasing or decreasing the risk of type 2 diabetes mellitus is still poorly understood. Most findings indicated a dose–response relation, with diabetes risk reduction for

higher levels of coffee consumption. Generally, four or more cups of coffee per day have generally been associated with a lower risk of type 2 diabetes mellitus, whereas for lower levels of consumption, results are controversial. Cross-sectional studies have highlighted that high coffee consumption is associated with lower postload plasma glucose concentrations among persons without diabetes and a lower incidence of impaired glucose tolerance and type 2 diabetes mellitus. In short-term metabolic studies, caffeine intake acutely reduces insulin sensitivity, exaggerating the blood glucose response to glucose loads. This action is probably related to the ability of caffeine to exert adenosine receptor antagonism or increase catecholamine levels. Also, decaffeinated coffee consumption has been associated with a reduction of risk of type 2 diabetes mellitus. Caffeinated and decaffeinated coffee consumption has a beneficial effect on insulin sensitivity because both reduce plasma C-peptide concentrations. However, the beneficial effect of decaffeinated coffee on glucose metabolisms requires further study. Chlorogenic acid and quinides are also probably involved in the reduction of risk of type 2 diabetes mellitus because they act on glucose homeostasis in animal models of diabetes. In humans, the components of coffee other than caffeine may exert beneficial effects on glucose metabolism. However, long-term studies on glycemic control are needed.

The previously considered harmful effects of coffee on the lipid profile depend on how the beverage is prepared. In fact, boiled coffee has higher concentrations of cholesterol-increasing compounds, classified as diterpenes, such as cafestol and kahweol. They are extracted from coffee beans by prolonged contact with hot water, while brewed/filtered coffee has a much lower concentration of cafestol and kahweol because of the much shorter contact with hot water and the retention of diterpenes by filter paper. Accordingly, it has been observed that the consumption of boiled coffee dose-dependently increase serum total and low density lipoprotein (LDL) cholesterol concentrations, whereas the consumption of filtered coffee results in very little change in serum cholesterol.

Regarding the association between coffee consumption and the risk of stroke, various findings have led to inconsistent results. Two recent meta-analyses have concluded that moderate coffee consumption has a preventive effect on stroke, and this is probably due to the healthy effect of coffee on the main risk factors for stroke, including hypertension, cardiovascular diseases, and diabetes mellitus. Regarding heart failure, a meta-analysis has indicated that there is a modest inverse association between moderate coffee consumption and the incidence of heart failure. Although potential modifiers of the coffee–heart failure relationship are not known, it is possible that the beneficial effects on blood pressure and other cardiovascular risk factors contribute to positively affect the risk of heart failure. In light of these findings, the current heart failure prevention guidelines have suggested a revision of the warning about coffee consumption, due to the evidence showing that coffee may provide a moderate protection against the incidence of heart failure.

Effects on Cancer

The relationship between coffee and cancer raises great interest, and in fact, more than 500 papers in the last decades have estimated the association between coffee consumption and the occurrence of cancer at 11 organ sites. Some studies found out that coffee consumption is associated with a reduced risk of hepatocellular, kidney, and, to a lesser extent, premenopausal breast and colorectal cancers, while it is unrelated to prostate, pancreas, and ovarian cancers. Since the results obtained are often controversial, in 2007, the World Cancer Research Fund (WCRF) conducted a comprehensive analysis of diet and cancer, using a standardized approach to review the overall evidence. This report showed an inverse relationship between coffee and the risk of pancreatic cancer and kidney cancer. This influence on cancer development appears to be related to the chemical composition of coffee and probably due to compounds able to influence the risk of cancer. For instance, caffeine can both stimulate and suppress tumors, depending on the species and the phase of administration. The consumption of caffeine is inversely related to hepatocellular injury. A population-based case–control study in the United States highlighted that caffeine was associated with a lower prevalence of abnormal alanine aminotransferase activity. Some other compounds, such as cafestol and kahweol, show anticarcinogenic properties, including the induction of phase II enzymes involved in carcinogen detoxification and the reduction of bile acid secretion associated with colon carcinogenesis. Polyphenols and chlorogenic acid are characterized by antioxidant effects. In particular, chlorogenic acid reduces blood glucose levels in rats and increases insulin sensitivity. Chronic hyperinsulinemia and insulin resistance are confirmed markers of high risk for some cancer sites. Moreover, caffeic acid inhibits DNA methylation in human cancer cells: this epigenetic regulation represents an important mechanism, because tumor cells are hypermethylated. In fact, DNA hypermethylation is often associated with the inactivation of genes and pathways involved in the tumorigenic process, including cell cycle regulation, inflammatory and stress response, and apoptosis. In addition, coffee-mediated stimulation of the Nrf2/ARE signaling pathway induces an increase in defense mechanisms against chemical stresses.

The influence of coffee consumption on the main types of cancer is reported in **Table 3**.

Effects on Neurodegenerative Diseases

Coffee has been shown to reduce the risk of Alzheimer's dementia and other diseases of the central nervous system, including Parkinson's disease.

A trend towards a protective effect of caffeine on Alzheimer's dementia has been frequently reported. Coffee may be the best source of caffeine to protect against Alzheimer's dementia due to a component in coffee that synergizes with caffeine to selectively enhance plasma cytokines. A quantitative review of four studies, despite heterogeneous methodologies and results, indicated that coffee consumption is inversely associated with the risk of Alzheimer's dementia, compared to nonconsumers.

Coffee and caffeine intake have also been associated with a reduced risk of Parkinson's disease, especially in men, in a number of prospective and case–control studies. Caffeine reduces the loss of dopaminergic neurons in animal models,

Table 3 Coffee influence on cancer development

Type of cancers	*Coffee effect*
Breast cancer	Coffee consumption has an inverse association with breast cancer, in particular among premenopausal women. Premenopausal women with a breast cancer 1, early onset (*BRCA*1) or breast cancer 2, early onset (*BRCA*2) gene mutation who habitually drank six or more cups of coffee per day experienced a 70% statistically significant reduction in breast cancer risk
Colorectal cancer	Coffee is a protective factor against colorectal cancer. This action is due to the presence of cafestol and kahweol that regulate the excretion of bile acids and neutral sterols into the colon
Prostate cancer	No association between prostate cancer risk and cumulative lifetime daily coffee consumption, duration of daily drinking, and age when daily drinking was started has been established
Ovarian cancer	No association between coffee and ovarian cancer has been found. However, some studies showed a modest inverse relationship between caffeine intake and ovarian cancer. This effect is probably related to caffeine modulation of estrogen level circulation
Pancreatic cancer	No association between coffee and pancreatic cancer has been found. A reduced risk was apparent among men who drank at least three cups of coffee per day
Liver cancer	Coffee consumption appears to reduce the risk of liver cancer. Probably, coffee polyphenols may maintain a relatively lower iron status and therefore reduce the risk of liver injury
Kidney cancer	Coffee consumption is associated, but not significantly, with a lower risk of kidney cancer. This action is probably due to the fact that caffeine has a diuretic effect by blocking antidiuretic hormone and antioxidant compounds reduce oxidative damage to DNA, proteins, and other molecules. Moreover, coffee consumption may reduce the risk of kidney cancer by improving insulin sensitivity

and its neuroprotective function is attributed to the antagonism on adenosine 2A (A2A) receptors in the brain, which are being increasingly targeted by anti-Parkinson therapies in clinical trials.

Other Effects

Coffee consumption is also associated with various other health effects. For instance, coffee may reduce the risk of depression. Additionally, coffee may improve asthmatic symptoms, probably through caffeine, which is a methylxanthine bronchodilator. Coffee may also enhance physical performance in sustained high-intensity exercise. Coffee may also prevent symptomatic gallstones and its consumption is associated with protection against some infectious and malignant diseases, particularly of the liver.

High levels of caffeine may increase urine output and urinary calcium and magnesium excretion, which have implications for bone health. Caffeinated coffee increases the risk of bone loss and fractures.

Effects on Mortality

Some studies have also investigated the association between coffee consumption and major causes of death, including heart diseases, respiratory diseases, stroke, injuries and accidents, diabetes, and infections. The results of these studies have been variable, and data are lacking to clarify the association between coffee drinking and mortality, to determine whether there is a dose–response relationship, and to assess whether associations are consistent across various subgroups. In a large, prospective cohort study, a dose-dependent inverse association between coffee drinking and total mortality was observed, after adjusting for potential confounders (smoking status in particular). Although the observational nature of the study does not prove a cause/effect relationship, the authors speculated that a plausible mechanism by which coffee consumption might have health benefits is the presence of antioxidant compounds, including polyphenols. The significant inverse associations of coffee consumption with death from all causes provide reassurance with respect to the concern that coffee drinking might adversely affect health.

Conclusions

The currently available overall evidence on cardiovascular effects related to habitual coffee consumption is largely reassuring. Moreover, coffee has a protective effect on cancer and neurodegenerative disease. Therefore, coffee can be included as part of a healthy diet. Many of coffee benefits probably derive from its caffeine content, but other substances may have an important role in health protection, particularly for their antioxidant effect.

It is very difficult to establish the factors responsible for coffee's beneficial or harmful effects. In order to better understand the function of these compounds, they should be isolated and utilized in a controlled experimental situation, using a well-established chemical balance of coffee components and at doses nutritionally achievable. Finally, it is also possible that coffee drinkers differ in other important dietary and sociological aspects from nonconsumers, and coffee use may be a surrogate marker of some other dietary or lifestyle risk factor.

Clinical trials need to verify the relationship between coffee and diseases, controlling coffee type, the original coffee beans, the roasting process, serving sizes, brewing process, and duration over a long period of time, with specific quantitative information (in mg kg^{-1} day^{-1}) on caffeine intake.

See also: Coffee: Analysis and Composition; Coffee: Decaffeination; Coffee: Types and Production; Fermented Foods: Fermented Vegetables and Other Products; Fermented Foods: Use of Starter Cultures; Functional Foods.

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Coffee: Types and Production

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Coffee Types

Coffee cultivation is spread all over the world, starting in Arabia (one species is called *Coffea arabica*, and a variety is 'Moka', named after an Arab village), passing through many countries, such as Ceylon (Sri Lanka), Java, India, the Philippines, Hawaii, and Vietnam, among others, some of which are important producers to this day. Coffee was introduced in America from plants previously adapted to the climate in Amsterdam and Paris and planted in Martinique, Suriname, and French Guiana, from where it was brought to Brazil. Nowadays, coffee is the second beverage widely consumed in the world, being overcome only by water, and Brazil is the largest producer in the world.

After oil, coffee is the most valuable traded commodity worldwide, with global retail sales estimated to be US$ 90 billion. Coffee is the major export product of some countries such as Brazil, Uganda, Burundi, Rwanda, and Ethiopia. About 70% of the world crop is grown on smallholdings smaller than 10 ha, and hence, it is often a family business that provides maintenance for over 25 million people worldwide. On a broader scale, the international coffee trade involves about 500 million people in its management, from cultivation to the final product for consumption.

The coffee tree belongs to the tribe Coffeeae in the family Rubiaceae. One hundred species are associated with the genus *Coffea*, but only two species are agroeconomically important, *Coffea arabica* and *C. canephora*. *C. dewevrei*, *C. congensis*, *C. eugenioides*, *C. kapakata*, *C. salvatrix*, *C. stenophylla*, *C. liberica*, *C. racemosa*, and others are primarily used in genetic crosses. Presently, arabica coffee accounts for about 63% of coffee produced, and robusta coffee 37%.

Coffea arabica (Arabica Coffee)

Coffea arabica was first described by Linnaeus in 1753. The best known varieties are 'Typica' and 'Bourbon,' but from these, many different cultivars have been developed, such as 'Caturra' (Brazil and Colombia), 'Mundo Novo' (Brazil), 'Tico' (Central America), the dwarf 'San Ramon', and the 'Jamaican Blue Mountain'. Arabica plant is a large bush with dark-green oval leaves. It is genetically different from other coffee species, having four sets of chromosomes rather than two. The fruits are oval and mature in 7–9 months; they usually contain two flat seeds (the coffee beans) – when only one bean develops, it is called a peaberry. Compared with robusta, arabica trees are generally less vigorous and productive with a higher cost of production and produce beans that contain about half the amount of caffeine. Arabica coffee produces a beverage with a typical sweet aromatic taste that can be consumed alone or blended with *C. canephora*.

'Catuai' and 'Mundo Novo' are the most traditionally cropped cultivars of *C. arabica*, but many others are also economically important worldwide. Since arabica coffee is often susceptible to attack by pests and diseases, resistance is a major goal of plant-breeding programs. Arabica coffee is grown throughout Latin America, in Central and East Africa, in India, and to some extent in Indonesia.

Coffea canephora (Robusta Coffee)

The term 'robusta' is actually the name of a widely grown variety of this species. It is a robust shrub or small tree growing up to 10 m height, but with a shallow root system. The fruits are round and take up to 11 months to mature; the seeds are oval in shape and smaller than those of *C. arabica*. Robusta coffee is harvested in West and Central Africa, throughout Southeast Asia, and to some extent in Brazil, where it is known as conilon. Robusta coffee constitutes a relatively new commercial crop, so there is a great potential for genetic improvement. 'Robusta' is the most widely cultivated variety of *C. canephora* in the world, so that the name of this variety is used to designate the common name of the species. Nevertheless, in Brazil, 'Conilon' (also known as 'Kouillou') is practically the sole cultivated variety of *C. canephora*.

Coffea canephora, produces a beverage that qualifies it to be used for soluble coffee production and to be blended with arabica coffee. Current research shows that in spite of giving beverage with different sensory characteristics of the arabica, robusta coffee produced and processed properly can be a source of desirable characteristics in the composition of blends with arabica.

Coffea liberica (Liberian Coffee)

Liberian coffee grows as a large strong tree, up to 18 m in height, with large leathery leaves. The fruits and seeds (beans) are also large. Liberian coffee is grown in Malaysia and in West Africa, but only very small quantities are traded.

Early planning of a coffee plantation has an important meaning and can ensure the success of the activity to be started. Because it is a permanent culture, mistakes in implementation may be impossible to fix, seriously compromising the venture. Thus, important aspects should be considered, such as available resources, proper choice of the area and cultivar, and the planting system and management.

Coffee cultivation and treatment involve the following steps: tree abatement; soil preparation; planting (small plants are usually grown in nurseries in the same or in external properties); treatment (soil correction, fertilizing, pest control, and terrain cleaning manually or with herbicides); fruit picking (ripe fruit is usually red and therefore called a berry; Figure 1); sieving to get rid of impurities; transportation; washing to remove pulp and membranes; sun drying, revolving grains with a rake, or mechanical drying through hot air blasting; hand separation of grains; and storing in silos and bagging.

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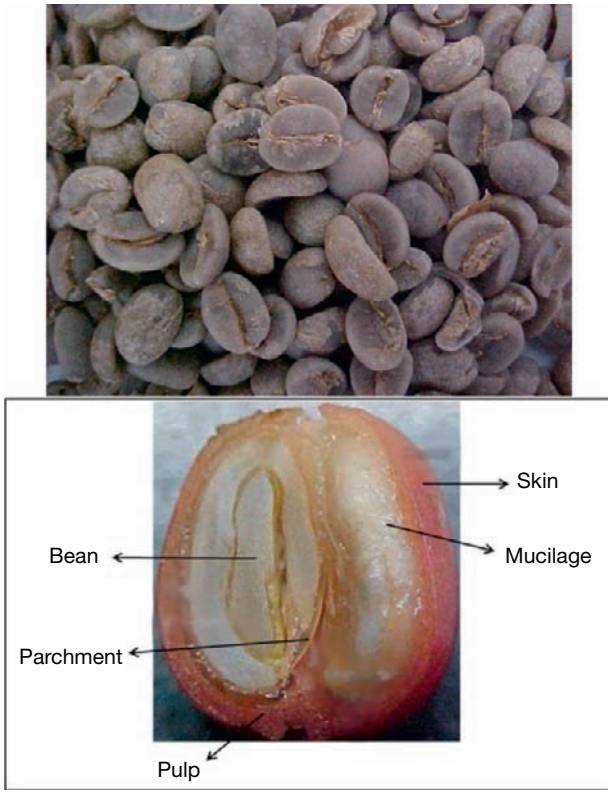

Figure 1 Green coffee (top) and longitudinal cut of the mature fruit coffee (cherry) (bottom) showing all the parts present in the coffee fruit.

Planting

One of the most important factors in coffee planting is the production of well-developed healthy plants of good quality. Seedlings must have a well-developed root system, have good root/shoot ratio, be acclimated to the sun, since coffee is grown in full sun, and be free of pest problems and nutritional deficiencies. However, the formation of good quality planting material depends primarily on the quality of the seeds.

Coffee Plant Propagation

Propagation by Seeds

Ripe red cherries of arabica coffees are collected and pulped, and the mucilage is removed by fermentation. The freshly picked coffee seeds (typically referred to as beans) can be either planted immediately or dried for later use. Coffee drying takes place on wire mesh trays in the shade. Coffee seeds with approximately 10–15% of humidity can be used up to a year or more if properly stored. Storing coffee beans correctly is essential for a longer seed life.

Growing Coffee Seeds in Poly Bags

Poly bags, made of black diothene (200 gauge), are commonly used and filled with a mixture of topsoil, well-rotted cattle manure, coarse sand, gravel, coffee pulp, and coffee husks. A ratio of three parts topsoil to one part coarse sand and one part cattle manure is often used. A top dressing of nitrogen is applied by incorporating 20 g urea in 5.0 l of water per meter of bed. The seedlings of arabica coffee can still be grafted franc or foot type. A variety of *C. canephora* is most commonly used as rootstock considering its resistance to nematodes. Tubes are conical containers, made of hard plastic (polypropylene). The production of seedlings in plastic pots in a greenhouse facilitates isolation of the nursery in relation to insects, reduces pest, preserves the integrity of the root system, and increases the ease of handling in producing grafted seedlings.

Coffee Cultivation

The basis of the success of the coffee activity relies on the adoption of good agricultural and processing practices, which can be considered as production processes based on recommendations predetermined aimed at satisfying performance of coffee farms, in an efficient way generating satisfaction and safety for the whole production chain.

Good agricultural practices for cultivation are based on three principles: food security; social and environmental responsibilities, necessary for the development of a solid strategy for the integration of production; and farm management capable of monitoring and controlling over the production process and when necessary making adjustments in the system (inputs, safety procedures, and management training).

On the other hand, consumers are concerned with aspects beyond the sensory quality of the product such as the preservation of the environment, improvement of income of the growers, and rural workers' safety. Aiming to attest if these requirements are met, several certifiers are responsible for guiding, inspecting, and certifying the compliance of these coffee requirements. The certification movement is growing by promoting improvements in the entire coffee production chain. Examples of accreditation in the culture of coffee are Fairtrade, Rainforest Alliance, Organic, and others.

The trend towards a global economy, a growing competition, and the search for technologies with higher productivity also has effects upon coffee cultivation. Mechanization is being disseminated and updated. Moreover, new methods of cultivation are introduced, among them being high-density cultivation, in which the distance between plants is being reduced. This modern method increases the number of coffee trees from 3000 or 4000 to 100 000 plants per hectare, with an increase in productivity of around 50% over the traditional method. This procedure is important for workers' health, since lower risks are involved and less herbicide is applied, especially after the third year. On the other hand, there is an increase in the frequency of tree cutting and higher demand for control of fungus disease in the plants.

Coffee Harvesting

Depending on the variety, it takes approximately 3–4 years for newly planted coffee trees to begin to bear fruit. The fruit (coffee cherry) turns a bright, deep red or yellow when it is ripe and ready to be harvested.

Harvesting is one of the most important steps influencing the coffee quality. Coffee processed from ripe cherries is naturally sweet and shimmering with floral and fruit notes. Coffee processed from unripe cherries may taste grassy, green, thin, or astringent and finally processed from overripe, shriveled cherries (sometimes called raisins) runs the risk of tasting fermented, musty, or moldy.

Harvesting coffee is particularly challenging because coffee fruit typically does not ripen uniformly; presenting more than one bloom, the same branch may simultaneously display ripe red cherries, unripe green cherries, and dry, overripe black cherries. Studies with coffee fruits in various stages of maturation showed that the highest quality of cherry fruit occurs in stage, ideal harvest. The coffee harvested early with a high percentage of green causes damage in the type of the coffee (quality) and drink and can also achieve a rate of 20% of losses on the final yield, in addition to affecting the appearance, roasting, type, and drink. On the other hand, the longer it stays in the coffee tree, the greater incidence of black rot grain or loss of dry mass and quality.

In regions where labor is inexpensive, where families pick their own small plots, or in mountainous areas, trees may be picked repeatedly, and only ripe fruits are harvested during each pass. In parts of the world where labor is scarce or expensive, coffee may be stripped from the trees in a single picking. Ripe, unripe, and overripe cherries are all gathered together, along with some leaves and twigs. Although sophisticated sorting methods can compensate to some degree for mass picking, no expedient is quite as effective as repeated, skillful handpicking.

Machines have been developed that selectively pick ripe cherries by vibrating the tree just vigorous enough to knock loose the ripe fruit while leaving the unripe fruit still attached to the tree. Such machines do not approach the selectivity of a good handpicker and are used only in regions of the world – Brazil, Australia, and parts of Hawaii – where labor is too costly to support handpicking. The main exception is Brazil, where the relatively flat landscape and immense size of the coffee fields have permitted mechanization of the process. Almost all fine coffee is still picked selectively by hand.

Harvesting Methods

Strip picked – the entire crop is harvested at one time. This can be done either by machine or by hand. In either case, all of the cherries are stripped off of the branch at one time.

Selectively picked – only the ripe cherries are harvested and they are picked individually by hand. Pickers rotate among the trees every 8–10 days, choosing only the cherries that are at the peak of ripeness. Since this kind of harvest is labor-intensive, and thus more costly, it is used primarily to harvest the finer arabica beans. At the end of the day, each worker's harvest is carefully weighed and each picker is paid on the merit of his or her work. The day's harvest is then combined and transported to the processing plant.

Coffee Processing

Coffee beans are the seeds of fruits that resemble cherries, with a red skin (the exocarp) when ripe. Beneath the pulp (the mesocarp), surrounded by a parchment-like covering (the endocarp), lies two beans, flat sides together. When the fruit is ripe, a thin, slimy layer of mucilage surrounds the parchment. Underneath the parchment, the beans are covered in another thinner membrane, the silver skin (the seed coat). Each cherry generally contains two coffee beans; if there is only one, it assumes a rounder shape and is known as a peaberry. Coffee beans must be removed from the fruit and dried before they can be roasted; this can be done in three ways, known as the dry, wet, and semidry fermentation methods. When the process is complete, the unroasted coffee beans are known as green coffee (Figure 1).

Dry Method

The dry method (also called the natural method) is the oldest and simplest method and requires little machinery. The method involves drying the whole cherry and the process is environmentally friendly because it produces low amounts of solid and liquid wastes, with no production of effluents with high organic matter content (Figure 2). The beverage quality depends of the proper processing. This coffee has their own market because the beverage is full-bodied and less acidic than coffee from wet process.

The method involves drying the whole cherry and, most often, is used after nonselective harvesting of different maturation stages of the coffee fruit. There are variations on how the process may be carried out, depending on the size of the plantation and the facilities available that interfere in the final quality obtained. The three basic steps, cleaning, drying, and hulling, are described later in the text.

Firstly, the harvested cherries are usually sorted and cleaned, to separate the unripe, overripe, and damaged cherries and to remove dirt, soil, twigs, and leaves. This can be done by winnowing, which is commonly done by hand, using a large sieve. Any unwanted cherries or other materials not winnowed away can be picked out from the top of the sieve. Ripe cherries can also be separated by flotation (by different density) in washing channels close to the drying areas.

The coffee cherries are spread out in the sun, either on large concrete or brick patios or on matting raised to waist height on trestles. As the cherries dry, they are raked or turned by hand to ensure homogenous drying. It may take up to 4 weeks before the cherries are dried to the 12.5% maximum moisture content, depending on the weather conditions. On larger plantations, machine-drying is sometimes used to speed up the process after the coffee has been predried in the sun for a few days.

The drying operation is the critical stage of the process, since it affects the final quality of the green coffee. Coffee that has been overdried will become brittle and produce too many broken beans during hulling (broken beans are considered defective beans). Coffee that has not been dried sufficiently will be too moist and prone to rapid deterioration caused by the attack of fungi and bacteria during the storage time.

The dried cherries are stored in bulk in special silos until they are sent to the mill where hulling, sorting, grading, and bagging take place. All the outer layers of the dried cherry are removed in one step by the hulling machine.

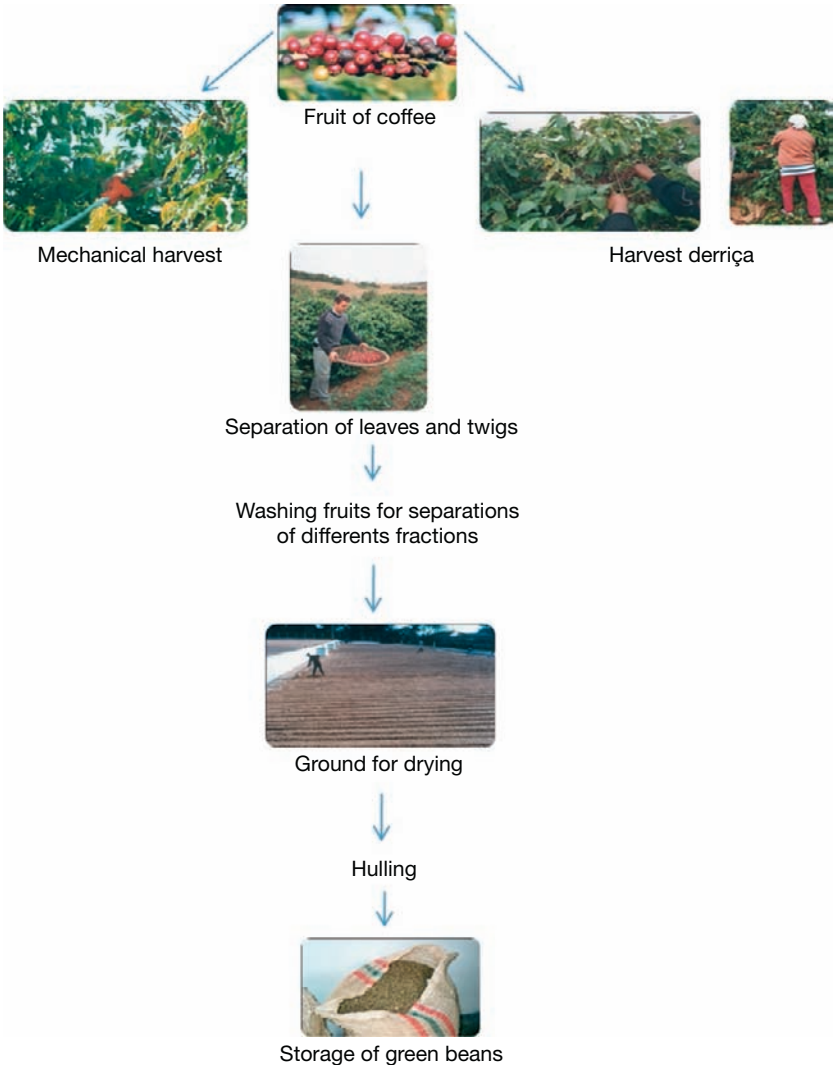

Figure 2 Main steps involved in dry or natural coffee fermentation. Reproduced from Schwan, R. F., Silva, C. F. and Batista, L. R. (2012). Coffee fermentation. In: Hui, Y. H. (ed.) *Handbook of plant-based fermented food and beverage technology*. Boca, Raton, FL: CRC Press, Chapter 42.

The dry method is used for about 90% of the arabica coffee produced in Brazil; Ethiopia, Haiti, and Paraguay; and India and Ecuador. Almost all robusta coffees are processed by this method. It is not practical in very rainy regions, where the humidity is too high or where it rains frequently during harvesting.

Wet Method

The wet method (also called the washed method) requires the use of specific equipment and substantial quantities of water (Figure 3). When properly done, it ensures that the intrinsic qualities of the coffee beans are better preserved, producing a green coffee, which is homogeneous and has few defective beans. Hence, the coffee produced by this method is usually regarded as being of better quality and commands higher prices. This method is used for all arabica coffee, except in Brazil, Ethiopia, and Yemen arabica. Just a few percentage of robusta coffee is processed in this way.

Even after careful harvesting, a certain number of partially dried and unripe cherries, as well as some stones and dirt, will be present among the ripe cherries. As in the dry method, preliminary sorting and cleaning of the cherries are usually necessary and should be done as soon as possible after harvesting. This operation can be done by washing the cherries in tanks filled with flowing water. Screens may also be used to improve the separation between the ripe and unripe, large and small, cherries.

After sorting and cleaning, the pulp is removed from the cherry. This operation is the key difference between the dry and the wet methods, since in the latter, the pulp of the fruit is separated from the beans before the drying stage. The pulping is done by a machine, which squeezes the cherries between fixed and moving surfaces. The flesh and the skin of the fruit are left on one side and the beans, enclosed in their mucilaginous parchment covering, on the other. The clearance between the surfaces is adjusted to avoid damage to the beans. Pulping operation should also be done as soon as

Figure 3 Main steps involved in wet coffee fermentation. Reproduced from Schwan, R. F., Silva, C. F. and Batista, L. R. (2012). Coffee fermentation. In: Hui, Y. H. (ed.) *Handbook of plant-based fermented food and beverage technology*. Boca, Raton, FL: CRC Press, Chapter 42.

possible after harvesting to avoid any fruit deterioration, which might affect the bean quality.

The pulped beans go on to vibrating sieves that separate them from any unpulped or imperfectly pulped cherries, as well as from any large pieces of pulp that might remain. From the screens, the separated pulped beans then pass through water-washing channels where a further flotation separation takes place before they are sent to the next stage.

Because the pulping is done by mechanical means, it normally leaves some residual flesh as well as the sticky mucilage adhering to the parchment surrounding the beans. The parchment has to be completely removed from the coffee beans to avoid contamination of products resulting from the mucilage degradation. The newly pulped beans are placed in large fermentation tanks in which the mucilage is broken down by natural and microbial enzymes until it is dispersible, when it can be washed away. Unless the fermentation is carefully monitored, the coffee can acquire undesirable, sour flavors. For most coffees, mucilage removal takes place between 24 and 36 h, depending on the temperature, thickness of the mucilage layer, and concentration of the enzymes; the end of the fermentation is assessed when the parchment surrounding the beans loses its slimy texture and acquires a rougher 'pebbly' feel. When the fermentation is complete, the coffee is thoroughly washed with clean water in tanks or in special washing machines.

Another version of this method is when the mucilage is mechanically removed obtaining the coffee called demucilated without the fermentation stage consequently with less final flavor. An intermediary process consists in obtaining the pulped beans with part of the mucilage and directly drying them (dry fermentation), obtaining the coffee called dehulled.

The wet parchment coffee at this stage has of approximately 57% moisture. To reduce the moisture to a maximum 12.5%, the parchment coffee is dried either in the sun, in a mechanical dryer, or by a combination of the two. Sun drying is done on extensive flat concrete or brick areas, known as patios, or on drying tables made of fine mesh wire netting. The beans are laid out in a layer of 2–10 cm and turned frequently to ensure even drying. Sun drying should take from 8 to 10 days, depending upon ambient temperature and humidity. Coffee dries more quickly if raised on tables because of the upward draft of warm air. The use of hot air drying machines becomes necessary to speed up the process in large plantations where, at the peak of the harvesting period, there might be much more coffee than can be effectively dried on the terraces. However, the process must be carefully controlled to achieve satisfactory and economical drying without any quality damage.

After drying, the wet-processed coffee, or parchment coffee as it is commonly known, is stored and remains in this form until shortly before export.

The final stages of preparation of the coffee, known as 'curing,' usually take place at a special plant just before the coffee is sold for export. The coffee is hulled, to remove the parchment, and then passes through a number of cleaning, screening, sorting, and grading operations, which are common to both wet- and dry-processed coffee. Electronic sorting machines may be used to remove defective beans that cannot be distinguished by eye.

The wet method is generally used for arabica coffees, with the exception of those produced in Brazil and the arabica-producing countries mentioned earlier in the text as users of the dry method. This method is rarely used for robusta.

Semidry Method

The semidry process (or pulped natural) is a variation of wet processing. It is an intermediate process between dry and wet processing and results in what are called pulped natural coffees. It combines a wet mechanical process to remove the pulp and the spreading out of depulped beans as a thin layer on cement patios for a period to allow further aerobic degradation of mucilage (dry process; Figure 4). Some studies have focused on the chemical properties of coffee beans under semidry processing and the correlation between those properties and beverage quality. No study has yet presented conclusive evidence of the level of influence of this process on the final quality of the beverage. However, it is possible to say that the qualities of coffee beverages made from pulped natural coffee lie somewhere between those obtained using dry and wet processing. The green beans produced via semidry processing are usually used in espresso blends.

Figure 4 Main steps involved in semidry coffee fermentation. Reproduced from Schwan, R. F., Silva, C. F. and Batista, L. R. (2012). Coffee fermentation. In: Hui, Y. H. (ed.) *Handbook of plant-based fermented food and beverage technology*. Boca, Raton, FL: CRC Press, Chapter 42.

Microorganisms: Quality and Safety in Coffee

The method of coffee processing impacts on the native microbiota and, therefore, with the groups of microorganisms involved during fermentation. In some regions or in certain producing years, because of the environmental conditions of coffee, the quality can be inferior because the microbiota changes to deteriorative microorganisms (like butyric and propionic acid bacteria). Nevertheless, in all processing methods (dry, wet, and semidry), the objectives of fermentation, involving bacteria and yeasts, are to remove the mucilaginous layer rich in polysaccharides (pectins) and to reduce the water content present in the fruits. Coffee seeds have all the precursors needed to generate typical flavor and aroma during the roasting operation but the natural microbiota during the fermentation/drying step confer special flavor in the coffee beverage. The microorganisms naturally present in the production environment use sugars in the pulp and mucilage and excrete organic acids and other metabolites that may affect the final sensory characteristics of the beverage. In addition, coffee fermentation and drying must be managed in order to control the growth of filamentous fungi that can give off-flavors and produce mycotoxins.

The three different methods for processing the fruit show quantitative and qualitative differences on the microbiota. The dry process has more microbial species than the other two processes (wet and semidry) because the whole fruits are processed. On the other hand, in the wet process, the skin and pulp are removed, and consequently, the epiphytic microorganisms are mechanically removed. The microbiota of the semidry processing is still not well known but bacteria, yeast, and filamentous fungi are detected in Brazil and India. The review for this topic has been published by Silva.

Microorganisms Present in Dry Process

The presence of organic acid from fermentation (acetic, lactic, butyric, and propionic acids) confirms the microbial action in the fermentation process for natural coffees. In the beginning of the process, the bacteria species predominate over yeast and filamentous fungi, but this changes when the water activity and physical–chemical conditions alter in the media and final phase of the fermentation. Some species identified were *Tatumella ptyseos*, *Pseudomonas putrefaciens*, *P. mirabilis*, *Enterobacter aerogenes*, *Acinetobacter*, *B. subtilis*, and *Paenibacillus amylolyticus*.

The bacteria present on the surface of the coffee cherries synthesize the acid that can migrate to the pulp and mucilage and can interfere with the organoleptic quality of the beans. The negative influence of bacteria on the quality of the coffee beverage is linked to the species present and not to population density. In natural coffee fruit rated very soft (i.e., as having optimal sensory quality), the bacterial population can represent up to 80% of the total microbiota during this process.

Yeasts become predominant as the water activity declines during the fermentation and drying periods, which can last up to 20 days. Twenty-one species of yeasts have been identified, including seven pectinolytic species (*Debaryomyces hansenii*, *D. polymorphus*, *Pichia anomala*, *P. holstii*, *P. burtonii*, *P. guilliermondii*, and *Arxula adeninivorans*). The presence of yeasts during the whole process contributes to the availability of the substrate and action of pectinases over the pulp and mucilage. The role of yeast may be related not only to the fermentation process but also to the control of filamentous fungi growth. Isolates belonging to the genera *Debaryomyces* and *Pichia* have demonstrated the ability to inhibit the growth of toxigenic fungi and may therefore have the potential for biological control.

Studies of natural coffee microbial populations always emphasize filamentous fungi isolation and identification, but the microorganisms present during the fermentation and drying periods are predominantly bacteria and yeasts. Therefore, the presence of filamentous fungi is not involved in the fermentation process, and in general, their presence decreases the sensorial and sanitary quality during the mycotoxin production, especially ochratoxin A (OTA).

Microbiota Present in Wet and Semidry Processing

The microbial diversity of coffee beans in wet processing is smaller compared with that in dry processing, because the

fermentation time is shorter (up to 48 h) and there is a more rapid pH decline from 6 to 4.3. This microbiota comprises few bacterial and yeast species. There is no report of filamentous fungi involved in wet fermentation. The mucilage adhered to beans is the substrate used by bacteria and yeast during fermentation. Higher microbial diversity in washed coffee fruits could be observed using molecular identification techniques that allow the detection of cultivable and uncultivable microorganisms.

The molecular identification of microorganisms associated with coffee showed that, in semidry process, the microbial succession occurred: bacteria dominated at the beginning of the process, but after 72 h, bacteria and yeast populations reach similar values (average 10^6 CFU g^{-1}) and after 192 h of fermentation, the yeast population predominates.

Currently, there is still doubt regarding the apparent bacterial role on coffee fruit fermentation. Recently, it was observed, in wet process, that the predominant population was lactic acid bacteria (LAB), especially *Weissella*, *Lactobacillus*, and *Leuconostoc* (*W. cibaria* and *W. soli*, *Lb. plantarum* and *Lb. brevis*, *L. mesenteroides*, *L. pseudomesenteroides*, and *L. citreum*). Thus, it is possible that the LAB in the wet processing are responsible for the degradation of the mucilage; however, this group of bacteria does not participate in the coffee fermentation in dry fermentation. It is possible that the anaerobic or low oxygen conditions present in wet fermentation favor the LAB development. The action of LAB allows the pH to fall, preventing the proliferation of other bacteria and promoting yeast growth. About the role of yeast in the fermentation of the depulped beans, some researchers do not believe the yeast action on the mucilage degradation. However, pectinolytic yeasts have been isolated during the wet processing of coffee in both robusta and arabica coffees. The fermentation also occurs due to the action of yeast, namely, *Kluyveromyces* sp., *Saccharomyces cerevisiae*, *Candida parapsilosis*, *C. tropicalis*, *C. pelliculosa*, *Torulopsis famata*, *Pichia anomala*, *P. kluyveri*, and *Hanseniaspora uvarum*. Some species like *Pichia kluyveri*, *P. anomala*, and *H. uvarum* showed an inhibitory effect on the growth of *Aspergillus ochraceus*, a fungus that is potentially toxigenic and is often isolated in coffee fruits and beans. Therefore, it is possible that yeasts play a dual role during the processing of coffee fruits: they facilitate mucilage degradation during fermentation and afford biological control.

The microbiota of the semidry processing is still not well known. A great diversity of prokaryotes and eukaryotes was detected using polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE) in addition to conventional techniques. PCR-DGGE allowed the detection of species not isolated from conventional techniques, such as *Enterobacter cowanii*, *Lactobacillus plantarum*, *Pantoea agglomerans*, *Bacillus macerans*, and an endophytic bacterium that could not be cultivated and the yeasts *S. cerevisiae* and *H. uvarum*. The presence of filamentous fungi was detected with populations below 10^3 CFU g^{-1} and only in recently harvested fruits, washed fruits, and washed fruit fractions and in the skin + pulp portion. The filamentous fungi species found were *Aspergillus* sp., *A. chevalieri*, *A. foetidus*, *A. niger*, *A. ochraceus*, *A. tubingensis*, *A. versicolor*, *Cladosporium* sp., *C. cladosporioides*, *C. macrocarpum*, *Cylindrocarpon* sp., *Eurotium chevalieri*, *Fusariella* sp., *Fusarium* sp., *F. chlamydosporum*, *F. lateritium*, *F. nivale*, *F. solani*, *F. sporotrichioides*, *Geotrichum* sp., *Mucor hiemalis*, *Penicillium* sp., *P. brevicompactum*, *P. commune*, *P. decumbens*, *P. fellutanum*, *P. implicatum*, *P. roqueforti*, *Phoma* sp., and *Ulocladium* sp.

Ochratoxin in Roasted Coffee

In agricultural products, such as coffee, interactions between different fungal species represent a natural phenomenon that affects the development of these microorganisms and their subsequent production of mycotoxins. Several studies have been undertaken to assess the growth of mycotoxigenic fungi and mycotoxin degradation. These interactions are influenced by environmental conditions, microorganism diversity, agricultural products, the type of processing, and the cultivation system used. Interactions between mycotoxigenic and nontoxic species can lead to the appearance of diseases and the production of different types of mycotoxins and their degradation. The results of these interactions are influenced by environmental conditions. OTA has been detected in various agricultural products, including coffee. Coffee drinks significantly contribute to the ingestion of OTA in the human diet. OTA is primarily produced by *Aspergillus* section *Circumdati* (*A. westerdijkiae* and *A. ochraceus*) and section *Nigri* (*A. carbonarius* and *A. niger*) species.

Coffee bean contamination with OTA may occur throughout the entire production chain and is directly related to the care and quality of the crop management, harvest, postharvest storage, and type of roasting.

The toxic effects of OTA appear to be related to its ability to inhibit protein synthesis by competing with phenylalanine in the reaction catalyzed by phenylalanyl-tRNA synthetase and other systems that require this amino acid. OTA also increases lipid peroxidation, leading to greater mitochondrial and cell damage. Furthermore, this mycotoxin is also considered nephrotoxic, cytotoxic, carcinogenic, teratogenic, and immunosuppressive and was classified by the International Agency for Research on Cancer (IARC) as a member of group 2B, that is, a possible human carcinogen.

Several studies have shown that the process of roasting is effective in reducing the OTA concentration, but there is still a lack of more conclusive studies about the effects of the various stages of roasting and grinding and methods of preparing the beverage on toxin stability. The type of roasting and particle size interfere with the residual content of OTA in the beverage. Therefore, the study helps to establish quality requisites for roasted and ground coffee production within national and international standards of quality, enabling value to be added to a traditional product of Brazilian agriculture.

See also: Caffeine: Characterization and Properties; Caffeine: Consumption and Health Effects; Coffee: Analysis and Composition; Coffee: Decaffeination; Coffee: Health Effects; Drying: Effect on Nutrients, Composition and Health; Drying: Physical and Structural Changes; Drying: Principles and Types; Mycotoxins: Occurrence and Determination; Oxidation of Food Components.

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Colon: Diseases and Disorders

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Functional Disorders Affecting the Colon

Constipation

Constipation is the most common gastrointestinal complaint in the general population. It is particularly prevalent in women, children, and older adults. The definition of constipation varies, but based on epidemiological studies, it is commonly defined as having a bowel movement frequency of 3 or less per week. However, reports of bowel movement frequency are usually inaccurate and often do not correlate with complaints of constipation. This observation led to a more extensive definition or the Rome III criteria (Rome III, last revised in 2006) for constipation that includes the presence of the following for a period of at least 6 months:

1. Must include two of the following:
   - Straining during at least 25% of defecations
   - Lumpy or hard stools in at least 25% of defecations
   - Sensation of incomplete evacuation for at least 25% of defecations
   - Sensation of anorectal obstruction/blockage for at least 25% of defecations
   - Manual maneuvers to facilitate at least 25% of defecations
   - Fewer than three defecations per week
2. Loose stools are rarely present without the use of laxatives.
3. There are insufficient criteria for irritable bowel syndrome.

The prevalence of constipation varies according to the criteria utilized for estimation. Overall, the prevalence of constipation in North America varies between 2% and 27%. In children, the worldwide prevalence ranges from 0.7% to 29.6%. In adults, the prevalence rises dramatically, especially after 65 years of age. In general, constipation can be regarded as disordered movement of stool through the colon or anorectum. However, secondary causes include neurological, metabolic, and endocrine disorders. Risk factors associated with constipation include advanced age, female gender, lower level of education, low socioeconomic status, nonwhite ethnicity, and use of certain medications. Cross-sectional studies, however, have failed to link low intake fiber and constipation.

The initial evaluation of the patient with constipation includes a careful history and physical examination. Laboratory evaluation, endoscopic evaluation, and radiological studies should be performed only in selected individuals if indicated.

Treatment of chronic constipation begins with lifestyle modifications, if indicated, and therapy with fiber. Osmotic and stimulant laxatives, stool softeners, emollients, and enemas sometimes are required to treat refractory intractable constipation. It is well recognized that fiber is important for normal defecation. This is due primarily to the ability of fiber to increase stool weight. The increased weight is secondary to the physical presence of the fiber, water held by the fiber, and increased bacterial mass from fermentation. Larger and softer stools increase the ease of defecation and reduce transit time through the intestinal tract, which may help to prevent or relieve constipation. In general, cereal fibers are the most effective at increasing stool weight. Wheat bran is considered the gold standard when it comes to fecal bulking. Overall, the recommended amount of dietary fiber is 20–35 $g\,day^{-1}$ coming from meals and supplements. The effect of fiber and low-digestible carbohydrates on gastrointestinal tolerance is a concern due to the development of symptoms like bloating and distention, leading to low compliance.

Irritable Bowel Syndrome

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by chronic abdominal pain and disturbed defecation without evidence of an organic etiology that explains the symptoms. IBS can be associated with significant emotional distress, impaired health-related quality of life, disability, and high health care costs. Psychosocial factors have an important role in modulating the illness experience and its clinical outcome.

The estimated prevalence of IBS in North America is approximately between 10% and 15%. Although it can affect both men and women of different age ranges, women and younger patients are more likely to be diagnosed. The clinical diagnosis of IBS is based on the Rome III criteria, which includes recurrent abdominal pain or discomfort for at least 3 days per month in the last 3 months associated with two of the following:

1. Improvement with defecation
2. Onset associated with a change in frequency of stool
3. Onset associated with a change in form (appearance) of stool

The clinical presentation of IBS comprises a history of chronic abdominal pain with variable intensity, locations, and periodic exacerbations; altered bowel habits ranging from diarrhea and constipation; and other gastrointestinal symptoms like bloating and increased gas production. Based on the symptoms, IBS is classified in four different types:

- IBS with constipation: hard or lumpy stools $\geq$25%/loose or watery stools <25% of bowel movements
- IBS with diarrhea: loose or watery stools $\geq$25% of bowel movements/hard or lumpy stools <5% of bowel movements
- Mixed IBS: hard or lumpy stools $\geq$25%/loose or watery stools $\geq$25% of bowel movements
- Unsubtyped IBS: insufficient abnormality of stool consistency to meet the previously mentioned subtypes

If performed, with the purpose of ruling out other conditions with similar symptomatology, routine laboratory workup is usually normal in IBS. Further workup should be done in the

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presence of alarming symptoms like unexplained weight loss, rectal bleeding, nocturnal symptoms, and abnormal laboratory results or family history of inflammatory bowel disease, celiac disease, or colorectal cancer.

The treatment strategy is based on the nature and severity of symptoms, their correlation with food intake and/or defecation, the degree of functional impairment, and the presence of psychosocial difficulties and psychiatric comorbidity affecting the course of the illness. If symptoms are mild and intermittent with low impact on the quality of life, treatment is usually aimed towards lifestyle and dietary modifications. However, if symptoms are moderate to severe in intensity or have a significant impact on the patient's quality of life, then adjunctive pharmacological therapy should be considered. Dietary modifications include the exclusion of gas-producing foods or foods that increase flatulence; the avoidance of lactose in patients with lactose intolerance; a diet low in fermentable oligo-, di-, and monosaccharides and polyols in IBS patients with bloating; and a gluten-free 2-week trial in patients with diarrhea-predominant IBS. Although the benefit of a high-fiber diet in IBS is controversial, its use should be considered in patients with constipation-predominant IBS. Lifestyle modification includes an increase in daily physical activity. If adjunctive pharmacological therapy is indicated, then it should be tailored according to the predominant IBS symptom. Treatment options in constipation-predominant IBS include osmotic laxatives (polyethylene glycol) and agents that enhance intestinal fluid secretion and transit, like intestinal chloride channel agonist (lubiprostone) and guanylate cyclase C agonist (linaclotide). In the case of diarrhea-predominant IBS, antidiarrheal medications, like loperamide or bile acid sequestrants, should be considered. As-needed basis antispasmodic agents are indicated for short-term relief of abdominal pain in patients with IBS. Patients with unremitting symptoms with associated psychiatric impairment may benefit from behavior modification techniques in conjunction with antidepressants.

Colonic Diverticulosis and Diverticular Disease

A diverticulum is a saclike protrusion or a herniation of the mucosa and submucosa through a defect of the muscularis. Location is over the mesenteric border of the colonic wall and its origin corresponds to the four sites of penetration of the bowel wall by the major branches of the vasa recta. Strictly speaking, it is truly a pseudodiverticula since it does not contain all four layers of the colonic wall, only the mucosa and submucosa. Diverticulosis is the term used to define the presence of colonic wall diverticulum. Diverticular disease is a broader term used to define symptomatic diverticulosis.

The exact incidence and prevalence of diverticulosis are not exactly known because the majority of patients are asymptomatic. It is known, however, that the prevalence of diverticulosis is age-dependent, $<20\%$ at age 40 and increasing to $>60\%$ by age 60. In addition, the distribution of diverticulosis also varies by geography. Around 95% of patients in Western countries have sigmoid diverticula. In Asian countries, the prevalence of diverticulosis is predominantly right-sided.

Environment and lifestyle are important factors associated with the development of diverticulosis. Increasing age, obesity, diet rich in meats and low in fiber, living in Western countries, and connective tissue diseases are factors that increase the risk of developing diverticulosis. On the other hand, high-fiber intake and living in predominantly rural areas decrease the risk. Equivocal or factor that do not affect the risk include gender, alcohol and coffee consumption, colorectal cancer, and polycystic kidney disease. Smoking has been associated with increased risk of perforated diverticulitis when compared with nonsmoking.

As mentioned earlier, diverticula develop at well-defined points of weakness in the colonic wall that correspond to where the vasa recta penetrate the muscularis. Although still unclear, abnormal colonic motility seems to be a predisposing factor in the development of diverticula. Diverticula can vary in number from one to literally hundreds. The typical size of a diverticulum is 3–10 mm in diameter, but they can exceed 2 cm. Gross descriptions of the diverticula show a thickened inner circular wall and shortening of the taeniae. Electron microscopy studies have demonstrated a normal size and number of muscle cells, but demonstrated an increase in elastin and collagen deposition between the muscle cells in the taeniae.

Clinically, most patients with diverticulosis remain asymptomatic throughout their lifetime, and many cases go undetected. On the other hand, diverticular disease is defined as symptomatic diverticulosis due to diverticular bleeding, diverticulitis, segmental colitis associated with diverticula, or symptomatic uncomplicated diverticular disease. Diverticular bleeding is characterized by painless hematochezia due to segmental weakness of the vasa recta associated with a diverticulum. Diverticulitis is defined as inflammation of a diverticulum that may be acute or chronic and uncomplicated or complicated by a diverticular abscess, fistula, bowel obstruction, or free perforation. Segmental colitis associated with diverticula or diverticular colitis is characterized by inflammation in the mucosa without involvement of the diverticular orifices. Symptomatic uncomplicated diverticular disease is characterized by persistent abdominal pain attributed to diverticula in the absence of macroscopically overt colitis or diverticulitis.

The diagnosis is based on clinical history and physical examination and aided by the use of laboratory workup, imaging, and endoscopic tools. Acute diverticulitis should be suspected in patients with lower abdominal pain and abdominal tenderness on physical examination, keeping in mind other causes like appendicitis, ischemic or infectious colitis, inflammatory bowel disease, and neoplasia.

Patients with acute diverticulitis can present with uncomplicated or complicated disease. The management of uncomplicated diverticulitis includes outpatient treatment with an oral antibiotic aiming at Gram-negative rods and anaerobe coverage, dietary modifications including a clear diet for 2–3 days, high-fiber intake and avoiding nuts and seeds, and if possible the use of topical anti-inflammatory medications. Generally, the elderly, immunosuppressed, patients with significant comorbidities, and patients with high fever or significant leukocytosis should be hospitalized. The treatment of acute complicated diverticulitis is based upon the specific complication: bleeding, perforation, obstruction, peritonitis, abscess, or fistula formation. Management involves hospitalization, bowel rest, and intravenous hydration and empiric antibiotics. Failed medical management and recurrent episodes of acute diverticulitis are indications for operative management.

Colonic Inflammation (Colitis)

Inflammatory bowel disease is discussed in a separate article.

Infectious Colitis

Infectious colitis or infectious diarrhea is a lethal but preventable problem that poses a significant health and economic burden. The majority of deaths occur in developing countries where sources of clean water and adequate sanitation amenities are not readily available. In addition, these are regions where the prevalence of malnourishment is high. In developing countries, most of the cases occur in travelers or secondary to outbreaks from food and water contamination. In these circumstances, mortality is rare and generally occurs in immunocompromised patients. Clinically, inflammation of the infected colon results in frequent, small-volume bowel movements, which may contain mucous and blood. Other presenting symptoms include fever, dyschezia, and tenesmus. Onset is often times acute, but can have a chronic duration. Treatment is frequently guided according to the identified pathogen. A description of the most common microorganisms responsible for infectious colitis is shown in Table 1. A detailed depiction of specific pathogens is described elsewhere.

Table 1 Common pathogens associated with infectious colitis

	Risk factor	*Symptoms*	*Diagnosis*	*Treatment*	*Complications*
Bacteria					
Campylobacter jejuni	Foreign travel	Fever; malaise; abdominal pain; diarrhea; hematochezia	Stool culture	Ciprofloxacin	Guillain–Barré; cystitis; pancreatitis; reactive arthritis; toxic megacolon; postinfectious IBS
Clostridium difficile	Antibiotic use; hospitalization	Diarrhea; fulminant colitis; asymptomatic carrier	Selective anaerobic stool culture; toxin immunoassay; PCR; cell culture cytotoxicity assay	First-line outpatient: metronidazole. First-line hospitalized: metronidazole, vancomycin	Ileus; toxic megacolon; postinfectious IBS; protein-losing enteropathy
Escherichia coli O157:H7	Contaminated food	Hematochezia; abdominal pain; fever	Toxin assay	Supportive care	HUS; ischemic colitis; TTP; toxic megacolon
Salmonella typhimurium	Contaminated food; foreign travel	Fever; abdominal pain; diarrhea; weight loss; hematochezia	Stool, blood, and urine culture	Ciprofloxacin; penicillin; quinolones; supportive care	HUS; toxic megacolon
Shigella spp.	Travel to endemic area	Fever; abdominal pain; diarrhea; tenesmus; hematochezia	Stool culture; PCR; DNA probe; serology	TMP/SMX; third-generation cephalosporins, fluoroquinolones; nalidixic acid; supportive care	HUS; appendicitis; erythema nodosum; meningitis; postinfectious IBS; toxic megacolon; protein-losing enteropathy; TTP; perforation
Yersinia spp.	Cold climate; winter months; contaminated food/water; undercooked pork	Abdominal pain; hematochezia	Stool culture; serology	Aminoglycosides; TMP/SMX; supportive care	Erythema nodosum; exudative pharyngitis; glomerulonephritis; carditis; polyarthritis; reactive arthritis; sepsis; perforation; appendicitis; toxic megacolon
Virus					
Adenovirus	AIDS; immunosuppression	Diarrhea	Viral culture; immunohistochemistry	Supportive	
Cytomegalovirus	IBD; immunosuppression	Nonspecific	Urine culture; immunohistochemistry; PCR	Ganciclovir; valganciclovir	

(*Continued*)

Table 1 (Continued)

	Risk factor	*Symptoms*	*Diagnosis*	*Treatment*	*Complications*
Herpes simplex virus	Anal intercourse; immunosuppression	Anorectal discharge; nonspecific	Intranuclear inclusion bodies on biopsy; PCR; immunohistochemistry; antibody in formalin-fixed tissue	Acyclovir; valacyclovir; famciclovir	
Protozoa					
Balantidium coli	Food/water contamination with pig excrement	Nonspecific	Identification of stool cyst, precyst, or trophozoites; trophozoites in muscle biopsy	Tetracycline; iodoquinol	Perforation; peritonitis
Cryptosporidium spp.	Public swimming pools; HIV; malnourishment; age <2 years	Chronic diarrhea	Microscopic stool analysis	Paromomycin; metronidazole; nitazoxanide	
Entamoeba histolytica	Foreign travel; pregnancy; malnourishment; immunosuppression	Diarrhea	Identification of stool trophozoites	Metronidazole and iodoquinol; paromomycin; nitazoxanide	Perforation; appendicitis; strictures; abscesses
Parasite					
Strongyloides stercoralis	Living in endemic areas	Hematochezia	Microscopic stool analysis; serology; biopsy	Thiabendazole	Perforation; emphysematous gastritis; appendicitis
Trichuris trichiura	Living in endemic areas (tropic/subtropics)	Diarrhea; abdominal pain; tenesmus; weight loss	Identification of eggs in stool	Mebendazole	Rectal prolapse; neurotoxicity

AIDS, acquired immunodeficiency syndrome; DNA, deoxyribonucleic acid; HIV, human immunodeficiency virus; HUS, hemolytic uremic syndrome; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; PCR, polymerase chain reaction; SMX, sulfamethoxazole; TMP, trimethoprim; TTP, thrombotic thrombocytopenic purpura.

Drug-Induced Colitis

Certain medications are often associated with gastrointestinal adverse effects ranging from nausea to severe colitis. Many medications are known to cause colitis and only a few will be discussed in this section.

Chemotherapy and radiation for underlying neoplastic conditions are well-known causes of neutropenia. In this setting, neutropenic colitis, necrotizing enteropathy, or typhlitis can occur. This is a serious condition clinically manifested as severe right lower quadrant abdominal pain and bloody diarrhea. Fever is indicative of sepsis, intestinal perforation, and peritonitis. The pathology of the affected colonic segment features transmural edema, mucosal inflammation, ulcerations, denuded epithelium, hemorrhage, and necrosis. Treatment includes supportive care in the intensive care unit, intravenous fluids and antibiotics, and surgical resection if indicated.

Drug-induced hypomotility is associated with the use of opioids, tricyclic antidepressants, and anticholinergic medications. The use of vincristine is associated with paralytic ileus. Intestinal hypomotility leads to luminal stasis and bacterial overgrowth.

Nonsteroidal anti-inflammatory drug-induced colitis has a clinical presentation similar to inflammatory bowel disease, presenting with generalized abdominal pain and diarrhea. The use of these drugs is also associated with the development of strictures and ulcerations, particularly in the right colon. Mucosal healing and symptom relief is usually seen after discontinuing the offending drug.

Alosetron, a serotonin receptor antagonist, is associated with the development of ischemic colitis (see the succeeding text). Dihydroergotamine is also associated with ischemic colitis secondary to prolonged vasoconstriction.

Chemical Colitis

Several agents are well-known causes of colonic inflammation. The type of damage is thought to occur from a hypertonic or direct toxic effect over the mucosa. The severity of damage is related to the concentration of the substance, the duration of exposure, and the presence of an underlying colonic disease. The classic example is the use of detergents or soaps for enemas. Other implicated substances include acetic acid, lye, hydrogen peroxide, water-soluble contrast agents, formalin, alcohol, vinegar, and glutaraldehyde. Soaps are alkaline agents that produce liquefactive necrosis, inflammation that ranges from mild to severe, as well as saponification of the colon. Damage from water-soluble contrast agents, mainly in the proximal colon, is thought to be secondary to its hypertonicity. Acute inflammation can be self-resolving without permanent damage. However, scarring, fibrosis, transmural inflammation, and perforation have been reported. Treatment is mainly supportive with intravenous fluids and antibiotics. Surgery is indicated in severe cases of extensive necrosis.

Ischemic Colitis

Intestinal ischemia is caused by a reduction in blood flow, which can be related to acute arterial embolic/thrombotic occlusion, venous thrombosis, or hypoperfusion of the

mesenteric vasculature causing nonocclusive ischemia. Colonic ischemia is a common disorder of the large bowel in the elderly and is the most common form of intestinal ischemic injury. The reason why the colon is more vulnerable to injury is because it receives less blood flow compared to the rest of the gastrointestinal tract. Perfusion to the colon can be compromised by changes in the systemic circulation or by anatomic or functional changes in the local mesenteric vasculature. The three main mechanisms responsible for colonic ischemia are as follows:

1. Nonocclusive ischemia: this is the most common cause comprising ~95% of the cases. This type of ischemia tends to affect the 'watershed' areas of the colon that include the splenic flexure and rectosigmoid colon.
2. Embolic and thrombotic ischemia: this is either from spontaneous emboli from proximal sources or from aortic instrumentation.
3. Mesenteric vein thrombosis: this is a rare cause of colonic ischemia, but when involved, it tends to affect the proximal colon.

The injury to the colon following an ischemic event is due to hypoxia and damage from reperfusion. The hypoxic component causes rapid injury over the colonic mucosal surface. Irreversible damage results from transmural necrosis and complete injury to the tissue. Reperfusion injury usually takes place in the setting of partial ischemia and it is secondary to the release of free oxygen radicals that promote further injury.

Patients with acute colonic ischemia present with a history of abdominal pain and tenderness of rapid onset, usually over the left lower quadrant, as well as increased urgency to defecate. Hematochezia is usually present within 24 h after the onset of pain. Laboratory work results suggestive of ischemia are leukocytosis, decreasing hemoglobin levels, and metabolic acidosis. Plain abdominal films are usually nonspecific, but the 'thumbprint' sign can be seen. Abdominal computed tomography is commonly the first test ordered. The typical finding of thickening of the bowel wall in a segmental pattern is suggestive but not specific for ischemia. The finding of hepatic portal venous gas, although rare, is associated with extensive bowel necrosis and predicts a high risk of mortality. Colonoscopy is another test modality that should be performed early and with minimal insufflation. Colonoscopy findings in the acute setting frequently include pale mucosa with petechial bleeding. Bluish hemorrhagic nodules may be seen representing submucosal bleeding; these are the equivalent to the 'thumbprint' sign detected on radiological studies. Ulcerations and cyanotic mucosa are indicative of chronicity. Arteriography is rarely helpful in the diagnosis of colonic ischemia. It is not always readily available, and patients with nonocclusive colonic ischemia are often markedly dehydrated and acidotic; therefore, if used, it should be after careful fluid resuscitation.

Supportive care including bowel rest, intravenous fluids, and continuous monitoring is the initial therapy in patients with colonic ischemia. Although there is no strong evidence for the use of antibiotics, they are recommended in cases of moderate to severe ischemia. Anticoagulant therapy is indicated for patients who develop ischemia due to mesenteric venous thrombosis or due to cardiac embolization. Urgent abdominal surgical exploration is required in patients with colonic infarction and necrosis followed by postoperative hemodynamic support and observation in the intensive care unit.

Microscopic Colitis

Microscopic colitis is an uncommon chronic inflammatory disease of the colon clinically manifested as chronic watery diarrhea, histological changes of chronic mucosal inflammation, and normal colonoscopy findings. Microscopic colitis is classified into two subtypes according to their microscopic findings:

1. Lymphocytic colitis: intraepithelial lymphocyte count >20 per high-power field
2. Collagenous colitis: colonic subepithelial collagen band >10 μm in thickness

The two subtypes are more common in patients aged 50–70, but cases have been reported in children and adolescents. Both also have a female predilection. Associated conditions include celiac disease, arthritis, and other autoimmune disorders like autoimmune thyroiditis and type 1 diabetes mellitus. Medications implicated as causative agents or triggers include nonsteroidal anti-inflammatory drugs. Smoking may play a role in the development of microscopic colitis due to its effect on colonic blood flow. The pathogenesis of microscopic colitis is unclear; however, it is likely to be multifactorial, involving mucosal immune responses to luminal factors in a genetically predisposed individual. Although both subtypes have a similar inflammatory cell response, it is uncertain whether they are related. Abnormal collagen metabolism leading to increased deposition and a defect in epithelial barrier function predisposing to increased intestinal permeability have been proposed as possible pathogenic explanations for collagenous and lymphocytic colitis, respectively.

Clinically, patients with microscopic colitis present with a history of chronic, watery, nonbloody diarrhea. Onset is often insidious. Bowel movement volume and frequency tend to decrease with fasting. Other symptoms include abdominal pain, urgency, incontinence, weight loss, and dehydration. Extraintestinal manifestations, like uveitis and arthralgia, can be encountered. Physical examination is usually unremarkable and routine laboratory studies are also normal. Colonoscopy examination is usually normal. Nonspecific abnormalities including patchy edema, erythema, friability, and an abnormal vascular pattern have been reported, whereas mucosal ulcerations in the ascending and transverse colons have been reported in a few patients with collagenous colitis.

Treatment is largely empiric. Evaluation of therapy is difficult, because both disorders usually exhibit a relapsing and remitting course over many years. No single agent works in all cases. Antidiarrheal agents like loperamide or bulking agents like psyllium and methylcellulose are associated with clinical improvement, but not histological remission. Other medications like 5-aminosalicylate, glucocorticoids, and bile acid resins, alone or in combination, improve diarrhea and inflammation in some patients. Trial with a 6–8 week course of budesonide has shown good results in patients with collagenous colitis. Ileostomy should be considered only as a last resort, but it appears to be effective in patients with disabling and refractory symptoms.

Diversion Colitis

Diversion colitis is an inflammatory process that occurs in the diverted segment of the colon and rectum after surgical diversion of the fecal stream. Although it has been reported to occur more commonly in patients with inflammatory bowel disease, diversion colitis has been found in patients who have undergone surgical diversion for many indications. The prevalence of diversion colitis is unknown and probably underestimated since a proportion of patients with histological changes of the diverted segment are asymptomatic.

The pathogenesis of diversion colitis is related largely to lumen nutrient deficiency in the form of short-chain fatty acids. Other luminal elements besides short-chain fatty acid deficiency are likely to play a role, but the nature of such factors is unknown.

The diagnosis is based on clinical history and endoscopic findings of erythema, friability, nodularity, edema, aphthous ulcerations, exudates, and frank bleeding. Histological findings range from lymphoid follicular hyperplasia and mixed mononuclear and neutrophilic infiltration to severe inflammation with crypt abscesses, mucin granulomas, and Paneth cell metaplasia. Crypt architecture tends to be preserved. Caution must be taken in patients with recurrent inflammatory bowel disease and *Clostridium difficile* infection should be ruled out.

The treatment of choice is restoration of colonic continuity that reverses symptoms and histological changes. If reanastomosis is not feasible and symptoms are moderate to severe, short-chain fatty acid enema with propionate, acetate, and butyrate mixed in a saline solution has been described as a treatment option.

Colonic Neuropathies: Hirschsprung's Disease

Hirschsprung's disease (HD) is an intestinal motor disorder that results from the congenital absence of ganglion cells in the rectum and distal colon. As a consequence, the affected aganglionic segment lacks receptive relaxation resulting in a functional obstruction clinically manifested as constipation.

The incidence of HD is ~1 in 5000 live births. The overall male to female ratio is of 4 to 1, although it can be nearly 1 to 1 when the entire colon is involved. In ~85% of the cases, the aganglionic segment is limited to the rectum and distal colon; in other cases, longer segments can be affected, and in 5%, the entire colon is affected, including the distal ileum.

HD results from a defect in the craniocaudal migration of neural crest cells during the first 12 weeks of migration. In addition, defective differentiation of neuroblasts into ganglion cells and accelerated ganglion cell destruction may also play a part. The etiology of HD also has a genetic basis. Hereditary patterns are not straightforward and at least eight genetic mutations have been identified. The predominantly affected gene is the RET proto-oncogene, accounting for nearly 50% of familial and 20% of sporadic cases with long-segment disease. Other involved genes include Down's syndrome cell adhesion molecule, endothelin 3, endothelin receptor type B, endothelin-converting enzyme, SOX10, PHOX2B, and the neuregulin genes.

Up to one-third of patients with HD suffer other syndromes or congenital anomalies. Down's syndrome affects up to 2–3% of patients with HD. Other monogenic syndromes include multiple endocrine neoplasia type 2, congenital central hypoventilation syndrome, cartilage–hair hypoplasia, Mowat–Wilson syndrome, Waardenburg syndrome type 4, Smith–Lemli–Opitz syndrome, and Bardet–Biedl syndrome. Approximately 20–25% of patients with HD have associated congenital anomalies that involve the heart, kidney, or urinary tract.

HD is usually diagnosed during the neonatal period in patients with symptoms of distal intestinal obstruction, but can present at any age. Delayed passage of meconium passed the first 48 h of life should raise the suspicion of HD. Abdominal distention, feeding intolerance, bilious emesis, and explosive expulsion of gas and stool after digital rectal exam are other clinical presentations in infants with HD. Some infants with HD may have normal stooling initially, especially if they are breast-fed, and then develop severe constipation when other foods are introduced into the diet. A life-threatening presentation is HD-associated enterocolitis, a severe diarrheal illness that requires a high index of suspicion and prompt management with aggressive fluid resuscitation, intravenous antibiotic anaerobic coverage, and rectal irrigations. In extreme cases, a diverting colostomy may be indicated. Older children with less severe disease may present with a chronic history of intractable constipation, abdominal distention, or failure to thrive. Interestingly, there appears to be no correlation between the severity of symptoms and the length of aganglionic segment.

The diagnostic steps depend on the level of suspicion for HD. Abdominal radiographs may show signs of distal intestinal obstruction. A contrast enema with barium may reveal a more dilated colon proximal to a narrower distal aganglionic colon. This radiological finding is called a 'transition zone' and it may not be evident in patients with long-segment HD. If not clearly identified, a postevacuation film 24 h later may reveal residual barium in the colon. Anorectal manometry is a useful screening test if performed by experienced personnel. The presence of internal anal sphincter relaxation, or rectoanal inhibitory reflex, with balloon distention excludes the diagnosis of HD. To confirm the diagnosis, a rectal biopsy should be done, safely using a mucosal suction technique. If tissue sample is inadequate, then a repeat full-thickness biopsy under anesthesia is warranted. The diagnosis is confirmed if submucosal ganglion cells are absent on light microscopy. Hypertrophied nerve trunks, positive acetylcholinesterase, and negative calretinin immunostains are supportive histopathologic findings.

Treatment is aimed at resecting the affected aganglionic segment, the creation of a neorectum in close proximity with the anus, and preserve sphincter function. Several surgical techniques have been developed over time and are performed in different stages. The decision depends on experience and clinical characteristics of the patient. Currently, the majority of cases are done over one stage with an operation known as the Soave endorectal pull-through.

The majority of patients usually do well after surgical correction having normal to near normal anorectal function and have a good quality of life. However, some bowel function abnormalities are commonly encountered. Constipation or intermittent obstructive symptoms can be caused by mechanical obstruction, residual aganglionosis, or increased internal anal sphincter pressure. Fecal incontinence is usually encountered after surgery. However, continence tends to improve with

age. Finally, despite surgical repair, patients are still at risk for developing HD-associated enterocolitis. Risk factors include the presence of a mechanical obstruction like an anastomotic stricture and patients with long-segment disease, suggesting that intestinal stasis may be a predisposing factor.

See also: Colon: Structure and Function; Diarrheal Diseases.

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Colon: Structure and Function

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Structure

Embryology

During embryogenesis, the mature intestine develops from all three germ layers: the endoderm that gives rise to the simple columnar epithelial cell lining of the surface of the small and large intestines, the mesoderm from which cells of the lamina propria and muscularis layers originate, and the ectoderm that gives rise to the enteric neurons and other components of the enteric nervous system.

The primitive gut develops around the fourth week of gestation from the primitive yolk sac, which is further divided into three parts: foregut, midgut, and hindgut. The embryological origin of the colon is dual. The midgut gives rise to the appendix, cecum, ascending colon, and proximal two-thirds of the transverse colon. The distal third of the transverse colon, the descending colon and sigmoid colon, the rectum, and the upper portion of the anal canal originate from the hindgut. The distal hindgut further divides into a ventral component that becomes the urogenital sinus and a dorsal component that forms the lower portion of the anal canal.

During the fifth week of gestation, the midgut rapidly elongates with the formation of the primary intestinal loop. The cecum originates as a small dilation of the caudal limb of the primary intestinal loop by the sixth week of gestation. It initially lies in the right upper quadrant and then descends to the right iliac fossa, placing the ascending colon and hepatic flexure in the right side of the abdominal cavity. The appendix originates from the distal end of the cecal bud, and its final position is frequently retrocecal or retrocolonic. When the caudal limb of the primitive intestine moves to the right side of the abdominal cavity, the dorsal mesentery twists around the origin of the superior mesenteric artery. After the ascending and the descending portions of the colon reach their final destinations, their mesenteries fuse with the peritoneum of the posterior abdominal wall, and they become retroperitoneal organs. The appendix, cecum, and descending colon retain their free mesentery. The transverse colon fuses with the posterior wall of the greater omentum.

Anatomy

Macroscopic features

The colon is a tubular structure that is continuous with the small intestine proximally at the ileocecal valve and ends distally at the anal verge. In the full-term newborn, the colon is ~30–40 cm in length, increasing approximately to 150 cm in adults. The caliber is greatest in the cecum and gradually diminishes as it reaches the rectum. The appearance of the colon differs from the small intestine in several ways. The outer longitudinal muscle fibers of the colon coalesce into three distinct bands termed taeniae coli that extend continuously from the base of the appendix to the rectum. The haustra are outpouchings of the colon that give it a characteristic sacculated appearance. The plicae semilunares are intermittent creases that are visualized as irregular folds over the luminal surface of the colon. In addition, small sacs of peritoneum filled with adipose tissue, the appendices epiploicae, are found on certain areas of the external surface of the colon.

The colon, as the small intestine, is enclosed by the mesentery. However, most of the colon is fixated in a retroperitoneal fashion, and only a small portion is suspended by mesentery. The transverse colon and sigmoid colon are suspended by mesentery. A small portion of mesentery termed mesocecum fixes the mobility of the cecum. The ascending colon, descending colon, and rectum are partially covered by mesentery on their anterior surfaces.

The cecum is the most proximal part of the colon. It lies in the right iliac fossa and projects downward as a blind pouch below the entrance of the ileum and projects superiorly to the ascending colon. It is a saclike structure ~6 cm long and 8 cm wide in adults. The vermiform appendix is a blind, narrow, elongated outpouching of the cecum, and its lumen is found ~2.5 cm inferior to the ileocecal valve. Its length varies from 2 to 20 cm, being longest during childhood.

The ascending colon extends from the cecum in a distal manner toward the posterior lobe of the liver where it makes a sharp turn and forms the hepatic flexure. It is covered in peritoneum; therefore, it is a retroperitoneal organ. The diameter of the ascending colon is narrower than the cecum, and its length reaches ~20 cm in adults.

At the hepatic flexure, the colon emerges medially and anteriorly into the peritoneum as the transverse colon. Distally, it curves acutely and inferior to the lower pole of the spleen, forming the splenic flexure. The transverse colon is the longest segment of the colon with measured lengths from 40 to 50 cm. It lies anteriorly to the stomach and small intestine. It is completely covered in mesentery termed the transverse mesocolon. It is the most mobile segment of the colon; in fact, it can lie at the level of the pelvis when a person assumes an upright position.

The descending colon, about 25–45 cm in length, arises distal to the splenic flexure. It travels downward and posteriorly and assumes a retroperitoneal position ending at the level of the left iliac crest.

Distally, the descending colon emerges at the pelvic brim from a retroperitoneal position as the sigmoid colon. This is an S-shaped segment of the colon that varies in length and is very tortuous and mobile. It is suspended by mesentery termed sigmoid mesocolon that shortens and disappears as it reaches the rectosigmoid junction.

The rectum extends distal to the sigmoid colon at the level of the third sacral vertebrae toward the anal canal, 2–3 cm in front and below the coccyx. In adults, the rectum measures 10–12 cm in length. It lies below the peritoneum and in close proximity to the pelvic structures.

The anal canal is ~5 cm in length in the adult and has a discrete upper and lower demarcation. The anorectal ring is located proximally and is composed of the upper portion of the internal anal sphincter, the longitudinal muscle of the rectum, the deep portion of the external sphincter, and the puborectalis portion of the levator ani muscle. Distally, the anal verge represents the transition of anoderm to true skin. The mucosa of the distal 3 cm of the rectum and the anal canal contains 6–12 redundant longitudinal folds called the columns of Morgagni. These columns are joined together by mucosal folds called the anal valves, which are situated at the dentate line. The muscularis mucosae disappears in the anorectal canal, and the inner circular coat of muscularis propria thickens to form the internal anal sphincter. The external anal sphincter surrounds the anal canal, and its fibers blend with those of the levator ani muscle to attach posteriorly to the coccyx and anteriorly to the perineal body.

Vascular supply

The superior mesenteric artery, a direct branch of the abdominal aorta, supplies oxygenated blood to the cecum, ascending colon, and proximal two-thirds of the transverse colon. The inferior mesenteric artery, also a branch of the abdominal aorta, supplies the distal third of the transverse colon, the descending colon and sigmoid colon, and the superior portion of the rectum as the superior hemorrhoidal artery. The middle and inferior hemorrhoidal arteries, which are branches of the hypogastric and internal pudendal arteries, respectively, supply the rest of the rectum and anal canal.

The superior and inferior mesenteric veins drain the same regions of the large intestine supplied by the corresponding arteries. The venous system of the anal region drains into the systemic and portal systems. The internal hemorrhoidal plexus drains the proximal anal region into the superior rectal veins and then into the inferior mesenteric vein, which then, together with the superior mesenteric vein, joins the splenic vein to form the portal vein. The external hemorrhoidal plexus drains the distal anal region through the middle rectal and pudendal veins into the internal iliac vein.

Lymphatic drainage

The lymphatic drainage of the large intestine follows their respective blood supply, first draining to the pericolic nodes, intermediate nodes, and ultimately preaortic nodes that surround the superior and inferior mesenteric arteries. The rectum and anal canal drain into the perirectal nodes, which then further drain into the iliac and inferior mesenteric nodes.

Innervation

The innervation of the large intestine, as well as the rest of the gastrointestinal tract, comes from the intrinsic nervous system and the extrinsic nervous system. The enteric nervous system constitutes the intrinsic nervous system of the gastrointestinal tract. It is derived from the neural crest cells and consists essentially of an equal to the 100 million neurons in the spinal cord. The nerve cell bodies of the enteric nervous system are located in different plexuses of the gastrointestinal wall, mainly in the submucosa and muscularis externa. The sympathetic and parasympathetic nerves constitute the extrinsic nerve supply. The proximal colon receives its sympathetic neuronal innervation from the celiac and superior mesenteric ganglia. The parasympathetic innervation comes from the vagus nerve. In the distal colon, the sympathetic innervation comes from the branches of the lumbar segment of the sympathetic trunk, whereas the parasympathetic nerves originate from the pelvic splanchnic nerves. The sources of innervation of the internal and external sphincters are different. In the anal canal, the internal anal sphincter receives inhibitory innervation from the intrinsic nervous system and from extrinsic input from the lumbar sympathetic and sacral parasympathetic nerves. The pudendal nerve innervates the external anal sphincter and other pelvic floor muscles.

Microscopic features

In general, the intestinal wall of the gastrointestinal tract is composed of the following four layers:

1. Mucosa
2. Submucosa
3. Muscularis externa
4. Serosa

Mucosa

The mucosa is the innermost layer of the colon. It is further subdivided into three distinct sublayers: the glandular epithelium, lamina propria, and muscularis mucosae, the outermost sublayer. Compared with the small intestine, the mucosa of the colon lacks villi. However, the glandular epithelium does contain crypts of Lieberkühn that extend down through the lamina propria to the muscularis mucosae. The glandular epithelium is composed of the following several cell types:

- Stem cells are pluripotential cells located at the base of the intestinal crypts. These cells give rise to all types of mature intestinal epithelial cells and additional stem cells to maintain their numbers. Cell differentiation is regulated by different signaling pathways (Wingless, Notch, bone morphogenetic protein, and Hedgehog) between epithelial cells and cells in the lamina propria. In the colon, terminal differentiation occurs in the upper one-third of the crypt, and most cells migrate onto the mucosal surface. On the surface of the colon, older cells are extruded to the intestinal lumen by a process termed anoikis and expelled out of the body by defecation.
- Absorptive cells, generally called enterocytes, are high columnar cells located at the base near the lamina propria. They have oval basal nuclei, an eosinophilic cytoplasm, and a periodic acid–Schiff-positive free surface. Enterocytes are composed of superficial microvilli that are more numerous on the small intestine compared with the colon and contain membrane-bound digestive proteins, transport proteins, and other cellular elements necessary for nutrient absorption. In the colon, absorptive cells are termed principal cells.
- Goblet cells are mucin-producing cells located in the crypts characterized by a narrow base and oval apical surface, flattened basal nucleus, and basophilic cytoplasm with mucin-containing granules. They are much larger in number in the colon compared with the small intestine. The ample amount of mucin produced by goblet cells in the

colon provides lubrication for adequate stool passage. Goblet cells also provide a protective barrier against shear stress. This same barrier is thought to bind surface antigens and inhibit their attachment to the epithelial surfaces.

- Paneth cells are pyramid-shaped cells located exclusively at the base of the crypts. They have a basal nucleus and a cytoplasm rich in eosinophilic granules. Their primary function is to secrete antimicrobial products conferring protection from enteric infection and contributing to the maintenance of the gastrointestinal antimicrobial barrier. In response to luminal bacteria and bacterial antigens, they secrete different substances like phospholipase A2, lysozyme, and α-defensins, the latter being the principal antimicrobial molecule secreted by Paneth cells.
- Enteroendocrine cells, also known as gut endocrine or neuroendocrine cells, are specialized, tall, columnar cells located in the crypts of the colon. They have a large nucleus and prominent cytoplasmic secretory granules, distributed mainly in the basal region of the cell. The enteroendocrine lineage consists of at least 15 different cell types that are categorized based on their morphology, specific regional distribution, and peptide hormone expression.

The lamina propria is a thin layer of connective tissue that provides support to the mucosal epithelial cell basement membrane. In the colon, the lamina propria contains solitary lymphoid follicles that extend to the submucosa, being more developed in the rectum. Furthermore, the lamina propria is rich in arterioles, veins, lacteals, and nerve fibrils and various cell types, including fibroblasts, lymphocytes, macrophages, eosinophils, mast cells, and neutrophils.

The muscularis mucosae is the outermost layer of the mucosa. It lies next to the submucosa and consists of an inner circular layer and outer longitudinal layer of smooth muscle cells (~3–10 cells thick). The colonic muscularis mucosae is thicker when compared with the small intestine, and the thickness increases progressively from the cecum to the anal canal.

Submucosa

The submucosa, located between the outermost layer of the mucosa and the muscularis externa, is made of connective tissue and several different cell types that include fibroblasts, lymphocytes, eosinophils, macrophages, plasma cells, and mast cells. The submucosa has a rich vascular supply and lymphatic drainage, and, because of its close proximity to the mucosa, it supports its electrolyte, fluid, and nutrient absorption. The enteric nervous system nerve cell bodies are located in the submucosal plexus, which is divided into two networks, Meissner's plexus, which lies closer to the mucosa, and Schabadasch's plexus, which lies adjacent to the inner circular muscle.

Muscularis externa

The muscularis externa is composed of smooth muscle cells arranged in two distinct layers: the outer longitudinal layer and the inner circular layer. As mentioned earlier, the outer longitudinal layer fibers form the taeniae coli, which run in parallel to the long axis of the colon throughout its entire length. Within the two smooth muscle layers, there is a nerve fiber plexus of ganglionated cells, termed Auerbach's plexus, that also forms part of the enteric nervous system.

It is important to mention a specific group of cells, the interstitial cells of Cajal, that are involved in the coordinated activity between the enteric nervous system, autonomic nervous system, and smooth muscle cells that direct gastrointestinal motility. The interstitial cells of Cajal are modified smooth muscle cells and are regarded as the pacemaker cells of the gastrointestinal tract. These cells form networks that are widely distributed within the submucosal, intramuscular, and intermuscular layers. Contractions of gastrointestinal smooth muscle are initiated by these specialized pacemaker cells. Propagation of contractions occurs as waves of membrane depolarization spread via gap junctions between adjacent smooth muscle cells.

Serosa

The serosa, an extension of the visceral peritoneum, is the outermost layer of the colon. It is formed by a layer of mesothelial cells supported by connective tissue or adventitia. Occasionally, scattered different cell types are found within the serosa like macrophages, fibroblasts, and mast cells.

Functions

Water and Electrolyte Transport

The colon plays an integral role in intestinal homeostasis by regulating the balance between water and electrolyte absorption and secretion. In general, colonic epithelial cells are primarily responsible for absorption, and the crypt cells are involved in both absorption and secretion.

The colon is a very efficient water-preserving organ. Normally, the colon receives between 1000 and 1500 ml of water daily. Approximately 90% of this volume is reabsorbed by the colon, and 100–150 ml is excreted in the feces. Passive diffusion along an osmotic sodium luminal gradient is the main mechanism in which water is transported along the colon via paracellular pathways. However, the transmembrane protein channels, termed aquaporins, also play a role in the transcellular movement of water. Of the colonic segments, the ascending colon has the greatest absorptive capability because of its longer exposure time to the large volume of small intestinal fluid.

Under normal conditions, the colon reabsorbs sodium and chloride and secretes potassium and bicarbonate. The recovery of sodium is one of the main functions of the colon. Approximately 130–140 mmol l^{-1} of sodium is delivered daily to the colon, and only 40 mmol l^{-1} is excreted in the stool. Under certain circumstances, the colon is able to salvage up to 800 mmol l^{-1} day^{-1}, preventing hyponatremia. Throughout the colon, the mechanism by which sodium is reabsorbed varies. The proximal colon exhibits sodium/hydrogen exchanger channels located in the apical surface of the epithelial cells. These channels are coupled with a chloride/bicarbonate exchanger channel. The distal colon and rectum have electrogenic sodium-specific channels. Both types of transport channels allow the passive diffusion of sodium along an electrochemical gradient. Once inside the cell, sodium is then exchanged for potassium via an energy-dependent pump on

the basolateral membrane of the epithelial cell. Aldosterone, short-chain fatty acids, α2 adrenergic agents, and somatostatin further enhance the absorption of sodium.

Chloride is also recovered from the colon. As mentioned earlier, the proximal colon has chloride/bicarbonate exchange channels that absorb chloride and secrete bicarbonate. The acidic environment of the colon enhances the absorption of chloride, and the secretion of bicarbonate is responsible for the alkaline pH of the feces.

Potassium is passively transported along a sodium gradient. However, potassium is actively secreted in the proximal colon via a transport channel and absorbed in the distal colon via an energy-dependent hydrogen/potassium exchanger. The final concentration of stool potassium is $\sim$50–90 mmol l^{-1}.

Urea entering the colon from the small intestine is metabolized by colonic bacteria and converted to ammonia, which is passively absorbed by the epithelial cells. Other sources of ammonia that is further absorbed come from dietary nitrogen, sloughed cells, and bacteria. Absorbed ammonia reaches the enterohepatic circulation and is delivered to the liver where it is reconverted to urea.

Colon Flora, Nutrient Absorption, and Fermentation

The human colon is one of the most diversely colonized and metabolically active organs in the human body. Up to 1000 different species of bacteria reside in the colon with microbial populations comprising $\sim 10^{11}$–10^{12} colony-forming units per gram of content. The colon provides a favorable environment for bacterial growth due to its slower transit time, readily available nutrients, and favorable pH. Generally, bacteria having an almost exclusive saccharolytic metabolism can be considered potentially beneficial. Such a metabolic profile is typical for lactobacilli and bifidobacteria.

The colonic flora salvages energy via fermentation of undigested carbohydrates that reach the colon. These substances include resistant starches, nonstarch polysaccharides, nondigestible oligosaccharides, and sugar alcohols. The end products of bacterial fermentation – mainly of soluble plant residues – are the short-chain fatty acids butyrate, propionate, and acetate and the formation of odorless gases, including hydrogen, methane, and carbon dioxide. Approximately 90% of these short-chain fatty acids are absorbed by the mucosa in the proximal colon via short-chain fatty acid/bicarbonate transport channels and other electrolyte-coupled transporters. An average of 400 mmol day^{-1} of short-chain fatty acids is produced in the colon, providing 5–15% of the total caloric needs. Although butyrate is the least abundant of the short-chain fatty acids, it acts as the primary energy source for colonic cell health, supplying up to 90% of its energy needs. In addition, butyrate serves as an antidiarrheal agent as it promotes the absorption of water, sodium, and chloride. Propionate and acetate reach the liver via the bloodstream and participate in gluconeogenesis and long-chain fatty acid synthesis, respectively.

The colon is also involved in the metabolism and absorption of other nutrients. Proteins, via fermentation, are converted in the distal colon to short-chain fatty acids, branched-chained fatty acids, amines, ammonia, phenols, indoles, and sulfurs. The human colon cannot absorb long-chain triglycerides. In turn, they bind to luminal calcium making oxalate readily available for absorption. In patients with short bowel syndrome, medium-chain fatty acids from supplements can be absorbed in the remaining colon providing caloric energy. Absorption of calcium is highest in the cecum. Calcium requires solubilization of calcium salts enhanced by the acidic environment of carbohydrate fermentation. In addition, propionate and acetate also enhance the direct absorption of calcium in the colon. Vitamin K, in its K_2 form, comprises a group of bacterial products termed the multiprenyl menaquinones. Vitamin K_2 is passively absorbed in the colon.

Motility of the Colon

The other important function of the colon is the transport of unused material into the rectal area where it can be expelled in a socially acceptable manner during defecation. Although undigested food takes <2 h to reach the colon, it may take as long as 2–5 days for it to be expelled as stool. The propelling function of the colon is accomplished by haustrations (segmental contractions) and mass movement (high-amplitude peristaltic contractions (HAPCs)).

HAPCs are defined as contractions of at least 80–100 mmHg, lasting 10 s and propagating for at least 30 cm (see Figure 1). HAPCs can reach amplitudes >200 mmHg. HAPCs originate in the proximal colon and migrate distally >95% of the time, usually stopping in the distal sigmoid colon.

Food ingestion has an important influence on colonic motility. The response lasts 2–3 h and is mostly composed of segmental contractions, accompanied by an increase in colonic smooth muscle tone and HAPCs, which have been described in pediatric patients. The colonic response to eating is influenced by the caloric content and meal composition. Fat and carbohydrate represent an important stimulant, whereas protein may have an inhibitory effect (Figure 2).

Defecation

Normal defecation is a complex process that involves a sequence of events initiated by the entrance of stool in the rectum. When the rectal wall becomes stretched by stool or gas, the internal anal sphincter relaxes (also known as the rectoanal inhibitory reflex), permitting a small amount of stool to enter the anal canal. An enteric descending inhibitory reflex mediates this process. At the same time, there is a brief contraction of the external anal sphincter, maintaining continence. Extrinsic cholinergic parasympathetic pathways in the pelvic nerves mediate this process. Sensory receptors in the proximal anal canal provide a distinction between solid or liquid stools and gas. The internal anal sphincter has a relaxation of longer duration when the rectal wall is distended by a larger volume, producing a conscious voluntary contraction of the external anal sphincter until the individual decides when it is socially appropriate to evacuate the intraluminal contents. Suppression of the defecation urge and rectal receptive accommodation allows for temporary storage of stool or gas in the rectum or retrograde transport back to the sigmoid colon.

When socially appropriate, an individual may voluntarily relax the external anal sphincter to allow defecation to proceed. Intra-abdominal pressure is elevated to aid in the expulsion

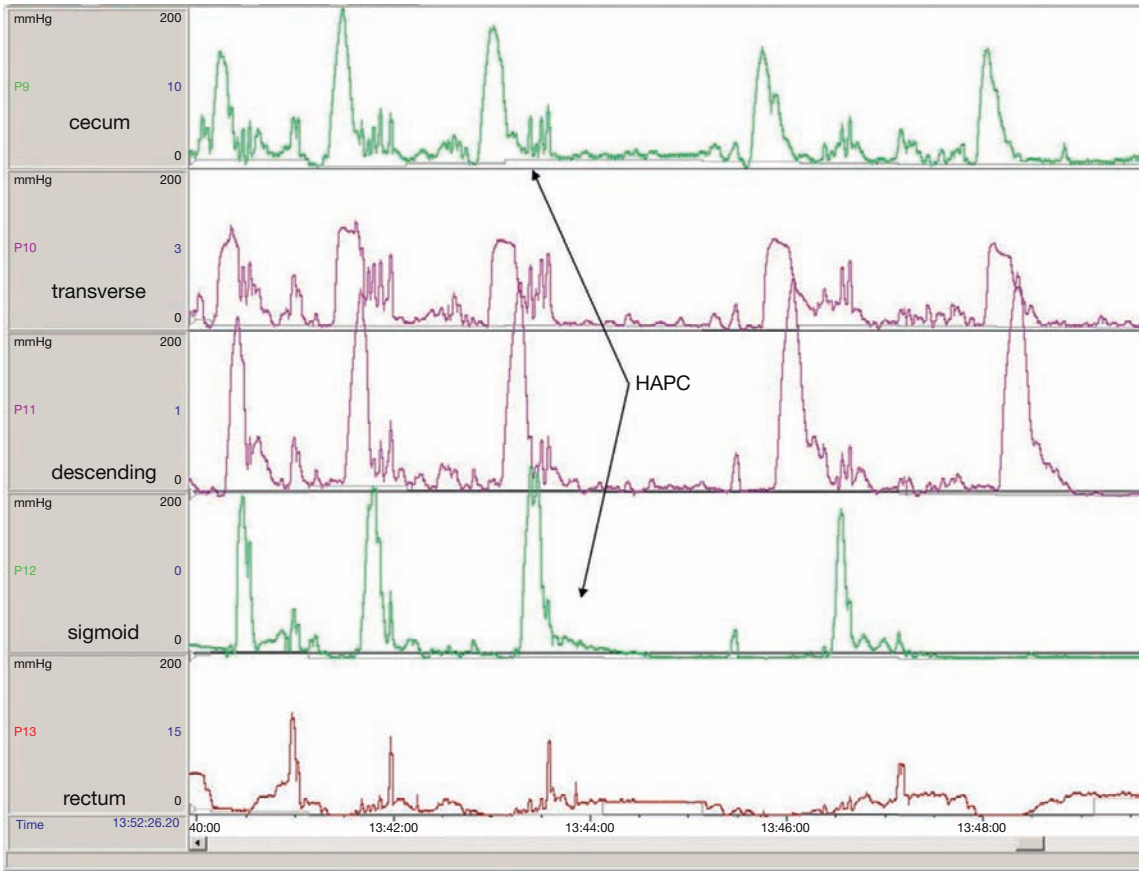

Figure 1 Normal colonic motility. Colonic motility in a child with functional constipation. Note the high-amplitude peristaltic contractions (HAPCs) that resulted after bisacodyl administration. Adapted and modified from Rodriguez, L. and Nurko, S. (2011). Gastrointestinal motility procedures. In: Wyllie, R., et al. (eds.) *Pediatric gastrointestinal and liver disease* (4th ed.), pp. 686–698. Philadelphia, PA: Elsevier.

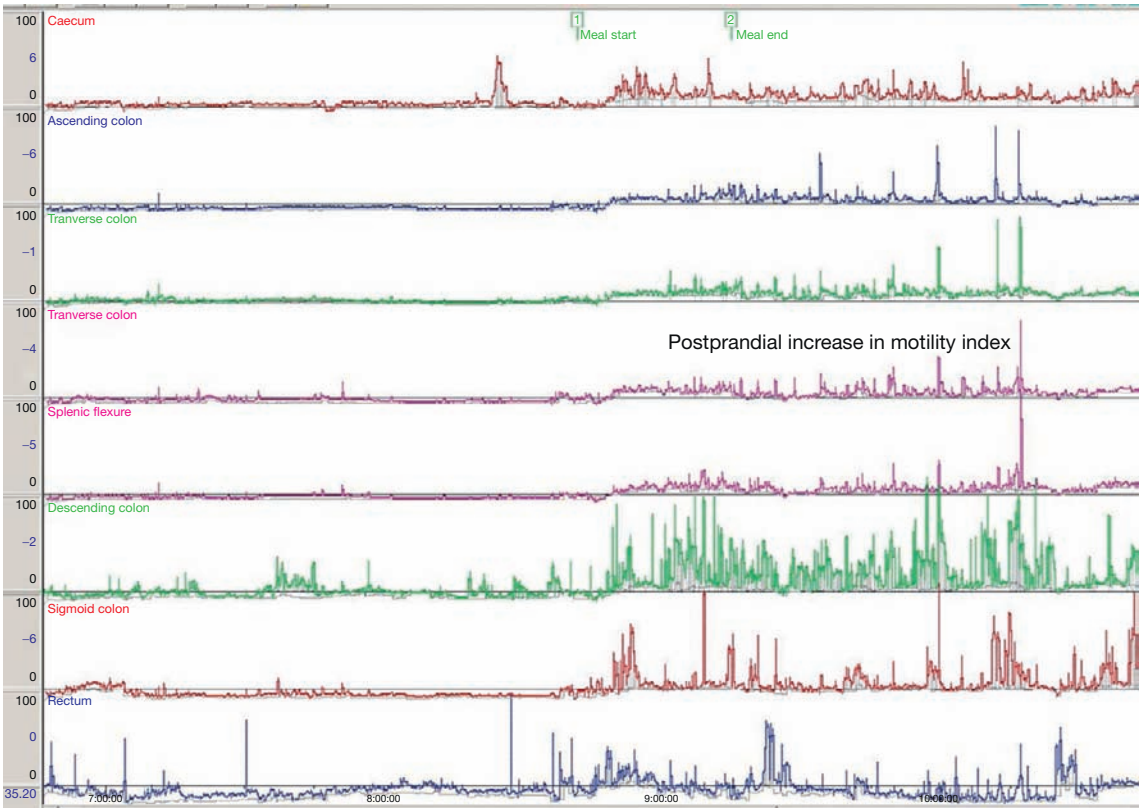

Figure 2 Normal postprandial colonic motility. This colonic motility shows the normal postprandial increase in the motility index. Adapted and modified from Rodriguez, L. and Nurko, S. (2011). Gastrointestinal motility procedures. In: Wyllie, R., et al. (eds.) *Pediatric gastrointestinal and liver disease* (4th ed.), pp. 686–698. Philadelphia, PA: Elsevier.

of feces. Evacuation is usually preceded by a deep breath, which moves the diaphragm downward. The glottis then closes, and contractions of the respiratory muscles on full lungs and abdominal muscles elevate both intrathoracic pressure and intra-abdominal pressure. Both activities serve to increase the anorectal angle, thereby reducing resistance to outflow. At the same time, the puborectalis muscles relax, further increasing the angle, the levator ani muscles contract, the perineum descends further, and stool is expulsed.

See also: Colon: Diseases and Disorders; Diarrheal Diseases.

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Colors: Health Effects

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Introduction

It is well known that color is one of the first criteria for identification and perception of food quality. This color is provided by pigments, natural in fresh foods and natural or synthetic in manufactured products. Even more, consumers associate food color with safety, quality, and good food processing, a reason why food industries spend high proportion of the product's cost to preserving or adding color.

Food colors or colorants are food additives added in order to make foodstuffs more visually appealing; to restore their original appearance if the visual acceptability has been damaged as a consequence of processing, storage, packaging, and distribution; and/or to add color to those foodstuffs otherwise colorless or colored differently.

Nevertheless, even if color is the obvious property for pigments, it is important to note that these molecules may have other biological properties. Due to this, natural pigments from plants are of growing interest as substitutes for synthetic dyes in the food and pharmaceutical industry, and they increase their added value if they possess positive effects on health. In most countries, the use of food additives (including colorants) is governed by strict regulation. The legislation specifies which colorant may be used, the source, the purity, and to which food and at what level they may be added.

This article reviews the main aspects of natural pigments and food colorants dealing with human health.

Food Natural Pigments

Different food natural pigments can be identified:

- Anthocyanins
- Betalains
- Carotenoids
- Chlorophylls
- Curcumin
- Myoglobin and hemoglobin

Pigments naturally formed during food processing have also been included in this section, as the melanoidins and the *Monascus* pigments (Figure 1).

Anthocyanins

The anthocyanins are part of a widespread group of plant constituents, known as flavonoids. The structure is based on a C15 skeleton consisting of a chromane ring bearing a second aromatic ring B in position two (anthocyanidin). The structure is complemented by one or more sugar molecules bonded at different hydroxylated positions (anthocyanins).

Six anthocyanidins (aglycones) are the most common in nature and consequently used as natural colorants (viz., pelargonidin, peonidin, petunidin, malvidin, cyanidin, and delphinidin).

Regarding their chemical stability, it is important to mention that the color of these pigments is highly affected by pH and four structures exist in equilibrium: the flavylium cation (colored) at low pH, displaced toward the uncolored forms (carbinol pseudobase and chalcone) or the quinoidal form (red/blue) as the pH value increases (Figure 2).

Anthocyanins provide the red to purple or blue color to pome fruits (apples, pears, chokeberries, etc.); stone fruits (apricots, cherries, peaches, and plums); small fruits (currants, strawberries, and all other berries); some tropical fruits (acerolas, lychees, mangos, etc.); other fruits such as grapes, pomegranates, and red oranges; cereals (rice, barley, sorghum, etc.); legumes (e.g., beans); roots, tubers, and bulbs (onions, potatoes, etc.); brassica crops (broccoli, red radish, etc.); and other crops (eggplant, perilla, rhubarb, tamarillo, etc.).

Due to the aforementioned chemical properties, anthocyanins, as food colorants, are used for red fruit drinks, sorbets, water ices, jellies, jams, gums, etc., at pH values below 4.

In recent years, several studies have shown that anthocyanins display a wide range of biological activities, including antioxidant, anti-inflammatory, and antimicrobial, and their consumption is associated with a diminished risk for different types of cancer owing to their antiproliferative, proapoptotic, and antiangiogenic effects. Also, they play an important role in blood vessels, platelets, and lipoprotein, able to reduce the risk of coronary heart diseases. Nevertheless, the absorption and metabolic fate of these pigments are still under study.

Their influence on prevention in visual performance and ocular diseases, their role in skin aging and UV-induced skin damage, and their neuronal and cognitive brain functions have also been reported, being of special interest as they are one of the fewer secondary metabolites able to cross the blood–brain barrier.

Betalains

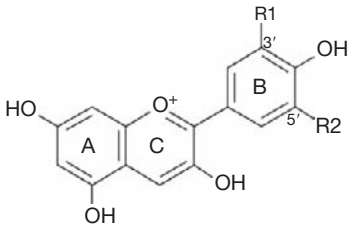
Betalains are water-soluble nitrogen-containing pigments, which provide red-violet (betacyanins) and the yellow (betaxanthins) colors to some fruits and vegetables. They are ammonium conjugates of betalamic acid with cyclo-DOPA and amino acids or amines, respectively.

With a focus on food, the scarce attention toward betalains may be due to the fact that red beet has long been considered the only edible betalainic source. However, other sources of betalains are species of the genus *Amaranthus*; other Cactaceae such as cactus fruits from *Opuntia* sp., *Hylocereus polyrhizus*, or *Myrtillocactus geometrizans*; and Swiss chard.

The most interesting applicable feature of betalains is the stability in the pH range from 3 to 7, when processed at low pasteurization, in the presence of ascorbic acid, and stored at low temperature, which makes these pigments suitable for their application in a wide range of low-acid and neutral foods.

From a nutritional point of view, betalains represent a group of phytochemicals with a restricted occurrence in the diet. Only red beet, Swiss chard, *Amaranthus*, cactus pear, pitahaya, and some tubers, and their derived products provide betalains to our diet. However, the use of betanin as food colorant and the plant betalain-enriched extracts in functional foods increases the consumption of this type of phytochemicals.

Betalains are bioavailable molecules, present as intact forms at systemic level, but new studies should be carried out in order to identify the complete pool of circulating compounds derived from betalain biometabolism once they are consumed.



Anthocyanidin	R1	R2
Pelargonidin	H	H
Cyanidin	OH	H
Peonidin	OCH_3	H
Delphinidin	OH	OH
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Figure 1 Structure of the main dietary anthocyanins.

Preliminary experiments on the biological and health-promoting properties of betalains have been focussed on their antioxidant properties due to their chemical and *in vitro* antiradical properties. However, their real physiological relevance has not been fully unraveled in intervention or clinical trials in humans. New studies conducted at the right physiological concentrations about the action of betalains and their circulating metabolites may open new ways of biological actions of these phytochemicals against human disorders.

Carotenoids

Carotenoids are the most widespread group of pigments in nature, responsible of the red, orange, or yellow colors of many vegetables and fruits. These molecules are isoprenoids that consist of eight isoprene units in a long polyene chain, which may extend from 3 to 15 conjugated double bonds.

β-Carotene is the most prominent member of the group of carotenoids. It belongs to the class of carotenoids named carotenes, together with lycopene. The group of xanthophylls is formed from the hydrocarbon carotenes by the introduction of oxygen functions, as lutein, α- and β-cryptoxanthin, and zeaxanthin.

Around 60 different carotenoids have been identified in fruits and vegetables.

At least 85% of our dietary lycopene comes from tomato fruit and tomato-based products. Major sources of β-carotene include green leafy vegetables, carrots, red peppers, oranges, potatoes, and broccoli. In a lesser extent, carotenoids are also present in eggs, poultry, and fish, especially when included in the animal feed. Lutein and zeaxanthin are highly present in spinach and kale, broccoli, peas, and Brussels sprouts, and they are the major carotenoids of corn and egg yolk; astaxanthin is the red pigment of crustaceans, salmon, and trout. Capsanthin and capsorubin are present in paprika flavoring. Bixin and norbixin are apocarotenoids present in annatto, a natural food coloring.

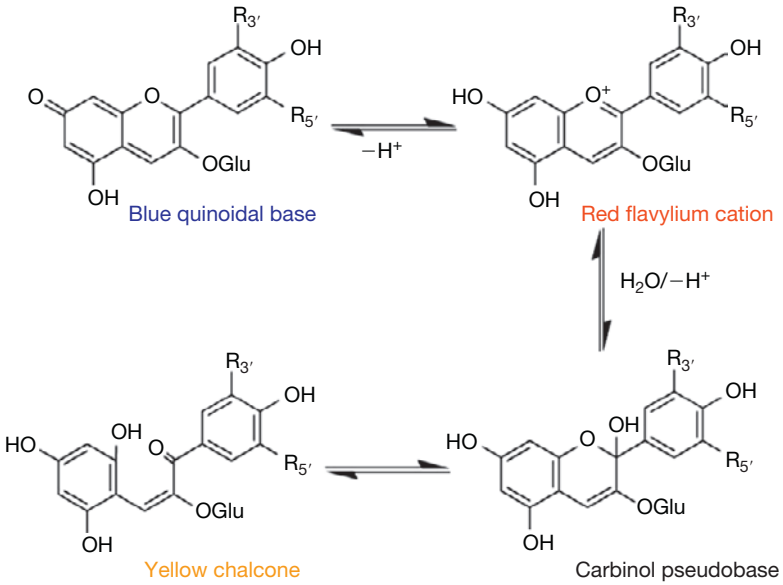


Figure 2 Structural transformations of anthocyanins depending on pH changes.

Bioaccessibility of carotenoids is limited and conditioned by different factors, as food processing and the composition and structure of the food matrix. Pressure cooking and stir-frying increase their bioaccessibility, whereas the presence of dietary fiber has a negative effect. Because of their lipophilic nature, the absorption of carotenoids is enhanced from a fatty diet.

Carotenoids have a broad range of positive effects on human health. The best established function is the provitamin A activity (vitamin A is important for many functions in the human body, being essential for normal growth and development, immune function, and vision), restricted to those molecules with β-ring end groups, as β-carotene, zeaxanthin, and β-cryptoxanthin.

Lutein and zeaxanthin are present in eye macula and might protect from light-induced damage and scavenge free radicals formed. Besides, UV protective effects have been reported in the skin after supplementation of β-carotene, due to the protection against photooxidation. Consumption of tomato products, rich in lycopene, is associated with reduced low-density lipoproteins (LDLs) oxidation implicated in vascular lesions, and low serum levels of lycopene have been related to increased risk of atherosclerotic events in middle-aged men. In relation to cancer, carotenoids have shown to inhibit cell proliferation and increase gap junctional communication. Epidemiological studies demonstrate that an increased consumption of a diet rich in carotenoids is associated with a diminished risk for different types of cancer, as it occurs with the high lycopene consumption and prostate cancer and lung and stomach cancer. However, the CARET (Beta-Carotene and Retinol Efficacy Trial) study showed that lung cancer was alleviated with β-carotene supplementation. It seems that the dietary consumption of a combination of carotenoids and other phytochemicals in fruit and vegetables is more effective in the protection against cancer than the intake of single supplements of any carotenoid.

Chlorophylls

Chlorophyll is the green pigment found in cyanobacteria and chloroplasts of algae and plants and is critical in photosynthesis as it allows plants to absorb energy from light.

The basic skeleton is composed of a large planar structure of four pyrrole rings joined together with a central metal atom of magnesium and a phytol hydrophobic group.

At least six chlorophyll molecules are known: chlorophylls a, b (higher plants), c, d, and e (algae) and bacteriochlorophylls (photosynthetic bacteria). The most widely distributed in plants is chlorophyll a.

Chlorophyllins are molecular analogs of chlorophyll more stable and also available as food colorants. They are hydrophilic, due to hydrolysis of the phytol tail, and the magnesium atom is replaced with other metal complexes as copper.

Chlorophylls are abundant in leafy fresh vegetables, as spinach and kale, lettuce, and basil. They are highly susceptible to degradation during processing. The most common reactions involve the loss of magnesium by heat or acidic conditions, forming pheophytin, and the green color turns to an olive green. Further decarbomethoxylation leads to the conversion of pheophytin into pyropheophytin. These changes are perceived by the consumer as a loss of quality. Storing the product at low temperatures and the immediate processing help to preserve the green color.

Native chlorophylls are poorly absorbed as such and are easily converted into Mg-free pheophytins during the gastric phase of digestion. Pheophytins are transferred to micellar fractions for their optimal uptake by human intestinal cells and likely represent the biologically relevant forms of these pigments.

Natural chlorophylls and chlorophyllins have been reported to display antioxidant activity, in models of lipid peroxidation, and antimutagenic and chemopreventive activities. Their porphyrin cycle can either scavenge free radicals or form a complex with carcinogens. Human studies on populations at high risk of developing carcinoma, because of toxin ingestion, have shown a reduction of urinary excretion of damaged DNA catabolites, without reported toxicity effects, possibly due to the formation of adducts of chlorophylls and mutagen, interfering in the bioavailability of the latter.

Curcumin

Curcumin is a natural colorant extracted from the rhizomes of *Curcuma longa* plant (turmeric). Three compounds have been isolated: curcumin, demethoxycurcumin, and bisdemethoxycurcumin that impart the yellow pigmentation to the plant and the products added, for example, curry.

The main pattern of consumption is in powder form, containing a mixture of the three curcuminoids, used as spice in Indian cuisine, to improve palatability and preservation of foods and to impart color to condiments and dressings.

Curcumin exhibits poor bioavailability, a major limit for its therapeutic efficacy and use in clinic. The major factors are its poor absorption due to insolubility in water, fast metabolism, extensive enterohepatic recirculation, and rapid systemic elimination in the bile and urine. The 40% of orally administered curcumin is excreted unchanged in the feces.

Curcumin is believed to have a wide range of biological effects. It has been shown to directly interact with numerous signaling molecules and activate/inhibit multiple regulatory proteins, including transcription factors, enzymes, and cytokines. In this way, curcumin may reverse conditions as insulin resistance, hyperglycemia, hyperlipidemia, and other inflammatory symptoms associated with obesity and metabolic diseases.

Because of its multitargeting properties, curcumin has exhibited activities against some types of cancer as colorectal and breast cancer. Histological improvement of precancerous lesions has been observed in bladder cancer, oral leukoplakia, and intestinal metaplasia of stomach, and a decrease in the number and size of polyps in skin lesions has been observed in phase I and phase II human clinical trials. In proinflammatory diseases as ulcerative colitis, Crohn's disease, pancreatitis, or arthritis, curcumin has shown promising effects, with high tolerability and scarce adverse effects. Efforts must be done to improve its poor bioavailability.

Melanoidins

Melanoidins are brown-colored compounds, with high-molecular-weight heterogeneous polymeric structure. These

products are formed through the Maillard reaction of sugars and amino acids at high temperatures and low water activity.

Information about the structure of melanoidins is scarce due to the high complexity and diversity of structures and the difficulty of isolation of pure compounds.

Melanoidins are present in a large range of products, such as barley malts, bread crust, bakery products, balsamic vinegar, tomato sauce, and coffee.

Little is known about the metabolic fate of melanoidins. They are largely resistant to digestion in the human gastrointestinal tract. The microbial gut degradation occurs for the less complex melanoidin structures that are absorbed and can be detected in urine in quantities up to 30% of intake.

Human studies have reported antioxidant activity of coffee or beer melanoidins after ingestion. Melanoidins protect against radical stress in the colon and behave as dietary fiber by promoting the growth of gut bifidobacteria. Besides, inhibitory effects of melanoidins on matrix metalloproteases implicated on tumor growth and development have been reported. They also have antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enterica* and could prevent the adhesion of *Streptococcus mutans* to the tooth surface playing a role inhibiting the development of dental caries.

Better knowledge of melanoidin structures would benefit the understanding of their biological properties.

Monascus

Monascus-fermented rice, also referred as red yeast rice (RYR), is a food product that is produced by inoculating fungi of the genus *Monascus* into steamed rice. *Monascus* fungi produce different main pigments: red colorants (rubropunctamine and monascorubramine), orange colorants (rubropunctatin and monascorubrin), and yellowish colorants (monascin and ankaflavin).

RYR (known as beni-koji in Japan) has been used for centuries in Eastern countries as China and Japan as natural food colorant and flavor as well as food preservative of meat and fish. Food examples are red rice vinegar, some types of tofu, Peking duck, Chinese pastries and rice wines as Japanese sake, imparting reddish color. The RYR is most often powdered and sold as such but the pigments may also be extracted.

Healthy effects have been reported for RYR as cholesterol lowering, antidiabetic activity, anti-inflammation, and prevention of osteoporosis, and some of these activities have been related to the pigments contained.

The red and orange pigments possess antibiotic activity against bacteria, yeast, and filamentous fungi. Both orange (rubropunctatin and monascorubrin) and yellow pigments such as monascin and ankaflavin have shown immunomodulatory and anti-inflammatory properties. In addition, *Monascus* pigments could prevent obesity development. Monascin and ankaflavin might reduce triglyceride accumulation and proliferation of preadipocytes. Cancer-chemopreventive activity has been reported for all pigments, specially monascin and ankaflavin, by inhibition of cancer cell progression and cytotoxicity of tumoral cells.

However, the RYR can be contaminated by citrinin, a hepatotoxic metabolite of *Aspergillus* and *Penicillium* species that has led to the prohibition of *Monascus* pigments use as food additive colorants in EU and United States, while in Asian countries, it is accepted under monitoring and the establishment of maximum limits for citrinin in foods.

Myoglobin and Hemoglobin

Meat color is due to the concentration of the pigments myoglobin and hemoglobin. Myoglobin is a metalloprotein composed of a polypeptide globin and a heme residue with iron ion.

Color changes in meat occur because of the susceptibility of myoglobin to alterations. Under low oxygen tension, iron in myoglobin exists in Fe^{2+} state and meat is visually purple-red. When it is exposed to oxygen for a short time, myoglobin binds oxygen producing oxymyoglobin, which is bright pink or red. Oxidization of iron to Fe^{3+} state produces metmyoglobin, visually brown or gray-brown. Further degradation of metmyoglobin gives green and dark brown pigments.

Biological function of myoglobin is related to storage of the oxygen brought by the hemoglobin for the requirements of living tissues. It binds reversibly O_2 and facilitates its transport to mitochondria. Myoglobin also scavenges reactive O_2 species and facilitates oxygen diffusion. An additional role of this protein is to bind nitric oxide in skeletal muscle as nitric oxide inhibits cytochrome c oxidase and thus impairs mitochondrial respiration.

Colorants Used as Additives in Food Industry

Fruit and vegetable juices can be added to foodstuffs but are considered ingredients rather than additives. Because of this, they do not carry an E number in the European Union (EU) market, turning the foodstuff into a more natural product. Accepted food colors and quantities allowed to be added to each foodstuff are regulated by national laws (except for the EU countries that share a common legislation).

According to the US Food and Drug Administration (FDA), all color additives shall be declared. Food colorings exempt from certification are those having generally a mineral, plant, or animal origin and are declared as 'artificial color,' 'artificial color added,' or 'color added.' Colors subject to certification are usually identified by the terms 'FD+C No.' All the 'FD+C' color additives have to be declared, and in order to save labeling space, they can appear with their names without the prefix 'FD+C No.'

This section will focus on natural pigments that are not a food in itself and are not normally used as a characteristic ingredient of foods, such as riboflavins, cochineal, vegetable carbon, and some minerals. Artificial food colors, which are produced by chemical synthesis, will also be reviewed.

Riboflavins

Riboflavin, also known as vitamin B2, occurs as yellow to orange-yellow crystals. It is designated by the E number E 101 and can be found both in its free form (E 101i) and as riboflavin-5′-phosphate (E 101ii). Riboflavin or vitamin B2 is present naturally in animal products (meat, milk, and eggs), vegetal ones (malted barley, nuts, and almonds), and yeast.

It is also used as food color in order to confer yellow shades to some foodstuffs such as dairy products and confectionary. It may be produced by chemical synthesis, biosynthesis from *Eremothecium ashbyii* (aka *Ashbya gossypii*), *Saccharomyces cerevisiae*, and *Bacillus subtilis*, among other microorganisms, or isolated from natural sources. Riboflavin-5′-phosphate is prepared.

The health effects of riboflavin are mainly determined by its role as a vitamin. Its physiological functions are described in other articles of this Encyclopedia.

Cochineal, Carmine, and Carminic Acid

Carminic acid is a red-crimson anthraquinone coloring matter that occurs naturally in some insects like cochineal (*Coccus cacti* L.). The food color cochineal or cochineal extract is obtained after hydroalcoholic extraction of bodies of insects. Carmine is a more concentrated solution containing, at least, 50% of carminic acid.

Cochineal-derived dyes are mainly used to confer red shades in alcoholic beverages, yogurts, juices, ice creams, and confectionary, although it can be also found in jams and some processed meat products.

Concerning its health properties, carminic acid may cause allergic reactions and even anaphylactic shock to a sensitive subset of populations. Nevertheless, it is not a significant hazard to the general population.

Vegetable Carbon

Vegetable carbon or carbon black is produced by steam activation of carbonized plant material. It is mainly used in confectionary and desserts, including dairy products.

No ADI has been established for vegetable carbon owing to its scarce toxicological data, despite evidence on its safety has been confirmed by the European Food Safety Authority (EFSA).

Food Colors from Mineral Origin

There is a group of food colors that are obtained mainly from mineral sources by diverse extraction processes. These food colors are titanium dioxide that provides white color and opacity; iron oxides, conferring yellow, red, or black shades; aluminum; silver; and gold. These colors are mainly used as surface coatings (e.g., iron oxides are present in sausage coatings). Gold and silver can also be included in expensive eccentric beverages. Calcium carbonate can also act as coating colorant providing white color, although its principal role as additive is to serve as anticaking agent, stabilizer, and acidity regulator. Although they are accepted in the EU, most of these cannot be used within the American market as they are banned by the FDA. On the contrary, some color additives as ferrous gluconate and ferrous lactate are accepted in the United States, but not at the EU, for the coloring of ripe olives.

Information on the dietary exposure to these minerals when used as food additives is very scarce (Table 1). Nonetheless, their contribution to the daily consumption of minerals may be considered as negligible attending to their restricted use. The absorption, distribution, metabolism, excretion, and safety of these mineral food colors follow the same patterns as when they are contained naturally in foods or they are present as contaminant due to food processing operations.

Azo Food Colors

Most of the synthetic additives used as food colors share a common chemical structure characterized by the presence of an azo functional group. In addition, many of these colors are sodium salts of sulfanilic acid. Tartrazine, Sunset Yellow FCF, Azo Rubine, amaranth, Ponceau 4R, Allura Red AC, Brilliant Black BN, Brown HT, Lithol Rubine BK, Orange B, and Citrus Red No. 2 are part of this group.

Tartrazine is the trisodium salt of 5-hydroxy-1-(4-sulfonatophenyl)-4-[(*E*)-(4-sulfonatophenyl)diazenyl]-1*H*-pyrazole-3-carboxylate. It provides orange-yellow color to some desserts and sweets, potato and corn chips, muesli, corn flakes, noodles, tartar sauce, mustard, bouillon cubes, and energy and sport drinks. Tartrazine is also available for consumer, as it is one of the few food additives that can be purchased at grocery stores. Sunset Yellow FCF, Orange Yellow S, or FD+C Yellow No. 6 is an orange-yellow azo dye present not only in soft drinks and desserts such as jellies but also in confectionary, jams, chips, biscuits, ice creams, instant noodles, and food decorations and coatings. Azo Rubine or carmoisine, a red dye, is present in nonalcoholic flavored drinks, sugar confectionary, and yogurts. Amaranth provides dark, reddish brown color to alcoholic beverages like aperitif wines just as to candied fruit, other confectionary products, yogurts, and ice creams. Ponceau 4R, Cochineal Red A, or New Coccine is a red color used mainly in soft drinks, confectionary, coatings, biscuits, ice creams, and fine bakery wares. Allura Red AC can be found in crisps, biscuits, nonalcoholic flavored drinks, drinks, desserts and yogurts, sweets, ice creams, and jams. Brilliant Black BN or Black PN and Brown HT are bisazo colors used to confer dark shades in a broad array of foodstuffs that are banned in many other countries outside the EU. Lithol Rubine BK is a red dye used exclusively in cheeses as surface coating. Orange B, banned in the EU market but allowed in the United States, is only used in sausage coatings. Citrus Red No. 2 is only for use in the United States to color orange skins.

Dietary exposure to azo food colors vary notably among these additives because of their differences regarding quantities used and pattern of consumption of foodstuffs containing them (Table 1).

Azo food colors are extensively metabolized by the gut microbiota to sulfanilic acid and aminopyrazolone after oral administration. On the basis of this fact, urine excretion of intact azo colors is negligible or very low (e.g., <5% for tartrazine), while their metabolites, mainly free and conjugated sulfanilic acid, are absorbed to a greater extent.

Regarding health effects of azo colors, controversial literature has been reported. Some cell/animal studies pointed out how tartrazine, Ponceau 4R, Allura Red AC, Brilliant Black BN, and Brown HT might affect neurodevelopment or exert carcinogenic properties. Nevertheless, adverse effects of these food colors on neurobehavioral development or cell proliferation should not be expected to occur in humans, according to EFSA. In general, there are no indications of any genotoxic/carcinogenic potential of (bis)azo dyes or their metabolites. On the

Table 1 Characteristics, acceptable intake, and exposure for food colors accepted in United States and EU

EU regulation[a]			*US regulation*[c]			
Name	*E number*	*MLU*[b]	*Name*	*Certification?*[d]	*ADI (mg kg body weight^{-1} day^{-1})*[e]	*Mean dietary exposure (mg kg body weight^{-1} day^{-1})*[f]
Curcumin	100	R	Turmeric	No	3	European children: 0.5–3.8 UK adults: 0.9
Riboflavins	101	q.s.	Riboflavin	No	0.5*	Men: 2 Women: 1.5
Tartrazine	102	R	FD+C Yellow No. 5	Yes	7.5	European children: 0.8–3.4 Hong Kong children: 0.35
Quinoline Yellow	104	R	D+C Yellow No. 10	Banned	0.5	Irish children: 2.7 Hong Kong children: 0.0
Sunset Yellow FCF/Orange Yellow S	110	R	FD+C Yellow No. 6	Yes	1	European children: 0.02–0.4 Hong Kong children: 1.1
Cochineal, carminic acid, carmines	120	R	Cochineal extract or carmine	No	5*	
Azo Rubine, carmoisine	122	R		Banned	4	European children: 0.3–2.5 Hong Kong children: 0.0 UK adults: 0.5
Amaranth	123	R		Banned	0.15	European children/adolescents: $1.6–4.0 \times 10^{-3}$, adults/elderly: $0.008–0.009 \times 10^{-3}$ Hong Kong children: 0.11
Ponceau 4R, Cochineal Red A	124	R		Banned	0.7	European children: 0.3–2.5 Hong Kong children: 0.06 UK adults: 0.5
Erythrosine	127	R	FD+C Red No. 3	Yes	0.1	Europe: Negligible Hong Kong children: 0.03
Allura Red AC	129	R	FD+C Red No. 40	Yes	7	European children: 0.8–3.4 Hong Kong children: 0.15 UK adults: 0.9
Patent Blue V	131	R		Banned	5	Toddlers: 1–4.5, children: 1.1–3.6 adolescents: 0.5–1.8, adults: 0.3–1.4, elderly: 0.2–0.6
Indigotine, Indigo carmine	132	R	FD+C Blue No. 2	Yes	5*	Hong Kong children: 0.0
Brilliant Blue FCF	133	R	FD+C Blue No. 1	Yes	6	European children: 0.5–3.4 Hong Kong children: 0.22 UK adults: 0.9
Chlorophylls, chlorophyllins	140	q.s.		Banned	Not limited*	
Copper complexes of chlorophylls, chlorophyllins	141	q.s.	Sodium copper chlorophyllin	No	15	
Green S	142	R		Banned	5	European children: 0.5–3.5 Hong Kong children: 0.0 UK adults: 0.9
Plain caramel	150a	q.s.	Caramel	No	Combined ADI: 300, with an ADI of 100 to E 150c	Toddlers: 19–105, children: 31–83, adolescents: 12–56, adults: 15–57, elderly: 9–35
Caustic sulfite caramel	150b	q.s.	Caramel	No		
Ammonia caramel	150c	q.s.	Caramel	No		
Sulfite ammonia caramel	150d	q.s.	Caramel	No		
Brilliant Black BN, Black PN	151	R		Banned	5	Below 5
Vegetable carbon	153	q.s.		Banned	No established	European children: 3–30 UK adults: 4
Brown HT	155	R		Banned	1.5	European children: 0.3–2.2 UK adults: 0.5
Carotenes	160a	q.s.	β-carotene	No	5*	European children: 0.03–0.22 UK adults: 0.06

(*Continued*)

Table 1 (Continued)

EU regulation[a]			*US regulation*[c]			
Name	*E number*	*MLU*[b]	*Name*	*Certification?*[d]	*ADI (mg kg body weight^{-1} day^{-1})*[e]	*Mean dietary exposure (mg kg body weight^{-1} day^{-1})*[f]
Annatto, bixin, norbixin	160b	R	Annatto extract	No	12*	
Paprika extract, capsanthin, capsorubin	160c	q.s.	Paprika, paprika oleoresin	No		
Lycopene	160d	R	Tomato lycopene extract/ concentrate	No	0.5*	
Beta-apo-8′-carotenal	160e	R	Beta-apo-8′-carotenal	No	0.05	European children: 0.5–3.4 UK adults: 0.9
Lutein	161b	R	Tagetes (Aztec marigold) meal and extract	No	1	0.6–2.2
Beetroot red, betanin	162	q.s.	Dehydrated beets (beet powder)	No	No established*	
Anthocyanins	163	q.s.	Grape color extract/skin extract (enocianina)	No	No established	Toddlers: 1.5–4, children: 1.5–4.7, adolescents: 1–2.5, adults: 0.7–1.9, elderly: 0.5–1.1
Calcium carbonate	170	q.s.	Calcium carbonate	Banned	No established	
Titanium dioxide	171	q.s.	Titanium dioxide	No	No established	
Iron oxides/ hydroxides	172	q.s.	Synthetic iron oxide	No	0.5*	
Aluminum	173	q.s.	Alumina	Banned		
Silver	174	q.s.		Banned		
Gold	175	q.s.		Banned		
Lithol Rubine BK	180	q.s.	D&C Red No. 6	Banned	1.5	European children: 0.02–0.06 UK adults: 0.01–0.03
Fast Green FCF	Banned	–	FD+C Green No. 3	Yes	25*	
	Banned	–	Ferrous gluconate/ lactate	No	0.8*	
	Banned	–	Orange B	Yes		
	Banned	–	Citrus Red No. 2	Yes		

[a]Data according to the Commission Regulation (EU) No 1129/2011 of 11 November.
[b]MLU, maximum level of use by EU laws; R, regulated on the basis of the foodstuff considered; q.s., 'quantum satis,' which means that no maximum level is specified and substances shall be used in accordance with good manufacturing practice.
[c]Data according to the Code of Federal Regulations Title 21 (Parts 73 and 74).
[d]If the color does not require certification, it follows the rules established at the Part 73 of the Code of Federal Regulations Title 21; if it requires, it follows Part 74 of Title 21.
[e]Acceptable daily intake (ADI) based on recent EFSA reevaluations of selected colors as food additives. Data with asterisk refer to values based on the Joint FAO/WHO Expert Committee on Food Additives (JECFA).
[f]Data based on the mean dietary exposure considering the maximum permitted levels (Tier 2) established by EFSA Panels after reevaluations of selected colors as food additives and on Lok et al.

other hand, the effects of Sunset Yellow FCF on the testis, sperm morphology, and sperm mobility remain unclear nowadays, which motived the reduction of the ADI for this azo dye.

Tartrazine has been identified to cause migraine, asthma, and certain cutaneous conditions (i.e., urticaria) in a very small fraction of the population due to intolerance reactions. Sensitive reactions after Sunset Yellow FCF, Azo Rubine, amaranth, Ponceau 4R, or Allura Red AC consumption may also take place in some individuals, overall when it is taken within blends of other synthetic food colors. Finally, behavior changes (hyperactivity, restlessness, and sleep disturbance) might appear in children following exposure to some azo colors in combination with other food additives like sodium benzoate. This fact would be restricted to specific sensitive individuals, and its clinical significance remains unclear.

Quinoline Yellow

It is a greenish yellow food dye, produced by chemical synthesis that can be found in sport and energy drinks, ices, and confectionary, among other products.

It has a limited absorption in mammal animal models, and the bulk of the dose is excreted unchanged via the feces. Nonetheless, this color additive is to some extent bioavailable, concerning some systemic effects found in long-term toxicity studies. Adverse sensitivity reactions (urticaria and rhinitis)

after intake of Quinoline Yellow have been reported although no conclusive results on the induction of sensitivity by this food color have been achieved. It may also have an adverse effect on activity and attention in children, similarly to some azo dyes.

Caramels

Caramel is a complex mixture of compounds resulting in a dark brown liquid or solid material formed by the controlled heat treatment of sugars. Caramelization is produced in the presence of ammonia, sulfite, alkalis, or combinations thereof, and caramels are classified, according to their processing, into plain caramel, caustic sulfite caramel, ammonia caramel, and sulfite ammonia caramel. Caramels are present in a plethora of foodstuffs such as beer, biscuits, chocolate, confectionary, cookies, crisps, ice creams, sauces, soups, vinegar, wood-aged spirits, and, overall, colas.

The main contributors to mean exposures (Table 1) to caramels in children through European countries are flavored drinks, bakery, and desserts, including dairy products. Non-alcoholic beverages, beer, cider, and confectionary account for the higher amounts of the four caramel colors in adults. Some population groups having high exposure to caramel color could exceed the ADI of this color.

Caramels are complex mixtures of poorly characterized compounds, and their bioavailability remains unclear.

Caramel food colors present low toxicity/carcinogenicity both in short-term tests and in chronic studies. However, certain low-molecular-weight compounds being part of these additives, such as furan and 5-hydroxymethyl-2-furfural (5-HMF), might have chemotoxic potential though their contribution to the toxicity of caramel colors has not been fully elucidated. There are no data concerning the long-term toxicity or carcinogenicity of caramel colors so far. In contrast to other synthetic additives, there are no reported cases of sensitivity reactions.

Triarylmethane Colors

Brilliant Blue FCF, Erioglaucine disodium, Green S or Lissamine Green B, and Fast Green FCF are synthetic triarylmethane dyes. They can be found mainly in ice creams and other dairy products, peas, and drinks. According to the higher level of intake of these dyes (Table 1), some adults may be above the ADI of Brilliant Blue FCF and Green S. Anyway, triarylmethane colors are poorly absorbed and mainly excreted unchanged in feces. Brilliant Blue FCF lacks significant genotoxicity effects, while data related to Green S are not available.

Other Synthetic Food Colors

Indigotine or Indigo carmine is a synthetic color, although it used to be extracted from plants of the genus *Indigofera*. Together with Patent Blue V, it is used in order to confer blue coloration to foodstuffs. Both blue colors are mainly used in coatings, ice creams, and confectionary. Erythrosine is exclusively used for cherry products in the EU and used also for sweets in other countries. In general, these food colors have low absorption and limited availability and are excreted almost completely via feces. Health effects of these colors are not worth mentioning as they are recognized as safe under proper manufacture practices and normal intakes.

See also: Caramel: Methods of Manufacture; Caramel: Properties and Analysis; Carotenoids: Physiology; Chlorophyll; Codex Alimentarius Commission: Role in International Food Standards Setting; Colors: Properties and Determination of Natural Pigments; Colors: Properties and Determination of Synthetic Pigments; Food Additives: Classification, Uses and Regulation; Food Allergies; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Riboflavin: Physiology; Riboflavin: Properties and Determination.

Further Reading

Commission Regulation (EU) No 1129/2011 of 11 November 2011 Amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by Establishing a Union list of Food Additives.

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Relevant websites

http://www.efsa.europa.eu – European Food Safety Authority (EFSA).

http://www.fda.gov/ – US Food and Drug Administration (FDA), Code of Federal Regulations – Title 21 – Food and Drugs.

http://www.food.gov.uk/ – U.K. Food Standards Agency (FSA).

http://www.mhlw.go.jp/english/index.html – Japanese Ministry of Health, Labour and Welfare.

http://www.nih.gov/ – US Department of Health & Human Services, National Institutes of Health (NIH).

Colors: Properties and Determination of Natural Pigments

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Abbreviations

APCI	Atmospheric pressure chemical ionization
DAD	Diode array detector
ESI	Electrospray ionization
HPLC	High-performance liquid chromatography
MS	Mass spectroscopy
SCC	Sodium copper chlorophyllin

Anthocyanins

Anthocyanins are water-soluble vacuolar flavonoid pigments. They are responsible for the orange, magenta, violet, and blue color of plant organs, including leaves, stems, roots, flowers, and fruits. Until now, 600 different anthocyanins are known mainly in the form of heterosides. The aglycone form, or anthocyanidin, is a polyhydroxy and polymethoxy derivative of flavylium (2-phenyl-1-benzopyrylium), which is usually bonded to a sugar moiety and can be possibly acylated by a phenolic or aliphatic acid (Figure 1). Among the 23 types of known anthocyanidins, only six are common in higher plants: pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin.

Many factors, that is, pH, heat, light, metals, and copigmentation, may affect their stability, color, hue, and density. Under acidic conditions, the eight conjugated bonds in anthocyanin structures carrying a positive charge on the heterocyclic oxygen ring are responsible for the intense red-orange to blue-violet color. They show a wavelength of maximum adsorption between 465 and 550 nm and a significant absorption in the UV range between 270 and 280 nm.

In vitro, under mildly acidic conditions (pH 3–6), the red and stable flavylium cation form predominates, while at pH 6 and above, it changes into the colorless carbinol pseudobase or chalcone forms and finally into the bluer and unstable quinonoidal base form (Figure 2). The larger the number of hydroxyl groups on the B-ring, the bluer the color is. Aromatic acylation causes a blueshift and stabilizes anthocyanins, whereas aliphatic acylation increases the stability and solubility. Self-association with flavonoids stabilizes anthocyanins and causes bluing and intensifying of color.

Sources and Production

Cyanidin, delphinidin, and pelargonidin glycosides are the most common in nature, being present in 80% of pigmented leaves, 69% of fruits, and 50% of flowers. The distribution of the six more common anthocyanidins in fruits and vegetables is the following: cyanidin (50%), delphinidin (12%), pelargonidin (12%), peonidin (12%), petunidin (7%), and malvidin (7%). 3-Glycosides occur about two and a half times more frequently than 3,5-diglycosides.

The major sources of anthocyanins are red fruits, some cereals, and root vegetables (aubergines, beans, cabbages, radishes, and onions).

Berries such as black currants and blackberries contain up to 2–4 g kg^{-1} fresh weight anthocyanins mainly in the skin. They are also present in apples, plums, and pears and in the flesh of cherries and strawberries. In particular, red grapes can contain up to 250 mg/100 g, while the concentration in red wines varies in accordance with the varieties of grapes employed and with the type of vinification and, especially, with aging. However, a medium value of around 500 mg l^{-1} of anthocyanins could be established in young wines.

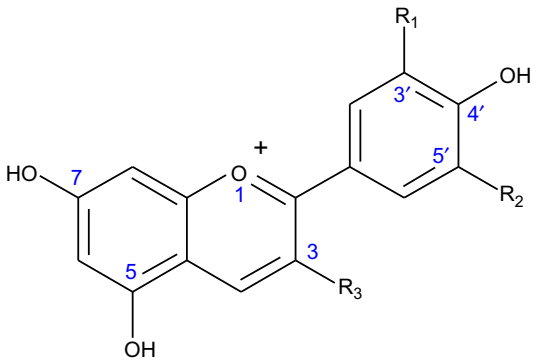
Actually, the European Union allows the use of anthocyanins as food colorants named E163 in acidic food or beverages including soft drinks, fruit preserves, sugar confectioneries, dairy products, frozen products, dry mixes, and alcoholic drinks. In particular, grape skins, a by-product of the wine industries, are the most common source for economic reasons along with the concentrated juice of black currants, strawberries, cranberries, elderberries, cherries, and red cabbages. Other less familiar sources include radishes, black carrots, and red and purple sweet potatoes.

Moreover, berry, grape skin, and seed extracts are commercialized as nutraceuticals and as dietary supplements for their antiaging effect.

Despite their great potential of application, anthocyanin extraction from natural sources is complex due to their relative instability.

Determination of Anthocyanins

Usually, anthocyanins are extracted under mild conditions with cold acidified polar organic solvents (i.e., methanol, ethanol, and acetone) at a composition of 70–80% in water. The acid employed is usually acetic acid, trifluoroacetic acid, or sulfured water in order to minimize the pH effects on the flavylium equilibriums. Then, the concentrated extract is purified by washing with hexane, petroleum ether, or diethyl ether to remove unwanted lipophilic compounds. Alternative techniques that reduce the use of organic solvents and maximize recovery with minimal degradation of the pigments are solid phase extraction and elution by solvent with increasing polarity, supercritical fluid extraction using CO_2 and polar organic modifiers as cosolvents, pressurized hot water



Anthocyanins	R1	R2
Pelargonidin-3-glucoside	H	H
Peonidin-3-glucoside	OCH_3	H
Cyanidin-3-glucoside	OH	H
Malvidin-3-glucoside	OCH_3	OCH_3
Petunidin-3-glucoside	OCH_3	OH
Delphidin-3-glucoside	OH	OH

Figure 1 Chemical structure of the six most common dietary anthocyanin 3-*O*-glucosides. Structural classification of the six anthocyanins (flavylium form). R_3 is a sugar moiety.

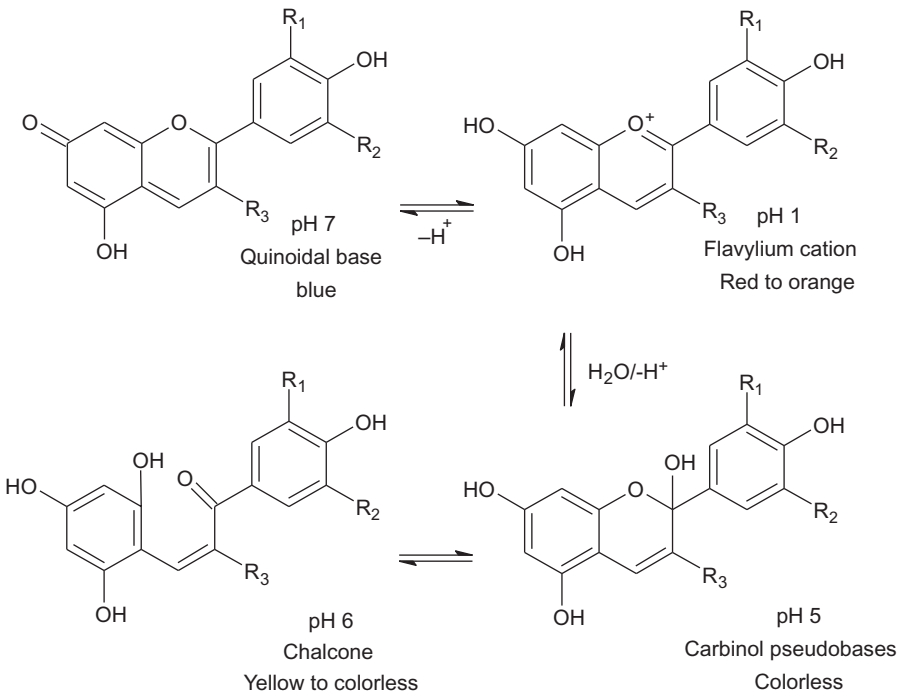


Figure 2 Structural transformations of anthocyanins upon pH changes.

extraction, microwave-assisted extraction with polar solvents, and countercurrent chromatography that allows fractionation and isolation of pure compounds for direct identification by mass spectroscopy (MS) and nuclear magnetic resonance.

Recently, enzyme-assisted extraction has been carried out for anthocyanin extraction from cherry, red grape, strawberry, black currant, and blueberry juice processing wastes and grape residues. Nowadays, some researchers have also developed cell cultures within plant species for the production of anthocyanins.

The analysis of anthocyanins is commonly carried out through high-performance liquid chromatography (HPLC) on octadecylsilane, polystyrene, or phenyl-bonded columns in reversed phase using gradient solvent systems of acetonitrile–water or methanol–water with a small amount of acid and a UV detector. Anthocyanins have a unique maximum absorption at 520 nm, and a diode array detector (DAD) performs a complete spectrophotometric scan on each peak as it elutes and provides a unique chromatogram for each compound. Recently, electrospray ionization–MS (ESI–MS) detection has been used allowing very low fragmentation and exact molecular weight determinations. Another common technique used for the separation of anthocyanins is capillary zone electrophoresis (CZE) with UV or ESI–MS detection using acidic media optimized via a phosphate buffer and a cationic surfactant.

Patterns of Consumption

Depending on the diet, the daily intake of anthocyanins in humans has been estimated to range between a few and 100 mg. The average anthocyanin intake in adults within the United States is about 12.5 mg day^{-1}. A higher consumption has been reported in European countries with a North–South gradient. This result is consistent with the Mediterranean diet including berries, other red- and blue-colored fruits, and red wine. Considerably, enhanced anthocyanin consumption of commercially available extracts is also suggested.

Availability, Absorption, and Metabolism

Anthocyanins are rapidly absorbed in the stomach and small intestine reaching a maximum plasma concentration (C_{max}) of between 1.4 and 200 nm for doses of 10–720 mg of anthocyanins. The C_{max} is reached between 45 min and 4 h after ingestion of an anthocyanin-containing meal depending on its composition. In addition, anthocyanins are rapidly eliminated in urine after 1.5–6 h of ingestion. Since the proportion of absorbed and excreted anthocyanins is $<0.1\%$ of the ingested amount, there is a paradox about the metabolic fate of a very high percentage of the ingested anthocyanins that needs to be explained.

In both blood and urine, they appear as intact, methylated, glucuronide derivatives and in sulfoconjugated form. Interestingly, the glycosides do not appear to be easily hydrolyzed to aglycones.

The reasoning of the paradox is that the bioavailability of anthocyanins compared with other flavonoids is influenced by physicochemical characteristics such as size, lipid/water solubility, and pK_a; by food matrix effects like the presence of lipids; or by interaction with other compounds in the meal. Moreover, the changes of pH and the presence of digestive enzymes, biliary acids, and microbiota along the gastrointestinal tract can greatly affect the chemical stability of anthocyanins and their metabolism.

Anthocyanins occur under cationic form in the gastric lumen, but whether they are adsorbed through binding to some specific protein transporters, it is still unknown. On the other hand, the absorption of anthocyanin glycosides in the small intestine, mainly in the form of carbinol pseudobase, may involve specific glucose transporters. Another possible mechanism could be the hydrolyzation of anthocyanins by brush border enzymes prior to passive diffusion of the aglycone. Finally, large amounts of unabsorbed anthocyanins and derivatives excreted in the bile from the enterohepatic circulation can reach the colon. Enzymes of the gut microbiota can also induce metabolization including methylation, sulfation, and conjugation with glucuronic acid and breaking of glycoside linkages. These compounds may undergo adsorption and further metabolization in the liver.

Moreover, the low stability of anthocyanins in the pH of the intestine and the activity of the intestinal microbiota are at least partially responsible for the conversion of anthocyanins into more stable small phenolic acids, such as protocatechuic acid, or other molecules of unknown structure.

Therefore, the overall anthocyanin bioavailability should result from the contribution of the amount that crosses all physiological barriers in all their possible forms: native, degradation products, metabolites, and anthocyanin derivatives.

Moreover, several intact anthocyanins and some metabolites have been detected in many animal tissues including the liver, the kidneys, the brain, the lungs, and the eyes.

As a consequence, what is left to explore is whether the biological effects associated with anthocyanins are due to their native forms or to some specific metabolites arising after adsorption.

Betalains

Betalains are water-soluble nitrogen-containing pigments responsible for the yellow-orange-red colors of flowers and fruits belonging to the angiosperm order Caryophyllales. However, in Caryophyllaceae and Molluginaceae, they are replaced by anthocyanins, and interestingly, the two pigment families are mutually exclusive. Betalains comprise 55 structures: the red-violet betacyanins and the yellow-orange betaxanthins that are ammonium conjugates of betalamic acid with *cyclo*-DOPA and amino acids and amines, respectively. Although more than 50 types of betacyanins are known, betanidin is the most common aglycone that can be glycosylated or acetylated on one or both C5 or C6 hydroxyl groups (**Figure 3**). Further glycosylation of the 5-*O*-glucoside and esterification with hydroxycinnamic acids are also very common.

Betacyanins display a wavelength of maximum absorption in the UV range between 270 and 280 nm due to the *cyclo*-DOPA structure and a second adsorption maximum in the visible range at about 535–538 nm depending on the solvent system. Acylation with hydroxycinnamic acids produces a third maximum at about 300–330 nm, whereas aliphatic acid

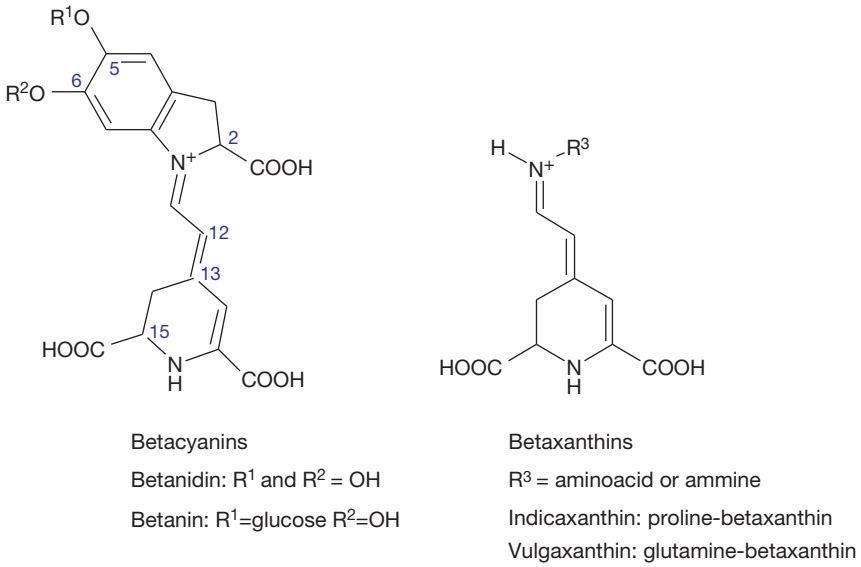


Figure 3 Basic structure of betacyanins (left) and betaxanthins (right).

derivatives are not distinguishable from their corresponding betanidin glycosides. Betacyanins and betaxanthins are cations below pH 2.0, zwitterions at pH 2.0, monoanions between pH 2.0 and 3.5, and stable bisanions above pH 3.5 and 7.5.

Sources and Production

The major dietary sources of betalains are not only cactus pear and red and yellow beetroot but also colored Swiss chard and grain or leafy amaranth (Table 1). The major commercially exploited betalain crop is red beetroot (*Beta vulgaris*) because the extract contains mainly betanin, which is approved as a red food colorant E162 in different low-acid and neutral food commodities (pH 3–7) from beverages to candies and from dairy to cattle products by both the European Union and the United States. Betalains are more stable as food colorants over a broader pH range than anthocyanins, although their use is still limited, being not always edible.

For example, cactus fruit betalains cover a broader color spectrum without negative flavor impact as compared with red beet. An additional advantage to cactus fruits is their minimal soil and water requirements, as it is regarded as an alternative culture for the agricultural economy of arid and semiarid regions.

Determination of Betalains

Plant cells and tissue cultures are alternative sources of betalains that ensure continuous supplies of easily extractable products with uniform quality and yield, independently from their geographic location and minimizing risks of political interference. The most widely used plant *in vitro* systems are cell suspensions, hairy roots, and tissue cultures. Extraction of betalains is commonly performed with an aqueous organic solvent (i.e., methanol); however, the addition of ascorbic acid in the extraction medium is recommended to slightly lower the pH, which stabilizes betacyanins and inhibits possible oxidation by polyphenol oxidases.

HPLC with C18 in reversed phases and UV detection is the method of choice for the analysis of betalains. Elution is performed by a gradient solvent system using methanol and phosphoric acid at pH 2.75 in ion suppression mode. Two different wavelengths were used to monitor selectively the red and yellow pigments (538 and 476 nm). Another common method of detection is ESI–MS in positive mode.

Patterns of Consumption

Betalains represent a group of phytochemicals with a restricted occurrence in the diet since their plant food sources are quite reduced. Only red beet, Swiss chard, amaranth, cactus pear, pitaya, and some tubers and their derived products provide betalains to our diet.

However, the use of betanin as a food colorant and the plant betalain-enriched extract in functional foods may increase their consumption.

Availability, Absorption, and Metabolism

Despite the scarce studies on bioavailability and metabolism of betalains, they all show that these pigments are bioavailable. In particular, cactus pear fruit or red beet juice indicaxanthin and betanin, which represent the adducts of betalamic acid with proline and cyclo-DOPA-5-*O*-β-glucoside, respectively, are reported to reach plasma concentrations of mM order, which is quite higher compared with that of polyphenols. Simulated gastrointestinal adsorption studies have indicated that indicaxanthin and betanin are absorbed passively by solvent drag through paracellular junctions of intestinal epithelial cells without any metabolic transformation and circulate as unconjugated molecules. However, in the case of betanin, its bioavailability appears to be limited by a digestive instability and

Table 1 Dietary natural pigments and their occurrence in food expressed as mg of compound per 100 g of fresh weight (FW) of food

Natural pigments	*Dietary sources*	*Concentration mg/100 g FW*
Anthocyanins		
Malvidin glycoside	Blueberry	115–148
	Red grape	2–19
Cyanidin glycoside	Blackberry	176–312
	Red grape	2–5
	Red cabbage	281–363
Petunidin glycoside	Blueberry	58–86
	Red grape	0.2–2
Peonidin glycoside	Cranberry	59–86
	Red grape	6–15
Pelargonidin glycoside	Strawberry	17–23
	Red radish	71–130
Delphinidin glycoside	Black currant	255–411
	Red grape	0.3–2
Betalains		
Betanin, isobetanin, vulgaxanthin I	Red beet	40–60
Betaxanthin	*Opuntia ficus-indica*	11–54
Betacyanin	*Opuntia ficus-indica*	5–25
Carotenoids		
Lycopene	Watermelon	5–14
	Tomato	1–13
α-Carotene	Pumpkin	0–7
	Carrot	3–6
β-Carotene	Carrot	4–21
	Pumpkin	1–15
	Spinach	3–5
	Apricot	0.5–4
	Red pepper	1–2
Lutein	Spinach	6–8
	Virgin olive oil	0.4–1
	Lettuce	1–5
	Red pepper	0–8
β-Cryptoxanthin	Red pepper	0.2–0.4
Chlorophylls		
Chlorophyll a and b	Spinach	79–125
	Green bean	5–7
	Green pea	5–13
Pheophytin a	Virgin olive oil	0.2–4

by an intestinal efflux mechanism, thus reducing the absorption by around 35% of betanin intake. Moreover, its intestinal permeation may be influenced negatively by the food matrix. Since unknown metabolites that may fit with conjugates or microbial metabolites are also detected in urine, there is the need to develop further studies on their effects on health.

Carotenoids

Carotenoids comprise more than 700 lipid-soluble, yellow-orange-red pigments with a broad distribution from bacteria to higher plants. They are found in chloroplasts of leaves and green fruits along with chlorophylls and in chromoplasts of flowers, fruits, roots, and seeds. The carotenoid backbone consists of eight isoprenoid units linked covalently to create a symmetrical polyene chain with multiple conjugated double bonds (Figure 4). Cyclization of the end groups occurs in hydrocarbon carotenes, whereas the introduction of oxygen functions gives rise to xanthophylls. Carotenoids are highly prone to chemical degradation during food processing and storage due to the effects of light, oxygen, and heat.

Sources and Production

Green vegetables, such as broccoli, spinach, and lettuce, contain lutein as their main pigment (45%), β-carotene (35–30%), violaxanthin (15%), neoxanthin (10%), and small amounts of α-carotene, β-cryptoxanthin, zeaxanthin, and antheraxanthin. Yellow and red fruits and vegetables (carrots, watermelon, potatoes, and tomatoes) have a more distinctive carotenoid distribution with high amounts of β-carotene. Tomatoes and watermelon are rich in red lycopene. Yellow and orange fruits (pumpkins, oranges, and peaches) contain primarily xanthophyll esters and β-carotene.

Vegetable oils, in particular palm oil, and some spices like red pepper (capsanthin and capsorubin) and saffron (crocin and crocetin) are good sources of carotenoids.

Animal tissues (salmon, trout, and shrimp) contain the orange-pink astaxanthin depending on their intake of phytoplankton (macro- and microalgae), which biosynthesize carotenoids. Egg yolk is also rich in lutein and zeaxanthin depending on their foraging.

Actually, few carotenoids are used as food colorants: α-, β-, and γ-carotene (E160a), bixin, norbixin, annatto (E160b), capsanthin, capsorubin, paprika (E160c), lycopene (E160d), β-apo-8-carotenal (E160e), ethyl ester of β-apo-8′-carotenal (E160f), lutein, and astaxanthin.

Some plant and seafood by-products, algae, and microorganisms are eco-friendly and low-cost sources of carotenoids. Moreover, production from algae and microalgae has many advantages, such as easier extraction with higher yields and no lack of raw materials or limited seasonal variation. The main carotenoids derived from algae are listed in Table 2.

Determination of Carotenoids

The extraction of carotenoids is generally carried out with organic solvents or supercritical fluid extractions. In recent years, in order to increase the recovery efficiency, a novel approach based on enzymatic extraction has been developed for marigold (lutein) and tomato products (lycopene). Recently, biotechnological productions or metabolic engineering of carotenoids using microorganisms and crop plant's cell cultures such as potatoes, tomatoes, rice, and maize has also been exploited.

The most common method for the analysis of carotenoids is HPLC with UV detection on a C18 or C30 reversed phase in isocratic or gradient elution mode using various mixtures of solvents (methanol, acetonitrile, and tetrahydrofuran) with the addition of butylated hydroxytoluene. Carotenoids absorb strongly between 400 and 500 nm (all-*trans* isomers), while *cis* and *cis–trans* isomers also exhibit absorption at around 320 nm. Another sensitive detection technique employed is

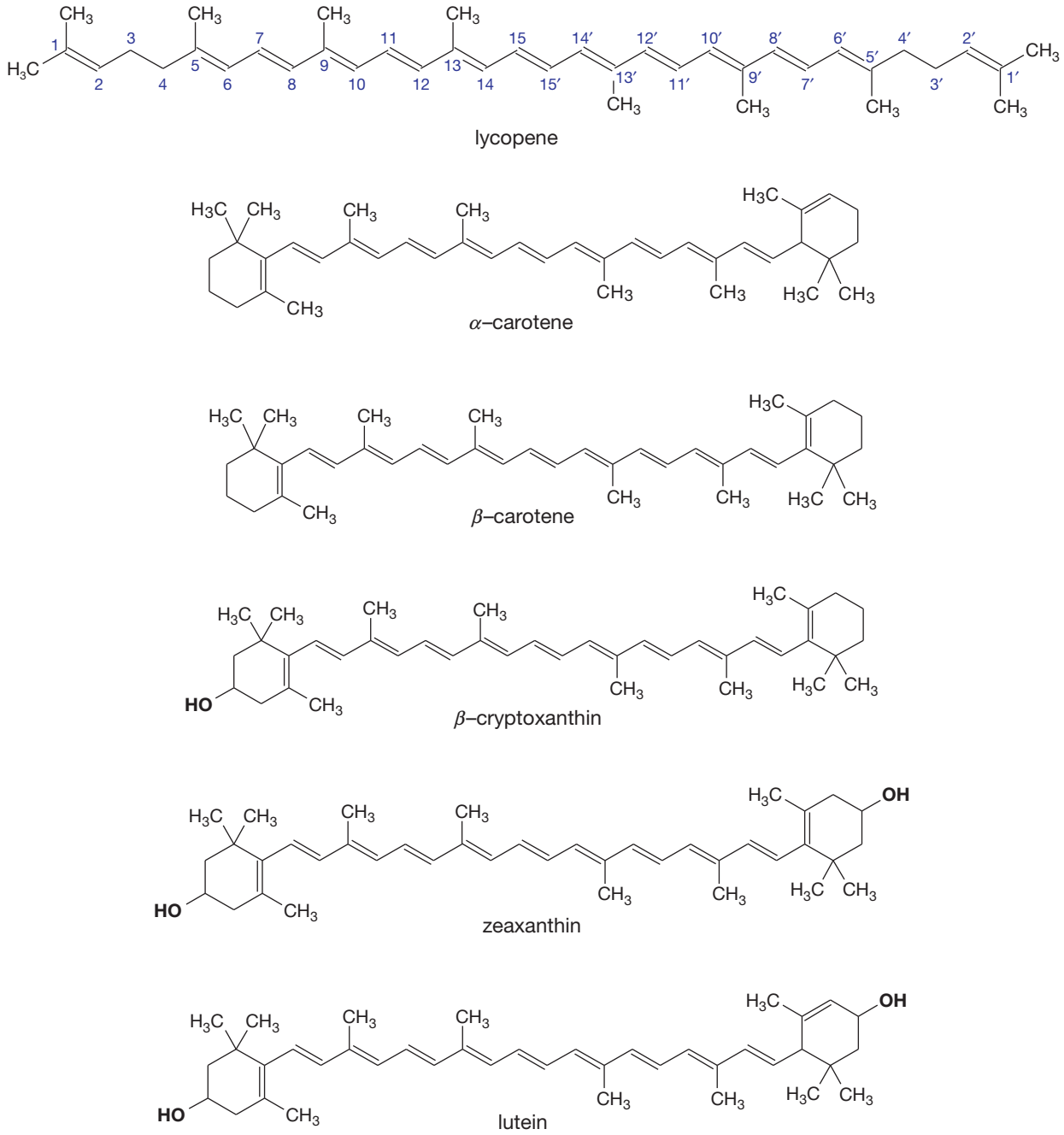


Figure 4 Chemical structure of the most common dietary carotenoids.

the atmospheric pressure chemical ionization (APCI)–MS in positive and negative mode. A very promising method for fast analysis of carotenoids is capillary electrochromatography on stationary phases similar to those of HPLC.

Patterns of Consumption

In developed countries, 80–90% of carotenoid intake comes from vegetable and fruit consumption. A European North–South gradient for the intake of some carotenoids within and among European countries has been reported since southern (Mediterranean) countries (i.e., Greece, Italy, Portugal, and Spain) consume greater amounts of fruits and vegetables than northern countries (i.e., the United Kingdom, Ireland, and Scandinavian countries).

Availability, Absorption, and Metabolism

The carotenoid bioavailability is affected by the food matrix, because fruit carotenoids seem to be more bioavailable than

Table 2 Natural pigments (mg/kg of dry weight (DW)) in seaweed

Natural pigments	*Sources*	*Concentration mg/kg DW*
Carotenoids		
β-Carotene	*Laminaria digitata*	63–336
	Fucus serratus	80–173
	Fucus vesiculosus	80–95
Fucoxanthin	*Laminaria digitata*	468
	Laminaria japonica	178–213
	Fucus serratus	495–720
Zeaxanthin and lutein	*Ascophyllum nodosum*	Up to 100
Chlorophylls		
Chlorophyll a and b	*Laminaria digitata*	1250
	Ascophyllum nodosum	565–1030

those in vegetables where they are linked to proteins. Moreover, ripening, food processing, and cooking increase their availability due to the disruption of the food matrix. Heating also induces isomerization from the *trans*-form, which is mostly found in raw vegetable materials, to the *cis* one that is easily adsorbed.

Being lipophilic compounds, carotenoid absorption takes place in the small intestine and requires the formation of mixed micelles with biliary salts. As a consequence, carotenoid bioavailability is enhanced by dietary fat, while it is decreased by soluble fiber. It is estimated that the consumption of at least 5 g of fat in a meal is required for suitable adsorption.

The chemical structure of carotenoids plays an important role in micellization. As a matter of fact, xanthophyll esters exhibit more fat solubility and are more available than free xanthophylls and apolar carotenes.

The intestinal assimilation of carotenoids seems to occur by simple or facilitated diffusion involving a membrane receptor. The bioavailability of lutein (79%) was found to be higher than that of lycopene (40%) and β-carotene (27%), and it was suggested that about 90% of β-carotene, lutein, and lycopene contained in fruits and vegetables are adsorbed. The increase of bioaccessibility of carotenoid formulations in hydrophilic matrices has also been developed.

After the intestinal uptake, carotenoids are packed into chylomicrons, which are then excreted into lymphs. Once taken up by the liver, carotenoids are exported to various tissues by lipoproteins like low density lipoproteins (LDLs) or high density lipoproteins (HDLs). In particular, carotenes are transported by LDLs, whereas xanthophylls by HDLs, so they will accumulate differentially in certain tissues depending on the receptor density for LDLs or HDLs.

Chlorophylls

Chlorophylls are green lipid-soluble pigments consisting of a substituted tetrapyrrole with coordinated magnesium in the center. The skeleton of chlorophyll derives from further esterification of the porphyrin macrocycle by phytol, a diterpene alcohol (Figure 5). Chlorophyll a and b are predominant in

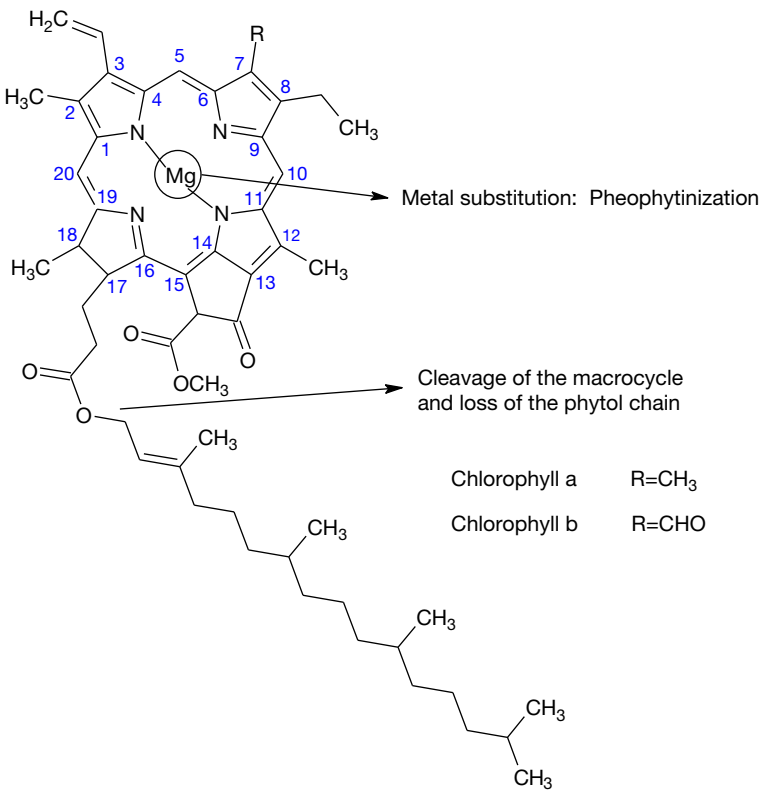


Figure 5 Chemical structure of dietary chlorophyll derivatives.

higher plants, whereas chlorophyll c, d, and e derivatives are found throughout various photosynthetic algae and diatomic species (brown, red, and yellow-green algae). Furthermore, several additional classes of bacteriochlorophylls have been isolated in photosynthetic bacteria. In plants, chlorophyll a is typically found in higher amounts than chlorophyll b by a 3-to-1 margin. The difference between chlorophyll b and a is the presence of an aldehyde residue instead of a methyl group at position 7. As a consequence, it results in chlorophyll a being a blue-green pigment with a λ_{max} of 660–665 nm and chlorophyll b being a green-yellow pigment λ_{max} of 642–652 nm. Chlorophylls occur in chloroplasts where they act as light-harvesting pigments during photosynthesis. Their distribution and content in fruits and vegetables depend on several factors including species, agroclimatic conditions, pre- and postharvest treatments, and type and degree of food processing. In fact, chlorophylls are relatively unstable and easily oxidized and converted to numerous derivatives responsible for the yellow discoloration of processed products.

Sources and Production

Commonly consumed green vegetables contain chlorophyll concentrations by up to fivefold higher than carotenoids. In some species, chlorophylls can exceed 1000–2000 mg kg^{-1} wet weight. Dietary chlorophylls include a large number of derivatives with structural differences affecting the color and the commercial and nutritive values of foodstuffs.

Fresh fruits and vegetables contain mainly chlorophylls a and b, which are both converted to olive-brown Mg-free pheophytin and yellow pyropheophytin during severe thermal processing or acidification common in canning operations. During blanching operations, chlorophyll undergoes the endogenous enzymatic cleavage of phytol forming the water-soluble chlorophyllide that can further degrade to Mg-free pheophorbide. However, in order to overcome the yellow discoloration of vegetables before thermal processing, metal-free derivatives can be converted to the stable Zn chlorophylls via the regreening process.

Chlorophyll extracts can be added as food colorants in jam, jelly, candy, ice cream, and several other products, although its application is limited because of the lability of the coordinated magnesium and the associated color change. To overcome this problem, a commercial-grade sodium copper chlorophyllin (SCC) has been developed. This is a mixture of Cu–chlorine derivatives prepared by the saponification of chlorophyll with NaOH, followed by a replacement of the Mg^{2+} atom by Cu. Since the copper complex is not absorbed by the body and is removed in its entirety as an excretion product, it is considered to be safe and is permitted to be used in most countries as the food additive E140. However, the concentration of free ionizable copper in the coloring must be kept below 200 mg kg^{-1} under current regulations.

Determination of Chlorophylls

Chlorophylls are commonly extracted from edible plants, nettle, grass or alfalfa, silkworm droppings and mulberry leaves, and, recently, marine microalgae. Traditionally, chlorophyll is extracted with organic solvents (methanol, ethanol, and acetone) under mild conditions. The chlorophyll extract is then mixed with dioxane and then precipitated through a dropwise addition of water in order to remove carotenoids. An alternative and safer technique is the supercritical fluid extraction with carbon dioxide, which is suitable for the isolation of thermolabile compounds and provides a solvent-free highly pure extract.

Chlorophyll pigment separation and identification are commonly achieved by HPLC with DAD (660 nm) and fluorescent detection (excitation 440 nm and emission 660 nm) on a C18 reversed phase using a gradient solvent system starting with methanol and ending with an ether–methanol mixture. Ammonium acetate is usually added as a suppression ion and ion-pairing reagent for the analysis of polar chlorophyll derivatives. An improved technique of detection is APCI–MS in both positive and negative modes. CZE with laser-induced fluorescence detection offers selectivity and sensitivity for the identification of the fraudulent addition of E140 to refined olive oil.

Patterns of Consumption

Compared with other food pigments, the dietary consumption of chlorophyll is really significant if we consider its natural abundance in green fruits and vegetables, the derivatives formed through food processing and cooking, and the increasing use of commercial-grade chlorophylls. In Mediterranean countries, a good source of chlorophylls is also virgin olive oil, although its chlorophyll content and profile are affected by both agronomic and technological factors, whereas, in East Asian culture, edible marine algae are an important source accounting for more than 10% of the Japanese diet with an average consumption of 1.4 kg per person per year.

Availability, Adsorption, and Metabolism

Considering that chlorophyll derivatives span a broad range of polarity from lipophilic (natural chlorophylls) to highly water-soluble (SCC) and have variable sensitivities to pH, they have different digestive behaviors and routes of absorption.

In vitro digestion models revealed that, due to the acid pH of the gastric lumen, both chlorophyll a and b are converted into pheophytins as the rate of pheophytinization is higher for chlorophyll a (97% of pheophytin a and 61% of pheophytin b). However, the type of food matrix also affects the digestive stability, and it seems that oily matrix exerts a slightly protective effect against pheophytinization. During the gastric phase, allomerization reactions involving C13 oxidation by triplet molecular oxygen can also take place, whereas de-esterification of pheophytins to give pheophorbides does not occur.

As for carotenoids, the adsorption of lipophilic chlorophylls by the small intestinal epithelium requires dispersion and solubilization into micelles for transport and incorporation through enterocytes. The bioavailability of the dephytylated chlorophyll derivatives is up 65-fold higher than that of the phytylated pigments. Where phytylated chlorophyll derivatives show passive absorption by simple diffusion, the dephytylated ones are passively absorbed by facilitated diffusion in the lower range of concentrations tested. In contrast to the lipophilic chlorophyll derivatives, the water-soluble SCC derivatives do not require association with bile

salt micelles, and the subsequent intestinal cell uptake seems to proceed predominantly by a facilitated process with absorbed SCC derivatives efficiently effluxed back to the luminal compartment.

The extent in which both natural lipophilic chlorophyll and SCC derivatives are metabolized and secreted into circulation is, however, currently unknown.

Curcumin

Curcumin is a yellow oil-soluble pigment found in the popular Indian spice turmeric deriving from the rhizomes of *Curcuma longa*. Curcumin, along with desmethoxycurcumin and bisdesmethoxycurcumin, belongs to curcuminoid, a group of polyphenolic pigments distributed not only in the *Curcuma* but also in the *Zingiber* species. Curcumin is a bis-α, β-unsaturated β-diketone and exists in at least two tautomeric forms, keto in acidic and neutral solutions and stable enol in alkaline medium. It is practically insoluble in water at acidic and neutral pH but soluble in alkali. It is stable at high temperatures and in acids but unstable in alkaline conditions and in the presence of light.

At pH 3–7, curcumin acts as an extraordinarily potent H-atom donor and it is a potent antioxidant, while above pH 8, curcumin acts mainly as an electron donor, a more typical mechanism for the scavenging activity of phenolic antioxidants. Due to the presence of two phenolic groups and one active methylene group, it also binds metals, particularly iron and copper, and can function as an iron chelator.

Sources and Production

Curcumin (diferuloylmethane) has been identified as the most bioactive constituent (2–8%) of the turmeric herb extract along with the other two curcuminoids. Curcumin is used as a food preservative and as a yellowish colorant. Turmeric has been also used in traditional medicine as a household remedy for various diseases. Nowadays, the health properties (neuroprotection and chemoprevention and cancer prevention) of curcumin and curcuminoids are gaining an ever-growing attention also in medicinal and clinical industries.

Commercial curcumin, generally extracted from the dried root of *Curcuma longa*, contains ~77% diferuloylmethane, 17% desmethoxycurcumin, and 6% bisdesmethoxycurcumin.

Determination of Curcumin

The extraction of curcuminoids is generally carried out using organic solvents. In particular, the efficiency of the solvent extraction presents the following pattern: methanol > acetonitrile > ethyl acetate. The efficient extraction of curcuminoids is obtained by also using supercritical CO_2 modified with 10% ethanol. On a massive scale, the microwave-assisted extraction technique allows the rapid and selective recovery of curcuminoids in a few minutes. Recently, there is an increased interest in the isolation of the individual curcuminoids and study of their various biological activities.

In this context, a high-speed countercurrent chromatography preparation with or without pH zoning has been developed in order to separate multigram quantities of curcumin and other curcuminoids from curcumin or turmeric powder while maintaining a high level of purity.

The main methods for the quantitative determination of curcumin include UV spectrophotometry (curcuminoids exhibit strong absorption between 420 and 430 nm in organic solvents), HPLC, and capillary electrophoresis. HPLC with diode array detection at 420 nm on C18 reversed phase in a gradient elution mode is the most widely used method without considering its limited sensitivity and low selectivity. HPLC–MS is very sensitive and has advantages over other methods in obtaining the structural information of analytes. However, it requires nebulization and vaporization steps, ionization of samples, and expensive instruments, which make this procedure unavailable in many laboratories. Therefore, a simple method with high sensitivity and low costs is still needed for the determination of these curcuminoids. HPLC coupled with electrochemical detection (ECD) is a highly sensitive and selective technique for the determination of redox substances. In recent years, the ECD method has also been reported for curcuminoid detection.

Patterns of Consumption

Curcuminoids have a restricted occurrence in nature; however, a higher consumption has been reported in Korean diets. Curry (37.24–617.98 $\mu g\,g^{-1}$), especially curry powder, mustard, candy, pickle, and snack food types, exhibits the highest curcuminoid content among various food items in Korean markets.

In Europe, curcumin is known as E100 food additive (turmeric or curcumin), which is widely applied to several food items (e.g., smoked fish, savory snacks, sauce, mustard, and seasoning) and to fabrics.

Availability, Absorption, and Metabolism

Many clinical studies have shown that curcumin has a very low bioavailability after oral assumption because of its poor solubility in aqueous media and its rapid metabolism and elimination. When orally administered, curcumin is absorbed in the gut and is present in the general blood circulation after being largely metabolized as glucuronide/sulfate conjugates. Unfortunately, whether curcumin metabolites are as active as curcumin itself remains unclear. In order to improve the bioavailability, novel delivery strategies such as lipid and polymeric nanoparticles, liposomes, and phospholipid complexes have been developed. Concomitant administration of adjuvants like piperine that interferes with glucuronidation has been undertaken.

Hemoglobin and Myoglobin

Myoglobin and hemoglobin are both hemeproteins responsible for the storage and transport of oxygen in many aerobic organisms, respectively. The characteristic red color of these proteins is due to the heme group (iron protoporphyrin IX) consisting of a porphyrin ring with a centrally located iron atom (Fe^{2+}). Myoglobin is a globular protein of about 153 amino acids (about 17 000 $g\,mol^{-1}$), holding the heme in a

protein pocket, while hemoglobin consists of four myoglobin-like subunits each containing a heme. The porphyrin ring structure held in the confines of the protein accounts for four of the six coordination sites available on the iron atom. These four bonds are with pyrrole nitrogens, while the fifth coordinates with the proximal histidine 93. A sixth site is available to reversibly bind oxygen and other small ligands. The presence of the ligand and the valence of iron dictate the pigment color of myoglobin and hemoglobin. A distal histidine 64 also influences the color dynamics by affecting space relations within the hydrophobic heme pocket.

Myoglobin is the dominant pigment in muscles and hence responsible for most lean meat color of land mammals. Therefore, four major chemical forms of myoglobin influence meat color: oxymyoglobin, deoxymyoglobin, metmyoglobin, and carboxymyoglobin.

Deoxymyoglobin occurs when no ligand is present at the sixth coordination site and the heme iron is ferrous (Fe^{2+}). This results in the purplish-red or purplish-pink color typically associated with vacuum-packaged products and muscles immediately after being cut.

Oxygenation occurs when myoglobin is exposed to oxygen and is characterized by the development of a bright cherry-red color.

Discoloration or browning results from oxidation of both ferrous myoglobin derivatives to ferric iron (Fe^{3+}) myoglobin called metmyoglobin. Reduction of metmyoglobin is crucial to meat color life and greatly depends on muscle oxygen scavenging enzymes, thus reducing enzyme systems, and the NADH pool, which is limited in postmortem muscles. Carboxymyoglobin is a relevant chemical state of myoglobin because of the current increased interest in packaging with low levels of CO. Moreover, combustion during cooking in a gas oven generates small quantities of CO or NO that bind to the myoglobin, forming stable pink compounds.

Sources and Production

The myoglobin concentration varies widely among different types of muscles, and it correlates with the oxidative capacity of the muscle cells within the species. Muscles involved in sustained repetitive actions, like breathing (diaphragm), contain higher concentrations of myoglobin than less-used muscles. The lateral line muscles of fish, which are used for sustained, low-power movement, are redder than the muscle groups used for short bursts of high-power movement. The pectoralis muscles of chickens, used for power takeoffs, are paler than the leg muscles. Moreover, the myoglobin concentration differs from species to species according to the age of the animals. The meat from older animals will be darker in color because of the myoglobin level increase with age due to the myoglobin loss of its affinity for oxygen.

Moreover, the processing and packaging conditions of meat also affect the concentration of myoglobin, thus the color of meat.

Determination of Hemoglobin and Myoglobin

The main methods for the quantitative determination of myoglobin and hemoglobin include UV spectrophotometry (myoglobin and hemoglobin exhibit absorption peaks between 400 and 600 nm) and colorimetric analysis. Colorimetric CIE (L*, a*, and b*) values are largely used to monitor color changes on meat surfaces. Moreover, size-exclusion HPLC combined with reversed-phase HPLC has been applied in order to obtain the separation of hemoglobin and myoglobin peptic hydrolysates.

See also: Authenticity of Food; Carotenoids: Occurrence, Properties and Determination; Carotenoids: Physiology; Chlorophyll; Colors: Health Effects; Colors: Properties and Determination of Synthetic Pigments; Mass Spectrometry: Principles and Instrumentation.

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Colors: Properties and Determination of Synthetic Pigments

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General Considerations

Food industry has developed rapidly in recent years by producing a large amount of processed foods for consumers. These consumers select processed foods, having little time to prepare them. The main challenge today for the food industry is to satisfy the growing consumers' demands, who want food to be attractive, to taste good, to be healthy, and to be at a reasonable price. This has led food manufacturers to develop technologies, in which there is almost no food recipe that does not contain food additives.

Synthetic pigments (SPs) are among the first listed food additives, standing out as one of the widest used additive classes for food industry. Apart from providing important properties to the finished product, such as color uniformity and stability during processing and storage, SPs are used to enhance sensory response in concordance with consumers' desire. This is leading to a state of good mood, with the desire to consume more. SPs have been introduced as food colorants once they were synthesized by chemists, at first without any suspicion of their negative health effects. Produced on industrial scale, they could be used in a variety of purposes and areas, including food processing, often without taking into account the possible threat posed to human health, especially when they are present in excess or consumed for a long period. Until a few decades ago, there was no a rigorous control on the use of these pigments in foods. Over the years, due to health side effects arising from the consumption of foods containing pigments, in the population appeared a great concern over their possible adverse effects. Inevitably, laws were issued to protect the consumer and to regulate the addition of SPs in foods. Therefore, only a short list of SPs was compiled under certain condition and limits in use; a maximum daily intake was also established, either for each SP alone or in their binary mixtures.

To check the fulfillment of the laws relating to the addition of SPs in food and to take action when problems arise, advanced analytic methods able to determine with accuracy these compounds at low concentrations are needed. This article will present an overview of properties and determination of SPs, where the term 'pigment' refers to any synthetic compound that can give color to food and nonfood.

Short History

Pigments have been known to people since prehistoric times, at that time being used to decorate the walls or to mark their passing. In the very beginning, the number of pigments was limited, because most of them were obtained only from natural sources (earth, plants, animals, etc.). Earth pigments, *yellow ochre*, *red ochre*, *white chalk*, and *black*, were the most famous ones, being easily accessible from their environment and with a very simple technology for their preparation. Only by grinding these colored earths to a fine powder and then mixing them with some animal fats, people could apply these materials on different surfaces, resulting in latter paintings.

Even around 3000 BC in Egypt, pigments were used for painting. Also mineral *malachite*, *azurite*, and *cinnabar* pigments followed by a new color obtained by mixing more natural minerals, *Egyptian blue*. The Egyptians also were able to produce insoluble pigments by mixing organic dyes extracted from plants with hydrated clay or tannin, the procedure that was called lake making. Almost at the same time, the Chinese civilization developed *vermilion* (mercury sulfide), a new bright red pigment produced by burning mercury and sulfur.

In antiquity, Greeks manufactured two new pigments based on lead: first full white opaque color, the *white lead*, and the *red lead*, which was used also in cosmetics as powder. Today, lead pigments were banned for use by the general public due to their toxic potential. Romans have benefited from the existing pigments obtained before, fortifying the palette with a very expensive pigment obtained from small mollusks, the *Tyrian purple*, affordable only by the very rich people, being also used to dye the togas of Roman emperors. In the same period of time, the cinnabar was brought from the Spanish mined and used by Romans.

During the Middle Ages, the palette of pigments was enriched by discovering innovative combination and processing for other ores and rocks. Thus, a wide range of pigments were created, such as *bone white*, *green malachite* or *green earth*, and *lazulite* (*Lapis Lazuli*). In this period, the most important blue in the Middle Ages was ultramarine and also the most expensive pigment known so far. Due to its price and having a strong blueness, ultramarine has been predominantly used to decorate sacred subjects, especially for the Virgin Mary who was graced in blue.

In the eighteenth century, the synthetic chemistry revolutionized the field of pigments. The chemically synthetic pigment *Prussian blue* (blue lake) manufactured in 1704 by Heinrich Diesbach can be considered as the first modern pigment. However, it must be also related the synthesis of picric acid in laboratory almost in the same time (1771), used as yellow dye in a silk fabric. Since then, the chemistry has spent almost three centuries and succeeded to synthesize thousands of organic pigments in the finest quality ranges, which are very much used in a variety of industries. So, early in nineteenth century, once the Industrial Revolution started, the pigment industry has flourished and new permanent pigments were produced: cobalt pigments (in blue, green, violet, yellow, and red colors), synthetic ultramarine (less expensive than genuine ultramarine, but with identical properties), chrome yellow, genuine emerald green (a copper acetoarsenite compound, potentially lethal), synthetic iron oxides (known as Mars colors), and synthetic organic pigments mauveine, discovered by Perkin in 1856, alizarin crimson, and others.

Throughout the twentieth century, continuing apace until today, many new pigments – based on the discovery of new

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chemical elements and on complex organic synthesis – were produced. An important place is taken by azo pigments, known especially by commercial names (Hansa Yellows, Winsor Yellow, Monastral Blue, Allura Red, etc.), because the scientific definition has been quite difficult to practice.

With strict reference only for foods, they were colored since ancient times by using naturally occurring pigments from vegetable and mineral sources, Egyptians, around 1500 BC being the first who used plant extracts as pigments in order to improve the appearance of candy. Three millennia ago, saffron, spice with a high nutritional value, was used to color certain foods and to cure some diseases in Greece. In early 300 BC, wine was also colored with saffron, and in the next period, many other food have improved their appearance by adding various natural pigments, like flower petals, marigold, spinach, parsley, and indigo. Starting with the twentieth century, SPs began to be produced on industrial scale and gradually started to be also used for food. At the very beginning, SPs were used without any regulation, although some of them presented a great toxic potential and were even poisonous (pigments based on lead, arsenic, and mercury complexes). As a result of such findings, a careful assessment of the SPs used in food started, their use was regulated, and only some of them were considered safe and approved for use in food. First foods for which the use of artificial pigments was authorized (yellow *pigments)* were butter and cheese in 1881 in the United States. Today, worldwide, more than ever in history, SPs are used for all kinds of food, drugs, and cosmetics. Nevertheless, as chemical substances, they are strictly regulated and monitored, being the subject of many clinical studies.

Synthetic Food Pigments Used in Food Industry

Color is the attribute most commonly used as base for the quality assessment, a natural color showing to the consumer a high-quality food product, while a pale color may indicate an inferior-quality product. For example, orange in its various forms is associated with aroma, pleasant taste, and energy. Therefore, SPs, provided by industry in a full range of colors, have gained great importance. Making food more attractive for consumers, SPs became always present components in almost every processed food, starting with fruit juices, fast food, dressings, ice cream, cakes, and so on. More than that, added color serves as a kind of code that allows to identify in the easiest way the food products such as candy flavors.

Standing out as one of the widest food additive classes, synthetic food pigments are used in food processing for many specialized applications, especially to keep or to improve the perceived color of fresh and processed foods, being known that colors are a clue to the flavor to be savored. In particular, synthetic food pigments are used not only to provide color to colorless food but also to produce 'fun' foods for children, who are thus exposed, without being aware, to the most undesirable effects of this type of food consumption.

In food processing, manufacturers are tempted to use synthetic food pigments because they are more attractive than natural ones, with a number of advantages: high stability during food processes, exposure to light or storage, more intense and uniform color, easy production by synthesis, a wide variation of colors, and convenient prices. Unfortunately, these colors were sometimes used not in a reasonable quantity, without taking into consideration the possible harmful effects on human health, in particular to most vulnerable members of the population (children). Moreover, cases have been identified when the synthetic dyes were used to disguise grave defects of the low-quality food.

Despite possible adverse effects on human health and on the environment, today, SPs are widely used in the food production and other goods, representing more than 60% of all the dyes produced annually.

Effects of Synthetic Food Pigments on Human Health

The dangers on human health through using SPs in food have been a source of controversy for decades, which continues today. On one hand, nowadays, the use of SPs by food industry has grown constantly, becoming a necessity with the increasing need for processed food. The manufacturers' opportunity to use a wider range of SPs considerably increased due to high complexity of food matrices, notwithstanding possible harmful effects on the health of humans. On the other hand, a growing concern of all consumers regarding food safety relates to the presence of the SPs in food and drinks, because these chemical compounds have been suspected to cause serious health troubles, complaints regarding their mutagenic and carcinogenic risks being also reported.

The most incriminated synthetic compounds as carcinogenic are azo pigments, due to the presence in their molecule of azo linkage, which do not occur naturally. This linkage is the most labile portion of azo dye molecule and can be easily broken enzymatically (i.e., metabolic cleavage), inducing the oxidative stress that leads to formation of free radicals in mammals, including man. Therefore, numerous toxicological and metabolic researches on animals and humans have been performed to assess the effects of food colors on human health, and almost all these studies have shown that synthetic food pigments are unhealthy substances for consumers, especially for children. Many research results show that SPs may cause mainly DNA damage, which then manifests differently. Thus, allergies and changes in hepatic and renal parameters, behavioral problems and attention disorder (particularly in children), respiratory problems (asthma), and heart troubles have been reported especially if azo dyes were present in food (Tartrazine-E102, Sunset Yellow-E110, Allura Red AC-E129, Ponceau 4R–E124, etc.). Azo dyes are also incriminated for their mutagenic potential, especially in the development of intestinal and kidney cancers. Also, there are some SPs that have been banned for food use due to toxic side effects of the breakdown products of these chemicals.

Studies on the dangerous effects of the presence of synthetic food pigments on humans are nonconclusive, and some of them are contradictory. Most toxicological studies showed that adverse health effects are observed when foods containing SPs are consumed in large quantities or when there is an extended exposure over a period of time. But effects on certain sensitive groups of children cannot be excluded.

Although a lot of issues still remain unknown, the majority of toxicological research results agree that the presence of color

additives in foods, in limited amounts, as required by law, does not present serious health hazard. On the other hand, there are highly sensitive people to the presence of unnatural food ingredients that often exhibit severe negative reactions, even in a very limited exposure to such chemicals. Therefore, it is necessary to create consumer awareness regarding the possible ill effects of these food azo dyes and, generally, to pay attention to the type and concentration of each chemical substance added to food, which is compulsory to be written clearly on the respective label.

Current Legislation

Globally, there is a growing consumers' concern surrounding food quality, the food naturalness, the ecological origin of raw materials, the processing technology, packaging, labeling, transportation, and storage. However, in recent decades, there has been an increasing demand of processed foods, and along with it is a high increase in the use of food additives, including SPs. Under the circumstances, the consumer's anxieties regarding the presence of SPs in food were stressed, and the legislation came into force to prevent their unsafe and fraudulent uses. Strict rules were imposed, and the most dangerous SPs were banned in most countries. It was established that the introduction of any SP in food must be subject to premarket approval, and only a limited number of permitted SPs were certified. For each food color, a maximum admitted limit per unit of food was established, and furthermore, limits for simultaneous presence of two or more pigments were foreseen.

There is no common legal framework valid in all countries for the use of SPs in food. In the European Union, the legislation for the use of food additives has been harmonized across all countries, the Council Directive 94/36/EC (1994) being the major law on food colors. Among other food safety regulations, in this directive, the color additives are termed as *E numbers* (from E 102 to E181), abbreviation given to their easy usage as shorter than chemical denominations. A list of permitted colors with maximum allowable levels in various food categories and of colors that have restricted application is annexed here. For example, the maximum accepted level in food for tartrazine is of 100 ppm ($\mu g\,ml^{-1}$) and for Sunset Yellow is 50 ppm, when used individually and no more than 100 ppm in binary mixtures. The purity criteria for colors must also be taken into account by law, these aspects being stipulated in Commission Directive 95/45/EC. In the United States, the Code of Federal Regulations is the body that restricted the use of color food additives in a number of seven water-soluble dyes and six insoluble lakes. Here, SPs are denoted as FD&C colors; for example, tartrazine is known as FD&C Yellow 5.

It should be mentioned that most other countries have their own rules and regulations for the use of synthetic food colors in various applications. For example, Sunset Yellow and Allura Red AC are permitted food pigments by European law, but there are European countries where these colors are banned due to their potential allergic reaction.

Moreover, the synergetic effect due to the simultaneous presence of all types of additives in food has not been investigated yet. Nor the prolonged consumption of synthetic foods containing multiple additives with disastrous consequences on the health of humans, an effect that may occur immediately or over time, was not taken in the study to date.

The establishment of a general legal framework for the use of SPs in foods to be available around the world is a difficult task, although it would be particularly useful. Also, a cumulative additive food content regulation (in terms of synergy effects) is not yet covered so far.

SP Classification and Properties

SPs are substances that absorb the light in the visible spectrum and have the ability to uniformly impart color to a food or nonfood due to the presence in their molecule of at least one chromophore group and of conjugated system with double and single bonds. They are liquid or powdered chemical substances, many of which are petroleum by-products. For thousands of pigments produced through chemical synthesis, in literature, there are reported several types of classification, taking into account various criteria.

As chemical compounds, SPs may be inorganic or organic compounds. These two categories can be further subdivided into other classes. Thus, organic SPs, as the main topic of this work, can be classified in four main classes, according to their chemical structure: azo, nonazo, fluorescent, and miscellaneous pigments. SPs can be also divided into 'lakes' or 'dyes.' But the most common classification system for SPs is drawn according to their use: artwork and industrial paints, architectural, automotives, textiles, cosmetics, printing inks, plastics, pharmaceuticals, foods, etc.

Synthetic organic pigments display a wide variety of physical and chemical properties such as color, light and heat stability, solubility in water or organic solvents, and reactivity; depending on the class they belong to, but generally, SPs within a chemical class display similar properties. However, in the same class, there is the possibility to create some differentiations intentionally through fine synthesis for quality improvement, especially for artwork.

Synthetic Food Pigments

Synthetic food pigments, also called dyestuffs, have the capacity to give color to the food in a stronger and longer-lasting manner than natural pigments. They can be easily identified in food in several ways. Each pigment is listed at international level with five-digit C.I., according to Color Index Generic Numbers, and at European level, through 94/36/EC Directive, by an *E number*, which has the correspondent FD&C colors in the United States. Synthetic food pigments are also represented by their meaningful commercial names, such as Patent Blue V and Indigo carmine.

Synthetic food pigments could be subdivided into two major classes: azo food pigments and nonazo food pigments, but often, they are categorized by their predominant shade, such as pigments yellow, red, or blue.

Azo food pigments

Azo pigments are synthetic compounds that contain in their molecular structure one or more azo groups (—N═N—), being the oldest and most used pigments in food. The base

for the development of azo pigments was the discovery of diazo reaction in the laboratory of Peter Griess in 1858, when the first azo compound was produced, and very rapidly after these, organic chemists have discovered new azo pigments.

The most representative food monoazo pigments permitted by the EU are the following: *tartrazine* E102, C.I. 19140; *Sunset Yellow* E110, C.I. 15985; *carmoisine* E122, C.I. 14720, *amaranth* E123, C.I. 16185, *Ponceau 4R* E124, C.I. 16255; *Red 2G* E128, C.I. 18050; and *Allura Red AC* E129, C.I. 16035 (Figure 1).

Azo pigments are purely of anthropogenic origin, being generally obtained through a relatively simple organic synthesis carried out in two steps, when two organic compounds are required for the coupling and azo linkage formation, respectively, the diazotization of a primary aromatic amine is followed by coupling. Because they have a long scientific name, in most of the cases, azo pigments are known by their market name. For example, the azo pigment 3-carboxy-5-hydroxy-1-(4′-sulphophenyl)-4-(4′-sulfophenylazo) pyrazole trisodium salt is known as tartrazine, disodium 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonate is Sunset Yellow, disodium 6-hydroxy-5-((2-methoxy-5-methyl-4-sulfophenyl) azo)-2-naphthalenesulfonate is Allura Red AC, and so on.

Class of nonazo food pigments allowed in the European Union (EU) includes four main groups: the triarylmethanes (with *Patent Blue V* E131, C.I. 42051; *Brilliant Blue* FCF E133, C. I. 42090; and Green S E142, C.I. 44090), the chinophthalon derivative (*Quinoline Yellow* E 104, C.I. 47005), the xanthenes (*Erythrosine* E127, C.I. 45430), and the indigo colorants (*Indigo Carmine* E132, 73015). But the representative class for food colorants is azo food pigments, and they are currently the market leaders.

Synthetic food pigments can be used either alone or in binary mixtures for obtaining a large spectrum of colors, such as *tartrazine* and *Sunset Yellow*, *tartrazine* and *Erythrosine*, and *Allura Red AC* and *Ponceau 4R*. Usually, manufacturers choose a pigment that is very effective, or they choose a pigment as a base for other color combinations, as appropriate for the type of food product.

Synthetic Food Pigment Determination

Potential health hazards due to the presence of SPs in food have attracted considerable attention, and much effort has been devoted to the development of methods for their detection and analysis. These issues have become very topical in terms of current legislative constraints, the literature reporting an impressive number of papers dealing with the determination methods for synthetic food pigments. From the outset, it must be said that the determination of SPs is often a difficult task not only because they are complex chemical compounds of relatively low concentration in food but also because they are in a very complex food matrix sample. The analysis becomes more difficult, or even impossible, for those synthetic

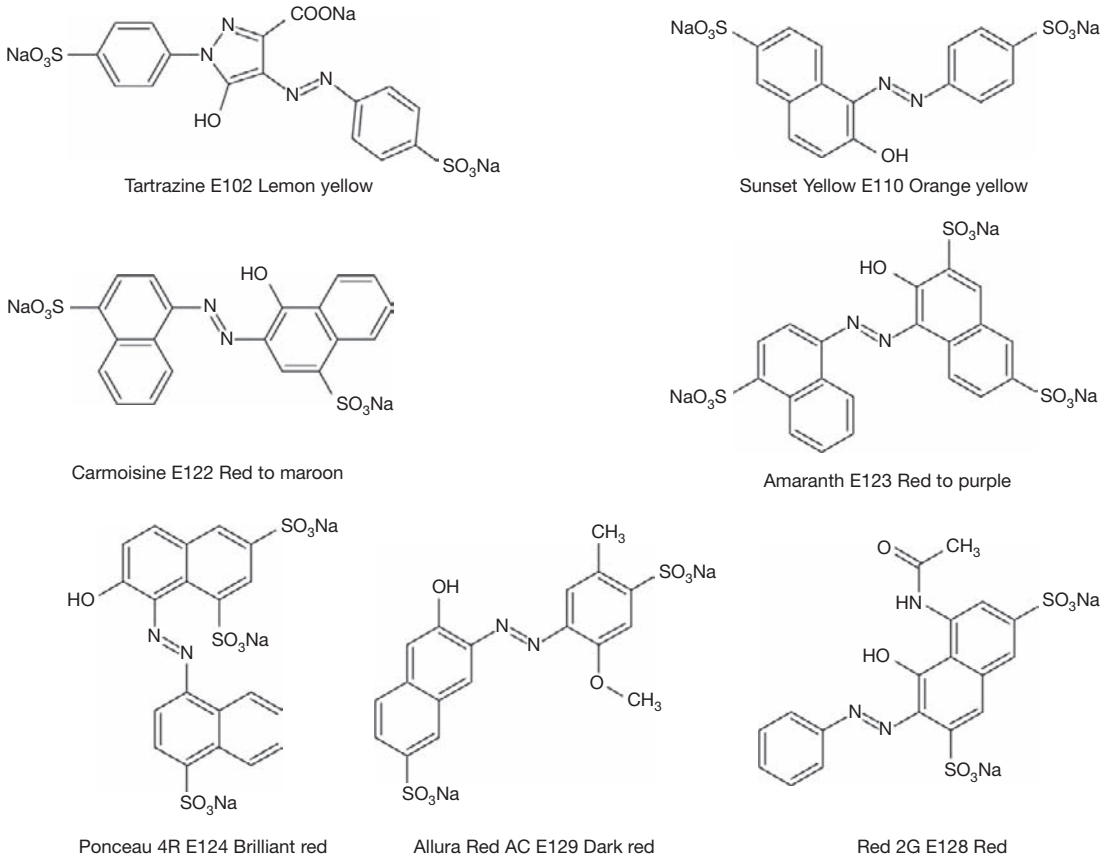

Figure 1 Chemical structures and predominate color for seven most important food azo pigments.

organic pigments that differ only in terms of structure, with a different position of their substituents on the benzene ring. Nevertheless, a variety of analytic techniques for the identification and determination of SPs have been developed, traditional analytic techniques and most advanced ones being reported in the literature. In the following, the most commonly used of these methods in SPs analysis will be briefly reviewed.

Spectral Methods

Spectral methods were the first methods used in SP examination; the analysis of pigments from tube paints was and is commonly performed by ultraviolet–visible spectrometry. Due to the possible presence in the same sample of the natural pigments and of other numerous interferences, absorption molecular spectrometry, in its classical form, was applied mainly to individual pigment analysis and had a relatively small number of applications for analyzing mixtures of SPs. Thereafter, this method has supported numerous improvements in terms of selectivity, especially by the application of chemometrics, and simultaneous analysis of two or more SPs has been reported. From the beginning, infrared spectroscopy had a bigger potential to be used in the analysis of SPs, dry or in solution, although the presence of fillers and extenders as main components in sample matrix makes analysis particularly difficult. Therefore, more important results have been reported after improving sensitivity of IR method by using, as sample, the extracts of SPs. Undoubtedly, the most important achievement in the field was the development of new FTIR technique, by which nearly all SPs could be identified against the spectra of pure compounds that have a characteristic molecular *fingerprint*. Further studies have recommended Raman spectroscopy as a good technique when analyzing synthetic organic pigments from artworks, either individually or in mixtures (up to twenty-one dry azo pigments can be analyzed simultaneously). Compared with FTIR, Raman spectroscopy has the advantage of sharper peaks that do not overlap each other, making much easier the identification of each pigment from a complex sample. However, FTIR and Raman spectroscopies are complementary techniques, together succeeding to overcome almost any problem that may arise in the pigment analysis.

Spectral methods described in the preceding text have reported just few applications in the determination of SPs in real food samples, which include especially the analyses in soft drinks. The reason for this is the high complexity of food samples; almost always, an additional extractive step in sample preparation is needed in order to avoid the spectral overlap, and therefore, more time for analysis is required. New achievements in the simultaneous determination of SPs, such as Sunset Yellow, tartrazine, and Allura Red AC, in soft drink powders have been recently reported by the use of derivative signals of the ratio spectra and the involvement of chemometrics. The obtained linear response covers tens of ppm ($\mu g\ ml^{-1}$) starting with 2 $\mu g\ ml^{-1}$ up to 60 $\mu g\ ml^{-1}$, so the spectral methods may be used for SP control in soft drinks.

Electrochemical Methods

Many SPs possessing electroactive properties have led researchers to develop electrochemical methods for their determination, especially for azo pigments. Differential pulse voltammetry (DPV) and cyclic voltammetry, using different working electrodes, are the most common electrochemical techniques used for the investigation of SPs. Depending on the respective pigment, voltammograms show specific anodic or cathodic peaks that allow the identification and quantification, the peak current being in directly proportionality to the pigment concentration. Figure 2 presents the DPV curves for tartrazine at different concentrations ($mmol\ l^{-1}$) on glassy carbon electrode at pH = 3, where linear dependence of anodic and cathodic peaks with the tartrazine concentration is observed in the upper inset.

Numerous studies revealed that voltammetric methods are selective and sensitive techniques, with very good detection limits of 0.80 $mmol\ l^{-1}$ for common azo pigments. Also, voltammetric techniques have proved to be not only powerful

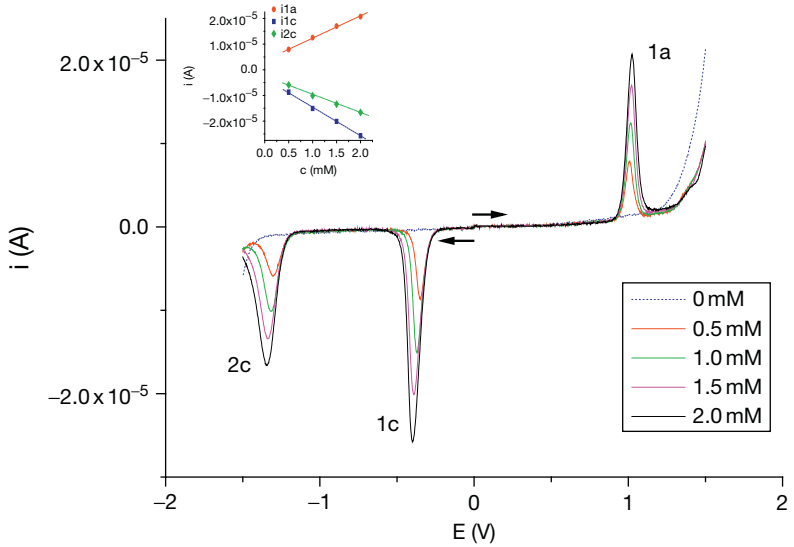

Figure 2 DPV curves for tartrazine; upper inset – dependences of anodic (i1a) and cathodic peaks currents (i1c) on tartrazine concentration (c).

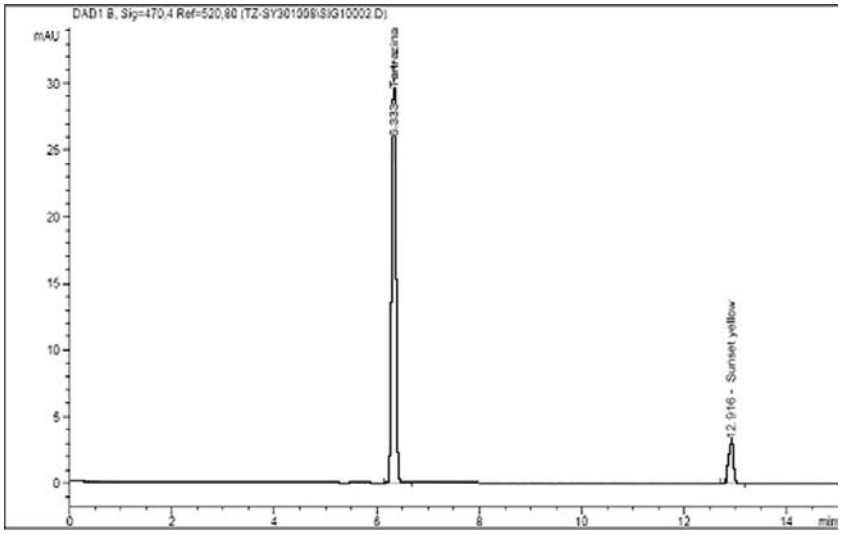

Figure 3 Chromatogram for an orange juice sample on Hypersil 5 μ MOS C_8 column. HPLC system *Agilent Series 1100*-DAD detection.

tools in the SP investigation that offer analytic options for their analysis but also good way to determine the degradation products because they are able to establish certain degradation processes that are likely. Moreover, they can make distinction between synthetic and natural pigments of the same color because the latter are not electroactive (do not have azo group in their molecule).

Although voltammetric methods are simple, sensitive, and less expensive than spectrometric ones, they do not have a broad use in the control of SPs in food, mainly due the same reasons as mentioned in the preceding text. However, the latest breakthroughs in the use of new materials for electrodes are a promising outlook in simultaneous determination of SP in food, particularly in beverages, and these methods are a hopeful alternative to classical approaches to the analysis of SPs.

Chromatographic Methods

Compared with other analytic methods used in the analysis of SPs in food, chromatographic methods are by far the most attractive for achieving the requested performance criteria, either under research requirements or under routine analysis. These methods offer the possibility to analyze SPs with high accuracy and precision, with a very good detection limit ($\mu g\ ml^{-1}$), without a prior step of sample preparation. In order to detect the presence and content of SPs in foodstuff, many separation techniques have been developed and applied, such as thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC) capillary electrophoresis, gas chromatography, high-performance liquid chromatography (HPLC), including ion chromatography and ion-pair chromatography, reporting valuable results. Among these methods, TLC and HPLC have been widely used for food pigment analysis in various food samples, such as fruit-flavored drinks, alcoholic drinks, fruit yogurts, jams, sweets, sugar confectionery, spice, and brightly colored foods. Mainly, TLC aimed to develop rapid methods for the separation and identification of complex mixtures of pigments. This method, with new improved version of reversed-phase HPTLC, has been successfully applied in the analysis for mixture up to 9 pigments on the same chromatographic plate. Recently, HPTLC has been used in the investigation of chili, paprika, and curry powders for the presence of illegal pigments, such as Sudan I–IV, Sudan Red B, Sudan Red G, Para Red, Butter Yellow, and Toluidine Red. All these pigments are classified as carcinogenic substances and are unauthorized substances in foodstuffs under the EU Food Regulations. Reversed-phase HPLC method with diode-array detection in the isocratic elution mode became one of the most valuable approaches for simultaneously determination of complex mixtures of SPs from food matrices and only requires a simple pretreatment of the sample. This can consists in a solvent extraction or in a filtration and sonication. A chromatogram for tartrazine and Sunset Yellow determination in an orange soft drink sample is presented in Figure 3.

HPLC method has reached today a new level of performance by further advances in instrumentation and column technology, this method being known as ultrahigh-performance liquid chromatography (UHPLC). This chromatographic technique brought significant increases in speed, with a very good resolution and sensitivity, UHPLC technology having the capacity to solve almost every problem raised in food pigment analysis.

In this brief overview of methods for determining the SPs, a special role is played by analytic methods based on liquid chromatography coupled with mass spectrometry, considered as unrivaled techniques in the identification and quantification of SPs in food. Performance criteria of these coupled methods, especially the selectivity, provide unambiguous identification and accurate determination of SPs in complex matrices at trace levels, without the need of laborious pretreatment procedures.

Conclusions

Dangers to human health caused by synthetic food pigments are in the spotlight, food safety experts raising numerous

concerns regarding the use of SPs as food colorants. Consequently, the European authorities have established the legal framework for reevaluation of azo pigments as food additives. The aspects raised in this article have highlighted few issues regarding safety and quality of food and drinks that are of high priority worldwide:

1. The need to reduce as much as possible the human health risks due to the presence of chemical ingredients in food by using natural ones. More and more people have become increasingly aware of the dangers of foods that contain artificial pigments, and such foods became less popular, polls indicating global preference for natural food colors.
2. The requirement to test and evaluate the safety of any SP before being used in food with the strict revision of already permitted SPs in food and the law enforcement for taking action if problems are found.
3. The necessity to review the food additive science by running projects on the subject and to develop advanced analytic techniques for the presence of the colors additives in food. It is unanimously recognized that determination of synthetic organic pigments is not easy to perform because of their high coloring power and of similarity in the structure; therefore, there is a critical demand for new development of analytic procedures addressed to improve the determination of SPs in food. Thus, an accurate assessment of SPs in foods can ensure the compliance of legal requirements, in many countries, synthetic food colors being banned in the interest of public health. However, it should be understood that actually, food technology passes through colors, but at the same time, it must go to people's health. Therefore, it is necessary to try to make sense of SPs by setting universal standards.

See also: Allergies: Public health; Amaranth; Butter: Properties and Analysis; Cancer: Diet in Cancer Prevention; Carcinogenic: Carcinogenic Substances in Food; Colors: Health Effects; Colors: Properties and Determination of Natural Pigments; Consumer Protection Legislation; Food Additives: Classification, Uses and Regulation; Food Allergies: Occurrence and Analysis; Food Allergies; Mutagens; Quality Control in Food Processing; Storage Stability: Mechanisms of Degradation.

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Condensed Milk

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Introduction

Since ancient times, people have been aware that milk could only be kept fresh for a short while and that it was only available in the immediate vicinity of a cow. In the thirteenth century, Marco Polo reported that the Tatars were able to condense milk. He stated that 10 pounds (4.5 kg) of milk paste was carried by each man, who would subsequently mix the product with water. However, this report probably refers to the soft Tatar curd *katyk*, which can be made into a drink (*ayran*) by diluting it, and therefore it refers to a fermented, not fresh, milk concentrate. Nicolas Appert condensed milk in France in 1820, and Gail Borden Jr. did the same in the United States in 1853, in reaction to the difficulty of storing fresh milk for more than a few hours. While returning from a trip to England in 1851, Borden was devastated by the death of several of his children, apparently from poor quality milk obtained from shipboard cows. Borden was inspired by the vacuum pan he had seen being used by Shakers to condense fruit juice, and he was at last able to reduce milk without scorching or curdling it. Even then, his first two factories failed, and only the third, built in New York with his new partner, Jeremiah Milbank, produced a usable milk derivative that was long-lasting and needed no refrigeration.

The first person to preserve milk in a concentrated form was Nicolas Appert, who, in the early nineteenth century, concentrated milk by boiling it in a water bath over a fire, then pouring it into glass bottles after cooling, and sterilizing the final product by heating the bottles for 2 h in a boiling water bath. Two inventions made in the second half of the nineteenth century resulted, essentially, in the process that is still used today. Gail Borden (1856) patented the process to evaporate milk, which uses low pressure to make sweetened condensed milk. In 1884, John B. Meyenberg patented a process for sterilizing concentrated milk in tinned cans, which were rotated under pressurized steam, allowing for a relatively short sterilization time. A brief account of the historical developments of condensed milk manufacturing is conveyed in Table 1.

Condensed Milk

Condensed dairy products are value-added milk products with extended shelf life. Fresh milk is clarified and standardized to a suitable level of fat, and it is then heat treated at 85–90 °C for several seconds. This heating process acts as a hurdle, which destroys the majority of microorganisms. It also decreases fat separation and inhibits oxidation. The water content of the milk is reduced due to evaporation. Condensed milk products have several advantages over fresh milk, such as they require less storage space, they retain high quality, they preserve milk's valuable surplus nutrients, and they reduce transportation costs. These products can be used under adverse conditions such as wars, epidemics, or earthquakes or other natural disasters when fresh milk is unavailable. They are also suitable for specialty food products designed for people such as sportsmen, convalescents, or older individuals.

Evaporated milk (EM) is sterilized unsweetened condensed milk, and this product is available commercially. It is prepared from fresh milk from which a sizable (almost half) amount of water is evaporated. Then the product is homogenized and canned, and the cans are subjected to the thermal process of sterilization (Figure 1). In the event of limited availability of fresh milk, milk powder is used in part or all of the solids-not-fat (SNF). Similarly, the fat portion can be obtained from anhydrous milk fat (AMF). If the product is prepared using only milk powder and AMF, it is called *recombined evaporated milk* (REM). In addition to milk, skim milk, and cream, sweet cream buttermilk is also frequently used as a source of some of the milk solids. Because sweet cream buttermilk contains a large portion of the fat globule membrane lipids in milk, it improves the heat stability and the flavor of products, particularly REM. The properties of REM resemble those of EM.

There is no provision under the Codex Alimentarius standards to distinguish a product made from milk powders and AMF from that made from fresh milk. But legislation in some nations has set limits on the use of powders (e.g., EU legislation requires that no more than 25% of the milk solids in regular unsweetened condensed milk are derived from milk powder). There is an emerging trend in some countries to prepare EM using vegetable fats (most frequently, palm oil) instead of milk fats; this trend could be due to an attempt to reduce costs or simply a local preference to not use milk fats. The product thus prepared is called 'filled' EM. Oils high in polyunsaturated fatty acids, such as soybean or sunflower oil, are used in food products to lower blood cholesterol. The oxidation stability of the fats used in REM is of paramount importance.

To improve milk's heat stability during thermal processing, sodium orthophosphate is added. In addition, calcium carbonate, soy lecithin, or calcium chloride is added to help improve milk's heat stability. Polyphosphates are also added to ultra-high-temperature (UHT)-sterilized evaporated milk to control defects such as age gelation of the product. The Codex sets a maximum limit for an addition of 0.2% on an individual basis, or 0.3% if a combination is used.

When the liquid product is mixed with a proportionate amount of water, EM becomes the rough equivalent of fresh milk. However, it requires half the space of its nutritional equivalent (fresh milk), which makes EM attractive for transportation purposes as it can have a shelf life of months or even years, depending on the fat and sugar content. EM is a safe and

Table 1 Historical developments in condensed milk manufacture

Year	*Developments in condensed milk manufacturing*
1357	Marco Polo (Mongolia) described the method for preparing a pasty milk concentrate, which was mixed with water
1809	Nicolas Appert (France) described a method for preserving milk by condensing it
1856	Gail Borden Jr. commercially developed a method of condensing milk; he is known as 'the Father of the process of milk condensing'
1858	Borden's milk, sold under the Eagle Brand, gained popularity for its purity, durability, and affordability
1864	Borden's New York Condensed Milk Co. constructed the New York Milk Condensery, the largest and most advanced condensery, in Brewster, New York
1883	The vacuum pan was markedly improved due to advancements in scientific knowledge in the field of sterilization
1884	John B. Meyenbarg (Switzerland) experimented to create the process to sterilize condensed milk using steam under pressure. He conceived the idea of making condensed milk without the addition of sugar or other preservatives and without storing it under refrigeration
1906	The Federal Food & Drug Act of 1906 changed the name 'evaporated cream' to 'evaporated milk'
1911	Nestlé constructed the world's largest condensed milk plant in Dennington, Victoria, Australia
1914	Professor O. F. Hunziker, Head of the Department of Dairy at Purdue University published a book titled *Condensed Milk and Milk Powder*. Professor Hunziker and others with the American Dairy Science Association standardized and improved condensery operations in the United States and other countries
1958	Plate-type evaporators were developed
1961	Anand Milk Producer's Union Ltd. (AMUL), in India, started the first ever commercial production of sweetened condensed milk in that country

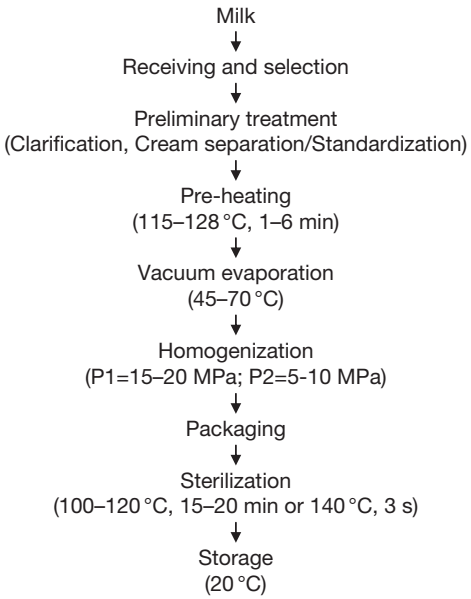

Figure 1 Flow diagram for the preparation of condensed milk.

reliable substitute for perishable fresh milk, and it was very popular before the invention of refrigeration. It can be easily shipped to locations devoid of resources for safely producing or storaging milk. Today, households across the world use it most often to prepare desserts and in baked goods due to its characteristic flavor.

The Codex Alimentarius standards, which are the most common in international trade, prescribe a minimum of 7.5% milk fat and 25% total milk solids (TMS) in EM. Similarly, legislation in various nations has set limits for the composition of EM. According to the traditional British standard, EM requires a minimum of 9% milk fat and a minimum of 31% TMS, while the U.S. standard prescribes a minimum of 7.9% milk fat and 25.9% TMS. Thus, the concentration of EM ranges from 2.0 to 2.5. The Codex allows the protein content in the SNF to be adjusted, with a minimum of 34%, by adding lactose, milk permeate, or milk retentate. However, additions that change the ratio of casein-to-whey protein are not allowed, with the local legislations in some countries still forbidding standardization of the protein.

Method of Evaporated Milk Preparation

The method to manufacture sterilized and UHT-sterilized EM is shown in Figure 1. The major processing steps are discussed in the following sections.

Preheating

Fresh milk is heat treated before it is concentrated. This treatment is mainly to increase the concentrated milk's heat stability. The homogenized full-cream EM cannot be sterilized if the milk is not subjected to preheating. Generally, milk is preheated at 110–130 °C for about 1–3 min in a continuous flow to yield EM with the highest heat stability. Preheating also acts as an important hurdle for microbial growth as it inactivates microbial spores. For REM, preheating is carried out by the powder manufacturer, before concentration and drying. This allows the powder to be reconstituted at the predetermined concentration for REM.

Concentration of milk

Modern multistage falling film evaporators with energy efficient designs are invariably used for milk concentration. Overconcentration should be avoided to prevent the risk of lowering the milk's heat stability. Similarly, overconcentration also requires higher energy use and lowers the production capacity. Based on the continuous measurement of the refractive index or density of the concentrate, the total solids (TS) content is adjusted at the point where the product leaves the evaporator. The final standardization of fat and TS is usually carried out between the concentrating and sterilizing processes. The milk, which is concentrated by reverse osmosis (RO), can also be used to prepare unsweetened condensed milk. EM produced by RO has virtually the same composition and properties as that produced by evaporation, but the industrial application of RO for this purpose appears to be very limited.

Homogenization

The milk is homogenized as this process prevents coalescence of fat globules and reduces the rate of creaming while the product is stored. The homogenization pressures up to about 5 MPa generally have little effect on or slightly increase heat stability, but higher pressures result in a large decrease in heat stability. The concentrated milk is homogenized immediately after it leaves the evaporator. A higher homogenization temperature results in a smaller fat globule size at the same pressure with better heat stability.

Heat stability test

During preparation of EM, it is obvious that the heat stability of the product varies from batch to batch. Stabilizers are added to regulate the heat stability. The amount of stabilizer needed also varies to a great extent. The quantity of stabilizers to be added is determined by adding different amounts of the stabilizers to a series of cans containing the product. The cans are subsequently sterilized, and the contents are checked for heat stability. The amount of stabilizer that results in the optimum properties is added to the total batch.

Cooling and cold storage

After homogenization, EM is cooled and stored, and then a predetermined quantity of stabilizer (dissolved in water) is added; this quantity is determined by the results of the stability test. At this stage, prolonged storage should be avoided to prevent microbial growth. Final standardization of fat and TS can be carried out at this stage, taking the amount of water that is added with the stabilizer into account. The cold storage of unsterilized EM for more than 24 h considerably increases the risk of the sterilized product's age gelation.

EM is expected to be stored for longer period, which requires various conditions so that the product's quality does not deteriorate during storage. The storage temperature is one major factor determining the shelf life of the product. Condensed milk and EM are stored at about 10–15 °C. Storage at low temperatures such as 0 °C or below may lead to sugar separation in the condensed milk, leading to sandiness; this sugar separation is caused by the formation of very large lactose crystals. A very low storage temperature also increases the viscosity of the product, which may be beneficial up to a certain point, but it also affects the product's body and texture characteristics. It has been shown that commercial EM remains acceptable even after 2 years when stored below 15 °C, but it deteriorates rapidly when stored at 21 °C or above. The humidity of the storage space should also be kept low (below 50%) to check the spoilage of cans and labels. Inversion of the cans during storage minimizes fat separation in EM.

Packaging

One of the characteristic features of EM is its long shelf life at ambient temperatures; this feature places high demands on the packaging materials. The packaging materials should meet the criteria of mechanical resistance and permeability to water, light, gasses, and hydrophobic components. Those parts that are in direct contact with the product must be made of food-grade materials. The can is the most widely used container for EM, with a standard content of 170 or 411 g (6 or 14.5 oz, respectively). Mechanically sealed cans are widely used for the packaging of concentrated milk products. Modern tinned plate is coated with a layer of a polymer to prevent the dissolution of the tin and iron into the product. In some Western European countries, glass bottles with twist-off caps are used to package EM used as a coffee creamer. Aluminum foil-lined milk cartons and single-portion cups made of aluminum or polystyrene are also widely used. The EM packaged in translucent (white) polystyrene single-portion cups usually develops an oxidized flavor due to the permeability of this material to both oxygen and light.

Sterilization

Sterilization not only kills all microorganisms but also inactivates all microbial spores that may germinate during the storage of EM. The native milk enzymes are inactivated during preheating. While selecting the milk to prepare as concentrated milks, its excellent microbiological quality should be noted. The enzymes produced by microorganisms (especially psychrotrophs) should be absent because they are thermostable, and even sterilization is insufficient to inactivate them. Heating is carried out in continuous sterilizers. Several machines have been designed to fill cans with concentrated milk. In a commonly used system, known as 'vent hole type,' concentrated milk flows through a small aperture (3 mm or 1/8 in. diameter) into cans. After filling, the cans are sealed carefully because the seal has to withstand the heat of the sterilization process. It is also essential that the cans be filled as quickly as possible to avoid contaminating the milk. The opening in the cans is usually soldered by a mechanical finger. The filled and sealed cans are then tested for leaks by plunging them into a hot water tank. If any of the cans rise or give off air bubbles, they are discarded as unfit for sterilization. Modern sealing processes are carried out so rapidly that a large number of cans may be sealed in 1 min. In this process, open or sanitary-type cans are used. The concentrated milk fills the open can, one end of which is already seamed, and, after filling, the other end of the can is seamed by an automatic seaming machine. Cans are sterilized in a horizontal rotating system with rotary air locks, while the bottles are sterilized in hydrostatic sterilizers. These are highly energy-efficient systems because they have considerable heat regeneration.

Defects in Evaporated Milk

Condensed milk and EM are products with a prolonged shelf life. They have typical body, texture, and sensory properties by which they are identified. These properties must be such that the product is fit for sale immediately after production and does not alter during a reasonable period of storage. The product should be physically, chemically, and microbiologically fit for human consumption until the end of the storage period. Routine examination of the product soon after its manufacture as well as during its storage may be carried out to judge the quality of the product. If a defect is noted, care should be taken to eliminate the defect in subsequent batches. Therefore, it is important to know the expected defects, the probable reasons for their occurrence, and the preventive measures used to avoid these defects. The defects that may occur in condensed milk are classified as microbial defects and nonmicrobial defects.

Microbial defects

For all sterilized milk products, there is continuous risk of postprocessing contamination. It may be through microleaks in the cans or during the filling process. The growth of *Bacillus stearothermophilus*, a thermophilic spore-forming bacteria, in an evaporator is one of the most important causes of product defects. Prolonged running time of the evaporator or improper cleaning of the equipment may lead to an appreciable spore count in the concentrated milk. Inactivation of these spores during sterilization is limited, and the residual spores may germinate and grow if the product is sold in tropical countries, where storage temperatures may exceed 40 °C. A brief account of microbial defects follows.

Gassy fermentation/bloats: Gas can form in cans and barrels of condensed milk, causing them to bulge or burst. Gas-producing yeasts are the cause of many types of gaseous fermentations. The source of the contamination may be the raw milk, inferior quality sugar, or unhygienic factory conditions (e.g., improperly cleaned and sanitized equipment and filling machines). To avoid this microbiological defect, good quality raw milk and a proper preheating temperature should be used. In addition, only good quality sugar without any yeast contamination should be used. Proper sanitary conditions should be maintained during the manufacture and packaging of condensed milk. The containers should be filled fully, with little space for air or oxygen.

Bacterial thickening: Condensed milk thickens progressively during storage because of microorganisms, which produce rennet-like enzymes. These organisms are easily destroyed during the preheating process. An optimum sugar ratio also inhibits the growth of the microorganisms. In addition, low-temperature storage helps to reduce the bacterial thickening.

Mold buttons: This defect occurs during storage due to mold contamination. Small reddish-brown pieces of curd about ¼ in. to ¾ in. in diameter form on the product's surface, causing localized coagulation. These pieces of curd are caused by the mold *Aspergillus repeno*, which produces a rennet-like enzyme that causes localized clotting. High-temperature storage promotes the growth of the organism. The milk may be infected with the organism during the concentration process. To avoid this defect, scrupulous cleaning and care of dairy equipment is essential. Because this organism does not grow at low temperatures, storing condensed milk at a lower temperature will prevent this defect.

Nonmicrobial defects

These defects are of chemical or physical origin, and they are briefly described in the following paragraphs.

Sandiness: Good quality condensed milk should possess a smooth homogenous texture and be pleasant to the palate. Sometimes, however, the milk may be gritty and contain a large number of large lactose crystals. The solid particles are so large that the product lacks smoothness. This defect is readily detected by an average consumer. *Sandy*, *rough*, *grainy*, *granular*, and *gritty* are the terms used to describe this defect. If an excess amount of sugar is used to manufacture condensed milk, the sugar particles may also crystallize out and cause sandiness. To check this defect, condensed milk must be cooled so that a large number of minute sugar crystals are formed, which results in a smooth texture. An optimum storage temperature is also essential to avoid this defect. High viscosity also delays crystal formation; therefore, it is necessary to rapidly cool the condensed milk in the initial stages. Correct cooling and induced rapid crystallization with the correct amount of seed lactose will help to avoid this defect.

Age thickening: Thickening of the condensed milk is the most common defect seen in sweetened condensed milk. This defect varies markedly in its intensity from slight gelation to a firm and thick consistency. The defect becomes progressively more intense with storage, especially at room temperature or above. The preheating temperature of the milk and the degree to which the milk is concentrated have been observed to have profound effects on age thickening. With high packaging and heating temperatures, there is a greater tendency for the product to thicken early. With increasing concentration of milk solids, the thickening tendency becomes more marked. To avoid early thickening of condensed milk, an optimum preheating temperature should be maintained. Sugar should only be added at the end of the milk condensing process, and the product should preferably be stored at a temperature below 15 °C. With the addition of the proper type of stabilizers, age thickening may be decreased to a great extent.

Brown color: Usually brown color discoloration is associated with age thickening, both of which become progressively more intense with storage. The acidity and storage temperature determine the rapidity of this change. This defect may be avoided by storing the condensed milk at reasonably low temperatures.

Other defects in condensed milk include some flavor defects such as rancid, tallowy, and metallic. Following good manufacturing practices; using good quality raw materials, good quality milk, and good packaging; and storing the product at a low temperature may help to avoid these defects.

Uses of Condensed Milk

Milk is converted into EM to preserve its nutrients, which should not perish during months or even years of storage without refrigeration, and EM can be easily transported over long distances. For countries with low milk production, especially in the tropics, EM is a general-use milk product. In other markets, EM is used for specific purposes such as in coffee and tea or for cooking. As fluid milk, EM is consumed after 1:1 dilution with boiled water. This yields a product with a slightly higher SNF content and fat:SNF ratio than those of the regular 3.5% fat full-cream milk. Some consumers prefer the flavor of sterilized milk to that of pasteurized or UHT-sterilized milk. The quantity of EM required to give coffee, cocoa, or tea a milky flavor and a white appearance is relatively small. The products of the Maillard reaction in EM give coffee, cocoa, or tea a yellowish hue, which is preferred over the grayish hue obtained if UHT-sterilized or pasteurized milk is added. EM is obtained from homogenized milk, so it is recommended for infant feeding because of the formation of a soft curd in the stomach. The smooth consistency of EM makes it an important ingredient in several types of puddings, sauces, and gravies. Owing to its high TMS content, it is used to manufacture ice

cream. It is also used to prepare chocolate, as well as bakery and confectionery products. In addition, it is diluted with milk and cream to produce coffee creamer.

See also: Dairy Products: Dietary and Medical Importance; Milk Powder; Milk: Role in the Diet; Preservation of Foods; Protein: Food Sources; Sterilization of Foods.

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Consumer Protection Legislation

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Introduction

Access to safe food is an essential precondition for the life of every human being. In this respect, the United Nations (UN), the Food and Agriculture Organization (FAO), and the World Health Organization (WHO) recognized that access to safe and adequate food in terms of nutritional values forms a right of each individual (Article 11, International Covenant on Economic, Social and Cultural Rights). Complex legal frameworks are needed to ensure the functioning of food markets and protect consumers from risks associated with food.

As exposure to health risks in food as well as consumer expectations varies vertically (e.g., from nation to nation, culture to culture, and region to region) and horizontally (according to the scientific knowledge on risks available), the amount of regulation needed for the effective realization of the right to food largely depends on the circumstances of the respective food markets and available scientific knowledge. Consumer protection regulation on food and health has to be different in different countries and regions and react to new insights from science. In addition, different legal cultures with a view of enforcement and application of the law require nuanced solutions according to the respective legal context.

Food safety is becoming more and more a global challenge by virtue of its economic implications as well as its public health impact. Effective regulation of consumer protection on markets is hence under threat, as consumer protection regulation designed for a specific regional food market increasingly governs other food markets, which are not necessarily embedded in the same prerequisites.

In order to realize the right to food, states and increasingly private standard setters have on the one hand to find an optimal level of regulation and on the other hand to design regulation that attracts safe foodstuffs to their markets.

Food Law and Consumer Protection

Consumer protection legislation takes various forms, which cut across commonly known distinctions in law, and are applied according to their own distinct economic policies and access to justice policies. When protecting from risks involved with foods, this legislation forms consumer protection legislation in the functional area of food law:

(1) Classical state-centered regulation encompasses civil law, criminal law, and administrative law. Civil law protection provides consumers rights within a contractual relationship, for example, by allowing consumers to remedy a contract for unsafe foods. Criminal law imposes sanctions, for example, on food producers for selling unsafe foods. Administrative law typically imposes market entry restriction, for example, by demanding an authorization procedure for foodstuff.

(2) In a market-driven global food sector, the importance of governments as regulators decreases and is increasingly replaced by private standard setters and enforcers. The law of private standards has hence become an increasingly important area for consumer protection legislation in the global food chain.

(3) In consumer protection legislation in federal, supranational, or international legislation such as in the EU or World Trade Organization (WTO), consumer protection on food markets typically follows a subordinate role. In these regimes, protection of the consumer is used primarily to satisfy other objectives such as the fostering of cross-country trade or forming the product of a spillover from legislation of the supply side of markets. However, the more these legislative systems mature into autonomous systems, the more state-centered their regulation becomes.

Consumer Protection in Food Law

As consumer protection legislation differs according to the legal regime in foods to be applied, consumer protection food legislation needs to be viewed according to the logics of the underlying legal regime. According to constraints of space, this piece will hence present an overview of consumer protection legislation of what we consider to be the most important legal food regimes in the world as well as the area of private standards.

Consumer Protection Legislation in International Food Law

In International Food Law, consumer protection in food markets is mainly sought by establishing food safety standards. These are then incorporated and enforced in other regulatory regimes such as national law or private standards. To this end, the FAO and the WHO established in 1963 the Codex Alimentarius Commission (CAC). The work of the CAC has resulted in a vast collection of internationally agreed food standards that are presented in a uniform format, the 'Codex Alimentarius.' Most of these standards are of a vertical (product-specific) nature. They address all principal foods, whether processed, semi-processed, or raw. Standards of a horizontal nature are often called 'general standards,' like the General Standard for the Labelling of Prepackaged Foods.

According to this general standard, the following information shall appear on the labeling of prepackaged foods:

- The name of the food (this name shall indicate the true nature of the food)
- List of ingredients (in particular if one of a list of eight allergens is present)
- Net content
- Name and address of the business

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00194-X

- Country of origin where omission could mislead the consumer
- Lot identification
- Date marking and storage instructions
- Instructions for use

In addition to the formally accepted standards, the Codex Alimentarius includes recommended provisions called codes of practice or guidelines. Most notably are, for example, the 'Code of Ethics for International Trade in Food' and a set of hygiene codes like the 'Recommended International Code of Practice, General Principles of Food Hygiene' and the 'Hazard Analysis and Critical Control Point System and Guidelines for its Application.'

The 'constitution' of the Codex Alimentarius is the procedural manual. The procedural manual gives not only the procedures and format for setting Codex Alimentarius standards and guidelines but also some general principles and definitions. The principles relate among other things to the scientific substantiation of the work of the Codex Alimentarius and the use of risk analysis for food safety.

Consumer Protection Legislation in WTO Law Concerning Food

The WTO is primarily concerned with removing barriers to trade between its member countries. (The WTO was established on 1 January 1995 by the Agreement Establishing the WTO as the result of the so-called Uruguay Round of trade negotiations and signed in Marrakesh on 15 April 1994 (WTO Agreement). The WTO is the institutional continuation of the General Agreement on Tariffs and Trade (GATT, 1947).) It does so by establishing equal treatment of all trading partners as the norm. As different consumer protection legislations form barriers to trade, they require an exception from the general application of liberalizing the WTO law. To this end, Art. XX(b) of the GATT recognizes that certain exceptions to free trade can be necessary to protect social values like health and (food) safety. This opens up a leeway for members of the WTO to legitimately regulate consumers' health and safety of foods. The Agreement on Technical Barriers to Trade (the TBT Agreement) and the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement) further substantiate these requirements. The SPS Agreement ensures that countries only apply measures to protect human and animal health (sanitary measures) and plant health (phytosanitary measures) based on the assessment of risk or, in other words, based on science. The measures must be scientifically justified and they may neither be discriminating nor constitute disguised barriers to international trade. The SPS Agreement incorporates, therefore, the main safety aspects of foods in trade. The TBT Agreement covers all technical requirements and standards (applied to all commodities), such as labeling, that are not covered by the SPS Agreement. Therefore, the SPS and TBT Agreements can be seen as complementing each other.

If the measures are in conformity with international standards, no scientific proof of their necessity is required. These measures are by definition considered to be necessary. The most important international standards regarding SPS are set by the so-called three sisters of the SPS Agreement: the Codex Alimentarius Commission, the International Office of Epizootics (OIE) (the abbreviation follows the French spelling), and the Secretariat of the International Plant Protection Convention. The standards on food and food safety are mainly found in the Codex Alimentarius. (The agreement on TBT treaty has similar articles.)

Consumer Protection in Private Food Law

Private standards stipulate rules that food businesses impose upon each other in contractual relations (from a strategic business viewpoint). Legal food safety systems limited by national borders and political compromise are often ill-suited to reflect consumers' and suppliers' demands in the food chain. For this reason, corporations increasingly choose to develop their own food safety standards. These standards form 'uniform' food safety management systems, which lay down detailed requirements for service providers and producers. Such private food standards have been so widely adopted by retailers in particular that they have emerged as the primary mode of governance in global food supply chains. While voluntary, the widespread adoption of private food standards means that these standards have become *de facto* mandatory as a precondition for participation in the majority of global supply chains. This initially applied primarily to export markets. In the developing world retailer capture of food markets in recent years has left few alternatives, even in a domestic context.

Under such private standard regimes, suppliers have to obtain certification and demonstrate compliance with the respective quality management system, verified by independent audits. The variety of standards demanded by customers often requires suppliers to obtain multiple certificates. As their differences are mainly bureaucratic in nature, this is an unnecessary and costly burden. In order to cope with this shortfall, the Global Food Safety Initiative (GFSI) makes a move to level or mutually recognize these different standards as far as possible. To this end, the GFSI approves certification regimes, which then lead to worldwide acceptance ('certified once, accepted everywhere').

Consumer Protection Legislation in American Food Law

The United States of America

In the United States, consumer protection legislation in food law is shared between the federal and the state level. However, since President Roosevelt signed the Pure Food and Drug Act and the Meat Inspection Act into law in 1906, consumer protection in food law became increasingly federalized. Along with the federalization of consumer protection law, the federal Food and Drug Administration (FDA) matured into an ever more powerful regulative body in the United States. However, as the power of criminal law remains with the States, federalization of consumer protection food laws embraced private and administrative laws only.

Consumer protection food legislation in the United States was to a large extent triggered by the cheating practices of the food industry. As a reaction to this, US consumer protection

food legislation is largely occupied with the prevention of adulteration of foods in order to 'promote honesty and fair dealing in the interest of consumers' (21 U.S.C. Sec. 341). This is why the concept of 'adulteration,' unlike, for example, in EU law, takes a prominent position in consumer protection legislation in the United States (21 U.S.C. Sec. 342). The large bunch of legislation hence seeks to prevent such adulteration by ensuring that consumers can trust in the information they receive about the properties of the respective food. To this end, the FDA first and foremost regulates the labeling of food (see especially 21 U.S.C. Sec. 343 and the health claims requirements). Adulteration shall also be prevented by regulating, for example, the manufacture, processing, or packing of foods (see, e.g., 21 U.S.C. Sec. 348–350).

Important legislation (selection): Federal Food, Drug, and Cosmetic Act (FD&C Act); Federal Meat Inspection Act; Federal Trade Commission Act; Filled Milk Act; Import Milk Act; Public Health Service Act; Trademark Act of 1946; Reorganization Plan 1 of 1953; Poultry Products Inspection Act; Fair Packaging and Labeling Act; National Environmental Policy Act of 1969; Controlled Substances Act; Controlled Substances Import and Export Act; Egg Products Inspection Act; Lead-Based Paint Poisoning Prevention Act; Federal Advisory Committee Act; Government in the Sunshine Act; Federal Anti-Tampering Act; Sanitary Food Transportation Act; Mammography Quality Standards Act (MQSA); Bioterrorism Act of 2002; Project BioShield Act of 2004; FDA Food Safety Modernization Act (FSMA) (still pending).

Internet reference address: http://www.fda.gov/RegulatoryInformation/Legislation/default.htm.

Canada

As Canada is a federal system, it is in this respect in many ways comparable to the US legal system. Consumer protection legislation in food law is likewise shared between the federal and the state level. However, with the enactment of several federal laws with regard to consumer protection in food law (most notably, the Canadian Food and Drugs Act (CFDA) and the Safe Food for Canadians Act; for an overview, see list at the end of this subarticle), food legislation became increasingly federalized. Along with the federalization of consumer protection law, the Canadian Food Inspection Agency matured into an ever more powerful regulative body in Canada. Unlike in the United States, the federalization of consumer protection food legislation embraces also criminal law.

The main concern of the early consumer protection food legislation was adulteration of liquor. Hence, like US consumer protection food legislation but for a different reason, Canadian consumer protection food legislation is historically likewise occupied with the prevention of adulteration of foods. However, after modernization, Sec. 3 and Sec. 4 of the CFDA (R.S.C., 1985, c. F-27) are much more up to date than its US counterpart: such as in many other jurisdictions, Sec. 4 obliges food sellers as a general rule to ensure that the food on the market shows certain properties. In particular, food needs to be not poisonous or harmful or unfit for human consumption. If it is, Sec. 22 et seqq. of the act empowers several Canadian authorities to take action.

Important legislation (selection): Agriculture and Agri-Food Administrative Monetary Penalties Act; Appropriation Acts; Canada Agricultural Products Act; Canadian Food Inspection Agency Act; Consumer Packaging and Labelling Act; Feeds Act; Fertilizers Act; Fish Inspection Act; Food and Drugs Act; Health of Animals Act; Meat Inspection Act; Plant Breeders' Rights Act; Plant Protection Act; Safe Food for Canadians Act; Seeds Act.

Internet reference address: http://www.inspection.gc.ca/about-the-cfia/acts-and-regulations/eng/1299846777345/1299847442232.

Latin America

Consumer protection legislation in food law in Latin America is as diverse and vivid as the continent. Although there have been several attempts made in the past decade, however, until the time of writing, only some countries such as Brazil have developed a fully fledged food regulation. However, an all-encompassing Latin American approach to consumer protection food law that one could present in an encyclopedia such as this is nonexistent.

Consumer Protection Legislation in European Food Law

In Europe, consumer protection food legislation has to be evaluated in view of the specific features of different legal entities. Food law is largely harmonized at the supranational level of the EU with the result that member states of the EU do not have a strong say in consumer protection food legislation. Countries such as Switzerland, which are not part of the EU, also increasingly follow the example of EU regulation in food.

The European Union

There is no general authorization procedure for foodstuffs in the EU. Art. 14 (1) of the General Food Law (Regulation (EC) 178/2002, GFL), however, stipulates that "food shall not be placed on the market if it is unsafe." Hence, liability of producers as well as legitimate intervention of authorities depends on whether the respective food passes an 'unsafety' test. The Unfair Commercial Practices Directive 2005/29/EC ensures that consumers are not subject to fraudulent or deceptive practices, adulteration, and other practices, which may mislead the consumer (Art. 8 GFL). Product liability for foodstuffs is regulated by Directive 85/374/EEC (see Art. 21 GFL). If there is scientific uncertainty about the unsafety of foods, authorities may nonetheless take precautionary measures according to the precautionary principle (see Art. 7 GFL).

Compliance with numerous food processing and other market regulation is generally monitored by member state authorities. They are, however, interwoven in a dense communication network between member state authorities and the European Food Safety Authority (EFSA). The EFSA monitors the work of member state authorities. Together with the Commission, it has also extended competences in food crisis management and information regulation.

According to the principle of mutual recognition (also known as the *Cassis de Dijon* principle), foods that are lawfully put on the market in one member state can and should be

allowed access to the markets in other member states if member states cannot provide good justifications why they should not. In this sense, consumer protection food law is local and travels with the food. However, with the enactment of the horizontal General Food Law, the principle of mutual recognition is increasingly on the decline. In case intervention is needed in order to enforce consumer protection rules, the Court of Justice of the European Union has established a preference for information-related over content-related measures. That is why a wide array of measures available to authorities are information-related in nature. Health claims and novel foods even need authorization.

Genetically modified foods and other novel foods fall under a different regulatory regime. They need authorization as a general rule. The authorization decision is taken by the European Commission. It is based on risk assessment by the EFSA.

Important legislation (selection): Regulation (EC) No 178/2002 laying down the general principles and requirements of food law; Regulation (EC) No 510/2006 of 20 March 2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs; Regulation (EC) No 852/2004 on the hygiene of foodstuffs; Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin; Regulation (EC) No 854/2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption; Directive 2002/99/EC laying down the animal health rules governing the production, processing, distribution and introduction of products of animal origin for human consumption; Regulation (EC) No 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules; Regulation (EC) No 1829/2003 on genetically modified food and feed; Regulation (EC) 1924/2006 on nutrition and health claims made on foods.

Internet reference address: http://europa.eu/legislation_summaries/food_safety/general_provisions/index_en.htm.

Switzerland

Since 2010 and pursuant to Art. 16a (1) of the Loi fédérale sur les entraves techniques au commerce, products lawfully marketed in the EU or the European Economic Area may also be marketed in Switzerland. Switzerland has thereby autonomously expanded the Cassis de Dijon principle to their home country. However, in order to successfully market foodstuffs marketed in the EU in Switzerland, they still require authorization by the competent Swiss authority.

Internet reference address: http://www.bag.admin.ch/themen/lebensmittel/10380/index.html?lang=en.

Consumer Protection Legislation in Asian Food Law

China

China is a multilevel system, whereby state laws interplay with local regulation. However, there are strong desires and preparatory works for a basic food law modeled after EU or US standards. Although since 2009 China has enacted horizontal food laws after the model of Western legal systems, at the time of writing, food legislation in China is nonetheless still primarily scattered along the different levels of Chinese legislation. Although the food legislative system in China is modernizing fast, consumer safety regulation still mainly follows the classical approach of administrative control.

China knows three different levels of regulation. The first and highest level concerns laws passed by the National People's Congress. Here, the main act concerned with consumer protection in foods is the horizontal Food Safety Law of the People's Republic of China. The China FDA is the main focal point of the administration and supervision of food (including food additives and health food, the same in the succeeding text) safety at this level. Other important legislations include the Food Hygiene Law, Product Quality Law, Agricultural Product Quality and Safety Law, and Consumer Rights Protection Law. The second level embraces legal instruments issued by the State Council or the provincial People's Congress. They are primarily concerned with the administration of pesticides and veterinary drugs, inspections of slaughterhouses and abattoirs, and the regulation of genetically modified organisms. Third-level regulation embraces decrees or provisions formulated by ministries of the national or provincial government(s). The most important decrees concern practices on food processing, decrees on foods for particular populations, and decrees specific to the management of food poisoning and street foods.

Important legislation (selection): Food Hygiene Law; Product Quality Law; Agricultural Product Quality and Safety Law; Consumer Rights Protection Law.

Internet reference address: http://eng.sfda.gov.cn/WS03/CL0756/ (CFDA); http://www.fas.usda.gov/gainfiles/200903/146327461.pdf (unofficial translation of the Food Safety Law of the People's Republic of China).

India

Indian food law focuses on strengthening both food security and food safety. In 2013, the Indian National Food Security Act was signed into law. This act creates legal entitlements to subsidized food grains for large parts of the Indian population.

In 2006, a new Food Safety Standards Act was put in place. The Ministry of Health and Family Welfare implemented this act through the Food Safety and Standards Regulation, 2011. The act and the regulation are the domain of the Food Safety and Standards Authority of India (FSSAI).

The law covers topics such as prevention of adulteration, hygiene, and labeling for packaged food including health claims, permitted food additives and colors, and microbiological requirements.

All domestic food operators are required to be licensed by the FSSAI.

Important legislation (selection): Food Safety Standards Act, 2006; Food Safety and Standards Regulation, 2011; Indian National Food Security Act, 2013.

Internet reference address: http://www.fssai.gov.in/

Japan

Consumer protection food regulation is based on the Food Safety Basic Act such as other more specific legislations. Food safety policy in Japan is conducted in accordance with the

general internationally recognized deviation of risk management and risk assessment. While the Food Safety Commission is responsible for scientific risk assessment, the Ministry of Health, Labour, and Welfare and the Ministry of Agriculture are in charge of risk management.

Important legislation (selection): Food Safety Basic Act, 2003; Food and Sanitation Law; Business Control and Poultry Inspection Law.

Internet reference address: http://www.fsc.go.jp/english/index.html

Consumer Protection Legislation in South African Food Law

South Africa is governed by not only a pluralist system, whereby state laws interplay with local regulation at a vertical level, but also by the laws of different societies, tribes, and governmental regulation, which interplay horizontally. This is reflected in legal acts as well as control of consumer protection food legislation. The most important act is the Foodstuffs, Cosmetics and Disinfectants Act, 1972. Several other acts accompany the regulatory framework in this respect.

Important legislation (selection): Foodstuffs, Cosmetics and Disinfectants Act, 1972; Meat Safety Act, 2000; Agricultural Products Act, 1990; Liquor Products Act, 1989; Health Act, 1977.

Internet reference address: http://www.doh.gov.za/healthtopics.php?t=Food%20Control

See also: Allergies: Public health; Cured Foods: Health Effects; Food and Agriculture Organization of the United Nations; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Legal Requirements for Food Hygiene; Quality Control in Food Processing; Risk Assessment of Foods and Chemicals in Foods.

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Controlled Atmosphere Storage: Applications for Bulk Storage of Foodstuffs

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Introduction

Fruits and vegetables are biological systems that once harvested deteriorate rapidly because they continue metabolic processes. To diminish the rate of senescence, vegetable products must be handled adequately. Low-temperature storage is the most effective and simplest way to reduce metabolic processes causing deterioration. Nevertheless, the combination of low temperatures with preservation factors that avoid the action of oxygen involved in many enzymatic reactions is recommended to extend shelf life while preserving the quality of foods. Although the use of packages under normal atmospheric conditions protects foods from environmental factors such as light, physical, and biological contaminants, vegetable products are still in contact with oxygen.

Controlled atmosphere (CA) and modified atmosphere (MA) are two technologies used to change the gas composition of the surrounding environment with the aim of reducing metabolic reactions, maintaining the freshness, and extending the shelf life of foods. In these technologies, the concentration of oxygen, carbon dioxide, ethylene, and/or nitrogen of the surrounding atmosphere of foods is changed. While in CA the CO_2 and O_2 levels are constantly monitored and adjusted, in MA, the gas concentration changes due to metabolic processes of the fruits and vegetables, and this concentration is not controlled during storage. As a consequence, in MA, the rate of change depends on the respiration rate of the product, package permeability, and storage conditions. Due to the gas control in CA, it is a more costly method, but it is more appropriate for long-term storage products.

CA and MA can be applied once the product is packaged or also be used during the bulk storage where the gas composition inside the storage rooms is changed according to the requirements of foods stored. The use of CA in bulk storage extends the shelf life of foods, avoids microbiological reactions, and controls pests including insects, rats, and mice. Some of these effects are achieved due to the capacity of CA to reduce ethylene biosynthesis retarding ripening. During the storage of fruits and vegetables, the level of O_2 is generally lowered to diminish ethylene production, and the level of CO_2 is often increased to decrease the action of ethylene. The concentration of gases in CA storage rooms is monitored by infrared gas analyzer to measure CO_2 and by a paramagnetic analyzer for O_2.

The use of inadequate preservation conditions in CA storage can cause negative effects on the quality of foods; very low levels of O_2 can result in fermentation causing off-flavors related with ethanol and acetaldehyde production. This can also decrease the synthesis of volatile compounds affecting the sensory quality of foods. This contribution is focussed on the preservation of foods by CA bulk storage.

Metabolic Processes of Fruits and Vegetables Affecting Their Shelf Life

After harvesting, fruits and vegetables continue having metabolic processes like respiration, transpiration, and production of ethylene. These physiological activities are responsible for the ripening and senescence of vegetable products; thus, adequate postharvest handing is necessary to delay changes maintaining quality. The rate of these processes determines the shelf life, and they depend on food composition, physiological characteristics, and storage conditions such as temperature and relative humidity. Respiration oxidizes carbohydrates by using the atmospheric oxygen generating ATP, CO_2, and water. The ATP produced is used in many ripening reactions; thus, the control of the respiration rate is a way of controlling the rate of senescence. Storage at low temperatures decreases the activity of enzymes involved in ripening. Diminished levels of oxygen reduce respiration and the action of enzymes like polyphenol oxidase and peroxidase. The application of CA at low O_2 levels combined with reduced temperature is a way of decreasing senescence of foods. Transpiration is the process of water loss causing wilting, shriveling, shrinkage, softening, flaccidity, limpness, loss of crispness, juiciness, and loss of nutritional quality in fruits and vegetables. Ethylene regulates many aspects of growth, development, and senescence of vegetable products and can be physiologically active at very low concentrations. Transpiration and ethylene production can be controlled by adequate storage conditions.

CA Storage Definition and Principles

The desired atmosphere in CA storage is first achieved by replacing the normal gas composition by purging some gas or by the metabolism of fruits and vegetables, and then, it is maintained by lowering the rate of gas input. The gas input rate is determined by the room capacity, its degree of gas tightness, humidity, temperature, and metabolism of the stored foods.

CA technology generates products that remain turgid because the lower O_2 concentration inhibits or retards the activity of enzymes that act on cellular membranes avoiding texture changes. CA also expands the climacteric and postclimacteric periods; delays senescence during storage due to the reaction between O_2 and ethylene; reduces microbial degradation due to the decreased O_2 levels and the increased CO_2 concentrations; improves the color stability due to a low effect on chlorophylls; allows obtaining foods with similar nutritional and sensory characteristics than the fresh product due to the low effect on acidity, sugars, and vitamins; and controls proliferation of pests and molds at low O_2 or high CO_2 atmospheres.

CA constraints are related with the high costs of storage, the special seals required in the storage room to avoid gas leakage, and the longtime of exposure needed (8–12 days). Another disadvantage is the variability in the concentration of gases required for the storage of foods that depends on many factors such as the type of fruit or vegetable, species, stage of maturity, medium, and climate in which they were grown. Biochemical and anatomical differences, including the size of intracellular spaces and the rate of gas diffusing through the food structure, also influence the variability in gas concentrations. Another disadvantage of CA is that at inadequate storage conditions, undesirable changes like fermentation can appear. For example, apples and pears stored at very low O_2 concentrations presented changes in flavor and color and changes in hardness and darkness of skin due to high concentrations of CO_2. The need for low temperature and high relative humidity to enhance CA effectiveness is also considered as constraints of CA storage.

Main Gases Used in the CA Storage of Foods

There are many gases to be controlled in order to reduce the metabolic processes of foods, microorganisms, or pests in CA storage. The control of both O_2 and of CO_2 levels can be used in order to have synergetic or additive effects to extend the shelf life of foods. The election of either low level of O_2 or high concentrations of CO_2 depends on factors such as the time of treatment, design of the room used, cost of gases, and systems available to control them.

The use of CO_2 prevents the growth of aerobic bacteria and fungi and avoids the oxidation of some compounds. The increase in CO_2 level has different effects in fruits and vegetables such as decrease of synthetic reactions in climacteric fruit, delay in the initiation of ripening, inhibition of some enzymatic reactions, decrease of production of some organic volatiles, modification of the metabolism of some organic acids, reduction in the rate of pectin breakdown, inhibition of chlorophyll breakdown, production of off-flavor, induction of physiological disorders, retarding of fungal growth, inhibition of the effects of ethylene, inhibition of postharvest development, retention of tenderness, and decrease of color loss. Oxygen is necessary for the growth of aerobic bacteria and enzymatic or chemical reactions in foods. Therefore, its concentration may affect the product quality. However, it should not be completely eliminated because it would cause the growth of anaerobic bacteria causing fermentation. Decreasing O_2 levels reduces respiration rate, decreases oxidation, delays ripening of climacteric fruit, extends shelf life, delays breakdown of chlorophyll, reduces rate of ethylene synthesis, changes fatty acids synthesis, reduces degradation rate of soluble pectin, decreases off-flavors, and alters texture. Nitrogen is another gas used in CA, normally as filler instead of CO_2. It is innocuous and when it is used in packages, it may prevent the collapsing. It is also used in some cases instead of oxygen as a nonreactive gas to avoid decomposition and oxidation of lipophilic compounds.

Methods Used to Control Gas Levels in CA Storage

CA storage rooms are built with structures capable of supporting the gases used, and they are provided with the adequate system to produce the atmosphere and the best-available method to maintain the atmosphere composition for the required time. In CA storage, foods are placed into an insulated store room whose walls and doors are gastight. CA rooms are made from metal-faced insulated panels, which are fitted together with gastight locking devices. Doors are usually sealed by having rubber gaskets around the perimeter, which correspond to another rubber gasket around the door jamb of frame to avoid leaks. Static and dynamic are the two ways used to produce the atmosphere needed in CA storage rooms. In the static method, the product generates the atmosphere and it is maintained by ventilation and scrubbing. In the dynamic system, the gas concentrations are supplied by flushing gases. There are also different ways to control the gas levels in CA rooms. Systems such as external burners, liquid or gaseous nitrogen, gas separator systems, and hypobaric storage can be used to control the O_2 levels. For the removal of CO_2 scrubbing systems based on NaOH, hydrated lime ($Ca(OH)_2$), water, activated charcoal, and molecular sieves have been implemented. Heated catalyst scrubbers, ethylene-absorbing beads, and UV light are used to control the ethylene levels (Table 1).

CA Storage of Fruits and Vegetables

The recommended storage conditions for fruits and vegetables under CA are highly variable because they depend on many factors. Factors such as species, cultivars, anatomical differences, initial gas concentration in the storage room, temperature, humidity, degree of ripeness, ethylene concentration, and pre- and postharvest conditions influence the level of gases used. Thus, there are many recommended conditions for the storage of foods.

Temperature is one of the factors that more influence the required concentration of CO_2 and O_2 for the CA storage of foods. The effect of temperature on delaying the ripening of foods by decreasing the rate of reactions is well known. Nevertheless, due to the diversity of factors influencing, when selecting the conditions for the storage or certain food, it will be necessary to evaluate their effects on food quality in terms on color, texture, nutritionals changes, and microbiological safety.

Increasing the CO_2 levels can have different effects on the biochemical and physiological changes associated with ripening such as the production of ethylene that can be inhibited, increased, or not altered. In the first case, CO_2 acts by displacing ethylene from its receptor side, delaying fruit ripening; CO_2 concentration lower than 1% favors ethylene production. The oxygen levels also influence the effects of CA storage on ripening of fruits by inhibiting or lowering the action of ethylene and also altering its synthesis. Lowering O_2 to more than 8% reduces the binding of ethylene to its receptor influencing its actions and therefore ripening. O_2 is required in the final step of ethylene biosynthesis from methionine. Oxygen levels below 5% greatly reduce ethylene production of fruits by inhibiting the ACC oxidase enzyme activity. Very low levels of O_2 can cause fermentation of foods with consequent development of off-flavors, while very high levels of CO_2 can cause texture changes. It was reported that apple (Delicious) stored at 1 °C in 0.5% O_2 + 0.2% CO_2 for 30 days developed high concentrations of ethanol and acetaldehyde and that at 0% O_2, the respiration rate of cucumber stored at 10 °C or 20 °C increased because of fermentation. CA storage of mango in 1% O_2 produces off-flavors and skin

Table 1 Systems used to control the O_2, CO_2, and ethylene levels in CA storage

System	*Characteristics*
O_2 control	
External burners	The level of O_2 in the storage room is reduced by the combustion of propane or natural gas generating CO_2 and water vapor. They usually require a CO_2 scrubber to control the levels of this gas and cooling systems to reduce the temperature of air before and after combustion. They are classified as open flame or catalytic burners. The open flame burner does not allow air recirculation because of the risk of extinguishing the flame by the O_2 depleted recirculating airstream. Catalytic burners provide more complete combustion reducing O_2 levels to 3%; in addition, they have lower operation cost despite being more expensive to install
Flushing with liquid or gaseous N_2	O_2 levels are reduced by directly flushing N_2 in the storage room until the desired O_2 level is achieved. Liquid N_2 is injected into the room through spray headers that atomize the N_2
Gas separator systems	Gas separator systems decrease the O_2 levels while increasing the N_2 concentration from 97% to 99%. They are classified as pressure swing absorption (PSA), hollow fiber membrane separators (HFMS), and high-temperature ammonia cracking systems (HTAC) PSA system compresses dry air and once filtered forces it through a bed of pelletized carbon material, which absorbs O_2 and enhances the N_2 levels HFMS systems are based in the different diffusion rates of gases through membranes. CO_2 and O_2 have much higher permeation rates than N_2; thus, the levels of CO_2 and O_2 decrease, while the level of N_2 increases In the HTAC system, high-temperature anhydrous NH_3 gas reacts with air from the CA room, yielding hydrogen and inert N_2 gas. Further, hydrogen reacts with the O_2 to yield water vapor
Hypobaric storage	Hypobaric pumps are used to reduce the pressure of the room with consequent decreasing in O_2 levels
CO_2 control	
NaOH	NaOH dissolved in water can be used to remove CO_2 in the storage room by circulating the air in open tubes. The amount of CO_2 removed is proportional to the NaOH exposure time
Hydrated lime scrubber ($Ca(OH)_2$)	$Ca(OH)_2$ is one of the simplest and most effective systems for controlling the level of CO_2. $Ca(OH)_2$ is placed in an insulated and airtight box outside the storage room to which it is connected by two pipes in which one of them allows air from the storage room to enter the box that contains the lime $Ca(OH)_2$ can also be placed on bags or pellets that react with CO_2 yielding calcium carbonate, water, and heat maintaining it at level lower than 1%. This methods required space to place the bags and is inefficient because 80% of the lime is not used
Water CO_2 scrubbers	A brine solution is pumped or sprayed over the evaporator coils in the CA room. The brine absorbs CO_2, due to the pressure difference between the incoming water and the atmosphere in the CA room. The corrosion caused by the brine solution may be prevented by using dry cooling units
Activated charcoal and molecular sieve scrubbers	The air from the CA room is blow through an absorbing unit reducing the CO_2 levels, and then it is forced back to the CA room. When saturated with CO_2, the absorbent must be reactivated by pursing it with air
Ethylene control	
Heated catalyst scrubber	The air is forced thought ceramic pickings used as heat exchangers removing up to 87% of the ethylene from the air. The catalyst is electrically heated and the high temperature promotes the oxidation of ethylene to CO_2 and water vapor. This system requires energy for heating and cooling
Ethylene absorbing beads	Small particles of aluminum silicate impregnates with potassium permanganate are placed in a sealed unit. Air from the CA room is circulated through the unit and the ethylene reacts with the permanganate changing the color of the beds when saturated with ethylene
UV light	The UV light reacts with O_2 to form ozone, which eliminates ethylene

Source: Thompson, A. K. (2010). CA technology. In: Thompson, A. K. (ed.) *Controlled atmosphere storage of fruits and vegetables* (2nd ed.), pp. 26–42. London: CAB International; Rennie, T. J., Vigneault, C., Deell, J. R. and Raghavan, S. G. (2003). Cooling and storage. In: Ramaswamy, H. S., Vijaya Raghavan, G. S., Chakraverty, A. and Mujumdar, A. S. (eds.) *Handbook of postharvest technology: cereals, fruits, vegetables, tea, and spices*, pp. 505–538. New York: CRC Press.

discoloration, but the storage at 12 °C in 5% CO_2 + 5% O_2 was possible for 20 days. With 3% O_2 + 6% CO_2, mango did not present significant fermentation.

CA including either reduced O_2 or elevated CO_2 not only generally decreases the loss of provitamin A but also inhibits the

biosynthesis of carotenoids. Concentration of 5% CO_2 caused loss of carotenes, while 7.5% CO_2 or higher favors their synthesis. CA storage with 2% O_2, air + 12% CO_2, and 2% O_2 + 12% CO_2 had no effect on quality attributes of sliced peaches over 7 days of storage, while the visual quality in color of persimmon slices was slightly enhanced by the treatments containing 12% CO_2. Peach slices stored in 12% CO_2 had a lower content of β-carotene and β-cryptoxanthin. The various carotenoids found in persimmon slices responded differently to the tested atmospheres; storage in 2% O_2 or 12% CO_2 tended to result in lower retinol equivalents after 8 days storage at 5 °C, but the loss was not significant for fruit stored under 2% O_2 + 12% CO_2. In the case of other vitamins like vitamin C, it was noticed that 2% O_2, air + 12% CO_2, or 2% O_2 + 12% CO_2 had no significant effect on total ascorbate content for both fresh-cut strawberries and persimmons.

The effects of CA on texture and other sensorial attributes of some fruits and vegetables have also been evaluated. CO_2 concentrations above 4% with 15–20% O_2 retard the softening of kiwifruit, and this effect increased as the CO_2 content increased from 4% to 10%, but without additional effects at CO_2 content above 10%. Low O_2 levels (2–3%) with 3–5% CO_2 further delayed the rate of kiwifruit softening and increased storage life up to 3–4 months. Although CA increases storage life of kiwifruit, the magnitude of the effects varied from year to year. Contamination of the storage atmosphere by as little as 0.1 μl^{-1} ethylene severely reduced the effectiveness of controlled atmosphere storage in maintaining kiwifruit firmness, even at 0 °C. It was showed that Valencia oranges can be treated with 0.5%, 0.25%, or 0.02% O_2 at 5 °C or 10 °C for up to 20 days without detrimental effects on their appearance, nutritional value, and flavor, while 60% CO_2 at 5 °C could injure oranges, and 7–12% O_2 + 0–5% CO_2 retards decay and toughening of asparagus. Elevated CO_2 concentrations resulted in firmer strawberries, while low O_2 did not affect texture demonstrating that although strawberry is highly perishable, under high CO_2 condition, the firmness and shelf life could be maintained. Off-flavor developed after 3 days of storage at 20% CO_2, but it decreased when fruit was subsequently held for 24 h at 20 °C. Similar results were obtained using low O_2 (2%, 1%, and 0.5%) or elevated CO_2 (10%, 15%, and 20%) concentrations, and their combinations reduced respiration and ethylene production rates of strawberry stored at 2 °C and fruits maintained their color and firmness. The rate of color change in cucumber could be delayed with high CO_2 and low O_2 in the storage atmosphere, but 10% CO_2 could impair their flavor.

The storage of mamey at 15 °C in 1% CO_2 + 5.6% O_2 + 89.3% N_2 for 2 weeks retarded their maturation, and it was recommended to store onion at 5% CO_2 + 3% to reduce sprouting and root growth. The use of 1–5% O_2 + 14% CO_2 allows to reduce the levels of crown rot in bananas as compared with those stored in air, and this effect continued even during subsequent ripening in air at 25 °C, and the storage of cauliflower at 10%, 15%, or 20% O_2 the leaf yellowing and rot caused by *Alternaria brassicicola* is delayed, but it caused injury inside the stem and vegetables developed off-flavors and odors after cooking. Some other recommended CA storage conditions for fruits and vegetables are present in Table 2.

Some products are also classified according to the minimum concentrations of O_2 and maximum level of CO_2 required for storing. Most fruits and vegetables have a threshold level of O_2 and CO_2 to cause injury that includes problems such as off-flavor development, discoloration, off odors, alcoholic taste, browning, and softening. Some minimum concentrations of O_2 and maximum level of CO_2 required for storage of some foods are presented in Table 3.

Table 2 Recommended CA storage conditions for some fruits and vegetables

Product	*O_2 (%)*	*CO_2 (%)*	*Temperature (°C)/relative humidity (%)*	*Shelf life*	*Variety*
Apple	0.02			12 days	
	1.5	Up to 3			
	1.5	<0.5	0	9 months	Gala
	<1	2	3.5–4	23 weeks	Gala
	3	3	0	9 months	Golden Delicious
	1.5	Up to 3	0		Golden Delicious
	3	1			Golden Delicious
	3	3	0	8.5 months	Golden Delicious
	0.8–2.5	0.8–5	0.5–2	8–10 months	Granny Smith
	1.5	1.5	0.5	9 months	Red Delicious
	1–2.5	1–3	−0.5 to 1	8–10 months	Red Delicious
Blueberry	0			14–21 days	
Cherry	0.02			25 days	
Mango	0.1–0.2			5 days	
	5	5			
	5	10	10/90		
	3–5	5–10	10–15		
Nectarine	0.02			14 days	
Orange	0.02			16 days	
	5–10	0–5	5–10		
	5–10	0–5	5–10		
Papaya	0.2–0.4			3 days	
		10	18		

Table 2 (Continued)

Product	*O_2 (%)*	*CO_2 (%)*	*Temperature (°C)/relative humidity (%)*	*Shelf life*	*Variety*
	2–5	5–8	10–15		
	3–5	5–10	10–15		
	2–5	5–8			
Peach	0.02			40 days	
	2	5			
	1–2	3–5			
Pear	0.02			14 days	
	>2	<5			Bartlett
	2–2.5	0.8–1			
	1–1.5	<0.1			
Strawberry	0.25			10 days	
	10	15–20			
	5–10	15–20	0–5		
	1.5	1.5		3 weeks	
Apricot	2–3	2–3			
	0.3		0 or 6	37 days	Reale d'Imola
Avocado	2	10	5.5	4 weeks	
	2–5	3–10	10–13		
Banana	3	5	14		
	10–13.5				Green bananas
	2	8	15		Green bananas
	3	5–8	15		Green bananas
Lemon	5	5–10			
	5–10	0–10	10–15		
	0	10		6 months	
Asparagus	5–10	5–15	20	4.5 days	
	5	9	0/95		
	>10	<10	5		
	20	5–10			
Cabbage	3	3	0		Red and Savoy
	3	0–3	0		White
	2.5–5	2.5–5	0		
	3	5	0		White
Celery	2–4	2–5			
	3	5	0		
Cucumber	3–5	5–7			
Lettuce	3	2		1 month	
	2–5	0			
	1–3	0			
	1–3	0			
Onion	1–2	0			
	3	5	0/65–75		
	0–1	0–5	0–5		
Tomato	3–5	3–5	10–15		
	1.5	0			

Source: Ke, D. and Kader, A. A. (1990). Tolerance of 'Valencia' oranges to controlled atmospheres as determined by physiological responses and quality attributes. *Journal of American Society of Horticultural and Science* **115**(5), 779–783; Ke, D. and Kader, A. A. (1992). Potential of controlled atmospheres for postharvest insect disinfestation of fruits and vegetables. *Postharvest News and Information* **3**(2), 31N–37N; Ke, D., Goldstein, L., O'Mahony, M. and Kader, A. A. (1991). Effects of short term exposure to low O_2 and high CO_2 atmosphere on quality attributes of strawberries. *Journal of Food Science* **56**, 50–54; Ke, D., Van Gorsel, H. and Kader, A. A. (1990). Physiological and quality responses of "Bartlett" pears to reduced O_2 and enhanced-CO_2 atmospheres and storage temperatures. *Journal of the American Science for Horticultural Science* **155**, 435–439; Thompson, A. K. (2010). Recommended CA storage conditions for selected crops. In: Thompson, A. K. (ed.) *Controlled atmosphere storage of fruits and vegetables* (2nd ed.), pp. 116–191. London: CAB International.

CA Storage of Grains and Animal Products

CA can be also used to store grains and cereals for the control of insect's proliferation. Similar to CA storage of vegetable products, CA storage of grain involves the alteration of the proportion of normal atmospheric gases to give an atmosphere inadequate for the development of insects. CA in grain can cause lethal and sublethal effects. Sublethal effects include delayed or disturbed development, other deleterious phenotypic effects, and behavioral phenomena. Thus, the low levels of O_2 and high concentrations of CO_2 used in CA represent an alternative way to the use of poisonous gases for the control of

Table 3 Minimum O_2 level and maximum CO_2 concentration (%) recommended for the CA storage of some foods

Minimum O_2 level	*Examples*
0.5	Nuts, dried fruits, and vegetables
1.0	Pear, apple, broccoli, mushroom, garlic, onion, and minimally processed vegetables
2.0	Apple, pear, kiwi, apricot, plum, cabbage, peach, cherry, nectarine, papaya, strawberry, pineapple, melon, cabbage, lettuce, and cauliflower
3.0	Avocado, carrot, tomato, persimmon, pepper, cucumber, and artichoke
5.0	Citrus fruits, pea, asparagus, potato, and sweet potato
10	Potato
Maximum CO_2 level	*Examples*
2	Apple (Golden Delicious), pear, grapes, tomato, pepper, and lettuce
5	Apple, peach, nectarine, orange, avocado, banana, mango, kiwi, couliflower, pear, and papaya
10	Grapefruit, lemon, lime, pineapple, cucumber, zucchini, broccoli, asparagus, potato, onion, and garlic
15	Strawberry, raspberry, blueberry, cherry, mushrooms, brocoli, and spinach

Source: Fellows, P. J. (1996). *Food processing technology*. Cambridge: Woodhead Publishing; Kader, A. A. and Ke, D. (1994). Controlled atmospheres. In: Paull, R. E. and Armstrong, J. W. (eds.) *Insect pests and fresh horticultural products*, pp. 223–236. Wallingford: CAB International.

insects. CO_2 in high concentrations kills insects acting directly as fumigant, while low O_2 levels have asphyxiate effects acting on all the development stages of the insect without detrimental effects on foods but with dangerous results to human, especially low levels of oxygen and high contents of carbon dioxide. High levels of CO_2 are often more effective to control insect than low levels of O_2 because they act more quickly and in addition require a less stringent standard of gastightness. The time required to kill insects depends on the species and developmental stage, temperature, O_2 and CO_2 levels, and air humidity; an initial level of 70% CO_2 maintained above 35% for 10 days is appropriate for complete insect control at temperatures above 20 °C. Oxygen level below 1.2% for 1 week at temperatures above 35 °C or more than 24 weeks at 15 °C has been proposed for complete insect control in Hass avocados. Sunrise papayas and Keitt mangoes can be stored at 0.5% O_2 and 50% CO_2 for 1, 2, and 5 years at 20 °C. These treatments could have some potential application as insecticides for quarantine insect control, on the basis of fruit tolerance, insect mortality, and cost. There are some studies about the CA storage of animal products; for whole pork loins divided into roasts and subjected CO_2 at 1 °C for up to 21 days, a strong inhibition on microbial growth as well as a marked residual effect during posttreatment storage in air was observed. In the evaluation of the quality of whole ungutted bigeye tuna during bulk storage in CA with 60% CO_2 + 15% O_2 + 25% N_2 and 40% CO_2 + 40% O_2 + 20% N_2, at day 33 of storage, none of the lots were rejected based on pH, trimethylamine nitrogen, total volatile base nitrogen and histamine, or the tasting panel scores. The control and CA lots were rejected after inspection (sensorial analysis) at 13 and 22 days, respectively. Fish muscle undergoes a series of changes during storage under atmospheres and conventional chilled storage, resulting in deterioration and loss of quality.

Legislation

Act No. 228 of 1959 for fruit and vegetable storage by CAs from the State of Michigan, the United States, indicates some definitions, specifications for the construction of storage room, and general conditions for the adequate handling of fruits and vegetables stored under CA. The regulation No. 530 of this act specifies the conditions for the CA storage of apples. The Ontario Regulation 95/97 also gives some specifications for the CA storage of apples. Section 3–502.12 of the Food Code (FDA Food Code 2009: Annex 6 – Food Processing Criteria) provides the criteria that are to be met in the HACCP plans of those operators who are conducting reduced oxygen packaging operations. According to the European Union directive No. 95/2/EEC, food stored under CA must have that indicated in the label and gases used must be treated as additives.

See also: Fumigants; Fungicides; Insect Pests; Spoilage: Bacterial Spoilage; Spoilage: Yeast Spoilage of Food and Beverages.

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Controlled Atmosphere Storage: Effect on Fruit and Vegetables

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Introduction

Controlled atmosphere (CA) storage of fruits and vegetables refers to storage in atmospheres with reduced levels of oxygen and/or relatively high carbon dioxide concentrations, at low temperature and high relative humidity (RH). The modified atmospheres are created in an air tight room, and they are continuously monitored and maintained throughout the storage period.

CA storage is an appropriate and practical technology for prolonging the shelf life and maintaining the quality of fruits and vegetables on a large scale. CA storage reduces the metabolic activity of the produce, allowing the control of ripening and senescence, which reduces weight loss, delays softening and anthocyanin formation, inhibits browning and decay, and favors flavor and chlorophyll retention.

CA storage is most effective on climacteric fruit such as apples, pears, apricots, mangos, tomatoes, bananas, and papayas, and it has great potential for preserving vegetables with high respiration rates. It is less effective on nonclimacteric fruit and horticultural products with slow respiration rates. Climacteric fruits should be subjected to CA at the earliest possible stage after harvest. Nonclimacteric fruits, such as pineapples, grapes, and limes, have greater flexibility in the afterripening process.

Oxygen concentration is lowered to the desired level in the storage room by first flushing with nitrogen gas or liquid nitrogen, and then injecting carbon dioxide from a gas generator. The overall result is that oxygen concentration declines and carbon dioxide concentration increases in the atmosphere around the stored commodities.

CA Storage Variables

The response of fruits and vegetables to CA storage varies with species, cultivar, growing conditions before harvest, prestorage treatments, maturity or ripeness stage at harvest, ethylene sensitivity, concentration of gasses, temperature in the storage room, and storage time.

The distinctive characteristic of the CA technique is that the storage variables must be rigorously controlled. Thus, to achieve optimum storage conditions for fruits and vegetables, the main variables to be considered are temperature; RH; the concentrations of oxygen, carbon dioxide, and ethylene; and storage duration (Figure 1).

Temperature

Cold storage is the most used method for maintaining the quality of fruits and vegetables. Lower temperatures lead to slower respiration rates and reduce other metabolic or degradation reactions. Temperatures are usually kept between 1 and 3 °C in CA storage, but the accurate optimal temperature depends on the produce and cultivar, as well as other characteristics of the produce.

The storage of fruits and vegetables at the lowest temperatures would seem to be the most beneficial for long-term storage. However, some products, especially tropical and subtropical fruits, show cold stress (termed 'chilling injury') when exposed to low but not freezing temperatures. Chilling injury implies physiological and biochemical disorders. It causes membrane breakdown and the release of metabolites, such as amino acids, sugars, and mineral salts, and enzymes from cells, resulting in enzymatic reactions and the development of harmful microorganisms. Some chilling injury symptoms are surface pitting, discoloration, internal breakdown, flesh browning, failure to ripen, loss of flavor, and decay (Table 1). The severity of the injury increases with lower temperatures and longer durations of chilling exposure.

The beneficial effects of using low-oxygen and high-carbon dioxide atmospheres to reduce the development of chilling injury have been reported. The effectiveness of CAs in reducing chilling injury varies with the commodity, maturity, gas composition, and, above all, the storage temperature. The critical temperature for chilling injury varies with the commodity and cultivar. For instance, it occurs at temperatures below about 12–13 °C in tropical produce and close to 0 °C in the majority of the other products.

Relative Humidity

The RH is also an essential CA storage factor to maintain the quality of fruits and vegetables. Most of these products should be maintained at high RH in the CA room, close to the saturation point (95%), to delay the ripening of different commodities; to maintain the freshness, flavor, and commercial weight of the commodities; and to restrict the development of chilling injury symptoms in sensitive tropical fruits.

However, low RH in the storage room induces the loss of moisture from living tissues (transpiration) that, in turn, causes weight loss in the stored fruits and vegetables. These changes deteriorate the produce appearance (color), texture (shrinking, wrinkling), juiciness, flavor, and nutritional quality. The impact of RH on the water loss of the stored product is strongly related to the temperature and air velocity in the storage room. Other factors, such as variety and maturity, are also important, however.

On the other hand, at high RH (100%), water condensation on the product may occur, providing an ideal environment for the growth of decay organisms. An RH between 85% and 95% is recommended for the storage of fresh produce, in order to restrict weight loss and microbial spoilage. Apples, cherries, peaches, pears, plums, apricots, lemons, berries, litchi, and avocados are among the fruits that need high humidity levels, and the vegetables that require these conditions include asparagus, broccoli, cabbage, carrots, cauliflower, celery, beans, peas, radishes, and corn.

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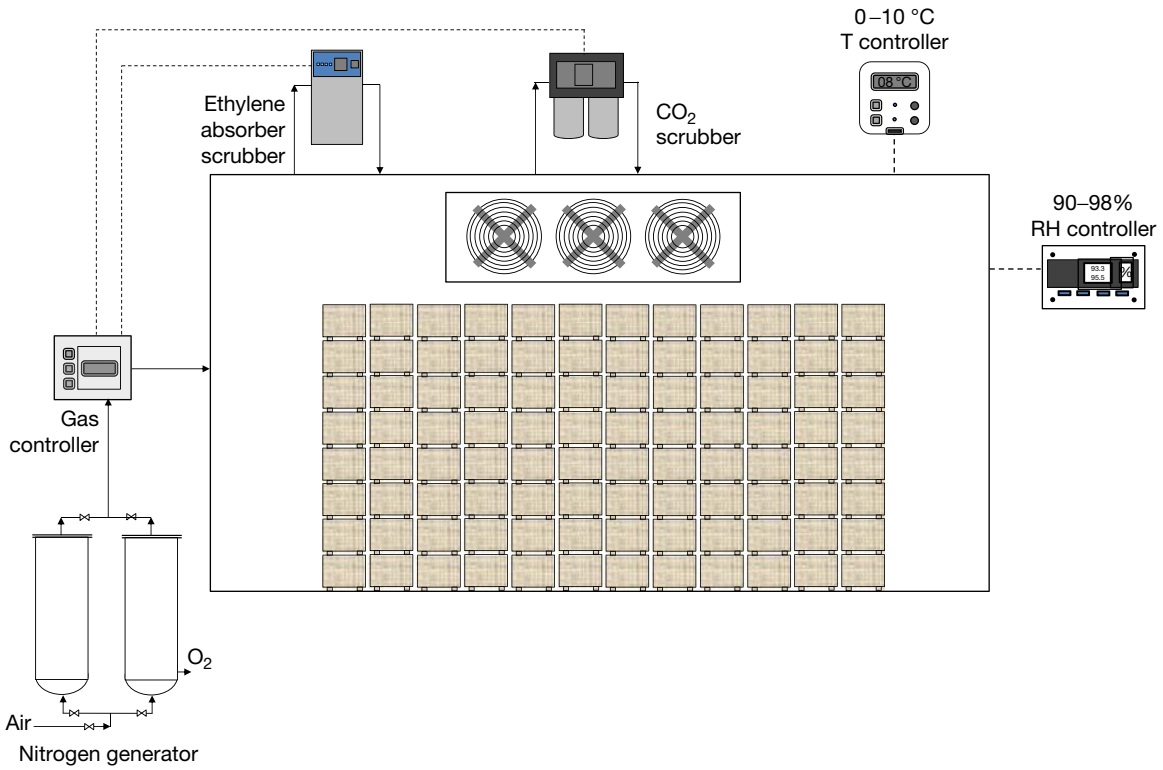

Figure 1 Cold storage room.

Table 1 Possible effects of CA storage conditions on fruits or vegetables

	Effect of storage condition	
Storage condition	*Optimal condition*[a]	*Out-of-range condition*
Decrease temperature	● Minimizes metabolic activity ● Minimizes respiration rate	Very low temperature causes: ● Chilling damage ● Freezing damage ● Browning ● Decay
Decrease O_2 level	● Reduces aerobic respiration rate ● Reduces degradation of color, texture, flavor, and vitamins ● Reduces production rate and action of ethylene ● Delays ripening of climacteric fruit	Very low concentrations induce: ● Fermentation ● Off-flavor ● Alcoholic taste ● Failure to ripen
Increase CO_2 level	● Minimizes aerobic respiration ● Inhibits the effect of ethylene ● Delays ripening ● Reduces discoloration due to enzymatic reactions ● Decreases the production of some organic volatiles ● Reduces the rate of breakdown of pectin and chlorophyll ● Retards fungal growth on the crop	Very high concentrations induce: ● Fermentation ● Drowning of tissue ● Softening ● Off flavor ● Decay

[a]Optimal and out-of-range O_2 and/or CO_2 concentrations depend on the commodity, cultivar, duration of storage, and interactions between O_2 and CO_2.

To assure the beneficial effects of CA storage, the RH needs to be monitored and controlled. Humidity is monitored with a hygrometer or psychrometer, and it is controlled by different means: operating a humidifier in the produce environment, wetting the storage room floor, regulating air movement, maintaining a difference between the air and the evaporator of 0.5–1 °C. At high RH, a small fluctuation in temperature can induce water condensation on the product surface and promote decay.

Oxygen and Carbon Dioxide

It has been demonstrated that, when the storage conditions of fruits and vegetables change from the normal air composition (78% N_2, 21% O_2, and 0.03% CO_2) to an environment with low concentrations of O_2 (1–10%) and high concentrations of CO_2 (1–20%), a reduction in the rates of metabolic activity, respiration, and ethylene production is induced. The effects of reduced oxygen and elevated carbon dioxide on metabolic

changes and respiratory activity are additive. The prime result of the metabolic modification is that the stored commodity maintains the quality characteristics (color, firmness, soluble solids, acidity, vitamins, and freshness) for a longer storage time. In addition, properly maintained levels of O_2 slow down microbial deterioration and inhibit enzymatic browning for some products, such as apples, lychees, and bananas.

However, storage in a CA environment may have adverse effects at too high carbon dioxide concentration (e.g., >3% for red delicious apples and apricots; >30% for cherries) or too low O_2 concentration (e.g., <1% for avocados and bananas). If the O_2 and CO_2 levels are not tolerated by fruits and vegetables, physiological disorders and loss of product may occur. For instance, low O_2 concentration changes the respiration of apples, pears, and tomatoes from aerobic to anaerobic, producing an accumulation of alcohols and aldehydes, which are responsible for undesirable flavors. The extent of these injury symptoms is also a function of exposure time and temperature.

On the other hand, elevated CO_2 concentrations (>5%) may affect the anthocyanin stability and color in red fruits such as strawberries, or it may inhibit the enzymatic activity, due to the high solubility of CO_2 in water and the consequent generation of carbonic acid and lowering of pH. Thus, the success of CA storage technology depends on convenient levels of gas being achieved and maintained in room storage.

Ethylene

Ethylene (C_2H_4) is a natural gaseous plant hormone, which is produced by fresh fruit and vegetables and released into the atmosphere around the fresh product. It increases the respiratory activity of most fruits and vegetables, which, in turn, determines the evolution of their physical and chemical characteristics (appearance, texture, taste, and color) and, consequently, their storage-life.

Controlling ethylene gas in the storage room preserves the commodity quality parameters and freshness, and it extends the life cycle, allowing the produce to be maintained for a longer storage time.

The rate of ethylene production in fruits undergoing ripening is determined by the ability of the fruit to synthesize 1-aminocyclopropane-1-carboxylic acid (ACC) and to convert ACC to ethylene, which is controlled by the activity of ACC synthase and ACC-oxidase.

The ethylene-releasing rate of commodities is highly dependent on the temperature and the concentrations of oxygen and carbon dioxide. For instance, the optimum temperature for ethylene production is 30 °C for apples, 32 °C for some plum cultivars, and 20 °C for peaches, nectarines, and pears.

The O_2 and CO_2 concentrations in the fruit play important roles in the biosynthesis and action of ethylene. Ultra-low oxygen and elevated carbon dioxide concentrations inhibit the activity of ACC synthase and ACC oxidase and suppress ethylene production in fruits such as apples and pears.

Ethylene induces the quality attributes of fruits and vegetables: color development and softening in apples and pears, the greening of citrus, and increases in soluble solids, organic acids, aroma, and flavor in honeydew melons. However, reduced produce quality can be related to the ethylene hormone. For example, this hormone promotes chlorophyll destruction in lettuce and cucumbers; yellowing and dropping of leaves in *Brassica* species; softening, pitting, and development of off-flavor in peppers and watermelons; and browning and discoloration in eggplant pulp.

The removal of ethylene from the CA room can retard the development of undesirable disorders. Preventing ethylene accumulation or ensuring an environment free of ethylene can be accomplished through the ventilation of the cold room, through the use of oxidizing agents (potassium permanganate or ozone), or through the filtration of the air.

Recommended CA Storage Conditions

The inherent variability of fresh commodities and their responses to storage conditions complicate the establishment of the optimal CA. Tables 2 and 3 show recommended temperature, RH, and oxygen and carbon dioxide concentrations for the storage of several fruits and vegetables. However, the recommended CA storage conditions found in literature for a specific fresh produce should be refined through experimentation, empirical observations, and mathematical modeling.

Table 2 Controlled atmosphere conditions for some fruits

Fruits	T *(°C)*	*RH (%)*	*O_2 (%)*	*CO_2 (%)*
Apricot	−0.5 to 0	90–95	2–3	2–3
Apple	0–1	90–95	1–3	1–4
Avocado	5–13	90–95	2–5	3–10
Banana	12–15	90–95	2–5	2–5
Fig	−1 to 0	90–95	5–10	15–20
Guava	5–15	90–95	5–8	2.5–5
Kiwifruit	0	90–95	1–2	3–5
Lemon	12–14	90–95	5–10	0–10
Mamey	1–14	90–95		5–10
Mango	12–14	90–95	5–10	0–10
Orange	3–8	90–95	5–10	0–5
Pineapple	7–13	85–90	3–5	5–8
Prickly pear	6–8	90–95	2	2–5
Mamey sapote	10–15	70–80	10–15	5–10
Strawberry	4	90–95	5	15

Table 3 Controlled atmosphere conditions for some vegetables

Vegetables	T *(°C)*	*RH (%)*	*O_2 (%)*	*CO_2 (%)*
Asparagus	0–1.5	95–100	7	7
Beans, green	8	95	3	3
Bell peppers	7–14	90–95	2–3	5
Broccoli	0	95–100	1–2	5–10
Cauliflower	0	95–98	2	5
Celery	0	98–100	2–4	3–5
Cucumbers	10–13	95	3–5	10
Garlic	0	65–70	1–3	5–15
Jicama	13–18	65–70		5–10
Lettuce	0–5	98–100	1–3	7–10
Mushrooms	0	95–98	3	10
Onions	0	65–70	3	5–7
Peas	0	95–98	2–3	2–3
Radish	0	95–100	1–2	2–3
Tomatoes	13–15	90–95	4	5

Conclusions

To date, controlled atmosphere storage is the unique technology that can assure long-term storage of fresh fruits and vegetables. Quality of fruits and vegetables is maintained through the application of specific CA storage conditions to each commodity, and control of the gas composition, temperature, and relative humidity of the environment. In addition to these factors, metabolism changes of fruits and vegetables are been considered to establish the optimal storage conditions. This is leading to the development of interactive storage systems, the next generation of storage systems.

See also: Apples; Ascorbic Acid: Properties, Determination and Uses; Avocado; Controlled Atmosphere Storage: Applications for Bulk Storage of Foodstuffs; Flavor Enhancers: Characteristics and Uses; pH: Principles and Measurement.

Further Reading

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Convenience Food

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Introduction

Convenience food is one of the major food trends in Western societies. However, it is not a new phenomenon. In Ancient Rome, the urban population mainly ate convenience food. The Romans did not even have a choice; their apartments did not include a kitchen so they had to obtain their food from cook shops in the streets. One reason for such a lifestyle was the high cost of firewood; a more practical reason was to prevent fires that could destroy whole quarters or cities. Today, there are different reasons for using convenience food: People want to save time and minimize their physical and mental efforts, and an increasing number of people no longer know how to cook a meal from scratch, so they are dependent on convenience food products.

Definition

There are many definitions of convenience food in the literature. However, most of them include the three aspects of time, effort, and skills. An early definition from 1979 by Traub and Odland defines convenience food as: "Any fully or partially prepared foods in which significant preparation time, culinary skills, or energy inputs have been transferred from home kitchen to the food processor or distributor." Today, the definition of convenience food not only includes the preparation stage of planning, buying, and preparing meals but also incorporates the following stages of consumption and cleanup.

The meaning of using convenience food products has changed dramatically over the decades. In 1950, for example, Haire presented two identical shopping lists to his participants, with the exception of ground coffee on one list and instant coffee on the other. Participants had to characterize the women who bought the items on the lists. Women who bought instant coffee were described as lazy, unable to manage their household, unthrifty, and just not good wives in general. It would be very interesting to replicate this study today; the results would certainly be quite different.

Classification Systems

Several different classification systems for convenience foods have been proposed, for example, classifying them on a scale ranging from zero convenience to full-service convenience, on the basis of the level of prior processing, or on the level of preparation required after industrial processing. Later classification systems have become more complex, for example, a matrix with two levels of prior processing and three levels of preparation and an even more complex matrix with four levels of shelf life and four levels of preparation. All of these classification systems were developed theoretically, and some of them were subsequently validated with consumer data. In the author's own research, the opposite approach was used. They started with food frequency data from consumers and conducted a principal component analysis to identify convenience food categories. Four components emerged: (1) highly processed food products (e.g., frozen ready-made meals), (2) moderately processed food products (e.g., chilled fresh tortellini), (3) single components (e.g., marinated meat), and (4) salads (e.g., cut and washed salad in bags).

Most of these classification systems show that the overall category of convenience foods is very heterogeneous, with some products being only slightly processed and others being heavily processed. There is a continuum of products being processed, and every convenience food product can be indexed at some level of processing. What level of processing is required for consumers to label a product a convenience food is not clearly defined. Today, hardly anybody would label bread, yogurt, and cheese convenience foods, although according to most definitions, they fall into this category. There are other products, such as gravies and soups, that some consumers would call convenience foods, while other consumers would not. And then, of course, there are products like ready-to-heat meals or frozen pizzas that every consumer considers convenience foods.

Overriding Trends

A major trend fostering convenience food consumption that is often discussed in the literature is the increasing participation of women in the workforce. The reason seems plausible: dual-income households have higher incomes but less time. Thus, they are willing to trade some of their money back into time by purchasing convenience food products. Surprisingly, most studies investigating the relationship between female participation in the workforce and convenience food consumption have found no evidence for a positive correlation. Only by applying attitudinal measures it was possible to find some evidence. For example, it was shown that meal preparers with a workload of more than 30 h per week were more convenience-oriented than those with a workload of less than 9 h a week.

Another overriding trend that results in higher convenience food intake is the increasing number of single-person households that can be observed in most Western countries. Although single people have more time available, they are not inclined to use that time cooking for themselves. An unpublished analysis by the author confirms that people living in single-person households consume more highly and moderately processed convenience food products than people living together with at least one other person. For salads, no significant difference was found, indicating that almost everyone buys cut and washed salads in bags. Finally, people from multiperson households consume more single components than those living in single-person households. This seems plausible, since with single components, one still has to cook

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and prepare a meal. Together with less-structured mealtimes and the replacement of traditional meals with snacks for all households, the increase in single-person households gives convenience foods a strong boost.

Whereas the causalities for the effects of the increasing female participation in the workforce and the increasing number of single-person households are straightforward, the causality for the decline of cooking skills is not clear. Do people use convenience foods because they lack cooking skills, or did the emergence of convenience foods cause the decline in cooking skills? The fact is that today, many consumers lack cooking skills and therefore depend on convenience foods. The author's own research shows that a lack of cooking skills is indeed a significant driver for convenience food consumption. The more people lack cooking skills, the more they consume highly and moderately processed food items. For salads and single components, cooking skills did not reach significance, which makes sense, as foods from these two categories require much less skill if one wants to prepare them from scratch than foods from the former two categories.

Drivers of Convenience Consumption

To identify the drivers of convenience food, the author conducted a comprehensive study. A sample of $N = 918$ participants completed a survey that included 17 common convenience food items and 14 potential drivers and seven sociodemographic questions. Usage of the various convenience food items differed greatly ranging from salad cut and/or washed, which was consumed on average 73 times per year, to instant pasta, which was only used 2.6 times per year. As mentioned in the preceding text, convenience food items were grouped into four categories based on a principal component analysis: highly processed items, moderately processed items, single components, and salads. For each category, the potential drivers were analyzed separately using regression analysis.

Age is the most important driver for convenience food consumption. The older the consumer, the less convenience food is eaten. This was true for all four categories. There might be two reasons for this: First, after retirement, people have more time available and might spend that time cooking, and second, older people are more accustomed to cooking from scratch from their younger days and may simply continue this habit. Another important driver is an affinity for naturalness. People who pay attention to the naturalness of food do not like processed food items. Naturalness was significant for all categories but salads. This seems plausible since cutting and washing do not affect the naturalness of a food item and, thus, people concerned with food's naturalness can buy these convenience products. Another strong driver is nutritional knowledge. This was significant for highly processed foods, single components, and salads. People with a high level of nutritional knowledge are capable of evaluating what is good for their health. Most highly processed foods and single components contain many additives and preservatives that concerned consumers might judge as unhealthy. For salads, the reason might be the cut and/or washed salad's sensitivity to fungi and bacteria. Most moderately processed foods, on the other hand, are fresh products (e.g., readily bought sandwiches and warm pizza bought/delivered) that are equivalent to self-prepared foods. Therefore, consumers with a high level of nutritional knowledge have no reason not to buy them.

Three more drivers turned out to be significant for two of the four categories: the presence of children in a household, a desire to avoid waste, and cooking skills. Having children reduces the consumption of highly and moderately processed food items. In these categories, one finds the typical ready-made meals that are convenient for single-person households. In a family household, it might be more efficient to prepare meals from scratch because of the higher number of persons living in these households. Another reason might be that parents are concerned about their children having health issues and therefore try to avoid processed foods containing a great deal of additives and preservatives. People who try to avoid food and packaging waste consume less moderately processed food items and less single components. For moderately processed foods, the issue of food waste may be the motivating factor, as these foods usually come in portions that are too large for one person. For single components, it might be the packaging waste, as most of these items are extensively wrapped. A lack of cooking skills is again a driver for highly and moderately processed food items. People with a lower level of cooking skills are not able to prepare the foods in these two categories by themselves and therefore depend on processed foods.

Each category features several drivers that are specific to it. For highly processed food items, these are working status and sociability. People working only part-time or not working at all consume more highly processed foods than people working full-time. This phenomenon has been observed in other studies as well. For example, a snacking consumer segment was identified that was less likely to be well educated and more likely to be unemployed. Sociability refers to an attitude of enjoying eating together with others. The more people display such an attitude, the fewer highly processed food products they consume. This is because in most households, it is still not appropriate to serve ready-made meals when inviting, for example, friends for dinner. For moderately processed food items, education is a specific driver. The more well educated people are, the more moderately processed food products they consume. This seems plausible since well-educated people are likely to have busy jobs but still want to eat rather healthy. Therefore, they go for the moderately processed foods that are freshly prepared, like a sandwich or other to-go foods. For single components, there are three specific drivers: value for money, household size, and physical effort. Value for money refers to a smart shopper mentality, that is, consumers who try to get the best value for their money. It seems that single components, such as marinated or crumbled meat and fish, have good value for their money since smart shoppers buy more of these food items. Household size is also positively related to the consumption of single components. This category also contains foods like frozen french fries and croquettes, things that are prepared easily and things that children really like. This might explain the correlation with household size. Physical effort is the third specific driver for single components. Everyone who has prepared french fries from scratch can understand why people who want to minimize the physical effort of preparing meals buy more foods from the single components category. Finally, two specific drivers emerged for

salads. Consumers who think that convenience foods are too expensive for what one gets buy fewer convenience salads. It seems that, particularly for salads, the surcharge does not seem justified. Some people are not willing to pay extra for merely having their salad already cut and washed. The second driver for salads is an attitude of considering the kitchen to be the woman's domain. The more people think that housekeeping is the woman's responsibility, the more convenience salads they consume. Salads might be regarded as a rather female food product, and therefore, people with such an attitude might want to avoid preparing these foods.

It is also just as interesting to look at the potential drivers that did not turn out to be significant. Surprisingly, variables related to time and effort, such as time-saving, time pressure, and mental effort, did not predict convenience food consumption, with the exception of the physical effort for single components, as discussed earlier. The reason for this might be that convenience foods are now omnipresent. They have crept into our daily lives without us noticing; think of gravy, for example. Today, everyone uses convenience food products. Therefore, these time- and effort-related variables do not differentiate consumers' convenience consumption. Of course, this does not mean that time and effort are not important reasons to use convenience foods; it is just that they do not differentiate consumers and therefore do not turn out to be significant predictors. Instead of having reasons to use convenience foods, it seems that all consumers use them and only consumers who have explicit reasons not to use these items, such as an affinity for naturalness and concerns resulting from a high level of nutritional knowledge, will abstain from consuming them. In other words, the standard is to use them, and people only avoid using such products if they have reasons not to consume them. Besides these time- and effort-related variables, three more potential drivers did not prove significant. For cooking involvement, the same reasoning mentioned earlier might apply. Regardless of the level of cooking involvement, convenience food products are used. Income was also insignificant. One explanation could be that many convenience foods are not really that expensive. For example, a ready-made frozen pizza is usually even cheaper than a self-made pizza when adding up the costs of the ingredients. If one includes the preparation time required to cook a pizza from scratch, it is even worse. More surprising is that concerns about a healthy diet did not predict convenience food consumption. Many researchers found that consumers have an unfavorable image about convenience food. However, it seems that when it comes to a single convenience food product, consumers might not judge it to be unhealthy; they only have an unhealthy image when they think about the marketing category of convenience food in general.

Consumer Segments

In 2007, Buckley and colleagues conducted a comprehensive survey to identify convenience food lifestyle segments. A total of $N=779$ respondents were included in the cluster analysis that was run over 20 factors related to convenience food consumption. The authors identified four convenience food lifestyle segments: (1) food connoisseurs, (2) home meal preparers, (3) kitchen evaders, and (4) convenience-seeking grazers.

For food connoisseurs, convenience is not important. They like to experiment in the kitchen, and eating for them is a social event that is done together with family or friends. They eat proper meals and do not snack in between these meals. They like to take their time when cooking, they are not in a hurry, and they do not feel time pressure. Therefore, they are less likely to buy and consume convenience foods. Food connoisseurs are not very price-sensitive; in fact, they like to spend money on food. For them, cooking is not only a woman's task; male food connoisseurs like to cook either alone or together with their wives. Consumers belonging to this segment think that convenience foods are rather unhealthy and not very attractive in terms of their value for the price. This segment comprises 26% of all consumers, has an average age of about 40 years, and includes the highest proportion of men.

Home meal preparers are similar to food connoisseurs but even less convenience-oriented. They have plenty of time resources to shop and prepare foods and to clean up after a meal. This is the segment that purchases the fewest convenience food products; they prefer preparing a meal from scratch. Home meal preparers are well organized when it comes to shopping for food. They pay attention to freshness, and they try to get good value for the price they ought to pay. Meal preparation activities are a family endeavor as is the intake of meals. A microwave is usually not found in these households. As with food connoisseurs, home meal preparers regard convenience food products as rather unhealthy and expensive. This segment makes up 25% of consumers, most of whom are older and retired, and is more or less balanced in regard to gender.

Kitchen evaders comprise the first of two highly convenience-oriented segments. They use convenience foods to make their lives easier and to avoid food waste. With convenience food, they just buy what they need so nothing is spoiled. If they cooked from scratch, they would have ingredients left that they would have to then throw away. Kitchen evaders are busy people who are always under time pressure. Therefore, they do not want to plan their meal preparation activities; they decide what to eat spontaneously while they are shopping. They are neither interested in product information, nor do they want to try new foods. Instead of having proper meals, they often snack during the day. They are used to eating alone and display a high level of individualism in their consumption behavior. Kitchen evaders have a positive attitude toward convenience food products and think that these products offer good value for their price, are healthy, and save them a lot of time. This segment consists of 16% of all consumers, is below the population's average age, and is rather balanced when it comes to gender.

The last segment is the convenience-seeking grazers. Similar to kitchen evaders, they are very convenience-oriented and are also similar in many other aspects. Unlike kitchen evaders, they are organized in their meal preparation activities and more price-sensitive. Although this segment has the highest proportion of females, convenience-seeking grazers are more likely to think that the kitchen is the woman's task. When it comes to the trade-off between freshness and convenience, they opt for convenience. Similar to kitchen evaders, they are time-stressed, use the microwave, often eat alone, and snack in between meals. Interestingly, convenience-seeking grazers perceive convenience foods as the least beneficial for time-saving. However, they have a positive attitude toward convenience

foods and think that they have good value for their money. One-third of consumers fall into this segment, and most of them are between 30 and 50 years of age.

Healthy Convenience Food

Convenience food has a reputation of being unhealthy. However, the food industry has recently started combining the convenience food trend with the trend of healthy eating by producing healthy convenience foods and healthy ready-made meals. In a tasting test of two rather healthy ready-to-heat meals – one with chicken and rice and the other with salmon and pasta and both with a large portion of vegetables – overall liking was found to be the strongest predictor of the likelihood of buying for both meals. Sociodemographic variables were only relevant for the salmon meal, whereas for the chicken meal, no such effects were observed. It seems that consumers are not ready to make trade-offs when it comes to taste. The food industry's challenge will be to develop healthy and tasty convenience foods, not just one or the other.

Another study segmented consumers based on their health-related motive orientations and found two segments to be particularly inclined to choose ready-made meals. For the first segment, vitality and energy were the main terms used to describe health. They want to keep their bodies in good condition to live active lives and to explore new things. The second segment perceives health mainly in terms of achievement and outward appearance. They want to look good, stay slim, and be successful. Individuals from both segments were rather young, with most of them having no children. This paints a picture of who will be buying healthy convenience food products. It is the young, busy consumers who are successful in what they are doing and who lead active lives.

Convenience food products will surely become healthier in the years and decades to come. Consumers' demand for healthy food is steadily rising, and the trend toward increased convenience will not stop. The ability to bring these two major trends together will be decisive for the success of a particular convenience food producer. Consumers will benefit from this development by obtaining healthy food and saving time, which will improve their work–life balance.

See also: Adolescent Nutrition; Appetite Control in Humans: A Psychobiological Approach; Chilled Foods: Effects on Shelf-life and Sensory Quality; Chilled Foods: Modified Atmosphere Packaging; Chilled Foods: Packaging Under Vacuum; Chilled Foods: Principles; Consumer Protection Legislation; Controlled Atmosphere Storage: Applications for Bulk Storage of Foodstuffs; Controlled Atmosphere Storage: Effect on Fruit and Vegetables; Cooking: Domestic Techniques; Obesity: The Role of Diet; Preservation of Foods; Preservatives: Food Use; Stabilizers: Types and Function.

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Cooking: Domestic Techniques

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Introduction

As with all forms of cooking, domestic cooking is intended to improve the palatability of the food, making it more appetizing. Unlike industrial food preparation and catering or food service, people who may not have any technical knowledge of what is happening from an engineering or biochemical point of view carry out domestic cooking in the home. By definition, cooking raises the temperature of the food, which results in a number of simultaneous and interrelated processes that influence flavor, texture, appearance, nutrient content, and food safety. The different techniques for domestic cooking reflect the way in which the temperature of the food is raised (Figure 1).

Clearly, there are two basic ways in which energy can be applied to a foodstuff to achieve a rise in temperature. The traditional route is by contact with a heated medium, which causes heat to flow to the surface of the food and, subsequently, to the center through conduction. An alternative route is to apply electromagnetic radiation. Of the two types of electromagnetic radiation commonly used in domestic cooking, infrared radiant heating (grilling) employs short-wavelength radiation that is only able to penetrate a couple of millimeters below the surface of the food. The inner regions of the food are heated by conduction. Microwaves have longer wavelengths that are able to penetrate deep into foods, generating heat *in situ*, albeit in nodes requiring food to be rotated during cooking to ensure even distribution of heat.

In many cases, energy transfer during cooking is not via a single mechanism. For example, ovens absorb and emit infrared energy and baked food heat through a combination of convection and radiation. Similarly, barbecues emit infrared radiation and generate hot combustion gases, which flow around the food, heating it by convection.

General Considerations

Surface Heat Transfer

The crucial difference between the different techniques is what happens at the surface of the food.

When a solid food is placed in a hot fluid, there is a stagnant layer of fluid around the food. This boundary layer acts as an insulating barrier that slows the flow of heat from the fluid to the food.

Air is a good insulator (thermal conductivity, 0.024 W m^{-1} K^{-1}); thus, a boundary layer consisting of air slows significantly the flow of heat from an oven to the food (Figure 2(a)). One way of reducing the thickness of this boundary layer is to agitate the heating medium. In the case of fan-assisted ovens, air is forced to circulate, thereby impinging on the surface of the food and physically reducing the thickness of the boundary layer (Figure 2(d)). The surface heat transfer coefficient in a fan-assisted oven is about 10 times greater than in a conventional oven.

Heat travels by conduction through static boundary layers. Heating media that are better conductors of heat than air will increase the rate of heat flow to the surface of the food. Water has a thermal conductivity of 0.573 W m^{-1} K^{-1}, and the rates of convective heat transfer during boiling and simmering are, therefore, considerably faster than in ovens at the same temperature (Figure 2(e)). However, the maximum temperature attainable with water is limited by its boiling point. Alternative liquid heating media, such as oil, have thermal conductivities in the same region and can operate at temperatures around 180 °C. Hence, rates of heat flow to the surface in frying are considerably greater than in boiling. This is also attributable in part to physical agitation of the boundary layer caused by generation of water vapor at the surface of the food, which is in contact with the hot oil (Figure 2(g)).

When saturated steam is used as the heating medium, heat flow to the surface of the food is simultaneously accompanied by condensing of the steam. The dramatic volume change during condensation results in fresh steam flowing to occupy the void, thereby maintaining a virtually negligible boundary layer (Figure 2). However, if the steam is not saturated, the non-condensing air accumulates at the surface of the food, forming an insulating layer. In fact, as little as 6% air in steam reduces surface heat transfer by 90%.

Rate of Heating

During heating, the rate at which heat flows into any food depends on its shape and size and the temperature difference between the food and the heating medium as well as the rate of heat transfer between the food and the heating medium.

Obviously, large surface areas allow more heat flow than small surfaces. Shapes with a higher surface-to-bulk ratio, such as spherical foods, will heat quicker than other shapes (e.g., slabs). Consequently, considering how rapidly heat penetrates to the center of two 'spherical' potatoes of different sizes, evidently, it takes longer for heat to penetrate to the center of a larger potato. There are two reasons for this: greater distance for heat to flow and a smaller area to mass ratio.

The driving force in any heating operation is the difference in temperature between the food and the heating medium. The greater the differences, the faster the rates of heat transfer. In the case of domestic cooking, foods heat by unsteady-state heat transfer. That is to say, the temperature difference reduces as the temperature of the food rises, and hence, the driving force lessens as the process ensues.

The physical properties of the food and the heating system contribute to the heating rate. Factors such as how easily the heat is able to flow through the insulating surface boundary

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<table>
<tr><td colspan="5">Energy transferred by contact with a heated:</td><td colspan="2">Energy transferred by electromagnetic radiation</td></tr>
<tr><td rowspan="2">Solid</td><td colspan="2">Liquid</td><td colspan="2">Gas or vapor</td><td colspan="2">Typical wavelength</td></tr>
<tr><td>Oil</td><td>Water</td><td colspan="2">Steam</td><td colspan="2">Air convection</td><td>0.03 mm</td><td>300 mm</td></tr>
<tr><td></td><td></td><td></td><td>Atmospheric</td><td>Pressure</td><td>Natural</td><td>Forced</td><td></td><td></td></tr>
<tr><td>Griddling</td><td>Frying</td><td>Boiling
Simmering</td><td>Steaming</td><td>Pressure cooking</td><td colspan="2">Roasting/baking</td><td>Grilling</td><td>Microwave</td></tr>
<tr><td colspan="7">All these techniques achieve surface heating: heat flow to center by conduction</td><td>Heat generated throughout</td></tr>
</table>

Figure 1 Classification of domestic cooking techniques.

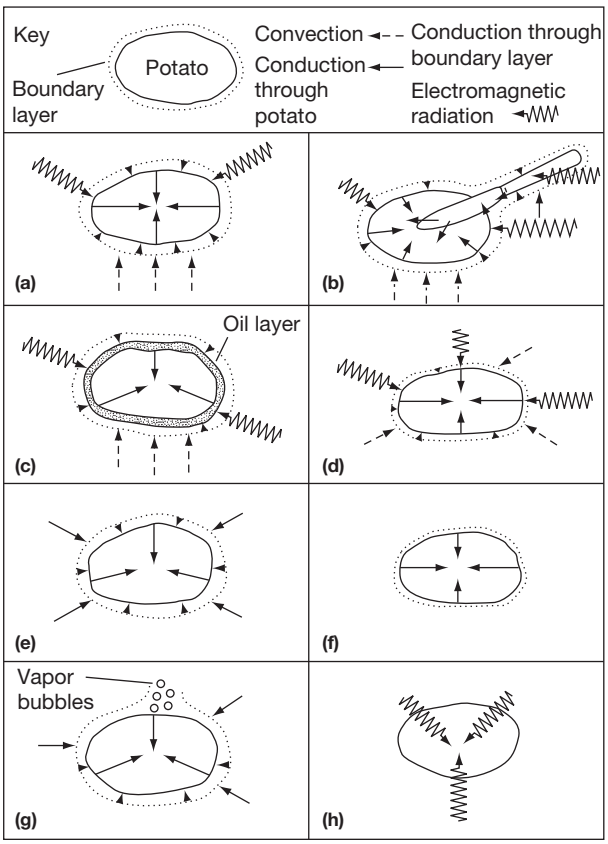

Figure 2 Eight ways to cook a potato: (a) baking, (b) baking with a good conductor (e.g., knife) inserted, (c) roasting, (d) forced convection (fan-assisted oven), (e) boiling, (f) steaming or pressure cooking; (g) frying (chipped), (h) microwaving.

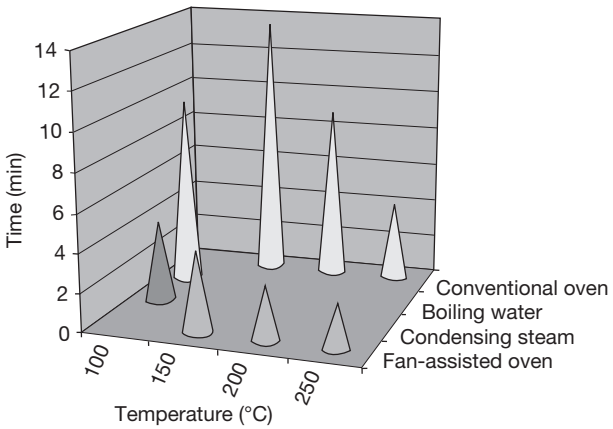

Figure 3 Predicted time for the center of 2 cm diameter potatoes to reach 80 °C.

layer and through the bulk of the food by conduction toward the center are influential.

There are methods for predicting rates of heating based on the physical properties of materials and heating systems. Figure 3 illustrates factors that influence the rate of heating. Time taken for a food of fixed shape and size to reach a particular temperature has been used as a measure of heating rate. In Figure 3, 2 cm diameter spherical potatoes starting at 5 °C have been used. Identical potatoes are placed into different heating media at designated temperatures and the time taken for the core temperature to reach 80 °C predicted. The temperature (80 °C) has been chosen as a realistic temperature for many types of meat and vegetables and one that will achieve gelatinization of starch in a food like a potato.

Clearly, short cooking times can be achieved in a number of ways; working at higher temperature achieves more rapid cooking, as does selecting a cooking process with a high surface heat transfer coefficient. Hence, a fan-assisted oven is faster than conventional ovens because they reduce the thickness of the insulating surface boundary layer around the food.

Cooking by Electromagnetic Radiation

Infrared

As with the other traditional forms of cooking, grilling (broiling) causes the surface of the food to heat, and the center heats by conduction. Absorption of infrared radiation at the surface is proportional to the difference between the fourth power of the radiation source temperature and the surface of the food. However, foods absorb infrared radiation to differing extents depending on their absorptive capacity (e.g., black body, 1; perfect reflector, 0; and water, 0.96).

Conventional grilling and barbecuing are restricted by the fact that radiant energy is supplied from one direction; cooking of all sides is only achieved by frequently turning the food, for example, on a spit.

In practice, infrared heating is involved in many cooking techniques, such as baking and roasting. In these cases, oven walls heat up by convective heat transfer and then emit infrared radiation.

Microwaves

Microwaves penetrate deep into most foods; their sinusoidal electromagnetic waveform causes dipoles (e.g., water) to oscillate, which in turn generates heat (Figure 2(h)). As microwaves pass through a food material, energy is lost; although heating occurs within the food, it is greatest at the surface. The actual temperature achieved within the food depends on several factors, including the frequency of the radiation, strength of the field, dielectric loss factor of the food, free moisture present, and the shape of the food.

Quality Changes during Cooking

General Considerations

Arguably, there are only two basic cooking methods: wet and dry. Wet cooking includes boiling, simmering, steaming, pressure cooking, stewing, and poaching. During wet cooking, the surface temperature of the food does not exceed the boiling point of water (usually 100 °C, but up to about 120 °C in domestic pressure cookers); consequently, the surface remains moist. Dry cooking includes frying (deep and shallow), baking, roasting (in an oven with natural or added fat), grilling (broiling), barbecuing, and griddling. The surface temperature during dry cooking may well exceed 100 °C, leading to evaporation of moisture and a dry or crisp surface.

Whatever is being cooked, it is worth bearing in mind that most foods are biological in origin and many are still respiring up to the time of cooking. The rise in temperature during cooking can result in a disruption of structural integrity and the termination of normal metabolic activity.

We eat a diverse range of foods, each with unique quality criteria. Since changes that occur during cooking are dependent on both the temperature achieved and how long food is held at each temperature, product quality is constantly changing. Most of the changes taking place during domestic cooking can be described by first-order Arrhenius kinetics (i.e., after a finite activation energy is reached, rate of change is proportional to a rate constant, which is affected exponentially by temperature). Activation energy and the rate constant vary for different quality factors; moreover, any particular factor may behave differently in each food (e.g., thiamine is destroyed more rapidly during cooking of rainbow trout than in a buffered solution with the same time–temperature profile). If a food is cooked on the basis of one quality characteristic (e.g., color or texture) and if the time–temperature treatment is varied from an established procedure, other quality parameters may be suboptimal. For example, microwaved meat may not develop the same flavor as conventionally roasted meats.

Textural Changes

No single mechanism is responsible for the texture of foods, yet changes in protein structure and solubilization of polysaccharides (and some proteins) are the two primary phenomena involved.

Heat causes the tertiary structure of proteins to unwind. Heating for long periods or at high temperatures frequently results in irreversible changes in tertiary structure, termed denaturation, which causes changes in functional properties that may or may not be desirable. If the protein is highly charged, the uncoiled amino acid chains tend to repel one another increasing the affinity for water and enhancing solubility.

If the protein is reasonably close to its isoelectric point, the amino acid chains tend to attract one another, with hydrophobic interactions and hydrogen bonds, forming a network of chains. This association behavior results in a reduction in the amount of water associated with protein and the following consequences: a loss of protein solubility; precipitation of the protein from solution, a solid structure (thermal setting or gelation); loss of water-holding capacity accompanied by an aqueous exudate from the product (often observed when cooking meats); shrinkage as both the aforementioned occur; and increased opacity of the food (e.g., egg white).

Some proteins undergo reversible thermal transitions when heated. For example, collagen (and its partially hydrolyzed derivative, gelatin) is solubilized when heated. Its prevalence in connective tissue means this has important implications in the tenderness of cooked meat and is responsible in part for the softening action of long-duration stewing.

Solubilization is also a process that affects polysaccharide material between cell walls of plants. Dissolution of pectin results in softening of plant tissues. Addition of sodium bicarbonate enhances solubilization of pectin, through production of the sodium salt, and displaces calcium ions that are naturally chelated in the structure. Another mechanism commonly involved in providing rigidity in plant tissues is turgor. Denaturation of proteins present in the cell membranes causes termination of osmoregulation and softening of the tissues. Deliberate protein denaturation is the aim of blanching, brief

exposure to temperatures around 85 °C with the intention of destroying flavor-modifying enzymes prior to home freezing.

Starch is present in many plant foods, and heating starch with an ample supply of water leads to gelatinization. The granules swell as they absorb water and the crystalline regions are disrupted. This has both a softening effect and a thickening effect, which is why gelatinized starch provides the viscosity of many sauces. The light, open texture of baked flour products is due to the presence of gas or air cells in the prebaked dough or batter. During heating, the gases expand aided by the generation of additional gas from chemical leavening agents (e.g., sodium bicarbonate) and increased vapor pressure of the water present. Finally, the liquid matrix is heat-set by denaturation of proteins and starch gelatinization. In the case of popcorn, the impervious grain coat acts like the wall of a pressure vessel. As the grain is heated, the water vapor pressure increases until the wall ruptures. The vapor flashes off, opening out the endosperm, which then heat-sets.

A crisp outer surface is often associated with dry cooking because temperatures in excess of 100 °C result in moisture being lost from the surface.

Color and Flavor Changes

With the exception of added colors sometimes used in domestic cooking (e.g., saffron or cochineal), two types of color change result from domestic cooking: modification of natural pigments present in the raw materials and browning reactions. Most natural colors tend to be relatively unstable when cooked. In addition to these chemical reactions, in wet cooking, loss of natural color may result from leaching of water-soluble pigments into the cooking medium.

Metalloporphyrin colors are found in foods of both animal origin and plant origin. Myoglobin, the pigment found in muscle, undergoes changes color from red purple to brown when heated; this is as a result of certain amino acids in the globin protein becoming coordinated with the central iron atom. Chlorophylls are also prone to lose their color when heated in acid conditions. The change from bright green to a grayish green is the result of demineralization of the central magnesium atom from the molecule. Other natural pigments exhibit sensitivity to pH. Anthocyanins, for example, change from red (in acid conditions), to colorless (when neutral), to blue (when alkaline). This can result in color changes when heat disrupts cells and allows these water-soluble pigments to mix with the cooking water.

Browning reactions tend to occur at low levels of available water. They are, therefore, common in dry cooking where the surface layers may dry out. Caramelization occurs when sugars are heated, particularly in the presence of acids or alkalis, and is important in both sugar and flour confectionery. The most famous of these reactions is the Maillard browning, which comprises the reaction between carbonyl groups (as found in reducing sugars) and amino groups. The reaction gives rise to a variety of brown compounds and characteristic aromas. Simple mixtures of one amino acid and one reducing sugar have been shown to produce distinctive aromas, reminiscent of particular foods (e.g., glucose and cysteine heated at 180 °C for 30 s smells of puffed wheat, yet after 3 min, it smells of overroasted meat). Generally speaking, volatile components are lost during cooking, and this can result in a loss of the uncooked flavor. Since the end user carries out domestic cooking, loss of volatiles is not completely wasted and may act as an appetizer.

Sustained heating of dry surfaces can result in carbonization, usually accompanied by smoke. In such circumstances, the surface browns and then blackens as the food burns; such products are usually regarded as spoiled.

Nutritional Changes

Although the act of cooking involves raising the temperature of the food, the term is frequently undifferentiated from other food preparation procedures, many of which commonly precede cooking. Peeling and trimming are two such operations that can lead to substantial loss of available nutrients, by cutting unsightly portions off the food and throwing them away.

Nutrient loss during cooking is attributable to two basic routes: thermally induced chemical reactions and leaching of nutrients into the cooking medium.

Many nutrients are thermally unstable, and when heated, concentrations fall exponentially with time. Obviously, different nutrients have unique rates of destruction. The most sensitive are ascorbic acid (vitamin C) and folic acid, both of which can be completely destroyed by domestic cooking. Of the essential amino acids, lysine is the least stable and up to 40% may be lost by domestic cooking practices. In general, rapid cooking methods, using high temperatures for short periods or microwaves, cause less nutrient destruction than long-duration, low-temperature cooking methods such as stewing. High-temperature short-time processes, such as frying, can result in similar levels of vitamin C as in raw potatoes.

In addition to thermal decomposition, nutrients can be lost through reactions with each other. For example, proteins will participate in the Maillard reactions, particularly when ε-amino groups are present.

Sodium bicarbonate is sometimes added to vegetables for its softening effect. Unfortunately, its addition leads to the destruction of vitamin C as well as chemically modifying proteins, lowering their biological value.

Cooking processes are not entirely detrimental from a nutritional point of view. For example, frying can result in an uptake of oil, resulting in increased energy density of foods.

Safety Aspects

A common misconception is that domestic cooking is performed purely to improve the gastronomic experience to the detriment of biological value. In fact, domestic cooking can make safe products that would otherwise contain harmful or toxic components.

Harmful components in foods arise in the form of naturally present toxins (e.g., cyanides in kidney beans and cassava) or compounds that interfere with digestion, effectively making the food less nutritious (e.g., trypsin inhibitors found in many legumes). The presence of pathogenic microorganisms may lead to infections, while other microorganisms produce toxins, both of which result in food poisoning. Cooking is

effective at destroying or removing many of these harmful components. However, some toxins are relatively heat-stable (e.g., the toxin produced by *Staphylococcus aureus*) and may not be destroyed by temperatures and times incurred during domestic cooking. Domestic cooks without formal training may be unaware of the risks involved in eating particular foods or to the neutralizing effects of cooking. A further risk arises from cross contamination of cooked food by raw ingredients, resulting in the potential for fresh growth of pathogenic microorganisms.

See also: Arsenic: Properties and Determination; Browning: Non-enzymatic browning; Colors: Properties and Determination of Natural Pigments; Starch: Structure, Property, and Determination.

Further Reading

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Copper: Physiology

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Properties, Production, and Industrial Uses of Copper

Copper (Cu) is a transition metal with atomic number 29. Cu has 29 isotopes, two stable isotopes (^{63}Cu and ^{65}Cu), and 27 radioisotopes. The most abundant isotope is ^{63}Cu that accounts for approximately 69% of naturally occurring Cu. Cu has a face-centered cubic crystal structure. Pure Cu is reddish orange in color, malleable, and a good conductor of heat and electricity.

Cu is naturally present in the Earth's crust and is found in the environment as a metal and in various minerals (e.g., cuprite and malachite). Concentrations can vary from less than 50 ppm to more than several thousand ppm in certain areas. Most of the extracted Cu comes from sulfide ores. Both natural and anthropogenic activities release Cu into the environment. Natural events include volcanic eruptions, windblown dusts, forest fires, and decaying vegetations. Mining, milling, and metal productions are common human activities that discharge Cu into the environment. Mean Cu concentrations in air range from 5 to 200 ng per cubic meter in rural and urban areas. Combustion of fossil fuels is a major contributor to airborne Cu.

In 2013, the largest producers of Cu were Chile, China, Peru, the United States, and Australia. Chile was the top producer with an estimated production of 5 700 000 tons. Cu is used in a variety of industrial applications. Major uses are in electrical wires, plumbing, roofing, and industrial machinery. Cu is also combined with other elements such as zinc and tin to make the alloys brass and bronze, respectively. These alloys are used in applications that require a harder material than pure Cu.

Analytic Measurement of Copper

The most commonly used methods for determining Cu content in food and environmental and biological samples are flame or graphite furnace atomic absorption spectrometry (AAS) and inductively coupled plasma–atomic emission spectroscopy (ICP-AES). In AAS, the sample is atomized by a flame or graphite tube (electrothermal). Quantification of Cu (or other elements) is made by determining the absorption of element-specific optical radiation (light) by free atoms in the gaseous state. ICP-AES produces excited atoms and ions using inductively coupled plasma. The atoms emit electromagnetic radiation at characteristic wavelengths and the intensity of the emission is related to the concentration of the atoms in the sample. For both AAS and ICP-AES, standards with known analyte concentration are used to derive the concentration of the analyte in the sample. Prior to Cu determination by AAS or ICP-AES, samples often need to be prepared for analysis. The sample preparation procedure depends on the specific sample and can involve acid digestion, filtration, and acidification. Additional methods that can be used for the measurement of Cu include inductively coupled plasma-mass spectrometry (ICP-MS), anodic stripping voltammetry, x-ray fluorescence, neutron activation analysis, photon-induced x-ray emission, and gas/liquid chromatography.

Reference Values for Copper

Nutrient reference values for Cu have been established by scientific bodies. Table 1 shows recommended intakes and tolerable upper intake levels (UL) for Cu by life stage group established for North America (i.e., Canada and the United States) and the European Union. Depression in Cu status induces a number of quantifiable physiologic changes. Despite this, no single indicator was deemed sufficient for deriving the estimated average requirement (EAR) for Cu as part of the North American Dietary Reference Intakes due to inconsistencies in results from studies measuring similar biomarkers. For adults aged $\geq$19 years, four indicators were used to establish the EAR: serum Cu concentration, serum ceruloplasmin concentration, erythrocyte Cu/Zn superoxide dismutase (SOD1) activity, and platelet Cu concentration. The EAR for adults range from 0.7 to 1 mg day^{-1} and the recommended dietary allowances (RDA) range from 0.9 to 1.3 mg day^{-1}. EAR and RDA are the same for males and females and these reference values are higher for pregnancy and lactation. It was determined that there were inadequate data to separately establish EAR for infants, children, and adolescents. For infants 0–12 months, adequate intakes were established based on mean Cu intake of infants fed with mainly human milk. For children and adolescents aged 1–18 years, the EAR were derived by extrapolation from the adult EAR. The European Union established population reference intakes that are slightly higher than the North American RDA.

There are limited data on the effects of high intakes of Cu in healthy people. Chronic high intakes of Cu result in the accumulation of Cu in the liver that can lead to liver damage. Acute ingestion of large amounts of Cu salts in drinking water has been reported to cause gastrointestinal symptoms; however, liver damage was selected as the more relevant endpoint to establish the UL for Cu. A no observed adverse effect level (NOAEL) of 10 mg day^{-1} was established for adults based on data showing no liver injury in adults consuming 10 mg of supplemental Cu daily. North America applied an uncertainty factor (UF) of 1 to the NOAEL to derive a UL of 10 mg day^{-1} because of the low prevalence of liver damage from Cu exposure in human populations with normal Cu homeostasis and other evidences indicating the absence of adverse effects with Cu intakes of 10–12 mg day^{-1} from foods. The European Union set a more conservative UL of 5 mg day^{-1}, applying a UF of 2 to account for variability to Cu toxicity within the normal population. It was also concluded that the UL is not

Table 1 Reference values for copper[a]

	North America[b]				European Union[c]	
Life stage group	*AI (mg day^{-1})*	*EAR (mg day^{-1})*	*RDA (mg day^{-1})*	*UL (mg day^{-1})*	*PRI (mg day^{-1})*	*UL (mg day^{-1})*
0–6 months	0.2	–	–	–	–	–
6–11 months	–	–	–	–	0.3	–
7–12 months	0.22	–	–	–	–	–
1–3 years	–	0.26	0.34	1	0.4	1
4–6 years	–	–	–	–	0.6	2
4–8 years	–	0.34	0.44	3	–	–
7–10 years	–	–	–	–	0.7	3
9–13 years	–	0.54	0.7	5	–	–
11–14 years	–	–	–	–	0.8	4
14–18 years	–	0.685	0.89	8	–	–
15–17 years	–	–	–	–	1	4
19–50 years	–	0.7	0.9	10	1.1	5
51–70 years	–	0.7	0.9	10	1.1	5
>70 years	–	0.7	0.9	10	1.1	5
14–18 years (P)	–	0.785	1	8	1.1	–
19–50 years (P)	–	0.8	1	10	1.1	–
14–18 years (L)	–	0.985	1.3	8	1.4	–
19–50 years (L)	–	1	1.3	10	1.4	–

[a]AI, adequate intake; EAR, estimated average requirement; L, lactation; P, pregnancy; PRI, population reference intake; RDA, recommended dietary allowance; UL, tolerable upper intake level.

[b]Values obtained from Food and Nutrition Board, Institute of Medicine (2001). Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press.

[c]Values obtained from the Scientific Committee on Food, European Food Safety Authority (2006). Tolerable upper intake levels for vitamins and minerals. Parma, Italy: European Commission Publications Office; Scientific Committee for Food, Commission of the European Communities. (1993). Nutrient and energy intakes for the European community. Luxembourg: Office for Official Publications of the European Communities.

applicable during pregnancy and lactation because of limited data relating to these life stages. Given the paucity of data on Cu toxicity in healthy infants, children, and adolescents, a UL was not set for infants and UL for children and adolescents were derived by extrapolation from the adult UL.

Sources of Copper Exposure

For humans, the main sources of Cu exposure are food, Cu-containing supplements, and drinking water. Intake of Cu through dermal routes or inhalation is minimal for most people. Table 2 shows the Cu content of foods rich in Cu obtained from the Canadian Nutrient File. Foods have been separated into different food categories, and the Cu content per serving size and 100 g of the food source have been provided. Organ meats, seafood, nuts, and seeds are rich in Cu. Consumption of one serving of foods highest in Cu content is sufficient to meet the daily Cu requirements for an adult.

Usual Cu intakes from food and supplements for the US population were determined in the third National Health and Nutrition Examination Survey (1988–1994). For men and women aged 19–70 years, mean Cu intakes from food alone ranged from 1.54 to 1.70 and 1.13 to 1.18 mg day^{-1}, respectively. Mean Cu intakes increased to 1.79–1.85 mg day^{-1} for men and 1.32–1.45 mg day^{-1} for women when Cu intake from supplements was also considered. Cu intakes for most people were above recommended intakes and below the upper limits. However, usual intake of Cu from food and supplements at the 5th percentile was below the EAR for women of several age groups. Mean Cu intakes from food for adults in European countries range from 1.0 to 2.3 mg day^{-1} for men and 0.9 to 1.8 mg day^{-1} for women.

Health-based and aesthetics-based guidelines for Cu in drinking water have been established. The US Environmental Protection Agency (US EPA) has set a maximum contaminant level goal of 1.3 mg l^{-1} to protect against gastrointestinal symptoms. The World Health Organization (WHO) has set a guideline value of 2.0 mg l^{-1} to protect against acute gastrointestinal effects and to provide a margin of safety for populations with normal Cu homeostasis. Canada has set an aesthetics-based guideline for Cu in drinking water of $\leq$1.0 mg l^{-1} to ensure palatability and to minimize staining of laundry and plumbing fixtures. The European Union standard for maximum concentration of Cu in drinking water is 2 mg l^{-1}.

Cu concentrations in natural water (e.g., rivers, lakes, and oceans) are relatively low (median: 4–10 μg l^{-1}). Drinking water in some instances can make a substantial contribution to total Cu intake. Tap water from dwellings with Cu piping can contain much higher concentrations of Cu. A number of factors can affect the amount of Cu dissolution from Cu piping accounting for the large variability in Cu concentrations. Tap water of a first-draw sample is more likely to contain higher amounts of Cu than a flush sample since the water has sat in the plumbing for some time. In a monitoring survey carried out in Ontario, Canada, communities, the Cu concentration in consumed water averaged 0.176 mg l^{-1} with a range of 0.020–2.02 mg l^{-1}. It can be estimated that an adult consuming 1.5 l of water would have a daily Cu intake of 0.264 mg

Table 2 Copper content of foods

Food	*Serving size*	*Copper per serving (mg)*	*Copper per 100 g (mg)*
Organ meats			
Veal/beef/lamb/pork/chicken liver (cooked)	75 g	0.476–11.3	0.635–15.1
Pork kidney (braised)	75 g	0.500	0.683
Beef kidney (simmered)	75 g	0.423	0.564
Liver pate	75 g	0.300	0.400
Seafood			
Oyster (boiled or steamed)	75 g	1.10–5.68	1.47–7.57
Lobster (boiled or steamed)	75 g	1.46	1.94
Squid (breaded and fried)	75 g	1.41	1.89
Crab, octopus, clam, crayfish (boiled or steamed)	75 g	0.466–0.886	0.621–1.18
Nuts and seeds			
Sesame seeds (whole, dried)	60 ml	1.5	4.08
Flaxseeds (whole and ground)	60 ml	0.809	1.90
Cashew nuts (dry roasted)	60 ml	0.771	2.219
Pumpkin/squash/sunflower seeds	60 ml	0.623–0.734	1.08–1.28
Brazil nuts, hazelnuts, walnuts, pine nuts	60 ml	0.413–0.619	1.16–1.74
Legumes			
Meat substitutes	1 serving	1.10–1.34	0.733–0.893
Fermented soybean products (natto, tempeh)	175 ml	0.863	0.667
Pink lentils (boiled)	175 ml	0.714	0.399
Soy/white/kidney/navy beans	175 ml	0.283–0.507	0.166–0.283
Peanuts (oil-roasted)	60 ml	0.438	1.30
Meats			
Quail (cooked)	75 g	0.444	0.592
Veal (varied cuts)	75 g	0.161–0.360	0.215–0.480
Cured pork (ham, bacon)	75 g	0.115–0.313	0.153–0.417
Vegetables			
Mushrooms (cooked)	125 ml	0.415–0.686	0.504–0.895
Heart of palm (raw)	85 g	0.547	0.644
Potato with skin (boiled or baked)	1 potato	0.379	0.253
Fruit			
Prunes	60 ml	0.205	0.612
Avocado	½ fruit	0.191	0.190
Raisins	60 ml	0.133	0.362
Milk and alternatives			
Soy beverages	250 ml	0.424	0.165
Goat cheese	50 g	0.366	0.732
Chocolate milk	250 ml	0.172	0.065
Grains			
All bran ready-to-eat cereal	30 g	0.318–0.343	1.06–1.14
Kamut (cooked)	125 ml	0.225	0.248
Quinoa (cooked)	125 ml	0.14	0.191
Others			
Dark chocolate	40 g	0.408–0.704	1.02–1.760

Source: Data obtained from Health Canada. Canadian Nutrient File. 2010. Available at www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/index-eng.php.

from drinking water. A Swedish study found that the median daily intake of Cu from drinking water for young children was 0.32 mg. It should be noted that considerably higher Cu concentrations that exceed standards have been reported in drinking water from a small percentage of dwellings. Consuming water with high concentrations of Cu concomitant with a Cu-rich diet could result in total Cu intakes exceeding the UL.

Copper Bioavailability

Dietary interactions of Cu with a number of food constituents have been described, yet under most circumstances, Cu bioavailability is minimally affected by the composition of the diet. It is well established, however, that consumption of large quantities of zinc can decrease Cu absorption and lead to Cu deficiency. In fact, decreased Cu status was the endpoint selected by the Institute of Medicine in establishing the UL for zinc for adults. High iron intakes may also interfere with Cu absorption in infants. The amount of Cu ingested greatly affects Cu absorption. When a diet is low in Cu (0.4 mg day^{-1}), up to 75% of the Cu is absorbed, whereas only 12% is absorbed when the diet is high in Cu (7.5 mg day^{-1}). Notably, even though fractional Cu absorption decreases with higher Cu intakes, the absolute amount of Cu absorbed is greater.

Whole-Body Copper Metabolism

In healthy people consuming a balanced diet, overt Cu deficiency and toxicity are rare because of the body's elaborate systems that maintain Cu homeostasis over a moderate range (~10-fold) of exposures. The intestine is the primary site for Cu absorption. Ingested Cu from foods and Cu from digestive fluids (e.g., pancreatic and biliary secretions) present in the intestinal lumen are absorbed by enterocytes by the Cu permease, Cu transporter 1 (Ctr1). Ctr1 is present on the brush-border surface and transports reduced Cu^{1+}. The reduction of Cu^{2+} to Cu^{1+} prior to transport by Ctr1 is not well understood, but may involve Dcytb or Steap2. Cu exits the basolateral side of absorptive enterocytes in a process mediated by the action of the P-type ATPase, ATP7A. Once absorbed, Cu is transported bound to carrier proteins (albumin and α2-macroglobulin) to the liver where it is subsequently released into the circulation for transport to tissues.

The liver is a main organ for controlling Cu balance and maintaining circulating Cu concentrations. The body responds to increases in Cu by eliminating endogenous Cu into the bile in a processed mediated by the P-type ATPase, ATP7B. Biliary Cu excretion is a key homeostatic mechanism for ridding the body of excess Cu and preventing Cu overload. Renal excretion plays a minor role. In healthy people consuming a normal Cu diet, urinary Cu excretion is low (<0.1 mg day^{-1}).

Intracellular Copper Trafficking

Cu is a redox-active metal. Free Cu can participate in Fenton-type reactions and lead to the production of reactive oxygen species that can damage cellular components. The cell has evolved an overcapacity to sequester Cu and it has been estimated that there is less than one free Cu atom per cell. Ctr1 plays a major role in the uptake of cellular Cu, although Ctr1-independent Cu uptake systems exist. Once inside the cell, Cu is transferred to a family of proteins called Cu chaperones and possibly other small Cu-binding proteins (e.g., metallothioneins and GSH). Cu chaperones deliver Cu to specific Cu-requiring enzymes by directly transferring the Cu to the apo-cuproprotein by protein–protein interaction. This direct transfer prevents the accumulation of free Cu that can otherwise engage in deleterious reactions. Metallothioneins provide the cell with additional capacity to bind Cu under conditions of high Cu exposure.

The Cu chaperone for SOD1 (CCS) transfers Cu to SOD1 in the cytoplasm and intermitochondrial space. COX17 is required for incorporating Cu into cytochrome *c* oxidase (CCO) in the mitochondria along with other Cu chaperones including Cox11, Sco1, and Sco2. ATOX1 delivers Cu to ATP7A and ATP7B. When cellular Cu levels are normal, ATP7A and ATP7B are largely localized to the trans-Golgi network and function to transport Cu into the lumen of the trans-Golgi network for incorporation into nascent secretory cuproenzymes such as ceruloplasmin, lysyl oxidase, peptidylglycine α-amidating monooxygenase, and dopamine β-hydroxylase. When cellular Cu levels rise, ATP7A and ATP7B translocate in cytoplasmic vesicles from the trans-Golgi network to the cell surface to expel Cu from the cell.

ATP7A is expressed in the intestine and most other tissues. The tissue expression pattern of ATP7B is more restricted, with high expression in the liver. ATP7A mediates Cu efflux from enterocytes into the plasma by a process that involves fusion of Cu-loaded vesicles with the basolateral membrane of enterocytes. ATP7B mediates Cu excretion into the bile by a mechanism involving fusion of Cu-loaded vesicles with the canalicular membrane of hepatocytes. Cu metabolism MURR1 domain-containing protein 1 (COMMD1) has been shown to interact with ATP7B and may facilitate the excretion of Cu by ATP7B into the bile canaliculus. Mutation of the *COMMD1* gene in dogs has been associated with Cu toxicosis.

Cu transporter 2 (Ctr2) shares sequence similarity with Ctr1 although its function in mammalian cells is less understood. Ctr2 is localized to cytoplasmic vesicles and a small proportion has been detected on the plasma membrane. Studies in yeast support a role for Ctr2 in the mobilization of Cu from intracellular vesicles to the cytosol. Recently, Ctr2 has been shown to play an important role in regulating the function of Ctr1 by controlling the accumulation of a truncated form of Ctr1 lacking the Cu-binding ectodomain. $Ctr2^{-/-}$ mice were found to be defective in the accumulation of the truncated form of Ctr1 and showed increased tissue Cu that accumulated as intracellular foci.

The role of other Cu-binding proteins such as the prion protein (PrP^c) and amyloid precursor protein (APP) in Cu metabolism is not well understood. Some data suggest that the APP may be involved in regulating Cu levels in the brain.

Copper-Dependent Enzymes

Cu has been known to be an essential nutrient for almost a century. Cu is required by organisms in small quantities and therefore considered a trace mineral. Total body Cu content in an adult is approximately 80 mg and most (half to two-thirds) is located in the skeleton and muscle because of their relative size. The brain, liver, heart, and kidneys have the highest Cu concentrations.

The ability of Cu to cycle between its oxidized cupric (Cu^{2+}) and reduced cuprous (Cu^{1+}) forms is critical for its biological function. As an essential component of Cu-dependent enzymes (also called cuproenzymes), Cu functions as a catalytic cofactor carrying out single-electron transfer reactions. Cuproenzymes present in mammals and their physiological function are listed in Table 3. Cuproenzymes function in various physiological processes such as antioxidant defense (SOD1 and extracellular superoxide dismutase (SOD3)), iron homeostasis (ceruloplasmin, hephaestin, and zyklopen), mitochondrial respiration (CCO), development of connective tissue (lysyl oxidase), melanin biosynthesis (tyrosinase), catecholamine production (dopamine β-hydroxylase), peptide hormone processing (peptidylglycine α-amidating monooxygenase), and oxidative deamination (amine oxidases).

Copper Deficiency and Toxicity

Symptoms of Cu deficiency are a result of reduced activity of cuproenzymes. Symptoms of overt Cu deficiency in mammals

Table 3 Physiological function of mammalian cuproenzymes

Cuproenzyme	*Physiological function*
Amine oxidases	Oxidative deamination
Ceruloplasmin (ferroxidase)	Ferrous iron oxidation
Cytochrome *c* oxidase	Reduction of molecular oxygen
Dopamine β-hydroxylase	Norepinephrine production
Hephaestin	Ferrous iron oxidation
Lysyl oxidase	Elastin cross-linking
Peptidylglycine α-amidating monooxygenase	Peptide C-terminal α-amidation
Superoxide dismutases (SOD1 and SOD3)	Superoxide disproportionation
Tyrosinase	Dopaquinone production
Zyklopen	Ferrous iron oxidation

include hypochromic anemia, neutropenia, thrombocytopenia, and hypopigmentation. Structural and functional abnormalities occur in the skeleton and cardiovascular system and depressed immune function and neurological abnormalities are also observed. Overt Cu deficiency is rare in humans consuming a balanced diet. Subpopulations at increased risk for Cu deficiency included preterm infants fed with cow's milk (or other milk-based formulas low in Cu), individuals with malabsorption syndromes (e.g., celiac disease), and gastric bypass patients. Cu deficiency has also been observed in patients receiving total parenteral nutrition. Consumption of high amount of zinc can also predispose individuals to Cu deficiency. Cu toxicity has been observed with overzealous use of Cu-containing supplements and following ingestion of contaminated water with high concentrations of Cu.

Genetic Disorders of Copper Metabolism

The best characterized genetic disorders of Cu metabolism are Menkes syndrome and Wilson's disease. Menkes syndrome is an X-linked recessive disorder that results from mutations in the *ATP7A* gene leading to impaired intestinal Cu absorption and a systemic Cu deficiency. Symptoms include growth failure, mental retardation, weak muscle tone, and brittle hair. A milder form of the disease termed occipital horn syndrome has been described. Occipital horn syndrome is characterized by calcium deposits in the occipital bone, coarse hair, and loose skin and joints.

Wilson's disease is an autosomal recessive disorder that is caused by mutations in the *ATP7B* gene. In Wilson's disease, Cu accumulates in tissues, namely, the brain and liver, resulting in neuropsychiatric symptoms and liver damage. Indian childhood cirrhosis, idiopathic Cu toxicosis, and endemic Tyrolean infantile cirrhosis are rarer diseases of hepatic Cu accumulation in infancy and childhood that have a genetic predisposition. Manifestation of these diseases is often associated with high intakes of Cu from contaminated food or water.

Evaluation of Copper Status

The most commonly used biomarkers for routine assessment of Cu deficiency are plasma Cu concentration and plasma ceruloplasmin (Cp) protein concentration or activity. Most Cu in the circulation is bound to Cp (>90%) and therefore changes in plasma Cu closely follow changes in Cp. A limitation of both these biomarkers is that they are affected by conditions unrelated to Cu status including inflammation, age, gender, hormone use, and pregnancy. In addition, strong homeostatic mechanisms act to maintain plasma Cu and Cp within a normal range, which likely makes these indicators insensitive to mild reductions in Cu status. Several studies have shown changes in biochemical indicators in response to Cu restriction in the absence of changes in plasma Cu or Cp.

Erythrocyte SOD1 enzymatic activity and protein content decrease in experimental animals with Cu deficiency. Enzymatic activity is a better measure of Cu deficiency than protein level given that inactive apo-SOD1 accumulates in Cu-deficient cells. Erythrocyte SOD1 activity has been shown to be lower in Cu-deficient patients, in subjects consuming a low Cu diet and in people taking high-dose zinc (a Cu absorption antagonist) supplements. Limitations of erythrocyte SOD1 activity as an indicator of Cu deficiency are the absence of a simple, standardized assay with a reference interval and the high intra-individual variation. Erythrocyte SOD1 activity is also affected by certain diseases and conditions.

Platelet Cu concentrations may response more quickly to change in Cu intakes and be more sensitive to changes in Cu status compared to other conventional biomarkers. In establishing requirements for Cu in North America, diets that decreased serum Cu and Cp concentrations were considered deficient in Cu. If the diet did not alter serum Cu and Cp, but reduced platelet Cu concentration, the diet was considered to be marginally adequate in Cu.

The search for more sensitive, specific, and noninvasive biomarkers of Cu status that can be easily measured in humans is ongoing given that traditional biomarkers may be insensitive to small changes in Cu status and have other disadvantages. Cu deficiency induces many biochemical changes. Changes in cuproenzymes, Cu-binding proteins, and markers of immune function and bone metabolism have been proposed as potential indicators for assessing Cu status. One promising biomarker is CCS. Studies in rodents and cattle have shown that CCS protein increases in cells in response to Cu deficiency. The mechanism by which CCS is regulated by Cu has been elucidated. Cu binding to CCS decreases its stability and increases its rate of degradation by the 26 S proteasome. Under Cu-deficient conditions, CCS is more stable and protein levels increase. Since CCS is responsive in erythrocytes, it can be measured in a small blood sample and is therefore appealing for use in humans. Since SOD1 protein decreases and CCS protein increases with Cu deficiency, the CCS:SOD1 protein ratio has been proposed as a measure of Cu deficiency. The CCS:SOD1 ratio has the advantage of increasing the magnitude of the change in some situations compared to assessing CCS alone. A study in rats has shown that erythrocyte CCS protein decreases with Cu overload suggesting that CCS may also be useful as a biomarker for Cu excess. Furthermore, CCS mRNA in peripheral blood mononuclear cells has been shown to decrease in response to Cu supplementation in people with high Cp concentration. Other promising biomarkers for assessing Cu deficiency include serum and tissue activity of peptidylglycine α-amidating monooxygenase and CCO activity in platelets.

Presently, liver Cu concentration is considered the 'gold standard' for diagnosing Cu overload, but the invasiveness of a liver biopsy precludes its use in routine assessment. The non-Cp-bound Cu and protein-free Cu in plasma are promising indicators for assessing Cu excess. Protein-free Cu in serum or plasma was found to be higher in patients with untreated Wilson's disease compared to treated patients. New research has revealed that the relative exchangeable Cu, defined as the exchangeable Cu to total serum Cu ratio, is a promising test for diagnosis of Wilson's disease.

Acknowledgment

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Cream: Clotted Cream

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Introduction

Clotted cream is a thick dairy cream with a nutty flavor, a golden crust of grainy texture, and high fat content (a minimum of 55%, but an average of 64%). Historical records have it that the clotted cream was first produced in Afyon province (Western Turkey) known as Afyon Kaymag in which buffalo milk was used to manufacture the same. British clotted cream also has a long and intriguing history, and it was suggested by Alan Davidson that the arrival of Phoenician traders to Cornwall around 2000 years ago may have introduced the tradition of clotted cream. In the nineteenth century clotted cream was considered a better source of nourishment as compared to 'raw' cream because the latter was liable to go sour easily and when consumed without any heat treatment caused several health problems. Consumers used to prefer clotted cream over raw cream due to its nutritive value, characteristic aroma, and flavor. The European Union made an application in 1993 called 'Cornish clotted cream' to obtain a protected designation of origin for the production of cream by the traditional method in Cornwall. Clotted cream is produced on a day-to-day basis in many parts of European countries, which gives a regular income to many small producers. Clotted cream is creamier than ordinary cream, with a unique flavor developed as a result of milk sugar caramelization and perhaps a slight butteriness. Buffalo milk is preferred over cow's milk for manufacturing of clotted cream as it is high in fat, protein, lactose, total solids, mineral, and vitamin contents, which gives the final products its characteristic rich aroma and flavor. Qashta is a type of thick cream from the Eastern Mediterranean region where, due to its higher amount of fat and protein than buffalo milk, ewe's milk is preferred.

Traditionally, clotted cream was produced by straining cow's or buffalo milk. After filtration, the milk was left to stand in a shallow pan in a cool place for several hours, which facilitated the cream to rise to the surface, which is followed by heating either over hot cinders or in a water bath for hours and then cooled. Yellow clots that formed on the surface of the milk were then skimmed off with the help of a long-handled cream-skimmer known as a reamer or raimer in Devon. By the mid-1930s, the modern processing technique had become famous in Devon because using a cream separator actively separated the cream from the milk using centrifugal force, which helped to produce a larger quantity of clotted cream than the traditional method from the same amount of milk in much less time. English luxury clotted cream has a lower fat content of 48% and is generally used as a topping for desserts, cakes, and muffins, but it is also suited for savory recipes, including pasta and sauces. In bakery products like 'butter shortbread' and 'clotted cream biscuits' clotted cream along with butter is used in the formulation to give the bread and biscuits their distinctive rich and soft texture.

The characteristic flavor of clotted cream is developed due to slow heating of cream for longer duration. The color of the clotted cream can vary from a pale off-white to golden yellow color, with a smooth texture similar to cream cheese. Clotted cream production provides a lot of employment. Clotted cream is called 'malai' in India and 'Devonshire cream' in the Western world. Malai is made by the heating of nonhomogenized milk to about 80 °C (180 °F) for about an hour and then cooled a long time in undisturbed condition. Finally, a thick yellowish layer of fat and coagulated proteins are formed on the surface, which are skimmed off, and the process is repeated to recover most of the fat until it reaches a minimum fat content of about 55%.

Regular consumption of clotted cream was usually believed to be bad for health due to its higher amount of fat. According to the United Kingdom's Food Standards Agency, a 100 g cup of clotted cream provides 586 kcal, roughly equivalent to a 200 g cheeseburger.

Manufacturing Methods of Clotted Cream

In the traditional manufacturing technique, raw buffalo milk, which is preferred for producing clotted cream, is bolted by cheesecloth twice and transferred into copper containers. The milk is heated on a low flame and is continued until the milk reaches temperature of about 90 °C and the milk is held for 30 min; this stage is called 'göbek baglama.' After heating and holding of milk at 90 °C for 30 min, the milk is cooled at room temperature overnight. The cream layer formed on the top of the milk is collected by releasing the milk by perforating the side of the container with a pin. Fresh milk is again added to the previous day's heat-treated cream, which is again slowly heated to 90 °C for 45 min and again slowly cooled to room temperature and left in the cool room for about 4–6 h, followed by refrigeration for 6–8 h. The clotted cream layer formed on the surface is separated out and then inverted into a plastic can, which is packed and sealed before sale.

Today, manufacturers are following two methods for production of clotted cream, that is, (1) farmhouse production method and (2) commercial production method. In the farmhouse production method, milk is usually strained into a rectangular stainless steel tray followed by centrifugal separation of cream with the help of a centrifugal separator. The cream obtained after centrifugal separation is then subjected to heat treatment either by using a forced air convection oven maintained at 80–90 °C for 50–60 min or by scalding the cream in a 'bain marie' type equipment, which operates like a huge water bath in which the hot water is circulated until the final temperature of about 77–88 °C is reached with a holding period of 45–50 min. After slow heating of cream, the cream is allowed to cool for the next 24 h without any disturbance. Finally a golden-color crust that has a fat content of 55–60% is formed on the surface and is separated out with great caution (Figure 1).

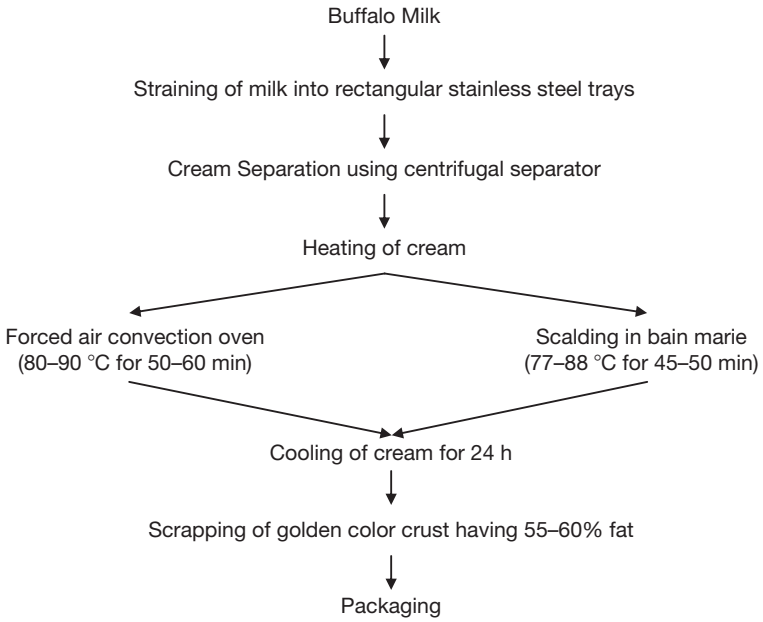

Figure 1 Farmhouse production method for manufacturing of clotted cream.

Commercial manufacturers of clotted cream produce the product by using either a 'float method' or 'scald method.'

In the float method, double cream is floated on milk or skim milk in large jacketed trays and are heated up to 80–85 °C by circulating steam or hot water into the jackets, and the cream is given a holding period of 40–60 min. After slow heating of cream, it is allowed to cool followed by refrigeration to set a golden-color crust, which is scrapped off and packaged into containers (Figure 2).

Cream with a fat content of about 56% is usually used as a starting material for producing clotted cream by the 'scald method.' The cream is heated at 80 °C for 40–60 min with the help of a plate heat exchanger and cooled to below 7 °C and left undisturbed for 12–14 h. It is then packaged for sale in polystyrene or polypropylene pots (Figure 3).

The scald cream method is very similar to float method, but the milk layer is removed and a layer of cream that has been mechanically separated to a minimum fat level is used. In the United Kingdom the resultant cream is allowed to be equivalent to pasteurized cream for legal purposes. A high level of hygiene standards is to be maintained when the processing temperatures are lower than those used in the standard pasteurization process.

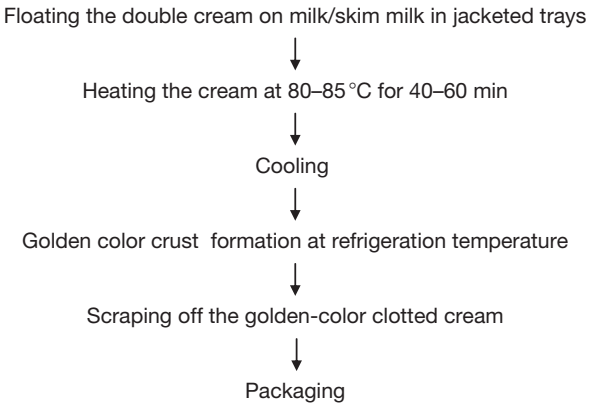

Figure 2 Float method for manufacturing of clotted cream.

Chemistry of Flavor and Golden Crust

As clotted cream is a traditional product, the literature available is limited, but several scientists have studied different aspects, which include microbiological quality, chemical properties, and suitability of packaging materials for enhancing the shelf-life. One interesting study on clotted cream was reported by Senel in which he discussed the effect of carbonyl compounds and free fatty acids on aroma and flavor. The formation of aroma and flavor compounds is the result of the chemical and biochemical transformations of milk components and may be divided into four categories: (1) nonvolatile acids (lactic and pyruvic); (2) volatile acids (formic, acetic, and butyric); (3) carbonyl compounds (acetaldehyde, acetone, and diacetyl); and (4) miscellaneous compounds (certain amino acids, constituents formed by thermal degradation of protein, fat, or lactose). Apart from imparting characteristic flavor to the clotted cream, carbonyls, lactic acid, and free fatty acids also play a vital role in imparting shelf-life of the product. Carbonyl compounds and free fatty acids in clotted cream produced from buffalo milk were found to be much different when compared with other dairy products. The characteristic flavor of clotted cream may also be attributed to the slow heating treatment of cream for a long time, which causes caramelization of milk sugar. Golden-color crust of clotted cream may be attributed to high carotene levels in the milk, which is normally present in the grass eaten by buffalo.

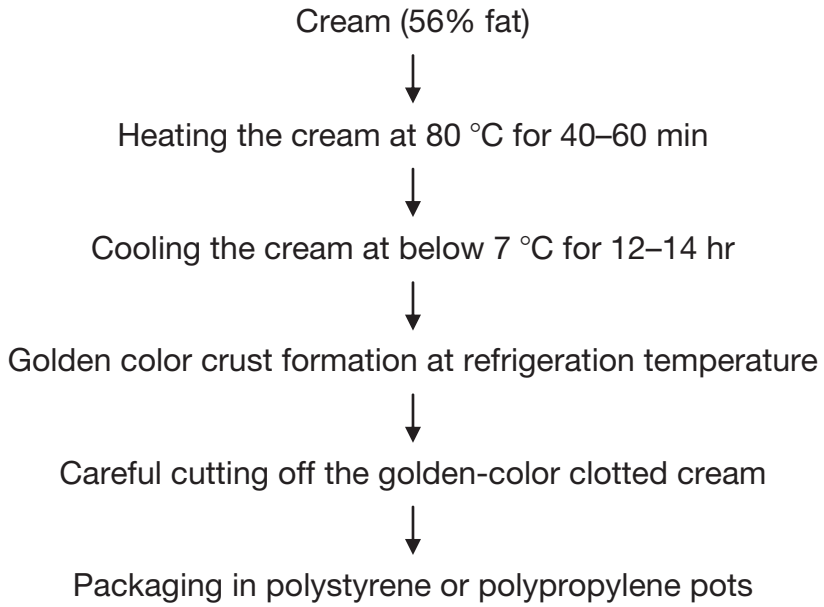

Figure 3 Scald method for manufacturing of clotted cream.

Figure 4 MasoSine pump ideal for clotted cream.

Recent Status of Clotted Cream

The largest commercial producer of clotted cream in United Kingdom (Cornwall) is Rodda's, founded in 1890, which is now able to produce more than 25 tons per day. Clotted cream is believed to have quality as it requires longer manufacturing time. Commercial manufacturers of clotted cream are using modern equipment to manufacture the product under hygienic conditions, both at a large and small scale. Rodda's company in 2012 installed a MasoSine sanitary process pump (Figure 4) supplied by Watson Marlow Pumps Group to carry out the production of clotted cream under hygienic conditions. As a by-product, from every 100 imperial gallons of milk, 94 imperial gallons of skimmed milk are produced, which can be further used to manufacture different dairy products.

Uses of Clotted Cream

Clotted cream is also known as 'baked cream' and is mostly used in the bakery industry as a topping and also in culinary dishes.

Cream Tea

In most of the countries, an essential part of the 'cream tea' is a clotted cream. In Cornwall, clotted cream is served on scones with different fruit flavors of jam, along with a cup of tea. Langage Farm in Devon has started a campaign for the protection of origin of 'Devon cream tea' as similar to 'Cornish clotted cream,' where scones are spread with jam or lemon curd and then topped with clotted cream, which is served with afternoon tea and known as 'Devonshire tea.'

Confectionery

On many occasion clotted cream is used as a supplement to hot or cold desserts. Clotted cream, especially Devon clotted cream, which is less yellow in color due to the lower carotene levels, is regularly used in different baked products. Clotted cream is used in most of the southwest part of England for ice cream and fudge production. One of the products, in which bread is topped with clotted cream and golden syrup honey, is called 'thunder and lightning.' Clotted cream can be spread as a topping over any baked treat, like English muffins, biscuits, croissants or muffins, ice cream, cake, trifle or pudding, or crusty bread, and it also serves a purpose of filling in cakes.

Savory Dishes

Clotted cream is used in savory dishes and can also be incorporated into mashed potatoes or scrambled eggs. Clotted cream, along with rich cheddar sauce and mustard, can be used as a dip with its unique richness of flavor and texture. A moist scone can be split like a shortcake biscuit, topped with berries and clotted cream, and served as a dessert. Clotted cream is also used in some of the creamy savory dishes like risotto, pasta, curry, quiche, or gratins. In some parts of England, barley is used instead of rice for preparing risotto or rice pudding, which adds a unique texture and slightly nutty flavor. The addition of clotted cream to this dish enriches and enhances the creamy texture.

Risks Associated with Consumption of Clotted Cream

Clotted cream might be quite delicious, but on health grounds it cannot be suggested as a daily diet due to its high amount of saturated fat. The market for clotted cream has not expanded beyond certain parts of the world as it has a very short shelf-life and hence is difficult to export. Clotted cream is often associated with a high number of microbiological counts, which makes it vulnerable for its consumers. To obtain a good quality of clotted cream apart from hygienic conditions, factors like status of healthy animals, hygienic milking, manufacturing tools and equipment, and hygienic practices during production and storage of clotted cream must be considered. *Brucella abortus* was first reported in March 1966 from a market sample of clotted cream, and aerobic spore-forming bacteria like *Bacillus subtilis* are more prominent. Researchers have found that cooling for a long time during manufacturing of clotted cream and contamination during handling of packing are the main reasons for such a high number of microbial load. To deliver a safe product to the consumers and to reduce the level of spoilage bacteria, acceptable heat treatment must be applied during the production of clotted cream, and apart from this the clotted cream must be produced in a clean environment under hygienic conditions.

See also: Cream: Types of Cream; Ethnic Foods; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Heat Treatment: Effect on Microbiological Changes and Shelf Life; Heat Treatment: Principles and Techniques; Lactose; Milk: Processing of Milk; Milk: Role in the Diet; Spoilage: Bacterial Spoilage; Traditional Foods.

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Cream: Types of Cream

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Introduction

Cream is one of the most important dairy products. It was traditionally considered a luxury product, but it is now readily used in many forms and for a variety of purposes. For example, it is a primary raw material for the manufacture of table butter and for preparing *ghee*, a fat-rich Indian dairy product. It is also used as an ingredient in sweet and savory dishes, such as ice cream, soup, custard bases, and cakes. Cream is a concentrated emulsion of milk lipid globules in skimmed milk, and it is separated from milk either by gravity or centrifugal force. Different types of creams are primarily classified according to their fat content (g/100 g) as double cream (45–50%), cream or full cream (30–40%), single or half cream (15–25%), coffee cream (15–18%), and light coffee cream (<10%). According to the standards of the United Nations Food and Agriculture Organization (FAO), cream is classified as followed, based on fat content:

(a) Cream: 18–26%
(b) Light cream (or coffee cream): >10%
(c) Whipping cream: >28%
(d) Heavy cream: >35%
(e) Double cream: >45%

The fat-based standards for various types of cream vary in individual countries, and diverse names are used to describe different creams. So, it is not possible to provide a uniform international definition or universally accepted classification system. However, Table 1 provides the regulations for the fat content of cream products in major cream-producing countries. Cream products are also classified according to their final uses (e.g., whipping cream, coffee cream, or cream liqueur) or by processing method (e.g., pasteurized cream, ultra-high-temperature (UHT)-treated cream, frozen cream, dried cream, and cultured or sour cream).

The physicochemical properties of cream are affected by several factors, such as the state of the lipid globules and the milk lipid globule membrane, the concentration of lipid globules, the type and concentration of nonfat milk solids in cream (e.g., proteins, salts, and added emulsifiers and stabilizers), the temperature of the cream, and the physical handling of the cream (e.g., pumping, aeration, and agitation), which can cause the disruption or agglomeration of the globules. Table 2 provides the chemical composition of cream.

Technology of Cream Separation

The basic principle of cream separation, whether by gravity or centrifugal methods, is based on the fact that milk fat is lighter than the skim milk portion. At 16 °C, the average density of milk fat is 0.93 and skim milk 1.036. Thus, when milk, which is a mixture of fat (as cream) and skim milk, is subjected to either gravity or centrifugal force, the cream and skim milk stratify or separate from one another by virtue of their differing densities. Separating the cream phase from the milk, standardizing the cream to the desired fat content, and heating the cream to increase its shelf-life are universal to the manufacture of (almost) all industrially processed cream products.

Gravity Methods

When milk is allowed to stand for some time, there is a tendency for the fat to rise. The velocity, or rate, at which the fat globules rise is described by the following equation, which is known as Stoke's Law

$$V = 2G/9^{*}(ds - df)r^{2}/n$$

Where,
V=velocity or rate at which a single fat globule rises, G=acceleration due to gravity, ds=density of skim milk, df=density of fat, r=radius of fat globule and n=viscosity of skim milk.

According to Stoke's Law, the velocity is increased by:

(i) An increase in the radius of the fat globule
(ii) An increase in the difference in the densities of skim milk and fat
(iii) A decrease in the viscosity of skim milk

However, in practice, the important factors affecting the rate of cream rise using gravity methods are as follows:

Table 1 Standards for the lipid content of cream in some countries

Country	*Cream type*	*Lipid content (g/100 g)*
Australia and New Zealand	Cream	18–40
France	Light cream	12–30
	Cream	30–40
Germany	Coffee cream	10–30
	Whipping cream	30–40
The Netherlands	Cream	10–30
	Whipping cream	30–40
United Kingdom	Half cream	12–18
	Single cream	18–35
	Whipping cream	35–48
	Double cream	48–55
United States	Half and half cream	10–18
	Light cream	18–30
	Light whipping cream	30–36
	Heavy cream	36–45

Source: Hoffmann, W. (2002). Cream products. In: Roginski, H., Fox, P. F. and Fuquay, J. W. (eds.) *Encyclopaedia of dairy sciences*, pp. 551–557. London: Academic Press.

Table 2 Chemical composition of cream

Constituents	*Percentage*		
Fat	30	25.00	50.00
Water	64	68.20	45.45
Serum: protein	02.4	02.54	01.69
Lactose	03.5	03.71	02.47
Minerals/ash	00.4	00.56	00.37
Total solids	36	31.80	54.55
Nonfat solids	06	06.80	04.55

(i) *Size of fat globules*: As the size of the fat globules increases, the rate at which the cream rises also increases. (Thus gravity creaming occurs faster for buffalo milk than it does for cow milk because of the larger size of fat globules in buffalo milk.)
(ii) *Temperature*: As temperature increases, viscosity decreases and, in turn, velocity increases.
(iii) *Clumping*: A clump or cluster acts like a single globule, in so far as movement through skim milk is concerned. Therefore, the effective r^2 is increased, which then increases velocity.
(iv) *Addition of adhesives*: Adhesives ultimately help increase the rate at which fat globules rise.

Centrifugal Methods

When milk enters the rapidly revolving bowl of the cream separator, it is immediately subjected to a tremendous centrifugal force, which is 3000–6000 times greater than gravitational force. While both the fat and skim milk are subjected to the centrifugal force, the difference in density affects the heavier portion (i.e., skim milk) more intensely than the lighter portion (i.e., cream). Centrifugal separators contain stacks of conical disks with vertically aligned distribution holes through which whole raw milk is introduced. Under the influence of a centrifugal force, the sediment and lipid globules in the milk settle radially outward or inward, respectively, in the separation channels. Solid milk impurities of high density rapidly settle out to the outside of the separator, and they are then collected in the sediment space. Because the cream is less dense than skimmed milk, it moves inward toward the axis of rotation, and it is removed through an axial outlet. Skimmed milk moves outward beyond the disk stack and is removed to a skimmed milk outlet through a channel between the top of the disk stack and the conical hood of the separator bowl. Therefore, the separation of cream from whole milk involves the concentration of lipid globules, followed by the removal of the cream from the skim phase to produce two streams, skimmed milk and cream, the latter of which commonly amounts to 10% of the total throughput. This method allows rapid and efficient separation, and although very high centrifugal forces are applied, not all lipid globules are removed. Skim milk usually has a residual fat content of ~0.1 g/100g, which primarily consists of lipid globules <1 μm in diameter (Figures 1–3).

Factors Influencing the Fat Percentage of Cream

A brief summary of the factors influencing the fat percentage of cream by centrifugal separation is given below:

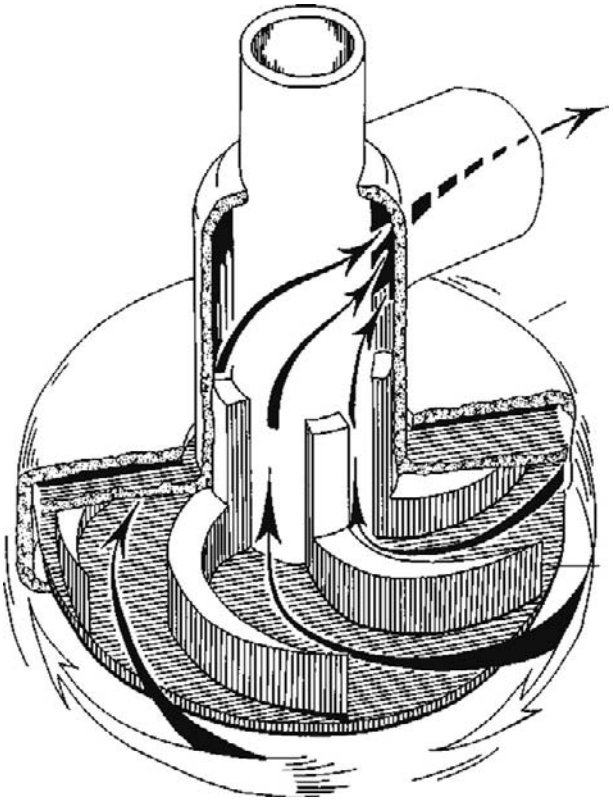

Figure 1 Paring chamber and disk of a semienclosed separator.

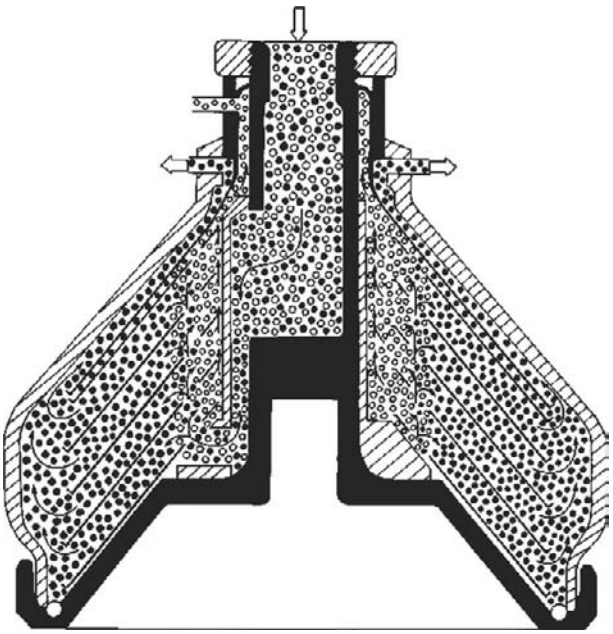

Figure 2 Open-design cream separator.

(a) *Position of the cream screw*: The cream screw/outlet consists of a small, threaded, hollow screw pierced by a circular orifice through which the cream emerges. This screw can be driven IN or OUT, thus bringing it nearer to, or away from, the center of rotation. Under normal conditions, the

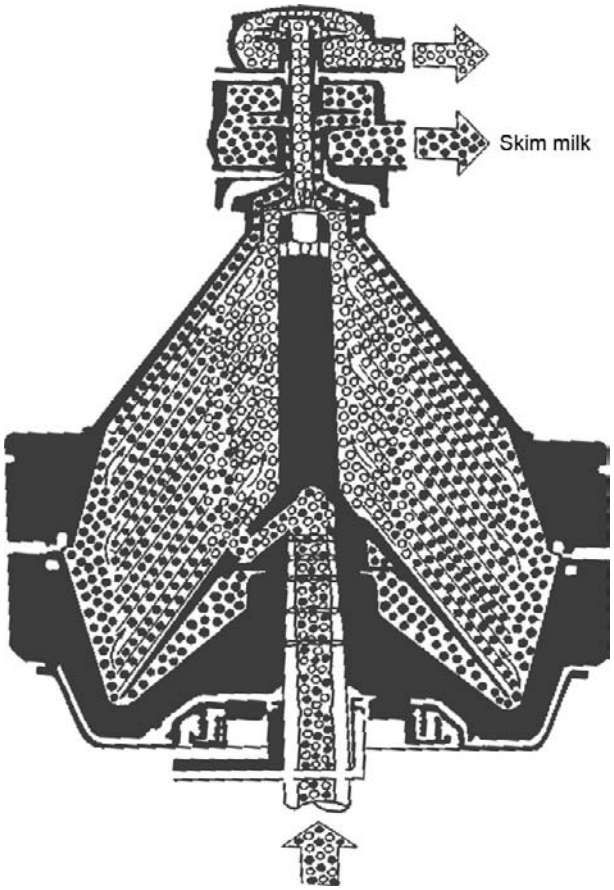

Figure 3 Hermetic-design cream separator.

ratio of skim milk to cream is 90:10. Changes in the separation procedures affect the fat percentage in the cream. Altering the position of the cream screw can change the ratio of skim milk to cream. Therefore, when the cream screw is moved IN toward the axis of rotation, a higher fat percentage in the cream is obtained, and vice versa; this is because the force that tends to discharge cream through the orifice is decreased.

(b) *Fat percentage in milk*: Because all of the fat in milk is present in cream, the cream from the separation of high-fat milk has a higher fat content than that from low-fat milk. Higher fat content in cream is obviously consistent with higher fat percentage, and vice versa.

(c) *Speed of the bowl*: Higher bowl speeds exert a greater centrifugal force, resulting in the rapid flow of skim milk from the bowl. An increase in bowl speed increases the skim milk discharge, leading to a higher fat percentage in the cream.

(d) *Rate of inflow of milk*: When the rate of inflow increases, the discharge from the cream outlet increases, as the skim milk discharge remains constant (with constant centrifugal force); more cream containing the same amount of fat results in a lower fat percentage and vice versa.

(e) *Temperature of milk*: Lowering the temperature increases the viscosity of both cream and skim milk, but the viscosity of cream increases more than that of skim milk. Thus, the quantity of cream discharged is reduced because of bowl-clogging, resulting in a higher fat percentage.

(f) *Amount of water or skim milk added to flush the bowl*: The addition of more water or skim milk will cause an increase in the amount of cream produced, which, with the same fat content, will show a lower fat percentage.

Standardization of Cream

Because traditional cream separators cannot be accurately operated to produce a cream of specific fat content, it is usually necessary to produce cream with a higher fat content. This cream is collected and mixed in cream tanks from which the cream was sampled for fat percentage. The fat percentage is determined and then reduced via the addition of skimmed milk in a process called standardization, which is necessary for producing cream with a fat content close to a predefined value. Direct in-line standardization is usually combined with separation, and equipment that automatically monitors and controls the fat content of cream is used for this purpose. On discharge from the separator, the skim and cream streams are mixed, and the proportion of cream included determines the fat content of the cream prepared.

Fat content must be as close to the stipulated value as possible; too high a fat content results in economic losses for the processor, whereas too low a fat content may result in cream products not meeting legal requirements. During standardization, the temperature of cream can be over 40 °C, so bacterial growth may occur. Thus, it is essential that standardization is carried out rapidly, to prevent cream being held at these temperatures for unnecessarily long periods. Subsequent pasteurization and cooling steps must also follow rapidly.

Pasteurization of Cream

Cream is pasteurized chiefly to inactivate enzymes and microorganisms, thus extending the shelf-life of cream. It renders a safe product for the consumer. The effects of heating largely depend on the intensity of the treatment. To pasteurize cream, either high-temperature short-time (HTST) pasteurization or UHT sterilization is commonly applied. The high fat content of cream protects microbes during heating, necessitating a more severe heat treatment than that required for the pasteurization of milk. For the HTST pasteurization of cream, the International Diary Federation recommends heating at 75 °C, for 15 s for cream containing $<$ 20 g fat/100 g, or to $>$80 °C for 15 s, for cream containing $>$20 g fat/100 g.

When cream is UHT-treated, the quality of the raw material is particularly important, owing to the longer shelf-life of this product. The UHT processing of cream involves heating the product to 135–150 °C for a few seconds, whereby minimal chemical, physical, and organoleptic changes occur in the cream, but spoilage and pathogenic microorganisms are inactivated. The UHT treatment of cream does not necessarily produce a sterile product, as some bacterial spores, especially those of *Bacillus* spp., can survive UHT treatment. The indigenous milk enzymes, lipoprotein lipase and proteinase, cause lipolysis and proteolysis, respectively, during storage.

Neutralization of Cream

The neutralization of sour cream refers to the partial reduction of its acidity. It is done to avoid the excessive fat loss in butter milk that results from churning highly acid pasteurized cream. When pasteurizing sour cream, the casein curdles, thereby entrapping fat globules, and as a bulk of curd goes in buttermilk, this causes high fat loss. Neutralization also guards against the production of an undesirable off-flavor in cream.

In addition, neutralization improves the storage quality of the product. The neutralizer used should never be dried when added to cream. So it is dissolved in clean potable water and then diluted to a 10% solution, before being distributed with vigorous steering at 29–32 °C. The cream acidity is checked to know whether it is correctly neutralized or not.

Homogenization of Cream

The main objective of homogenization is to prevent, or at least minimize, the creaming phenomenon. However, the alteration of the characteristics of cream products or the production of new product structures is also possible by selecting the appropriate homogenizing parameters. Although homogenization is essential for all types of single and half cream, and may be applied to double cream, it can have negative effects on cream functionality.

Ripening of Cream

Ripening is a process for fermenting cream, and it may be coupled between pasteurization and churning, during which cream undergoes changes in flavor, aroma, and texture. Ripening cream results in the improved and uniform flavor of butter and enhances churning. Diacetyl is a major flavor compound in the ripened cream, and a large number of other compounds also contribute at levels above and below the individual sensory threshold where synergistic interaction may occur.

Cream Products

Whipping Cream

Whipped cream is valued by consumers for its taste and texture, and it is often considered a luxury product. The whipped product is created by beating air into cream to form a stiff-foamed product, which has many applications, including those in desserts and cakes.

Cream is first standardized to the desired fat content, which is generally between 30 and 40 g/100 g. Stabilizers may be added to the standardized cream before heat treatment. The pasteurization of cream is commonly carried out at 80 °C, and then the cream is cooled, aseptically packaged, and distributed. Pasteurized cream has a shelf-life of <3 weeks at refrigeration temperature. The need for a cream with an extended shelf-life has led to the production of UHT whipping cream, which, after the optional addition of stabilizers, is heated at >135 °C for a few seconds. As a result, a shelf-life of several months can be obtained.

However, the physical shelf-life of such cream is limited, owing to the fact that the creaming of lipid globules occurs, so a homogenization step needs to be applied to reduce lipid globule size and minimize the separation of the globules during storage. However, homogenization impairs the whipping properties of cream, so choosing the optimal conditions for the homogenization of UHT whipping cream represents a trade-off between maintaining optimal whipping characteristics and minimizing the separation of lipid globules during storage. Following homogenization, UHT-treated whipping cream is cooled, packaged aseptically, and distributed.

Process of whipping

Whipping involves beating air into the cream, resulting in the formation of a coarse foam, which contains air bubbles with an average diameter of 150 μm. These are rapidly covered by milk proteins, which stabilize them against collapse. A large proportion of the protein absorbed on the bubble surface is formed by the surface-active β-casein, which is present in large quantities in nonmicellar form at the low temperatures at which cream is commonly whipped, but other caseins and whey proteins are also found at the bubble interface. On further whipping, air bubble size is reduced approximately threefold, and milk lipid globules displace some of the proteins from the bubble interface. On adsorption at the bubble interface, the globules shed their globule membrane material from the contact area with the air bubble in the process, thereby creating an air–lipid interface.

Prolonged whipping leads to the stiffening of the cream, as a result of the creation of a network of partially coalesced lipid globules. Partial coalescence may be induced as a result of mechanical damage to the milk lipid globules during whipping. This process is enhanced by the presence of large lipid crystals in the globules. The partial coalescence of the lipid globules can also result from the collapse of air bubbles in the foam. Because the globules have shed their original membrane material on the area that interacts with the bubble interface, the collapse of air bubbles leads to partially uncovered globules, which are extremely susceptible to partial coalescence. Eventually, a stiff foam results, in which the air bubbles are surrounded and stabilized by a network of coalesced lipid globules. A network of coalesced lipid globules in the serum phase of the foam adds structure and stability and prevents the collapse of the whipped cream.

Effect of processing parameters on whipping characteristics

Various processing steps can significantly affect the whipping characteristics of cream. These characteristics of cream obtained from the same milk can vary considerably, just by use of a different separator for separating the cream, and the overrun of whipped cream increases with the increasing temperature of separation in the range 25–50 °C. Homogenization is required to prevent the creaming of lipid globules in UHT whipping creams. It increases the whipping time of the cream and reduces the overrun and stiffness of the whipped cream. These changes are related to the membrane of homogenized lipid globules primarily composed of caseins. Compared to globules, they are less susceptible to absorption onto the air

bubble interface and shear-induced aggregation, however. A thickening agent such as K-carrageenan is generally added at around 0.01% to prevent creaming during storage.

To improve the whipping characteristics of homogenized cream, emulsifiers may be added to the cream. Heat treatment does not drastically affect the whipping properties of cream per se, but it is commonly applied in conjunction with homogenization because highly heat-treated creams generally have poor whipping properties.

Sour Cream

Sour cream is a heavy-bodied, ripened cream of high acidity (0.6% lactic acid), clean flavor, and smooth texture made by inoculating sweet, pasteurized, and homogenized cream with a culture of lactic acid bacteria and allowing fermentation to proceed until these desired qualities are obtained. It should have a white-to-yellowish, slightly creamy appearance; clean, slightly acidic, rich flavor; and clean, milk-sour, flavorful taste.

Method of manufacture

Sweet cream is standardized to get 18–20% milk-fat. It is then pasteurized, homogenized, and chilled to 15–20 °C, and the final fat content is set. It is inoculated with a lactic acid bacterial starter (i.e., lactic acid/butter culture) at 2–4% and 24 °C, before being fermented until the desired qualities are obtained. During the acid production, the homogenization clusters flocculate, resulting in a highly viscous cream. To increase the firmness, rennet or permitted thickening agents are sometimes added to the sweet cream. When the pH reaches 4.5, the cream is further cooled with gentle stirring and then chilled to 2–4 °C and packed. Alternatively, souring may be applied to the package. Fermentation, which takes 14–24 h, may occur in filled cream containers to produce set-style cultured cream, or in a fermentation tank from which cream containers are subsequently filled. Both types of fermentation have disadvantages. Following in-tank fermentation, the cream has to come in contact with the production equipment again, because it has to be further processed, thereby increasing the possibility of recontamination and reduction in the viscosity of the cream. Consequently, the need for the addition of hydrocolloids increases. Although set-style cultured cream is thicker, it tends to become inhomogeneous during the long fermentation at ambient temperatures.

Cream may also be chemically acidified, using glucono-δ-lactone or food-grade acids. This method has several advantages over conventional culturing practices, including the elimination of culture-handling problems, improved production efficiency, and quality control. Chemically acidified sour creams have a similar appearance and texture to those of cultured sour creams, but the latter have superior flavor characteristics. Sour cream is mainly used in prepared foods.

Coffee Cream

Coffee cream is a shelf-stable product with a fat content of >10%. It is homogenized and UHT-processed, filled aseptically, or sterilized in the container. It is a popular product that is mainly used for whitening coffee, as well as for imparting a pleasant flavor to coffee. It is also used in the preparation of food and drinks, and for direct consumption. Coffee cream has a minimum shelf-life of 4 months at room temperature, and it normally contains 10–12 g fat/100 g, and, less often, 15–20 g fat/100 g. Its shelf life is similar to the shelf life of UHT milk. The important quality attributes of coffee cream are taste, whitening power, and stability in hot coffee.

Following separation and standardization, the cream is heat-treated at 90–95 °C and cooled to 6 °C. Stabilizers such as phosphates or citrates may be added to increase the pH and to reduce the concentration of ionic calcium, thereby reducing the susceptibility of casein micelles to aggregation during the sterilization process or on addition to hot coffee. Cream is subjected to two-stage homogenization and sterilization. Traditionally, coffee cream was sterilized in cans or bottles, but since 1996, continuous-flow sterilization in a UHT plant, followed by aseptic packaging, has largely replaced the former process. Both homogenization steps operate at a total pressure of 20 MPa and a temperature of 70 °C. Flow-sterilized coffee cream is subsequently cooled to 25 °C and aseptically filled, usually into plastic cups (7.5–15 g capacity) or preformed cups or cans (100–200 g capacity).

Importance of homogenization

Coffee cream must be homogenized. This prevents a fat layer or fat plug from forming in the container, thus improving taste, whipping power, and stability. It has a direct influence on the flocculation stability of coffee cream. When sterilizing cream in the pack, homogenization has to take place before the sterilization, which again is a double stage process (20 MPa) using the same pressure. Flocculation of cream in hot coffee is due to casein precipitation.

Desirable attributes of coffee cream

In addition to good sensory properties, the two main criteria for assessing the quality of coffee creams are as follows:

Stability of the emulsion: This is the ability of the particulate constituents (primarily lipid and protein particles) to remain evenly dispersed throughout the coffee cream during its long shelf-life.

Whitening effect: This is the ability to form a milky, homogenous mixture on addition to coffee, irrespective of the composition, brewing conditions, and temperature of the coffee.

Like UHT milk, homogenized UHT creams exhibit a gelation phenomenon, generally attributed to the activity of heat-resistant proteolytic enzymes, after prolonged storage.

One important defect with respect to coffee creams is the phenomenon known as 'feathering.' Feathering is the formation of macroscopic aggregates containing proteins and lipids through the acid-induced flocculation of partially protein-covered lipid globules in hot coffee drinks. Although aggregates always form in hot coffee, the visible aggregates (i.e., ≥100 μm) render the coffee cream defective, because the consumer commonly perceives such aggregates as an indication of the sourness of the product. The susceptibility of coffee cream to feathering is often referred to as its coffee stability.

Cream Liqueur

Cream liqueurs are a group of liqueurs that contain milk cream as an ingredient. The roots of cream liqueur lie in home-made products that can be traced back for centuries, such as the Scottish product Atholl Brose. Atholl Brose consists of milk cream and honey, to which whisky, steeped with oatmeal to impart a pleasant nutty flavor, is added. In the second half of the twentieth century, commercial-scale attempts to produce Atholl Brose and other home-made cream liqueurs largely failed, until 1974, when Baileys Irish Cream Liqueur became the first commercialized cream liqueur. The most famous cream liqueurs are the Irish product Baileys Irish Cream and the South African product Amarula.

Composition of cream liqueur

In terms of commercial practice, a standard cream liqueur does not exist. Each manufacturer decides the preferred components and their concentrations. This flexibility enables a product of desired organoleptic properties and a sufficiently long shelf-life. Table 3 shows the composition of a standard cream liqueur, which appears to represent most commercially available products. The 14 g/100 g concentration of ethanol in the product equates to 17 g/100 ml ethanol. Of the ingredients, sodium caseinate is used as an emulsifier, and citrate is added to prevent serum separation during storage.

Technology of cream liqueur

Although there is no universal manufacturing process for cream liqueur, the principle steps of mixing the ingredients and homogenizing the cream base are universal. The sodium caseinate can be dissolved in hot water (85 °C), after which time the other ingredients are added to produce the cream base. Ethanol may be added to the cream base either before or after homogenization. The function of homogenization is to reduce the lipid globule size, thereby prolonging the physical stability of the product by preventing creaming and/or the formation of the cream plug on storage. A general rule-of-thumb for homogenization efficiency is that >98% of the milk lipid globules should have a diameter <0.8 μm.

Maintaining the quality of cream liqueur

As with other cream products, the shelf-life of cream liqueurs has two dimensions, the microbiological shelf-life and the physicochemical shelf-life. The microbial shelf-life of cream liqueurs causes little concern, because human pathogens are unable to grow in this medium of high ethanol and sugar content.

Table 3 The composition of a standard cream liqueur

Component	*Concentration (g kg^{-1})*
Milk fat	160
Sucrose	195
Sodium caseinate	30
Nonfat milk solids	14
Total solids	399
Ethanol	140
Water	461

Source: Banks, W. and Muir, D. D. (1988). Stability of alcohol-containing emulsions. In: Dickinson, E. and Stainsby, G. (eds.) *Advances in food emulsions and foams*. London: Elsevier Applied Science.

Physicochemical defects in cream liqueurs

Different types of physicochemical defects have been noted in cream liqueurs:

(a) *Cream plug formation*: Cream plug formation refers to the accumulation of fat in the neck of the bottle during storage, with the fat unable to be redispersed into the product through shaking or agitation. Cream plug formation results from insufficient homogenization. As a result, lipid globules rise to the top, which may ultimately lead to a cream plug. Because the plug cannot be redispersed on shaking or agitation, it is likely that the creamed lipid globules have aggregated or coalesced. The extent of cream plug formation correlates positively to the proportion of large lipid globules in the product, and it can be prevented by increasing the homogenization pressure. Homogenization efficiency may also be increased, reducing creaming, by the inclusion of low levels (0.5 g/100 g) of low-molecular-weight surfactants in the cream liqueur. Cream plug formation is likely enhanced at low pH levels (<6.0), high concentrations of ionic calcium, and low concentrations of emulsifier, and it can also be increased by temperature fluctuations during storage.

(b) *Serum separation*: The gelation of cream liqueur results in syneresis, leading to the separation of a serum layer. The shelf-life of cream liqueur is the number of days the product can be stored at 45 °C before noticeable serum separation, and it increases in a sigmoidal manner with increasing pH. The use of washed cream or anhydrous milk fat improves the shelf-life of cream liqueurs, and the same is true for the addition of the calcium-chelating agents such as trisodium citrate. The addition of citrate reduces the concentration of ionic calcium and accordingly increases the shelf-life of cream liqueurs by up to two orders of magnitude. Replacing caseinate with whey protein concentrate also increases product stability. Furthermore, the use of sorbitol as a carbohydrate component significantly increases the shelf-life of cream liqueurs.

(c) *Precipitation*: Although the addition of citrate to cream liqueur enhances its stability against serum separation, it also causes a defect in the form of a slightly granular precipitate, consisting primarily of calcium and citrate, at the bottom of the bottle. The precipitate is particularly noticeable on storage at above ambient temperatures, because the solubility of calcium citrate decreases with increasing temperature. As a result, the amount of citrate added to cream liqueur becomes a trade-off between preventing serum separation and minimizing the risk of precipitate formation.

Other Cream Products

Clotted Cream

Clotted cream is prepared by heating cream to 77–88 °C (170–190 °F) in shallow pans and then allowing it to cool

slowly. The surface layer consists of dotted cream, which is skimmed off and strained. Clotted cream is exceedingly rich, containing 60–70% milk fat. This fat is in a finely emulsified condition, which renders it easily digestible. The product has a peculiar boiled taste and rough appearance, and it exhibits a white-flaked surface. The average composition of clotted cream is 67.50% milk fat, 4.90% protein; 1.00% lactose, 0.50% ash, and 26.10% water.

There is no standardized method for preparing clotted cream. Several systems differ in obtaining the raw cream, thus resulting in considerable variation in the texture, flavor, and appearance of the finished product. The flavor and physical consistency of cream depend on the acidity of the original milk, the temperature of scalding, and the time allowed for scalding.

Canned or Sterilized Cream

Canned or sterilized cream generally possesses a peculiar flavor because of its processing, as well as a high viscosity due to homogenization. The texture should be smooth, and the cream should be free from lumpiness and separation of serum. Sterilization spoils its whipping quality. The fat content is about 20–25%, and the nonfat solids content may vary between 6.5% and 9.5%. The various steps in manufacture are as follows:

(i) Fresh, sweet cream is standardized to 20% fat.
(ii) Pre-Standardized cream is heated to 80 °C, without holding.
(iii) Cream is double-homogenized at 80 °C, using 2500–3000 psi in the first stage and 500 psi in the second stage.
(iv) Cream is immediately cooled to 16 °C, preferably over a surface cooler using brine.
(v) Cream is filled into tin cans (or bottles) and immediately sealed.
(vi) Filled cream is sterilized in retorts employing 15 min for coming-up, 12–15 min for holding at 118 °C, and 15 min for cooling to room temperature.

Plastic Cream

Plastic cream is more viscous than any other type of cream. Its texture resembles paste. Its fat content is between 65% and 85%, and it can be used directly for the manufacture of butter-oil. Plastic cream is obtained by (i) reseparating normal cream (30–40%) in a normal cream separator or (ii) separating milk in a specially designed plastic cream separator. In both of the above cases, the initial product is pasteurized at about 71–77 °C for 15 min and cooled to 60–66 °C before separation.

Conclusion

Cream is an extremely versatile product with applications that extend far beyond the dairy sector. Cream products have been traditionally regarded as luxury products, and their popularity may be affected in the current climate by consumer concerns about high fat intake. Given recent advances in our understanding of milk and cream constituents and their interactions with other ingredients, the development of reduced fat cream products with properties equivalent to their traditional high-fat counterparts should be possible.

See also: Buffalo Milk; Butter: Manufacture; Coffee: Health Effects; Coffee: Types and Production; Cream: Clotted Cream; Dairy Products: Dietary and Medical Importance; Ghee.

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Cured Foods: Health Effects

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Introduction

Curing refers to the technological process involving the addition of salt and nitrates and/or nitrites to different types of raw materials to produce the so-called cured food products. The origin of the word 'curing' roots in the Latin word 'cure,' translating as 'means of healing, remedy,' and was used for products processed that way because they were known to be safer and no longer capable of transmitting any disease. Curing is no doubt one of the earliest processing technologies for preservation of different foodstuff, whose origin probably dates backs to ancient Egypt, being well known and described by the ancient Greeks and Romans. In even earlier times, basic processing technologies consisted of adding salt to different raw food materials, such as fish, cheese, and meats, thereby leading to a stable food product that could be preserved at room temperature. The main preserving effect of salt relies on the decrease of water activity; however, in the past, the addition of sea salt involved the unintentional addition of nitrates, whose effects on preservation were not understood until later. Nevertheless, 'saltpeter,' a salt efflorescence very rich in nitrates, was used in ancient China and India, and its effects on producing a stable meat color were also well known. Nowadays, curing is undoubtedly the technological process most commonly used for transforming meats, including fermented, dry-cured, smoked, cooked, or canned products.

The technological and safety effect of curing salts is well known and appreciated, and it is thoroughly explained elsewhere in this encyclopedia. To synopsize, curing leads to the development of a stable red color in meats due to the formation of a stable pigment through the reaction of myoglobin with nitric oxide (NO), which in turn is formed from nitrite. Furthermore, the antioxidant effect of nitrites is well known, and its addition helps to control the formation of compounds showing rancid flavors involved in warmed-over off-flavor development in cooked and reheated meats. In addition to this effect on flavor, nitrates and nitrites are known to positively influence the overall flavor of cured products, although their exact involvement has not been entirely elucidated yet. But it is perhaps the antimicrobial effect associated with nitrate/nitrite usage that is the most outstanding property. The presence of a certain amount of nitrites inhibits the germination of *Clostridium botulinum* spores and also helps in the control of the growth of other pathogenic and spoilage bacteria in meat products. Therefore, nitrate and nitrite addition is among the most effective and reliable hurdles for avoiding some of the most serious microbial poisonings.

However, in spite of all these positive effects, the use of nitrate and nitrite in food processing has been controversial over the past 40 years, and this has been mainly due to the toxicity of nitrite itself and to the further reaction with amino compounds to form *N*-nitroso compounds, some of which, like nitrosamines (NAs), have been shown to be potential carcinogens. On the other hand, the intake of nitrate and nitrite, through their transformation into NO, has also been shown to have a protective role in some cardiovascular diseases.

The balance between all these effects has been thoroughly researched for quite some years, but still, there is no clear final scientific outcome supporting either the total avoidance of these preservatives or their indiscriminate use. Accordingly, food legislations in most countries have restricted their use to some specific products and to limited amounts. While meat companies claim that their use is absolutely necessary for obtaining safe products, other groups ask for their total banning from food processing.

This article attempts to collect evidence pertaining to the potential harmful or health-promoting effects of the addition of nitrate and nitrite to food (especially meat) products.

Formation of *N*-Nitroso Compounds

The major concern for the use of nitrate/nitrite in food is related to the involvement of nitrite in the formation of carcinogenic *N*-nitroso compounds, especially NAs, and their subsequent effect as known carcinogenic compounds. These *N*-nitroso compounds can be formed either in the food during processing and prior to consumption or in the human body, due to the ingestion of nitrates or nitrites. The basic chemistry for the formation of NAs is explained elsewhere, but basically, it involves the formation of a nitrosating agent, typically formed from nitrites, which further reacts with a secondary or a tertiary amine (Figure 1). This takes place faster in acidic conditions, although it can also happen in an alkaline medium in the presence of some carbonyl compounds. In the case of added nitrates, it would involve a previous reduction of nitrates to nitrites, which is also favored not only under acidic conditions but also by microbial activity. Such kind of routes can be produced not only in the food itself but also in the human body, as a consequence of the ingestion of amines and the nitrite from the food or through interaction with saliva.

The concern about the involvement of curing salts in the formation of carcinogenic compounds commenced in the 1950s and 1960s, with several studies highlighting the potential implication of nitrite in the formation on NAs. This fact was also linked to the occurrence of different types of cancer. In 1970, a report paper published in Nature by Lijinsky and Epstein reviewed the role of NAs in the development of cancer. Due to the implication of nitrite and amino compounds in the formation of these carcinogenic compounds, the authors concluded that the best way to avoid the risk of NA intake was to eliminate any of these precursors. Since amino compounds are hardly avoidable in most foods, the final indication toward a banning of curing salts was quite clear.

Thus, there are different aspects that should be considered within the controversy of nitrates/nitrites as food additives.

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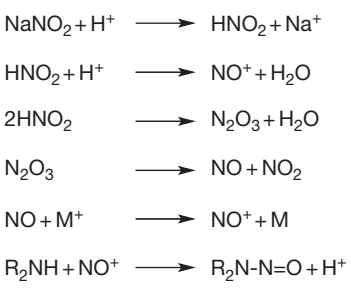

Figure 1 Formation of nitrosamines (M/M^+ are transition metals ions like Fe^{2+}/Fe^{3+} or others). Adapted from Honikel, K. O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science* **78**, 68–76, with permission.

First, it seems clear that several *N*-nitroso compounds have been characterized as being potential carcinogenic compounds, but it is important to know which ones and at what level. Then, the actual involvement of nitrates and nitrites in the formation of NAs, both in the food and in the human organism, and other factors affecting their formation in the food, should also be considered as very few compounds can be considered totally harmless. It is the dose and the intake conditions that make them more or less dangerous. Furthermore, the actual contribution of cured foods as a source of nitrates, nitrites, and *N*-nitroso compounds in the diet should also be taken into consideration. Finally, there is the question about the link between consumption of cured products and the incidence of cancer.

As far as the role of NAs in the development of different types of cancer, their high carcinogenic potency has been proven in animal experiments on many different species, and virtually no species has been found to be resistant to their cancer-inducing potential. In fact, the metabolic activation of NAs into DNA-damaging electrophilic agents is very similar in all animals and tissues, resulting in the induction of similar mutagenic DNA lesions. So, although direct proof of carcinogenicity to humans is not available, the evidence for these compounds to be considered human carcinogens is overwhelming. However, each NA seems to have a different ability to induce cancer. In fact, the volatile NAs seem to be more carcinogenic, while nonvolatile ones appear to be not as carcinogenic, or not directly carcinogenic; nevertheless, some of them can be further transformed into volatile versions. Among those frequently found in cured products, *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosopiperidine (NPIP), *N*-nitrosomorpholine, *N*-nitrosomethylbenzylamine, and *N*-nitrososarcosine have shown strong evidence of being carcinogenic, while *N*-nitrosothiazolidine, *N*-nitrosoproline, or *N*-nitrosothiazolidine-4-carboxylic acid seems to either be noncarcinogenic or show a low carcinogenic potential. Despite the type of compound, the maximum allowed level for the sum of all NAs in cured meat products has been set to a limit of 10 $\mu g\ kg^{-1}$ of total volatile NAs in the United States, while no maximum limits have been established within the EU as of yet. Nevertheless, the actual levels leading to the development of a cancerous lesion can be very different from one NA to another, and in the case of humans, it is mostly unknown. To make things even more complex, it seems that NAs show a higher effect as carcinogens in laboratory animals when applied in repeated small doses as compared with larger single applications. This situation would in fact mimic the exposure of humans to traces of NAs on a daily basis.

The presence of nitrate and nitrite enables the formation of NAs in cured products, not only cured meats but also cheese and some fish products. The level and type of NAs found in cured products are very variable. In cooked cured meat products, the amount of secondary amines produced when the product is heated is rather low, since they are processed using fresh meat. The presence of free secondary amines in meat is mainly due to microbial metabolism, and thus, their formation involves high microbial counts, the presence of specific conditions, and the factor of time. On the other hand, due to the high reactivity of nitrite, in raw cured meat products, the amount of residual nitrite is rather low, and moreover, there is no heating step during processing, which would otherwise boost nitrosation reactions. As a consequence of all this, in meat products, the two most frequently determined NAs (NDMA and NPYR), with the production conditions and additive levels allowed nowadays, are found at levels rarely higher than 5 $\mu g\ kg^{-1}$. Other common NAs in cured meat products are NDEA and NPIP, and they also reach similar or slightly lower levels. Cured fish products and cheese usually show lower levels of these same NAs, even though some specific fermented and salted fish products, especially from Southeast Asia, may show very high amounts of NDMA, NPIP, and NPYR, most likely due to their elevated content of biogenic amines. There have been several attempts to address how much the different types of food consumed in a regular diet contribute to the total intake of nitrate, nitrite, and *N*-nitroso compounds (or NAs). Beer, cured meats, and fish products have been highlighted as the main sources of NAs in several countries. Nevertheless, the levels found in cured products are usually low and within established safe limits, except for exceptional circumstances (whether these limits are actually safe in the long term for humans is largely unknown, but they have been established using animal model systems).

Different processing factors may enhance the NA content in cured products: nitrate and nitrite content, pH, cooking temperature, presence of secondary amines or presence of ascorbate or tocopherols, etc. A deep knowledge about how these factors influence the formation of NAs may enable the development of processing protocols aimed at controlling the formation of such carcinogenic compounds.

It seems clear that the amount of nitrate and especially nitrite positively influences the formation of NAs, and in fact, it has been suggested that their formation is proportional to the square of the nitrite concentration. As far as nitrate is concerned, an almost linear relationship between the amount of added nitrate and the content of NDMA and other volatile NAs has been found in several cured meat products and in cured cheese. Such a relationship between nitrate and nitrite content and amount of NAs is the reason behind the limitation in the use of these curing salts in most food products all around the globe and also the voluntary reduction by many food manufacturers. These controls have led, over the past 20 years, to a reduction in the NA content of many common cured meat

products. Nevertheless, when comparing the actual levels of NAs in commercial cured meat products in Denmark, where the national normative only allows a maximum level of 60 mg kg^{-1}, with those in meat products from other EU members, in which the maximum allowed level is more than twice the Danish level (Table 1), differences were very scarce. In fact, some studies have indicated that there is a positive, but not necessarily linear, correlation between the amount of nitrite added into the food and the amount of NAs formed in the final product.

In some countries, the legislation for organic products does not allow the use of nitrates and/or nitrites in the processing of cured meat products. In fact, consumer preferences for organic and natural foods are largely based on concerns about additives used in conventionally produced foods. Consumers are willing to pay significantly higher prices for organic foods. Strangely, many of these organic processed meats (the term 'cured' is not allowed in US legislation, because of the lack of nitrate and nitrite addition) show similar properties to the conventional ones in terms of color and oxidative stability. In most of these products, producers are using spices or plant extracts naturally rich in nitrates, like celery, spinach, and beetroot. Among these, celery is perhaps the most popular, due primarily to its lack of negative impact in relation to color and flavor tainting. The nitrate content of celery powder may be higher than 20 g kg^{-1}. Nevertheless, in this type of product, the actual final amount of curing salts is usually lower than in conventional cures, which could be a concern relative to microbiological safety.

Considering that nowadays the addition of nitrite to cured products remains one of the only plausible strategies to ensure the inhibition of *C. botulinum* spore germination, a further reduction in allowed nitrite levels for cured meat could imply a safety risk. Therefore, other strategies for reducing the formation of NAs would be of enormous interest from a public health perspective. Indeed, ascorbic acid, erythorbic acid, and tocopherols have shown to have a remarkable decreasing effect on the formation of NAs in different types of cured meats. They are capable of inhibiting NA formation by competing with the precursor amine for available nitrosating agents. During curing at a regular meat pH, ascorbic acid reacts faster than secondary amines, such as proline, with the nitrosating agent N_2O_3, thereby preventing the manner in which NA formation occurs. Ascorbate reacts with nitrite 240 times more rapidly than ascorbic acid, and it is, therefore, the preferred candidate of the two. In fact, ascorbate is commonly used in the processing of cured meat products, with the aim of helping both the formation of a stable red pigment through its positive effect on the nitrosation of myoglobin and the prevention of the formation of NAs during processing and subsequent storage. Some lipid-soluble salts of ascorbic acid have been shown to have an even better effectiveness in preventing the formation of NPYR during the frying of bacon. Gamma irradiation has also been shown to have promising effectiveness in reducing the NA content in cured meat products. However, this type of technology is far from being popular in most countries right now, particularly within the EU.

Heating is well known to be one of the factors boosting the formation of NAs during the processing of cured meat products and also during cooking before consumption. For example, raw bacon is mostly free of NAs, but after cooking, and especially after frying, their levels considerably rise. In fact, when analyzing the effect of heating on the NA content of different foodstuffs, a general trend of higher levels of NAs in products with higher processing temperatures has been found. However, in a recent study in which NA content was corrected for moisture loss, the trend was not the same for all NAs: while NPIP increased in almost all cured meat products due to heating, other volatile NAs showed either an increase or a decrease depending on the product. Such varying results could be due to the specific temperatures used for cooking, since very high temperatures could lead to a decrease in the content of certain NAs due to evaporation.

Cured products with a high initial level of secondary amines, usually present due to microbial metabolism, run a higher risk for the formation of NAs during processing. In many of these products, such as meat, cheese, or fish, there is no additional heating of the product before consumption, which in a way limits the formation of NAs. However, it should be considered carefully that some of these products are actually heated when being used as part of the recipe of another foodstuff, such as pizza, pasty, and soups. In some fish products in

Table 1 Maximum allowed levels for nitrate and nitrite (mg kg^{-1}) in some cured products by the EU legislation (EU Commission Regulation, No. 1129/2011)

Type of product	*Nitrite (E249/E250)*	*Nitrate (E251/E252)*	*Observations*
Non-heat-treated processed meat	150	150	
Heat-treated processed meat	150		Except sterilized meat products ($F_o > 3.00$)
Heat-treated processed meat	100		Only sterilized meat products ($F_o > 3.00$)
Wiltshire bacon and similar products	175	250	Maximum residual amounts
Dry-cured bacon and similar products	175	250	Maximum residual amounts
Dry-cured ham	100	250	Maximum residual amounts
Salchichón, chorizo, saucisson sec		250	
Ripened cheese		150	Only hard, semihard, and semisoft cheese
Whey cheese		150	Only cheese milk of hard, semihard, and semisoft cheese
Cheese products		150	Only hard, semihard, and semisoft ripened products
Dairy analogues		150	Only dairy-based cheese analogue
Pickled herring and sprat		500	

In all cases, it refers to the maximum added amount, except when otherwise specified.

which the initial content of free amines is enormous, curing implies a very high risk for the formation of unacceptable amounts of NAs. In fact, addition of nitrites to fish and seafood is banned or severely restricted by most food legislation.

Some unusual NAs, such as *N*-nitrosodi-*n*-butylamine and *N*-nitrosodibenzylamine, have also been detected in cured meat products packaged in elastic rubber nettings, formed as a result of the interaction of the residual nitrite in the cured meats with amine additives in the rubber nettings. NPIP in cured products due to the simultaneous presence of nitrite and pepper can occur either by the oxidative cleavage of the amide bond of piperine and a subsequent nitrosation of piperidine or by a direct nitrosation of the already available piperidine.

In addition to the formation of NA in the food, nitrates and nitrites are also involved in their formation *in vivo* in the human body, mainly due to the acidic conditions of the stomach. Gastric nitrosation proceeds through reaction between amines from the food and nitrosating agents derived from the diet, both nitrites present directly in the food and nitrates reduced to nitrites in the mouth. In fact, nitrate is resorbed in the intestine and excreted through the saliva; thereafter, it is partially reduced to nitrite by nitrate reductase-containing bacteria in the oral cavity. Some studies have suggested that around 90% of ingested nitrite comes from the saliva. Nitrite intake in most countries mainly comes from cured meats, while the main source of nitrates is green leafy vegetables. In fact, most global food legislation has limited the use of nitrates for cooked cured meat products, so that the actual amount of nitrite can be more precisely controlled (**Table 1**). Moreover, there has been a trend toward a reduction in allowed levels of nitrate and nitrite in cured meats, so that nowadays, the residual levels are much lower than they used to be 30 years ago. Nevertheless, EU legislation still allows the use of nitrate in several traditional cured meat products and in cured cheese (**Table 1**). In the case of traditional cured meat products, processors claim that without the addition of nitrates, it would be difficult to achieve the features that consumers expect in this type of products, while in cheeses, the allowance is based in the prevention of early blowing during the processing of less acidic varieties. The use of such high levels of nitrates allowed in pickled herring by the EU normative is remarkable, and as such, usage entails a risk for NA formation.

Nitrosation in the intestinal track seems to be markedly diminished by the presence of ascorbate, so that its presence in the food would limit the *in vivo* formation of NAs. However, some findings suggest that in the presence of fat, ascorbate could actually enhance the formation of NA in the stomach. The parallel intake of polyphenols or other plant compounds with antioxidant activity could also help in the reduction of *in vivo* nitrosation, while other studies suggest that heme from meat could play a role in boosting nitrosation in the large intestine. Without question, more research is needed in this area, which will potentially allow designing cured food product formulas targeted at reducing nitrosation of amines, both in the food and *in vivo*.

As discussed previously, the potential involvement of cured products in the development of cancer caused by *N*-nitroso compounds could be due to both the formation of NAs (and other *N*-nitroso compounds) during product processing and storage and their contribution to the total nitrate and nitrite intake, which would be in turn related to *in vivo* nitrosation. Indeed, the role of NAs in cancer was already described in the 1950s. Later, in the 1960s and 1970s, several papers linked the consumption of cured meats to the development of different types of cancer. Although subsequent studies failed to confirm that nitrite intake caused cancer, the Assembly of Life Sciences in the United States was prompted to commission a report on the toxicity of nitrate and nitrite, which was only partially reassuring. In 2003, the Joint FAO/WHO Expert Committee on Food Additives stated that the epidemiological studies showed no consistently increased risk for cancer with increasing consumption of nitrate. Subsequent epidemiological studies have similarly failed to show a convincing link between nitrate or nitrite intake and cancer. Nevertheless, the International Agency for Research on Cancer (IARC) published a monograph in 2010 in which it was concluded that ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans. However, this statement does not specifically refer to cured products. In fact, and as explained earlier, the major sources for nitrate and nitrite intake in most countries are saliva, vegetables, and water.

More specifically related to cured products, different epidemiological studies relating the consumption of cured meats or fish with a higher risk for the development of different types of cancer have been published and have been widely communicated in the media. Moreover, the World Cancer Research Fund published guidelines for cancer prevention, one recommendation being to limit the consumption of red meat and eliminate processed meat consumption entirely. Although not specifically mentioned, it is assumed that such a risk is attributed to curing, although other factors specifically linked to the processing of meats could also be involved. Such claims have been very controversial, and a number of papers and articles have been published highlighting the fact that such published claims have lacked epidemiological evidence. In fact, it has been pointed out that studies linking the consumption of processed meat to cancer seriously failed to completely consider several additional factors that can contribute to chronic disease, including participants' behavior as to alcohol and tobacco use, exercise, weight, and access to health care.

At any rate, if processed meats are truly involved in a higher risk of colorectal cancer, it is unlikely that this is due to residual nitrate or nitrite, because green leafy vegetables show much higher levels and seem to show no epidemiological relationship to cancer. Moreover, both nitrate and nitrite are formed endogenously and recycled with the saliva. Nevertheless, some studies suggest that the concomitant ingestion of heme and nitrosation agents could boost the endogenous formation on *N*-nitroso compounds in the colon. Others have hypothesized that *N*-nitrosamides, rather than NAs, could be related to the development of some types of cancers, but little is known about their actual content or their specific involvement in this type of disease.

Other Potential Harmful Effects of Cured Foods

Cases of infant methemoglobinemia as a consequence of well-water with a high nitrate content occurred in the past, and that is the reason why the maximum allowed amount of nitrates in

water is regulated in most countries. In fact, it is nitrite that causes methemoglobinemia, and in this sense, it could be thought that the consumption of food containing residual nitrite, like cured foods, could somehow be related to this type of disease. Nevertheless, the levels of dietary nitrites causing this condition are much higher than those that can be found in cured foods. Yet, there are reports linking cases of methemoglobinemia to the consumption of cured products with high levels of nitrites. For example, in one such incidence dating from the 1950s, an outbreak of methemoglobinemia occurred after consuming wieners and bologna containing more than 5000 ppm of nitrite. At any rate, episodes of cured food involved in cases of acute nitrite poisoning are most likely due to processing mistake.

Potential Beneficial Effects of Nitrate and Nitrite

Nitrate conversion into nitrite, and subsequently into NO, is behind the potential positive health effects of intake of these inorganic salts. Most of these possible effects have been shown for vegetable extracts, very rich in nitrates, and it is unlikely that any significant effect could be due to the residual levels of nitrate or nitrite found in cured products. Nevertheless, as a part of the diet, cured products no doubt contribute to the total intake of nitrate and specially nitrite, since they are estimated to be the third source of these salts, after vegetables and water.

NO is an endogenously produced modulator of vascular tone. Indeed, as a consequence of eating a meal rich in nitrates, a subsequent fall in both systolic pressure and diastolic pressure has been described. It seems clear that the reduction of ingested nitrate into nitrite is behind this effect, since in an experiment in which subjects had to eliminate their saliva after nitrate ingestion, the nitrite peak in blood disappeared and so too did the effect on blood pressure. NO also plays a key role in vascular homeostasis by maintaining vessels in their relaxed state; in experiments carried out in humans, dietary nitrate supplementation attenuated the impairment in endothelial function seen in ischemia reperfusion injury. NO also plays a role in the inhibition of platelet adhesion and aggregation. In this sense, dietary supplementation with either nitrate or beetroot juice has resulted in significant inhibition of platelet aggregation.

Widespread media attention has focussed on the positive effect of nitrate on muscle efficiency and fatigue resistance. In different experiments, a short-term dietary supplementation with sodium nitrate resulted in improved muscular efficiency and a reduction in oxygen consumption during submaximal exercise in healthy subjects. NO has numerous physiological signaling functions that may impact on muscle fatigue and performance: regulation of blood flow, muscle contractility, glucose and calcium homeostasis, and mitochondrial oxygen consumption. The reduction of nitrite to NO is facilitated when oxygen availability is limited, which agrees with the fact that nitrate supplementation appears to be particularly effective in enhancing performance in hypoxia and ischemia. Recent studies suggest that nitrate supplementation may specifically improve calcium handling and force production in type II muscle fibers and result in preferential distribution of blood flow to type II muscles. Given the importance of type II fiber recruitment and metabolism to performance in high-intensity intermittent exercise, and the likelihood that tissue hypoxia develops to a greater extent during such exercise compared to continuous low-intensity exercise, these recent studies therefore provide a clear rationale for nitrate supplementation to enhance performance during multiple sprint sport.

See also: Cancer: Diet in Cancer Prevention; Food Additives: Classification, Uses and Regulation; Nitrites and Nitrates.

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Cystic Fibrosis, Nutrition in

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Definition

Cystic fibrosis (CF) is a multiple organ system disease, affecting the lungs and pancreas. Nutritional status is impaired by inadequate intake, increased energy needs, and pancreatic insufficiency. Pancreatic insufficiency results in maldigestion and malabsorption of nutrients and fat-soluble vitamins. Pancreatic enzyme supplementation and optimizing nutritional deficiencies prevent growth failure and improve other outcomes in patients with CF, including quality of life, resistance to infection, and chronic lung disease prevention, leading to longer life expectancy in patients with this disease.

CF is a result of the defects in the CF transmembrane conductance regulator (CFTR), which is responsible for the excretion of salt, in turn creating viscous secretions in multiple organ systems. For decades, CF was thought to be a disease only of childhood given the lower life expectancy. Largely because of improvements in nutrition, the average life expectancy of patients with CF has advanced well into adulthood.

Clinical Manifestations

Defects in the CFTR result in multisystem disease including lung, liver, and gastrointestinal (GI) diseases, notably pancreatic insufficiency. While the majority of CF mutations cause lung disease, growth and nutritional status are closely tied to lung disease and affected by the type of CF mutation. Body mass index (BMI) being maintained at $\geq 50\%$ for age has been shown to correlate with the percent predicted forced expiratory volume in 1 s of greater than or equal to 90%, which is an excellent marker of lung function. For CF patients ages greater than or equal to 20 years, it is recommended that women maintain a BMI at or above 22 and that men maintain a BMI at or above 23.

Nutritional failure in CF is multifactorial. Insufficient production of pancreatic enzymes leads to fat malabsorption, which can be exacerbated by bile salt abnormalities if there is concurrent liver disease. Progressive pulmonary infection leads to increased work of breathing and therefore increased caloric needs. Chronic lung infection can reduce appetite and lead to an inflammatory catabolic process in the body. Other factors affecting nutrition include CF-related diabetes mellitus, altered motility of the GI tract, and small bowel bacterial overgrowth.

Management

CF derived its name from the cysts and fibrosis noted in the pancreas of patients with CF. The pancreas is responsible for secreting enzymes that aid in the absorption of nutrients including fat-soluble vitamins A, D, E, and K. In order to counteract this malabsorption, pancreatic enzyme replacement therapy is used. Lipase is the enzyme responsible for fat absorption. Pancreatic enzyme replacement is based on the dosage of lipase in the supplement (500–2500 lipase units per kilogram of body weight per meal or $<10\,000$ lipase units per kilogram of body weight per day). In addition, fat-soluble vitamin supplements are routinely given. The CF Foundation (CFF) supports that higher energy intake is needed for improved weight gain. In order to achieve energy intake of 110–200% of the healthy population, the CFF makes several recommendations with the goal being a high-calorie unlimited fat meal.

For children ages 1–12 with CF, intensive behavioral and nutritional counseling is recommended to promote weight gain. For those with growth deficits and adults who are having difficulty with maintaining weight gain, oral or enteral nutritional supplements are recommended.

Fat-soluble vitamins A, D, E, and K are supplemented in all children with CF. Supplements are started at diagnosis including asymptomatic infants and those without pancreatic insufficiency. Standard ADEK supplements tend to overestimate vitamin A and underestimate vitamins D and K. This can be problematic, for example, in CF patients who also have CF-related liver disease, as vitamin A toxicity can affect the liver.

Vitamin A deficiency is rare except at the time of diagnosis. Vitamin A is necessary for vision, gene expression, growth, and immune function. Available forms of vitamin A include preformed vitamin A (retinol and retinoic acid) and provitamin A (alpha-carotene and beta-carotene). Vitamin A levels are monitored via serum retinol and retinol-binding protein. Vitamin A toxicity is possible in CF patients and is marked by bone mineral loss and liver abnormalities.

Vitamin D deficiency in common in CF. Vitamin D helps the body use calcium from the diet and deficiency can lead to poor improper bone mineralization. Vitamin D_3 (cholecalciferol) is contained in most supplements and is the form produced in the skin by sunlight. The CFF recommends vitamin D_3 versus vitamin D_2 (ergocalciferol) because a small study showed it achieved better target 25-hydroxyvitamin D. Levels are checked annually at the end of winter. Bone disease in CF results from decreased mineral density, which worsens with age, severe lung disease, and malnutrition. There is also an increase in fracture rates and kyphosis in young adults with CF. In addition to vitamin D deficiency, chronic corticosteroid use and reduced weight-bearing activity can contribute to bone disease.

Vitamin E is an antioxidant and its deficiency could contribute to inflammation and lung disease in CF. Vitamin E deficiency leads to delayed stretch reflexes, cerebellar ataxia, and peripheral neuropathy. Some studies have shown a correlation between vitamin E status, polyunsaturated fatty acid (PUFA) status, and inflammation in CF. Eight different forms of vitamin E exist, the most common form being alpha-tocopherol acetate. The recommended intake for CF patients is 20 times the general recommended intake. Serum vitamin E is influenced by serum lipid levels.

Vitamin K deficiency is associated with coagulation abnormalities and bone disease. Vitamin K is found in green vegetables, and vitamin K status is monitored by a serum prothrombin time. As intestinal bacteria are a source of vitamin K, supplementation should be provided during courses of antibiotic, a common occurrence in CF patients.

Essential fatty acids are long-chain PUFAs and include omega-3 and omega-6 fatty acids. Their deficiency may contribute to an inflammatory state including scaly dermatitis, alopecia, and growth failure and tends to be more common in infants. Routine supplementation is not recommended at this time. However, a systematic review showed that supplementation with omega-3 fatty acids improved several markers of lung disease.

CF patients are prone to hyponatremic dehydration under conditions of heat stress secondary to sodium losses through sweat. Sodium chloride supplementation is used especially in warm months or climates and in infants.

The CFF recommends a trial of zinc supplementation for children <2 years not growing well. Dermatitis can be a symptom of zinc deficiency.

Iron deficiency has been noted in several studies of CF patients and is monitored yearly by serum hemoglobin and hematocrit.

Oral supplements are used in CF but are often less effective because they may displace ordinary food. A systematic review showed that calorie-protein supplements are not superior to monitoring and dietary advice from a health professional and a nutritionist. Enteral nutrition is generally started via gastrostomy tube for patients with growth failure. Although there are no randomized control trials to support this, enteral nutrition is thought to improve and maintain lung function in patients with CF. For toddlers, a combination of daytime feeds, daytime meals, and nighttime continuous feeds is used. Concentrated formula delivers more calories in less volume for overnight feeds to help prevent excessive nocturnal urination.

In conclusion, CF is a disease in which nutritional status is highly important to overall disease outcomes. It is important to monitor nutritional status regularly, looking at growth and overall digestive issues including signs of malabsorption, and to screen for nutritional deficiencies. Fortunately, advances in these nutritional measures over the past several decades have resulted in improved life expectancy for this chronic disease.

See also: Calcium: Physiology; Calcium: Properties and Determination; Fats: Classification and Analysis; Malnutrition: Concept, Classification and Magnitude.

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D

Dahi

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Introduction

Indian curd, known as *dahi*, is a well-known fermented milk product consumed by large sections of the population throughout the Indian subcontinent, either as a part of the daily diet or as a refreshing beverage. This product finds its name in the ancient Hindu scriptures and is used in several of Ayurvedic formulations (traditional Indian medicinal system) because of its potential therapeutic properties. The beneficial aspects of *dahi* and its products have been often investigated. *Dahi* prepared from buffalo milk is white in color and possesses a firm body and slightly granular texture, whereas *dahi* prepared from cow milk is less firm and smooth in texture. *Dahi* may be consumed directly, either sweetened or salted and spiced. It is also consumed with other foods such as rice and wheat loaf. *Dahi* has assumed a special place in the daily diet of Indian populations, who prefer to take *dahi* once or twice a day in morning or evening meals. In 2010–11, the production of *dahi* was estimated at almost 8% of the total dairy production in India. Since conversion of milk into *dahi* is an important intermediate step in the manufacture of indigenous fat-rich dairy products like butter and ghee, it can be said that over 40% of the total milk production in India is converted into *dahi*. *Dahi* is also used as a vehicle to incorporate probiotics, and several varieties of *dahi* are developed for the benefit of consumers, like fruit *dahi*, sweetened *dahi*, whole-milk *dahi*, and skim-milk *dahi*. Though nowadays, *dahi* is mostly sold in polythene pouches and in set form in HDPE containers, traditional *dahi* was being sold in earthen pots. *Dahi* sold in pouches does not have the three-dimensional network structures that are typical of set *dahi*, whereas the products sold in the rigid plastic containers possess a firm body and smooth texture that appeals to consumers. Its typical flavor is the foremost quality attribute of *dahi* that determines its acceptance. The pleasant flavor of *dahi* is attributed to its diacetyl content, which is produced by citrate-fermenting lactic acid bacteria (LAB).

Definition

According to the Bureau of Indian Standards (BIS), *dahi* is a product obtained by lactic fermentation of cow or buffalo milk or mixed milk through the action of single or mixed strains of LAB. This definition does not include milk coagulated by the addition of acids and milk-coagulating enzymes. As per the Prevention of Food Adulteration Act of India (PFA, 1988), *dahi* or curd is a product obtained from pasteurized or boiled milk by souring naturally or otherwise by adding harmless lactic acid and/or other aroma-producing lactic acid bacterial cultures. *Dahi* may contain additional cane sugar. It should have the same percentage of fat and nonfat solids as the milk from which it is prepared. Where *dahi* or curd, other than skimmed-milk *dahi*, is sold or offered for sale without any indication of the class of milk, the standards prescribed for *dahi* prepared from buffalo milk should apply. This is an equivalent of yogurt made from boiled milk after inoculation through back-slopping, which consists of the leftover *dahi* from the previous lot. However, it differs from yogurt in having less acidity.

Starter Cultures Used for the Preparation of *Dahi*

In the production process of *dahi*, inoculation with small quantities of the desired fermenting microbiota, that is, through back-slopping or via the starter culture, takes place. The most important microorganisms in starter cultures are the homofermentative and heterofermentative LAB. The *dahi* starter cultures can be classified into two groups, according to the characteristics of the microorganisms: mesophilic and thermophilic cultures.

Mesophilic starter cultures are used at temperatures varying between 16 and 30 °C (Table 1). The characteristics of *dahi* starter cultures and their metabolic products are given in Table 2. The most typical representatives are the homofermentative *Lac. lactis* subsp. *lactis* and *Lac. lactis* subsp. *cremoris*. These are similar, though the former is able to produce free amino acids, which are believed to stimulate the growth of *Lac. lactis* subsp. *cremoris* strains. Another important species in the mesophilic starter category is the homofermentative *Lac. lactis* subsp. *lactis* biovar *diacetylactis*, which is able – besides lactic acid production – of fermenting citric acid into a number of other compounds such as diacetyl and carbon dioxide. However, at a pH below 5, carbon dioxide and a number of compounds similar to diacetyl, but nonaromatic, are produced. Heterofermentative aroma-producing starter cultures, like *Leu. mesenteroides* subsp. *dextranicum* and *Leu. mesenteroides* subsp. *plantarum*, are also able to ferment citric acid, that is,

Table 1 Designation of *dahi* based on types of starter cultures

Sr. no.	Designation	Cultures used	Remarks
1.	Sweet *dahi*	*Lactococcus lactis* subsp. *lactis*, single or in combination with *Lac. lactis* subsp. *cremoris*,	With or without aroma producers like *Leuconostoc mesenteroides* subsp. *dextranicum*, *Leu. mesenteroides* subsp. *plantarum*, *Lac. lactis* subsp. *lactis* biovar *diacetylactis*
2.	Sour *dahi*	*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*	Single or in combination with or without aroma producers

Table 2 Characteristics of starter cultures used in *dahi* making and their principal metabolic products

Starter organisms	Metabolic products	Lactose fermentation
Lactobacillus delbrueckii subsp. *bulgaricus*	Lactate, diacetyl, acetaldehyde	Homofermentative
Lactococcus lactis subsp. *lactis*	Lactate	Homofermentative
Str. salivarius subsp. *thermophilus*	Lactate, diacetyl, acetaldehyde	Homofermentative
Lactococcus lactis subsp. *cremoris*	Lactate	Homofermentative
Leuconostoc mesenteroides subsp. *dextranicum*	Lactate, diacetyl	Heterofermentative
Lac. lactis subsp. *lactis* biovar *diacetylactis*	Lactate, diacetyl, CO_2	Heterofermentative

into carbon dioxide, diacetyl, and some other products. Unfortunately, leuconostocs may reduce diacetyl into nonaromatic compounds again. The intensity of reduction varies between the various representatives of the strains.

Homofermentative thermophilic starter microorganisms, like *L. delbrueckii* subsp. *bulgaricus* and *Str. salivarius* subsp. *thermophilus*, are used to obtain mildly sour *dahi*. These starters play an important role in producing a good quality *dahi* having a firm and uniform consistency with a sweet aroma and acidic taste.

Classification of *Dahi*

Broadly speaking, *dahi* may be classified into two types:

- *Dahi* for churning into *desi* (indigenous) butter
- *Dahi* for direct consumption

The *dahi* for direct consumption is further classified into the following subtypes:

- (i) Whole milk *dahi* and (ii) skim-milk *dahi*
- (i) Sweet (or mildly sour) *dahi*, (ii) sour *dahi*, and (iii) sweetened *dahi*

Technology of Sweet and Sour *Dahi* Manufacture

The flow diagrams showing the steps involved in manufacture of sweet (or mildly sour) and sour *dahi* are given in Figure 1.

Preliminary Treatment of Milk

The preparation of the basic mix involves selection of milk, preheating, filtration/clarification, and standardization of the milk. The different methods for standardization indicate the possibilities that exist for either increasing or decreasing the various milk constituents, especially the fat percent. However, the choice of any particular system is primarily governed by the following factors:

(i) Cost and availability of the raw materials
(ii) Scale of production

The composition of *dahi* varies considerably. The average composition of *dahi* made from buffalo milk is delineated in Table 3. Commercial *dahi* tends to contain around 14% of milk solids.

The use of ultrafiltration to concentrate the solids in cow milk and skim milk is being considered as a feasible alternative. The importance of total solids stems from the improved consistency imparted to the *dahi* coagulum, an improvement that is carried further in the homogenization stage by employing a pressure of 2500 psi. Homogenization of milk imparts a smooth texture to the coagulum. It also reduces the chances of whey separation. Homogenization is followed by a final heat treatment of 80–90 °C for a prolonged period of 20 min. The effect of this drastic heat treatment can be summarized as follows:

- The bacterial load in the milk is reduced, and hence, the starter culture has less competition from the contaminating microorganisms.
- There is a denaturation of whey proteins (albumins and globulins) and an aggregation of the casein micelles into a three-dimensional network. The network traps the whey proteins, and the *dahi* coagulum produced subsequently is rendered more viscous.
- There is a reduction in the amount of oxygen in the milk, and as normal *dahi* cultures are microaerophilic, the lowered oxygen tension encourages their growth.
- Some limited damage to the milk proteins may occur during heating, and the breakdown products can stimulate starter activity.

Fermentation of Milk

The acidification of milk during the manufacture of *dahi* is an important biochemical process, which must be carried out under controlled conditions in special incubators and/or

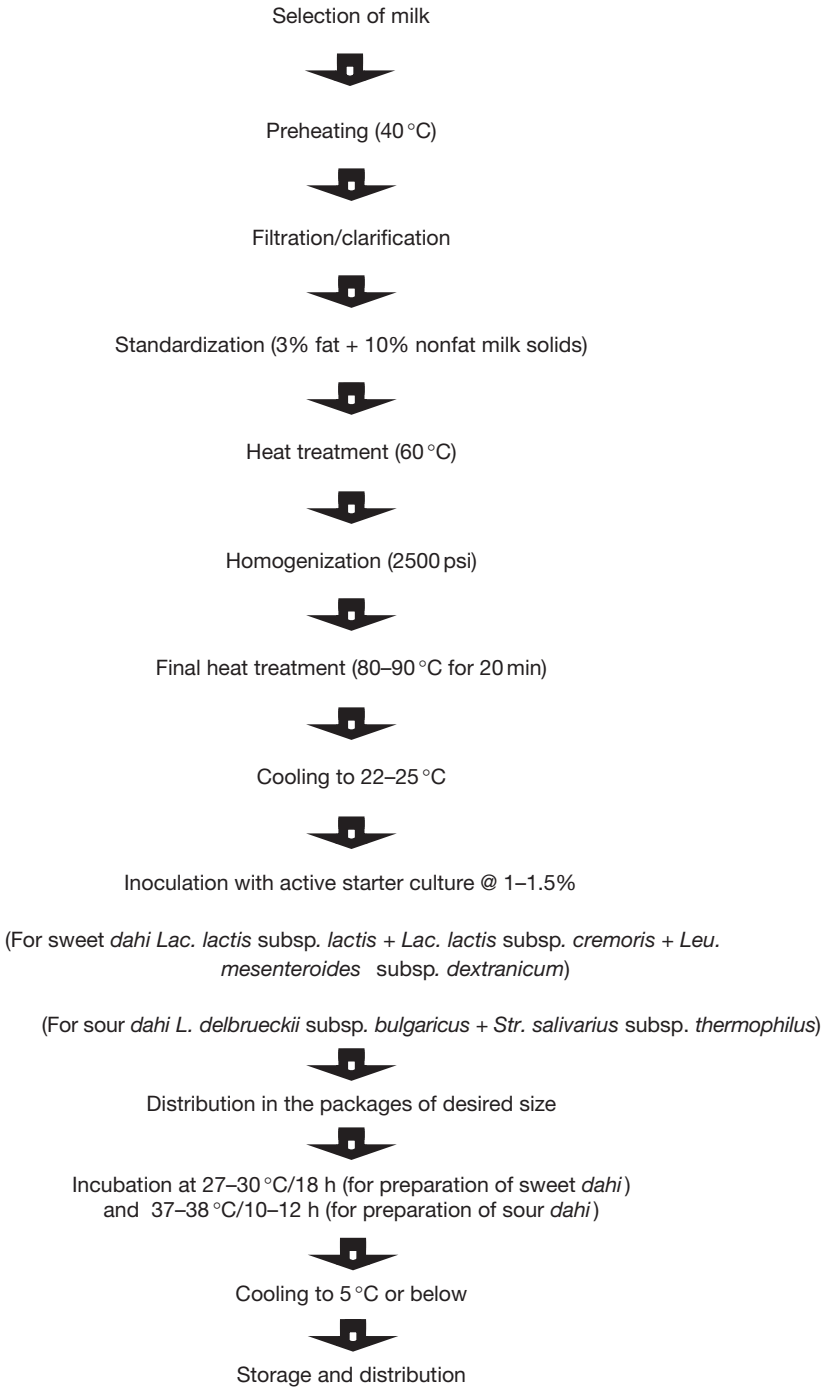

Figure 1 Flow diagram for the preparation of *dahi*.

fermentation tanks. Fermentation tanks are used as incubators only, and they are usually insulated in order to maintain appropriate temperature. The processing of the milk and the cooling of *dahi* is carried out in other equipment on the production line. Cabinets are also used for this purpose. These comprise a small insulated room, which is divided into compartments, and most incubators of this type are multipurpose chambers capable of circulating hot or cold air. Hot air is circulated during the fermentation period followed by cold air during the cooling stage. Sometimes, these cabinets are used as incubators only, and the *dahi* can be cooled in refrigerated cold storage. In the case of mildly sour *dahi* manufacture, the temperature is maintained at 27–30 °C, while for sour *dahi*, it is 37–38 °C.

Table 3 Average composition of buffalo milk *dahi*

Constituents	*%*
Moisture	85–88
Fat	5–8
Protein	3.2–3.4
Lactose	4.6–5.2
Ash	0.70–0.75
Lactic acid	0.5–1.0
Calcium	0.12–0.14
Phosphorus	0.09–0.11

Cooling of *Dahi*

After the incubation period is over, *dahi* is cooled in order to control the level of lactic acid in the product. The rate of cooling can affect the structure of the coagulum. Thus, very rapid cooling can lead to whey separation, and this is due to a too rapid concentration of protein filaments, which, in turn, affects their hydrophilic properties.

Microbiological Quality of *Dahi*

In general terms, *dahi* can be regarded as 'hygienically safe.' The reason for this confidence stems from the level of acidity present around 0.7–1.0% (lactic acid). Under this situation, potential pathogens such as *Salmonella* spp. will be largely inactive. Similarly, coliforms will be unable to survive the low pH encountered. Inhibition of some pathogens may be reinforced by the production of antimicrobial substances by the *dahi* starter organisms. Several studies have confirmed that certain LAB isolated from *dahi* can produce bacteriocins effective against several of the human Gram-positive pathogens. As far as the microbiological standards for *dahi* are concerned, the maximum acceptable number of yeasts and mold and coliforms of 100 and 10 cfu g^{-1}, respectively, is accepted by the BIS for both the sweet and sour types of *dahi*. In both types, it is mandatory that a phosphatase test should be negative.

Technology of Sweetened *Dahi* (Misti *Dahi*) Manufacture

Misti *dahi* (syn. sweetened *dahi*, red *dahi*, *payodhi*) is a traditional sweetened fermented milk product of the Eastern region of India. It is prepared on cottage scale by *halwais* (sweetmeat makers) every day to cater to local demands. Traditionally, milk added with cane sugar is continuously heated in an open pan at simmering temperature (68–70 °C) for 6–7 h to concentrate and develop an intense cooked flavor and brown color, besides increasing the viscosity and inducing other physicochemical changes. After cooling to about 40 °C, the mix is inoculated with a commercial misti *dahi* starter culture, which generally comprises a mixed lactic culture like *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, and some aroma-producing organisms like *Lac. lactis* subsp. *cremoris*, as mentioned in the case of mildly sour and sour *dahi*. Wide variations in total solids content (27–43%), nonfat milk solids (11–16%), and sucrose (13–19%) in the market samples of misti *dahi* sold in Kolkata city have been reported. Many flavor defects such as overly fruity, alcoholic, highly acidic, and flat and textural defects like gassiness, weak body, wheying off, and thick crust on top surface were observed in most of the market samples. In view of the increasing nationwide demand for this product and the growing interest of the organized dairy plants for large-scale manufacture, the technology was developed to suit industrial-scale production. This technology is accepted and invariably adopted for dahi manufacture on large scale. Manufacturing technology for industrial production of misti *dahi* is given in Figure 2.

To prepare Misti *dahi*, fresh buffalo milk is standardized to 3.5% fat and 9% nonfat milk solids, and it is then heated to 65 °C in a plate heat exchanger and homogenized at a pressure of 56 kg cm^{-2} (one stage). Milk is concentrated to 1.44-fold in a vacuum evaporator. After adding cane sugar, the milk is heated at 85 °C for 10 min to generate cooked flavor. The mix is cooled to 40 °C before inoculation with a commercially available lactic culture (e.g., LF-40). In some cases, sugar, caramel, jaggery, and artificial colors are added to impart brown color. The inoculated mix is aseptically distributed into presterilized polystyrene containers of desired size and mechanically transferred to an incubation chamber at 40 °C. During the curd formation, milk must remain stationary. In all postfermentation activities, the gel should be subjected to a minimum amount of external influences. Diacetyl is the predominant flavor component, but microquantities of acetoin, acetic acid, and carbonyls are also present. Off-flavors in this product can be traced back to poor-quality raw milk, the use of contaminated cultures, high incubation temperature, and contaminated packages.

Rheological Properties

Consistency, firmness and viscosity of *dahi* are important textural parameters that govern the quality of the product. These attributes can be measured objectively by a texture analyzer or cone penetrometer. The texture analyzer works on the principle that a cylindrical steel probe penetrates into the product samples and experiences resistance during the penetration. The resistance offered by the *dahi* sample (5 °C) during the penetration of the probe up to a specified distance is recorded as firmness (newtons) of *dahi*. A cone penetrometer is a simple and useful instrument for determining the firmness or hardness of food materials. In this instrument, a conical metal probe is allowed to freely fall into the sample for a specified time, for example, 5 s. Then, its fall is arrested by an electromagnetic mechanism, and the depth of penetration is measured on a dial scale and expressed as (×0.1) mm. The higher the depth, the softer the *dahi* sample must be. Since *dahi* is generally a soft material, a metal probe would be too heavy for the purpose, so a rubber conical probe can be used.

Factors Affecting Rheological Properties of *Dahi*

Rheological parameters of *dahi* are significantly influenced by the heat treatment of the milk used for *dahi* making. It has been reported from the texture analyzer parameters that by the heat treatment of milk, the firmness of *dahi* increases from 0.039 N in no heat treatment (control) to 0.110 N in *dahi* prepared

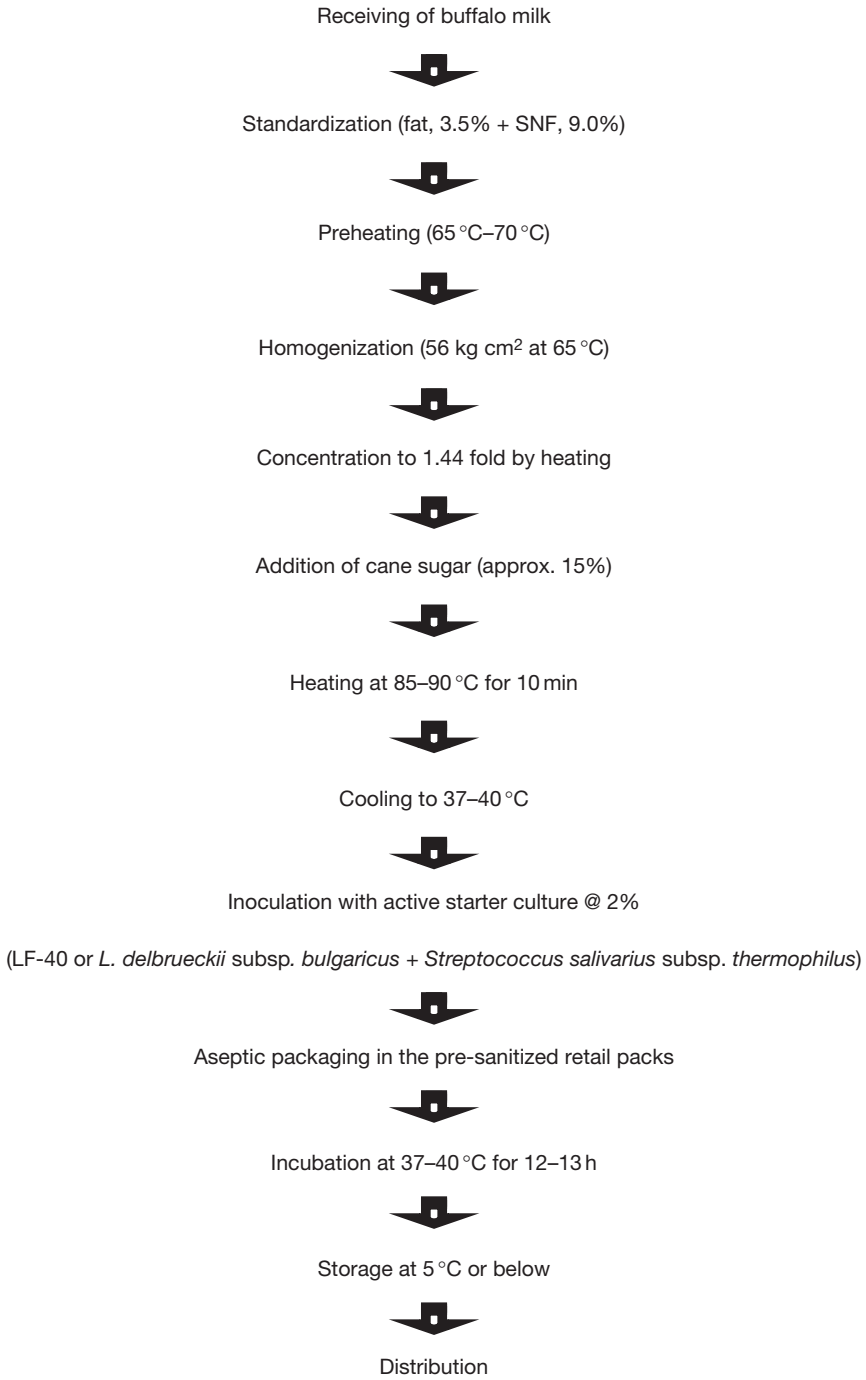

Figure 2 Flow diagram for preparation of Misti *dahi*.

from boiled milk. The low-temperature, long-time (LTLT) treated milk *dahi* has a 0.070 N firmness. Similar observations were recorded with consistency value, but the LTLT treated (0.33 N s) and boiled-milk (0.31 N s) *dahi* display almost the same consistency, yet much higher than that of the control (0.071 N s). Index of viscosity values are lower for control *dahi* (0.0012 N s), whereas those for LTLT treated milk *dahi* and boiled-milk *dahi* are 0.007 and 0.012 N s, respectively. This trend is also reflected in parameters measured by cone penetrometer.

The effect of a heat treatment of milk on the rheological parameters can be attributed mainly to the denaturation of proteins. In milk, casein exists in a colloidal state, which is sustained by calcium bridges between the casein micelles. The whey proteins exist in a soluble state and have no interaction with casein micelles. But by heat treatment of milk, the whey proteins get denatured and involved in interactions with the casein micelles without affecting the calcium bridges. As intensity of heat treatment given to milk increases, casein–whey protein interactions take place. However, during the

fermentation process, the calcium bridges between the protein particles dissolve because the calcium goes from the colloidal state to solution state, as the acidity rises and pH decreases. It also results in the slow precipitation of the casein–whey protein particles. The precipitated casein–whey protein particles arrange themselves in specific manner forming three-dimensional networks, which impart firmness to *dahi*. This network can easily be broken by subtle forces of agitation, but once broken, the network cannot be built back. However, part of the original viscosity may be recovered during storage, which is known as thixotropic character.

The sticky character acquired by the curd during fermentation is responsible for the thixotropic character and can be attributed to mucilaginous substances produced by starter bacteria. This is the reason why it is widely believed in the subcontinent that the milk should not be disturbed while setting of milk is in progress otherwise firm curd would not form. Disturbing the milk during setting means disturbing the network being formed during acid production. A firm, well-set curd is most preferred than stirred curd in India. However, most of the curd sold is stirred curd because of convenient processing steps, but manufacturers are realizing the people's preference for set curd, so set curd with a thick, firm consistency is now gaining market. In this regard, the extent of heat treatment of milk and use of starter cultures, which produce not only lactic acid and diacetyl but also texturizing compounds (heteropolysaccharides), are important. Some of the workers in this field have advocated application of inulin and texturizing substances for improving the consistency of *dahi*, whereas others used xylooligosaccharides for the purpose. The observations of these workers show that apart from heat treatment, external polysaccharides could also be employed to enhance the consistency of products like *dahi*.

Effect of Heat Treatment on the Syneresis and Acid Development in *Dahi*

Syneresis is the separation of whey from the curd. The extent of syneresis is indicative of the quality of *dahi*. Consumers prefer *dahi* with no visible whey separation. The presence of separated whey indicates excessive fermentation and stored *dahi*. During fermentation, changes take place with regard to the milk proteins, affecting water retention. Consumers tend to think that the curd showing whey separation is not fresh and hence not good. *Dahi* prepared from raw milk shows visible whey separation measured as 7.7 ml after centrifugation, whereas *dahi* prepared from milk pasteurized by LTLT shows a syneresis value of 7.2 ml, which can also be visualized. However, boiled-milk *dahi* exhibits no visible whey separation and shows firm consistency.

When well-set curd is disturbed, the whey flows through the channels in the network and finally separates out. The partially denatured proteins hold more moisture or whey, so syneresis is less in the curds prepared with high heat-treated milk (boiling). In the case of raw milk, the whey proteins are in native state so they possess low water-binding ability; hence, more syneresis is expected to take place in that curd. In LTLT treated milk, the whey protein denaturation has just begun so water binding is slightly larger than in raw milk, leading to less syneresis. In boiled milk, the proteins hold more moisture, giving low syneresis values. Thus, the firmer the curd, the lower the syneresis.

Effect of Heat Treatment on Sensory Quality and Acceptance of *Dahi*

The acceptability of *dahi* samples is generally measured on a 9-point hedonic scale. It is observed that the boiled-milk *dahi* scores highest in all the sensory attributes. Milk that does not receive any heat treatment results in *dahi* with low sensory scores that fall in the range of around 5–6, that is, 'liked slightly.' LTLT heat-treated *dahi* scores better than raw-milk *dahi* indicating that flavor, body, and texture improve by heat treatment. By heat treatment, undesirable microorganisms are killed and LAB grow with less competitive inhibition. When milk is boiled, the same happens, but because of protein–protein interaction, the body and texture improve further, which will synergistically enhance the flavor attribute. For these reasons, the overall acceptance scores are highest for the boiled-milk *dahi*.

The intensity of heat treatment given to milk influences the extent of denaturation of whey proteins. It is stated that during *dahi* manufacturing, heating of milk (90–95 °C/5 min) denatures whey proteins, which consist of α-lactalbumin and β-lactoglobulin. The denatured whey proteins interact with casein micelles by coating on their surface. The denatured whey proteins have a good water-binding ability, so they enhance the thickness of the curd. They also help in sustaining the three-dimensional structure of *dahi*. It is known that whey proteins denaturation begins at 65 °C and would almost completely be denatured (93–95%) by heat treatment of milk at 80 °C for 45 min. The water-binding ability of proteins is maximal only when they are partially denatured, that is, when the protein structure is not completely damaged. This is because of exposure of hydrophilic groups, which were otherwise remaining embedded inside the tertiary or quaternary structure. But when milk is intensely heat-treated, as in heat sterilization, that is, more than 10 psi, then whey proteins are completely denatured, their secondary structure is fully straightened out, and the hydrophilic groups are lost; as a result, the proteins lose water-binding ability. So, sterilization of milk is detrimental to the curd firmness. It is now clear that boiling the milk without holding will not lead to complete damage to the secondary structure of whey proteins, but results in only partial uncoiling leading to more number of hydrophilic groups being exposed. Thus, water-binding ability is increased.

Role of *Dahi* in Complementary Feeding

Conversion of milk into *dahi* helps to extend the shelf life of milk, thereby allowing storage and transport. In fact, the generation of lactic and short-chain fatty acids from lactose and the consequent fall in pH inhibit the growth of many pathogenic microorganisms. This technology has been widely practiced throughout India for a long time.

Dahi is nutritionally similar to unfermented milk, except that some of the lactose is broken down to glucose and galactose, which are further fermented, some vitamins are

Table 4 Mineral and vitamin content of *dahi*[a]

Sr. No.	*Constituents*	*Milk*	Dahi
1.	Mineral matter (g)	0.8	0.8
2.	Calcium (mg)	118	149
3.	Phosphorous (mg)	96	102
4.	Vitamin A (I.U.)	4.6–5.2	96.0
5.	Thiamine (μg)	55	49
6.	Riboflavin (μg)	167	157
7.	Nicotinic acid (μg)	96	86
8.	Biotin (μg)	29.0	3.2
9.	Pantothenic acid (μg)	202	183
10.	Folic acid (μg)	161	178
11.	Vitamin B_{12} (μg)	0.15	–
12.	Ascorbic acid (mg)	1.4	1.3

[a]Values given per 100 g of the product.

synthesized, and the bioavailability of Ca and P is enhanced due to increased acidity and release of some amino acids. This makes *dahi* like other fermented milks an important food in cases of lactose tolerance. In very young children, lactose intolerance is often due to an impaired activity of intestinal lactase following intestinal pathologies. Children with lactose intolerance could receive dahi and other fermented milks without showing the classical clinical signs of impaired absorption, such as intestinal bloating and gas production. In addition to that, lactase activity of live microorganisms present in *dahi* also remains after ingestion in the gastrointestinal tract, improving lactose digestion. The use of special milk formulation for lactose-intolerant subjects could not be affordable in poor regions in which the use of *dahi* can be a valid alternative. Although *dahi* is primarily regarded as a useful source of protein and minerals, vitamins from the original milk or secreted by the starter culture bacteria are usually present in detectable quantities (**Table 4**). The nutritional value of milk protein is self-evident, but it is important that the level in *dahi* is due to the concentration of milk solids, higher than in liquid milk, as is the concentration of minerals such as calcium and phosphorous. Furthermore, the low pH of *dahi* tends to make the latter more accessible for absorption through the intestinal wall.

Dahi is indeed able to enhance the absorption of some minerals, such as calcium and nonheme iron, as a result of its lower pH. The increased calcium absorption is related to both a direct effect of the acidification of intestinal lumen and the reduction of the mineral complex formation with other dietary components. If *dahi* is consumed at mealtimes, the lactic acid is likely to have a positive effect on the absorption of iron from other foods. The bioavailability of iron is more important than the total amount of iron in the diet. In contrast to breast milk, iron from unmodified cow's milk is poorly absorbed, as a result of the high content of protein and the low content of vitamin C compared with commercial infant formula. Furthermore, the early introduction of unmodified cow's milk can cause blood loss from the intestinal tract, thereby having a negative influence on iron status, especially during the first 6 months of life and also during the second half of infancy.

A number of health benefits have traditionally been attributed to *dahi* and products made from it, which have been used to prevent a wide range of diseases including atherosclerosis, allergies, and gastrointestinal disorders. Although empirical findings are yet to be supported by controlled studies, initial results from investigations into the antibacterial, immunologic, antitumor, and hypocholesterolemic effects of *dahi* consumption suggest potential benefits.

See also: Acidophilus Milk; Fermented Foods: Composition and Health Effects; Fermented Foods: Origins and Applications; Fermented Foods: Use of Starter Cultures; Lactic Acid Bacteria; Malnutrition: Prevention and Management; Probiotics; Protein: Digestion, Absorption and Metabolism; Single Cell Proteins; Yogurt: Dietary Importance; Yogurt: The Product and its Manufacture.

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Dairy Products: Dietary and Medical Importance

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Introduction

Milk is a widely disseminated beverage worldwide and is essential to the diet of several millions worldwide because it provides important macro- and micronutrients. Dairy products are also important food staples that provide protein and micronutrients in a rather concentrated form.

Milk and dairy products are perceived as nutritionally relevant foods, mostly because their calcium, protein, phosphorus, etc., content promotes skeletal, muscular, and neurological development. However, their relatively high saturated fat proportion (milk fat contains about 70% of saturated fatty acid (SFA); myristic and palmitic acids combined account for ~50%, while the rest is mostly short- and medium-chain fatty acids, including oleic acid) often raises issues in terms of adverse effects on cardiometabolism. However, the vast majority of recent epidemiological and intervention studies indicate that dairy products do not adversely affect surrogate markers of cardiovascular disease and cardiovascular prognosis. Conversely, short-chain SFA from milk and its derivatives are – apparently – benign or even beneficial with regard to inflammation. Extensive calcium intake is often frowned upon because of potentially noxious actions on coronary calcification. However, accumulated epidemiological evidence suggests that calcium does not play major roles in coronary calcification and its intake is inversely associated with blood pressure. Therefore, the hypothesized association between calcium intake and cardiovascular risk is not supported by current data and, actually, the converse might be true. In addition, some milk components such as *trans*-fatty acids, tripeptides, calcium, phosphorus, lactoferrin, and oligosaccharides might exert useful – though as yet unproven – physiological actions. Finally, milk is an efficient vehicle for fat- and lipid-soluble nutrient absorption because milk fat is highly dispersed in very small micelles; this makes milk an excellent matrix for functional foods.

In this article, the latest published evidence on milk, dairy products, and human health is summarized.

Milk, Dairy Products, and Cardiometabolism

Cardiovascular disease is the leading cause of death in the Western world and its incidence can be modulated by diet. As mentioned, milk is often perceived as a double-edged sword chiefly because of its SFA content. In addition, milk and dairy products contain cholesterol (approximately 80 mg/100 g). Therefore, several people worry that the intake of either one might theoretically have detrimental effects on their cholesterol concentrations. The net result is that cardiovascular patients or individual at risk (real or perceived) often stops using milk and its derivatives precisely to improve their prognosis. In this respect, it is noteworthy that the real contribution of dietary cholesterol to cardiovascular risk is being debated; the current consensus is that it depends on individual, inherited ability to synthesize versus absorb cholesterol. Also, the extent and the exact nature of the role of SFA in cardiovascular disease onset and development are being reexamined; moreover, genetic background is a predictor of the response to SFA intake.

The first review on the association between dairy products and circulating cholesterol concentrations has been published by St-Onge et al. In that paper, the authors mention an old study carried out in the African Maasai ethnic group. Maasais consume large amounts of milk, which, in that study, was inversely correlated with cholesterolemia. The same authors proposed milk as an inhibitor of cholesterol synthesis. Other publications expanded on the cholesterol-lowering effects of both milk and skimmed milk (which were previously suggested by Hepner et al.). In their review, St-Onge et al. hypothesized that the cholesterol-lowering effects of milk are due to the intestinal microbial fermentation of indigestible carbohydrates (see succeeding text), which would positively alter the microbiota, inhibit cholesterol synthesis, and interfere with cholesterol enterohepatic circulation, in turn lowering cholesterolemia.

A recent study performed by Høstmark et al. in 18 770 subjects examined the association between cheese consumption and circulating concentrations of high-density lipoprotein (HDL) cholesterol (which was positive) and triacylglycerols (which was negative), in different age groups. The authors attributed this effect to the fatty acid composition of cheese and its bacterial content, although this hypothesis needs confirmation.

One of the questions arising from these studies is whether various dairy products have different effects on cardiovascular risk markers. In this respect, one study equicalorically (20% of total calories, normalized for lactose and casein) compared the effects of milk, cheese, and butter, for 3 weeks. Cheese slightly increased low-density lipoprotein (LDL) cholesterol, whereas the effects of whole milk and butter were similar. These data have been replicated by Biong et al., who reported that consumption of cheese induced a lower increase of cholesterol concentrations than that of an identical amount of fat from butter. Along these lines, Nestel et al. administered 40 g day^{-1} from either cheese or butter to mildly hypercholesterolemic subjects. Total and LDL cholesterol increased significantly – after 4 weeks – in the butter group as compared with the cheese one. Some authors suggest the inclusion of modest amounts of cheese in the diets of mildly hypercholesterolemic subjects, even though current guidelines recommend otherwise. Indeed, in a study by Hjerpsted et al., 13% of total daily calories were replaced with either 143 g of cheese or 47 g of butter (with the same lipidic content), for 6 weeks, in a randomized crossover trial on healthy subjects. The result indicates that cheese does not increase LDL cholesterol compared with the run-in period; rather – as compared with butter – cheese induces a significantly lower increase in total (5.7%) cholesterol and LDL (6.9%) cholesterol.

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Mechanistically, several hypotheses have been to explain the differential effects of cheese and butter on cholesterolemia. One such hypothesis is that calcium – whose concentrations are higher in cheese than in butter – binds to fatty acids in the intestine and forms insoluble soaps, thereby decreasing fat absorption. This hypothesis is corroborated by the higher-fat fecal excretion recorded in the cheese group with respect to the butter one. Also, the higher protein and probiotic content of cheese might theoretically contribute to its almost neutral effect on plasma cholesterol. However, it should be noted that the study by Tholstrup et al. reported a lack of difference in cholesterolemic effect of the diets containing whole milk and butter. In summary, whole milk likely raises LDL-cholesterol concentrations similar to butter, and its use by hypercholesterolemic patients or by individuals with elevated cardiovascular risk should be considered with caution. *Trans*-fatty acids are also a matter of concern in cardiometabolism, and laws are being approved to eliminate them from the diet. However, dairy *trans*-fatty acids might have different physiological actions than the industrial ones. As an example, conjugated linoleic acid is being promoted as healthful even though solid scientific evidence of its putative health effects is lacking. Yet, there are efforts to increase its concentrations in bovine milk.

Two of the most researched issues in cardiometabolism are oxidative stress and inflammation, because they play major roles in the onset and development of the metabolic syndrome (MetS).

Two large studies, that is, the Nurses' Health Study and the Multi-Ethnic Study of Atherosclerosis, reported an inverse association between a prudent diet that included low-fat dairy products and biomarkers of inflammation. In addition, Australian study reported an inverse association between MetS and type II diabetes incidence and dairy consumption in 1807 and 1824 patients, respectively. In particular, those who had the highest consumption level of dairy products witnessed a MetS risk reduction of 59%. A similar association was recorded when the incidence of diabetes was computed. These publications triggered a series of investigations aimed at clarifying mechanisms of actions and whether this association was casual or causal. Zemel et al. supplemented obese patients with skimmed milk for 28 days and recorded a significantly lower oxidative stress and inflammation. In addition, a 12-week diet administered to MetS patients that included 3.5 servings/day of milk and/or yogurt reduced oxidative stress markers after 7 days, as compared with an isocaloric diet that provided 0.5 servings/day of dairy products. After 12 weeks, decreased systemic inflammation was also recorded in the dairy group though not in the control arm.

It is noteworthy that the observed decrease in oxidative stress and inflammation markers was independent of body weight changes and became apparent shortly after the administration of dairy products. This suggests causation and an active role of milk and dairy products in reducing two hallmarks of the MetS.

A recent meta-analysis of eight randomized and controlled studies in overweight or obese subjects concluded that dairy product consumption does not negatively impact on biomarkers of inflammation. Yet, further ad hoc studies on the effects of milk and dairy products on inflammation are warranted before solid conclusions can be drawn.

Cancer

Cancer is the second cause of death in industrialized countries (and in some of them, it is actually number one). Milk and dairy products contain compounds that have suggested, in *in vitro* studies, as chemopreventive. Some epidemiological studies have been carried to ascertain whether milk and dairy product use is associated with cancer incidence. For example, Kampman et al. published an observational study in which 331 men and 350 women have been followed for 4 and 8 years, respectively, and no association between milk and fermented milk consumption and colorectal cancer was found. Other such studies yielded mixed outcomes: some of them found positive associations between dairy use and prostate and ovary cancers, whereas others found the converse when the incidence of colorectal, lung, or breast cancers was evaluated. A very large prospective study by Park et al. analyzed the diets and cancer incidence of 293 907 men and 198 903 women, with a follow-up of 7 years. In this study, calcium and dairy product use was inversely associated with gastrointestinal (GI) (especially colorectal) cancer incidence. In particular, the reduction of GI cancer risk was of 16% for men and of 23% for women. Other cancers were also inversely associated with milk and cheese consumption, namely, those of the head and neck, esophagus, stomach, colon, and bladder. As mentioned, milk contains putatively chemopreventive compounds such as calcium, vitamin D, and conjugated linoleic acid that might explain these observations. *In vitro*, calcium inhibits cell proliferation and stimulates differentiation and apoptosis in the GI tract and in the mammalian gland. As mentioned earlier, calcium also binds to fatty acids and biliary salts in the intestine, therefore lessening their potentially noxious effects on carcinogens the mucosa. Accordingly, two European studies reported an inverse association between milk use and colorectal cancer. In the first one, 45 241 subjects have been followed for 12 years, and their use of yogurt was monitored and plotted against colorectal cancer incidence, showing an inverse association that was stronger in men. A systematic review by Aune et al. took into account 19 cohort studies and reported a significantly inverse association between milk, total dairy products yet not cheese consumption, and colorectal cancer incidence. On the other hand, calcium also interacts with vitamin D and with insulin-like growth factor-1, which would increase prostate cancer risk, as shown by Park et al.

Concerning breast cancer incidence, one report by Knekt et al. found a significantly inverse association between milk use and breast cancer incidence in 4697 Finnish women. Shin et al. also published a cohort study, in which they did not find any association between dairy, calcium, or vitamin D consumption and breast cancer incidence in postmenopausal women. Conversely, in premenopausal women, a significant reduction of breast cancer risk as associated with low-fat dairy products was reported, suggesting that hormonal status might modulate the effects of milk and its derivatives on breast cancer, via unknown mechanisms. A large meta-analysis of more than

20 studies did not find any association between breast cancer incidence and dairy product use. These results have been subsequently confirmed. Conversely, Dong et al. performed a meta-analysis on 1 063 471 participants in 18 prospective cohort studies and reported an inverse association between dairy – though not milk – use and breast cancer, notably in premenopausal women and when low-fat products were separately computed. In summary, based on accumulated data, there is currently no indication that milk and dairy use favors or contrasts the incidence of breast cancer.

The association between milk use and bladder cancer incidence has also been analyzed. One meta-analysis by Mao et al. reported that high milk consumption was associated with a 16% reduction of bladder cancer risk; this inverse association was stronger in Asian subjects than in North American and was not found in Europeans. Also, the association depended upon the kind of dairy product that was analyzed. Another meta-analysis did not find any correlation among variables, after computing 14 studies on milk (4879 cases of bladder cancer) and six studies on dairy products (3087 cases), for a total of 324 241 subjects.

When other cancers, including lung, ovary, esophagus and stomach, and oropharyngeal cancers, are taken into account, no clear association between milk consumption and incidence emerges. Conversely, positive associations between dairy use and prostate cancers have been published and several mechanisms of action have been proposed to explain this hypothetical effect.

Milk as a Vehicle for Bioactive Molecules

Fat is found in milk in extremely small micelles: the average diameter of the fat globules in homogenized bovine milk is 1–3 μm; estimations indicate that 1 g of fat in milk is dispersed in 10^{10-12} micelles, that is, a very wide surface of about one square meter. Therefore, it is conceivable that lipid-soluble compounds, such as added fatty acids, liposoluble bioactive molecules, and vitamin E, are highly bioavailable when ingested with milk (Figure 1). In addition to exogenous compounds (which render some milks functional foods), milk naturally contains biologically active molecules. One example is that of oligosaccharides, which are not metabolized along human GI tract, so they reach the colon intact where they serve as specific nutrients for probiotic strains. Even though bovine milk contains only trace amounts of these potentially healthful molecules, some researchers are working toward introducing human milk oligosaccharides in that of transgenic animals. One current issue is the lack of suitable commercial standards for bovine oligosaccharides; consequently, we can only identify approximately 70 fully annotated oligosaccharides in human milk and approximately 40 in bovine milk. It is noteworthy that human milk is rich in oligosaccharides, which have been proposed as important contributors to child development, largely because they are prebiotics and help in creating a healthy microbiota. Indeed, neutral oligosaccharides – namely, the monomer *N*-acetylglucosamine and fucose – are essential to the development of the correct microbiota of breast-fed neonates, because of their immunomodulating actions. Also, acidic oligosaccharides (where the monomer is sialic acid) help in preventing the adhesion of pathogens to the intestinal mucosa. Interestingly, bovine milk also contains these oligosaccharides, which are abundant in colostrum. Fruits and vegetables also contain oligosaccharides and some of them have been synthesized; yet, those from milk are peculiar in that they exhibit a branched rather than linear structure. Moreover, they contain fucose and sialic acid, which are almost absent in other oligosaccharides. This structural difference might confirm milk oligosaccharide activities that are different than those of synthetic or vegetal origin. It must be underscored that the concentration of oligosaccharides in bovine milk decreases in a time-dependent fashion: ad hoc investigations are being carried out to stabilize oligosaccharides in milk or to formulate them as nutraceuticals or as probiotic components of functional foods.

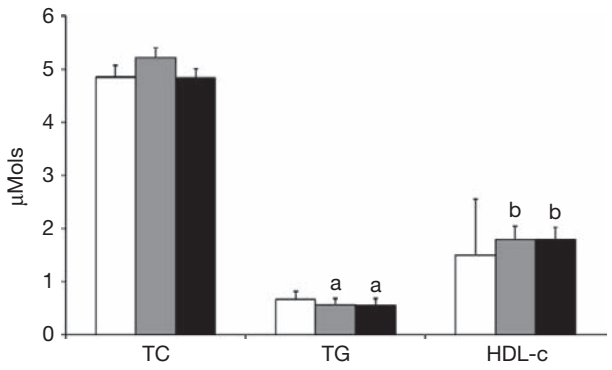

Figure 1 Milk is an efficient vehicle for omega 3 fatty acid administration. In addition to high bioavailability of added fatty acids (and, in this case, of vitamin E), this functional food improves lipid surrogate markers of cardiovascular disease. Values are the means ± SD. T_0, initial values; T_3, after 3 weeks of treatment; T_6, after 6 weeks of treatment. Values marked with letters are statistically different from those at T_0 as follows: $^{a}p < 0.05$; $^{b}p < 0.005$. Data are from Visioli et al. (2000), where full details of the study are provided.

In terms of dairy products, it is noteworthy that some kinds of cheeses, namely, those infected with *Penicillium* such as Roquefort, Stilton, and Gorgonzola, contain high amounts of andrastins A–D, which – *in vitro* – are potent inhibitors of farnesyltransferase, a key enzyme in cholesterol synthesis. Other peptides formed during ripening-induced proteolysis might further contribute healthful – albeit as yet unexplored – properties that would partially explain the paradoxically relatively low incidence of cardiovascular disease (CVD) in high-cheese-use countries.

The rapidly growing market of functional foods indicates milk as a suitable vehicle for bioactive compounds: as mentioned, milk is an excellent vehicle for fat-soluble molecules. Indeed, milk and some dairy products such as yogurt provide an excellent matrix for the administration of bioactive molecules, for example, phytosterols, and are being marketed to target population groups, pending appropriate regulatory evaluation. Nowadays, any supermarket exhibits a large number of fortified milks and dairy products, some of which do indeed contribute to well-being.

In summary, either fortified or 'natural' milk and dairy products contain several compounds – even though often present in low concentrations – that might in the future be exploited for pharmanutrition applications.

Conclusions

While milk and dairy products are often regarded as double-edged sword because of their high saturated fat content and abundance of important micro- and macronutrients, accumulated evidence suggests that their use within the frame of a balanced diet might be advantageous from a health viewpoint. It is also worth reminding that milk and dairy products account for a substantial proportion of calories and macro- and micronutrients in several populations worldwide. Therefore, their nutritional value is quantitatively relevant on a global scale.

Of course, subjects with cardiometabolic disturbances should consume dairy products in moderation and should discuss their use with their physician/nutritionist. Even though it is difficult to single out the health contribution of a particular food item, future studies might further elucidate the role of milk and dairy products in human health and disease.

See also: Acidophilus Milk; Bioactive Peptides in Foods; Butter: Properties and Analysis; Cheese: Composition and Health Effects; Fatty Acids: *Trans* Fatty Acids; Fermented Foods: Fermented Milks; Food Intolerance: Lactose Intolerance; Lactose; Milk: Role in the Diet; Obesity: The Role of Diet; Yogurt: Dietary Importance.

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Date Palm: A Wealth of Healthy Food

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Introduction

Date is one of the healthiest foods in the world. It has been part of the people's life in the desert and a way of surviving arid conditions. Although about three-fourths or more of the fruit consists of sugars, there are many other nutritious components such as minerals, vitamins, antioxidants, and dietary fibers. The diversity of date palm cultivars, species, and clones adds to the wealth of this tree. The different date fruit types, whether soft, semidry, or dry, provide consumers with many choices and the ability to grow palm trees in a variety of climates. Even the edibility of the fruit varies from those that could be consumed at full coloration, as a mature fruit, called the bisr or khalal stage, to others that must be converted to the rutab stage or the ripe fruits. The mature fruit could be yellow or red (rarely orange) but not necessarily free of soluble tannins while the ripe fruit is full of phenols and tannins in a polymerized form with a consumable taste. However, the initiation of ripening means the initiation of rutab development, and the fruit becomes soft and edible. Firm texture of the rutab fruits could be maintained by applying the polyamine putrescine. The predominant sugar in dry dates is sucrose because the invertase enzymatic activity is low due to low water or high temperature stress that inhibits such activity. It was found that when a reduced rate of gain in fresh weight and volume occurs, a decrease in the rate of gain in reducing sugars and a rapid increase in accumulation of sucrose and total sugars take place. Thus, sucrose accumulation starts late in the season when the weather is usually hot, and the rate of sucrose inversion to reducing sugars at this stage varies in different date cultivars. The predominant sugars in soft dates are glucose and fructose as in Zaghloul and Barhee dates.

Description of Dates

Date palm tree is a dioecious plant where male and female flowers are born on separate palms. The tree has only one point of vegetative growth represented in the apical meristem. Date palm cultivars have specific leaf morphology whether in the distribution of leaflets (pinnae) or their angle in the three dimensions. The fruit is a berry that develops from one of three carpels found in each of the pistillate flowers. Fruit shape is usually more or less oblong or ellipsoidal. The seed is bony called the pit and about three times as long as it is wide, and it contains mainly cellulose. Female trees need artificial pollination to obtain a fair fruit set. Variations in pollen source could affect some fruit characteristics outside the embryo and the endosperm such as fruit length and size, the pulp to pit ratio, color intensity, and even the time of ripening in marginal-production areas. This has been known as the metaxenic effect of pollen grains. When pollination is prevented or when fertilization fails, all or one of the three carpels of the pistillate flowers will develop into a parthenocarpic fruit. These are called parthenocarpic single or triplets, respectively. The parthenocarpic triplets are hollow, whereas the parthenocarpic singles contain quasi-degenerated seeds. Both types of parthenocarpic fruits are found in different cultivars at various ratios, especially when failure occurs in pollination and fertilization. Data on parthenocarpic fruits showed the same pattern of growth, though at a different rate. They change their color at the same time as seed-bearing fruits but do not reach maturation, if at all, at the same time.

It was reported that the date fruit has five phases that can be distinguished. The Arabic terms for these different stages have been used in the literature. The hababouk stage of slow growth rate begins from fruit set and continues 4–5 weeks. In this time, the content of moisture in the fruit is 85–90%. This stage is followed by a rapid increase in fruit weight ranging from 6 to 10 weeks (the kimri stage), then a declining rate of growth (the depression phase of the kimri stage) until reaching to the bisr or khalal stage, which is fruit coloration. Fruit skin is relatively thin at the early stages of hababouk and kimri, which could not resist the exerted water pressure leading to cracking. Similarly, the fruit tip at the initiation of the khalal stage could crack due to the increase in water absorption while there is a reduced rate of water loss because the relative humidity is high. That is one of the factors that creates a driving force for water loss.

During the khalal stage, the fruit starts to turn from green to yellow (or red), weight gain is slow, but sucrose content increases while moisture goes down to 50–55%. In some cultivars such as Barhee and Zaghloul, tannins start to precipitate and lose their astringency. The khalal stage could be extended by applying a calcium formulation after harvest and modifying the method of packaging. During the fourth stage, namely the rutab stage, dates become half ripe and soft and turn to light brown color while the sucrose turns to invert sugars. Fruit moisture is about 35–40%, whereas the astringent taste disappear. Finally, during the tamr stage, dates become semidry or dry contain about 50% sucrose (or higher if temperature is high and water content is low). Usually the fruit moisture ranges between 20% and 25%.

Types of Cultivars

Dates can be marketed in three types, namely, soft dates, semidry, or dry. The division is based on the texture, water content, appearance, and their heat summation units. Soft dates are distinguished by their soft flesh, high moisture content (more than 30% reaching to 70%), and high sugar content, especially glucose and fructose. Examples of soft dates are Barhee, Halawy, Hayany, Zaghloul, and Samany. Semidry dates have a relatively firm flesh, fairly low moisture (20–30%), and high sugar content especially reducing and sucrose in similar amounts ranging from 35% to 37%. This

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type includes Sewi, Al-Amry, Al-Ajlani, Dayri, and Khadrawi. Furthermore, dry dates have a hard texture, low water content (< 20%), and great sugar content, especially sucrose. Examples are Zahdi, Sayer, and Sackouttti. Moreover, soft dates could be edible at the khalal (fully colored) or at the initiation of the rutab stage depending on their soluble tannins content at this stage because they have to lose their astringent taste. It is suggested that the rate at which soluble tannins are converted to insoluble tannins varies within cultivars, making some of them edible as fresh juicy dates. Brownish color at the rutab stage is ascribed to date phenolase that works on substances such as catechin or the dactylifric acids. Furthermore, heat requirements vary among date cultivars. Soft dates require relatively lower summation of heat units as compared with semidry and dry heat. These requirements are calculated from full bloom to maturity and estimated to be 2500, above 2500–3150, and above 3150–6000 heat units for soft, semidry, and dry dates, respectively.

Production of Different Areas

In history date palm cultivation has been extending along desert areas and around the rivers. The tree favors high temperature and sunshine. Thus, date palm trees are confined to arid and semiarid regions. No wonder that date palm figures were part of ancient history and civilizations. It is reported that date palm is currently grown for fruit production used for local consumption or trade in 37 countries around the world, in addition to few other countries growing the crop on small areas. Almost all parts of the tree have been beneficial whether as an energy source or providing shade and shelter against the harsh conditions in the desert regions. Many traditional by-products have been made ranging from hand fans to furniture. According to FAO (2011), Table 1 illustrates the production areas of date palm in the leading countries.

The data in Table 1 indicated that the greatest production of dates is in United Arab Emirates even though there are much larger areas grown with date palm in other major producing countries such Saudi Arabia, Iraq, and Iran.

Date palm germplasms may exceed 5000 cultivars and clones. Most clones are less productive and susceptible to diseases than known-commercial cultivars. Many threatened clones and cultivars are subjected to extinction due to the spread of Fusarium fungi in Morocco, Tunisia, and Algeria. This is why there has been a narrow genetic diversity in these regions such as Deglet Noor in Tunisia and Algeria, although it represents over 65% of the total population, and Boufeggous-O-Moussa and Jihal in Morocco.

Table 1 Cultivated area and the total production of date palm in the leading countries

	2011 Data	
Countries	*Cultivated area (ha)*	*Total production (tons)*
Egypt	41 652	1373 570
Iran	154 274	1 016 608
Iraq	123 230	619 182
Saudi Arabia	172 297	1122 822
Oman	31 348	268 011
Morocco	70 433	117 867
Tunisia	51 000 (Feddans)	180 000
The United States	3399	30 209
United Arab Emirates	41 159	2 391 643

Source: *FAO statistics handbook* (2011). Date Palm Statistics.

The cultivated area has increased by 0.3 million hectares over the previous decade. The largest date palm areas are located in the Asian continent, which include the Middle Eastern producing countries, followed by Africa, North whereas in the Americas and Europe, the production of date palm covers only a few thousands hectares. The date crop has been also established in California, Arizona, and Mexico. The common required temperature is 35 °C necessary for an optimum development of pollen and low relative humidity for fruit setting and ripening. Date palm trees grow in nearly rainless regions at 9–39 O North latitude, which are represented by the Saharan and Southern fringe of the Near East.

The increase in relative humidity around the bunches causes some physiological disorders such as checking at the early kimri stage or black nose by reaching to initiation of maturity (the khalal stage). In both disorders, the rate of water absorption is greater than its loss from the fruit, which creates a pressure on fruit skin, resulting in surface cracking.

With regard to the distribution of date palm, this crop cultivation is spreading in both the old world (Near East, North Africa, Spain) and the new world (Australia and the American continent), where dates are grown and consumed in large quantities.

The distribution of date palm according to the latitude for both Northern and Southern Hemispheres is between 10° N (Somalia) and 39° N (Elche/Spain or Turkmenistan). Favorable areas are located between 24 and 34 (Morocco, Algeria, Tunisia, Libya, Egypt, Iraq, and Iran). In the United States, date palms are found between 33 and 35° N. Because of the climatic factors, the date palm will grow but will not fruit properly outside these defined geographical limits. Altitude is very important because it imposes the availability of water and the temperature limits that largely determine the distribution of date palm in the world. Date palm grows well from 392 m below sea level to 1500 m above with an altitude range of 1892 m.

Consumption of Dates as a Food

Dates have been the essential food in many regions around the world, especially arid zones of the old world and part of the diet all year-round. Even in the new world and Europe, date fruits have attracted the attention of the public and sport dieticians due to the high glucose and fructose content in semidry and soft dates. It was reported that one pound of dates (453 g) supplies the human body with 5.33 kJ of physiological energy.

A comparison was made to show the relative value of four types of food (cow's milk, meat, honey, and orange juice) against date fruits. The comparison showed that protein content in dates was very close to that of cow's milk, but greater than that found in honey or fresh orange juice. Meanwhile, the

date fruits were superior to all the other four types of food in potassium. Furthermore, calcium in dates was three times greater than that found in cow's meat and 15 times greater than that in honey while about half of the calcium content in the cow's milk. Moreover, phosphorus content in 100 g of dates was about two-thirds of that found in cow's milk but three times more than that found in honey. Iron, on the other hand, is much more in date fruits when compared with cow's milk, honey, or orange juice. Iodine content, as an essential element for the gland activity, in dates is equal to that found in honey or fresh orange juice. Moreover, sodium, as an important element required for water balance in the human body and that affects muscles and the nervous system, is contained in dates in smaller amounts than that found in cow's milk or meat but greater than that found in honey or fresh orange juice. Even carotenes in dates are much greater than that found in cow's milk or orange juice. These carotenes are important antioxidants for providing protection against cancer.

Dates are also an excellent source for carbohydrates, proteins, fats, dietary fibers, essential minerals, and vitamins. Edible dates have been classified into four stages termed kimri, khalal (bisr), rutab, and tamr.

These represent the astringent green, mature full-colored, soft brown, and hard raisin-like stages of development, respectively. For human consumption, in general, dates become edible at the khalal stage where the fruit color changes to a yellow or red tone, depending on the cultivar, over a period of 3–5 weeks. Fruits are rarely orange in color at the khalal stage. Few date cultivars could be consumed at the khalal stage such as Zaghloul, Samany, and Barhee because they are not astringent at this stage, and soluble tannins do not represent a problem. However, many date cultivars and clones, especially in the Arabian Peninsula need to initiate the rutab stage to get rid of the astringent taste. At the rutab stage, dates begin to soften in about a 2–4 week period due to an increased loss of moisture content and an increase in enzymatic activity of some cell wall hydrolyses such as pectinase and polygalacturonase. The conversion to the rutab stage in some cultivars is required to obtain the insoluble form of tannins. During ripening (rutab development) there is a sharp reduction in both the amount and concentration of soluble tannins indicating the possibility of ceasing their accumulation together with breakdown and/or a conversion into insoluble form during development and ripening. It was also reported that soluble tannins are polymerized when converted to the insoluble form and lose their ability to compete or bind to the binding sites of ethylene. Date fruits are also heavily consumed at the tamr stage whether semidry or dry. Dates are drier and rather firm in texture at this stage, and their color turns to a darker tone. The lower water content of dates at the tamr stage enhances their storability and shelf life. Dates, however, continue to ripen after harvest, exhibiting a characteristic respiratory rise before ripening preceded by a spike in ethylene production, which accelerates the process. The most important category of accumulated proteins involved in membrane and cell wall degradation are polygalacturonase and pectinesterases that ensure the softening of the fruit. There are also some other proteins that are responsible for the pigment degradation and color change during various stages from kimri to the tamr stages. Thus, physiological changes that occur in dates during ripening begin with the recognition of external signals, including ethylene spike that occurs before the respiratory rise.

Nutritional Value of Dates

The high content of carbohydrates in date fruit, especially sugars that reach to 88%, may lead to the impression that little is left to contribute to the nutritional value of dates. However, the fruit is full of essential nutrients such as potassium, phosphorus, sodium, zinc, manganese, magnesium, copper, iron, fluorine, and selenium. Dates are a great source of potassium that helps in the maintenance of a healthy nervous system and in balancing the body's nervous system. Phosphorus functions with calcium to help with bone strength and growth. Moreover, selenium is important for cell growth and repair. Iron is essential to production of red blood cells, which carry all the nutrients to cells throughout the body. As an example, the macro- and microelements of the two well-known cultivars, Hallawi and Zahdi, were reported (on a dry weight basis) in Table 2.

The amount of sodium in dates is low but ideal when looking at the recommended amount per day for an individual (more than 2.4–2.0 mg day^{-1}). Consuming too much sodium may increase the risk of heart disease and hypertension. Thus, dates are perfect for a healthy lifestyle because they contain reducing sugars, low sodium, no fats or cholesterol, great potassium, and calcium contents in addition to dietary fibers. The dietary fiber content of widely retailed dates was estimated at 4.4% with 3.2% insoluble and 1.2% soluble. Others reported a greater percentage of dietary fibers ranging from 6.4% to 11.5%. Variations were reported to be dependent on

Table 2 The macro- and microelements content of two commercial date cultivars

	Cultivars			*Cultivars*	
Macroelements	*Hallawi*	*Zahdi*	*Microelements*	*Hallawi*	*Zahdi*
Calcium	184	207	Iron	5.26	10.37
Phosphorus	16	14	Manganese	5.86	5.16
Potassium	854	887	Copper	2.77	2.75
Sodium	14	5	Zinc	1.39	0.74
Chlorine	260	342	Cobalt	0.76	0.95
Magnesium	56	59	Fluorine	0.20	0.12

Source: Yousef, A. and Kado, A. (1982). *Chemical composition of four Iraqi date cultivars*. Baghdad: Agriculture and Water Resources Research Center.

Table 3 Variations in sugar percentage within different date types

	Sugars (%)		
Date cultivar	*Total sugars*	*Reducing sugar*	*Nonreducing (sucrose)*
Zaghloul (soft)	80.70	79.50	1.20
Ajlany (semidry)	78.80	35.40	43.40
Sakoutti (dry)	78.70	14.40	63.30

Source: Ibrahim, A. M. and Kholaif, M, N. (1998). *Date palm production and hasbandry* (in Arabic). Egypt: Al-Maaref Press.

Table 4 Vitamin content of two commercial dry date cultivars (mg per 100 g)

	Date cultivars	
Vitamins	*Hallawi*	*Zahdi*
Thiamine (B_1)	99	80
Riboflavin (B_2)	173	167
Biotin (H)	4.63	5.74
Folic acid (folacin)	57	63
Ascorbic acid (C)	3.56	2.41

Source: Yousef, A. and Kado, A. (1982). *Chemical composition of four Iraqi date cultivars*. Baghdad: Agriculture and Water Resources Research Center.

the stage of ripeness. Six to seven dates, approximately 100 g, consumed daily by an adult would provide 50–100% of the recommended daily fibers uptake. Thus, dates proved to be a better snack than others containing artificial preservatives. Fluorine in dates proved to be essential to protect against tooth decay. It is even added to toothpaste, whereas selenium plays an important role in protecting against cancer and stimulating the immune system.

Dates are very rich in carbohydrates, especially sugars such as fructose, glucose, mannose, maltose, and other nonreducing sugars such as sucrose. The percentage of the reducing sugars, namely glucose and fructose, depend on the type of dates (dry, semidry, or soft) and on the cultivar. In Deglet Noor, for example, the predominant sugar was sucrose, whereas in Allig it was reduced sugars, the more abundant sugar. This trend was early reported because it was shown that rutab dates had much less sucrose than reduced sugars. This difference was ascribed to the potential presence of high invertase activity in Allig dates. A similar trend of sugars was reported for Zaghloul, Ajlany, and Sakoutti as soft, semidry, and dry dates, respectively (Table 3).

The high increase in sugars reflects on the energy produced by 1 kg of apricot, banana, orange, and dates to be 520, 970, 480, and more than 3000 calories, respectively. Meanwhile, 1 kg of cooked rice, wheat flour, or meat (without fats) produces 1800, 2295, and 2245 calories, respectively.

Dates are also rich in many vitamins such as B-carotene (vitamin A), thiamine (B_1) and riboflavin (B_2). The contents, however, vary with the cultivar and the stage of ripeness. For example, vitamin C content (ascorbic acid) in rutab dates is greater than dry dates. Ripe fruits were reported to contain a substantial amount of carotenoids including lutin and various forms of β-carotene with the total content of carotenoids decreasing toward the final ripening stages and in storage. Furthermore, dates contain a moderate amount of folic acid. Table 4 reports vitamin contents of two date cultivars on a dry weight basis, namely Hallawi and Zahdi.

With regard to phenolic compounds in dates, it was found that dates contain a number of phenolics such as phenolic acids, hydroxycinnamates and flavonoids including tannins. These compounds are beneficial for human health as antioxidants and maintaining a specific cellular homeostasis. Phenolics have also been considered a defense mechanism against pathogens.

Concerning dietary fibers in dates, it was reported that six to seven dates (about 100 g) consumed daily by an adult would provide 50–100% of the recommended daily need.

Seeds of date also contain beneficial constituents that could be utilized for animal feeding. They contain carbohydrates (62.5%), fibers (16.2%), oils (8.5%), proteins (5.2%), ash (1.1%), and moisture (6.5%). Date seeds also represent a source of sterols and alkali-soluble polysaccharide. The seeds yield a yellow green moisturizing oil rich in essential fatty acids such as lauric (8%), myristic (4%), palmitic (25%), stearic (10%), oleic (45%), and linoleic (10%) as well as traces of caprylic and capric acids, suitable for use in soap and cosmetic products.

The health benefit of dates is a relatively new important aspect of date palm. It was found that alterations induced by cerebral ischemia were significantly attenuated by 15 days of pretreatment with methanolic extract of date fruits (100 and 300 mg kg^{-1}), whereas a 30 mg kg^{-1} dose was insignificant in this regard. These results suggested the possible use of dates against bilateral common carotid artery occlusion induced oxidative stress and neuronal damage. In another recent study they investigated the role of date Palm fruit extract in protection against oxidative damage and hepatotoxicity induced by subchronic exposure to dimethoate (20 mg $kg^{-1}day^{-1}$) in rat liver. Such chemical induces an excessive production of free radicals that are responsible for several cell alterations in the organism. The study provided evidence that pretreatment with date palm extract restored the liver damage induced by dimethoate as revealed by inhibition of hepatic lipid peroxidation and prevention of oxidative stress induced hepatotoxicity. Moreover, another important beneficial health aspect of date palm extract was found with rat experiments where such extract was able to significantly reduce the frequency of defecation and decreased gastrointestinal motility. It was concluded that date palm extract may contain some pharmacologically active substances with antidiarrheal properties.

See also: Adolescent Nutrition; Anemia: Prevention and Dietary Strategies; Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Ascorbic Acid: Properties, Determination and Uses; Berries and Related Fruits; Browning: Enzymatic Browning; Diarrheal Diseases; Dietary Fiber: Determination; Fatty Acids: Essential Fatty Acids; Food and Agriculture Organization of the United Nations; Fructose: Sources, Metabolism, and Health; Fruit Juices; Fruits of Tropical Climates: Biodiversity and Dietary Importance; Functional Foods; Lauric Oils; Malnutrition: Prevention and Management; Meat: Structure; Mediterranean Diet; Milk: Sources and Composition; Phenolic

Compounds: Occurrence, Classes, and Analysis; Phospholipids: Properties and Occurrence; Protein: Food Sources; Snack Foods: Types and Composition; Sucrose: Dietary Importance; Sugar Alcohols; Tannins; Traditional Foods; Vitamins: Overview.

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Diarrheal Diseases

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Epidemiology and Global Burden of Diarrhea

The WHO defines diarrhea as the passage of three or more loose or liquid stools per day, or more frequently than is normal for the individual. Diarrhea is the leading cause of mortality among children under 5 years of age accounting for one in ten child deaths worldwide annually. It disproportionally affects young children in low- and middle-income countries (Figure 1). In 2010, there were 1.731 billion episodes of diarrhea (36 million of which progressed to severe episodes) and in 2011, an estimated 700 000 deaths were due to diarrhea. Incidence and mortality from diarrhea and pneumonia vary by age (Figure 2) with the burden of disease mainly concentrated in the younger age groups. The epidemiology of childhood diarrhea and pneumonia overlap, likely because of shared risk factors, such as undernutrition, suboptimum breastfeeding, and zinc deficiency. Children are at the highest risk during the first 2 years of life, with 72% of deaths being secondary to diarrhea. Diarrheal mortality rates are declining by about 4% annually with disease incidence decreasing from an estimated 3.4 episodes/child-year in 1990 to 2.9 episodes/child-year in 2010. The highest burden of disease has remained consistent with respect to age in the 6–11-month-old age group having the highest incidence. The burden of mortality due to diarrhea in children less than 5 years of age in 2010 was highest in the WHO regions of Africa and Southeast Asia. It is estimated that a third of severe diarrhea episodes is preventable by vaccination (i.e., against rotavirus and cholera). Furthermore, under nutrition is a key underlying risk factor for morbidity and mortality associated with both diarrhea and pneumonia. Diarrhea disease severity in children under-five is mostly mild (~65% cases for which no care was sought/no dehydration) lasting about 4 days or moderate (~35% cases for which care was sought/any dehydration on presentation) with only 0.5% of cases being severe (cases for which care was sought, with severe dehydration on presentation) and lasting 8 days. Older children and adults have predominantly mild episodes (95%) with 4.95% being moderate and 0.05% being severe. Among individuals 16 years or older, severe episodes typically last ~3 days and cause dehydration in ~93% of cases.

Morbidity and Risk Factors for Diarrhea

Despite reduction of diarrheal mortality by use of oral rehydration therapy, persistent global burden of enteric infections results in high morbidity of up to 43% of stunted growth, affecting one-fifth of children worldwide and one-third of children in developing countries. Children suffering from diarrhea during the first 2 years of life might have on average an 8 cm growth shortfall and 10 IQ point decrement by the time they are 7–9 years old. The proposed link between enteric infections and child growth and development is a double burden of enteric infections and malnutrition, and the potential link of both of these factors to obesity later in life is an interrelated 'triple burden' (Figure 3). Important risk factors for diarrhea include lack of exclusive breastfeeding in infants younger than 6 months, undernutrition and zinc deficiency. While evidence suggests that vitamin A deficiency increases the risk of severe diarrhea, it is not an important risk factor for the incidence of diarrhea. Associations of specific water and sanitation risk factors (i.e., unwashed hands and poor water quality) with diarrhea morbidity and mortality have been shown. However, for some outcomes such as inappropriate excreta disposal, poor availability of evidence permits only rough estimates of risk. In a stratified subgroup of infants aged less than 6 months, acute lower-respiratory-tract infections are a risk factor for diarrhea. Measles is an established risk factor for diarrhea. A study from the 1980s showed that with moderate coverage of the measles vaccine (45–90%) in infants, the incidence of diarrhea could be reduced by 0.6–3.8%, and diarrhea mortality reduced by 6–26% in children younger than 5 years. Table 1 shows the effect sizes for specific confirmed biological risk factors for diarrhea.

Diarrhea Classification and Pathophysiology

Diarrhea can be classified based on duration as prolonged/acute (1–13 days) and persistent (14 days or more). Prolonged diarrhea can further be classified as prolonged/acute watery diarrhea which is loose or watery stools at least three times in a 24 h period or prolonged/acute invasive diarrhea which is gross blood (by history or inspection) in the stool of less than 14 days duration, typically accompanied by fever. Diarrhea can be secondary to one or more of the following pathophysiological processes: secretory, osmotic and inflammatory diarrhea. *Secretory diarrhea* occurs when there is a significant increase in the volume of intestinal fluid output which exceeds the reabsorptive ability of the gastrointestinal epithelium. Research initially done in patients with cholera revealed that while the secretory diarrhea in cholera does lead to significant fecal water and electrolytes loss, the gut still retains the ability to reabsorb water and electrolytes due to an intact Na-coupled solute cotransport mechanism. Chloride-mediated secretion is stimulated by second messengers in diarrheal infections secondary to *Vibrio cholerae* 01 and 139, Shigella, Salmonella, *Escherichia coli* strains and other bacterial pathogens who produce enterocyte receptor binding pathogens, causing chloride-mediated secretion stimulated by second messengers (e.g., cAMP, cGMP, and calcium). It is important to recognize that there may be a combination of osmotic and secretory diarrhea such as in the case of Rotavirus where there enterotoxin mediated secretory diarrhea along with osmotic diarrhea secondary to enterocyte

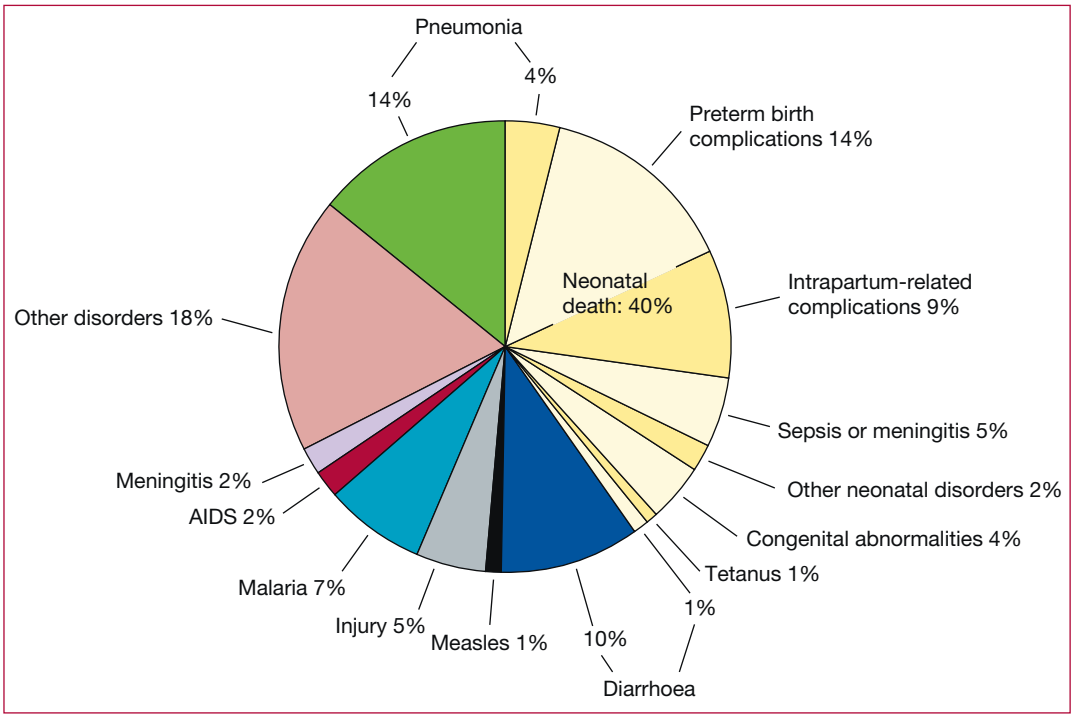

Figure 1 Global causes of childhood deaths in 2010, diarrhea kills 2195 children daily – more than malaria, measles, and AIDS combined. (Note – new figures show that neonatal component may be 44%). Reproduced from Liu, L., Johnson, H.L., Cousens, S., et al. (2012). Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet* **379**(9832), 2151–2161.

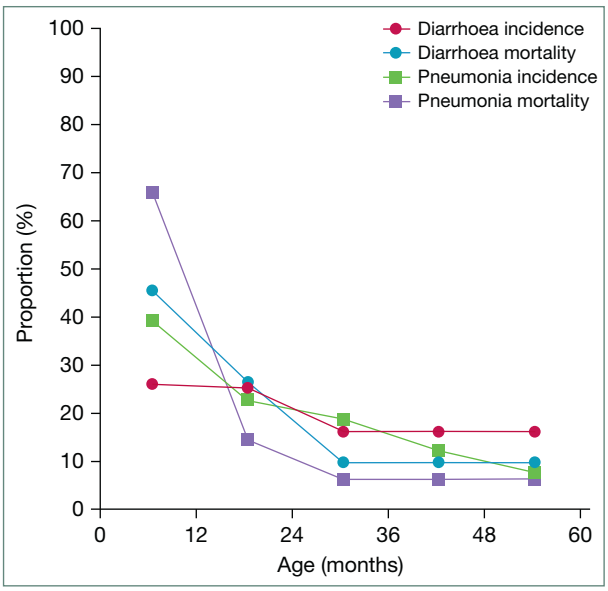

Figure 2 Distribution of cases of and deaths from diarrhea and pneumonia in children aged 0–4 years. Reproduced from Walker, C.L., Rudan, I., Liu, L., et al. (2013). Global burden of childhood pneumonia and diarrhoea. *Lancet* **381**(9875), 1405–1416.

damage. Phosphorylation-induced activation (by increase in cAMP, cGMP, or calcium) of these channels can be set off by exogenous or endogenous substances. Exogenous mediators include bacterial toxins such as cholera or shiga toxins, or parasites. Endogenous mediators can be endocrine, such as vasoactive intestinal peptide or serotonin, or released by the immune system, such as histamine, serotonin, prostaglandins, or interleukin-1. Lastly, increased intestinal permeability due to loss of cellular tight junctions can lead to increased intestinal fluid loss (Figure 4).

Poorly absorbed osmotically active substances in the gut lumen retain fluid, pull water and secondarily ions to maintain osmotic equilibrium with body fluids – this is the key underlying process behind *osmotic diarrhea*. This can occur secondary to an absorptive defect or enzyme deficiency from pancreas or intestinal wall causing maldigestion and/or malabsorption. An osmotic gradient is created that can only be neutralized by maintaining excessive amounts of water within the intestine. Examples include, malabsorption with congenital or acquired lactase deficiency, gluten-sensitive enteropathy (celiac disease) and maldigestion, as seen in pancreatic insufficiency (Figure 5).

Gastrointestinal barrier disruption by bacteria (Salmonella, *E. coli*, Campylobacter), viruses (rotaviruses, coronaviruses), and protozoa (Cryptosporium, Giardia) in addition to idiopathic inflammatory bowel diseases are common causes of *inflammatory* diarrhea. Immune cells after activation release inflammatory mediators and cytokines which stimulate secretion and activate enteric nerves. Subepithelial myofibroblasts destroy the basement membrane by metalloproteinases, damaged enterocytes are extruded, and villous atrophy develops followed by regenerative crypt hyperplasia in the small intestine and colon. These surfaces are covered with immature enterocytes that typically are deficient in the brush border enzymes and transporters necessary for absorption of nutrients and water.

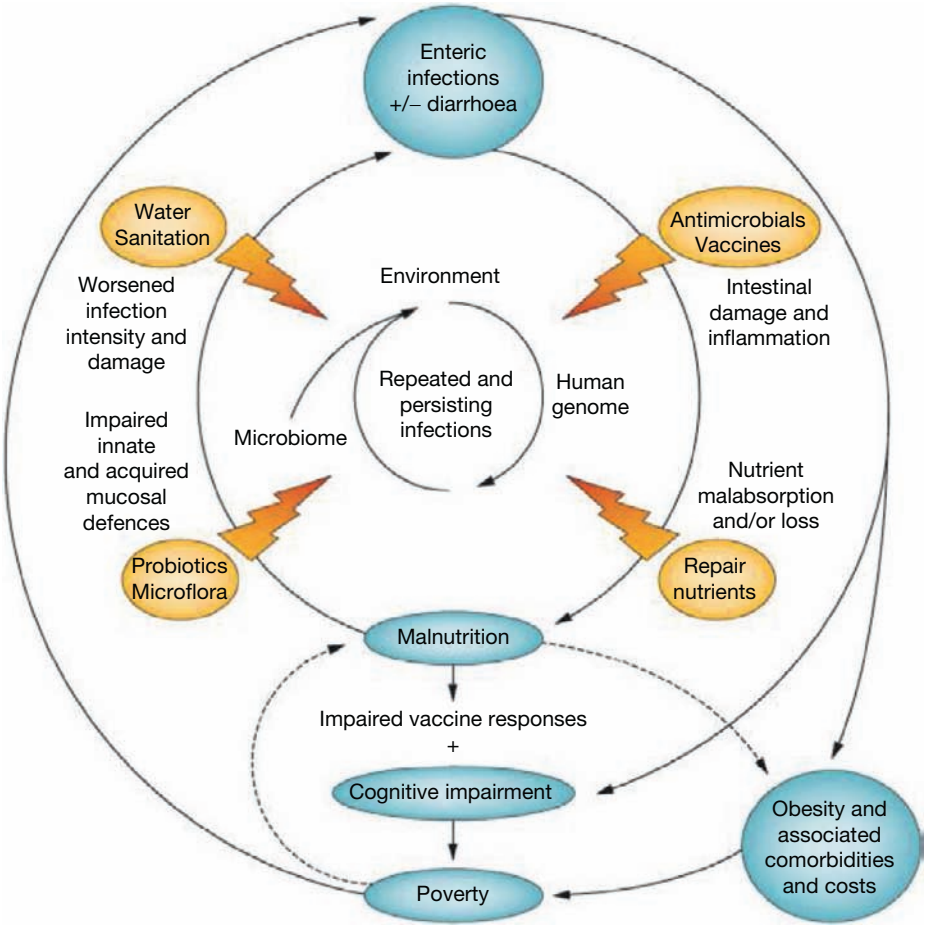

Figure 3 The vicious cycles of diseases of poverty. Enteric infections, especially in the first 2–3 years of life, with or without overt diarrhea, can predispose an individual to malnutrition and stunted growth through multiple mechanisms. Stunting by 2 years of age, in turn, is associated with impaired cognitive development that extends into later childhood and even adulthood and adult productivity. In addition, malnourished children experience both greater frequency and duration of diarrheal illnesses, and, documented in animal models, heavier infections. The latter is documented with Cryptosporidium and with enteroaggregative *E. coli*. Finally, enteric infections or stunting can predispose to obesity and its comorbidities of diabetes, hypertension, cardiovascular disease, metabolic syndrome and burgeoning healthcare expenditures, contributing to individual and societal poverty in vicious cycles. Reproduced from Guerrant, R.L., DeBoer, M.D., Moore, S.R., Scharf, R.J., and Lima, A.A. (2013). The impoverished gut – a triple burden of diarrhoea, stunting and chronic disease. *Nature Reviews Gastroenterology & Hepatology* **10**(4), 220–229.

Etiology of Diarrhea

Major etiologies for prolonged/acute and persistent diarrhea are outlined in Table 2. The most predominant cause of diarrhea in developing countries is infectious (viral, bacterial, parasitic). A systematic review of articles published between 1990 and 2011 reporting etiological agents causing diarrhea in hospitalized children less than 5 years of age showed that rotavirus, calicivirus, enteropathogenic, and enterotoxigenic *E. coli* cause more than half of all diarrheal deaths in this age group globally. A recent 3-year prospective study in four sites in Africa (the Gambia, Mali, Mozambique, Kenya) and three in Asia (India, Bangladesh, Pakistan) showed the most attributable cases of moderate to severe diarrhea in children aged 0–59 months was secondary to infection with rotavirus, Shigella, ST-ETEC, cryptosporidium, and enteropathogenic *E. coli*. Table 3 shows a comparison of viral, bacterial, and parasitic etiologic agents of diarrhea in developing countries and the United States. There is a seasonality in the transmission patterns of infectious agents in developing countries with bacterial diarrheas peaking in the hot months and viral diarrheas typically occurring throughout the year with some peaking in the cooler months. More than one pathogenic agent can exist with 11% (in community studies) and 12% (in hospital based studies) of diarrhea cases documented as having two or more enteric pathogens. Transmission of infectious agents causing diarrhea is primarily 'fecal–oral' in the setting of poor hand washing hygiene, unsanitary water and dirty food preparation. The dose of infectious agent required to cause diarrhea also matters with low infectious dose (e.g., shigella, giardia, rotavirus, cryptosporidium) can be transmitted by person-to-person contact and high infectious dose (e.g., salmonella, *E. coli*, vibrios) usually transmitted by water or food.

Table 1 Risk factors with direct biological links to diarrhea

	Morbidity	*Mortality*
Not exclusively breastfeeding (0–5 months; vs. exclusive breastfeeding)		
Partially breastfed	RR 1.7 (95% CI 1.0–2.8)[a]	RR 4.6 (95% CI 1.8–11.8)[a]
Not breastfed	RR 2.7 (95% CI 1.7–4.1)[a]	RR 10.5 (95% CI 2.8–39.6)[a]
No breastfeeding (6–23 months; vs. receives any breast milk)	RR 1.3 (95% CI 1.1–1.6)[b]	RR 2.2 (95% CI 1.1–4.2)[a]
Underweight (vs. >−2 WAZ for morbidity and >−1 WAZ for mortality)		
−2 to <−1 WAZ	–	OR 2.1 (95% CI 1.6–2.7)[c]
−3 to <−2 WAZ	RR 1.2 (95% CI 1.1–1.4)[d]	OR 3.4 (95% CI 2.7–4.4)[c]
Less than <−3 WAZ	–	OR 9.5 (95% CI 5.5–16.5)[c]
Stunting (vs. >−1 HAZ)		
−2 to <−1 HAZ	–	OR 1.2 (95% CI 0.9–1.7)[c]
−3 to <−2 HAZ	–	OR 1.6 (95% CI 1.1–2.5)[c]
<−3 HAZ	–	OR 4.6 (95% CI 2.7–8.1)[c]
Wasting[c] (vs. >−WHZ)		
−2 to <−1 WHZ	–	OR 1.2 (95% CI 0.7–1.9)[c]
−3 to <−2 WHZ	–	OR 2.9 (95% CI 1.8–4.5)[c]
<−3 WHZ	–	OR 6.3 (95% CI 2.7–14.7)[c]
Vitamin A deficiency (vs. not deficient)		RR 1.5 (95% CI 1.3–1.8)[e]
Zinc deficiency (vs. not deficient)	RR 1.2 (95% CI 1.1–1.2)[f]	RR 1.2 (95% CI 1.0–1.6)[f]

Abbreviations: RR, relative risk; WAZ, weight-for age *z*-score; OR, odds ratio; HAZ, height-for-age *z*-score; WHZ, weight-for-height *z*-score.

[a]Mix of cohort, observational, and case–control studies. Adjusted risk relations from individual studies used when available.

[b]Mix of cohort, observational, and case–control studies. Adjusted risk relations from individual studies used when available. Data only available for ages 6–11 months for this risk relation.

[c]Prospective datasets adjusted for socioeconomic and non-nutritional determinants of mortality.

[d]Mix of cohort, observational and case–control studies; analysis done with unadjusted risk estimates.

[e]Meta-analysis of observed risk reductions from supplementation trials.

[f]Meta-analysis of observed risk reductions from randomized controlled trials.

Source: Walker, C.L., Rudan, I., Liu, L., et al. (2013). Global burden of childhood pneumonia and diarrhoea. *Lancet* 381(9875), 1405–1416.

Diagnosis and Management of Acute/Prolonged and Persistent Diarrhea

Despite clinical clues, determining the causative agent of diarrhea in an individual patient on the basis of clinical grounds alone can be difficult. Both acute/prolonged and persistent diarrheas have similar initial evaluation and management. This chapter focuses on assessing the severity of illness and the need for rehydration. A brief history and directed clinical exam can help determine this and further guide the management.

History

Specific points in history include, time of onset of diarrhea, frequency, amount, consistency, and contents (blood, mucous, pus) of stool, associated signs and symptoms (fever, abdominal pain, nausea, vomiting, bloating, flatus, tenesmus), recent travel, dietary history, exposure to pets or cattle, drug history (specifically antibiotics and laxatives), pertinent past medical and surgical history, family history and day care attendance. For persistent diarrhea, the age of symptom onset and a detailed dietary history with relation between onset of symptoms and introduction to new foods is relevant.

Physical Examination

The initial physical exam focuses on signs and symptoms of dehydration (discussed below) and sepsis (fever and toxic look) which are major complications of acute and chronic diarrhea and require urgent management. Nutritional assessment is necessary and includes height, weight, vital signs, and global nutritional appearance.

Assessing Dehydration and IMCI Guidelines

Early recognition of dehydration, which is the main cause of mortality, is the most critical part of diarrhea management. Loss of water and electrolytes (sodium, chloride, potassium, and bicarbonate) via liquid stools, emesis, sweat, urine, and breathing leads to dehydration if left unreplaced. The WHO suggests assessment of dehydration on a scale of three (Figure 6).

1. Early dehydration with no signs or symptoms,
2. Moderate dehydration notable for thirst, restless, or irritable behavior, decreased skin elasticity, sunken eyes,
3. Severe dehydration when symptoms become more severe with diminished consciousness, lack of urine output, cool, moist extremities, a rapid and feeble pulse, low or undetectable blood pressure, and pale skin in the setting of clinical shock.

Lab Evaluation

For acute diarrhea, maintaining adequate intravascular volume and correcting fluid and electrolyte disturbances take priority over the identification of the causative agent. Stool cultures are usually unnecessary for immune-competent patients who present within 24 h after the onset of acute, watery diarrhea. Lab testing including

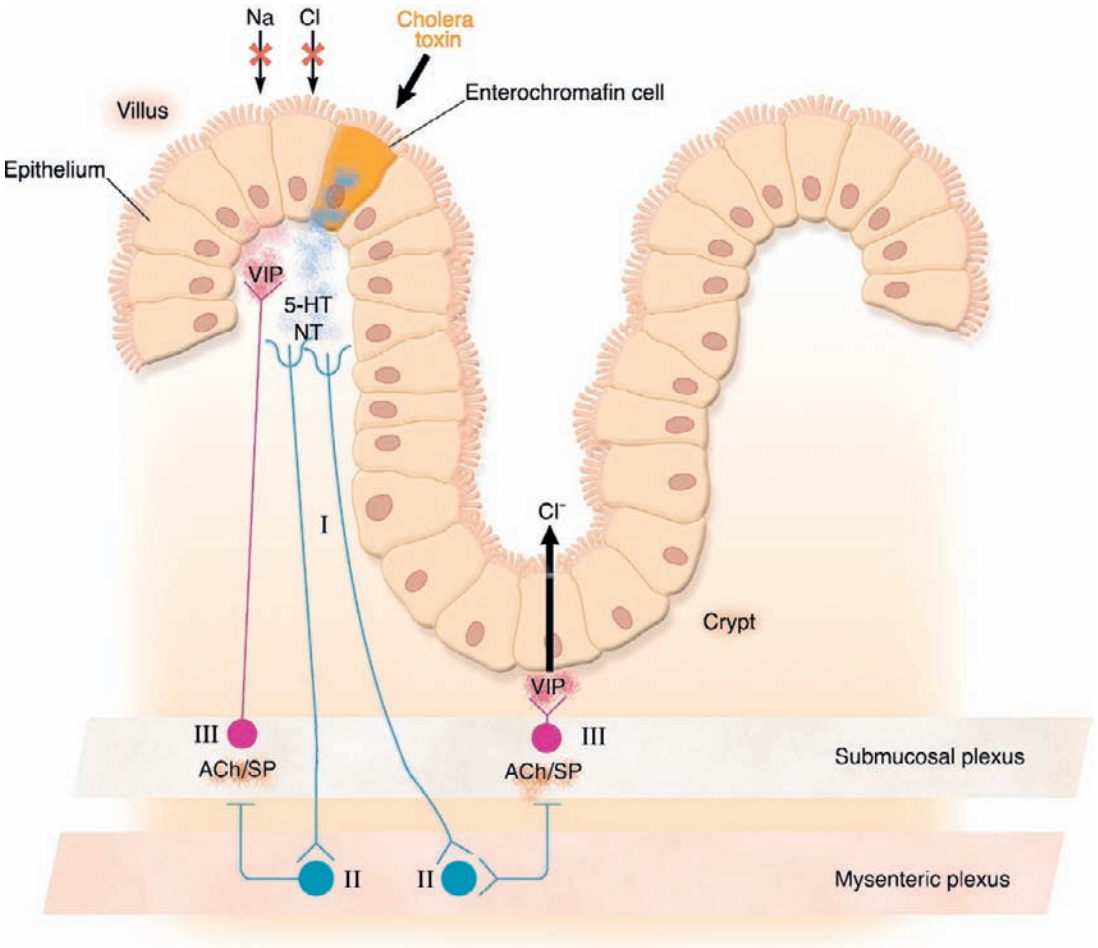

Figure 4 Pathophysiology of secretory diarrhea: diarrhea secondary to cholera toxin cholera toxin induces secretion, in part, through activation of an intramural neural reflex. Toxin attaches to enterochromaffin cells in the epithelium, causing an increase in (cAMP) there. In response to the latter, serotonin (5-HT), neurotensin (NT), and possibly additional peptides are released. These activate afferent neurons (I), whose axons course from close to the epithelium to the myenteric neural plexus, where they connect with interneurons (II) that, in turn, through release of acetylcholine (ACh) and/or substance P (SP), activate secreto-motor neurons (III) in the submucosal neural plexus. Axons from these secreto-motor neurons reach the epithelial surface, in both the villus and the crypt regions, releasing VIP and thereby stimulating secretion in crypts and inhibiting nutrient-independent salt absorption in villi. Reproduced from Field, M. (2003). Intestinal ion transport and the pathophysiology of diarrhea. *The Journal of Clinical Investigation* **111**(7), 931–943.

stool studies, blood tests, intestinal imaging, and immunologic and serologic evaluation are indicated in bloody diarrhea, high fever (>104 F), systemic illness, severe, or prolonged diarrhea, history suggestive of food poisoning, recent history of travel overseas or possibility of immune dysfunction. Supplementary laboratory studies, including serum electrolytes, to assess patients with acute diarrhea usually are unnecessary.

Treatment Approach

Fluids Resuscitation

Dehydration is the primary cause of death in patients with diarrhea and effectively addressing this is the cornerstone of diarrhea treatment. Oral rehydration solution (ORS), developed in the 1960s and 1970s by researchers in South Asia, is a simple cost-effective solution containing glucose and electrolytes that can be used to prevent and treat dehydration due to diarrhea of any cause across all ages. Development of the ORS took advantage of the glucose-coupled intestinal sodium transport mechanism discovered in the 1960s by Robert Crane. Absorption of sodium and glucose molecules across the luminal membrane is facilitated by the protein sodium glucose co-transporter 1 (SGLT1). Although nutrient-independent sodium absorption across the intestinal epithelial brush border membrane is impaired in diarrhea, cotransport of sodium and glucose is preserved. This allows absorption of sodium and thus water as provided by ORSs (Figure 7).

Once in the enterocyte, the transport of glucose into the blood is facilitated by GLUT2 (glucose transporter type 2) in the basolateral membrane with the Na+K+ATPase provides the gradient that drives the process. Key in the success of use of ORS for rehydration is the fact that these physiological processes stay intact, even in the setting of severe diarrhea.

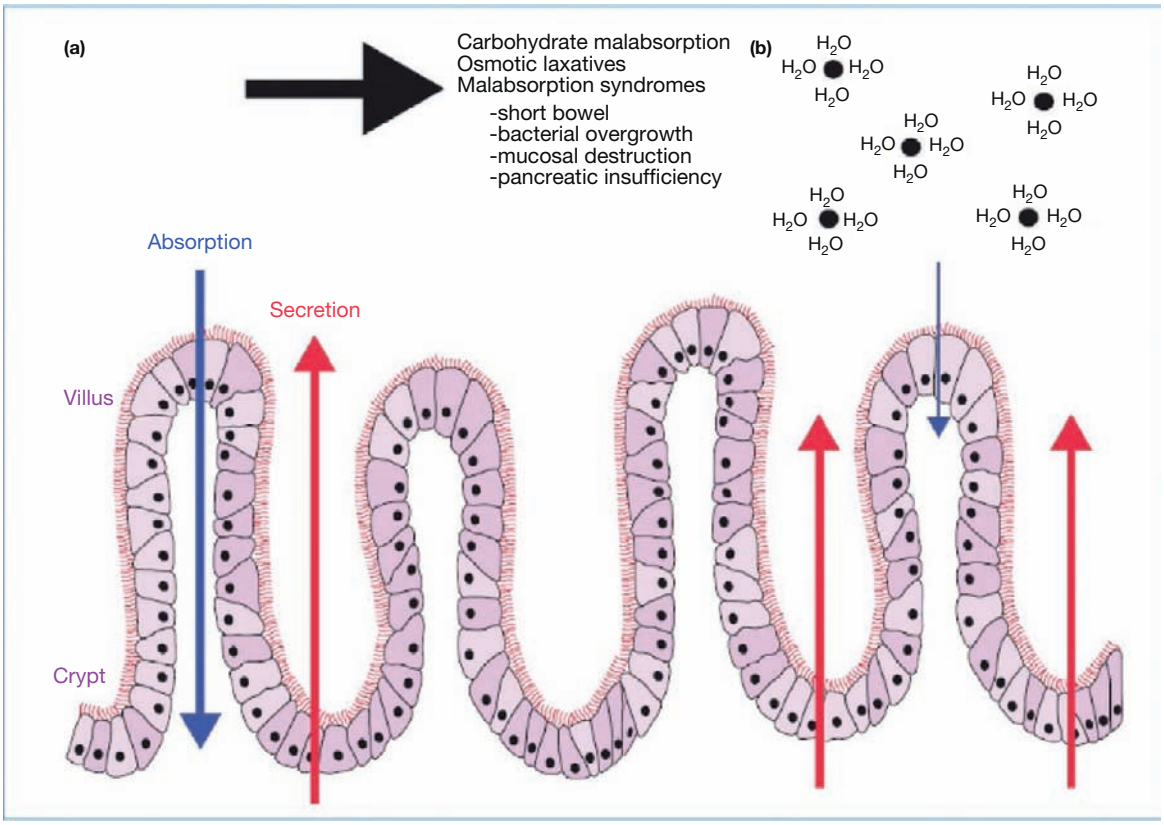

Figure 5 Pathophysiology of osmotic diarrhea. The figure demonstrates the pro-secretory effects of osmotic agents on intestinal fluid movement. (a) Represents the normal luminal environment and (b) represents the effects of osmotic agents such as non-absorbed carbohydrates. Image reproduced from the Internet http://cancerlink.ru/?page_id=1612 – free image source.

The original ORS recommended by the WHO in the 1970s had a formulation with a total osmolarity of 311 mmol l^{-1} (Table 4) but subsequent investigations lead to the current WHO recommendations from 2004 of a low osmolarity ORS (total osmolarity of 245 mmol l^{-1}, decreased concentrations of glucose and sodium). The current formulation when compared to the original formulation is associated with decrease in stool volume by about 25% when compared to the original World Health Organization/United Nations Children's Fund (WHO/UNICEF) ORS solution, reduced vomiting by almost 30%, and reduced need for unscheduled IV therapy by more than 30%. A 2010 review by Munos and colleagues assessed 205 studies and showed that universal coverage with ORS would reduce diarrhea-related deaths by 93%. There are two phases to treatment: rehydration and maintenance. In the rehydration phase, there is quick replacement of the fluid deficit over 3–4 h to achieve clinical hydration. In the maintenance phase, maintenance calories and fluids are administered with reintroductions of enteral feeding with an age-appropriate unrestricted diet, including solids. In children with mild to moderate dehydration, 50–100 ml of ORS/kg body weight during 2–4 h should be administered to replace the estimated fluid deficit, with additional ORS administered to replace ongoing losses. In the setting of severe dehydration, there is an emergent immediate need for IV rehydration. Lactated Ringer's solution, normal saline, or a similar solution should be administered (20 ml kg^{-1} body weight) until pulse, perfusion, and mental status return to normal.

Zinc Supplementation

Zinc deficiency is widespread among young children in low- and middle-income countries. It contributes to mortality and morbidity related to diarrhea, malaria, pneumonia, and restricts growth. Children with diarrhea suffer due to poor baseline dietary zinc intake with aggravated net losses of zinc during the period of illness. The WHO and UNICEF has recommended zinc for the treatment of diarrhea since 2004. Evidence suggests that zinc administration (20 mg per day until the diarrhea ceases) reduces the duration, severity and hospital admissions associated with diarrheal episodes in children in developing countries. Zinc for the treatment of diarrhea is estimated to decrease diarrhea mortality by 23% as well as significantly reducing the severity and duration of diarrheal episodes in children as well as a reduction in treatment failure or deaths in diarrheal episodes of longer duration (>14 days).

Diet

Current WHO guidelines for the management and treatment of diarrhea in children strongly recommend continued feeding alongside appropriate management. An extensive review on

Table 2 Differential diagnosis for prolonged/acute and persistent diarrhea

Prolonged/acute diarrhea (1–13d)	
Infectious	Viral (rotavirus, Norwalk, enterovirus, calcivirus); bacterial (*E. coli*, Shigella, Salmonella, Yersinia, Campylobacter, *C. difficile*, *V. cholera*, Aeromonas); Protozoa (Giardia, cryptosporidium, *E. histolytica*)
Allergy	Short exposure to allergen, challenge to known allergen
Toxic	Drug side effects, acute abdomen with diarrhea as presenting symptom, intussusception
Extra-intestinal infections	Respiratory, urinary, sepsis
Persistent diarrhea (>=14 days)	
Infantile protracted diarrhea with villous atrophy	Post infectious, food allergy, malnutrition congenital histological dysmorphism (e.g., tufting enteropathy, microvillus inclusion disease)
Infectious	Bacterial, parasitic
Inborn errors of metabolism	Familial chloride diarrhea, sodium–hydrogen exchange defect, abetalipoproteinemia and hypobetalipoproteinemia, Galactosemia, Tyrosinemia, Acrodermatitis enteropathica
Carbohydrate malabsorption	Congenital (lactase deficiency, glucose–galactose malabsorption, sucrase–isomaltase deficiency, fructose malabsorption) secondary (acquired mono and disaccharidase deficiencies)
GI organ pathology	Small intestine (celiac disease, tropical sprue, Whipple's disease, intestinal lymphangiectasia, eosinophilic gastroenteropathy, enterokinase deficiency); short bowel syndrome; ischemia; lymphoma; motility disorders (for e.g., small bowel overgrowth); pancreas (all conditions with exocrine insufficiency for e.g., cystic fibrosis); liver (all conditions leading to cholestasis, bile salt deficiency)
Immune defects	Isolated IgA deficiency, SCIDS, AIDS, autoimmune enteropathy
IBD	Crohn's disease, ulcerative colitis
Fecal impaction with overflow	Hirschsprung's disease, anorectal malfunction, functional constipation
Dietary	Overfeeding, nondigestible carbohydrates
Others	Toddler's diarrhea, Munchausen by proxy, factitious diarrhea, non-GI causes (tumors – APUDoma, ganglioneuroma, neuroblastoma)

Source: Robert Wyllie, J.S.H. (2011). *Pediatric gastrointestinal and liver disease* (4th ed). Diarrhea.

Table 3 Comparison of viral, bacterial, and parasitic etiologic agents of diarrhea in developing countries and the United States

Etiologic agent	*Developing country*	*United States*
Viral		
Rotavirus	Important	Very important
Noroviruses	Probably important	Important
Enteric adenoviruses	Minor	Probably important
Bacteria		
Enterotoxigenic *E. coli*	Very important	Minor
Campylobacter	Important	Important
Shigella	Important	Minor
Salmonella	Variable	Important
Enterohemorrhagic *E. coli*	Minor	Important
Parasites		
Cryptosporidium	Important	Minor
Giardia	Minor	Minor
Strongyloides	Minor	Minor
Entamoeba histolytica	Minor	Minor

Source: Table modified from Lanata, C.F., Fischer-Walker, C.L., Olascoaga, A.C., Torres, C.X., Aryee, M.J., Black, R.E., and Child Health Epidemiology Reference Group of the World Health Organization and UNICEF (2013). Global causes of diarrheal disease mortality in children <5 years of age: a systematic review. *PLoS One* **8**(9), e72788.

feeding strategies and food-based interventions in children younger than 5 years with diarrhea in low- and middle-income countries showed that although illness duration was shorter and risk of treatment failure 47% lower in children with acute diarrhea who consumed lactose-free diet, there was no effect of lactose avoidance on stool output or weight gain. WHO recommends the following:

- An age-appropriate diet regardless of the fluid used for ORT (oral rehydration therapy)/maintenance
- More frequent breastfeeding or bottle feeding – special formulas or dilutions are unnecessary
- Age-appropriate fluid resuscitation
- Frequent, small meals throughout the day (six meals/day)
- Energy and micronutrient-rich foods (grains, meats, fruits, and vegetables)
- Increasing energy intake as tolerated following the diarrheal episode
- Avoid: canned fruit juices and other high-sugar food and drinks – these are hyperosmolar and can aggravate diarrhea.

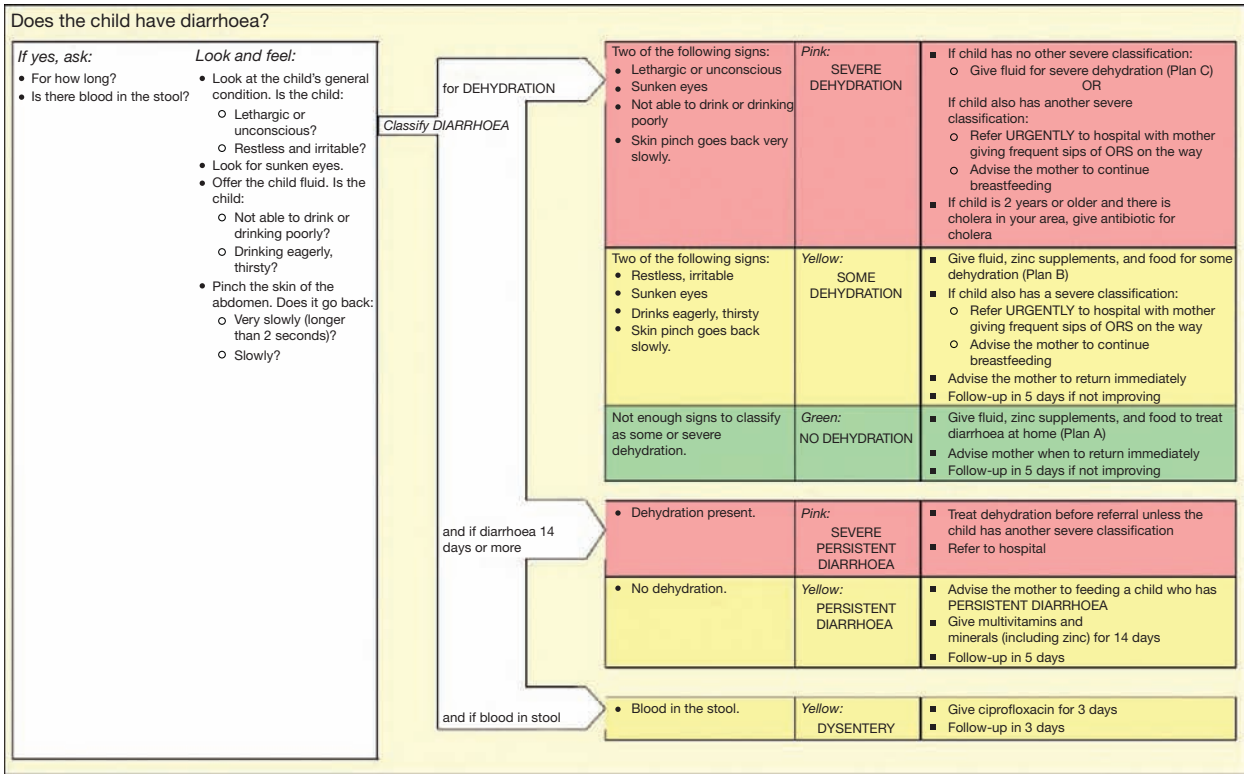

Figure 6 IMCI flow chart for diarrhea in children under 5 years of age. Reproduced from WHO (2014). *Maternal, newborn, child and adolescent health*. IMCI Chart Booklet. Page 3/76.

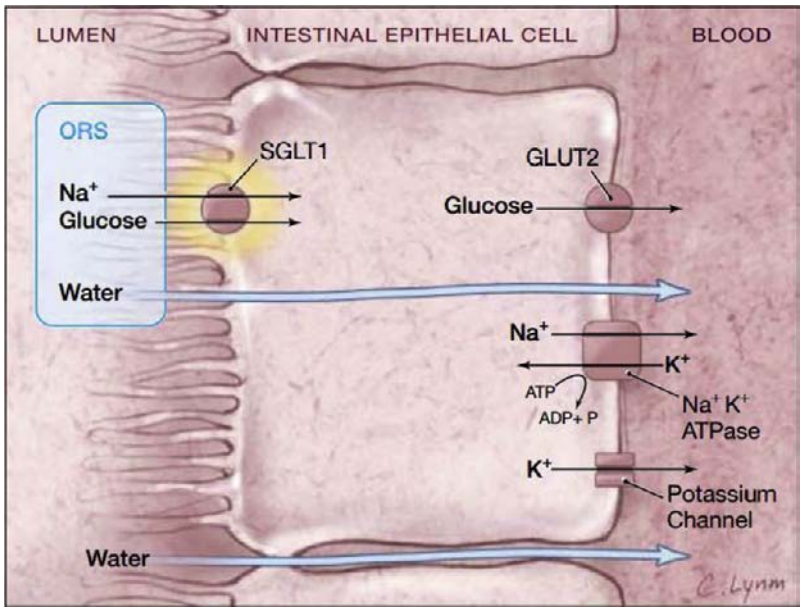

Figure 7 Coupled transport of sodium and glucose in intestinal epithelial cells. Reproduced from Duggan, C., Fontaine, O., Pierce, N.F., et al. (2004). Scientific rationale for a change in the composition of oral rehydration solution. *JAMA* **291**(21), 2628–2631.

Antibiotics for Cholera, Shigella, and Cryptosporidium

Antibiotics should not be used for relatively mild, self-limited infectious diarrhea to minimize development of drug resistance. When the diarrhea is hemorrhagic, seriously dehydrating, associated with serious systemic signs and symptoms, or of longer than 5 days' duration without improvement, antibiotic use is appropriate. A review by Traa and colleagues assessed the effectiveness of WHO-recommended antibiotics – ciprofloxacin, ceftriaxone, and pivmecillinam – for the treatment of dysentery, and concluded that antibiotics can be expected to

Table 4 Composition of standard and reduced-osmolarity WHO ORS

	Standard WHO (1975)	*Reduced-osmolarity WHO (2002)*
Glucose (mmol l^{-1})	111	75
Sodium (mequiv. l^{-1})	90	75
Potassium (mequiv. l^{-1})	20	20
Chloride (mequiv. l^{-1})	80	65
Citrate (mmol l^{-1})	10	10
Osmolarity (mOsml l^{-1})	311	245

Abbreviations: ORS, oral rehydration solution; WHO, World Health Organization.
Source: Duggan, C., Fontaine, O., Pierce, N.F. et al. (2004). Scientific rationale for a change in the composition of oral rehydration solution. *JAMA* **291**(21), 2628–2631.

decrease diarrhea mortality attributable to dysentery by more than 99%. There is evidence to recommend antibiotic use for reduction of morbidity and mortality due to cholera, Shigella spp. and cryptosporidium – Das et al. showed that in the developing country setting antibiotic management of cholera resulted in a 63% reduction in rates of clinical failure and a 75% reduction in rates of bacteriological failure. They also showed that antibiotic management of Shigella resulted in an 82% reduction in rates of clinical failure and a 96% reduction in rates of bacteriological failure. Antibiotics for treatment of cryptosporidiosis reduced mortality by 76%, rates of clinical failure by 52%, and rates of parasitological failure by 38%. The empiric antibiotic regimen should be determined by host factors, severity of illness, local resistance patterns, and history of travel to an area with increased resistance.

Probiotics

Probiotics are defined by the joint Food and Agriculture Organization/WHO Working Group as 'live microorganisms that when administered in adequate amount concern a health benefit on the host.' Recent Cochrane systematic reviews on the use of probiotics as an adjunct therapy to ORS have shown that probiotics shorten the duration of diarrhea and reduce stool frequency but the observation remains inconclusive due to few trials with small numbers of participants. Most commonly used probiotics in trials include Bifidobacterium, two genera of lactic acid bacteria (Lactobacillus and Streptococcus) and the yeast Saccharomyces. There is insufficient evidence to recommend their widespread use at this time.

Antiemetics

Vomiting associated with diarrhea causes significant additional physical discomfort and is of concern for both parents and physicians. Additionally, vomiting can limit the success of ORS and can result in increased use of intravenous (IV) rehydration, need for prolonged emergency department stays and hospitalizations. A recent systematic review evaluated seven RCTs which used ondansetron, metoclopramide, dimenhydrinate, and dexamethasone in children aged 0–12 years. They found that use of antiemetics significantly reduced the incidence of vomiting and hospitalization by 54% and reduced IV fluid requirements by 60%. A 2011 Cochrane Review on the use of antiemetics for reducing vomiting related to acute gastroenteritis in children and adolescents concluded that oral ondansetron increased the proportion of patients who had ceased vomiting and reduced the number needing IV rehydration and immediate hospital admission. IV ondansetron and metoclopramide reduced the number of episodes of vomiting and hospital admission, and dimenhydrinate as a suppository reduced the duration of vomiting. Antiemetics are effective for the management of gastroenteritis in children and have the potential to decrease morbidity and mortality burden due to diarrhea.

Breast Feeding

Breast milk provides various immunological, psychological, social, economic, and environmental benefits, and is therefore recommended as the best feeding option for newborn babies and young infants in developing countries, including HIV-infected populations. Lamberti and colleagues reviewed 18 studies from developing countries reporting the effect of breastfeeding on diarrhea morbidity and mortality. The investigators estimated that not breastfeeding was associated with a 165% increase in diarrhea incidence and mortality in 0–23-month-olds.

Vaccines for Rotavirus

Rotavirus is the most common cause of severe dehydrating diarrhea in infants worldwide. Rotavirus vaccines represent an important preventive approach to reducing the burden of diarrheal disease due to rotavirus. Since 2006 two rotavirus vaccines are currently licensed for vaccinating infants in North and South American, European, and Eastern Mediterranean countries: RotaTeq® (Merck) and Rotarix® (GlaxoSmithKline). RotaTeq®/RV5 – is a live, oral vaccine that contains five reassortant rotaviruses developed from human and bovine parent rotavirus strains and is given orally in three doses at ages 2, 4, and 6 months. Rotarix®/RV1 is a live, oral vaccine licensed in 2008 for use in the United States that contains a human rotavirus strain, this is given orally in two doses at ages 2 and 4 months. This first dose of either vaccine is most effective if it is given before a child is 15 weeks of age and children should receive all doses of rotavirus vaccine before they turn 8 months old.

In the global health setting, there is evidence suggesting that rotavirus vaccine efficacy may vary by setting due to regional differences in circulating rotavirus vaccine strains, and reduced efficacy of oral vaccines in countries with high burden of malnutrition and enteric infections. A meta-analyses by Munos et al. assessed the effectiveness of two currently marketed vaccines the pentavalent human-bovine reassortant rotavirus vaccine and monovalent attenuated human rotavirus vaccine in Phase III trials in European and Latin American sites for both vaccines, as well as the United States (including Navajo and Apache populations) and Taiwanese sites for the pentavalent vaccine. The effectiveness studies of the monovalent and pentavalent vaccines were conducted in northern Australia and Nicaragua, respectively. The authors estimated that these rotavirus vaccines could prevent

74% of rotavirus deaths and 47–57% of rotavirus hospitalizations. Data published from a randomized, placebo-controlled, multicenter trial in South Africa and Malawi evaluating the efficacy of a live, oral rotavirus vaccine in preventing severe rotavirus gastroenteritis showed that the rotavirus vaccine significantly reduced the incidence of severe rotavirus gastroenteritis among African infants during the first year of life. The ROTAVAC rotavirus vaccine study group recently published results from a Phase III clinical trial using ROTAVAC which is a new rotavirus vaccine that consists of a strain of the virus that was isolated, manufactured, and tested in India. Infants aged 6–7 weeks received three doses of oral human-bovine natural reassortant vaccine (116E) with efficacy results from the study showing the vaccine to be effective and well tolerated in Indian infants.

Vaccines for Cholera

Although case management with oral rehydration therapy has substantially improved case-fatality rates for cholera, the infection can still kill rapidly, especially in outbreak settings. Old-generation injectable cholera vaccines, abandoned since the 1970s because of their restricted effectiveness and local side effects. However, studies have shown vaccines to be effective in reducing risk of cholera infection in children younger than 5 years by 52%, increase in vibriocidal antibodies by 124% and a relative risk of one or more adverse events of 1.4. Such evidence for the effectiveness of oral cholera vaccines makes them good candidates for cholera control in endemic areas.

Improved Water Provision, Use, Sanitation, and Hygiene Promotion

Maintaining water quality (protection or treatment of water at source or point of use) is more effective than improving water supply (improved source of water or improved distribution, or both). Cairncross and colleagues estimated the effect of water, sanitation, and hygiene strategies and estimated risk reductions for diarrhea of 48% for hand washing with soap, 17% with improved water quality, and 36% with proper excreta disposal.

Community-Based Approaches

Although evidence confirms the efficacy and effectiveness of many interventions, many interventions are not accessible to people in need; hence, the focus on delivery strategies has increased. One method of community-based case management is to provide these amenities through community health workers with home visitation and community-based sessions for education and promotion of care seeking. These approaches have been assessed and concluded that that community-based interventions are associated with a 160% significant increase in the use of ORS and an 80% increase in the use of zinc in diarrhea. Furthermore, findings showed a 9% increase in care seeking for diarrhea and noted a 75% significant decline in inappropriate use of antibiotics for diarrhea.

Global Action Plan for Pneumonia and Diarrhea

The 2013 Lancet Childhood Pneumonia and Diarrhea series reviewed existing preventive and therapeutic interventions that could have a role in reducing the morbidity and mortality burden in children younger than 5 years due to diarrhea and pneumonia. The interventions with the maximum effect include breastfeeding, ORS, and community case management. The series concluded that despite a large global burden childhood diarrhea and pneumonia deaths are avoidable and 15 interventions delivered at scale can prevent most of these deaths (Figures 8 and 9). Estimates modeled with the Lives Saved Tool showed that if the interventions are scaled up by 80% in the 75 countdown countries, 95% of diarrheal, and 67% of pneumonia deaths in children younger than 5 years can be prevented by 2025. Scaling up of diarrheal and pneumonia interventions would cost US$ 6.715 billion, only $2.9 billion more than present levels of spending. Assessment is needed of the cost-effectiveness of these interventions in national health delivery frameworks. We have a clear idea of effective interventions for both diarrhea and pneumonia and with community health-worker programs increasingly being employed to reach underserved populations, real opportunities exist to scale-up community advocacy/education programs, early case detection and management strategies.

The series also included summary recommendations after a series of consultations with several hundred front line public health practitioners in target countries were held to identify key barriers to scaling up of evidence-based interventions to reduce pneumonia and diarrhea mortality. Critical barriers included: absence of national coordination within ministries and other stakeholders to deliver interventions, insufficient financial resources, inadequate training and support for health workers, poor systems for monitoring and assessment of key programmatic indicators and sporadic availability of key commodities. The final list of recommendations included: (1) Improve coordination between various groups working on preventive and treatment interventions to control pneumonia and diarrhea. (2) Substantially increase resources for child survival programs, with an emphasis on pneumonia and diarrhea control efforts. (3) Enhance efforts to attract, train, and retain a competent work force of caregivers. (4) Invest in better systems for harmonization of the collection of essential programmatic indicators, and ensure that this information is shared throughout the system. (5) Strengthen supply systems that deliver essential commodities. In summary, the cost to achieve the end of preventable deaths from pneumonia and diarrhea by 2025 is estimated to be around US$ 6.715 billion. Despite large reductions in child mortality between 2000 and 2010 (both in all-cause mortality, and specifically diarrhea and pneumonia associated), these diseases remain causes for preventable deaths. The achievement of the fourth Millennium Development Goal and the longer-term target of reduction of child mortality to 20 deaths or fewer per 1000 live births in all countries by 2035 requires significant decreases in mortality from both diarrhea and

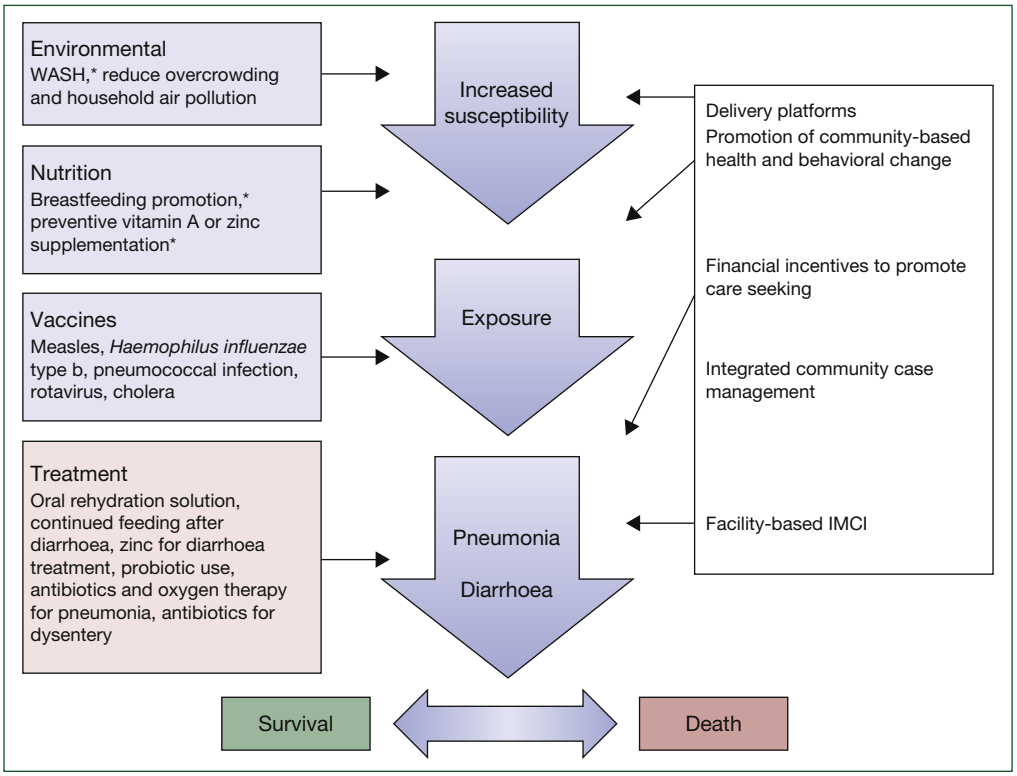

Figure 8 Conceptual framework of the effect of interventions for diarrhea and pneumonia – WASH, water, sanitation, and hygiene; IMCI, Integrated Management of Childhood Illness. Asterisk (*), interventions common to both diarrhoea and pneumonia. Reproduced from Bhutta, Z.A., Das, J.K., Walker, N., et al. (2013). Interventions to address deaths from childhood pneumonia and diarrhoea equitably: what works and at what cost? *Lancet* **381** (9875), 1417–1429.

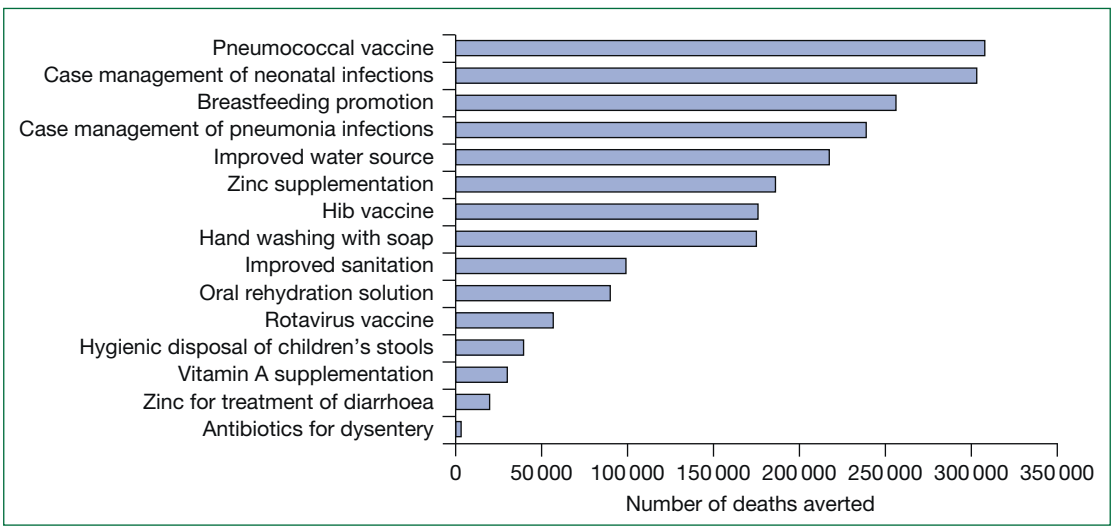

Figure 9 Sequential effect of 15 individual interventions on deaths due to diarrhea and pneumonia Haemophilus influenzae type b – Hib. Reproduced from Bhutta, Z.A., Das, J.K., Walker, N., et al. (2013). Interventions to address deaths from childhood pneumonia and diarrhoea equitably: what works and at what cost? *Lancet* **381**(9875), 1417–1429.

pneumonia. Our knowledge on diarrhea has drastically evolved over the past five decades and the present challenge is to implement these evidence-based strategies for reducing morbidity and mortality due to diarrhea.

See also: *Clostridium botulinum*; *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; *Clostridium*: Occurrence and Detection of *Clostridium perfringens*; Colon: Structure and Function; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; *Escherichia coli* and Other *Enterobacteriaceae*: Occurrence and Detection; Malnutrition: Concept, Classification and Magnitude; Malnutrition: Prevention and Management; Nutrition and Infection; Probiotics; *Salmonella*: Detection; *Salmonella*: Properties and Occurrence; *Salmonella*: Salmonellosis; Sanitization; *Staphylococcus*: Food Poisoning; World Health Organization; Zinc: Physiology and Health Effects; Zinc: Properties and Determination.

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Dietary Exposure Assessment

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Introduction

Food contains, naturally, intentionally, or unintentionally, a wide range of substances that may constitute a health hazard. In this context, risk assessment provides the scientific foundation upon which the risk analysis process is built. Risk assessments are performed in a four-step process: hazard identification, hazard characterization, exposure assessment, and risk characterization. The first stage of a risk assessment, hazard identification, is primarily a question of identifying the effects that are considered as adverse, irrespective of the dose needed or the specific mechanism involved to elicit this effect. The next step, hazard characterization, is centered on the quantification of these effects, whereas dietary exposure assessment is the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, or physical agents via food. Risk characterization is the final stage of risk assessment that integrates information from exposure assessment and hazard characterization into advice suitable for use in decision-making.

Dietary exposure assessments are often performed according to the fit-for-purpose principle with the consequence that there is little harmonization across disciplines with each having their own agreed international guidelines. In order to calculate reliable estimates of the amounts ingested through the diet of a specific chemical substance, three elements have to be taken into account: (1) levels and fate of the chemical in food, (2) food consumption patterns, and (3) integration of these elements to determine exposure. In all three areas, the limitations of the approaches currently used lead to uncertainties that can cause either an over- or underestimation of real intakes and thus of risk.

Presence and Levels of Chemicals in Food

A central aspect in the assessment of exposure is the concentration of the hazardous substance in food as consumed. Factors influencing the variability of the chemical concentration levels in ready-to-eat food products are of environmental origin or related to agricultural and storage practices, food processing, and cooking habits. Quantitative information on the level of a chemical in food can be obtained through analytic determination. Inaccurate measurements can lead to results differing significantly from the real concentration of the chemical in food when the amount is determined analytically. Above all, food sampling procedures for subsequent analysis can critically determine how close the measured value is to the real value. Aspects such as climate, ripeness, and soil conditions are likely to influence the absence or presence of an environmental or agricultural contaminant chemical, and this should be considered when food products are sampled for analysis and methods are chosen. For example, the presence of mycotoxin or fungicide residues in crops and derived foods is related to climatic differences and year-to-year variation. A further source of uncertainty is therefore linked to the possible effects of food processing, like washing, peeling, or cooking, on the chemical of interest, due to both industrial and household preparations.

Most of the analytic determinations of a chemical in a food that are used in dietary exposure assessments are carried out within monitoring and surveillance programs. In these cases, the concentration of a defined chemical in a food is commonly assessed by means of random stratified sampling plans or targeted sampling. The first tries to obtain a representative picture of chemical levels present in food, whereas the second is aimed at sampling those products expected to contain higher levels in a cost-effective way. Targeted data are often collected for enforcement purposes in response to specific problems. They should be used with caution in dietary exposure assessments, as they may not be representative of all foods available for sale. However, clearly representative data are often not available, and targeted data have to be used as an alternative resulting in more conservative estimates of exposure. A major limitation of using monitoring data is that often, not all commodities entering the food chain are monitored. This information might therefore not be sufficient to estimate exposure from all possible dietary sources.

After representative samples are taken, the accuracy, specificity, and sensitivity of the analytic methods used are also important. Generally, analytic methods used for screening purposes (e.g., for enforcement of maximum residue levels (MRLs) or maximum levels) tend to be less precise and are not suitable to detect residues at low levels. This is to a large extent due to the fact that the objective of analytic methods used for screening purposes is to separate the compliant from the noncompliant results. For accurate dietary exposure assessments, the limits of detection (LOD) and quantification (LOQ) should be as low as technically possible. Different recommendations exist for the handling of nondetected or nonquantified values when assessing exposure. In the substitution method, the most commonly used approach, three different scenarios are considered: the lower bound obtained by assigning a value of zero to all the samples reported as less than the LOD or LOQ, the middle bound by assigning half of the LOD or LOQ, and the upper bound by assigning the LOD or LOQ as the sample result. Other factors influencing the final result are the accuracy of the method, laboratory-to-laboratory variation, repeatability, and recovery.

A complementary tool to the monitoring and surveillance programs relies on the total diet studies (TDS), which are designed to collect representative information on the chemical occurrence in food as consumed by the population. In the framework of the TDS, both sampling frame and analytic techniques and performances are adapted in order to fit for the purpose of dietary exposure assessment.

Besides these two main sources of data, chemical concentration data can also originate from experimental trials,

legislated maximum permitted levels (MPLs), MRLs, and usage levels as reported by manufacturers. In the framework of pre-regulatory exposure assessment (e.g., pesticides), the primary sources of residue data in food are the supervised trials. These data must be submitted by the applicants in support of the registration of a pesticide. The trials are usually performed by a manufacturer and simulate a maximum registered use scenario. The trials are designed to determine the maximum residue concentration that may be present in food or feed at the earliest point at which these food commodities could enter into commerce and are used to establish legally enforceable residue limits.

When evaluating the presence and levels of chemicals in food, it is valuable to distinguish between substances intentionally added, like additives, flavorings, or enzymes, from others, such as contaminants. In the case of chemical substances intentionally added to food at various processing steps, the amounts that may be added are usually regulated by MPLs or MRLs. They are defined in the food legislation for each food category to ensure product safety. MPLs or MRLs can be used for a preliminary assessment of exposure in order to identify those substances for which acceptable or tolerable intake limits may be exceeded in a worst-case scenario. Data on the use patterns of chemical substances intentionally added to food are difficult to obtain. Information on the concentrations in brand foods is considered to be commercially sensitive and is therefore not widely available. Additives, like all other ingredients, must be included on the product label in descending order by weight at the time of manufacture. This may provide information on the simple presence and in some cases on the amounts used, but the collection of such data from labels requires major efforts.

Occurrence levels can also be obtained from the producers. These levels range from the MPL to nothing at all. Manufacturers use the chemicals, for example, an antioxidant, at the lowest effective levels in a given formulation; unnecessarily high addition is usually avoided for cost reasons and prohibited by the requirement to work according to 'good manufacturing practice.' Proposed usage levels provided by the industry are often the only available information in pre-marketing situations.

Flavoring substances do not follow the same regulation as food additives. Individual flavor components are usually not specifically indicated on the label beyond the statement that flavorings are used and maximum levels for these substances were not established. Another category of substances that are intentionally added during food processing is that of food enzymes, which are primarily used as processing aids.

Food Consumption Patterns

When assessing exposure, three different sources of information regarding food consumption can be used: food supply data, data from household consumption surveys and from individual dietary surveys, and data from duplicate diet and biomarker studies from other sources of exposure data, whereby these measures reflect both the consumption of food and the concentration of the chemical in these foods.

Disappearance data provide gross annual estimates of the national availability of food commodities. Food supply data are calculated in food balance sheets (FBSs), which are accounts, on a national level, of the annual production of food, changes in stocks, imports and exports, agricultural use, and industrial use. The result is an estimate of the average supply per head of the population, irrespective of, for instance, age or gender. Food supply data refer to food availability, which gives only a crude (usually overestimated) impression of the potential average consumption. Food losses prior to consumption, due to processing, spoilage, trimming, and waste, may not be adequately accounted for. As a result of their long history, FBSs are especially used for assessing trends over time. International FBSs are prepared and published by Eurostat and the FAO. They are compiled in the so-called GEMS/Food cluster diets, which are used by joint FAO/WHO expert meetings while performing international risk assessment.

Food available at the household level may be estimated from budget surveys and consumption surveys. The first type of survey gives information on the purchases of food in terms of expenditure and is used for economic policy. In household consumption surveys, the amounts of food and drinks brought into the household are also recorded, but not usually the meals consumed out of home. In general, household surveys do not provide information on how food is handled within the household or on actual consumption by its members. Data on the quantity of and/or expenditure on food maybe collected by record keeping, by interviews, or by both methods.

The data from dietary surveys among individuals more closely reflect actual consumption. To collect dietary intake data at an individual level, several methods can be used. The methods can be divided into two categories: recall and record methods. Recall methods reflect past consumption, varying from intake over the previous day (24 h recall) to usual food intake (dietary history or food frequency). Record methods collect information on current intake over one or more days.

The food frequency method consists of a questionnaire containing a given list of foods, for which subjects are asked to estimate the habitual frequency of consumption during a specified period of time. The 24 h dietary recall is the most common recall method used. During the interview, an individual recalls actual food intake during the preceding day. Food quantities are usually assessed by the use of household measures, food models, or picture books. The interviewer obtains information during a face-to-face interview or by telephone. In the weighed record technique, the subject is taught to weigh and record all foods and beverages immediately before eating and to weigh and describe any leftovers. The weighed method differs from the estimated record where subjects do not use a scale but estimate the portion sizes using picture books or household measures. The record is usually done over 3–7 days.

The duplicate diet method differs from the other methods in that the intake estimation does not rely on chemical data from other data sources. The concentration is measured by the chemical analysis of the duplicate diet collected.

The quality of the results of a food chemical exposure assessment will depend largely on the quality of the food consumption data used in the assessment. Collecting food consumption data in surveys and in duplicate diet samples for exposure assessment can alter the diet of study participants, resulting in a bias in intake estimate. Intake may also be altered

if participants change their diet due to fatigue or loss of interest. It is important to highlight that many dietary surveys reported energy intakes consistently lower than would be expected from the estimated basal metabolic expenditure and physical activity. This problem of underreporting of energy intakes is widespread and is one of the major biases in exposure assessments based on dietary surveys.

Another important issue is the food coding system. In dietary assessment, food products have to be identified and thereafter categorized in line with the purposes of the study. For this, national and international systems exist, but with regard to exposure assessment, unconventional categorization systems are often needed. An important limitation is that analyses of residues in cereals, fruits, and vegetables are mainly conducted in raw agricultural commodities (RACs) including peel and nonedible parts. Either processed or prepared foods are not monitored or the number of samples is very small. This is due to the fact that in the legislation, the limits of residues are mainly set for RACs, and the purpose of monitoring is usually to assess compliance, rather than exposure.

In order to estimate acute exposure, it is sufficient to record food intake for only one day, including details of the foods consumed per meal and details on the time of each eating occasion. However, food consumption data for several days per subject are required in order to assess chronic exposure. Information on food consumption for a few days may not actually represent usual intake. However, statistical techniques can be used to estimate usual intake, provided that two or more repeated and independent days are available, although consecutive days cannot be considered as independent days.

Methods Used in Dietary Exposure Assessment

Up to 15 years ago, assessment of exposure was mainly based on the estimates obtained by combining MPLs with mean food consumption data for all chemicals. However, mean consumption is not informative in relation to subjects consuming high levels of one or more food categories ('high consumers') who are more likely to ingest hazardous chemicals in excessive quantities. The methodologies now adopted to assess exposure from diet take into special consideration nonaverage individuals and in particular those who consume relatively large quantities of foods containing higher concentrations of substances that may potentially lead to a health risk. The definition of high-level consumers varies, it is normally the 90th, 95th, 97.5th, or 99th percentile of the distribution of individual intake values. A high percentile, rather than the maximum value, is usually chosen because maximum intakes are unlikely to be maintained over long periods of time and hence are not representative of high-level intakes in relation to chronic exposure. The reliability of estimates depends on the sample size available and should be considered when choosing the high percentile value to retain for the exposure assessment. It is also important to highlight that the definition of high-level consumers is a major issue since, in practice, it defines the proportion of the population that would have to exceed the 'at-risk dose' before risk mitigation measures are to be considered to reduce exposure levels. This is therefore both a scientific and a political/ethical question.

Ideally, dietary exposure to chemical substances should be assessed by combining data on the concentration in all food products with data on their consumption. Unfortunately, with the exception of duplicate diet studies, exposure assessments are not performed on the basis of consumption, occurrence, and concentration data related to the same individuals within a population. Therefore, assessments of exposure to dietary components will usually require some degree of modeling to attempt to create a representation of the real-life exposure situation. There are a number of different models for combining or integrating the consumption data with the residue/concentration data. The method chosen usually depends on a number of factors, including the purpose of the assessment (target chemical, population group, and degree of accuracy required) and the availability of data. In general, it is considered neither cost-effective nor necessary to collect detailed food consumption and chemical concentration data for every hazardous substance. A stepwise procedure is commonly used to minimize estimation costs and focus resources on the most important issues. The stepwise approach to the dietary exposure assessment of food chemicals is such that as the accuracy of dietary exposure assessments increases, the cost of collecting adequate data and resources needed to undertake the assessments also increases. The aim of the stepwise procedure is to target dietary exposure estimates to chemicals that might be of health concern for 'average consumers' or individuals belonging to certain at-risk groups. If the estimated exposure to a given pesticide residue, food additive, veterinary drug residue, and contaminant exceeds its safety limit (e.g., acceptable daily intake (ADI)), a more accurate method of dietary exposure assessment should be applied. Exposure is therefore first assessed by using methods following a deterministic approach based on conservative assumptions. Especially at this stage, the methodologies adopted to assess exposure from the diet take into special consideration 'high consumers.' However, for high consumers in particular, food consumption data are not commonly available at the individual level. Consequently, highly conservative methods are used. It is important to remind that these methods are not suitable for predicting actual exposure since they are designed to cover the worst-case scenario. Nevertheless, they are useful and inexpensive screening tools for identifying those substances for which safety limits of intake may be exceeded.

The so-called budget method is the most known screening method; it is above all used in the field of food additives. This method uses theoretical food consumption data and MPLs for additives. The budget method is based on the physiological upper limit to the amount of food and drinks that can be consumed each day and thus of food additives. For beverages, this is assumed to be 100 ml, and for solids, 25 g kg^{-1} b.w. per day. A further assumption is that only a certain proportion of the diet is likely to contain food additives. The default proportion of beverages and solid food that could contain the additive is typically assumed to be 25% but varies depending on the additive in question and the population under consideration. A model diet is, for example, used for the assessment of exposure to feed additives and veterinary drug residues. It assumes the daily consumption of 300 g of muscle (from mammals, birds, and/or fish), 100 g of liver (from mammals and/or birds), 50 or 90 g of fat (from mammals or birds, respectively), 50 or 10 g of kidney (from mammals or birds, respectively),

1500 g of milk, and 100 g of eggs. Screening methods are also used for assessing acute exposure; an example is the international estimated short-term intake, which is used in the case of pesticide residues. This method requires data on the consumption of large portions (usually the 97.5th percentile from the single-day consumption data among consumers) together with typical unit weights of the edible part of the commodities and the body weights of the population associated with the food consumption data. The baseline assumption is that a consumer may eat a large portion (high-level consumer at the top end of the distribution curve among consumers only) of a food that may contain a high residue level, as derived from supervised field trials. The acute assessment is conducted for each commodity separately as it is considered unlikely that a consumer will eat two or more different commodities with a large portion size within a short period of time and that those commodities have the highest level of the same pesticide.

When screening methods cannot rule out a safety concern, more refined methods should be applied. With access to food consumption survey information, actual mean and high percentile consumption can be used as inputs into an exposure model. Such calculations can be further refined by splitting the population into age groups and by using the average body weight within each group to estimate the mean and high percentile consumption per kg body weight. A further refinement is possible when food consumption is available at the individual level. In such cases, chemical concentrations and food consumption can be directly matched for each subject and the respective food or food category and a distribution of total exposure estimates produced, where any percentile of interest can be calculated. Dietary exposure estimates are in the majority of cases produced by considering the consumption of all foods and all subjects involved in the dietary surveys, but estimates for 'consumers only' are also calculated under specific circumstances.

The method applied in any dietary exposure assessment should be clearly stated and reproducible. Information about the model and data sources used, assumptions, limitations, and uncertainties should also be documented. When assessing the exposure, the contribution of variability (heterogeneity) and true uncertainty (lack of knowledge) should be as far as possible distinguished. Although it may be challenging to identify all the uncertainties and to distinguish them from the real variability, each scientific output should describe the types of uncertainties encountered and considered during the different risk assessment steps and indicate their relative importance and influence on the assessment outcome.

Over the past years, to get a more realistic view of exposure to hazardous substances, risk managers are becoming more interested in probabilistic modeling. In contrast, probabilistic approaches are typically more resource-intensive than deterministic approaches, but they permit the characterization of the variability and uncertainty that may exist in such exposure estimates and thus facilitate more meaningful and realistic assessments. The results, however, are only as good as the input data, algorithms, and assumptions. The critical aspects of the food consumption and chemical concentration data used to assess deterministic estimates must therefore be taken into account also when using a probabilistic approach. Moreover, the impact of the assumptions should always be tested carefully and the results should be fully documented. A modeling tool must be structured so that all algorithms and assumptions inherent to the model can be identified and validated. Sensitivity analyses should be used to set priorities for risk reduction measures and to define the main sources of uncertainty in order to plan further studies and improve the exposure estimates.

In addition, more and more concerns stand in the risk resulting from the combined exposure to multiple chemicals. One challenge is to identify which chemical mixtures the population are exposed. Developments in the research field are under way to address this challenge. Once the mixtures of interest are defined, methodologies to assess combined exposure in a risk assessment perspective do not differ greatly from the ones previously described for a single chemical. The main issue, especially when assessing acute exposure, is to address the co-occurrence of the chemicals composing the mixture in foods. Future developments in this direction mainly require adaptation of the occurrence data collection for multiple chemicals in same food samples.

Finally, there is also an increasing interest for the potential use of biomarkers for assessing exposure to food chemicals. It needs to be recognized that this approach takes account of exposure from all sources, including nondietary sources. It is thus important to understand to what extent such other sources exist and are likely to be relevant, especially if attempting to compare the results of such measurements with dietary exposure estimates assessed by means of conservative models. A number of methods for specific individual food chemicals are currently being developed, and it seems that this trend will continue.

See also: Dietary Surveys: National Food Intake; Food Classification and Description; Food Composition Databases; Risk Assessment of Foods and Chemicals in Foods.

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Dietary Fiber: Bran

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Introduction

Anatomically, a cereal grain is a one-seeded fruit, called caryopsis, in which the fruit coat (or pericarp) is fused with the seed coat (cuticle or testa) to form what we refer to as the cereal bran. The outermost layer of the cereal kernel is thus the pericarp or the ripened ovary wall, which lacks cytoplasm and has lignified walls and is dead at harvest ripeness. The pericarp is composed of the outer epidermis, hypodermis, parenchyma, intermediate cells, cross cells, and tube cells. The seed coat (arising from the integuments) and an underneath pigment strand cover the seed completely and control water migrations between the enclosed seed and the pericarp cells. Beneath the seed coat is the aleurone layer, or the outermost layer of the endosperm tissue, which is the only endosperm tissue that will be alive at maturity. Unlike the starchy endosperm cells, the aleurone layer cells have large prominent nuclei that are not crushed during milling. The bran (Figure 1) represents these outer parts of the grains including the pericarp, the testa, and the aleurone layer. These are all fibrous layers containing cellulose, hemicellulose, lignified materials, and a wide range of bioactive compounds. Commercial bran preparations contain variable amounts of the starchy endosperm and germ depending on the variety of cereal and the milling process (Figure 2). The amount of endosperm in the commercial bran will depend on the shape of the kernel and the extraction rate with, for example, rye being more irregular in shape than wheat and, therefore, more difficult to mill to prepare 'pure' bran fractions. The relative distribution of bran, endosperm, and germ in selected cereal kernels is given in Table 1.

Composition and Properties

Brans from different cereals are different in the shape and hardness of their particles, chemical composition, color, flavor, and other sensory properties. The color of the cereal bran may vary from white, yellow, green, blue, black, and purple red depending on the variety and the type and amounts of pigments as well as the inclusion of starchy endosperm. For example, oat bran is lighter in color than rye, wheat, rice, and barley brans. Wheat bran is more yellow in color, barley bran followed by oat bran has more redness, while rye bran is greener in color. All cereal brans have a pleasant flavor with the exception of rye bran, which imparts an unpleasant feeling of grittiness, roughness, and lack of smoothness and creaminess.

Table 2 presents the major and most important constituents of cereal brans. The major chemical component in cereal brans is dietary fiber, which is defined by the American Association of Cereal Chemists as consisting of "the remnants of edible plant cells, polysaccharides, lignin and associated substances resistant to (hydrolysis) digestion by the alimentary enzymes of humans." Dietary fibers in cereal brans include water-insoluble and water-soluble types, where wheat and rye brans contain 1–5% soluble fiber, rice bran contains about 10% soluble fiber, while oats and barley contain about 30–40% soluble fibers. The insoluble dietary fiber is composed of cellulose, hemicelluloses, and small amounts of klason lignin. The hemicellulosic fraction is mainly composed of arabinoxylan (or pentosan) structured as β-(1→4)-xylopyranosyl backbone with α-L-arabinofuranosyl residues linked to the main chain as (1→2) or (1→3) side units. This hemicellulose fraction also contains side units of D-glucuronic acid or oligosaccharide side chains of galactose or xylose, and some arabinofuranosyl side units contain ferulic acid. Because of the complex structure of this fraction, it is often referred to as a heteroxylan. It is believed that the heteroxylan fraction acts as a fill in the spaces between the cellulosic microfibrils and forms a cross-linked structure with other cell wall components, via di- and triferulate bridges, that act as a cement solidifying the cell wall structure and enhancing its insolubility. Klason lignin is a complex three-dimensional hydrophobic polymeric network of aromatic p-hydroxyphenyl, guaiacyl, and syringyl units resistant to chemical and enzymatic degradation. Soluble dietary fiber is composed partly of soluble arabinoxylan, fructan, and mainly highly soluble and viscous β-glucan, which is a linear polysaccharide that consists only of β-D-glucopyranosyl units joined by either (1→3)- or (1→4)-β-D-linkages, hence the name mixed-linkage (1→3), (1→4)-β-D-glucan.

Cereal brans contain considerable amounts of minerals and vitamins and contribute considerably to the recommended daily intake (RDI) of some of these nutrients (Table 3). For example, wheat bran is a good source of the B vitamins, including niacin (19% of RDI/100 g), vitamin B_6 (18% of RDI/100 g), riboflavin and thiamine (10% of RDI/100 g), and folates (B_9) and pantothenic acid (B_5) (6% of RDI/100 g), and it provides small amounts of the lipid-soluble vitamins E and K. Wheat bran is also a rich source of eight important minerals being exceptionally high in manganese (161% of RDI/100 g), and very high in magnesium (43% of RDI/100 g), selenium (31% of RDI/100 g), and phosphorus (28% of RDI/100 g), and still a good source of iron (16% of RDI/100 g), zinc and copper (14% of RDI/100 g), and potassium (9% of RDI/100 g). Rice bran has higher values and oat bran significantly less values compared to wheat bran.

Besides fiber, cereal brans are rich sources of a wide range of bioactive secondary metabolites including phytic acid, tocopherols and tocotrienols, carotenoids, γ-aminobutyric acid, octacosanol, squalene, unsaturated fatty acids, phytosterols, and phenolic compounds. The phenolic components of cereal brans have received special interest because of their antioxidant properties. These phenolic compounds (including free aglycones, soluble conjugates, and polymeric and insoluble-bound forms) differ widely in their structures and water solubility. The free phenolic compounds are mainly present within

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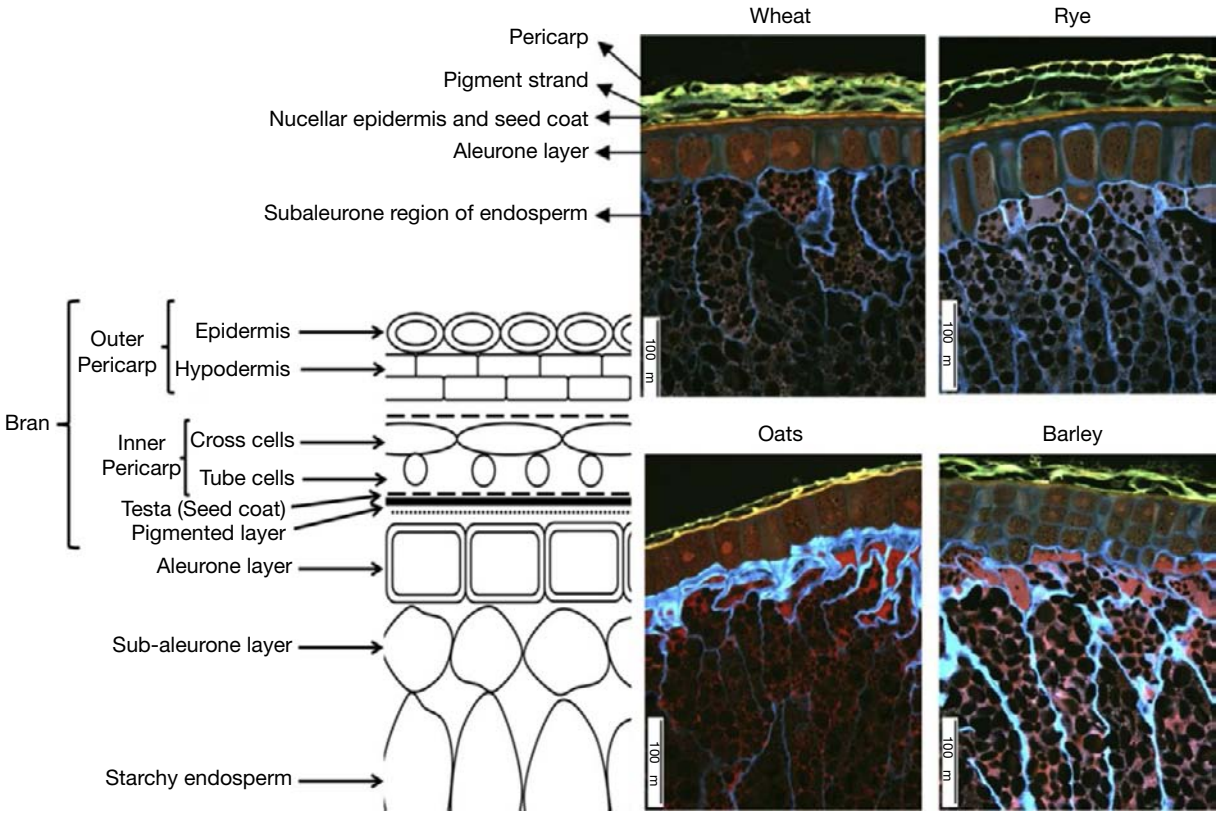

Figure 1 Scanning electron micrographs showing the outer parts of wheat, rye, oats, and barley grains (modified from Dornez, E., Holopainen, U., Cuyvers, S., et al. (2011). Study of grain cell wall structures by microscopic analysis with four different staining techniques. *Journal of Cereal Science* 54, 363–373, with permission from Elsevier) and a schematic demonstration of the component cells of a cereal bran composed of the fruit coat (the pericarp) and the seed coat (testa). Technical bran fractions obtained by milling usually contain a great part of the aleurone layer and some amount of the subaleurone layer and the starchy endosperm depending on the cereal grain and the milling process.

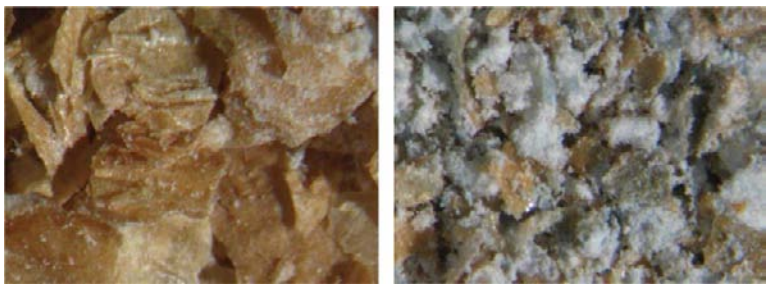

Figure 2 Microstructures of unstained wheat bran (left) and rye bran (right) showing that the rye bran contained higher amount of the starchy endosperm due to the difficulty in separating this anatomical fraction during milling. Reproduced from Kamal-Eldin, A., Laerke, H. N., Bach Knudsen, K. E., et al. (2009). Physical, microscopic and chemical characterisation of industrial rye and wheat brans from the Nordic countries. *Food & Nutrition Research* 53, 1–11.

the plant cell vacuoles, the soluble esters or conjugates are esterified to sugars and other low-molecular-weight components, while the polymeric proanthocyanidins and the insoluble-bound forms are covalently bound to cell wall structural components such as cellulose, hemicellulose, lignin, pectin, and rod-shaped structural proteins. Analysis of 51 Canadian wheat cultivars revealed a variation in the contents of phenolic compounds (expressed in μg gallic acid equivalents/g of bran) with the contents of free phenolic compounds extractable with 80% ethanol ranging 850–1750 and the content of bound phenols ranging 2300–5400 with the total phenolic content ranging from 3400 to 6700 and averaging c.5200. The main phenolic compounds in nonpigmented cereal brans are the phenolic acids ferulic and p-coumaric acids, which are found mainly linked to dietary fiber. An indicative detailed high-performance liquid chromatography analysis is available for rice bran (Table 4), and it shows that pigmented brans of cereals contain much higher levels of phenolic compounds imparted by flavonoids, anthocyanins, and proanthocyanidin pigments. Consequently, brans from

pigmented cereal cultivars have higher antioxidant activities compared with brans from nonpigmented cereals.

A group of important bioactive compounds in cereal brans include sterols, steryl esters, and steryl ferulates (the steryl ferulates in rice bran are referred to as oryzanols), which are particularly interesting because of their cholesterol-lowering properties. Cereal brans are also characterized by their contents of tocotrienols and policosanol waxes (mixture of long chain alcohols) believed to have anticarcinogenic effects. Cereal brans also contain considerable amounts of phytic acid, which is not only known for its antioxidant properties but also considered an antinutritional compound as will be discussed later. Wheat and rye kernels are characterized by their contents of alkylresorcinols, which can be used as specific intake biomarkers for whole-grain and bran-rich diets including these cereals.

Health Effects

Cereal brans provide an invaluable source of soluble and insoluble dietary fiber for human nutrition. It is believed that fiber intake of 30–40 g day^{-1} is desirable and that half of it could come from cereal products and the other half from fruits and vegetables. Soluble and insoluble dietary fibers are appreciated for several health benefits including weight and diabetes management, lowering of blood cholesterol, regulation of blood glucose levels, modulation of the immune system, and anti-inflammatory effects leading to lower risk of heart disease and cancer. Insoluble dietary fibers (cellulose, some arabinoxylans, and lignins) are effective in enhancing the feeling of fullness, increasing stool size, and helping reduce constipation and hemorrhoids. Soluble fibers (some arabinoxylan and β-glucan) form a gel in the intestine and increase the water content of the stool. Cereal dietary fibers also improve microbial fermentation, promote the growth of beneficial bacteria,

Table 1 The relative proportions of bran, endosperm, and germ in selected cereal grains

	Relative percentage (wt%)		
Cereal grain	*Pericarp*	*Endosperm*	*Germ*
Wheat (*Triticum aestivum*)	4–6	91–93	2–4
Rice (*Oryza sativa*)	2–3	95–96	2–3
Maize (*Zea maize*)	5–6	80–85	10–15
Sorghum (*Sorghum bicolor*)	6–7	83–85	9–10
Pearl millet (*Pennisetum glaucutn*)	7–9	75	15–18

Table 3 Minerals and vitamins in the three commercially available cereal brans

Component	*Wheat bran*	*Oat bran*	*Rice bran*
Minerals (mg/100 g FW)			
Calcium	73	58	57
Iron	10.6	5.4	18.5
Magnesium	611	235	781
Phosphorus	1013	734	1677
Potassium	1182	566	1485
Sodium	2	4	5
Zinc	7.3	3.1	6.0
Vitamins (μg/100 g FW)			
Thiamine	523	1170	2753
Riboflavin	577	220	284
Niacin	13 578	934	33 995
Vitamin B_6	1303	165	4070
Folate (vitamin B_9)	79	52	63
Vitamin E	1490	1000	4920
Vitamin K	1.9	3.2	1.9

Source: USDA National Nutrients Database (http://ndb.nal.usda.gov/).

Table 2 Important chemical components in samples of cereal brans (g/100 g DM)

Cereal bran	*Wheat*	*Rye*	*Oats*	*Barley*	*Rice*
Proximate composition					
Crude protein	16.9	16.5	17.4	13.8	13.7
Ash	5.5	4.6	1.7	4.9	7.8
Fat	5.6	4.3	12.3	4.9	22.4
Dietary fiber	40.0	42.1	17.3	26.3	45.2
Starch, soluble carbohydrates, and other minor components	32.0	32.5	46.6	50.1	10.8
Dietary fiber components					
Arabinoxylan	22.4	21.5	6.4	10.3	9.9
β-Glucan	2.2	4.3	9.0	7.8	trace
Cellulose	9.3	5.6	2.2	Unknown	19.0
Klason lignin	3.3	4.1	3.5	Unknown	Unknown
Fructan	2.8	6.6	–	Unknown	Unknown
Antinutritional factors					
Phytic acid	4.2	2.7	2.7	4.0	4.3
Polyphenols	0.32	Unknown	0.05	0.65	0.58
Tannins	0.30	Unknown	0.62	0.34	0.07
Oxalates	0.04	Unknown	0.03	0.03	0.04
Saponins	0.26	Unknown	0.20	0.32	0.30

Source: Kamal-Eldin, A., Laerke, H. N., Bach Knudsen, K. E., et al. (2009). Physical, microscopic and chemical characterisation of industrial rye and wheat brans from the Nordic countries. *Food & Nutrition Research* 53, 1–11; Kaur, S., Sharma, S., and Nagi, H.P.S. (2011). Functional properties and anti-nutritional factors in cereal bran. *Australian Journal of Food and Agro-Industry* 4, 122–131.

Table 4 Classification and contents of phenolic compound in rice bran (mg/100 g DM)

	Pigmented rice bran			*Nonpigmented rice bran*		
Phenolic compound	*Soluble*	*Insoluble*	*Total*	*Soluble*	*Insoluble*	*Total*
Phenolic acids						
Gallic acid	1.18–8.3	1.69–4.06	5.4	0.05–0.48	0.20–2.1	1.35
Protocatechuic acid	1.98–9.7	6.04–24.5	16.2	0.33–1.07	0.04–0.72	0.94
p-Hydroxybenzoic acid	1.25–26.4	4.0–52.5	31.2	0.25–1.39	0.06–6.73	3.71
Vanillic acid	1.09–16.6	7.2	54.8	0.28–1.64	0.02–0.34	0.86
Syringic acid	0.07–5.59	–	–	0.07–0.58	0.08–0.21	0.36
Chlorogenic acid	9.79–15.4	–	–	0.11–1.55	0.21	1.05
Caffeic acid	2.78–25.3	11.1	25.6	0.14–0.79	0.04–0.15	0.45
p-Coumaric acid	1.64–6.2	16.8–144	46.2	0.10–1.57	8.1–74.2	42.2
Sinapic acid	0.65–5.0	15.0–25.9	22.8	0.04–0.60	0.20–3.47	2.06
Ferulic acid	2.95–19.1	15.3–94.8	96.6	1.37–8.69	11.9–225	120.3
Cinnamic acid	–	–	–	0.02	0.07	0.09
Ellagic acid	5.5–12.2	–	–	4.2	–	4.2
Total			298.8			177.5
Flavonoids						
Luteolin	18.4	–	18.4	–	–	–
Apigenin	7.8	–	7.8	0.04	0.32	0.4
Tricin	11.9–193	–	101	0.50–4.86	0.65–2.85	3.3
Quercetin	1.4–6.3	–	3.4			
Isorhamnetin	0.04–0.67	–	0.2			
Total			130.8			3.7
Anthocyanins						
Peonidin-3-*O*-glucoside	11.4–534	–	124	0.04–2.6	–	0.96
Cyanidin-3-*O*-glucoside	9.1–2640	–	700	7.36	–	7.36
Cyanidin-3-*O*-galactoside	2.93–50	–	28.3	–	–	–
Cyanidin-3-*O*-rutinoside	3.17–96.6	–	55.5	6.19	–	6.2
Total			908			14.5
Proanthocyanidin residues						
Catechin	5.8–48.7	–	20.9	0.55–2.35	–	1.4
Epicatechin	46.5	–	46.5	–	–	–
Total			67.4			1.4
Grand total phenolics			1405			197

Source: Goufo, P., and Trindade, H. (2014). Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. *Food Science and Nutrition* 2, 75–104.

and increase the production of short-chain fatty acids in the large intestine. Scientists are still in search for the putative mechanisms behind the beneficial health benefits of cereal fibers. In a metabolomic study, the intake of a high-fiber rye diet was found to increase the concentrations of the ribose metabolites ribitol and ribonic acid and the tryptophan metabolite indole acetic acid, and it was speculated that high-fiber diets increase the synthesis of serotonin and reduced hunger. A high intake of bread of whole-grain rye with added rye bran by males in the early stage of prostate cancer was found to elevate plasma betaine and dimethylglycine and 3-hydroxybutyric acid and acetone, which is explained by a shift in energy metabolism from anabolic to catabolic state. The increase in betaine explains the reduction of homocysteine and the increase of dimethylglycine and methionine.

The intake of high levels of cereal brans exposes individuals to higher intake of some antinutritional factors. The antinutrient phytic acid is known to act as a powerful chelating agent that forms insoluble complexes with divalent cations such as calcium, magnesium, zinc, and iron, thereby reducing their bioavailability. Phenolic compounds also chelate metals and reduce their bioavailability. As shown in Table 4, the content of polyphenols varies widely in wheat, rice, barley, and oat brans. Rice bran contains higher levels of phenolic compounds and oxalates followed by wheat, barley, and oat bran.

Food Applications

Brans are major side streams generated in the milling of cereal grains by the industry. Brans of wheat, oats, and rice are widely commercially available and are well characterized with respect to their composition and properties, while brans of other cereal grains are much less known. Cereal brans can be added to a number of foods to increase their fiber content and to improve their value as functional foods (the US Institute of Medicine, 2002 recommended that the intake of total dietary fiber for children and adults should be above 25 g day^{-1}). Cereal brans can be used as ingredients in a number of healthy food products including breads, cookies, crackers, snack foods, beverages, sauces, dairy products, imitation cheeses, meats and meat analogues, and many other products. One application

area for wheat bran is its incorporation in pasta manufacturing as it was shown that the addition of 25–30% of common wheat bran to a commercial pasta preparation increased its dietary fiber to a level similar to that obtained by using whole-grain durum flour and provided pasta with very good sensory quality and lower losses of dry matter and higher resistance to overcooking compared with whole-grain durum pasta. Arabinoxylans, from wheat and rye brans, may be added as thickening agents in gluten-free bread, and β-glucan may be added to contribute viscosity to, for example, yogurts and other dairy and nondairy products. The sensory and nutritional value of cereal brans may be improved by fermentation, which adds taste and flavor and removes phytic acid and some of the antinutritional factors. An important product from rice bran is rice bran oil, which is high in γ-oryzanols and tocotrienols and well known for its cholesterol-lowering properties.

In this article, the main characteristics and properties of cereal brans have been discussed. Cereal brans can be exploited as ingredients in a number of functional foods aiming for control of energy intake and health benefits against diabetes, coronary heart disease, and some cancers. The possibilities for applications in food products are wide and unlimited and offer the industry new opportunities for the production of innovative and healthy food products having high contents of fiber and associated bioactive compounds.

See also: Barley; Carbohydrate: Digestion, Absorption and Metabolism; Cellulose; Cereals: Dietary Importance; Cereals: Types and Composition; Dietary Fiber: Determination; Oats; Rice: Types and Composition; Wheat: Grain Structure of Wheat and Wheat-based Products.

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http://www.aaccnet.org/initiatives/definitions/Pages/OatBran.aspx – Association of Analytical Cereal Chemists.

http://www.aaccnet.org/initiatives/definitions/Pages/DietaryFiber.aspx – Association of Analytical Cereal Chemists.

Dietary Fiber: Determination

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Introduction

This article presents a brief overview and comparison of commonly utilized methods for quantifying dietary fiber in food. There are, fundamentally, only two types of methods. Both rely on the removal of nonfiber components by a combination of physical, chemical, and enzymatic procedures prior to measurement of dietary fiber. The gravimetric methods measure dietary fiber by weighing the residue, and the gas–liquid chromatographic (GLC) methods measure dietary fiber by hydrolyzing a treated sample with acid to sugar monomers prior to quantification by GLC. Although, on the surface, both types of methods appear similar, there are fundamental practical differences that have come about because of variations in the individual method steps and differences in the choice of reagents and enzymes. These practical differences have significant consequences since they determine the final composition of dietary fiber as measured by each method.

Procedures have been described for isolating and analyzing dietary fiber in specific foods as well as analyzing carbohydrate polymers that are not digested in the small intestine and are not identified as dietary fiber by the current Association of Official Analytical Chemists (AOAC) methods. This article, however, will focus on practical methods that are applicable to the routine analysis of a wide range of foods. Most total dietary fiber (TDF) methods also allow for the quantification of different fiber fractions such as soluble fiber, insoluble fiber, hemicelluloses, cellulose, and lignin. In this article, only the methods that measure TDF are reviewed. This is partly because of space limitations and partly because the individual fiber components have not yet been positively associated with human physiological effects. For example, soluble and insoluble fiber can be readily measured, but these designations have little physiological meaning since the digestive properties and physiological benefits associated with some soluble fiber can be found in some insoluble fibers and the physiological benefits associated with insoluble fibers are also found with some soluble fibers. In addition, some processing techniques can also partly depolymerize insoluble fiber that may then be found in the soluble fiber fraction.

The early work of Van Soest focussed on measuring ruminant fiber (mainly hemicellulose, cellulose, and lignin), but these procedures could not be used to measure TDF because they would have underestimated the TDF content of human foods by missing a significant proportion of the soluble dietary fiber. All the modern methods incorporate steps to measure soluble fiber. This is important since it can represent a significant proportion of the TDF of a food. The chemical complexity and physical variability of the dietary fiber fraction in various foodstuffs as well as the various food-processing techniques have contributed to the difficulties encountered in devising adequate dietary fiber methods. Many of the present-day TDF procedures are based on the pioneering work of Southgate.

Ideal reference standards do not exist for dietary fiber analysis. Purified fiber components and unpurified standardized 'reference samples' are both used as standards of analysis, but the former group rarely represents the complex dietary fiber matrix found in foods and the latter group remains variable in composition, structure, and properties.

Historical Perspectives on Dietary Fiber Values

Several different measures of dietary fiber and dietary fiber fractions have been reported for foods over the last 150 years. It is important to put these values into some sort of context to understand the differences between these measurements and the implications for human dietary fiber research.

The crude fiber method was standardized in the late nineteenth century in Germany. It was initially developed to analyze ruminant feeds and silage. It is a highly empirical method that uses sequential extractions with hot dilute acid and alkali. The crude fiber residue contains no soluble fiber and retains only about 15% of the total hemicelluloses, 50–100% cellulose, and 10–50% lignin. The variability in the method and the variable losses of various dietary fiber fractions mean that crude fiber values are of little value in human nutrition.

Acid detergent fiber (ADF) represented the first method that reproducibly maintained specific dietary fiber fractions. ADF is obtained by refluxing in 1 N mol l^{-1} sulfuric acid containing cetylmethylammonium bromide detergent followed by filtering and washing. This fraction contains mostly cellulose and lignin.

Neutral detergent fiber (NDF) represents the insoluble fraction of TDF. It is obtained by refluxing in a solution of sodium lauryl sulfate followed by filtration and washing. NDF includes most of the cell wall material but excludes the soluble β-glucans and soluble hemicelluloses. It includes the ADF fraction as well as the insoluble hemicelluloses. It thus underestimates TDF because it does not measure soluble dietary fiber.

In some older tables, TDF values are reported as the sum of NDF and pectin (estimated TDF) but, as shown in Figure 1, this value is not always accurate. Figure 1 also presents the relationship between insoluble dietary fiber (essentially NDF but using porcine pancreatic α-amylase to remove starch; see succeeding text) and TDF (as determined by the rapid health protection branch (HPB) method; see succeeding text) determined for the same foods. The insoluble dietary fiber underestimates the TDF value by ~40%, but this is not constant. The differences between TDF and insoluble dietary fiber represent actual differences measured on the same food sample, but the differences between the estimated TDF and the actual TDF

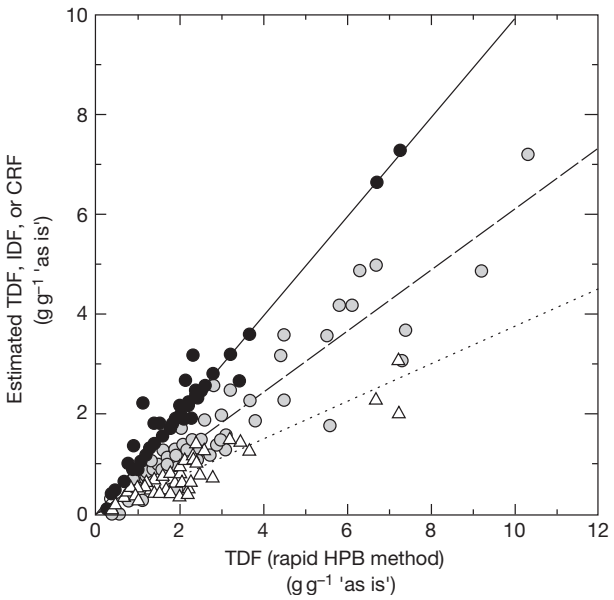

Figure 1 Relationship between crude fiber (CRF; white triangles), insoluble fiber (IDF; gray circles), estimated total dietary fiber (TDF, total of pectin + neutral detergent fiber (NDF); black circles), and TDF values for different types of foods. The TDF and CRF values are from the Canadian Nutrient File (Health Canada 1997; Mongeau et al., 1989). TDF and IDF values were determined by the rapid HPB method (AOAC 992.16, see text). The solid line represents a 1:1 correspondence. The dashed line (IDF) and the dotted line (CRF) are regression lines with the intercept fixed at the origin. Insoluble dietary fiber is essentially NDF but includes a treatment with porcine pancreatic α-amylase. The NDF + pectin values are ultimately from the USDA Nutrient Database (1999).

values were performed at different times with different procedures using different food samples so that procedural variations may account for some of the differences. For example, it is known that some previously determined NDF values were measured after incubation at lower (suboptimal) temperatures that may have given higher and more variable values due to incomplete starch digestion.

In addition to the relationships described earlier, Figure 1 shows the relationship between crude fiber (CRF) and TDF (as determined by the rapid HPB method) for foods from the Canadian Nutrient File. Like the insoluble dietary fiber values, the CRF values significantly underestimate the TDF values; however, in this case, the variability is largely due to the CRF method. Figure 1 shows no consistent relationship between TDF and CRF or insoluble dietary fiber (and thus NDF). Thus, it is not possible to predict TDF values from either CRF or insoluble dietary fiber: a complete TDF measure is required.

Gravimetric Methods

Gravimetric methods use enzyme and/or chemical treatments to remove material that is digestible in the small intestine. Ideally, these treatments should mimic the digestive processes to arrive at a sample that is representative of the material entering the large intestine of humans. Because dietary fiber represents the structural components of plant cell wall material, the weight of the residue is corrected for the presence of noncombustible materials (such as minerals) and corrections may also be applied for components that are incompletely digested by the procedure (such as protein). The remaining weight is considered to be dietary fiber. In order to measure the remaining material gravimetrically, it must be filtered through glass crucibles of defined porosities. It is, therefore, important when preparing the sample to ensure that it is not too finely ground. The use of a Wiley mill avoids these problems and minimizes particle loss during filtration. Many different gravimetric methods have been published with many variations in reagents, temperatures, and incubation times. However, relatively few of these methods have been approved by the AOAC after rigorous testing and interlaboratory verification. Three AOAC-approved methods will be discussed in detail in this section: the rapid HPB method, the Prosky method, and the Lee method. Before presenting these methods, we will first discuss the detergent system.

The Detergent System

When introduced in 1963, the detergent system represented a great improvement over the CRF method, and it is still in use today for measuring insoluble dietary fiber. It has been widely used to measure silage and ruminant feed fiber. The system includes the NDF and the ADF procedures, which use hot detergent to remove digestible material and soluble fiber. The detergent system discriminates among the insoluble fibers – hemicelluloses, cellulose, and lignin. A chelating agent in the neutral detergent solution permits the solubilization of insoluble pectin. When NDF and ADF procedures are performed sequentially, the difference in weight between the NDF and the ADF extracts is an estimation of the insoluble hemicellulose fraction. The ADF residue contains lignin, cellulose, and (if present) cutin. Lignin can be measured by gently oxidizing the ADF residue with potassium permanganate. A 72% sulfuric acid treatment hydrolyzes cellulose leaving cutin, phlobaphenes, and the Maillard products.

NDF and Porcine α-Amylase

Since water-insoluble dietary fibers represent a major fraction of TDF, the NDF procedure represents a simple method that can form part of an overall procedure for measuring TDF in human foods. Nonfiber materials such as starch, most protein, and any fiber-like artifacts remaining in the NDF residue can be efficiently removed by rapid treatment with α-amylase from porcine pancreas (A-3176, Sigma Chemical Co., St Louis, MO); the rapid treatment consists of 5 and 60 min incubations at 55 °C and is at least as effective as the 18 h treatment at 37 °C originally proposed by Schaller. The source of the enzyme is specified since pancreatin and other preparations of porcine pancreas α-amylase are much less efficient. Unpurified porcine α-amylase has unique amylolytic and proteolytic activities when applied after the neutral detergent extraction step, making it an ideal enzyme to use for measuring insoluble dietary fiber.

The heat-stable α-amylase from *Bacillus subtilis* has also been used to digest starch after the neutral detergent extraction

step, but several potential problems arise with this enzyme. It may contain fiber-digesting enzymes that could lead to an underestimation of the cellulose and hemicellulose fractions. On the other hand, it does not completely remove the nonfiber material (such as starch), which will lead to an overestimation of the lignin and possibly the hemicellulose fractions. The final value, therefore, represents a compromise between these two competing factors. Table 1 shows that, for shredded wheat, the NDF residue obtained after treatment with *B. subtilis* α-amylase contained 30% more material than that obtained after treatment with porcine pancreas α-amylase. This residue apparently contained three times more lignin and four times more protein. Both the NDF residue and the apparent lignin content were also higher in samples of bran flakes. Other problems are apparent with the *B. subtilis* α-amylase. For example, toasting whole wheat bread increased the *B. subtilis* α-amylase-measured NDF and lignin values by two- and fivefold, respectively (Table 1), but no such differences were noted with the porcine pancreas α-amylase. The efficacy of the porcine pancreas α-amylase treatment has been verified in various foods and food products and confirmed by other researchers.

In addition to its demonstrated capabilities in avoiding artifactual increases in the estimation of various dietary fiber fractions, porcine pancreas α-amylase enzyme also facilitates rapid filtration in subsequent steps (starch and protein tend to clog the crucibles). The neutral detergent–α-amylase combination is advantageous for research analyses since the neutral detergent solution discriminates well between plant and bacterial cell walls. This is important because bacterial cell walls interfere with the fiber analysis of fecal samples.

Other modifications from the original Van Soest NDF method include the deletion of decalin, 2-ethoxyethanol, and sulfite (to maximize the recovery of lignin).

Table 1 Effect of two sources of α-amylase on the apparent neutral detergent fiber (NDF) and lignin content of breakfast cereals and bread (g 100 g^{-1}, dry weight basis)

	Porcine pancreas α-amylase[a]			Bacillus subtilis *α-amylase*		
	NDF	*Lignin*	*Protein*[b]	*NDF*	*Lignin*	*Protein*[b]
Shredded wheat	10.2	0.8	0.4	13.4[c]	2.7[c]	1.7[c]
Bran flakes	10.1	0.9		15.8	2.4	
Whole wheat bread[d]	5.2	0.3		5.9	0.4	
Whole wheat bread, toasted[d]	5.4	0.4		10.1	2.2	

[a]α-Amylase from porcine pancreas (Sigma Chemical Co., catalog no. A-3176).
[b]Protein measured in NDF residue (N × 6.25).
[c]From Van Soest, P. J. (1978). Fiber analysis tables. *American Journal of Clinical Nutrition* **31**, S284.
[d]From Mongeau, R and Brassard, R. (1980). Rapid digestion of starch and artifact fibre in the measurement of neutral detergent fibre of cereal products. *Getreide Mehl und Brot* **34**, 125–127.

Rapid HPB Method

The rapid HPB method described here is the AOAC No. 992.16 method. In order to obtain a measure of the TDF in a sample, one needs only to combine the insoluble dietary fiber from the NDF + porcine pancreas α-amylase treatment with a specifically tailored method for measuring soluble fiber. In the rapid HPB method, the soluble fiber procedure was devised to maximize the recovery of soluble fiber while excluding protein and other precipitable, nonfiber materials. This latter characteristic is important if one wishes to avoid time-consuming protein determinations that are part of other procedures. The soluble fiber procedure includes a gelatinization step in acetate buffer at pH 4.5 (121 °C) followed by a treatment with heat-stable α-amylase (100 °C) to remove any starch. Starch gelatinization is important to ensure complete starch hydrolysis. The soluble material is separated from insoluble fiber by filtration and is treated at 60 °C with amyloglucosidase (to finish starch digestion by hydrolyzing branched residues) and then with protease (to hydrolyze the peptide bonds of protein). The soluble fiber is then precipitated in 80% ethanol at room temperature; the sugar and amino acid residues remain soluble in ethanol.

Acetate buffer at pH 4.5 favors the exclusion of protein and appears to prevent the degradation of fiber components that may occur at high temperatures. This does not occur as readily at the relatively higher pH values used by other TDF procedures (e.g., phosphate buffer at pH 7). The heat-processed reference standards extracted from apple and carrot, and used in some collaborative studies, may show lower TDF values due to the loss of pectin during analysis. The differences are less apparent when expressed on a 'per gram fresh weight' basis. Without a method for accurately measuring all cell wall components, it is difficult to provide an absolute compositional measure of the constituents of dietary fiber as measured by different methods. However, the use of differential procedures to obtain fiber fractions (ADF and NDF), coupled with sulfuric acid digestion, permanganate oxidation, and total nitrogen analysis, allows an estimate of the relative contribution of several plant components to the final TDF value.

Figure 2 shows a comparison between several methods for measuring dietary fiber and the relative amounts of the components retained by each method. The rapid HPB method retains lignin (a structural polyphenol), the cell wall-associated protein, and a small amount of starch. The significant amount of starch retained by the Prosky method (see succeeding text) is readily apparent when TDF values are measured in legumes. Table 2 shows a comparison of four different methods for measuring TDF in green peas. The starch in the residue was estimated by subtracting the values from the total nonstarch polysaccharides (NSPs) + lignin measured by the Englyst procedure. The table also shows the effect of adding porcine pancreas α-amylase.

The method is considered 'rapid' when compared to other fiber methods, particularly when the Fibertec E (for soluble fiber) and the Fibertec I (for insoluble fiber) are used. For example, the maximum number of duplicate determinations using the Prosky TDF method is 20 per week, while 44 duplicate TDF determinations per week are possible using the rapid HPB method. This evaluation takes into account the time required for preparing the reagents and performing the

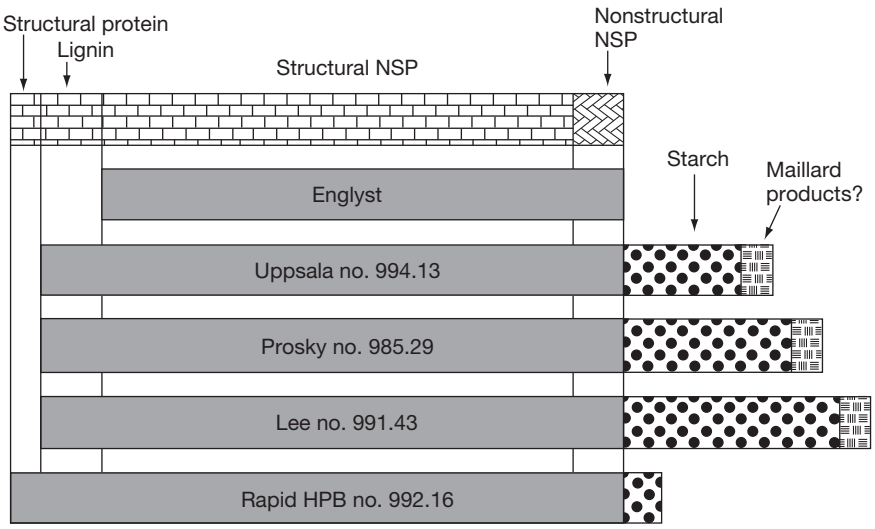

Figure 2 Material measured as dietary fiber by unmodified methods for all food categories. The surface area of the box is proportional to its total dietary fiber (TDF) value. It shows whether the method includes lignin and structural fiber protein or if it excludes starch and the Maillard products. The cell wall material corresponds to the structural nonstarch polysaccharides (NSP), lignin, and structural protein. The larger amounts of starch retained by the Theander, Prosky, and Lee methods are most evident when measuring TDF in legumes. AOAC numbers for each procedure are indicated. Adapted from Mongeau, R. (1995). HPB, Health Protection Branch, Health Canada.

Table 2 Total dietary fiber (TDF) in boiled dried green peas as measured by four different methods with or without added porcine pancreas α-amylase

Method	*TDF on a 'per g dry weight basis' (%)*	*Estimated starch content (%)*
Rapid HPB method, separate	9.9 ± 0.1	2.7
Prosky TDF method	19.0 ± 0.2	11.8
Prosky TDF method + porcine pancreas α-amylase	12.3 ± 0.3	5.1
Lee TDF method	18.8 ± 0.3	11.6
Lee TDF method + porcine pancreas α-amylase	12.3 ± 0.3	5.1
Englyst method (NSPs + lignin)	7.2 ± 0.2	
Englyst method (omit DMSO)	10.4 ± 0.1	3.2

NSPs, nonstarch polysaccharides; DMSO, dimethyl sulfoxide.
Starch content estimated by subtracting the NSP + lignin value from the TDF estimate.
Source: Mongeau, R. and Brassard, R. (1994). Comparison and assessment of the difference in total dietary fiber in cooked dried legumes as determined by five methods. *Journal of AOAC International* **77**, 1197–1202.

analysis, but not the time required for preparing the samples (freeze-drying and grinding).

Both separate and sequential rapid HPB methods are available and Table 3 presents a comparison between them. In the sequential method, the insoluble residue generated during the soluble fiber determination is treated with neutral detergent and porcine α-amylase yielding the insoluble fiber residue. Thus, a single sample generates both insoluble and soluble fiber measurements to give the TDF value. The separate method requires two samples for analysis. Using a single sample to measure TDF gives a more accurate and reliable measurement because it eliminates potential cross contamination that could give rise to falsely high TDF readings. For example, if the insoluble fiber fraction contains some small amount of soluble fiber, it will be counted twice in the final value (once in the insoluble fraction and once in the soluble fraction). The far right column of Table 3 shows that the TDF values are comparable when measured by either procedure and demonstrates the variability in the procedure.

In a comparison of 28 foods from various categories, the rapid sequential HPB method agreed well with the Prosky TDF method (slope = 0.99, intercept = −0.11, $r^2 = 0.985$) and with the Englyst GLC method (slope = 1.03, intercept = 0.15, $r^2 = 0.989$). For comparison with the Englyst method, it was necessary to measure lignin in each food and add its weight to the total NSP (obtained from the Englyst analysis). This gave a TDF value that was comparable to that measured gravimetrically. The regression parameters indicate that the rapid HPB method and the Prosky TDF method retain similar material in the final fraction (Figure 2). However, the negative intercept shows that the Prosky TDF method gives a higher value than the rapid HPB method when measuring foods containing low amounts of fiber.

The Prosky, Lee, and Asp Methods

The Prosky TDF method has also been referred to as the AOAC method since 1985. The Prosky, Lee, and Asp methods are similar but differ in the sources and types of enzymes as well as in the type and pH value of the buffers. The TDF values from all three methods are comparable (see Table 2 for a comparison between the Prosky and the Lee methods for green peas). In the Prosky and Lee TDF methods, duplicate samples are treated with α-amylase, protease, and amyloglucosidase to remove digestible material. Four volumes of 95% ethanol are

Table 3 Comparison of total dietary fiber (TDF) values by gas–liquid chromatography (GLC: nonstarch polysaccharides (NSPs) + lignin) or gravimetric methods (on a g 100 g^{-1} dry weight basis)

				Gravimetric TDF values[c]		
Food	*NSP*[a]	*NSP*[b]	*Permanganate lignin*	*Prosky*	*Rapid separate*[d]	*Rapid sequential*
Apple	9.9–14.7	13.4	0.8	14.6	14.1	14.6
Beans, green	27.8–32.2	30.6	2.1	33.7	31.3	29.1
Bread, white wheat	2.6–3.3	3.0	0.6	3.5	3.0	3.9
Carrot	22.8–28.9	23.5	1.8	25.4	23.4	23.4
Corn kernels, canned	6.7–8.9	5.9	1.0	7.7	7.8	7.6
Cornflakes	0.7–2.4	1.1	<0.1	2.5	1.1	2.6
Flour, white wheat	3.0–3.7	2.6	<0.1	3.0	3.9	3.6
Oats, rolled	7.1–9.5	8.2	0.9	10.5	11.0	9.6
Peas, canned	20.4–22.3	–	–	–	23.3	20.9

[a]Range of values reported by Anderson and Bridges (1988), Englyst and Cummings (1989), Englyst et al. (1995), and Marlett (1992).
[b]NSP as determined by the Englyst GLC method.
[c]TDF as determined by the Prosky method and the rapid separate and sequential methods.
[d]The separate method required analyzing two separate samples to obtain the TDF value.

then added and the precipitate filtered through Celite C-211 (Thermo Fisher Scientific, Mississauga, Ontario, Canada). The residue contains a substantial amount of nitrogen requiring a total nitrogen analysis (N) and subsequent back-calculation to correct for protein content (N content × 6.25). One of the duplicates is also analyzed for ash as in other gravimetric analyses, and this, too, is subtracted from the residue weight. It is proposed that the use of this protein conversion factor leads to overestimation of dietary fiber (Table 4). The additional protein determination step prevents this method from being considered rapid. As pointed out earlier and in Tables 3 and 4, the final insoluble dietary fiber residue can retain significant amounts of starch. In a simplified version of the Prosky method, the protease step is skipped since protein is measured in the final residue. This means that a larger amount of protein is subtracted as compared with the original Prosky procedure and this may contribute to some variability. The nature of the Celite used in different laboratories has also been considered as a source of variability. Other modifications have also been proposed to improve the precision of the Prosky method. The method comparisons in Tables 2 and 3 were confined to the results obtained with the original version of the Prosky method.

Table 4 Polysaccharide and unidentified material in the Prosky total dietary fiber (TDF) method for selected food groups

	Prosky TDF method residue (g 100 g^{-1} dry matter)		
Food	*Starch*	*Total polysaccharides*	*Unidentified material*
Bread	1.0	5.1	0.8
Other cereals	0.3	3.8	1.0
Green vegetables	1.5	25.6	4.6
Other vegetables	1.8	10.2	7.5
Canned vegetables	2.3	12.9	4.0
Fresh fruit	0.2	8.6	3.5
Fruit products	0.1	2.5	1.5
Nuts	0.4	6.7	2.5

Values represent TDF measurements after correction for ash and 'protein' (N × 6.25). Part of the unidentified material represents lignin but the values are too large to be accounted for as lignin only.
Source: Englyst, H. N., Quigley, M. E., Englyst, K. N., Bravo, L. and Hudson, G. J. (1995). Dietary fibre. Measurement by the Englyst NSP Procedure. Measurement by the AOAC Procedure. Explanation of Differences Medical Research Council & University of Cambridge. Report of a Study Commissioned by MAFF, UK.

Complete Polysaccharidic Components (GLC Methods)

Southgate published the original TDF method that required the hydrolysis of fiber into its individual polysaccharidic components. In Southgate's method, TDF represented NSPs plus lignin, but the method also provided values for hemicellulose and cellulose content. The method used 85% (v/v) alcohol to precipitate soluble starch residues followed by gelatinization and starch digestion with an appropriate enzyme. The original enzyme used by Southgate (takadiastase) had both amylolytic and proteolytic activities. However, in the 1970s, this enzyme became unavailable and other enzymes were used as replacements. It is apparent that some of these replacement enzymes are often less effective (Tables 2 and 3) but the α-amylase from porcine pancreas appears to be adequate. The original Southgate method measured the NSPs colorimetrically, but now, more specific methods are available such as GLC.

The more modern chemical methods use a combination of enzyme and/or chemical treatments to remove digestible material. The residue is then hydrolyzed by acid prior to the measurement of the sugar content. The acid treatment needs to be severe enough to assure complete hydrolysis but mild enough to prevent monomer degradation. The neutral fiber polysaccharidic constituents are measured chemically, usually by GLC after derivatization to a measurable form (e.g., by forming alditol acetates). Chemical derivatization is not required when high-performance liquid chromatography is used to measure the residue concentration. Acidic sugars (uronic acids) are measured colorimetrically. The total NSP content is obtained by appropriate calculations and TDF is calculated as the sum of

NSPs + lignin. The main GLC methods have been published by Anderson, Englyst, and Theander and their coworkers. Marlett used a modified Uppsala method. The GLC methods are different in several aspects, including the starch gelatinization step, enzyme treatments, and conditions of acid hydrolysis. This explains why the methods may be in agreement for some foods, but not for others.

The Englyst Method

Like all GLC methods, the Englyst method measures NSPs (neutral sugars + uronic acids). However, unlike other methods, the Englyst method is completely applicable to processed foods because it makes use of dimethyl sulfoxide (DMSO) during the gelatinization step to disperse the starch completely. This is followed by treatment with a heat-stable α-amylase and by a mixture of pullulanase–pancreatin in acetate buffer at pH 5.2. This treatment completely removes all the starch, leaving only the nonstarch polypeptides (Figure 2). The total insoluble and soluble NSPs are then precipitated in 80% ethanol and collected by centrifugation prior to hydrolysis with sulfuric acid. Neutral sugar residues are measured by GLC as alditol acetates and uronic acids are measured colorimetrically. According to Englyst and Cummings, the method can be performed in approximately the same amount of time as the Prosky TDF method. However, the GLC instrument must be equipped with an autosampler for the comparison to be valid. A rapid version of the Englyst method also exists with the sugar components measured colorimetrically, but this does not represent a substantial reduction in assay time when compared to the GLC method with an autosampler.

The Englyst method is the only dietary fiber method that does not include lignin. The role of noncarbohydrate components (which include lignin) in *in vivo* fiber digestion may be significant. There is some debate over whether some of the starch and structural protein should be considered as dietary fiber. These components would have to be measured by other methods if they were included in the TDF value.

The Uppsala Method

In the Uppsala method, there is a provision for an initial extraction in 80% ethanol if the sample contains very large amounts of mono-, di-, and oligosaccharides. This extraction appears important in reducing any coprecipitation of nonpolymeric sugars and fiber. Starch is digested in acetate buffer at pH 5.0 by incubating with a thermostable α-amylase and then with amyloglucosidase. DMSO is not used and starch may be less efficiently dispersed and, therefore, less efficiently removed (Figure 2). After reprecipitating in ethanol and centrifuging, the residue is hydrolyzed with sulfuric acid. As in the Englyst method, neutral polysaccharides are analyzed as alditol acetates by GLC and uronic acids by decarboxylation with special equipment or by colorimetry.

Unlike the Englyst method, the Klason lignin is measured gravimetrically. Potential errors can arise when lignin is measured independently and added back to NSPs. For example, the lignin fraction may include other nondigested fiber materials (such as cellulose), which would then overestimate insoluble fiber. This was apparently the reason behind an apparent lignin value of 0.9–3.5% (dry weight basis) measured in apples, peas, or a food composite. This value is suspect because low permanganate lignin values (obtained after the treatment of the insoluble fiber residue with porcine pancreas α-amylase and subsequent workup to obtain an ADF residue) were obtained, which were independent of any food-processing method (similar to the values in Table 1). Marlett had discussed the necessity of measuring lignin and the problems related to its measurement.

The Anderson Method

As in the Englyst method, the Anderson GLC method uses DMSO to disperse starch prior to hydrolysis. The starch is then digested with porcine pancreas α-amylase in acetate buffer at pH 5.2. After precipitation with four volumes of ethanol, the residue is extracted with water at 100 °C to obtain soluble and insoluble fiber fractions. Following centrifugation, the soluble fiber from the supernatant and the insoluble fiber from the pellet are hydrolyzed with sulfuric acid. The Klason lignin is measured as the loss in weight of the nonhydrolyzed insoluble residue after ashing.

Comparison of GLC and Gravimetric Methods for Measuring TDF

As already indicated, there is general agreement between the rapid HPB method and the Prosky and Englyst methods. The regression coefficients were 0.985 and 0.989 for the Prosky and Englyst methods, respectively. Other comparisons have also shown good agreement between values. For example, in a comparison of 25 different foods, TDF values as measured by the rapid HPB method agreed with those reported by Anderson and Bridges (modified Englyst method) and with those reported by Marlett (modified Uppsala method). However, more direct comparisons are needed to determine the potential problems with each method.

Table 3 presents a comparison of the TDF values expressed on a 'per gram dry weight' basis to magnify differences that may be partly hidden when the values are expressed on a 'per gram fresh weight' basis. In order to compare TDF values, the permanganate lignin values need to be added to the NSP values. Three different levels of variability are apparent from the data shown in Table 3. The range of NSP values obtained from three different laboratories (using three slightly different methods and different food samples) illustrates, in part, the intermethod variability. In addition, there is some interlaboratory variability, as shown by a comparison of four laboratories as well as an inherent variability in the samples (including stage of maturation of the product). Direct comparisons are only possible for the results in the five right-hand columns. These samples were composites based on winter and summer collections of up to 20 individual foods over 30 months in different locations. The results show that, when lignin is included in the final TDF value, there is good agreement between GLC and gravimetric methods for the indicated foods.

Nutritional Labeling and Analysis

It is difficult to decide on a single method for measuring the dietary fiber content of all foods. The challenge is to find a

single method that works adequately for all food groups and is independent of sample processing. Several candidates are possible, but to a large extent, the final decision will reflect the definition of dietary fiber. The dietary fiber definition must not be driven by available methodologies but must be arrived at independently of methodological considerations. As shown in Figure 2, it is possible to make a case for including structural protein, lignin, structural, and nonstructural NSPs, as well as some starch. Each method includes variable amounts of all these components. In addition to these complications, it is also possible to separate TDF into insoluble and soluble components. Once again, each method uses different procedures to measure these fractions so that the measured content of insoluble and soluble fiber will differ from laboratory to laboratory. Consumption of these different fiber fractions has not yet been related to definite physiological effects, although there appears to be a role for viscous soluble dietary fiber in reducing the risk factors for cardiovascular disease. The role of starch and other nonstarch polymers not traditionally associated with dietary fiber has yet to be determined as well. Therefore, the following discussion deals only with practical considerations in choosing a TDF method for food labeling.

Equipment

A method requiring simple, inexpensive equipment would be the most widely applicable because it would be more acceptable to nations unwilling or unable to spend considerable sums of money to monitor labeling. In general, the gravimetric methods require simpler equipment and the rapid HPB method is less costly than other gravimetric procedures because it does not require elemental nitrogen analysis. The Englyst colorimetric method can be completed with a simple spectrophotometer, although this method is less robust than the GLC method. Except for the Englyst method, all methods require a muffle furnace for ashing the samples. Although the gravimetric systems require simpler equipment, a hot-water supply is needed and analyses are greatly aided by purchasing semiautomated filtering/refluxing equipment that would increase the cost. However, these pieces of equipment are usually less expensive to maintain than GLCs.

Operator Time

Because it does not include a protein determination, the rapid HPB method requires the least amount of operator time per duplicate determination. Methods that require sugar derivatization and analysis by GLC tend to be longer than the gravimetric methods, especially when lignin measurement is included. The time required for these methods is greatly reduced if the GLC system includes an autosampler. Semiautomatic equipment is available for the gravimetric determinations, but filtering time can be slow, especially for samples that contain a considerable amount of starch.

Lignin

The Englyst method is the only one that does not include lignin in the final TDF value. Most researchers consider lignin to be an important dietary fiber constituent, and, consequently, most methods include a measure of the lignin content. In the GLC methods, the adoption of the Klason method (residue after sulfuric acid digestion) for measuring lignin means that other nonfiber materials such as the Maillard products may be included in the lignin fraction. This could lead to overestimation of the lignin content and, consequently, overestimation of the insoluble dietary fiber and TDF values. Lignin may be more accurately measured by a combination of NDF + porcine pancreas α-amylase and ADF treatments prior to permanganate oxidation. This would, however, increase the time required for analysis as well as increase the amount of sample needed per assay.

Independence from Food Processing

Different methods include different amounts of starch in the final TDF estimate (Figure 2). The only method that does not include any starch is the Englyst method because it completely solubilizes the starch with DMSO prior to enzymatic hydrolysis. There are several different types of starch, some of which arise from food preparation such as retrograde starch, and so the inclusion of starch in a method could be problematic. An example of the potential for problems comes from measuring the TDF values in cooked and uncooked rice and potatoes (high-starch foods) using the Prosky method. The Prosky method gave higher TDF values in the cooked foods. Problems were also noted with roasted peanuts where nonfiber material was included with TDF as well as toasted whole wheat bread (Table 1). Lower cooking temperatures (i.e., 95 °C compared to 100–120 °C) artificially increased the TDF content of white navy beans when the Prosky and Lee methods were used. Some of the problems may have been related to the choice of enzyme used to degrade starch, as was observed for toasted whole wheat bread (Table 1).

In general, the Englyst method is independent of the effects of food processing because it uses DMSO to aid in removing starch and nonpolymeric sugars. This helps exclude food preparation artifacts, such as retrograde starch, as well as the Maillard products, from the final analysis, but it also means that lignin is not included. It also means that lignin is excluded from the final analysis. Other methods that rely on GLC identification of NSPs include the Klason lignin, but this analysis has other problems (see preceding text). Of the gravimetric methods, the rapid HPB method appears to be independent from food processing and, at the same time, includes lignin in the final value.

Precision

Method variability pooled over all foods has been measured in a US Department of Agriculture study using 25 duplicate food samples. Different laboratories were asked to measure TDF in the samples without knowing their composition or source. The coefficient of variation for a single observation was 3.0% with the rapid HPB method, 4.7% with the Prosky TDF method, and 8.4% for the Uppsala method. This represents an estimation of the intralaboratory precision for selected unprocessed and processed foods. More interlaboratory work comparing precision of several methods is needed.

Remaining Problems

The general agreement between the different methods for measuring fiber is encouraging because it opens the door to universal acceptance of a dietary fiber method. However, many problems remain. First, an alcohol precipitation step is used to separate fiber material from nonfiber material. This step usually follows starch digestion and is designed to precipitate sugar polymers. It is possible that some of the fiber components are not completely precipitated and that some sugars released by starch digestion are coprecipitated. This may vary with the method and may be the source of some of the intermethod variability. In addition, the selectivity of this step in separating sugar polymers from nonpolymeric compounds has been questioned. In particular, polymers such as oligofructose (actually a mixture of oligomers and polymers) do not precipitate during the subsequent centrifugation. The 'dietary fiber' nature of these polymers is currently being debated.

Second, structural fiber protein may represent up to 10% of dietary fiber in immature plant tissues. It is impossible, at the present time, to quantify this protein (although the rapid HPB gravimetric method presumably includes it). Except for the rapid HPB method, this fraction is excluded from the reported TDF values.

Third, the colorimetric method for the determination of uronic acid values (included in most GLC methods) needs to be reexamined. This is because the uronic acid content of vegetables was shown to be higher when measured by a decarboxylation method.

Fourthly, there are a small number of foods where significant method disagreement occurs. For example, for soya bean fiber isolate, the NSP value using the Englyst GLC method is lower than the TDF value measured gravimetrically. The reasons for these discrepancies need to be examined in order to determine the exact TDF composition measured by each method. These comparisons will greatly improve fiber methodology.

Finally, although both acetone and ether have been used to remove fat from the samples prior to analysis, their efficacy is questionable and only the defatting procedure of Bligh and Dyer is efficient enough to defat all food samples completely. Caution is warranted when substituting defatting procedures since TDF methods have been validated with their respective defatting methods and substitutions may produce unexpected results. For example, the degree of lignin removal with the Bligh and Dyer procedure is unknown. There is some question as to the necessity for complete defatting since the methods were validated with a wide variety of food samples, all with different fat contents. It may be necessary to reexamine the importance of fat removal prior to analysis in samples containing high amounts of fat.

Conclusion

Comparable TDF values can be obtained for many foods using any of the methodologies discussed earlier. Some foods are more problematic and require a careful choice of methodology. Significant causes of error have been identified, including the potential precipitation of incompletely digested starch and the measurement of the Klason lignin, particularly in processed foods. The inclusion of the Maillard products in the Klason lignin may significantly increase the final TDF value. The following treatment is recommended: NDF, porcine pancreas α-amylase, ADF, and permanganate treatment. This procedure gives permanganate lignin values that are independent of food processing and, when added to NSPs determined by GLC, provides a detailed and known measurement of dietary fiber. The rapid HPB method, including the neutral detergent and porcine α-amylase treatments, has several advantages and would serve as a good method for food labeling.

See also: Cellulose; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Starch: Sources and Processing; Starch: Structure, Property, and Determination.

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Dietary Fiber: Energy Value

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Caloric Value in Human Nutrition

All food contains energy, which is needed by the body to fuel metabolic processes. This energy is obtained through the oxidation of macronutrients (carbohydrate, fat, and protein) and is partly captured as chemical energy in compounds such as adenosine triphosphate (ATP). These 'high-energy' compounds are used to drive many processes, including the biosynthesis of cellular components, the maintenance of ion concentrations across membranes, the initial phases of catabolic pathways, cell replication, mechanical function, etc. The large amount of energy released by the hydrolysis of ATP ($\sim$7 kcal mol^{-1} under cellular conditions) is captured by coupling its hydrolysis to other chemical reactions to drive them to completion.

The energy captured as ATP equivalents is one end of a long chain of digestive and metabolic processes that starts as the gross energy of the macronutrients in ingested food (Figure 1). Significant losses in energy occur during the digestive process. These can be identified and quantified as losses due to incomplete digestion and absorption (loss in fecal energy to give apparent digestible energy; DE_{app}), losses to fermentation gases and urinary nitrogen energy (to give apparent metabolizable energy), and losses associated with the digestive process itself and with the absorption and storage of food (to give net energy). Calculating the metabolizable energy from a food requires the collection of all excreta (including methane and hydrogen gases). For substances that are fermented, extra information is also required as discussed later in this article. Net energy values are more difficult to estimate and require some assumptions about digestive processes (see section on factorial models of fermentation, eqn [19]).

Metabolizable energy values are the commonly reported food energy values found on product labels. These were initially determined in the 1900s by a German scientist who was a pioneer in this area. To honor his name, these factors are commonly referred to as 'Atwater factors.' Both general and specific Atwater factors exist. The specific values were measured in individual foods. The general values (4 for carbohydrate, 9 for fat, 4 for protein, and 7 for alcohol) were calculated from the specific Atwater factors using average intakes of mixed diets. The more modern food energy values, as contained in tables such as the US Department of Agriculture Handbook #8, represent specific Atwater factors as modified by Merrill and Watt. Although we refer to these factors as metabolizable energy values for macronutrients, the values for carbohydrate and fat are, in reality, DE_{app} values. This is because urinary and gaseous energy losses were considered as insignificant for these macronutrients. In the case of the protein Atwater factors, urinary energy losses were taken into account, but gaseous energy losses were, again, assumed to be negligible. The Atwater factors for fat, protein, and carbohydrate represent the total energy available for metabolism, and this heat should be equivalent to the energy measured with a whole-body calorimeter.

Digestible sugars, fats, and proteins are readily absorbed in the small intestine by physiological and biochemical processes that capture the maximum energy from these food components. These macronutrients are virtually completely absorbed by the small intestine with little energy finding its way into the large intestine. In fact, with diets high in digestible sugars, fats, and proteins, most of the energy entering the large intestine comes from small intestine secretions and from the sloughing off of dead intestinal cells. It is the relative simplicity of the digestive process coupled with the high degree of absorption that allows the comparatively easy metabolic energy calculation for these macronutrients.

Not all food components are readily digested and absorbed in the small intestine. Nonstarch long-chain polysaccharides such as those associated with plant cell walls are not digested by human enzymatic secretions. Many other carbohydrates are not well absorbed by the small intestine. These pass into the large intestine where they are anaerobically fermented by the bacterial population that resides there. For the sake of clarity, the terms 'undigested' and 'resistant' will be used here to refer to the degradation processes occurring in the upper gastrointestinal tract (mostly by host digestive enzymes), and the term 'fermentation' and related words will be used to refer to the bacterial degradation processes occurring in the lower gastrointestinal tract. The energy losses associated with fermentation are not as readily measured, and so calculation of the metabolizable energy for fermentable food components is not as straightforward as it is for sugars, fats, and proteins. The present chapter outlines different methods for obtaining the metabolizable energy of food components not digested and absorbed in the small intestine.

A short note about the methods used to determine macronutrient content of foods is in order. In some countries like the United States and Canada, the carbohydrate content is determined by subtracting the fat, protein, water, and ash content of the food from the total weight (difference method). This measurement includes both 'available carbohydrate' (i.e., carbohydrate that can be digested in the small intestine) and dietary fiber. Other countries, such as Great Britain and Australia, measure 'available carbohydrate' directly using chromatographic methods. The carbohydrate values from this latter method do not include dietary fiber. Specific Atwater factors used in the United States and Canada take into account some (but not all) of the energy losses associated with dietary fiber because they rely on the apparent digestibility of carbohydrate to calculate food energy. This approach overestimates energy availability from mixed diets. In Europe, the general Atwater factor for carbohydrate (4 kcal g^{-1}, 16.7 kJ) is used to calculate

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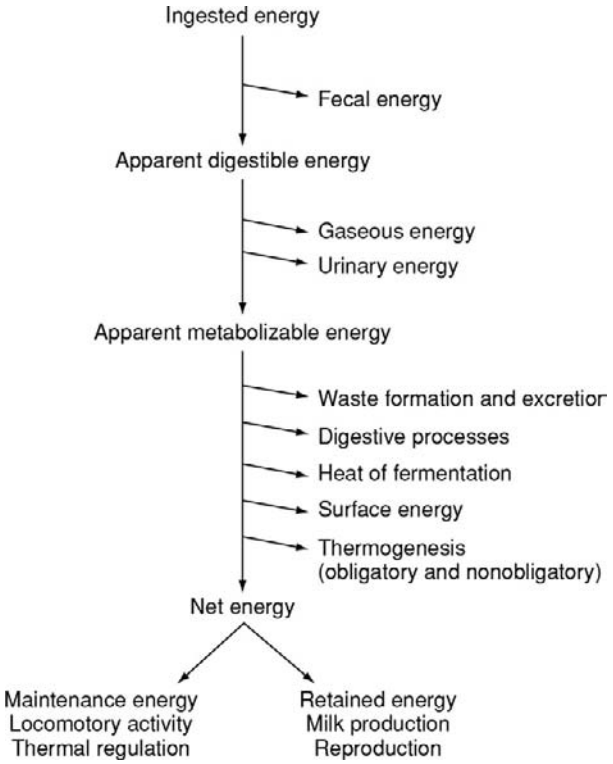

Figure 1 Diagrammatic representation of the energy losses associated with various stages of digestion and the resulting determined energy values.

carbohydrate energy after subtracting the dietary fiber content. This method underestimates the energy of mixed diets. When 'available carbohydrate' is measured directly, one must also measure dietary fiber and use a dietary fiber-specific energy value to calculate the energy contribution from dietary fiber.

Digestible Energy and Metabolizable Energy: Calculations

Digestible Energy

As noted earlier, the digestible energy of a substance is the difference between the ingested energy and the energy excreted in the feces. This is, in principle, a simple definition but gives rise to two different digestible energy values depending on how the measurement is made: the apparent digestible energy and the partial digestible energy. We begin by defining IM_s as the ingested mass of a measured substance FM_s as the amount of the substance of interest. This substance can represent any dietary material, but for the purposes of this article, we will assume that it escapes digestion in the small intestine and reaches the large intestine where it may (or may not) be fermented. If we further define recovered in the feces, the apparent digestibility (D_{app}) can be calculated as

$$D_{app} = (IM_s - FM_s)/IM_s \quad [1]$$

The apparent digestible energy value (DE_{app}) is simply the apparent digestibility multiplied by the heat of combustion (ΔH_c) of the material:

$$DE_{app} = D_{app} \times \Delta H_c \quad [2]$$

The DE_{app} takes into account only the energy loss associated with incomplete digestion of a single food component and not any potential interaction of the component with macronutrients. If the food component is fermented, part of its energy value will be captured and utilized by the host, part of the remaining energy will appear in the feces, and part will be lost to other processes (discussed in the succeeding text). Thus, the DE_{app} overestimates the true digestible energy.

Partial digestible energy values (DE_{part}) describe the influence of a dietary substance on the energy digestibility of the whole diet. As such, they represent a more complete picture of the energy intake and excretion associated with ingestion of a particular food component. Calculating DE_{part} values requires measurement of overall energy balance. The first step is to define the energy digestibility (D_{energy}) of the overall diet as

$$D_{energy} = (IE_T - FE)/IE_T \quad [3]$$

where IE_T is the total ingested energy and FE is the fecal energy. Partial digestible energy values are determined by adding a varying amount of a food component (as a supplement) to an unchanging basal diet. The energy digestibilities of the unsupplemented and supplemented diets are then determined (eqn [3]) and are plotted as a function of the fraction of ingested energy that is derived from the supplement (IE_S/IE_T). If the supplement has a lower digestibility than that of the overall diet, the graph should be a straight line with a negative slope – the energy digestibility of the overall diet will decrease with increasing amounts of the supplement. The partial indigestibility of the supplement (S_D) is given by

$$S_D = \Delta D_{energy}/\Delta(IE_S/IE_T) + (1 - D_{energy,0}) \quad [4]$$

where $D_{energy,0}$ is the energy digestibility of the basal (unsupplemented) diet. The DE_{part} is obtained by multiplying the partial digestibility (1 – the partial indigestibility) of the supplement by its heat of combustion:

$$DE_{part} = \Delta H_c \times (1 - S_D) \quad [5]$$

Partial and apparent digestibilities can differ by a considerable amount. In general, $DE_{part} < DE_{app}$ because DE_{part} includes energy losses that are not accounted for by DE_{app} measurements. This is illustrated by an experiment that compared the DE_{app} and DE_{part} values for the fiber component of hard red wheat bran (bran is ~40% fiber). Measurement of the diet and fecal fiber content and the diet and fecal energy content gave a $DE_{app} = 1.82 \pm 0.17$ kcal g^{-1} (7.6 kJ g^{-1}) and a $DE_{part} = 0.45 \pm 0.10$ kcal g^{-1} (1.9 kJ g^{-1}). The DE_{app} value shows that, for every gram of wheat bran fiber ingested, $4.15 - 1.82 = 2.33$ kcal (9.7 kJ) of energy was lost to feces in the form of fiber. The DE_{part} value shows that $1.82 - 0.45 = 1.37$ kcal (5.8 kJ) of extra energy was lost to the feces in addition to that lost from fecal fiber excretion. Thus, only $0.45/1.82 \times 100\% = 25\%$ of the energy of the fermented wheat fiber was potentially retained by the rats (see later in text for other calculations on the metabolizable energy value of wheat fiber). These calculations show the importance of determining the total energy losses in a digestion experiment involving fermentable substances.

Metabolizable Energy

Like digestible energy values, it is possible to define two different metabolizable energy values. Livesey (1993) called the Atwater metabolizable energy values 'apparent metabolizable energy' values (ME_{app}) because they do not take into account all the energy losses normally associated with metabolizable energy measurements:

$$ME = IE - (FE + UE + GE) \quad [6]$$

where UE is the urinary energy and GE is the gaseous energy. If a strict parallel between digestible energy and metabolizable energy is to be made, one could also define ME_{app} as $DE_{app} - (UE + GE)$. In essence, this is the definition used by Atwater with some assumptions. For fat and carbohydrate, it was assumed that GE and UE were negligible. For protein, it was assumed that GE was negligible but UE was not.

Partial metabolizable energy values (ME_{part}) can also be calculated in a fashion analogous to DE_{part} values. In this case, we define the total energy loss as

$$\sum E_{losses} = FE + GE + UE \quad [7]$$

Once again, we define the energy metabolizability of the total diet as

$$M_{energy} = \left(IE_T - \sum E_{losses}\right)/IE_T \quad [8]$$

and use this value to define a partial energy 'nonmetabolizability' (S_M):

$$S_M = \Delta M_{energy}/\Delta(IE_S/IE_T) + \left(1 - M_{energy,0}\right) \quad [9]$$

where $M_{energy,0}$ is the energy metabolizability of the basal (unsupplemented) diet. The ME_{part} is then given by

$$ME_{part} = \Delta H_c \times (1 - S_M) \quad [10]$$

Equations that minimize the error structure for experimentally determining DE_{part} and ME_{part} are also available.

Like DE_{part} values, ME_{part} values describe the influence of a part of the diet on the energy metabolizability of the whole diet. For protein and fat, it is thought that $ME_{part} > ME_{app}$ because fecal excretion of bacterial nitrogen and fat will decrease the ME_{app} value. Note that energy losses to bacterial fat and nitrogen are not simply due to the loss of macronutrient mass. Energy is required for the *de novo* bacterial synthesis of fat- and protein-containing macromolecular structures, which are then excreted in the feces. This energy is also lost. For carbohydrates, it is thought that ME_{part} and ME_{app} are equivalent since bacteria do not contain appreciable amounts of carbohydrate. For carbohydrates that escape digestion in the small intestine and are partially or completely fermented, like those in dietary fiber, $ME_{app} > ME_{part}$ because of the extra energy losses that occur during fermentation. This energy loss is the subject of this article.

Metabolizable or Net Energy?

The discrepancies between ME_{app} and ME_{part} energy values mean that a more accurate system for measuring food metabolizable energy must be employed for diets containing appreciable amounts of undigested and fermented carbohydrates as the greatest differences occur with these food components. Many methods can be used to obtain the ME values of food components that are not digested by the small intestine. Whole-body calorimetric measurements (in a chamber where an individual can be placed for experimentation) can be used to determine the energy value directly, estimates of the metabolizability can be obtained from mixed diets, body energy measurements can be made in laboratory animals and related back to the metabolizable energy of the food, and theoretical calculations can provide a general model for estimating metabolizable energy.

Large differences exist between the energy losses associated with the digestion of macronutrients (fat, carbohydrate, and protein) and undigested and fermented carbohydrates. Figure 2 shows an example of the various processes that occur in the gastrointestinal tract during the consumption of a partially digested and partially fermented food. Because the final energy value is net of all major losses of energy, many researchers have called it a net metabolizable energy (NME). This should not be confused with the net energy values used for livestock, which represent different energy measurements. Also, it is not the net energy of Figure 1, which incorporates many more energy losses. The NME is a more practical measure of food energy for undigested and fermented substrates because it takes into account energy losses that do not occur for fat, carbohydrate, and protein. Use of this value would allow comparisons between the energy values of different food components.

Mathematical Models for Undigested and Fermented Compounds: Sugar Alcohols

It is technically difficult and expensive to measure all stages of the fermentation process and determine the energy losses

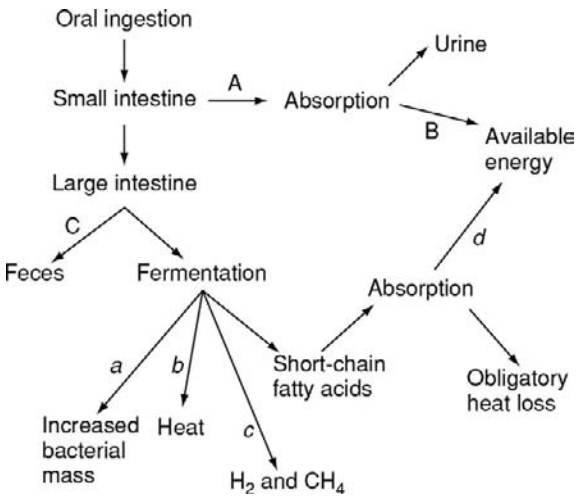

Figure 2 Scheme showing various stages of digestion of a macronutrient that is partially digested in the small intestine and partially fermented in the large intestine. The letters represent fractions of the initial amount that proceed down various pathways (see text).

associated with each step. This is true of human whole-body calorimetric measurements since analysis requires a complete energy balance profile for each subject (see succeeding text). A more simplistic approach involves the construction of a mathematical model of the digestive events in the large intestine. This approach has most commonly been applied to the energy value of sugar alcohols but can also be extended to other partially or completely fermentable carbohydrates.

Common dietary sugars (such as glucose, galactose, and fructose) have a ketone or aldehyde group that allows cyclization to a hexose or pentose ring structure. Reduction of this carbonyl group to an alcohol produces a sugar alcohol that cannot cyclize. The resulting sugar alcohols are not well absorbed in the small intestine so that a significant percentage can pass intact into the large intestine. Isomalt (Palatinit®), an equimolar mixture of two disaccharide alcohols (α-D-glucopyranosyl-1,1-D-mannitol and α-D-glucopyranosyl-1,6-D-sorbitol), is an example of a sugar alcohol that is virtually unabsorbed in the small intestine. It passes intact into the large intestine, where it is rapidly and completely fermented by the bacterial population that resides there. As such, it represents a model for a theoretical dietary fiber that is completely fermented in the large intestine and can be used as a basis for constructing a mathematical model.

A good mathematical model seeks to define all losses that occur during fermentation of ingested material and assign reasonable factors to these losses. Figure 2 presents the scheme for such a model. Fermentable substances provide carbon units for bacterial growth and reproduction and serve as metabolizable substrates to meet the colonic bacterial population's energy requirements for maintenance and growth. Four major products result from anaerobic colonic fermentation: increased bacterial mass (factor *a*, Figure 2), heat energy loss due to fermentation (factor *b*, Figure 2), methane and hydrogen gas (factor *c*, Figure 2), and short-chain fatty acids (SCFAs). Humans can only use SCFAs as an energy source. Thus, the metabolic energy yield of the sugar alcohol has been reduced by the losses to bacterial mass, as well as the heat and gas produced during fermentation. It has also been reported that SCFAs may produce significantly less ATP per gram of absorbed SCFA than glucose, so an additional energy loss to this difference in metabolic efficiency should also be included (factor *d*, Figure 2).

The fermentation model of Figure 2 can be expressed as an equation that incorporates the four major processes by which energy is lost during fermentation. The equation describing the scheme of Figure 2, as it applies to sugar alcohols, was first developed for the Nutrition Council of Holland and has been recognized by several authors as an alternative method for calculating the energy value of sugar alcohols. For partly fermentable substances, it can be written as

$$\mathrm{NME} = \Delta H_c \times [(A \times B) + \alpha \times (1 - A - C) - \beta] \qquad [11]$$

where ΔH_c is the heat of combustion of the substance in question, A represents the fraction of the ingested substance absorbed in the small intestine, B represents the fraction of absorbed substance that is metabolized, and C represents the fraction of the ingested substance that is excreted intact in feces (Figure 2). The factor α represents the proportion of energy derived from fermentation of the substance. It can be calculated from eqn [12]:

$$\alpha = (1 - a - b - c) \times d \qquad [12]$$

The factor β was not present in the original equation and represents the extra energy lost due to the presence of the fermentable carbohydrate. This can come about through binding to macronutrients, changes in osmotic balance, alterations in transit time, changes in intestinal viscosity, inhibition of digestive enzymes, inhibition of macronutrient uptake, or increased sloughing of intestinal cells. As discussed earlier (see section on DE_{part} and ME_{part}), measuring the appearance of an undigested food component in the feces is not sufficient to account for all of the energy losses that can occur when a diet is supplemented with a fermentable carbohydrate. In a practical sense, it is impossible to differentiate between any 'extra' energy lost to the feces (β) and the energy lost due to increased bacterial mass that results from fermentation (factor *a*). The solution is to set $\beta = 0$ in eqn [11] and measure total fecal output.

Using the mathematical model of eqns [11] and [12] requires a knowledge of factors *A*, *B*, and *C*, which are obtained from experimental evidence. For sugar alcohols, values for factor *A* have been obtained from ileostomy patients or by direct sampling using multiple lumen tubes. Factor *B* (the amount of sugar alcohol metabolized) has been estimated indirectly by measuring urinary sugar alcohol content (the amount of sugar alcohol not metabolized).

In the original factorial model paper, the Nutrition Council of Holland held α to be constant for all sugar alcohols and estimated it at 0.5. This value included fermentation energy losses resulting from increased microbial mass excreted in the feces ($a \approx 0.2$), loss of H_2 and CH_4 ($c = 0.03$–0.08), heat produced by microbes ($b = 0.02$–0.05), and higher heat loss during utilization of SCFAs as compared with glucose ($d = 0.80$–0.85). Summing these factors gives a value of 0.40–0.53. The value of 0.5 was adopted because the Nutrition Council of Holland did not want to suggest that the value was known with high precision (they could have used 0.46–0.47 as the average) and because they wanted to include some energy lost to increased cell proliferation in the large intestine caused by increased fermentation. Thus, the slightly higher value of 0.5 was chosen as an estimate of the total losses during fermentation. In a later review of the value of α, it was determined that the individual factors that contribute to α should give a value closer to 0.6. However, in the same review, the author noted that the factorial procedure overestimates energy values to the extent that it takes no account of osmotic effects of the sugar alcohol. Thus, the results of energy values calculated by the Dutch method are probably fairly accurate. Many researchers agree that the value of 0.5 represents a reasonable estimate of the fermentation process.

For completely fermentable substrates that are not absorbed by the small intestine, like sugar alcohols, the MNE value is $0.5 \times \Delta H_c$ because $A = 0$ and $C = 0$. As indicated earlier, the value of β is set to 0, and any 'extra' energy lost due to ingestion is incorporated into the factor α, which has been estimated at 0.5. Thus, for the sugar alcohol isomalt (which is completely fermented and not absorbed by the small intestine), the energy value is ~ 1.9 kcal g^{-1} (8 kJ g^{-1}, approximately half the heat of combustion). For completely fermentable dietary fibers, the $0.5 \times \Delta H_c$ rule of thumb

represents an upper limit of the NME since dietary fiber is known to bind fats and cholesterol as well as inhibit carbohydrate absorption. Thus, dietary fiber may interact with other macronutrients to decrease their metabolizable energy values. This will give a lower NME value for dietary fiber since there will be a higher excretion of fecal energy.

When all the energy losses are included, the energy value of dietary fiber can be small compared with the ingested value. For example, measurements with hard red spring wheat fiber give NME values of ~ 0.3 kcal g^{-1}, even though the total ingested energy is ~ 4 kcal g^{-1}. In the case of hard red spring wheat bran, much of the energy is lost to excretion; wheat bran dietary fiber is incompletely fermented so that $\sim 60\%$ is lost directly to the feces. In addition, a significant loss of energy comes from dietary fiber interactions with macronutrients plus other losses due to the presence of wheat bran fiber (β). Finally, energy is lost during the fermentation process itself. The energy value of hard red spring wheat fiber is not typical of other dietary fibers, as shall be shown in the following sections (Table 1).

Whole-body Calorimetric Measurements: Lactitol as a Model

Indirect calorimetry (a type of whole-body calorimetry where all gas consumption and gas exhalation are continuously monitored) can be used to determine energy expenditure through the use of equations relating total O_2 consumption, CO_2 expiration, and urinary nitrogen excretion to energy utilization. One such equation is

$$\text{Heat production} = 16.175\,O_2 + 5.021\,CO_2 - 5.987\,\text{urea N} - 4.5\,H_2 \quad [13]$$

In addition to total energy production, indirect calorimetry provides information on macronutrient utilization through the nonprotein respiratory quotient (RQ). The RQ is the ratio of CO_2 expired to O_2 consumed during a defined period. The theoretical RQ values are known for fat, carbohydrate, and protein oxidation, making it possible to determine total fat, carbohydrate, and protein oxidation. When protein oxidation is estimated from nitrogen excretion, fat and carbohydrate oxidation can be calculated as follows:

$$\text{Nonprotein RQ} = 1.00 \times \text{carbohydrate oxidation} + 0.707 \times \text{fat oxidation} \quad [14]$$

However, caution is needed when estimating fuel oxidation from nonprotein RQ values, since the RQ itself can vary considerably depending on the fat and protein mixture being oxidized. In addition to the problem of obtaining an accurate picture of fat and carbohydrate oxidation from nonprotein RQ values, it is also not possible to distinguish between the oxidation of carbohydrate + fat and the oxidation of SCFAs produced by carbohydrate fermentation. When measuring the apparent metabolizable energy value of a macronutrient that represents a small proportion of the total diet, there is a high potential for error when macronutrient oxidation profiles are measured. However, theoretical calculations show that there is much less error associated with measuring energy loss.

An example of the use of indirect calorimetry for measuring the metabolic energy of completely fermentable substances is the determination of the net energy value for lactitol, a disaccharide sugar alcohol (4-*O*-(β-D-galactopyranosyl)-D-sorbitol). The total energy balance was measured during the experimental period in the calorimeter. The retained energy of each subject was then calculated as

$$\text{RE(retained energy)} = \text{IE}_\text{T} - (\text{fecal E} + \text{urine E} + \text{gaseous E} + \text{expended E}) \quad [15]$$

The energy needed to maintain energy balance (metabolizable energy for maintenance; ME_m) could then be determined as

$$\text{ME}_\text{m} = \text{metabolizable energy intake} + \text{negative RE} - 1.11 \times \text{positive RE} \quad [16]$$

The factor 1.11 represents the cost of depositing body energy and was measured in an earlier experiment. The difference in ME_m on a diet supplemented with sucrose versus one supplemented with lactitol is a measure of the energy lost from lactitol and can be expressed as the change in ME_m adjusted for the metabolic weight of the individuals (weight$^{0.75}$):

Table 1 Potential error associated with oxidation of 50 g of a carbohydrate versus fermentation and oxidation of the released short-chain fatty acids[a]

Substrate	*RQ*	*Oxidation substrates (mol)*[b]	*O_2 consumed (mol)*	*H^{eq}*[c]	*kcal per 50 g*
50 g of sucrose	1.00	0.146 CHO	1.753	5.012	250.6
50 g of lactitol	0.943	0.409 (CHO) + 0.099 (fat)	1.113 (CHO) + 0.269 (fat)	4.981 (CHO) + 4.682 (fat)	153.1
50 g of lactitol	0.943	0.508 SCFA	1.382	4.663	144.5

[a]The table illustrates the potential error involved in measuring the apparent metabolic energy value of a completely fermentable supplement (lactitol) by indirect calorimetry. This was done by calculating the apparent metabolic energy value using two different methods. It was assumed that lactitol was fermented to a mixture of SCFAs followed by complete oxidation to CO_2 and water plus fermentation gases. In an indirect calorimeter, oxidation of SCFAs can be mistaken as oxidation of fat and carbohydrate in a 0.195:0.808 ratio. Oxidation of these substrates would give 153.1 kcal per 50 g. Oxidation of the SCFA directly would give 144.5 kcal per 50 g of original lactitol. Thus, an error of $\sim 6\%$ can occur when indirect calorimetry is used to measure the metabolic energy value of lactitol. The yield of SCFAs from lactitol and the proportion of acetate, propionate, and butyrate (115.1:35.38:24.5 per 100 mol of glucose) represent weighted averages of literature values for ruminants. As such, these calculations approximate the actual error associated with indirect calorimetry, which varies from individual to individual.

[b]Equivalent mol of sucrose or SCFAs obtained from 50 g of substrate.

[c]H^{eq} values from Livesey, G. and Elia, M. (1988). Estimation of energy expenditure, net carbohydrate utilization, and net oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. *American Journal of Clinical Nutrition* 47: 608–628. The H^{eq} value for glucose was used for carbohydrate and the H^{eq} value for the fat mixture of an omnivore diet was used for fat. The H^{eq} value for SCFAs represents a weighted average.

$$\Delta ME_m = (ME_m/weight^{0.75})_{lactitol} - (ME_m/weight^{0.75})_{sucrose} \quad [17]$$

and the NME can then be calculated as

$$NME = ME_{part} - \Delta ME_m/(IS/weight^{0.75})_{lactitol} \quad [18]$$

where ME_{part} is obtained from eqn [10] and IS is the ingested mass of the supplement (lactitol). The ME_m values are divided by the metabolic weight to account for differences in metabolic mass. Using this procedure, a value of 2.4 kcal g^{-1} (10 kJ g^{-1}) for lactitol was obtained. Note that some measure of the energy expenditure is required in this experiment (eqn [15]). The authors relied on diaries and ankle actometers and corrected to equal actometer counts. They acknowledged that this was not very satisfactory; there is a potential 15% error in the final net energy value for lactitol.

Metabolizable Energy Value of Dietary Fiber in Mixed Diets

Up to this point, this article has focussed on undigested and fermented food components as a general group of substances. This section will deal specifically with dietary fiber, a partly fermented food component that passes through the small intestine unacted upon by human digestive enzymes. Dietary fiber is a mixture of nonstarch polysaccharides and lignin that are structural components of plant cell walls.

As indicated earlier, it is not appropriate to use metabolizable energy values for dietary fiber since many more energy losses are associated with fiber digestion as compared with the digestion of fat, carbohydrate, and protein. Equations have been developed to take account of these extra losses and provide a method to calculate the NME value of a fermentable dietary component when the fecal energy losses are known. These equations show that approximately one-half of the potential energy is available to the host when undigested carbohydrates are completely fermented. This value is widely accepted.

Knowledge of the energy yield from the fermented portion of dietary fiber is not sufficient in and of itself to allow the calculation of dietary fiber NME since a significant proportion of the 'fermentable' dietary components are not fermented but appear in the feces. In addition, some 'extra' energy (factor β, eqn [13]) may be lost during digestion because of the intimate physical interaction between dietary fiber and the food matrix. The aim of these studies is to determine a general NME value for dietary fiber that can be used to calculate the metabolizable energy of mixed human diets. To overcome these problems, two solutions have been employed. The first solution involved determining individual digestibility factors for specific foods and using these to calculate metabolizable energy intake. This method has the advantage that macronutrient digestibility is measured directly for specific foods and multiplied by the specific heats of combustion when available. In this method, the calculated digestible energies of the diets are close to the measured values for mixed diets since the final value reflects the sum of the individual components. This method has some problems. In the system of Merrill and Watt, the digestibility of carbohydrates was determined by difference, so accurate values for carbohydrate digestibility are lacking. This also means that a digestibility value for the fermentable food component portion of the carbohydrate fraction (as determined by difference) is lacking. It is, therefore, not possible to calculate the metabolic energy of a food of known composition since values for undigested and fermented components are not available. This limits the usefulness of this system to foods that have been measured. In addition to this problem, the NME value is appropriate for undigested and fermented food components, whereas the digestible energy value is not. Thus, this system does not allow any correction for energy lost when food components are resistant to digestion but fermented in the lower gut.

The second solution is to use an average digestibility value determined by examining typical mixed diets. This can be calculated by two separate methods: using a factorial approach and using an empirical model. Factorial models relate total protein, fat, carbohydrate, and dietary fiber intake to digestible energy through an equation of the type:

$$\begin{aligned} ME(kca) = {} & 4.0 \times protein(g) + 9.0 \times fat(g) + 3.75 \\ & \times carbohydrate(g) + NME_{DF} \\ & \times dietary\ fiber(g) \end{aligned} \quad [19]$$

In the development of eqn [19], carbohydrate was determined directly (not determined by difference) and is expressed as monomeric units. Dietary fiber must also be measured directly through an approved, reproducible method that is not affected by food processing. The factors 4 (protein) and 9 (fat) are the general Atwater factors derived from mixed diets. The value of 3.75 for carbohydrate is slightly lower than the general Atwater factor of 4. This reflects the fact that the carbohydrate is expressed as the total monomeric weight.

The value of NME_{DF} (the NME for dietary fiber) comes from an analysis of the dietary fiber digestibility of mixed diets and the loss of energy to feces in mixed Western diets. This value is determined as follows. Firstly, the DE_{part} value for dietary fiber is obtained by comparing the digestible energies of mixed diets to the metabolizable energy calculated by eqn [19]. The apparent digestible and metabolizable energies for carbohydrate and fat are identical since the macronutrient digestibilities were accounted for, and urine and gaseous losses of these components were negligible. Protein losses are corrected for urine nitrogen and protein digestibilities. The digestible energy for dietary fiber (DE dietary fiber) was estimated by

$$\begin{aligned} DE\ dietary\ fiber &= \Delta H_c \times D_{app} \times (1 - a) \\ &= 4.1\ kcal\ g^{-1} \times 0.7 \times 0.7 \\ &= 2.0\ kcal\ g^{-1} (8.4\ kJ\ g^{-1}) \end{aligned} \quad [20]$$

The apparent digestibility of dietary fiber is around 0.7. The value of factor a in eqn [20] needs to be defined for dietary fiber. For sugar alcohols, the value is ~0.2 (see preceding text). A value of 0.3 has been adopted for dietary fibers in humans. This value reflects the fact that the majority of energy loss estimates lie between 0.2 and 0.4 kcal $kcal^{-1}$ of fermented energy. As already mentioned, a value of 0.2 was obtained when measuring lactitol energy utilization in human subjects. It has also been suggested that between 20% and 30% of the energy in undigested and fermented carbohydrates is mikuutilized by bacteria in humans. A value of 0.2 kcal $kcal^{-1}$ of fermented material has been measured in rats fed with an α-amylase-resistant pea starch, and a value of 0.4 kcal $kcal^{-1}$ of fermented material has been measured in rats fed with guar gum. In human diets with high intakes of dietary fiber, an increased fecal energy output of 0.3

and 0.39 kcal kcal^{-1} of energy fermented, respectively, has been measured. Overall, these data suggest that about 0.3 kcal of energy is lost to the feces per kcal of energy fermented. Thus, $1.0 - 0.3 = 0.7$ kcal kcal^{-1} is retained.

As indicated earlier, the digestible energy value does not give an accurate picture of the energy losses that occur during fermentation of undigested food components. Correction factors must be included for energy losses to the heat of fermentation (factor *b*), losses to fermentation gases (factor *c*), and the relative efficiency of utilization of SCFAs (factor *d*). Correcting the digestible energy for these values gives

$$\begin{aligned} \text{NME}_{\text{DF}} &= \Delta H_c \times D_{\text{app}} \times 0.54 \\ &= 4.1 \text{ kcal g}^{-1} \times 0.7 \times 0.54 = 1.5 \text{ kcal g}^{-1} \end{aligned} \quad [21]$$

Equation [21] shows that the NME value for dietary fiber is approximately 1.5 kcal g^{-1}. Equation [21] applies strictly to dietary fiber because the ΔH_c value and digestibilities were determined for dietary fiber. It is useful for calculating the metabolic energy values of mixed diets because the digestibilities were determined for mixed diets. It is comparable, therefore, with the general Atwater factors of 4, 9, and 4 for carbohydrate, fat, and protein.

Empirical models represent another method for determining total energy intake. Empirical models are, in general, superior to the factorial models for a number of reasons, the most important being that the empirical models are much more robust to errors in carbohydrate, fat, and protein determinations as well as to the use of general Atwater factors. An example of an empirical equation is as follows:

$$\begin{aligned} \text{ME} = 0.96 &\times \text{total energy(kcal)} - 2.5 \text{ kcal g}^{-1} \\ &\times \text{dietary fiber(g)} - 12 \text{ kcal g}^{-1} \\ &\times \text{ingested nitrogen(g)} \end{aligned} \quad [22]$$

Equation [22] relates the metabolizable energy to total energy, protein, and dietary fiber only. The factor 0.96 represents the metabolizability of mixed diets in the absence of dietary fiber. The value of 2.5 kcal g^{-1} represents the NME energy loss associated with ingestion of dietary fiber. This value is therefore the difference between the heat of combustion of dietary fiber and its NME_{DF} value (determined in the preceding):

$$\begin{aligned} 2.5 \text{ kcal g}^{-1} &= \Delta H_c - \text{NME}_{\text{DF}} \\ &= 4.0 \text{ kcal g}^{-1} - 1.5 \text{ kcal g}^{-1} \end{aligned} \quad [23]$$

A version of eqn [22] was tested for bias and accuracy of prediction by comparison with 43 human diets containing varied intakes and sources of dietary fiber. The equation showed no bias over a wide range of dietary fiber intake (2–93 g daily). By necessity, this test was performed by comparing DE_{part} values because ME_{part} values cannot be measured directly for fermentable food components. This analysis provides further evidence for a general NME for dietary fiber of 1.5 kcal g^{-1}.

Overall Conclusion

Eating food components that are not digestible but that are fermentable lowers the amount of metabolizable and digestible energy available from food. This occurs because of two different processes. Fermentable food components, like dietary fiber, are not 100% fermentable, and so the NME for these components is lower than that of protein, carbohydrate, and fat. In addition, fermentation itself is associated with many different energy losses, including losses to increased bacterial mass, the heat of fermentation, fermentation gases, and a lower efficiency of host utilization of SCFAs. Because the digestion of fermentable food components is so different from that of macronutrients, special equations are needed to determine the NME yield.

It is possible to utilize data from several sources to obtain an estimate of the relative losses that occur during fermentative digestion and back-correct to obtain an unbiased estimate of the energy value of dietary fiber. Taking into account all these energy losses, an NME value for dietary fiber of 1.5 kcal g^{-1} (6.3 kJ g^{-1}) has been calculated. This appears to accurately predict energy losses associated with mixed Western diets when analyzed using either factorial or empirical models. Thus, the NME of 1.5 kcal g^{-1} (6.3 kJ g^{-1}) can be used in a fashion similar to the general Atwater factors of 4, 9, and 4 for protein, fat, and carbohydrate to predict the metabolizable energy of mixed Western diets. Individually measured NME values for fiber in whole foods may differ from this value since dietary fiber is fermented to different extents and is intimately associated with the food matrix, which may limit macronutrient digestibility. Thus, individual measurements are needed for direct comparison and may be required for specific types of foods.

See also: Dietary Fiber: Bran; Dietary Fiber: Determination; Dietary Fiber: Physiological Effects.

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Dietary Fiber: Physiological Effects

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Introduction

The term 'crude fiber' dates from the nineteenth century, when it was used to describe the material that remained after rigorous nonenzymatic hydrolysis of animal feedstuffs. The importance of similar undigestible residues in human foods was recognized in the early 1970s by the physician and epidemiologist Hugh Trowell, but he also argued that the crude fiber figures then available for foods had little physiological significance and were of no value in the context of human diets. He therefore coined the term 'dietary fiber' for what he and others came to define as "The sum of lignin and the plant polysaccharides that are not digested by the endogenous secretions of the mammalian digestive tract." In practice, these residues contain resistant starch molecules, but for many years, a narrower definition, which included only 'nonstarch polysaccharides' (NSP), found favor, particularly in the United Kingdom. However, the most recent CODEX definition (2009) states that "Dietary fiber means carbohydrate polymers with 10 or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans." This definition, which has been widely adopted internationally, includes both resistant starch molecules and cell wall polysaccharides, together with lignin and a few other minor components. Certain isolated or synthetic polysaccharides can also be included in the definition, so long as they have been shown to exert beneficial physiological effects. Dietary fiber thus includes a large and complex mixture of polysaccharides that share few properties other than resistance to digestive enzymes. The presence of undigested cell wall fragments and dispersed polysaccharides in the intestinal lumen can alter physiological processes throughout the gut, but the effects of different fiber components depend upon their varied physical and chemical properties during digestion and their varying susceptibilities to degradation by bacterial enzymes in the colon. It is important to realize that a single analytic value for the fiber content of a food is a poor guide to its physiological effects. This article will review the main mechanisms of action of resistant polysaccharides in the alimentary tract and their implications for human health.

Fiber in the Alimentary Tract

Food is conveyed through the alimentary tract by the rhythmic muscular activity of peristalsis. Digestive enzymes are released into the lumen at intervals to facilitate the breakdown of macromolecules into fragments that can then be absorbed and utilized by the body. The earliest stages of digestion begin in the mouth, where food particles are reduced in size and lubricated with saliva, which also contains the digestive enzyme salivary amylase. In the stomach, any large particles that remain are broken down by rigorous muscular activity in the presence of hydrochloric acid and proteolytic enzymes. The need to disrupt and disperse intractable food particles and cell walls delays gastric emptying, and since cell wall polysaccharides help to determine the texture of many plant foods, and hard foods tend to be chewed more thoroughly than soft foods, the presence of dietary fiber in unrefined foods may begin to regulate digestion at a very early stage. Apples, for example, tend to be consumed more slowly than apple juice, and the absorption of sugar from apples is also slower. Similarly, the rate at which the products of starch digestion are absorbed from cubes of cooked potato has been shown to be much slower when they are swallowed whole than when they are chewed normally. The nutritional significance of such effects is not entirely clear, but they may limit the rate at which glucose from some foods enters the circulation.

Small Intestine

The small intestine is the largest of the digestive organs, with the greatest surface area, as befits its role as the main site of nutrient absorption. The semiliquid products of gastric digestion are released at intervals into the small intestine and propelled away from the gastroduodenal junction by peristaltic movements at about 1 cm min^{-1}. The hydrolysis of proteins, triglycerides, and starch continues within the duodenum and upper jejunum, under the influence of pancreatic enzymes. The final stages of digestion occur at the mucosal surface, where the products are absorbed into the circulation, along with water and electrolytes, via the specialized epithelial cells of the intestinal villi. Muscular activity in the small intestinal wall, together with rhythmic contractions of the villi, ensures that the partially digested chyme is well stirred. In adults, the first fermentable residues from a meal containing complex carbohydrates enter the colon ~4.5 h after ingestion. When a solution containing indigestible sugar is swallowed without food, it reaches the colon about 1.5 h earlier than when the same material is added to a solid meal containing dietary fiber. The effects of dietary fiber on transit through the small intestine are complex and difficult to study. Experiments with human subjects suggest that though coarse bran added to experimental meals delays gastric emptying, it may accelerate small intestinal transit. However, there is also some evidence that phase separation occurs, so that bran may accumulate in the distal ileum without any delay in transit of the liquid phase to the colon. Viscous polysaccharides such as guar gum appear to have little effect on small intestinal transit in humans.

Carbohydrate Metabolism

In his early work, Trowell proposed that dietary fiber was a major factor in the prevention of type 2 diabetes. Indeed, he

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argued that this condition was largely unknown in Western Europe prior to the introduction of mechanized flour milling because until then, the consumption of unrefined carbohydrate foods had favored slow absorption of glucose, which placed less strain upon the ability of the pancreas to maintain glucose homeostasis. Whatever its merits, this hypothesis stimulated a great deal of research on the effects of cell wall polysaccharides on the digestion and absorption of carbohydrates, and this has undoubtedly shed light on the role of dietary fiber in the management of diabetes. The 'glycemic index' (GI) quantifies the appearance of glucose in the bloodstream after ingestion of a carbohydrate-rich food. To determine the GI for a specific food, fasted subjects are given a test meal containing a standardized quantity of carbohydrate. The area under the blood-glucose curve in response to the test meal, divided by that produced by an equal quantity of a reference carbohydrate such as glucose, gives a ratio that, expressed as a percentage, is the GI. When glucose is used as the standard, unrefined foods containing dietary fiber often have a GI substantially lower than 100%.

The resistance of plant cell walls to digestion during passage through the gut varies considerably from one food to another, and any cell walls that remain intact in the small intestine will impede the access of pancreatic amylase to starch. Even when enzymes and their substrates do come into contact, the presence of cell wall polysaccharides may slow the diffusion of hydrolytic products through the partially digested matrix in the gut lumen. Pulses tend to give particularly low GI values, probably because legume seeds have relatively thick cell walls that resist destruction during processing and cooking. This particular effect of fiber on GI cannot be predicted from simple analytic values for total fiber because it reflects the structure, as opposed to the absolute quantity, of cell wall polysaccharides within the food.

Many studies on postprandial glycemia have been conducted using isolated fiber supplements added to glucose test meals or to low-fiber sources of starch. They demonstrate that, contrary to Trowell's original hypothesis, wheat bran and other insoluble cell wall materials have little effect on glucose metabolism. However, certain polysaccharides, such as guar gum, pectin, and oat beta-glucan, which form viscous solutions in the stomach and small intestine, do reduce the GI of foods to which they are added. Highly viscous food components may delay gastric emptying but the primary mechanism of action appears to be suppression of convective stirring in the fluid layer adjacent to the mucosal surface. The rapid uptake of monosaccharides by the epithelial cells tends to reduce the concentration of glucose in this boundary layer, so that absorption from the gut lumen becomes rate-limited by the relatively slow process of diffusion. The overall effect is to delay the assimilation of glucose and hence suppress the glycemic response to glucose or starchy foods. A similar mechanism probably inhibits the reabsorption of cholesterol and bile salts in the distal ileum, and this may account for another important physiological effect of viscous polysaccharides such as oat beta-glucan, which is to reduce plasma cholesterol levels in humans.

Mineral Metabolism

Polysaccharides and phenolic components of cell walls often contain polar groups that can interact with ionized solutes in the gastrointestinal contents. Such molecular binding effects do occur *in vitro* and might, in principle, restrict the availability of nutrients and other substances for absorption in the small intestine. Iron, zinc, and calcium are absorbed relatively poorly from the human diet, and there have been some concerns that high intakes of dietary fiber might exert an adverse effect on human mineral nutrition. However, studies with subjects who, for clinical reasons, have had the colon removed and the small bowel brought to the abdominal surface (ileostomists) suggest that neutral polysaccharides do not bind minerals to any significant extent. Charged polysaccharides such as pectin can bind mineral cations, but fermentation of cell walls in the colon probably releases iron and calcium into the lumen, whence they may be salvaged by colonic absorption. There is little objective evidence that dietary fiber per se has an adverse effect on mineral metabolism. Furthermore, consumption of highly fermentable oligosaccharides such as inulin has been shown to improve calcium absorption in humans and animal models, apparently because the production of short-chain fatty acids stimulates active mineral transport in the colon.

The effect of phytate (myoinositol hexaphosphate) is generally considered to be more significant than that of fiber. Phytate is often present in close association with cell wall polysaccharides in unprocessed legume seeds, oats, and other cereals. Phytate does exert a potent binding effect on minerals and has been shown to reduce the availability of magnesium, zinc, and calcium for absorption in trials with human volunteers. Phytate levels in foods can be reduced by the activity of endogenous phytase, by hydrolysis with exogenous enzymes, or by fermentation. Dephytinized products may be of benefit to individuals at risk of suboptimal mineral status.

Large Intestine

The large intestine, which is located distal to the small bowel, salvages energy from food residues and from endogenous proteins and carbohydrates that have escaped digestion and absorption. Its other main role is to form and store feces. Fecal microorganisms degrade undigested starch and many of the polysaccharides that comprise dietary fiber, to yield the volatile fatty acids butyrate, propionate, and acetate. Whereas the foregut of ruminants is adapted to provide a watery, nutrient-rich environment for bacterial fermentation, it is the colon that provides the main site of permanent bacterial colonization in the human alimentary tract. The development of rapid and inexpensive gene sequencing technology has revolutionized the study of this gastrointestinal microbiota in recent years, and the gut microbiome, as it is now known, derives much of its nutrition from dietary fiber. The metabolic activities of the gut bacteria are now thought to exert a major influence on human metabolic health, and the effects of diet on the composition of the microbiome are a major focus of interest.

Absorption and metabolism of short-chain fatty acids derived from carbohydrate fermentation provides an important route for the recovery of energy from undigested polysaccharides. Butyrate functions as a major fuel for the colonic mucosal cells, whereas propionate and acetate are absorbed and metabolized systemically. The other major breakdown products of carbohydrate fermentation are hydrogen, methane, and carbon dioxide, which together comprise flatus gas.

Excess gas production is said to cause distention and pain in some individuals, especially those attempting to increase their fiber consumption, but this is probably caused more by fermentation of oligosaccharides such as stachyose and verbascose, found, for example, in legume seeds, rather than the cell wall polysaccharides themselves. The proximal colon contains about 200 g of dilute fecal material, portions of which are transferred at regular intervals from the right colon into the transverse and distal segments for partial dehydration and storage. The pattern of motility in the large intestine is similar in principle to that of the rest of the alimentary tract, but the rate of transit is slower. In healthy individuals, stools are passed with a frequency varying from once or twice a day to once every 2–3 days.

Fecal Bulk and Composition

The ability of dietary fiber to prevent cancer and various degenerative diseases of the alimentary tract was proposed by Denis Burkitt, who based his hypothesis largely on the concept of fecal bulk. His field observations in Africa, where cancer and other chronic bowel diseases were rare, suggested that populations consuming traditional rural diets rich in vegetables and cereal foods produced bulkier, more frequent stools than persons living in the industrialized West. Burkitt argued that consumption of highly processed cereals in industrialized societies led to chronic constipation and that this caused prolonged high pressures both within the colonic lumen and also within the lower abdomen as a result of straining to pass hard stools. This in turn was thought to increase the risk of various diseases of muscular degeneration including varicose veins, hemorrhoids, hiatus hernia, and colonic diverticula. Furthermore, infrequent defecation was thought to cause prolonged exposure of the colonic epithelial cells to mutagenic chemicals that could initiate cancer. Although Burkitt's overall hypothesis for the beneficial effects of fecal bulk is undoubtedly an oversimplification, it has never been comprehensively refuted. There is relatively strong epidemiological evidence for a protective effect of dietary fiber against colorectal cancer, and although there is no conclusive evidence from randomized controlled trials, this association provides one of the main reasons for recommendations to maintain relatively high levels of fiber consumption in the United Kingdom (30 g day^{-1} in adults) and elsewhere.

It is certainly true that the consumption of dietary fiber is a major determinant of both fecal bulk and bowel habit, but the magnitude of the effect depends upon the type of fiber consumed. Soluble cell wall polysaccharides such as pectin are readily fermented by the microflora, whereas lignified tissues such as wheat bran tend to remain partially intact in the feces. Both classes of dietary fiber can contribute to fecal bulk but by different mechanisms. The increment in stool mass caused by wheat bran depends to some extent on particle size, but in healthy Western populations, it has been shown that for every 1 g of wheat bran consumed per day, the output of stool is increased by between 3 and 5 g. Other sources of dietary fiber also favor water retention. For example, ispaghula, which is a mucilaginous material derived from *Psyllium*, is used pharmaceutically as a bulk laxative. Soluble polysaccharides such as guar and oat β-glucan are readily fermented by anaerobic bacteria, but solubility is no guarantee of fermentability, as is illustrated by modified cellulose gums such as methylcellulose, which is highly resistant to degradation in the human gut. Fermentation reduces the mass and water-holding capacity of soluble polysaccharides considerably, but the bacterial cells derived from them do make some contribution to total fecal output. Thus, although all forms of dietary fiber exert some fecal bulking effects, their magnitude cannot be deduced from a single analytic measurement of total fiber content.

Apart from increasing the availability of energy from dietary fiber, the major effect of bacterial fermentation is to regulate the physical and chemical properties of the intraluminal environment. The production of metabolites by the fecal microbiome is strongly influenced by both the level and type of dietary fiber consumed by the host, and it is conjectured that a high consumption of fiber favors a less carcinogenic chemical environment in the fecal stream. Most colorectal carcinomas develop progressively from precancerous lesions called adenomatous polyps. The gradual transition from a normal crypt via a precancerous lesion to a malignant tumor is associated with a progressive loss of differentiation, deregulation of cell growth, and an accumulation of mutations in genes associated with the control of cell proliferation and death. These somatic mutations are assumed to be caused primarily by mutagenic chemicals in the feces that have been shown to be carcinogenic in animals. These include heterocyclic aromatic amines created during the cooking of meat at high temperatures and *N*-nitrosamines derived by bacterial metabolism from dietary proteins. It has not been established conclusively that these substances cause colorectal carcinogenesis in humans, but there is strong circumstantial evidence that they do and it is prudent to adopt nutritional strategies to reduce their concentration in the colon.

The realization that colorectal carcinogenesis is a prolonged multistage process involving changes to a complex array of genes raises new questions about interactions between the colonic epithelial cells, dietary fiber, and other constituents of the fecal stream. Bile acids, which are produced by the liver and secreted into the gut lumen, where they facilitate the digestion and absorption of fat, are known to increase the rate at which colonic mucosal cells divide. This is particularly true of secondary bile acids (e.g., deoxycholic acid and lithocholic acid) produced by bacterial metabolism of primary bile acids in the colon. It has been proposed that bile salts act as tumor promoters in the human colon, accelerating the carcinogenic process initiated by fecal mutagens. High-fat diets tend to stimulate the release of bile acids and increase their concentration in the feces, and this may partially explain the adverse effects of Western diets on the risk of bowel cancer in human populations. However, increased fecal bulk will tend to dilute the concentration of bile salts and reduce their residence time. Furthermore, recent studies suggest that fermentation of dietary fiber shifts the composition and metabolic activities of the fecal microbiome so as to inhibit the production of secondary bile acids. The nonfermentable particulate components of plant cell walls may also provide a finely dispersed solid phase on to which bile acids can bind, so that their concentration in the aqueous phase of the feces is reduced still further.

Cellular Physiology

Fermentation of carbohydrates lowers the pH of the feces and increases the concentration of short-chain fatty acids in contact with the mucosal cells. There is evidence that these changes modify the intraluminal environment in such a way as to regulate the birth and death of the mucosal cells. Programmed cell death (apoptosis) is a mechanism whereby damaged cells are removed selectively from a tissue in an orderly manner that does not cause inflammation or general tissue destruction. This may well provide a defense against the survival of cells carrying tumorigenic mutations. Apart from the role of butyrate as an essential source of energy for the colonic mucosa, it also suppresses proliferation, increases differentiation, and induces apoptosis in many types of tumor cell grown *in vitro*. Tumor cell lines established from adenomatous polyps and carcinomas from human colon undergo increased apoptosis in the presence of butyrate, at concentrations close to those that occur *in vivo*. Unabsorbed starch and fermentable components of cereal fiber may provide a source of increased butyrate, which helps to suppress the proliferation of tumor cells and increase the likelihood of their deletion from the tissue by apoptosis. If so, much may depend on the rate of fermentation. Sugars and small oligosaccharides probably disappear too rapidly after entering the colon to provide a supply of butyrate to the distal colorectum, whereas lignified plant cell walls and some types of resistant starch that are slowly fermented may deliver butyrate efficiently to more distal regions of the colonic mucosa.

Adverse Physiological Effects

There are few well-authenticated adverse effects of dietary fiber. Some cases of pediatric malnutrition caused by very high fiber intakes have been reported, but this problem appears to be confined to grossly unbalanced diets. Similarly, the few cases of gastrointestinal obstruction in the literature appear to stem from very abnormal intakes of wheat bran or from swallowing nonhydrated supplements of viscous polysaccharides intended for consumption as a drink. There is some evidence that patients with a history of gastrointestinal surgery are at increased risk of obstruction due to abnormally high intakes of cereal bran.

Sources of Fiber

As should now be obvious, although all plant foods provide some form of dietary fiber, not all components of fiber have the same physiological effects. In the United Kingdom, about 47% of total fiber intake is obtained from bread of various types and from breakfast cereals. The level of fiber in bread depends upon the extraction rate, which is the proportion of the original grain used to make flour. White flour has an extraction rate of about 70% and contains about 3% NSP, whereas 'wholemeal' (100% extraction) flour contains about 10% NSP. Wheat bran, which contains about 40% NSP, can be added to the diet as a supplement, but it usually requires processing to increase its palatability. One of the lasting effects of the dietary fiber hypothesis has been to increase the variety of high extraction cereal products in the market place. Such products tend to contain primarily insoluble, poorly fermentable polysaccharides, and they provide an effective way of increasing fiber intake to increase stool bulk. Oats, rye, and barley contain higher quantities of soluble fiber than wheat. Oat bran in particular is an important source of β-glucan, which has been shown to reduce plasma low-density lipoprotein cholesterol levels in humans. A further 45% of total fiber intake in the United Kingdom comes from fruits and vegetables. Typically, the levels of NSP in fruits and vegetables are between 1% and 5% of fresh weight, and the polysaccharides are mostly soluble pectins and arabinogalactans, which are readily fermentable in the large bowel. For reasons described earlier, soluble fiber from vegetables and fruit does not have the bulk laxative effect of cereal bran, but it does provide fermentable carbohydrate for fermentation in the colon.

See also: Cancer: Diet in Cancer Prevention; Carbohydrate: Digestion, Absorption and Metabolism; Dietary Fiber: Determination; Phytic Acid: Properties, Uses, and Determination.

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Dietary Fiber: Properties and Sources

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Background

Although dietary fiber has been known for more than 2000 years under various terms (e.g., bran and roughage), the term 'dietary fiber' first appeared in 1953 and referred to hemicellulose, cellulose, and lignin. The term 'fiber' is somewhat misleading since only a fraction (cellulose) of dietary fiber is fibrillar in nature. To correct this misnomer, other terms (e.g., plantix) have been proposed, but despite these efforts, the term 'dietary fiber' has survived.

This section deals with several different aspects of dietary fiber, including its determination and many of its physiological effects. Most of this information is relatively new and is the result of progress in research brought about in the last 30 years. The first part of this article will focus on the nature and composition of dietary fiber, its properties, and examples of sources of dietary fiber. This is followed by a definition of dietary fiber. Although this should be a relatively straightforward description, there are many different viewpoints concerning the nature and physiological effects that dietary fiber should have. These will be discussed in relationship to the physiological effects of dietary fiber.

Chemical Structure

The composition and structure of dietary fiber differ from plant to plant. It is also a function of the portion of the plant that is edible and the stage of maturation and is largely composed of the cell wall (structural) components that give the plant physical stability. As such, it is made of highly interlinked sugar-based and phenolic-based polymers (hemicelluloses, pectic substances, phenolics, glycoproteins, and proteoglycans) in a matrix of amorphous structure with some enmeshed cellulose microfibrils. The cell wall components are intimately linked together through various linkages including protein–sugar bonds.

Polysaccharide Fiber Components

Dietary fiber comprises carbohydrate and noncarbohydrate polymers; most of these are structural components. The carbohydrate polymers are often described as nonstarch polysaccharides (NSPs) to differentiate them from the (relatively) easily digested starch components of food. Figure 1 shows the proportion of the monomeric constituents of fiber polysaccharides from three food categories (11 cereals, 13 fruits, and 11 vegetables) and from canned mushrooms, peanuts, and baked white beans. As can be seen, arabinose is a major NSP component of cereals, peanuts, and beans. Xylose is present in large amounts only in cereals. Pectic substances (uronic acids) represent a substantial part of fruit, vegetable, peanut, and bean NSPs. Mushroom NSP is formed mostly of glucose residues. These results were based on the analysis of composites made of up to 20 individual foods purchased over a 30-month period using the Englyst gas–liquid chromatography method. A more recent version of this method would have given a higher proportion of xylose relative to glucose.

The different monomeric residues of Figure 1 are the basic units that form the major NSP macromolecular constituents shown in the upper part of Table 1. Cellulose and β-glucans are generally unbranched polymers containing mostly glucose residues. The β-glucans have mixed β-1,3 and β-1,4 interresidue linkages and are a constituent of dietary fiber from barley and oats. The glucose residues of cellulose are joined by β-1,4 linkages. In addition to their resistance to the enzymes of the human digestive tract, these linkages allow greater inter- and intrapolymer hydrogen bonding than the α-1,4 linkages of starch do. This allows the formation of tightly condensed crystalline regions within the cellulose microfibrils. Cellulose includes structurally amorphous regions where the presence of other sugars can be found. The final cellulose structure is a function of the glucose and nonglucose contents: the degree of cellulose crystallization is inversely proportional to the nonglucose residue content. Cellulose is a minor fraction of the total cereal dietary fiber but is a major fraction of dietary fiber in other foods. The proportion of cellulose in the dietary fiber of a food is not reflected by the glucose content of the NSP fraction, since glucose is also a major component of other fractions such as β-glucans and hemicelluloses.

Xylose is a part of the primary chain of hemicelluloses and gums and the secondary chain of gums and pectin. Arabinose is in the primary chain of gums and the secondary chain of hemicelluloses and pectin. Hemicelluloses constitute a major fraction of dietary fiber; this fraction is largely made of arabinose and xylose in cereals and of xylose and glucose in fruits and vegetables (Table 1). Pectic substances are prevalent in citrus fruits. They are also present in vegetables, legumes, and cereals in small amounts (Table 1). Other polysaccharide constituents are mainly represented by galactose or mannose in bananas. Nonstructural NSPs represent a small portion of dietary fiber. The monomeric NSP composition is of limited usefulness in predicting the properties and functions of dietary fiber. This is because the structural architecture of dietary fiber plays a large role in many of its physiological properties, and this structure cannot be predicted by its composition. Indeed, two dietary fibers with a similar component profile (e.g., wheat bran and corn bran) can have clearly different structures, even under optic microscopy, and can show different behaviors in the gastrointestinal tract.

Nonsaccharide Fiber Components

The minor components of dietary fiber that are not polysaccharides and, hence, not NSPs also play an important

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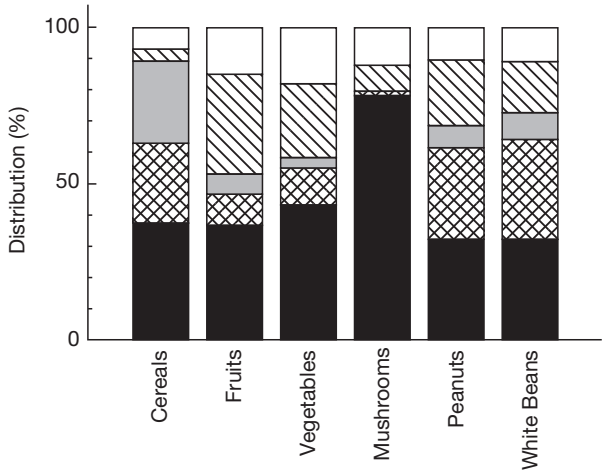

Figure 1 Distribution of the main polysaccharide constituents of dietary fiber from some foods and food categories. The values are means of $n=11$ cereals, $n=13$ fruits, and $n=11$ vegetables. Other values are for $n=1$. ▪: glucose; ⊠: arabinose; ▩: xylose; ⧅: uronic acid; and □: other sugars.

Table 1 Main macromolecular constituents of dietary fiber

Constituent	*Fruits and vegetables*	*Cereals*	*Legumes*
Polysaccharides			
Hemicelluloses			
Xyloglucans	X		X
Glucuronoxylans	X		
Arabinoxylans		X	
Glucuronoarabinoxylans		X	
Galactomannans			X
Cellulose	X	X	X
β-D-Glucans		X	
Pectic substances (pectin)	X	X	X
Others			
Lignin		X	
Phenolic esters	X	X	
Protein		X	
Glycoproteins	X		X

structural role. Table 1 shows the distribution of the nonsaccharidic polymers that are intimately associated with dietary fiber structure. These polymers include glycoproteins (fruits, vegetables, and legumes), proteins (cereals), phenolic esters (cereals), and lignins (lignified tissues of fruits, vegetables, and cereals). Lignin is of special interest because of its role in slowing down the fermentation of dietary fiber. It is a complex group of phenyl propane polymers formed by the condensation of aromatic alcohols and is especially important in conferring structural stability. As such, highly lignified tissues are found in the stems of plants such as trees and bushes and in the stalks of cereals. Lignification occurs at the expense of water and pectin as cells differentiate and mature. This increases cell wall rigidity in critical areas. Since edible plant tissues are consumed when relatively immature, their cells are largely undifferentiated and, largely, unlignified. Wheat bran and the small seeds covering strawberries are examples of lignified tissues. In most fruits and vegetables, lignin represents only a small part of dietary fiber, although it can be as high as 4% of dry matter in mature pears.

Cutin, waxes, or suberin may also be present in some tissues. These compounds are mixtures of lipids, proteins, and carbohydrates that form the waterproof covering and cuticle on the outer cell wall of plants. They are especially resistant to digestion and fermentation. As such, they impair the digestibility of other cell wall components and are usually found in the feces.

Structure

Models of primary cell walls of plants were often obtained from nonfood materials such as wood and tobacco. Because the cell wall material forms a large part of these plants, the models provide a wealth of information on the interactions between cell wall components such as carbohydrates and lignin and protein and carbohydrates. More recent reports have provided some structural information on the cell walls of plant foods. These reports have shown that, in carrots, protein–protein or protein–polysaccharide linkages may contribute to the formation of a rigid, inextensible cell wall. Other data have shown that protein may form up to 10% of cell walls in immature plants and appears to be important for structural cohesion. The major part of the flesh of fruits and vegetables contains undifferentiated types of cell wall that have significant amounts of highly branched hemicellulosic types of polysaccharides. The cellulose content is usually low, and cellulose is laid down in a more oriented arrangement in the matrix. This may reflect stresses on the plant. The cell walls are often rich in pectic substances and contain ~10% protein, as described earlier. These cell walls are thin and elastic with the cell form maintained by osmotic pressure.

In contrast, the outer layers of many seeds and nuts contain thick lignified cell walls that are difficult to break. These serve as mechanical protection to the seed within and as a vapor barrier to prevent desiccation.

Physical Properties

Soluble and Insoluble Dietary Fibers

It is possible to separate the total dietary fiber (TDF) into soluble and insoluble components based on solubility in water, but quantifying the amount of soluble fiber remains problematic. This is because the conditions utilized to measure the proportion of soluble fiber differ among laboratories (e.g., time, temperature, pH, and type of buffer; see the section '**Definition (Based on Function and Structure)**'). It should be noted that the distinction between soluble and insoluble fibers is somewhat artificial, since it is difficult to predict the actual fiber solubility in the gastrointestinal tract.

For many years, it was thought that soluble fiber was not commonly found in foods. Reports on pectin, guar, legume fiber, and oat bran appeared in the early 1960s, and currently, it is estimated that about a third of the daily TDF intake is soluble fiber. Pectic polyuronides are the major soluble dietary fiber components of vegetables and fruits, but the exact composition varies from plant to plant. When measuring dietary

fiber, one must also take into account the method of food preparation because the soluble fiber content of a fiber source is influenced by the method of food preparation. For example, some polyuronides are susceptible to depolymerization at the high temperatures used to cook the foods. Similarly, some insoluble fiber is susceptible to depolymerization at higher temperatures and may be found in the soluble fiber fraction.

In addition to the distinction between soluble and insoluble fibers, viscosity may also be an important fiber characteristic. This is because viscous fibers modestly lower blood cholesterol and triacylglycerol concentrations and attenuate the postprandial glucose response. It is thought that the increased viscosity of the intestinal lumen reduces the rate of diffusion of glucose, cholesterol, and triacylglycerols to their respective receptors along the intestinal wall, reducing absorption. The results of a recent meta-analysis using several different clinical studies showed a statistically significant lowering of plasma triacylglycerols and cholesterol in subjects fed for at least 14 days with a diet supplemented with oats, psyllium, pectin, or guar gum, but the effect was modest: a decrease of $\sim 1\ \mathrm{mg\ dl^{-1}\ g^{-1}}$ of soluble fiber was observed. If we consider that an individual with moderately higher blood cholesterol has a total serum cholesterol value of $\sim 300\ \mathrm{mg\ dl^{-1}}$, the change brought about from the consumption of viscous, soluble fibers is on the order of 0.3%. Considering that oat bran contains about 20% fiber by weight and only half of this is soluble, one can calculate that 10 g of oat bran per day is needed for each 0.3% change in total cholesterol.

Insoluble dietary fiber is, by definition, the fraction of the TDF that is not soluble in hot buffer solution. However, the measured proportion of insoluble fiber can vary depending on the dietary fiber methodology. Insoluble dietary fiber consists primarily of hemicellulose, cellulose, and lignin. It is often incorrectly believed that insoluble fiber is not fermented to any great extent by the bacteria present in the large bowel. However, actual measurements of fiber fermentability have shown that a significant proportion of insoluble dietary fiber is fermented. This is true even for wheat bran, which has a relatively lower insoluble fiber fermentability of between 30% and 40% (~90% of the wheat bran TDF is insoluble dietary fiber). The fermentability of oat bran is even greater – up to 80% of the insoluble fiber (approximately half of the TDF) is fermented in the human large intestine. The degree of fermentability is a property unique to each dietary fiber and depends largely on the nature and the structural arrangement of the fiber components and also on other physical characteristics such as particle size. For example, the structure of coarse wheat bran favors a high water-holding capacity (see later) and a slow rate of fermentation. This permits the microtrapping of slowly released fermentation gases, increasing fecal bulk and stimulating defecation.

Water-Holding Capacity

Dietary fiber holds water by adsorption and absorption. Some water is also retained outside the fiber matrix (free water). The particle size, chemical composition, and structure of dietary fiber influence the water-holding capacity. The *in vitro* water-holding capacity cannot be used to predict the impact of highly fermentable fiber on colonic function.

Table 2 Effect of grinding on the properties of wheat bran insoluble fiber[a]

Mesh sieve	*Aperture*[b] *(μm)*	*MPS (μm)*[c]	*WHC*[d]	*Glycocholate binding*[e]
As is		800	9.5 ± 0.1	26.5 ± 1.5
20	840	420	8.1 ± 0.1	24.9 ± 1.2
40	420	280	6.6 ± 0.2	23.8 ± 0.8
60	250	180	5.8 ± 0.1	22.1 ± 1.0
80	175	160	5.6 ± 0.1	21.5 ± 0.3

[a]Residue after neutral detergent and porcine pancreatic α-amylase treatments.
[b]Aperture size of sieve used with a Wiley mill, intermediate model.
[c]MPS, geometric mean particle size.
[d]WHC, water-holding capacity, grams of water per gram of insoluble fiber, using the centrifugation method; mean ± SEM of three measurements.
[e]Grams of glycocholate bound per 0.2 g of insoluble fiber; mean ± SEM of four measurements.

Although lettuce fiber isolates retain much water, the water-holding capacity expressed in grams of water held by fiber per gram of edible portion of food has been found to be higher for wheat bran fiber (4.5 g) and carrot fiber (2.1 g) than for that of lettuce and various other foods (0.3–1.3 g). The water-holding capacity of fiber from the bran of ready-to-eat breakfast cereals is positively correlated ($r \geq 0.85$ and $P < 0.05$) with its mean particle size (MPS): the water-holding capacity of 160 μm MPS wheat bran fiber is 59% of that of 800 μm MPS wheat bran fiber (Table 2). A large portion of the water held by wheat bran fiber appears to be free water.

The water-holding capacity has a significant effect on fecal output and stool hardness, as discussed earlier. These factors are important physiological effects of wheat bran and contribute significantly to its laxative effects.

Bile Salt Binding and Serum Cholesterol Reduction

There is considerable interest in the cholesterol-reducing properties of dietary fiber. This is because the influence of serum cholesterol (low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, in particular) on cardiovascular disease is considerable. Three different mechanisms have been proposed to account for the serum cholesterol-reducing properties of dietary fiber. As discussed earlier, a certain soluble viscous fiber may modestly lower cholesterol by increasing luminal viscosity and preventing cholesterol (re)absorption. Acetic and propionic acids, produced by the fermentation of dietary fibers in the gut, are thought to inhibit liver cholesterol synthesis at the metabolic level. Dietary fibers also have the ability to bind cholesterol directly: deconjugated bile salts are bound to pectic substances by hydrogen bonding, and lignin appears to bind bile salts through hydrophobic interactions. The actual *in vivo* mechanism is unknown, and it is likely that different fibers operate through different combinations of all three mechanisms to influence blood cholesterol levels.

In vitro evidence suggests that the physical form of the fiber may also play an important role in binding bile salts. In commonly ingested ready-to-eat cereals, glycocholate binding ($r = 0.90$, $P < 0.001$) and taurocholate binding ($r = 0.86$, $P < 0.05$) have been positively correlated with the MPS of the

neutral detergent fiber (NDF). Reducing the particle size of wheat bran NDF from 800 to 160 μm reduces its glycocholate binding by 19% (Table 2). The bile salt-binding capacity of fiber isolates, although method-dependent, may reflect events in the terminal small intestine. Purified fiber fractions may be poor (cellulose) or strong (lignin) binders. Some rice bran appear to have a high capacity to bind bile salts.

Human cholesterol-lowering studies have linked the consumption of dietary fiber with lower total plasma cholesterol and increased LDL/HDL ratios in addition to reductions in serum triglycerides. Studies such as these have suggested that cardiovascular risk may be reduced by consuming particular types of foods high in dietary fiber, most notably whole cereal grains. However, it should be noted that coronary heart disease (CHD) is a complex disease and is affected by many factors. For example, the reduction in CHD observed in a recent study was larger than would be expected from the beneficial effects of soluble dietary fiber on serum cholesterol levels only. Thus, reductions in CHD are likely to be the result of many factors, one of which is TDF intake.

Cation Exchange Capacity

Cation exchange is partly dependent on the presence of uronic acid in the nonesterified form. The preparation of the fiber material may decrease the number of nonesterified carboxyl groups and the apparent cation exchange capacity. Wheat bran contains little uronic acid, and its cation exchange capacity is mainly due to diffusion within the dietary fiber network. The cation exchange capacity of fiber from different sources is difficult to compare, unless it is expressed per edible portion. According to the affinity of minerals for carboxylic acid groups, cabbage and coarse wheat bran show a high cation exchange capacity compared with pectin.

There has been some concern that the ability of dietary fiber to bind minerals (as measured *in vitro*) may lead to mineral deficiencies in individuals consuming high-fiber diets. In North American diets, calculations show that mineral intakes far exceed the potential binding capacity of dietary fiber so that no need for concern arises. In addition, minerals that are bound to fibers or that are trapped in the (as yet undigested) cell wall matrix may not be absorbed in the small intestine but could be partially released and absorbed in the colon when the fiber is degraded by bacteria. Absorption of minerals in the colon has been suggested as a mechanism for accounting for increased mineral absorption in rats fed with fructooligosaccharides. This may explain why cation adsorption has not been consistently related to mineral bioavailability. However, wheat bran fibers can permanently bind heavy metal ions to decrease their toxicity.

Viscosity and Gelling Properties

As mentioned earlier, certain soluble fibers, such as oat β-D-glucans, are viscous when dissolved in water, while others, such as pectins, show gelling properties. These fibers influence gastric emptying and absorption rates in the small intestine. Direct measurement of the viscosity produced by concentrations of fiber likely to be used in diets has shown little effect of low-methoxy pectin, a slight increase with wheat bran, and a significant increase with high-methoxy pectin. Some of the nonstructural dietary fiber components can also increase viscosity.

Microbial Degradation (Fermentability)

Most dietary fiber remains undegraded until it reaches the large intestine, where the extent of fermentation depends on the source and several other factors, including the physical structure of the fiber, presence of specific components in the fiber matrix, nitrogen source, bacterial adaptation, and transit time. It is generally accepted that soluble fiber is almost completely fermented in the large intestine, but unfortunately, it is also generally believed that insoluble fiber is not fermented. Actually, both insoluble and soluble fibers are extensively fermented. On average, 70–80% of the TDF from mixed diets (e.g., fruits, vegetables, legumes, and most cereals) is degraded by colonic bacteria. Since insoluble dietary fiber is the major dietary fiber fraction, representing approximately two-thirds of the TDF in Western diets, this means that insoluble fiber is highly fermented. Indeed, as indicated in the preceding text, 70–80% of the insoluble fiber from oat bran and up to 40% of the insoluble dietary fiber from wheat bran are fermented.

There is some indication that fermentability depends on particle size, with small particle sizes being more readily fermented, but this has not been consistently reported, and the effect appears modest. In rats fed with purified diets containing 15% hard red wheat bran, NDF fermentability was largely unchanged (34–36%) when the MPS varied from 1275 to 394 μm. Similar trends were observed when American Association of Cereal Chemists soft and hard wheat bran were fed at various MPSs. In these experiments, 37.3–37.6% and 30.0–32.8% of the NDF were fermented, respectively.

Interaction between Structure and Physiology

Food Processing

The physicochemical properties of dietary fiber per se are dependent on the chemical composition and the structural characteristics of the fiber. However, as noted earlier, knowing the former does not allow one to predict the latter. When considering the potential *in vivo* effect of dietary fiber, one must also take food processing practices into consideration. Many different types of food processing practices are currently employed. Foods can be boiled, canned, frozen, blanched, parboiled, extruded, adiabatically extruded, and milled. All processes can have an effect on dietary fiber content, as measured by current methodologies. For example, heat treatment can break glycosidic linkages in dietary fiber polysaccharides. This can solubilize some of the insoluble fiber and reduce the TDF content if the polymers are broken down into small molecular mass fragments. Heat treatment can also reduce the overall length of the polysaccharide chains to lower viscosity and the water-holding capacity of the fiber. The pentosans in dietary fiber can react with amino acids such as lysine (Maillard reaction) and form new polymers that do not behave physiologically like dietary fiber. The boiling of vegetables increases the apparent TDF content, but this seems to be due to the loss of nonfiber components to the water.

It is often difficult to predict beforehand the effect of a food processing method on the TDF and the distribution between insoluble and soluble fractions. For example, extrusion cooking of some cereal bran apparently increases its water-holding capacity *in vitro*, although this does not translate into a change in fecal water-holding capacity *in vivo*. Extrusion cooking of wheat bran not only causes small decreases in the TDF and insoluble dietary fiber but also gives rise to small increases in soluble dietary fiber. Processing of rice (abrasion or parboiling) removes the seed coat and the majority of the dietary fiber. Some collapsing of the fiber structure also occurs during food preparation and/or mastication due to the reduction of particle size, and the fiber structure of sources such as spinach may be modified by cooking.

Milling is known to significantly affect TDF fermentation in the human colon. Cereal grains have a tough outer seed coat that would be completely resistant to digestion if the cereals were not milled prior to consumption. After milling, the thin-walled dietary fiber structures from the endosperm are partly disrupted and, thus, easily broken during fermentation. Milling, therefore, not only makes the endosperm accessible to the digestive enzymes in the upper digestive tract but also makes the dietary fiber more accessible to colonic bacteria. However, milling can also negatively affect the physiological properties of the dietary fiber in grains. While coarse grinding and adequate mastication appear to be sufficient for the digestion of cereals, fine grinding disrupts much of the cell wall architecture, and further milling can even interfere with its determination by gravimetric methods. Table 3 shows the effect of milling on the dry bulk volume of wheat bran, as measured in a volumetric cylinder. The volume of the coarse bran was reduced from 5 ml g^{-1} to <2 ml after grinding in an M5 Wiley mill using a 0.5 mm screen. Fiber from different sources ground under the same conditions may behave differently during milling, so that their resulting particle sizes may be different.

As discussed earlier, the particle size of unfermented wheat bran fiber may be related to colonic function and fecal characteristics in humans and laboratory animals. Finely ground cellulose or even wheat bran, for example, may have a constipating effect, whereas coarsely ground wheat bran prevents constipation. The influence of particle size on fecal bulking has been measured directly in rats. When rats were fed with diets containing 12% hard wheat bran, the fecal wet density was 0.796 ± 0.010 (mean $\pm$ SEM, $n = 12$) in the coarse bran group (geometric MPS: 850 μm) but increased to 0.888 ± 0.013 in the fine bran group (geometric MPS: 308 μm). This increased density was not due to a change in fecal weight but was due to a decrease in fecal volume, paralleling the effect demonstrated for bran in Table 3. In ready-to-eat breakfast cereals, the fiber MPS has been found to vary between 350 and 2000 μm. Increased particle size normalizes gastrointestinal transit time, delays gastric emptying, and may also affect the extent of insoluble fiber fermentation. Although dietary fiber has many positive effects on the digestive tract, excessive milling of dietary fiber to produce small particles in the range of 7–70 μm may have negative effects. Smaller particles do not exhibit the transit time normalization associated with larger bran particles, and the persorption of a small amount of very fine fiber particles may occur in some regions of the intestinal wall. Contrary to persorbed starch granules, persorbed fiber particles are not degraded in the blood and may impose an increased workload on the kidneys. Data on persorption are scarce, and much work is needed on this subject.

Table 3 Effect of grinding on dry bulk of wheat bran (ml g^{-1}): duplicate measurements

Sieve aperture[a]	*Bran A*[b]	*Bran B*	*Bran C*	*Bran D*
As is	3.1	3.4	5.1	3.5
2.0 mm (10 mesh)	2.7	2.4	2.8	2.1
1.0 mm (18 mesh)	2.2	2.1	2.4	2.0
0.5 mm (35 mesh)	1.8	1.8	1.9	1.7

[a]Using an M5 Wiley mill. Mesh sieve equivalence is indicated in parentheses.
[b]Bran A was American Association of Cereal Chemists-certified soft white wheat bran; bran B–D were soft or hard wheat bran from other sources.

Studies with apples, apple puree, and apple juice have highlighted the link between the physical integrity of the cell wall and the beneficial effects of unprocessed dietary fiber, in this case, a lowering of the glycemic index of a food (the rise in blood glucose that accompanies the ingestion and digestion of a carbohydrate-containing food). Only the unprocessed apple had a reduced glycemic index; the apple puree, containing the physically degraded dietary fiber, was similar to apple juice in response. This demonstrates the importance of the physical encapsulation of available macronutrients within the fiber matrix. This effect has also been observed with rolled oats where higher glycemic indexes were observed with more finely cut oats. Studies such as these show that the integrity of the cell wall is important in bringing about beneficial physiological effects, even in the presence of viscous, soluble fiber types (β-glucans in the cell wall; see the section '**Bile Salt Binding and Serum Cholesterol Reduction**').

In Vitro Measurements

In vitro properties of fiber isolates have been used to estimate their potential *in vivo* effect. However, *in vitro* data should be used with caution. The measurements are influenced by the methods used to follow digestion *in vitro* and by the methods used to prepare the fiber. Other confounding influences are also present: the fiber residue obtained *in vitro* may have been altered by heat or chemicals or may still contain residual digestible materials. For these reasons, *in vivo* conditions generally result in a more extensive digestion than that measured *in vitro*.

Digestive Events

An important consideration when examining the physiological effect of dietary fiber is the fact that dietary fiber is concentrated and denuded only in the terminal small intestine. This means that the dietary fiber that is partially associated with digestible materials in the jejunum and duodenum may have different physical properties from those of an entirely denuded fiber matrix at the terminal ileum. In the large intestine, most of the soluble fiber and a large but variable portion of insoluble dietary fiber are degraded by the colonic microflora. As already

discussed, fermentation plays an important role in mediating many of the physiological effects associated with dietary fiber.

Definition (Based on Function and Structure)

In the early 1970s, it became evident that cellulose represents only a small part of the TDF content of foods. Prior to this, there was a tendency to equate dietary fiber with cellulose because that is what was measured using the old crude fiber method. While this helped reinforce the use of the term 'dietary fiber' (cellulose has a fibrillar structure), cellulose represents only a small part of the TDF content of foods. Thus, at that time, new methodologies were emerging, and it was clear that there was a need for a dietary fiber definition that was linked to the actual physicochemical composition of dietary fiber.

It is in this context that the dietary fiber hypothesis appeared, a few years after Cleave's hypothesis in 1956, which related refined foods and Western diseases. The dietary fiber hypothesis thus appeared as a refocusing of Cleave's original ideas, reversing the original emphasis to become a diet high in unrefined edible plant material that is protective against Western diseases of the bowel. The dietary fiber hypothesis is important in understanding the 'dietary fiber concept,' since it links botanical structure to digestive processes and health outcomes – mainly noninfective colonic diseases such as constipation, diverticular disease, irritable colon, ulcerative colitis, appendicitis, hemorrhoids, polyps, and cancer of the large bowel. It is this link to these and other health outcomes that has characterized much of the work in the dietary fiber field over the last 30 years. The first dietary fiber definition in 1972 sought to describe the plant structures and components that were responsible for mediating these health benefits: "that portion of food which is derived from cellular walls of plants which is digested very poorly by human beings... Fibre is composed largely of cellulose,... hemicelluloses, pentosans, pectin and lignin."

There are many different definitions of dietary fiber. However, they all include certain specific and critical aspects. First and foremost, a definition must include the fact that dietary fiber corresponds mostly to the structural material in the edible part of plant foods in the usual human diet. This has been consistently reflected throughout the decades by the inclusion of the term 'plant cell wall' when referring to dietary fiber (Table 4). The term 'dietary fiber' itself is something of a misnomer, since dietary fiber is not fibrillar (except for cellulose). To reflect this fact, many other terms have been proposed over the years, including unavailable carbohydrate and plantix. None of these terms have succeeded in replacing 'dietary fiber.'

Another important aspect of any dietary fiber definition is that it is resistant to human digestive secretions (including enzymes) and not absorbed in the small intestine. The observation that dietary fiber increased fecal weight initially gave the impression that dietary fiber was resistant to both human digestive secretions and bacterial digestion in the lower gut (fermentation). While it is true that dietary fiber is not digested and absorbed in the small intestine, a large proportion of dietary fiber is fermented in the colon. The extent of this fermentation is a function of several different factors (see the section 'Microbial Degradation (Fermentability)'), meaning that the extent of fermentation cannot be used as a criterion to define dietary fiber.

Using 'resistance to endogenous digestive secretions' alone as a criterion may be confusing. For example, some dietary fiber may be partly degraded by acids in the stomach, while

Table 4 Use of the term 'plant cell wall' in defining dietary fiber over time

Year	*Text*	*Reference*
1885	The 'cell membranes' of Rubner	Trowell, H. C. (1975). In: Burkitt, D. P. and Trowell, H. C. (eds.) *Refined carbohydrate foods and disease*, p. 43. London: Academic Press
1929	The 'skeletal framework of the plant'	McCance, R. A. and Lawrence, R. D. (1929). The carbohydrate content of foods. *Medical Research Council special report series* No. 135, pp. 1–173. London: HM Stationary Office
1981	The 'remnants of plant cells'	AOAC Fiber Consensus (1981). *Association of Official Analytical Chemists 95th Annual Meeting*, October 1981, Washington, DC
1985	'Derived from plant cell walls and not digested by human alimentary enzymes'	Trowell, H., Burkitt, D. P. and Heaton, K. (eds.) (1985). *Dietary fibre, fibre-depleted foods and disease*. London: Academic Press
1990	The 'structural plant cell wall composed of polysaccharides and lignin'	Eastwood, M. (1990). Function of dietary fibre in the large intestine. In: Southgate, D. A. T., Waldron, K., Johnson, I. T. and Fenwick, G. R. (eds.) *Dietary fibre: chemical and biological aspects*, pp. 211–219. Cambridge: Royal Society of Chemistry
1995	'The "cell wall material of plant foods" responsible for the physical form and texture of unprocessed plant foods'	Heaton, K. W. (1995). The dietary fibre concept – time for a re-evaluation? In: Sørensen, A., Bach Knudsen, K. E., Englyst, H. N., Gudmand-Høyer, E. and Nyman, M. (eds.) *Metabolic and physiological aspects of dietary fibre in foods: recent progress in the analysis of dietary fibre, COST 92*, p. 15. Luxembourg: Commission of the European Commission
1995	The 'plant cell walls' are the common characteristic of plant foods that constitute a high-fiber diet associated with beneficial effects	Cummings, J. H., Hudson, G. J., Quigley, M. E. and Englyst, H. N. (1995). The classification and measurement of dietary carbohydrates. In: Sørensen, A., Bach Knudsen, K. E., Englyst, H. N., Gudmand-Høyer, E. and Nyman, M. (eds.) *Metabolic and physiological aspects of dietary fibre in food. Recent progress in the analysis of dietary fibre in food, COST 92*, pp. 17–36. Luxembourg: European Commission
1999	'The cell walls of edible plant tissues in the traditional human diet'	Mongeau, R., Scott, F. W. and Brassard, R. (1999). Definition and analysis of dietary fiber. In: Cho, S. S., Prosky, L. and Dreher, M. (eds.) *Complex carbohydrates in foods*, pp. 305–316. New York & Basel: Marcel Dekker

some small intestine-digestible material can reach the colon where bacterial fermentation occurs. In addition, a definition based on the resistance to digestibility by endogenous digestive secretions could be problematic if the material is not specified as coming from the edible part of plant foods. Indeed, such a definition could be erroneously interpreted as meaning that dietary fiber is any material resistant to *in vivo* digestion or even as any material measured as dietary fiber by a specific dietary fiber method. Among the materials to be excluded from the dietary fiber definition are 'ruminant fiber' and the products formed during processing and/or cooking. There have been requests to include some other plant components and manufactured polymers, and this is a subject of much debate. Among the criteria that could be used to accept or reject a material as dietary fiber, only 'edible plant cell wall' is specific enough to allow an absolute definition. In addition, it links current dietary fiber definitions to the original 'dietary fiber concept,' which recognized that a diet high in minimally processed edible plant foods provided many health benefits. Confusion often arises because of the overlapping characteristics and physiological effects of the different 'candidate' materials with those of a dietary fiber 'reference.' Examples of 'candidates' are polydextrose (a randomly bonded synthetic glucose polymer), inulin and fructooligosaccharides (naturally occurring glucose–fructose oligomers and polymers), and resistant starch (formed by food processing methods and largely undigested in the small intestine). This overlapping means that it becomes virtually impossible to distinguish between fiber and nonfiber material based on resistance to digestion alone. Debates such as this started shortly after dietary fiber became popular in the 1970s. They are still ongoing, as demonstrated by the publication of a recent international survey of 147 professionals. While a complete discussion of the merits of either side is beyond the scope of this article, it is important to remember the origins of the dietary fiber definition and the dietary fiber hypothesis that firmly linked fiber to physiological outcome.

The definition of dietary fiber was first centered on the 'original dietary fiber concept' that linked the consumption of 'plant cell walls' in unrefined, traditional foods to positive physiological outcomes. Table 5 shows two definitions that were proposed after the turn of the millennium. These two definitions have taken the original ideas of the dietary fiber hypothesis into account and included a reference to some specific physiological effects in humans.

While the newer definitions of dietary fiber specifically mention the fact that dietary fiber has physiological benefits, the list is rather specific and deals primarily with effects observed with viscous fibers (lowering postprandial serum glucose and lowering serum cholesterol) or effects brought about from partial fermentation and water-holding capacity (laxation). These effects are well supported by clinical experiments with laboratory animals and with humans. However, the original dietary fiber concept related dietary fiber intake to the diseases of Western man, including heart disease, cancer, and obesity. As mentioned earlier, the link between viscous dietary fiber and heart disease is supported in the literature through its effect on serum cholesterol and triglycerides, but the link with other Western diseases has been much harder to demonstrate. It has been well established that eating patterns that favor foods high in dietary fiber and low in red meat significantly reduce the risk of cancer, heart disease, and obesity. However, it has been relatively difficult to isolate this effect to dietary fiber consumption per se. Part of the problem comes from the almost exclusive reliance on epidemiological studies to demonstrate any association. There are several reasons why epidemiological studies are generally favored. First, these studies provide data on free-living humans, rather than inbred laboratory animals. Second, these studies follow the long-term effect of dietary fiber consumption on health outcomes. Third, the sample size can vary from a few hundred (case control studies) to many thousands of individuals (prospective cohort studies). This gives the studies good statistical power and lends weight to the conclusions. However, epidemiological studies also suffer from a number of shortcomings including problems with assessing food intake for the past 2–3 years (case control studies) or with monitoring food intake 2–3 times over a period of 5 years and using this to predict disease outcome 15 years later (prospective cohort studies), problems that arise from the use of incomplete dietary tables to calculate dietary fiber intake, and problems arising from the use of inappropriate methods for correcting energy intake.

The most striking conclusion from epidemiological studies is a failure to provide support for an inverse correlation between dietary fiber intake and colon cancer. This concept has long been entrenched in the literature largely because of studies with laboratory animals that have provided much data in favor of a protective effect of dietary fiber. The animal studies are supported by plausible physiological and biochemical mechanisms that can account for the observed results. However, the epidemiological data are inconsistent in its support for an inverse relationship between colon cancer and dietary fiber intake, and clinical trials that examined the effect

Table 5 Recent proposed definitions of dietary fiber

Year	*Text*	*Reference*
2001	'. . .the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine. . .promotes beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or glucose attenuation'	American Association of Cereal Chemists (2001). Report of the Dietary Fiber Definition Committee to the Board of Directors of the American Association of Cereal Chemists. 2001. The definition of dietary fibre. *Cereal Foods World* **46**, 112–129
2002	'Dietary Fiber consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants. Functional Fiber consists of isolated, nondigestible carbohydrates that have beneficial physiological effects in humans'	National Academy of Sciences (2002). *Food and Nutrition Board. 2002. Dietary reference intakes. Proposed definition of dietary fiber*. Washington, DC: National Academy Press

of diet on the reappearance of colon polyps (colon cell growths that have a 1/20 chance of forming a cancerous tumor) found no protective effect of dietary fiber. Even though the link between adenomatous polyps and cancer is not straightforward, these studies show that more research is needed to elucidate the relationship between the known physiological benefits of dietary fiber and cancer.

Sources of Dietary Fiber

The TDF content of a food depends on many factors, including (1) the plant variety, (2) the stage of maturity when harvested, (3) the plant-growing conditions, and (4) the method of food preparation. Although not all the methodological issues have been completely resolved with respect to measuring dietary fiber, there is now general consensus on the TDF content for a majority of foods.

The names of 49 common dietary fiber sources are provided in Table 6. The foods are classified according to their TDF content expressed on an 'as is' basis. Since some methodological variability is inevitable, the letters 'E,' 'M,' and 'P' in the right-hand column designate which methods would give TDF values in the range shown. Other methods not included in the comparison are likely to agree with these values. The letter 'E' refers to NSPs as measured by the Englyst GLC procedure and includes a lignin value measured separately with potassium permanganate. The letter 'M' refers to the Mongeau rapid method, and the letter 'P' refers to the Prosky method. The last two methods are gravimetric and include lignin in the final determination. Table 6 shows a considerable agreement between methodologies: 33 of 49 foods are designated 'EMP,' meaning that all three methods give similar values. In addition, three foods are designated 'MP' and 13 foods 'M' only because sample size limitations precluded measurement with other methods. The TDF values are based on winter and summer collections of up to 20 individual foods over 30 months in different locations.

Table 6 Sources of dietary fiber

TDF[a]	*Foods*	*Methods*[b]
0.1–0.5	White rice, cooked	M
	Orange juice from concentrate	M
0.6–0.9	Lettuce	M
	Cucumbers	EMP
	Pineapple, melons (cantaloupe)	EMP
1.0–2.2	White bead	EMP
	Corn kernel (canned), potatoes (boiled), green beans (boiled), cabbage (raw), celery (raw), onion (cooked), green peppers (raw)	EMP
	Cauliflower (cooked), tomatoes	M
	Apple, bananas, orange, pears, strawberries, cherries, grapefruit, plums	EMP
	Apple pie, blueberry pie, pasta (cooked)	M
2.3–3.0	Rye bread	M
	Mushroom ($E = 2.0$)	MP
	Beets (canned), rutabaga (raw), carrots (raw), broccoli (raw)	EMP
	Blueberries ($E = 1.9$)	MP
3.1–4.3	Brown rice	EMP
	Raisins (seedless)	M
4.4–6.4	Bran muffins, wheat cereal, wholemeal bread	EMP
	White beans (baked)	EMP
	Kidney beans (baked), peanut butter	M
6.5–7.3	Peanuts	EMP
	Green peas (boiled)	M
8.0–9.3	Oatmeal cereal	EMP
9.4–10.3	Shredded wheat	EMP
12.3–13.1	Bran flakes	EMP
15.5–15.8	Oat bran	EMP
40.6–40.9	AACC hard wheat bran ($E = 36.4$)	MP

[a]TDF, total dietary fiber range in grams per 100 g on an 'as is' basis.
[b]Methods: E, Englyst GLC method plus potassium permanganate lignin; M, Mongeau rapid gravimetric method; and P, Prosky gravimetric method.
Source: Mongeau, R. and Brassard, R. (1989). A comparison of three methods for analyzing dietary fiber in 38 foods. *Journal of Food Composition and Analysis* **2**, 189–199; Mongeau, R., Brassard, R. and Verdier, P. (1989). Measurement of dietary fiber in a total diet study. *Journal of Food Composition and Analysis* **2**, 317–326.

Most fruits and vegetables contain low amounts of dietary fiber (1.0–2.2% TDF) on an 'as is' basis. Legumes contain intermediate amounts of dietary fiber (around 4% TDF), and cereals contain between 1–2.2% TDF (corn kernels) and 15.5–15.8% TDF (oat bran). The dietary fiber content of foods is greatly influenced by the moisture content. Thus, cereal TDF values are high because these foods contain little moisture (up to 10% at most), whereas fruits and vegetables contain 80–90% moisture on an 'as is' basis. The values of Table 6 are reported as a percentage of weight, but when calculating dietary fiber intake, it is important to factor in the serving size (30 g for breakfast cereals, 50 g for lettuce and raisins, and 125 g for vegetables and fruits). For example, 1 serving of bran flakes will provide ~4 g of dietary fiber, 1 serving of lettuce will provide 0.35 g of TDF, 1 serving of raisins will provide about 1.8 g of TDF, and 1 serving of corn will provide about 2 g of TDF.

See also: Cellulose; Dietary Fiber: Bran; Dietary Fiber: Determination.

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Dietary Practices

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How to Build the 'Perfect Diet'?: A Methodological Approach

From the era of the 'fathers' of medicine, like Aesculapius, a large attention has been given to a correct diet as the best way to maintain health. During the last century, we have sampled many data on diets or specific diet components that could have positive or deleterious effects on health. Nevertheless, what kinds of data are available?

Preclinical Data

Preclinical data are those derived from test performed in cellular models or in animals. They are usually carried out on single components of the diet or of a nutrient and furnish information on the pharmacological activities of foods or food components. Even if the obtained results are usually of scientific interest, the proposed models do not adequately represent the complexity of human diets and human metabolism, so they can only constitute a basis for further research on humans.

Epidemiological Data

Epidemiological data relate the assumption of specific dietary components or specific dietary patterns with the risk of developing a disease. They are actually the milestones on which we built dietary guidelines and furnish dietary advices. They are particularly relevant when obtained from large population samples followed up for years with a standardized methodology. The main limitations of epidemiological data are the following:

- They are population-specific: It is not immediately true that a protective habit for a population is also protective for a different one because other factors could be involved, such as other dietary components, physical activity, environment, and genetics/genomics. Moreover, the dietary questionnaires used in various studies are frequently different, and they are not always validated in different populations.
- They are usually 'old': During the time in which the study is carried out (from years to decades) and the data are analyzed, the dietary and behavioral habits of the population could have changed. A typical example is the one of the Mediterranean diet, codified on the basis of the southern Italian habits in the 1950s, which are totally different from the current ones.
- They are usually related to a lifelong exposition to a specific diet or diet components. Nonetheless, observing that the lifetime exposition to a diet component seems to be protective against the development of a given disease does not immediately mean that it will be protective when introduced/increased in the diet starting from the middle or old age.

Clinical Data

Data derived from clinical trials represent the best source of evidence. They clearly show how changing a diet pattern or increasing/reducing a specific diet component is associated with a modification of biological parameters (usually a laboratory or instrumental biomarker) in humans. The main limitations of clinical trials are the large number of concomitant parameters to be controlled (both in the diet and outside the diet) and the lack of long-term large trials with hard outcomes (morbidity or mortality).

Systematic Reviews/Meta-analyses/Metaregressions

Finally, we could 'cumulate' the evidence derived from epidemiological studies or clinical trials in order to obtain a general conclusion on a specific dietary exposition or intervention on specific parameters of human health. These analyses, when available, usually constitute the basis for new guidelines, even if their value could not be absolute for the single subject or a specific subpopulation. Then, their conclusion does not always reflect a final answer to a scientific question, especially when the available trials are strongly heterogeneous as it regards design, number, and kind of subjects enrolled and other study characteristics. Thus, from all these data, we still have no definitive idea about the 'perfect diet' to be adopted by the general population in order to conduct a healthy and prolonged life, but we can highlight some milestones.

General Rules for the General Population

Large-scale unfocused interventions on lifestyle habits have been proved to be needed but hardly cost-effective. The best way to plan dietary suggestions in order to improve the global health of a whole population, apart from the individual needs, is to attentively detect, between the most recent scientific literature, the most evidence-based milestones of a healthy diet and healthy lifestyle, avoiding in particular to focus on intermediate outcomes, but rather concentrating on morbidity and mortality. Moreover, the suggestions have to be as short, clear, and simple as possible, feasible by the subjects, and possibly transversally useful to prevent the most common chronic diseases in a population. To do this, a possible way consists on focusing our attention on the results of the largest available systematic reviews and meta-analyses.

Overall, the main recent meta-analysis of clinical data suggests the following:

- Water is a milestone of a healthy diet.
- Any excessive energy intake has to be avoided: Since preclinical data show that a mildly reduced energy intake is per se associated with an increased life span, an adequate

energy intake is probably overall more relevant than any other dietary indications.

- Increased sodium intake was associated with an increased risk of stroke (~25%), stroke mortality (~60%), and coronary heart disease mortality (~30%).
- A diet with a low glycemic index (GI) and glycemic load (GL) is associated with a reduced risk of developing type 2 diabetes (T2D) and coronary heart disease (CHD). In particular, the risk of developing T2D is 33% higher in subjects in the highest quintile of energy-adjusted GI than those in the lowest one. Participants who consume a combination diet high in GI or GL and low in cereal fiber have an ~50% higher risk of T2D. GL appears to be more relevant than GI in increasing the risk of CHD (~50%) and stroke (~20%) in a dose-dependent manner.
- Overall, nonrigid vegetarian diets (neither vegan nor fruitarian) are associated with a lower risk of developing cardiovascular diseases, cancers, and autoimmune diseases.
- A diet rich in cereal and vegetable fiber is associated with a dose-dependent reduction of developing cardiovascular disease (~9% per 7 g per day) and various gastrointestinal (~10–40% per 10 g per day) and some nongastrointestinal cancers. The total intake of fruit and vegetables had a negative association with risk of asthma in adults and children (~45%).
- Red meat is per se not significantly associated with total or cause-specific mortality; conversely, the intake of processed meat was associated with a 30% higher rate of cardiovascular disease (per 50 g per day) and higher cancer mortality. Preservatives are the notable difference; the calculated blood-pressure effects of sodium differences (~400% higher in processed meats) explain most of the observed higher risk. ~3% of deaths could be prevented if all participants had a processed meat consumption of less than 20 g per day. Moreover, increasing the intake of red meat of more than 0.50 servings per day is associated with an increased risk of developing T2D of ~55% while reducing the intake of the same amount leads to a decreased risk of ~15%.
- The highest category of fish consumption (i.e., $\geq$4 times per week) is associated with the greatest risk reduction in CHD (~20%). In dose-response analysis, each additional 100 g serving of fish per week was associated with an ~5% reduced risk.
- There is a linear association of consumption of total dairy products, low-fat dairy products, cheese, and yogurt and the risk of T2D. The relative risk reduction is ~5% and ~10% for 200 g per day total and low-fat dairy consumption, respectively. The relative risk reduction is ~20% and ~10% for 30 gper day cheese and 50 g per day yogurt consumption, respectively.
- Dietary consumption of saturated fatty acids and cholesterol is not significantly associated with an increase in cardiovascular disease risk, if not because usually assumed with high-energy diet.
- Extreme quintiles of total trans fatty acid intake (3–10 g per day) are associated with an increased risk of ~20% of CHD and ~25% of fatal CHD.
- The consumption of nuts is inversely associated with fatal CHD (~25% less risk), nonfatal CHD (~20% less risk), and T2D (~10% less risk).
- Coffee intake is associated with a lower risk of mortality for every cause (high vs low category of coffee consumption: RR = 0.86). In particular, this seems to be related to a reduction in the risk of developing cardiovascular disease, T2D, and some cancers too. Moreover, coffee consumption seems associated with a lower risk of developing seropositive rheumatoid arthritis, urolithiasis, and Parkinson's disease.
- High-quality chocolate intake is associated with a reduced risk of developing stroke of ~20%.
- Low alcohol intake (from every source, not necessarily from red wine) is associated with a reduced risk of CHD (~20%), total stroke (~15%), ischemic stroke (~20%), and stroke mortality (~30%), but heavy alcohol intake is associated with an increased risk of total stroke (~20%). Moreover, compared with lifetime abstainers, the RR for T2D among men is most protective when consuming 22 g per day alcohol and becomes deleterious at just over 60 g per day alcohol. Among women, consumption of 24 g per day alcohol is most protective and becomes deleterious at about 50 g per day alcohol. Furthermore, low alcohol intake also seems to be associated with a lower risk of developing rheumatoid arthritis, urolithiasis, and Parkinson's disease. However, even a low alcohol intake is a risk factor for (among others) gout, atrial fibrillation, colorectal adenoma development, and upper aerodigestive tract cancer mortality.
- Potassium intake is associated with a reduced risk of developing stroke (~20%).
- Dietary magnesium (per 200 mg per day increment) is associated with a 22% lower risk of CHD. The association of dietary magnesium with fatal CHD is nonlinear, with an inverse association observed up to a threshold of ~250 mg per day, compared with lower intakes.
- For total calcium intake, each 300 mg per day increase is associated with an approximately 8% reduced risk of colon cancer. For supplementary calcium, each 300 mg per day increase is associated with a 9% reduced risk of colon cancer.

Overall, the available evidence suggests that a complete balanced 'Mediterranean-like' antioxidant and anti-inflammatory diet, mainly based on fresh vegetables and whole-grain carbohydrates (nearly vegetarian, not necessarily vegan), should be advised to most of the people in order to preserve and promote their health (Box 1). Most of the successful diets (Mediterranean, Asian, Atkins, and zone/dissociated) are low-

Box 1 Milestones of a Healthy Diet

- Total energy intake proportional to physical activity
- Low salt intake
- Carbohydrates with low glycemic index as the main source of energy
- Very low intake of simple sugars
- Large intake of water, fresh vegetables, legumes, and berries
- Fish and nuts and moderate amount of nonprocessed meat
- Moderate doses of dairy products (preferably fermented, rich in probiotics, and low-fat)
- Coffee, high-quality dark chocolate, and low quantity of alcohol (from either wine or beer) not forbidden

energy diets fundamentally based on these principles. Low-carb/high-protein diet could be considered for a short period in selected overall healthy subjects to reduce body weight and related cardiovascular risk factors, but then, a standard balanced diet has to be recovered as soon as possible. Extreme diets (high protein, vegan, etc.) have to be avoided, especially on the long term for the risk of developing deficit of intakes (for instance, of essential amino acids and some vitamins) or excessive intakes (for instance, of nonessential amino acids) of specific micro- and macronutrients.

Finally, all the potential suggestions previously reported increase their potential effect on global health if associated with a strong message on dramatic reduction of active and passive exposition to cigarette smoking and on an improvement of everyday physical activity amount.

The main advantage of this approach is that the same lifestyle suggestions seem to be useful to reduce the risk of cardiovascular disease, obesity, type 2 diabetes, nonalcoholic liver fatty acids, age-related degenerative maculopathy, the most frequent cancers, some degenerative neurological diseases, and rheumatologic diseases.

A factor that has to be considered is that a healthy diet is often slightly but significantly more expensive than an unhealthy diet, so, when formulating suggestions, the availability of the foods and their cost always have to be kept in mind. For instance, even if Asian goji berries have a very high antioxidant power, it is nonsense to recommend their consumption in Europe, where various much less expensive berries with high antioxidant power are available.

It is also relevant to remember that some of the previously reported suggestions do not count for every subject. For instance, obese, diabetics, hypertriglyceridemics, and patients affected by hyperuricemia have to consume fruits in moderation since they are a relevant source of energy and induce the synthesis of uric acid.

Another factor to be considered is the modality of cooking. Some foods (in particular, vegetables) have not to be cooked in order not to lose vitamins and minerals. The best ways to cook are with steam or on hot stones, less salting, boiling, or baking. Pasta and rice have to be boiled just the time to make them chewable (slightly underdone), but not overcooked, in order not to increase their glycemic index. Salt has to be limited at minimum and possibly substituted with spices (e.g., pepper, chili, and aromatic herbs), lemon juice, or vinegar. Vegetable oils (preferably extra virgin olive oil, less sunflower oil, corn oil, or rice oil), rich in n-6 fatty acids, which can be useful in the prevention of cardiovascular disease, have to be used without reaching their smoking point, that is, before they are peroxidized. Anyway, even though they are 'healthy,' vegetable oils constitute a relevant source of energy and thus have to be consumed in moderation. Animal fats have to be avoided; however, they are always preferable than modified trans-hydrogenated fats.

The Individual Approach

Mind over Food and Eating Disorders

If we only had our core systems such as the brain stem and hypothalamus, we would not probably have many of the problems linked to food, such as obesity and related diseases or eating disorders. The communication between the internal system of fuel availability and the hypothalamus, where hunger and the satiety centers are located, is regulated by a homeostatic control system through hormones capable of crossing the blood–brain barrier. In this way hunger, satiety, metabolic functions, and fat reserves are adjusted in the short and long terms.

The main molecules involved in homeostatic control are *leptin* and *adiponectin* (produced by the adipose tissue), *ghrelin* (produced by the stomach), *peptide YY* (produced by the intestinal cells), and *neuropeptide Y* and *agouti-related protein* (produced by the hypothalamus), where ghrelin and neuropeptide Y stimulate the appetite, while leptin and peptide YY inhibit the appetite.

With respect to cardiovascular disease risk, adiponectin is a 'good' adipokine due to its anti-inflammatory, antiatherogenic, antidiabetic, and cardioprotective effects and the promotion of good endothelial function. Leptin, on the contrary, could be considered a 'bad' adipokine given that it promotes insulin resistance, high blood pressure, atherosclerosis, MI risk, vascular inflammation, VSMC hypertrophy and proliferation, oxidative stress, and endothelial dysfunction.

Our modern lifestyle puts pressure on the homeostatic system. Many factors such as stress, anxiety, depression, and nervousness could influence our body weight. Palatable food high in fat, sugar, and salt can increase dopamine in the subcortical mesolimbic system (the ventral tegmental area, nucleus accumbens, amygdala, and hippocampus). This generates an intense pleasure and can corrupt neural functions of the brain system involved in hedonic, motivational, and cognitive processing. For this reason, it has been suggested that there is a possibility for the development of a food addiction. Further studies are needed to better describe these mechanisms and to understand how to utilize the modern neuromarketing to promote the consumption of healthy food.

An abnormal relationship with food can result in an *eating disorder* (ED). The main consequences of EDs are malnutrition, obesity, a worse quality of life due to the obsessive thoughts related to food, and increased mortality. They are commonly rare in the general population; the prevalence of all EDs is about 5%. Patients who suffer from an ED tend to deny the disease and avoid treatment. The derivation of EDs is psychological. The causes could be genetic or they could derive from other pathologies such as anxiety, depression, and alcoholism. The Diagnostic and Statistical Manual of Mental Disorders (5th edition) distinguishes EDs as anorexia nervosa and bulimia nervosa, which are common in adolescent women; binge eating disorders typical in men and adults; and not otherwise specified EDs. The therapy for EDs consists in a team treatment carried out by different professionals: psychiatrists, psychologists, nutritionists, nurses, and other medical professionals. The nutritional rehabilitation of an ED should provide a gradual modification of eating habits. The expectation is often not a complete cure, rather the improvement of the nutritional status and the quality of life of the individual.

Clinical Nutrition and Morbidities

Many diseases require a personalized nutrition for their care. The diet thus becomes an integral part of the therapy. If you

think you are suffering from one of these conditions, you should contact medical professionals and a nutritionist.

Overweight and obesity

The key *strong recommendations* for overweight and obesity recently published in the *Journal of the American College of Cardiology* are as follows:

- Advising overweight and obese individuals who would benefit from weight loss to participate for ≥ 6 months in a comprehensive lifestyle program that assists participants in adhering to a lower calorie diet and increasing physical activity through the use of behavioral strategies
- Prescribing on-site, high-intensity (i.e., ≥ 14 sessions in 6 months) comprehensive weight loss intervention
- Using a very-low-calorie diet (≤ 800 kcal day^{-1}) only in limited circumstances where a medical supervisor is required
- Advising overweight and obese individuals who have lost weight to participate in a long-term (≥ 1 year) comprehensive weight loss maintenance program

Cardiovascular disease and dyslipidemia

In order to lower the levels of total cholesterol and LDL cholesterol in heart diseases, the *American College of Cardiology* and the *American Heart Association* recommend normalizing body weight and reducing the consumption of saturated fats, cholesterol, and trans fats. Saturated fats are found primarily in dairy products, meats, and certain oils (coconut and palm oils). Trans fats are found in many snacks and fried foods and are listed on labels as 'partially hydrogenated oils.' The intake of soy protein and functional foods rich in phytosterols could play a positive role in reducing heart diseases. In order to lower triglycerides, it is useful to reduce the consumption of alcohol, simple sugars, and carbohydrates. Finally, a rise in unsaturated fats and constant physical activity could help increase our HDL cholesterol levels. However, in our attempts to cut 'bad fats,' we should be careful not to increase the intake of other potentially dangerous nutrients, such as sugars. Heart health is also linked to the adequate introduction of protective foods, such as fruits and vegetables, recommended by the World Health Organization in an amount of 400 g per person per day. Cholesterolemia being strongly genetically determined, recent guidelines also suggest to include in the everyday diet some specific nutraceuticals and functional foods in order to further reduce the LDL cholesterol plasma levels.

Diabetes

The *American Diabetes Association* recognizes the integral role of nutrition therapy in overall diabetes management. For individuals with type 1 diabetes, participation in an intensive flexible insulin therapy education program, using the carbohydrate counting meal planning approach, can result in improved glycemic control. A simple diabetes meal planning approach, such as portion control or healthy food choices, may be better suited for individuals with type 2 diabetes. In summary, people with diabetes should

- limit or avoid the intake of sugar-sweetened beverages;
- reduce sodium to less than 2300 mg per day, with additional reductions individualized for those who have a high blood pressure;
- eat fatty fish at least 2 times (two servings) per week (they do not benefit from the use of omega-3 (EPA/DHA) supplements for the prevention or treatment of cardiovascular diseases);
- not use vitamin or mineral supplements without proved deficiencies (additionally, there is no evidence supporting the use of cinnamon or other supplements).

Food allergies and food intolerances

Whereas a food allergy causes an immune system reaction, a food intolerance does not. In some cases, an allergic reaction to food can be serious, even deadly. Food intolerance symptoms generally appear gradually and are limited to gastrointestinal effects, whereas food allergy symptoms are more severe. If you have a food allergy, you will need to avoid the allergenic food completely. If you have a food intolerance, you may generally be able to eat a small amount of the food in question. *Lactose intolerance* is a common example of an enzymatic intolerance, which is due to a lack of lactase needed to digest lactose. *Celiac disease* is a permanent intolerance to gluten, a protein found in oats, wheat, spelled, Kamut®, barley, and rye. Indeed, the consumption of gluten, also in small quantities, can cause several more or less serious consequences. The *nonceliac gluten sensitivity* is today a still poorly defined condition. The aim of the scientific community is to test and find a definition of the specific markers and precise diagnostic criteria.

Inflammatory bowel diseases

Patients with inflammatory bowel diseases (IBDs) should generally avoid foods that worsen symptoms, eat smaller meals in frequent intervals, drink adequate fluids, and avoid caffeine and alcohol. IBD patients should eliminate dairy if they are lactose-intolerant, limit excess fat, reduce simple carbohydrates, and reduce high-fiber foods during the inflammatory phase.

Chronic kidney disease

People with a chronic kidney disease (CKD) need to make changes to their diet such as limiting fluids; eating a low-protein diet; limiting sodium, potassium, phosphorous, and other electrolytes; and paying attention to malnutrition. The diet can be changed if the kidney disease worsens and during dialysis.

Neurodegenerative diseases

Neurodegenerative diseases (NDs) are hereditary and sporadic conditions, characterized by a progressive nervous system dysfunction. They include Alzheimer's disease and other dementias, such as Parkinson's disease. The following guidelines suggest how to prevent and delay the symptoms:

- The intake of saturated fats and trans fats should be reduced to the lowest amount possible.
- Vegetables, legumes, fruits, and whole grains should replace meats and dairy products.
- Vitamin E should come from foods, rather than from supplements.
- A reliable source of vitamin B_{12} should be part of one's daily diet.

- If using multiple vitamins, choose those without iron and copper and consume iron supplements only when directed by your physician.
- Those who desire to minimize exposure to aluminum should avoid the use of cookware, baking powder, or other products that contain aluminum.
- Aerobic exercise should be included in an individual's routine.

Conclusion

A global healthy diet associated with a healthy lifestyle is advisable for the general population and has to be founded on some basic suggestions. The assumption of fresh foods, without the addition of salt, in amounts proportional to the energy expenditure, would be sufficient to prevent the largest part of chronic diseases in Western countries. Only subjects with specific diseases or characteristics should have a structured individual diet support.

See also: Alcohol: Metabolism and Health Effects; Aluminum: Properties, Presence in Food and Beverages, Fate in Humans, and Determination; Appetite Control in Humans: A Psychobiological Approach; *Bacillus cereus* and Other *Bacillus* sp. Causing Foodborne Poisonings, Detection of; Barley; Berries and Related Fruits; Bioactive Peptides in Foods; Cereals: Types and Composition; Cheese: Composition and Health Effects; Cholesterol: Factors Determining Blood Cholesterol Levels; Cocoa: Composition and Health Effects; Coffee: Health Effects; Colon: Diseases and Disorders; Dairy Products: Dietary and Medical Importance; Eating Disorders; Energy: Intake and Energy Requirements; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fatty Acids: *Trans* Fatty Acids; Fish: Dietary Importance and Health Effects; Food Allergies; Fructose and High-Fructose Corn Syrup; Fructose: Sources, Metabolism, and Health; Glucose: Glucose Intolerance; Hypertension and Diet; Legumes in the Diet; Magnesium; Meat: Role in the Diet; Nutritional Epidemiology; Nuts: Health Effects; Obesity: The Role of Diet; Olive Oil: Its Role in the Diet; Potassium: Properties and Determination; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Retinol: Properties and Determination; Satiety; Spices and Flavoring Crops: Uses and Health Effects; Starch; Vegetable Oils: Dietary Importance; Vegetarian Diets; Vinegar.

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Introduction

Dietary reference intakes (DRIs) are a set of nutrient-based reference values that provide quantitative recommendations by gender, age, life stage, or physiological condition (such as pregnancy or lactation) for nutrient intakes of individuals living in the United States and Canada. The reference values include the estimated average intake (EAR), the recommended dietary allowance (RDA), the adequate intake (AI), the tolerable upper intake level (UL), and, for energy providing nutrients, the acceptable macronutrient distribution range (AMDR). DRIs provide nutrient standards that are used to shape various public policies, such as nutrient standards for school lunch programs, congregate and delivered elderly meal programs, and guidelines for the women, infants, and children supplemental feeding program. The DRIs are the basis for developing dietary advice for patients requiring medical nutrition therapy and to establish the daily values displayed on the nutrition fact panels of packaged food and supplements. They are the foundation for formulating the Dietary Guidelines for Americans.

History

The DRIs replaced the RDA, a nutrient standard used in the United States from 1941 to 1989 and a similar Canadian standard. These newer values were developed because of changes in science, limitations of the existing RDA, and the need to consider the role of nutrients in preventing dietary deficiency diseases as well as their associations with diet-related chronic degenerative diseases and toxicities. Starting in 1994, expert committees examined the scientific data on requirements of nutrients for these functions. The Standing Committee on the Scientific Evaluation of DRIs coordinated the effort under the direction of the Food and Nutrition Board, Institute of Medicine (IOM) of the National Academy of Sciences, and Health Canada.

Indicators Used to Establish DRIs

Many possible functional indicators and health outcomes exist and are examined by the DRI committees, including chronic disease indicators. The DRIs are based on the relationship between a nutrient and a single functional indicator of the health outcome of interest. These functional indicators may involve intake of a nutrient such as vitamin D and a status biomarker such as 25-OH-D of known sufficiency for bone health outcomes. Alternatively, for other nutrients such as ascorbic acid, the functional biomarker is a plasma ascorbic acid level above the threshold, 0.2 mg dl^{-1}, which is adequate to prevent scurvy. For example, night blindness is associated with vitamin A deficiency and its prevention is a desirable health outcome. The ability of the retina to adapt to dim light depends on AIs of vitamin A (retinal). It plays an important role in rod function during dim light. Without an adequate retinal supply, the functions of the rods become compromised in dim light settings, and if chronic vitamin A deficiency occurs, it can result in night blindness.

Definitions of DRIs

Table 1 summarizes definitions of the DRIs. After the choice of a criterion for nutrient adequacy is established, the existing experimental data are analyzed and summarized. The estimated average requirement (EAR) is the amount of a nutrient that meets the requirement of a specific criterion of adequacy (as determined by the functional indicator) for half of the healthy individuals within a defined age, gender, and life stage. There must be enough scientific evidence available to set an EAR for a nutrient and to calculate an RDA. The RDA is the average daily dietary intake that meets the nutrient requirements of nearly all healthy individuals of a specific age, gender, life stage, or physiological condition for the outcome of interest. If the statistical distribution of the amounts of nutrient intake needed to achieve the EAR, for example, the desired level of the functional indicator for the specific population, is determined to be normal (Gaussian) (Figures 1 and 2), and the standard deviation (SD) can be determined, then the following calculation is used:

$$\mathrm{RDA} = \mathrm{EAR} + 2 \times \mathrm{SD}$$

When there are insufficient data or variability in order to calculate an SD for the nutrient, a symmetrical distribution is assumed and then a coefficient of variation (CV) of 10% EAR is usually used. The calculation then becomes

$$\mathrm{RDA} = \mathrm{EAR} + 2 \times \mathrm{CV} \text{ or } \mathrm{EAR} + 2(0.1 \times \mathrm{EAR})$$

Note: the CV is usually set at 10% unless variability is known to be greater.

When nutrient requirements are not normally distributed, as, for example, is the case for iron, then statistical transformation of the data is required before the EAR and RDA are set.

When insufficient evidence is available to determine an EAR and RDA, then an AI is derived to provide an intake recommendation. The AI is a recommended daily intake of a nutrient that is based on observational and experimental data or observations of average intakes of a group or groups of presumably healthy people. The AI represents an informed decision about what seems to be an appropriate intake for an individual based primarily on epidemiological rather than an experimentally determined value and it should be used more cautiously than the RDA.

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Table 1 Terms used in US reference standards

Term	*Definition*
Estimated average requirement (EAR)	The amount of an individual nutrient that meets the requirement for a specific criterion of adequacy for half of the healthy individuals within a defined age, gender, or life stage
Recommended dietary allowance (RDA)	The average daily dietary intake level that meets the nutrient requirement of nearly all healthy individuals of a specific age, gender, life stage, or physiological condition
Adequate intake (AI)	The nutrient intake amount that is set when decisive data on the requirement are lacking in order to set an EAR and RDA
Tolerable upper intake level (UL)	The highest level of daily nutrient intake that is likely to pose no risk of adverse health effect to almost all individuals
Acceptable macronutrient distribution range (AMDR)	A range of intakes for macronutrients expressed as the proportion of total energy intake that is associated with chronic disease reduction and provides adequate amounts of the nutrient while maintaining energy balance
Estimated energy requirement (EER)	The requirement for energy of healthy adults with a body mass index (BMI) in the normal range of 18.5 and 25
Total energy expenditure (TEE)	Set of equations that estimate the energy expenditure needed to maintain current body weight and activity levels

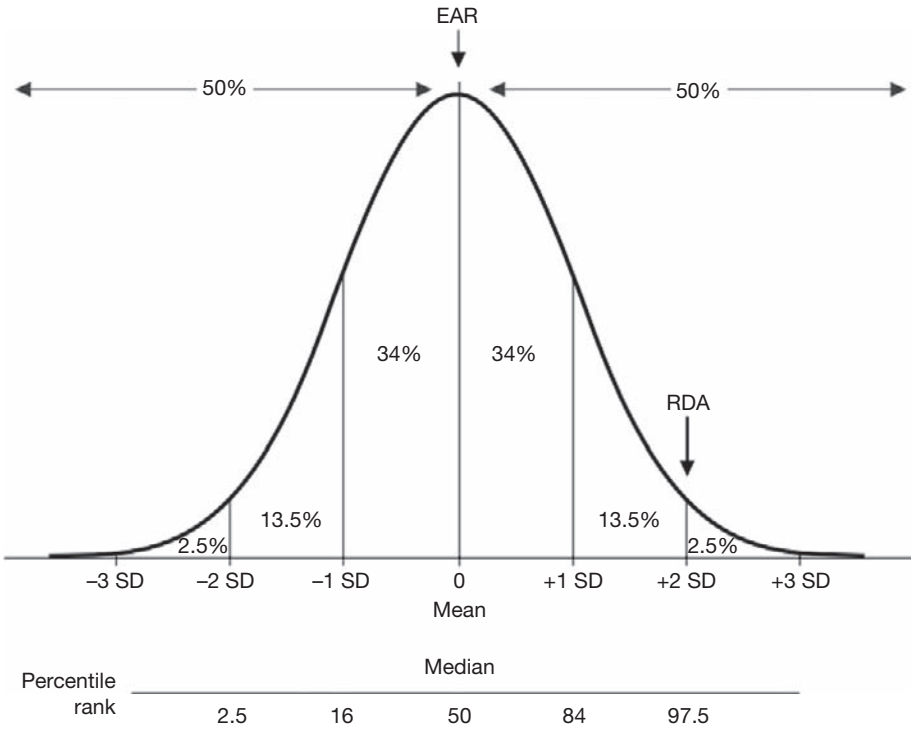

Figure 1 The normal distribution of a nutrient requirement and placement of the EAR and RDA. Reprinted with permission from *Dietary reference intakes: the essential guide to nutrient requirements*, 2006 by the National Academy of Sciences, courtesy of the National Academies Press, Washington, DC.

Table 2 presents the RDA and AI for vitamins, and Table 3 presents the same values for mineral elements. Table 4 presents the RDA and AI for total water and macronutrients.

Little experimental data are available on nutrient requirements for children, and thus, the EAR, RDA, and UL are based on extrapolations from adult values. For setting an EAR, the extrapolations for infants include scaling down the requirements by a specified power to adjust for metabolic differences or adjustments based upon reference body weights. The AIs are estimated by observing the amounts of nutrients eaten by healthy infants or children.

The AMDR provides acceptable high and low limits of protein, carbohydrate, and fat ($n-6$ and $n-3$ polyunsaturated fatty acids) expressed as percent of energy needs. It is based on some intervention trials but primarily on epidemiological studies indicating that an imbalance in macronutrients can increase risk of several diet-related chronic degenerative diseases including coronary heart disease, obesity, type 2 diabetes, and some cancers. Table 5 presents the AMDRs. One of the key features of the AMDR is that it gives an upper- and lower-level boundary for the range since there is an increased risk resulting from chronic intakes both above and below it. Note that the

Table 2 Dietary reference intakes (DRIs): recommended dietary allowances and adequate intakes, vitamins (Food and Nutrition Board, Institute of Medicine, National Academies)

Life stage/ group	*Vitamin A (μg day^{-1})*[a]	*Vitamin C (mg day^{-1})*	*Vitamin D (μg day^{-1})*[b,c]	*Vitamin E (mg day^{-1})* [d]	*Vitamin K (μg day^{-1})*	*Thiamin (mg day^{-1})*	*Riboflavin (mg day^{-1})*	*Niacin (mg day^{-1})*[e]	*Vitamin B_6 (mg day^{-1})*	*Folate (μg day^{-1})*[f]	*Vitamin B_{12} (μg day^{-1})*	*Pantothenic acid (mg day^{-1})*	*Biotin (μg day^{-1})*	*Choline (mg day^{-1})*[g]
Infants														
0–6 months	400*	40*	10	4*	2.0*	0.2*	0.3*	2*	0.1*	65*	0.4*	1.7*	5*	125*
6–12 months	500*	50*	10	5*	2.5*	0.3*	0.4*	4*	0.3*	80*	0.5*	1.8*	6*	150*
Children														
1–3 years	**300**	**15**	**15**	**6**	30*	**0.5**	**0.5**	**6**	**0.5**	**150**	**0.9**	2*	8*	200*
4–8 years	**400**	**25**	**15**	**7**	55*	**0.6**	**0.6**	**8**	**0.6**	**200**	**1.2**	3*	12*	250*
Males														
9–13 years	**600**	**45**	**15**	**11**	60*	**0.9**	**0.9**	**12**	**1.0**	**300**	**1.8**	4*	20*	375*
14–18 years	**900**	**75**	**15**	**15**	75*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	25*	550*
19–30 years	**900**	**90**	**15**	**15**	120*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	30*	550*
31–50 years	**900**	**90**	**15**	**15**	120*	**1.2**	**1.3**	**16**	**1.3**	**400**	**2.4**	5*	30*	550*
51–70 years	**900**	**90**	**15**	**15**	120*	**1.2**	**1.3**	**16**	**1.7**	**400**	**2.4**[h]	5*	30*	550*
>70 years	**900**	**90**	**20**	**15**	120*	**1.2**	**1.3**	**16**	**1.7**	**400**	**2.4**[h]	5*	30*	550*
Females														
9–13 years	**600**	**45**	**15**	**11**	60*	**0.9**	**0.9**	**12**	**1.0**	**300**	**1.8**	4*	20*	375*
14–18 years	**700**	**65**	**15**	**15**	75*	**1.0**	**1.0**	**14**	**1.2**	**400**[i]	**2.4**	5*	25*	400*
19–30 years	**700**	**75**	**15**	**15**	90*	**1.1**	**1.1**	**14**	**1.3**	**400**[i]	**2.4**	5*	30*	425*
31–50 years	**700**	**75**	**15**	**15**	90*	**1.1**	**1.1**	**14**	**1.3**	**400**[i]	**2.4**	5*	30*	425*
51–70 years	**700**	**75**	**15**	**15**	90*	**1.1**	**1.1**	**14**	**1.5**	**400**	**2.4**[h]	5*	30*	425*
>70 years	**700**	**75**	**20**	**15**	90*	**1.1**	**1.1**	**14**	**1.5**	**400**	**2.4**[h]	5*	30*	425*
Pregnancy														
14–18 years	**750**	**80**	**15**	**15**	75*	**1.4**	**1.4**	**18**	**1.9**	**600**[i]	**2.6**	6*	30*	450*
19–30 years	**770**	**85**	**15**	**15**	90*	**1.4**	**1.4**	**18**	**1.9**	**600**[i]	**2.6**	6*	30*	450*

31–50 years	**770**	**85**	**15**	**15**	90*	**1.4**	**1.4**	**18**	**1.9**	**600**[i]	**2.6**	6*	30*	450*
Lactation														
14–18 years	**1200**	**115**	**15**	**19**	75*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*
19–30 years	**1300**	**120**	**15**	**19**	90*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*
31–50 years	**1300**	**120**	**15**	**19**	90*	**1.4**	**1.6**	**17**	**2.0**	**500**	**2.8**	7*	35*	550*

Note: This table (taken from the DRI reports; see www.nap.edu) presents recommended dietary allowances (RDAs) in **bold type** and adequate intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level, sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a group. It is calculated from an estimated average requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stages and gender groups is believed to cover the needs of all healthy individuals in the groups, but the lack of data or uncertainty in the data prevents one from specifying with confidence the percentage of individuals covered by this intake.

[a]As retinol activity equivalents (RAEs). 1 RAE = 1 μg retinol, 12 μg β-carotene, 24 μg α-carotene, or 24 μg β-cryptoxanthin. The RAE for dietary provitamin A carotenoids is twofold greater than retinol equivalent (RE), whereas the RAE for preformed vitamin A is the same as the RE.

[b]As cholecalciferol. 1 μg cholecalciferol = 40 IU vitamin D.

[c]Under the assumption of minimal sunlight.

[d]As α-tocopherol. α-tocopherol includes *RRR*-α-tocopherol, the only form of α-tocopherol that occurs naturally in foods, and the *2R*-stereoisomeric forms (*RRR*-, *RSR*-, *RRS*-, and *RSS*-α-tocopherol) that occur in fortified foods and supplements. It does not include the *2S*-stereoisomeric forms (*SRR*-, *SSR*-, *SRS*-, and *SSS*-α-tocopherol), also found in fortified foods and supplements.

[e]As niacin equivalents (NE). 1 mg of niacin = 60 mg of tryptophan; 0–6 months = preformed niacin (not NE).

[f]As dietary folate equivalents (DFE). 1 DFE = 1 μg food folate = 0.6 μg of folic acid from fortified food or as a supplement consumed with food = 0.5 μg of a supplement taken on an empty stomach.

[g]Although AIs have been set for choline, there are few data to assess whether a dietary supply of choline is needed at all stages of the life cycle, and it may be that the choline requirement can be met by endogenous synthesis at some of these stages.

[h]Because 10–30% of older people may malabsorb food-bound B_{12}, it is advisable for those older than 50 years to meet their RDA mainly by consuming foods fortified with B_{12} or a supplement containing B_{12}.

[i]In view of evidence linking folate intake with neural tube defects in the fetus, it is recommended that all women capable of becoming pregnant consume 400 μg from supplements or fortified foods in addition to intake of food folate from a varied diet.

[j]It is assumed that women will continue consuming 400 μg from supplements or fortified food until their pregnancy is confirmed and they enter prenatal care, which ordinarily occurs after the end of the periconceptional period – the critical time for formation of the neural tube.

Source: Reprinted with permission from *Dietary reference intakes for calcium*, *phosphorous*, *magnesium*, *vitamin D*, *and fluoride* (1997); *Dietary reference intakes for thiamin*, *riboflavin*, *niacin*, *vitamin* B_6, *folate*, *vitamin* B_{12}, *pantothenic acid*, *biotin*, *and choline* (1998); *Dietary reference intakes for vitamin C*, *vitamin E*, *selenium*, *and carotenoids* (2000); *Dietary reference intakes for vitamin A*, *vitamin K*, *arsenic*, *boron*, *chromium*, *copper*, *iodine*, *iron*, *manganese*, *molybdenum*, *nickel*, *silicon*, *vanadium*, *and zinc* (2001); *Dietary reference intakes for water*, *potassium*, *sodium*, *chloride*, *and sulfate* (2005); and *Dietary reference intakes for calcium and vitamin D* (2011) by the National Academy of Sciences, courtesy of the National Academies Press, Washington, DC. These reports may be accessed via www.nap.edu.

Table 3 Dietary reference intakes (DRIs): recommended dietary allowances and adequate intakes, elements (Food and Nutrition Board, Institute of Medicine, National Academies)

Life stage/ group	*Calcium (mg day^{-1})*	*Chromium (μg day^{-1})*	*Copper (μg day^{-1})*	*Fluoride (mg day^{-1})*	*Iodine (μg day^{-1})*	*Iron (mg day^{-1})*	*Magnesium (mg day^{-1})*	*Manganese (mg day^{-1})*	*Molybdenum (μg day^{-1})*	*Phosphorus (mg day^{-1})*	*Selenium (μg day^{-1})*	*Zinc (mg day^{-1})*	*Potassium (g day^{-1})*	*Sodium (g day^{-1})*	*Chloride (g day^{-1})*
Infants															
0–6 months	200*	0.2*	200*	0.01*	110*	0.27*	30*	0.003*	2*	100*	15*	2*	0.4*	0.12*	0.18*
6–12 months	260*	5.5*	220*	0.5*	130*	**11**	75*	0.6*	3*	275*	20*	**3**	0.7*	0.37*	0.57*
Children															
1–3 years	**700**	11*	**340**	0.7*	**90**	**7**	**80**	1.2*	**17**	**460**	**20**	**3**	3.0*	1.0*	1.5*
4–8 years	**1000**	15*	**440**	1*	**90**	**10**	**130**	1.5*	**22**	**500**	**30**	**5**	3.8*	1.2*	1.9*
Males															
9–13 year	**1300**	25*	**700**	2*	**120**	**8**	**240**	1.9*	**34**	**1250**	**40**	**8**	4.5*	1.5*	2.3*
14–18 years	**1300**	35*	**890**	3*	**150**	**11**	**410**	2.2*	**43**	**1250**	**55**	**11**	4.7*	1.5*	2.3*
19–30 years	**1000**	35*	**900**	4*	**150**	**8**	**400**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.5*	2.3*
31–50 years	**1000**	35*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.5*	2.3*
51–70 years	**1000**	30*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.3*	2.0*
>70 years	**1200**	30*	**900**	4*	**150**	**8**	**420**	2.3*	**45**	**700**	**55**	**11**	4.7*	1.2*	1.8*
Females															
9–13 years	**1300**	21*	**700**	2*	**120**	**8**	**240**	1.6*	**34**	**1250**	**40**	**8**	4.5*	1.5*	2.3*
14–18 years	**1300**	24*	**890**	3*	**150**	**15**	**360**	1.6*	**43**	**1250**	**55**	**9**	4.7*	1.5*	2.3*
19–30 years	**1000**	25*	**900**	3*	**150**	**18**	**310**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.5*	2.3*
31–50 years	**1000**	25*	**900**	3*	**150**	**18**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.5*	2.3*
51–70 years	**1200**	20*	**900**	3*	**150**	**8**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.3*	2.0*
>70 years	**1200**	20*	**900**	3*	**150**	**8**	**320**	1.8*	**45**	**700**	**55**	**8**	4.7*	1.2*	1.8*

Pregnancy															
14–18 years	**1300**	29*	**1000**	3*	**220**	**27**	**400**	2.0*	**50**	**1250**	**60**	**12**	4.7*	1.5*	2.3*
19–30 years	**1000**	30*	**1000**	3*	**220**	**27**	**350**	2.0*	**50**	**700**	**60**	**11**	4.7*	1.5*	2.3*
31–50 years	**1000**	30*	**1000**	3*	**220**	**27**	**360**	2.0*	**50**	**700**	**60**	**11**	4.7*	1.5*	2.3*
Lactation															
14–18 years	**1300**	44*	**1300**	3*	**290**	**10**	**360**	2.6*	**50**	**1250**	**70**	**13**	5.1*	1.5*	2.3*
19–30 years	**1000**	45*	**1300**	3*	**290**	**9**	**310**	2.6*	**50**	**700**	**70**	**12**	5.1*	1.5*	2.3*
31–50 years	**1000**	45*	**1300**	3*	**290**	**9**	**320**	2.6*	**50**	**700**	**70**	**12**	5.1*	1.5*	2.3*

Note: This table (taken from the DRI reports; see www.nap.edu) presents recommended dietary allowances (RDAs) in **bold type** and adequate intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level, sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a group. It is calculated from an estimated average requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stages and gender groups is believed to cover the needs of all healthy individuals in the groups, but the lack of data or uncertainty in the data prevents one from specifying with confidence the percentage of individuals covered by this intake.
Source: Reprinted with permission from *Dietary reference intakes for calcium*, *phosphorous*, *magnesium*, *vitamin D*, *and fluoride* (1997); *Dietary reference intakes for thiamin*, *riboflavin*, *niacin*, *vitamin* B_6, *folate*, *vitamin* B_{12}, *pantothenic acid*, *biotin*, *and choline* (1998); *Dietary reference intakes for vitamin C*, *vitamin E*, *selenium*, *and carotenoids* (2000); and *Dietary reference intakes for vitamin A*, *vitamin K*, *arsenic*, *boron*, *chromium*, *copper*, *iodine*, *iron*, *manganese*, *molybdenum*, *nickel*, *silicon*, *vanadium*, *and zinc* (2001); *Dietary reference intakes for water*, *potassium*, *sodium*, *chloride*, *and* sulfate (2005); and *Dietary reference intakes for calcium and vitamin d* (2011) by the National Academy of Sciences, courtesy of the National Academies Press, Washington, DC. These reports may be accessed via www.nap.edu.

Table 4 Dietary reference intakes (DRIs): recommended dietary allowances and adequate intakes, total water and macronutrients (Food and Nutrition Board, Institute of Medicine, National Academies)

Life stage/ group	*Total water*[a] *(l day^{-1})*	*Carbohydrate (g day^{-1})*	*Total fiber (g day^{-1})*	*Fat (g day^{-1})*	*Linoleic acid (g day^{-1})*	*α-Linolenic acid (g day^{-1})*	*Protein*[b] *(g day^{-1})*
Infants							
0–6 months	0.7*	60*	ND	31*	4.4*	0.5*	9.1*
6–12 months	0.8*	95*	ND	30*	4.6*	0.5*	**11.0**
Children							
1–3 years	1.3*	**130**	19*	ND[c]	7*	0.7*	**13**
4–8 years	1.7*	**130**	25*	ND	10*	0.9*	**19**
Males							
9–13 years	2.4*	**130**	31*	ND	12*	1.2*	**34**
14–18 years	3.3*	**130**	38*	ND	16*	1.6*	**52**
19–30 years	3.7*	**130**	38*	ND	17*	1.6*	**56**
31–50 years	3.7*	**130**	38*	ND	17*	1.6*	**56**
51–70 years	3.7*	**130**	30*	ND	14*	1.6*	**56**
>70 years	3.7*	**130**	30*	ND	14*	1.6*	**56**
Females							
9–13 years	2.1*	**130**	26*	ND	10*	1.0*	**34**
14–18 years	2.3*	**130**	26*	ND	11*	1.1*	**46**
19–30 years	2.7*	**130**	25*	ND	12*	1.1*	**46**
31–50 years	2.7*	**130**	25*	ND	12*	1.1*	**46**
51–70 years	2.7*	**130**	21*	ND	11*	1.1*	**46**
>70 years	2.7*	**130**	21*	ND	11*	1.1*	**46**
Pregnancy							
14–18 years	3.0*	**175**	28*	ND	13*	1.4*	**71**
19–30 years	3.0*	**175**	28*	ND	13*	1.4*	**71**
31–50 years	3.0*	**175**	28*	ND	13*	1.4*	**71**
Lactation							
14–18 years	3.8*	**210**	29*	ND	13*	1.3*	**71**
19–30 years	3.8*	**210**	29*	ND	13*	1.3*	**71**
31–50 years	3.8*	**210**	29*	ND	13*	1.3*	**71**

Note: This table (taken from the DRI reports; see www.nap.edu) presents the recommended dietary allowances (RDAs) in **bold type** and adequate intakes (AIs) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level, sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a group. It is calculated from an estimated average requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, an AI is the mean intake. The AI for other life stages and gender groups is believed to cover the needs of all healthy individuals in the groups, but the lack of data or uncertainty in the data prevents one from specifying with confidence the percentage of individuals covered by this intake.

[a]Total water includes all water contained in food, beverages, and drinking water.

[b]Based on g protein per kg of body weight for the reference body weight, for example, for adults 0.8 g kg^{-1} day^{-1} body weight for the reference body weight.

[c]Not determined.

Source: Reprinted with permission from *Dietary Reference intakes for energy*, *carbohydrate*, *fiber*, *fat*, *fatty acids*, *cholesterol*, *protein*, *and amino acids* (2002/2005) and *Dietary reference intakes for water*, *potassium*, *sodium*, *chloride*, *and sulfate* (2005) by the National Academy of Sciences, courtesy of the National Academies Press, Washington, DC. The report may be accessed via www.nap.edu.

Table 5 Dietary reference intakes (DRIs): acceptable macronutrient distribution ranges (Food and Nutrition Board, Institute of Medicine, National Academies)

	Range (percent of energy)		
Macronutrient	*Children, 1–3 years*	*Children, 4–18 years*	*Adults*
Fat	30–40	25–35	20–35
n−6 polyunsaturated fatty acids[a] (linoleic acid)	5–10	5–10	5–10
n−3 polyunsaturated fatty acids[a] (α-linolenic acid)	0.6–1.2	0.6–1.2	0.6–1.2
Carbohydrate	45–65	45–65	45–65
Protein	5–20	10–30	10–35

Macronutrient	*Recommendation*
Dietary cholesterol	As low as possible while consuming a nutritionally adequate diet
Trans-fatty acids	As low as possible while consuming a nutritionally adequate diet
Saturated fatty acids	As low as possible while consuming a nutritionally adequate diet
Added sugars[b]	Limit to no more than 25% of total energy

[a]Approximately 10% of the total can come from longer-chain *n*−3 or *n*−6 fatty acids.

[b]Not a recommended intake. A daily intake of added sugars that individuals should aim for to achieve a healthful diet was not set.

Source: Reprinted with permission from *Dietary reference intakes for energy*, *carbohydrate*, *fiber*, *fat*, *fatty acids*, *cholesterol*, *protein*, *and amino acids* (2002/2005) by the National Academy of Sciences, courtesy of the National Academies Press, Washington, DC. The report may be accessed via www.nap.edu.

AMDR for an individual can only be calculated once the total energy expenditure (TEE) is estimated and that it is not based on experimental data.

The UL is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effect to almost all individuals. These are limits set for safety reasons and are not derived from a continuous distribution like the EAR that permits statistical manipulation of the probabilities of exceeding the value. UL is determined using a risk assessment model adapted for nutrients that is set based on expert judgment for the adverse effect of interest for the nutrient. Table 6 presents the UL for vitamins, and Table 7 presents the UL for mineral elements (Figure 2).

The other DRIs describe energy intakes. They include the resting energy requirement, or REE, and the TEE, an estimate of the energy expenditure needed to maintain current body weight and activity levels. There is no RDA for energy since an excess above one's energy requirement would result in weight gain. An individual's resting energy expenditure only describes needs at rest. The estimated energy requirement (EER) is the estimated average dietary intake associated with an individual of a specific sex, age, height, weight, and activity level. The EER values are determined from equations that reflect the energy expenditure of individuals of normal body composition using data from doubly labeled water experiments. The EER and TEE are the same under conditions other than growth, pregnancy, or lactation; the EER prediction equations also account for the deposition of new tissue during pregnancy or growth and production of milk in the case of lactation. Many different equations are used to estimate the TEE or REE, depending on the individual's height, weight, age, and current activity level. Some use a multiplier reflecting physical activity level, ranging from 1 for sedentary, to 1.48 for moderately active, to higher for the very active. For hospitalized patients, different multipliers are sometimes used to account for metabolic stresses due to illness. All of these equations are approximations but useful to obtain a rough rule of thumb on energy needs.

The DRIs are based on a 'reference person' of a given weight and body composition. Those references are based on typical characteristics of American and Canadian citizens in these regards. They present reference values for each nutrient by age, sex, and physiological condition (pregnancy or lactation) for healthy people.

Applications

The DRIs are used to assess nutritional adequacy and make recommendations for dietary planning of individuals and groups. They are also used as standards for establishing nutrient recommendation for those who are ill. The DRIs for individuals suffering from disease only change when a specific nutrient requirement is affected by a disease or condition.

Assessment

The DRIs are used to assess the adequacy of nutrient intakes of individuals and populations from recalls or records of usual intake for micronutrients, protein, essential fatty acids, and carbohydrates. They are also used as a rule of thumb for assessing the intakes of individuals, but caution is needed. Usual intake and not a single day's intake must be considered. Moreover, since an individual's requirement is never known, only qualitative statements about adequacy are possible. The EAR is the average requirement for a group, but by definition, it does not meet the requirements of half of the population. In contrast, the RDA meets the requirements of virtually all individuals. Usual intakes at or above the RDA mean a low probability of inadequacy since the RDA is calculated to meet 97–98% of the needs of the population. Note that intakes below the RDA may be sufficient for some individuals whose requirements are low. However, we never know if a given individual's requirement is high or low. The likelihood of nutrient inadequacy increases as usual intakes fall further and further below the RDA, but intakes below the RDA cannot be assumed to be inadequate.

Although definitive statements cannot be made, mean intakes of an individual below the EAR very likely need to be improved because the probability of inadequacy is by definition at least 50%. An individual's observed mean intake between the EAR and the RDA probably needs to be improved, because the probability of inadequacy is more than 50 but less than 97%. Only if an individual's intake has been reported for a large number of days can one have a considerable degree of confidence that the diet is inadequate. For nutrients without an EAR or RDA, those who have intakes above the AI can be considered to have reasonably good intakes. The AI is an intake at or above which there is low probability of inadequacy. Therefore, many intakes are already likely to be in excess of requirements; an intake that is low by the AI standard may still be perfectly adequate.

The UL is the usual intake above which there is an increased risk of adverse effects for almost all individuals in the general population. When assessing nutrient intake, the UL can only show the likelihood of adverse effects from usual (chronic) intakes above the UL, not intake on a single day. It is important to note that with most nutrients, the UL applies to all sources of dietary intake, but for one nutrient, magnesium, only pharmacological sources are included.

Dietary assessment of an individual is only one component of a complete nutritional status evaluation. It should be combined with clinical, biochemical, and anthropometric data to provide a valid picture of nutritional status, especially in those who are significantly different than the 'reference person.'

The goal of assessing nutrient intakes of a population using the DRI is to determine the prevalence of inadequate or excessive nutrient intakes within it. There are several methods available to estimate usual intake distributions from dietary intake data on two or more days' worth of intakes. More information and a link to software are available through Iowa State University at http://www.cssm.iastate.edu/software/side/. The statistical techniques are well worked out, and today, monitoring of dietary adequacy and excess is done on an ongoing basis in the US National Health and Nutrition Examination Survey.

Planning

The primary goal of planning an individual's diet is to devise an intake pattern that ensures nutrient adequacy while also minimizing risk of excess and maximizing individual

Table 6 Dietary reference intakes (DRIs): tolerable upper intake levels, vitamins (Food and Nutrition Board, Institute of Medicine, National Academies)

Life stage/ group	*Vitamin A (μg day^{-1})*[a]	*Vitamin C (mg day^{-1})*	*Vitamin D (μg day^{-1})*	*Vitamin E (mg day^{-1})*[b,c]	*Vitamin K (μg day^{-1})*	*Thiamin (mg day^{-1})*	*Riboflavin (mg day^{-1})*	*Niacin (mg day^{-1})*[c]	*Vitamin B_6 (mg day^{-1})*	*Folate (μg day^{-1})*[c]	*Vitamin B12 (μg day^{-1})*	*Pantothenic acid (mg day^{-1})*	*Biotin (μg day^{-1})*	*Choline (g day^{-1})*	*Carotenoids*[d]
Infants															
0–6 months	600	ND[e]	25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6–12 months	600	ND	38	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Children															
1–3 years	600	400	63	200	ND	ND	ND	10	30	300	ND	ND	ND	1.0	ND
4–8 years	900	650	75	300	ND	ND	ND	15	40	400	ND	ND	ND	1.0	ND
Males															
9–13 years	1700	1200	100	600	ND	ND	ND	20	60	600	ND	ND	ND	2.0	ND
14–18 years	2800	1800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
31–50 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
51–70 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
>70 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
Females															
9–13 years	1700	1200	100	600	ND	ND	ND	20	60	600	ND	ND	ND	2.0	ND
14–18 years	2800	1800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
31–50 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
51–70 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
>70 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND

Pregnancy															
14–18 years	2800	1800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
31–50 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
Lactation															
14–18 years	2800	1800	100	800	ND	ND	ND	30	80	800	ND	ND	ND	3.0	ND
19–30 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND
31–50 years	3000	2000	100	1000	ND	ND	ND	35	100	1000	ND	ND	ND	3.5	ND

Note: A tolerable upper intake level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to a lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, and carotenoids. In the absence of a UL, extra caution may be warranted in consuming levels above recommended intakes. Members of the general population should be advised not to routinely exceed the UL. The UL is not meant to apply to individuals who are treated with the nutrient under medical supervision or to individuals with predisposing conditions that modify their sensitivity to the nutrient.

[a]As preformed vitamin A only.

[b]As α-tocopherol; applies to any form of supplemental α-tocopherol.

[c]The ULs for vitamin E, niacin, and folate apply to synthetic forms obtained from supplements, fortified foods, or a combination of the two.

[d]β-Carotene supplements are advised only to serve as a provitamin A source for individuals at risk of vitamin A deficiency.

[e]ND = not determinable due to the lack of data of adverse effects in this age group and concern with regard to the lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

Source: Reprinted with permission from *Dietary reference intakes for calcium*, *phosphorous*, *magnesium*, *vitamin D*, *and fluoride* (1997); *Dietary reference intakes for thiamin*, *riboflavin*, *niacin*, *vitamin* B_6, *folate*, *vitamin* B_{12}, *pantothenic acid*, *biotin*, *and choline* (1998); *Dietary reference intakes for vitamin C*, *vitamin E*, *selenium*, *and carotenoids* (2000); *Dietary reference intakes for vitamin A*, *vitamin K*, *arsenic*, *boron*, *chromium*, *copper*, *iodine*, *iron*, *manganese*, *molybdenum*, *nickel*, *silicon*, *vanadium*, *and zinc* (2001); and *Dietary reference intakes for calcium and vitamin D* (2011) by the National Academy of Sciences, courtesy of the National Academies Press, Washington, DC. These reports may be accessed via www.nap.edu.

Table 7 Dietary reference intakes (DRIs): tolerable upper intake levels, elements (Food and Nutrition Board, Institute of Medicine, National Academies)

Life stage/ group	*Arsenic*[a]	*Boron (mg day^{-1})*	*Calcium (mg day^{-1})*	*Chromium (μg day^{-1})*	*Copper (μg day^{-1})*	*Fluoride (mg day^{-1})*	*Iodine (μg day^{-1})*	*Iron (mg day^{-1})*	*Magnesium (mg day^{-1})*[b]	*Manganese (mg day^{-1})*	*Molybdenum (μg day^{-1})*	*Nickel (mg day^{-1})*	*Phosphorus (g day^{-1})*	*Selenium (μg day^{-1})*	*Silicon*[c]	*Vanadium (mg day^{-1})*[d]	*Zinc (mg day^{-1})*	*Sodium (g day^{-1})*	*Chloride (g day^{-1})*
Infants																			
0–6 months	ND[e]	ND	1000	ND	ND	0.7	ND	40	ND	ND	ND	ND	ND	45	ND	ND	4	ND	ND
6–12 months	ND	ND	1500	ND	ND	0.9	ND	40	ND	ND	ND	ND	ND	60	ND	ND	5	ND	ND
Children																			
1–3 years	ND	3	2500	ND	1000	1.3	200	40	65	2	300	0.2	3	90	ND	ND	7	1.5	2.3
4–8 years	ND	6	2500	ND	3000	2.2	300	40	110	3	600	0.3	3	150	ND	ND	12	1.9	2.9
Males																			
9–13 years	ND	11	3000	ND	5000	10	600	40	350	6	1100	0.6	4	280	ND	ND	23	2.2	3.4
14–18 years	ND	17	3000	ND	8000	10	900	45	350	9	1700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2500	ND	10 000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
31–50 years	ND	20	2500	ND	10 000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
51–70 years	ND	20	2000	ND	10 000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
>70 years	ND	20	2000	ND	10 000	10	1100	45	350	11	2000	1.0	3	400	ND	1.8	40	2.3	3.6
Females																			
9–13 years	ND	11	3000	ND	5000	10	600	40	350	6	1100	0.6	4	280	ND	ND	23	2.2	3.4
14–18 years	ND	17	3000	ND	8000	10	900	45	350	9	1700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2500	ND	10 000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
31–50 years	ND	20	2500	ND	10 000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
51–70 years	ND	20	2000	ND	10 000	10	1100	45	350	11	2000	1.0	4	400	ND	1.8	40	2.3	3.6
>70 years	ND	20	2000	ND	10 000	10	1100	45	350	11	2000	1.0	3	400	ND	1.8	40	2.3	3.6

Pregnancy																			
14–18 years	ND	17	3000	ND	8000	10	900	45	350	9	1700	1.0	3.5	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2500	ND	10000	10	1100	45	350	11	2000	1.0	3.5	400	ND	ND	40	2.3	3.6
31–50 years	ND	20	2500	ND	10000	10	1100	45	350	11	2000	1.0	3.5	400	ND	ND	40	2.3	3.6
Lactation																			
14–18 years	ND	17	3000	ND	8000	10	900	45	350	9	1700	1.0	4	400	ND	ND	34	2.3	3.6
19–30 years	ND	20	2500	ND	10000	10	1100	45	350	11	2000	1.0	4	400	ND	ND	40	2.3	3.6
31–50 years	ND	20	2500	ND	10000	10	1100	45	350	11	2000	1.0	4	400	ND	ND	40	2.3	3.6

Note: A tolerable upper intake level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to a lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B_{12}, pantothenic acid, biotin, and carotenoids. In the absence of a UL, extra caution may be warranted in consuming levels above recommended intakes. Members of the general population should be advised not to routinely exceed the UL. The UL is not meant to apply to individuals who are treated with the nutrient under medical supervision or to individuals with predisposing conditions that modify their sensitivity to the nutrient.

[a]Although the UL was not determined for arsenic, there is no justification for adding arsenic to food or supplements.

[b]The ULs for magnesium represent intake from a pharmacological agent only and do not include intake from food and water.

[c]Although silicon has not been shown to cause adverse effects in humans, there is no justification for adding silicon to supplements.

[d]Although vanadium in food has not been shown to cause adverse effects in humans, there is no justification for adding vanadium to food and vanadium supplements should be used with caution. The UL is based on adverse effects in laboratory animals and these data could be used to set a UL for adults but not children and adolescents.

[e]ND = not determinable due to the lack of data of adverse effects in this age group and concern with regard to the lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake.

Source: Reprinted with permission from *Dietary reference intakes for calcium*, *phosphorous*, *magnesium*, *vitamin D*, *and fluoride* (1997); *Dietary reference intakes for thiamin*, *riboflavin*, *niacin*, *vitamin* B_6, *folate*, *vitamin* B_{12}, *pantothenic acid*, *biotin*, *and choline* (1998); *Dietary reference intakes for vitamin C*, *vitamin E*, *selenium*, *and carotenoids* (2000); *Dietary reference intakes for vitamin A*, *vitamin K*, *arsenic*, *boron*, *chromium*, *copper*, *iodine*, *iron*, *manganese*, *molybdenum*, *nickel*, *silicon*, *vanadium*, *and zinc* (2001); *Dietary reference intakes for water*, *potassium*, *sodium*, *chloride*, *and sulfate* (2005); and *Dietary reference intakes for calcium and vitamin D* (2011) by the National Academy of Sciences, courtesy of the National Academies Press, Washington, DC. These reports may be accessed via www.nap.edu.

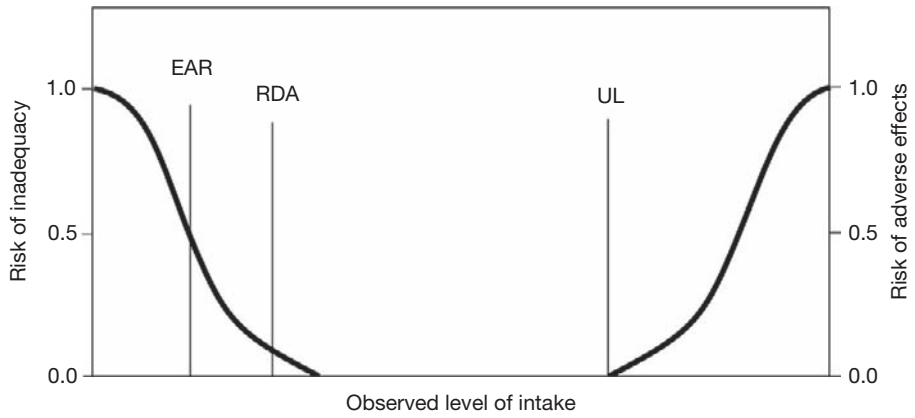

Figure 2 The relationship between different descriptors of dietary reference intakes. Reprinted with permission from *Dietary reference intakes: the essential guide to nutrient requirements*, 2006 by the National Academy of Sciences, courtesy of the National Academies Press, Washington, DC.

preferences, including meal patterns, culture, religious beliefs, and taste. Perfectly adequate diets from the nutrient standpoint that are not eaten are nutritionally useless. When planning for individuals, the nutrient goal should be at least the RDA or AI to minimize risk of inadequacies. There is no increased risk of adverse effects when an individual's intake is greater than the RDA as long as it remains below the UL. Similarly, when planning for populations or particular groups, it is important to ensure that virtually all individuals exceed the EAR without exceeding the UL.

Many tools are available for diet planning. The USDA provides daily food plan guides (http://www.choosemyplate.gov/myplate/index.aspx) by food groups for those with varied daily calorie needs. Additionally, the USDA periodically issues the Thrifty Food Plan (http://www.cnpp.usda.gov/USDAFoodPlansCostofFood.htm), which is a guide to selecting foods and menus for individuals using Supplemental Nutrition Assistance Program benefits to maximize the amount and nutritional value of food while receiving benefits.

Challenges, Additional Research, and Future Steps

The development of the DRIs has been important in improving national assessment and monitoring of dietary intakes and planning of federal and other programs, but research challenges remain.

Tolerable Upper Intake Level

The UL is the level of nutrient intake below which adverse health effects are unlikely. Adverse events have not been collected routinely on a systematic basis in studies of high levels of nutrient intakes requirements, and therefore, there are often very little data upon which to base the UL. Moreover, dose–response studies are rarely done at high levels, and in some cases, they would be unethical, yet in order to accurately determine a health risk associated with an intake above the UL, it is necessary to know the dose–response relationship between chronic intake of the nutrient and the observed adverse effect chosen. More data are needed in order to estimate dose–response curves for most nutrients. A second problem with the UL is that the level of severity of the adverse effects upon which the UL is based varies greatly from one nutrient to another. For example, exceeding the UL on a chronic basis for pyridoxine is a major concern since it may produce irreversible nerve damage. In contrast, exceeding the UL for choline is likely of lesser concern since the UL is based on a fishy body odor. A final problem with the UL is its characteristics; it is based on a toxicological model with many safety factors that are based on expert judgments but not necessarily on empirically determined data. The UL is a defined as a sharp cutoff and is difficult to manipulate from the statistical standpoint.

Protein Requirement

The current DRI for protein is based on the analysis of nitrogen balance studies, which have many limitations. Nitrogen balance may give falsely high estimates because it is easy to overestimate nitrogen intake and underestimate excretion due to incomplete collections and difficult to measure dermal and miscellaneous nitrogen losses that vary greatly under some environmental conditions. Protein requirements can also be determined using other techniques, such as stable isotopes, carbon balance, and amino acid flux. However, before new methods are adopted, there is a need to conduct comparative studies using the proposed techniques to harmonize them with the nitrogen balance studies of requirements.

One alternative technique that has been proposed to estimate requirements of essential amino acids is the indicator amino acid oxidation method to determine indispensable amino acid (IDAA) requirements in humans. It is based on the idea that when one IDAA is deficient for protein synthesis, then the excess of all other IDAAs will be oxidized, including the amino acid marked with a stable isotope. Proponents claim that this method is robust, minimally invasive, more rapid, and sensitive to changes in amino acid intake than nitrogen-based studies. Studies using it have identified amino acid requirements that are much higher than those based on nitrogen balance.

Better Experimental Data on Subgroups

Many of the DRIs for specific life stage groups have been extrapolated from adults, due to the lack of data on the specific nutrient and life stage group. More studies of these groups are needed in order to set more accurate DRIs.

DRIs for Nonnutrient Bioactives?

Nonnutrient bioactive constituents may have significant health benefits. They include food components such as fiber, carotenoids, glucosinolates, and flavonoids, among others. With the exception of fiber, DRIs are not established for these components.

There are many challenges in determining recommendations for consumption of bioactives including their variable bioavailability and bioactivity, errors in intake measurement, and health benefits based on surrogate biomarkers of effect rather than health outcomes. Historically, much of the discussion around setting recommendations for bioactives was about the paucity and quality of scientific data available. Phenols, polyphenols, and flavonoids were excluded from consideration by the DRI panel at the time the DRIs were last revised because of the lack of sufficient data. Since then, there has been a substantial increase in research and available data, although randomized clinical trials of intakes of these constituents with health outcomes are still few in number. Some other types of guidance may be more appropriate than to establish DRI for them.

Chronic Disease Endpoints

More attention needs to be paid to methods for linking nutrient intakes to chronic disease endpoints/outcomes and setting recommendations based on these.

Updates and Harmonization

It may be time for reevaluation of the DRI development process. Perhaps, it should establish criteria for which nutrients are most in need of revision and then concentrate on revising one nutrient at a time rather than many nutrients. At present, the Food and Nutrition Board and Health Canada have received nominations for many nutrients thought to be in need of revision, of which four were selected for further consideration (vitamin E, sodium, magnesium, and omega 3 fatty acids). It remains to be seen what nutrients will be chosen. Finally, there is a need to harmonize efforts on setting requirements with other authoritative bodies in Europe, Australia, New Zealand, and elsewhere, to avoid needless overduplication of work.

See also: Elderly: Nutrition Requirements; Energy: Intake and Energy Requirements; Fatty Acids: Determination and Requirements; Infants: Nutritional Requirements; Pregnancy: Metabolic Adaptations and Nutritional Requirements; Protein: Requirements.

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Relevant Websites

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Dietary Surveys: National Food Intake

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Introduction

National food intake surveys are surveys that monitor the food consumption of groups of individuals that are representative for a national population or important population groups within a country. National food intake surveys provide insight into the consumption of foods, the intake of energy and nutrients, and the exposure to potentially harmful chemical substances. Regular repetitions of national food intake surveys show dietary trends in a population.

Apart from national food intake surveys, other sources of national food data are available in countries. These data from food balance sheets and household budget and expenditure surveys are sometimes used as a surrogate for food intake data. However, each of them provides information about a different level in the flow of food production to consumption and on different aggregation levels. Food balance sheets give information on the national per capita food supply, and household budget surveys focus on food acquisition at the level of households, sometimes supplemented with foods acquired out of home. The data of food balance sheets and household budget surveys are thus not directly comparable with data from food consumption surveys (see Figure 1). Only national food intake surveys allow to estimate the population distributions of consumption of foods, intake of energy and nutrients, and exposure to chemicals and to do this for subgroups within a population such as by gender and age groups.

Many countries conduct national food intake surveys. For the European Union, food intake data of 22 member states are available in the comprehensive food consumption database of the European Food Safety Authority (EFSA). The number of countries that carry out food intake surveys on a regular basis rather than incidentally is however limited. The most well-know long-standing national food intake surveys are probably those in the United States, starting from the National Food Consumption Survey in 1935 to the ongoing dietary surveys as part of the National Health and Nutrition Examination Survey (NHANES).

This article highlights major uses of national food intake data, important aspects about the design and methodology of food intake surveys and their European harmonization, limitations associated with food intake surveys, and future directions.

Major Uses and Stakeholders of National Food Intake Data

Data from national food intake surveys together with data on the composition of foods and information on nutritional status are needed to develop and evaluate health, nutrition, and food policy. Examples are the evaluation of diet from a health perspective, simulating the impact of dietary policy measures, describing trends in food and nutrient intakes, and deriving optimal levels of food fortification. Moreover, the food intake data are essential to develop food frequency questionnaires, dietary indexes, and food-based dietary guidelines.

Similarly, data from national food intake surveys in combination with data on contaminants, additives, or chemical constituents in foods are essential for the assessment of exposure to hazardous substances, as part of the risk assessment process. This information is also useful for the evaluation of new food legislation. Examples are dietary exposure assessment, derivation of safe maximum fortification levels, communication of risks associated with the food chain, estimations of the effects of particular diseases caused by infectious agents that can be transmitted between animals and humans, and identification of core foods for total diet studies.

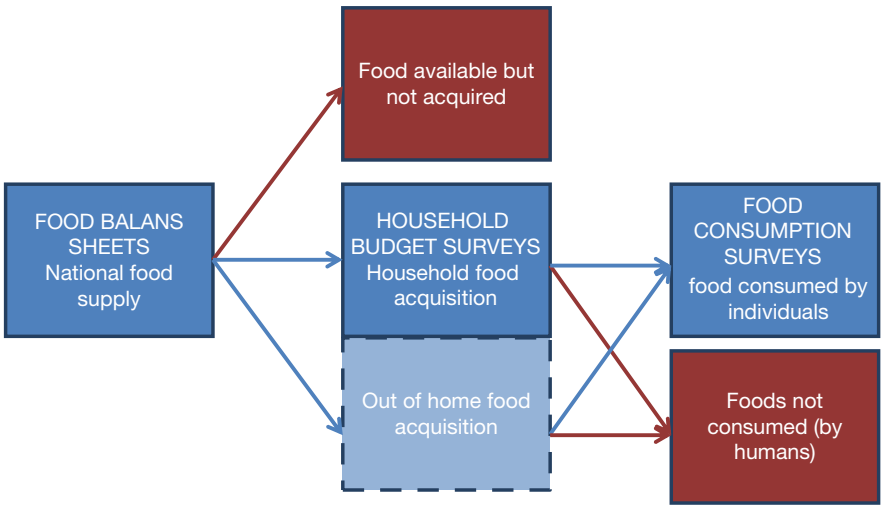

Figure 1 Relationships between food supply data, food acquisition data, and food consumption data.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00237-3

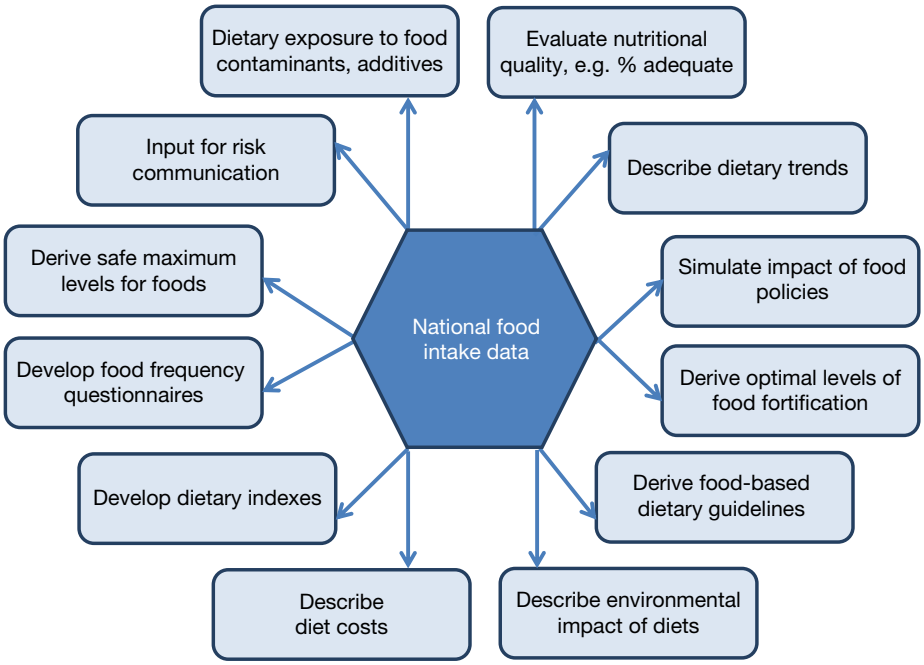

Figure 2 Examples of uses of food intake surveys.

Additionally, food intake data can be used for the assessment of the environmental impact of diet or to describe dietary costs (see Figure 2).

Stakeholders of national food intake data are ministries of health, ministries of agriculture, food industry, dieticians, patient organizations, branch organizations, journalists, universities, and national and international health and food safety organizations like the World Health Organization (WHO), Food and Agriculture Organization (FAO), and EFSA. Scientists in public health or nutrition institutes, food safety agencies, universities, and research departments of the larger food industries often have an interest to conduct specific statistical analyses in national food intake databases themselves.

Study Design, Data Collection, and Handling

Two features are critical for the success of national food intake surveys. The first critical aspect is to obtain a study population that is representative of the target population. The second challenge is to collect food intake data that are valid and precise and contain sufficient detail for their purposes.

Study Population

When planning a national food intake survey, the first step to be taken is defining the target population. The target population is the entire set of individuals to which findings of the survey are to be extrapolated. Typically, a broad age group within a national population is defined as the target population for a food intake survey. The target population may not be the same as the study population, that is, the population that can be defined accurately and reached in a study. In order to precisely define the study population, it is necessary to agree on exclusion criteria. Institutionalized subjects are often not included in food intake surveys as their inclusion makes the sampling procedures and/or the field surveys too complicated. Also, language barriers are commonly used as exclusion criteria. In order to obtain a representative study population for the target population, excluding subjects due to language barriers or other practical reasons should be kept to a minimum.

The sampling frame is the list of units from which the sample is drawn. The availability of sampling frames varies from country to country. For national food intake surveys, the most complete lists can be chosen among population registers, electoral lists, and census lists. Alternative sampling frames are telephone lists, lists of schools or day care centers, market research panels, and lists of medical centers or general practitioners. Great care must be taken when making decisions on the sampling frame, particularly with regard to bias due to undercoverage. Overcoverage of the sampling frame normally can be detected and dealt with during the fieldwork. Specific subgroups within a population might need a different sampling frame, since electoral list is only suitable for adults and list of day care centers or schools is especially suitable for young or older children, respectively.

Sampling from the sampling frame can be done in various ways. It is considered a good survey practice to use a sampling design that yields a probability sample. Under such a design, each sample unit of the sampling frame has a nonzero probability of being selected. It does not necessarily require that every person has the same probability of being selected. Stratified sampling, for example, sampling by subgroups of age and gender, is often conducted in food intake surveys to ensure that there are a sufficient number of people in each subgroup to allow meaningful analysis. For practical reasons, cluster sampling or multistage sampling can be applied. In cluster

sampling, first, a random sample of naturally occurring units or groupings within the population is taken (e.g., areas, cities, or schools). Then, either all members of the chosen clusters or a random selection from among them is included in the sample. Multistage sampling is an extension of cluster sampling where a hierarchy of clusters are chosen going from larger to smaller.

The required size of a food intake survey population depends on the variability in intake, the statistics of interest (i.e., mean, percentiles, or variance), and the level of precision needed. Information on intake variability (from previous studies) is therefore needed in order to determine the number of subjects to include in a survey. In the European Food Consumption and Validation (EFCOVAL) project, the sample size per country was estimated to be, on average, equal to 2000 subjects for a desired precision of 5% in nutrient and food group intake. In simple random samples, high percentiles can be assessed with sufficient accuracy if the minimum sample sizes for the 95th, 97.5th, and 99th percentiles can be estimated to be equal to 160, 320, and 800, respectively. When results are presented for different subgroups within a study population, the minimum sample size has to be available for each subgroup.

The expected response rate should be taken into account in determining the sample size for achieving a certain number of actual participants. In the case of recruitment in waves through a year, adjustments in the number of invited participant can be made according to the actual response rates for specific strata in previous waves. Moreover, it is important to collect information on nonrespondents in order to eventually correct for lack of representativeness of the study population for the target population. For active nonparticipants, it is possible to administer a concise nonrespondent questionnaire (among those willing); for passive nonparticipant with which no contact was established, only information from the sampling frame can be used (see **Figure 3**).

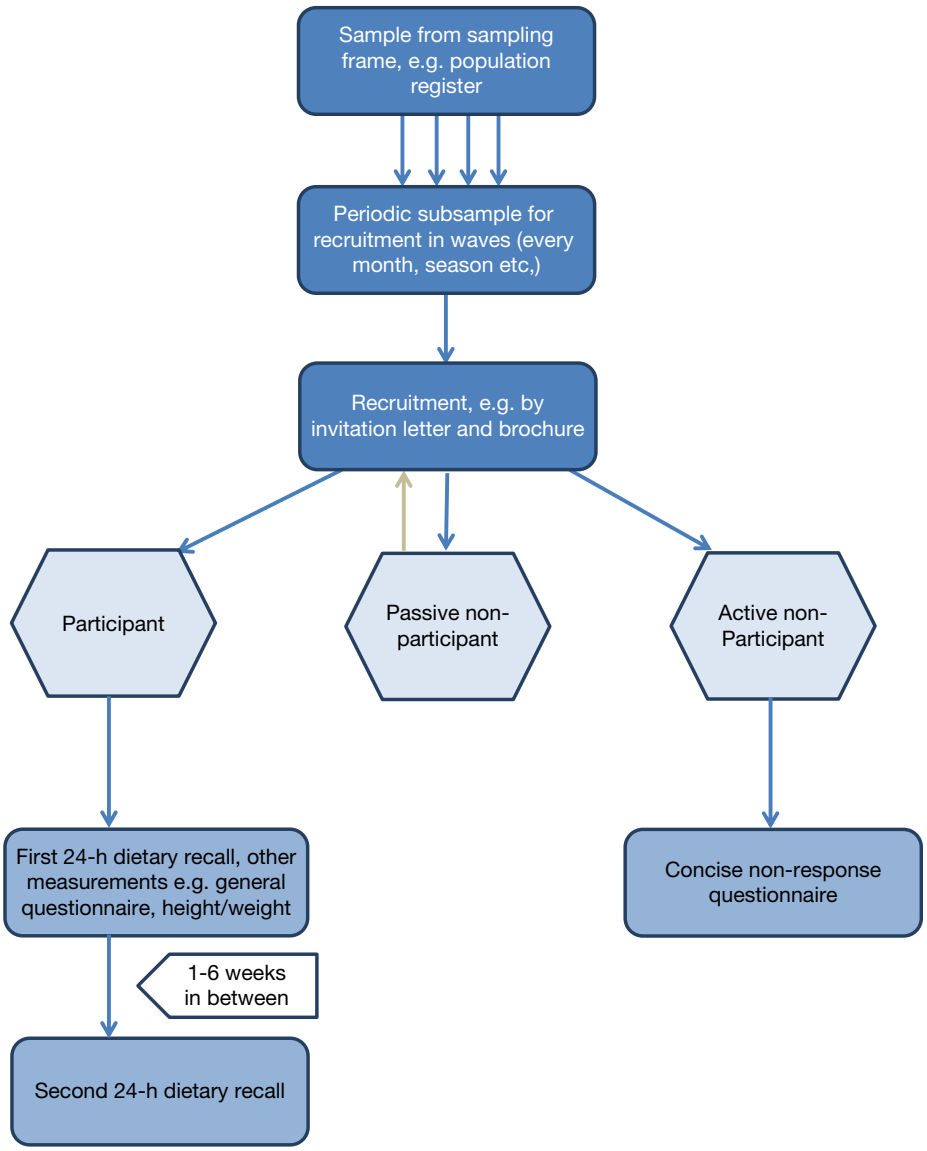

Figure 3 Example of the logistic flow of a food intake survey.

Dietary Assessment

Traditionally, four main methods of dietary assessment are distinguished. These are food records, dietary recalls, food frequency questionnaires, and dietary history questionnaires. Food frequency questionnaires capture usual intake, but they are not quantitatively precise and thus cannot estimate the percentage of the population with inadequate intakes or too high intakes. This was clearly demonstrated in a biomarker-based validation study in the United States, the Observing Protein and Energy Nutrition (OPEN) Study. The traditional dietary history also captures usual intake but is extremely labor-intensive and involves a high subject burden with an interview of several hours, whereas the modern version of a dietary history method has similar problems to food frequency questionnaire with estimating intake quantitatively. Therefore, food frequency methods and dietary history methods are not suitable for national food intake surveys.

Well-conducted food records and dietary recalls are better able to quantify food intake for the specific days they cover. Since usual intake is of interest for food intake surveys, at least two repeated nonconsecutive food records or dietary recalls are required together with statistical modeling of usual intake. With the statistical modeling, the within-person day-to-day variation in the intake data can be accounted for. For foods that are important contributors of nutrients and that are consumed by less than 50% of the population, additional information on the frequency of consumption has added value. Examples of these products are types of dietary supplements and liver.

Whether a food record or dietary recall is the preferred dietary assessment method for food intake surveys depends on the survey population. In populations with notably low response rates, the preferred method is the method with less respondent burden; this is the dietary recall. The dietary recall method is also preferred in populations with high illiteracy levels. In other populations, food records might be preferred. The food record is a prospective method and therefore does not have the disadvantage that it relies on memory such as the retrospective 24 h dietary recall method. However, it is well-known that people change their diets because of keeping a record of everything that is consumed. For harmonized pan-European food intake surveys, EFSA advises to apply dietary recalls as dietary assessment method, particularly because of the lower burden to the participants.

In order to obtain intake data that are usable for many purposes, it is important to collect information on all foods, beverages, and dietary supplements consumed. The information should contain a sufficient level of detail for the different purposes, for example, information on fat content, sweetening (sugar and/or artificially sweetened), fortification, preparation method, conservation method, and package material. Brand name information can help to ascertain aspects like sweetening and fortification that are often not known by consumers. It is also necessary to estimate the consumed amount for each eaten food. There are different options, which can be used in combination: estimation in household units like glasses or spoons, estimation in natural units, for example, for eggs or apples, estimation in commercial units for products like chocolate bars and sweets, estimation using food models or food photographs, and estimation in gram or milliliter.

To conduct a high-quality standardized 24 h dietary recall, it is advised to use a computer-administered software for 24 h recalls. With the software, the interviewer is guided by automatic response routing to ask tailored questions and prompts for specific foods. Using multiple steps, the system helps to ensure completeness and consistency across interviewers. There are automated links to food lookup tables and data quality checks. It is of major importance to test any software before applying it in a survey; preferably, a validation study should be conducted. Examples of carefully developed and extensively tested and validated software are the five-step multiple-pass method used in the NHANES in the United States and GloboDiet, previously known as EPIC-Soft, used in food intake surveys of Austria, Belgium, France, Germany, Malta, the Netherlands, and Switzerland.

Specific subgroups within a population might need a different dietary assessment method. For example, a 24 h dietary recall is less suitable for young children in case the child is often not together with the parent or caretaker that is interviewed. Also for older adults with a higher likelihood of impaired short-term memory, a 24 h dietary recall is less suitable. In both age groups, a dietary record or a food diary and 24 h dietary recall could be an option. For less developed countries, more emphasis is needed for feasible and robust methods.

Supplemental Information to be Collected from Participants

Depending on the specific aims of a food intake survey, other information is collected besides the dietary data. All food intake surveys collect information on background characteristics of the participants, usually including sociodemographic information and information on dietary practices. This enables to stratify the results by sociodemographic subgroups and to judge the representativeness of the study population.

Because of the high prevalence of overweight, additional information on physical activity and anthropometric information like height, weight, and waist circumference is also collected in many food intake surveys. Information on body weight is also of prime importance if chemical exposure from the diet is to be compared with health limits. These health limits are usually expressed per kilogram body weight. Moreover, some surveys collect information on health and others on food choice determinants, biological marker for nutritional status, or aspects important for microbiological risk associated with food consumption. It is outside the scope of this article to detail methods for collecting these data.

Administration Protocol

The study design must consider that in order to capture the interseasonal variability in consumption patterns, subjects must be uniformly distributed over the four different seasons and each seasonal sample must represent each survey stratum (e.g., on age and sex). This can be done by recruitment of participant in waves throughout the year (see Figure 2). It is of prime importance to monitor response and the completed number of participants and to adjust procedures if response or the completed number of participants falls behind.

The survey calendar must be organized in such a way that all days of the week are represented proportionally in the food consumption data. Specific emphasis on an adequate proportion of weekdays and weekend days at population group level is needed, since this is the main source of variation in intake over the days. For food intake surveys with nonconsecutive dietary records or recall, it is advised to have an interval of 1–6 weeks in between both administrations. More than 6 weeks in between, which might be an option theoretically, might result in losing study participants and is therefore not advised.

The administration of the interviews is usually done face to face, although in some surveys, telephone interviews are conducted. Face-to-face interviews can be conducted at a study center or at the home of the participant. The latter has the advantage that consumed foods and used household measures are available to show to the interviewer. Interviewers should preferably be trained dietitians or nutritionist; although, with more extensive training on consumed foods and recipes, also interviewers with other backgrounds are used in practice. During the survey period, refresher trainings are recommended. Moreover, it is of prime importance to monitor the quality of the interviewers and provide them feedback to improve their work.

Data Cleaning and Analyses

After data collection, data cleaning is an important phase. It is recommended to start data cleaning immediately after the first data are received from the interviewers, rather than waiting until the survey period is finalized. Data cleaning should include checking for missing, inconsistent, and extreme values and considering data-related remarks made by interviewers. When the food intake data are checked, further handling can include linkage to nutrient databases for foods and dietary supplements, databases with concentrations of chemicals in foods, or other food-related databases. More information on nutrient databases can be found in this encyclopedia.

Subsequently, statistical analyses can be performed. In these analyses, survey weights can be used to make the data representative for the target population. A commonly applied correction technique for lack of representativeness is weighting adjustment. It assigns an adjustment weight to each survey respondent. Persons with characteristics that are underrepresented get a weight larger than one, and those in overrepresented groups get a weight smaller than one. In the computation of survey results, not just the values of the variables are used, but the weighted values.

When usual intake is of interest, intake or exposure per person per day should be calculated and then usual intake modeling should be applied. In these models, the within-person variation in intake is removed from the data. Various tools to model usual intake exist. In the Statistical Program to Assess Dietary Exposure (SPADE), usual intake can be modeled for the combination of intake from foods and drinks as well as dietary supplements measured on two specific days. In the Monte Carlo Risk Assessment (MCRA) tool, usual exposure to chemical substances can be modeled, dealing, for example, with occurrence data below the detection limit and having the option to check the input data quality.

An important step in data analyses is evaluation of dietary intake against dietary reference values for nutrients and against health limits for food chemicals. Procedures for proper evaluation are described elsewhere in this encyclopedia.

Limitations

Two major challenges of food intake surveys are the internal validity and the external validity.

Internal validity of national food intake surveys is never perfect, since diet cannot be measured without error; both random error (including day-to-day variation) and systematic bias (social desirability) are present in the data. Important aspects within dietary recalls are the correctness of the reported foods (both omissions and intrusions occur), the completeness of food description, and the correctness of portion size estimation. In practice, dietary assessment based on self-reporting usually leads to underestimation of energy intake. Moreover, dietary assessment in combination with using food composition databases has additional limitations for specific components in the diet. This is, for example, the case for dietary exposure assessment of mycotoxins. Since the concentration of mycotoxins in foods is very variable due to, for instance, weather and storage conditions, duplicate diet studies or total diet studies are better equipped to estimate mycotoxin exposure. Another example is estimation of sodium intake. This is difficult because of the large variation in sodium concentrations in processed foods and recipes, as well as the difficulty of estimating discretionary salt intake. Dietary assessment of sodium (rather than urinary excretion) does however enable identification of important dietary sources of sodium, which can inform public health interventions to lower sodium intake.

What is important is whether the dietary assessment method is suitable for providing useful analytic measurement for a given purpose and context. For many purposes and in many contexts, 24 h dietary recall data from national food intake surveys proved to be useful in helping to address important research and policy questions, despite their known errors. Strategies to optimize the impact of food surveys are to have more transparency of raw research data, consistent data-staging techniques, improved data analysis, and reporting indicators for dietary intake quality such as indicators for underreporting.

External validity is impacted by low response rates that often occur. In practice, response rates between 26 and 97 are observed in national food intake surveys, with the majority between 42% and 71%. Low participation rates and selective nonparticipation may cause bias to the survey results based on participants alone. It is known that respondents and nonrespondents might differ in their socioeconomic and demographic status as well as in their health and lifestyle behaviors. Therefore, for achieving a representative study population, it is of utmost importance to use all means to obtain the highest response rate possible. Strategies to optimize response rates are choosing data collection methods with a lower burden for the respondents, providing a suitable incentive, flexibility in recruiting and participation (times available for the interview, second call if a no-show, etc.), personal contact in recruitment, and awareness of the study by media coverage.

Harmonization of National Food Intake Data Across Countries in the European Union

National food intake surveys in Europe are heterogeneous with respect to dietary assessment methodology and number of days for which dietary data are collected. This hampers the comparison of results across countries, for example, in dietary exposure assessment using the data of the comprehensive food consumption database such as conducted by EFSA. Therefore, EFSA stimulates European Union member states to collect national food intake data in a harmonized way and prepared guidance for this in 2009 with an update in 2014. Moreover, EFSA provides seed money to countries that conduct food intake surveys in accordance with EFSA guidance. This is called the EU Menu process. The guidance of EFSA was partly based on the results of previous European research projects, such as summarized in the succeeding text.

In the European Food Consumption Survey Method (EFCOSUM) project, recommendations for reliable and comparable pan-European collection of food intake data were formulated. As the most suitable method to collect internationally comparable data on population means and distributions of actual intake, the 24 h recall was recommended, to be conducted at least twice. This would also allow for the estimation of usual intake by a modeling technique that separates intra- and interindividual intake. GloboDiet, at that time called EPIC-SOFT, was considered the most suitable software for a standardized data collection in a pan-European survey. For a number of micronutrients, that is, vitamin D, folate, sodium, iron, and iodine, the use of biomarkers was recommended.

Subsequently, the EFCOVAL project further developed and validated the pan-European food consumption method recommended by the EFCOSUM project. The validation study confirmed that the repeated 24 h dietary recall using GloboDiet in combination with a food propensity questionnaire and modeling of usual intake is a suitable method for a pan-European food intake survey. This conclusion applied to healthy adults and possibly to children aged 7 years and older. As next step, it was recommended to provide and standardize an implementation plan to facilitate this methodology in European countries. The plan should account for maintenance and updates, sampling designs, national surveillance programs, tailored capacity building and training, and linkage to food composition and occurrence databases.

The objective of the PILOT-PANEU project was to develop, test, and evaluate the applicability of tools and procedures for conducting a pan-European food intake survey including adolescents, adults, and elderly people. The dietary assessment methodology consisted of two nonconsecutive 24 h recalls performed with GloboDiet methodology. The consortium made a number of recommendations for the improvement and further development of the tools and procedures. Assuming that the recommended modifications are made, the tools and procedures developed and tested were considered suitable for the EFSA EU Menu process in adolescents, adults, and the elderly.

Similarly, in the 'Pilot study for the Assessment of Nutrient intake and food Consumption Among Kids in Europe' (PANCAKE), the feasibility of tools and procedures for a pan-European food intake survey among children 0–10 years was tested. This included two alternative dietary assessment methods. One method consisted of a 3-day food diary, which was checked with a parent, and data were entered afterward using GloboDiet. The alternative method consisted of two nonconsecutive 1-day food diaries followed by GloboDiet completion interviews. Both dietary assessment methods with related tools and administration protocols were evaluated as feasible. The administration protocol with two 1-day food diaries with completion interviews was judged to offer more advantages for the EFSA EU Menu survey in children 0–10 years. It offered more complete description of the consumed foods, was more suitable to estimate usual intake, and resembled more the method in adults.

Future Directions

In the future, it is important to improve data collection methods and to collect food intake data in connection to other information.

First, improving data collection methods should focus on methods that have a small participant burden or that are attractive to participants, so that response rates increase. The use of modern technologies might help for this purpose, although for some population groups, it might not be appropriate. Additionally, future data collection methods should be less labor-intensive than current data collection methods, since the costs of the current methods are high and increase because of the dynamic and increasingly complex food market. Examples of promising data collection methods are online 24 h dietary recalls, devices that automatically take pictures of all food consumed, and apps for food records using bar codes and food pictures for portion sizes, or automatic recognition of photographed consumed food and volumes. These promising technologies need to be extensively tested in the general population and important subgroups of the population and validated positively before they can be incorporated in food intake surveys. At the moment, various interesting developments and validation studies with modern technologies are ongoing.

Second, it has added value to conduct national food intake surveys in connection to biomarker and other data collection. Combining with nutritional status monitoring and collecting information of biomarker of food intake allow for better interpretation of food intake results and provide insight in the validity of the results. Combining with data collection on other lifestyle information has the advantage that risk groups with multiple unhealthy lifestyles can be identified. Moreover, in order to get insight in ways to improve dietary intake, it is an asset to collect food intake data in combination with information on determinants of food choice, the food environment, and economical aspects of diet and health. Such integrated food intake surveys ask for ingenious study designs so that the participant burden can be kept acceptable, and not all information has to be collected from each participant.

Conclusions

Conducting regular national food intake surveys is important for the development and evaluation of nutritional and food safety policies. The current best practice in food intake surveys

is to obtain a representative sample from a national population register. For each participant, at least two nonconsecutive food records or 24 h dietary recalls combined with a short food frequency questionnaire need to be collected supplemented with at least sociodemographic background information. Statistical modeling should be used to estimate usual intake. For pan-European harmonization of food intake surveys, dietary recalls rather than food records are advised. Standardized collection of 24 h dietary recalls with much detail can best be done using validated software such as GloboDiet.

Overall, study preparation and data collection and data handling of food intake surveys with much detail in the food description are labor-intensive and therefore costly. Using the collected dietary data to their full potential will however lead to a return on investment. Potential uses are manifold. Moreover, investments can become smaller if the same methods are used repeatedly and shared with other groups. Output and lessons can increase if the collected data are easily discovered, user-friendly to access and understand, and freely available. The trend toward open data helps here.

This article described that two features are critical for the success of national food intake surveys. The first is the internal validity or validity of the collected food intake data. The second is the external validity or the representativeness of the survey population for the target population. In the practice of food intake surveys, we have to deal with suboptimal situation that diet cannot be measured without error and decreasing response rates. Future directions should address both these challenges and time and cost-efficiency.

See also: Dietary Exposure Assessment; Dietary Practices; Dietary References: US; Food Composition Databases.

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Drying: Effect on Nutrients, Composition and Health

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Introduction

In their natural state, most foods contain a significant quantity of water. In foods with a high water activity (a_w), spoilage and/or pathogenic microorganisms can grow; in addition, degradative physicochemical changes occur more rapidly in high a_w foods. One of the most effective methods of increasing both the shelf life and safety of foods is through dehydration. Traditional drying methods, such as sun-drying, are slow and manual in nature, and can incur losses in yield due to difficult-to-predict factors relating to the local climate and indigenous wildlife. These traditional methods of dehydration are still successfully used in developing countries and also in the production of artisan or minimally processed food. However, the modern food supply chain is a global one, and the increased affluence of consumers has resulted in a strong demand for the year-round availability of a diverse range of foods. In industrial countries, this demand has contributed to the general adoption of mechanized drying technologies (e.g., spray- and freeze-drying) and a proliferation of food powders.

Food powders are nutrient-dense, low-volume products that can be easily transported nationally and internationally. The drying process itself can also be harnessed to encapsulate nutrients deemed to have specific health benefits, such as probiotics, fatty acids, and phytochemicals. These encapsulated nutrients can be stored for use as versatile ingredients in the fortification of a wide range of foods, which could allow their targeted delivery to a larger proportion of the world population. In this article, the influence of the drying process and powder storage on the stability of key nutrients in different food systems is discussed. Certain dried foods, such as herbs and spices, are omitted, as the low levels at which they are consumed were considered to render their nutritional contribution to the average diet negligible. A comprehensive overview of the effects of drying on a range of foods and nutrients is provided, including fruits, fruit juice, vegetables, egg, milk, wine, algae, infant formulas, enteral formulas, bacteria, and plant-derived oils. This overview is based on recent research, which has primarily focused on the effect of drying conditions (e.g., temperature and flow rate) and postdrying storage conditions (e.g., relative humidity and light exposure) on the stability of key nutrients. The effect of bulk density on nutrient dosage in nutritional powders and the role of accelerated stability tests and fortification overages in formulation design, two increasingly important topics in modern manufacturing practices for dried foods, are also highlighted before a general closing discussion on the link between dried foods and health.

Effect of Drying on Nutrients

Changes in Macronutrients During Drying and Powder Storage

Much of the focus of this article is on the changes that occur in the micronutrients present in foods during drying and subsequent storage. Although reactions occurring between macronutrients, such as those involving proteins and carbohydrates during nonenzymatic browning, are of nutritional consequence, individual macronutrients in food are generally not impacted to a significant degree by the drying process alone. In this section, the changes that occur in the macronutrient fraction are discussed briefly. The factors influencing the degradation of macronutrients during the drying of foods are also summarized in Table 1.

Protein

Protein is naturally present in many common dried products, including milk and egg powders, and can play a significant role in the encapsulation of lipids and probiotics. In addition, advances in processing technology have resulted in an increased availability of dairy-derived (whey protein and casein) and plant-derived (soy and rice) protein powders, bars, and beverages, which are popular product categories among young adults engaged in athletic activities; on the other hand, an aging or 'graying' global population is likely to result in increased instances of diseases such as pathological sarcopenia, which is accompanied by muscle wastage. In both cases, consumption of adequate quantities of protein is essential to aid in the process of muscle synthesis. Modification of the structure of proteins (i.e., denaturation and aggregation) has been associated with altered biofunctional properties such as allergenicity and digestibility, but these effects are generally not attributed to the drying process itself. Additionally, these structural modifications can occur in predrying processing, such as the pasteurization and evaporation treatments applied to some dairy systems. In the presence of carbohydrates, however, proteins may undergo structural modifications due to the Maillard reaction, a nonenzymatic browning process. This aspect is covered in '**Carbohydrate**.'

Carbohydrate

Carbohydrates are important structural components in dried food matrices. In powders, they are frequently the primary encapsulating material for other nutrients (e.g., fatty acids and probiotics). Degradative changes associated with carbohydrates of nutritional consequence are mostly limited to the generation of Maillard reaction products (MRPs) through nonenzymatic browning reactions between reducing sugars and amino acids. The generation of MRPs has been linked with increases in cytotoxicity, mutagenicity, and carcinogenicity; however, MRPs have also been associated with positive effects such as increased antioxidant activity. In addition to health effects, MRPs are associated with color and flavor development in foods. However, while the sensory attributes that Maillard browning imbues on certain foods, such as bakery and confectionary products, are often desirable, browning in food powders is typically considered a defect. Investigations of MRP generation in infant formula powders have been frequent, as

Table 1 General properties of the main macronutrients in foods related to the influence of drying on their nutritional quality

Macronutrient name(s)	*Main degradative reaction*	*Drying-induced changes*	*Storage-induced changes*	*Key influencing factors*	*Some potential adverse nutritional consequences*
Proteins	Denaturation	Negligible	Negligible	Predrying thermal processes (e.g., pasteurization and evaporation)	Protein digestibility⇓ Protein allergenicity⇑
Carbohydrates/ proteins[a]	Browning	Minor	Significant	Drying technology, drying temperature (spray-drying), feed composition (type of sugar), storage conditions (relative humidity, temperature, light exposure), crystallization of sugars	Cytotoxicity⇑ Mutagenicity⇑ Carcinogenicity⇑ Lysine levels in food⇓
Lipids	Oxidation	Significant	Significant	Emulsion state (e.g., fat globule size distribution, emulsifier type), encapsulation efficiency, protein/fat ratio, crystallization of sugars	Cytotoxicity⇑ Genotoxicity⇑ PUFA[b] biofunctionality⇓

[a]Nonenzymatic browning requires amino acids in addition to carbohydrates.
[b]PUFA, polyunsaturated fatty acid.

they are nutritional products high in both lactose and protein. MRPs can be generated during spray-drying, with reports highlighting the impact that spray-drying has had on infant formulas, resulting in greater loss of lysine than observed under processes of sterilization; however, it is also worth pointing out that higher concentrations of MRPs have also been measured in sterilized infant formulas compared with spray-dried formulas. Different drying techniques and feed compositions can influence the physical properties (e.g., porosity and morphology) and chemical behavior (e.g., crystallization and water sorption) of the dried material during storage, which in turn can influence the generation of MRPs. MRP generation also occurs in powders during storage. Environmental conditions during the storage of infant formula powders (e.g., relative humidity, temperature, and light and oxygen exposure) have a strong impact on the rate of generation of MRPs.

Lipids

Lipids are a primary contributor to the energy density and texture of foods. Lipid-containing foods are typically emulsions, consisting of lipids dispersed in a continuous water phase (O/W emulsion). Emulsified systems are intrinsically unstable, and to improve their stability, multiple strategies are employed, including the reduction in fat globule size through physical disruption (e.g., indent homogenization and microfluidization), increasing the viscosity of the continuous phase (e.g., addition of hydrocolloids), and the addition of amphiphilic molecules that adsorb at the oil–water interface and reduce interfacial tension (e.g., lecithin). When emulsions are dried, the continuous phase is removed and a solid carbohydrate/protein matrix forms around the fat. Effective encapsulation reduces the levels of free fat at particle surfaces and is dependent on the properties of the emulsion to be dried, the nature and level of the encapsulant, and the drying conditions. Oxidation of specific components in the lipid fraction, namely, fatty acids and cholesterol, is associated with losses in nutritional quality and is discussed in more detail in '**Fatty acids**' and '**Cholesterol**,' respectively.

Changes in Micronutrients During Drying and Powder Storage

Fatty acids

Consumption of foods that are rich in polyunsaturated fatty acids (PUFAs) has been associated with numerous health benefits. Soybeans are an example of a food that is naturally rich in PUFAs. Microwave-assisted drying has been reported to result in less PUFA degradation in soybeans compared to forced-convection drying, and PUFA degradation was independent of drying temperature if soybeans were dried to the same final moisture content. It has been demonstrated that sun-drying or freeze-drying resulted in higher retention of seaweed PUFAs compared to oven-drying. Although drying can result in PUFA degradation, it may be preferable to canning as a method of preserving seaweed, with one study reporting higher levels of total PUFAs in five dried seaweeds compared to two canned varieties.

Infant milk formulas are frequently fortified with PUFAs, as long-chain PUFAs are abundant in human milk and are believed to contribute to the development of cognition and visual perception. Infant formulas contain metals that are known to be pro-oxidizers, such as iron. Iron-binding proteins, such as lactoferrin, have been shown to protect against oxidation when added to infant formulas. Methods for the encapsulation of PUFA-rich oils (e.g., fish oil) have been developed, but the protective effect of the encapsulant can diminish during processing, and oxidation of PUFAs remains a challenge.

It has been reported that fortification of infant formula with microencapsulated fish oil resulted in higher peroxide values after 18 months. Reduced oxidation has been shown in infant formulas where casein was the dominant protein, compared to whey protein-dominant formulas, while higher levels of carrageenan or lecithin have also been demonstrated to reduce oxidation in infant formulas. Higher levels of PUFAs, elevated storage temperatures, and long storage duration generally result in increased oxidation.

There is ongoing research into the potential for the encapsulation of plant-derived oils that are high in PUFAs, such as walnut and chia oils, using spray-drying, with some recent

research showing that homogenization of a chia oil emulsion had a greater impact on the effectiveness of sodium caseinate–lactose encapsulation than the temperature at which the emulsion was spray-dried. Encapsulation of oils by spray-drying in the presence of hydroxypropyl methylcellulose, with or without rosemary oil (antioxidant), improved the oxidative stability of walnut oil but resulted in greater oxidation in chia oil; it was suggested that the poor stability of encapsulated chia oil was linked to chemical damage caused during spray-drying.

Cholesterol

The oxidation of cholesterol with a concomitant increase in the levels of cholesterol oxidation products (COPs) can occur during the manufacture of egg powders, which is a concern as COPs have been linked with disease in humans. In other dried products, such as spray-dried milk, cream, and infant formula powders, the levels of COPs are less of a concern. High outlet air temperatures and the generation of nitrogen oxides in a gas-fired air heater during spray-drying resulted in increased generation of COPs. In addition, COPS can form during storage of egg powders, but this can be controlled by the presence of antioxidants in the powder, such as ascorbyl palmitate and tocopherols. Spray-dried egg powders contained more COPs than freeze-dried varieties, with the generation of COPS being higher in those powders produced from liquid whole egg than liquid egg yolk alone.

Phytochemicals

Phytochemicals are plant-derived substances, such as polyphenols and flavonols, which are purported to have various bioactive properties. Studies have found that the levels of phenolic compounds in dried kale were highest when freeze-drying was used compared to air-drying, while blanching before drying led to reduced levels in kale dried by either method. Recent research has shown that Irish brown seaweed dried in a hot air oven at different temperatures (25, 35, or 40 °C) increased the levels of phytochemicals as drying temperature increased and that increasing temperature of rehydration of dried Irish brown seaweed in water results in faster rehydration times but lower levels of phenols.

It has been shown that drying conditions have no impact on the phytochemical contents of apricots, currants, or prunes, but do have an effect on grapes, while sun-drying has little effect on the levels of phenolic compounds in Italian bell peppers. A comparison of air- and freeze-drying for their ability to retain phytochemicals in physiologically dropped, unmatured citrus fruits reported that while air-drying was better at retaining flavonols, freeze-drying exhibited superior retention of phenols.

A phenol-rich ingredient, named piraltin, has been manufactured by freeze-drying red wine; piraltin contained ~70% of the polyphenols in the wine and was alcohol-free. Phenol levels in spray-dried bayberry juice, with maltodextrin as encapsulant, were nearly 100% of those in the original juice; when stored at 4 °C, the levels of polyphenols in the bayberry juice powder only decreased by <10% at the highest a_w studied (0.44); in addition, air-drying of apple pomace was more effective than vacuum-drying at preserving the phytochemical content. The use of maltodextrins with low dextrose equivalents and spray-drying at low inlet temperatures resulted in lower phenol losses during drying of extracts of phenols from black currant pomace. Encapsulation of a phenol extract derived from olive pomace by spray-drying at different inlet temperatures (130 or 160 °C), feed flow rates (5 or 10 ml min^{-1}), and maltodextrin levels (100 or 500 g l^{-1}) has also been studied, with the highest polyphenol levels at an inlet temperature of 130 °C, a feed flow rate of 10 ml min^{-1}, and a maltodextrin level of 100 g l^{-1}; it was also found that the powder underwent the least degradation of phenols when stored in dark conditions at low temperatures.

Probiotics

Probiotics are live bacteria or yeasts that are believed to have a beneficial role in the maintenance of gut health and the prevention of gastrointestinal diseases. In addition, a growing body of evidence supports a role for probiotics in the promotion of neurological function. Common probiotic strains, which are frequently added to foods in dried form, include *Bifidobacterium* (*B.*) and *Lactobacillus* (*L.*).

Adapting *L. paracasei* by growing it in a stressful environment (high heat or salt) resulted in a 16–18-fold increase in stability when the bacteria were inoculated into milk and dried. Similar findings have been reported for *L. casei* Nad and *L. plantarum* 8329 spray-dried with skim milk. When *L. salivarius* subsp. *Salivarius* was freeze-dried in distilled water, only 1% of viable bacteria remained; however, when the same probiotic was dried in a medium consisting of skim milk, glucose, and trehalose, >83% of viable bacteria remained; the survival of the freeze-dried probiotic during storage was best at the intermediate levels of moisture, which were studied (2.8–5.6%). Researchers have spray-dried *L. paracasei* inoculated in a skim milk/yeast medium using an inlet temperature of 175 °C and an outlet temperature of 68 °C and reported that ~15% of the probiotic was lost during drying; when cheddar cheese was fortified with the powder, the researchers found that the probiotic population grew during ripening.

The survival of *Saccharomyces boulardii*, a probiotic yeast, was greatest at acid pH and high inlet temperature, deemed by the authors to be due to optimal protein crust formation around the yeast in these conditions. Encapsulation of *B. animalis* subsp. *lactis* BB-12 in sweet whey resulted in reduced viability at acid pH and no difference in salt tolerance (bile or NaCl); when the probiotic was added to a refrigerated dairy dessert, it showed a consistently higher viability during 6 weeks of storage. Freeze-drying of *B. longum* 1941 in different protein–sugar encapsulants revealed that milk proteins (skim milk, sodium caseinate, and whey protein concentrate) outperformed soy proteins and that sugar alcohols (glycerol and mannitol) outperformed maltodextrins, in terms of postdrying viability and acid and bile tolerance. Experiments where single droplets containing *L. plantarum* A17 in addition to skim milk, whey protein isolate, lactose, or trehalose were dried revealed that the dairy ingredients were capable of protecting the bacteria to a greater degree than the sugars during drying, due to the formation of a crust that limited the thermal load applied to the encapsulated bacteria.

An interesting approach to the drying of probiotics is their codrying in the presence of prebiotics. Prebiotics are carbohydrates that cannot be digested by humans, but that can be used as a nutrient source by probiotics, thereby stimulating the

growth of the latter. For this reason, they are sometimes collectively referred to as 'synbiotics.' Recent studies have shown that *Bifidobacterium* species can be effectively encapsulated using inulin, oligofructose, oligofructose-enriched inulin, or polydextrose, while *Lactobacillus* species have been encapsulated using fructooligosaccharide. Other combinatory approaches at improving the manufacture of probiotics include the use of spray–freeze-drying, which combines spray-drying and freeze-drying technologies and has primarily been used in the production of biopharmaceuticals. Spray–freeze-drying was successfully used to dry *L. casei*, with 98% of the probiotic surviving the drying process.

Nucleotides

Nucleotides in the diet are thought to contribute positively to immune function, gastrointestinal health, and absorption of other micronutrients. Nutritional sources of nucleotides mainly include mammalian milks. It has long been known that human milk has innately high levels of nucleotides, and this has resulted in efforts to humanize infant formulas through fortification with nucleotides. Significantly lower levels of adenosine 5′-monophosphate and cytidine 5′-monophosphate have been measured in reconstituted skim milk powder compared to raw milk, indicating that the spray-drying process may cause degradation of nucleotides. It was also reported by the same group that pasteurized milk had lower levels of these nucleotides, and as the skim milk powders were pasteurized and evaporated before drying, it is difficult to ascertain the influence of the drying step in isolation; it is also worth noting that in-container sterilized and powdered milks were the only samples to contain significantly lower levels of orotate. A recent study found that pasteurization of human milk actually increased the levels of free nucleotide monophosphates; on the other hand, high-pressure processing caused a decrease.

Casein-dominant infant formulas have been shown to have naturally higher concentrations of nucleotides compared with whey protein-dominant formulas, whether the latter had been fortified or not. Additionally, the same author indicated that minimal degradation of nucleotides occurred in the powders studied during 1 year of storage. Recently, nucleotides in 11 infant formulas were analyzed and it was found that the majority had levels that were in general agreement with their label claim; however, one formula was reported to have nucleotide levels that were 90% less than what the label claims, indicating major losses during processing. More research is required to identify the potential role of spray-drying and other processing steps on the degradation of nucleotides in infant formulas.

Vitamins

Foods such as fruits and vegetables are widely known to be important natural sources of vitamins, while formulated products such as infant formulas are increasingly fortified with vitamins. Vitamin D_3, which is now routinely added to milk and infant milk products, was shown to undergo negligible degradation in the production of spray-dried milk. Vitamin C was shown to degrade during the air-drying of tomatoes, with higher drying temperatures resulting in greater losses; immersion in salt or sugar solutions prior to drying was found to inhibit this degradation. An inverse relationship between air-drying temperature and vitamin C levels in both tomatoes and papaya has also been reported.

Decreases in vitamins A, E, and C were measured during storage of infant formula powders for up to 18 months; the greatest losses were reported for vitamin C, and levels of all vitamins decreased more rapidly when powders were stored at elevated temperatures (40 °C vs. 25 °C). Tracking the degradation of vitamins A and E in infant formula powders after the packaging had been opened revealed that, while the levels of these vitamins did decrease over 70 d storage, the levels remained within regulatory limits. Enteral formula powders underwent reductions in vitamins A, B_1, and E during storage for up to 6 months at 30 °C that were sufficient to compromise the ability of those formulas to satisfy their label claims for RDAs, particularly in adverse conditions (high a_w and lengthy storage). Efforts to increase the retention of vitamins in powders by advanced drying techniques (nanospraying or electrospraying with encapsulants) have recently been investigated.

Minerals

The greatest potential for loss of minerals in products intended for drying is when the feed is in its liquid state. Mineral fallout in neutral-pH liquid products (e.g., cow milk, soy milk, and infant formula) can be caused by the use of insoluble calcium salts such as calcium carbonate, phosphate, and citrate, but this is less of a concern in acid products such as fruit juices. The stability of insoluble salts in liquids can be improved through the use of thickeners to increase the viscosity of the solvent or micronized forms of calcium that sediment less rapidly. Mineral loss from food during drying or during postdrying storage has not been demonstrated to be an issue affecting the nutritional quality of foods.

Other Considerations When Drying Nutritional Formulations

Influence of Bulk Density on Nutrient Dosage in Nutritional Powders

Powdered protein supplements are currently very popular among the physically active. In addition, powders are available that, once reconstituted, are intended to function as complete meal replacers, where they are the sole source of sustenance for the consumer. Critically, these products require the consumer to add a defined quantity of powder to a defined quantity of water to create a beverage with defined levels of key nutrients. In products such as these, infant formula powders being another example, the manufacturer must ensure that the powder has a consistent bulk density so that the consumer can scoop the correct quantity of powder into the liquid. In spray-drying, bulk density is influenced primarily by the viscosity of the feed, degree of aeration of the feed, type of atomizer, and agglomeration and/or instantization. If pneumatic conveying is used to transport powders within a manufacturing facility, additional care must be taken to prevent agglomerate breakage, which can result in deviation of bulk density from specified values. The manner in which bulk density can affect nutrient dosage in these products is illustrated in Figure 1.

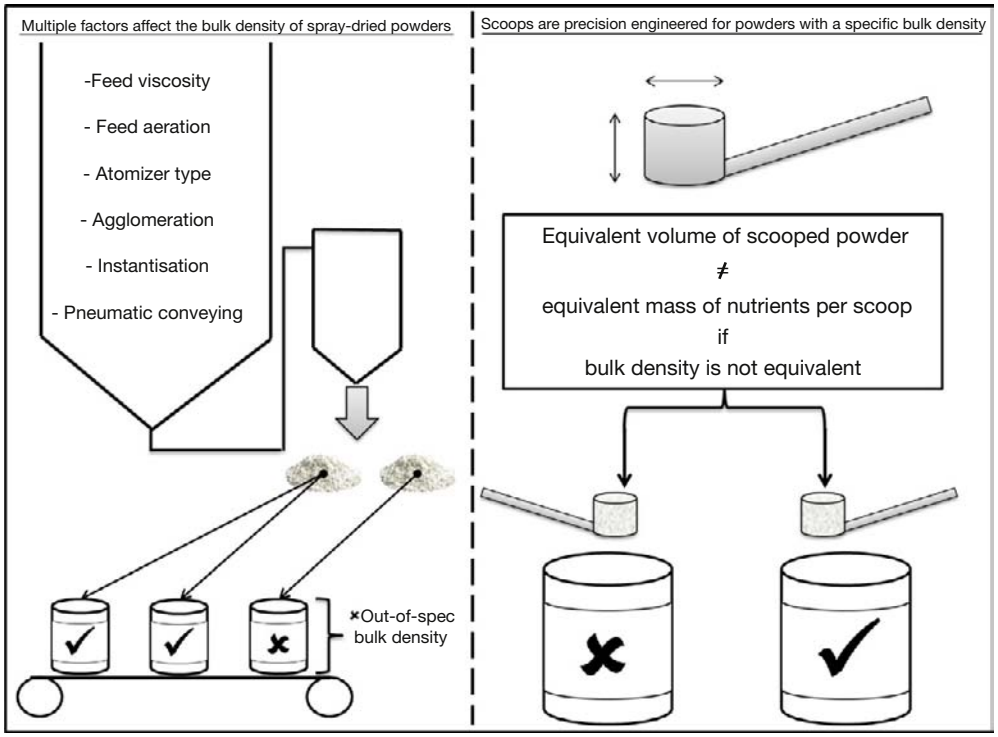

Figure 1 Influence of bulk density on nutrient dosage in spray-dried nutritional formulations, such as infant formula and meal replacers.

Role of Accelerated Stability Tests and Fortification Overages

In the development of new nutritional products, the final formulation and shelf life are determined by the integration of nutrient and physical stability testing data. For extended shelf life powders, the long shelf life necessitates the implementation of accelerated stability testing programs that complement traditional ambient stability tests. The principal environmental factors that are modulated during accelerated stability tests are temperature, light exposure/intensity, and relative humidity. Typical ambient and accelerated stability testing storage conditions for powders are 25 °C/60% relative humidity and 40 °C/75% relative humidity, respectively. For sensitive nutrients (e.g., B vitamins and PUFAs), the formulation overages required are normally determined based on measured losses during processing and stability testing. The principal factors affecting the overages required for individual nutrients vary but are generally determined by inherent stability of the nutrient, regulatory/quality limits, choice of drying technology and operating parameters, packaging material, transport and storage conditions, and the required shelf life. Some general considerations for nutrient stability testing are that ambient and accelerated programs should run in parallel and stability data should be generated using the actual commercial packaging material in all types and sizes that are manufactured. These concepts are illustrated in Figure 2.

Drying and Health: Overview and Future Perspectives

Most foods, in their natural, high-moisture state, are prone to rapid deterioration due to microbial spoilage and/or chemical degradation. Spoiled food is unpalatable and potentially toxic and generally has poor nutritional value. Dried foods and food powders have shelf lives that far exceed those of undehydrated varieties, and it may take many years before any spoilage eventually occurs. It would be a mistake to simply compare the nutritional value of a freshly harvested food with the same food recently dried, because the relative dynamics of degradation of each over time are also an important factor determining their potential health-giving properties.

For certain nutrient-dense foods, it may not be possible to produce them in certain regions of the world, or where they can be produced, the supply may be contaminated or otherwise untrustworthy; in cases such as this, dried foods are a low-volume product that can be transported from a reliable source at low cost and low environmental impact. Some of the research that has been highlighted in this article has demonstrated that the nutritional value of foods can indeed depreciate when being transformed into a dried product; however, many of these studies have also shown that this effect can be minimized or even eliminated if the drying process is carefully controlled.

The consumption of bioactive substances (e.g., phytochemicals, probiotics, and PUFAs) in sufficient quantities to extract their purported benefits is difficult to achieve in many regions, particularly for the socioeconomically disadvantaged. Encapsulation by drying affords the possibility of a range of bioactive-fortified foods becoming commercially available. The availability of such products may alleviate the burden on individuals to consistently source and acquire the requisite foods, which may be prohibitively expensive or otherwise inaccessible. Moreover, it is thought that targeted food fortification strategies may be needed to alleviate region-specific

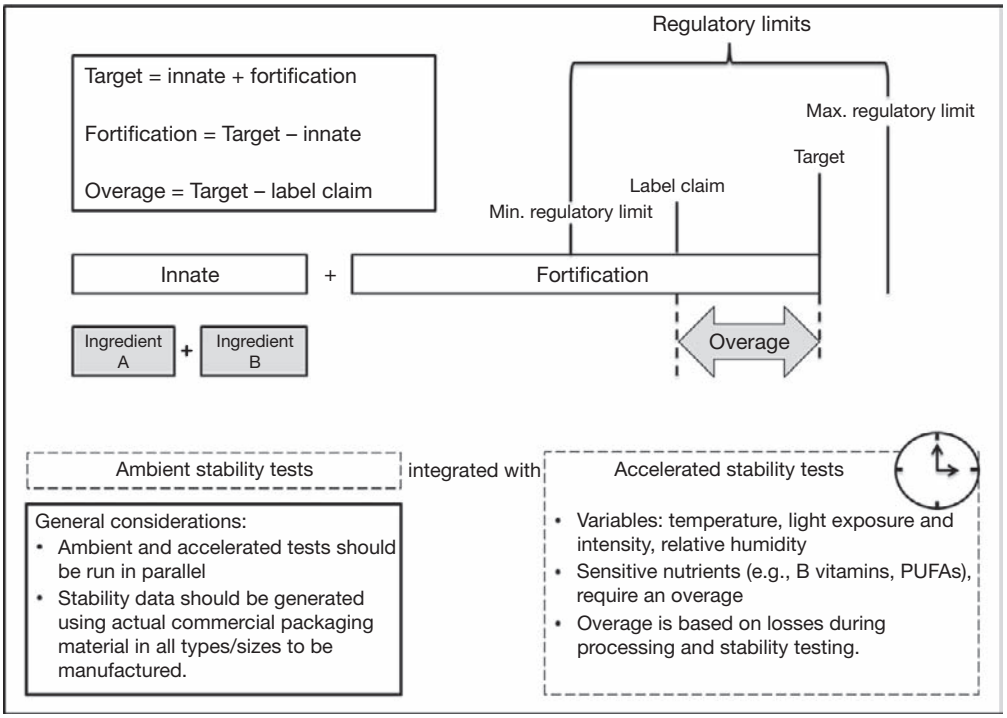

Figure 2 Principles of stability testing and fortification overages for sensitive nutrients during formulation of nutritional product.

deficiencies in certain vitamins, for example, vitamin D, the status of which is thought to be lower in northern latitudes due to a lack of sunshine; the effective incorporation of such fat-soluble vitamins into stable food systems is likely to require effective encapsulation technology.

Dried food is a valuable alternative to fresh food, where the latter is unsafe or unavailable. The nature of a given food needs to be considered during the design of appropriate processes for its dehydration. In general, dried foods should be stored in cool (<25 °C), dark environments and preferably in sealed containers, to prevent degradation of nutrients. Encapsulation processes for the manufacture of different bioactive substances have been reported as being successful by many researchers. The challenge now is to incorporate these ingredients into real food matrices, ensure their stability, and verify that they can have a positive influence on the health of the modern consumer.

See also: Antioxidants: Role on Health and Prevention; Ascorbic Acid: Physiology and Health Effects; Ascorbic Acid: Properties, Determination and Uses; Berries and Related Fruits; Beverage: Health Effects; Beverage: Patterns of Consumption; Bifidobacteria in Foods: Health Effects; Bioavailability of Nutrients; Browning: Non-enzymatic browning; Carbohydrate: Digestion, Absorption and Metabolism; Dairy Products: Dietary and Medical Importance; Drying: Physical and Structural Changes; Drying: Principles and Types; Eggs: Composition and Health Effects; Elderly: Nutrition Requirements; Fatty Acids: Fatty Acids; Fish Oils: Composition and Health Effects; Food Fortification: Rationale and Methods; Freeze Drying: Effects on Sensory and Nutritional Properties; Freeze-drying: The Basic Process; Functional Foods; Heat Treatment: Principles and Techniques; Infants: Nutritional Requirements; Lactic Acid Bacteria; Maillard Reaction; Milk Powder; Milk: Processing of Milk; Milk: Role in the Diet; Oxidation of Food Components; Papayas; Pasteurization: Effect on Sensory Quality and Nutrient Composition; Phenolic Compounds: Occurrence, Classes, and Analysis; Prebiotics; Preservation of Foods; Probiotics; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Solanaceous Fruits Including Tomato, Eggplant, and Peppers; Sports Nutrition; Storage Stability: Mechanisms of Degradation; Storage Stability: Shelf Life Testing; Vitamins: Overview; Whey and Whey Powders: Production and Uses; Whey and Whey Powders: Protein Concentrates and Fractions.

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Drying: Physical and Structural Changes

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Introduction: Useful Concepts in Food Drying

Food is an important part of our life, and all types of food need to be preserved one way or another for a number of reasons, drying being the most important and effective way of preservation. Fresh fruits and vegetables contain high amounts of water, which results in microbial damage. This reduces the shelf life of foods substantially, unless it is processed efficiently. A considerable volume of fruit and vegetables is lost every year because of microbial spoilage. The moisture in a food product can be reduced to an acceptable level using several thermal dehydration techniques to minimize the possibility of microbial damage. In addition to this, the cost for packaging, transportation, and storage for dried product is substantially less compared to canned or frozen food products. Nevertheless, drying is a highly energy-intensive unit operation because of the high latent heat of evaporation of water (2260 kJ kg^{-1} in free state); hence, the cost of reducing moisture to a very low level is expensive. There have been several developments in recent years in the field of thermal dehydration to enhance drying rates, reduce energy usage, and minimize environmental impact. However, in the case of food products, the quality of the dried product is more important, although other issues such as energy usage and environmental effects of a selected dryer/drying system cannot be neglected.

The moisture present in solids can be classified as bound or unbound moisture. The bound moisture exerts vapor pressure less than that of the pure liquid and the moisture in excess of bound moisture is the unbound moisture. When wet solids are subjected to thermal dehydration, two processes occur simultaneously: removal of surface moisture by evaporation and transfer of internal moisture to surfaces through diffusion and its subsequent evaporation. The removal of surface moisture depends on external conditions such as temperature, relative humidity of drying air, surface area of the wet material available for drying, airflow, and pressure. On the other hand, the removal of internal moisture depends on the physical nature of the solids present, temperature, and amount of moisture present. This largely decides physical and structural changes during the drying of any material. The variation in the airside variables such as airflow, temperature, and relative humidity becomes less important, except when increasing the heat transfer rates to the material. In case of food products, drying generally occurs in falling rate period as most of the moisture present is bound moisture; hence, physical and structural changes in products are quite common. This happens as a result of an excessive moisture gradient from the product interior to the surface, especially during the final stages of drying. This causes overdrying and shrinkage, which, again, result in structural damage.

Drying is a very complex heat- and mass-transferring process with several rate processes, including physical and chemical transformations. Other factors, which are responsible for these complexities, are different types of feedstocks, throughput, quality requirements of a particular product, and, very importantly, the existence of hundreds of dryer types. Therefore, selection of dryer or drying system is a crucial consideration in order to satisfy the demands for a particular application. Interested readers may refer to either the *Handbook of Industrial Drying* or *Mujumdar's Practical Guide to Industrial Drying* for a detailed discussion on classification and selection of dryers. This article will discuss, in short, some conventional and advanced drying techniques and their importance with respect to physical and structural changes in food products.

Importance of Drying in Foods: Benefits and Quality Importance

Fruits and vegetables – in particular – have many positive health effects. This food grouping has a high fiber content and other useful components that help control blood glucose levels, reduce cholesterol, and probably reduce the risk of cancers (colon especially). They also contain antioxidants, vitamins, and minerals, which also have positive health effects. It is important to preserve all nutritional qualities of such food products in their dried state as closely as they are in their native state.

Other important quality attributes are physical and structural changes, which include shrinkage, puffing, glass transition, rehydration capacity, density, and mechanical properties. These properties depend on several factors, such as pretreatment process prior to drying, dryer conditions (such as temperature, pressure, and, to some extent, relative humidity of drying medium), type of drying medium, type and extent of heat input, and physical state of the product to be dried. The selection of drying conditions and dryer type will depend on the desired and expected properties of the product. The following section presents some of the important physical and structural properties of dried products:

Shrinkage is a change in volume as a result of loss in moisture during drying. Shrinkage is an important consequence of drying, which determines the rate of drying and quality of the dried material to great extent. Shrinkage influences product structure and the rehydration ability of a dried food product. Shrinkage occurs as a result of capillary collapse due to the evaporation of water occupying the capillaries. Shrinkage can be either isotropic or anisotropic. Isotropic shrinkage is defined as uniform shrinkage of all geometric dimensions of a material. In anisotropic shrinkage, the extent of shrinkage of all geometric dimensions of a material is uneven or nonuniform in nature. As discussed earlier, the outer layer of the material loses moisture much faster than the interior; hence,

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the surface layer shrinks. If the surface moisture is removed very fast, but the diffusivity or the migration of internal moisture to the surface is too slow, then an exterior layer, which is essentially impermeable to moisture diffusion, is formed. This phenomenon is known as *case hardening*. In food drying, case hardening is quite common and selection of proper drying conditions is very important; however, it may be desirable in some instances for some products.

Cracking is another phenomenon that occurs during the drying of foods. In this situation, the product surface shrinks during drying, causing tensile stress at the surface and compressive stress inside the material. When the tensile stress exceeds the material strength, cracking occurs. The cracking of material during drying needs to be avoided as it results in loss of material and consumer unacceptability. In the case of grains, cracking usually leads to material loss during milling and may also result in bacterial attack during storage. The internal *microstructure* of food products is also very important as it can have a profound influence on its nutritional value, rheology, and textural attributes. As the drying can substantially affect the internal structure of foods, it is necessary to understand the effect of various parameters on product microstructure. The *rehydration ratio* is defined and based on the mass of material after drying and its corresponding mass after soaking in water.

Drying rates can have dramatic effects on structure of the dried products as drying can cause case hardening. This consequently causes the surface to crack, which in turn allows the vapor generated within the body to flash out and cause poor appearance and nonuniform drying. This is undesirable. This phenomenon is product-dependent, and hence, laboratory testing is necessary. Discoloration of the product surface can also occur. Furthermore, gradients in porosity can lead to a nonhomogeneous product. The use of radiative heat input can also result in crust formation if the flux is too high. Drying at elevated temperatures, if it is elevated enough under the operating pressure used, can cause internal boiling of the liquid. The internal boiling leads to hollow particles in spray-dried powders. This again enhances the porosity, thereby resulting in better rehydration ability. The following sections present the effects that operating parameters and dryer types have on the physical and structural properties of dried foods.

Influence of Various Drying Parameters on Physical and Structural Food Modifications

Pretreatment

A pretreatment of wet product prior to drying is very important for foods. This is used to achieve physical or chemical changes in order to enhance the drying rates and product quality. Blanching and osmotic treatment are used for several food products, especially for fruits and vegetables. During osmotic dehydration, a product to be dried is treated with an osmotic solution; water from the food material migrates outward and is replaced by the solute from the osmotic solution. The presence of this solute also serves as a preservative. Although osmotic dehydration is mainly useful to improve product quality, including color, microstructure, and enhanced drying rate, however, it may sometimes have adverse effects on physical characteristics. Table 1 shows the impact that pretreatment has on the physical properties of fruit products.

Table 1 Use of pretreatment on physical properties of dried fruit

Product	*Type of pretreatment*	*Findings*
Kiwifruit slices of different initial textures (firm, medium and soft)	Sucrose syrup (70% w/w) for 2 h at 25 °C	Improved texture
Apple, banana	Sucrose solution (50% w/w) followed by air-drying at 70 °C	Porosity of the osmotically treated product decreased as a result of solid gain; however, it prevented color deterioration; increases the maximum stress and maximum deformation of the dehydrated product
Papaya	Osmotic dehydration followed by hot-air (HA) drying of papaya with a sucrose solution (65°Brix) with syrup to fruit mass ratio of 4:1	Better maintenance of cell structure of papaya when OD with additives was used followed by HA drying
Salak fruit	Thermal pretreatment (blanching)	Increase in the cross-sectional area of cells, which affects the water sorption characteristics

Recently, various approaches have been employed to enhance the process of osmotic dehydration. The use of microwave (MW), pulsed electric fields, ultrasound, and high hydrostatic pressure has resulted in enhanced osmotic dehydration rates. It has been shown that the ultrasound pretreatment improves the chewability of products such as dried sea cucumber (low hardness and high springiness) and results in a higher rehydration ratio as a result of microchannels formed during ultrasound pretreatment. The use of pulsed vacuum and ultrasound has also been shown to improve water removal rate, favorable glass transition temperature, and color. Some physical pretreatments include freezing, thawing, bruising, pinning, drilling holes on surface (to ease moisture diffusion), and extrusion (to increase the surface area). Each one of these techniques has been used selectively to either enhance drying rates or improve final product quality.

Temperature and Humidity

As explained earlier, the external drying conditions determine removal of surface moisture; however, the diffusion of internal moisture determines the drying rate when internal moisture needs to be removed. It was also highlighted that the drying of most food products shows a falling rate period; hence, the increase in temperature can elevate internal moisture diffusion.

However, the use of very high temperatures may harm the quality of a dried product. Several studies have been carried out in an attempt to understand the effect of temperature and initial moisture on the physical properties of material. In general, low-temperature drying results in lower shrinkage and higher rehydration compared with drying at a higher temperature, as it results in crispy crust formation. Some studies also observed increases in porosity with increase in temperature, which resulted in better preservation of the structure at intermediate temperatures. This is due to the fact that both the longer drying time at lower temperatures and the use of very high temperatures can result in development of wrinkled structure. Figure 1 shows scanning electron microscopy (SEM) photographs of carrot dried using hot-air drying; higher temperature affects the pore diameter. In the case of some products, such as gelatin films, crystallinity and glass transition temperature are important. The lower drying temperature can result in higher crystallinity and higher glass transition temperature of the dried product, which is important for better water absorption and lower levels of hysteresis.

The relative humidity of a drying medium also has an effect on physical properties of the dried material. In addition to temperature, the humidity also decides the rate of drying. If the drying medium is highly saturated with moisture, then the drying rates are slower; hence, either temperature rise or reduction of relative humidity is necessary. Besides this, relative humidity can also affect product structural properties if the glass transition temperature is affected by higher relative humidity, especially products containing sugars.

Pressure

The pressure used during drying can have considerable influence on the physical structure of the dried material. A dryer can be operated either at atmospheric, at subatmospheric, or with variable pressure cycles. Most commonly used dryers operate at atmospheric pressure. However, if there is a restriction on the drying temperature used, because of heat sensitivity of a particular product, then drying needs to be carried out at subatmospheric pressures. The moisture can be removed at lower temperatures when vacuum is used, although operating a dryer at subatmospheric pressure increases processing cost considerably. Drying under vacuum can open up voids within a material with a nonrigid structure. This increases the porosity and enhances rehydration ability. This has been observed by a number of researchers using a variety of drying methods, such as freeze-drying (FD), MW vacuum drying, and superheated steam drying. Some researchers have used variable pressures to achieve porous structures, such as successive pressure drop (déshydratation par détentes successives, DDS) and controlled instantaneous pressure drop (détente instantanée contrôlée, DIC). All these ideas and related findings will be discussed in more detail in the section on advanced drying techniques in this article.

Drying Medium

The choice of drying medium for food products is very important as it can have considerable effects on product quality. The most commonly used drying medium is air as it is the least

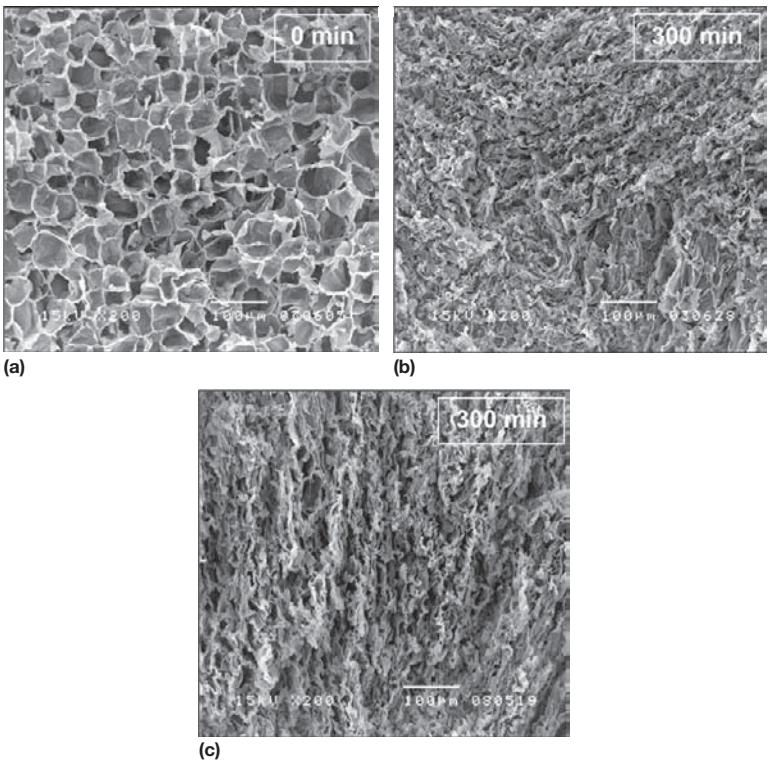

Figure 1 SEM photographs showing cross section of carrot cube undergoing hot-air drying (a) fresh sample; (b) hot-air drying at 60 °C; (c) hot-air drying at 80 °C.

expensive to use. However, the use of air can damage product quality in terms of browning (as a result of unwanted enzymatic or nonenzymatic reactions when in the presence of oxygen), shrinkage, porosity, and texture. Modified atmospheric drying can be carried out by replacing air with an inert medium such as carbon dioxide, nitrogen, or steam. This is very useful for many products, especially food products. The use of superheated steam was proposed in 1990s and has been developed over the years as an important drying technique for foods. There are several published reports on use of inert atmosphere during drying, although the system needs to be a closed cycle in order to minimize the volume of the drying medium used, thereby reducing processing cost. An extensive review on drying of foods using heat pump dryers has been published, which addresses the use of inert atmosphere for drying. This article elaborates on the improvement of quality attributes of dried products, such as reduction in browning, increased porosity, and production of less firm products compared with products obtained using heat pump drying (HPD) employing air. The improved structure of the products dried using an inert atmosphere also resulted in faster rehydration. It is certainly useful to use an inert atmosphere for foods, but one needs to consider several aspects such as added cost, improvement in quality, and possible returns.

Mode of Heat Input

The classification of dryers based on the mode of heat input is most common. Dryers are classified as direct and indirect. Direct dryers, also known as convective dryers, are by far the most commonly used in industry, although efficiencies are low. In direct dryers, the drying medium contacts the material to be dried directly and supplies the heat required for drying by convection. Drying gas temperatures may range from 40 °C to very high temperatures, the temperature selected being dependent upon the food material to be dried. As described earlier, the selection of dryer temperature is very important in order to minimize structural food damage. As described previously in this article, the drying of foods falls in the falling rate period. Hence, one can use intermittent heat supply at the end of the drying when the moisture transport from inside to the surface is very slow.

On the other hand, indirect dryers involve the supply of heat to the wet solids without direct contact with the heat transfer medium. The heat is transferred from the heat transfer medium to the wet solid by conduction, and the use of vacuum or gentle gas flow is necessary in order to remove the evaporated moisture. Heat transfer surfaces may range in temperature from 40 °C (as in FD) to very high temperatures; the selection of which is dependent upon the specific application. The application of indirect dryers in foods generally uses vacuum as most of the foods processed are heat-sensitive.

Heat can also be supplied by radiation (using electric or natural gas-fired radiators) or volumetrically by placing the wet solid in dielectric fields in microwave or radio-frequency (RF) ranges. These options, especially MW dryers, are worth considering as devices to speed up food drying in the tail end of the falling rate period in order to reduce changes to physical structure as a result of prolonged drying times. Some of these dryers and related applications are discussed in the following sections.

Effect of Drying Methods

It is extremely important to select an appropriate dryer or drying system for each food product as most are highly heat-sensitive. An incorrect choice of drying system and/or drying conditions adversely affects the physical and nutritional properties of the dried food. This section discusses conventional and recently developed drying techniques, as well as their effects on the physical structure of foods.

Conventional Drying Techniques

There are several techniques traditionally used for food drying, such as open sun drying, solar cabinet drying, and hot-air drying; however, these techniques result in unacceptable physical and nutritional properties of foods. The products from these dryers can be used to partly satisfy domestic demands, although one cannot sustain the ever-growing international markets. Table 2 lists several conventional dryers used for food drying. This list is based on type of feedstock used, although the dryers can be classified based on several other parameters.

For particulate materials, a variant of fluid bed is the most popular choice because of the requirement of very high heat and mass transfer rates as a result of vigorous mixing between particles and drying medium. A major limitation of fluidized bed dryers can be particle size and the feedstock particle size distribution. In the case of some food products, attrition results in unacceptable size distribution. In fluidized beds, the nonuniform distribution of drying gas also results in nonuniform quality of drying products in terms of shrinkage, moisture content, and rehydration ratio. Traditionally, tray drying is popular, but throughput and energy efficiency are major issues. Drying time can be very long, in the case of tray dryers, and hence, it can have adverse effects on product quality, especially with respect to physical structure. Though continuous tray dryers were also used, these dryer types are not popular anymore.

Spray drying is one of the techniques used extensively in food-related industries for liquid feedstocks. As heat contact time is very short and the rate of evaporation is high, spray drying produces a high-quality product, although cost may also be high due to energy consumption. The liquid droplets

Table 2 Classification of conventional dryers based on the type of feedstock employed

Type of feedstock	*Dryer types*
Particulates	Tray, fluidized bed dryers, belt conveyor, vibrated bed, rotary, screw conveyor, vacuum, freeze
Sheets, extruded material	Impingement, infrared, microwave, conduction
Liquids	Spray, variant of fluidized bed dryer with a bed of inert particles, drum
Paste	Paddle, fluidized bed, drum, rotary

containing solids are dried in seconds as a result of the highly efficient heat and mass transfers. The final product from a spray dryer is in the form of powder. However, granules or agglomerates are conveniently produced by combining a fluidized bed dryer or by recycling the fine particulates. There are several factors that decide the physical form of the spray-dried product, mainly atomization, chamber design, the type of contact between drying gas and droplets, and drying air conditions. SEM images of dried product are generally used to examine particle morphology. The use of very high temperatures can result in crust formation. There are several reports on SEM analysis of spray-dried food products, such as skimmed milk, fruit juices, and other extracts showing the effect of operating parameters, atomization, and chamber design on morphology of dried powder. Figure 2 shows SEM images of spray-dried powder of fermented mixed juice of carrot and watermelon at different operating conditions.

Spray drying is also commonly used in the food industry for microencapsulation. Microencapsulation is defined as a process by which one material, or a mixture of materials, is coated or entrapped within another material or system. The coated material is called the active or core material, and the coating material is called the shell, wall material, carrier, or encapsulate. It is necessary to obtain a rigid structure to protect the active ingredients trapped within the carrier. The selection of drying conditions and the type of carrier and its composition are extremely important in order to achieve efficient microencapsulated material.

Other techniques for drying of liquid materials are drum drying and drying on a bed of inert particles, which has grown in popularity in recent times. For sheets and extruded food products, mainly belt conveyors or batch tray dryers are used in combination with infrared (IR), microwave, or conductive heating. In the case of pasty materials, paddle dryers or fluidized bed of inert particles are more commonly used.

Recently Developed Drying Techniques

Each traditional drying technology described earlier can have numerous variants, which possess some unique characteristics. It is highly unusual to have a variant that only presents advantages without limitations. So, final selection is always a compromise based on the product specification. Selection of dryers is a very complex process. There are several reasons why few truly innovative technologies find commercial adoption, the most important being quality in terms of physical structure of the dried material. The following are some of the more recently developed drying techniques and their influence on the structural properties of the final dried products.

Heat pump drying

Although HPD is not a very new technology, there has been continuous improvement in the design and application of HPD technologies for food drying. HPD consists of a refrigeration cycle to recover heat from dryer exhaust air and reheat it to the required temperature before it enters back into the dryer. Detailed discussion on HPD and its operation can be found elsewhere. The HPD system was traditionally used for the recovery of heat; however, HPD is used in food drying to improve product quality by utilizing some of the other advantages it offers. The primary advantages of employing the HPD system include providing a wide range of operating conditions (humidity and temperature), possibility of using inert gas as drying medium, and combining with other heat modes. For heat-sensitive food products with long drying times, HPD is very useful as drying can be carried out using relatively lower temperatures in combination with dehumidified air. As presented in several published studies, the use of inert gas has resulted in better physical properties of the dried material. A closed-loop HPD system can substantially reduce the operating cost, as the same inert gas can be recycled, with some top-up as necessary. Some of the applications of inert gas food drying include drying of apple, ginger, guava, papaya, lecithin, etc.

The most common HDP system used is mechanical compression with low coefficient of performance. Some research has focused on improving process efficiency, including multistage heat pumps, chemical heat pumps, or use of heated pipes. The chemical heat pump has good commercial potential but needs thorough research. Another way of improving the performance of heat pump systems is using external heating modes such as microwave, RF, IR, or solar energies. This is useful during the final stages of drying in order to minimize possible damage to the physical structure of the foods. Solar-assisted HPD is extremely useful for agricultural products. Another approach of reducing product damage, as well as reducing the overall cost of drying, is by varying the operating cycle of HPD, such as through intermittent or time-dependent approaches. For more detailed discussion of recent technological advances and possible R&D opportunities in HPD, readers may refer to related book chapter and review articles listed in reference.

Refractance window drying

The refractance window drying is a relatively new method used to convert pulverized fruits and vegetables, pastes, and also thin slice of fresh fruits. In this technology, the IR energy coming from hot water (90–97 °C) is used to dry the thermally sensitive food materials. An IR-transparent plastic film is essentially allowed to float on top of thin layer of heated water body. The IR energy refracted through the plastic film due to the presence of 'window' of moisture in the product across the film is responsible for drying. The conduction of heat through the thin plastic film also assists with drying. This is a relatively energy-efficient system as the IR only gets refracted when the wet product is present across the film. The refractance gradually declines as the product dries. The loss of heat in this system (in the absence of product) is due to conduction. It is reported that this drying method preserves the color, texture, and antioxidant contents compared with other drying methods of similar heat intensity. This method is attracting increased research attention.

Filtermat spray drying

This is a relatively newly developed drying method (GEA Technologies, Denmark) in which spray drying is combined with Filtermat drying. This technology is especially designed to dry difficult-to-dry sugar-rich products. The Filtermat component involves a porous conveyor with controlled linear speed. The drying section is usually divided into different sections. The partially dried particles are deposited on this Filtermat, and

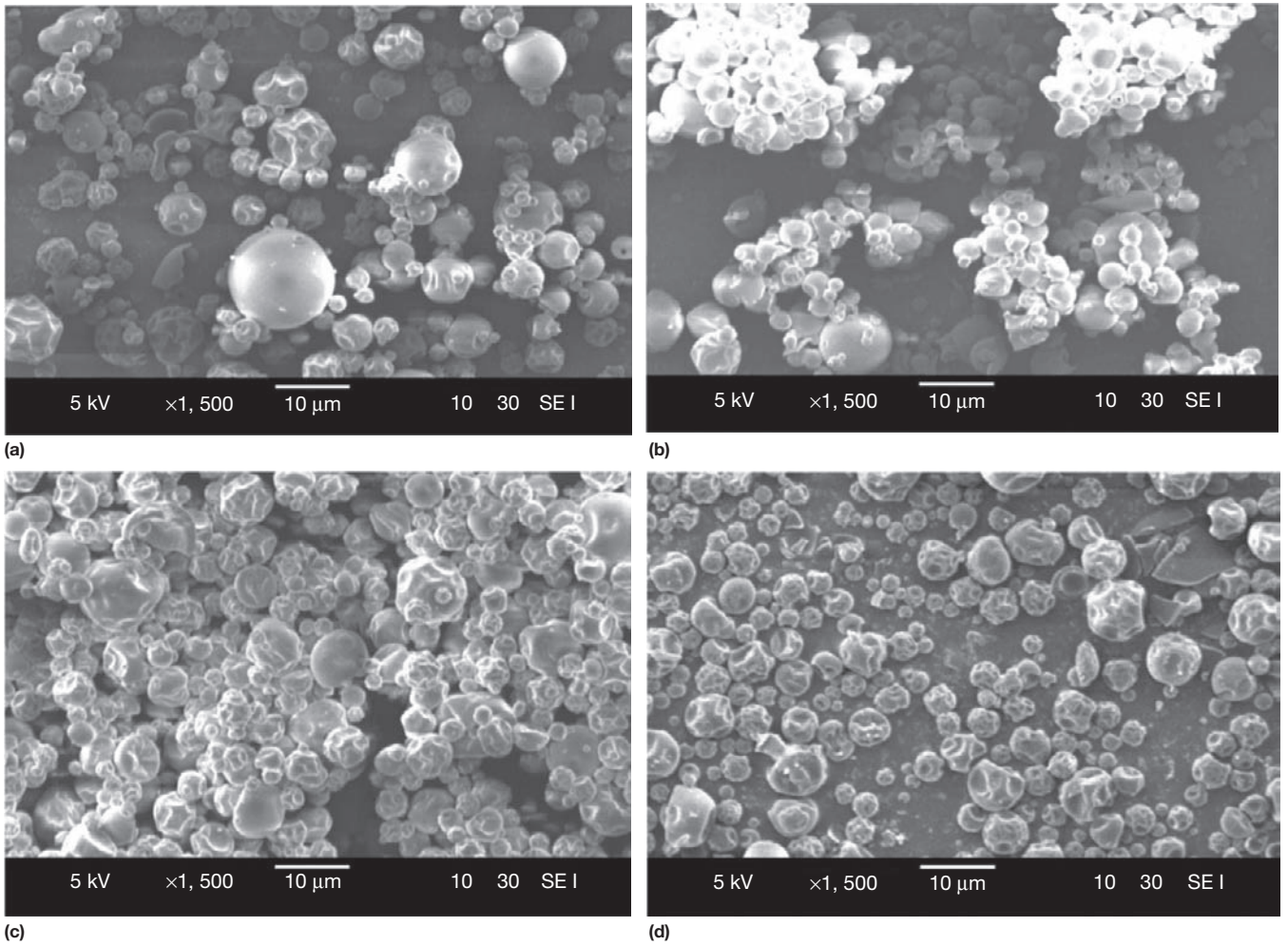

Figure 2 Scanning electron micrographs of spray-dried powder illustrating the variation in particle size and surface roughness when produced at different operating conditions (T, temperature; F, flow rate; P, pressure): (a) $T = 140\ ^{\circ}C$, $F = 3.5$ ml min^{-1}, $P = 2$ bar, maltodextrin = 17.5%; (b) $T = 160\ ^{\circ}C$, $F = 5$ ml min^{-1}, $P = 3$ bar, maltodextrin = 10%; (c) $T = 140\ ^{\circ}C$, $F = 3.5$ ml min^{-1}, $P = 2$ bar, maltodextrin = 12.5%; and (d) $T = 120\ ^{\circ}C$, $F = 5$ ml min^{-1}, $P = 1$ bar, maltodextrin = 15%. Reproduced from Mestry, A. P., Mujumdar, A. S. and Thorat, B. N. (2011). Optimization of spray drying of an innovative functional food: fermented mixed juice of carrot and watermelon. *Drying Technology* **29**(10), 1121–1131.

the hot air is allowed to pass through the pores of the conveyor and through the interstices of the deposited mass of particles. In the second stage, dehumidified cold air is used to further dry and cool the product. The final product requires certain degree of grinding. The difference in this method over fluidized bed drying is that the particles are not fluidized. The deposited agglomerates are allowed to dry as thin layer of porous cake. This type of dryer can be suitable for producing baby formula powders.

Intermittent drying

Intermittent drying is an attractive concept due to its potential to enhance energy efficiency and achieve better product quality as a result of uniform drying. In intermittent drying, drying airflow, temperature, or humidity is varied, or other alternative parameters such as system pressure and type of external heat source (microwave, IR, or RF) are varied with time in order to suit the requirements. Sometimes, cycles of different heating modes can be used. The main objective of intermittent drying is to allow internal moisture to migrate to the surface of the material during nonactive period – known as the tempering period. During the active period, the heat is supplied, while during tempering period, the supply of drying medium is completely stopped. As the moisture content at the surface is increased during tempering period, the rate of drying increases substantially during the following active period. However, as the drying rate is very small during the tempering period, the overall drying time may increase slightly, although this is compensated by the overall energy consumption.

Another intermittent drying approach is through the application of a stepwise change in operating conditions. This is especially important in the case of food products, as drying rates are very slow during final processing stages. This is due to the fact that drying is diffusion-controlled in the final stages; hence, the effect of external conditions is negligible. The use of this stepwise strategy of changes in the operating conditions is very useful as a lot of energy can be saved. At the same time, the product being dried is exposed to severe drying temperatures for a shorter time, and this results in less product shrinkage and less physical product damage. The concept of intermittent drying can be applied to various food products, using different drying methods, including the use of a tray dryer, conveyor dryer, vibrating bed dryer, and fluidized bed dryer.

Variable pressure methods

Drying can be carried out using either a controlled instantaneous pressure drop method or a cyclic pressure drop method. In a standard controlled instantaneous pressure drop (DIC) treatment, the product is subjected to high pressure, generally using gas or steam, while temperature is maintained by one of the various means available. This is followed by an instantaneous drop in pressure, usually assisted by vacuum technologies, which leads to autovaporization of water and sometimes volatiles. This process can be easily controlled and results in very good product quality, especially with respect to product expansion ratio and porosity and also in the enhancement of product texture. Swell drying is a combination of hot-air drying and the DIC texturizing process. The reduction in drying time, using swell drying, results in the significant enhancement of product quality. In a dehydration process, followed by successive decompression (DDS), the thermosensitive product undergoes a series of cycles, during which it is placed at a particular pressure for a defined time, followed by an instantaneous drop in processing pressure. The product is maintained at lower pressures for some time, before the following cycle begins, and each decompression step results in partial removal of water by autovaporization. The amount of water removed depends on various factors, such as operating conditions and the type of water present in the material dried.

The major advantage of these techniques is the considerable reduction in drying time that results compared to normal vacuum drying or hot-air drying techniques used for food products. The reduction in drying time always helps control the physical properties of dried products. The techniques of instantaneous and cyclic pressure drop have been successfully applied to various food products, including grains, fruits, vegetables, and fish. The DIC method is specifically used to tackle the problem of product shrinkage.

Superheated steam drying

Superheated steam is an attractive drying medium for some food products since the net energy consumption can be minimized if the exhaust (also superheated steam) can be utilized elsewhere in the plant. The quality of superheated steam-dried products tends to be better than that from conventional hot-air dryers. Superheated steam also allows pasteurization, sterilization, and deodorization of food products. This is particularly important for food and pharmaceutical products that require a high standard of hygienic processing. In addition, drying rates are higher in the case of superheated steam drying, in both constant and falling rate periods and under certain conditions, which are important to control in order to create the structural changes required in foods. Mujumdar had discussed the principles, advantages, and limitations as well as diverse applications of superheated steam drying technologies in a number of publications, including the *Handbook of Industrial Drying*.

Some products are not stable at 100 °C, then one option is to lower the operating pressure. The application of low-pressure superheated steam drying is used for products, such as potato chips, carrot, and longan. SEM images shows considerable improvement in structure (porosity), less shrinkage, and high rehydration ratio compared to hot-air drying. As an example, SEM images of fresh and dried carrot using hot air and the application of low-pressure superheated steam drying (LPSSD) are shown in **Figure 3**. Although one can obtain good dried product quality at low pressures, the process is still not popular due to the requirement for large equipment owing to low drying rates, but perhaps it may be necessary to use supplementary heat sources in order to improve the process. Much research and development is needed in this area in order to progress the drying technologies discussed.

Advances in FD

FD is undoubtedly the most acceptable drying technique for foods because it probably generates the best product quality, as

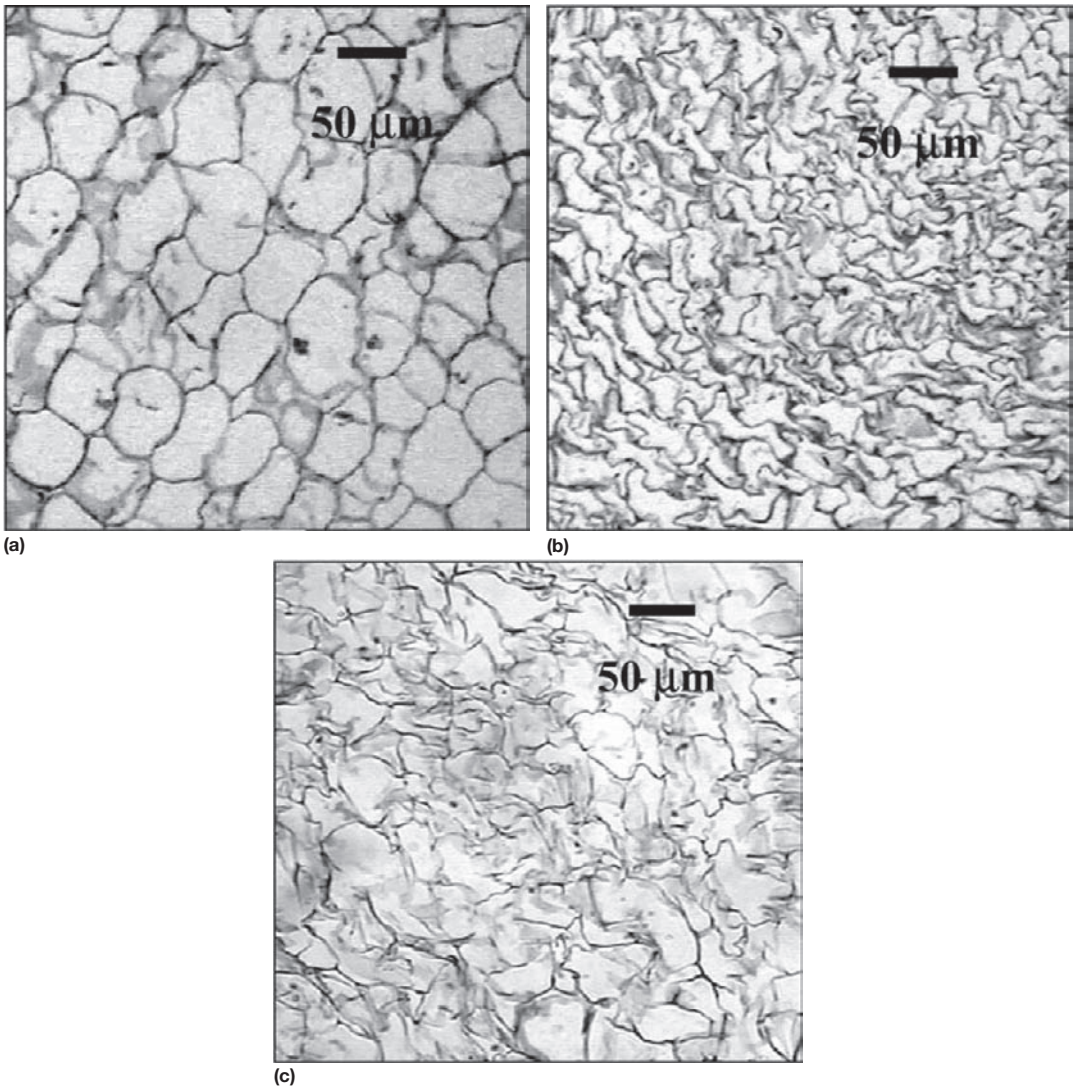

Figure 3 Microstructure of (a) fresh carrot, (b) carrot dried until reaching equilibrium moisture content using hot-air drying, (c) carrot dried using low-pressure superheated steam drying. Reproduced from Kerdpiboon, S. and Devahastin, S. (2007). Fractal characterization of some physical properties of a food product under various drying conditions. *Drying Technology* **25**(1), 135–146.

the frozen product is not allowed to melt during drying. It prevents undesirable shrinkage and produces materials with high porosity, ultimately resulting in better product rehydration properties. There are several reports in the literature showing SEM images of freeze-dried products, clearly indicating that FD enhances powder microstructure compared with that produced using conventional hot-air drying. However, the industrial application of FD for food drying is limited because of the high associated processing cost. There has been significant effort to develop new techniques in order to achieve similar product quality obtained from FD, although it is difficult to achieve some characteristics, which only FD can achieve such as rehydration, color, and texture. The cost of FD can be reduced by improving the drying rates using external heating, but without allowing the product to melt. This can be achieved by either using magnetic and electric fields, using atmospheric FD, or using microwave in combination with FD (MFD). Application of ultrasound during the freezing stage also greatly improves the drying rate and product quality of freeze-dried products. A review on the use of electric and magnetic fields during freezing and their application to FD suggests better control on the ice crystals and, ultimately, on product quality. The use of microwave FD for products such as fruit, vegetable, and some marine-derived products has resulted in better drying rates and product quality, particularly in relation to product microstructure, shrinkage, and rehydration ratio. Figure 4 shows comparison of SEM images of banana chips dried using FD and microwave FD. A detailed discussion on FD and technological advances in the area can be found elsewhere in this book.

Hybrid drying

In the case of some food products, many conventional drying techniques may not provide the expected final moisture contents at sufficiently acceptable drying rates. In such cases, a

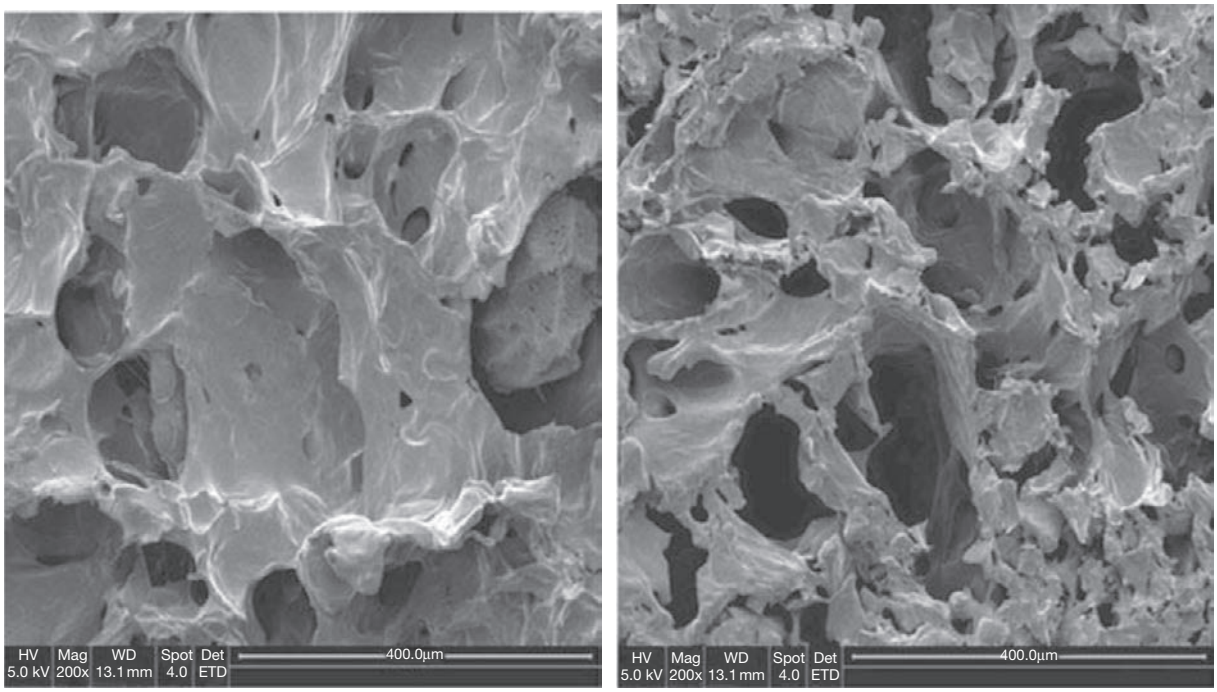

Figure 4 Scanning electron micrographs of (a) FD banana chips and (b) MFD banana chips. Reproduced from Jiang, H., Zhang, M., Mujumdar, A. S. and Lim, R.-X. (2013). Analysis of temperature distribution and SEM images of microwave freeze drying banana chips. *Food Bioprocess Technology* **6**, 1144–1152.

combination of dryers or drying technologies can be used. In recent years, hybrid drying is popular as it can reduce drying time substantially and provide more flexibility. Hybrid drying systems may adopt more than one drying technique or more than one heating mode in the same dryer. Microwave drying offers advantages in enhancing drying kinetics, precise control, fast start-up and shutdown times, quality of dried product, smaller footprint of equipment, etc. IR drying is also useful in removing final traces of product moisture at a faster rate. These techniques are typically combined with other drying methods to overcome the limitations of uneven heating, resulting in unacceptable physical structure, for example, overdrying at the corners and edges of products.

Spray FD is another hybrid drying technique used to prepare highly porous particles. This technique involves the spraying of a liquid feed in a cryogenic atmosphere, followed by FD of these frozen droplets. The technique of spray FD has been used for selected food application; however, necessary research and development work is needed to minimize associated costs.

Concluding Remarks

Drying of foods is an extremely important commercial and preservation process, and the approach used to dry foods will impact differently upon the physical and structural properties of the products in question. Drying rate and operating conditions can have considerable effect on structure of the dried product. To a certain extent, the structure can be controlled by selection of an appropriate drying technique and manipulation of operating conditions, although it is necessary to take into consideration cost-effectiveness and suitability of each method employed. Appropriate changes in existing drying systems and the use of some innovative dryers can give improved quality of dried foods such as porosity, rehydration ratio, texture, and microstructure.

New drying technologies such as microwave or RF-assisted drying, pulse combustion drying, intermittent batch drying, and superheated steam drying at subatmospheric pressure may be applied in specific cases to obtain desired product characteristics under optimal drying conditions. Experimental validation at laboratory or pilot scale is necessary as it is not possible to predict the effect of drying conditions on product characteristics with high reliability.

See also: Drying: Principles and Types; Freeze Drying: Effects on Sensory and Nutritional Properties.

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Drying: Principles and Types

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Introduction

Drying is one of the oldest methods used for food preservation. In fact, it is quite evident that initially, drying occurred by chance and under natural conditions. Most of the raw material used as food comes from biologically active sources. Consequently, very few foodstuffs are inorganic in nature, such as table salt. Since water is needed for life, it implies that most raw materials destined for food consumption have a high water content and, ultimately, high water activity (a_w) values. Water activity is a thermodynamic term that is related with the degree of freedom of water.

Although the first objective for using drying was food preservation, nowadays, drying is also used to reduce food volume and/or weight in order to optimize its storage and transportation (e.g., milk powder) and to avoid the need for refrigeration or freezing conditions for preservation (e.g., dry-cured ham) and is used for food product development (e.g., snacks).

Food drying occurs when the water activity of the air surrounding food is lower than its a_w. One characteristic of the drying process is that the liquid water of food is transferred to the gaseous state in drying air at temperatures below the boiling point through an evaporation process.

The water activity of the air surrounding food is equal to its relative humidity (RH) divided by 100 and is usually lower than the original a_w value of the animals and plants used as food sources. Thus, when an animal or plant dies, a natural drying process occurs, during which the water activity value of food naturally decreases, thereby making it more stable.

Some characteristic water activity values of raw and dried food products are presented in Table 1.

Sometimes, drying is used in combination with other preserving techniques. The most common technique is addition of solutes, which contribute to further a_w reduction due to their interaction with the water present in food. The most widely used solutes are NaCl and sucrose. NaCl is normally used with meat and fish products (e.g., dry-cured ham and dry-salted cod), while sucrose is habitually employed with processed fruit products, like jams and marmalades.

Water Activity and Stability

The water activity of food is related with its stability. The original water activity of plants and animals is high enough to allow reactions that permit life (such as solute movement and biochemical reactions). This fact implies that when an animal or plant dies, the spoilage process begins quickly since high a_w values would promote microbial growth and allow chemical and biochemical reactions to proceed because of solute movement and the activity of natural reagents and enzymes.

Reduced a_w of food implies lower kinetics of spoilage reactions, and a time comes when the spoilage reaction stops altogether when a low enough a_w point has been reached within the food.

The only exception to this behavior is during fat oxidation. The kinetics of fat oxidation is initially diminished with a reduction in a_w of food, until a value (usually in the 0.2–0.3 range) is reached when oxidation kinetics begins to increase due to the direct exposure of fats to oxygen as a consequence of the removal of most of the water molecules that create a protective layer on the fat surface.

Thus, as a general pattern, the preservation behavior of food increases with the reduction of water activity, up to 0.2–0.3.

Water Activity and Equilibrium

As mentioned in 'Introduction,' water activity is a thermodynamic concept. This means that, in the absence of other forces, movement of water will occur from points with a higher a_w to points with a lower a_w. It is important to bear this in mind, since a very common mistake is to consider that food systems remain at equilibrium when the moisture at all points is the same, instead of the true condition, which is when the a_w of all its points is the same.

So, food also tends to its internal equilibrium and with the water activity of its surrounding air, which is the basis of the drying processes.

At equilibrium, the water activity at each point within the food is the same and equals that of the surrounding air. Within the range of high water activity values, predictive equations can be used to estimate the water activity of foods depending on their composition: water and solute concentrations. Within this range, food behaves similarly to the liquid phase contained within its structure, and models developed for nonionic or ionic solutes can be used, for example, Norrish for nonionic solutes, Pitzer and Bromley for ionic solutes, or Ross for a combination of solutes. If water activity values are low, predictive equations for solutions are not useful because the interaction of water with nonsoluble food compounds plays a major role. This is why sorption isotherms must be used, which represent the water activity of food versus the water content of food on a dry basis. The main disadvantage of using sorption isotherms is that they must be experimentally determined for each food type, owing to the variance in food composition and structure. Figure 1 shows a typical sorption isotherm, which relates the moisture of a certain food with a particular water activity.

The sorption isotherm can also be used to determine the equilibrium moisture of a certain food with the air used for drying, which is achieved at the end of a certain drying process if the air RH remains constant.

In commercial situations, the drying process usually finishes far from equilibrium conditions with the drying air because the drying rate is very low close to equilibrium.

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Table 1 Characteristic water activity values of raw and dried food products

a_w *raw food*	a_w *dried food*
Raw fruit and vegetables	*Dried fruits and vegetables*
Apples: 0.99	a_w within the 0.6–0.7 range
Bananas: 0.98	
Figs: 0.97	
Grapes: 0.98	
Oranges: 0.99	
Carrots: 0.99	
Tomatoes: 099	
Fresh meat: 0.99	Dry-cured ham[a]: 0.87–0.92
	Salami[a]: 0.82
	Beef jerky[a]: 0.80
Fresh fish: 0.99	Dry-salted cod[a]: 0.65–0.75
Milk: 0.99	Milk powder: 0.20
	Aged cheese[a]: 0.87–0.89
Coffee: 0.99	Instant coffee: 0.20

[a]The final a_w of these products is due not only to drying but also to the combined effect with salt.

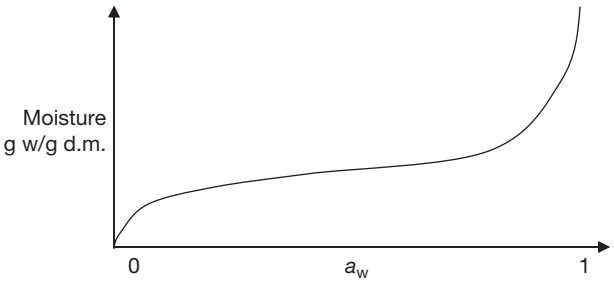

Figure 1 Typical sorption isotherm (w, water; d.m., dry matter).

When drying moist products begins, the decreased water activity in food is due mainly to the concentration of the solutes present in the food liquid phase. A time arrives when the liquid phase disappears and the interaction of water molecules with the solid compounds (insoluble or precipitated) of food occurs, and this plays a key role in the water activity of food.

Water Activity of Air

The water activity of air is equal to its RH divided by 100. On most occasions, the water activity of air is lower than the water activity of raw material used as food, which means that a natural drying process occurs, usually when food is stored without protection. Nevertheless, the reduction of air RH is usually desirable to increase the difference of a_w of food and air to enhance the drying process.

A number of actions can be performed to modify relative air humidity, of which the most common is to increase air temperature. This is why most drying techniques imply heating air by different means. Air RH is the water vapor pressure of air at a certain pressure divided by the saturated vapor pressure of air at the same pressure, at the system's temperature multiplied by 100. There is a direct relationship between saturated vapor pressure of air and its temperature (eqn [1]), and its value increases when temperature rises, so the water activity of air reduces:

$$p_{ws} = e^{(77.345+0.0057\ T-7235/T)}/T^{8.2} \quad [1]$$

where p_{ws} = water vapor saturation pressure (Pa), e = the constant 2718, and T = dry bulb temperature of moist air (K).

Nonetheless, heating air contributes not only to reduce its water activity but also, consequently, to increase the gradient between food and air. Air heating implies an increase in the kg of water that each kg of dry air can take from food. Finally, an increase in transport coefficients occurs because they are temperature-dependent, usually following the Arrhenius equation pattern.

Apart from using air heating to reduce water activity, the direct removal of water from air can be used if food products are sensitive to high temperatures. In this case, water removal can be done by passing air through hygroscopic media or by water condensation at temperatures below the dew point of air, followed by heating.

Understanding air thermodynamics is the most important in drying processes.

Water Transport Through Drying Processes

As previously highlighted, drying occurs if the water activity of food is higher than that of air and, more specifically, if the water activity on the food surface is higher than the surrounding air. Thus, a typical drying process implies transporting the water inside food towards its surface, evaporating air on the food surface, and transporting water vapor from the food surface to the bulk of the drying air:

- Transporting water inside food: transport of water inside food can occur in the liquid state (by diffusion, capillary forces, etc.) or as vapor.
  - The movement of water in the liquid state usually occurs in products with a free liquid phase. This is a fast process that implies food shrinkage equal to the volume of water lost from the food product.
  - The movement of water in the vapor state during drying usually occurs when food shrinkage is lower than the volume of water lost during drying. This implies that voids with a gas phase inside food appear. Thus, water needs to be in the gas state to leave food. A consequence of this type of movement is that the drying rate is very low.
- Transporting water inside drying air: transporting water inside air usually occurs by convection, since drying air is normally at a rate in the dryer that ensures convection. On rare occasions, water vapor moves by diffusion and is usually associated with storage conditions more than drying conditions.

In a real process, the transported water inside food is equal to the transported water inside drying air. In most drying processes, the limiting factor defining the drying rate is the transport inside the food itself, because the food structure is more limiting to water transport than air. This implies that the drying rate decreases with time, since reduced moisture inside food

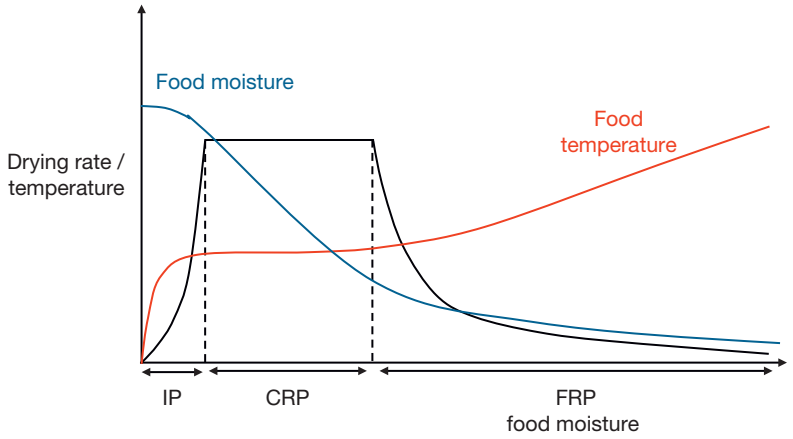

Figure 2 Typical drying curve (IP, initial period; CRP, constant rate period; FRP, falling rate period).

implies a lower transport coefficient (higher viscosity, higher shrinkage, smaller quantity of liquid phase, lower water activity, etc.). This period is called the falling rate period (FRP). The typical shape of this period is shown in Figure 2.

In products with a large amount of water, which can be easily evaporated, a constant rate period (CRP) can be observed at the beginning of drying. This is typical in the drying processes of liquid food, such as coffee or milk.

Finally, a very short drying period can be observed at the very beginning of the drying process, with an increasing drying rate (initial period) due to both the initial increase in temperature on the food surface and the mobilization of the water in the food product.

Figure 2 shows what can be considered a whole drying rate curve with the three typical periods.

Product and Process Variables Affecting Drying

In accordance with previous explanations, the mass transfer rate in drying processes is dependent on water activity gradients, which is the driving force of the process, and the mass transfer coefficients, which are dependent on the properties of the air or product involved in the drying process (eqn [2]):

$$V_i = K_i \cdot A \cdot (a_{w1} - a_{w2})/\Delta x \qquad [2]$$

where V_i = mass transfer rate in the i phase (food, interphase, or air), K_i = mass transfer coefficient in the i phase, A = mass transfer area, a_{w1} = water activity of point 1, a_{w2} = water activity of point 2, and Δx = distance.

The mass transfer rate equation can be applied to the transport of the water inside food (V_f), in the interphase of food with air (V_{int}), and in bulk air (V_a).

In a drying process, it can be considered that the following equality $V_f = V_{int} = V_a$ is fulfilled. The only exception to be considered is when case hardening occurs on the food surface because transporting the water in the food interior can be faster than on the food surface, with consequent moisture accumulation below the crust.

Thus, product and process variables are those that influence the value of the mass transfer coefficients (K), mass transfer area (A), distance (Δx), and water activity gradients ($a_{w1} - a_{w2}$):

- Product variables:
  - Type of surface: Most food that is dried is non-homogeneous (exceptions occur in slurries, liquid, or minced food). It is usual to find a surface that is resistant to water transfer, such as skin in tomatoes and grapes or skin and fat layers with hams. Sometimes, surface treatments are performed on food to enhance water transfer through the food surface, for example, treatment with acids, skin and fat perforation, or skin removal.
  - Internal food structure: The internal structure of foods has a direct influence on the transport coefficient of the water inside food. For instance, the presence of an intact cellular structure or internal fat layers represents barriers to water transport. Sometimes, treatment of food is possible to reduce the presence of internal barriers, such as freezing and thawing of cellular structures or milling, thereby leading to a product with a higher mass transfer coefficient.
  - Food shape and size: The mass transfer process inside food is limited by the maximum distance that water must cross to leave food (Δx_{max}) – the radius in a sphere or cylinder and half-thickness in a layer dried through both sides. If possible, the shape and size of food should be modified to reduce the aforementioned maximum distance. The variable-specific surface (surface/volume) is related with the total time needed to dry a product since the water contained in a certain volume leaves through the product surface; thus, the drying process is faster when the specific surface is higher. The higher the A value for the same sample volume, the higher the V value.
  - Temperature: Usually, the transfer coefficient of a product is exponentially temperature-dependent. This means that an increase in the temperature of the food product results in an increased mass transfer coefficient. There are some exceptions, mainly due to discontinuities in product structure as a result of temperature. This happens in meat products containing fat pieces. If temperature rises, fat can melt and an additional barrier inside food appears, which results in a slower drying process.
- Air variables:
  - Temperature: As mentioned earlier, the mass transfer coefficient (K) is dependent on temperature, which is

higher for rising temperatures. Thus, an increase in air temperature implies a higher mass transfer rate in air.
  - Air velocity: The mass transfer coefficient depends on air velocity since a rise in this value implies increased turbulence. Nevertheless, a time arrives when the water transfer rate does not increase at faster air velocities, because the limiting transfer rate of the process becomes the water transfer through the food interior.
  - RH: As previously explained, the water activity of air equals its RH divided by 100. For the same a_w of food, lowered RH implies that the a_w gradient increases, with the consequent rise in the water transfer rate. Nevertheless, case hardening can occur at a low RH in air due to excess food surface drying, which can slow the drying process.
- Type of drying: Apart from the product and air variables, there are other processing conditions that strongly influence the result of the drying process, such as type of contact of air and food, heating procedures, pressure, and movement. These processing conditions are closely related with drier design and are also related with the physicochemical properties, size, and shape of the food and/or with other food characteristics. This point will be explained in more detail in section '**Drying Equipment and Techniques (Types of Drying)**.'

Temperature and Drying

In most drying processes, air is heated by solar energy or by any other source of energy, such as electric resistance, or mainly through the combustion of gas, oil, or food industry by-products. As mentioned earlier, the rise in temperature results in lower RH of air (reduced a_w), an increase in the maximum capacity of air to take water vapor per kg of dry air, and higher mass transfer coefficients.

Nevertheless, temperature has a direct influence on the quality of dried food and is, therefore, a very important variable.

- Food temperature during drying: When food comes into contact with hot air, there is a tendency to equalize its temperature. Nevertheless, if food is very moist and the water inside food is transferred very quickly, food remains in the CRP, and the food temperature tends to equal the adiabatic saturation temperature, which, usually, is significantly lower than air temperature. This is because the energy transported from air to the food surface, due to temperature gradients, is used completely for changing its state from liquid to gas, and it returns to air as latent heat in water vapor.

When the food surface becomes dry and food is unable to transport as much water as drying air demands, it is known as the FRP, and the temperature on the food surface begins to rise to air temperature.

Temperature affects food properties in some ways:

- Vitamins and other nutritious compounds can be affected by high temperatures.
- Food can be cooked at a high temperature (this can happen with fruit and vegetables and meat and fish products).
- Microorganisms can grow if food is maintained at high a_w values for long periods of time at temperatures within the 3–40 °C range (e.g., in dry-cured ham production).
- Temperature can affect the Maillard reaction, sugar caramelization, etc.

These are some examples of changes that can occur in food because of air temperature, and a detailed analysis of the effect of drying temperature on the type of food and the processing conditions utilized is recommended.

Drying Equipment and Techniques (Types of Drying)

There are many types of driers, which are usually optimized for each product type. The following is a list of the characteristics of dryers to be considered for design purposes:

- Air contact with food: Air can flow in parallel or perpendicular to food through the bed of the product to be dried.
- Stirring food: Food can be static or stirred throughout drying. Stirring the product implies a faster, more homogeneous drying process. Stirring the product can be done mechanically or by means of the drying air.
- Air rate: The air rate is an influencing parameter in the drying process. Nevertheless, using a very high air rate can lead to stirring of products (fluid bed dryers) or even to product transport while drying (pneumatic drying).
- Heating system: The most common procedure is heating air before it enters the drying chamber by means of a heat exchanger. There are other alternatives, such as intermittent heating inside the drying chamber by means of a heat exchanger, radiation heat, and use of microwaves to heat the food product.
- Use of solar energy to heat air in drying processes: solar dryers can range from very simple (solar drying through direct exposure to the sun) to very sophisticated systems, where the only difference with other dryers is that solar energy is used, which can accumulate in thermal fluids and be used in a heat exchanger with or without being combined with other sources of energy.
- Pressure: Although the drying processes imply working at atmospheric pressure, the use of a vacuum pressure to improve the drying kinetics, by reducing heat damage, is an alternative in dryer designs.
- Air recirculation: Partial air recirculation is a usual practice to increase the dryer's energy yield due to the increased amount of water removed per kg of dry recirculated air.
- RH control: In most dryers, no control of RH exists. Nevertheless, controlling RH is an option, which is used mainly when drying at low temperatures.

There are many possible combinations of all the aforementioned parameters, and consequently, many different types of dryers exist. Nevertheless, the list in the succeeding text describes the most widely used dryers in the food industry:

- Spray dyer: It is a dryer for liquids. Liquid is sprayed at the top of a cylinder, and droplets are dried on their way to the bottom of the dryer, which is conical in shape.
- Drum dryer: It consists of one or two cylinders that are heated in their interior. The food to be dried (mainly viscous

products, such as potato puree or hydrolyzed baby food) is dosed onto the drum surface and dried by contact with the hot drum surface. The sheets of drum-dried product are removed from the cylinder surface and milled.
- Drying chambers: It is for small production batches or very long drying processes. Discontinuous drying is possible using drying chambers. Food products can be arranged on trays (e.g., apricot halves) or individually hung (e.g., dry-cured ham).
- Belt dryer: The products to be dried are continuously transported inside the drying chamber placed on a conveying belt. This can be used with many types of products, such as breakfast cereals covered later with honey and washed fruits and vegetables.
- Rotary drier: Food comes into contact with heated air on its way through a cylinder that rotates to facilitate the movement of food and to facilitate it coming into contact with the air circulating inside the cylinder. It can be used with products that are hard enough to resist impacts inside the cylinder or when there is no need to maintain the food shape and/or structure, such as seeds before solvent oil extraction.
- Fluid bed dryer: Food floats in the air that circulates upward. When food is dry enough, due to its lowered density, it is transported by air to the collecting unit.
- Pneumatic dryer: Food is transported and dried simultaneously by a fast air current. It can be employed for foods such as sugar and flour.
- Column dryer: These are vertical dryers used with free-flowing products, such as cereals. The products to be dried are placed into the column at the bottom and descend by gravity, while they are well mixed with hot air by various procedures, such as perforated cylinders.
- Superheated steam dryers: These dryers use superheated steam instead of air. This offers the potential for heat recovery and also enables operations with alternative heat sources while providing enhanced safety and improved environmental benefits.
- Paddle dryer: It is a dryer where heat is transferred by contact with heated paddles that are also used to stir food. It can be used with powdered, granulated, or pasty products (e.g., milk powder).
- Other types of drying: flash drying, freeze drying, and vacuum drying.

When talking about drying food, we commonly refer to the operation that implies loss of water due to gradients of a_w. This implies water passing from liquid to vapor by evaporation at temperatures below the boiling point. Nevertheless, some drying processes are based on the change of state of water due to boiling. The most well-known drying techniques based on this are the following:

- Flash drying: Food is heated at temperatures above the boiling point at atmospheric pressure. Water is maintained in the liquid state because high pressure is used. When the system is decompressed, water from food immediately evaporates, which leads to a porous, dry product, such as inflated rice for breakfast cereals.
- Vacuum drying: This process is based on lowering the water boiling temperature through use of low pressure. This results in the temperature of food being lowered in the process, thereby resulting in less thermal damage. One example is dried sausages. In this process, air scarcely moves due to its removal as a vacuum is used.
- Freeze drying: This is a particular vacuum drying situation based on water sublimation from frozen food by applying vacuum conditions. The drying temperature is very low, and some original food properties are well maintained, such as freeze-dried coffee extracts.

Changes in Product Properties Due to Drying

Drying implies not only reducing the a_w of food but also altering the characteristics of food products. As regards texture, hardness usually increases because the dry matter concentration increases. If moisture is low enough, the product can pass from a glassy to a vitreous state at room temperature, which implies a dramatic change in textural properties. If very fast drying is employed, case hardening can occur due to the very strong dehydration of the food surface, which leads to a hard and very dry surface and is an important barrier for water transport.

Drying also affects food structure due to shrinkage, as well as other properties such as color and flavor. Sometimes, these changes are positive, but may be negative in others, which depends on the product and the objective of the drying process.

See also: Drying: Effect on Nutrients, Composition and Health; Drying: Physical and Structural Changes; Freeze Drying: Effects on Sensory and Nutritional Properties; Freeze-drying: The Basic Process; Preservation of Foods.

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Eating Disorders

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Eating Disorders Are Motivated Behavioral Disorders

Eating disorders are motivated behavioral disorders and are characterized by (i) increasingly driven and ritualized behaviors that include dieting, purging, excessive exercise, and intermittent binge eating, (ii) narrowing of the behavioral repertoire with increased time spent on disordered eating behaviors, (iii) excessive preoccupation with food, (iv) progressive functional impairment, and (v) ambivalence toward, or frank avoidance of, treatment. Individuals diagnosed with eating disorders typically exhibit body dissatisfaction and a tendency to base self-evaluation on weight and shape.

As behaviors such as dieting or binge eating are repeated, learning and the conditioning of environmental cues and feeling states associated with disordered eating result in increasingly automatic and inflexible behavioral response patterns. Over time, repetition of these abnormal behavioral patterns deregulates homeostatic hunger and satiety signaling between the gut and the brain altering levels of neuropeptides and hormones such as cholecystokinin, pancreatic polypeptide, leptin, ghrelin, glucagon-like polypeptide-1, and opioid peptides. Some of these secondary changes are increasingly believed to further contribute to the maintenance and driven quality of disordered eating. In Anorexia Nervosa (AN), for example, starvation results in both physiological and psychological consequences that perpetuate the anorectic state. The landmark 1940s Ancel Keys' Minnesota Starvation Experiment focussed on the effects of starvation in 36 male conscientious objectors. These men developed many of the symptoms and behaviors typical of AN including depressed mood, apathy, preoccupation with food, eating rituals, increased obsessionality, and an overvalued concern with ascetic goals. Some exhibited increased drive to exercise and many developed binge eating behavior during the refeeding phase of the experiment.

Although unable to mimic all aspects of eating disorders, experimental animal models including the activity-based model of AN and rodent models of Bulimia Nervosa (BN) that alternate intermittent food restriction with access to palatable food have confirmed that physiological consequences of sustained or intermittent food deprivation can alter hunger and satiety signaling, influencing food reward and eating behavior.

Diagnostic Criteria of Eating Disorders

The Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-V), provides the specific criteria to identify symptoms and diagnose individuals with an eating disorder.

Anorexia Nervosa

The primary features of AN include a significantly low body weight driven by caloric restriction and/or purging behaviors coupled with an intense fear of fatness or weight gain and the presence of body image disturbance such that the individual either places undue emphasis on the importance of their weight or shape or minimizes that his or her weight is dangerously low. A 'significantly low body weight' is defined as less than what would be expected given the individual's age, sex, health status, and developmental level. Both the World Health Organization (WHO) and the Center for Disease Control (CDC) define the lower level of 'normal' weight in adults as a body mass index (BMI) of 18.5 $kg\,m^{-2}$, and suggested BMI ranges for assigning a level of severity to AN in the DSM-V are as follows: mild (BMI $>$ 17 $kg\,m^{-2}$), moderate (16–16.99 $kg\,m^{-2}$), severe (15–15.99 $kg\,m^{-2}$), and extreme (BMI $<$ 15 $kg\,m^{-2}$). Fear of fatness need not be verbally acknowledged if the patient is of significantly low weight and consistently engages in behavior that prevents weight gain. Patients may recognize that they are too thin but remain overly concerned with the size of specific body parts, such as the stomach, hips, or thighs. Frequent or obsessional body checking and weighing are often used to evaluate weight and shape.

Individuals with AN are divided into two subtypes based on an assessment of disordered eating behaviors in the past 3 months. Patients who engage solely in restricting behaviors and excessive exercise, without regular (weekly) binge eating or purging behaviors, are diagnosed with AN, restricting subtype, whereas those whose restricting behaviors are accompanied by at least weekly binge eating and/or purging behaviors are diagnosed with the binge eating/purging subtype of AN. The 12-month prevalence rate among females is low (0.4%) and approximately 10% of cases are male. Of note,

AN has the highest standardized mortality rate of any psychiatric condition with fivefold risk of any cause of death and 18-fold risk of death by suicide compared to an age-matched female population.

Bulimia Nervosa

BN is characterized by recurrent episodes of binge eating and inappropriate compensatory behaviors. A binge is defined as eating a larger amount of food than typically normal in a discrete period of time, associated with a sense of loss of control over eating. Lack of control is defined as the inability to refrain from eating or to stop eating once an individual has begun. Compensatory behaviors are employed to prevent weight gain and include self-induced vomiting; misuse of laxatives, diet pills, or diuretics, restricting, and excessive exercise. Both the binge eating episodes and compensatory behaviors must occur on average at least once a week for 3 months. As in AN, excessive emphasis on body shape and weight as a measure of self-evaluation and self-esteem is a core criterion of BN. Individuals with BN are typically of normal weight or overweight; if significantly underweight, the diagnosis of AN-purging subtype trumps the diagnosis of BN. Within BN, the level of severity depends on the frequency with which individuals engage in compensatory behaviors and ranges between mild (1–3 episodes per week), moderate (4–7 episodes per week), severe (8–13 episodes per week), and extreme (14 or more episodes per week). BN is slightly more common than AN, with 12-month prevalence rates of 1–5% in females. Similar to AN, male patients represent approximately 10% of cases.

Binge Eating Disorder

Binge Eating Disorder (BED), defined as recurrent consumption of large amounts of food in a fairly short period of time accompanied by feelings of loss of control (without the compensatory behaviors seen in BN), has recently been changed from a provisional diagnosis in need of further research in the *Diagnostic and Statistical Manual of Mental Disorders*, 4th Edition (DSM-IV), to a unique psychiatric condition in the DSM-V. Binge episodes are associated with eating until uncomfortably full, eating rapidly, eating alone, eating in the absence of hunger, and/or feeling disgusted with oneself after binge eating. The DSM-V requires that at least three of these five features accompany binge episodes on average once a week or more for 3 months to make a diagnosis. Binge episodes differ from overeating in that they are often secretive, associated with shame, and occur in the context of negative affect and cognitions.

BED is associated with decreased quality of life and increased lifetime co-occurrence of medical (e.g., irritable bowel syndrome, fibromyalgia, type 2 diabetes, and obesity) and psychiatric disorders, including major depressive disorder, bipolar disorder, anxiety disorders, substance use disorders, and body dysmorphic disorder. Although not all individuals with BED are overweight or obese, approximately 30% of those seeking weight loss treatment meet criteria for BED in comparison to 2–5% prevalence rates in community samples. BED is particularly prevalent among those pursuing bariatric surgery, ranging from 10% to 50%, and preoperative BED may indirectly predict postsurgical weight loss mediated by postoperative eating behavior.

Other Eating Disorders

Avoidant/Restrictive Food Intake Disorder (ARFID) is an eating or feeding disturbance in which individuals avoid or restrict their oral intake of food in the absence of an underlying medical condition. This disturbance results in one or more of the following: significant weight loss, or in children, a failure to meet expected weight gain, nutritional deficiency, dependence on oral nutritional supplements or enteral feeding, or a significant impairment in psychosocial functioning. The avoidance or restriction of food in ARFID is not in response to scarcity of food or cultural and developmental norms such as picky eating in preschool-aged children. Typically, the avoidance/restriction results from a lack of interest in eating or food, or an avoidance of eating due to sensory characteristics of food, such as texture or smell. Unlike other eating disorders, individuals with ARFID do not experience a disturbance in the perception of body shape or weight.

In addition to these disorders, DSM-V distinguishes a fifth broadly defined category of Other Specified Feeding or Eating Disorders. The clinical presentations of these syndromes may be behaviorally similar to AN, BN, or BED and cause significant impairment and distress in the individual's life. However, the symptoms do not meet full diagnostic criteria for one of the earlier mentioned conditions, or there is insufficient information to make a diagnosis. Examples of these disorders include atypical AN, subclinical BN or BED, purging disorder, and night eating syndrome. In atypical AN, all the criteria for AN are met; however, despite significant weight loss, the individual's weight is within or above the normal range. Subclinical BN or BED may be diagnosed when the individual engages in binge eating or inappropriate compensatory behaviors less than once a week and/or for less than 3 months. Purging disorder is characterized by recurrent purging behaviors with the goal of preventing weight gain in the absence of binge eating. Night eating syndrome is defined as recurrent episodes of nocturnal eating in which an individual consumes an excessive amount of food after an evening meal or will eat after awakening from sleep. These episodes of eating are not in response to external influences or related to a medical or psychiatric disorder. Finally, unspecified Feeding or Eating Disorder may be diagnosed if a patient presents with symptoms of disordered eating that cause significant distress or impairment in functioning and is most commonly used when there is insufficient information to determine a diagnosis.

Disordered Eating Behaviors

Certain disordered eating behaviors are observed cross diagnostically. Further, these behaviors may occur in nonclinical samples and potentially signify the need for early intervention. Though restricting behaviors are thought to be primarily associated with AN, they are seen in other eating disorders as well, especially BN. Individuals who engage in restricting behaviors attempt to limit their dietary intake with the goal of weight loss or to prevent weight gain. Common restricting behaviors

include reducing the amount and frequency of meals and food portions, avoiding calorie-dense foods, and narrowing one's food repertoire such that consumption is limited to specific low-calorie 'safe' food items. In addition, individuals will often engage in prolonged periods of fasting. For example, after a binge episode, individuals may forgo food for an extended period of time to prevent weight gain.

Binge eating and purging behaviors also occur trans-diagnostically. Binge eating episodes are defined as consuming an unusually large amount of food (about two meals worth) in a discrete period of time coupled with a sense of loss of control over eating. When a sense of loss of control is associated with eating small amounts of food, this is termed a subjective binge episode. Subjective binge episodes are more frequently reported by patients with binge eating/purging type of AN than by those with BN. Common purging behaviors include self-induced vomiting and laxative, enema, or diuretic abuse. Uncommon but important additional purging behaviors employed in the service of weight loss by individuals with eating disorders can include insulin omission in type I diabetics and wasting expressed breast milk in postpartum mothers. The frequency of purging behavior use varies, with some individuals purging exclusively after binge eating episodes, whereas others may purge after consuming small amounts of food or after every meal.

Additional behaviors are observed across all eating disorder diagnoses. These include chewing and spitting and grazing. Chewing and spitting out food before swallowing is common, often associated with a sense of lack of control over eating, and can involve large amounts of food and feelings of shame/guilt afterward. This behavior may be utilized as a way to taste 'forbidden foods' without ingesting calories. In other cases, chewing and spitting is best conceptualized as an alternative to bingeing and purging and is perceived as less medically risky, with lower likelihood of electrolyte abnormalities or other medical complications than vomiting. Grazing is the unplanned, repetitive ingestion of small amounts of food that may occur in a compulsive manner, such as repeatedly eating from a container of food when walking past it. Although grazing is frequently observed in nonclinical and eating disorder samples, when accompanied by a sense of loss of control over eating, it is associated with increased psychopathology. Grazing behavior has received significant attention in the bariatric surgery literature, particularly since grazing postsurgically is associated with suboptimal weight loss and/or weight regain, and efforts are currently under way to determine the best way to define grazing both clinically and for research purposes.

Excessive exercise is a frequently observed behavior employed in the service of weight loss among individuals diagnosed with AN or BN and is commonly defined by dysfunction in the quality and quantity of exercise. Unlike normal exercise, excessive exercise manifests as a strict and compulsive adherence to exercise regimes, preoccupation with exercise, feelings of guilt if exercise is not completed, and exercising despite illness, injury, or physical complications. These behaviors are utilized to prevent weight gain, influence one's shape and/or weight, or reduce feelings of distress related to food consumption. In addition, individuals may engage in increased frequency, duration, and intensity of exercise. Excessive exercise is associated with increased psychological distress, psychopathology, and risk of relapse.

Laboratory Studies of Food Consumption/ Macronutrient Selection

Laboratory studies of eating behavior have consistently shown that individuals with eating disorders will engage in disordered eating under controlled laboratory conditions. Compared to healthy controls, patients with AN select fewer food items, eat fewer calorie-dense foods, and have a lower total caloric intake in laboratory test meals in which they are presented with a large variety of foods of different calorie densities. Macronutrient selection primarily consists of carbohydrates ($\approx$55%) and protein ($\approx$30%), with limited caloric intake from fat ($<$20%). Following treatment, weight-restored patients with AN show increased caloric intake in test meals but continue to eat fewer calorie-dense foods and have a more limited repertoire of food items compared to healthy controls. Further, studies of implicit and explicit liking (pleasure associated with food) and wanting (desire for food) show that current AN and recently weight-restored patients with AN report decreased explicit and implicit wanting of higher-calorie food items compared to healthy controls. Of concern, avoidance of calorie-dense foods and limited food choices are associated with increased risk of relapse in this population.

Laboratory studies of binge eating typically offer participants either single-item or multi-item food arrays and instruct participants to 'eat a normal meal' (meal paradigm) or 'eat as much as you would like' (binge paradigm). Individuals with BN and obese individuals with BED consume significantly more calories and significantly more calories from fat than healthy controls using normal meal and binge eating test paradigms. Patients with BN also eat more rapidly than controls during laboratory binge eating episodes and report higher caloric intake for binge eating episodes than individuals with BED. Binge size is positively correlated with BMI in patients with BED, but this has not been observed in BN samples.

Nutritional and Medical Complications of Eating Disorders

Complications of eating disorders are best thought of as consequences of starvation, purging behaviors, or both. Those individuals most at risk are therefore those with the binge/purge subtype of AN, who are both underweight and engage in purging behaviors. Medical complications can affect all body systems and several are severe and potentially life-threatening.

Cardiovascular complications of starvation include severe bradycardia and orthostatic hypotension, which can result from fluid restriction, vagal instability, or decreased venous return attributable to atrophy of peripheral muscles. A frequent cause of orthostatic hypotension in individuals who purge is dehydration resulting from vomiting or laxative or diuretic abuse. In very low weight patients, starvation-induced atrophic cardiomyopathy can lead to mitral valve prolapse as the mitral valve becomes too big for the heart. Cardiac arrhythmias are often the result of electrolyte imbalances but can also be due to abuse of caffeine or ephedra in the service of weight loss.

Of all metabolic complications, the most common and the ones of highest concern are hypokalemia, because of its

association with lethal heart rhythms, and severe hypoglycemia. Acid–base imbalances include metabolic alkalosis in individuals who vomit and metabolic acidosis in laxative abusers. Less frequently, starved patients may develop hypomagnesemia, hypocalcaemia, or hyponatremia. The latter can be due to sodium depletion in starvation, diuretic abuse, or water intoxication. Excessive water ingestion is motivated by a desire to increase satiety, make weight, or 'eliminate toxins.' Severe hyponatremia or hypoglycemia can result in seizures as the presenting symptom. Oral repletion of electrolytes is the safest initial route to stabilize patients, although adjunctive judicious intravenous repletion may be indicated in severe electrolyte deficiency.

Gastrointestinal complications are very common in eating disorders. One study found that 98% of eating disorder inpatients met criteria for a coexistent functional gastrointestinal disorder (FGID), one-half of whom reported three or more FGIDs. The most commonly reported of these were irritable bowel syndrome, functional abdominal bloating, functional constipation, functional heart burn, functional dysphagia, and functional anorectal pain. Even in a nonclinical college student population, severity of dieting has been associated with increased abdominal symptoms.

State-dependent consequences of starvation, including delayed gastric emptying and delayed gastrointestinal transit times, are associated with early satiety and abdominal discomfort, which impede patients' efforts to increase their food intake. Gastrointestinal symptoms in AN dramatically improve with refeeding alone. Delayed gastric emptying is also well documented in BN, and a recent study found that gastric emptying normalized faster in restricting subtype of AN than in those individuals with AN who also engaged in binge/purge behaviors. The use of proton pump inhibitors or metoclopramide may be of some help in select cases; however, improvement is limited without concomitant increase in nutritional intake and weight gain. Gastrointestinal complications arising from laxative abuse can include colonic dysmotility, development of hemorrhoids, and rectal prolapse.

In individuals who purge by vomiting, enamel erosion of the lingual surface of the teeth, parotid gland enlargement, and reflux esophagitis are common, and there is risk for a Mallory–Weiss tear presenting with hematemesis. Acute gastric dilatation is a potentially life-threatening complication of refeeding in which marked dilation of the stomach can result in infarction by compression of the mesenteric artery or rupture of the stomach. It is best treated conservatively with nasogastric decompression. Both pancreatitis and transaminitis can be seen as consequences of starvation and both may transiently worsen with refeeding before improving. High cholesterol is common in AN and has been attributed to depressed cholesterol-binding proteins or to low triiodothyronine levels that inhibit cholesterol breakdown.

Starvation is associated with multiple reversible endocrine abnormalities. These include hypercortisolemia, growth hormone resistance, low insulin-like growth factor 1 (IGF-1), hypothalamic amenorrhea, and sick euthyroid syndrome. All are reversible with refeeding and hormone replacement therapy is not indicated. As the typical age of onset of AN is peripubertal, there are developmentally relevant medical complications besides amenorrhea. Both stature and breast development can be stunted, and catch-up growth is limited following recovery from prolonged adolescent AN. This information may be helpful in motivating a reluctant teen to gain weight.

Osteopenia and osteoporosis occur rapidly in AN with up to 50% of patients demonstrating abnormal DEXA scans within 6 months of diagnosis. Osteoporosis is one of the most common, serious, and irreversible complications of AN and is thought to result from the combination of low estrogen, high cortisol, and low IGF-1. Unlike in menopause, estrogen is not protective of bone loss in AN, whereas refeeding clearly improves bone density in adolescent AN and prevents continued bone loss. Most experts agree that hormone replacement should not be routinely prescribed in AN as it results in monthly withdrawal bleeding, which exacerbates any underlying nutritional anemia and can contribute to the mistaken belief by patients that their reproductive function is normal. Prescription of calcium and vitamin D is recommended as well as weight-bearing exercise following weight restoration. Data on bisphosphonates are still lacking, and caution is warranted in using these compounds in women of reproductive age since they leach from bone during pregnancy and there is concern about their teratogenic potential.

Besides nutritional anemia, at low weights, hematologic complications of severe AN include hypocellular bone marrow on biopsy and pancytopenia. Leukopenia in severely cachectic patients is associated with immunocompromised status and increased risk of opportunistic infections, and thrombocytopenia can cause easy bruising or bleeding.

Renal complications are not common, but can include impaired creatinine clearance in AN binge/purge subtype relative to AN restricting type. This usually reverses with refeeding although occasional cases with renal failure attributed to laxative abuse are well described. Renal failure is presumed to be the result of chronic hypokalemia and chronic intravascular volume depletion.

Brain changes in AN are most notable for the development of atrophy, visible on MRI, which reverses at least partially with refeeding. Patients with AN have also been found to have state-dependent neuropsychological deficits in a number of domains including set shifting, delayed discounting, gambling, and attention.

In general, information on medical complications may serve as a therapeutic tool to increase patient engagement in treatment. Reviewing lab results and providing patients copies of any abnormal tests or scans while supportively but clearly stating that these are medical consequences of their behavior may help increase motivation to change and engage patients in behavioral treatment. Somatically preoccupied patients may be in denial about their eating disorder and be overly concerned about functional gastrointestinal symptoms. These patients often avoid eating disorders specialty treatment, presenting instead to medical providers with somatic complaints that are consequences of their eating behavior. In such cases, the clinician can maintain an agnostic stance about diagnosis while formulating behavioral treatment as physiological retraining aimed at reinstating normal homeostatic mechanisms of hunger and satiety. This explanation may be more acceptable to these patients than an eating disorder diagnosis. Educating family members on how they can work with health-care providers to help contain the patient's behavior and how this will ultimately return control to the patient is also helpful.

Treatment of Eating Disorders

Levels of Care

Treatment options for patients diagnosed with eating disorders include outpatient individual, family, or group psychotherapy, partial hospitalization, and residential or inpatient treatment. Inpatient hospitalization and partial hospitalization are reserved for the most severe cases, and a high proportion of these patients have very low BMI and are diagnosed with AN. These patients may require hospitalization for medical stabilization, refeeding, and monitoring cardiac irregularities and dehydration or for co-occurring psychiatric conditions such as depression with suicidal ideation. Patients with purging behaviors may be admitted due to electrolyte imbalances associated with frequent vomiting or laxative abuse especially hypokalemia.

Generally, the first step for patients diagnosed with an eating disorder is outpatient evaluation and treatment. While a wide variety of therapies have been used with this population, the most efficacious outpatient therapy modalities include family-based therapy (FBT) for adolescent patients with AN and cognitive behavioral therapy (CBT) for patients diagnosed with BN and BED. When indicated, admission should ideally be to a behavioral specialty program for the treatment of eating disorders as these programs are capable of helping patients interrupt unhealthy behavioral patterns and normalize eating with the help of an expert multidisciplinary team using a structured behavioral protocol and group therapy approaches. Inpatient treatment goals include medical stabilization, normalization of eating behaviors, weight restoration in low weight patients, and relapse prevention. Length of stay varies by patient and is influenced by a number of factors including co-morbidity, BMI at admission, insurance coverage, patient dropout, and rate of weight gain. For underweight patients with AN, a goal weight is determined by the treatment team (generally a BMI of 20 or above for adults). Most expert programs are able to achieve rates of weight gain of 1–2 kg per week.

Pressure from insurance companies to decrease inpatient hospital-based costs has resulted in expansion of residential and partial or day hospitalization programs. Partial hospital programs may be stand-alone treatment centers or hospital-based step-down programs integrated with an inpatient unit. Patients in partial hospital programs typically engage in treatment activities such as supervised meals and individual and group psychotherapy and may also participate in meal-based occupational therapy sessions such as restaurant outings, meal preparations, and grocery shopping in order to practice newly acquired social eating skills in real-world settings.

Nutritional Rehabilitation

Nutritional rehabilitation includes weight restoration as well as normalization of eating behaviors. Clinically, this requires patients to consume appropriate quantities of foods of a variety of calorie densities without engaging in binge eating or compensatory behaviors, such as purging or excessive exercise. Though not the sole focus of treatment, weight restoration is the primary goal of treatment for AN as it is associated with superior outcomes including long-term weight maintenance, correction of medical complications, and improvement in both eating disorder psychopathology and comorbid psychiatric symptomatology. Weight restoration is also associated with decreased frequency of disordered eating behaviors, reduced likelihood of readmission, and increased rate of recovery at both short and long-term follow-up and is often used as a marker of readiness to progress to a lower level of treatment.

Refeeding Syndrome and Medical Monitoring

Refeeding syndrome comprises a constellation of potentially life-threatening metabolic and clinical changes that result from rapid oral, enteral, or parenteral feeding. These are most likely to occur with parenteral refeeding and least likely with oral nutritional rehabilitation. In the absence of access to an expert behavioral program capable of restoring weight in patients through oral refeeding alone, the use of enteral feeds may be indicated to initiate weight restoration. Enteral feeding should not be the initial choice however as it does not address the fear of consuming calorie-dense foods central to the phenomenology of AN. Furthermore, although some specialty programs advocate use of supplementary nasogastric night feeds, the outcomes of these programs are not superior to those of expert behavioral programs that rely on oral nutrition alone. Finally, parenteral feeding should be resorted to rarely if at all given the high risk of line sepsis and refeeding complications especially in those with AN who are severely malnourished and immunocompromised.

The hallmark of refeeding syndrome is hypophosphatemia, which occurs within 1–3 days of an increase in food intake. Hypophosphatemia results from the intracellular movement of phosphate for the formation of ATP and other anabolic demands and places patients at risk for potentially lethal cardiac arrhythmias. Postprandial hypoglycemia is also common during refeeding as is the development of peripheral edema, which can lead in the extreme to congestive heart failure, ileus, or anasarca.

Edema is most common in individuals who abuse laxatives or diuretics and who self-induce vomiting once they stop purging. Most edema can be managed conservatively; however, chronic volume depletion from severe purging can result in pseudo-Bartter's syndrome marked by secondary hyperaldosteronism, salt retention, hypokalemia, and marked edema. Judicious use of spironolactone can alleviate severe edema. In low weight patients who do not purge, dependent edema during refeeding can result from low albumin, fragile capillaries, and third spacing of fluid into the interstitial compartment. Care should be taken not to rapidly replete intravascular fluid as this can worsen edema in these cases. For patients who abuse laxatives, these can be stopped abruptly and need not be tapered, although compliance with laxative discontinuation is difficult outside a structured inpatient setting. When compliant, however, most patients do well with a bowel regimen of a stool softener and bulk laxative, with the possible addition of an osmotic laxative agent as well as education and reassurance that their bowels will normalize over a period of a few weeks.

Other potential risks of refeeding include two very serious neurological complications associated with severe AN, Wernicke's encephalopathy due to thiamine deficiency and central pontine myelinolysis, which most commonly results from overly aggressive correction of hyponatremia. Wernicke's encephalopathy is especially a risk in patients with comorbid alcohol abuse. Thiamine should be repleted IV or IM in patients who are severely malnourished (BMIs of <14) before initiating refeeding or administering IV dextrose.

Since relief from medical symptoms is a common goal for both patient and provider, formulating behavior change as the path to symptom relief can make weight gain and treatment more acceptable to patients and help engage them in therapy. It is important to appreciate that medical complications can play a sustaining role in the maintenance of eating disorders.

Psychological and Behavioral Treatment

Family-based therapy

FBT based on the 'Maudsley model' is generally considered to be the most efficacious treatment for AN in adolescent patients with less than 3 years of illness. The Maudsley model of family therapy consists of training parents to take control of their sick child's eating schedule. Utilizing an agnostic stance toward the origin of AN, parents are informed of the grave nature of their child's illness and are encouraged to exclusively focus on refeeding their child, usually via the provision of high-calorie meals and supplements, meal and bathroom supervision, and the development of a system of rewards and consequences to increase compliance with eating. Regular family therapy sessions are used to support parental refeeding efforts and improve communication skills among family members. As symptoms begin to remit, patients gradually regain privileges based on their ability to make appropriate food choices and maintain their goal weight. When compared to ego-oriented individual therapy (focussed on building autonomy and improving self-mastery), FBT has been associated with better rates of weight gain and an increased likelihood of resuming menstrual functioning, which may lower the risk of osteoporosis. Following achievement of recovery, FBT has low relapse rates of 10% compared to 25–30% often reported following inpatient hospitalization, though the latter group may include more severe cases. Attrition is also much lower with only 10–15% of patients dropping out of treatment compared to drop-out rates of 50% or more from other treatment modalities.

Exposure and response prevention

A potentially promising yet understudied intervention in AN is exposure with response prevention (EX/RP). EX/RP in AN targets eating related anxiety by exposing patients to *in vivo* eating challenges while preventing them from engaging in ritualized compulsive behavior. Patients are encouraged to practice these skills between sessions and monitor their anxiety and compulsive rituals, including excessive exercise. Weight-restored inpatients with AN who received 12 sessions or EX/RP demonstrated increased caloric intake in a laboratory test meal compared with inpatients who received a cognitive-based intervention following weight restoration. In particular, EX/RP may be beneficial in relapse prevention as it focuses on achieving normative eating practices in real-world settings.

Psychological treatment for BN and BED

Fortunately for patients diagnosed with BN, outpatient treatment recommendations are quite clear: CBT for BN, a form of psychotherapy, was given the top grade from the National Institute for Health and Clinical Excellence in 2004. The goal of CBT for BN (CBT-BN) is to disrupt the diet–binge eating cycle by regulating the eating pattern, introducing avoided foods into the diet, and eliminating restrictive behaviors. It further addresses cognitive aspects of BN, including excessive concern about weight and shape, perfectionism, rigid thinking, and low self-esteem. It can be delivered in a variety of formats, including individual and group therapy, as well as in a guided self-help version. A recent treatment review reported that one-third to one-half of all patients treated with CBT-BN endorsed abstinence of behavioral symptoms (e.g., binge eating and purging behaviors) at the end of treatment and that these gains were generally maintained 1 year post treatment. CBT-BN was shown to be more effective than fluoxetine alone, and patients reported increased satisfaction with CBT-BN compared to medication. In a meta-analysis of treatment for BN, CBT-BN demonstrated superiority to nutritional counseling, supportive therapy, and interpersonal psychotherapy (IPT), a form of treatment that helps individuals resolve interpersonal problems and overcome their eating disorder indirectly by better managing negative feelings associated with interpersonal conflict. Although research on an eating disorder-specific model of IPT is in a much earlier stage than research on CBT, IPT has yielded comparable recovery rates to CBT in the long term for BN.

Although many individuals with BED are overweight or obese and seek weight loss treatment, CBT for BED (CBT-BED) is the most studied and established treatment for BED. Like CBT-BN, CBT-BED focuses on disrupting the binge eating cycle by promoting normative and structured eating patterns, developing skills for managing urges/triggers to binge, and encouraging the use of cognitive strategies to tackle problematic ways of thinking that maintain binge eating. A meta-analysis examining psychological and pharmacological treatments for BED found that psychotherapy and structured self-help based on CBT were superior to pharmacotherapy and weight loss treatment. Pharmacotherapy and weight loss treatment produced medium and moderate effects on binge eating, respectively. Individual versus group formats of CBT-BED do not significantly differ when examining their effect on reductions in binge eating frequency and recovery and relapse at follow-up. When examining predictors of outcome in CBT-BED, rapid response during early treatment (the first four therapy sessions) is predictive of more favorable long-term treatment outcomes. Furthermore, those who cease binge eating during treatment tend to lose the most weight in the long but not in the short term; therefore, it is generally agreed that treating binge eating should be the first phase of treatment in BED before treating weight.

Conclusion

Eating disorders are motivated behavioral disorders marked by serious morbidity and mortality. A range of restricting, binge

eating, and purging behaviors occur across diagnoses and are accompanied by body image disturbance in AN and BN and often in BED, although body image disturbance is notably absent in ARFID. Medical and nutritional complications may be severe and life-threatening and include cardiac arrhythmias, electrolyte imbalances, pancreatitis, transaminitis, anemia, and osteoporosis. FGIDs are also common in this population, with the majority of patients presenting with one or more FGID. Discussion of medical complications with patients may serve to increase motivation to engage in treatment. Behaviorally oriented treatments that emphasize weight restoration or maintenance, as well as normalization of eating behaviors, are associated with the best outcomes. In particular, FBT for adolescents with AN and CBT for individuals with BN and BED are the best evidenced current treatments. Weight restoration in AN and the resumption of normative eating practices across diagnoses are associated with decreased rates of relapse.

See also: Adolescent Nutrition; Anemia: Causes and Prevalence; Appetite Control in Humans: A Psychobiological Approach; Enteral Feeding; Glucose: Metabolism and Regulation; Malnutrition: Prevention and Management; Obesity: Causes and Prevalence; Obesity Management; Osteoporosis; Parenteral Nutrition; Satiety.

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Eggs: Composition and Health Effects

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Egg Composition

The basic components of eggs are the white and the yolk, which differ greatly in their ratio of fluid, physical characteristics, and nutrient content. A current existing misconception is that the egg white is more healthful than the yolk due to its high protein content. However, the egg yolk is a much more important source of nutrients, some of which exert protective effects that go beyond nutrition. While eggs from a range of different birds are produced for human consumption, this article will present data relevant to chicken eggs – the most common and significant type of egg consumed in the human diet. The egg white is composed of 88% water, 11% protein, 0.2% fat, and 0.8% ash, while the egg yolk is made of 48% water, 17.5% protein, 32.5% lipids, and 2% ash. Regarding micronutrient composition, one large egg contains 186 mg cholesterol, 126 mg choline, 0.2 mg riboflavin, 0.5 μg vitamin B12, 24 μg folate, 0.1 mg vitamin B6, 41 IU vitamin D, 270 IU vitamin A, 0.5 mg vitamin E, 99 mg phosphorus, and 0.9 mg iron. These nutrients are distributed between the egg white and the yolk. The percent distribution of the different nutrients is presented in Table 1.

Eggs have been reported to be a nutrient-dense food with high-quality protein, which is present in both the egg white and the yolk. Kwashiorkor and other types of malnutrition have been correlated with lower egg consumption in underdeveloped countries. Eggs can also be part of weight loss diets due to their effect in suppressing appetite and decreasing caloric intake during the next 24 h.

In addition to its cholesterol content, a highly controversial topic regarding heart disease risk, the egg yolk also contains the carotenoids lutein and zeaxanthin, which are key antioxidants in protecting against oxidative damage, low-density lipoprotein (LDL) oxidation, and age-related macular degeneration (AMD). Further, the egg yolk has other micronutrients absent from the egg white including iron, vitamin E, and vitamin A. Egg yolk is also a good source of choline, a nutrient that plays a major role in normal fetal development and cognitive function and may possibly protect against Alzheimer's disease. Therefore, eggs contain major nutrients that are important throughout the life cycle, in addition to other components that have been shown to protect against chronic diseases such as AMD, sarcopenia, and Alzheimer's disease. However, the perceived concern of eggs being a source of dietary cholesterol is highly associated with decreased consumption and dietary recommendations in the United States limit and target eggs as a source of dietary cholesterol.

Egg Sources and Production

Eggs are produced in many countries throughout the world. As of 2006, China was the largest producer of eggs, followed by the United States, India, and Japan. The number of cases of eggs produced in United States in 2012 was 223.7, 58% of which were allocated to retail, 31.9% for food preparation, 9% for institutions, and 3.8% for export. There are a number of factors that determine egg production, including time of the year, color of the eggshells, and health-related concerns due to eggs' cholesterol content. However, health concerns are decreasing as the public becomes more aware of the healthful benefits of eggs and the lack of correlation between egg intake and heart disease. A closely controlled breeding program results in maximum egg production. Other factors that need to be considered are (1) laying more eggs early although in the long run, this can result in smaller eggs; (2) resistance to disease, and (3) favorable genetic factors. Today, the Single White Leghorn hen dominates the egg industry although in certain regions such as New England, consumers prefer the brown-shelled eggs; thus, the Rhode Island Red, New Hampshire, and Plymouth Rock hen breeds predominate in this region.

The production of eggs can be classified into four main categories: (1) conventional cage eggs, (2) cage-free eggs, (3) free-range eggs, and (4) organic eggs. In the first category, hens live in communal cage systems, which vary depending on the size of the bird and the facility. These eggs are normally collected by an automatic collection system. For the second category, hens live on indoor floor operations and are housed in a barn or poultry house, and they may be allowed to roam outdoors. Depending on the farmer, egg collection may or may not be automated. In the third category of free-range hens, animals are raised outdoors or have access to the outdoors; shelter is provided during inclement weather. All of these housing systems participate in handling and care practices. Organic eggs are produced according to specific standards that exist in many countries. For example, in the United States, the United States Department of Agriculture (USDA) dictates the organic standards in relationship to methods, practices, and substances used in the production of crops. In addition, antibiotics and growth hormones are prohibited. All organic systems are cage-free. It is important to note that the nutrient content of eggs is not affected by the way the hens are raised. Other important facts regarding production are that only 5% of eggs come from cage-free systems and they are generally more expensive. In addition, eggs from conventional systems have been found to have lower bacterial levels than that those from cage-free or free-range hens. Eggs need to be produced under the best sanitary conditions since the moment an egg is laid; physical and chemical changes begin to occur that can reduce freshness.

Patterns of Consumption

Eggs are consumed throughout the world in various forms. Item penetration for eggs in the United States is 93%, which

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Table 1 Percent distribution of the various components of eggs between the white and yolk[a]

Component (% of total)	*White*	*Yolk*
Weight	66	34
Volume	40	60
Calories	15	52
Water	64.7	35.3
Protein	57	43
Fat	0.6	99.4
Cholesterol	0	100
Vitamin A	0	100
Vitamin E	0	100
Vitamin D	0	100
Riboflavin	66.6	33.3
Folate	4	96
Choline	0.4	99.6
Selenium	40	60
Iron	0	100
Lutein	0	100
Zeaxanthin	0	100

[a]These percentages do not take into account the egg shell.
Source: http://www.eggnutritioncenter.org/.

means that eggs can be found in almost every household. According to a consumer research conducted by Miller-Zell, 85% of consumers view eggs as very or somewhat healthy. This research also concluded that individuals are more likely to consume eggs if they are told how to prepare nutritious dishes with eggs rather than providing information on the nutritional benefits of eggs.

According to the USDA's Economic Research Service 2000 data, it was estimated that more than 70 billion eggs were eaten in the United States, corresponding to an annual 250 eggs per person. These data include not only eggs that were eaten individually as main dishes but also eggs that were incorporated into other foods as ingredients or beverages. According to USDA Continuing Survey of Food Intakes by Individuals data, eggs are used in more than 900 different foods in the United States. These data are supported by the fact that the primary dietary sources of eggs are processed foods or mixed dishes that use eggs or egg components as ingredients. This is true regardless of whether individuals are eating at home or away. Findings from USDA surveys also revealed that per capita consumption of eggs increases from low- ($<$185% of poverty line) to high-income ($\geq$300% of poverty line) groups and that men consume more eggs than women on average. Further, per capita egg consumption increases with overweight status and obese weight status in adults.

Nutrients in Eggs

Cholesterol

Cholesterol, a sterol and a lipid-soluble compound, has essential functions in the body, among which are to provide permeability and fluidity to cell membranes and to be the precursor of bile acids, steroid hormones, and vitamin D. Despite these key functions, an excess of cholesterol in the body leads to a number of metabolic dysregulations that can result in an accumulation of cholesterol in vital organs including the liver, adipose tissues, and heart arteries leading to fatty liver, inflammation, and atherosclerosis. However, it is important to make a clear distinction between dietary cholesterol and cholesterol in our bodies. Since our body produces about 78% of the cholesterol handled on a daily basis, dietary cholesterol only comprises 22% of the total. Therefore, it is important to make a clear distinction between dietary cholesterol and circulating cholesterol or cholesterol accumulated in diverse organs.

A large egg contains between 186 and 230 mg of cholesterol, which has resulted in the perception that eggs, being a high source of this dietary component, may lead to heart disease risk and other problems. In the next paragraphs, some strong evidence that the cholesterol in eggs should not be a concern for healthy populations is presented.

Health effects

Epidemiological studies

Epidemiological studies do not support an association between egg consumption and coronary heart disease (CHD) risk. This could partly be explained by the presence of other nutrients in eggs that are known to be protective against oxidative stress and dyslipidemias. In addition, the response to dietary cholesterol is highly variable. Some individuals respond by having no changes in plasma cholesterol following a dietary challenge as high as three eggs per day for 12 weeks, while others could have a higher response than 0.05 mmol l^{-1} for each additional 100 mg of dietary cholesterol, which is considered the average response. However, all those individuals who are responders to a dietary cholesterol challenge have increases in both LDL cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), resulting in a maintenance of the LDL-C/HDL-C ratio, a central marker of CHD risk.

The main epidemiological studies addressing the effects of specific foods and nutrients on the development of chronic disease – including Framingham Heart Study, the Nurses' Health Study, National Health and Nutrition Examination Survey (NHANES), and the Lipid Research Clinics Prevalence Follow-up Study – do not support a relationship between dietary cholesterol/egg intake and increased risk for CHD. Further studies conducted in Japan in which more than 9000 individuals were evaluated reported an inverse association between egg consumption and heart disease risk. There are, however, some epidemiological data reporting that egg intake can adversely affect heart disease risk, but these are mostly in people with documented heart disease or diabetes. In summary, the preponderance of the epidemiological evidence from the past 14 years does not support a relationship between egg intake and risk for CHD.

Clinical studies

A number of clinical studies conducted in recent years have demonstrated that eating two or three eggs per day for 4–12 weeks does not increase the risk for heart disease. Individuals from these studies did not have changes in their LDL/HDL ratio or even had improvements in this ratio; this is possibly due to the consistent finding that egg intake increases HDL. Further, studies have been conducted with individuals having metabolic syndrome, a population with an increased risk for both diabetes and heart disease. These individuals were

prescribed a carbohydrate-restricted diet (25% of total energy) and were randomly allocated to consume either three eggs per day for 12 weeks or the equivalent amount of egg substitute. After the intervention, individuals consuming the eggs did not have increases in LDL, but they presented a consistent increase in HDL; therefore, there was an improvement in the LDL/HDL ratio. Another finding in all these clinical interventions is that egg intake favors the formation of the large LDL, which is less atherogenic, and the large HDL involved in reverse cholesterol transport.

Cholesterol and dietary recommendations

A very interesting observation is that a number of countries including the European Union, Japan, Korea, India, Canada, and New Zealand, among others, do not have an upper limit for dietary cholesterol in their guidelines; thus, their dietary guidelines do not restrict egg consumption. In the United States, both the USDA and the American Heart Association consider that consuming one egg per day is appropriate for healthy populations, which still results in a cautionary and confusing message for most individuals. It is very clear that most of the recently published data support the lack of effect of whole egg consumption on heart disease. Dietary recommendations should not target the elimination of a food that not only has a substantial amount of nutrients but also provides additional health benefits. In fact, a recent report on the management to reduce cardiovascular disease states that "There is insufficient evidence to determine whether lowering dietary cholesterol reduces LDL-C," a statement that clearly supports both recent epidemiological data and clinical studies.

Protein

Eggs are known to be one of the best sources of high-quality protein. The 2010 USDA National Nutrient Database for Standard Reference reported that one large egg contains 6.3 g of protein on average. Egg protein is distributed between the egg yolk and egg white portions, with egg white containing more protein than egg yolk. On average, a large egg containing 6.3 g of protein in total will provide 3.6 g of protein in the egg white, in addition to 2.7 g of protein in the egg yolk. In total, one egg provides approximately 13% of one's recommended daily intake for protein.

Egg protein composition

Eggs are a rich source of essential amino acids and are particularly abundant in glutamine, leucine, and asparagine/aspartic acid. The majority of egg protein can be found in the white (57% of total protein) rather than in the egg yolk (43% of total protein).

Egg whites contain a number of different proteins that exert a range of biological and antimicrobial activities. The predominant protein in egg whites is ovalbumin, comprising approximately 54% of total egg white protein. Conalbumin, the second most predominant egg white protein, binds metal ions, whereas flavoprotein and avidin are known to bind riboflavin and biotin, respectively. A number of egg white proteins have proteinase inhibitor activity, including ovomucoid, ovomacroglobulin, and ovoinhibitor. Ovomucin possesses antiviral properties, whereas lysozyme exerts antimicrobial *N*-acetylmuramidase enzyme activity to disrupt Gram-positive bacteria cell walls. Together, these proteins protect eggs against microbial contamination and spoilage.

Egg yolk additionally contains a variety of proteins that have biological functions. The predominant proteins in egg yolk fractions include lipovitellins, livetins, and phosvitin. Lipovitellins represent HDL in egg yolk, whereas livetins correspond to proteins found circulating in chicken blood, such as serum albumin, α_2-glycoprotein, and γ-globulin. Phosvitin contains a relatively high amount of phosphorous and strongly binds multivalent cations such as iron and calcium. The ability of phosvitin to bind heavy metal ions supports antioxidant activity and protects against lipid oxidation. Egg yolks additionally contain antibodies referred to as immunoglobulin Y, which have promising therapeutic potential in human and veterinary medicine.

Health effects

Eggs confer a number of health effects due to their protein content. High-quality protein from eggs supports skeletal muscle synthesis and maintenance, which is particularly important in athletes and aging populations to protect against sarcopenia. Individuals who consume eggs for breakfast have also been shown to have increased satiety and consume fewer calories throughout the day. Compared to consumption of a bagel breakfast, men who consumed eggs had lower plasma levels of glucose, insulin, and ghrelin – an appetite-regulating hormone. An additional study found that whole egg intake during carbohydrate restriction improves insulin resistance as determined by the homeostasis model assessment of insulin resistance (HOMA-IR) equation. Therefore, eggs represent a nutrient-dense addition to low-glycemic and weight loss diets.

The antimicrobial activity of egg white proteins may also confer anti-inflammatory properties to humans. For example, egg white-derived hen egg lysozyme has been shown to reduce intestinal inflammation in an experimental model of colitis. Egg intake has also been shown to reduce proinflammatory markers in the plasma of overweight and metabolic syndrome subjects, including C-reactive protein, monocyte chemoattractant protein-1, and tumor necrosis factor-α. Conversely, individuals with an egg allergy display hypersensitivity and immune reactivity to egg-derived proteins, thus initiating a proinflammatory immune response.

Lutein and Zeaxanthin

Egg yolks are an important source of two highly bioavailable antioxidant carotenoids, lutein and zeaxanthin. Since lutein and zeaxanthin are embedded in the lipid matrix, these carotenoids have been shown to be more bioavailable in eggs when compared to vegetable sources, despite a concentration of 200–300 μg per egg versus concentrations up to 1500 μg per serving of spinach, broccoli, or kale. Table 2 shows the content of lutein and zeaxanthin in different food sources including eggs.

Biological roles of carotenoids

Egg yolks are an important source of highly bioavailable lutein and zeaxanthin, two carotenoids that are selectively removed from circulation by the eyes and protect against AMD and

Table 2 Lutein concentration in selected foods

Food	*mg/serving*
Kale (raw)	26.5/1 cup
Kale (cooked)	23.7/1 cup
Spinach (raw)	3.7/1 cup
Spinach (cooked)	20.4/1 cup
Collards (cooked)	14.6/1 cup
Turnip greens (cooked)	12.2/1 cup
Green peas (cooked)	4.1/1 cup
Corn (cooked)	1.5/1 cup
Broccoli (cooked)	0.8/1/2 cup
Broccoli (raw)	1.3/1 cup
Romaine lettuce (raw)	1.1/1 cup
Green beans (cooked)	0.9/1 cup
Papaya (raw)	0.3/1 large
Egg (cooked)	0.2/1 large
Orange (raw)	0.2/1 large

Data from U.S. Department of Agriculture, Agricultural Research Service, USDA Nutrient Data Laboratory. http://www.ars.usda.gov/nutrientdata.

cataract formation. This selection is indicative of an important photoprotective role. These carotenoids exert their action by absorbing blue light and reducing its ability to reach the retina by an estimated 40–90%, thereby reducing the generation of reactive oxygen species, which damage photoreceptors and retinal pigment epithelium. The concentration of these carotenoids in the retina can be measured by quantifying macular pigment density in the eye.

Health effects

Epidemiological studies also indicate an inverse relationship between intake of lutein and zeaxanthin and both cataract and AMD. A major clinical trial, Age-Related Eye Disease Study 2 demonstrated that lutein and zeaxanthin are more protective against AMD than other supplements including β-carotene. In addition, studies conducted in animal models have shown potent antioxidant properties of lutein as documented by its effects in decreasing the susceptibility of LDL to oxidation, reducing liver injury, protecting against early atherosclerosis development, and reducing oxidative stress in the liver and eyes. Cell studies have also shown that lutein or zeaxanthin supplementation protect eye lens protein, lipid, and DNA from oxidative damage.

Studies evaluating the effects of egg intake on plasma carotenoids have demonstrated a substantial increase of plasma lutein and zeaxanthin following egg consumption. Lutein and zeaxanthin circulate in plasma transported by LDL and HDL. Egg intake has been demonstrated to result in the formation of larger LDL and HDL particles. These larger particles facilitate the circulation of lutein and zeaxanthin in plasma and support the findings of increased plasma concentrations of these carotenoids. More importantly, studies conducted in both hypercholesterolemic and healthy individuals have demonstrated that macular pigment density is significantly increased following egg consumption indicating that eggs could be used to protect against AMD.

Choline

Choline is a water-soluble organic compound and natural amine that has a wide variety of biological functions. The Food and Nutrition Board of the Institute of Medicine first established Dietary Reference Intakes for choline in 1998 after recognizing its role as an essential nutrient. The body is capable of *de novo* choline synthesis via hepatic catabolism of phosphatidylcholine; however, this process fails to adequately meet daily nutrient needs.

Choline in the diet

Despite choline being essential in our diets, many individuals fail to consume adequate amounts.

According to population-based data from NHANES, 2003–04, it has been estimated that only one in ten Americans (adults and older children) is meeting the adequate intake guidelines for daily choline intake.

Eggs are known to be one of the best sources of dietary choline, in addition to beef and chicken liver, beef steak, milk, cod, and peanut butter. According to the 2010 USDA National Nutrient Database for Standard Reference, one large, raw, fresh egg contains approximately 126 mg of choline. The majority of choline is located in the yolk, which on average is estimated to contain 116 mg of choline, with approximately 0.4 mg in the egg white.

Biological roles of choline

Choline provides structural integrity to cell membranes, in addition to playing roles in cell signaling, muscle functioning, neurotransmitter synthesis, and hepatic transport of lipids to the circulation via lipoproteins. In addition, choline is essential for proper fetal development, particularly in regard to brain and spinal cord development. This is attributed to the role of choline in folate metabolism and its effects on regulating neural stem cell proliferation and apoptosis. However, this biological role of choline has also led to its association with an increased risk of developing certain types of cancer.

Deficiency in choline can result in fatty liver and muscle damage, whereas choline deficiency during pregnancy increases risk of fetal neural tube defects. Reduced intake of choline has also been associated with increased plasma homocysteine due to a weakened capacity to handle methionine, resulting in an increased risk of developing cardiovascular disease. Given its significant neurological roles, choline is also thought to protect against the development of Alzheimer's disease and dementia while promoting integration of learning and memory.

Health effects

A number of health effects have been attributed to diets rich in choline, with eggs often serving as one of the primary dietary sources of choline across the world. Diets higher in choline have been shown to be protective against neural tube defects and cognitive impairments. Choline ingestion from egg intake has also been shown to undergo metabolism to trimethylamine *N*-oxide by gut microflora, which has been associated with an increased risk of major adverse cardiovascular events. However, choline-rich diets are also associated with lower levels of homocysteine, suggesting a protective effect against cardiovascular disease. Diets high in choline, in addition to egg intake, have also been associated with increased risk of developing lethal prostate cancer. Conversely, dietary choline and

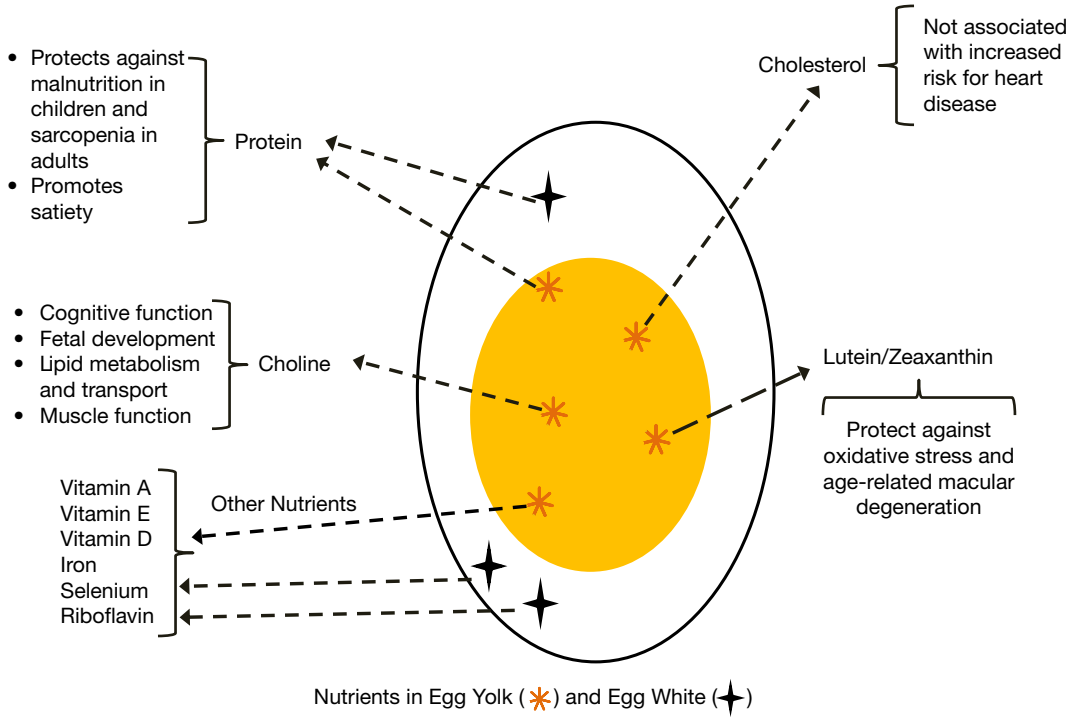

Figure 1 The nutrient content of egg yolk (cholesterol, protein, lutein/zeaxanthin, choline, vitamin A, vitamin E, vitamin D, selenium, riboflavin, and iron (*)) and egg white (protein, selenium, riboflavin, and iron (†)). The specific function of each nutrient as related to health is described.

egg intake has been shown to be protective against the development of breast cancer.

Other Nutrients in Eggs

Eggs are also a good source of fat-soluble vitamins including vitamin A, vitamin E, and vitamin D in addition to iron, all of which are located in the yolk. Since they are part of the lipid matrix of eggs, they can be more easily incorporated into micelles and be absorbed into the intestine where they are incorporated into chylomicrons and distributed throughout the body. Eggs are also good sources of riboflavin and selenium, which are distributed between the egg white and the yolk. One large egg provides 5% of the daily requirements of vitamin A and 5% of the daily requirements of iron. It is also a good source of vitamin E. In a study conducted in children, where they were allocated to consume either two whole eggs per day for 4 weeks or an equivalent amount of egg whites, dietary records indicated that the amount of vitamin E they consumed was almost three times more during the whole egg period.

The nutrients present in eggs and their contribution to nutrition and health are depicted in Figure 1.

Conclusions

Eggs are a rich source of key nutrients and should therefore be considered part of a healthy diet. Not only eggs provide a well-known source of high-quality protein, but also they contain dietary constituents that promote health across the life spectrum, protecting individuals against chronic disease. Evidence derived from epidemiological data and clinical studies support the notion that there is no association between egg consumption and heart disease risk. The yolk is a good source of choline that could be beneficial for pregnant women, for fetal and infant development, and for protection against Alzheimer's disease in the elderly.

All populations of children, young adults, and the elderly should benefit from the excellent protein quality of eggs that supports optimal growth in children and adolescents, while additionally protecting the elderly against sarcopenia. In addition, the carotenoids lutein and zeaxanthin protect against AMD and oxidative stress. Overall eggs should be part of a healthful diet.

See also: Adolescent Nutrition; Antioxidants: Characterization and Analysis; Cholesterol: Properties, Processing Effects, and Determination; Choline: Properties and Determination; Functional Foods; Mediterranean Diet.

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Eggs: Use in the Food Industry

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Introduction

The majority of eggs produced in the world are sold in-shell for consumers to utilize in the home in traditional ways or for baking. In the United Kingdom, only 17% of eggs produced go into egg products, and in the United States, the figure is nearer 30%.

Traditionally, surplus eggs not required for the shell egg market (normally the smaller sizes) and downgraded eggs were turned into egg products. Today, farmers have specific contracts to produce eggs for further processing, and any egg that is damaged cannot be used for this purpose. There are essentially three types of egg products, liquid, dried, and frozen, with small markets for peeled, hard-boiled, and hard-boiled pickled eggs. The main products are used widely in the bakery and confectionery trades along with other industries. Figure 1 illustrates the many uses to which processed whole eggs can be put and also shows the way in which whole eggs can be used to produce value-added products for the food industry without going through the process of pasteurization with or without subsequent drying. Value-added products in the United States are often made with pasteurized eggs.

The world trade in liquid eggs is in excess of 110 000 tonnes traded annually, some 80% of which are traded between EU member countries. Outside of this area, the only significant major market is Japan, which purchases around 15 000 tonnes a year. The Netherlands is the number one exporter closely followed by Belgium, both of them accounting for about 45% of the total world trade.

Some 28 000 tonnes of dried eggs is marketed internationally. Japan and European countries are the major buyers, with the United States being the major seller.

When setting up an egg processing plant, it should ideally be located close to its supply of eggs. A constant supply of eggs is important in the efficient operation of the plant. The correct location enables the eggs to arrive at the plant in good condition, thus ensuring that the resulting product is of good quality.

Liquid Egg Products

The production of liquid egg products takes place in specialist, large plants specifically designed for the purpose. Such a plant could process in excess of 36 million eggs a year. Mechanical equipment is used to break the eggs automatically and, if necessary, separate the egg yolk from the albumen. Such systems can handle up to 108 000 eggs an hour with electronic detectors to make sure that no yolk ends up in the white at the time of separation. A temperature of 10 °C is optimum for breaking and separating.

Further processing of eggs into liquid product is not a means of disposing of poor-quality eggs. In fact, the increasing concern about food safety and quality has meant that only surplus first-quality eggs and uncracked seconds are used in breaking plants.

At the time of breaking, each egg will be visually inspected, either automatically or by an operator, to ensure that no

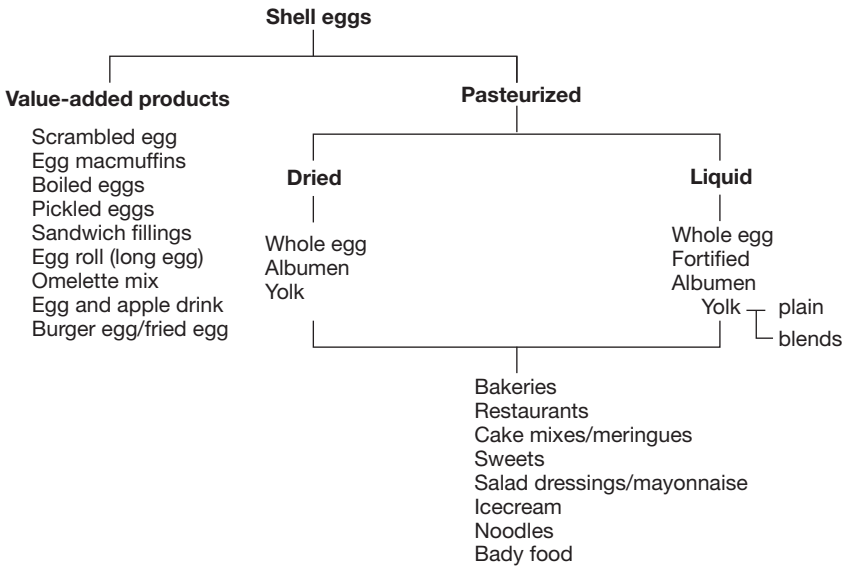

Figure 1 Eggs in the food industry.

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undesirable material is passed into the further processing line. Any eggs seen to be unsuitable will be rejected from the system at this stage and disposed of. The contents from the broken eggs are then passed through a sieve to remove any remnants of shell, the chalazae, which are the strands of protein that hold the egg yolk in place, and any other undesirable material.

About 50% of total liquid egg production in the United States is in the form of whole egg (yolk and albumen), and some may be fortified by the addition of extra yolk to increase the ratio of yolk to albumen over and above that found in a normal egg. The balance is divided between production of albumen and that of yolk. Liquid yolks may be sold as plain yolk or special blends such as sugared yolk or salted yolk. Not only such products meet the needs of specific customers but also the addition of these agents to yolk helps to maintain the consistency of the yolk, as without these natural additives, it tends to separate into a thin liquid surrounding a congealed core if it is frozen. The whole egg tends to be standardized to a solids content of 24.7%. The separate egg white should not contain more than 0.03% yolk on a liquid basis, and yolk tends to be standardized to a solids content of 43%.

In the past, all these products would have been frozen rapidly in a blast air freezer and held in this form until used. However, there has been a tendency in large-scale plants to chill the products and handle them as a fresh food, transporting them in refrigerated vehicles to their point of use. In this case, whole eggs and yolk liquids are chilled to 4.4 °C and egg albumen is chilled to <7 °C. In this form, the shelf life of the product is only about 4 days, but the costs of canning and freezing are avoided. Freezing is still adopted for small-scale use of liquid eggs.

Bakeries as a group are the largest users of whole eggs and separate albumen. Mayonnaise manufacturers and salad dressing makers use large quantities of salted yolks, and ice cream makers use sugared yolks. Noodle makers and processors of baby foods use plain yolks.

Liquid eggs or egg products are pasteurized (Figure 2) during their manufacture to prevent organisms such as salmonella from becoming a major health hazard during their subsequent use and particularly after the eggs are removed from storage. Legislation to enforce this was introduced in the United Kingdom in 1964 and in the United States in 1966. The pasteurization process in the United Kingdom involves subjecting the product to a temperature of 64.4 °C (148 °F) for 2.5 min for whole eggs. Comparable figures for the United States are 60 °C (140 °F) for 3.5 min. Albumen is more susceptible to heat, and so, a slightly lower temperature is used, whereas the reverse is the case for yolk, where any salmonella is more heat-resistant. The alpha-amylase test is routinely used to test for adequate pasteurization.

Traditionally, liquid egg products were packed in metal cans or transported by large tankers. Today, modern packaging machinery enables the product to be put in large plastic bags contained in rigid outers, and each bag can contain 500 kg of liquid egg, which is drained from the container by means of a tap inserted at the bottom. The bag is then disposed of and the outer is returned to the manufacturer to be refilled. The product can be put into smaller cartons (Figure 3) that can be supplied to retail outlets for direct sale to consumers or larger cartons for supplying to hospitals and institutions. This is an expensive way of producing liquid eggs because of the cost of the packaging. In these cases, liquid whole eggs would be used for making scrambled eggs and omelets, for example.

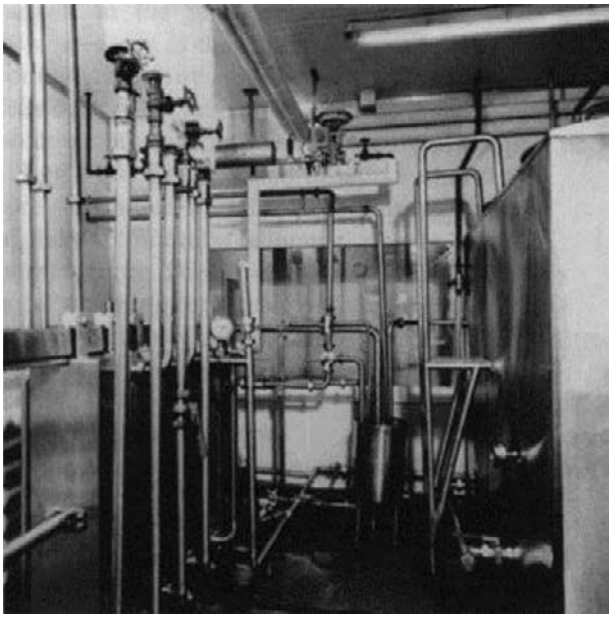

Figure 2 A liquid egg pasteurization plant. Courtesy of Oasters–Fridays (Cranbrook) Ltd.

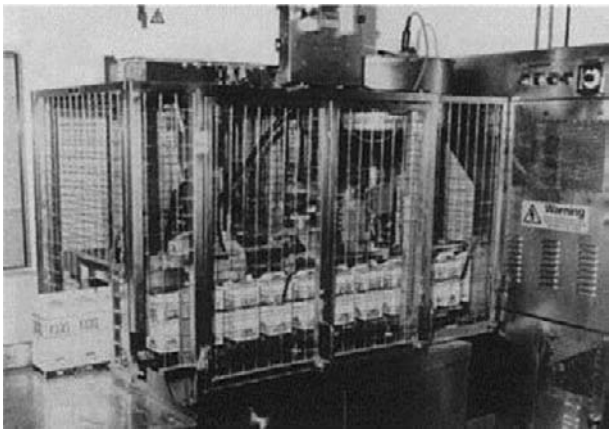

Figure 3 Liquid egg being packed into small cartons for retail sale. Courtesy of Oasters–Fridays (Cranbrook) Ltd.

Over the last 10 years, the production of egg products has been seen as the way in which any decline in egg consumption can be reversed. Consequently, a more diverse range of products are now available, including burger eggs, fried eggs, and meringue mix.

Freezing of Liquid Egg

If the liquid egg is going to be frozen, to maintain a quality product, it is important that the freezing process should be rapid and that the frozen product should be stored at a low temperature. Cans of chilled eggs should be subjected to an air

blast at −30 to −40 °C to ensure total freezing within 24 h, producing a uniform product without coagulation of the yolk. Subsequent storage should be at −10 to −20 °C.

Production of Egg Powder

The production of dried egg products is a further step beyond pasteurization. Spray drying is now the most common method for producing egg powder. However, some egg white is pan-dried and some whole egg is freeze-dried. Dried egg products have a number of advantages, which are listed in the succeeding text.

(1) They can be handled and stored with ease and at low cost.
(2) They are ready to use immediately with no thawing.
(3) They are easy to handle in a hygienic way.
(4) They are easy to remove from the container without scraping.
(5) No bacterial growth can occur in powder at room temperature, provided it is kept dry.
(6) There is good uniformity.
(7) There is precise control over the amount of water used in formulation.
(8) There is no loss when used because the dried egg is usually added directly to the batch.
(9) No special transfer or storage equipment is needed.
(10) Because the moisture content is reduced from around 74% to 2–4% by weight, there is a reduction in weight and volume and a concentration of food value.

A possible disadvantage of powder compared with liquid egg is a loss of 'fresh flavor' and a loss of certain functional properties such as aerating power, unless treated with nonreducing sugars prior to heating. This, however, limits its use because of the sweetened nature of the powder.

There are now several different types of dried egg products available that can be summarized under four major headings: dried egg white, dried plain whole egg and yolk, dried blends of whole egg and yolk with carbohydrates, and special types of dried egg products.

The growth in egg drying is well illustrated by reference to the United States. Prior to 1941, <1% of the eggs produced went to drying plants. Government purchases stimulated drying operations to such an extent that 20 400 tonnes were produced in 1941, 107 000 tonnes in 1942, 118 000 tonnes in 1943, and 146 000 tonnes in 1944. The current annual volume is over 180 000 tonnes. A 30 dozen case of shell eggs will yield about 18 kg of liquid whole egg or 4.5 kg of dried product containing 2.5% moisture.

The handling of shell eggs prior to drying is no different from the handling of eggs that are to be kept liquid. The liquid material is put through a clarifier to remove any bits of shell and is then screened to remove the chalazae and vitelline membranes. Pasteurization is important, not only to control salmonella infection but also to preheat the liquid so as to ensure a low-moisture powder that will not show any scorching. A typical egg-drying plant is shown in Figure 2.

The liquid egg is then pumped under a pressure of 17 250–34 500 kPa (2500–5000 lbf in^{-2}) to nozzles through which it is released into a large chamber where it immediately comes into contact with a stream of air that has been heated to temperatures of 120–175 °C. The enormous surface area created by atomization causes instantaneous evaporation of most of the moisture from the egg material and the formation of powder that falls to the floor as a fine powder, while the moist air passes on out of the drying chamber at a temperature of 65–71 °C. It is important that the spray dryer is designed so that the product stays at relatively low temperatures throughout the drying system and that the dried product is removed from the hot air stream as rapidly as possible.

Modern dryers produce a powder in which the moisture content does not exceed 2%. The temperature of the powder as it leaves the dryer may be 65 °C or higher, and this must be reduced quickly to <29 °C if the product is to have good keeping qualities. Since the powder is extremely hygroscopic, the temperature must be reduced by contact cooling, rather than by exposure to cold air. Two different powder-cooling methods are commonly used: first, pneumatic conveying with cooling air and, second, mixing the powder with carbon dioxide in the form of dry ice. After cooling, the powdered eggs are then packed immediately in sealed containers. Many of them are packed in carbon dioxide to remove the oxygen and lower the pH value in order to improve the keeping quality of the product.

Egg white can be dried in pans, rather than through a spray technique. The pan-dried egg white can be in the form of flake or granular albumen or can be milled to a powder form. This form of egg albumen tends to have good whipping properties once reconstituted.

The chief users of dried egg products are cake mix manufacturers, sweet makers, and manufacturers of meringue powders and, to a lesser extent, mayonnaise makers. Most dried egg products made for commercial use are packed in polythene-lined boxes or drums. A common pack size would contain 25 kg of product.

Quality of Dried Egg Products

The quality of dried egg or dried egg products can be affected by a number of factors. Important are the quality of the eggs broken out to make the original product, handling methods, sanitation practices, conditions during processing, pasteurization procedures, drying, and conditions under which the products are held in storage. Glucose (0.3–0.5%) is removed from the liquid egg white prior to drying by fermentation using a yeast or bacterial culture or by oxidation to gluconic acid using a glucose oxidase–catalase enzyme system. With glucose removed, dried egg whites are completely stable. If glucose is not removed, the product would be unstable because reducing groups from the glucose would combine with amino acids in the protein, leading to a condensation reaction that would be followed by browning and the development of insoluble proteins. There could also be the development of off-odors and the loss of some functional properties during storage. Plain whole egg and yolk can be pasteurized and dried without removal of the glucose, and consequently, these are the least stable of the egg products.

The physical properties important in relation to dried egg products are bulk density, dispersibility, solubility, and reconstituted viscosity.

Storage of Dried Egg Products

One of the real advantages of dried egg products is their ease of storage. Most are relatively stable when stored at room temperature. Dried egg whites can be held under almost any storage condition for an indefinite period of time. Dried products containing whole egg and yolk should be under refrigeration if held for long periods of time. Some of these are relatively stable at room temperature, particularly those where the natural glucose has been removed prior to drying.

Other Uses of Eggs in the Food Industry

Although liquid eggs and dried eggs still account for the majority of eggs used in the food industry, there are other products, which have evolved during the last 10–15 years, that are commercially available.

The mass production of hard-boiled eggs has developed in recent years. Normally, white eggs are used in the process, rather than brown, because the shells can be more easily removed from white eggs than from brown, resulting in less damage to the finished product. In countries where brown egg production predominates, such as the United Kingdom, this means that white eggs may have to be imported for the process from countries such as Denmark. However, in recent years, flocks of white egg-laying hens have been established in the United Kingdom specifically to supply the processing industry.

Again, only good-quality eggs are used in the process so that the final product is also of good quality. Normally, in European Union countries, small eggs would be used (under 53 g) for boiling as there is little demand for these sizes of eggs in the shell egg market.

The eggs are gradually boiled as they move around a trough arrangement containing high-temperature water, with the objective of them being hard-boiled by the time they have completed a single circuit of the trough. The eggs are then removed, deshelled, and cooled. Once hard-boiled, the eggs can be used for a number of products. They can be pickled in jars in plain vinegar or with spices. They can be used in pies or salads and a major use in this form is in airline meals. Eggs damaged during the boiling or shelling processes would not necessarily be discarded. They can be diverted to the manufacture of sandwich fillings, such as egg mayonnaise, which can be packed into 5 kg tubes for distribution to sandwich manufacturers and the catering trade.

Problems associated with large-scale boiling of eggs, apart from that of deshelling, include optimizing the temperature for both the boiling and cooling processes, effluent discharges, and the prevention of black rings occurring at the junction between the yolk and the albumen. This is associated with the breakdown of sulfur-containing amino acids in the albumen producing hydrogen sulfide, which reacts with iron released from the yolk to form iron sulfide.

Hygiene Measures

In all egg processing plants producing egg products for human consumption, a strict code of practice relating to hygiene is essential. Conventional egg products must now meet strict chemical, physical, and functional specifications, which include moisture, fat, protein, ash, glucose, reconstituted viscosity, whipping ability, and functional performance in the foods in which they are used. Also included are microbiological standards for total plate count, coliform, yeast and mold, *Escherichia coli*, salmonella, coagulase-positive staphylococcus, and *Clostridium perfringens*.

Many plants have a resident food technologist responsible for the implementation of a quality control program using an in-house quality control and microbiology laboratory. Every batch of product produced would be tested for contamination, and production plants would be swabbed twice a week to test for the presence of undesirable organisms within such a program.

See also: *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Clostridium*: Occurrence and Detection of *Clostridium perfringens*; Drying: Principles and Types; Eggs: Composition and Health Effects; Freezing Theory.

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Elderly: Nutrition Requirements

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Introduction

It is well known that the population of elderly people is expanding rapidly; it has been estimated that by 2030, 70 million people will be age 60 years or older, and, by 2050, the population older than age 65 is projected to be 88.5 million. The 85+ population is anticipated to grow from 4.2 to 6.6 million in 2020, then to 8.9 million by 2030, to 19 million by 2050. An increase in the prevalence of disabilities and diseases is one of the natural consequences that accompany advanced aging.

Functional impairment in older adults is associated with poor nutritional status or malnutrition, which is frequently also associated with the perception of quality of life, particularly among women. Disability is associated with frailty and frailty is linked to loss of muscle strength and weight. Frail individuals are not always cachectic but may be overweight or obese. Data from the Health and Retirement Study, a prospective study conducted by the University of Michigan, demonstrate that excess body weight is associated with less Activities of Daily Living (ADL) and Instrumental Activities of Daily Living (IADL) impairment. The challenge of determining nutritional requirements in elderly individuals is associated with an increasingly diverse impact of physiological aging and a lack of adequate and appropriate standards against which nutritional status can be measured.

Energy Requirements

As humans age, there are changes in body composition; lean body mass, total body water, and bone density decrease over time. Fat stores may increase or remain the same, but fat compartments shift and tend to become denser around major organs. These body composition changes contribute to a decrease in the energy required to maintain homeostasis including body weight and function. To avoid excess weight gain, caloric intake must be reduced. The Baltimore Longitudinal Study of Aging demonstrated a decrease in energy intake from 2700 kcal at age 30 to 2100 kcal by age 80 in male subjects. This observation is important because as basal energy needs decrease and activity becomes more sedentary, less energy substrate is required to maintain lean body mass and support energy expenditure. However, reducing total caloric intake can increase the individual's at risk for a deficient intake of other essential nutrients.

Decreased energy expenditure is associated with chronic disease and increasingly sedentary behavior. There is a decrease in consumption of calories with age as well as a decrease in energy expenditure. However, if older adults do not reduce their caloric intake as they age and become more sedentary, weight gain results. Weight gain may serve to further exacerbate disabling conditions; however, heavier patients may have better survival and recovery from atherosclerotic heart disease, stroke, and other potentially life-threatening conditions.

Requirements for energy to maintain weight are often linked to energy consumption, but research indicates that this might not be reasonable in estimating energy needs for elderly individuals. Prediction equations for energy requirements do not give reliable estimates of energy requirements in elderly people, and indirect calorimetry should be employed when actual energy needs must be defined rather than grossly estimated. The clinical challenge is to continue to meet all nutrient requirements in a smaller caloric base of food consumed regardless of weight.

Data indicate that the relationship between obesity and mortality becomes stronger with age. Perhaps, more pervasive is the role that obesity has in functional consequences; older adults who are physically limited because of obesity (body mass index of ≥ 30) tend to be more sedentary. Sedentariness contributes to weight gain, more severe obesity, and possibly mortality.

Undernutrition

Undernutrition, a deficit of essential nutrients, including energy substrate and protein, is a common finding in older adults. In elderly individuals, measuring nutritional status is somewhat challenging because there are no population reference standards against which to compare. Alternate approaches to measure undernutrition or malnutrition abound, but it has been well documented that undernutrition exists and is a therapeutic challenge in older adults regardless of their health status. In elderly people, loss of reserve capacity is associated with undernutrition. Reserve capacity is the ability of an organism to respond to trauma, acute illness, or other physiological insults; for patients who lose this ability, an insult may lead to a rapid decline. Weight loss has been associated with adverse outcomes.

Calorie Restrictions

Evidence that caloric restriction increases life span in research models ranging from single-cell organisms to nonhuman primates has had positive results. Evaluating the animal calorie restriction models in humans is challenging due to the poorly defined mechanisms of calorie restriction and longevity and the potentially difficult ethical issues in human research. Skepticism about the positive effects of calorie restriction in humans is related to the real-life, nonlaboratory, controlled environment situation under which people live.

Protein Requirements

Protein intake tends to be highest in young adults and progressively declines with advancing age. The decrease in protein intake is likely to be the result of reduced energy intake and not a change in the proportion of energy consumed as protein. Data indicate that although the acceptable macronutrient

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distribution range for protein is 10–35% of energy, very few older adults usually consume more than 20% of their energy intake as protein. In normal aging, changes in body composition occur that result in a shift toward decreased muscle mass and increased fat mass.

The loss of fat-free mass with advancing age theoretically results in a relatively lower protein requirement (per kilogram of body weight) compared to a younger adult. However, at present, there is no consensus regarding the protein needs of elderly persons, although there is some research suggesting that the Recommended Dietary Allowances (RDAs) should be higher than the current level. Short-term nitrogen balance studies are inconclusive, whereas the limited longer-term nitrogen balance studies suggest that the RDA is not sufficient to completely meet the metabolic and physiological needs of elderly persons.

Fat Requirements

A minimum of 10% of total energy intake should be derived from fat to ensure an adequate dietary intake of fat-soluble vitamins and essential fatty acids. Recommendations for dietary fat intake are the same for older adults as for younger adults: 30% or less of total diet with saturated fats providing from 8% to 10%, polyunsaturated fats being ~10%, and monounsaturated fats making up the difference. The recommendation for dietary cholesterol intake is 300 mg day^{-1} or less. Total cholesterol is generally considered an important risk factor for coronary heart disease; however, the impact of cholesterol profiles in elderly people is unknown. In a 5-year prospective study of 4066 men and women older than age 71 years, elevated total cholesterol levels were associated with a similar pattern of death from coronary heart disease as seen in younger adults. The subject group with the lowest total cholesterol levels (<160 mg dl^{-1}) had the highest incidence of preexisting cardiovascular disease, the highest risk factors for cardiovascular disease, the highest indexes of poor health, and the highest crude coronary heart disease mortality. Currently, it appears that elevated total cholesterol levels remain a risk factor for death from coronary artery disease in elderly persons. High-density lipoprotein cholesterol fraction values <35 mg dl^{-1} predict coronary heart disease mortality and occurrence of new events in individuals older than 70 years. Specific recommendations for dietary fat modifications must be made on an individual basis based on a complete profile of cardiovascular and stroke risk factors.

Carbohydrate Requirements

Carbohydrate is the third major macronutrient; it includes the simple sugars and complex carbohydrates. Requirements for dietary carbohydrate generally approach 55–60% of total energy intake. The complexity of dietary carbohydrate can be as important as the percentage of calories contributed by carbohydrate-rich food. Consumption of food with the indigestible dietary fiber removed can contribute to gastrointestinal disorders commonly encountered in older adults, including constipation, diverticular disease, diabetes, and hyperlipidemia.

Dietary fiber, mainly composed of plant polysaccharides and lignin, is resistant to human digestive enzymes. One example of such fiber, wheat bran, increases fecal bulk and decreases gut transit time and intraluminal pressure within the colon. These effects help to reduce the incidence of constipation and the formation of colonic diverticula. However, soluble fibers such as gums and pectins increase the viscosity of intestinal contents, increase gut transit time, and decrease the rate of small intestinal absorption. Consumption of fibers such as guar, pectin, and tragacanth leads to reduced insulin secretion in normal subjects, increased carbohydrate tolerance in diabetics, improved lipid profiles, and reduced glycosylated hemoglobin (HbA1c). The mechanism of the positive effect seems to be related to delayed gastric emptying and reduced rate of absorption of carbohydrate in the small intestine. Purified fibers such as guar, pectin, oat bran, and high-fiber foods, including cereals, starchy vegetables, and beans, have been reported to lower serum lipids. Decreases in total cholesterol, LDL cholesterol, and triglycerides without changes in HDL cholesterol have been reported. At present, it seems prudent to have a daily dietary fiber intake of 25–35 g day^{-1} and to include a variety of fibers from fresh fruits, vegetables, legumes, and whole-grain products. Every effort should be made to encourage older adults to have a varied dietary intake of carbohydrate-containing foods daily. This strategy will increase the likelihood of adequate consumption of micronutrients as well.

Fluid Needs in Older Adults

In older adults, fluid intake often does not receive the attention it deserves. Inadequate fluid consumption can lead to rapid dehydration, which has its own potentially risky consequences including hypotension, constipation, nausea, vomiting, mucosal dryness, decreased urinary output, elevated body temperature, and mental confusion. Total body water decreases with age, shrinking from 75% body weight in infants to 55% body weight in elderly adults associated with the reduction in total lean body mass.

Older adults are at risk of dehydration, particularly when there are climate challenges (extreme heat or cold). Water acts as a thermal buffer to protect the individual from hypo- or hyperthermia. Elderly people have decreased sweating and changes in other thermoregulatory responses, making them more vulnerable to periods of environmental shifts in temperature. Insensible losses through skin can range from 0.3 l h^{-1} when sedentary to 2.0 l h^{-1} with exercise; this may lead to a loss as high as 6 l day^{-1} in extreme heat. Decreased plasma volume and osmolality can result in death during periods of extreme environmental heat. Adequate hydration in older adults affects their ability to be physically active; cognition, alertness, and short-term memory are affected by mild dehydration. The challenge comes from the fact that thirst mechanisms and sensitivity are compromised in elderly people. The decrease in fluid intake is the result of alterations in mechanisms that control thirst sensitivity, particularly a decrease in osmoreceptors and baroreceptors and secretion of vasopressin.

Environmental factors, hormonal changes, climate (temperature and humidity), availability of water and food, medical problems, diuretic abuse, polypharmacy, and avoluntary decrease of fluid intake may all affect fluid balance. Along

with disordered thirst control mechanisms and inadequate intake of fluid for maintenance, older adults who have fever, infection, immobility, dementia, coma, fluid loss due to hemorrhage, diarrhea, vomiting, incontinence, or diabetes insipidus or have acute illness can become chronically dehydrated. The kidneys' ability to concentrate urine or efficiently conserve water also can affect fluid balance.

Many older adults, particularly those who are institutionalized, are not consuming adequate amounts of fluid. Although the usual stated requirement for fluid intake is eight 8 ounce glasses of fluid per day, data to support this recommendation are lacking. Actual need is probably in the range of six 8 ounce glasses of fluid per day.

Methods commonly used to determine fluid needs in individuals include 30 ml kg^{-1} body weight with a minimum of 1500 ml day^{-1}, 1 ml $kcal^{-1}$ consumed, or 100 ml kg^{-1} for the first 10 kg of actual weight, 50 ml kg^{-1} of the next 10 kg actual body weight, and 15 ml kg^{-1} of remaining weight. The first method (30 ml kg^{-1} body weight) approximates fluid needs most accurately in institutionalized elderly persons; additional research needs to be conducted to better estimate actual needs of older adults. Strong and chronic encouragement for oral fluid intake might be necessary to maintain hydration status.

Vitamin Requirements

Water-Soluble Vitamins

Vitamin B_1 (thiamine)

Some studies suggest that older adults might have a higher requirement for vitamin B_1, but lower energy requirements for older adults may contribute to an increase in requirement. Based on what is presently known, the recommended amount appears to be adequate for all adults independent of age. Depending on income, race, and socioeconomic status, up to 70% of elderly adults have low levels of vitamin B_1 intake.

Vitamin B_2 (riboflavin)

Vitamin B_2 acts with its coenzymes flavin mononucleotide and flavin adenine dinucleotide in many electron transfer reactions. Because of the many different roles of the flavoenzymes in intermediary metabolism, the important role of riboflavin (such as in antioxidant defense or in the modulation of homocysteine metabolism) is often forgotten. Dairy products and cereal products are, for older adults, the most important sources of riboflavin. In various populations around the world, the high prevalence of riboflavin deficiency is mainly caused by an inadequate intake of dairy products.

There might be a role for riboflavin in the pathogenesis of age-related cataracts because this vitamin might be important as a photosensitizing agent. Despite the potential involvement in some disease states, there is no evidence that there is a higher need for vitamin B_2 in older adults.

Niacin

Data about niacin status in elderly adults are very limited. Niacin intake in elderly adults varies widely and is associated with the variables of socioeconomic background, race, age, health status, and institutionalization. Nutritional intake of niacin is related to the mortality of elderly people; however, this relation does not necessarily imply causality. The nutrient density of niacin was adequate in nursing home diets so that low intakes become the major determinant of recommendations for this vitamin. For niacin, low dietary intake is key to a niacin-insufficient diet. Encouragement of higher energy and protein consumption is the best strategy to increase niacin status because this approach improves niacin and protein intake.

Vitamin B_6

The intake of vitamin B_6 varies widely among elderly persons as a function of age, socioeconomic level, and state of health. Studies report intakes below the RDA in up to 50% or even 90% of elderly populations. Even in healthy, free-living, elderly adults, 61% of women consumed <1 mg of vitamin B_6 per day, and 54% of elderly men consumed <1.1 mg (corresponding to 55% of the 1989 RDA). Between 10% and 50% of the US population older than 51 years consumed less than the Estimated Average Requirement (EAR) of vitamin B_6, <1.4 mg day^{-1} for men and <1.3 mg day^{-1} for women. (The EAR corresponds to the daily nutrient intake that is estimated to meet the requirement of half the healthy population of the corresponding age group.)

It has been suggested that an age-related increase in activity of the alkaline phosphatase, considered to be of major importance in the degradation of pyridoxal-5-phosphate (PLP), and low intakes of vitamin B_6 are the major causes of age-related decline in plasma PLP levels. Abnormalities of vitamin B_6 can be corrected by the ingestion of vitamin B_6 supplements in most elderly persons. A few studies report the inability of some older adults (up to 20%) to correct their biochemical vitamin B_6 status on supplementation. These observations are suggestive of age-related changes in vitamin B_6 requirements of older adults. The effects of diseases and comorbidities seem to be the likely causes of unresponsiveness to supplements. The bioavailability of vitamin B_6 from food is low; the RDA, based on the intake vitamin B_6 from food sources, might be even higher than assumed. The present recommendations do account for these alterations of the metabolism with aging.

Both retrospective and prospective studies have described a relationship between the risk of coronary artery disease, myocardial infarction, and the intake of vitamin B_6. This relationship is, however, strongest for folic acid.

Folate

Since the 1989 RDA was published, the recommended intakes of folate have been increased for older women and men. Folate intakes in older adults vary considerably as a result of diet, but most elderly people ingest amounts close to the RDA. Before fortification of cereal grains with folate (i.e., before 1 January 1998), the prevalence of low folate intakes varied between 0% and 50%. The median intake in the population at large before mandatory cereal folate fortification was 250 μg day^{-1}, which is well below the present recommendation. The average folate intake has increased by $\sim$80–100 μg day^{-1} due to the fortification of cereals.

A high intake of alcohol alone or in combination with an inadequate diet is one of the most important factors contributing to clinical folate deficiency. In elderly subjects, the presence of both low folate intake and elevated plasma homocysteine level represents a rather prevalent clinical situation. Elevated

levels of plasma homocysteine are mainly attributable to an impairment of folate, vitamin B_6, and/or vitamin B_{12} status. In most people, folate seems to be the major determinant of plasma homocysteine levels. Perhaps, homocysteine is only a surrogate factor and its relationship to etiologies of chronic disease is more complicated. Presently, the best strategy to manage progression of chronic disease risk is the consumption of a well-balanced diet.

As a result of the US government-mandated folic acid fortification of cereal grain products, a shift in the distribution of folate intakes and consequently the biochemical status of folate occurred. It has been reported that a higher supplemental folate intake ($>400\ \mu g\ day^{-1}$) was associated with an increased cognitive decline in elderly subjects. This effect was noticeable in older people who also had a vitamin B_{12} deficiency or low vitamin B_{12} plasma concentration as reported in other studies. Adequate intake (AI), whenever possible, from food, based on present guidelines should be more strongly encouraged, but it should be remembered that larger amounts of folate from fortified foods and/or supplements are not good for everyone.

Vitamin B_{12}

Vitamin B_{12} requirements for older persons (i.e., >51 years) can be obtained through consumption of vitamin B_{12}-fortified foods or vitamin B_{12}-containing supplements. Fortified foods and supplements contain crystalline vitamin B_{12} and absorption is not affected by aging. Intake of vitamin B_{12} in free-living elderly people varies, and ~0–50% of older adults have been reported to have (dietary nonsupplemental) intakes below the present recommendation. Dietary intake varies in older adults as a function of food choices. Nearly 70% of vitamin B_{12} intake in elderly subjects is from meat, eggs, and fish.

It has been shown that atrophic gastritis, common among older adults, decreases the bioavailability of dietary vitamin B_{12}. In food, vitamin B_{12} is bound to a protein carrier and must be released before it can be absorbed. Several mechanisms can lead to the malabsorption of protein-bound vitamin B_{12} in atrophic gastritis. Because of decreased or lack of gastric acid production, protein digestion is impaired, and vitamin B_{12} cannot be released from its protein carrier. Another potential factor in atrophic gastritis is that bacterial overgrowth in the upper gastrointestinal tract can bind vitamin B_{12} and make the vitamin unavailable. However, it has been shown that malabsorption of the protein-bound vitamin B_{12} in subjects with atrophic gastritis can be reversed by treatment with tetracycline; the absorption of crystalline vitamin B_{12} is not impaired in atrophic gastritis. Most elderly people can absorb vitamin B_{12} from fortified food. Vitamin B_{12} deficiency could be a major undetected problem in apparently healthy elderly people since the symptoms of deficiency are vague. There is a controversy over the lower limit of normal plasma levels of vitamin B_{12}, so elderly subjects with low plasma levels of vitamin B_{12} should be monitored more closely.

'Pernicious dementia' and other changes in cognitive function caused by a deficiency of vitamin B_{12} have been described in the literature. Patients with different forms of cognitive impairment may have impaired vitamin B_{12} status; however, this is mainly the result of low intakes. It has been reported that anemia in old age is more likely related to folate deficiency than to vitamin B_{12} deficiency. The new Dietary Reference Intakes (DRIs) for vitamin B_{12} have been increased compared to earlier recommendations because a large fraction of elderly people malabsorb this vitamin from food sources (i.e., protein-bound vitamin B_{12}); it is recommended that individuals older than 50 years consume foods fortified with B_{12} or take a B_{12} supplement.

Biotin

Biotin deficiency occurs rarely; the recommendation for the AI for biotin has been set at $30\ \mu g\ day^{-1}$ for all adults independent of age, but there are hardly any data about elderly adults. Since aging and excessive alcohol consumption can be linked to an alteration in biotin metabolism, it is likely that elderly individuals may be biotin-insufficient. Present evidence, although minimal, suggests that biotin status is not a major concern for older adults.

Vitamin C (ascorbic acid)

Vitamin C deficiency disease, scurvy, is rare but as new metabolic properties of vitamin C are described, an AI of vitamin C-rich foods remains a key for health maintenance, especially in elderly adults. There are no age-related changes in absorption and metabolism at median level intakes; it can be assumed that any deficiency is related to poor intake. Dietary vitamin C intakes vary mainly as a function of the presence of illness associated with overall poor nutrient intake, institutionalization, and age.

The intake of different dietary supplements, especially including vitamin C, has increased and contributes considerably to the nutrient intake of large segments of the elderly population. Institutionalization, hospitalization, and illness lead to a decrease of vitamin C intakes contributing to low plasma levels.

It has been reported that lower ascorbic acid levels are seen more frequently in elderly men than in elderly women; sex differences in plasma ascorbic acid concentration might be caused by a lower tubular reabsorption of vitamin C in elderly men. Healthy centenarians had significantly lower vitamin C plasma concentrations because of lower intakes.

Low levels of vitamin C in healthy elderly people can be corrected easily by the administration of oral ascorbic acid supplements, but when stopped, there may be a rapid decrease in the blood levels. Elderly people with low vitamin C levels do not necessarily show clinical symptoms, so it has been concluded that there is no real need to raise the ascorbic acid levels in the blood through supplementation. Because of the functions of vitamin C, this conclusion might be incorrect. In view of what is known or not yet known about the significance of low plasma levels, a continuous, adequate daily intake should be ensured.

Ascorbic acid can be important in the pathogenesis of chronic diseases of aging. Ascorbic acid has been found to be potentially important in the prevention of atherosclerosis, cancer, senile cataract, lung diseases, cognitive function, and various degenerative diseases. Vitamin C supplements are promoted to decrease lipid levels, thereby reducing the risk of atherosclerosis. However, a 5-year vitamin C supplementation trial had no markedly favorable effects on the serum lipid and lipoprotein profile in an adult population.

Vitamin C supplements do not prevent any of the chronic diseases of aging, but neither do pharmacological doses of vitamin C have a protective effect. The current recommendation for ascorbic acid seems to be adequate for older adults, although some individuals might find it difficult to achieve this intake level from diet alone. Nevertheless, intake should be strongly encouraged in elderly individuals who are institutionalized, ill, or of lower socioeconomic status. An increased consumption of fresh fruits and vegetables should be encouraged in elderly people.

Fat-Soluble Vitamins

Vitamin A

Meeting vitamin A needs by an increased ingestion of carotenoids might actually be a safe strategy for older adults. The vitamin A intake of many elderly people is below the present recommendations, but their vitamin A plasma levels remain well within normal limits. Vitamin A intake varies widely, and up to 70% of elderly people, depending on age, income, sex, race, geographic location, and socioeconomic status, have vitamin intakes below two-thirds of the 1989 RDA.

There is evidence that vitamin A status is generally not impaired in elderly people. In the Women's Health Study, vitamin A status was associated with an increased risk of hip fractures only in postmenopausal women with vitamin D deficiency. This is a well-known phenomenon and bone symptoms are a known feature of vitamin A toxicity. Nevertheless, it is probably wise not to encourage high vitamin A intakes in older adults, especially in the presence vitamin D deficiency. A more reasonable strategy for older adults is to get vitamin A from a higher intake of carotenoid-containing foods. An increased intake of carotenoids from fruits and vegetables was found to be associated with a decreased cardiovascular mortality in elderly patients.

High vitamin A supplement use by older adults might contribute to toxicity in elderly individuals. Acute hypervitaminosis A is rare, but chronic toxicity can be seen in this age group. Increased consumption of carotenoids from food and vegetables to improve vitamin A status should be encouraged in older adults.

Vitamin D

For men and women older than 70 years, the AI is 15 μg day^{-1} (600 IU). Skin synthesis is important compared to intake from food sources. Cutaneous synthesis of vitamin D ranges between 80% and 100% of the total vitamin D content of the body. It has been suggested that, with adequate sun exposure, dietary sources of the vitamin become unnecessary. Fortified foods, such as milk, contain considerable amounts of vitamin D, which would cover the needs of older adults. Some of these products are, however, not regularly consumed by elderly people or are consumed only in small quantities. Even a marine-based diet (e.g., salmon, sardines, and mackerel) seems to be insufficient for the maintenance of an adequate vitamin D status.

Data reported from the NHANES III survey indicate that older adults (71 years and older) report low intakes of vitamin D from food. Food fortification can lead to an improved vitamin D nutriture in elderly people.

Dietary vitamin D intake is usually not able to prevent vitamin D insufficiency especially during the winter months when people have low sun exposure. Plasma and serum levels of 25-hydroxyvitamin D_3(25[OH]D_3) decline with age in all populations. Along with low intake, decreased synthesis in the skin, decreased 1-α-hydroxylation in the kidney, decreased sunlight exposure, decreased plasma-binding capacity for vitamin D and vitamin D metabolites, and drug/nutrient interactions all contribute to inadequate vitamin D nutriture. Dietary fortification, enrichment of food, and ingestion of vitamin D supplements have a high potential for improving vitamin D status; the consequences are prevention of bone loss and decreased fracture risk.

Vitamin E

Age-related changes resulting from oxidative reactions are found in most organ systems; vitamin E and other antioxidants would seem to have a role to play in aging and the aging process. However, the consequences of a deficiency do not necessarily imply that pharmacological doses of antioxidant vitamins will have a protective effect on aging and related diseases. Nevertheless, all large supplementation trials have led to disappointing results regarding the role of vitamin E in the modulation of age-related diseases. Current evidence suggests that there is no impairment of vitamin E absorption with aging.

Because of the antioxidative properties of vitamin E, the effects of the vitamin in the prevention of atherosclerosis, cancer, cataracts, central nervous system disorders such as Alzheimer's and Parkinson's disease, immune function, impaired glucose tolerance, and many more conditions have been proposed. Despite all these potential relationships, the intake of high doses of supplements cannot be recommended.

Vitamin K

Data about vitamin K status in older adults are scarce. Poor vitamin K status has been associated with different chronic diseases involving calcification processes (e.g., osteoporosis and atherosclerosis). It is assumed that a dietary intake of 1 μg kg^{-1} of body weight per day should be sufficient to maintain normal blood clotting in adults. Recommendations are based on the maintenance and function of normal clotting mechanisms. In elderly people who have atrophic gastritis with a bacterial overgrowth, vitamin K status does not appear to be affected when assessed by measuring the resistance to warfarin therapy. Intake of vitamin K varies over a wide range, primarily due to diet composition. Studies indicate that up to one-third of elderly adults do not consume the recommended amount of phylloquinone.

Although the function of vitamin K is usually associated with blood coagulation, research conducted during the past few years identified vitamin K as an important factor in bone metabolism and thus in the development of osteoporosis. Combined treatment of vitamin K_2 and bisphosphonates in postmenopausal women with osteoporosis demonstrated that the combination treatment is more effective in the prevention of new vertebral fractures than a single treatment of bisphosphonate alone. A high dose of vitamin K might have positive effects on fracture risk. More studies are needed to clarify the

effect of vitamin K on bone health and at this time do not point to any specific recommendation regarding an ideal intake of vitamin K for bone-related issues.

Minerals

Sodium, potassium, calcium, and magnesium are considered essential minerals for humans. Older people may have challenges in maintaining levels of these minerals within normal limits because of decreases in renal function, by other renal mechanisms designed to conserve or excrete excessive amounts of these minerals, and certain medications or disease states. The DRIs and the *Dietary Guidelines for Americans* serve as guides for the intakes of these nutrients from foods and supplements (e.g., for calcium). It has been suggested that the dietary intakes of sodium are too high and the intakes of potassium, calcium, and magnesium are too low among older adults.

Sodium

Dietary sodium is available through many sources, particularly processed, preserved, and canned foods. It is actually a greater challenge to avoid dietary sodium for individuals on sodium restrictions.

Potassium

Age alone does not appear to affect the ability to maintain the concentration gradient of potassium. Intracellular potassium stores can be depleted in a variety of clinical conditions commonly seen in elderly patients (metabolic and respiratory acidosis, congestive heart failure, cirrhosis, and uremia) with serum potassium concentrations remaining within normal limits. Dietary potassium is found in a wide variety of foods, and there are rarely disturbances of potassium status due to dietary factors.

Calcium

Both hypocalcemia and hypercalcemia occur with more frequency in older adults because specific disease conditions that cause challenges to calcium balance are more common in elderly people. More importantly, the age-related loss of bone calcium occurs in both sexes, but more commonly in women, it is related to a variety of variables such as diet, hormone levels, bone density, and other factors. Nevertheless, this loss leads to the development of osteoporosis and an increased risk of fractures. Dietary factors play an important role in the age-related loss of bone calcium. In older individuals, calcium intake is generally low and is associated with reduced absorption of calcium. Lack of vitamin D (hypovitaminosis D) is often associated with increased age. Because vitamin D is the major regulator of intestinal calcium absorption, the cumulative effect of a calcium intake deficit and an inadequate intake and generation of active vitamin D is a negative calcium balance. This stimulates parathyroid hormone (PTH) release, increasing bone calcium resorption and risk for osteoporotic fractures, a major cause of morbidity and mortality in the elderly population.

Magnesium

Poor magnesium status has been associated with numerous age-related conditions. Gastrointestinal disorders and alcohol consumption are the most commonly encountered problems in older adults. Hypomagnesemia produces neuromuscular and psychiatric symptoms, including neuromuscular hyperirritability, tetany, seizures, muscle weakness, vertigo, gross tremors, and mental changes (e.g., irritability and aggressiveness). Linking these symptoms with hypomagnesemia may be elusive because serum levels will generally remain within normal limits.

Barriers to Maintaining Adequate Nutrition

Ingesting an adequate diet may be affected by a variety of other secondary factors associated with advancing age. Oral health status; swallowing difficulties; changes in vision, smell, and taste; socioeconomic factors; polypharmacy; drug/nutrient interactions; cognitive status (delirium, depression, and dementia); and functional status all constitute potential barriers to maintaining adequate nutritional status and the consumption of a nutrient-dense, nutritionally adequate diet.

For elderly adults who are living on fixed income such as social security or other retirement incomes, food can be a significant part of their living expenses. Living conditions, cooking facilities, transportation, and seasonal variations in the price of specific foods, for example, fresh fruits and vegetables, can have an impact on nutritional status.

Polypharmacy is a problem among many elders who are taking multiple medications. There are often drug/drug, drug/nutrient, and drug/herbal product interactions that may affect appetite, taste, the efficacy of medication treatments and may be the etiology of gastrointestinal disturbances. A review of the prescription, over-the-counter, and herbal remedies is warranted on an annual basis to minimize the effect that drugs may have on the nutritional status of an elderly person.

Cognition may have a profound impact on dietary intake; dementia, delirium, and depression will affect appetite, the perception of food, activity level, and the ability to feed oneself. Activity level, and need for energy calories, may be quite impacted by dementia, with some individuals becoming hyperactive and others becoming very sedentary.

Depression may have a longer and more profound effect on nutritional status than dementia. Depression has an impact on appetite, weight loss, and poor oral intake. It may be associated with a broad range of problems associated with chronic disease, medications, grief, loneliness, and others.

Conclusion

Encouraging older adults to eat more complex carbohydrates rather than simple carbohydrates can contribute to adequate dietary fiber intake. Reduction of saturated fat, with an increased emphasis on consumption of poly- and monounsaturated fats, and decreased overall fat intake compensate for lower basal energy requirements and a decreased activity level often encountered in older adults. Providing adequate protein

of high biological value is essential in older adults as is meeting fluid needs. Attention to overall fluid intake in healthy free-living, chronically ill, functionally dependent, or acutely ill elderly people is key to maintaining optimal health in any person, but particularly important for this age group. Needs for these macronutrients (carbohydrate, fat, protein, and fluid) can be met with awareness of individual requirements, imagination, and attention to daily intake.

Not all micronutrients are affected by aging although chronic disease may be a factor in the adequacy of intake, digestion, absorption, or metabolism of various vitamins and minerals. The water-soluble vitamins (B complex and vitamin C) require attention because they cannot be stored and are involved in many metabolic pathways that affect overall health and nutritional status. Among the fat-soluble vitamins, vitamins A and D require some attention. Vitamin D has been an issue in recent years due to indications that many adults have low levels of dietary vitamin D, particularly older adults.

Barriers to adequate consumption of a nutritious diet including, but not limited to, cognitive status, oral health, deficits in sensory perception and functional status, socioeconomic factors, swallowing disorders, polypharmacy, and other changes that occur with age must all be assessed. Screening older adults for potential risks for nutrition problems is a highly recommended, cost-effective, and preventive strategy.

The specific recommendations concerning nutrient intakes and food-based dietary guidelines for older adults – with particular emphasis on guidelines for promoting physical activity among older and prevention of chronic noncommunicable diseases – can contribute to healthy aging.

See also: Antibiotics and Drugs: Drug–Nutrient Interactions; Beverage: Health Effects; Bioavailability of Nutrients; Dietary References: US; Energy: Intake and Energy Requirements; Malnutrition: Concept, Classification and Magnitude; Malnutrition: Prevention and Management; Osteoporosis; Protein: Requirements; Single Cell Proteins; Trace Minerals and Trace Elements.

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Emerging Foodborne Enteric Bacterial Pathogens

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Introduction

Compared to clinical infectious diseases, the number of currently recognized foodborne pathogens seems small. In part, this is due to general procedures of food production, for example, cooking, acidification, and dehydration, which over the centuries and in different cultures have been refined and become a self-proved effective means of food preparation. However, with changes in the sourcing of food ingredients, the need for large-scale food preparation practices and longer storage periods along with changes in eating habits has ensured a continuation of food-related illnesses. Such infections are an important cause of morbidity and mortality worldwide and have a major impact on public health. The true worldwide burden of diseases caused by foodborne pathogens is largely unknown. At best, we have data indicating trends in infections for a few pathogens in a few number of developed countries.

The European Food Safety Authority has defined 'emerging risk to human, animal and/or plant health' as a risk resulting from a newly identified hazard to which a significant exposure may occur or from an unexpected new or increased significant exposure and/or susceptibility to a known hazard. Hence, in order to detect such risks, adequate surveillance and monitoring programs need to be in place. There are many food safety monitoring systems at national and international levels. These are summarized in Table 1. Examples are the 'Rapid Alert System for Food and Feed' (RASFF) in Europe and WHO–INFOSAN. In general, these systems identify problems such as the increased reported occurrence of a foodborne hazard retrospectively. Consequently, any intervention is initially reactive instead of predictive.

In order to determine the global health burden of foodborne illness, the WHO set up the Foodborne Disease Burden Epidemiology Reference Group. This is composed of internationally renowned experts in a broad range of disciplines relevant to global foodborne disease epidemiology; http://www.who.int/foodsafety/foodborne_disease/ferg/en/index3.html. They have estimated that in 2005, 1.8 million people died from diarrheal disease, and these were mainly attributable to contaminated food and water sources. In the United States, foodborne illnesses affect up to 80 million people and cost an estimated US$5 billion on an annual basis. Additionally, our understanding of such infections is changing with the recognition of previously unrecognized and previously well-recognized pathogens, which have increased in their prevalence or become associated with new food vehicles.

The major organisms associated with foodborne disease are bacteria due to their ease of isolation and identification compared to viruses and fungi. However, with improved global surveillance systems, as well as improved isolation and identification methods, previously unrecognized foodborne infectious organisms are being recognized. With a global economy and the international transportation of food, the need to attribute human infections to food vehicles is warranted for their control.

The study of emerging foodborne enteric pathogens is both exciting and daunting at the same time. The opportunity to recognize and define an emergent pathogen leading to its control and reduced risk is an admirable goal, yet to publicize the emergence of an enteric pathogen based on limited information and initiate action could be regarded as scaremongering and alarmist. Given the limited number of organisms, which are routinely monitored in food, it may take considerable time before there is sufficient body of evidence of its significant presence such that it might be argued that by the time the link between the clinical presentations and food as a vector has been established, the organism will no longer be 'emerging' and therefore outside the scope of the study. The word 'emerging' implies the emergence *de novo* of the pathogen, yet for some, the term 'recently recognized' is more appropriate as the organism had been previously described, but the link with foodborne disease may be new. Some organisms in this article are 'emergent' or 'recently recognized' pathogens for which food has been established as a vector, but some will not be so strongly associated but could be foodborne. It may be argued that the latter should not be included – however, this begs the question of how will we ever know unless we look. Our powers of microbial epidemiology are still mainly limited to bacteria as we are still largely culture-dependent. In time, next-generation sequencing (NGS) will enable real-time genomic analysis of infections, but for now, our future predictions remain misty and unclear.

Illness caused by foodborne pathogens represents an important economic and public health burden worldwide. Based on a yearly case estimate and recent US census data, an estimated 25% of the US population is affected by foodborne illness each year. Viral foodborne pathogens are thought to contribute to ~50% of all foodborne illness cases in the United States. Rates of foodborne illness in many developed countries are similar. While exact numbers are difficult to estimate, it is thought that the burden of foodborne illness in developing countries is even higher.

Since 1996 in the United States, FoodNet has been an active surveillance program for laboratory-confirmed infections due to seven commonly reported bacterial foodborne pathogens: *Salmonella*, shiga toxin-producing *Escherichia coli* O157, *Shigella* spp., *Vibrio* spp., *Yersinia* spp., *Campylobacter* spp., and *Listeria monocytogenes*. This surveillance covers 15% of the US population. Over the period 1996–2005, there had been 121 536 cases including 552 deaths, for which 215 and 168 were due to were among persons infected with *Salmonella* and *Listeria*, respectively. Useful as this surveillance is, it does not cover the myriad of foodborne pathogens and toxigenic agents.

In the United States, there are an estimated 9.4 million cases of foodborne illness, which can be attributed to 31 known

Table 1 Early identification systems for emerging foodborne hazards

Country	*Organization*	*Activity*
The United Kingdom	FSA	Food Standards Agency (FSA)
The Netherlands	ISIS	Day-to-day changes in frequency of all communicable diseases
European	European Centre for Disease Prevention and Control (ECDC)	Provides a structured and systematic approach to the control of communicable diseases and other serious health threats in partnership with national health protection agencies. Issues warnings to EU member states through the early warning and response system (EWRS)
	Rapid alert system on food and feed (RASFF)	Centralized reporting of food hazards (physical, chemical, biological)
	EU: FVO	Food and Veterinary Office (FVO) promotes effective control systems in food safety and checks on compliance
The United States	CDC: FoodNet	Determines the exact burden of foodborne diseases, monitors foodborne disease trends in the United States, and relates foodborne disease to specific foods
	CDC: eFORS	Electronic Foodborne Outbreak Reporting System
	CDC: PulseNet	National network of public health laboratories that undertake pulsed-field gel electrophoresis analysis of bacteria that may be foodborne
International	WHO: GPHIN	Global Public Health Intelligence Network
	WHO: INFOSAN	International Network of Food Safety Authorities Network disseminates urgent information
	OIE	Office International des Epizooties (OIE) sets standards for sanitary practices in the diagnosis of diseases in animals and animal products. This includes the World Animal Health Information System (WAHIS)

After Marvin, H. J. P., Kleter, G. A., Prandini, A., Dekkers, S., and Bolton, D. J. (2009) Early identification systems for emerging foodborne hazards. *Food and Chemical Toxicology* **47**, 915–926.

pathogens (2011 data). Of these, 14 pathogens account for 95% of the illnesses and hospitalizations and 98% of deaths. The associated costs are estimated as $14.0 billion (ranging from $4.4 to $33.0 billion) of which 90% are attributable to five pathogens: nontyphoidal *Salmonella enteric*, *Campylobacter* spp., *L. monocytogenes*, *Toxoplasma gondii*, and norovirus. However, there were an additional estimated 38.4 million cases (80%), which were due to 'unspecified agents, including known agents with insufficient data to estimate agent-specific illness, known agents not yet recognized as causing foodborne illness, substances known to be in food but of unproven pathogenicity, and unknown agents.' These unattributed cases resulted in ~71878 hospitalizations and 1686 deaths. Although this author is not claiming that all these cases are due to emerging foodborne pathogens, the article illustrates that there is still scope for previously unrecognized pathogens to be discovered. Toxin-induced gastroenteritis due to *Bacillus cereus* is a well-recognized foodborne pathogen, yet the lack diagnostic tools means its incidence is highly likely to be underestimated and not an emergent pathogen as such.

The microbiological safety of food is dependent upon multiple variable 'from farm to fork' along the food chain. The combined advances in the Internet and culture-independent (i.e., DNA sequencing) methodologies are enabling improvements in monitoring and surveillance, though the scenario is constantly changing with increased global transportation of foods and food ingredients, aging populations, and changes in eating habits. Against this background, this article reviews the current situation regarding emergent enteric foodborne pathogens. In particular, reference will be made to previously unrecognized vehicles, for example, fresh produce, and serious issues to public health following the acquisition of antimicrobial resistance. Additionally, previously unknown foodborne pathogens, many of which are zoonotic, are being recognized through improved methods of surveillance and identification.

This article will not consider well-recognized foodborne pathogens: *Campylobacter jejuni*, *Salmonella enterica* serovars, *E. coli* O157 and O104, *Vibrio cholerae*, *V. parahaemolyticus*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Clostridium perfringens*, *Staphylococcus aureus*, and *Bacillus cereus*. For information on these organisms, please consult the specific chapters as listed at the end under 'See also.' Such organisms are often formally controlled via microbiological specifications whether in international policies or producer–distributor agreements. Essentially, improvements in the microbiological safety of foods have been largely driven by public demand in response to disease outbreaks, and consequently, less-recognized pathogens are overlooked. Focussing on a particular potential source of infection will invariably lead to discoveries, as evidenced by the emphasis of bats as vehicles of bacterial disease, which has opened a whole field of previously unrecognized bacterial and virus route of transmissions. Awareness and surveillance of viral foodborne pathogens are generally poor and primarily limited to norovirus, hepatitis A, and rotaviruses. Although many foodborne parasites are recognized, only *Ascaris*, *Cryptosporidia*, and *Trichinella* are effectively monitored in foods. Their epidemiology of such parasites through the food chain is poorly understood. The issues of viral and parasitic foodborne pathogens are not considered in this article due to the lack of information.

Bacterial Enteric Pathogens

Aeromonas Species

Description: *Aeromonas* genus, composed of 17 hybridization groups and 14 phenospecies
Phenotypic differentiated groups: *A. hydrophila*, *A. caviae*, and *A. sobria*
Clinical presentations: Traveler's diarrhea
Sources: Water, fish, shellfish, meats, dairy products, and fresh vegetables
Detection methods: Various culture media, for example, starch ampicillin agar (SAA) and BIBG

The genus *Aeromonas* was first proposed in 1936 for rod-shaped Enterobacteriaceae, which possessed a polar flagellum. Since then, there have been many taxonomic revisions, and currently, it forms the family Aeromonadaceae. They are Gram-negative, rod-shaped, facultative anaerobic, nonspore-forming bacteria, which are widely distributed in the soil and aquatic environments. Members of the genus are divided into three phenotypically differentiated groups: *Aeromonas hydrophila*, *Aeromonas caviae*, and *Aeromonas sobria*. Currently, the genus is composed of 17 DNA hybridization groups or genomospecies and 14 phenospecies.

The *Aeromonas* genus includes psychrophiles and mesophiles and is associated with various diseases of warm- and cold-blooded animals. They have been isolated from fish, shellfish, meats, dairy products, and fresh vegetables. However, only few foodborne outbreaks have been documented. Some studies have shown that some motile *Aeromonas* species are becoming food- and waterborne pathogens of increasing importance. They have been associated with several foodborne outbreaks and are progressively being isolated from patients with traveler's diarrhea.

A. hydrophilia is present in all freshwater environments and in brackish water. It has frequently been found in fish and shellfish and in market samples of red meats (beef, pork, and lamb) and poultry. The organism is able to grow slowly at 0 °C. It is presumed that given the ubiquity of the organisms, not all strains are pathogenic. Some strains of *A. hydrophila* are capable of causing illness in fish and amphibians as well as in humans who may acquire infections through open wounds or by ingestion of a sufficient number of the organisms in food or water. *A. hydrophila* may cause gastroenteritis in healthy individuals or septicemia in individuals with impaired immune systems or various malignancies. *A. caviae* and *A. sobria* also may cause enteritis in anyone or septicemia in immunocompromised persons or those with malignancies.

There is controversy as to whether *A. hydrophila* is a cause of human gastroenteritis. The uncertainty occurs because volunteer human feeding studies (10^{11} cell dose) have failed to demonstrate any associated human illness. However, its presence in the stools of individuals with diarrhea, in the absence of other known enteric pathogens, suggests that it has some role in disease and has been included in this survey of foodborne pathogens. Similarly, *A. caviae* and *A. sobria* are putative pathogens associated with diarrheal disease, but as of yet, they are unproved causative agents. General symptoms of *A. hydrophilia* gastroenteritis are diarrhea, abdominal pain, nausea, chills and headache, dysentery-like illness, and colitis. Additional symptoms may include septicemia, meningitis, endocarditis, and corneal ulcers.

Two types of gastroenteritis have been associated with *A. hydrophila*: a cholera-like illness with severe diarrhea and a dysenteric illness characterized by loose stools containing blood and mucus. The infectious dose of this organism is unknown. *A. hydrophila* may spread throughout the body in the bloodstream and cause a general infection in persons with impaired immune systems. Those at risk are individuals suffering from leukemia, carcinoma, and cirrhosis and those treated with immunosuppressive drugs or who are undergoing cancer chemotherapy.

A. hydrophila produces a cytotonic enterotoxin, hemolysin, acetyl transferase, and phospholipase. Together, these virulence factors may account for the organism's pathogenicity. The detection of genes responsible for *Aeromonas* virulence is a vital tool in establishing the potential pathogenicity of the bacteria, as these virulence genes may act alone or in synergy in the establishment of infections.

A number of selective and differential isolation media have been developed for the recovery of *Aeromonas* species from the environment, foods, and clinical specimens. Since comparative studies demonstrate that no single medium results in the optimal recovery of aeromonads, combinations of different isolation media and methods are used including direct plating, membrane filtration, and multiple tube tests for determining most probable numbers. SAA and bile salts–inositol–brilliant green (BIBG) agar with initial enrichment in alkaline peptone water (APW) or tryptose broth containing ampicillin (TSB-30, ampicillin 30 mg l^{-1}) are recommended simultaneously with commercially available media such as *Aeromonas* medium (Ryan's medium). Starch glutamate ampicillin penicillin (SGAP-10) medium has been used to detect aeromonads from foods. The isolation of aeromonads from contaminated samples such as feces requires the use of selective and differential media such as MacConkey agar, cefsulodin–irgasan–novobiocin (CIN) agar, and blood agar with ampicillin (10 mg l^{-1} ampicillin). To facilitate recovery of aeromonads from highly contaminated samples such as feces, enrichment broths such as APW are incubated overnight and subcultured onto CIN agar and blood agar with ampicillin. Culture plates are then incubated aerobically at 35 °C for 24–48 h. *Aeromonas* species produce distinctive colonies, with or without hemolysis on blood agar. Colonies are screened by carrying out oxidase test and identified using biochemical methods or commercially available bacterial identification kits.

Arcobacter Species

Description: *Arcobacter butzleri*, *A. cryaerophilus*, and *A. skirrowii* are the primary species of interest.
Clinical presentations: Persistent and watery diarrhea.
Sources: Contaminated drinking water and food.
Detection methods: mCCDA and CAT.

Campylobacter jejuni and *C. coli* are well recognized as the major causes of human gastroenteritis. Meanwhile, a number of other *Campylobacter* species and related organisms have also been recognized as causing animal if not human cases of gastroenteritis. These include *Campylobacter concisus* and *Arcobacter* species. While there are a relatively few reported cases of infection

due to *Arcobacter*, this is probably due in part to the lack of routine use of appropriate isolation media.

A number of reviews on *Arcobacter* spp. have demonstrated that the organism is a cause of human pathogen and is associated with food products. One of the first significant studies of *Arcobacter* started in 1995 in Belgium where the WHO Centre for *Campylobacter* specifically looked for non-*jejuni*/*Campylobacter coli*-like organisms (CLOs) in fecal samples. It is plausible that *A. butzleri* and *A. cryaerophilus*, which were first isolated from aborted bovine fetuses and later from porcine fetuses, will in the future be more fully recognized as of significant human importance. Currently, there is increasing awareness of their role as veterinary pathogens, but there are relatively few human cases.

The *Arcobacter* genus was formerly known as aerotolerant campylobacters and CLOs. But improved methods of taxonomic evaluation have led to the recognition of the genus *Arcobacter*. There are currently 17 recognized *Arcobacter* species, and these have been isolated from various sources. Of particular interest are *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*, as these have been associated with human cases of diarrhea, the probable transmission routes being through the ingestion of contaminated drinking water and food. These three species are also veterinary pathogens causing porcine abortions. *A. butzleri* serotypes 1 and 5 are regarded as the primary human pathogens; however, no epidemiological studies have yet shown the transmission of the organism through the food chain to humans. The situation is, however, reminiscent of *C. jejuni* and *L. monocytogenes*. These organisms were recognized as veterinary pathogens many years before the medical microbiologists used suitable isolation media to samples from patients suffering from gastroenteritis. *Arcobacter* species have been isolated from a range of food sources (**Table 2**) using a range of methods, some of which may favor certain species more than others. Water may also be a vector of *Arcobacter* transmission to animals and humans.

In general, *Arcobacter* spp. are more aerotolerant and have lower growth temperature limit than *Campylobacter* spp. On blood-based agars, *Arcobacter* spp. produce round, off-white or grayish colonies, 2–4 mm in diameter after 3 days of incubation. The colonies are generally small, nonpigmented, and convex with entire edges. Swarming has been reported on fresh agar. Brain Heart Infusion Agar supplemented with 0.6% (w/v) yeast extract and 10% (w/v) blood agar has been used recently for routine culturing.

Table 2 Incidence of *Arcobacter* in animals and animal products

Source	*%*	*n*	*Site*
Pigs	0–89.9	299[a]	Ground pork
	7	100	Pork
Cows	1.5	68	Minced beef
	2.2	90	Beef
Poultry	96.8	125	Chicken carcasses
	24.1	220	Turkey meat
Ducks	50	10	Carcasses
	52	170	Carcasses
Water	100	10(4)[b]	

[a]Only tested for *A. butzleri*.

[b]All samples were positive using FISH, whereas *Arcobacter* was only isolated from four samples.

Adapted from Forsythe, S. J. (2006). *Arcobacter*. In: Motarjemi, Y. and Adams, M. (eds.), *Emerging foodborne pathogens*. Cambridge: Woodhead Publishing, references therein.

Arcobacter isolates can be presumptively identified by their shape (small comma-shaped or spiral rods) and motility (darting or corkscrew motility). They can be easily distinguished from *Campylobacter* and related genera by their ability to grow in air at 25 °C. The main phenotypic traits used for *Arcobacter* species differentiation are catalase activity, nitrate reduction, cadmium chloride susceptibility, microaerophilic growth at 20 °C, and growth on MacConkey agar and in the presence of 3.5% NaCl and 1% glycine. Organic acids and amino acids are used as carbon sources. Hydrogen is not required for growth. All *Arcobacter* isolates hydrolyze indoxyl acetate. A simple diagnostic characteristic useful for the presumptive identification of *Campylobacter jejuni*, *C. coli*, and *Arcobacter* spp. is the cadmium chloride test.

Arcobacters appear resistant to antimicrobial agents typically used in the treatment of diarrheal illness caused by *Campylobacter* spp., for example, erythromycin, other macrolide antibiotics, tetracycline, and chloramphenicol. Isolation of arcobacters requires selective media such as mCCDA and CAT. Identification can subsequently be achieved using 16S rRNA probes and genotyped.

The occurrence of arcobacter-related diseases may be underestimated due to the lack of surveillance and optimized detection procedures. Examination of human and veterinary clinical specimens for the presence of *Arcobacter* species is rarely performed, and in most cases, suboptimal procedures are used. In addition, little is known about the risk factors for human infection. The most extensive study to date on this matter was undertaken on a total of 67 599 stool samples over an 8-year period. *A. butzleri* was the fourth most common CLO isolated. It was more frequently associated with symptoms of a persistent and watery diarrhea than *C. jejuni*.

The isolation of *A. butzleri* was associated with cases of persistent and watery diarrhea and less associated with bloody diarrhea compared to *C. jejuni*. The pathogenicity and virulence mechanisms of *Arcobacter* spp. are still poorly understood and have only been studied in *A. butzleri*, *A. cryaerophilus*, *A. skirrowii*, and *A. cibarius*. Analysis of the *A. butzleri* strain RM 4018 genome shows the presence of several putative virulence genes in the organism, such as *ciaB*, *cj1349*, and *cadF*. These are homologous to genes associated with pathogenicity in other closely related organisms. The *ciaB* gene encodes an invasion protein injected directly into the cytoplasm of the host cells through a secretion system. The *cj1349* gene encodes for proteins that enable adhesion to host cells by binding specifically to fibronectin, and the CadF protein also induces the internalization of bacterial cells by the activation of GTPases.

Campylobacter concisus

Description: *Campylobacter concisus*, a fastidious *Campylobacter* species

Clinical presentations: Isolated from healthy and diarrheal individuals

Sources: Contaminated food and water

Detection method: No specific method available to date

Despite considerable efforts in surveillance and improvements in isolation methods, only about half of the reported intestinal infectious cases are attributable to a named organism. While a large number of cases are related to *C. jejuni* and *C. coli* infections, there is evidence that other *Campylobacter* species may also be causative agents. Epidemiological evidence is poor, but it is reasonable to assume that more fastidious *Campylobacter* species, such as *C. concisus*, will not be recovered as efficiently as the better understood *C. jejuni*. In common with other campylobacters, *C. concisus* is a Gram-negative, curved, microaerophilic bacterium. It can be isolated from the human oral cavity and fecal samples from healthy and diarrheic patients. As a consequence, its primary pathogenic potential is uncertain. In many diarrheal cases, *C. concisus* is isolated as the only potential intestinal pathogen, and hence, the need to determine its role in human enteric disease is evident.

Studies to date suggest *C. concisus* is composed of various genotypes, which may vary in their pathogenic potential. However, considerable further work is needed to determine the risk of *C. concisus* to human health and vehicles of infection. Given the habitat of the organism as being in the intestinal tract of healthy individuals, it is plausible that contaminated food and water could be the sources of infection. The organism has been included here to raise awareness of this emerging pathogen and its possible contribution to foodborne infections.

Cronobacter spp., Formerly *Enterobacter sakazakii*

Description: *Cronobacter* genus. Seven recognized species proposed: *C. condimenti*, *C. dublinensis*, *C. malonaticus*, *C. muytjensii*, *C. sakazakii*, *C. turicensis* and *C. universalis*. Gram-negative, frequently motile, facultative aerobic rods; members of the Enterobacteriaceae.

Clinical presentations: Infections in all age groups, particularly neonates, infants <6 months of age, and the elderly. Life-threatening symptoms in neonates and infants <6 months of age include meningitis, necrotizing enterocolitis, and respiratory infections.

Sources: Infections in neonates associated with contaminated reconstituted powdered infant formula (PIF). Ubiquitous in nature, particularly plant material. Human carriage.

Detection methods: Cultivation on chromogenic agar, followed by sequencing of *fusA* or *rpoB* gene for phylogenetic analysis.

Cronobacter is possibly the archetypal recently recognized bacterial pathogen, which has been associated with foodborne infection, albeit via one particular food source: PIF. The considerable progress in better understanding and control of this organism is undoubtedly due to policy issues. Using seven-loci multilocus sequence analysis, the close relatedness of the *Cronobacter* species is shown in Figure 1. According to phylogenetic analysis, these major divisions formed about 41 million years ago, and further, host adaptation has occurred as will be considered later with the specific case of *C. sakazakii* sequence type 4.

Short timeline: Pre-2002, various outbreaks in neonatal intensive care units and sporadic cases reported.

2002 Outbreak at the University of Tennessee investigated by CDC and FDA. Fatal case linked to the use of powdered formula
FDA *Cronobacter* (and then *E. sakazakii*) detection method; Bacteriological Analytical Manual

2004 1st FAO–WHO risk assessment meeting on microbiological safety of PIF
Development of first chromogenic differential agar

2006 2nd FAO–WHO risk assessment meeting
ISO/TS 22964 method released

2008 3rd FAO–WHO risk assessment meeting
Revised Codex Alimentarius Commission guidelines for microbiological specifications for PIF released, to include *Cronobacter* as a named pathogen
Cronobacter genus defined, composed of 5 species

2009 Open-access seven-loci multilocus sequence typing (MLST) scheme established to replace ambiguous 16S rDNA analysis; www.pubMLST.org/cronobacter

2010 First whole sequence for *C. sakazakii* published; isolate from the University of Tennessee outbreak

2011 *C. sakazakii* sequence type 4 (clonal lineage) association with neonatal meningitis determined
C. condimenti and *C. universalis* recognized

2012 First pan-genome analysis of *Cronobacter* genus based on 14 genomes
Revised FDA method of detection; Bacteriological Analytical Manual

2013 www.pubMLST.org/cronobacter expanded to include the following:
(a) Tax-MLST established, extension of MLST to include genotyping of *ompA* and *rpoB*
(b) *Cronobacter* BIGSdb searchable repository for all published *Cronobacter* sp. genomes established

Before 1980, yellow-pigmented *Enterobacter cloacae*-like strains were called *Enterobacter sakazakii*. Despite earlier sporadic cases of neonatal and infant infections, the organism was highlighted following an outbreak in a university hospital in Tennessee (the United States). One neonate died from meningitis, two died from suspected infections (tracheal aspirate-positive), and seven were colonized (six fecal-positive and one urine-positive) with the same strain according to pulsed-field gel electrophoresis (PFCE) profiling. The source was attributed to powdered formula. Since such neonates only have a limited number of exposure routes, the FAO–WHO instigated three risk assessments that have directly contributed to the 2008 Codex Alimentarius Commission revisions in the microbiological specifications of PIF. Prior to this, the number of Enterobacteriaceae (except for *Salmonella*) permitted in PIF was <100 cfu g^{-1}. These specifications had not been revised since 1979. However, following the concern of the serious implications following *Cronobacter* infections of neonates, the microbiological criteria changed such that *Cronobacter* should not be detectable in 10 g test volume quantities of PIF.

In order to implement the specifications, improved understanding of the organism and development of detection methods are needed. The focussed attention showed *Enterobacter sakazakii* should be regarded as a separate genus and was

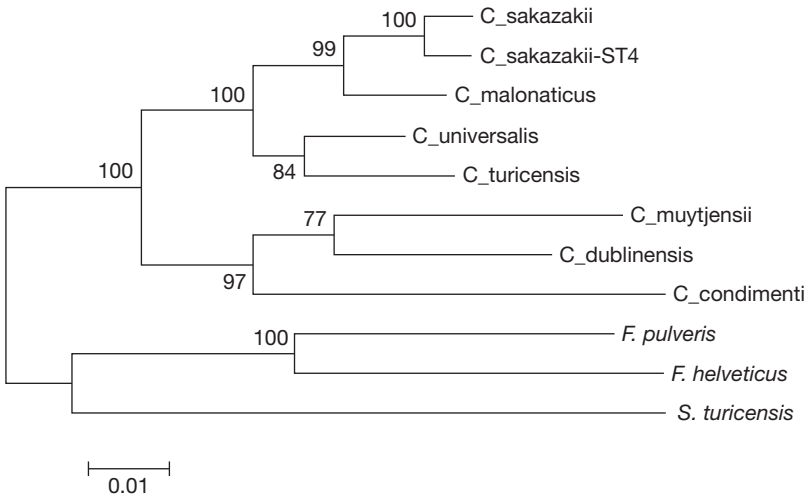

Figure 1 Maximum likelihood tree of the seven-loci multilocus sequence typing (3036 base pair concatenated length) for the members of Cronobacter, Franconibacter, and Siccibacter genus, showing the sequence type for each species type strain and the neonatal meningitis-associated sequence type 4. The tree was drawn using MEGA5.2 (http://www.megasoftware.net) with 1000 bootstrap replicates. Adapted from Holy, O. and Forsythe, S. J. (2014). Cronobacter species as emerging causes of healthcare-associated infection. *Journal of Hospital Infections* **86**,169–177.

Table 3 Isolation of *Cronobacter* spp. from throat swabs of outpatients according to age groups

	Patient age (years)								
Year	*<1*	*1–4*	*5–9*	*10–14*	*15–44*	*45–64*	*65–74*	*>75*	*Total*
2005	1	5	1	1(1)	2	0	2	0	12(1)
2006	(1)[a]	1(2)	0	5	2	0	(3)	(2)	8(8)
2007	2	9	0	2	2(1)	(1)	(1)	(1)	15(4)
2008	0	1(1)	2	2(1)	0	0	(2)	(1)	5(5)
2009	(2)	4	1	2	1	(2)	1	(1)	9(5)
2010	0	5	2(1)	0	(2)	1(1)	(2)	2	10(6)
2011	(1)	0	2	0	0	0	0	0	2(1)
Total number of *Cronobacter* isolates	3(4)	25(3)	8(1)	12(2)	7(3)	1(4)	3(8)	2(5)	61(30)
% of isolates/age group	4.9	41	13.1	19.7	11.5	1.6	4.9	3.3	100.0
Number of patients sampled[b]	808	3404	2651	1867	18223	10744	3888	3538	45123
Incidence of *Cronobacter* isolates/1000 patients sampled	8.7	8.2	3.4	7.5	0.5	0.5	2.8	2.0	2.0

[a]Numbers in parentheses indicate additional isolates from normally sterile sites, sampled according to the clinical presentation of the patient.
[b]Total number during the period 2005–2011.
Reproduced from Holy and Forsythe (2014).

named *Cronobacter*; in 2013, there were seven recognized species. Only four of which are associated with human infections: *C. sakazakii*, *C. malonaticus*, *C. turicensis*, and *C. universalis*. It is highly probable that the number of infections has probably been unreported due to misidentification. The species can be grouped, with the mostly clinically relevant being group 1 (*C. sakazakii* and *C. malonaticus*, which form the majority of clinical isolates in all age groups) and group 2 (*C. turicensis* and *C. universalis*, which have been less frequently reported). The other species are primarily environmental commensals and are probably of little clinical significance. Table 3 shows an age profile of *Cronobacter* isolated using throat swabs from over 45 000 outpatients during the period 2005–2011. The organism was isolated from every age group, with a higher frequency from children < 14 years of age.

In 2011, Joseph and Forsythe announced the strong association between neonatal meningitis cases and the clonal lineage of *C. sakazakii* ST4. This was established using retrospective study of 41 clinical strains from 1953 to 2008, collected from seven countries. This association was confirmed later in the year by the analysis of a number of highly publicized cases in the United States. The reason for the association of one sequence type out of >200 STs in the genus is unclear as no particular virulence traits have been determined in *C. sakazakii* ST4. However, it is known that this sequence type is frequently (~24% of strains) isolated from the infant formula and milk powder manufacturing plants in Australia, Germany, Switzerland, and Ireland and therefore may represent a particularly persistent clonal variant, resulting in increased neonatal exposure.

Cronobacter can invade human intestinal cells, replicate in macrophages, and invade the blood–brain barrier. The route of infection is probably through attachment and invasion of the intestinal cells. Whole genome sequencing has revealed a large

number of plausible virulence factors, though many require further laboratory studies for confirmation. These are summarized in the succeeding text, and further details can be obtained from the original publications. Due to the increasing number of *Cronobacter* genomes being sequenced, for up-to-date information, the reader should consult the *Cronobacter* BIGSdb, which is a searchable (BLAST) repository of all published *Cronobacter* genomes (see 'Relevant Websites').

A number of fimbria clusters have been identified in the genomes of *Cronobacter* species. Many fimbria clusters are common to all species, though there are two interesting exceptions. *C. sakazakii* is the only *Cronobacter* species encoding for β-fimbriae, whereas the genomes of the other species encode for curli fimbriae. This may reflect evolution to the host ecosystem. A number of iron assimilation mechanisms have been found in *Cronobacter* species, which might enable the organism to utilize iron from breast milk and infant formula. Five putative type VI secretion system clusters have been identified *Cronobacter* sp. genomes. These may be involved in adherence, cytotoxicity, host-cell invasion, growth inside macrophages, and survival within the host. It has been proposed that the outer membrane proteins ompA and ompX have roles in *Cronobacter* penetrating the blood–brain barrier. The mechanism (s) leading to the destruction of the brain cells is unknown and could in part be a host response. The organism also encodes for a number of hemolysins.

C. sakazakii is unique in the *Cronobacter* genus in its utilization of exogenous sialic acid, and this may have clinical significance. The ability to utilize sialic acid could be a major evolutionary host adaptation since the compound is found in breast milk, mucin, and gangliosides. Sialic acid is also an ingredient in PIF due to its association with brain development. *C. sakazakii* is also able to grow on the ganglioside GM1 as a sole carbon source. Levels of endotoxin due to the presence of heat-stable lipopolysaccharide (LPS) have been measured in infant formula. In rat pups, LPS enhances the translocation of *Cronobacter* across both the intestines and the blood–brain barrier and therefore indicates that its presence in infant formula could increase the risk of bacteremia in neonates. It has been speculated that frequent LPS contamination of PIF (known to disrupt tight junctions) might contribute to the invasiveness of *Cronobacter* across the blood–brain barrier. The organism also has a number of heavy metal resistance factors and biofilm formation, which might enable it to resist disinfectants in food production environments.

Due to the high profile and changes in microbiological specifications for PIF for target age <6 months, a number of isolation and identification methods were developed post-2002.

Isolation of the organism from PIF largely resembles that for *Salmonella*, with the use of stages for preenrichment, enrichment, presumptive isolation, and confirmatory tests. It should be noted that the FDA and ISO methods for the detection of *Cronobacter* are not up to date with respect to the taxonomy of the genus and not all species will necessarily be recovered by these techniques. In fact, a well-known phenotyping kit manufacturer still retains the name *Enterobacter sakazakii* instead of *Cronobacter* spp. in its online database. In addition, *E. helveticus*, *E. pulveris*, and *E. turicensis* have been used as negative control cultures as they were regarded as close relatives. In 2013, they were reclassified as Cronobacter and therefore should have been positively detected, however in 2014 there were removed from the genus and formed the new genera Franconibacter and Siccibacter (Figure 1).

It should be noted that the emphasis of controlling *Cronobacter* spp. in PIF has given the false impression that this is the only route of infection. In fact, the *C. malonaticus* type strain was isolated from breast abscess, there is human carriage and the organism is recovered from a wide range of foods (Table 4), and the organism has been isolated from the nasogastric feeding tubes of neonates not receiving reconstituted infant formula.

Enteroaggregative *Escherichia coli*

Description: The enteroaggregative *Escherichia coli* (EAEC) are a pathotype within the *E. coli* species. Gram-negative, facultative rods. The genus is a member of the Enterobacteriaceae family.

Source: Contaminated food.

Clinical presentations: Watery diarrhea with or without blood and mucus, abdominal pain, nausea, vomiting, and low-grade fever can be persistent.

Detection methods: No specific isolation procedures are available.

Table 4 Survey of dry food products and food ingredients from which Cronobacter spp. have been isolated

Food product or ingredient	*Number of positive samples*	*Total number of samples*	*%*
Powdered infant formula	2	82	2
Follow-up formula	3	91	3
Dry infant food	24	199	12
Milk powder	3	72	4
Milk powder and derived products	1	55	2
Starches	40	1389	3
Corn, soy, wheat, and rice	14	78	18
Rice flour	6	16	38
Saengsik	41	86	48
Dry food ingredients	15	66	23
Herbs and spices	40	122	33
Spices	14	71	20
Nuts	2	2	100
Instant soups	2	13	15
Tea	3	5	60
Confectionary	3	42	7
Chocolate products	11	37	30
Seeds	14	34	41
Dried fish (inc. shrimp)	13	50	26
Sunsik	17	36	47
Tofu	4	11	36
Desiccated foods	18	115	16

Adapted from Forsythe, S. J., Dickins, B., and Jolley, K. A. (2014). Cronobacter, the emergent bacterial pathogen Enterobacter sakazakii comes of age; MLST and whole genome sequence analysis. *BMC Genomics* **15**, 1121 and references therein.

The well-known gastrointestinal bacterium *Escherichia coli* includes a number of pathotypes defined according to their clinical presentation or adherence pattern on Hep-2 cell line. EAEC self-aggregate and form a biofilm on intestinal mucosa cells (Hep-2 cell line) with a 'stacked-brick' adherence phenotype. The first EAEC infection case was described in 1987 in a child with acute diarrhea in Lima, Peru. These days, EAEC is estimated to be the second most common cause of traveler's diarrhea. Unconfirmed reports have linked EAEC with the development of irritable bowel syndrome. EAEC is a heterogeneous cluster of *E. coli* strains, which is an important cause worldwide of acute or persistent diarrhea in children and adults. The reason for its inclusion in this article is that since the late 1990s, EAEC has received increasing attention as a cause of acute, often persistent, and watery diarrhea and malnutrition in children and HIV-infected persons living in developed countries.

EAEC outbreaks have been described in children and adults in the United Kingdom and France. The carriage of the organism by children <5 years of age was higher in those with a history of diarrhea. Similarly in Brazil, EAEC was found to be more frequently associated with diarrhea in children <2 years of age. There has been an increasing rise in the proportion of childhood diarrheal cases in which EAEC are implicated, and this indicates that EAEC are important emerging agents of pediatric diarrhea. It is believed that EAEC not only may be causing acute and persistent diarrheal disease but also may be persisting in the human intestine subclinically, inducing chronic inflammation in the absence of dysentery. This persistent infection could result in chronic disruption of gut function resulting in malnutrition and restricted childhood development.

EAEC route of transmission can be through contaminated food. In Italy, two consecutive EAEC outbreaks (affecting 24 individuals) were linked to contaminated unpasteurized cheese. A study of Mexican tabletop sauces identified 44% of sauces from Guadalajara (Mexico) contained viable EAEC cells, compared to 0% of sauces in restaurants in Houston (the United States). In the largest reported outbreak so far, 2697 (40.6%) Japanese children who consumed infected school lunches had severe diarrhea, and EAEC was found in 10% of cases. Serotyping of EAEC is not applicable in the diagnosis of diarrheagenic *E. coli* infections. In the United Kingdom, 97 EAEC strains were serotyped to 40 different O types, and in another study, 93 out of 143 EAEC strains could be serotyped to 47 different serotypes. Finally, many of the EAEC strains autoagglutinate and so are described as nontypable or O rough.

EAEC pathogenesis involves three stages: (1) adherence to the intestinal mucosa by aggregative adherence fimbriae and adherence factors, (2) increased production of mucus that enhances EAEC adherence to the surface of enterocytes, and (3) the release of toxins and elicitation of an inflammatory response, mucosal toxicity, and intestinal secretion. The best-studied virulence factor is *AggR*. This is a transcription activator carried on pAA, a large 60 MDa plasmid. *AggR* controls the expression of fimbriae (adherence factors), a dispersin protein, and at least two pathogenicity islands on the EAEC chromosome. Dispersin is an antigenic antiaggregative protein, which is encoded by the *aap* gene in the pAA plasmid. It is regulated by *aggR*, modulates fimbrial adhesion, and facilitates penetration of the microorganism through intestinal mucus by binding to LPS and altering the electrostatic properties of the EAEC outer membrane surface. Although several other virulence factors are detectable using PCR probes, none exhibit 100% specificity to EAEC strains. Other putative virulence factors include a yersiniabactin system, a complex carbohydrate-specific lectin, enterotoxins, and cytotoxins. The large *E. coli* O104 outbreak in 2011 was due to a hybrid EAEC.

Escherichia albertii

Description: *Escherichia albertii*, species closely related to *E. coli*. Gram-negative, facultative nonmotile rods. The genus is a member of the Enterobacteriaceae family.
Sources: Human feces, birds, animals, and environment.
Clinical presentation: Diarrhea.
Detection methods: No specific isolation procedures are available. May originally be identified by routine diagnostic protocols as enteropathogenic *E. coli* (EPEC) or enterohemorrhagic *E. coli* (EHEC).

Little is known about the host or geographic distribution of *E. albertii;* however, it is carried by ~1% of wild birds and is associated with mass mortality events among birds in the Northern Hemisphere and the cause of mortality in captive birds and poultry. The author does not know of any confirmed cases of foodborne infections due to this organism or even isolation from food. Nevertheless, the organism has been tentatively included in this article as an emerging enteric foodborne pathogen based on its association with poultry and misidentification with EPEC and EHEC in order to raise awareness.

The recognition of *Escherichia albertii* as an emerging human enteric pathogen typifies the value in reevaluating previous identification schemes and anomalies. The organism has previously been misidentified as eae-positive *Hafnia alvei*, as well as strains of eae-positive *E. coli*, *E. coli* serotype O86, EPEC, and EHEC.

In 1991, clinical isolates of *H. alvei* were found to differ from representative *H. alvei* strains by phenotypic and genotypic assays. This variation included the carriage of an intimin gene homologous to the *eae* gene encoding for the attaching-effacing phenotype of EPEC. Following the use of 16S rDNA sequence analysis and DNA–DNA hybridization, the new *Escherichia* species, *Escherichia albertii*, was proposed for the previously *eae*-positive *H. alvei* strains. In addition to the possession of intimin, *E. albertii* also produces a cytolethal distending toxin. Using MLST and virulence gene sequences (*eae* and *cdt*), it is proposed that the *E. albertii* lineage includes *Shigella boydii* (serotype 13) and other previously nontypable clinical isolates. This lineage shared a common ancestor with *E. coli* and *Shigella* pathogenic groups ~28 million years ago (mya), and also the *E. albertii* lineage was established before the radiation of pathogenic *E. coli* and *Shigella*. In essence, *E. albertii* is not a newly emerged pathogenic variant of *E. coli–Shigella*, but a previously unrecognized pathogen, which was confused with other more familiar, closely related organisms.

E. albertii strains are nonmotile, fermented D-glucose (with gas), D-mannitol, and D-mannose, but does not ferment lactose

or other sugars. All strains are positive for the *eaeA* gene, but the presence of other virulence-related genes is variable, namely, *cdtB*, *phoE*, *ehxA*, and *stx2f*. Therefore, there is variability within this lineage, which may cause confusion and hinder accurately identifying *E. albertii* from other members of the *Escherichia* genus. Although the number of accurately recorded outbreaks due to *E. albertii* is uncertain, it is plausible that some outbreaks have been misattributed to *E. coli* as shown in the reassessment of a food poisoning outbreak of gastroenteritis.

Plesiomonas shigelloides

Description: *Plesiomonas shigelloides* is the only recognized species in the genus *Plesiomonas*. Gram-negative, facultative aerobic rods; oxidase-positive. The genus is a member of the Enterobacteriaceae family.
Clinical presentations: Three forms of gastroenteritis, (i) a secretory, watery type; (ii) an invasive, dysentery-like type; and (iii) a subacute or chronic form, lasting between 2 weeks and 3 months.
Sources: Freshwater and estuarine water. Wide range of hosts: amphibians, birds, fish, and animals.
Detection methods: No specific isolation procedures are available.

P. shigelloides is the only species in the genus *Plesiomonas* and is the only oxidase-positive member of the Enterobacteriaceae family. It is recognized as an emerging water- and foodborne enteric pathogen and a major cause of traveler's diarrhea in China and Japan. The organism can cause three types of gastroenteritis: (i) a secretory, watery type; (ii) an invasive, dysentery-like type; and (iii) a subacute or chronic form lasting between 2 weeks and 3 months. There are additional reports of extraintestinal infections: meningitis in neonates, bacteremia, sepsis, and septic shock with high fatality rates. The natural environment for the organism is freshwater and estuarine water. It is also found in amphibians, birds, fish, and animals. Outbreaks are generally related to the consumption of contaminated seafood (crabs, oysters, and fish) or untreated water.

The pathogenicity of *P. shigelloides* is poorly understood, although there are reports of secreted toxins *in vitro*: cholera-like toxin, thermostable and thermolabile toxins, β-hemolysins, and cytotoxin complex. The somatic antigen of *P. shigelloides* may also play a role in pathogenicity, since the gene encoding the most common type, O17, shares almost complete identity with the smooth antigen gene of *Shigella sonnei*. An acute foodborne outbreak in Cameroon was postulated to be due to a preformed toxin in the food, due to the short incubation period.

There is no specific medium for the direct isolation of *P. shigelloides*. In general Enterobacteriaceae media, the organism produces nonlactose-, nonsucrose-fermenting colonies. The organism does not grow on thiosulfate–citrate–bile salts–sucrose medium, but does grow on CIN medium (normally used to isolate *Yersinia* and *Aeromonas* species), producing opaque colonies without a pink center indicating that mannitol is not fermented. It has a minimum growth temperature of 8 °C.

Providencia alcalifaciens

Description: Gram-negative rods. The genus is a member of the Enterobacteriaceae family.
Clinical presentation: Traveler's diarrhea.
Sources: Contaminated food and water.
Detection method: A selective medium has been formulated but possibly not evaluated.

Providencia alcalifaciens is primarily recognized as possible cause of traveler's diarrhea in developing countries. But in 1996, there was a large outbreak of foodborne infection causing acute gastroenteritis, involving 290 patients, in Fukui, Japan. No previously recognized enteropathogens were detected in the fecal samples of the patients. However, PFGE-indistinguishable *P. alcalifaciens* strains were recovered from 7 of 18 samples. Additionally, a specific antibody against the isolated strain was found to be elevated in the patients' sera. *In vitro* virulence studies of the strains using the Caco-2 cell line showed strains could invade human intestinal cells and fluid accumulation in rabbit ileal loop experiments. Together, these results indicate that *P. alcalifaciens* was probably the causative agent of the outbreak.

A selective medium for the recovery of the organism from feces has been published. But to the author's knowledge, no large-scale trials of method evaluations have been undertaken.

Shigella sonnei

Description: Gram-negative facultative aerobic rods. The *Shigella* genus is a member of the Enterobacteriaceae family.
Clinical presentations: Dysentery, fever, and stomach cramps.
Sources: Water and food contaminated with feces and flies.
Detection methods: No specific isolation procedures are available.

Shigella spp. can be regarded as pathotypes of *Escherichia coli* with the ability to invade the human gut mucosa and cause dysentery. Infection is due to the ingestion of the organism via fecally contaminated food or water and can be due to poor personal hygiene by food handlers. *S. sonnei* is well known as a primary cause of dysentery in developed countries and is now emerging as a problem in the developing world. It is replacing the more diverse *Shigella* species *S. flexneri* in areas that are undergoing economic development and improvements in water quality.

Whole genome sequence analysis shows that the current *S. sonnei* population descends from the most recent common ancestor <500 years ago. Evidence to date indicates the species primarily diversified in Europe into four distinct lineages with unique characteristics. This was followed by the spread and establishment of a single, rapidly evolving, multidrug-resistant lineage into Asia, Africa, and America, termed 'pandemic lineage III.' This lineage is now a dominant cause of dysentery in many endemic areas of the world.

The emergence of *S. sonnei* has been attributed to the acquisition of virulence plasmid pINV B, encoding the *Plesiomonas shigelloides*-related O antigen. This has been difficult to confirm since the plasmid is easily lost during laboratory subculturing prior to analysis. Similarly, it has been proposed that previous exposure to *P. shigelloides* (via contaminated water) may offer some protection from *S. sonnei* infection since the O antigens are indistinguishable and cross-react. This could account for the increased incidence in *S. sonnei* infections following improvements in water quality due to the reduced passive cross protection by exposure to *P. shigelloides*.

Ones to Watch

Antibiotic Resistance of Recognized Foodborne Bacterial Pathogens

A detailed consideration of antibiotic resistance and their widespread use in human and veterinary medicine, agriculture, and aquaculture animal husbandry and veterinary usage are outside the scope of this article. Nevertheless, antibiotic-resistant enteric foodborne pathogens are an emerging public risk. Antimicrobial resistance has been described in well-known pathogens, some of which are foodborne: *Salmonella*, *Campylobacter*, *Shigella*, and *Vibrio* spp., methicillin-resistant *Staphylococcus aureus*, *E. coli*, and enterococci. The resistance of animal- and food-associated bacteria to antimicrobial agents has been repeatedly reported. Bacterial resistance inevitably develops to each new class of antibiotics. A variety of different antimicrobial resistance phenotypes result from the acquisition of external genes that can provide resistance to an entire class of antimicrobials. These genes are frequently associated with large transferable extrachromosomal DNA elements, plasmids, on which there may be other mobile DNA elements such as transposons and integrons.

Plasmids in the incompatibility group IncA/C are of considerable interest with respect to multiple antibiotic resistance in enteric pathogens of humans and animals. Although these large, low-copy number plasmids have been known for over 40 years, they appear to be of increasing relevance in the spread of antibiotic resistance. The plasmids have at least three hotspots for the integration of mobile genetic elements. They were first identified from multidrug-resistant *Aeromonas hydrophila* and *Vibrio* spp. in cultured fish. This coincided with the use of antibiotics for cultured fish. A survey of clinical isolates of *Salmonella* newport from 1940 to 2000 revealed that IncA/C plasmids and their multiresistance phenotype emerged after 1980, the reservoir being environmental bacteria, followed by the acquisition of antibiotic gene modules according to antibiotic exposure. IncA/C plasmids which have acquired the bla_{NDM-1} gene encoding the New Dehli metallo-β-lactamase have been disseminated in clinical *E. coli* and *K. pneumoniae*.

Another issue with increasing antibiotic resistance is the coresistance to microbiocides, such as triclosan and quaternary compounds. It has been reported that exposure of *Salmonella enterica* and *Escherichia coli* O157 to sublethal concentrations of antibacterial agents contributed to their development of adaptive resistance to both biocides and antibiotics. Benzalkonium chloride-resistant *Salmonella enterica* serovar Virchow showed elevated resistance to chlorhexidine. *E. coli* O157 acquired high levels of resistance to triclosan after only two sublethal exposures and, when adapted, repeatedly demonstrated decreased susceptibilities to various antimicrobial agents, including chloramphenicol, erythromycin, imipenem, tetracycline, and trimethoprim. However, the specific mechanisms of such coresistance remain poorly understood.

Revelations from Whole Genome Sequencing

Clinical and food microbiology is moving from being culture-dependent to culture-independent. This gives the promise of earlier recognition of attributable organisms. In order to achieve this, there needs to be (a) accessibility to centralized information and (b) consistent interpretation of the DNA sequence. From mid-2013, the FDA has started to sequence 100 000 foodborne pathogens, with 30 completed in December 2013. The genome sequence data will have open access and are being deposited in the National Center for Biotechnology Information and hence shared with other countries for international surveillance.

The major advantages of culture-independent testing are that it is usually faster than culture-based methods and can simultaneously provide subtyping information as required for epidemiological studies and accessible for further analysis. MLST can be predicted from the genome sequence and is not limited to the conventional seven loci. BIGSdb has been implemented for a number of bacterial pathogens such as *Campylobacter jejuni* and *Cronobacter* spp. A number of retrospective foodborne outbreaks have been studied using NGS although as yet few in real time (Table 5).

Conclusions and Future Studies

Compared to clinical infectious diseases, the number of currently recognized foodborne pathogens seems small. In part, this is due to general procedures of food production, which over the centuries have been refined and self-proved effective means of food preparation, for example, cooking, acidification, and dehydration. However, with changes in sourcing of food ingredients, large-scale food preparation practices, longer storage, and changes in eating habits have ensured a continued of food-related illnesses. The major organisms associated with foodborne disease are bacteria due to the ease of isolation and identification compared to viruses and fungi. However, with improved global surveillance systems, as well as improved isolation and identification methods, previously unrecognized

Table 5 Foodborne outbreaks investigated using whole genome sequencing, genomic epidemiology

Organism	*Source*	*Size of outbreak*	*Comment*	*References*
Salmonella Typhimurium	Seven egg farms	193 cases	Retrospective	Hawkey et al.
Salmonella Montevideo	Spiced salami	300 people in 44 US states	Retrospective	Allard et al. and Lienau et al.
E. coli O104	Bean sprouts	>3600 infections and >40 deaths in Germany and >100 in other countries	Real time	Mellmann et al.
L. monocytogenes 1/2a	Ready-to-eat meat products	22 deaths and at least 57 illnesses	Retrospective	Gilmour et al.

foodborne infectious organisms are being recognized. With a global economy and transportation of food, the need to continue to attribute human infections to food vehicles is warranted for their control. This article considers a range of viral and bacterial enteric pathogens, which may be foodborne. Some like *Cronobacter* are currently associated with one food product, reconstituted infant formula, and was previously unrecognized as a bacterial genus, whereas others such as *Shigella* are far better understood. Some recently recognized enteric pathogens are due to increased awareness (such as *Cronobacter*); others are adaptations of existing pathogens. Selective pressures due to the use of antibiotics will drive further changes in the microbiota, which may have severe consequences yet in the future. Being able to predict the future emergence of foodborne enteric pathogens is not possible at this time because of their diversity in virulence and pathogenicity properties, their ability to adapt, the diversity of foods, and the variation in susceptibility to infection by the human population. The best available approach to recognizing the emergence of a foodborne enteric pathogen emergence is through improved surveillance schemes leading to prompt, informed, and appropriate level of control. In part, this will be met by the expansion of genomic epidemiology whereby cases and outbreaks are investigated using whole genome sequencing.

The recognition, control, and monitoring of potential enteric pathogens require a reliable and robust detection system. It is probable that as newly described enteric pathogen is studied, then its description and taxonomy are likely to change as hitherto, it was poorly described and any strains in culture collections could be the more easily recovered variants and only a subpopulation of the taxonomic unit.

It is clear that the major challenge ahead is to establish effective communication between public health, veterinary, and food safety experts, which will bring together multidisciplinary skills and multipathogen expertise. Such collaboration is essential to monitor changing trends in foodborne infections, in order to detect emerging pathogens and to predict their risk to human health such that appropriate control measures can be implemented.

See also: *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; *Clostridium*: Occurrence and Detection of *Clostridium perfringens*; *Escherichia coli* and Other *Enterobacteriaceae*: Occurrence and Detection; Food Poisoning: Classification; Food Poisoning: Epidemiology; Food Poisoning: Tracing Origins and Testing; Foodborne Pathogens; *Salmonella*: Detection; *Salmonella*: Properties and Occurrence; *Salmonella*: Salmonellosis.

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Relevant Websites

www.pubMLST.org/cronobacter – Searchable (BLAST) repository of all published Cronobacter genomes.

Emulsifiers: Types and Uses

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Introduction

Surfactants, also called surface-active agents, are molecules that migrate to the interface between two phases (solid, liquid, or gas). The three most common two-phase systems in food products are emulsions, foams, and dispersions (Table 1). Emulsions are composed of two immiscible liquids. One liquid is dispersed within the other as individual droplets and is called the discontinuous phase, while the other liquid surrounds the droplets and is called the continuous phase. The emulsion behaves like the continuous phase. The typical food emulsions are oil-in-water (o/w), which consists of discontinuous droplets of oil dispersed in a continuous water matrix, and water-in-oil (w/o), which is composed of discontinuous droplets of water dispersed in a continuous oil matrix. When describing emulsions, the 'oil' component may be actual oil or some other ingredient that is not soluble in water. Examples of o/w emulsions are homogenized milk and ice cream, while common w/o emulsions include margarine, salad dressing, and mayonnaise. Emulsions can be formed mechanically by shaking or stirring; however, they are not stable for long periods of time and will separate into the two separate components relatively quickly. Emulsifiers are a type of surfactant that is used to stabilize emulsions.

Types of Emulsifiers

There are many types of emulsifiers. They are classified according to (1) charge in aqueous systems, (2) solubility, (3) hydrophilic/lipophilic balance (HLB), and (4) functional groups that compose them.

Table 1 Interfaces in food systems

Food system	*Continuous phase*	*Discontinuous phase*
Emulsion	Liquid	Liquid
Foam	Liquid	Gas
Dispersion	Liquid	Solid

Charge in Aqueous Systems

There are four classifications of emulsifiers based on the charge of the hydrophilic group when it solubilizes in water. They may be anionic, cationic, nonionic, or amphoteric. Anionic emulsifiers form negatively charged ions. They are effective in the neutral to alkaline pH range and are also sensitive to ionic strength and so do not function well in high salt-containing systems. The salt shields the negative charges so the anionic emulsifiers become ineffective and the emulsion breaks down rapidly. Sodium stearoyl-2-lactylate (SSL) is an example of an anionic emulsifier. Cationic emulsifiers form positively charged ions in water and are functional in the acidic pH range. They are somewhat toxic and so are not used as food additives. Nonionic emulsifiers have no net charge and form no ions. They are highly effective and are compatible with all other types of emulsifiers. They are relatively insensitive to pH and salt concentrations. Mono- and diglycerides (MDGs) are nonionic. Amphoteric emulsifiers form both positively and negatively charged ions when dissolved in water. They are not affected by pH values except some are not effective near the isoelectric point (pH at which protein is uncharged). Lecithin is an amphoteric emulsifier.

Solubility

The solubility of an emulsifier governs the type of emulsion that will be formed. The phase in which the emulsifier is least soluble becomes the continuous phase. A key attribute of emulsifiers is that they are amphiphilic. This means one end of the molecule is lipophilic and the other end is hydrophilic (Figure 1(a)). The lipophilic end, referred to as the tail, is usually a long-chain fatty acid taken from a food grade fat or oil. It is not soluble in water but is soluble in oil and is called

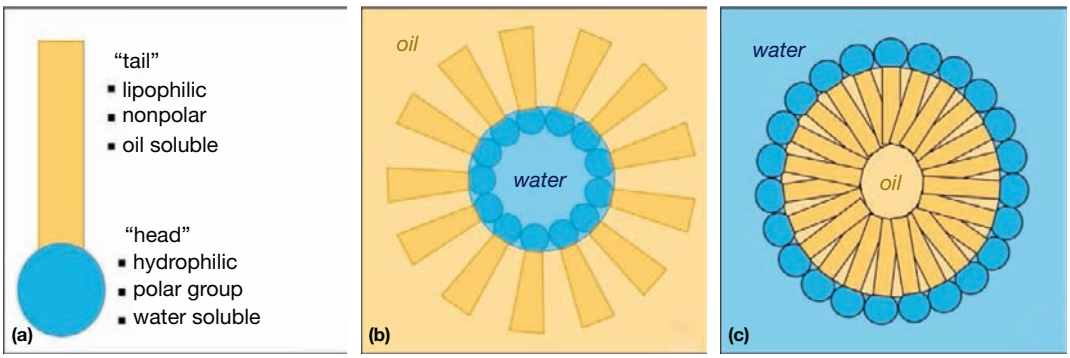

Figure 1 Emulsifier molecules contain a lipophilic 'tail' and a hydrophilic 'head' (a). Orientation of the emulsifier molecules in water-in-oil (b) and oil-in-water (c) emulsions.

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oil-loving. The hydrophilic end is often referred to as the head and is polar. Thus, it easily dissolves in water and is termed water-loving. Emulsifiers that are easily soluble in water (more hydrophilic) produce o/w emulsions. The emulsifier interacts strongly with the water, thus reducing the surface tension of the water so that it approaches zero. The water no longer forms droplets and becomes the outer (continuous) phase of the emulsion, leaving the oil dispersed as discrete droplets (inner or discontinuous phase) to form an o/w emulsion (Figure 1(b)). Lipophilic emulsifiers are not water-soluble and interact with the oil phase. As a result, the surface tension of the water is not significantly reduced so it remains as discrete droplets in the oil, producing a w/o emulsion (Figure 1(c)).

Hydrophilic/Lipophilic Balance

Several methods of classifying emulsifiers are available. The most commonly used is HLB. HLB is an index of the solubilizing properties of emulsifiers and indicates the type of emulsion (o/w or w/o) that the emulsifier is best suited for (Table 2). HLB values can be calculated based on the molecular structure of the emulsifier or determined empirically. The values range from 0 to 20, but some emulsifiers have been shown experimentally to have higher values. Emulsifiers with low HLB values are more oil-soluble (lipophilic), while those with higher values are more water-soluble (hydrophilic). In general, emulsifiers with HLB values of 3–6 are lipophilic and best suited for w/o emulsions. Emulsifiers in this range include MDGs and propylene glycol monostearate (PGMS). Hydrophilic emulsifiers with HLB values of 10–18 are best suited for o/w emulsions. Examples include diacetyl tartaric acid esters of monoglyceride (DATEMs) and lecithin. The effectiveness of emulsifiers is affected by processing conditions and ingredients such as sugar, salt, and protein. It has been reported that the strength of the interaction is more important than the number of reactive groups in determining the hydrophilic properties of emulsifiers. However, HLB can provide an estimate of the hydrophilic nature of various emulsifiers and serves as a useful guide for classifying and selecting emulsifier systems. Selecting the ideal emulsifier or combination of emulsifiers often requires experimental work.

Table 2 Hydrophilic/lipophilic balance (HLB) values of common food emulsifiers

Emulsifier	*Abbreviation*	*HLB value*
Mono- and diglycerides	MDGs	4
Glycerol monolaurate		7
Ethoxylated monoglyceride	EMG	13
Diacetyl tartaric acid esters of monoglyceride	DATEMs	9
Succinylated monoglyceride	SMG	5
Lactylated monoglyceride	LMG	3
Sodium stearoyl-2-lactylate	SSL	22
Propylene glycol monostearate	PGMS	3
Sorbitan monostearate		6
Sodium stearoyl-2-lactylate	SSL	15
Polysorbate 65		11
Polysorbate 80		15
Sucrose monostearate		5
Calcium stearoyl-2-lactylate	CSL	8

Functional Groups

Food emulsifiers are created by alcoholysis or direct esterification of edible fatty acids taken from animal or vegetable sources with polyols (i.e., glycerol, propylene glycol, and sorbitol). Further processing by reaction with ethylene oxide or esterification with organic acids produces a wide range of emulsifiers with differing properties. The most commonly used food emulsifiers include MDGs, stearoyl lactylates, sorbitan esters, polyglycerol esters, sucrose esters, and lecithin. They find use in a wide array of food products (Table 3).

MDGs are the most commonly used food emulsifiers, composing about 75% of total emulsifier production. The largest use is in yeast-raised baked goods to increase shelf life, followed by cake and cake icings. Another major use is in margarine manufacture. MDGs are produced by heating triglycerides and glycerol with an alkaline catalyst. The

Table 3 Common food emulsifiers and uses

Emulsifier	*Food products*
Mono- and diglycerides	Bread, cake, pasta, frozen dessert, icing, topping, peanut butter, margarine, dehydrated potatoes, shortening, coffee whitener, and pasta
Glycerol monolaurate	Bread, whipped topping, frosting, glaze, and cheese products
Ethoxylated monoglyceride	Bread, whipped topping, icing, frozen dessert, and coffee whitener
Diacetyl tartaric acid esters of monoglyceride	Bread, extruded products, icing, margarine, and salad dressing
Succinylated monoglyceride	Bread
Calcium stearoyl-2-lactylate	Bread, egg whites, and dehydrated potatoes
Sodium stearoyl-2-lactylate	Bread, pasta, dehydrated potatoes, and coffee whitener
Propylene glycol esters	Cake, whipped topping, dehydrated potatoes, and shortening
Sorbitan esters	Whipped topping, cake, cake mix, cocoa, icing, filling, and coffee whitener
Polysorbate 60	Whipped topping, cake, cake mix, cocoa, icing, filling, coffee whitener, shortening, salad dressing, and edible oil
Polysorbate 65	Ice cream, frozen custard, ice milk, sherbet, frozen dessert, icing, cake, cake mix, whipped topping, filling, and coffee whitener
Polysorbate 80	Ice cream, frozen custard, ice milk, sherbet, frozen dessert, gelatin mix, shortening, baked goods, bakery mixes, filling, icing, topping, and frying oil
Sucrose esters	Bread, bakery mixes, frozen dessert, whipped milk products, and ice cream
Lecithin	Baked goods, chocolate, cooking spray, instant foods, and margarine

resulting product is a mixture of about 45% monoglycerides, 45% diglycerides, and 10% triglycerides. The monoglyceride concentration can be increased to about 90% using molecular distillation to produce distilled monoglycerides. MDGs can also be processed to produce emulsifiers with various functionalities. Ethoxylated monoglycerides (EMGs) are formed by treating monoglycerides with ethylene oxide gas. DATEMs are produced from the reaction of monoglyceride with diacetyl tartaric acid anhydride. Succinylated monoglycerides (SMGs) result from the reaction of distilled monoglycerides with succinic anhydride. Stearoyl lactylates are the reaction products of stearic acid and lactic acid, which are further converted into the calcium or sodium salts and become calcium stearoyl-2-lactylate and SSL. SSL is one of the most common anionic emulsifiers used in the food industry. Sorbitan monostearate, a sorbitan ester, is produced by reacting sorbitol and stearic acid. It can then be reacted with ethylene oxide to produce polyoxyethylene sorbitan monostearate (polysorbate 60), polyoxyethylene sorbitan tristearate (polysorbate 65), and polyoxyethylene sorbitan monooleate (polysorbate 80). Polysorbate 60 is the most widely used in the group. Polyglycerol esters result from reacting fatty acids with polymerized glycerol of three to ten molecules. Sucrose esters are derived from sucrose and edible tallow and consist of the mono-, di-, and triesters of sucrose with fatty acids. Emulsifiers that solidify into the stable alpha crystalline form are called alpha-tending and include acetylated monoglycerides (AMGs), lactylated monoglycerides (LMGs), and propylene glycerol monoesters (PGMEs). Lecithin is a mixture of phosphatides including phosphatidylcholine, phosphatidylethanolamine, and inositol phosphatide. It is a natural component found in animal and vegetable products. Egg yolk, which contains about 20% phospholipid, is well known for its emulsifying ability. It is too expensive to isolate lecithin from egg yolk; therefore, most commercial lecithin is derived from soybeans, which contain about 2% lecithin. Many other emulsifiers and emulsifier blends are available for specific applications.

Mechanism of Action in Food Products

An o/w emulsion will be used as an example of how an emulsion is formed. When water and oil are mixed vigorously (i.e., whipped, stirred, and shaken), the oil will form into individual droplets dispersed within the water. The oil droplets are the discontinuous phase because they are separated by the water. The water (also referred to as the aqueous phase) is the continuous phase where each water molecule is touching another water molecule. Although an emulsion was formed, it is not stable. Within a short period of time, the oil droplets will bump into each other, and the two droplets will merge into a single, larger droplet. This is called coalescence. These larger oil droplets have less surface area and higher surface tension. Ultimately, all of the oil droplets will coalesce and rise out of the water to form into a separate layer floating on top of the water. The rate at which the oil droplets rise out of the emulsion is inversely proportional to their size; thus, larger droplets rise faster than smaller droplets. In the case of a w/o emulsion, the water is present as individual droplets (discontinuous phase) within the continuous oil phase. Emulsifiers are added to promote emulsion formation and stabilization.

Emulsifiers do not create emulsions. An emulsion is typically created using mechanical means such as mixing or stirring. However, one of the roles of emulsifiers is promoting the formation of smaller oil droplets during emulsion creation, which slows the rate of coalescence. When the appropriate emulsifier is added into the oil and water mixture, the molecules orient at the interface between the water and the oil droplets. The hydrophilic heads are attracted to the water, whereas the lipophilic tails are repelled by the water and attracted to the oil. As a result, the lipophilic tail will react with the oil droplet, while the hydrophilic head will react with the water at the surface of the oil droplet; thus, it reacts at the interface between the two immiscible phases. When this happens, the surface tension of the oil droplet decreases considerably, which decreases the surface tension and allows the droplets to be subdivided into smaller droplets during mixing. The effectiveness of lowering surface tension varies between different emulsifiers.

The second role of emulsifiers is stabilizing the emulsion once it has formed. This is the main reason that emulsifiers are used in food systems. Even though smaller droplets are more stable, emulsion stabilization by emulsifiers is not directly the result of their ability to lower interfacial tension and allow for the creation of smaller droplets. Rather, emulsifiers stabilize emulsions by accumulating at the interface of the two phases where they form a barrier that prevents the droplets of the discontinuous phase from coalescing. The mechanism by which the barrier forms is determined by the properties of the emulsifier. One mechanism of stabilization is due to like-charged particles repelling each other. Anionic emulsifiers carry negative charges that repel each other to keep the droplets apart. SSL, SMG, and DATEM are anionic emulsifiers. They are not highly effective in systems with high salt levels because the salt shields the charge, which suppresses the repulsive forces and leads to rapid coalescence and breakdown of the emulsion. Although not used in food systems, cationic emulsifiers carry positive charges that also repel each other to stabilize the emulsion. In nonfood systems, anionic and cationic emulsifiers should not be used in combination as the opposite charges would interact, thus rendering the emulsifiers inactive. Another form of stabilization is employed by emulsifiers that contain a large hydrophilic portion such as EMG. The large hydrophilic head holds a large amount of water and creates a layer of 'bound' water around the droplet, which prevents coalescence. These emulsifiers are not sensitive to salt. Simple steric hindrance is a third type of stabilization. The emulsifier forms a solid layer at the interface, which physically prevents coalescence even if the droplets touch each other. This occurs in alpha-tending emulsifiers such as PGME.

Food Uses of Emulsifiers

In the food industry, the term 'emulsifier' is used generically and includes surfactants that are not technically classified as emulsifiers. Additives categorized as emulsifiers in the food industry provide a broad range of functions in a wide range of food

products including emulsification, stabilization, antistaling, crumb softening, foaming, fat crystal modification, mouthfeel, agglomeration, defoaming, wetting, solubilizing, and stickiness control. Many food products contain emulsifiers that perform many different functions (Table 3). A few are discussed here.

Dairy Products

Emulsifiers are added to ice cream to shorten the freezing time, improve whipping, and produce a dry, stiff texture that melts slowly. The ice cream mix is an o/w emulsion. Emulsifiers stabilize the emulsion and prevent the fat globules from agglomerating in the mix. When the mix is churned into ice cream, the emulsifiers function to destabilize the natural milk protein film that surrounds the milk fat globules so they can surround the air bubbles and stabilize into a frozen foam. MDGs, sucrose esters, and polysorbates are commonly used in ice cream.

Nondairy whipped toppings are whipped cream replacers made using vegetable fats. They may be frozen or in pressurized cans. In these products, emulsifiers are added as destabilizers to promote aeration. In this role, the emulsifier causes the fat globules to agglomerate during whipping. This is done to create a fat network in the continuous aqueous phase, which stabilizes the air bubbles and imparts stiffness to the foam. Polysorbate 60 and alpha-tending emulsifiers such as LMG and propylene glycol monoesters are commonly used in the production of commercial nondairy whipped toppings.

Coffee whiteners are liquid or powdered cream replacers. Emulsifiers are added to improve their dispersion in hot coffee. Typical emulsifiers used include distilled monoglycerides, DATEM, polysorbate 60, and sorbitan monostearate.

Salad Dressings

Mayonnaise is an o/w emulsion that contains at least 65% vegetable oil. The emulsion is stabilized by the high level of lecithin in the egg yolk. FDA standards of identity do not allow the addition of other emulsifiers.

Pourable salad dressings are w/o emulsions that contain at least 30% vegetable oil. Typically, consumers shake the bottle to form the emulsion before pouring the dressing over their salad. Polysorbates and DATEMs are commonly used to enhance emulsification when the bottle is shaken.

Margarine

Margarine is a w/o emulsion containing about 80% fat. It is usually made with MDGs of long-chain fatty acids and lecithin. The MDGs promote the formation of the emulsion, while the lecithin is added to increase the solubility of the MDGs in the oil blend. Distilled monoglycerides with a high concentration of unsaturated fatty acids and lecithin are used in low-calorie spreads, which are also w/o emulsions that contain at least 50% water and 40% fat.

Shortening

Shortening consists of a fat blend that contains emulsifiers. All-purpose shortenings contain about 1% distilled monoglycerides. High-ratio or emulsified shortenings, for use in cakes where the levels of sugar and liquid are present at higher levels than the flour, contain several emulsifiers including distilled monoglycerides, LMG, and PGME. These shortenings allow better air incorporation during mixing and bind more sugar and water.

Pasta

SSL and distilled monoglycerides are added into pasta products to aid in extrusion. They are also used in pasta for soups or canned products to reduce stickiness and improve the texture of the cooked product.

Baked Products

In the baking industry, the term 'emulsifier' is used to describe a broader group of surfactants, which includes those that are active in foams (air-in-water) and dispersions (liquid-in-solid) and in emulsions (liquid-in-liquid). Thus, the term emulsifiers will be used in this section to encompass the common surfactants used in baked products.

Doughs and batters are complex systems that contain multiple interfacial interactions that occur simultaneously, some of which change forms as the products are heated. Emulsifiers play many different roles in these products. Most batters and doughs contain a polar, continuous phase that consists of an aqueous (water) solution of salts, sugars, water-soluble proteins, and other solubilized formula ingredients, which can be a component of an emulsion, a foam, and a dispersion simultaneously in a single batter or dough. For example, the continuous aqueous phase may form an emulsion in which a lipid in some form (oil or solid fat) constitutes the nonpolar, discontinuous phase. It may be the continuous phase in an emulsion with a nonpolar, discontinuous polar discontinuous phase of lipid in some form (oil or solid fat). Almost all batters and doughs contain a foam of the continuous aqueous phase with a discontinuous phase of air bubbles, which are incorporated during mixing. Furthermore, the continuous aqueous phase makes a dispersion with the insoluble formula ingredients, which include the starch and gluten in the flour.

In cake batters, distilled monoglycerides and alpha-tending emulsifiers such as AMG and PGMS function as aerating agents to speed up the whipping rate and facilitate the incorporation of many small air cells. This leads to a more stable emulsion and results in a cake with a larger volume and fine, uniform crumb grain. Alpha-tending emulsifiers are critical in cakes made using a one-stage mixing procedure or prepared from boxed cake mixes where liquid shortening or oil is used because they form a solid film at the oil–water interface, which stabilizes the emulsion and also keeps the liquid oil from breaking the emulsion or foam that is prepared during mixing.

Emulsifiers used in yeast-leavened bread products are also active in the solid–liquid interface (dispersion) where they function as crumb softeners and dough strengtheners. As crumb softeners, they interact with starch granules and gluten protein polymers to impart a softening effect on the crumb grain and extend the shelf life of the product. Monoglycerides are the primary crumb softener used in bread. Other common crumb softeners include DATEM, sucrose esters, and PGMS.

Dough strengtheners interact with the gluten protein and alter the rheology of the dough to produce a stronger gluten structure. Anionic emulsifiers such as DATEM, SSL, SMG, EMG, and polysorbates are good dough strengtheners. Some of the emulsifiers used in baking have only one or the other of the functionalities, while others have both. For example, SSL functions mainly as a dough strengthener, but it also plays a minor role promoting the emulsification, incorporation, and subdivision of air cells during mixing and crumb softening.

See also: Aeromonas; Bread: Chemistry of Baking; Food Additives: Classification, Uses and Regulation; Rheological Properties of Food Materials; Stabilizers: Types and Function; Triacylglycerols: Structures and Properties.

Further Reading

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Relevant Websites

www.emulsifiers.org – European Food Emulsifiers Manufacturers Association (EFEMA).
www.faia.org.uk – Food Additives and Ingredients Association (FAIA).

Energy Metabolism

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Regulation of Energy Metabolism

Energy metabolism represents the most important body function and has an effect on energy expenditure. It is paramount to basal metabolism, growth, and physical activity in humans. Energy derived from nutrients drives the critical cellular functions in humans and is essential for survival. Using complex biochemical pathways, chemical energy stored in the macronutrients (carbohydrate, fat, and protein) is transformed into other forms of energy such as heat and *adenosine triphosphate*. The regulation of metabolism at the cellular level relies on a complex neurohormonal system, which responds to a variety to stimuli and controls the utilization of substrate in individual cells. The overall aim of this regulatory process is to maintain energy balance, and it is important to understand these pathways that convert food into usable fuel.

The importance of the central nervous system (CNS) in the regulation of body weight has been known for the past 100 years. Several factors have been identified, including mechanical, hormonal, neural, and metabolic, that act as peripheral signals in sending information to the CNS to regulate substrate metabolism. The CNS processes these signals and integrates a response to modify the control mechanisms that maintain energy balance. It has been proposed that energy balance is maintained by the regulation of body fat through a feedback mechanism with specific processes mediated. In these processes, the hormones leptin and insulin send signals to the CNS about the body fat stores and participate in body weight control. Both leptin and insulin cross the blood–brain barrier and interact with receptors at the hypothalamus; two types of neurons in this area have been described to have opposite effects (anorexigenic vs. anabolic) to the action of leptin and insulin concentrations, and it is by this mediating effect that energy balance is achieved.

Other factors have been described in the CNS regulation of energy balance, including signals from the gastrointestinal tract and from the nutrients (i.e. glucose, fatty acids, and amino acids). Among the satiety hormones secreted by the gut in response to presence of substrate are cholecystokinin, amylin, peptide YY, apolipoprotein A, glucagon-like peptide-1, and ghrelin. The concentrations of the different substrates send feedback to the CNS about the energy status of the body. The observation that lower or higher blood glucose levels induce changes in feeding behavior led to the well-known fact that the CNS uses several sugar molecules as signals to regulate food intake. Although neurons get most of their energy supply from the oxidation of glucose rather than fat, studies in animals have demonstrated that administration of fatty acid synthase inhibitors produces anorexia and weight loss. Recently, studies have shown the effect of intake of different amounts of amino acids in the central regulation of satiety, with diets high on protein having an effect of decreasing food intake. On the other hand, administration of several amino acids (i.e., leucine and valine) has been shown to have opposite effects on food intake.

In summary, the CNS is an important and vital component of the regulation of energy metabolism. Hormonal secretion and feedback regulation by the CNS, gastrointestinal factors, and substrate intake and metabolism are important in achieving energy homeostasis.

Components of Energy Expenditure

In order to understand energy balance, it is important to review the total energy expenditure (TEE) during health and illness. In healthy individuals, TEE can be partitioned into three main components: basal metabolic rate (BMR), thermogenesis, and physical work. BMR represents 60–70% of TEE, while thermogenesis represents 10%, and physical work accounts for 20–30% of TEE. During conditions of stress, the proportions of these components vary depending on the severity of the insult, amount of substrate intake, and physical activity. Diet-induced thermogenesis (DIT) is the amount of energy required to absorb, process, and store nutrients and represents an increase in energy expenditure with respect to postabsorptive state (Figure 1). BMR is difficult to measure. However, indirect calorimetry (IC) allows a measurement of a close approximation of this value and has been described in more detail later in this article. Resting energy expenditure (REE) is the energy expenditure measure by IC in true resting state. Energy supply greater than two to three times the REE gives rise to a DIT that increases from 10% up to 20% after the first week of illness with resultant increases in energy expenditure and higher ventilatory and cardiocirculatory demands on the patient; therefore, it is important not to supply excessive calories in relation to measured energy expenditure or BMR during the acute phase of the injury or surgical stress. With age, energy expenditure is reduced and this in addition to decreased exercise activity explains the increase in weight gain commonly seen during adulthood. On average, the rate of basal metabolism per body weight is reduced from infancy to adulthood by 1.5–2 times (Figure 2).

For many years, the concept of the increased energy expenditure seen in infants has been attributed to the energy cost of growth. In early infancy, the growth velocity is maximal at approximately 6 months of age; this process slows down considerable by the age of 1 year. It has been described in infants less than 6 months that approximately 7–8% on the energy used by the body is utilized for growth purposes; this amount decreases to less than 2% of the energy expenditure by the time the child becomes older than 1 year of age when the BMR reaches about 55 kcal kg^{-1} day^{-1}. There is a reduction in the rate of basal metabolism that starts after the age of 3 years. This change is explained by changes in body composition and an uneven growth of organs, for example, the metabolic rate of

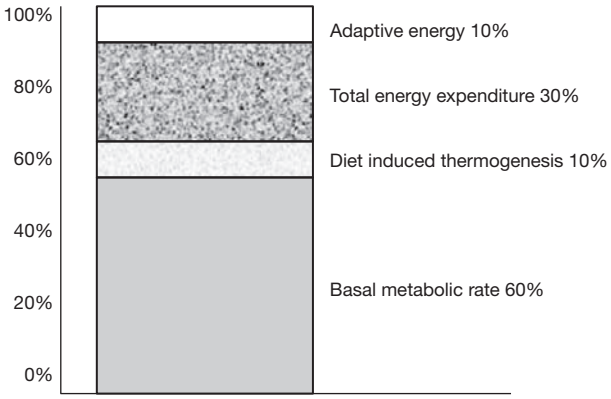

Figure 1 Components of energy expenditure.

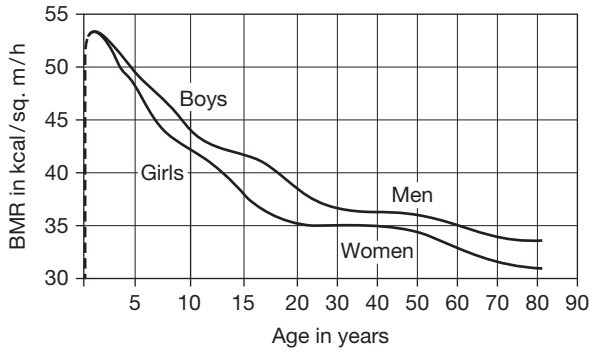

Figure 2 Change in basal metabolic rate with age. Reproduced with permission from Mitchell, H. H. (1962). *Comparative nutrition of man and domestic animals*, vol. 1, pp. 3–90. New York: Academic Press.

the brain represents 12% of the total in the newborn, while in adults, it is only 2% of their body mass. The last peak of BMR occurs at the age of 14 years, and after this period, the BMR is stabilized at the level of an adult.

BMR defined as the energy expenditure after a 12 h fasting period represents 50–70% of TEE, and it does decrease over time by approximately 1–2% per decade. Many studies have reported that the BMR is lower after adjustment for fat-free mass in older individuals. The two changes that could explain a lower metabolic rate during aging is the relative increase of fat mass in relation to the decrease of fat-free mass. Multiple studies have indicated that BMR is lower in the elderly when compared to rates in younger individuals even after taking into consideration changes in body composition associated with age. BMR defined as the energy expenditure after a 12 h fasting period represents 50–70% of TEE, and it does decrease over time by approximately 1–2% per decade. Many studies have reported that the BMR is lower after adjustment for fat-free mass in older individuals. The two changes that could explain a lower metabolic rate during aging are the relative increase of fat mass in relation to the decrease of fat-free mass. Multiple studies have indicated that BMR is lower in the elderly when compared to rates in younger individuals even after taking into consideration changes in body composition associated with age. There is a reduction in the rate of basal metabolism that starts after the age of 3 years. This change is explained by changes in body composition and an uneven growth of organs, for example, the metabolic rate of the brain represents 12% of the total in the newborn, while in adults, it is only 2% of their body mass. The last peak of BMR occurs at the age of 14 years, and after this period, the BMR is stabilized at the level of an adult.

An important component of energy expenditure is the energy dissipated by physical activity; this expenditure in addition to the BMR and the thermal effect of food represents the TEE. The explanation for the decline in TEE with age is not well understood. With aging, there is an increase in body fat mass, making body composition changes an important reason for this change. Another important factor in creating energy imbalances and eventually weight gain is the ability to oxidize fat, with an increase in body fat stores if oxidation of fat is decreased and vice versa.

For many years, the concept of the increased energy expenditure seen in infants has been attributed to the energy cost of growth. Growth velocity (as measured by grams/day or grams/kg/day) is highest in neonates and early infancy and starts to slow down in the second half of infancy. In infants less than 6 months, approximately 7–8% on the TEE is allocated for growth purposes; this amount decreases to less than 2% of the energy expenditure by the time the child becomes older than 1 year of age when the BMR reaches about 55 kcal kg^{-1} day^{-1}.

In summary, multiple studies have shown that dysregulation of energy intake occurs in healthy older individuals increasing the risk of energy imbalance; in addition, studies have indicated a dysregulation of energy expenditure and substrate oxidation associated with aging that results in changes in body weight. In periods of sickness, when energy expenditure varies and the individual is dependent on artificial nutrition, it is important to be aware of the balance between energy intake and requirement.

Metabolic Response to Stress

The profound metabolic response to critical illness, including injury, surgical stress, or inflammation, is not always predictable and varies in intensity and duration between individuals.

The energy cost imposed by this metabolic response may be proportional to the severity and duration of the stress but cannot always be accurately estimated. Importantly, nutritional support itself cannot alter the metabolic stress response. Failure to recognize existing nutritional deficiencies and provide adequate nutrition support during the acute phase of the illness may exacerbate preexisting malnutrition or result in new nutritional deficiencies. Both overfeeding and underfeeding should be avoided in order to decrease metabolic imbalances and development of malnutrition in critically ill patients. The nutritional plan should be individualized and customized to each patient during their hospital admission. A complete understanding of the metabolic events involved in the metabolic stress is essential for planning appropriate nutritional support plans in critically ill patients.

The hormonal and cytokine profile of the stress response to critical illness is characterized by increased serum levels of insulin, glucagon, cortisol, catecholamines, and proinflammatory cytokines. Elevated serum counterregulatory hormone

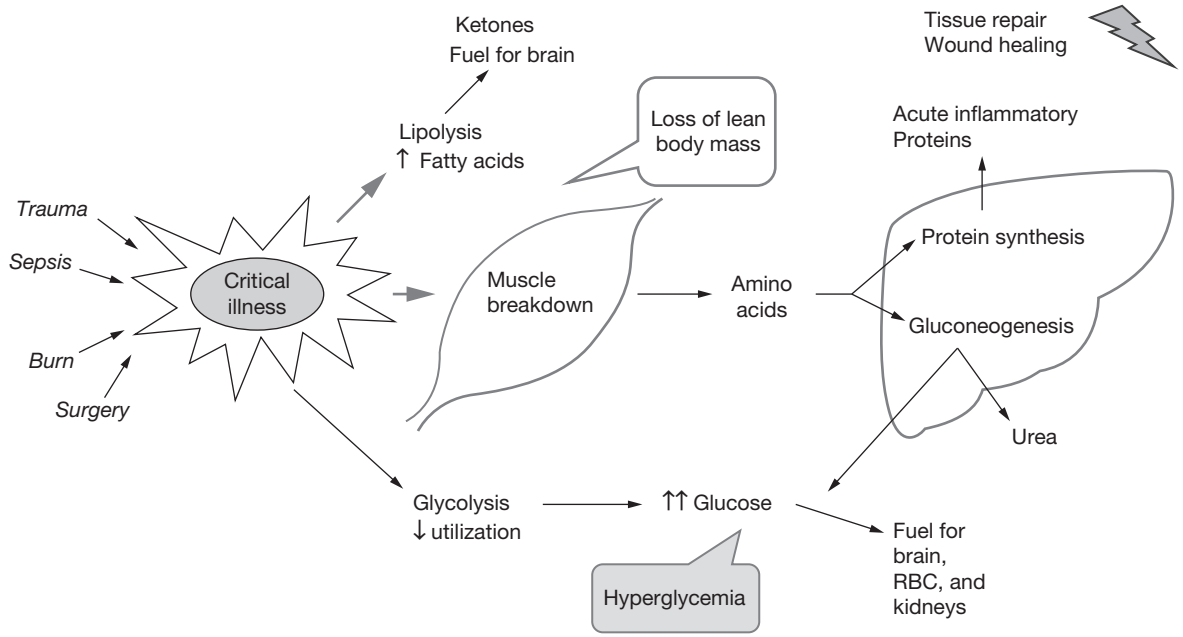

Figure 3 Basic pathways of the metabolic stress response to injury. Reproduced with permission from Mehta, N. and Jaksic, T. (2008). The critically ill child. In: Duggan, C., Watkins, J. B. and Walker, W. A. (eds.) *Nutrition in pediatrics* (4th ed.), pp. 663–673. Hamilton, ON: BC Decker.

concentrations induce insulin and growth hormone (GH) resistance, resulting in the catabolism of endogenous stores of protein, carbohydrate, and fat. This autocannibalism ensures a steady supply of essential substrate intermediates and energy necessary to support maintenance energy and micronutrient needs in addition to driving the ongoing metabolic stress response. The *sine qua non* of this acute metabolic response is a hypermetabolic, hypercatabolic state resulting in the loss of endogenous muscle stores with increased glucose and free fatty acid oxidation, elevated energy expenditure, and increased protein breakdown (Figure 3).

Among the changes at the CNS seen during the acute metabolic response are alterations in the hypothalamic–anterior pituitary axes, resulting in increased cortisol secretion, elevated concentrations of GH, increased reverse T_3 secretion, increased prolactin, and decreased testosterone. The degree of alterations in the levels of these hormones is proportional to the severity of the insult and produces significant changes in body homeostasis. Cytokines are endogenous proteins that act as mediators of local and systemic injury or inflammation. They are synthesized by several tissues and white blood cells in response to stimulation by endotoxin and viral particles. These mediators are critical in modulating a number of responses by promoting tissue repair, prioritization of substrate for protein synthesis, and anti-inflammatory actions. This response when augmented secondary to an overwhelming insult or inadequate response by the host immune response can lead to an increased risk of injury-induced morbidity and mortality. Tissue injury elicits the release of proinflammatory cytokines (TNF-α, interleukin (IL)-1, IL-6, and IL-8). Once these peptides are released by activated macrophages and endothelial cells, signals are enacted to release anti-inflammatory cytokines, mainly IL-10. Many clinical studies have reported the use of drugs aimed to attenuate the proinflammatory response or medications to augment the anti-inflammatory reaction with mixed results, highlighting the inherent difficulties in the control of the inflammatory response.

During the acute metabolic response, there is a significant increase in the concentrations of the counterregulatory hormones (oppose the anabolic effects of insulin) including catecholamines, glucagon, and cortisol. The actions of catecholamines include an elevation of the metabolic rate, hyperglycemia induced by hepatic glycogenolysis, and suppression of pancreatic secretion of insulin. The known actions of glucagon are glycolysis and gluconeogenesis with a resultant elevation in the serum concentrations of lactate and alanine; this results in the endogenous regeneration of glucose. The effects of cortisol are at the muscle level with significant proteolysis and elevation of metabolic rate in conjunction with the action of catecholamines. The other important effect of cortisol is the increase in gluconeogenesis by the incorporation of alanine and glutamine from muscle proteolysis and gut sources, respectively. Also, cortisol has been shown to increase insulin resistance and oppose the anabolic effects of insulin.

It has been postulated that the attenuation of the effects of anabolic hormones during the acute metabolic response represents a compensatory mechanism. Insulin is a potent anabolic hormone with multiple actions including glycogenesis, lipogenesis, and protein synthesis. When a state of increased insulin resistance is present, glucose production, lipolysis, and proteolysis are increased, conditions usually seen in critically ill adults and children. Recent evidence suggests an anti-inflammatory effect of insulin administration; this finding has been reported in animal and clinical studies. GH, also known as somatotropin or somatropin, is a peptide hormone that stimulates growth, cell reproduction, and regeneration in humans and other animals. GH is a stress hormone that raises the concentration of glucose and free fatty acids and stimulates

production of insulin-like growth factor 1 (IGF-1). The actions of GH are aimed toward anabolic conditions, and its synthesis and secretion are stimulated by IGF-1. During stress conditions, the secretory pattern of GH and IGF-1 is suppressed by a cytokine-mediated mechanism. Clinical studies in children with burns have reported that the exogenous administration of recombinant GH has increased protein synthesis and nitrogen balance and improved clinical outcomes.

Substrate Adaptations During the Acute Metabolic Response

The changes in substrate homeostasis seen after the acute metabolic response are aimed to preserve life, despite the fact that in many instances, this response is maladaptive. During periods of starvation not associated with stress, endogenous mobilization of adipose stores is the main source of energy for metabolic purposes. This mobilization of body fat stores is preceded by the use and eventual depletion of glycogen stores; the time it takes for glycogen reserves to be depleted is usually few hours for infants and children and up to 24 h in adults. It is during this initial period of lack of substrate intake that the energy needs are supplied mainly with glycogen and protein. After a period longer than 48–72 h, an increase in fat store mobilization is ensued with subsequent utilization of fat as the sole source of energy, with the exception of tissues that are dependent mainly on glucose for metabolic fuel. Also, a decrease in energy expenditure is seen aimed to preserve lean body mass. Unfortunately, patients with limited or smaller protein stores (i.e., children and malnourished adults) will be metabolically compromised in less time.

The acute metabolic response seen with injury, trauma, and sepsis results in important alterations in substrate metabolism. In addition to the use of glycogen stores, gluconeogenesis is increased and provides the bulk of carbohydrates for the glucose-dependent tissues; protein breakdown provides substrate for the synthesis of acute phase reactants and gluconeogenesis. The hyperglycemic response seen is secondary to the uncontrolled need to synthesize new glucose and to the increased insulin resistance at the peripheral tissues associated to the dysfunction of the intracellular glucose transport protein 4 (GLUT4).

The acute metabolic response in the critically ill subject is hypercatabolic resulting in proteolysis, increased protein turnover, and decreased protein synthesis. These changes have been shown by the use of stable isotopes in studies that included patients with sepsis and extensive burns. The response of the acute ill patient in terms of protein catabolism is different from the subject under starvation conditions in that the former has negative protein balance secondary to an exaggerated proteolysis as opposed to decreased synthesis in the starved subject. This catabolic response is not attenuated by the provision of adequate nutrition support and persists until the acute process is resolved. Much of this loss of lean body mass as it relates to commonly measured outcomes, including length of intensive care unit (ICU) stay and length of hospitalization, has been debated by the experts. Even though the exaggerated catabolic response is not reversed by the provision of substrate in the form of glucose, protein, and fat, adequate supplementation of protein is recommended to increase protein synthesis and attenuate the net negative protein balance.

Glucose is the primary fuel for the CNS and minimal amounts are required to maintain function. Provision of glucose in excess of the capacity of oxidation, usually 8–10 mg kg^{-1} min^{-1} in neonates and 4–6 mg kg^{-1} min^{-1} in adults, has been associated with lipogenesis, hepatic steatosis, and hyperglycemia. It has been reported that up to 150% of the glucose oxidized under stress conditions is provided by gluconeogenesis, and this response is not attenuated by the exogenous administration of glucose. Exogenous administration of insulin does not increase the rate of glucose oxidation in critically ill patients because the rate of maximal glucose oxidation has been reached in addition to increased endogenous levels of insulin. The resultant hyperglycemia seen in these patients is a result of a combination of factors including increased glucose oxidation and hepatic glucose production and decreased peripheral uptake of glucose.

Assessment of Energy Requirement for the Hospitalized Patient

Methods to Estimate Energy Needs

Adequate nutrition support is important giving the high prevalence of malnutrition in the hospitalized patient. Several studies have reported malnutrition rates ranging from 30% to 50% upon admission to the hospital, with more than 50% of these already malnourished patients having a worsening nutritional status if it is left untreated. It has been reported that up to one-third of hospitalized adults patients will develop malnutrition after admission to the hospital. Malnutrition has been reported to worsen outcomes, including immune suppression, infection, longer hospital stay, increased morbidity, and decreased survival. Critically ill children differ in their energy needs from healthy children in terms of underlying metabolism and growth, comorbidities, and preexisting energy reserve, and therefore, it is difficult to estimate energy needs in this population. In addition, frequent monitoring of energy expenditure is necessary to accommodate any variations in energy expenditure throughout the course of illness. In the absence of metabolic equipment to measure energy needs in the ICU, caution should be used when reference values are used to estimate energy needs in this population.

Reference values

The BMR reference values include the following:

1. The Harris–Benedict equation, (Harris–Benedict 1919) one of the most widely used to estimate BMR. This equation is widely used in adults and has never been validated for children.
2. The Food and Agriculture Organization, the World Health Organization, and the United Nations University (FAO/WHO/UNU). These equations were based on data derived from 6100 individuals under a variety of conditions and represent BMR.
3. Talbot published guidelines in 1938 for the estimation of BMR based on measurements made in children. These values were based on studies performed by the author.

Table 1 Prediction equations

Equation	*Gender*	*Age*	*Basal metabolic rate estimate*
Harris–Benedict		Children	[22.1 + (31.05 × Wt) + (11.6 × Ht)]
	Female		[665.0955 + (9.5634 × Wt) + (1.8496 × Ht) − (4.6756 × age)]
	Male		[66.473 + (13.7516 × Wt) + (5.0033 × Ht) − (6.755 × age)]
FAO/WHO/UNU	Female	0–3 years	[(61 × Wt) − 51]
		3–10 years	[(22.5 × Wt) + 499]
		10–18 years	[(12.2 × Wt) + 746]
	Male	0–3 years	[(60.9 × Wt) − 54]
		3–10 years	[(22.7 × Wt) + 495]
		10–18 years	[(17.5 × Wt) + 651]
Schofield	Female	0–3 years	[(16.252 × Wt) + (10.232 × Ht) − 413.5]
		3–10 years	[(16.969 × Wt) + (1.618 × Ht) + 371.2]
		10–18 years	[(8.365 × Wt) + (4.65 × Ht) + 200.0]
		18–30 years	[(13.623 × Wt) + (2.83 × Ht) + 98.2]
	Male	0–3 years	[(0.167 × Wt) + (15.174 × Ht) − 617.6]
		3–10 years	[(19.59 × Wt) + (1.303 × Ht) + 414.9]
		10–18 years	[(16.25 × Wt) + (1.372 × Ht) + 515.5]
		18–30 years	[(15.057 × Wt) − (0.1 × Ht) + 705.8]
Estimated energy requirements	Female and male	0–3 months	[89 × Wt − 100] + 175
		4–6 months	[89 × Wt − 100] + 56
		7–12 months	[89 × Wt − 100] + 20
	Female	3–8 years	135.3 − [(PA × 10 × Wt) + (30.8 × Age) + (934 × Ht)] + 20
		9–18 years	135.3 − [(PA × 10 × Wt) + (30.8 × Age) + (934 × Ht)] + 25
	Male	3–8 years	88.5 − [(PA × 26.7 × Wt) + (61.9 × Age) + (903 × Ht)] + 20
		9–18 years	88.5 − [(PA × 26.7 × Wt) + (61.9 × Age) + (903 × Ht)] + 25

FAO, Food and Agriculture Organization; WHO, World Health Organization; UNU, United Nations University. Basal metabolic rate estimate in kcal per day; weight (Wt) in kg; height (Ht) in cm; physical activity (PA).

4. Schofield equations. These equations were based on data from the FAO/WHO/UNU report with some additional data (Table 1). It is important to mention that the use of these reference values in critically ill patients could lead to underfeeding or overfeeding because of the variability of the metabolic state of patients during their stay in the ICU.

Correction factors

The use of stress and activity factors in addition to the BMR reference values has been reported to overestimate and underestimate energy needs in critically ill patients, reinforcing the concept that IC should be used as a strategy to measure energy needs in this population of patients. Several studies conducted in critically ill patients on mechanical ventilation that used different BMR equations (i.e., Harris–Benedict, Schofield, and Talbot) and correction factors of 1.3 and 1.5 to estimate energy needs were found to have significant differences when compared to measured energy expenditure by IC, emphasizing the concept that IC is the only method to accurately measure energy needs in critically ill patients.

Predictive equations

The use of regression equations based in multiple variables (sex, weight, height, body temperature, heart rate, inotrope dose, sepsis, days of admission to the ICU, minute ventilation, etc.) has been reported in both adults and children admitted to the ICU and has shown to be more accurate than reference values when compared to measured energy expenditure by IC. Caution should be given when using predictive equations because of the dynamic nature of the metabolic condition of critically ill patients.

Methods to Measure Energy Needs

The measurement of energy expenditure in humans has been attempted in the last century by physiologists. These methods include direct calorimetry, IC, and the isotope dilution technique. The use of direct calorimetry requires the measurement of heat produced by the body while the subject is placed in an insulated and tight chamber. The principles used are similar to those in a bomb calorimeter (Figure 4). IC is the method by which metabolic rate and substrate utilization are estimated from respiratory gas exchange measurements and urinary nitrogen excretion. It has been regarded as the gold standard for accurate measurement of REE. IC measures whole body oxygen consumption (VO_2) and carbon dioxide production (VCO_2). Then, the VO_2 and VCO_2 values are converted to a caloric equivalent based on equations developed by Weir: $REE = [VO_2\ (3.941) + VCO_2\ (1.11)] \times 1440$. The use of IC and urinary nitrogen allows the calculation of 'net' oxidation rates for carbohydrates, protein, and fat by the use of the Conzolazio formulas. The VCO_2/VO_2 ratio is known as respiratory quotient (RQ), and it is well known that the conversion of glucose to fat elevates the RQ and reflects the proportion of substrate utilization in the body. The npRQ represents the ratio of glucose and fat utilization by excluding the participation of protein and varies in value from 0.70 to 1.0, with values >1.0 indicating net fat biosynthesis from glucose (lipogenesis).

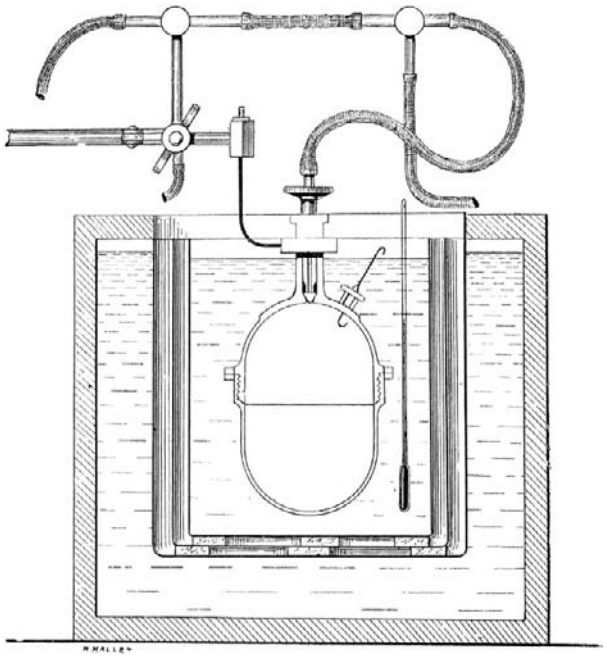

Figure 4 The bomb calorimeter. This diagram shows the bomb calorimeter designed by the French chemist Pierre Eugene Marcellin Berthelot (1827–1907). It is used to measure the heat of chemical reactions. Artwork from the tenth volume (second period of 1892) of the French popular science weekly 'La Science Illustree.' From the Science Photo Library.

The correct interpretation of IC results implies an understanding of the assumptions and technical considerations of this methodology. There are several sources of error and many technical difficulties in applying this methodology in the ICU including (1) model of calculation and assumptions, (2) calorimetric factors used, (3) leak around endotracheal tube, (4) inspired oxygen concentration above 0.60, (5) use of high levels of positive end expiratory pressure, (6) unstable gas analyzers, (7) inability to reach steady state, and (8) human factors. Studies in mechanically ventilated adults and children have concluded that the use of an abbreviated IC protocol of 3–5 min duration is enough to achieve steady state and obtain reasonable accuracy, while other authors have concluded that energy expenditure results of a 30 min IC test are comparable to 24 h test results.

The potential useful clinical applications of IC in critically ill patients can be summarized as follows: (a) assessment of energy expenditure in patients who fail to adequately respond to estimated nutritional needs, (b) assessment of energy expenditure in patients with single or multiple organ dysfunction who need prolonged ICU care and artificial nutritional support, (c) assessment of the effects induced by artificial nutrition on the cardiocirculatory and respiratory systems in mechanically ventilated patients with acute and chronic respiratory failure, and (d) monitoring of VO_2 while weaning from mechanical ventilation. Targeted measurements of REE are currently recommended and are included in the American Society for Parenteral and Enteral Nutrition (ASPEN) Clinical Guidelines: Nutrition Support of the Critically Ill Child. In summary, IC has brought understanding of how energy is utilized during critical illness; this has yet to be translated into improving patient outcomes. Studies examining the role of simplified IC technique, its role in optimizing nutrient intake, its ability to prevent overfeeding or underfeeding in selected subjects, and the cost–benefit analyses of its application in the ICU are needed.

TEE that takes into account physical activity can be measured using doubly labeled water technique (DLW). The isotope dilution technique uses stable isotopes (^2H_2O, $H_2{}^{18}O$, and $NaH^{13}CO_3$) to measure energy expenditure. The DLW method is based on the differences in turnover rates of ^2H_2O and $H_2{}^{18}O$ in body water. The DLW technique has been validated against IC and is now considered to be a gold standard for measurements of TEE under free-living conditions. Sources of measurement error include analytic inaccuracies in the mass spectrometric determination of isotopic enrichment, biological variations in the isotope enrichment, isotopic fractionation during formation of carbon dioxide and during vaporization of water, the calculation of total body water, and the assumption or calculation of the 24 h RQ. The use of the DLW method is not possible in the critically ill child because of the fluid shifts and imbalances.

The carbon dioxide production (VCO_2) during respiration has long been used as an index of substrate oxidation and energy expenditure. The isotopic dilution technique allows the ^{13}C from infused labeled bicarbonate ($NaH^{13}CO_3$) tracer to be diluted by metabolically produced carbon dioxide. By measuring the degree of isotopic dilution in expired air or blood, VCO_2 rates can be calculated. The assessment of energy expenditure must involve knowledge of the amount of energy released per liter of carbon dioxide produced, or the energy equivalents of CO_2, which constitutes the food quotient, which serves as a surrogate for RQ under conditions of nutrient balance. Two important concerns in relying on this technique relate to (1) errors in quantifying the tracer dose of labeled bicarbonate and (2) the possibility that the labeled bicarbonate does not adequately trace total CO_2 formed in the mitochondria. Another limitation is the need to know accurately the labeled bicarbonate correction factor(s) required for the physiological condition investigated (recovery factor). In summary, isotopic tracer techniques and IC should be considered as complementary techniques, in particular since the tracer techniques require the measurement of carbon dioxide production obtained by IC. However, it should be kept in mind that the assessment of substrate oxidation by IC may involve large errors in particular over a short period of time. By using IC, energy expenditure is calculated with less error than substrate oxidation rates.

In summary, energy needs must be carefully evaluated during hospitalization particularly during the course of critical illness by measuring the energy expenditure. If IC is available and the patient meets the conditions for a measurement test, IC provides an accurate way to measure REE. IC could be used in specific patient populations (targeted IC; Table 2) to prevent unintended underfeeding and overfeeding. In the absence of measured REE, equations to estimate energy needs may be used, but caution should be exercised in order to avoid incorrect estimation.

Table 2 Criteria for high risk for metabolic alterations and candidates for targeted indirect calorimetry per American Society for Parenteral and Enteral Nutrition (ASPEN)

1. Underweight (BMI <5th percentile for age) or at risk for overweight (BMI >85th percentile for age) or overweight (BMI >95th percentile)
2. Children with >10% weight gain or loss during ICU stay
3. Failure to consistently meet prescribed caloric goals (for >5 days)
4. Failure to wean or need to escalate respiratory support
5. Need for muscle relaxants for >7 days
6. Neurological trauma (traumatic, hypoxic, and/or ischemic) with evidence of dysautonomia
7. Oncological diagnoses (including children with stem cell or bone marrow transplant)
8. Children with thermal injury
9. Children requiring mechanical ventilatory support for >7 days
10. Children suspected to be severely hypermetabolic (status epilepticus, hyperthermia, systemic inflammatory response syndrome, dysautonomic storms, etc.) or hypometabolic (hypothermia, hypothyroidism, pentobarbital, midazolam coma, etc.)
11. Any patient with ICU length of stay >4 weeks may benefit from indirect calorimetry to assess adequacy of nutrient intake

BMI, body mass index; ICU, intensive care unit.

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Energy Provision for the Hospitalized Patient

In the critically ill patient, the goal of nutrition support is to provide adequate needs and prevent underfeeding and overfeeding. Multiple complications do occur attempting to convert a catabolic condition in an anabolic state including increased carbon dioxide production, hyperglycemia, hypertriglyceridemia, and fatty liver. Recent studies in critically ill adults indicate worse outcomes occur with low energy intake in patients with either low or very high body mass index. The inability to deliver adequate energy intake, resulting in negative protein–energy balance, has been associated with increased morbidity and mortality rates in adult ICU patients. Cumulative energy deficit also is associated with an increasing number of complications including increased length of ICU and hospital stay.

The magnitude of the stress response and the presence of malnutrition amplify the complications associated with undernutrition in the management of the critically ill patient admitted to the ICU. Nitrogen deficits accrue over short periods of time; therefore, it is important to remember this situation when malnourished patients in the ICU develop an acute metabolic stress response (i.e., sepsis and injury), because the level of nitrogen deficit to be tolerated is less compared to patients with the same level of stress and normal nutritional status; thus, delaying nutrition support in the malnourished patient beyond 5–7 days can potentially increase morbidity and mortality in this population. Another important aspect in the provision of nutrition support to patients admitted to the ICU is the prescription of overnutrition. This excessive nutrition comes as carbohydrates and protein. Hyperglycemia secondary to increased insulin resistance in addition to oversupply of dextrose can impose additional respiratory work with eventual development of fatty liver. Also, excessive amounts of protein in patients with underlying renal disease increase the risk for uremia-associated complications.

Enteral nutrition should be the feeding method of choice in critically ill patients because it replicates the normal pattern of nutrient consumption and hormonal homeostasis, maintains and improves gastrointestinal integrity, and reduces the incidence of multiorgan failure. Early enteral nutrition appears to be well tolerated in the general ICU population and is associated with early attainment of nutritional goals. The impact of early enteral nutrition and optimal energy balance might be most relevant in patients with preexisting malnutrition, who cannot afford added nutritional worsening during the course of the acute illness. Enteral nutrition is preferable to parenteral nutrition, but if enteral nutrition route is contraindicated or not tolerated, parenteral nutrition should be started after patient condition has stabilized. If enteral nutrition is partially tolerated or the rate of advancement is slow and does not cover energy and protein needs, then supplemental parenteral nutrition should be considered.

Recent studies in critically ill patients have found an association between the use of parenteral nutrition and increased morbidity and mortality. Since critically ill patients are highly dependent on substrate availability, have lower reserves, and are at risk of malnutrition, parenteral nutrition should be considered. Current recommendations agree on initiating parenteral nutrition if enteral nutrition has failed or it is contraindicated. If low-volume enteral nutrition is started and is inadequate to cover nutritional needs, supplemental parenteral nutrition should continue and be gradually decreased as enteral nutrition is advanced until full volume of enteral feeds covering nutritional requirements is achieved.

The use of clinical practice guidelines developed by multidisciplinary expert consensus and based on clinical evidence can help to improve nutrition support practices in the ICU. Most recent clinical guidelines for the critically ill patient by the American Society for Parenteral and Enteral Nutrition recommend to provide energy intake equivalent to BMR; this is based on extensive studies that reported measured energy expenditure in the patient admitted to the ICU. Regarding the provision of protein supplementation during periods of stress, current recommendations are to supplement 15–20% of the total nutrient intake to be provided as protein, with protein intakes of 1.5–2.0 g kg^{-1} day^{-1} for adult patients and 1.5–3 g kg^{-1} day^{-1} for infants and children.

The nutritional assessment of critically ill patient upon admission to the ICU should aim to identify those with existing malnutrition, and a tailored and individualized nutritional plan should be implemented in order to prevent underfeeding and overfeeding in these patients. Serial assessment of energy expenditure, by using IC, will guide energy intake and prevent cumulative imbalances. The most important aspect of the provision of nutrition support during periods of stress is the preservation of lean body mass; the amount of lean body mass has been shown to be associated with mortality. There are multiple barriers to the implementation of adequate nutrition with many of these barriers being preventable. Implementation and utilization of clinical nutrition guidelines may help achieve nutrition goals in the hospitalized patient.

See also: Amino Acids: Metabolism; Carbohydrate: Digestion, Absorption and Metabolism; Cholesterol: Absorption, Function and Metabolism; Fatty Acids: Metabolism; Fructose: Sources, Metabolism, and Health; Glucose: Metabolism and Regulation; Protein: Digestion, Absorption and Metabolism.

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Energy: Intake and Energy Requirements

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Introduction

Energy intakes (EIs) that meet energy requirements (ERs) can in principle be defined from physiological considerations of energy balance. Thus, when EI from food and drink matches total energy expenditure (TEE), there is energy balance with the body neither gaining nor losing energy. This means that either EI or TEE measured at energy balance could form the basis of a reference EI or ER value. In practice, measurement of either EI or TEE is not a simple task.

Energy Content of Foods

Within the International System of Units (SI), the unit of energy is joule (J) and the energy content of food is normally in kilojoule (kJ = 10^3 J) and megajoule (MJ = 10^6 J). The thermochemical calorie is also still widely used especially in the United States and is equivalent to 4.184 J (1 kcal = 4.184 kJ).

Food energy content can be determined in terms of the maximum amount of potentially available energy by measuring the heat released after its complete combustion to carbon dioxide and water in a bomb calorimeter. This is the gross chemical energy content (GE) of all organic food constituents of which the macronutrients carbohydrate, fat, protein, and alcohol account for the main components. However, not all GE from food is available for human metabolism because of losses during food utilization at the level of digestion, metabolism in the intestine, especially in the colon, and postabsorption metabolism. This means that the available energy to the organism, defined as metabolizable energy (ME), is the difference between GE intake and all losses in feces, in urine, a small amount in sweat, and in postprandial increases in metabolism. Energy losses in feces largely represent incomplete digestion and the energy content of bacteria produced in the colon, whereas energy losses in urine and sweat are due primarily to the urea content, which represents the incomplete catabolism of protein. The value quoted as the energy content of foods on food labels and in the food composition tables is the ME content. Historically, the ME content of food was calculated from the amounts of protein, fat, carbohydrate, and alcohol in the food (determined by chemical analysis) using specific energy conversion factors called the Atwater factors, which are estimates of the energy content of each macronutrient and alcohol, numerically rounded for practical purposes. These energy values are 17 kJ g^{-1} (4.0 kcal g^{-1}) for protein, 37 kJ g^{-1} (9.0 kcal g^{-1}) for fat, 17 kJ g^{-1} (4.0 kcal g^{-1}) for carbohydrates, and either a rounded value of 29 kJ g^{-1} (7.0 kcal g^{-1}) or an unrounded value of 6.9 kcal g^{-1} for alcohol. However, there are several issues that complicate the calculation of ME and that can result in differences between agencies in how ME is calculated.

For fat, the GE content will vary slightly according to the chain length and degree of saturation of constituent fatty acids in dietary triglycerides. Also, the ME will be less than the GE because of some loss as fecal fat. In practice, because dietary fat incorporates a mixture of different fatty acids, these differences, which could influence the GE, are ignored, and it is usually assumed that the digestibility is ≈95%. As a result, ME content of dietary fat is assumed to be 95% of GE, that is, 37 kJ (9.0 kcal g^{-1}), the general Atwater factor.

For carbohydrates, there are two complicating factors. Firstly, the GE of carbohydrates in food varies according to their molecular form, that is, glucose, disaccharides, and starch. The GE per unit weight is 15 kJ (3.6 kcal g^{-1}), 16 kJ (3.8 kcal g^{-1}), and 17 kJ (4.0 kcal g^{-1}), respectively. FAO has recommended that a conversion factor of 16 kJ g^{-1} (3.8 kcal g^{-1}) should be used when carbohydrate is expressed as monosaccharide equivalents and 17 kJ g^{-1} (4.0 kcal g^{-1}) when determined by direct chemical analysis of carbohydrate, that is, the general Atwater factor, and in the United Kingdom, this method is used. However, in the United States, carbohydrate is calculated from total dry weight of food minus protein, fat, and ash, and this will include unavailable carbohydrate (i.e., dietary fiber) and other noncarbohydrate components (e.g., lignin, organic acids, tannins, waxes, and Maillard products) in the calculated value. As a result, for a mixed diet, total EIs can be 12% higher and carbohydrate intake 14% higher when carbohydrate by difference is used in dietary analysis compared with available carbohydrate analyzed directly. Thus, estimates of the extent of underreporting in dietary surveys in the United States are often lower than those in the United Kingdom.

Secondly, carbohydrates include nonstarch polysaccharides (NSPs) and resistant starch; these may also provide energy through fermentation in the colon to form short-chain fatty acids. Some of these products are absorbed into the bloodstream or used locally by colonocytes as energy, and a conversion factor of 8 kJ g^{-1} (1.9 kcal g^{-1}) has been suggested by FAO. However, in the United Kingdom, the food composition tables ignore energy from NSPs and resistant starch and assume all carbohydrates are completely absorbed so that the ME is calculated using the general Atwater factor of 17 kJ g^{-1} (4.0 kcal g^{-1}), which is carbohydrate expressed as monosaccharide.

For protein, the GE can vary according to the amino acid content and the ME according to its digestibility and the amino acid content, which determines its nitrogen content and the amount of urea produced and excreted per gram of protein. These differences especially the digestibility can be large so that highly digestible animal proteins like in egg may yield 40% more ME than less digestible plant proteins. In practice, the ME content of dietary protein is assumed to be 17 kJ (4.0 kcal g^{-1}), the general Atwater factor, on the basis of an assumed loss of urinary energy as urea. This will be a relatively accurate value because dietary protein usually represents a mixture of several animal and

plant protein sources. Because the immediate metabolic fate of amino acids absorbed into the body includes deamination and other reactions such as gluconeogenesis that contribute to the postprandial increase in heat production (the thermic effect of food (TEF)), it has been suggested that this should also be accounted for in a value defined as the net metabolizable energy (NME). However, this value is not generally used and the TEF is usually assumed to be part of energy expenditure.

For alcohol, the ME is assumed to be 29 kJ (6.9 kcal)/g consumed, the unrounded general Atwater factor. Alcohol is oxidized completely and does not directly add to body energy stores. There is a component of postprandial thermogenesis following alcohol consumption with reported values ranging between 9% and 28%, but this is not included in the ME.

Energy Intakes

The amount of food energy consumed is assessed as part of national nutrition surveillance in many countries and in the course of many studies of diet and health interactions. However, it is well known that accurate measurement of dietary EI is difficult. Moreover, the extent of the unreliability of the various methods is not as widely appreciated as it should be. There are a number of ways in which food intakes can be measured, and the likelihood that the various methods are accurate can be tested to some extent by modeling intake values with predicted values of TEE to see if the values are physiologically sensible. This comparison is a valid test because on a daily basis, differences between EI and TEE are likely to be small and not usually measureable.

At the national population level, the FAO compiles data on food balance sheets, converted to per capita overall food energy supply. Clearly, this measure is unlikely to be useful in identifying variation in intakes between subpopulations or influences of age, gender, and lifestyle. Furthermore, food wastage at all points in the food supply chain will result in an overestimate of per capita overall food energy supply. This is most likely to have increased over recent decades through wastage, especially at the prehousehold level. An analysis of these data in the United Kingdom shows little change between 1961 and 1990 (at about 13.4 MJ/day/per capita); thereafter, however, this intake increases by 5% to the year 2000, and by a further 2% to 2009, eventually to reach 14.3 MJ/per capita/day. This value is higher than would be expected and modeling shows it to be an overestimate of EIs by about 40%.

At the household level, records of household food purchases adjusted for number and ages of household members enable a better estimate of per capita EI. In the United Kingdom, such records have been collected annually on a complete cross section of the British population from 1950. This is now the Expenditure and Food Survey, a 2-week diary record of all expenditure by each household member over the age of 7 years in about 5000 UK households. Such records are subject to both underestimation through underreporting of purchases and overestimation through food wastage in the home, although attempts are made to minimize the former and account for the latter error. Currently, reported values are 9.58 MJ/per person/day, which are 20–30% lower than values in 1955. This value is slightly lower than the lower range (25th centile) of the predicted current UK all-adult population TEE.

At an individual level, food EIs can be measured directly from questionnaires of food intakes recorded as the frequency of consumption of lists of individual food types, i.e., food frequency questionnaires; recorded as a recall of intakes over the previous 24 h, as dietary records collected over a number of days (e.g., 4–7 days), or most accurately as records of weighed food intakes over periods of up to 7 days. In national surveys such as NHANES (the US National Health and Nutrition Examination Survey) and NDNS (the UK National Diet and Nutrition survey), a balance is struck between likely accuracy and the numbers of subjects who can be studied. Currently, for the UK NDNS, 4-day dietary records are used with annual measurements in a rolling program collecting anthropometric and biochemical measures of nutritional status as well as food intakes. There has been a downward trend in the mean reported daily intakes for total energy for adult men and women from 1987, with the most recent value indicating 7.86 MJ day^{-1}. In fact, this value is much lower (and unphysiological) than would be predicted by simple modeling of likely TEE, almost certainly due to underreporting.

Energy Expenditure as the Basis for ERs

From the earlier, it is quite clear that measurement of EI is exceedingly difficult and unlikely to provide information that can be reliably transformed into dietary reference values (DRVs) for energy. In any case from what is known about the regulation of energy balance, it is clearly more physiologically appropriate to base the derivation of energy reference values on TEE than on EI. This is because, in humans, it appears that EI is finely regulated through the appetite mechanism to enable EI to match TEE, rather than the other way round. TEE comprises the basal metabolic rate (BMR), energy expended at rest in the postabsorptive state and at thermoneutrality, energy expended in physical activity (physical activity energy expenditure (PAEE)), and other components such as postprandial thermogenesis (TEF) and thermogenesis associated with growth. TEE does not appear to include a significant component of regulatory EE responsive to EI, that is, dietary-induced thermogenesis, apart from the heat increment of feeding that is associated in a near stoichiometric way with postprandial metabolism, mainly amino acid oxidation. The diet-related thermogenesis associated with uncoupled fat oxidation in brown adipose tissue mitochondria, which is a feature of rodent metabolism, is not believed to be quantitatively important in humans after the first months of life apart from a small, recently identified component of cold-induced nonshivering thermogenesis. Thus, it is TEE, mainly a function of size (BMR) and lifestyle (PAEE), that can be identified as the physiological determinant of the ER: that is, the ER can be predicted specifically as the rate of TEE plus any additional needs for growth, pregnancy, and lactation.

ERs for What Purpose and for Whom?

Given the context of target populations exposed to an obesogenic food supply and built and social environment, to which it is genetically susceptible, and that have become overweight, all recent definitions of the ER refer to healthy body weights.

For example, in the United Kingdom, the ER is defined as "the amount of food energy needed to balance energy expenditure in order to maintain body size, body composition and a level of necessary and desirable physical activity consistent with long-term good health. This includes the energy needed for the optimal growth and development of children, for the deposition of tissues during pregnancy, and for the production of milk during lactation consistent with the good health of mother and child." This specifically 'prescriptive' approach involves identifying ER values for infants, children, and adults in relation to best estimates of healthy body weights: for children, growth standards and reference weights that are below current UK values and, for adults, a weight associated with a minimum risk of mortality, that is, W equivalent to a BMI of 22.5 kg m^{-2} at actual heights. This is below the current average UK population BMI of 27 kg m^{-2}. Application of ER values derived in this way to under- or overweight groups will tend to mediate weight change toward the desirable range. The question of including within a prescriptive ER, sufficient EI to balance a 'desirable' level of PAEE, is also an important consideration given that much of the population is thought to be sedentary. Achieving this in practice, however, is much more difficult since any advice to overweight populations to consume more energy carries obvious risk. In practice, advice on desirable physical activity is usually given separately including information on the additional energy needed for various amounts of additional PAEE.

In the public health context, ER values can be defined as DRVs for food energy that provide a best estimate of the food energy needs of a population and its subgroups and represent criteria against which to judge the adequacy of their food EIs. For all nutrients other than energy, the DRV is identified as the reference nutrient intake (RNI), a value at the upper bound of the reference ranges, that is, the estimated average reference value (EAR) + two standard deviations, ensuring low risk of deficiency for any individual. However, for dietary energy, the DRV is defined differently: that is, as the EAR, itself; this is because an RNI defined as earlier would represent an excess EI for the majority of the population, leading to weight gain and obesity in the long term. In contrast, the EAR represents an intake associated with similar probabilities of excessive and insufficient EIs for any individual within the population. Because EIs and the requirement are highly correlated by virtue of the appetite mechanism's adjusting EI to match ER, such probabilities are much lower than would be expected, that is, $<50\%$. Thus, in adults, the DRV for energy is the EAR, that is, the intake associated with a healthy body weight at existing height and levels of physical activity. During infancy and childhood, the requirement also has to meet the needs for healthy growth and development, whereas during pregnancy and lactation, the requirement must meet the needs for development of a healthy baby, supporting adequate lactation while minimizing postpregnancy weight gain.

Utilizing Doubly Labeled Water-Derived Measurements of Energy Expenditure to Derive ERs

Measurement of TEE can be achieved by several methods. Short-term measurements under highly defined conditions can be made by calorimetry with direct calorimetry, measurement of heat loss from the subject in a calorimeter, the most accurate method. Indirect calorimetry, the most commonly used approach, measures oxygen consumption and/or carbon dioxide production from which TEE is calculated using standard formulas. The doubly labeled water (DLW) method is generally recognized as the most accurate measure of free-living TEE currently available. The DLW method is a minimally invasive stable isotopic technique of measuring carbon dioxide (CO_2) production in free-living subjects over a period of several weeks. After a measured dose of DLW (i.e., water containing the stable isotopes of hydrogen, ^2H, and oxygen, $^{18}O_2$), samples of saliva or urine are taken over 2 weeks, and the rate of dilution of the two isotopes in these samples is measured. As water is lost from the body, the two isotopes are lost and the enrichment of both ^2H and $^{18}O_2$ falls. However, $^{18}O_2$ in water exchanges with the oxygen in CO_2 because of the carbonic anhydrase reaction such that there is an additional loss of $^{18}O_2$ as $C^{18}O_2$. As a result, $^{18}O_2$ is lost from the body faster than ^2H with the difference providing a measure of the CO_2 production rate. TEE is then estimated from this rate after assigning an energy value to CO_2 calculated from the assumed average respiratory quotient value. This in turn is estimated from the macronutrient composition of the dietary intake. The method provides more accurate measures of TEE than other noncalorimetric methods such as heart rate monitoring and has dramatically improved our understanding of human energy expenditure.

However, making use of DLW-derived data is not straightforward with either of two main approaches that can be employed. The first involves a definition of the ER in which TEE is expressed in a factorial way. TEE can be considered as comprising three components, BMR, TEF, and PAEE. With TEF assumed to be about 10% of EI, then for subjects at energy balance (TEE = EI), $TEE = BMR + 0.1 \times TEE + PAEE$. However, PAEE will vary not only with the extent of physical activity but also as a result of body weight. Thus, an alternative way of expressing TEE is to normalize it for the BMR assuming this captures influences of weight age and gender by expressing it in terms of multiples of the BMR as the physical activity level (PAL), that is,

$$ER \equiv TEE = PAL \times BMR$$

BMR is predictable with adequate accuracy from anthropometric variables (weight, age, height, and gender). PAL is assumed to include just PAEE although any TEF will also be included. In the past, PAL has been derived from time-allocated lists of activities for which energy costs are known and PAL values or ranges have been identified that equate to lifestyles. Thus, in free-living populations, PAL can range from <1.3 in immobile subjects to between 3 and 4.7 for limited periods of time in soldiers on field exercises or elite endurance athletes, for example. Within the general population, however, the overall range of PAL values for individuals in energy balance and leading sustainable lifestyles is between 1.38 for the most sedentary and 2.5 for the most active. TEE can be predicted from BMR and a lifestyle-related value of PAL. DLW data can identify PAL values in population groups. In this case, BMR can be measured separately or predicted so that the values for TEE determined by the DLW method can be expressed in terms of PAL. This has allowed a much better understanding of the range and variation in PAL as a function of lifestyle.

The second approach models the DLW-derived TEE values against anthropometric characteristics of the reference population in regression equations, allowing TEE and ER values to be predicted for population groups: for example, children or adults. However, regression modeling does not easily allow for inter- and intraindividual variation in PAEE. Because of this, committees using this approach have had to make provision for the likely variation in PAEE within the population group by introducing multilevel ordinal variables derived from PAL data in the regression model to modify the total height and weight contribution to TEE.

Limitations of the Factorial Model of TEE

The increasing amount of reported DLW measurements of TEE has in fact allowed a detailed analysis of the various factorial models, which have all been shown to be unsatisfactory in terms of being able to predict TEE of population groups according to their lifestyles. This is because values predicted for individuals or groups according to their lifestyles do not correspond well with measured, DLW-based values. In fact, most DLW or whole body calorimeter studies show considerable variation in TEE between individuals involved in similar activities. A likely explanation for this is indicated by 24 h calorimeter and DLW studies showing a wide variation in PAL values (from 1.15 to 1.7) in subjects with restricted activity. Furthermore, these 24 h values correlate with free-living DLW-measured PALs (1.35–2.15). Such observations have led to the development of the concept of spontaneous physical activity (SPA). SPA is body movements associated with activities of daily living, change of posture, fidgeting, and 'a propensity for locomotion.' SPA is a highly reproducible, within-individual familial trait; it accounts for a considerable fraction of between-individual variation in TEE and displays an inverse relationship with future weight gain. Nonexercise activity thermogenesis is another term used to describe a somewhat extended definition of this phenomenon. Other individuals have been identified who exhibit marked reductions in activity, that is, to levels approaching the BMR, after periods of intense activity. The extent and variation in SPA, currently not quantifiable within population groups, calls into question the predictability of PAL from lifestyle information as currently collected, especially with the precision previously assumed.

A New Approach to the Factorial Model

The recent UK report on energy DRVs adopted an alternative approach to the use of PAL values in the definition of TEE and the ER of target population groups. In this report, it was concluded that when there are sufficient DLW measurements of TEE available for a particular population group to enable it to serve as a reference, the most useful information is the distribution of PAL values within that reference population. The assumption is made that the reference population groups are sufficiently demographically similar to the target population and that the DLW data are extensive enough and representative of the reference population. In this case, the distribution of PAL values observed in the reference population in terms of 25th, median, and 75th centiles can be used to predict TEE and hence the ER as a function of BMR for the less active, average, and more active members of the target population group. The principle is shown in Figure 1. The restriction of the range of ERs to just three values is clearly a major departure from previous approaches for adults. However, in most cases only the median population value will be used. It is in principle similar to the method adopted by FAO in their recommendations for children. However, it is an approach that recognizes the reality that prediction of rates of TEE for individuals or population groups is inherently uncertain and that, in practice, most health professionals use a very restricted range of ER values.

Identifying Suitable PAL and EAR Reference Values for Adults

Identifying reference, DLW-derived PAL values is difficult because most published studies contain only small numbers of subjects recruited in various ways for various reasons, that is, not necessarily representative. For example, in a recent meta-analysis of DLW data of adults, 4971 individuals were identified. However, these subjects derived from 183 cohorts with a median cohort size of only 14 and with only 10% having >70 subjects. If few of the individual cohorts are representative of the general population, then it cannot be assumed that combined data sets will be representative either. In contrast, DLW studies of energy expenditure have been reported for two large, randomly selected, urban-population cohorts, with a combined population of 929 men and women aged 30–69 years. The two study populations were quite similar, both being recruited from the Washington, DC, metropolitan area. They were judged to be a reasonable match to the UK population in terms of the extent of overweight and obesity (mean BMI values of 27.2 kg m^{-2}: current UK values 27 kg m^{-2}) and ethnicity. PAL was evaluated for the whole cohort and the median and distribution of PAL values within each cohort did not differ and were not influenced by gender. For the combined cohort ($n = 929$), the distribution, shown in Figure 1, was skewed, with subjects clustered at the lower end of the range. PAL values of 1.49, 1.63, and 1.78 were identified for the 25th, median, and 75th centiles, corresponding to sedentary, low, and moderate activity. Neither median value nor distribution of PAL values for BMI categories differed significantly, and the regression of PAL on BMI was nonsignificant ($p = 0.64$ for slope: $R^2 < 0.1\%$). This suggests that with increasing BMI, the decrease in extent of PAEE is matched by its increasing energy cost. PAL fell slightly with age, although age explained only 0.4% of the variance, that is, falling from PAL values of 1.69 at 30 years to 1.63 at 70 years. Although the minimum age was 30, PAL values of the youngest subjects aged ≤35 years did not differ from those aged >35 years suggesting that the lack of 18–30 years of age is unlikely to represent a significant source of bias when the data set is used to represent PAL values for all adults. Derived EAR values for adult men and women are shown in Table 1.

Identifying Suitable PAL and EAR Reference Values for Infants, Children, and Adolescents

For infants, most recent committees have calculated energy reference values from the energy deposited in new tissue plus TEE. The energy costs of tissue deposition have been calculated

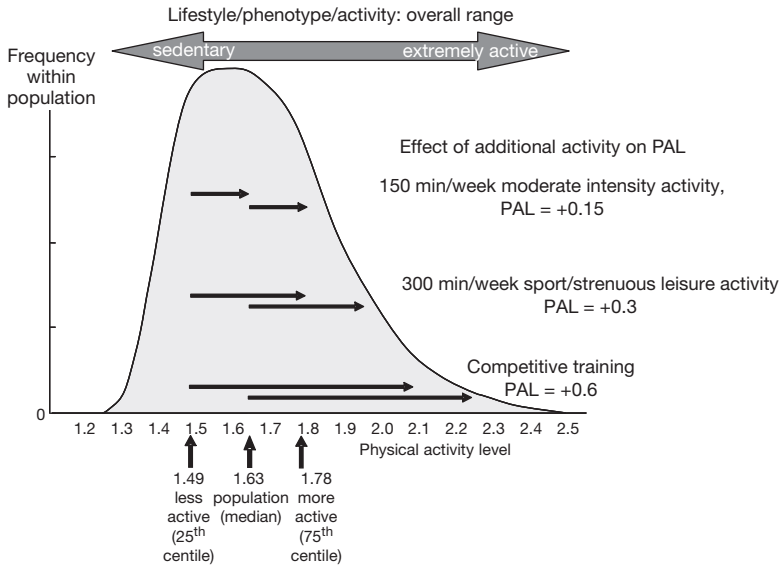

Figure 1 Schematic representation of energy expenditure and consequent energy requirements of the adult population as a function of the physical activity (PAL) value. From the UK report Dietary Reference Values for Energy 2011, Scientific Advisory Committee on Nutrition London TSO 2012. The distribution of PAL values is that of the reference population identified as appropriate to define the UK dietary reference values for energy. The likely increases in PAL (and consequent energy needs) shown for various activities include the current UK recommendations that adults participate in at least 150 min of moderate intensity activity per week: an increase in PAL of 0.15. These increases in activity are shown as they would affect initial PAL values at the 25th centile and median values, with the increases indicated by the length of the arrows. Although for those with PAL values at the 25th centile, the recommendations for increased activity will only increase PAL to the median level, this can still be expected to have health benefits. The increases shown are only approximate. They will vary to a small extent with the initial PAL, that is, with the intensity of the activity replaced, and will be slightly less than the values shown, especially for subjects with high initial PAL values. The equivalent values in terms of energy are shown in **Table 1**.

Table 1 Estimated average energy requirements for adults (MJ day^{-1})

				EAR values (MJ day^{-1})		
	Height (cm)	*Weight (kg)*	*BMR (MJ day^{-1})*	*Less active*	*Population*	*More active*
Men	175	69.2	6.7	10.0	10.9	11.9
+30 min moderate intensity activity, 5 days per week: +PAL = 0.15					+1	
+60 min active sport five times per week: +PAL = 0.30					+2	
+intense aerobic exercise in competitive training: +PAL = 0.60					+4	
Women	162	58.7	5.4	8.0	8.7	9.5
+30 min moderate intensity activity, 5 days per week: +PAL = 0.15					+0.8	
+60 min active sport five times per week: +PAL = 0.30					+1.6	
+intense aerobic exercise in competitive training: +PAL = 0.60					+3.2	

Values shown for illustration are prescriptive aimed for a healthy body weight equivalent to a BMI of 22.5 kg m^{-2} at mean adult heights for the UK population. BMR is calculated from the Henry prediction equations (Henry, C. J. (2005) Basal metabolic rate studies in humans: measurement and development of new equations. *Public Health Nutrition* **8**:1133–1152), based on weight, age, sex, and height. EAR values calculated as BMR × PAL where PAL = 1.49 (less active), 1.63 (population), and 1.78 (more active). Also shown are additional daily energy needs associated with the selective amounts of increased activity shown in **Figure 1**.

Source: Values derived from the UK report, Dietary Recommendations for Energy 2011, Scientific Advisory Committee on Nutrition London TSO 2012.

from an analysis of the body composition of healthy infants during normal growth. Longitudinal DLW studies of TEE over the first 2 years of life have been performed in healthy, well-nourished, nonstunted infants, born at full term with adequate birth weight, who have been either breast-fed or formula-fed. These measurements have allowed the development of simple prediction equations for TEE as a function of weight for breast-fed, formula-fed, and mixed-fed infants, in the latter case by predicting TEE values intermediate between the breast-fed and formula-fed values. EAR values for energy have then been calculated from the sum of TEE and energy deposition for breast-fed, formula-fed, or mixed-fed infants. For the United Kingdom, EAR values are calculated from weights of children growing along the trajectory of the UK-WHO Growth Standards. Although it is recognized that exclusive breast-feeding is highly unlikely to continue beyond the recommended first 6 months of life, in most recent reports, EAR values have been calculated by this model for the first 12 months of life for each mode of feeding (adopted during the first 6 months) and tabulated for boys and girls at monthly intervals. This is because after 12 months, a different calculation of the EAR is used as described later. For boy infants, EAR values increase

from 2.03, 2.36, and 2.17 MJ day^{-1} for breast-fed, formula-fed, and mixed-fed infants at 1 month to 3.2, 3.3, and 3.25 MJ day^{-1} at 12 months, respectively. For girl infants, EAR values increase from 1.82, 2.16, and 1.97 MJ day^{-1} for breast-fed, formula-fed, and mixed-fed infants at 1 month to 2.91, 3.05, and 2.98 MJ day^{-1} at 12 months, respectively.

For children and adolescents, the same principle used to derive EAR values for adults is used based on TEE derived from PAL × BMR. However, because of potential changes in age in both body composition and behavior that may influence TEE, the starting point for derivation of EAR values is an analysis of changes in PAL with age for boys and girls. In fact, while there are a large number of published DLW studies of infants, children, and adolescents, not all of them allow individual PAL values to be calculated, and most of the studies involve small numbers (average $n = 20$). Thus, only a limited analysis of the distribution of PAL values at each age and each gender can be performed. In fact, PAL values were obtained for 170 studies (3502 individual measurements, 2082 females, and 1420 males), with reported values for every age from 1.5 to 18 years allowing mean study values for boys and girls to be examined as a function of age, as shown in Figure 2. PAL values were not influenced by gender, but did increase with age; this effect is driven by low values for preschool children and some groups of preadolescents. As a result, all studies were grouped into three age categories ≤3, >3 to <10, and 10–18 years. Whether this age increase in PAL is a physiologically or behaviorally real phenomenon once children enter schooling is not clear since several studies of children aged 5–10.5 years show individual PAL values that vary markedly (e.g., from 1.19 to 2.34). It is possible therefore that schoolchildren from a very early age exhibit a similar very wide range of activities and PAL values as observed in adults.

EAR values for children also include the cost of energy deposition during growth, which is substantial in the first year of life, tapering off thereafter. Thus, for preschool children, schoolchildren, and adolescents, growth costs are much less, and the energy deposited can be accounted for by a simple +1% adjustment of PAL (PAL × 1.01), which results in acceptably low levels of error. This resulted in adjusted median PAL values for the three age groups of 1.40, 1.58, and 1.75. These values were used to define the population EAR values of children as a function of BMR predicted for age, weight, height, and gender. Population EAR values for energy as a function of age are shown in Figure 3. The gender differences reflect the slightly higher BMR values for boys due to differences in weight and body composition.

Energy Reference Values for Pregnancy

It is customary to calculate the ERs for pregnancy and lactation as increments to be added to the mother's EAR. Ideally, women should begin pregnancy at a healthy body weight, and this raises the question as to whether ER values for pregnant women should be calculated as for nonpregnant women on their healthy body weight (i.e., a BMI of 22.5 kg m^{-2}) or on their actual body weights. It is the case that women who are overweight or underweight at the beginning of pregnancy are at risk of poor maternal and fetal outcomes, although this is not completely understood. Because of this uncertainty, it is usual practice to adopt a precautionary approach and not to make ER recommendations aimed at weight loss for

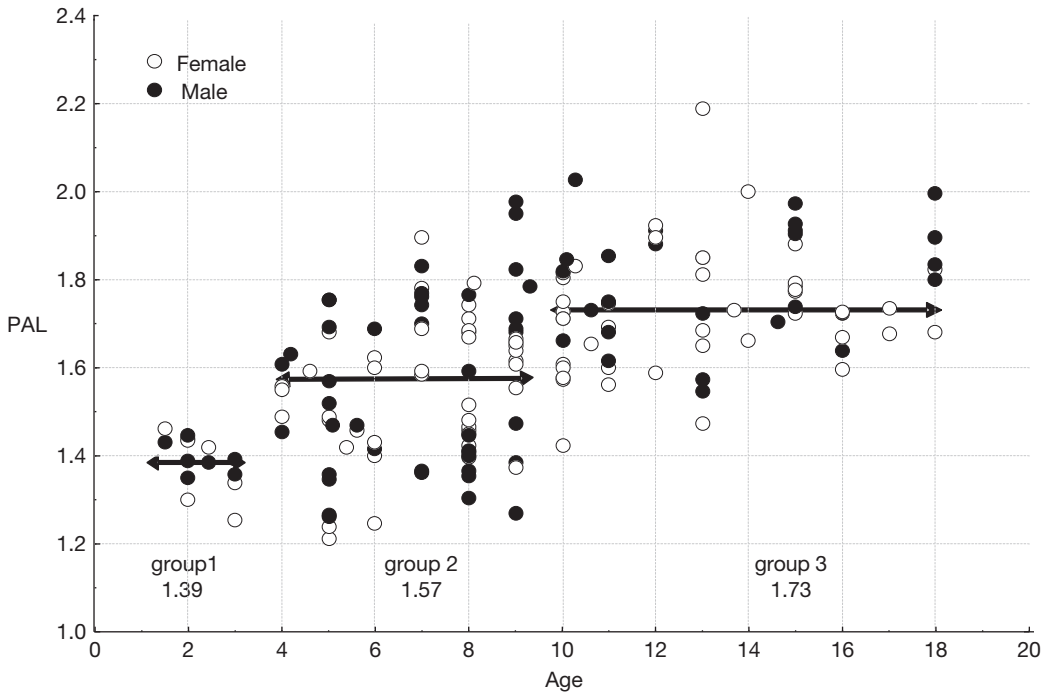

Figure 2 PAL values for energy (MJ day^{-1}) for infants, children, and adolescents. From the UK report Dietary Reference Values for Energy 2011, Scientific Advisory Committee on Nutrition London TSO 2012. Median values for the indicated age ranges are shown. Each point represents a single age group reported in a single publication or in publications, which list mean values for each of several age groups. Boys and girls are shown separately.

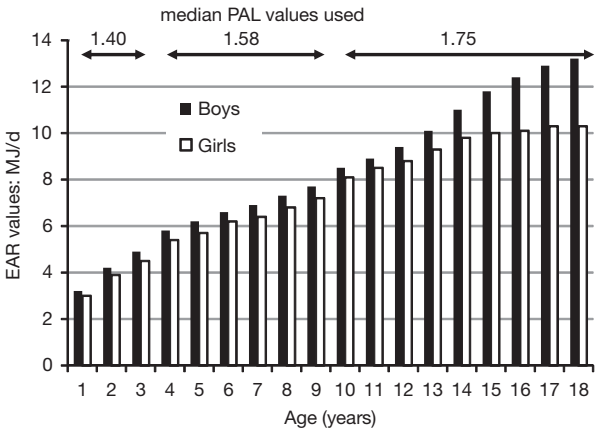

Figure 3 EAR values for energy (MJ day^{-1}) for infants, children, and adolescents. Values derived from the UK report Dietary Reference Values for Energy 2010, Scientific Advisory Committee on Nutrition London TSO 2012. Values are calculated from BMR × PAL values shown for the various age groups and are prescriptive values calculated for healthy weights at each age. PAL values are median values for the published DLW data. BMR values are calculated from the Henry equations (reproduced from Henry, C. J. (2005). Basal metabolic rate studies in humans: measurement and development of new equations. *Public Health Nutrition* **8**, 1133–1152), using weights and heights indicated by the 50th centiles of the UK-WHO Growth Standards (ages 1–4 years) and the UK 1990 reference for children and adolescents (Royal College of Paediatrics and Child Health (2011) UK-WHO Growth Charts: early years. Available at http://www.rcpch.ac.uk/growthcharts).

overweight pregnant women. For this reason, the recent UK report identified EARs for pregnancy and lactation as estimates of the incremental EIs likely to be associated with healthy outcomes for mother and child that should be added to EAR values calculated at actual preconceptional body weights.

As to the amount of extra food energy required for pregnant women, the key issue is that of identifying the extent to which the demands for gestational weight gain (GWG) due to growth of the products of conception and of maternal weight can be met by adaptive physiological and behavioral changes of the mother as opposed to increased dietary EIs. A balance needs to be struck between allowing for optimal fetal growth and avoiding the excessive weight gain likely to occur with overestimates of energy needs. In this context, the recent UK report concluded that, in general, it is unlikely that women require extra energy in the first trimester of pregnancy and compensatory reductions in PAEE during the second and third trimesters are likely to reduce the demand for extra EI at this time. Furthermore, although careful studies of energy expenditure and deposition indicate an apparent need for additional energy, such calculations include maternal fat deposition, which is not a determinant of birth weight. Indeed, on the one hand, longitudinal body composition studies have indicated that some fat deposition remains 6 months postpartum and may contribute to weight gain in women through successive pregnancies. This is undoubtedly undesirable. On the other hand, there is strong evidence that inadequate GWG is associated with decreased birth weight and fetal growth (small for gestational age). Recommendations for constraining weight gain may also be inappropriate for pregnancies in vulnerable groups such as teenagers. On this basis, it was concluded that the EAR for pregnancy should be limited to an additional intake of 0.8 MJ day^{-1} (191 kcal day^{-1}) during the last trimester.

Energy Reference Values for Lactation

The energy needs for lactation can be calculated from DLW measurements of energy expenditure and the amounts and composition of breast milk and estimated energy mobilization from tissue stores. On the basis of a milk energy output of 500 kcal day^{-1} and an average weight loss of 0.8 kg month^{-1}, which is equivalent to 170 kcal day^{-1}, this would imply the need for an increment of 330 kcal day^{-1} (or 1.38 MJ day^{-1}) for the first 6 months during which time exclusive breast-feeding is recommended.

After the first 6 months, the energy cost of lactation will depend on the amount of breast milk consumed by the infant, which is likely to be diminishing. The need for additional maternal EI will also be modified by maternal body composition, so recommendations for additional intakes are generally not identified for this period of the infant's life.

Conclusion

Historically, reference values for dietary food energy needs developed from crude observations of food EIs to an assumed detailed and extensive understanding of the energy costs of human activities. However, the advent of DLW studies of actual energy expenditure has revealed that the assumed ability to predict accurately the energy needs of individuals or population groups with certainty was overly optimistic. Because of this, the most recent national report has markedly simplified the estimation of energy needs identifying only three activity levels for any population group. These are a population EAR value based on a PAL value of 1.63 and BMR at weights equivalent to a BMI of 22.5 kg m^{-2} at individual heights. Given that for UK men and women, mean heights are 175 and 162 cm, respectively; this equates to 10.9 (2605) and 8.7 (2079) MJ day^{-1} (kcal day^{-1}), respectively, as shown in Table 1. For the chronically ill or for immobile elderly men and women, a lower PAL value is appropriate with the 25th centile PAL value (PAL = 1.49), corresponding to ER values of 10.0 (2393) and 8.0 (1915) MJ day^{-1} (kcal day^{-1}). For men or women responding to advice to generally increase activity, the 75th centile PAL value (PAL = 1.78) is more appropriate and corresponds to ER values of 11.9 (2848) and 9.5 (2274) MJ day^{-1} (kcal day^{-1}).

In each case, these values are prescriptive in terms of supporting a healthy body weight. Thus, for the overweight or obese, that is, the majority of the UK population, the population ER values are less than weight-maintaining EIs and should allow for some degree of weight loss. However, the recommendations are not prescriptive in terms of physical activity although the extent to which PAL values will be higher for those specifically engaged in additional physical activity can be predicted as shown in Figure 1, with daily energy increments shown in Table 1. Thus, for those engaged in 30 min of moderate, intensity activity for 5 or more days of the week, 60 min of active sport five times per week, or intense aerobic

exercise associated with training for competitive sport, the ER will increase by an amount equivalent to 0.15, 0.3, or 0.6 PAL units, respectively. Importantly, the principle of a reference population distribution of PAL values as the basis for EAR values allows for simple updates as appropriate DLW data accumulate.

See also: Dietary References: US; Dietary Surveys: National Food Intake; Energy Metabolism; Food Composition Databases.

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Relevant Websites

http://www.cdc.gov/nchs/nhanes.htm – NHANES, National Health and Nutrition Examination Survey.

http://www.efsa.europa.eu/ – EFSA European Food Safety Authority.

http://www.food.gov.uk/the-website-of-the-food-standards-agency – Food Standards Agency.

https://www.gov.uk/government/statistics/national-diet-and-nutrition-survey-results-from-years-1-to-4-combined-of-the-rolling-programme-for-2008-and-2009-to-2011-and-2012 – NDNS, National Diet and Nutrition survey.

Enteral Feeding

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Definition

Enteral nutrition (EN) can be defined as a set of therapeutic procedures applied to solely or partially replace or supplement oral feeding for maintenance or recovery of nutritional status of malnourished or eutrophic patients in hospital, ambulatory or home settings, through the enteral (gastrointestinal tract) administration of commercial or noncommercial diets composed by controlled predefined or estimated amount of isolated or combined nutrients and specially formulated and developed for use by enteral probes or oral intake, aiming at the synthesis or maintenance of tissues, organs, or systems.

Relevance

EN is part of specialized nutritional therapy, which also includes parenteral nutrition (PN) (endovenous nutritional supply). Its main relevance is to restore or maintain the nutritional status of patients in order to prevent malnutrition. It has long been known that malnutrition is associated with increased morbidity and mortality in hospitalized patients.

Both EN and PN allow achieving the protein-calorie requirements and the minimum daily requirement of vitamins and minerals. When the gastrointestinal tract is structurally and functionally intact, it is preferred to use EN. When you cannot reach 60% of caloric needs by EN, we must consider the associated use of PN.

There are metabolic, safety, cost/benefit, and mainly physiological benefits when providing EN. The supply of nutrients through the digestive system maintains intestinal anatomy and its natural microbiota. These benefits allow the improvement of the intestinal immune system and the decrease of infectious complication risk in surgical patients.

Indications

EN should be indicated in situations in which the digestive tract is fully or partially functional and when oral intake is insufficient to reach two-thirds to three-quarters of the daily nutritional needs, mainly in malnutrition conditions. The main indications for EN use are listed in Table 1. In the other hand, EN is relative or temporarily contraindicated in some conditions, such as gastrointestinal tract obstruction, diarrhea and vomiting refractory to drug therapy, short bowel syndrome with severe malabsorption, severe paralytic ileus, high-output intestinal fistulas, massive bleeding from the gastrointestinal tract, severe malabsorption syndrome, and inability to access gastrointestinal tract.

Planning

Route of Feeding

After the indication of the route of feeding, the time by which EN will be required should be estimated to then proceed with the best access choice. Particularly for surgical patients, the estimate must comprise the time of pre- and postoperative or perioperative (both) nutrition. At least 10 days of preoperative EN are associated with improved nutritional status in moderate malnourished patients. However, patients with severe malnutrition associated with other clinical comorbidities may require longer EN treatment, particularly those with benign diseases.

Short-term EN (less than 6 weeks) is achieved using a nasogastric feeding tube (in gastric, duodenal, or jejunal position). Insertion of nasogastric feeding tubes in gastric or postpyloric position can be done manually or with endoscopic or fluoroscopic assistance and at the bedside in most cases. Although rare, complications can occur during the passage of a nasogastric feeding tube, such as pneumothorax, empyema, mediastite, esophageal perforation, bleeding and gastric perforation, injury to teeth, tracheobronchial injury, and arrhythmias.

For long-term EN (more than 6 weeks) gastric or jejunal nutrition ostomies are preferred. Gastrostomy and jejunostomy nutrition can be performed through open or percutaneous surgery using endoscopic, radiological, or laparoscopic techniques. Planning for the nomination and selection of the EN access is provided in Scheme 1.

After the choice of a nasogastric feeding tube or ostomy, it should be decided where the distal end of the feeding tube will be placed in gastric or intestinal position. Gastric access can be obtained with nasogastric or gastrostomy and the postpyloric access through nasojejunal feeding tube, gastrojejunostomy, or jejunostomy. Some criteria applied to determine the placement of a nasogastric feeding tube include the speed of gastric emptying, gastroparesis, use of drugs inhibiting gastric and digestive motility, and risk of pulmonary aspiration.

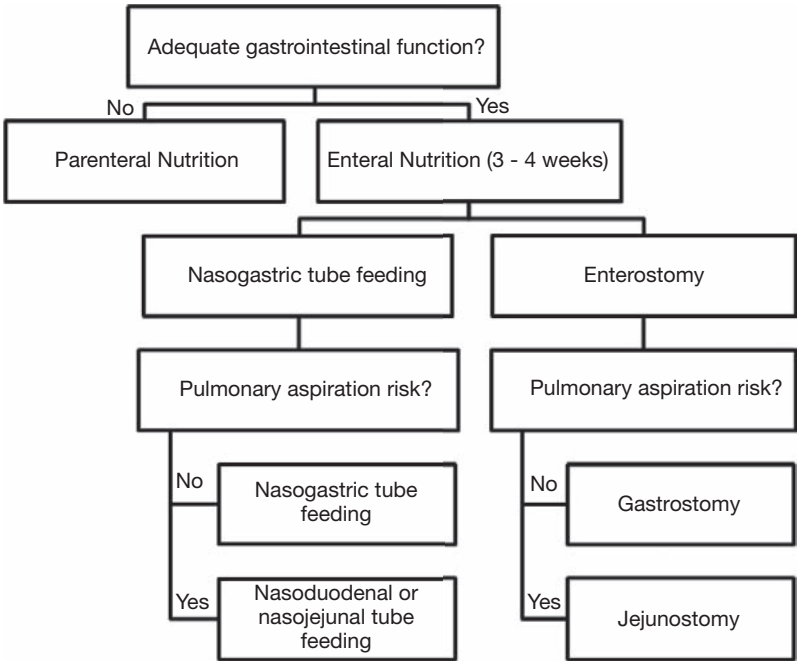
Formulation

The EN can be administered intermittently or continuously. Selection of pathway for EN administration and the type of infusion to be adopted will influence its formulation design. This also involves determining the total period for diet administration, the volume to be infused, infusion rate, if gravity drip will be used, and in which form it will be provided (infusion pump or by bolus). Table 2 outlines the programming of EN according to feeding tube positioning in pre- or postpyloric location.

Enteral formulations should be nutritionally complete when used as exclusive nutrition or as a supplement to patients with normal oral ingestion; or nutritionally incomplete when

Table 1 Enteral nutrition indication for adult patients

Clinical setting	*Recommendation*
Cancer	Less than 60% of energy needs intake for 5 consecutive days
	Dysphagia
Elderly	Severe neurologic dysphagia
	Emotional depression at hyporexia phase
	Contraindicative for terminal dementia
Surgery	High nutritional risk (5–7 preoperative days)
	Head and neck cancer (early – 24 h postoperative)
	Gastrointestinal cancer (early – 24 h postoperative)
	Severe trauma (early – 24 h postoperative)
	Malnutrition at the surgery period (early – 24 h postoperative)
	Insufficient oral intake to achieve basal energy expenditure for more than 10 postoperative days (early – 24 h postoperative)
Heart disease	Loss of body weight (6%) in 6 months (heart cachexia)
Crohn disease or ulcerative colitis	Nutritional supplementation higher than 600 kcal day^{-1} (consider)



Scheme 1 Planning for indication and pathway selection of enteral nutrition.

used only as a supplement nutrition. The evaluation of the digestive and absorptive capacity of the patient should be performed for better enteral formula selection (Scheme 2).

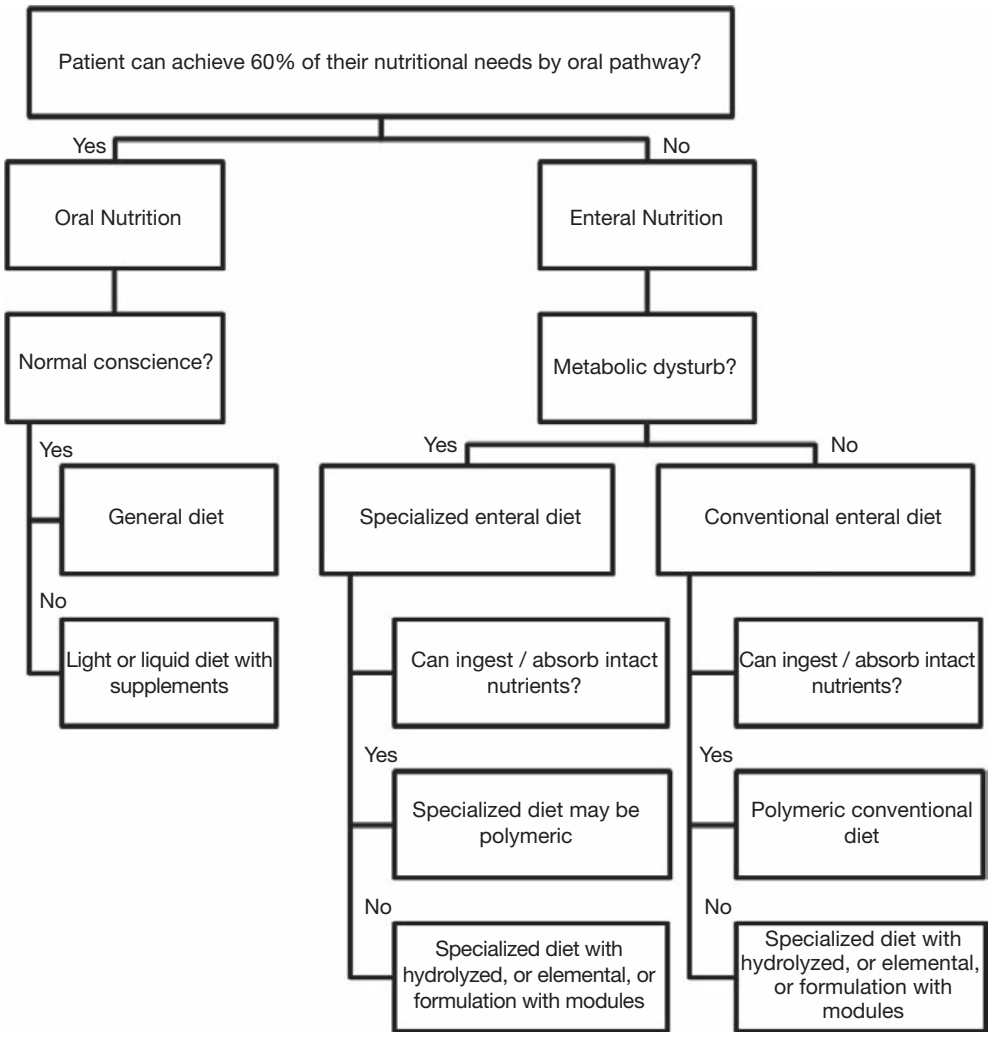
Several enteral formulations are based on fresh food, processed food, or both fresh and processed food. Therefore, nutrients comprising EN are generally the same constituents of a normal diet, consumed by the oral route, including carbohydrate (40–60% total energy needs), protein (14–20% total energy needs), fat (15–30% energy needs), and fiber (40–20 g l^{-1}). Different factors should be considered to facilitate the choice of the most appropriate enteral formulation for patients with EN indication, such as caloric density, osmolarity and osmolality, administration pathway, source and complexity of nutrients, and disease.

The EN caloric density (kcal ml^{-1}) should be based on the patient's total calorie needs versus the volume of enteral diets to be administered per day. Enteral diets with higher energy density have a lower amount of water, which can range from 690 to 860 ml l^{-1} diet. The categorization of enteral formulas, according to its energy density, is shown in Table 3.

Vitamin and mineral supply varies according to the specific needs of the patients and their disease. In the specific nutritional needs, you should evaluate the indication of additional micronutrient supplementation, even when the formulation, per se, achieves those values recommended by the Recommended Dietary Allowance (RDA). Clinical nutritional patient evaluation should include objective and/or subjective indicators to identify, as early as possible, any risk of specific

Table 2 Programming of EN according to feeding tube positioning

Tube feeding position	*Volume*	*Osmolality*	*Fractionation*	*Administration time*
Stomach	Allows high-volume supply	Hyperosmolar solutions are tolerated, but the higher solution osmolality the slower stomach emptying	Depends on the total volume/day and patient tolerance. Lower fractionation (four to six times per day) and higher volume in each supply may be used	About 120 drops per min (or time (min) = total volume (ml)/6) from the beginning of therapy
Postpyloric	During intermittent supply, the volume should not exceed 300 ml h^{-1} in adapted patients	Better tolerance for formulations with less than 550 mOsm l^{-1}; dripping of hyperosmolar solutions should be strictly controlled by using infusion pump	Continuous or intermittent fractionation, generally between six and eight supplies per day in each 3 h	Initial phase: 60 drops/min (or time (min) = total volume (ml)/3); 'adapted' phase: 120 drops per min (or time (min) = total volume (ml)/6)



Scheme 2 Planning for selection of enteral diets.

micronutrient deficiency for it to be immediately corrected and/or prevented.

Some specialized and very specific formulations to particular clinical situation (e.g., renal failure) are insufficient in some vitamin and mineral supply. Therefore, EN dietary planning attends to the need for supplementation or not of these micronutrients. For the long-term use of incomplete enteral feeding, the supplemental vitamins and minerals should be indicated.

Table 3 Categorization of enteral formulas according to its energy density

Energy density	*Value (kcal ml^{-1})*	*Formula*
Very low	<0.6	Sharply hypocaloric
Low	0.6–0.8	Hypocaloric
Standard	0.9–1.2	Normocaloric
High	1.3–1.5	Hypercaloric
Very high	>1.5	Sharply hypercaloric

In patients with malabsorption syndromes, investigate the possible fat soluble vitamins (A, D, E, and K) deficiency to correct it shortly. There is a lack of specific vitamin and mineral recommendations for critically ill patients. However, in such a condition, the needs of antioxidant nutrients are increased due the oxidative stress, and it is recommended to supplement vitamins A, C, and E, zinc, and selenium.

EN osmolality (mmol l^{-1} solution) and osmolality (mOsm kg^{-1} water) are associated with its digestive tolerance. Although the stomach tolerates diets with higher osmolality, more distal portions of the gastrointestinal tract respond better to isosmolares formulations. Therefore, hyperosmolar diets infused by gastrostomy or nasogastric feeding tube have better digestive tolerance when compared with administration by postpyloric or jejunal probes.

The nutrients that most affect the osmolality of a solution are simple carbohydrates (mono- and disaccharides), which have greater osmotic effect than the higher molecular weight carbohydrates (starch); minerals and electrolytes, due the property of its dissociation into smaller particles (e.g., sodium, potassium, and chloride); hydrolyzed proteins; crystalline amino acids; as well as medium-chain triglycerides, because they are more soluble than long-chain triglycerides. The more hydrolysates components contains the formulation, the higher its osmolality.

Enteral diets should not exceed the value of the renal solute load tolerated by the kidneys (800–1200 mOsm, in normal situation). Renal solute load can be calculated by adding 1 mOsm for each mEq of sodium/potassium/chloride, and 5.7 mOsm (adults) or 4 mOsm (children) for each gram of protein from its formula. Special attention should be given to critical clinical situations, such as sepsis, postoperative, polytrauma, and severe burn, where the urine becomes very dense, with high osmolality (around 500–1000 mOsm kg^{-1}), even under appropriate hydration.

Importantly, the influence of the medication osmolality is usually neglected. The mean osmolality of liquid medications administered orally or by feeding tube ranges from 450 to 10 950 mOsm kg^{-1} water. Certain manifestations of gastrointestinal intolerance may be related to the medication, although it is often attributed to enteral formulation.

In specific clinical situations, there may be demands for change in the types of nutrients used; the quantity and/or form these should be presented. In such cases, nutritional therapy becomes more specialized. These adaptations involve changes from simple source of nutrients used until its physicochemical and structural modifications. Thus, specialized formulations for enteral use may provide different sources of vitamins, minerals, carbohydrates, lipids, and proteins, and these nutrients may be presented in their entirety or hydrolyzed (wholly or partly) structure.

Some specialized EN formulations are part of immunonutrition. The immunonutrition is a nutritional intervention that explores the particular activity of various nutrients in alleviating inflammation and modulating the immune system, in which are included the omega-3 fatty acids, arginine, glutamine, nucleotides, and antioxidants. There is a current consensus that perioperative immunonutrition can beneficiate elective surgical patients, especially those malnourished patients submitted to major gastrointestinal surgery. In these patients, administration of enteral diets containing n-3 PUFA, nucleotides and arginine contributes to decrease postoperative infectious and noninfectious complications and must be initiated 5–7 days preop (500–1000 ml day^{-1}) and maintained in the postoperative period.

Although the benefit of using this enteral formula combining different nutrients with immunomodulatory functions is well established in surgical patients, data are lacking to confirm or guide the effective and safe use of enteral diets containing isolated immunonutrients in different clinical populations, including arginine and glutamine. In hemodynamically stable condition, arginine may offer immunologic and metabolic benefits, but its participation in the synthesis of nitric oxide may constitute a potential risk for septic patients. Enteral glutamine should be considered to treat burn patients and trauma victims, but there is not sufficient evidence for its use in critically ill patients with failure of multiple systems.

Other nutrients that may compose specialized EN formulations include the branched chain amino acids (BCAAs). BCAAs provide primary fuel for skeletal muscle during stress and sepsis. Therefore, leucine, isoleucine, and valine may be added to specialized EN formulas as supplemental metabolic sources to attend the metabolic needs of skeletal muscle during hypermetabolic conditions.

Administration

EN should be initiated as soon as possible to prevent intestinal atrophy and its associated complications (mainly infection) due the lack of nutrients, a concept known as early EN. In critically ill patients, EN should be started within the first 24–48 h after admission. Under the metabolic point of view, the use of early EN can prevent excessive secretion of catabolic hormones by inhibiting the increase in serum cortisol and glucagon and maintain nutritional status to prevent loss of body weight and muscle mass and negative nitrogen balance.

However, the benefits of early EN do not extend to all patients, and its efficient use is restricted by the need for hemodynamic stability and adequate tissue perfusion and visceral oxygenation. Therefore, some clinical conditions, such as paralytic ileus, abdominal distention, nausea, and vomiting, may hinder the selection of potential candidates to benefit from early EN. The points that limit the indication of early EN remain to be clearly defined, with consensus and controversy points, which can give rise to different clinical behaviors.

When the nasogastric feeding tube is placed in the stomach, the concern about the dose and infusion rate is not so relevant due the local adaptation mechanisms. Intermittent gastric administration (30–60 min each one) can be initiated with the volume of 60 ml in its total concentration and progress up to 250 ml each four hours, respecting tolerance and nutritional purpose; whereas continuous infusion can be initiated

with the volume of 10–40 ml h^{-1} in its total concentration and progress from 10 to 20 ml every 8–12 h, as tolerated.

For postpyloric (duodenum or jejunum) access, attention must be increased because fast dripping can cause colic and diarrhea, with decreased caloric use and patient injury. With the technique of continuous duodenal infusion, the dose and rate to be employed correspond to those described for the intragastric placement, with the difference that the concentration of the diet should be iso- or hypotonic.

Monitoring

Nasogastric or by ostomy feeding is associated with mechanical, gastrointestinal, infectious, pulmonary, and otorhinolaryngologic complications, which is prevented or treated by monitoring patients appropriately. Aspiratory pneumonia is considered the most serious complication in EN. It can occur due to excessive diet supply, delayed gastric emptying (with potential risk for neurological disability of the reflex mechanisms for vomiting protection) and paralytic ileus.

Aspiration of gastric residue may be useful to evaluate its emptying and to avoid the risk of regurgitation and aspiration, despite a lack of consensus for its routine use. For gastric residues larger than 200 ml (nasogastric feeding tube) or than 100 ml (gastrostomy) associated with abdominal discomfort and distension, the EN must be must interrupted and the patient clinically and radiologically investigated. When not associated with digestive symptoms, it is recommended to delay the diet for an hour and reassess the gastric residual volume. Some drugs, such as erythromycin, metoclopramide, bromopride and donperidona can be used to accelerate gastric motility.

Among the EN-associated complications, diarrhea diagnosed by three or more liquid bowel movements per day also takes a prominent position. Specialized anamnesis can be useful for the differential diagnosis of diarrhea during EN to exclude other potential etiologies, such as infectious and/or inflammatory gastroenterocolites.

Conclusion

As part of specialized nutritional therapy, EN is not free of complications, but its use ensures the maintenance of the intestinal anatomical integrity, tropism, and natural microbiota that would be impaired when providing PN. Moreover, considering that the gastrointestinal tract and liver process the nutrient before its reaching the systemic circulation, EN is very effective for maintaining the homeostasis of amino acids pool, as well as the muscle mass. Currently, EN is preferred to PN because 'when the gut is functioning and can be used, it should be used,' and it is extensively applied around the world to effectively maintain or reestablish patient nutritional status. Furthermore, EN-related complications may be avoided or minimized through the adequate nutritional planning (including suitable choice of enteral access and placement, administration form, and formulation design) and strict patient monitoring.

See also: Amino Acids: Determination; Amino Acids: Metabolism; Carbohydrate: Digestion, Absorption and Metabolism; Diarrheal Diseases; Dietary Fiber: Determination; Dietary Practices; Dietary References: US; Energy: Intake and Energy Requirements; Energy Metabolism; Fatty Acids: Determination and Requirements; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fatty Acids: Metabolism; Fish Oils: Composition and Health Effects; Malnutrition: Concept, Classification and Magnitude; Malnutrition: Prevention and Management; Nutrition and Infection; Protein: Digestion, Absorption and Metabolism; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Proteins: Chemistry, Characterization, and Quality; Storage Stability: Mechanisms of Degradation; Storage Stability: Shelf Life Testing; Vitamins: Overview.

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http://www.espen.org/ – ESPEN - The European Society for Clinical Nutrition and Metabolism.

https://www.nutritioncare.org/– ASPEN - The American Society for Parenteral and Enteral Nutrition.

Enzymes: Analysis and Food Processing

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Proteins with catalytic properties are called *enzymes*. They are the key molecules responsible for the correct functioning of living organisms. Because of their catalytic properties, enzymes have long been used as crucial tools in many food technologies. Their deliberate use started in Neolithic times, at the dawn of human food processing, when they were employed in the first intentional fermentation processes (1). Since then, humans have used starters or rennets containing beneficial enzymes.

As with all proteins, enzymes are composed of 20 amino acids linked by peptide bonds, which, from a chemical point of view, are a subtype of the amide bond. The character of the side chains of amino acids (polar, apolar, negatively or positively charged, H-bond donor or acceptor) defines most of the intrinsic and extrinsic interactions of the enzyme molecule and its interactions with the surrounding medium. Thus, the character and spatial arrangement of the amino-acid side chains define the structural and catalytic properties and specificities of enzymes. The classification of enzymes, as proposed by International Union of Biochemistry and Molecular Biology, organizes enzymes according to the reactions they catalyze (3). In this way, enzymes are classified into six main categories:

- EC 1: Oxidoreductases
- EC 2: Transferases
- EC 3: Hydrolases
- EC 4: Lyases
- EC 5: Isomerases
- EC 6: Ligases

According to a study carried out by the Freedonia Group Inc. in 2003 (9), the global enzyme industry was worth $5.8 billion US dollars in 2010, and it is forecast to rise 6.8% annually to $8 billion US dollars in 2015. The Freedonia study covers the total sales of enzymes, including carbohydrases, proteases, polymerases, nucleases, and lipases, for use in various markets, such as biotechnology, food, and pharmaceuticals. It was pointed out in Freedonia study that three important factors drive the growth of the world enzyme market:

- Novel advances made in production biochemistry, fermentation processes, and recovery methods have resulted in the affordable large-scale production of enzymes.
- The development of various applications employing enzymes has greatly increased market demand.
- Enzymes are capable of catalyzing many different reactions, enhancing their collective commercial usefulness.

Despite increased growth in world demand for enzymes, only approximately 200 of the 4000 types of enzymes are commercially available on the global market.

Of these 200 enzymes, about 20 are produced in industrial quantities. The various difficulties impeding the industrialization of enzymes are as follows: (i) it is currently difficult to find enzymes with high yield, high activity, and high stability; (ii) enzymes used for drug and food purposes must pass through multiple phases of clinical trials and government approval, such as those conducted by the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), which is both time-consuming and costly; and (iii) enzymes for other purposes, such as detergents, require strict testing and approval by government sectors dealing with environmental safety. Not all the classes of enzymes are used in food processing with equal intensity. Some of the key food enzymes are described below, grouped according to function (e.g., processing carbohydrates or proteins).

Processing Carbohydrates

α-Amylase, EC 3.2.1.1

Synonyms: 1,4-alpha-D-glucan glucanohydrolase.

α-Amylase catalyzes the endohydrolysis of 1,4-α-D-glucosidic linkages in polysaccharides containing three or more 1,4-α-linked D-glucose units.

Uses and applications: α-amylases of different origins (4, 5) serve as processing aids in the production of alcoholic beverages, starch hydrolysis, brewing, bread- and sugar-making, and fine bakery.

Origins: *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Aspergillus oryzae*, barley malt, porcine pancreas.

Amylases are used extensively in the food, textile, detergent, paper, and pharmaceutical industries. α-Amylases are also used as sugarcane or sugar beet processing aids, solving the viscosity and raw sugar filtration problems typical to starches. The specific ability to hydrolyze the internal α-(1,4) links in amylose and amylopectin, yielding shorter, less viscous polysaccharide chains, has made α-amylases attractive for use in sugarcane and sugar beet processing. Although different sources of α-amylase are available, enzymes of microbial origin are generally preferred because of their advantages, such as cost-effectiveness, efficient production, and consistency. Most commercial α-amylases are produced from *Bacillus* species. These amylases are thermophilic, thermostable, and acidic enzymes isolated from *Bacillus* strains, and they are widely used in starch processing.

Their unique activities, efficiencies, and stabilities are typically specific to the *Bacillus* species from which the enzymes originated, unless they were genetically modified or produced. Overall, α-amylase performance is affected by several variables: pH range, calcium requirements, temperature stability, hydrolytic activity, Rt, and resistance to osmotic conditions, that is Brix tolerance. Because of the diverse functionality of commercial α-amylases, their optimal application in the sugarcane industry requires a full understanding of their activities and potential to hydrolyze different forms of starch under various industrial conditions.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00257-9

α-Amylases [α-1, 4-glucan glucohydrolase] are among the most important enzymes used for the direct conversion of starch to glucose, which can be further fermented into ethanol. It breaks successive bonds from the nonreducing end of the straight chains producing glucose. The production of ethanol from starch requires two steps, enzymatic hydrolysis followed by fermentation, which are performed by two different organisms (bacteria and yeast).

The quantitative increase in glucoamylase secretion and ethanol production requires strain improvement, because the quantities produced by the wild strains are often too low.

Strain improvement in industrial applications is generally achieved by mutation with agents such as methylnitronitrosoguanidine(MNNG), ethidium bromide, and ultra violet light. Production strains that are mostly used in industrial fermentations are developed by repeating random mutations followed by selection.

A common substrate for α-amylase production by solid-state fermentation (SSF) is wheat bran. Enzyme extracted from these substrates contains various impurities, including a significant amount of melanine-like product-coloring matter, which can interfere in the juice clarification process, if the enzymes are used in crude form. Usually, α-amylases are purified from these colored extracts by different purification steps. The purification of the targeted enzymes should be such that the number of steps are minimized in order to be cost-effective, because the yields and the number of operational steps are inversely proportional to each other. Often α-amylases are partially purified by activated charcoal in a single step.

α-Amylases are calcium-containing enzymes, binding at least one calcium ion per monomeric unit. The calcium ions convey the resistance to pH, temperature, proteolysis, and denaturation by different agents and by heat. The thermostability of α-amylase is increased greatly by bound calcium ions. Recently, many calcium-fortified fruit juices have been proposed as healthy and nonfat alternative foods, especially for children. Therefore, many fruit juices are clarified with use of α-amylase in presence of calcium chloride.

α-Amylase has been found to be a possible causative allergen for protein contact dermatitis (PCD), however. Main activity assay: One unit will liberate 1 mg of maltose from starch in 3 min at pH 6.9 and 20 °C. The α-amylase assay uses ethylidene-pNP-G7 as the substrate. Once the substrate has been specifically cleaved by α-amylase, the smaller fragments produced can be acted upon by α-glucosidase, which causes the ultimate release of the chromophore, which can then be measured at 405 nm.

β-Amylase EC 3.2.1.2

Synonyms: 1,4-α-D-glucan maltohydrolase. β-Amylase catalyzes the exohydrolysis of 1,4-α-D-glucosidic linkages in polysaccharides, resulting in the successive liberation of maltose units from the nonreducing ends of the chains.

Uses and applications: Partial delactosylation of milk and other dairy products. B-Amylase is also employed in the starch and alcohol industries.

Sources: *Kluyveromyces lactis*, barley, wheat grains.

β-Amylase is a member of family 14, which comprises glycosyl hydrolases, and it catalyzes the release of successive β-maltose from the nonreducing ends of α-1,4-linked oligo- and polyglucans.

β-Amylase is a major component of so-called diastatic power (i.e., the combined α-amylase, β-amylase, debranching enzyme, and α-glucosidase activities) of malt. Exclusive maltose production is utilized by the industry for the production of maltose-rich syrups and the nondigestible sweetener maltitol.

β-Amylases have been found not only in various plants, such as sweet potato, barley, wheat, and soybean, but also in some bacteria. β-Amylase, alone or the combination with transglucosidase treatment, can also be used to enhance the slow digestion properties of starch. For amylopectin with a higher proportion of short chains, the amylopectin structure itself slows enzymatic digestion, due to its higher branch density and short chain length, which are more difficult for the amylolytic enzymes to digest. Starch is the major reserve polysaccharide of higher green plants, and it is the second most abundant natural biopolymer on earth after cellulose. Starch is also the main energy-providing material in cereal- and tuber-based food products for the majority of the world population. In human nutrition, the proper rate of glucose release and absorption from digesting starch plays an important role in nutrition, helping to maintain adequate energy levels. According to the rate and extent of starch digestibility, starch is generally classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), or resistant starch (RS). RDS induces a fast increase in blood glucose and insulin levels, whereas SDS is slowly digested throughout the small intestine, with a low glycemic response. Recent studies have shown that obesity and related metabolic diseases, such as diabetes mellitus and cardiovascular diseases, may be associated with the long-term consumption of foods with high RDS contents, which is positively correlated to a high glycemic index and will cause a substantial fluctuation of glucose homeostasis regulatory hormones and high stress to the regulatory system. Therefore, improving food quality with higher amounts of SDS is becoming an area of interest for academia and industry. Generally, starches differ in digestion rates. β-Amylase hydrolysis is important for making sugar syrup and brewing beer and liquor derived from starch. Thanks to the application of β-amylolysis, the amount of SDS can be more than doubled.

β-Glucanase from *Talaromyces emersonii* belongs to the family of hydrolytic enzymes. This food enzyme catalyzes the hydrolysis of 1,3 linkages or the β-1,4 glucan of the cereals used in the production of beer, and it helps reduce the viscosity of musts caused by barley glucans.

Main activity test: According to Bernfeld (1955), the rate at which maltose is released from starch is measured by its ability to reduce 3,5-dinitrosalicylic acid. One unit releases one micromole of β-maltose per min at 25 °C and pH 4.8.

Xylanase EC 3.2.1.8

Synonyms: endo-1,4-β-xylanase.

Uses and applications: bread making, special bread, the production of maize starch and alcohol through fermentation.

Origins: *Aspergillus oryzae*, *Bacillus subtilis*, *Bacillus subtilis*, and *Trichoderma longibrachiatum*.

Xylanase hydrolyzes the β-1,4 linkages-D-xylosidic inside of chains of xylans and arabinoxylans releasing reducing sugars.

Hemicelluloses are hydrolyzed by the cooperative action of a group of enzymes, and the main enzyme involved in the depolymerization of xylan is endo-β-1,4-xylanase. The β-1,4-endoxylanases (1,4-β-D-xylan xylohydrolase, EC 3.2.1.8), which are produced by algae, protozoa, molluscs, crustaceans, insects, seeds of terrestrial plants, bacteria, and many fungal species belonging to the genus *Aspergillus*, are commonly found in nature. However, the xylanase enzymes of filamentous fungi are potentially the most interesting for industrial uses, because they are produced in greater amounts compared with those of yeast and bacteria. These enzymes can be used in monogastric feeds in order to hydrolyze nonstarch polysaccharides such as β-glucan and arabinoxylan that are found in vegetables, thus increasing feed conversion, body weight gain, and the yield of production (4, 5). To date their use was broadened to many processing industries, such as pulp, paper, food, and textile. Of the many reported applications for xylanase, its use as a food supplement has played an important role for monogastric animals, because it can improve the utilization of nutrients.

Xylanases are used in the baking industry to stabilize dough, to make dough flexible, and to improve gluten strength. Xylan is the principal hemicellulose, which is the major plant cell wall polysaccharide component. Arabinoxylans are important in the formation of gluten and other networks in dough, and it has been reported that the nonwater-extractable arabinoxylans exert a negative effect on bakery products, whereas the water-extractable forms of arabinoxylans, with medium to high molecular weights, have a beneficial effect.

Xylanases have been added to dough to accelerate the process of bread making by helping to breakdown polysaccharides in the dough. Enzyme activity is effective in dough by cleaving arabinoxylan chains and thus modifying their functions. Moreover, xylanases contribute to the decrease of dryness and stiffness of the dough with increasing elasticity, extensibility, and coherency, as well as increases in volume and decreases in bread density. Xylanase treatments also result in higher moisture retention and the improvement of sensory perception of bread. Dough characteristics and bread quality improve significantly in response to xylanase treatments during tempering, as compared to application during mixing.

Main activity test: Xylanase activity is tested using an assay based on the release of dyed fragments from 4-*O*-methyl glucuronoxylan dyed with Remazol Brilliant Blue dye or p-pydroxybenzoic acid hydrazide measured at 410 nm.

Glucose Oxidase EC 1.1.3.4

Synonyms: β-D-glucose:oxygen 1-oxidoreductase

Uses and applications: gluten strengthening, gelation of the soluble arabinoxylans in the flour.

Origins: *Aspergillus niger*.

Glucose oxidase can be used for strengthening the dough. Glucose oxidase catalyzes the oxidation of b-D-glucose to form D-glucono-1,5-lactone and hydrogen peroxide. Glucose oxidase has a gluten-strengthening effect by inducing the formation of protein–protein bonds that strengthen the protein network and thereby strengthen and stabilize the dough. Glucose oxidase catalyzes the oxidation of glucose into gluconolactone, with the concomitant reduction of the oxygen to hydrogen peroxidase. Five mechanisms are known, concerning the effects of the sugar oxidase enzymes, as follows: (i) generation of disulfide bridges between the proteins, (ii) formation of di tyrosines, (iii) blocking the effect of glutathione, (iv) gelation of the soluble arabinoxylans in the flour, and (v) alteration of the equilibrium between the different enzyme systems present in the dough.

Main activity assay: Glucose oxidase can be utilized for the enzymatic determination of D-glucose in solution. As glucose oxidase oxidizes β-D-glucose to D-gluconolactate and hydrogen peroxide, horseradish peroxidase is often used as the coupling enzyme in glucose determinations. Although glucose oxidase is specific for β-D-glucose, solutions of D-glucose can be quantified, because α-D-glucose will mutorotate to β-D-glucose as the β-D-glucose is consumed by the enzymatic reaction. Thus, this enzyme is also used in biosensors that measure the amount of glucose in blood.

Processing Proteins

Rennet/Chymosin EC 3.4.23.4

Synonyms: chymosin, rennin.

Uses and applications: Rennet or chymosin is used for cheese making and has highly specific milk-clotting activity relative to its proteolytic activity.

Origins: Fourth stomach of the suckling calf and fungi such as *Mucor miehei*, *Mucor pusillus*, and *Endothia parasitica*.

Aspartic proteases (EC 3.4.23.X) are endopeptidases having two aspartic acid residues that are critical for catalytic activity within their active sites. They are an important class of proteases, which are widely used as milk-coagulating agents. Chymosin (EC 3.4.23.4), the principal aspartic protease used for cheese making, has highly specific milk-clotting activity relative to its proteolytic activity.

Chymosin has broad specificity, similar to that of pepsin A, and it clots milk through the cleavage of a single Ser-Phe105-Met-Ala bond in κ-casein. By predominant cleavage of the Phe105–Met106 bond of bovine κ-casein, chymosin has high specificity for splitting the caseinomacropeptide from the casein micelles, and therefore, it induces milk clotting. Because they are responsible for the hydrolysis of the κ-casein chain in casein micelles of milk, these enzymes are used as milk coagulants in cheese preparation. Because cheese consumption is growing all over the world, the production of different cheese varieties has been increased. Consequently, the availability of calf rennet is limited, and now, calf rennet only covers 20–30% of the world demand for milk coagulant.

Calf rennet obtained from the fourth stomach of a suckling calf is used all over the world, except in India, for the manufacture of almost all varieties of cheeses. However, due to the acute shortage of calf rennet, a substitute for calf rennet has been developed from fungal sources such as *Rhizomucor miehei*, which produces acid protease for the manufacture of goat and sheep cheeses. Substitute proteases are also derived from plants such as wild thistle (*Cynara cardunculus*), and recombinant rennet-chymosin is used for milk coagulation. Chymosins can be obtained from various sources, such as animals, plants, and fungi. The properties of different chymosins have been reviewed by Kumar et al. Chymosins, the major component

of rennet (milk clotting enzyme), are the acid proteases produced in the fourth stomach of milk-fed ruminants.

Although the cost has been reduced due to the biotechnological preparation of recombinant chymosins, natural alternatives are still preferable. However, the unavailability of calf stomachs and ethical problems associated with animal slaughter have forced a search for other alternatives to calf chymosin. In this regard, various microbial alternatives are used for chymosin production, but not all of these sources are suitable for the production of quality cheese, because they often generate a bitter taste. Recombinant chymosin production has been achieved in different microorganisms in response to the consumer demand for vegetarian and vegan cheeses. Buffalo, sheep, goat, and rabbit are believed to be good sources of rennet. Bovine (*Bos tarus*), buffalo (*Bubalos bubalis*), and lamb (*Ovis platyurea*) chymosins have been characterized extensively.

Substitutes from microbial sources would be more interesting, considering the stable availability of these sources and the low cost due to the possibility of using cheap substrates for fermentation. It has been reported that microbial coagulants represent 53% of the demand for milk-clotting preparations. Chymosins from microbial origins have found well-defined applications in cheese manufacturing. Some microbial chymosins from fungi such as *Mucor miehei*, *Mucor pusillus*, and *Endothia parasitica* have been purified, and their enzymatic properties have been investigated in detail. These fungal enzymes have been used in large-scale cheese production since the 1960s. Apart from low cost and stable characteristics, chymosins from fungi are also better accepted by people whose eating habits and religious beliefs exclude the use of animal enzymes or recombinant rennets.

Main activity test: Skimmed milk solution is incubated at 35 °C for 10 min. Then 0.5 ml of rennet is added to the solution and mixed immediately. The time from adding rennet to milk coagulation is recorded.

Transglutaminase EC 2.3.2.13

Synonyms: Transglutaminase, factor XIIIa, fibrinoligase, fibrin-stabilizing factor, glutaminylpeptide γ-glutamyltransferase, polyamine transglutaminase, tissue transglutaminase, *R*-glutaminyl-peptide:amine γ-glutamyl transferase, (TGase; protein-glutamine gamma-glutamyltransferase), fibrinoligase, glutaminylpeptide gamma-glutamyltransferase, polyamine transglutaminase.

Uses and applications: Stabilization of the gluten network in dough, reconstitution of matrix meat products.

Origins: Blood coagulation factor XIII, pig/bovine liver, *Streptoverticillium moboarense*.

Transglutaminases are the enzymes used for the stabilization of the gluten network in dough, allowing manufacturers to overcome the lack of emulsifiers and produce good bakery products from frozen dough. It is also used for the reconstitution of meat products.

Transglutaminase is an efficient and promising food enzyme because it catalyzes acyl transfer reactions, deamidation, and crosslinking (polymerization) between protein intra- or interchain glutamine (acyl donor) and lysine (acyl acceptor) peptide residues. The reactions promoted by this food enzyme create profound changes in the proteins composing food matrices, leading to improved texture and stability in terms of temperature, syneresis, emulsifying properties, gelation, and increased water-binding capacity, without changing the pH, color, flavor, or nutritional quality of food.

Protein cross-linking may be carried out with help of transglutaminase (TG) either prior to or simultaneously with the addition of rennet. Enzymatic cross-linking affects both the primary and the secondary stages of rennet coagulation. The impact of protein cross-linking on the primary enzymatic phase is mainly related to increased coagulation times due to the inhibition of caseinomacropeptide release. Consequently, a higher extent of protein cross-linking may lead to reduced gel firmness. The simultaneous reaction of TG and rennet results in significantly increased yield due to the enhanced lactoserum binding of the gel network stabilized by additional covalent bonds resulting from transglutaminase action. In conclusion, cross-linking has a strong impact on the entire coagulation process, leading to rennet gels with modified functional properties.

Transglutaminases may even render food protein more nutritious because of their potential use for the incorporation of essential amino acids in proteic matrices. Microbial transglutaminase (MTGase), an extracellular enzyme from the class of transferases, is produced commercially via traditional fermentation by the microorganism *Streptoverticillium moboarense*. This food enzyme, which acts in a wide range of pH and temperature (pH 5.0–8.0, optimum temperature of 50 °C, but with activity between 40 and 70 °C), is Ca^{2+} independent, and its activation does not require special cofactors. This enzyme was also granted *generally recognized as safe* (GRAS) status by the FDA in 1998, as well as approval from European (French and Danish) food control agencies, making MTGase very attractive for the food industry. According to existing reports, native and MTGase-modified proteins differ only in the number of bonds between glutamine and lysine residues.

Several studies have tested a variety of food proteins as substrates for MTGase: milk and whey proteins, soy globulins, myofibrillar proteins, albumins, and others. One of the main industrial applications of MTGase is in the restructuring and consolidation of meat products. Through the cross-links MTGase catalyzes, the transglutaminase increases stability, improves texture, and decreases losses during cooking, giving the restructured meat product an appearance resembling that of intact muscle. In the dairy industry, transglutaminase has been used in the production of yogurt, in which it has been largely used for the prevention of syneresis during whey separation, because of its capacity to increase the gel water-holding capacity. Despite transglutaminase's innovative potential in the formulation of various food products, it remains underexploited for industrial uses, particularly in products of plant origin.

Considering the food industry's significant interest in using food-grade transglutaminases because of their specificity, mild reaction conditions, and very low risk of toxin formation, understanding how transglutaminases modify the functional properties of food proteins is the most important step in promoting their industrial use in novel, reconstructed food products.

MTGases belong to the class of transferases widely known to modify the functional properties of proteins in food systems. The main mechanisms of their action are polymerizations that result in many functional and biophysical changes, including changes in protein hydrophobicity. MTGases decrease protein

solubility, inducing protein gelation. They also affect emulsification, foaming, viscosity, and water-holding capacity, all of which depend on radical decreases in cross-linked protein solubilities. Because MTGase promotes the polymerization of proteins via decarboxylation of free amino acids, it could be used to suppress the formation of biogenic amines in foods containing high amounts of free amino acid, such as the meat of certain fish (e.g., mackerel, tuna, etc.).

Given that the majority of plastics are nonbiodegradable and their excessive use has led to serious environmental problems, industry is seeking alternative natural sources for those materials. Therefore, reticulated fish skin and scale gelatines, in combination with soya proteins, could be used to produce edible and biodegradable films, improving food product quality and reducing waste.

Among the materials used to prepare edible films, gelatine has been extensively utilized because of its relative abundance and excellent film-forming ability. Recently, edible films based on fish gelatin have received increasing attention due to concerns about mad cow disease and foot-and-mouth disease. Transparent films with good mechanical properties can be prepared from the fish scale gelatin extracted at pH 5. However, gelatin films have poor water resistance properties, because of the highly hygroscopic property of gelatin. Additionally, the mechanical properties of gelatin films decrease when in contact with the surfaces of foods with high moisture contents. These properties restrict the application of edible films prepared from unmodified native gelatin only.

It was reported that the physical properties of protein films could be improved by adding transglutaminase, which is considered to be a safe and effective cross-linking agent. The addition of soy protein isolate (SPI) and its coreticulation with TG could also greatly improve the swelling property of gelatin films.

Main activity assay: Most tests rely on measuring the absorbency caused by OPA-reactive Lys on the casein during the transglutaminase action.

Papain EC 3.4.22.2

Synonyms: Papainase, papaya peptidase I.

Uses and applications: Papain is a processing aid for the production of protein hydrolyzates as aromatic bases, aswell as plant protein hydrolyzates. Papain is also used in the brewery and in meat tenderization.

Origins: *Carica papaya* (papaya), *Ananas comosus* (pineapple).

Papain is a cysteine protease with wide specificity, cleaving the peptide bonds of basic amino acids, such as leucine and glycine. It also hydrolyzes esters and amides. Papain will digest most protein substrates more extensively than the pancreatic proteases do.

The ability of papain to improve beef meat tenderness is attributed to the increased degradation of myofibril proteins and the disruption of muscle fiber structures, because of its proteolytic action. The main components of meat, myofibrils, mainly consist of actin, myosin, and various accessory proteins and connective tissue, which consists of various collagen subtypes and elastin. The relative proportions of these components influence meat structure and texture and, thus, the relative tenderness of a meat. It is known that the disruption of these components of meat structure in various ways, including the partial hydrolysis of meat proteins, contributes to tenderness. The potential of exogenous proteases, such as papain, to tenderize meat has been exploited for a long time.

Main activity assay: A titrimetric determination of the acid produced during the hydrolysis of benzoyl-L-arginine ethyl ester (BAEE) serves to measure papain activity. One unit hydrolyzes one micromole of benzoyl-L-arginine ethyl ester per min at 25 °C and pH 6.2, under the specified conditions.

Asparaginase EC 3.5.1.1

Synonyms: Colaspase.

Uses and applications: Asparaginase is used in the deamidation of food preparations containing L-asparagine and carbohydrates, which are cooked at temperatures above 120 °C, such as bread and other cereal products, as well as fried products based on potatoes, generating polyacrylamide. Asparaginase is an enzyme that catalyzes the hydrolysis of asparagine to aspartic acid.

Origins: *Aspergillus oryzae*.

Main activity assay: Asparaginase hydrolyzes asparagine to generate aspartic acid, which can be detected colorimetrically ($\lambda = 570$ nm) or fluorescently (Ex/Em = 535/590 nm) using a coupled enzymatic reaction.

Processing Lipids

Lipases EC 3.1.1.X

Uses and applications: Cheese production, oil and egg industries, bakeries.

Origins: Pancreas, *Fusarium venenatum*, *Fusarium oxysporum*, hybrid between *Thermomyces lanuginosus* and *Fusarium oxysporum*.

The importance of lipases (triacylglycerol acylhydrolases, E) is undeniable, and the impact of this group of enzymes is clearly seen in their widespread application in many industries, including organic synthesis, paper manufacturing, oleochemistry, dairy, cosmetics, perfume, biosensors, and detergents.

Lipases catalyze the hydrolysis of fats into fatty acids and glycerol at the water–lipid boundary, as well as the synthesis of esters from fatty acids and glycerol at the water-insoluble substrate boundary. The unique capability of lipases to react only at the interface between aqueous and nonaqueous phases distinguishes them from esterases. Lipases hydrolyze the ester bonds in triglycerides, yielding diglycerides, monoglycerides, and fatty acids, and they catalyze the hydrolysis of ester bonds in sn-1 diacyl phospholipids to form 2-acyl-1-lysophospholipid and fatty acids. This improves the properties of dough and the quality of bread, leading to a finer and more homogeneous crumb structure.

Phospholipases are also used in cheese production, as well as the oil and egg industries. Engineered phospholipase C may be used for the efficient degumming of edible vegetable oils; the enhancement of the natural emulsifying properties and heat stability of yolk and/or whole egg; the enzymatic hydrolysis of vegetable lyso lecithins into lecithins in order to improve their emulsifying properties; and the degumming of vegetable oils during refining, which eliminates phospholipids

from the oil during the purification step, in order to obtain satisfactory taste and quality, provide better storage stability, and facilitate the rest of the manufacturing process.

Lipases often have high chemo-, region-, and enantioselectivity, so that they are considered to be one of the most important industrially utilized biocatalysts. The current trend in lipase research is to focus on microbial lipases rather than lipases derived from animals and plants, because lipases from microorganisms have several advantages, including the ability to catalyze diverse reactions, produce high yields, and reduce production costs. In addition, microorganisms have the advantage of relative ease of genetic manipulation. Other major advantages of microbial lipases include (i) structural stability in organic solvents, (ii) independence from cofactors, (iii) catalyze reactions utilizing a wide range of substrates, and (iv) high enantioselectivity. A detailed description of bacterial and fungal lipases, as well as a brief account of lipase purification techniques and screening methods, can be found in a recent review by Nagarajan.

Omega-3 polyunsaturated fatty acids (ω3 PUFA) from fish oils promote well-established health and anti-aging benefits that justify their use as functional ingredients in dietary supplements, healthy foods, and nutraceutical products. Among them, eicosapentaenoic (EPA, C20:5) and docosahexaenoic acids (DHA, C22:6) continue to receive particular attention because of their numerous biological properties and positive effects on human health. In addition to recognized benefits for the prevention of cardiovascular diseases, EPA and DHA were reported to protect from inflammation-mediated disorders, including obesity and diabetes, as well as Alzheimer's and related neurodegenerative diseases.

Human metabolism exhibits a limited ability to synthesize ω3 PUFAs. The dietary supply of preformed compounds therefore appears to be an essential alternative. However, the practical use of such lipids as food ingredients is often limited by their high susceptibility to oxidation, which is responsible for the undesirable off-flavor and odor of rancid oils associated with a loss of nutritional value. Various solutions can be implemented to minimize these degradation pathways, and the pathway most commonly used by manufacturers is the addition of antioxidants. Intensive research has been pursued on natural phenolic antioxidants issued from plants. Many studies reported that such antioxidants are highly effective at protecting ω3-enriched food products from oxidation. In addition, some works pointed out the interest of formulations mixing ω3 PUFAs and phenolic compounds for the prevention of Alzheimer's disease and the treatment of obesity through lipid-lowering effects. Another approach involves bringing together ω3 lipids and phenolic compounds in a single entity. Such an association was shown to improve the stability of highly oxidizable fatty acids, while facilitating the solubilization of phenolic compounds in lipid phases. Additional effects could include the increased bioavailability of phenols, as well as cumulative and even synergistic biological activities. In addition, PUFA phenolic esters were reported to exhibit higher antimicrobial, antioxidant, and antiinflammatory activities than native phenols do.

Fatty-acid phenolic esters are preferentially produced by enzymatic bioprocesses based on the use of lipases that exhibit high selectivity towards polyfunctional substrates and mild reaction conditions, as compared to chemical synthesis pathways. Most often, esterification reactions were carried out in dry organic solvent, but few studies reported the possibility of processing without any solvent.

The synthesis of biodiesel by lipase-catalyzed reactions has also become a matter of great commercial interest due to its environmental friendliness over petrodiesel fuel.

The main advantages of using biodiesel are that it is biodegradable, can be used without modifying existing engines, and produces less harmful gas emissions, such as sulfur oxide. Chemically, biodiesel is a mixture of alkyl esters with long-chain fatty acids, and it is typically made from nontoxic, biological resources such as vegetable oils, animal fats, or even used cooking oils. In this sense, due to the low miscibility of the substrates, the application of ultrasound irradiation to this reaction system may lead to high reaction rates. Although the rates of triglyceride conversion to fatty-acid alkyl esters are known to be slower than the alkali-catalyzed routes, enzymatic catalysis has attracted much attention, because it is more ecofriendly and has the potential for industrial implementation, due to the simplicity of the product-refining process and the use of lower reaction temperatures. In fact, the key advantage of an enzymatic biodiesel process is that triglycerides, as well as free fatty acids, can be efficiently transformed into biodiesel under the same mild conditions, and the byproduct, glycerol, can be easily recovered as part of a high-quality, high-value stream, without any complex process. The high cost of enzyme production remains the major obstacle to the commercialization of enzyme-catalyzed processes, but considerable progress has been made to address it, including the use of solvent-tolerant commercial and noncommercial immobilized lipases and new immobilization technologies, making catalyst reutilization possible.

From *Fusarium venenatum*, *Fusarium oxysporum*, hybrid between *Thermomyces lanuginosus* and *Fusarium oxysporum*

Main activity assay: Lipase hydrolyzes a triglyceride substrate to form glycerol, which may be quantified enzymatically by monitoring a linked change in the OxiRed probe absorbance at 570 nm. Other spectrophotometric methods for lipase activity determination use synthetic lipase substrates transformed via enzyme-catalyzed hydrolysis into spectrophotemetrically detectable products. The predominant substrates are p-nitrophenyl and naphthyl esters of the long chain fatty acids, as well as thioesters. The lipolysis of the p-nitrophenyl esters (e.g., laurates, palmitates, oleates) gives rise to yellow-colored p-nitrophenol measured at 405–410 nm.

Other Food Enzymes

Phytases EC 3.1.3.8 X

Uses and applications: Degradation of antinutritional factors (phytates).

Origins: *Bacillus* spp., *Aspergillus ficum*, *Aspergillus niger*, *plants*.

Phytases catalyze the hydrolysis of phytate molecules, known as antinutrients, which complex certain essential minerals, and it is used in bread making to reduce the content of the bread phytates.

Phytates (myo-inositol-1,2,3,4,5,6-hexakisphosphates) are widely thought to play a significant role in decreasing the bioavailability of minerals by chelating cations and forming complexes, which may be insoluble or otherwise unavailable under physiological conditions. A number of studies, including animal experiments and human trials, have been done on the negative effects of phytates, and these studies clarify that dietary phytate has a negative impact on the bioavailability of mineral ions (i.e., Zn^{2+}, $Fe^{2+}/3^{+}$, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Cu^{2+}). Additionally, phytate has also been reported to form complexes with proteins at both low and high pH values, and the complex formations may alter the protein structure, which induces changes in the physicochemical and functional properties, enzymatic activity, and proteolytic digestibility of proteins (or enzymes). Thus, it is necessary to partially, or even completely, eliminate the antinutritional effect of phytates when phytate-rich products are produced or used. There are several processing techniques, such as soaking, germination, malting, fermentation, ultrafiltration, and ion exchange columns, which can be used to decrease the inhibitory effect of phytate on mineral absorption. In addition, phytase (myo-inositol hexakisphosphate phosphohydrolase), which catalyzes the conversion of phytate to inositoland inorganic phosphate, can also be used to reduce phytate in food processing. Some research studies describe the application of phytase in food processing to reduce phytate in products such as white wheat rolls, cereal porridges, wheat bran flour, glandless cotton flour, bread, corn wet milling, and rice bran protein isolate. It was reported that 85% of phytates in soybean meal and 67% of phytates in cottonseed meal could be hydrolyzed by *Aspergillus ficuum* phytase at 50 °C and pH 4.0–5.5.

Phytic acid (PA) can be degraded by phytase enzymes, leading to an increase in the bioavailability of zinc and other minerals. PA degradation was reported to occur during the fermentation of cereals such as wheat, which contain endogenous phytases, and on the addition of exogenous phytases during the preparation of cereal flours for complementary feeding. Both processes require long preparation in slurry form, however, with adjustments to pH and temperature for efficient dephytinization. A simpler procedure, suitable for in-home fortification mixtures, would be to add the phytase to the cereal porridge at the time of consumption so that PA degradation could take place during the digestive process. This would then have a positive effect on zinc and iron adsorption.

Phytate can be hydrolyzed in the gastrointestinal tract (GIT), at least to some degree, by the intrinsic phytases present in feeds originating from plants, endogenous phytases, or possibly by microbial phytase from the gut microbiota present in the GIT. More recently, however, phytases have been commercially produced from microbial sources and are often included in pig and poultry diets to improve the availability of minerals, such as Ca and P, as well as other nutrients, ostensibly through greater phytate hydrolysis. Several studies have been published examining the efficacy of dietary microbial phytase supplementation on Ca and P availability in pigs, but these studies largely focus on disappearance across the total tract, while largely overlooking apparent ileal mineral absorption. There is a lot of information about the fate of dietary phytate P and total P and Ca throughout the GIT of pigs, particularly in response to the presence of dietary microbial phytase and in relation to corn–soybean meal diets.

Sources: *Aspergillus niger*, plants.

Main activity assay: The assay is based on the enzymatic hydrolysis of sodium phytate under controlled conditions via the measurement of the amount of orthophosphate released. The phytase activity is expressed in phytase units (FTU). One phytase unit (FTU) is defined as the amount of enzyme that liberates 1 µmol of inorganic phosphorus per minute from 0.0051 mol/l sodium phytate at 37 and pH 5.50, under the conditions of the test.

Legislation Concerning the Safe Use of Food Enzymes

With very rare exceptions, largely limited to their allergenic properties, the consumption of most food enzymes does not present a particular danger. However, some of these enzymes in active form, such as certain proteases and lipases, may have detrimental effects on the cell membranes of human skin or the GIT. Thus, safe practice requires that all enzymes used in food formulations should be either inactivated, denatured, or eliminated before the end-product stage. Because the great majority of food enzymes are the mixtures of various components present in their production media including other enzymes, present sometimes in even greater amounts that a desired enzymatic product, and an array of other compounds, the safety of the product should conform to current security and health standards, assuring safety for consumers.

The first legislation addressing security verifications for food enzymes was voted on and enforced in Denmark and in France. These laws, in turn, inspired European legislation for securing the safety of foods created with help of industrially produced enzymes (12, 13). In most cases, if there is no considerable history of safe use, as in the case of rennet, the new enzymatic processing aid should fulfill many sanitary requirements, such as microbiological, toxicologic, mutagenic, carcinogenic, and allergenic safety.

See also: Biscuits, Cookies, and Crackers: Chemistry and Manufacture; Bread: Breadmaking Processes; Bread: Chemistry of Baking; Cheese: Chemistry and Microbiology; Enzymes: Functions and Characteristics; Fermented Foods: Fermented Milks; Whey and Whey Powders: Fermentation of Whey; Wines: Champagne and Sparkling Wines – Production and Effervescence; Wines: Wine Production.

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Enzymes: Functions and Characteristics

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Enzymes: Concept and History

Enzymes are biological macromolecules that act as facilitators (catalysts) of every metabolic reaction that takes place in or is produced by the action of living organisms. With the exception of a small group of catalytic RNA molecules, all enzymes are proteins, and their activity depends on their native protein conformation. The function of enzymes is to increase the speed (rate) of biological reactions so that they occur in a time scale useful for organisms. An example of enzyme action can be taken from how bread supplies us with energy. The essential component of bread is starch, a polymer of glucose. When we eat bread, digestive enzymes splice starch down to free glucose molecules, which then undergo a chain of enzymatic reactions to be finally converted into CO_2 and H_2O. The overall balance of these reactions is highly exergonic, and the energy liberated sustains our physical and mental activity. However, if we keep a piece of bread in a container, it can remain for years without any noticeable change. The same reactions that occur in our organism are thermodynamically possible and may occur in the container, but in the absence of enzymes they take place at an extraordinarily low speed that makes them useless to sustain life. Enzymes are therefore agents whose role is to reduce the time of chemical reactions.

Although virtually all enzymes are proteins, there are many cases in which the protein needs another chemical component (cofactor) to perform its catalytic activity. This cofactor may be an inorganic ion, such as Fe^{2+}, Mg^{2+}, Mn^{2+}, or Zn^{2+}, or a more complex organic molecule, named coenzyme, such as coenzyme A. When the coenzyme is tightly bound to the protein, it is called a prosthetic group, such as the heme group. The protein part is called apoenzyme or apoprotein, and the complete, catalytically active enzyme with both components is called holoenzyme.

The use of enzymes by humans dates to the earliest times of civilization, far before the time when the concept of enzyme could be conceived. Ancient enzyme uses were associated with the production of certain types of foods and beverages. The use could be indirect, as in the production of foodstuffs resulting from microbial fermentation, such as bread, wine, beer, or vinegar. It could also be direct as in cheese production from milk, using renin obtained from the stomachs of ruminants. Of course, early civilizations were not aware of the nature or function of the substances that they used in the elaboration of enzyme products, which was based on empirical observations and folklore.

It was during the nineteenth century that the development of biochemistry provided an emerging picture of the nature and properties of enzymes. In 1833 Anselme Payen and Jean-François Peroz described the isolation of an amylolytic substance from germinated barley that they called diastase. Two years later, the chemist Jöns Jacob Berzelius coined the term catalysis (1835), referring to the property of certain substances to accelerate chemical reactions. One year later Theodor Schwan discovered the digestive enzyme peptidase. However, it was not until 1876 that Wilhelm Kühne purposed the term 'enzyme' derived from the Greek word 'zymé,' which means ferment. In 1894 Emil Fischer proposed the lock-and-key theory to explain the mechanism of enzyme action. In 1897, the brothers Hans and Eduard Buchner demonstrated that the transformation of glucose in ethanol can be carried out by chemical substances (enzymes) present in cell free extracts.

During the first half of the twentieth century, the chemical nature of enzymes and their mechanism of function were elucidated. In 1903, Victor Henri published the first mathematical model describing the process of enzyme action (enzyme kinetics). Ten years later, in 1913, Leonor Michaelis and Maud Menten published their very well-known mathematical model of enzyme kinetics relating the velocity at which an enzymatic reaction proceeds with the concentration of substrate. In 1926 James B. Summer purified and crystallized the enzyme urease isolated from jack beans. His work provided first evidence that enzymes are proteins. This was confirmed in the next few years by the work of John Northrop with digestive enzymes pepsin, trypsin, and chymotrypsin. Around 1950, two key contributions were determinant for understanding the structure and function of proteins in general, and enzymes in particular. Frederick Sanger's work with insulin showed that proteins are composed by chains of amino acids linked by peptidic bonds. Based on Sanger's discovery, Kaj Linderstrøm-Lang made an accurate description of the shape and organization of the architectural elements that compose protein molecules. Already in the second half of the twentieth century, around 1960, John Kendrew and Max Perutz using X-ray crystallography resolved the first three-dimensional structure of proteins. Although they worked with globular, noncatalytic proteins, myoglobin and hemoglobin, their work pioneered subsequent studies linking protein structure and enzymatic function. Enzyme studies were fostered by the onset and development of genetic engineering techniques in the second half of the twentieth century. As a result of this development, it is possible nowadays to obtain in large amount and pure form almost any enzyme from any organism.

One-Gene–One-Enzyme

In 1941 George Beadle and Edward Tatum published a highly influential article in which they proposed the so-called one-gene–one-enzyme hypothesis. At that time, the principles of Mendelian inheritance were understood, and it was known that enzymes were proteins, but the chemical nature of the genetic material remained a mystery. Beadle and Tatum worked with the bread fungus *Neurospora crassa*, looking for genetic mutations causing metabolic defects. The selected mutants required the addition of a specific supplement,

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typically an amino acid (e.g., arginine or leucine) to restore their capability to grow. These, called auxotrophic mutants, were therefore impaired in the metabolic process – pathway – leading to the biosynthesis of the required compound. The genetic analysis of a group of mutants requiring the same supplement showed that they were affected in different genes controlling successive steps – chemical reactions – of the same pathway. From this, Beadle and Tatum concluded that each gene determines the existence of an enzyme that catalyzes a specific biochemical reaction.

Later discoveries rendered this theory an oversimplification. Presently, we know that there are many genes that do not encode enzymes as they have a regulatory function; other genes encode nonenzymatic proteins. There are also enzymes that are composed by more than one polypeptide and are therefore encoded by more than one gene. Nevertheless, with many exceptions, Beadle and Tatum's insight was essentially true and established an early, first connection between the genome and the thousands of biochemical reactions that take place in a living organism.

Structure and Function of Enzymes

The working functionality of a molecule resides in its chemical composition and spatial conformation – structure. Enzymes are molecular machines able to catalyze chemical reactions. They are built by at least one polypeptide chain, and their structure derives from the corresponding particular sequence of amino acids. The general features of protein structure were first proposed by Linderstrøm-Lang and later confirmed by crystallographic analysis of Kendrew, Perutz, and the work of Linus Pauling. The structure of a protein results from a hierarchical organization of the molecule at four levels. The basic level – primary structure – is determined by the specific linear sequence of the amino acids that compose the polypeptide chain. Stretches of amino acids bound in a polypeptide chain, depending on their composition, can take an apparently random conformation – coil or loop – or adopt particular patterns. These may have the shape of a helix – α-helix – or a zigzagged extended conformation of chain segments (β-strands) interconnected laterally with the shape of a sheet – β-sheet. These patterns constitute the following – secondary structure – level. Every polypeptide chain, with its distinct secondary structure elements, adopts a specific three-dimensional arrangement that is referred to as tertiary structure. Often, proteins are made of more than one polypeptide chain – monomer – which can be identical or different. The specific association of two or more monomers in proteins constitutes its quaternary structure (Figure 1). Although the amino acids of a polypeptide chain are linked by covalent union between the amino group of one amino acid and the carboxyl group of the next – peptide bond, the secondary, tertiary, and quaternary structures are maintained by numerous, usually noncovalent, forces such as hydrogen bonds, salt bridges (electrostatic), and hydrophobic interactions. Proteins contain secondary structure (α-helix and β-chain) elements connected by coil regions of different length. Generally, the secondary structure elements cluster at the internal hydrophobic core of the protein, whereas the connecting coils are at the surface. A relatively small three-dimensional pocket within the overall structure of an enzyme constitutes the catalytic site, which is formed by the amino acid residues directly involved in catalysis and those that participate in binding the substrate. In a way, it could be considered that the secondary structure elements, which are evolutionary conserved among similar, phylogenetically related proteins – homologues, are mostly involved in maintaining the protein framework, whereas some of the loops build the catalytic pocket, providing substrate specificity and enough flexibility for the conformational changes occurring in the enzyme during the catalytic process.

Presently, two methods are used for protein structure determination, X-ray crystallography, and nuclear magnetic resonance (NMR). Analysis of X-ray diffraction patterns provides a very accurate picture of protein structures with a resolution up to 1 Å. A drawback of this technique is that it requires the protein to be in crystalline form. Protein crystals are often difficult to grow, and sometimes their quality is not good enough to obtain the three-dimensional structure with sufficient accuracy. Around 1970, NMR was applied to the resolution of protein structures thanks to the work of Kurth Wüthrich. NMR analysis is carried out using protein solution samples, much easier to prepare than crystals; however, the practical use of this technique is still limited to proteins of relatively small size. Progress made during the last decades has yielded a huge number of protein structures, most of them enzymes, available at the Protein Data Bank (http://pdb.org/). A highly useful complement to the experimental techniques are bioinformatics tools, such as SWISS-MODEL (http://swissmodel.expasy.org/) or I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER/), which allow predicting the three-dimensional structure of a protein from its amino acid sequence, on the basis of existing homology with other proteins of known structure.

Name, Types, and Classification of Enzymes

Enzymes are named by adding the suffix '-ase' to a term describing or referring to their activity. For instance: citrate synthase, peptidase, lactase, etc. Exceptions to this rule reflect the circumstance that the enzyme was named before the specific reaction that catalyzes was known. This was the case for enzymes such as pepsin or lysozyme. Before a systematic way for naming enzymes was adopted, the same enzyme could be named differently according to the context where it was studied. Conversely, different enzymes could receive the same name. This created misunderstanding and made difficult the interpretation of published results. The situation became unbearable when the rapid growth of biochemical research increased enormously the number of enzymes that were identified and characterized. In 1956, the International Union of Biochemistry (IUB) appointed a commission, the International Commission on Enzymes, or simply the 'Enzyme Commission' (abbreviated EC), whose mission was to establish a rational methodology for the classification and nomenclature of enzymes. The adopted classification was based on chemical activity (function). Each enzyme was

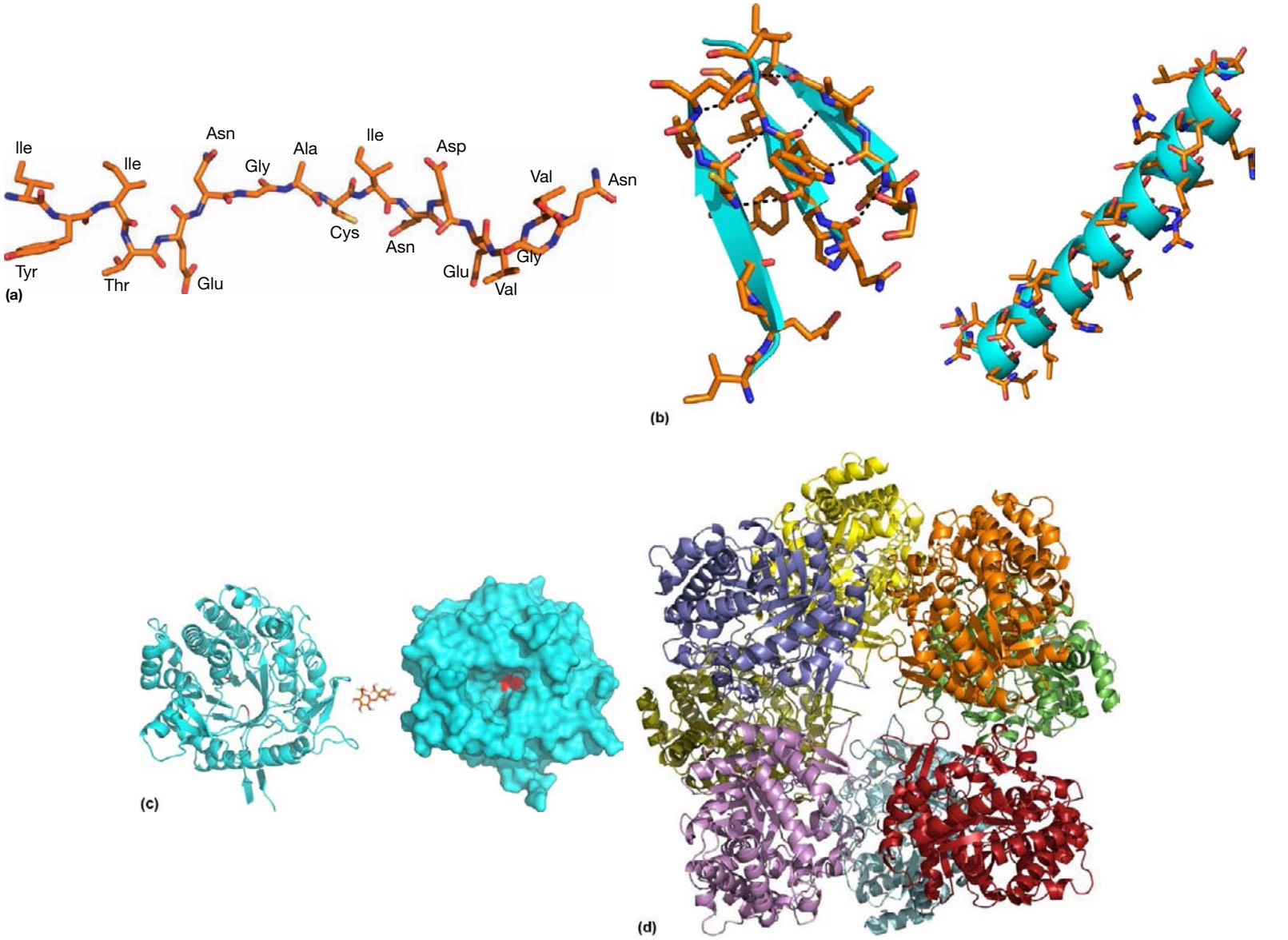

Figure 1 (a) Primary structure fragment of the enzyme β-glucosidase A (BglA) from *Paenibacillus polymyxa*. The amino acidic residues are identified with a three-letter code. (b) Two different secondary structure elements from BglA: a parallel β-sheet (left panel) and an α-helix (right panel). Hydrogen bonds connecting residues of different β-strands in the β-sheet are indicated by dashed lines. (c) Tertiary structure of a BglA polypeptide (monomer). The structure represented on the left is an α/β-barrel, made of an inner cylindrical (barrel-shaped) β-sheet, surrounded by α-helixes. On the right side, a 'surface' representation of the monomer shows the contour of amino acid residues that are fully accessible to the solvent. Catalytic residues are colored in red. A molecule of the enzyme's substrate, the disaccharide cellobiose, is represented in the center of the figure. (d) Quaternary structure of BglA. This enzyme is an octamer of identical monomers (PDB ID: 1BGA).

assigned a classification number (EC number) and a systematic name to identify the reaction that it catalyzes. In addition, for traditional or practical reasons, enzymes often have a common or trivial name. The classification proposed by the EC established three hierarchical levels: classes, subclasses, and sub-subclasses. Six classes of enzymes were considered: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Within each class, subclasses, and sub-subclasses were established attending specific characteristics of the reaction being catalyzed, such as the nature of the substrate and the chemical groups involved in the reaction. EC numbers are composed of four set of digits, separated by dots. The first, second, and third sets of digits refer to the class, subclass, and sub-subclass. Finally, the fourth digit identifies individual enzymes within a given sub-subclass. As an example, lactase is the common name of the enzyme that breaks the disaccharide lactose, the milk sugar, into its two monosaccharide components, glucose and galactose. The systematic name of the enzyme is β-D-galactoside galactohydrolase and its EC number is EC 3.2.1.23. The number 3 specifies the class name (hydrolase). The second number – 2 – the subclass (glycosylase). The third number – 1 – indicates the enzyme's property of hydrolyzing *O*- and *S*-glycosides. The fourth digit – 23 – identifies this particular enzyme as β-galactosidase. Nowadays, around 6500 enzymes, virtually the full set of enzymes involved in the metabolism of living organisms, are known. An updated view on enzyme names and classification is given by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (http://www.chem.qmul.ac.uk/iubmb/enzyme/).

Proteins are classified on the basis of their structural similarity. At the level of tertiary structure, that is, the three-dimensional appearance of the protein, natural polypeptides tend to adopt characteristic shapes that arise from different combinations of secondary structure elements. These shapes can be classified at different levels into a defined number of classes (http://scop2.mrc-lmb.cam.ac.uk/). Enzymes are protein molecules whose functions – catalytic properties – are determined by their structure, which in turn depends on their sequence. On the other hand, resemblance at the primary structure level (amino acid sequence) reflects obvious evolutionary relatedness. Therefore, systematic classification of enzymes on the basis of their structure affords information about both their phylogenetic relationship and their function. A particular case of structural classifications of enzymes is the Carbohydrate Active Enzymes Database (http://www.cazy.org/), which provides comprehensive information about all sorts of enzymes related to carbohydrate metabolism.

How Enzymes Work

Most biochemical reactions are energetically favorable. However, they would take place extremely slowly without the existence of a catalyst. This is because the chemical conversion of a substrate into a product undergoes an intermediate state (transition state), which has a higher energy than the substrate. The term *activation energy* (Ea) is used to describe the energy required to reach this transition state and is related to the reaction rate (the higher the Ea, the lower the reaction rate). It may also be described as a kinetic barrier that needs to be surpassed for the reaction to proceed. The role of enzymes is to supply an environment that greatly increases the chances for the reaction to occur, by decreasing the energy of the transition state and therefore the Ea. The environment for the catalysis is provided by the active site. The interaction between specific amino acid residues of the enzyme, in a particular three-dimensional configuration, and substrate is mediated by multiple weak interactions and allows that the substrate is recognized unmistakably among other similar molecules. Once the substrate is bound to the enzyme, catalytic amino acid residues transiently react with the substrate, assisting its transformation into the final product through a more energetically favorable transition state than that occurring without an enzyme. It is important to highlight that the enzyme, as any other catalyst, is not consumed during the reaction and recovers its original form when the reaction is completed. Enzymes act as molecular machines able to break, ensemble, or transform molecules. They have evolved as highly specialized tools, which explains the existence of as many different enzymes as metabolic reactions.

Enzyme Kinetics

Enzyme kinetics is the discipline that studies how enzymatic reactions take place, the rate at which they occur, and the influence of environmental conditions in the reaction process. The study of the rate of the reaction and its connection with the other most critical factor, the available amount of the substrate, is essential to understand enzyme action. In 1913 Michaelis and Menten published, in German, a paper whose title translated to English is 'The Kinetic of Invertase Action.' In this paper, which became a milestone of biochemistry, the authors established the relationship between the velocity of an enzymatic reaction and the concentration of the substrate according eqn [1]:

$$V_0 = V_{max}[S]/K_m + [S] \quad [1]$$

In this equation, V_0 (initial velocity) is the rate at which the reaction proceeds at a given substrate concentration, V_{max} is the maximal speed that the enzyme achieves (at saturating substrate concentrations), [*S*] is the substrate concentration, and *K*m is the Michaelis–Menten constant, which reflects the affinity of the enzyme for the substrate (the lower the *K*m, the higher the affinity). Its value corresponds to the concentration of substrate at which the reaction rate is half of the maximum. Michaelis and Menten deduced their equation from the study of the enzyme invertase that splits the disaccharide sucrose into its two constituent monosaccharides, glucose and fructose. This reaction was a convenient model system to study the behavior of an enzyme because the conversion of substrate into products was easily monitored by physical techniques. When a beam of polarized light traverses a sugar solution, the polarization plane is rotated, either to the right – dextro rotation – or to the left – levo rotation. This optical activity can be measured with a polarimeter, and the measured value correlates with the concentration of the sugar in the solution. Sucrose and glucose are dextrorotatory, whereas fructose is levorotatory. Because optical activity of fructose is higher than that of glucose, the enzymatic reaction can be followed

by measuring the rotation of light from right to left. Using this procedure, Michaelis and Menten determined the rate at which sucrose was transformed by the action of the invertase. The correlation that they found between the speed of reaction and sucrose concentration fitted a hyperbolic curve. The reaction rate increases as the substrate increases with an asymptotic tendency to a maximum (V_{max}; **Figure 2(a)**) according to eqn [1]. The study of many other enzymes in the following years confirmed the general value of the Michaelis–Menten model, even though there are enzymes that show different kinetics. This is the case, for instance, of allosteric enzymes, in which the interaction between enzyme and substrate is affected by another molecule – effector – that regulates the activity of the enzyme. In 1934, Hans Lineweaver and Dean Burk described a transformation of the Michaelis–Menten equation (eqn [2]). In the graphical representation of the transformation, the function of $1/V_0$ versus $1/[S]$ is a straight line (**Figure 2(b)**), where the intercept with the x axis is $1/V_{max}$ and the slope is K_m/V_{max}.

$$1/V_0 = (K_m/V_{max})\cdot 1/[S] + 1/V_{max} \qquad [2]$$

Linear regression analysis of experimental data by means of the Lineweaver–Burk, or double reciprocal, plot soon became a useful, albeit inaccurate, tool to easily calculate enzyme kinetic parameters (K_m and V_{max}) from experimental determinations. Currently, the availability of computational tools has facilitated nonlinear regression analysis, using directly eqn [1], and this is the preferred method to obtain more accurate values of K_m and V_{max}. However, the Lineweaver–Burk plot is still useful to corroborate the Michaelis–Menten behavior of an enzyme or to study the effect of different types of enzyme inhibitors.

The Effect of Physical and Chemical Agents on Enzyme Activity

Like most chemical reactions, enzyme-catalyzed reactions proceed faster when the temperature increases. This happens because an increase of the system's energy accelerates the molecules in the reaction, increasing the probability of interaction between the chemical components. However, in the case of enzymatic reactions the boosting effect of temperature is limited by the stability of the enzyme. Each enzyme has an optimal temperature at which it shows maximal activity. This is because enzymes structure is maintained by multiple noncovalent weak interactions such as hydrogen bonds, electrostatic, and hydrophobic interactions, which are influenced by physicochemical conditions, such as temperature. High temperature disrupts the network of interactions that holds the molecular architecture of enzymes. If the change is drastic, the protein structure disassembles, and the enzyme losses its activity irreversibly. This process is called heat denaturation. Enzymes show different degrees of sensitivity to temperature. In general terms, as a result of evolution, they are adapted to the temperature conditions in the natural habitat of the producing organism. For instance, human enzymes work at a temperature of around 37 °C. Enzymes from hyperthermophilic archaea function at temperatures of around 100 °C at which virtually all human enzymes are denatured. On the other hand, enzymes from psychrophilic organisms work optimally at temperatures close to 0 °C at which human enzymes are structurally stable but inactive. Another critical parameter for enzyme activity is pH, which affects mainly electrostatic interactions holding the enzyme structure. As happens with temperature, there is a pH at which the enzymes work optimally and a working range at which they remain active. If the pH increases or decreases outside the working range, the enzyme structure can be affected irreversibly.

Chemical compounds can affect enzyme activity. As mentioned earlier, some inorganic ions may be required as cofactors for the activity of some enzymes. Some compounds may bind reversibly or irreversibly to critical sites in the protein structure (within or outside the catalytic pocket), reducing or abolishing enzyme activity. Other compounds can react with a functional group of the enzyme leading to chemical modifications (e.g., oxidation, carbonylation) that may cause (reversible or irreversible) inactivation. A special case of inhibitors are those called 'suicide inactivators.' These are compounds of

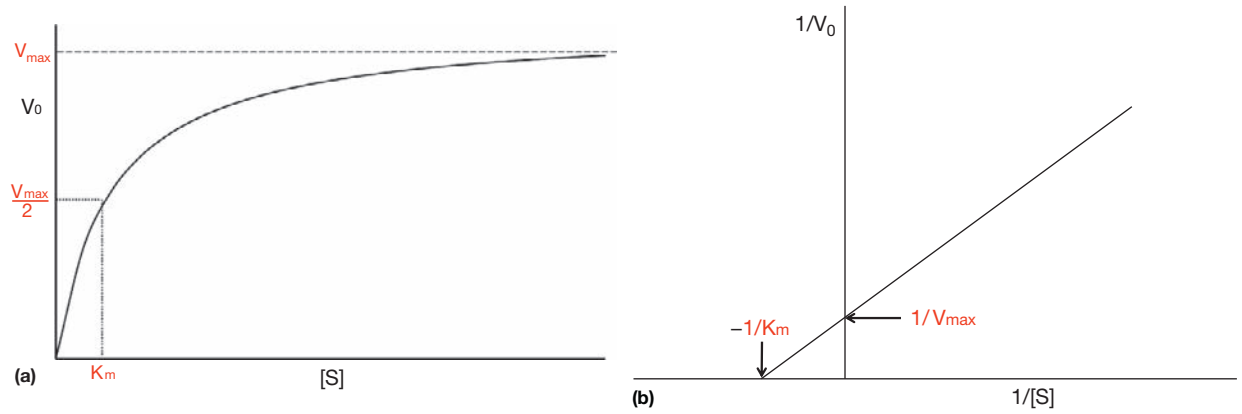

Figure 2 Michaelis–Menten kinetics. (a) Variation of the initial rate of the reaction (V_0) with the substrate concentration ([S]). The asymptote corresponding to maximum velocity (V_{max}), achieved at saturating substrate concentrations, is indicated with a dashed line. The Michaelis constant (K_m) value, which is indicative of the affinity of the enzyme for the substrate, corresponds to the substrate concentration for which the enzyme initial rate is half of V_{max}. (b) Lineweaver–Burk or double reciprocal plot of Michaelis–Menten kinetics.

chemical structure similar to the enzyme's substrate, which bind to the active site and are converted into a reactive molecule, which interacts irreversibly with one of the catalytic residues. These kinds of inhibitors are very useful research tools as they help to explain the mechanism of catalysis, can facilitate the crystallization and structural analysis of enzymes, and in some instances can be used as drugs in therapeutic treatments.

Enzyme Regulation

A simple living organism such as the bacterium *Escherichia coli* contains around 4000 genes that direct the synthesis of a fairly equivalent amount of proteins, most of which are enzymes. In humans, the number of protein-encoding genes is around 35 000. Not all proteins encoded in a genome are needed at the same time or in the same quantity. Because protein synthesis is a highly costly process for living cells, living organisms have developed regulatory mechanisms that supply the cells with the proteins that are required at a certain moment and in a convenient amount. Regulation is exerted at different levels in the flux of information, which goes from DNA to mRNA and from mRNA to protein. Genes can be classified into two categories, according to their expression rate. There are genes required at all times, for example those encoding enzymes acting in central metabolic pathways. These are called housekeeping genes, and they are expressed in a constitutive – continuous – way. For other genes, which are required just at certain stages, the first level of regulation is transcription. The synthesis of a regulated mRNA may be increased in the presence of a signal molecule called inducer. The consequent effect of increasing the encoded enzyme is called induction. An alternative mechanism is gene repression, in which the transcription of a gene is decreased or suppressed by the presence of a molecule called a repressor. As consequence of these two mechanisms the presence or absence of individual enzymes and even the levels at which they are present in the cells is controlled. Another level of regulation takes place on the life span of the mRNA molecule. In some cases, mRNAs translation responds to environmental signals through the interaction of specific sequences (UTRs, for untranslated regions) with protein factors. In other cases, as long as an mRNA is present in the cytoplasm, it continues to be translated by the ribosomes into the encoded protein. Different mRNA species differ in their intrinsic stability. As a general rule, mRNAs from highly regulated genes are more unstable than mRNAs from housekeeping genes and tend to disappear quickly. Even though an mRNA is eliminated, the enzyme resulting from its translation will remain present in the cytoplasm for a certain time, depending on its intrinsic stability. If its action is no longer required, its activity is generally suppressed by a mechanism of inhibition that can be exerted in different ways. One type of competitive inhibition is caused by a molecule that competes with the substrate for the active site of the enzyme. Often the inhibitor is the product of the enzymatic reaction. The action of the inhibitor depends on its concentration and on the affinity that the enzyme has for it. The effect is reversible because an increase on the concentration of the enzyme's substrate will make difficult the access of the inhibitor to the active site. Another type of reversible inhibition is noncompetitive or allosteric. In this case, the inhibitor binds to a region of the enzyme other than the active site, called the allosteric site.

Industrial Enzymes

Since early times of the development of biochemistry, researchers realized the industrial potential of enzymes. In 1907, the German company Röhm and Haas commercialized for the first time an enzyme preparation. The product, marketed with the name OROPON, represented a revolutionary innovation for leather bating, as an alternative to traditional procedures, which used animal excrements in the process. The innovation resulted from the work of Otto Röhm, who had studied the properties of enzymes like trypsin, isolated from pig pancreas, and its digestive effect on skins and furs. A few years later, in 1914, the Röhm and Haas firm launched BURNUS, the first enzyme containing detergent for laundry. One century after these precursory accomplishments, enzymes have innumerable applications in the food, chemical, and health industrial sectors. Enzymes are becoming an advantageous alternative to an increasing number of industrial chemical processes. Enzymes are natural, nontoxic, biodegradable, efficient, and highly specific catalysts that operate under mild conditions. Therefore, the use of enzymes generally represents an eco-friendly – green – alternative to conventional procedures. Current global production of industrial enzymes can be estimated to represent a value between 4 and 5 USD billion. Danish company Novozymes dominates world market with a share that approaches one-half of the total. Currently, other important European players are DSM in The Netherlands and BASF in Germany. In the United States, DuPont has achieved an important role in the enzyme business after the acquisition of Danisco and Genencor. The main area of enzyme application is the food and feed sector, which consumes over one-third of enzyme's production, closely followed by detergents and cleaners. Biofuel (ethanol), pulp and paper, textile, leather, and pharmaceutical industries also make extensive consumption of enzymes. Starch processing represents a major field for enzyme application. Simple sugars, monosaccharides, resulting from the enzymatic hydrolysis of starch carried out by α-amylase and glucoamylase, are used as sweeteners in different types of foods and beverages and are also used by biofuel industries, as fermentable material to produce ethanol. Traditional food industries like bakery and brewery also make extensive use of enzymes. In bakery, amylolytic enzymes are used to increase the amount of fermentable sugars, increasing loaf volume. Xylanases help dough handling and improve crumb structure. Proteases digest the gluten, improving dough texture. Lipases are used to obtain an emulsifying effect that reduces bread staling. In brewing, amylases are used to increase fermentable sugars. Glucan hydrolyzing enzymes (β-glucasases) have an important application in reducing the viscosity of beer, making for easier filtration. Proteases prevent turbidity and chill-haze formation in beer. ALDC (α-acetolactate-decarboxylase) prevents the formation of diacetyl, a compound that is formed as a natural product of yeast metabolism but has undesirable off-flavor characteristics. The detergent industry is the largest single market for

enzymes. Proteases and lipases are included in detergent formulations to eliminate stains of different chemical nature. Cellulases help to remove substances attached to cotton fibers. Enzymes improve different operations in the pulp and paper industry such as bleaching, pitch control, and deinking. Xylanases, lipases, cellulases, and laccases are used for this purpose. Enzymes have numerous applications in the pharmaceutical and health care industries. An example is the use of glucose oxidase in sensor strips to monitor glucose level in serum.

Most industrial enzymes are produced by microorganisms. Producer strains of fungi and bacteria are grown under well-defined conditions in either submerged or solid state fermentation. Genetic engineering techniques are routinely used to achieve homologous or heterologous expression of genes encoding enzymes of interest in suitable host strains and to enhance the performance of the producing strains. Production procedures are different for intracellular and secreted enzymes. In the first case, the cells of the producing microorganism need to be disrupted to release the enzyme, which may require expensive purification methodologies such as preparative chromatography. Secreted enzymes are recovered from the culture medium by filtration. The use of enzymes for food applications requires GRAS (generally recognized as safe) status granted by the U.S. Food and Drug Administration (FDA), the European Food Safety Authority (EFSA), or the views of qualified experts.

Extremozymes

Life thrives in extreme conditions. Living organisms can find niches at temperatures higher than 50 °C (thermophilic), or even higher than 100 °C (hyperthermophilic), below 20 °C (psychrophilic), extreme acid or alkaline conditions at pH lower than 3 (acidophilic) or higher than 9 (alkaliphilic), or under extreme salinity (halophilic). Organisms capable of living in such environments have enzymes that must endure extreme conditions. These 'extremozymes' have unique properties that could be used for many purposes. Thermophylic and hyperthermophylic enzymes show an increased number of interactions (hydrophobic, salt bridges, disulfide bonds) to maintain the protein structure at high temperatures. They are valuable tools for the degradation of complex polysaccharides (such as starch or lignocellulosic materials) or substrates whose solubility is greatly increased at high temperatures. In contrast, psychrophilic enzymes have weaker protein interactions to ensure sufficient flexibility to sustain enzymatic activity at low temperatures. Psychrophilic enzymes are used in detergents and other textile-related applications. Acidophilic or alkaliphilic enzymes usually fall in the thermophilic category and can be used for similar purposes. Production of extremozymes at industrial scale is still a challenge because generally extremophilic organisms are not easily culturable, and often the genes coding for their enzymes are not well expressed in conventional hosts. Nonetheless, this is an active research field because extremozymes may find countless applications in various industrial fields.

See also: Enzymes: Analysis and Food Processing.

Further Reading

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Escherichia coli and Other *Enterobacteriaceae*: Food Poisoning and Health Effects

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Introduction

The family Enterobacteriaceae consists of a heterogeneous group of rod-shaped, gram-negative bacteria, varying in their ecological niche, host range, and pathogenic potential for humans, animals, insects, and plants. Members of the family are aerobes or facultative anaerobes, are motile by peritrichous flagella or nonmotile, do not form endospores or microcysts, and are chemoorganotrophic with both respiratory and fermentative metabolisms, and most grow well at 22–35 °C. Foodborne pathogens in the family Enterobacteriaceae generally do not demonstrate an atypical heat resistance, and they are inactivated at temperatures lower than the pasteurization temperature for milk (72 °C for 15 s). The family Enterobacteriaceae contains more than 50 genera and a large number of species, and there are several human pathogens within this family. Important foodborne pathogens in the family Enterobacteriaceae include *Cronobacter* spp. (primarily *C. sakazakii*), *Escherichia coli*, *Salmonella enterica*, *Shigella* (*boydii*, *flexneri*, *sonnei*, and *dysenteriae*), and *Yersinia* (*enterocolitica* and *pseudotuberculosis*). In the United States, ~3.6 million cases of foodborne illness are caused each year by bacterial pathogens, and ~1.5 million of domestically (the United States) acquired foodborne cases are due to diarrheagenic *E. coli*, *Salmonella*, *Shigella*, and *Yersinia*. Thus, four genera within the family Enterobacteriaceae cause ~40% of bacterial foodborne illness in the United States. The 2011 data from the European Union indicate that the pathogens *Salmonella*, *Campylobacter*, and pathogenic *E. coli* account for 48% of the outbreaks and 27% of the cases of the foodborne disease. This chapter will focus primarily on pathogenic *E. coli* categories, with some discussion also on other foodborne pathogens in the family Enterobacteriaceae.

Intestinal Pathogenic *E. coli*

E. coli is a commensal organism that makes up part of the normal flora of the intestinal tract of humans and animals. *E. coli* belongs to the family Enterobacteriaceae and is a gram-negative, facultatively anaerobic, non-spore-forming, rod-shaped bacterium. Not all strains of *E. coli* are harmless, and some strains have acquired virulence mechanisms, making them pathogens. Some of these strains cause diarrhea and other intestinal diseases, while other *E. coli* pathogens induce disease at nonintestinal sites.

Enterohemorrhagic *E. coli* (Also Known as Shiga Toxin-Producing *E. coli*)

In 1977, Konowalchuk and coworkers in Canada discovered that culture filtrates from some *E. coli* strains produced a cytopathic effect on Vero cells (African green monkey kidney cell line). Since most of the strains producing this effect were from infants with diarrhea, they speculated that the toxin produced by these strains, which they referred to as verotoxin, had a role in the genesis of diarrhea. The *E. coli* that produced verotoxins were referred to as verotoxigenic *E. coli* or VTEC. In 1983, O'Brien and LaVeck purified the toxin from Konowalchuk's prototype strain, *E. coli* O26:H11 H30, and showed that it was structurally and antigenically similar to Shiga toxin produced by *Shigella dysenteriae*, and they named the toxin, Shiga-like toxin. In 1983, there were two outbreaks of hemorrhagic colitis (HC) in the United States, and it was reported by Riley and coworkers that they were linked to eating undercooked hamburgers contaminated with *E. coli* O157:H7, a rare *E. coli* serotype that was later shown to produce verotoxin. Subsequently, a link was established between VTEC infection and development of hemolytic uremic syndrome (HUS). The term enterohemorrhagic *E. coli* (EHEC) was coined by Myron Levine at the University of Maryland and refers to Shiga toxin-producing *E. coli* (STEC)/VTEC strains that have the same clinical, epidemiological, and pathogenic features associated with the prototype EHEC organism *E. coli* O157:H7.

STEC/EHEC O157:H7 and certain non-O157 STEC serogroups are major foodborne pathogens that cause diarrhea, HC, and HUS, and occasionally, death results in infected individuals. A conspicuous feature of STEC O157:H7 and non-O157 STEC intestinal pathology is the intestinal attaching and effacing (A/E) lesion, which is dependent on a type 3 secretion system (T3SS). The secretion system is encoded on a pathogenicity island, the locus of enterocyte effacement (LEE). The T3SS structure forms a pore complex that spans from the inner bacterial membrane to the host cellular membrane and enables the bacteria to inject bacterial effector proteins directly into the eukaryotic host cells following attachment. The effector proteins subvert eukaryotic cell processes and enable the pathogen to modulate the host environment to induce bacterial infection and disease symptoms. Among the non-O157 STEC, there are both LEE-positive and LEE-negative serogroups. For example, STEC serogroups O26, O45, O103, O111, O121, and O145 are generally LEE-positive and T3SS-dependent, whereas serogroups O1, O2, O5, O8, O48, O73, O76, O87, O91, O113, O118, O123, O128, O139, and O174 may be LEE-negative and T3SS-independent. However, only a few of the several hundred non-O157 serotypes have been tested to determine their LEE and T3SS status.

The *eae* (*E. coli* attaching and effacing) gene located on the LEE encodes the production of intimin at the bacterial outer membrane. Tir (transmembrane intimin receptor), also encoded by *eae*, is translocated by T3SS to the host cell membrane; Tir allows attachment of the bacterial intimin protein to the host cell

membrane leading to an intimate attachment of the bacterium with the host cell. The intimate attachment is characterized by the A/E lesion with localized loss of the microvilli (effacement) and an accumulation of polymerized actin pedestals beneath and surrounding the adherent bacterial cell. The bacteria remain extracellular during infection. There are non-O157 STEC that are LEE-negative and do not produce A/E lesions; however, these strains may produce severe diarrhea, HC, and even HUS, but their virulence mechanisms have been poorly studied. Other genes, such as the Saa (STEC agglutinating adhesion) and Iha (IrgA homologue adhesion), are also carried by STEC serogroups that may play a role in attachment; however, additional research is needed to determine the function of these genes.

The most critical virulence factor in STEC is a bacteriophage-encoded cytotoxin similar to the Shiga toxin produced by *Shigella dysenteriae*. In *E. coli*, the *Shigella* Shiga toxin was initially called Shiga-like toxin but was renamed to Shiga toxin 1 and Shiga toxin 2. STEC produce two Shiga toxins: Stx1 and Stx2. Stx2 has ~60% sequence homology to Stx1. There are a number of genetic variants for *stx*1, including *stx*1a, *stx*1c, and *stx*1d, and for *stx*2, there are seven variants, *stx*2a to *stx*2g. Stx2a is a more potent toxin than Stx1; the LD_{50} (in mice) of Stx1 is >1000 ng, whereas the LD_{50} of Stx2a is 6.5 ng. Stx1 and Stx2 are AB_5-type toxins; the B-pentamer of the holotoxin binds to globotriaosylceramide (Gb_3) present on host microvascular endothelial cell surfaces (the kidney, intestine, and brain) followed by endocytosis of the holotoxin. A subunit of the A portion of the toxin is an *N*-glycosidase that acts on the 28S RNA of the 60S ribosomal subunit leading to the inhibition of protein synthesis and apoptosis of endothelial cells, particularly those of the kidneys with resultant damage of the renal endothelial cells. There are swelling of the renal cells and detachment of the cells from the basement membrane and formation of fibrin thrombi in the kidney capillaries. Narrowing of the capillary lumens leads to a reduced blood supply to glomeruli with a loss of kidney function resulting in HUS. HUS is characterized by hemolytic anemia (destruction of red blood cells), acute kidney failure (uremia), and low platelet count (thrombocytopenia).

Early symptoms of STEC infection include watery diarrhea and abdominal cramps. Approximately 90% of confirmed STEC infections result in HC, and 5–15% of the HC cases progress to HUS. The mortality rate for HUS ranges from 5% to 10%. A few patients suffer long-term consequences affecting the gastrointestinal tract, liver, cardiovascular system, or central nervous system (CNS); however, most patients recover without major consequences. Very young and elderly patients show the most susceptibility to complications and death due to HUS. Antibiotics are not recommended in the treatment of STEC-infected patients and can increase the risk for development of HUS due to toxin release from lysed STEC cells. Fluid hydration may be necessary in diarrheic patients. Patients with severe bloody diarrhea may need blood transfusions, and in cases of HUS, dialysis may be necessary.

The main reservoir for STEC is the intestinal tract of cattle. However, other ruminants, including sheep, goat, buffalo, guanaco, deer, and elk, and nonruminants, including cats, dogs, pigs, horses, rabbits, and chickens, also can carry STEC. Contact with cattle, the cattle environment, and food products originating from cattle provides major risk factors for STEC infection to humans. Recreational, drinking, and irrigation waters contaminated with cattle feces can be sources of STEC. Infected individuals can transmit STEC person-to-person, and food may be contaminated by cross contamination or by an infected food preparer. In the United States, STEC O157:H7 was declared an adulterant in beef in 1994 after a large outbreak linked to contaminated ground beef occurred, and recently, STEC serogroups O26, O45, O103, O111, O121, and O145 were declared adulterants, since these serogroups cause the majority of the non-O157 STEC infections and illness similar to that caused by STEC O157:H7. For the period 2009–13 in the United States, O157:H7 STEC outbreaks were associated with foods, including spinach (1 outbreak), Romaine lettuce (1), Lebanon bologna (1), beef (3), hazel nuts (1), cheese (1), and prepackaged cookie dough (1). STEC serogroup O26 was associated with outbreaks linked to ground beef and raw clover sprouts. An outbreak linked to STEC O121 involved frozen food products, and O145 was associated with two outbreaks, one linked to Romaine lettuce and the other due to an unknown source. Outbreaks are often associated with STEC O157:H7, whereas non-O157 STEC are more often associated with sporadic cases and less often with outbreaks. However, it is now estimated that non-O157 strains cause a greater number of illnesses than *E. coli* O157:H7.

Based on the Centers for Disease Control and Prevention's FoodNet program in the United States for the years 2000–10, STEC O157:H7 was the cause of 5688 infections (74.0%), whereas non-O157 STEC caused 2006 infections (26.1%). The top six non-O157 STEC caused 1416 cases (18.4%). Serogroup O26 was responsible for 5.8% of STEC cases, followed by serogroups O103 (5.0%), O111 (4.2%), O121 (1.4%), O45 (1.2%), and O145 (0.8%). However, estimates of the total STEC foodborne infections indicate that the non-O157 serogroups cause 64% of the STEC disease cases in the United States. In the European Union, in 2011, nine STEC serogroups were responsible for 4022 cases of disease. STEC O157:H7/H− was responsible for 54% of the STEC cases, and STEAEC (Shiga toxin-producing enteroaggregative *E. coli*) O104 caused 26% of the disease cases. Serogroup O26 accounted for 7% of the cases. Serogroups O103, O91, O145, O128, O111, and O146 were responsible for fewer cases in the descending order. In 2010, nine serogroups (in the descending order, O157, O26, O103, O145, O91, O63, O111, O128, and O146) accounted for 2110 cases; STEC O157:H7 caused 71% of the STEC illnesses followed by 12% for serogroup O26. There was no report of STEAEC cases in the European Union in 2010. Of 504 isolates of STEC isolated for the period 2001–09 in Australia, the following serogroups were the most common: O157 (58%), O111 (13.7%), O26 (11.1%), O113 (3.6%), O55 (1.3%), and O86 (1.0%). The information on STEC isolates from patients in the United States, the European Union, and Australia indicate that serogroup O157 was the most common cause of STEC illness.

Strict hygiene during animal slaughtering, vegetable and fruit harvesting, product handling, shipping, and food preparation is critical in controlling and preventing STEC-induced disease.

Enteropathogenic *E. coli*

Enteropathogenic *E. coli* (EPEC) are an important cause of persistent and potentially fatal diarrhea in infants and young

children in developing countries. EPEC possess the LEE pathogenicity island similar to the Shiga toxin-producing *E. coli* but do not produce Stx. The formation of A/E lesions is characteristic of EPEC and is dependent on LEE island genes encoding T3SS, which acts in a similar way to the secretory system in STEC and allows intimate attachment of the bacteria to the host cell. LEE genes encode for effector proteins that subvert host cellular processes and induce gastrointestinal symptoms. Non-LEE-encoded genes produce effector proteins that inhibit phagocytosis, activate innate immune responses, and have a role in colonization and pathogenic responses. The *E. coli* adherence factor plasmid (pEAF) has the *bfp* operon, which encodes the bundle-forming pilus. The EPEC strains that carry the *eae* and *bfpA* genes are termed typical EPEC, whereas the atypical EPEC strains have the *eae* gene but lack *bfpA*. The typical EPEC strains are a major cause of infantile diarrhea in developing countries but are rare in the developed world. Diarrhea manifests as a severe watery stool with a large amount of mucus but is rarely bloody. The atypical EPEC induce a less severe illness. Treatment consists of prevention of dehydration induced by diarrhea. Humans appear to be the only reservoir for typical EPEC, whereas both animals and humans are reservoirs for atypical EPEC. Contaminated food and water have been associated with EPEC diarrhea. EPEC infections are generally sporadic, and food vehicles are often unknown. Raw chicken and beef, cold pork, meat pie, and other foods, as well as water, have been implicated in a few outbreaks. However, any foods exposed to the feces of an infected human may induce diarrhea.

Enteroinvasive *E. coli*

Enteroinvasive *E. coli* (EIEC) induce an invasive inflammatory colitis with copious watery diarrhea. EIEC are closely related to *Shigella* species but do not produce Shiga toxin. A number of EIEC strains contain the *sen* gene, which encodes for *Shigella* enterotoxin-2 (ShET-2). ShET-2 is translocated to the host cell via T3SS and may participate in the inflammation of the intestinal epithelial cells. The virulence effector genes, the *sen* gene, and T3SS genes are located on a large plasmid, pInv. EIEC does not carry the LEE pathogenicity island. When ingested, EIEC reach the colon, gain access to the colonic submucosa through the M cells, and are then taken up by macrophages. The bacterial cells escape from the macrophages and invade and replicate in the colonocytes. Bacterial virulence effectors are secreted via T3SS into the host cells, which enable the bacteria to evade the immune system and to promote cell-to-cell dissemination. The role of T3SS in EIEC is internalization of the bacteria into the host cell; A/E lesions are not seen in EIEC infection. EIEC-contaminated food and water are associated with the illness. EIEC infections caused by EIEC are rarely detected in the United States; however, a large outbreak occurred in Texas in 1992 in which 370 people became ill after consuming guacamole. Foods are most likely contaminated by infected food handlers, and EIEC infections may also occur via person-to-person transmission. Outbreaks associated with EIEC in several countries have been reported with water, imported cheese, vegetables, and potato salad as the vehicles of infection. Rehydration therapy and antibiotics are recommended as treatments for EIEC infection. Since humans are the only known reservoir of EIEC, it is probable that most EIEC infections are due to food prepared by an ill individual.

Enterotoxigenic *E. coli*

Enterotoxigenic *E. coli* (ETEC) infection induces a watery diarrhea, which may be a mild, self-limiting disease or a severe purging cholera-like disease. The infection may attack children in developing countries or other areas with poor hygiene and unclean water. In addition, ETEC is a major cause of traveler's diarrhea. The organism colonizes the surface of the small bowel and produces enterotoxins, which induce the diarrhea. ETEC elaborate two types of plasmid-encoded enterotoxins: heat-labile enterotoxins (LT-1 and LT-2) and heat-stable enterotoxins (STa and STb). LT-1 is an AB_5 toxin closely related to cholera toxin. LT intoxication of host intestinal cells leads to permanent activation of adenylate cyclase with increased levels of cyclic AMP (adenosine monophosphate). Cyclic AMP activates protein kinase A, leading to phosphorylation of ion channels and secretion of electrolytes into the intestinal lumen. The increased gut osmolarity leads to a watery diarrhea. STa is a small, single-peptide toxin, which binds to the host cell guanylate cyclase, leading to increased cyclic GMP (guanosine monophosphate) levels. Chloride secretion is increased, and NaCl absorption is inhibited. The increased osmolarity in the intestinal lumen results in diarrhea. ETEC strains may produce LT, ST, or both. Rehydration therapy and antibiotics are recommended as treatments for ETEC infection. Humans are the reservoir of ETEC, and ingestion of food or water contaminated by an infected person is often the likely cause of illness. Foods implicated in infections include Brie cheese, curried turkey, mayonnaise, crab meat, deli foods, and salads.

Enteroaggregative *E. coli*

Enteroaggregative *E. coli* (EAEC) include a heterogeneous population of *E. coli* strains that do not produce LT or ST and that adhere in a stacked brick pattern to HEp-2 cells and to each other. These strains cause a watery diarrhea in both industrialized and third-world countries. Diarrhea can be of relatively long duration ($\geq$ 14 days) in some cases. EAEC infects children and immunocompromised individuals, and these pathogens are an important cause of traveler's diarrhea. The major features of EAEC pathogenesis are colonization of the intestinal mucosa with mucoid biofilm formation, elaboration of enterotoxins, and mucosal inflammation. The plasmid (pAA)-encoded AAF (aggregative adherence fimbriae) and AggR (regulator) are required for colonization; however, AAF is not present in all EAEC strains, and therefore, other adhesins are likely involved in EAEC colonization. Adhesion is followed by secretion of Pet (plasmid-encoded toxin) and EAST1 toxins (plasmid-encoded EAEC heat-stable toxin 1). EAST1 is a small polypeptide enterotoxin that induces diarrhea by secreting cyclic GMP. The *pet* gene is located on pAA and encodes Pet, a heat-labile protein toxin belonging to the serine protease autotransporter family. Pet is an enterotoxin inducing cytoskeletal changes and epithelial cell rounding. In addition, the enterotoxin ShET-1 may be found in some EAEC. However, the role of enterotoxins in EAEC-induced diarrhea is not clear. EAEC strains that show the stacked brick phenotype and that

contain pAA are termed typical EAEC, whereas those strains that have the stacked brick phenotype but lack pAA are termed atypical EAEC. Both atypical and typical strains induce diarrhea. Rehydration therapy and antibiotics are recommended as treatments for EAEC infection. Ingestion of food has been associated with EAEC-induced diarrhea; however, the reservoir of the organism is unknown. Salsa in Mexico, unpasteurized cheeses in Italy, and school lunches in Japan have caused outbreaks of EAEC-induced illness. It is probable that infected food handlers are the source of the organism.

Diffusely Adherent *E. coli*

Diffuse adherence is characterized by uniform attachment of bacteria to HeLa or HEp-2 cells, whereas localized adherence is when bacteria attach in groups to only a few sites on the cultured cell surface. Diffusely adherent *E. coli* (DAEC) are a poorly characterized heterogeneous population of *E. coli* showing diffuse attachment to cell surfaces and are believed to be a cause of diarrhea. The DAEC pathotype colonizes the small bowel and may be responsible for watery diarrhea in children. Diffuse adherence is mediated by both fimbrial (Dr and F1845) and afimbrial (Afa) adhesions. A secreted autotransporter toxin (SAT) has been demonstrated in DAEC strains associated with diarrhea. SAT induces lesions on tight junctions of epithelial cells causing increased permeability and may be a virulence mechanism in DAEC. However, the mechanism of diarrhea induction in DAEC strains is not certain.

Shiga Toxin-Producing Enteroaggregative *E. coli*

The Shiga toxin-producing enteroaggregative *E. coli* (STEAEC) may be a newly emerging pathotype of *E. coli*. The serotype O104:H4 caused a large outbreak associated with close to 4000 cases of illness, over 800 cases of HUS, and over 50 deaths in Europe in 2011. This pathotype is unusual in that the organism carried the pAA-virulence plasmid (and its virulence genes) of EAEC strains and a *stx*2-harboring prophage. Thus, this STEAEC strain presents the stacked brick phenotype of EAEC and production of Shiga toxin. The STEAEC strain is not a pathogenic *E. coli* with new virulence factors but is rather a pathogen that has combined the virulence properties of the EAEC with the production of Shiga toxin of the STEC pathotype. The strong morbidity and mortality induced by this strain may be due to its strong adherence to the host cell leading to higher levels of Stx transfer. The serotype O111:H2 with both Stx2 and EAgg (AggR) was reported in 1997 as a cause of a case of HUS, and in 2012, an outbreak induced by serotype O111:H21 positive for Stx2c and *aggR* occurred in Ireland. Therefore, it is probable that the transfer of the Shiga toxin gene into other EAEC serotypes will produce STEAEC serotypes that may cause serious outbreaks of Shiga toxin-induced HC and HUS in the future.

Recent Outbreaks in the United States due to *E. coli* Pathotypes

For the years 2009–10, the Centers for Disease Control and Prevention reported that there were 58 outbreaks due to STEC serotypes (53 due to STEC O157:H7 and 5 due to non-O157 STEC) with 651 total cases. In 2009, there were three outbreaks due to ETEC with 85 cases, and in 2010, EPEC caused one outbreak with 7 cases. The vehicles of infection were not provided. There were no reported outbreaks caused by EIEC, EAEC, or DAEC for 2009–10.

Nonintestinal Pathogenic *E. coli*

The nonintestinal pathogenic *E. coli*, which are a cause of illness outside of the gut, are termed extraintestinal pathogenic *E. coli* (ExPEC). The intestinal pathogenic *E. coli* (IPEC) are obligate pathogens, whereas the ExPEC are facultative pathogens that reside in the gut of a fraction of individuals and behave as harmless commensal organisms. In general, the IPEC have well-defined virulence traits, whereas ExPEC strains are very diverse and have few common virulence factors among them. Thus, it is difficult to define ExPEC by virulence properties. At the present time, ExPEC and nonpathogenic *E. coli* cannot be differentiated by molecular epidemiological techniques, and therefore, there is a limited understanding of the biology of ExPEC.

Urinary tract infections (UTIs) are common bacterial infections, particularly in women, and extract extremely high health costs in terms of morbidity, treatment, and loss of productivity. Uropathogenic *E. coli* (UPEC) are ExPEC that cause ~80% of community-acquired UTIs and ~30% of nosocomial UTIs. The UPEC reside in the gastrointestinal tract and can be transferred to the urinary tract. Infection of the urethra is termed urethritis, that of the bladder is termed cystitis, and that of the kidneys is termed pyelonephritis. Individuals at risk for acquiring UTIs include the very young, elderly, females, pregnant women, and individuals who are immunocompromised or who are undergoing urinary catheterization. The UPEC possess a number of virulence traits that make them efficient pathogens of the urinary tract such as specific adhesins; several toxins including hemolysin, cytotoxic necrotizing factor type 1 (CNF1), and a protease toxin (SAT); and iron acquisition systems. The primary reservoir for UPEC is the human intestinal tract; however, the original source (e.g., possibly avian, food, or environmental source) of UPEC is not clear.

The pathology leading to UTIs by UPEC includes adherence and colonization, evasion of host defenses, and damage to host cells and tissues. The UPEC are transferred from the rectum to the periurethral area and ascend the urethra into the bladder. Type 1 fimbriated UPEC attach to the bladder epithelial cells and trigger apoptosis and exfoliation, as well as invasion and multiplication of UPEC in the bladder cells. If the UTI is not treated, the organisms may ascend the ureters and induce pyelonephritis. Pyelonephritis can cause irreversible kidney damage, kidney failure, and death. Some individuals may have high bacterial counts in the urine but are asymptomatic. Treatment of UTIs is becoming more complicated due to increased resistance of UPEC to antimicrobial compounds.

Neonatal meningitis *E. coli* (NMEC) strains are ExPEC that represent the most frequent cause of gram-negative meningitis in the newborn. Bacterial meningitis is an inflammation of the meninges with high morbidity and mortality. Severe neurological sequelae occur in 30–50% of infants who survive

meningitis. The neonate (neonatal period is the first 28 days after birth) acquires NMEC during birth or from the environment, and the organisms invade and multiply in the bloodstream. The NMEC pathogen then crosses the blood–brain barrier (BBB). The BBB is a structural and functional barrier made up of the brain microvascular endothelial cells (BMECs), which separates the CNS from the vascular component of the body. The BBB regulates the transition of macromolecules into the CNS and protects the CNS against entry of microbes and toxins that may be present in the blood.

There are several stages of NMEC pathogenesis: bacteremia, binding of bacteria to the surface of the BMEC, and invasion of the BMEC followed by invasion of the meninges and the CNS.

Meningitis is associated with bacteremia counts of $>10^3$ CFU ml^{-1} blood. Outer membrane protein A (OmpA), K1 capsule (carried by the predominant strains of *E. coli* that cause neonatal meningitis), and *O*-lipopolysaccharide are necessary for survival and multiplication of NMEC in the circulatory system. OmpA and FimB (fimbriae) of NMEC bind to BMEC, and the organism invades the BMEC via a transcellular traversal mechanism in which the bacteria penetrate the cells without disrupting them. OmpA, Ibe proteins, and CNF1 induce actin cytoskeletal rearrangement and formation of microvillus-like protrusions, which facilitate entry of the organisms into the BMEC. NMEC leave the BMEC and invade the meninges and CNS, and then, they multiply and induce the release of proinflammatory compounds. In addition, there are brain edema and increased intracranial pressure with meningitis and neuronal injury. Neonatal meningitis is treated with appropriate antibiotics.

Avian pathogenic *E. coli* (APEC) are responsible for a number of economically important poultry diseases. Interestingly, some human ExPEC and some APEC strains have similar phylogenetic backgrounds and share some of the same virulence genes. This relationship between ExPEC and APEC suggests that APEC may be zoonotic pathogens that can lead to human diseases such as UTIs or neonatal meningitis through the ingestion of poultry meat. Recent studies support the role of chicken meat as a potential source of organisms inducing UTIs to humans; however, food has not been shown to be related to neonatal meningitis.

Other Enterobacteriaceae

Cronobacter sakazakii

The genus *Cronobacter* consists of seven species, namely, *C. sakazakii*, *C. malonaticus*, *C. turicensis*, *C. dublinensis*, *C. muytjensii*, *C. condimenti*, and *C. universalis*. *Cronobacter* species are gram-negative, motile by peritrichous flagella, non-spore-forming, facultatively anaerobic, rod-shaped bacteria. *Cronobacter* species are more thermotolerant when compared with other genera in the Enterobacteriaceae family, and they can grow in temperatures over a range of 6–47 °C. The reservoir of *Cronobacter* is unknown; however, plants are a possible source, and the organisms have been isolated from a wide range of food products. Most *Cronobacter* species have been associated with human illness; however, the majority of the infections in infants and neonates have been associated with *C. sakazakii*, and this species has been the most studied. Infants that ingest rehydrated powdered milk formula contaminated with *C. sakazakii* suffer bacteremia, meningitis, and necrotizing enterocolitis; mortality is greater than 50%. Infections among immunocompromised adults, particularly the elderly, have been reported. The outer membrane protein, OmpA, is an important virulence factor in the development of meningitis in newborn rats. OmpA-positive *Cronobacter* cross the intestinal barrier, multiply in the blood, and invade the brain endothelial cells. The pathogen crosses the BBB and eventually causes meningitis. *Cronobacter* lacking the *ompA* gene do not bind to intestinal epithelial cells, do not cross the intestinal barrier, and do not lead to pathology. Other potential virulence factors include biofilm formation and membrane-associated efflux pumps that expel a broad range of inhibitory compounds. However, it is clear that knowledge of the virulence mechanisms of the *Cronobacter* species is limited.

Shigella

The *Shigella* species are small, gram-negative, nonmotile, facultatively anaerobic rods, which do not produce spores or capsules. The optimum growth temperature is 37 °C. The organisms are the causative agents of bacillary dysentery (shigellosis). All of the species except *Shigella sonnei* have several serotypes. Humans are the natural host of the *Shigella* species. The organisms are transmitted via the fecal–oral route by infected individuals and contaminated food and water. Shigellosis is an invasive infection of the colon that ranges from short-term watery diarrhea to inflammatory bowel disease. In inflammatory bowel disease, watery diarrhea proceeds to bloody mucoid stools, abdominal cramps, and tenesmus. In healthy individuals, shigellosis is self-limited and resolves without sequelae. In malnourished infants and young children in developing countries, shigellosis can result in acute, life-threatening complications. HIV-infected and immunocompromised individuals may suffer persistent diarrhea and malnutrition if infected by *Shigella*. Cellular invasion and spread of infection involve invasion of the enterocytes, intracellular multiplication, intra- and intercellular spread, killing of the host cells, and tissue damage. *Shigella dysenteriae*, but not other *Shigella* species, produce Shiga toxin. Shiga toxin is encoded chromosomally in *Shigella dysenteriae*, whereas it is phage-encoded in STEC. Similar to STEC, infection by *Shigella dysenteriae* can induce HUS. The virulence of *Shigella* is dependent on a virulence plasmid encoding T3SS components. T3SS is necessary for invasion and secretion of virulence factors into intestinal epithelial cells leading to inflammatory destruction of the epithelial lining of the intestine. Rehydration therapy and antibiotics are used to treat shigellosis.

Salmonella enterica

Salmonella spp. are rod-shaped, gram-negative, non-spore-forming, predominantly motile with peritrichous flagella, chemoorganotrophic, and facultatively anaerobic. The range of temperature for growth is 2–54 °C, and the optimum is 35–37 °C. There are more than 2000 different serovars based on flagellar, carbohydrate, and lipopolysaccharide characteristics. *Salmonella* causes disease in both animals and humans. Serovars Typhi, Paratyphi, and Sendai cause enteric fever, whereas most

serovars cause enterocolitis and diarrhea. The elderly, infants, and immunocompromised individuals are at the highest risk for *Salmonella* infections. Ingestion of food or water contaminated with nontyphoidal *Salmonella* is followed by crampy, abdominal pain, diarrhea, nausea, and vomiting. Enterocolitis in children is marked by increased severity of inflammation, bloody diarrhea, long duration, and increased risk of complications. Salmonellosis is self-limited in immunocompetent hosts. A small number of infected individuals develop a sequela known as reactive arthritis. Important virulence genes are carried on the *Salmonella* pathogenicity islands (SPI-1 through SPI-5). Other virulence traits include the virulence plasmid (pSLT), adhesins, flagella, and proteins involved in biofilm formation. Antibiotics are recommended in severely ill patients; however, it is important to realize that antibiotic resistance is common in the salmonellae. Hydration therapy is necessary in cases of severe diarrhea.

Yersinia

Yersiniosis is a self-limiting gastrointestinal disease in immunocompetent individuals; however, in young children and immunocompromised individuals, the disease is more severe and may result in death. Sequelae such as reactive arthritis may occur after infection. The organism is rod-shaped, gram-negative, psychrotrophic (can grow at 4 °C), and facultatively anaerobic. Yersiniosis is a foodborne disease caused mainly by *Y. enterocolitica* and *Y. pseudotuberculosis*, and the flea-transmitted *Y. pestis* rarely causes foodborne illness. Pigs are the major reservoir of *Y. enterocolitica*; pig tonsils are a common site of *Yersinia* colonization. The absence of the virulence plasmid (pYV) is associated with lack of pathogenicity in *Y. enterocolitica*. pYV encodes the *Yersinia* adhesin YadA, which mediates mucus and epithelial cell attachment and host cell invasion. A T3SS is also encoded by pYV and is involved in the injection of virulence-related effector proteins, the *Yersinia* outer membrane proteins (Yops), into the host cell. The Yop proteins interfere with host signal induction (evasion of immune responses), disrupt host actin cytoskeleton, and induce apoptosis in host cells. Chromosomal genes encoding the invasion protein (Inv) and the attachment-invasion locus (Ail) have a role in cell binding and invasion by *Yersinia*. The most common vehicle for yersiniosis is contaminated food, including meat, oysters, fish, raw milk, and cheese. Antibiotics are used to treat yersiniosis.

See also: Diarrheal Diseases; Emerging Foodborne Enteric Bacterial Pathogens; *Escherichia coli* and Other *Enterobacteriaceae*: Occurrence and Detection; Food Poisoning: Classification; Food Poisoning: Epidemiology; Food Poisoning: Tracing Origins and Testing; *Salmonella*: Detection; *Salmonella*: Properties and Occurrence; *Salmonella*: Salmonellosis; Shigella; *Yersinia enterocolitica*: Properties and Occurrence; *Yersinia enterocolitica*: Detection and Treatment.

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Escherichia coli and Other *Enterobacteriaceae*: Occurrence and Detection

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Enterobacteriaceae

All members of the *Enterobacteriaceae* possess some common bacteriological features. They are gram-negative, rod-shaped facultative anaerobes. Bacterial isolates are classified as being either motile by means of peritrichous flagella or nonmotile, being devoid of any flagella. Members of this family are generally 0.3–1.0 × 0.6–6.0 μm in size and are non-spore-forming. They can ferment glucose and are catalase-positive (except for *Shigella dysenteriae*) and oxidase-negative, and they reduce nitrate to nitrite. Table 1 provides a summary of the general characteristics of some of the members of this family.

Enterobacteriaceae family members are associated with a wide range of disease (Figure 1). Illnesses associated with members of the *Enterobacteriaceae* include wound infections, urinary tract infections (UTI), gastroenteritis, meningitis, pneumonia, septicemia, and hemolytic uremic syndrome (HUS). While some of these species are true pathogens, others are regarded as opportunistic.

Members of this bacterial family can grow readily on a variety of microbiological culture media. They are also widely dispersed throughout the environment being found in soil, water, plants, and the gastrointestinal tract of animals and humans.

The Genus *Escherichia*

The genus *Escherichia* was described originally by Theodore von Escherich who first discovered *Escherichia coli* in 1885. This bacterial genus contains five species with *E. coli* being the best known. Other species include *E. albertii*, *E. fergusonii*, *E. hermannii*, and *E. vulneris*. A number of species are pathogenic; however, the majority are commensals or opportunistic pathogens.

Features associated with isolates of the E. coli *genus*

Escherichia coli cells are 0.5 μm in length and 2.0 μm in diameter. Optimal growth occurs at a temperature of 37 °C, though some strains can grow at temperatures up to 49 °C. The optimal range of pH is between 6.0 and 7.0, with few being able to grow at a pH <4 or >9. *E. coli* grown on MacConkey agar forms small, red/pink, smooth, and circular colonies. When further assessed, these are motile by means of peritrichous flagella (Table 1).

Environments known to contain E. coli

These bacteria can be found in the gastrointestinal tract of warm-blooded animals in which it colonizes within hours of birth. Strains can also be found throughout the environment, in water, food, and soil. Most *E. coli* isolates are harmless and play an important role as constituents of the normal biota or microbiome found in a healthy human intestinal tract. They aid digestion in the gut, produce vitamin K_2, and prevent pathogenic bacteria from thriving in the environment of the intestine.

Relevance of E. coli *to public health*

Pathogenic strains of *E. coli* cause a variety of illnesses such as food poisoning with severe stomach cramps, watery or bloody diarrhea, vomiting, and nausea. Severe complications include kidney failure, seizures, and stroke. The main route of transmission is through *fecal–oral contamination*, due to poor hygienic conditions.

E. coli has a very fluid genome that enables the uptake of many different mobile genetic elements. This genomic fluidity has had a large impact on the evolution of pathogenic serovars with phages, plasmids, and pathogenicity islands playing a role in their diversity and evolution. This has resulted in the emergence of pathogenic strains that are associated with their own virulence factors and disease symptoms. Pathogenic strains are classified into a number of pathotypes as follows:

- Verotoxigenic *E. coli* (VTEC)
- Enterotoxigenic *E. coli* (ETEC)
- Enteropathogenic *E. coli* (EPEC)
- Enteroaggregative *E. coli* (EAEC)
- Enteroinvasive *E. coli* (EIEC)
- Diffusely adherent *E. coli* (DAEC)

Pathotypes of *Escherichia coli* Important to Human Health

Verotoxigenic E. coli

VTEC strains are defined by the production of cytotoxins that disrupt protein synthesis within host cells. These toxins are referred to as either verotoxins (*vt*) due to their activity against Vero cells *in vitro* or Shiga-like toxin 1 (*stx*1) and Shiga-like toxin 2 (*stx*2) due to their similarity to the Shiga toxin produced by *Shigella dysenteriae*.

Dissemination of these strains occurs *via* the *fecal–oral* route arising from substandard hygienic conditions. An infectious dose can be as low as 10–100 cells. Ruminant animals are an important reservoir for VTEC. Infections can occur through contact with an infected individual, directly from an animal shedding the organism or its fecal matter, *via* the consumption of a contaminated food containing the bacterium or through the consumption of contaminated water.

Symptoms of a VTEC infection can range from mild diarrhea to severe bloody diarrhea. Potentially fatal complications arising from VTEC infections include HUS and thrombotic thrombocytopenic purpura.

Enterohemorrhagic *E. coli* (EHEC) is a subset of VTEC that is classified as pathogenic to humans. Usually, strains are classified as EHEC when they contain a verotoxin-encoding gene in combination with an *eae* gene that encodes intimin, an outer membrane protein that is involved in attachment to epithelial cells. Some *eae*-negative strains may also be EHEC.

Table 1 Typical characteristics of *Enterobacteriaceae*

Features	*Escherichia coli*	*Salmonella*	*Yersinia*	*Cronobacter*	*Enterobacter*	*Citrobacter*
Size (μm)	0.5 × 2.0	3.4 × 0.7	0.8 × 1–2	3 × 1	0.6 × 2–3	1 × 2–6
Motility	Motile	Motile	Nonmotile (between 25 and 35 °C)	Motile	Motile	Motile
Optimal Temperature or range (°C)	37	30–37	28	6–45	30–37	37
pH range	6–7	6.5–9	4–10	5–10	4.4–9	5–8
Flagella	Peritrichous	Peritrichous	Peritrichous	Peritrichous	Peritrichous	Peritrichous
Encapsulated	Yes	Yes	Yes	Yes	Yes	No
Pathogen category	Major	Major	Major	Opportunistic	Opportunistic	Opportunistic

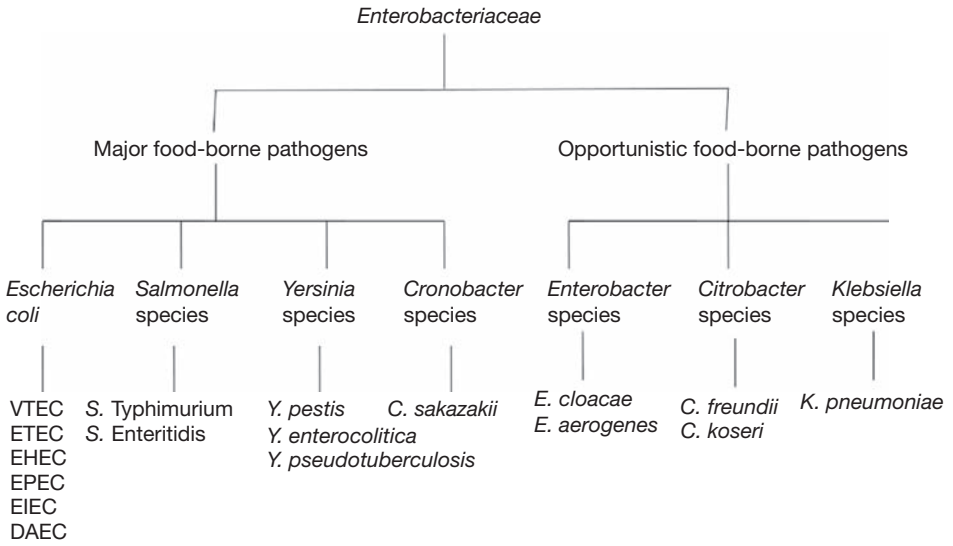

Figure 1 *Enterobacteriaceae* of importance to public health.

They produce highly potent toxins and can form attaching and effacing lesions on epithelial cells, a characteristic typically associated with EPEC strains. In terms of public health, *E. coli* O157 is one of the most commonly detected EHEC serotypes, although other serotypes are also being recognized.

Enteropathogenic E. coli

EPEC strains are noted for their ability to produce attaching and effacing (A/E) lesions. In this case, bacteria attach to the host epithelial cell membrane disrupting the cell surface resulting in the effacement of microvilli at the site of adherence. EPEC contains a 35 kbp pathogenicity island called the locus of enterocyte effacement, which encodes genes responsible for the attaching and effacing lesions.

These strains are further classified into typical enteropathogenic *E. coli* (tEPEC) and atypical enteropathogenic *E. coli* (aEPEC) based on the presence and absence of the *E. coli* adherence factor plasmid that is only found in tEPEC strains. tEPEC is a well-documented cause of gastroenteritis in infants. aEPEC is more prevalent than tEPEC in both developed and developing countries and is an important risk factor in juvenile endemic diarrhea and diarrheal outbreaks.

Outbreaks of EPEC infection have been linked to the consumption of contaminated drinking water as well as some meat products. In the case of tEPEC, humans are thought to be the only reservoir while both animals and humans are reservoirs for aEPEC.

Enterotoxigenic E. coli

ETEC strains are characterized by their expression of enterotoxins and the presence of host-specific fimbriae required for attachment to intestinal cells. Two types of toxins, heat-stable (ST) and heat-labile (LT) toxins, are produced by ETEC. Different strains can harbor either one or both of these toxin-encoding loci. The toxins act to stimulate the intestinal lining leading to an increase in fluid secretion resulting in diarrhea.

These pathogenic *E. coli* are the leading cause of traveler's diarrhea. Symptoms range from mild to severe cholera-like illness characterized by watery stool(s), vomiting, and stomach pains lasting up to 3 days. The infectious dose is estimated at 10^8 cells but is lower in immunocompromised patients.

ETEC is mainly transmitted through food and water contaminated with human and animal feces. In humans, ETEC

infections are widespread though rarely life-threatening and usually self-limiting. It is a major pathogen in animals and can even cause death.

Enteroinvasive E. coli

These bacteria are characterized by their ability to invade epithelial cells and disseminate from *cell to cell* while replicating within. EIEC differ from typical *E. coli* in that they are nonmotile and cannot decarboxylate lysine nor ferment lactose. EIEC strains possess an invasion plasmid that encodes the virulence genes conferring EIEC invasive capacity.

EIEC strains are highly related to *Shigella* species at the genetic, biochemical, and pathogenic levels. They cause a syndrome identical to bacillary dysentery more often linked to *Shigella* species, with profuse diarrhea and high fever. The infectious dose of EIEC is estimated at 10^6 cells whereas that of *Shigella* is similar to EHEC, in the range of ten to several hundred cells.

There are no known animal reservoirs of EIEC. Transmission usually occurs through contact with infected humans through the fecal–oral route. EIEC infections can also occur through ingestion of contaminated food or water.

Enteroaggregative E. coli

EAEC strains are characterized by their ability to adhere to epithelial cells in an aggregative manner resulting in a *stacked-brick pattern*. They do not secrete LT or ST enterotoxins. EAEC pathogenesis involves adherence to the intestinal mucosa resulting in increased production and deposition of a mucus biofilm leading to mucosal toxicity due to inflammation and cytokine release. EAEC strains can cause persistent infection by evading the immune system of the host.

These organisms are associated with acute or persistent diarrhea with little or no fever and no vomiting. They are especially prevalent among children in developing countries. EAEC strains are considered emerging pathogens, as VTEC O111:H2 strains isolated from a HUS outbreak in France also possessed genetic markers of EAEC *E. coli*. EAEC outbreaks have been linked to infant food including formula, milk, and water.

In the summer of 2011, a novel strain of *E. coli* O104:H4 emerged and caused a serious food-borne outbreak in Lower Saxony, Germany. This outbreak was associated with serious illness such as bloody diarrhea with a high incidence of HUS. Epidemiological investigations reported 3816 cases with 845 instances of HUS and 54 deaths. At first, *E. coli* O104:H4 was thought to be an EHEC strain, but it was later identified as an EAEC that had acquired the ability to produce Shiga toxin.

Diffusely adherent E. coli

DAEC strains are characterized by their adherence to Hep-2 cells in a diffuse pattern. Approximately 75% of these bacteria express the Afa/Dr family of adhesins, but not the typical virulence genes of other *E. coli* pathotypes.

DAEC are a major cause of UTI and can cause watery diarrhea typically without blood but with vomiting. Infection is characterized by the growth of fingerlike cellular projections on the surface of infected epithelial cells that envelop the bacteria. Sources associated with DAEC infections include contaminated food such as undercooked beef, contaminated water, and contact with infected animals.

Enterobacteriaceae of Importance to Human Health

The genus Salmonella

This bacterial genus was named after Daniel Elmer Salmon following the discovery of the type strain *Salmonella enterica* in 1885. The *Salmonella* genus contains three species, *S. bongori*, *S. enterica* (including six subspecies), and *S. subterranea*. The genus is subdivided into more than 2500 serovars based on antigenic features. *Salmonella enterica* and its serovars are most commonly associated with human disease.

Features associated with isolates of the Salmonella *genus*

Salmonella cells are 2–3.4 μm long and 0.7–9.0 μm in diameter, with rounded ends. Most have peritrichous flagella, though *S. subterranea* has one lateral flagellum. Optimal growth occurs within a pH range from 6.5 to 9.0 and across a temperature range of 30–37 °C. *Salmonella* species do not grow at pHs <3.5 and >9.5 nor at temperatures <10 °C and >42 °C. *Salmonellae* are acid-tolerant and can grow at lower pH limits of 3.7–4.4. *Salmonella* species can produce hydrogen sulfide from thiosulfate. The motility of *Salmonella* isolates is strain-dependent and specific to the host environment.

Environments known to contain Salmonella

Salmonella species are isolated from aquatic and sedimentary environments where they can multiply though these are regarded as transitional environments between host infections. Some strains such as *S. subterranea* are specifically adapted for living in soil. Salmonellae possess the ability to survive in low-moisture conditions and are associated with many food-borne outbreaks especially in the ready to eat food market.

Relevance of Salmonella *to public health*

Salmonella species cause illnesses including typhoid fever, paratyphoid fever, and food-borne illnesses. These bacteria are zoonotic pathogens, being present in (food-producing) animals from which they can transfer to humans. *S. enterica* subspecies *enterica* serovars Typhimurium and Enteritidis are commonly reported members of this genus. These bacteria can exhibit resistance to one or more antibiotics, compromising treatment in some infected individuals.

Nontyphoidal salmonellosis is food-borne, and consequently, the food chain is an important route of infection for humans. *Salmonella* typhimurium is one of the serovars most often associated with cases of food-borne infections. Clinical signs associated with salmonellosis include diarrhea, dehydration, fever, and abdominal pain that can last for up to 7 days. There is a greater risk of infection in the young, the elderly, and immunocompromised individuals.

The genus Yersinia

Yersinia pestis (the 'plague bacillus') was first identified by Alexandre Yersin in 1894 and classified as *Pasteurella pestis* soon thereafter. Von Logham reclassified *Pasteurella pestis* and *Pasteurella rodentium* into the new genus of *Yersinia* in 1944. The name was accepted as an official genus in 1980. The genus *Yersinia* contains 17 species, of which only 3 are known to be

pathogenic to humans. These include *Y. pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis*.

Features associated with isolates of the Yersinia *genus*

Yersinia species form gray-white translucent colonies after 24 h of growth on blood agar plates. After 48 h on MacConkey agar, they form clear colonies. *Yersinia* strains are nonmotile at temperatures between 25 and 35 °C (Table 1). Y. *enterocolitica* and *Y. pseudotuberculosis* are psychotropic, being capable of growth at temperatures of 4 °C or even lower.

Environments known to contain Yersinia

Rodents are the natural reservoirs of *Yersinia* species, although other mammals can occasionally serve as hosts. *Yersinia pestis* can be transmitted to humans subcutaneously through flea bites and can become airborne during pandemics. Due to the ability of enteropathogenic *Yersinia* species to grow at low temperatures, refrigerated foodstuffs can become contaminated with these organisms. Swine and wild animals are common reservoirs.

Relevance of Yersinia *to public health*

The pathogenic species of *Yersinia* have been linked with a wide range of illnesses in humans including Crohn's disease, yersiniosis, mesenteric lymphadenitis pseudoappendicitis, and systemic infectious disease, commonly known as plague.

Y. pestis is the causative agent of the plague, which has been responsible for three human pandemics throughout history. These are the Justinian plagues, recorded from the sixth to eighth centuries, the Black Death from the fourteenth to nineteenth centuries, and the modern plague from the nineteenth century to the present time.

Y. enterocolitica is primarily a food-borne pathogen found in some food-producing animals such as pigs and other mammals. After ingestion of contaminated water or food, *Y. enterocolitica* colonizes the intestine causing yersiniosis, an acute gastrointestinal condition. Symptoms include fever, abdominal pain, vomiting, and diarrhea. Treatment usually consists of an aggressive course of antimicrobial chemotherapy.

Y. pseudotuberculosis is the least common of the three *Yersinia* pathogenic strains. It causes an illness characterized by fever and acute abdominal pain arising from mesenteric lymphadenitis, an inflammation of the lymph nodes.

The genus Cronobacter

Originally, members of this bacterial genus were described as yellow-pigmented *Enterobacter cloacae* being subsequently reclassified as *Enterobacter sakazakii* in 1980. A further and more recent reclassification was reported in 2007 with the acceptance of the new genus name, *Cronobacter*, based on a polyphasic study of a large collection of these isolates. Currently, the genus *Cronobacter* contains seven species, namely, *C. sakazakii*, *C. malonaticus*, *C. turicensis*, *C. muytjensii*, *C. dublinensis*, *C. condimenti*, and *C. universalis*.

Features associated with isolates of the Cronobacter *genus*

Cronobacter are 3 μm long and 1 μm in diameter. *Cronobacter* species produce two colony morphotypes, one being described as glossy and the other having a matte texture. Some 80% of strains identified produce a yellow pigment on tryptone soya agar at 25 °C. These strains can grow over a wide range of temperatures from −5 °C to 44–47 °C depending on the isolate. Optimal growth occurs in pH ranging from five to ten with some isolates being more susceptible to acid exposure. *Cronobacter* have a notable tolerance to desiccation, being able to withstand dry conditions for up to 2 years in infant formula. *Cronobacter* strains are motile by means of peritrichous flagella.

Environments known to contain Cronobacter

Cronobacter has been isolated from a variety of food and environmental sources such as dairy products, dried meats, powdered infant formula (PIF), households, livestock facilities, food manufacturers, and PIF production facilities.

Relevance of Cronobacter *to public health*

Cronobacter species are regarded as opportunistic pathogens linked with life-threatening infections in neonates. Clinical presentation can include necrotizing enterocolitis, bacteremia, and meningitis. These organisms can also infect older adults, although these cases tend not to be life-threatening.

The genus Enterobacter

The genus *Enterobacter* was accepted in 1960 with *Enterobacter cloacae* being designated as the type species. With the application of modern typing techniques, nine species are currently recognized in this genus. These are *E. cancerogenus*, *E. aerogenes*, *E. mori*, *E. ludwigii*, *E. kobei*, *E. soli*, *E. asburiae*, *E. cloacae*, *and E. hormaechei*.

Features associated with isolates of the Enterobacter *genus*

Enterobacter species are motile by four to six peritrichous flagella. Some are encapsulated (Table 1). Generally, these bacteria are 0.6–1.0 × 2–3 μm in size. *Enterobacter* species produce round, iridescent, flat, nonpigmented, irregular-edged colonies, when grown on nutritive agar. The optimum growth temperatures are between 30 and 37 °C, though they can grow in temperatures up to 44 °C.

Environments known to contain Enterobacter

Enterobacter can be found on human skin, plants, soil, water, sewage, intestinal tracts of animals, including humans, dairy products; and clinical specimens such as feces, urine, blood, sputum, and wound exudates.

Relevance of Enterobacter *to public health*

Enterobacter species are considered opportunistic pathogens, rarely causing disease in healthy individuals. The species *E. cloacae* and *E. aerogenes* are the main pathogens in the genus. These organisms are associated with bacteremia, lower respiratory tract infections, skin and soft tissue infections, UTIs, endocarditis, osteomyelitis, arthritis, and ophthalmic infections. Outbreaks involving *E. cloacae* have been reported in the ICUs of hospitals.

E. cancerous is rarely associated with human infections and is considered a plant pathogen.

The genus Citrobacter

The genus *Citrobacter* was first proposed in 1932. *Citrobacter* contains 11 species: *C. amalonaticus*, *C. farmeri*, *C. braakii*,

C. freundii, *C. gillenii*, *C. murliniae*, *C. sedlakii*, *C. werkmanii*, *C. youngae*, *C. koseri*, and *C. rodentium*.

Features associated with isolates of the Citrobacter *genus*

Citrobacter species are 1.0 × 2.0–6.0 μm in size. They are found either singly or in pairs, are devoid of a capsule, and are motile. *C. freundii* form small, circular, convex dark pink colonies on MacConkey agar. Rough or mucoid forms have also been reported. *Citrobacter* species grow optimally at a temperature of 37 °C.

Environments known to contain Citrobacter

Citrobacter are found in a variety of environmental sources, including soil and water, and in the human intestines. They are rarely the primary source of illness, though some strains can cause infections of the urinary tract, sepsis, and infant meningitis.

Relevance of Citrobacter *to public health*

Citrobacter species are not regarded as significant etiological agents in human disease. *C. freundii* and *C. koseri* have mainly been isolated from urinary and respiratory tract infections. *Citrobacter* can cause septicemia in patients that display a number of predisposing factors. *Citrobacter* have also been found to cause meningitis, septicemia, and pulmonary infections in neonates and young children, and some of these cases have been linked to contaminated batches of PIF. *Citrobacter* is considered an opportunistic pathogen.

Detection of Members of the *Enterobacteriaceae* Family Using Conventional and Molecular Methods

Conventional microbiology

Conventional microbiological-based techniques make use of morphological and biochemical differences between genera and bacterial isolates recovered from different sources. Selective and differential bacterial culture media for enrichment and isolation of strains have been developed.

Examples of detection using bacterial morphology and reactions on specific culture media

Macroscopic morphology can aid with the identification of features visible to the naked eye. It describes colony appearance in terms of its shape, texture, pigment, speed of growth when incubated, and the growth pattern. Microscopic morphology allows for an examination of individual bacterial cells for their shape, size, Gram stain, and acid-fast reactions to aid identification. The goal is to achieve an initial presumptive identification of the bacterium on the culture plate.

The application of specially designed culture media is a method commonly used to aid the identification of key pathogens of significance to public health. As an example, *E. coli* O157 must be identified quickly due to its association with low infectious doses. Its impact on human health makes it the focus of numerous commercial diagnostic companies. Many of these exploit the inability of VTEC O157:H7 strains to ferment the carbon source contained in sorbitol MacConkey agar. When grown on this media, VTEC O157:H7 produces a colorless colony distinguishing it from other organisms on the same plate. However, more recently, some *E. coli* O157 have been identified with the ability to ferment sorbitol so other means for confirmatory identification must be developed and applied. Non-O157 strains may sometimes be differentiated through the use of Chromocult and Rainbow agar on the basis of their colony color. However, currently, there are no suitable commercial agars available for this purpose.

Similarly, *Salmonella* species are cultured from food (after a period of resuscitation) and from clinical samples directly onto selective agar media, usually xylose–lysine–deoxycholate agar, and incubated at 37 °C for 24 h. Colonies can be preliminarily classified based on their morphology.

Examples of diagnostic biochemical tests

Commercially available biochemical galleries exploit the expression or otherwise of different phenotypic characteristics to identify bacteria. Some of these tests exploit the biochemical capacity of an isolate in terms of its ability to ferment a range of sugars, susceptibility to various antibacterial agents including drugs, and the capacity to metabolize complex polymers. In addition, some strategies will allow for the assessment of the motile nature of an isolate (see **Table 1** in the preceding text).

Molecular-based diagnostic microbiology

Polymerase chain reaction

The polymerase chain reaction (PCR) is an *in vitro* version of the natural DNA replication process that occurs within all living cells. It can be applied to rapidly amplify a discrete segment of genomic DNA. Characteristically, double-stranded DNA is denatured, separating it into two discrete strands. Primers, short oligonucleotide sequences of synthetic DNA, hybridize to opposite DNA strands, and, following the addition of a thermostable DNA polymerase and the four dNTPs, a round of synthesis is initiated, extending the DNA from the synthetic primer on each strand. Up to 30 reiterated cycles of denaturation, annealing, and extension are completed within a typical PCR reaction, amplifying the original DNA target exponentially.

In a more recent development of the conventional PCR approach, quantitative *real-time* PCR (qPCR) emerged. This strategy enables quantification of target-specific amplicons. In this approach, a housekeeping gene is chosen to act as a normalized comparator against which a variable DNA target is measured, resulting in quantification of the latter. qPCR can be applied for the quantification of individual genes and bacteria of importance to human health.

As an example, VTEC organisms can be detected by targeting their unique virulence genes, including *stx* and *eae* (as described earlier). Real-time PCR assays have been developed that allow the detection of the major VTEC-associated genes enabling the differentiation of strains in *real time*. These targets can be combined into multiplex assay platforms that can facilitate the detection of several different VTEC serogroups simultaneously.

Various PCR-based methods for the detection of *Cronobacter* strains exist. These include a conventional 1,6-α-glucosidase-based PCR and a *dnaG*-based qPCR that can detect all seven of the *Cronobacter* species. When used in combination with a species level PCR assay, individual species of the genus can be identified. This PCR method used the *rpoB* gene for which specific primers have been designed for each species.

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) makes use of suitable restriction enzymes to digest bacterial DNA at a limited number of sites within the genome, producing large or macro-DNA fragments that can be separated based on their size. The careful separation of these DNA fragments is achieved through the application of a periodical alternating electric field to a gel matrix. Comparison of these restriction profiles can indicate whether or not isolates are epidemiologically linked. PFGE has been used to aid with the tracking of many food-borne outbreaks and is regarded as the *gold standard* in molecular subtyping. PFGE has been standardized between different laboratories allowing for the comparison of PFGE profiles. PulseNet is an initiative that was originally developed at the CDC that facilitates the global tracking of food-borne pathogens.

Multilocus sequence typing

Multilocus sequence typing is a genome-based protocol used for the characterization of bacterial species by sequencing internal regions of typically seven housekeeping genes. To enable accurate sequencing, fragments of between 450 and 500 base pairs are used. Bacterial species display sufficient polymorphism within the chosen genes to assign an identifier to the alleles detected. By combining the sequences of (usually up to) seven loci, a species-specific allelic profile can be generated for each bacterial isolate.

Optical mapping

Optical mapping is a recently developed technique that generates high-resolution, ordered, whole-genome restriction maps from single DNA molecules independently of available sequence information. Purified DNA is isolated from a bacterium of interest and digested with a suitable restriction endonuclease. The DNA is then stained, scanned, accurately measured, and subsequently assembled into a complete genome restriction map. These maps can be compared to discover genetic differences, such as insertions or deletions between strains. Optical mapping can be used to distinguish between different species and isolates.

Optical mapping was applied to distinct *E. coli* O104:H4 German outbreak isolates in order to characterize differences. In this case, regions of differences contributing to the pathogenicity of this unusual strain were identified.

Whole-genome sequencing

Genome sequencing involves the decoding of a bacterium's complete genetic code, more specifically the determination of nucleotide order, within the chromosome. The technology underpinning this approach has evolved following a number of advancements over the years, and it is the most powerful analytic approach to molecular determination.

Next-generation sequencing

Next-generation sequencing (NGS) or high-throughput sequencing describes a number of sequencing technologies in common use. These approaches generate large volumes of sequence data at relatively low cost, thereby facilitating the rapid sequencing of DNA and RNA. This technology is significantly quicker and cheaper than the traditional Sanger-based technology.

A number of NGS technologies now exist for this purpose, such as

- Illumina (Solexa) sequencing,
- Roche 454 sequencing,
- Pacific Biosciences single-molecule real-time sequencing,
- SOLiD sequencing,
- Life Technologies Ion Torrent sequencing.

Despite the differences in the chemistries used, all the methods in the preceding text share some common features such as the random fragmentation of the template DNA, amplification of the target to be sequenced on a solid surface such as a bead or glass slide, and direct *step-by-step* detection of each nucleotide, following incorporation.

Sequencing technologies provide for the determination of the base order from thousands of small fragments that are subsequently assembled to generate a consensus genome sequence. Once assembled, this draft sequence must then be checked to confirm that correct assembly has taken place, followed by careful gene annotation and importantly the discovery of features of interest, including the identification of resistance and virulence genes along with other phenotypic data on a molecular basis.

NGS is a high-resolution and therefore accurate and reliable means of bacterial characterization.

In the 2011 German outbreak mentioned earlier, NGS technology facilitated the whole-genome characterization at an early stage of this incident. An *E. coli* O104:H4 outbreak isolate and a historic *E. coli* O104:H4 HUS isolate from 2001 were characterized using Ion Torrent's NGS in combination with optical mapping. Within 62 h, draft genomes were available enabling the genes to be examined. It was found that the strains carried genes associated with both EAEC and EHEC. Hence, it was proposed that the outbreak strain was a highly pathogenic hybrid of EAEC and EHEC strains.

See also: Emerging Foodborne Enteric Bacterial Pathogens; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; *Salmonella*: Detection; *Salmonella*: Properties and Occurrence; *Salmonella*: Salmonellosis; Shigella.

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Essential Oils: Isolation, Production and Uses

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Background

Essential oils are volatile, aromatic components of herbs and spices and have been used since ancient times in flavoring, food preservation, medicine, and perfumery. Some well-known aromatic and spice plants from which essential oils are produced commercially are shown in Table 1. The plant families Lamiaceae and Apiaceae are particularly rich in aromatic plants. The essential oil can be found in the flowers, leaves, roots, rhizomes, fruits, seeds, wood, and resins of these plants. On the surface of leaves and calyces, the essential oil is located in specialized cells called glandular trichomes.

Essential oils are synthesized by plants, as secondary metabolites, and are bioactive compounds with biological properties. They are considered to play a role in plant protection against herbivores making them unpalatable; in the attraction of pollinators; in plant protection against fungal, viral, and bacterial infections; in communication and signaling between other plants; and in allopathy by reducing the competition of neighboring plant species, among others. The essential oil content of plant material can vary with season, bioclimatic zone, growing conditions, and stress.

Essential oils can easily be isolated from plant material by hydrodistillation and are liquids at room temperature. They are a complex mixture of terpenes and terpenoids, which make up the characteristic aroma of each plant, and it is the composite effect of all the constituents present in it. They can be present from a mere trace up to around 95% of the essential oil. Most often, main components are precursors, intermediates, and end products of a particular biosynthetic pathway, and their relative proportions may depend on the time of year or the developmental stage of the plant. Terpenes are chemical compounds of low molecular weight, which are made up of isoprenoid units consisting of 5 carbon atoms, and these can join together to form units of C10 mono-, C15 sesqui-, C20 diterpenes. Oxygenated terpenes or terpenoids can be aldehydes, alcohols, ketones, acids, esters, ethers, and others.

Natural biodiversity is reflected by plants and their essential oils. It is possible to have several different species producing an essential oil with the same composition and major component (same biosynthetic pathway), for example, carvacrol in 'oregano' plants: *Origanum vulgare* subsp. *hirtum* ('Greek'), *O. onites* ('Turkish'), and *Thymbra capitata* ('Spanish' oregano). On the other hand plants of the same species, for example, Mentha spicata can have completely different aromas: 'peppermint' (menthol), 'spearmint' (carvone), 'wild mint' (piperitone and piperitenone oxide), and 'lavender' (linalool and linalyl acetate). These are known as chemotypes, plants of the same species having terpenoids arising from different biosynthetic pathways.

A return to the use of natural products in everyday life has stimulated interest in different aspects of essential oils and prospective uses in medicine, cosmetics, and the food industry. This interest is reflected in the marked increase in research publications on essential oils over the last twenty years (Figure 1) focused primarily on not only essential oil composition and biological properties but also isolation methodologies, species variation, chemotaxonomy, and others.

Isolation

The volatile nature, general insolubility in water, and solubility in organic solvents are properties of essential oils that can be exploited when designing isolation methods. The nature of the plant material and the lability of essential oil components should also be considered. Traditional and commercially most commonly used methods are distillation using water and/or steam, cohobation, maceration, enfleurage, and cold pressing. However, new technologies are continually being developed in order to decrease extraction times and energy costs.

Traditional Technology

Hydrosteam distillation

Distillation using water and/or steam is the most widely used and cost-effective method for the production of the majority of essential oils worldwide. It involves the application of heat and moisture to vaporize and release the essential oils from the plant material followed by the cooling of the vapor mixture and the separation of the oil from the water. Although the boiling point of most essential oil components generally ranges from 150 to 300 °C, they can be evaporated with steam/boiling water at 100 °C, as the combined vapor pressures of two immiscible liquids will be equal to atmospheric pressure at a lower temperature than that at which the essential oil will vaporize.

Hydrodistillation is the most simple of processes and has been used by man for centuries for the extraction of essential oils and is used mostly by small-scale producers. The plant material is almost completely covered by water in the chamber. The water is boiled using an external heat source, and the essential oils together with the steam are condensed and separated. Essential oil content of aromatic plants is determined using a Clevenger-type distillation apparatus, according to the European Pharmacopoeia. Disadvantages of hydrodistillation are that the process is slow and requires energy for heating, distillation rates may vary if the heat source is not controlled, and the direct heat source may cause charring of plant material in the base of the chamber. Prolonged contact of essential oils with the hot water can cause the hydrolysis of some essential oil constituents, for example, esters can hydrolyze to acids and alcohols. However, advantages of hydrodistillation are high yields of essential oil and good recovery of constituents. It is simpler and faster than steam distillation.

In steam and water distillation, direct contact with the boiling water is avoided by supporting the plant material on a grid. Advantages are that the process is faster and requires less fuel

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00261-0

Table 1 Some well-known aromatic plants and spices used commercially for their essential oil

Plant family	*Common (and scientific name)*
Anacardiaceae	Mastic (*Pistacia lentiscus* var. *chia*)
Annonaceae	Ylang-ylang (*Cananga odorata*)
Apiaceae	Dill (*Anethum graveolens*), parsley (*Petroselinum crispum*), anise (*Pimpinella anisum*), coriander (*Coriandrum sativum*), cumin (*Cuminum cyminum*), caraway (*Carum carvi*)
Asteraceae	Chamomile (*Chamaemelum nobile* and *Matricaria chamomilla*), tarragon (*Artemisia dracunculus*), yarrow (*Achillea millefolium*)
Burseraceae	Frankincense (*Boswellia* sp.)
Caprifoliaceae	Valerian (*Valeriana officinalis*)
Lamiaceae	'Greek' oregano (*Origanum vulgare* subsp. *hirtum*), peppermint (*Mentha* x *piperita*), spearmint (*Mentha spicata*), sage (*Salvia officinalis*), lavender (*Lavandula* sp.), rosemary (*Rosmarinus officinalis*), basil (*Ocimum basilicum*), marjoram (*Origanum majorana*), lemon balm (*Melissa officinalis*), pennyroyal (*Mentha pulegium*), thyme (*Thymus* sp.), patchouli (*Pogostemon cablin*)
Lauraceae	Bay (*Laurus nobilis*), cinnamon (*Cinnamomum* sp.)
Myristicaceae	Nutmeg (*Myristica fragrans*)
Myrtaceae	Clove (*Syzygium aromaticum*), eucalyptus (*Eucalyptus* sp.), wintergreen (*Gaultheria* sp.)
Oleaceae	Jasmine (*Jasminum* sp.)
Piperaceae	Black pepper (*Piper nigrum*)
Poaceae	Citronella grass (*Cymbopogon nardus*, *C. winterianus*)
Rosaceae	Damask rose (*Rosa* x *damescena* and *R. centifolia*)
Rutaceae	Lemon (*Citrus limon*), bergamot orange (*C. bergamia*)
Santalaceae	Sandalwood (*Santalum* sp.)
Schisandraceae	Star anise (*Illicium verum*)
Zingiberaceae	Cardamom (*Amomum* sp. and *Elettaria* sp.), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*)

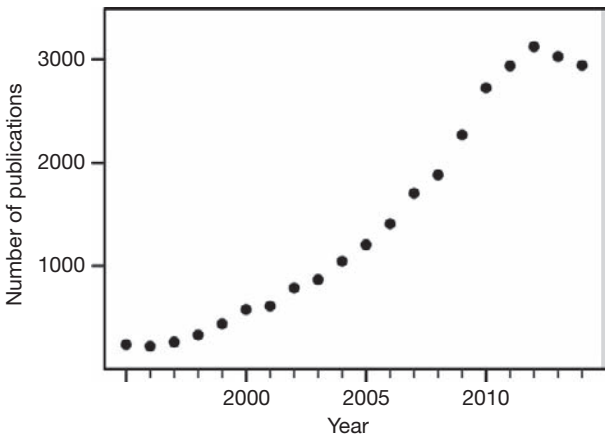

Figure 1 Trends in essential oil publications: 1995–2014. Web of Science (all databases), Thomson Reuters (2015) Thomson Reuters Web of Science. Available online: http://apps.webofknowledge.com (2015) (Accessed 30 January 2015).

than hydrodistillation while giving high reproducible yields. The method is used in developing countries as portable field distillation units, combining simple construction, ease of operation, and low cost with capacities of 100–2000 kg plant material.

In direct steam distillation, steam is generated in a boiler (external source) that is then passed through the plant material, which is supported on a grid in the chamber. Steam at atmospheric pressure has a temperature of 100 °C; however, the use of high-pressure steam correspondingly increases the temperature enabling a more rapid and complete distillation of essential oil. The advantages are reduced fuel costs due to higher thermal efficiency and suitability for large-scale production (1–3 ton plant material). However, setup costs are quite high and stills should be of stainless steel.

Distillation with cohobation: most essential oils have very low solubility in water; however, the solubility of rose oil, for example, is quite high. To avoid the loss in the condensing water, this water is returned to the still when using water and steam distillation for redistillation.

Solvent extraction

In the perfume industry, essential oil production is carried out using volatile solvents such as petroleum ether and hexane at 50 °C. This temperature is lower than that of distillation reducing the possibility of degradation of thermally labile compounds, and thus, essential oils are considered to have a more natural odor. After filtration, the essential oil is recovered by reducing the volume of solvent under vacuum evaporation; however, this can lead to severe losses of essential oil volatile components. Solvent extraction is advantageous when plant material is very limited (e.g., single leaf analysis) or where the major essential oil constituents are of interest rather than the essential oil content. Disadvantages are that nonvolatile compounds may be coextracted and that toxic solvent residues remain in the extract and can then contaminate food and fragrances. The process is also more expensive than distillation and creates waste disposal problems for the used solvents.

Enfleurage

Enfleurage is the extraction of essential oils using cold fat and has been used traditionally in the production of perfumes from fresh flower petals with low essential oil content, for example, jasmine and violets. The process has limited application on a large scale as it is very labor-intensive and has been superseded by modern processes such as extraction with volatile solvents. Dry flower petals are placed on glass plates coated with a thin layer of a high-quality odorless fat. Emitted essential oils are absorbed by the fat, and after extraction (in the order of 24 h or more hours), the petals are removed (defleurage) and replaced by fresh petals. This is an accumulative process that is repeated until the end of the flowering season. The essential oil that is absorbed in the fat is subsequently extracted with alcohol.

Cold pressing (expression)

This mechanical method is almost exclusively used in the production of citrus fruit (e.g., lemon, orange, and bergamot) essential oils. Pressure is used to crush the peel (pericarp) by rolling the fruit against sharp objects. Essential oil glands located in pits on the pericarp are punctured, releasing the essential oil, which is washed away with water. The essential

oil and water are separated by centrifugation. The process is carried out at low temperature so there is no thermal degradation of the essential oil components. Essential oil can be extracted before or after the extraction of the juice. Traditionally, expression was carried out by hand.

New 'Green' Technology

In recent years, research has centered on designing more efficient extraction procedures, which give higher yields (process intensification) with lower energy consumption and lower ambient operational temperatures. Conventional steam distillation and solvent extraction procedures although being generally simple have disadvantages of requiring long extraction times and large volumes of solvents. Industries are moving towards the application of greener nonconventional technologies with an emphasis on safer, sustainable, environmentally friendly, and more economical protocols. These nonconventional technologies include supercritical fluid extraction (SFE), solid-phase microextraction (SPME), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE), which are increasingly finding applications at a pilot and industrial level. It is interesting to see whether the trends in essential oil publications with respect to methods of isolation (MAE, solvent extraction, SPME, SFE, and hydrodistillation) for the period 1995–2014 (Figure 2) follow the trends in the development of new nonconventional technologies. Hydrodistillation is by far the primary isolation method reported in publications that would appear to be a result of the simplicity and low cost of the method. However, rising trends are also observed for SFE and SPME as might be expected.

Supercritical fluid extraction (SFE)

Carbon dioxide is a supercritical fluid when it is subjected to temperatures and pressures above its critical temperature (304.25 K) and pressure (7.39 MPa). It then expands like a gas but has the density of a liquid. Supercritical CO_2 is an important industrial solvent, which is odorless, colorless, nonflammable, and of low toxicity and environmental impact, compared to other organic solvents used in essential oil extraction, such as hexane and acetone, which are toxic and flammable. Other advantages of supercritical CO_2 extraction are relatively low temperature, shorter extraction times, selectivity, solvating power that varies with temperature and pressure enabling fractional extraction, absence of toxic residues that remain in extracts, absence of oxidation of essential oil compounds, ease of isolation of analytes from the supercritical CO_2 by pressure reduction (evaporation), and CO_2 recycling by condensation.

For essential oil extraction by SFE fractionation, supercritical CO_2 at temperatures of 40–50 °C and a pressure of about 11 MPa is passed into a heated extraction column packed with plant material. Usually, the plant material is incubated for a period of time (static mode) followed by a flow of new fluid (dynamic mode). The supercritical CO_2 with dissolved compounds is subjected to pressure reduction to remove the CO_2. Isolated essential oils may contain small amounts of other coextracted compounds such as cuticular waxes.

Solid-phase microextraction (SPME)

SPME is a rapid, solvent-free sample extraction technique. The SPME apparatus consists of a fiber holder and assembly containing a 1–2 cm thin, fused-silica retractable fiber coated with a thin polymeric coating such as polydimethylsiloxane (stationary phase). The sample is placed in a vial sealed with a septum. The fiber is extended through the needle into the headspace above the sample. The volatile analytes establish an equilibrium between the sample matrix, the gas phase above the sample, and the polymeric coating on the fused-silica fiber to which the analytes adsorb. After a given extraction time, the fiber is withdrawn and can be inserted directly into the injection port of a capillary GC column where the volatile analytes are rapidly, thermally desorbed. As no solvent is injected, there is no solvent front to mask component peaks

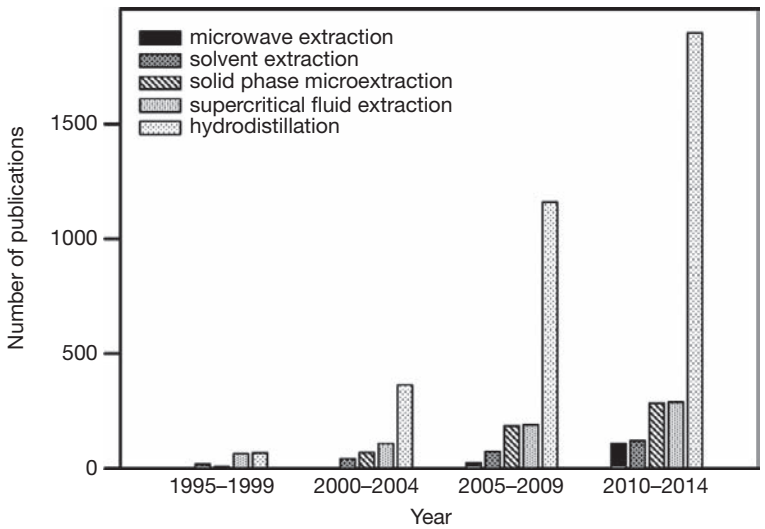

Figure 2 Trends in essential oil publications with respect to methods of isolation (microwave-assisted extraction, solvent extraction, solid-phase microextraction, supercritical fluid extraction, and hydrodistillation) for 5 year periods from 1995 to 2014. Web of Science (all databases), Thomson Reuters (2015) Thomson Reuters Web of Science. Available online: http://apps.webofknowledge.com (2015) (Accessed 30 January 2015).

eluting early from the column improving detection limits and resolution. SPME is used in the analysis of flavor and fragrance components in foods and beverages and is easily automated. It can be applied to very small samples and also to living systems.

Ultrasound-assisted extraction (UAE)

Ultrasound (US) has been used traditionally in biochemistry as a procedure for the production of plant extracts, as it causes the rupture of cell membranes and the release cell contents into the medium. The main factors that cause cell breakage are the water jets and shock waves caused by the collapse of cavitation bubbles generated by US. Part of the US pressure wave is dissipated as heat. The application of US to essential oil isolation enhances the homogenization of the plant material and thereby decreases the extraction time and the total energy consumed in the process. Analytes are more concentrated in the extract. The process is clean with no residues left in the extract. Combined with other extraction procedures, it can improve yield, selectivity, and product quality.

Recently, it has been shown that UAE using large-scale multiple transducer flow reactors operating at high power density ($700\,W\,l^{-1}$) promotes process intensification in the isolation of essential oils as compared to classical maceration in hydroalcoholic extracts. US has also been used to improve hydrodistillation extraction (sono-Clevenger) by greatly reducing extraction time without reducing yield or essential oil quality. Combined US and hydrodistillation extractions have been implemented on an industrial scale.

Microwave-assisted solvent extraction (MAE)

MAE is an extraction technique that combines microwave radiation technology with traditional solvent extraction. Microwave radiation is used to heat the solvent and plant material. Advantages of MAE are shorter extraction times (minutes rather than many hours), the use of less solvent, higher extraction yield, higher-quality products with lower cost, and lower energy consumption and CO_2 emissions when compared to Soxhlet or solvent extraction with stirring. Apparatus is simpler and less expensive than other methods such as SFE. Under optimized conditions (solvent volume, power, and heating time), the quality of essential oil and yield obtained are similar to those obtained by hydrodistillation.

Solvent-free microwave extraction (SFME)

Solvent-free microwave extraction (SFME) combines microwave heating and 'dry' distillation (no added solvent or water) under conditions of atmospheric pressure and 100 °C. Isolation and concentration of volatile compounds are performed in a single step. The internal heating of water within the plant material swells the plant cells and leads to rupture of the essential oil glands. Essential oil is evaporated by the *in situ* water of the plant material, and the distillate is passed through a cooling system outside the microwave. Excess water is returned to the extraction vessel to restore the *in situ* water of the plant tissue. SFME significantly reduces the time, energy, and plant material required for extraction. The reduction in extraction time and small amounts of water utilized decrease the likelihood of the hydrolysis, esterification, and/or oxidation of essential oil compounds.

Production

Production technology should be optimized and standardized in order to improve the overall yield and quality of the essential oil product. Raw plant materials, extraction yield and extraction time, and quality (composition, color, and texture) are important factors for industrial production, which looks for efficient, fast, and economic processes. Subsequently, storage conditions and containers should be optimized for maximum shelf life.

The quality of the raw plant material and an adequate supply for industry can be guaranteed by sustainable agricultural practices. Indiscriminate collection from the wild can lead to the reduction of wild resources, loss of biodiversity, and plant material of inconsistent quality. Cultivation of selected plants with high essential oil and biomass yields and of desired chemical composition (quality) is preferable. Harvesting times and postharvest drying and processing conditions must also be optimized and standardized so as to minimize any deterioration in quality and yield (appearance and loss of essential oil).

Extraction procedures should be carried out in stills and vats made of noncorrosive materials (e.g., stainless steel and glass) of optimal design and under optimal standardized conditions (temperature and time of extraction) for reproducible results. Plant materials such as seeds, roots, and wood may need to be crushed, ground, or soaked prior to extraction. Distilled essential oils with a high moisture content must be clarified using desiccants or on an industrial scale by high speed centrifugation. Moisture, oxygen, high temperature, and light adversely affect the storage life of the essential oil.

Essential oil composition (quality) can be analyzed using gas chromatography (separation of compounds) coupled with detection methods such as mass spectrometry (MS). For example, the gas chromatographic analysis of oregano (*Origanum vulgare* subsp. *hirtum*) essential oil (Figure 3) indicates many component peaks and the presence of four main components p-cymene (5), γ-terpinene (6), thymol (9), and the major component carvacrol (10). Some characteristics of these components are given in Table 2.

The standardization and quality control of essential oils ensure product quality and safety in worldwide markets. Standards are important and aid in the detection of adulterated and synthetic essential oils (addition of synthetic compounds and mixing with cheaper lower-quality oils, or solvents, to increase bulk). Standardization is an important reference for industry providing the properties of the natural components of products.

The International Organization for Standardization technical committee ISO/TC 54, *Essential oils*, has developed 1. specific monographs for quality standardization of all essential oils; 2. standardized analytic methods to control the quality of international standards of analytical methods and specifications; 3. requirements for transport, labeling, and marking; and 4. nomenclature and botanical names, with the aim of expanding global trade in essential oils and ensuring their quality and safety. ISO/TC 54 aims to 1. facilitate the global trade in essential oils, 2. enhance the quality of essential oils on the market, 3. protect the health of essential oil consumers, and 4. enhance the safety of essential oils. Many essential oils (or components) demonstrate toxicity and/or are hazardous in pregnancy and for persons with asthma or epilepsy and thus should be treated as medicines. The

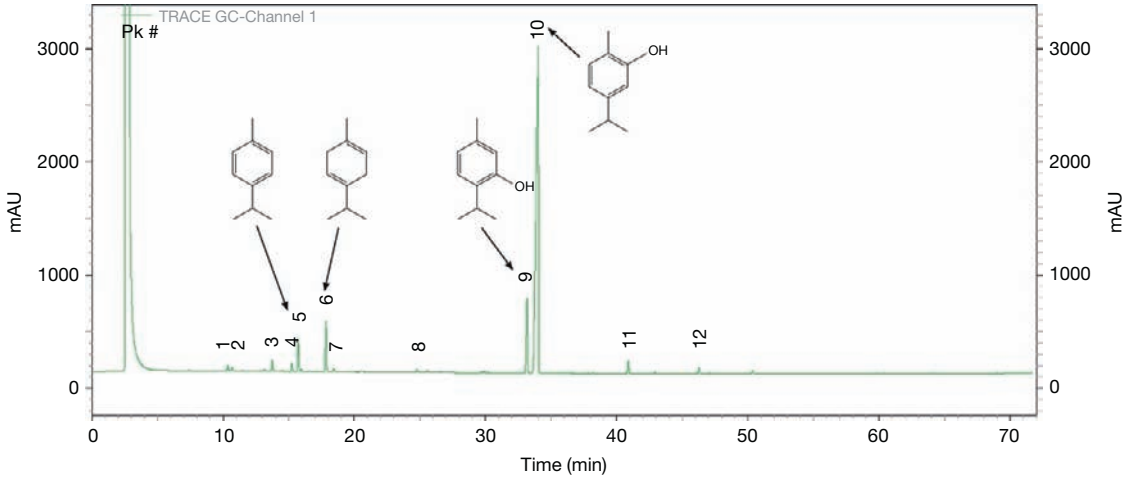

Figure 3 Gas chromatograph of *Origanum vulgare* subsp. *hirtum* (oregano) essential oil. Experimental details: the oil was analyzed by GC-FID using a ThermoQuest Trace GC with an HP-5MS capillary column (5% diphenyl-, 95% dimethyl- polysiloxane, non-polar, 30m × 0.25 mm ID × 0.25 μm film thickness) coupled with a flame ionization detector. Carrier gas: helium. Flow rate: 1.0 ml min^{-1}. Column temperature was 60 °C for 5 min, increased 2 °C min^{-1} until 150 °C and then increased 6 °C min^{-1} until 280 °C. The injector and detector temperatures were 240 °C. Kovats indexes were calculated by comparison of the relative retention times of the separated essential oil components with those of an homologous series of C_8-C_{26} n-alkanes standards.

Table 2 Some characteristics of the four main compounds of oregano essential oil analyzed by gas chromatography (see **Figure 3**)

Peak number	*Compound name*	*Kovats index*	*Molecular formula*	*Formula weight*	*Chemical abstract service number*
5	p-Cymene	1024	$C_{10}H_{14}$	134	99-87-6
6	γ-Terpinene	1059	$C_{10}H_{16}$	136	99-85-4
9	Thymol	1290	$C_{10}H_{14}O$	150	89-83-8
10	Carvacrol	1299	$C_{10}H_{14}O$	150	499-75-2

Compiled from data given in Adams, R. P. (2007). Identification of essential oil components by gas chromatography/mass spectrometry (4th ed). Allured Publishing Corporation: Carol Stream, IL

committee has also participated in the revision of and new European Pharmacopoeia monographs for essential oils.

Uses

Essential oils contain many bioactive compounds (e.g., terpenoids), which, apart from their fragrance and flavor, also show a wide range of biological activities and properties. For example, some of these properties are antibacterial, antifungal, antioxidant, insecticidal, insect repellent, acaricidal, larvicidal, anthelmintic, anti-inflammatory, cytotoxic, antibiotic, anticarcinogenic, analgesic, and local anesthetic. As such, these natural products from more than 300 essential oils are widely used in the food and beverage industry; in the pharmaceutical industry and medicine; in perfumery, cosmetics, and healthcare products (toiletries); and in veterinary science and alternative medicine (aromatherapy). As the uses are many, it is useful to examine them on a general level and then focus on some recent advances relevant to food and health.

Food Industry

About 60 % of essential oil production is used in the food industry primarily for flavoring and seasoning of food products such as cured meats, alcoholic and soft drinks, ice cream, and confectionary, among others. Essential oils also represent a source of natural antimicrobials for food preservation. Carvacrol, a major component of oregano essential oil, has many beneficial bioactivities in the preservation of food, which include antioxidative properties in food (fats and oils); the inhibition of foodborne human pathogenic bacteria, fungi, and parasites in human foods (eggs, meat and poultry products, milk, and leafy vegetables) and animal feed; and also the inhibition of microbial and fungal toxin production. The many health promoting effects of carvacrol create a potential for its use as a multifunctional food in pure and encapsulated form and in edible films.

Pharmaceutical Industry

Essential oils are generally not recommended for internal use undiluted due to their toxicity, nor for direct application to the skin, with the risk of dermatitis, allergic reaction, or skin photosensitization. However, topical application of some essential oils such as lavender and oregano when diluted in plant oils aids in the healing of wounds and burns. Essential oils are used as analgesics, expectorants, decongestants (menthol and eucalyptus), antiseptics, flavors to neutralize unpleasant tastes, and others.

Recently, some essential oil constituents such as geraniol have shown potential antitumor effects, inducing growth inhibition and apoptosis in cancer cells. Mice fed with diets supplemented with geraniol (25–75 mmol kg^{-1}) showed a dose- and time-dependent growth inhibition of tumor growth *in vivo* together with an induction of apoptosis. It also reduced cholesterogenesis. The doses of geraniol were nontoxic to the mice; the mevalonate biosynthetic pathway was inhibited. As such, geraniol could be a potential candidate for cancer chemotherapy.

Animal Feed Supplements and Animal Health

Essential oils have been evaluated for their effects on growth performance and meat quality of animals raised for meat production (e.g., poultry, pigs, and rabbits) as a safe and natural feed supplement alternative to the overuse of nontherapeutic antibiotics (growth promoters) and the subsequent development of antibiotic resistance in bacterial pathogens of humans and animals. For example, the supplementation of quail (*Coturnix coturnix*) diets with juniper oil (100 and 150 mg kg^{-1}) caused a significant increase in live weight, live weight gain, and carcass yields. Further, the dietary juniper oil reduced thiobarbituric acid levels in raw thigh meat samples in storage and exhibited antioxidant activity, preventing lipid oxidation in stored meat. Generally, essential oils as animal feed supplements can protect against the colonization of pathogenic microorganisms in the gut, reduce the fermentation process and the production of toxic metabolites, and have a positive effect on gut microbiota and animal welfare.

Many essential oil constituents (e.g., trans-cinnamaldehyde, thymol, carvacrol, citronellal, neral, linalool, and limonene) are important insect repellents and are effective against mosquitoes, fleas, ticks, flies, and lice, which can transmit infective diseases such as malaria.

Acknowledgment

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See also: Essential Oils: Properties, Composition and Health Effects; Flavor Enhancers: Characteristics and Uses; Spices and Flavoring Crops: Fruits and Seeds.

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Essential Oils: Properties, Composition and Health Effects

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Sources and Production

Essential oils are a group of plant secondary metabolites and are obtained only by steam distillation (according to ISO rule 9235) from different plant organs especially from aromatic plants. Those volatile compounds vary in odor and flavor. The manner of this distillation method is important because it determines the quality of the essential oil. Inappropriate production can result in the loss of bioactivity. Essential oils are concentrated liquids of complex mixtures and contain a lot of bioactive compounds.

Patterns of Consumption

Essential oils are lipophilic compounds, which are able to cross membranes very easily. Hence, essential oils are well absorbed not only from the intestine but also through the skin and the lungs. Because of their transdermal and pulmonary absorption, they are used for many medical applications in ointments, balms, and bath additives.

Availability, Absorption, and Metabolism

A high amount of essential oils is rapidly absorbed after dermal, oral, or pulmonary administration and crosses the blood–brain barrier. They are able to interact with the receptors in the central nervous system (CNS) and to affect biological functions such as relaxation, sleep, and digestion. Essential oil constituents can be metabolized by the liver in the form of polar compounds following limited phase I enzyme metabolism by conjunction with glucuronate or sulfate. Furthermore, they can be exhaled via the lungs as volatiles or as CO_2. Sulfate and glucuronide forms have been detected in urine and in plasma. Because of the fast metabolism and short half-life of active compounds, a minimum risk of accumulation in the body is suggested. The respiratory tract exerts the most rapid way of entry, followed by the dermal pathway. Essential oils and their metabolites can also be absorbed and delivered to the body through oral ingestion.

Health Effects

Essential oils and especially their constituents have a lot of health effects such as antibacterial, antiviral, and antifungal activities. Further fields of applications are anticancer therapy and therapies for cardiovascular and nervous system disorders. Additionally, they are used to reduce the level of cholesterol and regulate the glucose level. Besides, they are useful in the treatment of gynecological diseases. Essential oils are commonly used in the food and cosmetic industries.

Antioxidative Properties of Essential Oils

Nutritional properties of foodstuffs are affected by oxidation of oils. Today, the tendency goes towards using natural compounds such as essential oils for producing functional food. A present study has tested *Cinnamon zeylanicum* and *Zataria multiflora* Boiss as two natural preservatives. The use of this essential oil prevents oxidation rate and reduces preliminary and secondary oxidation products compared with butylated hydroxyanisole (BHA). Foods, which contain fat and oils, can be oxidized slowly during storage. As a result, different oxidation products occur, which cause rancidity and reduction of the sensory properties of foodstuffs. Lipid oxidation and fungal growth reduce the shelf life of food products. To solve these problems, manufacturers use antioxidants and preservatives. In the majority of cases, the producers of foodstuff use synthetic additives such as BHA and butylated hydroxytoluene as antioxidants, but the even low toxicity of those products has restricted their use. Recently, aromatic plants and spices serve as a source of biologically active substances such as antioxidants in foodstuffs. Today, the focus is on natural antioxidants that can replace synthetic additives that might be carcinogenic. Cinnamon, which belongs to the *Lauraceae* family, provides a variety of oils with different aroma characteristics. This aromatic plant contains the highest phenolic contents and strongest antioxidant activity. The main components of cinnamon are cinnamaldehyde and methyl eugenol. Studies have shown good antioxidative and antifungal activities of *Cinnamon zeylanicum* compared with control samples. The components that are responsible for antiradical activity of cinnamon are eugenol, cinnamaldehyde, cinnamic acid, and 1,8-cineole. The antifungal nature of cinnamon belongs to the high phenolic contents especially carvacrol, thymol, cinnamaldehyde, and eugenol. Food manufacturers need antioxidants that resist at high temperature during baking. Studies have been shown that concentrations of 500 ppm of *Cinnamon zeylanicum* essential oil can be used instead of BHA. Foodstuff, which contains this oil, might have nutritive and functional advantages compared to BHA. Consumption of foodstuff, which includes natural additives, can help us to prevent health disorders caused by oxidation, for example, aging, atherosclerosis, and cancerogenesis.

Inhibition of Foodborne Pathogens by Essential Oils

Foodborne illness that is caused by microorganisms is a growing public health concern. The percentage of people that are suffering from foodborne illness are up to 30%. Unsafe food is responsible for illness and results in several deaths every year. Nowadays, there are an increased concern of food safety and a demand from customers of natural products, which are free from synthetic additives. As a result, the use of natural antimicrobial compounds, such as essential oils, herbs, and spices for food, is gaining interest. Essential oils offer a relatively safe status and a potential for multipurpose functional use.

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Especially essential oils from the *Lamiaceae* and *Apiaceae* families exert bactericidal properties against microorganisms that contaminate food products; therefore, they are considered as natural food preservatives. Additionally, the widest spectrum of action as well as the greatest antibacterial activity is shown by phenolic compounds, for example, thymol and carvacrol. Above all, *Thymus vulgaris* L., *Origanum vulgare* L., and *Satureja hortensis* L. play an important role. The phenylpropanoid eugenol, which is a phenolic compound, is also known for its antimicrobial properties, and eugenol occurs especially in the essential oils of clove and of cinnamon leaves. All these essential oil compounds, especially as carvacrol, thymol, eugenol, cinnamaldehyde, and cinnamic acid, are used in food to control natural spoilage process and to prevent growth of microorganisms.

Moreover, essential oils could be used for the reduction of biogenic amines and as a flavoring agent in Gouda cheese, which has a positive health effect. Biogenic amines affect physiological functions such as immune response, brain activity, and gastric acid secretion. However, if biogenic amines occur in high amounts in food, they can cause health damage. Different concentrations of the essential oil from *Z. multiflora* Boiss (*Lamiaceae*) were added to milk, to test the effect of this oil on biogenic amines production such as tyramine and histamine and microbial counts in Gouda cheese. Thus, the concentration of tyramine and histamine was significantly reduced at the end of the maturation period in comparison with the control group. The higher the concentration of the essential oil, the higher was the decrease in biogenic amines and microbial counts. *Z. multiflora* essential oil was most effective against yeasts, but there was only a low reduction in *Enterobacteriaceae* counts.

Essential oils and spasmolytic activities

Present studies have shown promising spasmolytic activities from *Cuminum cyminum* L. Generally, cumin is used as an antioxidant and flavor compound. Besides these properties, it also acts as an antiseptic, analgesic, anti-inflammatory, and sedative and is used against stomach disorders, diarrhea, and spasms. The antispasmodic activity of the hydrodistilled fruit extract of *Cuminum cyminum* was tested on isolated of guinea pig ileum. At the beginning, concentration-dependent responses of acetylcholine – which induces such spasms – on pig ileum were recorded and then a distinctive antispasmodic activity was shown. The anticholinergic drug atropine was used as the standard antispasmodic agent. These results proved that acetylcholine alone causes contraction of ileum, but when acetylcholine was given in combination with the hydrodistilled fruit extract, a significant decrease of contraction was noted. This high degree of spasmolytic activity was caused by blocking the cholinergic receptors.

Essential Oils and Anticancer Activities

Cancer is a worldwide disease that can occur at any age and it is the second cause of mortality particularly in low-income countries. Until 2030, the cancer mortality could increase by 50% to reach 15 million worldwide. There is a relationship between the production of reactive oxygen species and the origin of oxidation and inflammatory, facts which can lead to cancer. Oxidative stress acts as a DNA-damaging agent, which furnishes an increase of the mutation rate within cells and promotes oncogenetic transformation. ROS can activate signaling pathways that contribute tumor development through the regulation of cellular proliferation, angiogenesis, and metastasis. It is worth to note that many cytotoxic molecules of plant origin that possess a chemopreventive potential are used in chemotherapy, for example, thymoquinone as an active ingredient of *Nigella sativa* L. (*Ranunculaceae*). It should be considered that 30–40% of all kinds of cancer can be prevented with a healthy lifestyle and dietary measures, and it is obvious that nutrition has an impact on the cancer process. There are dietary components, which are able to promote cancer progression, and there are some that act as chemopreventive agents. *N. sativa* exerts a positive effect as mediating inflammation and cancerous cell growth especially to treat colon cancer. Nuclear factor-kappa B activation was decreased by thymoquinone in a dose-dependent manner. The chemotherapeutic effect of this substance is comparable with the synthetic 5-fluorouracil. The inhibiting effect of the essential oil from *N. sativa* on colon carcinogenesis may be linked with the suppression of cell proliferation in the colon mucosa. The essential oil of black cumin seeds is known for its cytotoxic and apoptotic/necrotic properties in a cancer cell line. An animal study has shown that the addition of *N. sativa* seeds to the diet is able to protect against oxidative stress, inflammatory response, and carcinogenesis. Furthermore, this study reported that the daily intake of these seeds (and thus of the volatile oil) results in the reduction of chromosomal aberrations and damaged cells. Additionally, animal models showed that thymoquinone blocked angiogenesis *in vitro* and *in vivo*, thus preventing the growth of cancerous cells. In conclusion, the anticancer and anti-inflammatory properties of *N. sativa* and its essential oil containing thymoquinone may be attributed partly to the suppression of NF-kappa B activation pathway. This essential oil can also be considered as a potential immunosuppressive agent. Because of its nutritional quality, *N. sativa* is a potential source of diet-based strategies, which may play a role in improving human health.

Besides, monoterpenes that are components of nearly all essential oils and especially of citrus fruits can be used to prevent cancer. They show ideal chemopreventive properties, such as antitumor activity, commercial availability, low cost, oral bioavailability, and low toxicity. Because of this reason, monoterpene studies are proceeding in human clinical trials for chemotherapeutic activity. Several dietary monoterpenes are known for their antitumor activity. It has been shown that (+)-limonene exerts chemopreventive activity against rodent mammary, skin, liver, lung, and fore-stomach cancers. (+)-Limonene, the main constituent of orange oil, for example, is widely used as a flavoring agent for fruit juices, soft drinks, ice cream, and so on. The monoterpenes (+)-limonene and perillyl alcohol and their metabolism products showed a high degree of oral bioavailability.

Essential oil constituents such as citral are also known for their anticancer potential. Citral can be obtained from herbs such as lemongrass, melissa, and verbena. Citral consists of two isomeric acyclic monoterpene aldehydes, namely, geranial (*trans*-citral, or citral A) and neral (*cis*-citral, or citral B). This key component is commonly used as a food additive. Studies showed that concentrations of 44.5 μM citral induced

apoptosis in cancer cell lines, a concentration that is comparable to the one of citral in a cup of tea prepared from 1 g of lemongrass. Apoptosis is linked with DNA fragmentation and induction of caspase-3 catalytic activity, whereby the αβ-unsaturated aldehyde group is responsible for this apoptotic effect and might be a core structure for the design of pro-apoptotic drugs.

Essential oils are also able to inhibit the formation of *N*-nitrosodimethylamine in vegetables. Vegetables are rich in nitrate that is a precursor of nitrite. Crops contain different concentrations of nitrate, dependent on the species, genetic, and environmental factors and cultivation conditions, whereas the concentration of nitrite in plants is commonly very low. *N*-Nitroso compounds such as nitrosamines that are formed by *N*-nitrosation of secondary amines with nitrite in an acidic environment act as strong carcinogens. Several foods contain the precursors of this reaction. Meats, cured meat products, and smoked fish are sources of secondary amines. Nitrite often occurs in hams and sausages as an antimicrobial or coloring substance. Essential oil constituents, such as carvone and limonene, are able to activate the glutathione S-transferase that reduces the risk of cancer. The terpene hydrocarbon (+)-limonene, which represents one of the major compounds of citrus essential oils, exhibits anticarcinogenic properties against mammary, lung, stomach, and skin cancer. It has been reported that the essential oils of citrus show an inhibitory effect on the formation of *N*-nitrosodimethylamine. Especially *Citrus junos* Siebold ex Tanaka (*Rutaceae*), also known as yuzu, which is a popular sour citrus fruit in Japan, shows an inhibitory effect on the formation of *N*-nitrosodimethylamine. Because the Japanese diet is richer in vegetables than in other countries, their intake of nitrate is very high, and thus, they have a higher risk to suffer from stomach cancer. Bacteria in saliva have an influence on the *N*-nitrosodimethylamine formation, because they induce the reduction of nitrate. Although the rate of formation of nitrosamines is proportional to the square of nitrite concentration, it has been considered that nitrite in vegetables especially cabbage, celery, spinach, and mint is not mainly responsible for the formation of *N*-nitrosodimethylamine. In most cases, oral bacteria are responsible for the reduction of nitrate into nitrite. Terpene hydrocarbons such as myrcene, α-terpinene, and terpinolene show an inhibitory effect and cause the decrease of *N*-nitrosodimethylamine formation. In conclusion, yuzu oil showed an inhibitory activity on the formation of *N*-nitrosodimethylamine in the presence of vegetables and saliva.

Essential Oils and Diabetes

Essential oils from different plants can also have a positive effect on diabetes and hypertension. Nowadays, there is a tendency to manage type-2 diabetes and hypertension with natural sources. There are two major ways to handle this: the scavenging of free radicals and the inhibition of key enzymes, which are involved in starch digestion such as α-amylase and α-glucosidase. By those enzymes, starch is converted into glucose, thus increasing its concentration. Inhibition of these enzymes leads to a delay of the absorption of glucose followed by a moderate postprandial blood glucose elevation. There is also a relationship between diabetes and an increased generation of free radicals and a defective antioxidant defense system. Additionally, oxidative stress is involved in the diabetogenic process. For that reason, antioxidant-rich foods have a good dietary intervention in the management of this disease. The use of the essential oil of black pepper seeds in relation with diabetes and hypertension is known in folk medicine. Aromatic spice plants such as black pepper show good antioxidant properties, due to phenolic contents of the essential oil, which possesses antimicrobial, antihypertensive, anticonvulsive, and sedative activities. The essential oil is extracted from the seeds and leaves from black pepper and contains mono- and sesquiterpenes, besides phenolic compounds that are effective in preventing diabetes in animal models. The essential oil of black pepper is established for its radical scavenger abilities and its ferric-reducing antioxidant activity attributed to the presence of pinene and 1,8-cineole. Another therapeutic approach is the inhibition of the starch-metabolizing enzymes. In this case, the activity of α-glucosidase is more strongly inhibited by the essential oil than the one of α-amylase. This circumstance has a therapeutical purpose towards synthetic α-amylase and α-glucosidase inhibitors. The use of those natural products is important in preventing some side effects of the synthetic inhibitors. The essential oil of black pepper can inhibit also the angiotensin-1-converting enzyme (ACE) activity. This fact is a therapeutic approach in the treatment of hypertension, which is one of many complications associated with type-2 diabetes. *In vitro*, the ACE activity was inhibited in a concentration-dependent manner by the essential oil, because it is able to block the conversion of angiotensin-1 into the vasoconstrictor angiotensin-2 that causes the hypertension.

Essential Oils and Mood Disorders

Furthermore, some essential oil constituents, such as (*E*)-methyl isoeugenol (MIE), is often used as food flavor. This essential oil from *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum (*Myrtaceae*) leaf has calming properties. This ubiquitous food additive appears attractive for the treatment of mood disorders. Studies demonstrated the anxiolytic- and antidepressant-like activities of MIE, suggesting the participation of serotonergic pathways. Among psychiatric diseases, mood disorders are most common, that is, widespread with a prevalence of up to 20% worldwide. Because of the low remission rate and the high rate of nonresponse to current treatment, it is necessary to develop new therapeutic agents, such as consumption of functional food. With this basic food nourishment as well as health benefits are covered. Today, aromatic plants have been largely explored as functional ingredients in the pharmaceutical and food industries. The treatment of neural disorders with naturally occurring food flavors, such as MIE, seems to be more acceptable than the use of pharmacotherapies. Oral administration of MIE showed an increase in sleep duration and a soothing activity of the CNS. An antiseizure property of MIE was hypothesized too, because it acts similar to diazepam, a CNS-calming compound. This hypothesis is further supported by the antiseizure properties of aromatic compounds with a similar chemical structure as MIE, such as methyleugenol, eugenol, and 1-nitro-2-phenylethane.

Besides MIE, in diets, the widely consumed essential oil of lemon is also known for its antidepressant-like effect and

therefore acts as functional food ingredient. Because of many side effects, such as nausea and anorexia, by using conventional medicaments against depression, there is a growing interest in the search of new effective antidepressants, such as functional foods that worldwide are used in the kitchen. Animal studies have been shown that the volatile oil from lemon has increased the metabolic turnover of dopamine in the hippocampus and of serotonin in the prefrontal cortex and striatum. The oral administration of lemon essential oil does not show any toxic effects or an influence on weight, blood, and organs of rodents. Upon oral administration of 400 mg kg^{-1} lemon essential oil, the dopaminergic activity in the striatum and the hippocampus was significantly enhanced on account of an increased concentration of dopamine and a decreased turnover. It is known that depressed patients suffer from a dysfunctional 5-hydroxytryptamine (5-HT) system; thus, selective 5-HT reuptake inhibitors enhance the activation of various 5-HT receptor subtypes. In conclusion, the essential oil of lemon has a great potential to be used as an antidepressant functional food ingredient.

Essential Oils and Osteoporosis

Furthermore, essential oils can also be useful in the treatment of osteoporosis. This disease is a major health problem in aging humans – especially women – when low bone mass leads to osteoporotic fractures. The bone turnover is increasing and it comes to a marked decrease in trabecular bone mineral density (BMD) and bone mineral content. Osteoporotic fractures reduce the quality of life of the patient and they are a burden to health care. Hence, it is desirable from medical and economical points of view to prevent the loss of bone mass. A nutritional approach would be an inexpensive opportunity to prevent low bone mass. Studies have shown that essential oils from sage, rosemary, and thyme and essential oils from pine, juniper, and eucalyptus inhibit or at least retard the activity of osteoclasts. The essential oil of pine is the most potent one and also the bitter orange-peel oil is very potent, for which monoterpenes, such as thujone, eucalyptol, camphor, borneol, menthol, and thymol, are responsible. Studies have shown that pine oil reduces the loss of trabecular BMD in animal models, and *in vitro* studies report that these monoterpenes directly inhibit the osteoclast activity. (1*R*,3*R*,4*S*)-(−)-Menthol that is the most widely used monoterpenic alcohol in human nutrition inhibits bone resorption *in vivo* and *in vitro*, but the latter effect is weak. Also, the consumption of monoterpene-rich fruits and vegetables is linked with a greater BMD in humans. Animal studies have shown that vegetables, salads, and herbs, as a part of human nutrition, are able to inhibit bone resorption. Essential oils exert a positive effect on the bone metabolism when they are added to food. Therefore, essential oil-containing herbs are possible candidates for a dietary approach to osteoporosis.

Essential Oils and Their Effect on the Gastrointestinal Tract

Essential oils are also famous for their positive effect on the gastrointestinal tract. *Mentha x piperita* L., which belongs to the *Lamiaceae* family, contains a volatile oil with the major compound (−)-menthol. This essential oil is used against irritable bowel syndrome, because of its influence on gastric motility. Peppermint tea is commonly used against acute and chronic gastritis, enteritis, and disturbances of the GI tract by the antispasmodic effect of menthol. Furthermore, peppermint tea is commonly used as an antiemetic in pregnant women, besides ginger and cannabis. This volatile oil shows the same effect as 5-HT_3 receptor antagonists, 5-HT_4 receptor agonists, and anticholinergics. Those receptors are also involved in the pathogenesis of irritable bowel syndrome. Peppermint oil and (−)-menthol are able to completely inhibit 5-HT_3 receptor in a concentration-dependent manner by relaxing the smooth muscle and antagonizing the serotonin-induced stimulation. This antagonistic effect on the 5-HT_3 receptor channel influences in a positive manner the disturbed motility during symptoms of irritable bowel syndrome and emesis. The cationic influx through 5-HT_3 receptor channels was inhibited by peppermint oil and its main constituent (−)-menthol. The influence of peppermint essential oil and (−)-menthol leads to the reduction of serotonin-induced contraction in animal models. In addition, they possess a direct relaxant effect.

Essential oils often are used as food additives to complement present therapies of gastritis and peptic ulcers. This disease is caused by an increased density of *Helicobacter pylori* in the gastric mucosa. A nutritional approach can help people with asymptomatic gastritis to manage the infection and to decrease the development of the disease. Those essential oils that exhibit the strongest bactericidal potential against *H. pylori* P1 were also active against other *Helicobacter* strains. The most effective single essential oils constituent against *H. pylori* are carvacrol, isoeugenol, nerol, citral, and sabinene. The widespread infection with *H. pylori* is well known as the major etiologic factor in chronic active type B gastritis, gastric ulcers, and gastric cancer. Nowadays, a triple therapy such as the combination of proton pump inhibitor and two antibiotics is common, but increasing antibiotic resistance is also responsible for an eradication treatment failure. Therefore, there is a growing interest to find alternative treatments, for example, the development of new nutritional approaches. Especially patients with an asymptomatic gastritis profit from this nutritional approach.

Essential Oils and Alzheimer's Disease

Essential oils can also be used in the treatment of Alzheimer's disease, the most common cause of dementia in the aged population. It results in cognitive decline and mental deterioration that is the result of massive and progressive loss of neurons from different regions of the brain. Disorders of the CNS are linked with neurotransmitter disturbances and insufficiencies in cholinergic functions. Cholinesterase is responsible for the hydrolyzation of choline, and if there is a high concentration of this enzyme, then a lower concentration of choline in the synaptic gap results. Investigations have shown that there is a relation between increased levels of cholinesterase enzymes and Alzheimer's disease. This fact leads to the hypothesis that this cognitive decline in patients is linked to the progressive cholinergic degeneration. Hence, promising approaches for the treatment of Alzheimer's disease are cholinesterase inhibitors that are able to enhance the level of cholinergic neurotransmitters in the brain. Acetylcholinesterase (AChE) and

butyrylcholinesterase (BChE) are the major cholinesterase enzymes that are responsible for the decreasing choline levels. Today, Alzheimer's disease patients are treated with AChE inhibitors, synthetic compounds that reveal some toxicity during prolonged use. Hence, there is a great demand for alternative drugs to treat Alzheimer's disease. It has been suggested that dietary supplements with antioxidants and free radical scavengers may decrease the mild cognitive impairment of Alzheimer's disease. A high amount of plants has been traditionally used for the enhancement of cognitive function and alleviation of several symptoms linked with Alzheimer's disease. Nowadays, essential oils of edible plants are considered to inhibit AChE and BChE. Those volatile oils are of great interest, because of their availability, few side effects, low toxicity, and their biodegradability. Studies have shown that the essential oils of *Citrus aurantifolia* (Christm.) Swingle (*Rutaceae*), *Alpinia galanga* (L.) Willd. (*Zingiberaceae*), and *Melissa officinalis* L. (*Lamiaceae*) possess the highest inhibitory activity against AChE, whereas *Melissa officinalis*, *Citrus aurantifolia*, and *Ocimum gratissimum* L. (*Lamiaceae*) exhibited the strongest inhibitory properties against BChE. Therefore, the leaf essential oil of *Citrus aurantifolia* with the constituents (+)-limonene, (−)-camphor, citronellol, *cis*-ocimene, and 1,8-cineole is a promise for the prevention and treatment of Alzheimer's disease, because it inhibits both enzymes. 1,8-Cineole that is one of the most potent AChE inhibitors also occurs in other plants such as *Rosmarinus officinalis* L. (*Lamiaceae*).

Essential oils and atherosclerosis

The essential oil of sage could be used as food ingredient with anti-inflammatory and antiatherogenic properties. Cholesterol deposition in the intima of large- and medium-size arteries together with a chronic inflammatory process leads to atherosclerosis. Oxidized low-density lipoproteins play a key role in early inflammation. This type of LDL that is not recognized by the LDL receptor is taken up by the scavenger receptors in monocytes–macrophages and endothelial cells. Hence, it comes to the accumulation of cholesterol in macrophages and to the formation of foaming cells. Moreover, oxidized LDL is able to induce the expression of proinflammatory cytokines such as TNF-α, Il-1β, and IL-6 in macrophages and endothelial cells. Essential oils from spices, which are added to food, present various biological activities, such as antioxidant and anti-inflammatory properties. As mentioned earlier, the oxidation of LDL results in this atherosclerotic lesion and the initiation of the inflammatory cascade. Therefore, oxidized LDL is able to induce the expression of proinflammatory cytokines, such as IL-1β, TNF-α, and IL-6 from monocytes and macrophages. IL-1β and TNF-α play an important role in the initial amplification of the inflammatory response. Moreover, they are able to induce the expression of adhesion molecules by endothelial cells and promote secretion of different cytokines and chemokines by monocytes. Furthermore, they are involved in the process of foam cell formation, especially by means of induction of growth factors. Activated macrophages, lymphocytes, fibroblasts, and vascular smooth muscle cells produce during stimulation IL-1 and TNF-α. The inflammatory cytokine IL-6 plays an important role in the development and progression of atherosclerosis. It has been reported that the essential oil of *Salvia officinalis* L. (*Lamiaceae*) is able to inhibit COX-2, thus exerting anti-inflammatory properties. Also, supercritical extracts of *Salvia officinalis* are effective inhibitors of oxidized LDL and induced proinflammatory cytokines. This sage extract mainly consists of camphor, borneol, and 1,8-cineole that is a strong inhibitor of TNF-α, IL-1β, and IL-6 production. Borneol and camphor also exhibit excellent anti-inflammatory properties. Therefore, sage extract has a great potential to be used as an anti-inflammatory agent to prevent atherosclerosis.

See also: Essential Oils: Isolation, Production and Uses; Herbs: Composition and Dietary Importance.

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Ethnic Foods

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Introduction

Consumers of ethnic foods often have distinctive cultural characteristics, including language, social norms, and behaviors that provide them with a sense of connectedness. From a cultural standpoint, ethnic foods serve as the familiar link with the past and help those who are accustomed to them to maintain and promote among peers their ethnic identity.

In countries like the United States, with a very diverse ethnic population, the production, distribution, and consumption of ethnic foods are an integral feature of the overall American culture.

Native Americans are direct descendants from the original population groups distributed throughout the country before the European colonization. Since then, they have contributed to the cultural dimensions of 'ethnic foods' with their food production developed around the 'three sisters' (corn, beans, and squash) system. Moreover, they developed a rich and highly nutritive traditional cuisine with many similitudes to those found in Central America. For example, in Guatemala, the 'three sisters' system was termed as the 'milpa.'

During and after the colonization, several waves of immigrants from Europe, Africa, and Asia brought from their countries of origin their ethnic foods and dishes. Then, their cuisine evolved from the homeland traditional dishes to the modified versions of ethnic cuisines that currently exist in these modern times.

Similarly, more recent waves of immigrants from Central and South America, the Caribbean, and the four corners of the globe contributed to the rich diversity of the foods present today in US households.

Several cooking techniques and eating styles from the original traditions brought by original, earlier, and new American settlers were exchanged and adopted by them. Traditional dishes like tacos, tamales, pizza, and noodles and Chinese dishes (e.g., lo mein) gave origin to new dishes, different from their native, original versions. Those ethnic food transformations are likely to continue as the American population becomes more diverse and interrelated, with evolving sense of taste, flavor, and smell for what they term as 'ethnic foods.'

Ethnic Diversity of the American Population

The United States has a very diverse population. This diversity was long in existence in the precolonial times with more than 300 tribal groups of Native Americans. Since then, the United States had transformed into a 'plurality nation.' This pluralism is reflected in the ancestry of the early settlers in the country, which are those that contributed to the ethnic diversity of the American cuisine. In the current times, those from German (16%), Irish (11%), English (8%), and Italian (6%) descents are the largest ethnic groups that contributed to the diversity of the white population (see **Figure 1**). The Census Bureau defines as white those having origins in any of the original peoples of Europe, the Middle East, or North Africa.

Furthermore, it is the plurality of the more recent immigrants, along with that from the African-American population that has given this country the ethnic diversity observed in the early periods of the twenty-first century. According to the 2010 Census Bureau, the total population reached about 309 million people. The racial and ethnic heterogeneity of this population is represented in **Table 1**. As seen in this **Table 1**, whites were the largest group (75%). Other ethnic groups include black or African-Americans (14%), Asians (6%), American-Indian and Alaska Natives (2%), and Native Hawaiian and other Pacific Islanders (0.4%). Based on ethnicity, which is reported by the Census Bureau as having or not Latino or Hispanic background, 16% of the total US population is of Latino or Hispanic origin.

This diversity of the major racial and ethnic groups in the United States is reflected in the large number of ethnic groups that, because of their geographic, socioeconomic, and cultural origins, have contributed to the rich, mixed, and festive ethnic foods that every day are present in the American households.

Ethnic Foods Present in the American Homes

Based on the geographic, climate, and, particularly, the plurality of the American population, one could find an extraordinarily rich ethnic cuisine in most households across the country. Most ethnic cuisines have been influenced by centuries of food exchanges between indigenous groups and immigrants from all over the world. As result, the American cuisine is a unique multiethnic blend. No single eating pattern has prevailed over all the others, and no ethnic or cultural group has emerged with an unchanged food pattern. However, some ethnic cuisines are more popular than others, mainly due to the high number of immigrants from the latitudes where those ethnic foods and traditions originated.

In general, foods that are regarded as ethnic foods by a cultural group vary over time and with the degree of acculturation of the group consuming them. Even within major race and ethnic subgroups, many variations in ethnic foods exist. Some foods only bear a passing resemblance to the original cuisine from which they were derived. However, all of them fulfill important cultural roles for the diverse American population. For example, in the United States, pizza, tacos, chili with meat, and chicken chow mien are very different from the Italian, Mexican, and Chinese dishes that inspired them. Hispanic-Americans with a Mexican-American heritage differ in their food preferences from Puerto Ricans, Colombians, and Chileans, whose eating patterns involve a blend of Spanish, African, Native Central/South American, and continental North American influences. Similarly, Asian-Americans of Filipino, Japanese, or Chinese ancestry have very different food choices and traditions. Among the over 300 different Native

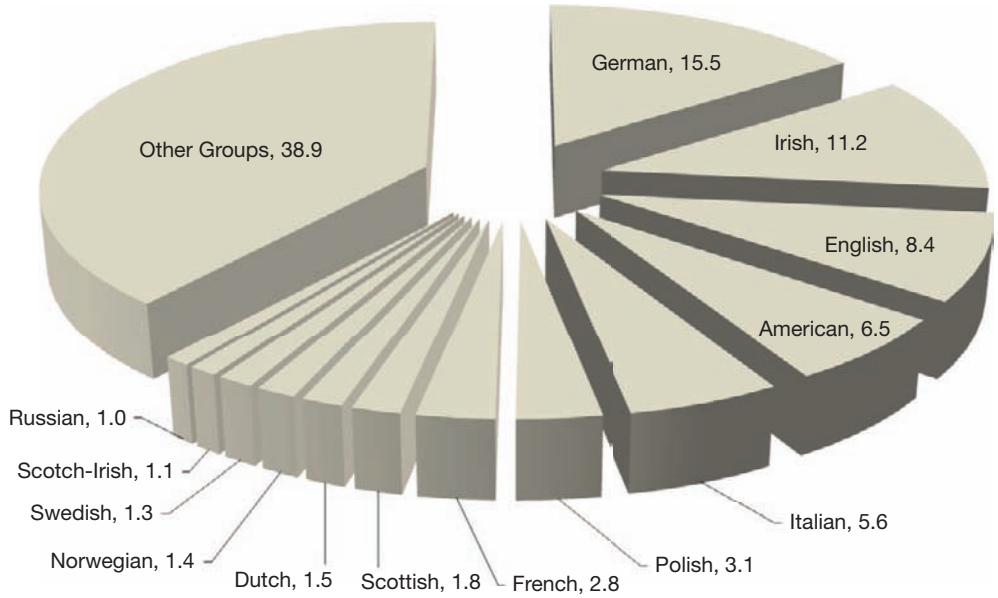

Figure 1 Ancestry of the American population, based on the 2010 US Census data. Reproduced from US Census Bureau. (2010). 2010 American Community Survey. American Fact Finder: People Reporting Ancestry.

Table 1 Racial and ethnic population groups in the United States

Race and Latino or Hispanic origin	*Number*	*Percent*[a]
Total population	308 745 538	100.0
Race categories		
White	231 040 398	74.8
Black or African-American	42 020 743	13.6
Asian	17 320 856	5.6
American Indian and Alaska native	5 220 579	1.7
Native Hawaiian and other Pacific Islander	1 225 195	0.4
Some other race	21 748 084	7.0
Latino or Hispanic ethnicity (*from any race categories*)	50 477 594	16.3

[a]Percentages estimated from the total population.

Source: US Census Bureau. (2010). 2010 Census Briefs. Overview of Race and Hispanic Origin: 2010.

Table 2 Examples of ethnic cuisines and representatives dishes commonly eaten by the American population

Ethnic cuisine	*Examples of popular dishes*
Puerto Rican	Rice with pigeon peas, tostones (fried green plantains), habichuelas (stewed pink beans), pasteles (similar to tamales), and rice pudding
Mexican	Tamales, tortillas (corn- or wheat-based), tacos, enchiladas, guacamole, and mole poblano
Italian	Pizza, risotto, spaghetti, lasagna, ciabatta bread, cheeses, and wines
Japanese	Sashimi, sushi, soba, sukiyaki, yakitori, and miso soup

American groups, the staples and other foods that they regard as unique and distinctive to their cultures also vary.

The traditional 'Mediterranean' diet varies in terms of foods and seasonings included in their cuisine. These differences are reflecting the European and North African Mediterranean locations and cultural norms of the Mediterranean countries where the Mediterranean diets originated. These traditional diets include liberal amounts of fruits, vegetables, legumes, grains, and fish. High amounts of monounsaturated compared with saturated fats, low amounts of meats, and moderate consumption of alcohol are also observed. Although there is a debate about the extent to which such a regimen is exportable, some 'Mediterranean' ethnic foods have become very popular in America. Examples of those foods include olives and olive oil, breads, couscous, pastas, artichokes, arugula, grapes, dates, figs, almonds, chickpeas, basil, bay leaf, and parsley.

The ethnically diverse cuisines found in the United States include the contributions of most ethnic groups. Those groups brought with them their native recipes and some of their traditional food ingredients. And then, throughout the times, they modified and adjusted those recipes to the availability and cost of foods and seasonings in order to make them acceptable by the general population. Table 2 contains a short list of examples of ethnic cuisines present in the US households.

Germans, for example, brought and modified hot dogs and hamburgers. Italians, with their basic ingredients wheat, tomato sauce, and olive oil, also influenced the American foodways. They introduced classic dishes such as pizza and spaghetti, to the point that the Italian cuisine is the third most popular in the country.

Ethnic cuisines of Asian origins, particularly represented by Chinese and Japanese ethnic dishes, adapted to the American palate. Chinese-American foods represent the second most popular ethnic cuisine in the United States. Those ethnic cuisines contributed with several unique ethnic dishes. Noodles-based dishes and a large variety of vegetable dishes (e.g., broccoli and tomatoes) are part of the incorporation of non-Chinese foods added to satisfy the American preferences.

Table 3 Useful sources to consult on ethnic cuisines, ethnic foods, and food behaviors

Description of sources	*Electronic sites*
Scientific journals	
Journal of the Academy of Nutrition and Dietetics	http://www.andjrnl.org/
Nutrition Today	http://journals.lww.com/nutritiontodayonline/pages/default.aspx
American Journal of Clinical Nutrition	http://ajcn.nutrition.org/
Journal of the American College of Nutrition	http://americancollegeofnutrition.org/content/the-journal
Journal of Nutrition	http://jn.nutrition.org/
Appetite	http://www.journals.elsevier.com/appetite/
Public Health Nutrition	http://journals.cambridge.org/action/displayJournal?jid=PHN
Journal of Food Composition and Analysis	http://www.journals.elsevier.com/journal-of-food-composition-and-analysis/
Ethnicity and Disease	http://www.ishib.org/wordpress/?page_id=1388
British Food Journal	http://www.emeraldinsight.com/journal/bfj
Proceedings of the Nutrition Society	http://journals.cambridge.org/action/displayJournal?jid=PNS
Organizations, programs, and other resources	
Dietary Guidelines for Americans	http://www.health.gov/dietaryguidelines/
US Food Classification: MyPlate	http://www.choosemyplate.gov/food-groups/
USDA Cultural and ethnic food and nutrition resources	http://www.nal.usda.gov/fnic/pubs/ethnic.pdf
Society for the Study of Ingestive Behavior	http://www.ssib.org/web/
American Institute of Wine & Food	http://www.aiwf.org/
Center for Food Safety	http://www.centerforfoodsafety.org/about-us
Center for Science in the Public Interest	http://www.cspinet.org/
Oldways Preservation Trust	http://oldwayspt.org/
Sally's Place	http://www.sallybernstein.com/
Slow Food USA	http://www.slowfoodusa.org/
UN Food and Agriculture Organization (FAO)	http://www.fao.org/home/en/
FAO food-based dietary guidelines	http://www.fao.org/nutrition/nutrition-education/food-dietary-guidelines/en/

From Latin America, Mexicans contributed a large variety of ethnic foods. An extensive list of Mexican foods, including chili peppers, chocolate, black beans, tacos, 'burritos,' and tamales, is part of the rich offerings found across the country. Currently, the Mexican cuisine is the most popular ethnic food category in the country. Because the large Mexican population in the United States, along with their rich and varied traditional cuisine, the general American population has incorporated many of those Mexican ethnic foods into their eating habits, diversifying the culinary landscape of 'American' ethnic foods.

Ethnic food preferences and choices are important aspects of the cultural heritage they represent. Some of the many sources available on food-related aspects of culture are summarized in Table 3. It includes the cultural anthropology literature on foodways, reports, and nutrition information materials that describe foods, their uses, and related traditions.

Ethnic Differences in Food Classification Systems

Ethnic groups vary in their foodways, social and physical contexts in which food preparation, serving, and eating occur. Table 4 describes the factors that are thought to be involved in the development of ethnically distinctive cuisines and ethnic foods. Nutritional anthropologists subscribe to various theories on how ethnic food patterns have developed (see Table 5). No one theory appears to predominate over the other.

Individuals in different ethnic groups have different ways of categorizing foods, which are sometimes at variance with medical views of foods as sources of nutrients that are based on physiological and biochemical characteristics. They may also differ in their attitudes toward medical care and their health behaviors and in their beliefs about the therapeutic

Table 4 Factors involved in the development of ethnically distinctive cuisines

Factors	*Examples*
Ecological factors	Geography, climate, range of native plants and unusual species in area
Individual factors	Philosophical, religious, and moral attitudes
Social factors	Social structures, social relationships, social status, migration patterns (both voluntary and involuntary)
Economic factors	Trade, food prices, food distribution, imports, and exports

Table 5 Theories on how ethnic food patterns developed

Theory	*Comments*
Environmentalism	Ethnic food traditions are determined primarily by environmental and external influences
Cultural determinism	Humans mold their environments and diets quite independently of their physical surroundings
Cultural ecology	Both culture and environment are important
Cultural history	Historical influences on how foods have evolved and developed over time
Functionalism	Use of foods to identify or please people socially and how foods are used in society

Table 6 Some ways in which foods are classified in different cultures

Food classification system	*Descriptions*
Food habits	'Core,' secondary core, and peripheral foods
Gastronomic	Foods versus nonfoods, edible versus nonedible
Religious/ philosophical	Sacred versus profane, allowed versus forbidden
Opposing categories or properties	Hot versus cold, yin versus yang, or other ways of 'balancing food intakes to prevent or cure disease'
Medicinal	Foods for the prevention of diseases or as treatment for certain diseases or psychological states
Social	Symbolic of a culture or belief system
Emotional significance	Foods for the sick, party foods
Nutritional	Macro- and micronutrients
Hygienic	Wholesomeness, cleanliness, freedom from environmental contaminants, microorganisms, or other undesirable elements

Source: Kaufman-Kurzrock, D. L. (1989). Cultural aspects of nutrition. *Topics in Clinical Nutrition* **4**(2), 1–6.

and preventive properties of foods and the appropriate balance between different foods. For example, there are systems in various cultures that categorize foods into medicinal versus nonmedicinal, hot versus cold, or yin versus yang. These are classification schemes based on properties other than nutrient content (see Table 6). Food classification systems may affect nutritional status because they cause the exclusion of healthful foods or food groups from the diet, encourage consumption of harmful items, or produce a false sense of security among eaters that all of their nutritional needs are met when in fact they are not.

Dietary Assessment of Ethnic Food Consumption Patterns

Familiarity with ethnic foods is important for dietary assessment, particularly if they are imports from other countries. The foods themselves, their names, composition, portion sizes, and frequency of consumption vary. Accurate assessment of nutrient intakes depends on a good knowledge of these factors. Some ethnic foods may be especially rich sources of nutrients or other food constituents, such as phytochemicals.

During the last 40 years, Americans experienced increases in a series of nutritional and health threats associated with unhealthy eating practices, including obesity, type 2 diabetes, hypertension, cardiovascular diseases, and certain types of cancers. To halt those detrimental effects, people, in general, are responding favorably to the new policies and recommendations to improve eating and health-related practices. For some groups, those recommendations are being implemented with the use of ethnic foods considered healthy alternatives. To confirm those beliefs, food and nutrition experts need to analyze the growing number of ethnic foods that are being incorporated into the already diversified, ethnic cuisine practiced by Americans. The application of tools and techniques for dietary assessment may be needed in order to provide appropriate nutritional guidance for those groups adapting new ethnic foods.

One challenge in evaluating diets that include many ethnic foods is the appropriate choice of methods for dietary assessment. Methods such as semiquantitative food frequency questionnaires are often designed to capture intakes of important nutrients for those who conform to the predominant food culture. These are often inappropriate for subgroups with very different dietary patterns. Subgroup members may call the foods by different names, consume items not included on the food lists, or vary in amount and frequency of consumption. The use of such instruments to assess intakes of individuals with unconventional dietary preferences can lead to highly erroneous conclusions. Subgroup-specific food frequency questionnaires that take account of these factors are sometimes available, and when they are, these should be used. For example, well-validated ethnic-specific food frequency questionnaires have been developed for Japanese and Chinese-Americans living in Hawaii and for Puerto Ricans living in the Northeast.

Another potential problem in the dietary assessment of ethnic cuisines is that nutrient databases for converting dietary intakes into nutrients for ethnic foods may not be available. In research studies on individuals who use many ethnic foods, nutrient databases that include entries for ethnic favorites are needed to obtain complete information about intakes. Whenever possible, food tables that are specific to the country or ethnic group should be used. Country-specific tables of food composition and nutrient databases are becoming increasingly available, and these are the best option. For individuals who eat largely ethnic cuisines in countries where the parent food culture is different, special food composition tables for the ethnic foods that are most popular in mainstream diets are usually unavailable. Even for ethnic foods that are included in nutrient databases, food composition information may be incomplete, inappropriate, or lacking. Moreover, nutrient values for foods that are rarely consumed outside of the ethnic group may be absent, especially if the ethnic group is small. This is a challenge with many local foods consumed by Alaskan natives for which there is a gap in nutrient composition of those foods.

The dietary assessment task is more difficult when no country or ethnic-specific food tables are available. Recipes made from locally available ingredients or imported foods may differ in their food composition from recipe values in food tables used in Western countries. For example, a typical curry dinner as served in India, Ghana, and England varies greatly in its nutrient composition. For this reason, country-specific food consumption tables are needed.

Ethnically Appropriate Dietary Planning and Interventions

The consumption of ethnic foods is usually interpreted as a cultural phenomenon. However, it has physiological implications. Sometimes, alterations in eating habits are necessary to achieve nutritional needs, but this is a difficult goal to achieve. Food habits are among the oldest and most deeply entrenched aspects of many cultures, and they cannot be easily changed.

Table 7 Examples of the importance of ethnically appropriate nutrition interventions

Resources for nutrition intervention	*Electronic sites*
National Heart, Lung, and Blood Institute (NHLBI): Materials for American Indian/Alaska Native Audiences	http://www.nhlbi.nih.gov/health/healthdisp/an.htm
NHLBI: American Indian Health	http://americanindianhealth.nlm.nih.gov/index.html
NHLBI: Materials for Asian American/Native Hawaiian/Other Pacific Islander	http://www.nhlbi.nih.gov/health/healthdisp/aapi.htm
NHLBI: Materials for Latino/Hispanic Americans	http://www.nhlbi.nih.gov/health/healthdisp/lat.htm
NHLBI: On the Move to Better Heart Health for African Americans	http://www.nhlbi.nih.gov/health/resources/heart/african-american-index
USDA: Cultural and ethnic food and nutrition resources	http://www.nal.usda.gov/fnic/pubs/ethnic.pdf
US Department of Health and Human Services. Office of Disease Prevention and Health Promotion: Dietary Guidelines for Americans	http://www.health.gov/dietaryguidelines/
US Department of Agriculture. US Food Classification: MyPlate	http://www.choosemyplate.gov/food-groups/
Oldways Preservation Trust: Inspiring good health through cultural food traditions and lifestyles	http://oldwayspt.org/
Illinois State University: Healthy habits at the holidays	http://stories.illinoisstate.edu/student-affairs/health-promotion-and-wellness/healthy-habits-holidays-2/
Mayo Clinic. Mediterranean diet: a heart-healthy eating plan	http://www.mayoclinic.org/healthy-living/nutrition-and-healthy-eating/in-depth/mediterranean-diet/art-20047801
Winham, D.M., 2009. Culturally tailored foods and CVD prevention. *American Journal of Lifestyle Medicine* **3**, 64S	http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2782861/
Lemacks, J. et al., 2013. Interventions for improving nutrition and physical activity behaviors in adult African American populations. *Preventing Chronic Disease* **10**, 120256	http://www.cdc.gov/pcd/issues/2013/12_0256.htm

When they are changed, they often produce unexpected and unwelcome reactions.

There is no simple formula for success, but ethnic specificity and cultural sensitivity are important ingredients in crafting successful nutrition planning and interventions. Table 7 provides some examples from the recent literature of ethnically appropriate nutrition interventions.

The expertise of nutritional anthropologists who specialize in cultural anthropology as it relates to food and foodways is helpful. Some general principles that must be kept in mind are discussed in the succeeding text.

Some foods are more important than others to eaters and are the base of their food systems. Those foods constitute a continuum or gradient that consists of the 'core diet,' which includes universal, regular staple foods, the 'secondary core' of foods that are used widely but not universally, and 'peripheral' foods that are used infrequently or during specific holidays or celebrations. Such groupings are useful in planning nutritional interventions since they often suggest opportunities for making acceptable changes and possible pitfalls that need to be avoided. Food habits are always difficult to change. The most resistant habits of all involve foods in the core diet, especially those that are also viewed by the eaters as ethnically appropriate and vital to group cohesion.

The language used about foods and the names of favorite foods themselves must be used in recruitment. Ethnic-specific nutrition information, recommendations, dietary guidance, and education are also vital if interventions are to be appropriate to the dietary patterns and culture of intended recipients. In many countries, ethnicity, socioeconomic status, educational level, geographic location, and religious and ethical views are often confounded. As these other factors vary, so will the appropriateness of the intervention.

The importance of including ethnic foods in determining the ultimate acceptance of nutrition interventions varies within and between ethnic groups. In general, those who are least acculturated to the larger mainstream society, those with language barriers, the ill, the geographically isolated, the economically disadvantaged, traditionalists, and the religiously conservative may regard ethnic foods as particularly important. Those who are innovators and desire acceptance in the larger culture may regard ethnic foods as less important. The degree to which new foods are incorporated into eating styles varies depending on contact between cultures, food availability, trade, and social status of the group introducing the food and philosophical, religious, and individual factors. Immigrants differ in the extent to which they wish to remain unassimilated in their foodways and true to their native cuisines. Ethnic borrowing and mingling rather than rigid preservation of unique eating traditions often occur.

Ethnic-specific food-based dietary guidelines and graphics to guide food choices present nutritionally appropriate food patterns in a culturally acceptable manner for many ethnic groups. Many guidelines are currently available. They have the advantage of including ethnic foods as a part of overall diets. These guidelines are helpful as starting points for developing population-based nutrition education materials for healthy persons. Graphics that depict appropriate food choices have the additional advantage of being understandable even to those who may be illiterate.

Special attention must be paid to the ethnic appropriateness of nutritional counseling for those who are ill or in especially vulnerable groups from the physiological standpoint. Therapeutic diet manuals are sometimes available or obtainable that can be helpful in designing diets that provide medical nutrition therapy that is culturally appropriate.

Additional useful techniques for crafting nutrition information, education, and counseling interventions include the use of ethnic and regional terms for foods and recipes. Interventions should be adapted to be culturally appropriate. Food preparation and cooking demonstrations can be used to show how to incorporate recommendations into existing food patterns. Nutrition education and counseling should include attention to ethnic issues. Community social structures in the ethnic group are helpful to make contacts with the target ethnic groups.

Those who develop nutritional interventions that involve food enrichment, fortification, or special supplements must also pay attention to the role of ethnic foods and food patterns in the diet. In many cultures, the target groups for such efforts are those who are nutritionally vulnerable because of their age, sex, and socioeconomic status. And they are likely to have eating practices that are deeply embedded in their culture. Nutrition counselors must remember that care of the sick is heavily intertwined with food and health beliefs, social relationships, and acculturation. In summary, nutrition workers must avoid cultural biases and ethnocentricity in dealing with their clients in both dietary assessment and planning.

See also: Amaranth; Authenticity of Food; Bananas and Plantains; Cashew Nuts; Cassava: The Nature and Uses; Date Palm: A Wealth of Healthy Food; Food–Herbal Medicine Interface; Legumes in the Diet; Quinoa.

Further Reading

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Extrusion Cooking: Chemical and Nutritional Changes

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Introduction

The Extrusion Cooking of Foods

Extrusion cooking has become one of the most prominent technologies for thermal processing of food for a number of reasons. It was adapted from the plastic industry and first used for food processing in the 1920s to cook and model cereals in several forms. It evolved in the 1970s to the high-shear, high-pressure, high-temperature machines, which has become ever since the major process used throughout the food industry to generate expanded ready-to-eat products and textured vegetable protein. In the 1990s, modulated double-screw extruders were developed, and more feed materials with distinct water content could be processed. However, the most successful configuration for extrusion cooking is still the one that through a high shear at high temperature and pressure in a single screw creates the final texture by orienting and cross-linking the food macromolecules in a tridimensional stable structure, generating a wide variety of food products. In this extruder configuration, the feed material, rich in carbohydrates and proteins and with a low moisture content, is conveyed through an Archimedes screw within a barrel forming a molten mass that is expelled through a die. During this process, the molten mass experiences elevated shear, temperature, and pressure, with resident times at high temperatures of <5 s. Extrusion is thus a high-temperature short-time (HTST) food process method that inhibits most of the biological events but preserves most of the nutritive value of the processed food. In addition, the elevated pressure, temperature, and shear promote fragmentation of the feed material and orientation of the biopolymers dissolved in the molten mass until the exit of the equipment. The high pressure inside the equipment keeps the overheated water, typically around 150 °C, in the liquid state within the molten mass. At the die exit, it then flashes off because of the sudden drop of pressure, and the mass cools down. The biopolymers' filaments that hitherto flowed into the molten mass undergo an intense cross-linking, creating a supramolecular network that stabilizes the final structure of the extruded product. For this reason, extrusion has been widely used to cook and model food in various shapes, generating a great number of commercial convenient products. These extruded products are usually stable, with a long shelf life, microbiologically safe, and with an imparted texture, which increases their acceptability. They can be ready-to-eat products or ground and becoming ingredients that added to diverse products' formulation.

Because the extrusion cooking process employs elevated temperatures for short periods, usually below 5 s, several beneficial effects are expected in the extruded food. Nonetheless, some concern was also devoted to the potential deleterious effects of this processing to the nutritive value of foods. The reader should refer to the 'Further Reading' section to several reviews, books, and websites on the extrusion cooking technology. This article will describe the most common chemical changes throughout the extrusion cooking process and how they affect the nutritive value of the extruded food.

The More Frequent Chemical Transformations in Extrusion Cooking

Cross-Linking and Chemical Changes in Proteins

The diverse composition of the monomers that make up proteins enables a wide spectrum of interactions between proteins and other macromolecules. In general, the conditions of extrusion cooking promote raw materials' native protein dissociation, denaturation, orientation, and cross-linking at the exit of the equipment. Besides this macromolecular organization and because of the high temperatures that a protein experiences inside the equipment, extrusion cooking has been supposed to produce some potentially toxic amino acid and peptide derivatives, as it was described for other thermal processes. For example, under alkaline conditions, amino acids may cross-link during food thermal processing. As the extrusion cooking process destabilizes the tertiary and quaternary structures of the proteins by a combination of increased shear and temperature within the extruder, protein–protein interactions and amino acid cross-linking may occur. Proteins rich in alanine, lysine, cysteine, and ornithine are more prone to protein–protein interaction under specific conditions.

Thermal alkaline processes transform alanine into dehydroalanine, which may then react either with lysine to form lysinoalanine, or with cysteine to form lanthionine, or with ornithine to form ornithoalanine. Lysinoalanine is the most common of these compounds, which interferes with growth and induces changes in nucleus size and cytoplasm volume of kidney tubules cells in rats. However, all studies on the extrusion cooking process showed that there is less lysinoalanine formed in the extruded foods as compared with similar products prepared by traditional cooking methods. The majority of reactions of protein cross-linking observed throughout the extrusion process participate in the formation of the supra molecular structure that stabilizes and texturize the extruded products. Extrusion is safe as to the production of toxic compounds derived from thermal decomposition of amino acids.

The intensity of these cross-links may also be dependent on the other components from the food matrix and on the protein functionality. The content and type of protein may produce distinct structure resistance after extrusion as observed when amaranth or chickpea flour are treated with sodium glutamate and inosinate. The extrusion cooking of amaranth pasta mixtures may also cause a decrease of protein solubility in different denaturant solvents in the presence of starch; and all this knowledge may be used for technology improvements. However, none of these changes affect the nutritive value of the final extrusion product nor produce any health threat.

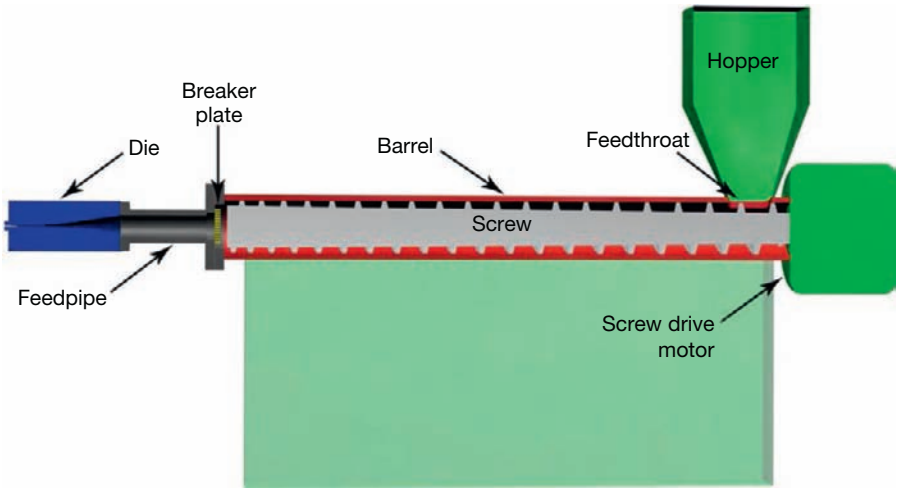

Figure 1 Schematic representation of a typical single-screw extruder. Ambient pressure and temperature built up to the typical maxima of 5 MPa and 150 °C, respectively, from the feed hopper to the die end. The residence time of the feed material at these conditions is typically below 5 s. Public domain picture. Author: Magnus Manske. http://commons.wikimedia.org/wiki/User:File_Upload_Bot_(Magnus_Manske).

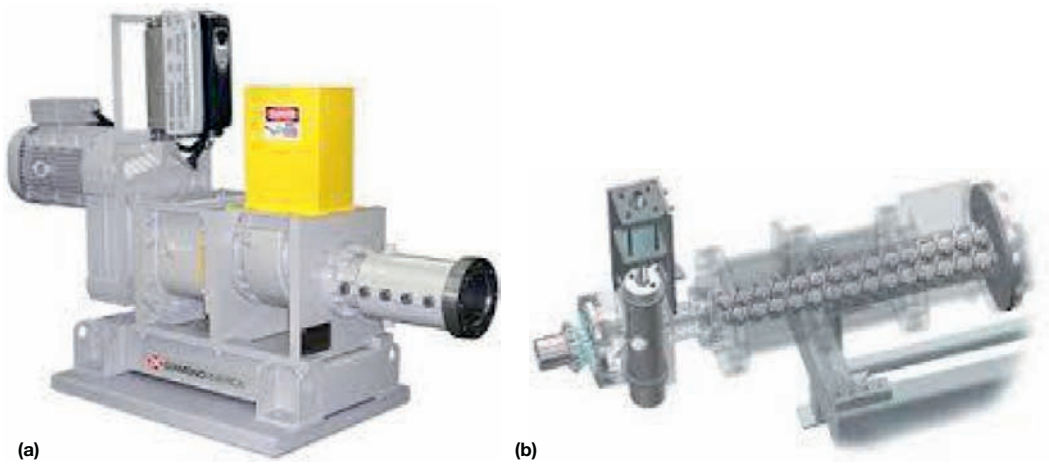

Figure 2 Examples of single-screw (a) and double-screw (b) extruders. From: Schweizer, J. P. (2011) The Right Extruder: Design Considerations - http://www.foodmanufacturing.com/articles/2011/05/right-extruder-design-considerations.

Extrusion of bovine rumen protein with 3.8% of residual lipid, for example, produced more structured extrudates with higher content of disulfide linkages and noncovalent interactions than those with less lipid content. Lung protein shows similar results, indicating an important role of the lipid, especially phospholipid content. These findings are consistent with some of the proteins becoming inaccessible upon thermal treatment and with the reorganization of the macromolecules at the die end favoring disulfide and noncovalent interactions. Again, these changes only affect texture with no effect on the nutritive value of the extruded food.

Chemical Changes in Carbohydrates

Extrusion cooking is a process where occur moderate carbohydrate hydrolysis and intensive starch gelatinization through cleavage of intermolecular hydrogen bonds. For this reason, extrusion causes an increase in water absorption until the rupture of starch granules, which occurs during this process, creating a viscous mass. The action of high pressure, high shear, and high temperature, combined with the moisture content and the amylose of the raw material, may subject the processed material to significant changes.

One of the most important modifications undergone by the material rich in carbohydrates and proteins, after extrusion, is expansion. The rate of expansion is very important for an extruded product, and for most of the cereal starches, such as corn, wheat, and rice, the product has a maximum expansion when subjected to temperatures between 150 and 200 °C. The gelatinized starch, the cellular protein, and the cellulose encompass a complex that decisively influences the product's expansion ability after the mass passes through the die.

The extrusion cooking purpose is to transform starchy raw materials, whose main feature in their original form is an insoluble granular material, in a crunchy, fluffy, expanded final product that easily breaks. Therefore, gelatinization rate

Figure 3 Examples of snacks and biscuits (a), breakfast cereals (b), and pet food (c), produced by single-screw extrusion cooking. Promotional photographs. Source: (a) http://www.kishanagrotech.com/; (b) http://www.clextral.com; (c) http://www.diytrade.com.

measurements and the effects of extrusion are essential. Thus, some physical tests are used, including viscosity, product expansion in the die, shear strength, amylograph tests, consistency index, and expansion ratio. There are also chemical tests, such as gelatinization index, water absorption index (WAI), water solubility index (WSI), and aqueous ethanol-soluble carbonate and water-soluble carbohydrate.

During the extrusion cooking process at high temperatures between 120 and 180 °C and pressures from 3 to 16 MPa, partial starch hydrolysis may occur. It appears that because of thermoplastic treatment, starch flour has a variation in its color; the milk-white flour at temperatures between 120 and 135 °C turns dark brown at 150–180 °C. At temperatures of 120–135 °C, starch becomes completely gelatinized, but at this point, hydrolysis does not occur yet. This will be only achieved at higher temperatures and pressures. The consequence of this high-temperature and high-pressure combination is the cleavage of 1,2-glycoside bonds of sucrose, 1,4-glycoside bonds of raffinose, and 1,4-glycoside bonds of maltodextrins. The higher the extrusion temperature and lower moisture content of the raw material, the greater is the bond cleavage within the molecules with high molecular weight.

For evaluation of the final extruded product, two factors are quite important and shall be taken into account: the WSI and the WAI. The conclusions found by many studies on these properties were that the WAI increases for a wide variety of starch products with the temperature rise in the extruder barrel. The lower is the material initial moisture content used for extrusion, the higher is the extrudates' WSI.

The extrusion of starchy raw materials results in important changes in product viscosity after dissolution in water. This property is relevant for a technological point of view, but its impact on nutritional properties is not clear. All processes that increase starch solubility may increase its glycemic index (GI). However, several cooking processes for amaranth showed that the GI of starch of the extruded amaranth was not the highest one. Even increasing starch solubility, the extrusion produces a complex network made of starch and protein that may hinder access of amylolytic enzymes to the food matrix. A more detailed account of the impact of extrusion on the GI is in Section **Changes in GI** of this article.

Chemical Changes in Lipids

Generally, the raw materials used throughout the extrusion process contain low amounts of lipids. The friction necessary for mechanical energy transfer to the extruded material is essential for food extrusion. Large amounts of lipids cause slippery at the barrel and impair the process.

Some lipid hydrolysis occurs in lipid extrusion by lipase action. However, even high levels of free fatty acids in foods have no toxicological effects on humans, although organoleptic and storage quality of the extruded foods may be impaired. The proper combinations of humidity and heat are responsible for reducing the lipase activity in the extrusion process, decreasing the factors favoring free fatty acids' production. The inactivation of enzymes that occur during the extrusion process promotes increased shelf life of the final product.

Fatty acids' oxidation may also occur in a limited extent. Some conditions in the extrusion cooking process that may affect the rate of lipid oxidation, which are similar to the ones found in any other process, are listed in the succeeding text:

(1) The rate of oxidation increases with the amount of oxygen present and the surface area of the food exposed.
(2) Higher temperatures produce faster reaction rates.
(3) Oxidation rate is retarded at intermediate moisture contents of water activity (Aw = 0.3), but the rate is higher at water activities below 0.1 and above 0.55.
(4) Light and metals act as prooxidants.
(5) The presence of antioxidants reduces the rate of oxidation by terminating free radicals or by sequestering metals.

Chemically, the double bonds of unsaturated fatty acids can easily react with free radicals and oxygen. Oxygen adds to a carbon–carbon double bond in a chain of unsaturated fatty

acids forming hydroperoxides. These compounds break down rapidly in the presence of some substances, factors, and process conditions that affect the rate of lipid oxidation. Contents up to ~5% of total lipids improve expansion ratio and texture, whereas the formation of *trans* fatty acids at this lipid concentration is almost insignificant. As the lipid content of the raw material for extrusion is typically below this content, extrusion negligibly affects lipid oxidation.

Other Chemical Changes

During the extrusion cooking process, there is a strong structural molecular rearrangement. The intense disturbance considerably influences the shape and texture of the extruded products. One such chemical change that occurs is the amylose–lipid complex formation. The interest in amylose–lipid complexes focused on their technological importance in starchy food systems. A consequence of these chemical changes is the low lipid extraction using solvents after extrusion.

At low hydration levels, the linear component of starch, amylose [α 1–4]-glucose-linked polymer, behaves as a flexible random coil with stretches of left-handed helical segments in aqueous solution. Stable secondary conformations, known as A-, B-, and V-amylose, are formed both in ionic solutions and in solvents of lower polarity. In the presence of lipids, the amylose chain undergoes a conformational change to V-amylose, characterized by the interaction of the aliphatic lipid tails with the hydrophobic core of the polysaccharide, forming a very stable imperfect helix.

These amylose–lipid complexes turn up when the material is between its melting and glass transition temperatures. The barrel temperature and moisture within the feed material are determinant in the process; the water acts as a plasticizer, and the decrease of moisture content increases the temperature. The presence of other components in the food matrix increases the melting temperature of starch–lipid complexes, which are also present at high barrel temperatures.

Moreover, the presence of triglycerides, diglycerides, and different fatty acids' saturation in the lipid fraction may result in lower expansion, decreased degree of gelatinization of starch, reduced dough viscosity, distinct dough flow properties, and decrease of the hardness of the extrudate.

Nutritional Consequences of the Chemical Changes

Increasing Digestibility of Amino Acids

Comparable to other HTST processes, extrusion is the preferred choice regarding nutrient retention, destruction of antinutritional factors, microorganisms, and capability of producing different textures. However, the intense high-pressure homogenization can break down chemical bonds and promote new chemical reactions that alter the protein digestibility. Mild heat treatment of vegetable proteins generally improves digestibility, and with increasing severity of temperatures, protein digestibility decreases.

In some studies, the decrease of the true digestibility was evident after extrusion. The process caused protein denaturation with formation of insoluble compounds through new protein–protein interactions that were responsible for the stabilization of the structure. Intermolecular disulfide and other hydrophobic bonds appear to be the major type of linkages that occurs during extrusion and influence extrudate characteristics. These results occurred due to previously hidden amino acid residues becoming exposed and free to react with other food components. The exposure of hydrophobic residues reduces the solubility of extruded protein in aqueous systems, decreasing digestibility.

However, most studies have demonstrated that the protein digestibility improves because of the denaturation of protein, which renders them more susceptible to digestion by proteolytic enzymes. Extruded cowpea beans, a protein-rich legume, showed an increase of 55.9% digestibility measured *in vitro* as compared to the raw grain. Thus, extrusion enhances the digestibility of proteins through denaturation and by inactivation of protease inhibitors.

Loss of essential amino acids

The amount of essential amino acids is an important indicator of nutritional quality of extruded products. Process conditions of extrusion influence the digestibility of the proteins and affect the availability of certain amino acids. Thus, no doubt that lysine is the most unstable amino acid during the extrusion process. Several chemical reactions, combined or not, are responsible for amino acid losses, such as protein denaturation, Maillard reaction, and protein cross-linking.

Increasing energy input and decreasing water cause significant reduction in the availability of several amino acids. Loss of unstable amino acids in the extrusion cooking process was reported in the literature to be 30–40% lysine, 7–21% arginine, 15% histidine, 8–21% cystine, 14% methionine and tryptophan. During extrusion cooking of maize grits, below 14.5% water, there was also a decrease in cystine. Only minor losses were found in other amino acids.

The nature of the extruded protein also influences the extent of the loss of the available amino acids. In meat sources of protein, bovine rumen, for example, extrusion did not affect the concentration of very sensitive essential amino acids like cystine and methionine, and the occurrence of any other limiting amino acid in the extruded protein was not observed.

Maillard reaction

The Maillard reaction is one of many chemical reactions that occur during the extrusion cooking process and has important nutritional and functional consequences. Known as non-enzymatic browning, Maillard reaction is actually a series of reactions with a wide variety of compounds produced as a result. This chemical reaction is characterized by the binding of a reducing sugar (glucose, fructose, lactose, or maltose) and a free amino group on an amino acid, usually the epsilon-amino group of lysine.

Because of this, the main nutritional loss related to this reaction is the quantity of available lysine. Data reported throughout the literature indicated that small peptides from the matrix can also be involved in this reaction. Another indication of Maillard reactions is the dark color of the extruded material, due to the formation of melanoidins and other reaction products; however, a darker color is not necessarily due to the imperative presence of these materials.

Extrusion cooking appears to cause lysine losses that do not exceed those for other methods of food processing (loss range 3–13%), but appears to depend on the raw material used. In semolina pasta, for example, lysine is involved to loss of a greater extent with respect to other amino acids in the range of 43.9–62.0%. In food with lysine limitation, this loss is important for the nutritional quality.

Extreme conditions of temperature (>180 °C) and rotation (>100 rpm) in combination with low moisture (<15%) promote the breakdown of poly- and oligosaccharides and release reducing units of the molecule. On the other hand, an increase in feed rate promotes less lysine loss due to a residence time decrease and heat transfer to the mixture. Conditions of higher pH, reducing sugar hydrolysis in the extrusion of mung bean and cowpea flours, are also favorable for lysine retention.

For quality control purposes, the amount of available lysine in an extrudate may be quantitatively measured through several methods. The reaction of free lysine with fluorodinitrobenzene is one of these. Total lysine may also be measured after acid hydrolysis of the protein, and the amount of unavailable lysine can be calculated.

Although researchers are still unsure of the precise mechanisms by which acrylamide is formed in foods, there is a strong relationship between Maillard reaction and acrylamide formation on extruded products. Temperatures above 120 °C, very low moisture content of 5%, higher asparagine content, and reducing sugars are favorable parameters. It has been suggested that the butanedione, one of the several dicarbonyl compounds formed during the Maillard reaction (Strecker degradation), in the presence of free asparagine can produce high amounts of acrylamide. On the contrary, glycine, L-lysine, and L-cysteine added to formulation can reduce the formation of acrylamide (95%, 91%, and 87%, respectively), and they can be used as additives of the extrusion process.

Hydrolysis of Carbohydrates

Redistribution of soluble and insoluble fiber

Few studies have been performed about the effect of extrusion cooking or other processes on dietary fiber. The degradation of the components of dietary fibers may occur in processes that involve shear. It is known that the glycosidic bonds of cellulose can be broken during dry grinding in ball mill. It is also known that degradation in the colon of the fiber is inversely proportional to its particle size. Therefore, the disruption and homogenization of the particles due to the intense mechanical working during extrusion could leave the dietary fiber more available for fermentation in the colon.

The thermoplastic extrusion causes structural changes in biopolymers, with significant transformation in polysaccharides. Because of this, the structure and composition of the fiber fraction experience a shift in the molecular weight of constituent polysaccharides. In general, one observes an increase between 10% and 15% in the content of the soluble dietary fiber (SDF) fraction at the expense of the insoluble one. Enzymatic or acid treatments can increase this proportion, being an additional tool in achieving higher levels of soluble fiber.

A very important effect brought about by the extrusion process is the degradation of phytic acid, in addition to rearranging components of total dietary fiber (TDF), which would improve the nutritional properties of food after this process. The heat applied during the extrusion process modifies the texture of raw foods, which can consequently alter the composition and the structure of these components.

In some foods, such as oat bran, the extrusion process improves the functional properties of SDF. When one compares the SDF raw oat bran with SDF-extruded oat bran, some characteristics have been changed in the extruded product. They are the increased aggregate formation, the increased gelatinization temperature, the increased solubility index, the water absorption capacity, and water retention solvent. An increase in the apparent viscosity and the consistency coefficient is also observed, besides a decrease in the flow behavior index, a decreased radial expansion, an increased bulk density and breaking strength of extrudates, and an improvement in foam ability.

Studies have also reported an increase in TDF of barley flour after extrusion. This is due to the increase either in one or in both fractions of fiber. These changes in the profile of dietary fiber during the extrusion process can be explained by the resistant starch formation that is determined as dietary fiber, 'enzyme-resistant indigestible glucans,' formed through transglycosidation, and a structural transference from insoluble to SDF.

Digestibility of starch

Many changes may be observed in the functional properties of the starch due to the extrusion process. These changes can be monitored by physical and chemical methods in the starch granules and their components. They include the determination of textural and rheological properties, which are related to the digestibility of the starches and availability of glucose. Among the many food processing methods, the extrusion cooking is the one that mostly increases the rate of starch digestibility, due to a decrease of particle size of the final product. The variation and the digestibility rate will depend on some parameters, such as amylose and amylopectin ratio, the feed material particle size, the process temperature, the moisture of the raw material, and the screw speed and geometry.

During extrusion, starch is exposed to high shear forces that physically cleave glycosidic linkages, forming fragments of lower molecular weight. Inside the extruder, the starch granule is compressed and will slowly turn into a homogeneous and molten mass where the disappearance of granular and crystalline structure is observed. However, the extent to which the starch will break down depends upon the intensity of treatment. The degree of digestibility of starch is an important property of the extrudate, and it appears that the *in vitro* digestibility of starches is significantly higher after the extrusion process.

The structural integrity of the starch granules is lost due to increased shear action and molding in the extruder barrel. This makes the starch more available for enzymatic action. Nevertheless, low levels of starch digestibility observed in some extruded foods with a high starch content can be attributed to the formation of amylose–lipid complex, starch–protein, and the limited availability of water, which impairs the starch digestibility during the enzymatic hydrolysis. The decrease in the grain size distribution results in increased surface area and a higher degree of hydrolysis.

Extrusion potentially produces greater increase in starch digestibility than any other processing methods. However, some studies comparing four processing method – popping, roasting, flaking, and extrusion – found that the rate of hydrolysis of starch is greater by popping, roasting, and peeling than by extrusion.

Changes in GI

Values of the GI of several products and the respective glycemic response can be influenced for many factors; and processing plays a very important role among them. When the cellular structure of the starch granule is preserved even after heat processing, the final product keeps its GI, which is lower than when the starch granule is broken. Nongelatinized starches are less digested by humans, so heat processing is beneficial for a complete starch digestion. After the heating and shearing during the extrusion process, the starch granules are cooked and broken down, and the crystalline structure of the pellet is reduced, resulting in partial gelatinization and depolymerization. This will result in a viscous fluid of starch dispersion in water. Extrusion processing conditions such as temperature, moisture, and screw speed, among others, will affect the physical and chemical structures of starch and, subsequently, the IGs of the final extruded products. Therefore, snack amylaceous products usually have higher GIs than the corresponding raw materials.

Most of the extruded products are basically composed of cereals. Thus, they have a high GI. Various studies have been performed to increase the dietary fiber content after extrusion, thereby lowering their glycemic response. They shed some light on the mechanisms by which dietary fiber lowers the glycemic response. In these studies, the fibers seem to coat the starch granules, protecting them against the action of the amylolytic enzymes and reducing the degradation of starch, eventually producing a low glycemic response. There is also a physiological effect of the soluble fiber fraction, increasing gut fluid viscosity and reducing glucose absorption and hence the GI.

With increased dietary fiber content, it is possible to enhance the amount of slowly digestible starch components in processed food with reduced rate and degree of glycemic response. The use of whole-wheat flour instead of the refined flour in the extrudates reduces the amount of rapidly digestible starch components and increases the quantity of slowly digestible starch in extruded cereal-based breakfast. In the extrusion process, starch is subjected to high shear forces that chemically break glycosidic linkages forming fragments of lower molecular weight. Thus, this smaller branched starch forms new linkages, which are more resilient and, therefore, present lower GI. These structures are considered resistant starch and greatly contribute to the TDF content of the extruded food product.

Loss of Essential Fatty Acids and Vitamins

Extrusion of food can be a possible adverse effect with the destruction of vitamins during processing. The loss or retention of vitamins in extruded foods can vary according to the type of food, screw rpm, die diameter, moisture content, residence time at high temperatures, time and temperature of processing, and throughput. In general, in lower temperatures, the losses are minimal. In usual extrusion conditions at high temperatures for a short time and with quick cooling of the product during output, a relatively small loss of most of the essential fatty acids and vitamins is observed. For example, there are a 95% retention of thiamine and a small loss of riboflavin, pyridoxine, niacin, and folic acid in cereals at temperatures as high as 154 °C.

Vitamins A and C are normally lost by oxidation and heat. The retention of vitamin C is about 70% in extruded foods, while the retention of vitamin A ranged from 50% to 140% after extrusion. These differences are due to the intrinsic susceptibility of each vitamin and because vitamin A fortification of extruded products is performed through a series of β-carotene derivatives with distinct heat stability.

Other Nutritional Changes

Extrusion can maintain the levels of the lycopene in extruded enriched snacks, if the source used to enrich is the tomato skin powder.

The extrusion process can reduce the content of tannin that sometimes is undesirable in food. The extrusion of flaxseed, for example, showed 77% reduction of tannins.

A 13–35% reduction in phytate content occurs after the extrusion of a wheat bran–starch–gluten mix. Phytate is known to reduce absorption of iron and other essential minerals.

Fortification of extruded products must be performed carefully. The added minerals should have high bioavailability, they should also be homogeneously mixed with food ingredients, and these must be compatible with the processing. Calcium salts, for example, reduce the expansion of snacks. The mineral used may also be compatible with the postextrusion storage. The bioavailability of the iron is generally not affected by the extrusion process, and highly bioavailable iron is observed after processing of leguminous seeds.

Overview of the Extrusion Cooking Effects on Nutritional Value

In short, the extrusion process is widely used throughout the food industry during recent years; and there are several nutritional aspects of this thermal processing that should be taken into account.

Among the positive aspects, the following can be featured:

(a) The possibility of developing a vast range of versatile products ready for consumption, with a long shelf life, since various enzymes are inactivated, as lipases and lipoxidase.
(b) The destruction of antinutritional factors and microorganisms is effective and the final product is safe with respect to the production of toxic compounds derived from thermal decomposition of amino acids.
(c) The transformation of starchy materials in a crunchy, fluffy, expanded product that easily breaks with a final pleasurable product with texture.
(d) The enhancement of the digestibility of proteins through denaturation and by inactivation of protease inhibitors.

(e) The promotion of an increase of SDF fraction content and degradation of phytic acid, in addition to rearranging components of TDF.
(f) Extrusion process promotes a greater increase in starch digestibility than any other processing methods.

The negative aspects of extrusion are minimal but should be considered:

(a) Extruded snack products tend to have a higher glycemic response in comparison with the raw materials, although the increase in the dietary fiber content of the extruded products can lower their glycemic response.
(b) Vitamins are destroyed during processing, with vitamin C being the most affected, but in lower temperatures, the losses are minimal.
(c) The availability of several amino acids is reduced; lysine is the most unstable amino acid during the extrusion process.
(d) There is a strong relationship between Maillard reaction and acrylamide formation on extruded products.

See also: Amaranth; Browning: Non-enzymatic browning; Cooking: Domestic Techniques; Extrusion Cooking: Principles and Practice; Fatty Acids: Fatty Acids; Functional Foods; Oxidation of Food Components; Proteins: Chemistry, Characterization, and Quality; Quality Control in Food Processing; Starch: Modified Starches; Starch: Sources and Processing.

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http://www.wisegeek.com/what-is-extrusion-cooking.htm – wiseGeek.

Extrusion Cooking: Principles and Practice

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Extrusion-Cooking Technology

Extrusion technology, which is well-known in the plastics industry can be modified for a wide variety of extrusion-cooking applications in the food and agriculture industries, for which it produces 'engineered' food and special feed. Generally speaking, the extrusion cooking of raw vegetable materials involves the extrusion of ground material under barothermal conditions, with controlled moisture content, so that the shear energy, which is exerted by the rotating screw and/or additional heating of the barrel, heats the food material to its plasticizing or melting point. In this changed rheological status, the food is conveyed under high pressure through a die or a number of dies, and the product expands to its final shape. As a result, the extrudates have physical and chemical properties that are very different from those of the raw materials. Food extruders, which are often called extrusion cookers, belong to the family of high-temperature short-time equipment, because they perform under high pressure (up to 20 MPa) and high temperatures (up to 200 °C) for only a short time (typically tens of seconds). This form of processing restricts unwanted damage to nutrients such as proteins, amino acids, vitamins, and enzymes. The process conditions and raw materials used have a strong impact on the food and feed properties during extrusion cooking, however, and they can drastically influence the final product quality.

A modern food extruder is a process reactor that creates different extrudates by varying screw layout, mixing elements, clearances in the gaps, and the installed motor power and barrel heating, in order to control the dough reaction. For example, altering the process parameters and the combined effects of heat and shear affects the degree of starch gelatinization or denaturation of proteins in the presence of water.

Extrusion cooking is now used for the manufacturing of a diverse array of foodstuffs (Figure 1), including the following:

Figure 1 Various extrusion-cooked foodstuffs and feedstuffs.

- Direct-extruded snacks, such as ready-to-eat (RTE) breakfast cereals, differing in shape, color, taste, and texture
- Snack pellets: products destined for fried or hot air–expanded snacks, precooked pasta
- Baby food, precooked flour, instant concentrates, functional components
- Pet food, aquafeed, feed concentrates, and calf-milk replacers
- Texturized vegetable protein or meat analogs (mainly from soybeans)
- Crisp bread, bread crumbs, emulsions, and pastes
- Confectioneries: different kinds of sweets, gums, and jellies
- Barothermally processed products for the pharmaceutical, chemical, paper, and brewing industries

Extrusion cooking can use raw materials that have not previously displayed great economic importance (e.g., legumes) or have been regarded as waste. Of practical importance, the process can also be implemented with relatively low effort, does not require excessive capital investment, and uses equipment that can be employed for multiple applications. The energy consumption of food extruders is in the range 0.08–0.20 kWh kg^{-1}. Thus, in many cases, food extruders provide a cost-competitive alternative to other machines used for food and feed manufacturing.

The direct extrusion of cereal snacks provides a good example of extrusion-cooking technology. Figure 2 presents the standard installation used in this process.

Preparation of Raw Material

Direct-extruded snacks are most often manufactured from popular cereal-based materials, such as corn, wheat, and rice. Depending on its quality, the material must be properly ground and weighed, according to the recipe, and mixed thoroughly before being fed to the extruder. If preconditioning is required, water and/or steam is added to the material before mixing.

Extrusion Cooking

Extrusion cooking is based on the concept that, after the material leaves the die, the sudden decrease in pressure allows the superheated water in the material to turn into steam, leading to a rapid expansion of the material. This expansion is responsible for the porous structure of the extrudate, with the structure shaped by bundles of molten protein fibers. The melt mass leaving the extruder generally takes the shape of the extruder dies. A rotary knife is installed outside the die, in a lengthwise arrangement, and the appropriate setting of the knife speed controls the product length. This allows for the production of different, fancy shapes of extrudates, such as balls, stars, shells, and rings, even when using a simple single-screw food extruder. The technology for the production of each shape requires an appropriate distribution of temperature, pressure,

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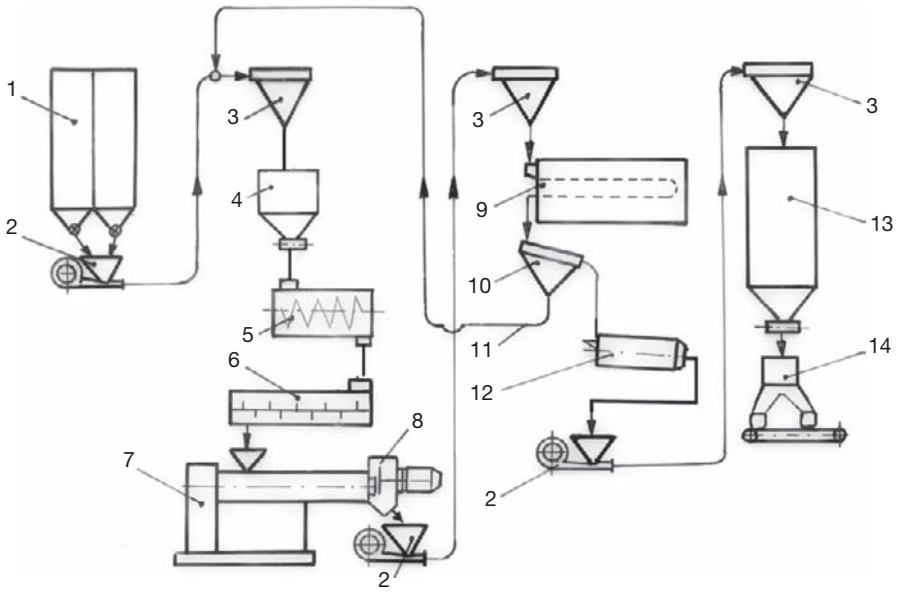

Figure 2 A diagram of a standard installation for the production of direct-extrusion snacks. 1, raw materials; 2, pneumatic conveyer; 3, collector; 4, weight; 5, mixer; 6, preconditioner; 7, food extruder; 8, cutter; 9, dryer; 10, screen; 11, recycling of dust; 12, coating drum; 13, finished product; 14, packaging.

and moisture content in the material during processing. Therefore, the operator must be focused on flexibility and precise control, especially in regard to the thermal process. Frequently, the process for the manufacturing of specific products must be developed empirically.

Drying, Coating, and Packing

After extrusion, the extrudates are dried to a moisture content of about 6–8% and cooled when necessary. Drying can be performed with simple rotating drums installed with electric heaters, or it can use a gas-operated hot air installation working at temperatures just above 100 °C. Larger installations rely on belt dryers, heated by gas-fired heat exchangers or steam, with hot air circulating through the unit sections. Cooling takes place at the ambient temperature, as air flows through the perforated belt of the cooler device. The flavor and vitamin-coating step is often integrated in drum dryers. Flavor additives, liquored or powdered, come in a wide variety of forms, from smoked meat flavor to paprika aroma.

Popular direct-extrusion breakfast cereals include coated cereal balls, rings, or shells in an icing sugar coating. The industrial production of this type of product requires the use of an additional high-performance drum dryer or belt dryer, so that the application of the coating is a continuous process, thus maintaining fixed product quality.

Quality Parameters

The literature has described the influence of various extruder variables, such as screw speed, die geometry, screw geometry, and barrel temperature, on product quality. A number of measuring methods are used to obtain the desired physical properties of extrudates. The most important are bulk density, water sorption, wet strength, dry strength, cooking loss, and viscosity behavior after extrusion. A more detailed description of the properties of starches and proteins also requires additional chemical data, including dextrose equivalents, reaction rate constants, and data describing sensitivity to enzymatic degradation. The food engineer and technologist must be able to forecast the relationship between these properties and their dependence on the extruder variables. Therefore, it is necessary to provide a (semi)quantitative analysis of biopolymers in the extrusion-cooking process, which can be done by adopting an engineering point of view, with the extruder considered to be a processing reactor.

Food Extruders

As previously mentioned, extrusion cooking involves machines in which the main operative body is one screw or a pair of screws fitted in a barrel. During barothermal processing, the material is mixed, compressed, melted, and plasticized in the end part of the machine (Figure 3). The range of physical and chemical changes in the processed material principally depends on the parameters of the extrusion process and the construction of the extruder.

There are many conventional methods for classifying food extruders, but the most practical accounts for the following three factors.

- The method of generating mechanical friction energy converted during extrusion into heat: autogenic, isothermic (heated), polytropic (mixed)
- The amount of mechanical energy generated: low-pressure extruders producing relatively limited shear rate and high-pressure extruders generating large amounts of mechanical energy and shear

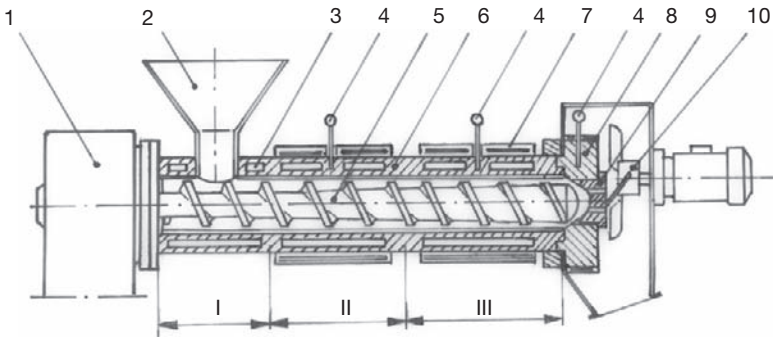

Figure 3 A cross section of a single-screw food extruder: 1, engine; 2, feeder; 3, cooling jacket; 4, thermocouple; 5, screw; 6, barrel; 7, heating jacket; 8, head; 9, dies; 10, cutter; I, transport zone; II, compression zone; III, melting and plasticizing zone.

- The construction of the plasticizing unit, with both the barrel and the screw designed as a uniform, integrated body or fixed with separate modules

In 1946, in the United States, single-screw extruder were developed to cook and expand corn and rice snacks. In combination with an attractive flavoring, this product type is still popular, and the use of single-screw extruder equipment to produce such snacks is, in principle, is still the same. A wide variety of extruder designs can be employed for this purpose. The old method of cutting preshaped pieces of dough out of a sheet with roller-cutters is also still in use, however, because the complicated shapes of snacks require very expensive dies and die heads for cooking and forming extruders. Although modern techniques offer improved control of mass flow in food extruders, new extruders with better mixing and a more steady mass flow often show advantages over the traditional single-screw equipment. In the mid-1970s, twin-screw extruders were introduced for the combined process of cooking and forming of food products, partly as an answer to the restrictions of single-screw extruder equipment, because twin-screw extruders provided a largely forced flow. Twin-screw extruders also tended to produce better results on scale-up from the laboratory extruder types in use for product development.

During the processing of biopolymers, nearly all chemical changes in food are irreversible. Continued treatment after such an irreversible reaction in an extruder should be a process controlled for temperature, time, and shear, leading to a series of completely different functional properties of the produced food. From a rheological point of view, two facts are the most important. First, non-Newtonian flow behavior usually occurs in food extruders. Second, chemical reactions occur during the extrusion-cooking process (e.g., gelatinization of starch or Maillard reactions), strongly influencing the viscosity function. The rheological behavior of the product, which is relevant to the modeling of the extrusion process, has to be defined directly after the extruder screw tip, before expansion has occurred, in order to determine the influence of water losses, the consistency of the material, and temperature effects due to the flashing process that occurs directly after leaving the die.

Today, the food extruder is integrated into the technological line as a single- or twin-screw reactor, with the preheating step performed in a specially built preconditioner. The forming task of the extruder can also be separated from the heating and shearing steps. In this case, the final shaping and forming are done in a second, well-optimized post-die forming extruder, which largely processes the food at a lower water level than is present in the first reactor extruder. This method produces cooked, preshaped, unexpanded food pellets, which can be used for the production of RTE cereal flakes (e.g., popular corn flakes) or as half product in the manufacturing of fried snacks.

Single-Screw Food Extruders

The design of single-screw extruders is relatively simple. In food extruders, sticking effects are prevented by the force of friction between the material and the barrel wall, and this friction is facilitated by the suitable grooving of the inside of the barrel (longitudinal or spiral grooves).

These devices process relatively easy materials characterized by a high friction coefficient, such as maize or rice grits (i.e., basic materials for the production of direct extrusion snacks or breakfast cereals). For these materials, the use of the simplest autogenic extruders is often sufficient, even at a low ratio of screw length L to diameter D (L/D) = 4–6. The main disadvantages of single-screw extruders are poor mixing (as a result, materials should be mixed before feeding) and limited efficiency, especially when multicomponent mixtures of raw materials are used. Smooth positive movement of the material in a single-screw food extruder depends on the actual drag flow, which is caused by the screw geometry and its rotation minus the so-called back flow.

The operator must strictly follow the technological regime during operation, which is determined experimentally or given by expert technicians. A number of issues must be taken into consideration in daily extruder operations:

- The moisture content and particle-size distribution of the raw material mixtures must be properly chosen and homogeneous. This prevents shooting or blocking in the extruder and ensures the desired quality of the extrudates.
- Intensive cooling of the barrel (e.g., with cold water) contributes to a lower processing temperature and increases the friction inside the material. It must be correlated with the quality requirements of the extrudate. A temperature drop in the material raises its viscosity and positively contributes to the extruder's performance.
- The blocking of a few die holes results in a sudden increase in pressure and leads to a powerful back flow, or even

lockout of the machine. In such a case, immediately cleaning the blocked holes with a thin tool might correct the problem. If this does not help, the operator must stop the machine and dismantle the die. Postponing the disassembly of the die with a plasticized material inside it may lead to permanent damage to the equipment during the next start-up.

- The smallest holes in the die cause a higher resistance during the extrusion of the material, because smaller openings increase pressure and reduce the extruder's output, given the higher incidence of back flow.
- Applying a plasticizing screw with a greater L/D ratio can generate more extrusion pressure resulting from a longer fully filled screw, which leads to improved plasticizing of the material and reduced back flow.
- The loss of a defined clearance between the barrel surface and the screw flights hampers the movement of the material, reduces friction and pressure, and causes poor operation of the machine, which means poor product or no product at all.

Twin-Screw Food Extruders

These machines are much more complex in terms of design but much more universal in operation. However they are more expensive. Nonetheless, twin-screw extruders have become very popular with producers of extrusion-cooked foodstuff, because of their ability to process a wider range of materials (e.g., viscous and hard-to-break materials) and their lower energy consumption, broadening the list of products the machines can produce. Corotating food extruders are also used to a greater extent because of their high productivity and efficient material transportation, mixing, plasticizing, and extrusion. The self-wiping and intermeshing flights of the screws effectively force the material forward, and, as a result, no material is locked in the space between the surface of the barrel and the screw. Popular corotating twin-screw food extruders most often produce RTE breakfast cereals, pet food, and aquafeed.

The physical processes and heat exchange associated with material transfer in twin-screw food extruders are more complicated than they are in single-screw extruders. Readers who wish to learn more about the engineering aspects of extrusion can find detailed information in the literature. The extrusion-cooking technique is fairly complicated, and its proper use requires considerable expertise on the part of the operators. Yet, when operators are properly trained, extruders can allow food companies to vastly broaden their product offerings.

Counterrotating twin-screw food extruders are special-purpose devices. In these machines, the screws rotate slowly, but they can mix the material effectively, and their work resembles a positive-displacement pump, generating high pressure on the screws in the barrel's closed C-shaped chamber. This approach is especially suitable for high viscosity material. The back flow of material in these extruders is very small because of the tiny clearances between the screws and the barrel. They are mainly used for the production of confectionery and chewing gum, as well as the processing of fiber- and cellulose-rich materials.

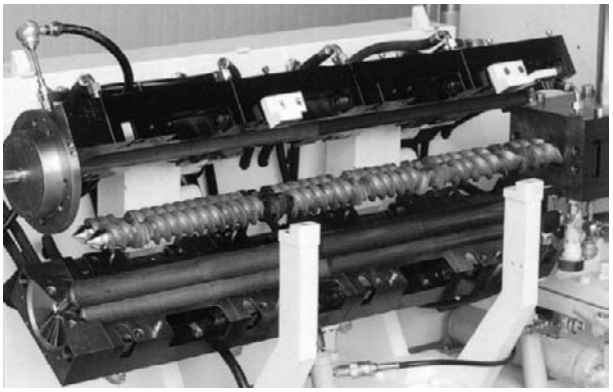

Figure 4 Modern multifunctional corotating twin-screw food extruder with an axially open barrel.

Twin-screw food extruders now have a modular construction, with the screws built from several different elements mounted on the screw shaft. These elements handle transporting, mixing, compressing, and melting the material (Figure 4). By properly setting these elements, the operator can control the behavior of material inside the extruder, thus influencing the scope of the physical and chemical processes during the extrusion-cooking process.

Extruders often produce a wide range of products, from simple maize snacks to protein-based food. Snack production requires high pressure and mechanical energy at a low L/D ratio. Protein-based material needs long processing times, with many intermediate stages applied. Modular screws allow for these possibilities, either by the disassembly of the barrel units in combination with the replacement of the screws or by changes in the screw lay-out. Some food-extruder producers offer a mobile feeder installed on the feed ports between the center and the end of the barrel. In such cases, distance rings are placed on the screw shafts ahead of the final set of screw elements.

During the operation of extruders, the engine load must be maintained at an adequate level (maximum 80–85%). As a result, the screw's rotational speed, torque, and energy consumption (expressed in kWh per 1 kg of product) must be controlled. The proper choice of extruder machinery for the type of production is also important for a successful operation. Yet, no established and strict recommendations currently exists regarding the range of temperature in different zones of the plasticizing section of food extruders. Some temperature ranges can be recommended during the processing of certain raw materials, but, in the case of a particular machine, these ranges should be verified empirically, or they should be based on the manufacturer's recommendations.

In the daily operation of food extruders, the start-up and close-down of production causes difficulties. This is an important job for the operator because it is relatively easy to block the machinery. Cleaning the plasticizing unit, after it has stopped, requires patience and time. If the stop procedure is incorrect, permanent damage to the equipment can occur. On the other hand, the start-up procedure is relatively uniform. After the extruder heats up and reaches the desired temperature in individual zones of the plasticizing section, the engine begins running at one-third of the nominal rpm level. Highly moisturized material is then fed into the machine. Subsequently, the

machine performance is increased step by step, while the operator closely watches the outflow of material and the engine load. After a few minutes, the machine reaches its nominal operation parameters. The close-down must be done in the reverse order, and finally, the operator completely cleans the machine after taking down the die head, using some coarse material (e.g., oats) for the final cleaning of the plasticizing section. The machine will not restart when the working parts and the head are dirty.

Producers of extruded foodstuffs now prefer to fully control the production process. In the case of high-volume production rates, this level of control is indispensable, because only a properly programmed control system is fast enough to govern the production flow, responding immediately to failures of the working equipment. Modern food extruders can easily replace many conventional food-processing units, and these extruders are capable of helping the industry develop new series of products. The market expects new foodstuffs that are fancy in shape, taste, and raw material composition, as well as attractive from an economic point of view. As previously mentioned, extrusion cooking can texturize proteins from vegetable sources to create textures resembling those of meat, allowing for healthier (low fat), palatable, and more sustainable products. Extrusion-cooking technology can meet these expectations, but food technologists need specialized knowledge.

See also: Acrylamide; Aerated Foods; Bread: Types of Bread; Cereals: Types and Composition; Cooking: Domestic Techniques; Extrusion Cooking: Chemical and Nutritional Changes; Functional Foods; Heat Treatment: Principles and Techniques; Maize; Rheological Properties of Food Materials; Snack Foods: Role in Diet; Snack Foods: Types and Composition; Starch: Modified Starches; Starch: Sources and Processing; Starch: Structure, Property, and Determination; Wheat: Grain Structure of Wheat and Wheat-based Products.

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Famine, Hunger, and Undernourishment

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Introduction

At present, the worldwide population is estimated to be around 7 billion people. During the twentieth century, however, it is estimated that more than 70 million died due to food crises. Nowadays, at the height of the twenty-first century, the food crises (famine) continue as a global humanitarian tragedy. These crises are recognized and are mainly located in zones of the African continent, such as in the Horn of Africa where an endemic form persists. This does not mean, however, that other regions of the world are free from the risk of food crises. Generally, the risk persists primarily in developing countries with deficient economic transformations combined with problems of political instability, natural disasters, wars, and/or social conflicts. Moreover, these crises persist in concrete zones that are poorly integrated into worldwide trade, as well as in urban and rural areas whose public services lack the capacity to attend the poorest and most vulnerable groups. However, although these determinants can be found in different parts of the world and in distinct moments, the current food crises concentrate mainly in sub-Saharan Africa and some regions of South Asia.

Hunger, be it transient or endemic, is a historical reality whose incidence continues being decisive at the present time. Although the statistics on malnutrition and food insecurity have greatly improved in the last decade, the most recent reports of the Food and Agriculture Organization of the United Nations (FAO) on the state of food insecurity in the world indicate that much remains to be done. Hunger has fatal repercussions in the life of millions of people; as part of the process, it deprives its victims of health, causes massive migrations to other countries as people seek sustenance, and aggravates and/or causes illnesses such as malnutrition, AIDS, tuberculosis, and malaria. In addition, it is both a source and a consequence of instability, poverty, and underdevelopment as attested to by the fact that the majority of armed internal conflicts happen in countries with precarious food conditions.

One must ponder: Why, if it was possible to end famine in hunger-prone countries such as Russia, China, India, and Bangladesh during the twentieth century, due to the technical advances in food production, do people still to continue dying of hunger? Surely, this is because hunger is a phenomenon much more complex than it seems on its face. In any case, it would be an error to think that death from starvation or famine is homogenous; in fact, the causes and consequences of food insecurity greatly differ between countries and even between different regions within the same country. As such, a general theory explaining the periods of hunger and famine cannot exist, and thus, each one of these periods should be treated in a unique manner.

Definitions for the Discussion of Famine

To better understand the context of famine, it is necessary to clarify some concepts associated to the term. Distinctions need to be made among hunger, malnutrition, famine, and food insecurity.

Hunger

Hunger is defined here as a condition resulting from an individual's inability to eat sufficient food to lead a healthy and active life. It compromises a series of feelings, emotions, and behavioral changes, not easily measurable in themselves that result from disruption in an individual's access to food. Naturally, hunger is a recurring feature of food insecurity of households and their members in developing countries.

Malnutrition

Undernutrition, by contrast, is defined as the measureable nutrient deficiencies in a diet that can lead to illness (lack of energy, retardation, and blindness) or death. The symptoms themselves may not be recognized as indications of nutrient deficiencies since interactions between undernutrition, care behavior, and diseases are complex.

Famine

Famine is a widespread and extreme hunger that for individuals results in a drastic loss of body weight and an increase in morbidity. At the community level (as an interaction of these two symptoms), a rise in the death rate and massive social dysfunction and dislocation result. Famine is a process of socioeconomic crisis, relatively prolonged over time, and consists in the progressive impoverishment of the most vulnerable groups

and the deterioration of their systems of sustenance, with an increase in massive hunger. The process also implies population movements, the propagation of epidemics, community degradation, and, in severe cases, an increase in mortality that results more from related epidemics than to starvation, itself. Therefore, food crises (famine) are a catastrophic split of social, economic, and institutional systems that provide food for production, feeding, and consumption. For many years, these crises have been considered as anomalies, crises that could be resolved in a brief period of time and for which the previous situation existing before the crisis could be reestablished. Nowadays, this vision of a transient stage has been dispelled, as it is known that food crises (famine) can destroy not only lives but also future development of the community/region.

Food Insecurity

Food insecurity is a situation that exists when people lack secure access to sufficient amounts of safe and nutritious food for normal growth and development and an active and healthy life. It may be caused by the unavailability of food, insufficient purchasing power, inappropriate distribution, or inadequate use of food at the household level. Food insecurity, poor conditions of health and sanitation, and inappropriate care and feeding practices are the major causes of poor nutritional status. Food insecurity may be chronic, seasonal, or transitory.

Famine Mortality

The most serious manifestation of starvation is increased mortality, although food crisis does not occur with the same intensity or in the same way in all cases. Mortality data associated with food crises also reveal that they do not affect all people equally. From a biological and socioeconomic focus, the highest mortality rates are seen in vulnerable groups. First affected are children under five and the elderly living in poor conditions and in remote rural areas far from the cities. However, data recorded in the great famines indicate a complex interaction between biological vulnerability and socioeconomic vulnerability. The latter is closely associated with the cultural, social, and economic contexts of each region or country.

The death toll in the severe famines is only approximate estimates. The reason for not being able to set an exact number of deaths is mainly due to the lack of official records of births and deaths in some developing countries as well as basic demographics of the population (population census and mortality rates during nonfamine years). On the one hand, excess mortality rates are estimated for population groups monitored by the national population census, and the rates are compared to the 'normal' population before the famine. On the other hand, mortality is a parameter that does not assess the total demographic impact of famine, as the falling birth rate may have consequences on population size. This increased mortality in famines is due to two main factors. First, there is the effect of starvation that brings about weight loss and the subsequent undernutrition that puts individuals at increased risk for diseases. Second, as a result of undernutrition, there is increased susceptibility to infectious diseases as a result of weakened immune systems. Furthermore, there is increased exposure to epidemics unrelated to food (e.g., typhoid, cholera, measles, and malaria) due to the lack of sanitation and hygienic conditions caused by displacement and overcrowding in unsanitary settings and the lack of potable water. Both circumstances imply that refugee camps are locations that generally register the highest mortality rates.

Although the overall data indicate that famines throughout history have produced a high number of deaths, in relation to the national population of the countries where they occur, this has rarely exceeded 2–3% of the total population. During twentieth century, there were between 70 and 80 million deaths. From the recorded data, a pattern of geographic and seasonal appearance can be established. It seems that over the decades, famines have been happening in the northern hemisphere (Russia) and Asia (China, India, and Bangladesh), moving toward sub-Saharan Africa, where even today, it remains endemic. In addition, this geographic shift has been associated with a decrease in mortality. During the pre-1960 period, the nine famines that occurred in Russia, China, and India led to a total of 66 million deaths. However, the 16 famines that have occurred since the 1960s in Africa have caused 10 million deaths. This reduction is due to several advances made during the twentieth century. In the first half of this period during the colonial administration, vulnerability to famine in Asia, and to a lesser extent in Africa, decreased because of improved communications and transportation. Markets that were previously isolated were linked with larger markets, and warning systems were established and policies implemented to help those affected by famine. In some colonized countries, decolonization brought disparate effects. On the one hand, in countries like India, decolonization produced greater government involvement in the eradication of hunger through policies of job creation or the delivery of humanitarian aid, both of which led to excellent results. On the other hand, in many African countries, decolonization was associated with the *de novo* appearance of famines, induced by increases in political and economic instability and civil wars. Moreover, in many cases, drought and the lack of water for crops worsened the problems of hunger in many of these countries. Thus, during the 1980s and 1990s, some countries that had never been prone to famine such as Sierra Leone, Zaire, Angola, and Mozambique and others that had previously suffered famine such as Sudan and Ethiopia suffered major famines due to armed conflict and/or drought. Today, in the Horn of Africa, the equation *drought + war = famine* is still very much in vogue.

Current State of Global Food Insecurity

FAO's latest estimates indicate that reducing global hunger continues on the right track. It is estimated that approximately 805 million people were chronically undernourished during 2012–14. These figures represent a decline of more than 100 million in the last decade and of about 209 million over the period 1990–92. However, one in nine people worldwide still lacks sufficient food to lead healthy and active lives. The vast majority of malnourished people live in developing countries (791 million people), although it is in these same countries where most of the improvements in the last two decades have occurred (FAO). The decrease in the proportion of hungry people has been approximately 7.4 percentage points from the period 1990–92 to 2012–14, which has reduced the proportion of people affected by hunger from 18.7% to 11.3% (Figure 1).

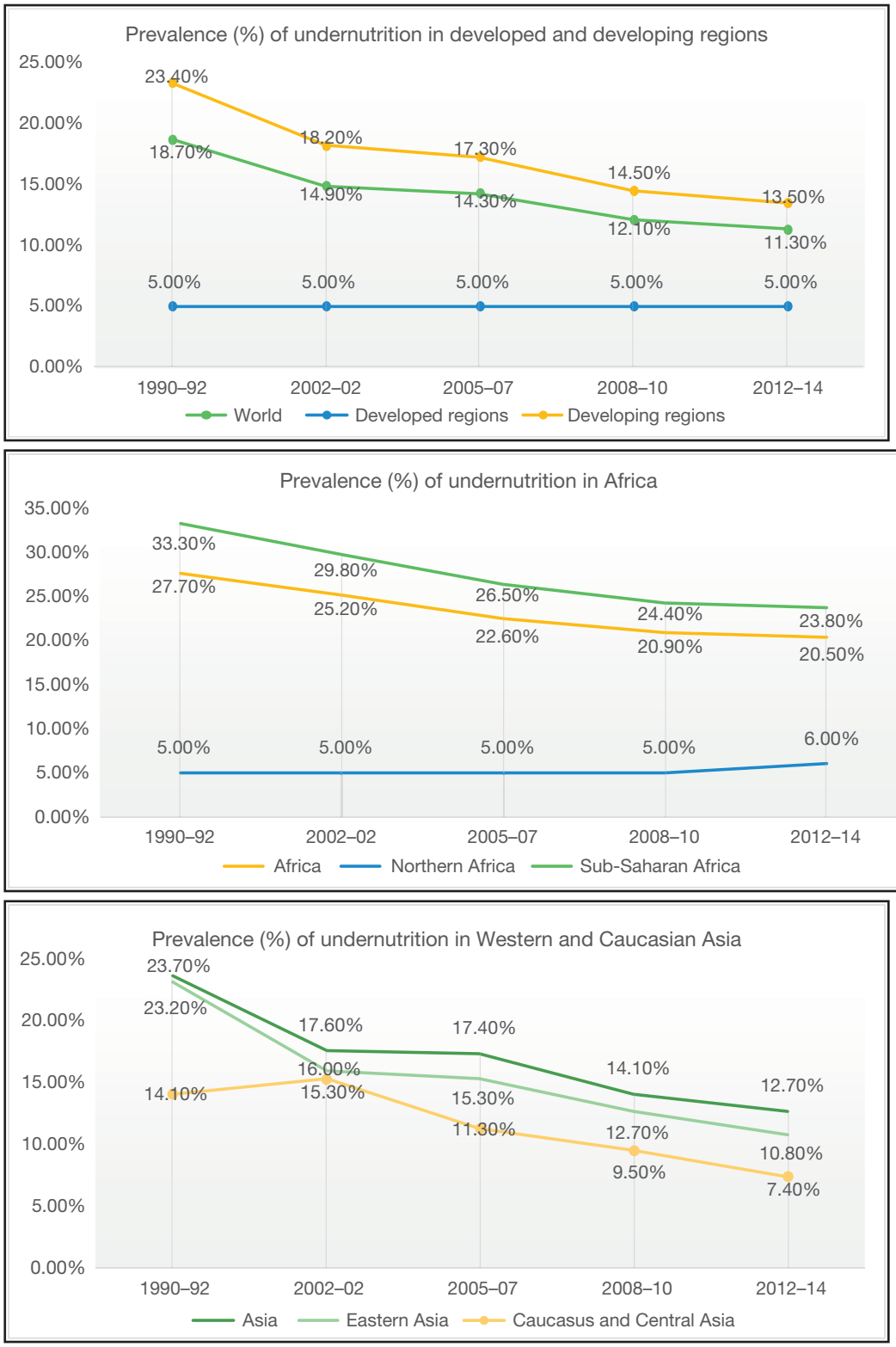

Figure 1 Trends in undernutrition prevalence rates.

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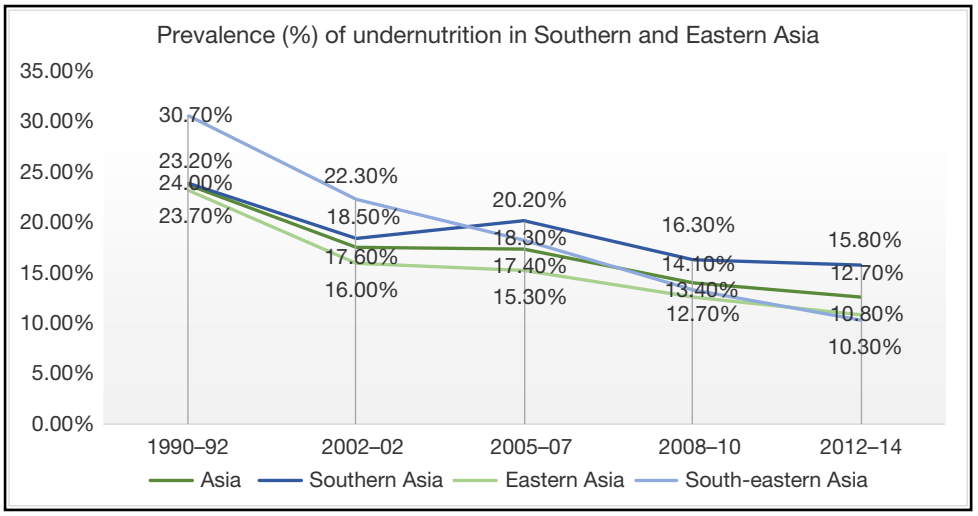

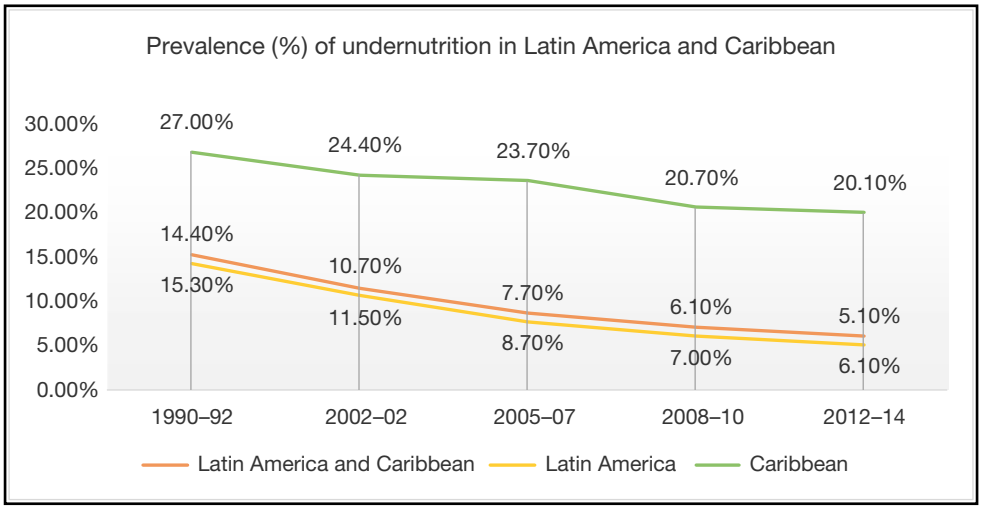

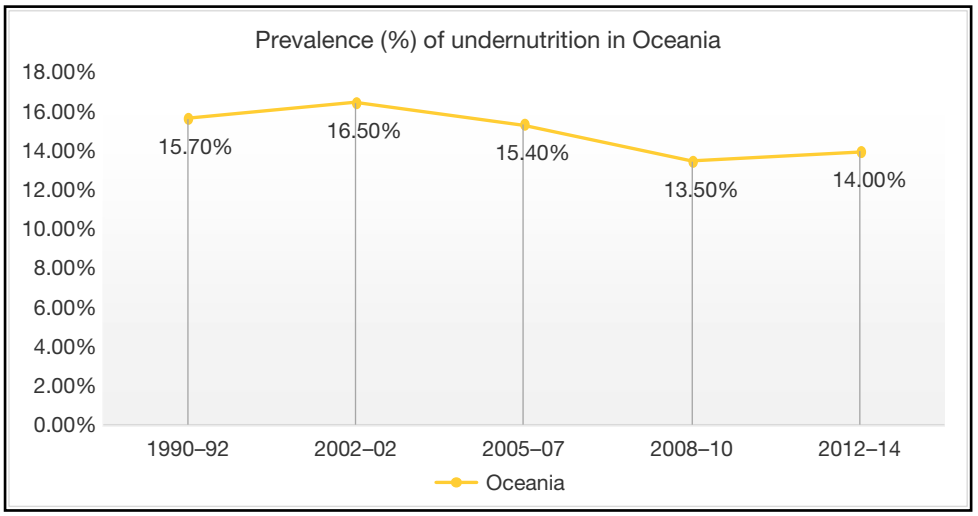

Figure 1—Cont'd

This decline has been much more significant in developing countries, where for the same period, the decrease was almost 10 percentage points (from 23.4% in 1990–92 to 13.5% in the period 2012–14). Therefore, it seems that the hunger goal stated in the Millennium Development Goals (MDGs) for 2015, "reduce by half the proportion of undernourished people" is within reach, but not the objective of the World Food Summit (WFS) of "halving the number of chronically underfed people." According to estimates, it appears that by 2015, the proportion of undernourished people would be 12.8%, a rate 1.1 percentage points higher than that expected by the MDG (11.7%). However, according to experts, increasing efforts in the most affected areas (sub-Saharan Africa and South and West Asia) would make it possible to accelerate the pace of reducing hunger. Nevertheless, the estimate of people affected by hunger in 2015 is around 791 million, a far cry from the 500 million that constituted the goal of 1996 World Food Summit.

Africa

In Africa, there are a total of 226.7 million undernourished people (20.5% of undernourished people worldwide). The data show that progress in reducing hunger in Africa, mainly sub-Saharan area, has not been sufficient. Still, there have been significant improvements from the period 1990–92 when the prevalence of undernutrition in sub-Saharan area was 33.3% and the prevalence for the period 2012–14 was 23.8%. In total, it is estimated that in the period 2012–14, the number of undernourished people in sub-Saharan area was 214.1 million people. The lack of political stability and wars are the major causes for less progress in sub-Saharan Africa compared to other at-risk regions. A different situation is observed in North Africa, where the prevalence of undernutrition was observed to be less than 5% in all periods since 1990–92; during the period 2012–14, there was an increase in undernutrition reaching 12.6%, for a total of 12.4 million undernourished people. This increase rate can be attributed mainly to the inclusion of Sudan into the region of North Africa.

Asia

Globally, Asia has a total of 525.6 million undernourished people, representing 65% of the world's undernourished population. Although globally, Asia is close to meeting the Millennium Development Goals on hunger, it is the area with the highest numbers of undernourished individuals, and there are significant differences between the regions that make up this area. The Caucasus and Central Asia, East Asia, and Southeast Asia are the regions where most progress has been observed over the last 20 years. The decreases in undernutrition rates have been over 50% for West Asia (from 23.2% in the period 1990–92 to 10.8% in 2012–14). In Caucasus and Central Asia, the decreases had been from 14.1% in 1990–92 to 7.1% in 2012–14, and a 66.5% decrease was observed in the case of Southeast Asia (30.7% in 1990–92 and 10.3% in 2012–14). Still and all, it is worthy to note that in the West Asia region, there is a total of 161.2 million undernourished people.

Moreover, the worst scenarios are observed in West and South Asia. In the region of South Asia, although progress since 1990–92 has been seen, undernutrition rates of 15.8% (with a total of 276.4 million people having food access problems) are still far from the Millennium Development Goals. The situation is worse in West Asia where, albeit undernutrition rates are not as alarming, there is much concern about the developments in recent periods where rates have gone from 6.3% in 1990–92 to 8.7% in 2012–14. This increased prevalence of undernutrition is also reflected in the number of undernourished people, increasing from 8 million in 1990–92 to 18.5 million in 2012–14, showing an increase of more than 10 million undernourished individuals. Deterioration of the economies and high political instability are the main causes of the increase in rates in recent years in the West Asia region.

Latin America and the Caribbean

The Caribbean and especially Latin America presented the most progress of all the developing regions in relation to increased food security. This region has already met the Millennium Development Goals and is close to achieving the target set by the WFS. In total, the region has in 2012–14 an undernutrition rate of 6.1% (a total of 37 million undernourished people), a decrease from the 68.5 million in 1990–92. Latin America's progress in improving food security has been faster than in the Caribbean. In Latin America, the prevalence of food insecurity for 2012–14 is 5.1%; in the last 20 years, the total number of undernourished people has decreased from 60.3 million in 1990–92 to 29.5 million in 2012–14. In the Caribbean, this reduction has been slower, declining from 27% in 1990–92 to 20.1% in 2012–14.

Oceania

Oceania is the region that has the least number of undernourished people in the world (1.4 million representing 0.17% of the world's undernourished population). The prevalence of undernutrition has varied little since 1990–92, showing only a 1.7% decrease. The main concern in the area is that the rates of undernutrition have been accompanied by a greater burden of overweight and obesity. As such, the region suffers from a considerable double burden of malnutrition.

Theories of Hunger

Hunger as a collective phenomenon has always existed, which throughout history has involved different regions of the planet and during given periods of time. It is a result of various factors: poor harvests, wars, economic crises, and natural disasters. The hunger of our time has the peculiarity that it is concentrated in certain regions and countries; it now can be considered as chronic and endemic, that is, not only due to natural disasters or wars. For this reason, it is important to study the theoretical frameworks that have been developed to explain the worldwide phenomenon. In recent decades, several different explanatory paradigms have emerged, attempting to explain the causes of famines. These can be grouped into five distinct models:

Model 1. Demographic Theories (Malthusian and Neo-Malthusian Theories)

The relationship between hunger, production and food availability, and the population is the core of the reductionist

Box 1 Criticisms of the Malthusian Theory

(a) Malthus wrote thinking of the English agricultural situation in the eighteenth century when it was based on subsistence agriculture.
(b) Malthus failed to foresee the agricultural, transport, industrial revolution, and demographic transition.
(c) Geographically marginal land is not necessarily less fertile, even if it is the furthest away.
(d) Famine did not act as a 'Malthusian leveler.'

theories of Malthus and the neo-Malthusians. The focus of these theories was based on the fundamental consideration that the amount of food production (production or food availability) and the number of people (population) are the only two variables that determine the global food situation and therefore the existence – or not – of hunger in the world. From the relationship between both elements, the acceptance of hunger as a fact that has to happen consistently was derived, since the number of people grows geometrically, whereas the amount of available food grows arithmetically. In this formula, Malthus affirmed that in order to avoid hunger, a decrease in the growth rates of the population was essential so as to increase food availability. Currently, there are some authors who keep the Malthusian theory alive, and they conceive hunger as a result of the competitive relationship between the two variables mentioned earlier: food production and population growth and poverty. In other words, according to these principles, the causes of hunger are the lack of food and high rates of population growth. Based on these theories, the balance between food availability and population would be restored through famines, epidemics, mass migration, and reduced fertility. These theories were popular in the 1960s and 1970s when the perception that the world was running out of food resources was on the rise. Today, Malthusian theories have evolved with new arguments that differ from the original theory. New proposals differ from the original Malthusian argument because the constraint on population growth is represented not only by arable land and food scarcity but also by the availability of water, energy supply, and raw materials, as well as by land and air pollution. From this point of view, the neo-Malthusian approach is strongly supported by increasing the awareness of the need to protect the environment and its resources. The Malthusian approach is susceptible to four criticisms outlined in Box 1.

Despite these critiques, these theories could still be considered quite current as they focus on the safety and availability of natural resources and on nonpolluted air and water, thus emphasizing the environmental and social constraints of our planet.

Model 2. Climatic Theories (Based on the Decrease in the Availability of Food or Food Availability Decline)

The food availability decline (FAD) approach assumes that famines are caused by a sudden reduction of per capita food supply. It is usually triggered by natural disasters (drought, floods, pest infestation, etc.), wars, and epidemics that lead to a contraction of the food supply. As a consequence, food prices go up and people, who are not able to bear such an increase, consume less calories and nutrients. Consequently, the most vulnerable individuals start reducing their food consumption because of rising prices. In the case of prolonged exposition to a crisis, this may culminate in increased mortality due to starvation and infectious diseases. This model points out the insufficient production and availability of food as the main causes of famines and starvation. This approach implicitly assumes an equal division of the available food, but unfortunately, such an assumption fails to reflect reality. The FAD hypothesis implies that food security is essentially a matter of expanding food availability. As a solution, it argues to increase the supply and consequently the availability of food. This solution has been strongly criticized by Amartya Sen. However, he recognizes that famines can be caused by food availability decline and he also assumes this hypothesis for its entitlement approach.

Model 3. Food Intervention Decline

This new framework, the so-called food intervention decline (FID), originates from the recent and growing awareness that governments, and more generally political institutions, humanitarian agencies, and nongovernmental organizations (NGOs), have the responsibility to protect all citizens by promoting direct public interventions. This model approach argues that people starve because food policies and services fail to guarantee a sufficient level of nutrition. When these policies and services decline or disappear, persons start suffering and severe undernutrition may occur and consequently famine appears. This approach identifies as main actors all those institutions that are responsible for the production and implementation of measures to secure nutrition and healthy feeding.

The FID approach suggests a number of different policies that may be broadly grouped into two categories, depending on their target. One constitutes the policies established to prevent undernutrition in at-risk zones. Examples are improving the conditions of public health and the warning systems to prevent the crisis, reducing social inequalities and social cohesion, applying policies of price protection for basic foods, and fortifying the systems of education, health, and social services. The other category includes policies establishing fast-responding systems to deal with the appearance of food crises. Examples consist of introducing free or price-reduced food consignments into markets, establishing policies to control prices and subsidize the food supply, creating food reserves (i.e., cereals), carrying out direct interventions, and improving communication and transport infrastructures.

All these interventions should be implemented in a planned and carefully thought-out manner in accordance with the nature of the problems and the underlying causes of the famine. One of the main risks is to damage economies with abundant food supplies, further depressing food prices affecting agricultural incomes; the outcome could be further reducing the disadvantaged population's purchasing power.

Model 4. Sen's Food Entitlement Approach

As a response, mainly to the theories of Malthus and the neo-Malthusians, some authors elaborated theories that determined that famines and hunger were not produced by a lack of food in the market, but rather by the poverty of given social sectors that deprived them of food access. These criticisms gave

place to a new approach, known as food entitlement, proposed by the Indian economist Amartya Sen in his famous 1981 work *Poverty and Famines*. This was decisive for the formulation of the concept of household food security, which focused more on families than on countries (the concept of national food security was changed to household food security). The entitlements are the capacities that a family has to access food by legal means, be it by production, purchase, or receiving food in the form of donations from the state or the community. Sen demonstrated in diverse studies that famine was not frequently due to a lack of supplies, but rather to a sudden loss of entitlements in poor families, until reaching a point that food requirements could not be met. He understood famine as a temporary convulsion of the economic system. According to this theory, the political answer to famines resided in the struggle against the poverty. One of the advantages of Sen's theory on famine is that it is not a simple theory, but a model that is generalizable and can explain different models of famine. In some way, Sen's model assumes as its own the models of FAD and of FID. In the first case, it understands that the descent in food availability can increase the price of foods and, as such, leads to impaired food entitlements. FAD's role is assumed to be one more factor out of many that are related to entitlements. In the same way, when Sen's model suggests to adopt measures to improve food purchases and food assistance, it assumes the hypotheses of the food intervention decline (FID) model.

The fundamental criticism of Sen's theory is that it assumes that famine is like a sudden collapse in food consumption that causes a particularly virulent form of starvation and involves widespread death. That is to say, it would be considered as a onetime and sporadic event, which is distinct from hunger and whose appearance is characterized by an increase in mortality due to starvation. In contrast, authors like Amrita Rangasami and Alex de Waal have shown that famine is not so different from hunger, but rather is a process that begins with hunger as its starting point and, in the most severe cases, can lead to massive death. In fact, the majority of famines nowadays are characterized by a not so extreme starvation, thanks to the palliative mechanisms of the family's distinct coping strategies. At present, it is not the increase in mortality that is the most worrisome factor, but rather the misfortune and social perturbation that is caused. As such, the victims perceive hunger not so much as a life-threatening biological process but rather as a socioeconomic process of impoverishment that threatens their systems of livelihood. Therefore, it understands that Sen's definition of famine only refers to the last phase of the famine and not to the entire process. In fact, authors like Purusottam Nayak criticize Sen for limiting his explanation to the immediate cause of famine, such as the loss of entitlements, but not including background causes or dynamics once the process is initiated. That is to say, Sen explains how famines are produced but does not explain why or what causes them to happen.

Another critical vision of his theory addresses the excessively economic vision of the problem, which centers only on the loss food access and on subsequent starvation, but omits other essential factors of famines like the impact of epidemics on increased mortality, violence, wars, and population movements or social destruction. Sen also contemplated famine victims as passive subjects, ignoring their own capacities for implementing coping strategies to mitigate the effects of famine. Sen himself indicated that his theory had some limitations, which could be summarized in two aspects: on the one hand, a failure to recognize individuals as socially embedded members of households, communities, and states and, on the other hand, a failure to recognize that famines are political crises as much as they are economic shocks or natural disasters.

Sen's theory, when centering on circumstantial economic appearances, specifically on the relationship between markets and people, tends to ignore the structural, historical, and political factors. Therefore, his theory is soundly criticized for limiting itself to measures of a technical nature, suggesting solutions such as providing employment. Thus, resources are generated to help those that have temporarily lost their entitlements or both, but it does not contemplate structural transformations that would eliminate the underlying structural vulnerability.

Model 5. Complex Emergencies (Humanitarian and Political Emergencies)

From the mid-1980s, certain authors posed a distinct vision of the etiology of famines, based on political causes instead of economic or demographic determinants. Authors such as Alex de Waal, David Keen, and Mark Duffield are the maximum exponents of this explanatory political model of famines. For these authors, the victims of the famine are defined not by a lack of economic but rather political power. Famines are seen as political phenomena derived from the lack of government initiatives and even of policy practices causing famines, such as ethnic persecution, forced population displacement, and denying humanitarian aid.

Famine is a result of the failure of a 'political contract' between the country's leaders and the population. This agreement would impose obligations on the politicians, certain obligations to guarantee the right to food. When this tacit agreement exists, the apparition of famine can generate grave political consequences, even including the downfall of government or sanctions for its leaders; and as such, there is keen interest in impeding the appearance of famines. In those countries that do not guarantee the civil rights of their citizens, famines do not concern governing leaders, and therefore, the fight against hunger is not a priority. This phenomenon has mainly been observed in countries with authoritarian regimes like Russia, China, or Ethiopia in the decades of the widespread famines or in civil wars where such a political agreement does not exist, as seen in distinct countries of the Horn of Africa. Besides, these authors indicate that in any famine, there are some sectors that become impoverished, but there are also those that become wealthy, such as traders, landowners of big agricultural farms, and soldiers taking advantage of the hunger-weakened poor. Therefore, the analysis of famines should also be done from the perspective of those that exert political, economic, or military pressure, to benefit from the transfer of resources.

When famine appears as a result of military conflicts, it may be the case where hunger in this context is being used as a weapon of war. Thus, war becomes the main cause of famines, as it destroys crops and food production systems, generates massive migrations, paralyzes health services, prevents or hinders possible family coping strategies, and prevents the distribution of humanitarian aid. From this perspective, famine is

not so much the unavoidable consequence of a crisis, but rather a deliberate aim caused and sustained by determined social powers for taking ownership of resources from the most vulnerable. This allows for the clear definition of political responsibilities in famines and goes beyond the mere economic factors proposed by Sen or the demographic changes defended by Malthus. This new approach understands that the famines are unleashed by a lack of political responsibility on the part of governments and for their noncompliance with fundamental human rights, especially the right to food.

Synthesis of the Famine Models

In short, none of these approaches are sufficient in and of themselves to explain the nature of famines. On the one hand, the Malthusian and neo-Malthusian explanations have lost strength due to the fact that their suppositions are no longer valid, although it is true that the concern for the environment and resources of the planet is in the center of current political debate.

On the other hand, the economic approach is for the time being what dominates among the authors. The vision of famine is seen as a form of economic disaster heightened by some natural catastrophe (drought, floods, etc.) and facilitated by the lack of policies to protect markets, prevent hunger, and intervene in states of emergency. By not considering the political aspects in relation to wars or the related violence of armed conflicts and by not respecting human rights, the vision of famine is reduced to a technical problem that requires technical measures for improving the efficiency of food systems.

Finally, the political approach goes beyond mere economic circumstances and deals with famine as a political problem of not respecting fundamental human rights, of power conflicts and wars, of repressing designated population groups, and of the passivity of the international community in confronting the issues. In these cases, the solution demands interventions not only that are of an economic nature and addressing productive market shares but also that focus on the international level to respect human rights.

Conclusions

Nowadays, already well into the twenty-first century, many people from different parts of the world continue suffering from famine. The famine seen today is different from the widespread famines of the past, as political, economic, and social contexts have changed. Although the triggers and effects are similar, the causes are much more extensive and much more complex. Famine appears as a complex phenomenon of factors, distinct in nature: economic, political, environmental, and social. This multiplicity of factors confirms the high complexity of the problem and therefore also hampers the election of the best responses in each case. Therefore, the policies of prevention and action against famine should take on a holistic approach and consideration for all these aspects. For this reason, although nowadays, famines are more predictable (as they are processes that occur over months or even years, this allows for early detection) and preventable (since they are a result of economy or policies and, as such, are a product of human factors that can be corrected), they are much more difficult to resolve or counteract simply by means of developing structural policies that guarantee the disappearance of hunger.

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Fat Replacer

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Introduction

Although many nutrition recommendations remain controversial, there is consensus among health and nutrition professionals in the developed world that the ratio of saturated fatty acids to poly- and monounsaturated fatty acids in the diet should be decreased, and intake of dietary cholesterol should be limited. It is further agreed that consumption of calories from fat should not be more than 30% of the daily total and that consumption of cholesterol should be restricted to <300 mg day^{-1}. It is suggested that those with elevated Low-density lipoprotein (LDL) cholesterol levels or cardiovascular disease should restrict saturated fats to 7% of calories. Prominence is also given to increasing the intake of fruits, vegetables, and grains and modifying the type and amount of fat consumed. The emphasis is on achieving an overall healthful pattern of eating rather than on limiting the focus to merely achieving goals for macronutrient composition.

In the food supply, 30% of the total fat comes from meat, poultry, and fish, and 25% comes from baked goods and other processed grain products. Milk products and fats/oils account for 18% and 11% of the total fat, respectively. Collectively, these food categories account for 84% of the fat in the food supply. Although the type of fat in these contributors to fat intake varies, the effort to reduce total fat and calorie intake has focused largely on reducing the fat content of foods in these categories. The effects of fat on cardiovascular disease are so well documented that they have been recognized as major factors in the etiology of cardiovascular disease. Dietary treatment to prevent obesity and high blood cholesterol levels have become the cornerstone of the National Cholesterol Education Program, which has been designed to help prevent coronary heart disease. Advocates of this program are boosted by reports that coronary heart disease rates can be decreased by dietary interventions and by a recent report that changes in lifestyle, including diet, can bring about regression of severe coronary atherosclerosis after a year without the use of lipid-lowering drugs. Obesity is strongly associated with the development and severity of both diabetes and hypertension, both of which are major risk factors for cardiovascular disease. Due to this, the consumer demand for low-fat foods has encouraged research on reducing the fat content of foods. Problems of inferior organoleptic and physical properties in these products suggested the use of fat replacers (FRs) to provide the desirable qualities.

Definition of FR

FR is defined as a carbohydrate-, protein-, or fat-based compound that replaces one or more of the functions of fat to reduce calories in foods. A broader scheme of FR is presented in Table 1.

FR can be classified as synthetic fat substitutes (FS), emulsifiers or surface active agent, starch derivatives, maltodextrins, hemicelluloses, β-glucans, bulking agents, microparticulates, composites, and functional blends. FR can be further categorized into the following groups:

FS: a synthetic compound designed to replace fat on a weight-by-weight basis, usually having a similar chemical structure to fat but resistant to hydrolysis against digestive enzymes.
Fat mimetics (FMs): a substance that can imitate physical or organoleptic properties. FMs are also referred to as 'texturizing agents.' It requires a high water content to achieve its functionality.
Fat analogs: are compounds with many of the characteristics of fat but have an altered digestibility and altered nutritional value.
Fat extenders: optimize the functionality of fat, thus allowing a decrease in the usual amount of fat in the product.

Some of the applications of FR along with their usage levels are given in Table 2.

Carbohydrate-Based FRs

Carbohydrate-based FMs incorporate water into a gel-type structure, resulting in lubrication and flow properties similar to that of fat. Carbohydrate-based FRs are categorized into starch-derived, cellulose-based, fiber-based, gum-based, and others.

Starch-derived FRs

Native starch is widely used in food and industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent, and water-retention agent. Starches from different plant sources such as wheat, maize, rice, and potato have received extensive attention.

Resistant starch

Commercial resistant starches contain about 20–60% total dietary fiber. Resistant starch is insoluble, having a low water-holding capacity (WHC) and is most suitable for cereal-grain-based products with lower levels of moisture content. Resistant starch is generally used as an FR in baked goods, extruded snacks, pasta, breakfast cereals, and beverages.

Maltodextrin

Maltodextrin is a nonsweet, nutritive saccharide polymer, with a dextrose equivalent (DE) of <20. Starches from botanical sources like corn, potato, wheat, rice, tapioca, sago palm, barley, and sorghum can be used as raw material. Maltodextrins

Table 1 Classification of fat replacers by nutrient source, functional properties, and use in food

Type of fat substitute	*Nutrient source (energy density)*	*Functional properties*	*Use in food*
Derived from carbohydrate			
Polydextrose	Water-soluble polymer of dextrose (1 cal g^{-1})	Bulking and retaining moisture	A wide range of foods, including baked goods, confections, and frozen desserts
Modified food starch	A variety of starch sources (1–4 cal g^{-1})	Modifying texture, gelling, thickening, and stabilizing	Processed meats, salad dressing, baked goods, frozen desserts, etc.
Dextrin and maltodextrins	A variety of starch sources (4 cal g^{-1})	Modifying texture and bulking	Baked goods, dairy products, salad dressing, sauces, and spreads
Gums and pectin	Xanthan, guar, locust bean, carrageenan, alginates, and fruit	Retaining moisture, mouthfeel and modifying texture	Wide range of products, including baked goods, sauces, and salad dressings
Cellulose	Various plant sources (virtually noncaloric)	Modifying mouthfeel, texture, and pourability	Dairy products
β-Glucan	Soluble fiber extracted from oats (1–4 cal g^{-1})	Adding body and texture	Baked goods and a variety of other food products
Derived from protein			
Microparticulated protein and modified whey	Denatured or microparticulated protein from egg/milk (1–4 cal g^{-1})	Modifying mouthfeel	Dairy products, spreads, and bakery products
Derived from fat			
Olestra	Sucrose polyester with triglycerides (noncaloric)	Modifying texture and mouthfeel	Savory snacks (stable for fried foods)
Caprenin and salatrim	Caprylic, capric, behenic acid, and glycerine, or triglyceride of short- and long-chain fatty acids (5 cal g^{-1})	Simulating properties of cocoa butter	Confections, baked goods, and dairy foods
Mono- or diglycerides	Derived from vegetable oil and emulsified with water (9 cal g^{-1})	Adding moisture and modifying texture and mouthfeel	Baked goods, vegetable dairy replacers

Source: Judith, W. R. (2002). Fat substitutes and health an advisory from the Nutrition Committee of the American Heart Association. http://dx.doi.org/10.1161/01.CIR.0000019402.35632.EB.

Table 2 Fat replacers and their food applications

Type of fat replacer	*Usage level (%)*	*Food application*
Modified starch (potato)	21	Bakery dough
Maltodextrin	40–45	Chocolate
Maltrin M040	20	Low-calorie pudding
Maltrin M100	10–28	Butter-like product
Polydextose	20	Peanut butter
HPMC	13	White chocolate bar
Pectin	0.18	Nonfat frozen dessert
Pectin (Slendid) and maltodextrin	0.5–3 + 0–10	Fruit spread
Microcrystalline cellulose + pectin	0.05–0.5 + 0.1–10	Fruit spread
Microcrystalline cellulose + κ-carrageenan (93:7)	25	Yogurt and cottage cheese
Carrageenan gels	15	Frozen dessert
κ-carrageenan	10–14	No-fat coffee creamer
λ-carrageenan	0.2	Whipped toping
Carrageenan + maltodextrin	1–3 + 0–10	Fruit spread
Xanthan gum	0.18	Fat-free cookie
	0.3	Low-fat spread
Pectin + xanthan gum	0.63 + 0.28	Fat-free fruit bar
Starch, pectin, guar gum, and gelatin mixture	1.29	Low-calorie yogurt
Simplesse	2–20	Fruit spread
Sucrose fatty acid polyester (Olestra)	10	Low-calorie cheese
Ethoxylated glucoside tetraoleate	13	Cake mix
	10	Icing mix
	56.25	Mayonnaise
	50	Salad dressing
	80	Margarine
Ethoxylated glucoside tetrastearate	25	Plastic shortening
	13	Cake mix

Compiled from various sources: Roller (1996), Sharma and Ganeshkumar (1996), Akoh (1998), Sharma et al. (1998), Sandrou and Arvanitoyannis (2000), Chavan and Prajapati (2009), Tiwari (2005).

Table 3 Maltodextrin-based fat replacers

Trade name	*Type*	*Usage level (%)*	*pH stability*	*Temperature stability (°C)*	*Applications in food products*	*Manufacturer*
Paselli A2	Potato	20	3–7	25–212	Dressings, sauces, spreads, frozen desserts, toppings, cakes, and butter	Avehe American Inc., NJ, USA
Stellar, Instant-Stellar	Corn	20–25	4–6	25–200	Baked goods, frostings, fillings, gravies, sauces, and dairy products, confectionary	A. E. Staley Manufacturers, USA
Maltrin M040, M100, M150	Corn	20	3.5–7.5	300	Bakery and snack foods, beverages, confectionary, dairy products, and salad dressings	Grain Processing Corporation, USA
Amalean I, Amalean II-Instant	Corn	1–8	3–7	25–200	Cheesecake, spoonable salad dressings, icings, cheese spreads, and desserts	American Maize Product Co., USA
N-oil, Instant-N-oil	Tapioca	25	3–8	25–225	Confectionary, soup, frozen desserts, spoon/pourable sauces, gravies, and puddings	National Starch Manufacturers, USA
N-Lite LP, N-Lite B/L, N-Lite D, N-Lite CL	Maize	25–30	3–8	25–200	Salad dressings, soups sauces, bakery products, dairy products, yogurt	National Starch Manufacturers, USA
Oatrim	Oatflour	3–6	>3	Retort	Dairy products, cereal, baked goods, and meat products	Rhone-Poulenc Food Ingredients, USA
Oat-fiber White Tan (770, 780, 782)	Oat fiber	3–6	All	All	Processed meats, ice-cream, butter-coated products and deep fried foods, and chocolate	Williamson Fiber Products, County Cork, Ireland

Compiled from various sources: Roller (1996), Sharma and Ganeshkumar (1996), Akoh (1998), Sharma et al. (1998), Sandrou and Arvanitoyannis (2000), Chavan and Prajapati (2009), Tiwari (2005).

are used as an FR in dairy products, confectionary, frozen desserts, cereal baked goods, and meat products due to their ability to form soft, spreadable, thermoreversible gels with melt-in-the-mouth properties. Low-DE maltodextrin is usually added at a level of 1–5% in liquid foods to impart a full-bodied texture and mouth coating, whereas it can substitute about 25–35% of fat in cookies. Starches are usually hydrolyzed to a DE's value from 0 to 100, and as the DE increases, browning reaction, freezing point depression, hygroscopitity, sweetness, solubility, and osmality increase, whereas viscosity, cohesiveness, film-forming ability, and ability to prevent large crystal formation decrease. Maltodextrin with low DE has similar properties to those of native starch and can be used as an FR (Table 3).

Maltodextrins based on potato are low DE ($\leq$5), are cold soluble, and provide low viscosity in the form of solution, but at high concentration (>20% w/w) they are unstable and tend to gels, which are plastic, and spreadable, which provides shortening-like texture. Potato maltodextrin is produced by solubilizing the slurry by jet cooking and treating it with enzymes until the required degree of hydrolysis is achieved (Paselli SA2, C* Pur 01906). Potato starch contains amylose molecules with longer chains as compared to corn and wheat, which retrograde less readily, thereby reducing the tendency to cause turbidity and an undesirable texture in foods. Paselli SA2 is applied in products such as low-fat mayonnaise, sauces, imitation cheese, and frozen desserts, whereas C* Pur 01906, is used in baked goods. Preprepared gels consisting of one part maltodextrin and three parts water provide 1 kcal g^{-1}.

Corn maltodextrin is obtained from limited hydrolysis of cornstarch. Maltrin M040, a product of Grain Processing Corp., Muscatine, Iowa, is a spray-dried corn maltodextrin (DE=5) and is able to partially or totally replace fat in margarine, frozen desserts, salad dressings, and extruded cereals and snacks. Tapioca maltodextrin is obtained by heating tapioca starch in the presence of hydrochloric acid, which yields a gel and is characterized by a bland flavor, smooth mouthfeel, and a texture similar to that of hydrogenated fat, for example, N-oil, N-oil II. N-oil is used as a 20–35% aqueous solution, contributes only 1 kcal g^{-1}, and is used in products like salad dressings, puddings, and margarine.

Oat maltodextrin is obtained by partial enzymatic hydrolysis of oat flour, oat fiber, or oat-bran. Oat maltodextrin (Otrim-1, Otrim-5, Otrim-10) gels are useful for fat sparing in food and food products like yogurt, baked products, and meat products. Oatrim-5, invented and patented by the USDA at the National Center for Agricultural Research, is the only carbohydrate-based FS and contains the unique combination of β-glucan and low-DE maltodextrin. Wheat-based maltodextrin is produced by microfibrillation of wheat fibers and maltodextrin, for example, Vitacel, produced by Rettenmaier, is composed of 70% wheat fibers and 30% maltodextrin. Vitacel can be used instead of hydrocolloids as a thickening and binding agent for stabilization of emulsions, foams, and liquid media.

Disadvantages associated with the usage of maltodextrins is that the amylopectin present in it has a tendency to retrograde slowly, giving rise to a phenomena of setback in low-fat spoonable salad dressings. Low-DE maltodextrins also suffer from freeze–thaw stability and unreliable heat and acid stability. Energy output of all the maltodextrin-based FMs is about 4 kcal g^{-1}.

Polydextrose

Polydextrose was invented by Hans Rennhard at Pfizer Central Research Laboratories, United States, in the mid-1970s.

Table 4 Microcrystalline cellulose and polydextrose-based fat replacers

Trade name	*Type*	*Usage level (%)*	*pH stability*	*Applications in food products*	*Manufacturer*
Avicel RC/CL	Cellulose gel	2–5.0	>3.5	Salad dressings, dips, spreads, bakery, products, dairy products, meat products	FMC Co., PA, USA
Tabulose	Powdered MCC	0.5–20	–	Mayonnaise, salad dressings, sauces, dietary products, bakery products, imitation cheese products	Blanver Farmoquimica Ltd., USA
Novagel RCN-10; RCN-15	MCC + Guar gum	0.5–5.0	–	Bakery and snack foods, beverages, confectionary, dairy products, salad dressings	FMC Corporation, PA, USA
Polydextrose, Litesse, Litesse II	Polymer of glucose with sorbitol and citric acid (89:10:1)	5%	5–6	Pastry, confectionary products, dressings, spreads, bakery fillings, toppings, chilled desserts	Pfizer Inc., New York, USA
Sta-Lite	Polydextrose (fast dissolving)	–	–	Confectionary and bakery products	A. E. Staley Manufacturing Company, IL, USA

Compiled from various sources: Roller (1996), Sharma and Ganeshkumar (1996), Akoh (1998), Sharma et al. (1998), Sandrou and Arvanitoyannis (2000), Chavan and Prajapati (2009), Tiwari (2005).

Polydextrose is composed of randomly cross-linked glucose polymers with all types of glucosidic bonds, although 1–6 bonds are predominant (min. 90%), sorbitol end-groups (max. 2%), and monoester bonds with citric acid. Polydextrose exists in five forms viz., coarse powder, fine powder, type N, type K, and type F. Polydextrose is used as a low-calorie bulking agent that can replace all or part of the sugars and some of the fats in foods while maintaining a pleasant texture and mouthfeel. Polydextrose can also be used as a humectant, texturizer, thickener, stabilizer, and cryoprotectant (Table 4). Litesse®, a white- to cream-colored powder that has a pH (10% solution) of 2.5–3.5 and melts above 130 °C, is clear, bright, and nonsticky; exhibits no crystallization; and supplies only 1 kcal g^{-1}. According to the FDA, Litesse® may be used in frozen dairy desserts, baked goods, confections, frostings, salad dressings, gelatins, puddings, fillings, hard and soft candy, and chewing gum. Litesse® II, specifically developed for light foods, provides a higher level of sugar and fat replacement. The FDA has recognized polydextrose as a 'carbohydrate.'

Cellulose-based FRs

Cellulose is a polymer based on β-(1→4)-linked D-glucose. The most widely available cellulose-based FR are microcrystalline cellulose (MCC) and methylcellulose (MC) gums.

Microcrystalline cellulose

Developed in 1964, is a nonfibrous form of cellulose, ranging in size from 25 to less than 1 μm. MCC is composed of anhydroglucose units linked through a β (1–4) glycosidic bond, which is hydrophilic, linear, and high molecular weight, and it accounts 75–95% by weight. The remaining 5–20% is soluble in nature (Table 4).

MCC is produced using α-cellulose, which is composed of paracrystalline and crystalline regions of microfibrils by employing acid depolymerization and agglomeration, which is followed by drying to give porous particles of MCC. In case of colloidal grade, soluble hydrocolloids such as carboxymethyl cellulose, xanthan gum, or alginate are added to prevent aggregation during drying. On proper dispersion, the cellulose crystallites set up a three-dimensional network that is highly thixotropic, is temperature stable, and adds body, while imparting a clean mouthfeel. Functionality of MCC is affected by different factors like adequate shear, order of addition, hard water/electrolytes presence, and pH of the food. MCC, when used in cakes at levels from 2% to 4%, improves the sponginess, increases cake volume, provides better handling properties, and creates improved product appearance. In doughnuts, powdered grade MCC (1–3%) can reduce the fat absorption. MCC is indigestible, and FDA regulations permit a maximum addition of 1.5% (w/w) of finished ice cream, with an exception in the case of fruit sherbets, wherein a maximum of 0.5% (w/w) can be added.

MC gums

Hydroxypropylemethyl-cellulose (HPMC) and MC are gums derived from chemical modification of cellulose derived from wheat, maize, potato, and/or rice. The cellulose is made soluble by adding sodium hydroxide and then treating with methyl chloride to form MC and along with propylene oxide to give HPMC, which is further dried using hot air, followed by grinding and packaging. MC and HPMC are described in terms of degree of substitution (DS) and molar substitutions (MSs). DS is the amount of substituent groups on the anhydroglucose units of cellulose, and MS is the average number of molecules of substituent that have been substituted per anhydroglucose unit. DS and MS affect physicochemical properties of MC gums like water retention, sensitivity to electrolytes, dissolution temperatures, and gelation characteristics. Methoxy substitution in MC and HPMC corresponds to a DS range of 1.49–2.00 and 3.0–12.0%, respectively. In fried foods MC and HPMC cause reduction in fat uptake, lower caloric value, improve cooking economy, provide more moisture retention, and provide improved yield. In cake and yeast-leavened doughnuts, oil reduction of about 26–28% can be achieved along with enhancement of air entrainment and promotion of uniform and fine cell size in crumb structure. In liquid foods like sauces

and dressings, MC gums act as a stabilizer and provide pourability, texture, and viscosity. In frozen dairy products, their film-forming property, thickening capability, and lubricity mimics the feel of fat. MC is labeled either as 'methylcellulose' or E-461 and HPMC as 'hydroxpropyl methylcellulose' or E-464.

Dietary fiber-based FRs

Dietary fibers can be used to replace fat due to their wide range of technological functionalities, and soluble fibers, such as pectin and gums, possess a higher WHC of almost 20 times their weight of water as compared to cellulosic fibers. WHC is affected by concentration, temperature, presence of certain ions, and pH of the food system. Fibers, such as pectin, gums, β-glucans with mixed bonds, and polysaccharides extracted from algae, form highly viscous solutions, whereas that of inulin is minimal. The capacity of a fiber to bind fat depends on the porosity, which is useful for avoiding excessive absorption of frying fat in batters. Dietary fiber also acts as a protective agent against cardiovascular diseases, diverticulosis, constipation, irritable colon, colon cancer, and diabetes.

Pectin

Pectin is a complex mixture of polysaccharide composed of a galacturonan backbone of which variable proportions can be methyl-esterified and is usually obtained from citrus fruit and apple. D-galacturonic acid units are linked by α-(1, 4)-glucosidic bonds. Commercial pectins are divided according to the degree of esterification into low methocxyl (LM) pectins and high methoxyl (HM) pectins. HM pectins can also be chemically amidated to obtain low methyl-esterified and amidated (LMA) pectins. HM pectins are used mainly in sugar-acid gels, whereas LM and LMA pectins are used in pectate gels. Functionality of pectin is influenced mostly by the molecular weight and the level and distribution of methyl-esters. Slendid®, manufactured by Copenhagen Pectin, Denmark, is produced by extraction from plant, purification, and isolation of the pectin followed by a controlled desertification with the help of either acid or base. Currently, it is used for fat sparing in confectionary products, dairy products, and bakery fillings. To mimic the physical and sensory characteristics of emulsified fat, the particle size of 25–50 μm and usage level of 1.5–4.0% is recommended. It is possible to reduce the fat content in a frankfurter from 25% to 35% to 3–5% by using a wet preparation of 2% Slendid® 110. Pectin gives a trophic effect, increases villus height and crypt depth in the small intestine, stimulates intestinal cell proliferation and activity of brush border membrane enzymes, and stimulates short-chain fatty acid production in the cecum. Pectin gives a net energy of 9 kJ g^{-1} on digestion. In the United States, pectin is given a Generally Recognized As Safe (GRAS) status, whereas in the European Union it is given a designation of E440 as a food additive.

Inulin

Inulin is an oligomer found in plants such as *chicory* and *Jerusalem artichoke* and has a sweetening power of 30–65% that of sucrose and a high degree of polymerization (DP) of 2–60. Inulin is produced by extracting inulin from chicory roots by diffusing the roots in hot water followed by refining and last spray-drying the concentrate. At a concentration of 40–45%, it forms a gel or crème having a fatty creamy feel with characteristics like high water binding, stability against freeze thaw, and inhibition of syneresis in mayonnaise and salad dressings. Inulin with lower a DP of 25 is generally available for high-performance fat replacement. Inulin crème has been successfully applied in fat-reduced table spreads, frozen desserts, cheese products, meat products, fillings, sauces, and meat replacers (Table 4).

β-glucan

β-glucan is a cell wall polysaccharide present in oat, barley, and other grains, and recently yeast β-glucan has been used as a FR in mayonnaise. β-glucan preparation is used to partially substitute for vegetable oil in low-fat products such as salad dressings, ice creams, yogurts, and cheese. β-glucans can also be used for their therapeutic values, as they are found to be immunomodulators, antitumorogenic, and antiviral agents for treatment of hypercholesterolemia and stabilization of glycemia.

Bacterial cellulose

It is produced by some *Acetobacter*, which is a high-value raw material for producing dietetic and dessert foods due to its high WHC. Instead of xanthan gum, bacterial cellulose at a concentration of 3% can be used in ice creams. Bacterial cellulose improves the quality of pastry products by reducing their stickiness.

Z-trim

Z-trim refers to zero calories and can be used for replacing fat and some of the glycemic materials (starches, sugars, and syrups). It provides an aqueous gel fiber structure without taste and imparts a smooth texture. Z-Trim gels also provide insoluble fibers to be used separately or with other fibers such as Oatrim. Z-Trim gel can be used for considerable reduction in calories depending on the amount of fat and carbohydrates replaced in food formulations.

Gum-based FRs

Gums are hydrocolloids, which are long-chain, high-molecular-weight polymers that dissolve or disperse in water. These gums are used along with a combination of other ingredients viz. starches, bulking agents, emulsifiers, and flavor to yield a finished product similar to a full-fat system. Viscosifying and gelling property are affected by temperature, pH, solvent quality, ionic strength, and presence of specific ions. Hydrocolloids usually come from: (a) plant materials such as seaweed, seeds, roots, and tree exudates; (b) microbial biosynthesis; and (c) chemical modification of natural polysaccharides.

Locust bean gum

It is derived from carob seed, *Ceratonia Siliqua* and consists of D-Mannopyronosyl backbone with attached D-galactopyranosyl units existing in a ratio of 4:1. It is having a limited solubility in cold water but on heating to 80 °C for 10 min it hydrates fully, resulting in a highly viscous pseudoplastic solution. Prolonged heating, high rate of shear, and the time of heating can irreversibly degrade the locust bean gum solution.

Guar gum

Guar gum or guran is the endosperm of the seed *Cyampsis tetragonolobus* containing a backbone of (1→4)-β-D-mannopyranosyl units, with every second unit bearing a (1→6)-α-D-galactopyranosyl unit. Lower mannose:galactose ratio (1.5–2) in guar gum indicates that there are few intermolecular interactions, and consequently, less heat is required for solubilization (i.e., at 25–40 °C). Guar gum is usually utilized at a concentration <1% and is stable between pH 3.5 and 9.0.

Protein-Based FRs

Protein-based FR is derived from proteins found in eggs, milk, and other foods (Table 5). The concept of using proteins as FRs is related to proteins with a particular spheroidal structure, ranging from 0.1 to 2.0 μm in size and is usually produced by microparticulation process.

Simplesse® (NutraSweet, Deerfield, IL) was the first FR developed with protein and has received a GRAS status to be used in frozen dessert. It contains 53% protein and is a microparticulated spray-dried powder that mimics emulsified fat by forming a dispersed phase of particles that are free to move independently. It is a multifunctional dairy ingredient made from whey protein concentrate (WPC) that undergoes a unique microparticulation process that results in uniform, spherical, and deformable protein particles, much like fat globules, which range in size from 0.1 to 3 μm. This process helps to reduce the tendency to aggregate the spherical droplets and form gel on heating. These droplets roll around on the tongue, providing a smooth, creamy texture without chalkiness or graininess. Simplesse increases the opacity of the product by forming small spherical droplets of protein that allow more scattering of light. Simplesse retains the biological property of the protein used, so individuals who are allergic to egg or milk proteins can experience allergic reactions to it.

'Dairy Lo,' a thermally denatured WPC, interacts with water and prevents iciness, provides opacity, controls viscosity, and stabilizes air cells along with a fat-sparing effect in dairy product when used at a level of 2–5%. The formulations supplemented with WPC showed greater elasticity, firmness, chewiness, and gumminess compared to the control sample, thus contributing to the texture of the fat-free dairy desserts. Some of the other protein-based FRs available in the market are K-Blazer®, Ultra-Bake™, Ultra-Freeze™, and Lita®.

Fat-Based FRs

Fat-like substances, which are resistant to hydrolysis by digestive enzymes, comprise another major category of materials being promoted as partial or full replacements for oils and fats in bakery and other food products.

Structured lipids

A structured lipid is a triglyceride obtained by the hydrolysis and random transesterification of medium-chain triglyceride (MCTs) and long-chain triglyceride. Structured lipids provide approximately half of the calories of the normal edible oil. Caprenin, an MCT derived from coconut, palm kernel, and canola oil, mimics the physical properties of cocoa butter or confectionary fat. Another example of MCT is Neobee M-5, derived from coconut oil, which prevents bloom formation in chocolate and sticking of uncoated nutritional bars to packaging material by migrating to the surface of these products. Salatrim (short- and long-chain acyltriglyceride molecules) is a 'family' of triacylglycerols produced by the interesterification of highly hydrogenated vegetable oils with triacylglycerols of acetic and/or propionic and/or butyric acids. Salatrim preparations are available to emulate cocoa butter, as well as for use in baked products and filled dairy products.

Table 5 Protein-based fat replacers

Product name	*Type*	*Applications in food products*	*Manufacturer*
Simplesse®	Microparticulate whey protein concentrate	Dairy products, spreads, bakery product, salad dressing, dips, mayonnaise, frostings, sauces, soups	CP Kelco
Dairy-Lo®	Modified whey protein concentrate	Milk/dairy products, baked goods (cheesecake), frostings, salad dressing, mayonnaise-type products	Cultor, New York, NY
K-Blazer®	A blend of protein, food starch, gums, and emulsifiers	Salad dressing, mayonnaise, frozen dessert, sour cream, baked goods, and dairy products	Kraft Food Ingredients Corp, Memphis, TN
Lita®	Corn zein	Baked goods	Opta Food Ingredients, Inc., Cambridge, MA
Leancreme™	Microparticulated whey protein	Baked goods	SPX-APV brand
Optipep™	Hydrolyzed whey protein concentrates and isolates	Yogurts, ice cream, cheese, and drinking milk	Carbery
N-Flate	Nonfat milk, gums, emulsifiers, and modified starch	Sports nutrition products and nutrition bars	
ULTRA-BAKE™	Starch, modified vegetable protein, and xanthan gum	Cake mixes, salad dressing, icings, glazes, desserts, ice cream, and ground beef	
ULTRA-FREEZE™	Egg-white, milk protein, corn syrup solids, and modified starch	Baked goods	

Compiled from various sources: Roller (1996), Sharma and Ganeshkumar (1996), Akoh (1998), Sharma et al. (1998), Sandrou and Arvanitoyannis (2000), Chavan and Prajapati (2009), Tiwari (2005).

Sugar polyesters

Olestra is a mixture of hexa-, hepta-, and octaesters of sucrose prepared by esterifying sucrose with long-chain fatty acids isolated from edible fats and oils. Olestra can be liquid or solid at room temperature based on the fat source in the sucrose polyester. Olestra has the organoleptic and thermal properties of fat and thus it can serve as a zero-calorie replacement for fat in a variety of foods like savory snacks such as potato and corn chips.

Esterified propoxylated glycerol

Esterified propoxylated glycerol (EPG) is a family of propylene oxide derivatives with a structure similar to that of natural fat. It is been claimed that EPG is resistant to enzymatic hydrolysis and that it can be substituted for fats and oils in products such as table spreads, frozen desserts, salad dressings, and bakery products.

Dailkyl dihexadecymalonate

A fatty alcohol ester of malonie and alkylmalonic acids, dailkyl dihexadecymalonate (DDM) in combination with soybean oil has been used to produce potato and tortilla chips. DDM has also been tried in products like mayonnaise and margarine.

Trialkoxytricarballate

A tricarballylie acid, esterified with fatty alcohol, is being evaluated as an oil substitute to produce acceptable margarine and mayonnaise-based products.

Safety of FRs

The use of FRs as a food additives to reduce the fat content in food products also raises the concern of consumers' safety. The safety of the currently used FRs is ensured by the GRAS status by the FDA. FRs could be used and consumed by the consumers in large quantities in fat-less or reduced-fat products. Many of the carbohydrate-based, protein-based, and fat-based FRs have not shown major health concerns except for polydextrose and Olestra. Polydextrose can have a laxative effect, which requires a labeling disclaimer when present at specified levels. Olestra may cause leaky and fatty stools and loss of fat-soluble vitamins. In general, there is limited evidence at the present time on the long-term adverse consequences associated with the consumption of these or any other reduced-fat foods by adults. However, further research is required to study the safe use of these products by children and adults and to evaluate fully their long-term health effects.

Conclusion

Due to the increasing demand for low-fat food products, reduced-calorie and low-fat food markets are showing a dynamic growth. As pointed out in the beginning of this article, the FR can mimic one or more roles of fat in food products. Classification of an FR usually is based on the nutrient source. They provide the functional and sensory qualities of fats in foods and are absorbed and metabolized normally. The introduction of olestra, which is a nonabsorbable FS that can affect nutrient absorption, raises questions about its potential effect on overall health. Some research suggests that individuals who consume a diet that is reduced in fat and calories and includes use of fat-modified products have a better overall nutrient profile than do individuals who do not use any fat-modified products. The recent increase in the availability of FRs in the market raises questions about the cumulative impact of using them in multiple food products and the potential interaction with medications and food ingredients. Within the context of a healthy dietary pattern, FRs, when used judiciously, may provide some flexibility in dietary planning, although additional research with clinical trials is needed to fully determine the longer-term health effects.

See also: Fats: Classification and Analysis; Fatty Acids: Determination and Requirements; Fatty Acids: Fatty Acids; Food and Agriculture Organization of the United Nations.

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Fats: Classification and Analysis

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Introduction

Fats are essential nutrients and concentrated sources of energy of the diet, supplying about 9 kcal g^{-1}. They are soluble in most organic solvents, such as hexane and ethyl ether, but basically insoluble in water.

By classical definition, fats are referred to total lipids, which can be classified into two different fractions depending on if they form or fail to form soaps when blended with sodium hydroxide, considered as saponifiable and unsaponifiable fractions, respectively. The different compounds that can be part of the saponifiable fraction are shown in the next scheme.

The unsaponifiable matter contains terpenic (sterols, 4-methylsterols, 4,4′-dimethylsterols, carotenoids, tocopherols, and tocotrienols) and aliphatic compounds (saturated and unsaturated hydrocarbons and fatty alcohols).

The different ways of analysis of the fat compounds are going to be described in the succeeding text.

Saponifiable Fraction

Triacylglycerols

Triacylglycerols consist of the trihydric alcohol glycerol esterified with fatty acids. They represent the main lipid fraction of foods. For the analysis of molecular species of triacylglycerols, chromatographic and spectrometric methods are available. However, stereospecific determination of positional distributions of fatty acids in them remains a substantial technical challenge. Nowadays, the most used methods for their analysis are HPLC and GC.

Reversed-phase HPLC

Good resolution of triacylglycerols by reversed-phase high-performance liquid chromatography is possible. The retention times are dependent on the total number of carbon atoms of the three fatty acids of the molecule, with each double bond reducing the effective chain length of the species by the equivalent of about two carbon atoms.

The most frequently used columns have been those with phases of the octadecylsilyl (C18, ODS, or ODS-2) type, since stationary phases similar in chain length to the fatty acyl chains maximize the interactions and give a higher efficiency. Silver ion HPLC has been also used. The 250×4.6 mm column with 5 μm packing, the 100×3 mm column with a 3 μm packing, and the 100×2.1 mm column with 1.7 μm packing have been used, the last one being used in terms of saving time and solvent and with no loss of resolution.

Referring to the mobile phases, the most used are

- acetonitrile–acetone,
- acetonitrile–isopropanol,
- acetonitrile–dichloromethane,
- acetonitrile–ethanol–hexane,
- acetonitrile–tetrahydrofuran.

They improve the solubility of triacylglycerols and have an effect on the selectivity and efficiency of the separation. The detector used and the nature of triacylglycerols may determine the choice of mobile phase and the choice of an isocratic or a gradient system. For example, acetonitrile–acetone mixtures are not well suited to saturated components of higher molecular weight, since they tend to precipitate out of solution. For such purpose, the acetonitrile–dichloromethane combination may be the best available. Isocratic eluants are utilized with detectors in which the change in the composition of the mobile phase affects the obtained signal, as in the case of the refractive index (RI) detector. The gradient of elution can be used with detectors such as UV and evaporative light scattering detectors (ELSDs).

The column temperature should be fixed and controlled to achieve reproducible retention times. With low temperatures, solubility effects may come into play, and the more saturated molecular species may precipitate out of solution.

The detection systems for quantitative analysis of triacylglycerol species separated by HPLC have been RI, UV, evaporative light scattering, and mass spectrometry detectors.

One of the most used for the mentioned purpose is the RI detector, in which the RI changes with the chain length and with the unsaturation number. The response factor is considered the same (1) for all triacylglycerols in the practice. This detector is affected by the temperature and composition of the mobile phase, and due to this, the analysis needs to be carried out with thermostated cells and isocratic elution. In some cases, flow gradients can be used. Another limitation of this detector is its low sensitivity. However, RI detection gives good results and is one of the most adequate for the quantitative analysis of these compounds.

It has been reported that UV detection can be used to get good quantitative data at wavelength between 200 and 220 nm, where the ester bond absorbs. This detector shows the highest sensitivity. Nevertheless, the absorption of several solvents used in the mobile phase, like acetone, the unsaturations, and the conjugation of these unsaturations are a problem for the use of this detector in triacylglycerol analysis.

An alternative to the detectors mentioned is the ELSD, which is universal and simple. This shows the disadvantages that a calibration method must be taken into account and that the linear range is between 50 and 250 μg. Below 50 μg, the sensitivity decreases, which is a problem for the analysis of the minor compounds. But this detector shows the advantages that no solvent peak appears and the resolution of the peaks is good and it is not affected by the changes in the solvents of the mobile phase.

For both detecting and identifying molecular species of triacylglycerols, mass spectrometry is used, showing a good sensitivity, although a careful calibration is also needed.

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The choice of injection solvent is another important practical point that should be mentioned. Samples should be injected in a small volume of the mobile phase (5–10 µl). Hexane is not employed as injection solvent because a single component can emerge as double peaks due to its properties, which are so similar to those of the stationary phase that it competes with this for the solute molecules.

High-temperature GC

Nowadays, the use of high-temperature GC with flame ionization detection for the separation and quantification of triacylglycerols is a good alternative to HPLC, solving its problems.

Triacylglycerols are not at all easy to volatilize for gas chromatography purposes since their molecular weight is quite high. The injection procedure is of great importance to avoid any sample discrimination and achieve good quantification. Modern gas chromatographs usually come equipped to achieve such purposes with cold on-column injection, split injection, and/or programmed temperature vaporizer injection. The use of cold on-column injection is one of the most desirable to reduce or eliminate thermal decomposition (e.g., the pyrolysis of the most unsaturated triacylglycerols), mass discrimination, and other undesirable effects associated with classical split/splitless injection. Using this injection technique, the injector temperature should be lower than the solvent boiling point. The new split/splitless injectors minimize mass discrimination loss and make possible to apply this technique to triacylglycerol analysis with similar results to those obtained by using cold on-column injection.

Another aspect that needs to be addressed is the nature of the stationary phase. The temperature stability of this is highly important. The most stable to high temperatures is the nonpolarity phases of the dimethylpolysiloxane type, which permit separation by chain length. However, high-polarity or polarizable phases allow remarkable separations by both carbon atom number and degree of unsaturation, but they are less stable to high temperatures. Columns coated with polar stationary phases, such as cyanopropyl silicone, are capable of resolving triglyceride mixtures even better than nonpolar phases, but they cannot reach temperatures over 300 °C. The polarizable phenyl methyl silicone phases are capable of enduring temperatures of about 360–370 °C for a long time, separating triacylglycerols by both chain length and the number of unsaturation. The fused silica capillary DB-17ht column (30 m × 0.32 mm i.d. and 0.15 µm film thickness) shows a really good resolution for the different molecular species of triacylglycerols.

Detection is carried out by using a flame ionization detector (FID), and the response factors need to be considered, which depend on a certain extent on the ratio of the carrier gas.

The purity and nature of the carrier gas, flow rate, rate of temperature change, and whether the carrier gas is maintained at constant flow or constant pressure rate are other practical factors that have to be considered.

An example of triacylglycerol analysis by GC-FID is shown in Figure 1.

Diacylglycerols and Monoacylglycerols

Diacylglycerols are esters of the trihydric alcohol glycerol in which two of the hydroxyl groups are esterified with fatty acids, and they are found as 1,2- and 1,3-isomers. They are generated by acidic and enzymatic hydrolyses of triacylglycerols during the transformation and storage of fats, and 1,3-isomers can be generated from 1,2-diacylglycerols due to an isomerization process, since the 1,3 species is more thermodynamically stable. In tissues, 1,2-diacylglycerols can also be generated from phosphatidylinositol (PI) by the action of phospholipase C. The determination of molecular species of diacylglycerols in fats can be accomplished by a previous extraction and purification by column chromatography or solid phase extraction (SPE) and a subsequent chromatographic method, GC being the most appropriate for that purpose. An aliquot of the fat in hexane and a suitable internal standard (e.g., 1,3-dipalmitoyl-glycerol and dinonadecanoine) are added prior to

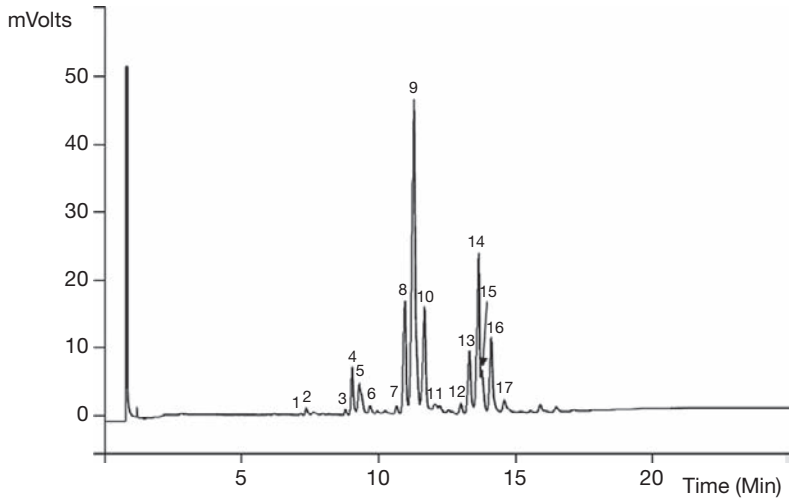

Figure 1 Chromatogram of the triacylglycerol profile of an Iberian pig subcutaneous fat sample, where 1, PPP; 2, MOP; 3, PPS; 4, POP; 5, POPo + PLP; 6, PLPo + MLO; 7, PSS; 8, PSO; 9, POO; 10, PLO; 11, PLL + PoLO; 12, SOS; 13, SOO; 14, OOO; 15, SOL; 16, OOL; 17, OLL. Reproduced from *Journal of Agricultural and Food Chemistry*, 2012, **60**, 1645–1651.

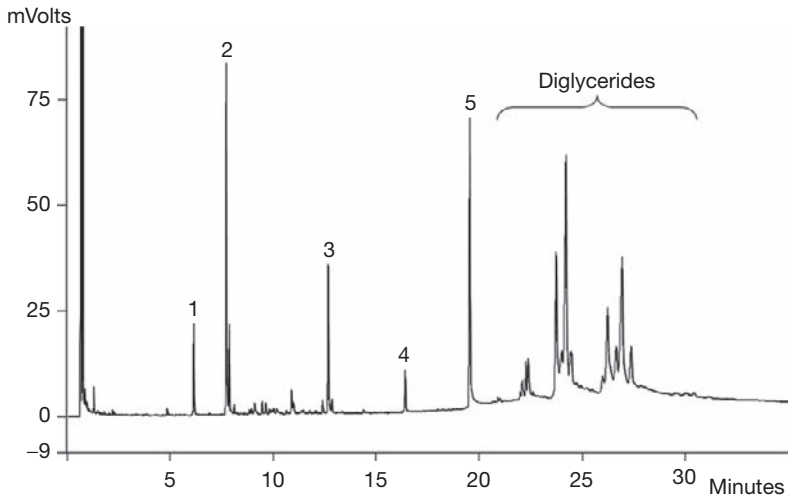

Figure 2 Gas chromatogram of the polar fraction from 0 to 35 min corresponding to the subcutaneous fat of Iberian dry-cured ham. Labeled peaks are identified as follows: 1, palmitic acid; 2, oleic acid; 3, 1-monoolein; 4, cholesterol; 5, 1,3-dimyristin (IS). Reproduced from *Journal of Agricultural and Food Chemistry*, 2007, **55**, 10953–10961.

the purification step, in which silica or diol SPE columns are utilized.

Purification comprises several steps using different solvent systems. The first is carried out passing a solution to eliminate the major compounds (e.g., hexane/methylene chloride/ethyl ether (89:10:1)) through the column, which is discarded. In the second step, the diacylglycerol fraction is obtained by passing a solution, generally chloroform/methanol (2:1), that is evaporated to dryness in a rotary evaporator under reduced pressure, and the residue is treated with 200 µl of the silylating reagent and left at room temperature for a few minutes, obtaining the corresponding trimethylsilyl derivatives of 1,2- and 1,3-diacylglycerols. Monoacylglycerols are also extracted with the diacylglycerol fraction, and they can be analyzed together by GC using a proper temperature program and a proper column. An example of this is shown in Figure 2.

The analysis of the silylated fraction is performed by GC-FID using thermostable midpolarity GC columns (e.g., fused silica capillary DB-17ht polyamide-coated column, 50% phenyl methylpolysiloxane, 65% phenyl- and 35% dimethylpolysiloxane, and 65% methyl phenyl silicone). The peak of 1,2-diacylglycerols appears before their corresponding 1,3-isomers.

With respect to monoacylglycerols, they are at trace levels in fats, and their analytic determination is carried out with a procedure similar to diacylglycerols, trimethylsilyl derivatization, and GC-FID analysis but with nonpolar capillary columns, or their combination as it has been mentioned in the preceding text.

Phospholipids

Phospholipids are a class of lipids that have two fatty acyl molecules esterified at the sn-1 and sn-2 positions of glycerol and a head group linked by a phosphate residue at the sn-3 position. They have amphipathic property since the head group forms a hydrophilic region that determines the type of phospholipid, and the fatty acyl side chains are hydrophobic.

Two different categories are distinguished: glycerophospholipids (phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), and cardiolipin (CL)), the derivatives of glycerol, and sphingophospholipids (sphingomyelin: SPH), those that do not contain glycerol.

Phospholipids have been determined traditionally by gravimetric methods and spectroscopic methods, like molecular or atomic absorption spectroscopy. However, these are not usually used nowadays.

The thin-layer chromatography (TLC) technique using adsorbent (silica) coated on glass strips is used for the separation of phospholipids as a separation technique previous to the quantification of these by another procedure or even for their direct quantification by densitometry or thincography. The type of mobile phase used and whether the TLC is in one or two dimensions are critical in the separation of the different groups. This technique has some disadvantages, such as a limited capacity to resolve the phosphatides in their different molecular species, techniques of detection more or less complex with significant errors in the quantification, and a relatively long time period required for analysis.

Nowadays, the method used for the separation and determination of phospholipid classes is the HPLC. Silica columns are the most used for such purposes since this can give excellent results and is relatively inexpensive and robust. Nevertheless, amino columns have been also used, but they need to be periodically regenerated with ammonia solutions. So, they are not widely used.

The most used detectors in the study of phospholipid classes are the UV and the ELSD. The response of UV detector is dependent on the degree of unsaturation in the molecule, and different wavelengths have been used, but the most frequently used are 205 and 208 nm (absorption of the ester bond). Besides, in these detectors, another problem is the absorption of some mobile phases at these wavelengths. That is why there are solvents that cannot be used with these detectors, like chloroform and acetic acid. So, the quantification of

phospholipids using this detector is difficult. On the other hand, ELSD gives a stable flat baseline and is very sensitive, and its response is not dependent on the number of double bonds in the molecule, showing the advantage of simplicity and universality. To use this detector in quantitative analysis, the response factors of the phospholipids must be calculated, due to the minor components.

Referring to the mobile phases used, an isocratic eluant has rarely been used. With a UV detector, an isocratic mode or a linear gradient can be applied. Binary gradients can be employed using ELSD, and the following have been used in several works: (a) chloroform–methanol–ammonium hydroxide and (b) chloroform–methanol–water–ammonium hydroxide. More complicated gradients of elution with three or four eluants have been used, but to a lesser degree than the binary ones. A wide range of different mobile phases have been used to separate the different phospholipid classes, since unfortunately not all phases have the same effectiveness for the different types of samples and the composition of the mobile phase can become critical for each type of matrix. Binary gradients that have been successfully used with different types of matrix are (a) chloroform–methanol–ammonia solution and (b) chloroform–methanol–triethylamine–water. An example is shown in Figure 3.

The use of reversed-phase HPLC allows the separation of the different molecular species of each phospholipid class. Normally, the different phospholipid classes are separated and collected by HPLC or TLC. One or two columns in series can be used depending on the resolution desired and the mobile phase used. Acetonitrile–methanol–water is the most commonly used mobile phase in reversed-phase HPLC using an RP18 column.

The last thing to mention about phospholipids is that they can be purified previously to their chromatographic analysis using SPE to isolate them, and the best results are obtained when silica columns are employed, allowing a high recovery.

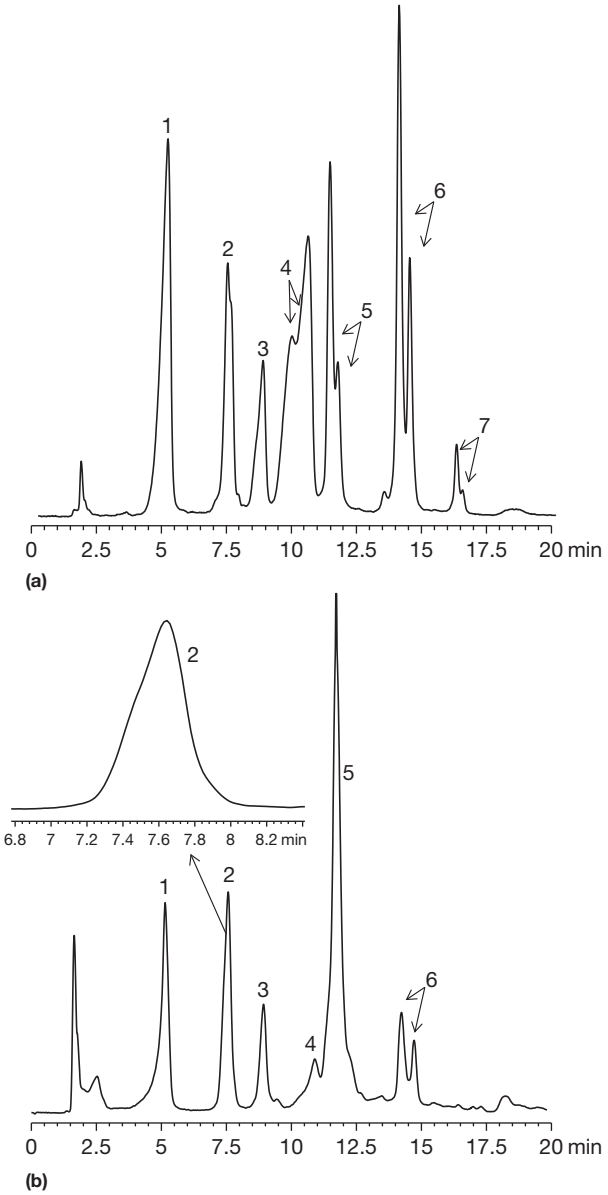

Figure 3 HPLC chromatogram of phospholipid fractions (a) from the standard solution and (b) from the subcutaneous fat of Iberian pig. 1, Cardiolipin; 2, phosphatidylethanolamine; 3, phosphatidylinositol; 4, phosphatidylserine; 5, phosphatidylcholine; 6, sphingomyelin; 7, lysophosphatidylcholine. Reproduced from *Journal of Chromatography A*, 2011, **1218**, 3453–3458.

Waxes

Waxes are organic compounds that consist of long alkyl chains. They are insoluble in water but soluble in organic nonpolar solvents. The waxy material of the fats can include primary and secondary alcohols, diols, ketones, β-diketones, triacylglycerols, hydrocarbons, sterol esters, and aliphatic aldehydes.

Silica gel column chromatography, TLC, SPE, and HPLC (using silica) have been used to separate them from other lipid constituents and to isolate individual classes of waxes for more detailed analysis. On the other hand, high-temperature gas chromatography following trimethylsilylation has been used in recent works, often in combination with mass spectrometry, to achieve simultaneous identification and quantification of the various molecular species.

Semipolar columns can be used for their analysis, although a better resolution is obtained with phenyl methyl silicone columns, which can endure higher temperatures.

All the methods for the determination of waxes consist of a separation from other lipid constituents using silica gel column chromatography, SPE, or HPLC and subsequent quantification using GC. In the official method, the waxes are determined by separating them according to the number of carbon atoms. The fractionation step is carried out by chromatography on a hydrated silica gel column after the addition of the internal standard (e.g., lauryl arachidate). The eluted fraction is recovered and subsequently injected on-column in a GC capillary column. SE-54 or SE-52 semipolar columns can be used, although a better resolution can be achieved with phenyl methyl silicone columns, which can tolerate higher temperatures.

Waxes can be determined simultaneously with fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography, which is a method widely applied in laboratories.

A method based on through oven transfer adsorption–desorption (TOTAD) interface is another analytic

alternative. The lipid extract, with an internal standard diluted in *n*-heptane, is injected directly with no sample pretreatment step other than filtration. The wax ester fraction is separated from the triglycerides by normal-phase liquid chromatography, and the TOTAD interface transfers it to the GC to be analyzed. This method allows an automatic analysis of different wax esters, being simpler and faster than other methods, although its resolution is poorer.

A method in which samples are prepared by normal-phase liquid chromatography has also been proposed for the analysis of wax esters, but the precolumn is attached to the inlet of the column in the GC × GC instrument by means of a press-fit connector, the first dimension being performed with a PS-255 column (20 m × 0.25 mm i.d.) and the second dimension with a SOP-50 (50% phenyl polysiloxane) (1.5 m × 0.15 mm i.d.).

Unsaponifiable Fraction

The main classes of compounds that constitute the unsaponifiable fraction of food are hydrocarbons, carotenes, tocopherols, tocotrienols, linear fatty alcohols, triterpenic alcohols, methyl sterols, and sterols. Other components that are part of the unsaponifiable fraction are carotenoid pigments, triterpenic dialcohols, diterpenic alcohols, and phytol, but they are not always present in food matrices.

The unsaponifiable fraction is got doing a saponification with ethanolic potassium hydroxide at high temperature. After this, distilled water is added to the solution, and the mixture is extracted several times with portions of a nonpolar solvent such as hexane, heptanes, diethyl ether, chloroform, or their mixtures thereof. The unsaponifiables will remain in the nonpolar phase, while the fatty acid salts will partition to the polar phase. The nonpolar extracts are combined and washed several times with portions of a mixture of ethanol–water (1:1), until the wash reaches neutral pH. Finally, the hexane solution is dried under a stream of nitrogen or by rotoevaporation and redissolved in an appropriate amount of solvent for cleanup by either TLC or SPE or directly for derivatization. The fractionation of the different classes present in the extract can be carried out by HPLC. In Figure 4, there is an example of the fractionation using the HPLC technique. The same preparative procedure is utilized for the analysis of all unsaponifiable components. The trimethylsilyl derivatives (except for the hydrocarbons) of the purified fractions are analyzed using nonpolar capillary gas chromatography columns. On the other hand, a direct analysis of the silylated unsaponifiable components obtained from different food products can be carried out using a thermostable polar gas chromatography column (65% phenyl–35% dimethylpolysiloxane).

Hydrocarbons

In the natural lipid systems of food matrices, hydrocarbons are present in quite small amounts, and most of them have an odd number of carbon atoms. Cyclic, polycyclic aromatic, and ramified hydrocarbons are also present in small amounts.

A good separation of hydrocarbons is provided by silica TLC of natural lipids using a mobile phase of *n*-hexane-diethyl ether (7:3, v/v), eluting just after the solvent front. To improve the isolation of the unsaponifiable fraction of lipids, TLC and liquid chromatography are being replaced by HPLC methods, and HPLC-GC off-line methods that allow isolation and quantification of the hydrocarbon fraction have been reported. The column used for such purposes is a silica column and the mobile phase is hexane/ethyl acetate. SPE is also used, like in the case of polycyclic aromatic hydrocarbons, which are considered as contaminants.

After their purification, the hydrocarbons are analyzed by GC-FID or GC-MS, the last technique allowing their simultaneous identification. For polycyclic aromatic hydrocarbon analysis, low-bleed and low-polarity columns have been used, like DB-XLB columns. Linear, branched, and terpenic hydrocarbon analyses are performed using nonpolar columns of (95%) dimethyl–(5%) diphenylpolysiloxane type. An example of hydrocarbon analysis by GC-FID is shown in Figure 5.

Alcohols and Sterols

Linear alcohols are constituted by a homologue series of primary fatty alcohols that generally contain 20–30 carbon

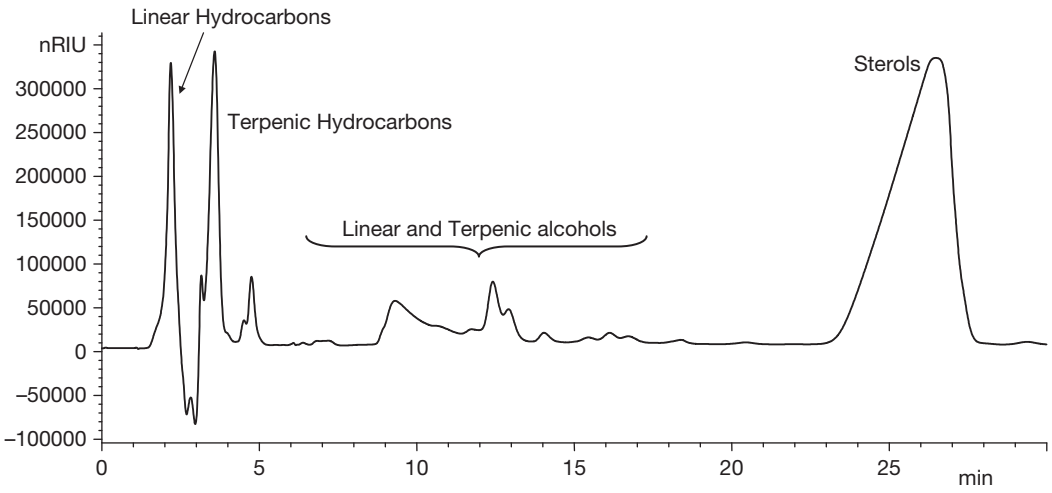

Figure 4 HPLC chromatogram of unsaponifiable fraction from the subcutaneous fat of Iberian pig.

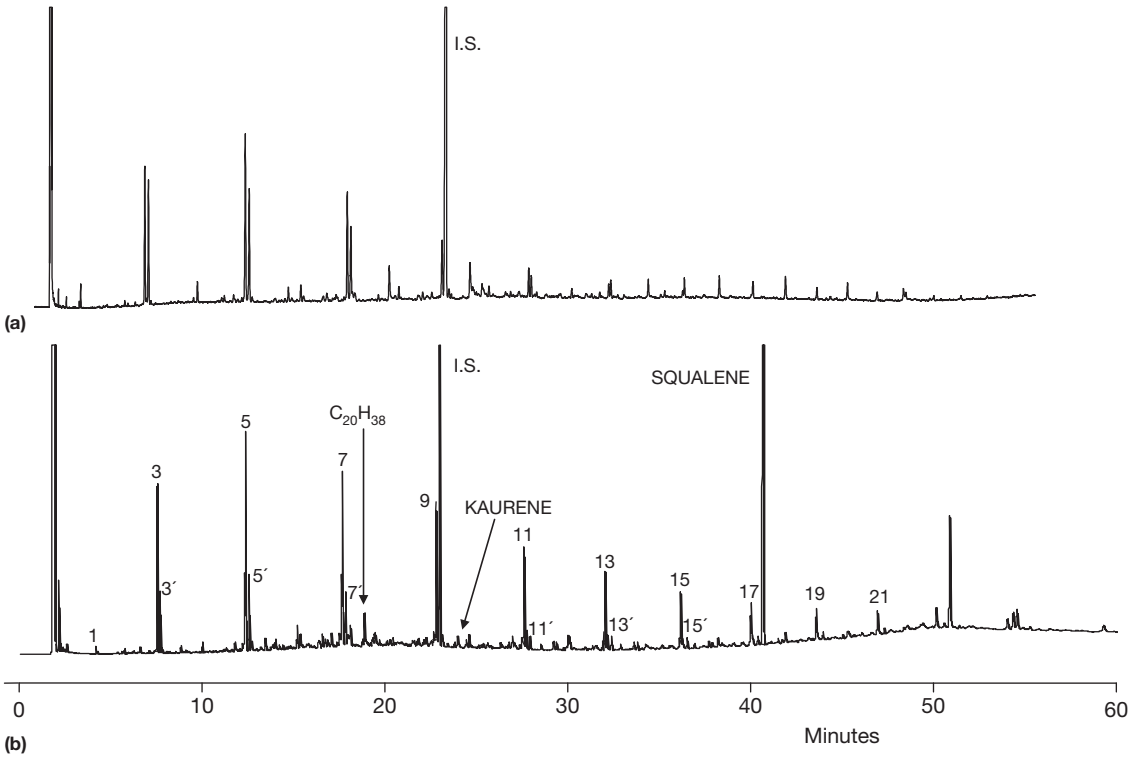

Figure 5 Chromatograms of hydrocarbons obtained from the subcutaneous fat of Iberian pig by LC (a) and HPLC (b). Peaks: 1, 1-dodecene; 3, 1-tetradecene; 3′, tetradecane; 5, 1-hexadecene; 5′, hexadecane; 7, 1-octadecene; 7′, octadecane; 9, 1-eicosene; 11, 1-docosene; 11′, docosane; 13, 1-tetracosene; 13′, tetracosane; 15, 1-hexacosene; 15′, hexacosane; 17, 1-octacosene; 19, 1-triacontene; 21, 1-dotriacontene.

atoms. The triterpenic alcohols have a steroid structure and are present at different levels in vegetal lipids. These are also known as 4,4′-dimethylsterols.

Both linear and triterpenic alcohols are often badly separated in silica TLC. To achieve a better TLC separation, a multiple development technique with a slightly different mobile phase for the second development can be carried out. Then, they are determined using the GC technique.

Sterols are steroid alcohols and they occur naturally in plants (phytosterols), animals, and fungi. Cholesterol (cholest-5-en-3β-ol) is by far the most abundant sterol in animal tissue. This can be esterified to long-chain fatty acids (cholesterol esters). These cholesterol esters are much less polar than free cholesterol. Cholesterol oxidizes slowly in tissues or foods forming a range of different products with additional hydroperoxy, epoxy, hydroxy, or keto groups. These compounds are called oxysterols, cholesterol oxides, or cholesterol oxidation products (COPs). Enzymatic methods from commercially available kits are used to determine the cholesterol content. It is necessary to hydrolyze the cholesterol ester fraction to determine the total cholesterol content.

In the analysis of sterols, these are first isolated from lipid extracts by TLC or column chromatography, following hydrolysis if necessary. Individual components can then be determined by GC, often after conversion to trimethylsilyl ether derivatives to give sharper peaks. The addition of an internal standard allows obtaining accurate qualitative and quantitative results by GC. The most common internal standards for sterol quantification are 5α-cholestane, dihydrocholesterol (5α-cholestan-3β-ol), epicoprostanol (5β-cholestan-3α-ol), and betulin. The internal standard is typically added to the sample prior to alkaline or acidic hydrolysis, undergoing the same extraction conditions as the sterol fraction, or it can be added after extraction but prior to derivatization.

Acidic hydrolysis is necessary to free the sterols when the sample has a complex protein or carbohydrate matrix and/or steryl glycosides. If this is needed, the internal standard is added to the weighed sample, and then, it is hydrolyzed with hydrochloric acid at 100 °C. After the acidic hydrolysis, lipids are extracted using a nonpolar solvent. After solvent removal, saponification of the lipid fraction is carried out as described in the preceding text.

Once saponification has been done, the unsaponifiable fraction may contain other lipids besides sterols including hydrocarbons, carotenoids, tocopherols, free fatty acids, and other triterpenes. For the unsaponifiable fraction of a refined fat or oil sample, it is usually acceptable to proceed with derivatization and GC analysis without further sample cleanup, and no problems with interference by these compounds are presented. However, in other types of samples, the sterol fraction needs to be purified. For such purpose, silica, C18, and aminopropyl SPE cartridges have all been used.

Nowadays, sterols are usually analyzed as trimethylsilyl ethers, since their volatility, peak shape, and response factors improve. Direct injection of nonderivatized sterols results in broader peaks and a lower FID response. The silylating reagents used are N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and N-methyltrimethylsilyltrifluoroacetamide (MSTFA), as well as mixtures of pyridine, hexamethyldisilazane, and trimethylchlorosilane (9:3:1, v/v/v). Derivatization reactions may proceed

completely in several minutes (about 15 or 20 min) at room temperature or heated to complete the reaction when the hydroxyl group on the sterols is hindered in any way.

After derivatization, sterols can immediately be analyzed by GC or after carefully evaporating the derivatization reagents with nitrogen at low temperatures to prevent any sterol ethers and redissolving them in an organic solvent, like chloroform, to an appropriate concentration before injecting. Split mode injection is usually used and the injector temperature usually set from 250 to 300 °C. Fused silica capillary columns with polysiloxane phases of low to midpolarity are used for their analysis. The most common column phase used for sterol analysis is composed of (95%) dimethyl–(5%) diphenylpolysiloxane. A nonpolar phase of (100%) dimethylpolysiloxane can be used, but a good separation of sterol peaks and their respective stanol peaks is not achieved with this column phase. Midpolarity columns (14% cyanopropyl-phenyl-methylpolysiloxane) have been suggested for a better resolution of Δ5-saturated and Δ5-unsaturated sterols.

For the identification of individual components of sterol fraction, mass spectrometry may be required.

HPLC can be employed for the analysis of sterols, using UV and mass spectrometric detection for their quantification and identification, respectively. An HPLC-/atmospheric pressure chemical ionization-MS method has been developed for the analysis of these compounds, in which no previous derivatization is needed. Several stationary reverse phases (C8, C18, and zirconia-based adsorbent) have been studied for this purpose, and the best separation of sterols has been obtained with the C8 phase. Increasing the column temperature accelerates their elution markedly. Nevertheless, different mobile phases have been used.

Rigorous attention in the analysis of the COPs should be attempted because they tend to be present at rather low levels, and there is a danger of further oxidation or side reactions during the analytic process. Both hot and cold saponifications, using either ethanolic or methanolic potassium hydroxide (KOH), have been used in the analysis of COPs. Their enrichment can be carried out using either silica or aminopropyl SPE. Besides, the sample should be protected against light, oxygen, and high-temperature exposures, since they promote lipid oxidation. HPLC and GC techniques have been used for the analysis of COPs, but GC has given better results than HPLC. Samples are derivatized to trimethylsilyl ethers using BSTFA, Tri-Sil reagent (Pierce Chemical Co., Rockford, IL), or the mix of hexamethyldisilazane–trimethylchlorosilane–anhydrous pyridine (3:1:9, v/v/v) prior to GC and GC-MS analyses. Fused silica open tubular capillary columns coated with (5%) phenyl methyl silicone and columns coated with (100%) dimethylpolysiloxane, such as DB-1, have been used for their analysis. Similar results are obtained using DB5-MS and DB1-MS columns. Although GC has been the most used technique for the analysis of COPs, some HPLC methods have been reported. A method using reversed-phase LC/atmospheric pressure chemical ionization mass spectrometry for the successful determination of cholesterol and its oxidized compounds in processed foods such as pork, beef, chicken, and eggs has been described. Diode array ultraviolet detection and laser light scattering detection (LLSD) have been compared, concluding that the use of LLSD in the HPLC of cholesterol and its oxidized products could provide a reliable tool for the determination of these compounds, since the use of this allowed the detection of cholestanetriol and α- and β-epoxides. Between the different internal standards used for their quantification are 5α-colestane, betulin, 19-hydroxycholesterol, and betamethasone-17, 19-dipropionate.

4-Methylsterol and 4,4-dimethylsterol fractions are analyzed in a similar way to other sterols.

Tocopherols and Tocotrienols

These compounds consist of a polar chromanol ring and a hydrophobic 16-carbon side chain attached to the ring via the C2 atom. The difference is that tocopherols contain saturated phytyl side chains, while tocotrienols have isoprenyl side chains with three double bonds. They display antioxidant properties and are active as vitamins (vitamin E), making them particularly important for human health. They are amphipathic and lipid-soluble and are easily oxidized when subjected to heat, light, and alkaline conditions. Tocotrienols are present in small amounts in food lipids. However, palm oil, grape-seed oil, and the annatto lipid fraction contain a relatively high amount of these components.

The determination of the tocopherols and tocotrienols as trimethylsilyl derivatives can be carried out on a short GC column of medium polarity (OV-17), with both flame ionization and mass spectrometric detection, within a short time of analysis, achieving a very good separation of the two classes of components. However, the methods that are usually recommended involve HPLC with fluorescence detection.

These compounds can be extracted using solvent or alkaline hydrolysis extractions. Supercritical fluid or pressurized liquid extractions are alternative techniques to traditional solvent extraction methods that may be used to extract tocopherols and tocotrienols.

HPLC is the most widely used technique for the analysis of these compounds since they are stable under HPLC conditions and easy to dissolve in appropriate solvents, and there are several detectors that can be combined with this technique to detect them, fluorescence detection and UV ($\lambda = 290–300$ nm) detection being the most commonly used. Aside from this, both normal-phase chromatography and reversed-phase chromatography can be applied.

Normal-phase using silica columns is commonly applied when all eight tocopherols and tocotrienols need to be separated, achieving better selectivity when hexane is used with a relatively strong polar modifier 1,4-dioxane in the mobile phase than with a weak modifier such as tert-butyl methyl ether.

However, reversed-phase might also be utilized when only some vitamins are of interest or when mixtures of fat-soluble vitamins and free and esterified tocopherols and/or tocotrienols have to be separated.

The external standard method is usually used to quantify tocopherols and tocotrienols because it is difficult to find a compound that would not interfere with their analysis.

See also: Fatty Acids: Determination and Requirements; Fatty Acids: Fatty Acids; Triacylglycerols: Characterization and Determination;

Triacylglycerols: Structures and Properties; Vegetable Oils: Composition and Analysis.

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Relevant Website

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Fats: Production and Uses of Animal Fats

SB Smith and DR Smith, Texas A&M University, College Station, TX, USA

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Introduction

The composition of lipids in fatty tissues of meat varies in response to diet, the time at which the lipids are deposited, and adipose tissue depot. The fatty acid composition of pork is especially sensitive to dietary manipulation, whereas that of beef and lamb is affected primarily by the age of the animal and fat depot, although fatty acid composition of ruminant tissues can be modified by dietary means. For example, pasture or grass feeding causes fat depots to be high in saturated fatty acids, whereas grain feeding increases the concentration of monounsaturated fatty acids.

Between 45% and 50% of the entire live weight of livestock species does not enter directly into the human food chain. The by-products of animal slaughter and fabrication include muscle trim and a large quantity of the carcass fat (especially subcutaneous and internal fat depots) that are not used in processed meats. In addition, many internal organs are included in total by-products. Altogether, these by-products contain approximately 20% extractable fat. With rendering, some of this extractable fat can be recovered and converted into more useful and profitable materials such as lard or tallow, allowing part of carcass fat to reenter the human food chain. The quality, and especially the fatty acid composition of the rendered materials, determines the fate of the finished product.

Sources of Lipid in Meat

There are four sources of lipid in meat that are used for meat processing or rendering: the muscle fibers; subcutaneous fat; intermuscular (seam) fat between muscle groups; and intramuscular (interfascicular and marbling) fat. Very lean beef, pork, or lamb, in which all subcutaneous and intermuscular fat has been removed, contains approximately 1% extractable lipid. At the other extreme, even lean trim from Japanese Black (or wagyu) cattle can contain over 40% extractable lipid. This extraordinary concentration of lipid can be attributed largely to lipid within the muscle.

Fatty Acid Composition of Subcutaneous, Intramuscular, and Muscle Lipids

Lipids from fat, lean trim, and organs destined for rendering contain the saturated fatty acids palmitic acid (16:0) and stearic acid (18:0) and their delta-9-desaturase products, the monounsaturated fatty acids palmitoleic acid (16:1$n-7$) and oleic acid (18:1$n-9$). Palmitic acid is produced by the combined activities of acetyl-coenzyme A carboxylase and fatty acid synthase, whereas stearic acid is produced by the addition of two carbons to palmitic acid via a fatty acid elongase. Palmitoleic acid and oleic acid are the products of the delta 9 desaturation of palmitic acid and stearic acid, respectively, catalyzed by the enzyme stearoyl-CoA desaturase (SCD). Palmitic, palmitoleic, stearic, and oleic acids are always present in all tissues of animals (Figures 1 and 2), but the relative concentration of each fatty acid is dictated primarily by the activity of the enzyme SCD.

The polyunsaturated fatty acids typically consumed by livestock species consist primarily of linoleic acid (18:2$n-6$) and α-linolenic acid (18:2$n-3$). In the rumen of cattle, sheep, and goats, polyunsaturated fatty acids are extensively isomerized to *cis*-9, *trans*-11 conjugated linoleic acid (CLA) and other CLA isomers (Figure 3). However, the concentration of CLA isomers in ruminant tissues does not exceed 2% of total fatty acids because CLA is almost quantitatively hydrogenated to stearic acid in the rumen, although small portions of *trans*-vaccenic acid and other fatty acid *trans*-isomers escape the rumen and are absorbed in the gastrointestinal tract. For this reason, ruminant tissues are relatively high in stearic acid and naturally occurring *trans*-fatty acids.

The contribution of both *de novo* fatty acid synthesis and fatty acid desaturation to the fatty acid composition of fat depots is especially apparent in bovine fat (Figure 1). Stearic acid constitutes more than 80% of the fatty acids available for absorption from the duodenum in beef cattle, yet the most abundant fatty acids in bovine fat are palmitic acid (the product of *de novo* synthesis) and oleic acid (from desaturation of stearic acid). Typically, abdominal fat is more highly saturated than subcutaneous or intramuscular fat. In beef cattle, intramuscular fat is also higher in stearic acid than is subcutaneous fat (Figure 1). Unlike the situation for beef, intramuscular fat from pork contains less stearic acid than does subcutaneous fat (Figure 2).

As monogastrics, swine have adipose tissues with fatty acid composition that more closely resembles that of dietary fatty acids. Thus, diets enriched with oleic or linoleic acid will cause the lipids associated with pork to become similarly enriched with these fatty acids. Polyunsaturated fatty acids are especially susceptible to oxidation during long-term storage or rendering. This results in the formation of undesirable aldehydes and ketones, causing the rendered fat to be less acceptable. Feeding pigs diets enriched with polyunsaturated fatty acids also causes the carcasses to be oily and may lead to production problems such as soft pork bellies. Enrichment of carcass lean trim and fat with oleic acid, which is a solid at typical freezer temperatures, does not appear to accelerate lipid oxidation.

The majority of the fatty acids are stored as highly nonpolar triacylglycerols (Figure 4). Triacylglycerols coalesce into the large lipid vacuoles that are the central features of adipocytes. The triacylglycerol structure consists of a glycerol (i.e., three-carbon alcohol) backbone containing three fatty acids in ester linkages. The glycerol primarily is derived from glycerol-3-phosphate, which in turn is derived from the metabolism of glucose or, in the liver, from the phosphorylation of free

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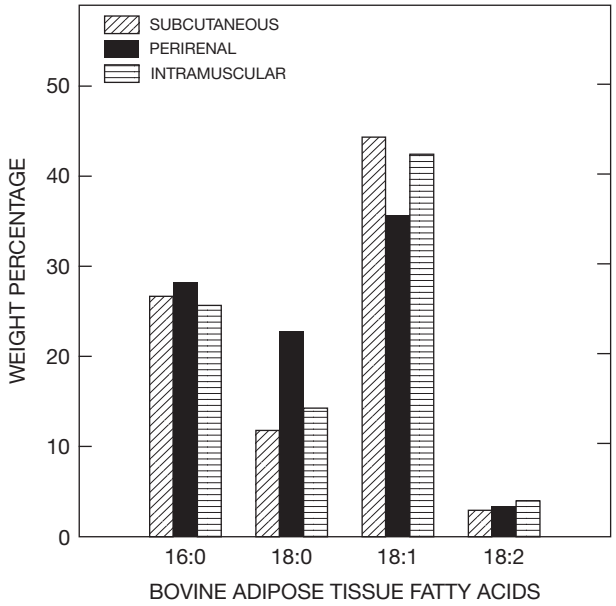

Figure 1 Fatty acid composition of bovine adipose tissues. Reprinted with permission from Rule, D. C., Smith, S. B. and Romans, J. R. (1995). Fatty acid composition of muscle and adipose tissue of meat animals. In: Smith, S. B. and Smith, D. R. (eds.) *Biology of fat in meat animals: current advances*, pp. 144–165. Savoy, IL: American Society of Animal Science.

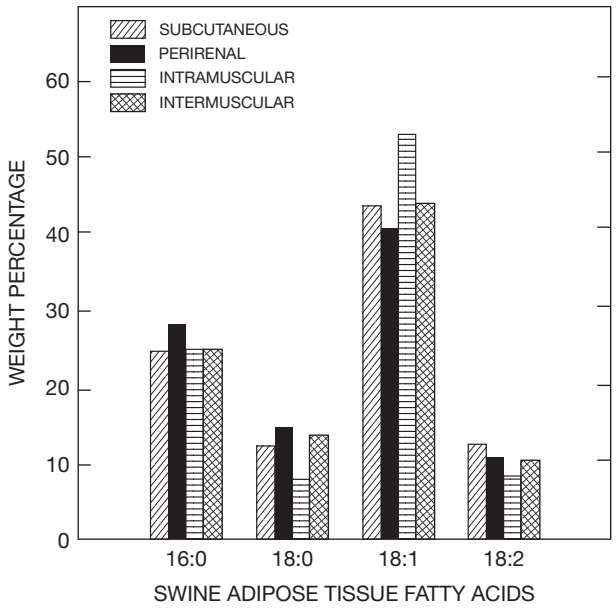

Figure 2 Fatty acid composition of porcine adipose tissues. Reprinted with permission from Rule, D. C., Smith, S. B. and Romans, J. R. (1995). Fatty acid composition of muscle and adipose tissue of meat animals. In: Smith, S. B. and Smith, D. R. (eds.) *Biology of fat in meat animals: current advances*, pp. 144–165. Savoy, IL: American Society of Animal Science.

glycerol by glycerol kinase. In adipose tissue and intestinal mucosal cells, 2-monoacylglycerol (derived from partial hydrolysis of triacylglycerols) provides a portion of the carbon backbone for triacylglycerol synthesis.

Desaturation of Fatty Acids

Whereas ruminal hydrogenation of unsaturated fatty acids makes it difficult to enrich beef with unsaturated fatty acids, desaturation of fatty acids available for digestion in the duodenum ensures that the plasma, muscle, and fat depots are not overly enriched with saturated fatty acids, especially stearic acid. The activity of SCD has been demonstrated in the liver, muscle, subcutaneous fat, and intestinal mucosa of pigs and cattle. The greatest SCD activity is located within the subcutaneous fat. However, substantial activity also is observed in intestinal mucosal cells, where it may function to reduce the concentration of stearic acid in chylomicrons and very-low-density lipoproteins produced within mucosal cells. In this manner, SCD activity within mucosal cells largely influences the composition of fatty acids available for deposition in other tissues.

Fatty Acid Composition and Melting Points of Lipids

Longer-chain fatty acids have higher melting points than shorter-chain fatty acids, and more saturated fatty acids have higher melting points than unsaturated fatty acids. Because of the abundance of stearic and palmitic acids, animal fats are typically solids at room temperature. At 90–100 °F, rendered pork fat will separate with a liquid upper layer and solid material in the lower layer. The solid portion is known as stearin (from the Greek for animal fat) and is composed primarily of triacylglycerols enriched with stearic acid. The liquid portion is composed of the glycerol backbone in the form of esters with the less saturated fatty acids, primarily oleic acid, and is known as lard oil or olein (from the Latin for oil).

Due to differences in fatty acid composition, the melting points of fat from different species vary. Lamb fat typically has the highest melting point, followed by beef fat and then pork fat. With a high polyunsaturated fat content, poultry fat has the lowest melting point of the four species (80–110 °F). Related to the higher internal temperature, the internal fats have higher saturated fatty acid content and correspondingly higher melting points. The fat in the outer layers of the animal body is less saturated with lower melting points. This corresponds to the lower temperature near the body surface and the need to maintain a more liquid state. For example, beef kidney fat has a melting point of 104–122 °F, whereas the external subcutaneous fat has a melting point of only 89–110 °F. Beef brisket subcutaneous fat has an unusually low melting point (77 °F), due to its high concentration of oleic acid. Pork leaf fat has a melting point of 110–118 °F; pork back fat has a melting point of 86–104 °F. Tallow and lard melting points are standardized as titers, which are the minimum temperature at which fat congeals. For edible tallow, the minimum titer is 105 °F, whereas for edible lard, the minimum titer is 100 °F.

Major differences in fatty acid composition are observed across fat depots of beef carcasses (Figure 5). Brisket fat contains over 6% palmitoleic acid and less than 10% stearic acid, whereas subcutaneous fat overlying the flank contains less than 3% palmitoleic acid and more than 15% stearic acid. The highly consistent, negative relationship between palmitoleic acid and stearic acid demonstrated in several studies indicates that the concentrations of these fatty acids are coordinately

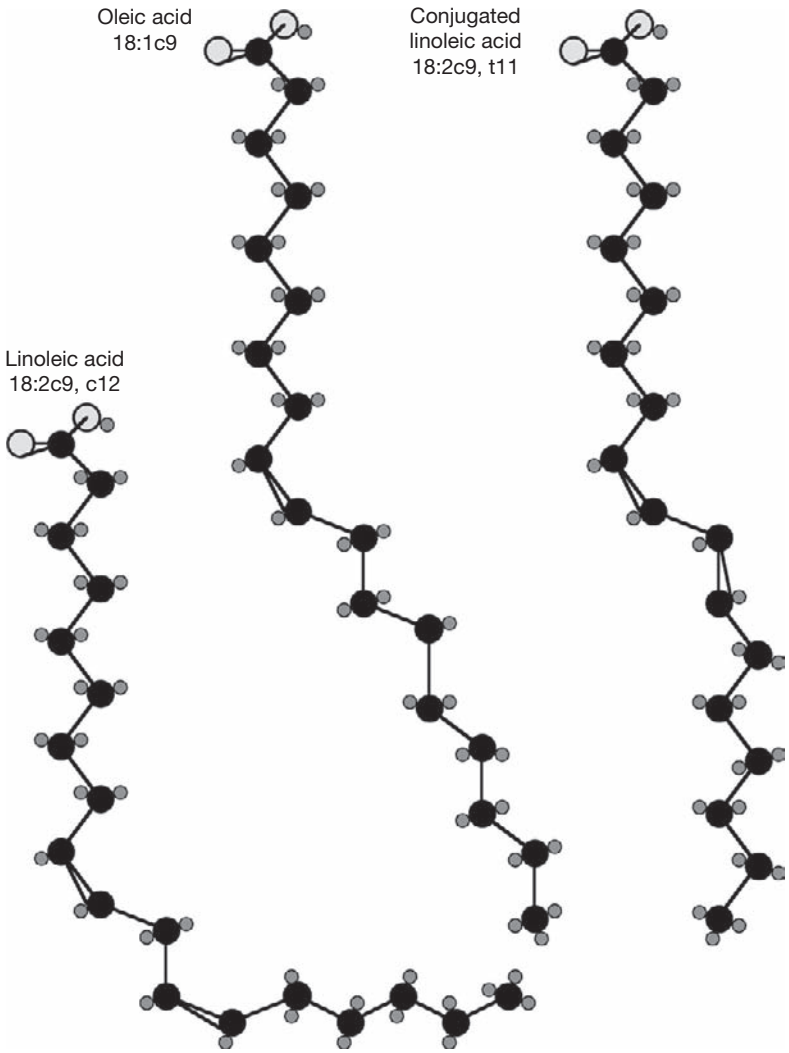

Figure 3 Structures of linoleic acid, oleic acid, and the *cis*-9,*trans*-11 isomer of conjugated linoleic acid. Large filled circles represent carbon; large shaded circles represent oxygen; small shaded circles represent hydrogen.

controlled by SCD. The concentration of stearic acid in adipose tissue lipids is the primary determinant of lipid melting points (Figure 6), and even lipids extracted from fat depots of the same carcasses can exhibit a remarkable range of melting points. These differences in lipid melting point can have major practical importance.

Procedures for Rendering Fats

Early rendering involved the addition of water to the by-products in an open kettle or the injection of steam into a sealed autoclave. This type of process is referred to as 'tanking' and could be used for both edible and inedible products. The fat produced by this method was relatively light in color, but the increased presence of added water resulted in an elevation in the free fatty acid content, reducing the quality and making the product more susceptible to oxidation. Therefore, for economic reasons, dry rendering is most commonly used today.

In the past, each slaughter facility had its own steam-jacketed kettle and rendered its own fat. Although the kettle was jacketed in steam, the by-products were cooked in their own juices with no added water, and therefore, this was considered a dry rendering method. Currently, in the United States, there has been a general shift to centralized rendering operations that collect animal by-products and ship them to a central rendering facility.

Rendering fat involves applying heat, extracting moisture, and separating the fat. To accomplish this, by-products are first ground into a consistent particle size and then cooked at 115–145 °C for up to 90 min. With the heating process, microorganisms such as bacteria and viruses are inactivated (an advantage over other waste product disposal methods). While the by-products are cooked, the jacket steam pressure must be controlled, the mixture agitated, and the temperature monitored for the desired end point. Over the years, there have been advances in the uses of the rendered products and in the rendering methods. Today, more energy-efficient, continuous

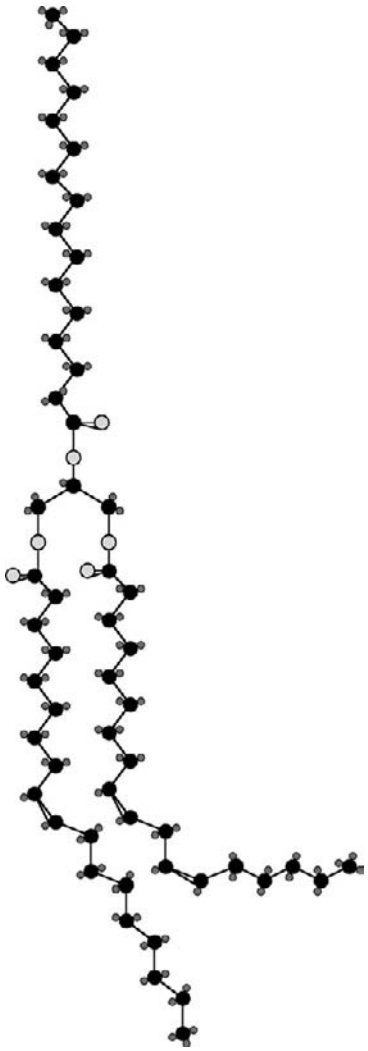

Figure 4 Typical structure of a triacylglycerol: *sn*−1 fatty acid (on left), oleic acid; *sn*−2 fatty acid, palmitic acid; *sn*−3 fatty acid, linoleic acid. This triacylglycerol would be common in porcine adipose tissue. In bovine and ovine adipose tissues, palmitic acid would occupy the *sn*−1 position and stearic or oleic acid would occupy the *sn*−2 position. Large filled circles represent carbon; large shaded circles represent oxygen; small shaded circles represent hydrogen.

processes are used to allow the reuse of process vapors to preheat or dry the materials. Filtering and bleaching systems, as well as refining equipment to remove free fatty acids, may be part of the rendering process.

During rendering, the melted fat floats to the top of the unit by virtue of its lesser density, whereas protein and bone solids settle to the bottom of the rendering kettle. Traditionally, the rendered fat then was skimmed from the top of the rendering kettle. This has been replaced with a screw press that draws off the melted fat, and the melted fat is stored and/or transported in tanks.

Chemistry of Rendered Fats

Rendered fats consist primarily of triacylglycerols (Figure 4). The characteristics of the rendered fat are determined by the composition of fatty acids attached to the glycerol backbone,

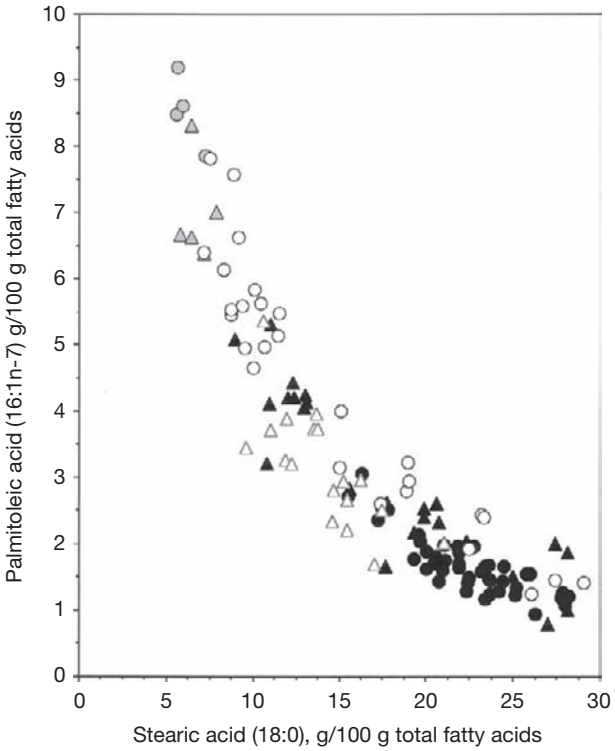

Figure 5 Palmitoleic acid (16:1*n*−7) plotted as a function of stearic acid (18:0) in subcutaneous fat. Lipids were extracted from eight subcutaneous fat depots, taken from 50 carcasses of unknown background. Lipids from brisket fat contained the greatest concentration of palmitoleic acid and least stearic acid, whereas lipids from flank fat contained the least palmitoleic acid and the most stearic acid. Reproduced with permission from Turk, S. N. and Smith, S. B. (2009). Carcass fatty acid mapping. *Meat Science* **81**: 658–663.

which in turn is determined by the source of the raw materials used in rendering. The most abundant fatty acids of animal fat triacylglycerols are palmitic, stearic, and oleic acids, typically comprising 20–25%, 10–30%, and 30–55%, respectively, of the total fatty acids in lean and fat trim. Significant quantities of linoleic and α-linoleic acids are contained in triacylglycerols, especially in rendered poultry fat. Lipids from ruminant tissues also contain measurable amounts of odd-chained fatty acids, branched-chain fatty acids, *trans*-fatty acids, and conjugated fatty acids, all of which are products of ruminal fermentation. Lard and tallow also contain free fatty acids (primarily oleic acid), but these must be no more than 0.5% in edible lard and 0.75% in edible tallow.

When the rendered fat contains more saturated fatty acids, the fat is referred to as hard fat. It is more solid at room temperature and has a higher melting point due to its high concentration of stearic acid. When the rendered fat contains more unsaturated fatty acids, the fat is referred to as a soft fat, which is not as solid at room temperature due to a greater abundance of oleic acid.

Oxidative Rancidity

Rendered fat high in polyunsaturated fatty acids will react chemically with oxygen, resulting in off-flavors and odors

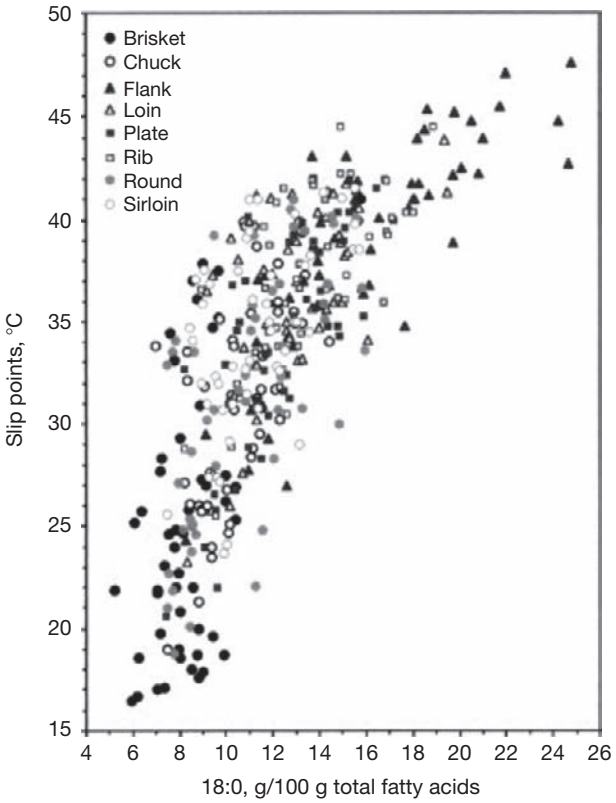

Figure 6 Lipid melting point (slip point) plotted as a function of stearic acid (18:0) in lipids from eight subcutaneous adipose tissue depots. Lipids from brisket adipose tissue had melting points less than 17 °C, whereas lipids from the flank had melting points greater than 45 °C. Reproduced with permission from Turk, S. N. and Smith, S. B. (2009). Carcass fatty acid mapping. *Meat Science* **81**: 658–663.

known as rancidity. Oxidative rancidity is catalyzed by the presence of heat, light, salt, and iron, as well as other elements. Because rancidity is not chemically defined or quantifiable, measurements of oxidation products are often used to indicate quality of the product. One such test is the peroxide value test that measures the milliequivalents (me) of peroxide per kilogram fat. A low peroxide value (<10 me) indicates a nonrancid fat. Lard and leaf lard (from pig abdominal fat) should have a peroxide value no higher than 5 to be considered high quality.

Fate of Rendered Fats

The US rendering industry produces an estimated 0.9 billion kg edible tallow, 1.5 billion kg inedible tallow, 0.6 billion kg yellow grease, 0.1 billion kg lard, and 0.6 billion kg poultry fat. Tallow is primarily derived from rendered beef fat. Choice white cooking grease is derived from pork fat. Yellow grease (not to be confused with off-colored white cooking grease) is restaurant-quality grease or cooking oil and may be from blended sources. Rendered fat provides concentrated sources of energy for use in feed for poultry, aquaculture, and pets. Other uses of rendered fat include soap, candles, and biodiesel.

Conclusions

Fatty acid composition of animal fats varies with animal species, diet, and fat depot. Stearic acid is the primary determinant of lipid melting points, although high concentrations of linoleic acid can effectively reduce melting points. Rendered fats have a great deal of functionality, depending on the source of the fat and the separation techniques. Rendering provides an outlet for animal by-products that would otherwise be unusable. Differential rendering of fat trims that vary in melting points improves the functionality and usefulness of the rendered fat.

See also: Adipose Tissue: White Adipose Tissue Structure and Function; Beef; Fats: Classification and Analysis; Fatty Acids: Fatty Acids; Fatty Acids: Metabolism; Triacylglycerols: Structures and Properties.

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Fatty Acids: Determination and Requirements

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Introduction

Fatty acids, with a few rare exceptions, are the main components of oils or fats. Generally, fatty acids are combined as esters, and they are the main compounds of lipids. The most common ester composed of fatty acids is the triacylglycerol, which is a combination of glycerol and three fatty acids.

According to the last definition, acetic acid would be the first of the fatty acids, but most scientists do not consider it that way. They start the monocarboxylic aliphatic series of saturated fatty acids with the propanoic or butanoic acid. That series extends to an acid with 30 carbon atoms (melissic acid). Usually, fatty acids have an even number of carbon atoms, but there are fatty acids that have an odd number of carbon atoms, such as the common margaric acid, with 17 carbon atoms. In general, the aliphatic chain is linear, but in some cases, fatty acids have branched chains: isoacids and anteisoacids. The unsaturated fatty acids are very important along the aliphatic chain, and we can distinguish them based on characteristics of the double bond: *cis* or *trans* (oleic acid or elaidic acid), position, number (linoleic acid, arachidonic acid), and possible conjugation. Most of the unsaturated fatty acids are *cis* and they have no conjugation. The *trans* form appears more or less frequently in the comestible oils and/or fats when they have been subjected to high temperatures (the energy supplied to the *cis* fatty acids allows isomerization). Because of this, the degree of thermal change in oil and/or fat can be measured using *trans* fatty acid content. Natural oils and/or fats with *trans* fatty acids exist, and an example is the fat of ruminants' milk.

Some of the natural fatty acids show different unsaturation to the double bond: ethylenic bonds, a cycle of three (cyclopropane or cyclopropene) or five (cyclopentene) carbon atoms (sterculic acid and Chaulmoogric acid). Between the fatty acids with saturated cycles, the most common have structures of the type: dihydrosterculic ([9-10] methylen, octadecanoic; 9D, 10L, *cis*). The ring of three carbon atoms makes a plane, and the substituents can be put above or under it. In that case, the first carbon atom of the cycle has four valences linked to four different radicals: —H, $-(CH_2)-COOH$, $-CH_2R_1$, and $-CHR'_1(CH_2)[(CH_2)_7CH_3]$. The next carbon atom bonds in a similar manner: —H, $-(CH_2)_7CH_3$, $-CH_2R_2$, and $-CHR'_2$. The two hydrogens attached to these carbon atoms can be on the same side of the plane (*cis*) or on opposites sides (*trans*). Moreover, two different forms exist for the same isomer, one being the mirror image of the other, depending on the position of the radicals of each carbon atom.

Most of the fatty acids only have the acid role. Nevertheless, in nature, fatty acids with other roles exist, because an organism has synthesized them through a specific biochemistry (e.g., ricinoleic acid, with an OH group; licanic acid, with a ceto group). Due to the action of the oxygen, the mechanics of oxidation and autoxidation produce oxidized fatty acids. The oxygenated functional groups that are part of natural fatty acids are hydroxy, ceto, oxo, and epoxy. Among the components of the natural wax, we can find α-, or 2-, hydroxy acids and β-, or 3-, hydroxy acids, which are also part of bacteria lipids. Among the monohydroxy natural acids, there are acids with different chain lengths and saturations, such as the mentioned ricinoleic acid. Among the polyhydroxy acids, there are fatty acids (most of them saturated) with chain lengths between 14 and 24 carbon atoms (most of them from 16 to 18 carbon atoms), having 2–5 OH groups. The most common positions for these groups are the Ω carbon and the positions adjacent to the unsaturations, when the fatty acid includes them.

Fatty Acid Analysis

Classical Methods

Before the emergence of gas chromatography (GC), the methods for determining the fatty-acid composition and the attributes of fat were basically volumetric methods based on some physical–chemical attribute of the fatty acids that were part of fats. Since the advent of GC, some of these analytical methods are still in use. The determination of fatty acids is the primary goal of fat analysis.

Saponification value. This value is defined by the quantity (in milligrams) of potassium hydroxide needed to saponify 1 g of fat. The value shows us the total amount of fat that is contained in the sample, or the combined volume of free an esterified fatty acids, and, in some ways, this value also shows us the lengths of fatty acids in oils and fats.

Reichert–Meissl index and Polenske index. The first index is a volumetric method that denotes the amount of sodium hydroxide needed to neutralize the fatty acids included in 5 g of fat, which can be dissolved into and swept away by water vapor. So, this index indicates the type and quantity of volatile fatty acids (with short chains). The second index refers to insoluble fatty acids in water.

Iodine value. Organic compounds with unsaturation show the property of adding halogens, as well as other compounds, in double bonds. This property is used for the quantitative determination of unsaturation; determining the number of double bonds identifies the type of unsaturated fatty acids contained in an oil or fat. This parameter is determined by using sodium thiosulfate to titrate the iodine excess that remains after the addition to the double bond, with clear starch serving as the indicator.

Chromatographic Methods

We have seen that some of these rates are connected to chain length and the number of double bonds, in the fatty acids.

After the advent of chromatographic methods, such as GC, the previous methods went out of favor, because GC can

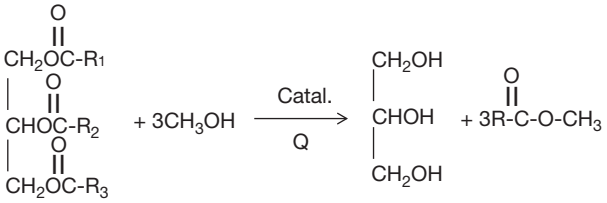
provide more information than the volumetric methods can. GC can also be applied to the analysis of fatty acids and their esters. In general, for fatty acids, direct GC is not commonly used. Instead, a fatty-acid derivatization using methyl esters is carried out, followed by their chromatographic analysis. It is only used for very short-chain acids that are very difficult to be esterified, such as formic acid, acetic acid, propionic acid, butyric acid, and valerianic acid.

The knowledge of the composition, both in quantity and quality, of the fatty acids in oil and fat has always been one of the main issues, because of its importance in oil and fat characterization and the determination of their possible adulteration. The methods for isolating and derivatizing these compounds and the selection of the most appropriate techniques for their determination have been discussed in many scientific publications. The following section describes the GC analysis of these compounds and how they should be prepared for such analyses.

Most of the time, the fatty acids are not free; when they are, they occur only in small amounts. They usually form esters, most often with glycerol, to produce glycerides (mono-, di-, and tri-acylglycerols) and phospholipids. They can also form esters with aliphatic alcohols of linear structure (waxes) or terpenic structure (terpene and sterol esters).

Derivatization of fatty acids

For fatty acid analysis by GC, these compounds have to be analyzed as volatile esters (usually methyl). The usual way to derivatize fatty acids is by forming their methyl esters, because they are more volatile and less polar than free acids, and this makes them easier to elute in chromatographic columns. The reaction that takes place is as follows:



This interesterification reaction involves slow kinetics. The presence of water slows down the reaction ratio and decreases the output. The reaction has to be accelerated by means of catalysis and/or by increasing the temperature to be efficient. The most common methods for preparing the fatty-acid methyl esters use a methanolic medium with alkaline catalysis, with acid catalysis, with alkaline and acid catalysis, or with diazomethane.

Methylation in a methanolic medium with alkaline catalysis

Methylation with sodium methylate is the most widely used method. A solution of sodium methylate in methanol, prepared via the direct reaction of metallic sodium with methyl alcohol, is added to the oil sample. Reflux heating cleans and homogenizes the solution, thus dissolving the methyl esters in the reaction medium. Then, they are extracted by adding a solvent, such as hexane or diethyl ether, and a watery solution of sodium chloride at 10% to separate both phases without their emulsioning. This method presents some drawbacks. Even though the reaction takes place with high yield, there is always the danger that the sodium salts of fatty acids (soaps) might form in the alkaline medium. In addition, free fatty acids do not methylate properly when this procedure is used. To overcome this disadvantage, the fatty matter can be treated with sodium methylate in a methanol solution inside a closed tube and heated. Because of the increased pressure inside the tube, the temperature rises above the boiling point of methanol, up to 85–90 °C. This increases the reaction yield. The heating has to take place for a period of no less than 2 h, however.

Another procedure that gives good results is methylation with potassium hydroxide. It consists of treating a solution in hexane of fat with a KOH 2N methanol solution, shaking it, and letting it decant. It is rapid and has the advantage of not needing heating, which requires eliminating the volatilization losses of acids with chains shorter than C12. This method is used for the methylation of milk fat, and it is widely used to avoid the isomerization of unsaturated fatty acids (analysis of *trans*-isomer fatty acid), but free acids do not derivatize well when this technique is applied; therefore, its use is not advisable for high-acidity oils.

Methylation in a methanolic medium with acid catalysis

Transesterification with methanol can also be catalyzed in an acidic medium. A common procedure consists of making oil react with a hydrochloric acid solution in methanol in a proportion of 2–3%. The acid has to be previously dried in a closed tube at 100 °C for about 1 h. Also with this procedure, it has been observed that the reaction is not complete. The esters are extracted with hexane before being injected.

A variation of the method involves adding a sulfuric acid solution in methanol and hexane and then heating by reflux for 90 min. This method increases the output of the reaction, thanks to the dehydrating action of the sulfuric acid, shortening the time employed in preparing the sample.

Lewis's acidic catalysis is another procedure that efficiently uses methylation with methanol that has been catalyzed with boron trifluoride. The sample is reflux-heated with a methanolic solution of sodium hydroxide until it is homogenized (complete formation of soaps). Next, a methanolic solution of boron trifluoride is added, and the heating continues for a few moments. The esters are extracted by adding a saline solution with heptane, and, thus, the saponification process is not necessary for fatty acids.

Methylation in a methanolic medium with alkaline and acid catalysis

As noted above, methylation in an alkaline medium, besides being incomplete, has the disadvantage that oil or fat can produce soaps (to saponify), and free acids do not methylate well in the alkaline medium. On the other hand, glycerides do not methylate properly in an acidic medium. Thus, a widespread method to improve the yield of the methylation reaction consists of combining both forms of catalysis.

Usually, the sample first undergoes alkaline methylation with reflux heating. The medium is then neutralized and subsequently acidified by means of the methanolic solution of sulfuric acid, before being reheated by reflux. This process

guarantees the hydrolysis of the soaps that might have been formed during the first step and the esterification of the free acids.

Another procedure consists of first treating the sample with a methanolic solution of potassium hydroxide, heated by reflux. Then, dimethyl sulfate is added, and the solution is shaken and heated again. The reaction is monitored using bromocresol green as the indicator. When the reaction is complete, the indicator changes from blue to yellow. Finally, a methanolic solution of sulfuric acid is added to separate the phases. The extract of methyl esters can be injected after purification with alumina.

Methylation with diazomethane

The apparently fast methylation using the diazomethane procedure has been used for free acids. The sample, after being dissolved in methanol, is treated with an ethereal solution of diazomethane, which has been prepared at the very moment of the reaction and under a nitrogen stream. The process is fast, but it requires the previous preparation of the diazomethane from *N*-methyl-*N*-nitroso-*p*-toluene-sulfonamide and 2-(2-ethoxyethoxy) ethanol in an alkaline medium. Diazomethane is toxic and explosive; therefore, some safety measures must be taken.

Formation of methyl esters without the previous extraction of the fat

In some cases, the analyst might be in trouble when undertaking the study of the composition of fatty acids, either because the amount of oily material on hand is minimal or because of extraction selectivity. Another factor to be considered is the possibility that the alteration of the fat during the study itself can induce the analysts to make serious mistakes in their conclusions. Some techniques have recently been developed in order to carry out the interesterification of some oily materials without the previous extraction of the fat. Even though this method can also be useful for oil transmethylation, its main advantage is that it can be directly applied to fresh tissues containing a considerable amount of water, such as olives, without the need for them to be previously dried, as in the aforementioned methods. In this method, both methylation with methanol and acid catalysis are carried out during a dissolving phase. The following reactive mixtures have been successfully used:

1. Methanol:heptane:benzene:2,2-dimethoxypropane:sulfuric acid (37:36:20:5:2, by volume)
2. Methanol:heptane:toluene:2,2-dimethoxypropane:sulfuric acid (39:34:20:5:2, by volume)
3. Methanol:heptane:tetrahydrofurane:2,2-dimethoxypropane:sulfuric acid (31:42:20:5:2, by volume)

The working temperature is 80 °C, and the process takes place in a closed tube. Both extraction and transmethylation are carried out in a single phase because the working temperature is the same for both. After the reaction takes place, the sample is cooled at room temperature, and two different phases occur. The organic (superior) phase containing the methyl esters is then ready for chromatographic analysis. This method is extremely interesting and allows for a great deal of possibilities, such as system automation when there are many samples to be analyzed.

GC analysis of the methyl esters of fatty acids

Once fatty acid methyl esters have been obtained by means of any of the methods mentioned previously, GC analysis can be undertaken with different aims. The first focus, however, is the study of the different types of chromatographic columns and the analysis of the results that can be obtained by using each of them.

The material used to prepare packed columns is the main factor determining the nature of the separations that can be achieved. Liquid phases, such as SE-30, OV-1, JXR, or OF-1, separate fatty acids according to their molecular weight; when the amount in the stationary phase is low, however, fatty acids can also be separated by their unsaturation. Phases such as Apiezón-L separate by unsaturation for the same chain length, with the previous elution of the unsaturated components.

The polyester liquid phases are quite appropriate because they allow for the separation of esters of the same chain length, from 0 to 6 double bonds, eluting the unsaturated components after their corresponding saturated compound. These phases can be classified into three groups:

1. High-polarity phases, such as polymers of ethylene glycol succinate (EGS), diethylene glycol succinate (DEGS), and copolymers of the former with a methylsilicone (EGSS-X™), CP-Sil 84™, and CP-Sil 88™
2. Medium-polarity phases, such as polyethylene glycol adipate (PEGA), polybutanediol succinate (BDS), and EGS copolymers with a high proportion of methylsilicone (EGSS-Y™)
3. Low-polarity phases, such as polyneopenthylglycol succinate (NPGS), EGS with a phenyl-silicone (EGSP-Z™), Carbowax 20M™, and Silar 5CP™

EGSS-X™, EGSS-Y™, and newer phases of equivalent polarity are widely accepted as the most useful representatives of the first and second groups, respectively, because of their relatively high thermal stability, particularly in work with packed columns. The phases from the group of highest polarity are stable at temperatures above those possible with EGSS-X™, and they produce excellent separations of polyunsaturated fatty acids. **Figure 1** shows the analysis using columns of 15% of EGSS-Y™ in a 100–120 mesh silanized and acid-washed support.

The low-polarity phases are mainly utilized in wall-coated open tubular (WCOT) and support-coated open tubular (SCOT) capillary columns because the saturated and monoenoic compounds of the same chain length are separated rather poorly when these phases are used in packed columns. At the same time, the quality and nature of the separations are related to the quantity applied to the stationary phase support. For example, low polyester levels are advisable for eluting the methylesters of long-chain polyunsaturated fatty acids at low temperatures. The relative retention time of fatty acid esters (generally 16:0 or 18:0) tends to shorten as the amount of liquid phase in the support decreases. Unfortunately, variation in the retention times for the esters of fatty acids is inevitable when the column ages through use, as the stationary phase polymerizes further from the column.

The nature of the support also influences the quality of the separations. Silanized and acid-washed materials are inert, and the separations are controlled only by means of the liquid phase. A minute particle size (160–120 mesh) is the best for analytical columns.

Some losses of esters of polyunsaturated acids partly respond to the use of very active supports or aged columns and to the transesterification by the polyester liquid phase, which is caused by the remnants of the catalyzer used in the fabrication of the polyester.

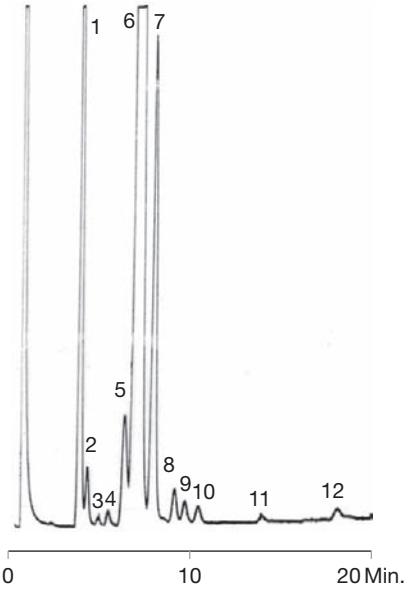


Figure 1 Chromatogram of fatty-acid methyl esters in packed column EGSS-X. 1: Palmitic; 2: palmitoleic; 3: margaric; 4: margaroleic; 5: stearic; 6: oleic; 7: linoleic; 8: linolenic; 9: arachidic; 10: gadoleic; 11: behenic; 12: lignoceric.

The use of WCOT columns of fused silica has led to remarkable improvements in separations with higher resolution, higher analytical precision in quality and quantity, and a higher sensitivity, as well as a shortening of analysis time and a reduction in the preparation of analysis procedures. Polyglycol phases based on Carbowax 20M™, FFAP™, Supelcowax-10™, and SP-1000™ seem to have been the most utilized in recent years. Figure 2 shows a chromatogram of olive oil fatty acids carried out in a SP-2380 capillary column (60 m, 0.25 mm, i.d. 0.25 μm film thickness).

Stationary phases are the same as those used in packed columns; the only difference is that, instead of impregnating a support, they wet the interior wall of the capillary tube. Most phases are now chemically bonded to the tube wall, which decreases loss in the column and, in turn, increases duration and temperature resistance and improves the resolution.

Trans-*fatty-acid determination*

Trans-fatty-acid isomers should be almost completely absent in oils and fats, because they are formed during technological treatments such as partial catalytic hydrogenation; the content and distribution of the *trans* isomers depend on the hydrogenation parameters (i.e., temperature, hydrogen pressure, and the kind of catalyst). Thus, the quantification of *trans* fatty acids is of great relevance to the authentication of oils and fats.

Thin-layer chromatography with silver nitrate

Since its introduction in 1962, thin-layer chromatography (TLC) using silica gel coated with silver nitrate has been very useful for lipid analysis, because fatty acids can be separated according to the number of unsaturations, the geometric configuration of the double bonds, and even the position of the double bond. Mobile phase systems using diethyl ether or

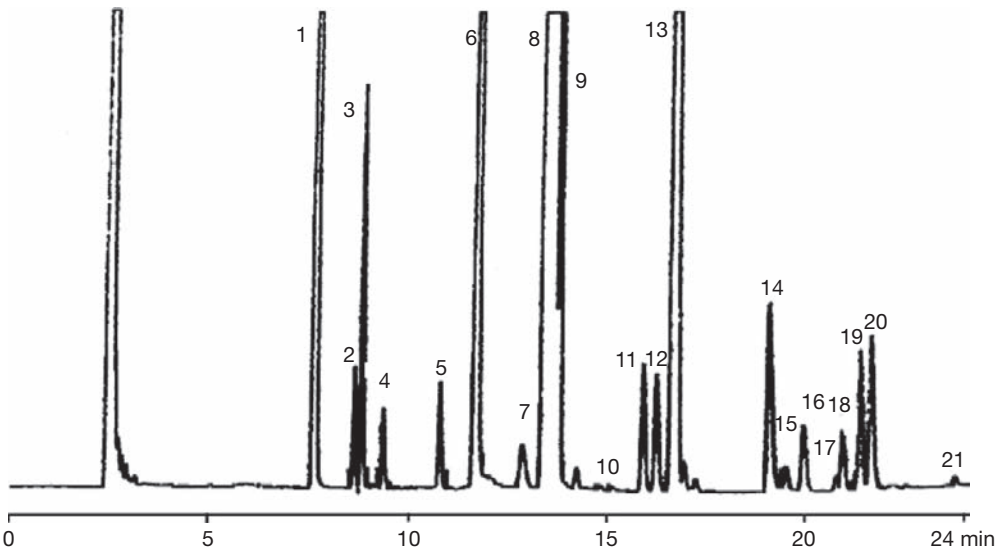


Figure 2 Chromatogram of fatty-acid methyl esters in capillary column SP-2380, 60 m. 1: palmitic; 2: *trans* palmitoleic; 3: *cis* palmitoleic; 4: margaric; 5: margaroleic; 6: stearic; 7: elaidic; 8: oleic (ω9); 9: oleic (ω7); 10: linoleic (9t, 12t); 11: linoleic (9c, 12t); 12: linoleic (9t, 12c); 13: linoleic (9c, 12c); 14: arachidic; 15: linolenic (9t, 12c, 15t); 16: linolenic (9c, 12c, 15t); 17: linolenic (9c, 12t, 15c); 18: linolenic (9t, 12c, 15c); 19: linolenic (9c, 12c, 15c); 20: gadoleic; 21: behenic.

hexane are more useful for separating general kinds of methyl esters of the fatty acids, although it is not possible to resolve them if they have three or more double bonds.

In fact, separations with more than four double bonds are not easy, and mixtures of hexane/ diethyl ether 90:10 (v/v) divide compounds with two double bonds. The obtained bandwidths are visualized under ultraviolet light, after revealing them with 2′,7′-dicloroflurosceine, and the compounds with zero to two double bonds are eluted with diethyl ether or chloroform. The chloroform/methanol 9:1 is necessary to recover completely unsaturated compounds.

Monoenoic natural fatty acids, which contain *trans* double bonds, can be studied through separation of *cis* compounds by TLC with silver nitrate eluted with hexane/diethyl ether (9:1, v/v) as a mobile phase and eluted together with saturated compounds. With care, it is also possible to separate positional isomers of the unsaturated fatty acids by TLC with silver nitrate. With a high concentration of silver nitrate (in the order of 30%) and developing with toluene at low temperatures (−5 to 25 °C), the methyl esters 6-; 9-y 11-*cis*-octadecenoic are well-separated.

Infrared spectroscopy methods

Infrared spectroscopy was used to determine the amount of *trans* isomers of fatty acids, replacing chromatographic absorption methods with silver nitrate, until the appearance of capillary columns of great length (60–100 m) and very polar stationary phase, which have allowed the determination of these compounds. First, to carry out analysis using infrared spectroscopy, fatty-acid methyl esters must be produced using some of the mentioned procedures, before measuring absorption at wavelengths of 10.36 μm. Before any measurements are carried out, it is necessary make a calibration with standard solutions, however.

GC methods

The determination of *trans* fatty acids has been carried out by infrared spectroscopy. However, the use of GC with great-length capillary columns allows the determination of the percentage of *trans* fatty acids, as well as the separation of individual fatty-acid esters of a given chain-length that differ in degree of unsaturation and in the positions of the double-bonds (e.g., two isomers of 18:1 and of 18:3 are separated). In the analysis of the methyl esters of polyunsaturated fatty acids in oil, it is important to note that the shorter the distance between the last double bond and the end of the molecule, the longer the retention time of the isomer. A good correlation exists between the data supplied by GC and infrared instruments.

To determine the composition of *trans* fatty acids by GC, fatty-acid methyl esters should be prepared by means of any previously mentioned procedure. However, it has been observed that, for samples with low *trans*-isomer contents, methylation methods using heating produce an increase in those isomers. Therefore, for this determination, cold methylation with methanolic potassium hydroxide or diazomethane is recommended. In addition, different injection temperatures have been studied because there is always the possibility that *cis*-fatty-acid isomerization might occur in the chromatographic injector. The results of the analysis of a virgin olive oil sample with low *trans*-isomer content (Table 1) are similar for methods B and C, whereas method A yields results that are slightly higher in elaidic acid (18:1t), which is in agreement with the preference for isomerization in an acidic medium. On the other hand, a remarkable increase in elaidic acid seems to occur as the injection temperature increases.

Table 1 Effect of injection temperature and methylation method on the elaidic and linoleic (9c, 12t) acid content of a virgin olive oil

	Method[a]		*Method*[b]		*Method*[c]	
T °C injector	200	300	200	300	200	300
C18:1 9t	0.026	0.042	0.017	0.030	0.016	0.024
C18:29c,12t	0.012	0.090	0.012	0.013	0.015	0.013

[a]Methylation in a methanolic medium with alkaline and acid catalysis.
[b]Methylation with sodium methylate.
[c]Methylation with potassium hydroxide.
Source: León-Camacho, M. and Cert Ventulá, A. (1994). Recomendaciones para la aplicación de algunos métodos analíticos incluidos en el reglamento cee 2568/91 relativo a las características de los aceites de oliva y de orujo de oliva. *Grasas y Aceites* **45**, 395–401.

After numerous injections, an analyst might also observe an increase in the amount of elaidic acid that results from the catalytic action of the residues accumulated in the injector. On the contrary, the cleaner the injector, the lower the amount of acid that will result. Consequently, cold methylation is used, with a solution of potassium hydroxide in methanol at a temperature no higher than 225 °C.

Regarding GC analysis, the methods indicate that the amount of injected sample must be such that a peak of arachidic acid (C20:0) should represent over 20% of the full scale, although it must not be higher than 50%. It has been proved that overlapping between the peaks corresponding to both elaidic and oleic acid and a quantitative distortion, either through discrimination or saturation of the detector, can occur.

The column used by GC analysis should show a chromatographic profile similar to that presented in Figure 2, because the linolenic acid has a slightly inferior retention time than the eicosenoic acid. The use of columns of lesser polarity produces the appearance of peaks that interfere in the determination of the *trans* isomers of linolenic acid. These peaks can resemble ethyl or other esters. The position of the *trans* isomers, therefore, has to be precisely determined by means of the analysis of a refined oil sample.

An HP-88 capillary column, coated with 88% cyanopropyl aryl siloxane (100 m × 0.2 mm i.d × 0.2 μm film thickness), or a Varian Chrompack CP-Sil 88 column, coated with cyanopropyl polysiloxane (100 m × 0.25 mm i.d. × 0.2 μm film thickness), is able to provide an accurate quantitation of *trans*-fatty-acid isomers previously derivatized as methylesters. A better peak resolution is obtained if the carrier gas is hydrogen instead of helium. Furthermore, no silver-ion prefractionation of *trans* fatty acid (by TLC, SPE, or HPLC) is required prior to GC analysis.

Free-fatty-acid determination

Acidity

Acidity was determined through a volumetric method based on fatty-acid neutralization using a nonaqueous solution of sodium or potassium hydroxide and an acid–base indicator

(usually phenolphthalein) to determine the final point of the titration.

Gas-chromatographic method

Free fatty acids, together with mono- and diacylglycerols, form a part of the fat polar fraction, and their analysis can be carried out using a methodology for suitable separation and quantification. This fraction is isolated by solid-phase extraction using diol columns and 1,3-dimyristin as the internal standard. A solution of hexane:methylene chloride:ethyl ether (89:10:1 v/v) is first applied to the column to eliminate triacylglycerols. Subsequently, a portion of chloroform:methanol (2:1 v/v) is applied to the column and collected. This fraction contains free fatty acids and mono- and diacylglycerols, and it is evaporated to dryness in a rotary evaporator under reduced pressure. The residue is silylated and left at room temperature for 15 min. Then, the residue is injected into the GC system using a DB-17HT column and a flame ion detector. In the chromatogram, the free fatty acids appear in first place, followed by monoacylglycerols and subsequent diacylglycerols. An example of this can be observed elsewhere in this encyclopedia.

See also: Fats: Classification and Analysis; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fatty Acids: *Trans* Fatty Acids; Triacylglycerols: Characterization and Determination; Triacylglycerols: Structures and Properties; Vegetable Oils: Composition and Analysis.

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Fatty Acids: Essential Fatty Acids

B Lands, College Park, MD, USA

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The Nature of Staple Foods

As human society slowly changed from hunter-gatherer lifestyles to more agricultural and urban lifestyles, food from capture or culture remained the source of nutrient energy needed for physical activity and of essential amino acids (EAAs) and essential fatty acids (EFAs) needed for growth and healthy tissue functions. Plants make all of these needed nutrients, and they also contain the vitamins and minerals that sustain healthy human life. Local community decisions about soil and climate conditions have influenced the shift from opportunistic capture to deliberate culture of a food, but the logical basis by which people selected a regional staple is not appreciably evident in any written history. Eventually, domestication of plants and animals led to regional foods that were available on a more reliable basis with less expenditure of human energy. Having sufficient food energy (in carbohydrate or fat) is one obvious feature of a staple food plus its stability on storage over time. Awareness of EFA actions seems to have played no role in creating the markets for staple foods that were well established by the mid-twentieth century.

Vitamins and essential nutrients were not discovered until the twentieth century after trial and error experiences had already led to established staple foods with adequate protein (EAA) to successfully support healthy human growth and development. However, knowledge of EFA abundance and action remains a topic of growing awareness and concern among the biomedical community in the twenty-first century. Current knowledge of metabolism and physiology of EFAs indicates that some traditional ethnic lifestyles unknowingly developed healthier daily supplies of EFA than others. Now, careful use of recent information about EFA is likely to give new insights and priorities for current staple foods.

Plants make the essential 18-carbon polyunsaturated fatty acids (PUFAs) that are essential for growth, development, and function of healthy animals and humans. Figure 1 shows how two different desaturases (not found in animals) convert nonessential omega-9 ($n-9$) oleic acid into the omega-6 ($n-6$) and omega-3 ($n-3$) acids. The 18-carbon polyunsaturated acids, predominantly linoleic acid (LA) and alpha-linolenic acid, are made and stored in plant tissues. They are not made in animals for which they are essential vitamin-like nutrients. Small amounts of two other EFAs, stearidonic acid and gamma-linolenic acid, are formed in a few plants by the action of a $\Delta 6$ desaturase on the $n-3$ or $n-6$ precursor. A similar desaturase enzyme occurs in animals.

Table 1 shows recent annual worldwide production of major plant food staples along with the balance of their $n-3$ and $n-6$ EFAs. The Omega-3–6 Balance Scores are derived from the difference in milligrams per kilocalorie (kcal) of eleven different $n-3$ and $n-6$ nutrients in a food item. A negative value means that there is more $n-6$ than $n-3$ nutrient, whereas a positive value indicates more $n-3$ than $n-6$ nutrient. The grains listed at the top of Table 1 have high contents of carbohydrates, which provide food energy. Grains also have sufficient protein to provide needed EAAs. Importantly, the dry forms of grains are stable over long storage times and can be transported fairly easily. The legumes in the center of Table 1 have twice the amount of protein. The dried seeds also are stable over long storage times and can be transported fairly easily. The plant foods at the bottom of the table have a higher water content and lower nutrient density that limits their distribution away from local markets. In the absence of a scientific rationale favoring any particular staple food, convenience, historical precedent, and financial marketing considerations tend to drive the choice of staples among different communities.

Ethnic Food Traditions Affect EFA Intakes

The low magnitudes of many Omega-3–6 Balance Scores in Table 1 show that most major plant staples have relatively balanced amounts of $n-3$ and $n-6$ EFAs. However, the very large negative values (-36 and -59) for the processed oils from these staples indicate a presence of much greater amounts of $n-6$ than $n-3$ nutrients. These values show how important nutritional differences can occur between a food and its processed product. Later sections in this article note some unintended consequences of processing staple foods into forms not used earlier during the development of human societies.

The ability of plants to make their own carbohydrates, amino acids, fatty acids, and vitamins from their surrounding supply of nitrogen, carbon dioxide, minerals, and solar energy allows each plant staple to have gene-determined predictably consistent composition. The nutrient composition of most plant-based foods has remained fairly constant throughout the transition from prehominid times to the present. However, some cultivars developed by selective breeding have specialized differences in composition and yield. Some high-oleic, low-linoleic seed oils come from selected sunflower and safflower strains, but they are not yet widely marketed. The major change in staples as human populations increased was a shift from the plant foods at the bottom of Table 1 toward increased use of the staple commodities near the top. Plants at the bottom of Table 1 were more common in tropical Africa during early hominid times and may have been major foods during early human evolution. Humans began to culture grains as staples only about 10 000 years ago.

Soybeans and peanuts have much more fat than the other plant staples, and they provide a profitable opportunity to separate the oil from the protein to be stored and marketed separately. Food oils also can be recovered as by-products from other commercial activities (e.g., cottonseed oil or lard). Over 150 million tons of food oils were produced in 2012 (Table 2).

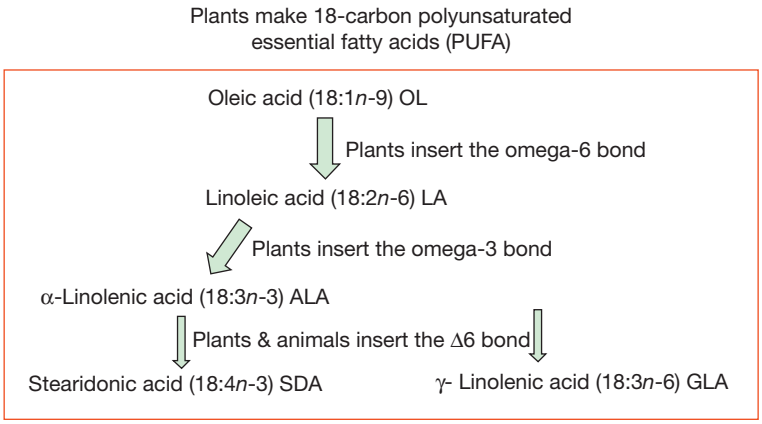

Figure 1 Plants make 18-carbon polyunsaturated essential fatty acids (PUFAs).

Table 1 Production and composition of plant staples

Staple food name	*Annual production (million tons)*	*Protein (%)*	*Carbohydrate (%)*	*Fat (%)*	*Omega-3–6 Balance Scores*
Corn, maize	823 Mt	9.4	76	4.7	−3 oil, −59
Rice, white	690 Mt	7.1	79	0.7	−0.3 oil, −36
Wheat	685 Mt	12.6	71	1.5	−2 oil, −54
Sorghum	66 Mt	11.3	79	3.3	−4
Millet	30 Mt	11	73	4.2	−5
Oats	24 Mt	17	66	6.9	−6
Soybeans	231 Mt	36	30	20	−17 oil, −59
Peanuts	45 Mt	26	16	49	−29 oil, −36
Beans	23 Mt	22	62	1.4	−0.3
Peas	11 Mt	24	64	1.2	−1
Cassava	233 Mt	1.4	38	0.3	−0.1
Sweet potato	110 Mt	1.6	20	0.1	−0.5
Yam	52 Mt	1.5	28	0.2	−0.4
Plantain	34 Mt	1.3	32	0.4	−0.1
Taro	7 Mt	0.5	26	0.1	−3

Table 2 Production and composition of food oils and fats

Staple food name	*Annual production (million tons)*	*Omega-3–6 Balance Scores*
Palm oil	53	−10
Soybean oil	41	−59
Rapeseed oil	24	−12
Sunflower oil	15	−74 (−4, high oleic)
Margarine	14	−69 to −21
Cottonseed	5	−59
Butter	5	−1
Coconut oil	3	−2

The introduction of hydrogenated vegetable oils at the start of the twentieth century began providing cheap semisolid shortenings and margarines (Omega-3–6 Balance Scores = −21 to −69) that partially replaced lard (−10) and butter (−1) for household and commercial food preparation. Food marketing messages about food oils built new cultural lifestyles by the late twentieth century, and regional ethnic differences in food habits gave way to global corporate marketing priorities. Importantly, the new staple food oils and fats continue to be adopted without open public discussion and rigorous evaluation of the health impact of the relative contents of $n-3$ linolenate and $n-6$ linoleate EFAs.

Animal Metabolism Has Competing Actions of $n-3$ and $n-6$ EFAs

When humans and animals eat plants, the EFAs are metabolized by desaturase and elongase enzymes (see Figure 2) that convert the 18-carbon PUFAs into 20- and 22-carbon highly unsaturated fatty acids (HUFAs). Most of the enzymes are indiscriminate, allowing the $n-3$ and $n-6$ forms of EFA to compete similarly for reaction during each metabolic step. As a result, the relative abundance of a competitor affects the amount of conversion that occurs. The proportions of $n-3$ and $n-6$ in the HUFA accumulated in human and animal tissues predictably reflect the proportions of competing $n-3$ and $n-6$ PUFA in the foods eaten. In contrast to what occurs in plants, the EFA composition in human and animal tissues is determined by both genetics and environment.

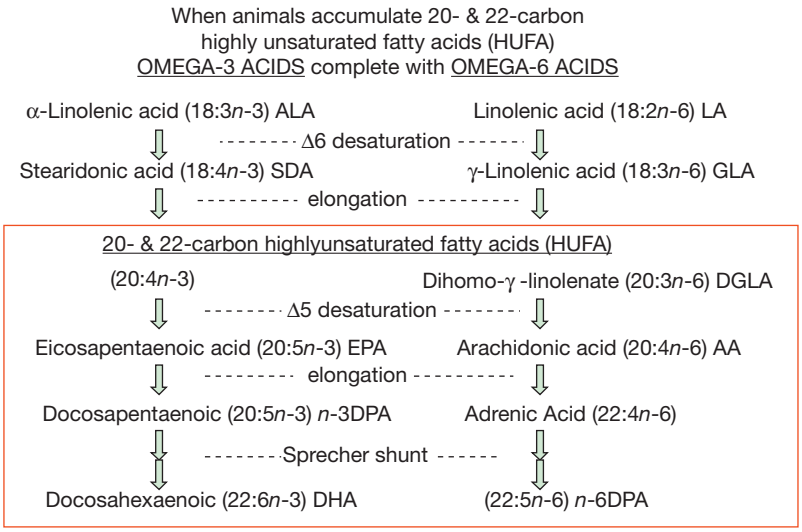

Figure 2 When animals accumulate 20- and 22-carbon highly unsaturated fatty acids (HUFAs), the omega-3 acids compete with omega-6 acids.

Table 3 Production and composition of animal staples

Staple food name	*Annual production (million tons)*	*Protein (%)*	*Carbohydrate (%)*	*Fat (%)*	*Omega-3–6 Balance Scores*
Meat, pig	109	22	0	4	−4
Meat, chicken	93	20	0	3	−8
Meat, cattle	63	20	0	9	−3
Cheese	17	23	3	30	0
Fish, carp	25	18	0	6	+14
Fish, freshwater	7.5	19	0	1	+18
Clams	5.0	15	0	1	+14
Oysters	4.7	6	3	2	+50
Fish, tilapia	4.5	20	0	2	+7
Shrimp	4.3	20	0	1	+19
Salmon	3.2	20	0	6	+73 (+28, farmed)

The Sprecher shunt noted at the bottom of Figure 2 involves an elongase that acts faster with the $n-3$ than the $n-6$ intermediate during a complex set of multiple metabolic steps coordinated between subcellular regions, which gives 22-carbon acids with a Δ4 double bond. This process leads to a greater abundance of 22:6$n-3$ (DHA) than 22:5$n-6$ ($n-6$DPA).

An interesting human genetic haplotype that has faster Δ6 and Δ5 desaturation activities was apparently selected favorably among humans in central and western Africa where plant staples were like those at the bottom of Table 1. Faster conversion of $n-3$ and $n-6$ PUFAs to tissue HUFAs makes African-Americans more responsive than Caucasians or Asians to the high proportions of $n-6$ nutrients in typical American diets. Health-related consequences of these different nutrient responses are noted later in this article.

By domesticating animals, agriculture provided a continual supply of animal-based staple foods that do not require wide-ranging efforts of hunting and gathering. Rather, the meat, milk, and cheese acquired from livestock are eaten with short storage times. However, one important (and often ignored) nutritional aspect of these foods is that the EFA present in domesticated animals is controlled by the EFA that the farmer provides to the animal. As a result, different farming environments produce different balances of $n-3$ and $n-6$ EFAs in the animal product. The food chain that provides EFA to humans merits continuing careful attention and management.

Public attention to animal-based staple foods (Table 3) is focussed primarily on protein content, even though plants do provide appreciable protein. Animals store little carbohydrate, and their energy reserves are mainly stored in fats that have mostly nonessential palmitic, stearic, and oleic acids. Farmers feeding grains to their domesticated animals are adding EFA that accumulate in the animal fat. The negative Omega-3–6 Balance Scores for pig, chicken, and cattle reflect the impact of feeding grains (scores near −3 to −6) in contrast to leaves, grass, and foliage (scores near 0 to +3). The milk, cheese, and yogurt produced in high alpine meadows have more positive scores than those from Japan (where there is limited pasture acreage).

In the same manner, high positive scores for aquatic animals reflect the impact of the algal-based food web in the aquatic environment. At present, wild and domestic fisheries worldwide annually provide fish at a level of 91 million tons (Mt) by capture and 67 Mt by culture plus aquatic plant capture of 1 Mt and culture of 24 Mt. Fisheries remain the

last large hunter-gatherer enterprise on Earth, and they are increasingly being 'managed' by people hoping to sustain some suitable level of capture for the growing human population.

Eating captured seafoods provides humans with much more $n-3$ than $n-6$ EFAs, and those $n-3$ EFAs are predominantly long-chain $n-3$ HUFAs rather than the 18-carbon PUFAs found in domestic plants. However, modern large-scale intensive culture of seafood can involve feeding grain and plant foods that shift EFA proportions away from those normally found in the less intensive natural algal-based food web of oceans and lakes. These commercial, financial factors strongly affect the scores for the cultured staples listed in Table 3. As a result, scores in the table are conditional average values subject to differing environmental variations. As wild-caught seafoods continue to have increasing societal pressures to diminish their capture, the issue of what balance of $n-3$ and $n-6$ EFAs occurs in cultured animal staples will become an increasingly serious topic to manage carefully.

Highly Unsaturated Fatty Acids Form Bioactive Mediators

While linolenic acid is not a dietary essential for plants, it has important physiological actions in plant survival. This occurs by a lipoxygenase-catalyzed oxidative conversion of the acid to the plant hormone, jasmonate, which optimizes plant growth, development, and immunity. For animals and humans, the linolenic acid and LA obtained in foods also undergo oxidative conversion to many potent bioactive derivatives that optimize growth, development, and immunity in animals.

One of the first recognized signs of severe EFA deficiency in laboratory animals was an impaired epidermal water barrier essential to effective growth and survival in low humidity. Two lipoxygenases, ALOX12B and ALOXE3, participate in forming ceramide and hepoxilin derivatives that strengthen the epidermal barrier. Fortunately, the amount of EFA in the diet needed to prevent deficits in the barrier and in growth is $<0.3\%$ of food energy. Because nearly all edible staples can provide this amount, EFA deficiency is very rare among humans.

An associated biomarker of EFA deficiency is the occurrence of more than 50% of tissue HUFA in the form of the $n-9$ HUFA, eicosatrienoic acid. This happens when the supply of dietary 18-carbon EFA is too low to compete effectively with the large amount of endogenous oleate for the elongation and desaturation enzymes that form the $n-3$, $n-6$, and $n-9$ HUFAs. Again, most dietary staples provide enough EFA to maintain the $\%n-9$ in HUFA of tissues below 2% of HUFA. In fact, the frequent occurrence of undetectable levels of $n-9$ HUFA in blood samples from Americans may indicate that they are eating much more EFA than is needed for good health.

One of the most important features of EFA action in humans is the oxidative conversion of HUFA into a large group of potent hormonelike mediators called eicosanoids. These induce signaling actions by many different specific receptors that occur on nearly every cell and tissue of the body. Such signaling is essential for coordinating tissue responses, and excessive signaling can shift healthy physiology toward pathophysiology. Potent hormonelike mediators formed by the oxidative action of cyclooxygenases are prostaglandins (PGs) and thromboxanes (TXs). Mediators formed by lipoxygenases include leukotrienes (LTs), hydroxyeicosatetraenoic acids (HETEs), hydroxyoctadecadienoic acid (HODE), lipoxins (LXs), resolvins (Rv's), hepoxilins (Hx's), maresins (Ma's), and protectins (PDs). Cytochrome P450 enzymes oxidize HUFA to bioactive epoxides (EET, EEQ, and EDP). Also, endocannabinoids are EFA derivatives with potent actions that regulate neural signaling functions.

After the recognition of EFAs in 1929, biomedical research on their actions progressed slowly until the introduction of sensitive gas chromatographic methods by 1960 that gave detailed insights to EFA occurrence and metabolism. Soon thereafter, the 1964 discovery that $n-3$ and $n-6$ HUFAs can form eicosanoids with potent physiological impacts led to an explosive expansion of biomedical research as shown in Figure 3. This interest was fueled by intensive pharmaceutical company interest in developing new drugs to moderate excessive actions of $n-6$ bioactive mediators formed by a set of metabolic steps called 'the arachidonic acid cascade.' While the pace of publication of papers on eicosanoids per se slowed after 1990, interest in the physiological impacts of $n-3$ and $n-6$ essential nutrients on a wide range of human health conditions continues to expand.

The Balance of $n-3$ and $n-6$ HUFA Actions Affect Health

The essential $n-6$ linoleic acid is a very potent and effective nutrient at very low intake levels. However, in the absence of $n-3$ nutrients, it seems to have a very narrow therapeutic window that moves readily from healthy physiology to pathophysiology. The window of safe efficacy is widened by dietary $n-3$ nutrients. That makes the balance between $n-3$ and $n-6$ EFA nutrients a vital aspect of healthy human physiology. The

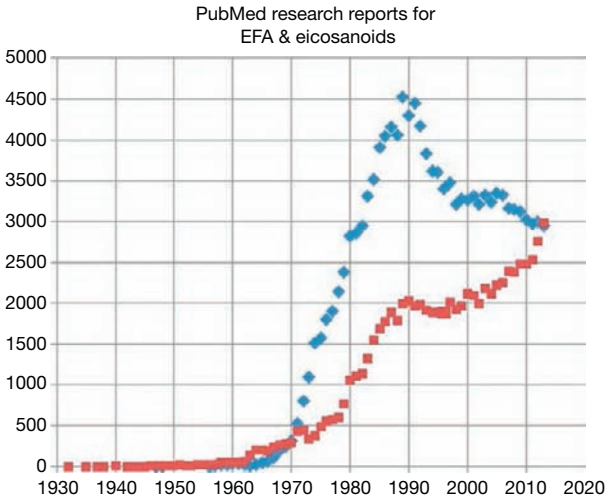

Figure 3 Research reports for EFA and eicosanoids. Squares indicate annual reports retrieved by PubMed for the combined terms: essential fatty acids or linoleic or linolenic or arachidonic or eicosapentaenoic or docosahexaenoic. Diamonds indicate annual reports retrieved for the term eicosanoids.

paradox of a therapeutic window involves both benefit and harm from an agent.

Such a situation is apparent in dose-related actions of aspirin-like NSAIDs. Increasing amounts diminish harmful actions of $n-6$ eicosanoids, whereas doses above the level that gives benefit can begin to impair needed eicosanoid-mediated actions and increase the risk of adverse side effects. While low-dose aspirin is widely used to decrease the risk of $n-6$ thromboxane-mediated heart attacks, high doses of NSAIDs have well-recognized adverse actions. In the case of dietary $n-6$ linoleate, intakes from 0.05 to 0.50 en% increasingly meet the need for essential tissue functions, whereas intakes above those amounts increase the likelihood of $n-6$-mediated overreactions with well-recognized adverse side effects that we treat with medications. Such a transition from benefit to harm is not evident for the dietary $n-3$ nutrients that compete with and moderate the $n-6$ nutrients.

Figure 4 gives the context for the impact on health conditions by $n-3$ and $n-6$ nutrients and their accumulated tissue HUFA. The proportions of $n-3$ and $n-6$ nutrients determine the proportions of $n-3$ and $n-6$ accumulated in tissue HUFA. This makes the $\%n-6$ in HUFA a useful biomarker for average dietary EFA intakes. When the stored $n-3$ and $n-6$ HUFAs are released in the tissues, they are converted by cyclooxygenases to prostaglandins. Slower rates of the formation of $n-3$ than $n-6$ prostaglandins and weaker actions of the $n-3$ than $n-6$ mediators at selective receptors cause greater proportions of $n-3$ than $n-6$ HUFA to give less severe signaling (and pathophysiology). These dynamics are evident in cardiovascular disease (CVD) and heart attacks that are so prevalent in Americans. Excessive $n-6$ mediator signaling leads to greater inflammation and oxidative stress in atherosclerotic vascular wall plaques and in greater thromboxane-activated platelet-mediated thrombosis. Such conditions are less frequent in people with 50% $n-6$ in HUFA than with a typically American value of 78% $n-6$ in HUFA. In this way, the $\%n-6$ in HUFA is a useful biomarker for health risk assessment (HRA).

When stored $n-3$ and $n-6$ HUFAs are released in the tissues, they are also converted by lipoxygenases to leukotrienes. A 10- to 20-fold slower formation of $n-3$ than $n-6$ cysteinyl leukotrienes gives less intense signaling in bronchopulmonary conditions like asthma and chronic obstructive pulmonary disorder. Also, the 50-fold weaker actions of the $n-3$ leukotriene B5 (LTB5) compared to $n-6$ LTB4 at its selective receptor give much less intense amplification of inflammatory conditions in people with 50% $n-6$ in HUFA than with 78% $n-6$ in HUFA. The potent amplification of immune-mediated inflammatory conditions by $n-6$ LTB4 (rather than $n-3$ LTB5) causes many widespread and diverse impacts on human health. These effects are mediated by increasing the formation and action of inflammatory cytokines and chemokines, which can further amplify many harmful health conditions.

A growing number of biomedical research reports (Figure 3) link imbalanced $n-3$ and $n-6$ EFA intakes and excessive $n-6$ eicosanoid actions to unwanted health conditions, including arthritis, asthma, atherosclerosis, bone loss, cancer growth, heart attacks, depression, and suicide; length of hospital stays; classroom disruptions; oppositional behavior; unproductive workplace behavior; and annual healthcare costs.

Three examples in Figure 5 show how the biomarker for the balance of EFA intake, the $\%n-6$ in HUFA, provides a useful HRA measurement for predicting the risk of several unwanted conditions. Coronary heart disease (CHD) mortality rates per 100 000 people are much higher for people with more than

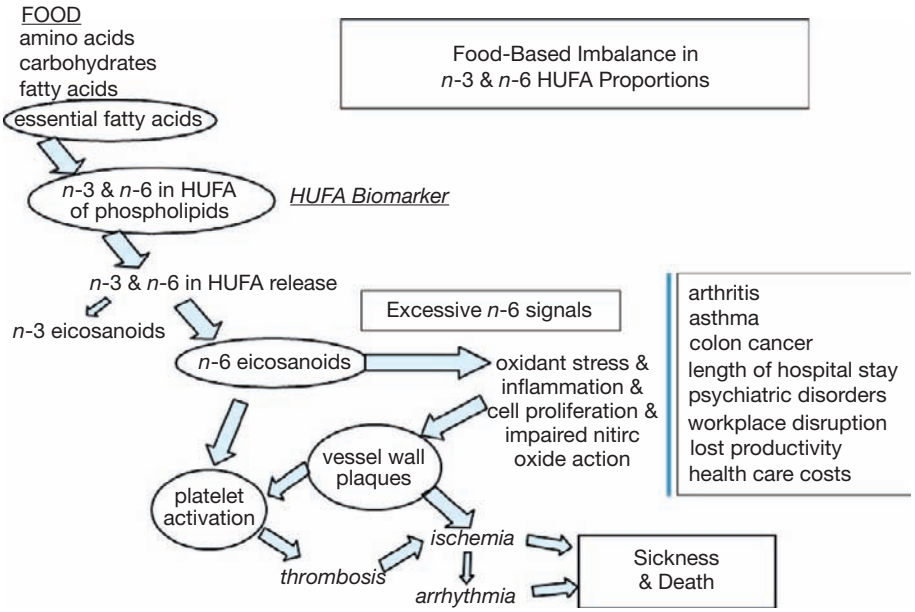

Figure 4 Food-based imbalances in $n-3$ and $n-6$ HUFA proportions. The HUFAs accumulated in phospholipids are released by phospholipase and form eicosanoids that amplify oxidative and inflammatory processes, which lead to many unwanted health conditions. Modified from Lands, B. (2014). Historical perspectives on the impact of $n-3$ and $n-6$ nutrients on health. *Progress in Lipid Research* **55**, 17–29. Internet: http://www.sciencedirect.com/science/article/pii/S0163782714000253, with permission.

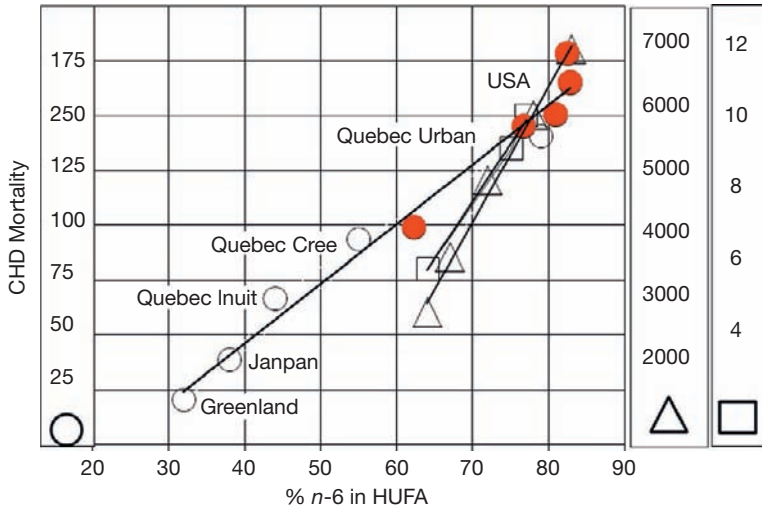

Figure 5 The risk of adverse conditions is higher for people with more than 50% *n*-6 in HUFA CHD mortality rates (per 100 000 people) for different ethnic groups, indicated by circles, with closed circles representing the quintiles in the large US MRFIT study. Annual healthcare claims (in $US) from diverse American groups are noted with triangles. Average hours per day of pain in a randomized, controlled clinical trial are noted with squares.

50% *n*−6 in HUFA than for those with less. People in the lowest quintile of the large US MRFIT study had an estimated HRA value near 62%, and they had nearly half the incidence of CHD as those in the upper four quintiles. A similar relationship was seen for CVD in two large clinical studies. Fragmentary evidence of annual healthcare claim costs from diverse American groups with HRA values ranging from 82% to 64% shows lower annual expenses for people with lower HRA values. In a recent randomized, controlled clinical trial that carefully lowered the intake of competing omega-6 nutrients while increasing the omega-3 nutrients, the HRA value went from 77% to 61% *n*−6 in HUFA, and the average hours of pain per day was lowered by 44% in 3 months.

Omega-6 Eicosanoids Amplify Transient Insults into Chronic Injuries

The chronic inflammatory damage to the blood vessels during atherogenesis is a well-recognized precursor of thrombotic heart attacks. These conditions are known to be made worse by the actions of *n*−6 eicosanoids. However, the contribution of *n*−6 EFA to this situation has not been widely recognized in public health messages that focus on predictive risk factors related to food energy toxicity. High food energy density and caloric imbalances were recognized long ago to accompany higher risks of CVD, although explicit causal mechanisms that need to be prevented remained uncertain. Decreasing associated predictive factors without removing the major causal factor seems unethical as it creates a sense of risk removal while leaving the causal factor undiminished to continue causing harm and a need for treatment.

Many people regard obesity, the visible biomarker of an imbalance in food energy intake and expenditure, to have a causal role in the risk for CVD. Elevated blood cholesterol levels, long recognized to be associated predictively with CVD, are also regarded by many to be a cause of CVD. Massive financial investments in biomedical studies have gathered evidence to support or disprove both hypotheses. However, a clear causal mechanism by which these two biomarkers cause death has not yet been identified and prevented.

Food energy per se poses little health problem if it enters the body's metabolism at a rate slow enough to be expended by the normal biological actions of work, exercise, or building body tissues. Unfortunately, modern lifestyles include much time spent sleeping, eating, driving a car, riding public transit, standing in line, using computer or Internet, reading, and watching television, activities that tend to burn only 200 cal $3\,h^{-1}$. As a result, a 1200 kcal meal (commonly consumed when eating out) will provide an extra 1000 kcal during the next 3 h that the liver will likely convert to triacylglycerols and cholesterol (Figure 6). An important dimension of food is energy density (kcal g^{-1}) that causes an apparently small amount of food to deliver many calories per mouthful and easily give many more kcal per hour than is expended. Figure 6 shows some of the biomarkers and mediators for the resulting food energy toxicity and how the food energy per hour interacts with the food *n*−6 per *n*−3 nutrients.

The 1984 NIH Cholesterol Consensus Conference noted that elevated blood cholesterol results from a food energy imbalance. The conference urged that the first step in the treatment of people who have blood cholesterol levels between 220 and 280 mg dl^{-1} should be diet therapy and caloric restriction, because weight loss and moderate physical exercise can lower the blood cholesterol biomarker. It also urged that the use of drugs to lower blood cholesterol should be used only after using the most rigorous diet modification appropriate for the individual. The conference added that "even when use of drugs seems appropriate, it is important to stress that maximal diet therapy should be continued."

Because high food energy density is associated with both CVD and elevated blood cholesterol levels, blood cholesterol has a logical predictive association with CVD. Even though the association does not prove causality, the public perception is that cholesterol causes CVD deaths. However, results from a large, long-term multiethnic study indicate that cholesterol's predictive relationship may occur only to the degree that people have more *n*−6 HUFA than *n*−3 HUFA and the resulting

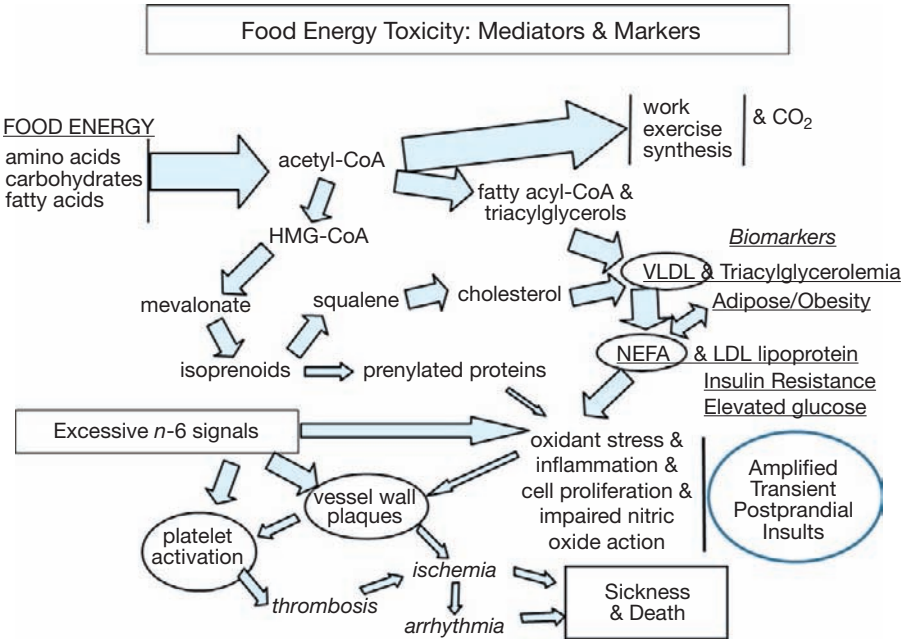

Figure 6 Food energy toxicity: Markers and mediators. Food energy that is not converted to work and CO_2 forms cholesterol and triacylglycerols that are released to plasma as very-low-density lipoproteins (VLDLs). Hydrolysis of VLDL produces nonesterified fatty acids that cause transient postprandial insults that are amplified into fatal chronic disorders by excessive *n*-6 eicosanoid actions. Modified from Lands, B. (2014). Historical perspectives on the impact of *n*-3 and *n*-6 nutrients on health. *Progress in Lipid Research* **55**, 17–29. Internet: http://www.sciencedirect.com/science/article/pii/S0163782714000253, with permission.

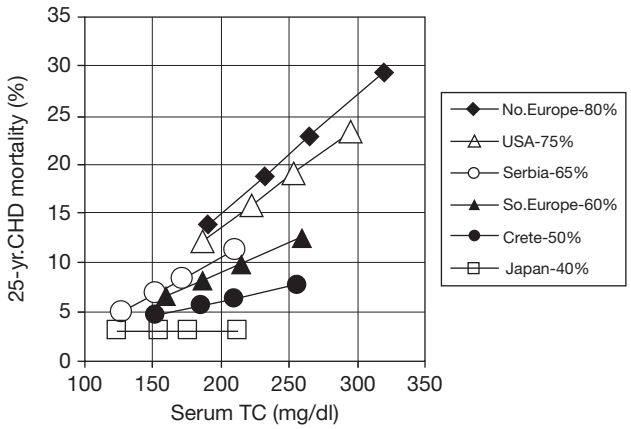

Figure 7 HUFA imbalance controls risk prediction by a food energy biomarker. Fatal heart attack risk may be predicted by blood cholesterol levels only to the degree that *n*-6 exceeds *n*-3 HUFA. Modified from Lands, B. (2014). Historical perspectives on the impact of *n*-3 and *n*-6 nutrients on health. *Progress in Lipid Research* **55**, 17–29. Internet: http://www.sciencedirect.com/science/article/pii/S0163782714000253, with permission.

excess of *n*−6 eicosanoid mediators amplifies transient insult of food energy toxicity into chronic inflammatory plaques with their prothrombotic actions. Figure 7 shows that blood cholesterol levels have no clear relationship to death rates in Japan, where the *n*−3 and *n*−6 HUFAs are well balanced.

Cholesterol has a strong relationship with CVD for people in northern Europe or the United States, where dietary habits maintain the HUFA balance near 75–80% *n*−6 in HUFA. In these people, every large meal with its transient postprandial insult (Figure 6) has a higher probability of producing chronic inflammatory conditions. This evidence can help the public turn its attention away from decreasing the noncausal predictive biomarker of cholesterol toward decreasing the causal biomarker of the %*n*−6 in HUFA. This will likely lead to a successful preventive nutrition intervention, which has eluded the public for so long.

Using Knowledge of EFA Contents for Effective Preventive Nutrition

The previously mentioned discussion of causal mechanisms by which EFA balance affects health creates a high priority for informing individuals of their current HUFA balance and the

balance of $n-3$ and $n-6$ nutrients in the foods they typically eat. With such information in hand, people can begin to reverse the unintended consequences that come from including certain staples in their diet. An important step in this approach was to describe the quantitative way by which eleven major dietary $n-3$ and $n-6$ EFAs affect the accumulated balance of HUFA in tissues. An empirical equation describing this process was developed in 1992 and followed in 2011 by the development of Omega-3–6 Balance Scores. The scores compress data on milligrams per kilocalorie for 11 different EFAs in a food into a single value that indicates the likely impact of the food on the HRA measure, $\%n-6$ in HUFA. Positive values indicate that the food will increase the $\%n-3$ in HUFA, and negative values indicate that the food will increase the $\%n-6$ in HUFA.

Hundreds of vegetables like potatoes, onions, and cabbage have scores near 0, indicating a balance between $n-3$ and $n-6$ nutrients. However, potato salad (-21), potato chips (-33), fried onion rings (-11), and coleslaw (-14 to -33) provide much more $n-6$ than $n-3$ because of added food oils (-36 to -80) and mayonnaise (-32 to -68). While most staple foods have balanced EFA, current processing and marketing procedures have unknowingly altered the balance toward harmful health consequences. Of the top 100 foods eaten by Americans (average score $=-6.2$), eleven have high negative scores between -17 and -50, and only five foods have positive scores ($+2$ to $+5$). No seafood (e.g., solid white tuna, $+46$; wild salmon, $+73$; and herring, $+70$) is on the list.

A hundred legumes (peas and beans) have scores near 0, but soybeans (-17) and peanuts (-29) have very negative scores plus a high energy density from their oil content (**Table 1**). These two legumes and their oils may cause Americans to have 76–80% $n-6$ in HUFA in contrast to a value near 60% for people in Mediterranean coastal villages. Some unintended consequences of such regional food habits that affect HRA values are reflected in **Figure 5**. Annual healthcare costs may be much less for people with HRA values near 50% compared to those with values near 80%. Public health experts estimate that more than half of American healthcare costs are for preventable health conditions, and many of those conditions are worsened by excessive $n-6$ eicosanoid actions. Public recognition that dietary $n-6$ linoleate in the absence of $n-3$ nutrients has a very narrow (to almost nonexistent) therapeutic window may begin to build a broader awareness of benefits from foods that help people NIX the 6 and EAT the 3.

See also: Cholesterol: Factors Determining Blood Cholesterol Levels; Ethnic Foods; Fats: Production and Uses of Animal Fats; Fatty Acids: Metabolism; Fish: Dietary Importance and Health Effects; Food–Herbal Medicine Interface; Legumes in the Diet; Lipoproteins; Mediterranean Diet; Nutritional Epidemiology; Phospholipids: Physiology; Risk Assessment of Foods and Chemicals in Foods; Soy Beans: Dietary Importance; Vegetable Oils: Dietary Importance.

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Fatty Acids: Fatty Acids

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From Dietary Fats to FAs

Fatty acids (FAs) are the compounds of animal and vegetable fats and oils, also found in cell membranes as a component of phospholipids. They represent a class of long hydrocarbon chain molecules (C4 to C28 atoms) with a terminal carboxylate group. The common dietary source of FAs is dietary fats, which are degraded to the final digestive products monoglycerides and free FA. In the body, most FAs are stored as triacylglycerols (TGs), the uncharged esters of glycerol and three FAs.

The term fats or lipids is a common name for large spectra of chemical compounds, including TGs, phospholipids, and cholesterol, originating from the organism or from food. The main lipids in humans, such as TGs or less abundant glycerophospholipids, glycoglycerolipids, and sphingolipids, are the esters of FAs and different alcohols. TGs constitute 90% of dietary lipid and provide 30–40% of daily energy intake in the Western world. In addition, ordinary diet contains a small amount of phospholipids and cholesterol esters (also having FAs in their structure) and free cholesterol.

The digestion of TGs is dynamic and complex process supported by water-soluble digestive enzymes, bile acids, and motion of the digestive tract, predominantly taking place in the small intestine. Lingual lipase from saliva is the first enzyme meeting fats in the mouth. Its enzymatic activity, together with physical action of chewing, and emulsifying activity of phospholipids represent the first step in the digestion of TGs. As a result, the fats form the tiny droplets and become more accessible to the digestive enzymes. Aided by stomach's churning and contractions, gastric lipase stars with the breakdown of TGs into diglycerides and FAs, but almost complete TGs digestion occurs in the small intestine. Due to the action of the bile, lipid droplets in the intestine became emulsified increasing the surface area over a thousandfold and therefore being more exposed to lipase activity. Pancreatic lipase is the most important fat digestive enzymes. It acts on TGs at water–lipid interfaces of bile micelles and with the assistance of colipase accesses the TGs in the inner lipid core to hydrolyze the ester links in positions 1–3. The final products of fat digestion are monoglycerides and free FAs, which are absorbed into intestinal epithelial cells.

Triacylglycerols, Phospholipids, and Cholesterol

TGs are the uncharged esters of glycerol and three FAs. On one hand, they are the predominant type of lipid ingested by humans and consequently the primary source of free FAs; on the other, they are synthesized in humans by two main biosynthetic pathways, the *sn*-glycerol-3-phosphate pathway in liver and adipose tissue and a monoacylglycerol pathway in the intestines. FAs derived from triacylglycerols are absorbed into enterocytes to be reesterified into TG. Packed with proteins and phospholipids, TGs in the form of lipoprotein particles, chylomicrons, enter the lymphatic system and the blood to be transported from the small intestine to other tissues.

The phospholipids, the main component of biomembranes, are present in different foods but mostly in fats, oils, meats, nuts, wheat germs, and egg yolks. Their structure is similar to TGs, with a phosphorus molecule in the location of one FA. Phospholipids from the diet are cleaved at position 2 by pancreatic phospholipase A_2, releasing the lysophospholipids and the free FAs.

Ordinary diet also contains a small amount of cholesterol, originating from animal sources (meat, egg yolks, diary fats, and butter). Cholesteryl esters, in which cholesterol is esterified with long-chain FAs (usually linoleic acid (LA)), are the main form of cholesterol in the diet. The pancreatic enzyme cholesterol esterase hydrolyzes cholesteryl esters to free cholesterol and FA. Cholesterol by itself is a steroid compound that does not contain FAs, but shows some physical and chemical characteristics of fat, like fat origin and fat-like metabolism. Comparing to phospholipids and TGs, it is poorly absorbed from the diet.

There are plenty of different FAs occurring in dietary fats, as well as in plant and animal tissues. To be better described, they are named and classified according to their composition, structure, and properties.

FAs Nomenclature

The nomenclature of FAs relies on three main FA characteristics:

- The length of the hydrocarbon chain
- The degree of unsaturation (double bond)
- The position of the double bonds in the chain

The systematic name for a single FA is the name of its parent hydrocarbon with the final 'e' substituted with 'oic.'

Parent hydrocarbon	Fatty acid
Octadecane	Octadecanoic C18

The number of double bonds is given with the prefix 'en,' 'dien,' 'trien,' 'tetraen,' and so on, before 'oic.'

C18 with:	Name
No double bond	Octadecanoic
One double bond	Octadecanoic
Two double bonds	Octadecadienoic
Three double bonds	Octadecatrienoic

The position of double bond is derived from numbering of FA carbon atoms that starts at the carboxyl terminus as a C1. Carbon atoms 2 and 3 are often referred to as α and β, respectively. In the same manner, the carbon farthest from carboxyl terminus (C atom from methyl group) is called the ω (**Figure 1**).

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00277-4

The symbol Δ with the superscript number (Δ^n) is used to represent the position of double bond, where n indicates lower-numbered carbon of each pair. For example, the *cis*-Δ^9 means that there is a *cis*-double bond between carbon atoms 9 and 10; *trans*-Δ^2 means that there is a *trans*-double bond between carbon atoms 2 and 3.

Altogether, the systematic name for a single FA (common name arachidonic acid (AA) and scientific name 5,8,11, 14-eicosatetraenoic acid) is derived like this:

Total carbons	20
Double bonds	4
Double bonds positions	$\Delta^{5,8,11,14}$
Systematic name	$20{:}4\Delta^{5,8,11,14}$

Alternatively, FA could be named according to a double bond distance from the ω carbon atom, which is in that case numbered as 1. The known examples are ω-3 and ω-6 FAs.

FA Composition and Structure

FAs vary in the length of their carbon chains, mostly having an even number of carbons, typically between 12 and 24. Their hydrocarbon chain may be saturated or it may contain one or more double bonds separated by at least one methylene group (Figure 2).

Based on the number of carbon atoms, FAs are as follows:

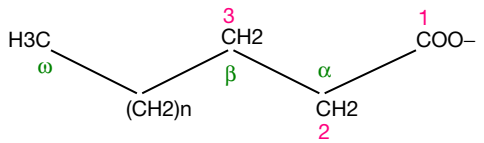

Figure 1 The numbering of carbon atoms in fatty acid hydrocarbon chain.

Short-chain fatty acids	<8 carbons
Medium-chain fatty acids	8–14 carbons
Long-chain fatty acids	≥16 carbons
Very-long-chain fatty acids	>22 carbons

According to the degree of desaturation, FAs are as follows:

Saturated fatty acids (SFA)	No C–C double bonds
Unsaturated fatty acids (USFA)	At least one C–C double bond
Monounsaturated fatty acids (MUFA)	Only one C–C double bond
Polyunsaturated fatty acids (PUFA)	Two or more C–C double bonds

All double bonds in naturally occurring FAs are in the *cis*-conformation, with two pieces of the carbon chain on the same side of the molecule, both 'up' or both 'down.' Thus, due to *cis*-double bond (Figure 3), the carbon tails of unsaturated FAs have the kinks.

The *trans*-conformation mostly appears by the conversion of naturally occurring *cis*-bonds, when unsaturated plant oils are used for frying. In *trans*-bond, two pieces of the molecule are on opposite sides of the double bond, one 'up' and other 'down,' across from each other. The most common SFAs, MUFAs, and PUFAs are listed in Table 1.

There is also a group of FAs, *branched-chain FAs*, with acyl branch(es) connected to the hydrocarbon chain (Figure 4). Normally, their carbon chain is saturated with an odd number of carbon atoms, and the branch is a methyl group usually giving an even number of carbons in total. Branched-chain FAs are common constituents of the bacterial lipids. They also represent the 1–2% of total FAs in mammalian tissues, derived mainly from bacteria from ruminant intestine, while they are very rarely found in the integral lipids of higher plants.

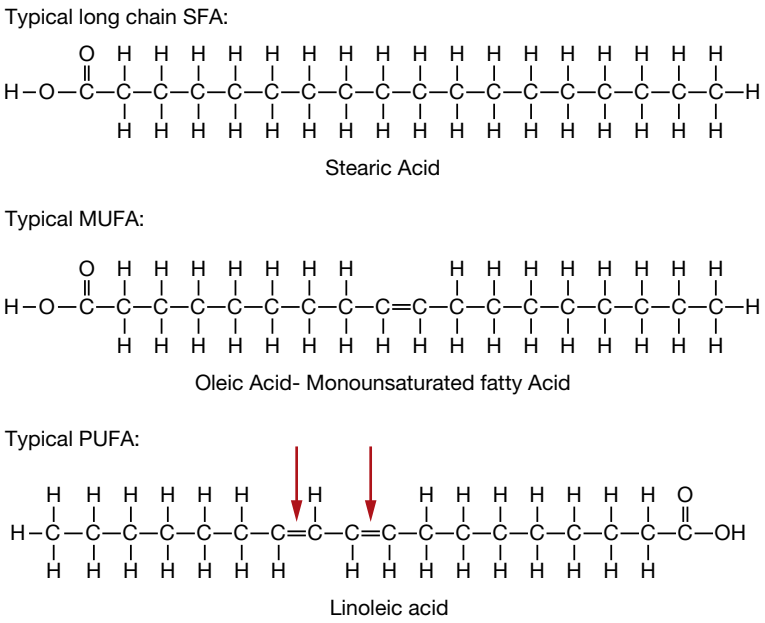

Figure 2 The structural formulas of typical long-chain saturated fatty acid, monounsaturated fatty acid, and polyunsaturated fatty acid.

FAs with the ring in the carbon chain structure are *cyclic FAs* (Figure 5). They are derived from appropriate monounsaturated FAs, such as oleic, linoleic, and linolenic acids. The ring is commonly cyclopropene or cyclopentene. Cyclopropane FAs (CPA-FAs) and cyclopropene FAs (CPE-FAs) have three-member carbocyclic ring. They are found in bacteria and some plants, especially in the seeds of plants belonging to families: Bombacaceae, Malvaceae, Sterculiaceae, Leguminosae, and Ranunculaceae.

Properties of FAs

The properties of FAs and lipids derived from them are markedly dependent on their structure, especially on FAs' chain length and degree of desaturation. The difference occurs in the temperature of melting point, fluidity at room temperature, water solubility (or hydrophobicity), and susceptibility to oxidation and reduction.

The temperature of melting point is influenced by FA structure in the way that it is lower for unsaturated FA compared with saturated FA of corresponding size. For example, the melting point of palmitic acid (C16) is 63 °C, whereas that of palmitoleic acid (C16, which contains one *cis*-double bond) is 0 °C. It happens because the straight hydrocarbon chain of saturated FA favors highly ordered molecules packing, increasing the melting point of FA. On the other side, the *cis*-double bond(s) introduces a kink in the shape of unsaturated FAs, preventing FA molecules to be closely packed and therefore leading to melting point decrease. In opposite, due to its different orientation, the *trans*-double bond favors again linear molecular shape, which helps molecules to pack together in a stable repeating array, resulting in the higher melting point of *trans*-double bond isomers. For example, the *trans*-double bond isomer of oleic acid (elaidic acid) has a melting point of 45 °C and *cis*-oleic acid 13 °C. The fatty acyl chains interact more strongly as the chain length extended with each newly added –CH_2-, increasing the melting points of long-chain FAs compared with shorter-chain FA. In addition, the FA melting points decrease with chain branching, being higher for FA with odd-number carbon chains than for FA with even-number carbon chains. The FA fluidity at room temperature is in strong connection with their melting point. SFA and their derivatives are usually in solid state at room temperature, while MUFA, PUFA, and their derivatives are liquid.

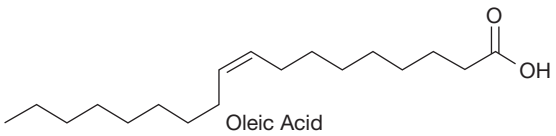

Figure 3 Oleic acid structure with one *cis*-double bond and a kink in hydrocarbon chain.

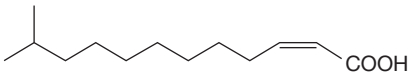

Figure 4 The structure of typical branched-chain fatty acid.

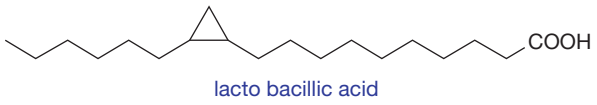

Figure 5 The structure of typical cyclopropane cyclic fatty acid.

Table 1 Examples of saturated, monounsaturated, and polyunsaturated fatty acids

Common name	*Chemical structure*	*Scientific notation C: double bonds*
Caprylic acid	$CH_3(CH_2)_6COOH$	8:0
Capric acid	$CH_3(CH_2)_8COOH$	10:0
Lauric acid	$CH_3(CH_2)_{10}COOH$	12:0
Myristic acid	$CH_3(CH_2)_{12}COOH$	14:0
Myristoleic acid	$CH_3(CH_2)_3\mathbf{CH}=\mathbf{CH}(CH_2)_7COOH$	14:1*n*−5
Palmitic acid	$CH_3(CH_2)_{14}COOH$	16:0
Palmitoleic acid	$CH_3(CH_2)_5\mathbf{CH}=\mathbf{CH}(CH_2)_7COOH$	16:1*n*−7
Stearic acid	$CH_3(CH_2)_{16}COOH$	18:0
Oleic acid	$CH_3(CH_2)_7\mathbf{CH}=\mathbf{CH}(CH_2)_7COOH$	18:1*n*−9
Vaccenic acid	$CH_3(CH_2)_5\mathbf{CH}=\mathbf{CH}(CH_2)_9COOH$	18:1*n*−7
Linoleic acid	$CH_3(CH_2)_4\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}(CH_2)_7COOH$	18:2*n*−6
α-Linolenic acid	$CH_3CH_2\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}(CH_2)_7COOH$	18:3*n*−3
Arachidic acid	$CH_3(CH_2)_{18}COOH$	20:0
Arachidonic acid	$CH_3(CH_2)_4\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}(CH_2)_3COOH$	20:4*n*−6
Eicosapentaenoic acid	$CH_3CH_2\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}(CH_2)_3COOH$	20:5*n*−3
Behenic acid	$CH_3(CH_2)_{20}COOH$	22:0
Erucic acid	$CH_3(CH_2)_7\mathbf{CH}=\mathbf{CH}(CH_2)_{11}COOH$	22:1*n*−9
Docosahexaenoic acid	$CH_3CH_2\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}CH_2\mathbf{CH}=\mathbf{CH}(CH_2)_2COOH$	22:6*n*−3
Lignoceric acid	$CH_3(CH_2)_{22}COOH$	24:0

The water solubility of FA also depends on the length of hydrocarbon chain. In general, FAs are poorly soluble in water, with the increase of hydrophobicity parallel to chain extension. Even more, the solubility of FA is additional hindered by their tendency to form micelles and monolayers in aquatic environment. However, FA became more water-soluble when present in the form of potassium or sodium salts, as well as in the medium with increasing pH. At the same time, FAs will be well dissolved in nonpolar solvents such as ethanol and chloroform.

In terms of the chemical susceptibility, SFAs are very stable, whereas USFAs react more easily. Here states the rule: the more double bonds, the greater reactivity.

Like other carboxylic acids, FAs can be esterified due to the exchange of groups attached to the FA carboxyl. In biological systems, this type of reactions converts FA to TGs and vice versa. Ester exchange reactions, with TG as a starting point, are also used in the manufacture of TGs enriched in medium-chain FAs, methyl esters, glycerol, or biodiesel.

Despite hydrophobicity, in aquatic environment, at suitable temperatures and pressures, FA can undergo hydrolysis, as used for the industrial production of glycerol and soaps. However, FA hydrolysis usually needs to be catalyzed by acid, base, or lipase. Lipases are enzymes that hydrolyze FAs from lipid species (e.g., TGs and phospholipids) *in vivo*. They favor the reaction of FAs with Δ9 double bond, when compared to Δ4, Δ5, or Δ6 double bond. Being active in milder conditions (less solvent and lower temperature and pressure), lipases are used for large-scale reactions in the manufacturing of confectionary fats and nutritional products (cocoa butter).

Both the double bonds and adjacent allylic carbons of FA alkyl chain are susceptible to oxidation, with higher susceptibility following higher degree of unsaturation. When exposed to air (oxygen), unsaturated FAs undergo a process of auto-oxidation, which is a free-radical chain reaction giving the allylic hydroperoxides as a final product. The process becomes much faster in the presence of light (photo-oxidation), natural pigments (chlorophyll, hematoporphyrins, and riboflavin), and dyes (erythrosine and methylene blue). *Ex vivo*, oxidation leads to the deterioration of unsaturated oils and fats, reducing their nutritional quality. Still, due to antioxidants in their content (tocopherol), most vegetable oils can resist this process. *In vivo*, enzyme-catalyzed oxidation is the initial step in the production of eicosanoids and jasmonates.

The FA alkyl chain is susceptible to reduction at both carbon–carbon double bond and the carboxyl group of FA. From nutritional point of view, attention should be given to catalytic partial hydrogenation. This is a process introducing H atoms in the double bonds, accompanied by a variable degree of *cis*- to *trans*-isomerization. Edible vegetable oils are transformed by partial hydrogenation to the solid form of margarines. The arising content of *trans*-FA in margarines is a subject of concern because of their atherogenic properties.

There are some other reactions involving FAs but mainly used for specific industrial handling and therefore not additional disused.

FA Metabolism

FAs in the body are organized in two forms: free or unesterified and esterified. In the plasma, free FAs (5% of total FA) are bound to albumin and to a lesser extent to globulins and lipoproteins. Most of the FAs in circulation (95% of total FA) are in the form of triglycerides, cholesterol esters, and phospholipids. In biological systems, FAs usually contain an even number of carbon atoms, mostly 14–24.

Synthesis of Saturated FA

Synthesis of FAs takes place in the cytosol of the liver, adipose tissue, lung, and brain. Precursor of all carbon atoms is acetyl CoA generated by oxidative decarboxylation of pyruvate, degradation of amino acids, and β-oxidation of FA.

Prior to the FA synthesis, malonyl CoA is formed from acetyl CoA by the action of acetyl CoA carboxylase, which has biotin as prosthetic group. The reaction of carboxylation is carried out in two stages. The first step is the formation of a carboxybiotin with the presence of ATP and the second step involves CO_2 transfer to acetyl CoA and formatting of malonyl CoA:

$$HCO_3^- + ATP + Enzyme - Biotin \rightarrow Enzyme - Biotin - CO_2^- + ADP + P_i$$

$$Enzyme - Biotin - CO_2^- + acetyl\ CoA \rightarrow malonil\ CoA + Enzyme - Biotin$$

After synthesis of malonyl CoA, a series of four reactions in repeating cycle follows: condensation, reduction, dehydration, and reduction, which lead to the extension of the FA chain.

The FA synthesis starts with covalently binding of acetyl CoA and malonyl CoA to the sulfhydryl group of an acyl carrier protein (ACP) and formation of acetyl ACP and malonyl ACP. Enzymes that catalyze these reactions are acetyl transacylase and malonyl transacylase. Afterward, the reaction of condensation follows, in which acetyl ACP and malonyl ACP form acetoacetyl-ACP (β-ketoacyl ACP) under the action of acyl-malonyl-ACP synthase (β-ketoacyl-synthase) and CO_2 is released. In the third step, acetoacetyl-ACP is reduced to D-β-hydroksybutyryl-ACP, with NADPH as a reductant, by the action of β-ketoacyl-ACP reductase. The following is the process of dehydration: D-β-hydroksybutyryl-ACP to α, β-*trans*-butenoyl-ACP (crotonyl-ACP) in the presence of 3-hydroxyacyl-ACP dehydratase. Finally, crotonyl-ACP is reduced to butyryl-ACP by enoyl-ACP reductase (Table 2). In the following reactions, butyryl-ACP reacts with malonyl-ACP and the cycle is repeated.

The synthesis of FA is continued up to palmitoyl-ACP (a total of 7 cycles) that is then cleaved to palmitate and ACP under the influence of thioesterase:

$$Palmitoil - ACP + H_2O \rightarrow palmitate + ACP$$

The syntheses of SFA, of up to 16 carbons in length, are catalyzed by the enzyme system that is called the fatty acid synthase (FAS). Mammalian FAS is a homodimer, found in the cytoplasm, consisted of the 250-kDa subunits containing three domains each. Domain 1 includes enzymes acetyl transacylase, malonyl transacylase, and acyl-malonyl-ACP, while domain 2 contains ACP, β-ketoacyl-ACP reductase, β-hydroxyacyl-ACP dehydratase, and enoyl-ACP reductase. Domain 3 contains thioesterase enzymatic activities. Unlike the eukaryotes where FAS is a large polypeptide chain with seven different enzymatic activities in two catalytic centers, in bacteria, FAS is a multienzyme complex consisting of six different enzymes plus a separate ACP.

Table 2 Reaction in fatty acid synthesis

Reaction	*Enzyme*
Acetyl CoA + ACP ↔ acetyl − ACP + CoA	Acetyl transacylase
Malonyl CoA + ACP ↔ malonyl − ACP + CoA	Malonyl transacylase
Acetyl − ACP + malonyl − ACP ↔ acetoacetyl − ACP + ACP + CO_2	Acyl-malonyl-ACP synthase
Acetoacetyl − ACP + NADPH + H^+ ↔ D-β-hydroxybutyryl − ACP + $NADP^+$	β-Ketoacyl-ACP reductase
D-β-Hydroxybutyryl − ACP ↔ crotonyl − ACP + H_2O	3-Hydroxyacyl-ACP dehydratase
Crotonyl − ACP + NADPH + H^+ → butyryl − ACP + $NADP^+$	Enoyl-ACP reductase

The final product of FA synthesis by FAS complex, which utilizes NADPH as reductant, is palmitate. In summary, stoichiometry for synthesis of palmitate can be presented with the following reaction:

$$8\ \text{acetyl CoA} + 14\ \text{NADPH} + 7\ \text{ATP} \rightarrow \text{palmitate} + 14\ \text{NADP}^+ + 8\ \text{CoA} + 7\ \text{ADP} + 7\ \text{Pi}$$

FA synthesis occurs in the cytosol using acetyl CoA, which is generated in the mitochondria. Since the transport of acetyl CoA through the mitochondrial membrane is not possible, acetyl CoA is transferred to the cytosol with the use of citrate. Citrate, formed in the mitochondrial matrix, in reaction of acetyl CoA and oxaloacetate, passes across the inner mitochondrial membrane and enters the cytosol. In the cytosol, under the influence of ATP citrate lyase, citrate is cleaved to acetyl CoA and oxaloacetate.

Synthesis of Long-Chain FA

FAs in the cells, produced by FAS or taken up from the diet, may be elongated into long-chain FAs containing 18 carbon atoms or more. Synthesis of long-chain PUFA in mammalian tissues is performed in the endoplasmic reticulum using 4 enzymes; three of these are on cytosolic side of the ER membrane and the fourth is embedded in the membrane. The formation of long-chain FAs starts the same as with FAS, with the reaction of malonyl CoA and acyl CoA and formation of β-ketoacyl CoA, in the presence of elongases. This is followed by the reactions of reduction, dehydration, and repeated reduction to form the acyl chain that is extended with 2C atoms. *Three enzymes are involved*: 3-ketoacyl CoA reductase, 3-hydroxyacyl CoA dehydratase, and 2,3-*trans*-enoyl CoA reductase. In mammals, condensation reaction is catalyzed by enzymes of elongation, known as elongases of very-long-chain fatty acids (ELOVLs). There are seven ELOVLs including elongases 1, 3, 6, and 7 involved in the elongation of SFA and MUFA and elongases 2, 4, and 5 involved in the elongation of PUFA. Microsomal cell vesicles containing the endoplasmic reticulum fraction possess the highest enzyme activity for acyl CoA elongation.

Unsaturated FAs are synthesized by introducing a double bond at a specific position on the acyl chain of long-chain FAs, using the enzyme acyl CoA desaturase. These desaturases are present in the membrane of endoplasmic reticulum and use fatty acyl CoA as substrates and cytochrome b_5 as an electron donor. The desaturases can be divided into two distinct families referred to as stearoyl CoA desaturases (SCDs) or Δ9 desaturase and FA desaturases (FADS), which included Δ6 and Δ5 desaturases.

The conversion of saturated to monounsaturated FA is carried by Δ9 desaturase. This enzyme introduces the first *cis*-double bond at position 9, 10 from the carboxyl end of FAs. The reaction is catalyzed by three enzymes: NADH-cytochrome b_5 reductase, cytochrome b_5, and Δ9 desaturase:

$$\text{NADH} \rightarrow \text{FAD} \rightarrow \text{cyt } b_5 \rightarrow \text{desaturase}$$

$$\text{Stearoyl CoA} + \text{NADH} + H^+ + O_2 \rightarrow \text{oleoyl CoA} + \text{NAD}^+ + 2H_2O$$

The syntheses of PUFA are catalyzed by Δ6 desaturase and Δ5 desaturase. The Δ6 desaturase can introduce a double bond at position Δ6 of the essential FAs of the $n-6$ (LA C18:2$n-6$) and $n-3$ family (α-linolenic acid (ALA) C18:3$n-3$) (Figure 6). After the reactions of desaturation and elongation by Δ6 desaturase and elongase, Δ5 desaturase introduces another double bond at position Δ5 of 20-carbon chain in 20:3$n-6$ and 20:4$n-3$ (Figure 6). The synthesis of docosahexaenoic (DHA) (22:6$n-3$) is carried out from the 22:5$n-3$ through two successive elongations, accompanied by Δ6 desaturation of 24:5$n-3$ in 24:6$n-3$, and followed β-oxidation in peroxisomes to DHA (Figure 6).

Opposite to plants, mammals lack Δ12 and Δ15 desaturases, which introduce double bonds at carbon atoms beyond C9 in the FA chain, and cannot synthesize LA and ALA *de novo*. Thus, these FAs are essential for mammals and must be obtained from food.

Catabolism of FAs

FA Activation

FAs primarily enter a cell via FA protein transporters on the cell surface. FA transporters include FA translocase, tissue-specific FA transport proteins, and plasma membrane-bound FA-binding protein. Depending on the tissue and its metabolic demand, FAs in cell can be converted to either triacylglycerols or membrane phospholipids or oxidized in the mitochondria for energy production. Before this processes, FAs must be activated to acyl CoA. The reaction of activation, taking place on the outer mitochondrial membrane, is catalyzed by acyl CoA synthase (or FA thiokinase) to form an acyl adenylate. In the second reaction, sulfhydryl group of CoA attacks the acyl adenylate and forms acyl CoA and AMP:

$$\text{R} - \text{COO}^- + \text{ATP} + \text{HS} - \text{CoA} \leftrightarrow \underset{\text{acyl adenilate}}{\text{R} - \text{CO} - \text{AMP}} + \text{PPi}$$

$$\text{R} - \text{CO} - \text{AMP} + \text{HS} - \text{CoA} \leftrightarrow \underset{\text{acyl CoA}}{\text{R} - \text{CO} - \text{CoA}} + \text{AMP}$$

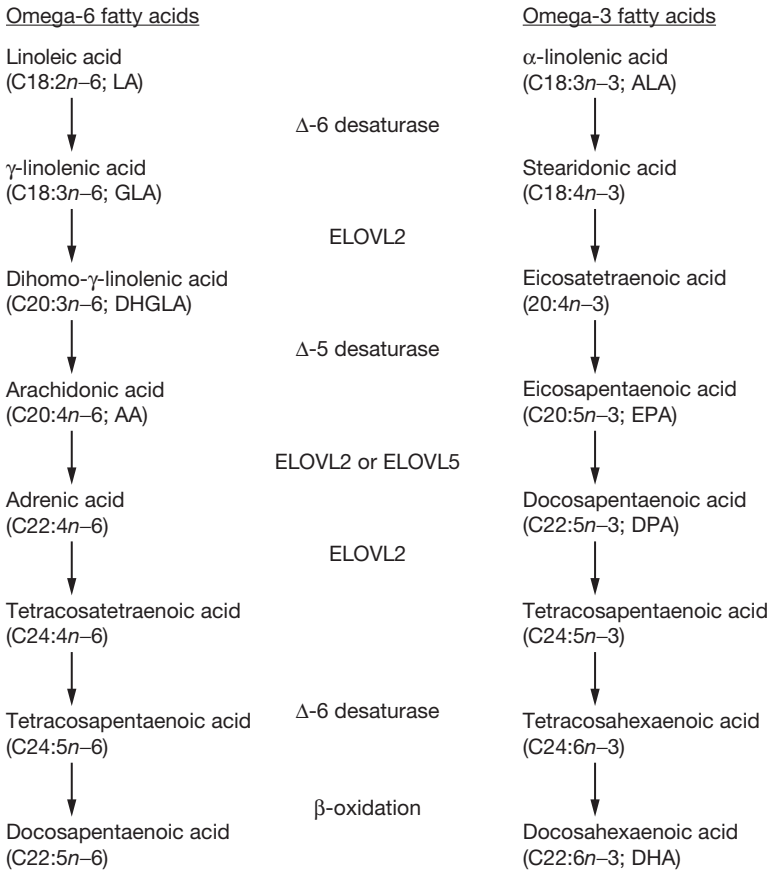

Figure 6 Biosynthesis of long-chain fatty acids.

Transport of FA into Mitochondria

The process of FA activation occurs at the outer mitochondrial membrane, whereas the process of β-oxidation of FA takes place in the mitochondrial matrix. Short- and medium-chain acyl CoA are transported into the mitochondrial matrix directly, but the transfer of long-chain acyl CoA through inner mitochondrial membrane requires a special transport mechanism. Carnitine and three proteins are included in the FA transport into mitochondrial matrix. In the first reaction, the acyl CoA reacts with carnitine and forms acyl carnitine. Reaction is catalyzed by carnitine acyltransferase I (also called carnitine palmitoyltransferase I, CTP-I) located in the outer mitochondrial membrane. An acyl carnitine is transported across the inner mitochondrial membrane by a translocase, with simultaneous transfer of carnitine from the matrix to cytosolic side. In the mitochondrial matrix, acyl carnitine is converted back to acyl CoA in a reaction catalyzed by carnitine acyltransferase II (CTP-II), releasing the carnitine. The acyl CoA then undergoes the process of β-oxidation.

Oxidation of FA

FA β-oxidation is the process by which FAs are broken down to produce energy. The β-oxidation of SFA is a process that involves a cycle of four repeated reactions: oxidation by flavin adenine dinucleotide (FAD), hydration, oxidation by NAD^+, and thiolysis by CoA, resulting in FAs shortened for two C atoms. The first step is the oxidation of the acyl CoA to *trans*-Δ^2-enoyl CoA catalyzed by enzyme acyl CoA dehydrogenase. The second reaction is the hydration of the *trans*-double bond in the presence of enoyl CoA hydratase and the formation of the L-3-hydroxyacyl CoA. The third reaction leads to the conversion of hydroxyl group at C3 into a keto group by L-3-hydroxyacyl CoA dehydrogenase and generates NADH. The final step is the cleavage of Cα-Cβ-bond in the reaction of thiolysis (Claisen ester cleavage), by using a thiol group of the second molecule CoA, in the presence of β-ketothiolase and the formation of acetyl CoA and acyl CoA shortened by two C atoms (Table 3).

Acyl CoA then undergoes another process of β-oxidation. At the final step of this cycle, an FA with an even number of carbons yields two molecules of acetyl CoA. The complete oxidation of palmitoyl CoA requires seven repeated cycles and stoichiometry of oxidation is as follows:

$$\text{Palmitoyl CoA} + 7\text{FAD} + 7\text{NAD}^+ + 7\text{CoA} + 7\text{H}_2\text{O} \rightarrow 8 \text{ acetyl CoA} + 7\text{FADH}_2 + 7\text{NADH} + 7\text{H}^+$$

The $FADH_2$ and NADH produced during the process of FA β-oxidation are used in the electron transport chain to produce ATP. Thus, the complete oxidation of a molecule of palmitate yields 106 molecules of ATP.

Additional enzymes, isomerase and reductase are needed for oxidation of USFA. The process of MUFA degradation begins the same way as degradation of SFA. However, *cis*-Δ^3-enoyl CoA that is formed in the third round cannot be

Table 3 Reaction in fatty acid oxidation

Reaction	*Enzymes*
Acyl CoA + E − FAD → *trans* − Δ^2 − enoyl CoA + E − $FADH_2$	Acyl CoA dehydrogenase
trans − Δ^2 − enoil CoA + H_2O ↔ L-3-hydroxyacyl CoA	Enoyl CoA hydratase
L-3-Hydroxyacyl CoA + NAD^+ ↔ 3-ketoacyl CoA + NADH + H^+	L-3-Hydroxyacyl CoA dehydrogenase
3-Ketoacyl CoA + HS − CoA ↔ acetyl CoA + acyl CoA $_{(\text{shortened by } C_2)}$	β-Ketothiolase

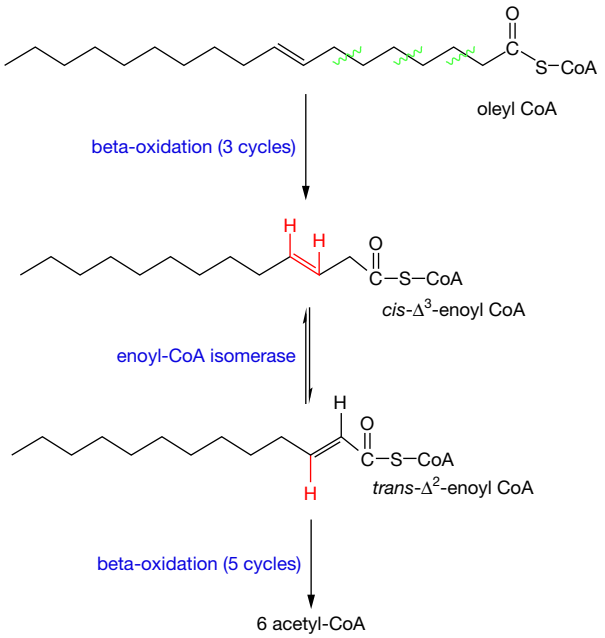

Figure 7 β-Oxidation of monounsaturated fatty acid.

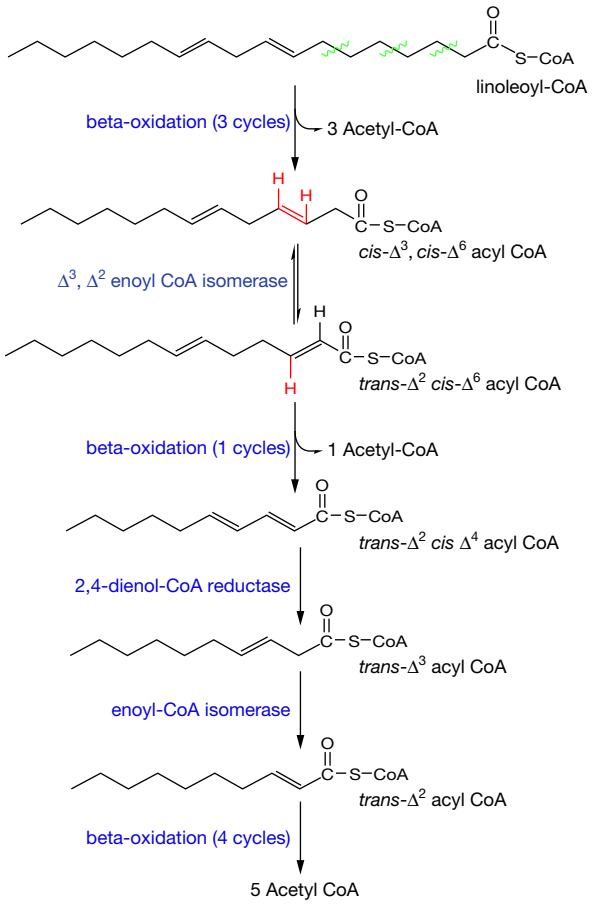

Figure 8 β-Oxidation of polyunsaturated fatty acid.

substrate for acyl CoA dehydrogenase. Therefore, isomerase catalyzes *the conversion* of *cis*-double bond between C3 and C4 into *trans*-Δ^2 double bond and forms *trans*-Δ^2enoyl CoA (Figure 7). This product is a regular substrate in FA oxidation pathway and the process can be continued.

For oxidation of PUFA, a reductase is needed. After three cycles of β-oxidation of linoyl CoA *cis*-Δ^3, Δ^6-enoyl CoA is formed. The *cis*-Δ^3 double bond is converted into a *trans*-Δ^2 double bond by an isomerase. In another round, an acyl CoA with a *cis*-Δ^4 double bond is produced, and its dehydrogenation yields a 2,4-dienoyl intermediate. This intermediate is not a substrate for enoyl CoA isomerase, therefore it must first be converted into *trans*-Δ^3-enoyl CoA by the enzyme 2,4-dienoyl CoA reductase, in the presence of NADPH. In the next step, enoyl CoA isomerase converts *trans*- Δ^3-enoyl CoA into the *trans*-Δ^2 form, a common intermediate in the β-oxidation pathway. (Figure 8). In the FA, β-oxidation pathway produces one acetyl CoA from each cycle of β-oxidation. This acetyl CoA then enters the citric acid cycle. The citric acid cycle, also known as the tricarboxylic acid (TCA) cycle or the Krebs cycle, is the final common pathway for the oxidation of acetyl CoA derived from carbohydrates, fats, and proteins into CO_2 and H_2O to generate a form of usable energy. The NADH and $FADH_2$ produced by both β-oxidation and the TCA cycle are used by the electron transport chain to produce ATP.

The final products of β-oxidation of FA with odd-number C atoms are the 3-carbon propionyl CoA and acetyl CoA. Propionyl CoA enters the citric acid cycle and is converted into succinyl CoA.

Acetyl CoA, which is formed in the process of β-oxidation, enters the citric acid cycle and forms citrate with oxaloacetate, if it is available. In cases of increased lipolysis, as in unregulated diabetes mellitus or in fasting, when oxaloacetate is used to form glucose, the molecules of acetyl CoA form acetoacetate and 3-D hydroxybutyrate, known as ketone bodies. Synthesis of ketone bodies, acetoacetate, hydroxybutyrate, and acetone occurs normally under all conditions, but their production is dramatically increased during starvation. Ketone bodies produced in the mitochondria of liver cells diffuse into the blood that then transports them to peripheral tissues. They are

normal fuels of respiration and very important as sources of energy. The acetoacetate is a major fuel source for the heart muscle and kidney cortex and has the advantage over the glucose, as energy source. Also, under conditions of starvation, the brain may provide even 75% of the energy from the acetoacetate, by using β-ketoacyl CoA transferase, which converted ketone bodies back to acetyl CoA.

The process of β-oxidation is also performed in the peroxisomes that have a major role in the degradation of very-long-chain FAs. In these vesicles, the end product of oxidation is octanoyl CoA. Therefore, the products of β-oxidation are transferred from peroxisomes to mitochondria and this transport is provided by carnitine. Acyl CoA oxidases in peroxisomes are different from those in mitochondria because they transfer the hydrogen to oxygen to produce H_2O_2 instead of producing $FADH_2$. The H_2O_2 is broken down to water by catalase. In peroxisomes, enzymes for α-oxidation, which are necessary for oxidation of some FAs with methyl branches, are also present. Peroxisomes lack the Krebs cycle and cannot degrade the acetyl CoA to produce CO_2 and water.

In microsomes, very-long-chain FAs are oxidized to form eicosanoids and epoxy and hydroxy FAs. Thus, prostaglandins, thromboxanes, and leukotrienes known as eicosanoids are formed from arachidonic and eicosapentaenoic acid by enzymes cyclooxygenases and lipoxygenase. Eicosanoids act as paracrine and autocrine hormones and each has specific effect in the cells. They are involved in the regulation of local inflammation, but they also modify the blood pressure, aggregation of blood platelets, and cardiac function. Additionally, eicosanoids inhibit the mobilization of FAs from adipose tissue and are included in the functions of the central nervous system and the contraction of smooth muscles.

Regulation of FA Metabolism

The availability of free FAs and the rate of utilization of acetyl CoA are the crucial factors that control FA oxidation. The release of FAs from adipose tissue is stimulated by glucagon and epinephrine and inhibited by insulin. The metabolic process that FA will undergo depends on cellular energy state.

If the energy charge of the cell is low, FA will be degraded by β-oxidation reactions to yield acetyl CoA, $FADH_2$, and NADH. The process of β-oxidation is mainly allosteric-regulated, which means that each of the β-oxidation enzymes is inhibited by the specific fatty acyl CoA intermediate it produces. Malonyl CoA is an essential metabolic intermediate in the regulation of FA oxidation. A decrease in malonyl CoA level results in an increased activity of CPT-I in mitochondria in the heart and muscle, while an increase in malonyl CoA level inhibits CPT-I activity. CTP-I catalyzes the commitment step in FA oxidation, which links acyl CoA molecules to the hydroxyl group of carnitine. In addition, activities of two other enzymes included in β-oxidation pathway are allosteric-regulated; 3-hydroxyacyl CoA dehydrogenase is inhibited by NADH and thiolase by acetyl CoA.

The synthesis of FA is carried out if the energy charge is high in the cells and when the level of FAs is low. Enzyme acetyl CoA carboxylase plays an essential role in regulating FA synthesis and degradation. This process is hormone-regulated. Insulin activates carboxylase, while its activity is inhibited by glucagon and epinephrine. In addition, levels of citrate, palmitoyl CoA, and AMP within a cell also control acetyl CoA carboxylase. The most important regulator of FA synthesis is the level of citrate in the cytosol. Citrate stimulates acetyl CoA carboxylase to form malonyl CoA and allows the synthesis of FAs. On the other hand, palmitoyl CoA, as the end product of FA synthesis by FAS, inhibits acetyl CoA carboxylase. AMP is also included in the regulation of an acetyl CoA carboxylase activity. AMP activates AMP-dependent protein kinase, which then performs the phosphorylation of carboxylase and inhibits its enzymatic activity.

There is adaptive control of FA metabolism regulation. The rates of synthesis and degradation of the enzymes involved in the FA synthesis change within a few days, depending on dietary habits, such as fasting and low-fat or high-carbohydrate intakes.

FAs, Human Nutrition, and Health

The general grouping of FAs as SFA, MUFA, and PUFA is usually applied, when their effects on human health are discussed. This approach is based on the assumption that major groups of FAs are associated with different health effects. However, despite the similarities of chemical structure, individual FAs within the same group may have distinct biological properties and physiological activity.

Major SFAs in the diet are myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0) that originate mainly from animal fats. When predominant food sources of total fats are milk and coconut oil, most SFAs range from C4 to C18. The SFAs with shorter chain, such as butyric (C4:0), caprylic (C8:0), capric (C10:0), and lauric (C12:0) acids, have positive health effects through the prevention of cancer and antiviral and antibacterial activity. On the other side, lauric, myristic, and palmitic acids are known to have thrombogenic and atherogenic effect. Long-chain SFAs are abundant in animal fats. Their negative effect is related to increase in total and LDL cholesterol leading to obesity and/or coronary disease. However, in the case of stearic acid, no atherogenic effect has been shown. Moreover, recent results indicate the proapoptotic effect of stearic acid in some cancer diseases.

Predominant MUFA in the diet is oleic acid (C18:1$n-9$), mostly supplied from olive oil. Oleic acid demonstrates large spectra of biological activities being connected to many health beneficial effects. On the cellular level, as a compound of membrane phospholipids, oleic acid increases membrane fluidity and transport, stimulates enzymatic activity, and regulates the activity of membrane receptors and signal transduction and transcription of some genes. It is associated with the decrease of plasma total cholesterol, LDL cholesterol, and triglyceride concentrations, having positive effect in coronary artery disease. The reduced risk of cardiovascular and neurodegenerative diseases as found in Mediterranean diet is also connected to higher oleic acid content in this type of feeding. Even it is much less present in ordinary diet, the palmitoleic acid (16:1$n-7$) is interesting because of its pro-lipogenic activity in the adipose tissue. Due to this activity, palmitoleic acid could be beneficial in the protection of other tissues and organs from fat accumulation and consequent lipotoxicity. On the other side, its

antiapoptotic activity is undesirable in uncontrolled proliferation of cancer cells. In diets with common use of rapeseed and mustard seed oil, the major MUFA is erucic acid (C22:1$n-9$). No negative health effects of erucic acid consumption have been reported in humans.

Major PUFAs in the diet are LA, ALA, and long-chain PUFA such as AA, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and DHA. LA and ALA are essential FAs, which must be supplied from dietary sources. LA, usually found in sunflower and soybean oil, is significant as a precursor of PUFA $n-6$ family, while ALA, abundant in linseed oil, is precursor of $n-3$ PUFA. At the same time, long-chain PUFA can be provided from the dietary sources. The main foods containing $n-6$ PUFA AA are meat, poultry, and eggs, while fatty fish and fish oils are the main sources of $n-3$ PUFA, EPA, and DHA. The $n-6$ and $n-3$ PUFAs have different roles in human's physiology and health. The AA is involved in the biosynthesis of pro-inflammatory eicosanoids, having pro-inflammatory activity in stress and injury. In opposite, eicosanoids derived from $n-3$ PUFA and EPA are anti-inflammatory, protecting organism from inflammatory diseases (lupus, arthritis, and asthma), heart attack, and stroke. In addition, EPA acts as an inhibitor of synthesis of pro-inflammatory cytokines. Due to the stimulatory effect on neuro- and synaptogenesis, DHA is crucial for optimal neural function. Even more, $n-3$ PUFAs by decreasing lipid level and blood pressure, improving cardiac and endothelial function, and manifesting antiplatelet and anti-inflammatory effects have a crucial role in the control of cardiovascular risk factors. In general, effects of PUFA are dependent on $n-3$ and $n-6$ PUFA ratio, which is about 1:10 in modern dietary habits compared with 1:4 as recommended in terms of the health benefits. One or two fish meals weekly could probably improve this situation.

The human diet contains a certain amount of *trans*-FA, mostly 18:1 *trans*-isomers, derived from partially hydrogenated or high-heat-treated vegetable oils, as well as produced by bacteria from ruminant intestine. The increased intake of *trans*-FA is associated with the HDL cholesterol decrease, an increase of LDL cholesterol, and the higher risk of coronary heart disease and atherosclerosis. The minor proportion of 'unusual' FA, as FA with branched-chain or non-methylene-interrupted double bond structure, is also present in the diet. In the case of increased concentration, the overall effects could be potentially harmful for human health.

See also: Fats: Classification and Analysis; Fatty Acids: Essential Fatty Acids; Fatty Acids: Metabolism; Fatty Acids: *Trans* Fatty Acids.

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Fatty Acids: Metabolism

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Fatty Acid Structure, Classification, and Nomenclature

A fatty acid is a hydrocarbon (acyl) chain with a carboxyl group at one end and a methyl group at the other. A large number of fatty acids exist in nature because the hydrocarbon chain may be of varying lengths (i.e., containing a varying number of carbon atoms linked together) and may have different degrees of unsaturation (unsaturation refers to the presence of double bonds between carbon atoms within the hydrocarbon chain). The terminal carboxyl group is reactive and readily forms ester links. Ester links between fatty acids and alcohol groups on glycerol or cholesterol produce acylglycerols (e.g., mono-, di-, and triacylglycerols are often called mono-, di-, and triglycerides) and cholesteryl esters, respectively. Traditionally, the carbon of the terminal carboxyl group has been referred to as carbon 1 with the carbon next to it (carbon 2) being referred to as the α carbon and the methyl terminal carbon as the ω (sometimes called *n*) carbon. The most abundant fatty acids in the human diet and in the human body have straight hydrocarbon chains of an even number of carbon atoms, although branched-chain, odd-numbered, and substituted fatty acids do exist. Fatty acid chain lengths vary from 2 to 30, and based upon chain length, fatty acids are classified as short chain (2–6 carbon atoms), medium chain (8–12 carbon atoms), and long chain (14 or more carbon atoms); sometimes, fatty acids of 20 or more carbon atoms are referred to as very long chain. Fatty acids are also classified according to the degree of unsaturation (i.e., the number of double bonds in the hydrocarbon chain). Fatty acids containing double bonds in the hydrocarbon chain are called unsaturated fatty acids; a fatty acid containing two or more double bonds is called a polyunsaturated fatty acid or PUFA. Saturated fatty acids do not contain double bonds in the hydrocarbon chain.

Fatty acids have systematic names that are based upon the number of carbons in the hydrocarbon chain (Table 1). However, complications arise for the naming of unsaturated fatty acids because there are multiple possibilities for the position of double bonds within the hydrocarbon chain and because each double bond may be in the *cis* or *trans* configuration. Therefore, when naming an unsaturated fatty acid, it is important that the exact positions of double bonds and their configurations be specified. Traditionally, the position of double bonds was denoted by identifying the carbon number (counting from carbon 1 (the carboxyl carbon)) on which each double bond occurs. Thus, octadecadienoic acid, an 18-carbon fatty acid with *cis* double bonds between carbons 9 and 10 and carbons 12 and 13, is correctly denoted as *cis*-9, *cis*-12-octadecadienoic acid or as *cis*, *cis*-9,12-octadecadienoic acid. More recently, an alternative shorthand notation for fatty acids has come into frequent use, particularly within the nutrition community. This relies upon identifying the number of carbon atoms in the hydrocarbon chain and the number of double bonds and their position. Thus, octadecanoic acid is notated as 18:0, indicating that it has a hydrocarbon chain of 18 carbons and does not contain any double bonds. Unsaturated fatty acids are named simply by identifying the number of double bonds and the position of the first double bond counted from the methyl terminus (with the methyl, or ω, carbon counted as number 1) of the hydrocarbon chain. The way the first double bond is identified is as ω−*x*, where *x* is the carbon number on which the double bond occurs. Therefore, *cis*, *cis*-9,12-octadecadienoic acid is also known as 18:2ω−6. The ω−*x* nomenclature is sometimes referred to as *n*−*x* (e.g., 18:2*n*−6). In addition to these nomenclatures, fatty acids are often described by their common names (Table 1), which sometimes reflect a common origin of the fatty acid, for example, palmitic acid (16:0) from palm oil and oleic acid (18:1*n*−9) from olive oil. In most PUFAs, each double bond is separated by a methylene (–CH_2–) group. However, this is not always the case, and in some PUFAs, the double bonds are conjugated (i.e., the two double bonds are separated by only one single bond). Figure 1 shows the structure of several 18-carbon fatty acids indicating the position of the double bonds in the hydrocarbon chain and how this is reflected in their naming.

Most commonly occurring unsaturated fatty acids contain *cis* rather than *trans* double bonds. *Trans* double bonds do occur in some intermediates in the fatty acid biosynthetic pathway, in ruminant fats (e.g., cow's milk), in plant lipids, and in some seed oils, and they may also be introduced by exposure of *cis* unsaturated fatty acids to high temperatures such as when oils are used for deep fat frying. *Cis*, but not *trans*, double bonds produce a kink in the molecule so that the molecular shape of unsaturated fatty acids containing *cis* double bonds is distinct from that of saturated (and *trans* unsaturated) fatty acids. This has an effect on the physical properties of the fatty acid. The melting point of a fatty acid is affected by its chain length, its degree of unsaturation, and whether the double bonds are *cis* or *trans*. Fatty acid melting point increases with increasing chain length and decreases with increasing degree of unsaturation. Thus, among the 18-carbon fatty acids, stearic acid (18:0) has a higher melting point than oleic acid (18:1*n*−9) and oleic acid has a higher melting point than linoleic acid (18:2*n*−6). For a given chain length and number of double bonds, a *trans* fatty acid has a higher melting point than a *cis* fatty acid. Thus, elaidic acid (*trans* 18:1*n*−9) has a higher melting point than oleic acid. Therefore, the fatty acid composition of a fat-rich substance will determine whether that substance is solid or liquid at room temperature. Consequently, lard, beef tallow, and butter are solid at room temperature because they contain a high proportion of long-chain saturated fatty acids, while coconut oil and corn oil are liquid at room temperature because they contain a high proportion of medium-chain saturated fatty acids and PUFAs, respectively. Melting point can be altered by the modification of fatty acid composition. Hence, to produce margarine from

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Table 1 Nomenclature of fatty acids

Systematic name	*Trivial name*	*Shorthand notation*
Saturated		
Ethanoic	Acetic	2:0
Propanoic	Propionic	3:0
Butanoic	Butyric	4:0
Hexanoic	Caproic	6:0
Octanoic	Caprylic	8:0
Decanoic	Capric	10:0
Dodecanoic	Lauric	12:0
Tetradecanoic	Myristic	14:0
Hexadecanoic	Palmitic	16:0
Octadecanoic	Stearic	18:0
Eicosanoic	Arachidic	20:0
Docosanoic	Behenic	22:0
Tetracosanoic	Lignoceric	24:0
Monounsaturated		
cis-9-Hexadecenoic	Palmitoleic	16:1*n*−7
cis-11-Octadecenoic	Vaccenic	18:1*n*−7
trans-11-Octadecenoic	*trans*-Vaccenic	t18:1*n*−7
cis-9-Octadecenoic	Oleic	18:1*n*−9
trans-9-Octadecenoic	Elaidic	t18:1*n*−9
cis-11-Eicosenoic	Gadoleic	20:1*n*−9
cis-13-Docosenoic	Erucic	22:1*n*−9
cis-11-Docosenoic	Cetoleic	22:1*n*−11
cis-15-Tetracosenoic	Nervonic	24:1*n*−9
Polyunsaturated		
cis-9, *cis*-12-Octadecadienoic	Linoleic	18:2*n*−6
All-*cis*-6, 9, 12-Octadecatrienoic	γ-Linolenic	18:3*n*−6
All-*cis*-9, 12, 15-Octadecatrienoic	α-Linolenic	18:3*n*−3
All-*cis*-6, 9, 12, 15-Octadecatetraenoic	Stearidonic	18:4*n*−3
All-*cis*-11, 14, 17-Eicosatrienoic	Mead	20:3*n*−9
All-*cis*-8, 11, 14-Eicosatrienoic	Dihomo-γ-linolenic	20:3*n*−6
All-*cis*-5, 8, 11, 14-Eicosatetraenoic	Arachidonic	20:4*n*−6
All-*cis*-5, 8, 11, 14, 17-Eicosapentaenoic	Timnodonic[a]	20:5*n*−3
All-*cis*-7, 10, 13, 16-Docosatetraenoic	Adrenic	22:4*n*−6
All-*cis*-4, 7, 10, 13, 16-Docosapentaenoic	Osbond	22:5*n*−6
All-*cis*-7, 10, 13, 16, 19-Docosapentaenoic	Clupanodonic[b]	22:5*n*−3
All-*cis*-4, 7, 10, 13, 16, 19-Docosahexaenoic	Cervonic[c]	22:6*n*−3

[a]Commonly called eicosapentaenoic acid.
[b]Commonly called docosapentaenoic acid.
[c]Commonly called docosahexaenoic acid.

vegetable oil, the process of hydrogenation is used to 'harden' the oil: vegetable oils are hardened by reacting them with hydrogen gas at about 60 °C in the presence of a catalyst, which results in the conversion of some of the double bonds to single bonds. This process can also convert *cis* to *trans* double bonds.

Fatty Acid Biosynthesis

Biosynthesis of Saturated Fatty Acids

Saturated fatty acids can be synthesized from carbohydrates and from proteins because the basic building block is acetyl-coenzyme A (acetyl-CoA) produced from sugar and amino acid metabolism. Acetyl-CoA is usually produced within mitochondria, while fatty acid biosynthesis occurs in the cytoplasm. Thus, there is a transport system whereby mitochondrial acetyl-CoA condenses with oxaloacetate to form citrate (catalyzed by citrate synthase) – this is actually a reaction within the citric acid (Krebs) cycle – and the citrate is transported across the mitochondrial membrane to the cytoplasm. Here, citrate is cleaved to produce oxaloacetate (which is converted to malate and transported back into the mitochondria) and acetyl-CoA.

De novo synthesis of fatty acids is catalyzed by a multienzyme complex called fatty acid synthase. The growing fatty acid hydrocarbon chain is built up by successive addition of two-carbon units. These are donated by malonate – a three-carbon intermediate – linked to acyl carrier protein (ACP) through the latter's sulfhydryl group. Malonyl-ACP is formed from malonyl-CoA that is, in turn, formed from acetyl-CoA. Acetyl-CoA carboxylase catalyzes conversion of acetyl-CoA to malonyl-CoA (Figure 2). This is the rate-limiting step of fatty acid biosynthesis and is subject to hormonal regulation (e.g., it is upregulated by insulin). The malonate group of malonyl-CoA now condenses with ACP to produce malonyl-ACP. In the

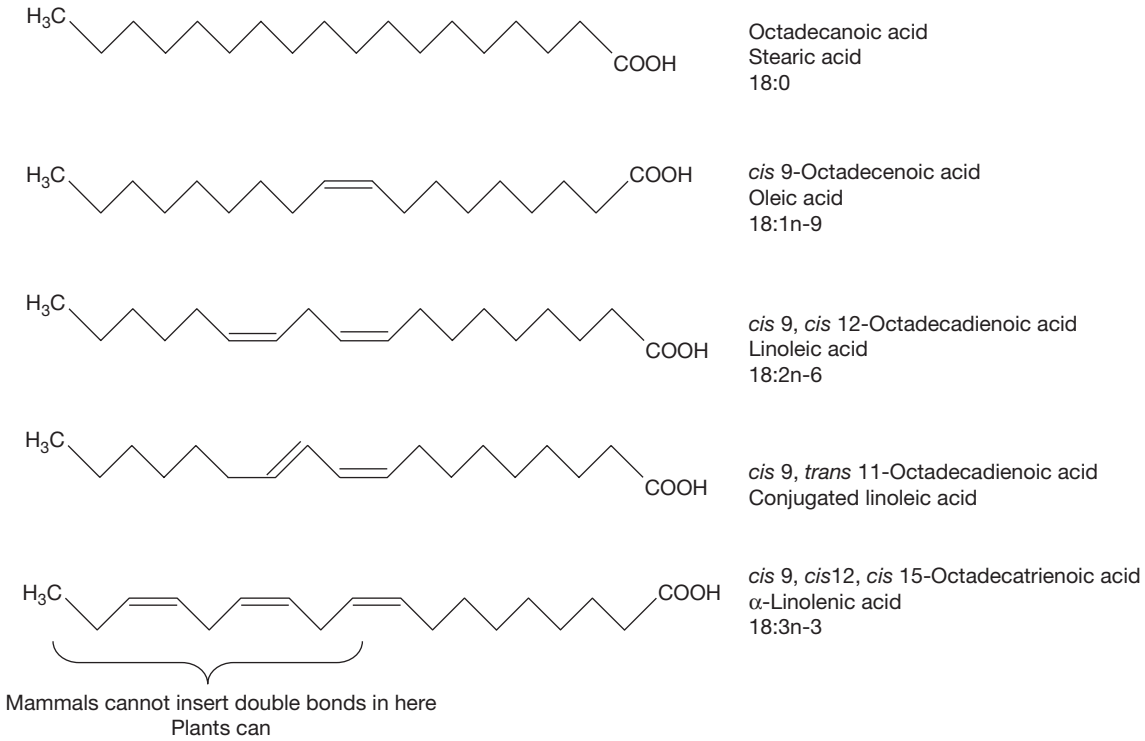

Figure 1 Representation of the structure of five different 18-carbon fatty acids demonstrating the basis of different nomenclatures.

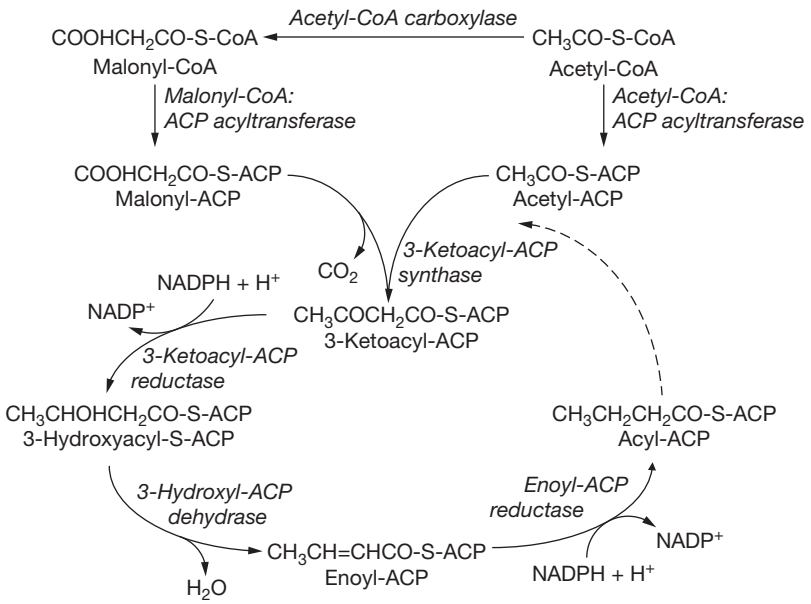

Figure 2 The pathway of *de novo* fatty acid biosynthesis catalyzed by the multienzyme fatty acid synthase complex. Malonyl-ACP (acyl carrier protein) acts as the donor of a two-carbon unit to the growing acyl chain. The acceptor in the first step is acetyl-ACP, and in the subsequent steps, it is the acyl-ACP produced during the previous round of reactions. The usual end product of the process is palmitoyl-ACP.

initial step of fatty acid biosynthesis, malonyl-ACP is condensed with acetyl-ACP to form the four-carbon acetoacetyl-ACP with the loss of one carbon as CO_2 (Figure 2). There follows a sequence of hydrogenation (reduction), dehydration, and hydrogenation (reduction) steps to produce the four-carbon butyryl-ACP (Figure 2). The cycle is now repeated with butyryl-ACP taking the role of acetyl-ACP from the first cycle to ultimately produce a six-carbon acyl-ACP. The cycle is repeated seven times to produce the 16-carbon palmitoyl-ACP, which is hydrolyzed from the ACP to yield palmitic acid. The

reductive steps in the fatty acid biosynthesis pathway use NADPH derived from the pentose phosphate pathway. The overall reaction for palmitic acid biosynthesis is

$$\text{Acetyl-CoA} + 7\ \text{malonyl-CoA} + 14\text{NADPH} + 14\text{H}^+ \rightarrow \text{palmitic acid} + 7\text{CO}_2 + 8\text{CoA} + 14\text{NADP}^+ + 6\text{H}_2\text{O}$$

It is important to note that enzymes exist in some tissues that act to release fatty acids of shorter chain length than palmitic acid from the *de novo* biosynthetic pathway. For example, in the mammary gland of some species, there are enzymes that are responsible for the release of the medium-chain saturated fatty acids such as caprylic acid (8:0) and capric acid (10:0) that are characteristic of the milks of those species.

In eukaryotes, longer-chain saturated fatty acids (actually the fatty acyl-CoA esters are formed) are produced from palmitic acid (actually palmitoyl-CoA is the substrate) by elongation reactions (the enzymes catalyzing these are termed elongases), which occur on the endoplasmic reticulum or in mitochondrial membranes. The mitochondrial system uses acetyl-CoA as the two-carbon donor, and there is a requirement for NADH or NADPH or both depending upon the tissue location. However, the main location for fatty acid elongation is the endoplasmic reticulum and this system uses malonyl-CoA as the two-carbon donor and NADPH. Elongation converts palmitic acid to stearic acid, and in neuronal tissue, large amounts of behenic (22:0) and lignoceric (24:0) acids are produced through elongation reactions. Thus, a large number of fatty acid substrates may be elongated, and seven elongase enzymes, which differ in their selectivity for fatty acids of different chain lengths and degrees of unsaturation, have been identified. Elongases 1, 3, 6, and 7 preferentially catalyze the extension of saturated and monounsaturated fatty acids, while elongases 2, 4, and 5 act mainly on PUFAs (see Further metabolism of linoleic and α-linolenic acids). Elongation of palmitic acid to stearic acid is catalyzed by elongase 6. Elongases 1, 3, and 7 catalyze the elongation of stearic acid to lignoceric acid, while only elongases 1 and 3 can further elongate lignoceric acid.

Biosynthesis of Monounsaturated Fatty Acids

The endoplasmic reticulum is a site for the introduction of double bonds ('desaturation') into fatty acids. The pathway used is almost universal, having been identified in bacteria, yeasts, algae, higher plants, protozoa, and animals. Examples are the conversion of stearic acid (18:0) to oleic acid (18:1$n-9$) and of palmitic acid to palmitoleic acid (16:1$n-7$) by insertion of a *cis* double bond between carbons 9 and 10; once again, it is the fatty acyl-CoA that is the substrate for these reactions that use NADPH and molecular oxygen. Because the double bond is inserted between carbons 9 and 10 counting from the carboxyl end of the hydrocarbon chain, the desaturase enzyme is known as delta-9 desaturase (Δ9-desaturase), although sometimes, this enzyme is referred to as stearoyl-CoA desaturase or SCD.

Biosynthesis of PUFAs

Biosynthesis of linoleic and α-linolenic acids

All eukaryotes and some bacteria can produce PUFAs. Plant enzymes normally introduce a new double bond between an existing double bond and the terminal methyl group of the hydrocarbon chain, whereas animal enzymes normally introduce a new double bond between an existing double bond and the carboxyl group (see Figure 1). Insertion of a double bond between carbons 12 and 13 (counted from the carboxyl carbon) of oleic acid yields linoleic acid (18:2$n-6$). The enzyme that catalyzes this reaction is called Δ12-desaturase (Figure 3). Linoleic acid can be further desaturated by insertion of a double bond between carbons 15 and 16 (counted from the carboxyl carbon) by Δ15-desaturase to yield α-linolenic acid (18:3$n-3$) (Figure 3).

Linoleic and α-linolenic acids are the simplest members of the $n-6$ and $n-3$ families of fatty acids, respectively. As indicated earlier, mammals lack the enzymes that introduce double bonds at carbon atoms beyond carbon 9 in the acyl chain (counting from the carboxyl carbon). Because these include the Δ12- and Δ15-desaturases, this means that mammals cannot synthesize linoleic or α-linolenic acids. Since these fatty acids are required by mammalian cells, they are termed essential fatty acids, and there is a need for their consumption in the diet.

Further metabolism of linoleic and α-linolenic acids

Although mammalian cells cannot synthesize linoleic or α-linolenic acids, they can metabolize them by further desaturation and elongation; desaturation occurs at carbon atoms below carbon number 9 (counting from the carboxyl carbon). Linoleic acid can be converted to γ-linolenic acid (18:3$n-6$) by Δ6-desaturase, and then, γ-linolenic acid can be elongated (by elongase 5) to dihomo-γ-linolenic acid (20:3$n-6$) (Figure 3). Dihomo-γ-linolenic acid can be further desaturated by Δ5-desaturase to yield arachidonic acid (20:4$n-6$) (Figure 3).

Using the same series of enzymes as used to metabolize $n-6$ PUFAs, α-linolenic acid is converted to timnodonic acid, which is more commonly known as eicosapentaenoic acid (20:5$n-3$) or EPA (Figure 3). In mammals, the pathway of desaturation and elongation occurs mainly in the liver, although it is also likely to occur in other cells and tissues that have specific requirements for very-long-chain PUFAs. The desaturation enzymes use NADPH and molecular oxygen and are located on the endoplasmic reticulum. The genes encoding Δ6- and Δ5-desaturase are known as fatty acid desaturase 2 and 1 (*FADS2* and *FADS1*), respectively.

It is evident from the pathway shown in Figure 3 that there is competition between the $n-9$, $n-6$, and $n-3$ fatty acid families for metabolism. The Δ6-desaturase reaction is rate-limiting in this pathway. The preferred substrate for Δ6-desaturase is α-linolenic acid, followed by linoleic acid followed by oleic acid. However, because linoleic acid is much more prevalent in most human diets than α-linolenic acid, metabolism of $n-6$ fatty acids is quantitatively the more important. In the absence of intake of linoleic and α-linolenic acids, metabolism of oleic acid is enhanced resulting in the accumulation of mead acid (20:3$n-9$), which is normally only found in tissues in trace amounts (Figure 3). Appearance and accumulation of mead acid are taken to indicate dietary essential fatty acid deficiency. The activities of Δ6- and Δ5-desaturases are regulated by nutritional status, hormones, and feedback inhibition by end products; for example, high-fat diets and insulin downregulate the activities of these enzymes.

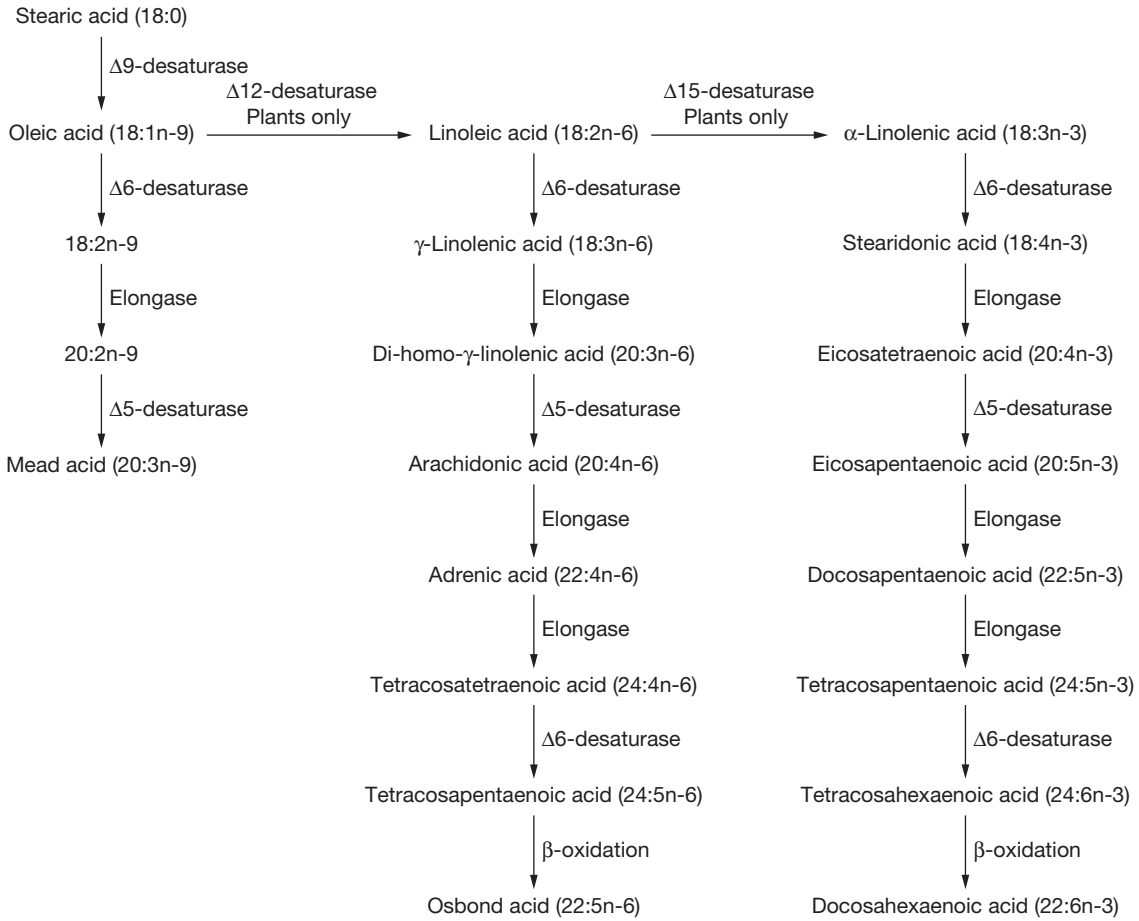

Figure 3 The pathways of biosynthesis of *n*−9, *n*−6, and *n*−3 unsaturated fatty acids.

Furthermore, polymorphisms in the *FADS1* and *FADS2* genes have been associated with differences in the status of *n*−6 and *n*−3 PUFAs, presumably due to the polymorphisms resulting in different capacities of the pathway.

Further conversion of arachidonic acid to osbond acid (22:5*n*−6) and of EPA, to cervonic acid, more commonly known as docosahexaenoic acid (22:6*n*−3) or DHA, occurs by a complex pathway. This involves chain elongation catalyzed by elongase 5, a second chain elongation catalyzed by elongase 2 or 5, desaturation by Δ6-desaturase, and then removal of two carbon atoms as acetyl-CoA by limited β-oxidation in peroxisomes (Figure 3).

Fatty Acid Incorporation into Complex Lipids

Fatty Acids Are Esterified into Complex Lipids

Fatty acids do not usually exist in high concentrations in 'free' form; most often, they are esterified into acylglycerols like mono-, di-, and triacylglycerols, into phospholipids or similar complex lipids, or into cholesteryl esters. Fatty acids in foods are mainly present in triacylglycerols, since this is how plants and animals store fatty acids, although some dietary fatty acids are present in phospholipids since these make up cell membranes and many foods (e.g., meat) include intact cells. Humans and other mammals store fatty acids esterified into triacylglycerols in adipose tissue. Phospholipids are important structural components of all cell membranes and phospholipids contain fatty acids esterified at their s*n*−1 and s*n*−2 positions. Fatty acids circulate in the bloodstream mainly as esterified lipids within lipoproteins. For example, dietary fatty acids are carried in the bloodstream mainly as triacylglycerols within chylomicrons, while very-low-density lipoproteins carry triacylglycerols of hepatic origin (e.g., carrying fatty acids from hepatic *de novo* biosynthesis that have been incorporated into triacylglycerols). Low- and high-density lipoproteins are the main cholesterol-carrying lipoproteins in the bloodstream, and the majority of this cholesterol is present as fatty acid cholesteryl esters. Within these lipoproteins, the hydrophobic triacylglycerols and cholesteryl esters are within the core, which is surrounded by a phospholipid monolayer, which serves to solubilize the particle within the aqueous environment of the bloodstream.

Incorporation of Fatty Acids into Triacylglycerols

The glycerol 3-phosphate pathway

Glycerol 3-phosphate is produced from glycerol in a reaction catalyzed by glycerol kinase:

$$\text{Glycerol} + \text{ADP} \rightarrow \text{glycerol 3-phosphate} + \text{ADP}$$

There follows stepwise transfer of fatty acyl groups from fatty acyl-CoA onto position 1 and then position 2 of glycerol 3-phosphate (Figure 4). The first acylation is catalyzed by acyl-CoA/glycerol phosphate 1-*O*-acyltransferase, known as GPAT, while the second acylation is catalyzed by acyl-CoA/1-acyl glycerol phosphate 2-*O*-acyltransferase, known as LPAT (because 1-acylglycerol 3-phosphate is also called lysophosphatidic acid, hence lysophosphatidate acyltransferase). GPAT has marked specificity for saturated fatty acyl-CoA, whereas LPAT shows specificity toward mono- and dienoic fatty acyl-CoA. Different isoforms of GPAT and LPAT are found on the endoplasmic reticulum and outer mitochondrial membrane, and this distribution differs between tissues. The product of these two reactions is 1,2-diacylglycerol 3-phosphate (also known as phosphatidic acid). Phosphatidic acid can also be formed by phosphorylation of 1,2-diacylglycerol by diacylglycerol kinase using ATP as the phosphate donor. The next step of the glycerol 3-phosphate pathway of triacylglycerol synthesis is the formation of 1,2-diacylglycerol by the hydrolysis of phosphatidic acid by phosphatidate phosphohydrolase. The final step of this pathway is fatty acylation of the now vacant third position of the diacylglycerol catalyzed by diacylglycerol acyltransferase, known as DAGAT. This enzyme has a broad specificity for fatty acyl-CoAs.

The dihydroxyacetone phosphate pathway

Dihydroxyacetone phosphate is an intermediate in glycolysis. The first reaction in the dihydroxyacetone phosphate pathway for triacylglycerol synthesis is fatty acylation at position 1 catalyzed by dihydroxyacetone phosphate acyltransferase (Figure 4). Although this enzyme is found in mitochondria, peroxisomes appear to be a more important location. The next step is the reduction of the keto group to form 1-acylglycerol 3-phosphate, linking into the glycerol 3-phosphate pathway of triacylglycerol synthesis. This reduction occurs on the cytosolic side of the peroxisomal membrane and uses NADPH. Further acylation requires transfer of the 1-acylglycerol 3-phosphate to the endoplasmic reticulum.

The monoacylglycerol pathway

The main function of this pathway appears to be resynthesis of triacylglycerols of dietary origin following their partial digestion in the intestinal lumen. The 2-monoacylglycerol produced during this hydrolysis is fairly resistant to further digestion and appears to be absorbed intact. The pathway involves stepwise transfer of fatty acyl groups from fatty acyl-CoA to positions 1 and 3 of the 2-monoacylglycerol (Figure 4). The first reaction is catalyzed by monoacylglycerol acyltransferase, which appears to have a preference for esterifying position. The second reaction is catalyzed by diacylglycerol acyltransferase, which is specific for 1,2-diacylglycerols (i.e., for esterifying position 3).

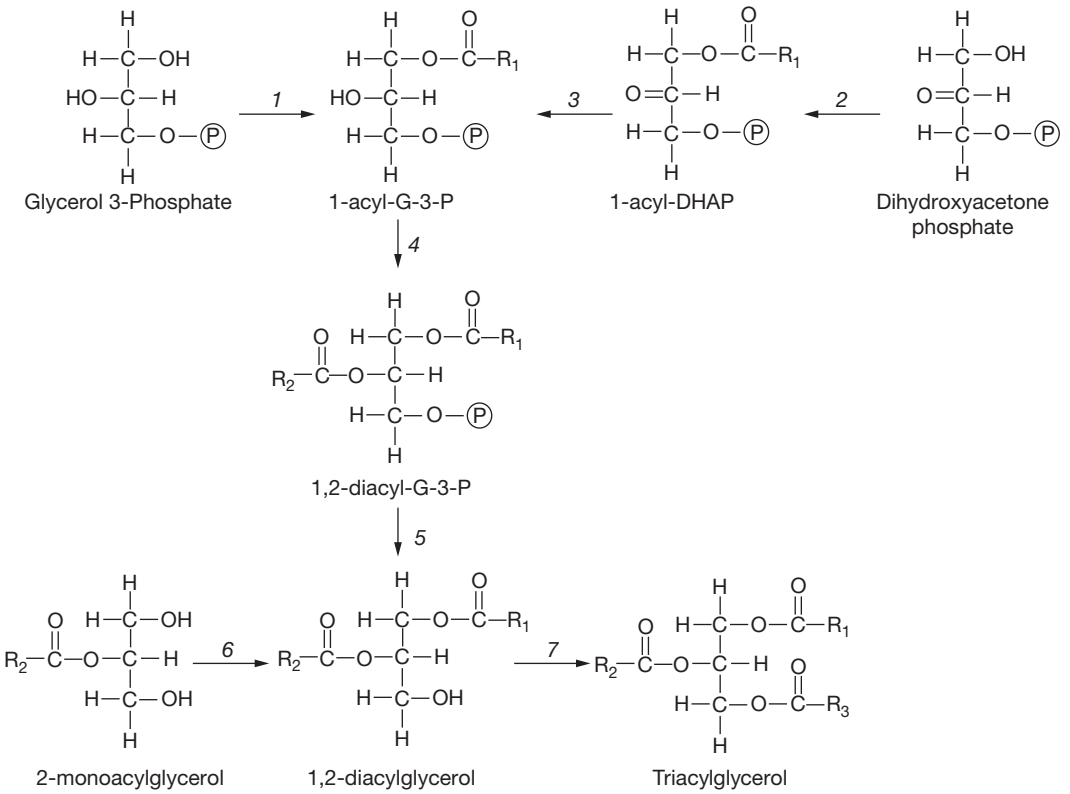

Figure 4 The pathways of triacylglycerol biosynthesis. Enzyme: 1, glycerol 3-phosphate acyltransferase (GPAT); 2, dihydroxyacetone phosphate acyltransferase; 3, acyl-dihydroxyacetone phosphate reductase; 4, lysophosphatidate acyltransferase (LPAT); 5, phosphatidate phosphohydrolase; 6, monoacylglycerol acyltransferase; 7, diacylglycerol acyltransferase. R_1 and R_2 represent fatty acyl chains; P represents phosphate.

Incorporation of Fatty Acids into Phospholipids

Cell membrane phospholipids and related structures

All cells are bordered by a membrane composed of a lipid bilayer that has both structural and functional roles. The most common membrane lipids are based upon a glycerol backbone with various moieties linked to the hydroxyl groups on the glycerol; these are termed glycerolipids or glycerides. In higher animals, phosphoglycerides (these are usually called 'phospholipids') predominate, whereas in plants, glycosylglycerides are more important. In mammalian systems, the most common phospholipids are the 1,2-diacylphosphoglycerides, which have fatty acyl groups esterified at positions 1 and 2 of the phosphoglyceride and usually another moiety (termed the 'head group') esterified to the phosphate group, which is at position 3. These include phosphatidic acid, phosphatidylcholine (also called lecithin), phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol. Phospholipids with ether, rather than ester, linked fatty acids at position 1 (and sometimes at position 2) also exist. These include plasmalogens, which have an ether linkage at the s$n-1$ position and an ester linkage at the s$n-2$ position. In mammals, the s$n-1$ position is typically derived from 16:0, 18:0, or 18:1$n-9$ fatty alcohols, while the s$n-2$ position is most commonly occupied by a PUFA. The most common head groups present in mammalian plasmalogens are ethanolamine (called plasmanylethanolamines) or choline (called plasmanylcholines). Platelet-activating factor has an s$n-1$ ether-linked acyl group, an s$n-2$ acetyl group, and choline as the head group. Sphingolipids are membrane lipids based on sphingosine rather than glycerol. These may include fatty acyl groups or fatty acyl derivatives as side chains.

The fatty acid compositions of membrane lipids are usually characteristic for the cell and membrane type, but may change with the cell cycle, with age, in response to stimuli or to changes in the environment or the diet; these changes may have functional consequences. As many as 40 different fatty acids can be incorporated into the s$n-1$ or s$n-2$ positions of a phospholipid. Under normal conditions, the membrane bilayer is in a 'fluid' state, meaning that membrane proteins and lipids can migrate within the plane of the membrane. The fluidity of a membrane is strongly influenced by the fatty acid composition of its constituent lipids. In turn, membrane fluidity may affect cell function. This may be because fluidity, and hence protein movement, is required to establish appropriate interactions between proteins or between proteins and lipids.

Phospholipid biosynthesis

Phospholipids are formed from the parent compound phosphatidic acid, mentioned earlier as an intermediate in the pathway of triacylglycerol biosynthesis. There is variation in the way in which the polar head groups are attached to the phosphate of phosphatidic acid. Phosphatidic acid is the precursor of phosphatidylglycerol, phosphatidylinositol, and diphosphatidylglycerol, while the dephosphorylated derivative of phosphatidic acid 1,2-diacylglycerol is the precursor of phosphatidylcholine and phosphatidylethanolamine (Figure 5).

Fatty Acid Release from Complex Lipids

Fatty Acid Release from Triacylglycerols

Lipases are enzymes that hydrolyze fatty acids from acylglycerols; lipases are a subclass of esterases and they are widespread in nature being found in microorganisms, plants, and animals. Because there are a large number of possible acylglycerol substrates that have different tissue distributions, there are a number of different lipases with differing specificities and locations. Triacylglycerol lipases typically split the fatty acids esterified at positions 1 and 3 leaving the 2-monoacylglycerol intact. Hydrolysis of the latter will usually require a 2-monoacylglycerol lipase. Different triacylglycerol lipases have different fatty acid specificities, meaning that different triacylglycerol substrates are hydrolyzed at different rates and to different extents. In animals, triacylglycerol lipases can be classified as either extracellular or intracellular. Extracellular lipases are secreted from the cells in which they are synthesized and they are involved in hydrolyzing triacylglycerols present in the extracellular environment enabling cellular uptake of the liberated fatty acids. Extracellular lipases include pancreatic lipase, involved in hydrolyzing dietary triacylglycerols in the intestinal lumen; lipoprotein lipase, involved in hydrolyzing lipoprotein triacylglycerols in the circulation; hepatic lipase, involved in hydrolyzing lipoprotein triacylglycerols in the hepatic sinusoids (the liver's equivalent of capillaries); and endothelial lipase, which has both triacylglycerol lipase and phospholipase activities. The best characterized intracellular triacylglycerol lipase is hormone-sensitive lipase found in adipocytes. Hormone-sensitive lipase acts on the surface of the triacylglycerol droplet stored in the adipocyte to release stored fatty acids that can be delivered to the circulation as 'free' fatty acids (or more correctly as nonesterified fatty acids (NEFAs)) from where they are made available to tissues for β-oxidation (see succeeding text). As its name suggests, hormone-sensitive lipase is under tight regulation by various hormones, including adrenaline, noradrenaline, glucagon, and insulin. Insulin acts to inhibit its activity. In contrast, adrenaline and glucagon increase the activity of lipoprotein lipase. Therefore, through its concerted actions on the activities of lipoprotein lipase and on hormone-sensitive lipase, insulin acts to make available fatty acids from circulating lipoproteins for storage in adipose tissue and to retain those fatty acids held there in stored triacylglycerols (Figure 6). Note too that through its actions on glucose transport into adipocytes, insulin makes available the precursor for the synthesis of the glycerol 3-phosphate that is required for triacylglycerol synthesis. In contrast, an increase in adrenaline or noradrenaline concentration, for example, during exercise, or in glucagon concentration, for example, during fasting, enhances the activity of hormone-sensitive lipase, thus promoting release of NEFAs from adipose tissue (Figure 6). The bulk of these will be transported in the bloodstream noncovalently bound to albumin and will be destined for energy generation via β-oxidation in tissues like the skeletal muscle, heart, and liver (see succeeding text). Hormone-sensitive lipase also has cholesterol esterase activity. Adipose tissue also contains a lipase that is more active on monoacylglycerols than triacylglycerols.

Fatty Acid Release from Phospholipids

Phospholipases are enzymes that selectively remove different components of the phospholipid molecule including the fatty

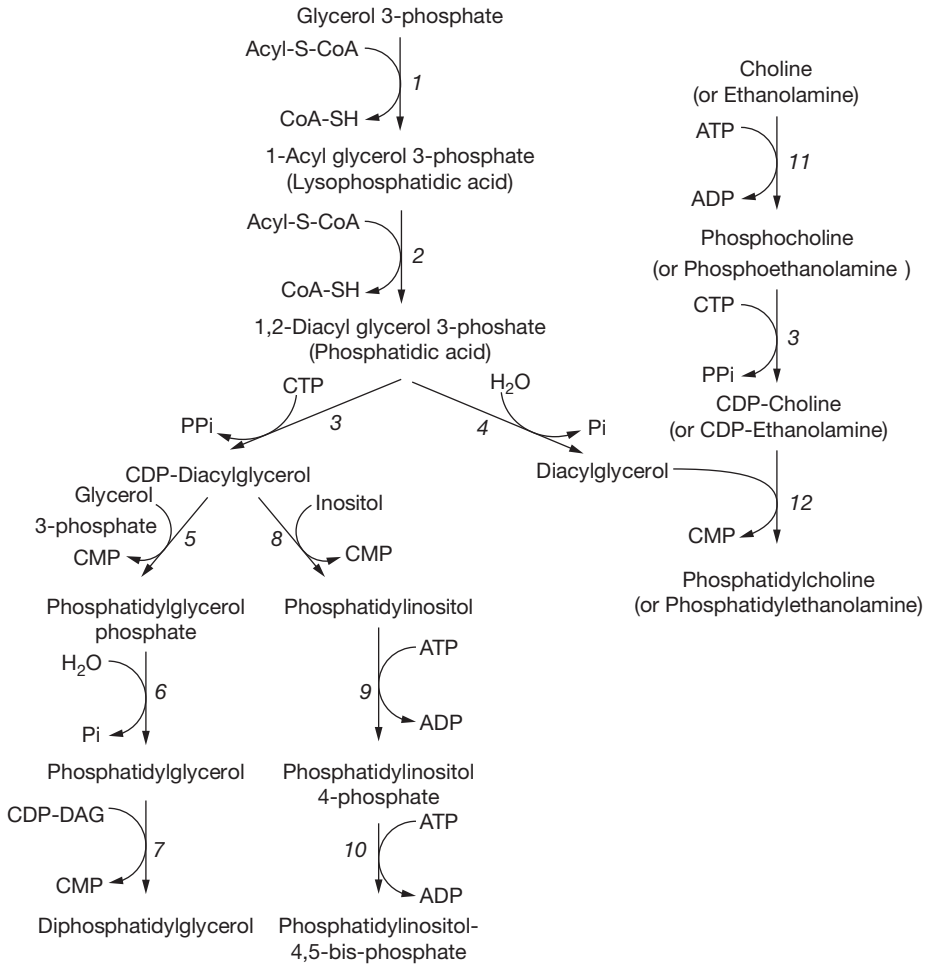

Figure 5 The pathways of phospholipid synthesis. Enzymes: 1, glycerol 3-phosphate acyltransferase; 2, lysophosphatidate acyltransferase; 3, cytidylyltransferase; 4, phosphatidate phosphohydrolase; 5, phosphatidylglycerol phosphate synthase; 6, phosphohydrolase; 7, cardiolipin synthase; 8, phosphatidylinositol synthase; 9, phosphatidylinositol 4-kinase; 10, phosphatidylinositol 4-phosphate 5-kinase; 11, choline (or ethanolamine) kinase; 12, choline (or ethanolamine) phosphotransferase.

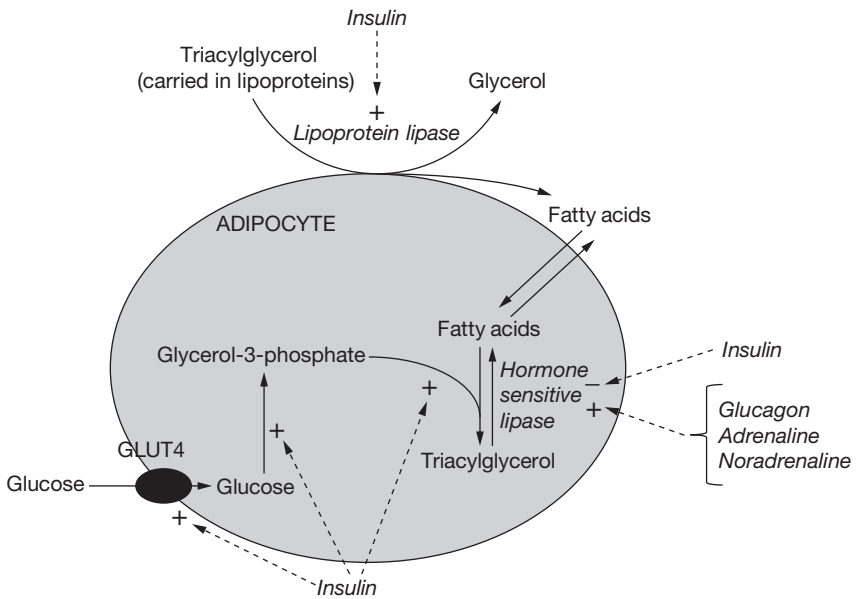

Figure 6 Scheme depicting triacylglycerol and fatty acid metabolism in adipocytes, the roles of lipoprotein lipase and hormone-sensitive lipase, and the regulation by hormones including insulin. GLUT4, glucose transporter type 4.

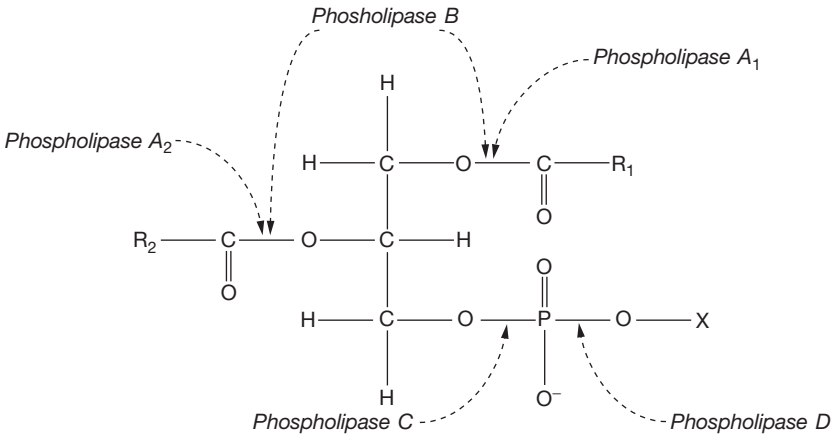

Figure 7 Sites of action of different phospholipase enzyme families toward a phospholipid substrate. R1 and R2 depict fatty acyl chains; X depicts the phospholipid base.

acyl groups at positions 1 and 2 or the base ('head group') or the entire phosphobase moiety. Phospholipases are classified according to their site of action on the substrate phospholipid (Figure 7). Type A phospholipases yield a monoacylphosphoglyceride. Phospholipase A_1 removes the fatty acid at the s*n*−1 position, while phospholipase A_2 removes that at the s*n*−2 position. The phospholipase A_2 enzymes are divided into four groups based upon their properties. Phospholipase C yields a 1,2-diacylglycerol and the phosphobase moiety, while phospholipase D yields 1,2-diacylglycerol 3-phosphate (phosphatidic acid) and the base. Type B phospholipases can remove fatty acids from both s*n*−1 and s*n*−2 positions, but these enzymes are quite rare. Phospholipases are important in the digestion of dietary phospholipids, in cell membrane remodeling, in generation of second messenger molecules, and in provision of PUFAs for the production of bioactive lipid mediators (see later section).

Fatty Acid β-Oxidation

β-Oxidation is the major metabolic pathway by which energy is released from fatty acids. The rate of fatty acid β-oxidation is controlled by the intracellular concentration of 'free' (i.e., unesterified) fatty acids, which in turn is determined by their concentration in the blood, so that a rise in the concentration of circulating NEFAs increases fatty acid oxidation in the tissues that can use them (most aerobic tissues but not the brain). NEFAs become important energy sources during starvation, endurance exercise, and other situations where carbohydrate supply is limiting.

Prior to β-oxidation, the fatty acid substrate is converted to its CoA ester derivative:

$$\text{Fatty acid} + \text{ATP} + \text{CoA} \rightarrow \text{acyl-CoA} + \text{AMP} + \text{PPi}$$

This reaction is catalyzed by a cytosolic acyl-CoA synthase; there are several such synthases with different specificities according to fatty acid chain length.

Fatty acid β-oxidation occurs in the mitochondrial matrix, and therefore, the fatty acid substrate (in the form of fatty acyl-CoA) needs to be transported across the outer and inner mitochondrial membranes that are not permeable to fatty acids or fatty acyl-CoAs with a hydrocarbon chain longer than 12 carbons. Shorter-chain fatty acids can cross both the outer and the inner mitochondrial membranes. Medium- and long-chain acyl-CoAs require a special transport mechanism to cross the mitochondrial membranes. Translocation of these is a carnitine-dependent process involving the coordinate action of isoforms of carnitine palmitoyltransferase (CPT) on the mitochondrial outer and inner membranes (Figure 8). CPT1 forms the fatty acylcarnitine at the outer mitochondrial membrane, and this is then transported to the intramembrane space via porin. The fatty acylcarnitine is transported through the inner mitochondrial membrane via acylcarnitine translocases in exchange for free carnitine. Once across the inner mitochondrial membrane, the fatty acyl-CoA is reformed by CPT2 (Figure 8). CPT1 is the rate-limiting step for mitochondrial β-oxidation. It is inhibited by malonyl-CoA, and thus, conditions that promote malonyl-CoA synthesis (e.g., the fed state) suppress fatty acid β-oxidation (and promote *de novo* fatty acid biosynthesis). Conversely, conditions that promote a decline in malonyl-CoA concentrations (e.g., fasting, starvation, exercise, and insulin resistance) will act to promote fatty acid β-oxidation (and suppress *de novo* fatty acid biosynthesis). These actions at the level of CPT1 form part of integrated whole body metabolic responses to changes in prevailing hormonal conditions, for example, in fasting-to-fed and fed-to-fasting transitions.

Fatty acid β-oxidation itself involves the progressive removal of two-carbon units, as acetyl-CoA, from the carboxyl end of the fatty acyl-CoA substrate in a series of four reactions that act sequentially and repeatedly (Figure 9). Each round of the cycle generates $FADH_2$ and NADH in addition to acetyl-CoA and an acyl chain that is two carbons shorter than the original. The latter reenters the cycle. Thus, complete β-oxidation of palmitoyl-CoA will generate eight acetyl-CoA, seven $FADH_2$, and seven NADH molecules:

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7\text{FAD} + 7\text{NAD}^+ + 7\text{H}_2\text{O} \rightarrow 8\ \text{acetyl-CoA} + 7\text{FADH}_2 + 7\text{NADH} + 7\text{H}^+$$

The acetyl-CoA produced by β-oxidation is normally oxidized in the citric acid (Krebs) cycle, while the $FADH_2$ and

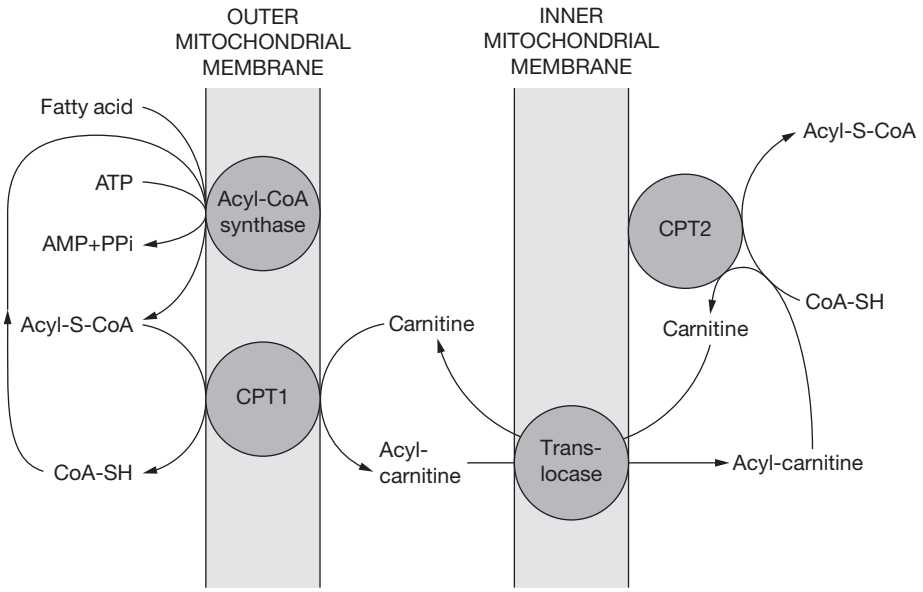

Figure 8 The carnitine palmitoyltransferase (CPT) system for transport of fatty acyl-CoA across the mitochondrial membranes into the matrix prior to β-oxidation.

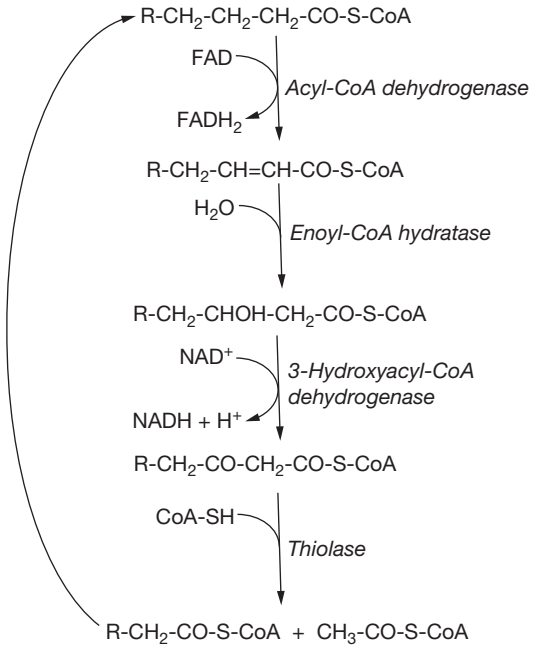

Figure 9 The pathway of β-oxidation of fatty acyl-CoA. Each cycle of four reactions produces acetyl-CoA and an acyl-CoA shortened by two-carbons that then reenters the cycle.

NADH pass their electrons to the mitochondrial electron transport chain. Hence, fatty acid β-oxidation generates a large amount of ATP per mole of fatty acid and per mole of fatty acid carbon oxidized:

$$\text{Palmitoyl-CoA} + 23O_2 + 131\text{Pi} + 131\text{ADP} \rightarrow \text{CoA} + 131\text{ATP} + 16CO_2 + 146H_2O$$

Because the activation of palmitate to palmitoyl-CoA consumes two ATP equivalents, the net gain per molecule of palmitic acid oxidized is 129 ATP. This is equivalent to approximately 37 kJ or 9 kcal of energy per gram of fatty acid oxidized, over twice the energy yield for carbohydrate or amino acid oxidation.

Entry of acetyl-CoA into the citric acid (Krebs) cycle releases CoA, and this is needed to maintain β-oxidation. This entry of acetyl-CoA requires a supply of oxaloacetate for it to condense to so producing citrate (the 'first' reaction of the cycle catalyzed by citrate synthase). The supply of oxaloacetate comes from pyruvate via the enzyme pyruvate carboxylase. Pyruvate carboxylase is activated by acetyl-CoA, which indicates a lack of oxaloacetate. In turn, the pyruvate is produced by glycolysis. Thus, some glucose metabolism is required to support continued fatty acid β-oxidation. The conversion of pyruvate to oxaloacetate is a type of anaplerotic reaction and, because of this requirement for some glucose metabolism to support fatty acid β-oxidation, it is said that "fats burn in the flame of carbohydrates."

The β-oxidation pathway outlined earlier operates for straight-chain saturated fatty acids with an even number of carbon atoms in the chain. Other specific enzymes are required for the oxidation of branched-chain, odd-numbered, or unsaturated fatty acids. β-Oxidation of odd-chain fatty acids yields propionyl-CoA.

Although the process of β-oxidation was originally thought to occur exclusively in mitochondria, a second β-oxidation system occurs in peroxisomes. Fatty acid transport into peroxisomes involves the CPT system, which similar enzymes to mitochondrial CPT2 and regulation via malonyl-CoA. Compared with mitochondrial oxidation, peroxisomal β-oxidation has a much broader substrate specificity and is especially active toward very-long-chain fatty acids and also toward many fatty acids with less common structural features, fatty acid derivatives, and more complex lipids. Acyl-CoA oxidase is an important enzyme involved in catalyzing the first step of peroxisomal β-oxidation. Peroxisomal β-oxidation is involved in

the biosynthesis of DHA from its precursor 24:6*n*−3 (Figure 3) and in the conversion of DHA to EPA, a process sometimes called retroconversion. Peroxisomal β-oxidation also seems important in metabolism of other very-long-chain fatty acids like erucic acid. Defects in acyl-CoA oxidase or in peroxisomal β-oxidation cause clinical features such as mental retardation, seizures, and leukodystrophy because of the inability to metabolize certain very-long-chain fatty acids that become elevated in the circulation if not metabolized.

Ketone Body Synthesis

Ketone bodies are produced using acetyl-CoA derived from fatty acid β-oxidation in the liver under specific metabolic conditions. The two ketone bodies are acetoacetate and β-hydroxybutyrate. Ketone body biosynthesis occurs in mitochondria and the pathway is shown in Figure 10. Two molecules of acetyl-CoA are condensed to form acetoacetyl-CoA, which is then conjugated to another molecule of acetyl-coA to form 3-hydroxy-3-methylglutaryl-CoA (HMGCoA), which is also an intermediate in *de novo* cholesterol biosynthesis. HMGCoA is then cleaved to release one molecule of acetyl-CoA and one molecule of acetoacetate. Some of the acetoacetate is reduced to β-hydroxybutyrate. Both acetoacetate and β-hydroxybutyrate are readily soluble in the bloodstream and are easily taken up by extrahepatic tissues, including the brain. Some acetoacetate may be nonenzymatically decarboxylated producing acetate, a volatile ketone that can appear in the breath.

In target tissues, β-hydroxybutyrate is oxidized back to acetoacetate, which is then converted to its CoA ester. This is cleaved to yield two molecules of acetyl-CoA, which can enter the citric acid (Krebs) cycle for complete oxidation. Thus, hepatic ketone body synthesis allows the transfer of fatty acid carbon to other tissues for oxidation and energy generation. This is especially important for the brain, which cannot utilize NEFAs as an energy source, but which can readily use ketone bodies.

Hepatic ketogenesis is promoted in times of carbohydrate shortage. As described earlier, glucose metabolism is required to support continued entry of acetyl-CoA into the citric acid (Krebs) cycle. However, during times of limited carbohydrate availability, the liver diverts pyruvate and oxaloacetate into gluconeogenesis (i.e., synthesis of glucose) and away from the citric acid (Krebs) cycle; hence, anaplerosis is not supported. This creates a metabolic challenge to supporting the recycling of CoA from acetyl-CoA back to fatty acids to enable their β-oxidation. A solution to this is to divert acetyl-CoA into another pathway that frees up the CoA to enable continuation of β-oxidation. Ketogenesis, which converts acetyl-CoA to acetoacetate and β-hydroxybutyrate, is this solution, and furthermore, it produces metabolic substrates that are able to be used by the brain when glucose is limiting.

Ketone bodies are synthesized during fasting (even after overnight fasting), starvation, and prolonged exercise; in

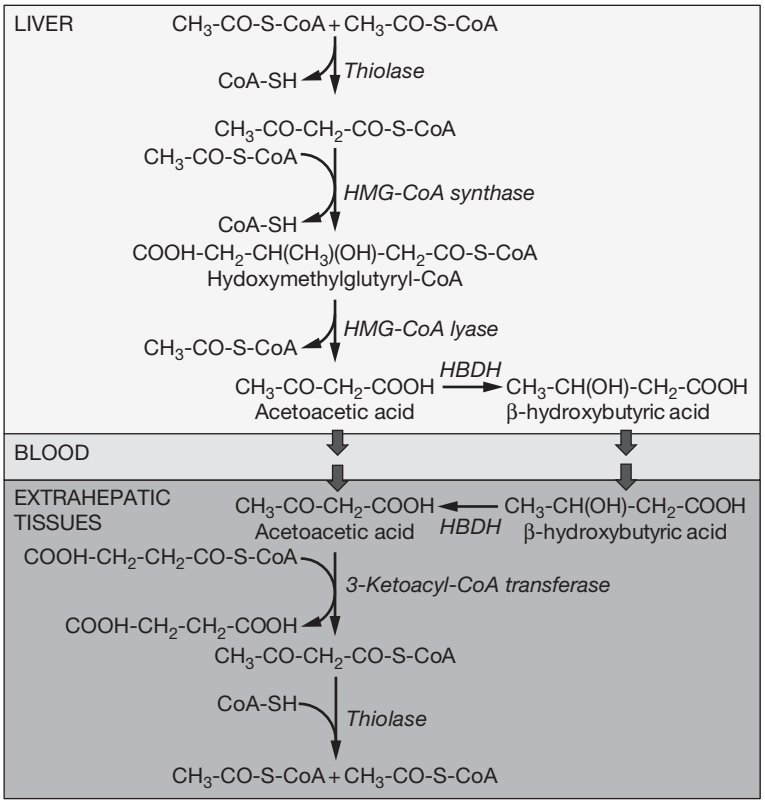

Figure 10 The pathways of ketone body synthesis (ketogenesis) and oxidation. HMG, hydroxymethylglutaryl; HBDH, β-hydroxybutyrate dehydrogenase.

response to high-fat, low-carbohydrate diets (these are sometimes called 'ketogenic diets'); and also by people with diabetes. In the latter case, the marked elevation in blood and brain ketone body concentrations can be pathological.

PUFAs as Substrates for Synthesis of Bioactive Lipid Mediators

One of the key functional roles of PUFAs is as substrates for the biosynthesis of bioactive mediators. These are oxidized derivatives of PUFAs that are usually produced by enzyme-catalyzed reactions, although some of them can be formed non-enzymatically. Linoleic, dihomo-γ-linolenic, arachidonic, eicosapentaenoic, osbond, clupanodonic, and docosahexaenoic acids have all been shown to give rise to such mediators, and it seems likely that all 18-, 20-, and 22-carbon PUFAs can act as substrates for their formation.

Among the possible PUFA precursors, arachidonic acid is usually the most prevalent in cell membranes, and consequently, the most well described of these families of PUFA-derived mediators are the eicosanoids formed from arachidonic acid (eicosa is a prefix that denotes that a structure contains 20 carbons). These include prostaglandins (PGs) and thromboxanes (TXs), which together are termed prostanoids, and leukotrienes (LTs), lipoxins (LXs), hydroperoxyeicosatetraenoic acids (HPETEs), and hydroxyeicosatetraenoic acids (HETEs). Prior to synthesis of eicosanoids, arachidonic acid is released from the s*n*−2 position of membrane phospholipids usually by the action of certain phospholipase A_2 enzymes (often cytosolic group IV phospholipase A_2), although pathways involving phospholipase C and diacylglycerol lipase also release arachidonic acid. The free arachidonic acid then acts as a substrate for cyclooxygenase (COX; e.g., COX-1 and COX-2), lipoxygenase (LOX; e.g., 5-LOX, 12-LOX, and 15-LOX), or cytochrome P450 enzymes (Figure 11). COX enzymes lead to PGs and TXs (2 series when formed from arachidonic acid), LOX enzymes to LTs and LXs (4 series when formed from arachidonic acid), and cytochrome P450 enzymes to HETEs and other derivatives (Figure 11). Eicosanoids act through binding to specific receptors, usually G protein-coupled receptors, and thus, they act in a hormonelike manner working to control cell and tissue responses. The range of activities of the eicosanoids produced from arachidonic acid is extremely broad and includes actions involved in controlling platelet aggregation, blood clotting, inflammation, immune function, smooth muscle contraction, renal function, neuron function, bone turnover, and tumor cell proliferation. The physiology and pathophysiology of arachidonic acid-derived eicosanoids are widely explored.

One feature of increased dietary intake of the very-long-chain *n*−3 PUFAs EPA and DHA is that they partly replace arachidonic acid at the s*n*−2 position of cell membrane phospholipids. The consequence of this is decreased availability of arachidonic acid as a substrate for eicosanoid synthesis. EPA and DHA may also inhibit arachidonic acid metabolism. These effects of EPA and DHA are thought to be important in preventing adverse pathological effects of eicosanoids produced from arachidonic acid like thrombosis (excess blood clotting), chronic inflammation, and tumor promotion and are thought to underlie several of the health benefits of EPA and DHA and foods that contain them like fatty fish. EPA is also a substrate for the synthesis of PGs and TXs (3-series) and LTs (5-series) but, in general, these are biologically weak compared to the arachidonic acid-derived eicosanoids.

Although PGs, TXs, and LTs have been known for decades, a relatively recent discovery has been of new families of bioactive oxidized derivatives of EPA and DHA. These derivatives,

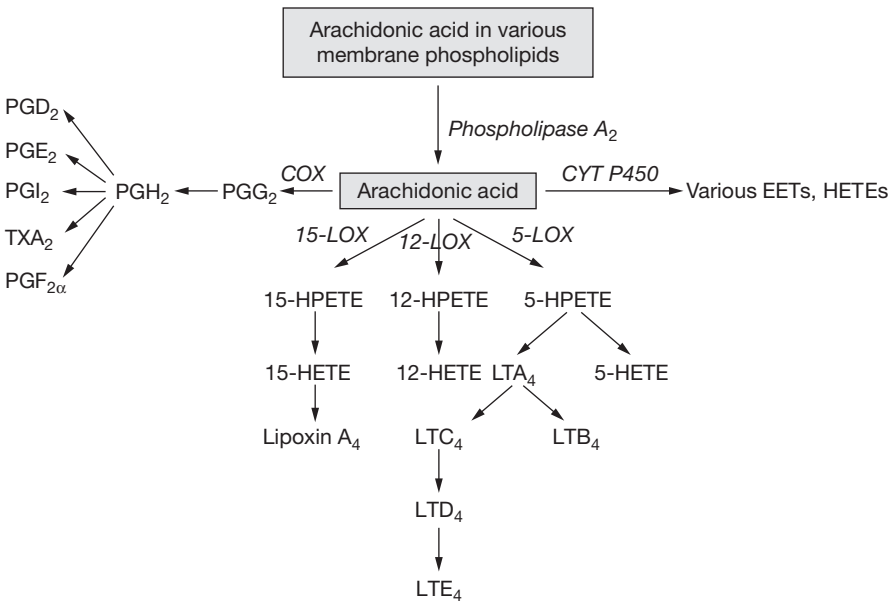

Figure 11 Overview of the pathways of conversion of arachidonic acid to eicosanoids. COX, cyclooxygenase; CYT P450, cytochrome P450; ETE, eicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane.

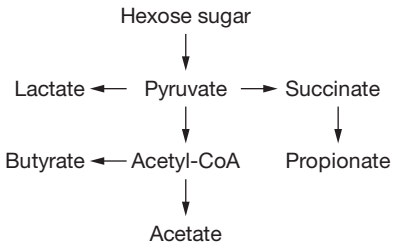

Figure 12 Brief overview of the pathway of biosynthesis of short-chain fatty acids from fermentation of simple sugars.

termed resolvins, protectins, and maresins, have been most thoroughly explored in the context of inflammation, where they have potent activity in resolution ('turning off') of ongoing inflammation. Hence, they have been termed specialized proresolving mediators. E-series resolvins are produced from EPA and D-series resolvins, protectins (sometimes called neuroprotectins), and maresins from DHA. Synthesis of these mediators involves the COX and LOX pathways often across more than one cell type, with different epimers being produced in the presence and absence of aspirin, and they act via specific G protein-coupled receptors. The full range of the biological activities of resolvins, protectins, and maresins has not yet been explored.

Short-Chain Fatty Acid Metabolism

Short-chain fatty acids are volatile fatty acids produced mainly by microbial fermentation. Their principal relevance to human physiology is that they are produced in the proximal colon by microbial fermentation of oligo- and polysaccharides that have escaped digestion by mammalian enzymes further up the gastrointestinal tract (**Figure 12**). There are also some short-chain fatty acids in ruminant milks. The major short-chain fatty acids are acetic, propionic, and butyric. They are very soluble and are absorbed easily from the gut lumen into the bloodstream. In humans, acetate is usually the major short-chain fatty acid present in the circulation. Increased dietary intake of substrates for short-chain fatty acid producing microbes increases their production resulting in higher concentrations in the gut contents and in the bloodstream. Short-chain fatty acids can be oxidized to produce energy, which is considered important for cells of the gut lining, while they can also be used as substrates for the synthesis of longer-chain fatty acids and ketone bodies.

See also: Adipose Tissue: White Adipose Tissue Structure and Function; Energy Metabolism; Fatty Acids: Determination and Requirements; Fatty Acids: Essential Fatty Acids; Fatty Acids: *Trans* Fatty Acids; Fish Oils: Composition and Health Effects; Ketone Bodies; Lipoproteins; Olive Oil: Its Role in the Diet; Phospholipids: Physiology; Triacylglycerols: Structures and Properties; Vegetable Oils: Dietary Importance.

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Fatty Acids: *Trans* Fatty Acids

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Introduction

The relationship between dietary fat type and cardiovascular disease risk was first identified soon after the turn of the twentieth century. Since that time, the predominance of data indicates that saturated fat is associated with the increased risk of cardiovascular disease, whereas unsaturated fat (monounsaturated or polyunsaturated) is associated with the decreased risk of cardiovascular disease. These conclusions come from both observational studies and interventional studies and have mechanistic underpinnings. However, within the category of unsaturated fatty acids, there are two subtypes that have been demonstrated to have unique effects with respect to cardiovascular disease risk: fatty acids with the first double bond in the omega-3 position of the acyl chain, associated with decreased risk, and fatty acids containing one or more double bonds in the *trans* configuration, associated with increased risk. The focus of this article will be restricted to fatty acids containing at least one *trans* double bond.

Terminology

The term '*trans* fat' typically refers to edible fats that contain *trans*-fatty acids. The term '*trans*-fatty acid' refers to fatty acids that have at least one double bond in the *trans*, as opposed to the more comment *cis*, configuration, in their acyl chain (Figure 1). *Trans* double bond containing fatty acids can be either monounsaturated or polyunsaturated. Edible fats contain a wide range of *trans*-fatty acid isomers, varying in the location of the *trans* double bond(s) and the number of *trans* double bonds in each acyl chain.

Food Sources of *Trans*-Fatty Acids

Ruminant Fat

Trans-fatty acids occur naturally in animal fat as a result of anaerobic bacterial fermentation in the rumen. These *trans*-fatty acids are absorbed from the gut and ultimately distributed throughout the fat depots of the animal. Hence, animal fats, such as meat and dairy fats, contribute to the *trans*-fatty acid content of the diet. Of note, currently dietary guidelines to reduce cardiovascular disease risk recommend limiting intake of animal fat due to the relatively high concentration of saturated fat. When these guidelines are adhered to, so too is *trans*-fatty acid intake from this source limited.

Partially Hydrogenated Fat

Another major dietary source of *trans*-fatty acids is from partially hydrogenated fat. The *trans* double bonds are formed during the partial hydrogenation of liquid oil, typically vegetable oil, which result from the breaking of *cis* double bonds and their random reformation, some of which form *trans* double bonds. In their unmodified state, vegetable oils are made up of fatty acids with only *cis* double bonds. Vegetable oils are partially hydrogenated to increase their viscosity by changing them from a liquid state to a semiliquid or solid state and to extend their shelf life by decreasing their susceptibility to oxidation. Both of these properties are favorable to the food industry.

Appearance of Partially Hydrogenated Fat in the Food Supply

The initial impetus for developing the process of partial hydrogenation of fat was to find an inexpensive substitute for butter. The French chemist Paul Sabatier first developed the process of partial hydrogenation around the turn of the twentieth century. This process involved a nickel catalyst. Shortly thereafter, the German chemist Wilhelm Normann developed a fat hydrogenation process that involved hydrogen gas.

The use of partially hydrogenated fat accelerated from the 1960s to the 1980s as food producers responded to public health recommendations to shift away from saturated fatty acids, particularly animal fats and tropical oils, to unsaturated fatty acids. At the time, partially hydrogenated fats appeared to be a good substitute for fats high in saturated fatty acids because their starting materials, vegetable oils, particularly vegetable oils high in polyunsaturated fatty acids. In addition, relative to vegetable oil in its natural state, partial hydrogenation resulted in a product with more favorable stability and functional properties, similar to that of solid animal fat. The incorporation of these fats into the food supply led to an increased *trans*-fatty acid intake. During this period, there was little concern about the effects of *trans*-fatty acids on lipoprotein profiles.

Estimating *Trans*-Fatty Acid/Partially Hydrogenated Fat Intake

The *trans*-fatty acid content of foods is incompletely captured in most nutrient databases, in part due to the rapidly changing use of partially hydrogenated fat. Changes in use of partially hydrogenated fat, primarily their decline, have been prompted by evolving labeling regulations and legislative mandates. Hence, there is likely a systematic misestimation of *trans*-fatty acid intake when using food frequency questionnaires or food records.

Plasma total *trans*-fatty acid, plasma phospholipid *trans*-fatty acid, and erythrocyte membrane *trans*-fatty acid concentrations have been used as a biomarker of *trans*-fatty acid intake, from both rudiment and partially hydrogenated fat sources. The usefulness of this approach is limited to estimating relative intakes within or among populations. An

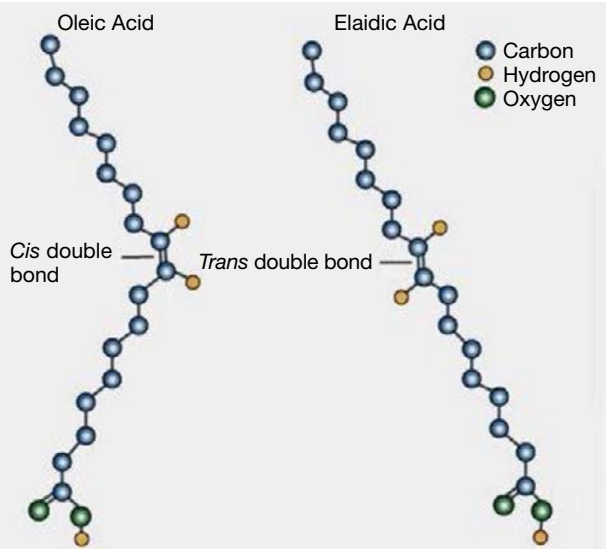

Figure 1 Structure of fatty acids containing a *cis* (left) and *trans* (right) double bond.

additional option for estimating relative *trans*-fatty acid intake from partially hydrogenated fat is to measure plasma dihydrophylloquinone concentrations. Dihydrophylloquinone is the hydrogenated form of vitamin K and not naturally present in vegetable oil. It is formed as an unintended consequence of partial hydrogenation. However, as with biomarkers for *trans*-fatty acid intake in plasma or erythrocyte membrane, the data only provide an assessment of relative intakes within and among populations.

More recently, an attempt to distinguish between the effects of ruminant and partially hydrogenated fats using the plasma proteome is being considered. At this time, it has met with limited success. In the development phase are attempts to identify potential protein biomarker panels for specific *trans*-fatty acids. This approach is based on changes in the regulation of proteins elicited by the addition of elaidic acid to HepG2 cells.

Current Sources and Trends in Dietary *Trans*-Fatty Acid Intake

Sources

The major source of *trans*-fatty acids in the food supply has come from foods made with partially hydrogenated fat, primarily commercially prepared fried and baked products. Over the past decade, the *trans*-fatty acid intake in the United States has declined. This trend has been prompted by a number of factors, including legislative changes at the federal and local levels and grassroots pressure to remove partially hydrogenated fat from the food supply. In the mid-2000s, the Food and Drug Administration mandated that by 2006, the *trans* fat content of packaged food must be listed in the Nutrient Facts label. This ruling appears to have prompted a re-formulation of some products. Between 2005 and 2010, the US Department of Agriculture documented a dramatic decline in the *trans* fat content of newly introduced foods and a rise in the use of 'no *trans* fat' claims on food packages. By 2011, it was estimated that about 66% of the products surveyed had lower *trans*-fatty acid content than previously. Of those, the vast majority, 82%, had <0.5 g per serving of *trans*-fatty acids. Overall, the mean *trans*-fatty acid content of the products surveyed decreased by 49% between 2007 and 2011. Of note, at the time mandatory *trans* fat labeling was enacted, the Food and Drug Administration ruled that products containing <0.5 g *trans* fat per serving could be labeled as 0 g *trans* fat or *trans* fat-free.

Around that same time that the FDA mandated *trans* fat labeling on packaged foods, some US localities restricted the use of partially hydrogenated fats by restaurant chains. As a result, in one such city (New York City), between 2006 and 2008, the proportion of restaurants using partially hydrogenated fat declined from 51% to 2%. More recently, in late 2013, the Food and Drug Administration published a preliminary determination that partially hydrogenated fat, which is currently included on the Food and Drug Administration's 'generally recognized as safe' list, will be removed. A final ruling is expected to become available in 2015. If put into effect, this ruling will likely precipitate additional product reformulation in a variety of food categories and result in a further decline in the *trans*-fatty acid content of commercially prepared foods.

Trends

Although it is difficult to assess the potential public health impact of changes in the *trans*-fatty acid content of the food supply, fragmentary data suggest that the legislative mandates in Scandinavia and the United States have contributed to a secular decline in *trans* fat intake in those places. For example, it has been estimated that between the years 2000 and 2009, plasma *trans*-fatty acid concentrations in non-Hispanic, white adults living in the United States declined by 50%. In the Framingham Offspring Study cohort, between 1999 and 2006, erythrocyte *trans*-fatty acid content declined by 23%. Similar downward trends have been seen in Canada, the United Kingdom, Costa Rica, and Western Europe, but *trans*-fatty acid intake remains high in Eastern Europe and appears to have increased in Greece, North Africa, the Middle East, and South Asia.

Trans-Fatty Acids and Lipoprotein Profiles and Metabolism

Lipoprotein Profiles

Early work in the 1950s through 1970s evaluating the effect of *trans*-fatty acids on plasma cholesterol concentrations suggested a neutral effect. When differences were observed, they were attributed, in part, to decreases in the level of polyunsaturated fatty acids and increases in saturated fatty acids. In the early 1990s, reports started to appear suggesting that, similar to saturated fatty acids, *trans*-fatty acids increased plasma low-density lipoprotein (LDL) cholesterol concentrations and, in contrast to saturated fatty acids, *trans*-fatty acids did not increase high-density lipoprotein (HDL) cholesterol concentrations. Taken together, these changes resulted in a less favorable LDL cholesterol/HDL cholesterol ratio with respect to cardiovascular disease risk. Subsequent work confirmed these findings and, with few exceptions, demonstrated a dose–response relationship between dietary *trans*-fatty acids and LDL cholesterol concentrations. A trend toward increased triglyceride concentrations resulting from *trans*-fatty acid intake has been reported in some studies.

Other Cardiovascular Disease Risk Factors

Small, dense LDL particles have been associated with a higher risk of cardiovascular disease than large, buoyant LDL particles. Dietary partially hydrogenated fat, compared to saturated or polyunsaturated fat, has been reported to shift LDL particle distribution from the large, buoyant particles toward small, dense LDL particles. Changes in fasting insulin and glucose concentrations resulting from dietary partially hydrogenated fat have been reported to be small and, hence, would not be predicted to have a large impact on glucose homeostasis. No significant effect of partially hydrogenated fat intake on C-reactive protein concentrations, lipoprotein (a), blood pressure, visceral adiposity, metabolic syndrome, inflammatory factors, and susceptibility of LDL particles to oxidation has been reported. When effects have been observed, they tend to be small and occur at relatively high intakes of *trans*-fatty acids, and the data are not as consistent as the effects on LDL and HDL cholesterol concentrations.

Trans-Fatty Acid Metabolism

Changes in *trans*-fatty acid intake are associated with proportional changes in tissue levels. For example, plasma and erythrocyte *trans*-fatty acid content declines when intake declines, making these useful measures for assessing trends in *trans*-fatty acid intake. These data also suggest that humans have adequate capacity to metabolize *trans*-fatty acid isomers.

Early work that focussed on identifying the mechanism by which *trans*-fatty acids elevate LDL cholesterol concentrations led to the conclusion that higher rates of *de novo* cholesterol synthesis were not a contributing factor, leading to the speculation that an impairment in the catabolic pathway was involved. This speculation was confirmed with the use of stable isotope-labeled leucine to characterize lipoprotein kinetics in response to partially hydrogenated fat intake. Relative to butter (saturated fatty acids) and soybean oil (polyunsaturated fatty acids), partially hydrogenated fat increased LDL concentrations by decreasing the catabolic rate, resulting in a larger pool size. Little effect of partially hydrogenated fat on LDL production rate was observed. In contrast, partially hydrogenated fat lowered HDL concentrations by increasing the catabolic rate, resulting in a smaller pool size. Again, little effect on HDL production rate was observed. Additional work on the effect of *trans*-fatty acids on HDL metabolism indicated that differences in cholesteryl ester transfer protein activity, phospholipid transfer protein activity, or the fractional esterification rate of cholesterol in HDL did not account for the differences in observed concentrations.

In some cases, dietary partially hydrogenated fat has been reported to increase plasma triglyceride concentrations. In an attempt to determine the mechanism by which *trans*-fatty acids alter plasma lipoprotein profiles, the effect of partially hydrogenated fat on triglyceride uptake by adipose tissue was estimated by measuring plasma acylation-stimulating protein (ASP) concentrations. The data indicated that a diet high in partially hydrogenated fat resulted in lower plasma ASP concentrations compared with a diet high in polyunsaturated fat. Differences in remnant-like particle concentrations, measured in both the fasting and nonfasting states, did not account for differences in triglyceride concentrations.

Trans-Fatty Acid and Cardiovascular Risk

The negative effect of partially hydrogenated vegetable oil and the resulting *trans*-fatty acids on plasma lipoprotein profiles bears out consistently in multiple research studies conducted throughout the 1990s. A meta-analysis of prospective cohort studies and retrospective case-controlled studies resulted in an estimated 2% increase in energy intake from *trans*-fatty acids is associated with a 23% increase in the relative risk of coronary heart disease. Similar estimates on the basis of multiple trials have been made for the substitution of *trans*-fatty acids for saturated, monounsaturated, and polyunsaturated fatty acids, all of which appear to have a more favorable effect on LDL cholesterol and HDL cholesterol concentrations, and the total cholesterol/HDL cholesterol ratio.

The relationship of dietary *trans*-fatty acids and cardiovascular disease risk has been well documented and reviewed. Recent work supports these earlier findings. *Trans* 18:2 isomer intake has been associated with less favorable indexes of heart rate variability in adults from two cohorts: the Cardiovascular Heart Study, involving older subjects (mean age 72 years), and a cohort of younger Portuguese subjects (mean age: 19 years). *Trans* 18:1 isomers were associated with more favorable heart rate variability in the older, but not the younger, cohort. Similarly, a study of middle-aged and older Chinese volunteers concluded that higher erythrocyte 18:2 *trans*-fatty acid concentrations were associated with dyslipidemia, whereas higher 18:1 *trans*-fatty acid concentrations were associated with lower risk of type 2 diabetes. In the Nurses' Health Study, *trans*-fatty acid intake, estimated from food frequency questionnaires, was not associated with sudden cardiac death. However, when the data were assessed on the basis of women with and without coronary heart disease, *trans*-fatty acid intake was positively associated with sudden cardiac death in those women who had clinical atherosclerosis. Data from the Reasons for Geographical and Racial Differences in Stroke (REGARDS) cohort, a US-based prospective cohort study of white and black women and men, indicate that *trans*-fatty acid intake, as assessed using food frequency questionnaires, was associated with higher all-cause mortality. Using plasma phospholipid profiles from two cohorts, the Cardiovascular Health Study and the Estrogen Replacement and Atherosclerosis Study, *trans*-fatty acids were associated with increased cardiovascular disease risk.

Ruminant Versus Partially Hydrogenated Fat and Cardiovascular Disease Risk

Some observational data have suggested that dietary *trans*-fatty acids derived from partially hydrogenated fat are more closely associated with cardiovascular disease risk that dietary *trans*-fatty acids derived from ruminant fat. However, these data have not been consistent.

The difference between ruminant and partially hydrogenated fat sources of *trans*-fatty acids is reflected in the relative distribution of *trans*-fatty acids isomers. Conjugated linolenic acid and vaccenic acid predominate in ruminant fat. The results of clinical studies comparing these two sources of *trans*-fatty acids have been mixed. Some have reported no or only small differences between ruminant and partially

hydrogenated fat sources of *trans*-fatty acids on lipoprotein profiles, whereas others have reported that partially hydrogenated fat results in less favorable lipoprotein profiles than ruminant fat. Interpreting these data can be challenging, because in many studies, the level of ruminant fat *trans*-fatty acid isomers achieved in the test diets could not be achieved with current forms of ruminant fat in the food supply.

Conclusions

The data supporting a negative effect of dietary *trans*-fatty acids on cardiovascular disease risk are consistent. The primary dietary sources of *trans*-fatty acids include partially hydrogenated fat and ruminant fat. Due to legislative changes and public pressure, intake from the former source has declined over the past decade. Adherence to current recommendations to restrict the intake of saturated fat from meat and dairy products would result in reduction in *trans*-fatty acid intake from the latter source. These factors have contributed to a secular decrease in the United States and some other countries worldwide. The adverse effect of *trans*-fatty acids on plasma lipoprotein profiles is consistent; the effect of *trans*-fatty acids on other cardiovascular risk factors is less clear. Observational data consistently demonstrate an adverse effect of *trans*-fatty acids and partially hydrogenated fat on cardiovascular disease risk. Yet to be resolved is the issue of whether *trans*-fatty acid isomers predominating in ruminant and partially hydrogenated fats have differential effects in terms of health outcomes. This uncertainty should not detract from a clear and consistent public health message to restrict the intake of *trans*-fatty acids. Recent trends in the food supply toward less use of partially hydrogenated fat have accelerated this change.

See also: Fatty Acids: Fatty Acids; Fatty Acids: Metabolism; Soybean Oil; Vegetable Oils: Composition and Analysis; Vegetable Oils: Dietary Importance.

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Fermented Foods: Composition and Health effects

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Introduction

Fermentation is a biotechnological process used by man since, at least, the Neolithic period (around 10 000 years BC). The fermentation process is carried out by microorganisms (filamentous fungi, yeast, bacteria, or a combination of them) in anaerobic conditions and consists of the conversion of fermentable carbohydrates into end-metabolites such as organic acids, alcohols, and carbon dioxide. Louis Pasteur defined the fermentation process as 'la vie sans l'air,' which means 'life without air.'

Although the main aim for its application was initially food preservation and an increase in the shelf life, other effects such as the improvement of safety, nutritional value, and organoleptic quality of the foods are simultaneously achieved.

The discovery of microorganisms in the nineteenth century enabled the improvement of the production of fermented foods using specific starter cultures that can be added in a large number to accelerate and steer the fermentation process and to obtain a broad variety of fermented foods with different organoleptic and nutritional properties. Spontaneous fermentation is still used in some cases, such as in traditional foods and foods in which the microorganisms responsible for the fermentation are not well known. The microorganisms that can be used as starter cultures because of their proved safety, technological role, and/or beneficial use are gathered in lists elaborated by different institutions such as the International Dairy Federation (IDF), the European Food and Feed Cultures Association (EFFCA), the European Food Safety Authority (EFSA), and the Food and Drug Administration (FDA). Recently, an updated inventory of microorganisms used in food fermentations covering a wide range of food matrices (dairy, meat, fish, vegetables, legumes, cereals, beverages, and vinegar) was presented. The inventory of microbial food cultures (live bacteria, yeasts, or molds used in food production) contains 195 bacterial species and 69 species of yeasts and molds. There are two main fermentation processes: alcoholic fermentation, which results in the production of ethanol and is carried out predominantly by yeasts, and lactic acid fermentation, which gives rise to a great variety of products, occurs mainly in dairy products and cereals, and is carried out by lactic acid bacteria (LAB). Also, acetic acid and alkali fermentation processes take place in alcoholic products and in fish and seeds, respectively.

In the last years, special attention has been paid to the nutritional and health effects of fermented foods derived from microbial metabolic activities. This article is mainly focussed on these aspects. A brief revision of the composition of the different groups of fermented foods and the currently known main health aspects related to the biocompounds resulting from the fermentation process is reported.

Fermented Foods: Composition and Nutritional Value

Approximately one-third of human food intake corresponds to fermented foodstuffs. The major groups of fermented foods worldwide are dairy products, beverages (beer, wines, cider, coffee, and tea), and cereal-based products (bread, pancakes, and donuts) and several fermented foods derived from meat, fish, fruits, and vegetables.

The nutritional and organoleptic properties that characterize each type of these products depend on the composition of the food matrix, the type of microorganism responsible for the fermentation, and the processing conditions.

Dairy Products

The group of dairy products encompasses some of the most typical fermented foods, especially in Europe, North America, and the Middle East and also in Africa and Southeast Asia. The most common subcategories are cheeses and acid-fermented milks.

There are more than 1000 different types of cheeses elaborated with milk from different animal species (cow, sheep, goat, camel, and buffalo) and different maturation degrees, giving rise to a wide variety of textures and flavors. From a nutritional point of view, cheeses are in general high-density products with high energetic value and significant fat, protein, vitamin B, and calcium contents. Also, if they are subjected to large ripening processes, high amounts of biogenic amines (BAs) might be present. These compounds, resulting from microbial decarboxylation of amino acids, may generate toxic effects such as headache and hypertensive crisis and also act as inhibitors of histamine-detoxifying enzymes (as monoamine oxidase). Selection of appropriate starter cultures (decarboxylase-negative activity) has been suggested as a good strategy to try to reduce BA formation during fermentation processes.

Acid-fermented milk (e.g., yogurts and kefir) have different nutritional profiles depending on the ingredients used: type of milk (full fat vs. skimmed milk), added sugars or flavors, etc. The LAB responsible for their fermentation give rise to a degree of acidity that inhibits the growth of other types of microorganisms. In addition, they can also produce several bacteriostatic compounds and compounds responsible for the characteristic organoleptic properties of these types of products. One of the main changes that occur during the fermentation of milk is the conversion of lactose (milk sugar) into the more digestible lactate. Effectively, there are a growing number of individuals that are intolerant to lactose, which benefit from the fermentation of milk. There are other beneficial effects related to the intake of LAB (*Lactobacillus* and *Streptococcus*) and *Bifidobacterium* that are also used as starter cultures in fermented milk. Some of these microorganisms are considered as probiotics and can be positively related to an improvement of the intestinal tract health, an enhancement of the immune system, a decrease in the prevalence of allergy in susceptible individuals, and even a risk reduction of certain cancers. As for cheeses, fermented milk are also rich in calcium and B vitamins.

Fermented Beverages

Different beverages result from the fermentation process of vegetable products. Wines, beers, and cider have a rich composition that is dependent on the fermentation process (predominantly carried out by yeasts) and also on the raw materials (grapes or fruit juice and barley or other cereals) that serve as substrates. Ethanol is a common end product in beverage fermentation and has been classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC). However, there are several epidemiological studies suggesting that moderate consumption of alcohol reduces overall mortality, mainly from coronary diseases. In general, the positive effects of alcoholic beverages are due to the presence of multiple polyphenols, bioactive compounds present in the vegetables or even their metabolites as a consequence of the fermentation process. Wine, being the richest in very active polyphenols such as resveratrol, is associated, when consumed in moderate amounts, with a decreased incidence of cardiovascular disease, hypertension, diabetes, and certain types of cancer. These positive effects could also be due to the presence of other phytochemicals, such as melatonin and phytosterols. Additionally, moderate consumption of beer has been associated with these effects to a lesser degree, probably because of its lower amount of polyphenols. Nevertheless, beer is rich in nutrients such as carbohydrates, amino acids, minerals, and vitamins. In the case of distillate drinks (spirits), they hardly have other interesting compounds apart from ethanol and the aromatic compounds responsible for their organoleptic properties. However, it is worthy to note that favorable changes in several cardiovascular biomarkers (higher levels of high-density lipoprotein cholesterol and adiponectin and lower levels of fibrinogen) provide indirect pathophysiological support for a protective effect of moderate alcohol use on coronary heart disease, independently of its source of supply.

Tea and coffee constitute another interesting group of fermented beverages characterized by their supply of stimulating compounds. It should be pointed out however that, although microorganisms (basically fungi) are involved in their processing, the main changes are due to endogenous polyphenol oxidase activity and other biochemical reactions that take place during fermentation. Although the intake of caffeine, theophylline, and theobromine has been associated with lifestyles related to risk of cancers, those beverages have shown some beneficial effects. Tea is the second most popular beverage in the world after water. Around 75% of tea consumed worldwide is black tea (obtained from the fermentation of green tea). There are many studies demonstrating protective effects of tea over chronic diseases as cardiovascular pathologies, and also, there are some studies, although not conclusive, demonstrating a chemopreventive effect. These effects are related to the high amounts of polyphenolic compounds, especially catechins. Effectively, during the fermentation process to obtain black tea, catechins are converted in a great extent to polymeric complexes known as theaflavins and thearubigins, which seem to be also effective in the prevention of cardiovascular disease. Coffee is very rich in several biological compounds as caffeine, diterpenes, chlorogenic acids, and melanoidins, which may affect human health. The health benefits of coffee consumption regarding the cardiovascular system and metabolism mostly depend on its antioxidant compounds; meanwhile, diterpenes and caffeine may produce harmful effects by raising lipid fraction and affecting endothelial function, respectively. The variety of coffee species, roasting degree, and brewing method determine the final composition and the derived effects.

Fermented Meat and Fish Products

Meat fermentation processes in combination with drying and smoking probably developed empirically over 5000 years ago and have given rise to a broad variety of products: dry, semidry, mold-ripened, and undried products all around the world. The fermentation process is carried out basically by LAB and coagulase-negative staphylococci. Fermented meat products have the nutritional advantages related to the intake of meat, which is a significant amount and quality of proteins depending on the type and percentage of raw meat used in the formulation, iron (50–60% in the heme form), zinc, and vitamin B. On the other hand, they have some arguably negative aspects related to a generally high amount of fat (with a high saturated fat proportion), cholesterol, and sodium. Moreover, the presence of nitrites/nitrates used as nitrificants (curing process) in their formulations has to be taken into account. As the result of the fermentation process (due to proteolytic and lipolytic activities of microorganisms in concertation with the action of meat enzymes), a wide variety of compounds with significant impact on the sensory properties are formed: amino compounds, aldehydes, alcohols, acids, alkanes, alkenes, ketones, and even BAs. Some of these compounds have been pointed out as functional compounds, in particular some of the peptides. Furthermore, there are studies that have pointed out that fermented sausages could be considered as probiotic foods although much more studies are needed to establish the actual health benefits. Moreover, different studies trying to improve the composition of fermented sausages in order to get healthier products are being carried out.

Fermented fish products are especially consumed in Asia and Oceania countries. These products include very popular sauces and pastes, which are used as condiments and ingredients in a great variety of processed foods. They are rich in proteins and amino acids but sometimes also in BAs, which have to be controlled. Also, they have in general high amounts of salt (around 20–25%, except for a traditional product sikhae, which contains 6–8%).

Cereal-Based Fermented Products

Cereals are a good microbial substrate, so the fermentation process takes place in a natural way involving mixed cultures of yeast, bacteria (in general LAB), and fungi (*Aspergillus*, *Fusarium*, *Penicillium*, and *Trichothecium*, among the most frequent). Fermentation of cereals gives rise to very interesting changes in composition, namely, a decrease in the level of carbohydrates and nondigestible oligosaccharides, a synthesis of some amino acids, an increase in the availability of the vitamin B group, and a degradation of phytate (a quelant of iron, zinc, and calcium). As a consequence of the fermentation of wheat and rye flour by LAB (sourdough process), a reduction in the starch digestibility is noticed, leading to a lower glycemic

index. This effect is mainly attributed to the formation of organic acids (lactic acid), to the greater release of peptides and amino acids, or to the increment in the amount of free phenolic compounds, among other factors. All these changes improve the nutritional properties and even can lead to the development of functional products. Effectively, the products formed as a consequence of the fermentation process and the presence of some compounds with health beneficial effects, as it is the case of β-glucan (especially present in oat), make fermented cereals a group to be taken in consideration in the context of functional foods. Moreover, cereals have also been evaluated as good substrates for the growth of probiotic strains, giving rise to some new probiotic foods (e.g., Yosa and Dogik) with supposed beneficial effects, although these aspects are still a research challenge. Furthermore, the fact that cereal-based fermented products are major contributors to energy intake in developing countries has to be considered.

Fermented Fruits and Vegetables

Fresh vegetables and fruits are recommended in a healthy diet because they are fundamental sources of vitamins, minerals, dietary fiber, and biocompounds as phytosterols (hypocholesterolemic effects) and polyphenols (antioxidant properties). However, there are some traditional and emerging fermented products resulting from lactic acid fermentation that represent a suitable way for increasing the consumption of vegetables and fruits. For instance, kimchi is a traditional fermented vegetable product of Korea, which is consumed in winter when the dietary intake of fresh vegetables might be more difficult. Other examples include sauerkraut, cocoa, fermented olives, pickles, and fermented radish.

Bioactive Compounds Derived from the Fermentation Processes

Fermentation leads, among other changes, to the release and/or synthesis of different bioactive compounds that are responsible for some of the health benefits attributed to this type of foods.

Table 1 gathers some of the most common bioactive compounds resulting from fermentation processes and the foods in which they are present.

Bioactive Peptides

Fermentation is one of the mechanisms by which bioactive peptides can be present in foods. Effectively, a number of papers describe the release of these compounds during fermentation of a wide variety of foods, ranging from milk, legumes, meat, soybean, to sourdough. These compounds, formed by 2–20 amino acid sequences, have been related to several physiological functions able to influence health.

Antihypertensive properties have been described for IPP (isoleucine–proline–proline) and VVP (valine–proline–proline) tripeptides released during the fermentation of milk by different proteolytic strains of *Lactobacillus helveticus* (CP790, LBK-16H, and ASC474), *Streptococcus thermophilus*, and *Saccharomyces cerevisiae*. The formation of these and other peptides can be affected by fermentation time and temperature and by pH and calcium concentration in medium. The main mechanism involved in their biological activity deals with the inhibition of ACE (angiotensin-converting enzyme). It has been postulated, for instance, that after a 2-week consumption of 150 ml day^{-1} of fermented milk, hypertensive patients reduced systolic blood pressure approximately by 7 mmHg. Other studies do not clearly support this finding. Nevertheless, according to the EFSA, more studies are needed to conclude about the cause–effect hypotensive activity. In addition, not only the production but also the stability of bioactive peptides has to be taken into account during fermentation.

Antioxidant, antimicrobial, and immunomodulatory properties and anti-inflammatory potential have also been described for peptides formed during the fermentation of milk and other dairy products such as kefir or cheese.

Soybean is also an important source of bioactive peptides. Thus, the proteases and peptidases of *Bacillus*, *Aspergillus*, and *Rhizopus* strains have been shown to hydrolyze soy proteins into active hypotensive peptides. In addition, soy-fermented products such as nattō and tempeh have yielded antioxidant peptides. Less explored sources of bioactive peptides are lentils or porcine skeletal muscle proteins, whose fermentations by *Lactobacillus plantarum* or *Lactobacillus sakei* and *Lactobacillus curvatus*, respectively, were able to release also ACE inhibitory peptides during *in vitro* studies.

Exopolysaccharides

Exopolysaccharides (EPSs) are homopolymers or heteropolymers with a wide diversity of structures, capable of modifying the sensory properties of foods. Some strains of LAB species (*Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactococcus lactis*, *Lactobacillus plantarum*, *Lactobacillus casei*, etc.) are EPS producers, and in fact, one of these bacteria, *Leuconostoc mesenteroides*, excretes dextran, authorized as a novel food ingredient to be used in bakery products (Decision 2001/122/EC). Besides its technological property as thickener in bakery and dairy products, prebiotic potentialities have been associated with this polymer, as gut microbes metabolize it to produce propionic acid, which is able to reduce cholesterol and triglyceride levels. In addition, EPSs have generally been suggested as blood cholesterol reducers, antioxidants, immunomodulators, and antitumor and antiulcer agents. One of the most studied strains for the antitumor activity of its EPS has been *Lactobacillus plantarum* 70 810, whereas the EPS from *Lactobacillus helveticus* MB2-1 with whey as matrix was associated with strong scavenging activity in *in vitro* tests. Kefir grains are also able to release EPSs from soy milk. However, there is a lack of clinical evidence demonstrating these putative prebiotic characteristics and beneficial effects after oral administration of EPS in functional foods.

Arabinoxylans

During the fermentation of cereal-derived processed food such as bread or beer, the enzymatic hydrolysis of arabinoxylans, yielding arabinoxylan oligosaccharides, can occur. These compounds have been associated with prebiotic and other health effects (improved gut transit, reduced serum triglyceride and

Table 1 Nonexhaustive list of examples of compounds produced during fermentation of foods

Compound	*Health effect*	*Substrate*	*Microorganisms*	*References*
Peptides	Hypotensive	Lentils	*Lactobacillus plantarum* CECT 748 T	Torino et al. (2013)
Peptides	Hypotensive	Soybean meal	*Bacillus subtilis natto*	Wang et al. (2013)
Peptides	Hypotensive	Fermented milk	*Lactobacillus helveticus* LBK-16H	Seppo et al. (2003)
Peptides	Hypotensive	Fermented milk	*Lactobacillus helveticus 1188*	Elfahri et al. (2014)
Exopolysaccharide	Antitumor	Chinese pao cai (fermented cabbage)	*Lactobacillus plantarum* 70 810	Wang et al. (2014a,b)
Exopolysaccharide	Scavenging activity	Whey	*Lactobacillus helveticus* MB2-1	Li et al. (2014)
Phenolic compounds	Antioxidant activity	Buckwheat, wheat germ, barley, and rye	*Lactobacillus rhamnosus* and yeast *Saccharomyces cerevisiae*	Dordevic et al. (2010)
Phenolic compounds	Antioxidant activity	Red cabbages	*Lactobacillus plantarum* ATCC 8014	Hunaefi et al. (2013)
Folates, free phenolic acids, total phenolic compounds, lignans, and alkylresorcinols	Varied	Germinated rye	*Saccharomyces cerevisiae*	Katina et al. (2007)
Isoflavone aglycone	Improvement of glucose metabolism, antioxidant activity, alleviation of hormonal disorders in postmenopausal women, etc.	Soy milk	*Lactobacillus rhamnosus* C6	Hati et al. (2015)
GABA	Hypotensive	Lentils	*Lactobacillus plantarum*	Torino et al. (2013)
GABA	Hypotensive	Cheeses	*Lactobacillus brevis* CECT 8183, CECT 8181, and CECT 8182 and *Lactococcus lactis* subsp. *lactis* CECT 8184	Diana et al. (2014)
CLA	Varied	Pickle	*Lactobacillus plantarum*	Liu et al. (2011)
CLA	Varied	Milk	*Lactococcus lactis* (best among 155 LAB cultures)	Ashish et al. (2012)
Ubiquinone	Antioxidative	Jeotgal	*Bacillus subtilis*, *Leuconostoc mesenteroides*, *Pediococcus halophilus*, *Torulopsis* sp., *Sarcina* sp., *Saccharomyces* sp.	Pyo and Oh (2011)
Folate	Vitamin	Oat bran	*Saccharomyces cerevisiae* and *Candida milleri*	Korhola et al. (2014)
Vitamin B_2	Vitamin	Pasta and bread	*Lactobacillus plantarum*	Capozzi et al. (2011)
Probiotic mixture	Modification of activity of the brain regions that control the central processing of emotion and sensation	Fermented milk product with probiotic (FMPP)	*Bifidobacterium animalis* subsp. *lactis*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactococcus lactis* subsp. *lactis*	Tillisch et al. (2013)
Probiotics	Gut health	Fermented foods	Mainly *Lactobacillus* and *Bifidobacterium* strains	Smug et al. (2014)

cholesterol levels, and immunomodulating and antioxidative effects), although they have not been extensively studied yet.

Polyphenols and Flavonoids

Health beneficial effects are attributed to polyphenols and flavonoids present in vegetable products. These compounds are predominantly found as glycosides in nature. However, their bioavailability in the organism increases when they are not glycosylated. Some food-fermenting LAB can remove glycoside residues by means of enzymatic activity (α-rhamnosidase, β-galactosidase, and β-glucosidase), resulting in increased bioavailable bioactive compounds. Some examples of LAB causing the hydrolysis of healthy phenolic compounds are the following: *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactobacillus brevis*, and *Pediococcus pentosaceus*. Strains of these

LAB species have been shown to hydrolyze oleuropein, the most prevalent polyphenol present in olives, to which some of the beneficial effects of unrefined olive oil are attributed. It has been described as an antioxidant and with antitumor and anti-inflammatory properties.

Soy fermentation induces a relevant increase in free isoflavones (daidzein, glycitein, and genistein) from their conjugated forms (daidzin, glycitin, and genistin). β-Glucosidase activity during soy fermentation has been described for selected strains of *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Enterococcus faecium*. As soy isoflavones are structurally similar to estrogen, similar health effects to these compounds have been reported. They have been postulated to play a role in the prevention of cardiovascular disease, metabolic syndrome, and type 2 diabetes; in relieving postmenopausal symptoms such as osteoporosis; or in the decrease in risk of breast and prostate cancers. However, in addition to evidences from *in vitro* biochemical tests and also from epidemiological studies, mostly done in populations consuming high amounts of soya, long-term interventional studies are needed to conclude on the health effects of these compounds. In this sense, the EFSA has considered that, based on the currently available studies, the following claimed beneficial health effects lack a clear enough cause and effect relationship: maintenance of normal blood LDL cholesterol concentrations, protection of DNA, proteins and lipids from oxidative damage, maintenance of bone mineral density, and reduction of vasomotor symptoms associated with menopause.

In the case of cereals, it has been shown that fermentation by *Lactobacillus rhamnosus* and *Saccharomyces cerevisiae* can increase antioxidant activity and total phenolic content of buckwheat, wheat germ, barley, and rye as compared with their unfermented counterparts. Thus, tailored fermentation to improve the antioxidant capacity of resulting foods can be accomplished.

γ-Aminobutyric Acid

γ-Aminobutyric acid (GABA) is a nonprotein amino acid that has several well-known physiological functions including antioxidant, antihypertension, and antidepression effects. Although most of the clinical applications of GABA are theoretical, based on data extrapolated from drug interventions, there are some experiments in humans and mostly in animal studies, in which fermented foods have shown these physiological effects. A significant decrease in blood pressure was noticed after the administration of fermented milk rich in GABA to mild hypertensive patients (using *Lactobacillus casei* strain Shirota and *Lactococcus lactis* YIT 2027) and also in rats (with *Lactobacillus paracasei* subsp. *paracasei* NTU 101 and *Lactobacillus plantarum* NTU 102). Administration of products fermented with the fungus *Monascus* to rats demonstrated their antidepressant effect.

LAB are known food-associated GABA producers, based on their glutamic acid decarboxylase activity, which is the *enzyme* that catalyzes the decarboxylation of glutamate to GABA. *Lactobacillus brevis* gives particularly high GABA production yields compared with other LAB. A wide range of GABA-enriched fermented food products have been described, including cheeses and other dairy products (e.g., koumiss and diverse fermented milk), soy sauces, kimchi, sourdough, black raspberry juice, red seaweed beverage, and fermented fish and meat. Also, model fermentation systems aiming at an increase in GABA and other bioactive compounds have been successfully assayed using lentils and kidney bean extracts as matrices.

Conjugated Linoleic Acid

Conjugated linoleic acid (CLA) (C18:2, with c9, t11 and t10, and c12 being the most common isomers) is naturally present in milk. In addition, several *Lactobacillus*, *Propionibacterium*, *Bifidobacterium*, and *Enterococcus* strains extensively synthesize CLA during the fermentation of milk, whereby the final concentration is influenced by the processing conditions. Often, CLA is considered as a healthy compound, to which several potent physiological properties were attributed, including anticarcinogenic, antiobese, antidiabetic, immunomodulator, and antihypertensive effects. However, other studies point out as well an increase in oxidative markers after CLA supplementation, and controversial results on glucose metabolism or liver function problems have been obtained. Thus, safety concerns regarding the use of CLA in humans persist and need further investigation, with better experimental designs that will clarify the mechanisms of CLA activities. In this sense, the EFSA has considered that, with the studies currently available, the previously mentioned claimed beneficial health effects for these molecules have no clear-enough cause–effect relationship.

Vitamins and Cofactors

The (over)production of vitamins by LAB provides a very attractive approach to improve the nutritional composition of fermented foods. Fermentation can indeed modulate the vitamin content of foods in diverse ways, via either increases or decreases. For instance, yeast fermentation has been shown to increase the folate content in the production process of bakery goods based on wheat, oat, and rye. In the particular case of rye, the level of folate was increased up to sevenfold and that of free phenolic acids up to tenfold after germination and fermentation by *Saccharomyces cerevisiae*. Not only yeasts but also LAB (*Lactobacillus plantarum* riboflavin-overproducing strains) have been successfully used for the preparation of bread and pasta with enhanced levels of vitamin B_2. In milk, folate levels can significantly increase after fermentation with different LAB, as shown for milk fermented with *Lactobacillus acidophilus* ATCC 4356 and *Streptococcus thermophilus* MC. Also, higher amounts of ubiquinone have been found in Korean and Japanese fermented foods than in unfermented foods, due to their diverse microbiota during the process of fermentation (LAB, yeast, and fungi). Foods such as kimchi and jeotgal (a fermented fish) may show high values of this compound. The deficiency of CoQ10 has been implicated in several clinical disorders, including chronic heart failure and hypertension.

Polyols

Other beneficial compounds that might result from fermentation processes are polyols. Modulation of the redox balance

during sugar metabolism by LAB can result in the synthesis of sorbitol, mannitol, and xylitol, which play an important role in foods as reduced caloric sweeteners and anticariogenic agents.

Fermented Foods as Probiotics

Probiotics are 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.' Several *Lactobacillus* and *Bifidobacterium* species have been investigated and even commercialized, and certain strains of *Enterococcus*, *Propionibacterium*, and *Bacillus* species and also from the yeast *Saccharomyces boulardii* have been considered as microorganisms with probiotic properties. Some of the health benefits traditionally associated with probiotic consumption include the improvement of intestinal tract health (contributing to the control of irritable bowel syndrome and inflammatory bowel disease, the suppression of pathogens, and the control of travelers' diarrhea or antibiotic-caused diarrhea), enhancement of the immune system, synthesis and enhancement of the bioavailability of nutrients, reduction of symptoms of lactose intolerance, reduction of serum cholesterol levels, and reduction of the risk of certain cancers. Several specific criteria should – arguably – be fulfilled by a microorganism to be considered a probiotic: it should remain viable during the shelf life of the product, withstand transit through the GI tract, and stabilize the intestinal microbiota.

One of the difficulties dealing with confirming the health benefits associated with probiotics is the fact that their biological effects are strain-specific, which makes the data comparison among studies difficult. In addition to this, the lack of evidence for cause–effect relationship has caused the EFSA to reject probiotic health claims. In many of the opinions given by the EFSA on probiotics, it was argued that the outcomes measured did not equate a beneficial physiological effect. Nevertheless, some EU member states have national nutrition guidelines or recommendations that include either probiotics or fermented milk with live bacteria.

Due to the fact that some fermenting microorganisms have probiotic properties, they have been used as starter cultures in attempts to formulate healthier foods. A consensus document elaborated in 2014 makes a reflection about whether the probiotic framework should include traditional fermented foods containing live microbes or not. According to the latter opinion, it is not always possible to clearly distinguish the health contribution of the pure cultures from that caused by the consumption of fermented foods. Consequently, it has been concluded that conventional fermented products are best described as 'containing live and active cultures' but should not be called probiotic. Nevertheless, fermented foods containing live cultures might also qualify as a 'probiotic' if they meet the criteria for that category.

Conclusion

Fermented foods include a wide variety of foodstuffs, whose composition strongly determines their nutritional value. Additionally, several metabolites resulting from fermentation processes are currently being researched as potential functional compounds that contribute to the health properties of this type of foods. Among others, compounds such as bioactive peptides, EPSs, arabinoxylans, polyphenols and flavonoids, and GABA can be highlighted.

See also: Acidophilus Milk; Alcohol: Metabolism and Health Effects; Alcohol: Properties and Determination; Beer: Fermentation; Beer: History and Types; Beer: Raw Materials and Wort Production; Beverage: Health Effects; Bifidobacteria in Foods: Health Effects; Brandy and Cognac: Consumption, Sensory and Health Effects; Bread: Breadmaking Processes; Bread: Types of Bread; Cheese: Chemistry and Microbiology; Cheese: Composition and Health Effects; Cheese: Processing and Sensory Properties; Cheese: Types of Cheese – Medium; Cheese: Types of Cheeses – Hard; Cheese: Types of Cheeses – Soft; Cured Foods: Health Effects; Dairy Products: Dietary and Medical Importance; Fermented Foods: Fermented Meat Products; Fermented Foods: Fermented Milks; Fermented Foods: Fermented Vegetables and Other Products; Fermented Foods: Origins and Applications; Fermented Foods: Use of Starter Cultures; Tea: Health Effects; Wines: Wine and Health; Yogurt: Dietary Importance; Yogurt: The Product and its Manufacture.

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Fermented Foods: Fermented Meat Products

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Introduction: From Necessity to Delicacy

Fermented meat products have been known as highly appreciated foods throughout the ages. They are found in many regions worldwide as a part of traditional diets, where they are perceived as attractive gastronomic entities contributing to cultural and geographic distinctiveness (Table 1). In Europe, fermented meats can be traced back to, at least, classical antiquity where they served to preserve meat after slaughter. The production of dry-fermented sausages, consisting of seasoned and salted minced meat that is stuffed into intestinal casings, has been documented in historical sources as a Roman craft. The technique was, however, largely based on the art of dry ham production developed by the Celts and Gauls, consisting of the salting and drying of hind legs of wild boars and pigs. Over the years, fermented meats came to exist in an overwhelming variety, characterized by large differences in ingredients, shape and caliber, and processing conditions. Several types of meat have been applied, including pork, beef, horse, donkey, deer, poultry, and ostrich. Well-known examples of dry-fermented sausages include different types of *salami* (e.g., *salame Brianza*), *chorizo* (e.g., *chorizo de Pamplona*), and *saucisson sec* (e.g., *rosette*). In addition, some fermented meats are semidry or even moist, such as German *mettwurst*, which have distinctive texture properties but are generally displaying a decreased stability. Besides Europe and its expansion to the New World, Asia also has a remarkable tradition of meat fermentations, although this has been less documented and explored. Yet, all local fermented meat specialties have origins in more or less remote pasts, with unique stories to tell, but eventually trace back to the same ancestral need for meat preservation. In fact, meat fermentation originally took off as an empirical preservation method of a primary commodity, in a similar manner as the preservation of milk led to the production of cheese and fermented milks of all sorts.

Due to its highly proteinaceous, energy-dense, and overall nutritious character, meat originating from hunted or domesticated animals has been a strategic foodstuff for most of human history and continues to be so. Nonetheless, before the introduction of the cold chain, the perishable nature of meat made it difficult to achieve prolonged storage in times of scarcity. The stabilization of meat through salting and fermentation therefore led to large logistic benefits. The end products became stable to such a degree that they could even be used as trading goods and provisions for travelers and soldiers, widely circulating throughout the European continent and facilitating expeditions and military campaigns. Clearly, preservation is no longer the most important *raison d'être*, at least in the Western world. Still, the convenience factor and unique sensory properties of fermented sausages continue to provide them with a steady share of the meat market. Over the centuries, the basic ingredients have remained the same, that is, minced meat, fat, curing salt, and spices. These ingredients are stuffed into casings, from either intestinal or synthetic origin, and subsequently subjected to a microbial fermentation process, in most cases followed by a drying stage for further stabilization and maturation of flavor complexity (Figure 1).

Meat Fermentation as a Spontaneous Process

As for all raw and salted meat products, the endogenous activity of meat enzymes, in particular proteases and lipases, contributes to the aging and ripening of the product being subjected to drying. In contrast to dry-salted hams, the original production of dry-fermented sausages involved a desirable and spontaneous acidification of the meat matrix that serves as an extra preservation hurdle in addition to the primary salting and drying, mostly because a comminuted mixture of meat trimmings and fat particles is less stable than whole muscle. Fermentative acidification to pH values that are usually situated between 4.8 and 5.2 inhibits the growth of undesirable microorganisms that are present on the raw meat and denatures the salt-solubilized protein fractions to a characteristic sliceable and gel-like texture.

Under appropriate temperature conditions (e.g., between 18 and 28 °C), the combination of curing salt and anaerobic conditions that are prevailing in the sausage casings strongly select for specific and desirable microorganisms. As a result, the initial microbial species diversity that is present on the raw materials is narrowed down within a couple of days to a few persistent microbial groups. Largely dominating bacteria are certain mesophilic species of *Lactobacillus*, in particular *Lactobacillus sakei* and, albeit to a lesser extent, *Lactobacillus curvatus* or *Lactobacillus plantarum*. Other lactic acid bacteria that may occasionally be present but do not or only marginally affect the production process of most traditional, spontaneously fermented sausages include enterococci, lactococci, pediococci, and leuconostocs. In addition to the dominance of meat-associated lactobacilli, a considerable subdominant population consists of catalase-positive cocci, mostly coagulase-negative staphylococci and sometimes kocuriae. Depending on the applied raw materials and processing conditions, different species of catalase-positive cocci may be encountered during spontaneous meat fermentations. In most cases, *Staphylococcus equorum*, *S. saprophyticus*, and/or *S. xylosus* are prevailing. Yet, minor levels of, for instance, *S. carnosus*, *S. sciuri*, *S. simulans*. *S. succinus*, and *S. warneri* are not uncommon. Whereas lactic acid bacteria chiefly cause acidification through lactic acid production, catalase-positive cocci prevent oxidative deterioration, generate stable nitrosomyoglobin pigments via nitrate reductase activity, and contribute to an attractive aroma via amino acid and fatty acid conversions. These precursor amino acids and fatty acids are liberated via protease, peptidase, and lipase activities by muscle enzymes, although minor contributions by bacteria have also been described.

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Table 1 Illustrative but nonexhaustive list of some different types of well-known fermented sausages and their particularities

Type	*Country of origin*	*Particularities*
Mediterranean dry-fermented sausages	Italy (e.g., salami), France (e.g., rosette), Spain (e.g., fuet), etc.	Large variety of recipes and types, often heavily seasoned with pepper, garlic, and herbs; mildly fermented, extensively dried and matured, and mostly molded
Chorizo	Spain	Contains paprika; variable drying periods and moist contents but often semidry and smoked; high-end variants contain Iberico pork
North European dry-fermented sausages	Germany, Norway, Belgium (saucisson d'Ardennes), etc.	Large variety of recipes and types; usually rather acid and often heavily smoked without molding, although salami-type sausages are also produced
Teewurst, mettwurst	Germany	Moist and spreadable pork sausages that are only moderately or not dried; usually smoked and no molding; highly perishable
Hungarian salami	Hungary	Salami-like but with an unusual combination of intense smoking and molding
Lap Cheong	China, Vietnam, etc.	Different types of dried and usually smoked pork or poultry sausages; sometimes containing rice and seasoned with soy sauce, rose water, or sake

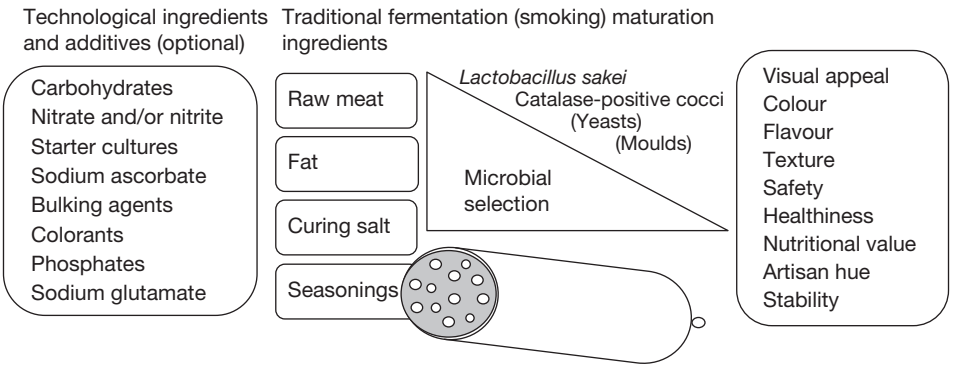

Figure 1 Common ingredients used in the meat fermentation process and targeted quality characteristics of the end products.

Flavor maturation of fermented sausages thus correlates with the amounts of branched-chain aldehydes and alcohols from the conversion of branched-chain amino acids and branched-chain and straight-chain methyl ketones derived from microbial β-oxidation of fatty acids.

Besides bacteria, yeasts and molds can be present in some sausage types. Yeasts, such as *Debaryomyces hansenii*, are probably of a rather minor importance in flavor development but could accentuate certain aromas. In contrast, molds are crucial elements in the elaboration of south European fermented sausages where they develop as a desirable outside layer on the product surface after fermentation, during which they generate additional aroma and contribute to color stabilization through catalase activity, oxygen consumption, and shelter against light. The mycobiota of fermented sausage is diverse, but *Penicillium* is usually the predominant genus (e.g., *Penicillium nalgiovense*). In traditional northern European fermented sausages, molds are mostly undesirable. This is due to the rather humid climate conditions in these regions, which are unsuitable for proper, controlled, and safe mold development. As a consequence, such products are frequently smoked after fermentation, as an empirical manner to add additional preservative action and to prevent the unbalanced growth of detrimental molds. Meanwhile, the smoked flavor became a desirable sensory attribute for northern European consumers. In addition, northern European varieties of fermented sausages are generally more acidified to obtain enhanced microbial inhibition, since they are usually more moist and, hence, more vulnerable to hazards than their drier southern counterparts.

Technological Optimizations

Over the years and particularly since the mid-twentieth century, several technological adaptations have been introduced, as to adjust the traditional meat fermentation process to the requirements of an ever industrializing and globalizing world. This led to the emergence of large factories in the 1960s, producing fermented meat products of consistent quality and safety on an industrial scale. Adaptations range from the simple addition of ascorbate as an antioxidant and of sugar and manganese as bacterial stimulants, over the introduction of climate chambers and microbial starter cultures for improved control, to a multitude of cost reduction attempts via the use of artificial casing and additives of all sorts. Also, technological adaptations have been brought in to reduce the potential generation of toxic compounds, for instance, by dipping sausages in a sorbate solution to avoid undesirable mold growth and by optimizing potentially hazardous smoking procedures.

One of the key breakthroughs has been the gradual introduction of controlled-climate chambers in the 1950s, which enable a rigid control of temperature and relative humidity. Accordingly, the unpredictability of fluctuations in weather

conditions can be ruled out, and a more precise tuning to optimal process requirements can be obtained. This is particularly important not only for a correct drying process but also to steer fermentation and molding.

More or less in the same period, another major advancement has been achieved through the development and addition of starter cultures. In the 1940s, Jensen and Paddock explored the use of *Lactobacillus* cultures onto raw meat, which was followed by inoculations with *Pediococcus cerevisiae* in the United States and *Micrococcus* M53 in Europe. Such starter cultures permit to mostly overcome the problem of failed fermentations, which may be occurring in traditional processes where producers rely on a spontaneous development of the indigenous meat microbiota. Meat starter cultures nowadays usually consist of a predefined microbial consortium of lactic acid bacteria for acidification purposes, mostly *Lactobacillus sakei* and/or *Pediococcus* species, accompanied by selected catalase-positive cocci, mostly *S. xylosus*, *S. carnosus*, and/or *Kocuria varians*. These combined cultures are usually lyophilized and added as a powder that has been resuspended in water to the meat batter prior to stuffing and fermentation. In addition, a surface mold culture may be applied to control the molding process during maturation. As a result of starter culture addition, the production process is accelerated, and end products are more uniform. In North America, further speeding up has been achieved by increasing the fermentation temperature from the traditional range below 28 °C toward temperatures above 35 °C. As a result of this enhanced fermentation temperature, pediococci take over from the mesophilic lactobacilli. Even faster processes have been obtained with chemical rather than microbial acidification, mostly by using glucono delta-lactone or encapsulated lactic acid as acidulants.

Yet, the ever-increasing attempts to speed up fermentation and maturation and to minimize ingredient and processing costs have been counterbalanced by severe losses in product quality. Flavor, for instance, is the result of a complex and delicate interplay between endogenous meat enzymes, microbial action, and physicochemical effects. The dynamics of this interplay depend strongly on the process conditions, in particular the allowed time frame for biochemical conversions and the rate of acidification. On the one hand, cathepsin D, which is a major meat proteinase acting on the myosin and actin fractions, is activated by acidity (with optimal activity at pH 4.5). On the other hand, low pH values strongly inhibit the metabolic performance of meat microorganisms, including their production of peptidyl peptidases and aminopeptidases and their enzymatic conversions of amino acids and fatty acids. With respect to the postfermentation processing, too short maturation periods may impede the proper flavor-generating chemical changes that lead to fatty acid oxidation products and Strecker degradation of amino acids.

In conclusion, excessive acceleration of the production process, on the level of both fermentation and maturation, is known to compromise flavor development to a large extent, despite the obvious advantages related to standardization and cost reduction. As a technological response to partially conceal cost-induced quality losses on the level of texture, color, and flavor, several additives have been introduced in the meat fermentation process. Commonly applied examples include the use of bulking agents (e.g., milk powder), phosphates, sodium glutamate, added flavors (e.g., liquid smoke flavorings), colorants (e.g., cochineal), and sorbates.

From a safety point of view, controlled use of nitrite and/or nitrate salts as additives to fermented meats has become a common practice, as these curing compounds contribute to the inhibition of meat-associated pathogens. Moreover, they are involved in the generation of a typical cured flavor, inhibit lipid oxidation, and lead to stable color formation via the nitrosylation of myoglobin. Yet, because of their involvement in carcinogenic nitrosamine formation, maximum levels have been introduced by regulation. In addition, their chemical conversions can be tuned technologically, for instance, by adding the reductant ascorbic acid, which is to be added in levels exceeding 550 ppm in the United States.

Understanding the Metabolic Functioning of Meat Microorganisms

Improving the meat fermentation process to yield even more attractive end products is a matter not only of muscle biochemistry but also of a better insight into the microbial community dynamics and metabolism. Although the overall roles of the fermented meat microbiota are well understood, the more subtle aspects of their metabolism need further exploration. For instance, recent whole-genome analyses of fermented meat bacteria have identified specialized metabolic repertoires that may explain why certain community members are so strikingly dominant. The specialized adaptation by *Lactobacillus sakei* to the fermented meat environment has been linked to specific metabolic properties via genome analysis, metabolite kinetics, modeling, and gene expression studies. Consequently, the use of arginine and nucleosides by *Lactobacillus sakei* as alternative, nonglucose compounds as energy sources that naturally occur in meat has been suggested as relevant for bacterial dominance. This property has also been shown for coagulase-negative staphylococci, possibly explaining differences on the inter- and intraspecies levels. Variability in competitiveness between coagulase-negative staphylococci is particularly intriguing, since this forms the basis for the eventual selection of a specific bacterial diversity associated with a certain product. As a result, repercussions on the overall bacterial metabolic activity and hence the final sensory quality are to be expected. It has, for instance, been shown that differences in sensitivity to pH lead to different growth or survival kinetics, when comparing *S. carnosus* to the more acid-sensitive *S. xylosus*. Also, the replacement of a smoking treatment by molding may cause a shift from *S. saprophyticus* to *S. equorum*, possibly because of pH increases. As different staphylococcal species display variations in both the absolute and specific production of volatile compounds, this can affect sausage aroma measurably. Enhanced technological control on the staphylococcal community could thus represent an opportunity for tailored flavor modulation.

Interactions between microorganisms, the enzymes of the muscle tissue, the applied ingredients, and the process technology determine the quality, safety, and stability of the end products. New metabolic insights will thus contribute to process optimization and the further development of the so-called functional starter cultures, which should lead to improved

sausage fermentation with respect to flavor, safety, processing, technology, or health, as compared with traditional starter cultures that are merely aiming at standardization and processing speed. Such novel insights are to be obtained from the use of state-of-the-art methodologies, for instance, omics approaches, which are increasingly being used in the domain of food science and technology.

Toward a New Generation of Fermented Meat Products?

Although the meat fermentation process has been dramatically optimized through technological innovations, several challenges are still to be undertaken. Preferably, these strategies need to make use of 'natural' options, avoiding the use of commonly shunned chemical additives. Some of the existing concerns have to do with the safety and health implications upon consumption of the end products.

First, several incidences with foodborne pathogens have been reported despite the fact that acidification, salting, and drying are important microbiological hurdles. Problems are sometimes encountered due to the presence and ingestion of *Salmonella* and *Escherichia coli* or enterotoxins produced by *Staphylococcus aureus*. *Listeria monocytogenes*, although not linked to foodborne illness by fermented meat consumption till now, is also of concern. Several countries, such as the United States, have adopted a zero-tolerance policy with respect to the presence of this bacterium in ready-to-eat foods. This is problematic because *Listeria monocytogenes* has been shown to survive several meat fermentation processes and can sometimes be recovered from nonheated European end products. In general, the presence of foodborne pathogens may be due to high loads in the raw materials or failing production processes. Novel strategies that could eliminate potential residual levels of undesirable bacteria are being sought for. The use of lactic acid bacteria starter cultures that produce natural antimicrobials, in particular bacteriocins, has been frequently explored. Although this mode of action offers considerable potential against *Listeria monocytogenes*, bacteriocins by lactic acid bacteria are generally inefficient toward gram-negative pathogens. Meat-compatible microorganisms with targeted antimicrobial actions, in particular toward *Salmonella* and *E. coli*, would thus offer considerable food safety advantages. Other microbial concerns are related to the use of the fermentative microbiota itself, since certain lactobacilli, staphylococci, and enterococci present during fermentation may be producing biogenic amines. Likewise, surface molds may in principle be toxicogenic. This is particularly of concern during meat spontaneous fermentation processes, since commercial starter cultures have normally been screened to demonstrate the absence of biogenic amine, mycotoxin, and antibiotic formation.

A second major concern is related to the potential implications of fermented meat consumption on human health. Although meat products are characterized by a high intrinsic nutritional value, mostly due to their high contents of quality protein, iron, zinc, and vitamins, some unease has been expressed with respect to their fat and sodium contents. Although the mostly pork-based fat fraction is rather unsaturated and not necessarily problematic, high-fat products are energy-dense and do not meet the current market demand for low-fat foods. More importantly, fermented meat products have been charged for their potential association with the risk of colon cancer. The World Cancer Research Fund specifically advises to avoid all processed meats as part of its ten recommendations to combat cancer. The mechanisms for this association are not fully understood, but a part of the effect could be due to the use of mostly red muscle and the presence of nitrate and/or nitrite in the curing salt. Several attempts have already been made to partially counter all aforementioned concerns, although this frequently implies loss of sensory excellence. Reduction of sodium results in a loss of flavor intensity and texture development, whereas the replacement of potassium and/or magnesium may sometimes lead to metallic or bitter taste defects. Likewise, fat reduction leads to poor mouth feel and aroma losses, whereas fat replacement by, for instance, vegetable oils often results in texture setbacks. Also, the increased production of poultry-based fermented meat products or products based on meat replacers, as an attempt to counter the consumption of red muscle, is leading to end products that display weaker hedonistic profiles. Up till now, attempts to lower the added nitrite levels have been limited to the indirect addition of nitrate via vegetal fractions such as celery or spinach extracts, which is then further converted to nitrite by the meat staphylococci. Although this may be useful for labeling purposes, this approach is mainly aiming at concealment and does not contribute to the health debate.

In an endeavor to counter the negatively perceived health status of fermented meats, several trials have been made to include health-promoting compounds in the original formulations. Examples include the use of probiotics, prebiotics, and other fibers, lycopene, antioxidants, olive oil, and calcium and attempts to increase the content of conjugated linoleic and linolenic acids. Although commercial examples exist, technological and legislative bottlenecks often remain, and questions arise on the conceptual and nutritional relevance of such products.

Paradoxically, part of the innovation of fermented meat products is based on the reintroduction of artisan features. Such features are increasingly used in marketing strategies for contemporary fermented meat products, in a process that can be categorized as 'reemergence of tradition.' The use of labels that claim artisanship and tradition is largely employed to give a feel of trustworthiness in a globalizing food market in crisis.

Conclusions

Despite the fact that fermented meats are rooted in an age-old tradition, several innovations and technological adaptations have been introduced over time. The advent of modern food technology has greatly improved processing speed and efficiency, although in many cases, a too pronounced focus on cost reduction has resulted in inferior sensory quality. As a result, several opportunities can be identified to improve the quality of fermented meats, especially when produced on a cost-effective industrial level. Further research, based on state-of-the-art methodologies and taking into account the combined disciplines of microbiology, meat science, and

processing technology, is needed if any breakthroughs are to be achieved. In addition, the nutritional aspects of fermented meat consumption will likely become more important in the future. Finally, sociological approaches and consumer studies may help to better understand the complex relationship between apparently conflicting demands for traditional characteristics and innovative elements in fermented meats.

See also: Bacteriocins; Beef; Biogenic Amines: Toxicology and Health Effect; Cancer: Diet in Cancer Prevention; Carcinogenic: Carcinogenic Substances in Food; Cholesterol: Factors Determining Blood Cholesterol Levels; Cured Foods: Health Effects; Drying: Physical and Structural Changes; Drying: Principles and Types; Fat Replacer; Fermented Foods: Composition and Health Effects; Fermented Foods: Origins and Applications; Fermented Foods: Use of Starter Cultures; Foodborne Pathogens; Functional Foods; Ham: Dry-cured Ham; Horse Meat; Hypertension and Diet; Iron: Physiology of Iron; Lactic Acid Bacteria; *Listeria*: Properties and Occurrences; Low-fat Foods: Types and Manufacture; Low-salt Foods: Types and Manufacture; Meat: Conversion of Muscle into Meat; Meat: Eating Quality and Preservation; Meat: Role in the Diet; Meat: Structure; Mycotoxins: Occurrence and Determination; Nitrites and Nitrates; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Oxidation of Food Components; pH: Principles and Measurement; Pork Meat Quality, Production and Processing on; Poultry: Processing; Prebiotics; Preservation of Foods; Preservatives: Classifications and Analysis; Preservatives: Food Use; Probiotics; Protein: Food Sources; *Salmonella*: Properties and Occurrence; Skeletal Muscle; Smoked Foods: Principles and Production; *Staphylococcus*: Occurrence and Properties; Traditional Foods.

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Fermented Foods: Fermented Milks

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Introduction

A chronological appraisal of the origin of fermented milks (FMs) (e.g., Bulgarian milk, Yogurt, Kefir, Koumiss, Laben, Dahi, Skyr, and sour milk) shows that they date back to early civilizations around 10 000 BC. As the way of life of humans changed, it led to the domestication of the cow, sheep, goat, buffalo, and camel. A wide range of indigenous FM products are traditionally made in rural areas worldwide. Most of these products rely primarily on spontaneous fermentation due to the presence of indigenous microflora (mainly lactic acid bacteria, LAB) in the milk. In the Asiatic Steppes, containers made from animal hides containing microorganisms from the previous production batch are used during the manufacture of traditional Koumiss, Bulgarian milk, etc. This reliance on containers or utensils to provide an initial inoculum of starter organisms is widespread.

During the past 90 years, considerable attention has been directed sporadically on benefits derived from the consumption of milk products containing LAB. The role of FM in human nutrition is well documented. Man knew the virtues of fermented foods even during the early days of civilization. In earlier days, these foods were produced by natural fermentations with the main objective of preserving milk.

In many areas of the world, the fermentation process is initiated by the adventitious indigenous microorganisms or through 'backslopping' with mixed or unknown cultures. Often, this type of fermentation may be slow or unpredictable. Nevertheless, it affords a vital means of preserving highly perishable foods, especially where refrigeration is lacking. In Europe, Asia, and Africa, sour milk was known as being more stable and advantageous than fresh milk. It preserved the high-quality nutrients present in milk in a relatively more stable form.

From prehistoric times, man learned to use milk as food. Though the origin of the art of preserving milk by lactic acid fermentation has been lost in antiquity, the biochemical and microbiological knowledge of fermentation is of comparatively recent date. In Indian subcontinent, the conversion of milk into Dahi in every household by souring with the leftover of previous day's sour milk has been a common practice ever since the Aryans inhabited the land. In this way, the life and utility of milk nutrients were extended.

Today, the practice of preserving milk by fermentation has become a routine household technology, and FMs are almost compulsory in this subcontinent. In developed countries, fermentation is initiated with pure starter cultures, which have predictable performance potentials. Recent scientific and technological advances in starter culture management and process control have yielded a variety of products. These products are with superior chemical, physical, nutritional, and sanitary qualities.

A number of FMs, cereal-based products, and beverages are prepared through the action of LAB. Nearly every civilization has consumed FMs. Over the past two decades, the per capita consumption of FMs has increased more than 45-fold due to their image as nutritious and healthful foods. Likewise, the per capita production and consumption of cheese have recorded notable growth in the United States, Europe, and Indian subcontinent. Thus, FMs have been and still are of extreme importance in the nutrition of people throughout the world. The FMs like Acidophilus milk, Bifidus milk, Yogurt, Kefir, Koumiss, Leben, and Skyr are also used as dietary adjuncts. The FMs represent an important component of functional foods and value-added probiotic products.

Nowadays, intense research efforts are under way to develop dairy products into which probiotic organisms are incorporated to make them more valuable. This article provides an overview of the different types of FMs and the starter cultures used in their preparation and health benefits, which can be derived by the consumers through their regular intake.

Raw Materials

The milk of several species, including cow, buffalo, goat, sheep, mare, camel, and reindeer has been used for the preparation of FMs. Each type of FM has its own characteristic texture, body, flavor, and composition, depending on the type of milk used, the type of starter cultures (LAB, with or without aroma producers, and yeasts), and the procedures followed in its preparation. The FMs popularly used in various countries may be classified broadly into four categories: (1) moderately sour types with a pleasant aroma associated with diacetyl, for example, cultured milk; (2) sour and very sour types owing to high acid production, for example, yogurt and *Dahi*; (3) ethanol in addition to lactic acid, for example, Koumiss and Kefir; and (4) probiotic FMs, for example, acidophilus milks especially prepared with human strains of *L. acidophilus*.

Definition

FM is a milk product obtained by the fermentation of milk, where milk may have been manufactured from products obtained from milk with or without compositional modification as limited by the provisions in law, by the action of suitable microorganisms resulting in reduction of pH with or without coagulation (isoelectric precipitation). These starter microorganisms shall be viable, active, and abundant in the product to the date of minimum durability. If the product is heat-treated after fermentation, the requirement for viable microorganisms does not apply.

Types of FM

Concentrated FM

Concentrated FM is an FM of which the protein has been increased prior to or after fermentation to minimum 5.6%. Concentrated FMs include traditional products such as Stragisto (strained yogurt), Leben, Ymer, and Ylette.

Flavored FMs

Flavored FMs are composite milk products, which contain a maximum of 50% (m m^{-1}) of nondairy ingredients (such as nutritive and nonnutritive sweeteners, fruits, and vegetables as well as juices, purees, pulps, preparations and preserves derived therefrom, cereals, honey, chocolate, nuts, coffee, spices, and other harmless natural flavoring foods) and/or flavors. The nondairy ingredients can be mixed in prior to or after fermentation.

Drinks Based on FM

Drinks based on FM are composite milk products, obtained by mixing FM with potable water with or without the addition of other ingredients such as whey, other nondairy ingredients, and flavorings. Drinks based on FM contain a minimum of 40% (m m^{-1}) FM. Other microorganisms than those constituting the specific starter cultures may be added.

Starter Cultures Used in the Preparation of FM

Although the evolution of the process was strictly intuitive, the production of FM products became an established pattern of preservation; since the early 1900s, defined microorganisms have been used to prepare these products on a large industrial scale. The microorganisms that are employed in FMs (including probiotic products, alcoholic/lactic beverages, cultured cream, and products containing molds) and the cheese industry are used singly, or in pairs or multiples, or in a mixture, thus giving the industry the opportunity to manufacture different products. Certain FMs are characterized by specific starter culture(s) used for fermentation; Table 1 depicts the starter cultures used in preparation of FM.

Nutritional Significance of FMs

Lactobacilli use the nutrients in milk for their own metabolism and growth and multiply from 1 to 10 million cells per milliliter. The microorganisms are present in the FM not only as viable cells but also as autolyzed cells, which give rise to primary and secondary metabolites and enzymes, which they have produced during fermentation and storage.

The nutritive value (NV) of an FM depends on the availability and digestibility of nutritive constituents and the changes in these constituents due to microbial growth and fermentation processes (Table 2).

Similarly, the NV of a fermented food also depends on the nutrient content and bioavailability of the nutrients. Impact of fermentation of milk on its constituents is depicted in Table 3. Generally, the energy value of FM is similar to that of the milk from which it was prepared, yet it is claimed that the FM is more nutritious (the composition of milk and yogurt is given in Table 4). Fermentation enhances availability of some of the nutrients for absorption, as shown below:

Effect of Fermentation on Milk Constituents

Milk Proteins

The proteins in milk are of excellent biological quality, and both caseins and whey proteins are well endowed with essential amino acids (EAA). The fact that the protein content of FM, like yogurt, is often elevated by concentration or addition of skim milk solids means that it is an even more attractive source of protein than liquid milk. It is important that the proteins in FMs are totally digestible due to the fact that the starter organisms cause some degree of initial proteolysis. The extent of this breakdown depends on the strains of bacteria being employed, but, in general, at least some release of amino acids and peptides can be expected during incubation and storage.

FM products contain significantly higher levels of free amino acids than milk, due to the heat treatment and proteolytic activity of the starter organisms. The native milk proteins, which form a hard curd in the stomach, are converted into a soft curd containing finely dispersed casein particles resulting from the action of starter organisms in the FM. The FM proteins are useful for children, old people, and those with stomach ulcers, because the soft curd in FM is easier to digest than the milk (Table 3).

It has been observed that the rats fed with diets containing FM products had higher feed consumption, greater weight gains, and a more efficient utilization of their feed. Some medical practitioners advocate the use of *Dahi* and buttermilk by children not only for treatment of common intestinal ailments but also for their general nutrition. In tropical countries like India, where milk is exposed to heavy bacterial contamination, it is better to convert it into FM in order to preserve its NV.

Milk Fats

Although much of the FM sold in industrialized countries is produced from skim milk, traditional materials have always contained some 3–4% milk fat. It has been proposed that the digestibility of fat is improved via the action of the bacteria present during the fermentation process, but whether this effect really exists is highly debatable. The overall energy content of FM reflects both the fat content of the milk from which it was made and whether or not there has been the addition of ingredients such as cream and sugar. Humans have a double requirement for lipids. It acts as storage fat, which is composed of saturated fatty acids, and serves as a source of energy or protection for vital organs.

In addition to this, it also plays an important role by acting as structural fat, which, with proteins, forms many of the essential membranes in animal cells, particularly in areas like brain and nerve cells. These are essential to many physiological processes. Some essential nutrients are fat-soluble and are

Table 1 Starter cultures used for various fermented milks

Sr. no.	*Fermented milks*	*Starter cultures used*
1.	Yogurt	Symbiotic cultures of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*
2.	Acidophilus milk	*L. acidophilus*
3.	Kefir	Starter culture prepared from kefir grains, *L. kefir*, species of the genera *Leuconostoc*, *Lactococcus*, and *Acetobacter* growing in a strong specific relationship. Kefir grains constitute both lactose-fermenting yeasts (*Kluyveromyces marxianus*) and nonlactose-fermenting yeasts (*Saccharomyces unisporus*, *Sac. cerevisiae*, and *Sac. exiguus*)
4.	Koumiss	*L. delbrueckii* subsp. *bulgaricus* and *Klu. marxianus*. Other microorganisms than those constituting the specific starter culture(s) specified above may be added
5.	Bulgarian buttermilk	*L. delbrueckii* subsp. *bulgaricus*
6.	Dahi	Thermophilic lactic cultures *Str. salivarius* subsp. *thermophilus* *L. delbrueckii* subsp. *bulgaricus* Mesophilic lactic cultures *Lactococcus lactis* subsp. *lactis* *Lac. lactis* subsp. *cremoris* Citrate fermentators *Leuconostoc mesenteroides* subsp. *citrovorum*, *Leu. dextranicum*
7.	Shrikhand	*Str. salivarius* subsp. *thermophilus* *L. delbrueckii* subsp. *bulgaricus*
8.	Lassi	*Str. salivarius* subsp. *thermophilus*, *L. delbrueckii* subsp. *bulgaricus* *Lac. lactis* subsp. *lactis* *Lac. lactis* subsp. *cremoris* *Leu. mesenteroides* subsp. *citrovorum* (any two LAB and one citrate-fermenting culture organism)
9.	Cultured buttermilk	*Lac. lactis* subsp. *lactis* or *Lac. lactis* subsp. *cremoris* and *Str. cremoris* subsp. *diacetylactis*
10.	Leben	*Lac. lactis* subsp. *lactis* *Str. salivarius* subsp. *thermophilus* *L. delbrueckii* subsp. *bulgaricus* Lactose-fermenting yeasts
11.	Cheese(s)	*Lac. lactis* subsp. *lactis*, *Lac. lactis* subsp. *cremoris*, *Lac. lactis* subsp. *diacetylactis* *Str. salivarius* subsp. *thermophilus* *L. delbrueckii* subsp. *bulgaricus* *Propionibacterium freudenreichii* subsp. *shermanii* *Penicillium roqueforti*, *Pen. camemberti*, etc.
12.	Yakult	*L. casei* strain Shirota
13.	Filmjolk	*Lac. lactis* subsp. *lactis* or *Lac. lactis* subsp. *cremoris* and *Leu. mesenteroides* subsp. *citrovorum* or *Leu. mesenteroides* subsp. *dextranicum*
14.	Viili	*Lac. lactis* subsp. *cremoris* *Lac. lactis* biovar. *diacetylactis* *Leu. mesenteroides* subsp. *cremoris* and *Geotrichum candidum*
15.	Skyr	*Str. salivarius* subsp. *thermophilus* *L. delbrueckii* subsp. *bulgaricus*

Table 2 Fermentation induced changes in milk constituents

Constituents	*Changes*
Milk proteins (3–4.5%)	Coagulated into a smooth curd with casein particles finely dispersed. Partially peptonized (0.1–0.7%) and utilized for microbial growth, resulting in the buildup of microbial cell proteins, increase in nonprotein nitrogen, and release of peptides and amino acids
Lactose (4.5–5%)	Partially utilized (1–2%) by starter bacteria producing mainly lactic acid (0.6–2%), volatile acids, flavor compounds, and CO_2 may also be formed by heterofermentative types. Alcohol and CO_2 may be produced by lactose-fermenting yeasts
Milk fat (3.5–7%)	Fermentation leads to partial digestion of lipids
Mineral matter (0.7–0.8%)	No direct appreciable change
Vitamins	No change in fat-soluble vitamins A, D, E, and K. Slight decrease in B-complex vitamins depending on the strains used

Source: Khedkar, C. D., Khedkar, G. D., Chavan, N. V. and Kalyankar, S. D. (2003). Fermented milks: Dietary importance. In: *Encyclopaedia of Food Science and Nutrition* (2nd ed.). London: Elsevier.

Table 3 Composition of milk and yogurt (per 100 g)

Nutrients	*Whole milk*	*Whole milk plain yogurt*	*Low-fat plain yogurt*	*Very low-fat/low-calorie plain yogurt*	*Greek yogurt*	*Plain fromage frais*
Energy (kcal)	66	79	56	41	115	113
Protein (g)	3.1	5.7	5.1	4.3	6.4	6.8
Fat (g)	3.9	3.0	0.8	0.2	9.1	7.1
Calcium (mg)	115	200	190	130	150	89
Zinc (mg)	0.4	0.7	0.6	0.4	0.5	0.3
Phosphorus (mg)	92	170	160	110	130	110
Magnesium (mg)	11	19	19	13	12	08

Source: Khedkar, C. D., Khedkar, G. D., Chavan, N. V. and Kalyankar, S. D. (2003). Fermented milks: Dietary importance. In: *Encyclopaedia of Food Science and Nutrition* (2nd ed.). London: Elsevier.

Table 4 Effect of fermentation on vitamin content (μg/100 g)

Vitamins	*Whole milk*	*Buttermilk*	*Sour cream*	*Yogurt*	*Cottage cheese*
Thiamin	30	34	35	44	21
Riboflavin	170	154	149	214	165
Niacin	100	58	67	114	128
Pantothenic acid	300	275	360	591	215
Vitamin B_6	40	34	16	49	68
Folacin	6	Traces	11	11	12
Vitamin B_{12}	0.40	0.22	0.30	0.56	0.63

Source: Khedkar, C. D., Khedkar, G. D., Chavan, N. V. and Kalyankar, S. D. (2003). Fermented milks: Dietary importance. In: *Encyclopaedia of Food Science and Nutrition* (2nd ed.). London: Elsevier.

found primarily in foods that contain fat. These nutrients are essential fatty acids and fat-soluble vitamins. The LAB possess lipase activity evidenced by the lipids in the cultured products being partially digested. Their assays for lipase activity, however, were performed using culture organisms with tributyrin emulsions as substrate, and they did not prove that the bacterial lipases acted on lipids in the cultured products. A significant increase in free fatty acids over the starting milk has been observed in FM. The lactic acid fermentation and enzymatic action on amino acids also produce small quantities of volatile fatty acids (Table 3).

Milk Carbohydrates

The milk carbohydrate, lactose, causes intestinal problems in a number of persons who are lactose-intolerant. These persons are deficient in the intestinal enzyme, ß-galactosidase, and thus must restrict their intake of milk and dairy products. Milk does not contain this enzyme. Lactose intolerance is normally defined as the occurrence of gastrointestinal symptoms after administration of single test dose, about 50 g lactose in aqueous solution. In a large proportion of world's population, ß-galactosidase activity is low or absent. This enzyme is present in sufficient quantities in lactic FMs. Consequently, the lactose level in FM can be lower than milk, although this is not always the case, as skim milk powder or nonfat milk solids are sometimes added during the manufacture of some of these products.

During fermentation, 20–50% of the lactose in milk is hydrolyzed to its component monosaccharides, glucose and galactose, by the starter culture organisms. The production of ß-galactosidase increases during the fermentation of yogurt, reaching a maximum after four hours of incubation. This enzyme may be released from the microbial culture during digestion, indicating that it may be present in the intestine of persons consuming FM. Lactose-intolerant subjects had a much lower rise in blood glucose and thus a much lower lactose utilization efficiency. A lower lactose content would presumably help tolerance of the product to those with a reduced ability to digest lactose. But clearly the explanation is not this simple because FM with fairly high lactose content is also better tolerated by such individuals than the equivalent amount of lactose in milk. Lactic acid is produced as a by-product of the fermentation process. It acts as a preservative. It influences the physical properties of casein curd by inducing a finer suspension, which promotes digestibility of casein. It also improves utilization of calcium and other minerals and inhibits the growth of potentially harmful bacteria.

Milk Vitamins

Milk is a rich source of vitamins, particularly A, D, and some B-complex vitamins. Breed, diet, climate, geographic location, age and stage of lactation, and other factors can influence the vitamin content of the milk. This, in turn, will affect the vitamin content of the FM. There is conflicting evidence about whether there is an increase, decrease, or no change in level of vitamins during the fermentation of milk. The various manufacturing treatments may affect the content of labile vitamins while there is metabolism of vitamins by the LAB during the log phase of their growth and subsequent synthesis by the same bacteria. International Dairy Federation gave an indication of the changes due to heat treatment, fermentation, and storage.

The vitamin content of FM varies with the type of milk used (particularly the fat content of the milk, which influences the amount of vitamin A and other fat-soluble vitamins present), the strain of bacteria, and the fermentation conditions. Vitamin C is heat- and light-labile, but it is more stable in the acid

conditions of FM than normal milk. Levels of some B-complex vitamins are reduced due to the requirement of some of the LAB. Losses of up to 90% of vitamin B_{12} have been reported with specific bacterial strains, but losses are not always so large and can be reduced considerably by the use of 'supplementary culture' capable of synthesizing significant amounts of vitamin B_{12}. On the other hand, several cultures are able to synthesize the B vitamin, folic acid, increasing the level of this vitamin present in the final product, for example, *Prop. freudenreichii* subsp. *shermanii*, which is used as a starter culture in some of the cheeses, is known to synthesize vitamin B_{12}.

In the current scenario, when there are concerns about the folate intake of women during the early stages of pregnancy, this aspect may prove to have nutritional advantage. Increase in vitamin B and P (riboflavenoid) group vitamins has been observed in Kefir due to the activity of yeasts and acetic acid bacteria. Fermentation has been reported to increase folic acid in buttermilk, sour cream, Yogurt, Bifidus milk, Koumiss, *Dahi*, lassi, laben, skyr, and kefir. Effect of fermentation on the vitamin content in some of the FMs is given in Table 4.

Milk Minerals

Most experiments performed recently did not confirm the improved absorption of calcium because of the more acidic pH in the intestine following consumption of FM. When bioavailability of essential minerals and trace elements was investigated in rat experiments using diets based on milk, yogurt, and pasteurized yogurt and a commercial diet, it was observed that bioavailability (as measured by intestinal absorption, urinary excretion, and bone content) of calcium, phosphorus, magnesium, and zinc from all diets was superior than the commercial diet. In experiments with albino rats, availability of calcium and phosphorus from FM was increased by about 7% and 11%, respectively, compared to milk. The results are explained by the fact that colloidal calcium complexes and lactic acid, both present in FM, enhance calcium absorption.

Bioactive peptides derived from the tryptic hydrolysates of casein known as caseinophosphopeptides (CPP) possess physicochemical properties that enable chelation of various bi- and trivalent minerals to be carried out, thereby enhancing mineral solubility in the lower small intestine. It has been suggested that moderate and exchangeable binding of calcium to CPP is responsible for the high absorbability of calcium from milk. Results of experiments conducted at Melbourne Dental School, the University of Melbourne, have proved that there is a significant reduction in the tooth decay in laboratory animals that are fed with CPP (Table 3).

Formation of Single-Cell Proteins in FMs

In recent years, the nutritional importance of microbial single-cell proteins (SCPs) has received considerable attention. When FM is consumed, a large number of bacterial cells (about 10^7–10^9 CFU g^{-1}) and the products released from these cells enter the digestive tract. Most of the living cells are inactivated in the stomach by the action of hydrochloric acid and pepsin. It has been estimated that about 60% of the SCP nitrogen content (excluding cell wall material and nucleic acids) can be utilized by the body. The EAA content of SCP in FM ranges from 2.0 to 6.5 mg/100 g of product. The SCPs of some bacteria were found to be rich in methionine, lysine, and cystine. Studies on amino acid patterns of acid hydrolysates of SCP have revealed the presence of all the known amino acids. The SCPs of mixed cultures contained higher concentrations of many amino acids than those found in cells of individual cultures.

There were also considerable species variations in the pattern of amino acids in the SCP. When the cells were subjected to proteolytic enzymes simulating to some extent the conditions during passage in the gastrointestinal tract (GIT), significant differences were found between species with regard to the extent of hydrolysis and concentration of EAA released. Cells of *L. acidophilus* and mixed cultures of *Str. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus* released the highest concentrations of EAA. The biological activity of many EAA in FM protein is much higher than that of corresponding amino acids in milk. These results indicate that the SCPs of starter bacteria consumed along with FM are likely to be hydrolyzed partially or fully in the digestive tract, releasing EAA, and some of the starter cultures are most useful than others in this respect.

Biological Value of FMs

The cumulative effect of the available EAA from milk proteins and SCP is reflected in the protein quality (biological value) of the total protein content isolated from FM prepared with different cultures. The protein quality of *Dahi* was assessed by microbiological assay procedure using *Str. zymogenes* as the test organism. It was found to be higher (3–30%) than that of milk used for preparing *dahi*. Samples prepared by using mixed cultures of *Lac. lactis* subsp. *lactis*, *Lac. lactis* subsp. *cremoris*, *Str. salivarius* subsp. *thermophilus*, and *L. delbrueckii* subsp. *bulgaricus* gave higher values than those containing other cultures. Incorporation of the culture of *Prop. freudenreichii* subsp. *shermanii* along with the starter cultures caused a further improvement in protein quality. It has also been found that there is an increased secretion of digestive enzymes by salivary glands when stimulated by the curd particles and that the FM proteins are twice as digestible as milk proteins.

Biopeptides in FMs

Several of the peptides derived from milk proteins represent 'extranutritional' substances, which display significant physiological influence. There are several modes of release of these peptides from precursor proteins or synthesis thereof from simpler molecules. The starter and nonstarter bacteria are commonly used in the manufacture of fermented dairy products. They take advantage of their proteolytic system, which contains at least 16 peptidases. During the microbial fermentations involved in the manufacture of FM, milk proteins undergo controlled proteolysis under the combined influence of native microflora (comprising of some spore producers) and the starter bacteria.

Additionally, proteinases produced by the starter organisms could complement the complex enzyme system of starter microorganisms, leading to the accumulation of beneficial peptides. The angiotensin-I-converting enzymes inhibiting peptides derived from casein are termed as casokinins, whereas those derived from whey proteins are called as lactokinins. Cheeses and processed cheese products, therefore, offer the potential for developing specialized functional food products displaying prophylactic properties, besides their natural nutritional virtues. This represents a hitherto unexplored area of food research, which has tremendous future potential, in both academic and commercial perspectives.

The folklores regarding the nutritional, healthful, and therapeutic aspects of FM have been fully substantiated through recent biochemical, nutritional, and physiological research. With the growing awareness of beneficial attributes of FM products, there has been a tremendous growth of market for probiotic FM products in the European countries, Japan, and developing countries. Prospectus for the genetic modification of starter organisms to enhance the NV and probiotic attributes has opened challenging vistas for the new product development and diversification. The FM, as a potential carrier for natural source of biological peptides, is a fascinating area for technological development.

FMs in the Health-Aware Era

When milk is fermented to obtain any FM, it has billions of live microbial cells, some of which can sustain the drastic conditions in the stomach and impact the GIT microflora. The human GIT microbiota, has been investigated since the beginning of microbiological studies, when Antonie van Leeuwenhoek, the father of microbiology, investigated the microorganisms in his own stools. The human GIT microbiota comprises trillions of microbes distributed in various niches throughout the intestinal tract and is one of the most complex microbiological ecosystem on Earth. The host and its microbiota have coevolved together and considering the staggering numbers and diversity.

There have been major scientific advances especially in human intestinal microbiology in the recent past. Early studies were limited to description of the culturable microbes, which, as we now realize, made up only a minority of the GIT microbes. Due to the development of molecular biological techniques over the last two decades, microbes can now be detected and studied to a large extent, without the need for culturing.

The vast extent of human GIT microbiota is only being revealed by virtue of the advancement of modern molecular biological tools. It is stated that 90% of the DNA of GIT is contributed by this microbiota. The number of microorganisms within the intestine greatly exceeds human cells, resulting in one of the most diverse and dynamic microbial ecosystems. Associations among the microbes and between the microbiota and the host have a profound influence on all concerned. The GIT offers various alcoves with nutrients, those ingested and spawned by the host, and a relatively nonhostile environment to the microorganisms. The microbiota plays essential roles in a wide variety of therapeutic, nutritional, developmental, and immunologic processes and therefore notably contributes to the well-being of the human beings. During the last two decades, specific bacterial isolates capable of surviving and establishing in the GIT known as probiotics have been extensively used to modulate the microbiota of GIT to benefit the consumers. Today, there is swaying evidence for probiotics in prevention and/or treatment of a number of GIT-related ailments, especially for reducing attack of diarrhea, providing relief for lactose-intolerant human beings and also atopic diseases. In order to rationally use synbiotics or other functional foods as therapeutic agents, in-depth knowledge of the structure, dynamics, and function of the bacterial populations of the GIT microbiota is crucial.

Studying the microbial ecology of the intestinal content involves the determination of the abundance and diversity of the microorganisms present, their activity within this niche, and their interactions with each other and their host. The GIT microbiota have been extensively investigated by culture-based methods, but our knowledge about the culturable fraction of this community is limited. It is due to the challenges of obtaining pure cultures of GIT inhabitants, which are hindered by the largely anaerobic nature of this community and the paucity of suitable enrichment strategies to simulate intestinal conditions.

The advent of molecular techniques based on 16s ribosomal (rRNA) gene analysis is now allowing a more complete assessment of this complex microbial ecosystem by unraveling the extent of diversity, abundance, and population dynamics of this community. The techniques like metagenomics have extended our view of those microorganisms that have proved difficult to culture and that play an important role in gut physiology. This huge intestinal microbial reservoir is estimated to contain above 1500 bacterial species and as much as 10^{14} cells. Besides studying the diversity, it is essential to identify these microbes based upon their ecophysiological traits, that is, those that are functionally active versus those that are effectively redundant and play little or no role at a particular time or at a given site of the intestinal tract. Thus, in the present era of health consciousness, the FMs have opened a new vista for multifarious development of these products as dietary adjuncts.

Future Developments in FM Industry

Mechanization and Automated Controls

In the era of aggressive marketing, a strong trade name and a good company image are of utmost importance in order to succeed as a food producer in the increasingly competitive global food market of the FMs. One of the essential aspects when building and protecting a brand name is to produce and deliver a product that is consistent in quality. Consumers expect their favorite product to have the same taste and texture every time it is consumed, regardless of when or where it was purchased and under what circumstances it was manufactured.

In just a few decades, the fermented dairy product industry has developed from small production units operated by a number of skilled dairy personnel, performing most of the operations manually, to large-scale production lines where predetermined product quality and production efficiency are

equally essential for success. Mechanization and the introduction of automation and production management systems have major roles in these developments. The main advantage of having an automated FM production line is that each procedure in the operation is repeated in exactly the same controlled manner every time, which ensures high precision of repeatability and safety.

At present, the rapid developments in computer-assisted information technology and electronics facilitate much more than just the process control to be built into an automated production system, for example, production management systems with work execution, data collection and data analysis, and integration to local or global business systems. The benefits from such developments in the FM industry are not only improved quality, safety, and control but also increased possibilities to map, visualize, analyze, develop, and optimize the product and the process as well as the production management and the production facilities.

Food Safety Aspects

The LAB are ubiquitous in fermented and nonfermented foods and are common components of the human commensal microflora. This long history of human exposure and consumption has led to the reasonable conclusion that they are generally safe. Attention has also been focussed on their possible role as probiotic bacteria, promoting beneficial health effects. There have, however, been a number of reports of human infections caused by LAB and these are reviewed. In most cases, the source of the infection was the commensal LAB flora rather than ingested bacteria, and the patient had some underlying disease or predisposing condition.

Even opportunistic pathogens, the LAB, with the notable exception of the enterococci, are much less successful than a number of other members of the commensal microflora. The use of new strains for probiotic use is likely to require more detailed evidence for their safety, particularly if the strains have been genetically modified or have been derived from animals. Procedures that have been proposed for assessing the safety of new strains are described. Consumers around the world are raising their voices on this issue, and local health authorities are introducing regulations that require all food producers to ensure that food is handled appropriately and safely. All these demands from a global consolidating food industry drive the development of tools that are accurate, competent, and effective that can assist the human brain and body when it comes to managing a food processing enterprise in most advantageous way.

Future Prospectus

Anyone walking around a supermarket will notice the prominence given to dairy products like cheeses, and FMs appear to be prominent dietary components. The sheer variety and consistent quality of such products soon become evident as well and attribute to the technical and scientific expertise of the manufacturers. How markets will develop in the future remains to be seen, for the consumers' tastes are not always predictable. In order to meet such changes in demand, what is important is that fundamental knowledge about the fermentation of milk continues to expand, for only then can product diversity and quality achieve the standards that consumers deserve.

See also: Acidophilus Milk; Cheese: Chemistry and Microbiology; Fermented Foods: Composition and Health Effects; Fermented Foods: Origins and Applications; Fermented Foods: Use of Starter Cultures; Functional Foods; Lactic Acid Bacteria; Malnutrition: Prevention and Management; Probiotics; Protein: Digestion, Absorption and Metabolism; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Single Cell Proteins; Vitamins: Overview; Yogurt: Dietary Importance; Yogurt: The Product and its Manufacture.

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Fermented Foods: Fermented Vegetables and Other Products

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Lactic Acid Bacteria as Cell Factories for the Production of Health-Promoting Compounds

Food fermentations are typically driven by microbial consortia, which may lead to significant changes in the health-promoting properties of fermented foods. Besides the potential direct role of food-borne microorganisms on the gut microbiota ecosystem, their indirect role in the modification of the bioavailability of certain food elements may also have a biogenic effect. The latter depends on the specific metabolic traits of the microorganisms, whereby several biochemical mechanisms allow them to fulfill the role of efficient cell factories for the synthesis and release of health-promoting compounds. As a result, fermentation may lead to a marked increase of the concentration of vitamins or amino acids, a higher bioavailability of phytochemicals and minerals, and an improvement of the nutritional qualities of foods by increasing digestibility and removing antinutrients (e.g., oxalate, protease and α-amylase inhibitors, lectins, condensed tannins, and phytic acid). The proteolytic system of lactic acid bacteria may also contribute to the liberation of bioactive peptides. Based on the previously mentioned considerations, lactic acid fermentation represents a simple and valuable biotechnology to exploit the health-promoting properties of fruits and vegetables.

Fruits and Vegetables as Carriers for Probiotic Microorganisms

Raw, minimally treated, and, especially, fermented vegetables are the vehicles of high-value nutrients and new lineages of commensal bacteria that interact with the gastrointestinal resident microbiota. Few and, in some cases, controversial *in vivo* studies have shown the direct causal relationship between microbes conveyed by fermented vegetable foods and gut microbiota. The consumption of fermented kimchi, for instance, delivers ~8 log cfu g^{-1} of autochthonous lactic acid bacteria and can lead, after administration of about 60 g day^{-1} for 2 weeks, to a positive effect on the number of beneficial human intestinal microbiota (*Lactobacillus* sp. and *Leuconostoc* sp.). *In vivo* intervention studies based on short-term consumption of diets, consisting entirely of plant foods, have shown that food-borne bacteria associated to diet can transiently colonize the gut. Although there is still debate on the link between fermented vegetables and other fermented foods on the one hand and the gut microbiota on the other hand, it is conceivable that ingestion of fermented foods is a source of beneficial microbes that remain viable during transit and at least interact with the gastrointestinal microbiota. More than other food ecosystems, raw fruits and some vegetables possess intrinsic chemical and physical parameters that, for some traits, mimic those of the human gastrointestinal tract, such as the very acid environment, buffering capacity, presence of antinutritional factors (e.g., tannins and phenols), and high concentration of indigestible compounds (fiber, inulin, and fructo-oligosaccharides). Indigestible molecules protect probiotic microorganisms from the acidic environment of the stomach and are a source of nutrients for the microbiota, which positively influences bacterial survival. Plant tissues have a microstructure composed of intercellular spaces, stomas, lenticels, capillaries, irregularities naturally occurring on the surface tissue, and tissue lesions. These sites favor microbial internalization and offer protection. Thus, raw fruits and vegetables may harbor functional strains able to survive gastric and intestinal fluids and capable of adhering to the gut epithelium and hydrolyzing dietary constituents, which cannot be metabolized by the host.

Microbiota of Raw Vegetables and Fruits

Vegetables and fruits, providing a variety of nutrients, are attractive hosts for microbes that colonize surfaces (epiphytes) and tissues (endophytes). Type, availability, and concentration of substrate; buffering capacity; competing microorganisms; and natural plant antagonisms provide a unique environment harboring a rather constant microbiota. However, farming and storage conditions may influence the composition and abundances of microbial communities found on raw vegetables and fruits. The microbial population of these raw matrices fluctuates between ~5 and 7 log cfu g^{-1}, which may include spoilage (e.g., *Erwinia carotovora*), pathogenic (e.g., *Listeria monocytogenes*), and beneficial (i.e., lactic acid bacteria) microbes. As also shown by deep sequencing approaches (high-throughput pyrosequencing analysis of the 16S rRNA gene), the microbiota of fruits is dominated by yeasts and fungi, and that of vegetables mainly consists of bacteria, especially aerobes (e.g., pseudomonads, enterobacteria, and coryneforms). Lactic acid bacteria are only a relatively small part (2–4 log cfu g^{-1}) of the autochthonous microbiota of raw vegetables and fruits. Hetero- and homofermentative species belonging to species of *Leuconostoc*, *Lactobacillus*, *Weissella*, *Enterococcus*, and *Pediococcus* have been variously identified, depending on the species of vegetables. Nevertheless, *Weissella cibaria*/*W. confusa* and, especially, *Lactobacillus plantarum* are the most frequently found species. *Lactobacillus plantarum* is considered to be a ubiquitous and metabolic versatile bacterium, which is largely found in fruits and vegetables. Recently, fructophilic lactic acid bacteria (FLAB) were described as bacteria that prefer fructose to glucose as growth substrate. They are found in fructose-rich niches such as fruits and fermented foods made from fruits and flowers. Only two *Lactobacillus* spp. have been classified as fructophilic until now, that is, *Lactobacillus plantarum* and *Lactobacillus kunkeei*. The latter has originally been isolated from grape juice. All other FLAB were grouped into the genus *Fructobacillus*, which contains *Fructobacillus durionis*,

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F. ficulneus, *F. fructosus*, *F. pseudoficulneus*, and *F. tropaeoli*. All these species are phylogenetically related to the genera *Leuconostoc*, *Oenococcus*, and *Weissella*. *F. pseudoficulneus* was isolated from figs and banana. FLAB species are separated into two groups: obligately and facultatively fructophilic. Obligately FLAB grow well on fructose, whereas external electron acceptors such as fructose, pyruvate, and oxygen are needed for glucose metabolism. Molecular typing of lactic acid bacteria isolated from vegetables and fruits is useful to group them into clusters that reflect their distribution in the raw matrix and, presumptively, their way of entry. Besides, intraspecific differentiation is the important preliminary step to select starter cultures.

Fermentation of Vegetable Products

When favorable conditions of anaerobiosis, water activity, salt concentration, and temperature occur, raw vegetables and fruits may be subjected to spontaneous lactic acid fermentation. Alcoholic fermentation may take place concomitantly. Usually, Gram-negative bacteria are inhibited starting from the early stage of fermentation. Spontaneous fermentation may be optimized through back-slopping, commonly used for sauerkraut production (i.e., inoculation of the raw material with a small quantity of a previously performed successful fermentation). Hence, back-slopping results in the dominance of the best-adapted strains and represents a way of using a selected microbial consortium to shorten the fermentation and to reduce the risk of fermentation failure. The same effect is achieved through the use of starter cultures, especially when these are selected within the autochthonous microbiota. In either cases (spontaneous and with starters), the composition of the microbiota completely changes, showing high cell densities of lactic acid bacteria towards the end of the fermentation ($\sim$9 log cfu g^{-1}). Under these conditions, humans are solely exposed to beneficial bacteria without the need for drastic processing.

Nowadays, the use of starter cultures is increasing in vegetable fermentation technology. Yet, contrary to other fermented foods (e.g., dairy, meat, and baked goods), only few cultures are used for fruit and vegetable fermentations, with *Lactobacillus plantarum* as the most frequently used one. It has been shown that the use of selected autochthonous lactic acid bacteria as starters, when contrasted to allochthonous/commercial cultures or to spontaneous fermentation, guaranteed the prolonged shelf life of fermented vegetables and fruits and maintained agreeable nutritional, rheological, and sensory properties. These effects may be the consequence of the modified profile of organic acids, the synthesis of lactic and acetic acids, and the metabolism of free amino acids. Recently, a number of vegetables and fruits were successfully subjected to fermentation by selected autochthonous lactic acid bacteria (Table 1). Adaptation to the intrinsic features of the raw matrices guarantees high survival ($\sim$8–9 log cfu g^{-1}) during fermentation and storage (c.40 days), whereas allochthonous strains show longer latency phases of growth and acidification with respect to selected autochthonous strains. Autochthonous cultures may also synthesize bacteriocins to improve competitiveness and contribute to food safety.

Main Fermented Vegetable Products

Many fruits and vegetables are processed through lactic acid fermentation for the production of a wide range of traditional fermented products in various worldwide regions, mainly in the Mediterranean area. Sauerkraut, kimchi, and fermented cucumbers are the most studied lactic acid-fermented

Table 1 Examples of vegetables and fruits successfully subjected to fermentation by selected autochthonous lactic acid bacteria

Vegetables and fruits	*Autochthonous lactic acid bacteria*	*Effects*
Almagro eggplant	*Lactobacillus brevis*, *Lactobacillus plantarum*	Synthesis of most preferred end products with regard to sensory properties
Leek	*Leuconostoc mesenteroides* and *Lactobacillus plantarum*	High microbiological quality, extensive consumption of carbohydrates, and synthesis of various end products
Carrots, French beans, or marrows	*Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and *Pediococcus pentosaceus*	Rapid decrease of pH, marked consumption of fermentable carbohydrates, and inhibition of Enterobacteriaceae and yeasts
Red and yellow peppers	*Lactobacillus plantarum*, *Lactobacillus curvatus*, and *Weissella confusa*	Rapid decrease of pH, marked consumption of fermentable carbohydrates, inhibition of Enterobacteriaceae and yeasts, high firmness, high color indexes
Pineapple	*Lactobacillus rossiae* and *Lactobacillus plantarum*	Inhibition of yeasts, maintaining agreeable sensory and nutritional features
Sweet cherry puree added of stem infusion	*Pediococcus pentosaceus* and *Lactobacillus plantarum*	Evident consumption of organic acids (e.g., malic acid) and free amino acids, especially throughout storage and preservation of phenolic compounds
Tomato juice	*Lactobacillus plantarum*	Synthesis of exopolysaccharides and high viscosity; preservation of elevated values of ascorbate, glutathione, and total antioxidant activity during storage; synthesis of volatile compounds having a positive log odor value (e.g., 3-methyl-3-butan-1-ol, 2,3-butanedione, and 3-hydroxy-2-butanone)
Kimchi	*Lactobacillus sakei*	Mild acidifying properties, synthesis of volatile compounds (diacetyl, acetoin, acetaldehyde, secondary alcohols, esters, and lactones) affecting the sensory properties

vegetables mainly due to their commercial importance. The manufacturing protocols for some traditional fermented vegetables are described in the following.

Fermented Olives

Table olives are one of the main pickled products with industrial importance. The trade standard applying to table olives of the International Olive Oil Council defines table olives as "the sound fruit of varieties of the cultivated olive trees (*Olea europaea* L.) that are chosen for their production of olives whose volume, shape, flesh-to-stone ratio, fine flesh, taste, firmness and ease of detachment from the stone make them particularly suitable for processing; treated to remove their bitterness and preserved by natural fermentation; or by heat treatment, with or without the addition of preservatives; packed with or without covering liquid." Table olive processing has been a very old tradition in all Mediterranean countries and, from the beginning of this century, has become popular all over the world. The high nutritional value of table olives is well known, since they contain several nutritional components (e.g., vitamins E and A and polyphenols), which largely depend on the olive variety, the maturation stage of the olive fruit, the cultivating conditions, and the processing method. Because of the bitterness caused by the phenolic glycoside oleuropein, raw olives are not suitable for consumption without any processing step. The main commercial preparations, namely, (a) treated green olives (Spanish style), (b) naturally black olives (Greek style), and (c) ripe olives processed by alkaline oxidation (Californian style), are among the first three processing methods defined by the standard. Spanish-style olive manufacture combines a lye treatment with a NaOH solution (1–3.5% w/v) and a fermentation step. It has been generally established that lactic acid bacteria are responsible for the fermentation of treated olives. However, lactic acid bacteria and yeasts compete for the fermentation of natural olives. The predominant bacterial genus is *Lactobacillus*. Other genera of lactic acid bacteria have also been isolated to a lesser extent (e.g., *Weissella*, *Pediococcus*, *Enterococcus*, and *Leuconostoc*). Yeasts play a minor role, though they may contribute to the flavor and aroma of table olives. According to the Greek-style process, the fermentation occurs in a brine solution with high NaCl content (6–15% w/v), which promotes the growth of yeasts (mainly *Candida* spp., *Debaryomyces* spp., *Pichia* spp., and *Rhodotorula* spp.). The predominant lactic acid bacteria genera are the same as the ones involved in Spanish-style processing. Californian-style processing is based mostly on a lye treatment to remove the bitterness and may include only a weak fermentation step.

Microbial starter selection, bacteriocin production, availability of nutrients, probiotication, and adjustment to determinants affecting the fermentation process (e.g., pH, temperature, NaCl, and polyphenol content) are some of the main current challenges for the table olive industry.

Sauerkraut

Sauerkrauts, widely consumed in many European countries and in the United States, are manufactured by spontaneous lactic fermentation of white cabbage (*Brassica oleracea* L. var. *capitata*). After removal of the core and outer leaves, fresh cabbage is shredded and mixed with 2–3% (w/w) salt before allowing for natural fermentation. Cabbages are quickly surrounded by brine and covered with plastic sheeting draped over the tank, to ensure air exclusion. Sauerkraut production typically relies on a sequential microbial process that involves hetero- and homofermentative lactic acid bacteria, such as *Leuconostoc* spp. and *Weissella* spp. in the early phase and *Lactobacillus* spp., *Lactococcus lactis*, and *Pediococcus* spp. in the subsequent phases. *Lactobacillus plantarum* prevails during late fermentation, leading to further acidification and a final pH of ~3.5–3.8. Sauerkraut brine is an important by-product of the cabbage fermentation industry and may be used as a substrate for the production of carotenoids by *Rhodotorula rubra*. Sauerkraut fermentation lasts several weeks, depending on the fermentation conditions, sensory attributes, the autochthonous microbiota of cabbages, and the related sugar contents. Secondary fermentations, such as those carried out by yeasts, are undesirable.

Kimchi

Kimchi is a Korean traditional fermented vegetable made from Chinese cabbage (beachu), radish, green onion, red pepper powder, garlic, ginger, and fermented seafood (jeotgal), which is traditionally made at home and served as a side dish at meals. Kimchi is a generic term indicating a group of traditional lactic acid-fermented vegetables in Korea. Kimchi fermentation traditionally represents an efficient method of preserving the fresh and crispy texture of vegetables during winter, when fresh vegetables are not available. Actually, kimchi is a globally popular food, and its market size is gradually increasing. Thanks to the physiological effects of its ingredients and its microbial metabolites, kimchi is widely perceived as a health-promoting food. Vegetables are soaked with water, cut, and placed in the salt solution (5–7%) for 12 h, until the salt content in the tissues reaches the value of ~2.0–4.0% of the total weight. Further, vegetables are rinsed several times with freshwater and drained. The mixed ingredients are then placed in fermentation vessels, covered with macerated cabbage leaves, and pressed by stones to ensure anaerobic conditions. Kimchi fermentation is a temperature-dependent process. It ripens in 1 week at 15 °C but takes only 3 days at 25 °C. Low temperature (2–6 °C) is nevertheless preferred in kimchi fermentation to prevent the production of strong acidity and overripening, as well as to extend the period of optimum taste. The time for the fermentation of kimchi may thus vary from few days to several months, depending on the temperature and concentration of NaCl. Kimchi fermentation is carried out by various autochthonous microorganisms. The microbiota is largely affected by the fermentation conditions (e.g., anaerobiosis, ingredients, salt concentrations, and temperature). Generally, *Leuconostoc mesenteroides* and *Lactobacillus plantarum* have been identified as the predominant species, but several studies suggest that lactic acid bacteria contributing to kimchi fermentation also include *Leuconostoc citreum*, *Leuconostoc gasicomitatum*, *Lactobacillus brevis*, *Lactobacillus curvatus*, *Lactobacillus sakei*, *Lactococcus lactis*, *Pediococcus pentosaceus*, *W. confusa*, and *W. koreensis*.

Pickled Cucumbers

The manufacture of pickled cucumbers requires that fully ripe cucumbers are washed and drained and, in some cases, sliced.

Further, regular-shaped cucumbers are dipped into brine (5–7% of NaCl). Industrial fermentations are carried out by the spontaneous growth of lactic acid bacteria. The fermentation lasts 2–3 weeks, depending on the temperature (20–27 °C), which allows to reach a final pH of ~3.1–3.5. Because of the high concentration of NaCl, the growth of *Leuconostoc mesenteroides* and other less salt-tolerant species is inhibited. Only homofermentative lactic acid bacteria, such as *Pediococcus pentosaceus* and *Lactobacillus plantarum*, are responsible for fermentation. Strictly heterofermentative species are undesirable. The synthesis of CO_2 leads to texture defects and bloater damage, with serious economic losses. Since CO_2 results also from the cucumber respiration or the malolactic fermentation by *Lactobacillus plantarum*, fermentation vessels are sometimes purged with inert nitrogen gas. Salt, consumption of fermentable carbohydrates, and low values of pH prevent undesirable secondary fermentations by yeasts and other lactic acid bacteria (e.g., *Lactobacillus buchneri*), which often cause cucumber spoilage. Blanching of cucumbers is also used to decrease the concentration of NaCl (4% or less), partially inhibit the autochthonous microbiota, and improve the texture, through the inactivation of endogenous pectin esterase and glycosidases. Partial or complete replacement of NaCl by $CaCl_2$ has been suggested as an efficient method to prevent softening.

Household-Level Fermentations and Traditional Products

Besides the semi-industrial and globally popular products, a large variety of fermented vegetables are manufactured around the world at household level as fundamental components of the daily diet, representing a culture heritage. Perishable and seasonal raw plant substrates are fermented into edible products using indigenous methods of biopreservation. Lactic acid fermentation is the simplest way to preserve fruits and vegetables and helps in solving with the issue of food security. A brief description of some traditional fermented vegetable products from different regions of the world is reported later in the text.

Africa

Fermented foods have a long history in Africa. Substrates used in fermented plant food production include vegetables, fruits, seeds, cereals, roots, and tubers. Some fermented products, usually obtained from carbohydrate-rich raw matrices, serve as main course meals or as beverages, while others, resulting from the fermentation of protein-rich seeds, are used as food condiments.

Fermented seeds are an important cheap source of dietary protein. Examples of traditional fermented seeds used as flavoring agents include iru (fermented *Parkia biglobosa* seeds), ogiri (fermented *Citrullus vulgaris* seeds), dawadawa (fermented soybean (*Glycine max*) seeds), and okpehe (fermented *Prosopis africana* seeds). Often, *Bacillus* species are predominant during these fermentations.

Pickled lemons are widely consumed in North Africa. Fermentation helps in dealing with the issue of seasonality. Cured lemon, called msayer or msir, is consumed in Morocco, while lamoun makbouss are pickled lemons consumed in Libya and Egypt. According to Moroccan style, the salted lemons are stored in sealed glass container, kept in a cool dry place for 4–6 weeks, to allow the leakage of the lemon juice and the dissolving of salt. The resulting strongly salty and acidic brine promotes the growth of lactic acid bacteria and yeast (e.g., *Candida parapsilosis* and unclassified Saccharomycetales).

Cassava (*Manihot esculenta* Crantz) is one of the most important root crops in many African countries and ensures the energetic food security for many millions of people. Sweet varieties of cassava may be consumed directly, whereas bitter varieties are traditionally processed into a wide range of traditional products: gari (partially gelatinized granular cassava meal), fufu (pounded boiled cassava), lafun (fine powdery cassava), kokonte (dried cassava flour), kivunde (retted cassava), and cingwada (retted cassava). Lactic acid fermentation may significantly prolong the short shelf life (only 5 days after harvesting) of raw cassava and may have a role in cyanogen reduction in bitter cassava. *Lactobacillus plantarum* typically dominates the fermentation. Some *Lactobacillus plantarum* strains isolated from fermented cassava are able to hydrolyze resistant starch, thereby increasing the energy density.

Asia

Plant-based foods represent the core of the daily diet in Asian countries, which are the first in the world for per capita consumption of fruits and vegetables. Food fermentation in Asia is strongly dependent on the availability of seasonal vegetables. Ethnic fermented foods are linked to the Asian culture and tradition, and hundreds of different forms of fermented vegetables are prepared by the different ethnic groups. A wide range of substrates is subjected to fermentation, which includes vegetables, fruits, legumes, rice, and bamboo shoots. Processing is still carried out at household-scale through spontaneous fermentations (e.g., alcoholic, lactic, propionic, and acetic acid fermentations).

Gundruk is a fermented leafy vegetable commonly prepared by the Nepalis of the Himalayan region prepared during the months of December to February, when the weather is less humid and there is a wide supply of vegetables. For gundruk preparation, fresh leaves of local cabbage, mustard, and cauliflower are wilted for 1–2 days. The dried leaves, after a mild crushing procedure, are soaked briefly in hot water and hand-pressed in an earthen pot to remove surplus water. Then, they are kept in a warm and dry place for 15–22 days. Generally, a spontaneous fermentation is started by *Lactobacillus brevis* and *Pediococcus pentosaceus* and dominated by *Lactobacillus plantarum* in the late stage. After a desirable fermentation, gundruk is removed and sun-dried. Other examples of Asian fermented leafy vegetables include hum-choy, produced in the south of China from a local vegetable called gay-choy; anishi, goyang, and ziang-sang, made in northeast India from local leafy vegetables; sunki, prepared from red turnip leaves in Japan; and pak-gard-dong, produced in Thailand from mustard.

Khalpi is a fermented pickle consumed in the Himalayan region. For its preparation, mature and ripened cucumbers are cut and sun-dried for 2 days. Then, they are fermented into sealed bamboo vessels for 4–7 days at room temperature.

Khalpi is eaten as a pickle after mixing with mustard oil, powdered chilies, and salt. The microbiota of khalpi is characterized mainly by *Leuconostoc fallax*, *Lactobacillus brevis*, and *Pediococcus pentosaceus* in the early stage and *Lactobacillus plantarum* in the last stage of fermentation.

Sinki is prepared from radish taproots, and it is very popular in Nepali communities. The protocol for its manufacturing is similar to that of gundruk. The fermentation may last up to 30–40 days and is commonly started by *Lactobacillus fermentum* and *Lactobacillus brevis* and later replaced by *Lactobacillus plantarum*.

Pickled fruits, such as lime, lemon, and mango, are very popular in Asia. Burong mangga is a green mango pickle produced in the Philippines. For its manufacturing, peeled and sliced fruits are fermented in brine for ~3 days. Lactic acid bacteria involved during burong mangga fermentation include mainly *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Pediococcus cerevisiae*, and *Lactobacillus plantarum*.

Tempoyak is a traditional Malaysian fermented condiment made from the pulp of the durian fruit (*Durio zibethinus*). Fermentation is carried out by lactic acid bacteria, such as *Lactobacillus brevis*, *Lactobacillus mali*, *Lactobacillus fermentum*, *Lactobacillus* durianis, and *Leuconostoc mesenteroides*.

Kombucha is a refreshing beverage obtained by the fermentation of sugared tea by a symbiotic mixture of bacteria (acetic acid and lactic acid bacteria) and yeast. Native of China, kombucha is currently common around the world. The many benefits of kombucha for health were reported, such as anticancer activity, detoxifying effects, and antibiotic activity. Its properties are mainly attributed to the acidic composition, to the capacity of glucuronic acid to bind to toxin molecules, and to the content of B-complex vitamins and phenols.

South America

Water kefir, also known as tibicos or sugar kefir, is a probiotic beverage native of Mexico and currently common worldwide. Water kefir is obtained through fermentation of sweetened water with a symbiotic culture of lactic acid bacteria, bifidobacteria, and yeast. Figs and lemons are traditionally added for additional flavor and nutrients. Microorganisms are introduced through water kefir grains (polysaccharide matrix in which microorganisms are imbibed), handed down from household to household. Water kefir grains are thought to have originated from fermented sap on the pads of the *Opuntia cactus*.

Eastern Europe and Middle East

Researchers and food manufacturers have become increasingly interested in Turkish fermented nonalcoholic beverages due to their value as probiotic source. Shalgam, for instance, is a lactic acid-fermented beverage that is very popular in Turkey. Its manufacturing requires that bulgur flour, sourdough, and salt are mixed with water and left to ferment by lactic acid bacteria and yeasts for 3–5 days at room temperature. Then, vegetables (e.g., carrots) and water are added to the fermented product of the first stage. Fermentation is carried out in wooden barrels for 3–10 days, mainly by *Lactobacillus plantarum*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus fermentum*, and *Lactobacillus brevis*, although yeasts such as *Saccharomyces cerevisiae* may contribute to flavor development. Hardaliye is a Turkish grape-based nonalcoholic traditional beverage. Pressed grapes and cherry leaves are mixed with pressed mustard and fermented in barrels at room temperature for 5–10 days. After incubation, the mixture is filtered and kept in a cool place. The predominant lactic acid bacteria species are *Lactobacillus paracasei* subsp. *paracasei* and *Lactobacillus casei* subsp. pseudoplantarum.

Innovative Plant-Based Fermented Products

During the last decade, innovation in food biotechnology has led to an increase in the number of new products with functional claims for an estimated growth rate of 28% per year. This is paralleled by a rise in the amount of scientific papers dealing with this topic. The global market of functional foods is a growing field of the food industry, driven by modern consumer's demand for foods that may improve well-being and reduce the risk of disease. The ongoing trend of vegetarianism, the increasing prevalence of lactose intolerance, and the cholesterol effects of dairy products drive the research towards nondairy functional foods. The suitability of several vegetables and fruits as raw materials for the manufacturing of functional products has opened new perspectives. There is a strong interest in the development of plant-based functional foods, as juices, smoothies, and yogurt-mimicking beverages. Lactic acid fermentation represents an innovative and attractive approach to improve the nutritional, functional, and hedonistic features of plant material, although several applications often originate from or are inspired by ethnic food (e.g., Asian). In order to develop novel fermented functional foods, implementation strategies relate to the exploitation of microbial functionalities, the testing of novel formulations with ingredients that either are of natural origin or originate from industrial by-products as functional ingredients, and the fortification with probiotics and prebiotics.

Fruit and Vegetable Juices

One of the most attractive opportunities is the development of lactic acid-fermented fruit and vegetable juices, also named 'lacto-juices.' Since the latter have flavor profiles that are pleasing to all age groups and are perceived as healthy and refreshing beverages, they promise a considerable market value. Fruit and vegetable juices are ideal substrates for functional beverage making, due to their content of essential nutrients. Furthermore, formulation with different substrates may provide different combinations of health-promoting components (e.g., antioxidants, dietary fiber, minerals, and vitamins). The technological options for the production of fermented vegetable juices mainly include (i) spontaneous fermentation by autochthonous microbiota, (ii) fermentation by selected starter cultures added to raw vegetables, and (iii) fermentation of mildly heat-treated vegetables by starter cultures. Selection of lactic acid bacteria that are able to adapt to the inherent features of each raw material (e.g., availability of nutrients and physicochemical features) may assure fermented vegetable juices with optimal characteristics. Fruits and vegetables tested in novel lacto-juices include watermelon, sapodilla, carrot, beetroot,

pepper, parsley, lettuce, lemon, cabbage, spinach, tomato, pomegranate, blackcurrant, orange, grapes, and sweet potatoes. Pomegranate juices fermented with selected strains of *Lactobacillus plantarum* have, in general, improved health-promoting and sensory properties. Besides higher phenolic concentration and antioxidant activity, a positive immunomodulation activity distinguishes fermented pomegranate juices from unfermented ones. Fermentation by autochthonous lactic acid bacteria also positively affects the health-promoting and sensory properties of tomato juice, leading to improved viscosity, color, and, especially, ascorbic acid, glutathione, and total antioxidant contents. A fermented juice based on vegetables, seeds, and sprouts of germinated lentils and cowpeas was proposed as potential functional beverage, due the content of ACE-inhibiting substances, bile acid-binding activities, and hemagglutinating activities. Fermented carrot juice, in addition to being microbiologically stable, has good sensory properties and a potentially high nutritional value, due to the improved iron solubility and overall mineral availability. The preservation of pigments and the synthesis of health-promoting substances, such as aglycones and β-carotene, have been shown in lactic acid-fermented beetroots and sweet potatoes. Red beets were also evaluated as a potential substrate for the production of probiotic beet juice by four species of lactic acid bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, and *Lactobacillus plantarum*). Although the probiotication of several fruit and vegetable juices with lactic acid bacteria and bifidobacteria seems promising, some cultures may negatively affect the sensory properties of the juices or show sensitivity towards the low pH of the raw matrices and postfermented products, fermentation metabolites, processing temperature, and gastrointestinal tract conditions. The addition of yeast autolysate (e.g., spent brewer's yeast) into the juices may increase the number of lactic acid bacteria during fermentation, reduce the time of fermentation, and enrich the juices with amino acids, vitamins, minerals, and antioxidants. Thus, a proper selection of the fruit matrices and probiotic strains and, if required, the addition of other ingredients are decisive steps to produce a palatable beverage that may be consumed regularly, providing functional benefits.

Fruit and Vegetable Smoothies

As an alternative to the consumption of fresh fruits and vegetables, the food industry offers the manufacture of smoothies, which consist of mixtures of fruits and vegetables processed to pulp or puree. Recently, a novel protocol for the manufacture of smoothies was set up, based on lactic acid fermentation of white grape juice and *Aloe vera* extract mixed with fruit and vegetable purees. Lactic acid fermentation by autochthonous selected starters positively affects the content of antioxidant compounds and enhances the sensory attributes of smoothies. An alternative technological option for processing sweet cherries involves the lactic acid fermentation of cherry puree supplemented with cherry stem infusion, as to exploit the nutritional, antioxidant, and sensory features of the raw materials. Likewise, broccoli puree fermented by *Lactobacillus* spp. for the production of functional foods is enriched in phenolic derivatives with increased bioavailability and biological activity.

Minimally Processed Fruits and Vegetables

Minimally processed fruits and vegetables are products obtained by trimming and/or peeling and/or cutting and packaging and are characterized by convenience and ability to maintain their freshness. Fermented pineapple slices by autochthonous lactic acid bacteria, also without any heat treatment, is an example of a shelf-stable minimally processed fruit with elevated numbers of viable lactic acid bacteria and agreeable nutritional and sensory properties. Lactic acid fermentation may promote the storage of peppers at room temperature for extended shelf life. A protocol that includes blanching at 85 °C for 2 min, fermentation at 35 °C for 15 h in brine (1%, w/v) with selected autochthonous starters, and heat treatment at 85 °C for 15 min allows the storage at room temperature for 30 days with better texture and sensory properties compared to the 'unstarted' peppers. Moreover, the survival of *Lactobacillus plantarum* and *Lactobacillus fermentum* strains on pineapple pieces has been demonstrated throughout storage. A protective effect against relevant food-borne pathogens was also successfully reported. Artichokes may act as a valuable vector, as they are capable of supporting viable bacteria for at least 90 days. The anchorage of strains to artichokes also improves the survival of probiotic microorganisms during gastrointestinal digestion.

Fruit- and Vegetable-Based Yogurt-Like Products

The so-called vegetable milks might be used as raw materials to develop yogurt-mimicking products. They are mainly obtained from soy, cereals, and nuts. Other minor raw materials may be hemp, sunflower, lupin seeds, and tubers (e.g., tiger nuts). Although the production of yogurt-mimicking products may differ according to the raw material and the microbial starters, the main steps are (i) milk conditioning to the optimal growth temperature of the starters, (ii) inoculation and incubation procedures, and (iii) cooling to 4 °C. Protein coagulation and serum separation during storage may cause physical stability problems. The addition of hydrocolloids, such as xanthan gum, modified starches, pectin, and cellulose derivatives, promotes physical stability. *In situ* production of oligosaccharides and exopolysaccharides by lactic acid bacteria positively affects the texture, since these compounds act as emulsifiers or stabilizers and increase viscosity and mouthfeel. Prebiotic compounds (e.g., starch and fibers) are sometimes added to promote the survival of probiotic starters, due to their nutritional contribution and protective action, since they act as protective barriers within the human gastrointestinal tract. The most popular vegetable milk derives from soy. Soy-based beverages have a huge potential as functional foods, since they may have a role in the prevention of some chronic degenerative diseases, are poor in cholesterol and saturated fats, and contain high levels of quality protein and amino acids, dietary fiber, magnesium, phosphorus, vitamin K, riboflavin, thiamine, folic acid, isoflavones, and other flavonoids. The low concentration of soluble carbohydrates of soy milk may limit the growth and acidification of lactic acid bacteria and make the addition of glucose and fructose indispensible. Probiotic bacteria have been successfully incorporated in soy milks, with a positive impact on the intestinal ecosystem. Nut-derived milks also represent suitable raw substrates to develop healthy yogurt-mimicking beverages, since they are

rich in mono- and polyunsaturated fatty acids, proteins, dietary fibers, phytosterols, phenolics, vitamins, and minerals. Probiotication of hazelnut milk through fermentation with *Lactobacillus rhamnosus* and *Streptococcus thermophilus* has been successful, ensuring high probiotic survival throughout cold storage (≈ 108 cfu ml^{-1}) and an *in vitro* simulation of the digestion process (≈ 105 cfu ml^{-1}). Minor raw materials, such as tuber milk, may also be exploited for the production of yogurt-mimicking products. Fermented tiger nut milk with good nutritional, chemical, and sensory properties was produced through pasteurization at 90 °C for 15 min and fermentation at 45 °C for 18 h using mixed cultures of *Lactobacillus plantarum*, *Lactococcus lactis*, and *Lactococcus thermophilus*.

Conclusion

Fermented fruits and vegetables are associated with several social and cultural aspects of different human communities. Due to their high nutritional value, fermented vegetable foods and beverages are estimated to constitute about one-third of the foods daily consumed worldwide. Studies have shown that fruits and vegetables may serve as a suitable carrier for probiotics. In addition, fermented fruits and vegetables contain a large range of prebiotic compounds that can stimulate the growth of beneficial bacteria. Therefore, exploitation of the axis 'fermented foods – human health' based on plant fermentations is a promising and conceivable strategy for the future. Basic understanding of the relationship between food, beneficial microorganisms, and human health can be important to improve food quality and to prevent disease.

See also: Fermented Foods: Composition and Health Effects; Fermented Foods: Use of Starter Cultures; Fruit Juices; Functional Foods; Lactic Acid Bacteria; Probiotics.

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Fermented Foods: Origins and Applications

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What Is Fermentation? Food Fermentation Versus Biochemical Fermentation

Fermentation is one of the oldest methods of food preservation and preparation; however, the word has many meanings. It is derived from the Latin word *fermentum*, which is the past participle of *fervere* (to boil). A first microbiological definition relied upon the expression 'la vie sans air' by Louis Pasteur (life in the absence of air, in particular oxygen). In biochemistry, fermentation is thus a microbial metabolism that produces energy from carbohydrates in the absence of oxygen. Alternatively, fermentation can be defined as an incomplete oxidation of sugars in the absence of oxygen that uses an internal organic compound as an electron acceptor. In 1957, Prescott and Dunn defined it "a process in which chemical changes are brought about in an organic substrate, whether carbohydrate or protein or fat or some other types of organic materials through the action of biochemical catalysts known as enzymes, elaborated by specific types of living organisms." Based on the latter broad definition, fermentation describes any biological process whether oxygen is present or not, that is, the production of wine, vinegar, yogurt, beer, table olives, and many other foods, as well as of different organic fine chemicals (e.g., antibiotics, amino acids, citric acid, and monosodium glutamate). The main trait of a fermentation is the conversion of complex organic substrates into simpler compounds with the production of chemical energy as adenosine triphosphate.

At least three kinds of fermentation can be distinguished: (i) solid-state (e.g., tempeh), (ii) liquid-state (or submerged) (e.g., fermentation of corn steep liquor), and (iii) semisolid-state, where the product is submerged (e.g., *shoyu* or soy sauce). Moreover, fermentation can be spontaneous and uncontrolled (raw materials get fermented by microbiota that are indigenous or that originate from the equipments and environment) or controlled and inoculated (the fermenting microbiota mostly originates from the inoculation of starter cultures). Controlled fermentation is based on either a monoculture (using a single and pure strain) or multiculture (using biculture or multiculture strains of microorganisms or mixed inocula). The definition of spontaneous or controlled fermentation is closely related to the definition of starter cultures; a starter culture consists of microorganisms that are inoculated directly into food materials to overwhelm the existing microbiota and bring about desired changes in the finished product. These changes may include novel functionality, enhanced preservation, reduced food safety risks, improved nutritional or health value, enhanced sensory qualities, and increased economic value. Table 1 lists the most important microorganisms of starter cultures.

Fermentation is at the basis of the production of fermented foods, which can be defined in different manners:

1. Foods that are fermented until at least one of the constituents has been subjected to the action of microorganisms for a period, so that the final products have often undergone significant changes in physicochemical composition and sensory scores, due to microbial and enzymatic changes
2. Foods that have been subjected to the action of microorganisms or enzymes, so that desirable biochemical changes cause significant modification
3. Palatable and wholesome foods prepared from raw or heated materials by microbial fermentation

There are at least 5000 major and minor or ethnic fermented foods; they generally bear the 3A or A^3 connotation that pinpoints acidic, alkaline, or alcoholic traits. Thus, the most important sensory and physicochemical properties of fermented foods are as follows:

1. Acidic taste (low pH and lactic acid fermentation) (e.g., yogurt, fermented milks, gundruk, kimchi, and table olives)
2. Alkaline nature (high pH) (e.g., kinewa, dawadawa, and pidan)
3. Alcoholic taste (moderate-to-high concentration of ethanol) (e.g., wine, beer, sake, and pulque)

There are different classifications of fermented foods, relying upon different criteria. Hereby, we propose a simple classification (Table 2) based on three questions:

1. Is the fermented food an alcoholic product/beverage?
2. What is the raw material?
3. Which kind of starter culture was applied?

Historical and Cultural Aspects

Our ancestors already consumed food products that had been subjected to food fermentation; they did not know the role of microorganisms, but they recognized the preservative and nutritional qualities of fermented foods, as well as their palatability. We do not know when humans began to use fermentation intentionally, but archaeological data suggest an intentional fermentation of honey, fruits, and rice about 10 000 years ago. Moreover, we have some evidence of bread and cheese making, as well as of grape fermentation, around 6000–7000 BC.

One of the first descriptions of a fermented food is the Hymn to Ninkasi by Sumerians; it dates back *c.*1900 years BC and describes the production of beer. Fermented foods were widespread in all the ancient populations and there is an important document for the production of fermented foods by ancient Indian: the sacred book Rig Veda reported on the production of a fermented beverage to gain immortality. For centuries, fermentation has been an empirical process until Louis Pasteur described the role of microorganisms in 1857. Table 3 proposes a short historical overview of fermentation from the Neolithic era to the twentieth century.

Table 1 Starter cultures

Bacteria	Lactic acid bacteria (*Lactobacillus* spp., *Lactococcus* spp., *Streptococcus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Leuconostoc* spp., *Oenococcus oeni*), *Propionibacterium* spp., *Bifidobacterium* spp., acetic acid bacteria (*Acetobacter* spp., *Gluconobacter* spp.), *Bacillus* spp., *Brevibacterium linens*, catalase-positive cocci (coagulase-negative *Staphylococcus* spp., *Kocuria* spp.)
Yeasts	*Saccharomyces cerevisiae*, *Sacc. pastorianus*, *Zygosaccharomyces* spp., *Candida* spp., *Pichia* spp.
Molds	*Penicillium camemberti*, *P. roqueforti*, *P. chrysogenum*, *P. nalgiovense*, *Aspergillus oryzae*, *Rhizopus microsporus* subsp. *oligosporus*

Table 2 Classification of fermented foods

Nonalcoholic fermented foods	*Alcoholic fermented foods*
Fermented vegetables	Nondistilled mild alcoholic beverages produced by amylolytic starters[a]
Fermented soybean and legumes	Nondistilled and filtered alcoholic beverages produced by amylolytic starters
Fermented cereals	Distilled alcoholic beverages produced by amylolytic starters
Fermented milks	Alcoholic beverages produced using human saliva as amylolytic starter
Fermented fish	Alcoholic beverages produced by mono-fermentation
Fermented meat	Alcoholic beverages produced from honey
Fermented root/tuber products	Alcoholic beverages produced from plants
Miscellaneous products	Alcoholic beverages produced from malting or germination
	Alcoholic beverages produced from fruits without distillation
	Distilled alcoholic beverages without amylolytic starters

[a]Ethnic preparations containing filamentous fungi able to hydrolyze starch.

Table 3 Historical overview of fermentation

When	*What*
7000 BC	Cheese- and breadmaking practiced
6000 BC	Winemaking
3500 BC	Breadmaking in Egypt
2000–3000 BC	Beer fermentation in Egypt and Near East
1900 BC	The Hymn to Ninkasi by Sumerians describes the production of beer
1500 BC	The sacred book of Hindus (Rig Veda) describes Soma, a fermented beverage for immortality, although in Indian subcontinent, fermentation has been used since 6000 BC
1000–2000 BC	Preparation of fermented meat by Babylonians
300 BC	Preparation of fermented vegetables in China
54 BC	Julius Caesar reports cheddar cheese being produced in Britain
48 BC	Sausage making introduced into Rome by Caesar
277	Emperor Probus introduces wine in Alsatia
617	Kumyss, an ethanol-containing fermented mare's milk product, is introduced in Asia
1070	Roquefort cheese discovered
1383	A brewery, later named as Löwenbräu in 1552, opens in Bavaria
1637	The Gekkeikan Sake Co. begins producing sake in Kyoto, Japan; today, it is the world's largest producer of sake
1697	Introduction of white grape in Southern California
1698	Dom Pierre Pérignon (a French monk) develops a method to retain carbon dioxide in wine (production of Champagne)
1857	Pasteur realizes that fermentation is a microbial process
1864	Production of Heineken beer in Amsterdam by using a customized yeast
1873	Lister isolates *Lactococcus lactis* from milk
1881	Publication of some papers on the preparation of koji and the production of sake
1907	Publication of book *Prolongation of Life* by Metchnikoff describing therapeutic benefits of fermented milks
1900–30	Use of defined cultures for fermentation
1950	Identification of bacteriophages in New Zealand
1996	*Saccharomyces cerevisiae* genome sequenced
2001	*Lactococcus lactis* genome sequenced

Not surprisingly, the ancient roots of fermented foods have led to the incorporation of the concept in cultural and religious frameworks. For instance, fermented foods are mentioned in many parts of the Holy Bible, thus suggesting that they must be already known by those civilizations that lived during that time. In the Bible, we found that Noah planted a vineyard immediately after the floodwaters had receded; later, he was described as drunk and naked (Genesis 9, 20). Later in Genesis (18, 8), three angels visited Abraham who offered them some refreshments, including curds. The most important mention of

fermented foods in the Bible was in the Passover story: Hebrew slaves were thrust out of Egypt and could not tarry; therefore, dough could not rise. Fermented foods also played a major role in the New Testament. The first miracle of Jesus was at a wedding in Cana (John 2, 1–11), where he turned water into wine. Another important miracle was the feeding of 5000 men with five loaves of bread and two fishes (John 6, 5–15). Finally, the sacrament of Holy Communion is represented by bread and wine.

Effects of Fermentation on Food Properties

Biosafety and Preservation

As mentioned earlier, food fermentation originally emerged as a way to preserve perishable foods when few other preserving methods existed (e.g., salting). However, even today, preservation is still an important feature of fermented foods. Often, this effect is related to the microbial end products of fermentation (lactic acid, acetic acid, ethanol, etc.), the resulting decrease of pH up to the threshold value of 4.5 in the case of acidic fermentations, the production of specific antimicrobial compounds (such as bacteriocins and antibiotics), and competition for nutrients. Fermentation is thus responsible for the inhibition not only of spoiling microorganisms, such as *Pseudomonas* spp. but also of foodborne pathogens. The latter include *Staphylococcus aureus*, *Clostridium botulinum*, *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Bacillus cereus*, *Mycobacterium tuberculosis*, *M. bovis*, *Brucella abortus*, and *Br. melitensis*.

Safety of fermented foods is also linked to the ability of fermenting microorganisms to remove or decrease the content of harmful compounds from the raw materials. Examples include the hydrolysis of limarin (a cyanogenic glucoside found in the leaves and roots of plants such as cassava) by *Lactobacillus* spp. and the degradation of mycotoxins (e.g., aflatoxins and ochratoxin A) by lactic acid bacteria and *Saccharomyces cerevisiae*.

Flavor Generation

Aroma compounds produced throughout fermentation comprise different molecules, synthesized through the degradation of proteins, lipids, and carbohydrates by the concerted actions of microorganisms and the endogenous enzymes of the raw materials. The most important groups include acids, alcohols, aldehydes, esters, lactones, amines, pyrazines, and sulfur compounds.

Acids

This group comprises fatty acids from the hydrolysis of lipids, short-chain fatty acids (SCFA) produced from the degradation of amino acids or carbohydrates, and organic acids (lactic, acetic, and succinic acid), which are the major or minor products of some pathways of carbohydrates.

Long-chain fatty acids ($C > 12$) do not contribute to aroma because they have a high threshold in the perception value, while aroma is strongly affected by SCFA and organic acids. Besides their effects on aroma, acids also contribute to taste and overall flavor complexity.

Alcohols

The contribution of primary and secondary alcohols is very important to aroma development; they can be produced throughout different pathways: (i) carbohydrate metabolism (ethanol is the end product of the primary metabolism in many yeasts and a minor end product in some heterofermentative lactobacilli), (ii) amino acid metabolism (amino acids undergo an oxidative deamination and produce α-keto acids, which are the intermediate compounds to produce aldehydes and alcohols), (iii) reduction of methyl ketones from fatty acid metabolism, and (iv) degradation of long-chain fatty acids (linoleic and linolenic acids) by lipoxygenase and hydroperoxide lyase enzymes.

Aldehydes

Aldehydes are usually produced through two different routes: (i) amino acid deamination or transamination, giving rise to α-keto acids, which are then decarboxylated into aldehydes; and (ii) Strecker degradation of amino acids. Aldehydes are generally intermediate products, as they are converted into alcohols or acids; however, their contribution to aroma is significant.

The most important aldehydes recovered from fermented foods are ethanal, 2-methyl-propanal, 2-methyl-butanal, and benzaldehyde.

Esters

Esters are produced through the esterification between SCFA and alcohols (generally ethanol). They play a significant role in fermented beverages, as they have a very low perception threshold in water (some ppb). The most important notes are melon, pear, banana, apricot, pineapple, floral, rose, honey, and wine.

Lactones

Lactones are cyclic esters produced by the condensation of an alcohol and acid function of the same molecule. They play a significant role in the aroma development of some fermented fruit, dairy, and meat products. They are generally described as fruity notes (peach, pear, apricot, and coconut) or yielding a milky and creamy flavor; the most important compounds are δ- and γ-octalactone, δ- and γ-decalactone, and δ- and γ-dodecalactone.

Amines and nitrogen compounds

Amines come from the decarboxylation of amino acids; these pathways could be a great threat as they can produce biogenic amines, whose toxic effects are well known. An important pathway is the synthesis of L-glutamine from L-glutamic amino acid, reported in yogurt and *miso*.

Pyrazines

Pyrazines are heterocyclic compounds containing nitrogen; they are responsible for different flavors (nutty, roasty, and toasty), depending on the alkyl group. The two most important pyrazines in fermented foods are 2,5-dimethylpyrazine (abundant in Camembert cheese) and tetramethylpyrazine (abundant in Chinese black vinegar); threonine is the precursor of 2,5-dimethylpyrazine, whereas tetramethylpyrazine is produced from acetoin and ammonium.

Sulfur compounds

Sulfur compounds are end or intermediate products of the routes of degradation and synthesis of some amino acids, mainly methionine. The flavor of these compounds has been described as similar to garlic, cabbage, cooked cabbage, cauliflower, and cooked cauliflower; moreover, they have a very low perception threshold.

Color

Optical perception is one of the main sensory traits of importance for food quality, as many consumers decide to buy a product for its appearance. It is related to three different categories: color, cesia, and spatial properties. Color is linked to the optical power spectral properties of the stimulus detected by observers, while cesia includes transparency, translucence, gloss, luster, haze, lightness, opacity, and matt and depends on the properties of reflecting, transmitting, or diffusing light. Finally, spatial properties rely upon the angle of observation and the optical properties of surface.

The color of a fermented food is strongly affected by its composition, storage, and physical parameters (e.g., pH and Aw). Microorganisms can also play an important role. The latter is, for instance, clear in the case of dry-cured fermented sausages; their bright red color is due to nitrosylmyoglobin ($MbFe^{II}NO$) formation, in which an axial ligand nitric oxide (NO) is coordinated to the central Fe^{II} in the myoglobin heme group. In such products, color development depends on the reduction of nitrate into nitrite and subsequently into NO. Meat-associated coagulase-negative staphylococci are able to reduce nitrate contained in the curing salt into nitrite, using a membrane-bound nitrate reductase. This nitrite is then further reduced to yield NO, which takes place under acidic conditions or can be performed by some coagulase-negative staphylococci. Thus, nitrate reduction is an important trait for the selection of strains of *Staphylococcus xylosus* and *Staphylococcus carnosus* intended as starter cultures for dry-cured fermented sausage production.

Aside from fermented meats, starter cultures or indigenous microbiota play a significant role in the color development of different types of fermented foods. For example, microbial metabolism is important for the color of mozzarella cheese, surface-ripened cheeses, and blue-veined cheeses. Some kinds of mozzarella cheese are used for baking (pizza); thus, the presence of residual galactose (a monocarbonyl) can modify the color of melted cheese due to its participation to Maillard reactions. The selection of galactose-positive lactobacilli could thus minimize mozzarella browning. As another example, *Brevibacterium linens* is associated with smear surface-ripened cheeses; it produces yellow-to deep-orange colonies, due to the synthesis of carotenoids. Some other important microorganisms for cheese color are the following: *Brevibacterium aurantiacum*, *Corynebacterium casei*, *Microbacterium gubbeense*, *Staphylococcus saprophyticus*, *Debaryomyces hansenii*, *Candida* spp., *Penicillium roqueforti*, and *P. camemberti*.

Rheology

Fermentation can significantly affect the rheological properties of foods through some main routes, including the production of exopolysaccharides (EPS) and the gelation of casein and other proteins. EPS are extracellular polysaccharides of microbial origin; they might be both bound to the surface of microbial cells and secreted in the extracellular environment in the form of suspension or mucilage. They exhibit thickening, thixotropic, and pseudoplastic properties in yogurt and in some other fermented (dairy) products (e.g., desserts, and dressings). Another important effect of fermentation on rheology relies upon the gelation of casein and whey proteins; this change is associated with the isoelectric point, but the changes in the electric charges may affect the overall structure of proteins and consequently their functionality.

Fermented Foods and Health

Food fermentation is often associated with the production of some biologically active compounds with a potential impact on health, usually referred as nutraceuticals. They can be synthesized through different pathways, for example, oxidation, reduction, hydrolysis, condensation, and isomerization. Table 4 reports some examples of biologically active compounds produced throughout fermentation.

Table 4 Nonexhaustive list of biologically active compounds and their producer microorganisms in fermented foods

Compound	*Producer microorganisms*
EPS: homopolysaccharides (α-D-glucans, β-D-glucans, fructans, polygalactan) and heteropolysaccharides	*Leuconostoc mesenteroides* subsp. *mesenteroides*, *Ln. mesenteroides* subsp. *dextranicum*, *Streptococcus mutans*, *Str. sobrinus*, *Str. thermophilus*, *Pediococcus* spp., *Lactobacillus kefiranofaciens*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. casei*, *Lb. acidophilus*, *Lb. helveticus*, *Bifidobacterium* spp.
Angiotensin-1-converting enzyme (ACE)-inhibiting peptides	*Lactobacillus. helveticus*, *Lb. rhamnosus* GG, *Lb. delbrueckii* subsp. *bulgaricus*, *Lactococcus lactis* subsp. *diacetylactis*, *Str. thermophilus*, *Saccharomyces cerevisiae*
Bacteriocins (e.g., reuterin, nisin, sakacin, and plantaricin)	*Lactobacillus reuteri*, *Lb. acidophilus*, *Lb. brevis*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. helveticus*, *Pediococcus* spp., *Lb. sakei*, *Lb. plantarum*, *Lactococcus lactis* subsp. *lactis*
Peptides with cholesterol-lowering activity	*Bifidobacterium* spp.
Conjugated linoleic acid (CLA)	*Lb. acidophilus*, *Bifidobacterium* spp.
Vitamins (group B)	*Lactobacillus* spp., *Bacillus* spp., *Saccharomyces cerevisiae*
Phenolic antioxidants	*Acetobacter* spp., species of *Saccharomyces*, *Schizosaccharomyces*, *Kloeckera*, *Zygosaccharomyces*, *Brettanomyces*, *Pichia*
Antibiotics	*Streptomyces* spp.

An interesting topic is the effect of fermentation on health. Some authors found that health disorders were characterized by chronic and low-grade oxidative stress; moreover, they suggested the existence of an 'intestinal inflammatory microbiome,' significantly contributing to altered mood via intestinal permeability, systemic LPS burden, and even direct-to-brain microbe communication. This kind of inflammatory microbiome is enhanced by some Western habits, for example, high-fat and high-sugar or low-nutrient-value foods. 70–80 years ago preliminary studies in rodents and humans pinpointed the consequences of inflammatory microbiome on health; nowadays, it is possible to find some answers toward the potential of fermented foods. It is well known that fermentation can magnify protein quality and the bioavailability of vitamin B, zinc, and magnesium; these compounds exert a significant effect on mental health as they act on mood regulation. Moreover, there is a strong evidence on the fact that some lactic acid bacteria, mainly some strains of *Lb. plantarum*, provide a strong protection toward oxidation. Fermentation of herbal extracts by *Lb. plantarum* was shown to preserve phenols and vitamin C, thus enhancing the free scavenging effect of fermented foods; a similar phenomenon was also found in fermented soy milk by lactobacilli and/or bifidobacteria.

A frontier goal is the production of bioactive compounds; some of these compounds act on immune system, influence glycemic index, possess anti-inflammatory activities, and to some extent can show beneficial mental activities. One of the most important compound is GABA (gamma-aminobutyric acid); the use of synthetic GABA has a limited benefit due to adsorption issues, while the consumption of fermented foods containing GABA produced in situ by *Lb. hilgardii* contributes to the reduction of blood pressure and of anxiety. In addition, GABA-rich red yeast rice has been reported as a strong antidepressant.

Fermentation could act on health toward its effect on flavonoids; some authors suggested the ability of some starter cultures to produce a biotransformation of phenols and this phenomenon is the leading cause of a beneficial shift in growth stimulation of 'good bacteria,' mainly bifidobacteria.

A particular promising aspect is provided by the possible influence of fermented foods on mental health, related to the emerging and promising trend of nutritional psychiatry. The effects of fermented foods on mental health could be due to three leading factors: (i) the presence of specific nutrients, (ii) the ingestion of beneficial microorganisms, and (iii) the modulation of the intestinal microbiota. Ingestion of beneficial microorganisms may have the potential to affect and improve the body responses to some stressful conditions via a whole set of direct and indirect actions, including the following:

1. Direct protection of intestinal barrier
2. Influence on local and systemic antioxidant status and reduction in lipid peroxidation
3. Production of neurochemical compounds, such as GABA
4. Indirect influence on neurotransmitter or neuropeptide production
5. Prevention of stress-induced changes in the intestinal microbiota
6. Activation of neural pathways between the gut and brain
7. Reduced production of cytokine
8. Modulation of neurotrophic chemicals
9. Limitation of carbohydrate malabsorption
10. Improvement of nutritional status
11. Inhibition of gastric and intestinal pathogens
12. Limitation of overgrowth of bacterial growth in the small intestine
13. Analgesic properties

Moreover, some placebo-controlled researches suggest that beneficial microbes can influence mood and fatigue, decrease anxiety and perception of stress, and improve mental outlook; these effects could be linked to their ability to increase peripheral tryptophan levels and alter dopamine and serotonin turnover in the frontal cortex and in the limbic system. Besides the mere ingestion of beneficial microorganisms as such, fermented foods can also display the ability to modulate the qualitative composition of the intestinal microbiota. In 1986, one of the first studies focusing on the effect of traditional dietary patterns examined the differences in the fecal microbiota of rural Japanese versus Canadian urbanites. The researchers found higher counts of *Lactobacillus* spp. and *Bifidobacterium* spp. in the Japanese group that maintained a traditional diet rich in fiber, fermented foods, fish, and vegetables. Several studies have confirmed these preliminary data and have reported a high diversity of fecal microbiota in people consuming fermented foods, whereby the improvement of intestinal balance has been linked directly or indirectly to brain functionality.

A final remark on the most important result on the effect of fermentation on mental health: 3 years ago, some authors were able to show a direct correlation between the administration of a strain of *Lb. pentosus* var. *plantarum* (isolated from kimchi, a traditional fermented cabbage) and hippocampal production of brain-derived neurotrophic factor in mice with a protective effect toward scopolamine-induced memory deficit. This result is a challenge for the future, as it shows that the effects of fermented foods on mental health are not hypothesis, but possibilities.

Concluding Remarks

Fermentation is one of the oldest method of food preservation; moreover, the consumption of fermented foods affects both well-being and cultural traits of life, as it is a part of religion, society, and tradition all over the world. Apart from the effect of fermentation on safety and quality of foods, a new trend is the focus on the link between fermentation and mental health; the expected outcomes of this frontier goal could be a great threat and a challenge for future. We are only scratching the surface in our understanding of the relationship between potentially beneficial microbes and brain health; going inside this phenomenon could lead to some interesting findings and possibilities.

In conclusion, fermented foods are a significant part of our life and culture, and we could presume that discovery, optimization, and focus on fermentation were and will be some leading factors of mankind evolution.

See also: Bacteriocins; Beer: History and Types; Bifidobacteria in Foods: Health Effects; Bioactive Peptides in Foods; Biogenic Amines; Cheese: Chemistry and Microbiology; Ethnic Foods; Fermented Foods:

Composition and Health Effects; Fermented Foods: Use of Starter Cultures; Functional Foods; Lactic Acid Bacteria; Preservation of Foods; Probiotics; Traditional Foods; Yeasts.

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Fermented Foods: Use of Starter Cultures

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Background

Fermentation has been used to extend the shelf life of foods for over 6000 years. Fermented foods have played an important role in the diets of almost every society throughout the world and are known to offer a variety of benefits. While initially utilized for food preservation, fermentation has numerous benefits that include the improvement of sensory characteristics, acceptability, nutritional value, and safety of foods while also providing diversification to the diet. The number of fermented food products is nearly limitless.

Fermentation is the anaerobic metabolism of carbohydrates by microorganisms. The production of many frequently consumed foods and beverage such as bread, cheese, yogurt, sauerkraut, beer, and wine involves fermentation. Numerous lesser known fermented foods are used in cultural foods throughout the world. The popularity of fermented foods is increasing due to their health benefits and technological advancements that allow for their production on an industrial scale.

A starter culture is a preparation of microbiological cultures that performs or initiates fermentation. Starters usually consist of a cultivation medium, such as grains, seeds, or nutrient liquids that have been well colonized by the microorganisms used for the fermentation. Initially, starter cultures needed to be prepared just prior to use; today, they may be frozen and lyophilized or dried and prepared on an industrial scale.

The choice of starter culture is dependent upon the substrate or raw material being fermented. A starter culture may consist of bacteria, molds, yeasts, or a combination thereof. Lactic acid bacteria are of critical importance in fermentation. Desirable properties of starter cultures include rapid acidification, predictable fermentation processes, desirable sensory characteristics (including taste, texture, aroma, and consistency), and the reduction of harmful microbiota. The first isolation and cultivation of starter cultures occurred in 1890 for cheese and sour milk in Denmark and Germany. Modern technological advancements have led to the development of functional starter cultures. A functional starter culture provides at least one functional characteristic to improve the food safety, nutrition, or quality of the food product.

Purpose of Fermentation

Fermentation serves five primary purposes:

Preservation: Historically, fermentation has predominantly been used as a method of food preservation. Fermentation allows the preservation of substantial amounts of food through lactic acid, alcohol, acetic acid, and alkaline fermentations.

Diet enrichment: Fermentation enriches the diet through the development of a diversity of flavors and textures in food substrates. Examples of this include carbon dioxide production by fermentation in leaven bread and the formation of eyes in cheese or foam in beer and buttermilk.

Biological enrichment: Fermentation enables biological enrichment of food substrates with protein, essential amino acids, and vitamins.

Detoxification: Fermentation can reduce toxic or undesired food components in foods, such as cyanide, phytic acid, and oxalic acid.

Efficiency: Unlike other means of food preservation, fermentation decreases cooking times and cuts back on fuel requirements.

Types of Fermentation

There are three primary types of fermentation most used in traditional and industrial fermentation practices around the world: lactic acid fermentation, alcohol fermentation, and alkaline fermentation.

Lactic Acid Fermentation

Lactic acid fermentation, commonly referred to as lactofermentation, is one of the most common and easiest methods of home preservation. Lactic acid fermentation was a method used to preserve dairy products, vegetables, and meat for extended periods of time before the advent of refrigeration and modern canning practices and today is also utilized in industrial fermentation. Lactic acid bacteria such as *Lactobacillus* spp., lactococci, *Streptococcus thermophilus*, and leuconostocs are examples of lactic acid bacteria that have the ability to convert sugars into lactic acid. Lactic acid inhibits the growth of subsequent and potentially harmful bacteria of other species. It also creates favorable conditions for yeast activity, a property that is utilized in the production of wine and beer.

The chemical reaction of lactic acid fermentation can be written as

$$C_6H_{12}O_6 \rightarrow 2C_3H_6O_3 + 2CO_2 + 2ATP$$
$$\text{Glucose} \rightarrow \text{lactic acid} + \text{carbon dioxide} + \text{energy}$$

Alcohol Fermentation

Alcohol fermentation, also referred to as ethanol fermentation, is the anaerobic process in which yeasts convert simple sugars such as glucose, fructose, and sucrose into cellular energy where ethanol and carbon dioxide are produced as metabolic end products. While yeasts typically function under aerobic conditions, they are also capable of anaerobic respiration, in which sugars are metabolized in the absence of oxygen.

The net amount of alcoholic fermentation by yeast can be written as

$$C_6H_{12}O_6 + \text{zymase} \rightarrow 2C_2H_5OH + 2CO_2$$
$$\text{Glucose} + \text{zymase} \rightarrow \text{ethanol} + \text{carbon dioxide}$$

The first step in alcoholic fermentation involves the process in which the enzyme invertase breaks the glycosidic linkage between glucose and fructose molecules. Each glucose molecule is then broken down into two pyruvate molecules via the chemical process known as glycolysis. Glycolysis causes the reduction of two NAD^+ molecules to NADH. Lastly, two ADP molecules are converted to two adenosine triphosphate (ATP) and two water molecules via substrate-level phosphorylation. The last step uses the enzymes pyruvate decarboxylase and alcoholic dehydrogenase. Zymase is the enzyme complex that catalyzes ethanol fermentation.

Yeast fermentation

The most commonly used microorganism for food production domestically is *Saccharomyces cerevisiae*, also known as 'baker's yeast.' This species was selected due to its ability to produce a large volume of carbon dioxide and a pleasant flavor and to be shelf-stable during storage. When mixed with bread dough, the resulting product is sugar-ferment. Pure cultures of *S. cerevisiae* are isolated and maintained in a laboratory and then inoculated into a medium that often contains molasses and corn steep liquor. (Corn steep liquor is a by-product of wet-milling corn. It is a viscous substrate that contains carbon, nitrogen, and mineral salts.) The inoculated medium is then incubated and aerated, and yeast cells are removed by centrifugation and then washed and mixed with starch or cornmeal. They can be pressed into yeast cakes or freeze-dried to extend their viability.

Yeasts are common epiphytic residents on the surfaces of fruits, vegetables, and cereals. It is the fermentation activity of these yeasts that will lead to their eventual spoilage. Storage and drying decrease the amount of yeast present. Dry flours can also harbor yeasts, though less than whole grains (cereals) that have not had their hull removed.

Alkaline Fermentation

Alkaline fermentation occurs when the protein in a food is broken down into amino acids and peptides. During alkaline fermentation, ammonia is released and pH increases. Alkaline fermented food products are primarily found in Asia and Africa. Examples include natto, Thai thua-nao (and kinema–Thai thua-nao), kinema (made from cooked soybeans), dawadawa (made from African locust beans), ugba (made from African oil beans) kawal (made from fresh legale leaves), and pidan (made from fresh poultry eggs). Spontaneous cultures are most frequently used to make these foods; however, pure cultures can also be used.

Combination Fermentation

Combination fermentation results from the presence of multiple fermentative organisms. Mold-ripened cheeses and salami, cocoa, vinegar, certain fermented milks, and sourdough bread are examples of products that use multiple types of fermentation. Specifically, vinegar is produced using both yeast and acetic acid bacteria. Sorghum beer in Africa is made through lactic acid and alcoholic fermentation. Cocoa is made using lactic acid bacteria, acetic acid bacteria, and yeast.

Traditional Versus Commercial Fermentation

The earliest record of fermentation dates as far back as 6000 BC, long before microorganisms were discovered. Nearly every civilization since has included at least one form of fermented food in its culinary heritage. In some cases, fermentation is a critical component of food safety beyond preservation. Links between food fermentation and human health have been found as early as ancient Rome and China. As cultures and times have changed over the centuries, so have the ways in which we process and preserve foods through fermentation.

Traditional Fermentation and Starter Cultures

Fermentation can occur either through traditional methods or via industrial production. Early methods of fermentation occurred in small batches and involved spontaneous fermentation from microorganisms present on raw goods (i.e., vegetables, fruits, milk, meat, and grains) under the proper conditions. The outcome and taste of the fermented product in traditional fermentation is dependent on the quality and range of microbiota contained in the natural starter culture. For other fermentation processes, inoculation is necessary and a small sample of a previously fermented product is saved and used as inoculate for new raw material. Referred to as backslopping, this method is still used today in the making of some artisanal cheeses, kombucha, sourdough, and sauerkraut.

Commercial Fermentation and Starter Cultures

While methods of traditional fermentation are still being used in various cultures today, the majority of fermented goods are now produced through large-scale industrial techniques. With the discovery of microorganisms, recent insight into their genetics, metabolism, and interaction with raw material has allowed for the ability to improve products and the process of fermentation by using specifically designed and isolated starter cultures. Bread, alcohol, and vinegar were among the first to be 'produced' from starter cultures in the nineteenth century, later leading to the industrialization of the process. Dairy and meat fermentation followed a century later. Today, manufacturers of fermented foods can either use highly concentrated ready-to-use starter cultures or propagate the starter culture in factory production. The decision to use one starter culture method over another depends on various factors including economic value, the number of products produced, the degree of automated fermentation, and the level of microbiology expertise. Variations in commercial starter cultures include direct vat inoculates, frozen or freeze-dried cultures, or starter cultures formulated with stabilizer and carrier genes. Lactic acid bacteria constitute the majority of the volume and value of modern starter cultures. A commercial culture starter with direct inoculation results in the highest level of safety and yield flexibility.

Fermented Foods and Starter Cultures

Due to the variation of raw materials, starter cultures, and fermentation conditions, there are a wide array of fermented food products available. Fermented products are often categorized by the food category of the raw material. See Table 1.

Health Benefits

Fermented foods, as a group, are known to be highly nutritious, and a vast number of health benefits associated with consuming fermented foods are well documented.

Predigestion

As bacteria and yeast feed on the food in their immediate environments, they release enzymes that break down molecules into smaller ones, releasing energy for cellular metabolic use in the form of ATP along the way. Thus, as the larger polysaccharides and protein molecules that hold cells together are degraded, large food particles are ultimately digested into usable pieces. In this way, microorganisms partially digest and physically break down food before it is consumed. Different types of foods are metabolized and broken down in different ways. With grains, gluten is cleaved to nonallergenic fragments. With dairy, predigestion occurs as lactic acid bacteria convert lactose into lactic acid. (For those with a milk allergy, while lactic acid is acceptable to consume, lactic acid starter cultures may contain milk.) With bean ferments such as soy products, enzymes convert proteins into amino acids, which are more digestible and readily assimilated by humans. Lastly, with meats and fish products, bacterial and fungal enzymes help tenderize foods.

Removal of Antinutritional Factors

Fermentation is known to remove a number of harmful and toxic compounds from foods by several means of detoxification. Most notably, fermentation increases the nutritive quality of plant foods by metabolically reducing certain types of antinutrients found in foods such as cyanide, phytic acid, and oxalic acid. For example, the fermentation process that produces kawal, a Sudanese meat substitute, removes harmful toxins from cassava leaves, ensuring them safe for human consumption. Phytates, or phytic acids, are the phosphorus-bound organic acids that protect grains, beans, nuts, and other seeds from premature germination and are well-known antinutrients that can cause mineral deficiencies and digestion issues over time. Common health effects of a phytate-rich diet include tooth decay, lack of appetite, digestive problems, and nutrient deficiencies. For regular consumption of phytate-containing foods in the absence of negative health effects, it is necessary to remove the phytates and other antinutrients through processing techniques such as sprouting, soaking, and fermenting.

Table 1 Nonexhaustive list of types of fermented foods, including the final product, raw material, and type of starter culture

Food category	*Product*	*Raw material*	*Starter culture*
Bean	Tempeh	Soybean	Molds (*Rhizopus oryzae* or *Rhizopus oligosporus*)
	Miso	Soybean	Koji (*Aspergillus oryzae*)
	Natto	Soybean	*Bacillus subtilis* var. natto
	Soy sauce	Soybean	Mold (*Aspergillus*), lactic acid bacteria
Grains/cereals	Bread	Various cereals	Yeast, lactic acid bacteria
	Injera	Teff	Lactic acid bacteria and yeast
	Beer	Various cereals	Yeast (*Saccharomyces*)
	Sorghum beer	Sorghum	Yeast (*Saccharomyces cerevisiae* and *K. marxianus*), lactic acid bacteria
	Sake	Rice	Lactic acid bacteria, yeasts (and occasionally mold)
Vegetables	Kimchi	Cabbage	Lactic acid bacteria
	Sauerkraut	Cabbage	Lactic acid bacteria
	Pickled vegetables	Cucumbers, olives, and more	Lactic acid bacteria
Fruits that underwent alcoholic fermentation	Wine	Grape juice	Yeast, lactic acid bacteria
	Hard cider	Apple juice	Yeast
	Vinegar	Grapes	Acetic acid bacteria (*Acetobacter*)
Meat	Fermented sausage	Sausage	Lactic acid bacteria, catalase-positive cocci (and sometimes molds)
Dairy	Yogurt	Milk	Lactic acid bacteria
	Kefir	Milk	Lactic acid bacteria and yeast
	Cheese	Milk	Lactic acid bacteria (and sometimes propionic acid bacteria, brevibacteria, and molds)
	Pima	Milk	Lactic acid bacteria
Tea	Kombucha	Tea	Lactic acid bacteria and yeast
Honey	Mead (honey wine)	Honey	Yeast
Root	Tanzanian kivunde	Cassava	Lactic acid bacteria

Probiotics and Gastrointestinal Immune Function

Probiotics refer to microorganisms that when ingested in adequate amounts offer health benefits to the host and host physiology. Lactic acid bacteria and bifidobacteria are thought to promote healthy gut bacteria; this is why lacto-fermented foods (fermented dairy foods such as yogurt and dietary supplements) containing these bacteria are considered to have probiotic characteristics. Research suggests that ingestion of probiotic-containing 'health' and functional foods can alter and improve the human gastrointestinal microflora and host immunity. In the absence of mechanistic data, exactly how particular bacterial species contributes to immune system function is not completely understood; however, improvements may include nonspecific defenses against infection, regulation of the balance of T-helper type 1 and T-helper type 2 cells, and increased activity of white blood cells. Some probiotics have been shown to produce antimicrobial compounds, affect immune response or prevent autoimmunity, and/or alleviate lactose intolerance in humans.

Enhancement of Micronutrients and Functional Compounds

During the process of predigestion, vitamins are produced and excreted by the yeast and/or bacteria. In particular, fermentation can increase the amount of several vitamins (e.g., niacin, thiamine, and biotin) in some plant and animal products. In addition to increasing vitamins and their absorption availability in humans, fermentation produces a unique array of micronutrients or functional compounds not found in raw foods. One example of this involves the phytochemical glucosinolate, which is broken down into anticarcinogenic compounds as a result of cabbage fermentation.

While the literature on the health benefits of fermented foods continues to grow, there is still much to be understood. For example, the understanding of how ingestion of live cultures affects, mediates, and regulates physiological processes in the human body is still elementary.

Risks of Fermented Food Consumption

Although the benefits of fermented food consumption have been well documented, biogenic amine content, the possibility of antibiotic resistance in fermented meat products, and the uptake of ethanol and high sodium content are among the risks associated with the overconsumption of fermented foods.

Biogenic Amines

While lactic acid bacteria produced during food fermentation are typically considered to be nontoxic and nonpathogenic to humans, some species of lactic acid bacteria have been found to convert amino acids into potentially harmful amine-containing compounds referred to as biogenic amines. Overconsumption of foods containing a high level of amines is known to have toxicological effects. High levels of biogenic amines are found in a wide array of foods including fermented food products (fish, meat, dairy products, and vegetables) and fermented beverages (wine, beer, and cider). Of the many biogenic amines, histamine and tyramine are thought to be the most important amines occurring in fermented foods. Minimizing or avoiding the presence of amine producing organisms can be achieved through the use of fresh, hygienic raw food materials and, if applicable, addition of microbial controls. Furthermore, precautions should be taken against organisms intended for use in starter cultures confirming non-production of biogenic amines and the ability to withstand production and storage conditions.

Antibiotic Resistance

The emergence and rise in antibiotic-resistant bacterial strains in food products, specifically in meat products, is an ever-increasing public health concern. Starter cultures help drive fermentation in meat products by decreasing fermentation time and improving the overall quality and safety of the products. However, bacteria used as starter cultures, including lactic acid bacteria, are known to pose safety risks by way of transmission of antimicrobial resistance genes. Fermented meat meant for consumption is typically not heat-treated and thus can serve as a direct route for antimicrobial-resistant bacterial transmission from food to human gut microbiota. Studies have found antibiotic resistance in lactic acid bacteria from meats and meat products, with particular mention of strains involved in sausage fermentation such as *L. plantarum*, *L. curvatus*, and *L. sakei*.

Other Risks

As foods ferment, or decompose, certain waste by-products are produced by the bacteria that break down our food. Of the various by-products, alcohol is one by-product that can lead to ethanol uptake due to overconsumption of fermented foods. Fermented foods may also contain excess sodium and not be appropriate for individuals on a low-sodium diet.

Future Possibilities

Biotechnology is advancing the understanding of fermentation and starter cultures rapidly. Pangenomics, or the analysis of the genetic sequences of members of the same species, is now possible with the reduction in cost and time of DNA sequencing. Pangenomics compares complete genome sequences of members within the same species. This opens vast new possibilities to understand and improve industrial starter cultures, which can help with strain screening, strain improvement, safety, and process improvements. For example, thousands of tons of commercial starter cultures are used every year to produce cheese, yogurt, and other dairy products. Further developments and understanding fermentation and starter culture technology will deepen our awareness of this fascinating field.

See also: Fermented Foods: Composition and Health Effects; Fermented Foods: Fermented Meat Products; Fermented Foods: Fermented Milks; Fermented Foods: Fermented Vegetables and Other Products; Fermented Foods: Origins and Applications.

Further Reading

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Fish Oils: Composition and Health Effects

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Introduction

Fats and lipids constitute an important part of the daily diet, and they have a number of essential biological functions. Lipids have an important structural function in cell membranes, they act as energy storage in the cell, they work as signaling molecules, and some of them even act as a kind of hormones. Lipids and fats constitute a broad range of molecules among which triacylglycerols (TAGs) and phospholipids are the most important in our diet. TAGs are composed of a glycerol backbone on which three fatty acids are esterified. Phospholipids can be divided into three classes: glycerophospholipids, ether glycerolipids, and sphingophospholipids. Glycerophospholipids represent the most widespread phospholipid class, and they differ in their polar head groups esterified to the sn-3 position of the glycerol backbone. Fatty acids occupy the two other positions (sn-1 and sn-2) on the glycerol backbone. Fatty acids can be classified into saturated, monounsaturated, and polyunsaturated fatty acids (PUFAs), depending on the number of double bonds in the fatty acid. PUFA can be classified into omega-3 and omega-6 fatty acids, which can be further subdivided into various subclasses. Marine lipids are a major source of the two very long-chain omega-3 fatty acids eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3), while plant oils such as flaxseed oil are a major source of alpha-linolenic acid (ALA; C18:3 n-3), which has a shorter carbon chain and fewer double bonds. The molecular structure of EPA and DHA is shown in Figure 1. EPA and DHA are not naturally found in plant oils. These two fatty acids have a wide range of well-established health benefits including lowering of triglyceride levels in the blood and reducing the risk of arrhythmia. ALA can be converted to EPA and DHA in the human body, but for people on a normal Western diet with a medium to high intake of omega-6 fatty acids, this only happens to a low extent. The conversion of ALA to EPA and DHA is particularly low in men. This article will provide an overview of the major sources of EPA and DHA and will also discuss the health benefits of marine oils in the different stages of life.

Sources and Production

Sources of Fish Oils

Marine oils refer to fish body oils, fish liver oils, crustacean oils, marine mammal oils, and cephalopod oils. Fish body oils are the predominant product in this category representing almost all of the production with small amounts of marine mammal and squid oils. The fish body oils are sourced from pollack, salmon, tuna, hoki, sand eel, mixed white fish, catfish, dogfish, sprat, horse mackerel, sardines/pilchards, and mackerel, among others. The fatty acid compositions of some of the important fish body oils are shown in Table 1. These fish oils are usually extracted from fatty fish in connection with the production of fish meal. The fish that are processed to produce crude fish oil (and fish meal) can usually be categorized as follows:

(1) Offal and waste from the edible fisheries, for example, cutting from filleting industry. The cuttings or trimmings from the edible fisheries constitute ~50% of the fish biomass intended for food use. These by-products are composed of 15% heads, 14% frames, 4% skin, and 17% viscera (including the livers), and they represent a potential of about 42–44 million tons of waste. Only a portion of that potential resource is currently utilized.
(2) Fish of a quality that is not high enough to make the fish suitable for human consumption.
(3) Fish types that are not considered acceptable or aesthetically pleasing for human consumption. The latter are caught especially for reduction to fish meal and fish oil. This category represents 15–20% of the landings or approximately 16–21 million tons of fish. This raw material, which is landed for direct production of marine oil and fish meal, is 100% utilized.

In the EU, the so-called hygiene legislation requires that fish oils intended for human consumption must be produced from fish that are fit for human consumption, meaning that some of the raw materials that have previously been used for fish oil production for human consumption can no longer be used.

The predominant source of fish liver oils is cod liver. Other sources are hake, halibut, and shark. Fish liver oils constitute <3% of the total production of fish oils.

The other species used for the production of marine oils include krill, squid, and to a lesser extent marine mammals.

The main producers of marine oils are Japan, Scandinavia, Chile, Peru, and Japan (Table 2). The most important countries in the 'Others' category include Russia, Vietnam, and China.

The total annual world production of fish oil during the last 10 years has been ~1–1.25 million tons. The annual production in Chile and Peru and thereby also the total world production are greatly affected by the El Niño events, which can reduce production significantly.

Most of the fish oil is going into salmonid production in Norway, Chile, Canada, and various European countries, but an increasing proportion is being used for dietary supplements and functional foods as will be discussed later. Due to the growth in both aquaculture and direct human consumption of fish oil, it is expected that the total demand for fish oil may soon exceed the production. However, as already indicated earlier, a large proportion of the cuttings and trimmings from the fish industry are currently being wasted and could be utilized for fish oil production. Moreover, ~25–30 million tons of fish is discarded annually and it represents another potential source of fish oils.

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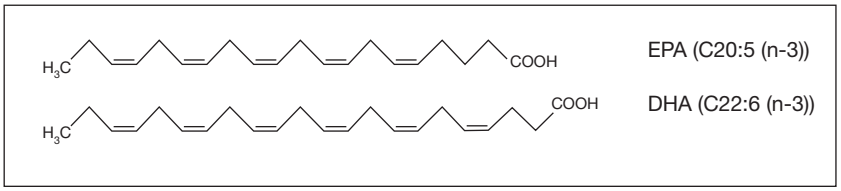

Figure 1 Molecular structure of EPA and DHA.

Table 1 Sources of fish oil and their fatty acid compositions

Fish species	*Capelin*	*Herring*	*Norway pout*	*Mackerel*	*Sand eel*	*Menhaden*	*Sardine/ pilchard*	*Horse mackerel*	*Anchovy*	*Sprat*
Main sources	Barents Sea N. Atlantic	N. Atlantic N. Sea, Norwegian Sea, Pacific Ocean	N. Sea, N. Atlantic, Barents Sea	N. Atlantic Pacific Ocean, N. Sea	N. Sea	US East Coast, Gulf of Mexico	Off S. Africa, Chile, Peru, Japan, Atlantic coasts of Canada and the United States	S. Africa Pacific coast of South America	Off S. and W. Africa, Chile, Peru, and Mexico (Pacific coast)	N. Sea
Fatty acids										
14:0	7	7	6	8	7	9	8	8	9	–
16:0	10	16	13	14	15	20	18	18	19	16
16:1	10	6	5	7	8	12	10	8	9	7
18:1	14	13	14	13	9	11	13	11	13	16
20:1	17	13	11	12	15	1	4	5	5	10
22:1	14	20	12	15	16	0.2	3	8	2	14
20:5	8	5	8	7	9	14	18	13	17	6
22:6	6	6	13	8	9	8	9	10	9	9
Total (principals)	86	86	82	84	88	75	83	81	83	78

Source: Allen, D. A. (1995). Fish oil compositions. In: Hamilton, R. J. and Rice, R. D. (eds.) *Fish oil. Technology, nutrition and marketing*. Wycombe: PJ Barnes & Associates, pp. 95–108.

Other Sources of EPA and DHA

Anchovy and sardine oils are the major sources of EPA and DHA products. These two species are currently responsible for 80% of the omega-3 products on the market. Fish liver oils, salmon, tuna, algae, or single-cell oils, krill, and yeast account for the remaining 20%. However, the production of EPA and DHA from alternative sources such as microorganisms and plants is expected to grow and is therefore further discussed in the succeeding text.

Several microorganisms are capable of producing EPA and DHA including lower fungi, bacteria, and marine microalgae. Marine microalgae are able to accumulate high amounts of omega-3 PUFAs and they are therefore regarded as a very promising source. Algal oil contains higher levels of DHA, for example, up to 52% compared to fish oil.

Microalgae are cultivated either in photoautotrophic or in heterotrophic production systems. EPA and DHA produced in heterotrophic production systems have been available on the market for several years. Currently, DSM uses *Schizochytrium* sp. and *Crypthecodinium cohnii* for the production of DHA. They have obtained novel food approval from the European Commission for using the *Schizochytrium* sp. oil in products such as dairy products, spreads, dressings, breakfast cereals, and food supplements. The *C. cohnii* DHA oil is used in several infant formula products. The advantage of the heterotrophic production system is that it can take place in closed fermentation systems. In contrast, photoautotrophic algae have to be produced in open systems as they require the presence of light. If the production is carried out in closed photobioreactors, the scale-up of the production is to some extent limited by the ability to effectively introduce light. However, recent development in photobioreactor technologies has reduced this problem, and commercial EPA and DHA products produced in photobioreactors or a combination of photobioreactors and raceway ponds are available.

Genetically modified organism (GMO) plants that can produce EPA and DHA are an alternative source that is being

Table 2 Global fish oil production in major countries, 10-year average

	1000 metric tons	
EU 25[a]	48.32	4.76%
Scandinavia	207.58	20.44%
Peru	274.25	27.00%
Chile	161.74	15.93%
The United States	76.68	7.55%
Japan	112.49	11.08%
Morocco	30.31	2.98%
Canada	5.15	0.51%
Mexico	18.65	1.84%
Panama	8.81	0.87%
Ecuador	5.78	0.57%
S. Africa	5.55	0.55%
Others	60.27	5.93%
Total	1015.58	

[a]Denmark and Sweden included in Scandinavia and not EU27.
Source: Bimbo, A. (2013). Sources of omega-3 PUFA. In: Jacobsen, C., Horn, A. F., Sørensen, A.-D. M., Nielsen, N. S. (eds.) *Food enrichment with omega-3 fatty acids*. Cambridge, England: Woodhead Publishing Ltd. pp. 27–107.

intensively investigated these years. A major challenge is to obtain satisfactory yields. Commercial products are expected to occur on the market within the next 5 years. The biotech company Monsanto has developed a GMO soybean plant that can produce stearidonic acid (SDA) (C18:4 n-3). The advantage of this fatty acid is that it has one double bond more than ALA. This means that SDA is more efficiently converted in the human body to EPA than current plant sources (ALA) because it bypasses a step in the conversion process. The SDA oil is the first step towards EPA and DHA from GMO plants.

Patterns of Consumption

Among the different omega-3 fatty acids (EPA, DHA, and ALA), marine oils were the most used form in 2007. It thus constituted ca.86% of the ingredients market. A consumer study conducted in 2008 showed that 67.0% of the existing users of omega-3 products identified marine oils as the most preferred source of omega-3.

The marine oils were mainly consumed as dietary supplements, that is, gelatin capsules and to a lower extent liquid supplements. The total consumption of EPA- and DHA-rich oils was ~100 000 metric tons in 2010 and increased to 123 800 tons in 2013. It has been predicted that total global human consumption will increase to 241 000 tons by 2020.

Among the marine oils, fish oils are consumed as triglyceride oils (80% of total sale), low concentrates (2.3% of total sale), middle concentrates (5.2% of total sale), and high concentrates (2.6% of total sale).

As previously mentioned, commercial algal DHA oils are now on the market. However, in 2007, algal DHA oils only accounted for 1.3% of the volume share in omega-3 fatty acid market. However, with more producers entering the market and thereby increasing availability, the consumption of algal oil is expected to increase. Algal oils are primarily used in infant formulas as this application sector accounts for ~70% of the volume. The remaining 30.0% is consumed as dietary supplements and functional foods.

The most natural way of consuming EPA and DHA is of course via fish consumption, and therefore, a few comments on the contribution of fish consumption to EPA and DHA intake are given in the succeeding text. A single lean fish meal, such as one serving of cod, could provide about 0.2–0.3 g of EPA and DHA. On the other hand, a single oily fish meal, such as one serving of salmon or mackerel, could provide 1.5–3.0 g of these fatty acids. However, in most Western countries, the fish intake is low and the intake of lean fish is more common than of oily fish. For example, the median intake of fish was only 16 g fish per day in Denmark during the period from 2003 to 2008. Among the Danes, ~25% eat <5 g fish per day. Consequently, intakes of EPA and DHA among adults in several Western countries may be as low as 0.1–0.2 g day^{-1}. The International Society for the Study of Fatty Acids and Lipids suggests a minimum intake of 500 mg day^{-1}. In Australia and New Zealand, the recommended intake is 430–610 mg day^{-1}. Recently, the Food and Agriculture Organization (FAO) of the United Nations and the European Food Safety Authority (EFSA) recommended a minimum intake of 250 mg EPA + DHA per day for adult males and for nonpregnant/nonlactating adult females. For pregnant or lactating females, the minimum intake was recommended to be 300 mg EPA + DHA per day of which at least 200 mg should be DHA. They also made recommendations for DHA intake for infants aged up to 2 years. For children, the recommendations for EPA + DHA in mg per day were 100–150 for those aged 2–4 years, 150–200 for those aged 4–6 years, and 200–250 for those aged 6–10 years.

Absorption and Metabolism

Absorption and metabolism of omega-3 fatty acids that have been orally consumed follow the same routes as other dietary fatty acids. In brief, they are hydrolyzed from the TAGs or phospholipids, absorbed into enterocytes, and reesterified back into TAGs that are assembled into chylomicrons (lipoproteins). The chylomicrons are released into the lymphatic circulation and then enter the bloodstream. Omega-3 fatty acids from chylomicron TAGs will primarily be stored in adipose tissue, but a minor part of the omega-3 fatty acids will be cleared by the liver. Here, they may be oxidized, metabolized to other omega-3 fatty acids, or resecreted into the bloodstream.

Several studies have investigated whether EPA and DHA from fish are more available than from fish oil supplements in the form of TAGs or ethyl esters. In a recent study, the bioavailability of EPA + DHA (3.1–3.6 g day^{-1}) as reesterified triacylglycerides, ethyl esters, or free fatty acids was compared with that of natural fish oils (triacylglycerides) in a human study. Omega-3 fatty acids were taken as capsules. EPA and DHA contents were lowest in capsules with natural fish oils, but this was adjusted for by the number of capsules taken per day. The bioavailability was highest (124%) in the reesterified triacylglycerides compared with natural fish oil, whereas the bioavailability from ethyl esters was inferior (73%). Free fatty acid bioavailability (91%) did not differ significantly from

natural fish oils. The availability of EPA and DHA from TAG has also been compared with that from phospholipids. Although several studies seem to suggest that EPA and DHA may be more bioavailable when ingested from phospholipids, more studies are needed to finally confirm this conclusion.

In the human body, different cells and tissues have different fatty acid compositions. The EPA and particularly the DHA contents differ significantly among these pools. EPA content is usually lower than the DHA content. Tissues such as parts of the brain and eye have a particularly high level of DHA. This is due to the fact that DHA plays an important role in maintaining a normal function of these tissues. Importantly, supplementation with omega-3 PUFA results in the increased amounts of EPA and DHA in plasma lipids, platelets, erythrocytes, leukocytes, colon tissue, cardiac tissue, etc. Several studies have reported an almost linear relationship between EPA and DHA intake and the resulting EPA and DHA contents of plasma phospholipids and of platelet phospholipids.

Health Effects

Mechanisms of Action

This section will discuss the health beneficial effects of EPA and DHA in the different stages of human life. As an introduction, the different mechanisms through which these fatty acids may exert their effect will be reviewed. Figure 2 provides an overview of the most well-understood mechanisms, but more studies are needed to fully understand the mechanisms behind all the known health beneficial effects of EPA and DHA.

Several studies suggest that the beneficial role of EPA on inflammatory response and platelet aggregation is related to eicosanoids produced in the body from EPA. Eicosanoids are potent chemical messengers that have well-established roles in the regulation of inflammation, immunity, platelet aggregation, smooth muscle contraction, and renal function. EPA is a precursor of the 3-series prostanoids TXA_3 and PGI_3, whereas the omega-6 fatty acid arachidonic acid is a precursor of TXA_2 and PGI_2. TXA_2 and TXA_3 are both prothrombotic, but TXA_3 is less prothrombotic than TXA_2. In contrast, PGI_2 and PGI_3 are equally antithrombotic. Hence, an improper balance between omega-3 eicosanoids and omega-6 eicosanoids can lead to an imbalance in inflammatory response and thrombosis.

EPA and DHA are also precursors for another group of metabolites, namely, resolvins. These lipid mediators have been demonstrated in cell culture and animal feeding studies to have potent anti-inflammatory, inflammation resolving, and immunomodulating effects. A higher intake of EPA and DHA will lead to increased synthesis of resolvins.

The EPA and DHA content of membrane phospholipids influences physical structure and fluidity of the membrane and the structure and composition of important regions of the membranes, which are called rafts. These rafts play a role for receptor action and for the initiation of intracellular signaling pathways. Together, the fluidity of the membrane and raft structure influences the activity of membrane proteins including receptors, transporters, ion channels, and signaling enzymes. In turn, intracellular signal transduction and transcription factor activation may be altered and gene expression modified. This may affect the regulation of inflammatory

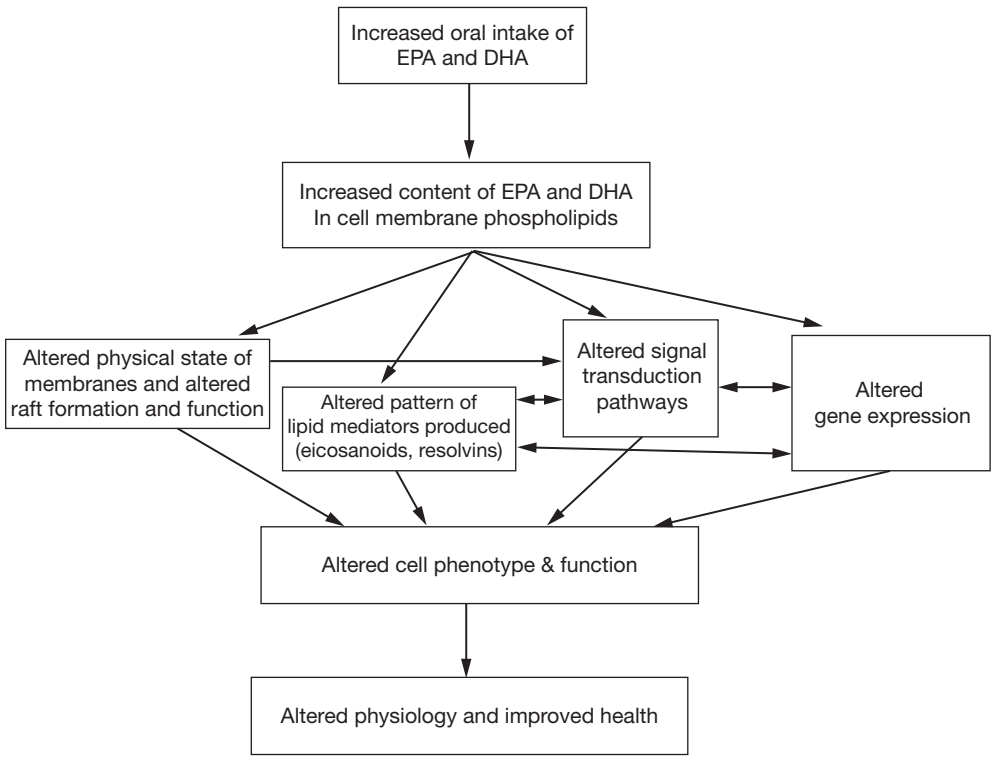

Figure 2 Overview of underlying mechanisms behind health beneficial effects of EPA and DHA. Reproduced from Calder, P. C. (2012). Mechanisms of action of (n-3) fatty acids. *The Journal of Nutrition* **142**, 592S–599S.

processes, the regulation of lipid metabolism, and insulin sensitivity.

The anti-inflammatory effects of EPA and DHA may also be related to their binding to the membrane G protein-coupled receptor called GPR120, which is a lipid-sensing receptor highly expressed in proinflammatory macrophages. When macrophages are stimulated to activate their proinflammatory pathways, this can be inhibited by pretreatment with EPA and DHA via GPR120. A similar receptor dependency for the insulin-sensitizing, anti-inflammatory effects of EPA and DHA was observed in mice deficient in the *GPR120* gene. This suggests that GPR120 is the functional receptor/sensor for these effects of omega-3 PUFA. The same signaling system functions in the hypothalamus. This results in decreased food intake, weight reduction, and improved insulin sensitivity.

Effects of EPA and DHA during Pregnancy and Lactation

PUFAs are preferentially transferred to the fetus at the expense of the mother's own physiological stores. This preferential transfer is termed biomagnification. Maternal arachidonic acid is preferentially consumed by the fetus primarily in early pregnancy followed by DHA in the third trimester and postpartum during lactation. DHA is important for the development of the nervous system including the brain of the fetus. The primary rationale for supplying sufficient intakes of omega-3 PUFA for mothers during pregnancy is therefore to support the optimal health and brain development of the baby during the neonatal period as well as providing for the health and physiological needs of the mother. Maternal omega-3 PUFA intake under the present dietary conditions seems to be inadequate to keep up with the increased demand for omega-3 PUFA during pregnancy. Therefore, it has been suggested that pregnant women should increase their intake of DHA and that infant formulas for both preterm and term infants should contain DHA. Infant formulas with DHA are now available in several countries.

As previously mentioned, high levels of DHA are needed in the brain and retina (eye) for optimal neuronal functioning (learning ability and memory) and visual acuity. Therefore, high levels of DHA in breast milk are necessary in order to ensure proper development of these functions in the infant. The amount of DHA in breast milk is dependent on the intake of the mother during lactation. Hence, consumption of DHA provides a fairly rapid and marked improvement in the DHA level found in breast milk.

Effects of EPA and DHA during Infancy

The body of literature suggests that long-chain PUFAs are important to the growth and development of infants. Fleith and Clandinin concluded in a review that a target DHA level of 0.4% of total fat for preterm infants is desirable and that slightly higher levels than necessary for term infants may be needed. Several studies have demonstrated that addition of omega-3 PUFA to infant formula results in better performances in various tests for visual acuity, mental development, and motor activity including body control, large muscle coordination, manipulatory skills of the hands and fingers, and ability to recognize objects by touch.

Effects of EPA and DHA during Childhood

Unfortunately, there are limited reports on the relationship between fatty acid consumption and health outcomes in children >2 years. One study found that toddlers consuming formula with 130 mg DHA per day for 60 days had fewer adverse events (e.g., strep throat, cough, and upper respiratory infection) and a lower incidence of respiratory illness compared with toddlers consuming the formula without DHA. Other studies of the impact of maternal fish or omega-3 consumption on childhood outcomes have not been able to demonstrate any effects. The relationship between cognitive development of 4-year-old children and their DHA levels has recently been studied, and no association between umbilical venous plasma or red blood cell phospholipid DHA and arachidonic acid levels and cognitive outcomes was observed. There are methodological limitations of the existing randomized controlled trials and epidemiological studies. These limitations include the challenges in quantifying fatty acid exposure and measuring neurodevelopmental outcomes. Therefore, more studies are required to understand the importance of omega-3 intake during childhood.

Effects of EPA and DHA during Adulthood

In adults, low intake of omega-3 PUFAs has been associated with many disease states, including cardiovascular, inflammatory/autoimmune, neurodevelopmental, and psychiatric disorders.

Several studies have demonstrated a beneficial effect of omega-3 PUFA intake on fatal and nonfatal myocardial infarction. An overall decrease of risk of suffering a cardiovascular event of any kind of 10%, a decrease of risk of cardiac death of 9%, and a decrease of coronary events (fatal or nonfatal) of 18% upon intake of omega-3 PUFA have been reported, and it was concluded that marine omega-3 PUFAs are effective in preventing cardiovascular events, cardiac death, and coronary events, particularly in persons with high cardiovascular risk. Moreover, marine omega-3 PUFA may positively affect coronary disease risk factors by decreasing plasma TAG levels, improving platelet function, and lowering blood viscosity.

Omega-3 PUFA has also been found to reduce incidence of joint tenderness and morning stiffness in patients suffering from rheumatoid arthritis. Intake of omega-3 PUFA also appears to offer some protection against asthma, cystic fibrosis, and Crohn's disease. These diseases are all related to a malfunctioning immune system.

The ability of omega-3 PUFA to prevent and treat cancer has been much discussed. A recent review concluded that omega-3 fatty acids are likely to have multifaceted roles in both prevention and treatment of colorectal cancer and other cancers. A review by Gerber was less conclusive. Thus, it concluded that there is only limited evidence from observational studies on colorectal, prostate, and breast cancers on a possible role of omega-3 fatty acids in cancer prevention. Nonetheless, the author stated that there is an increasing biological plausibility of the beneficial anti-inflammatory effect of omega-3 fatty acids on cancers.

Several studies have investigated the role of DHA in Alzheimer's, schizophrenia, and mood disorders. A review by

Hegarty and Parker found that studies investigating the efficacy of omega-3 fatty acid supplementation for mood disorders have provided inconsistent results. They concluded that more research is required before omega-3 supplementation can be firmly recommended as an effective treatment for mood disorders. It has been reported that both arachidonic acid and DHA levels were low in the brain of patients with schizophrenia but that there is only limited evidence that supplements may reduce symptoms. Despite the limited level of evidence for the potential preventative effects of omega-3 fatty acids in mental diseases, the role of DHA in brain function is recognized and a cause and effect relationship has been established as concluded by the EFSA. This also means that health claims on the role of DHA in brain function are allowed. Foods bearing such a claim should contain 250 mg DHA in one or more servings. EFSA has recognized a similar role of DHA in maintaining normal vision, and also, in this case, foods must contain 250 mg DHA in one or more servings to bear the claim (EFSA).

Negative effects of omega-3 PUFA have also been reported. In a recent case–cohort study on 834 men diagnosed with prostate cancer, of which 156 had high-grade cancer, it was found that when compared with men in the lowest quartiles of blood plasma EPA, DPA, and DHA, men in the highest quartile had increased risk of low-grade and high-grade prostate cancer. The reported increased risk of low-grade prostate cancer was 44%, and the reported increased risk for high-grade prostate cancer was 71% for the highest quartile blood plasma omega-3 PUFA group. However, this study has been criticized for not being specifically designed to look at the exact relationship between omega-3 fatty acid intake and prostate cancer as it was an observational study and not a cause/effect study. Another criticism was that plasma phospholipid fatty acids, as measured in this study, are not a good index for long-term intake and can be influenced dramatically by a single meal or even the timing of a fish oil supplement. Moreover, there was no standardization of the omega-3 PUFA quality or quantity consumed by the study subjects. Some were dietary and some were supplementation. Therefore, more research is needed to further study if high intakes of EPA and DHA can promote prostate cancer.

Effects of EPA and DHA during Aging

Studies investigating the role of omega-3 PUFA in healthy older adults are limited. However, there are studies showing that elderly people who consume higher levels of fish as a source of DHA/EPA exhibited a slower rate of decline in their cognitive scoring with age. Other studies have shown that aging individuals who utilized fish oil supplementation appeared to show improved retention of their cognitive functioning in old age. Studies in elderly individuals (65–98 years of age), who were free of known cerebrovascular disease at baseline, exhibited a significantly lower risk of developing a stroke during 12 years of follow-up with increased fish consumption as a source of DHA/EPA. The lowest risk (by 30%) was found in those aging individuals who consumed fish at least five times per week. Moreover, omega-3 PUFAs have been demonstrated to inhibit hepatic triglyceride synthesis, decrease inflammation, and inhibit platelet aggregation in the elderly. Another study suggested that elderly women may be more sensitive to the immunologic properties of omega-3 fatty acids. They found that fish oil supplementation (2.4 g day^{-1} for 3 months) reduced interleukin (IL)-6 in young women (aged 23–33 years) by 30% and in older women (aged 51–68 years) by 60%. Total IL-1β synthesis was reduced by 48% in young women in comparison to 90% in older women, whereas tumor necrosis factor was reduced by 58% in young and 70% in older women.

Conclusions and Future Trends

Consumer awareness about health beneficial effects of omega-3 PUFA is increasing, and this is expected to lead to increasing consumption and thereby increasing demands for marine oils in the coming years. The production of fish oils from traditional resources has stagnated, and therefore, increasing supply must come from new resources such as by-products and discards from the fish industry as well as from microalgae, bacteria, yeast, and GMO plants.

The health benefits from omega-3 PUFA are many and in several cases well documented. However, more studies are needed to obtain more documentation for possible beneficial effects on the prevention of diseases such as mental disorders and cancer particularly in healthy subjects. Likewise, more research is needed to understand the long-term effects of omega-3 PUFA during pregnancy, infancy, and childhood.

See also: Fatty Acids: Determination and Requirements; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fatty Acids: Metabolism; Fish: Fish in the Human Diet; Fish Oils: Production and Properties; Functional Foods; Infants: Nutritional Requirements; Lipoproteins; Shellfish: Characteristics of Crustaceans and Mollusks.

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Fish Oils: Production and Properties

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Introduction

The total world fisheries and aquaculture production was 158 million tons in 2012, of which 136.2 tons were used for human consumption and 21.7 million tons of the catch and aquaculture production was used for fish oil and fish meal production. The global fish oil production is estimated to be about 1 million tons per year (0.980 tons in 2012 according to FAO). Out of the estimated 1 million tons of fish oil, about 70 000 tons is estimated to be used for human consumption.

Peru and Chile are the largest producers of fish oil (around 295 000 tons in 2012), followed by Norway and Denmark (140 000 tons in 2012) and Iceland (around 176 tons in 2012). Norway is the largest global importer of fish oil (approximately 200 000 tons) followed by Chile, United Kingdom, and Canada.

Marine Sources for Fish Oil Production

Marine sources used for fish oil and fish meal production can be divided into four categories:

(1) Fish caught especially for the production of oil and meal. This includes species such as anchovy, jack mackerel, capelin, menhaden, and blue whiting. By-catches from other fisheries can also be included in this category.
(2) By-products/rest raw material from the processing industry (both fisheries and aquaculture).
(3) Cod liver (from *Gadus morhua* L. or other gadidae species).
(4) Other marine resources such as seal, krill, micro algae, etc.

Raw Material Quality

The quality of the fish oil depends on the sorting, storage, and handling of the raw material. Fish rest raw material is especially vulnerable to spoilage and degradation because it contains fractions like viscera and blood with high amounts of endogenous enzymes. Lipases and phospholipases will lead to formation of free fatty acids (FFAs) and reduction in quality and stability of the lipids. Prolonged storage of the rest raw material before processing has been shown to lead to an increased concentration of FFA, thus lowering the quality of oil produced from herring and pollock rest raw material. Proteolytic enzymes will cause a reduction in the molecular weight of protein, thus reducing the functional properties. Rest raw materials are also susceptible to microbial spoilage and it is important that they are handled as a raw material intended for human consumption. To ensure high quality oil, it is important to process the raw material within a short time.

There are some regulations for the raw material for fish oil if it is going to be used for human consumption or feed. If the whole fish is going to be used for fish oil production for human consumption, the freshness criteria are based on the total volatile basic nitrogen (TVB-N), which shall not exceed 60 mg of nitrogen/100 g.

Storage and transport must be carried out under hygienic conditions by using tanks of stainless and mild steel construction. The catch has to be chilled within 24 h. Fishing of pelagic species used for fish oil and meal production is mainly done by purse seiners and the fish is stored in bulk in refrigerated sea water (RSW) tanks at −1.5 °C on-board. Whole fish being processed within 36 h must have an acceptable freshness criteria based on TVB-N. Rest raw material may be used directly after production at the slaughter lines to produce oil or may be chilled and transported in containers to the production site. Organic acids can be added to the rest raw material to conserve the raw material before further processing (production of silage). However, the use of non-food-grade acids will result in fish oils that can be used in feed and not for human consumption.

Fish Oil Production

Fish oil and fish meal are traditionally produced by the wet rendering method. The first step is mincing of the raw material (whole fish or by-products), followed by cooking. The cooked material is separated into different fractions, resulting in production of crude fish oil and fish meal. A simplified scheme of the oil and meal production is given in Figure 1.

Cooking

Minced whole fish or by-products are transferred to a continuous cooker and steam heated to 90–95 °C for approximately 10–20 min. The heating will cause protein coagulation and rupture of fat depots, resulting in liberation of oil and water. The cooker usually consists of a long, steam-jacket cylinder where the raw material is moved by a heated rotary screw conveyer. The procedure normally takes around 20 min; however, if a scraped surface heat exchanger is used, the heating time can be reduced to less than 2 min.

Pressing

After cooking, the heated material is transferred to a screw press where the liquid ('press liquor') is squeezed from the solid phase ('press cake') in order to remove as much liquid as possible. Optimal pressing will lead to a higher oil yield and a lower amount of oil in the press cake; thus, a fish meal with low oil content. The press cake liquid consists of water, dissolved material, and oil, while the press cake consists of

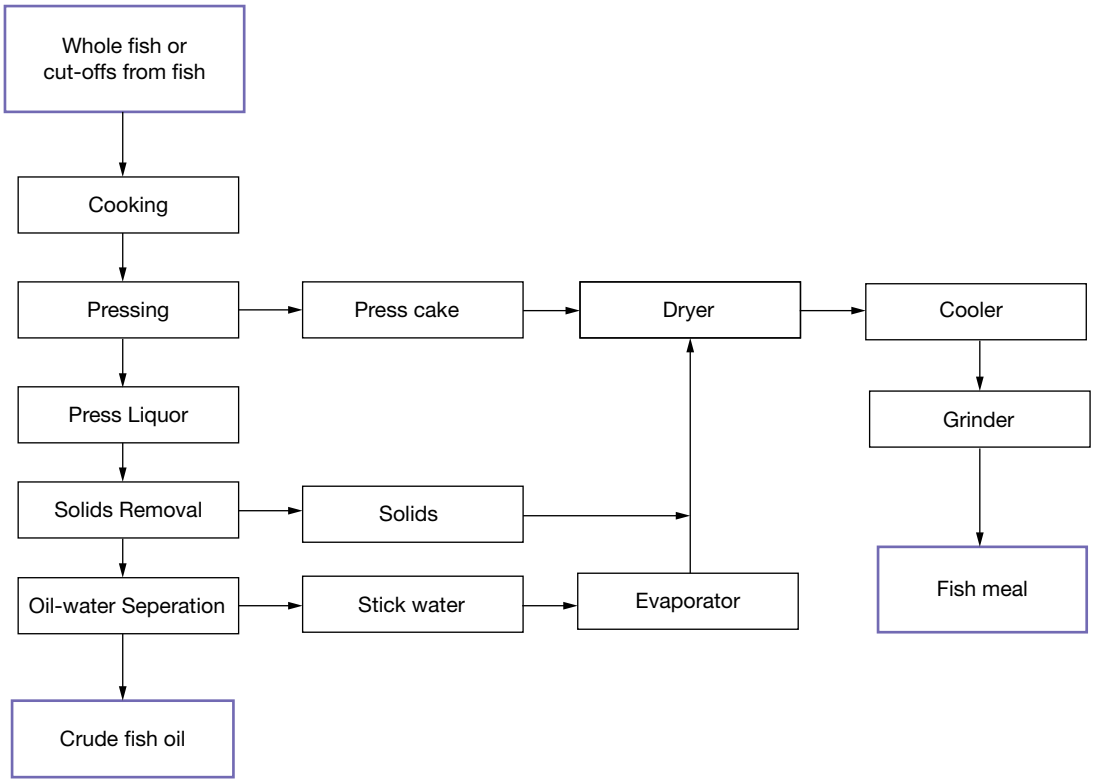

Figure 1 Simplified scheme of oil and meal production.

60–80% of the oil free dry matter. The press cake is further processed into fish meal.

Separation of Press Liquid

Before the oil and water is separated, the press liquid is passed over a vibrating screen (with 5–6 mm perforation) to remove unwanted coarse particles of fish and bones. Different processing solutions based on the fractions' different specific gravity can be used for separation of the press liquid (oil, water, and remaining solids). First, a decanter (horizontal centrifuge) can be used to remove fine suspended solids from the liquor in order to obtain an oil and water phase suitable for further separation. The oil and water phase is transferred to a centrifuge for separation into oil and stick water (water soluble components). The oil is pumped to a polishing centrifuge while the stick water is transferred to the evaporator.

An alternative separation technique is to use a tri-canter which enables the separation of three fractions (sludge, oil, and stick water) at the same time. However, industry experience indicates that it is difficult to produce three high quality fractions and in many cases one of the fractions has to be downgraded in order to produce the other high quality fractions.

Oil Polishing

A polishing centrifuge can be used to remove the final traces of moisture and impurities in the oil. This reduces the amount of pro-oxidants in the oil, thus increasing the oil stability during storage. After polishing, the oil is pumped into storage tanks. The produced oil is called *crude fish oil*.

Drying of Press Cake

Sludge separated from the press liquor is mixed with the press cake together with the concentrated stick water. The press cake is then dried to reduce the moisture content to a level low enough to hinder microbial growth. Two types of driers are used in the fish meal industry: direct and indirect driers. In direct driers, the press cake is in direct contact with the hot gases, while in indirect driers the heat is transferred to the cake through a retaining wall. Indirect driers are mainly used in the fish oil industry.

Enzymatic Protein Hydrolysis

New processes for production of oil based on enzymatic protein hydrolysis are being developed and applied in the industry. Enzymatic protein hydrolysis is based on the use of commercial proteases for proteolytical cleavage of peptide bonds, facilitating the degradation of fish tissue and release of oil. The advantages of using enzymatic hydrolysis compared to the wet rendering method could be a protein product with better biological, digestible, and functional properties. In addition, the use of lower cooking temperature (50–60 °C) compared to the wet rendering method (90–95 °C) can

give an oil of higher quality (low oxidation status) and stability.

For processing of whole fish and by-products by enzymatic hydrolysis, a hydrolysis stage has to be implemented after the material is minced. The reaction can take place in either a tank reactor or a screw-mixing pipe reactor. Heated minced raw material is transferred to a hydrolysis tank and mixed with water (usually in a 1:1 ratio) and commercial proteolytical enzymes (0.1–1 wt% of raw material weight). The reaction proceeds for 30–90 min (hydrolysis time), followed by enzyme inactivation at 90 °C for several minutes.

Several factors can influence the hydrolysis process: substrate type and properties, enzyme type and properties, and processing conditions including temperature, pH, hydrolysis time, and amount of added water. These factors are important for product yield and quality and need to be controlled during processing.

Krill Oil Production

The krill oil industry has developed and optimized the production process for krill, resulting in different processing steps and end products (oil, meal, pasta, etc.). Processing details are often confidential and little is published in the literature. The most common process is to pump live krill onboard the factory trawler were the krill is frozen or directly processed into krill meal. The dried meal can be transported to shore and used to extract krill oil by solvent extraction.

Production of Refined Fish Oil

Crude fish oils contain minor amounts of water and unwanted substances as phospholipids, mono- and diacylglycerols, FFAs, cholestereol, proteinaceous compounds, trace metals, pigments, and primary and secondary oxidation products. The crude oil needs to go through different refining steps before it can be used for human consumption. The general objective of refining of oils is the removal of impurities and unwanted products which cause the crude oil to have an unattractive color or taste or can cause harmful metabolic effects. The refining process can include the following steps:

- Neutralization by addition of citric acid or caustic soda: removal of mainly FFAs, but also oxidation products, phospholipids, pigments, trace metals, and residual soaps
- Washing with hot water: removal of water-soluble material, oxidation products, trace metals, and soaps
- Winterization
- Bleaching by using bleaching earth and activated carbon: removal of pigments, peroxides, phospholipids, soaps, trace metals, sulfur compounds, persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), retinol, tocopherol, and astaxanthin
- Deodorization (steam stripping): removal of volatile compounds, aldehydes, ketones, POPs, FFAs and pigment decomposition products
- Short path distillation (SPD): further reduction of POPs, removal of cholestereol, primary and secondary oxidation products, FFAs, and polymeric materials

Which refining steps are included and in which order can vary between the different fish oils processors.

Neutralization

Neutralization is carried out to remove FFAs (lowering the oils' acid value) and reduce the content of phospholipids, pigments, and trace metals. FFAs can cause darkening of the oil, foaming, and possible smoke during heating. During neutralization, the oil is mixed with caustic soda, resulting in the formation of soaps that can be separated from the crude oil. The oil is heated to the optimum processing temperature of 80–95 °C before caustic soda is added. Hot water is then added to the solution, mixed, and the soapy wash water is removed by using a separator. Washing with water will also remove any water-soluble components present in the oil such as decomposition products of proteins, free amino acids, trimethyl amines and histidine, oxidation products, and phospholipids.

Winterization

Winterization is carried out to reduce the amount of high melting point triacylglycerols and waxes in the crude oil. The process is carried out in tanks with inert conditions and the oil is slowly chilled to 0–2 °C allowing the formation of triacylglycerol crystals with higher melting points to settle and be removed by filtration. The winterization process prevents clouding of the oil at refrigeration temperatures.

Bleaching

The bleaching step removes pigments, but will also further reduce the content of FFAs, residual trace metals, and oxidation products (peroxides) that are left in oil after neutralization. In addition, the process will also remove soaps and sulfur-containing materials. Bleaching by using activated carbon reduces the residual traces of environmental pollutants such as dioxins, furans, and PAHs. However, the process will also reduce the content of desirable natural antioxidants present in the oil as retinol, tocopherol, cholecalciferol, and astaxanthin. The process is carried out by adding bleaching earth and/or activated carbon to the oil through a dosing unit. It is performed at 130–150 °C for 30–60 min under vacuum.

Deodorization

The deodorization step is carried out to remove as much as possible of the unwanted volatile compounds (secondary oxidation products such as aldehydes, ketones, etc.) that are responsible for the unwanted odor and taste of the oil. However, possible nonvolatile secondary and tertiary oxidation products that are formed during processing will not be removed

by deodorization. The oil is steamed under vacuum at a high temperature (150–250 °C) to remove the volatiles. The process is also called stripping, desorption, or steam distillation when oil is mixed with stripping gas to facilitate the mass transfer of the volatiles to the gas phase. The gas phase is continuously removed from the liquid phase. Deodorization should be carried out at a maximum temperature of 180 °C to reduce the risk for polymerization and geometrical isomerization.

Molecular (Short Path) Distillation

An additional step can be added to the refining line to reduce trace amounts of POPs such as dioxins, PCBs, and furans. The use of short path distillation will also lead to the reduction of odor compounds, FFAs, oxidation products, and polymers formed during decompostion/oxidation of the oil. Short path distillation is also used in the production of omega-3-acid ethyl esters.

Fish Oil Properties

Physical Properties

Many of the physical properties of fish oil are dependent on the composition of fatty acids and on the nature of their incorporation into acylglycerols. Density is one of the characteristic that determines quality of the oil and is usually in the range 920–930 kg m^{-3}. Density increases as the mean molecular weight decreases (i.e., with higher saponification values) and the degree of unsaturation increases. Fish oil is a liquid above 10 °C and can solidify below this temperature. The viscosity of fish oils at 20 °C ranges between 60 and 90 cP and decreases exponentially with increasing temperature, the regression equation being $121e^{-0.028T}$ where T is in the range of 20–90 °C.

Fish color can vary depending on the species of fish used and processing conditions.

Chemical Properties

Fish oil is usually made up of triacylglycerols, which often account for more than 95% of the lipids in the oil. The characteristic of a specific triacylglycerol is dependent on the fatty acids composition. However, some fish oils, for example, shark oil, can comprise 40–50% ether linked glycerides. Some fish species can have wax-esters as dominant storage lipids. Wax ester–rich oils generally contain lower levels of omega-3 LC PUFA.

Fatty Acid Composition

The fatty acid composition of fish oils is complex and variable. The composition of fish oils in terms of fatty acids is usually determined by chromatographic methods. The fatty acids tend to contain an even number of carbon atoms and double bonds are of the 'cis' configuration.

The most abundant fatty acids in fish oils are: 14:0 – myristic; 16:0 – palmitic; 16:1 $n-7$ – palmitoleic; 18:1 – oleic acid, 20:1 $n-9$ gondoic, 22:1 $n-11$ – cetoleic, 20:5 $n-3$ – eicosapentaenoic and 22:6 $n-3$ – docosahexaenoic fatty acids. The triacylglycerols consist of fatty acids esterified at three different positions on a glycerol molecule. This distribution of fatty acids in the glycerol molecule is more or less unique for different types of natural fat. An indication of approximate composition of various fish oils are given in Table 1. In most of the fish oils the omega-3 LC PUFAs, mostly EPA and DHA, generally make up 10–35% of the total fatty acids. Fatty acids such as 20:1 and 22:1 are present in significant parts in oils produced from fish such as herring, mackerel, and capelin, caught in northern waters.

Other Chemical Constituent

Fish body and liver oils contain other chemical components such as vitamins. Vitamin D tends to concentrate in the liver of fish. Therefore, body oils contain less vitamin D compared to liver oils. The content of vitamin D depends on the species, age, size, sex, nutritional conditions, and spawning state of the fish. The liver oils also contain vitamin A. Vitamin E is not synthesized by fish; therefore, vitamin E content is dependent on the fish diet. The most abundant form of vitamin E is alpha tocopherol with the concentration being higher in the liver than body oils. The level of tocopherol in fish oil is reduced during refining and deodorization and generally less than 100 ppm is found.

Oxidative Stability

Because fish oils usually contain long chain polyunsaturated fatty acids, they are highly susceptible to oxidation. In addition to fatty acid composition, the oxidative stability of oils also depends on the amount of prooxidants and antioxidants in the oils and storage conditions such as temperature, light, and oxygen availability. Anoxic production and storage of oils prevent against oxidation. Generally, each 10 °C decrease in storing temperature doubles the shelf-life of the oil. Trace amounts of prooxidants (catalysts of lipid oxidation) such as transition metals are usually present in fish oils. Therefore, in order to reduce oxidation, antioxidants that inactivate metals or lipid radicals are added to the fish oils. Encapsulation of n-3 fatty acids reduces the contact between lipids and oxygen and contributes to protection against oxidation. Packaging in material nonpermeable for light and oxygen also contributes to protection against oxidation.

Lipid oxidation is a dynamic process creating a complex mixture of primary, secondary, and tertiary products. Because several products are intermediate; they are formed and then broken down again during marine oil processing, and there is not always a linear increase in the oxidation-products with time of oxidation.

The oxidative state of oil is defined as the sum of all oxidation products present. Traditionally, an estimate of primary (peroxide value) and secondary products (anisidine value) is applied.

PV analysis usually is performed using the iodometric titration method—that is, the one referred to both by Ph. Eur.

Table 1 Composition of major fatty acids of selected fish

Fatty acid	*Percentage composition*								
	Farmed Atlantic salmon[a]	*Tuna*[a]	*Jack mackerel*[a]	*Atlantic cod liver*[b]	*Atlantic menhaden*[b]	*South American anchovy*[b]	*Sardine/ pilchard*[c]	*Capelin*[c]	*Herring*[c]
14:0	4.2	3.9	7.3	3.3	7.3	7.5	8	7	7
16:0	15.7	17.6	15.7	13.4	19	17.5	18	10	16
18:0	4.2	4.1	3.1	2.7	4.2	4	nd	nd	nd
16:1 *n*−7	5.1	5.4	5.1	9.6	9.1	9	10	10	6
18:1 *n*−9	16.5	12.4	9.9	23.4	13.2	11.6	11	14	13
18:1 *n*−7	3.5	2.4	2.9	nd	nd	nd	nd	nd	nd
20:1 *n*−9	3.3	1.3	8.3	7.8	2	1.6	4	17	12
22:1	2.5	0.5	5.8	5.3	0.6	1.2	3	14	20
18:2 *n*−6	6.6	1.9	1.7	1.4	1.3	1.2	nd	nd	nd
20:5 *n*−3	7.1	12.4	10.9	11.5	11	17	18	8	5
22:6 *n*−3	15.7	27.8	11.5	12.6	9.1	8.8	9	6	6
22:5 *n*−3	3.9	1.7	2	1.6	1.9	1.6	nd	nd	nd

[a]Nichols (2007).
[b]Ackman (2006).
[c]Bimbo (1990).

(Monograph 2.5.5 Method A) and by GOED (AOCS procedure Cd 8–53).

Anisidine value (AV) determines the amount of aldehydes (secondary oxidation products) formed as a result of breakdown of lipid peroxides in the oils. The method is based on the color reaction between the aldehydic compound and the p-anisidine (AOCS procedure Cd 7–58, Ph.Eur. Monograph 2.5.36).

Oxidative stability of oils is usually difficult to determine. The effect of different antioxidants on the oxidative stability of fish oils can be determined by accelerated oxidative stability methods such as the oxidative stability index (OSI). The OSI method is an American Oil Chemist Society (AOCS) approved method that determines the relative resistance of fat and oil samples to oxidation. The instrument measures the conductivity increase in deionized water as a result of the secondary products' formation during the oxidation of the oil. The Schaal oven test is another accelerated method for determining the stability of lipids and the effect of antioxidants. The test is based on the measurement of weight gain of lipid samples over time at a chosen temperature. The increase in the weight is a result of oxygen binding to the unsaturated fatty acids (i.e., peroxidation). Active antioxidants will delay the onset of peroxidation and, therefore, the weight gain, compared to a control (no antioxidants).

Quality Criteria for Marine Oils for Human Consumption

The chemical composition and quality of crude oils depend on both the production process and the quality of the raw material. This will influence the properties of the oils in regard to edible properties and technical applications. No standard quality criteria are set for the crude oils, but an overview of the typical chemical and physical properties of the crude oils has been reported by Bimbo in 'Guidelines for Characterizing Food-grade Fish Oil' shown in Table 2. Several tests are used to evaluate the quality, moisture content, and impurities. The quality criteria for crude oils are mainly set by the buyer of the oils depending on their range of application; feed, nutraceuticals, functional foods, or pharmaceuticals.

Table 2 Chemical properties of crude oils

Quality guidelines for crude oils	
Moisture and impurities (%)	Usual basis 0.5 up to maximum of 1
Fatty acids (% oleic acids)	Range 1–7, usually 2–5%
Peroxide value (mequiv. kg^{-1})	3–20
Anisidine value	4–60
TOTOX (2PV + AV)	10–60
Iodine value	95–200 (depending on fish source)
Color (Gardner scale)	Up to 14
Iron (ppm)	0.5–7.0
Copper (ppm)	Less than 0.7
Phosphorus (ppm)	5–100

According to the European Food Safety Authority (EFSA), it is difficult to give an exact recommendation for the maximum tolerable PV and AV levels in crude oils because no literature is available on the relationship between oxidation values in the crude oil and in the final oil. However, the quality of the crude

Table 3 Recommended criteria by the European Pharmacopeia (Ph.Eu) and the Global Organization for EPA and DHA (GOED) for oxidation status in refined marine oils intended for human consumption

	[a]*Ph.Eur.7.0 (2011) (maximum values)*		[b]*GOED monograph (2006) (maximum values)*	
Type of oil	[c]*Peroxide value*	[d]*Anisidine value*	[e]*Peroxide value*	[f]*Anisidine value*
Omega-3 rich fish oil (type 1)	10	30	5	20
Omega-3 rich fish oil (type 2)[g]	5	15		
Omega-3-acid triglycerides	10	30		
Omega-3-acid ethyl esters 60	10	20		
Omega-3-acid ethyl esters 90	10	20	None	None

oil will decide the range of application and how extensive the refining that is required.

At present, no adequate international, European, or national legislation standard for origin, quality, and/or composition of marine oil for human consumption is available. The European Pharmacopeia (Ph.Eur) has developed standards with quality criteria for some types of refined marine oils intended for human consumption; cod liver oil, farmed salmon oil, omega-3 rich fish oils, omega-3-acid triglycerides, omega-3-acid ethyl esters 60, and omega-3-acid ethyl esters 90. In addition, both the Ph.Eur and the Global organization for EPA and DHA have prepared recommendations for the evaluation of oxidation status in refined fish oils and set maximum values for the quality parameters. An overview is given in **Table 3**.

See also: Fish: Processing; Fish Oils: Composition and Health Effects.

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Fish: Dietary Importance and Health Effects

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Introduction

According to the Food and Agriculture Organization (FAO) of the United Nations, the worldwide production of seafood reached 149 million metric tons (MMT) in 2010. This production was divided between capture fisheries at 89 MMT and aquaculture at 60 MMT, and 128 MMT was utilized for human consumption.

Fish consumption varies greatly between countries and is influenced by several factors. Availability in forms of production (capture and aquaculture) and trade (imports and exports) is often the main variable. Factors affecting availability within different countries are geographic location and political conditions. Production is also largely dependent on climatic conditions, as extreme weather conditions such as El Niño affect both capture fisheries and the production of aquaculture feeds. The consumption pattern is also influenced by cultural variables, such as religion and dietary traditions.

Statistics from the FAO show that the general fish consumption in the world has increased over the last 50 years, from 9.0 to 17.8 kg capita^{-1} year^{-1}. This increase is recognizable in most countries. However, the variation between countries is large. For instance, in countries with no coastline, such as Mongolia, the average fish consumption is 0.4 kg capita^{-1} year^{-1}, while island states, like Faroe Islands, have an average yearly consumption of 87.7 kg capita^{-1}.

Most of the literature concerning fish consumption does not distinguish between fish and other seafood, such as crustaceans and mollusks. In the following, unless otherwise specified, fish and other seafoods are hence treated as one.

Composition and Nutritional Advantages

The biodiversity and compositional variety among species make it difficult to conclude on uniform nutritional characteristics valid for all types of fish. In the following, the nutrients particularly associated with fish consumption will be emphasized.

Lipids and Fatty Acids

Lipids are the main energy storage in the human body and also important constituents of brain tissue and other biological membranes. Fatty acids are built up of a carbon (C) chain of typically 4–22 C-atoms, with a methyl group at the proximal end and a carboxyl group at the distal end. The C-atoms are bound together by single or double bonds and the fatty acids are termed thereafter as saturated (zero double bonds), monounsaturated (one double bond), or polyunsaturated (two or more double bonds) fatty acids. The 'omega' indication refers to the location of the first double bond counting from the methyl end of the C-chain. If the first double bond is located between C-atoms 3 and 4, the fatty acid is an omega-3 fatty acid, and if the first double bond is located between C-atoms 6 and 7, it is an omega-6 FA. Two fatty acids are essential to humans, that is, they cannot be synthesized *de novo* in the body. These are linoleic acid (LA, 18:2n − 6) and α-linolenic acid (ALA, 18:3n − 3). These fatty acids are not crucial per se but are metabolized through a series of chain elongation and desaturation to the very long arachidonic acid (ARA, 20:4n – 6), eicosapentaenoic acid (EPA, 20:5n – 3), and docosahexaenoic acid (DHA, 22:6n − 3). The two former act as precursors to fatty acid hormones, called eicosanoids, while the latter has been proven essential for brain development. Depending on whether the eicosanoids originate from omega-3 or omega-6 FA, they have distinctly different qualities. In general, eicosanoids derived from ARA are proinflammatory and prothrombotic, whereas eicosanoids derived from EPA are less inflammatory and even anti-inflammatory. The optimal dietary ratio between omega-6 and omega-3 fatty acids is 2–5/1, depending on the disease in question. The change in diet towards more processed foods with high intake of omega-6 vegetable oils has shifted the omega-6/omega-3 ratio of the current western diet to 15–17/1. LA and ALA are competing substrates for the enzymatic endogenous synthesis of n − 6 and n − 3 long-chain PUFAs. The high dietary content of LA results in a more predominant endogenous production of ARA at the sacrifice of EPA and DHA. Hence, the only way for humans to achieve sufficient amount of EPA and DHA is directly through the diet. Fish contain high amounts of EPA and DHA due to direct transfer up the food chain from phytoplankton that produce them *de novo*. A daily intake between 200 and 500 mg EPA and DHA is usually recommended. This may be achieved by eating fish, preferably fatty fish twice a week. It is also recommended that energy from fat should be around 30% and that saturated fat should contribute to not more than 10% of the total energy. Fish is divided into three groups depending on their fat content: Lean fish, such as cod and saithe/pollock, have fat content less than 2%; medium fatty fish, such as catfish, redfish, and tuna, have a fat content between 2 and 8%; and fatty fish, such as salmon, herring, and mackerel, have a fat content higher than 8%.

Proteins

Fish was initially regarded as a healthy food choice because it is a low-fat protein source. Nonetheless, in the last half century, the positive health effects of seafood have generally been associated with the marine omega-3 fatty acids EPA and DHA, leaving the benefits of proteins and peptides disregarded. Proteins are molecules consisting of one or several polypeptide chains composed of amino acids bound together by peptide bounds. Twenty different amino acids constitute proteins, and of these, nine are essential to humans. The essential amino acids are histidine, isoleucine, leucine, lysine, methionine,

phenylalanine, threonine, tryptophan, and valine. The quality of a protein source is determined from the extent it meets our requirement of essential amino acids, regarding both the amount of essential amino acids and the extent we can absorb and utilize them. Fish contains 12–24% protein depending on species. The proteins contain all essential amino acids in sufficient amounts to cover our daily requirement, and fish is therefore recognized as a complete protein source. The digestibility of fish protein is excellent, and the lower content of connective tissue indicates that fish protein is more easily digestible compared to meat protein from terrestrial animals.

Carbohydrates

Together with fat and protein, carbohydrates are the third dietary source of energy. Fish contains almost no carbohydrates, while crustaceans and mollusks contain only small amounts. Seafood is therefore not a source of carbohydrates.

Vitamins and Minerals

Vitamins and minerals are also essential nutrients, although not for energy. The daily requirements of these components are hence substantially smaller than those of the energy-providing nutrients. Herein, the vitamins and minerals abundant in fish will be discussed. Fish is an important source of the water-soluble vitamin B_{12}. In addition, fatty fish is also rich in the lipid-soluble vitamins A and D. Fish is a good source of several minerals, and whereas only iodine and selenium are more abundant in fish than in terrestrial animals, fish may also be an adequate source of other minerals such as zinc, iron, magnesium, and calcium.

Vitamin A

Vitamin A is a collective term including compounds with biological effect as retinol. Carotenoids are provitamins as they are transformed into retinol in the body. Vitamin A is important in fetal development and for several functions in the body such as reproduction, vision, skin, immune system, and epithelial integrity. In Western countries, vitamin A deficiency is rare, whereas it is a major problem in developing countries. The earliest sign of vitamin A deficiency is reduced growth and night blindness. Recommended daily intakes are presented as retinol activity equivalents (RAE), where 1 RAE equals 1 μg retinol, and are 900 μg for men and 700 μg for nonpregnant and nonlactating women. While eggs and carrots are the main sources of vitamin A, fatty fish, particularly fish liver and fish oils, are also good sources. Fish contain vitamin A in its retinol form that is more easily absorbed than the carotenoids.

Vitamin D

Vitamin D is crucial in development and maintenance of the bone tissue and skeleton by ensuring adequate concentrations of calcium and phosphate in plasma and extracellular tissue. Deficiency of vitamin D can lead to low calcium and phosphate concentration and softening of the bones known as rickets in infants and osteomalacia in adults. Humans synthesize vitamin D when exposed to sunlight, but in northern latitudes, the synthesis is inadequate and additional intake through diet is necessary. The current daily recommendation of vitamin D is 10 μg per day. Good dietary sources of vitamin D are fish liver oil, fatty fish, and fortified foods. Vitamin D content in fish varies greatly, but a portion of fatty fish such as herring or mackerel will ensure the recommended daily intake.

Vitamin B_{12}

Vitamin B_{12}, or cobalamin, is one of the eight vitamins in the B-group. It has a key role in normal brain functioning and nervous system and in blood formation. The most common deficiency symptom is hence anemia. A special variety of anemia, called pernicious anemia, is the result of a hereditary condition where lack of intrinsic factor leads to reduced uptake of B_{12}. The daily requirement of B_{12} is low, around 2 μg, and one portion of 100 g fish will generally cover the recommended daily intake. In addition, most fish also contain some amounts of the other B vitamins.

Iodine

Iodine is a trace element essential in the synthesis of the thyroid hormones detrimental for growth and metabolism. Deficiency of iodine is the largest deficiency disease worldwide, causing goiter (thyroid enlargement), growth inhibition, and mental retardation (cretinism). The recommended daily intake of iodine is 150 μg. Fish, especially marine fish, are good sources of iodine and one portion of cod will cover the daily recommendations. Furthermore, iodine is the simplest micronutrient to fortify and is usually added to dietary salt.

Selenium

Selenium is another trace mineral essential for humans. Being a constituent of the antioxidative enzyme class glutathione peroxidases (GPx), it is regarded as one of the most important endogenous antioxidants. In addition, selenium is central in the thyroid hormone regulation, and a diet deficient in selenium may therefore lead to, and even intensify, some of the deficiency characteristics seen in iodine deficiencies. The recommended daily intake is 40–50 μg. Seafood is recognized as a good source of selenium, especially in areas where the soil is depleted of this trace mineral.

Bioactive components

In recent years, an increased focus has been directed towards food components with beneficial health effects beyond that of nutritional value, namely, bioactive compounds. The bioactivity in question may be linked to a vast range of physiological conditions, such as oxidative stress, hypertension, cancer, obesity, and infections. Likewise, the compounds may possess very different chemical properties. Some of the compounds and bioactivities related to fish will be discussed further.

Peptides

Peptides are parts of proteins, ranging from two to several amino acids. The bioactivity is based on the sequence and composition of typically up to 30 amino acids occasionally occurring as free peptide in foods, but commonly within their parent proteins. Within proteins, side groups of amino acids are buried and the peptides have no bioactivity. Hence, they need to be released through fermentation, food processing, proteolysis, or gastrointestinal digestion to exert any activity. Several bioactivities have been linked to fish peptides,

although three main activities are well documented, namely, antihypertensive effect, antioxidative activity, and antiobesity effect.

Taurine

Taurine (2-aminoethanesulfonic acid) is the end product of the sulfuric amino acid metabolism, and although it contains a sulfonic group instead of a carboxyl group, it is considered as an amino acid. However, it is not bound in proteins, but exists as an exclusively free amino acid in most human tissues, the concentrations being especially high in the brain, retina, skeletal muscles, heart, and leukocytes. Its participation in several physiological processes, such as osmoregulation, neuro- and immunomodulation, and bile salt formation, has been well documented. In addition, together with its precursors methionine and cysteine, it is involved in protection from oxidative stress reactions. Lately, several reviews have discussed the relationship between taurine and several other diseases, such as cardiovascular disease (CVD), hypertension, and diabetes.

The endogenous synthesis of taurine is dependent on a series of enzymes and the efficiency of the production is species-dependent. In humans, it is quite inefficient and in certain situations, the endogenous production may be inadequate. Taurine is therefore characterized as a conditionally essential amino acid that should be provided through dietary intake. The main dietary source of taurine is seafood, levels being higher in mollusks and crustaceans than in fish.

Other bioactive components

Other bioactive components frequently found in foods are ubiquinone, carotenoids, polyphenols, and phytosterols. Of these, carotenoids are considered important in relation to consumption of seafood. Carotenoids are a class of antioxidative compounds, with some chemical resemblance to vitamin A, and some of them may be chemically converted to this vitamin. The most abundant carotenoid in seafood, especially in crustaceans, is astaxanthin. This compound is produced by microalgae as a defense towards UV light and may be found in decreasing concentrations upward in the marine food web. Within the fish, it acts as an endogenous antioxidant, protecting it from autoxidation, and it is also believed to possess similar protective effects in humans. Phytosterols are cholesterol-like compounds abundant in plant materials and to a certain degree in mollusks. In fish, these compounds have traditionally not been present. However, in line with the increasing inclusion of plant materials in aquaculture feeds during the last years, an increasing amount of phytosterols has been detected in farmed fish.

Health Effects of Fish Consumption

Numerous studies have been performed in order to evaluate a potential causal relationship between fish consumption and chronic diseases. Such studies make up the foundation of evidence-based decisions and are combined by the world's large health organizations in order to establish official dietary advice for consumption of fish. There are two main approaches when studying this field, epidemiological and experimental studies, and there are advantages and disadvantages associated with both study types. A combination of studies will probably return the most solid foundation for advice.

In brief, in epidemiological studies, human populations are studied in order to describe and explain health and disease. The main advantages of these studies are that they range over a long period of time, the data material is very large, there are few restrictions in diet, and diseases can be included as end point. The main drawbacks are that they are poorly controlled and that the sources of error are many. Among epidemiological studies, several subtypes are defined: descriptive studies, ecological studies, migrant studies, case–control studies, cohort studies, randomized controlled studies, and meta-analysis.

Experimental studies are performed in a more controlled environment than are epidemiological studies. The test participants can either be human volunteers, but most experimental studies are performed in animals (*in vivo*) or in cell-based systems using human or animal cells grown in the laboratory (*in vitro*). There are also subtypes of experimental studies: human feeding studies, live animal models, *in vitro* studies, and studies of biological pathways.

The world's large health organizations, such as the World Health Organization (WHO), the World Cancer Research Fund (WCRF), the American Heart Association, and the Academy of Nutrition and Dietetics, have defined different categories describing the causal relationship between exposure/intake and morbidity, and each category has different demands of the strength of documentation. There are slight differences in the definitions of the criteria between the various organizations, but the strength of documentation is the same. The main categories used by WHO and WCRF 'Convincing relationship,' 'Probable relationship,' 'Limited relationship – suggested,' 'Limited relationship – no conclusion,' and 'Substantial effect on risk unlikely.'

The evidence-based documentation is often presented in matrices, one for each food type and disease group. Potential harmful effects/risks on intake of food are also included. The associations presented in the matrices are documented relationships and meet the demands of convincing relationship or probable relationship. Inclusion of evidence from more than one study type, no substantial unexplained heterogeneity within or between studies and control of confounding factors, and random or systematic errors are among the qualifications to be met in order to end up in one of these two categories.

Cardiovascular Diseases

The vast majority of studies regarding fish consumption and chronic diseases are related to CVDs. CVD is a collective term encompassing a group of disorders of the heart and blood vessels and is the largest cause of morbidity and a major cause of premature death worldwide. The two most frequent disorders are coronary heart disease and cerebrovascular disease (stroke), affecting blood vessels of the heart and brain, respectively.

There are several risk factors associated with the development of CVDs. Some are nonmodifiable, such as age, gender, and heredity. Yet others are modifiable, such as smoking, hypertension, unfavorable blood lipid composition, physical inactivity, obesity/overweight, and diabetes mellitus. The latter ones are recognized as lifestyle-related risk factors and may be

influenced by dietary habits. Concurrent presence of several risk factors is common and certain risk factors may in combination increase the risk of CVD considerably. Although there are several independent risk factors for the development of CVDs, causal relationship between their presence and actual disease has not been established for most of them.

CVD and Fish Intake

The vast majority of studies regarding fish intake and health benefits have been focused on their content of long-chain omega-3 fatty acids. An increased intake of these fatty acids, especially EPA, will lead to replacement of ARA in cell membranes and red blood cells. As previously mentioned, eicosanoids may be formed from both ARA and EPA. However, the properties of different eicosanoids are substantially different depending on the originating fatty acid. As mentioned previously, eicosanoids formed from ARA are proinflammatory and prothrombotic, whereas eicosanoids formed from EPA are less potent and show anti-inflammatory and antithrombotic properties. This may have impact on the atherosclerotic process. In addition, an increased intake of LC-PUFAs has been shown to improve the stability of plaques.

It is well documented that a high intake of fish reduces the level of triglycerides in the blood and, hence, attenuate the effects of dyslipidemia. The effects on blood cholesterol levels are not as consistent.

The relationship between the intake of LC-PUFAs and blood pressure has also been studied, but also here, the results are inconclusive. Some studies imply that hypertensive patients may benefit from a high intake of these fatty acids, while the same effect is not seen in persons within the normal range of blood pressure. Several fish species are also considered good sources of potassium that is essential for water and electrolyte balance and the normal functioning of neurons and other cells. Dietary potassium may lead to loss of sodium, and increased dietary intake of potassium has been associated with a decrease in blood pressure.

However, intake of fish may affect blood pressure through yet another mechanism, that is, via peptides released through gastrointestinal digestion. Numerous *in vitro* studies and animal models have shown that fish peptides exhibit blood pressure-reducing effects by inhibition of ACE. A few human studies have confirmed these findings.

Fish Intake and Other Chronic Conditions

Although not as frequent as for CVDs, studies on the correlation between fish intake and other positive health effects have also been performed. Among the studied effects are cognitive development and disorders in later life, cancer, allergy, and other immunologic conditions, along with lifestyle-related conditions, such as type 2 diabetes. Due to large heterogeneity between studies, the conclusions regarding these conditions are not as convincing as for CVDs, but some of the main suggestions and findings will be presented here.

Cognitive disorders: Development and later life

The fatty acid DHA is thought to be of great importance for neurodevelopment and most studies regarding this subject are focused on this fatty acid. However, the number of potential confounders in studies regarding cognitive development in early life is substantial. This makes it extremely challenging to draw conclusions from such studies. Despite this, most studies conclude that a high maternal intake of fish is beneficial for overall cognitive development of the offspring and that neither maternal nor postnatal supplementation with long-chained n − 3 fatty acids provides additional effects.

When cognitive decline and dementia, including Alzheimer's disease, have been investigated in relation to fish and n − 3 intake, the results are also inconclusive. In some studies, there seem to be a trend, although not significant, that fish intake, but not n − 3 supplementation, may offer a certain protection against dementia. Other studies find no correlation at all.

Cancer

Lifestyle and dietary habits have been related to several types of cancer, in particular the cancers of the gastrointestinal system. As a natural result of this, the relationship between fish intake and these cancers has been examined. For most of these cancer forms, there is no convincing or probable relationship associated with fish intake.

Allergy and immunologic reactions

Also asthma, eczema, and other atopic conditions in children have been investigated in relation to both maternal fish intake and fish intake during the first year. Although the results are inconclusive, eczema seems to be the only condition where fish intake may have positive effects. Both maternal fish intake and early introduction of fish during the first year seem to positively affect the risk of this condition.

Type 2 diabetes

Type 2 diabetes is recognized as a lifestyle-related disease. As dietary habits are a part of the factors related to the development of these diseases, the intake of several food items, including fish, has been investigated in order to discover potential correlations. So far, the number of studies regarding fish intake and type 2 diabetes is small and the heterogeneity between them is large, and hence, no correlation has yet been identified.

Adverse Health Effects

Increased fish consumption has long been recommended due to the well-documented health benefits. Nevertheless, fish as other foods can accumulate environmental pollutants and heavy metals that may reduce the overall nutritional value and alter consumer's health. In order to lower the risk of such detrimental effects, the Joint WHO/FAO Expert Committee on Food Additives has compiled guidelines and set limit values for assumed safe intakes of most contaminants. These limits, termed tolerable weekly intakes (TWI), are based on experimental data and equal the amount of a compound that may be consumed weekly during the total life span of a human being without risk of adverse health effects. In cases where experimental data are insufficient or lacking, a provisional tolerable weekly intake (PTWI) is set. The unit of both TWI and PTWI is μg or mg kg^{-1} body weight (BW) per week.

The contaminants most relevant for fish consumers are heavy metals and persistent organic pollutants (POPs). Pathogens and marine toxins can undoubtedly result in negative health effects. These risks may however be eliminated through proper cooking and are therefore not discussed here.

Heavy Metals

One definition of heavy metals is that they are elements with atomic weights ranging from 63.5 to 200.5 and specific gravities greater than 4.0. Small amounts of heavy metals occur naturally in the environment, but the vast amount of them may be attributed to human activities such as industrial emissions. Some of the heavy metals, such as iron, copper, and zinc, are essential for the human body in low doses. Others, such as arsenic, cadmium, lead, and mercury, have no known essential function in man. Common for all of the heavy metals is that they are toxic if consumed in excess, but the level of toxicity varies greatly between metals and which chemical state they are in. They have great affinity to ligands in cellular proteins and bind more easily to these than do other metals present in the body. A result of this may be inhibition of enzymatic reactions. As the elimination rate is slower than the absorption rate, heavy metals accumulate in the body.

Arsenic

Arsenic is used in a number of industrial processes including processing of glass, pigments, textiles, and ammunition. Small amounts are also used in pesticides and feed additives. High levels of arsenic are found both in the groundwater in several areas of the world and in the oceans. The toxicity of inorganic arsenic is far greater than the organic form and the PTWI of 15 $\mu g\,kg^{-1}$ BW is hence related to this form. Most of the registered adverse health effects related to arsenic are also related to the inorganic form and include peripheral vascular disease and various cancers. Fish is recognized as the most significant dietary source of arsenic, however, mainly in its less toxic organic form.

Cadmium

The main sources of exposure of cadmium are through commercial fertilizers and atmospheric pollution. Galvanizing processes and batteries are other sources. The absorption of cadmium is generally low but may increase in the presence of calcium or proteins. The main target organs for cadmium are the kidney and liver and toxic effects of it are related to these organs. Such effects include increased excretion of glucose, amino acids, and calcium, along with increased gluconeogenesis. The PTWI of cadmium is 7 $\mu g\,kg^{-1}$ BW. Shellfish are recognized as one of the food sources containing the highest level of cadmium.

Lead

Lead is abundant in nature, but also environmental contamination contributes to the total amount present. During the last half of the twentieth century, addition of lead to gasoline led to a sharp increase in contamination. Introduction of unleaded gasoline has turned this trend and lead contamination is now decreasing. The absorption of lead is around 5–15% in adults but slightly higher in children. The toxicity of lead is among other things linked to its capability of competing with essential metals for binding seats on enzymes and for inhibiting the transport of important ions such as calcium. Lead poisoning may affect the central nervous system, kidneys, and blood-producing organs. Lead is classified as a carcinogenic substance and may affect fertility. There is currently no PTWI for lead. In contaminated areas, seafood has been shown to contain high levels of lead, shellfish more than finfish.

Mercury

Mercury exists naturally in the environment, but industrial emissions are a significant contributor. It exists in elemental, inorganic, and organic forms, where the organic form is the more toxic. In aquatic environment, inorganic mercury is converted by microorganisms to methyl mercury (MeHg), which accumulate in the aquatic food chain. Humans are therefore exposed to MeHg through consumption of large predatory fish, of both marine and freshwater origins. The harmful effects of MeHg were first discovered after large-scale industrial poisonings in Japan in the 1950s and in Iraq between 1955 and 1972. These events demonstrated the harmful effects of MeHg primarily on the nervous system and in particular neurodevelopmental effects of fetuses. The PTWI for MeHg is 3.3 $\mu g\,kg^{-1}$ BW. Due to its well-documented negative developmental effects, specific dietary advice has been made for pregnant and lactating women. These state that these groups should not consume pike at all, perch above 25 cm, and trout and char above 1 kg. Dietary advice for other population groups is to not consume these species more than once a month. All groups are advised to minimize consumption of other large predatory fish such as swordfish, shark, king mackerel, and tuna.

Persistent Organic Pollutants

POPs are a large group of organic substances in which one or more hydrogen atoms have been substituted with halogens. The most important substances regarding fish consumption and adverse health effects are polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (flame retardants). None of these substances occur naturally, but are formed when hydrocarbons and halogens are exposed to high temperatures in incineration or other industrial processes. The decomposition rate is very slow due to the lack of enzyme systems able to break carbon–halogen bindings. They are lipophilic in general and the lipophilicity increases with increasing degree of substitution. The combination of these two factors leads to bioaccumulation in the marine food web, especially in fatty fish.

Dioxins and PCBs

The chemical structures of PCDD, PCDF, and PCBs are quite similar, as all of them contain two phenol rings. Hydrogen atoms are attached to these phenol rings, eight in PCDD and PCDF and ten in PCB. All of the hydrogen atoms may be substituted with chlorine atoms, making a total of 75 possible variants of PCDDs, 135 different PCDFs, and 209 PCBs. Twelve of the 209 PCBs, those without chlorine substitution in the ortho-position or those containing only one chlorine in

ortho-position, are characterized as dioxin-like PCBs. These act on the same toxicological pathway as dioxins, that is, via the aryl hydrocarbon receptor, and share more or less the same toxic properties as the dioxins. The dioxin-like PCBs are included in the term 'dioxins' in the following section. The non-dioxin-like PCB will not be discussed further here.

Some of the common adverse health effects that may be caused by these compounds are skin reactions (chloracne), damage of the neurological and immunologic systems, organ damage, enzyme induction, carcinogenic effects, and teratogenic effects.

A common system for evaluation of total toxicity of these compounds has been prepared, in which each compound has been given a toxic equivalency factor (TEF) related to the most toxic substance 2,3,7,8-tetrachlorodibenxodioxin, whose TEF is 1. In general, PCBs are less toxic than PCDDs and PCDFs. The total amount of dioxins in a sample is reported as toxic equivalents (TEQ) and is found by multiplying the concentration of each compound with its respective TEF and finally adding them up. The TWI for dioxins is currently 14 pg TEQ kg^{-1} BW.

Due to the bioaccumulation of dioxins in the aquatic food chain, seafood is recognized as a significant dietary source of dioxins. However, dioxins are also found in most other foods, and studies of food consumption patterns show that the daily intake of dioxins is approximately equal in most populations regardless of the seafood intake.

From time to time, the issue of high levels of dioxins in farmed fish is brought forward. The assumption is that levels of these compounds may be high in farmed fish due to the use of unrefined fish oil in the aquaculture feeds. However, the substitution of fish oil with plant oils during the last years has actually led to a decrease of dioxins in the farmed fish, and currently, the levels of these compounds are actually higher in wild species.

The prevalence of dioxins in the environment has decreased during the last decades, partly because of changes in industrial processes and partly because of a ban introduced in the 1980s on the usage of PCBs in new products. However, due to their slow decomposition rates, these compounds will be present in the environment for several years to come.

Polybrominated diphenyl ethers (flame retardants)

This is a group of compounds with similar chemical structures as PCBs, the difference being that the substituting atoms are bromine instead of chlorine. Their primary function is to serve as flame retardants, and as opposed to PCBs, their use and presence in the environment are increasing. Their toxicological properties have not been studied to the same extent as the dioxins, but several recent reviews have suggested that their use should be restricted.

Risk versus Benefit Analysis

Fish and seafoods have been extensively evaluated for their risks and benefits. Balancing risks and benefits is important, particularly in countries with high *per capita* seafood consumption such as Japan, Portugal, Iceland, and Maldives. Several meta-analyses have been published evaluating the risks and benefits associated with seafood consumption. An example of a risk–benefit analysis is the one performed by the Joint WHO/FAO Expert Committee in 2010 where the increased risk of dying from cancer caused by dioxins versus the reduced risk of CVD mortality due to EPA + DHA by consumption of equal amounts of fish was evaluated. The evaluation criteria were changes in total mortality per million people by intake of one fish meal per week for a lifetime. An example of such an analysis is for farmed Atlantic salmon, 600 lost lives to cancer versus 39 800 avoided deaths due to CVD, corresponding to a ratio between benefits and risks of approximately 66:1.

Conclusions

Fish and other seafoods are excellent sources of several important nutrients, and it is concluded that a high intake of these food items lowers the risk of CVD. Although not as consistent, positive health effects related to fish consumption and other chronic diseases have been confirmed. Despite being a source of several contaminants, the benefits of seafood consumption outweigh the potential risks, and hence, the conclusion is clear; the general seafood consumption should be increased.

See also: Antioxidants: Role on Health and Prevention; Bioactive Peptides in Foods; Cadmium: Properties and Determination; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fatty Acids: Metabolism; Fish: Fish in the Human Diet; Fish: Processing; Fish Oils: Production and Properties; Food and Agriculture Organization of the United Nations; Hypertension and Diet; Infants: Nutritional Requirements; Iodine: Physiology; Low-fat Foods: Types and Manufacture; Mediterranean Diet; Oxidation of Food Components; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein: Requirements; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Proteins: Chemistry, Characterization, and Quality; Shellfish: Role in the diet; Trace Minerals and Trace Elements.

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Fish: Fish in the Human Diet

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Seafood Sources and Production

Countries of Origin for Seafood

According to the Food and Agriculture Organization of the United Nations, in 2012, China was the major seafood exporting nation to the world (14% by value), followed by Norway at 7% by value, Thailand (6% by value), and Vietnam (5% by value). In 2012, the FAO ranked China as the top fishing country by quantity and value, followed by Indonesia, the United States, India, and Peru (primarily due to anchoveta). China is also the leading producer of aquacultured species in the world at 41.1 million tonnes, and its production is nearly 2.5 times that of its wild capture. In fact, in 2008, Asia started producing more farmed fish than wild catch, contributing 54% of the world's production in 2012, with Europe at a distant second at 18% and other continents below 15%. Other countries important to aquaculture are India (4.2 million tonnes), Vietnam (3.1 million tonnes), Indonesia (3.1 million tonnes), and Bangladesh, Norway, Thailand, Chile, Egypt, and Myanmar, each supplying less than 2 million tonnes. All these countries supply 88% of the world's production.

The most captured species is anchoveta, followed by Alaska pollock, skipjack tuna, Atlantic herring, and chub mackerel. The FAO monitors 1600 harvested marine species, but 40% of the total marine catch comes from 23 species.

In 2012, shrimp continued to be the most important commodity traded in world commerce in terms of value, accounting for about 15% of the total value of internationally traded fish proteins. Other main groups of exports are salmon and trout (more than 14%), groundfish at 9% (e.g., hake, cod, haddock, and Alaska pollock), and tuna (8%).

In 2012, about 73% of the total fishery imports in value were in developed countries, with the United States and Japan accounting for 27% of the total. The European Union represented 36% of the total world imports, which include intraregional trade among its members, and thus is the largest market in the world.

The total global capture fisheries production for 2012 is 91.3 million tonnes, while aquaculture was 66.6 million tonnes for a total of 157.9 million tonnes.

Patterns of Consumption

According to the FAO, in 2012, about 86%, or 136.1 million tonnes, of the total fishery production was used for direct human consumption. The remaining 14%, or 21.7 million tonnes, goes primarily for fish meal and fish oil. About 46% of seafood for human consumption is eaten in live or fresh form.

In 2011, the global per capita annual consumption of seafood was estimated by the FAO at 18.9 kg, with fish accounting for 16.7% of the global population's intake of animal protein and 6.5% of all proteins consumed. Globally, fish provides 3.0 billion people with almost 20% of their average per capita intake of animal protein and 4.9 billion people with 10% of such protein. Table 1, based on FAO data, is a look at the role of seafood in supplying protein to the diet. Interesting and somewhat predictable from Table 1 are the countries and continents that rely on land sources of animal protein. Fourteen countries have a consumption of fish/animal proteins greater than 50% (i.e., the British Virgin Islands, Ghana, French Guiana, Guadeloupe, Martinique, Bangladesh, Cambodia, Indonesia, Maldives, Sri Lanka, Kiribati, the Solomon Islands, Tokelau, and the Wallis and Futuna Islands). Seafood contribution to the diet is reduced by consideration of other nonanimal proteins as food sources, but the countries of the British Virgin Islands, Guadeloupe, Maldives, Kiribati, Tokelau, and the Wallis and Futuna Islands remain strong at >30% of fish/total proteins.

Health Effects

Omega-3 Fatty Acids in the Diet

Omega-3 fatty acids (omega-3s) are long-chain fatty acids (LCFAs) that contain two or more double bonds and are also known as polyunsaturated fatty acids (PUFAs). The beneficial effects of PUFAs vary depending on the length and structure of the fatty acid. The two most recognized PUFAs are omega-3 and omega-6, both of which are important for optimal growth, development, and health, and these are essential in the diet because the human body does not make omega-3 and omega-6 fatty acids.

Three of the most well-researched omega-3s include DHA (22 carbons in length with 6 double bonds) and EPA (20 carbons in length and 5 double bonds) and alpha-linolenic acid (ALA; 18 carbons in length and 3 double bonds). Both EPA and DHA are LCFAs primarily found in salmon, sardines, trout, tuna, and herring. ALA is a short-chain fatty acid (SCFA) and is found in plant sources, such as nuts, hempseeds, flax, and canola and soybean oils.

While all of these PUFAs are important for optimal health, the human body can utilize DHA and EPA much more efficiently than ALA. Because ALA is a SCFA, enzymes are necessary to convert ALA to a more bioavailable LCFA. While a small percentage of ALA can be converted to EPA (approximately a 5–15% conversion rate depending on the individual's body efficiency), there is little to no conversion of ALA to DHA.

Omega-3 PUFAs are important for heart health and brain development (discussed in the succeeding text). EPA and DHA help to lower inflammation, improve blood cholesterol levels, and reduce the risk of death from heart attacks and stroke.

The dietary recommendations for omega-3s from global organizations for the general adult population are as follows:

- World Health Organization (WHO) and FAO: Aim for 250 mg of EPA and DHA each day.
- International Society for the Study of Fatty Acids and Lipids (ISSFAL): To reduce the risk of cardiovascular disease, consume a minimum of 500 mg of DHA + EPA (combined) each day.

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Table 1 Seafood contribution to protein supply by continents and by selected nations, particularly those with % fish/animal protein greater than 50%

Nations by continent	*Per capita supply (kg)*	*Fish proteins (g per capita per day)*	*Animal proteins (g per capita per day)*	*Fish/animal proteins (%)*	*Fish/total proteins (%)*
Africa	10.4	3.0	15.4	19.4	4.6
Ghana	22.9	9.0	17.3	51.9	14.5
Morocco	12.5	4.0	24.2	16.7	4.2
Nigeria	17.1	4.4	10.3	43.1	7.1
The Seychelles	59.3	17.0	35.8	47.6	22.2
North America	21.7	5.3	69.4	7.6	4.8
Canada	22.3	5.8	57.9	1.0	5.6
The United States	21.7	5.2	70.7	7.4	4.8
Latin America/the Caribbean	9.9	2.7	41.8	6.5	3.3
Brazil	10.6	2.7	51.0	5.4	2.9
The British Virgin Islands	28.6	7.4	11.9	61.9	59.8
French Guiana	17.0	4.0	6.2	64.7	20.7
Guadeloupe	21.7	6.2	11.1	55.6	38.9
Martinique	12.6	3.8	7.5	50.3	25.7
Asia	21.4	5.8	25.5	22.9	7.7
Bangladesh	19.7	5.5	9.8	56.2	10.0
Cambodia	40.5	12.8	19.3	66.3	19.8
China	33.5	8.1	36.2	22.4	8.6
Indonesia	28.9	9.6	17.4	54.8	15.6
Japan	51.7	17.9	48.1	37.3	20.4
Korea, the Democratic People's Republic of (North Korea)	9.4	2.6	10.0	25.8	4.6
Korea, the Republic of (South Korea)	60.4	16.7	43.1	38.7	17.5
Maldives	164.0	52.2	72.1	72.4	48.4
Sri Lanka	26.3	9.2	16.3	56.1	15.5
Thailand	26.3	8.5	24.2	35.3	14.4
Vietnam	33.6	8.7	31.8	27.3	11.2
Europe	22.0	6.6	57.5	11.6	6.5
The Faroe Islands	85.5	22.4	51.9	43.2	28.0
Iceland	89.9	28.0	96.2	29.1	21.3
Norway	53.4	15.1	64.4	23.4	13.8
Portugal	57.1	15.3	68.9	22.1	13.7
Spain	43.0	13.0	65.2	19.9	12.6
Oceania	25.1	6.4	61.9	10.3	6.7
Kiribati	74.1	22.5	37.5	59.9	30.0
The Solomon Islands	35.0	10.8	18.7	57.5	18.6
Tokelau	54.0	15.5	25.7	60.2	43.2
The Wallis and Futuna Islands	61.5	17.4	28.9	60.0	43.8

Source: FAO, 2012. ftp://ftp.fao.org/FI/STAT/summary/FBS_bycontinent.pdf -FAO (2012). Statistical collections, Summary tables of fishery statistics: capture, aquaculture, commodity and food balance sheets.

- NATO workshop on omega-3 and omega-6 fatty acids: 300–400 mg EPA + DHA each day.
- World Gastroenterology Organization: 3–5 servings of seafood each week.

Table 1 documents the variable importance of seafood per capita, as a percent of animal protein and as a percent of total protein in the world, including those countries where the percent of fish/animal proteins is greater than 50%. In a Harvard study of dietary fats and oils, the global mean intake of omega-3s is about 163 mg per day, far lower than the recommendations in the preceding text. This study considered 113 countries and a population of 1 630 069 or 82% of the global population. Worldwide, omega-3 intake from seafood has increased in the past 20 years by 24 mg per day. By 2020, nearly 75% of all deaths and 60% of all disability-adjusted worldwide will be attributable to noncommunicable diseases including cardiovascular diseases, type 2 diabetes, obesity, and cancers. Cardiovascular disease particularly can be better controlled by omegas.

Seafood is the premiere dietary source of omega-3s DHA and EPA. According to the FAO, Oceania boasts the highest per capita seafood consumption in the world, followed very closely by Europe (Table 1). Countries who consume the least amount of seafood include Africa and the Near East. With the exception of islands with large fishing communities, seafood consumption tends to be greater in developed countries as compared with developing countries.

Beneficial Nutrients of Seafood

Seafood is a nutrient-dense food naturally rich in protein, vitamins (especially B and D), minerals, and heart-healthy omegas. Seafood is grouped with meat, poultry, eggs, nuts, legumes, and seeds as major contributors (greater than 50%) of protein, niacin, zinc, and vitamin B_6 to the diet by the Institute of Medicine (IOM) and as an important source of greater than 10% of vitamins E and B_{12}, thiamine, and riboflavin and phosphorus, magnesium, iron, copper, potassium, and linoleic acid. Higher levels of the antioxidant selenium and the omegas EPA and DHA and generally lower levels of saturated fats compared with meat, poultry, eggs, etc., are unique to seafood, notes the IOM. In fact, says the IOM, depending on the preparation method, seafood is lower in total and saturated fats and cholesterol than some more frequently selected animal protein sources such as lean and fatty cuts of beef, pork, and poultry. Certain species of seafood (e.g., canned salmon with bones) may also be a good source of calcium.

Heart Health Benefits of Eating Seafood

Omega-3 fatty acids have been shown to be cardioprotective. While the exact mechanism of how these fatty acids benefit heart health is largely unknown, omega-3s decrease triglyceride levels and risk of arrhythmias (abnormal heartbeats), slow the growth rate of atherosclerotic plaque, and slightly lower blood pressure, according to the American Heart Association (Washington, DC). Omega-3s from seafood may also reduce the risk of sudden cardiac death.

Recent studies by the FAO/WHO have shown that modest intake of omega-3s (approximately 250–500 mg EPA + DHA per day) in various populations in the United States, Europe, Asia, and Australia lowers the risk of dying from coronary heart disease. The magnitude of the protective effect of eating seafood about twice each week is a 36% reduced risk of heart disease death. For this reason, public health organizations and experts around the world advise adults to consume at least 2–3 servings of seafood each week to help meet heart health recommendations for omega-3s.

The Mediterranean-style diet has long been touted as a heart-healthy diet. To determine whether seafood in the Mediterranean diet is beneficial to heart health, researchers examined the seafood intake of more than 3000 men and women in the Attica region of Greece. In this region, about 88% of men and 91% of women consumed fish or shellfish at least once a week. Fish intake was positively correlated to reduced levels of inflammatory markers related to cardiovascular disease.

Brain Benefits of Eating Seafood

The need for the nutrients in fish is especially high during pregnancy. During the last trimester, a fetus' brain and nervous system rapidly develops, requiring about 65 mg per day of DHA. The heightened demand for DHA continues to 2 years of age.

While the benefits of omega-3s on brain development during infancy and early childhood are well documented, there is limited evidence that omega-3s from seafood intake during later adulthood may help reduce the risk of dementia. In a study that looked at nearly 15 000 older adults (aged 65 years or older) in low- to moderate-income areas in 11 countries (including China, India, Cuba, Venezuela, Mexico, Peru, and the Dominican Republic), there was a beneficial effect of seafood on dementia in this population in all countries except India. More research is needed, but the effect of omega-3s on brain health throughout the lifetime is promising.

Mercury in Seafood

Essentially, all seafood includes traces of mercury, a compound that originates primarily from natural volcanic activity and thermal events in oceans. Although some mercury has been contributed by human activity, studies show that mercury in the oceans has remained virtually unchanged over time.

The amount of mercury equated with serious illness has only been recorded in two international industrial accidents and poisonings in Japan and Iraq in the 1950s–1970s. While the levels of mercury present in those instances are on a scale dramatically different than the levels seen in commercial seafood, the devastating effects of the poisonings, specifically on developing babies, piqued the scientific community's interest in studying cultures who eat seafood-rich diets. People from two island areas, the Faroes and Seychelles, were followed to determine the health effects of regular, high-level seafood consumption. Subclinical cognitive deficits were found among children of women in the Faroes who ate pilot whale, a mammal with a nutrient and contaminant makeup unlike seafood, during pregnancy. No pattern of harm was found among children of women who ate seafood during pregnancy in the Seychelles. Subsequent adjustment of the Faroes data to isolate the effects of seafood from whale meat also showed no harm from eating fish.

See also: Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fish: Dietary Importance and Health Effects; Fish: Processing; Fish Oils: Composition and Health Effects; Fish Oils: Production and Properties; Food and Agriculture Organization of the United Nations.

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Fish: Processing

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Introduction

Marine species are known to provide high proportions of important constituents for the human diet such as: (i) nutritional and digestive proteins including high levels on essential amino acids; (ii) lipid soluble vitamins (namely, A and D); (iii) microelements (I, F, Ca, Cu, Zn, Fe and others); and (iv) highly unsaturated fatty acids. Among these components, the lipid fraction is now the subject of a great deal of attention due to its high content on ω-3 polyunsaturated fatty acids (PUFA).

In spite of this profitable and valuable composition, wild and farmed marine species are known to be highly perishable products. In this sense, it should be stressed that such food products arise from poikilothermic organisms with a high water and non-protein-nitrogen content, a soft muscle and skin structure, and low collagen content. Further, the highly unsaturated lipid composition has been shown to be especially prone to lipid oxidation, which leads to important sensory and nutritional quality losses.

Marine products can be obtained through a wide range of technological processes. In order to minimize the above-mentioned deteriorative behavior, different strategies have been applied for years such as cooling (chilling, freezing and frozen storage), heating (cooking, canning, smoking), and water activity reduction (salting, drying). Among marine products, there is an increased demand for fresh products and represent very high proportions in fish production and human consumption. In order to commercialize fresh seafood, refrigerated and chilled storage have been widely employed. In spite of the reduction in temperature, it has been shown that quality loss could be produced by the following damage pathways: (a) endogenous enzyme activity; (b) microbial decomposition; (c) lipid oxidation. Consequently, significant deterioration of sensory acceptance and loss of nutritional value have been detected which lead to a strong effect on the commercial value. The rate of alteration depends on factors such as the nature of the species, size, lipid content, feeding state at the moment of capture, importance and nature of the microbial load, and storage temperature.

With a view to retarding quality deterioration and improving the storage stability of seafood, refrigeration and chilling technologies have constantly been performed. In some cases, a combination of strategies has been applied, so that each one provides different and complementary advantages (or barriers to spoilage) to ensure quality retention. In connection with the above-mentioned trend of fresh seafood consumption, public health concerns have become an issue requiring careful attention, not only to ensure sensory acceptability and nutritional value, but also safety, as the major challenges faced by marine food trade and technologists. The potential health risks associated with seafood, together with the high demand far from local fishing ports or farms, entails the need for advanced preservation techniques to be addressed continually.

The present article provides an overview on commercial technologies suitable for application in the manufacture of fresh marine products. In it, traditional and advanced strategies are overlooked.

Preliminary Steps to Storage

It is well known that prior to any technological treatment, steps like catching, harvesting, slaughtering and handling can strongly affect the quality of marine species. Such steps, carried out either in farm, on vessel or ashore, are intended to: (i) prevent physical damage; (ii) remove blood, digestive juices, slime and feces, and (iii) avoid bacterial contamination.

Soon after catching/harvesting, the fish begins to stiffen, giving rise to the rigor mortis period. This state in marine species generally has a shorter duration than in mammals. It starts 1–7 h after death; its peak in slaughtered individuals, kept in ice, lies between 5 and 22 h after death. In this period, various physiological reactions in the animals, as the use of energy reserves in the muscle and liver, and change in the acid–base balance are produced. These reactions, by changing the conditions in the tissues, affect the enzyme activity in postmortem fish and the rate of freshness degradation. Physical activity at or before slaughter affects the flesh quality negatively; as the muscle metabolism is predominantly anaerobic, rapid reduction in ATP and pH is produced, resulting in formation of lactic acid. Stressed individuals may develop shorter rigor mortis periods, and a softer muscle texture which is more susceptible to the undesirable separation of muscle blocks (gaping phenomenon). In connection to farm handling, different commercial methods of slaughtering have shown to cause varying stress and aversive behavior. Whatever method is used, the important point is to carry it out efficiently and fast. Killing requires special consideration in terms of both the welfare of the species and the quality of the final product. A prolongation of the rigor mortis period is of great economic importance.

The precise nature and speed of deteriorative changes during the on-board or ashore handling may vary with marine species, and within one species according to endogenous (anatomical, physiological, genetic, chemical composition) and exogenous (previous feeding availability, temperature, season) factors. Fish corresponding to the 'top of the catch' should be of higher quality than those caught during the beginning of the trip. Handling of fish with gaff hooks picks or forks should be avoided. Breaks in the skin and unsightly holes in the flesh quickly introduce spoilage bacteria and accelerate quality deterioration. Fish should not be exposed to direct sunlight or to the drying effect of wind, but should be carefully and safely cleaned and washed, and then cooled down as quickly as possible. Any careless treatment or delay in reducing the

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temperature of fish will have a marked effect on their potential keeping time.

A common on-board treatment for white fish is gutting. Guts are known to be a reservoir of powerful digestive enzymes and bacteria that can spoil the flesh, resulting in unacceptable odors and flavors. As a result of gutting, many studies report an extension of shelf life during marine species refrigeration. For small-sized fatty species however, the process of gutting has shown to be impracticable and may create new cut surfaces for potential bacterial contamination and lipid oxidation development and would not benefit the shelf life.

Traditional Refrigeration Systems

Common Flake Ice

Refrigeration to about 0 °C by using common flake ice (chilled storage) is the preservation method most extensively used to cool marine species rapidly and to extend their shelf life. Under such conditions, the normal storage life of cold water species immediately after post mortem is 1–2 weeks, while species from warm tropical waters keep somewhat longer. Ideally, aquatic species should be in contact only with ice and not with each other. Finally, much of the care exercised by fishermen in handling and stowing the catch may be wasted unless good standards of cleanliness, hygiene and sanitation are maintained at all times.

The rate at which heat can be removed from marine foods during flake ice chilling depends on several parameters. In the case of packaged products, size and shape will affect the rate of heat transfer while in the case of unpacked products special attention should be paid to the relative humidity conditions, with a view to prevent dehydration of the food material. Although pure ice melts at 0 °C, the temperature of material stored in crushed ice may be slightly lower. This effect is due to the salts present in the flesh, and to the seawater on the surface of the carcasses, which cause some of the ice to melt and extract heat from the surroundings.

Quick and efficient flake ice chilling of the seafood to the lowest temperature practicable without actually freezing the flesh is essential if spoilage is to be kept to a minimum. Rapid chilling also aids bleeding and results in more attractive fillets. Besides, contributing to the cooling, the flow or meltwater also washes away bacterial slime, spoilage products and any residual blood and thus helps to preserve the fresh appearance and smell. Room temperature should be kept above 0 °C, so that a good flow of melting water is maintained; however, if this temperature is too high, the weight of necessary ice will be larger.

Chilled and Refrigerated Seawater

Chilled (CSW) or refrigerated (RSW) seawater are the terms commonly used to describe seawater which has been cooled just below 0 °C. RSW holding of marine species is accomplished by filling a hole with seawater and then recirculating it through refrigerated coils. As RSW requires complicated pumping and filtering systems, it may be replaced by CSW, a system where sufficient fresh seawater ice is taken and eventually mixed first with water and then with fish.

Both RSW and CSW are considered to be able to extend the shelf life of fish and shellfish due to the lower storage temperatures achieved. Both hydro-cooling systems have led to a wide range of commercial applications for both on-board and the in-land use, being excellent media for refrigerating and transporting whole carcasses while still at sea and for the road transport of bulk quantities. Thus, the following advantages compared to traditional flake icing have been described: (i) faster cooling; (ii) less stress on food pieces; (iii) the possibility of a lower temperature; (iv) faster handling of large quantities of aquatic material; (v) a longer storage time in a few cases.

However, some negative effects on sensory and nutritional qualities have been encountered, while its on-board handling (pumping, filtering, and so on) has also found some economical and technical difficulties. Such inconveniences when compared with flake ice employment can be resumed as follows: (i) excess of salt intake, (ii) water absorption by species of low lipid content; (iii) partial water-soluble protein loss; (iv) presence of anaerobic bacteria responsible for putrefaction; (v) appearance modification in parameters such as gills, skin and eyes.

Addition of Chemical Preservatives

One previous or simultaneous handling to refrigerated storage has been salt treatment. Such step consists of direct addition of NaCl to marine species or to the ice used to cool them or immersion of seafood material in a brine solution that is followed by the cooling treatment. The preservative effect of salt is due to a decrease in water activity, which leads to lesser potential for microbial attack, and enhancement of functional properties, leading to an increase of the shelf life time. As a result, salt is absorbed by the flesh and imparts a desired flavor to the finished product. Salting has an additional beneficial effect as it toughens the skin and prevents its adhesion to any surface. Salting also brightens the appearance of marine individuals by removing any remaining slime. Salt treatment has mostly been targeted to species that are further processed by any other technological treatment.

However, some bacteria have shown to be able to multiply in a high concentration of salt, especially when the solution carries substantial quantities of oil, blood and pieces of gut. Another problem is that salt contact with fish has been reported to enhance lipid oxidation of the highly unsaturated lipids, protein denaturation, and texture changes. NaCl is said to act as prooxidant by enhancement of the prooxidant effect of chelatable iron ions widely present in fish muscle, especially in the dark one.

Among factors limiting acceptability of crustaceans, melanosis development plays a very important role. In the presence of oxygen and polyphenoloxidase, the monophenolic compounds are first hydroxylated to o-diphenols and then oxidized to o-quinones, which react non-enzymatically with other compounds, giving rise to pigments of high molecular weight and dark color. Sulfites and SO_2 have been applied as dipping solutions in the crustacean industry as being very effective as inhibitors of such enzymatic browning by interfering with the polymerization of quinones. Thus, such preservatives have successfully been applied to delay melanosis reactions in crustaceans, thereby increasing shelf life.

Ozone is a powerful antimicrobial agent that is suitable for application in seafood in the gaseous and aqueous states. Literature resumes most research where its employment at the same time as flake ice has led to significant increases in sensory quality and shelf life of marine species. As an explanation, molecular ozone or its decomposition products rapidly inactivate microorganisms by reacting with intracellular enzymes, nucleic material and other components. In contrast to these positive effects, ozone is also reported to be a powerful oxidant, and has accordingly been said to negatively affect phospholipids, PUFA and membrane proteins. Its employment is recommended only under optimized conditions.

Advanced Strategies in Refrigeration

Super-chilling Systems: Slurry Ice

Various types of cooling systems have been used for 'super-chilling' (−4 °C to 0 °C) of seafood products. Due to the low storage temperatures achieved, such systems have permitted a significant bacterial growth delay and accordingly extend the seafood shelf life. Super-chilling techniques have been described as being especially useful when the fishing banks are so distant that storage in flake ice does not guarantee an accurate preservation of the fish quality at landing; it can either be used prior to traditional chilled distribution or throughout the storage and distribution process. However, super-chilling of muscle food can result in partial freezing, which may lead to negative changes such as drip loss and decreased water-holding capacity; in addition, enzyme activity may increase due to higher concentrations of soluble matter in unfrozen water and improved access of enzymes to substrate.

Newer chilling systems have enabled the storage at subzero temperatures of fish products through the addition of salts and other compounds to ice-water mixtures. These are called 'slurry ice systems,' 'water-binary systems' or 'liquid ice.' Slurry ice can be defined as a mixture of ice particles finely dispersed in an aqueous solution composed of water and other solid components (namely, NaCl) used to decrease its freezing point and achieve temperatures lower than 0 °C, but always above the initial freezing point of fish material. Two phases co-exist in slurry ice: 25–30% of the mixture consists of small spherical ice crystals, although this percentage can be modified according to specific applications, and the remaining 70–75% is water.

Compared with traditional flake icing, new slurry systems show a wide number of advantages which can be summarized as follows: (a) the lower temperature slows down degradative biochemical and microbiological reactions, (b) faster heat exchange that leads to faster cooling, (c) more efficient cooling by complete coverage of external surface, (d) its flow exerts a surface-washing effect, (e) size and geometry of slurry crystals limit the physical damage, (f) its fluid nature allows automation of handling and distribution, (g) versatile technique which can be combined with other preservative strategies.

Meanwhile, a main disadvantage has been identified. Thus, the temperature employed should not decrease until partial freezing of the product takes place; otherwise, sensory quality (cloudy eyes, development of dull color, etc.) could be diminished. Consequently, it is specially recommended that the slurry ice users take good advice from manufacturers in order to apply, in each case, the best combination of parameters involved (optimization pre-step): salt content, storage temperature, liquid–solid ratio and marine species size.

In the scientific literature, reports referring to the application of slurry ice systems to marine products have been extensive in the last decade. In such research, the need to recognize the main degradation processes involved in quality loss is essential. Thus, slurry ice has been applied satisfactorily at different stages: on-board storage and pre-cooling, in-land storage system, slaughtering and storage in farming production and as a treatment prior to other technological processing (chilling storage under flake ice in retail, frozen storage, cooking and canning). In such studies, comparative analysis with traditional flake ice employment was undergone, taking into account changes produced in sensory, microbiological, physical and chemical properties of marine foods.

Packaging Technologies

Different kinds of advanced packaging technologies have been proposed and applied successfully in the latest decades.

The modified atmosphere packaging (MAP) is an increasingly popular food preservation technique that involves the total or partial removal of air contained in the package followed by reintroduction of an altered or modified atmosphere consisting of other gasses, usually carbon dioxide, nitrogen, oxygen alone or in combination. It is specially recommended to preserve quality and to maintain hygienic, sanitary and sensory characteristics of perishable products such as chilled marine products. The carbon dioxide is the most important gas because of its bacteriostatic and fungistatic properties, this property being increased by increasing its concentration in the surrounding atmosphere. The extension of shelf life by using MAP has shown to depend on species, fat content, initial microbiological quality, handling, slaughtering, storage temperature, gas nature and mixture composition, packaging materials, gas-to-product volume ratio, presence of additives, and combination with other hurdle technologies.

It must be taken into account that gas level in MAP could change with time due to dissolution of gasses into the muscle tissue and/or microbiological and biochemical reactions; therefore, experiments are often conducted under controlled atmosphere packaging (CAP) condition for studying the real effect of the different gas constituents. Throughout the storage in CAP, a more stable gas composition is ensured and the accumulation of off-odors is avoided. These advantages make the use of CAP of great interest for marine species preservation on deep-sea fishing boats.

Vacuum skin packaging (VSP) is a technique which was developed to overcome some of the disadvantages of the traditional vacuum packaging. It is characterized by the instantaneous heating of the upper packaging film immediately before its descent over the food surface. The VSP concept relies upon a highly ductile plastic barrier laminate which is gently draped over a food product, thereby molding itself to the actual contours of the product to form a second skin, which is discarded when consuming the seafood. The product's natural shape, color and texture are highlighted and, since no mechanical pressure is applied while drawing the vacuum, soft or delicate products are not crushed or deformed.

Active packaging is an innovative concept in which the package, the product, and the environment interact to extend the shelf life or enhance the safety or sensory properties. This technology includes packaging materials and edible films and coatings which contain preservative substances and also techniques modifying the atmosphere within the package. Additives can be incorporated in different ways, according to the marine food encountered. It must be stressed that incorporation of preservatives into the packaging instead of its inclusion into the bulk has been made for preventing surface microbial growth or lipid oxidation. Moreover, a controlled release of the agent from the surface to the inner of the food can assure the presence of the agent in the food throughout storage. On the contrary, when the preservative is added into the food, it could be bounded to food components or react with other additives or food components losing its activity. All forms of active packaging have received remarkable attention in recent years, improving due to the important role involved in extending food shelf life.

Most of the films used are manufactured with synthetic plastic materials but for environmental reasons, an increasing attention is being paid to active edible films and coating as a result of their biodegradable properties. Proteins, lipids and polysaccharides are the main biopolymers used to produce edible films. Two materials and their combination have recently attracted most interest. On one side, gelatin from marine fish species as a renewable material; on the other side, chitosan obtained from natural chitin not only because of its film-forming capabilities but also because of its antioxidant and antimicrobial properties. It has been shown that films composed of proteins or polysaccharides are sensitive to moisture; however, films composed of lipids are more moisture resistant but are opaque and susceptible of oxidizing. Accordingly, actual trend is to use an optimized combination of biopolymers.

Addition of Natural Preservatives

Sodium and potassium salts of low molecular weight organic acids such as acetic, lactic, citric and sorbic have been used to control microbial growth, improve sensory quality and extend shelf life of many food systems including marine ones. These preservatives are able to control the development of spoilage bacteria and also possess antibacterial activity against various foodborne pathogens such as *Staphylococcus aureus*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Escherichia coli* and *Clostridium botulinum*. Moreover, these compounds represent a relevant choice because of their easy availability, low commercial cost and wide range of permitted concentration for their use.

Since ancient times, spices and herbs have been added to foods as seasoning additives due to their aromatic properties. They inhibit microbial growth of Gram-positive and Gram-negative bacteria, yeasts and molds and also exhibit useful antioxidant activity. Herbs of the Lamiaceae family, mainly oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*), have been reported as having significant preservative capacities. The active compounds responsible for such activity in spices are primarily phenolic components of the essential oil fraction. However, their use in foods as preservatives is limited because of flavor considerations, since effective doses may exceed sensorial acceptable levels.

As already mentioned in the previous section, chitosan is a modified, natural carbohydrate polymer derived by deacetylation of chitin. As one of the most abundant natural polymers present in insects, fungi and crustaceans, it is produced from shell wastes with different deacetylation grades and molecular weights and therefore, different functional properties and biological activities. In particular, antimicrobial and antioxidant activities have shown to depend on its molecular weight and concentration. It was demonstrated that chitosan inhibited the growth of many spoilage and pathogenic bacteria and also yeast and molds; additionally, it may retard lipid oxidation by chelating ferrous ions released during storage by proteins such as myoglobin, hemoglobin and ferritin, thus eliminating pro-oxidant activity of ferrous ions or preventing their conversion into ferric ions.

Live tissues contain a complex oxidation/reduction system which controls the oxidative reactions needed for life. However, during the post-mortem stage of marine species a significant number of reactions occur affecting the oxidative equilibrium of fish muscle, so that the endogenous reducing system (alpha-tocopherol, glutathione, coenzyme-Q, ascorbic acid) is consumed. It is therefore necessary to provide exogenous antioxidants during feeding of cultivated fish and/or during processing/storage of seafood. Such antioxidants have been classified as chelators, reductants or free radical scavengers and represent an effective and profitable methodology for stabilizing seafood products or ω-3 PUFA rich products. Recently, consumers have rejected synthetic antioxidants because of their possible carcinogenicity, so that their replacement by natural ones has been widely developed; the disadvantages are their higher cost and lower effectiveness.

In recent years, the inclusion of plant extracts as a source of natural antioxidants has been a common practice in the food industry. The radical scavenging capacity of some natural compounds such as polyphenols which are known to be present in many fruits and vegetables, as well as their corresponding by-products, has resulted in their use as bioactive antioxidant compounds in food material susceptible to lipid oxidation. Purified natural compounds such as procyanidins, flavonoids and terpenoids, have shown higher antioxidant activity than their corresponding crude extracts. Hydrophilic compounds offer advantages when compared with lipophilic ones as food additives since the former can easily be incorporated into food systems by application into aqueous solutions.

Biopreservation

The term biopreservation is defined as the use of a natural or controlled microflora and/or its antimicrobial metabolites to extend the shelf life and improve the safety of food. This term is linked to lactic acid bacteria (LAB), a group of Gram-positive bacteria which are often used in food to ensure its safety, preserve its quality and develop new flavors. However, and unlike starter cultures, protective cultures used for biopreservation purposes should modify the sensory features of the seafood product as little as possible. Certain LAB species and strains have been shown to exert strong antagonistic activity against spoilage and pathogenic microorganisms such as

Listeria, *Clostridium*, *Staphylococcus* and *Bacillus* spp. Such antagonistic effect is caused by a decrease in the pH of the food – mainly motivated by the production of lactic acid and other organic acids – the competition for nutrients, and/or the production of antimicrobial metabolites.

Due to these benefits, LAB can be used as protective cultures to outgrow competitor microorganisms such as certain spoilage and pathogenic bacteria, with the subsequent benefits in terms of food safety. In addition, several LAB species and strains are able to produce bacteriocins. These molecules are synthesized by the ribosomes, released extracellularly as bioactive peptides or peptide complexes with a bactericidal or bacteriostatic effect on other closely and non-closely related species, while the producer bacterial cell exhibits specific immunity to the action of its own bacteriocin. Members of the genera *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Carnobacterium* and *Enterococcus* are known to secrete bacteriocins, being especially relevant for the biopreservation of raw and processed seafood products the two latest genera.

Bacteriocins as food preservatives may be used for biopreservation purposes either by using the bacteriocinogenic strain as a protective culture or by using the extracellular extracts of the producing bacteria in the food formulation. LAB bacteriocins or bacteriocin-producing strains can be utilized either alone, in combination with one another, or in combination with other antimicrobials to improve the preservative effect.

Among bacteriocins, nisin is the most studied. It has been found efficient for the inhibition of *Listeria* and other spoilage and pathogenic bacteria in marine foods. Some bacteriocins (e.g., lactocin S) are much more slower acting and have different inhibition spectra against spoilage and pathogenic bacteria. Thus, combining fast- and slow-acting bacteriocins, and broad and narrow inhibition spectra, the food remains safe longer. Bacteriocin-producing *Enterococci* are widespread in nature, and they have also been isolated from a variety of seafood products. One of the principal bacteriocins produced by *Enterococci* isolated from fish is enterocin P, which shows a good antimicrobial activity against *L. monocytogenes*, *S. aureus* and other spoilage and pathogenic bacteria such as *Bacillus* spp.

An alternative to antimicrobial agents in the prevention and management of fish diseases in aquaculture facilities is the use of probiotics added to the animal feeds. Some protective cultures of LAB have been reported to exhibit probiotic activity due to their positive contribution to the healthy microflora of the human gut. Moreover, these probiotic LAB have also been introduced into animal feeds due both to their contribution to the health of farmed animals as well as for their activity for the biological control of fish pathogens in aquaculture facilities. The possible mechanisms of action of probiotics are the production of compounds that are inhibitory towards pathogens, competition with targets microorganisms for nutrients and energy, competition with undesirable species for adhesion sites, enhancement of the immune response of the animal, improvement of water quality, and interaction with phytoplankton. It should be highlighted that the probiotic LAB strain introduced in the food chain through the animal feed must also be safe for humans. Accordingly, all microbial strains intended to be used as probiotics in animal feeds must be evaluated and authorized before being used as ingredients in feedstuffs.

High Hydrostatic Pressure Processing

Since the nineties of the last century, high hydrostatic pressure (HHP) processing has been recognized as a powerful strategy to provide attractive, nutritive and safety foods. HHP technology has been shown to retain the sensory and nutritional properties of foods while inactivating microbial populations and leading to shelf-life extension and food safety enhancement. Among different food sources, seafood have proven to be easily compressed and accordingly, and be well suited for HHP processing, normally applied in the 100–600 MPa range.

From a physical point of view, an increase in pressure has an important effect on the molecules, for they get closer to each other. HHP treatment has been reported to follow the Le Châtelier principle; consequently, any reaction or phenomenon in equilibrium accompanied by a decrease in volume can be enhanced by the HHP treatment. Another interesting aspect is that this treatment operates according to the isostatic rule; in compliance with this, pressure is instantaneously and uniformly transmitted throughout the whole food matter. From a chemical point of view, HHP treatment can lead similar changes than does the thermal processing. Thus, the covalent bonds are not broken but the weak energy bonds like hydrogen and hydrophobic bonds can be irreversibly modified. This means that low-molecular-weight food components (responsible for nutritional and sensory characteristics) would not be affected, whereas high molecular weight components (proteins whose tertiary structure is important for functionality determination) are sensitive.

Concerning HHP application to seafood, previous research accounts for a wide range of studies focused on protein denaturation analysis, specially concerning the activity inhibition of deteriorative endogenous (namely, calpains, cathepsins, lipases, phospholipases) and bacterial enzymes. This research topic has been related to all kinds of marine products, both from wild and cultivated sources, and assessing the straight relationship with sensory and nutritional value. HHP conditions applied have been reported to lead to reversible or irreversible damages in proteases, this leading to a marked inhibition of their action; on the contrary, HHP treatment can also disrupt lysosomal membranes and cause the enzyme release into the fish muscle and provoking a subsequent hydrolytic activity increase. The need for HHP conditions optimization is reported to be imperative to attain important shelf life increases in fish products.

Concerning the lipid fraction, changes as a result of HHP treatment of marine foods have also been studied, although not in a so exhaustive way as for the protein fraction. Thus, lipid oxidation has shown to increase as a result of HHP treatment; however, the possible prooxidant effect of HHP treatment on muscle lipids has proved to be eliminated if a previous water washing of the muscle was applied or if a complexation compound (EDTA, e.g.) was added. Consequently, iron-bound protein denaturation during HHP treatment has been reported to facilitate a free metal ion content increase which would be responsible for lipid oxidation in fish meat after HHP treatment. The effect of the HHP treatment on other kinds of marine chemical components (vitamins and minerals) can be considered very scarce.

Finally, it is worth to point out that this technology has shown potential application in the seafood industry when

combined with other processing strategies. This accounts for surimi and kamaboko production, cold-smoked fish preparation and when assisting freezing, thawing and thermal processing. A beneficial effect on quality retention has also been observed when an HHP treatment is used prior to refrigerated, chilled or frozen storage of fish products; as above mentioned, deteriorative hydrolytic and oxidative (namely, peroxidases, lipoxygenases) endogenous enzymes would be HHP-inactivated for a further stabilization of the marine product.

See also: Acids: Natural Acids and Acidulants; Antioxidants: Characterization and Analysis; Bacteriocins; Biofilms; Chilled Foods: Effects on Shelf-life and Sensory Quality; Chilled Foods: Modified Atmosphere Packaging; Chilled Foods: Packaging Under Vacuum; Chilled Foods: Principles; Controlled Atmosphere Storage: Applications for Bulk Storage of Foodstuffs; Essential Oils: Isolation, Production and Uses; Lactic Acid Bacteria; Oxidation of Food Components; Preservation of Foods; Preservatives: Food Use; Probiotics; Quality Control in Food Processing; Spoilage: Bacterial Spoilage; Storage Stability: Mechanisms of Degradation; Storage Stability: Shelf Life Testing.

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Flavor Enhancers: Characteristics and Uses

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Introduction

What is a Flavor Enhancer?

A flavor enhancer is a chemical compound that amplifies and intensifies the flavor impact of other flavor compounds in the mouth when food is consumed. Flavor enhancers are added to food to potentiate its flavor and taste; they are regarded as food additives and not flavorings in many countries. They are defined in European legislation as "substances which enhance the existing taste and/or odor of a foodstuff." It is often thought that flavor enhancers are tasteless; this is not the case as those in common use in the food supply have their own gustatory sensations, which are specific and recognizable as discreet tastes in their own right.

The most commonly used flavor enhancers are the sodium salt of the amino acid glutamic acid, monosodium glutamate (MSG), and the nucleotides disodium-5′-inosinate (IMP) and disodium-5′-guanylate (GMP). The use of these compounds is regulated in various parts of the world, for example, in Europe, they have been assigned E-numbers. Flavor enhancers permitted for use in food are detailed in Table 1.

MSG, IMP, GMP, and other flavor enhancers initiate the 'umami' effect on the palate, which is associated with a savory taste; umami is the Japanese word for delicious or succulent and was the fifth taste sense to be discovered. The functions of the five taste sensations recognized today are considered to be

- sweet (detection of carbohydrate for energy balance),
- sour (protection from acidic and potentially harmful substances),
- bitter (protection from toxic substances),
- salty (regulation of blood salt levels),
- umami (detection of the nutritional/protein content of food, which serves as a marker for adequate protein nutrition).

The umami taste produces salivation releasing gastric juices containing enzymes that help to digest protein to release essential amino acids in the gut. MSG taste also induces insulin secretion in anticipation of an increase in blood amino acids.

Historical Background

Glutamic acid was first isolated in 1866 by the German chemist Karl Ritthausen from an acid hydrolysate of wheat gluten, and he named it after its source. Forty-two years later, in 1908, MSG was discovered by the Japanese chemist Kikunae Ikeda in the broth derived from kombu, *Laminaria japonica*, a kelp-like seaweed used for centuries as a flavorful broth in Japanese cuisine. He patented the discovery and marketed MSG as a table condiment and flavor enhancer through a newly founded company, Ajinomoto, which roughly translated means 'essence of taste.' The Ajinomoto company remains a major force in the market for MSG today.

IMP, the disodium salt of inosinic acid, was discovered in 1913 by Shintaro Kodama a Japanese chemist and student of Kikunae Ikeda, in bonito flakes, traditionally used in Japan as a flavoring agent. Bonito is a sardine-like fish of the *Scombridae* family, common in the seas around Japan. Inosinic acid had been known for a long time since Justus van Liebig's mid-nineteenth century work on beef broth, but its significance as a flavor enhancer had not been recognized.

GMP, the disodium salt of guanylic acid, was discovered by the Japanese chemist Akira Kuninaka in 1960 in the broth derived from shiitake mushrooms used by the Japanese for hundreds of years to flavor foods. Guanylic acid had been known since 1898 when the Norwegian medical chemist, Ivar Christian Bang, elucidated the structure during the course of his work on pancreatic nucleic acid.

Japanese researchers have been instrumental in identifying the significance of MSG, IMP, and GMP as flavor enhancers through their study of the flavorings properties of the traditional Japanese products kombu, bonito, and shiitake mushroom. It is not surprising therefore that the Japanese word 'umami' is used to describe the taste sensation, which was recognized as a taste sense long before its discovery by workers at University Of Miami School Of Medicine in 2000. The team, led by Nirupa Chaudhari, discovered a receptor that interacted with glutamate in a manner consistent with the umami taste sensation and established that umami is a fundamental taste quality similar to sweet, sour, bitter, and salty.

Characteristics of Flavor Enhancers

Chemical Properties of MSG

Glutamic acid is an α,γ-amino dicarboxylate with the structure depicted in Figure 1(a). The monosodium salt (MSG) can form an α-salt or a γ-salt; the α-salt is shown in Figure 1(b).

At the correct pH, the compound has the potential to form a loosely bound cyclic tautomer between the two carboxylate functions Figure 1(c).

Compounds with related chemical structures have also been found to exhibit an umami effect such as monosodium D,L-threo-ß-hydroxy glutamate Figure 1(d), monosodium D,L-homocystate (e), monosodium L-aspartate (f), and the salts of two unusual amino acids, monosodium L-tricholomate (g) and monosodium L-ibotenate (h).

The relative umami contributions measured from these compounds is shown in Table 2.

Chemical Properties of Nucleotides

A nucleotide combines a nucleobase, a pentose sugar (ribose or deoxyribose), and at least one phosphate group. IMP is the disodium salt of inosinic acid (Figure 2), which has an

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Table 1 Flavor enhancers permitted for use in food

EU E-number	*Flavor enhancer*	*Australasian number*	*US FEMA GRAS number*[a]
Glutamic acid and its salts			
E620	Glutamic acid	620	FEMA 3285
E621	Monosodium glutamate (MSG)	621	FEMA 2756
E622	Monopotassium glutamate	622	
E623	Calcium diglutamate	623	
E624	Monoammonium glutamate	624	
E625	Magnesium diglutamate	625	
Nucleotides			
E626	Guanylic acid (guanosine monophosphate)		
E627	Disodium guanylate (disodium guanosine-5′-monophosphate GMP)	627	FEMA 3668
E628	Dipotassium guanylate		
E629	Calcium guanylate		
E630	Inosinic acid (inosine monophosphate)		
E631	Disodium inosinate (disodium inosine-5′-monophosphate IMP)	631	FEMA 3669
E632	Dipotassium inosinate		
E633	Calcium inosinate		
E634	Calcium-5′-ribonucleotides (mixtures of guanylates and inosinates)		
E635	Disodium-5′-ribonucleotides (mixtures of IMP and GMP)	635	

[a]FEMA, flavor and extract manufacturers; GRAS, generally recognized as safe.

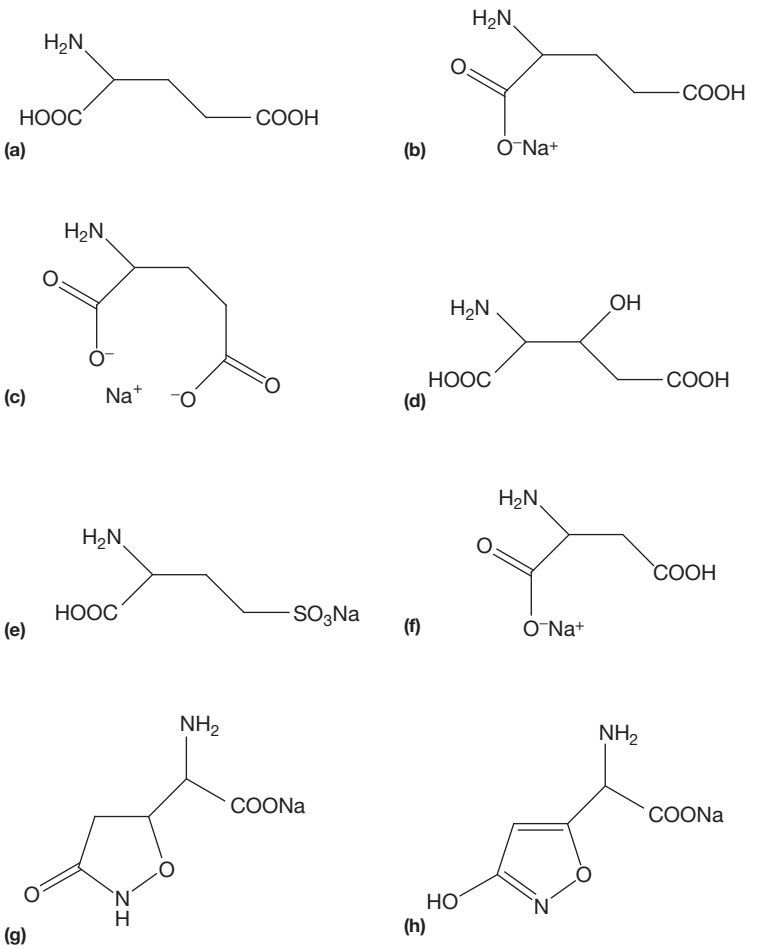

Figure 1 Chemical structures of glutamic acid and other amino acid salts producing umami flavor.

important role in metabolism. It is formed by the deamination of adenosine monophosphate (AMP). GMP is the disodium salt of guanosine monophosphate and consists of a phosphate group, the pentose sugar ribose, and the nucleobase guanine (Figure 2).

When the nucleobase is a purine moiety with a hydroxyl function on the 6-carbon of the purine ring and there is a phosphate ester on the 5′-carbon of the ribose moiety, an umami taste is imparted. Both IMP and GMP possess these chemical features (Figure 2). If the ribose moiety is phosphorylated on the 2′ or 3′ positions, the compounds are tasteless. Phosphorylation on the 5′carbon of the ribose ring is essential for umami taste.

Physical Properties

MSG, IMP, and GMP are all crystalline solids and are nonhygroscopic; consequently, they can be stored for long periods of time at ambient temperature without their appearance or quality changing. The solubility of flavor enhancers is important when they are blended into food formulations. MSG is much more soluble in water than IMP or GMP. However, IMP has a much higher solubility in vinegar than either MSG or GMP; GMP becomes gelatinous in acidic solutions, such as vinegar.

Glutamate is not decomposed during normal cooking processes. IMP and GMP are stable in aqueous solution, but under acidic conditions (pH 3.5 and below) and at high temperatures, decomposition takes place and umami taste is lost. Phosphatase enzymes, occurring naturally in plant and animal materials, remove the phosphate group from IMP and GMP. Consequently, during food processing, IMP and GMP should be added after deactivation of these enzymes by heat.

Table 2 The relative umami contributions measured from the compounds shown in Figure 1

Umami substance	*Relative umami intensity*
MSG	1.00
Monosodium D,L-threo-ß-hydroxy glutamate	0.86
Monosodium D,L-homocystate	0.77
Monosodium L-aspartate	0.077
Monosodium L-tricholomate	5–30
Monosodium L-ibotenate	5–30

Source: Yamaguchi, S., (1979). The umami taste. In: Boudreau, J. L. (ed.) *Food taste chemistry*, ACS Symposium Series 115. Washington, DC.

Measurement of Flavor Enhancers in Foods

A range of methods has been used for the analysis of glutamate in food products: spectrophotometric, derivative HPLC, HPLC with UV detection, fluorescence detection, GC, paper chromatography, and potentiometric methods.

Standard methods for analysis of nucleotides are based on liquid chromatography with UV, fluorescence, and mass

Table 3 Glutamate content of various foods

Food	*Glutamate content (mg 100g^{-1})*
Kombu (kelp)	1608
Parmesan cheese	1680
Emmentaler cheese	308
Cheddar cheese	182
Chicken	22
Beef	10
Pork	9
Alaskan king crab	72
Shrimp	20
Scallop	140
Sweet corn	106
Tomato	246
Potato	10
Spinach	48
Cabbage	50
Green peas	106
Shiitake mushroom (fresh)	71
Soy sauce (Korea)	1246
Fish sauce (Vietnam)	1370
Natto/soybeans (Japan)	136
Douche/soybeans (China)	476
Soumnara/locust beans (West Africa)	1700

Source: Ninomiya, K., (1998). Natural occurrence. *Food Review International* 14, 177–212.

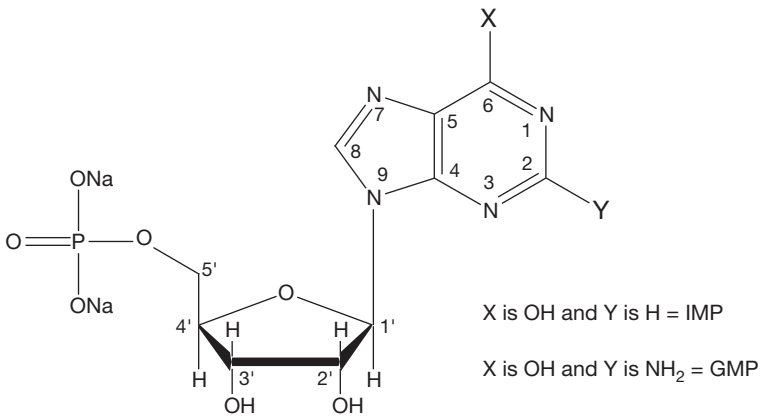

Figure 2 Chemical structures of nucleotides disodium inosinate (IMP) and disodium guanylate (GMP).

spectrometer detection. Other specialist techniques, such as reverse-phase HPLC and ion-pairing HPLC, are also available.

Threshold Levels and Synergy

Studies by a number of groups of researchers into the threshold levels of the three major umami inducers have produced broadly similar results; the average detection threshold levels are shown in Table 4. When IMP and GMP are combined with MSG, there is a synergistic enhancement such that the 'umami' taste is increased to an extent that would not be expected from the sum of their individual effects; food manufacturers use combinations of MSG and nucleotides to increase the 'umami' taste of savory products.

There is also a synergistic effect between the nucleotides; a 50:50 mixture of IMP and GMP reduces the threshold of the two flavor enhancers by roughly 50%. However, the combination of low levels of MSG with the individual nucleotides produces a dramatic change to their threshold detection levels. Conversely, the addition of a small quantity of IMP to MSG lowers the detection threshold of MSG, but the effect is less dramatic (Table 5).

The nucleotides have lower thresholds than MSG; when used commercially, the prices of the nucleotides are approximately ten times higher than MSG, so exploiting the synergistic effect in food products has been aimed at lowering cost by adding small amounts of nucleotides to MSG. Table 4 shows that low concentrations of IMP and GMP can significantly reduce the amount of MSG applied to a food to produce an equivalent umami effect (Table 6).

In practice, manufacturing methods for the two 5′-nucleotides results in equal quantities of IMP and GMP being produced, which has led suppliers to offer 50:50 mixtures of IMP and GMP, and it is this mixture that is used in combination with MSG to produce the synergistic flavor enhancement of food products. Nucleotides were commercially introduced in 1960, and due to their higher flavor potency, they have increasingly been replacing MSG or reducing the amount used in foods by exploiting the synergy phenomena.

The Japanese have exploited synergy in their cuisine for centuries. For example, miso soup is made by producing a dashi stock to which miso paste is added. The dashi stock usually consists of a combination of kombu (MSG source) and bonito (IMP source). The miso paste is produced by fermenting soybeans, barley, or rice with a koji fungus, *Aspergillus oryzae*, followed by treatment with a lactic acid bacterium, which leads to the formation of high levels of glutamic acid and its salts.

Flavor Enhancers and Salt Reduction

The intake of sodium in the diet has been linked to hypertension and increased risk of cardiovascular disease. Currently, the daily adult intake of sodium is approximately three times the recommended daily allowance, and therefore, regulatory authorities are recommending a reduction in sodium intake to 2.4 g (6 g salt) per day. Food companies are investigating a range of strategies for reducing sodium or salt content of foods, and some of these involve the use of flavor enhancers to increase the salty taste of foods so that lower concentrations of salt can be used to achieve the same effect. For example, glutamic acid can be used to mask the bitter taste of potassium chloride (added as a salt replacer), and IMP and GMP can be used in conjunction with sodium acid sulfate (US Patent 2013/0171327A1) to reduce salt content of food by between 30% and 50%.

Table 4 Content of IMP, GMP, and AMP in various foods

Food	*IMP content (mg 100g^{-1})*	*GMP content (mg 100g^{-1})*	*AMP[a] content (mg 100g^{-1})*
Beef	70	4	8
Pork	200	2	9
Chicken	200	5	13
Squid	ND	ND	184
Tuna	286	ND	6
Bonito	285	–	Trace
Snow crab	5	4	32
Scallop	ND	ND	172
Tomato	ND	ND	21
Green peas	ND	ND	2
Shiitake mushroom (dried)	ND	150	–
Porcini mushroom	ND	10	–
Oyster mushroom	ND	10	–
Morel mushroom (dried)	ND	40	–

[a]AMP = adenosine monophosphate.

Source: Yamaguchi, S. and Ninomiya, K., (2000). The use and utility of glutamates as flavouring agents in food. *Journal of Nutrition* 130, 921S–926S.

Table 5 Average detection threshold concentrations for nucleotides in the presence of different MSG concentrations

Flavor enhancer	*Threshold levels (water) (%)*	*In 0.1% MSG solution*	*In 0.01% IMP solution*
IMP	0.012	0.0001%	–
GMP	0.0035	0.00003%	–
MSG	0.03	–	0.002%

Source: Kuninaka, K., (1966). Recent studies of 5′-nucleotides as new flavor enhancers. In: *Advances in Chemistry Series*, No 56, 261–274, American Chemical Society.

Table 6 The effect of increasing the ratio of MSG to IMP or GMP on relative umami intensity

Ratio of		*Relative umami intensity*	*Ratio of*		*Relative umami intensity*
MSG	*IMP*		*MSG*	*GMP*	
1	0	1.0	1	0	1.0
1	1	7.0	1	1	30.0
10	1	5.0	10	1	18.8
20	1	3.5	20	1	12.5
50	1	2.5	50	1	6.4
100	1	2.0	100	1	5.4

Source: Matheis, G., (2007). Flavour Modifiers. In: Ziegler, E and Ziegler, H., (eds.), Flavourings: production, composition, applications, regulations, (2nd ed.), Wiley.

Food Occurrence

Flavor enhancers are used widely in conjunction with flavoring materials throughout the food supply chain for a number of reasons:

- To improve the palatability and enjoyment of foods
- To standardize foods and reduce natural variations in flavor
- To mask bitterness in foods
- To create brand recognition
- To create variety across a brand such as the range of flavors found in snack foods
- To create novelty and unique flavor delivery effects
- To create cultural identity
- To serve as a vehicle to deliver nutraceuticals (molecules that benefit health)

Glutamic Acid and its Salts

Glutamate is naturally present in virtually all foods especially in meat, fish, shellfish, cheese, and tomatoes. It is present not only as its sodium salt but also as its potassium, calcium, and ammonium salts depending on which cations are available to combine with glutamic acid. The highest concentrations are found in kombu, Parmesan cheese, Roquefort cheese, soy sauce, fish sauce, yeast extract, and tomatoes. The concentration of glutamates increases considerably after ripening of certain foods, such as Parmesan cheese and dry-cured ham. Free glutamates exist in certain cheeses and dry-cured meats, in tomato products, and in soy sauce. Glutamic acid in the bound form is a major constituent of food proteins (plant and animal), and free glutamic acid is naturally present in most foods providing a mild umami taste compared to MSG (Table 3).

Patterns of consumption

It is estimated that the average person consumes around 17 g of glutamate in free and bound forms (in protein) every day. It is important in brain function, and there are considerable amounts of both free and bound glutamates in our bodies at any one time – the body produces around 40 g d^{-1} to maintain normal physiological functioning. Free glutamate consumed as MSG equals around 1/1000th of the total glutamate present in the body. Individual consumption ranges from around 0.5 to 1 g d^{-1} in Western countries, but in Japan and Korea, it is typically three times this level rising to around 4–5 g d^{-1} in China, where MSG is widely used in cooking.

Nucleotides

Nucleotides are naturally occurring substances that are ubiquitous in foods especially meat, fish, shellfish, and mushrooms. GMP is present at the highest concentrations in shiitake mushrooms, while IMP is found at the highest concentration in bonito flakes (Table 4).

Pressure on the food industry to remove E-number compounds, such as flavor enhancers, from food products has led to a search for alternatives. Yeast extracts contain significant amounts of the nucleotides IMP and GMP as well as varying amounts of glutamate salts. Other sources of MSG are hydrolyzed vegetable proteins (HVPs), tomato powder or purees, and soy sauce. These materials do not require E-numbers on the label and therefore satisfy the 'clean label, additive-free' strategy adopted many food companies.

Manufacturing

Fermentation

Ajinomoto has been producing amino acids by fermentation for many years. It dominates the MSG market with a market share of 34% (200 000 tons per annum). Cheil Jedang Group in Indonesia and Daesang in Korea are also major producers of MSG using fermentation technology. Production capacity and demand for MSG are greater than for any other amino acid. It is manufactured via fermentation of corn, sugarcane, or tapioca using the bacterium, *Corynebacterium glutamicum*, which is capable of producing high yields of L-glutamic acid from sugar. Ammonia is used as the nitrogen source and oxygen is provided by passing compressed air into the fermenting vessel. The L-glutamic acid is released by the microorganism into the fermentation medium and recovered by downstream crystallization.

In addition to the amino acid MSG, these companies also produce the nucleotides disodium inosine 5′-monophosphate (IMP) and disodium guanosine 5′-monophosphate (GMP) by fermentation. They have developed microbial strains, such as *Escherichia* or *Bacillus*, capable of overproducing MSG and nucleotides. These overproducing strains have been developed using molecular biological techniques and tools, such as gene disruption and amplification and efficient gene expression systems. IMP and GMP can also be produced by the enzymatic degradation of RNA with 5′-phosphodiesterase; however, economically, this process does not compete with fermentation.

The use of fermentation to produce these nucleotides and MSG can satisfy the vegetarian market with umami flavor enhancers derived from plants. Fermentation also offers a route to natural flavor enhancers; there is an enormous consumer demand for natural foods and beverages in the United States and Europe, and the requirement for natural flavorings to satisfy this is increasing.

Health Effects

Absorption and Metabolism of Flavor Enhancers

Glutamate is absorbed from the gut by an active transport system specific for amino acids. This process can be saturated and competitively inhibited and is dependent on sodium ion concentration. Glutamic acid is a nonessential amino acid, but it is used in the synthesis of L-arginine, which is an essential amino acid for protein synthesis in cells.

Evidence from human subjects, rats, and nonprimates indicates that free glutamate acts as a signal to regulate protein intake and nutritional status. Glutamate plays an important role in the body's disposal of excess or waste nitrogen. It undergoes deamination, an oxidative reaction to produce ammonia, which is then excreted predominantly as urea, and transamination with pyruvate to yield oxaloacetic acid. This effectively allows nitrogen from the amine groups of amino

acids to be removed, via glutamate as an intermediate, and excreted from the body in the form of urea.

Glutamate is also an excitatory neurotransmitter in the vertebrate nervous system; it is stored in vesicles at chemical synapses. Nerve impulses trigger release of glutamate from the presynaptic cell, and glutamate receptors in the postsynaptic cell bind glutamate and are activated. Because of its role in synaptic plasticity, glutamate is involved in cognitive functions like learning and memory in the brain.

Nucleotides are synthesized within the cells of the body but are also ingested in foods. They are the essential monomeric building blocks of DNA and RNA for cells in the body, but they also function as cofactors and coenzymes for cellular messengers aiding with communication between the nucleus and the cytoplasm.

Most dietary nucleotides are rapidly metabolized and often excreted. However, at times of rapid growth, limited food supply, disease, stress, and immunologic challenges, dietary nucleotides become essential for healthy development and are incorporated into tissues. Under normal conditions, sufficient nucleotides are available through internal metabolic and catabolic processes: either the *de novo* synthesis of nucleotides or the reuse of nucleotides from dead cells (the 'salvage pathway'). Dietary nucleotides are reported to have significant effects on lymphoid, intestinal and hepatic tissues, and lipid metabolism. The nucleotides can serve as nucleic acid precursors and exogenous sources of purine and pyrimidine bases. Dietary purines are not significantly incorporated into hepatic nucleic acids, but pyrimidines are. Both are taken up by intestinal cells with excess purines converted to uric acid.

Health Effects of Glutamate

Chinese restaurant syndrome

Controversy regarding the use of MSG in foods stems from a letter to the *New England Journal of Medicine* in 1968, which speculated that MSG could be the cause of adverse reactions (a burning sensation in the neck, chest tightness, nausea, and sweating) following consumption of food in Chinese restaurants. This became known as Chinese restaurant syndrome (CRS) and resulted in a backlash against the use of MSG and its subsequent removal from many foods. After a considerable period of safety evaluations, the Joint FAO/WHO Expert Committee on Food Additives, the EU Scientific Committee for Food, and the US Food and Drug Administration all concluded that the levels of MSG added to food do not represent a hazard to health.

Investigation of the 'syndrome' itself has yielded no clear causative agent to date. A double-blind controlled challenge of individuals claiming to suffer from the syndrome failed to confirm MSG as the causative agent. Other studies have found that allergic-type reactions after Asian meals are more often due to other ingredients, such as shrimp, peanuts, spices, and herbs. More recently, CRS has been linked to the pathogen *B. cereus* and the practice of reheating rice in Chinese restaurants. *B. cereus* is a spore former and a toxin producer; when rice is heated, the vegetative *B. cereus* cells are destroyed but the spores survive. On cooling, they germinate, grow, and produce an emetic toxin in the product during storage. Reheating the rice prior to serving does not inactivate the toxin, which is insensitive to heat. Consuming rice containing a 'trigger' level of the toxin will produce severe food poisoning, but lower levels could cause CRS symptoms.

Table 7 Concentrations of free glutamate in the milk of different mammals

Free glutamate in milk (mg 100g^{-1})	
Human	21.6
Chimpanzee	38.9
Rhesus monkey	4.6
Cow	1.9
Sheep	1.4
Mouse	2.2
Cat	2.6
Rat	1.6
Rabbit	4.6

Source: *Sweet*, *sour*, *bitter*, *salty*, *umami – It's simply a matter of taste*. Published by the Umami Information Center, Tokyo, Japan.

Glutamate in human infant milk

Human and primate milk is rich in free glutamate, about 20 times the amount found in cow's milk. So glutamate is one of the first tastes we experience in life (Table 7).

Glutamate is the most abundant free amino acid found in human milk and that of other primates. It is not found at significant levels in the milk of other species. Glutamate in human milk is at its highest concentration straight after birth and is important for inducing the newborn infant to feed.

Work in Japan with newborn infants has shown them to produce 'satisfied expressions' when fed solutions of sugar and vegetable soup containing MSG and 'dissatisfied expressions' when fed vegetable soup without MSG. Despite the clear importance of MSG to infant nutrition, its addition to baby milk is still banned following the CRS fallout. The synergistic effect between the nucleotides IMP and GMP with MSG could be important for the health of neonates.

Health Effects of Nucleotides

Although they do not fit the exact definition of a prebiotic, dietary nucleotides act like prebiotic agents, demonstrating immunomodulating and direct beneficial effects on the composition of gut microflora. Nucleotides can also be described as 'functional foods' because they have a potentially positive effect on health beyond basic nutrition.

Nucleotides in infant milk

Rapidly growing infants require nucleotides for protein synthesis and cell growth. This is particularly important in the gut, where cell turnover is high and nucleotides are constantly required to replace cells. The gut also plays a key role in the immune system: some 70–85% of the body's immune system is located there. Nucleotides are increasingly considered by nutrition experts to be essential nutrients at times when the endogenous supply is insufficient to meet elevated demands, such as under conditions of rapid growth.

A large number of studies have found that nucleotide supplementation of infant formula milk has beneficial effects on

health. Human milk contains more nucleotides than cow's milk; thus, many infant formula manufacturers add small amounts of nucleotides as an ingredient to make the formula match maternal milk more closely. A high nucleotide intake has been suggested as an explanation of some of the benefits of breastfeeding compared with formula feeding. The European Society for Paediatric Gastroenterology, Hepatology and Nutrition coordinated a study with an International Expert Group, which produced a medical position paper in 2005 on Global Standard for the Composition of Infant Formula. The paper recommends the addition of nucleotides to infant formulas at a maximum total content of 5 mg 100 $kcal^{-1}$ (as well as maximal levels of 2.5 mg 100 $kcal^{-1}$ cytidine 5′monophosphate (CMP), 1.75 mg 100 $kcal^{-1}$ uridine 5′monophosphate (UMP), 1.5 mg 100 $kcal^{-1}$ AMP, 0.5 mg 100 $kcal^{-1}$ GMP, and 1.0 mg 100 $kcal^{-1}$ IMP). No adverse effects have been reported to date in healthy infants fed nucleotide-supplemented formula milk.

The beneficial effects of nucleotide addition to infant formula include improved composition of gut microbiota, decreased risk of diarrhea, increased weight gain and head growth, and increased immunity following vaccinations against *H. Influenzae* type b, polio, and diphtheria toxoid in formula-fed infants. For example, one study carried out in 2005 by a team including the Institute of Child Health in London and the Universities of Nottingham and Dundee found that supplementation of infant formula with 31 mg nucleotides l^{-1} had a prebiotic effect on colonic microbiology by improving the ratio of *Bacteroides–Porphyromonas–Prevotella* group to *Bifidobacterium* spp. *Bifidobacterium* spp. predominate in the large intestine of breastfed infants (where they are suggested to confer health benefits by protecting against infection from enteropathogenic organisms), whereas formula-fed infants are more frequently colonized by *Bacteroides–Porphyromonas–Prevotella* spp. The study concluded that the addition of nucleotides to infant formula milk could be important for optimizing the diet of formula-fed babies.

Studies in other mammals have revealed a similar picture. The supplementation of the diet of weaning piglets with nucleotides has shown a variety of beneficial effects. When piglets are weaned, they can suffer severe stress due to a change in diet, a lack of immunity, a change in gut flora, and a marked reduction in food consumption. This can lead to a reduction in the length of the villi on the intestine wall and decreased intestinal development. Enterocytes in the gut have a high level of metabolic activity, and their renewal is constant, with an average life span in the intestine of a piglet of around 4–5 days. Piglets are born without a functional immune system, relying on maternal antibodies present in colostrum and milk for defense against disease. The internal immune system of the piglets develops as the concentration of antibodies in the maternal milk decreases. This development is not complete at weaning, and consequently, farmers can be faced with health problems, such as diarrhea, in postweaned piglets. This problem can be significantly reduced by supplementation with nucleotides.

Nucleotides improve the production parameters of animals by enhancing the development of the immune system and the intestinal mucosa of piglets and reducing the stress that the animals may suffer as a result of diseases or weaning. Animals fed nucleotide-supplemented diets generally show enhanced resistance to viral, bacterial, and parasitic infections and have an increased number of lymphocytes per villus. The effect is long-lasting and most pronounced when piglets receive nucleotides preweaning. Nucleotides fed at an early stage of life enhance the development of intestinal structures and microflora leading to an increase in the active surface of the intestinal tract, facilitating the digestion of feed and the uptake of nutrients. The feed conversion ratio is thus improved. The effects of nucleotides are dose-dependent; the final weight, the daily weight gain, and the improvement of the feed conversion ratio are dependent on the dosage of the nucleotides in the feed.

Sows can also benefit from a nucleotide-supplemented diet. When nucleotides are added to sow feeds, there is an increase in the number of litters per year, the litter size, and the number of piglets weaned. The farrowing rate was increased and the number of sows returning to service was reduced. The uniformity of the litter was also improved.

Role of Nucleotides in Immune Response

Dietary nucleotides have been shown to be required for normal immune defense in adults. In two separate double-blind clinical studies, the patients fed a diet containing nucleotides had an improved immune function compared with patients receiving a nucleotide-free diet. In addition, infectious complications and length of hospital stay were reduced by nucleotide supplementation of postoperative cancer patients compared with a control group.

T lymphocytes appear to require dietary nucleotides for normal maturation and function. Host resistance to bacterial and fungal infections was decreased in mice on nucleotide-free diets; addition of RNA or uracil prevented this vulnerability to infection. Dietary RNA is required to restore lost immune function after protein deprivation. Adult rats receiving diets containing nucleotides (yeast, RNA, and arginine) showed accelerated healing of ulcers. Investigations in animal models and clinical literature suggest that administering dietary nucleotides helps to minimize infectious complications, improve clinical outcomes, and provide cost-effective nutritional support.

Exogenous nucleotides have also shown promise as dietary supplements to enhance immunity and disease resistance of fish produced in aquaculture. Research indicates that they can improve growth of fishes in early stages of development, enhance larval quality via broodstock fortification, alter intestinal structure, increase stress tolerance, and modulate innate and adaptive immune responses. Fishes fed nucleotide-supplemented diets generally have shown enhanced resistance to viral, bacterial, and parasitic infection.

Role of Nucleotides in Brain Function

Studies indicate that nucleotides play an important role in cell-to-cell communication through P2 purinoceptors. Some P2 purinoceptors are activated by nucleotides and couple to intracellular second-messenger systems through specific proteins (heteromeric G-proteins). This represents strong evidence for

extracellular nucleotide signaling and communications in glial cells in the CNS.

Other studies have suggested that nucleotide supplements can reduce the deterioration of brain tissue and memory and prevents aging. Aging mice given a nucleotide-supplemented diet performed better in a learning capability test than the control group of mice. The nucleotides were thought to stimulate glial cell proliferation, neurotubule endothelial cell proliferation, and the growth of nerve axons by interaction with other growth factors. They also suppressed the spongy degeneration phenomenon of the brain stem reticular formation, which is closely related with learning capability and memory disorder. In an earlier study, memory-deficient senescence-accelerated mice and mice with dementia showed improved memory with dietary nucleoside and nucleotide supplementation.

Effects of Nucleotides on Liver Function

A balanced mixture of nucleosides (molecules comprising a pentose sugar and a base) and nucleotides may improve hepatic function in rats and promote earlier restoration of nitrogen balance following liver injury according to one recent study. In another report, increased hepatic cholesterol and lipid phosphorous and serum bilirubin levels and decreased liver weight and glycogen were observed when feeding nucleotide-free diets to weaning mice in comparison with mice receiving nucleotide-enriched diets.

Effects of Nucleotides on Cholesterol

Feeding a nucleotide-supplemented formula diet to rats resulted in increases in long chain polyunsaturated fatty acids in their plasma. Furthermore, when rats were fed diets supplemented with nucleotides (AMP, CMP, GMP, IMP, and UMP), the concentrations of long chain polyunsaturated fatty acids on red blood cell membranes were enhanced compared to the control group. Dietary nucleotides may have the potential to modify the conversion of essential fatty acids into their long chain derivatives, which have been shown to lower cholesterol and reduce the risk of heart disease.

Evolutionary Significance of Nucleotide Flavor Enhancers

The fact that nucleotides and MSG have a strong umami flavor as well as many health benefits may not be coincidental. It would be a clear evolutionary advantage to favor foods that guaranteed healthy development of infants as well as resistance and immunity to disease.

Concluding Remarks

Flavor enhancers have had a bad press in recent years and as a result food manufacturers have been removing them from many food products. However, humans enjoy the flavor of these compounds and have a specific taste for them; removing flavor enhancers from food reduces its palatability as well as the market share for the food companies concerned. Because flavor enhancers are as essential to savory foods as sugar is to sweet foods, alternatives, such as yeast extracts and HVPs, have been developed that naturally contain high levels of MSG, IMP, and GMP.

Over the past decade, many discoveries have been made showing the importance of flavor enhancers, in particular the nucleotides, for health. Benefits are especially significant for young animals including human infants. It is probably not surprising, therefore, that animals possess a specific taste receptor forged by evolutionary processes to detect flavor enhancers as part of a survival strategy. Education of the public about the health benefits of these food additives may change attitudes and lead to the more judicious and considered use of flavor enhancers in food products to promote health as well as flavor.

See also: Amino Acids: Determination; Appetite Control in Humans: A Psychobiological Approach; Cheese: Types of Cheeses – Hard; Fermented Foods: Composition and Health Effects; Fermented Foods: Origins and Applications; Fish: Dietary Importance and Health Effects; Fish: Fish in the Human Diet; Food Additives: Classification, Uses and Regulation; Functional Foods; Meat: Role in the Diet; Milk: Role in the Diet; Mushrooms and Truffles: Role in the Diet; Poultry: Processing; Prebiotics; Shellfish: Role in the diet; Soy Beans: Dietary Importance.

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Folic acid and Folates: Physiology and Health Effects

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The Vitamin Folate and Folic Acid

The generic term folate refers to a large number of mainly reduced vitamers (Figure 1) with similar chemical and nutritional properties to pteroyl-L-(mono)glutamic acid (folic acid). The term folic acid denotes the fully oxidized more stable synthetic compound of this B vitamin, which is used for food fortification and in pharmaceutical preparations. Most of the naturally occurring folates in foods are polyglutamyl vitamers of tetrahydrofolate, which are susceptible to interconversion and oxidative degradation. Oxidative cleavage of the molecule results in loss of the vitamin activity.

The vitamin participates, as a coenzyme, in one-carbon transfer reactions in the body, for example, purine biosynthesis and amino acid interconversions. A good folate status is, therefore, essential for normal cell division. Folates have a recognized role in the prevention of megaloblastic anemia and of neural tube defects (NTDs) in the fetus, such as spina bifida. A good status is also linked to several other health benefits, for example, a reduced risk for certain cancers, cardiovascular diseases (CVDs), and neuropsychiatric disorders. However, in several European countries, the estimated dietary folate intake is below nutritional recommendations. To bridge the gap between these recommendations and actual folate intake, mandatory folic acid fortification has been implemented in many different countries, for example, the United States, Canada, and Chile.

Food Sources for Folate

Folate is an essential micronutrient that has to be obtained through diet. Typical folate sources are leafy green vegetables, pulses, liver, yeast, citrus fruits, nuts, and some berries. Native folate content in food is usually low, ranging from a few to some hundred parts per million.

Traditionally, food folate composition data derive from the microbiological assay using *L. rhamnosus* ATCC7469. In order to differentiate and quantify individual folate forms, HPLC methods are also used. Depending on the chosen quantification method, sample pretreatment is more or less laborious but always a crucial step. Thorough quality control and documentation during all analytic steps is required. However, conflicting folate data also arise through variation in folate content in foods of different origins, because climate, growing area, cultivar, and maturity also affect folate content.

Foods are grouped according to their folate content into rich (>100 μg per serving), good (50–100 μg per serving), and moderate (15–50 μg per serving) sources. Rich sources of folate are dried legumes with a folate content up to 600 μg/100 g (cowpeas and chickpeas) and green leafy vegetables, which reach up to 100 μg/100 g (spinach). Other vegetables and fruits, except strawberries, which are considered a good folate source with 65–100 μg/100 g, are moderate sources. Cereals are important sources since they are consumed frequently in high amounts in many countries. A general rule is that wholegrain products or products with a high extraction rate contain more folate than the starchy endosperm. The cereal fractions bran, germs, and aleurone flour are rich folate sources. Different folate forms are found in food. In cereals and legumes, both formyl and methyl vitamers are predominant, whereas in vegetables and fruits, mainly 5-methyltetrahydrofolate is found.

Effects from Food Processing

Storage and processing of foods, under both household and industrial conditions, affect folate content. Most foods are processed before consumption and common processing methods include thermal processing, freezing, fermentation, soaking, germination, and drying.

Since most native food folates are unstable, the folate content of many processed and stored foods is considerably lower than in the raw material. Food processing techniques and storage decrease the folate content to a variable extent (Table 1), and vitamin retention is affected by the amount of soluble oxygen and protective antioxidants (e.g., ascorbic acid) present in the food. Data regarding folate retention are inconsistent and depend on processing conditions.

Food fortification is the addition of nutrients to food above levels normally present. For folic acid, this practice is governed differently as either mandatory or voluntary fortification. In the United States and Canada, cereal-grain products are fortified at 140 μg/100 g, aiming to provide an additional 100 μg folic acid on top of dietary folate intake. Other folic acid-fortified products – offered in some countries through voluntary fortification – are beverages (juices, soft drinks, and mineral water), snacks, and table salt. Also, dairy products were considered as vehicles for folic acid fortification. In retention trials with model food systems, synthetic folic acid exhibited retention several-fold higher than native food folates. However, processing techniques can also enhance folate content (Tables 2 and 3). For example, milling sequesters cereal fractions rich in folate and bioprocessing techniques; fermentation and germination can also lead to enhanced vitamin content.

Germination is a biological process used to obtain a typical flavor and texture in foods, during which folate is synthesized *de novo* for cell differentiation and growth. Germination of cereal seeds and legumes results in up to a sixfold increased folate content, depending on germination conditions (temperature and time) and plant variety (Figure 2). Soaking for just a few hours or overnight increases folate content by up to half.

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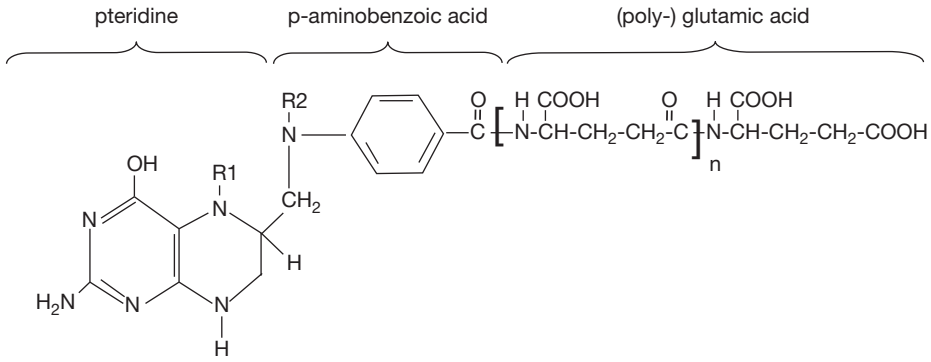

Folate vitamer	R1 at N^5	R2 at N^{10}
Tetrahydrofolate, H_4folate	H	H
5-methyltetrahydrofolate, 5-CH_3-H_4folate	CH_3	H
5-formyltetrahydrofolate, 5-HCO-H_4folate	HCO	H
10-formyltetrahydrofolate, 10-HCO-H_4folate	H	HCO
5,10-methylenetetrahydrofolate, 5,10-CH_2-H_4folate	-CH_2-	
5,10-methenyltetrahydrofolate, 5,10-CH^+-H_4folate	-CH=	

Figure 1 The structure of polyglutamyl tetrahydrofolates.

Table 1 Processing that decreases folate content in foods

Process	*Food*	*Folate loss (%)*
Boiling	Various vegetables	20–80
Steaming	Various vegetables	0–40
Blanching	Various vegetables	40–90
Canning	Various vegetables	0–90
Pasteurization	Tomato puree	15
	Milk	10
Oven-baking	Bread	15–70
	Fish	30–45
	Meat	35
Chill + reheating	Various vegetables	25
Frozen + reheating	Ready-to-eat meals	5–50
Soaking + boiling	Legumes	30–70
Blanching + freezing	Legumes	30–85
Blanching + tinning	Various vegetables	80–90
Storage, frozen	Various vegetables and fruits	0–20
Storage, chilled	Juice	0–5
	Fermented milk	0
	Bread	15
Storage, ambient temperature	Various fruits	0–30
	Bread	30

Table 2 Processing that increases folate content in foods

Process	*Food*	*Folate increase*
Germination	Rye grains	1.6–6-fold
	Wheat grains	3–6-fold
	Legumes	1.8–3.5-fold
Fermentation	Wheat bread	0.6–2-fold
	Milk	3–4-fold

Table 3 Novel functional foods with increased folate content by the addition of germinated plant ingredients

Food	*Description*	*Folate increase (%)*
Bean stew foul	A canned stew prepared from soaked and boiled fava beans, consumed in Arabic countries. The novel product is prepared from germinated fava beans before canning	50
Baladi/pita bread	A flat wheat bread consumed in Arabic countries. Wheat grains are germinated, dried, and milled. 50% of wheat flour is replaced by germ flour	40
Biscuits	Wheat flour biscuits are baked replacing 25% of flour with germinated chickpea flour	570
Rye muesli	Malted rye grain flakes are produced from germinated rye and subsequent freeze-drying	250
Wheat bread	Wheat bread was leavened with a *Saccharomyces cerevisiae* strain (CBS7764) and cultured in defined medium	300–500
Fermented milk products	Several fermented milk products are prepared by fermentation with specific starter cultures and addition of fruits	200

During fermentation, raw materials undergo changes in composition, flavor, and texture. Fermentation of cereals and legumes, spontaneous or with selected microorganisms, is a common food preparation method in many African and Asian countries, although making bread is also a type of fermentation. Folate concentration increase depends on process conditions. Increases during dough fermentation can be attributed

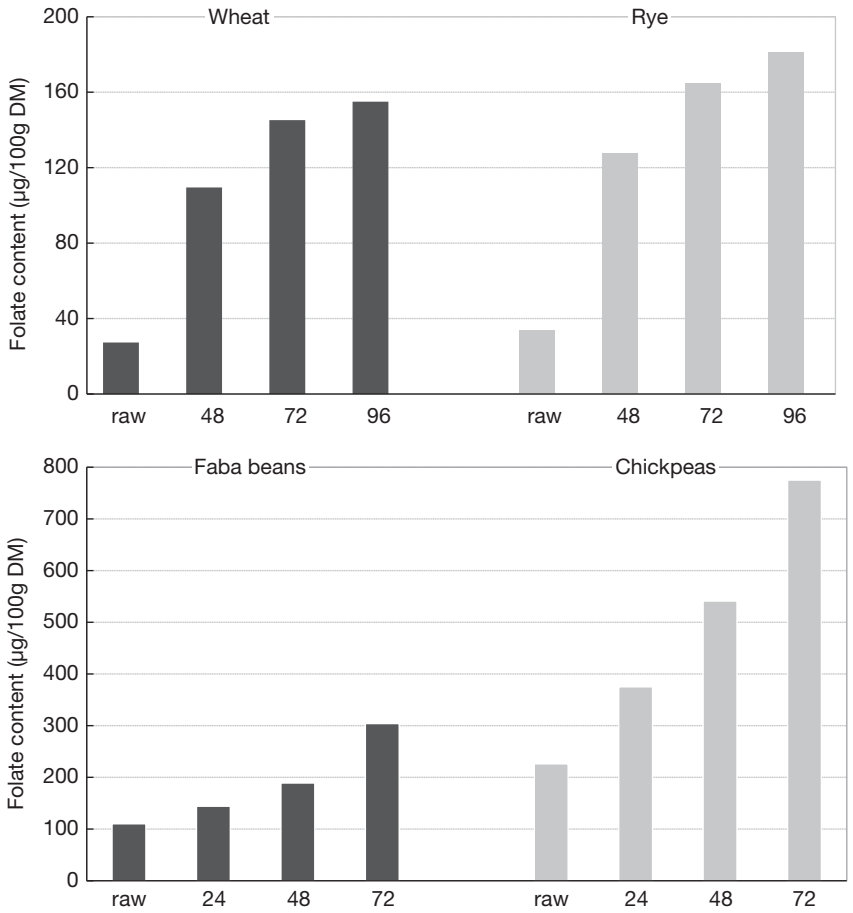

Figure 2 Folate content (μg/100 g DM) in cereals (wheat and rye) and legumes (fava beans and chickpeas) after germination at 25 °C for 24, 48, 72, or 96 h.

to the contribution from yeast cells and folate synthesis during their growth. Fermentation of bovine milk (e.g., with *L. rhamnosus* ATCC7469) increases folate content from ~4–8 μg/100 g to around 20 μg/100 g, although the increase in folate content depends on the lactic acid bacteria strain. Increased folate in hard cheese is chiefly caused not only by a reduction in the water content but also to a certain extent by the starter culture organism.

Dietary Intake of Folate

It has been estimated that populations in several European countries have a dietary folate intake of below 200 μg day^{-1} to around 300 μg day^{-1}. While these intakes prevent folate deficiency, which can result in megaloblastic anemia, they are below recommendations aiming to maintain or achieve a good folate status population-wide. With current dietary food habits, only few individuals reach the desired dietary intake (350 μg day^{-1} or greater). Raising folate intake, and in view of the low folate native content in food, it would seem necessary to develop strategies to increase folate intake. Fortification policies are thought to be an efficient tool for increasing average folate intake, and mandatory or voluntary folic acid fortification of staple foods has been introduced in several countries.

Suboptimal folate intakes observed in European populations are, commonly, attributed to the naturally low food content, processing losses, and the low bioavailability of natural food folates. Also, data from the US National Health and Nutrition Examination Survey (NHANES) suggest that 33–66% of the population was at risk of low serum and erythrocyte folate before the introduction of mandatory fortification of cereal-grain products and ready-to-eat cereals in the United States and Canada in 1998.

US and Canadian dietary reference intakes for folate, and other micronutrients, aimed to include the concept of optimal intakes and the associated long-term health benefits, going beyond simply avoiding deficiency diseases. Therefore, recommended intake levels were increased to 400–600 μg day^{-1} for women of childbearing age and during pregnancy and lactation. Many European countries also adapted this approach, and an aim of EURRECA – an EU-funded Network of Excellence – was to harmonize best practice guidance for setting science-based micronutrient recommendations including folate/folic acid. Reports from the United States and Canada suggest that in most population groups, folate status (serum folate and erythrocyte folate) improved during the postfortification period. The argument in both countries for

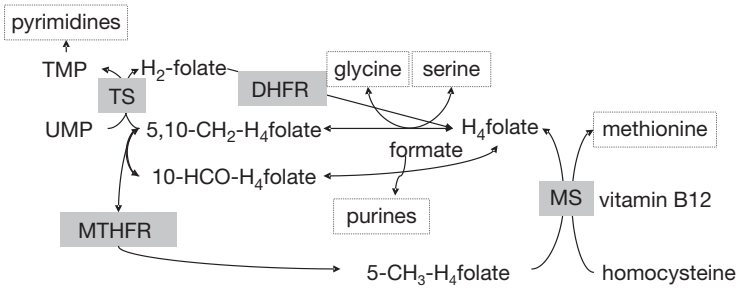

Figure 3 Role of folate in nucleotide synthesis and methylation reactions. Enzymes marked in gray and their end products in boxes. DHFR, dihydrofolate reductase; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; TMP, thymidine monophosphate; TS, thymidylate synthase; UMP, uridine monophosphate.

introducing mandatory folic acid fortification in 1989 was to reduce the prevalence of NTD. This aim was achieved as indicated by numerous epidemiological data, but mandatory folic acid fortification remains controversial in some countries.

In most countries, the recommended dietary allowance for folate in adults is 300 μg day^{-1} and, for women of childbearing age, up to 400 μg day^{-1}. In the United States, dietary recommendations are expressed in terms of dietary folate equivalents to account for differences in the bioavailability of food folates and folic acid from fortified foods. For pregnant and nursing women, recommendations are 500 and 600 μg day^{-1} folate to maintain erythrocyte folate. Several national health authorities recommend pregnant women should consume folic acid supplements during early pregnancy (before pregnancy and up to week 12). The US Food and Nutritional Board set an upper safe level for folic acid intake at 1 mg day^{-1}, which is also applied in other countries.

Folate Absorption and Metabolism

Dietary folate occurs mainly in the reduced polyglutamate forms. At physiological concentrations, reduced folate is deconjugated to its monoglutamate form by intestinal γ-glutamyl hydrolase before being absorbed by an active carrier-mediated process in the small intestine and being released into the blood stream via the hepatic portal vein. Folic acid has to undergo reduction for which, as shown by recent research, the human mucosa cell has limited enzymatic capacity. Indeed, it has been suggested that humans are dependent on hepatic reduction and methylation of folic acid from portal venous blood. The dominant folate form in circulating systemic blood is 5-methyltetrahydrofolate, and when present in an oral dose at higher concentrations, folate monoglutamate vitamers also pass through cell membranes by passive diffusion. The liver contains about half the body pool of folate. Up to 20% of folate absorbed by either mechanism is retained by the liver during first passage, depending on the hepatic folate status. The remainder is transported, via systemic circulation, to body tissues; tissue folates are retained in their polyglutamate form.

The biochemical function of this vitamin in cells of mammals is based on the different oxidation states of folate, because each has an essential role as one-carbon donors or acceptors. During nucleotide synthesis, folate supplies carbon units for *de novo* purine and pyrimidine bases. Folates act also as coenzymes in the methylation of proteins as well as the synthesis of neurotransmitters and phospholipids (Figure 3). A good folate status is, therefore, essential for normal cell division, and requirement increased during, for example, the growth, pregnancy, and lactation. Suboptimal folate status periconception has been shown to increase the risk of birth defects, specifically NTDs. Severe folate deficiency causes megaloblastic anemia, which is still observed in some Asian and African countries, although seldom seen in the United States and Europe.

Genetic polymorphisms can affect folate metabolism, decreasing enzyme activities and/or increasing folate requirements. Also, insufficient vitamin B_{12} can reduce the activity of a key enzyme (methionine synthase), resulting in cellular deficiency of tetrahydrofolate.

In the United States and Canada, mandatory folic acid fortification at 140 μg/100 g was anticipated to increase the average daily folate intake in women of childbearing age by an additional 100 μg while preventing excess intake by other population groups. Folate status in US populations has improved postfortification, resulting in less than 5% at risk due to increased dietary intake (>100 μg folic acid per day). However, in fastened blood samples from certain population groups, unmetabolized folic acid has been reported in addition to plasma folate 5-methyltetrahydrofolate suggesting excess intake. Various hypotheses regarding potential risks associated with this long-term exposure to high intakes of folic acid are being scrutinized (e.g., progression and growth of preneoplastic cells or subclinical cancers), and the issue of folate bioavailability and bioactivity has again gained importance.

Folate Bioavailability

The term bioavailability is commonly defined as the fraction of an ingested nutrient available for storage and metabolism under normal physiological function. Bioavailability is affected by host-related factors (e.g., folate status, health, age, sex, gastrointestinal function, and genetic factors), while the chemical nature of the ingested folate, food matrix, or food processing can influence bioavailability extrinsically. Therefore, determining folate bioavailability is still challenging. The numerous approaches for assessment of folate bioavailability from individual foods, meals, and whole diets have specific advantages or limitations and often provide conflicting

information on folate bioavailability. Only limited folate bioavailability data for vegetables, fruits, cereal products, and some fortified foods are available.

Common estimates for the bioavailability for food folate from a mixed diet are half that of folic acid, but this should be considered a rough estimate, as the bioavailability of food folate is reported to vary between 30% and 98%. Discrepancies in data about folate bioavailability can be explained by the nature of the foods or meals tested, which may affect vitamin stability and absorption, intersubject variation, different study designs and reference doses, and differences in postabsorptive metabolism among individual folate forms.

The bioactivity of dietary folates is commonly studied in 4–16-week intervention trials assessing changes in folate status parameters. Several 4-week trials with, for example, five-a-day fruits and vegetables, spinach, or yeast intervention did not improve folate status. Trials up to 16 weeks with similar and fortified foods (e.g., five-a-day or 500 g fruits and vegetables; aleurone, rye, or wholemeal breads; or a breakfast meal according to recommendations) or folic acid supplements resulted in improved folate status.

For assessment of short-term bioavailability or acute absorption of folate, several different methods have been used (Table 4). In many trials, the acute absorption of food folate has been found to be incomplete compared with folic acid.

The plasma area under the curve (AUC) approach is based on the assumption that postdose plasma folate concentration over time corresponds to the absorbed folate dose. Commonly, relative folate absorption of a single dose of food folate is compared with a reference dose (folate or folic acid). Sometimes, apparent folate absorption is estimated using biokinetic modeling. However, because of differences in metabolic handling of folic acid and reduced folate, folic acid is not an appropriate reference dose for bioavailability studies with food folate. Therefore, current bioavailability data should be interpreted carefully considering the limitations of the study design or model systems used. It has been suggested that 20–90% of supplement and food folate is absorbed.

Using the oral–fecal balance technique, folate studies in healthy ileostomy subjects, who do not possess colon microflora affecting fecal folate excretion, often in combination with the AUC technique, suggest folate absorption is inversely correlated with folate content in stomal effluent, and folate bioavailability from foods or a meal is ~50–90%.

Estimation of folate bioavailability using urinary folate excretion ratios is based on the assumption that excretion of labeled folate from an oral dose corresponds to that of a labeled intravenous reference dose. Relative folate bioavailability of food folates and synthetic folic acid ranged from 35% to above 100%, which indicates the human body handles folate vitamers differently. Similarly, whereas data on folate bioavailability were in the past thought to be contradictory, several studies now suggest reduced (food) folates and synthetic folic acid are metabolized differently.

Table 4 Methods to estimate the bioavailability and bioactivity of folate in humans

Study design	*Principle; advantages (+), disadvantages (−)* *Tested doses/foods*	*Finding*
Intervention	Intervention with test food and/or synthetic folic acid, compared to control group with no folate dose, commonly over 4–16 weeks (+) Information on the bioactivity of given dose from supplement/food/diet (−) Laborious, expensive, time-consuming, possible problems with compliance	
	Fruits, vegetables, spinach, yeast (1–4 weeks)	No effect on status
	Fruits, vegetables, aleurone, rye, or wholemeal breads, a breakfast meal (>4 weeks)	Improved or maintained status
Plasma area under the curve (AUC)	Postdose AUC corresponds to absorbed folate dose (+) Information on the acute/apparent absorption of given dose from supplement/food/diet (−) Laborious, expensive, requires strict standardization of test days, requires careful choice of reference dose, sometimes use of nonphysiological doses/large test food portions	
	Synthetic folate compounds, fruits, vegetables, dairy products, breads	Apparent absorption of 20–90%
Urinary folate excretion ratios	The urinary excretion of labeled folate from an oral dose corresponds to that of a differently labeled intravenous reference dose (+) Information on relative absorption from given dose of supplement/food (−) Labeled folate compounds required	
	Folic acid-fortified cereals-grain products, synthetic folates	Relative absorption of 35–155%
Oral–fecal balance technique	Folate absorption is inversely correlated with folate content in stomal effluent (in ileostomy subjects) (+) When combined with AUC technique, it enables safer estimation by complementation of folate excretion data with plasma data on folate absorption; when combined with AUC and stable isotope technique, it enables distinguishing between labeled folate from the dose and unlabeled endogenous folate from body stores (−) Laborious, expensive (labeled folate compounds required)	
	Fermented milk, spinach, yeast, fortified bread, breakfast meal	Absorption of 50–90%

Table 5 Health effects of folate: relation between folate intake and status with clinical and functional outcomes

Clinical/functional outcome		*Comment*
Pregnancy and birth outcomes	• *Maternal* folate status and intake are inversely associated with neural tube defects	• Strong evidence
	• Levels and means of recommended dietary folate or supplemental folic acid for protective effect	• Insufficient inconclusive data
	• Association of nonneural birth defects and folate status and intake	• Inconclusive data
Hematological status	• Mandatory folic acid fortification increases average folate intake and improves folate status	• Strong evidence
	• Detection of unmetabolized folic acid in circulating plasma after ingestion of folic acid fortificant or supplement	• No data regarding implications on human health
Neurological status	• Adequate folate intake or status protects from poor cognitive function and several neurological disorders in the elderly	• Probable
	• Folate intake is inversely associated with Alzheimer's disease and depression prevalence	• Probable
Cardiovascular diseases (CVDs)	• Adequate dietary folate intake is inversely associated with severe and subclinical CVD outcomes	• Suggestive
	• No adverse effects of dietary folate or folic acid supplements	• Probable
Cancer	• Dietary folate is inversely associated with risk for colon and colorectal cancer	• Probable
	• B vitamin supplementation including folic acid does not increase risk for colorectal cancer	• Probable
	• Dietary folate is inversely associated with risk for breast cancer	• Probable
	• Dietary folate is inversely associated with risk for bladder cancer, prostate cancer, or pancreatic cancer	• Insufficient inconclusive data

Health Effects of Folate

The vitamin folate attracted both scientific and public interests in recent years (Table 5). The role of folate in the prevention of megaloblastic anemia and of NTDs in the fetus is well established. National mandatory fortification programs of cereal-grain products or staple foods in the United States, Canada, and several other countries were launched with the public health goal of reducing the incidence of NTD-affected pregnancies, and this goal was successfully achieved. However, data regarding a particular level and means of intake for folate by food and/or folic acid supplement or fortificant are inconsistent and insufficient.

Further, a health benefit associated with a good folate intake or status is suggested to be decreased risk for CVDs.

Less consistent is the evidence regarding the protective role with respect to certain forms of cancer. Folate intake and status are inversely correlated with the risk for colon cancer. Findings regarding breast cancer are inconclusive and evidence is lacking regarding other cancers.

Also unclear is, for example, the impact of folate status on neuropsychiatric disorders, such as, dementia and Alzheimer's disease (Table 5).

Summary and Future Trends

This article summarizes briefly information on folate in the context of food and nutritional sciences. Folates have been recognized regarding their health benefit. With respect to the gap between current dietary folate intake and nutritional recommendations, further research is warranted regarding optimal level and means for intake of folate and folic acid in order to provide safe, tasty, healthy, and convenient foods.

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Food Additives: Classification, Uses and Regulation

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Food additives are natural, semisynthetic, or synthetic substances or products of biotechnology, present in edible products at levels varying usually between a few mg kg^{-1} and 1% by weight. They are used either directly or indirectly during processing, treatment, packaging, transport, or storage of a food for one or more technological purposes, such as prolonging shelf life, sweetening, modifying or stabilizing consistency, enhancing taste, and enhancing or preserving color.

Directly used food additives are substances intentionally added to foodstuffs in controlled levels. The main purposes of their use are food preservation and food appeal, convenience, or processing improvement. Indirectly used food additives are substances that become part of an edible product, usually at very low levels, as a result of processing or packaging.

Classification of Food Additives

Food additives are classified in many functional classes. The Codex Alimentarius Standard CAC/GL 36-1989 includes in section 2 a table with the following additive functional classes:

- Acidity regulators, as additives that control the acidity or alkalinity of a food
- Anticaking agents, as additives that reduce the tendency of food components to adhere to one another
- Antifoaming agents, as additives that prevent or reduce foaming
- Antioxidants, as additives that prolong the shelf life of foods by protecting against deterioration caused by oxidation
- Bleaching agents, as additives used to decolorize food (except flour)
- Bulking agents, as additives that contribute to the bulk of a food without having a significant contribution to its available energy value
- Carbonating agents, as additives used to provide carbonation in a food
- Carriers, as additives that dissolve, dilute, disperse, or otherwise physically modify a food additive or nutrient without altering its function (and without exerting any technological effect themselves) in order to facilitate its handling, application, or use of the food additive or nutrient
- Colors, as additives that add or restore color in a food
- Color retention agents, as additives that stabilize, retain, or intensify the color of a food
- Emulsifiers, as additives that form or maintain a uniform emulsion of two or more phases in a food
- Emulsifying salts, as additives that rearrange proteins in order to prevent fat separation during the manufacture of processed food
- Firming agents, as additives that make or keep tissues of fruits or vegetables firm and crisp or interact with gelling agents to produce or strengthen a gel
- Flour treatment agents, as additives added to flour or dough to improve its baking quality or color
- Flavor enhancers, as additives that enhance the existing taste and/or odor of a food
- Foaming agents, as additives that make it possible to form or maintain a uniform dispersion of a gaseous phase in a liquid or solid food
- Gelling agents, as additives that give a food texture through the formation of a gel
- Glazing agents, as additives that, when applied to the external surface of a food, impart a shiny appearance or provide a protective coating
- Humectants, as additives that prevent foods from drying out by counteracting the effect of a dry atmosphere
- Packaging gases, as additives that are introduced into a container before, during, or after its filling with a food with the intention of protecting the food, for example, from oxidation or spoilage
- Preservatives, as additives that prolong the shelf life of foods by protecting against deterioration caused by microorganisms
- Propellants, as additives that expel a food from a container
- Raising agents, as additives or combination of additives that liberate gas and thereby increase the volume of a dough or batter
- Sequestrants, as additives that control the availability of a cation
- Stabilizers, as additives that make it possible to maintain a uniform dispersion of two or more components
- Sweeteners, as additives other than sugars (mono- or disaccharides) that impart a sweet taste to a food
- Thickeners, as additives that increase the viscosity of a food

Food additives are classified into both direct or secondary direct food additives and indirect food additives according the US Code of Federal Regulations (CFR). Direct food additives are divided into eight categories. Flavorings and nutrients are two of these categories. Secondary direct food additives are divided into four categories. In two of these categories, enzymes and solvents are included. Food colors are classified in certified colors and colors exempt from certification.

Regulation of Food Additives

Food additive use is regulated by international or national authorities taking into account that they are safe and technologically indispensable. Each approved food additive has been assessed for its toxicity with the aim to determine the 'no observed adverse effect level' and the 'acceptable daily intake' (ADI). The latter is taken into account by establishing its maximum use levels in various food groups. An additive to which has been allocated an ADI 'not specified' may be used in foodstuffs with 'no limitations other than current good

manufacturing practice.' Identity and purity criteria for each permitted food additive, maximum use level at the different commodities in which it may be used, and food categories or individual food items in which the use of each permitted food additive is not acceptable or where use should be restricted have been allocated in the Codex General Standard for Food Additives (GSFA, Codex STAN 192-1995). This standard has been used as a starting point for developing the European Union system that regulates food additive use.

Food additive use in European countries is harmonized by Regulation (EC) No 1333/2008 of 16 December 2008. This regulation, which replaces previous directives and decisions concerning food additives permitted for use in foods, harmonizes also the use of food additives in food additives, food enzymes, food flavorings, and nutrients. The following are not considered to be food additives:

- Substances used for the purpose of imparting flavor and/or taste or for nutritional purposes
- Substances not consumed as food itself but used intentionally in the processing of foods, which remain as residues in the final food and do not have a technological effect in the final product (processing aids)
- Food enzymes
- A number of food ingredients, such as ammonium chloride, chewing gum bases, caseinates, casein, edible gelatin, gluten, inulin, and protein hydrolysates and their salts
- Products containing pectin that are derived from dried apple pomace or peel of citrus fruits or quinces
- Physically modified starch, starch modified by acid or alkali treatment, starch modified by bleaching and dextrinated or roasted starch, starch treated by amylolytic enzymes, sugars used for their sweetening properties, and white or yellow dextrin

Commission Regulation (EU) No 1129/2011 amends Annex II to Regulation (EC) No 1333/2008 by establishing a union list of food additives approved for use in foods and conditions of their use. Part B of this list includes colors (40 substances), sweeteners (16 substances), and additives other than colors and sweeteners (266 substances). Union list of food additives, whose use is permitted in food additives, food enzymes, food flavorings, and nutrients, is replaced in Annex III to Regulation No 1333/2008 by the text of Annex to Commission Regulation (EU) No 1130/2011. Specifications for all food additives listed in Annexes II and III to Regulation No 1333/2008 are laid down in the Annex to Commission Regulation (EU) No 231/2012.

The use of food colors and other food additives in the United States is regulated by the Food and Drug Administration (FDA). Food additives are divided into generally recognized as safe (GRAS) substances, prior-sanctioned substances, and regulated direct or indirect additives. The FDA is involved to ensure that GRAS substances and prior-sanctioned substances, which were in use in foods prior to the Food Additives Amendment in 1958 to the 1938 Food, Drugs, and Cosmetics Act, are tested by applying the most modern toxicological methods. Title 21 of the CFR includes a number of parts concerning food additives. Parts 170 and 171 provide general information on food additive regulation. Food additives permitted for direct addition to food for human consumption and secondary direct food additives permitted in food for human consumption are listed in parts 172 and 173, respectively. Indirect food additive regulations are presented from parts 174 to 178. Parts 181 and 182 refer to prior-sanctioned food ingredients and GRAS substance use. Parts 70 and 71 present general information on color additive regulation. Colors exempt from certification and colors subject to certification are listed in parts 72 and 73, respectively.

Approved Food Additives and Their Use

The following description of direct food additives used in foods is based on their functional classification.

Acidity Regulators

These additives, mainly acids or their salts, hydroxides, and oxides, are used as acidifiers, buffering agents, or pH adjusting agents that affect rheological properties, taste, and texture of foods, enhance the ability of food to form gel or to absorb water for swelling, or contribute to food shelf life extension. They are mainly used in bakery products, chewing gum, chocolate and cocoa products, confections, dairy products, fruit and vegetable products, nonalcoholic beverages, and wine, usually at levels in accordance with GMP. Citric acid and phosphoric acid are the most commonly used acidifiers.

Anticaking Agents

These substances, mainly inorganic calcium and magnesium salts (carbonates, ferrocyanides, phosphates, and silicates), fatty acid calcium and magnesium salts, oxides (magnesium oxide and silicon dioxide), cellulose, and soybean hemicellulose, are added in foodstuffs containing hygroscopic ingredients to inhibit the formation of aggregates or lumps. They are added, at a level of 1% or less, in

- dried powdered foods,
- foods in tablet and coated tablet form,
- food supplements such as in capsules or tablets,
- rice,
- salt and salt substitutes,
- sliced or grated hard, semihard, or semisoft ripened and unripened cheese products,
- processed cheese products,
- sugar confections,
- spreadable fats,
- tabletop sweeteners in powdered form or in tablets, and
- whey cheese products.

Antifoaming Agents

Dimethylpolysiloxane, which is the most commonly used antifoaming agent, is mainly added in foods, such as alcoholic and nonalcoholic beverages, batters, broths, canned or bottled fruits, confections, dairy spreads, fat spreads, food supplements, frying fats, jams, jellies, marmalades, and soups, at a level of 10 mg kg^{-1}. Mono- and diglycerides of fatty acids, microcrystalline wax, and calcium alginate are other substances used as antifoaming agents.

Antioxidants

These additives protect foods against oxidative deterioration caused by color and aroma defects. They are oil- or water-soluble substances used also to avoid an earlier oxidative deterioration in fatty foods because naturally occurring antioxidants may be often destroyed during processing. Antioxidants may be divided into chain-breaking antioxidants, preventive antioxidants, reducing agents, and oxygen scavengers. Chain-breaking antioxidants are natural or synthetic phenolic substances used for the inhibition or retardation of lipid oxidation caused by free radical reactions. Metal chelating agents (sequestrants), as the most important preventive antioxidants, are used often in combination with chain-breaking antioxidants for retardation of lipid hydroperoxide decomposition catalyzed by polyvalent metal ions and also for the inhibition of ascorbic acid oxidation. Reducing agents are used to transform lipid hydroperoxides in inactive products by nonradical reactions, to inhibit color changes in foods, or to regenerate chain-breaking antioxidants. Oxygen scavengers are, at least, substances used for removing oxygen from food packaging (e.g., glucose oxidase).

Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), extracts of rosemary, gallic acid esters, tert-butylhydroquinone (TBHQ), and tocopherols are the most important chain-breaking antioxidants used in foods. Tocopherols are used in oils, fats, and foods for infants and young children. Extracts of rosemary are added in animal fats, fish oils, fats and oils used in the manufacture of nonheat-treated and heat-treated foods, and vegetable fats and oils containing polyunsaturated fatty acids more than 15% (w/w) of the total fatty acids. They are also used in dehydrated potato products; fine bakery wares; mustard; nonheat-treated and heat-treated meat products; nut butters; nut spreads; potato-, cereal-, flour-, or starch-based snacks; processed eggs and egg products; processed nuts; seasonings and condiments; sauces; and soups and broths. Gallic acid esters, TBHQ, BHA, and BHT are added in animal fats, fish oils, fats and oils used in the manufacture of heat-treated foods, and frying fats. They are also used in cereal-based snack foods, chewing gum, dehydrated broths, dehydrated meat, dehydrated potatoes, dehydrated soups, processed nuts, sauces, and seasonings and condiments. The addition level of chain-breaking antioxidants other than tocopherols varies between 10 and 400 mg kg^{-1}. Tocopherols are added at levels in accordance to GMP.

Ascorbic acid, which is the most important water-soluble antioxidant, acts as a metal chelating agent, as an oxygen scavenger, or as a reducing agent. It is utilized for protecting flavor constituents against oxidation in fruit and vegetable juices or nectars and beer, due to its oxygen scavenging activity, and also for improving flour or dough baking quality. Ascorbic acid and its salts are also used in dehydrated milk; foods for young children; fresh and heat-treated processed meat, unprocessed fish, crustaceans, and mollusks; and unprocessed and processed fruit and vegetables. Fatty acid esters of ascorbic acid are mainly used in fats and oils, for improving their oxidative stability, dehydrated milk, and foods for young children and infants. Isoascorbic (erythorbic) acid and its sodium salt are used in heat- and nonheat-treated cured meat products to reduce the amount of added nitrous acid salts. These additives stabilize also color in deep-frozen and frozen fish with red skin, preserved meat products, and preserved or semipreserved fish products.

Colors

Colors used to enhance the appearance of foods are synthetic, semisynthetic, or natural water-soluble or water-insoluble substances. Approved color additives are

- mainly synthetic water-soluble azo and triphenylmethane dyes,
- natural water-soluble (anthocyanins, betanin, and carminic acid) or water-insoluble (beta-carotene, trans-β-apo-8′-carotenal, bixin, capsanthin, chlorophylls, chlorophyllins, copper complexes of chlorophylls/chlorophyllins, curcumin, lutein, lycopene, and riboflavins) colors,
- water-insoluble colors that are products of biotechnology (beta-carotene and lycopene),
- caramel,
- metals (aluminum, gold, and silver),
- minerals (calcium carbonate, iron hydroxides, iron oxides, and titan dioxide), and
- vegetable carbon.

Metals are used in confection external coatings. Most natural colors, caramel, minerals, and vegetable carbon are used at levels in accordance with GMP. Bixin and lycopene are used at levels varying between 10 and 50 mg kg^{-1}. Synthetic colors, curcumin, lutein, and trans-β-apo-8′-carotenal, are used at levels of 50–500 mg kg^{-1}. The use of food colorants is permitted in a great number of foodstuffs, mainly in alcoholic beverages, chewing gum, confections, edible ices, fine bakery wares, jams, jellies, marmalades, mustard, processed meat, processed fish and fishery products, sauces, and seasonings.

Emulsifiers and Emulsifying Salts

These additives, which are used as plasticizers, dispersing agents, suspending agents, clouding agents, density-adjusting agents, crystallization inhibitors, or melding salts, are natural or synthetic surface-active agents widely used to stabilize w/o and o/w food emulsions in margarine, mayonnaise, or ice cream; to prevent fat separation in sausages; to inhibit 'fat blooming' in chocolate or starch retrogradation in bread; to improve baked product volume, crumb structure of bread, and rheological properties of chocolate; to dissolve instant powders and spice extracts; and to achieve a characteristic consistency in ice cream. The relative strength of hydrophilic and lipophilic groups present in the molecule of a surface-active agent (HBL value) is related to its main industrial application. Surface-active agents suitable for stabilization of w/o and o/w emulsions have an HLB value varying between 3 and 6 and between 8 and 18, respectively, while surface-active agents having an HLB value varying between 7 and 9 are mainly utilized as food humectants.

The most commonly used food emulsifiers are lecithin; mono- and diglycerides of fatty acids and their esters with acetic, citric, lactic, and mono- and diacetyl tartaric or tartaric

acids; polyglycerol fatty acid esters; polyoxyethylene sorbitan fatty acid esters (polysorbates); propylene glycol fatty acid esters; sorbitan fatty acid esters (Span's); stearoyl-2-lactylate salts; and sugar esters. Ammonium phosphatide, fatty acid salts, glycerol esters of wood resin, quillaia extract, and thermally oxidized soybean oil interacted with mono- and diglycerides of fatty acids are other approved emulsifiers. Additives with emulsifying properties, which are mainly utilized as food humectants, stabilizers, thickeners, and/or sweeteners, are modified starches; lactic acid salts; ortho-, di-, tri-, or polyphosphoric acid salts; propylene glycol; polysaccharides; sugar alcohols; triacetin; and triethyl citrate. The use of emulsifiers is permitted in a great number of foodstuffs, including foods for infants, babies, and young children. These additives are utilized in bread, cheese, chewing gum, chocolate and cocoa products, confections, edible ices, fat or oil emulsions, fine bakery wares, flavored fermented milk products, flavored nonalcoholic beverages, heat-treated processed meat products, liqueurs, other alcoholic beverages, processed cheese, sauces, and soups. Lecithin, modified starches, mono- and diglycerides of fatty acids, and esters of mono- and diglycerides of fatty acids are added in foods, excluding food for infants and young children, at levels in accordance with GMP. The rest of the emulsifiers are added at levels varying between 100 and 10 000 mg kg^{-1} or mg l^{-1}.

Firming Agents

Calcium and aluminum salts of inorganic acids are the main firming agents. Calcium chloride is the most commonly used, mainly in canned and bottled fruit and vegetables, fruit compotes, jams, jellies, marmalades, and similar spreads. Its use, at levels in accordance with GMP, aims to prevent the collapse of fruit tissues by forming a calcium pectate gel around them and, therefore, to protect them against softening during processing. Aluminum sulfate, aluminum sodium sulfate, and aluminum ammonium sulfate are used in the manufacture of candied, crystallized, or glace fruits, at levels of 200 mg kg^{-1}, for coating fruits with an aluminum pectate film before they are candied in saccharose or glucose syrups.

Glazing Agents

The most important substances used as glazing agents are natural or synthetic waxes, such as beeswax, candelilla wax, carnauba wax, hydrogenated poly-1-decene, microcrystalline wax, montan acid esters, oxidized polyethylene wax, and shellac. All these additives are used for surface treatments of some entire fresh fruits. Beeswax, candelilla wax, carnauba wax, and shellac are also used in chewing gum; chocolate products; coffee; confections; potato-, cereal-, flour-, or starch-based snacks; fine bakery wares coated with chocolate; and processed nuts. Microcrystalline wax and hydrogenated poly-1-decene are used in chewing gum and confections. Glazing agents, with the exception of carnauba wax and hydrogenated poly-1-decene, are used in food at levels in accordance with GMP (about 4000 mg kg^{-1}). Carnauba wax is used at levels varying between 200 and 1200 mg kg^{-1}. The maximum use level for hydrogenated poly-1-decene is 2000 mg kg^{-1}.

Humectants

These additives are hygroscopic substances that control water activity because its reduction preserves texture and enhances food stability against deterioration caused by microorganisms. They are used in a great number of intermediate moisture foods, which have a water activity ranging from 0.6 to 0.9, such as cereal products; cheese; chewing gum; confections; dietary foods; dried fruits; fermented milk products; food supplements; jams; jellies; marmalades; meat products; potato-, cereal-, or flour-based snacks; processed eggs and egg products; processed fishery products; processed nuts; salads; sandwich spreads; sauces; soups and broths; and seasonings and condiments, at levels in accordance with GMP. The most commonly used humectants are sugar alcohols, which are multifunctional substances. Other multifunctional substances used as humectants are glycerol; glycerol triacetate; polydextrose; propylene glycol; salts of ortho-, di-, tri-, and polyphosphoric acids; and polysaccharides used as stabilizers and thickeners, such as alginic acid and its salts, agar, and carrageenan.

Preservatives

These substances inhibit metabolic pathways responsible for microorganism survival by the attack of microbial cell membranes or by reaction with functional groups of microbial enzymes. They are mostly used to protect fresh prepared foodstuffs with a low microbial load against deterioration, because the reduction rate of microorganism population depends on their levels. Their use depends also on microorganism species present in a food, food pH value, and food storage temperature. Preservatives suitable for use in foods are organic acids, such as acetic, benzoic, propionic, and sorbic acids, and their salts, *p*-hydroxybenzoic acid esters and their sodium salts, and inorganic acids and/or their anhydrides or salts, such as borax, boric acid, carbon dioxide, nitric acid salts, nitrous acid salts, sulfur dioxide and sulfurous acid salts, lysozyme, natamycin, nisin, hexamethylenetetramine, and dimethyl dicarbonate. Both inorganic and organic acids are effective in their undissociated form. They show, therefore, their highest antimicrobial activity at low pH values and they are preferably used in acid foods. The maximum use level of preservatives other than acetic acid and its salts and carbon dioxide varies between 3 and 4000 mg kg^{-1}. Acetic acid, acetic acid salts, and carbon dioxide are used at levels in accordance with GMP.

Sorbic acid and its sodium, potassium, and calcium salts show antimicrobial activity mainly against molds and yeasts. Foods that may be preserved using these additives are bakery wares, alcoholic beverages (alcoholic drinks with less than 15% alcohol, aromatized wine-based drinks, aromatized wine-product cocktails, cider, fruit wine, made wine, mead, and wine), cheese, cheese products, dehydrated and concentrated frozen or deep-frozen egg products, emulsified sauces, fat emulsions, flavored drinks, and processed fruit and vegetable products. Sorbic acid salts are also used for surface treatment of unpeeled fresh citrus fruits, ripened cheese, and cheese analogues.

Benzoic acid and its sodium, potassium, and calcium salts show antimicrobial activity against molds, yeasts, and aerobic

bacteria. Benzoic acid salts are often used in combination with sorbic acid salts, because they are more effective against lactic acid bacteria, for the preservation of chewing gum; confections, excluding chocolate and cocoa products; dietary foods; food supplements; jams; jellies; liquid eggs; liquid fruit and herbal infusion concentrates; liquid broths; liquid soups; liquid tea concentrates; marmalade and similar fruit-based spreads; mustard; olive-based preparations; olives; processed fish or fishery products; salads and sandwich spreads; sauces; seasonings and condiments; tabletop sweeteners in liquid form; and vegetables in vinegar, oil, or brine.

Methyl and ethyl esters of *p*-hydroxybenzoic acid and their sodium salts show antimicrobial activity against molds, yeasts, and many bacteria. They are mainly used in combination with either sorbic acid salts or sorbic and benzoic acid salts due to their higher effectiveness against Gram-positive bacteria. In comparison with sorbic or benzoic acid salts, they are effective in a wide pH area due to their lower polarity. Foods that may be preserved using these additives in combination with sorbic and benzoic acid salts are potato-, cereal-, flour-, or starch-based snacks; processed nuts; decoration, coating, and fillings of confections; and jelly coatings of meat products.

Sulfur dioxide and sulfurous acid calcium, potassium, and sodium salts show a stronger antimicrobial activity against bacteria than against molds and yeasts. They are often used in combination with sorbic and/or benzoic acid salts. These additives are also used, at levels higher than that needed for food preservation, as antioxidants, for the inhibition of food enzymatic and nonenzymatic browning, or as bleaching agents. Therefore, sulfurous acid salts are added in food at levels varying between 10 and 2000 mg SO_2 per kg. Foods containing these additives are mainly alcoholic beverages; analogues of meat or fishery products based on protein; confections; flavored drinks; lemon juice; mustard; potato-, cereal-, flour-, or starch-based snacks; processed and unprocessed fishery products; processed and unprocessed fruit and vegetables; sugars; syrups; and whole or concentrated fruit juices for special uses. The presence of these preservatives at levels higher than 10 mg kg^{-1} or mg l^{-1} must be indicated on the label because they cause asthma symptoms in special groups of consumers.

Potassium and sodium salts of nitric and nitrous acids are utilized in nonheat-treated processed meat and cured meat products to inhibit the growth of anaerobic bacteria. They are also effective as color retention agents. Nitrous acid salts are also used in heat-treated processed meat products. The maximum use levels of nitrous acid salts vary between 50 and 150 mg kg^{-1}. Nitric acid salts are added in meat products, at levels varying between 10 and 300 mg kg^{-1}; in milk used for manufacturing ripened cheese and ripened cheese products, at a maximum level of 150 mg kg^{-1}; and in pickled sprat and herring, at a maximum level of 500 mg kg^{-1}.

Propionic acid and its sodium, potassium, and calcium salts are used in baked products, such as breads, rolls, and fine bakery wares with a water activity of more than 0.65, at levels varying between 1000 and 3000 mg kg^{-1}. They prevent mainly bread ropiness caused by the action of *Bacillus subtilis*. These additives are often used in combination with more effective preservatives against mold, such as sorbic acid salts.

Sequestrants

The most important metal chelating agents used in foods are calcium disodium salt of ethylenediaminetetraacetic acid (EDTA) and citric acid. Both substances also find use as color retention agents. Calcium disodium salt of EDTA is used in fats for frying; fat spreads with a fat content of 41% or less; emulsified sauces; canned, frozen, or deep-frozen crustaceans; canned fish; and heat-treated processed meat. Citric acid is used in fats and oils to improve oxidative stability. Other approved sequestrants are citric and tartaric acid salts; ortho-, di-, tri-, and polyphosphoric acid salts; phosphoric acid; and tartaric acid.

Stabilizers and Thickeners

These additives modify the physical properties of water. They provide many functions to foods acting as binders, bodying agents, bulking agents, colloid, emulsion or foam stabilizers, fat substitutes, flavor fixatives, gelling agents, rheology modifiers, suspension agents, and texturizers. Approved stabilizers and thickeners include

- natural polysaccharides obtained from seaweed and plant materials (agar, alginates, carrageenan, carob bean gum, cellulose, guar gum, gum arabic, karaya gum, konjac glucomannan, pectins, soybean hemicellulose, tara gum, and tragacanth gum),
- chemically synthesized polysaccharide derivatives (cellulose derivatives and chemically modified starches) or semisynthetic materials that are manufactured by microbial fermentation (gellan gum and xanthan gum), and
- additives, such as beta-cyclodextrin; fatty acid esters; ortho-, di-, tri-, and polyphosphoric acid salts;, polydextrose; and sugar alcohols.

Citric acid salts, fatty acids, invertase, tartaric acid salts, and triethyl citrate are also utilized as stabilizers in food emulsions. Glycerol, polyethylene glycol, polyvinyl alcohol, pullulan, and talc are also additives used as thickeners.

Most additives used as stabilizers and thickeners are added in foods, excluding infant, baby, and young children foods, at levels in accordance with GMP. Maximum use levels have been established for gums, such as karaya gum, cassia gum, and cross-linked cellulose gum; ortho-, di-, tri-, and polyphosphoric acid salts; propylene glycol alginate; and soybean hemicellulose. These substances are added in concentrations that vary between 0.01% and 4% by weight.

Sweeteners

These natural or synthetic substances, which are mainly used in products with no added sugar or in energy-reduced edible products to imprint a sweet sensation, are classified into nutritive or nonnutritive sweeteners. Sugar alcohols, such as erythritol, isomaltitol, lactitol, maltitol, mannitol, sorbitol, and xylitol, are utilized as nutritive sweeteners. The most commonly used nonnutritive sweeteners are artificial substances, mainly acesulfame potassium, aspartame, aspartame–acesulfame salt, cyclamic acid and cyclamic acid salts, saccharin and its salts (saccharins), sucralose, neohesperidin dihydrochalcone, and neotame.

Other nonnutritive sweeteners used in foods are also steviol glycosides and thaumatin, substances that are isolated from natural sources. Two or more nonnutritive sweeteners are often used in combination for a synergistic intensification of sweetness or for the elimination of their side or posttaste effects.

Sugar alcohols are used as sugar substitutes in food commodities suitable for diabetics because their metabolism is insulin-independent. These substances, with the exception of xylitol, are less sweet than sugar and have a low-calorie value varying between 0.2 kcal g^{-1} (erythritol) and 3.0 mg kg^{-1} (maltitol). They are used for sweetening purposes, at levels in accordance with GMP, in dietary foods, food supplements, and sugar-free or low-calorie foods, such as candies and other confections, cereal-based products and breakfast cereals, chewing gum, chocolate and cocoa products, desserts, edible ices, fine bakery wares, jams, jellies, marmalades, mustard, and sauces.

Nonnutritive sweeteners are added in foods at low or very low levels because they are 30–3000 times sweeter than saccharose. These substances have a taste profile that differs slightly from that of saccharose. They are often used in combination with low-calorie bulking agents. Their use, at levels varying between 5 and 6000 mg kg^{-1} or mg l^{-1}, is permitted in the same food products where sugar alcohols are used and also in other sugar-free or low-calorie foods, such as alcoholic and nonalcoholic beverages (alcohol-free beer, flavored drinks, fruit and vegetable nectars, mixtures of alcoholic drinks, and spirits with less than 15% alcohol, etc.); broths; potato-, cereal-, flour-, or starch-based snacks; processed nuts; salads; sandwich spreads; soups; several fruit and vegetable products; and sweet–sour preserves and semipreserves of fish or fishery products. Neohesperidin dihydrochalcone, neotame, and thaumatin are used as flavor enhancers, at levels not exceeding 5.0, 2.0, and 0.5 mg kg^{-1}, respectively, in some food commodities and food supplements.

Epilogue

The most important changes in legislation during the last decade are the use of additives that are products of biotechnology and the more detailed description of conditions for use of authorized additives in the different categories and subcategories of foods. The tendency of food industry to produce a variety of products with special sensory or technological properties resulted in the use of a greater number of different additives than in the past. There is, however, a trend to limit the level of a number of additives with a low ADI (e.g., nitrous acid salts).

See also: Ascorbic Acid: Properties, Determination and Uses; Emulsifiers: Types and Uses; Gums: Properties and Uses; Preservatives: Food Use; Stabilizers: Types and Function.

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www.efsa.europa.eu/en/topics/topic/additives.htm – European Food Safety Authority: Food additives.

Food Allergies

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Glossary

Basophil Blood cell that contains mediators of allergic reactions.

Food allergy Illness occurring in some individuals upon ingestion of certain foods that elicit an abnormal immune response in that individual to certain food components.

IgE A class of immunoglobulins (antibodies) involved in allergic reactions.

Mast cell Cell present in many tissues that contains mediators of allergic reactions.

Introduction

Food-allergic individuals in the population experience adverse reactions upon ingestion of foods that are safe and nutritious for the vast majority. Individualistic adverse reactions to foods can occur through a variety of mechanisms including food allergies, metabolic food disorders also known as food intolerances, and food sensitivities. The most serious of these illnesses are food allergies. Food allergies affect a small percentage of the population. Food allergies are caused by an abnormal immunologic response to a particular food or food component, usually a naturally occurring protein component of the food. The most common form of food allergy occurs through an immunoglobulin E (IgE)-mediated mechanism. IgE-mediated allergies are known as immediate hypersensitivity reactions because the symptoms ensue within minutes to a few hours after ingestion of the offending food. However, cell-mediated food allergies, known as delayed hypersensitivity reactions, can also occur with symptoms typically beginning 24–72 h after ingestion of the food. Delayed hypersensitivity reactions to foods are poorly understood with the possible exception of celiac disease.

IgE-Mediated Food Allergy

IgE-mediated food allergies have emerged as an important public health concern in recent decades. While the number of affected individuals is relatively small, IgE-mediated reactions can be quite severe, even life-threatening, in some affected individuals especially if a significant quantity of the offending food is inadvertently ingested. Furthermore, the degree of tolerance for the offending food is small, making the implementation of safe and effective avoidance diets more difficult.

Mechanism

IgE-mediated allergies occur in two phases: sensitization followed by elicitation. While all humans have IgE antibodies, the majority will not produce IgE antibodies specific to a particular allergen. However, in susceptible individuals, allergen-specific IgE antibodies are produced in the body in response to exposure to a specific food protein (allergen) during the sensitization phase. The IgE antibodies are highly specific and will recognize only a specific portion (known as an epitope) of the food protein that they are directed against. Occasionally, IgE antibodies produced against one particular protein in a specific food will confer sensitivity to another food either because the food is closely related or because it shares a common epitope with the allergenic protein. Some food proteins are more likely to elicit IgE antibody formation than others. While exposure to the food is critical to the development of allergen-specific IgE antibodies, exposure will not invariably result in their development even among susceptible people. Many factors including the susceptibility of the individual, the age of exposure, the immunogenic nature of the food and its constituent proteins, and the dose, duration, and frequency of exposure are likely to influence the formation of allergen-specific IgE antibodies.

Once produced, allergen-specific IgE antibodies become attached to the membrane surfaces of two types of specialized cells, mast cells, that are found in many different tissues and basophils, which are found in the blood. The sensitization process is complete once the IgE antibodies are attached to the membrane surfaces of the mast cells and basophils, activating those cells that are then ready to respond to subsequent exposure to that specific food allergen. Sensitization to a particular allergen distinguishes allergic individuals from non-allergic individuals. Normally, dietary exposure to allergenic food proteins elicits no immunologic response whatsoever, known as oral tolerance. Even among individuals susceptible to the development of food allergies, dietary exposure to most food proteins results in oral tolerance. Most food-allergic individuals will become sensitized to relatively few food allergens.

Once the mast cells and basophils are sensitized, subsequent exposure to the allergen initiates the elicitation phase of the allergic response. In this phase, the food allergen cross-links two IgE molecules on the surface of the mast cell or basophil. The interaction between the allergen and the allergen-specific IgE elicits the release of a host of mediators of allergic disease, which are either stored in or formed by the mast cells and basophils. Several dozen different physiologically active mediators are released from the mast cells and basophils including histamine, prostaglandins, and leukotrienes. The mediators are responsible for the symptoms associated with allergies through interactions with receptors in various target organs of the body. The interaction of a small amount of allergen with the allergen-specific IgE antibodies

results in the immediate release of comparatively large quantities of the various mediators into the bloodstream and tissues. Thus, exposure to extremely small amounts of allergens can elicit symptoms.

With food allergies, ingestion is the most common route of exposure to the allergenic food. However, symptoms can also be provoked by exposure through the respiratory tract or the skin. Often, responses to cutaneous or respiratory exposures to food allergens are localized to those target organs. Although consumers can respond to cutaneous or respiratory exposures to allergenic foods, occupational food allergies are known to occur in food industry workers who are exposed by inhalation or skin contact to certain allergenic foods. Baker's asthma associated with inhalation of wheat flour dust among bakery workers is a prime example. Workers with occupational food allergies on cutaneous or respiratory exposures can sometimes eat the offending food with no provocation of symptoms.

Symptoms

IgE-mediated food allergies occur with a short onset time between the ingestion of the offending food and the onset of symptoms. Because the mediators released from the mast cells and basophils can interact with receptors in a number of different tissues in the body, numerous symptoms can be associated with IgE-mediated food allergies as shown in Table 1. Most food-allergic individuals suffer from a few of the many possible symptoms. Symptoms tend to be more severe with higher exposure doses. However, exposure to even trace quantities can elicit noticeable reactions due to the large release of mediators.

Some of the symptoms of IgE-mediated food allergies, for example, the gastrointestinal manifestations and asthma, are not particularly definitive. Thus, clinical diagnosis is sometimes rather difficult. Anaphylactic shock is, by far, the most serious manifestation of food allergies and involves gastrointestinal, cutaneous, and respiratory symptoms in combination with hypotension and cardiovascular complications. Death can ensue within minutes of the onset of anaphylactic shock. Very few individuals with food allergies are susceptible to anaphylactic shock after the ingestion of an allergenic food.

Oral allergy syndrome (OAS) characterized by symptoms confined to the oropharyngeal region (pruritus, angioedema, and urticaria) is the most common and possibly the mildest form of IgE-mediated food allergy. OAS is associated with the ingestion of various fresh fruits and vegetables. In OAS, sensitization occurs to allergens present in pollen such as birch and ragweed. This pollen sensitization increases the likelihood of sensitization to specific foods. Although fresh fruits and vegetables contain comparatively low quantities of protein, sufficient exposure occurs to elicit the oropharyngeal symptoms. The fruit and vegetable allergens involved in OAS are apparently quite susceptible to digestive proteases in the gastrointestinal tract, so systemic reactions are rarely encountered in these foods. These allergens are also apparently heat-labile, since the heat-processed versions of these foods do not typically trigger OAS episodes. In some cases, a more stable allergen called lipid transfer protein has been implicated in allergic reactions to certain fruits, and these reactions are more likely to be systemic and severe.

In some individuals, allergic reactions to foods only occur when the foods are eaten coincident with exercise. Exercise-induced food allergies have occurred with numerous foods including shellfish, wheat, and celery. The symptoms of exercise-induced food allergies are individualistic, variable, and similar in nature to those involved in other food allergies. Exercise-induced allergies also occur apart from food ingestion. The mechanism of this illness is not well understood, although the involvement of IgE antibodies is apparent.

Table 1 Symptoms of IgE-mediated food allergies

Gastrointestinal symptoms	*Cutaneous symptoms*
Vomiting	Urticaria (hives)
Diarrhea	Dermatitis
Nausea	Angioedema
Respiratory symptoms	*Other symptoms*
Rhinitis	Anaphylactic shock
Asthma	Laryngeal edema

Sources

The most common allergenic foods are peanuts, tree nuts (almonds, walnuts, pecans, cashews, etc.), soybeans, cows' milk, eggs, fish, crustacean shellfish (shrimp, crab, lobster, etc.), and wheat. On a worldwide basis, these eight foods or food groups are responsible for perhaps 90% of all food allergies. In the United States, allergies to crustacean shellfish and peanuts are the most common. Peanut allergy tends to be more common in countries where peanuts are frequently consumed. Among infants and young children worldwide, cows' milk and eggs are the most common causes of food allergy due to their widespread use in the diets in this young age group. In the United States, peanut allergy is also relatively common among young children because of the popularity of peanut butter. The prevalence of any specific type of food allergy is related to some extent to cultural food preferences. For example, in Japan and Korea, buckwheat is a commonly allergenic food because of the popularity of soba noodles leading to frequent exposure to buckwheat. By contrast, buckwheat allergy is uncommon in the United States because buckwheat is not frequently eaten in the United States. Different regions of the world recognize different foods as commonly allergenic, although milk, eggs, fish, crustacean shellfish, peanuts, soybeans, tree nuts, and cereal grain sources of gluten (including wheat) are recognized in most countries. Molluscan shellfish (clams, oysters, squid, snails, etc.) and mustard are considered as commonly allergenic in Canada and the European Union. Sesame seed is identified as a commonly allergenic food in Canada, the European Union, and Australia/New Zealand. The European Union also recognizes celery and lupine as commonly allergenic. Mango is recognized as a commonly allergenic food in Taiwan.

Most foods have some potential to cause food allergies. Any food that contains protein has some potential to elicit an allergic reaction. The most common allergenic foods tend to be foods with high protein content that are frequently consumed. However, despite their frequent consumption and high protein content, beef, pork, chicken, and turkey are rarely allergenic.

Food Allergens

The vast majority of the allergens in foods are naturally occurring proteins. However, foods contain millions of proteins and most do not provoke allergic sensitization. Only a very small percentage of all food proteins are known to be allergens. Even within the commonly allergenic foods, only one or a few of the many proteins in those foods are identified as allergens. All proteins are capable of provoking immune reactions under selected circumstances of exposure, but some food proteins do not appear to elicit allergic sensitization. Some allergenic foods, such as peanuts, eggs, milk, and shrimp, contain multiple allergens, but other allergenic foods, such as cod and Brazil nut, appear to contain only a single major allergen. Major allergens are defined as proteins that elicit specific IgE formation in 50% or more of patients having that particular food allergy. Plant food allergens tend to fall into certain functional categories such as some of the pathogenesis-related proteins or certain classes of storage proteins. Legumins, vicilins, and 2S albumins are storage proteins that are major allergens in peanuts, cashews, and walnuts. Similarly, several panallergens appear to exist in allergenic animal species such as parvalbumin in fish and tropomyosin in crustacean shellfish. However, multiple allergens have been identified in other animal-based foods, including milk and eggs.

Prevalence

The overall prevalence of IgE-mediated food allergies in the United States has been estimated as high as 8%. The prevalence of food allergies is higher in infants and young children than in adults. The prevalence of allergies to specific foods is not precisely known, although surveys indicate that the prevalence of crustacean shellfish allergy approaches 2% of the US population, while peanut allergy affects 0.7–0.8% and tree nut allergy affects 0.6%. While milk allergy is relatively uncommon among adults, several studies in various countries have indicated that milk allergy affects about 2.5% of infants under the age of 3 years.

An increasing prevalence of food allergies is apparent in several developed countries, but the prevalence in developing countries is, as yet, rather poorly understood. Multiple factors could be involved in the increased prevalence. The prevalence of food allergies is higher in urban environments than rural areas perhaps because of the prevalence of bacteria in farm environments that promote oral tolerance over allergic sensitization. This observation has led to the promulgation of the hygiene hypothesis that attempts to associate the high degree of cleanliness in the modern-day home with an increased risk for allergic sensitization. Another factor is the increase in cesarean sections, which is associated with an increase in food allergies. This is understandable as the infant's intestinal tract is devoid of beneficial microbiota after cesarean births. Weaning practices (see section '**Prevention**') may be another important factor. Taken together, these factors suggest that the intestinal microbiota influences the development of food allergies, but these interactions are not yet well understood.

Persistence

Some IgE-mediated food allergies are persistent, while others are generally not. Cows' milk and egg allergies are more frequently outgrown than allergies to peanuts. The time period for the development of oral tolerance to milk and eggs ranges from a few months to several years from the time of the onset of the food allergy. More than 80% of food-allergic children develop tolerance to milk and eggs over a period of months to years. Peanut allergy tends to be very persistent, but perhaps 20% of peanut-allergic children do ultimately become tolerant. Even with milk and eggs, a few individuals do fail to achieve tolerance and become milk- or egg-allergic adults. Milk- and egg-allergic children may first become tolerant of certain processed forms of milk and eggs, for example, baked foods, while still sensitive to less heated forms of milk and eggs such as pasteurized milk and scrambled eggs.

Prevention

Strategies do exist to prevent the development of IgE-mediated food allergies in infants. Most infants have a low risk for the development of food allergies, so preventive efforts are typically focussed on those high-risk infants most likely to develop IgE-mediated food allergies. High-risk infants are those infants born to parents with histories of allergic disease of any type (pollen, mold spores, animal danders, bee venom, food, etc.). Several preventive strategies have been advocated for high-risk infants, although consensus on the optimal approach has certainly not been achieved. One strategy involves the exclusion of commonly allergenic foods such as cows' milk, eggs, and peanuts from the infant diet in the early years. This strategy can be accomplished by breast-feeding for an extended period, possible use of hypoallergenic infant formulas, and the exclusion of commonly allergenic foods from the diet of the infant at weaning and the nursing mother and possibly during pregnancy. While the exclusion of commonly allergenic foods from the environment of young infants, especially high-risk infants, seems logical and has been advocated, the efficacy of this approach has not been clinically established. In fact, recent evidence suggests that the early introduction of peanuts into the diets of young infants may actually lower the likelihood of sensitization to peanuts. Exclusive breast-feeding for extended periods of time may only delay, but not prevent the development of IgE-mediated food allergies.

Infants can occasionally be sensitized to allergenic foods through exposure to the allergens present in breast milk. Some food allergens are resistant to maternal digestion, are absorbed to some extent from the small intestine, and are secreted in breast milk leading to sensitization. Accordingly, maternal dietary avoidance of certain commonly allergenic foods, such as peanuts, during the lactation period is advocated to prevent sensitization through breast milk. However, the elimination of certain other commonly allergenic foods, such as milk and eggs, is not recommended for nursing mothers because these foods are usually considered to be too important nutritionally.

The use of probiotic bacteria during lactation may also help to diminish the likelihood of allergic sensitization. However, the results of clinical trials with probiotics have been mixed, and the use of probiotics is not yet commonly practiced in most countries.

Hypoallergenic infant formulas will also prevent the development of food allergies in high-risk infants. Hypoallergenic infant formulas, especially extensively hydrolyzed formulas,

are most often used to prevent reactions after the infant has been sensitized to milk. However, the use of a partial whey hydrolysate formula has been advocated for the prevention of the development of milk allergy in high-risk infants.

Current recommendations for weaning promote the prolonged avoidance of commonly allergenic foods such as peanuts. But, the early introduction of peanuts into the diet may actually induce oral tolerance and prevent later sensitization. At the present time, more clinical research is needed before any particular prevention strategy is advocated.

Diagnosis

Optimally, the diagnosis of food allergies should be conducted by an allergy specialist. Parental diagnosis and self-diagnosis are often incorrect leading to identification of the wrong foods or the identification of too many foods as allergens. First, the physician should take a careful history of the patient's adverse reactions, noting the foods eaten immediately before the onset of symptoms; the amount of various foods consumed; the type, severity, and consistency of symptoms; and the time intervals between eating and the onset of symptoms. Multiple episodes may need to be investigated to link a particular food to an allergic reaction. History alone can be sufficient to make the diagnosis in some situations if the cause-and-effect relationship is particularly compelling. If not, challenge tests with the suspected food(s) may be required to firmly establish the role of a specific food in the reaction. The double-blind, placebo-controlled food challenge is the most definitive clinical approach to document the cause-and-effect relationship with a particular food. A positive challenge test indicates that the food is associated with the adverse reaction but does not establish an IgE-mediated mechanism. The role of IgE can be established with either the skin prick test or use of a blood test for the presence of IgE specific to the particular food.

Treatment

The avoidance of the offending food is the primary means of treatment for IgE-mediated food allergies. With IgE-mediated food allergies, low amounts of the offending food can provoke reactions. Accordingly, the construction of a safe and effective avoidance diet can be difficult. Several common ingredients are derived from commonly allergenic foods such as casein or whey from milk, soy flour from soybeans, or wheat flour, gluten, or semolina from wheat. The avoidance diets must exclude such ingredients. The sources of ingredients derived from commonly allergenic foods must be labeled in the United States and some other countries. Refined oils from allergenic sources such as peanut oil and soybean oil appear to be safe for consumption by allergic individuals because these oils do not contain enough of the allergenic protein to provoke reactions. But, less refined oils, such as expeller-pressed or cold-pressed oils, may contain sufficient allergenic protein to pose a risk to allergic consumers. The careful reading and complete understanding of food labels is critical to the implementation of safe and effective avoidance diets. Packaged food manufacturers have the responsibility to assure that the label statements on packages are accurate. Occasionally, processing errors are made that result in the presence of undeclared residues of allergenic foods in a packaged food. The contamination of one food with another from the failure to adequately clean shared food processing equipment is one of the most common errors occurring in food manufacturing. However, restaurant and other food service meals present an even bigger challenge for food-allergic individuals. The accurate identification of all of the ingredients in food service and restaurant meals can sometimes be quite difficult, and such foods are not labeled. Furthermore, cross contamination can occur from the use of shared food preparation equipment (utensils, cooking surfaces, pots and pans, etc.). As a result, many inadvertent exposures occur among allergic consumers who are attempting to avoid their offending food(s).

Effective avoidance diets are further complicated by cross-reactions that can occur between different foods. Cross-reactions sometimes occur between closely related foods. Common examples would be cross-reactions occurring among the various species of crustacean shellfish (shrimp, crab, and lobster), among the various species of fish (cod, salmon, etc.), or, more rarely, between crustacean and mollusc-an shellfish (clam, oyster, squid, etc.). Cows' milk and goats' milk invariably cross-react as do the eggs of various avian species. Cross-reactivity is not so common among related species in plant foods. Frequently, individuals are allergic to both cashews and pistachios because these two tree nuts are quite closely related. However, among the legumes, many individuals are allergic to peanuts, but most of these individuals are not allergic to other legumes such as soybeans, lupines, peas, and green beans. A few individuals are cross-reactive between peanut and one or more other legumes. Lupine, a legume that is unfamiliar except in the EU and Australia, cross-reacts with peanuts in some allergic individuals. Cross-reactions can also occur between foods and other environmental allergens. The most common examples are the cross-reactions that occur between some fresh fruits and vegetables and certain pollen allergies in some individuals experiencing OAS and the cross-reactions that occur in a few individuals allergic to natural rubber latex with several foods including bananas and kiwis.

Pharmacological approaches can be used to treat the symptoms of allergic reactions. In particular, epinephrine (also known as adrenalin) is prescribed for individuals who experience life-threatening food allergies. The early administration of epinephrine after inadvertent exposure to the offending food can be life-saving for such patients. Antihistamines are also effective in treating the less serious symptoms of food allergies.

Immunotherapeutic approaches are under clinical development for the treatment of food allergies. In these approaches, patients are treated beginning with very small doses of the allergenic food over a period of some weeks until they are able to tolerate more typical doses of the food in their diets. The safety and long-term effectiveness of immunotherapy remain under active clinical investigation.

Minimal Eliciting Dose (Threshold)

IgE-mediated allergic reactions to food can be triggered by very low doses of the offending food in some individuals, although the range of minimal eliciting doses among the entire population of peanut-allergic individuals, for example, is quite wide (fractions of milligrams up to several grams). These

low-threshold doses for the offending food complicate effective avoidance as great care must be taken. The interaction of a rather small amount of allergen with IgE antibodies on the surfaces of mast cells and basophils stimulates the release of large quantities of biologically active mediators into the bloodstream and tissues. Accordingly, exposure to a small dose of the allergen can elicit a clinically significant reaction.

Thus, food-allergic individuals must be cautioned to exercise the complete avoidance of the offending food. However, clinical research has documented that doses do exist, below which allergic individuals will not experience adverse reactions. Although individual threshold data are rapidly emerging, population thresholds have not yet been promulgated by public health agencies. Population thresholds for specific allergenic foods could be used to guide food-allergic consumers in the construction of safe, effective avoidance diets and labeling and other allergen control strategies used by the food industry.

Occurrence of Allergic Reactions

The frequency of the occurrence of allergic reactions to foods among sensitized consumers is unknown. In all likelihood, particularly mild allergic reactions may go unnoticed by affected individuals. However, food-allergic individuals would definitely pay attention to more serious allergic reactions. While accurate records do not exist, tens of thousands of individuals seek treatment in hospital emergency rooms for food allergies each year in the United States. The factors leading up to these visits are poorly understood. Certainly, some unknown fraction of these visits occur among individuals who are experiencing an allergic reaction to food for the first time. But many such reactions are likely occurring among individuals who are aware of their food allergy and who should be avoiding the offending food. Avoidance diets take constant vigilance, and occasional errors are likely to happen.

Food allergy reactions can occur in any location where food is sold or eaten, although homes and restaurants are the most frequent locales. Several factors could lead to the occurrence of moderate to severe allergic reactions among susceptible consumers. Obviously, the food-allergic individual or their caregivers could make an error that leads to inadvertent ingestion of the offending food. Failure to read or understand food labels may also be a contributing factor in some instances. However, food manufacturers or restaurants may also contribute to food allergy reactions. Prevention of errors by food manufacturers or restaurants has become an important public health focus.

Food manufacturing errors can occur including the placement of the incorrect product in the package, the failure to adequately clean shared equipment, the use of rework (a common practice in certain segments of the industry involving the incorporation of leftover or misformulated quantities of a food product into subsequent batches of related products with identical or related formulations), and the inadvertent addition of an ingredient that is not supposed to be in the formulation. With packaged foods, the majority of food ingredients are declared on the ingredient statement on the label. These ingredient statements assist food-allergic consumers in their avoidance diets. Additionally, many food processors have begun to add precautionary labeling to food products, including terms such as 'made on shared equipment with peanuts' or 'may contain peanuts.' This sort of labeling alerts allergic consumers to the possible presence of hazardous residues of allergenic foods in the product.

Restaurants and other food service facilities represent a bigger hazard for food-allergic consumers because foods in restaurants are not labeled, and the waitstaff sometimes fail to provide correct information on menu components to allergic consumers. Additionally, errors can occur in food preparation including the use of shared cooking/serving equipment or surfaces or cooking media and mistakes or substitutions in adherence to recipes. Many adverse reactions have occurred in restaurants, school cafeterias, and similar settings.

Regulations

Public health agencies such as the US Food and Drug Administration regulate the labeling of packaged foods. US regulations require that ingredient statements on packaged foods identify the vast majority of the allergenic foods or ingredients sourced from such foods. While regulations differ around the world, the clear labeling of commonly allergenic foods is a key regulatory component in many countries.

Once regulations are established, they must be enforced. The enforcement of existing regulations is another task for the public health agencies, such as the US FDA. In the United States, one of the fastest-growing areas of recalls of packaged foods from the marketplace is the undeclared presence of allergenic foods. Improved methods for the detection of residues of allergenic foods have been pivotal to the investigation of allergic incidents and the enforcement of labeling regulations.

Detection of Allergenic Food Residues

Over the past several decades, methods have been developed to detect reliably the presence of residues of allergenic foods present in other foods. The food industry uses these detection methods to assure that their manufacturing practices are adequate to eliminate the presence of unwanted allergen residues in other foods. Where residues cannot be effectively and consistently eliminated, then precautionary labeling is often applied. Regulatory agencies can use the detection methods for the enforcement of labeling provisions on packaged foods and to investigate alleged reactions that might be attributable to the presence of an undeclared allergen in packaged foods. Commercial enzyme-linked immunosorbent assays or ELISAs with detection limits in the low ppm ($mg\,kg^{-1}$) range are available for the detection of residues of many of the most commonly allergenic foods including peanuts, eggs, and milk. Polymerase chain reaction assays are also available for the detection of DNA from allergenic food sources.

Effects of Processing on Allergenicity

Typically, food processing operations have little effect on the allergenicity of most foods since the allergenic food proteins tend to be remarkably stable to food processing conditions. Many food allergens are quite stable to thermal food processing. However, some of the allergens in fruits and vegetables are heat-sensitive. Additionally, food allergens tend to be

resistant to proteolysis allowing these proteins to survive digestive processes and arrive in the intestine in immunologically active form. Accordingly, food allergens may survive, in whole or in part, the acid and enzymatic hydrolysis methods used to prepare protein hydrolysates often used as food ingredients. Hypoallergenic infant formula is often manufactured with extensive hydrolysis of milk proteins, but that is one of the few examples where processing has an effect on eliminating or dramatically reducing allergenicity. The removal of protein in edible oil refining serves as another example.

Conclusion

While food allergies affect a small percentage of the population, reactions can range in severity from mild to life-threatening. Food allergies are caused primarily by naturally occurring proteins in foods. Food allergies can be triggered by ingestion of very small amounts of the offending food.

See also: Allergies: Public health; Colon: Diseases and Disorders; Food Allergies: Occurrence and Analysis; Food Colloids and Emulsions; Food Intolerance: Lactose Intolerance; Infants: Weaning.

Further Reading

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Food Allergies: Occurrence and Analysis

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Food Allergies and Occurrence of Food Allergens

Food allergies are adverse immunologic reactions that occur in previously sensitized subjects after the ingestion of the triggering foods or ingredients. In most cases, the causal agent (called antigen) is a single protein or a protein family.

The immunologic reaction is not direct toward the whole protein, but only to certain specific regions, called epitopes. If the immune system recognizes the amino acid sequence (the primary structure) of that region, the epitope is defined as 'linear.' They are usually short amino acid sequences (8–11 amino acids) that are stable to proteolysis in the gastrointestinal tract. When the immune system recognizes the tertiary structure of the epitope, it is defined as 'conformational'; these epitopes are usually less stable to food processing than linear epitopes, because denaturation modifies the tertiary structure of the protein preventing the recognition of the conformational epitope.

Food allergies can be IgE-mediated or not IgE-mediated, depending on the type of antibodies involved. In the classic IgE-mediated food allergy, only IgE antibodies are involved. Two different phases can be distinguished: the sensitization phase and the reaction phase. The sensitization phase occurs during the first exposition of the immune system to the antigen. When a food allergen crosses the intestinal barrier (due to some mucosal defects), the antigen-presenting cells (APCs) recognize the epitopes and they form with them the so-called major histocompatibility complex (MHC). In this way, B lymphocytes are induced to produce specific IgE toward the target epitopes. Specific IgE will bind to the mast cell surface, making them sensitized against the allergen. The reaction phase occurs when the immune system is exposed to the allergen at a later time: the previously sensitized mast cells immediately recognize the allergen and a strong immunologic reaction starts. After the allergen recognition, in fact, mast cells will degranulate releasing proinflammatory mediators (histamine, heparin, and cytokines) that will cause the characteristic allergic symptoms. The severity of these symptoms is highly variable among allergic subjects, ranging from mild gastrointestinal problems to anaphylactic shock. In food allergy, the gastrointestinal tract is not the only target of the symptoms, but also the respiratory tract is frequently involved. The main symptoms are acute urticaria, atopic eczema, gastrointestinal anaphylaxis, and oral allergy syndrome.

In non-IgE-mediated food allergies, other types of antibodies are involved, such as IgA and IgG. Also other cells of the immune system can be involved (e.g., T lymphocyte), so that they are sometimes called 'cell-mediated food allergies.' Usually, non-IgE-mediated food allergies show a less acute and more chronic behavior. The main target is always the gastrointestinal tract, whose damages lead anyway to systemic consequences due essentially to nutrient malabsorption. Classic examples of cell-mediated food allergies are food protein-induced enterocolitis syndrome, food protein-induced proctitis, enteropathy, and celiac disease. Among these, the highest prevalence can be attributed to celiac disease. This is an autoimmune enteropathy that develops in some genetically predisposed subjects after gluten consumption or the ingestion of related proteins of barley and rye. Gluten peptides cross the intestinal epithelium due to mucosal defects and enter the lamina propria. There, they are deamidated by the enzyme tissue transglutaminase 2 and recognized by the APCs that have the heterodimer HLA-DQ2 or DQ8 on their surface, forming the MHC. The complex is recognized by T lymphocytes, which induce the differentiation of B lymphocytes into plasma cells, that produce different types of IgA and IgG antibodies. These antibodies are directed not only toward external agents (antigliadin and anti-deamidated peptides) but also against endogenous components (anti-tissue transglutaminase and anti-endomysium). Moreover, T lymphocytes secrete proinflammatory cytokines that damage the intestinal epithelium structure.

Food allergies affect an increasing number of subjects, with a high variability among countries and for the type of allergen involved. Globally, 220–250 million people are estimated to suffer from food allergy. The disease is more diffused among children, because some food allergies generally disappear in adulthood. The prevalence of food allergies has been estimated to be around 1–3% in adults and 4–6% in children. Food allergens are present in an extremely wide variety of foods (of both vegetal and animal origins), but eight main groups can be identified. It is important to underline that often, allergic patients do not react to a single food, but cross-reaction phenomena are observed. In fact, sequence similarity among the homologous proteins of different species can lead to the recognition of the epitopes by IgE in sensitized subjects. This cross-reaction can be observed also for nonfood allergens, for example, birch pollen.

Among the products of animal origin, the most important allergenic foods are the following:

- *Crustaceans*. This class includes several important foodstuffs, such as crab, crayfish, lobster, prawn, and shrimp. Allergy is mainly caused by the muscle proteins tropomyosin and myosin light chain and by the enzyme arginine kinase. While invertebrate tropomyosin is highly allergenic, vertebrate tropomyosin can be considered safe. The prevalence is variable, with higher percentages where crustacean consumption (thus the exposition) is higher, for example, in Asian countries. Prevalence in the United States is around 2%.
- *Eggs*. The worldwide prevalence of egg allergy is around 1.6–3.2%, and the main proteins involved are contained in the egg white, rather than the yolk. Egg allergy is the second for prevalence (after milk allergy) in children. Ovomucoid (probably the most important allergen),

ovalbumin, ovotransferrin, and lysozyme have already been identified as clearly allergenic proteins.

- *Fish*. Fish allergies are less common than other allergies to seafood, such as crustaceans and mollusks. Most of food allergies directed toward fish have a small calcium-binding protein as target, called parvalbumin. Fish allergies affect around 0.4% of the US population, and it is more diffused in coastal areas (e.g., Norway), where fishing is more common and fish products constitute an important part of the diet.
- *Milk*. Cow's milk allergy is much more diffuse in children (2–3%; the most common allergy) than in adults, where prevalence is around 0.1–0.5%. Allergic reaction is caused by caseins, α-lactalbumin, and/or β-lactoglobulin, depending on the patient. The different sequences of the homologous serum protein and caseins in different animals (cow, sheep, and goat) allow sometimes the consumption of milk from a particular source by subjects allergic to other species. Some hard ripened cheese can be consumed in a moderate amount by some allergic subject, due to the proteolysis that induces the breakdown of the epitopes.
- *Mollusks*. This class includes gastropods, bivalves, and cephalopods. Tropomyosin (a muscle protein) is the generally recognized major allergen of this food category. Subjects allergic to mollusks are often allergic also to crustaceans, due to sequence similarity of tropomyosin. The approximate prevalence is around 0.5–2.5%, with higher percentages in Asia, where shellfish consumption is higher.

Among the products of vegetal origin, the most important are the following:

- *Celery* (*Apium graveolens*). Several allergens were identified, Bet v 1 homologous protein, chlorophyll a/b-binding protein, profilin, and a 55/58 kDa allergen. This type of allergy is pollen-related and it is more frequent in some European countries (Switzerland, France, and Germany), where celery is usually consumed as a raw vegetable.
- *Cereals containing gluten*. The prolamin and glutelin fractions of wheat (*Triticum* spp.), barley (*Hordeum vulgare*), rye (*Secale cereale*), and presumably oats (*Avena sativa*) induce a cell-mediated food allergy in celiac subjects. Gluten constitutes more than 10% (w/w) of wheat and is the main trigger of celiac disease, which affects about 1% of the population. Other cereal allergens are involved in IgE-mediated food allergies: nonspecific lipid transfer proteins, chloroform–methanol (CM)-soluble proteins, and gluten. ω_5-Gliadins are involved in wheat-dependent exercise-induced anaphylaxis, while CM-soluble proteins are the main trigger of baker's asthma, an occupational disease.
- *Lupine* (*Lupinus* sp.). The consumption of lupine is increasing in the last years due to its use as an alternative protein source for vegetarian or gluten-free foods or as a food additive. The main triggers of lupine allergy are conglutinins, more specifically vicilin-like protein (an acid 7S globulin), legumin-like protein (an 11S globulin), sulfur-rich 2S albumin, and basic 7S globulin. Prevalence is still largely unknown.
- *Mustard*. This category is composed of three different species: *Sinapis alba*, *Brassica juncea*, and *Brassica nigra*. Mustard is a spice widely used for the preparation of sauces and dressing, mainly in some Southern European countries such as France and Spain. The main proteins recognized by IgE of allergic patients are 2S albumin, 11S globulin, lipid transfer protein, and profilin. It is more common in France (up to 8.9%), where mustard is mostly grown and consumed.
- *Tree nuts*. This category includes different species: almond (*Prunus dulcis*), Brazil nut (*Bertholletia excelsa*), cashew (*Anacardium occidentale*), hazelnuts (*Corylus avellana*), pecan nut (*Carya illinoinensis*), pistachio (*Pistacia vera*), and walnut. Peanuts are not included, because they are considered legumes. Major allergens are not only 2S albumin, 7S globulin, and 11S globulin but also PR-10 protein, profilin, ribosomal proteins, nonspecific lipid transfer proteins, and oleosin. Prevalence is around 1% of the population.
- *Peanuts*. Major allergens of peanuts (*Arachis hypogaea*) belong to the cupin superfamily (7S vicilin-like globulin and glycinin) and to the prolamin superfamily (trypsin inhibitor and 2S storage protein). Prevalence of peanut allergy is 1–2% of the US population.
- *Sesame* (*Sesamum indicum*). Sesame is used for oil production and it has been recently introduced in the production of bakery products; it is more diffuse in the oriental countries. Allergy to sesame is not very diffused (0.1%) and it is directed against a 2S albumin and oleosins.
- *Soybeans* (*Glycine max*). Soybeans are rarely consumed as such, but they are used for the production of a wide variety of food products, especially as an animal protein replacer for vegetarian food. The triggering proteins of soy allergy are the soybean hydrophobic protein Gly m 1, defensin, profilin, PR-10 protein, β-conglycinin, and glycinin. Prevalence of soy allergy is around 0.2% of the population.

Legislation on Labeling of Foods Containing Allergens

The regulatory frameworks for the labeling of foods containing allergens are highly diverse around the world. In Table 1, the regulatory frameworks of several countries are reported, also including the Codex standard that in many legislative frameworks is used as reference.

Each legislation identifies priority allergens, even if the criteria of inclusion/exclusion in this list are often unclear. The priority allergens in the legislative frameworks reported in Table 1 are given in Table 2.

Five foods are common in all the frameworks: wheat cereals, eggs, milk, peanuts, and crustaceans. Excluding Japan, also fish, soy, and tree nuts occur in every list. These allergens are actually responsible for most of the adverse allergic reactions to foods. Anyway, within every group of foods, the exact description of the species can vary from country to country.

These legislations mostly address the labeling rules when the earlier foods or derivatives obtained from them are used as ingredients in food processing, with the purpose of warning potential allergic consumers of the presence of allergenic ingredients or additives. Most of the implemented regulations include the allergenic ingredient labeling in the preexisting labeling format for ingredients, but more and more, the regulatory frameworks also foresee the use of additional warning 'contains' statements, such as in the United States, Canada, and very recently EU.

Labeling always and only concerns the presence of an allergenic ingredient, with no indications on the amounts. This is

Table 1 Most important food allergen regulatory frameworks

Jurisdiction	*Legislative framework*
Codex Alimentarius	Codex General Standard for the Labelling of Prepackaged Foods (CODEX STAN 1-1985)
Australia	Australia New Zealand Food Standards Code – Standard 1.2.3 – Mandatory Warning and Advisory Statements and Declarations
Canada	Regulations Amending the Food and Drug Regulations (1220 – Enhanced Labelling for Food Allergen and Gluten Sources and Added Sulfites)
China	General Rules for the Labeling of Prepackaged Foods (GB7718-2011)
EU	Regulation No 1169/2011 and Annex II; EU Commission Directive 2007/68/EC and Annex IIIa; EU Commission Directive 2003/897/EC and Annex IIIa
Japan	Standards and Evaluation Division, Department of Food Safety, Pharmaceutical and Medical Safety Bureau, Ministry of Health, Labour and Welfare
The United States	Food Allergens Labeling and Consumer Protection Act of 2004

Table 2 Priority allergens in different regulatory frameworks

	Codex	*Australia*	*Canada*	*China*	*EU*	*Japan*	*The United States*
Wheat/cereals	X	X	X	X	X	X	X
Eggs	X	X	X	X	X	X	X
Milk	X	X	X	X	X	X	X
Peanut	X	X	X	X	X	X	X
Fish	X	X	X	X	X		X
Crustaceans	X	X	X	X	X	X	X
Soy	X	X	X	X	X		X
Tree nuts	X	X	X	X	X		X
Sesame		X	X		X		
Shellfish/mollusks			X		X		
Mustard			X		X		
Celery					X		
Lupine					X		

due to the enormous difficulty of setting commonly agreed thresholds for consumer protection, given the extreme diversity of the allergic subjects as far as sensitivities are concerned. The absence of agreed thresholds implies that no labeling of the format 'allergen-free' has been implemented yet, being unclear what the threshold level for the 'free' declaration is. A striking exception is the gluten-containing products: since a threshold safety level of 20 ppm has been agreed, whenever a food contains <20 ppm of gluten, it can be labeled 'gluten-free.'

All the earlier labeling rules only concern the use of allergenic foods as ingredients, whereas their adventitious presence as contaminants (the 'hidden' allergens) is usually not covered by the earlier legislations.

Impact of Food Processing on the Allergenic Properties of Food Allergens

During processing, food proteins can undergo a series of modifications, including protein unfolding and aggregation, chemical modifications, and proteolysis. Only in the most extreme cases, proteins are degraded in smaller peptides, but very often, they at least experience alterations in the tertiary and secondary structures, through heating, pH changes, or adsorption to surfaces. As a common consequence, many proteins in food matrices can also undergo aggregation. All these modifications have the potential to alter their allergenic potential, both directly, affecting the way in which the proteins present themselves to the immune system, and indirectly, affecting their resistance to gastrointestinal digestion. The length of treatment, pH, temperature, and the presence of other components potentially interacting with proteins (sugars and fats) all affect the kinetic of food protein denaturation and aggregation. Allergens might roughly be divided into processing-labile and processing-stable ones.

The best example of processing-labile allergens is represented by the proteins of the Bet v 1 family of plant allergens, since they often contain conformational epitopes easily lost upon protein unfolding. Thus, as a general rule, processing will reduce the allergenicity of proteins belonging to the Bet v 1 family, but not surprisingly, also the type of processing has a high impact. For example, cooking cherries usually reduces their allergenicity, whereas roasting hazelnuts has very little impact on their allergenic potential. This is likely due to the fact that heating in a wet environment, as it is cooking, helps denaturation, whereas heating in a dry environment, like roasting, is a very inefficient way to denature proteins. Moreover, not all the members of the Bet v 1 family are equally thermolabile, with some being more stable than others. Gly m 4 from

soy is more thermostable than Mal d 1 from apple, and this can relate to the observations that the former protein can trigger allergic reactions even in highly processed soy products.

On the other side, allergens of the prolamin superfamily exhibit a very stable structure, due to disulfide bridges providing resistance to a variety of denaturing conditions, including low pH, chaotropic agents, high temperatures, and high pressures. 2S albumins and lipid transfer proteins (LTPs) belong to this class, and thus they retain their allergenic properties also in processed foods. High thermal processing does not reduce allergenicity of peach juices or apple peels, if due to these proteins, and similarly, allergenicity to maize LTP is retained also after cooking polenta. These findings correlate with the observation that subjects allergic to these proteins often undergo very severe reactions. Thermal treatments also reduce allergenicity to milk whey proteins, by a proportion that it is usually related to the length and the harshness of the thermal treatment.

Besides proteins having thermally stable structures, there are proteins that are mostly recognized through linear epitopes; thus, even if unfolded or partially unfolded by thermal treatments, they fully maintain their allergenic potential. Two major proteins falling in these categories are milk caseins and wheat gluten.

The loss of the folded structure is not the only consequence of thermal treatments. Several chemical modifications can occur to the amino acid side chain upon heating, the most notable being the Maillard reaction between free amino groups (mostly from lysine residues) and aldehyde or ketone groups from sugars, which lead to further rearrangement giving rise to diversely glycated proteins. Glycated proteins might undergo cross-link and increase their IgE-binding abilities, leading to increased allergenic potential of modified proteins. This has been observed, for instance, in the case of peanut allergens Ara h 1 and Ara h 2 and shellfish tropomyosin. However, glycation of fruit allergens, on the other side, does not seem to increase their allergenic potential.

In conclusion, there are no clear rules on how the food processing impacts the allergenic potential of the food proteins. Some allergens show a decreased allergenic potential upon treatment, others are unaltered in their allergenicity, and some can even show an increase. It is anyway clear that the response of the single individuals to allergens can be highly affected by the processing, requiring more deep insights on the matter.

Digestibility of Food Allergens and Correlation with the Allergenic Potential

The analysis of many allergenic foods indicates that many, but not all, common food allergens share the characteristic of being resistant to gastrointestinal digestion. This means that a significant portion of the intact protein might survive intact for a long time in the hostile (for proteins) gastrointestinal environment, thus increasing the chances of triggering an immune reaction. Such resistance to enzymatic digestion is often (but not always) accompanied by a similar resistance to heat treatments or other denaturing processes, leading to the suggestive concept that a general 'robustness' of the structure is a requisite, increasing the chances for a protein to become an allergen.

For milk and wheat allergens, indeed, the resistance to pepsin digestibility was well related to the induction of an allergic response, being these allergens quite resistant to pepsin attack. When the susceptibility to pepsin was increased by reducing disulfide bonds, and the allergens become more readily digested by pepsin, as a consequence, their ability to induce an allergic response was also highly reduced. Even if not all allergens are perfectly resistant to pepsin, it is true that all food allergens are more resistant to pepsin hydrolysis than common plant proteins. In the standard conditions of pepsin assay, intact plant allergens or significant portions of them were found to resist from 8 to 60 min, whereas nonallergenic plant proteins never resisted for more than 15 s. Even if the exact duration of the resistance was subsequently challenged by further experiments, nevertheless, the basic finding that allergen proteins are generally more resistant to enzymatic digestion was confirmed. Further studies on the peanut allergen Ara h 2 demonstrated that food allergen structure is linked to the stability of digestion and in turn to its ability to elicit an allergenic response through binding to the IgE.

Further studies with more sophisticated digestion models strictly simulating the gastrointestinal fluids challenged again this view, demonstrating that food allergens might be more, or equally, or less susceptible to digestion as compared to nonallergenic proteins, so digestibility alone, or rather the resistance to it, is not per se a causal reason for the induction of an allergic response. Moreover, among allergens, the resistance to digestion is a poor predictor of the potency of the allergenic response. For example, ovalbumin, a major egg allergen, is degraded in simulated gastrointestinal fluids in <5 min, whereas lysozyme, a minor allergen, is stable for more than 60 min. Alpha-casein, a major milk allergen, degrades more rapidly than bovine serum albumin, a very minor allergen.

So, even if a certain resistance to digestion seems to be a common tract of many allergens, the resistance to digestion alone is not per se the reason why a protein becomes an allergen.

Analysis of Food Allergens

Analysis of food allergens is of outmost importance from two different points of view: first, for the detection of food allergens in food and, second, for the study of the molecular features of food allergens.

Detection of food allergens is of basilar importance for the safety of allergic subjects. Since the allergic response is highly variable among subject and the type of allergy, a defined 'safe threshold' for food allergens cannot be defined. Moreover, the dose–response effect is not proportional, and in some allergic subjects, even a small trace of the triggering food can provoke serious consequences, even anaphylactic shock. So, detection of food allergen requires very sensitive and accurate methods in order to ensure protection of allergic subjects. Different approaches can be used for the detection of food allergens, but two main categories can be identified: DNA-based methods and protein-based methods. The latter are based on immunoassays or on mass spectrometry methods.

DNA-based methods are indirect detection methods: they do not detect the allergen itself, but the DNA of the allergenic ingredient. These methods are based on a real-time polymerase

chain reaction (RT-PCR) and can be qualitative (present/absent) or quantitative. In this latter case, it is necessary to have a suitable standard of the allergenic ingredient (at known concentration) to construct a calibration curve. The target genes do not necessarily encode for the specific target allergen, but they are species-specific for the organism of origin. Several problems arise from this; for example, different food ingredients cannot be distinguished, for example, chicken from egg or milk from beef. Detection can be achieved using fluorescent probes (gene-specific) or SYBR Green (nonspecific; it binds to double-stranded DNA). DNA can be extracted from the food matrix using different extraction procedures on the basis of the starting material. Briefly, cell membranes must be disrupted with detergents, and RNase treatment is necessary to degrade RNA that could interfere with the subsequent quantification and amplification. DNA is then purified using column for cleanup in which sugars, proteins, and salts are washed away. Primers (and eventually probes) are designed on a single- or multicopy gene of the target organism. Once a correct fluorescence threshold is fixed, the number of amplification cycles necessary to reach that value will be inversely proportional to the $\log_{10}$ of gene copies present in the sample. These methods are very sensitive and extremely specific (genotype is very stable) and able to detect $<0.001\%$ of allergenic ingredient (ten DNA copies are enough to achieve a correct amplification). Better performance is obtained in raw food matrices, because during food processing, DNA can degrade due to heat treatment or hydrolysis processes. DNA degradation can affect both DNA extractability from the matrix and DNA length: if the target gene is hydrolyzed, allergen content can be underestimated or even not detected. The different stability of nucleic acids and protein to technological treatments can lead to controversial results: DNA can be still detectable even when the allergenic protein is denatured and not anymore allergenic (false-positive), or, on the opposite, DNA could not be present (or it can be present in too short fragments to be amplified) but the allergen could still be intact. PCR-based methods were developed for a wide variety of allergenic foods: cashew, peanut, hazelnut, celery, sesame, lupine, mustard, pea, pecan nut, pistachio, soy, walnut, etc. A lot of ready-to-use kits are also available on the market and easily employable in routine analysis.

Protein-based methods can rely on the recognition antigen–antibody and they can be classified into ELISA (enzyme-linked immunosorbent assay) tests and LFD (lateral flow device) tests. These methods can be direct, if the target protein is the allergen, or indirect, if they detect a specific protein of that species that is not the allergenic one. They can be qualitative or quantitative: In this latter case, a standard of the allergen is required for the construction of the calibration curve. The limit of quantification varies in function of the analyte and of the food matrix, but usually, it is around a few ppm. ELISA tests can be further divided into two types: sandwich assays and competitive assays. In both cases, it is necessary to extract the protein fraction from the food matrices, and the protocol varies depending on the allergen type and the food matrix; usually, the use of denaturing and reducing agents is required. In sandwich ELISA, the wells are coated with monoclonal (highly specific) or polyclonal (less specific but sometimes more sensitive) antibodies specific for the target protein. When the protein extract is added, the antibodies on the well surface will recognize the antigens, while other compounds are washed away. The detection is achieved by means of a second antibody that will recognize a second epitope on the previously bound protein. This latter antibody is functionalized with an enzyme catalyzing a chromophore reaction. So, the absorbance can be measured spectrophotometrically and it is directly proportional to the amount of antigen. Sandwich-type immunoassays require thus that two epitopes are present on the same polypeptide, and this can lead to an underestimation of the allergen content in the case of foods undergone to a certain degree of hydrolysis. To overcome this problem, some competitive ELISA assays were developed. In this type of assay, the protein extract is incubated with specific antibodies that will recognize the antigen. Then, the mixture is added to antigen-precoated wells, where the remaining free antibodies will bind to the fixed antigen. Other compounds are washed away and the detection is achieved using a second antibody that will recognize the first one. The secondary antibody is functionalized with an enzyme catalyzing a chromophore reaction. So, the absorbance can be measured spectrophotometrically and it is inversely proportional to the amount of antigen. Finally, in LFDs, a drop of the protein extract is placed at the beginning of a strip where it will contact specific antibodies previously adsorbed. The antibodies will bind to the antigens and the complex will migrate for capillarity toward a second line, where another series of antibodies is placed. These antibodies will recognize the antigen–antibody complex previously formed, and the reaction can be monitored colorimetrically. They are only semiquantitative but useful for the on-line monitoring of cleaning processes. Protein-based assays are simple, easy, and fast, so they find broad application in quality control laboratories. Some limits to the detection can arise in the case of processed foods, where protein denaturation can strongly reduce their extractability and thus their detectability. A lot of commercially available kits are on the market for quite all the type of allergens. The different extraction protocols and the different antibodies used in every kit lead to a sometimes high interkit variability, with serious issues for the safety of allergic subjects and for the legal issues.

In the recent years, the development of mass spectrometry also had an impact in the field of allergen analysis. The technique is based on the enzymatic digestion of a protein extract or of the whole-food matrix. Then, specific marker peptides are identified for the allergenic protein. This method is direct and with a specificity comparable with that of DNA-based methods. The method can be not only qualitative (detection of the marker peptide) but also quantitative. In this latter case, a standard is necessary and the quantification can be carried out in two different ways. If there is availability of the standard of the allergenic protein, it can be digested in matrix in different concentrations in order to obtain a calibration curve. The lack of the protein standard can be overcome by direct synthesis of the marker peptide, which can thus be used for the quantification. The implementation of multiple reaction monitoring methods allows us to obtain limit of detection and of quantification comparable to those of RT-PCR and ELISA assays. At the moment, the use of mass spectrometry detection of food allergens is more suitable for confirmation analysis than for routine, since it requires very expensive equipment and trained specialists.

See also: Proteins: Chemistry, Characterization, and Quality.

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Food and Agriculture Organization of the United Nations

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History and Purpose of the Food and Agriculture Organization

The Food and Agriculture Organization (FAO) of the United Nations is an autonomous agency within the United Nations (UN) system. The organization had a membership of 180 countries in 2001.

In 1943, world leaders met at Hot Springs, Virginia, the United States, and determined to lay a framework for peace through international cooperation in food and agriculture. The meeting, the UN Conference on Food and Agriculture, decided on the establishment of permanent organization in the field of food and agriculture and set up an interim commission to prepare a specific plan. This commission worked for 2 years and prepared the constitution of the FAO. The signing of the constitution and the opening of the first FAO Conference were arranged to take place in Quebec, Canada, on 16 October 1945. Representatives from 44 nations met at this date and established the FAO, the first of the UN agencies. As the commemorative tablet in Canada states, "Thus for the first time nations organized to raise levels of nutrition and to improve production and distribution of food and agricultural products." The FAO's goal is enshrined in its motto, "*Fiat Panis* – Let there be bread."

The FAO Conference is the governing body and is composed of all member nations. It meets every 2 years and is a major forum for the discussion of food and agriculture issues. It is able to take concerted action on issues of concern to its members. Recent initiatives include the adoption of the World Food Security Compact and the International Code of Conduct on the Distribution and Use of Pesticide.

The FAO Conference elects a council to which a series of committees, such as the Committees on Commodity Problems, Fisheries, Agriculture, Forestry, and World Food Security, report. Many bodies have been set up by the FAO Conference and Council to foster cooperation in particular subject areas. In adopting the International Undertaking on Plant Genetic Resources in 1983, for example, the FAO Conference provided for the establishment of the Commission on Plant Genetic Resources to guide the FAO on policy and to monitor the implementation of the undertaking. The Commission on African Animal Trypanosomiasis was established in 1979 to coordinate a large-scale FAO program to control the disease. Another example is the cooperation of the FAO with the World Health Organization (WHO) through the Joint FAO/WHO Codex Alimentarius Commission, which has issued some 200 international food standards.

Along with these bodies, the FAO organizes conferences and meetings on particular issues. For example, the FAO-serviced World Food Conference in 1974 resulted in a series of measures to promote world food security. In December 1992, the first International Conference on Nutrition (ICN) was held in the FAO's headquarters in Rome. It was jointly sponsored by the FAO and the WHO. At this conference, the World Declaration and Plan of Action for Nutrition were unanimously adopted, committing governments to prepare their National Plans of Action for Nutrition containing attainable goals and measurable targets based on the principles and relevant strategies agreed at the ICN. World leaders assembled in Rome in November 1996 for the World Food Summit aimed at renewing global commitment to the fight against hunger. The FAO called the World Food Summit in response to widespread undernutrition and growing concern about the capacity of agriculture to meet future food needs.

Besides being a major forum for the discussion of food and agriculture matters, the FAO is an information center collecting data related to its field of work. It also acts as an adviser to governments, being a useful source of independent advice on agricultural policy and planning, on the administrative and legal structures needed for development, and on ways of ensuring that national development strategies are directed toward rural development and the alleviation of poverty and malnutrition. Its role as a development agency should be highlighted: the FAO gives direct, practical help in the developing world through technical assistance projects in all areas of food and agriculture (including fishery and forestry). These field projects strengthen local institutions, assist research and training, and develop and demonstrate new techniques.

Food Security and Nutrition

The idea of international action to achieve world food security was launched by the FAO in early 1973. The international undertaking in world food security contains a set of general principles for stockholding, special assistance to developing countries (including aid to increase production), the exchange of information, and arrangements for consultations. The concept has developed further. Today, the objective of the world food security concept is to ensure that all people at all times have both physical access and economic access to the basic food they need. Its more specific aims are related to production, with special emphasis on low-income food-deficit countries, stability in the flow of supplies, and the problems of access to available supplies on the part of needy nations and social groups.

The search for policies that would cope with food shortages and surpluses has been one of the main threads in FAO history. Various activities contributed to the preparation of such a policy, including the following: expanding production, improving nutrition, stabilizing prices, obtaining a fair return for producers, and coping with famine.

Nutrition was a dominant theme at the Hot Springs Conference, with the adoption of eight resolutions dealing exclusively with various aspects of nutrition, far more than on other subjects. There is no single formula for curing malnutrition. The best approaches vary, according to local and national

circumstances. The development of policies for food and nutrition is largely a question of orienting sectoral activities to have an impact on nutrition. Thus, from the mid-1970s, the FAO worked closely with a number of governments on the incorporation of nutritional objectives into development plans.

A mass of information on every aspect of food and agriculture (including production, supply, demand, prices, and technology) is needed if government planners, traders, scientists, farmers, and technologists in every country in the world are to make rational decisions on planning, investment, marketing, research, or training. The FAO provides that essential information for food policy planning. For example, the Global Information and Early Warning System on Food and Agriculture monitors production conditions and food supply prospects worldwide and issues warnings when there are indications of trouble. The role of world food surveys cannot be overemphasized in the assessment of world nutrition status. The FAO prepares these surveys for collecting essential data on the world's food problems. These data are analyzed and distributed to all member countries and concerned bodies as reference data for the elaboration of food policies at world or national levels. At the time of writing, the latest survey is the sixth one, published in 1996.

In addition to its tasks of collecting, analyzing, and disseminating information, advising governments on food and agriculture (including fishery and forestry) policy and planning, and providing opportunities for governments to meet and discuss food and agriculture problems, the FAO carries out a major program of technical advice and assistance for the agricultural community on behalf of governments and development funding agencies. The FAO is involved at every stage of agricultural development, from demonstrating new cultivation techniques to subsistence farmers to advising governments on how to achieve more stable and equitable international trade in agricultural commodities.

Food Control

In the past several decades, nations have experienced extensive changes and faced new or greater problems in assuring adequate national food security, trade, and health. This trend is continuing for the following reasons: intensification of farm production and the centralization of food manufacturing; the development of large urban markets remote from the agricultural production areas; markets and consumer demands for consistent supply of safe, uncontaminated, and good-quality food and food products; the economic dependency of many countries on revenues from agricultural food exports; and the rapid growth in global food trade.

Within the overall mandate of the FAO with regard to production, distribution, and marketing of food, and for nutrition improvement, the necessity of providing a safe, wholesome, and nutritious food supply, and the protection of this supply from losses, waste, deterioration, contamination, and adulteration, is implicit. It is interesting that the Hot Springs Conference noted that the FAO would play a role in ensuring "protection of the public, through the medium of pure-food laws, against impurities for adulteration and against unfair competition and undesirable trade practices." It recommended that the FAO should "assist governments to extend and improve standards of nutrient content and purity of all important foods." In view of this, the FAO has developed programs to assist governments to orient their country policies and programs so as to include nutrition improvement and food protection as specific objectives.

Food safety and quality start at the farm level and continue throughout the processing and distribution chain to storage and final preparation by the consumer. Good agricultural, processing, distribution, and marketing practices are essential to ensure consumer protection. Now, more than ever, food control programs are needed in order to assure that foods are safe for consumption and are sold to the consumer on fair terms; furthermore, such programs should help to reduce food losses caused by improper handling or spoilage of raw materials, prevent 'dumping' practices of shipping nonstandard (low-quality or contaminated) food products to countries that do not have the capability of food control, and increase the earnings of foreign currency necessary for the implementation of development plans. Food control, including standards, is therefore an integrated part of the programs developed by the FAO.

Issues relating to food safety and food control draw considerable public attention and represent an important concern for governments, consumers, and the industry alike. They need to be addressed at the national level by adequate legislation, which should be developed in cooperation with all interested sectors and implemented efficiently.

The Economic and Social Development Department of the FAO and especially its Food and Nutrition Division (ESN) are the main bodies responsible for the programs oriented to guarantee food quality and safety and consumer protection. Within this division, the Food Quality and Standards Service focuses on activities in the field of food control. These activities are implemented through the Food Standards Group, which provides the Secretariat for the Codex Alimentarius Commission of the Joint FAO/WHO Food Standards Programme, and the Food Quality and Consumer Protection Group, which assists developing countries in the formulation and development of effective food control programs and gives scientific and technical support to the Codex Alimentarius Commission. Technical assistance provided by the FAO in food control is based upon the fundamental elements that all effective programs must consider:

- A basic food law complemented by detailed regulations that provide for correct hygienic practices in food and agricultural production and processing; the establishment of food standard and codes of practice; the correct use of food additives, pesticides, etc.; and the proper use of labeling to avoid consumer deception
- An effective administration of such laws and regulations through an organization, which includes technical officers, inspectors, laboratory personnel, and compliance officers, together with the necessary infrastructure to carry out their tasks adequately
- A well-informed consumer, aware of the problems related to food control, especially with respect to good handling practices and storage
- Food producers, processors, and handlers who cooperate with government agencies in assuring food quality and safety

In order to ensure food security, facilitate trade, and protect consumers, the development of the following activities receives special consideration from the FAO:

- Support of developing countries in strengthening their national strategies for quality and safety, food control systems, and contamination monitoring programs
- Preparation of food legislation, regulations, and standards on a national, regional, or international basis accordingly to meet changing consumer demands (e.g., food irradiation and biotechnology), new food ingredients (e.g., vegetable proteins), and new food safety concerns (e.g., veterinary drug residues and radionuclides)
- Technical assistance in training for food control inspectorate and laboratory personnel and for food control management staff

Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission

The Hot Springs Conference noted the role of government services in ensuring the protection of the public. It recommended that the FAO, when established, should "consider the formulation and adoption of international standards to facilitate and protect the interchange of such products between countries, and agree upon international methods of determination."

In 1958, work was begun by the FAO/WHO Committee of Government Experts, in collaboration with the International Dairy Federation, on the establishment of the Code of Principles concerning Milk and Milk Products and compositional standards for milk products. A further development that took place in 1958 and that was to prove to be of great importance to both the FAO and the WHO in introducing an international program for the elaboration of food standards was the creation of a body known as the Codex Alimentarius *Europaeus*. In 1961, the Council of the European Codex Alimentarius adopted a resolution proposing that the work should be taken over by the FAO and WHO. From that moment, matters moved quickly, and the Codex Alimentarius Commission was established in 1962 by the decision of the FAO Conference and the WHO Executive Board.

The basic objectives were precisely those put forward at Hot Springs: removal of nontariff trade barriers caused by differing national food legislation, ensuring fair practices in international trade, and protection of the consumer against health risks and fraud. The commission assumed the role of an international forum for elaborating standards for foods moving in international trade and for providing guidance to countries wishing to create their own national food laws and regulations.

The Uruguay Round of multilateral trade negotiations concluded at Marrakesh in April 1994. It established the new World Trade Organization (WTO). Among the agreements were the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement) and the Agreement on Technical Barriers to Trade (the TBT Agreement). Both of these agreements are relevant in understanding the international dimensions of food. The SPS Agreement encourages the use of international standards, guidelines, or recommendations where they exist and identifies those of Codex Alimentarius (relating to food additives, veterinary drugs and pesticides residues, contaminants, methods of analysis and sampling, and codes and guidelines of hygienic practices), as consistent with provisions of the SPS. Thus, the Codex Alimentarius standards serve as a benchmark for a comparison of national sanitary and phytosanitary measures. The TBT Agreement also recognizes international standards where they exist. It requires that technical regulations on traditional quality factors, fraudulent practices, packaging labeling, etc., imposed by countries not be more restrictive on import products than they are on products produced domestically.

Membership of the Codex Alimentarius Commission is open to all member nations and associate members of the FAO and/or WHO. In 30 years, the commission has grown from the original 30 countries that were present at its first session in 1963 to 165 member countries in 2001. The distribution of member countries is as follows: Africa, 41; Near East, 19; Asia, 21; Europe, 40; Latin America and the Caribbean, 31; North America, 2; and Southwest Pacific, 11. The commission has an executive committee and 29 subsidiary bodies. All of these bodies, like the commission, are intergovernmental in character. The composition of the executive committee is such as to ensure an adequate representation of the various geographic areas of the world to which the members of the commission belong. The executive committee makes recommendations to the commission regarding the general orientation of the program of the work.

The main activities of the commission have been concerned with the development of international worldwide standards for foods as well as other texts on related matters, their publication in the Codex Alimentarius, and the registration of acceptances by member governments of standards. In addition, the commission, through its subsidiary bodies, is concerned with the elaboration of advisory codes of practice, which are adopted within the Codex Alimentarius. The commission determines priorities and initiates and guides the preparation of draft standards, whether by its own subsidiary bodies or with the assistance of other appropriate organizations.

During its lifetime, the Codex Alimentarius Commission has elaborated more than 230 individual commodity standards, 37 codes of hygienic and technological practices, and maximum residue limits (MRLs) for pesticides covering more than 3000 potential pesticide plant and animal combinations. The Codex Alimentarius itself contains standards on the following: processed, semiprocessed, or nonprocessed food products; raw materials used in manufacturing; labeling; nutrition information; food additives; contaminants; pesticide residues; veterinary drug and hormone residues in food; methods of analysis; sampling; codes of practice; and guidelines and other recommendations. All of the previously mentioned have been published in 16 volumes of the Codex Alimentarius.

Labeling

Food labeling is the primary means of communication between the producer and the consumer. One of the most vexatious problems that exporters of foodstuffs are likely to encounter is the wide disparity between different countries'

requirements with regard to labeling. The work of Codex Alimentarius in devising and issuing the General Standard for the Labelling of Prepackaged Foods must be regarded as a landmark, a major achievement, in international recommendations for food legislation.

The Codex Alimentarius has issued the Guidelines for the Production, Processing, Labelling, and Marketing of Organically Produced Foods. These guidelines set out the principles of organic production at farm, preparation, storage, transport, labeling, and marketing stages and provide an indication of accepted permitted inputs for soil fertilizing and conditioning, plant pest and disease control, and food additives and processing aids. For labeling purposes, the use of terms inferring that organic production methods have been used are restricted to products derived from operators under the supervision of a certification body or authority.

Nutrition Information

Consumer demand for nutrition information is growing, stimulated by the media, whose interpretation and presentation of the views of nutrition scientists and on national policies are not always balanced or accurate. Few consumers have any systematic training in nutritional matters or easy recourse to reliable information sources. The natural concern for the family health leads them to use the media as their information source. The problem faced by the Codex Alimentarius was how to regulate it so that accurate and balanced information could be presented without creating a rigid system. The Guidelines on Nutrition Labelling have been issued to ensure that nutrition labeling is effective:

1. Providing the consumer with information about a food so that wise choices can be made
2. Providing a means for conveying information of the nutrient content of a food on the label
3. Encouraging the use of sound nutrition principles in the formulation of foods that would benefit public health
4. Providing the opportunity to include supplementary nutrition information on the label

Nutrition labeling should not describe a product or present information that is in any way false, misleading, deceptive, or insignificant in any manner, to ensure that no nutritional claims are made without nutrition labeling.

Food Additives

The use of food additives is becoming more important in the production and worldwide distribution of food. As the demand for variety increases, and with it the desire to purchase goods from distant lands, the importance of food additives will increase, rather than diminish. None the less, this is a topic of great consumer concern, and the Codex Alimentarius has always treated it as such through the Codex Committee on Food Additives and Contaminants (CCFAC). The CCFAC considers technological needs on the one hand from information supplied by Codex commodity committees. On the other hand, it applies safety considerations based on the reports of the Joint Expert Committee on Food Additives. The CCFAC follows a risk-based approach to develop the General Standard for Food Additives, which includes a list of additives that can be used in accordance with good manufacturing practice and specific levels of use for a number of additives for which an acceptable daily intake (ADI) has been established.

Contaminants

The term 'contaminants' is used to denote the presence in food of traces of undesirable elements of substances, for example, traces of lead, cadmium, polychlorinated biphenyls, or even radioactive fallout. The CCFAC is responsible for establishing permitted maximum or guideline levels for individual food additives, for contaminants (including environmental contaminants), and for naturally occurring toxicants in foodstuffs and animal feeds. The establishment of maximum levels for contaminants is a complex subject, and there are specific difficulties to address in order to reach an agreement at the international level in this area. Sometimes, the complete or updated risk assessment is lacking, especially exposure assessment, and there is a need to update the scientific information. In addition, legislation in member countries differs widely, especially between exporter and importer countries, and it is difficult to come to a consensus; there are also several technical problems to be addressed, especially as regards methods of analysis and sampling or inspection procedures.

Pesticide Residues

The use of pesticides is essential in agriculture and horticulture if crop yields are to be economically viable and, indeed, if crops are to be successful. The fact that residues can remain in the food is another topic of considerable consumer and even wider concern. There is well-justified concern that an unrestricted and uninformed use of these substances could damage health. The Codex Committee on Pesticide Residues, on the basis of the work of the FAO/WHO Joint Meeting (Committee of Experts) on Pesticide Residues (JMPR), has established MRLs.

MRL is the maximum concentration of a pesticide residue (expressed as milligrams per kilogram) recommended by the Codex Alimentarius Commission to be legally permitted in or on food commodities and animal feeds. MRLs are based on good agricultural practice data, and foods derived from commodities that comply with the respective MRLs are intended to be toxicologically acceptable.

Codex MRLs that are primarily intended to apply in international trade are derived from estimations made by the JMPR as follows:

1. Toxicological assessment of the pesticide and its residue.
2. Review of residue data from supervised trials and supervised uses including those reflecting national food and agricultural practices. Data from supervised trials conducted at the highest nationally recommended, authorized, or registered uses are included in the review. In order to accommodate variations in national pest control

requirements, the Codex MRLs take into account the higher levels shown to arise in such supervised trials, which are considered to represent effective pest control practices. Consideration of the various dietary residue estimates and determinations at both the national level and the international level in comparison with the ADI should indicate that foods complying with the Codex MRLs are safe for human consumption.

Food Hygiene

Poor food hygiene is a major cause of human illness. Most hygiene problems are caused by faulty handling of food and, in the case of processed or packaged food, often occur after manufacture and packaging. There are powerful reasons for the great attention paid by the Codex Alimentarius to food hygiene. In matters of hygiene, it is especially true that good practices and economic benefits go hand in hand. The establishment of effective good manufacturing practice as regards hygiene is relatively simple in principle but is far from simple in practice.

The Codex Alimentarius has issued a code of practice and the General Principles of Food Hygiene. These general principles lay a firm foundation for ensuring food hygiene. The document follows the food chain from primary production through to final consumption, highlighting the key hygiene controls at each stage. It recommends a hazard analysis and critical control point (HACCP)-based approach, wherever possible, to enhance food safety. The HACCP system, which is science-based and systematic, identifies specific hazards and measures for their control to ensure the safety of food. HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing.

Principles for the establishment and application of microbiological criteria for foods have been approved by the Codex Alimentarius Commission. These principles are intended to give guidance on the establishment and application of microbiological criteria for foods at any point in the food chain from primary production to final consumption.

Current Issues

Matters related to biotechnology are at the center of considerable debate in many countries and also at the international level. In the framework of Codex Alimentarius, the commission decided to establish the Task Force on Food Derived from Biotechnology, in order to ensure a comprehensive approach and to consider safety, nutrition, and all relevant aspects of genetically modified organisms (GMOs) and food produced from GMOs.

Several food safety issues are related to animal feeding, especially contamination of feed with mycotoxins and other contaminants. This question drew the attention of the media owing to the recent occurrence of contamination with dioxins in feeds, but there are also several other issues, including the bovine spongiform encephalopathy. The commission decided to establish a specific Task Force on Animal Feeding to consider all aspects that can affect food safety. Another important emerging issue in relation to animal production is antimicrobial resistance in bacteria, and in view of its complexity, it should be addressed through a multidisciplinary approach. It is currently under consideration by the Committee on Food Hygiene and the Committee on Residues of Veterinary Drugs in Foods and is also relevant to the work of the task force.

Future Directions

The fundamental objective of the Codex Alimentarius Commission is to establish sound internationally agreed guidelines for national food control systems based on the criteria of consumer health protection and fair practices in trade, taking into account the needs and special concerns of all countries.

The new recognition and status that Codex Alimentarius standards, guidelines, and other recommendations acquired under the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement) also brought new challenges and responsibilities, including the need to ensure that its standards and related texts are based on scientific principles and meet the needs and mandate of the organization. The WTO Agreement on Technical Barriers to Trade is also of great relevance, given the significance of the provisions pertaining to product description, labeling, packaging, and quality descriptors for consumer information and fair practices in trade. Although quality provisions are fundamentally driven by the market, the CAC has an important role in ensuring that provisions relating to quality are sound and based on the criteria of essentiality and do not constitute disguised barriers to trade.

See also: Codex Alimentarius; Legal Requirements for Food Hygiene.

Further Reading

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ENCYCLOPEDIA OF FOOD AND HEALTH

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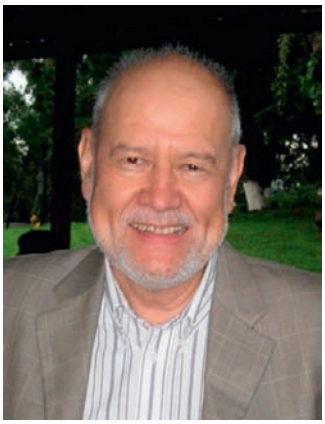


Benjamin Caballero is professor of International Health and of Maternal and Child Health (Bloomberg School of Public Health), and professor of pediatrics (School of Medicine) at Johns Hopkins University.

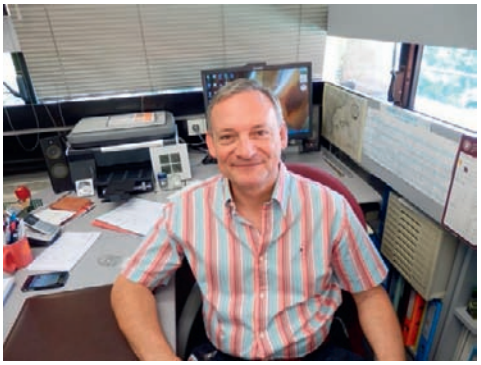
He obtained his MD from the University of Buenos Aires, his MSc in biochemistry from the University of San Carlos, and his PhD in neuroendocrine regulation from MIT, in Cambridge, MA. He started his academic career as assistant professor of pediatrics at Harvard Medical School and director of the Nutrition Unit of Boston Children's Hospital, and subsequently became the founding director of the Center for Human Nutrition at Johns Hopkins University, in Baltimore.

Prof. Caballero has focused his research on child nutrition and health in developing countries. In particular, he has explored the combination of undernutrition and overweight that has become increasingly prevalent in low- and middle-income countries. He was a member of the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences, USA, and of a number of expert panels created by the Institute, including the Dietary Reference Intakes (DRI) Committee, the Expert Panel on Macronutrient Requirements, and the Childhood Obesity Task Force. He was also a member of the Dietary Guidelines for Americans Advisory Committee, of the Scientific Advisory Board of the Food and Drug Administration (FDA), and of a number of advisory committees of the National Institutes of Health (USA).

He is the editor-in-chief of the *Encyclopedia of Food Sciences and Nutrition*, a 10-volume work on food production, consumption and biological effects. He is also editor-in-chief of the *Encyclopedia of Human Nutrition*, which received the Book of the Year Award from the British Medical Association. His *Guide to Dietary Supplements* summarizes the current scientific basis for the use of mineral and vitamin supplements. His book *The Nutrition Transition: Diet and Disease in the Developing World* explored the impact of demographic and economic development on diet- and lifestyle-related diseases in developing countries. His book *Obesity in China* summarizes research conducted in rural and urban China to track the impact of socioeconomic development on health outcomes. He is also coeditor of a widely used textbook on human nutrition, *Modern Nutrition in Health and Disease*.

He is a member of the Spanish Academy of Nutritional Sciences, and a Fellow of the American Society for Nutrition and of the Royal Society of Medicine (UK). Recent awards include the Donald Medearis Lectureship from the Massachusetts General Hospital/Harvard Medical School, the Mataix Prize for lifetime achievements in nutrition science from the Spanish Academy of Nutritional Sciences, the Ancel Keys Prize for achievements in international public health, and the Thompson–Beaudette Lectureship from Rutgers University.

Paul Finglas joined the Institute of Food Research in 1981 and is currently head of the Food Databanks National Platform and Research Leader in Food and Health at the Institute (http://www.ifr.ac.uk/science/platform/FD/default.html). He has, for most of his science career, been involved in a wide range of research in food composition and analysis, and the nutritional effects of micronutrients in food and health research. Paul has considerable experience of co-coordinating both national and international projects (e.g., EuroFIR, TDS-EXPOSURE, Bacchus and QualiFY (all EU FP7), and is currently of the spin-out EuroFIR AISBL, a non-profit international association based in Belgium, from one of these projects. Paul has a broad range of experience in science publishing and is currently editor of the journals *Food Chemistry* and *Trends in Food Science and Technology*, and was one of the coeditors for the *Encyclopedia of Food Science and Nutrition* (2nd Ed.). Paul has a degree in chemistry from Aston University in Birmingham and has published over 150 publications on a wide range of topics in food science and nutrition.



Fidel Toldrá holds a BSc in chemistry (1980), high degree on food technology (1981) and PhD in chemistry from the University of Valencia (1984). Professor Toldrá was a Fulbright postdoctoral scholar at Purdue University in West Lafayette (US, 1985–86) and visiting scientist at the University of Wisconsin-Madison (1991 and 1995), and the Institute of Food Research-Bristol (UK, 1987). Currently, he is research professor at the Instituto de Agroquímica y Tecnología de Alimentos (CSIC), in Paterna, Valencia (Spain). He is also associate professor of food technology at the Polytechnical University of Valencia.

Prof. Toldrá has focused his research on food biochemistry and its relationship with nutrition, quality and safety. He has filed 12 patents, directed 22 PhD thesis and published over 245 manuscripts in recognized scientific journals and more than 115 chapters of books. His h-index is 41. Prof. Toldrá has authored two books and edited/co-edited more than 30 books for major publishers like CRC Press, Wiley-Blackwell, Elsevier and Springer.

Prof. Toldrá is the European editor of *Trends in Food Science and Technology* (2005–) and associate editor of *Meat Science* (2014–); he was the editor-in-chief of *Current Nutrition & Food Science* (2005–2012), section editor of the *Journal of Muscle Foods* (2009–2010) and guest editor of 12 special journals issues. He is a member of the editorial boards of *Food Chemistry*, *Food Analytical Methods*, *Journal of Food Engineering*, *Journal of Food and Nutrition Research*, *The Open Nutrition Journal*, *The Open Enzyme Inhibition Journal*, *Recent Patents in Agriculture*, *Food and Nutrition*, *Food Science & Nutrition* and *Current Opinion in Food Science*.

He has been a member of the Scientific Panel on food additives, flavorings, processing aids and materials in contact with foods (periods 2003–2008) and the Scientific Panel on flavorings, enzymes, processing aids and materials in contact with foods (2008–2015) of the European Food Safety Authority (EFSA) acting as Chairman of the Working groups on Irradiation (2009–2010), Processing Aids (2011–2014) and Enzymes (2010–2015). He was a member of FAO/WHO group of experts to evaluate chlorine-based disinfectants in the processing of foods (2008–2009). He was a member of the Executive Committee of the European Federation of Food Science and Technology (EFFOST, 2002–2009). He is a Fellow of the International Academy of Food Science and Technology (IAFOST, 2008) and of the Institute of Food Technologists (IFT, 2009–). He received the Iber Award on Food and Cardiovascular Diseases (1992), the Institute Danone award in Food, Nutrition and Health (2001), the International Prize for Meat Science and Technology from the International Meat Secretariat (2002), GEA award on R+D activity from the Valencian Community (2002), and the Distinguished Research Award (2010) and Meat Processing Award (2014), both from the American Meat Science Association.

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Siân Astley has worked extensively with individuals and organizations throughout Europe from a variety of disciplines including research, food and biotech industries, and the media. She is the author of more than 300 popular science articles for magazines and trade publications as well as 25 peer-reviewed papers, and she was awarded her Diploma in Science Communication in 2009 (Birkbeck University of London). After 14 years as a bench-scientist, Siân became communications manager for NuGO, one of the first FP6 networks of excellence, and was the European communications manager for the Institute of Food Research in Norwich (UK) until April 2012. Currently, she is the training and communications manager for the European Food Information Resource (EuroFIR AISBL) supporting training within EU-funded research projects and networks, and communication of research activities.

David J. Baer is a supervisory research physiologist with the US Department of Agriculture's Beltsville Human Nutrition Research Center located in Beltsville, Maryland. He serves as the research leader for the Center's Food Components and Health Laboratory and also serves as the director of the Center's Human Studies Facility.

Dr. Baer conducts controlled dietary intervention studies to investigate the relationship between diet and the risk for chronic degenerative diseases, especially cardiovascular disease, cancer, and diabetes in people. His research also includes studies on the health impacts of weight gain and determining the calorie content of foods. Some of the dietary interventions he has investigated include the effects of different types of protein, fats and fatty acids, fiber, margarine, butter, plant sterols, salad dressings, nuts, whole grains, berries, alcohol, and tea on overall nutrition and health. In addition to dietary intervention studies, Dr. Baer is involved in research studies to validate food survey methodologies and in developing new methods for dietary assessment.

Dr. Baer earned his bachelor's degree from the University of Illinois and his master's and doctorate in nutrition from Michigan State University.

Marina Carcea was awarded a master degree in agricultural sciences at the University of Pisa, Italy "cum laude" in 1980, and a PhD in food science also "cum laude."

She is currently a senior researcher in the Research Center on Food and Nutrition of the Council for research in agriculture and analysis of agricultural economy (CRA-NUT formerly INRAN National Research Institute on Food and Nutrition) and she was the director of the Cereals Research Programme in INRAN. CRA-NUT is a primary research institute in Italy under the egis of the Ministry of Agriculture. Dr. Carcea joined INRAN in 1989 after having worked in Italian and English universities (Queen Elizabeth College, King's College, and University of London) and after a two-year contract with the Food and Agriculture Organization (FAO) of the United Nations (UN), Rome.

She has a vast experience in the field of research on foods, cereals in particular. In recent years, her main research interests have been: chemical characterization and study of the functional properties of cereal components; study of the interactions between components and of the interrelationships between the biochemical properties of components and the technological properties of the raw material and derived products; development of new, cereal-based products; development of methods to assess technological parameters of the raw material; nutritional value of cereals; and developments of protocols for quality assurance of cereals, food authenticity.

She has taken part and/or co-ordinated several research projects within national or international programs (European Commission, FAO) involving several institutions. She is the author of more than 160 scientific publications, mostly in international journals, eight book chapters, and two scientific books. She delivered lectures on her research activity at about 150 national and international congresses and she seats in several national and international committees (Italian Ministry of Agriculture, Codex Alimentarius, and European Commission) regarding food and nutrition topics. She is also a member of the editorial board of scientific journals.

From 1994 to 2006, she has also been a lecturer of food science and technology at the University of Tor Vergata, Rome, Italy.

She is a founding member of AISTEC, the Italian Association of Cereal Science and Technology. Since 1996, she is an elected member in the Executive Committee of the same association and since 2009, president of the association.

Since 2000, she is the Italian National Delegate of the International Association for Cereal Science and Technology (ICC) and she was also the president of the same association for 2011–2012.

In 2004, she was the first woman to be awarded the International Harald Perten Prize for her excellent research achievements in the field of cereal science and technology.

She is also a member of the Georgofili Academy in Florence, Italy.

Lawrence J. Cheskin graduated from Dartmouth Medical School and completed a fellowship in gastroenterology at Yale–New Haven Hospital. He is an associate professor of health, behavior, and society at the Johns Hopkins Bloomberg School of Public Health, with a joint appointment in International Health–Human Nutrition, and in medicine (GI) at the Johns Hopkins University School of Medicine. Dr. Cheskin is also a founder and director of the Johns Hopkins Weight Management Center, a comprehensive treatment program for obesity.

In his research, Dr. Cheskin has studied the effects of medications on body weight, the gastrointestinal effects of olestra, how cigarette smoking relates to dieting and body weight, and the effectiveness of lifestyle and dietary changes in weight loss and weight maintenance.

He is also the author of four books: *Losing Weight for Good*, *New Hope for People with Weight Problems*, *Better Homes and Gardens' 3 Steps to Weight Loss*, and *Healing Heartburn*. Dr. Cheskin has appeared on television news programs and lectured to both professional and lay audiences on the topics of obesity and weight control.

Nigel Cook is a graduate of the University of Dundee. After postdoctoral research in the Universities of Aberdeen and Leicester, he moved to the Central Science Laboratory (now the Food and Environment Research Agency (FERA)) at the Food Science Laboratory, Torry, Aberdeen in September 1994, before relocating to new facilities in York. At FERA, he studies the transmission of pathogens, particularly enteric viruses, through foods and the environment. He has a visiting professorship at the Katholieke Universiteit Leuven in Belgium. He is a councilor of the International Association for Food and Environmental Virology. He is a project leader within the standardization working group ISO TC34 SC9 WG6, currently developing a standard for detection of *Cryptosporidium* and *Giardia* on berry fruits and leafy green vegetables. He was a coordinator of the European Framework 7 project "Integrated monitoring and control of foodborne viruses in European food supply chains (VITAL)," and a chair of COST Action 929 "A European Network for Environmental and Food Virology" from 2006 to 2010. Between 2009 and 2014, he was a member of various European Food Safety Authority's Working Groups preparing opinions on the risk of foodborne viruses, and represented the European Communities on the Codex Committee on Food Hygiene Working Group developing *Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food*. He was a member of the UK Advisory Committee on the Microbiological Safety of Food's Viral Infections Subgroup. He was the founding editor of the journal *Food and Environmental Virology*, published by Springer.

Luca Simone Cocolin graduated in 1994 in food science with a grade of 110/110 and remark followed by food biotechnology PhD studies from 1995 to 1998. In February 1999, he defended his thesis acquiring the title of PhD in food biotechnology. From 1998 to 2001, he received a scholarship from the Friuli Venezia Giulia region (Italy). From November 1, 2001, he was an assistant professor at the University of Udine, Faculty of Agriculture, Food Science Department, Italy, and in October 1, 2006, he became an associate professor at the University of Torino, Italy. In January 2014, he had the habilitation for full professor and from June 2015, he is the full professor in food microbiology at the University of Torino.

From September 2008, he is an executive board member of the International Committee on Food Microbiology and Hygiene (ICFM) part of the International Union of Microbiological Societies (IUMS) (http://www.icfmh.org/). From January 2008, he is the editor-in-chief of the *International Journal of Food Microbiology* and he is a member of the editorial board of *Applied and Environmental Microbiology*, *Food Analytical Methods*, *Frontiers in Food Microbiology*, and *Frontiers in Nutrition and Food Science Technology*. He regularly reviews paper for *Food Microbiology*, *Meat Science*, *Journal of Applied Microbiology*, and *Letters in Applied Microbiology*. He is a co-author of more than 180 papers on national and international journals and he attended national and international congresses with oral and poster presentations. On Scopus (www.scopus.com, consulted on March 2015) he has 172 documents reviewed, which were cited 3520 times, resulting in an h index of 33.

His scientific interests comprise:

development, optimization, and application of molecular methods for the detection, quantification, and characterization of foodborne pathogens;
study of the microbial ecology of fermented foods (mainly sausage, cheese, and wine) by using culture independent and dependent methods;
bioprotection: molecular characterization of bacteriocin production and its study *in vitro* and *in situ*;
selection of new putative probiotics from artisanal fermented foods; and
study of the human microbiome and its influence on human health.

Christopher Duggan, for the past 25 years, has been performing clinical trials in the fields of pediatric nutrition, gastroenterology, and global health. His early work centered on the management of diarrheal diseases in children, including trials that demonstrated the feasibility and efficacy of oral rehydration solutions (ORS) for diarrhea management in the United States and globally. In collaboration with colleagues at Harvard TS Chan School of Public Health and Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania, Dr. Duggan and colleagues are evaluating the efficacy of micronutrient supplementation in infants and young children born to women with or at risk of HIV infection. Recent studies include the development of new biomarkers of environmental enteric dysfunction as well as the evaluation of nutritional status on neurodevelopment. With colleagues at St John's Research Institute in Bangalore, India, he is evaluating the efficacy of maternal vitamin B12 supplementation on biochemical and clinical parameters during pregnancy and infancy. He is a course co-director of the Bangalore, Boston Nutrition Collaborative (http://bbnc.globalhealth.harvard.edu). Past and present research support has come from the National Institutes of Health, the Gates Foundation, and the World Health Organization.

In addition to his global health research interests, he is a pediatric gastroenterologist and a nutrition physician at Boston Children's Hospital where he directs the Center for Nutrition (http://www.childrenshospital.org/nutrition). He is a medical director of the Center for Advanced Intestinal Rehabilitation, one of the largest centers in the United States for the care of children with intestinal failure/chronic diarrhea syndromes (http://www.childrenshospital.org/cair). He is also the course co-director of an inaugural Harvard College course "Nutrition and Global Health" and mentors undergraduate, graduate, and postdoctoral students at HMS and HSPH (http://www.hsph.harvard.edu/nutrition-and-global-health/).

He is a professor of pediatrics at Harvard Medical School and a professor in the Department of Nutrition at the Harvard TS Chan School of Public Health (http://www.hsph.harvard.edu/faculty/christopher-duggan/).

Jed William Fahey's current research concerns elucidating the mechanisms of how plants protect themselves against unfavorable and stressful conditions, and how this understanding can be translated to chemoprotection of eukaryotic mammalian systems. This work draws on elements of natural product chemistry, enzymology, nutritional epidemiology, and clinical research in order with isothiocyanates (e.g., sulforaphane) and glucosinolates. His work led to the discovery that broccoli sprouts are an exceptionally rich and consistent source of phytochemicals that induce the detoxification of carcinogens, and to the development of methods for their detection and for assessing their metabolism in humans. He discovered that one of the inducers, sulforaphane, has potent antibiotic activity against *Helicobacter pylori*, a causative agent of peptic ulcer and stomach cancer, and followed up with trials in animals and in *H. pylori*-infected humans. Ongoing collaborations examine the effects of broccoli, Moringa, and the other plants and their phytochemicals against a range of chronic diseases. Dr. Fahey has for years taught courses in chronic disease prevention and nutrition at both medical and public health schools.

Manuel Franco is an associate professor at University of Alcalá in Madrid (Spain) where he leads the social and cardiovascular epidemiology research group (http://www3.uah.es/cardiosocialepi/). He is also an adjunct professor at Johns Hopkins University (Baltimore).

Prof. Franco's work focuses on the social determinants of cardiovascular diseases and its major risk factors as diet. His methodological interests include the measurement of the urban environment and large social and economic changes in relation to cardiovascular health. He is the lead investigator of the Heart Healthy Hoods, study funded by the European Research Council, that will study the urban environment in relation to cardiovascular health in Madrid (http://hhhproject.eu/). This longitudinal study will be collecting neighborhood level data (via audits, Google Street view, photovoice, and qualitative methods) and linking them to clinical outcomes collected from patients enrolled at the City of Madrid primary healthcare clinics. Prof. Franco trained in Spain and Germany to obtain his MD and obtained his PhD from Johns Hopkins Bloomberg School of Public Health working with Dr. Ana Diez-Roux in the MESA study on food environment and dietary patterns. He has published over 30 international high impact articles and collaborates with universities in the United States, Europe, and Latin America.

Maria Glibetic is a research director of Centre of Research Excellence in nutrition research, Institute for Medical Research in Belgrade, University of Belgrade, Serbia, and member of executive board of directors for food data association EuroFIR AISBL. She is an experienced basic and nutritional scientist with over 250 scientific publications and presentations. Maria has considerable experience in leading national and international projects and since 2006, she participated in nine EU funded projects including EuroFIR, EURRECA, BaseFOOD, CHANCE, BACCHUS, and ODIN. Maria and her team are responsible for the creation of the first online national food database, for designing food data management system, and for the development of different nutritional tools for intake analysis. She was a principal leader of many nutrition intervention studies evaluating the plant bioactive component effects on human cardiovascular health. She leads postgraduate department for integrated nutritional sciences at University of Belgrade, where she teaches two courses.

Linda Harvey obtained her PhD from the University of East Anglia, UK. She is currently the head of the Human Nutrition Unit at the Institute of Food Research, Norwich, UK. Her research interests include micronutrient requirements, bioavailability, and metabolism.

Ronald Jackson received his bachelor's and master's degrees from Queen's University and doctorate from the University of Toronto. His time in Vineland, Ontario, and subsequent sabbatical at Cornell University, redirected his interest in *Botrytis* toward viticulture and enology. As part of his teaching duties at Brandon University, he developed the first wine technology course in Canada. For many years he was a technical advisor to the Manitoba Liquor Control Commission, developing sensory tests to assess candidates of its sensory panel, and was a member of its external tasting panel. He is the author of *Wine Science: Principles and Applications, 4th edition* (2014), *Wine Tasting: A Professional Handbook, 2nd edition* (2009), *Conserve Water, Drink Wine*, and chapters and technical reviews in other multiple books and encyclopedia. He is retired in Bronte, Ontario, but remains active writing, cycling, doing yoga, and traveling, as well as being a fellow in the Cool Climate Viticulture and Oenology Institute, Brock University, St. Catharines, Ontario, Canada.

Joe P. Kerry is a senior college lecturer and the head of the food packaging research group in the School of Food and Nutritional Sciences at University College Cork (UCC). He received his doctorate in microbiology at University College Galway in 1995. Prof. Kerry is also a qualified member of the Institute of Packaging. He is very involved in national and international research projects both at fundamental and applied levels. Primary research interests address various aspects of food packaging, shelf-life stability, food composition, and numerous aspects of food quality, particularly in relation to muscle foods. He also has very strong links with industry and his research team assists companies in relation to many aspects of new food product development. He has over 220 publications in peer-reviewed international journals, over 300 presentations at major international conferences, along with several other significant publications. His expertise includes use and manipulation of modified atmosphere packaging systems for use with foods, use of extrusion technology for the manufacture of food products/packaging materials, and applications and sensor/new technology developments within the area of food packaging, especially in the area of smart packaging.

Frédéric Leroy, after studying bio-engineering at Ghent University, obtained a PhD in applied biological sciences at the Vrije Universiteit Brussel in 2002, where he continued his academic career at the research group of Industrial Microbiology and Food Biotechnology (faculty of sciences and bio-engineering sciences). As associate professor, his lecturing activities include courses in food science and technology (i.e., "Nutrition," "Technology of animal products," "Food microbiology and ecology," and "Quantitative and predictive microbiology"). Dr. Leroy's research primarily deals with the ecology and functional roles of bacterial communities in (fermented) foods, in particular with respect to the generation of quality, safety, and/or nutritional and health advantages. Focus is mostly on meat products, but other food systems are also being studied, including fermented milks and sourdough breads. In addition, his research interests relate to elements of tradition and innovation in foods, both from a technological and societal point of view.

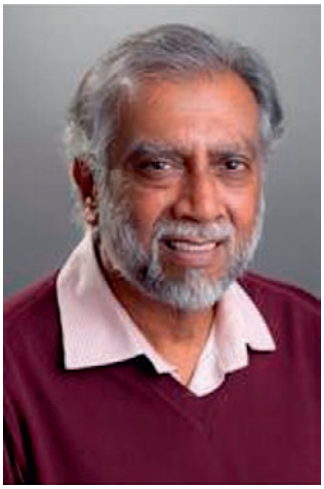
Catherine M. Logue completed her undergraduate and postgraduate degrees in Ireland and earned a PhD in meat microbiology from the University of Ulster, UK in 1996. Dr. Logue was a faculty member at North Dakota State University from 1999 to 2011 rising through the ranks of assistant to associate and full professor. In 2011, she re-located to Iowa State University's College of Veterinary Medicine and is a professor of veterinary microbiology and preventive medicine. Dr. Logue is also the director of faculty and staff advancement and equity for the college. Her research interests focus on foodborne pathogens of food animals and the contamination of meat and meat products destined for human consumption. Her research studies the detection, isolation, and characterization of a range of foodborne pathogens such as *Salmonella*, *Campylobacter*, *Listeria*, *Escherichia coli*, and methicillin-resistant *Staphylococcus aureus* (MRSA) in poultry, bovine, and swine. She also focuses her research on antimicrobial resistance in commensals and pathogens of production animals. She has been an author and a co-author on more than 90 peer-reviewed papers and book chapters as well as more than 150 abstracts and presentations at national and international meetings.

F. Xavier Malcata graduated in chemical engineering in 1986 from the University of Porto (Portugal), received a PhD in chemical engineering/food science from University of Wisconsin, Madison (USA) in 1991, and his habilitation in food science and engineering by Portuguese Catholic University in 2002. He was the dean of College of Biotechnology of Portuguese Catholic University, the chairman of Portuguese Society of Biotechnology, Portuguese representative at VI and VII European Union Framework Programs of research and development, expert for European Food Safety Agency, and a co-ordinator of Portuguese Engineering Accreditation Board in chemical engineering for Northern Region. He is currently a full professor at University of Porto.

His major research interests have focused on technological improvement of traditional Portuguese foods and upgrade of byproducts thereof, development of nutraceutical ingredients and functional foods, design and optimization of enzymatic reactors for edible oil processing, characterization of plant proteases toward cheese and whey cheese manufacture, production of starter and nonstarter cultures from adventitious microflora, and optimized application of unit operations to food processing.

With an academic career of independent research and teaching for more than two decades, Prof. Malcata published more than 450 papers in refereed journals worldwide, wrote 11 books, and prepared more than 45 chapters for edited books. Among many international distinctions, he was

recipient of Ralph H. Potts Memorial Award in 1991 by American Oil Chemists' Society (AOCS, USA), Foundation Scholar Award – Dairy Foods in 1998 by American Dairy Science Association (ADSA, USA), Young Scientist Research Award in 2001 by AOCS, Canadian/International Constituency Investigator Award in physical sciences and engineering in 2002 and 2004 by Sigma Xi (USA), Danisco International Dairy Science Award in 2007 by ADSA, Scientist of the Year Award in 2007 by European Federation of Food Science and Technology (the Netherlands), Samuel C. Prescott Award in 2008 by Institute of Food Technologists (IFT, USA), International Leadership Award in 2008 by International Association for Food Protection (IAFP, USA), Elmer Marth Educator Award in 2011 by IAFP, Distinguished Service Award in 2012 by ADSA, and William V. Cruess Award in 2014 by IFT. He has been elected for the honor societies of food science (Phi Tau Sigma, USA), scientific research (Sigma Xi, USA), and engineering (Tau Beta Pi, USA). He was also elected for fellow of IFT, ADSA, AOCS, and International Academy of Food Science and Technology.



Gopinadhan Paliyath is a professor at the Department of Plant Agriculture, University of Guelph, and the research program director for "Food for Health," under the UG/OMAFRA partnership. Dr. Paliyath is a biochemist and has an interest in various aspects of fruits and vegetables, specifically the nutraceutical components and their mechanism of action. He obtained his BSc Ed degree (botany and chemistry) from the University of Mysore, MSc degree (botany) from the University of Calicut, and PhD degree (biochemistry) from the Indian Institute of Science, Bangalore. Subsequently, he did postdoctoral work at Washington State University, University of Waterloo, and University of Guelph. Dr. Paliyath's research is focused on the biochemistry of plant senescence, specifically pertaining to postharvest biology and technology of fruits and vegetables. Investigations on the role of phospholipase D (PLD) in membrane homeostasis and signal transduction have led to advances in the understanding of the mechanism of membrane deterioration that occur during stress and senescence. Another aspect of his research is focused on understanding the mechanism of action of food components in disease prevention. The efficacy, bio-accessibility, bioavailability, and molecular mechanisms of action of nutraceuticals in fruits and processed products in relation to their cancer-preventive and anti-inflammatory actions are being investigated using mammalian cell lines, and animal model systems.

Dr. Paliyath has developed technologies and products for enhancing the shelf life and quality of fruits and vegetables based on PLD inhibition. R&D activities relevant to the industry sector include: (1) optimization of an enhanced freshness formulation for application to various fruits, vegetables, and flowers; (2) developing methods for nutraceutical carriers that would enhance the functional food quality and delivery (e.g., stabilizing lycopene in tomato juice, sauce, etc., for health beneficial effects); and (3) developing novel technologies to enhance the cancer-preventive ingredients in fruit products, etc. Patents awarded include: (1) # 6,514,914 (US) and 2,298,249 (Canada); (2) #7,198,811 (USA), 4141387-1 (Japan), 260738 (Mexico), 1469736 (Turkey), 028 284763 (China), and 223077 (India). The patents describe the use of nanoformulations based on hexanal and other generally regarded as safe (GRAS) ingredients for enhancing the shelf life and quality of fruits, vegetables, and flowers by pre or postharvest treatments. These technologies are currently being evaluated for extending shelf life and quality of mango in India and Sri Lanka with the assistance of the Canadian International Food Security Research fund. The collaboration involves researchers from Canada, India (Tamil Nadu Agricultural University), and Sri Lanka (Industrial Technology Institute).

Dr. Paliyath is also the research program director for the food and health theme-related activities under the OMAF/MRA/University of Guelph research partnership. He serves on the editorial board of several journals. He is a member of American Chemical Society and Canadian Society of Plant Biologists.

(Total-refereed publications in journals – 92; patents and intellectual properties – 2; disclosures – 4; chapters in books – 27; nonrefereed contributions – 10; research reports – 28; conference proceedings – 88; edited books – 9; book reviews – 6 (Google Scholar: h index – 31, i10 index – 68, citations – 4332; RG score – 35.63)).

Yolanda Picó is a full professor of nutrition and food science at the University of Valencia since 1998. She is currently the head of the research group on food and environmental safety of the University of Valencia. Her research interests are the development of new analytical methods to determine organic contaminants in food and the environment, identification of unknown compounds by liquid chromatography–mass spectrometry, micro-extraction separations, and environmental and food safety. To the date, she is the author of nearly 200 peer-reviewed papers, 170 scientific papers in journals of SCI, 25 book chapters, and editor of four books on food and environmental safety.

Vieno Piironen is a professor of food chemistry at the Department of Food and Environmental Sciences, University of Helsinki, Finland. She received her PhD in food chemistry at the University of Helsinki in 1987 and has approximately 35 years of experience in food research and education on bachelor, master, and PhD levels. She has participated actively in international research and education projects and networks. Her research has focused especially on chemical and nutritional properties, reactions and analysis of lipids, vitamins, and other bioactive compounds. Research on vitamins has been active from the beginning of 1980s. She has studied both lipid- and water-soluble vitamins; their chemical and nutritional properties and importance in foods and diets as well as factors influencing vitamin levels. In addition, development of analytical methods for different vitamers as well as validation and harmonization of the methods through international collaboration have been among the priorities. Recent collaboration projects have focused on enhancing vitamin contents in cereal-based foods by plant breeding, utilization of vitamin-rich grain fractions, and bioprocessing. Currently, the research focus lies on investigating microbial *in situ* synthesis of folate, vitamin B12, and other B vitamins in cereal and legume matrices as a means to improve nutritional quality of foods and to develop new food applications. In lipid research, different lipid classes and their chemical and enzymatic reactions in food matrices are studied. Diverse methods are used to study proceeding of oxidation from primary products to monomeric oxides, volatiles, and polymerization products and to study possibilities to control oxidation. Controlling enzymatic reactions leading to off-flavors in cereal and legume matrices is also among the interests. Phytosterols and their conjugates have been studied as natural food components belonging to the dietary fiber complex. On the other hand, questions related to sterol enrichment such as oxidation susceptibility and mechanisms as well as factors affecting oxidation reactions have been of interest. She has also studied nutrients and anti-nutrients in legumes and more recently started research on utilization of high value components in microalgae. She has approximately 160 papers in international journals and a number of other publications.

David Rodríguez-Lázaro is a doctor in veterinary medicine (DVM), specialized in food science (BSc and MSc) and molecular microbiology (PhD). He is a senior scientist at ITACyL and an assistant professor of microbiology at the University of Burgos. He has performed research stays in the Danish Institute for Food and Veterinary Research (Denmark), the University of Prague (Czech Republic), the Food and Environmental Research Agency (UK), and the University of Bristol (UK). He was a Leverhulme visiting professor in the Institute of Advanced Sciences in the University of Bristol during the years 2004 and 2005 and Marie Curie research fellow in the faculty of medical and veterinary sciences in the University of Bristol (UK) until 2007.

His research interest is focused on the establishment of reliable, quantitative molecular strategies for detection of important food-borne pathogens from environmental sources and various types of foodstuffs, the characterization of the prevalence of the main foodborne pathogens in food and food-related environments, and the development of emergent food preservation processes and their effects in the microbial virulence. He has participated in a number of coordinated EU-funded projects such as PROMISE, BASELINE, VITAL, FOOD-PCR, SACROHN, and MONI-QA, having established active links with the leading European research groups working in food safety.

He has published more than 100 international scientific papers and book or book chapters regarding food safety. He is currently a member of the editorial board of *Applied and Environmental Microbiology*, *International Journal of Food Microbiology*, *Food and Environmental Virology*, and *International Journal of Food Contamination* and the editor-in-chief of the journal *Food Analytical Methods*. He was awarded with the XV Jaime Ferrán Award in 2013 by the Spanish Society for Microbiology for his promising scientific career in microbiology.

Turid Rustad is a professor and the head of the food science group at the Department of Biotechnology, Norwegian University of Science and Technology. The main research focuses on the biochemistry of marine raw materials, the relationship between biochemistry and quality, and changes in raw material properties during processing. Studies of enzymatic activities in different raw materials have been linked to studies of changes in the biochemistry of these raw materials. She has worked with characterization of composition and enzymatic processes in a wide range of different raw materials, such as fish, fish by-products, and zooplankton in relation to different storage and processing methods such as chilling, heating, superchilling, and frozen storage.

Noel W. Solomons has lived and worked in Guatemala for 40 years. He was born and educated in Massachusetts in the United States. As a young child, he became an amateur naturalist and was a nature counselor at various summer camps; this would guide him to a career in science. In his young adulthood, he would participate in the civil rights and anti-war movements, only to become disillusioned by the intractable nature of the injustice elements in the fabric of American society. As a physician by graduate training, he performed his university studies at Harvard College and Harvard Medical School; it was during overseas electives in his medical training that he visited Peru and Colombia and committed to an expatriate life trajectory outside of his homeland. Clinical training included a residency in internal medicine and infectious diseases at the Hospital of the University of Pennsylvania and specialization in gastroenterology and clinical nutrition at the University of Chicago. He became a resident of Guatemala in 1974 as an affiliated investigator at the Institute of Nutrition of Central America and Panama. He would later commute for eight years to a faculty position in the Department of Nutrition and Food Science of the Massachusetts Institute of Technology. Assuming a full-time Guatemala commitment in 1985, he co-founded the Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM) where he remains its scientific director. Over 40 local university theses have been completed by Central American students in that institution as well as an equal number of master's degree research projects from international students from Europe, and North and South America. He has supervised doctoral dissertations for 12 PhD candidates from the United States, Canada, Germany, Spain, and the Netherlands through CeSSIAM.

Dr. Solomons has 332 publications indexed on Medline. In addition, he has edited two books and contributed over 100 articles, reviews, editorials, and commentaries in nonindexed venues and over 50 book chapters. These are dedicated to the scientific and academic interests of his career including: clinical nutrition; human growth and body composition; lactose maldigestion; dietary intake, nutritional status, intestinal absorption, and food fortification related to various micronutrients (vitamins, trace elements, and essential fatty acids); complementary feeding; nutrition in aging and chronic disease; and the interaction of malnutrition and infection.

Among the honors bestowed upon Dr. Solomons are the International Nutrition Prize of the International Union of Nutritional Sciences and the Kellogg Prize of the Society for International Nutrition Research. He is a fellow of the American Society of Nutrition. He is an academic member of the Guatemalan Academy of Medical, Physical and Natural Sciences and the Spanish Academy of Nutrition and Food Science. He was the awardee of the 2010 National Medal for Science and Technology for Guatemala.

He has been a visiting professor in university courses in Mexico, Peru, Brazil, Indonesia, and Spain. He currently holds adjunct professorial appointments at the Boston University School of Public Health, and the Friedman School of Nutrition Science and Policy and the Department of Community Medicine and Public Health, both at Tufts University. He is a founding board of directors, member of the Hildegard Grunow Foundation in Munich and the Essential Nutrient Foundation of Singapore. Finally, Dr. Solomons is a coordinator for Central America of the Nevin Scrimshaw International Nutrition Foundation in Boston, and an associate editor for the Foundation's *Food and Nutrition Bulletin*. He serves on editorial boards for ten scientific journals.

Maria Tsimidou is a professor of food chemistry and the head of the Laboratory of Chemistry and Technology in the School of Chemistry at the Aristotle University of Thessaloniki (AUTh), Greece. Her teaching is food chemistry, analysis, quality control, and food legislation. Research interests are related to virgin olive oil chemistry, quality and authenticity, saffron chemistry, authenticity and quality, antioxidant activity of plant extracts and constituents, new sources of targeted bioactive compounds (squalene, carotenoids, and phenols), and analytical procedures for their determination. She has published many research papers, review articles, and contributions to scientific books and encyclopedias on the above-mentioned topics. Currently, she is an associate editor in the *European Journal of Lipid Science and Technology* and chairs the COST ACTION FA1101 "Saffronomics."

Jorge Welti-Chanes earned his degree in biochemical engineering (1976) and master of science in food engineering (1978) at Tecnológico de Monterrey (ITESM, Mexico), later he moved to Spain to perform his doctoral studies in chemistry, in the area of food technology, obtaining his degree at the University of Valencia. He is currently the national director of graduate studies at School of Engineering and Sciences at Tecnológico de Monterrey also is professor and researcher in the areas of biotechnology and food at the same institution. He started his academic activity in 1976 as a university professor of ITESM, has additionally been a full professor at the National Polytechnic Institute (IPN, Mexico) and the University of the Americas, Puebla, Mexico (UDLA). He has an experience of 37 years as a teacher and university researcher, 20 of which were spent in combination with the development of administration work in education, science and technology. In the UDLA, he was teaching in the Departments of Chemistry and Biology and Chemical Engineering and Food, in the latter was responsible for the leadership for a period of a year and subsequently became dean of the School of Engineering (1986–1988). From January 1989 to June 2002, he was an academic vice chancellor at UDLA. He has published 14 books and has over 200 scientific publications in refereed journals and books, has given more than 250 presentations at international conferences. He is an associate editor of the journals *Food Engineering Reviews* and *Journal of Food Science* and participates as a member of the editorial boards of *Journal of Food Engineering* and *Current Opinion in Food Science*. In May 2011, he received the Life Achievement Award by the International Association for Engineering and Food (IAEF), for his career as a researcher and academic worldwide, and in January 2014, the Romulo Garza Award from the Tecnológico de Monterrey for the impact of their research work and as recognition for being one of the most productive researchers in the life of Tecnológico de Monterrey. He has been the president of ISOPOW and IAEF and is the currently president of the International Society of Food Engineering (ISFE).

Peter J. Wilde graduated in biophysics at the University of East Anglia in 1985 and has been researching the colloidal and interfacial properties of food systems at the Institute of Food Research (IFR) for over 25 years. IFR is the only publicly funded UK research institute that focuses on the underlying science of food and health to address the global challenges of food security, diet, and health, healthy aging, and food waste. IFR is the one of eight institutes that receives strategic funding from the Biotechnology and Biological Sciences Research Council (BBSRC). It also receives funding from government agencies and departments, the EU, charities, and industry, from the UK and overseas.

Pete's research expertise is the interfacial behavior of proteins and other surface active components in food relevant systems. The aim is to determine how the molecular and interfacial processes control the functionality of foams and emulsions. Currently, the functional aspects of his research have focused on improving the dietary impact of emulsified foods. These include fundamental studies on how interfacial layers control emulsion rheology to develop novel fat reduction strategies; the design of interfacial structures to control lipid digestion to promote satiety or the delivery of fat-soluble nutrients and drugs; and to determine the physico-chemical role played by the salivary film in perceiving fat content in emulsions. The impact of this research will be to aid the rational design of foods with enhanced nutritional benefits to address the global challenges of obesity, type 2 diabetes, and other major diet-related conditions.

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HOW TO USE THE ENCYCLOPEDIA

All articles in the encyclopedia are arranged alphabetically as a series of entries.

1. Contents

Your first point of reference will likely be the contents. The complete contents list appears at the front of each volume providing volume and page numbers of the entry. We also display the article title in the running headers on each page so you are able to identify your location and browse the work in this manner.

2. Cross-references

The majority of articles within the encyclopedia have an extensive list of cross-references that appear at the end of each article, for example:

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Iron: Biosynthesis and Significance of Heme; Iron: Physiology of Iron.

3. Index

The index provides the volume and page number for where the material is located, and the index entries differentiate between material that is a whole article; is part of an article, part of a table, or in a figure.

4. Contributors

A full list of contributors appears at the end of volume 5.

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INTRODUCTION

Until a few decades ago, virtually all known health effects of foods were related to their content of essential nutrients. The clinical description of most diet-related illnesses mirrored the signs of essential nutrient deficiencies, such as pellagra, beriberi, and others. Consequently, the key public health concern regarding diet was ensuring that everyone consumed enough food. It was only in the past 50 years that large-scale epidemiological observations began to associate chronic diseases like diabetes and cardiovascular disease with nonessential diet constituents such as saturated fat, fiber, and cholesterol. Taking advantage of the emergence of digital informatics, these studies were able to manipulate increasingly large sets of data and provide, for the first time, a picture of the secular changes in the health of large populations and its association with what they ate regularly. These findings progressively shifted the concern from eating enough to avoiding excessive consumption of certain foods. Eating enough was replaced by eating well.

But it turned out that defining how to eat well is far more complex than defining minimum needs of essential nutrients. First, there is no single paradigm to study those relationships, given the wide variety of biological mechanisms and the long exposures involved. Second, many of the experimental models used to define essential nutrient needs are not applicable to the study of long-term effects of diets in free-living populations. And it is now clear that experiments with isolated dietary compounds do not reflect the actual effects of the complex food matrix we consume daily. Finally, while the discovery of essential nutrients and their role in health was the domain of a few specialties speaking a common language (primarily biochemists and physiologists), the study of the long-term effects of whole diets in humans must of necessity involve epidemiologists, social and behavioral scientists, food scientists, clinicians, policy experts, etc., making far more difficult the development of consensus and foundational concepts.

It is thus not surprising that today we have still not achieved a stable consensus on how to eat 'well.' Furthermore, while few nonscientists would care about the minimum requirement of a vitamin to sustain life, there are plenty of opinions among nonscientists on how to eat 'well.'

Our goal in preparing this encyclopedia has been to contribute to the understanding of that complex diet–health relationship by providing a multidisciplinary, integrative and accurate source of information. We aim to serve the needs not only of established and in-training scientists, but also of the increasingly important group of professionals who are key to disseminate and sustain the practice of science: journalists, science writers, science administrators, fund raisers, donors, and policymakers. In preparing this work, we had the enormous advantage of working with one of the publishers with the most extensive expertise in major reference works, Elsevier. This first edition builds on the impressive breadth of knowledge of over 922 authors and on the tireless work of our editorial advisory board. We are very grateful to all of them.

Benjamin Caballero
Paul Finglas
Fidel Toldrá

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Food Classification and Description

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Importance of Food Classification and Description

The preparation of reliable data on food requires precise identification of foods. Even data of good quality can be a source of error if they are derived from foods that are not clearly defined. Foods in databases are usually defined by a food ID, which should be its unique identifier in the database, by a food name, by food classification, and by food description.

A simple food name is not sufficient, as it can be inadequate or ambiguous to those who are not closely acquainted with the local language and culture. For example, the English term 'sweetbread' refers to an offal product and not pastry, while 'mincemeat pie' is sweet pastry and not a meat dish. A common name may, moreover, be misleading when the same name is used for different foods in different regions or for foods having different scientific names. For example, the name 'endive' is used for *Cichorium endivia* L. in the United Kingdom but for *C. intybus* L. in France and the United States. The situation is further confused by homonyms, synonyms, identical brand names for different products, and different culinary or technological terms. It is therefore necessary to include further information, through food classification and description, to define a food item, and to retrieve information about it.

As most databases employ different methods of identifying foods, it is difficult to exchange data between countries, between organizations within the same country, or even between workers in the same institution. This article reviews existing food classification and description systems used to define foods in databases with the view that an international understanding of standardized food classification and description could solve many of the problems arising from the misidentification of foods.

Food Classification Systems

Classification is a structure that organizes concepts into a hierarchy, from broad to narrower terms, according to common characteristics. For example, the food 'milk' can be classified under 'dairy products' and 'wine' under 'alcoholic beverages.' Classification thus facilitates information retrieval. Many national and international systems have been created to classify foods, but these exist in parallel and are rarely compatible. It is important to keep in mind that classification depends on the point of view of its creation and that food classification systems are reporting-level (e.g., as commodity, as purchased, and as eaten) and purpose-specific. The following are questions to ask when examining a food classification system:

- What is the purpose of the classification?
- At what level are the foods recorded?
- What do we want to classify: foods as consumed, ingredients, commodities, prepacked foods, etc.?

Food Classification Systems According to Purpose

A variety of food classification systems have been developed to describe food habits or to fulfill requirements set by regulatory bodies. Classification systems are often standardized, as they may be based on legal documents, the most standardized being 'vocabularies.'

National food classification systems for databases and surveys

Nutrient intake assessments are undertaken at a national and regional level to assess population nutritional status. Food composition databases and consumption studies thus use country-specific food classification systems, based on national criteria. This is mainly due to legal aspects and traditions, besides the economic and cultural importance of foods. For example, there is a separate group for coconut products in the food composition tables of the Pacific Islands and a group for edible insects in the Thai food composition database. Food classification systems for use in nutrient intake assessments are therefore country- and time-specific, reflecting known food consumption patterns at the time of the study.

Regional food classification systems for food composition databases

Some attempts have also been made to create regional classification systems for food composition databases. A good example of this is the EuroFIR (European Food Information Resource) classification used in European food composition databases, overcoming language and cultural differences. The EuroFIR classification was created by mapping classifications used in national food composition databases and food consumption surveys in Europe; definitions of food groups were based on Eurocode (see succeeding text). EuroFIR food classification is currently used to link over 29 European datasets, covering over 29 000 foods.

International food classification systems for products in commerce

Harmonized Commodity Description and Coding System

The World Customs Organization's Harmonized Commodity Description and Coding System (HS), also called tariff nomenclature, is an international nomenclature for the classification of products in commerce. It is used to classify traded goods on a common basis for customs purposes and for generating trade statistics. The trade coding system is agreed upon internationally and implemented in all countries. In Europe, it corresponds to the European Combined Nomenclature (CN). The coding system comprises 20 sections, four of which apply to foods, and the classification is based on the terms of the headings (e.g., 'leeks and other alliaceous vegetables'). HS lists products corresponding to customs tariffs of foods as purchased, that is, single, unprocessed foods, and therefore lacks details of prepared foods and food products. The PRODCOM

classification (see succeeding text), based on CN, provides statistics on the production of manufactured goods.

Global Product Classification (GS1 GPC)

Global Product Classification (GPC) is the classification system for the GS1 Global Data Synchronization Network (GDSN), which is the most widely used supply chain standards system in the world. GPC allows manufacturers, distributors, and retailers to group products in the same way to ensure data synchronization in the network. GPC is essentially a nonhierarchical system, with the addition of higher hierarchies (segment, family, and class) provided for information purposes. GPC 'bricks' define categories of similar products and can be further characterized by brick-specific attributes. Examples of bricks are 'milk and milk substitutes (perishable),' 'milk and milk substitutes (frozen),' and 'milk and milk substitutes (shelf-stable).' GPC categories thus reflect food origin and shelf life and potential for microbiological contaminants.

International food classification systems for regulatory purposes

These classification systems are based on commodities' similar potential for containing certain additives or pesticide residues and contaminants.

CIAA Food Categorization System and codex food categorization system (GSFA)

The CIAA Food Categorization System is a European-approved classification, originally developed by the Confederation of the Food and Drink Industries of the EEC. It is a hierarchical food classification system, designed to serve as an allocation tool for food additives as a basis for their authorization at the European community level. The CIAA Food Categorization System lays the basis for the codex food categorization system and the food classification used in the food additive 'positive lists' in the Nordic countries.

The Codex General Standard for Food Additives (GSFA) is maintained by the Codex Alimentarius Commission, established by the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO). Inspired by the CIAA Food Categorization System, foods are classified into 16 main food categories and then subcategories, according to additive authorizations. When the use of an additive is permitted in a certain category, it is automatically permitted in all its subcategories, unless otherwise stated. Both classifications cover all foodstuffs, even those where additives are not allowed. There are special categories for prepared dishes, which may contain the additives allocated to the categories to which their ingredients belong. These classifications are food additive-driven and hence emphasize processed foods, as the methods of preparation and conservation of foods condition the use of certain additives. Since these classifications deal with foods as marketed, they are interesting in a food consumption context and have been suggested as a useful starting point for an international system of food classification for nutrient risk assessments.

Codex Classification of Foods and Animal Feeds (CCPR) and General Standard for Contaminants and Toxins in Food and Feed (GSCTFF)

The FAO/WHO Codex Alimentarius classification of food and animal feed commodities was developed by the Codex Committee on Pesticide Residues (CCPR). The classification is intended to be as complete a listing of food commodities in trade as possible, classified into groups on the basis of the commodities' similar potential for pesticide residues. In Europe, it corresponds to the EC Classification of Products of Plant and Animal Origin, a listing food and feed products to which the maximum levels for pesticide residues apply.

The Food Categorization System of the Codex Alimentarius General Standard for Contaminants and Toxins in Foods and Feed (GSCTFF) uses the system that was developed in the framework of the CCPR but extends it to include processed products at type or group level. The GSCTFF is intended to promote harmonization of grouping commodities with a similar potential for residues for which a maximum residue limit can be set. In GSCTFF, manufactured foods containing ingredients of both plant and animal origins are classified according to their main ingredient. To be used effectively on an international level, both classifications need to be reviewed to include a wider variety of foods, particularly those from regions other than Europe and North America.

Global Environment Monitoring System (GEMS/Food)

The WHO GEMS–Food Contamination and Monitoring System (GEMS/Food) informs governments, the Codex Alimentarius Commission, and other relevant institutions on the levels and trends of contaminants in food and their significance with regard to public health and trade. GEMS/Food hazard identification is used to set priorities for Codex Alimentarius committees such as the Codex Committee on Food Additives (CCFA), Codex Committee on Contaminants in Foods (CCCF), and Codex Committee on Pesticide Residues (CCPR). The GEMS/Food code is based on a hierarchical classification with 2 levels. The first level (WHO Food Group) corresponds to broad categories usually reported in food consumption surveys; these categories are expected to be valid for all types of diets around the world and should allow for an international dietary exposure assessment. The second level (WHO Food Identifier) corresponds to the detailed food descriptors used in the earlier-mentioned Codex Alimentarius committees and to particular foods, processed or not and analyzed as purchased or as consumed. GEMS/Food reports include mapping of local food identifiers with the 2 WHO levels of classification.

Food classification systems for international dietary monitoring

FAO food balance sheet

Food balance sheets are compiled by the FAO and EUROSTAT on an annual basis. A food balance sheet (FBS) presents a comprehensive picture of a country's food supply during a specific reference period. It shows for each food item (i.e., primary commodity and a number of processed commodities) the areas of supply and its utilization. The food supply available for a specific period is the result of the total quantity of

foodstuff produced, added to the total quantity imported, and adjusted to any change in stocks during this period. FBS expresses food consumption in kilogram per head per year for broadly defined food groups (e.g., bovine meat). They have the advantages of providing a harmonized set of data for all member states, being carried out on an annual basis, and providing information for member states who do not have national food consumption surveys.

PRODCOM classification for household budget surveys

All Household Budget Surveys (HBSs) in the EU member countries use the PRODCOM food classification scheme based on the European CN and transformed by EUROSTAT. The EU DAFNE project on HBS enhanced the PRODCOM classification scheme, placing more emphasis on goods such as food and improving comparability with other international food consumption classifications. Even so, the HBS classification schemes only cover products as obtained in a household (purchased or grown) and not foods as consumed.

Eurocode, European Food Groups (EFG)

An attempt was made in Europe to create a master list of foods and codes that could be used in dietary studies in all participating countries. The Eurocode food codes were, however, not specific enough to replace national codes in food composition or consumption databanks, especially for nutritional calculation or for exposure assessment. Moreover, Eurocode's monohierarchical classification of foods suffered from inconsistencies in rules for assigning food items to specific food groups (particularly for mixed foods) and from incoherencies with respect to standards set by Codex Alimentarius and/or the European Commission, thus making its use difficult for governmental bodies.

A nonhierarchical classification, EFG, was then developed, in an attempt to standardize food classification at main group level and permit international comparisons of the results of food consumption and food availability surveys. For example, 'fruit juice' is a food group separate from 'fruit' and 'beverage' in EFG. When creating the EFG system, major classification schemes used for recording food intake were compared, and 33 food groups were chosen as the 'least common denominators' of the food classification schemes, using the definitions of the corresponding food groups from Eurocode.

EPIC-SOFT food classification

The European Prospective Investigation into Cancer and Nutrition (EPIC) studies 500 000 people in ten European countries, where detailed data on diet and lifestyle are obtained by questionnaires, using EPIC-SOFT software. The EPIC-SOFT food classification system in 17 main groups and underlying 124 subgroups was used to classify the 1500–2000 items reported in the EPIC study. Because of the importance of this study in Europe, national consumption surveys often map to EPIC-SOFT food classification.

Food Classification Systems According to Reporting Level

Different survey types/methodologies will achieve different levels of detail. Individual surveys, HBS, and food balance sheets all record, to varying detail, food as eaten/prepared or as raw commodities. Foods exist on all reporting levels, but characteristics of the food change from level to level, so systems capturing this information are food level-specific and cannot cover several groups at the same time:

- Food composition databases and individual consumption surveys record foods at the intake level (food as consumed). Examples of international intake-level classification systems are EPIC-SOFT and EuroFIR food classification.
- Foods are recorded at ingredient level (prepacked foods, foods as purchased, and raw foods) in HBS, GEMS/Food surveys, CIAA and GSFA Food Categorization Systems for food additives, GS1 GPC for products in commerce, and food classifications for comparing consumption surveys (e.g., EFG and Eurocode). Food composition databases also include foods at the ingredient level.
- Classification systems that record foods at raw commodity level include food balance sheet classification, harmonized commodity system (HS), Codex CCPR and CCFAC, and GEMS/Food (**Figure 1**).

In order to compare consumption between countries, data from national dietary surveys (where foods are reported as consumed) need to be extrapolated to ingredient level using standard recipes and yield factors. Data collected at ingredient level (or extrapolated from input level) can be extrapolated to raw commodity level using processing, hydration, or equivalence factors. For example, quantities of bread consumed (intake level) can be extrapolated to quantities of flour (ingredient level), which in turn can be extrapolated to quantities of wheat (raw commodity level). However, it is impossible to extrapolate the opposite way, that is, from raw commodity level to ingredient level or from ingredient level to intake level.

Food classification systems for prepared food items, foods as purchased/ingredients, and raw commodities need to be linked through a system of processing and yield factors. Recommendations exist, but practices are very different, and some countries do not use such factors. Other countries have computerized programs, customized to their national dietary survey data collections, to undertake these tasks. In the future, it would be useful to have an international database of standard processing and yield factors available online.

Level of Detail in Food Classification

The level of detail required in a food classification system used in dietary exposure assessments depends on whether it is a nutrient of interest or hazard occurrence in food. Food classification systems for use in nutrient intake and food additive assessments include codes for processed foods and some foods as consumed in the system, whereas those for contaminant/agricultural and veterinary drug residue assessments are mainly based on raw commodities. The level of detail required in food classification is also determined to some extent by the purpose of the assessment, whether it is for reporting nutrient intake results from a dietary survey, for reporting nutrients and/or hazardous chemical results from a total diet study, or for food regulatory purposes (i.e., food standards).

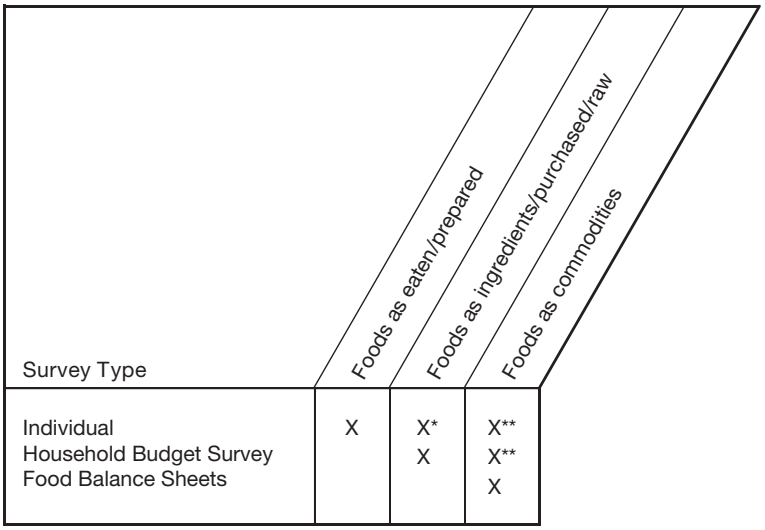

Figure 1 Survey reporting levels.

Food Description Systems

Food classification and food description have different goals, and this leads to different appearances of the systems. A classification system tends to group foods with similar characteristics; it is a tool of the end user of data. A description system, on the other hand, is a tool of the data originator, who wants to give a description of the food, as precise as possible, without the necessity of aggregating them.

Free Text Food Description

In 1987, the International Network of Food Data Systems (INFOODS) food nomenclature and terminology committee prepared Guidelines for Describing Foods, in order to provide a framework for the exchange of data between data sources and compilers of food composition databases. The INFOODS Guidelines constitute a broad, multifaceted, and open-ended mechanism designed to capture all information that might be available and that might be of some use to someone. The guidelines first propose criteria for deciding whether a food is 'single' or 'mixed' and then provide sets of descriptive facets for these two classes of foods. It is an open-ended, free-text food description system, a listing of features or entities that might influence the composition of food and that collectors of data should be encouraged to record. It does not include controlled terms to standardize description and facilitate data retrieval. The system is useful for recording food description that cannot be systematized, but has not been widely used in food databases.

Food Description Thesauri

Consistent indexing and retrieval can be attained using thesauri, in which vocabulary control is achieved by deliberately restricting the scope of terms and by displaying hierarchical relationship. A thesaurus is a controlled indexing language, formally organized so that a priori relationships between concepts (e.g., 'broader' and 'narrower') are made explicit. Concepts can be further organized into a scheme of viewpoints or facets, that is, clearly defined, mutually exclusive, and collectively exhaustive aspects, properties, or characteristics. To facilitate indexing and retrieval, many thesauri allow polyhierarchical relationships; for example, the descriptor FIELD CORN can appear under several broader terms: sugar-producing plant, starch-producing plant, oil-producing plant, and grain. In addition to broader and narrower terms within a facet, thesaurus descriptors may be given synonyms, related terms, definitions, and scope notes. The use of underlying codes renders a thesaurus more robust, allowing spelling changes and translations. Due to its flexible structure, a thesaurus can be amended by adding new viewpoints and by including more details within facets. A faceted thesaurus is thus well adapted to systematically describe features of foods.

AGROVOC thesaurus

An example of a successful implementation of a faceted thesaurus is AGROVOC, a controlled vocabulary covering all areas of interest to FAO, including food, nutrition, agriculture, fisheries, forestry, and environment. AGROVOC was first developed in the early 1980s, as a thesaurus to support uniform indexing of the FAO bibliographic catalog. To date, AGROVOC contains over 32 000 concepts organized in a faceted hierarchy, and each concept may have labels in up to 22 languages. However, its facets and terms are not sufficient or specific enough to describe food products as consumed.

LanguaL™ thesaurus

Work on the LanguaL™ thesaurus was started in the late 1970s by the Center for Food Safety and Applied Nutrition (CFSAN) of the US Food and Drug Administration (FDA) as an ongoing cooperative effort of specialists in food technology, information science, and nutrition. Since then, LanguaL™ has been developed in collaboration with the US National Cancer

Institute (NCI) and, more recently, its European partners. The European LanguaL™ Technical Committee has administered the thesaurus since 1996.

LanguaL™ is a multilingual thesaurus using faceted classification, constructed according to ISO standards. LanguaL™ allows foods to be described by a set of standard, controlled terms chosen from 14 facets characteristic of the nutritional and/or hygienic quality of a food, such as biological origin and technological treatments, plus parallel systems of food classification (e.g., EuroFIR, Codex GSFA, and GS1 GPC). The thesaurus contains about 7000 descriptors, many with definitions, links to authoritative sources, scope notes prescribing use, synonyms, and related terms. The thesaurus is language-independent at concept level: each of its descriptors possesses an underlying code that points to equivalent terms in different languages (9 languages in 2012). A stand-alone software program, LanguaL™ Food Product Indexer, assists food description by providing examples of already-indexed foods. The LanguaL™ thesaurus is used by databases in the United States (USDA National Nutrient Database for Standard Reference) and Europe (EuroFIR network), thus facilitating links between food databases and contributing to coherent data exchange.

Comparison of Food Description Systems

The two major food description systems used in food composition databases are thus the LanguaL™ thesaurus, with well-defined terms, and the INFOODS Guidelines, in free text. A comparison of these two systems was carried out by INFOODS regional data center coordinators in 1998. In this evaluation, LanguaL™ scored better in relation to addressing issues of barriers of language and culture, but maintenance of descriptors and codes was judged to be time-consuming. The INFOODS Guidelines scored better in relation to 'friendliness' and local usefulness by conventional users of food composition data. Free-text food description is simpler and quicker to use and allows information that is more detailed and thus not limited by insufficiencies in the choice of terms from a thesaurus.

The demand for a language-independent thesaurus (like LanguaL™) and the requirement for a practical, in-the-field system (like the INFOODS Guidelines) for food description in databases have led to attempts to link complementary systems to create a minimum set of standards and a harmonized approach for identifying foods worldwide.

Combined Approaches for Classifying and Describing Foods

FDA International Interface Standard for Food Databases

In the early 1990s, the USFDA developed an 'International Interface Standard for Food Databases' (IIS) in order to facilitate retrieval of information from food databases and to improve and standardize food descriptions. The IIS includes food names, LanguaL™ descriptors, free-text description in INFOODS facets, recipe information, and parallel food classification systems. Although it has not obtained international acceptance, IIS is an invaluable step toward the definition of the relevant types of metadata in the domain of food databases.

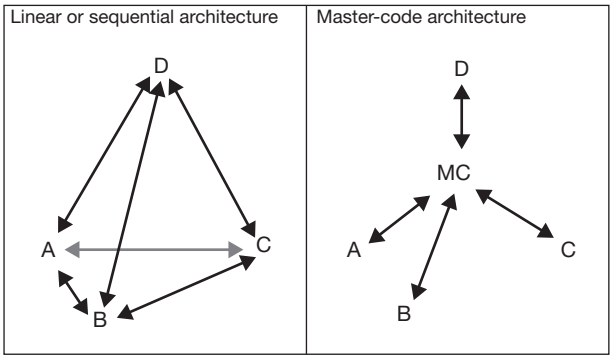

Figure 2 Architecture for code translation systems.

Mapping Between Food Classification/Description Systems

Food composition and consumption data are scattered over many food databases, each having its own system for identifying and describing foods. Combining data from two or more databases thus becomes difficult and time-consuming. It is possible, however, to create links between food identification systems in these databases, by 'mapping' one system to another. There are two ways of mapping between systems: a linear (sequential) way and using a so-called master code, which are illustrated in Figure 2.

In linear translation, a code from one system must be associated with that of the second, with that of the third, etc. Then, each code of the second system must be associated with one of the third. In the master-code approach, one coding system serves as an internal master code with which all the others are directly associated. For this to work, the master code must be more detailed than any of the other classification/description systems. All of this should be transparent to the end user, with software carrying out master-code lookups in the background. The master-code solution is flexible and allows for additions in the future, simply by providing two files: one catalog file with the new code list and one cross table file with the master code on one side and the new code on the other side. This technique was used in the CARE Food Safety project to link Italian, German, and Codex Alimentarius food codes. Because of its level of detail and use in food composition databanks, LanguaL™ has also been presented as a good potential choice for such a central mapping master-code structure.

EFSA FoodEx2

In an effort to introduce standardized reporting of food data, the European Food Safety Authority (EFSA) has recently created a food classification and description system – FoodEx2 – based on classification systems already in use for exposure studies. As an additional challenge, FoodEx2 intends to handle food at commodity, ingredient, and consumption levels. FoodEx2 consists of a detailed list of individual food items aggregated into food groups and broader food categories. The system includes parallel hierarchies, aggregating food items and food groups in different ways according to the needs of specific food safety domains: general reporting, chemical

exposure, pesticide residues, zoonoses, veterinary drugs, and animal feeds.

The use of a food list, rather than just classification, is not only a strength but also a weakness. The food list approach allows easier mapping to classification systems already in use for exposure studies but lacks sufficient detail to replace food codes in national consumption surveys and food composition databases. Moreover, the food list approach does not allow queries on new criteria; for example, a search for foods containing soy would not yield food items where 'soy' is not in the food name, for example, coffee whitener.

To provide additional information, the FoodEx2 food classification/list is complemented by facet descriptors. Some FoodEx2 facets and descriptors are straightforward and based on the LanguaL™ thesaurus, for example, food source (LanguaL™ facet B) and partly consumed/analyzed (LanguaL™ facet C). In these cases, links to the LanguaL™ thesaurus should be made clearer, as LanguaL™ is already used in major food composition databases. Other FoodEx2 facets seem to have been added to highlight specific safety risks, overlap with other facets, and lack the rigor prescribed in the ISO standard. FoodEx2 should be reviewed and provided with scope notes and examples to facilitate accuracy and consistency of food indexing.

Conclusions

All classifications are purpose-driven, created for a specific purpose and fit for use. This is a source of both strength and weakness. A classification system often cannot be used for another purpose than the one it was designed for or be used for foods at all levels (foods as eaten, ingredients, and commodities). Classifications are often contradictory, and their very existence shows that there can be no single, satisfactory, international classification system. In other words, there is no single food classification system that can serve all the needs of every database compiler. It is necessary to include food description.

The first breakthrough in international food classification and description has been the clear recognition of the advantages of using a multifaceted approach for identifying foods in food-related databases (composition and consumption). The second breakthrough has been the recognition of the need to include alternate classification/description systems. Criteria for such a combined system were laid down by the IIS: encompassing several parallel complementary schemes, structured, robust to accommodate different national languages, flexible for use by all users and for all types of foods, specific enough to avoid misclassification, adequately documented, and internationally acceptable.

The challenge of establishing a multifaceted system that can enable end users to analyze data held in different food-related databases was also the focal point in the 2010 EFSA Scientific Colloquium on Food Classification. The symposium recognized that no single system existed to meet all the potential end user needs and that it was not possible to develop a unique system to meet all requirements. At the same time, it concluded that the use of a common food classification and description system could provide a central linking system (master code) between existing systems to promote more accurate dietary exposure calculations in Europe and also at a worldwide level. Despite its shortcomings, the proposed FoodEx2 system can be seen to represent a major step toward harmonized food classification and description.

See also: Codex Alimentarius Commission: Role in International Food Standards Setting; Codex Alimentarius; Dietary Exposure Assessment; Dietary Surveys: National Food Intake; Food Composition Databases; Nutritional Epidemiology; World Health Organization.

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Food Colloids and Emulsions

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Introduction

Food is essential for life. It provides the micro- and macronutrients necessary to maintain body homeostasis. The concept of 'eating to live,' however, has changed dramatically through history, especially in modern affluent societies where eating is related to lifestyle choices, social identity, or pleasure and has become more than simply a means to acquire essential energy and nutrients. Against this challenging background, the food industry aims to develop novel food products, which simultaneously are healthy, tasty, and cheap. It is now belatedly recognized that some of the aforementioned, perhaps conflicting, needs can be addressed by understanding the 'microstructure' of food products as it evolves during processing, storage, and consumption. When viewed under the microscope, many of today's food products reveal a complex hierarchical structure with different coexisting phases organized on a wide range of spatial length scales from molecular to microscopic (Figure 1). Moreover, food microstructure is a dynamic concept. It changes continuously with time and the rate of change depends on many factors including composition, the environmental conditions (temperature, pH, mechanical forces, etc.), and the specific nature of the ingredient interactions. The relevance of the time and length scale to food functionality depends to some extent on the origin of the food materials but more importantly on the phenomena one is interested in. Digestion of lipids in emulsions, for example, depends on the size of the oil droplets with small droplets digested in the ileum instead of the duodenum, which impacts on the satiety. The example mentioned earlier highlights that some, if not most, of the transport, diffusion, and dissolution phenomena relevant to food functionalities (nutritional, sensory, and physicochemical) involve fat crystals, polymer networks, and oil droplet that all fall within the definition of colloids. The study of foods from a colloidal prospective extends the concept of 'microstructure' to a consideration of the nature of the interactions between the constituent elements of the food material, which are typically dominated by entropy changes rather than chemical bonds. Thus, understanding the physicochemical mechanism behind the formation and stability of colloidal structures is paramount for the design of food structure, which consider, together with the classic needs (texture and mouthfeel), the impact upon wellness and health, including modulated digestion to trigger different physiological responses.

Food colloids and emulsions can represent a significant proportion of a consumer's everyday diet, as they are often contained in a range of products such as spreads, condiments, cook-in sauces, dressings, deserts, and ice creams. In many current products, these colloids and emulsions are high in fat or can be structured with sugars leading to weight problems in the general public. In order to address these issues, the future of food colloids and emulsions is likely to focus on fat, salt, and carbohydrate reduction, as well as on the targeted delivery of nutrients.

The use of complex emulsion and colloid structures (single or multiple emulsions, fluid gels, mixed gels, etc.) offers an attractive range of tools to engineer healthier foods without compromising the organoleptic properties of the product. In order to design the appropriate microstructures and functionality, an understanding of the conditions experienced by foods in the human gastrointestinal tract is required.

Colloidal Aspects of Food Digestion

The starting point to understand the importance of food colloids in the general 'food–health' relationship requires a sound appreciation of what happens to foods once they enter the body (Table 1). Food structures evolve after food is eaten, initially as a result of oral processing and also during mixing and transport within the human gastrointestinal (GI) tract, which largely rely on its aqueous phase. In the mouth, food is essentially broken down through mastication, where the maximum hardness is limited by the chewing force (~50–150 N). As food is broken down and mixed with saliva, a thin film of food is formed between the tongue and the palate. As such, lubrication of the oral surfaces becomes progressively the most important factor determining the sensory perception of food. Saliva also affects food behavior as a result of mixing and dilution, the amylase in saliva starting the digestion of starches in the food, and the polymeric molecules (e.g., mucin) that may cause flocculation of particles or oil droplets. Food materials respond differently to the environment in the mouth depending on both their structure and composition and as such impart different sensorial properties. Oil-in-water emulsions stabilized by starch particles can undergo coalescence during oral processing as the starch is hydrolyzed by the amylase, which has been found to enhance the oral fat perception. After 15–20 chewing strokes, the food has been processed to a stage where bolus has been formed and can be swallowed. After swallowing, food enters the stomach where the digestion process starts. This is an acidic environment where a significant amount of food structure is broken down by added enzymes (e.g., pepsin and gastric lipase) and electrolytes (e.g., $Na+$, Cl^-, and Ca^{2+}). The time taken for complete digestion depends on the original food structure, with liquids being digested more rapidly than semisolids. Understanding the gelation of polysaccharides and/or protein–polysaccharide electrostatic interactions in acidic conditions relevant to the stomach environment is essential to develop fluid meals, which form gels in the stomach, thus delaying gastric empty and prolonging satiety. Upon leaving the stomach, the partially digested food, called 'chyme,' enters the small intestine where hepatic, pancreatic, and gallbladder secretions containing enzymes and phospholipids are introduced to continue digestion and allow

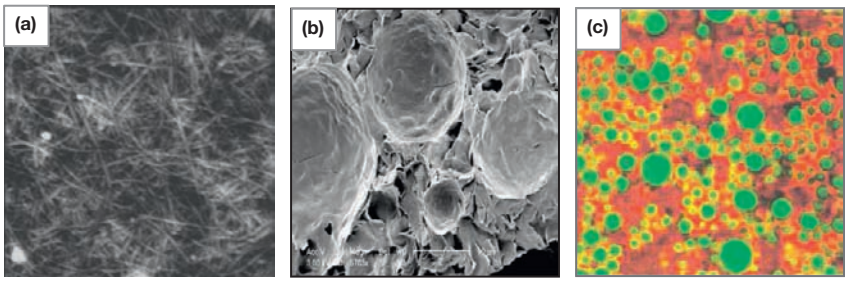

Figure 1 Examples of food microstructures: (a) acid-set hydrated gellan gel (image size 2×2 μm). Reproduced with permission from Gunning et al. (1996) Investigation of gellan networks and gels by atomic force microscopy. *Macromolecules* **29**, 6791–6796. ACS. (b) Cyro-SEM of a cocoa butter emulsion indicating a continuous fat phase with water droplets dispersed within. Reproduced with permission from Norton et al. (2009). Development and characterisation of tempered cocoa butter emulsions containing up to 60% water. *Journal of Food Engineering* **95**, 172–178. Elsevier. (c) Confocal micrograph (image width 80 μm) of low-fat mayonnaise showing oil droplets (green) and swollen starch phase (red). Reproduced with permission from Norton et al. (2006). Product/process integration in food manufacture. Engineering sustained health. *American Institute of Chemical Engineers Journal* **52**, 1632–1640. Wiley.

Table 1 Summary of the digestion steps

Digestive step	*Mechanical actions*	*Enzymatic/biochemical condition*	*Colloidal aspects*
Mouth	Mechanical breakdown Mixing with saliva	pH 6–7 Amylase Mucin	Interaction with salivary protein Emulsion droplet flocculation Phase inversion Rheology/lubrication
Stomach	Mixing Sieving Pumping	pH 1.5–3 Pepsin, trypsin, lipase	Enzymatic breakdown of droplets (interfacial) Oil droplets coalescence/emulsification Acid-induced gelation
Small intestine	Low-intensity mixing (peristaltic movement)	pH 6–7.5 Pancreatic secretions, bile salts, glucosidase	Displacement of protein from emulsion interface Swelling of biopolymer particles Mass transfer
Large intestine	Longitudinal contraction	pH 5.5–6 Microflora fermentation	Adhesion mechanism of bacteria

nutrient absorption. The presence of biosurfactants (e.g., bile salts) facilitates emulsification of lipids by adsorbing at the droplet surface or displacing some of the original surfactant favoring the access of lipase to the lipid. The presence of hydrocolloids can slow the digestion of starch, proteins, and lipids either by reducing mass transfer or by restricting access of enzymes to substrates. As such, nutrient uptake will take place toward the end of the small intestine causing the hormonal response known as ileal break, which slows gastric emptying. In the large intestine, the remaining undigested food is fermented by bacteria. This releases key health-promoting compounds for the host. Inclusion of additional health-promoting agents, self-structuring for satiety, and the limitation of nutrient absorption have all been developed from this knowledge.

Emulsions Stability and Functionality

Emulsions are essential structural components in many foods and they consist of mixtures of immiscible liquids (generally oil and water), with one dispersed within the other as droplets. The droplets usually have diameters that lie somewhere between 0.1 and 100 μm. In its simplest form, an emulsion is one of two different types, oil-in-water (e.g., mayonnaise) or water-in-oil (e.g., margarine), and the oil phases are nearly always a triglyceride in food emulsions. The mechanisms by which the droplets are formed by the different emulsification processes are now fairly well understood. Traditionally, the two fluids are mixed using high-energy input devices, breaking the dispersed phase fluid down to small droplets, within the continuous phase fluid, until the size range of the droplets approximates that desired for the final product. Droplet formation results in the enlargement of the interfacial area between the two phases, which carries a large positive interfacial energy. As such, the formation of droplets is thermodynamically unfavorable and emulsions tend to destabilize over time to minimize the interfacial area. Stability is provided by a layer of surface or interfacial active material, generally amphiphilic in nature (e.g., protein and surfactant), which lowers the interfacial tension and prevents instability phenomena, such as flocculation and coalescence through the mechanism of electrostatic and steric stabilization. The structure and physical

properties of the interfacial layers depend on the types, concentration, and the conformation adopted at the interface by the adsorbed surfactant. Proteins, such as β-lactoglobulin, once adsorbed, undergo unfolding and rearrange their secondary and tertiary structures to expose hydrophobic residues to the oil phase. Interfacial unfolding impacts on the mechanical properties of the adsorbed layer, as unfolded protein interacts with their neighbors forming a cohesive elastic structure around the droplets. On the other hand, small molecule surfactants form a compact interfacial layer, which stabilizes the droplets by charge repulsion or the Gibbs–Marangoni mechanism. Because of the greater reduction in the interfacial energy compared to protein, surfactants, given sufficient time and at sufficiently high concentrations, may completely displace proteins from the interface when both are present. Figure 2(a) shows atomic force microscopy images of the planar interface consisting of a protein film to which a nonionic surfactant (Tween 20) is added. The surfactant adsorbs at the defects in the protein network; these nucleated sites then grow, compressing the protein network. At sufficiently high surface pressure, the network fails, releasing the protein from the interface (orogenic displacement). The two-dimensional phase separation is markedly different when ionic surfactants are considered, which involves the formation of many small surfactant-rich domains, but limited domain growth (Figure 2(b)). Ionic surfactants are likely to form (electrostatic and hydrophobic) complexes with the adsorbed protein molecules and potentially interfere with the ability of protein to efficiently interact with their neighbors, thereby providing more gaps at which they may be adsorbed. Displacement and changes to the interfacial structures by surface-active compounds produced by the body are an integral part of the lipid digestion process. Bile salts are biosurfactants produced by the liver and stored in the gallbladder. They adsorb at the surface of emulsion droplets entering the intestine promoting the binding of colipase and pancreatic lipase to the droplet surface, which allows triglyceride hydrolysis to take place. The morphology of the protein displacement taking place when bile salts are added differs from those observed previously with conventional surfactants (Figure 2(c)). The different molecular structure of bile salts (e.g., derived from cholesterol) from the synthetic surfactant and the conformation they adopt at the interface certainly play a role. The process of the lipolysis reaction depends on the surface activities of the interfacial material coated on the emulsion droplets. Rational strategies for limiting lipid digestion stem from observations of the nature of the displacement of proteins from interfacial structures by bile salts. One way is to use surfactants that are strongly surface-active and do not interact with bile salts, thus resisting displacement and preventing adsorption of the lipase.

Pickering Stabilization

Submicron-sized colloidal particles also adsorb at the oil–water interface forming a rigid interfacial film of densely packed particles, which provide steric hindrance to droplet–droplet coalescence (the Pickering stabilization). Droplets that are insufficiently covered, however, tend to coalesce as 'naked' interfaces cannot repel colliding droplets. The effectiveness of particles against destabilization (e.g., coalescence) stems from their strong adsorption to the liquid–liquid interface. The energy required to remove such particles from the interface can be several orders of magnitude higher than the thermal energy, and it ultimately leads to irreversible interfacial adsorption. The energy of desorption (ΔG_d) is related to the size of the particle (r), the interfacial tension between the two phases (γ_{ow}), and the contact angle (θ) that the particle makes with the interface:

$$\Delta G_d = \pi r^2 \gamma_{ow} (1 - \cos\theta)^2 \quad [1]$$

Several factors determine whether or not particles will stabilize an interface in a satisfactory manner including particle wettability, size, shape, and particle–particle interactions. Among these, the parameter of choice, which dictates the position of the particles at the liquid–liquid interface and hence the interfacial curvature, is the particle wettability, which is best defined by the contact angle. Hydrophilic particles, with a contact angle (measured through the water phase) of $\theta < 90°$, are preferentially wetted by water and therefore tend to stabilize O/W emulsions. On the other hand, particles of $\theta > 90°$ tend to stabilize W/O emulsions (Figure 3). Particles that are completely wetted by either water or oil (contact angle = 0° or 180°) are not likely to adsorb at the interface. In addition to wettability, effective Pickering stabilization is obtained when the adsorbed particles are at least an order of magnitude smaller than the emulsion droplet they coat, due to the effect that particle size has on the energy of adsorption ($E \propto r^2$). The

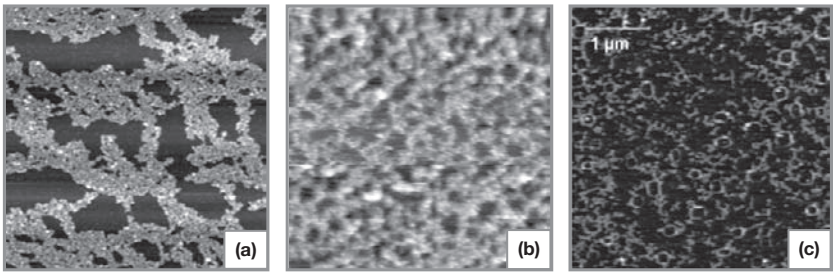

Figure 2 Atomic force microscopy of spread β-lactoglobulin films being displaced, at the air–water interface, by (a) nonionic Tween 20, $\Pi = 25.0$ mN m^{-1} (image size 4 × 4 μm), (b) anionic SDS $\Pi = 36.0$ mN m^{-1} (image size 1 × 1 μm). Reproduced with the permission from Gunning et al. (2004). The effect of surfactant type on protein displacement from the air–water interface. *Food Hydrocolloids* **18**, 509–515. Elsevier. (c) Bile salts, $\Pi = 26.9$ mN m^{-1}. Reproduced with the permission from Maldonado-Valderrama et al. (2008). Interfacial characterization of β-lactoglobulin networks: displacement by bile salts. *Langmuir* **24**, 6759–6767. ACS.

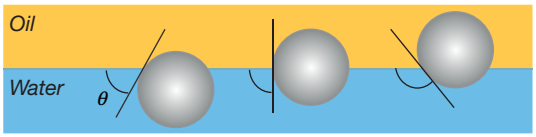

Figure 3 Effect of contact angle on the interfacial position of a solid particle.

wettability of the particle can be tailored via the adsorption of surfactant on the particle surface, which can modify the surface properties, and thus particle hydrophobicity to favor adsorption at the liquid–liquid interface. Surfactants can also displace particles from the interface depending on surfactant type and concentration. In the case of oil-in-water emulsions, the presence of a water-soluble surfactant will eventually result in the displacement of particles as their concentration in solution increases due to the greater affinity they have for the interface. On the contrary, the presence of oil-soluble surfactant, independently from its concentration, does not result in particle desorption. Many food products contain colloidal particles in their formulations and some of them participate in the stabilization of emulsions by adsorbing at the interface. Examples include cocoa particles in emulsion-based chocolate fillings, crystallized emulsion droplets in ice cream, and fat crystals in margarine. Fat crystals tend to favor the stabilization of oil-continuous emulsions by increasing interfacial viscosity or by forming a crystalline shell of sintered crystals around water droplets. The tendency to adsorb at the oil–water interface depends on the size as well as on the polymorphic form of the crystal. Tristearin α-crystals (contact angle between 20° and 30°), for instance, are more hydrophilic than β-crystals (contact angle 0°); therefore, they tend to absorb at the interface. However, surfactants are usually employed to increase crystal polarity, thus favoring adsorption. Once at the interface, crystals may form a rigid layer of sintered crystals, which contributes to the stability of the emulsion but more importantly allows the entrapment of electrolytes and other osmotically active components, independent of the magnitude of the applied osmotic pressure gradient. Release, in this case, is triggered by increasing temperature. At higher temperatures, crystals melt, and as a result, emulsion stability is compromised eventually leading to complete entrapped active (e.g., salt) release.

Hydrocolloids

A large number of biopolymers, mainly polysaccharides, routinely used in food formulations as thickening (e.g., guar gum) or gelling agents (e.g., carrageenan) to control mouthfeel, are grouped under the technical term of 'hydrocolloids.' The term 'hydrocolloids' or 'hydrophilic colloids' arises as these polymers have a marked affinity for water, due to the presence of a large number of hydroxyl (–OH) groups. In addition, when in solution, they exhibit the properties of a colloid due to their large size as macromolecular entities when compared to water molecules. As mentioned earlier, many hydrocolloids are polysaccharides, which typically contain several hundred of thousands of monosaccharide units. The position of the linkage between adjacent sugars and the orientation of the bonds to the glycosidic oxygen (axial or equatorial) dictate the overall shape (conformation) of the individual chains and the way in which they interact with one another. This has a profound effect on the physical state of polysaccharides and consequently on their physiological role as constituents of digest. In the case of starch, a direct consequence of the α $(1\rightarrow4)$-linkages in the polyglucose sequences of amylose and amylopectin is the formation of coaxial double helices, which are densely packed to form crystalline spherical granules. Because of its crystalline structure, starch is insoluble in cold water, and it is far less susceptible to breaking down by human digestive enzymes (i.e., resistant starch). On heating, however, the amylopectin helices melt and lose its crystalline structure; the granules swell with the release of disordered amylose in a process known as gelatinization. As a result of this order–disorder transition, starch molecules become highly hydrated and susceptible to enzymatic hydrolysis. An important use of polysaccharide chain when present in food systems as disordered coils is to increase the viscosity of the solution by restricting the flow of the solvent. This is strongly dependent on the molecular weight and conformation of the polymer in solution as well as on its concentration. Figure 4(a) shows changes in specific viscosity, η_{sp}, defined as

$$\eta_{sp} = \frac{\eta - \eta_s}{\eta_s} \qquad [2]$$

where η is the viscosity of the solution and η_s is the viscosity of the solvent, as function of concentration for a solution of methylcellulose. In dilute solutions, the individual coils are free to move independently and generate viscosity by tumbling around and interfering with the flow of the solvent. The viscosifying capacity at such low concentrations is correlated to the volume that the molecules occupy in solution (hydrodynamic volume), which is a consequence of its molecular weight, branching chains, and affinity with the solvent. As the polymer concentration is raised, these molecules begin to come into contact with one another and to overlap and interpenetrate one another. The formation of entangled network of interpenetrating chains occurs at a critical concentration known as coil overlap concentration (C*) and is accompanied by a sharp increase in the concentration dependence of viscosity. With increase in concentration, shear-thinning behavior is often observed (Figure 4(b)). At low rates of deformation (i.e., low shear rates), there is sufficient time for entanglements, pulled apart by the imposed stresses, to be replaced by new entanglements with different chains, and as a result, the viscosity remains constant (the Newtonian plateau). However, at higher shear rates, where there is insufficient time for new entanglements to form, the extent of coil overlap is decreased and the viscosity decreases (shear thinning).

Hydrocolloids Gelation

While all hydrocolloids thicken and increase the viscosity of aqueous dispersions, some of them also have another major property of being able to form gels. The formation of a cohesive network requires enough ordered association (as in the solid state) or cross-linking to hold the three-dimensional network together, but there must also be sufficient residual

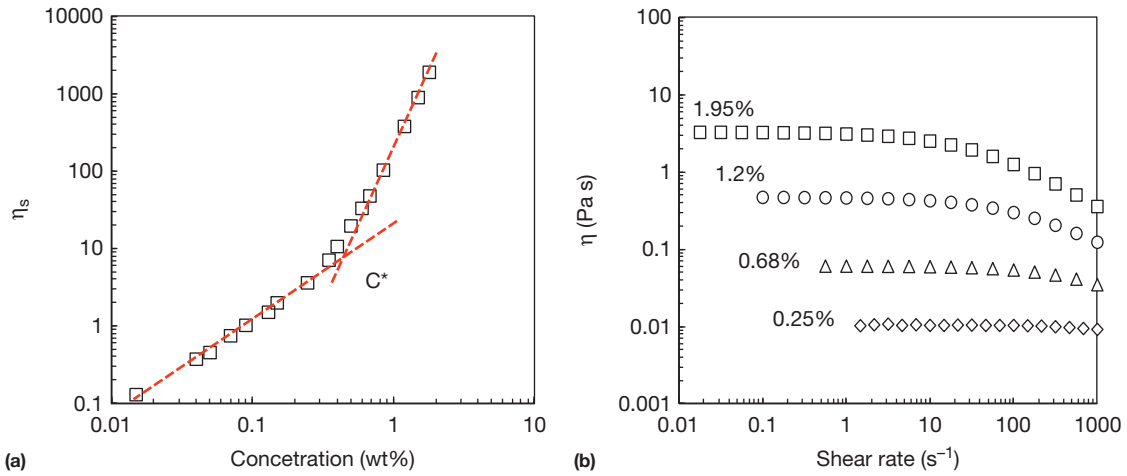

Figure 4 (a) Variation of specific viscosity (25 °C) with concentration for methylcellulose. (b) Viscosity curves for aqueous solution of methylcellulose at 25 °C.

disorder (as in solution) to maintain hydration and prevent the network collapsing to a close-packed solid. This requires that the number of ordered association known as 'junction zones' must terminate at some point so that the remaining unordered segments of the polymer chain are free to participate or not in other junction zones with other polymer chains. The minimum length can vary substantially from one polysaccharide to another but is typically in the range 8–20 residues. Termination of junction zones usually results from structural irregularities that include changes from one type of residue to another type in the polysaccharide chain or branch points, which can inhibit ordered packing and promote solubility. Charge also promotes solubility by introducing electrostatic repulsion between the chains. Negatively charged polysaccharides may, however, associate forming junction zones in the presence of cations that can bind to the chains and balance their charge (e.g., κ-carrageenan). The physical characteristic of the network depends on several factors including the number and length of the 'junction zones' and the number of molecules in the junction zones. The latter can vary across the different hydrocolloids; junction zones may be formed by two (e.g., alginate) or more polymer chains (e.g., κ-carrageenan), which has a direct impact on the rigidity of the gel. In addition, there are also various types of conformations present in ordered junction zones in gels. These include helical structures, such as the coaxial double helices found in carrageenan and agar gels, which form aggregated assemblies incorporating cations to balance the charge on the polymer chains and the 'egg-box' structure found in alginate gels. The thermal stability of the gels is also a direct consequence of the nature of their junction zones. Gelatin melts at low temperatures because the junction zones are only bound by relatively weak hydrogen bonds. On the other hand, it is possible to make alginate gels that do not melt on heating because of the strength of calcium bridges in the junction zones. Knowledge of the conditions required for gelling of particular hydrocolloid dispersions and the characteristics of the gels produced are very important aspects in the design of hydrocolloid-based foods, which might convey properties in the GI tract, related to promotion of health. These include the effect that gelation has on the GI transit time, where an extended residence time in the stomach produces a 'sensation of fullness' and hence a potential way for appetite control. With gelation, the greater sensation of satiety is thought to be a combination of viscosity increase and mechanical properties of the gel. Strong gels would be less likely to be broken up by the grinding action of the stomach, which is reported to apply forces in the order of 0.65 N. Gelation can be triggered by the physiological conditions found in the gastric lumen, that is, low pH and body temperature. Low-acyl gellan, a polysaccharide obtained from *Aeromonas elodea*, forms a threefold double helix structure (similar to that of carrageenan) at low temperature. Lowering the pH promotes gelation by reducing the charge density, thus suppressing electrostatic repulsion between individual helices. As such, in acidic environments similar to those present in the stomach, low-acyl gellan forms strong elastic gels where maximum gel strength is observed between pH 3 and 4 (Figure 5). The rate of gelation is likely to play a major role in determining the effect of intragastric gelation on satiety. The rate of gelation should be sufficiently fast to allow structuring while the digesta stay in the stomach. However, if the gelation rate is too fast, only part of the digesta will be trapped in the gel structure, thus decreasing the impact on satiety. This requires a detailed understanding of the relationship between the fine chemical structure and the physicomechanical properties of the chosen hydrocolloids to generate the desired gel microstructures.

Biopolymer Mixtures

Mixtures of biopolymer are frequently used in food to impart specific flow behaviors, texture, and appearance to the final food product, for example, low-fat spreads, salad dressings, sauces, and ice creams. The structures and morphologies that such mixtures develop in relation to their functionalities are the direct consequence of the thermodynamic compatibility of the macromolecular components and the interfacial phenomena. On the basis of the interaction taking place

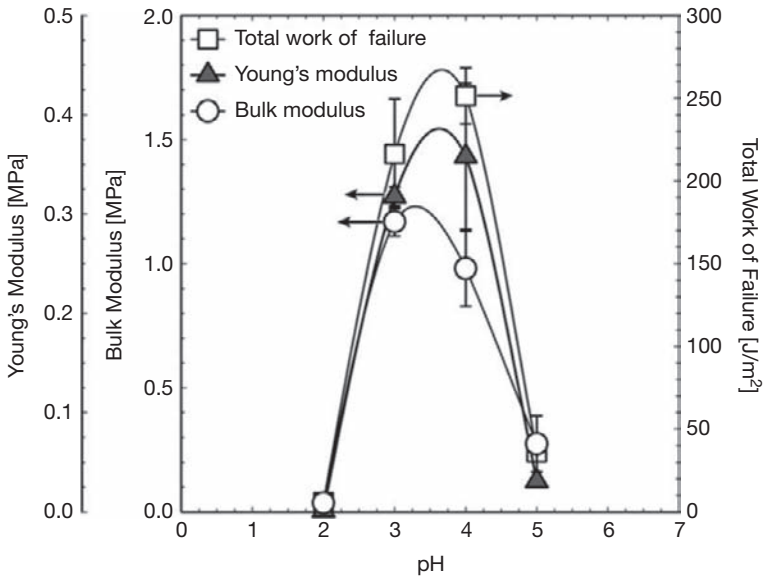

Figure 5 Young's and bulk moduli and total work of failure for 2% gellan acid gels formed under different pH environments. Reproduced with permission from Norton et al. (2011). Acid gelation of low acyl gellan gum relevant to self-structuring in the human stomach. *Food Hydrocolloids* **25**, 1105–1111. Elsevier.

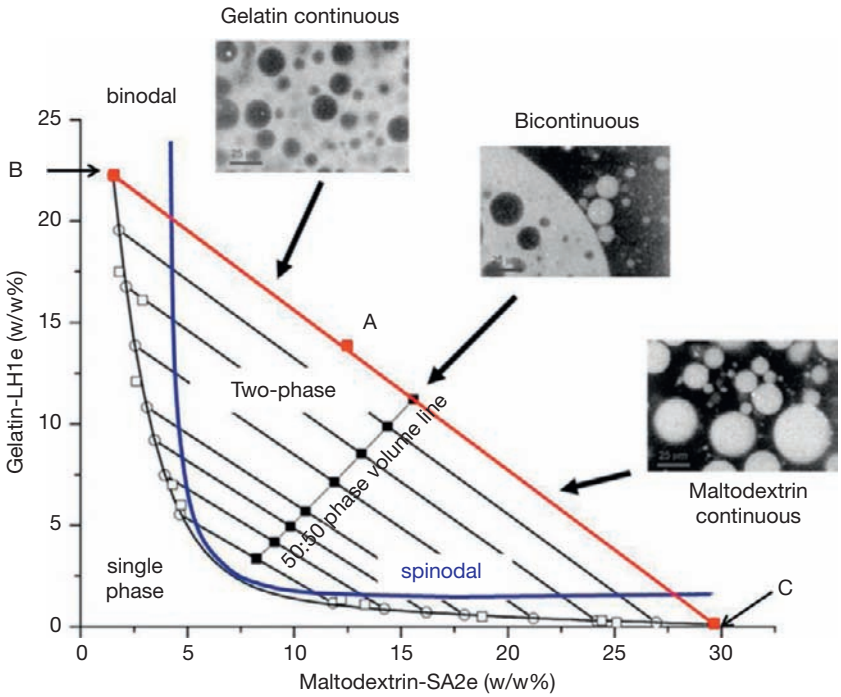

Figure 6 Phase diagram of a mixture of gelatin-LH1e and maltodextrin-SA2e. Confocal laser scanning micrographs show the phase morphology (light phase is protein rich). Reproduced with permission from Norton and Frith (2001). Microstructure design in mixed biopolymer composites. *Food Hydrocolloids* **15**, 543–553. Elsevier.

between the two polymers, it is convenient to distinguish two types of phase separation: segregative and associative. In the case of associative phase separation, the two polymers, usually oppositely charged polyelectrolytes, will interact and collect in the same phase. The result is a system in which one phase is enriched in both polymeric components existing in equilibrium, with another phase poor in both. If the interaction is repulsive, then, above certain polymer concentrations, the two polymers will separate in two phases each containing primarily one biopolymer with only a small amount of the other. A phase diagram (Figure 6) is used to determine the composition of polymers needed to induce phase separation in

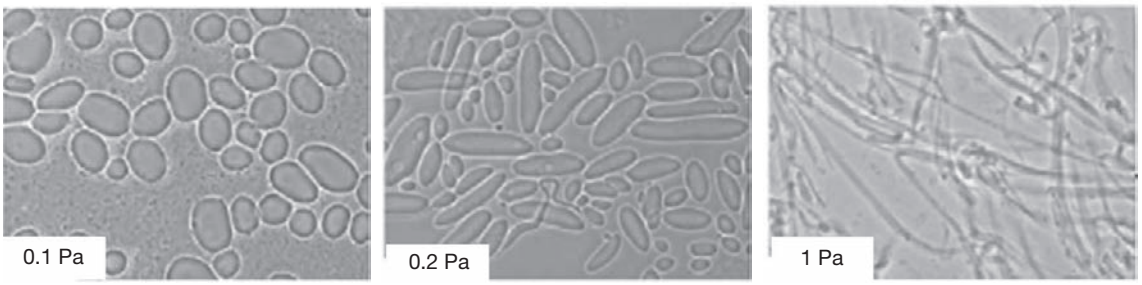

Figure 7 Different particle morphologies produced by increasing the shear regimen during gelation of various biopolymer mixtures. Reproduced with permission from Norton et al. (2006). Fluid gels, mixed fluid gels and satiety. *Food Hydrocolloids* **20**, 229–239. Elsevier.

an aqueous biopolymer mixture. Phase diagrams are usually constructed by plotting the polymer concentrations as an x–y plot as the solvent in the mixture often constitutes more that 90% of the sample. The binodal curve shows the cosolubility profile of the two biopolymers in water. It divides the miscible region at low polymer concentrations (single-phase region) from immiscible region at higher polymer concentrations (two-phase region). The compositions of the phases formed are determined by a number of factors, including the solvent condition, the temperature, and the molecular weight of the two polymers. Molecular weight (M_w) plays a direct role on the phase compositions, since higher M_w polymers have a smaller entropic term, so that increasing the M_w of one of the components will have the effect of increasing the concentration. Temperature also has a major effect on the phase separation. A reduction in temperature reduces the entropy of mixing leading to an increase in incompatibility and, more importantly, often inducing molecular ordering, which has the potential to increase the effective molecular weight of the components. The way in which the phase morphology initially forms and then evolves follows essentially two types of mechanisms: nucleation and growth and spinodal decomposition. Microstructure of biopolymer solutions whose composition lies within the two-phase region (spinodal line) takes the form of suspensions of droplets (~2–20 μm) of one component in a continuous phase of the other, similar to that of conventional oil-in-water emulsions (Figure 6). In fact, these structures have been found to depend on the same physical principles (droplet breakup and coalescence) as conventional emulsions. Once the droplets have formed, they can start to ripen through droplet coalescence, and if the process is allowed to continue undisturbed, then bulk phase separation is expected. A competing event in these systems is the gelation of one or both phases, which arrests the process of ripening of the phase structure. Another factor, unique to mixed biopolymer mixtures in determining the structure, is the low interfacial tension between the separated phases due to the fact that the main component of the two phases is water. A surface tension of $\sim 10^{-5}\ \mathrm{Nm}^{-1}$ is generally measured, which is about three orders of magnitude below that normally measured for an oil-in-water system. The low interfacial tension and the high viscosity of the two phases impact on the timescales involved in the recovery from the effects of applied shear, which is slow. Therefore, the combination of gelation with a suitable sample flow history can be used to kinetically trap flow-induced structures, the morphology of which is determined by both the thermal history and process shear conditions. Examples of the anisotropic structures, which can be generated, include ellipsoidal gel particles and fiber-like structure (Figure 7). These asymmetrical particles exhibit different rheological properties to those possessing a spherical phase structure, for example, higher viscosity at lower phase volumes and complex flow patterns. Although biopolymer mixtures have been long considered to control of oral breakdown, this is arguably only their main function in food. In principle, such formulations offer the promise of a dual stage and breakdown profile, with the second stage leading to structuring of material in the stomach or gut. Such properties are potentially beneficial in terms of enhancing the sensation of satiety or in controlling the uptake of food macronutrients.

Emerging Developments

Multilayer Emulsion

A powerful emerging approach to achieve effective control over emulsion stability and release of functional ingredients is the formation of a multilayer interface. Production of multilayer emulsions generally involves the sequential deposition of charged biopolymers onto an oppositely charged emulsion surface (Figure 8). Adsorption of the charged biopolymer occurs until saturation of the surface is obtained. The reversal of net charge on the interface, which creates an electrostatic repulsion between the surface and the nonadsorbed biopolymer in solution, prevents further adsorption. Charge reversal then also allows the adsorption of an additional oppositely charged biopolymer onto the surface. This process can be repeated a number of times to form oil droplets coated by laminated shells of three or more layers. Multilayer emulsions have been prepared from protein–proteins, protein–polysaccharides, phospholipids–polysaccharides, and surfactant–proteins. The properties of the interfacial multilayers, such as its thickness and permeability, are dictated by the strength of molecular interaction between adjacent layers. This is controlled by charge density of the adsorbing biopolymer and the prevailing solution conditions. Changes in pH in the GI tract can potentially be used to weaken electrostatic interactions between the layers and provide a means for the transport and release of bioactive compounds. Equally important is the ionic strength of the solution. The presence of salts

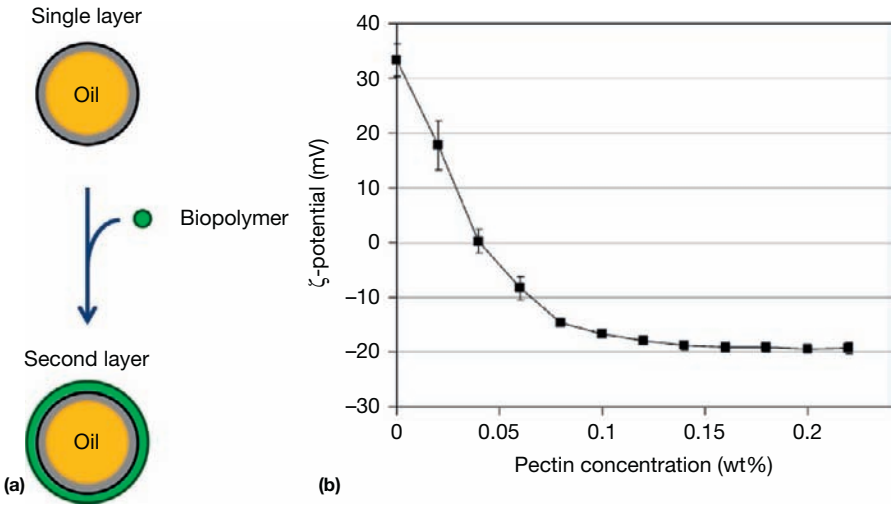

Figure 8 (a) Schematic representation of the adsorption of a layer of biopolymer onto droplets surface. (b) Change of the surface electrical charge of emulsion droplets stabilized by β-lactoglobulin (β-Lg) as function of pectin concentration (5 wt% corn oil, 0.45 wt% β-Lg, 5 mM imidazole/acetate buffer, pH 3.0) after dilution. Reproduced with permission from Moreau et al. (2003). Production and characterization of oil-in-water emulsions containing droplets stabilized by β-lactoglobulin – pectin membranes. *Journal of Agricultural and Food Chemistry* **51**, 6612–6617. ACS.

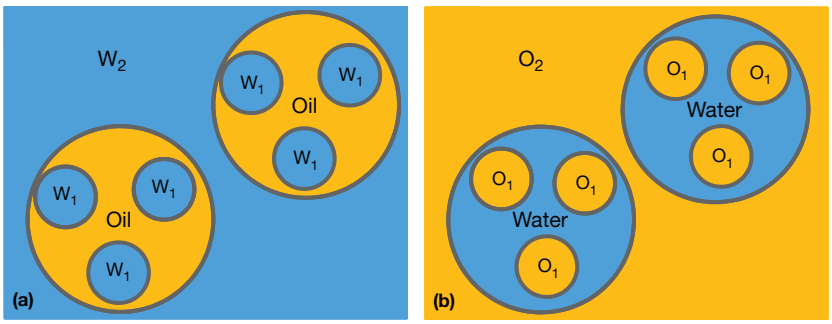

Figure 9 Schematic representation of (a) water-in-oil-in-water ($W_1/O/W_2$) emulsion and (b) oil-in-water-in-oil emulsion ($O_1/W/O_2$).

can reduce the strength of the electrostatic interaction between oppositely charged biopolymers influencing the stability and thickness of the layers. Multilayer coating can be, potentially, designed to make oil-in-water emulsion completely inaccessible to digestive enzymes in the GI tract so that they could be used to reduce the calorie content of foods while maintaining good sensorial acceptability. In addition, understanding the design rules that control the structure and instability of the laminated shells opens up a wide array of opportunities to engineer emulsion with multilayer membrane that responds to physiological stimuli (e.g., pH, enzymes, and salt) providing a means of selective triggering and release of functional molecules at desired sites through the GI tract.

Double Emulsions

Another emerging technology is double or duplex emulsions. These are emulsions that have a ternary structure where an inner emulsion is formed within an emulsion. There are two main types of such systems: water-in-oil-in-water ($W_1/O/W_2$) emulsions with water droplets inside oil droplets and oil-in-water-in-oil emulsions ($O_1/W/O_2$) with oil droplets inside water droplets (Figure 9). The potential benefit of such high-order structures is that they can be used to encapsulate health-promoting ingredients (e.g., peptides) whose unpleasant taste will not be perceived upon consumption as the internal phases will not come into contact with the sensory organs during oral processing. In addition to encapsulation, the use of $W_1/O/W_2$ emulsions in products such as mayonnaise allows the possibility of lowering the fat content as the dispersed oil phase itself contains water allowing a volumetric reduction of the oil without changes in the perceived oil phase fraction. Despite their commercial relevance, double emulsions are faced with a number of significant challenges, namely, the means by which the inner phase destabilizes during manufacture as well as during the subsequent storage. Production of double emulsions relies on two separate emulsification steps: a primary high-intensity mixing or valve homogenization to obtain the smallest possible droplets, followed by secondary emulsification under mild condition (e.g., membrane emulsification) to avoid any destabilization of the primary emulsion during the process. Instabilities on storage may occur at many different

levels: coalescence between internal phase droplets (W_1) or between outer phase droplets (W_2) and/or release of the internal phase (W_1) in the external one (W_2). The stability of duplex emulsions is highly dependent on the type and concentration of emulsifiers used. The use of polymeric emulsifier (e.g., whey protein isolate), on at least one interface, providing steric stabilization to the emulsion, markedly improves double emulsion stability against coalescence. Their large molecular size prevents them from migrating between interfaces. They will also not form reverse micelles, which can transport water and/or solute through the oil phase. Another common cause of instability comes from the large Laplace pressure (Π) of small inner droplets (W_1), resulting in a thermodynamic driving force for water to diffuse through the oil phase. In principle, the creation of a solid shell layer by the Pickering stabilization with fat crystals and subsequent sintering at the emulsion interface is an efficient way to prevent molecular migration through the interface resulting in highly stable double emulsions.

Fluid Gels

Many hydrocolloids such as egg albumin, whey protein, agar, alginate, and κ-carrageenan form microgel suspension ('fluid gel') if a suitable shear field is applied to the sample during gelation. This process can produce a material, which contains a high phase volume of spherical particles with droplet diameters in the 1–10 μm range (i.e., the same size range as oil droplets in many food emulsion systems). If the shearing process is not applied for the whole of the gelation process, then some cross-linking between gel particles can be induced resulting in a material with a yield stress, whose properties depend, among other things, on the concentration of the biopolymer used to produce the sample. By careful selection of the hydrocolloid and its concentration and by careful control of the shear and temperature/time profile, a range of textures and emulsion-type properties can be achieved. This is clearly an interesting colloidal structure with potential applications in many food products for fat replacement or for controlled release of other food ingredients such as sugars.

Colloidal Structure in Action: Where We Are?

Food colloids in many, if not most, food offer much potential for controlling their functionality. The structure and interfacial properties of colloids are critical to manipulate and control stability both before and after consumption. More questions arise as to how the dynamics and kinetics of nutrient uptake are influenced by food colloids structures (e.g., particle size and shape and self-assembled structures) where the timescales range from nanoseconds (enzyme reactions) to hours (transit time in the GI tract). Indeed, it is not yet possible to predict with confidence what will happen in the real systems until it has been studied previously. Further development in this area is needed to establish a solid link; on the one hand, between the biological mechanisms of digestion and, on the other, the engineering of food structures that can deliver desired physiological impacts.

See also: Appetite Control in Humans: A Psychobiological Approach; Emulsifiers: Types and Uses; Gums: Dietary Importance; Gums: Properties and Uses; Margarine: Composition and Analysis; Mayonnaise; Riboflavin: Physiology.

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Food Composition Databases

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Introduction

Food composition is the undertaking to determine and compile what is in the foods we eat and is the critical bridge between nutrition, health, and disease prevention and food production. This intersection is extensively described in the Dietary Reference Intakes prepared by the Institute of Medicine of the National Academies and the Dietary Guidelines for Americans prepared by the US Department of Agriculture (USDA) and the US Department of Health and Human Services and in similar documents from other countries. These include components essential to survival (protein, carbohydrates, fat, minerals, and vitamins) and compounds that have the potential to cause diseases, ranging from trace to excess concentrations. Of increasing interest are various bioactive compounds, which may have a protective effect against certain diseases such as cancer and coronary heart disease.

Food production incorporates agriculture to produce the raw materials and the processing and food science that turns raw commodities into the myriad of different foods available. These different processes impact the amounts of components in foods and the determination of the amounts of these components composes food composition databases or tables (FCDBs/FCTs). The uses of FCDBs are multidimensional and include the following: (1) evaluation and monitoring of nutritional status, (2) monitoring of changes in dynamic food supplies, and (3) inclusion of new information on the characteristics of commodity-level foods (growing conditions and feeding practices) and commercially prepared foods (brand differences), market share distributions, and reformulations. FCDBs are also important in world trade, particularly with the diversity of foods available from many countries. Increasingly, consumers want to know what is in the foods they eat. Foods, particularly commodity-level foods, are sold based on the content of certain components. In some cases, knowing the composition of a specific food can be used to uncover adulteration or other fraudulent activities. Many of these standards are established in the Codex Alimentarius – a set of international food standards, guidelines, and codes of practice.

History of Nutrition Monitoring and Food Composition

The study of the composition of foods and the connection to health and disease prevention has been a worldwide initiative for almost 200 years. Many diseases are considered preventable through diet and physical activity, so comprehensive information on the composition of foods is critical to health promotion and disease management. The first US FCTs were compiled by Atwater and Woods and were entitled 'Investigations on the Chemistry and Economy of Food.' This publication included data for 178 food items on five proximate components (water, protein, fat, total carbohydrates, and ash), kilocalories (called fuel in the Atwater table), and refuse. In subsequent decades, the USDA published a number of reports on the composition of foods, extending the number of foods and components covered. Researchers around the world expanded food composition research and data tables. In 1950, the USDA published Agriculture Handbook No. 8, which was updated in 1963 and again in section by food groups between 1976 and 1993. Concomitantly, data files were made available for use with various computer programs – first on punch cards and magnetic tape, followed by diskettes and CD-ROM. Today, the database is available on the Internet. In 1980, Release 1 of the USDA National Nutrient Database for Standard Reference (SR) was made available. The latest version, SR27 (2014), contains data for up to 150 components for nearly 8600 food items; the SR is available on NDL's website and is updated annually.

Types of Data

Knowing the composition of foods is essential to understanding the promotion of health and prevention of diseases. These data are used by researchers, nutrition policy makers, health professionals, the food industry (e.g., labeling, research, and development), and a wide consumer base. Electronic collection, storage, and rapid dissemination are standard, making currency and accuracy of the data more important than in the past.

There are a number of different types of data found in FCDBs/FCTs including analytic data. Analytic data are values determined by direct analysis of a food sample and in most cases are the highest quality data. Several types of information are essential if analytic data to be used in an FCDB/FCT. This includes a good sampling plan to ensure representative values, proper sample handling, and selection of the appropriate analytic method for the food matrix and component being analyzed and appropriate quality control materials to validate the results. The USDA's National Food and Nutrient Analysis Program (NFNAP) incorporates all of these features.

An accurate and comprehensive food description is critical for it to be added to the FCBD/FCT. A good description should include the name of the food, how it was processed (specifying any fortification or enrichment), how it is prepared, and any added ingredients. Basic commodities, for example, a plant or animal food, should include the cultivar or breed, whether it was from a wild or domesticated plant or animal, along with the scientific name. Color, maturity, and refuse should also be specified. It is also important to specify the edible portion and describe any refuse.

The average supermarket in the United States contains tens of thousands of unique food products; the latest version of the USDA National Nutrient Database for Standard Reference, SR27, contains over 8600 food items for up to 150 food

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components. It is not possible to analyze all foods in the food supply or even those reported in national food consumed, due to both the cost of the sample collection, preparation, and analysis and the resources needed to handle data. The USDA has used the key foods approach, which accounts for 75% of the intake for nutrients of public health significance, to identify and prioritize foods for analysis; key foods are determined by multiplying the reported consumption of foods by their respective nutrient concentrations, in order to prioritize foods for analysis.

Not all components are found in every food. For example, vitamin B_{12} and cholesterol are not found in plant foods, while fiber and vitamin C are not found in animal products. As such, it would not be a prudent use of resources to analyze these nutrients in such cases as the values can be assumed to be zero. The database compiler should document that decision in the FCBD/FCT. However, when considering processed foods, which are mixtures of many components, these assumptions do not apply. Furthermore, there are often additional exceptions to these general rules, and the database compiler must be cognizant of them.

- *Analytic data*: Analytic programs are funded by national governments (e.g., USDA) and through collaborations with other government agencies, the food industry, and academic institutions. Analytic data are generally the highest quality data and often include a prioritized list of foods, which are sampled nationally and analyzed using officially accepted analytic methods, sufficient number of samples to address nutrient variability, rigorous quality control procedures (both in handling of samples and in analysis of raw data), and appropriate statistical methods for data aggregation. At the USDA, a national sampling program, the NFNAP is a long-standing collaboration with the National Institutes of Health to expand and improve the quality of composition data for foods consumed by Americans. To date, composition profiles for approximately 2000 foods have been developed under this program.
- *Calculated data*: Missing values may be imputed from similar foods and adjusted for moisture content or normalized according to base nutrient, for example, fatty acids to total fat and amino acids to total protein. Missing values for multicomponent foods are often calculated through linear regression programs based on a list of ingredients in descending order from manufacturers or labels (with high-quality data for ingredients) and nutrient levels from the Nutrition Facts panel. When using values from the Nutrition Facts panel, issues may arise due to rounding permitted when calculating values for the label, for example, trans fat can be rounded to 0 if the value is less than 0.5 g per serving. This can result in underestimating the content when the values are calculated to 100 g, particularly with small servings.
- *Assumed zero*: In many cases, a specific nutrient is known not to exist in certain foods, for example, cholesterol in plant-based foods. In these cases, zeros may be used to populate missing fields.
- *Manufacturers' data*: Label data may be used to complete nutrient profiles where analytic data are not available.

As discussed in the preceding text, it is not possible to analyze every form of a food. Missing values can be calculated from other forms of the same food by adjusting for changes in moisture and fat content. If the food is processed or prepared, changes in nutrient content can be determined by the use of appropriate retention factors. Other procedures for calculating missing values have been developed. Another approach for calculating missing values is the use of a recipe for the food item of interest. Information on the quantity of the various ingredients (e.g., from recipes with specific preparation techniques and known ingredients and quantities) is entered into a computer program with adjustments for assumed moisture and fat losses/gains and for nutrient losses due to preparation practices (e.g., heat or exposure to air). This produces a nutrient profile for the food item.

For many processed foods, the ingredients are listed on the product label in descending order of content but their quantity is considered proprietary by the manufacturer and is not available. By using the label ingredient information, along with the Nutrition Facts panel, and the composition data in the SR, it is possible to use a linear programming calculation to estimate the proportion of the various ingredients in a particular food item. The ingredients and their proportions can then be used in the recipe program described in the preceding text to calculate a nutrient profile for the food item.

Types of FCDBs

There are a number of different types of FCDBs/FCTs available. Perhaps, the most common is a national database, which is developed for a specific country, such as the SR developed by the USDA's Nutrient Data Laboratory in Beltsville, Maryland. Release 1 of this database was made available in 1980 and the latest version, Release 27, was issued in 2014. The SR replaces printed tables issued as far back as 1892 and is complemented by an online search program, which enables users to look up the nutrient content of their food items. Development of national and regional FCDBs is recognized in Latin American countries, Europe, Canada, Asia, and Africa, in addition to the United States.

Regional FCDBs/FCTs are developed in areas where a number of small countries exist with similar climates and cuisines. An example is the West African FCT developed by the Food and Agriculture Organization (FAO) and other organizations. FCTs for population-specific groups (American Indians/Alaska Natives in the United States and indigenous peoples in Canada) have arisen to address specific eating practices of these groups and research on the health issues they face.

Special interest databases (SIDs) are often developed by database compilers. In the United States, these were developed for a number of various compounds (Table 1). In other cases, they may be included in appendix tables to an FCT, where values for all the foods in the main database are not available.

SIDs are developed for emerging nutrients of interest to researchers working on various diseases. The data from a number of these SIDs have been expanded, updated, and incorporated into the SR. As can be seen from the earlier table, these databases typically include several hundred food items. After incorporation into the SR, a particular nutrient may be considered appropriate to be included in the Food and Nutrient Database for Dietary Studies (FNDDS), which is used to assess the dietary intake of various nutrients in the US National

Table 1 Special interest databases developed by the USDA

Database	*Year*	*No. of foods*	*Compounds*
Sugars[a]	1987	522	Monosaccharides (galactose, glucose, and fructose), disaccharides (lactose, sucrose, and maltose), other sugars (trioses and tetroses), total sugar
Carotenoids[a]	1998	206	Alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein + zeaxanthin, lycopene
Selenium[a]	1992	506	Selenium
Trans-fatty acids[a]	1989–1993	214	Individual trans-fatty acids
Vitamin D[a]	1999	122	Vitamin D
Vitamin K[a]	1994	194	Vitamin K (phylloquinone)
Fluoride[a]	2005	427	Fluoride
Choline	2008	630	Betaine, glycerophosphocholine, phosphocholine, phosphatidylcholine, sphingomyelin, total choline[a]
Isoflavones	1999	128	Genistein, daidzein, glycitein
	2008	549	
Flavonoids	2003	220	Flavonols, flavones, flavanones, flavan-3-ols, anthocyanidins
	2007	385	
	2011	500	
Expanded flavonoids	2013	2926	Includes data for 29 compounds from isoflavone and flavonoid databases
Proanthocyanidins	2004	205	Monomers through polymers of flavan-3-ols

[a]Now included in the SR.

Health and Nutrition Examination Survey (NHANES). In these cases, values for missing data points are calculated using the procedures described in the preceding text.

There are a number of commercially available FCDBs developed for a variety of purposes. Some of these include databases for use in clinical studies such as the Nutrient Data System for Research developed by the Nutrition Coordinating Center at the University of Minnesota. Others are developed for dietitians to use in counseling their clients or for research and development groups at food companies to use in formulating their products. The National Nutrient Databank Conference has compiled a directory of these, the International Nutrient Databank Directory. Once the purpose of the database is established, parameters for the targeted population, specific foods and components, and strategies for gathering data are defined.

Criteria for FCDB

An FCDB/FCT should be representative for the population of interest. However, it is not possible to have data for the precise food consumed by an individual. In some clinical studies, matching food samples are prepared; one is consumed by the subject and the other is analyzed for its composition. This requires an enormous amount of resources and is not practical in most situations. Therefore, it is necessary to devise a sampling plan that will examine a sufficient number of samples with sufficient dispersion to ensure that any food consumed by an individual will fall within the universe defined by the FCDB/FCT values for the food. The USDA has developed a sampling plan, proportional to size, based on US Census data to identify sampling locations. Food sales data are used to identify specific brands of food items for purchase. The USDA typically samples in 12 locations and endeavors to select those brands that will account for 75–80% of the market. For some foods and/or nutrients where more variability is expected, a larger number of sampling locations might be desirable. In those cases, where one or two brands command a large share of the market for a specific food item, fewer samples may be acceptable.

While it is certainly desirable to include as many foods as possible to reflect the food supply, resource limitations often make this difficult or impossible. One must consider the cost/benefit of adding more foods, particularly if it requires new sampling and analysis. The FCDB/FCT should cover the most commonly consumed foods but must also consider the diversity of the population. Some foods may not be widely consumed across the entire national population but are important sources of various nutrients for certain population subgroups, either age, gender, or ethnically based.

When developing an FCDB/FCT, it is important to use good quality data. The USDA has developed a data quality evaluation system to evaluate analytic data. It looks at five key attributes: (1) sampling plan, (2) sample handling, (3) number of samples, (4) analytic method, and (5) analytic quality control. Each of these attributes is scored and summed to yield a quality index for the nutrient in each food. For an FCDB/FCT compiler to properly evaluate the data, it is critical that this information be included in journal articles and various reports.

One of the most critical pieces of information about a food is its description. Descriptions must be clear and unambiguous. They should include a complete description of the food including processing and preparation. For example, for a frozen food, the description should indicate if it is prepared or unprepared. A canned food should indicate if the entire can contents or just the drained solids were analyzed. If a food was fried, the type of oil used should be indicated. The FAO/INFOODS has prepared Guidelines for Checking Food Composition Data prior to the Publication of a User Table/Database.

Values should be presented in a consistent manner, for example, a given component should always be presented in the same units. In FCDBs/FCTs, data are presented per 100 g,

edible portion. This allows easy conversion to other household measures. Data are also presented on a wet-weight basis. While many analysts report results on a dry-weight basis, foods are consumed as prepared. When combining data from various sources, the developer will encounter a variety of units of measurement. Sometimes, a given component will be provided in milligrams and sometimes in micrograms. For the purposes of comparison with other FCDBs/FCTs, the developer should use the standard units of measurement conventionally used, for example, mg or ppm. The FAO/INFOODS has developed Guidelines for Converting Units. This publication also contains the recommended units for each component.

The source of the data should be clearly identified. If from a study sponsored by the FCDB/FCT developer, the name of the study and when it was conducted should be given. If data are used from a journal article, a full citation should be provided. If values were calculated, codes indicating how the calculations were performed should be developed and used. The USDA has a set of derivation codes that describe how the values were calculated (SR27 documentation). If values were calculated from another food, the identification number for that food should also be supplied. The FAO/INFOODS has prepared Guidelines for Food Matching, Version 1.2, which can be of assistance in this endeavor.

Users often wish to compare data from different databases. To do so, both databases must have clear, unambiguous descriptions. If combining multiple databases, care should be taken to ensure that food item identifiers are unique across the databases. This may require the use of a composite key, where one element identifies the specific database and the other element identifies the food item. The aforementioned comments regarding food description are of particular importance when comparing data across different FCDBs/FCTs.

Certain uses of FCDBs/FCTs, that is, dietary assessment or various clinical studies, require complete datasets with no missing values. The developer should be cognizant of the intended uses of the FCDB/FCT. The USDA maintains a hybrid system, where complete profiles for a selected set of nutrients are provided for all foods used in the What We Eat in America component of the NHANES. Other foods, not used for the NHANES, may contain fewer nutrients, in some cases, basically those found on a Nutrition Facts panel.

While the developer of an FCDB/FCT provides average values for dissemination, users frequently want to see the values behind the means. Some users may want to calculate different means more suitable for their needs. To do this, clear links between the mean values and the individual data points need to be provided.

International Databases

In 1983, international experts on food composition met in Bellagio, Italy. Based on their recommendations, the International Network of Food Data Systems (INFOODS) was formed in 1984. Initially, the INFOODS was established under the auspices of the United Nations University. Today, the FAO of the United Nations serves as the global coordinator. The INFOODS seeks to establish and maintain international standards and guidelines for the generation and compilation of food composition data. A series of regional data centers were established to facilitate interactions between member nations in each region in order to encourage the exchange of data and information on the composition of foods. A list of FCTs and databases was compiled and is available on the INFOODS website. These are organized by the regional data centers and many can be viewed online. A number have searchable databases that allow one to look up the composition of foods in the particular database.

International Guidelines

Books were published in the areas of nomenclature and food descriptions to facilitate data interchange among member countries. Since food composition as an academic discipline is not usually covered in detail, a number of training courses were held in the Netherlands and in other locations. Much of the information contained in these courses has been incorporated into an e-learning course available on the Internet. In recent years, the FAO/INFOODS has assembled a series of guidelines covering Food Matching, Checking Food Composition Data prior to the Publication of a User Table/Database, and Converting Units, Denominators, and Expressions.

The Guidelines for Food Matching grew out of an effort by the FAO to match data used by different coding schemata with that used by the FAOSTAT in order to update food balance sheets prepared by the FAO. It points out the need for accurate food descriptions when comparing data from different FCDBs or when using data from one database to impute values in another. When matching foods, various descriptive factors must be considered. These include processing and preparation, state of the food, color, biodiversity, maturity stage, refuse/edible portion, and fortification/enrichment.

The FAO/INFOODS Guidelines for Converting Units, Denominators and Expressions, Version 1.0, was published in 2012. The objectives of these guidelines are the following: (1) to provide a comprehensive list of conversions for standardization purposes, (2) to make users aware of possible difficulties concerning units and denominators, (3) to motivate users to pay more attention to units and expressions, and (4) to encourage data generators to publish all necessary data in order to be able to perform conversions and thus make their data available for the development of FCTs/FCDBs, in addition to research. While these guidelines include the common metric system conversions, such as micrograms to milligrams, they also include less common ones such as converting data on a dry-weight basis to a fresh-weight basis. Also included is information on handling edible portion coefficients.

Before releasing an FCDB/FCT, there are a number of quality control steps that must be undertaken to ensure a high-quality product. The FAO/INFOODS Guidelines for Checking Food Composition Data prior to the Publication of a User Table/Database was published in 2012 with input from many database developers around the world. Among these checks are making sure that, to the extent possible, all fields are complete. Another check is to make sure that various calculations are completed and correct, such as calculating carbohydrate by difference; calculating energy by multiplying protein, fat, and carbohydrate by the appropriate factors; and calculating

vitamin A from individual carotenoids. Other checks include ensuring values for related nutrients are complete. For example, if individual fatty acid data are available, there should also be values for total saturated fatty acids, total monounsaturated fatty acids, and total polyunsaturated fatty acids. Nutrient values should also be compared from release to release to make sure any changes in the values can be explained. The USDA has built these checks into its Nutrient Databank System and participated in the development of the FAO/INFOODS guidelines.

Future Needs: Nutrients, Foods, Technology, and Information Dissemination

Over the years, the SR has been expanded to include many more foods and nutrients (Table 2). Since 1998, over 2500 food items have been added to the SR. Nutrients have been added after identifying a need for the data by the scientific community and the availability of published data and a validated analytic method to determine the values. New or emerging nutrients of interests are typically first released as an SID (Table 1) and then moved into the SR. The typical SID contains data for several hundred food items, while over 3000 are supplied for use in the FNDDS. Therefore, if a nutrient is to be 'expanded' for use in the FNDDS, values have to be calculated for about 2500–3000 food items.

Many of these are for branded, multicomponent foods. Public–private partnership agreements to compile label and manufacturer data are evolving to allow the submission of current information through data portals. In most cases, the companies will provide data for those nutrients found on the Nutrition Facts panel. However, missing nutrients may need to be calculated through different techniques to populate the database with concentrations of important nutrients, which do not appear of the food label, for example, nutrients needed for the FNDDS. This will be accomplished with an enhanced version of the formulation module described in the preceding text. Other improvements will be made to the USDA's database infrastructure to better handle large amounts of data.

In the future, improvements to the database will enable users to see the values behind the mean values reported in the SR and SID. This will allow users to combine data in a variety of ways, generating data to meet their needs. A gateway is being planned to enable users to access all USDA food composition data, developed by various organizations within the department through a single portal. Potentially, this can be expanded to other federal departments to include their food composition data. No matter where the data originate, it will always be identified so the user can check back to the data's source.

Challenges

In general, it is a monumental task to reflect the food supply of a given country in a single FCDB and demand that data are current, comprehensive (nationally representative), and accurate. Tens of thousands of foods and tens of thousands of food components require systematic guidelines for the inclusion of foods and food components. The frequency with which the food industry reformulates commercially processed and packaged foods may be as often as every 6 months. Commercially processed foods and restaurant foods contribute to roughly half of the foods consumed by the US public, and this is a growing trend around the globe. Ingredient lists are becoming more complex and proprietary and the number of brand names, which represent a single commercially processed food, continues to grow. It is critical in describing a single highly consumed food that the majority of the market for that food is represented in the value and variability estimates (e.g., lot-to-lot and brand-to-brand variability). In addition, analytic methods must be developed for many compounds of emerging scientific interest. For commodity-level foods, capturing estimates of nutrient variability within a food (e.g., cultivar-to-cultivar, soil makeup, and trace elements) is highly dependent on a multitude of factors such as shipping and storage conditions, fertilizer use, and animal feeding practices. This information is not always available and assumptions are often necessary. The need to capture information on biodiversity in foods connects with the emerging concerns on the sustainability of the global food supply as we move into the future of a heavily populated planet. With the global food supply crossing country borders through import and export programs, characteristics on a more global scale – country of origin and related information – are needed. Products change over time and include, for example, stealth reductions in sodium (based on official recommendations) and fortification changes, such as folic acid (1994) in US grain-based products. Therefore, updates to the FCDB must be timely.

Table 2 Nutrients added to the SR since 1998

Release	*Year*	*Nutrient*
SR12	1998	Selenium
SR13	1999	Vitamin D
SR14	2001	Folic acid, food folate, DFE
SR15	2002	Vitamin A (RAE), retinol
SR16	2003	Individual carotenoids (5), vitamin K, alpha-tocopherol, total sugar
SR18	2005	Vitamin E, added, and vitamin B_{12}, added
SR19	2006	Total choline, fluoride
SR20	2007	Expanded total choline
SR22	2009	Revised and expanded vitamin D
SR26	2013	Tocotrienols

This article summarizes the state of FCDBs as they become as specific as tribe-specific foods and as broad as a global network of international databases. Comprehensive, high-quality databases are crucial in our understanding of health–disease relationships, population specific to global nutrition policies, and food production and national and international trade.

See also: Codex Alimentarius; Ethnic Foods; Food and Agriculture Organization of the United Nations; Food Classification and Description; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling.

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Food Environment

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Introduction: Population Determinants of Diet

Diet is a modifiable risk factor for an important number of diseases and conditions, such as cardiovascular diseases, certain types of cancer (e.g., colorectal cancer), hypertension, and diabetes. Underlying these effects are calorie imbalances and direct effects of certain dietary components. A healthy dietary pattern has been shown to reduce the risk of many of these diseases, increase well-being, and prolong life expectancy.

At the same time, trends in the last decades towards unhealthier dietary patterns have made public health practitioners and clinicians interested in understanding the main causes of these changes. The epidemic of obesity has been attributed, at least partially, to shifts in population diets. As stated by Geoffrey Rose in his 1985 paper 'Sick individuals and sick populations,' one can differentiate between two types of causes: the causes of cases and the determinants of incidence rates. The first, in this case, would look at why two individuals in the same population have different dietary patterns and try to discover which individual factors are different between them. The second type of cause would look at two different populations with different dietary patterns or at shifts in the same population over time. The determinants of incidence rates are the ones we need to study if we want to understand why dietary patterns have worsened over time and why some populations have higher rates of obesity or diabetes due to unhealthier dietary patterns. In summary, the determination of diet is a social behavior where availability, accessibility, affordability, and acceptability of different foods play a key role.

Mass influences on population diets include all components of the food system: from production and agriculture policies, processing, and transportation to marketing, retailing, and servicing. These mass influences are different across countries/regions and have changed over time within populations. Therefore, if we were able to understand these social determinants of population diets, we might be able to tackle the decreasing healthfulness of population diets and the associated increases in obesity and other metabolic diseases (especially diabetes). Effective policies to combat unhealthy diets go beyond the individual and need to take a population perspective.

One aspect of the food system that is amenable to policies and more proximal interventions is the local food environment. The places where we buy food and eat can exert great influences over individual dietary behaviors. Considering the large differences in local food environments within the same city, region, or country and the shifting patterns over time in food availability, accessibility, or affordability, local food environments represent a good example of a mass influence on population diets.

Definition of the Local Food Environment

The local food environment can be defined as all aspects of the local environment that influence dietary behaviors. According to Glanz et al., it encompasses three domains: the community food environment (food outlets and their accessibility), the organizational food environment (home, school, work, etc.), and the consumer food environment (availability, affordability, and acceptability). A further elaboration of this model includes macrolevel environment influences, such as societal norms and culture, industry, policies, and other systems related to the local food environment (such as the transportation system) (Figure 1).

The community food environment includes physical food outlets. These are both food retailing (food stores) and food service outlets (restaurants). Most research in this area has focused on different geographic measures that characterize the community food environment: count, count per surface (count/sq. mi. or count/sq. km.), count per population, etc. Moreover, the type and accessibility (both geographic, as in distance and availability of a transportation network, and temporal, as in business opening hours) of these outlets can be of interest on their own. Certain types of food stores are assumed to carry healthier foods (like supermarkets in the United States or fruit and vegetable stores in other locations), and some types of restaurants are assumed to be inherently unhealthy (fast-food restaurants).

The organizational food environment serves specific population groups, including workers (at worksites), students (at schools), and individual families (at home). Other parts of this domain also exist, like churches, nursing homes, and hospitals. Of these, the school food environment has probably received the most attention. Several intervention programs have aimed at improving school meals in many countries.

The consumer food environment includes socially determined influences on diet, such as availability, affordability, and acceptability of healthy foods. All these have been shown to exert influence on dietary behaviors and have been target of several interventions over the last decades. Measurement aspects of these three domains of the local food environment will be described in subsequent articles. While this is beyond the scope of this article, it is of great relevance to consider more upstream and downstream determinants of population diet. The effects of government policies (such as the Farm Bill in the United States and the Common Agricultural Policy (CAP) in the European Union or trade agreements such as NAFTA or the European Union in itself) can have enormous impacts on what type of foods is available and at what price. Industrial and agricultural practices are also of utmost importance, and they are interconnected with government policies. Food culture and other social norms that determine what type of foods is acceptable also steer dietary behaviors with great strength. The interplay between industry pressures,

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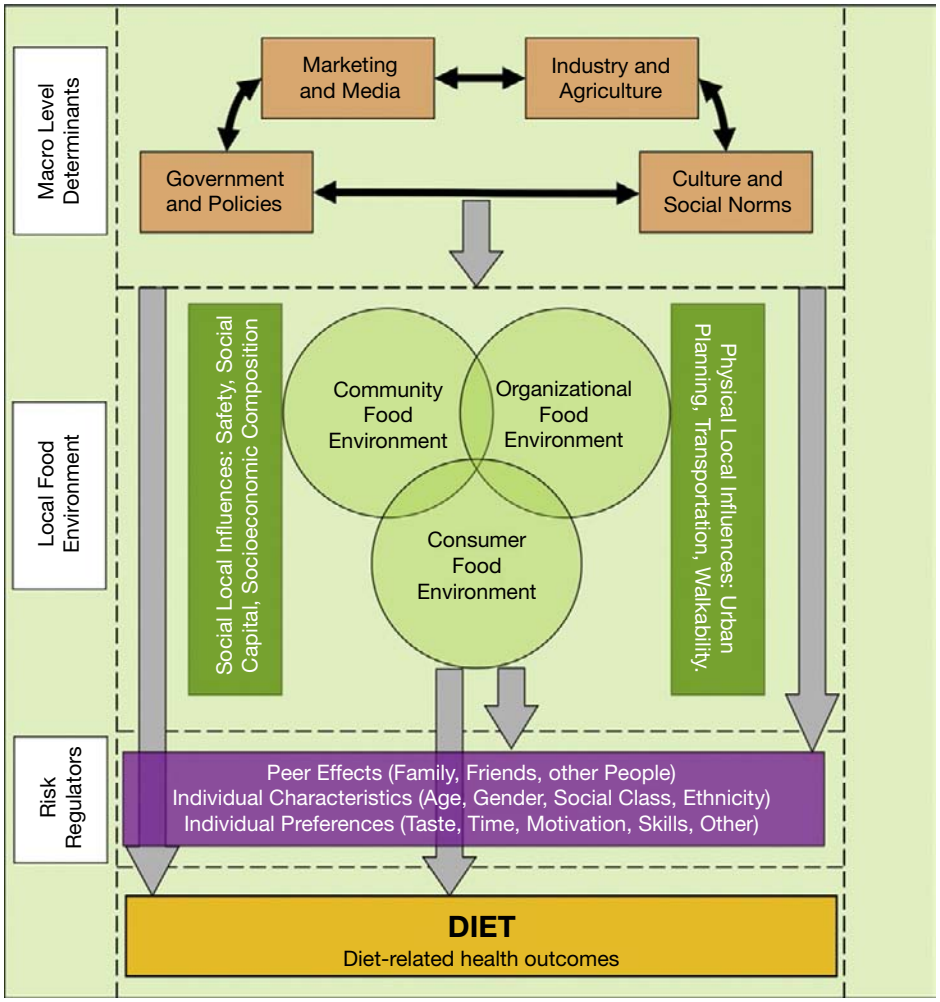

Figure 1 Framework to study the food environment and its effects on diet. Adapted from Glanz, K., Sallis, J. F., Saelens, B. E., Frank, L. D. (2005). Healthy nutrition environments: concepts and measures. *American Journal of Health Promotion* 19(5): 330–333; Story, M., Kaphingst, K. M., Robinson-O'Brien, R., and Glanz, K. (2008). Creating healthy food and eating environments: policy and environmental approaches. *Annual Review of Public Health* 29: 253–272.

government policies, and cultural aspects of food results in the food environments we see today.

Risk regulators of the effects of the food environment are also of great importance. Some of these are determined more upstream from the food environment and have more to do with cultural and societal norms (e.g., gender differences in eating preferences), while others are more proximal, like peer effects that have been shown to be a potent determinant of diet.

In the following headings, we will elaborate on the effects that the food environment has on diet and dietary-related conditions, ways to measure the food environment, and potential interventions upon it.

Food Environment and Eating Patterns

There is a growing interest in understanding the relationship between the local food environment and eating patterns, especially in identifying which aspects of this local food environment are the most determinant. However, this remains challenging due to conceptual and methodological issues (such as defining average dietary patterns or the assessment methods to use) and other complexities (such as dynamism of the food environment or the influence of individuals' characteristics).

Over the last 2 decades, several studies have looked at associations between the food environment and diet, finding associations in both directions, which may arise doubts on the understanding of this relationship. Larson et al. review found a positive association between a better access to a supermarket (or large grocery stores) and a healthier dietary intake. Franco et al. found a positive association between healthy dietary patterns and neighborhood availability of healthy foods. Other studies have found that a greater fast-food access is related to an unhealthier diet. However, most of these studies used cross-sectional study designs.

Three seminal systematic reviews assessed the evidence for the effects of the food environment on dietary intake. These studies showed consistent evidence for existing inequalities in food access in the United States and a greater availability to either healthy or unhealthy food items relating to a healthier or

unhealthier dietary intake. The scientific literature has also highlighted that studies suffer from great methodological limitations, a lack of longitudinal studies, and that there is a need to provide more standardized and validated measures when assessing this relationship between the local food environment and dietary intake.

When assessing diet regarding food environment, research is important to define how to collect dietary information and which appropriate measures are being used. Although there is no standardized measure of the overall diet available yet, some measures, such as the healthy eating index, have been created to describe diet quality. The most common dietary outcome measure is fruit and vegetable intake, due to the vast evidence linking fruit and vegetable consumption with a healthier status. Other dietary outcome measures include fast-food consumption, used to assess the relationship between unhealthy food exposure and a poorer dietary intake. Including multiple dietary outcomes may be the best way to study the food environment–diet relationship.

Influence of the Food Environment on Diet-Related Health Outcomes

Larson et al. and other seminal studies found that low-income, African-American, and rural communities tend to have a poorer food environment than white, urban communities in the United States. The food environment research has aimed then at understanding whether these food environment inequalities may affect health disparities in body weight and other diet-related health outcomes such as diabetes. These studies hypothesized that a higher access to supermarkets should be associated with a lower BMI, while a higher access to convenience stores or fast-food restaurants may be associated to higher BMI and obesity rates.

Different studies have provided mixed results: Supermarkets have been associated mostly with lower BMI indices and lower risk of obesity, and areas with higher density of fast-food restaurants tend to have higher obesity rates. However, most of these studies suffer form design and methodological problems: most were cross-sectional and were difficult to compare due to their variations in the food environment measures and analysis.

Although there are many health outcomes that might be affected by inequalities in the food environment, the most commonly health outcome measured to date is obesity. The evidence in the United States is mixed, which can be explained by the complexity of obesity. Therefore, better study designs and more accurate measures of the food environment are needed.

Measuring the Food Environment

The operational definition and measurement of the local food environment remains a major challenge in food environment research. Three main issues arise regarding the measurement of the local food environment: the conceptualization and definition of relevant features of the local food environment, the spatial scale to be used in the different studies, and the measurement methods to be used.

Following the conceptual model described earlier, adapted from Glanz et al., the three main domains of the food environment to be considered are the community food environment (focusing on food access), the consumer food environment (addressing availably, food quality, and affordability), and the organizational food environment (lunches in workplaces, schools, etc.). Given our focus on the local food environment especially as it relates to food stores or services, and its relation to individual dietary behaviors, we will leave out the organizational food environment. We define the main features of the food environment as food access, food availability, food affordability, and food quality. This terminology can slightly vary within the literature, and previous work has defined dimensions of the food environment research as availability, accessibility, affordability, acceptability, and accommodation. Acceptability refers to people's attitudes, and accommodation would refer to certain food stores' attributes such as opening hours and the type of payment being accepted.

Food availability refers to the actual foods that are available within a food store. Although there is no standardized measure of healthy food availability, the Nutrition Environment Measures Survey has shown great reliability and validity to measure the food availability of different types of healthy food items. However, these in-store audits are rather expensive and resource-consuming. Evidence in the United States has found that high-income neighborhoods do have a greater availability of healthy food choices than low-income areas, and most studies have found disparities in food availability by racial and socioeconomic characteristics. Studies in other countries have shown different results, including inverse gradients (low-income areas with higher healthy food availability) in studies in the United Kingdom. Conclusions about the association between this availability and dietary patterns are challenging due to this large heterogeneity of measurement methods.

Food accessibility measures usually rely on geographic information systems (GIS) and secondary databases, commonly commercial or business datasets (such as InfoUSA or ESRI's Business Analyst). The main limitations are that these data are usually cross-sectional and that there is no standardized categorization to determine the type of food store or restaurant. Food access can be operationalized as proximity to food outlets, density (e.g., count of supermarkets within the geographic boundary), or distance to the closest food store (straight Euclidean distance or network distance that more closely represents real distances for people). Several food store types (such as supermarkets or large grocery stores) have been considered as 'healthy' or 'low-BMI,' whereas others (e.g., convenience stores) have 'unhealthy' or 'high-BMI.' Therefore, food access is commonly considered as a proxy for healthy food availability. However, different studies have shown that the quality and cost of healthy food items do vary by store type, highlighting the needs for direct observations. Moreover, in an international context, the same type of store may carry a widely different balance of healthy and unhealthy stores. For example, studies in Southeast Asia or Central America reveal that supermarkets carry a much unhealthier balance of food compared to autochthonous stores.

An example of the study of food availability can be found in Figure 2. In this case, we have studied healthy food availability by the type of store in two cities in two very different food

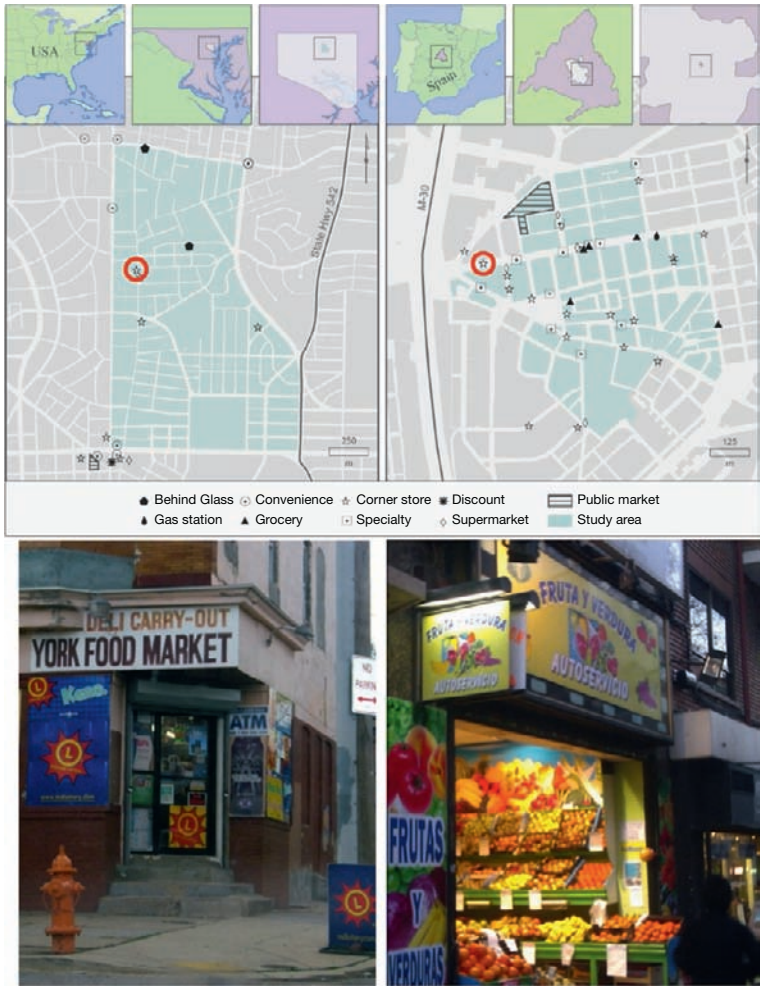

Figure 2 Comparison of two neighborhoods of similar relative socioeconomic status in Baltimore (the United States) and Madrid (Spain). Below, two stores (marked in map) in each neighborhood.

systems (Baltimore, the United States, and Madrid, Spain). As the maps show, the Madrid food environment is richer and has a greater variety of food stores, while several areas of the comparable neighborhood in Baltimore are underserved in terms of food stores. This highlights the intricate interconnections between the food environment and other aspects of the food systems like transportation networks or use of cars, urban form, and urban planning. The bottom part of Figure 2 highlights the two most common types of food stores in Baltimore and Madrid, respectively. The fruit and vegetable store is widely available in Madrid and carries mostly fresh produce, compared with Baltimore's corner stores carrying mostly processed foods.

Food affordability refers to the cost of foods. This can be measured also objectively or subjectively. Some studies use absolute (e.g., food baskets or food price indices of certain food items), whereas others rely on relative measures, such as food cost relative to income and purchasing power. Subjective measures focus on the perceived cost of food items for people. Several studies in the United States suggest that healthy food cost vs. unhealthy food cost varies by geographic areas and that there is no clear pattern regarding whether healthy or unhealthy food options are more expensive. These inconsistent findings may be explained by the large variety of measures being used to assess food affordability.

Food quality measures tend to be more subjective than the other food environment constructs (e.g., degree of bruising on fruits). It is usually measured by rating appearance and freshness of selected food items (commonly fruits and vegetables). Food quality has been found to vary by store type, with convenience or corner stores selling lower-quality foods than grocery stores or supermarkets. High-quality food has also been associated with socioeconomic status. However, this feature remains understudied and might be particularly context-relevant.

Features of the food environment can be measured both objectively and subjectively. Objective assessment methods usually include GIS measures or systematic social observation (e.g., in-store audit tools). Subjective measures, such as perceived food availability and food purchase behaviors, have been also used to deeply understand how residents interact with their local food environment. Additionally, other factors that may influence food-purchasing behaviors (e.g., time, transportation, and food preferences) need to be taken also into account when studying this relationship. A list of these measures is available at the National Institutes of Health National Cancer Institute

website on Measures of the Food Environment: Defining Measures (Instruments and Methodologies).

As a result of this use of different operational definitions and methodologies, comparisons across studies and drawing conclusions about the influence of the food environment on dietary intake or diet-related health outcomes remain a challenge. There is therefore a current need to develop better valid and reliable methods for measuring the local food environment to solve this lack of comparability and inconsistent results.

Interventions in the Local Food Environment

Food environment interventions have become a popular strategy to address the obesity epidemic and other diet-related health outcomes.

Several small-store intervention trials have been conducted in the United States and other countries to improve the food environment and associated dietary behaviors. A recent review by Gittelsohn et al. found that intervention strategies included increasing the availability of healthier foods (particularly produce) and point-of-purchase promotions (shelf labels and posters). Several of the trials reviewed showed significant effects for increased availability of healthy foods, improved sales of healthy foods, and improved consumer knowledge and dietary behaviors. Most effective food environment interventions were those that included actions both at the supply side (increased healthy food options) and at the demand side (included health communication components for both store owners and clients).

Food environment interventions within large grocery stores and supermarkets have been also published. Most interventions included combinations of point-of-purchase promotions and advertising of healthy food choices.

Finally, in order to create long-term, sustainable changes in our local food environments, it is crucial to include several steps in the development of food environment interventions:

1. Development of formative research to gain knowledge on the local food environment and the most appropriate implementation practices.
2. Identification of the local food environment-specific characteristics and selection of intervention domains and components.
3. Interventions should include wholesalers and distributors into multilevel food system change programs.
4. Clear and well-designed evaluation methods should be included in the intervention plans.
5. Engaging policy makers and main stakeholders is key to modify the food environment.
6. Conducting and evaluating pilot interventions.

Conclusions

The determination of diet is a social behavior where availability, accessibility, affordability, and acceptability of different foods play a key role. The places where we buy food and eat can exert great influences over individual dietary behaviors, so that local food environments represent a good example of a mass influence on population diets. Features of the food environment can be measured both objectively and subjectively. There is a current lack of comparability due to a huge heterogeneity in the use of operational definitions and methodologies. More standardized and reliable methods are therefore needed in order to understand the relationship between the food environment and dietary patterns and diet-related health outcomes. Food environment interventions have become a popular strategy to address the obesity epidemic and other diet-related health outcomes.

See also: Convenience Food; Dietary Exposure Assessment; Hypertension and Diet; Mediterranean Diet; Nutritional Epidemiology; Obesity: Epidemiology of; Obesity: The Role of Diet.

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Food Fortification: Rationale and Methods

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Introduction

To grow and flourish, a country requires a strong, healthy, and educated population. A country whose population is undernourished has higher rates of morbidity and mortality, and its human capital is diminished. Globally, undernutrition impedes the development of people and entire nations.

The nutritional status of a population is reflected most dramatically on the height and weight of its children. High rates of stunting (low height for age indicating chronic malnourishment), wasting (low weight for height indicating acute malnourishment), and underweight (low weight for age) among children in the developing world are the typical indicators of undernutrition and the greatest problems to be addressed.

A less visible but equally important measure of nutritional health within a population is the micronutrient status of the people. The consumption of vitamins and minerals is essential for growth, brain development, and immunity against disease. The vitamins and minerals used for fortification typically include vitamins A and D, folic acid and other B-complex vitamins, iodine, iron, and zinc. Among these, five nutrients – vitamin A, iodine, zinc, iron, and folic acid – are critical. When the intake or absorption of essential vitamins and minerals from the diet is inadequate (commonly referred to as 'hidden hunger'), individuals and communities suffer serious consequences, including learning disabilities, impaired work capacity, illness, and death. Although other indicators of global development have shown improvement, progress in reducing micronutrient malnutrition has been slow, leaving over 2 billion people suffering from a range of deficiencies.

Micronutrient deficiencies can be prevented and even eliminated if populations consume adequate quantities of the bioavailable forms of required vitamins and minerals on a continuous and ongoing basis. A number of complementary solutions are available to ensure adequate micronutrient intake. They include vitamin and mineral supplementation, food fortification, promotion of dietary diversification, homestead food production, and public health measures such as immunization and malaria and parasite control.

Food Fortification as Part of a Comprehensive Solution to Micronutrient Malnutrition

According to the *Codex Alimentarius* (a collection of internationally accepted food standards, codes of practice, and guidelines), food fortification or enrichment means the addition of one or more essential nutrients to a food (whether or not it is normally contained in the food) for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups. Food fortification is also permitted when it is necessary to restore nutrients lost during processing, handling, or storage or to maintain the nutritional equivalence of substitute foods (e.g., nondairy milk beverages). It could be a medium- to long-term solution to alleviate specific nutrient deficiencies. The World Bank has recognized that fortification and other interventions to control micronutrient deficiencies offer the largest opportunity to improve lives and accelerate development at such a low cost and in such a short time.

Food fortification involves the identification of commonly eaten foods that are centrally processed so that fortification could be dovetailed into the food production and distribution systems. This also means that fortification within the existing food patterns does not change the dietary practices of the population and so does not require special individual compliance. Staple foods and condiments are the obvious choice for fortification, given their consistent consumption by large sections of the population.

Food fortification is not a new concept. In the developed countries, food fortification policies have evolved in response to public health needs. The addition of nutrients to staple foods was introduced nearly 70 years ago as a means of dealing with micronutrient deficiencies prevalent in those countries where practices that began as short-term voluntary fortification policies later became mandatory. As a result, several nutritional deficiencies have been eliminated, partly because those nations have been fortifying widely consumed foods such as flour, salt, juices, margarine, milk, and sugar for decades. However, in many developing countries, fortification is much less prevalent because the choice of vehicles is limited.

Efforts are also under way to produce staple crops (such as rice, maize, sweet potato, cassava, common beans, and wheat) that are dense in minerals and vitamins through plant breeding and genetic modification – a process referred to as 'biofortification.'

Fortification Vehicles

Common food vehicles that can be fortified include wheat and wheat products, corn, rice, milk and milk products, cooking oils, salt, sugar, breakfast cereals, and condiments. As processed foods gain popularity and market reach in the developing world, they offer new channels for micronutrient delivery. Potential food vehicles can be sorted in a three-tier pyramid (Figure 1).

Staple foods at the base of the pyramid are cost-effective to fortify on a mass scale. Basic foods such as breads and biscuits, packaged cereals and flours, and dairy products are in the middle. Value-added foods such as condiments, snacks, candies, and convenience and ready-to-eat foods are at the top.

Fortifying less expensive staple foods at the base of the pyramid results in broader dissemination of micronutrients throughout the population – particularly to the poor. Also, fortifying foods at the base of the pyramid increases the likelihood of fortifying products through the other tiers of the pyramid, because staple foods are generally used to produce

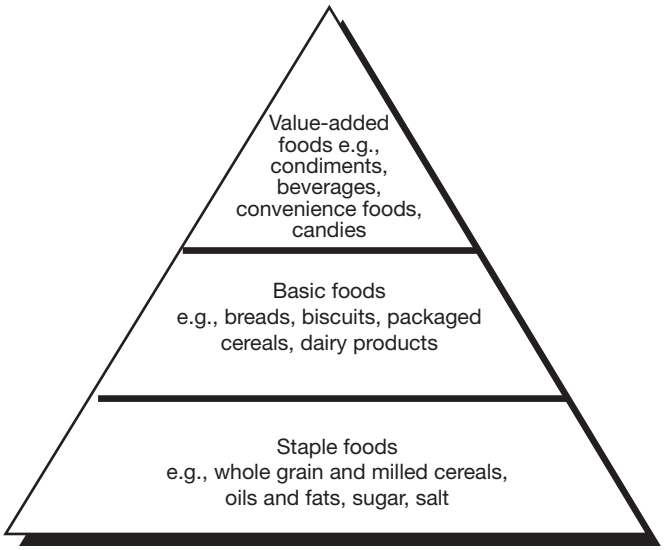

Figure 1 Food product pyramid.

Table 1 Examples of foods for fortification

Food vehicle	*Nutrients*
Salt	Iodine (and iron)
Wheat and maize flours	Iron, folic acid, vitamin A, and B vitamins
Rice	Iron, vitamin A, and B vitamins
Cooking oils and fats	Vitamins A and D
Sugar	Vitamin A
Condiments (sauces)	Iron
Milk	Vitamins A and D, iron
Complementary foods	Multiple vitamins and minerals

basic and value-added foods. Each food vehicle offers specific opportunities and constraints.

Table 1 provides examples of typical food vehicles and nutrients added. A multifaceted approach to fortifying more than one food vehicle is a good strategy, especially when a universally consumed vehicle is not available.

Cereals

Staples such as rice, corn, and wheat that are milled at centralized locations have the potential to reach large populations and are used in several countries as vehicles for multiple nutrients. When staple cereals are milled at the community level, it poses a challenge because of quality and safety constraints. For fortifying whole grain cereals like rice, there is now a technology to produce an extruded rice-shaped premix. Developed countries have long fortified breakfast cereals with a range of vitamins and minerals.

Fats and Oils

Cooking fats and oils offer an option to deliver fat-soluble vitamins such as A and D. While they are often centrally refined and packed, there is still the challenge of a large proportion being sold in loose unbranded form. Packaging in opaque containers is critical to protect vitamins from degradation.

Condiments

Salt, sugar, spices, and sauces are attractive carriers with great potential. They are consumed regularly and some are processed centrally. In Central America and some African countries, sugar has been a successful vehicle for vitamin A. Guatemala's sugar fortification program has virtually eliminated vitamin A deficiency, and big reductions have been seen in El Salvador and Honduras. Recent studies in India show the promise for salt double-fortified with iron and iodine.

Dairy Products

Fortifying milk with vitamins and minerals is an option where milk is processed in dairies.

Value-Added Products

Given global demographic shifts from rural to urban areas, a larger proportion of the population can now be reached via commercially processed foods and value-added products. However, the most vulnerable populations consume these higher-priced products (such as snack foods or beverages) only sporadically.

Type of Fortification Based on Population Reach

There are several types of fortification described in the succeeding text. Their respective suitability is based on the target population and the nature of industry compliance.

Mass Fortification

Foods widely consumed by the general population are fortified, where the population has an unacceptable public health risk of being or becoming deficient in specific micronutrients. Examples include the fortification of cereals, oils, vegetable fats, milk, sugar, and condiments.

Targeted Fortification

Fortification is aimed at specific subgroups of the population, thereby increasing the intake of that particular group, rather than that of the population as a whole. Examples are complementary foods for young children, food rations used in emergency situations, and foods aimed at preschool and school-aged children.

Infants and children under the age of 24 months have a different dietary pattern than adults. While exclusive breastfeeding is universally recommended for children up to the age of 6 months, industrially produced fortified complementary foods are recommended by pediatricians as an essential part of an infant's diet beyond the age of 6 months, complementary to breast milk and homemade foods.

Fortified infant cereals are commonly fed to young children in North America and Europe. Programs that supply fortified complementary foods to infants and young children have also been introduced in several developing countries. Where there are few centrally processed complementary foods, alternative mechanisms include home fortification in which micronutrient mixes in daily dose sachets have been successful.

Market-Driven Fortification

Food manufacturers take a business-oriented approach to fortify some products as value-added products, usually complying with the specifications for permissible nutrients and quantities in order to increase sales and profitability. Typically, such products are purchased by higher-income groups.

Mass and targeted fortification programs would focus on those micronutrients that are in the greatest need by the general population. Cost becomes a factor and it is important to have the collaboration of different stakeholders, particularly those in the government and the milling industries.

On the other hand, market-driven fortification might lean toward those micronutrients that have the greatest recognition and appeal to the intended market – usually the higher socioeconomic group that would purchase the premium products. Cost is generally not an issue in this situation.

Mandatory Versus Voluntary Fortification

Within a country, food fortification can be mandatory or voluntary. Mandatory fortification stipulates that certain foods be fortified with specified nutrients and levels. With voluntary fortification, the addition of micronutrients by food processors is permitted for certain foods following specified standards set by the government. Examples are the fortification of breakfast cereals and addition of calcium to orange juice in industrialized countries and oil fortification or soy/fish sauce in some developing countries. Mass fortification is usually mandatory, though not always. Examples are flour fortification and salt iodization. Targeted fortification can be either mandatory or voluntary, whereas market-driven fortification is voluntary.

Essentials of Food Fortification

The actual mechanics of adding the nutrients to the food are technically simple. The technology of food fortification also considers cost, bioavailability, sensory acceptability, and storage stability. In planning a food fortification program, there are essential technical considerations:

- The food used as a vehicle should be consumed in fairly constant amounts so that fortification levels can be accurately calculated. To do this, it is important to understand the consumption patterns of the food vehicle across the country. If it is for targeted fortification, the consumption pattern of the specific target group is needed.
- The chosen food should be processed centrally in large enough units to permit controlled fortification.
- The addition of the micronutrients should be compatible with the existing food production and distribution systems. There should be minimum loss of the nutrient during processing, storage, and final preparation of the food.
- There should be no change in taste, appearance, or color of the final product.
- The food should be affordable so that low-income groups, who are more vulnerable to malnutrition, consume it.

Depending on the food processing methods, adding micronutrients can be facilitated using different approaches to maximize the nutrient retention:

- Dry mixing – for cereal flours, powdered milk, and powdered beverages. This is one of the common mechanisms used for the addition of the nutrient premix to the food.
- Dissolution in water – for liquid milk, drinks, and fruit juices and for making bread, pasta, and cookies.
- Dissolution in oil – for the lipid-soluble vitamins to enrich oily products like margarine.
- Spraying – as in iodization of salt and in cornflakes where the nutrients do not withstand the cooking or extrusion step.
- Adhesion – as in sugar fortification where the vitamin A in powder form is adhered to the surface of the sugar crystal by vegetable oil.
- Coating – as in rice where the vitamins are sprayed over the grain as a coating.
- Extrusion and dry mixing – simulated rice-shaped premix of micronutrients produced through an extrusion process and dry-mixed with regular rice.
- Encapsulation – as in double fortification of salt where an iron compound is encapsulated to prevent interaction with iodine and the food matrix.
- Micronization – as in salt fortification where the particle size of an iron compound is reduced in order to increase its bioavailability.

Figure 2 depicts a simplified framework of how fortification of cereal flour takes place.

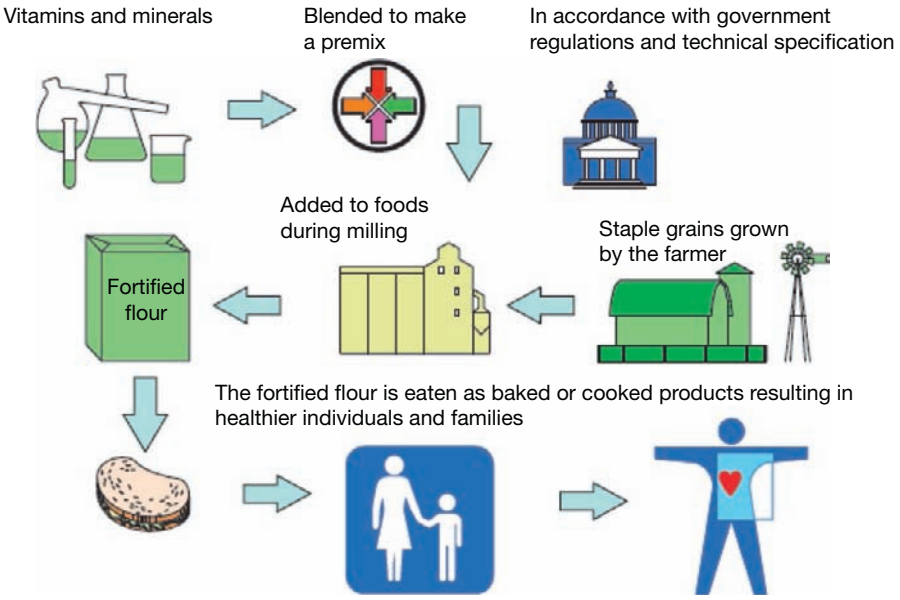

Figure 2 Process for fortifying cereal flour.

Table 2 WHO recommendations for average micronutrient fortification levels

			Fortification level (ppm) according to flour consumption (g day^{-1})			
Micronutrient	*Flour extraction rate*	*Compound*	*<75*	*75–149*	*150–300*	*>300*
Iron	Low	NaFeEDTA	40	40	20	15
		Ferrous sulfate	60	60	30	20
		Ferrous fumarate	60	60	30	20
		Electrolytic iron	NR	NR	60	40
	High	NaFeEDTA	40	40	20	15
Folic acid	Low or high	Folic acid	5.0	2.6	1.3	1.0
Vitamin B12	Low or high	Cyanocobalamin	0.04	0.02	0.01	0.008
Vitamin A	Low or high	Vitamin A palmitate	5.9	3	1.5	1
Zinc	Low	Zinc oxide	95	55	40	30
	High	Zinc oxide	100	100	80	70

Choice of Nutrients' Chemical Form

The chemical form of the nutrients being added (fortificants) influences the bioavailability, chemical stability, appearance, and homogeneity of the fortified food product. The choice depends in part on its solubility in the gastric juice and on its impact on sensory characteristics of the food itself. Table 2 presents the current World Health Organization recommendations for average micronutrient fortification levels based on flour extraction rates, type of fortificant, and estimated average per capita flour consumption.

The iron compounds used for fortification may be classified as follows: soluble in water, slightly soluble in water and highly soluble in dilute acid as in gastric juice, insoluble in water and poorly soluble in dilute acid, and chelated iron compounds. Highly water-soluble compounds like ferrous sulfate have excellent bioavailability and are suitable for a variety of applications but could create problems of unacceptable taste and color in the presence of high moisture and lipid contents. In such situations, less soluble forms of iron are chosen to ensure consumer acceptance. Due to its high bioavailability, use of sodium iron EDTA (chelated iron) is recognized more recently as the fortificant of choice for soy sauce, fish sauce, and whole-wheat/maize flours.

Research and development efforts have enhanced the effectiveness of fortification technology. In the case of vitamin A, work is ongoing to reduce loss of nutrient potency in storage through the addition of antioxidants and other stabilizers. Better refining procedures and packaging have significantly improved the stability of iodine compounds in salt and vitamin A in cooking oils. In the case of iron, stabilizers and absorption enhancers could be added along with the fortificant to retain it in an absorbable form or improve absorption. The structure of the iron compounds can also be modified to improve absorption.

Food Fortification Policy and Implementation

When a country or region is ready to implement food fortification, the process begins by identifying the commonly eaten foods that can act as vehicles for one or more micronutrients.

Food fortification aims to provide meaningful levels of the nutrient (usually 30–50% of the daily adult requirements) at normal consumption of the food vehicle. The levels also need to take into account variations in food consumption so that the safety of those at the higher end of the scale and impact on those at the lower end are ensured. Cost, bioavailability, sensory acceptability, and storage stability are some of the criteria that determine the best match between the nutrient and food vehicle.

The addition of vitamins and minerals to foods is typically regulated under food and drug laws that list nutrients that can be added to foods and specify food vehicles. The maximum and minimum amounts at which the specific nutrients can be added to each food are also specified.

Economics

The benefits of fortification can extend over people's entire life cycle. The economic benefits of food fortification include reduced morbidity, improved work capacity and cognitive ability, and, by preventing malnutrition, ultimately reduced health-care costs. Fortification leads to a healthier population and children will have a stronger ability to enhance academic performance. It can thus be one of the most cost-effective means of overcoming micronutrient malnutrition. The economics of food fortification has played an important role in its implementation in public policy.

The start-up cost for food fortification is relatively inexpensive for the food industry, and recurrent costs are rapidly passed on to the consumer. For example, adding iodine to salt costs between 2 and 7 cents per kg, which is $<5\%$ of the retail process of salt in most countries. The cost of fortifying a kilogram of flour with iron, folic acid, and other B vitamins varies between 0.2 and 0.3 cents. Even though absolute costs are low, the incremental cost could sometimes pose challenges. Premix is the single greatest expense in fortification programs, accounting for 70–90% of recurring fortification costs. In higher-income industrialized countries, such costs are generally absorbed into the consumer price of the fortified food.

However, this is not always the case for lower-income countries. Millers and food processors operate on thin profit margins in risky business environments, and poor consumers are highly price-sensitive and cannot necessarily absorb the additional costs of premix. As a result, in many instances, premix costs can be a serious deterrent to private sector involvement and compliance in fortification programs. Programs would need to maximize the impact of food fortification and make trade-offs as to which micronutrients to include, given the need for the industry to absorb these costs.

Standards, Regulations, and Quality Assurance

For governments to undertake effective food fortification activities, they must first ensure that laws and regulations provide legal authority and an adequate regulatory framework. Effective regulatory controls through established fortification standards protect consumers against the risk of purchasing and consuming nutritionally inadequate, deceptively mislabeled or misbranded, impure or unsafe foods. Regulations also create a level playing field for food manufacturers and sellers.

The quality assurance program includes controls for raw material quality, good manufacturing practices for production and packaging, and quality testing of the final product. Documentation and training are important components. Quality assurance and control systems should also be prioritized, as they provide ongoing assessment that food is being adequately fortified. Furthermore, regular assessment of a program's performance through the collection and dissemination of quality assurance and control data enables program managers to identify specific manufacturers who may require further support.

Partnerships for Sustainable Food Fortification

Through taking part in voluntary and mandatory food fortifications, food companies have played a historic role in protecting the people of the world's rich nations from the devastating effect of vitamin and mineral deficiencies. Effective and sustainable fortification will be possible only if the public sector (with a mandate and responsibility to improve the health of the population), the private sector (with experience and expertise in food production and marketing), and the social sector (with grassroots contact with the consumer) collaborate to develop, produce, and promote fortified foods.

Food fortification is perceived by the public and private sectors from slightly different angles. In the public sector, the addition of essential nutrients to foods is widely accepted because it is considered one of the cost-effective solutions to the challenge of nutritional deficiency among large segments of the population. However, for the private sector, there has to be a business case for food fortification, either because it is required by law or because it can be positioned as a value-added product. While the public sector is set up to work primarily for the public good, the private sector is not established for purposes of benevolence. Though public–private partnerships are conceptually appealing, it is important to recognize and respect the realities of the fundamental difference between the two sectors.

Consumer Awareness

Even when there is little or no cost differential between a fortified food and unfortified food, consumer awareness is an important issue for the acceptance of a newly fortified product. Consumers must be made aware of the benefits of fortified foods through a credible source. Effective communication to consumers is an essential but often overlooked part of effective fortification interventions. By demanding nutrient-fortified foods, consumers empower themselves to achieve their full social, physiological, and economic potential.

Monitoring and Enforcement

As food fortification programs expand around the world, there will be an increasing need to monitor the quality of the fortified food (in terms of its micronutrient content) and evaluate

its contribution to the alleviation of micronutrient deficiencies. Monitoring activities at various levels in the distribution chain – from production or importation to wholesale, retail, and household levels – should ensure that a fortified product contains a sufficient amount of the micronutrient and that it reaches the target population. Availability of data on anemia and nutritional deficiencies to policy makers is critical in advocating for a national fortification project.

As an indicator of effectiveness, a biological impact evaluation will determine whether the micronutrients are being well absorbed and are reducing the prevalence of deficiencies in the population. During evaluations, problems not identified in the initial stages may be identified.

For example, it is well recognized that folic acid fortification has profoundly improved the nutritional status of women of childbearing age and has a substantial effect on the original target by reducing neural tube defects. However, a need for protocols to evaluate large sections of the total population has been identified. The possibility remains that certain segments of the exposed population, such as those at risk of B12 deficiency, may benefit less and may even experience some adverse effects from an increase in folic acid intakes. (To avoid this potential problem and to improve the effect of folic acid fortification, it is recommended that B12 also be added along with folic acid during population-wide fortification.)

Global Experience with Food Fortification

Food fortification has played a major role in the health of the populations in several developed and developing countries over the last 70 years, and many nutritional deficiencies have been eliminated. Multiple studies have demonstrated the effectiveness of food fortification in eliminating micronutrient deficiencies.

The current low levels of iron deficiency in the United States are attributable to fortified sources. Almost one-quarter of iron intake in the US diet comes from fortified sources, especially flour products. In Canada, flour fortification with B vitamins began in Newfoundland in 1944. Within 4 years, deficiencies that were earlier found in nearly 20% of the population had dropped to negligible levels. After 1998, following the introduction of mandatory folic acid fortification of cereal-grain products in the United States, Canada, and Chile, there has been a 30–70% reduction of neural tube defects in newborn.

Over the past 20 years in the developing world, the fortification of a wide range of foods has grown rapidly. Adding iodine at an annual cost of no more than 10 cents per person has been especially successful. Since 2006, a significant population in more than 110 countries has access to iodized salt and nearly two-thirds of the salt consumed in the developing world is being iodized. This is protecting nearly 70 million newborn each year from the threat of mental impairment caused by iodine deficiency, reducing the incidence of goiter and iodine deficiency disorders and contributing to national productivity.

As of October 2013, globally, 78 countries have legislation to mandate the fortification of at least one major cereal grain. Of these, 77 countries fortify wheat flour, 12 countries fortify maize products, and 5 countries fortify rice. All 77 mandatory countries fortify wheat flour with at least iron and folic acid except Australia (which does not include iron) and Venezuela, the United Kingdom, and the Philippines (which do not include folic acid). Approximately 31% of the world's industrially milled wheat flour is fortified with at least iron or folic acid through these mandatory and voluntary efforts (Figure 3).

Building on the success of salt iodization, double fortification of salt with iodine and iron is gaining ground and can be integrated with established iodization processes. Double-fortified salt is currently being produced in India and has the potential to be distributed through commercial channels and

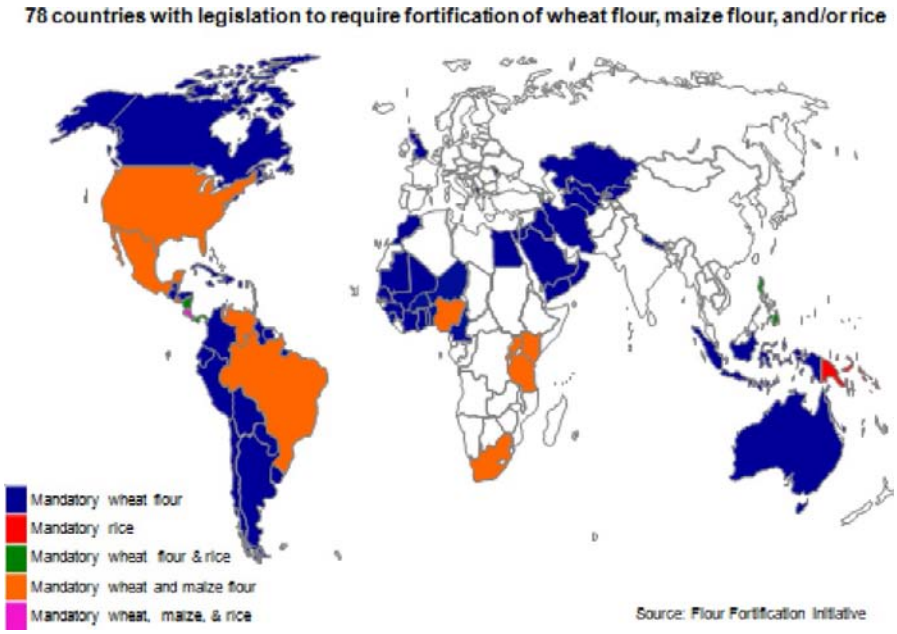

Figure 3 Countries with legislation requiring the fortification of wheat flour, maize flour, and/or rice.

public programs to reach economically weaker sections of the population in many countries.

Case Study: Folic Acid Fortification of Wheat Flour

By the early 1990s, there was convincing evidence that the daily provision of 400 mcg of folic acid to women at least 1 month prior to conception and in the early months of pregnancy helped prevent the birth of children with neural tube defects.

Several international studies called for public health measures to ensure that the diet of all women of childbearing age has an adequate amount of folic acid. The civic and public sectors led by the March of Dimes and US Centers for Disease Control and Prevention advocated for change in the US standard for enriched flour to include folic acid. In March 1966, the United States amended legislation to require folic acid, with full implementation by January 1998. Canada also made it mandatory shortly thereafter.

Following advocacy by several international agencies, including the Micronutrient Initiative, more than 60 countries have mandated the addition of folic acid to flour. Several evaluations in Canada and the United States have shown significant reduction in neural tube defects (NTDs) following flour fortification. The incidence of neural tube defects after fortification has fallen by 30–70% in countries where flour is fortified with folic acid.

Key features of successful fortification programs include the following:

- Strong political commitment and enforcement of regulations
- Early private sector involvement and willingness to comply with regulations
- Public sector backing, with endorsement of medical organizations
- Financial support by donors
- Active consumer education to raise awareness and promote demand

Ultimately, the long-term sustainability of fortification programs is ensured when consumers are willing and able to bear the additional cost of fortified foods.

Issues and Challenges

Notwithstanding the considerable progress in food fortification over the past decade, there are major challenges to ensure that undernourished people, especially in developing countries, receive meaningful amounts of micronutrients through improved access to fortified foods. For example, although significant success has been achieved with salt iodization, in many countries, the groups without access to iodized salt are those most vulnerable and in greatest need for protection against iodine deficiency. While the relatively easier task of getting the large- and medium-scale units to comply has been achieved, compliance by small- and some medium-scale salt producers continues to pose challenges. Thus, the strategies used to achieve 70% coverage of iodized salt globally will not necessarily result in addressing the challenge for the remaining 30% of the population.

The sustainability of fortification programs is complicated by the fact that national programs consist of numerous components, including the preliminary assessment of micronutrient deficiencies, development of fortification standards and legislation, procurement of fortification equipment by food manufacturers, implementation of communication and social marketing activities, execution of quality assurance and control systems, and assessment of health impact. Each component requires input and consensus from the government ministries, standards bureaus, industry partners, civil society, and international agencies that provide funding and technical support.

Within a national fortification project, challenges related to legislation, quality assurance and control systems, social marketing, and monitoring and evaluation can be addressed with modifications to project implementation.

Finally, it is imperative that local ownership and a plan for sustainability be built into each program, so that fortification efforts can be properly implemented and sustained. National mechanisms to reduce the cost of key inputs, such as the premix revolving fund in Uzbekistan, provide an example of how domestic financing can facilitate the adoption of fortification by numerous manufacturers. Funding and technical support from international development and public health agencies can help governments accelerate the expansion of their national fortification programs. Specifically, funding to purchase fortification equipment and to temporarily subsidize micronutrient premix is a key incentive to facilitate the adoption of fortification by food manufacturers.

The time needed for a fortification intervention to become effective in developing countries is likely to be much longer than in developed countries. Such vehicles as salt and flour are often processed in a large number of widely dispersed cottage-scale industries that are not professionally managed.

As food fortification policies evolve, the goal is to achieve a balance between addressing nutrient needs and preventing excessive nutrient intakes. The challenge is that food fortification levels are also influenced by pressures to harmonize national standards with other regulatory jurisdictions.

In addition to these programmatic challenges, there are differences in perceptions about fortification. While it is well established that food fortification has positive impacts on a population's health and well-being – easily outweighing any potential risk – historically, there has been public opposition (in some countries) to the addition of a foreign substance to food or water. It is important to understand different viewpoints but equally important to move forward, in a responsible way, with what is most beneficial to a large number of people whose lives would otherwise be compromised without essential vitamins and minerals in their diet.

The Way Forward for Food Fortification

A well-planned food fortification program can provide meaningful quantities of essential micronutrients to large populations on a permanent, cost-effective, and self-sustaining basis. Food fortification efforts must be integrated within the context of a country's public health and nutrition situation and be a clearly defined component of an overall micronutrient strategy that uses a combination of interventions to address key deficiencies. Fortification levels must meet meaningful proportions of nutrient needs without the risk of excessive intakes. It must be recognized that national fortification projects take time to establish and require ongoing support to respond to specific performance issues and broader shifts in market

structure and consumption patterns. From a management perspective, the long-term success of fortification projects requires that they must actively and continually be improved upon and modified in order to remain effective and relevant.

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Folic acid and Folates: Physiology and Health Effects; Iodine: Iodine Deficiency Disorders (IDD); Iodine: Physiology; Iron: Physiology of Iron; Malnutrition: Concept, Classification and Magnitude; Malnutrition: Prevention and Management; Nutritional Epidemiology; Pregnancy: Dietary Guidance for Pregnancy; Pregnancy: Metabolic Adaptations and Nutritional Requirements; Quality Control in Food Processing; Vitamins: Overview; World Health Organization.

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http://www.who.int/en – World Health Organization.

Food Fraud

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Definitions

No universal official definition exists for food fraud. In Table 1, the working definitions for food fraud proposed by different bodies and authorities over the last decade are quoted. The definitions for food adulteration or, better, economically motivated adulteration (EMA) are also given in the same table. Under this general term, intentional practices such as dilution, substitution, contamination, artificial enhancement, counterfeit, transshipment or origin masking, mislabeling theft, and resale of products are covered. The frequency of detection of such events varies dramatically among them (Figure 1) indicating that combating fraud needs international collaboration as it is repeatedly stressed in international fora by leading members of the Food Fraud Think Tank.

History and Occurrence

Food fraud has been widely evidenced in the everyday life of human communities over the millennia. Combat of food fraud and related punishments is the result of political decisions that reflect the character of each regime and is documented in written texts and ethical rules for what is fair trade and transaction. Mesopotamian, Hebrew, Egyptian, Greek, and Roman authorities had provisions on good practices, inspectors for the integrity of precious commodities or goods (grains, wine, etc.), and penalties and punishments for those that offended the public interest and often jeopardized the health of citizens. Rigorous were the punishments in the feudal system in medieval Europe according to many treatises on food adulteration. Islamic laws have been very specific for *haraam* transactions, food fraud and adulteration included. Similar was the case in the vast Chinese sovereignty over the centuries. The move from agricultural to industrial economies in Europe and the United States (eighteenth and nineteenth centuries) resulted in urbanization and the disengagement of people from food production for family needs. Adulteration of basic goods (bread, fats, wine, and honey) or precious goods (spices) for profit was, thus, steadily increased and supported by the advance in scientific knowledge on the composition of foods and properties of various chemicals. Indeed, food sophistication and its detection prevailed in parallel in those centuries. Thomas Wakley, Arthur Hill Hassall, and Harvey Washington Wiley are the personalities that played a crucial role in the endorsement of food regulations in Britain and the United States in the late nineteenth and early twentieth centuries. They fought against food adulteration using scientific means, by raising the public indignation against it, and succeeded in passing laws that considered the fraudulent food sales as criminal offense. The two great wars, the intermediate period of economic crisis and the subsequent division of countries into blocks of different ideologies and interests, delayed the harmonization of food law on a global scale. It was only after 1980 that the discussions for international standards were accelerated with emphasis to food safety and hygiene rules. Endorsement of new standards for food composition and food additives became a lesser priority as novel issues such as the advance of new technologies for food preservation (e.g., irradiation), food production through genetic modifications of organisms and biotechnology, cloning, and, more recently, food applications of nanotechnology captured the concern of both the society and authorities on health implications for generations to come. Currently, incidents in the global market are of growing concern as it is recognized that there is an inherent weakness within the food chain that allows the involvement of criminal organizations in food fraud, whereas the capacity of authorities is limited and action is rather taken *a posteriori* than *a priori*. Austerity programs even in developed countries, overpopulation, and continuous immigration of a large number of people form the background scenery. The two most recent events, the melamine and the horse meat scandals in China and the European Union, respectively, revealed the vulnerability of the food sector to fraud and the need to undertake strict measures. The Poison Squad initiative (1902) inspired the flying squad of the Danish Food Administration practice a century after (2006) to contest effectively illegal practices. The scientific community together with the international authorities takes advantage of informatics to organize databases with evidence for fraud practices and methods of analyses and prioritize foods for which more attention should be paid. Records in these databases may differ significantly depending on their source (e.g., scholar or mass media ones). Scholar references include mainly in-house adulteration experiments, carried out by the scientists to develop tools for food authentication. Real cases of adulterated products analyzed after the event is spotted by the authorities are also presented. The mass media records mostly cover what comes to their attention from the local or international authorities and are often misinterpretations of scientific information. All in all, latest tables based on these databases indicate that the most at risk of fraud products are olive oil, fish, organic foods, milk and dairy products, grains, honey and maple syrup, coffee, tea, spices (saffron, chili powder, and black pepper), wine, and certain fruit juices. Figure 2 illustrates events recorded in the United States and the rest of the world. Most of these categories of food products can be found in the early reports by F. Accum and A.H. Hassall, though the adulterants differ considerably.

Cases of EMA: Health Implications in the Twenty-First Century

Some cases with serious economic and health implications are briefly discussed in the succeeding text in alphabetical order. The means of detection are also stressed. The information is

Table 1 Definitions/content of 'food fraud' and related issues

Term	*Definition*	*Source*
Adulterated food	A legal term meaning that a food product fails to meet federal or state standards. Adulteration usually refers to noncompliance with health or safety standards. The act does not define or use the term 'economic adulteration' or 'economically motivated adulteration'	1
Economically motivated adulteration (EMA)	The fraudulent, intentional substitution or addition of a substance in a product for the purpose of increasing the apparent value of the product or reducing the cost of its production for economic gain	2
Intentional adulteration	May take several forms, including (1) acts of terrorism; (2) acts of disgruntled employees, consumers, or competitors; and (3) economically motivated adulteration not addressed by traditional Hazard Analysis and Critical Controls Point (HACCP) or other food safety systems, and likely will require different kinds of controls. The Food and Drug Administration (FDA) is taking the action described in this proposed rule to implement the intentional adulteration provisions in Sections 103, 105, and 106 of FSMA	3
Food fraud	A collective term used to encompass the deliberate and intentional substitution, addition, tampering, or misrepresentation of food, food ingredients, or food packaging, or false or misleading statements made about a product, for economic gain. Food fraud is a broader term than either the EMA defined by the FDA or the more specific general concept of food counterfeiting. Food fraud may not include 'adulteration' or 'misbranding,' as defined in the Food, Drug, and Cosmetic Act (FD&C Act), when it involves acts such as tax avoidance and smuggling	4
EMA	The fraudulent addition of nonauthentic substances or removal or replacement of authentic substances without the purchaser's knowledge for economic gain to the seller	5
Food fraud	Unlike the United States, the EU has no generally acknowledged definition of food fraud, the current EU legislative framework being largely focused on food safety. The only general guideline can be found in Regulation 178/2002 on the general principles and requirements of food law, which states that the labeling, advertising, presentation, and packaging 'shall not mislead consumers,' although in practical terms, the application of this provision varies largely among member states, and the number of controls in this area is extremely limited. As a result, food fraud remains largely undetected, especially when there are no public health or food safety implications. It is therefore difficult to determine the current scope of food fraud in the EU, although most parties contributing to this report indicate that it seems to be on the rise	6
Food fraud	Food fraud is committed when food is deliberately placed on the market, for financial gain, with the intention of deceiving the consumer. Although there are many kinds of food fraud, the two main types are the following: ● The sale of food that is unfit and potentially harmful, such as – recycling of animal by-products back into the food chain, – packing and selling of beef and poultry with an unknown origin, – knowingly selling goods that are past their 'use by' date. ● The deliberate misdescription of food, such as – products substituted with a cheaper alternative, for example, farmed salmon sold as wild and basmati rice adulterated with cheaper varieties, – making false statements about the source of ingredients, that is, their geographic, plant, or animal origin. Food fraud may also involve the sale of meat from animals that have been stolen and/or illegally slaughtered and wild game animals like deer that may have been poached	7
Food fraud	The deliberate placing on the market, for financial gain, foods that are falsely described or otherwise intended to deceive the consumer	8

Sources: 1, US Code §342; 2, Food and Drug Administration (FDA) 2009 & US Government Accounting Office (GAO) 2011; 3, FDA/Food Safety Modernization Act (FSMA); 4, Spink, J. and Moyer, D. C. (2011). *Journal of Food Science* **76**, 157–163; 5, US Pharmacopeia (USP) Expert Panel on Food Ingredient Intentional Adulterants; 6, European Parliament's Committee on the Environment, Public Health and Food Safety (2013/2091(INI); 7, Food Standards Agency (FSA), UK; 8, Europol and Interpol.

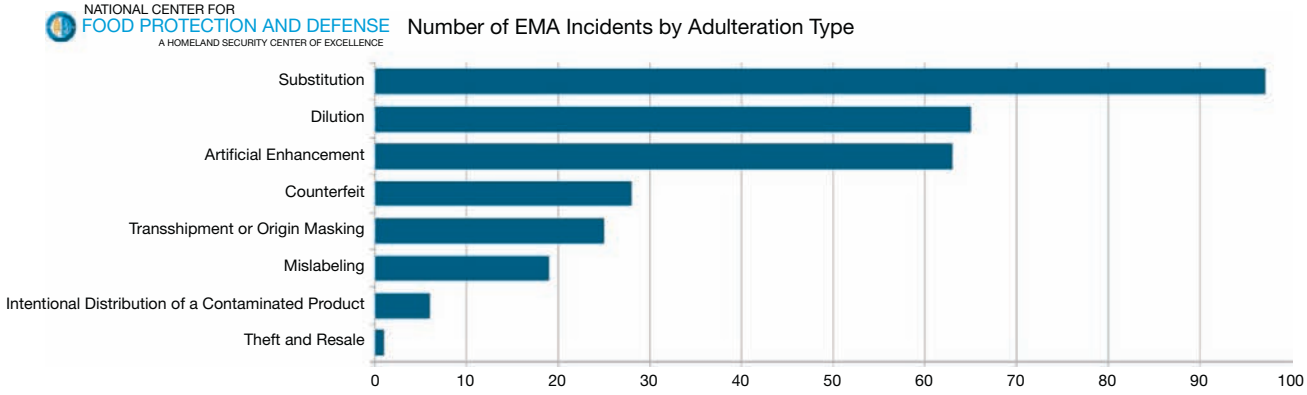

Figure 1 Number of EMA incidents by adulteration type. Courtesy: NCFPD EMA Incidents Database; http://www.foodfraudresources.com/tools.

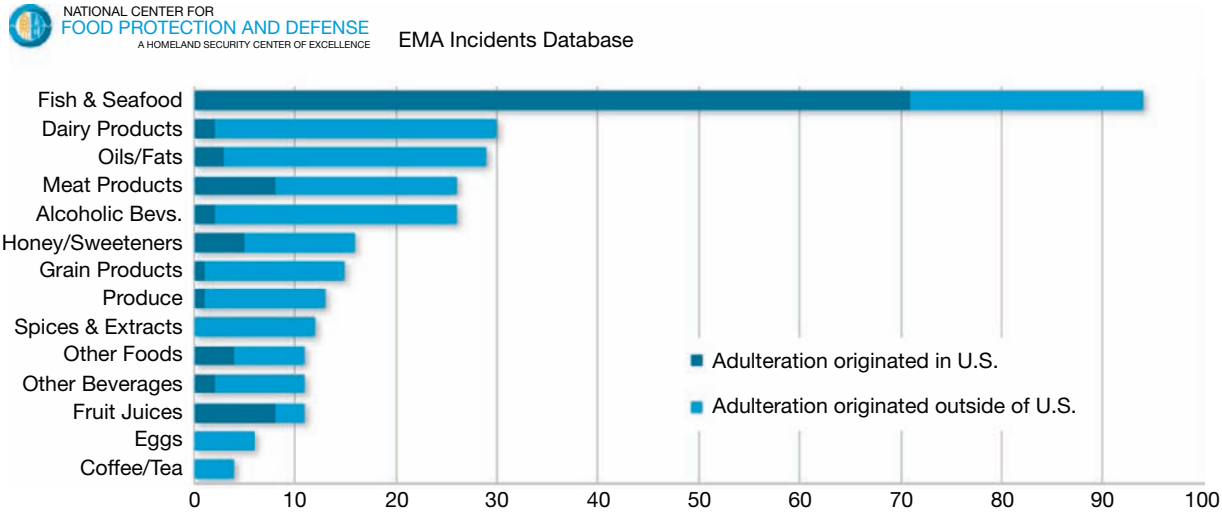

Figure 2 Frequency of EMA incidents in the United States and outside of the United States. Courtesy: NCFPD EMA Incidents Database; http://www.foodfraudresources.com/tools.

mainly obtained from food defense publications, project reports, presentations, and media reports available in databases that are web-accessible. The financial crisis, rising of food prices and consequent demand for cheap products, lack of focus of authorities to detect fraud, and lack of heavy penalties to fraudsters are the driving forces behind all of them.

Alcoholic Beverages

Methanol is the usual alcohol volume-increasing adulterant in such type of beverages. Between 2008 and 2011, bootleg liquor consumption in India led to hundreds of deaths. Cheap cough medicines, methanol, or even ammonium nitrate, high doses of which are quite toxic for humans, were among the commonest adulterants reported. In 2011, the UK Revenue and Customs officials seized 12 000 l of adulterated vodka that contained methanol. In 2012, 24 people died in the Czech Republic and Poland after consumption of liquor adulterated with methanol. The perpetrators and source of the adulteration are still unknown. Other incidents involve the addition of chemical substances with similar to alcohol symptoms. For instance, since 2012, adulteration of brand name alcohols with pills containing antiretroviral drugs (ARVs) and formaldehyde is under investigation in Kenya. ARVs are freely available and used to treat people infected with Human Immunodeficiency Virus (HIV), causing symptoms like those of a hangover. The material is cheap (about $26/60 pills) and used to produce alcohol, which is then sold for about $100. The alcohol is put in bottles bearing stolen labels.

Dairy Products

Besides the easily detected dilution of milk with water, the major adulterant type of the last decade became the addition of exogenous nitrogen source to cause a false-positive increase in the total protein content, which is usually extrapolated from its content using the Kjeldahl or Dumas methods. In 2005, a food poisoning scandal was revealed when dairy products from a Chinese company were found to contain hydrolyzed protein, which is produced during the leather softening process. Hydroxyproline, a hydrolysis product of animal collagen, was the key marker for the detection of this type of fraud. Two years later, the well-known melamine scandal emerged. Melamine cannot be metabolized in mammalian bodies and has resulted in sickness and deaths of human infants and animals in the last years. For instance, in 2007, more than 300 000 Chinese babies were hospitalized, and six died due to acute kidney injury, nephrolithiasis, and urolithiasis. All of them were found with distal tubular necrosis and intratubular crystals of melamine; some of them also suffered from chronic tubulointerstitial nephritis. All these symptoms were associated with the accumulation of melamine–uric acid or melamine–cyanuric acid crystals in the kidneys. During 2003–04, the fraudulent addition of sugar and starch in powdered milk, intended for use in infant formulas, caused the death of at least 13 infants in China. The formula was sold under multiple brand names so it was difficult to identify the counterfeit. Despite the detection of this type of adulteration, withdrawal of fraudulent products from consumption was difficult for a long time. In India, at least 2 tons of milk and 30 tons of dairy sweets containing urea, starch, formaldehyde, or hydrogen peroxide were destroyed by local authorities in 2011. Urea had been added to skimmed or expired milk powder to increase the total soluble solid value. Consumption of milk containing more than $\sim$70 mg dl^{-1} of urea can cause severe health problems for human beings. Other rich in protein foods and feeds have been found adulterated with illicit sources of nitrogen.

Fish and Seafood Products

Fish traded in fillets (salmon, trout, plaice, and lemon sole) or as fish fingers (crab) and caviar are the major target for EMA. Surimi, due to overexploitation of sources, is no longer a cheap substitute for crab sticks. DNA analysis seems to be the answer to speciation problems related to fish trade. Toxic chemicals

such as malachite green, chloramphenicol, and formaldehyde that had been used as a means to extend the shelf life of the fish products and claim 'freshness.' In all cases, the products were distributed in certain Asian countries (China and India). One case of intentional contamination has been reported in 2002 and involved the illegal exports of Baltic salmon with high level of dioxins from Sweden to other EU countries where it was sold in restaurants and grocery stores. Dioxins accumulate in the fatty tissues of the salmon, affecting the human reproductive system and causing cancer.

Fruit Juices and Concentrates

Citrus juices are frequently adulterated or misbranded. The price of grapefruit juice is always lower than that of orange juice, so their admixture can be expected. Misbranding of fruit juices made from imported concentrates remains as common practice over the decades. Widespread adulteration of drinks for athletes, juices, tea drinks, fruit jams, jellies, and syrups with phthalates such as diethylhexyl phthalate was discovered in 2011 by US federal agencies. The products had been exported from 34 Taiwanese companies to at least 22 countries abroad. Seven food companies in Taiwan were found to be involved in the scandal. Phthalates, added as clouding agents, had not been readily tested for, and the reaction to this adulteration was slow in the United States. Pomegranate juice, being a highly priced product that is frequently marketed for health-promoting action, is vulnerable to adulteration. In 2006, the product of a known company labeled as '100% pure pomegranate juice' with 'no added sugar or sweeteners' was found to contain high-fructose corn syrup and only trace amounts of pure pomegranate juice. It is still unclear whether the adulteration occurred at the source in the Middle East or by the manufacturer. A competing juice manufacturer uncovered the adulteration and brought legal action against the perpetrating company.

Grains, Cereals, and Products

The major cases of adulteration reported during the last decade involve the mislabeling of nonorganic grains as organic, addition of dyes and flavor enhancers in noodles or corn bun dumplings, and substitution of basmati rice by cheaper sources. In 2013, 100 000 tons of nonorganic grains being sold across Europe as organic was discovered in Italy. Fake companies and counterfeit certifications were used in transactions, and 16 people were arrested by the Italian police for the $72 million fraud.

Honey and Other Natural Sweeteners

Important types of adulteration of honey have to do with the mislabeling of the type and origin of the product, illegal feeding of bees with sugar, and thermal treatment that increases the content of hydroxymethylfurfural. Transshipment and origin mislabeling are top-rated issues within this category of products. From 2008 to 2012, Chinese honey declared as originating from other countries (such as Indonesia, Malaysia, Mongolia, Thailand, and Vietnam), or as sugar syrups other than honey, in order to avoid antidumping duties was imported to United States. The incident may have been uncovered as part of increased investigations into the evasion of antidumping duties in the honey trade by US Customs and Border Protection. Chinese honey has been found contaminated with chloramphenicol, an antibiotic that can cause a rare but serious condition called aplastic anemia in humans.

Meat and Products

Major counterfeit problems of the last decade involve the addition of antibiotics to organic meat and of banned synthetic dyes to pork to imitate beef's color. Injection of chicken with hydrolyzed porcine and bovine proteins that act as bulking agents of breasts is another common issue. In 2012, the Irish Food Safety Authority identified horse DNA in food products that were labeled as containing ground beef. The incident affected 13 European countries and various products, including beef burgers and frozen meals. Long supply chains and a wrong interpretation of the regulation about the supply of raw ingredients for ground beef may have contributed. The European Commission reacted by changing the classification of desinewed meat so that it could be no longer called 'meat,' but mechanically recovered meat. The discovery of undeclared horse meat led to concerns that phenylbutazone, a veterinary drug used as a painkiller, may have entered the human food chain. A joint risk assessment prepared by the European Food Safety Authority and the European Medicines Agency concluded that the illegal presence of residues of phenylbutazone in horse meat is of low concern for consumers due to the low likelihood of exposure and the overall low likelihood of toxic effects. Still, the prohibition of the use of phenylbutazone remained active. The two authorities pointed out the need of strengthening the traceability of horse meat in the food chain across the European Union including a horse passport system.

Olive Oil

Olive oil, a food staple in the warmer regions around the Mediterranean, has gained popularity among consumers from nonproducing countries all over the world. Moreover, expansion of olive tree cultivations to the southern hemisphere, the United States, Turkey, Iran, and China opened discussion on revision and updating of international standards for compositional data such as upper limits for linolenic acid content, trilinolenin, and other ECN 42 triacylglycerol concentrations and that of certain phytosterols to avoid adulteration with cheaper oils. Olive oil is often adulterated with seed oils and olive residue oil. Esterified oil, which is prepared by reesterifying low-grade olive oil or olive oil soap stock recovered from alkali refining, has also been used to adulterate olive oil products. More recent are the reports on fraud with desterolized or deodorized olive oil under mild conditions. Oils having a similar composition (hazelnut oil and high-oleic sunflower oil) or lower price (palm, avocado, and canola oils) have been used as common olive oil adulterants. The most recently reported cases of olive oil frauds are those where sunflower oil, artificially dyed with beta-carotene or copper complexes of chlorophyll (e.g., E141) to match the color of olive oil, was used as a substitute or diluent of the product. US federal, European, and Australian legislation were recently updated to strengthen official control. Extra virgin

olive oil, the most expensive commercial category, is also often misbranded as was experienced in the Chinese market for a lower-quality product labeled as a high-quality one from a Mediterranean country. 'Cold pressed' and protected designation of origin (PDO) labeling need specific certification to be trusted.

Spices and Extracts

Black pepper, cumin seeds, paprika, turmeric, curry, chili, and saffron are highly susceptible to fraud. According to official reports, saffron, the dried stigmas of *Crocus sativus* flowers, has been extensively adulterated during the last decade with other parts of the *C. sativus* flower, dyes, and other plant materials. Substitution by almost 90% per weight was discovered in commercial brands of high-quality saffron. A systematic problem with transshipment and mislabeling of Spanish saffron has been also reported during the last decade. Officials reported that only 1% of Spanish saffron on the marketplace is actually derived from Spain. Imports from countries such as Iran were used to either extend or replace Spanish saffron, which was sold for about double the price. Importers also falsified the Spanish label of authenticity. This appears to be a systemic problem. Spices diluted with lead chromate and lead tetroxide and substitution of Chinese star anise with toxic Japanese star anise are some of the most hazardous cases of spice fraud. In the last 15 years, the fraudulent addition of the synthetic Sudan dyes (I, II, III, and IV) has been reported as a means to artificially enhance the color of red- or orange-colored chili and curry powders imported mainly from India. Sudan (I), alone or in mixtures with other Sudan dyes, is used due to low cost and high stability. Sudan dyes are metabolized by the microflora of the human skin and those in the gastrointestinal tract to carcinogenic colorless amines. Since each type of the dye is degraded to different hazardous metabolites in the human body, the toxicity of which may be enhanced by their simultaneous presence in foods, it is of utmost importance to develop highly efficient analytic tools. Sudan dyes were found in a series of products (sauces) from different manufacturers in Great Britain.

Detection

Analytic techniques that provide physical, chemical, or biochemical information for foods are the front line to detect fraudulent practices and concomitantly dissuade their incidence. From the ones available, liquid and gas chromatographies (GC) and infrared spectroscopy (IR) seem to be the workhorses of official control laboratories. Mass spectrometry used alone or hyphenated to chromatography follows in popularity. Despite the high capital cost, its applicability in the near future is expected to increase in the food authentication sector due to its high efficiency. Electrophoretic and immunochemical techniques present more targeted applications. The potential of Raman and nuclear magnetic resonance (NMR) spectroscopies seems very promising. Most of the mentioned techniques offer multiple pieces of information in one experiment obtaining, thus, the fingerprint of a series of constituents. Unscrambling of the hidden information is then achieved through chemometrics; results are stored to form analytic databases. Last but not least, the advance of molecular markers for the certification of product provenance is a powerful tool to combat EMA.

Chromatographic Techniques

Chromatographic techniques provide speciation and sensitivity owing to the resolution of complex mixtures into their constituents. Data acquired with a detector (UV, mass spectroscopy (MS), etc.) are used for targeted or nontargeted analysis. In the latter case, the concentration of various constituents, peak areas/heights, or even the chromatogram as a continuous signal (after peak alignment with suitable algorithms) can be examined with chemometrics to detect adulteration.

High-performance liquid chromatography (HPLC) procedures for many classes of compounds or individual food components are available to check food adulteration. Oligosaccharide determination was shown to detect the addition of soy milk to cow's milk ($\geq 5\%$), corn syrups ($\geq 5\%$), and industrial syrups either directly added or via bee feeding ($\geq 10\%$) to honey. Using the ISO 11292 method for the determination of free and total carbohydrates in coffee (≥ 10 pmol) and detection of adulteration with wheat, soybean, their mixtures, or husks was proved to be feasible after chemometrics. Triacylglycerol analysis may serve detection of lower-quality seed oil addition (1–3%) to virgin olive oil and sesame oil, as well as of pork ($\geq 2\%$) in beef. A caseinomacropeptide was proposed as an index of cheese whey addition to milk (detection limit $= 1\ \mu g\ ml^{-1}$), whereas 29 amino acids assisted the detection of orange juice adulteration. Determination of tryptophan (limit of detection $= 1\ mg\ l^{-1}$) could serve as an index of soy sauce adulteration with acid-hydrolyzable vegetable protein. Phenolic compounds, namely, neoeriocitrin and naringin, are proposed markers of bergamot juice addition to lemon juice ($\geq 1\%$), whereas arbutin for pear juice addition to apple juice ($\geq 2\%$). The simultaneous detection of melamine ($5\ \mu g\ g^{-1}$) and the related triazine by-products ammeline ($5\ \mu g\ g^{-1}$), ammelide ($5\ \mu g\ g^{-1}$), and cyanuric acid ($90\ \mu g\ g^{-1}$) in wheat flour is of high importance for public health. HPLC coupled to GC was used to determine the addition of virgin (5%) and refined (10%) hazelnut oil in olive oil via filbertone detection. The coupling to IRMS (isotope-ratio mass spectrometry) improved the detection of honey adulteration with C4 and C3 sugars via determination of $\delta^{13}C$ values of the separated sugars. The recent development of ultrahigh pressure-operating (up to 15 000 psi) HPLC systems that enables analysis with sub-2 micron columns providing richer profiles in significantly shorter time is useful for fingerprinting as shown for pineapple, orange, and grapefruit juices and their admixtures detected with chemometrics down to 1%.

GC was the first strong tool for the detection of adulteration of oils and fats through fatty acid methyl ester (FAME), triacylglycerol, or sterol analysis. For example, the peak areas of FAMEs and squalene obtained with GC-MS were valuable for the accurate detection of extra virgin olive oil adulteration with sunflower, corn, peanut, and coconut oils with chemometrics and multivariate calibration model construction for the precise determination of the levels of adulterants. With a similar approach, the addition of lard or beef tallow ($\geq 5\%$) to

canola oil was possible. Currently, sophistication is also examined through the exploitation of volatiles fingerprint. Thus, the GC-MS determination of 9(E),11(E)-18:2 FAME traces was proposed for lower-quality deodorized olive oil addition to virgin olive oil and that of a lupane skeleton and lupeol for hazelnut oil presence (<4%). Inulotriose detection in honey indicated adulteration with high-fructose syrups of inulin, whereas that of difructose anhydrides the presence of high-fructose corn syrup and inverted syrup (≥5%). Solid-phase microextraction/GC-MS and chemometric treatment of the data estimated accurately 25% addition of apple puree to strawberry puree or detection of roasted coffee adulteration with roasted barley (≥1%).

Spectroscopic Techniques

IR is popular since analyses require no or minimal sample preparation, instruments are simple in operation, and the incorporation of Fourier transform facilitates the rapid and simultaneous measurement of various constituents in liquid and solid samples. Near-IR (NIR) (14 000–4000 cm^{-1}) penetrates more into the sample and can be used to examine samples in NIR transparent packing, water absorbance is not strong as in mid-IR (MIR) (4000–400 cm^{-1}), and portable equipment is available for on-site examination. The lower sensitivity to some minor constituents and the fact that due to the complex spectra multivariate regression is necessary for quantification are limitations. Nonetheless, to use the whole spectra (fingerprint), both preprocessing and chemometrics are necessary with NIR or MIR. Much research has been carried out in detecting adulteration of edible oils such as virgin olive oil (with high oleic or linoleic sunflower, pomace olive, canola, or hazelnut oils at level ≥10%), honey (with >7% beet invert sugar or >10% high-fructose corn syrup), milk (with ≥1 g l^{-1} urea and ≥5% starch, dextrin, urea, melamine, and ammonium nitrate), fruit juices (beet and cane invert sugars in apple juice with an error of prediction of 1.3% and 1.8%, respectively), and meat. Addressing a 'hot' issue, MIR could detect and quantify horse meat in minced lean beef at levels ≥2%.

MS can record the volatile profile from the headspace of a sample through proton transfer reaction (PTR-MS); multiple elements via ionization with inductively coupled plasma (ICP-MS); various nonvolatile constituents by direct infusion and electrospray ionization, by matrix-assisted laser desorption ionization (MALDI), or very recently via direct analysis in real time (DART) techniques; and isotope ratios (IRMS) after sample gasification. So far, the latter four have been applied to detect adulteration. MALDI is coupled to time-of-flight mass analyzer (TOF-MS) to determine macromolecules. Powdered milk addition to fresh cow milk (≥1%) via detection of diagnostic peptides related to thermal changes induced to milk proteins upon powder production and cow milk addition (≥5%) in sheep or goat milk using phospholipid markers and hazelnut oil (≥5%) to virgin olive oil by analysis of extracted polar components are few applications. DART/TOF-MS detected addition of pork to minced beef and vice versa at 10% via the triacylglycerol profile of isolated fat and hazelnut oil to extra virgin olive oil at 6% and 15% via the examination of the triacylglycerol and polar compound profiles, respectively. The $^{13}C/^{12}C$ ratio assisted the detection of low-cost sugar cane and high-fructose corn syrup addition in valuable natural products such as honey, royal jelly, mulberry pekmez, and most juices (except for pineapple). The $^{14}N/^{15}N$ ratio supplied additional information on the possible addition of yeast powder as a source of protein in royal jelly. Conversion of ethanol into the more stable ethylene and determination of nonexchangeable hydrogen ($^{2}H/^{1}H$) stable isotope ratio indicated ethanol addition in wine from other sources. The combination of $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratios diagnosed water addition to fruit juices and wines since plant water versus ground/rainwater is richer in ^{18}O and ^{2}H due to losses in ^{16}O and ^{1}H isotopes upon leaf evapotranspiration. IRMS can be coupled to an elemental analyzer or a chromatograph to increase the wealth of information.

Raman spectroscopy presents similarities to IR considering sample preparation. Analysis of samples already packed in plastic/glass packaging is possible, water interference is minimal, and portable equipment is available. Raman has been used to detect addition of soybean, rapeseed, sunflower, and corn oils (≥5%) in olive oil, high-fructose corn syrup and maltose syrup (10–40%) in honey (individually or as a mixture), and margarine (≥10%) in butter. Worthwhile is the detection of the toxic paraffin added in rice (>0.003), of melamine in milk (≥0.04%), and of banned horse meat at 25% in beef with the aid of chemometrics.

NMR spectroscopy provides simultaneous quantitative determination of compounds without prior separation (targeted analysis) or a fingerprint (nontargeted analysis) to be treated with chemometrics. Nontargeted ^{1}H and ^{13}C spectra examination detected hazelnut oil addition (≥8%) to olive oil. Targeted analysis with more elaborate sample preparation and chemometrics permitted admixing detection of olive–hazelnut, olive–corn, olive–sunflower, and olive–soybean oils (≥5%). Synthetic triacylglycerol detection in butter (≥1%) was obtained from the ^{13}C NMR spectra determination of the regioisomeric distribution of C4 between the sn-1,3 and sn-2 positions. Low-field NMR (60 MHz) was recently shown to detect sunflower or red palm oil and hazelnut oil addition in extra virgin olive oil down to 10% and 11%, respectively, and high-fructose corn syrup to blossom honey (≥10%) exploiting the ^{1}H spectra. NMR data fused with that of UV-vis have been used for Sudan III and IV detection in culinary spices. Site-specific natural isotope fractionation (SNIF)-NMR technique determining the relative deuterium and specific deuterium site locations in molecules easily detected sugar beet addition to orange juice, vanillin adulteration with a synthetic one from guaiacol or lignin or biotechnologically derived, and addition of molasses spirit to rice spirits (≥8%). SNIF-NMR is used officially (EC 2676/1990) to detect wine chaptalization.

Electrophoresis and Related Techniques

Protein electrophoresis to detect cow milk in goat milk (≥1%), bovine milk in caprine or ovine samples (≥5%), and soy milk in cow milk (≥5%) has been reported. Sodium dodecyl sulfate - Polyacrylamide gel electrophoresis (SDS-PAGE) detection of a glycomacropeptide was proposed as an index of whey addition to milk (≥1%). Electrophoretic patterns of the fish muscle myogens can be useful to detect surimi adulteration since they are characteristic of the species and remain unaltered during

processing/preservation. The sarcoplasmic protein electrophoresis is suitable for raw fish species identification, thus detecting substitution of species with less valuable ones when fishes are devoid of their external characteristics. However, in the case of heat-processed fish, this is feasible only for those species containing heat-stable parvalbumins.

Capillary electrophoresis (CE) progressively is gaining popularity due to high resolution, simplicity, versatility, and low operating costs despite its low reproducibility and sensitivity. The ratio of the sum of nonbovine β-lactoglobulins (ovine or caprine) to the total β-lactoglobulins obtained with CE-MS was useful to detect bovine milk addition in caprine or ovine milk ($\geq$5%). The CE-UV profiles of methanol-soluble paprika proteins facilitated detection of paprika addition ($\geq$5%) in high-quality smoked paprika 'pimentón de la Vera.' Nonprotein amino acid analysis with CE-MS made feasible the adulteration detection of virgin olive oil with soy oil ($\geq$2%). CE-MS detection of carnitines not present in virgin olive oil provided information for seed oil addition (2–10%) in virgin olive oil.

DNA analysis relying on polymerase chain reaction (PCR) and amplification using concepts such as restriction fragment length polymorphism, random amplified polymorphic DNA, and amplified fragment length polymorphism is the most suitable approach to verify the identity and quantity of species present in meat, dairy, and marine products. This is possible even after thermal processing because nucleic acids are more thermostable than proteins. Addition of pork meat to poultry ($\geq$1%) with a sensitivity of 0.1% was possible using cytochrome b and 12S rRNA as target genes for the two kinds of meat, respectively. Newly designed primers targeting porcine mitochondrial displacement permitted detection of pork without any cross amplification by other animal species with 10 pg of pig DNA limit of detection. The PCR assay was suitable in raw, cooked (60–100 °C), autoclaved, and microwave-treated samples. A pair of canine-specific primers detected minimum 0.1% of canine meat in frankfurter formulations comprising various animal and plant species and subjected to heating. Mitochondrial DNA fragments for horse, dog, cat, bovine, sheep, porcine, and goat minced meat differentiated species, and adulteration of 5%, 2.5%, 1%, and 0.5% in all combinations of possible admixtures was detected. Real-time PCR increasing the quantitative range of conventional PCR (from 1.5–2.0 log cycles to at least 5–6 log cycles) and employing fluorescent probes for visualization instead of electrophoretic separation and staining is faster and more sensitive. Thus, pork detection in beef burger formulations or commercial meatballs at $\geq$0.01% and surimi addition to soy-based vegetarian food at $\geq$1% have been reported using porcine cytochrome b, α-skeletal actin gene, and betaine aldehyde dehydrogenase 2 gene specific primers.

Immunochemical Methods

Enzyme-linked immunosorbent assay (ELISA) available in variable formats is the most popular. Although DNA methods are the most specific/sensitive for meat species identification, they require expensive equipment and training. Thus, ELISA is preferred for the routine analysis of numerous samples. Rate-limiting steps in application are the narrow availability of antibodies and standards and the generation of on-demand antibodies that can react normally at extreme pH, in the presence of high salt or solvent concentrations.

A monoclonal antibody-based ELISA assay permitted the detection of chicken and turkey meat added in cooked (100 °C, 15 min) red meat. The specificity of the 5D2 antibody to different species (pork, beef, lamb, deer, horse, duck, chicken, and turkey) and tissues (serum, gizzard, heart, and liver) examined showed that detection at <1% was feasible. Sandwich ELISA detected 0.05% added pork in chicken, 0.1% pork in beef mixtures, and 0.05% pork meal in soy-based feed, raw and heat-processed meat (132 °C, 2 h), and feed products. Test kits providing information for cooked, raw, and thermally processed meat and animal feed are available including horse meat detection at <1–2%. Available kits are also for milk and cheese examination to detect cow milk addition to goat or sheep milk, goat milk addition to sheep milk or cheese at levels 0.01–2%, and bovine casein in goat and sheep cheese at 0.5%. A useful application of ELISA is the determination of melamine in milk, milk powder, and feed samples via monoclonal antibodies produced by hybridoma technology, which provided high sensitivity and specificity.

Differential Scanning Calorimetry

Differential scanning calorimetry has been used to detect fats/oils and honey adulteration. It is fast and requires minimal or no sample preparation and no solvent use. The detection of hazelnut oil in olive oil ($\geq$5%) and high-oleic sunflower ($\geq$20%), refined olive, refined olive pomace, sunflower, and corn oils in extra virgin olive oil ($\geq$1–1.2%) has been described. Treatment of sample heating profiles with chemometrics permitted the detection of lard in admixtures with beef tallow or chicken fat ($\leq$1%). The linear dependence between enthalpy value of water phase transitions and its content can assist the detection of addition to butter ($\geq$5%), whereas measurement of the glass transition temperature (Tg) allows the determination of honey admixtures with industrial syrups (beet and isoglucose) at 5–10%.

Conclusions

Food fraud occurs since the very distant past. Rigorousness of authorities and advanced systems of detection are needed to increase trust in food manufacturers, retailers, and importers and ensure protection of public health.

See also: Alcohol: Properties and Determination; Authenticity of Food; Cereals: Storage; Cheese: Chemistry and Microbiology; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Codex Alimentarius; Codex Alimentarius Commission: Role in International Food Standards Setting; Dairy Products: Dietary and Medical Importance; Fats: Classification and Analysis; Fatty Acids: Determination and Requirements; Fish: Dietary Importance and Health Effects; Fish: Fish in the Human Diet; Fish Oils: Composition and Health Effects; Fish Oils: Production and Properties; Fish: Processing; Flavor Enhancers: Characteristics and Uses; Food Additives: Classification, Uses and

Regulation; Food Allergies; Food and Agriculture Organization of the United Nations; Food Poisoning: Tracing Origins and Testing; Fruit Juices; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Horse Meat; Legal Requirements for Food Hygiene; Mass Spectrometry: Applications; Meat: Conversion of Muscle into Meat; Meat: Eating Quality and Preservation; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Olive Oil: Its Role in the Diet; Preservatives: Classifications and Analysis.

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Food Intolerance: Lactose Intolerance

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Introduction

Lactose is a major disaccharide present in milk. It is composed of two simple sugars, glucose and galactose. Cow, goat, and buffalo milk contains less lactose than human milk. It has several applications in the food industry. Because of its physiological properties, it is widely used in sweets, confectionery, bread, and sausages; lactose provides good texture and binds water and color. To be absorbed, lactose needs to be hydrolyzed in the intestine by a β-galactosidase enzyme, lactase-phlorizin hydrolase, generally called lactase. This enzyme hydrolyzes lactose into the simpler sugars in order to digest and absorb the sugars. In adults with lactase deficiency (also called lactase nonpersistence (LNP)), lactose is not digested in the upper bowel and reaches the lower bowel, where it is fermented by gut microorganisms, which produce hydrogen, carbon dioxide, and methane gases. Undigested lactose also draws water into the intestinal lumen through its osmotic effect, which increases motility and can cause diarrhea. Symptoms include abdominal pain, bloating, and flatulence. Thus, low lactase levels cause lactose malabsorption (or lactose maldigestion). When lactose malabsorption gives rise to symptoms, this is called lactose intolerance (LI), that is, lactose malabsorption is the physiologic problem that manifests as LI.

Lactose maldigestion does not lead to symptoms of LI in all LNP subjects, and a small percentage of LNP subjects remain free of symptoms even after ingesting large amounts of lactose. Although rarely life-threatening, the symptoms of LI can lead to significant discomfort and disrupt the quality of life.

Males and females are equally affected by LI. Lactase deficiency in adults is a normal developmental phenomenon characterized by the down-regulation of lactase activity, which occurs soon after weaning in most ethnic groups. Lactose maldigestion increases with age during adulthood. Of those women who are lactose intolerant, 44% regain the ability to digest lactose during pregnancy. This is probably due to slow intestinal transit and bacterial adaptations during pregnancy.

Historic and Geographic Perspective on LI

The people with a low prevalence of lactose maldigestion (northwest Europeans and certain East African pastoral groups) have long traditions of consuming milk, much of it in lactose-rich forms. This suggests a geographic or cultural-historical hypothesis based on the assumption that in the hunting-and-gathering stage, human groups everywhere were like most other land mammals in their patterns of lactase activity. That is, in the normal individual, lactase activity would drop at weaning to low levels, which prevailed throughout life. With the beginning of dairy farming, however, significant changes occurred in the diets of many human groups. As a result, there may have been a selective advantage for those aberrant individuals who experienced high levels of intestinal lactase throughout life. In classic evolutionary terms, the condition of high intestinal lactase activity throughout life, or lactase persistence, would come to be typical of such a group.

Children of some ethnic groups (e.g., Thai) commonly lose lactase at 1–2 years of age while in others (e.g., Finns) lactase persists until later in life (10–20 years of age). According to some estimates, approximately 70% of the world's population has primary lactase deficiency. The frequency of lactose maldigestion varies widely among populations but is high in nearly all but those of Northern European origin.

Lactase deficiency in Europe has been reported to vary between 4% (in Denmark and Ireland) and 56% (in Italy). In South America, Africa, and Asia, more than 50% of the population is reported to have LNP, and, in some Asian countries, this rate is almost 100%. A study of gene-identified LI in a Dutch Caucasian elderly population is associated with lower dietary calcium intake and serum calcium levels but is not associated with bone mineral density or fractures. The paradox reinforces the complexity of the disease and the importance of biologic, genetic, and as yet undetermined factors in the etiology of osteoporosis. The lactase persistence trait is more common in populations that practice cattle herding and dairy farming, and it is related to genetic selection of individuals with the ability to digest lactose.

Definitions of Various Types of LI

- *Lactose intolerance* is a clinical syndrome of one or more of the following: Abdominal pain, diarrhea, nausea, flatulence, and bloating after the ingestion of lactose or lactose-containing foods. The amount of lactose that will cause symptoms varies among individuals, depending on the amount of lactose consumed, the degree of the lactase deficiency, and the form of food in which the lactose is ingested.
- *Lactose malabsorption* is the physiologic condition that manifests as LI and is attributed to an imbalance between the amount of ingested lactose and the capacity for lactase to hydrolyze the disaccharide.
- *Primary lactase deficiency* is attributable to a relative or absolute absence of lactase that develops in childhood at various ages in different racial groups and is the most common cause of lactose malabsorption and LI. It is also referred to as adult-type hypolactasia, LNP, or hereditary lactase deficiency. The most common type is found in ~70% of the world's population. In this condition, the production of

lactase declines over time, often beginning about 2 years of age; between the ages of 5 and 14 years, the majority of the population undergoes a genetically programmed reduction in lactase synthesis, resulting in only 5–10% of the lactase levels of infancy. This reduction, known as primary lactase deficiency, is not related to the continued intake of milk or lactose. Symptoms may not be experienced until late adolescence or adulthood, and the prevalence of this type of lactase deficiency in different ethnic groups is highly variable. Researchers have discovered that some people inherit genes from their parents that may cause a primary lactase deficiency.

- *Secondary lactase deficiency* is a lactase deficiency that results from a small bowel injury, such as acute gastroenteritis, persistent diarrhea, a small bowel overgrowth, cancer chemotherapy, or other causes of injury to the mucosa of the small intestine (e.g., celiac sprue, gluten-induced enteropathy, and tropical and non-tropical sprue). It can present at any age but is more common in infancy. Rotavirus infection may also be associated with diarrhea and LI in infants. Denudation of the brush border of the jejunal mucosa, which is associated with diarrhea, can lead to the loss of the other two disaccharides, maltase and sucrose. Continued diarrhea may also lead to severe complications such as monosaccharide intolerance. Other conditions that give rise to secondary lactose maldigestion include giardiasis and *Ascaris lumbricoides*. Tolerance to lactose can be improved by treating the underlying cause.
- *Congenital (Neonatal) lactase deficiency* is extremely rare. Affected newborn infants present with intractable diarrhea as soon as human milk or lactose-containing formula is introduced. Unless this is recognized and treated quickly, the condition is life-threatening because of dehydration and electrolyte loss. Teleologically, infants with this type of deficiency would not have been expected to survive before the twentieth century, when no readily accessible and nutritionally adequate lactose-free human milk substitute was available.
- *Developmental lactase* deficiency is now defined as the relative lactase deficiency observed among preterm infants of less than 34 weeks' gestation. Lactase activity usually develops late in pregnancy, so a preterm infant will not develop this enzyme function. Thus, greater the degree of prematurity, the greater the reduction in lactase activity in infants. This condition usually improves as the intestinal mucosa matures.

Pathophysiology of LI

Lactase is found most abundantly in the villus enterocytes of the jejunum (at the beginning of the small intestine), and it specifically only hydrolyzes dietary lactose into glucose and galactose for transport across the cell membrane. As it is found at the tip of the intestinal villi, it is therefore more vulnerable to attacks that cause cell damage than are other disaccharidases, which are located deeper. Absorption of lactose depends on the enzyme activity and the transit time of lactose through the jejunum mucosa. Insufficiency in lactase activity or its deficient state (hypolactasia) results in unabsorbed sugar, which attracts fluid (approximately triple the predicted amount) osmotically into the bowel lumen. Because the intestine cannot maintain a high electrochemical gradient between its contents and blood, there is an increase in the volume and fluidity of the gastrointestinal contents.

In addition to these factors, unabsorbed lactose reaches the colon, and fermentation is carried out by colonic microflora. As a consequence, gas is produced and monosaccharides that cannot be absorbed by the colonic mucosa are released, thus increasing the osmotic pressure and drawing more fluid into the bowel, resulting in symptoms such as flatulence, abdominal bloating, loose stools, abdominal cramps, and diarrhea (Figure 1). However, the development of symptoms depends on the amount of lactose ingested, intestinal transport, individual sensitivity, subjective expectations, stomach emptying frequency, and the colonic fermentation of lactose by microbiota. Sometimes there is a chance of the colonic flora producing toxins that, when reabsorbed into the body, cause harmful effects on a range of tissues, including neurons, heart cells, muscles, endocrine cells, and cells of the immune system.

Prevalence of LI

In most mammals, the synthesis of lactase declines to subtle levels after weaning. The phenomenon of the persistence of lactase production beyond weaning is seen only in humans, and it is more prevalent in the peoples of Northern Europe, which could be due to their regular consumption of milk and dairy products as part of their daily diet. However, it is not true with people in other parts of the world, where the regular intake of milk stops when breastfeeding ends. Although 70% of the world's population is deficient in lactase levels, not all suffer with LI symptoms because there are many nutritional, age, and genetic factors involved in their ability to tolerate lactose, creating variations between different individuals. The prevalence of LI in different age groups and pregnant women is discussed below.

In Children

Later childhood and adolescence is an important transition period for lactose digestion, data suggest based on diverse populations. Lactose persistence is observed in children younger than 3 years old; however, between 3 and 11 years, the beginning of genetically controlled LNP is recognized (Table 1). It has been observed that older children and young adults are increasingly unable to digest relatively small amounts of lactose. This results in increased symptom production, recognition of discomfort, and avoidance of lactose-containing products, which provoke symptoms.

A continuous decrease in lactase level is noted in children from 1 to 5 years old and through adolescence. The age of onset and its prevalence differ among various populations. Approximately 20% of Hispanic, Asian, and black children younger than 5 years show evidence of lactase deficiency and lactose malabsorption, whereas white children typically do not develop symptoms of LI until after 4 or 5 years of age. Recent molecular studies of lactase-phlorizin hydrolase (lactase) have correlated the genetic polymorphism of messenger RNA

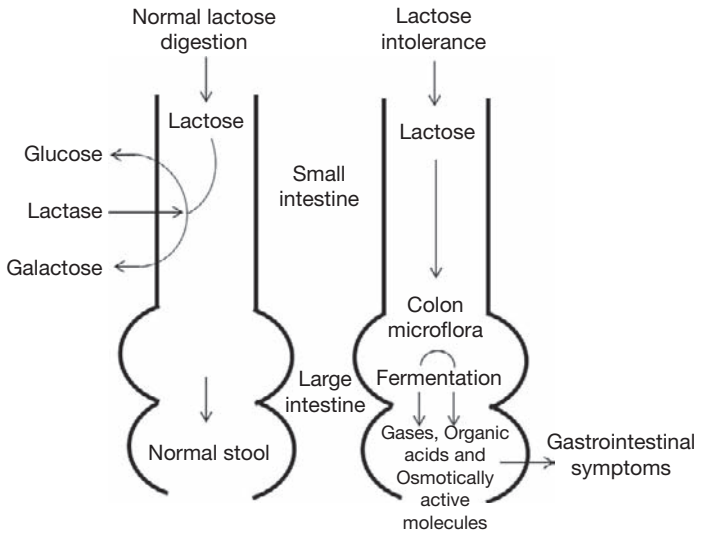

Figure 1 Lactose intolerance.

Table 1 Genetically determined lactase levels in healthy individuals by age and lactase persistence

	Lactase level	
Age lactase level	*Low lactase nonpersistent individual*	*High lactase persistent individual*
Fetal period	Low	Low
Birth	High	High
Weaning	Decline	High
3–12-year-old child	Reduced	High
Adolescent	Low	High
Adult	Lower	Average
Elderly	Lowest	Decline

Source: Paige, D. M. (2013). Lactose intolerance. *Encyclopedia of human nutrition* (3rd ed.), pp. 67–73. Oxford: Elsevier.

expression with persistence of lactase activity, demonstrating early loss (at 1–2 years of age) of messenger RNA expression and enzyme activity in Thai children and late (10–20 years of age) loss of activity in Finnish children.

Most lactase-deficient individuals experience onset of symptoms in late adolescence and adulthood. Because of the variation of dairy intake in each individual's diet and in the amount of lactose contained in different products, symptoms may vary and be modified by diet and milk-containing foods.

In Adults

There is some evidence that intestinal lactase activity does not continue to decline with age because there were no differences in the prevalence of hypolactasia between older and younger adults. However, the prevalence of hypolactasia is more common in adults than in children. The progressive increase in the prevalence of lactose maldigestion increases with age, reaching reported adult levels of approximately 70% of the world's adult population. The exceptions are the populations of Northern and Central Europe and some Middle Eastern populations as well as groups of primarily European descent in Australia, New Zealand, and North America. The highest rates of lactose maldigestion were in South America and Africa, with approximately 50–70% of the population affected, and especially some Asian countries, where almost 100% of the population was affected. The prevalence of LI varies significantly between different groups and selected populations (Table 2).

A recent Finnish study notes flatulence as the most severe symptom in maldigesters whereas abdominal bloating is most frequently reported by individuals self-identifying as lactose maldigesters. Moreover, microbiota may play a role in the presence and intensity of lactose-related symptoms. Data suggest that increased levels of colonic bacteria, as well as their diversity, may play a role, as a result of their increased fermentative capacity, in inducing the symptoms associated with LI.

Pregnant Women

Milk is considered to be one of the most nutritious foods during pregnancy, but the lactase levels in some individuals in a number of racial and ethnic groups may be insufficient to hydrolyze commonly consumed amounts of lactose, resulting in lactose maldigestion and possibly milk intolerance. The role of lactose digestion in pregnant women is of special interest because pregnant women are frequently the targets of nutrition education. LI in pregnant African American women was studied using the breath hydrogen response to 240 ml of low-fat (1%) milk. The researchers reported the prevalence of lactose maldigestion in early pregnancy (13–16 weeks), late pregnancy (30–35 weeks), and 8 weeks' postpartum as 66%, 69%, and 75%, respectively, compared to nonpregnant African American women (the control group), who had a prevalence of 80% (Table 3).

Less than 25% of pregnant lactose-maldigesting women reported any symptoms with 240 ml of low-fat (1%) milk. Implications for fetal growth and development remain to be answered by further study. However, symptoms may be further reduced when the milk is consumed with other foods. The level

Table 2 Prevalence of lactose maldigestion in selected populations

Population	*Country*	*% Lactose maldigestion*	*Population*	*Country*	*% Lactose maldigestion*
African American, 18–54 years	USA	75	General, 21–65 years	Finland	15
Asian, 23–39 years	USA	100	General, 20.3 years	Germany	70
Native American, 18–54 years	USA	81	General, 16–54 years	Chile	80
African American, 13–19 years	USA	69	Noncaucasian	Peru	94
Mexican, 18–94 years	USA	53	General, 38–49 years	Brazil	80
Vietnamese, 22–63 years	USA	100	Arab adult	Israel	81
Sicilian, 25 years average	Italy	71	General, male 14–34 years	Egypt	73
Northern, 28.7 years average	Italy	52	General, 15–78 years	Greece	45
Central, 36 years average	Italy	18	Bantu, 13–43 years	Uganda	100
Romani	Hungary	56	Yoruba, 13–70 years	Nigeria	83
Austrian, 22 years average	Austria	20	General, adult	India	61
General, 20.3 years average	Finland	17	General, 17–83 years	Korea	75
Aboriginal	Australia	84	General, 15–64 years	Japan	100

Source: Paige, D. M. (2013). Lactose intolerance. *Encyclopedia of human nutrition* (3rd ed.), pp. 67–73. Oxford: Elsevier.

Table 3 Lactose maldigestion[a] in pregnant and nonpregnant African American women

African American women	*% Lactose maldigestion*
Early pregnancy (13–16 weeks)	66
Late pregnancy (30–35 weeks)	69
Postpartum (8 weeks)	75
Nonpregnant	80

[a]Breath hydrogen rose 420 ppm following the consumption of 240 ml of low-fat (1%) milk containing 12 g of lactose following an overnight fast.
Source: Paige, D. M. (2013). Lactose intolerance. *Encyclopedia of human nutrition* (3rd ed.), pp. 67–73. Oxford: Elsevier.

of digestion and absorption of a range of nutrients in the consumed milk is unknown. It is necessary for pregnant women to discuss their ability to tolerate milk with a nutritionist; they should also ask for other food options.

Lactose, Calcium Absorption, and Bone Mineral Content

Recent evidence indicates that dietary lactose enhances calcium absorption and, conversely, that lactose-free diets result in lower calcium absorption. Thus, LI (and lactose-free diets) theoretically may predispose people to inadequate bone mineralization, a problem now recognized in many disorders affecting pediatric patients. The effect of lactose-free diets in childhood on long-term bone mineral content and the risk of fractures and osteoporosis with aging remains to be clarified. Calcium homeostasis is also affected by protein intake, vitamin D status, salt intake, and genetic and other factors, making long-term studies essential to determine the risks of each or all of these factors to bone health. In general, populations at risk for LI report a lower calcium intake as a result of the decline in the intake of milk and milk products. Data from National Studies in the United States indicate African American and Hispanic women have lower intakes of calcium compared to non-Hispanic women.

An Institute of Medicine Report concludes that the disparity in calcium intake "may be explained in part by the much higher prevalence of LI among African-Americans and Hispanics, sometimes resulting in their subsequent avoidance of milk." One solution to this problem is to educate lactose-intolerant groups about alternative calcium-containing foods, reinforce appropriate cultural patterns and dietary practices that include alternatives to milk, and identify other culturally acceptable calcium-containing foods.

Recent studies suggest that, in the future, genetic testing may be useful for identifying individuals at increased risk of lactase deficiency and consequent diminished bone mineral density, potentially allowing early intervention with dietary manipulation or nutrient supplementation. Recent research has even suggested that gene-replacement therapies might someday be available for susceptible individuals.

Diagnosis of LI

Usually, diagnosis can be made on the basis of the history, supported by dietary manipulation. Several tests have been used to diagnose LI (some of them are discussed below). Two formal tests, namely (a) the serum glucose level (lactose tolerance) test and (b) the breath hydrogen level test, are commonly used.

(a) *Lactose tolerance test*: In this test, a series of blood samples are obtained to measure blood glucose levels after the administration of approximately 1–1.5 g of lactose per kg of body weight. The test is positive if intestinal symptoms occur and the blood glucose level increases less than 20 mg dl^{-1} (1.1 mmol l^{-1}) above the fasting level. However, false-positive and false-negative test results occur in 20% of normal subjects because of the influence of variable gastric emptying and glucose metabolism.

(b) *Breath hydrogen test*: This test measures the rise in breath hydrogen after ingestion of 25–50 g of lactose. The test is based on the principle that carbohydrate in the colon is detectable in the pulmonary excretion of hydrogen and other gasses. A rise in breath hydrogen concentration greater than 20 ppm over baseline after lactose ingestion

indicates hypolactasia. Due to its greater accuracy, this test has been used more frequently than the lactose tolerance test, and it was found to be positive in 90% of patients with lactose malabsorption.

(c) *Stool acidity*: This test can be used to diagnose LI in small infants, for whom other forms of testing are risky or impractical.

(d) *Intestinal biopsy*: An intestinal biopsy can confirm LI following the discovery of elevated hydrogen in the hydrogen breath test. However, given the invasive nature of this test and the need for a highly specialized laboratory to measure lactase enzymes or mRNA in the biopsy tissue, this approach is used almost exclusively in clinical research.

Treatment of LI

Treatment of LI should not be aimed at reducing malabsorption but rather at improving digestive symptoms. Reduction of lactose intake rather than exclusion is recommended because most patients with self-reported LI can tolerate at least 12 g of lactose (equivalent to 250 ml of milk) without experiencing symptoms, and, if taken with other foods, up to 18 g of lactose can often be tolerated. It should be emphasized that the amount of lactose in tablets (<500 mg) is very unlikely to cause gastrointestinal complaints under any circumstances, whereas symptoms after intake of small amounts of dairy products should raise a suspicion of a true food allergy to a protein in cow's milk.

Observational studies report improvement of abdominal complaints with lactose restriction in up to 85% of IBS patients with lactose malabsorption. However, in randomized controlled trials, no significant improvement was found using dietary or enzyme-replacement treatments. This lack of efficacy in well-designed trials may be because lactose is only one of many poorly digestible, fermentable carbohydrates and dietary fibers in the diet. Therefore, for patients who have lactase deficiency, lactose restriction will rarely provide complete symptom relief.

Lactase enzyme replacement is another option although this changes the taste of food when mixed with the dairy products because the glucose and galactose produced by lactose digestion are sweeter than the original sugar. Varieties of preparations are available over the counter but may not be equally effective.

Fermented milk products, such as yogurt, dahi, bifidus milk, skyr, kumiss, and kefir, have been shown to be tolerated by lactose-intolerant individuals. The starter bacteria in these products partially digest the lactose into glucose and galactose (and the glucose to lactic acid); in addition, the semisolid state of these products slows gastric emptying and gastrointestinal transit, resulting in fewer symptoms of LI. The starter cultures (lactic acid bacteria) used in fermented milks alter the intestinal flora and may have beneficial effects in IBS patients that persist even after treatment.

Aged cheeses tend to have lower lactose content than other cheeses and, thus, may also be better tolerated. Predigested milk or dairy products with lactase are available in some countries and will often permit a lactose-intolerant individual to be able to take some or all milk products freely.

Finally, although lactase expression is not upregulated by lactose ingestion, tolerance may be induced by repeated lactose dosing due to adaptation of the intestinal flora.

Restricting lactose intake is a sensible treatment for LI, but patients should be reassured that absolute exclusion is unnecessary. If patients are unwilling to reduce milk intake, then lactase supplementation is appropriate.

Long-Term Effects of Lactose Restriction

Although restricting dietary lactose may improve gastrointestinal complaints, long-term effects of a diet free of dairy products may be of concern. Dairy products are the major source of calcium in many individuals. Current guidelines suggest a daily calcium intake of 1000 mg day^{-1} for adults, 1300 mg for adolescents, and 1200 mg for people more than 50 years old. Drinking three cups of milk per day has been recommended to obtain this amount of calcium.

There is also some evidence in the published literature that a low intake of dairy products is associated with a higher risk of fractures in women, although much less so in men. No study has addressed the safety and effectiveness of calcium replacement for patients with LI; however, it seems reasonable to recommend increasing calcium intake from other sources for patients who restrict their intake of dairy products and who have a low threshold for calcium replacements in the presence of other risk factors for osteoporosis.

Conclusion

LI is a perceived barrier for dairy intake. When the LI is due to lactose maldigestion, it is generally managed relatively easily, and thus one of the barriers impeding dietary adequacy is eliminated. Health professionals need to recognize that LI, whether real or perceived, should not be a significant barrier to improving dietary adequacy. They should understand also that LI becomes irrelevant if their patients are unlikely to drink milk, despite the relative ease with which symptoms can often be abated when they do so. Such individuals require special attention from nutrition professionals as it is not a simple task to achieve dietary adequacy without dairy, given contemporary foods and eating patterns. Further studies are required to provide high-quality evidence to support/compare the efficacy of various strategies adopted to treat LI.

See also: Allergies: Public health; Food Allergies: Occurrence and Analysis; Lactose.

Further Reading

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Food Labeling: Applications

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Food labels provide specific mandatory and voluntary information on the physical and nutritional properties of food products as well as instructions for their safe storage, preparation, and consumption. A food label can also be used to advertise, market, and promote a brand. Customers use the information on food labels as a guide in making decisions on which foods to purchase. Food labels are applied to prepacked food as single, large wraparound, or peel-and-seal labels. Food labels can also be printed directly onto the container or the product itself. As food manufacturing processes improve and food supply chains become more complex and globalized, more information is required on the durability and safety of food. Governance is needed to control the application of food labeling to effectively inform and protect the consumer, and this is achieved through international food laws and regulations. Such regulations prescribe the type and minimum amount of information that is to be provided and how it is to be presented.

Definition of Food Label (Labeling)

A food 'label' refers to the display of written, printed, or graphic material on the packaging, container, or wrapper directly surrounding a food product. The purpose of a food label is to provide information that will help the consumer make informed food choices. Traditionally label information would have been based on the physical character of the food (weight, net content, ingredient information, and identification of manufacturer). However with improvements in science and technology (e.g., food processing aids, additives) the ability of food processors to alter the characteristics of food has led to an increasing demand from consumers for more detailed nutritional and safety information. Food labels generally have a product name that describes the food; an ingredient listing, which will include any additives and allergens; nutritional information; net weight; country of origin (not mandatory for all food products); a durability date; and instructions for storage or cooking where necessary.

Food labeling refers to all food labels and any written, printed, or graphic material accompanying the food packaging. Food labels are also used to market a food product using comparative and noncomparative claims, nutrition claims, and health claims.

Food Labeling Legislation in Europe

In Europe the labeling of foodstuffs is coordinated throughout the European Union (EU) (28 member states to date) under the basic rule that the consumer must not be misled. Each member state has its own national Food Authority. The European Food Safety Authority, established in 2003, is an independent body that provides scientific advice and support for community legislation. Food law in the EU is governed by directives and regulations. Directives, on the one hand, prescribe a specific objective, and it is the responsibility of each member state to implement it and to define how to do so. A regulation, on the other hand, comes into effect as prescribed in all member states on a specific date, and its enforcement is enabled in each member state by the introduction of a national statutory instrument. The Food Information to Consumers (FIC) Regulation 1169/2011 came into force across Europe on 13 December 2014. Each member state introduced a statutory instrument to implement the regulation, which included guidelines for national authorities on enforcement and penalties.

Food Labeling Responsibilities in Non-EU Countries

Food labels must comply with regulations to protect consumers from inaccurate, misleading, or insufficient information that may result in deception, illness, or fatalities (e.g., due to anaphylaxis). Even though food distribution has become more globalized, the national rules and procedures are still very diverse. Codex Alimentarius is a collection of internationally recognized food standards and codes of practice to ensure food safety. Codex standards have been adopted into national legislation by many countries. However, bringing a new product to an international market requires detailed examination of the food regulations within the country of import, hence the relevant authorities for each country should be consulted accordingly. The United States Department of Agriculture (USDA) Foreign Agricultural Services also provides useful reports on food laws and regulations in 202 countries and regions (as of 01/01/2015). The prevailing food labeling regulations in Canada, Japan, China, India, the United States, Australia, and New Zealand are outlined next. These are subject to revision and should be checked for updates.

In Canada the Food and Drug Regulations, published in 2003, detail the labeling regulations for both food and drugs. These were amended in 2012 to include labeling of allergens, gluten sources, and sulfites. The responsibility for food labeling is shared by Health Canada and the Canadian Food Inspection Agency (CFIA). In Japan the Consumer Affairs Agency has published a single Food Labeling Standard, based on the Food Labeling Act (Act No. 70 of 2013), which is expected to come into force in April 2015. There will be a 5-year transition period to allow manufacturers to implement mandatory nutrition labeling on all prepacked foods. In China the general rules on Pre-packaged Food Labeling (GB7718) were implemented in 2005. China's Ministry of Health released the National Food Safety Standard for Nutrition Labeling of Prepackaged Food (GB 28050-2011) in 2011. In India a new regulatory authority was established in 2011, the Food Safety and Standards

Authority of India (FSSAI). The FSSAI requires prepackaged food to contain certain mandatory information as outlined in the Packaging and Labeling Regulations of 2011. One requirement specific to labels in India is the symbol to declare if a food is vegetarian or nonvegetarian.

In the United States, food labeling is regulated by the Food and Drug Administration (FDA) for all foods except meat, poultry, and egg products, which are regulated by the Food Safety Inspection Services. In the United States food products are governed by the Federal Food, Drug and Cosmetic Act (FD&C Act) of 1938 and the Fair Packaging and Labeling Act (1967). Nutrition labeling is required on most food labels since 1994 (Nutrition Labeling and Education Act). In 2014 the FDA put forward a proposal to revise the Nutrition and Supplement Facts Label. Food labeling in Australia and New Zealand must conform to the National Food Standards Code (Part 1.2) developed in 2002 by Food Standards Australia New Zealand (FSANZ). The Food Act 2003 implements the Food Standards Code and is enforced by the New South Wales Food Authority. FSANZ has been asked to review food labeling laws over a 3-year period, which ends in 2015, and make recommendations to Australian and New Zealand Food Regulation government ministers.

Mandatory Food Labeling

Voluntary labeling elements such as branding and marketing claims can dominate a label and relegate the presentation of essential mandatory information. To avoid this, the presentation of mandatory information is governed by legislation, and although there may be some regional differences from country to country, there are certain elements common to all food labels. In Europe, the FIC Regulation 1169/2011 came into force on 13 December 2014. Under the FIC regulation, food business operators at all stages of the food chain are responsible for providing mandatory information. This allows the end retailer to accurately label the food product for sale to the final consumer. Listed next are details of the 12 mandatory requirements that must be displayed on a European food label in a mandatory minimum font size. For the purpose of illustration, an example of how these are applied to specific food products is provided in each case; also included are some comparisons with other non-EU countries.

Product Name

The product name must not mislead the consumer. In Europe the name must be a legal, customary, or descriptive name. The name must inform the consumer of the nature of the product, but if the product is a recipe dish then it may also need an attached description to distinguish the contents from other similar products on the market. For example, Jam is a legal name with a reserved description, Mayonnaise is a customary name and recognized as a creamy sauce used as a condiment in all countries, Lasagne is a customary name in Europe but there may be recipe variations from country to country (vegetarian or meat), so a descriptor would also be required.

In the United States and Canada 'standards of identity for food' are the mandatory requirements that determine what a food product must contain to be marketed under a certain name. These standards ensure the label accurately displays what is inside the package. For example, Mayonnaise has a specific description under the Code of Federal Regulation (21 CFR 169.140). Only products that satisfy these conditions can use the name Mayonnaise.

List of Ingredients

In most countries the ingredients on a food label are listed by their common name in descending order by weight of the prepackaged product. The order or percentage must be calculated when added at the mixing bowl stage.

Some products may contain a compound ingredient – an ingredient made up of two or more ingredients, for example, the tomato sauce (peeled tomatoes, tomato juice, olive oil, salt) in a cheese pizza would be a compound ingredient. In Europe compound ingredients must be declared unless they are present in less than 2% of the final food product. In Australia compound ingredients must also be declared unless they make up less than 5% of the final product. However, in all countries, if an ingredient is present in less than 5% but a known allergen, then it must be declared.

Food additives

With an increased working population, busier lifestyles, and advances in manufacturing technology, food processing has increased dramatically since the nineteenth century. This has led to a requirement for alternative methods to aid food production for mass consumption. Food additives are substances added intentionally to foods to perform certain technological function, such as to sweeten, color, or preserve a food product. In European labels food additives must be identified by category followed by the specific name or European registration number, 'E number.' The E number is a code for substances that are approved by the EU for use as food additives on product labels. For example, in the ingredients listing of light mayonnaise (see Table 1), the category Preservative is listed followed by the preservative name potassium sorbate. This could also have been displayed as Preservative (E202). The E number can communicate information clearly across most languages. E numbers are also used on food labels in Australia, New Zealand, South Africa, and Israel. Only additives approved by the Ministry of Health, Labor and Welfare may be used in foods and beverages for sale in Japan. Under Japan's new Labeling Standard, if a

Table 1 Ingredient listing for light mayonnaise

Ingredients: Water, Rapeseed oil (25%), Pasteurized free range ***egg*** (6%), Modified maize starch, Spirit vinegar, Sugar, Salt, Preservative (potassium sorbate), Thickener (xanthan gum), Lemon juice concentrate, ***Mustard*** flavoring, Antioxidant (calcium disodium EDTA).

product is labeled for sale to the consumer, then the label must include the net content of each food additive and the name and address of the additive manufacturer in a separate line under the other ingredients.

China recently published an update on the standard for Food Additive usage (GB 2760-2014), which takes effect in May 2015. Under the new regulation, food categories have been modified, and there will be restrictions of the use of food additives as both flavoring and processing aids.

A small proportion of the population can have adverse reactions such as allergies or intolerances to certain food additives, be they derived from natural or synthetic sources. In addition, some food products may contain or be derived from genetically modified organisms (GMOs), which some consumers may not wish to consume. The labeling of sweeteners, colors, preservatives, and GMOs is discussed next.

Sweeteners

Sweeteners are ingredients that sweeten like sugar (sucrose), but may be low-calorie synthetic substitutes. Under EU legislation, food products containing a *sweetener* or *sweeteners* must include the statement 'with sweeteners' on the label accompanying the name of the food product. The relationship between sweeteners and negative health effects has been studied for many years by many different regulatory agencies, and after rigorous safety assessments and the establishment of safe levels of use, certain sweeteners have been approved as food additives. The low-calorie tabletop sweetener aspartame contains the amino acid phenylalanine. People who have the inherited disease phenylketonuria (PKU) are unable to metabolize phenylalanine effectively. If aspartame is listed in the ingredients by its E number, E951, then the label must also include the statement 'contains aspartame (a source of phenylalanine)' so that people who suffer from PKU can avoid consuming these products.

Colors

The color of a food product is an important part of the consumer's sensory experience of the product. Moreover, the color of a food can indicate whether it is fresh or stale, has a particular flavor, or contains certain ingredients. For example we expect a red apple to be sweet and a green apple to be sour. However, color can be lost during processing or storage of foodstuffs. Although the coloring of food has been carried out for over 100 years the artificial colors used in food today are subject to regulation, and only those established as being safe are permitted to be used in food. In Europe, studies by the University of Southampton linked certain artificial colors to hyperactivity in children. Although not banned under legislation, since 2010 any food or drink containing one or more of the so-called Southampton Six artificial colors (i.e., sunset yellow (E110), quinolone yellow (E104), carmoisine (E122), allura red (E129), tartrazine (E102), and ponceau (E124)) must carry the health warning 'may have an adverse effect on activity and attention in children.'

In the United States, the FDA divides food color additives into the two categories: those subject to certification (artificial colors) and those exempt from certification (natural colors). The FDA certified nine color additives that can be added to foods in the form of dyes (water-soluble) or lakes (fat-soluble). Certification-exempt additives are extracts from natural foodstuffs giving both color and sometimes flavor. In 2014 the FDA released a new list of color additives that are under review for safe use in foods, drugs, cosmetics, and medical devices. In Canada food additives are regulated under the Food and Drug Regulations and associated Marketing Authorizations. All permitted food additives are referenced in the Marketing Authorisation for Food Additives that may be used as Colouring Agents (SOR/2012-204).

Preservatives

Food can be preserved by physical or chemical methods or a combination of both. Physical methods of preserving food include refrigeration, dehydrating, freeze-drying, and UV-C radiation. Chemical preservatives are substances (natural or artificial) added to food to slow down food spoilage caused by bacteria, fungi, or yeasts. These preservatives can control contamination and help maintain food freshness and quality. In the ingredients listing for light mayonnaise (see **Table 1**), the category Preservative is listed followed by the preservative name potassium sorbate. This could also have been displayed as Preservative (E202). In Europe the preservative sulfur dioxide or sulfites at concentrations of more than 10 mg kg^{-1} in the final product is an allergen and must be highlighted in bold in the list of ingredients (can cause a reaction in people sensitive to asthma).

Genetically modified organisms

This is a controversial issue with consumers in Europe. Under the 1997 novel food regulation, genetically modified (GM) foods only required labeling if the GM content could be detected in the final product. This was updated by Regulation 1892/2003, whereby all food products that use GMOs at any stage of production are subject to labeling requirements, even if the GM content is not detected in the final product. However in reality this can be difficult to monitor so a threshold was introduced. Food products that have GM content equal to or less than the 0.9% threshold would not require labeling provided the presence is due to unavoidable cross contamination during or postharvesting.

Japan, Australia, Russia, Brazil, and China all require the labeling of GM foods on their prepacked products. The United States currently has a voluntary labeling system for GM foods. Consumer demand for transparency in the last 5 years has led to many individual states passing GMO labeling laws. Food industry groups are lobbying for the FDA to study GM traits and crops (compared to non-GM foods) and set standards for GM labeling, which they believe would build consumer support for biotechnology.

Allergens

Even though the list of allergens varies slightly from country to country, food regulatory authorities agree that for safety reasons allergens must be highlighted on prepacked foods. In Europe under the FIC regulation there are 14 allergens that, if present in prepacked food, must be highlighted in the ingredient listing in a font style that distinguishes it from the rest of the text. Most European manufacturers highlight (in bold or

capitals) the allergen itself or display the allergen in brackets immediately after the relevant ingredient. For example, in light mayonnaise the allergens egg and mustard are highlighted in *italics* (see Table 1).

The presentation of allergen information on nonprepacked food (i.e., loose food, food packed on the business premises at the consumer's request) is also mandatory. For some countries this presentation can be verbal but national legislation in Ireland and France requires the written display of allergen information for nonprepacked food for all businesses dealing with food, that is, restaurants, bakeries, farmers markets, hospitals, bars, crèches, fast-food outlets, distance selling, and so on.

In Australia there are nine common food allergens (peanuts, tree nuts, milk, eggs, fish, shellfish, wheat, sesame, and soy) that must be declared on packaging. FSANZ is currently working on the recommendation for the presentation of allergens emboldened both in the ingredients list and in a separate list. In Canada food labeling laws were amended in 2012 to include labeling of allergens, gluten sources, and sulfites (if present in more than 10 ppm). There are currently 13 allergens that must be identified if present on an ingredient listing. In Japan there are seven allergens (peanuts, milk, eggs, buckwheat, wheat, crab, shrimp/prawn) that require mandatory labeling and 20 allergens for which labeling is recommended. Similar to European labeling, if the product contains the same allergen in multiple ingredients (e.g., an egg salad would also contain eggs in the mayonnaise), the allergen must be listed each time after the ingredient.

Quantitative Ingredient Declaration

In the EU a quantitative ingredient declaration (QUID) is displayed as a percentage of the characterizing ingredients that are displayed on (or associated with) the name of the product. In the example (Table 1) light mayonnaise is made from a combination of rapeseed oil, egg yolks, vinegar, or lemon juice, therefore the primary ingredients – the rapeseed oil and egg – are QUID at 25% and 6%, respectively.

Net Quantity

Net quantity is required in metric units on all foods (FIC, Article 23). The units of volume for liquids are liters (l), centiliters (cl), or milliliters (ml), and the units of mass in the case of all other products are grams (g) or kilograms (kg).

Durability Dates

The date of minimum durability of a foodstuff is the date until which a food retains its specific properties when properly stored. There are two types of date markings, a best-before date or a use-by date. Most nonperishable foods with a long shelf life such as tinned beans or pasta, which do not require refrigeration, have a best-before date. A use-by date is required for foods that are highly perishable, may require refrigeration, and are likely after a short period to be an immediate danger to human health, for example, raw and cooked meats, milk, and yogurts. In Europe, under the FIC regulation, frozen products require a best-before date and a date of freezing on the label. Manufacturers use shelf life testing to calculate the durability date for each product. In Canada if fish has been frozen and thawed prior to sale, then it must include the words *previously frozen* on the label.

Storage

In Europe (FIC Regulation 1169/2011) storage conditions must be included if necessary to maintain the quality of the product. For a perishable product like the light mayonnaise example in Table 1, the label would read 'Storage: Keep refrigerated for 1 month after opening.'

Instructions for Use

Manufacturers must provide instructions for use for any foods that need specific preparations to avoid food poisoning and ensure they are safe. In Europe, uncooked meat and meat preparations intended for supply to the final consumer must bear a notice indicating that such products must be cooked before consumption. If cooking is necessary then the label must contain instructions on preparation, cooking, or reheating.

Business Name and Address

A food label must contain the name (or business name) and address of the manufacturer or seller within or outside the EU (FIC Regulation, 1169/2011). The contact details must be precise enough to enable a customer to contact the named agent where necessary. A website address or telephone number alone is not acceptable.

Country of Origin

In the EU, country of origin labeling is currently mandatory for certain products such as beef, fish, honey, olive oil, wine, fresh fruit, and vegetables, and after 1 April 2015, mandatory origin labeling will be extended to whole cuts from poultry, pork, sheep, and goats. The origin declaration of meat as an ingredient is currently under discussion in the European Commission. General labeling rules require that the origin of a product must be declared where its absence might mislead the consumer as to the true origin, whether due to misleading pictures, maps, or flags.

In the United States, country of origin labeling has been mandatory since 2013 for certain meat products (beef, pork, lamb, chicken, goat meat), wild and farm-raised fish and shellfish, perishable agricultural products, ginseng, peanuts, pecans, and macadamia nuts.

In Australia all packaged food must carry country of origin information. This can be presented as a statement identifying the country where the food was produced or grown or the country where the food was manufactured or packaged. In New Zealand the country of origin labeling is voluntary, but the contact details of supplier must be labeled should further information be required.

Table 2 Nutritional information for light mayonnaise[1]

	Light mayonnaise		
Nutrient	*Per 100 g Light*	*Per portion (per 15 g)*	*% RI[a] per portion*
Energy	1190 kJ/288 kcal	179 kJ/43 kcal	2.1%
Fat	28 g	4.2 g	6%
- of which saturates	2.4 g	0.4 g	2%
Carbohydrates	7.4 g	1.1 g	0.4%
- of which sugars	3 g	<0.5 g	0.6%
Protein	0.8 g	<0.5 g	1%
Salt	1.8 g	0.28 g	4.7%
Omega 3 fatty acids	2.5 g	0.4 g	

[a]Reference intake of an average adult is 8400 kJ/2000 kcal.
[1]All information, with the exception of % RI, were extracted from SuperValu Light Mayonnaise label with permission from Musgrave Retail Partner, Ireland.

Nutritional Declaration

In Europe a nutritional declaration is not mandatory on food labels until 13 December 2016, with some exceptions; that is, if making a nutrition or health claim or if a food label currently displays nutritional information, this must be updated to comply with the FIC format. The regulation prescribes the layout and sequence of the nutritional declaration (see light mayonnaise example in Table 2).

In Europe the mandatory information (energy, fat, saturated fat, carbohydrate, sugars, protein, and salt) must be presented per 100 g or 100 ml of finished product. Vitamins and minerals permitted for use in foods are listed in Annex III of the FIC Regulation 1169/2011. These can only be included if present in a significant amount (15% of the reference intake). Reference Intake (RI) has replaced the recommended daily allowance (RDA) and if used must be accompanied by the statement "reference intake of an average adult is 8400 kJ/2000 kcal." The nutritional information per portion and %RI is considered voluntary information but if included it must be presented as prescribed in the regulation.

In the United States nutritional information is presented under the heading 'Nutrition Facts' but the information is displayed as the amount per serving rather than per 100 g. The nutritional facts label is required on most prepacked food; for exemptions see further reading. After 20 years the FDA put forward a proposal in 2014 to revise the Nutrition and Supplement Facts Label. The new recommendation proposes removing 'calories from fat' and focusing on the total calories. Certain information will be emphasized (larger font and bold type) such as calories, serving sizes, and percentage daily value. The daily value is a single term based on the amount of nutrients (daily reference values) and the amount of vitamins and minerals (reference intake values) present in a food. The proposal moves the information on % daily value to the left of the column on the nutritional table. The FDA has also proposed changes to the serving size to realistically reflect how much people typically eat at a single sitting according to 2010 Dietary Guidelines for Americans. Added sugars will be included on the label; these are the sugars added during food processing. The update will require some changes in the mandatory declaration of certain nutrients if present in the food: Calcium and iron remain on the label, potassium and vitamin D must be included. However, vitamins A and C will now be voluntary. The consultation process closed in August 2014, and the information must be examined before a final ruling on proposed changes to the labeling regulation.

Canada introduced mandatory nutritional labeling for most prepackaged food in 2005. The Nutritional Fact Table presented information on the calories, 13 core nutrients, and % daily value of nutrients. In June 2014 the CFIA published the Food Labeling Modernization Initiative, which proposes changes to food labels based on recent scientific information. New guidelines on serving size based on current information, more information on sugars, and added sugars is presented either on the table or in the ingredients listing. Similar to the United States, there are changes in nutrients of concern to public health such as potassium and vitamin D, with removal of vitamins A and C from the table. The consultation process is now closed, and the information must be examined before a final ruling on the proposed changes to the labeling regulation. Prepacked food in Australia and New Zealand display a nutritional table very similar to the European model.

Front-of-pack nutritional labeling

Additional nutritional information can also be presented in Europe as a voluntary front-of-pack nutrition label (or nutritional lozenge) (see Table 3).

This was introduced to provide consumers with the most important information (energy, fat, saturates, sugars, and salt) at a glance. The United Kingdom introduced a traffic light color coding element to the nutritional lozenge whereby green was low, yellow was medium, and red was high in a particular nutrient. If this system was applied to the light mayonnaise example, then fat would register as yellow, and saturates, sugar, and salt would all be colored green. This traffic light system has been criticized by some EU states as being oversimplistic for foods with multiple components and for being discriminatory and undermining free trade in the EU. As a result, the use of the UK traffic light system is currently under investigation by the European Commission.

Table 3 Front of pack nutritional information for light mayonnaise (displaying nutrient values and the % reference intake per portion (15 g) of light mayonnaise)

Energy	Fat	Saturates	Sugars	Salt
179 kJ/ 43 kcal	4.2g	0.4g	<0.5g	0.28g
2.1%	6%	2%	<1%	4.7%

Energy per 100 g: 1190 kJ/ 288 kcal.

Alcoholic Strength

In the EU, beverages with more than 1.2% alcohol by volume must declare their actual alcoholic strength on the label. Canada, Australia, New Zealand, and China have similar labeling requirements for declaration of beverages more than 1.1%, 1.15%, and 1.2%, respectively. Japan and the United States do not specify the minimum amount but do require the alcohol content to be displayed on the label.

Minimum Font Size

Under the FIC regulation in Europe, all prepacked food labels must display the mandatory information in a minimum font size. In Europe if the surface area of the package is 80 cm^2 or more, then the font used must have a minimum *x*-height of 1.2 mm (Times New Roman size 8 approximately). If the pack's surface area is less than 80 cm^2 then the font may be displayed with an *x*-height of 0.9 mm (Times New Roman size 6 approximately). The minimum font size required for labels in Japan is 8-point for all characters. In the United States and Canada the letters and numbers on the principal display panel must not be less than one-sixteenth of an inch in height.

Claims on Food labels

Codex Alimentarius defines a claim as:

> Any representation which states, suggests or implies that a food has particular characteristics relating to its origin, nutritional properties, nature, production, processing, composition or any other quality.

Food and safety authorities around the world monitor and regulate claims displayed on food labels. In Europe nutrition and health claims are highly regulated. The Community Register contains all the claims submitted, but only the authorized claims (the EU positive list) can be used on food labels.

Nutrition Claims

A nutrition claim is made when a food highlights a specific benefit provided by a particular nutrient in the food product. Before a food label can make a nutrition claim, it must first prove that the nutrient in question is present (or absent) in a 'significant amount,' 15% of the DRI. To do this food products must complete a nutritional analysis. For example, a food label that displays the words 'source of vitamin C' must show that vitamin C was present in the final product at 15% of the RI. Comparative claims such as claiming a product is 'light' or 'low fat' must adhere to the definition and conditions of use outlined in Community Register database. If the product is a 'light' mayonnaise, then it must show that the fat content of the mayonnaise has been reduced by at least 30% when compared to a similar product. The light mayonnaise in Table 2 was more than 30% lower in fat (28 g) than a standard mayonnaise (79 g of fat).

Health Claims

A health claim is any claim that shows a relationship between food and health. There are three types of health claims authorized for use on food products in Europe: disease risk reduction claims and children's health and development (Article 14), general function health claims (Article 13), and claims based on new scientific evidence (Article 13.5). Most of the authorized general function health claims can be used if the particular nutrient in the food product fulfills the criteria required to make the claim. For example, the light mayonnaise (Table 2) contains omega-3 fatty acids, contributed from the egg used to make the mayonnaise. The omega-3 is presented in the table as 2.5 g per 100 g of mayonnaise. This was greater than the amount necessary (>0.3 g) and fulfills the criteria required to make the health claim 'Source of alpha-linolenic acid, which helps maintain a healthy blood pressure.'

Food Supplements

In the EU, food supplements, defined as 'foodstuffs,' are governed by the general labeling rules of the FIC regulation 1169/2011. However, under Food Supplement Regulation 1924/2006 there is some additional information on daily dosage and health warning statements that must also be included on the label. Food supplements can display nutrition and health claims on the label as long as the nutrient in the supplement is on the EU positive list and fulfills the criteria for each claim. It is important to have each food product scientifically analyzed (for shelf life testing and nutritional information) to substantiate any claims.

In the United States there are three claim categories, regulated by the FDA, that can be used on food labels: (1) health claims, (2) nutrient content claims, and (3) structure or function claims. Health claims such as 'adequate calcium throughout life may reduce the risk of osteoporosis' can be used if authorized by the FDA and meet the required criteria. Nutrient content claims 'low in sodium' or 'high in folate' must only be used with nutrients that have an established daily value and meet the criteria for the particular claim. Structure of function claims describe the role of a nutrient in maintaining normal structure of function in the human body, for example 'calcium builds strong bones.'

In Australia a new standard to regulate nutrition and health claims (currently voluntary) became law in 2013 with a 3-year transitional period for food businesses to comply. Under the new food standard, nutrition claims will need to meet certain criteria as set out in the standard. Health claims fall into two categories: general-level health claims, such as 'calcium is good for bones,' and high-level health claims, such as 'calcium may reduce the risk of osteoporosis.' General health claims can be based on one of 200 preapproved claims or self-substantiate the food health relationship claim by following the requirements in the Food Standard. As of October 2013 there were 13 preapproved high-level health claims. Health claims will not be permitted on foods high in saturated fat, sugar, and salt. They will only be permitted on foods that meet the nutrient profile scoring criterion. As in Europe, the manufacturer must substantiate that the claims are truthful and not misleading to the consumer.

See also: Authenticity of Food; Dietary References: US; Food Additives: Classification, Uses and Regulation; Food Allergies; Functional Foods; Legal Requirements for Food Hygiene; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Preservatives: Food Use.

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Food Poisoning: Classification

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What is Food Poisoning?

Unavoidably, in addition to nutrients, food also contains harmful substances that may be poisonous at low doses, and even chemicals that are harmless in small amounts, but may be damaging in large quantities. They may enter the food accidentally or intentionally, during growth, cultivation, or preparation. They can be formed in the food through the interaction of chemical components, accumulated during storage, or they may be concentrated from the natural components of the food. Microorganisms may be introduced directly to infected food from animals, workers, other foods, or the environment during food preparation. Poisonous substances may be produced by the growth of bacteria and molds in food. In some cases, the term food poisoning (or foodborne disease or foodborne illness) is used to denote gastrointestinal complications that occur following recent consumption of a particular food or drink. In a wider perspective, food poisoning, or food toxicity, is a term used to imply the adverse effects caused by the direct action of a food or food additive on a host recipient without the involvement of immune mechanisms.

Poisons: Toxins and Toxicants

Before the establishment of the food poisoning classification, several concepts should be defined. The term 'toxin' generally refers to toxic substances produced by biological systems such as plants, animals, fungi, or bacteria. They are generally metabolic products that have evolved as defense mechanisms for the purpose of repelling or killing predators or pathogens. The term 'toxicant' is used for toxic substances that are produced by, or are a by-product of, anthropogenic (human-made) activities.

These toxic substances can produce, according to their exposition and doses, different types of intoxications: acute (toxicity elicited immediately following short-term exposure to a harmful substance), subchronic (toxicity caused by repeated dosing over an extended period, but not one that constitutes a significant portion of the normal life span), and chronic (result of long-term exposure to a toxic substance).

Food Poisoning Classification

There are several considerations when classifying food poisoning. In the literature, different classification methods can be found, such as according to an incubation period, the chemical structures of toxics, the toxicological targets, or classifications related to the tolerance setting for toxic substances. Substances such as pesticide residues, drugs used in food-producing animals, heavy metals, chlorinated organics, nitrosamines, nitrosamides and N-nitroso compounds, mycotoxins, furan, and others have tolerance settings. We can find other substances, such as toxins present in fish and shellfish, preformed bacterial toxins, and others, for which tolerances may not be set.

In this article, a simple classification of five groups will be used to describe the toxic substances according to their origin:

- Biological pollutants
  - Bacterial intoxications
  - Bacterial infections
  - Foodborne animal parasites (protozoa, flatworms, roundworms, and tapeworms)
  - Viruses
  - Prions and transmissible spongiform encephalopathies
  - Mycotoxins
- Toxic substances of natural origin
  - Sea products
  - Higher plants
  - Higher mushrooms
  - Miscellaneous
- Chemical pollutants and/or residues
  - Inorganic substances
  - Organic substances
    - Environmental pollutants (Dioxins, dibenzofurans, and polychlorinated biphenyls)
    - Substances intentionally added to food (Pesticides and veterinary drugs)
- Food additives
- Toxic substances formed during food processing, preparation, storage, and intake.

Biological Pollutants

The biological pollutants, including foodborne bacteria, viruses, and microbes (pathogens) cause most illnesses in the world; this includes estimating the number of biological pollutants as causes of foodborne illnesses without a known cause. This group also includes foodborne animal parasites, prions, and mycotoxins. The last estimates made in the United States (2011) indicate that, each year, roughly one in six Americans (or 48 million people) gets sick, 128 000 are hospitalized, and 3000 die of foodborne diseases associated with these biological pollutants. Nontyphoidal *Salmonella*, *Toxoplasma*, *Listeria*, and norovirus caused the most deaths; nontyphoidal *Salmonella*, norovirus, *Campylobacter*, and *Toxoplasma* caused the most hospitalizations; and norovirus caused the most illnesses. Bacterial-related foodborne diseases can be classified as either intoxications or infections.

Bacterial intoxications

Foodborne intoxications result when toxins or poisons from bacteria are present in the ingested food. These toxins are generally odorless and tasteless, and they are capable of causing disease even if the microorganisms have been eliminated. *Clostridium botulinum* (in improperly canned foods),

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Staphylococcus aureus (in meat, eggs, and milk), and *Bacillus cereus* (in starchy foods) are the most important pathogens that cause bacterial intoxications. Table 1 shows the most important pathogens, their occurrence, main transmission routes, symptoms, and possible complications or chronic sequelae.

Bacterial infections

Foodborne infections result from eating a food containing a large amount of disease-causing microorganisms, which are capable of producing or discharging a toxin once they are ingested. Nontyphoidal *Salmonella* (in raw or undercooked food of animal origin), *Clostridium perfringens*, and *Campylobacter* spp. (in water and raw products) are the most important bacterial infections contributing to domestically acquired foodborne illnesses. Table 1 outlines information about the most important pathogens responsible for bacterial infections and their occurrence, main transmission routes, symptoms, and possible complications or chronic sequelae.

Foodborne animal parasites (protozoa, flatworms, roundworms, and tapeworms)

Parasitic Protozoa and Worms (MS 770 Zoonoses)

Protozoa important in food and water include *Cryptosporidium*, *Giardia lamblia*, *Entamoeba histolytica*, *Toxoplasma*, and *Cyclospora*. They are parasites, and exposure occurs via ingestion of water contaminated with fecal material from an infected animal or via ingestion of food that was irrigated or washed with contaminated water.

Cryptosporidium's most common symptom is profuse, watery diarrhea, along with nausea, vomiting, and cramping. Fever can also accompany these symptoms. As noted, the severity and duration of diarrhea usually is increased in immunodeficient people.

The symptoms from exposure to *Giardia lamblia* (*Giardia intestinalis*) generally consist of especially malodorous diarrhea, malaise, abdominal cramps, flatulence, and weight loss. Giardiasis is self-limiting in most people.

Entamoeba histolytica is capable of infecting a wide variety of hosts, which causes invasive disease in humans. When symptoms do occur, they usually begin within months after amoe-

Table 1 Foodborne bacterial intoxications and toxic infections

Pathogen	*Occurrence*	*Main transmission routes*	*Symptoms*	*Possible complications or chronic sequelae*
Bacterial intoxications				
Clostridium botulinum (MS 170–172)	Soil, water, plants, and intestines of animals and fish	Home-cured ham and sausages. Improperly canned foods, garlic in oil, tightly wrapped foods	Early symptoms from 4 h to 8 days after eating include unspecific gastrointestinal symptoms and paralysis, including paralysis of the breathing muscles.	Botulism is a serious, sometimes fatal, disease caused by a potent neurotoxin formed during growth of *C. botulinum.*
Staphylococcus aureus (MS 654–656)	Human and other animal skin, hair; in nasal passage and throat (staphyloenterotoxicosis; staphyloenterotoxemia)	Food handler to food (meat, meat products, poultry, egg products, salads, bakery products, milk and dairy products)	When ingested, the enterotoxin may rapidly produce symptoms (1–6 h), which commonly include nausea, abdominal cramping, vomiting, and diarrhea.	In more severe cases, dehydration, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur.
Bacillus cereus and other *Bacillus* spp. (MS 50-52)	*B. cereus* is widespread in the environment, often isolated from soil and vegetation	Cereals, rice, soups, vegetables, puddings, sauces, creams, other starchy foods	*Diarrheal type*: watery diarrhea, abdominal cramps, and pain. Emesis rarely occurs. *Emetic type*: nausea and vomiting	Other clinical manifestations of *B. cereus* invasion and infection include severe systemic and pyogenic infections, gangrene, septic meningitis, cellulitis, panophthalmitis, lung abscesses, infant death, and endocarditis.
Bacterial toxic infections				
Salmonella spp. (MS 606–608)	Intestines and feces of animals; *Salmonella enteriditis* in eggs	Raw or undercooked food of animal origin, mainly meat, poultry, eggs, and milk	Fever, headache, nausea, vomiting, abdominal pain, and diarrhea appearing 9–72 h after infection, lasting 1–7 days.	In ~2% of culture-proven cases, reactive arthritis may occur after ~3 weeks; also Reiter's syndrome
Campylobacter jejuni (MS 105–107)	Intestines of animals and birds, raw milk, untreated water, sewage sludge	Contaminated water, raw milk, raw or undercooked meat or poultry, or shellfish	Headache, fever, and muscle pain 2–5 days after ingestion; later severe abdominal pain, nausea, and diarrhea (sometimes bloody). May last 7–10 days.	May lead to reactive arthritis and neurological disorders in 2–10% of patients.

(*Continued*)

Table 1 (Continued)

Pathogen	*Occurrence*	*Main transmission routes*	*Symptoms*	*Possible complications or chronic sequelae*
Yersinia enterocolitica (MS 763–764)	Soil, water, often isolated from pigs, cats, dogs, birds, and beavers	Meats, oysters, fish, raw milk	Fever and abdominal pain, frequently also gastroenteritis with diarrhea and/or vomiting within 24–48 h after infection; may mimic appendicitis.	Can cause 'primary septicemia' in individuals with underlying chronic disease (e.g., diabetes, cirrhosis, AIDS, leukemia). This form is associated with 50% mortality.
Shigella spp. (MS 627)	Human intestinal tract, food, water	Person-to-person by fecal-oral route or fecal contamination of food handled by workers with poor personal hygiene	Diarrhea and dysentery (diarrhea with blood and mucus in the stools), vomiting, abdominal cramps, rectal pain and fever, appearing 12–50 h after infection and lasting a few days to 2 weeks.	Reiter's syndrome, reactive arthritis, and hemolytic uremic syndrome. Some strains are associated with fatality rates of 10–15%.
Vibrio parahaemolyticus (MS 724–725)	Contaminated fish and shellfish, especially oysters	Raw seafood or cooked seafood contaminated with raw product; improper refrigeration of seafood products	Diarrhea, abdominal cramps, nausea, vomiting, fever, and bloody diarrhea. Incubation period is 4–90 h. Duration of illness is 2–6 days.	Although the illness is generally mild or moderate, *V. parahaemolyticus* can also cause septicemia in susceptible people.
Mycobacterium bovis (MS 477)	Water, soil, cows, animal and human feces, nasal passage, saliva	Unpasteurized milk and dairy products; meat products	Fever, night sweats, fatigue, loss of appetite, and weight loss; other symptoms depend on the part of the body affected.	If lungs affected: chronic cough, blood-stained sputum, or chest pain If gastrointestinal tract affected: diarrhea, abdominal pain, and swelling. The disease may result in death, if untreated.
Brucella spp.	Symbionts and plant pathogens, intracellular animal pathogens, and environmentally ubiquitous bacteria	Unpasteurized milk and soft cheeses made from the milk of infected animals; brucellosis transmitted from animal hosts to humans highly contagious, but rarely transmitted from human to human	Intermittent fever, chills, sweating, weakness, malaise, headache, and joint and muscle pain	Complications may include symptoms of endocarditis or myocarditis, such as shortness of breath, arrhythmia, edema, or chest pain; meningoencephalitis, such as severe headache, stiff neck, confusion, or seizures; or spondylitis, such as back pain.
Vibrio cholera Serogroups O1 and O139 (MS 724–725)	Aquatic environments, freshwater and saltwater. It grows there naturally or can get into the water from the bowel waste of infected people (sewage).	Ingestion of water, ice; unwashed, contaminated food; and seafood that is raw, improperly cooked, or cross-contaminated by a raw product	Abdominal discomfort and diarrhea that may vary from mild and watery to acute, with rice-water stools. Vomiting also occurs in some cases.	Cholera gravis, the most severe form of cholera infection, is characterized by severe fluid and electrolyte loss from vomiting and profuse, watery diarrhea. Complications include tachycardia, hypotension, and dehydration.

bas first enter the body. The severity of the symptoms associated with intestinal amebiasis range from mild diarrhea to a severe, dysentery-like illness with mucus and blood in the diarrhea, as well as abdominal distention.

Toxoplasma gondii are ubiquitous parasites found around the world. In some locations, up to 95% of the local human population has been infected with *T. gondii*. People can become infected by different ways, such as eating raw or undercooked, contaminated meat—and not only meats, such as pork and beef, but also seafood, such as clams and oysters.

Cyclospora cayetanensis is more common in areas around the tropics, and people who travel to those areas are at

higher risk of getting this illness. Symptoms begin about a week after eating the contaminated food. They include diarrhea (sometimes explosive), loss of appetite, weight loss, cramps, bloating, nausea, and tiredness.

Among the worms, three types can be distinguished:

- Tape worms such as *Taenia* (exposure by eating undercooked pork or beef; these worms can cause diarrhea, nausea, pain, and a change in appetite)
- Roundworms, nematodes such as *Anisakis*, *Gnathostoma spinigerum*, *Trichinella spiralis*, *Ascaris lumbricoides*, and *Trichuris trichiura* (exposure by eating raw and undercooked fish; the symptoms range from tickling caused by a worm crawling up the throat to stomach or abdominal pain, nausea, vomiting, and diarrhea, from mild to severe, as well as allergic reactions)
- Flatworms, helminths, *Fasciola hepatica*, *Diphyllobothrium* species (found in raw and undercooked fish; symptoms usually are mild abdominal discomfort, diarrhea, and changes in appetite, and they may begin in about 10 days after ingestion; these kinds of worms can infect people who eat undercooked meat, especially from wild game)

Viruses (MS 727)

A number of different viruses, such as norovirus, the hepatitis A virus, the hepatitis E virus, rotavirus, and others, cause gastrointestinal illness in humans. Although norovirus usually causes a mild illness, it is a leading cause of foodborne deaths because it affects so many people. Examples of foods that have caused norovirus illnesses are fruits, vegetables, meats, and salads prepared or handled by an infected person. Oysters grown in contaminated water are another example. Norovirus infections are characterized by nausea, explosive vomiting, watery diarrhea, and abdominal pain. Hepatitis A virus can cause long-lasting liver disease and typically spreads via raw or undercooked seafood or contaminated raw produce. Infected food handlers are often the source of food contamination.

Prions and transmissible spongiform encephalopathy (MS 80)

In both humans and cattle, a disease-causing prion is a protein that has taken the wrong shape. Normally, the correctly shaped prion protein helps the brain and nerves work properly, but when it is the wrong shape, it can result in variant Creutzfeldt–Jakob disease (vCJD) in humans. Although this disease made headlines when it appeared in the mid-1990s, it is important to remember that, of the entire population of the world, only 217 cases of vCJD have been reported, and added safety regulations for feeding cattle appear to be working to prevent the disease.

Mycotoxins (MS 478-480)

Mycotoxins are secondary metabolites produced by fungi that infect a variety of crops, including cereals, nuts, spices, and, in some cases, fruits. The most important mycotoxins found in food are:

Aflatoxins. There are six different aflatoxins (B1, B2, G1, G2, M1, M2) produced by *Aspergillus flavus* and *Aspergillus parasiticus*. They cause hepatic cirrhosis and cancer, especially aflatoxin B1. They can occur in nuts (peanuts, almond, pistachios, and Brazil nuts), corn, other cereals, and milk.

Ochratoxins. The ochratoxins are a group of structurally related secondary metabolites produced by *Penicillium verrucosum* and *Aspergillus ochraceus*; ochratoxin A is the main and most toxic of these mycotoxins. It is nephrotoxic in almost all animals, producing hepatotoxic, teratogenic, and immunotoxic effects at higher doses. The ochratoxins can be present in barley, corn, wheat, rye, oils, seeds, coffee beans, wine, and poultry.

Tricothecenes. These mycotoxins are produced by the fungi *Fusarium* (such as *F. poae*, *F. sporotrichioides*, *F. graminareum*, and *F. culmorum*) and are subdivided into different categories, such as type A, which includes T-2 toxin and HT-2 toxin. The most important tricothecenes are type B, including deoxynivalenol and nivalenol. These toxins inhibit protein synthesis in the cells and induce apoptosis. The tricothecenes can occur in corn, barley, rice, wheat, milk, and other substances.

Fumonisins. Fumonisins are mycotoxins from *Fusarium* spp. They are present in corn and other cereal grains. Ingestion of fumonisin B1 during the first trimester of pregnancy causes neural tube defects and other disturbances in fetal sphingolipid profiles.

Zearalenone. Zearalenone, produced by *Fusarium graminearum*, are associated with hematoxicity, hepatotoxicity, estrogenic activity, and a decrease in fertility acting as an endocrine disruptor. Zearalenone can occur in corn, other cereal grains, soybeans, and animal feed.

Patulin. Patulin, produced by *Penicillium expansum*, causes embryotoxicity and chromosomal damage. It appears in spoilt fruits and fruit juices, especially apple juice.

Citrinin. This mycotoxin, produced by *Penicillium citrinum*, causes convulsions, paralysis, coma, gasps, and respiratory arrest, and it can be present in wheat, barley, rye, and oats.

Toxic Substances of Natural Origin (MS 698)

This group includes substances generated by animals and plants during metabolism or by microorganisms used in or present in food. Some of these natural substances are potent toxins with adverse and, in some cases, lethal effects. Clinical evaluation of human poisoning caused by this type of toxin is sometimes complicated by questionable identification of the plant or animal species of origin and the inability to quantify the patient's level of exposure.

Sea products

A variety of toxins are produced either by edible organisms (fish, shellfish) themselves or the sea plankton or algae that are ingested by the fish or shellfish. Consequently, these toxins can be classified as shellfish toxins (saxitoxins, gonyautoxins, okadaic acid, brevetoxins, domoic acids, and azaspiracid) and fish toxins (tetrodotoxin and ciguatoxin). The most common diseases caused by marine toxins in order of incidence are scombrotoxic fish poisoning, ciguatera poisoning, paralytic shellfish poisoning, neurotoxic shellfish poisoning, and amnesic shellfish poisoning.

Table 2 summarizes the sources of various natural toxins, as well as the sea organisms containing those toxins, their location, symptoms, and the toxic mechanisms involved in their toxicity.

Higher plants

Plant-based food products constitute an important source of nutrients and also a number of bioactive compounds whose effects may be considered as positive, negative, or both. Some of these substances have been considered to be endogenous plant toxins and/or antinutritional factors due to their adverse physiological effects on the humans or animals who frequently consume these foods. Nevertheless, their effects are generally reduced after the foods containing such compounds are adequately processed.

Nevertheless, a number of plants are potentially toxic because they contain cyanogenic glycosides from which HCN may be released by hydrolysis. Certain varieties of lima beans, bitter almonds, peach seeds, apricot, plum and cherry stones, cassava, and sorghum are among plants that produce large amounts of cyanide.

Furthermore, some legume species contain certain amino acids that can be associated with several diseases. Among them are human lathyrism (neurolathyrism and osteolathyrism), which has been associated with the presence of some amino acids in several species of *Lathyrus*, such as *L. sativus*.

Vicine and convicine are glucosides of divicine and isouramil (pyrimidine derivatives) that occur in *Vicia faba*. Their consumption by sensitive individuals, mainly in the form of fresh or dried beans, could lead to a disease known as favism. This disease is characterized by hemolytic anemia in people who are genetically deficient in the enzyme glucose-6-phosphate dehydrogenase.

Alkaloids (from the pyrrolizidine group, solanine group, or xanthine group) are present in different plants, such as solanine in potatoes (*Solanum tuberosum*), and they have mutagenic, carcinogenic, neurotoxic, and cardiotoxic effects in addition to inhibiting cholinesterase.

The term *antinutritional factor* means that a substance interferes with the bioavailability of nutrients, resulting in various activities such as inhibition of growth; a goitrogenic response; or damage to certain tissues, such as pancreas or

Table 2 Toxins in sea products (shellfish and fish), endogenous plant toxins, and antinutrients

Food toxins from the sea	*Toxins*	*Sources of toxins*	*Toxic mechanisms*	*Symptoms*
Shellfish toxins				
Paralytic shellfish poisoning (PSP) (shellfish)	Saxitoxins, gonyautoxins Occurrence: worldwide	*Alexandrium* spp., *Gymnodinium* spp.	Voltage-gated sodium channel I	Tingling of perioral area, gastrointestinal problems, numbness of extremities, disturbed muscle coordination, respiratory distress, paralysis
Diarrheic shellfish poisoning (DSP) (shellfish)	Okadaic acid Occurrence: worldwide	*Dinophysis* spp.	Inhibition of protein synthesis and phosphatases; tumor-promoting effects	Nausea, vomiting, diarrhea, and abdominal pain (30 min to several hours after ingestion)
Neurotoxic shellfish poisoning (NSP) (shellfish)	Brevetoxins Occurrence: USA, Caribbean, New Zealand	*Karenia brevis*, *Chattonella marina*, *Chattonella antiqua*	Neurotoxin acting via voltage-gated sodium channel 5	Nausea, numbness of perioral area, paresthesia, disturbed motor control, severe muscular pain
Amnesic shellfish poisoning (ASP) (shellfish)	Domoic acids Occurrence: North America, Europe	*Pseudo-nitzschia* spp.	Activation of the kainite glutamate receptor	Severe headache, loss of short-term memory, can be fatal
Azaspiracid shellfish poisoning (AZP) (shellfish)	Azaspiracids Occurrence: Europe	*Protoperidinium crassipes*	Voltage-gated calcium channel	Nausea, vomiting, severe diarrhea, and stomach pain
Fish toxins				
Puffer fish (*Takifugu niphobles*) Blue ring octopus (*Hapalochlaena*)	Tetrodotoxin Occurrence: Japan, South Pacific		Specific blocker of sodium channels	Paresthesia in mouth, dizziness, headache, muscular paralysis, and collapse
Amberjack (*Seriola spp.*) Moray (*Gymnotorax javanicus*)	Ciguatoxin Occurrence: tropical and subtropical	*Gambierdiscus toxicus*	Voltage-gated sodium channel 5, voltage-gated calcium channel	Gastrointestinal, neurological, cardiovascular, and general symptoms

(*Continued*)

Table 2 (Continued)

Food toxins from the sea	*Toxins*	*Sources of toxins*	*Toxic mechanisms*	*Symptoms*
Barracuda (*Sphyraena spp.*) Mackerel, Tuna, Bluefish, Bonito, Sardines, Anchovies	Scombroid Occurrence: worldwide		Transformation of histidine in histamine	Allergic reaction

Endogenous plant toxicants	*Toxins*	*Plant species*	*Toxic mechanisms*	*Symptoms*
Alkaloids (MS 20)	Pyrrolizidine group	Coltsfoot (*Tussilago farfara*)	Mutagenic, carcinogenic	Ascites, edema
	Solanine group	Potato (*Solanum tuberosum*)	Inhibition cholinesterases	Neurotoxicity
	Xanthine group	Coffee (*Coffea Arabica*)	Neurotoxic, cardiotoxic	Coffee syndrome
Cyanogenic glucosides	Amygdalin, dhurrin, linamarin	Bitter almonds, peach seeds, apricot, plum and cherry stones, cassava, lima bean, sorghum	Toxicity of cyanide (HCN) with high affinity to cytochrome oxidase	Acute: hyperventilation, headache, nausea, coma, death through cytotoxic anoxia Chronic: nerve injuries, konzo
Toxic amino acids	Hypoglycin A	*Blighia sapida*	Bind to coenzyme A, carnitine	Hypoglycemia
	Vicin, convicin	Horsebean (*Vicia faba*)		Favism
	DABA, β-ODAP	Vetch (*Vicia sativa*), grass pea	Decrease glutation in erythrocytes deficient in G6PD Bind to glutamate receptor	Lathyrism
Toxic lipids	Erucic acid	Rapeseed and mustard oils, and other seed oils of *Brassica* spp.	Oxidation of glutamate and inhibition of ATP synthesis in the cardiac mitochondria	Growth retardation, formation of fatty deposits in cardiac muscle
Saponins (MS 610)	Steroidal and triterpenic saponines (Glycyrrhizin)	Soybean, sugar beet, peanuts, spinach, broccoli, alfalfa, apples, potato, licorice	Surfactant activity, decomposing cellular membranes	Nausea, vomiting, diarrhea, dizziness, elevated blood pressure, cardiac arrythmia
Polyvalent antinutrients				
Lectins or hemagglutinins (MS 367)	Proteins, lipoproteins, or glycoproteins (i.e., ricin)	*Ricinus comunis,* Leguminosae Black beans, soybeans, lima beans, kidney beans, peas, lentils	Specifically bind to saccharide groups from cell membranes, affecting nutrient absorption; capable of agglutinating erythrocytes	Inflammation of intestinal mucosa, epithelial destruction, edema and lymphatic tissue hemorrhage
Tannins (MS 683)	Tannins that are hydrolyzable (gallic, digallic, ellagic, tannic acid) Condensed tanins (flavonoids)	Tea, coffee, grapes, wine, cocoa, tropical fruits	Bind proteins, inhibiting digestive enzymes and reducing iron bioavailability	Liver injury, esophageal cancers
Phytic acid (MS 544)		Bran, germ seeds, grains, legumes, nuts	Influence iron, magnesium, zinc, and copper absorption	Symptoms of iron and zinc deficiencies
Gossipol		Cotton seed oil	Interferences with iron and proteins	Toxic to erythrocytes *in vitro*

(*Continued*)

Table 2 (Continued)

Endogenous plant toxicants	*Toxins*	*Plant species*	*Toxic mechanisms*	*Symptoms*
Mineral assimilation				
Glucosinolates (MS 354)	Sinigrin, progoitrin, glycobrassicin after hydrolysis produce isothiocyanate (goitrin), thiocyanate, nitrile	*Brassica* spp. (cabagge, broccoli, brussels sprouts, cauliflowers, radishes)	Thiocyanate inhibits assimilation of iodine by the thyroid gland	Goiter
Oxalic acid	Oxalates	Spinach, tomatoes, tea, beet leaves	Influence calcium, iron, magnesium, and copper absorption	Kidney disease and stones, rheumatoid arthritis, gout
Enzyme inhibitors				
Protease inhibitors	Trypsin (Kunitz inhibitor; Bowman-Birk inhibitor)	Soybeans, beans, peas, beets, cereals	Bind the inhibitor to the enzyme, decreasing the nutritional value of proteins	Growth retardation, pancreatic hypertrophy
Amylase inhibitors	Stable proteins (phaseolamin)	Beans, wheat, cereals	Delay digestion and absorption of carbohydrates	

liver. However, an antinutritional factor might cause no symptoms if the food containing this factor is consumed at the normal level usually present in a varied diet. Among antinutritional factors present in higher plants are lectins, proteins or glycoproteins that agglutinate in the red blood cells of some blood groups. Some lectins, such as ricin, from *Ricinus communis*, are highly toxic.

Phytic acid and its salts are often considered to be antinutrients because they are capable of binding minerals, proteins, and starch. The adverse effects of phytates are related to decreasing mineral bioavailability. In addition, oxalic acid decreases calcium absorption in monogastric animals, and it influences the absorption of other minerals.

Enzyme inhibitors

Other types of antinutrients are enzyme inhibitors and some phenolic compounds that can reduce protein digestibility and mineral bioavailability, or interfere with the reproductive performance of experimental animals.

Table 2 shows a detailed list of toxins and antinutrients found in higher plants, indicating some of their characteristics and adverse effects, as well as their occurrence in plants.

Higher mushrooms

Worldwide, hundreds of mushroom poisonings are fatal each year. Toxins from higher mushrooms are typically classified according to the incubation period: short or long. A mushroom with a long incubation period is normally more dangerous than one with a shorter incubation period. Among mushrooms with long incubation periods are cyclopeptidic (*Amanita phalloides*), which causes the most fatalities, orellanic (*Cortinarius* spp.), and gyromitric (*Gyromitra* spp.); the syndromes produced by these mushrooms are the most important. The mushroom *Amanita phaloides* produces hepatotoxic cyclopeptides (amatoxins, phallotoxins) whose effects are to block the synthesis of mRNA and to inhibit RNA polymerase II. *Cortinarius orellanus* is a nephrotoxic mushroom that generates two types of toxins, called orellanines and cortinarines. Their main symptoms are nausea, paresthesia, anorexia, gastrointestinal disorders, headache, and renal insufficiency. The mushrooms *Gyromitra esculenta* and *Gyromitra giga* contain gyromitrin (methylethylhydrazine). After hydrolysis in the stomach, this toxin is converted into monomethylhydrazine (MMH). At first, MMH elicits intermittent gastrospasms and weakness, and, later, hepatic insufficiency and hemolysis that can lead to death.

Mushrooms with short incubation periods produce various types of symptoms, such as:

- *gastrointestinal* (incubation time: 1–6 h) due to mushrooms from genus *Entoloma*, *Omphalutus*, *Lactarius*, and *Boletus*, which produce toxins such as illudins, vinyl glycine, and bolesatine that cause vomiting, profuse diarrhea, and abdominal pain after ingestion.
- *inebriating* (30 min–2 h) due to *Amanita muscaria* or *Amanita pantherina*, which produce isoxazols, such as ibotenic acid and muscimol, that have toxic effects such as ataxia, excitotoxicity, and brain lesions.
- *cholinergic* (30 min–2 h) produced by the toxin muscarine present in mushrooms from the genus *Inocybe* or *Clitocybe*, which causes blurred vision, hypersalivation, lacrimation, and bronchial secretions.
- *hemolytic* (3–4 h) caused by repeated ingestion of *Paxillus involutus*, which may produce immunohemolytic anemia, with vomiting, diarrhea, abdominal pain, and decreased blood volume.
- *hallucinogenic* (30 min–1 h) caused by mushrooms from the genus *Psylocibe*, which produce psilocybin and its metabolite psilocin that creates visual and mental hallucinations, changes in perception, euphoria, and panic attacks.

- *coprinic* (10–30 min) generated by the toxin called coprine, which exists in mushrooms called *Coprinus atramentarius*. It is toxic only in the presence of ethanol because of the inhibition of ethanol in the metabolism. The symptoms are facial reddening, nausea, vomiting, malaise, agitation, and palpitations.

Miscellaneous

Biogenic amines are found in a wide range of foods, both animal- and plant-based foods. Serotonin, tyramine, dopamine, and norepinephrine are natural components of fruits and vegetables, such as bananas, tomatoes, avocados, potatoes, oranges, and spinach. In addition, much larger amounts of amines, mainly tyramine, are found in fermented foods such as aged cheeses. Usually, the presence of such amines in the diet has little effect on blood pressure because the amines are rapidly metabolically converted by monoamine oxidases (MAOs) and diamine oxidase, except in people who take drugs containing MAO inhibitors.

Avidin is an egg white protein that binds to biotin, resulting in the avidin-biotin complex, which is resistant to digestive enzymes, thus inhibiting the absorption of this vitamin by the intestine. This adverse effect may cause problems of biotin deficiency when large amounts of uncooked eggs are ingested. Cooking eggs denatures avidin, so its capacity to bind biotin is lost.

Thiaminase is an enzyme, found in raw fish, which splits the vitamin thiamin (B1) into two rings. Also, in plants, there are some phenolic compounds, such as tannins, that destroy thiamin.

Vitamins are bioactive substances that are necessary in small amounts. The ingestion of too much of a vitamin could result in hypervitaminoses, especially in the case of vitamin A. A continuous consumption of high doses of vitamin A can produce symptoms such as loss of appetite, tiredness, dermatitis, headache, and muscular and bone pain.

Chemical Pollutants

Inorganic substances

Most inorganic substances occur in nature in rocks, minerals, soil, water, and air, but, today, many of these substances can be readily found because anthropogenic activities have increased our environmental exposure to chemical elements, such as arsenic, which for centuries has been used as a poison; or heavy metals, such as cadmium, lead, mercury, and others. All of them are also present (at increasing levels) in food products such as fish, shellfish, vegetables, and edible mushrooms, with subsequent concern for food safety. These chemicals enter into the food chain from different sources. Some of them are geochemical pollutants that plants absorb from soil, while others are environmental pollutants that can be found in food because of carelessness in industrial and other activities carried out by humans. Table 3 compiles some of the most important inorganic elements, the foods in which they frequently appear, and their most remarkable effects. The toxic effects are due to different mechanisms, and they depend on the type of ingestion (acute or chronic).

Organic substances

In food, two types of organic substances with toxicological incidences can be found:

- Environmental pollutants, the most important of which are called *persistent organic pollutants* (dioxins, dibenzofurans, and polychlorinated biphenyls), are the unwanted by-products of industrial processes and waste incineration that accumulate in the environment and human body.

Table 3 Chemical pollutants

Inorganic substances	*Chemical species*	*Presence in food*	*Toxic effects*
No metals			
Arsenic (As) (MS 42–43)	As(III) more toxic than As(V)	Seafood, fish, meat, water	Acute: Diarrhea, abdominal cramps, nausea, vomiting, alteration of the cardiovascular system and central nervous system (CNS) Chronic: Carcinogen
Fluorine (F)	Fluorides (F^-)	Sodium chloride, sea fish, tea, water	Acute: Severe corrosive effect Chronic: Fluorosis of teeth
Radionuclides (MS 584)	Sr-90; I-131	Enter into food chain from water, plants, and animals after exposure in contaminated areas	Sr-90: Bone cancer, leukemia I-131: Damage to the thyroid gland
Heavy metals			
Lead (Pb) (MS 416–417)	Pb^{2+}	Water, food crops, pastures, meat, milk, fish, shellfish	Degenerative effects on nervous system, digestive tract, hematopoiesis system
Mercury (Hg) (MS 456–457)	Inorganic (Hg^+, Hg^{+2}, Hg^0) Organic (R-Hg^+, R-Hg-R')	Seafood (predatory species: shark, swordfish)	Necrotic damage in cells' renal tubules, disorders of the CNS, teratogen, brain atrophy
Cadmium (Cd) (MS 96–97)	Cd^{2+}	Food crops, cereals, tubers, rice, fish, shellfish	Osteomalacia, hypertonia, infertility, chronic pulmonary and renal insufficiency, carcinogen
Chromium (Cr) (MS 161–162)	Cr(II), Cr(III); Cr(VI) more toxic	Processed lean meat, full-grain products, cheese, brewer's yeast, legumes, spices	In high doses: Carcinogen and teratogen

(*Continued*)

Table 3 (Continued)

Organic substances	*Toxicant*	*Presence in food*	*Toxic effects*
Environmental pollutants			
Organochlorine compounds	Dioxins (TCDD), polychlorinated dibenzofurans (PCDF), chlorinated biphenyls (PCB), and brominated biphenyls (PBB)	From soil, plants, and water to food animals and fish (chicken, pork, salmon) and animal feed	Liver lesions, adrenal atrophy, kidney abnormalities, chloracne, carcinogen, adverse reproductive effects
Substances intentionally added to food			
Pesticide residues MS 534–535	Organochlorinated (OC; family of DDT, lindane, and cyclodienes) Organophosphates (OP) Carbamates Pyrethroids	Plant, fruits, water	Neurotoxic OC: Disruption of nerve impulse transmission by interfering with Ca^{2+} and Cl^{-} concentrations OP and carbamates are inhibitors of acetylcholinesterase Pyrethroids bind voltage-gated sodium channels
Veterinary drugs and feed additives	Adrenoceptor agonist (clenbuterol) Anthelmintic agent (closantel, ivermectin) Antimicrobial agent (antibiotics) Antiprotozoal agent (diclazuril, imidocarb) Beta-adrenoreceptor-blocking agent (carazolol) Glucocorticosteroid (dexamethasone) Growth promoter (trenbolone acetate, zeranol) Insecticide (cypermethrin, deltamethrin, trichlorfon) Production aid (abamectin, melengestrol, estradiol 17 β) Tranquilizing agent (azaperone) Trypanocide (isometamidium)	Milk, muscle, fat, skin, liver, kidney meat (pigs, sheep, cattle, rabbits, goats, chickens, turkeys, other poultry); regulated by the Maximum Residue Limits (MRL)	Direct toxic effects Immunologic effects Mutagen, carcinogen, teratogen Effects on intestinal biota (antibiotics) Endocrine disruption (production aid, growth promoters)

Inorganic and organic substances.

These compounds are highly toxic and can cause reproductive and developmental problems, can damage the immune system, and can interfere with hormones to cause cancer.

- Organic compounds intentionally added to food during food production are substances such as pesticides and veterinary drugs. They are found worldwide in the environment and accumulate in animal food chains. Their presence in food is regulated by Maximum Residue Limits (MRLs). Countries throughout the world have their own versions of MRLs.

Table 3 lists the foods that can contain these organic toxins as well as their toxic effects.

Food Additives

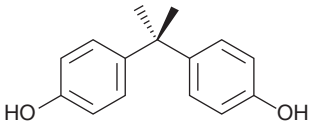
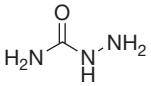
Food additives are substances intentionally added to food to perform specific functions, such as preservatives, bleaching agents, antioxidants, sweeteners, coloring agents, and flavoring agents, among others. Food additives are one of the most studied classes of compounds regarding their toxicity. Food additives can be classified according to their sources, such as from substances that are isolated from edible plants or from living material, substances that naturally occur in foodstuffs but are obtained by chemical synthesis, and substances that are not found in nature. In Europe, every additive has an E-number, and each is classified according to its function in food. As with any other substance used for food production, the toxicity of the additives must be assessed by different toxicological tests. In addition, some substances have to be revised, and, in some cases, they have been removed from the authorized list of additives. The most interesting additives from a toxicological point of view are preservatives, antioxidants, sweeteners, coloring agents, flavoring agents, and flavor enhancers (Table 4).

Toxic Substances Formed During Food Processing, Preparation, Storage and Intake

A variety of chemicals is formed during the food production process or during cooking. A large group of substances is generated in food products after high temperatures are applied during processing or cooking, or after heat is combined with several acid treatments (chloropropanols). Other toxic substances come into contact with food during the packaging process, such as phthalates or bisphenol A in plastics. Table 4 summarizes these kinds of substances. In this group, several toxic compounds can be formed in the human body after the

Table 4 Food additives and toxicants formed during food processing, preparation, storage, packaging, and intake

Additive group	*Substance*	*Use in food*	*Effects*
Preservatives	Benzoic acid; sodium benzoate (E 211)	Acidic foods such as salad dressings, carbonated drinks, jams, and fruit juices	Weight loss, diarrhea, internal bleeding (mice)
	Nitrite and nitrate	Meat, meat products	Methemoglobinemia, carcinogens, nitrosamine precursors
Antioxidants	Propyl gallate (E 310)	Used to retard or prevent rancidity in lard, edible fats, and oils	Estrogen antagonist; respiratory distress in asthmatics
	Butylated hydroxytoluene (BHT) (E 321)	Used to retard or prevent rancidity in lard, edible fats, and oils	Asthma, eczemas
	Butylated hydroxyanisol (BHA)	Commercial stabilizers (antioxidants) for food fortification or animal feeds	Metabolic inductor with increasing risk of mutagen formation
Sweeteners	Saccharin (E-954)	Sweetener most commonly used	Carcinogen: contradictory results
	Sodium cyclamate (E 952)	Sweetener, diet drinks, gums, dressings	After metabolization produces cyclohexylamine, carcinogenic; its use has been partially rehabilitated
	Aspartame	Diet products (soft drinks, jams, cereals)	Metabolized to Phe, Asp, and methanol; increased risk for phenylketonurics
Coloring agents	Amaranth (E-123)	Soft drinks, ice creams, salad dressings	Allergic reactions
	Tartrazine (E-102)	Cakes, deserts, cheese, liquors	Allergic and intolerance reactions, migraines, blurred vision
Flavoring agents	Methyl anthranilate	Used to imitate Concord grape flavor	Allergenic
	Diacetyl (2,3-butane dione)	Used for artificial butter flavoring in margarines or similar oil-based products	Bronchiolitis obliterans, a severe respiratory illness
Flavor enhancers	Monosodium glutamate	Used to supply meat-like flavor	Headache, drowsiness

	Production/origin	*Substances*	*Food*	*Effects*
Formed with high temperatures				
Polycyclic aromatic hydrocarbons (PAH) (MS 550)	Combustion products come into direct contact with food	Benzo(a)pyrene	Smoked and grilled meat	Carcinogenic
Heterocyclic aromatic amines (HAAs) (MS 377)	Generated in the crust of meat products as a result of heat treatment	Imidazoquinolin (IQ) and derivatives MeIQx, DiMeIQx, and Phe-P-1	Meat and fish cooked at temperatures higher than 150°C	Carcinogenic
Acrolein	Intermediate of production of acrylamide	$CH_2=CHCHO$	Foods with carbohydrates, vegetable oils, animal fats, and amino acids	Carcinogenic
Acrylamide (MS 5)	Reaction of asparagine with reducing sugars	$CH_2=CHCONH_2$	Bread, cookies, crackers, cereals, coffee	Carcinogenic
Furan	After heat treatment of certain foods		Brewed coffee, cereals, chili, salty snacks, soups containing meats	Possible carcinogen
Chloropropanols	Formed during production of hydrolyzed vegetable proteins (hydrochloric acid treatment at high temperatures)	3-chloropropane-1,2-diol (3-MCPD) 1,3-dichloropropanol (1,3-DCP)	Soups, gravy mixes, stock cubes, savory snacks, soy sauce	Carcinogenic
Packaging process				
Phthalates	Used in plastics with a wide range of applications	Di-n-butyl phthalate (DBP) Diethyl phthalate (DEP) Benzyl butyl phthalate (BBP)	Fatty foods (dairy, fish, meat, oils)	Endocrine disruptor affecting human sex hormone production
Bisphenol A	Polycarbonate and epoxy resins used in heating containers for foods, metal cans		Drinking water, leaches from plastic food and beverage containers, human breast milk	Endocrine disruptor affecting hormone production, mimicks estrogens
Semicarbazide	Formed by heating azodicarbonamide used as a foaming agent in plastic seals and food packaging materials		Food sold in glass jars, baby foods	Weakly carcinogenic

intake of several foods containing substances such as nitrites used as food additives (nitrocompounds such as nitrosamines and nitrosamides are considered to be potent carcinogens after metabolic activation).

Conclusions

Throughout history, people have identified a large number of potentially dangerous components present in food, ranging from substances with high acute toxicities to those with low acute toxicities but chronic adverse effects after continuous intake in the diet. Consequently, we need to classify all substances, of both natural and synthetic origin, in order to understand all the possible adverse effects that they produce after being ingested. The knowledge of their presence in food and the effects that they produce on the human body is the key to adequate food safety and healthy nutrition.

See also: Acrylamide; Aflatoxin: A Global Public Health Problem; Alkaloids: Properties and Determination; Alkaloids: Toxicology and Health Effects; ; Antibiotics and Drugs: Residue Determination; Antinutritional Factors in Legume Seeds: Characteristics and Determination; Arsenic: Properties and Determination; Arsenic: Toxicology and Health Effects; *Bacillus cereus* and Other *Bacillus* sp. Causing Foodborne Poisonings, Detection of; *Bacillus*: Occurrence; Biogenic Amines: Toxicology and Health Effect; Biogenic Amines; Cadmium: Properties and Determination; Cadmium: Toxicology; Campylobacter: Health Effects and Toxicity; Campylobacter: Properties and Occurrence; Campylobacter: Species Detection; Chromium: Physiology; Chromium: Properties and Determination; *Clostridium botulinum*; *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; *Clostridium*: Occurrence and Detection of *Clostridium perfringens*; Cobalt: Properties and Determination; Cobalt: Toxicology; Copper: Physiology; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; *Escherichia coli* and Other *Enterobacteriaceae*: Occurrence and Detection; Food Additives: Classification, Uses and Regulation; Foodborne Pathogens; Fumigants; Fungicides; Growth promoters: Characteristics and Determination; Heavy Metal Toxicology; Mycotoxins: Classification; Mycotoxins: Occurrence and Determination; Mycotoxins: Toxicology; Nitrites and Nitrates; Pesticides and Herbicides: Residue Determination; Pesticides and Herbicides: Types of Pesticide; Pesticides and Herbicides: Types, Uses, and Determination of Herbicides; Polycyclic Aromatic Hydrocarbons; Preservatives: Classifications and Analysis; *Salmonella*: Detection; *Salmonella*: Properties and Occurrence; *Salmonella*: Salmonellosis; Shigella; *Staphylococcus*: Detection; *Staphylococcus*: Food Poisoning; *Staphylococcus*: Occurrence and Properties; Toxins in Food: Naturally Occurring.

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Food Poisoning: Epidemiology

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Introduction

The World Health Organization defines a foodborne disease as "any disease of a toxic or infectious nature caused by consumption of contaminated water or food." Despite certain shades of meaning, the terms 'foodborne disease' and 'food poisoning' are frequently considered as equivalent.

Foodborne diseases are caused by a wide variety of pathogenic microorganisms, which enter into contact with humans through ingestion of food, and may be bacteria, viruses, protozoa, or prions. Different toxins must also be included as causal agents: natural toxins, such as poisonous plants or mushrooms; poisons incorporated into foods in their production, such as pesticides and heavy metals; or biological toxins produced in the food itself, such as *Staphylococcus aureus* toxin.

Food may become contaminated at various points between production, processing, and preparation, by a variety of mechanisms:

- Foods which are a primary source of microorganisms. For example, microorganisms present in the raw meat of some animals may persist if appropriate hygiene and heat treatment measures are not taken, or for example, the presence of *Salmonella* in eggs may be due to either internal contamination in the hen's ovary or to contamination of the shell by the bird's own feces.
- Foods contaminated by the water they grow in or are irrigated with. For example, fish and shellfish may be contaminated if they lived in contaminated waters, while fruit, vegetables, and cereals may become contaminated through irrigation with untreated water.
- Foods contaminated by processing techniques involving poor hygiene practices. For example, using contaminated equipment to process milk.
- Foods contaminated due to insufficient heat treatment. For example, the temperature of pasteurization eliminates the majority of vegetative forms of *Clostridium* spp., but not the spore forms.
- Foods contaminated through inappropriate manipulation after heat treatment, whether directly from workers' hands or through cross contamination between hands, equipment, or surfaces that are contaminated, or through defective conservation measures.

Foodborne diseases are common and do not usually result in severe illness, however they may produce considerable morbidity and mortality, and consequently social alarm, as happened in Germany in 2011 when there was an outbreak of more than 3800 cases of gastroenteritis caused by a Shiga toxin-producing *Escherichia coli* (O104:H4), with more than 20% of cases subsequently presenting hemolytic–uremic syndrome, followed by death in approximately 4.5% of cases.

The most common clinical presentation of these diseases is gastrointestinal symptoms (diarrhea, abdominal pain, vomiting), although they may also present extraintestinal symptoms such as neurological or systemic conditions. The severity of this entity may range anywhere between an asymptomatic or slight affectation not requiring medical care, to illness requiring hospitalization, and to death.

In general, the intensity of the clinical presentation of a foodborne illness is directly related to the concentration of microorganism or toxin present in the ingested food. The minimum microorganism concentration necessary to produce infection (minimum infective dose) may vary between 10 and 100 cells in the case of *Shigella* or *Campylobacter*, to as many as 10^5–10^6 cells in the case of *Salmonella enterica*. Even so, not all exposed individuals manifest symptoms, nor do the symptoms present with the same intensity. The infective dose may be affected by individual factors such as age, nutritional status, immunological status, the presence of underlying diseases, or by factors related to the microorganism such as virulence or its adaptation to the medium.

These diseases can present severe complications such as hemolytic–uremic syndrome from infection by *E. coli* O157:H7, Guillain–Barré syndrome, which appears following infection by *Campylobacter jejuni*, or reactive arthritis following an episode of salmonellosis. The clinical manifestations considered together with incubation period and duration of the illness are normally indicative of the agent involved, and information about these diseases may be found in other chapters of this encyclopedia.

An outbreak of a foodborne disease is considered to occur when symptoms affect two or more people who ate food or a meal at the same time and place. For certain diseases, however (e.g., botulism or gastroenteritis due to *E. coli* O157:H7), a single case is sufficient to launch an investigation and initiate the corresponding prevention and control measures.

Epidemiological Surveillance

Epidemiological surveillance of foodborne diseases is defined as the systematic collection, analysis and interpretation of information, which permits planning, execution and evaluation of the pertinent prevention and control activities.

Foodborne disease surveillance is not well-standardized globally, and it is executed using a variety of information sources, such as mandatory notification, outbreak investigations, microbiological notification, sentinel surveillance, hospital discharge, diagnosis registries, and death registries. Quality of surveillance data varies widely by information system, and care must be exercised in drawing conclusions based on comparisons between them.

Foodborne diseases are often under-reported:

- The majority of people with symptoms of acute gastroenteritis rarely seek medical care;

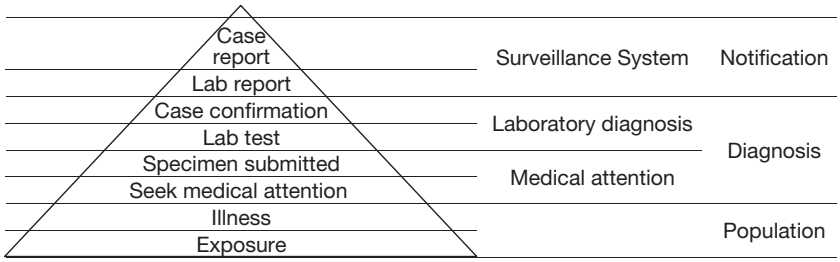

Figure 1 Foodborne disease information system. Adapted from CDC, www.cdc.gov.

- In many cases the physician does not request collection and analysis of biological samples;
- In some cases the laboratory receiving samples is unable to reach a diagnosis;
- It can be very difficult to connect separate patients of foodborne illness whose cases have sought medical care separately;
- The appropriate surveillance institution is not notified of the case.

As Figure 1 shows, information can get lost at different levels in the information system.

Knowledge about foodborne diseases is continually advancing, both in regard to identification of causal agents or vehicles of transmission, as well as in regard to prevention and control measures; hence the epidemiology of these diseases is not static, but rather constantly changing. In this sense, new challenges must be faced: emergence of new pathogens, or reemergence of known pathogens, the appearance of resistance to antibiotics, growth of population nuclei for which food supply demands must be met, population changes leading to a rise in numbers of vulnerable persons, changes in eating habits, changes in food production, or the increasing of international trade of food products.

Changes in Causal Agents

In the developed world, certain diseases common in the early twentieth century such as typhoid fever, cholera, brucellosis, or trichinosis, have now been controlled thanks, for example, to strategies of disinfection of water for human consumption, wastewater treatment, processes of food sterilization and pasteurization, or veterinary controls in livestock. These are now diseases which are documented sporadically and primarily in travelers to developing countries or to areas with poor hygiene conditions.

In recent decades, new pathogens, or new routes of transmission of known pathogens via food, have been identified. In these cases, questions always arise which must be answered by research, whether clinical (diagnostic criteria, clinical spectrum, treatments, complications, sequelae); microbiological (available diagnostic media); epidemiological (population incidence and/or prevalence, transmission routes, incubation periods, risk factors); or research related to prevention and control measures necessary (types of contaminated food, impact of food processing). For example, in the last 30 years, *E. coli* O157:H7 and the Norwalk virus have been identified as pathogens producing foodborne diseases.

Also, new variants of known microorganisms have spread over large geographical areas. This was the case with *Salmonella typhimurium* DT104, which first appeared in the 1990s among cattle in Europe and the United States but not Australia; and also the case with *Yersinia enterocolitica* serogroups O3 and O9, which was related to pork consumption and appeared in Europe and Japan in the 1970s and in the United States in the 1980s; or the case with *Vibrio parahaemolyticus* O3:K6 which appeared in Southeast Asia in the 1990s related to consumption of raw oysters, and which subsequently spread to the United States.

There has also been the appearance of resistance to antibiotics in known microorganisms, whether due to overuse of antibiotics by humans, in the case of those for which humans are the main reservoir (e.g., *Shigella* and *Salmonella typhi*), or through use of antibiotics in agriculture, in the case of those for which animals are the reservoir (quinolone-resistant *Campylobacter jejuni* or ceftriaxone-resistant *Salmonella*). All these have spread over large geographical areas in only a few years. For example, the highly resistant strain of *Salmonella* Newport (resistant to nine antibiotics) first appeared at a local level in the United States in 1999, and 3 years later, had spread nationwide. Another example is the outbreak of infection by *Salmonella* Heidelberg (resistant to four antibiotics) which was responsible for 136 cases in 34 states of the United States in 2011, ground turkey meat identified as the vehicle. Since resistance to antibiotics complicates treatment of microbe-vectored disease, prudent use of antibiotics, in both humans and animals is an urgent public health priority.

Recently, certain pathogens which act opportunistically have been identified as causing severe illness in vulnerable population groups (immunocompromised persons, the elderly, children), but which may be asymptomatic or cause only mild episodes of gastroenteritis in the healthy population. This is the case with *Vibrio vulfinicus*, which causes severe bacteraemia or necrotizing fasciitis in patients with alterations of hepatic function or iron overload, but rarely causes infection in healthy individuals.

Changes in the Population

In the last few decades, changes in population demographics and mobility have occurred which have affected the risk of contracting a foodborne disease. Population aging has led to a rise in numbers of people with more underlying diseases, with weakened immunological systems, with lower absorption of nutrients and higher levels of hospitalization and medical

treatment, who, consequently, are more susceptible to these types of infections. Due to advances in modern medicine, increased survival among persons with immunodeficiency, chronic diseases, or under aggressive treatments has also led to an increase in the numbers of people more susceptible to certain types of infections, including foodborne diseases. On the other hand, high population mobility, whether due to migratory processes, or to occupational, social, religious, or leisure-related activities, means that travelers are exposed to pathogens different from those of their country of origin, and that they may even transport pathogenic microorganisms to other countries.

Changes in people's eating patterns have also led to the emergence of new risks. The wide availability now of typical foods and traditional home-made dishes as store-bought products, and the new market of food imported from foreign countries, either as primary produce or as prepared dishes, has led to an increase in the variety of available foods. At the same time, this has meant an increase in the variety of pathogens which may affect the population. Hence, whereas at the beginning of the twentieth century it was extremely difficult to find, for example, a European who had tasted some product from Japan, such as sushi, now a century later it is difficult to find a European who has not tasted it.

Changes in Food Production

As a result of changes in the food industry, both foods and pathogens, have ceased to be local, spreading beyond country boundaries. In the past, foodborne disease outbreaks were processes which affected a limited number of people who had consumed some particular food together in the same meal and who approached the local medical services within a short period of time. Thus, it was a local process in which a common cause was easily identified. Nowadays, a food product may contain ingredients from very different geographical locations, and may be distributed widely around the world, making more difficult not only the investigation of a foodborne disease outbreak, but even identification of the outbreak. One of the first examples in modern times was that of the infection by *Cyclospora cayetanensis* detected in the United States and Canada due to consumption of food products which contained raspberries from Guatemala. A paradigmatic example is that of the use of herbs and spices. These are produced at a local level, commercialized worldwide, and are used as ingredients in many food products. Hence, their contamination can lead to foodborne disease outbreaks resulting from seasoning foods with contaminated lots of herbs or spices. Studies have been published relating these ingredients to different causal agents. For example, in 2007, fresh basil contaminated with *Salmonella* Senftenberg affected 51 people in the United Kingdom, Denmark, the Netherlands, and the United States; in 2009 and 2010, red and black pepper contaminated with *Salmonella* Montevideo affected 272 people from 44 different states in the United States.

New practices in livestock feeding have also been related to increased prevalence of diseases in humans. This was the case of bovine spongiform encephalopathy. Animals given feed enriched with meat and bone from other animals was presumed to have caused, from 1986 onward, an epidemic of bovine spongiform encephalopathy which affected approximately 178 000 animals in the United Kingdom and which, in 1996, was detected in humans as new variant Creutzfeldt–Jakob disease.

New population trends in the consumption of 'ecological' foods have led to a rise in the production of foods for which 'natural' fertilizers are used instead of chemical products, or untreated water, or even waste waters. These natural fertilizers are used to irrigate fruit, vegetables, and other green crops. These practices have contributed to an increase in the risk of contamination of these food products by pathogens, as was the case in a huge outbreak of infection by *E. coli* O157:H7 in 1996 that affected more than 9000 schoolchildren in Osaka, Japan, due to ingestion of white radish sprouts. Moreover, an increase in the consumption of fresh fruit and vegetables and changes in their processing have also led to an increase in outbreaks involving these types of foods.

New food handling techniques, whether in regard to processing, packaging, transportation, storage, or refrigeration, permit the supply of foods over large distances and over longer periods, although they do not completely eliminate the risk of containing pathogenic agents. For example, there have been reports of salmonellosis outbreaks related with prepared foods which only needed a final cooking in a microwave- or conventional oven, but where, in practice, this was not done sufficiently or completely. There are also reports of geographically extensive outbreaks related with improperly processed canned food contaminated by spores of *Clostridium botulinum*.

Epidemiology of Foodborne Disease

Worldwide, foodborne diseases are the cause of considerable morbidity and mortality. In the developed countries, they are considered an ever-growing public health problem, while in developing countries or war zones where food supply is a problem, foodborne diseases constitute one of the most critical public health problems.

National and international studies are attempting to estimate both incidence and patterns of foodborne diseases, yet there are still few good global estimates. These attempts to estimate incidence and patterns of foodborne diseases are frustrated by several factors. These diseases are clearly underreported, and those countries which perform surveillance of them use different information systems, making comparisons difficult. Also, the causes underlying foodborne illness vary widely with the geographical area under study, and surveillance is not always directed at the same causal agents. Moreover, the foods involved in transmission of infections are not the same in different geographical areas, nor are they processed and handled in the same way.

Country-level studies have been published which aimed to determine foodborne disease patterns. These studies do not use identical methodologies, thus, prudence must be exercised in comparing their findings. In Europe, data collected by the WHO Food Safety Department over the period from 1998 to 2001 suggest that infection by non-typhoidal *Salmonella* spp. is one of the most common foodborne infectious agents, with incidences ranging from 6.2 to 137 cases per 100 000 persons. Infection by *Campylobacter* spp. is another very common agent,

with incidences between 30 and 96.3 cases per 100 000 persons. Other bacterial infections present lower incidences: *Shigella*, between 0.9 and 7 cases per 100 000 persons; *E. coli* non-VTEC (verotoxin-producing *E. coli*) and *Clostridium botulinum* under 2 cases per 100 000 persons. Trends for both *Salmonella* spp. and *Campylobacter* spp. have continually risen over the last two decades.

It has been estimated that during the period 1996–2000 in the United Kingdom 1 724 315 cases of foodborne diseases occurred every year, with 21 997 hospital admissions and 687 deaths. Salmonellosis and infection by *Campylobacter* were the most common causes.

A study conducted in the United States during the period 2000–2008 estimated that every year, 31 major pathogens are responsible for 9.4 million cases of foodborne diseases, 55 961 hospital admissions, and 1351 deaths. The majority are caused by Norovirus (58%), non-typhoidal *Salmonella* spp. (11%), *Clostridium perfringens* (10%) and *Campylobacter* spp. (9%).

In Australia, a study conducted around 2002 estimated that a total of 5.4 million cases of foodborne diseases occurred every year, with an associated 3640 hospital admissions and 76 deaths.

New Challenges

Changes in the population and in different aspects of food production and consumption impose new challenges not only for foodborne diseases surveillance and research, but also for the prevention and control measures needed.

Thus, whereas in the past foodborne disease outbreaks were acute processes with high attack rates affecting population groups at a local level and related to events involving manipulation of food near the point of consumption, nowadays, geographically widespread outbreaks are becoming more common, involving low levels of contamination of foods that are widely distributed. In such cases, the outbreak may be detected through an unusual concentration of cases, by detection of some unusual pathogenic agent or through laboratory identification of some specific subtype.

In these new scenarios, the information systems necessary for epidemiological surveillance of these diseases must be more sophisticated in order to cope with such challenges. Various international initiatives have been developed with the goal of determining the pattern of these diseases at regional level: (1) The WHO Global Salm-Surv is an international program launched in 2000 which potentiates surveillance based on laboratory diagnoses, with the participation of various institutions in France, Denmark, and the Netherlands, as well as the United States, Canada, and Australia. (2) The WHO Sentinel Sites Project aims to assess patterns of foodborne diseases based on the setting up of various types of sentinel surveillance.

As examples of national-level networks, Enter-Net in the European Union is a surveillance network for two types of human intestinal infections (*Salmonella* and *E. coli* VTEC); FoodNet in the United States is a sentinel surveillance network for a specific list of foodborne pathogens; PulseNet (also in the United States) is a national network for molecular subtyping of pathogenic strains; and OzFoodNet (in Australia) is a national network for foodborne disease surveillance.

The more scattered presentation of foodborne disease implies a need to identify apparently sporadic cases that appear in more than one territory, as the result of contamination of foods which are widely distributed. In cases in which outbreaks may transcend local, or even national boundaries, it is necessary to develop public health strategies which include analysis of epidemiological data at regional, national, and international levels, the routine identification of microorganisms causing an outbreak by molecular epidemiology, and improvements in the communication of information between different epidemiological teams. Thus, more robust collaboration at local, regional, national, and international levels between experts in epidemiology, microbiology, and food safety may be the key to success in the investigation of a foodborne disease outbreak.

Identification of new pathogens, or of new transmission routes of known pathogens, represents a new challenge for surveillance systems. Both formal and informal surveillance strategies must be developed to permit early detection, and appropriate epidemiological research techniques must be developed in order to respond to questions that arise with the principle aim of establishing the corresponding prevention and control measures. Achievement of such responses will require considerable effort and dedication on the part of epidemiological surveillance teams.

Despite all these changes, the methodology of investigating a foodborne disease outbreak is not substantially different, although nowadays it must be more multidisciplinary, requiring participation of clinicians, epidemiologists, laboratory personnel, as well as experts in food safety, risk management, and communication. The process of investigating a foodborne disease outbreak represents a unique opportunity for obtaining information, especially when faced with diseases caused by new pathogens, or new transmission routes of known pathogens. Such investigations may contribute different types of information, depending on the population affected by the outbreak, from data on clinical affectation of patients, through to information about food production processes. Furthermore, basic surveillance data can provide more detailed information about affected cases.

Epidemiological study of the food-related origin of isolated gastroenteritis episodes can be considerably more difficult, since it implies identifying the common link between different cases being able to count on a laboratory diagnosis that confirms the common etiology. The growing use of surveillance networks such as Enter-Net in the European Union, PulseNet in the United States or OzFoodNet in Australia permits the identification of outbreaks that go beyond purely local settings. This makes it possible to differentiate sporadic cases from geographically scattered outbreaks.

Similarly, the necessary prevention and control measures must also be more sophisticated than measures applied in the past. Thus, while strategies such as disinfection of water for human consumption, treatment of waste waters, sterilization and pasteurization of foods, routine assessment of food handling practices, and human and animal vaccinations must be continued, it is also necessary to develop prevention and control measures to face new challenges involving the entire food

industry (from primary production to the consumer), contemplating national, or even international, levels of action.

Hazard Analysis Critical Control Point (HACCP) is a strategy which allows the identification, assessment and control of food safety from the start of production through to consumption. HACCP was originally developed in the 1960s by Pillsbury Co., for NASA, to strengthen the safeguards for food safety on manned space flights. When properly implemented, this has been a highly useful tool for the food production industry to monitor the processes. In this sense, inspections of finished food products have given way to the identification, along the entire food production chain, of a series of critical points that may be susceptible to improvement through the implementation of multiple prevention and control measures. This approach can be applied to small-scale production, as well as to large-scale manufacturing. It must, however, be complemented by microbiological analyses of the products, and by the maximum availability of epidemiological information.

Examples where HACCP has played an important role arose in 2000 in the United States and in 2009 in Europe. In the United States, a cluster of infection by *Salmonella enteritidis* was detected via molecular epidemiology, associated to consumption of an orange juice. Subsequently, 88 further cases of infection were detected in a total of eight states. A critical point was identified in the use of an alternative to pasteurization of the juice. As a result, these practices were modified in accordance with federal legislation. In two European countries (Hungary and Italy), local but persistent increases were detected in infections by *Salmonella* Goldcoast, which were eventually found to be related with contamination in the chain of production of pork products.

See also: Campylobacter: Health Effects and Toxicity; *Clostridium botulinum*; *Clostridium*: Food Poisoning by *Clostridium perfringens*; Diarrheal Diseases; Emerging Foodborne Enteric Bacterial Pathogens; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; Food Poisoning: Classification; Food Poisoning: Tracing Origins and Testing; Foodborne Pathogens; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; *Listeria*: Listeriosis; *Salmonella*: Salmonellosis; Shigella; *Staphylococcus*: Food Poisoning; Toxins in Food: Naturally Occurring; World Health Organization.

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Relevant Websites

http://www.cdc.gov/foodborneburden/ – Centers for Diseases Control and Prevention; Enter-Net; FoodNet; PulseNet; OzFoodNet.

http://www.ecdc.europa.eu/en/healthtopics/food_and_waterborne_disease/Pages/index.aspx – European Centre for Disease Prevention and Control.

http://who.int/foodborne_disease/en/ – World Health Organization.

Food Poisoning: Tracing Origins and Testing

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Introduction

Foodborne disease (food poisoning) has been defined by the World Health Organization as "a disease of infectious or toxic nature, caused by, or thought to be caused by, the consumption of food and water." It involves a large (>40) and diverse range of agents, the number of which continues to increase yearly as new agents are identified. Table 1 lists those agents currently considered to be involved in diseases transmitted by foods.

The main sources of many of these organisms are domestic and wild animals (including fish and birds). Enteroviruses, *Salmonella typhi*, and *Vibrio cholerae* are of human origin and *Clostridium botulinum*, *Bacillus* spp., and mycotoxigenic molds are general environmental contaminants.

Food poisoning is usually thought of as acute gastroenteritis (fever, abdominal pain, and diarrhea, often with vomiting) of the type caused by infectious disease-causing bacteria such as *Salmonella enterica* and toxin-forming bacteria such as *Staphylococcus aureus*. However, a number of the newly identified (often called 'emerging') food-poisoning microorganisms cause a wide range of nonintestinal symptoms. For example, *Listeria monocytogenes* causes illness ranging from a mild flulike illness to meningitis and life-threatening infections of the central nervous system. In addition, it has been recognized within the last 20 years that a number of classical gastroenteritis-causing bacteria, such as salmonellae and *Yersinia enterocolitica*, may cause extraintestinal infections as sequelae to intestinal disease; these include reactive arthritis, Reiter's syndrome, ankylosing spondylitis, and pericarditis. The wide range of symptoms caused by foodborne disease-causing agents, many of which mimic illness of a nonfood-poisoning nature, may cause some difficulty in identifying a food cause and establishing the extent of a foodborne disease incident.

This article considers the epidemiology of foodborne diseases, the procedures used for testing foods for the causative agents, tracing the source and cause of a food-poisoning incident, and the recall of contaminated food.

Epidemiology

When considering the sources of foodborne disease-causing microorganisms, it is important to recognize that virtually all raw agricultural products will from time to time be contaminated with low concentrations of such organisms, and as a result of cross-contamination and recontamination, they will also contaminate cooked and other processed food products. Although salmonella are regarded to be zoonotic, and thus of animal origin, they are frequently found in soils and water, and human carriers may be the source. Organisms of human origin, such as the previously mentioned enteroviruses and *Salmonella typhi*, may also contaminate various parts of the food chain, for example, via human carriers contaminating food during preparation for consumption or from human sewage contamination of foods during their growing, such as the contamination of vegetables with night soil or molluscan shellfish harvested from waters contaminated with raw human sewage.

Thus, the original source of an agent causing foodborne disease may be difficult or impossible to establish with any degree of certainty and it may only be possible, and in many cases only appropriate, to identify the circumstances and actual foodstuff that caused a particular food-poisoning incident. In many cases, this will be the result of poor food handling (including preparation in advance for consumption) allowing survival or growth of the pathogen. For example, whereas the main source of the contamination of foods with salmonellae and *Campylobacter* is poultry, provided the poultry is properly cooked, these bacteria will be killed, and if the cooked poultry is then protected from recontamination, they will not cause illness. Thus, while the source of salmonella contamination of cooked poultry is most likely to be the raw poultry, the cause of any salmonellosis arising from its consumption is most likely to be the result of poor hygienic practice during preparation. In this example, identification of the source of the original contamination is much less important than identifying the circumstances that led to the contamination of the cooked poultry. However, where contamination of a raw ready-to-eat food occurs, it is important to identify the source of the contamination to prevent its recurrence.

Further, when considering the cause of a foodborne disease incident, it is important to recognize that such illness is dose-related (the larger the concentration of the organism or the amount of toxin present in a food at the time of consumption, generally, the greater the severity and extent of illness within a group of persons consuming the contaminated food). It is seldom that all persons exposed to an infective or intoxicating dose of a pathogen will show disease symptoms and symptoms will often vary widely in those who are ill. The minimum concentration of an agent necessary to cause illness is frequently referred to as the minimum infective dose (MID). The MID of many food-poisoning bacteria is unknown, but it is generally accepted that the infective dose of classical causative agents of enteric disease, such as *Campylobacter*, *Salmonella typhi*, and *Shigella*, is 10–100 cells, whereas the MID of others such as the food-poisoning salmonella (now all classified as *Salmonella enterica*) is generally regarded to be of the order of 100 000–1 million cells for a healthy adult. However, the infective dose and hence the risk of illness arising from a contaminated food are affected by many factors. Factors that may reduce the infective dose include high virulence of a particular strain, age, health status of the individual exposed to the agent (in particular, immune status), nutritional

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Table 1 Foodborne disease-causing agents

Bacteria
Aeromonas hydrophila and *A. veronii* subsp. *sobria*
Bacillus cereus, *B. licheniformis*, and *B. subtilis*
Brucella melitensis, *B. abortus*, and *B. suis*
Campylobacter jejuni, *C. coli*, *C. lari*, and *C. upsaliensis*
Clostridium botulinum and *C. perfringens*
Escherichia coli (*EIEC*, *ETEC*, *VTEC*, *EAggEC*)
Listeria monocytogenes
Mycobacterium bovis
Salmonella enterica
Shigella dysenteriae, *S. flexneri*, *S. boydii*, and *S. sonnei*
Streptococcus zooepidemicus
Vibrio cholerae, *V. parahaemolyticus*, *V. fluvialis*, and *V. vulnificus*
Staphylococcus aureus
Yersinia enterocolitica
Mycotoxigenic molds
Protozoa
Cryptosporidium
Cyclospora
Giardia
Toxoplasma
Prions
vCJD
Viruses
Hepatitides A and E
Small, round-structured viruses (SRSV)
Dinoflagellates
Shellfish poisoning (PSP, DSP, NSP, ASP)

deficiencies in diet, and underlying disease. There is much evidence that there are in some food substances that protect infective agents from destruction by stomach acids. Thus, fats in high-fat foods such as full-fat cheeses, chocolates, and salamis may significantly reduce the infective dose of an agent; for instance, the infective dose of salmonellae may be only a few cells (<10) in these foods. Therefore, when considering the significance of a particular concentration of an agent in a food, in relation to it having caused illness, it is important to take these facts into consideration and also to remember that in any food collected after a food-poisoning incident, the numbers of microorganisms may have increased or decreased depending on how that food has been stored subsequent to the incident. With respect to microbial toxins, these are often more persistent in foods than their producing organisms but may increase in amount as the result of the presence of the producing organism or decrease as a result of biologically, chemically, or physically induced breakdown.

Case Histories

Hepatitis A and Frozen Raspberries

Twenty-one persons who consumed a mousse made from frozen raspberries developed jaundice found to be caused by the hepatitis A virus. Investigation of the incident suggested that contamination had occurred during harvesting or packaging of the raspberries. Cases of hepatitis had been reported in the area where the raspberries had been picked and the sanitary conditions in the places where the pickers were housed were found to be poor. Sanitary conditions were improved and basic food hygiene instruction was given to the pickers. There were no more reported incidents from this source.

Salmonella and Chocolate

The uncommon salmonella serotype *Salmonella napoli* caused 245 cases of salmonellosis among children. These were traced back to the consumption of contaminated chocolate bars from a single factory. The factory was very modern and clean but untreated river water was used for the water circulation system that kept the chocolate molten during transportation around the factory; *S. napoli* had previously been isolated from river water in the region of the factory. Contamination of the chocolate probably occurred as a result of microleaks in the pipes of the water circulation system, resulting in the seepage of water into the chocolate. Chocolate manufactures agreed to a code of hygienic practice following this incident.

Salmonella and Salami Sticks

A number of cases of salmonellosis, mainly among infants and children, occurred as a result of the consumption of salami sticks. The salami sticks were manufactured using traditional methods but with a very short maturation period. The short maturation period was not sufficiently long to destroy any salmonella present in the meat used in the manufacture of the salami. Thus, very small numbers survived. This survival was prolonged by the practice in retail outlets in the United Kingdom of storing the sticks in chill cabinets; no cases were reported in continental Europe where the sticks were stored at ambient temperatures. The manufacturers reviewed their manufacturing procedures, introducing a heating step in the process, thus guaranteeing the destruction of any salmonella that might survive the usual salami-manufacturing process.

Staphylococcus aureus and Pasta

Epidemiological and microbiological tracing attributed 47 cases of staphylococcal food poisoning, occurring in several European countries, to a single source of lasagna. The source of the problem was traced back to the contamination of the eggs used in the manufacture of the pasta and was believed to be associated with the hygiene of equipment used to transport the eggs. As a result of this incident, a code of practice was drawn up by the pasta industry requiring the use of pasteurized eggs in pasta manufacture and improved hygienic design of the equipment used for the preparation of the pasta.

Clostridium botulinum and Yogurt

After 27 cases of botulism were traced to the consumption of a locally produced yogurt containing hazelnuts, the source was identified as canned hazelnut puree; this was part of a batch of low-calorie products prepared using artificial sweetener. Unfortunately, the manufacturer of the hazelnut puree had not recognized that the heat process used for commercially sterilizing the normal high-sugar product was not suitable for the low-calorie product. Sugars reduce the available water (a_w) to a level at which spores of *C. botulinum* are unable to initiate

vegetative growth and are thus unable to grow and form botulinum toxin; the organism grew well in the sugar-free product that had a much higher a_w. The outcome of this incident was to inform producers of these and other products of the need to obtain expert advice on the required heat process before reformulating a canned food. Also, the national code of practice for low-acid canned foods was revised to clarify all requirements for the production of microbiologically safe low-acid canned foods.

Testing Foods for Foodborne Disease-Causing Microorganisms

Foods may be tested for foodborne pathogens for a variety of reasons; the main reasons are listed in Table 2.

Microbiological testing of foods has been widely applied by health authorities and industry as a means of identifying foods that have levels of microorganisms or their toxins in excess of those regarded to be acceptable and therefore are unsafe or unacceptable for human consumption for other reasons. Such testing was at one time believed to give adequate protection to the consumer and was the linchpin of microbiological control strategies practiced by governments and industry for much of the twentieth century. This was despite the recognition more than 30 years ago that such testing gave little or no assurance of microbiological safety or quality. This can be illustrated by reference to the most stringent testing plan applied internationally for examining baby food for salmonella. In this plan, a sample comprising 60 individual sample units is taken from a batch of baby food, and from each sample unit, an analytic sample unit of 25 g is taken and examined for salmonella (total analytic sample is thus 1500 g); the pass criterion is no detection of salmonella in any of the analytic units examined. Even when this pass criterion is met, there is 50% probability that up to 1% of the sample units in the food batch will be contaminated. Clearly, this degree of confidence is not acceptable, particularly when considering that such a sampling plan may be applied to test the acceptability of a baby food.

It is such considerations that have led to a total reappraisal in recent years of the value of such testing as a means of assuring the microbiological safety of a foodstuff. This has led to the wide-scale introduction of hazard analysis and critical control point (HACCP)-based control systems whereby the potential hazards associated with manufacture of a food in a particular processing plant are identified using a structured hazard analysis procedure and locations where these hazards can be effectively controlled are identified. These locations are called critical control points and means for their proper control (including means of monitoring that control is maintained) are identified.

Table 2 Uses of microbiological testing

Investigational testing	Suspect contaminated food
Compliance testing	Governmental regulations; industry standards and purchasing specifications
Monitoring	Authorities monitoring imported and domestic foods; industry monitoring products and processes
Verification	Good manufacturing practice and hazard analysis and critical control point

As a result of the introduction of HACCP as the prime means of assuring the microbiological quality and safety of foods, microbiological testing has become less important as a primary control function and increasingly is used mainly by industry for the purpose of verification of HACCP. However, where HACCP is not applied, the industry or a health authority may apply microbiological testing if there is a need to obtain a judgment about the acceptability of a foodstuff. For instance, it may be appropriate for use in a buying specification for a microbiologically sensitive raw material from a new supplier or by a health authority on a consignment of imported food, where microbiological testing is the most appropriate acceptance criterion. For such a purpose, it is important that statistically based sampling plans are used so that the degree of confidence in the result obtained is known; methods of analysis should also be agreed. One of the two types of sampling plans may be used for such acceptance sampling. The first and most widely used are the two- and three-class attribute plans established by the International Commission on Microbiological Specifications for Foods. Such plans not only were developed principally for testing foods in international trade for their acceptability but also are widely used by industry in buying specifications.

Two-class plans have a single limit that distinguishes between an acceptable concentration and an unacceptable concentration of a microorganism or toxin and is used in sampling plans for foodborne pathogens.

Three-class plans are used principally for determining the acceptability of levels of general microbial contaminants and indicator organisms and have two limits: One is a lower limit generally established by good manufacturing practice requirements and the other is an upper limit based on some index of unacceptable quality. The other main type of plan used is the standard variables plan that is based on knowledge of microbial distributions in batches of foods and can be used when such distributions approximate to log normality. This limits their use principally to industry, where their advantage over attribute plans is greater accuracy and hence greater confidence in decisions based on their use.

The choice of a sampling plan and testing procedures for investigative sampling that may, for example, be used to identify the level of contamination in a suspect consignment of food or to investigate food involved in a food-poisoning incident is more difficult than acceptance sampling. One major problem is that there will usually be no information on either the likely incidence of contaminated product units or the level of contamination; these factors set requirements for the number of units of product that should be examined and the size of the analytic unit that should be tested. In investigative sampling, unlike acceptance sampling, random sampling may not be the most appropriate procedure. For example, the location of the product units in relation to possible source of the contamination may suggest that cluster or stratified sampling may be more appropriate.

Tracing the Origins of a Foodborne Disease Incident

Investigation of the cause of an outbreak or case of foodborne illness will usually require a number of procedures depending on the type and circumstances associated with the incident.

These may include extensive testing of food and clinical samples for likely causative agents, the use of epidemiological analytic techniques to link illness to a common source if a number of cases of illness are involved, field investigations by public health and industry experts to find the cause, and specialist food-processing knowledge to identify what measures need to be taken to prevent its recurrence.

The tracing of the source and cause of an outbreak of foodborne disease associated with a well-defined event, such as a banquet, is usually a relatively easy matter. A list of foods consumed by the ill persons is compared against a list of items consumed by persons who are not ill, and using simple statistical correlation procedures, any significant differences in foods consumed by the two groups are identified and further investigated to find the most likely food source. Tracing the source of sporadic cases in a community is much more difficult, and in addition to an extensive use of well-founded epidemiological procedures, investigators need a certain amount of serendipity, backed up by specialist laboratory resources, in order to be successful.

Laboratory Investigations

Samples of suspect foods, together with clinical samples, should be collected and transported under conditions that protect them from external contamination and minimize changes in the concentration of any food-poisoning agent that may be present, for example, in sealed sterile containers in a suitably cool box. Details of the history of the sample, for example, where and when and by whom and how, together with information on symptoms of illness should be submitted to the laboratory as these will help in selecting the most appropriate method of analysis. On receipt by the laboratory, the samples should be documented, properly stored, and examined as soon as possible. Such examinations must be undertaken by laboratory staff who have been trained in the microbiological examination of foods as well as clinical specimens; they should be particularly knowledgeable of the analytic procedures to be applied. These should be performed in accredited laboratories operating to recognized good laboratory practice standards and using procedures and methods that have been approved by an appropriate accreditation organization. The microbiological examination of a foodstuff can be problematic and a number of pitfalls may trap the unwary or careless. For instance, examination of food specimens in the same room where clinical specimens are examined is bad practice; a heavy microbial load in a clinical specimen can easily cross-contaminate a food sample, where only light contamination with the same organism in the clinical specimen is sufficient evidence to implicate a food as the source.

Initial laboratory findings should always be interpreted with care. Isolates and where appropriate original specimens should be submitted to a reference laboratory with expertise in the suspect agent of concern in order to confirm an initial finding and, if appropriate, to apply modern 'fingerprinting' and typing methods such as DNA-based subtyping methods. There are many examples of foods being erroneously implicated in a food-poisoning incident because of poor laboratory practice. There have also been many examples where failure to identify a causative agent, or a food source, has been the result of failure of the laboratory to apply the correct isolation or detection method. The microbiological examination of foods is not an exact science, although continued improvements in detection techniques in recent years have considerably improved our ability to detect pathogenic agents in foodstuffs.

Epidemiological Investigations

Two main analytic tools are used by epidemiologists to trace the source of a foodborne disease incident: case-control studies and cohort studies. For both, the first stage is to review and document all available information. In an investigation of an outbreak, a questionnaire is prepared to detail when and what was consumed and symptoms of illness. This questionnaire is used in preliminary interviews with persons affected by the outbreak to obtain basic information as to the likely cause. A case definition is also agreed, that is, what symptoms should be searched for, as it is important when case searching (in order to identify the extent of an incident) that only the most likely cases are identified.

In a case-control study, a series of questions based on the preliminary questionnaire are asked to the persons identified as corresponding to the case definition (case group) and persons who have not been ill (control group). These two groups are matched as far as possible with respect to age, sex, socioeconomic group, and place of work or residence. Controls are often based on consultation with the general practitioners of the persons who have been ill. Based on information in the completed questionnaires, differences between items consumed by the ill and well groups are identified and any statistically significant differences subjected to further investigation. One problem with this procedure is that it is common in food-poisoning incidents to have symptomless cases and these may well be mistakenly identified as part of the control group.

In the analysis of the case data, the following are the most important:

1. Time to onset of illness. Based on knowledge of the incubation period of the illness, it is possible to determine the period of exposure to infection. This information may then be plotted as a histogram relating the frequency of cases chronologically to the date of onset of illness. This so-called 'epidemiological' or 'epidemic' curve can then be used to identify whether or not there has been secondary transmission of illness and whether or not it is a point-source outbreak, for example, a single meal, or a common-source outbreak, for example, a number of cases from the same source, such as a retail establishment, occurring over a particular time period.
2. The place where infection occurs. A map is drawn of known cases, and researchers look for relationships, such as clusters, that may assist in identifying a source.
3. The persons involved. Analysis of information on individual cases such as age, sex, medical history, occupation, home situation, and any travel history may also help one to form hypotheses as to the source of infection.

In a cohort study, a group of people is identified by some other means than illness. For example, this might be all the persons who attended a conference at which it is suspected that a meal was consumed that led to illness. The persons are divided into two groups according to whether or not they ate a particular

meal; the attack rate in the group eating the meal and that of the group not eating it is calculated. If there is found to be a statistically significant correlation for the consumption of a particular meal, the next stage is to determine the attack rates for persons eating and not eating a particular menu item. By this means, if successful, a particular menu item will be identified as the source of the agent that caused the illness.

Environmental Health Investigations

As part of an investigation into a foodborne disease incident, health inspectors may visit and investigate the identified source, for example, the place where a foodstuff implicated in a food-poisoning incident was manufactured or prepared for consumption. This may involve a detailed examination of the hygienic practices and the collection of product and environmental samples for laboratory examination. If an infectious disease is suspected, food handlers may be asked to submit samples for microbiological examination. Where a source of contamination has been identified, the health authority is most likely to require assurance that the condition that caused the contamination has been corrected prior to allowing the product to be returned to the marketplace. Such assurance may require evidence that checks have been done by a third party to confirm that satisfactory control measures are in place. If a HACCP plan is in place, this plan will need to be revalidated.

Withdrawal of a Suspect Food

If a distributed food is found by a health authority or a producer to be contaminated with a concentration of a pathogen or toxin exceeding a legal limit or exceeding a level generally regarded to be hazardous to health, this food should be withdrawn from sale as soon as possible and, if necessary, the public informed.

Before announcing the public withdrawal of a food, it should be ascertained, as far as is technically possible, that this food is unsafe to eat. Wherever possible, the results of microbiological analyses or evidence clearly linking cases of illness to an implicated food item should be available to the health authority and/or producer, as adverse publicity is likely to result from a public recall that may cause much damage to the reputation of the producer. The prudent producer may not wish to run the risk of even worse publicity arising from an incident occurring as a result of failure to inform the public and may voluntarily withdraw the product and inform the public of this.

Authorities will take different actions concerning the extent of a recall depending on a judgment as to the health risk involved by allowing food to remain on sale or in the hands of a consumer. Thus, a recall may only be required down to the distribution level, for example, distribution warehouse, if it is judged that any food remaining on sale is unlikely to cause further illness. A similar decision may well be made for a food containing a level of microorganisms exceeding a legal limit but where no illness has been reported. However, if it is judged that serious illness could result from product on sale or in the hands of consumers, recall down to retail level would be the minimum requirement and a public warning (via the media) would usually also be required. It is important that a recall is properly coordinated by the owner or supplier of the food; for this to be effective, a food company should have a documented recall procedure in place. As part of this procedure, a recall coordinator should be appointed whose role is to coordinate the recall, to liaise with the public and the authorities on progress with the recall, and to act as the spokesperson in contact with the media.

When carrying out a public recall, it is important that this is done as fast and thoroughly as possible. Thus, there will be the need for press statements via the media and communication to customers (and retailers) via e-mail or fax, advising them of the recall. In the case of a national or international recall, the health authorities will inform their contacts via established networks and, if a particular ingredient is involved, may work with the supplier to identify and advise producers who may be using this ingredient in further products. Effective coding of food batches and maintenance of records of distribution are essential requirements as production failures will inevitably occur from time to time.

See also: *Clostridium botulinum*; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; Food Poisoning: Epidemiology; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; *Listeria*: Listeriosis; Poultry: Processing; *Salmonella*: Properties and Occurrence; *Vibrio*: Types, Properties, and Determination; *Yersinia enterocolitica*: Properties and Occurrence; Zoonoses.

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Food Systems: Food Miles

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Introduction

The phase 'food system' encompasses all factors relating to food including all actors (people and groups) and processes and infrastructure associated with food production and its movement from farms to consumers. Food systems are also networks shaped by economic, social/political, and environmental circumstances. Food miles, a newer concept coined by Professor Timothy Lang, are the total miles food travels from the farm to the consumer. Food miles are one way to look at the environmental impact of food but are also a useful concept to help contemplate society's broader relationship to food and food producers. The average distance food travels has been increasing with current estimates at over 1500 miles. In Britain, a major study commissioned by the government estimated that in 2002, the annual social cost of increased transportation of food equaled £9 billion per year (about US$15 billion). This cost includes the increase in greenhouse gas emissions in addition to road maintenance and traffic congestion costs.

Many interesting food travel anecdotes exist. A 2007 British Broadcasting Corporation (BBC) story tells of Scottish langoustines (a small relative of lobster) being caught in Scottish coastal waters and then traveling to Thailand through the port of Rotterdam. After the langoustines arrive in Bangkok, they are shelled by hand and then travel to Holland where they are breaded. Next, the langoustines return to the United Kingdom for consumption, having traveled 17 000 miles. Most African agricultural products consumed in Europe or North America are processed in India or elsewhere in Asia before being distributed. One brand of Tanzanian coffee travels nearly 5000 miles just to be roasted and bagged. Cashew nuts harvested in the north of Ivory Coast are sent to India and Vietnam where the nut is extracted manually, sorted, and bagged before being shipped worldwide for consumption.

The concept of food miles has caught the public interest. There is a website (www.foodmiles.com) to help consumers estimate the distance their food travels in addition to providing other environmental information. There is disagreement regarding the use of food miles as an indicator for evaluating food systems. Some justify the use of food miles based on favoring local food production, which would minimize at least one environmentally related criterion (distance). The critics of food miles argue that it legitimizes a new form of market protectionism, it is too narrow of an indicator, and it is not a definitive environmental or economic measure.

Food System Definition

The phrase food system refers to all factors involved with food production, processing, distribution, storage, consumption, and associated waste management. The concept is often summarized by the image of the 'farm to fork' or 'field to table.' In the literature, many food system representations are frequently in the form of a chain or network. For any food system, there are products (inputs and outputs), actors (farmers, wholesalers, processors, distributors, consumers, and regulators), and modes of coordination (by the market, by standards, and by one or more agencies). A food system is always linked to a biophysical context (e.g., potential agronomic, climatic characteristics, or accessibility to seafood), an economic and social context (e.g., income, population, or eating habits), and a level of technological adoption (e.g., food preservation techniques).

Transition from Traditional to Modern Food System

One way to describe the food system is chronologically from the traditional to the modern system. The traditional food system fits the rural economies, where agriculture is the main industry. A large portion of the local agricultural production was consumed on the farm or in nearby villages, and only a small amount of agricultural surpluses (extra product) was sold on local markets. The farms were diversified, with little mechanization and little if any use of manufactured chemical inputs. Agricultural products were sold almost exclusively in their raw form (sometimes animals were sold live), at the farm, at local markets, or in small shops. There were no food safety standards or food traceability systems (method of tracking food back to the farm). Local diets depended on what was grown locally and would vary according to local production and the season. Famines might occur if the local supply was insufficient. There were few losses due to waste, but many losses would occur due to storage problems. Food systems similar to this traditional system are still found today in many developing countries where consumers rely on local foods.

Over time, farms and food operations have grown larger due to technological advances and mechanization. These factors favor large operations and lead to 'economy of size,' where average per unit costs normally decline with greater production. Lower average costs tend to increase profits, which create pressure for the food businesses to become larger. Small multicrop farms gave way to large specialized farms. Local wholesale markets began to shrink or disappear with the creation of worldwide 'grower-shippers' who grow crops in multiple locations to ensure year-round supplies and can deliver product virtually anywhere in the world. At the same time, small, locally owned grocery stores disappeared with the simultaneous growth in large supermarkets. This modern large-scale food system shall be called the conventional food system.

The conventional food system serves primarily urban and suburban economies in the developed world. This system brings food into areas where food is generally not produced. With this system, the economic importance of agriculture is low and farmers represent only a very small part of the country's population (frequently $<4\%$). The farming systems

are normally specialized with a high degree of mechanization and technology. Agricultural production is frequently concentrated in productive areas, which may be thousands of miles from the population centers.

With the conventional food system, consumers eat more processed food products and less raw and unprocessed foods. Products are transformed into specialized foods, which are sold through global food sales networks. Often, the processed food has higher fat and sugar levels and less fiber than raw or less processed foods. There are public and private food safety standards as well as food traceability. Within this system, food storage (including cold storage) is effective with minimal losses. Food losses are normally observed after the sales to the final consumer (e.g., if the food has reached its expiration date). Subject to the eating preferences of consumers, diets can be diverse because consumers have access to a vast number of products from around the world. Under the conventional system, consumers on average consume more meat than under the traditional food system due to taste preferences and lower costs of meats. The conventional food system can be credited with having widely reduced food insecurity, reduced the percentage of income spent on food, and also contributed to food deserts and obesity. A recent United Nations report states that unhealthy diets, which are partially due to the conventional food system, are now a greater health threat than tobacco.

Concerns Regarding Sustainability and Emergence of Alternative Food Systems

Although the conventional food system is productive and delivers massive quantities of inexpensive food, there are negative features. Farms under this conventional model are large and use more fertilizers and pesticides. Given the size and intensity of production, these systems are not sustainable and pose a threat to the integrity of ecosystems. Crop fertilizer use in the United States has been implicated in many cases of ground water contamination as well as the large ocean 'dead zone' where the Mississippi River empties into the Gulf of Mexico. In addition to the negative environmental aspects (negative externalities) of the conventional food system, critics also point to an adverse impact on employment and the community. Further, the global trade liberalization undertaken since the Uruguay Round of world trade negotiations has made the food system more sensitive to economic shocks. In assessing the sustainability of a food system, important considerations include the availability of food to all, system food security, and the ability to preserve natural resources. Although the ancient or traditional food system has evolved in much of the developed world into the conventional food system, there are still traditional food systems of varying sorts in many countries including the most developed. Most countries have multiple food systems existing side by side. Many cities have a local farmers' market operating within a short distance from a superstore selling thousands of food products. Since there are many alternative food systems frameworks, including farmers' markets and community-supported agriculture, systems differing from the conventional food system have become known as alternative food systems.

For the remainder of this article, we will compare and contrast the conventional food system and the alternative food system, as two ends of a spectrum. They will be our 'ideal types' for comparison along the approach developed by the sociologist, philosopher, and political economist Max Weber. An 'ideal type' is a simplified approximation of something that can be used for comparison or other analysis.

Comparisons Between the Conventional and Alternative Food Systems

A distinguishing feature of the conventional food system is its large scale and heavy use of mechanization and other technology. As mentioned before, technology and mechanization are accompanied by large production volumes and lower average costs. Large crop fields exist where frequently one crop is grown for as the eye can see. Large quantities of chemicals, pesticides, and fossil fuels are used. Processing and distribution centers are also large. Food in this model is increasingly processed, not only to meet the desires of consumers but also to lower costs and improve transportation and storage. As part of the food processing, considerable fats, salt, and sugars are added, while natural fibers are reduced, resulting in food that is generally considered less healthy.

Contrasted with the conventional food system, alternative food systems emphasize a connection between consumers and producers. They promote the economic and social benefits of local agricultural production, which can be processed locally by a small business. Alternative food systems are thought to have less detrimental impacts on the environment, generate more employment, and deliver food considered healthier than industrial food. Food produced in alternative food systems is usually considered to taste better than the same food produced under the conventional food system. In moving away from a conventional food production system, one is moving away from large-scale economies, and thus, one would expect higher costs and possibly higher energy use per pound of food delivered. However, when comparing costs of conventional versus alternative food systems, it is difficult to determine and include costs of increased obesity, fewer good jobs, and environmental costs associated with the conventional food system. In Table 1, several important features of the conventional and alternative food systems are compared.

The conventional food system is oriented toward efficiency and profits. Consequently, the conventional food system has been able to provide food in abundance and at overall declining inflation-adjusted prices. At the same time, it has been criticized for its environmental impacts due to intensive use of resources, externalities, and social impacts due to the concentration of actors and the resulting loss of jobs. The question of food healthiness and safety of the conventional food system is complex. On the positive side, the conventional food system is very efficient and delivers an enormous amount of food with relatively few food problems (on a per person basis). This is accomplished through the use of processing and preservatives, which limits food microbial contamination. As mentioned earlier, processed foods are high in sugar, salt, and fat, which contributes to an unbalanced diet, leading, in part, to increased obesity, food allergies, and other ailments. Another concern regarding the size (and speed of food movement) of the conventional food system is the challenge of monitoring food safety, because when there are cases of food contamination, many consumers can quickly become ill or die. Given the size

Table 1 Important food system features

Feature	*Conventional food system*	*Alternative food system*
Ways of improving farmer income	Specialization and growth; lower costs	Diversification and fewer intermediaries between the producer and the consumer to capture more value
Division of labor	High degree of labor specialization and labor concentration	Lower level of labor specialization and fragmented actors
Product quality	Uniform and homogeneous products fit best with the industrial framework; higher level of processing; consistent quality and overall safety record	Products are less homogeneous and often vary by producer; product less processed and thus fresher with better taste; product more often local and linked to geographic region (authentic)
Target markets	Mass markets and differentiated technological product (e.g., health food)	Markets are normally closer and differentiated by reference to the place of production (geographic identification), or by the farmer or artisan nature
Desired performance	Price competitiveness, improving productivity of labor and land through intensification and specialization	Competitiveness through quality (emphasis on taste and freshness); sustainability through crop rotation and growing multiple crops
Area requirements	Dense and concentrated in areas with high agricultural potential	Can be intensive but normally with some dispersion due to crop rotation or multicrop farm planning
Innovations	Technological	Organizational
Environmental performance	Efficient use of inputs; capable of meeting specific targets; improving carbon footprint; large disperse negative impacts due to production concentration	Goal of environmental sustainability in concert with vision of integrating all farming and food aspects as a system, including agronomy, animal welfare, worker/owner benefits, and rural development
Types of business qualifications	Traceability; government or private certification of production processes	Growing use of geographic identification as quality indicator; government or private certification of production process (such as US Organic)
Food Miles	On average long distance	On average less distance
Functions of agriculture	Primarily produce raw materials for industry	Multiple: produces food, maintains open spaces, provides jobs for rural people, and helps support rural economies

difference and the multiple dimensions of the conventional and the alternative food systems with respect to overall food healthiness and food safety, both systems have pros and cons, and a more detailed comparison is beyond the scope of this article.

The alternative food system improves upon many of the negative features of conventional agriculture including fewer negative environmental impacts and less processed food. Other benefits include a renewing of the social ties between those who produce the food and those who consume it. The alternative systems also employ fewer pesticides and have a better distribution of the food dollar (with more value going to the farmers). While many benefits of alternative food systems are noted, it has been criticized for its inefficiency and inability to address the food needs of the world's population as a result of low productivity and higher cost of food production. On the social level, consumers would need to cook more under the alternative food system and use more energy, as they would buy less processed products and more raw food products. In the next section of this article, we will discuss the assertion that lower food miles decrease the negative impact of a food system.

Does Reducing Food Miles Necessarily Improve the Environmental Performance of the Food System?

While no one disputes that food travels longer distances, the impact of food miles on energy consumption and greenhouse gas emissions has been the subject of extensive research. The conclusions of these studies are mixed. Most authors agree on how improvements might be made, such as using trains for transportation when possible and avoiding empty return truck trips. Some also conclude that the reduction of food miles can significantly reduce costs and also reduce the negative environmental impacts of food distribution. Others argue that the environmental impact of local food production and distribution is heavily penalized by low product volumes and inefficiencies. The critics also show that food transportation energy is a small portion of total food system energy and is less relevant.

How can these differences in researcher conclusions be explained? To begin with, the measurement of energy expenditure in food distribution presents many methodological difficulties. When considering the food system, how wide should the scope be? Will we consider differing sources of energy? What units of measurement will be used? Which energy expenditure value is used? Is the use of greenhouse gas emissions a good proxy for overall environmental impact? How can different situations be compared? Depending on the choices made, the results appear very different, sometimes to the advantage of the conventional food system, sometimes to its disadvantage. The review of existing literature shows numerous findings regarding food system energy usage and greenhouse gas emissions. Some consider the whole system, while others focus solely on distanced traveled. For tractability (being able to identify and aggregate all impacts), many studies reduce and

focus on one environmental criterion such as energy use or greenhouse gas emission per food unit (e.g., grams of CO_2 greenhouse gas emission per apple).

The Life Cycle Analysis Approach

The life cycle analysis (LCA) approach to evaluating food systems attempts to consider the environmental balance of the entire food system. For researchers using this approach, food miles are only one of many uses (expenditures) of energy. Other food system energy expenditures include the production technologies used to produce the food, as well as the energy to process and store the food. To illustrate, consider a comparison between vegetables grown closer to the equator where the sun provides enough energy for the vegetables to grow and ripen naturally and the same vegetables being grown in a greenhouse and sold locally. With the vegetables grown closer to the equator, less energy is needed in production, but more in transportation. With vegetables grown in greenhouses, more energy is needed in production and less in transportation. Various studies show that the gain in energy obtained from the sun in the production phase of the vegetables is much higher than the energy necessary for transportation.

Other examples exist where one or more strong efficiencies can outweigh transportation environmental costs. The government of New Zealand maintains that New Zealand lamb production has such a low carbon footprint that it more than offsets the environmental costs of ocean shipping. This low environmental impact is due to the sheep being rangeland-fed, the rangeland requiring little fertilizer and few pesticides, and also the high efficiency of container ship transportation. The New Zealand government concluded that the carbon footprint is generally lower if consumers in London, England, consume New Zealand sheep rather than English sheep.

Transportation by large container ships is very efficient with regard to fuel consumption per food unit, as 10 000 km by container ship is roughly equivalent to 400 km by truck for the same amount of food weight. There are efficiencies claimed in food processing also, as in the aforementioned case of the Scottish langoustines. The company's director maintains that a study shows a lower carbon footprint with peeling langoustines by hand in Thailand than with peeling by machine in Scotland.

The most common method of assessing the overall environmental performance of alternative food systems is LCA. This valuation method qualifies and quantifies all impacts of a product (or service) throughout its life cycle from production to distribution to the consumer (from the product's 'cradle to grave'). The LCA method has become a standard tool (ISO 14040) and has been employed in considerable research. LCA is considered a multicriteria method given the method's focuses on the overall impact of the product on the environment, energy, raw materials, water, emissions, waste production, etc.

To employ the LCA analysis, one first determines and evaluates the flow of raw materials and energy coming into and leaving each stage of the product's life. Next, environmental impacts are measured or estimated based on values (coefficients) calculated from previous research (e.g., truck manufacturer specifications on fuel use per mile). To allow comparisons, one determines and adopts 'functional units' for comparing products with the same function. In a recent study comparing the impacts of canned tomatoes (diced and paste) produced in California and distributed in Michigan with tomatoes produced and distributed in Michigan, the functional units adopted were kilogram of canned tomatoes (diced and paste). The results showed that overall the two scenarios had similar energy use and greenhouse gas emissions, but the California tomatoes had a much higher use of developed water sources. The impact of transporting the tomatoes from California to Michigan was offset by more productive California growing conditions including less soil amendments being required. An interesting result was that tomato paste contains approximately 4.5 times as much raw product equivalent than the diced tomato product. It was found that in some cases, using concentrated products for cooking would have lower environmental impacts than using the whole products. In this study, the environmental impact of transportation appears ultimately negligible, especially if transportation of the canned product is by train.

The previous examples illustrate the important finding that food miles represent only a small part of the energy expenditure (or environmental impact) of a food system. For the United States as a whole, a study published in 2000 estimated that transportation accounted for less than 20% of total energy consumed in the food system. Many studies regarding food system energy consumption conclude that the highest energy costs within the food system are not from transportation but from agricultural production, processing, and energy used by households to obtain, store, and cook their food.

LCA is a useful method used to identify all environmental impacts of a product or service. The LCA method provides an overall view but at the same time shows the complexities of the analysis since for each life step of the cycle, strengths and weaknesses can be highlighted. The LCA method is detailed and relies on current or recent estimates of impacts from other sources. Due to this reliance on other research, some authors consider LCA more of a decision support tool than a decision making tool.

Research on Food Miles

Although the distance traveled by food contributes moderately to the overall energy consumption of the food system, environmental impacts can be reduced by shortening travel. It is for this reason that various research projects have been conducted seeking to compare the respective impacts of long food chains (represented by the conventional food system) and short food chains (represented by the alternative food systems). There are two primary approaches to carrying out food mile studies and each has important methodological challenges. One approach is based on typical scenarios, while the other calculates specific food travel measurements.

Food miles studies based on evaluating typical scenarios begin with noting all supply chain stages through which the product will pass. Once these stages have been identified, the average distance the food travels between each stage is calculated (farm to wholesaler, wholesaler to processor, etc.) and assigned a transportation energy usage. The amount of energy used for each transportation leg is normally not directly

measured, but rather relies on average fuel consumption based on, for example, truck manufacturer specifications. In other cases, researchers rely on previously published results, such as those found in published LCA studies. This shows a methodological link between the LCA and a food miles scenario analysis.

Food miles studies based on actual measurements examine the specific miles traveled and energy or other environmental impacts for each stage of the supply chain from production to consumption. In a detailed example, Elmar Schlich and colleagues at Justus Liebig University in Gießen (Germany) calculated the total energy used at all stages in the supply chain for local German lamb compared with lamb imported from New Zealand. The New Zealand lamb is slaughtered and frozen on-site, transported by truck to a port, and then shipped to Germany on a container ship. While on the ship, the New Zealand lamb remains at its constant frozen temperature, and once in Germany, it is transported by truck to a distributor. The German regional lamb travels far fewer kilometers but travels in smaller vehicles and therefore has less fuel efficiency on a per kilogram bases. In all the cases studied by the research team, they concluded that the large transport distances traveled do not automatically imply high final energy consumption. From their point of view, these results highlight what they call 'ecology of scale,' where the concentration (production, transport, etc.) has a positive influence on any final energy consumption per unit produced or transported.

Most of the examples studied show a decreasing relationship between final energy utilization per food unit produced, and company size. Advantages do not automatically come from shorter food miles, but often come from logistical efficiencies due to large and specialized companies.

There is criticism of these works on multiple grounds. These studies could conclude that a country or region should abandon many traditional agricultural products in favor of products produced on the other side of the world. For example, it might be recommended that all of Europe abandon commercial apple production and only eat apples from Chile. One limitation is that frequently, all forms of energy are treated as the same, and this is questionable since all forms of energy do not have the same consequences for the environment. Moreover, some studies focus only on final energy and not all the elements used in the analyses of life cycle including air and water emissions.

Do food miles studies help us compare long food supply chains (conventional food system) with short food supply chains (alternative food system)? Not really, since under both the conventional and alternative food system scenarios, the product is assumed to be sold to the consumer via the same distribution method. This may not be the case since the consumer may acquire the product at a local farmers' market and not the grocery store. For these reasons, some researchers have focused on comparing the energy expenditure required for mass food distribution to local food distribution systems. These studies then incorporate another dimension – the path of the consumer. In order to deliver a product from field to plate, the food must be passed to the consumer, and this normally involves a smaller, less efficient, passenger vehicle. Often, the most expensive food travel leg in terms of energy is the 'last mile,' which is the trip made by the food consumer who inefficiently carries a relatively small amount of food in his or her personal vehicle. These results again led many authors to question the supposed energy savings from 'localism' in the food industry, and they show that short chains are often penalized by lower quantities sold, despite the short distances.

One unresolved issue regarding food miles studies is whether they have appropriately modeled the actual consumer trip patterns. In reality, some percentage of farmers' market shoppers are combining their visit with other trips ('trip chaining'). Thus, many farmers' market shoppers may not be making a special trip, but rather stopping at the market on the way home from his or her work, picking up a child, etc. According to a study in the Rhône-Alpes region (France), food distribution in a short supply chain can achieve good results when the choice of distribution sites allows consumers to avoid specific shopping trips.

Consumers seeking alternative food systems are thought to be more committed to environmental protection and thus more likely to consider low-impact methods of obtaining their food. More and more consumers are sensitive to the energy balance of their food choices and prefer less impact transportation, such as taking their bicycle to the farmers' market. If they visit a pick-your-own (or U-pick) farm to obtain food, it is frequently to also spend an afternoon outdoors and perhaps offer an experience for the children.

Conclusion

The scientific research on the impact of food miles on the energy requirements of the food system concludes that transportation has a low overall impact. This finding is due to the fact that for food, transportation energy is relatively small compared to energy required for production and processing. Therefore, the supposed benefits of 'eating local' in terms of energy consumption and other environmental measures are often questioned.

Thus, it is restrictive and probably scientifically unwise to assess the food system sustainability based solely on food miles. For this reason, many studies take a much broader perspective, along the lines of the LCA discussed earlier. A comprehensive approach would be similar to the LCA approach but be broader than only environmental (including energy) factors. Evaluating food systems should focus on the entire economic, social, and environmental benefits of each food system. Many argue that alternative food systems have other favorable indicators including a more equitable distribution of the value added (income) from farming, as well as a positive recognition of the family farm and farming as a desirable profession. Alternative agriculture can also be more harmonious with the development of the local economy, preservation of biodiversity, the greater 'naturalness' of products, etc. The sustainability of the food system must be studied in its entirety and cannot be reduced only to the extent of food miles or even energy consumption.

The methods of evaluating food systems are complex and a comprehensive analysis is even harder. How should we evaluate the benefit of local job creation in this era of disappearing jobs? Local agriculture also helps maintain open space and

contributes to biodiversity. An LCA that puts all measurements in terms of grams of food per kilowatt of energy is overly simplistic when trying to capture these multidimensional aspects. A study comparing bakeries on energy usage will likely conclude that the best bakery for reasons of efficiency is the large industrial bakery, and not the local artisanal bakery. However, there are non-energy use-related benefits to having a local bakery including great fresh bread and local employment, and it is a challenge to compare these multiple dimensions simultaneously.

In considering food miles, the length of the food system supply chain is important. Many alternative food systems have shorter food supply chains. This is quite a contrast to the lengthy food travel examples provided earlier under the conventional food system. The alternative food supply chains are still underdeveloped and significant improvements can be made. Urban sprawl pushes agricultural production further from the town or city center, thus increasing the distance between consumers and their food. Land prices near cities are also frequently prohibitive for farming. Urban and rural planning can play an important role by preserving farmland in the immediate outskirts of cities to promote local agriculture and also by promoting the location of food distribution sites within the urban centers.

Although of limited scientific use, food miles can be an important image that spurs consumers to reflect upon their relation with food. Consumer preferences do shape production practices as we have seen with the introduction and growth of free run and free range eggs. Perhaps we are seeing 'food miles' as a consumer focal point being replaced by the related 'local food' concept. This concept has been officially recognized and has gained national attention and support in the United States. In 2014, the US Department of Agriculture announced it will make a US$ 78 million investment in local and regional food systems, including food hubs, farmers markets, aggregation and processing facilities, and distribution services.

See also: Food Environment; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Traditional Foods.

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Foodborne Pathogens

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Introduction

Foodborne pathogens are one of the leading causes of illness and death in less developed countries. In developed countries, they are responsible for many millions of gastroenteritis cases every year. As the scale of globalization of food commodities and their associated supply chains continues to grow in response to ever-growing demands of consumers, the challenges posed to the scientific community dealing with methods of detection, surveillance programs, and intervention strategies for ways to reduce, control, or prevent these pathogens in the food supply chains also become more complex and frequent. Over the many decades that the discipline of food science has existed, including the areas of microbiology and chemistry, a huge amount of data and knowledge has been collected by researchers all over the world in response to these challenges. There are several articles within this book that describe in detail the foodborne pathogens currently of most concern. Of course, there is always the possibility that emerging and reemerging pathogens will proceed to contaminate the food chain and challenge researchers in the area, which is why there is a large amount of research still being done around the world to make methods for detection and enumeration more reliable, sensitive, and rapid. Some of the foodborne pathogens, which are described throughout this book, such as *Escherichia coli*, *Salmonella*, *Listeria*, and parasites, have been known agents of food poisoning for many decades, while some others such as hepatitis E virus (HEV) are emerging problems, at least in the sense that they have only been associated with foodborne outbreaks in recent times. These challenges have led to ever more sensitive detection technologies being employed: both qualitative and quantitative nucleic acid detection methods, for example, polymerase chain reaction (PCR), real-time quantitative PCR, digital PCR, and loop-mediated amplification, to name but a few.

The increase in the number of methods for detection of foodborne pathogens highlights the importance given to this area by government regulators and food manufacturers, but it also necessitates the need for harmonization and standardization of these methods in order for them to be fully compatible with current existing 'gold standard' culturing methods, many of which are still considered to be the most reliable. This is due to the fact they are tried and tested for many years and that, crucially, they only detect living organisms. There is, however, a general recognition in the food industry that the detection of nucleic acids and therefore the presence of certain pathogens within certain parts of the food supply chain signify that a failure of the manufacturing process has occurred, since the pathogen should not be present – whether alive or dead. There are of course exceptions to this, depending on which stage of the process is being monitored, but in recognition of this, there are major efforts in researchers' laboratories to find ways of detecting viable microorganisms apart from traditional culturing techniques, such as the use of propidium monoazide (PMA), which is reported to be able to distinguish live cells from dead cells. A light-induced photoreaction occurs that causes the binding of the PMA with the DNA, which is released when a cell dies. This then renders the DNA from the dead cells nonamplifiable by PCR, while the DNA protected within living cells is not affected. There is a mixed response to this technique, due to reproducibility issues; however, it appears to be the closest that researchers have come to answering the 'dead or alive' issue that has been a challenge in the molecular microbiology area for many years. Foodborne pathogens and the problems they cause within the food industry have been around for a long time, and there is no indication that this situation will decrease any time soon, so the need for continued research into the characteristics of these pathogens, how they survive, how they spread, their routes of contamination, and how they react with each other, continues to be answered. The following paragraphs provide a brief outline of some of the major foodborne pathogens described more thoroughly elsewhere in this book by experts in the field.

Aeromonas

The genus *Aeromonas* belongs to the Aeromonadaceae family and comprises Gram-negative, non-spore-forming, rod-shaped, facultative anaerobic bacteria. There is high intra- and interspecies genetic variability, resulting in the genus having a complex taxonomy. *Aeromonas* can be found in terrestrial and aquatic environments and some animals. *Aeromonas* species constitute a potential risk when consuming raw seafood. They are also commonly detected in raw vegetables and can be a risk. They are able to grow at low pH values or high NaCl concentrations and at refrigeration temperatures. They can therefore represent a risk in some processed foods and salads. *Aeromonas* species possess several virulence factors that might confer on the bacterium the ability to cause disease symptoms, although currently, knowledge of *Aeromonas* virulence mechanisms is incomplete. *Aeromonas* infections of the gastrointestinal tract may result in enteritis, which can vary from mild to severe with cholera-like symptoms. Septicemia and peritonitis can also be possible outcomes.

Bacillus

Bacillus spp. are ubiquitous in the natural environment and can be common contaminants of foods. They are rod-shaped, facultative anaerobic Gram-positive bacteria. They form dormant endospores when nutrients become scarce or when the population of the bacterium is high. When conditions become favorable, the endospores germinate and vegetative cells emerge. They are adapted to growth at a wide range of temperature and pH conditions and therefore can easily multiply in food matrices, where they can cause spoilage. *Bacillus* spp. can

contaminate a wide variety of foods including meats, milk, vegetables, and fish. Several species have been implicated as foodborne pathogens, the most common being *Bacillus cereus*. Foodborne outbreaks have mostly been associated with consumption of rice or rice products. Other foods, such as herbs and spices, potatoes, and pasta, have also been implicated. The spore-forming capacity can cause foods that have been left too long at room temperature or reheated to constitute a risk. During growth, *B. cereus* produces an emetic toxin, which can induce vomiting after ingestion, and an enterotoxin, which can induce diarrhea.

Campylobacter

Campylobacter species are curved- or spiral-shaped, microaerophilic Gram-negative bacteria. The most significant foodborne pathogenic campylobacters are *Campylobacter jejuni* and *Campylobacter coli*. These species live in the intestines of livestock, particularly poultry. Consumption of undercooked poultry products is a particular risk for infection by *Campylobacter*. Cross contamination of other foodstuffs from uncooked poultry products can also be a disease risk. *Campylobacter* causes a disease characterized by fever, abdominal pain, diarrhea, nausea, and vomiting. Postrecovery complications may arise and can include Guillain–Barré syndrome, arthritis, and inflammatory bowel disease. *Campylobacter* spp. possess several invasion and virulence factors including surface structures such as flagella. A toxin can also be produced.

Clostridium

Clostridium species are anaerobic spore-forming Gram-positive rod-shaped bacteria. These bacteria can tolerate a variety of environmental conditions, and the spores are tolerant to heating including boiling. There are two main foodborne pathogenic types: *Clostridium botulinum* and *Clostridium perfringens*. They cause differing disease syndromes from each other.

Clostridium botulinum

Clostridium botulinum is highly prevalent in the environment and can be found in soils and waters. It can also be present in the intestinal tracts of animals and can contaminate vegetables and fruits. The disease syndrome that it causes is commonly termed botulism. Foods associated with transmission of botulism predominantly include home-preserved (pickled and canned) foods particularly vegetables and cured meats. Honey has been implicated in cases of botulism in infants. *Clostridium botulinum* produces several types of toxin, and these can be lethal on ingestion of very small quantities. The toxin acts on the nervous system and can cause muscle weakness and paralysis; in serious cases, respiratory failure can occur. These conditions can be preceded by nausea, vomiting, and diarrhea.

Clostridium perfringens

Clostridium perfringens is prevalent in the environment and may be found on kitchen surfaces. Food can become contaminated during preparation. *Clostridium perfringens*, like *Clostridium botulinum*, produces several toxins, although these are not as hazardous as the botulinum toxins. The clinical symptoms of *Clostridium perfringens* food poisoning are generally mild and include abdominal cramps, nausea, and watery diarrhea.

Enterobacteriaceae

Enterobacteriaceae are Gram-negative, rod-shaped facultative anaerobes. They are either motile or nonmotile and are non-spore-forming. Enterobacteriaceae can cause a wide range of illnesses, which include wound infections, urinary tract infections, gastroenteritis, meningitis, pneumonia, septicemia, and hemolytic uremic syndrome. Not all are regarded as truly pathogenic – some are regarded as opportunistic. They are ubiquitous in the environment and have been detected in soil, water, plants, and the gastrointestinal tract of animals and humans. Due to its widespread distribution, it can easily contaminate the food chain. The family includes a number of important foodborne pathogens such as *Salmonella*; *Yersinia enterocolitica*; pathogenic *Escherichia coli*, including *Escherichia coli* O157:H7; *Shigella* spp.; and *Cronobacter* spp. Some members of the Enterobacteriaceae family also cause food spoilage such as *Erwinia* spp. and *Pectobacterium* spp. In the food industry, Enterobacteriaceae are commonly used as indicator organisms that can indicate poor hygiene practices or failure of a manufacturing process. The various members of the Enterobacteriaceae family are more thoroughly described throughout this book.

Listeria

Listeria is a genus of bacteria that contains ten species. *Listeria monocytogenes* and *L. ivanovii* are considered pathogenic microorganisms for humans and animals, respectively. *Listeria* spp. are Gram-positive, facultatively anaerobic, rod-shaped, and motile and are different to many other bacteria in that they have the ability to grow at low temperatures, such as refrigeration temperatures. They can be found widely in the environment, in soil, in water, and in some animals, such as poultry and cattle. They can also be found in raw milk products and processed meats and in ready-to-eat foods. They can often be found to persist in processing plants in areas such as drains. The infection caused by *Listeria* is known as listeriosis, and it can pose a major risk for pregnant women, older adults, and those with a weakened immune system. Listeriosis can lead to miscarriage, stillbirth, or lifelong health problems.

Staphylococcus

Staphylococci are Gram-positive, nonmotile, and catalase-positive bacteria. They are ubiquitous in the environment. The genus includes at least 40 species, most of them being harmless and normally residing on the skin, hair, and mucous membranes of humans and other animals. They can cause food poisoning when poor hygiene practices are used by food handlers and by improper storage temperatures. *Staphylococcus aureus* is the most pathogenic species; therefore, if foods become contaminated with *Staphylococcus aureus* and its subsequent toxin, there is a risk of contracting gastroenteritis as the toxin is resistant to heat and will not be destroyed by cooking. Many different types of food can potentially become

contaminated by *Staphylococcus*, especially those that have had a lot of handling and require little or no further cooking, for example, pasta, ham, salads, egg, tuna, potato, sandwiches, cream pastries, and other bakery products. Staphylococcal infection can cause a variety of symptoms including nausea, diarrhea, vomiting, severe stomach cramps, and a mild fever. Good personal hygiene is key to preventing contamination of foodstuffs, so are good hand washing techniques, not preparing foodstuffs if wounds or sores are visible on the hands or wrists, good cleaning practices in the kitchen, and proper storage of foods, especially perishable foods as already mentioned, at refrigeration temperatures. Antibiotic-resistant strains of *Staphylococcus* such as methicillin-resistant *Staphylococcus aureus* can cause life-threatening infections if they enter the bloodstream.

Yersinia

Yersinia are a group of Gram-negative, non-spore-forming, oxidase-negative, catalase-positive, lactose-negative, and facultative anaerobic rods (or coccobacilli). *Y. enterocolitica* is one of three human pathogenic species in the genus *Yersinia*. They are a genus in the family *Enterobacteriaceae*. They can be transmitted by the consumption of contaminated food products including vegetables, milk products, and meat. Optimum growth temperature is about 30 °C, and they, like *L. monocytogenes*, can grow at refrigeration temperatures. They can also survive the freezing process. Symptoms of *Yersinia enterocolitica* infection (yersiniosis) include diarrhea (which may be bloody in severe cases), mild fever, abdominal pains, and possibly vomiting.

Parasites

Parasites infect hosts and derive nourishment and protection from them. Many are transmitted through environmental pathways including soil, water, and person-to-person contact. Parasites live and reproduce within the tissues and organs of infected human and animal hosts and are often excreted in feces. A range of symptoms, dependent on the type, for example, protozoa and helminths, can be displayed by an infected person. They can cause a high burden of disease and suffering but are somewhat overlooked especially in the food industry, as compared with other causes of foodborne diseases such as bacteria and viruses. Parasite-contaminated foods can include undercooked pork, fruits and vegetables, fresh produce, and undercooked freshwater fish. A ranking exercise conducted in 2012 for foodborne parasites was performed in order of importance from a global perspective to identify the most relevant transmission vehicles. The top 8 ranked parasites include (in order of ranking out of 24 recognized parasites) *Taenia solium*, *Echinococcus granulosus*, *Echinococcus multilocularis*, *Toxoplasma gondii*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Trichinella spiralis*, and Opisthorchiidae.

Viruses

Viruses are obligate intracellular parasites that depend on a host cell to replicate. Unlike bacteria, they are unable to multiply in foods or the environment. They are known to be environmentally stable, with the ability to survive adverse conditions, and can be resistant to extreme pH conditions and enzymes in the gastrointestinal tract. Many have low infectious doses. They are generally transmitted via the fecal–oral route through contaminated foodstuffs, water, person-to-person contact, fomites, and aerosolization. Contamination of foods can often occur due to poor hygiene practices at all stages of the food supply chain including production, processing, and retail. Infection can occur by several routes including ingestion of foods that have been irrigated with contaminated water and that have been contaminated by infected food handlers. Education in good hygiene practices are thus a vital component in reducing the burden of illness associated with foodborne viruses. The foods most associated with viral contamination include fresh produce (e.g., strawberries, raspberries, and other berry fruits), bivalve molluscan shellfish (e.g., raw oysters), fruit juices, deli meats, and other ready-to-eat foods such as leafy greens and salad onions. The viruses most associated with foodborne diseases include human noroviruses (HNoVs), hepatitis A virus (HAV), HEV, and other enteroviruses. A summary of each is given in the succeeding text.

Human norovirus

HNoVs are considered to be the viruses that cause the greatest number of foodborne virus outbreaks worldwide. They belong to the Caliciviridae family and are one of five major genera within this family. They are nonenveloped and possess a single-stranded positive-sense RNA enclosed in a protein capsid. They can be divided into five genogroups (GI–GV), with GI, GII, and GIV being the genogroups that infect humans. Currently, there is no reliable/reproducible method of culturing noroviruses in the laboratory, which has delayed the development of potential control measures such as vaccines. HNoV is also known as 'winter vomiting bug,' and symptoms of the resulting gastroenteritis include vomiting, diarrhea, abdominal cramps, fever, and malaise. The symptoms of this self-limiting disease usually appear very rapidly and disappear just as fast; however, shedding of infectious virus can be prolonged, sometimes for weeks, which is perhaps why the transmission through food handlers who have returned to work, even though they may feel better, is a common route of infection. This highlights the need for scrupulous hygiene, especially for food handlers after using toilet facilities.

Hepatitis A virus

HAV is a positive-sense single-stranded nonenveloped RNA virus belonging to the Picornaviridae family of viruses. There are six genotypes (I–VI) with genotypes I–IV associated with human illnesses. HAV is transmitted mainly via the fecal–oral route and person-to-person contact. It is highly stable in the environment. It can withstand high levels of acidity. If infection occurs, there are no specific treatments apart from appropriate aftercare. Symptoms include jaundice, dark urine, fever, weight loss, and vomiting, and these can last up to 6 weeks. A vaccine is available, which provides immunity for up to and over 20 years. HAV-contaminated berry fruits (especially frozen berries) have been implicated in several large outbreaks.

Hepatitis E virus

HEV is a nonenveloped RNA virus and is a member of the genus *Hepevirus* in the Hepeviridae family. It is primarily transmitted through the fecal–oral route mainly via contaminated drinking water. It is found worldwide but has a high prevalence in South and East Asia. Symptoms of HEV infection are similar to that of HAV, including jaundice, loss of appetite, enlarged liver, abdominal pain, nausea, vomiting, and fever. In acute case, it can lead to acute liver failure and death. Pregnant women are at a greater risk of death from HEV. There is a 20% mortality rate in pregnant women in their third trimester. It has a long incubation period, and therefore, confirmation of its association with particular foodstuffs is difficult; however, during a recent study conducted in UK slaughterhouses, HEV RNA was detected in 13% of 40 pig feces and 3% of 40 pig liver samples. HEV RNA was not detected in 40 samples of muscle meat in a processing plant; it was found to be in 10% of 63 sausages purchased at retail. Other studies in the United States and Europe have shown similar findings. Ingestion of raw shellfish has been identified as the source of sporadic cases in endemic areas.

See also: Aeromonas; *Bacillus cereus* and Other *Bacillus* sp. Causing Foodborne Poisonings, Detection of; *Bacillus*: Occurrence; Campylobacter: Health Effects and Toxicity; Campylobacter: Properties and Occurrence; Campylobacter: Species Detection; *Clostridium botulinum*; *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; *Escherichia coli* and Other *Enterobacteriaceae*: Occurrence and Detection; Foodborne Viruses of Human Health Concern; *Listeria*: Detection; *Listeria*: Listeriosis; *Listeria*: Properties and Occurrences; Parasites in Food: Illness and Treatment; Parasites in Food: Occurrence and Detection; *Salmonella*: Detection; *Salmonella*: Properties and Occurrence; *Salmonella*: Salmonellosis; Shigella; *Staphylococcus*: Detection; *Staphylococcus*: Food Poisoning; *Staphylococcus*: Occurrence and Properties; *Yersinia enterocolitica*: Properties and Occurrence; *Yersinia enterocolitica*: Detection and Treatment.

Further Reading

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Relevant Websites

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http://www.fda.gov/food/foodborneillnesscontaminants/causesofillnessbadbugbook/default.htm – US FDA: Bad Bug Book.

http://www.food.gov.uk/ – Food Standards Agency.

http://www.foodsafety.gov/poisoning/ – Foodsafety.gov: Food Poisoning.

http://www.who.int/foodsafety/areas_work/foodborne-diseases/en/ – WHO: Food Safety.

Foodborne Viruses of Human Health Concern

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Introduction of Foodborne Viruses

Annually, in the United States, according to the Centers for Disease Control and Prevention and the report by Scallan et al., among the 31 pathogens that cause an estimated 9.4 million foodborne illnesses, human noroviruses (hNoVs) alone are reported to be responsible for an estimated 5.5 million 58% foodborne illnesses, 26% hospitalizations, and 11% deaths. Viruses have unique properties and are less complex and different from bacterial pathogens, being smaller in size (typically nM). Viruses are obligate intracellular parasites that are dependent on a host cell to replicate and therefore cannot multiply in foods or the environment, although they can survive in the environment outside their respective hosts. Foodborne viruses are generally environmentally stable, can survive adverse conditions, and are resistant to extreme pH and conditions of the gastrointestinal tract and its enzymes. They reportedly have low infectious doses, where as few as 10–18 human noroviral particles can cause illness per the report by Teunis et al.

Enteric viruses are reported to be transmitted through the fecal–oral route by contaminated food and water, via person-to-person, via contact with contaminated surfaces, and through aerosols. Viral contamination of foods often occurs due to poor hygienic practices of infected food handlers, where public education and enforcement of hygienic practices can play significant roles to help reduce outbreak incidence. Fecally polluted waters that flow into farmlands can contaminate crops and produce items, and also, shellfish can accumulate viruses in their gastrointestinal tract. These contaminated fresh produce and shellfish when eaten raw or undercooked can cause illness. In addition to fresh produce and filter-feeding shellfish, at-risk foods associated with viral outbreaks include juices, bakery items, deli meats, and other so-called ready-to-eat foods or undercooked foods. Viruses that are reported to be associated with foodborne and waterborne diseases include hNoVs, hepatitis A virus (HAV), rotaviruses (RVs), hepatitis E virus (HEV), adenoviruses (AdV), sapoviruses, astroviruses, Aichi virus (AiV), parvoviruses, and other enteroviruses.

Human Noroviruses

hNoVs are considered as the major causes of nonbacterial gastrointestinal outbreaks worldwide. HNoVs belong to Caliciviridae family that consists of five major genera, *Norovirus*, *Sapovirus*, *Vesivirus*, *Lagovirus*, and the recent addition of *Becovirus* or *Nebovirus* as reported by Scipiono et al. HNoVs were first reported to be discovered in an elementary school outbreak in 1968 in Norwalk, Ohio, where 50% of the students and teachers developed gastrointestinal illness, from which the name 'Norwalk-like' virus was derived by Albert Kapikian. HNoV transmission occurs all year round; however, outbreaks peak in winter and the so-termed winter vomiting disease is characterized by mild gastroenteritis and symptoms including fever, nausea, vomiting, abdominal cramps, and headaches. Recent reports indicate that emerging virulent strains are found to be capable of causing death in the elderly or immunocompromised. Their average incubation time is 24–48 h and the self-limiting symptoms last from 18 to 48 h, with virus shedding that occurs for 72 h (and even longer, >1 week). This is an important consideration for food handlers/workers, who need to maintain good hygienic practices to prevent transmission upon return to work after their symptoms subside. In addition to outbreaks associated with food settings, HNoV outbreaks have been reported to occur yearly on cruise ships, in elderly care facilities, and in closed environments.

Noroviruses are small nonenveloped containing a single-stranded positive-sense RNA that is enclosed in a capsid (outer shell protein), about 27–32 nm in size, with a buoyant density of 1.33–1.41 g cm^{-3}. HNoVs have icosahedral symmetry, with a capsid that comprises 90 capsomers protruding from the shell made of 90 dimers of capsid protein. The genome is about 7.3–7.7 kb long with three open reading frames (ORFs), namely, ORF1 that encodes a polyprotein that produces the RNA-dependent RNA polymerase and other proteins such as the viral protein genome-linked and N-terminal protein; ORF2 that encodes the major capsid protein, viral protein 1 (VP1); and ORF3 that encodes a minor viral structural protein, viral protein 2 (VP2). Based on sequence information, the genus *Norovirus* is further divided into five genogroups, GI, GII, GIII, GIV, and GV, and GI, GII, and GIV are reported to specifically infect humans. GIII includes bovine enteric calicivirus that is known to infect cattle, and GV infects mice. These genogroups are further subdivided into at least 32 genetic clusters to date. In GI and GII, 8 genotypes are currently recognized in GI and 19 genotypes are recognized in GII. HNoV GII.4 has been found to be the predominant cause of 70–80% outbreaks in the United States and Europe (mostly associated with health care settings).

HNoV is reported to bind to the histoblood group antigens in human intestinal epithelial cells, and the site of replication is considered as the upper intestinal tract by some researchers. Genetic determinants for these blood group antigens are known to play a role in susceptibility of an individual to HNoV infection. Human volunteer studies have shown that H type I antigen likely serves as the receptor for hNoV, encoded by the fucosyltransferase (*fut2*) gene where recessive individuals that lack the type I antigen are reported to be inherently resistant to HNoV infections.

With regard to survival, researchers have shown that HNoV GI and HNoV GII can persist in the environment for prolonged periods, depending on several factors including temperature and moisture. HNoVs are reported to survive at low pH of 2.7 and can retain infectivity after 60 °C for 30 min and after exposure to 20% ether at 4 °C for 18 h. Their resistance is found to be higher in foods and they are also reported to be

resistant to free residual chlorine of 0.5–1.0 mg ml^{-1} by researchers. HNoVs are known for their low infectious dose ranging from 18 to 100 virus particles. Teunis et al. reported that the infectious dose for Norwalk in human subjects ranged from 0.1 (at a dose of 10^3 NV genomes) to 0.7 (at 10^8 virus genomes). In a study by Atmar et al., the human infectious dose (50%) for Norwalk strain was reported to be approximately 1320 genomic equivalents as detected by RT-PCR.

The inability to cultivate HNoVs in the laboratory has hindered the development of vaccines as well as effective control measures. There is no vaccine available to date against HNoV infection. Cultivable surrogates such as feline calicivirus (FCV-F9) and murine norovirus (MNV-1) have been extensively used to understand the antigenicity of HNoVs and evaluate methods of inactivation. Recently, Tulane virus (TV) and porcine enteric sapovirus have been considered as alternate surrogates. The cultivable HNoV surrogate, FCV-F9 of the Caliciviridae family, is a small rounded, nonenveloped, single-stranded RNA virus with a 7.7 kb genome and has 3 ORFs, being similar to HNoVs in these features. However, as it is transmitted via the respiratory route and is also reported to be sensitive to low pH of 2–4 and pH 10 by Cannon et al. and other researchers, it may not completely mimic the survival of HNoV in the environment.

The other cultivable HNoV surrogate, MNV-1, is currently the only norovirus that replicates in the laboratory in murine macrophage RAW 264.7 cells. MNV-1 belongs to GV norovirus, with sequence similarity to GII noroviruses that mainly infect humans. MNV-1 is similar to HNoVs in immunologic, biochemical, genetic, and molecular properties, as MNV-1 is an icosahedral, nonenveloped, single-stranded RNA virus containing 3 ORFs and is 28–35 nm in size. Although it causes a different disease in mice, it is transmitted via the fecal–oral route similar to HNoVs. Unlike FCV-F9, it is reported to be less sensitive to pH within ranges of 2.0–10.0; however, the thermal inactivation parameter processes like pasteurization (at 63 °C) were reported in literature to be similar for both FCV-F9 and MNV-1.

Recently, TV representing a new genus *Recovirus* of the Caliciviridae family was reported to be isolated from the stool samples of a captive juvenile rhesus macaques (*Macaca mulatta*) at the Tulane National Primate Research Center. TV is 36 nm in diameter and icosahedral in symmetry, with a short genome of 6.7 kb, containing 3 ORFs without the poly(A) tail. Sequence homology and phylogenetic analysis of the nonstructural proteins of TV have revealed them to have the highest identity with HNoVs.

TV has been reported by several researchers to be readily grown *in vitro* in LLC-MK2 (rhesus macaque kidney) host cells. TV is also reported to bind to histoblood group antigens like HNoVs, suggesting that the antigenic determinants may be related and/or comparable to HNoVs. Studies have reported no significant differences in heat inactivation parameters between MNV-1 and TV, though MNV-1 was found to be more pH-stable and more tolerant to chlorine than TV. Therefore, the choice of appropriate cultivable surrogates for HNoVs currently remains debatable.

Regarding the application of cultivable surrogates for inactivation studies, 200 mg ml^{-1} sodium hypochlorite, 2% trisodium phosphate, ozone at 6.25 ppm, hydrogen peroxide vapors (30% w/w), heating at 72 °C, and high hydrostatic pressures of 450 MPa after 5 min at 20 °C were reported in literature to cause some though varying levels of inactivation of the cultivable HNoV surrogates, FCV-F9 and MNV-1. Even though inactivation strategies are based on the evidence obtained from surrogate studies such as the ones mentioned earlier in the text, this information may not be completely applicable to HNoV itself. Appropriate conclusions should be drawn from feeding studies using the actual HNoVs that have been subjected to the treatments described previously.

Treatment and Prevention Strategies for HNoV

There is no specific antiviral treatment against HNoVs. Gastroenteritis and dehydration can be treated by oral or intravenous fluid therapy. There is no vaccine currently available against HNoVs, the major barrier being the inability to culture them *in vitro*. Studies using human volunteers reinfected with HNoV after exposure showed these volunteers to be susceptible to the same strain as well as heterologous strains, suggesting either short-term immunity or none. HNoV spread and contamination can be prevented by proper sanitation and hygienic food handling practices or by appropriate treatment regimes such as heat, chemicals, or other processing conditions listed earlier in the text (refer to **Table 1** for recent outbreaks).

Hepatitis A Virus

HAV of the Picornaviridae family is a positive-sense single-stranded nonenveloped RNA virus ranging in size from 27 to 32 nm, similar to HNoVs. The genome is 7.5 kb in length and, unlike HNoVs, consists of a single ORF. The P1 region of the genome encodes three major viral structural capsid proteins, VP1, VP2, and VP3. P2 and P3 regions encode proteins required for RNA replication and the formation of virions. Only one serotype of HAV has been identified to date and a single exposure is reported to render lifelong immunity. However, genetic diversity in the strains worldwide led to their classification into seven genotypes designated I–VII that is known to infect either humans or simians.

The largest foodborne viral outbreak was reported by Halliday et al. where 292 301 cases of HAV were attributed to eating raw clams in Shanghai, China, in 1988. The average incubation period of HAV is long (unlike the short incubation period of HNoVs), about approximately 28 days with severe symptoms that can last for 4–6 weeks that include jaundice, dark urine, vomiting, fever, and weight loss. Among older children and adults, infection is usually symptomatic for 2 months, with jaundice occurring in 70% of patients. There are four phases reported for HAV infection: the first phase is the asymptomatic incubation phase, which lasts for 10–50 days, followed by three symptomatic phases, namely, the preicteric phase that lasts for several days to weeks, characterized by fatigue, nausea, loss of appetite, fever, dark urine, diarrhea, and pale stools; the icteric phase with jaundice; and the convalescent phase.

There are only a few strains of HAV that are cell culture-adaptable. These strains produce cytopathic effects causing cell death and hence used in research involving survival and inactivation studies.

HAV is also highly stable in the environment and reported by several research groups to retain infectivity in mineral water

Table 1 Brief list of some human norovirus outbreaks

Virus type	*Year*	*Sample type*	*Contamination source/location*	*Detection method*
Norovirus	2013	–	Unknown source (tourists), Yellowstone National Park	Epidemiology
	2012	Food	Imported frozen raspberries	Epidemiology
	2011	Stool sample	Pasta, raw vegetables/French military unit	RT-PCR
	2009	Clinical, food, environmental	Salad/German military base	RT-PCR
	2009	Stool	Water resort	RT-qPCR, epidemiology
	2009	Clinical	Cruise ship	RT-qPCR
	2008	Water	Municipal drinking water/Sweden	Epidemiology
		Food and clinical	Deli meat	Nested RT-PCR; sequencing
	2008	Clinical	University dining hall	Epidemiology; RT-PCR
	2003	Food and clinical	Oysters	Epidemiology; RT-PCR

Source: CDC (2013). http://www.cdc.gov/. Accessed 27th June 2013; Sarvikivi, E., Roivainen, M., Maunula, L., Niskanen, T., Korhonen, T., Lappalainen, M., Kuusi, M. (2012). Multiple norovirus outbreaks linked to imported frozen raspberries. *Epidemiology and Infection* **140**, 260–267; Filho, E. P., da Costa Faria, N. R., Fialho, A. M., et al. (2007). Adenoviruses associated with acute gastroenteritis in hospitalized and community children up to 5 years old in Rio de Janeiro and Salvador, Brazil. *Journal of Medical Microbiology* **56**, 313–319; Wadl, M., Scherer, K., Nielsen, S., et al. (2010). Food-borne norovirus-outbreak at a military base, Germany, 2009. *BMC Infectious Diseases* **10**, 30; Arvelo, W., Sosa, S. M., Juliao, P., et al. (2012). Norovirus outbreak of probable waterborne transmission with high attack rate in a Guatemalan resort. *Journal of Clinical Virology* **55**, 8–11; Wikswo, M. E., Cortes, J., Hall, A. J., Vaughan, G., Howard, C., Gregoricus, N., Cramer, E. H. (2011). Disease transmission and passenger behaviors during a high morbidity Norovirus outbreak on a cruise ship, January 2009. *Clinical Infectious Diseases* **52**, 1116–1122; Larsson, C., Andersson, Y., Allestam, G., Lindqvist, A., Nenonen, N., Bergstedt, O. (2013). Epidemiology and estimated costs of a large waterborne outbreak of norovirus infection in Sweden. *Epidemiology and Infection*, 1–9; Schwab, K. J., Neill, F. H., Fankhauser, R. L., et al. (2000). Development of methods to detect "Norwalk-like viruses" (NLVs) and hepatitis A virus in delicatessen foods: application to a food-borne NLV outbreak. *Applied and Environmental Microbiology* **66**, 213–218; Webby, R. J., Carville, K. S., Kirk, M. D., et al. (2007). Internationally distributed frozen oyster meat causing multiple outbreaks of norovirus infection in Australia. *Clinical Infectious Diseases* **44**, 1026–1031.

stored at room temperature even after 10 months, after a month at ambient temperatures on environmental surfaces, and for >4 h on hands, during prolonged storage of spinach leaves at 5.4 °C, and on romaine lettuce leaves after 12 days in a sealed-atmosphere plastic package at 4 °C.

HAV is also reported by researchers to survive at low pH of 3.75 over 4 weeks and at pH 1.0 even after 5 h, after 60 min at 60 °C. However, HAV is reported in literature to be inactivated in shellfish after heating at 85–90 °C for 1.5 min and at 450 MPa hydrostatic pressure for 5 min. These survival features play a significant role in the transmission of HAV.

Treatment and Prevention Options for HAV Infection

Once a person is infected with HAV, there are no known specific treatment options available. However, symptoms of HAV infection are reported to be alleviated by appropriate patient care that includes adequate hydration, nutrition (fatty foods are recommended to be avoided), rest, and antiemetics. For prevention, immunoglobulin therapy is reported to be effective when administered within 2 weeks of viral exposure (through passive immunity). As preventive measures, inactivated and heat-killed vaccines against HAV are commercially available, which provide immunity against HAV for almost >20 years or a lifetime. The US Centers for Disease Control and Prevention (CDC) recommend routine vaccination against HAV for children aged between 12 and 23 months. When economically feasible, vaccination of food handlers is also recommended to prevent HAV transmission and to prevent outbreaks (refer to Table 2 for recent outbreaks).

Hepatitis E Virus

HEV is a small nonenveloped 7.2 kb single-stranded positive-sense RNA virus, 32–34 nm in size, primarily transmitted through the fecal–oral route, usually through contaminated water. HEV of the genus *Hepevirus* in the Hepeviridae family has a genome containing three ORFs. ORF1 codes for a nonstructural polyprotein that forms helicase, protease, and a RNA-dependent RNA polymerase. ORF2 encodes the capsid protein and ORF3 encodes a replication and cytoskeleton synthesis phosphoprotein. Thus far, four genotypes of HEV have been reported with genotypes 1 and 2 affecting humans and genotypes 3 and 4 infecting swine.

The average incubation period for HEV infection is reported to be between 2 and 10 weeks, with two distinct phases of illness. The preicteric phase is known to be characterized by fever, anorexia, vomiting, and abdominal pain, while the icteric phase is characterized by jaundice and is self-limiting. Viral shedding is reported to occur 1–2 weeks before and 2–4 weeks after the symptoms appear. A significant rise in serum alanine aminotransferase and aspartate aminotransferase occurs, which is used as detection methods of liver injury. Due to its long incubation period, the association of HEV with particular foods has not been clearly established; however, recent studies report that HEV gt3 transmission is linked to uncooked pork meat consumption. There is no specific treatment except patient care as described earlier in the text for HAV symptom alleviation. PEGylated interferon administration has been reported for use in chronic infections with some level of moderate success. Vaccine trials have been under study to determine preventive measures (refer to Table 3 for outbreaks).

Rotavirus

RV of the Reoviridae family has a multilayered nonenveloped icosahedral capsid, ranging from 60 to 100 nm in size, and is easily distinguishable from HNoVs or HAV by electron microscopy. RV genome comprises 11 segments of double-stranded RNA that code for structural (VP1–VP8) and nonstructural

Table 2 Brief list of some hepatitis A virus outbreaks

Virus type	*Year*	*Sample type*	*Contamination source/location*	*Detection method*
Hepatitis A virus	2013	Food	Frozen berries in smoothies/Nordic countries	Epidemiology
	2013	Food	Antioxidant frozen berry and pomegranate mix	Epidemiology
	2011	Food	Sundried tomatoes	Epidemiology
	2011	Serum	Sushi bar, Japan	RT-PCR
	2009	Serum	Sun dried tomatoes	RT-PCR
	2009	Serum	Well water	Epidemiology, detection of anti-IgM
	2008	Serum/stool	Thai navy base	Detection of anti-IgM, RT-PCR
	2004	Clinical	Orange juice/Egyptian restaurant	RT-PCR
	2003	Serum	Green onions	Epidemiology, RT-PCR

Source: Gillesberg, S., Soborg, B., Midgley, S. E., et al. (2013). Ongoing multi-strain food-borne hepatitis A outbreak with frozen berries as suspected vehicle: four Nordic countries affected, October 2012 to April 2013. *Eurosurveillance* **18**, 20467; Carvalho, C., Thomas, H., Balogun, K., Tedder, R., Pebody, R., Ramsay, M., Ngui, S. (2012). A possible outbreak of hepatitis A associated with semi-dried tomatoes, England, July–November 2011. *Eurosurveillance* **17**, 200083; Tominaga, A., Kanda, T., Akiike, T., et al. (2012). Hepatitis A outbreak associated with a revolving sushi bar in Chiba, Japan: application of molecular epidemiology. *Hepatology Research* **42**, 828–834; Donnan, E. J., Fielding, J. E., Gregory, J. E., et al. (2012). A multistate outbreak of hepatitis A associated with semidried tomatoes in Australia, 2009. *Clinical Infectious Diseases* **54**, 775–781; Chen, J., Cheng, H. J., Zhang, L. J., Zong, J., Ma, H. L., Zhu, B. P. (2011). A hepatitis A outbreak caused by contaminated well water in a primary school of Jiangxi province, China, 2009. *Zhonghua Liu Xing Bing Xue Za Zhi* **32**, 1014–1017; Theamboonlers, A., Rianthavorn, P., Jiamsiri, S., Kumthong, S., Silaporn, P., Thongmee, C., Poovorawan, Y. (2009). Molecular characterization of hepatitis A virus causing an outbreak among Thai navy recruits. *Tropical Biomedicine* **26**, 352–359; Frank, C., Walter, J., Muehlen, M., et al. (2007). Major outbreak of hepatitis A associated with orange juice among tourists, Egypt, 2004. *Emerging Infectious Diseases* **13**, 156–158. Wheeler, C., Vogt, T. M., Armstrong, G. L., et al. (2005). An outbreak of hepatitis A associated with green onions. *New England Journal of Medicine* **353**, 890–897.

Table 3 Brief list of some rotavirus and hepatitis E virus outbreaks

Virus type	*Year*	*Sample type*	*Contamination source/location*	*Detection method*
Hepatitis E virus	2011	Serum	Italy	RT-PCR, detection of IgG and IgM by immunoassay
	2011	Food	Shellfish/England	RT-PCR
	2007	Blood	Uganda	RT-PCR
Rotavirus	2012	Clinical	Réunion, French island	RT-PCR
	2011	Clinical	Retirement communities	EIA, RT-PCR
	2011	Clinical	Intensive care unit/Brazil	RT-PCR
	2007	Clinical	Pediatric hospital/Mexico	ELISA
	1997	Stool, serum	Pediatric unit/toys	Epidemiology, EIA

RT-qPCR, reverse transcriptase quantitative polymerase chain reaction; EIA, enzyme immunoassay.

Source: Caillere, N., Vilain, P., Brottet, E., et al. (2013). A major outbreak of gastroenteritis in Reunion Island in 2012: first identification of G12 rotavirus on the Island. *Eurosurveillance* **18**, 20476; Cardemil, C. V., Cortese, M. M., Medina-Marino, A., et al. (2012). Two rotavirus outbreaks caused by genotype G2P[4] at large retirement communities: cohort studies. *Annals of Internal Medicine* **157**, 621–631; Ganime, A. C., Carvalho-Costa, F. A., Mendonca, M. C., et al. (2012). Group A rotavirus detection on environmental surfaces in a hospital intensive care unit. *American Journal of Infection Control* **40**, 544–547; Santibanez, G. S., Mayans, J. R., Saldana, N. G., et al. (2009). Outbreak of intra-hospital acquired rotavirus in a pediatric hospital in Mexico. *Salud Pública de México* **51**, 96; Rogers, M., Weinstock, D. M., Eagan, J., Kiehn, T., Armstrong, D., Sepkowitz, K. A. (2000). Rotavirus outbreak on a pediatric oncology floor: possible association with toys. *American Journal of Infection Control* **28**, 378–380; Garbuglia, A. R., Scognamiglio, P., Petrosillo, N., et al. (2013). Hepatitis E virus genotype 4 outbreak, Italy, 2011. *Emerging Infectious Diseases* **19**, 110–114; Crossan, C., Baker, P. J., Craft, J., Takeuchi, Y., Dalton, H. R., Scobie, L. (2012). Hepatitis E virus genotype 3 in shellfish, United Kingdom. *Emerging Infectious Diseases* **18**, 2085–2087; Teshale, E. H., Howard, C. M., Grytdal, S. P., et al. (2010). Hepatitis E epidemic, Uganda. *Emerging Infectious Diseases* **16**, 126–129.

proteins. These proteins form the multilayered capsid of the mature virus that encapsulates the core genome. The complete virion is called a triple-layered particle and is infectious. The structural proteins VP7 and VP4 make up the outermost core layer. Seven RV groups are reported from A to G, with groups A, B, and C reported to be associated with human infections and genotypes G1P8, G2P4, G3P8, G4P8, and G9P8 the most commonly identified worldwide.

RV is a common cause of acute gastroenteritis in children younger than 5 years. RV was first discovered by Bishop et al. using electron micrographs of intestinal and stool biopsies of pediatric gastroenteritis patients that showed a typical wheel shape and hence the *Latin* name 'rota' meaning wheel. The incubation period for human RV is reported to be around 1–3 days. The reported symptoms occur 48 h after RV infection that include watery diarrhea, abdominal pain, vomiting, and dehydration, with severe diarrhea resulting in high mortality rates worldwide. Tate et al. reported that in the 1980s, there were 870 000 deaths linked to RV infection globally, with current numbers around 450 000 per year, and developing countries such as the Democratic Republic of the Congo, Ethiopia, India, Nigeria, and Pakistan accounted for greater than half of total RV-related deaths, with India accounting for 22% (98 621) of deaths. Many RV outbreaks are known to occur due to contaminated water, unhygienic practices, and unsanitary conditions.

Human RV is also reported to survive and retain antigenicity after 5 mg ml^{-1} chlorine treatment for 60 min. Heating at wet (100 °C) and dry (60 °C) conditions for periods of 1–20 min and high-pressure processing (HPP) at 350 MPa at 4 °C have been reported by researchers to have some effects on RV titers. Commercial cranberry and grape juices at native pH and natural substances such as persimmon extract and wattle extract (0.5% solution) are also reported in literature to cause some reduction in RV titers.

Animal model systems have been developed to study and improve strategies for protection against infection from various RV serotypes/isolates. Two Simian RV strains SA11 and Rhesus rotavirus (RRV) are being used as reference strains worldwide and some of the currently available reassortment vaccines are reported to be based on the RRV strains. As a preventive measure against human RV infection, the World Health Organization recommends vaccination that is reported to have significantly reduced RV-related gastroenteritis and mortality (refer to **Table 3** for outbreaks).

Aichi Virus

AiV of the genus *Kobuvirus* in the Picornaviridae family was reported by Yamashita et al. to be first isolated in 1989 from patient stool samples in a gastrointestinal outbreak involving oysters in Aichi, Japan. AiV virions are icosahedral and 30 nm in diameter with three capsid proteins. AiV has a positive-sense single-stranded RNA and a poly(A) tail, with a single ORF encoding for structural proteins VP0, VP1, and VP3. The nonstructural proteins include 2A, 2B, 2C, 3A, 3B, 3C, and 3D. Three distinct genotypes of AiV are reported as A–C. AiV-related gastrointestinal cases have been reported from Asian, African, European, and South American countries indicating its geographic spread and prevalence. AiV is reported to survive (without reduction) HPP treatment of 600 MPa for 5 min, heating at 50 and 60 °C for 30 min, 10% chloroform, and MEM (pH 3.5) for 3 h. However, AiV is reported to show some inactivation by 240 mW s cm^{-2} of ultraviolet light treatment and suggested to be useful for AiV reduction on fresh produce like strawberries, green onions, and lettuce by some researchers.

Sapovirus

Sapovirus of the Caliciviridae family is a 7.5 kb positive-sense single-stranded RNA virus, 41–46 nM in diameter, that is known to infect humans and pigs. Sapporo virus (SaV), the human prototype, was reported by Atmar et al. as being first identified during an outbreak in Sapporo, Japan, in 1977 in an infant home. This virus has ten spikes on the outer surface; sapoviruses are divided into five genogroups. GI, GII, GIV, and GV infect humans and GIII infects pigs. Genomes of GI, GIV, and GV are reported to contain three ORFs and GII genomes are reported to contain two ORFs. RNA-dependent RNA polymerase and a major capsid protein (VP1) are encoded by ORF1, a small protein is encoded by ORF2, and ORF3 encodes for a protein whose function is currently unknown. Outbreaks of SaV are less frequent than HNoV; however, significant increases in SaV outbreaks have been reported to occur in various settings (closed populated environments) such as elementary schools, high schools, hospitals, and hotels. SaV is also reported to be associated with treated and untreated sewage and river water. The average incubation time is ~24–48 h with symptoms like fever, diarrhea, nausea, abdominal pain, chills, and malaise. Human SaVs like HNoVs are currently uncultivable.

Porcine sapovirus (Cowden strain), an enteropathogen that causes gastroenteritis in gnotobiotic pigs, is as another HNoV surrogate that is reported to be cultivated in the swine kidney cell line, LLC-PK. Both porcine SaV and HNoV have been reported by researchers to show similar resistance to HNoVs with regard to heat (50 °C for 30 min), treatment with sodium hypochlorite for 1 min, and pH ranging from 3.0 to 8.0. All these findings strongly support the use of porcine SaV as a surrogate to study HNoV.

Adenoviruses

AdV were reported to be first isolated from civilians and Army recruits who had respiratory disease. AdVs of the genus *Mastadenovirus* are 90–100 nm, 26–45 kb double-stranded DNA viruses enclosed in a nonenveloped, icosahedral shell. Fifty-two human AdV serotypes are reportedly proposed in literature according to Jones et al. based on their genomic, physical, and biological properties, which are further divided into six species, *Human adenovirus A to G*. The most widely associated with acute gastroenteritis are reported to include subgenera 'F' carrying AdV types 40 and 41 and 'A' carrying AdV types 12, 18, and 31. HAdV serotypes 40 and 41 are reported to be the second leading cause of viral gastroenteritis in children after RV.

Clinical manifestations of HAdVs reportedly range from upper and lower respiratory tract infections, pneumonia, conjunctivitis to gastroenteritis, and urinary tract infections. HAdVs are known to cause mild and self-limiting disease, and severe clinical presentations, if any, are known to include cystitis, enteritis, encephalitis, and pneumonia. Additionally, reported symptoms include diarrhea, vomiting, dehydration, and fever that develop after an incubation period of 8–10 days and last for 7–8 days, with shedding of virus until 7–14 days after infection.

Astroviruses

Astroviruses are small star-shaped, nonenveloped, single-stranded positive-sense RNA viruses, ~30 nm in size, that were reported by Appleton et al. to be first identified in children suffering from diarrhea in a maternity ward in England that year. Their name originates from the Greek word 'astron' meaning star, due to the five starlike projections on the virus. Astroviruses belong to the family Astroviridae that is divided into two genera: *Mamastrovirus* (which infect mammals) and *Avastrovirus* (which infect avians). Their genome is about 6.8–7.9 kb with a 5′ untranslated region and 3 ORFs, ORF1a, ORF1b, and ORF2. ORF1 encodes a RNA-dependent RNA polymerase and a protease, and the viral capsid is encoded by ORF2. Astroviruses are not easily cultivable *in vitro*; hence, their classification is mostly established and based on nucleotide and amino acid variability of ORF2. To date, there are eight known serotypes reported for human astroviruses (HAstV), and type 1 is reported to be the most prevalent. Along with the genetic diversity of ORF2, HAstV are further classified into two genogroups, A that includes

serotypes 1–5 and 8 and B that includes serotypes 6 and 7. When culturable, HAstV is reported to be grown using the human colon carcinoma host cell line (Caco-2).

HAstVs are reported to be a common cause of gastroenteritis in children with symptoms that develop 2–3 days after infection. These symptoms include diarrhea, vomiting, abdominal pain, headache, and dehydration. However, affected persons may not require hospitalization as symptoms are reported to be self-resolving. HAstV is known to infect the immunocompromised, elderly, and healthy adults, though children under 2 years are most susceptible. Astrovirus is also known to survive in the environment for long periods of time, including water and surfaces for 60 days at 4 °C and in water at 20 °C, and is reported to be resistant to 1 mg l^{-1} free chlorine by researchers. There is little evidence for foodborne transmission of AdV and astroviruses; however, given their fecal–oral route of transmission, infection upon consumption of contaminated food is quite likely to occur.

Parvovirus

Parvovirus of the Parvoviridae family is a small icosahedral, nonenveloped 5 kb single-stranded DNA virus, 18–26 nM in size. It was reported to be discovered in 1974 while testing for hepatitis B surface antigen, with a buoyant density of 1.39–1.42 g cm^{-3}. Three genera are known based on transcription maps, terminal repeats, and mode of replication: *Parvovirus* (autonomous), *Dependovirus* (replication with helper virus), and *Erythrovirus* (invasion of RBCs), and only *Dependovirus* and *Erythrovirus* are reported to infect humans. Parvovirus genome has 2 ORFs that code for viral proteins VP1 and VP2 (the major capsid protein) and nonstructural proteins.

Parvoviruses are also known to survive in the environment, and researchers have reported them to be resistant to pH between 3 and 9 and heat at 56 °C for 60 min. However, they are reported to be inactivated by formalin and gamma radiation. Parvoviruses have been linked to outbreaks involving shellfish. The 'cockle agent' parvovirus was reported by Appleton et al. to be associated with a large outbreak related with contaminated cockles in the United Kingdom that affected around 800 people.

Other Enteric Viruses: Enteroviruses, Toroviruses, and Picornaviruses

Enteroviruses

Enteroviruses of the Picornaviridae family are nonenveloped, ~7.2–8.4 kb positive-sense, single-stranded RNA viruses, 28–30 nM in size. They include polioviruses, coxsackie A and B viruses, and echoviruses; are transmitted by the fecal–oral route; and are associated with humans and animals. Their incubation period lasts from 3 to 7 days and can be transmitted 3–10 days after symptoms appear. They are typically culturable in the lab. Poliomyelitis linked to the consumption of raw milk in 1914 was the first recorded foodborne virus outbreak as reported by Jubb, and other enterovirus outbreaks are linked to contaminated shellfish, marine life, and freshwaters. Immunization against polioviruses worldwide, pasteurization of milk, and appropriate cooking have reportedly led to the decreased transmission of these enteroviruses.

Toroviruses

Toroviruses of the Coronaviridae family and genus *Torovirus* are positive, single-stranded RNA viruses (~28 kb in length) around 100–150 nM in size. They are known to be isolated from feces of children and adults with diarrhea (bovine, equine, and porcine toroviruses are also known in addition to human toroviruses). However, their role in human foodborne gastroenteritis remains unknown.

Picornaviruses

Picornaviruses of the Birnaviridae family are 35 nM, RNA viruses, associated with gastroenteritis in immunocompromised HIV-infected individuals, and reported to be detected in humans with and without diarrheal symptoms. The role of these viruses as well as that of toroviruses in human foodborne gastroenteritis is yet to be understood.

Overall Brief Summary of Detection Methods Used

Historically, foodborne virus detection was based on infectivity assays using live susceptible host cells to determine either cytopathic effect (TCID50) or plaque assays for plaque-forming viruses. However, these methods cannot be applied for HNoVs and wild-type HAV that are yet to be successfully and reproducibly propagated in mammalian cell-culture systems with established protocols. Immunologic-based assays (such as ELISA and immunoelectron microscopy) are used mainly for clinical detection using antibodies against known genotypes of HNoV or HAV. Their disadvantages include limited detection sensitivities, which make them unsuitable for detecting low viral loads, associated with food-related outbreaks. They are however suitable for clinical specimens where the viral load/titer is high.

The challenges associated with detecting low numbers of noncultivable HNoVs and HAV in foods include sample concentration, sample purification, and nucleic acid extraction with simultaneous removal of inhibitors that can negatively impact downstream detection as extensively reviewed before in literature (see 'Further Reading'). Rapid and sensitive robust detection methods are therefore needed to prevent food recalls and outbreaks. As the significant foodborne viruses are RNA viruses, reverse transcription PCR has reportedly been the 'gold standard' for their detection or PCR for the detection of DNA viruses. Typically, specific primers are used to prevent cross-reactivity from the food matrix. However, at times, degenerate primers are used to ensure detection of variants or newly emergent strains. For food samples, however, confirmation of RT-PCR products is needed and essential that requires time-consuming hybridization assays, restriction digestion of amplified products, nested PCR, or traditional sequencing. With the advent of real-time approaches, improved primer and probe design have enabled detection without the need for gel electrophoresis, where simultaneous amplification, detection, and confirmation occur. These approaches use intercalating dyes

such as SYBR green I (confirmation using melt temperature curves is needed), fluorescent hydrolyzing probes such as in TaqMan PCR, and hybridizing probes such as molecular beacons or FRET-PCR and also have been reviewed in literature (see 'Further Reading').

Isothermal nucleic acid amplification is another ongoing trend for rapid detection that eliminates the need for expensive thermocyclers that have been traditionally used in PCR-based approaches. Nucleic acid sequence-based amplification and loop-mediated isothermal amplification are isothermal approaches being researched for HNoVs, HAV, and other foodborne virus detection by several researchers worldwide. Biosensors and microarrays (lab-on-chip) also continued to be researched. As the field of 'omics' continues to advance and next-generation sequencing technologies become relatively inexpensive, it is very likely that these approaches/technologies will be routinely used and/or adapted for rapid foodborne virus detection in clinical and food environments. However, simultaneous advances in sample concentration and purification methods need to occur to match and parallel the advances in detection.

Future Directions for the Prevention and Control of Emerging Foodborne Viruses

Some of the described novel rapid detection methods are not routinely implemented in testing laboratories, due to the disadvantages associated with cost, labor, and sample preparation. It is important to note that novel approaches should not significantly increase the market price of tested products. Timely detection is one way to prevent contaminated food from being released to the market, followed by implementation of efficacious mitigation strategies to control and curb virus spread. Vaccination can help prevent viral spread, although vaccines are not available for some of the foodborne viruses. In the absence of vaccines, good sanitation and hygienic practices are the most important strategies that must be implemented to prevent viral disease transmission. If an outbreak does occur, rigorous control measures should be instituted to prevent transmission. In addition, emphasis must be placed on public education. As stated before by several researchers, together with improved monitoring and detection, effective mitigation and control, immunization, and foodservice and public education, there remains optimism among the global research community in attaining the goal of decreasing and/or preventing foodborne viral disease outbreaks.

See also: HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Immunoassays: Principles.

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Food–Herbal Medicine Interface

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Foods are generally seen as nourishing and providing the (human) body with the essential nutrition needed. On the other hand, medicines have a clearly defined role in the treatment and specific prevention of a disease. However, food items more and more become 'functionalized,' that is, they are marketed with a specific (preventive) health claim or with a functional claim, for example, as a sport supplement. 'Functionalities' like keeping a healthy skin and mental well-being are claims made for specific products and are incorporated into new brands of cereals, baked goods, drinks, dairy products, oils and fats, and snacks, as well as into products that are sold specifically to supplement an existing diet.

There is substantial scientific agreement that certain foods have beneficial effects on health. Examples include the following:

- Garlic, turmeric, ginger, and many so-called spices
- Anthocyanin- or flavonoid-rich species like grapes (red wine), cranberry, bilberries, and cocoa
- Carotenoid-rich species yielding vegetables including carrots and tomatoes

These have general health benefits, that is, not specific benefits that could be linked to treating or preventing a specific disease.

Products with acclaimed or well-demonstrated health benefits have become an important element of the food market. It is impossible to assess the total value of this segment of the food market since it is difficult to delineate and it is fragmented in a wide range of niches. On the other hand, it is clear that it is a fast-developing and changing market, which very heavily relies on incorporating 'novel' products generally derived from local or traditional knowledge. The most recent boom product seems to be chia (the seeds of *Salvia hispanica* L., Lamiaceae). Other botanical drugs, which in recent years were pushed onto the global markets, include goji (*Lycium barbarum* l., Solanaceae), mangosteen (*Garcinia mangostana*), and açai (*Euterpe oleracea* Mart., Arecaceae). Thus, in general terms, there are two essential themes that need to be covered in such an overview:

– The interface of food and medicine
– The development of local and traditional resources into global commodities

The following addresses these themes within the framework of the current regulatory mechanisms and ambiguities.

The Regulatory Framework

The regulatory framework governing such products varies from country to country and highlights that this situation is in fact far more complex. Here again, products sit at the (varying) borderline between what is classified as a food (or a food supplement) and what is classified as a medicinal product. For example, in the United States, herbal medicines are generally regulated by the US Food and Drug Administration (FDA) as 'dietary supplements' (www.fda.gov/Food/DietarySupplements/). In the European Union, on the other hand, there is a clear distinction between foods that are sold with a specific claim and (herbal) medicines. In December 2006, the regulation on nutrition and health (i.e., preventive) claims made on foods was adopted by the European Council and Parliament. For the first time, this regulation lays down harmonized rules across the European Union for the use of nutrition claims such as 'low fat' and 'high fiber' or health claims such as 'reducing blood cholesterol' (http://ec.europa.eu/food/food/labellingnutrition/claims/).

Using five botanical drugs commonly sold in five countries as an example, **Table 1** highlights the diverse regulatory status of, in botanical terms, very similar preparations. In some countries, a well-developed unique regulatory framework for herbal medicines exists (e.g., the listed medicines in Australia), while in other cases, no, or a much looser, regulation is used that generally combines food supplements and herbal medicines.

The terminology used in this area is complex and often confusing. For example, in the United States, a dietary supplement is a product taken orally that contains a 'dietary ingredient' intended to supplement the diet. The 'dietary ingredients' in these products may include vitamins, minerals, herbs or other botanicals, amino acids, and substances such as enzymes, organ tissues, glandulars, and metabolites. Dietary supplements can also be extracts or concentrates and may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, and powders (http://www.fda.gov/Food/DietarySupplements, accessed 7 August 2014). The more popular is nutraceutical. It is generally defined as a type of food substance that helps maintain health and prevents illness. This term is also used interchangeably with concepts like nutraceutical or functional food and food supplement. The concept of nutraceuticals was introduced in 1989 by the American medical doctor Stephen L. DeFelice claiming that "[t]he nutraceuticals revolution will lead us into a new era of medicine and health, in which the food industry will become a research-oriented one similar to the pharmaceutical industry" (DeFelice, 1995. *Trends in Food Science & Technology* 61, 59). He concludes that once the vastness of the potential nutraceutical market will be appreciated, "nutraceutical research will begin to flourish mightily owing to the energy and creativity of corporate capitalism" (p. 61).

However, these concepts are not identical. Functional foods are foods normally consumed in the diet that have scientifically been assessed to have health benefits. Dietary supplements are ingestible preparations purposefully added to the diet to benefit health but are not necessarily derived from foods (i.e., from plants, fungi, or animals consumed as food).

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Table 1 Examples of the regulatory status of some common products used as a functional food/nutraceutical and/or medicine

	The United Kingdom	*Australia*	*Russia*	*The United States*	*Germany*
Ginkgo	Food supplement/traditional medicine	Listed medicine[a];[a]	Food Supplement	Dietary supplement	Medicine
Echinacea	Medicine/traditional medicine/food supplement	Listed medicine[a];[a]	Medicine	Dietary supplement	Medicine
Cimicifuga	Traditional medicine	Listed medicine[a];[a]	Medicine/food supplement	Dietary supplement	Medicine
Siberian ginseng	Food supplement	Listed medicine[a];[a]	Medicine	Dietary supplement	Medicine/food supplement
Mangosteen	Food	Food	Food	Food/dietary supplement	Food

Traditional medicine – a product registered under the Traditional Herbal Medicinal Products Directive of the EU

[a]Listed medicine – according to the Australian regulations, a listed medicine has a lower level of claims and these medicines are not evaluated for efficacy, but product sponsors must hold evidence to support the claims they make about the product.

Nutraceuticals, on the other hand, are products with biological functions that are derived only from foods. Similar to dietary supplements, they generally are consumed in a form that resembles a medicinal product, and they usually are sold over-the-counter (OTC). These distinctions, however, are complicated by the fact that many substances fall within all three categories. For example, beta-carotene not only occurs naturally in fruits, vegetables, and grains but also is manufactured (e.g., extracted from plants or animals and purified) and sold as a dietary supplement and as a nutraceutical.

The Food–Medicine Interface and Commercial Development of (New) Products

All such products are often sold with remarkable health claims, such as being able to slow the aging process. However, these claims are problematic and difficult to substantiate. Since foods are not used in the treatment of diseases, in many regulatory systems, the claim that nutraceuticals can treat disease is deemed inappropriate for a food substance. In addition, in some 'therapeutic' areas, grossly fraudulent claims and adulterated products plague the market.

A now classical example of an unsuccessful development is *Hoodia gordonii* (Masson) Sweet ex Decne (Apocynaceae), first developed with the goal of commercializing a medicinal product and then, after this was not possible, a food supplement. *Hoodia pilifera* (L.f.) Plowes (Apocynaceae) and *H. gordonii* are succulent, slow-growing desert plants in southern Africa and have traditionally been used by indigenous groups to suppress thirst and hunger (especially during long stays in the desert). Since the nineteenth century, *H. pilifera* has been known to quench thirst. The discovery of this 'drug lead' has specifically been linked to the Khoisan people, but other groups could equally lay claim on this traditional knowledge. During the early 1960s, research started focussing on nutritional and safety aspects. In 1983, the appetite suppressant effect of the plant extracts was established. In the 1990s, phytochemical research at the Council for Scientific and Industrial Research (CSIR) of South Africa identified two hunger-suppressing pregnane glycosides from *H. gordonii*, which in 1997 were then patented. Very quickly, this patent attracted the industry's interest, and Phytopharm (a small UK-based company) took a lead in further developing it. The focus was on the extracts' and compounds' appetite suppressant and later their antidiabetic effects. In 1998, the product (labeled P57) was sublicensed to Pfizer and the company started clinical studies for treating obesity. The ultimate goal of this R&D effort was a *fully licensed medicine* on the basis of a characterized extract with a defined amount of the active constituent for the treatment of obesity. Developing the drug required considerable clinical and preclinical research, but in July 2003, Pfizer unexpectedly returned the license to Phytopharm. In late 2004, the food giant Unilever stepped in with the strategic goal of developing a *slimming food*, that is, a food supplement, and in 2007, Unilever, too, returned the rights, which has resulted in an end of any serious research and development on *H. gordonii* product

Without doubt, the preparation from *H. gordonii* studied decreased energy intake, which in turn can result in weight loss. However, the side effects including most importantly potentially lethal hypertension, loss of skeletal muscle mass, and also general feelings of gastrointestinal discomfort are so severe that such a product cannot be used as either a food supplement or a (herbal) medicine. Toxic effects could in fact have been predicted by the structure of the pregnane glycosides, but this was ignored during the preclinical, clinical, and later the food supplement development. Importantly, other crucial aspects for developing a product from a local knowledge-based herbal preparation had been ignored or started very late in the development process. *H. gordonii* is a traditional food and medicinal plant of the Khoisan but had been patented without their prior consent. Consequently, these groups and other stakeholders challenged this lack of intellectual and financial recognition, resulting in 2004 in a belated benefit-sharing agreement. On a positive note, this was, in fact, one of the first benefit-sharing agreements and gave the Khoisan a share of royalties derived from the sale of products containing the patented extract. The second unresolved problem had been the supply side of the crude drug. *Hoodia* species are slow-growing, succulent desert plants and the pregnane glycosides cannot be obtained synthetically. Also, the commercial goal has been the development of an enriched extract for use as a medicine or a food supplement. Clearly, the huge number of obese people in the world

would have resulted in an extremely high demand for the botanical starting material. This supply problem is directly linked to the reported high rate of adulteration of commercially available *H. gordonii* products. *Opuntia ficus-indica* (L.) Mill. (Cactaceae) is a cheap and easily available adulterant. Since it is the source of prickly pears, the cladodes are a common waste product of the fruit production. Adulteration is often the responsibility of unscrupulous companies that only have their short-term profit in mind, but it is also linked to the limited supply with *H. gordonii* and to uncontrolled and poorly managed value chains. The limited supply is due not only to the slow-growing nature of the desert plant but also to the need for a certificate based on the Convention on International Trade in Endangered Species (CITES) resulting in the need for permits to cultivate and export the crude drug and the overall high demand by consumers.

This example was presented in some detail in order to not only highlight the complex links between food and medicines but also demonstrate the need for a stringent regulatory framework for any product that makes specific preventive or curative health claims. In other cases, the problems are simply misleading or grossly inappropriate claims have been a problem, as exemplified in the case of açai (*Euterpe oleracea* Mart., Arecaceae), which has been one of the most highly praised nutraceuticals for the last years but which seems to be a boom and bust product. Overall, there is unconvincing and insufficient scientific evidence to validate the acclaimed uses.

Mangosteen (*Garcinia mangostana* L., Clusiaceae) highlights some examples of opportunities that arise out of developing high-value food supplements. It has a long history of use as a medicinal and food plant, mostly in Southeast Asia. Its fruit is an important food product, also used to treat skin infections and wounds and well known for its anti-inflammatory properties. It is also used to treat dysentery, different urinary disorders, cystitis, and gonorrhea. Commercial preparations based on *G. mangostana* are now distributed increasingly all over the world especially as 'liquid botanical supplements.' Central to the biological activity of the species are xanthones, polyphenolic compounds based on a skeleton of a xanthene-9-one with a range of significant *in vitro* biological activities. A serious weakness in our knowledge is the lack of intervention studies or clinical data and it is not yet clear to what extent the *in vitro* findings about pharmacological activities can be extrapolated to specific therapeutic benefits. However, a general use as a food supplement seems to provide adequate health benefits and these can be substantiated with some relevant data.

The general advice is that such products can supplement one's diet but that they cannot be a substitution for a healthy diet nor can they cure any disease. However, and most importantly, due to misleading commercial interests, this is often not spelled out clearly.

Let Food Be Thy Medicine and Let Medicine Be Thy Food

While the preceding text has been very much informed by a debate based on the different regulatory frameworks, increasingly, there also is a renewed scholarly debate about the borderline between food and medicine. Famously, the idea of "let food be thy medicine and let medicine be thy food" (attributed to Hippocrates, 460–377 BC) has continued to stimulate the debate about health, healthy living, and the interface of food and medicine. In the context of ethnopharmacology, Nina Etkin and Paul J. Ross led the discussion toward a (renewed) emphasis on the links between these two elements of human culture. It has been argued convincingly that we should set aside a place for food and try to understand its health-promoting effects. A diverse range of scholarly work has investigated food uses, people concepts about these foods, and a wide range of health benefits of such products and more recently assessed the globalization of local resources.

Taraxacum officinale (L.) Weber ex FH Wigg. (Asteraceae) is another example one can use to highlight the conceptual questions associated with the food–medicine interface. Clearly, dandelion is both a food and a medicine. Historically, it was very widely and is still consumed in salads. Also, in popular European medicine, it is considered to be a 'blood purifier.' It is employed for treating arthritis and rheumatism, as a mild laxative, and for skin conditions like eczemas. The German *Frühjahrskur* (a cleansing diet consumed in the spring) has a long tradition of use as an aqueous decoction or juice of the fresh plant. The therapeutic use is mainly based on the empirical findings over many centuries and certainly not, in a strict sense, evidence-based. Such cleansing could equally well be considered to be a 'health food' or a medical use. Some uses are clearly based on specific therapeutic expectations. The distinction between its medical and food uses is fluid and is clearly culturally constructed. They are linked to specific ways of preparing and consuming dandelion and most importantly to the context of the usage given.

This plant has given rise to some commercial food or herbal medical products. Until now, clinical studies on *Taraxacum officinale* are lacking. Some pharmacological investigations support the empirical local uses in particular to treat digestive disorders. Anti-inflammatory, anticarcinogenic, and antioxidative activities have also been reported. Various polyphenols and sesquiterpenes are responsible for these diverse effects, but not enough is known, and the concentration of individual constituents is rather limited. Hypothetically, a preparation enriched in these secondary metabolites could be developed, which then could potentially be exclusively marketed as a medicine.

There are numerous other examples, and Prendergast et al. provided an excellent, broad, and theoretical perspective on this topic, which is still relevant today.

The Changing Framework

There can be no sharp dividing line between food and medicine. Any such 'line' would be defined based on sociocultural, economical, and regulatory criteria. However, it needs to be ascertained that foods are essentially used to nourish the body by providing the essential nutrients. In a local and traditional context, such a usage would mostly have been limited to small groups of people. The globalization of such uses results in new challenges for maintaining high quality and safety (**Figure 1**).

With value chains of such products becoming more complex, often, the control and knowledge about the product's composition along with the value chain are lost and there is a dramatic increase in risks associated with adulteration or

other changes detrimental to the product's quality. Products claiming aphrodisiac, beautifying, body-building, and aging-preventing effects are a particular problem, since these claims seem to be mostly market-driven.

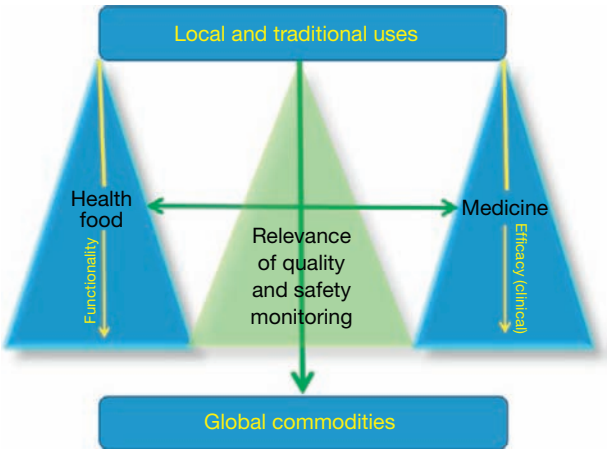

Figure 1 Idealized and simplified scheme highlighting the challenges in the development of locally and traditionally used resources into global commodities at the food–medicine interface. The relevance of quality and safety monitoring increases with a wider use of a commodity, which is generally paralleled by a loss of knowledge about the product's composition along with the longer and more complex value chains that develop with such globalized commodities. With a product's wider utilization, the requirements for quality and safety increase substantially. In the case of food supplements, the development of evidence-based functionalities is essential, while for herbal medicines, an evidence base needs to be built up.

While Figure 1 provides a general overview, Table 2 presents additional details focussing on the differences between healthy foods/food supplements and locally or globally used herbal medicines and the herbal medicinal products derived from them. There is a clear distinction between local (traditional) systems of knowledge and practice and the wider use of products derived from such knowledge in an increasingly globalized market, where most importantly these become commodities that are the end product of complex value chains. There are important differences between the food and the medicine sphere, and commodification is one of the main factors blurring the lines between products that in essence are used either as an element of nourishment or as a substance to cure an illness. While one plant may have both roles (albeit very often different plant parts are used), the context within a culture defines their use.

Health(y) food that has also entered mainstream discussions in many countries is again a popular concept. The global paradox is that the world is suffering from over- and undernutrition (starvation) simultaneously. One element in the debate is the global public health concern about obesity and diseases resulting from it. This has led to calls for dietary changes, including a reduction in caloric intake and modifications to the diets commonly consumed today in many countries especially linked to dietary changes that an excessive intake in saturated fat and sugar has caused. Clearly, this is not an area where the use of supplements needs to be prioritized, but where dietary changes are essential.

Maybe, the main call made within this article is for making the distinctions between foods and medicines clear and transparent. "*Let food be thy medicine and let medicine be thy food*" of Hippocrates is not a justification for blurring the lines between

Table 2 Differences between food supplements and herbal medicines in the context of developing global (or regional) commodities based on local (traditional) herbal medicines or health(y) foods

Health foods/food supplements	*Herbal medicines*
Local and traditional systems of knowledge	
Generally used in well-defined sociocultural and local context, for example, pleasure/enjoyment or as an element of rituals	Used based on a therapeutic need
Food with specific uses in order to keep a balanced self or a balanced community (holistic)	Therapeutic agents used to treat specific illnesses that may or may not be explained on the basis of 'natural' causes
Often not separated from other foods	Parts of complex healing practices and traditions and often a secondary element to, for example, a supernatural intervention
Used regularly	Used only when needed
Commercial (global) products	
Commodities in global or international and regional or local markets	
Not for nourishment but to supplement a diet with *preventive* (or generally poorly defined and substantiated therapeutic) claims	Therapeutic claims based on various levels of evidence (traditional use, well-established use, clinical evidence-based use)
Often poorly defined or very low doses	Mostly therapeutically active agents with a relevant dose
Functionally foods	Presented as medicines with a claim
Often used on a regular basis	Used only when needed (i.e., when a perceived health need exists)
Commonly used based on special expectation, for example, as an agent to 'maintain youth' and secure an 'ideal body' shape (slimming agents, athlete's diets)	Increasingly such products are seen to have a particular place in the treatment of minor; self-limiting diseases and major therapeutic claims are reserved for a minority of the herbal drugs used (i.e., for the products derived from them) as either an OTC product or one dispensed after medical or other health care interventions
Generally with limited regulations in terms of a consistent composition, quality, and therapeutic claims	Regulated as (herbal) medicines of various types or as 'supplements'

supplements and (herbal) medicines but a reminder that medical benefits are linked to changes in an adequate and health diet.

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Freeze Drying: Effects on Sensory and Nutritional Properties

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Introduction

The freeze-dryer is traditionally used to dehydrate high-value pharmaceutical products (medicines and therapeutics), biotechnological products (blood cells, plasma, viruses, vaccines, proteins, peptides, etc.), and limited food products (coffee, spices, meats, food ingredients, and dehydrated fruits) and most recently has been utilized to produce nanomaterials, specifically nanoparticles, nanospheres, or nanocapsules.

It is carried out at a low temperature, preserving the functionality, cell viability, or nutritional values even after water is removed in the dehydration process. Industries that are dealing with products with biological origin, especially those that have a high profit margin, find that freeze-drying is a good choice of dehydration to produce products of high quality providing better active ingredient retention, textural properties, and color preservation.

However, the operating cost of a freeze-dryer is very much higher than other dryers as it involves freezing and subsequent drying at vacuum pressure. Moreover, the drying time is long and thus requires high energy demand. Thus, freeze-drying is only commonly used in the pharmaceutical industry and the biotechnology industry where the product profit margin is high. It is not commonly used in the food industry except for those food products that have high value.

It is well known that improper processing of food products will inevitably lead to low-quality products. In the area of food drying, there are more than 500 types of dryers, utilizing different combinations of drying principles that one can select from for drying a food product. Often, for comparison of dryer performance, many characteristics and attributes of the dehydrated products are referred to, and these include drying kinetics, energy efficiency, and product quality, which includes color change, shrinkage, porosity, rehydration attributes, and nutritional values. This article covers the freeze-drying process and describes how it affects the sensory attributes and the retention of bioactive ingredients in the dehydrated products.

Brief Working Principle of Freeze-Drying

Freeze-drying is carried out by means of sublimation of solvent typically water in a frozen product at a low-temperature operating condition. As the dehydration process is carried out at low temperature in the absence of liquid water, deterioration, microbial activity, and chemical reactions are greatly reduced or eliminated, which in turn gives better product quality compared with products dehydrated by conventional methods.

As freeze-drying is operated at a low temperature, it is indeed suitable for drying heat-sensitive products. It involves three stages of operation: The first stage is freezing. At this stage, the operating temperature is reduced to a temperature that is below the melting point of the solvent (normally free water) in the drying materials. The solvent is frozen and becomes solid (ice if the solvent is free water). At this stage, bound water remains in the liquid phase. The second stage is primary drying where the pressure is reduced until vacuum is achieved. The solid form of the solvent is sublimed when the operating temperature is increased in vacuum condition. Theoretically, at the end of the primary drying, all free water is removed. Second drying involves the removal of bound water. The removal of bound water is accomplished by heating the product under vacuum.

Due to the high operating cost, freeze-drying is sometimes combined with other drying methods such as microwave drying in order to reduce its drying time. The application of electric and magnetic fields to the freezing and the freeze-drying process has been attempted by researchers to control the freezing process, and some commercial applications can be found.

Effect of Freezing-Drying on Sensory Attributes

Five sensory attributes are typically measured for freeze-dried food: color, shrinkage, texture, rehydration, and aroma. The aim is to preserve these qualities as close as possible to the fresh food products prior to freeze-drying. In linking the operation of a freeze-dryer to the sensory qualities of the final freeze-dried food, it will be important to distinguish the contributing effect of the initial freezing stage and the subsequent sublimation stage. The first stage is important as it determines the size of the ice crystals formed in the sample, which, in general, in terms of sensory attributes, determines the damage imposed by the frozen ice on the food material. Therefore, sensory attributes such as the texture may be affected in part by the initial formation of the ice crystals during the freezing stage. This in turn may subsequently affect the rehydration behavior of the dried food. The second sublimation stage is further exposed to the vulnerability of product collapse and thermal degradation of the food material. This is due to the exposure of the frozen sample to higher temperatures at this subsequent stage. The subsequent part of this section will describe in detail how each attribute is affected by the two stages of freeze-drying and the strategies to control them.

Types of Sensory Attributes

Color

Color change in air-dried food material is often associated with product browning or degradation. On top of the conventional enzymatic or nonenzymatic reactions leading to color change,

there are mixed reports on the effect of freeze-drying parameters on the color change of a freeze-dried sample. Color change could be due to pigment concentration during the freezing process. There is a chance where such color dissipates during rehydration. In the drying of fruit pulps, color change could also be attributed to the presence of pore structures in the freeze-dried sample, which scatters the reflected light. A similar observation was also reported for freeze-dried meat. A brief list on the different mechanisms of color change is given in a review in 'Further Reading' section.

Shrinkage or collapse

Shrinkage or collapse of the frozen food is the main effect during the sublimation stage as moisture is being removed. Lower sublimation pressure (higher vacuum) is generally associated with lesser shrinkage of the food material. There is a critical sublimation pressure, corresponding to the melting temperature of the sample, from which shrinkage will occur. It is important for the sublimation pressure to be lower than (or equal to) this critical value. In similar veins, lower pressure was also found to produce freeze-dried rice with lower bulk density and a more porous structure. Alternatively, this can be controlled by ensuring a low sublimation chamber temperature as observed in reports on the freeze-drying of agricultural products such as carrots, potatoes, apples, and bananas.

Therefore, it is important to prevent the sample from turning soft during the sublimation stage. Along this line, the concept of glass transition temperature has been introduced to semiquantitatively describe this softening state, in order to better control the shrinkage attribute of freeze-dried food products. This concept in relating the glass transition temperature to the collapse phenomenon can also be applied to foodstuffs with complex structures. The higher the sublimation temperature above the glass transition temperature of the sample, or the lower the glass transition temperature, the higher the extent of shrinkage. A list of glass transition temperatures for solid food and liquid food can be found in the reports cited in 'Further Reading' section.

Texture

Freeze-dried food samples are often rehydrated prior to consumption. However, several factors might change the texture even when water is being rehydrated into the dried sample. The formation of ice crystals in the initial freezing stage might break the cellular structure of the sample leading to softer textures. For soft materials, the sublimation stage might also lead to sample collapse, which will affect the texture attribute. Which aspect is dominant is certainly dependent on the materials considered and the operating conditions.

The texture of freeze-dried strawberries was found to be independent of the operating conditions of the sublimation stage. However, it was found that a slower freezing rate resulted in a significantly softer texture. This was because a slower freezing rate will result in larger ice crystal formation leading to higher cellular destruction during freezing. Such destruction on the integrity of a variety of fruit tissues will result in less firm fruit samples. In some applications, unique electromagnetic or magnetic freezing methods have been introduced to control the ice crystal formation. A review on such unique freezing approach can be found in 'Further Reading' section.

In the freezing of meat, the tenderness of the cooked rehydrated meat is a very important sensory attribute. One main cause for the loss of tenderness is the denaturation and possible aggregation of proteins during the sublimation stage. It is important to control the surface temperature of the meat sample to be lower than the denaturation temperature. Another factor that affects the tenderness is the loss of rehydration capability. It was found that higher rehydrated freeze-dried chicken breast meat produced a more palatable taste and texture.

Rehydration ratio

There are many reports in the literature to suggest that the rehydration ratio is predominantly affected by the initial freezing stage and is minimally affected by the sublimation stage. This is because the rehydration ratio is governed mainly by the size of the ice crystals formed in the initial freezing stage. Larger ice crystals produced at slower freezing rates will lead to a more porous structure. There are experimental reports showing that higher porosity resulted in better rehydration of the freeze-dried food. On the contrary, larger ice crystals might also lead to lower retention of the rehydrated water. This might lead to problems such as 'drippings,' which affect the texture of the cooked rehydrated meat.

For freeze-dried instant food, another sensory or perhaps a desired property of the freeze-dried product is the dissolution rate and wettability. In general, a slower freezing rate will potentially result in a faster dissolution rate of the final product due to the larger pores produced. This trend was observed in the freeze-drying of porridge and fruit pulps. For instant food containing proteins, another factor that has to be considered is the possible denaturation of the protein, leading to subsequent insolubility, should the sublimation temperature resulted in a high surface temperature of the freeze-dried sample.

Aroma

The retention of aroma is perhaps one main advantage in the freeze-drying of extracts. Such application can be found in the freeze-drying of certain higher market value coffee powder or other liquid food. A good review on the retention of aroma in freeze-dried coffee is given in 'Further Reading' section. In general, increasing the concentration of the dissolved solids in the liquid food reduces aroma loss during freeze-drying; this in effect reduces the diffusivity of the aroma compounds within the system restricting the transport out from the sublimating matrix. Another school of thought is that the retention of volatile compounds is due to molecular trapping of the compound in the amorphous and glassy matrix of freeze-dried liquid food. Apart from the liquid food, similar applications in retaining aromas in solid food can also be found in the freeze-drying of tea leaves as an alternative to oven-drying.

Typical Equipment Used for Testing the Sensory Attributes

Color

Color is typically analyzed using spectrocolorimeters. This form of analysis measures the reflected light from the freeze-dried sample at various wavelengths. The results from such an analysis are typically reported in terms of the three-color axis of 'L,' 'a,' and 'b' according to the International Commission on

Illumination. More information on this illumination system can be found in standard handbooks for food processing.

Shrinkage or collapse

This can be visually inspected qualitatively. Some qualitative sensory scale is introduced in the literature for plant-based products and meat/seafood products. For small freeze-dried particulates such as rice, the bulk density or the porosity of the particles can also be obtained as an indication of shrinkage or slight collapse of the internal structure. This can be undertaken with standard mercury porosimeters.

Texture

The standard method is to use penetration tests. This test involves pressing the freeze-dried sample with a probe and measuring the force imposed by the sample at different depths of the penetration. The freeze-dried sample is often rehydrated prior to the penetration test. The outcome from such a measurement would be the strain–displacement curves, which are used to analyze the mechanical strength of the sample.

As mentioned earlier, one approach that can be used to control the texture of the frozen food during the freeze-drying process is to use the glass transition temperature concept. The glass transition temperature at different water activities can be measured using a differential scanning calorimeter for liquid food, food pulps, or even solid food materials, which are to be freeze-dried. Such glass transition properties or the state diagrams of a material can also be developed using viscoelastic measurements.

Rehydration ratio

This is undertaken by measuring the mass of the freeze-dried food before and after soaking in water. The outcome from this test is normally interpreted in terms of the rehydration ratio: the ratio of the mass after water absorption to the mass of the sample after freeze-drying. In these tests, the temperature of the water is normally controlled, and it is sometimes of interest to measure the time it takes to achieve maximum rehydration, another parameter to delineate the rehydration behavior.

Aroma

The standard practice for evaluating aroma relies on human sensory evaluation. However, there are several chemical analysis methods that can be used to provide indicative quantitative measures of the aroma. This is done by measuring the concentration of the compound corresponding to the different aromas, which is of interest in their retention using standard chromatographic techniques. Along that line, a recent development in the food industry has also introduced the concept of an 'electronic nose' and is slowly gaining popularity in the industry.

Additional Note

The control of the sensory attributes was discussed earlier revolving around the freezing and the sublimation stages. The preparation of the sample to be frozen (pretreatment stage) will certainly also affect the control of these sensory attributes. Apart from controlling the freezing rate and the sublimation pressure and temperature, there are myriad pretreatment options and parameters and it is highly material-dependent. In general, fruit and vegetables can be blanched or soaked with organic or inorganic compounds before freezing. For meat products, excess fats will have to be removed prior to freezing. In liquid food, different additives can be included into formulations to induce stability and sometimes the flavor encapsulation capability of the freeze-dried liquid food. These additives may be added to improve the stability (preventing collapse) during the sublimation stage or to control the freezing stage and may even aid aroma retention. In certain scaled-up freeze-drying applications, the state of the initial fresh material also plays an important role more dominant than the effect of freeze-drying parameters. For example, it was found that the degree of ripeness in the zucchinis significantly affected the sensory attributes in contrast to the initially assessed freezing rate. This is an important factor for the freeze-drying of many agricultural products.

Effect of Freeze-Drying on Nutritional Values

The nutritional values of foods can be classified into the categories given in Table 1. The nutritional value changes due to drying (not limited to freeze-drying) are also summarized.

From Table 1, quality changes during the drying process include oxidation in the presence of oxygen, thermal degradation and heat denaturation due to high-temperature drying, enzymatic degradation, and Maillard browning. This results in lower retention of bioactive ingredients in the dehydrated products. In order to maximize the retention of the bioactive ingredients, selection of the proper drying technique is the key.

In this regard, freeze-drying, which is carried out at low-temperature condition, is considered as a good drying method for processing materials that are susceptible to heat denaturation and thermal degradation and Maillard browning that are due to high-temperature drying.

If a nutrient degrades in an aqueous condition, the time of contact with water will influence the retention of the nutrients in the dehydrated products. Thus, conventional freeze-drying may not be suitable as its drying time is typically long. However, one may combine freeze-drying with microwave drying in order to shorten the drying time. It can typically reduce the drying time by about 50–75% if compared with conventional freeze-drying. In certain cases, it has been reported that the retention of bioactive ingredients in the dehydrated food product by microwave-assisted freeze-drying is higher than the conventional freeze-drying.

Certain ingredients especially drugs are pH-sensitive; therefore, the pH profile will have to be carefully monitored during the freeze-drying process. Likewise, certain products are light-sensitive; hence, light exposure during the process should be minimized. This can be easily achieved by ensuring the freeze-drying chamber is covered to avoid light exposure. In general, the dehydration should minimize the time over which the degradations may occur and it is ingredient characteristic-specific.

Research finding about the effect of freeze-drying on the retention of bioactive ingredients and nutrients is well reported, and it is well known that freeze-drying is one of the best ways to remove water from a product while retaining its

Table 1 General nutritional value changes during drying

Nutritional value	*Changes during drying*
Calorie content	Does not change, but calorie per unit mass is increased due to removal of water
Fiber content	Does not change
Vitamin A	Fairly well retained under appropriate drying temperature range
Vitamin C	Mostly destroyed during hot-water blanching and high-temperature drying. It is also sensitive to oxidation
Thiamin, riboflavin, and niacin	Some losses during blanching but fairly good retention if the water used to rehydrate is also consumed
Minerals	Well retained during drying
Proteins	Undergo heat denaturation at relative high-temperature drying, susceptible to light oxidation, and may undergo enzymatic degradation
Lipids	May undergo enzymatic hydrolysis in the initial phase of drying. At low water activity, autooxidation of unsaturated fatty acids may cause rancidity
Carbohydrates	May undergo Maillard browning and changes in flavor under high-temperature drying. Sugar can be caramelized and produce darker pigments that give rise to severe color change issues
Antioxidants such as vitamin C, vitamin E, carotenoids, polyphenols, melanoidins, and indoles	May undergo thermal degradation or heat denaturation

bioactive ingredients. Often, freeze-drying is used as a benchmark for many drying experiments, especially in terms of bioactive ingredient contents. Some of the research findings on the effect of retention of bioactive ingredients are summarized in Table 2.

It should be noted that the freezing step during freeze-drying may damage or destroy some of the ingredients or lower the cell viability; therefore, protective agents are needed in order to reduce the effect of freezing on the quality of the product. It has been reported that the addition of protective agents such as trehalose, adonitol, betaine, glycerol, lactose, skim milk, dimethyl sulfoxide, sugars, and chlorogenic acids could improve the stability of the preserved ingredients and the preservation of other quality attributes such as color.

Concluding Remark

In controlling the sensory quality of freeze-dried solid food materials, the key lies in preserving the structure of the food during freezing and sublimation. The different structures formed during freeze-drying subsequently lead to changes in the rehydration ratio, the texture, the color, and also the taste of the food material. To a certain extent, the structure can be controlled by a combination of pretreatment procedures and manipulation of the freezing and the freeze-drying operating conditions. This is also true for preserving the quality of food pulps, extracts, or liquid foods that are dried. However, an added complexity or perhaps a manufacturing opportunity is in manipulating the formulation of the liquid food. This can be particularly useful in the preservation of flavors and aroma. Since freeze-drying is both capital- and energy-intensive operation due to the cryogenic temperatures and vacuum conditions required in this extremely slow sublimation drying process, it is cost-effective only for high-value and low-volume food products.

Table 2 Research findings about the effect of freeze-drying on the retention of bioactive ingredients

Materials	*Freeze-drying method*	*Research finding*	*Base case*
Apple cubes	Atmospheric freeze-dried	Higher antioxidant activity and polyphenol contents	Convective hot air
Apple slices	Microwave vacuum drying	Higher retention of vitamin C	Convective hot air
Apple slices	Freeze-drying	Higher antioxidant capacity, total polyphenol contents, and ascorbic acids Slightly higher than atmospheric freeze-drying	Convective hot air
Javanese pepper	Freeze-drying	Higher piper content	Oven-drying
Saffron (flavoring, coloring)	Freeze-drying	Higher safranal contents and crocin contents	Sun-drying
Ginger leaves	Freeze-drying	Higher total phenolic content, ascorbic acid equivalent antioxidant capacity, and ferric-reducing power	Microwave-, oven-, and sun-drying

See also: Browning: Enzymatic Browning; Browning: Non-enzymatic browning; Drying: Effect on Nutrients, Composition and Health; Drying: Principles and Types; Freeze-drying: The Basic Process; Oxidation of Food Components; Sensory Evaluation: Sensory Rating and Scoring Methods.

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Freeze-drying: The Basic Process

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Introduction

Freeze-drying or lyophilization is the operation whereby water is removed from a product by sublimation and desorption. The process consists in, firstly, freezing the product and then exposing it to high vacuum to sublimate the ice.

Lyophilization was known and used as a laboratory technology at the nineteenth century. However, it was after the Second World War when the first industrial applications of freeze-drying were observed. Originally, freeze-drying process was used only in the pharmaceutical industry to obtain antibiotics, cells, and other related products. It was not until the middle of the twentieth century that freeze-drying started to be used in the food industry. Currently, freeze-drying is considered as a valuable alternative to preserve foods.

Since sublimation is the direct transition from solid to gas state without passing through the liquid phase, it occurs only under specific conditions. Sublimation of water takes place when the vapor pressure of water and the temperature of ice are lower than those in the triple point, that is, 0.61 kPa (4.58 mm Hg or 0.006 atm) and 273.16 K (0.01 °C), respectively, as it is shown in the pressure–temperature phase diagram of water (Figure 1). Three major regions are illustrated in the phase diagram (Figure 1) separated by lines. Each region represents the solid (ice), gaseous (steam), and liquid thermodynamic states of water in a closed system. Each point along the separating lines denotes the combination of pressure and temperature where two states occur in thermodynamic equilibrium. In other words, AD line shows the solid–liquid equilibrium, DB line shows the liquid–gas equilibrium, and CD line illustrates the solid–gas equilibrium. The unique combination of pressure and temperature at which the three thermodynamic states of water coexist in equilibrium is shown by point D and it is called the triple point.

The major reason for using lyophilization rather than conventional drying methods among the food industry is because freeze-drying is carried out at low temperature, and consequently, thermal damage is minimized and heat-sensitive nutrients, such as vitamins, among others, remain within the product. Freeze-drying also preserves sensory and some structural and shape characteristics of foods. Likewise, this process has proved to be an alternative to obtain new ingredients and additives in the field of functional foods, ethnic foods, and in the development of new products with innovative characteristics. Nevertheless, freeze-drying is an expensive method and it is used only to obtain high value-added products or if the high quality of the food justifies its use. Moreover, compared with conventional air-drying, which dehydrate foods in a single stage, freeze-drying is an expensive process because it takes longer operation times and consumes large amounts of energy. Energy is required to freeze the product, to maintain the high vacuum in the system, to heat the frozen product to sublimate ice, and to condense water vapor.

Freeze-drying can also be carried out at low vacuum conditions, as well as at atmospheric pressure. However, to make sublimation possible without vacuum conditions, it is essential to produce a large difference between the vapor pressure of the environmental air and that of the food surface to make the sublimation transition. It is only possible by using very dried air all over the frozen food. The use of freeze-drying at low vacuum or at atmospheric pressure is of great interest since the application of vacuum is not necessary, and as a result, the operation costs are considerably reduced.

Freeze-Dryer Basic Components

Freeze-dryers are regularly designed with four basic components: a drying chamber, a vacuum pump, a heat source, and a condenser (Figure 2). The correct selection and operation of these components are critical to reach the benefits of the lyophilization process and depend on the requirements of each product.

The drying chamber is where the frozen product is placed; although the freezing process can be done within the chamber as a first step, in this case, the equipment must have a freezing system (not shown in Figure 2). However, the chamber must be of a vacuum-tight design, and the shelves in the interior require temperature control since heating and cooling processes take place inside the chamber. In addition, an adequate vacuum pump is required to remove the noncondensable gases from the chamber and, subsequently, reach the desirable vacuum level, that is, below 0.61 kPa (4.58 mm Hg or 0.006 atm). The heating source provides the latent heat of sublimation once the frozen product is already at high vacuum conditions. The temperature of the heating source may vary between 243.15 and 423.15 K (−30 to 150 °C; though, the latter being too high to be considered in practical applications) and essentially is determined by the glass transition temperature (T_g) and the moisture content of the food. Finally, the function of the condenser is to collect the water vapor released by the sublimation of ice within product, and it must have sufficient surface and cooling capacity to freeze all the steam generated during the sublimation process. The water vapor contacts the condenser surface and is turned into ice crystals releasing energy; afterward, crystals are removed from the system. Typically, the operational temperature of condensers in commercial freeze-dryers is around 208.15 K (−65 °C).

Freeze-Drying Stages

Freeze-drying is a complex operation and consists of three fundamental stages: initial freezing of the food, primary

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drying, and secondary drying. The initial freezing of the food is crucial on the size, shape, and ice crystal distribution; besides, the drying process and the final structure of the freeze-dried food depend on the freezing process. To understand the complete influence of freezing on all the process, it is necessary to comprehend the physicochemical changes taking place during the initial freezing stage.

Freezing

In the initial freezing stage, the food product is cooled to a temperature under its eutectic point, that is, the composition and temperature combination that produces the lowest point at which the product freezes. The purpose of this stage is to freeze all the mobile water originally present in the food. During the freezing stage, it is recommended to reach a food temperature lower than the glass transition temperature (T_g) for each moisture level. Above the T_g, food products are in an unstable rubbery or liquid state; however, below the T_g, products transform into a glassy state or amorphous solid.

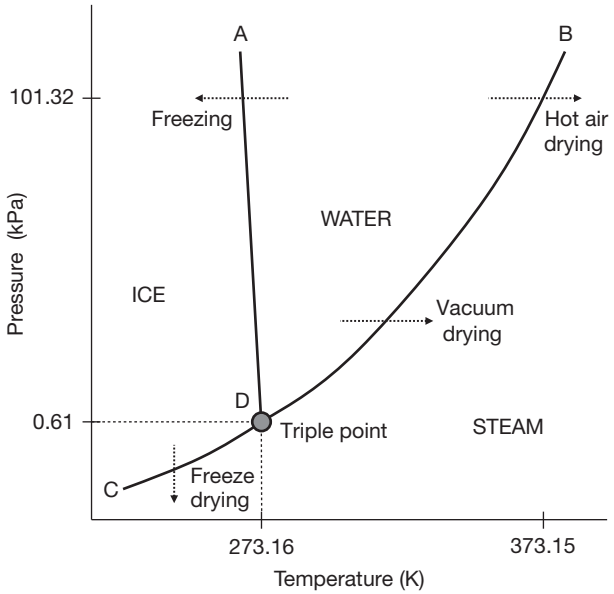

Figure 1 Pressure–temperature phase diagram of pure water in a closed system.

Drying Phases

As it was mentioned earlier in the text, the drying stage is performed in two phases. In the primary drying phase, the frozen product is heated under vacuum conditions to remove ice by sublimation, maintaining the food product below the T_g. Nevertheless, it is necessary to know that the T_g value is continuously changing as the moisture is decreased. If the temperature at the 'ice front' is higher than T_g, water is eliminated by both sublimation and evaporation processes. During the primary drying phase, ~90% of the water in the food is removed by sublimation process. The sublimated water in this phase is mainly composed of all the free water and some of the bound water in the food.

Finally, during the secondary drying phase, the unfrozen bound water is removed by desorption from the dried layer of the product. The result of this mass transfer process is a food product with moisture content between 1% and 3%. This final stage is achieved by increasing temperature and reducing the vapor pressure inside the chamber. The secondary drying requires up to 50% of the time required for the primary drying because of the low pressure of the residual bound water compared with that of the free water at the same process conditions (temperature and pressure), resulting in a long process. Once the secondary drying concludes, that is, when all the free water and bound water are removed, the freeze-drying process is complete.

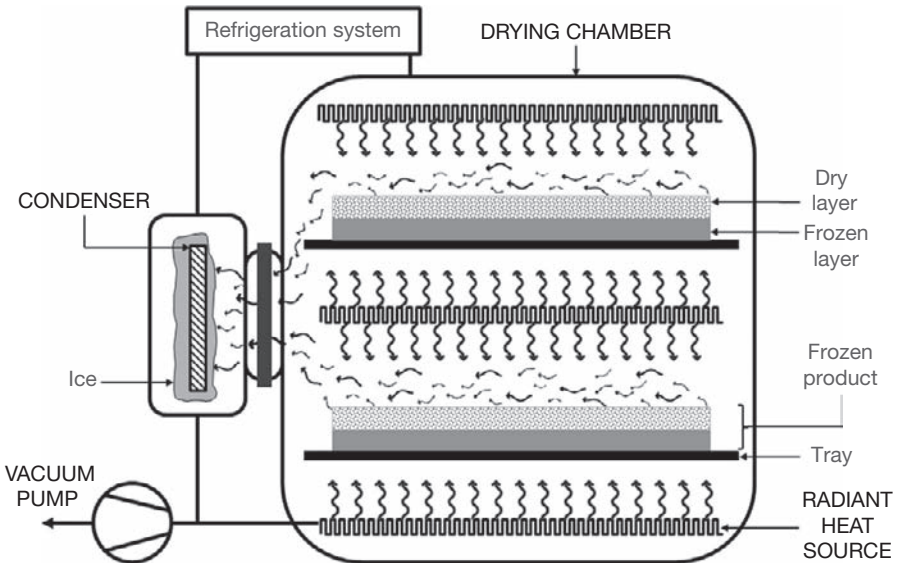

Figure 2 Basic components of a typical freeze-dryer system.

Heat and Mass Transfer

Freeze-drying is a complex operation where both heat and mass transfer processes occur in the interior of the food product. To generate vapor from the ice, energy in the form of heat is transferred to the sublimation zone. Unlike the mass transfer process, which is constantly taking place through the dried layer (mass flux), heat transfer (heat flux) can occur

(a) by conduction through the dried layer (Figure 3(a)) and in the opposite direction of the mass flux – in this case, near the food product is the heat source that irradiates heat to the dried layer;
(b) through the frozen layer (Figure 3(b)) – in this case, the frozen product is in direct contact with the heat source, and the heat transfer is by conduction; or
(c) within the frozen layer (Figure 3(c)) by heat generation using microwaves – in fact, microwaves are currently preferred as a heat source in freeze-drying due to their capability to infiltrate profoundly into the food product, resulting in a uniform and effective heating.

To illustrate the heat and mass fluxes through the freeze-drying operation, a food product with a slab shape is represented with the frozen layer and a dried layer in form of pores (Figure 4).

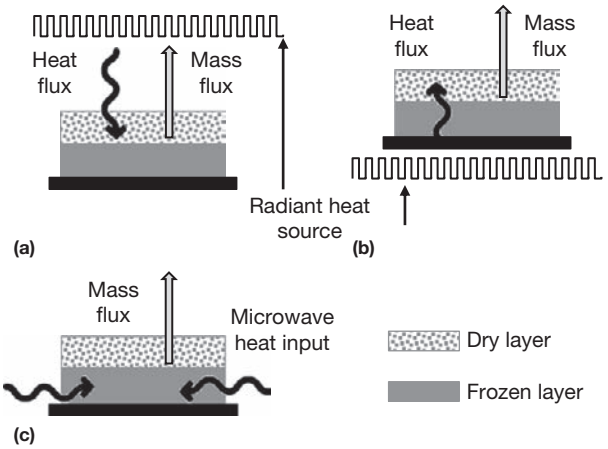

Figure 3 Heat and mass fluxes in different freeze-drying mechanisms.

This illustration assumes a one direction freeze-drying process. The ice front or sublimation point is the limit between the frozen layer and the dried layer. The ice front depends on time, and it is assumed to move at a uniform rate for mathematical and thermodynamic analysis. As ice within the frozen layer sublimates, the steam flows outside the food product, generating pores and channels in the slab.

Freeze-drying, as well as conventional air-drying, is a combined process between mass and heat transfer, and the sublimation rate can be calculated as described in eqn [1]:

$$G = \frac{A(p_f - p_c)}{R_d + R_s + R_o} \quad [1]$$

where G is the sublimation rate (kg s^{-1}); A is the area of the food slab surface (m^2); p_f is the partial vapor pressure of the ice inside the food, that is, the frozen layer; p_c is the partial vapor pressure of water in the condenser; R_d is the transfer resistance of the dried layer in the food slab; R_s is the transfer resistance of the space between the food and the condenser; and R_o is a constant that includes other resistance terms in the process.

At the same time, an external heating source provides the energy to sublimate the ice from the food slab. This energy is the latent heat of sublimation or sublimation enthalpy, ΔH_s. The sublimation rate can be described as a function of the rate of the heat transfer, q, and the sublimation enthalpy as shown in eqn [2]:

$$G = \frac{q}{\Delta H_s} \quad [2]$$

Heat and Mass Transfer Through the Dried Layer

Considering a simplified case where the food product to be dried is heated by radiation through the dried layer (Figure 3(a)), the temperature of the frozen layer is determined by a balance between the mass and the energy transfer; the geometry of the food slab allows no edge effects; the maximum slab surface temperature acceptable, T_s, is reached instantly; the heat flux is adjusted so the slab surface temperature remains constant through all the process; the partial pressure of the water inside the drying chamber is constant, p_s; and all the supplied heat is used to sublimate the ice; then, under

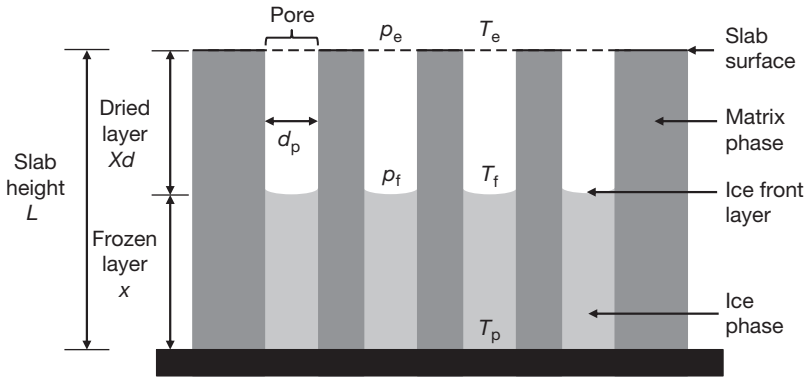

Figure 4 Representation of a freeze-dried food product Slab. d_p, the diameter of the pore; p_e, the partial vapor pressure of the slab surface; T_e, the temperature of the slab surface; p_f, the partial vapor pressure of the frozen layer; T_f, the temperature of the ice front layer; T_p, the temperature of the tray under the food product.

all these conditions, the heat transfer, q (kg m^2 s^{-3}, or J s^{-1}), at any instant is represented by eqn [3]:

$$q = \frac{Ak_d(T_e - T_f)}{Xd} \quad [3]$$

where the term Xd (or $L-x$) refers to the thickness of the dried layer (m) and is a time-dependent variable, k_d is the thermal conductivity of the dried layer (W m^{-1} K^{-1}), T_e is the temperature at the slab surface (K), and T_f is the temperature of the ice front (K).

The sublimation rate, G, can be expressed as shown in eqn [4]:

$$G = -\frac{dW}{d\theta} = \frac{Ab(p_f - p_e)}{Xd} \quad [4]$$

where b is the permeability of the dried layer (kg m^{-1} s^{-1} Pa^{-1}), p_e is the vapor pressure at the slab surface (Pa), W is the water weight of the food slab (kg), and θ is the sublimation time (s).

Since the dried layer thickness, Xd, is a time-dependent variable, the change of weight loss of the food product as a function of time, $dW/d\theta$, can be obtained by eqn [5], considering that inside the interface, between the frozen and dried layers, the moisture content drops from an initial value, m_e, to a final value, m_f:

$$-\frac{dW}{d\theta} = A\rho_s(m_e - m_f)\frac{dXd}{d\theta} \quad [5]$$

where ρ_s is the density of the food product solids on the tray (kg m^{-3}), m_e and m_f are the moisture content (dry basis), and the expression $A\rho Xd$ corresponds to the solids weight (kg). Making the sublimation rate in terms of weight loss from eqn [4] equal to eqn [5], the change of the dried layer thickness depends on both the partial pressure difference and the moisture content difference, as demonstrated in eqn [6]:

$$\frac{Ab(p_f - p_e)}{Xd} = A\rho_s(m_e - m_f)\frac{dXd}{d\theta}$$

$$Xd \cdot dXd = \frac{b(p_f - p_e)}{\rho_s(m_e - m_f)}d\theta \quad [6]$$

The heat transfer flux can be considered equal to the sublimation rate multiplied by the latent heat of sublimation as described by eqn [7]:

$$\frac{Ak_d(T_e - T_f)}{Xd} = \frac{Ab(p_f - p_e)}{Xd}\Delta H_s \quad [7]$$

In order to obtain a relation between the pressure and the temperature, eqn [7] is simplified, resulting in the following expression (eqn [8]):

$$p_f = p_e + \frac{k_d}{b\Delta H_s}T_e - \frac{k_d}{b\Delta H_s}T_f \quad [8]$$

It has been considered that p_e, ΔH_s, k_d, and T_e are constants; therefore, a linear equation that relates that p_f, and T_f is obtained as it is exemplified in Figure 5. Besides, in the same figure, the thermodynamic relationship between p_f and T_f is also shown (curve in Figure 5 can be obtained from any thermodynamics database for equilibrium vapor pressure of ice–vapor system). There is only one intersection point between the two curves, meaning that if the suppositions in the mathematical analysis are true, the temperature of the frozen layer, T_f, remains constant though the freeze-drying operation.

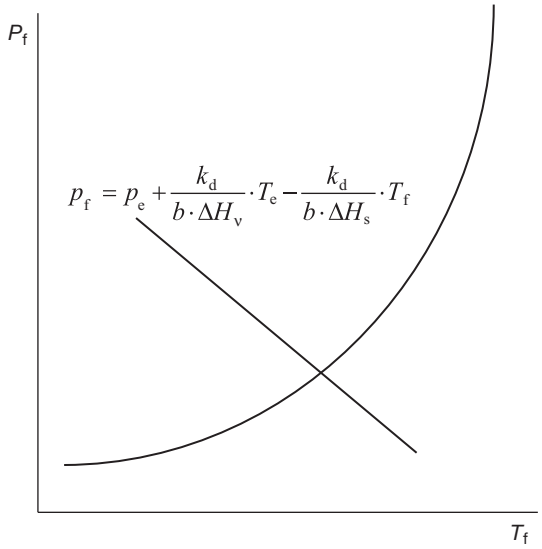

Figure 5 Thermodynamic relationships between pressure and temperature at the ice front layer.

Consequently, eqn [6] can be integrated to calculate the freeze-drying time, since it has only two variables Xd and θ, as demonstrated in eqn [9]:

$$\int_0^{L/2} Xd \cdot dXd = \frac{b(p_f - p_e)}{\rho_s(m_e - m_f)}\int_0^{\theta} d\theta$$

$$\theta_{fd} = \frac{L^2\rho_s(m_e - m_f)}{8b(\rho_f - \rho_e)} \quad [9]$$

where L is the food product slab height (m) and θ_{fd} is the freeze-drying process time. In the same way, due to the equivalence between the mass transfer and energy transfer, eqn [3] can be integrated to obtain the following expression (eqn [10]):

$$\theta_{fd} = \frac{L^2\rho_s(m_e - m_f)\Delta H_s}{8k_d(T_e - T_f)} \quad [10]$$

As it is illustrated in the two last equations, the freeze-drying process time depends on the following variables: the maximum slab surface temperature acceptable, initial and final moisture content, density of the food solids, latent heat of sublimation, food slab thickness, thermal conductivity of the frozen layer, and permeability of the dried layer.

Heat Transfer Through the Frozen Layer and Mass Transfer Through the Dried Layer

Heat can be transferred by conduction through the frozen layer when the food slab is in direct contact with the heat source, as it was exemplified in Figure 3(b). The heat and mass transfer calculations are similar to those in the preceding section; however, some considerations should be taken into account due to the direction of the heat flux and the position of the heat source. As mentioned earlier in the text, in a food slab,

considering no edge effects, the mass transfer of the water vapor is defined by eqn [4]; and the decrement and increment of the frozen and dried layers, respectively, depend on the temperature difference as shown in eqn [11]:

$$Xd \cdot \mathrm{d}Xd = \frac{b(p_f - p_e)}{\rho_s(m_e - m_f)}\mathrm{d}T \qquad [11]$$

In this case, the heat transfer through the frozen layer in the same direction of the mass flux through the dried layer is described by eqn [12]:

$$q = \frac{Ak_f(T_p - T_i)}{x} \qquad [12]$$

where T_p is the temperature of the heated tray under the food product slab, or the heat source plate in contact with the food (K); k_f is the thermal conductivity of the frozen layer (W m^{-1} K^{-1}); and x is the relative height position of the ice or sublimation front and is a time-dependent variable (m).

The relationship between the pressure and the temperature inside the interface of ice is more complicated. In the case of heat transfer through the dried layer, there is a linear relation between p_f and T_f (eqn [8]); however, in the case of heat transfer through the frozen layer, two additional variables, Xd and X, are involved (eqn [13]):

$$p_f = p_e + \left(\frac{k_f}{b\Delta H_s}\right)\left(\frac{Xd}{X}\right)(T_p - T_i) \qquad [13]$$

As it can be deduced from **Figure 4**, Xd is the difference between the slab thickness and the frozen layer height: ($Xd = L - X$). Therefore, eqn [13] can be modified as follows (eqn [14]):

$$p_f = p_e + \left(\frac{k_f}{b\Delta H_s}\right)\left(\frac{Xd}{L - Xd}\right)(T_p - T_i) \qquad [14]$$

Hence, the temperature and the pressure inside the interface are not really independent from the drying time, even if k_f, ΔH_s, Xd, b, p_e, and T_p remain constant. Then, p_f has to be evaluated as a function of Xd. The latter can be achieved assuming an Xd value and assessing its correspondent p_f. This process has to be repeated for several supposed Xd values until a function of Xd is reached. That is, $p_f = f(Xd)$. The function can be substituted in eqn [11] and integrated as demonstrated in eqn [15]:

$$\int_0^L \frac{Xd}{f(Xd) - p_f}\mathrm{d}Xd = \frac{b}{\rho(m_e - m_f)}\int_0^\theta \mathrm{d}\theta \qquad [15]$$

Equation [15] can be solved analytically, or if $f(Xd)$ is a complex function, numerical methods should be used.

Numerous mass and energy transfer models have been developed to describe the freeze-drying process. Most of the models are variants of the sublimation model, which only takes the subtraction of frozen water into account, and the sorption sublimation model, which includes both the frozen water and the bound water. Some of these models have proved to describe experimental drying rates and times successfully. Nevertheless, the application of most of the models has drawbacks, because several processing data and heat and mass transport properties of food products are required, such as water vapor concentration in the dried layer, porosity, mass diffusion, effective thermal conductivity, permeability, and diffusivity within the porous medium, among others.

Impact on Nutrients and Bioactive Compounds

Conventional heat-drying technology is based on the use of hot air to dehydrate the food products. The presence of high temperature, oxygen, and long operation time in conventional drying processes has an important effect on decreasing the sensory and nutritional characteristics of foods. The loss of fat-soluble vitamins (vitamins A and E) due to the oxidative reaction of lipids, the reduction of ascorbic acid and carotenes by heat degradation, the decrease of volatile and phenolic compounds, and the denaturation of heat-sensitive proteins by overheating, which may reduce the protein efficiency ratio and make them nonbioavailable for the organism, are some examples of the effects of heat-drying in foods. In fact, the problems for using heat-drying are not only the loss of nutrients but also the production of harmful substances to human health by different reactions, such as Maillard reaction, degradation of lipids, and Strecker degradation of amino acids.

On the other hand, freeze-drying operational conditions (low temperature and oxygen absence) make this technology an excellent alternative in food drying to prevent most of the high-temperature-related problems. Recently, freeze-drying has also been studied due to the positive effect on the extraction and bioavailability of different compounds, such as flavonoids and anthocyanins, in several fruit and vegetable products. Studies claim that the change in the tissue structures of fruits and vegetables during the freeze-drying process exposes the compounds of interest.

Although many studies have proved that the nutrient losses during the freeze-drying process and the benefits for freeze-drying foods are minimal, recent research results in this field have shown that the composition of some antioxidant compounds and antioxidant activity of several fruits may possibly be affected by freeze-drying. However, a number of operational variables influence the performance of the freeze-drying process and therefore the final product characteristics. The correct selection of the variables – freeze rate, heat flux, chamber vacuum, and temperature – should be enough to avoid any undesirable effect on food. Clearly, variables selection depends on the food and the final desirable characteristics.

Conclusions

Food industry trends are constantly changing. Currently, the increasing consumer demand to obtain secure food products with high quality, fresh-like attributes, and at the same time innovative is a priority for food processors. Therefore, food technologists are looking for alternative technologies with the objective of replacing conventional process operations. In these terms, freeze-drying has shown to be a good alternative for conventional heat air-drying, since it is capable of preserving different foods, food additives, and food ingredients, extending considerably the shelf life of food without losing their native sensory and nutritional properties.

It is well known that freeze-drying process maintains desirable characteristics; however, more research is needed to understand the role that water plays in each food when it is subjected to sublimation and to understand how the control of the operational variables results in high-quality freeze-dried products. The knowledge of the fundamentals of phase changes of water in foods and of the effect of the variables on the process effectiveness and cost can open new opportunities for the application of freeze-drying to obtain high-quality preserved foods and to avoid any possible loss of bioactive compounds in the food product.

See also: Drying: Principles and Types; Freeze Drying: Effects on Sensory and Nutritional Properties; Freezing Theory.

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Freezing Theory

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Nomenclature

A	Surface area, m^2
a_w	Water activity
Bi	Biot number, hR/k_f
c	Apparent specific heat (including latent heat of phase change), J kg^{-1} K^{-1}
c_f	Specific heat of frozen food (not including latent heat), J kg^{-1} K^{-1}
c_u	Specific heat of unfrozen food, J kg^{-1} K^{-1}
D_v	Diffusivity of water vapor in air, m^2 s^{-1}
D_w	Diffusivity of moisture in solid, m^2 s^{-1}
E_f	Freezing time shape factor (freezing time of infinite slab with the same smallest dimension/freezing time of solid object)
F	Frozen fraction
ΔG_v	Free energy change per unit volume due to crystallization ($\Delta G_v < 0$)
h	Heat transfer coefficient, W m^{-2} K^{-1}
H	Enthalpy, J kg^{-1}
i	Dissociation constant (van't Hoff factor)
J	Nucleation frequency, m^{-3} s^{-1}
j_w	Water diffusion flux, kg m^{-2} s^{-1}
k	Thermal conductivity, W m^{-1} K^{-1}
k_f	Thermal conductivity of frozen material, W m^{-1} K^{-1}
M_w	Molar mass of water, kg $kgmol^{-1}$
p_a	Water vapor pressure in the surrounding air, Pa
p_s	Water vapor pressure at the food surface, Pa
p_{atm}	Atmospheric pressure, Pa
p^*	Saturation vapor pressure of pure water, Pa
q	Heat flux, W m^{-2}
q	Heat flux vector, W m^{-2}
r	Particle (ice nucleus) radius, m
r_{cr}	Critical ice nucleus radius, m
R	Product radius or smallest half-dimension, m
R	Gas constant (8314 J K^{-1} kg mol^{-1} or Pa m^3K^{-1} kg mol^{-1})
t_f	Freezing time (time for the thermal center temperature to fall from T_i to T_c), s
t_{Plank}	Freezing time according to Plank's equation, s
T	Temperature, K
T_0	Freezing point of pure water (273.15 K)
T_a	Temperature of the freezing or thawing medium, K
T_c	Final product center temperature, K
T_f	Freezing point, K
T_{fm}	Nominal mean freezing temperature in Pham's (1986) freezing time method, K
T_g'	Glass transition temperature, K
T_i	Initial food temperature, K
T_s	Surface temperature, K
V	Product volume, m^3
w	Mass ratio of total moisture to dry solids, kg (kg dry solid)$^{-1}$
x_{sol}	Solute mole fraction
x, y, z	Space coordinates, m
∇	Gradient operator, m^{-1}
ϕ_g	Volume fraction of voids
λ_f	Latent heat of freezing (positive) per unit mass of food, J kg^{-1}
λ_{vl}	Latent heat of evaporation or sublimation (positive) per unit mass of moisture, J kg^{-1}
ρ	Density, kg m^{-3}
σ	Surface energy, J m^{-2}
θ	$T - T_0$, temperature in °C (for subscripts, see definitions of T)
τ	Tortuosity factor

Equilibrium Behavior of Ice and Water

Water in foods exists as an aqueous solution containing solutes such as minerals and sugars. As temperature falls below the freezing point, assuming that thermodynamic equilibrium is maintained at every instant, water molecules separate out forming ice crystals and leaving solute molecules behind.

Water's physical behavior is complex and unusual due to the peculiar shape and polarity of its molecules. The water molecule is V-shaped, with an oxygen atom bonded to two hydrogen atoms, the bonds being at an angle of 104.45° to each other. This is close to the value of the tetrahedral angle, and therefore, on freezing, the molecules are linked by hydrogen bonds with each other to form an approximately tetrahedral structure called hexagonal ice (or ice I), which has a lower packing density than liquid water. While most substances become denser as they cool and solidify, liquid water has a maximum density at 4 °C and expands by about 9% upon freezing. The hydrogen bonds are also responsible for the high latent heat of freezing (334 kJ kg^{-1}) and relatively high freezing points compared to species with similar molecular masses. At high pressures, the molecular arrangement of ice changes, giving rise to several distinct solid phases (Figure 1), all denser than liquid water. These other ice phases do not occur naturally on earth and have little or no implications for food preservation.

One interesting result of the expansion of water upon freezing is that, by Le Chatelier's principle, the freezing point of water decreases with increasing pressure (Figure 1) up to 207 MPa corresponding to $T_f = -22$ °C beyond which ice I is converted into ice III. This effect is used in pressure shift freezing and pressure-assisted thawing.

At atmospheric pressure, pure water freezes at 0 °C or 273.15 K (the thermodynamic freezing point). The presence of solutes depresses the water activity a_w and hence the freezing point T according to the relationship

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00329-9

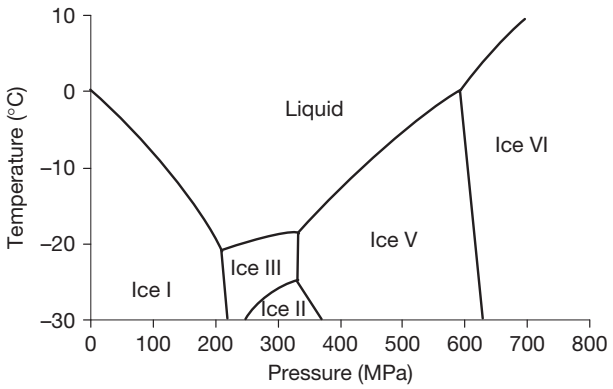

Figure 1 Ice phases.

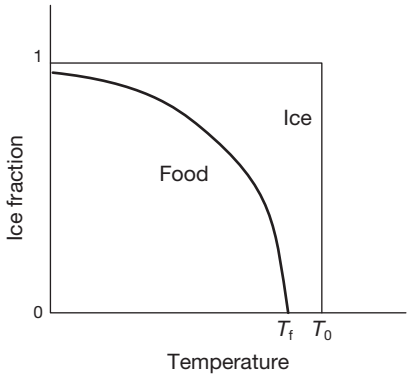

Figure 2 Frozen fraction as a function of temperature for ice and food.

$$-\ln a_w = \frac{M_w \lambda_f}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right) \quad [1]$$

Water activity depends on molar solute concentration x_{sol} and for dilute solutions, where Raoult's law applies, can be calculated from

$$a_w = 1 - i x_{sol} \quad [2]$$

Thus, from eqns [1] and [2],

$$-\ln(1 - i x_{sol}) = \frac{M_w \lambda_f}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right) \quad [3]$$

As ice freezes out, x_{sol} increases and eqn [3] becomes an equilibrium relationship between temperature and solute fraction in the remaining unfrozen solution. The latter depends on how much ice has frozen out; therefore, from eqn [3], we can derive a relationship between frozen fraction and temperature. A frequently used approximate expression is

$$F \approx 1 - \frac{T_0 - T_f}{T_0 - T}, \quad T \le T_f \quad [4]$$

where F is the fraction of unbound (freezable) water and T_f the initial freezing point. Some of the water may be 'bound' to the solid matrix and does not participate in the freezing process. The expression in the preceding text is plotted in **Figure 2**.

The freezing point of a solution reaches a minimum (the eutectic temperature) at the *eutectic composition*, beyond which the solute will solidify before the solvent (**Figure 3**). For a sodium chloride solution, the eutectic temperature is about −21.1 °C. The theoretical equilibrium freezing of an aqueous solution is illustrated on the phase diagram of **Figure 3**: the liquid solution (A) is cooled down until it reaches the freezing temperature at B. Pure ice forms (C) and continues to separate out as temperature falls, leaving a more and more concentrated solution that progresses from B to E, eventually reaching the eutectic temperature and composition E. As temperature continues to fall, the remaining water and solute will crystallize together. As will be seen later, various kinetic effects will intervene to modify this equilibrium freezing process in practice.

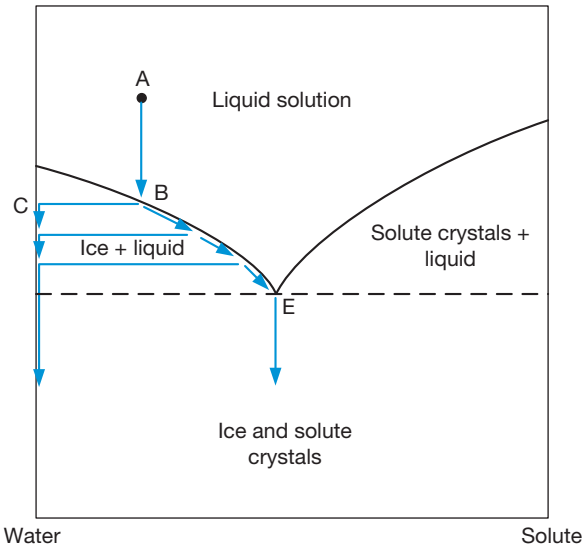

Figure 3 Phase diagram and theoretical equilibrium freezing of an aqueous solution.

Ice Nucleation

Nucleation is the spontaneous arrangement of a group of molecules into a crystal that is sufficiently stable to grow. At temperatures below the thermodynamic freezing point T_f, the crystalline phase has lower free energy than the liquid phase and is therefore the more stable phase. However, this is counterbalanced by the surface energy of the crystal, which predominates when the surface-to-volume ratio is high, that is, when the crystal is small, and causes the crystal to melt. Newly formed crystals, or nuclei, are therefore unstable even at temperatures somewhat below the freezing point, unless and until they reach a certain critical radius r_{cr}.

Homogeneous Nucleation

If a nucleus is surrounded on all sides by liquid, without any neighboring solid surface that may serve as support, the nucleation is said to be homogeneous. The nucleus or crystal can then be assumed to be spherical. Because the temperature is below the thermodynamic freezing point, the crystal phase will have lower free energy than the liquid phase. However, the creation of the crystal surface requires positive free energy. If the free energy change per unit volume due to crystallization is ΔG_v ($\Delta G_v < 0$) and the surface energy per unit area is σ, then the

free energy change associated with a change in the crystal radius dr (i.e., crystal growth) will be

$$\mathrm{d}\left(\frac{4}{3}\pi r^3\Delta G_v + 4\pi r^2\sigma\right) = \left(4\pi r^2\Delta G_v + 8\pi r\sigma\right)\mathrm{d}r \qquad [5]$$

For a nucleus to be able to grow, the free energy change associated with dr must be negative; therefore,

$$r \geq -\frac{2\sigma}{\Delta G_v} \qquad [6]$$

The expression on the right is the critical radius, r_{cr}. In general, therefore, freezing will not start at the thermodynamic freezing point, and the liquid will supercool below this temperature. As temperature continues falling, $-\Delta G_v$ will increase (i.e., the tendency to crystallize becomes greater) causing the critical radius to decrease, which increases the probability or frequency of stable nuclei being formed. According to classical nucleation theory, the homogeneous nucleation frequency J varies with temperature as

$$J \propto \exp\left[\frac{K}{T^3(T_f - T)^2}\right] \qquad [7]$$

The probability of nucleation happening in a given volume of water over a given time interval depends on both the nucleation frequency and the volume. In very small water droplets, nucleation can be substantially delayed until about $-41\,^{\circ}\mathrm{C}$, known as the homogeneous nucleation temperature. Some works have reported lower nucleation temperatures, $-45\,^{\circ}\mathrm{C}$ and even $-70\,^{\circ}\mathrm{C}$. Ice nucleation in foods is usually heterogeneous. In the lab, homogeneous nucleation can be produced in water-in-oil emulsions, which is a common method for studying it. It may also happen in foods such as butter, which contain droplets of water in a fatty matrix.

Heterogeneous Nucleation

In most foods, there are solids whose surfaces act as nucleation sites, causing heterogeneous nucleation. To be a good nucleation promoter, a surface must have a small lattice mismatch with ice, a low surface charge, and some degree of hydrophobicity, that is, it is not wetted by water. Heterogeneous nucleation occurs at temperatures much higher than homogeneous nucleation. Nucleation may also be facilitated by mechanical shocks, agitation, sound waves or ultrasound, or electric fields. The effect of sound/ultrasound is explained by the generation of cavitation bubbles that act as nucleation sites. An explanation for higher nucleation temperatures in an electrostatic field is that the free energy of the crystalline phase is lowered by the presence of the field.

Distributed Versus Surface Nucleation

In the freezing of solid foods, nucleation usually takes place first at the surface of the product where temperature is lowest. These ice crystals then grow into the product as dendrites, and no further nucleation will occur unless there are physical barriers that prevent the growth of existing crystals. In well-stirred liquids, such as in freeze concentration equipment, the product is at uniform temperature and therefore nucleation may take place throughout the material. Distributed nucleation may also take place in a solid that is cooled to a subfreezing temperature and then subjected to a triggering mechanism such as a shock wave, ultrasound, or and electric field. It also happens in pressure shift freezing, where a product is subjected to high pressure (up to 210 MPa), which lowers its freezing point, and then suddenly depressurized causing the freezing point to rise. As mentioned earlier, distributed nucleation may occur also in products where water exists as small droplets in a fatty matrix.

Extracellular and Intracellular Nucleation

In animal and plant tissues that are cellular, freezing usually takes place in the extracellular space, whose large continuous volumes facilitate the growth of crystals. As extracellular crystals form and grow, the extracellular solution becomes more concentrated, causing an osmotic pressure difference between intra- and extracellular spaces. Intracellular water diffuses through the cell membrane to deposit on the growing extracellular crystal, causing the cell to shrink and shrivel.

Whether intracellular nucleation occurs depends on the cooling rate. At slow cooling rates, water has plenty of time to diffuse out of the cell, and the intracellular concentration rises to almost keep up with the extracellular concentration; therefore, the cell is always at a temperature close to its freezing point. Due to the low degree of supercooling, intracellular nucleation will not occur. At very fast cooling rates, the freezing point in the cell falls much more slowly than the local temperature, resulting in a large degree of supercooling in the cell that facilitates intracellular nucleation according to eqn [7]. Intracellular freezing may also be facilitated by mechanical damage to the cell's membrane due to osmotic flux. Slow freezing rates result in large extracellular ice crystals, while rapid freezing produces more numerous but smaller crystals, both intra- and extracellular.

Crystal Growth and Recrystallization

Crystal Growth

Once the critical nucleus size is reached, it will grow. The crystal growth rate is a function of the local degree of supercooling $T_f - T$. At first, growth will be very fast, causing an almost instantaneous temperature rise due to latent heat release. When the local temperature approaches the freezing point, crystal growth will slow down and become controlled by the rate at which heat can be conducted away.

In aqueous solutions such as the liquid part of foods, crystal growth often takes the form of dendrites extending from the surface to the inside, due the phenomenon of constitutional supercooling. As the ice grows, solute is excluded from it and a concentration gradient will form in the liquid, decreasing the freezing point of the solution next to the freezing front. If an irregularity in the front protrudes into the unfrozen zone, it will be in contact with liquid at a lower concentration and hence higher freezing point. The degree of supercooling $T_f - T$, which drives crystal growth, is greater at the tip of the protrusion than at its base (Figure 4); hence, the protrusion will grow faster than the rest of the front and form a dendrite. Further

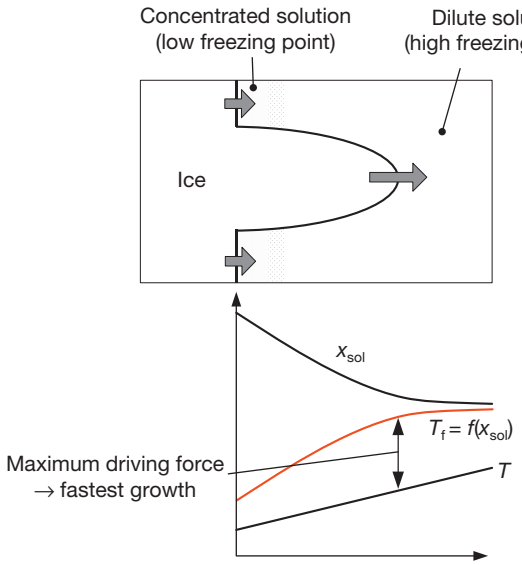

Figure 4 Top: Dendritic growth due to constitutional supercooling; bottom: profiles of solute mole fraction x_{sol}, local freezing point T_f, and temperature. Growth velocity (gray arrows) is approximately proportional to the driving force $T_f - T$; hence, growth is fastest at the tip of the dendrite.

instabilities may cause dendrites to branch and form complex, snowflake-like patterns, trapping solutes in an ice matrix.

Dendritic growth is more likely at faster cooling rate, because at slow cooling rates, solutes will have time to diffuse away and lessen or eliminate the constitutional supercooling effect. This has important implications in the freezing or freeze concentration of liquids such as fruit juice. At low freezing rates, solutes have time to diffuse away from the freezing front, and there is good separation, but at high growth rate, solutes and suspensions become trapped in the ice dendrite matrix. In cellular foods (meats and plants), dendrite growth may cause mechanical stresses and damages to cell membranes, resulting in increased drip loss.

Recrystallization

After freezing, crystals continue to change size and shape, although the process becomes slower the lower the storage temperature. This phenomenon is responsible for the increased graininess of ice cream stored for too long in a freezer. Temperature fluctuations are particularly conducive to recrystallization, as they cause molecules to leave the ice phase and redeposit on another crystal surface.

The principal mechanisms of recrystallization in frozen foods are isomass recrystallization, migratory recrystallization, and accretion. The driving force behind all forms of recrystallization is the minimization of surface energy. Isomass recrystallization happens when irregular crystals gradually take a spherical shape, which for a given mass has the smallest area and therefore lowest surface energy. Accretive recrystallization happens when adjacent surfaces merge, resulting in a reduction in the surface area. Migratory recrystallization happens because small crystals have higher specific surface energy than larger ones (eqn [5]) and are therefore less stable and thus smaller crystals gradually lose moisture to the larger ones and eventually disappear, leading to an increase in the mean crystal size.

The rate of recrystallization depends both on the activation energy for molecules to move from/to the crystal surface and on the diffusion rate from one crystal to another. With very high moisture foods where the crystals are almost contiguous, the process is controlled by the former, while in foods with lower moisture content, the latter will be the controlling factor and recrystallization will be much slower. For meat at temperatures well above the glass transition, an Arrhenius-type dependence has been proposed, with the recrystallization rate doubling every 9 K.

Glass Transition

At very high cooling rates, water molecules may not have time to rearrange themselves into ice crystals. They remain in the liquid phase, with the viscosity increasing until molecular motion becomes negligible, and the liquid vitrifies (turns into an amorphous solid known as glass). The glass transition temperature of pure water is generally believed to be about −137 °C (136 K). This temperature must be reached within milliseconds; therefore, vitrification is very difficult to achieve, especially for large specimen.

Foods consist of or contain aqueous solutions, which have higher glass transition temperatures than pure water due to the reduced mobility of water molecules and are therefore easier to vitrify. The glass transition temperature of the solution varies continuously from that of pure water to that of the solute, which can be quite high. After nucleation has occurred, water molecules separate out to form crystals of pure ice, leaving behind a more and more concentrated solution. Theoretically, as temperature gradually falls, the non-ice phase will also eventually reach its own crystallization point, and from that point onward, the two phases crystallize together as a eutectic mixture (Figure 3). In practice, due to a combination of high solute concentration and low temperature, the solution will become extremely viscous. Due to the very high viscosity, the molecules of the nonaqueous components will not be able to crystallize. These components remain amorphous, with their viscosity continuously increasing until they pass their glass transition point T_g' (point G′ in Figure 5). In practice, especially at fast freezing rates, there may be partial crystallization, resulting in an intermediate process path with a different glass transition temperature T_g (point G in Figure 5).

The glass transition temperature is an important property because food stored below this temperature will have reduced rate of quality loss, at least when the rate of damage is controlled by molecular diffusion. For example, ice recrystallization and water loss by sublimation can be expected to be reduced by storage below T_g'.

Devitrification is the formation of ice crystals in wholly or partly vitrified solutions; it may happen during storage and particularly during thawing, as higher temperatures during this stage facilitate the diffusion and rearrangement of water molecules. Devitrification is rare during food freezing, since most commercial freezing processes are not fast enough to cause vitrification.

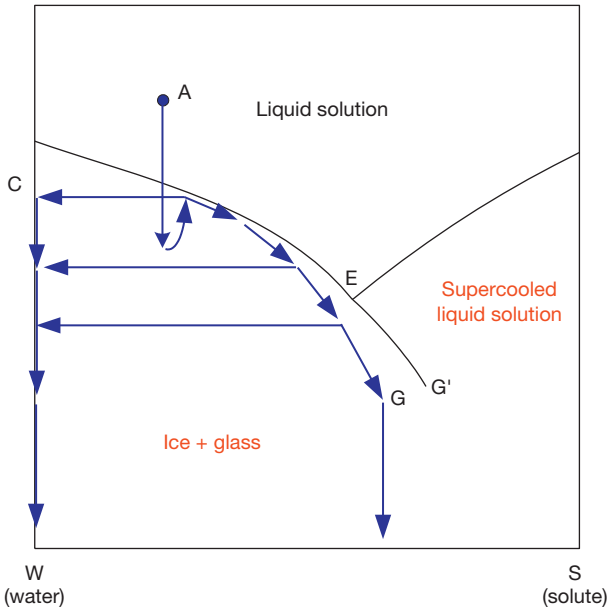

Figure 5 Freezing and glass transition in foods. A–E–G′: very slow freezing with maximum water crystallization up to G′; AG: fast freezing with partial crystallization of water.

Heat Transfer

Temperature Changes during Freezing

Once nucleation has started, the crystals grow very rapidly as neighboring liquid molecules deposit on the crystal's surface, releasing latent heat. The local temperature rises almost instantaneously until it reaches the thermodynamic freezing point, at which time crystal growth slows down and from this point onward can proceed only as fast as allowed by the rate at which heat can be conducted away from the ice crystals: this is the heat transfer controlled phase of freezing. If the liquid is well stirred so that its temperature is uniform, it will remain at the freezing point until it fully solidifies. The temperature therefore exhibits a freezing plateau. Once all the water has solidified, its temperature will fall again towards that of the surroundings. If the product cools and freezes uniformly without any spatial temperature variation, the evolution of temperature will be as shown in Figure 6. For pure water, the temperature curve (a) shows a clear horizontal plateau, while for a solution (b), the temperature falls continuously during phase change due to the increasing solute concentration (see eqn [1]), and the plateau is much less pronounced. For pure water, the cooling curve is thus divided into a precooling period, a phase change period, and a subcooling period, while for foods and other solutions, the latter two periods are less distinct.

Uniform freezing or cooling can only happen in a well-stirred liquid or when the product is very small and the freezing very slow, allowing temperature to equalize at every instant. In the freezing of most solid products, a significant temperature profile will develop, the surface being significantly colder than the core during the cooling and freezing process. After some sensible cooling, including supercooling, nucleation will happen at the surface, and the temperature near the surface jumps quickly to the freezing point. A layer of ice forms at the surface.

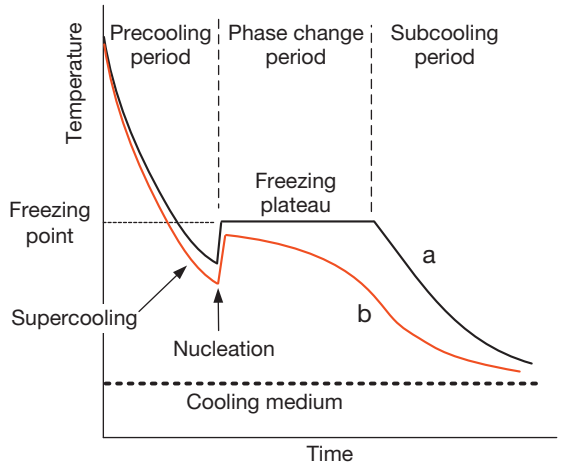

Figure 6 Temperature evolution during the cooling and freezing of a well-stirred liquid. (a) Pure water; (b) aqueous solutions.

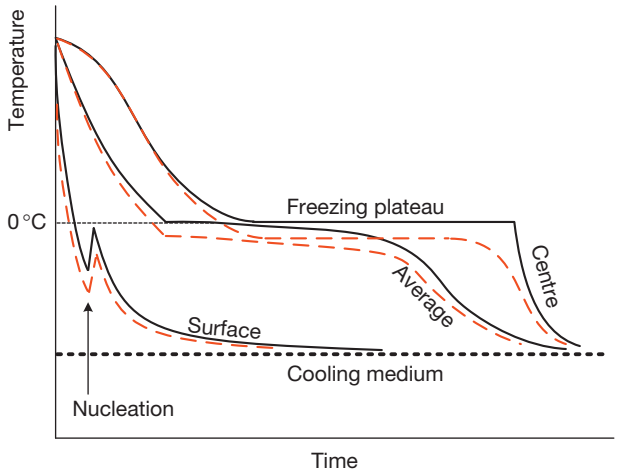

Figure 7 Stages in the freezing of still water (solid line) and that of a solid food (dashed line).

The interface between the frozen and unfrozen regions is called the freezing front. For pure water, the freezing front is quite sharp, and its temperature is the freezing temperature, while for foods and aqueous solutions, the front is diffuse. The outer, frozen region is below the freezing point, while the inner, unfrozen region is above it. Both regions continue to cool as heat is conducted from the inside to the surface. The freezing front gradually moves deeper into the product, usually by crystals growing inward from the surface. Unless freezing is extremely rapid or there are barriers to crystal growth such as plastic films or air gaps, once surface nucleation has occurred, crystal growth will become the principal or only mechanism, and no further supercooling or nucleation will occur.

Once the freezing front reaches the center, phase change is completed. Thereafter, some further sensible cooling will proceed until the ice is equilibrated with the external environment. Each point in the product has gone through the three stages of precooling, phase change, and subcooling but at different times depending on the distance from the surface (Figure 7).

The surface crosses the freezing temperature fairly quickly, while the center cools down to a near-freezing temperature and then remains in that vicinity, in an unfrozen state, for a certain duration. The center temperature still displays an apparent 'freezing plateau' similar to that in Figure 6, but its physical meaning is not identical, since in this case, the plateau starts some time after nucleation occurs at the surface and before phase change commences at the center. It means that, because the unfrozen core is surrounded by a freezing front whose temperature is the freezing point, any point in it (including the center) will tend towards that temperature but cannot cool any further until the freezing front has crossed it. For the product as a whole, there are no well-defined precooling, phase change, and subcooling periods. Nevertheless, we will see that these concepts will be adapted for the calculation of freezing time in solid foods.

Heat Transfer

In almost all food freezing applications, the freezing rate is determined by the rate at which latent and sensible heat can be transferred away from the food. Sensible heat is released throughout the food as it cools, while latent heat is released at the freezing front. In solid foods, these heats must be conducted to the surface and released to the surroundings. Heat conduction in a solid is governed by Fourier's law, which states that the heat flux vector $\mathbf{q}$ is proportional to the temperature gradient ∇T (or $\mathrm{d}T/\mathrm{d}s$ where s is the heat flow direction) and flows in the opposite direction to it:

$$\mathbf{q} = -k\nabla T \qquad [8]$$

where k is the thermal conductivity. The k-value of liquid water is around $0.57\ \mathrm{W\,m^{-1}\,K^{-1}}$, while that of ice is around $2.5\ \mathrm{W\,m^{-1}\,K^{-1}}$; therefore, frozen food has much higher thermal conductivity than unfrozen food. The thermal conductivity of frozen food can be approximately calculated from composition and frozen fraction (Figure 2) using theoretical models or empirical relationships. Figure 8 shows how the thermal conductivities of water and typical foods vary with temperature. A consequence is that under similar conditions, thawing takes much longer than freezing, since during freezing, heat must flow through a frozen outer layer with high thermal conductivity; while during thawing, heat must flow through a thawed outer layer with low thermal conductivity (Figure 9).

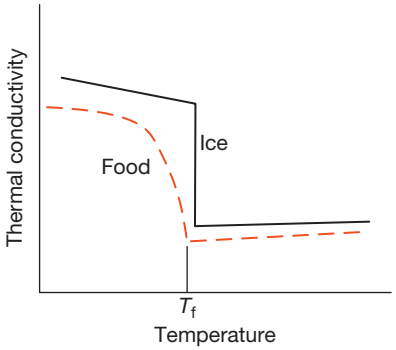

Figure 8 Variation of thermal conductivity with temperature for still water (solid line) and solutions or foods (dashed line).

An energy balance on an infinitesimal volume leads to the heat conduction equation

$$\rho c\frac{\partial T}{\partial t} = \nabla\cdot(k\nabla T) \qquad [9]$$

or in Cartesian coordinates

$$\rho c\frac{\partial T}{\partial t} = \frac{\partial}{\partial x}\left[k\left(\frac{\partial T}{\partial x}+\frac{\partial T}{\partial y}+\frac{\partial T}{\partial z}\right)\right] \qquad [10]$$

The apparent specific heat c includes the latent heat of phase change and is defined as

$$c \equiv \frac{\mathrm{d}H}{\mathrm{d}T} \qquad [11]$$

where H is the specific enthalpy of the product. Substituting this definition into eqn [9] gives the enthalpy form of the heat conduction equation, which is often used to calculate temperature changes by numerical methods:

$$\rho\frac{\partial H}{\partial t} = \nabla\cdot(k\nabla T) \qquad [12]$$

Typically, the apparent specific heat of water or foods varies with temperature as shown in Figure 10, with a sharp peak around the freezing point that represents the latent heat. For

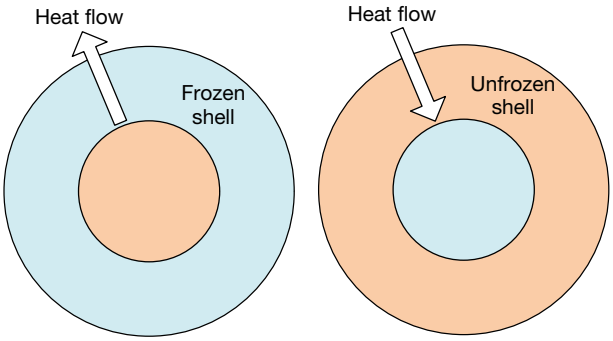

Figure 9 Heat flows through frozen layer during freezing and through thawed layer in thawing.

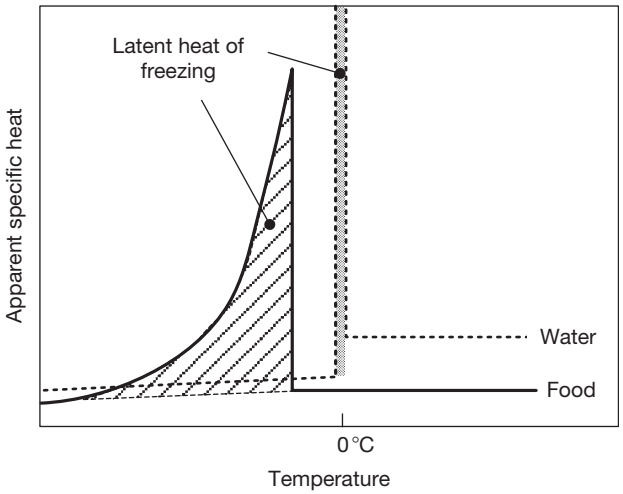

Figure 10 Variation of apparent specific heat with temperature for still water (solid line) and solutions or foods (dashed line).

pure water, the latent heat peak is infinitely tall with zero width, while for solutions and foods, the peak is spread out due to the gradual lowering of the freezing point as ice crystallizes out of the solution.

Freezing Time Calculations

Plank's Freezing Time

The calculation of freezing time has great practical importance as it governs the size of industrial freezers. For all industrial food freezing situations, heat transfer is the controlling factor for freezing rate and freezing time. Plank considers an infinite slab cooled by exposing both faces to a cold medium at temperature T_a. The following assumptions are made:

(a) The phase change takes place at a sharp temperature T_f, at which all the latent heat λ_f is released.
(b) At the surface, the heat flux is given by Newton's cooling law:

$$q|_{surf} = h(T_s - T_a) \quad [13]$$

where h is the heat transfer coefficient, which may include the effect of packaging.
(c) The sensible specific heat of the material is negligible.
(d) The thermal conductivity k_f of the frozen material is constant.

These assumptions lead to Plank's freezing time, t_{Plank}:

$$t_{Plank}(\text{slab}) = \frac{\rho\lambda_f}{T_f - T_a}\left(\frac{R}{h} + \frac{R^2}{2k_f}\right) \quad [14]$$

Plank's equation is inaccurate for practical food freezing problems because of its unrealistic underlying assumptions. However, it is very useful in helping visualize the effects of various physical parameters (product size, heat transfer coefficient, and thermal properties), and it is often used as a starting point to obtain better estimates of freezing and thawing times.

The Biot Number

Plank also derived expressions for the freezing times for an infinite cylinder and a sphere under the same assumptions as in the preceding text. Plank's solutions can be written in the following form for the three basic shapes:

$$t_{Plank} = \frac{\rho V\lambda}{hA(T_f - T_a)}\left(1 + \frac{Bi}{2}\right) \quad [15]$$

Bi is the Biot number:

$$Bi \equiv \frac{hR}{k_f} \quad [16]$$

The first factor (outside the parentheses) on the right of eqn [15] is the freezing time for an object with zero internal resistance to heat transfer, such as a well-stirred liquid, while the second factor, $(1+Bi/2)$, is a correction for internal resistance. During freezing, the latent heat flux has to cross a distance varying from 0 when the freezing front coincides with the surface to R when it reaches the center. The mean distance traversed is therefore $R/2$, and the average internal resistance to heat transfer is $R/2k_f$. The surface heat transfer resistance, which is due to the low velocity in the surrounding fluid next to the surface together with the thermal resistance due to any packaging, is given by $1/h$ (eqn [13]). Therefore, $Bi/2 = (R/2k_f)/(1/h)$ is an estimate of the ratio of internal to external (surface) resistances to heat transfer during the freezing process.

For extreme values of the Biot number, Plank's freezing time tends to the following limit:

- $Bi = \infty$ (negligible surface resistance, e.g., $h = \infty$, when the surface temperature is equal to the environment temperature at all times): the freezing time is proportional to the square of product size:

$$t_{Plank} = \frac{\rho\lambda R^2}{2k_f(T_f - T_a)} \quad [17]$$

- $Bi = 0$ (negligible internal resistance): temperature is uniform in the product at all times, and the freezing time is proportional to product size:

$$t_{Plank} = \frac{\rho\lambda R}{h(T_f - T_a)} \quad [18]$$

Knowledge of the Biot number helps in the optimization of heating and cooling processes. If it is much larger than 1 (i.e., if internal resistance is controlling heat transfer), then there is little use trying to improve heat transfer coefficient, say, by reducing the amount of wrapping or increasing air velocity, and the engineer should try to reduce the thickness $2R$ of the product (if possible). On the other hand, when $Bi \ll 1$, reducing the thickness will be less effective and efforts should be made to increase the heat transfer coefficient. Breaking up the food in smaller packages or pieces will increase the surface area to volume ratio A/V and hence reduce the freezing time, according to eqn [15].

Practical Freezing Time Calculations

Many empirical equations have been proposed for the prediction of freezing time. The most successful start from Plank's equation and apply approximate correction terms to take into account sensible heat effects and the gradual release of the latent heat. An example is Pham's approximate equation, which predicts freezing time with about 10% accuracy in most cases:

$$t_f = \frac{\rho R}{E_f h}\left(\frac{\Delta H_1}{\Delta T_1} + \frac{\Delta H_2}{\Delta T_2}\right)\left(1 + \frac{Bi}{2}\right) \quad [19]$$

where ΔH_1 and ΔT_1 are, respectively, the specific enthalpy change and temperature difference for the precooling period and ΔH_2 and ΔT_2 are those for the combined freezing–subcooling period, which may be calculated from

$$\Delta H_1 = \rho c_u(T_i - T_{fm})$$
$$\Delta T_1 = \frac{T_i + T_{fm}}{2} - T_a$$

$$\Delta H_2 = \rho[\lambda_f + c_f(T_{fm} - T_c)]$$
$$\Delta T_2 = T_{fm} - T_a$$

T_{fm} is a nominal 'mean freezing temperature,' and the following equation was proposed for most water-rich biological materials:

$$T_{fm} - T_0 = \theta_{fm} = 1.8 + 0.263\theta_c + 0.105\theta_a \quad [20]$$

where θ is temperature in °C.

In eqn [19], E_f is a shape factor (1 for slabs, 2 for infinite cylinders, and 3 for spheres). The equation is applicable to more complex shapes by using empirical shape factors. However, for more precise predictions, numerical solution of eqns [9] or [12] using methods such as finite element or finite volume is preferred.

Moisture Transfer during Freezing

Food cooling and freezing is usually accompanied by the movement of moisture. We have already seen how moisture movement may occur on the molecular scale (water molecules migrating towards crystalline surface) and on the cellular scale (molecules crossing cellular membrane by osmosis due to the growth of extracellular crystals). In this section, we consider moisture movement on the macroscopic scale.

Moisture movement is due to the difference in water potential between the product and the surrounding environment. When unwrapped wet food freezes in the air, there will be moisture transfer if the water vapor pressure in the air, p_a, is different from the equilibrium water vapor pressure at the food's surface, p_s. The latter is given by

$$p_s = a_w p^*(T_s) \quad [21]$$

where p^* is the saturation vapor pressure of pure water at surface temperature T_s and a_w is the food's water activity near the surface. The latter depends on moisture content and is usually close to unity for most fresh foods. Since the surface is warmer than the air, especially towards the beginning of the cooling–freezing process, $p^*(T_s)$, and hence p_s, is usually higher than p_a, and the product will lose moisture by evaporation or sublimation. The rate of moisture loss is much faster at the beginning, when the temperature difference and hence vapor pressure difference between surface and air are large, and rapidly falls as the surface cools down (Figure 7). Most of the moisture loss during freezing therefore takes place in the initial minutes.

Moisture loss at the surface sets up a moisture profile within the food, causing water to diffuse towards the surface to make up for lost fluid. The mechanism of moisture movement is different between dense foods and porous foods. In dense (nonporous) foods, moisture moves by molecular diffusion in the dense phase and the mass diffusion flux j_w is proportional to the moisture concentration gradient ∇w according to Fick's law:

$$j_w = -D_w \nabla w \quad [22]$$

When freezing happens, moisture movement practically stops, and there is no replenishment for the sublimated moisture. A drying front forms at or near the surface and gradually recedes into the product, leaving behind a thin desiccated layer, while there is little change in the moisture profile further inside.

In porous foods, moisture moves mainly in the pores by a process known as evaporation–condensation (or sublimation–condensation), which is driven by a temperature gradient. In this model, when a warm region of the food is connected to a colder region, moisture will evaporate or sublime from the warmer region and diffuses along the pore towards the colder region (usually near the surface), where it will condense into water or ice (Figure 11). The water vapor flux can be found from Fick's law.

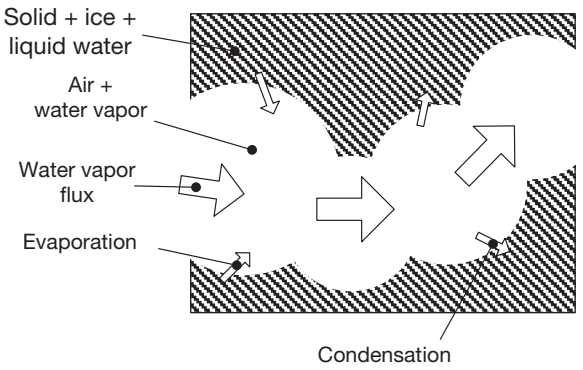

Figure 11 Evaporation–condensation in a porous food.

Evaporation–condensation causes drying of the food and is an important contributor to heat transport, accelerating the cooling rate, since water vapor carries substantial latent heat of vaporization. The contribution of this process to heat transport can be represented by an effective 'evaporation–condensation thermal conductivity' $k_{evap-con}$, given by

$$k_{eva-con} = \frac{\varphi_g}{\tau} \frac{\lambda_{vl} D_v M_w a_w}{RT} \frac{p_{atm}}{p_{atm} - a_w p^*} \frac{dp^*}{dT} \quad [23]$$

Evaporation–condensation can make a significant contribution to heat transport at above zero temperatures, but its effect becomes less at low temperatures, due to the low water vapor pressure.

See also: Chilled Foods: Principles; Freeze Drying: Effects on Sensory and Nutritional Properties; Freeze-drying: The Basic Process.

Further Reading

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Relevant Website

http://www.ceic.unsw.edu.au/staff/Tuan_Pham/coolfreeze.xls – An excel spreadsheet for the calculation of food cooling, freezing and thawing times using simplified methods.

Fructose and High-Fructose Corn Syrup

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Abbreviations

AMP	Adenosine monophosphate
apoB	Apolipoprotein B
apoCIII	Apolipoprotein CIII
ATP	Adenosine triphosphate
CVD	Cardiovascular disease
DHAP	Dihydroxyacetone phosphate
DNL	*De novo* lipogenesis
GLUT5	Glucose transporter 5
HFCS	High-fructose corn syrup
IRS1	Insulin receptor substrate 1
IRS2	Insulin receptor substrate 2
JNK	c-Jun NH_2 terminal kinase
LDL	Low-density lipoprotein
MCP-1	Monocyte chemoattractant protein-1
MTP	Microsomal triglyceride transfer protein expression
nPKC	Novel protein kinase
PAI-1	Platelet activator inhibitor-1
PFK	Phosphofructokinase
serine P	Serine phosphorylation
VLDL	Very-low-density lipoprotein

Properties and Sources of Fructose

Fructose has a fruity, sweet taste, with sweetness ratings between 130% and 180% (in part dependent on the serving temperature), compared with the standard, sucrose, rated at 100%. Both sucrose and high-fructose corn syrup (HFCS) are used extensively in foods to provide sweetness, texture, and palatability. These sugars also contribute to the appearance, preservation, and energy content of the food product.

The natural sources of dietary fructose are fruits, fruit juices, some vegetables, honey, and syrups. In these foods, fructose is found as the monosaccharide and also as a component of the disaccharide, sucrose (Table 1). However, the primary dietary source of fructose is in sugars added to foods and beverages. The top four categories that contribute added sugars to the Western diet are regular soft drinks, sugar and sweets including candies, grain-based baked goods, and fruit drinks. Many of these products are sweetened with sucrose and/or HFCS. HFCS is produced by hydrolyzing the starch in corn to glucose, using α-amylase (EC 3.2.1.1) and glucoamylase (EC 3.2.1.10). This is followed by treatment with glucose isomerase (EC 5.3.1.5) to yield a mixture of glucose and fructose. The process typically produces an HFCS composed of 42% fructose, 50% glucose, and 8% other sugars (HFCS-42). By fractionation, a concentrated fructose syrup containing 90% fructose can be isolated (HFCS-90). HFCS-42 and HFCS-90 are blended to produce HFCS-55, which is 55% fructose, 41% glucose, and 4% other sugars. HFCS-55 is the preferred sweetener used by the soft drink industry in the United States and a number of other countries, although HFCS-42 is also commonly used as a sweetener in many processed food products. Concentrated fruit juices, such as apple, pear, and white grape juices, are also used to sweeten beverages. The amount of fructose in fruit juices varies, as does its proportion with glucose, but the use of concentrated apple juice or blends of apple, pear, and grape juice can provide as much or more fructose relative to glucose at a ratio of 2:1, exceeding the fructose/glucose ratio close to 1:1 found in sucrose and HFCS. Nevertheless, considering the variety of sweeteners commonly available, it is likely that fructose provides close to 50% of the energy in added sweeteners.

Patterns of Fructose and HFCS Consumption

The addition of sweeteners and sugars to so many food products led to an increase in fructose consumption from the mid-1970s to the mid-2000s. However, sugars added to the diet are difficult to quantify accurately. The 2006 USDA MyPyramid Equivalents Database allowed for better estimates of added sugar in foods and beverages. The most recent comprehensive assessment of added sugar in the US diet used this database in combination with data collected during the NHANES surveys of 2003–06. The estimated added sugar consumption for the population as a whole averaged 83 g equiv. day^{-1}, adjusted for energy intake. For the majority who consumed between 5% and 20% of energy as added sugar, the intake ranged from 45 to 92 g equiv. day^{-1}. About 12.5% of the population consumed more than 25% of energy intake as added sugars, and in this group of high-sugar consumers, the mean added sugar consumption ranged between 160 and 233 g equiv. day^{-1}, with sugar-sweetened beverages contributing 90 or more grams equivalents of sugar per day. Overall, regular soft drinks contribute about 30% of the added sugar in the Western diet, a decline from the 33% contribution in 1999 when HFCS per capita availability was at its highest level. Many beverage manufacturers have reformulated soft drink recipes using cane sugar (sucrose) as the caloric sweetener. It is not known if this reduction in the use of HFCS by beverage manufacturers has directly affected the amount of fructose consumed in the Western diet. The best estimates of total fructose consumption from added sweeteners and natural sources have been done with NHANES survey data collected in 1999–2004. The total fructose consumption was about 40–75 g day^{-1} for adult men and 30–60 g day^{-1} for adult women, providing about 8–12% of energy intake for adults. A comprehensive analysis of fructose intake using more recent survey data is not currently available.

Table 1 Sucrose, glucose, and fructose contents of fruits, vegetables, syrups, and sweeteners[a]

Food item	*Serving size*	*Sucrose (g)*	*Glucose (g)*	*Fructose (g)*
Fruits				
Apple	1 medium	2.86	3.35	8.14
Banana	1 medium	2.82	5.88	5.72
Blueberries	1 cup	0.16	7.08	7.21
Cantaloupe	1/8 melon	3.00	1.06	1.29
Cherries	1 cup	0.18	7.71	6.28
Grapes	1 cup	0.24	11.52	13.01
Oranges	1 medium	5.99	2.76	3.15
Peaches	1 medium	4.66	1.91	1.50
Pears	1 medium	1.29	4.58	10.34
Plums	1 medium	1.04	3.35	2.03
Pineapple	1 cup diced	8.48	2.70	3.18
Raspberries	1 cup	0.25	2.29	2.89
Strawberries	1 cup	0.20	3.39	4.15
Watermelon	1/16 melon	3.46	4.52	9.61
Avocado	1 fruit	0.10	0.14	0.14
Vegetables				
Broccoli	1 cup	0.09	0.43	0.60
Carrots, baby	10 small	2.70	1.00	1.00
Corn, sweet	1 ear	1.85	0.45	0.43
Cucumber	1 cup	0.00	0.75	0.89
Onions	1 slice	0.44	0.74	0.44
Peas, green	1 cup	7.24	0.17	0.57
Potatoes	1 medium	0.36	0.70	0.58
Spinach	1 cup	0.02	0.03	0.04
Sweet potato	1 medium	3.28	1.25	0.91
Tomatoes	1 medium	0.00	1.54	1.69
Sugars/sweeteners/syrups				
Sugar, turbinado	1 tsp	4.56	0.00	0.00
Sugar, granulated	1 tsp	4.19	0.00	0.00
Sugar, brown	1 tsp packed	4.35	0.06	0.05
Honey	1 tbsp	0.19	7.51	8.60
Maple syrup	1 tbsp	11.26	0.47	0.18
Molasses	1 tbsp	5.88	2.38	2.56
Agave syrup	1 tbsp	0.00	2.58	11.52

[a]Values obtained from the USDA National Nutrient Database for Standard Reference, Release 27, accessed at http://www.ndb.nal.usda.gov.

Absorption of Fructose

Dietary fructose is ingested as the simple monosaccharide and also as part of the disaccharide, sucrose. Sucrose is hydrolyzed by sucrase (EC 3.2.1.10) at the intestinal brush border to yield one molecule of glucose and one of fructose. Glucose is rapidly absorbed via a sodium-coupled cotransporter and arrives at the liver via the portal circulation. Fructose absorption occurs by facilitated diffusion, enabled by a fructose-specific hexose transporter, GLUT5. This transporter is found in the jejunum on the brush border membranes. To maintain a favorable concentration gradient for continued fructose diffusion, it has been suggested that upon entry into the enterocyte, fructose is converted to fructose-1-phosphate, but it is not known if enterocytes are capable of this initial step of fructose metabolism. It is known that fructose enters the portal circulation from the enterocytes using the basolateral transporter, GLUT2, which also transports glucose and galactose. The expression of GLUT5 increases within hours of exposure to a fructose-enriched diet, indicating that the transporter is regulated by luminal signals, specifically fructose. However, consumption of a large amount of pure fructose can exceed the capacity of intestinal fructose absorption resulting in diarrhea. This problem is more prevalent in very young children with high intakes of dietary fructose and in whom GLUT5 expression is constrained by developmental stage. Although rare, isolated fructose malabsorption is a pediatric disease related to developmental limitations of GLUT5 expression. The malabsorption of fructose in adults is thought to be related to the insufficient upregulation of GLUT5, although changes in the gene expression or activity of GLUT5 have not been ruled out. Several studies have shown that when a single dose of 50 g of fructose is consumed by healthy adults, more than half experience malabsorption, and in some studies, malabsorption is also observed with a 25 g dose. Fructose malabsorption results in abdominal bloating, flatulence, and diarrhea. However, the intestinal absorptive capacity for fructose increases when it is consumed along with glucose as typically occurs with meals containing starch, sucrose, and/or HFCS. Thus, coingesting glucose to roughly balance fructose, as occurs when most fruits or sucrose is consumed, largely alleviates problems of fructose malabsorption. In addition, fructose absorption increases during sustained fructose consumption, suggesting adaptation to increased fructose intake.

Fructose Metabolism Impacts Lipid and Glucose Metabolism

Upon delivery to the liver via the portal circulation, the metabolic fates of glucose and fructose can diverge. Hepatic glucose metabolism is regulated by phosphofructokinase (EC 2.7.1.11), which is inhibited by adenosine triphosphate (ATP) and citrate when the hepatic energy status is elevated, thereby limiting the hepatic uptake of dietary glucose. This allows much of the ingested glucose arriving via the portal vein to bypass the liver and reach the systemic circulation leading to increased circulating glucose and glucose-stimulated insulin concentrations.

In contrast, the initial phosphorylation of dietary fructose is largely catalyzed by fructokinase (EC 2.7.1.4), which is not regulated by the hepatic energy status. This leads to unregulated fructose uptake by the liver, resulting in most of the ingested fructose being metabolized in the liver (Figure 1) and very little reaching the systemic circulation. This has been demonstrated in the 24 h fructose and glucose profiles in subjects who consumed fructose- or glucose-sweetened beverages with three meals. With the consumption of glucose-sweetened beverages, postmeal glucose peaks increased over fasting levels by 4–5 mmol; with consumption of the fructose-sweetened beverages, postmeal fructose peaks increased by less than 0.4 mmol. Other tissues that take up the small quantities of fructose that bypass the liver include the kidneys, skeletal muscles, and adipose tissues. The GLUT5 transporter is expressed in these tissues, but at relatively low levels.

Postprandial increases of plasma glucose and insulin concentrations are substantially lower when subjects consume fructose compared with isocaloric amounts of refined starch or glucose. The ability of fructose to lower postmeal glucose responses compared with an isocaloric amount of complex carbohydrates would be of potential benefit for maintaining glucose control in patients with diabetes or impaired insulin sensitivity. Indeed, many studies show isocaloric fructose replacement for other carbohydrates results in improvements

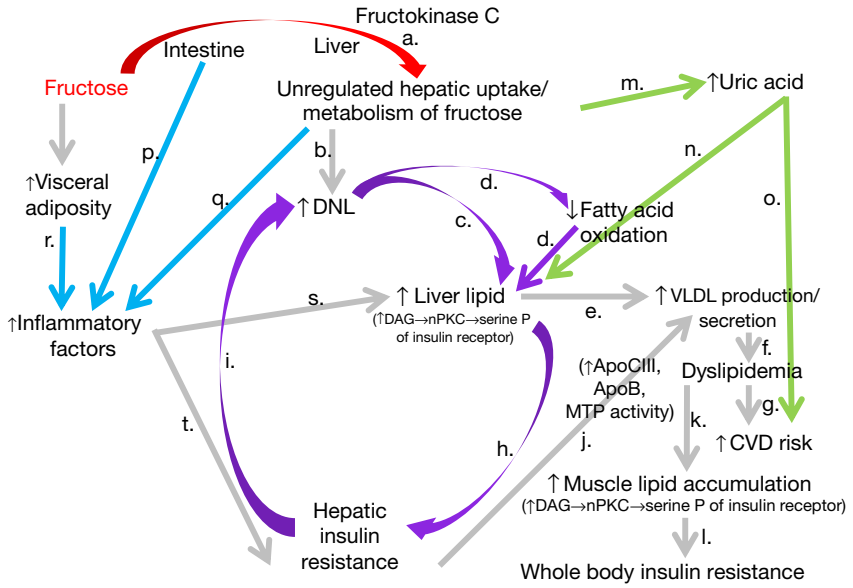

Figure 1 Potential mechanisms by which the consumption of fructose affects lipid metabolism and hepatic insulin sensitivity. Hepatic glucose metabolism is regulated by insulin and hepatic energy needs, and this allows much of the ingested glucose arriving via the portal vein to bypass the liver and reach the systemic circulation. In contrast, the initial phosphorylation of dietary fructose in the liver is largely catalyzed by fructokinase C, (a) which is not regulated by the hepatic energy status. This results in unregulated fructose uptake by the liver, with most of the ingested fructose being metabolized in the liver and very little reaching the systemic circulation. The excess substrate leads to increased *de novo* lipogenesis (DNL). (b) DNL increases the intrahepatic lipid supply directly, via synthesis of fatty acids, (c) and indirectly, by inhibiting fatty acid oxidation. (d) Increased levels of intrahepatic lipid content promote very-low-density lipoprotein (VLDL) production and secretion. (e) This leads to increased levels of circulating TG and low-density lipoprotein cholesterol (dyslipidemia (f)), risk factors for CVD. (g) Increased levels of hepatic lipids may also promote hepatic insulin resistance by increasing the levels of diacylglycerol, which activates novel protein kinase C (nPKC) and leads to serine phosphorylation (serine P) of the insulin receptor and insulin receptor substrate 1 (IRS1) and impaired insulin action. (h) Due to selective insulin resistance, DNL is even more strongly activated in the insulin-resistant liver DNL, (i) which has the potential to generate a vicious cycle (*purple arrows*). This cycle would be expected to further exacerbate VLDL production and secretion by increasing the intrahepatic lipid supply. Hepatic insulin resistance also exacerbates VLDL production/secretion (j) by increasing apoB availability and apoCIII synthesis and upregulating microsomal triglyceride transfer protein expression (MTP). This exacerbates and sustains exposure to circulating TG, leading to the accumulation of intramyocellular lipids in the skeletal muscle, (k) impaired insulin signaling, and whole-body insulin resistance. (l) Uric acid may also be involved in the metabolic dysregulation induced by fructose. The fructokinase-catalyzed phosphorylation of fructose to fructose-1-phosphate, which results in the conversion of adenosine triphosphate (ATP) to adenosine monophosphate (AMP) and a depletion of inorganic phosphate, leads to uric acid production via the purine degradation pathway. (m) High levels of uric acid are associated and may contribute to increased risk for development of fatty liver, (n) CVD, (o) and metabolic syndrome. Fructose exposure in the intestines (p) and liver (q) and fructose-induced increases of visceral adiposity (r) may promote inflammatory responses that further promote liver lipid accumulation (s) and/or impair hepatic insulin signaling (t).

in glycemic control in diabetic individuals. Also, fructose, in small quantities, increases the hepatic uptake of glucose and promotes glycogen storage, most likely by stimulating the activity of hepatic glucokinase (EC 2.7.1.2), and in individuals with type 2 diabetes mellitus, the addition of small amounts of fructose to an oral glucose tolerance test lowers the postprandial glycemic response, indicating improved glycemic control. However, given that one of the goals of maintaining tight glycemic control in diabetic patients is to lower their risk of cardiovascular disease (the major cause of death in these patients), it is extremely important to consider the potential effects of the unregulated hepatic uptake of high levels of dietary fructose beyond its benefits on short-term glycemic control.

The unregulated hepatic uptake of high doses of fructose results in increased production of lipogenic precursors, thereby leading to increased *de novo* lipogenesis (DNL) (Figure 1). DNL increases the intrahepatic lipid supply directly via synthesis of fatty acids and indirectly by inhibiting fatty acid oxidation. Increased levels of intrahepatic lipids promote increased production and secretion of very-low-density lipoprotein (VLDL), specifically VLDL1, the large VLDL particles that carry more TG than the smaller VLDL2 particles. The overproduction of VLDL1 has been proposed as the underlying defect that leads to dyslipidemia, that is, characteristic of patients with type 2 diabetes and metabolic syndrome.

Increased accumulation of intrahepatic lipids may also promote hepatic insulin resistance, possibly by increasing the levels of diacylglycerol, which activates novel protein kinase C (nPKC) and leads to serine phosphorylation (serine P) of the insulin receptor and insulin receptor substrate 1 (IRS1) and impaired insulin action. Due to selective insulin resistance, DNL is even more strongly upregulated in the insulin-resistant liver, which has the potential to generate a vicious cycle, that is, DNL increases liver lipids, which increases hepatic insulin resistance, which further increases DNL (Figure 1, purple arrows). This cycle would be expected to further exacerbate VLDL1 production and secretion by increasing the intrahepatic lipid supply. Hepatic insulin resistance may also indirectly increase VLDL production and secretion by (1) increasing apolipoprotein B (apoB) availability, the protein backbone of VLDL; (2) upregulating microsomal triglyceride transfer protein expression, which catalyzes the assembly of triglyceride and apoB into VLDL; and (3) increasing the production of apolipoprotein CIII (apoCIII), which promotes the conversion of VLDL2 into VLDL1.

Augmented VLDL secretion increases the exposure of the vasculature to triglycerides and apoB and can also lead to intramyocellular lipid accumulation. Increased intramyocellular lipid concentrations are correlated with reduced whole-body insulin sensitivity in humans. It is possible, but not definite, that this relationship is mediated by the same mechanism described for the development of hepatic insulin resistance: diacylglycerol-mediated activation of nPKC resulting in serine phosphorylation of the insulin receptor, IRS1, and other downstream phosphorylation events.

One of the most marked and significant effects of fructose consumption is increased 24 h exposure to circulating uric acid. As presented in the pathways in Figure 1, the mechanism by which fructose consumption leads to increased uric acid concentrations involves the unregulated uptake and metabolism of fructose. The first step in the metabolic pathway, the fructokinase-catalyzed phosphorylation of fructose to fructose-1-phosphate, results in the conversion of ATP to adenosine monophosphate (AMP) and a depletion of inorganic phosphate. The degradation of AMP through the purine degradation pathway leads to increased uric acid production. While the role of increased uric acid in the development of gout has been long known, more recent evidence suggests that uric acid is a potential mediator of metabolic disease, with a number of recent studies showing that it is both strongly associated and predictive of metabolic syndrome, fatty liver, and cardiovascular disease.

It is also possible that other factors such as inflammation and oxidative stress are contributors to, or possibly mediators of, the metabolic dysregulation induced by excessive fructose intake. Inflammatory responses to fructose could be mediated through its direct exposure to the intestine and/or liver. In animals, fructose consumption has been shown to increase the intestinal translocation of bacterial endotoxin, which is associated with hepatic tumor necrosis factor-α, and liver lipid accumulation. In isolated hepatocytes, fructose exposure, compared with glucose exposure, leads to the activation of c-Jun NH_2-terminal kinase (JNK), increased serine phosphorylation of IRS1, and reduced insulin-stimulated tyrosine phosphorylation of IRS1 and IRS2. In humans, the consumption of fructose, but not glucose-sweetened beverages, increases the circulating levels of some inflammatory markers including monocyte chemoattractant protein-1 (MCP-1) and platelet activator inhibitor-1 (PAI-1). Increases of visceral adiposity could also contribute to inflammation in response to the consumption of high levels of fructose.

While the roles of all the potential contributors to the adverse effects of excessive fructose consumption have yet to be determined, it is clear that the unregulated hepatic metabolism of fructose catalyzed by fructokinase, specifically fructokinase C, is essential. Fructokinase exists as two isoforms: fructokinase A, which is widely distributed and has low affinity for fructose, and fructokinase C, which is expressed primarily in the liver, intestines, and kidneys and has high affinity for fructose. It has been demonstrated that fructose-induced metabolic syndrome is prevented in knockout mice lacking both isoforms but is exacerbated in fructokinase A knockout mice compared with wild-type mice. These results demonstrate that the metabolism of fructose through fructokinase is necessary to generate adverse metabolic effects. The exacerbation of these effects in the mice lacking only fructokinase A demonstrates that fructokinase C is the key driver of the adverse effects induced by fructose. It also suggests that fructokinase A offers some protection against the adverse effects of fructose by allowing for some fructose metabolism in peripheral tissues, whereas in its absence, fructose delivery to the liver and flux through fructokinase C are increased, and the adverse metabolic effects of fructose are exacerbated.

Evidence for Metabolic Dysregulation by Excessive Fructose Intake

Providing nonhuman primates (rhesus monkeys) with a daily fructose-sweetened beverage along with ad libitum intake of a standard diet results in weight gain and increased body adiposity and produces several features of the metabolic syndrome, including dyslipidemia (increased triglycerides and

apolipoprotein CIII and decreased high-density lipoprotein concentrations) and insulin resistance. Interestingly, the adverse metabolic changes in this nonhuman primate model of diet-induced metabolic syndrome can be prevented by concurrent supplementation with a high dose of omega-3 fatty acids derived from fish oil. Direct experimental data from studies in human subjects also support many of the mechanisms described in the preceding text. Consumption of large quantities of fructose-sweetened, but not glucose-sweetened, beverages by older, overweight men and women for 10 weeks increased DNL, 24 h circulating triglycerides, LDL cholesterol, and apolipoprotein B concentrations and decreased fat oxidation and insulin sensitivity; these changes did not occur in the men and women consuming glucose-sweetened beverages. In this study, consumption of glucose-sweetened beverages with meals results in increased postprandial glucose excursions (i.e., glycemic index/load) and glucose-stimulated insulin secretion compared with a baseline high-complex-carbohydrate diet, while consumption of fructose-sweetened beverages produces lower postprandial glucose and insulin responses. Nonetheless, it was the diet with the fructose-sweetened beverages that resulted in increased visceral adiposity, produced unfavorable changes in circulating lipids, and decreased insulin sensitivity, indicating that it is the fructose, and not the glucose, component of dietary sugars that is primarily responsible for the observed adverse metabolic effects. In another study, young, healthy men who consumed moderate amounts of fructose for 3 weeks had lower hepatic insulin sensitivity (indexed by endogenous glucose production during euglycemic hyperinsulinemic clamp) than when they consumed moderate amounts of glucose. Whole-body insulin sensitivity was not affected in these men, which is consistent with the idea that fructose induces hepatic insulin resistance prior to the development of whole-body insulin resistance.

Recent studies indicate that adverse metabolic effects also occur when fructose is consumed in the form of sucrose or HFCS. Young, healthy subjects consuming 25% energy requirement as HFCS-sweetened beverages for 2 weeks exhibited significant increases of fasting LDL, non-HDL cholesterol and apoB, and postprandial triglycerides, which were comparable with those observed in subjects consuming fructose and greater than those in subjects consuming glucose. Liver lipids and cholesterol were significantly increased in overweight and obese men and women who consumed $1\,l\,day^{-1}$ of sucrose-sweetened soda (~20% energy requirements) for 6 months, compared with those consuming isocaloric amounts of low-fat milk, $1\,l\,day^{-1}$ aspartame-sweetened beverages, or $1\,l\,day^{-1}$ water. In this same study, the muscle lipid content was increased compared with the baseline only in subjects consuming sucrose. In a study in young healthy men, the total and LDL cholesterol increased after the consumption of moderate levels (~13% energy requirements) of dietary fructose or sucrose, but not glucose.

Fructose and Chronic Disease Risk

Numerous recent studies add to the considerable epidemiological evidence that increased sugar consumption is associated with metabolic disease, including cardiovascular disease, diabetes, fatty liver, hyperuricemia, and hyperlipidemia. Perhaps, the most compelling is the recent report that that there is increased risk of CVD mortality with increased intake of added sugar across quintiles. These data are of concern because the average level of added sugar consumption in the United States, 15% of daily calories, is associated with an 18% increase in risk for CVD mortality. Furthermore, recent data demonstrate that the consumption of beverages providing 0%, 10%, 17.5%, or 25% energy requirement from HFCS results in dose-dependent increases of established lipid/lipoprotein risk factors for CVD within 2 weeks in young men and women, and another study found that unfavorable changes in lipid risk factors are sustained after 6 months of sugar-sweetened beverage consumption of $1\,l\,day^{-1}$. These intervention studies provide mechanistic support for the epidemiological results.

Fructose Consumption, Body Weight, and Obesity

With the increase in fructose intake, primarily as sugar-sweetened beverages, occurring coincidently with the increase in the prevalence of being overweight and obesity at the end of the twentieth century, it is important to examine the evidence that links fructose consumption and body weight gain. In epidemiological studies, consumption of larger amounts of soft drinks and sweetened beverages is associated with greater weight gain in women and increased energy intake and higher body mass index in children. In experimental studies when fructose or sucrose-sweetened beverages are added to the diet, subjects do not compensate for the additional energy provided by these beverages by reducing energy intake from other sources, and total energy intake increases. In a controlled clinical trial, the consumption of large quantities of glucose- or fructose-sweetened beverages, along with the participants' usual diets ad libitum, led to a weight gain of about 1.5 kg after 8 weeks' exposure to either sugar. Of metabolic significance in this trial, intraabdominal (visceral) fat increased in those consuming fructose-sweetened beverages, while the increase in body fat was primarily due to an increase in subcutaneous fat in those consuming glucose-sweetened beverages.

Weight gain and the lack of caloric compensation when consuming sweetened beverages containing fructose may be explained by the lack of a significant effect of fructose ingestion on the secretion of hormones involved in the long-term regulation of food intake. Comparison of the effects of ingesting fructose- and glucose-sweetened beverages with meals indicates that fructose ingestion results in smaller increases in blood glucose and insulin concentrations following the meals. In addition, circulating leptin concentrations are lower, and the normal suppressive effect of meal consumption on ghrelin concentrations is attenuated with fructose beverages. Glucose, insulin, leptin, and ghrelin are all involved in the long-term control of food intake and body weight regulation through the central nervous system. Since these key signals are absent or reduced when fructose is consumed compared with equicaloric amounts of glucose, chronic consumption of a diet high in fructose could contribute, along with high levels of dietary fat and inactivity, to increased energy intake, weight gain, and obesity.

Inborn Errors of Fructose Metabolism

Several genetically based abnormalities in the fructose metabolism have been described in humans. Fructokinase deficiency

leads to high levels of fructose in the blood and urine. In the absence of fructokinase, fructose can be metabolized to fructose-6-phosphate by hexokinase (EC 2.7.1.1), although at a low rate. Consequently, no serious health problems are associated with this abnormality. In fact, fructokinase knockout in mice leads to attenuation of the metabolic dysregulation associated with high-fructose diets.

The aldolase A, B, and C enzymes (EC 4.1.2.13) catalyze the reversible conversion of fructose-1-diphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, and deficiencies in the A and B enzymes have been identified. Aldolase A is expressed in embryonic tissues and adult muscles. Possibly owing to the importance of this enzyme in fetal glycolysis, its deficiency results in mental retardation, short stature, hemolytic anemia, and abnormal facial appearance. There is no known treatment for aldolase A deficiency. Aldolase B is expressed in the liver, kidneys, and intestines, and a deficiency of this enzyme is more common and can be exhibited at first exposure to fructose during infancy or can have its onset in adulthood. Upon ingestion of fructose-containing foods, vomiting and failure to thrive are apparent. Hypoglycemia (in some cases severe), increased blood uric acid, and liver dysfunction also occur. While this disorder can be treated effectively by eliminating sources of fructose, sucrose, and sorbitol from the diet, completely eliminating these ubiquitous dietary components can be quite challenging. However, left untreated, severe organ damage can occur, presumably caused by the accumulation of fructose-1-phosphate, which not only is possibly toxic to liver and kidney cells but also results in the depletion of the cellular phosphate pool.

Deficiency of fructose-1,6-bisphosphatase (EC 3.1.3.11) is also considered a genetic disorder of fructose metabolism. This enzyme has a critical role in the enzyme complex regulating glycolysis and gluconeogenesis. Deficient individuals exhibit hypoglycemia, acidosis, ketonuria, hyperventilation, and often hypotonia and hepatomegaly. The urinary excretion of many organic acids is altered; notably, urinary glycerol is elevated and is useful in the diagnosis of this disease. The treatment includes avoidance of dietary fructose, sorbitol, and prolonged fasting.

D-Glyceric aciduria is caused by D-glycerate kinase (EC 2.7.1.31) deficiency. Only ten cases have been documented with symptoms ranging from none to metabolic acidosis, failure to thrive, psychomotor retardation, spastic tetraparesis, and seizures. The absence of significant symptoms in some suggests that this enzyme deficiency is essentially benign, and other associated enzyme deficiencies may underlie the more severe symptoms.

Summary

Fructose, a monosaccharide, is found in the food supply in fruits, vegetables, syrups, and sweeteners, usually paired with another monosaccharide, glucose. Consumption in Western diets is often higher than the recommended intakes. Excessive intake can lead to metabolic dysfunction and increased risk of chronic disease. The manifestation of metabolic dysfunction associated with fructose or HFCS consumption may be influenced by gender, the amount consumed, the food matrix in which it is consumed, and other dietary factors such as the energy level of the diet or nutrients or bioactives coingested with fructose. More research is needed to determine factors that exacerbate or mitigate the metabolic dysfunction associated with overconsumption of fructose or HFCS.

See also: Beverage: Health Effects; Glucose: Metabolism and Regulation; Obesity: The Role of Diet; Sucrose: Dietary Importance; Sucrose: Properties and Determination; Sugar Alcohols; Sweeteners: Classification, Sensory and Health Effects.

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Relevant Website

www.sugarscience.org – SugarScience Initiative.

Fructose: Sources, Metabolism, and Health

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Sources and Production

Fructose, from the Latin meaning fruit sugar, is a six-carbon sugar made by plants and has the same chemical formula ($C_6H_{12}O_6$) as glucose but is an isomer of that compound, with fructose having two hydroxymethyl groups, while glucose has one hydroxymethyl group. Although this is often represented in molecular drawings as occurring at the end of each linear molecule, the cyclic carbon ring is the more common form of both sugars. All plants in which fructose is found also synthesize glucose in varying proportion so that sugarcane and/or beet sugar contains sucrose, which is a disaccharide formed from equimolar amounts of fructose and glucose bound by a glycosidic linkage. Sugarcane is grown in warm climates and sugar beets in temperate and cooler areas, but both are used to produce the same sucrose. Other plants contain varying ratios of fructose to glucose, ranging from 3:1 in agave nectar to 2.5:1 in grapes, 2:1 in apples or pears, and 1:1 in dates and honey (which is made by bees from nectar of flowers). Some plants also synthesize small amounts of other sugars. So, commonly consumed fruits and their juices are the major dietary sources of fructose in excess of glucose.

Industrially, fructose is produced by isomerization of glucose derived usually from cornstarch and less so from sucrose. Pure crystalline fructose is used primarily as a specialty sweetener in that it is generally used in small amounts in any product. Even when part of a mixture of sugars, fructose provides other important functional properties to foods such as browning upon heating via the Maillard reaction, depression of freezing point, or being a humectant to keep baked products moist. There is considerable misunderstanding about the fructose content of high-fructose corn syrup (HFCS), which is discussed in the succeeding text in detail. Suffice it to say here that HFCS is usually 55% or 42% fructose with most of the remainder glucose plus small amounts of other sugars. The higher fructose content corn syrup is used for beverages and the lower content mostly for processed foods and baked products.

Patterns of Consumption

Isolated sugars are a relatively recent addition to the human diet. In the distant past, sporadic availability of fruit was the main source of concentrated sugars. Some estimates of the Paleolithic diet suggest that $\sim$20 g day^{-1} of fructose were consumed, but based on ripeness of various fruits and the fact that hunter-gatherers were often hungry, seasonal intake could have been several times that amount. In industrial societies, consumption of sugar was only a few kilograms per person per year until the mid-1800s when it began to increase linearly until a peak around 2000, with slight declines in some countries since then. Generally, the countries that ate more sugar recently have cut back, while other countries that consumed less have increased their intake.

Fructose is 20–40% sweeter than sucrose and is the sweetest of caloric sweeteners. Pure fructose is rarely used as a sweetener by itself. Ironically, it is commonly used in 'health foods' such as yogurt in the United States because health-conscious consumers want less added sugar but do not purchase such foods unless they are sweetened to accustomed tastes. At one time, it was thought that fructose was better for diabetics than sucrose since it did not raise blood glucose or require insulin for its metabolism by humans and crystalline fructose was sold in supermarkets for that purpose. However, fructose is no longer recommended as a preferred sugar for diabetics even though its intake is associated with lower glycemic response because there is increased risk of lactic acidosis, and the high cost of crystalline fructose prevented its incorporation into mainstream food products. Such advice was replaced by recommendations for moderate consumption of sucrose with recent emphasis on restriction of total carbohydrates for diabetics.

In the United States and a few other countries, commercial food processors use HFCS as a replacement for sucrose in many products so that the availability of sucrose and HFCS is approximately equal in the United States. The other nations are primarily Canada, Mexico, Hungary, Slovakia, Belgium, Bulgaria, South Korea, and Japan. Among these countries, the United States consumes about 25 kg year^{-1} per capita, Hungary about 15 kg year^{-1}, and the rest between 5 and 10 kg year^{-1}. Increases in use among these countries have come at different times and in many, quite recently. Few other countries have intake of HFCS greater than 1 kg year^{-1}. Despite the name HFCS, the most commonly used form of this sweetener, for soft drinks, contains 55% fructose and 42% glucose with the remainder other sugars; another type of HFCS for use in solid foods has 42% fructose. The name was coined to distinguish HFCS from regular corn syrup, which is comprised of 100% glucose. Although when HFCS was developed in the late 1960s, it was less expensive than sucrose whose price was twice the world average in the United States and remains so, the cost difference between the two sweeteners has been negligible for a number of years. HFCS is a liquid that is easier and less expensive to handle and move around a factory via pipes than is crystalline sucrose, which is very hydroscopic, leading to caking and increased labor expenditures.

Fructose is normally consumed covalently linked with glucose as sucrose or as monosaccharides in solution as HFCS, reflecting a general pattern of excessive sugar consumption by Americans; similar levels of sugar are consumed in New Zealand, Brazil, Argentina, Australia, Mexico, and Canada. Intake was almost 120 g day^{-1} for all people ages two and over in 2009–2010 according to the US National Health and Nutrition Examination Survey. The most significant sources of fructose, and all sugars, are soft drinks; sweetened fruit drinks; fruit and fruit juices; grain-based desserts like cookies, cakes

pies, or donuts; frozen desserts such as ice cream and frozen yogurt; candy; and sweetened ready-to-eat cereals. While the Dietary Guidelines for Americans distinguish between naturally occurring and added sugars, there are no metabolic differences between them; the emphasis on reducing the amount of added sugars is to encourage consumption of high micronutrient density foods, which will, in turn, be lower in energy content.

Availability, Absorption, and Metabolism

Fructose is highly available from almost all foods in which it is found, with the possible exception of some nuts, which contain trace amounts. As a monosaccharide, fructose is absorbed directly into the bloodstream from the small intestine as are glucose and galactose. If sucrose is ingested, contact with the brush border of intestinal epithelial cells results in immediate cleavage of most sucrose into its constituent monosaccharides via sucrase. While the traditional view of gastrointestinal physiologists has been that splitting of sucrose at the membrane of the epithelium is 100% efficient, recent studies make it clear that some sucrose is absorbed intact and is excreted in the urine, making urinary sucrose a potential biomarker for sugar consumption.

Intestinal epithelial cells contain the transporters GLUT-2 and GLUT-5; the former actively transports all monosaccharides, while the latter is specific for fructose and is dependent on a concentration gradient. Ingestion of fructose and glucose in roughly equal proportions, as is the most common scenario, facilitates uptake of both sugars. This is particularly relevant for study of fructose malabsorption. It is likely that the condition is overdiagnosed because free fructose in sufficient amount and form is rarely, if ever, encountered in a typical diet. When a 25 g bolus of fructose is used in a drink to evoke breath hydrogen, about half of healthy subjects exhibit evidence of malabsorption; when a 50 g dose is administered, the prevalence rises to 80%. The use of unrealistic amounts of fructose in solution to elicit microbial fermentation to breath hydrogen (which may not correlate with symptoms) is not a logical diagnostic pathway. This is not meant to minimize the fact that there are undoubtedly a large number of individuals who have gastrointestinal symptoms after consumption of large amounts of fructose. An excess of fructose over glucose is more likely to elicit symptoms of malabsorption. Hereditary fructose intolerance, resulting from mutations in the aldolase B gene, is quite rare with a prevalence of 1 in 20 000–30 000 people. While it is unclear what the true rate of fructose intolerance is in the general population, individuals with functional gastrointestinal disorders display a high rate of intolerance to fructose with half that group reporting symptoms. Well-controlled studies have shown a higher rate of intolerance to fructose than malabsorption, suggesting that symptoms are not entirely due to passage of unabsorbed fructose into the large intestine where it is fermented by the microflora.

Once absorbed, glucose is utilized by all cells of the body and can be stored as glycogen in the liver and muscles. In contrast, fructose is metabolized primarily in the liver and its uptake there depends on GLUT-2. An often unappreciated fact is that a considerable amount of fructose is converted to glucose by hepatocytes; isotopic tracer studies indicate that 30–50% conversion occurs in the liver within a few hours of consumption. This is postulated to occur via the reversible reaction between fructose 6-phosphate and glucose 6-phosphate; the latter can be used to produce glucose or be incorporated into glycogen (Figure 1).

Fructose is phosphorylated by fructokinase as a first step in its metabolism. Fructokinase exists in two isoforms – A and C – with the A isoform found in many tissues including the liver, muscles, intestines, and kidneys, while the C isoform is exclusively in the liver. At least half the metabolism of fructose occurs in the liver but certainly not all. Fructose 1-phosphate is acted upon by aldolase B to produce dihydroxyacetone phosphate (DHAP) and glyceraldehyde. The latter is acted upon by triokinase to make glyceraldehyde 3-phosphate (G3P). DHAP and G3P derived from fructose enter the pathway for glycogen synthesis just the same as intermediate products of glucose breakdown. However, these metabolites can also enter into the pathway for synthesis of triglycerides. It is well established that high fructose consumption can result in excessive pyruvate production, which yields increased acetyl CoA, leading to enhanced lipid biosynthesis. The same intermediates can also contribute to glycogen synthesis, and there are no known sensing mechanisms that allow the body to distinguish between metabolites derived from fructose versus glucose.

One measure of metabolic efficiency is diet-induced thermogenesis (DIT), which is the increase in energy expenditure following consumption of a meal. This reflects the costs of metabolism of a nutrient and has the practical value of decreasing the amount of energy available for weight gain. Most studies found a significant ~60% increase in DIT following ingestion of a meal containing fructose compared with one having glucose as the main sugar. These studies require abnormally large doses of the sugars so it is not clear what relevance they have to normal dietary patterns, but the increased thermogenesis indicates that fructose is used less efficiently than glucose for energy-yielding processes, likely because fructose is metabolized primarily in the liver, while glucose is oxidized in most cells. While these results reflect differences in consumption of pure fructose and glucose, there is no published information on the metabolic conversion of fructose to glucose, DIT, or other relevant pathways when mixtures of the two sugars alone or as sucrose are consumed. Although storage of sugars as fat is quite inefficient compared with storage of dietary lipids as fat, the increase in total sugar consumption throughout the twentieth century was followed by a decline in physical activity and accompanied by increased energy intake from all macronutrients that was not needed for maintenance, so the carbohydrates were converted to stored fat. At the present time, there are conflicting data on whether fructose intake leads to increased de novo lipid synthesis, but the metabolic biochemistry suggests that this process should be less efficient than the same process from glucose. Although there are claims on both sides of this issue, a meta-analysis comparing controlled feeding trials of fructose versus any other carbohydrate (which was either glucose or starch metabolized to glucose) found no effect of fructose in eucaloric trials and a small but significant increase in weight in hypercaloric experiments that could not be distinguished from the general effect of excess

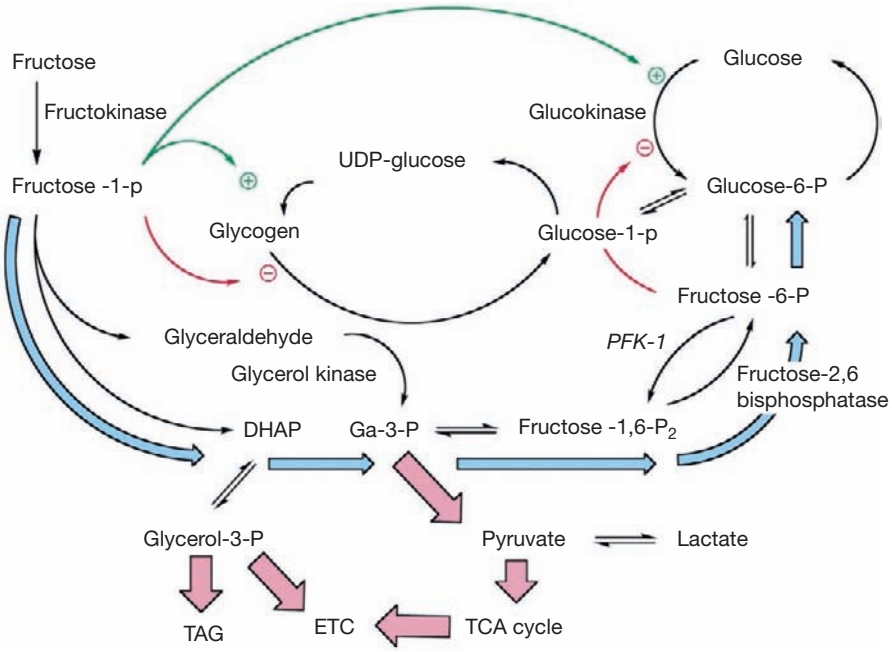

Figure 1 Overview of the major aspects of hepatic metabolism of glucose and fructose. Key points emphasized in the text of the original article: the two sugars converge at the level of the triose-phosphates (dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-P (Ga-3-P)). Conversion of fructose to the triose-phosphates is unidirectional, but fructose 1-phosphate is a positive effector of glucokinase and regulates glycogen synthesis by activating the synthase and inhibiting phosphorylase. The last effect may be different between species. Light blue arrows show the path of gluconeogenesis from fructose leading to glucose-6-P, which, in turn, can produce glucose or be incorporated into glycogen. Reproduced with permission from Feinman, R. D. and Fine, E. J. (2013). Fructose in perspective. *Nutrition & Metabolism* **10**, 45.

energy. Perhaps the most important conclusion of the meta-analysis was that the existing trials were of moderate to weak quality because they were too small and too short in time and had a number of other weaknesses in the methodology.

Health Effects

It is ironic that many research nutritionists do not know the nutrient content of many foods and many food scientists are not aware of the metabolic consequences of consuming common foods or nutrients. Since little pure fructose is consumed, there has been undue focus on HFCS, in part because of a lack of knowledge of its fructose content by nutrition researchers. As noted earlier, the name was coined to distinguish HFCS from conventional corn syrup rather than to indicate that it is unusually high in fructose in comparison with sucrose. In fact, much of the current interest in fructose is derived from the claim that the increase in obesity in the United States closely followed the increased use of HFCS. However, this claim did not take into account the fact that HFCS simply replaced sucrose in many processed foods nor was actual consumption by individuals considered so that the analysis suffered from what is termed the 'ecological fallacy' by attributing average population exposures to affected and unaffected individuals. More so, since the late 1990s, the use of HFCS has fallen in the United States, while obesity rates have plateaued for some groups and increased for others. But perhaps most importantly, obesity increased at a faster rate than in the United States in many countries of the world where virtually no HFCS is consumed, negating a causal relationship of that sweetener with weight gain.

In addition, there is also a small but vocal group of researchers who repeatedly publish comparisons of very high (greater than the 99th percentile of intake)-dose fructose with the same amount of glucose and suggest their results have important implications for what people should eat. There has also been concern expressed that the monosaccharides glucose and fructose in solution may have different effects from those of sucrose in a drink. However, particularly at acidic pH, sucrose dissociates into its constituent sugars within a few weeks so that most soft drinks actually contain more of the monosaccharides than the disaccharide when consumed. Furthermore, since almost all sucrose is rapidly split to its constituent sugars upon contact with the brush border of the intestinal epithelium, the body only sees mixtures of the two monosaccharides. There are, however, conflicting data that calories from liquids are not compensated for by reduction of other foods as is often observed with solid foods. Again, the existing controversy may have diverted researchers from studying true links to overconsumption.

Despite the information in the preceding text, researchers continue to study large doses (often $\geq$75 g or ~15% of daily energy requirement) of fructose in comparison with glucose in solution, neither one of which is consumed except under laboratory conditions. It is clear that consumption of the two sugars together has different effects, which are ameliorated in comparison with exposure to one of the monosaccharides, but a 75 g bolus of sugar in isolation is an obviously unusually heavy load that exceeds normal intake of either

monosaccharide from all sources in 1 day. Such doses of fructose or glucose result in increased endogenous lipid synthesis, increased plasma triglycerides, increased adipose tissue deposition, and weight gain. What these studies generally demonstrate is that fructose can be worse than glucose under specific conditions. However, these effects are generally not observed in a diet without excess calories and the two sugars are virtually never consumed in isolation. Adequate physical activity seems to protect against both short- and long-term effects of excessive fructose intake as does concomitant intake of fish oil, indicating the interactive effects of other dietary components and physical activity on health end points. The main issue with using such high amounts of fructose is that it violates the basic maxim of toxicology in that the dose makes the poison. Virtually all nutrients, essential or not, cause toxicity at high enough levels.

High levels of fructose feeding have been claimed to cause insulin resistance, the metabolic syndrome, and increase lipid synthesis. The main problem for interpreting such claims is the diet design in which extremely high levels of fructose are used and in which a very high-carbohydrate diet is fed. Therefore, it is difficult, if not impossible, to distinguish between a fructose-specific effect and the influence of a high-sugar or high-carbohydrate diet from the experiments in the literature. This is particularly problematic in people who are in excess energy balance, have increased baseline abdominal obesity, and/or have little habitual physical activity. All these conditions relate to the propensity to respond with appropriate levels of insulin postprandially and the insulin sensitivity of peripheral cells.

One of the shortcomings of virtually all controlled feeding studies on fructose is the relatively short time period of 4 weeks or less. If there were certainty that such time periods represented steady state for the metabolic consequence of interest, there would not be a controversy. But it is unknown if such values are in steady state or on a trajectory to worsen or toward adaptation. In other words, we have no experimental evidence to guide experimentalists in what is an adequate, let alone optimal, time frame for feeding studies of fructose. Most of the literature seems to be based on finding a statistically significant effect, rather than what is biologically meaningful.

It is well established that increasing total dietary carbohydrate results in elevated blood triglycerides, particularly when the carbohydrates are simple sugars. While some individual studies claim that fructose feeding is particularly hypertriglyceridemic, a meta-analysis of studies in diabetics indicates an effect in type 2 diabetics only, of fructose only in comparison with starch, only in studies that fed 60 or more grams per day, and in those studies of less than 4 weeks in length. Also, fluid fructose appeared to have greater negative effects than fructose from solid foods. At the same time, most of these studies reported that serum cholesterol concentrations were reduced in subjects given fructose.

Substitution of fructose for glucose or starch leads to reduced postprandial glycemic responses. The European Food Safety Authority (EFSA) currently allows a health claim in which food companies substitute fructose for sucrose or glucose because this reduces postprandial blood glucose responses even though the EFSA advisory panel acknowledged that high intakes of fructose may lead to elevated blood lipids, insulin resistance, and increased abdominal fat deposition.

A large number of animal studies have been conducted using fructose as the sole carbohydrate source, often at 65–70% by weight, particularly in rodents. These studies often report adverse health effects, including increased hepatic lipid synthesis and deposition, increased serum lipids, and increased weight gain. The rationale for such an approach is that untoward effects are magnified with this diet so that lower amounts must also be harmful. However, it should be obvious that reproducing an abnormal physiological response in an animal to an unrealistic diet means nothing for public health recommendations. The primary value of animal studies is in understanding mechanisms of developing disease, thereby providing a means to intervene in the process. In regard to fructose, there appear to be differences in hepatic metabolism of fructose between rodents and humans and between isolated hepatocytes exposed to fructose and the response of hepatic metabolism in a human fed fructose so the potential relevance of any of these studies is debatable.

Emphasis on potential adverse health effects of fructose has diverted scientific and public attention away from the real issue of excessive consumption of total sugars that usually represent excess calories, whether the sugar is added to foods or inherent in them. There is no credible evidence that obesity induced by overconsumption of sugar differs from that induced by excessive intake of fat, but it is also unclear if excessive sugar intake in weight-stable individuals has the same degree of effect as seen in overweight or obese subjects. Unfortunately, there is no information on either an optimal or maximal level of sugar that can be consumed without untoward health risks. It is clear that modern levels of sugar consumption are many times what we were exposed to for thousands of years. But simply because a lower amount of sugar was available in the distant past does not indicate that low level is what we should be eating now. The belief that humans have not evolved to metabolize the array of foods and nutrients that we have available now has support from some nutritionists and much of the general public but is clearly dismissed by evolutionary scientists who accept the fact that humans like other animals and plants continue to evolve as their environment changes.

See also: Carbohydrate: Digestion, Absorption and Metabolism; Fruit Juices; Fruits of Tropical Climates: Biodiversity and Dietary Importance; Maillard Reaction; Sweeteners: Classification, Sensory and Health Effects.

Further Reading

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Fruit Juices

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Introduction

Industrial production of fruit juices started in the United States around 1870. Concord grapes were juiced from their vines, and the juice was filtered, bottled, and then pasteurized in a hot water bath. The European fruit juice industry began in 1896 with the publication of detailed instructions for commercial production of unfermented fruit juices. That same year, apple and grape juices were first produced commercially in Berne, Switzerland. In the United States, nationwide expansion of commercial juice production developed about 1929, when economic necessity forced American consumers into an era of home canning, which included fruit and vegetable juices. During the 1930s, the development of pasteurization techniques and availability of lined cans afforded products of superior juice quality and an era of unprecedented growth for the industry.

During the 1940s, frozen single-strength fruit juices (i.e., juices produced by direct pressing of fruit, rather than by reconstitution of concentrated juice) were introduced to consumers in the United States. Frozen concentrated fruit juices were first sold in that market during 1945–46. In the same decade, orange concentrate, hot packed into small flat glass bottles, was made available in the United Kingdom to supplement vitamin C intake for children and pregnant women.

The development of freezing technology had a dramatic effect on the industry and was responsible for most of the early growth of the citrus industry in Florida and other producing countries such as Brazil. Frozen concentrated fruit juices for reconstitution and frozen concentrated orange juice (FCOJ), in particular, have now become the principal juice products of international commerce.

Single-strength juices, now known in the industry as 'not from concentrate' (NFC) or 'direct' juices, have entered the market more recently. These have been developed in two principal forms. Freshly pressed juices that have had no further processing other than chilling and have a very short shelf life of a few days are now available. Fresh juices that have been flash pasteurized and packaged in a very clean, but not aseptic, environment to give them a chilled shelf life of several weeks are also widely sold.

Single-strength and reconstituted juices and juice drinks can be packed under aseptic conditions in laminated containers and polyethylene terephthalate (PET) bottles. These can be stored at ambient temperatures for convenience and economy for producer and consumer alike. Aseptic packaging is also used for more economical storage and transport at ambient temperatures. The process is suitable for bulk single-strength and concentrated juices although freezing at about −18 °C remains the principal means of transporting and storing concentrated bulk juices.

Brazil and the United States are the main producing countries for orange juice. Germany, eastern European countries, and, increasingly, China are the principal sources for apple juice. Smaller producers are located more widely around the globe. The main consumer markets are the United States and Europe with increasing consumption in Asian countries.

Raw Materials

The early juice industry was often a salvage operation. The principal source of raw material was misshapen, poorly colored, or skin-blemished fruit unsuitable for the fresh, canned, or frozen fruit market. For many years now, raw materials have been selected and bred especially for suitability for juice production.

Variety and maturity of fruits are important factors affecting suitability for juice production. For some juices, such as citrus or grape, only a few varieties have traditionally been used to provide a distinctive flavor or to ensure freedom from unwanted characteristics. Developments in processing technology now enable the use of a wider range of varieties. Bitterness and other unwanted characteristics can now be removed or reduced by processing. When a range of varieties is available, blends are often used to achieve uniformity of flavor or analytic characteristics. Blends are sometimes perceived as having a better flavor or commercial advantage than a single varietal juice. For example, fruit having a high sugar content may be mixed with fruit from a different variety to achieve a desired sweetness/acidity balance. This balance, known as the sugar–acid 'ratio' is based on °Brix readings, that is, %w/w sugar, obtained with a refractometer, divided by the value for total acidity (%w/w) obtained by titration.

Field staff are often employed to advise growers on agronomic aspects of fruit production. The application of sprays to the growing crops and the coordination of harvesting with factory production schedules are important. Payment for raw materials is frequently based on specifications that are either official government grades or stated market standards. There is a smaller but significant demand for fruits and juices certified as organic or fair trade. To assist in juice authentication, an independent organization has been set up in Europe to inspect, validate, and register processing factories worldwide.

Composition and Fruit Juice Authenticity

Juice composition varies widely from country to country, with fruit variety and season. Much work has now been carried out, mostly in Europe, to develop sophisticated methods for authenticating fruit juices. Commercial databases can now be used to authenticate the principal fruit juice types from almost anywhere in the world. Fruit juice standards, particularly relating to juices reconstituted from concentrates, are now published by the German Industry (The RSK system), AIJN – European Fruit Juice Association, and Codex Alimentarius.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00334-2

Adulteration of fruit juices, particularly orange and apple, has been a significant issue in the past because of relative commodity price differentials between fruit juice solids and sucrose. Natural sugars are a major constituent of most fruit juices. The partial substitution of juice solids by sucrose or other bulk sugars from a variety of different sources can be an attractive commercial proposition. This can be achieved without affecting obvious juice quality. The addition of excessive amounts of pulp wash (see section '**Citrus Processing**') to citrus juices has also been commonplace despite being prohibited in some countries. The addition of malic acid to bland apple juice concentrates with low levels of acidity has also been used. This produces the more valuable high acid juice. In the case of more expensive red fruit juices, adulteration can take many forms including dilution with less expensive juices and by the addition of sugars.

A wide range of analytic techniques is now available to detect various forms of adulteration. The German 'RSK' system largely requires a fruit juice to meet a number of analytic characteristics. In other countries, new methods have been developed. For the detection of carbohydrate addition, measurements of their stable isotopes of carbon, hydrogen, and oxygen and comparison against a wide database have proved to be a powerful tool. The detection of small amounts of oligosaccharides that are characteristic of the added carbohydrate can also be useful in identifying adulteration. Other techniques that have proved valuable for specific applications include the examination of the amino acid and anthocyanin profiles of juices and the detection of added malic acid to apple juice. Many countries now use an industry-based voluntary screening of fruit juices sold to consumers. This is supported by inspection of producer factories and an audit trail of the supply chain for products. The net result has been a significant reduction in instances of adulteration although isolated occurrences still arise.

Manufacturing

Figures 1 and 2 set out the typical steps in the generalized production of citrus and soft fruit juices. Equipment and processes vary significantly for the type of fruit to be handled. In most major processing countries, harvested fruit is transported to the adjacent plant. For the production of 'freshly pressed juices' in some countries, including the United Kingdom, fruit is imported for local and often small-scale pressing and packaging.

Citrus Processing

Fruit of appropriate quality is washed, sometimes with a detergent, to remove soil and debris. It then passes over roller brushes and is rinsed and drained. Manual or automatic graders remove unwholesome fruit as the fruit passes over roller conveyors. It is then segregated automatically by size prior to juice extraction. Several types of extractors are available. Sizing and grading of each load of fruit, based on fruit color, size, shape, and weight, is increasing. This can be carried out by computer-controlled equipment. There is potential for total electronic grading and thus complete automated process control.

In one type of extractor (e.g., FMC Technologies Inc.), the fruit is located between two cups having sharp-edged metal tubes at their base and metal fingers that intermesh. The upper cup descends and the many fingers on each cup interweave to express the juice. The tubes cut holes in the top and bottom of each fruit. On further compression, the rag, seeds, and juice sacs are compressed into the bottom tube between the two plugs of peel. A piston moves up inside the bottom tube forcing the juice through perforations in the tube wall. A simultaneous water spray washes the peel oil expressed during extraction away from the peel as an oil–water emulsion. Peel oil is recovered separately from the emulsion usually by a centrifugal process. Each extractor unit has several cups of a single size, and in a typical factory installation, banks of extractors process different fruit sizes.

In another extractor type (e.g., Automatic Machinery and Electronics Inc. (AMC)), the individual fruits are cut in half as they pass a stationary knife. The halves are oriented in a vertical plane, picked up by synthetic rubber cups, and positioned across plastic serrated reamers. These revolve in a synchronized carrier in a vertical plane. As the fruit halves progress around the extractor turntable, the rotating reamers exert increasing pressure and express the juice. Oil and pulp contents in the juice increase with greater reaming pressure. Recoverable oil is removed in a separate step prior to juice extraction. Needle-sharp spikes prick the peel of the whole fruit, releasing oil that is washed away with water and recovered from the oil–water emulsion. Although most citrus juice extraction equipment is based on these two types of extractor, other types are used. Plants similar to that originating in the United States are now made in other countries.

During extraction of citrus juices, it is desirable to have as gentle an extraction pressure as possible. There should be minimal contact time between juice and pulp to reduce the amount of bitter substances expressed from the peel into the juice. The amount of suspended solids and pulp in citrus juice is controlled in a subsequent separation. This is effected by a finisher where a screw action is used to force juice through a rotating perforated screen and separate the larger pulp particles from juice. The oil level in the juice may, if required, be adjusted by vaporizing under a vacuum. Separated pulp is washed and finished several times to produce a solution. This may then be either added back to the juice to increase juice yield or concentrated to produce the so-called pulp wash solids. This material, also called water extract of orange solids, can be used as a base for cloudy beverages. The addition of pulp wash has been the subject of much debate in the issue of juice authentication. It is now generally regarded as acceptable if added back in an 'in-line' process in small ($<5\%$) quantities. Its addition offline is not generally now acceptable for authentic juice. Empirical methods for measuring pulp wash content have been developed using UV spectrophotometry.

Orange juice from the Navel varieties containing limonin and pulp wash solids can be excessively bitter. A commercial process developed in about 1992 for debittering these liquids involves the use of an ion exchange resin.

Concentration and Aroma Recovery

Most orange and grapefruit juice sold and transported internationally is frozen as concentrate, usually at 60–65 °Brix. Citrus

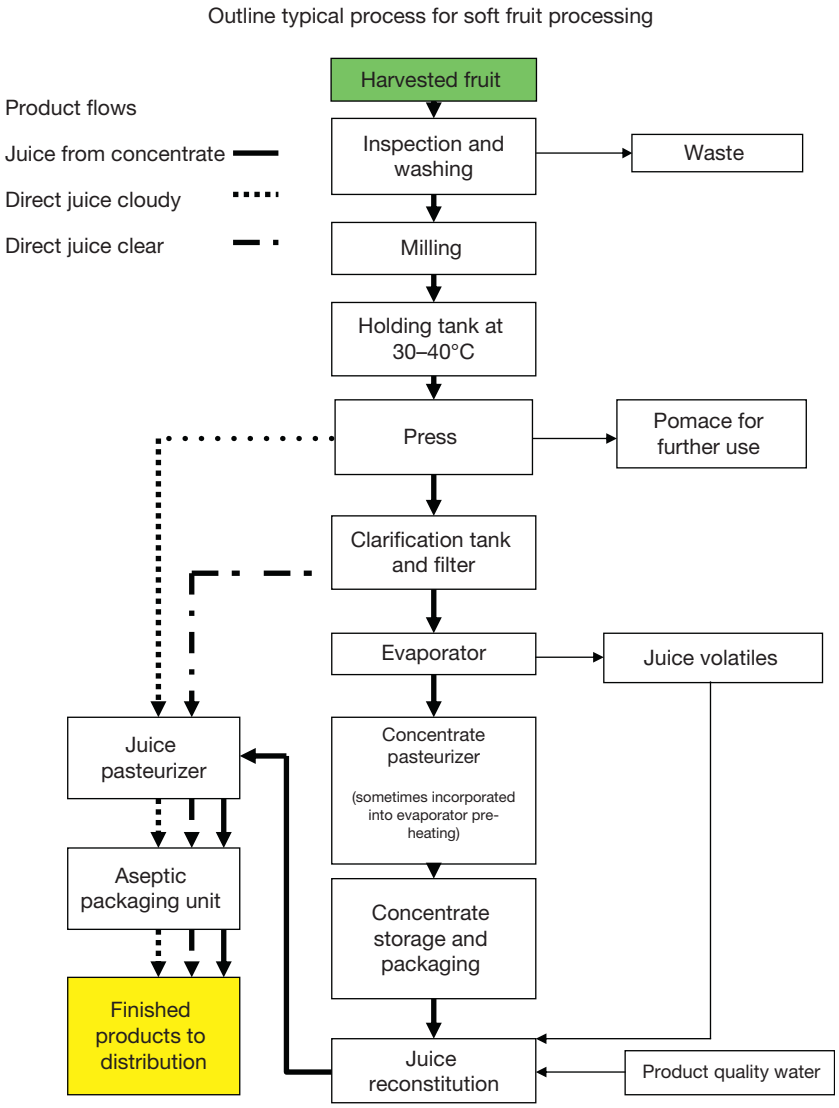

Figure 1 Outline typical process for citrus fruit processing.

juices are almost exclusively concentrated in a thermally accelerated short-time evaporator (TASTE). These evaporators are of optimized efficiency for the amount of water removed per kilogram of steam used. For example, in a seven-effect evaporator, 2.77 kg (6.1 lbs) of water is removed for each kilogram of steam used. Each effect consists of a bundle of tubes, with falling films of juice flowing inside. These are mounted inside a cylinder so that steam flowing into the first-effect cylinder can condense on the outside walls of the tube bundle. Heat transfer vaporizes a portion of the water contained in the juice. The hot water vapor thus produced is used to heat juice in the next effect and so on. This results in successively lower temperatures with each consecutive effect. The temperature will be around 98 °C in the hottest effect and reaches ~45 °C in the last effect. Concentrated juice is discharged from the last effect to a vacuum flash cooler yielding a product temperature of about 13 °C. The whole system is under vacuum with a progressively higher vacuum in each successive effect. This is sufficient to cause water evaporation.

During water removal, much of the desirable aroma characteristic of the citrus juice is carried off with the vapor, especially in the early effects. Essence recovery units are used routinely to condense the volatile flavor substances. This water-phase essence, industrially termed 'volatiles,' and an oil-phase essence oil are produced. These valuable commercial flavor fractions are saved separately. They either may be added back later to concentrated juice or may be used as separate flavor ingredients. Concentrate is cooled quickly and stored at or below −10 °C in bulk vessels. Most of the single-strength orange juice marketed worldwide is prepared by reconstitution and water-phase essence, and oil fractions may be added during this process. This restores flavor lost during concentration. Blended concentrates are reconstituted to between 11.2 and 11.8 °Brix juice. They are then pasteurized and chilled prior to packaging for the retail market. Aseptic packs of laminated material, glass, or PET bottles of 1.0 l or more are the most popular. In European markets, there is a code of practice requiring reconstituted orange juice to have a minimum of

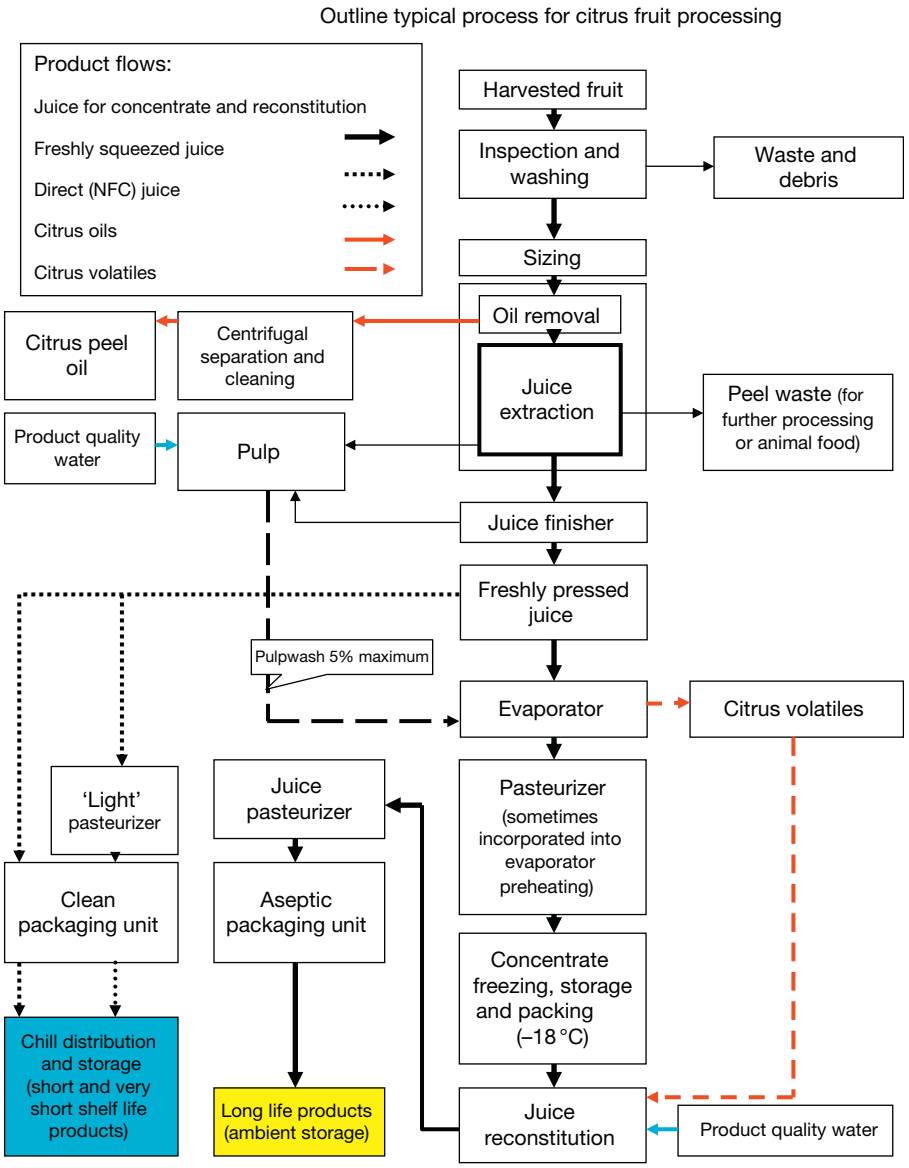

Figure 2 Outline typical process for soft fruit processing.

11.2 °Brix. In countries that follow the WHO Codex Alimentarius guidelines, the minimum must be 11.5 °Brix.

A newer citrus juice concentration process, requiring minimal heat treatment, has been applied commercially in Japan. Pulp is separated from the juice by ultrafiltration (UF) and pasteurized. Separated juice containing the volatile flavorings is concentrated at 10 °C by reverse osmosis. Concentrate and pulp are recombined to yield a product at a concentration of 42–51 °Brix. The flavor of this concentrate has been judged superior to that of commercially available concentrate and close to that of fresh juice. Clarified citrus juices (other than lemon or lime) are not regarded as acceptable to most consumers in Europe or the United States.

A further process using freeze concentration has also been developed and has been used on a very limited commercial scale. Fresh juice is cooled to below 0 °C, and the water crystals that form are removed and washed by more juice. This process is repeated four or five times until the residual juice has a concentration of around 45 °Brix. The juice is said to have flavor characteristics that resemble those of the fresh juice. The economics of the process are said not to be favorable for most markets and applications.

Pasteurized single-strength orange juice, NFC, often called premium orange juice in the United States, is increasing in sales worldwide. There has been a corresponding reduction in the retail market share of FCOJ in the United States. NFC juice requires special extraction and storage conditions to ensure high quality. Juices are expressed with extractors designed to produce juice having low oil content (<0.035%). Juice must be chilled and stored aseptically or in frozen blocks to provide a year-round supply to packers.

Unpasteurized orange and grapefruit juices also are increasingly popular products in the United States and Europe. Fruit must be processed under strict conditions of hygiene and the

juice kept just above 0 °C during its approximately 14-day shelf life. Year-round juice supply relies on the importation of fresh fruit from different growing countries according to the harvest season. It is often difficult to maintain consistency of flavor and Brix–acid ratio.

Lemon and Lime Juice

Lemons and Persian or Key limes can be extracted using similar FMC- and AMC-type extractors described earlier in the text. The juice can be concentrated in a TASTE evaporator, an APV Crepaco, Inc. evaporator, or other types of evaporators. An alternative method of manufacture for clarified lime juice to supply the UK demand for lime juice cordial is often used. Whole limes are crushed in a screw press and the resulting juice and pulp is allowed to stand for several days. This permits natural pectolytic enzyme activity to clarify the juice. Peel and pulp residues are then processed to recover lime oil. Separated juice can be concentrated in a suitable evaporator or by subjecting it to UF. This latter technique produces a six to seven times concentrate with an outstanding pale color. Lemon juice is widely produced in several European countries, notably Italy, Spain, and Greece as well as Argentina. It is generally available as either a clear or cloudy product. Lemon and lime juice concentrates are still available in Europe preserved with about 1500 mg kg^{-1} sulfur dioxide. These are typically used in making concentrated (dilute to taste) soft drinks. Clarified lime juice, made by mixing direct juice with filter aid prior to passing through a filter press, is the one clarified citrus juice that is a significant article of commerce in the United States. The pasteurized bottled juice is popular for drink mixes, punch bases, and fountain drinks.

Citrus Comminutes

In the United Kingdom in particular, there is a demand for comminuted citrus products, especially made from oranges and lemons. These are for use in the manufacture of 'whole fruit drinks' for consumption after dilution. Such products rely on the combination of juice, peel, and oil constituents to provide a much stronger flavor than can be obtained from juice alone. Early comminutes were produced by simply pulping the whole citrus fruit. Later developments take the various components from citrus processing including concentrated juices. They are then blended in required proportions, milled, and pasteurized. Citrus comminutes are often stored with added chemical preservatives for later use in the manufacture of soft drinks. UK regulations permit the limited carryover of sulfite preservatives from comminutes into appropriate end products.

Soft Fruit Processing

Fruit used for juicing must be sound, free from gross damage or contamination. The presence of mold or rot can lead to tainted juices or, especially in apples, the presence of toxic mold metabolites such as patulin. Mechanical harvesting can cause bruising and skin penetration, which may cause off-flavors or allow the growth of pathogenic bacteria such as *E. coli*. Mechanical harvesting is used extensively on apples and black currants, whereas berries are mainly picked by hand. Most temperate deciduous fruits, including apples, pears, grapes, berries, black currants, and red currants, are processed in a similar way. This typically involves milling the whole fruit and then pressing the juice from the resulting mash. Equipment for processing stone fruits such a peaches or apricots requires an additional stage to remove the stones prior to pressing. Many tropical soft fruits require specialized processing stages.

Milling

All fruit used for juicing must be thoroughly washed. A variety of methods are used to break the fruit to release juice. These methods depend on the structure of raw material, the clarity desired in the final juice, enzymatic discoloration, and breakdown of pectin.

Milling is essential for relatively hard fruit such as apples, whereas grapes and berries need only light crushing. Some soft fruits, such as raspberries and strawberries, are often received frozen and must be defrosted prior to processing. Grapes and other fruit harvested in clusters are mechanically destemmed prior to crushing. For crushing, a series of intermeshed arms mounted on two cylinders shred the grapes as they pass through to produce a mash.

The pulp from stone fruit, such as apricots, mangoes, and peaches, must be removed in a way that prevents damage to the stone or pit. These often contain components that can affect juice flavor and stability during storage. A mill for stoned fruit contains hard rubber-lobed wheels that rotate together to force the fruit down and strip most of the pulp from the intact stone. An alternative process for peaches involves halving and pitting the fruit in a Filper twist pitter. This pulps the fruit halves then heats the pulp to 82 °C in a steam cooker. A fruit disintegrator is then used to produce a puree, which is put through a finisher. Such pulpy fruit purees can be treated with sugar syrup to produce nectar drinks typically containing 25–50% juice.

Enzyme Treatment of Pulp

Because of their cell structure, some fruits are not easily juiced by milling and pressing. Commercial pectolytic enzymes are widely used to break down cell structure and degrade pectins in milled fruit to improve juice extraction efficiency. When clear products are required, enzymes may also be used to treat juice following pressing.

Press Aids

Fibrous materials are sometimes added to the fruit pulp to facilitate juice liberation or to provide a stable structure to withstand the application of pressure. Several types of press aid are used, for example, diatomaceous earth, coarse wood flour, rice hulls, and bleached or unbleached wood pulp. Diatomaceous earth and wood flour may be sprinkled over press cloths or added to the pulp before the cloths are filled for hydraulic pressing. This addition makes discharge of the spent cakes easier and also increases the yield of juice.

Pressing

There are a variety of fruit presses. Some presses are more suitable for one type of fruit than for others, but most can be used for any fruit with varying degrees of success. Some presses require the use of a press aid such as diatomaceous earth or wood chips. Total juice yield from the original fruit defines press efficiency. Additional yield can be obtained by adding a small amount of water to the press cake and pressing again. The denomination as 'pure juice' of such products is not permitted in many countries.

A simple rack-and-frame press uses heavy nylon or polypropylene woven cloth positioned in a wooden frame inside a rack. A measured amount of apple or other fruit mash is added from a hopper above the frame. The mash is leveled and the edges of the nylon cloth are folded over the mash to encase it and create a 'cheese.' The frame is removed, and a second rack is placed on top of the first cheese. This process is repeated until a stack of cheeses is prepared. A hydraulic ram then applies gradually increasing pressure on the stack and expresses the juice. A high yield of juice (~80%) can be obtained and no press aid is required. Because this process is labor-intensive, it is mostly used for small farm and pilot-plant operations.

The Bucher-Guyer horizontal rotary press is a highly automated batch process machine that requires no press aid. The press consists of a hydraulic piston inside a horizontal large rotating cylinder. Many flexible rods covered with a knitted synthetic fabric are arranged between piston face and the cylinder end. The rods have serrated surfaces to allow juice that passes through the fabric to flow to the discharge ends. Hydraulic pressure is applied to the piston for a preset time, which is retracted, and the cylinder is rotated to break up the press cake. This cycle is repeated several times before the press cake is removed from the cylinder and the press is cleaned. Juice yield for this horizontal rotary press can reach 84%; with secondary water addition, it may be increased to 92%.

The Stoll press uses a horizontal hydraulic ram and a chamber with screens on the sides that holds the mixture of fruit mash and press aid. Hydraulic pressure is applied to the mash first at 2750–3440 kPa (400–500 psi) and then at 17 200–20 600 kPa (2500–3000 psi) for preset times. Pomace is removed after each cycle. One person can operate several presses at once, each with a throughput of 4–5 ton fruit per hour.

A belt press commonly uses two belts of woven material arranged in a horizontal plane. Fruit mash is deposited on, and spread out evenly between, the two belts that converge and press the mash between them. The belts wrap around a series of successively smaller cylinders or pass between rollers (less efficient) for the pressing action. Juice yield for the wraparound belt press is typically 72% and increases up to 92% with secondary water addition.

Concentration and Aroma Recovery

Concentration of juice from soft fruit is best carried out using an evaporator that causes as little thermal degradation as possible. Equipment that permits recovery of volatile materials important to the aroma of the fresh fruit is preferred. Evaporators that use a high temperature for a short time and operate under a vacuum, such as the APV Crepaco falling film plate evaporator or the Alfa Laval centrifugal evaporator, are most commonly used. The TASTE, almost universally used for citrus, is rarely if ever used to concentrate soft fruit juices.

Multiple-effect falling film vacuum plate evaporators use a plate-and-frame heat exchanger to heat the juice to the required temperature for boiling under partial vacuum. The plate evaporator consists of multiple gasketed plates arranged in series within a compact frame. As feed juice flows in a single pass as a falling film, the water is vaporized on contact with steam-heated plates. It is discharged as a liquid–vapor mixture to a large volume separator. The concentrated juice flows out the bottom and the water vapors are used to heat the next effect or are condensed. For maximum energy efficiency, the warm concentrate stream is used to preheat feed juice. Falling film plate evaporators are especially useful for fruit juice that is heat-sensitive. This is because of short heat contact time, rapid start-up and shutdown times, and minimum juice loss from a small liquid hold up volume. Such evaporators can be used by a single processor for multiple fruit crops during the year.

Centrifugal evaporators are also suitable for concentrating fruit juices because they have very low heat contact times. The expanding flow centrifugal evaporator contains a stack of rotating, steam-filled hollow cones. As juice is sprayed onto the inside of the cones near the apex, centrifugal force spreads juice across the inner surfaces of the cones. The resulting concentrate is forced to the stationary walls of the evaporator and collected. Steam condensate on the outer surface of each cone is similarly forced out by centrifugal force. Thus, the heating surface in contact with the product is constantly heated with hot dry steam. Juice concentrate and steam condensate are continuously removed. Juice can be concentrated in one pass with a few seconds contact time.

There are two principal methods available for aroma recovery. In one method, a portion of the water is stripped from the juice prior to concentration. It is then fractionally distilled to recover a concentrated aqueous essence solution. Apple juice requires 10% water removal, peach 40%, and Concord grape 25–30% to remove volatile flavor as an essence. Fractional distillation affords an aqueous essence flavor solution of 100–200-fold strength. This means the essence is 100–200 times more concentrated in flavor than the starting juice. A second method of essence recovery is to condense the volatiles from the last effect of the evaporator; they are enriched in volatile flavor components.

Clarification

The appearance of fruit juices varies widely. Citrus and pineapple juices typically have high contents of insoluble pulpy solids and are almost always sold as cloudy juices. By contrast, cranberry, grape, and many apple juices have little or no suspended solids and are translucent. In some juices, such as citrus, many of the flavoring constituents adhere to the pulp, and the clarified juices lack flavor. Preservation of the natural pectin content by rapid heating and cooling of juice as soon after pressing as possible is necessary to inactivate the natural enzymes. This has been found to be particularly important in providing a stable cloud. The size of the suspended particles is another factor influencing flocculation. Particle size may be adjusted by screening, homogenization, colloid milling, or selective filtration.

In the production of opalescent or natural-type apple juice, ascorbic acid is often added to the fruit pulp before pressing or to the juice as it comes from the press. This is to retain more of the apple flavor. Ascorbic acid addition and pasteurization of the juice as soon as possible after pressing minimize oxidation of polyphenols, which cause browning and contribute to pulp flocculation.

In the production of clear juices, natural pectin present is degraded with added pectolytic enzymes. This facilitates filtration and minimizes subsequent precipitation or flocculation of pectic substances. Other sources of colloidal haze in filtered juices are polymerized tannins or compounds formed by the combination of tannins and proteins. Haze also may result from the denaturation of proteins. To overcome tannin precipitates, gelatin is sometimes added to the juice during enzyme digestion. The amount and type of gelatin added to form a floc must be carefully controlled because an excess of gelatin may cause a haze. Laboratory procedures are frequently conducted by adding graduated amounts of gelatin and tannin to samples of juice and noting the amounts required for greatest clarity. Such tests are frequently necessary because of the variation in the natural tannin and protein contents of the juice. The use of kieselsol, a synthetic silica sol, in combination with gelatin, or pectinase enzyme mixtures, increases the speed of the clarification process. This makes in-line clarification possible.

Treatment of juice with commercial pectolytic enzyme preparations may be carried out at low temperature for 15–18 h or at 40–50 °C for 1 or 2 h. If a higher digestion temperature is used, the juice may be clarified by rotary vacuum precoat filtration. This is followed by a small-cartridge or leaf-type filter to remove any filter aid. If juices are digested at low temperatures, sufficient time usually elapses for flocculation of the solids. Clear juice is then decanted and passed through a pressure-leaf filter. The filter aids used are diatomaceous earth of fine to medium grades depending on the clarity desired and the nature of the solids. Completeness of pectin destruction is of importance in maintaining good filtration rates and filtrate clarity.

The use of UF membranes is becoming increasingly popular for clarification of apple juice and is also used for lime juice. All particulate matter and cloud is removed, but enzymes pass through the membrane as part of the clarified juice. Thus, pasteurization before UF treatment to inactivate enzymes prevents later haze formation from enzymatic activity. Retention of flavor volatiles is lower than that using a rack-and-frame press but higher than that using rotary vacuum precoat filtration.

Natural style juices especially apple juice that contain cloudy components are increasing in popularity because these retain more fresh flavor if processed carefully. Optimum processing conditions involve chilling the fruit to 4 °C before milling. The addition of 500 ppm ascorbic acid to retard browning, pressing under nitrogen, and flash pasteurization of the juice as quickly as possible are also employed.

To avoid formation of tartrate crystals in bottled grape juice, filtered juice is stored at −2 °C, or just above the freezing point, for several weeks. This permits the deposition of potassium acid tartrate crystals. Prior to storage, juice is pasteurized at 88 °C and then cooled in storage at −2 °C before it is pumped into clean storage tanks. Unless care is taken in the sanitation of the pasteurization and cooling equipment, piping and tanks, yeast growth may cause alcohol production and/or generation of off-flavors.

Pasteurization

Fruit juices generally have pH values of 4 or less and spoilage is usually limited to yeast and mold growth with occasional bacterial contamination. Flash pasteurization minimizes flavor changes owing to heat treatment. For flash pasteurization, several methods are used. The most common is a plate heat exchanger with a separate holding tube. Typical flash pasteurization conditions for microbial stability are 85 °C for 15–30 s although a higher temperature, typically 95 °C, is usually regarded as necessary for complete enzyme inactivation. NFC juices are typically subjected to a temperature of between 90° and 95° for a few seconds. Juice treated in this way is then packed and stored cold but will normally have an extended but still limited shelf life.

An alternative, and widely used procedure, is to package juice cold in suitable containers. Pack and contents are then heated with hot water sprays in a tunnel, typically in three stages: preheating to around 40–50 °C, pasteurizing at around 70 °C for around 20 min, and then cooling.

Alternatively, filling the container with hot juice pasteurizes the package and the product, thus delivering product pasteurization. This technique carries a higher risk of spoilage of end products and is limited by the type of packaging available.

Aseptic packaging in flexible laminated multilayer plastic containers has become popular worldwide. The packaging materials used are economical and the product can be stored at ambient temperature. In such packaging, the juice must be heat-pasteurized, cooled to prevent heat damage to the package, and introduced into the package in a sterile atmosphere. The packaging material is fed from a reel and presterilized with hydrogen peroxide or other approved sterilant solution. It is then formed into the required shape and filled in the aseptic chamber. Aseptically packaged juice drinks typically have a shelf life of at least 12 months in temperate ambient conditions.

Alternative methods of pasteurization that are under development include irradiation with high-energy electrons or x-rays and the use of high-pressure pasteurization. In the latter process, batches of product in flexible packages are placed in a pressure vessel and subjected to pressures of around 600 MPa. Current processes are labor-intensive and limited by batch operation. Consequently, the process is limited to premium products where, for freshly squeezed product, shelf life can be extended by two or three times that of unprocessed juice.

Chemical Preservatives

The use of any chemical preservative in pure fruit juices is no longer permitted in most countries although labeling the product as a 'juice drink' can sometimes provide a loophole to facilitate the use of permitted chemical preservatives. Most governments require a listing of ingredients, including preservatives, on the labels of food and beverage containers. The most common preservatives used in beverages

containing fruit juice are the potassium and sodium salts of sorbic, benzoic, and sulfurous acids and also dimethyl dicarbonate ('Velcorin').

Freezing Preservation

Fruit juices can be preserved by freezing or dehydration but these processes are now little used for consumer products. Freezing is however widely used to ensure stability during transport of bulk juices.

Tropical Fruit

A wide range of tropical fruit juices are now available although blends are often more popular than individual juices. Pineapple juice has been the most popular tropical fruit juice consumed in the United States and Europe for many years. It has long been a by-product of the pineapple canning industry although now whole fruit for processing is grown in many countries including Thailand and Kenya.

Other tropical fruit juices are often considered too pulpy or are judged to have harsh or exotic flavors, which make 100% juice products unacceptable to many consumers. They are sometimes more acceptable as juice blends or nectars containing 25–50% juice or as blended fruit drinks where their strong flavors are diluted or modified. 'Smoothie' juices and drinks often based around banana pulp are also increasing in popularity.

Fruit Juice Drinks

Commonly, a juice drink contains around 5–10% of the named fruit juice, with added sugar or other sweetener, an acidulant, flavoring, and other minor ingredients as appropriate. The 1990 US Federal Nutrition and Labeling Act requires the declaration of juice content so that the consumer can make a more informed choice. Similar regulations now exist in EU countries. With cocktails and juice drinks, added sugars, acids, flavorings, colorings, and nutrients can be used to provide a wide variety of stable products of uniform quality and wide consumer appeal.

Notes

Mention of a trademark, trade name, or proprietary product is for the purposes of example or identification only and does not imply a warranty or guarantee of the product by the author or publisher. Other products may also be suitable.

See also: Acids: Natural Acids and Acidulants; Apples; Ascorbic Acid: Properties, Determination and Uses; Authenticity of Food; Berries and Related Fruits; Citrus Fruits; Food Fraud; Fruits of Tropical Climates: Biodiversity and Dietary Importance; Mangoes; Packaging: Aseptic Filling; Pasteurization: Effect on Sensory Quality and Nutrient Composition; Pasteurization: Principles and Applications; Spoilage: Yeast Spoilage of Food and Beverages; Yeasts.

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Fruits of Tropical Climates: Biodiversity and Dietary Importance

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Introduction

Tropical climates offer a wide array and diversity of fruits that have rich nutraceutical values and organoleptic qualities. These fruits are preferred by consumers mainly owing to their seasonal availability, traditional value, and rich mouthfeel. However, still, there are many diverse ranges of fruits that have remained underexplored for commercial utilization. The underexplored fruits are those that can be considered 'underexploited' (eaten by tribal or indigenous populations) or otherwise consumed by animals and birds in the wild. Exploring underutilized fruit crops can be opined to meet the nutritional necessities of underprivileged population and contribute towards enhancing the economic security and satiability in the developing region.

By and large, consumers prefer to eat fresh fruits to fulfill their daily requirements of energy and to gain adequate amounts of essential minerals and vitamins for a healthy living. However, available concentrations of bioactive compounds required to cater an individual's needs can depend on the fruit's maturity, harvesting stage, season, and the processing and preservation conditions adopted. Moreover, concentrations of the bioactive compounds and nutrients (amino acids, dietary fiber, and phenolic compounds) can vary within a fruit (e.g., in pulp, seeds, and pericarp), too. Recent years have witnessed a rapid expansion of ongoing research on fruit consumption, and it is a well-accepted fact that regular intake of fresh fruits can lower various risks associated with chronic diseases (cancer and cardiovascular diseases) and aging.

The climate in tropical countries is always warm and humid throughout the year. There are more than 100 tropical countries in the world, which have their own unique biodiversity encompassing a rich arena of fruits and vegetables. Some of the tropical countries like India, Malaysia, Australia, Thailand, Brazil, Vietnam, Cambodia, Kenya, Chile, Egypt, and Indonesia have already tried to tap the potential market of tropical fruits and have gained significant foreign exchequer, too. These countries have set up appropriate strategies to gain high exporting potential. The global production market of tropical fruits varies depending on the demand of consumers.

Tropical fruits can be categorized either as major or minor fruits. Some examples of major tropical fruits include mango, papaya, jackfruit, pineapple, dates, sapota, jambu, guava, and melons, while that of minor fruits include the exotic ones such as dragon fruit, durian, rambutan, passion fruit, star fruits, lychee, breadfruit, longan, noni, pulasan, and ackee. Most of these fruits are either consumed directly or converted into food products such as juice, jams, and jellies. Of late, fruit powders are also gaining wide popularity among consumers and manufacturers owing to the retention of natural flavor, color, and nutrition. An overview on the potential use of underutilized tropical fruits is depicted in Figure 1.

Agreeably, majority of the underexplored tropical fruits do not have much scientific information, and there is a major gap in scientific knowledge. Based on this, in the preceding text, we have attempted to highlight some of the most popular and exotic 'minor fruits,' which have some scientific evidence and information pertaining to their source, production, consumption patterns, and potential health-promoting effects.

Berberis (Family: Berberidaceae; *Berberis vulgaris*, *B. microphylla*, and *B. heterophylla*)

Berberis are a group of small berries, sweet and sour in taste. They are widely distributed in tropical regions (Asia, South America, and Africa) and scantly in temperate regions (Europe) of the world. There are several species of berberis; however, only a few of them are known to have food value. Commercially, these fruits are not popular but are consumed by some indigenous people in the growing regions. Ripe fruits are highly acidic (owing to vitamin C content) and impart a tingling sensation upon consumption. In the Indian subcontinent, fruits are used for culinary purposes (as a substitute for tamarind), while in Iran, they are used as flavoring agents during meat preparation. Some species (*B. heterophylla*, *B. microphylla*, and *B. darwinii*) are popular in Chile and Argentina, wherein they are used in the preparation of jellies and jams.

Most of the *Berberis* species have been used in traditional folk medicine, especially in the Afro-Asian regions. Phytochemicals such as isoquinoline alkaloid berberine (useful in treating a wide array of diseases including that of cancer) have been isolated from berberis. The nutritional and antinutritional qualities of the pulp and seed portions of edible berberis (*B. asiatica*; *B. aristata*; *B. lycium*; *B. jaeschkeana*; *B. pseudumbellata*) from the western Himalayas (India) have been reported to contain high fiber, protein, fat, tannins, and phytic acid.

Breadfruit (Family: Moraceae; *Artocarpus altilis* Parkinson Fosberg)

The breadfruit plant is a small evergreen tree with large leaves, widely distributed in the tropical and pantropical regions. The fruit has gained popularity in New Guinea, Indonesia, the Philippines, Taiwan, Hawaii, Malaysia, and India for its multiple culinary uses. A mature tree can yield more than 200 fruits in a season. Fruits are oval or oblong in shape (size 3–6 in., weighing ~1.5–2 kg) and covered with a thick pericarp (spines) and have latex exudates upon maturity. Fruit latex has medicinal value. Unripe fruits are green-colored while ripe are with yellow tinge (yellow color is due to the presence of papayotin and artocarpin enzymes). Consumers prefer to

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00337-8

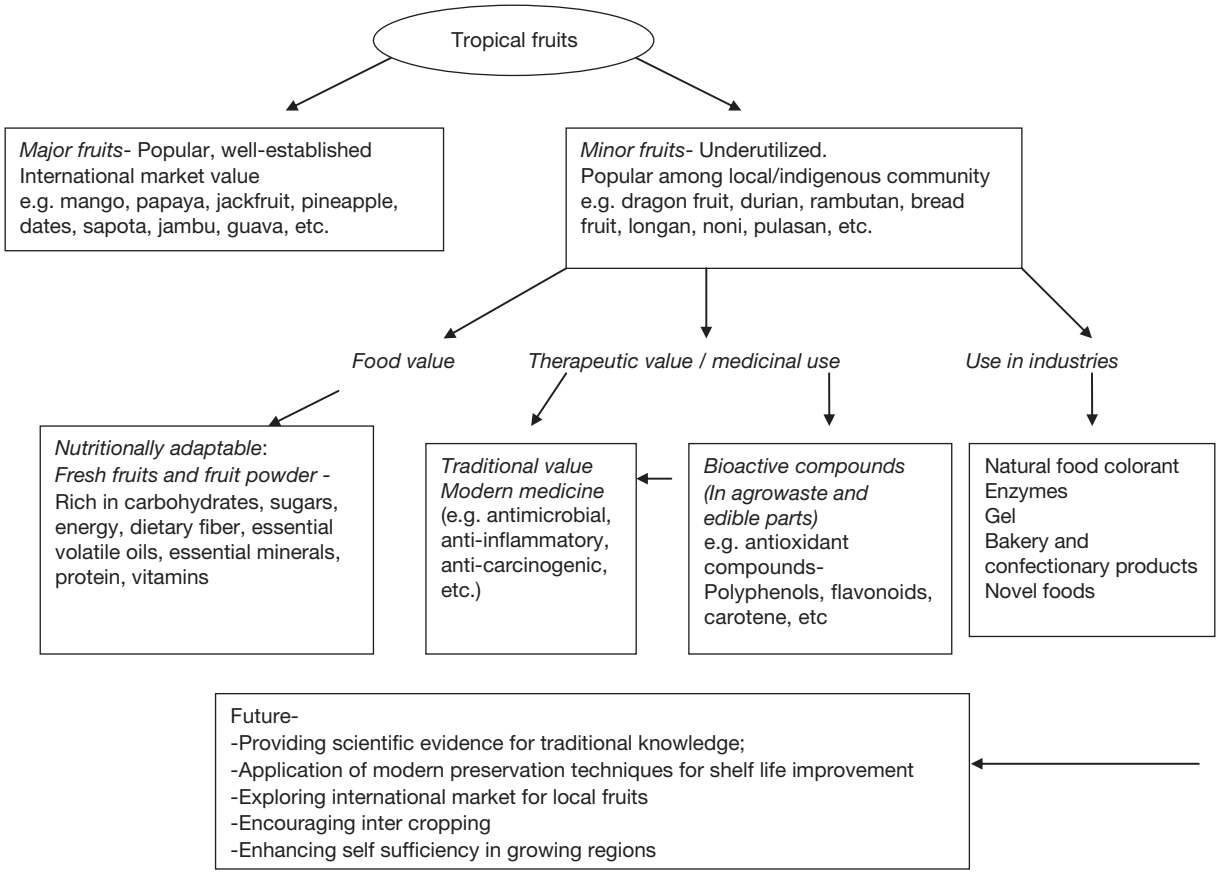

Figure 1 An overview on the potential uses of underutilized tropical fruits.

eat green and raw breadfruit flesh as vegetable. Seeds, though big, are less preferred to be eaten.

Fruits are rich in starch (~25%), and many starch-based food products are prepared by boiling, roasting, or baking. Raw breadfruits are prepared as curries, fermented and mashed with coconut milk, followed by baking in banana leaves. Nutritionally, breadfruits are rich in carbohydrates, fiber, protein, and essential minerals such as calcium and iron and ascorbic acid. Scientific reports on the medicinal value are scarce on this fruit. However, some traditional knowledge is available wherein breadfruit latex is used to treat sprains and broken bones; toasted flowers are used to treat toothaches (https://ntbg.org/breadfruit/uses/tree1.php; asses date: 18 February 2014).

Cashew Apple (Family: Anacardiaceae, *Anacardium occidentale* L.)

A small evergreen tropical tree (growing to 25–30 ft) produces highly valuable cashew nuts and the cashew apple. This tree has its origin in Brazil but is cultivated extensively in India and Vietnam. In fact, the cashew apple is botanically considered to be a false fruit (pseudocarp). The fruits are oblong-shaped (5–10 cm long) and brightly colored (yellow to orange and red, depending on the cultivar). Ripe cashew fruits are juicy with a unique taste (astringent) and a sweet and strong aroma. The cashew apple is consumed directly or is used to prepare alcoholic drinks (or rather country liquor such as that of feni).

Nutritionally, cashew apple juice is believed to be rich in vitamin C, carotenoids, and phenolic compounds. Traditionally (in Brazil), fruit juice is used as a remedy to treat sore throat and chronic dysentery. Alcohol produced from the ripe fruit is believed to reduce rheumatic pains. Cashew apple juice is reported to exhibit antioxidant, antimicrobial (antibacterial and antifungal), and antimutagenic and antitumor activities. High levels of carotenoids and ascorbic acid content in cashew apple products from Brazil have been reported. Besides, different varieties of cashew apples collected from different regions in Brazil showed them to be rich in β-carotene, β-cryptoxanthin, α-carotene, and 9-*cis*-+13-*cis*-β-carotene. Essential volatile oils from fruits of red and yellow cashew varieties showed oil from the red variety to be dominated by palmitic and oleic acids, while oil of yellow cashew was dominated by palmitic acid, furfural, 4-hydroxydodecanoic acid lactone, (*E*)-hex-2-enal, (*Z*)-hex-3-enol, and hexadecanol.

Durian (Family: Bombacaceae; *Durio* species; *Durio zibethinus*)

The durian plant is a native of the Malay Peninsula but is widely cultivated in Thailand, the Philippines, and Indonesia,

too. Durian fruits are seasonal and are popular as the 'king of fruits.' A matured tree bears fruit after 5–7 years of planting the seed, and fruits hung on a peduncle, which is firmly attached to the trunk and branch. The fruit is oblong to round-shaped, with the outer skin being light-green to brown in color (weighs between 1 and 3 kg, 15–16 cm in diameter, and 30–32 cm long). The yield per tree of durian is in the range of 15–800 fruits/season.

The fruit skin is covered with 1–2 cm long thick hexagonal spines and is very hard (55–66%). The edible part or aril is 22–30% encompassing big seeds (12–15%). Owing to a short shelf life, efforts are being made to commercialize durian flesh to convert into fruit powder. Even though farm varieties are popular, some of the wild cultivars (e.g., *D. kesudinarum* and *D. grandiflorus*) are also known to produce edible fruits, but these are less preferred owing to poor quality and taste. The durian fruit gets its popularity based on the distinctive odor, aroma, and taste.

Durian fruits are high in nutrition and bioactive compounds. The fruit pulp is a good source of protein, lipids, ash, fiber, and carbohydrates/sugars. Fatty acids contain the methyl esters of myristic acid, linoleic acid, oleic acid, 10-octadecenoic acid, palmitoleic acid, palmitic acid, and stearic acid.

Today, attempts are being made to prepare bakery products with durian flavor using the fruit powder. Novel bakery products such as jam, candy, ice cream, juice, and fruit shake are also being attempted. The fruit pulp is consumed directly or eaten with sticky rice. In order to retain the characteristic organoleptic qualities, in some countries, overripened fruits are fermented with salt or sugar to produce 'tempoyak' (acid-fermented durian) or 'lempok.' Dodol, a traditional sweet dish, is also prepared by cooking durian pulp with sugar. Some of the other traditional products like 'kuan' (pulp cooked with flour and sugar) are also prepared using durian fruit.

With regard to unripe or partially ripe fruits, they are boiled and consumed as vegetable, used to prepare soup, and converted into fruit bars or leathers. Reports are available wherein durian fruits have shown antiproliferative activities. Water-soluble polysaccharides from rinds of durian have been reported to have pharmaceutical value. The use of the durian fruit as a therapeutic agent to reduce cholesterol, in treatment of cardiovascular diseases, and as an antioxidant to prevent oxidative stress, obesity, and diabetic mellitus is also reported.

Rambutan (Family: Sapindaceae: *Nephelium lappaceum* L.)

This is a popular exotic fruit originated in Southeast Asia, extensively consumed and cultivated in Malaysia, Indonesia, Vietnam, the Philippines, India, Australia, and Thailand. There are several native and wild cultivars (more than 200) of rambutan. Fruits are oval in shape and possess smooth, hairy skin coverings, which are rather considered as soft spines. When raw (unripe), the hair color is green, but when ripe, they are bright red to maroon. However, depending on the cultivar, the color can be pink or yellow. The edible portion (flesh or aril part) is inside the skin (or hairy pericarp), which is juicy and sweet to acidic. This in turn encompasses a white translucent fleshy seed coat that covers an oblong-shaped seed. A single female plant (up to 20 m in height and 5–8 years old) can produce approximately 40–50 kg of fruit. As of today, Thailand is the leading exporter of rambutan (production rate: 6–7 lakh ton per annum), with earnings of more than 15 million US dollars.

Rambutan is consumed directly by opening up the peel. However, owing to huge amount of production, the fruits are processed into juice, jelly, and jam or are canned along with pineapple slices in a sweet syrup base. The seeds are poisonous but are consumed after roasting to treat diabetic patients, treat fever, and treat stomach upsets. In Malaysia, rind residues of the fruit are dried and used in local traditional medicine to treat common fever, flu, and dysentery. With regard to health-promoting activities and bioactive compounds, the pulp portion contained a significant amount of polyphenols. However, the rind is reported to have high antioxidant activity.

Rambutan seeds are reported to be rich in crude protein, fat, fiber, and ash. Seeds are rich in fatty acids such as oleic, arachidic, stearic, and palmitic acids. The presence of high amounts of arachidic acid is ought to contribute immensely to food and cosmetic applications.

Pulasan (Family: Sapindaceae; *Nephelium mutabile* Blume)

Pulasan is a tropical ornamental, drought-resistant fruit-yielding tree, encountered in regions of Southeast Asia (Malaysia, Thailand, the Philippines, and Indonesia). The fruits are seasonal and harvested twice annually. This fruit is confused with rambutan owing to its look-alike physical features (dark-red color, oval shape, and occurring in clusters). People prefer to eat the fruit directly, though fruit juice is also popular. The fruits are used as a flavoring agent in the preparation of ice creams, puddings, jellies, and jams. Traditional claims on body fat reduction upon consumption of this fruit and seeds do not have any scientific basis. The production rate and marketing statistics still remain undocumented. The flesh part is edible and is sweet and juicy. The seeds are edible and are eaten after roasting (tastes like almonds). A cocoa-like beverage is also prepared by the roasting of seeds.

Nutritionally, the fruits are documented to contain carbohydrate (16.6), fiber (0.5), ash (0.7), protein (0.9), and fat (0.1) (all in g/100 g) and vitamin C (10.80 mg/100 g) (http://www.nutriweb.org.my/cgi-bin/dbrecords.cgi?comp=Proximateandsubmit_do-=Viewandfood_no=106081andpart1=Raw+and+Processedandfname=Pulasanandgroup=Fruitsandmname=Pulasan, assessed date; 22 February 2014). Volatile constituents of the fruit contain aliphatic hydrocarbons such as pentadecane and aliphatic alcohols.

Mangosteen (Family: Clusiaceae; *Garcinia mangostana* L.)

Mangosteen, a native plant of Indonesia, is popular as the 'queen of fruits.' This is widely cultivated in Cambodia, India, Thailand, Malaysia, Sri Lanka, Vietnam, the Philippines, and Africa. The fruit contains maroon- to purple-colored thick skin

(pericarp or rind) with gummy latex. The edible portion (aril) consists of white flesh with tiny nonedible seeds. The edible portion of the fruit is sweet to sour in taste, juicy, and fibrous.

In traditional medicine, the fruits are recommended to be used to treat stomach ulcers and acidity. In addition, the rind portion is recommended to treat abdominal pain, diarrhea, inflammation, skin infection, and common wounds. Details of the processing of this fruit into food products are scarce. However, in local markets, mangosteen juice in a syrup pack is available. The mangosteen fruit encompasses rich amounts of phytochemicals and has high polyphenolic contents (phenolic acids, anthocyanin, proanthocyanidin, and xanthone) and cyanidin-3-sophoroside and cyanidin-3-glucoside. The rind portion of this fruit possesses antimicrobial, antioxidant, and antitumoral activities.

Nutritionally, the fruits contain 0.41 of protein, 17.91 of carbohydrates, and 1.8 of total dietary fiber (g/100 g of sample) and 2.9 mg/100 g of vitamin C (http://ndb.nal.usda.gov/ndb/foods/show/2348?qlookup=mangosteenandfg=andformat=andman=andlfacet=andmax=25andnew=1asses date: 23rd February 2014).

Identical to mangosteen, there is one more popular evergreen tree fruit referred to as the 'wild mangosteen.' This tree species is common in India, Sri Lanka, and other South Asian regions. The fruits are round to ovoid in shape (weighing 50–60 g) and are red- to maroon-(dark violet)colored when ripe, the pulp being sweet to sour in taste and astringent. Traditionally, juice prepared from fruit rind is believed to be of high medicinal value and is effective to treat digestive disorders, acidity, rheumatic pains, and constipation in humans. The rinds are sun-dried and are used in traditional food preparation (as acidulants) in parts of India to prepare sour curries and in the preparation of refreshing drinks.

Nutritionally, the rind portion contains 4% total sugars, 1.4% fat, 1% protein, and 1.7% tannins. The rind has a high antioxidant potential and contains anthocyanins such as cyanidin-3-glucoside and cyanidin-3-sambubioside. Dried rind is also reported to possess antibacterial and antifungal activities. Some of the bioactive compounds such as garcinol and isogarcinol have been isolated from the fruit. Fruit consumption is also linked with the prevention of leukemia cell lines (in humans) and possesses anticholinesterase and antiobesity activities.

Longan (Family: Sapindaceae; *Dimocarpus longan* Lour.)

The longan fruit is extensively cultivated in China, Indonesia, Malaysia, Vietnam, and Taiwan. The fruit is comparable with lychee fruits. The edible part (flesh) is inside a soft skin (pericarp) that is semitranslucent, juicy, sweet, and succulent with a pleasant aroma. The small, oval-shaped seeds are enclosed within the fruit flesh. The flesh portion contains polysaccharides such as D-galactopyranose, L-arabinofuranose, D-glucopyranose, and D-galacturonic acid. Phenolic compounds such as gallic acid, corilagin, flavone glycosides, and glycosides of quercetin and ellagic acids have been isolated from the pericarp. The pericarp, aril, and seeds exhibited antityrosinase, antioxidant, anticancer, and antiglycated activities. The pericarp portion also possesses antimicrobial (antibacterial and antiviral), anti-inflammatory, and anticarcinogenic properties.

Longan seeds though are nonedible (17%, w/w of fresh fruit); they are reported to be rich in polyphenols like gallic acid, grevifolin, corilagin, methyl brevifolin carboxylate, ethyl gallate 1-β-O-galloyl-D-glucopyranose, and 4-O-α-L-rhamnopyranosyl-ellagic acid and ellagic acid. Consumption of this fruit is reported to provide the benefits of antioxidant, antityrosinase, and immunomodulation activities. Longan fruit extract is also known to inhibit linoleic acid oxidation and free-radical scavenging activities against DPPH and hydroxyl radicals and superoxide anions.

Langsat (Family: Meliaceae; *Lansium domesticum* Corr. Ser.)

Langsat is a tropical, medium-sized, fruiting tree originated in Southeast Asia (Indonesia, the Philippines, Vietnam, and Malaysia). The fruits occur in clusters, are oval in shape (2–5 cm), and have a thin, golden-yellow-colored skin that can be peeled easily. The edible part is the inner juicy flesh (sweet and sour taste) that encompasses minute, flat, nonedible seeds (5–6 mm). The fruits are eaten raw without much processing. However, in local markets, fruit syrup is also popular, which is used to prepare refreshing juice. The actual production and marketing statistics of this fruit remain undocumented.

The outer skin part contains oleoresin, which is traditionally used in selected tropical regions to treat diarrhea. Seed extracts have insecticidal and antimalarial activities. Fruit extracts exhibited antityrosinase and antioxidant activity, too. The isolation of tetranortriterpenoids and triterpenoids from seeds has been linked with antimalarial activity against *Plasmodium falciparum*. The edible portion of the pulp is rich in carbohydrates, protein, fiber, and ascorbic acid.

Litchi (Family: Sapindaceae; *Litchi chinensis* Sonn.)

The litchi (or lychee) fruit is native to China and is extensively cultivated in warm tropical climates of Malaysia, Brazil, India, Bangladesh, Taiwan, and Japan. The fruits are similar to longan, with the edible part being the translucent aril, which are juicy and sweet to sour in taste. The pericarp is green when unripe and pink when ripe. The fruits are rich in phytochemicals, minerals, and dietary fiber. Litchi fruits are used by Chinese as a traditional remedy for a healthier heart, liver, and spleen.

Antioxidant activities in seeds are also reported. The seeds of litchi (*semen litchi*) are used to treat neuralgic pain. The pulp of litchi contains antioxidant compounds such as glycosides of quercetin and kaempferol. The pericarp encompasses antioxidant compounds (phenolic compounds, tannins, and anthocyanins) that exhibited high free-radical scavenging activity against DPPH radicals, superoxide anions, and hydroxyl radicals. In addition, the pericarp is reported to strongly hinder linoleic acid oxidation and show activity against hepatocellular

Table 1 Some reports highlighting the bioconstituents in selected tropical fruits

Fruit type	*Observations*	*Reference*
Berberis (*B. asiatica*; *B. aristata*; *B. lycium*; *B. jaeschkeana*; *B. pseudumbellata*)	High in fiber (pulp 7.0–8.1%; seeds 4.4–5.3%), protein (pulp 4.7–7.2%; seeds 5.9–8.5%), and fat (pulp 2.6–4.0%; seeds 4.6–5.3%) In seeds, tannin content varies between 1.4 in *B. jaeschkeana* and 2.4 mg/100 g in *B. asiatica* and phytic acid 2.2 in *B. lycium* and 9.9 mg/100 g in *B. pseudumbellata* In pulp, tannins ranged between 0.39 in *B. aristata* and 1.2 mg/100 g in *B. jaeschkeana* and phytic acid 0.37 (*B. asiatica*) and 6.0 mg/100 g (*B. pseudumbellata*)	Chandra et al.
Breadfruit (*Artocarpus altilis*)	Nutritionally, breadfruit contains carbohydrates (21.5–29.5%), fiber (1.08–5.5%), proteins (1.3–2.2%), lipids (0.2–0.86%), and ash (0.56–1.2%) and minerals such as calcium (0.05 mg/100 g), iron (2.4 mg/100 g), with ascorbic acid ranging between 15 and 30 mg/100 g	Morton
Cashew apple (*Anacardium occidentale* L.)	Cashew apple products such as concentrated juice, frozen pulp, nectar, ready-to-drink, and sweetened concentrated juice were found to contain ascorbic acid that ranged between 13.7 and 121.7 mg/100 g and total carotenoids that ranged from 8.2 to 197.8 μg/100 g	Assunção and Mercadante
	In: Different varieties of cashew apples collected from diverse regions in Brazil Identified compounds included β-carotene (16.6–67.9 μg/100 g), β-cryptoxanthin (7.7–64.4 μg/100 g), α-carotene (5.9–51.9 μg/100 g), and 9-*cis*-+13-*cis*-β-carotene (3.3–15.6 μg/100 g)	Assunção and Mercadante
	In: Red and yellow cashew varieties, the essential volatile oil in the red variety was dominated by palmitic (19.6%) and oleic (19.6%) acids, while oil of yellow cashew varieties was dominated by palmitic acid (11.4%), furfural (10.0%), 4-hydroxydodecanoic acid lactone (8.2%), (*E*)-hex-2-enal (7.2%), (*Z*)-hex-3-enol (6.2%), and hexadecanol (6.2%)	Maia et al.
Durian (*Durio* species: *Durio zibethinus*)	Durian fruits are high in nutrition and bioactive compounds. The fruit pulp is a good source of protein (1.47%), lipids (5.33%), ash (1.12%), fiber (3.08%), and carbohydrates (27.09%). The pulp contains methyl esters of myristic acid (2.52%), linoleic acid (2.20%), oleic acid (4.68%), 10-octadecenoic acid (4.86%), palmitoleic acid (9.50%), palmitic acid (32.91%), and stearic acid (35.93%)	Devalaraja et al. and Ho and Bhat
Rambutan (*Nephelium lappaceum* L.)	Fruit contains high polyphenols. However, the rind is reported to have a high antioxidant activity	Palanisamy et al.
	Rambutan seeds are reported to be rich in crude protein (7.80%), fat (40%), fiber (11.6%), and ash (1.22%). The seeds are rich in fatty acids such as oleic acid (40.3%), arachidic acid (34.5%), stearic acid (7.1%), and palmitic acid (6.1%). The presence of high amounts of arachidic acid is ought to contribute immensely to food and cosmetic applications	Zee
Pulasan (*Nephelium mutabile*)	Volatile constituents of the fruit contain aliphatic hydrocarbons (70.2%) such as pentadecane (61.4%) and aliphatic alcohols (19.4%)	Wong et al.
Mangosteen/Garcinia sp. family	Rind portion contains 4% total sugars, 1.4% fat, 1% protein, and 1.7% tannins. However, the seeds are good in triglycerides. The rind has a high antioxidant potential and is reported to have high anthocyanins (2.4%), mainly cyanidin-3-glucoside and cyanidin-3-sambubioside	Nayak et al.
Longan (*Dimocarpus longan*)	The flesh portion contains polysaccharides such as D-galactopyranose (33.7%), L-arabinofuranose (32.8%), D-glucopyranose (17.6%), and D-galacturonic acid (15.9%). Phenolic compounds such as gallic acid, corilagin, flavone glycosides, and glycosides of quercetin and ellagic acids have been isolated from the pericarp	Shi et al.
Langsat (*Lansium domesticum* Corr. Ser.)	The isolation of five tetranortriterpenoids and 11 triterpenoids from seeds exhibited antimalarial activity against *Plasmodium falciparum* (IC_{50} = 2.4–9.7 μg ml^{-1}) Besides, 1 g of edible portion of the pulp contains 9.5 g carbohydrates, 0.8 g protein, 2.3 g fiber, and 1 mg ascorbic acid. Fruit extracts exhibited antityrosinase and antioxidant activity, too	Saewan et al. and Morton
Litchi (*Litchi chinensis* Sonn.)	The presence of free, bound, and total phenols (range of 66.17–226.03, 11.18–40.54, and 101.51–259.18 mg of gallic acid equivalents/100 g, respectively) and the presence of free, bound, and total flavonoids in the range of 16.68–110.33, 10.48–22.75, and 39.43–129.86 mg of catechin equivalents/100 g, respectively, are reported	Zhang et al.

carcinoma and fat oxidation. The presence of free, bound, and total phenolics and total flavonoids has been reported.

In Table 1, some reports highlighting the bioconstituents identified in selected tropical fruits are depicted.

Future Perspectives

Majority of the edible tropical fruits possess rich nutraceutical value. Exploring these fruit crops can be opined to meet the nutritional requirements of the underprivileged population and contribute towards enhancing the economic security in the region. Besides, still, there are major gaps in the available database on the health benefits incurred on regular consumption of these fruits. Of late, fruit powders are also gaining wide popularity among consumers and manufacturers owing to the retention of natural flavor, color, and nutrition. This can be explored further to be used as a nutritional supplement, too. In addition, as the nonedible portions such as peels and seeds are also known to encompass rich nutraceutical value, further research studies are warranted in this direction.

See also: Berries and Related Fruits; Fruits of Tropical Climates: Dietary Importance and Health Benefits.

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Fruits of Tropical Climates: Dietary Importance and Health Benefits

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Introduction

Fresh fruits are an integral component of a balanced human diet in the tropical region. Though majority of the exotic tropical fruits are seasonal, their regular consumption is considered to provide rich health benefits. Traditional knowledge is available on the health-promoting effects on consuming certain fruits, thus supporting the need for enhanced intake of fruits. Scientific evidences and database information provide details of the traditional knowledge and clarify the claims made for the nutritional and therapeutic effects of the exotic fruits. Majority of the exotic fruits or their wastes have been reported to exhibit rich antioxidant, antitumor, and antimicrobial activities. Exotic fruits encompass rich amounts of phytochemicals such as carotenoids, polyphenolic compounds, vitamins, essential minerals, and fatty acids, which can efficiently reduce the incidence of chronic diseases and protect the human body against free radical-induced damages. Providing adequate information on bioactive compound composition in an exotic fruit not only is vital for research purpose but also can also be of use for local governments to recommend regular intake of a fruit as an additional supplement along with the normal food.

This article is in continuation with our earlier article and highlights more on the popular exotic fruits of the tropical and pantropical regions of the world. Focus is also laid on the biodiversity of the plant species, their composition, and the potential benefits incurred on regular consumption. Major challenges faced by the agro-based food industries with regard to production, marketing, and preservation of good-quality fruits are also discussed. In the following text of this article, we are highlighting on the remaining popular and exotic tropical fruits with relevance to their biodiversity and health-promoting effects.

Wolf Apple (Family Solanaceae, *Solanum lycocarpum*)

Wolf apples are smooth, globular berries (average 10 cm diameter) and are edible by both livestock and humans. Edible part is the flesh or pulp having several embedded seeds. Matured fruits are soft, aromatic, and sweet with skin and pulp being yellow in color (with red tinge when overripe). Generally, the fruit pulp is processed into jams.

With regard to nutritive value, these fruits are rich in carbohydrates, proteins, lipid, and ash with the absence of any dietary fiber. Fruits possess high antioxidant activities and are effectively used to treat hypoglycemia and dyslipidemia. Green fruits are rich in the steroid precursor 'solasodine,' and the fruit powder/flour is used to treat diabetic patients.

Rose Apple (Family Myrtaceae, *Syzygium malaccense* or *Syzygium samarangense*)

Rose apple is a common inhabitant of the Malaysian Peninsula and Indian subcontinent and parts of North Africa. Fruits are edible and bell-shaped (5–7 cm long, weighing around 35–36 g) with a unique flavor. Upon maturity, a single plant yields more than 700 fruits. Fruit skin (peel) is thin and occurs in different colors such as white, pink, pale green, red, mixture of green and red, and purple to red. The whole fruit is edible and the inner flesh (mesocarp and endocarp) are succulent and white in color, without any seeds.

Rose apple fruits are used in salads or as a snack food. *Rojak, a Malaysian salad delicacy of mixed fruits, is prepared using* pineapple, rose apple, fresh cucumber, mango, sugar, chili, lime bean curd, and paste of cuttlefish (or shrimp) in different proportions. Most of the people rely on eating rose apple when thirsty, owing to its high water content. Fruits are eaten raw, and owing to short shelf life, majority of the harvest is wasted.

There is a major gap in technological knowledge of utilizing this fruit for commercial purposes. Rose apple contains carbohydrates (6.5%), fiber (5.1%), protein (0.9%), lipids (0.1), and ash (0.5%). In Ayurvedic system of Indian medicine, the fruit is believed to improve brain and liver functioning. In addition, seeds are recommended to treat stomach cramps, dysentery, and diarrhea.

Sweetsop (Family Annonaceae, *Annona squamosal* L.)

Sweetsop (or custard apple) plant is a semideciduous tree, which is predominantly drought-resistant. Fruits are mainly preferred for their sweet taste and fragrance. Fruits are ovoid to conical (8–10 cm in diameter and length, weighing 1–2 kg), are pale green to yellow in color, and are thick-skinned (rind) with knobby, hexagonal sections. Pulp portion of matured, ripe fruits are eaten fresh. Unripe fruit are inedible and are astringent.

Pulp portion encloses thick inedible seeds. In some of the pantropical regions, leaves and seeds are used for their insecticidal properties. Sweetsop fruit are rich in carbohydrates/sugars, fiber, proteins, ash, and vitamin C. Fruits are also traditionally used as flavoring agent during preparation of ice cream in the Philippines, where marketing of sugar apple wine is popular.

Soursop (Family; *Annona muricata* L.)

Soursop is a tropical fruit, native to South American countries (Mexico, Brazil, Cuba, Peru, and Venezuela). However, this

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plant is widely cultivated in South East Asia, Pacific regions, and North Africa. Matured fruits are 20–25 cm long and weigh an average of 5–6 kg. Skin color is green and has prickles or soft thorns. The edible portion is the flesh, which is white-colored and encompasses inedible black seeds. Once mature, the fruits are harvested and allowed to ripe. However, fruits harvested when unripe and without reaching physiological maturity are of low quality and bitter in taste. Harvesting stage is determined when the skin color changes from dark green to dull yellow/green with their pulp being firm (firmness checked by thumb pressing).

Soursop fruits are either consumed directly or prepared into various food stuffs. This fruit is considered rich in dietary fiber and vitamins C, B_1, and B_2. The pulp portion is used to prepare juice, smoothies, nectar, candies, and fruit bars and as flavorings. In Malaysia and Indonesia, the fruits are used as an ingredient to prepare traditional foods like 'ais kacang' (shaved ice), 'dodol' (a sweet flavored dish), and refreshing fruit juice.

Nutritionally, the fruits contain carbohydrates (16.84 g/100 g), protein (1 g/100 g), total sugars (13.54 g/100 g), and total dietary fiber content (3.3 g/100 g; USDA, http://ndb.nal.usda.gov/ndb/foods/show/2459?qlookup=09315andformat=Fullandmax=25andman=andlfacet=andnew=1, assessed date 20 February 2014). Nearly 86% of the fruit is pulp with 3% being receptacle. Taste of soursop is attributed to the presence of sugars and organic acids (malic and citric acids) (0.65–0.85%). Studies have shown ripening stages to increase the total soluble sugars, ascorbic acid by 11-fold, malic acid by 7-fold, and citric acid by 3-fold. Fruit juice is rich in minerals like zinc, copper, and phosphorus. Besides, nearly 59 compounds were identified in the oil obtained from peel, leaves, and pulp.

Fruits have high polyphenol content, and regular consumption is linked with beneficial properties such as anticancer, anti-leishmanial, and antidiabetic activities. Contradictorily, consumption of soursop fruit is correlated with the development of neurodegenerative disease (Parkinson disease), which is attributed to the presence of annonacin in the seeds.

Gandaria (Family Anacardiaceae, *Bouea macrophylla* Griffith)

Gandaria or the plum mango (or kundangan fruit), a tropical, ornamental, evergreen fruit-bearing tree, is widely distributed in Malaysia, Thailand, Burma, the Philippines, and Indonesia. Both unripe and ripe fruits (approx. size: 3–5 cm, round to globose) are edible and are consumed directly or used for cooking purpose. Ripe fruit pulp is yellow to orange in color, sweet, aromatic, and juicy and has an orange-colored peel. Ripe fruits have been used by indigenous people to prepare thirst-quenching drinks or are converted to jam, syrup, and stewed. Unripe fruits have a green- to white-colored pulp and are sour with green skin. These are used in preparing curries or salads, and some of the traditional snack food like 'rojak' (fruit and vegetable mixed salad) and 'asinan' (fermented pickles).

Nutritionally, ripe fruits are high in protein, fat, carbohydrate, fiber, vitamin C, and lutein contents. However, no reports are available on the medicinal value of this fruit.

Pomelo [Family; Rutaceae; *Citrus maxima* (Burm.) Merr. or *Citrus grandis* L.)

Pomelo is a citrus plant grown in tropical regions of Asia. The fruits are large (up to 25 cm, weighing 2–3 kgs) with the skin being green- to yellow-colored when ripe. The inner edible flesh is pink-, white-, or cream-colored and sweet to sour in taste. In some cases, bitter and acidic fruits are also encountered. Pomelo fruits are rich in dietary fiber, sugars, and organic acids. Volatile fractions and flavor profile of different cultivars have shown the presence of nearly 50 aroma compounds comprising esters, terpene alcohols, unsaturated aliphatic aldehydes, terpene aldehydes, and nootkatone (a sesquiterpene ketone, a characteristic grapefruit flavor component) identified to impart flavor. Methanol extracts of fruit tissues are reported to exhibit antioxidant and antimicrobial properties. Coumarins, furanocoumarins, sitosterols, limonoids, and other volatile and nonvolatile compounds and limonin and nomilin, *p*-coumaric acid, oils, and caffeic acids have been detected. Isolated compounds have revealed inhibitory antimicrobial effects against *Escherichia coli*, *Bacillus subtilis*, *B. cereus*, *Salmonella enteritidis*, and *Staphylococcus aureus*. Additionally, antioxidant potential of the fruit peel and pulp and its fermented products is also reported. In addition, antidiabetic activity of the fruit extracts is known.

Kasturi Lime (Family Rutaceae, *Citrus microcarpa*)

Fruits of kasturi lime are oval in shape (1–2 in. diameter) with a thin, green skin and orange-colored flesh. Fruits look like a miniature of a lime fruit and are sour-sweet and aromatic. Kasturi lime is cultivated in Malaysia, Thailand, the Philippines, and Indonesia. This plant is an evergreen tropical columnar shrub (2–7.5 m tall) bearing few hundreds of fruits on maturity. *Citrus microcarpa* is reported to be a natural hybrid of mandarin and oval kumquat (*Citrus reticulate* × *Citrus japonica*) plants.

Fruit juice is used as a flavoring agent in selected local dishes and drinks (herbal tea), in marinades and beverages, and in salad dressings. Traditionally, fruits are used to treat coughs and minimize skin irritations and as a laxative. Kasturi lime is rich in bioactive and antioxidant compounds. Narirutin, a flavonoid, is found in abundance. Volatile components in fruit peel are dominated by limonene, β-myrcene, α- and β-pinene, β-phellandrene, and sabinene. Fruits contain ample amounts of volatile oil with the rind portion containing several aldehydes, beta-pinene, sesquiterpenes, linalool, tannins, and glucosides. Recently, 60 different types of volatile compounds have been identified in this fruit. As well, the presence of high levels of citric acids is reported. Antioxidant and hepatoprotective activities of the fruit (peel and extract) are also reported.

Identical to kasturi lime, there is one more fruit – calamondins (*Citrus madurensis*), a native to China. These fruits are

acidic, juicy, and orange-colored and are considered to be miniature of the popular tangerines. Fruits juice is used to prepare refreshing beverage and as flavoring agent during preparation of soups. In traditional Chinese medicine, fruit juice of calamondin is used to treat cough and also makes an excellent hair conditioner. However, scientific work reported on this fruit is still scarce.

Star Fruit (Family Oxalidaceae, *Averrhoa carambola* L.)

Star fruit is a popular tropical fruit considered to be native to India, Malaysia, China, Indonesia, Taiwan, the Philippines, Australia, and Florida. Fruits are oblong and angled (dissected longitudinally into 5–6 ridges) and are between 5 and 15 cm long, weighing 50–100 g. Fruits have a thin, waxy skin that is either green or orange to yellow in color. The whole fruit is edible and juicy. When sliced in cross section, fruits have the form of a 'star' and hence the name. The fruits are harvested when they are either light green, dark green, or yellow in color. In fact, two distinct classes of star fruits are cultivated: Small type that is light green is very sour but with high oxalic acids, and big type is sweet, mild-flavored, and with less oxalic acid.

In Ayurvedic medicine, fruit juice is recommended to aid in digestion and reduce high body temperatures and as a natural eye drop to improve vision. According to 'Yunani' medicine, ripe fruits reduce thirst and inhibit diarrhea and vomiting during food poisoning. Traditionally, in India, ripe fruits are used to stop blood hemorrhages. In Vietnam, fruit juice is used as an eye salve and for the amelioration of high blood pressure. In some countries, intake of juice is considered useful to relieve from excessive intake of alcohol. In Brazil, fruit juice is recommended as a diuretic and as a treatment for eczema.

Star fruits are used in fruit salads, jam preparations, clarified juices, jellies, and preserves. Local population in Malaysia cook star fruits with seafood or meat. In addition, salads prepared by mixing and stewing star fruits with apples, sugar, and cloves are also popular. However, in most of the growing regions, fruits are eaten raw with common salt and chili.

Star fruits are a major source of vitamins A and C and contain ample amount of calcium, folate, calcium, potassium, and oxalic acids. Star fruits are low in sugar and have phenol compounds such as epicatechin and proanthocyanidins. High levels of oxalic acids in star fruits (0.08–0.73 g/100 g) are also reported. However, reports are also available on intoxication and fatality on consumption of fresh star fruit juice.

Pitaya (Family Cactaceae, *Hylocereus undatus* Haw.)

Pitaya or the dragon fruit is believed to be an origin of Mexico and Central and South America. Today, this fruit is widely cultivated and has gained popularity in Thailand, Malaysia, Indonesia, China, Vietnam, Taiwan, Bangladesh, and Australia. Typical to a cactus plant, pitaya is an evergreen annual plant with cladode (as stem). Fruits are categorized with the presence of unique bracts on the thin skin of bright red (pink to purple)-colored fruits. Flowers and fruits of the plant are edible. Depending on the cultivar, the edible pulp portion can be either red (pink) (*H. polyrhizus*), white (*H. undatus*), or yellow (*H. megalanthus*) with small tiny black edible seeds. Overall, average weight of a fruit is approximately 300–350 g, and a single plant is estimated to produce up to six cycles of fruits annually.

Pitaya fruit is preferred by consumers owing to their unique taste, shape, and flesh color. Though pitaya is preferred to be eaten directly, intake as fresh juice is more popular in the growing regions. Pitaya-based products like jams and candy are commercially available in Malaysia and Indonesia. Wine preparation from red pitaya is also reported in Guangxi province of China. Pitaya fruit (both skin and flesh portions) is reported to contain water-soluble betacyanins, which are responsible for the intense red (dark pink/purple) color and its antioxidant activities. In addition, lycopene, vitamin E, and beta-carotene are reported in the pulp portion.

Fruits are high in total polyphenols, tannins, betalains, nonbetalainic compounds, and antioxidant compounds. Oligosaccharides from pitaya fruit are linked with prebiotic properties. Pitaya peel contains high amount of pectin and insoluble to soluble fiber. In addition, powder prepared from the fruit has potential to be used as natural food colorant. Significant amount of betacyanin pigment is reported from peel. Inhibition in the growth of melanoma cells is also reported from peel extracts of red pitaya. Intake of pitaya fruit is also linked with the reduction of dyslipidemia and promotion of wound healing capacity in diabetic rats. Seeds encompass essential fatty acids dominated by linoleic and linolenic acids.

Snake Fruit (Family Palmaceae, *Salacca edulis* Reinw)

Snake fruits are common in Malaysia, Indonesia, Brunei, and Thailand. The tree looks like palm tree, bearing fruits in bunch, which are ovoid in shape having a stretched (pointed) end. The outer skin of the fruit is red- to brown-colored and is covered by smooth, flexible scales. Snake fruit pulp is edible, sweet, and sour giving a tingling sensation in the mouth. The pulp of the fruit occurs in three lobes, enclosing a inedible seed.

Snake fruit are rich in total, soluble and insoluble dietary fibers; minerals such as sodium, potassium, magnesium, calcium, iron, manganese, zinc, and copper; and bioactive compounds such as polyphenols, flavonoids, flavanols, anthocyanins, vitamin C, total carotenoids, and β-carotenoids. Besides, nearly 46 volatile compounds have been identified in the fruit, dominated by 25% of methyl 3-hydroxy-3-methylpentanoate and 23.4% of methyl (E)-3-methylpent-2-enoate.

Persimmon (Family Ebenaceae, *Diospyros kaki* L. or *Diospyros* sp.)

Persimmon has an origin in China and Japan but is widely cultivated and marketed in Korea, Malaysia, and Thailand. Fruits are seasonal, round or spherical in shape (2–10 cm), and are borne on a small tree in few hundreds. The fruit pulp is edible, fibrous, and soft and is sweet. Ripe fruits are orange- to yellow-colored or red- to orange-colored. As the fruits are seasonal, using this fruit in dried form is traditionally recommended. The ripe fruits are mainly preferred to be eaten directly or are

cooked (after removal of skin) to produce various ethnic, traditional foods. A wide array of products are prepared using the fruits, which include pudding, jams, jellies, cookies, fruit salads, and ice creams. Unripe fruits also have culinary use.

Nutritionally, fruits contain high fiber and minerals like potassium, sodium, calcium, magnesium, iron, and manganese found in adequate quantities. Japanese persimmon is reported to contain 18.59% carbohydrates, 12.53 g/100 g total sugars, 3.6% dietary fiber, 0.19 g/100 g fat, 0.58 g/100 g protein, 0.33 g/100 g ash, and 7.5 mg/100 g vitamin C (USDA, http://ndb.nal.usda.gov/ndb/foods/show/2412?qlookup=09263andformat=Fullandmax=25andman=andlfacet=andnew=1; assessed date; 22 February 2014).

The presence of high amounts of polyphenols and antioxidant compounds is reported from the fruit. The major polyphenols include catechin, epicatechin, epigallocatechin, condensed proanthocyanidins, and *p*-coumaric acid. Reports available have also indicated the potential of this fruit to exhibit hypolipidemic or the cholesterol-lowering activity in rat models.

Passion Fruit (Family Passifloraceae, *Passiflora edulis*)

Passion fruit plant is a climber (vine) originated in South America and North African countries. They are abundant in Argentina, Australia, Brazil, India, Indonesia, Vietnam, and others. Fruits are seasonal; unripe fruits are green, while ripe fruits are cream to yellow to dark purple, with a thin and soft and firm skin with the pulp being sweet, aromatic, and juicy, having tiny seeds covered with mucilage.

Ripe fruits are used to prepare juice or are consumed directly with addition of little sugar. However, passion fruit has wide market applications and is used as flavoring agent in preparation of cake, ice cream, jam, deserts, smoothies, etc. Nutritionally, the fruits are rich source of dietary fiber, vitamin C, and lycopene. The subfractions and flavonoid constituents of the fruit are known to exhibit anxiolytic (antianxiety) properties. Administration of fruit peel extract is reported to reduce blood pressure in female spontaneously hypertensive rats and in humans without any adverse affects. Oral intake of peel extract of purple passion fruit is reported to reduce pain and stiffness along with improving physical function in people suffering from knee osteoarthritis.

Passion fruit extract is used in South American folk medicine to treat asthma, bronchitis, anxiety, and insomnia. Fruit peel extract has significant amounts of antioxidant compounds such as edulilic acid, cyanidin-3-*O*-glucoside, and quercetin-3-*O*-glucoside. Peel extract of purple passion fruit is reported to be beneficial to reduce asthmatic symptoms.

Tarap (Family Moraceae; *Artocarpus odoratissimus* Blanco)

Tarap fruits (green pedalai or marang) are a native of Borneo (Sabah) region in Malaysia but widely distributed in Brunei, the Philippines, Myanmar, Thailand, and Hawaii. The tree is identical to bread fruit tree and grows between 25 and 30 m tall. Fruits occur singly, weigh approximately 1–1.5 kg, and are 15–16 cm in length and 12–13 cm in diameter. Just like jackfruit, this fruit too has short, green- to yellow-colored spines, which are soft and brittle. Ripe fruits have strong odor, and the inner part of the fruit (flesh/pulp) is edible that is very sweet to sour and juicy. The pulp is divided into several segments (bulbs) enclosing seed.

In some households, young fruits are also cooked as vegetables. Just like jackfruit, this fruit is consumed directly. However, seeds are also edible and can be eaten after roasting or boiling. Traditional preparation includes mixing the flesh (with seeds intact) with corn flour and egg batter followed by deep frying. The flesh is also used as a flavoring agent of ice creams and cakes and fermented to obtain vinegar.

Tarap fruit flour contains high amount of carbohydrates with average levels of protein, ash, crude fiber, fat, and essential minerals such as potassium, calcium, sodium, and iron. Fructose is dominant in the flesh. In addition, antioxidant activities, cytotoxicity, and polyphenol content of different fruit parts are also reported by various researchers (**Table 1**).

Influence of Modern Food Processing Methods

Owing to short shelf life and being seasonal, tropical fruits require to be consumed fresh to retain their original taste, aroma, and flavor. However, exporting to regions where they are not cultivated is a task. Hence, application of modern processing and preservation techniques assumes high importance. If not fresh, then at least these fruits need to be processed (e.g., canning, syrup base, and jam) into edible forms to be used for marketing, especially during off-seasons. In tropical countries, a huge number of popular and underexplored tropical fruits need to be preserved for dietary use as they possess several health beneficial properties. Emerging and well-established technologies such as active packaging and combination of atmospheric packaging with natural botanicals could be explored for shelf life improvement of tropical fruits. Nonthermal food processing technologies like application of ultraviolet radiation (UV), electron beams, pulsed electric field, high-pressure processing, use of ultrasound, ozone, and minimal processing can be considered for preservation of fresh tropical fruits and their products.

Future

More research needs to be focused toward generating suitable statistical database on the exact production rate, and convertibility factor of the excess fruits harvested into a shelf stable product. Providing more scientific evidence on the nutraceutical value to health conscious consumers on lesser known fruits is a necessity to enhance the marketability and to attain self-sufficiency in the growing regions. Identifying novel bioactive compounds with potential therapeutic value can be explored. The by-products or waste generated from the inedible portions can be explored to generate some economical use. Developing low-cost postharvest techniques for preserving and improving shelf life of fresh produce needs to be explored.

See also: Annonaceous Fruits; Citrus Fruits.

Table 1 Bioconstituents of some selected tropical fruits

Fruits	*Observation*	*References*
Fruit of wolf (*Solanum lycocarpum*)	With regard to nutritive value, the fruits contains carbohydrates (5.74%), proteins (1.6%), lipids (1.6%), and ash (0.63%) with the absence of any dietary fiber Fruits exhibit high antioxidant activities (162.97 EC 50 μg DPPH/mL) with total polyphenols content being 35.58 g gallic acid equivalents/kg	Roesler et al. (2010) Silva et al. (1994)
Sweet sop (*Annona squamosa*)	Sweetsop fruit contains 12.78% carbohydrates, 4.72% fiber, 1.22% proteins, 3.88% lipids, and 1.37% of ash with an ascorbic acid level ranging between 15 and 44.4 mg AA/100 g	Silva et al. (1994)
Soursop (*Annona muricata*)	Fruits contain high polyphenol content (72 trolox equivalent antioxidant capacity and 42 gallic acid equivalents mg/100 g, respectively). Among the various compounds, aliphatic acids, esters (51%), and terpenes such as mono- and sesquiterpenes (β-caryophyllene 12.7%, 1,8-cineole 9.9%, linalool 7.8%, α-terpineol 2.8%, linalyl propionate 2.2%, and calarene 2.2%) were isolated from the essential oil of fruits	Lako et al. (2007)
Gandaria (*Bouea macrophylla* Griffith)	Nutritionally, ripe fruits have high protein (40–112 mg/100 g), fat (20–120 mg/100 g), carbohydrates (11–140 mg/100 g), fiber (600–140 mg/100 g), vitamin C (20–100 mg/100 g), and lutein (457 mg/100 g) contents	Morton (1987) Tee et al. (1997)
Pomelo (*Citrus maxima* or *Citrus grandis*)	In Malaysian (pink and white type) pomelo, juice contained sugars (mg ml^{-1}): fructose (11.02 and 13.41), glucose (13.13 and 10.43), and sucrose (49.91 and 56.51) in pink and white juices, respectively Besides, organic acids (mg ml^{-1}) detected were ascorbic (0.22 and 0.39), citric (14.15 and 0.14), malic (0.75 and 1.94), and succinic (0.05 and 0.39) acids and were in appreciable amounts in pink and white juice, respectively Volatile fractions and flavor profile of different cultivars have shown the presence of nearly 50 aroma compounds comprising esters, terpene alcohols, unsaturated aliphatic aldehydes, terpene aldehydes, and nootkatone that were identified to impart flavor in pomelo Methanol extracts of fruit tissues exhibited antioxidant and antimicrobial properties. Coumarins, furanocoumarins, sitosterols, limonoids, and other volatile and nonvolatile compounds were detected. The extract concentration providing 50% inhibition was dominated by mixture of limonin and nomilin (135 μg ml^{-1}), *p*-coumaric acid (105 μg ml^{-1}), oil (95 μg ml^{-1}), and caffeic acids (45 μg ml^{-1}). Isolated compounds revealed inhibitory antimicrobial effects against *E. coli*, *Bacillus subtilis*, *B. cereus*, *Salmonella enteritidis*, and *Staphylococcus aureus* Antioxidant potential of the fruit peel and pulp and its fermented products is also reported. Essential oil from peel had high amounts of total phenolic (214 mM) and flavonoid (134 mg QE/g of d.w.) contents compared to fruit pulp Antidiabetic activity of *C. grandis* extract treated with *Aspergillus saitoi* is reported. Studies conducted indicated hydroxylated products from fruit extract (after enzymatic and microbial treatments) to be useful toward developing pharmaceutical foods focused toward controlling blood glucose level in diabetic patients	González-Mas et al. (2011) Cheong et al. (2011) Mokbel and Suganuma (2006) Jang et al. (2010) Kim et al. (2009)
Kasturi lime (*Citrus microcarpa*)	Narirutin, a flavonoid, is found in abundance in *C. microcarpa* (2.89–4.60 mg g^{-1} dry weight) Volatile components in fruit peel contain limonene, β-myrcene, α- and β-pinene, β-phellandrene, and sabinene. Volatile oil constitutes between 0.9% and 1.06% of the components, with the rind portion containing several aldehydes, beta-pinene, sesquiterpenes, linalool, tannins, and glucosides. Recently, 60 volatile compounds and high levels of citric acid (5.0–5.52%) are reported in the fruit. Antioxidant and hepatoprotective activities of fruits are also reported	Bhat et al. (2011a) Cheong et al. (2012) Kawaii et al. (1999)
Pitaya (*Hylocereus undatus* Haw.)	Total phenolics (9.2–13.3 mg/100 ml), betalains (76.1–88.1%), nonbetalainic compounds (11.9–23.9%), and antioxidant capacity (TEAC) (24.5–36.1 ascorbic acid equivalents in mg/100 ml) in different genotypes are reported Whole fruit is also reported to contain high polyphenols (86.10 mg GAE/0.5 g) and tannins (2.30 mg CE/g) with EC50 (for DPPH) value of 2.90 mM ascorbic acid equivalents/g (all on d.w.) Seeds encompass essential fatty acids dominated by linoleic acid (48%) and linolenic acid (1.5%)	Esquivel et al. (2007b) Rebecca et al. (2010)
Snake fruit (*Salacca edulis* Reinw)	Snake fruit contains 3.0, 1.2, and 1.8 g/100 g (f.w.) of total, soluble, and insoluble dietary fibers, respectively. The fruits are reported to contain minerals such as sodium (254.3), potassium (12.473), magnesium (668.0), calcium (242.8), iron (13.22), manganese (11.44), zinc (11.41), and copper (3.71) mg kg^{-1} (d.w.) Snake fruits also contain polyphenols (8.46 mg gallic acid equivalent/g), flavonoids (0.313 mg catechin equivalent/g), flavanols (3.14 μg catechin equivalent/g), anthocyanins (9.74 mg cyanidin-3-glucoside equivalent/g), vitamin C (13.28 mg ascorbic acid/g), total carotenoids (2.2 μg g^{-1}), and β-carotenoids (1.17 μg g^{-1}) By using GC–MS and 1H NMR, nearly 46 volatile compounds have been identified in the fruit,	Gorinstein et al. (2011) Wong and Tie (1993)

(*Continued*)

Table 1 (Continued)

Fruits	*Observation*	*References*
	dominated by 25% of methyl 3-hydroxy-3-methylpentanoate and 23.4% of methyl (E)-3-methylpent-2-enoate	
Tarap (*Artocarpus odoratissimus*)	Fruit flour contains 75.75% carbohydrate, protein (6.58%), ash (2.84%), crude fiber (2.65%), and fat (1.89%). In addition, the flour is rich in minerals such as potassium (1.820 mg/100 g), calcium (6.00 mg/100 g), sodium (4.68 mg/100 g), and iron (0.68 mg/100 g) Fructose sugars are dominant in the flesh (5.8–13.7 g/100 g)	Masri et al. (2012) Tang et al. (2013)

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Fumigants

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Introduction

Fumigants are critical components in pest management programs because of their unique ability to diffuse throughout a common airspace in a commodity, substrate, or structure to eliminate a wide range of pests. Fumigation is especially useful for the treatment of soil prior to planting, raw and fresh agricultural commodities, processed food, and structures, which process, store, and transport these commodities, which are difficult to thoroughly and reliably disinfest by other means.

What Are Fumigants?

Fumigants are pesticides, which are in a gaseous state upon contact with the target pest. They differ from all other forms of pesticides, which are liquids, such as aerosols, emulsifiable concentrates, and solutions, or solids, such as wettable powders, suspension concentrates, dusts, and granules, upon contact with the pest. The size of a fumigant gas molecule is more than 1000 times smaller than that of an aerosol droplet or a dust particle. In addition, fumigants have high vapor pressure, often 10 000-fold greater than that of naphthalene (moth balls). The minute size of gas molecules and their high vapor pressure result in fumigants penetrating throughout the treated commodity, substrate, and structure to kill pests in areas inaccessible to liquid and solid formulations of pesticides. Commonly used fumigants and their major use patterns are described in Table 1.

Advantages of Fumigation

In addition to the penetrating capabilities described earlier, fumigation has other advantages. Fumigants can have broad toxicity, controlling a wide range of invertebrate and vertebrate pests. The appropriate fumigant may control all the pests infesting a commodity, substrate, or structure with one treatment. Therefore, fumigation can provide reliable elimination of pests where there is no tolerance for infestation, such as in quarantine treatments. Fumigation can also be the most rapid treatment to control pests.

Table 1 Commonly used fumigants and their uses

Fumigant	*Soil fumigation*	*Quarantine and preshipment*	*Commodity fumigation*	*Structural fumigation*[a]
Methyl bromide	X[b]	X	X[b]	X[b]
1,3-Dichloropropene	X			
Chloropicrin	X			
Metam sodium/potassium	X			
Dazomet	X			
Dimethyl disulfide	X			
Phosphine		X	X	X
Sulfuryl fluoride		X	X	X

[a]Includes mills, food processing facilities, and warehouses.
[b]Most uses phased out due to Montreal Protocol restrictions.

Fumigant Dosage

Fumigants are considered nonresidual treatments in that they do not leave residues to prevent future pest infestations. The commodity, substrate, or structural space to be fumigated is sealed prior to fumigant introduction to sufficiently confine the fumigant. The fumigant is confined for the planned exposure period to achieve the lethal dosage required to control the target pests, prior to the fumigant being actively or passively aerated. Therefore, the dosage of a typical fumigant is the product of its concentration (C) and exposure time (t). A Ct dosage can be achieved by varying either C or t to produce a toxic effect on the target pest. The model $C^n t = k$, where n is the toxicity index and k the dosage rate to produce a specific mortality level for a given species, describes the relationship of fumigant dosage to mortality. For fumigants like methyl bromide and sulfuryl fluoride, 'n' generally approximates one for typical exposure periods, such as 2–72 h. This means that concentration and time contribute equally to achieving a lethal dosage. Fumigant exposure period can be shortened if the concentration of fumigant is increased, or conversely, fumigant concentration can be decreased if the fumigant exposure period is increased.

Phosphine toxicity deviates from this model and the relationship between dosage and resulting toxicity in insects is not linear. Generally, longer exposures of insects to low concentrations of phosphine tend to produce greater toxicity than shorter exposures to high concentrations. Phosphine requires extended exposures, at least 1 day for phosphine gas and at least 2 days for metallic phosphides, to produce toxicity in target organisms. Research has documented in insects that after a minimum exposure period has elapsed, mortality for a given species become constant for a Ct dosage.

The dosage for a fumigant is based on numerous factors, including target species and life stage, temperature at the site of the target pest, fumigant formulation, atmospheric pressure, insect diapause, and selected insect resistance to fumigants. The initial concentration of fumigant to obtain this dosage is dependent upon the exposure period and the confinement of the fumigant during that exposure period. The confinement of the fumigant can be determined by measuring the fumigant concentrations in the fumigated space at two or more time intervals. These measurements determine if fumigant confinement is sufficient or if more fumigant needs to be added to achieve the required dosage. Tables listing required fumigant concentrations at sequential time intervals, based on target pest and temperature, are used for quarantine treatments when

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target pest eradication is essential. Calculation devices, such as handheld calculators or computer programs, are available with sulfuryl fluoride to calculate dosages, dosage accumulation, and amount of fumigant to apply.

Fumigant concentration measurements represent the target pest exposure to the fumigant, since fumigants disperse throughout the confined space. This is a unique attribute of fumigants, compared to other pesticides, that exposure of the target pests to the fumigant can be reliably measured to confirm that the required dosage has been achieved for control.

Mode of Action of Fumigants

The general toxicity of fumigants to a wide range of organisms indicates adverse effects on fundamental life processes at the cellular level. Methyl bromide is an active alkylating agent. The methylation and incapacitation of enzymes, involved in regulating cellular metabolism, and other bioorganic molecules may explain the broad toxicity of methyl bromide. Sulfuryl fluoride is metabolized into fluoride in animals. Low concentrations of fluoride are readily excreted; high concentrations of fluoride can be toxic. Excessive fluoride can interfere with various metabolic processes, such as inhibiting enzymes utilizing magnesium in the glycolysis cycle. Phosphine can act as a reducing agent that can interact with transition metals and therefore has the potential to disrupt the enzymatic activity of cellular proteins. Phosphine is known to disrupt mitochondrial energy metabolism leading to a decrease in ATP synthesis.

Safety in Use of Fumigants

Because fumigants are toxic to a wide range of organisms, including humans, stringent safety procedures are required for their use. Inhalation is the primary route of potential overexposure to fumigants. When handling fumigants, workers are required to wear respiratory protection when exposure to fumigant concentrations may occur that are unknown or are above permissible exposure limits. Additional personal protective equipment required to protect the applicator's eyes and/or skin during fumigant application is dependent upon the fumigant and its formulation. Equipment, capable detecting low fumigant concentrations, is used to monitor potential worker exposure and verify aeration of commodities or a structure following fumigation. Fumigated spaces are posted with warning signs during the fumigation. Regulatory authorities may require an exclusion zone be established around the fumigation site where unauthorized personnel are not permitted to enter. The size of the exclusion zone may vary based on the fumigant, concentration applied, application method, and confinement of the fumigant. Due to their toxicity, fumigants receive ongoing, intense regulatory review and oversight. The safety procedures and equipment used with fumigants have been extensively tested to validate that they provide the required protection for workers and the public.

Limitations on Development of New Fumigants

There are very few molecules that can fulfill the exacting requirements to be used as a fumigant. These requirements include being toxic to target pests under all typical temperature and exposure conditions, easily dispersed and aerated, rapidly penetrative into infested materials, essentially nonreactive to treated materials, leaving nontoxic or no residue, nonexplosive, nonflammable, and noncarcinogenic, mutagenic, or teratogenic for humans and having no adverse environmental effects. Many of the fumigants used in the first half of the twentieth century, such as acrylonitrile, hydrogen cyanide, and ethylene dibromide, subsequently lost their registrations in many countries due to their residues or extreme danger to people.

Methyl bromide was once the most widely used fumigant. Compared to other currently used fumigants, methyl bromide is toxic to the broadest spectrum of pest species and their life stages, typically delivering the desired control in 2–24 h. Methyl bromide does not corrode metal and is considered safe for food products due to minimal chemical reaction or residues. Methyl bromide has been commonly used in soil fumigations against pests such as nematodes, fungi, soil insects, and seeds of weed plants that affect the establishment and production of many crops. Methyl bromide's rapid, broad-spectrum efficacy is why it is used globally for quarantine and preshipment treatment of perishable commodities, including fruits, vegetables, and cut flowers, and durable commodities, including grain, legumes, and timber. Methyl bromide was also used to fumigate mills, food processing facilities, and structures that store and transport commodities.

Unfortunately, methyl bromide was identified as a chemical substance that contributes to the degradation of the stratospheric ozone layer in the Earth's atmosphere. In 1992, the fourth meeting of the 'Parties to the Montreal Protocol on Substances that Deplete the Ozone Layer' placed methyl bromide on the list of ozone-depleting substances that require regulation and an eventual worldwide ban on production, use, and emission, beginning in 2005 in developed countries and in 2015 in developing countries. The Montreal Protocol exempted quarantine and preshipment applications of methyl bromide due to the impact on international trade. Since 2005, critical use exemptions have been granted in developed countries where suitable alternative treatments did not exist; however, the amount of methyl bromide and number of exemptions granted have steadily decreased. The search for alternatives for methyl bromide has been a lesson that a myriad of other treatments are required to replace the versatility, reliable efficacy, ease of use, and economy offered by this fumigant. The characteristics of fumigants that make them effective (i.e., highly volatile, broad spectrum, high use rates, and toxicity) are basically the opposite of the characteristics most global regulatory agencies are looking for in new pesticides. These characteristics, plus the time and expense of developing a new pesticide, make it unlikely that a new fumigant with broad-spectrum efficacy similar to methyl bromide will be developed and registered with currently known technology.

Soil Fumigation

Preplant fumigation of soil has been a critical management tool for the production of many high-value crops. These crops include vegetables, fruit, potatoes, tobacco, and nursery stock that are grown in high production areas of the United States,

Spain, Italy, France, Australia, and Japan for domestic use as well as for export. In many cases, these crops could not be produced economically without managing multiple soil-borne pests such as nematodes, pathogens, and weeds that can devastate crop production if left unchecked. For example, nematode and disease populations can be so destructive that strawberries cannot be grown economically without fumigants in some areas of the United Sates (e.g., Florida and California) and Spain. The advantage of fumigants over traditional nonfumigant pesticides is that they provide extremely high levels of control throughout the critical root zone area of the developing crop. This fosters better root development during the early stages of crop growth and often results in higher yield production.

Prior to the Montreal Protocol, methyl bromide was the most effective and widely used soil fumigant for high-value crops. It was highly effective against a broad spectrum of soil pests including nematodes, insects, soil fungi, and seeds of weed species and enabled farmers to plant or transplant crops after a relatively short postfumigation interval. During the phase-out period of the Montreal Protocol, extensive research was conducted by many government and nongovernment organizations to find suitable alternatives. Initially, alternatives were available that provided solutions to control certain types of soil pests, but there was no single fumigant that had the efficacy, broad-spectrum control, or convenient use properties that methyl bromide provided. Solutions required combinations of fumigants that effectively controlled a broader spectrum of pests when used together. For example, 1, 3-dichloropropene and chloropicrin provided excellent nematode and disease control, respectively, when used individually. However, as methyl bromide was phased out, they were often used together to control a wide spectrum of nematode and pathogen pests. Metam sodium/potassium and Dazomet degrade in soil to form pesticidally active methyl isothiocyanate (MITC). The efficacy of MITC can be further enhanced by combination with other soil fumigants, such as applying metam sodium with 1,3-dichloropropene and/or chloropicrin. In the near term, viable solutions to methyl bromide will include combination products.

There were many novel alternatives tested during the phase-out period of methyl bromide. Some of these alternatives included steam heating, solarization, plant extracts, biologicals, crop rotation, and resistant cultivars. These alternatives had various levels of success, but none proved to be as effective and reliable against a broad spectrum of soil pests as methyl bromide.

One of the more productive areas of research during this phase-out period was in application technology. Application of soil fumigations often utilized plastic tarps. This reduced the emissions of fumigants and also made them more effective. New plastic films were developed that significantly reduced emissions and improved the performance of the fumigation. In addition, preplant applications of fumigants through drip irrigation systems were refined, which further reduced emissions and improved efficacy of fumigants.

Fumigation for Quarantine and Preshipment

One of the most important uses of fumigants is for disinfestation of commodities for quarantine and preshipment. Fumigants can offer rapid, reliable, measurable, and effective control of a broad range of target pests without adverse effects on the fumigated commodities. Disinfestation of exported commodities is essential to thwart the introduction of nonindigenous pest species into importing countries. The cost of damage and control associated with nonindigenous species introduced in the United States alone was estimated to be $137 billion per year in 2000.

Most countries have legislation restricting the importation of infested commodities. The International Plant Protection Convention (IPPC) is part of the Food and Agriculture Organization (FAO) and helps to communicate and standardize phytosanitary certification by different countries. More than 200 parties and their territories participate in the IPPC. They are required to establish, update, and make available lists of regulated pests. Fumigants have been an essential tool in preventing further dissemination of key regulated quarantine pests.

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is one of the world's most destructive fruit pests. Its larvae can feed on over 300 plant species, including deciduous, subtropical, and tropical fruits and some vegetables. Larval feeding in fruit creates an inedible mass. Many countries, such as the mainland United States and Japan, forbid the import of susceptible fruit without strict postharvest treatment having been applied by the exporter. Historically, susceptible fruits have been fumigated with methyl bromide. The probit-9 standard for quarantine efficacy (99.99683%) was developed using methyl bromide for treating tropical fruits with heavy infestations of fruit flies. Some countries have prohibited any use of methyl bromide, even though the quarantine and preshipment uses are exempted from the Montreal Protocol. Alternative treatments have been implemented. These include heat treatment (vapor heat, hot water, or forced hot air), cold treatments, insecticidal dipping, or irradiation. Heat treatment tends to reduce the shelf life of many fruits. Cold treatment can require up to 22 days depending upon the temperature and commodity. Irradiation is not accepted in many countries. Therefore, without the use of methyl bromide, imports may be restricted from areas where the fly occurs, potentially reducing trade opportunities for these countries.

There are relatively few stored-product insects which are quarantine pests, due to centuries of colonization and trade in which these pests were globally disseminated from food stores on ships. One exception is the khapra beetle, *Trogoderma granarium* Everts, which is a global quarantine pest that infests more than 96 commodities. Larvae of the khapra beetle can readily penetrate food packaging to contaminate and render food unfit for human consumption. The larvae can survive a wide range of environmental conditions and long periods without food, during which they enter diapause or retrogressively molt to survive. The establishment of the khapra beetle can create accessibility problems for commodities. Due to a high number of interceptions of khapra beetles early in 2011, the United States restricted rice imports to permitted commercial shipments in order to reduce potential number of introductions. Methyl bromide has been the fumigant of choice for treating infested commodities and subsequently infested warehouses, storage facilities, and even residences to eradicate incipient infestations of khapra beetle in the United States, Mexico, Australia, and other countries. Phosphine and sulfuryl

fluoride are being evaluated as replacement fumigants for control of khapra beetle.

An advantage of fumigation for treatment of infested commodities is that the equipment used for fumigant confinement, introduction, monitoring, aeration, and clearance testing is mobile and readily implemented in a suitable, secure location. Fumigation does not require extensive infrastructure setup, such as for irradiation facilities. This is demonstrated by the situation with the brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stal), a pest in Asia, which was introduced into the United States in the mid-1990s. The BMSB has more than 300 host plants including tree fruit, small fruit, grapes, vegetables, legumes, and ornamentals. Loss of value of commodities damaged by BMSB can be 80–90%. In 2010, this insect caused $37 million in damage to apple farmers throughout the Mid-Atlantic region of the United States. The BMSB is also is a structural nuisance, seeking harborage in buildings, vehicles, and other structures to overwinter as adults. Since December 2014, BMSB adults have been intercepted in shipments of vehicles, machinery, and equipment from the eastern United States to Australia, New Zealand, and the European Union. Treatment by heat or fogging agents was found unsatisfactory to eliminate BMSB in these items. A fumigation schedule using sulfuryl fluoride was implemented and found to be effective in eradicating the infestations. Prior to fumigation, items were sealed by a variety of configurations at port or at nearby locations to meet export schedules: tarping and/or tape-and-sealing the items to confine the fumigant, fumigating the items in sealed structures such as warehouses or shipping containers, or a combination of these methods.

It is important that any treatment applied to vehicles, machinery, and equipment has no adverse effects on these items. When applied according to label directions, sulfuryl fluoride will not affect electronics, including electronic control units, switches, relays, sensors, and touch screens, found in vehicles, machinery, and equipment. Sulfuryl fluoride is used to fumigate research laboratories, medical and manufacturing facilities, and mills and food processing facilities, which contain extensive state-of-the art electronics, and sensing and analytic equipment. Laboratory tests have documented no adverse effects on electronic equipment, including computers, when fumigated repeatedly with sulfuryl fluoride at dosages far exceeding the maximum allowed by labeling, at high temperatures (50 °C), and with equipment left operating during the fumigation. Qualitative assessments conducted by automotive engineers evaluating sulfuryl fluoride for fumigation of luxury automobiles for BMSB control determined that fumigation did not impart any odors or affect the appearance or texture of vehicle surfaces, including leather upholstery. Fumigation of vehicles, machinery, and equipment with methyl bromide for control of BMSB is an option but is not preferred due to the potential reactivity of methyl bromide with some materials containing sulfur, such as types of foam rubber, vinyl, and leather, to create persistent odors.

The rapid speed of treatment of commodities by fumigation to meet preshipment requirements is exemplified by the California walnut industry in the United States. The demand for in-shell walnuts is highest in European markets before St. Nicholas Holiday, December 6. Steamship delivery from California to Europe normally takes 30 days. Therefore, vessels shipping walnuts must set sail from California no later than November 6. The preferred variety for European in-shell walnut market is not ready for harvest until mid-October. This provides just a 3-week window for harvesting, processing, and shipping the walnuts. Walnut processors use vacuum fumigation chambers to expedite the disinfestation process of the Navel orangeworm, *Amyelois transitella*, and other field pests that could tunnel into walnuts to consume the nut meat. Sulfuryl fluoride is now used for this fumigation process and provides similar exposure time (4 h or less) and efficacy as methyl bromide. Other treatments do not penetrate the shell to control the target pests or may affect the flavor of the nut meat. Rapid heat treatment to control the in-shell pests causes rancidity in the fatty acids. The walnut industry is very important to the California economy, representing the fourth largest export from California in 2010, generating farm gate revenue of over $1 billion in 2011, involving 4000 growers and 82 processors, and employing 60 000 individuals directly and indirectly. Fumigation enables this industry to meet tight shipping schedules to offer a high-quality product in a competitive global market.

Commodity Fumigation

Fumigation can be the most effective method for disinfesting commodities when placed into storage after harvesting, which have been in storage for long periods of time, and prior to shipment for processing or export. Fumigation should be considered one tool in managing pest infestations in stored commodities. Other methods to control pest infestations include sanitation and application of approved insecticide surface treatments to storage containers prior to refilling, proper drying of commodities prior to storage, maintaining the required temperature and moisture content of commodities by aeration or other methods to prevent mold and insect growth, monitoring of pest presence through inspection and trapping, and then determining the best control strategy when pest activity reaches actionable thresholds.

Phosphine, or hydrogen phosphide, is one of the most commonly used fumigants globally for disinfestation of cereal grains, legumes, seeds, dried fruit and tree nuts, other durable food commodities, and many processed foods. Phosphine has the unusual property that it can be generated from solid formulations of metallic salts of aluminum phosphide and magnesium phosphide. These metallic phosphides react with water vapor in the treatment environment to generate phosphine. Metallic phosphides can be applied as pellets or tablets, which can be mixed with the commodity as it is loaded for storage, probed into or placed on the commodity, or placed on trays or paper adjacent to the commodity. Metallic phosphides can be applied in a diversity of sites to treat commodities in all types of storages, railcars, shipping containers, stationary truck cargo containers, and other vehicles, and in ship holds to treat grain during transit. Metallic phosphides are the fumigant of choice for treating commodities stored on farms. These solid formulations are simpler to apply compared to fumigants packaged as liquids under pressure in cylinders (methyl bromide, sulfuryl fluoride, or phosphine gas). The solid formulations, phosphine detection equipment, and respiratory protection

(cartridge mask for low phosphine concentrations) are inexpensive compared to the cost of other fumigants and cylinderized phosphine, the respiratory protection (self-contained breathing apparatus) required for methyl bromide and sulfuryl fluoride, and the detection equipment required for sulfuryl fluoride.

Metallic phosphides leave a solid residue of either aluminum or magnesium hydroxide and other inert ingredients. In bulk grains, this residue is inconsequential due to dilution during subsequent grain moving, handing, and processing. Metallic phosphides are available in a variety of formulations, such as permeable bags or blister packs, which allow for faster application of a premeasured dose and retrieval of spent material. These prepackaged, retrievable formulations can be applied to processed or ready-to-eat commodities where solid residues are not permitted and for countries that do not permit solid residues in raw commodities.

Phosphine can also be applied directly to a commodity as a gas from metal gas cylinders or phosphine generators. Dosage accumulation occurs more quickly because there is no delay in availability of phosphine as in the metallic phosphides. Phosphine gas can be applied at lower temperatures (0 °C) and to fresh commodities, such as certain fruit and vegetables. Current research is evaluating reducing the fumigant exposure time required for phosphine by combination with other gases, such as oxygen.

Globally, widespread and repeated fumigation of poorly sealed commodities has resulted in sublethal exposure and selection of resistance to phosphine. Phosphine resistance has been detected in key stored-product insects in more than 45 countries. For more than three decades, a monitoring and management program for phosphine resistance, funded and executed by a coalition of industry and government agencies, has been conducted in Australia. This program has demonstrated that phosphine resistance can be effectively managed by various methods, including intensive sanitation, use of grain protectants, better sealing and monitoring of fumigations, and increasing the phosphine dosage. Sulfuryl fluoride is used to treat commodities infested by insects known or suspected to have high levels of resistance to phosphine. Sulfuryl fluoride and phosphine have different modes of action, and phosphine-resistant insects are not cross-resistant to sulfuryl fluoride. The previously mentioned practices minimize insect infestation and damage to stored commodities, resulting in high-quality product available for use nationally and for international trade.

Sulfuryl fluoride was developed in the 1950s as an alternative to methyl bromide for control of drywood termites and other structure-infesting insects. Due to the phaseout of methyl bromide part of the Montreal Protocol, sulfuryl fluoride was further developed beginning in the late 1990s as a fumigant for durable commodities, including cereal grains, dried legumes, seeds, dried fruit and tree nuts, spices, and cocoa. Extensive research was required to indentify dosages for control of stored products pests, assess trace residues in fumigated commodities and develop residue tolerance thresholds, and verify that fumigation did not affect the taste of high-value commodities (dried fruit, tree nuts, and cocoa) or wheat kernel quality, milling quality, or properties of dough and baked goods (cookies, bread, and spaghetti). Sulfuryl fluoride received its first registration for commodity fumigation in 2004. Sulfuryl fluoride can be applied to similar sites as phosphine, except it is not permitted to treat railcars or ships that are in transit.

Other fumigants, such as ammonia, ozone, methyl iodide, carbonyl sulfide, propylene oxide (PPO), and ethyl formate, have been investigated as commodity fumigants as part of the phaseout of methyl bromide. These fumigants are effective in certain situations and have limitations. For example, PPO is used as a microbial sterilant for spices, cocoa powder, and processed nut meats. Due to its flammability, PPO is applied in special chamber or diluted with a propellant like carbon dioxide. PPO is generally effective in controlling the egg stage of insects, which can require higher dosages for control by other fumigants, such as sulfuryl fluoride. Ethyl formate occurs naturally in some foods and is designated as generally recognized as safe. Ethyl formate is also flammable and like PPO can be formulated with carbon dioxide to reduce flammability. This fumigant has poor penetration capabilities to control target insects feeding inside grain and other commodities. It also decomposes in water in commodities at a rate that is temperature-dependent.

Modified atmospheres are another method for control of commodity pests. Airtight or hermetic storage is used to create an environment in which insects die due to a decrease in oxygen through methods that are passive (insect respiration) or active (such as the use of a vacuum treatment or addition of carbon dioxide or nitrogen). The advantages of these treatments are that they do not create residues in commodities and do not have adverse environmental effects. The challenges are the cost of hermetic storage, maintaining the airtight capacity of the storage, and the time for treatment requiring one or more weeks.

Structural Fumigation

Fumigation should be considered one tool in managing pest infestations in mills, food processing facilities, and warehouses. Nonpesticidal methods to prevent infestations are extensive and include exterior and interior sanitation to remove food debris and pest harborage, a vegetation-free zone around the building exterior perimeters, and pest exclusion by sealing cracks and crevices, screening vents, and using air curtains to blow flying insects away from open entrances and loading docks. Monitoring of pest presence through inspection and traps (using lights, pheromones, or food lures) can determine when and where pest activity reaches actionable thresholds. The activity often can be controlled by additional sanitation or use of localized insecticide treatment.

Nonetheless, mills, food processing facilities, and warehouses are complex environments with many refugia for pests. Fumigation can provide whole structure control to treat areas not accessible for inspection, cleaning, or treatment with other insecticides. Sulfuryl fluoride was developed for this use pattern as a replacement for methyl bromide. As previously discussed, sulfuryl fluoride gas does not adversely affect electronics. Laboratory testing has confirmed that sulfuryl fluoride leaves no detectable residues on inert surfaces, such as metal, glass, and ceramic. Compared to methyl bromide, higher dosages of sulfuryl fluoride are required to control the egg stage of insects. Extensive field trials were conducted to validate the

efficacy of sulfuryl fluoride in these structures for control of target pests, and the sealing, fumigant introduction, and aeration methods provide required protection for workers and the public.

Phosphine is also registered for fumigation of mills, food processing facilities, and warehouses; however, metals including certain copper, brass, other copper alloy, gold, and silver are susceptible to corrosion by phosphine, especially at higher temperatures or relative humidity. In mills and food processing facilities with sophisticated electronics including sensors and computer monitoring systems, phosphine tends to be used as a spot treatment for selected equipment rather than as a whole structure treatment.

Other area-wide treatments commonly used in mills, food processing facilities, and warehouses for insect control include fogging (aerosols), mating disruption pheromones, and heat treatment. Fogging agents are liquids that are atomized and dispersed as fine particles, which look like a cloud. Fogging can offer inexpensive suppression of key stored-product insect pests. These treatments are rapid, requiring as little as 30 min to apply. Common active ingredients in fogging agents include dichlorvos and pyrethrins or pyrethroids that are used alone or in combination with insect growth regulators. Unlike fumigants, aerosol droplet size prevents distribution into obstructed harborages such as equipment or penetration into bulk commodities. The efficacy of this treatment varies by insect species, life stage, and insecticide active ingredient. Some fogging treatments are not permitted to contact food. Sanitation is a very important component of fogging, because food present during or after treatment can increase the survival of flour beetles (*Tribolium* spp.), a common stored-product pest.

Mating disruption pheromones can effectively suppress pest populations of selected species by interfering with their mating and thereby disrupting their life cycle. The pheromone must be dispensed for weeks before and during peak adult fight activity of the target pests. The penetration into commodities of these pheromones is unknown, and they do not kill active infestations of insects. Both mating disruption pheromones and fogging do not require the preparation and shutdown of facility operations as required with whole structure treatment by fumigation or heat.

Heat has been used for many decades for insect control. Heat has the benefit of no pesticide residues, no adverse environmental effects, and control of a wide range of insect pests if lethal temperatures can be obtained. Heat can require significant capital investment in heating equipment and facility modifications to withstand high temperatures for the required exposure period. High heat can cause raw and finished commodities to dry out, become rancid, degrade, and have affected handling characteristics. When possible, these commodities need to be removed or protected from exposure to high heat. High heat also can damage structural components, belts, electronics, lubricants, wood, plastics, lights, fire suppression systems, batteries, magnets, packaging, pressurized cans, and other heat-sensitive materials. When possible, these items need to be protected from high heat, removed prior to heating, replaced more often due to heat damage, or replaced with heat-resistant components. Heat does not distribute like fumigants and can stratify and sorb into concrete and other heat sinks. Temperatures need to be monitored throughout the heated space, and heat ducts and fans moved as needed to try to obtain homogenous heating. Heat also does not penetrate deeply into food residues, so sanitation prior to heating is important.

Importance of Pest Control for Food Safety

Postharvest pests such as cockroaches, mites, stored-product beetles, and moths can contaminate food with their body parts, cast skins, and feces. These contaminants contain allergenic materials that upon consumption cause IgE-mediated reactions ranging from dermal or respiratory reactions such as dermatitis and asthma to life-threatening anaphylaxis on sensitized individuals. These allergens are considered 'reasonably likely hazards' based on clinical reports proving allergenicity, evidence of ingestive effects, and recognition by medical authorities as an allergy hazard. Food products contaminated with these allergens may be subjected to a heating step in the manufacturing process; however, some of this allergenic material is heat-stable and can remain a threat to the consumer. All the earlier mentioned pests can be controlled by fumigation.

Cockroaches, including the American cockroach, *Periplaneta americana* (L.), and the German cockroach, *Blattella germanica* (L.), which can occur in food processing facilities and warehouses, have been shown to be capable of spreading foodborne pathogens. These pathogens include *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Staphylococcus* spp. Cockroaches can harbor pathogens in their bodies, especially in the lower digestive tract, for 14–16 days, and continue to excrete pathogens in their feces during that time. Postmortem survival of *Salmonella* in cockroach carcasses may last 60 days.

Conclusions

Fumigants are critical components in pest management programs because of their unique ability to diffuse throughout a common airspace in a commodity, substrate, or structure to eliminate a wide range of pests. The appropriate fumigant achieves this with no adverse effects to the treated commodity or structure. No other treatments provide such a broad spectrum of reliable control. Fumigants enable global trade of high-quality, pest-free commodities and are used to prevent food contamination by controlling pests that can transfer allergens and pathogens. Due to the toxicity of fumigants, fumigants will continue to receive intense regulatory scrutiny. Manufacturers and food industries will continue to work with regulators to validate that equipment and methods used for fumigant application meet required safety standards for workers, the public, and nontarget organisms.

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See also: Allergies: Public health; Cereals: Storage; Controlled Atmosphere Storage: Applications for Bulk Storage of Foodstuffs; Controlled Atmosphere Storage: Effect on Fruit and Vegetables; Foodborne Pathogens; Fungicides; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Insect Pests; Legal Requirements for Food Hygiene; Nuts: Health Effects; Pesticides and Herbicides: Residue Determination; Pesticides and Herbicides: Types of Pesticide; Pesticides and Herbicides: Types, Uses, and Determination of Herbicides; Pesticides and Herbicides; *Salmonella*: Properties and Occurrence; Shigella; *Staphylococcus*: Occurrence and Properties; Zoonoses.

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Functional Foods

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Introduction

During the last decades, the demand for health-improving foods and beverages has increased in parallel with the rising costs of health care, the increase in life expectancy, and the desire for higher life quality. In this context, functional foods offer a new kind of health message, promising specific effects by means of particular food components. A large number of definitions exist worldwide for 'functional foods,' referring to (processed) foods containing ingredients that beneficially influence specific body functions, in addition to being nutritious. However, we are dealing with a concept rather than a well-defined group of food products and a universally accepted definition is lacking.

Functional Foods: Origins and Definitions

The first written evidence of the existence of functional foods traces back to 1000 BC in China. Asia has a long tradition of attributing curative or therapeutic properties to foods and herbs, but such beliefs have been considered anecdotal and based on popular traditions. The term 'medical food' was frequently used in the literature of the Eastern Han Dynasty, about 100 BC. Another similar term, 'special foods,' was used in medical works of the Song Dynasty in AD 1000. With respect to the Western traditions, Hippocrates, the Greek physician who lived in VIth–Vth centuries BC, left a well-known phrase in his legacy: "Let food be your medicine and medicine your food." Even nowadays, this philosophy of food as a medicine is the basis of the paradigm of functional foods (see Figure 1).

Specific cases of functional foods were introduced in Japan in the 1980s. Three research programs were then funded by the Japanese government, consisting of the analysis and development of food functions, the analysis of physiological regulations of the food function, and the analysis of functional foods and molecular design. Those were national efforts to decrease the growing costs of health care. Consequently, Food for Specified Health Use (FOSHU) was established in 1991, as those foods were expected to have specific effects on health by addition of relevant constituents or removal of allergens. These effects were scientifically evaluated, and permission was granted to make claims regarding specific beneficial effects on health after their consumption, but only in the form of ordinary foods.

In the United States, the history of functional foods began in 1989 when 'designed foods' were formulated as foods that contained natural or enriched chemicals and biologically active nonnutrients from plants (phytochemicals) that were effective in reducing the risk of cancer. In 1989, the terminology of 'nutraceuticals' appeared, which were defined as substances that can be considered as food or part of a food and that provide medical or health benefits, including the prevention or treatment of a disease. Next, in 1991, 'functional foods' were defined as foods containing substances (natural or otherwise) for which there is evidence that, upon ingestion, a positive effect on health is provided. Since then, several exotic terms were launched, such as genetically designed food, pharmafoods, phytofoods, phytonutrients, phytogenic substances, performance foods, smart foods, therapeutic foods, foods with added value, genomic foods, phytochemical sources, superior foods, hypernutritive foods, and real foods.

In the European Union, the Concerted Action on Functional Food Science in Europe (FUFOSE) was coordinated by the International Life Science Institute through several meetings: France 1996, Helsinki 1997, and Madrid 1998. Finally, a consensus was reached in 1999, involving three major steps: (1) a critical assessment of the science base required to provide evidence that specific nutrients and food components positively affect target functions (biological responses) in the body, (2) an examination of the available science from a function-driven rather than a product-driven perspective, and (3) an elaboration of a consensus on targeted modifications of food and food constituents and on options for their applications.

According to the FUFOSE consensus document, a food can be regarded as 'functional' if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being or a reduction of risk of disease. Functional foods must remain foods and they must demonstrate their effects in amounts that can normally be expected to be consumed in the diet. Hence, they are not pills or capsules, but part of a normal food pattern. The beneficial effects could be either maintenance or promotion of a state of well-being or health or a reduction of the risk of a pathologic process or a disease.

A functional food can be a natural food or it can contain one or more specific components, which have functional influence on the health and well-being of the consumer. Such components can be added to food (e.g., omega-3 fatty acids), removed from food (e.g., fat from milk), naturally enhanced through special conditions (e.g., selenium-enriched garlic), or modified to provide health benefits (e.g., phytosterol-added probiotics). Importantly, functional foods do not necessarily induce a health benefit in all members of the population.

Overall, functional foods are often confused with the following related concepts:

(1) *Nutraceuticals*: products made from a food but sold as pill, powder, or other dosage forms not usually associated with food and shown to have beneficial or protective physiological properties against chronic diseases.
(2) *Pharmafoods*: a universally accepted term to designate products on the food–medicine borderline, which first emerged in Japan; they are also called 'border foods' as they claim drug-like preventive and therapeutic qualities.

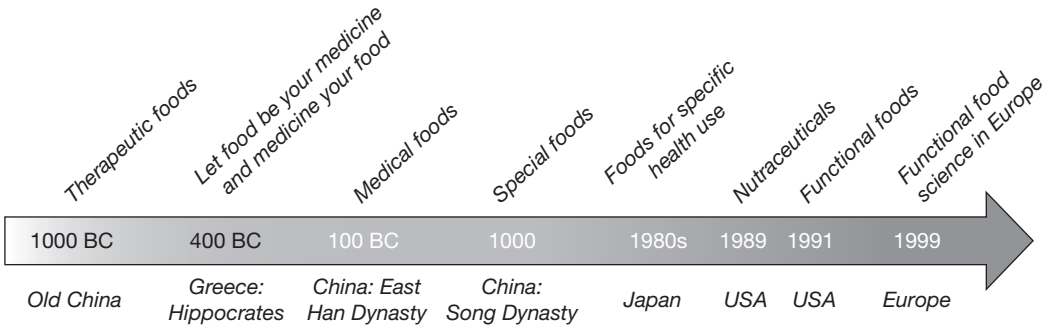

Figure 1 A historical picture of functional foods.

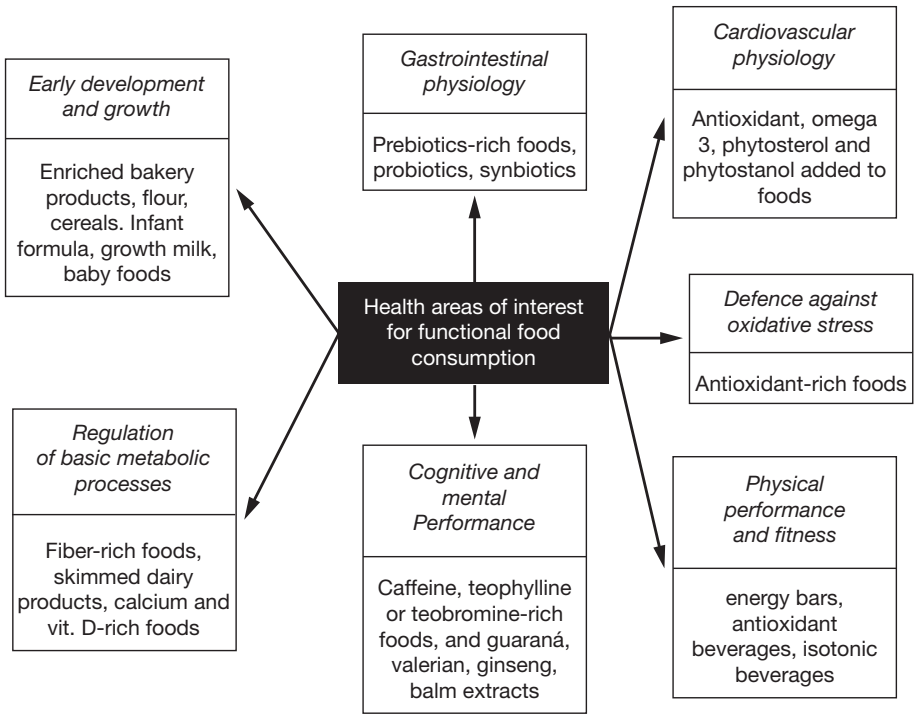

Figure 2 Health areas of interest for functional food consumption.

There are very few scientific publications on pharmafoods and even less about the possible interactions with drugs consumed by certain population sectors, such as children, pregnant women, and elderly people.

Potential Health Areas of Interest for Functional Food Consumption

Areas of human physiology that are relevant to functional foods are the following: early development and growth, regulation of basic metabolic processes, defense against oxidative stress, cardiovascular physiology, gastrointestinal physiology, cognitive and mental performance, and physical performance and fitness (Figure 2).

Early Development and Growth

Nutrition during early development has short-term effects on growth, body composition, and functions, as well as long-term effects. The course of pregnancy and childbirth and the composition of breast milk are influenced by the food intake. Pregnancy and the first postnatal months are a critical period for the development of the nervous system and sensory and cognitive abilities, as well as for the development of immune responses, bone mass, and intestinal growth and maturation. Therefore, several functional foods have been designed to provide an appropriate intake of nutrients to both mother and child, such as the addition of folic acid to bakery products, flour, and cereals to reduce neural tube defects in newborn infants. In addition, infant formula, growth milk, or baby foods are functional foods enclosed in this area.

Regulation of Basic Metabolic Processes

The control of glycemia and of food glycemic indexes and the control of the contents of starch, fiber, and fat are aspects of interest to prevent nontransmittable chronic illnesses as obesity, diabetes, dyslipidemia, and metabolic syndrome. Fiber-enriched foods or skimmed dairy products are functional foods enclosed in this area, but so is the fortification of foods with calcium and vitamin D as a strategy to improve bone mineral density.

Defense Against Oxidative Stress

Although oxygen is essential for human life, variable proportions of oxygen are daily converted in reactive oxygen species (ROS) in the mitochondria. ROS can alter the body structures through oxidative damage, that is, oxidizing proteins, peroxidizing lipids, modifying nucleic acids, and altering cell membranes. As such, they may be at the origin of illnesses as cardiovascular diseases (CVD), metabolic syndrome, anemia, several cancers, cataracts, decline of immune system, and degenerative diseases of the nervous system, as well as aging. The human body defends itself against ROS not only by means of endogenous antioxidants, such as superoxide dismutase, catalase, glutathione peroxidase, or glutathione reductase, but also by means of exogenous antioxidants, such as vitamins C and E, retinol and carotenoids, polyphenols, and minerals like selenium and zinc that are constituent of antioxidant metalloproteins. Antioxidant-rich foods can be consumed to enhance the body's antioxidant defenses and prevent or minimize several illnesses. Yet, an increase in the dietary antioxidant concentrations does not always lead to a decrease in illness and sometimes even to worrying pro-oxidative effects and cancer.

Cardiovascular Physiology

CVD are a major health concern in most countries. Major events are myocardial infarction, angina, stroke, and atherosclerosis. Most of the functional foods that promote cardiovascular health are designed to decrease fat contents from the original foods, mainly by decreasing saturated fatty acid or *trans* unsaturated fatty acid contents, as well as by adding omega-3 fatty acids, decreasing cholesterol contents, increasing phytosterol or phytostanol contents, or substituting cholesterol contents by phytosterols. Antioxidant-rich foods, including plant flavonoids, are also to be situated in this context of potential cardiovascular protection as they may inhibit LDL oxidation, modify the activities of immune-competent cells, and inhibit the formation of cell-to-cell adhesion factors. Recently, the use of vitamin K is being explored.

Gastrointestinal Physiology

The gut is an obvious target for the development of functional foods, because it is an interface between the diet and the body. The gut microbiota, together with the gastrointestinal immune system, provide the basis for a gut barrier that prevents pathogenic bacteria from invading the digestive tract and eventually the blood and the entire organism. Thus, preservation of the number and composition of the intestinal microbiota will be essential. Three strategies of functional foods have been addressed to promote healthier microbiota: prebiotics, probiotics, and synbiotics. Prebiotics are nondigestible food ingredients, mainly oligosaccharides, which stimulate growth and/or modify the metabolic activity of one or several bacterial species in the colon that potentially improve the gut health. They must not be hydrolyzed or absorbed in the upper part of the digestive tract, must be significantly transferred to the colon, and must be a selective substrate of probiotic microorganisms. Probiotics are live microbial food ingredients, mainly *Lactobacillus* and *Bifidobacterium*, combined or not, that, when ingested in sufficient quantities, have health benefits on the consumers by acting either directly or indirectly via interactions with the gut microbiota. Their major health benefits are alleviation of lactose intolerance and immune stimulation to reduce the incidence of digestive infections and also to reduce the incidence of precancerous lesions. Probiotics are only transient in the gut and do not become part of the gut microbiota. Therefore, regular consumption is necessary to maintain favorable effects. Synbiotics are mixtures of prebiotics plus probiotics addressed to increase survival of health-promoting bacteria in the gut. Probiotics are mainly – but not exclusively – applied in dairy foods, whereas prebiotics can be found in dairy products, breads and baked foods, salad dressings, and meat products.

Cognitive and Mental Performance

Some foods or food components could have effects on mood, cognition, or mental state, and these effects are characterized by a high degree of subjectivity, with large differences in response among people, mainly due to age and gender. Two types of effects can be distinguished: immediate effects (attention span, appetite, and satiety) and longer effects (changes in memory and mental processes). Carbohydrate-rich foods, through an elevation of glycemia, may influence mental performance by improvements in memory and decision time; however, high-carbohydrate meals could produce drowsiness. Generally, carbohydrates with a high glycemic index may lead to hypoglycemia due to excessive insulin responses. Yet, sucrose – the base of most of sweets – may also relieve distress and activate endogenous opioids, reducing pain perception. Tryptophan may decrease sleep latency and increase drowsiness and fatigue, likely due to its connection with serotonin metabolism. Alcohol affects most of the psychological and behavioral functions (stress, cognition, mood, and appetite), and its effects are dependent on the dose. Also, several food components, such as caffeine, theophylline, and theobromine, and plants, such as guarana, valerian, ginseng, and balm, show well-known effects on cognitive and mental performance.

Physical Performance and Fitness

Physical activity demands a high intake not only of energy but also of water, glucose, proteins, electrolytes, vitamins, and antioxidants. It is also well known that gastric emptying, and hence intestinal absorption, is influenced by physicochemical characteristics and temperature of foods. After a physical performance, it is necessary to replace glycogen reservoirs, enhance damaged muscular fibers, replace electrolytes and

water losses by sweating, and avoid oxidative stress and its subsequent mild hemolysis and immune cell depression that usually appear during and after the exercise. These changes are the rationale behind the development of functional foods such as energy bars, antioxidant beverages, or isotonic beverages.

Functional Food Consumption Worldwide

The biggest functional food markets are in Japan, Korea, and the United States, followed by Europe (see Figure 3). In the European Union, Spain, Finland, Cyprus, the Netherlands, and Sweden show high consumption of functional foods (see Figure 4). Obviously, consumer's acceptance and attitude toward functional foods determine the market size and success. While Americans accept and consume functional foods more easily, Europeans often seem to be more critical in their questioning of functional foods. It has been reported that Danish consumers have more negative attitudes toward functional foods than American and Finnish consumers. By contrast, Finnish consumers have the most positive attitudes toward functional foods. In addition, awareness of functional foods among French consumers seems to be lower than among Americans and Canadians.

Functional food consumption shows differences according to the age and gender of consumers. Functional foods like low-fat/skimmed milk, fruit juice, cereals, and probiotics are consumed by all age groups; however, cholesterol-lowering products and soy products are mostly consumed by adults and elders. Females are generally more interested in functional foods, but several products may be attractive for one or the other gender. Education has been pointed out as a factor contributing to healthy eating, and functional food users are usually more educated than the nonusers.

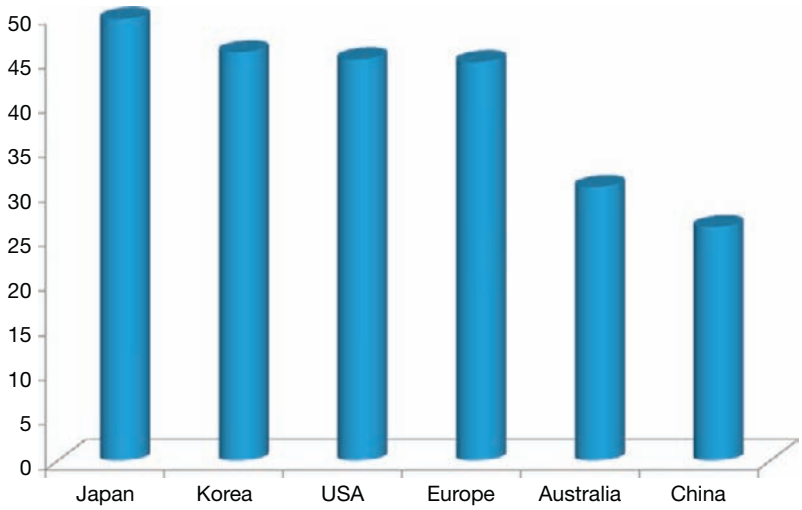

Figure 3 Proportion of functional food consumers worldwide.

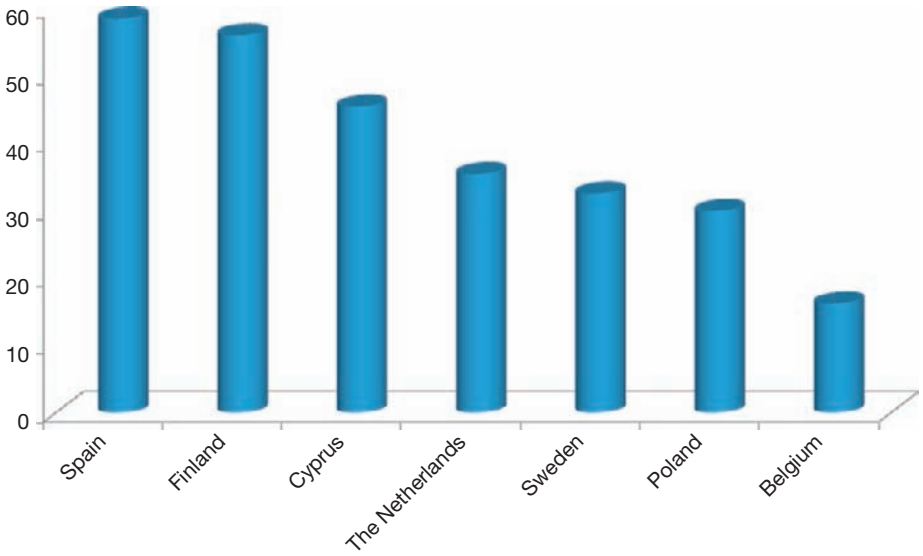

Figure 4 Proportion of functional food consumers in Europe.

Health status also may affect functional food uptake. Diet-related problems like high cholesterol, high blood pressure, and diabetes cause an increase in the consumption of cholesterol-lowering products. In several European countries, up to half of the consumers use functional foods to obtain a cholesterol-lowering effect. People with diet-related problems consumed cholesterol-lowering products in a higher proportion than those who did not have such problems; those with CVD consumed cholesterol-lowering products more frequently. Other main interests relate to blood sugar control and weight reduction.

Conclusions: Guide for Functional Food Consumption

Functional foods do not cure or prevent illnesses by themselves and are not essential to the diet. Functional foods must be viewed in the context of a healthy diet to exert their potential interest. Their inclusion in the diet may meet the following criteria:

- To incorporate functional foods in the diet, which add benefits according to the individual physiological state (pregnancy, lactation, physical activity, etc.).
- To consume functional foods in case of exclusion of certain foods by intolerance (e.g., fish, nuts, and milk), suboptimal intakes (e.g., vegetables and fruits), or low intakes (e.g., milk and dairy products and fish). In these cases, the inclusion of omega-3 fatty acid-enriched foods, vitamins or calcium-enriched milk and dairy products, or probiotics could help to lever the individual nutritional requirements.
- In chronic diseases (osteoporosis, CVD, obesity, diabetes, metabolic syndrome, etc.), the need of some nutrients may increase, and some bioactive substances contained in functional foods could exert some beneficial effects on health.
- The last consideration would refer to the preventive or health-promoting role of functional foods that, when included within a healthy diet, could help to get closer to optimal nutrition. Yet, this would require a considerable amount of further research using well-controlled methodology.

See also: Antioxidants: Characterization and Analysis; Beverage: Health Effects; Bioactive Peptides in Foods; Caffeine: Consumption and Health Effects; Fermented Foods: Fermented Milks; Food Fortification: Rationale and Methods; Genetically Modified Foods: Risk Assessment, Legislation, Consumer Behavior, and Ethics; Low-fat Foods: Types and Manufacture; Low-salt Foods: Types and Manufacture; Yogurt: Yogurt Based Products.

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Fungi

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Introduction to the Fifth Kingdom

In 2000, Bryce Kendrick introduced his book *The Fifth Kingdom*, inviting readers to "Imagine the picnic of your dreams. You and your loved one flat across the flower-filled meadow, coming to rest under the shade of a giant white pine. There you spread out the magic ingredients – the champagne (or wine, or beer if you prefer), the fresh crusty bread, the paté au truffle, the creamy camembert (or brie or roquefort) cheese. You may add other ingredients – many spring to mind – but up to this point your tryst is a tribute to the beneficial influence of fungi on our diet and our scenery."

Fungi make an enormous contribution to our daily lives. They represent a heterogeneous assemblage of eukaryotic organisms and are considered as a particularly versatile group with respect to their biotechnological applications in the areas of medicine, food production, pollution control, and agriculture. Recent studies on fungal biochemistry, plant pathology, medical mycology, and systematics are supported by progresses in genetics and molecular biology. Beginning from the end of the last century, an increased utilization of fungi as producers of recombinant proteins with therapeutic potential is seen. Due to their basic characteristics, fungi represent a special model for genetic investigations. These organisms can be easily cultured thanks to their ability to grow on a relatively simple medium. Also, their stable haploid phase can mutate and, in the presence of a sexual phase, mutants can be combined. Finally, due to their eukaryotic nature, they show many of the characteristics of human cells and can thus be used as a more useful model for cellular processes than bacteria.

From an ecological and environmental point of view, fungi play an important role as organic matter decomposers, but they can also cause disease in plants. Moreover, they can be used as beneficial fungi for agricultural purposes, in both organic or integrated crop protection approaches. Additionally, some harmful fungi such as *Candida* and *Aspergillus* are frequently associated with infections affecting immune-compromised patients, whereas other genera are known as environmental contaminants.

Once considered as primitive members of the plant kingdom, true fungi actually belong to the Fifth Kingdom that includes the subkingdom Dikarya (containing the phyla Ascomycota and Basidiomycota, i.e., fungi in which part of the life cycle is characterized by cells with paired nuclei), as well as the phyla Chytridiomycota and Zygomycota, which previously included the Glomeromycota, a group of fungi that forms an association with the roots of most plants. A group of parasitic organisms called Microsporidia, living inside animal cells, are now also included in the fungal kingdom, being considered a phylum basal to all fungi. Other fungi traditionally placed within the Zygomycota are distributed among several subphyla *incertae sedis* including Mucoromycotina, Entomophthoromycotina, Kickxellomycotina, and Zoopagomycotina. However, the classification system is still under modification as scientists increasingly rely on new methods. For example, Cryptomycota were described as a potentially new phylum within the kingdom.

Most fungi are filamentous, many grow as unicellular yeasts, and some primitive fungi as the Chytridiomycetes grow as individual rounded cells or dichotomous branched chains of cells. Dimorphic fungi can switch between the filamentous phase and the yeast phase in response to environmental conditions. The cells of filamentous fungi grow as an interconnected network of tubular, elongated, and threadlike structures called hyphae. The hyphae of higher fungi extend via tip (or apical) growth, which supports the invasive growth of saprobes and pathogens, facilitates long-distance transport in symbiotic mycorrhizal fungi, and, through aggregation, forms the basis for the development of multicellular reproductive structures.

The hyphal cell wall, composed by a combination of glucans and chitin, is a sort of exoskeleton that defines the shape and growth and protects the fungal protoplasts from the external environment. Some fungal species can grow as unicellular yeasts reproducing by budding or binary fission. In yeasts, the cell wall structure comprises polysaccharides such as β-glucans and proteins, mainly mannoproteins.

Fungi and yeasts have relatively simple nutritional needs and are metabolically heterotrophic. They can assimilate organic carbon and nonelemental sources of nitrogen. Sugars, from the simplest glucose to complex polysaccharides such as cellulose and lignin, are widely utilized for growth. Fungi cannot fix nitrogen and need to be supplied with nitrogen-containing compounds, either in inorganic form (e.g., ammonium) or in organic form (e.g., amino acids). In addition to urea that can be assimilated by some basidiomycetic yeasts, many fungi can grow on nitrate and nitrite being equipped with nitrate and nitrite reductases that allow conversion to ammonium.

Fungal cells characteristically display external digestion due to the release of many different enzymes and imbibe, rather than relying on the ingestion of nutrients. Their ability to absorb and grow on many substrates and to convert them in important secondary metabolites is noteworthy. Such versatile metabolic capability has attracted much interest towards fungi in view of their potential application in natural product chemistry and in the production of antibiotics and other bioactive metabolites. In addition, many fungi are currently used in several fermentation processes that lead to products of agro-food interest such as wine, beer, soy sauce, and several other food commodities.

Fungi are mostly haploid and can reproduce sexually and asexually through spores. Asexual spores, representing the end of vegetative growth, are produced by mitosis, whereas sexual spores, produced at the end of the meiotic division, can be produced upon or within specialized structures, sometimes closed in fruit bodies. Forms, details, method of production,

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00341-X

release, and dispersal of spores represent valid tools used to describe most fungal taxa.

For further information about detailed taxonomic, physiological, and metabolic aspects of these organisms, we refer to the series *The Mycota*, a comprehensive treatise on fungi as experimental systems for basic and applied research.

Fungal Plant Pathogens

Losses due to plant diseases have a dramatic impact on both the economical conditions of growers and the availability of food in areas where people's survival depends on plant production. Plant diseases are caused by both abiotic and biotic factors. Among the biotic factors, fungi are an important group of plant pathogens, since more than 10 000 fungal species have been identified as causing agents of plant diseases. They include obligate parasites (biotrophs) that need to live on their host plants for their whole life and nonobligate parasites that require a host plant for part of their life cycle but can complete their cycle on dead organic matter. Most biotrophs are generally specialized in the targeting of a limited number of plant hosts, while necrotrophic fungi can either grow on a wide range of host species or be specialized on a restricted range of hosts. Moreover, some fungi, defined as endophytes, are hidden inside their plant hosts in symbiotic association. They do not show any symptom of disease and confer tolerances to several kinds of biotic and abiotic stresses to their host plant. Other fungal plant pathogens can affect the level of growth of their hosts by inducing them to produce higher amounts of regulators or producing growth regulators themselves. The effects on plants of this overproduction include the development of cankers, galls, witches' broom, leaf curl, and stunting.

Fungal plant pathogens can also be distinguished based on the stage and part of the plant host that is attacked: seeds, seedlings, or adult plants and roots, leaves, shoots, stems, woody tissues, fruits, or flowers, respectively. Some fungi that kill seedlings can often also infect the roots of mature plants, mainly through wounds, and can cause lesions or death of a part or all of the root system and crown. Vascular wilt pathogens kill their host by infecting the plant through the roots or through wounds and growing into the xylem. The first symptoms of vascular wilt are a loss of turgidity in the plant leaves, often on one side of the plant or on a single branch, and vascular discoloration.

Leaf spot pathogens infect through natural plant openings such as stomates or by penetrating directly through the host cuticle and epidermal cell wall. In order to penetrate, fungi produce hydrolytic enzymes such as cutinases, cellulases, pectinases, and proteases, thus breaking down the host tissue. Specialized structures called appressoria, formed at the end of germ tubes, are used by other fungi as an alternative way to penetrate cell walls. Once inside the plant leaf, the fungus must obtain nutrients from the cells, which is often accomplished by killing host cells in case of necrotrophs. Many leaf-spotting fungi produce toxins that kill host cells, and this often produces a lesion surrounded by a yellow halo. If enough of the leaf surface is killed, or if the infected leaves drop prematurely, the photosynthesis activity of plants can be dramatically affected.

Other diseases, such as cankers, develop when the pathogen kills the phloem and vascular cambium in a woody host, causing death of the plant part that is encircled.

Rust fungi, so named because of the abundant orange spores formed on plants infected, are biotrophic pathogens that can infect, grow, and sporulate in living plant tissues. They can be devastating pathogens by reducing the photosynthetic surface and increasing water losses in the host plant. Rust fungi attack a wide range of plants and often require two different hosts in order to complete their life cycles. Finally, some diseases caused by fungi make plant products unfit for human and animal consumption since they produce toxic metabolites (mycotoxins) that contaminate field products, especially grains. These fungi are called mycotoxigenic fungi and will be discussed later.

Mycotoxigenic Fungi

The need of ensuring food safety to consumers is considered a main issue at worldwide level. Problems related to several kinds of food contamination harmful for human and animal health have been increasing in the recent years. Globalization and the development of an exchange-based worldwide economy have deeply influenced and enlarged the food market. However, at the same time, the increased marketing of food products increased the exposure to natural and chemical contaminants.

Among the emerging issues in food safety, the increase of plant diseases associated to the occurrence of mycotoxigenic fungal species is of major importance. As a result of their secondary metabolism, these fungi can produce mycotoxins, which are low-molecular-weight toxic compounds that represent a serious risk for human and animal health worldwide. They occur naturally and are the most prevalent source of food-related health risks in field crops, where approximately 25% of the global food and feed crop output is affected. Due to their broad range of biological activities, the consumption of mycotoxin-contaminated foods is a main issue in food safety worldwide. This is particularly so since a large proportion of the world's population consumes cereals as a staple food, which are the main targets for colonization by toxigenic fungi. The mycotoxin contamination of crops is generally regulated by two main factors: susceptibility of the host plant, on the one hand, and the geographic and climatic conditions, on the other hand. Mycotoxins are produced on the plants before the harvest due to toxigenic fungal contamination in the field and also at the postharvest stage, encompassing stages of the food chain (i.e., storage, processing, and transportation). Moreover, mycotoxins can also be accumulated in animal by-products, due to a carryover effect, as a consequence of the use of highly contaminated feed.

Main mycotoxin-producing genera are *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium*. Most of the mycotoxigenic species can produce more than a single mycotoxin, but a given mycotoxin can be produced by species that belong to different genera (Table 1). Factors that increase the stress status in plants, such as a lack of water and an unbalanced absorption of nutrients, and therefore reduce their immune system, can lead to a higher exposure to mycotoxin contamination. In addition, specific climatic conditions and environmental

Table 1 Main mycotoxigenic fungal species and genera contaminating food

Genus/species	*Main host plants*	*Mycotoxins*	*Food*
Alternaria			
A. arborescens	Wheat, tomato	AOH, AME, ALT, AAL toxin	Wheat by-products, tomato juice, and puree
A. tenuissima	Wheat	TeA, ATX-I, AOH, AME	Wheat by-products
A. alternata	Wheat, tomato	AOH, AME, ATX-I, ATX-II, ATX-III	Wheat by-products, tomato juice, and puree
A. infectoria	Wheat	Dibenzo-α-pyrones	Wheat by-products
Aspergillus			
A. carbonarius	Grape	OTA	Wine and grape by-products
A. ochraceus	Coffee	OTA	Coffee
A. flavus	Maize, nuts	AFB_1, AFB_2	Maize by-products, dried fruits
A. niger complex	Grape	FB_2, OTA	Wine and grape by-products
A. parasiticus	Maize	AFB_1, AFB_2, AFG_1, AFG_2	Maize by-products
Fusarium			
F. culmorum, *F. graminearum* complex	Wheat	DON	Wheat by-products
F. equiseti/*F. incarnatum* complex	Wheat	ZEA	Wheat by-products
F. poae	Wheat	NIV	Wheat by-products
F. sporotrichioides, *F. langsethiae*	Wheat	T-2, HT-2	Wheat by-products
F. verticillioides, *F. proliferatum* (*F. moniliforme*)	Maize	FB_1, FB_2	Maize by-products
Penicillium			
P. expansum	Apple	PAT	Apple juice
P. verrucosum	Wheat	OTA	Wheat by-products

AOH, alternariol; AME, alternariol monomethyl ether; ALT, altenuene; TeA, tenuazonic acid; ATX, altertoxin I, II, III; OTA, ochratoxin A; AFB, aflatoxin B (B_1 and B_2); AFG, aflatoxin G (G1 and G_2); DON, deoxynivalenol; ZEA, zearalenone; NIV, nivalenol; T-2, T-2 toxin; HT-2, HT-2 toxin; FB_1, fumonisin B (B_1 and B_2); PAT, patulin.

factors, as temperature and humidity, can influence the growth of mycotoxigenic fungi and eventually stimulate their ability to produce mycotoxins.

The *Alternaria* Genus

The *Alternaria* genus (*Pleosporales*, *Pleosporaceae*) includes species that are diffused worldwide, mainly as soil saprotrophs, and that can also survive on plant residues. Among different crops (e.g., oleaginous plants, vegetables, and fruits), *Alternaria* species can colonize the kernels of several minor cereals, especially wheat, barley, and sorghum, and release a number of toxic metabolites. *Alternaria* species cause postharvest decay of many vegetable products along the whole value chain, from the field, transportation, intermediate storage and processing, to the final (chilled) storage. Indeed, these organisms withstand low temperatures and can be responsible for the rot of several products during storage in refrigerators. Taxonomy of *Alternaria* spp. is not completely clear yet even though different types of molecular analysis have been used to support morphology. *Alternaria* species produce 71 known mycotoxins and phytotoxins, but only few occur naturally in food. They are divided in three main chemical groups: the dibenzo-α-pyrones, which include alternariol (AOH), alternariol monomethyl ether (AME), and altenuene (ALT); the tetramic acid group, including tenuazonic acid (TeA); and the altertoxin (ATX) group, of which the main members are ATX-I, ATX-II, and ATX-III. Moreover, other important relevant *Alternaria* mycotoxins are the AAL toxins and stemphyltoxin III, which are toxins produced in large amounts at the preharvest crop stage. Several of these *Alternaria* mycotoxins can have adverse effects on humans such as salivation, vomiting, erythema, convulsions, and gastrointestinal hemorrhage. However, due to a poor knowledge of the extent of their occurrence in food commodities, regulatory limits are still lacking.

The *Aspergillus* Genus

The genus *Aspergillus* (*Eurotiales*, *Trichocomaceae*) includes ubiquitous fungi that colonize a wide spectrum of habitats and are very common on stored grains and a wide range of foodstuffs. *Aspergillus* species have been taxonomically divided in sections that group closely related species. In particular, the sections *Flavi* and *Nigri* include species that colonize a wide range of plants of agrofood interest, where they can produce highly harmful mycotoxins such as aflatoxins, ochratoxins, and, to a very limited extent, fumonisins.

Aflatoxins (AFLs), including B_1, B_2, G_1, and G_2, are potent carcinogenic mycotoxins produced in a wide range of agricultural commodities such as maize, peanuts, and dried figs. The International Agency for Research on Cancer (IARC) classified aflatoxin B_1 as a group 1 carcinogen to humans. In addition to hepatocellular carcinoma, AFLs are associated with occasional outbreaks of acute aflatoxicosis leading to death shortly after exposure. The main species of concern for AFL contamination are *A. flavus* and *A. parasiticus*. The ability to produce AFL is highly conserved in some species but is highly variable in others. Almost all *A. parasiticus* strains produce AFLs, with only 3–6% of nonaflatoxigenic strains. In contrast, toxigenicity in populations of *A. flavus* is reported to be extremely diverse in terms of morphology and toxigenicity, and incidence of nontoxigenic strains depends on the strain, substrate, and geographic origin.

Ochratoxin A (OTA) is produced mainly by black aspergilli and *A. ochraceus* (occurring mainly on cereals and coffee beans) and acts as a potent nephrotoxin. It may be involved in the etiology of the human disease called Balkan endemic nephropathy and in the development of urinary tract tumors in humans. In 1993, IARC classified OTA as a possible human carcinogen (group 2B) and has been found in several commodities such as wine, grape juice, dried figs, rice, and coffee beans. The *Nigri* section, including species as *A. carbonarius* and *A. niger*, can often occur on grapes (Figure 1) and grape products where they can produce OTA. However, while *A. carbonarius* is a high and consistent producer (around 100% of the strains), only a limited percentage of strains of *A. niger* produce OTA.

Finally, fumonisins are also of health concern because they disrupt sphingolipid metabolism, can cause multiple diseases in experimental rodents and livestock, and are epidemiologically associated with several human diseases, including esophageal cancer. Some black aspergilli were recently shown to produce fumonisins, in particular species belonging to the *A. niger* aggregate. Therefore, the high occurrence of black aspergilli on raisins and grape must raise food safety concerns, because of the potential contamination of wines and grape by-products.

The *Fusarium* Genus

The genus *Fusarium* (*Ascomycetes*, *Hypocreales*) comprises a high number of plant-pathogenic species causing diseases in several agriculturally important crops, including cereals. Many of them produce a wide range of mycotoxins with an extraordinary chemical diversity. The ability of various *Fusarium* species to produce different classes of secondary metabolites combined with their ability to coexist in the same host and/or occur in quick succession has allowed these complexes to become 'invincible armadas.' Plant infections by *Fusarium* can occur at all developmental stages, from germinating seeds to mature vegetative tissues, depending on the host plant and *Fusarium* species involved.

Among the mycotoxins produced by *Fusarium* species, only a small number are considered important for human and animal health, including the trichothecenes and fumonisins that are under strict legislative control worldwide. Readers are referred to the excellent book *Fusarium Mycotoxins: Chemistry, Genetics and Biology*, compiled by Desjardins, for a complete review of *Fusarium* mycotoxins.

The trichothecenes are a family of epoxysesquiterpene metabolites, conveniently subdivided in two classes, based on their chemical structures: type A and type B, of which T-2 toxin and deoxynivalenol (DON) are the most common mycotoxins, respectively. The T-2 toxin is one of most acutely poisonous ones, produced mainly by *F. sporotrichioides* and *F. langsethiae*, two pathogens commonly occurring on minor cereals (e.g., barley and wheat). The illness associated with the consumption of cereals highly contaminated by T-2 includes nausea, vomiting, necrotic lesions, severe hemorrhagic diarrhea, and hemorrhagia in many of the body organs. The T-2 toxin is also a potent immunosuppressant because it causes irreversible damage to the bone marrow, leading to a characteristic reduction in white blood cells (aleukia). DON, which is the most important type B trichothecene, is much more common than the T-2 toxin in cereals such as wheat, barley, oat, and maize. It is produced primarily by *F. graminearum* and *F. culmorum*, the causal agents of *Fusarium* red ear rot in maize and *Fusarium* head blight (FHB) in wheat. DON is also known as vomitoxin because of its potent emetic properties and its action as a feed refusal factor and may still have a biological effect at very low concentrations. The acute phytotoxicity of trichothecenes and their occurrence in plant tissues suggest that these mycotoxins play a role in the pathogenesis of *Fusarium* in plants.

Fumonisins are a family of mycotoxins produced mainly by *F. verticillioides* and *F. proliferatum*, which are both very important pathogens of maize, where they cause the so-called *Fusarium* pink ear rot (Figure 2), and colonize a wide range of

Figure 1 Rotted grape berries colonized by black *Aspergilli*.

Figure 2 Rotted maize ear colonized by *Fusarium* spp.

agriculturally important plants worldwide. Fumonisins can cause the death of horses by massive necrosis of brain tissue, that of pigs by chronic accumulation of fluid in the lungs, and that of rats by necrosis of the liver. Moreover, they may also be the etiologic agents of esophageal carcinoma in humans. In 1993, the IARC evaluated these toxins as group 2B carcinogens. The role of fumonisins in phytotoxicity, pathogenesis, and other aspects of *Fusarium* ecology is still unclear.

The *Penicillium* Genus

The genus *Penicillium* (*Eurotiales*, *Trichocomaceae*) is maybe the most spread fungal genus in nature. Many of its species are ubiquitous and colonize a wide range of habitats. Generally, *Penicillium* species are considered saprotrophic fungi living on plant parts, soil, decaying organic substances, and plant residues. Moreover, many of the most common *Penicillium* species are associated not only with stored food and feed but also with animal dung and building materials, indoor air, and several other habitats. With respect to other mycotoxigenic genera, *Penicillium* species are less adapted to high temperatures. Many *Penicillium* show psychrophilic behavior and can therefore contaminate food stored at low temperatures, being able to grow at temperature around 0 °C. Some *Penicillium* species are plant pathogens acting on several fruit trees and producing harmful mycotoxins that can accumulate on several food products.

The taxonomy of this genus is difficult, mainly because of the enormous variability within the genus. Therefore, a polyphasic approach based on phenotypic characteristics (morphology and physiology), phylogeny, and secondary metabolite profiles (extrolites) is currently applied to create sections, which are useful taxonomic tools for the ecological and phylogenetic grouping of consistently related species. The genus *Penicillium* includes around 100 toxigenic species, and the range of known mycotoxins produced is wider than those of other toxigenic fungal genera such as *Aspergillus* and *Fusarium*. The *Penicillium* subgenus *Penicillium* includes the highest number of toxigenic species able to produce a large number of bioactive extrolites, including several mycotoxins. OTA is produced only by *P. verrucosum* and *P. nordicum*, with the first species being very common on cereals, mostly in cold environments, and the second species being a known contaminant of protein-rich foods, primarily found on dry-cured meat products.

Patulin, another important mycotoxin produced by *Penicillium* species, is a water-soluble polyketide lactone that occurs most often in apples spoiled by *P. expansum* or in products made from spoiled apples, such as apple juice, pies, and conserves. It has also been found in other fruits, including pears and grapes, and in vegetables, cereals, and cheese. Most of the information on the toxicity of patulin is derived from animal studies. At relatively high doses, patulin is acutely toxic in mice and rats, causing gastrointestinal lesions, distension, and hemorrhagia in the stomach and small intestine. It has been suggested that patulin is carcinogenic at low levels in the diet, but IARC has stated that there is insufficient evidence of carcinogenicity in animals or humans, other than at extremely high doses. Many other mycotoxins are produced by species of *Penicillium*. The most common are citrinin, considered nephrotoxic, penicillic acid, cyclopiazonic acid, citreoviridin, and PR toxin.

Beneficial Fungi

The consequences of the widespread and repeated use of chemical products in crop protection to control pests, plant pathogens, and weeds have become clear in recent years. It is generally recognized that some chemical pesticides contaminate groundwater and enter food chains, with an impact on a wide range of organisms. To make matters worse, fungicide-resistant plant pathogens have been developed as a result of the exasperated use of chemicals. Regardless of these problems, there is an urgent and pressing demand for food production in order to reply to an increasing world population. Consumers constantly require food and feed of high quality, cultivated according to an environmentally friendly framework and without chemical residues. All of this cannot be separated from the implications of new legislation on organic farming or the enforcement of improved integrated pest management strategies. The EU, for instance, has set rules for the sustainable use of pesticides to reduce the risks and impact of pesticide use on people's health and the environment.

From this point of view, exploitation of fungi as beneficial organisms is of increasing interest and trendsetting. Biological control via fungi is an exciting and rapidly developing research area with implications for plant productivity, animal and human health, and food production.

Many fungi can be used as biocontrol agents for plant disease management. The most extensively studied ones are *Trichoderma* spp., together with the more recently described *Sebacinales* genus. These fungi can control many different foliar, root, and fruit pathogens and dangerous organisms such as nematodes. Due to their excellent adaptability, broad spectrum, diversified mechanisms, fast growth, and simple nutritional requirements, *Trichoderma* spp. actually represent one of the most common active principles of many globally used commercial products. In addition to classical mechanisms, such as the production of inhibitory and lytic substances, mycoparasitism (Figure 3), and competition for nutrients and sites of infection, the most appealing aspect of these fungi is the ability to act as inducers of resistance in the host plants against biotic and abiotic stresses, in addition to physiological stresses such as seed aging. Many other positive effects of *Trichoderma* spp. on plants, such as the improvement of photosynthetic and respiratory efficiency, the enhancement of nutrient uptake, and the increase of nitrogen use efficiency, are considered as important as the disease-controlling aspect.

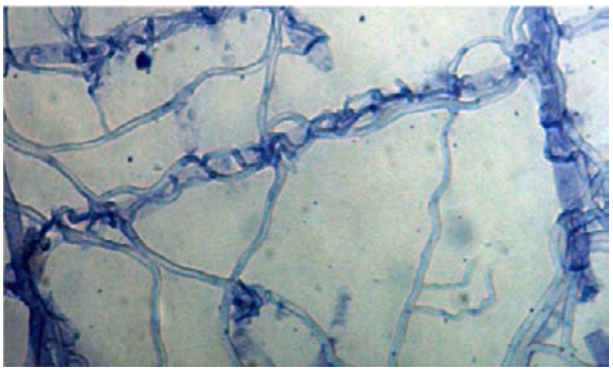

Figure 3 Coiling by *Trichoderma* spp. around hyphae of *Rhizoctonia solani*.

The most studied species within the *Sebacinales* is *Piriformospora indica*, able to promote plant growth due to the capability of colonizing host roots. This species is also able to induce resistance by upregulating some defense-related genes.

One of the most fascinating and important properties of fungi is the ability to produce a wide variety of secondary metabolites displaying a wide range of biological activities. Fungi commonly live in competitive environments, and it is generally accepted that their secondary metabolites play an important role in their ecology, with the most straightforward effect appearing in the regulation of the competitive and/or antagonistic interactions among fungal species. With their antifungal activity, many of these secondary metabolites have attracted great attention in order to satisfy the increasing need for new antifungal agents, not only in medicine but also in agriculture where new useful fungicides are being continuously sought for.

In this perspective, coprophilous fungi, a large group of saprotrophic fungi including more than 1200 species, mostly recorded from herbivorous dungs, receive increasing interest from both an ecological point of view and a biotechnological point of view. Taxonomy of these fungi is continuously being updated and unusual new species continue to be discovered: these fungi represent a productive reservoir of antibiotics, mostly of unknown structure and activity. Next-generation sequencing and automated gene prediction can preview the biosynthetic repertoire of these fungi, anticipating pathways for known and unknown antibiotics.

Beneficial Fungi and Food Safety

Mycotoxigenic fungi, such as *Aspergillus*, *Fusarium*, and *Penicillium*, pose serious problems and toxicological risks at preharvest and postharvest stage as well as in processed food products. Biological control using beneficial fungi (filamentous and yeasts) allows managing contamination by mycotoxins at both pre- and postharvest levels and can contribute to decontamination and/or detoxification of these dangerous compounds from food and feeds.

For instance, atoxigenic isolates of *Aspergillus* are widely used to prevent aflatoxin contamination of crops, such as nuts and maize, in several parts of the world. Field experiments have demonstrated that *Trichoderma gamsii* and *Fusarium equiseti* can reduce FHB incidence and DON contamination on wheat. The yeastlike *Aureobasidium pullulans* or the yeast *Metschnikowia fructicola* are able to reduce OTA contamination by *Aspergillus* sp. on grape and can degrade OTA to a less toxic compound *in vitro*.

Finally, patulin- and OTA-producing fungi on apples and pears can be controlled by the application of *Aureobasidium pullulans* or *Candida sake*, resulting also in a reduction of mycotoxin contamination.

Beneficial Fungi and Food Quality

Additional positive effect deriving from the application of beneficial fungi in plants concerns the improvement of nutritional factors in derived foods. An example is provided by the arbuscular mycorrhizal fungi (AMFs, Glomeromycota), able to establish a symbiotic relationship with the roots of most terrestrial plants. Interaction between plants and mycorrhizal fungi increases the crop yield and the disease resistance and also improves the intake of minerals and compounds with antioxidant properties. In lettuce, AMFs improve growth and nutritional quality (mineral nutrients, total soluble sugars, ascorbate contents, and quantities of anthocyanins, carotenoids, and phenolics). In tomato, this symbiosis enhances the nutritional and nutraceutical value of fruits by modifications of plant secondary metabolism that lead to increased levels of lycopene.

Bioactive fungal polysaccharides represent potential ingredients in food and nutraceuticals. Fungal polysaccharides comprise a large group of biopolymers mostly derived from edible mushrooms (maitake or shiitake) or yeasts. These polysaccharides are characterized by potential immune-modulating, anticancer, antimicrobial, hypocholesterolemic, hypoglycemic, and health-promoting properties. The fact they are obtained by edible mushrooms and yeasts makes them good candidates for the formulation of novel functional foods and nutraceuticals without serious safety concerns.

Conclusion

The fungal kingdom represents an impressive example of genetic biodiversity including organisms that can, from a human perspective, be either beneficial or harmful to human beings. Fungi can be considered as a perfect example of organisms that keep ecological balance and help to preserve the Earth from certain destructive human interventions.

See also: Fungicides; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto.

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Fungicides

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Introduction

Crop production is vulnerable to different pests and plant diseases and most of them are related to pathogenic fungal diseases. Fruits and vegetables are highly susceptible to fungal spoilage, both in the field and during postharvest storage. Significant fungi genera include *Pythium*, *Phytophthora*, *Fusarium*, *Penicillium*, *Alternaria*, *Botrytis*, *Geotrichum*, *Sclerotinia*, and *Rhizoctonia* spp. Important agricultural commodities, such as grain crops, are also venerable to fungal contamination particularly by *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* spp. In grains, molds are responsible for off-flavor formation and contribute to loss in dry matter through the utilization of carbohydrates as an energy source, degradation of lipids and proteins, and production of volatile metabolites and of allergenic compounds. Infection by *Botrytis cinerea*, *Plasmopara viticola*, and *Uncinula necator* is a significant problem in grape production worldwide.

Fungicides have been used for over 200 years to protect plants against diseases caused by fungi attack. From the remote beginnings, mainly to protect cereals and grapes, the number of crops and crop diseases treated, the chemicals available, the area and frequency of their use, and the effectiveness of treatments have increased enormously. Curiously, two of the first established products, copper-based formulations and sulfur, are still used widely and effectively, while several chemical families, such as phthalimides, dithiocarbamates (DTCs), dinitrophenols, and chlorophenyls, have been successfully used for more than 40 years. In the late 1960s and 1070s, a large number of more potent fungicides, of novel structure and mostly with systemic activity, were introduced, including 2-amino-pyrimidines, benzimidazoles, carboxanilides, phosphorothiolates, morpholines, dicarboximides, phenylamides, and sterol demethylation inhibitors (DMIs). Systemic fungicides have gradually replaced the older nonsystemic products, establishing higher levels of disease control.

In the 1980s, mostly analogs of existing fungicides were introduced, particularly DMIs, with improved properties. Since then, however, a number of novel compounds have been introduced commercially, including phenylpyrroles, anilinopyrimidines, quinone outside inhibitors (QoIs, including strobilurin analogs), benzamides, carboxylic acid amides (CAAs), phenoxyquinolines, oxazolidinediones, spiroketalamines, and scytalone dehydratase inhibitors, among others. Some recently developed products – unique in that they do not directly affect the pathogen itself – elicit a response from the host plant known as 'systemic acquired resistance' (SAR). These compounds basically mimic chemical signals in plants, activating plant defense mechanisms such as the production of thicker cell walls and antifungal proteins. The utility of SAR inducers has been limited so far, as many pathogens are capable of overcoming such defenses.

Fungicides remain vital for the effective control of plant diseases, which are estimated to cause yield reductions of almost 20% in the major food and cash crops worldwide. In this article, some of the most important issues related to fungicides, such as fungicide classification, environmental fate, reduction of fungicide residues in food, effects on health, maximum residue levels, and analytic methods for their determination, are discussed, from the viewpoint of analytical chemists.

Fungicide Classes

Fungicides can be classified according to different criteria but perhaps the most usually employed are the mode of action and the chemical group. Fungicides can also be classified according to where in the disease cycle they are active: (1) protectant or preventive, if they are effective prior to infection and the initiation of the disease cycle; (2) curative, fungicides that are effective against the fungus growing in the leaf tissue post the infecting spore germination; or (3) eradicant or antisporulant, that is, fungicides that are capable of stopping sporulation by the pathogen.

Fungicides kill fungi by damaging their cell membranes, inactivating critical enzymes or proteins, or by interfering with key processes such as energy production or respiration. Others impact specific metabolic pathways such as the production of sterols or chitin. When low concentrations of fungicides damage the cell, activation of one or more stress response pathways allows the cell to respond to the damage and survive. However, higher concentrations generally overwhelm the fungal stress response system leading to inhibition of growth or cell death.

A list of fungicides organized by the mode of action that also includes data on the target site, chemical group and name, and resistance risk of each compound is maintained by the Fungicide Resistance Action Committee (FRAC). All fungicides within a chemical group share a common mode of action and resistance mechanism, even though their chemical structures may be different. Two exceptions in the FRAC classification scheme are the 'multisite activity' fungicide class and the 'unknown' class of fungicides with unknown modes of action.

In this section, some of the most important fungicide chemical families are briefly discussed, given that from the food safety perspective, namely, in the development of analytic methods, this is one of the most pertinent classifications used.

Inorganic Compounds

Sulfur is a widely used fungicide by both conventional and organic farmers. It is one of the top pesticides in terms of quantities used accounting for more than 25%, both within the European Union (EU) and in California.

Copper-based fungicides, such as the Bordeaux mixture, $CuSO_4 + Ca(OH)_2$, have been intensively used in Europe since the end of the nineteenth century to control vine (*Vitis vinifera* L.) fungal diseases, such as downy mildew caused by *P. viticola*. Additionally, other copper compounds have been introduced including copper oxychloride ($3Cu(OH)_2 \cdot CuCl_2$), among others. Besides vineyards, copper-based fungicides have also been extensively used in hop fields and coffee, apple, and avocado orchards and in the cultivation of vegetables (e.g., tomatoes and potatoes).

Copper used to be one of the most applied pesticides, but it is currently used to a much lesser extent than sulfur. Sulfur is generally considered to be of low risk to humans, animals, and the environment, while copper is considered to have potentially toxic properties for certain nontarget organisms.

Dithiocarbamates

DTCs are a group of organosulfur fungicides characterized by a broad spectrum of activity against various plant pathogens, low acute mammal toxicity, and low production costs. Thus, DTCs are widely used in agriculture especially in combination with modern systemic fungicides to control resistances and to expand the spectrum of activity. As a consequence, DTCs are some of the most frequently detected pesticides in the EU, and this group also showed one of the highest frequencies in exceeding maximum residue limits (MRLs) in food commodities sampled during 2010 in the 27 EU member states, Iceland, and Norway.

DTCs can be classified into three subclasses depending upon their carbon skeleton: (1) dimethyldithiocarbamates (DMDCs) such as ziram, thiram, and ferbam; (2) ethylenebis (dithiocarbamates) (EBDCs) such as mancozeb, maneb, zineb, and metiram; and (3) propylenebis(dithiocarbamates) such as propineb. This distinction is important because many countries are restricting the use of EBDCs due to their toxicity.

Benzimidazoles

Benzimidazoles are a group of specific fungicides, introduced around 1970, that includes carbendazim, benomyl, and thiophanate-methyl as representatives. They inhibit β-tubulin assembly during mitosis and were first used to control gray mold (*B. cinerea*) and apple scab (*Venturia inaequalis*), but the pathogens rapidly developed field resistance, and they are now widely used in their relative crop segments because of their broad spectrum. Aside from their application in agriculture, benzimidazoles are used in animal and human health as anthelmintic and antiulcer drugs.

Though benomyl sales have decreased since registration was canceled in the EU and the United States, thiophanate-methyl meets the strict criteria for registration in Japan, the EU, and the United States, and its use has been increasing to control *Sclerotinia* rot of soybean in Brazil and deoxynivalenol levels on cereal grains in the EU.

Dicarboximides

The broad-spectrum dicarboximide fungicides, including procymidone, iprodione, and vinclozolin, were commonly used to control *Botrytis* on vines, fruits, and vegetables in the 1980s. Fungicides of this class have been used since the mid-1970s mainly to control fungi of the related genera *Botrytis*, *Sclerotinia*, and *Monilinia*.

Dicarboximides largely replaced benzimidazole fungicides, which in many situations were no longer effective due to resistance. Dicarboximide-resistant variants appear frequently in laboratory, and after about 3 years of intensive use, resistant strains were detected in the field. Vinclozolin was used worldwide on oilseed crops, vines, fruits, and vegetables. It was widely used in Europe until 2007 when a ban was implemented. The United States remains a major use area although uses were restricted in 2000 and is currently limited to canola and turf.

Concern about the toxicity of vinclozolin and/or its metabolites is related to its antiandrogenic activity and its ability to act as a competitive antagonist at the androgen receptor. Its terminal metabolite, 3,5-dichloroaniline, is also considered a potential carcinogen.

Triazoles

Modern systemic fungicides are typified by the triazoles. This group of fungicides is still the basis of cereal disease management strategies worldwide, particularly in Europe, North America, Australia, and New Zealand, where they are primarily mixed with strobilurins and with the new-generation pyrazole carboxamide SDHIs (succinate dehydrogenase inhibitors), introduced in 2010.

Azole fungicides are widely used in agriculture and in the treatment of human mycosis. Their antifungal activity is based on their ability to inhibit CYP51 (lanosterol 14-demethylase), a key enzyme for sterol biosynthesis in fungi. Several azole fungicides have been found to possess a common teratogenic potential to induce facial, axial skeleton, and limb defects, in laboratory animals tests. The inhibitory potency of triazoles is not limited to fungi and has been observed in several mammalian cytochrome P450-dependent activities, such as hepatic microsomial enzymes, accounting for the possible interference of azoles with the metabolism of other drugs.

Phthalimides

The phthalimides represented a major improvement over the previously used inorganic fungicides since they were more active, less phytotoxic, and easier to prepare by the user. The phthalimides captan and folpet are fungicides largely used in agriculture, namely, in grape production, and still provide very good powdery mildew control if properly applied. They have similar chemical structures, except that folpet has an aromatic ring unlike captan. Their half-lives in several matrices are very short, since they are readily broken down to tetrahydrophthalimide and phthalimide, respectively.

Phenylamides

Phenylamides (benalaxyl, metalaxyl, and metalaxyl M) were first introduced in 1977. They act specifically against oomycete pathogens and have no effect on other classes of fungi. The first cases of resistance occurred in 1980, against metalaxyl applied to cucumbers for the control of *Pseudoperonospora cubensis* in

Israel and applied to potatoes in several European countries for the control of late blight (*Phytophthora infestans*).

Anilinopyrimidines

After resistance to dicarboximides developed, the anilinopyrimidines (pyrimethanil, cyprodinil, and mepanipyrim) were introduced in the 1990s and have been used to control strains with multiple resistances to dicarboximides and benzimidazoles.

Pyrimethanil and mepanipyrim are particularly active against gray mold and apple and pear scab, whereas cyprodinil provides effective control of a wide range of cereal pathogens. Cyprodinil has been commercialized alone for cereals and as a mixture with fludioxonil against *B. cinerea* on grapes and vegetables.

Anilinopyrimidines inhibit elongation of germ tubes at low concentrations. Several amino acids, particularly methionine, reverse the fungitoxicity of the anilinopyrimidines. Biochemical studies suggest that pyrimethanil inhibits the biosynthesis of methionine, affecting cystathionine β-lyase.

Phenoxyquinolines

Quinoxyfen was introduced for the control of powdery mildew of cereals (*Erysiphe graminis*) and grapevine. In the EU, quinoxyfen was approved for use as a fungicide in 2004. Quinoxyfen inhibits spore germination and appressorium development and thus exhibits a preventive action toward powdery mildew pathogens. Because of its vapor redistribution and possibly its systemicity, quinoxyfen shows long-term protection.

Phenylpyrroles

Phenylpyrrole fungicides are derived from the antibiotic pyrrolnitrin, a secondary metabolite of *Pseudomonas pyrrocinia*. Fludioxonil is used in seed treatments and foliar sprays for the prevention of infection by *B. cinerea*.

Fludioxonil shows improved photostability and has very good activity against *B. cinerea*, *Monilinia* spp., and *Sclerotinia* spp. For use against foliar diseases, fludioxonil is commercialized alone or as a mixture with cyprodinil. Both fungicides play an important role in some biocontrol systems, particularly in the control of strawberry gray mold.

QoI-Fungicides (QoI, 'Strobilurins')

This class comprises fungicides from several different, but related, chemical groups (e.g., methoxyacrylates, methoxyacetamides, oximino acetates, and methoxycarbamates), which have a common antifungal mode of action, inhibiting electron transfer at the Qo site in mitochondrial complex III. Within 2 years after their introduction, marked loss of action against powdery mildew, associated with development of highly resistant populations, was observed in some European countries. Important resistant problems were subsequently observed in several target pathogens in wheat, apple, and bananas.

Strobilurins are natural substances isolated mainly from mushrooms (basidiomycetes). Strobilurin-A was isolated from the liquid cultures of *Strobilurus tenacellus*. These products are biosynthesized in fungus from phenylalanine via the shikimic acid cycle, and their discovery opened the door for new chemistry of synthetic fungicides. By applying quantitative structural activity relationship on the structures of natural strobilurins, many pesticide companies were able to synthetize analogs that are more efficacious and more stable fungicides.

The strobilurins were first commercialized in 1996 with the launch of azoxystrobin and kresoxim-methyl, and within only a few years, they were registered and sold in over 80 crops in 70 different countries. In 2008, sales of azoxystrobin alone were approximately US$ 900 million, making it the world's biggest selling fungicide.

Field application of the natural strobilurins was not possible because of difficulties in their large-scale production and their relative volatility and photochemical instability. Thus, efforts were directed at chemical optimization. Azoxystrobin, kresoxim-methyl, metominostrobin, and trifloxystrobin, some of the presently commercialized strobilurins, altogether have a wide spectrum of activity. This class of compounds possesses a unique spectrum of activity. Although the simultaneous control of pathogens belonging to different classes, such as powdery mildews (ascomycetes) and rusts (basidiomycetes), was already known for triazoles, strobilurins were able to control simultaneously powdery and downy mildews for the first time, which is particularly useful for crops such as grapevine as well as many others.

Other Chemical Families

Over the years, industrial research programs have resulted in the development of valuable crop protection compounds with many desirable properties including specificity, systemicity, curative and eradicant action, and high activity at low use rates.

As an example, SDHIs inhibit succinate dehydrogenase in complex II of the mitochondrial respiratory chain. Development of SDHIs can be traced back to three generations. The first generation (e.g., carboxin) was developed in the 1960s and used as a seed treatment against *Rhizoctonia* spp. Mepronil and flutolanil are representative of the second generation and were introduced in the 1980s. They are also active against basidiomycetes, particularly *Rhizoctonia* spp., and are used to control rice sheath blight in rice. The leading products of the third generation of SDHIs are boscalid and penthiopyrad, whose spectrum of efficacy has broadened to include ascomycetes.

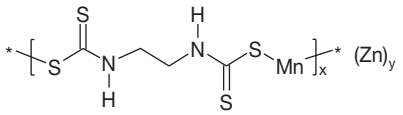
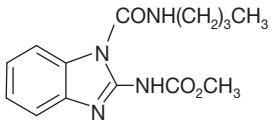
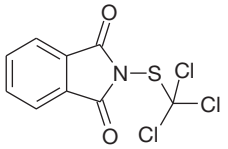
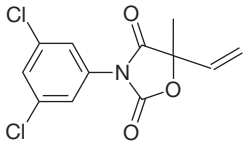
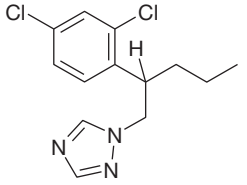
At present, the CAA fungicides used commercially include dimethomorph, flumorph, pyrimorph, benthiavalicarb, iprovalicarb, valifenalate, and mandipropamid. They specifically act against oomycete pathogens and probably have a common mode of action. Soon after dimethomorph was introduced in 1993, and despite recommendations to always use in combination with multisite fungicides, less sensitive populations of *P. viticola* were observed in a number of vineyards in France and Germany.

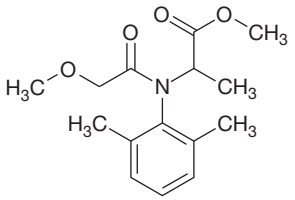
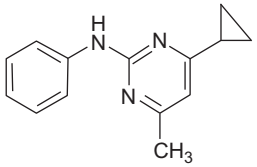
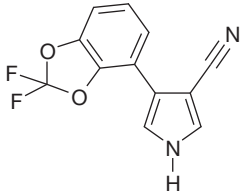
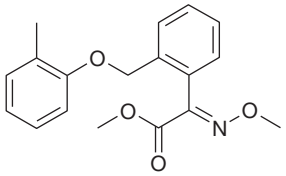
Representative compounds of some of the most important fungicide chemical families are presented in Table 1.

Environmental Fate

Synthetic organic fungicides belong to different chemical classes, and it is thus difficult to generalize their behavior in

Table 1 Physical–chemical properties of representative compounds of some of the most important fungicide chemical families

Chemical family	*Example*	*Mode of action*	*pK_a*	*Water solubility ($mg\ l^{-1}$, 20–25 °C)*	*Octanol/water partition coefficient log P (K_{OW})*	*Normalized soil organic carbon–water partition coefficient (K_{OC})*	*Aerobic soil half-life (days)*
Ethylenebis (dithiocarbamate)	Mancozeb 	Multisite action (M3)	10.3	6.2	1.80	2000	6–15
Benzimidazole	Benomyl 	β-Tubulin assembly in mitosis (B1)	4.48	2.9	1.37	1900	0.79
N-Trihalomethylthio (phthalimide)	Folpet 	Multisite action (M4)	-3.34 ± 0.20	0.8	3.11	143.9–304	8–14
Dicarboximide	Vinclozolin 	Osmotic signal transduction – MAP/histidine kinase (*os-1*, *Daf1*) (E3)	n.a.	2.6	3	100–735	3–21
Triazole	Penconazole 	C14-demethylase in sterol biosynthesis (*erg11/cyp51*) (G1)	1.51	73	3.72	2205	133–343

Phenylamide (acylalanine)	Metalaxyl 	Nucleic synthesis (RNA polymerase I (A1))	$\ll 0$	8400	1.75	30–300	10–40
Anilinopyrimidine	Cyprodinil 	Amino acid and protein synthesis (methionine biosynthesis (D1))	4.44	13	4.0	1706	20–60
Phenylpyrrole	Fludioxonil 	Signal transduction (MAP/histidine kinase, *Os-2*, *HOG1* (E2))	pK_a 1 < 0	1.8	4.12	75 000	140–350
Strobilurin	Kresoxim-methyl 	Target site and code: complex III, cytochrome bc1 (ubiquinol oxidase) at Qo site (cyt b gene) (C3)	< 0	2.0	3.4	219–372	<3

Data from Bermúdez-Couso et al. (2007); FRAC Code List 2014 (Fungicides sorted by mode of action); Komárek et al. (2010); Tomlin (1997); n.a. – not available.

the environmental compartments. In soils, fungicide fate is also significantly influenced by soil properties. Some fungicides have been detected in surface waters at concentrations higher than the EU regulatory limit, and it can be assumed that their extensive use poses a potential risk for the environment and human health. Therefore, the choice of fungicides should be performed carefully in accordance with good agriculture practices, physical–chemical characteristics of the soils, and climatic and hydrogeological regimes.

The fate of fungicides in soil is governed by processes that affect their persistence, such as chemical and microbial degradation, and processes that affect mobility, which involve adsorption, diffusion, absorption by plants, runoff, wind erosion, volatilization, leaching, and assimilation by microorganisms. The adsorption process is essential in the fate of pesticide in soils. The most common quantitative measurement of the sorption of organic pollutants by soils from aqueous solution is the soil organic carbon–water partitioning coefficient, K_{OC}. Thus, compounds with higher K_{OC} values will be less mobile than those with lower values.

Nearly 25% of the agrochemicals used in the world are chiral and this property can influence all aspects due to the possible different activities of stereoisomers. Despite having exactly the same physical and chemical characteristics, when exposed to a chiral environment, stereoisomers generally behave differently and this can lead to diverse activities, toxicologies, or degradation patterns.

Reduction of Fungicide Levels in Food

Pesticide residues are traces of pesticide active ingredients and metabolites that remain on food after being applied on the farm or in postharvest handling, including any specified derivatives, such as degradation and conversion products, metabolites, reaction products, and impurities considered to be of toxicological significance.

Pesticide degradation rates depend upon many factors, including characteristics of the compound and processing conditions. At high levels, pesticide residues can cause poisonings when ingested, but these types of poisonings are relatively rare. Cumulative effects of low-level exposures through pesticide residues in food have created concern and scientific and political debate for more than a century. Producing definitive answers on the effects of lifelong, low-level exposure to pesticides is extremely difficult and tests the limits of toxicology and epidemiology.

The common food processing operations along with the degree of pesticide residue removal, including fungicides, in each process have been reviewed. These processes included baking, bread making, dairy product manufacture, drying, thermal processing, fermentation, freezing, infusion, juicing, malting, milling, parboiling, peeling, peeling and cooking, storage, storage and milling, washing, washing and cooking, washing and drying, washing and peeling, washing peeling and juicing, and winemaking. Extensive literature review demonstrates that in most cases, processing leads to large reductions in residue levels in the prepared food, particularly through washing, peeling, and cooking operations.

Fermentation processes also influence pesticide residues in food. In most cases, fermentation leads to large reductions in original residue levels, with the formation of new pesticide by-products. The behavior of residues depends on the physical–chemical properties of the pesticide and the nature of the process. The presence of pesticides may decrease the growth rate of fermentative microbiota (yeasts and bacteria), which may cause stuck and sluggish fermentations. The observed changes have repercussions on several aspects of food sensory quality (physical–chemical properties, polyphenolic content, and aromatic profile) of fermented food.

The misuse of pesticides, including fungicides, may leave harmful residues in grapes after harvest that may pass to the must and eventually to the wine during fermentation, which involves a possible health risk. The proper use of pesticides does not cause a contravention of MRLs in grapes, and numerous studies have provided data on the dissipation rates of some pesticides in wine grapes and during winemaking. Azoxystrobin, cyprodinil, fludioxonil, and tebuconazole showed different disappearance rates, whereas pyrimethanil residues remained constant up to harvest. The residues of fenhexamid in grapes decreased rapidly to one-third of the initial concentrations after almost 1 week after the application. The order of dissipation rates in grapes for some fungicides widely used in vineyards was found to be vinclozolin > fenarimol > metalaxyl > penconazole > mancozeb.

Residues of boscalid, cyprodinil, dimethomorph, fenhexamid, metalaxyl, procymidone, and tebuconazole have been found in wine samples, being boscalid, cyprodinil, dimethomorph, and fenhexamid frequently determined pesticides in wine samples. Since some pesticides are less soluble, much higher residue concentrations were detected in cake than in crushed grapes and must (procymidone > cyprodinil > dimethomorph > boscalid > fenhexamid > metalaxyl > tebuconazole > azoxystrobin > quinoxyfen). The main processes that significantly reduced pesticide residues in the vinification of grapes are pressing and separation of lees from wine after alcoholic fermentation. In the processing of white grapes, the clarification of must is also an important factor in significantly reducing pesticide residues.

The influence of ten new-generation fungicides (ametoctradin, benthiavalicarb-isopropyl, boscalid, cyazofamid, dimethomorph, fenhexamid, kresoxim-methyl, mepanipyrim, metrafenone, and pyraclostrobin) on the fermentative activity of *Saccharomyces cerevisiae* yeast was evaluated in pasteurized red must. The presence of ametoctradin, dimethomorph, and mepanipyrim seemed to affect sugar-to-ethanol yield in the stationary phase. Additional experiments were carried out for these three fungicides in ecological red must from *V. vinifera* cv. *Tempranillo*. When the must was unfiltered, the fermentative activity of yeasts was unaffected by the presence of these fungicides. However, when the must was previously filtered, slight decreases of biomass and ethanol production, as well as in fruity aroma, were registered.

In the EU, some of the most frequently detected fungicide residues in food monitoring programs were propamocarb, thiabendazole, boscalid, DTCs, and imazalil. Together with chlorpyrifos (an insecticide), these substances were found in 5–25% of the samples analyzed.

In field experiments, enantioselective dissipation of penconazole occurred in head cabbage and pak choi, with the preferential degradation of (−)-penconazole, and resulted in an enrichment of the (+)-penconazole residue in the two

vegetables. However, the enantioselective behavior was not observed in cucumber and tomato. This was the first report of enantioselective behavior of penconazole.

Effects on Health

Regarding pesticides, which include fungicides, many health impact assessment studies have been carried out in the United States, Europe, and elsewhere in the past 15–20 years. In Europe, significant studies are also currently in progress to assess health effects caused by different types of environmental contaminants, including plant protection products. The large number and the chemical diversity of active substances and their different toxicity characteristics are of utmost importance. Although significant data gaps exist for a large part of the approved active substances, it has been found that many are positive for at least one health effect, after chronic and/or acute exposure, including carcinogenicity, reproductive, and neurodevelopmental disorders, as well as endocrine disruption.

Although not sufficiently in many cases, negative effects of some pesticides on human health, such as neurological dysfunctions and diseases, hypospadias, endocrine disruption, and increases in the incidence of cancers, have been reported.

Some fungicides can affect the endocrine system and interfere with important developmental processes in humans and wildlife. Endocrine-disrupting compounds represent a challenge, as their effects depend on both the level and timing of exposure, being especially critical when exposure occurs during development. In rat studies, fetal exposure to various chemicals with androgen receptor antagonist properties (e.g., the fungicides vinclozolin, procymidone, and prochloraz) causes disturbances of testosterone production and results in hypospadias, cryptorchidism, impaired fertility, and dysgenetic testicular histology. Studies with vinclozolin have suggested that this fungicide could be a potential comutagenic compound in human beings.

Some triazoles, including epoxiconazole and propiconazole, are also used as pharmaceuticals for treatment of fungal infections. Azole fungicides act by inhibition of enzymes necessary for the fungal cell membrane. Several members of this family have a number of toxicological risks, including carcinogenicity and reproductive toxicity. In mice, epoxiconazole and propiconazole have been reported as hepatocarcinogenic. Tebuconazole and epoxiconazole have shown antiandrogenic activity.

Exposure to pesticide residues, particularly during periods of rapid growth such as fetal life and infancy, may affect a child's health and development by increasing the risk of congenital malformations, cancer, malabsorption, immunologic dysfunction, endocrine disease, and neurobehavioral deficiencies.

Ethylenebis(dithiocarbamate) fungicides affect the thyroid gland and the lymphocyte genome, enhance the production of the thyroid-stimulating hormone, and probably cause higher prevalence of solitary nodules of workers. Mancozeb is also probably hormonally active as an estrous cycle-disrupting fungicide. Studies have suggested that seasonal application of mancozeb may cause changes of some immune parameters, but does not pose a significant immunotoxic risk for vineyard workers. After the exposure of workers to quinoxyfen, red, itchy rash with blistering was noticed.

The fungicides for which there has been evidence for endocrine disruption in humans and wildlife include fenbuconazole, pentachlorophenol, prochloraz, procymidone, pyrimethanil, tributyltin, trichlorophenate, triphenyltin, and vinclozolin.

In Europe, extensive legislation outlines global principles for including parent compounds and their metabolites in residue definition for risk assessment based on their significant contribution to the dietary risk (irrespective of their source) considering potential exposure from diet (abundance in food/feed items and drinking water and their relative amount) and relative toxicity.

Human health risks resulting from exposure to fungicides and mycotoxins via food have been compared, since they may be regarded as counteracting agents – fungicides are applied to protect crops from being infected by fungi and thus prevent the growth of mycotoxin-producing organisms but potentially lead to residues of the applied fungicides on or in the crop. An integrated probabilistic risk assessment model was used by the authors to compare the estimated health impacts on humans caused by crops contaminated with the fungicides spiroxamine and tebuconazole or with the mycotoxins deoxynivalenol and zearalenone. Based on the available data and the assumptions made, none of the four compounds was found to have impact on human health in the addressed scenarios.

Maximum Residue Levels

For food samples, pesticide residues have been regulated by several legislative authorities throughout the world, basically concerned with the quality, efficacy, and safety in the use of pesticides; however, there is not a global harmonized legislation. EU has set MRLs that are in general in the range of 0.01–10 mg kg^{-1}, depending on the combination commodity/pesticide. The lowest value is characteristic of banned compounds because it is considered as the minimum limit of detection (LOD) achievable. A value of zero is considered below the LOD because of the inaccuracies in the measurement methods available.

The more stringent regulations established for pesticides in food are those applied in food intended for infant consumption. Children and infants represent a vulnerable risk group of the population in terms of pesticide toxicity, and rigorous regulations have been set to protect them from dietary exposure to these chemicals. The EU specified the general MRL of 10 μg kg^{-1} for any individual pesticide residue in baby food.

Nevertheless, for most of the studied fungicides, MRLs have not been established in transformed products such as wine. For wines elaborated in the EU from September 2008, MRLs were established for boscalid (5000 mg l^{-1}), captan (20 mg l^{-1}), and pyraclostrobin (1000 mg l^{-1}). Other countries, such as Italy and Switzerland, have also established MRLs for some of the fungicides (vinclozolin, dichlofluanid, and boscalid in Switzerland at 1000 mg l^{-1} in all cases and quinoxyfen, boscalid, and pyraclostrobin in Italy at 10, 1000, and 50 mg l^{-1}, respectively).

In the United States, the Federal Food, Drug, and Cosmetic Act (FFDCA) authorizes EPA to set maximum residue levels,

or tolerances, for pesticides used in or on foods or animal feed.

Analytical Methods

The analysis of pesticide residues represents a basic instrument for the protection of human health and for trade and official control purposes. Despite pesticide trace analysis is being carried out since the 1970s, it still remains a challenge because different chemical classes may be present at low concentrations in complex matrices. Therefore, the development of multiresidue analytical methods with higher recoveries and lower limits of detection is an ongoing process, as well as incorporating the ultimate innovations.

Liquid chromatography coupled to mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS/MS) is increasingly applied for measuring pesticide residues, because the metabolites involved in residue definition are generally more polar than the parent pesticides, making LC-MS ideal for their detection. In addition, researchers are increasingly using ultra-high-resolution liquid chromatography (UHPLC) to enable simultaneous analysis of larger groups of pesticides and their degradation products as well as time-of-flight mass spectrometry (TOF-MS) and quadrupole time-of-flight mass spectrometry (QqTOF-MS) to identify new active substances, degradation products, and metabolites.

Analytical methods for determining fungicide residues involve several extraction and purification steps to remove the potentially interfering compounds that are generally present at higher concentrations than the pesticide residues. Solid-phase extraction (SPE); solid-phase microextraction (SPME); stir bar sorptive extraction (SBSE); the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method; hollow fiber liquid phase microextraction; matrix solid-phase dispersion (MSPD); and single-drop microextraction, among others, have been proposed for the extraction of fungicides as alternative to liquid–liquid extraction.

The trends in recent years have been toward the use of smaller sample sizes, more environmentally friendly and safer approaches, and faster and less laborious methods aiming at high recoveries and good precision.

Final Remarks

Chemical control will continue to play a central role in optimizing yields from the world's crops for the future, and fungicides are expected to remain as essential tools for plant disease management. The availability of compounds developed by exploring novel chemistry, the activation of plants' natural resistance mechanisms, and the use of natural products are expected to contribute to sustainable agriculture, providing benefits to users, environment, and society as a whole, not forgetting the continuation of studies on fungicide impacts on the environment and human health.

See also: Fungi; Pesticides and Herbicides: Residue Determination; Pesticides and Herbicides: Types of Pesticide; Pesticides and Herbicides.

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Game: Types and Composition

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Game Meat

In a global sense, game refers to wild animals and birds, with the term 'game' being used for culinary purposes to describe all birds and animals that are hunted for food. In the European Union (EU), wild game is defined in the EU Regulation (EC) No. 853/2004 as being "wild ungulates and lagomorphs, as well as other land mammals that are hunted for human consumption and are considered to be wild game under the applicable law in the Member State concerned, including mammals living in enclosed territory under conditions of freedom similar to those of wild game, and wild birds that are hunted for human consumption." The same legislation also defines 'small wild game' as being wild game birds and lagomorphs (rabbits and hares) living free in the wild and 'large wild animals' as land mammals, living free in the wild that do not fall within the small wild game definition. In the United States, wild game refers to wild land mammals (including those living within an enclosed area under conditions of freedom) that are hunted and wild birds. Large native game animals in the United States include antelope, buffalo, caribou, deer, elk, moose, and reindeer, while game birds include wild turkeys, wild geese, wild ducks, grouse, quail, pheasant, and other nondomesticated species of fowl.

Around the world, a vast number of wild animals are harvested for several reasons including for food purposes and sport hunting, with the type and range of animals hunted depending on climate, animal diversity, tradition, local taste, legislation, and local perception on what can or cannot be legitimately hunted. For example, in Europe, the most frequent big game species include red deer, roe deer, fallow deer, mouflon, and wild boar, while in South Africa, the hunted game species are mainly several African ungulates including springbok (*Antidorcas marsupialis*), blesbok (*Damaliscus pygargus phillipsi*), and kudu (*Tragelaphus strepsiceros*), among other antelopes. Game meat consumption is also related to the consumers' acceptability, which is influenced by several factors including cultural, religious, and ethnic aspects, organoleptic characteristics, price, and availability, among others.

Nowadays, besides being hunted as free-running animals in the wild, originally, wild species of animals are being raised, domestically or semidomestically, for sale. Farmed game is used not only for meat production but also for animal breeding, aiming at their subsequent release into the wild for restocking the game population in private and community hunting areas. According to the EU Regulation (EC) No. 853/2004, farmed game is defined as being farmed ratites (such as ostrich) and farmed land mammals other than domestic bovine, porcine, ovine, and caprine animals and domestic solipeds (single-hoofed, e.g., horse). With the exception of farmed rabbits, which are often produced in intensive systems, most of the other farm-raised game (such as deer, reindeer, and wild boar) is generally farmed using extensive systems. In the United States, species such as the American elk, wild boar, pheasant, and antelope (blackbuck (*Antilope cervicapra*) and nilgai (*Boselaphus tragocamelus*)) are being farm-raised, while in Europe, in particular in northern countries, the most common farmed-raised animals are deer (including several different species such as red deer (*Cervus elaphus*) and fallow deer (*Dama dama*)) and reindeer (*Rangifer tarandus*). Deer production is also a well-established industry in New Zealand. To distinguish farmed-raised game from wild free-running animals, some authors proposed to designate the first as venison, associating the second as being African game antelope. However, the designation 'venison' is most frequently used as a culinary term referring to meat not only from deer but also from other cervids such as reindeer, elk, and moose.

Although production and consumption data for farmed game are scarce, according to the Food and Agriculture Organization (FAO) Statistics Division, game meat production has been steadily increasing during the last decade (from approximately 1.59 million tons in 2000 to more than 1.99 million tons in 2012), which can possibly be related to a general increased consumption of game meat.

In fact, in the last years, game meat is increasingly perceived as a delicacy product; thus, its consumption in restaurants and at home dinners has also been increasing. This can be explained by several different motivations including the perception that game meat is healthier, its particular taste and flavor, the idea that game meat can be considered as a biological product (free-running animals without the use of anabolic steroids and other drugs), and the attraction for new experiences such as tasting exotic meats.

Game Meat: Sources and Production

Hunting has been practiced since ancestral times being one of man's main sources of food in prehistoric ages. Over millennia

of civilization, with the development of agriculture and domestication of animals, the importance of hunting and game meat as a means of survival radically decreased in most parts of the world. Nevertheless, in the present time and particularly in developing countries, a variety of different wildlife species is still sought as food as they inevitably remain a cheap source of protein for many population groups. In Africa and other regions of the globe, such as the Amazon in South America, bushmeat (according to the Convention on Biological Diversity, bushmeat is a term used to describe any terrestrial wild animal in tropical and subtropical countries harvested for subsistence or commercial purposes) remains of major importance either for consumption or to obtain revenue from trade. Although some recent data have been published on the bushmeat consumption in some parts of the world, such as in South America where consumption in indigenous communities can attain values of 35.8 to 191.6 kg per capita year, the level of consumption is very difficult to estimate due to several factors, such as unreported data concerning harvested animals, direct sales, trade in hidden markets, and illegal poaching. However, it is generally accepted that in rural African areas, bushmeat continues to represent a vital dietary item for a vast number of people although high variations across the continent exist. Apart from being consumed by local populations, game meat is also frequently tasted by tourists who visit those regions, although this represents a minor percentage of the total game meat consumed.

During the last decades, the potential of African ungulates for meat production is also being taken into account for meat exportation, with different wild animals that roam in large enclosures being harvested for this purpose. In 2005, it was estimated that South Africa exported the deboned meat from 160 000 game carcasses, with commercially harvested antelope being predominately the springbok (*Antidorcas marsupialis*, >80%), blesbok (*Damaliscus pygargus phillipsi*), and kudu (*Tragelaphus strepsiceros*), while other species such as blue wildebeest (*Connochaetes taurinus*), impala (*Aepyceros melampus*), and gemsbok (*Oryx gazella*) were exported in smaller numbers. Nowadays, most of the game meat harvested in Africa for exportation is obtained from game ranches with the promotion of legal wild meat production through game ranching being advocated as a way to conciliate wildlife conservation, rural food security, and community livelihood improvement. With the evolution of wildlife management, recurrent droughts, and the rising demand for tourism and safari hunts, wildlife ranching industry has been increasing in countries such as Zambia, Zimbabwe, Botswana, and Namibia, being most developed in southern Africa. In game ranching, wild animals roam free and are managed on natural vegetation although they are generally maintained in defined fenced areas. The incomes of game ranching arise mostly from sport/trophy hunting and ecotourism, though animal culling for meat production also generates relevant revenue, being also important to simultaneously control animal surpluses.

In the past 50 years, the farming of different game species has grown considerably worldwide possibly related to a higher consumption of game meat as it is generally perceived as a healthy food with distinctive and appreciated organoleptic qualities. To respond to an increasing consumer's demand for venison, in some countries such as New Zealand, farmed deer production has advanced to a level where typical livestock farming is applied, thus assuring a regular supply of quality meat. In New Zealand, red deer is the major farmed species, accounting for about 85% of the total farmed deer, which nowadays corresponds to approximately 1.1 million animals. According to the European Food Safety Agency (EFSA) data, among the cervids, the fallow deer and the red deer are the most common species farmed in Europe, with approximately 280 000 animals farm-raised in 2010, although less than half of these are slaughtered annually. In some European countries (Sweden, Norway, and Finland) and in Alaska, venison production is dominated by traditional reindeer farming, which is generally performed in a less-intensive system when compared to red deer farming since the animals are free-ranging in the forests/mountains instead of being enclosed in fenced areas. The animals that pasture extensively in herds may belong to different owners, being recognized by specific cut marks on their ears. In the United States and Canada, different cervid species are being farm-raised including the elk (*Cervus canadensis*), the fallow deer, the sika deer (*Cervus nippon*), the axis deer (*Axis axis*), and the white-tailed deer (*Odocoileus virginianus*).

Regarding small game animals, as referred, rabbits are generally farmed-raised mostly using intensive systems, while hares are hunted in the wild. Several game bird species, including migratory birds such as geese and ducks, are also frequently hunted worldwide, with a small number of species, such as pheasants, also being breed in captivity for restocking hunting areas or for boosting natural populations.

Chemical Composition of Game Meat and Nutritional Considerations

Nowadays, consumers are increasingly demanding for healthy and safe products as they are concerned about the food they eat. In general, consumers associate game to a healthier, thinner, and tasty meat arising from animals that are supposed to be free of hormones and drugs, such as antibiotics. In fact, game meat can generally be considered to be highly nutritious, as it is a valuable source of protein, minerals, and vitamins. Table 1 shows a compilation of data published on the proximate composition of different game meat. The values presented in Table 1 show that, considering the evaluated animals, protein contents are generally higher than 20%, with some species presenting levels around 25% protein. On the contrary, total fat content can be considered as being low, as it is generally inferior to 5%, with some species including different ungulates, lagomorphs, and game birds presenting levels less than 0.5% fat (Table 1).

Currently, it is of general agreement that total fat intake is certainly related to health risks; however, it is also accepted that concerning fat, both quantitative and qualitative aspects are important. Thus, the type of consumed fat or fatty acids is of critical importance. According to the 2010 FAO recommended dietary intakes for total fat and fatty acids, dietary fat should provide 20–35% of total energy and saturated fatty acids (SFA) should not exceed 10%. Individual SFA have different biological effects, with lauric, myristic, and palmitic acids increasing LDL cholesterol, while stearic acid is not considered to have such effect. Thus, the replacement of SFA (C12:0–C16:0) with polyunsaturated fatty acids (PUFA), whose consumption is

Table 1 Proximate composition of meats from different game species (g/100 g; mean values are given)

Animal species			*Proximate composition (g per 100 g)*			
		Samples	*Moisture*	*Protein*	*Fat*	*Ash*
Ungulates						
Kudu[a]	*Tragelaphus strepsiceros*	Longissimus dorsi muscle; males and females, total $n=18$	74.14–74.49	23.60–24.30	1.56–1.58	1.23–1.29
Impala[b]	*Aepyceros melampus*	9th–10th–11th rib cut; males and females, total $n=8$	70.2–74.0	18.9–20.0	1.2–4.3	4.4–4.6
Springbok[b]	*Antidorcas marsupialis*	9th–10th–11th rib cut; males and females, total $n=8$	70.4–75.3	17.4–18.4	2.5–5.3	4.2–4.3
Blesbok[c]	*Damaliscus dorcas phillipsi*	Longissimus dorsi muscle; 9th–10th–11th rib cut; males and females, total $n=73$	68.20–75.33	19.30–22.43	0.21–6.8	1.24–4.2
Common duiker[d]	*Sylvicapra grimmia*	Longissimus dorsi muscle; males, total $n=10$	71.41	25.71	2.12	1.29
Red deer[e]	*Cervus elaphus*	Longissimus dorsi muscle, total $n=10$	76.90	21.70	0.60	1.11
Fallow deer[e]	*Dama dama*	Longissimus dorsi muscle, total $n=10$	74.90	22.00	2.50	1.08
Roe deer[f]	*Capreolus capreolus*	Longissimus dorsi muscle; longissimus lumborum muscle, total $n=144$	71.40–74.4	22.82–25.70	1.0–2.12	1.29a
Elk[g]	*Alces alces*	Longissimus lumborum muscle; total $n=8$	—	22.72	1.33	—
Mouflon[h]	*Ovis ammon*	Longissimus dorsi muscle between the 10th and 13th ribs; males, total $n=18$	74.52–75.30	21.88–22.35	0.61 – 0.99	1.03 – 1.12
Wild boar[i]	*Sus scrofa*	Longissimus muscle, 12th/14th rib; longissimus lumborum muscle; male and female; total $n=97$	72.8 – 75.0	21.4 – 23.6	1.1 – 4.4	—
Leporidae						
Wild rabbit[j]	*Oryctolagus cuniculus*	Right leg, males and females, total $n=53$	74.86	23.71	0.20	1.18
Hare[k]	*Lepus europaeus*	Musculus longissimus thoracis et lumborum, total $n=33$	72.83	24.7	1.48	—
Game birds						
Wild duck[l]	*Anas platyrhynchos*	Breast, total $n=5$				
		Legs, total $n=5$	73.95 74.9	20.8 19.6	3.39 3.84	1.27 1.27
Quail[m]	*Coturnix japonica*	Musculus pectoralis major; males and females, total $n=144$	73.60–73.65	22.94–22.96	2.31–2.32	1.56–1.57
Pheasant[n]	*Phasianus colchicus*	Drumstick and breast, male, total $n=8$	73.09–75.14	22.22–25.37	0.13–0.40	1.26–1.35
Pigeon[o]	*Columba livia*	Breast and thigh, total $n=27$	66.52–70.59	20.56–23.61	4.32–7.85	0.98–1.48

[a]Mostert and Hoffman (2007).
[b]Van Zyl and Ferreira (2004).
[c]Van Zyl and Ferreira (2004); Hoffman et al. (2008).
[d]Hoffman and Ferreira (2004).
[e]Zomborszky et al. (1996).
[f]Zomborszky et al. (1996); Strazdina et al. (2012); Dannenberger et al. (2013).
[g]Strazdina et al. (2012).
[h]Ugarković and Ugarković (2013).
[i]González-Redondo et al. (2010).
[j]Mertin et al. (2012).
[k]Cobos et al. (2000).
[l]Sartowska et al. (2014).
[m]Franco and Lorenzo (2013).
[n]Pomianowski et al. (2009).
[o]Strazdina et al. (2012); Dannenberger et al. (2013).

associated with health benefits, has been recommended for decreasing the LDL cholesterol and total/HDL cholesterol ratio and decreasing the risk of coronary heart disease. A similar but lesser effect could be achieved by replacing SFA with monounsaturated fatty acids (MUFA). Although the 2010 FAO recommended dietary intakes do not mention a specific n-6/n-3 PUFA ratio, a daily intake ratio of 4:1 and a ratio of PUFA to SFA above 0.4 have been suggested by different authors.

Table 2 presents the fatty acid composition reported for different game species, including ungulates (African ungulates and cervids), lagomorphs, and game birds. As can be observed, in general, game meat presents a favorable fatty acid profile, with some game meats presenting low levels of SFA and/or considerably high levels of PUFA. Total SFA ranged from 22.2% in duiker to 51.12% in impala, with several large game animals presenting higher levels of stearic acid than

Table 2 Fatty acid composition of meats from different game species (relative %)

	Animal species													
	Ungulates											*Game birds*		
Fatty acids (%)	*Kudu*[c1] *(LDL*[a]*; n = 18)*	*Impala*[c2] *(LD*[a]*; n = 32)*	*Blesbok*[c3] *(LD; n = 65)*	*Springbok*[c4] *(LD; n = 19)*	*Common duiker*[c5] *(LD; n = 10)*	*Mouflon*[c6] *(LD; n = 18)*	*Red deer*[c7] *(ST*[a]*, TB*[a]*, LL*[a]*; n = 34)*	*Roe deer*[c8] *(L, LL*[a]*; n = 134)*	*Elk*[c9] *(LL; n = 8)*	*Wild boar*[c10] *(LD, SM*[a]*, LL; n = 104)*	*Wild duck*[c11] *(breast, legs; n = 5)*	*Quail*[c12] *(PM*[a]*; n = 108)*	*Pheasant*[c13] *(drumstick, breast; n = 8)*	*Pigeon*[c14] *(breast, legs; n = 27)*
C14:0	—[b]	1.10	—	—	0.75	0.93–2.63	4.57	0.8–1.32	2.44	0.8–2.92	0.27–0.70	0.872–1.017	0.52–0.53	0.54–0.92
C16:0	16.1–17.5	16.66	16.36	13.34–15.06	0.86	16.10–20.87	21.02	16.4–18.72	18.08	19.2–23.12	16.8–20.4	17.34–18.51	22.30–25.19	21.14–23.62
C16:1n7	0.52–0.69	0.35	—	0.03–0.2	18.58	1.03–1.45	6.66	1.95	4.35	4.27	0.94–2.22	3.45–3.69	5.66–5.82	7.27–11.25
C18:0	19.7–20.0	22.67	26.08	23.92–27.02	19.68	15.78–16.42	14.46	15.63–20.8	13.56	10.8–14.54	10.2–18.6	9.91–12.11	8.99–10.89	6.03–10.63
C18:1n9c	19.91–21.9	12.22	16.97	16.33–20.45	18.7	22.42–25.09	17.51	16.3–26.15	27.89	28.0–33.9	—	24.63–25.71	35.95–37.63	30.42–50.84
C18:2n6c	19.0–20.5	14.70	18.39	18.77–21.62	19.91	13.50–19.31	12.34	11.62–16.4	6.90	11.7–20.9	13.5–29.4	24.47–27.68	15.81–17.87	7.30–15.96
C20:0	0.11–0.20	0.21	0.30	0.28–0.40	0.81	0.12–0.14	0.06	0.02	0.08	0.09	—	—	—	0.00–0.17
C18:3n6	0.05–0.08	0.28	0.06	0.09–0.15	0.12	0.05–0.06	0.14	0.07	0.04	0.03	—	—	—	—
C20:1	0.06–0.10	0.5	0.05	0.08–0.12	0.23	0.40–0.47	0.13	0.04	0.04	0.24	—	—	0.17–0.20	0.10–0.34
C18:3n3	4.67–4.85	1.92	3.71	3.33–3.49	4.19	2.02–3.02	3.31	3.94–4.00	4.77	1.3–1.46	0.76–1.5	0.827–0.97	0.44–0.45	0.17–0.59
C20:2n6	0.12–0.15	0.49	0.04	0.22–0.28	0.29	0.08–0.09	0.1	0.07	0.45	0.26	—	—	—	—
C20:3n6	0.92–1.14	0.72	1.60	0.20–0.24	2.94	0.30–0.37	0.32	0.35	0.2	0.14	—	—	0.09–0.13	—
C20:4n6	7.74–8.44	9.80	10.00	7.63–9.30	7.83	6.24–8.42	4.25	5.00	4.59	2.02	5.35–17.6	6.63–7.703	4.51–4.85	0.01–4.03
C20:5n3	2.50–3.17	1.37	2.24	—	2.1	1.30–1.49	1.36	2.03	0.95	0.39	0.26–0.86	0.40–0.41	—	—
C22:4n6	—	—	0.26	0.21–0.37	0.31	0.18–0.29	—	—	—	—	—	—	—	—
C22:5n3	2.42–2.75	1.94	2.23	2.19–2.71	1.14	1.68–2.14	1.29	1.87	0.72	0.84	0.5–0.88	—	—	—
C22:6n3	2.06–2.5	1.05	—	0.94–1.26	1.09	0.44–0.47	0.2	0.39	0.32	0.08	1.07–2.44	0.09–0.10	1.21–2.75	—
Total SFA	35.9–37.7	51.12	43.59	38.40–42.69	22.24	35.20–42.24	42.13	40.9–42.13[c]	35.75[c]	32.8–41.76[c]	28.4–37.52	28.12–31.64	34.05–34.92	28.29–34.43
Total MUFA	20.48–22.74	14.82	17.53	16.67–20.99	37.51	27.82–30.99	26.56	21.1–26.56[c]	34.09[c]	36.2[c]–42.2	16.5–24.0	30.01–31.59	42.57–44.03	45.23–62.56
Total PUFA	39.53–43.6	34.06	38.87	36.34–41.64	40.26	27.63–36.98	23.38	23.48[c]–37.7	18.99[c]	17.26[c]–30.9	22.5–46.0	33.50–35.78	23.79–24.58	7.66–20.35
PUFA/ SFA	1.09–1.23	0.73	0.92	0.96–1.18	1.81[c]	0.70–1.08	0.68	0.68–0.92[c]	0.53	0.41–0.94	1.07[c]–1.17[c]	1.06–1.28	0.68–0.72	0.26–0.59
n-6/n-3	2.29–2.42	3.76	3.68[c]	2.83–3.44[c]	3.55[c]	1.05–1.48	2.75	2.07–2.8	1.72	4.81–8.7	8.98[c] – 10.0[c]-	25.23–26.09	7.22–14.20	21.07–69.59

[a]Muscles: LDL, longissimus dorsi et lumborum; LD, longissimus dorsi; ST, semitendinosus tendon; TB, triceps brachii; LL, longissimus lumborum; SM, semimembranosus muscle.

[b]Not reported.

[c]Calculated based on the published values. (1) Mostert and Hoffman (2007), (2) Hoffman et al. (2009), (3) Hoffman et al. (2008), (4) Hoffman et al. (2007), (5) Hoffman and Ferreira (2004), (6) Ugarković and Ugarković (2013), (7) Strazdina et al. (2012), (8) Strazdina et al. (2012), Dannenberger et al. (2013), (9) Strazdina et al. (2012), (10) Strazdina et al. (2012); Dannenberger et al. (2013), (11) Cobos et al. (2000), (12) Sartowska et al. (2014), (13) Franco et al. (2013), (14) Pomianowski et al. (2009).

those of palmitic acid (the last considered as being potentially atherogenic while the former having neutral effects on health). In general, a high proportion of PUFA was reported for the species presented in Table 2, in particular for kudu, springbok, common duiker, and quail. Pheasant, pigeon, and wild boar were reported to present a higher level of MUFA than of SFA. In general, data reported for the different game meats evidenced desirable ratios of PUFA/SFA and n-6/n-3 PUFA. Ratios of PUFA/SFA ranged from 0.25 to 1.81, with all meats presenting ratios above the recommended value of 0.4, with the exception of some samples of pigeon. The high variability of values reported for some species could possibly be related to, not only different factors, such as diet, but also genetic origin, age, sex, region, and climate. A high proportion of n-3 PUFA is also evidenced by the ratios n-6/n-3 PUFA, which were below the recommended ratio of 4:1, with the exception of game birds and some wild boar samples (Table 2). For ungulates, high levels of the essential α-linolenic acid (ALA, C18:3n3) were reported, ranging from 1.92 to 4.85%. According to FAO recommendations, the minimum dietary requirement of ALA for adults to prevent deficiency symptoms is >0.5% of total energy, while a dietary intake of 0.250 g–2.0 g per day of n-3 long-chain PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is recommended as part of a healthy diet.

Other Health Considerations

Microbiological Issues

The microbiological conditions of game carcasses and meat can vary widely. Carcasses from farmed game slaughtered and dressed at suitable abattoirs can present microbiological conditions comparable to that of carcasses from livestock, while those obtained from hunted animals will depend on several factors that can influence the possibility of carcass contamination and the potential growth of contaminating bacteria. These factors include the health of the animal, the types of microorganisms carried by each species (mainly on the hide and gastrointestinal tract), the circumstances in which the animal is killed, the conditions during evisceration in the field, the time before cooling, and the conditions under which the carcasses are transported, stored, and processed, among others. Currently, the vast majority of game, either hunted or farmed, is killed with a rifle or shotgun; thus, another important factor is the skill of the hunter as the anatomical shooting location is of critical influence for the carcass hygiene. For large game animals, such as deer, the hunters, generally, target the chest so the shot passes through the heart and lungs, though in deer culling, the animal is frequently shot in the head or neck to minimize the carcass damage. However, due to several reasons, such as the hunters' inexperience, the animal can be killed by shooting the abdominal area that can damage the gut, causing bacteria contamination. The skill of the hunter is also very important in the evisceration process to avoid damaging the gut.

Despite the increasing quality of hygiene standards in game meat harvesting and handling, human cases of infection can still occur. The relevant biohazards related to game meat, including parasites, bacteria, and viruses, can vary among countries according to the epidemiology and food consumption habits of each region. According to the EFSA Panel on Biological Hazards, *Salmonella* spp. in farmed wild boar and *Toxoplasma gondii* in farmed deer and farmed wild boar were recently ranked as a high priority for farmed game meat inspection, while *Trichinella* spp. in wild boar was ranked as low priority due to current controls, which should be continued.

Besides being a possible source of human infection due to foodborne diseases, wild game animals can also represent important reservoirs of zoonotic infections. In particular, due to wildlife contacts with domestic animals, there is an increasing concern regarding the possibility of disease transmissions.

Exposure to Lead from Gunshot

The majority of game animals are killed by gunshot, with hunters generally using lead-based bullet or shot ammunition, especially for large game animals. Lead is a nonessential and toxic metal that adversely affects a wide range of systems in the human body, with some effects being observed at very low levels of lead in the blood (PbB), such as neurocognitive and neurodevelopmental deficits in children and elevated systolic blood pressure and chronic kidney disease in adults. Thus, game meat obtained from animals shot with this type of ammunition can be a potential source of dietary lead for humans, representing a potential hazard. Previously, there was a general belief that the dietary lead exposure from game meat would be low because the mass of the projectile(s) remained in one large piece that either passed through the carcass or was easily removed, the meat around the wound channel is generally removed and if fragments of ammunition were visible in the meat, they are also discarded during food preparation or, at the most, consumers avoid swallowing them. However, recent studies showed that game meat can contain small and numerous bullet fragments, visible in radiographs, which can be widely dispersed relative to the wound channel, with this being particularly noticed if bullets impact with the animal's bones. Hence, several authors have been suggesting that lead exposure from game meat can pose a potential health risk, in particular for high-level consumers, such as subsistence hunting communities and the hunters' families.

Following the request of the European Commission, EFSA recently produced a scientific opinion on the risks to human health related to the presence of lead in foodstuffs. In this report, the Panel on Contaminants in the Food Chain concluded that the provisional tolerable weekly intake (PTWI) of 25 $\mu g\ kg^{-1}$ b.w. dietary lead, previously set by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA), was no longer appropriate as there was no evidence for a threshold for critical lead-induced effects. Based on data from different foods, game meat was among those that presented the highest levels of lead; thus, frequent consumers of game meat were included in the group with higher lead exposure levels. However, the impact of a specific diet, assuming a weekly meal of 200 g of game meat, did not appear to cause a greater risk regarding systolic blood pressure compared to other adult consumers. In general, the panel concluded that the risk of clinically important effects on either the cardiovascular system or kidneys of European adult consumers, at current levels of lead exposure, is low to

negligible, but for infants, children, and pregnant women, there is a potential concern for effects on neurodevelopment. However, it should be highlighted that the panel also referred that the possibility of effects on some consumers having a diet rich in game meat could not be excluded. In fact, different studies have been pointing out an association between game consumption and lead levels in the blood, although a lack of correlation was observed in some cases.

Adulteration of Game Meat and Processed Products

As referred, the consumption of game meat has been increasing in the last years. In general, game meat and products thereof are considered delicacy products and command higher prices compared to other meat products, thus being susceptible targets for frauds. Adulteration due to the fraudulent substitution of game by other lower-value meat species occurs mainly for economic reasons, with processed products being particularly prone to this type of unscrupulous practices. Therefore, the authentication of game meat and game meat products can contribute to avoid unfair competition among producers and to provide accurate information for consumers, being also important for safety and public health reasons, that is, in what concerns possible zoonoses affecting game animals and birds. So far, different methodologies, mainly relying on protein or DNA analysis, have been proposed for meat species identification purposes. However, in the last years, DNA-based methods have been elected for species identification as they present several advantages when compared to protein-based techniques, namely, the ubiquity of DNA in every type of cells, the higher stability of DNA molecules, and high specificity. Moreover, when evaluating processed meat products, protein-based methods present limitations, such as the possibility of protein denaturation and modification of specific epitopes associated with processing. Among DNA-based methods, polymerase chain reaction (PCR) is the most commonly used molecular technique due to its simplicity, fastness, specificity, and sensitivity. Until now, several different techniques have been proposed for verifying game meat and product authenticity, including species-specific PCR and real-time PCR, random amplified polymorphic DNA (RAPD) analysis, PCR–restriction fragment length polymorphism (PCR-RFLP) analysis, PCR sequencing, and DNA barcoding.

In RAPD, a DNA band pattern or fingerprinting is obtained by amplification using short arbitrary primers. The advantage is that species-specific patterns are generated without amplifying information about the gene fragments. However, it is difficult to obtain reproducible results and this technique is not suited for the identification of a targeted species in admixed meats or processed products. In PCR-RFLP, sequence variations within defined DNA regions are exploited to obtain characteristic restriction patterns. This technique has been proposed for the identification of different game meats with the successful identification of meats from closely related species, such as pork and wild boar meats. However, similarly to RAPD, this technique might not be applicable in complex and processed matrices since results may show a combination of miscellaneous restriction patterns that are difficult to understand.

Among the referred techniques, species-specific PCR is being increasingly used due to its simplicity, high specificity, and high sensitivity, being suitable for meat authentication studies, even in complex and processed foods. In this technique, a primary aspect consists of primer design targeting specific DNA fragments, which allows the specific identification of a target species in complex matrices containing a pool of heterogeneous DNA sequences. Even though the use of both nuclear and mitochondrial genes has already been described for the identification of game meat species, the latter offer the advantage of being several folds more abundant than nuclear DNA, thus improving sensitivity. More recently, quantitative analysis has been achieved by the use of real-time PCR since the target amplification is directly monitored along each amplification cycle. Data collection is performed by using fluorescent molecules able to provide a strong correlation and to measure minute amounts of DNA. Fluorescent compounds used in real-time PCR approaches for game meat identification include general dyes that intercalate DNA molecules, such as SYBR Green and EvaGreen, and different probe-based systems such as hydrolysis TaqMan probes. The use of dyes offers a suitable and less expensive alternative, while the use of probes that specifically bind within the target sequence adds specificity to the reaction and generally permits the use of smaller amplicons, which is especially useful for processed foods.

Although still few works report its application, DNA barcoding has emerged in the last years as a promising tool for the authentication of meats and meat products including game. In the near future, it is expected that newly and advanced technologies for high-throughput sequencing, namely, next-generation sequencing (NGS), coupled with DNA barcoding, can be applied to meat species identification, allowing the simultaneous identification of multiple species in complex matrices.

In spite of the utility of DNA-based methods in species identification for authenticity purposes, the majority of published works are devoted to the development of techniques with fewer referring to its application. Although some studies reported that several commercial processed meat products evaluated for authenticity purposes were in good agreement with the labeled game species, recently, other works reported a high incidence of species substitution and mislabeling in game meat products sold in different countries. These recent results suggest the existence of fraudulent practices, thus evidencing the importance of control and inspection programs and the need for systematically verifying labeling statements.

See also: Authenticity of Food; Ethnic Foods; Fatty Acids: Essential Fatty Acids; Protein: Food Sources; *Salmonella*: Properties and Occurrence.

Further Reading

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Garlic

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Garlic (*Allium sativum* L.) has been used as a food and medicinal herb for thousands of years. Ancient references to the use of garlic date to at least the third millennium BCE. Early records include recipes from Mesopotamian cuneiform tablets and medical remedies detailed in the Egyptian Ebers Papyrus. Garlic is, in a sense, a controversial plant. While praised by many for its unique culinary roles and possible health benefits, garlic has been reviled by others for its pungency. These opposing perceptions ultimately are grounded in the underlying biochemistry of garlic. The characteristic aroma and flavor of crushed fresh garlic arise from a number of relatively simple sulfur compounds. With cooking or processing, additional sulfur compounds are generated, which in turn modify not only aroma and flavor but also potential health effects. The many forms in which garlic is ingested, and their corresponding diverse chemical profiles, make the study of garlic-related health benefits an interesting and challenging field. The widespread consumption of garlic and the long history of its use in folk medicine drive the research attention it has received.

Garlic: Sources and Production

Although garlic is thought to have originated in Central Asia and the majority of production occurs in Asia, garlic is currently cultivated throughout the world. As of 2011, over 20 million metric tons of garlic was harvested globally (Table 1). About 80% of the world's garlic production was in China, with India, Egypt, Korea, Russia, Myanmar, Bangladesh, the United States, Argentina, and the Ukraine accounting for approximately another 12% of the total. Garlic production has increased significantly in recent years. Over the 10 years from 2001 to 2011, the global garlic harvest has more than doubled with over 90% of this increase accounted for by China (Figure 1). Common subspecies of garlic are the softneck (*A. sativum* L. var. *sativum*) and hardneck (*A. sativum* L. var. *ophioscorodon*) types. Softneck garlic is typically grown for sale in large supermarkets because it is better adapted for mechanized planting and harvesting and stores longer than hardneck garlic. Softneck garlic bulbs generally have more but smaller cloves than bulbs of the hardneck type. Hardneck garlic grows well in cooler climates and is preferred by some for what they perceive as stronger, more complex flavors. Elephant garlic (*A. ampeloprasum* L. var. *ampeloprasum*) resembles true garlic but is biochemically different and botanically is more closely related to leeks.

Composition of Garlic and Garlic Preparations

One key to understanding the possibilities of garlic for human health and the sometimes divergent results of scientific studies exploring the biological effects of garlic intake is to understand the various ways that garlic is processed and consumed, and to recognize that the biochemical compositions of various garlic products often differ significantly. Generally, garlic is consumed either as a whole food as part of a meal or as a dietary supplement consisting of distinctive garlic-derived organosulfur compounds, often produced by extraction and concentration processes. As a food, garlic is prepared in multiple ways. Dehydrated garlic is used as a condiment and is commonly incorporated into many processed foods as a flavoring agent. Fresh garlic is most frequently sliced, chopped, or crushed and may be incorporated into recipes in its raw form or cooked by boiling, sautéing, baking, or roasting. Other culinary techniques for preparing garlic include aging in vegetable oil and pickling. As dietary supplements, garlic and garlic-derived products may be formulated as tablets, capsules, or liquid extracts. Many garlic-derived dietary supplements have undergone an extraction process that significantly alters the chemical compositions of the products, with the notable exception of dried garlic powder that is biochemically similar to raw intact garlic. Extracted formulations include aged garlic extract, steam-distilled garlic oil, and oil-extracted garlic macerate.

Intact or Dehydrated Garlic

Garlic bulbs not only are well known for their unique sulfur compounds but also contain nonsulfur compounds not ubiquitously found in plants. Among these are fructans, steroid saponins, and selenium. Fructans are fructose polymers and comprise over 90% of the nonstructural carbohydrates in garlic. In contrast, garlic does not contain monosaccharides or starch (glucose polymers). Steroid saponins are a kind of natural detergent consisting of a hydrophilic glycoside moiety attached to a lipophilic steroidal group. Saponins may have a cholesterol-lowering effect. Garlic is a selenium accumulator, and as such, selenium levels in garlic are highly responsive to selenium fertilization. Organoselenium compound in garlic includes Se-methylselenocysteine, which metabolizes to methylselenol and thereby exhibits anticarcinogenic and antioxidant activities. Other nonsulfur garlic constituents occurring at appreciable levels include arginine, γ-glutamylphenylalanine, and allixin (3-hydroxy-5-methoxy-6-methyl-2-penthyl-4*H*-pyran-4-one), a phytoalexin that accumulates with long-term storage of garlic bulbs.

Much of the research into the bioactive compounds of garlic has centered on its sulfur compounds. The total sulfur content in garlic is about 0.35% of its fresh weight. Predominant sulfur compounds in raw intact or dehydrated garlic are alliin (*S*-allylcysteine sulfoxide), γ-glutamyl-*S*-*trans*-1-propenylcysteine, and γ-glutamyl-*S*-allylcysteine (Figure 2). In whole garlic, these compounds constitute about 0.5–1.4%, 0.3–0.9%, and 0.2–0.6% of the bulb, respectively. Sulfur compounds occurring at lesser concentrations include isoalliin (*S*-*trans*-1-propenylcysteine sulfoxide), methiin (*S*-methylcysteine

[†]Deceased

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Table 1 Top ten producers of garlic ranked by percent of total global production

Country	*Area harvested (ha)*	*Production (tons)*	*Global production (%)*
China	833 400	19 219 939	81.0
India	200 600	1 057 800	4.5
Egypt	12 145	295 845	1.2
Republic of Korea	24 035	295 002	1.2
Russian Federation	26 800	233 950	1.0
Myanmar	29 129	209 308	0.9
Bangladesh	41 997	209 153	0.9
The United States	10 180	190 690	0.8
Argentina	17 739	174 363	0.7
Ukraine	21 200	171 900	0.7

Source: Data are from the Food and Agriculture Organization Statistical Database (FAOSTAT) of the United Nations (2011). 'FAO statistics' faostat.fao.org.

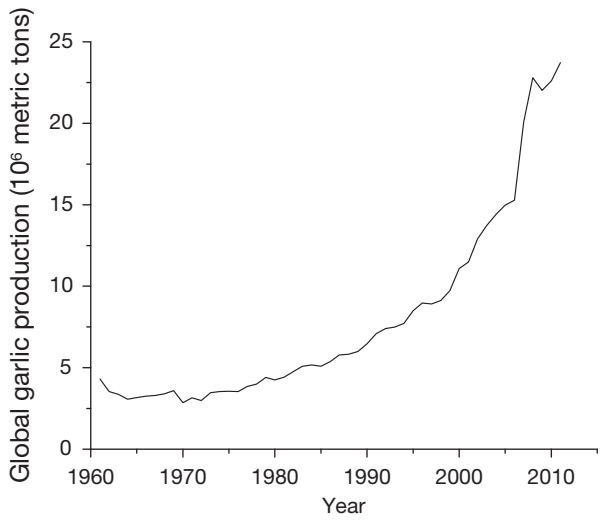

Figure 1 Global garlic production as reported in the Food and Agriculture Organization Statistical Database (FAOSTAT) of the United Nations (2011). FAO statistics faostat.fao.org.

sulfoxide), γ-glutamyl-*S*-methylcysteine, and γ-glutamyl-*S*-*cis*-1-propenylcysteine. *S*-Alkylcysteine sulfoxides such as alliin can be transformed to thiosulfinates when in the presence of water and the enzyme alliinase but are relatively stable in intact and dehydrated garlic.

Crushed or Cut Garlic

In intact garlic, alliinase is localized in vascular bundle sheath cells, whereas alliin is compartmentalized in mesophyll cells. Upon tissue disruption, the exposure of alliin to alliinase leads to the synthesis of allicin (diallyl thiosulfinate) in a matter of seconds. A similar enzymatic process occurs in the case of addition of water to garlic powder in which alliinase has been preserved. Allicin itself is moderately stable at room temperature. Allicin in undiluted crushed garlic has a half-life of about 2.4 days. Incubating crushed garlic in water extends the half-life of allicin, indicating the presence of garlic compounds that destabilize allicin in macerated cloves. In a process that is accelerated with increased temperatures, allicin decomposes through a series of steps resulting in polysulfides including diallyl disulfide and diallyl trisulfide, as well as allyl alcohol, propene, and sulfur dioxide. However, polysulfides are not present at significant levels in freshly crushed, room-temperature garlic.

Although γ-glutamyl-*S*-*trans*-1-propenylcysteine and γ-glutamyl-*S*-allylcysteine are far more stable than alliin under conditions of tissue rupture, they are metabolized as well, having a half-life of about 1 week. By the enzymatic activity of γ-glutamyl transpeptidase, they are quantitatively transformed to *S*-allylcysteine and *S*-1-propenylcysteine.

Aged Garlic Extract

This garlic formulation is produced by extraction of fresh chopped garlic in aqueous ethanol over the course of several months, after which the solution is filtered and concentrated. This process favors the generation of water-soluble compounds such as *S*-allylcysteine, *S*-*trans*-1-propenylcysteine, and *S*-allylmercaptocysteine with a concomitant decrease in the γ-glutamyl-*S*-alkenylcysteines. The aging process also yields smaller amounts of lipid-soluble diallyl sulfide, triallyl sulfide, diallyl disulfide, and other polysulfides. Allicin levels decrease rapidly although levels of alliin, which diffuses into the extract away from matrix-bound alliinase, change little for up to 2 years. Aged garlic extract is available in tablets and capsules as well as a liquid.

Steam-Distilled Garlic Oil

Steam treatment of crushed garlic causes allicin to react with itself, leading to a product high in mono- and polysulfides, in particular diallyl disulfide, diallyl trisulfide, allyl methyl disulfide, and allyl methyl trisulfide. In addition to allyl and methyl sulfides, 1-propenyl sulfides are also found in steam-distilled garlic oil. Allicin and other thiosulfinates as well as the γ-glutamyl-*S*-alkenylcysteines found in raw crushed garlic are not contained in garlic oil. Typically steam-distilled garlic oil is diluted in vegetable oil about 100–200-fold and is available in capsules.

Oil-Extracted Garlic Macerate

The extraction of homogenized garlic in soybean or other vegetable oils results in the decomposition of allicin to the oxygenated ajoenes and the ring-structured vinyldithiins. 2-Vinyl-4-*H*-1,3-dithiin constitutes about half of the sulfur

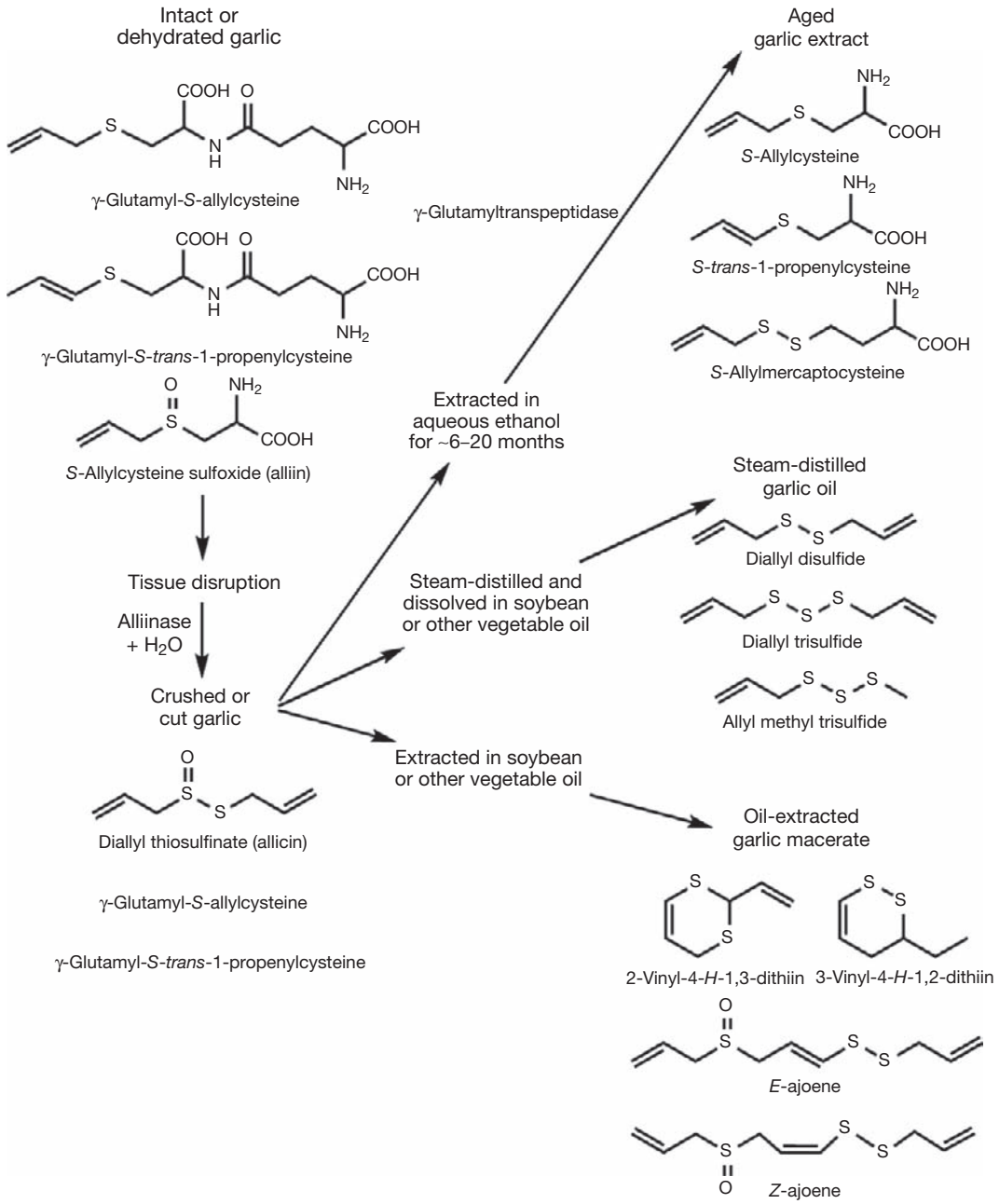

Figure 2 Major sulfur-based compounds present in garlic and in preparations derived from garlic.

compounds on a weight basis, whereas 3-vinyl-4-*H*-1,2-dithiin constitutes less than a quarter. Diallyl disulfide, diallyl trisulfide, and allyl methyl trisulfide are found at low levels in oil-extracted garlic macerate. Thiosulfinates and γ-glutamyl-*S*-alkenylcysteines do not occur in this product. This formulation also is generally sold as capsules.

Bioavailability, Absorption, and Metabolism

Most studies of the bioavailability and metabolism of garlic constituents have focussed on garlic's sulfur compounds. Although garlic and garlic formulations have been chemically well-characterized, current understanding of the fate of ingested potentially bioactive garlic compounds is far less complete. Allicin was once thought to be the principal sulfur-based compound responsible for the biological effects of garlic, but this is no longer the case. Despite considerable effort, no one has detected allicin or alliin or any of their metabolites *in vivo* in human blood, even following the consumption of 25 g of chopped raw garlic. Allyl methyl sulfide occurs in alveolar (lung) air after consuming allicin, strongly suggesting that allyl methyl sulfide is a metabolite of allicin present in the circulatory system. When incubated in heparinized human

blood, allicin disappears within five minutes and results in the generation of diallyl disulfide, which is stable for about an hour. Diallyl disulfide has not been observed *in vivo*.

The lack of bioavailability of allicin has been explained in a couple ways. One explanation is that allicin binds to protein and fatty acids in plasma membranes and is thus prevented from entering the circulation. A second explanation is that allicin may react with plasma glutathione to form allyl mercaptan (2-propanethiol), which is then rapidly converted via a methyltransferase and *S*-adenosylmethionine to allyl methyl sulfide. Alternatively, dithioallyl compounds such as allicin, diallyl trisulfide, diallyl disulfide, and ajoene also may react with free and protein-bound cysteines in the gut to form *S*-allylmercaptocysteine, with subsequent conversion in erythrocytes and epithelial cells to allyl mercaptan and then to allyl methyl sulfide. In rats given diallyl disulfide by gavage, allyl mercaptan, allyl methyl sulfide, allyl methyl sulfoxide, and allyl methyl sulfone were found in the stomach, liver, plasma, and urine. Allyl methyl sulfoxide and allyl methyl sulfone have not been reported in humans following garlic consumption.

Allicin is stable under gastrointestinal conditions but most likely does not reach target organs due to its binding to proteins and fatty acids and/or its rapid metabolism in blood. Nonetheless, the presence of allicin in the gastrointestinal tract is of interest because its metabolite allyl methyl sulfide may exert biological effects. Furthermore, the identities and bioactive properties of allyl methyl sulfide metabolites have not been elucidated, so the apparent absence of allicin in human blood does not in and of itself discount possible health benefits of consuming fresh garlic or allicin-producing garlic dietary supplements. Additionally, allicin exhibits antimicrobial effects and thus would be expected to modify intestinal microbial populations that in turn impact food digestion and human health. Generally, when raw garlic has been crushed and time allowed ($\sim$10–15 min) for alliinase to catalyze the hydrolysis of alliin to allicin prior to cooking and/or eating, allicin can be delivered intact to the gastrointestinal tract. However, in the case of dried garlic powder capsules in which alliinase is preserved during dehydration, the inhibition of alliinase by stomach acid may limit the conversion of alliin to allicin. Similarly in the case of enteric-coated garlic powders designed to pass through the stomach unmetabolized, simulated intestinal fluids have been shown to inhibit the production of allicin.

Allyl methyl sulfide is found in breath following consumption of garlic and garlic formulations, along with methanethiol and allyl mercaptan. However, only allyl methyl sulfide originates from the gut, whereas other metabolites result from oral metabolic processes. Measurement of allyl methyl sulfide in breath has been used as an indicator of allicin metabolism. The maximum concentration of breath allyl methyl sulfide following consumption of raw garlic occurs at about two hours and declines to undetectable levels by 30 h. Intake of ajoene, diallyl disulfide, diallyl trisulfide, and steam-distilled garlic oil also produces breath allyl methyl sulfide. In contrast, vinyldithiins, diallyl sulfide, and *S*-allylcysteine do not result in detectable breath allyl methyl sulfide. None of these latter compounds contains a dithio (S–S) group, which is thought to be necessary to generate allyl methyl sulfide. Allyl mercaptan, the immediate precursor to allyl methyl sulfide, is an exception.

S-Allylcysteine is found in plasma following intake of aged garlic extract, which is rich in *S*-allylcysteine. *N*-Acetyl allylcysteine has been found in urine following consumption of a garlic extract that was not chemically characterized. It was not described as aged garlic extract, however, and therefore probably was not high in *S*-allylcysteine. The precursor to *N*-acetyl allylcysteine may be γ-glutamyl-*S*-allylcysteine, which by the action of γ-glutamyl transpeptidase could be hydrolyzed to *S*-allylcysteine. *S*-Allylcysteine may be metabolized by two pathways. In one pathway, *S*-allylcysteine is acetylated by *N*-acetyl transferase, resulting in *N*-acetyl allylcysteine. Hepatic flavin-containing monooxygenases then oxidize *N*-acetyl allylcysteine to *N*-acetyl allylcysteine sulfoxide. In the second pathway, the flavin-containing monooxygenases act first upon *S*-allylcysteine, transforming it to *S*-allylcysteine sulfoxide (alliin), and *N*-acetyl transferase catalyzes the conversion of *S*-allylcysteine sulfoxide to *N*-acetyl allylcysteine sulfoxide. Other garlic γ-glutamylcysteines, which are abundant in intact, crushed, and dried garlic, may undergo similar metabolic processes.

Garlic: Health Effects

There is a rich history of garlic being used to prevent and treat health conditions as varied as asthma, cholera, and fever. With the rapid advancement of biological and medical science over the last half century, hundreds of studies have reported on the biological effects of garlic and garlic-related metabolites. Although the sheer number of studies devoted to garlic is impressive, there is a remarkable lack of clarity as to the roles of garlic in human health. The vast majority of studies have been conducted using cell culture (*in vitro*) or animals and signal that garlic intake may provide protection against cancer and cardiovascular disease. The results from cell and animal studies have not been consistently duplicated in human studies. Although all of the reasons for this are not known, often dose levels of garlic and garlic-related compounds used in cell and animal experiments do not mirror what is realistically achievable in humans. Moreover, the physiology of animals does not always well model human physiology, thus preventing the simple translation of results from animal studies to humans.

Results from human studies have been inconsistent with each other, suggesting the need to elucidate specific mechanisms of action induced by garlic and the specific garlic components that drive these mechanisms. The inconsistency of results from human studies may derive in part from the use of differing garlic formulations, doses, the durations of the studies, and outcome variables. Another important factor that may explain the inconsistencies of the effects of garlic and garlic formulations on health in humans is the variety and quality of experimental study designs. Epidemiological studies include prospective cohort and case–control (retrospective) studies. In prospective cohort studies, subjects are selected based on their garlic intake, and their disease status is monitored over a period of time that may extend to several years. In case–control studies, subjects are selected based on their disease status and attempts are made to correlate this status with prior garlic intake. In both prospective and case–control

studies, garlic intake is often self-reported and may not be accurate. Additionally, data interpretation may be confounded by the many forms of garlic and garlic formulations, the ways in which garlic is cooked, the varieties that are consumed, and the field and storage conditions to which cultivated garlic is exposed.

In contrast to epidemiological studies that are observational in nature, randomized controlled dietary intervention studies (randomized controlled trials) typically provide a known amount of garlic, garlic formulation, or specific garlic-derived compounds to control and intervention groups. Depending on the length and objectives of the study, the treatment response measured may be disease status or, alternatively, biomarkers that do not necessarily indicate the presence of a disease, but may otherwise reflect health status. The best-designed randomized controlled dietary intervention studies randomize to reduce baseline between-group variation, thoroughly characterize and report the dietary treatment, include an appropriate control group, administer a placebo to the control group in place of the dietary intervention of interest, and are double-blinded. In studies involving garlic and garlic formulations, the strong odor of the treatment often cannot be reproduced in a placebo and thus placebos are not always possible and subjects cannot always be blinded to the treatment they are receiving. Randomized controlled dietary intervention studies are particularly useful for determining the efficacy of a dietary intervention to affect a health outcome variable in a particular subject population. Epidemiological studies are valuable for hypothesis generation and often provide the foundation and impetus for designing and conducting randomized controlled studies.

Cancer Prevention and Treatment

There is a significant body of preclinical research aimed at understanding the roles of garlic and garlic formulations in the prevention and treatment of cancer. Preclinical studies have provided a means of elucidating the mechanisms by which garlic and garlic formulations may have anticancer effects. Xenobiotic metabolism may be affected by garlic compounds. Cytochrome P450 2E1, one of many phase 1 enzymes, which activate procarcinogens, is inhibited in culture by diallyl sulfide and its putative metabolites diallyl sulfoxide and diallyl sulfone. In rats, however, the phase 1 enzymes cytochromes P450 1A1, 2B1, and 3A1 were increased by garlic oil, diallyl sulfide, diallyl disulfide, and diallyl trisulfide, although P450 2E1 was decreased. Phase 2 enzymes modify activated carcinogenic intermediates to reduce toxicity and facilitate excretion. One type of phase 2 enzyme, the glutathione transferases, is upregulated in the livers of rodents provided diallyl disulfide or *S*-allylcysteine. These effects on phase 2 enzymes along with the inhibition of P450 2E1 are suggestive of an anticancer effect although the upregulation of P450 1A1, 2B1, and 3A1 (other phase 1 enzymes) by garlic polysulfides may not be desirable.

Besides influencing xenobiotic enzymes, garlic compounds may exert anticancer effects by arresting cell cycle progression, inducing apoptosis, inhibiting angiogenesis, or modifying histones. Treatment with diallyl disulfide, diallyl trisulfide, allicin, *S*-allylmercaptocysteine, or ajoene induces G2/M phase cell cycle arrest in a number of neoplastic cell lines. Garlic polysulfides induce apoptosis in several cancer cell lines but seem not to affect normal cells. The mechanisms for inducing apoptosis may include the regulation of the apoptotic Bcl-2 protein family and generation of reactive oxygen species. The inhibition of angiogenesis by diallyl sulfide and diallyl trisulfide seems to occur in part by suppressing vascular endothelial growth factor. Histone modification modifies gene expression by regulating access of transcription factors to DNA and may be protective against cancer. Modification of histones and associated inhibition of cultured neoplastic cells in response to allicin, allyl mercaptan, allyl methyl sulfide, diallyl disulfide, *S*-allylmercaptocysteine, and *S*-allylcysteine have been reported and involve hyperacetylation of histones H3 and H4. Other possible anticancer mechanisms resulting from garlic consumption include increased free radical scavenging and antioxidant activity.

In comparison to the abundance of preclinical studies, there are far fewer human studies investigating the effects of garlic and garlic formulations in cancer prevention and treatment. Many of the human studies are epidemiological studies. In the Iowa Women's Health Study, a prospective cohort study, 41 837 women of ages 55–69 were monitored for colon cancer incidence for 5 years. When the uppermost and lowermost consumption levels were compared, cancer incidence was inversely correlated with garlic intake, with an age- and energy-adjusted relative risk of 0.68. In the European Prospective Investigation into Cancer and Nutrition involving 368 010 women and 153 447 men, results after an average 6.5-year follow-up suggested that garlic and onion consumption reduced intestinal cancer, corresponding to a hazard ratio of 0.70 per 10 g per day increase. When 477 312 of these participants were followed up after 11 years, the decreased risk of intestinal cancer associated with garlic and onion intake was no longer present. An expert review panel organized by the World Cancer Research Fund and the American Institute for Cancer Research published a report in 2007 in which they judged that *Allium* vegetables, which include garlic, probably protect against stomach cancer. This conclusion was based on two cohort studies, 27 case–control studies, two ecological studies, and an intervention study utilizing allitridum (diallyl trisulfide) and selenium. Based on two cohort and six case–control studies investigating garlic consumption, the reviewers also concluded that garlic probably protects against colorectal cancer. Recent epidemiological studies have produced inconsistent results. An analysis of prospective data from the American Cancer Society Cancer Prevention Study (CPS) II Nutrition Cohort including 42 824 men and 56 876 women weakly supported a reduced risk of colorectal cancer in women, whereas in men, there was a slight increase in risk of colorectal cancer associated with past use of garlic supplements. In a prospective study encompassing cohorts of the Nurses' Health Study (121 700 women) and the Health Professionals Follow-up Study (51 529 men) followed for up to 24 years, garlic intake was not associated with the risk of colorectal cancer. The accumulation of both positive and null results suggests the need to identify groups most likely to benefit from garlic intake and the forms and doses of garlic that are most effective.

As with epidemiological studies investigating the effects of garlic on cancer, results from randomized controlled trials have

not always been consistent. In a double-blind randomized clinical trial, aged garlic extract increased natural killer cell number and activity in patients with liver, pancreatic, or colon cancer but did not increase their quality of life. In a different trial, after 12 months, aged garlic extract suppressed the emergence of colorectal adenomas, which are precancerous lesions of the large bowel. Another randomized double-blind trial tested the ability of a combination of aged garlic extract and steam-distilled garlic oil to reduce the incidence of precancerous gastric lesions. The study had 3365 participants and lasted for 7.3 years. Garlic supplementation did not affect the prevalence of precancerous gastric lesions or gastric cancer incidence compared to the control treatment. Synthetic allitridum in combination with selenium, however, decreased the risk of gastric cancer compared to placebo in an experimental population at high risk for gastric cancer. The garlic/selenium treatment was administered every other day for 1 month for each of three consecutive years to the intervention group of 2526, whereas members of the placebo group of 2507 received a corn oil capsule and starch tablet. Follow-up was at 10 years. In a trial to test the topical application of an ajoene-containing cream to basal cell carcinoma lesions, it was found that most tumors were reduced in size and Bcl-2 expression was decreased after twice daily treatment for 6 months. This response was attributed to the induction of apoptosis in the tumor cells.

Cardiovascular Effects

Investigation of the effects of garlic and garlic formulations on cardiovascular health has been another principal focus of garlic research. Numerous preclinical and clinical studies have investigated parameters such as plasma lipids, blood pressure, and platelet aggregation. A wide range of garlic preparations have been tested under varying experimental conditions. Several meta-analyses have been published that attempt to view and interpret the accumulated evidence and highlight significant trends.

Studies conducted in animals suggest that garlic and garlic preparations favorably modify total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Garlic formulations tested include allicin, dried garlic, boiled garlic, and garlic oil and, in most cases, have been provided at doses ranging from 2% to 5% of the total diet. Studies have been undertaken to determine possible mechanisms of lipid regulation. Activity of a key enzyme involved in cholesterol synthesis, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, was decreased in hens that had been given solvent-extracted garlic macerate or garlic oil for 24 days. In cultured hepatoma cells, garlic extract, diallyl sulfide, diallyl trisulfide, and allyl mercaptan inhibited cholesterol synthesis, perhaps by means of a mechanism in which inhibition of 4α-methyl oxidase led to feedback inhibition of HMG-CoA reductase.

Clinical trials investigating the effects of garlic and garlic formulations on lipid parameters have produced mixed results. In a particularly well-designed randomized controlled trial conducted through Stanford University, raw garlic, a commercial dried garlic powder product, a commercial aged garlic extract product, and a placebo were given to treatment groups each with 47–49 moderately hypercholesterolemic (LDL cholesterol of 130–190 mg dl^{-1}) subjects. Each of the treatments was extensively chemically characterized. The daily dose of the raw garlic was 4.0 g and the dried garlic product was provided in a similar quantity based on allicin generation. The provided dose of the aged garlic extract was 1.5–3 times the manufacturer's recommended amount. Subjects were randomly selected and assigned to treatment groups. The protocol entailed consumption of the dietary treatment 6 days per week for a total of 6 months, and lipid parameters were measured monthly. There were no significant treatment effects on total cholesterol, LDL cholesterol, HDL cholesterol, the total cholesterol/HDL cholesterol ratio, or triglycerides.

The results of the Stanford University trial do not necessarily extrapolate to other subject populations or to all garlic doses and formulations. It is possible that populations with higher LDL concentrations would respond favorably to the tested garlic treatments or to treatments consisting of higher doses. In a recent meta-analysis that included 39 randomized, placebo-controlled human trials investigating the effect of garlic on lipid parameters, it was concluded that when consumed for at least 2 months, garlic effectively reduced cholesterol by 17 ± 6 mg dl^{-1} and LDL cholesterol by 9 ± 6 mg dl^{-1} in humans with baseline total cholesterol levels >200 mg dl^{-1}, which is a higher baseline than that of the Stanford University trial. Twenty-four of the trials included in the meta-analysis used garlic powder, six used garlic oil, five used garlic extract, and two used raw garlic. This ~8% reduction in serum total cholesterol was deemed to be clinically relevant and associated with a 38% reduction in coronary events at age 50.

Garlic has also been studied as a means to regulate blood pressure. A meta-analysis that encompassed 11 randomized controlled trials assessed the efficacy of garlic to modulate blood pressure in normotensive and hypertensive subjects. Garlic treatments significantly reduced systolic blood pressure more than placebo by 4.6 mm Hg (95% CI: −7.36, −1.77) but diastolic blood pressure did not differ significantly. When stratified into normotensive and hypertensive groups, systolic blood pressure and diastolic blood pressure did not change in normotensive subjects but decreased significantly by 8.38 mm Hg and 7.27 mm Hg, respectively, in the hypertensive group. Dried garlic powder was used in nine of these studies, and aged garlic extract and distilled garlic oil were each used in the remaining two studies. A recent dose–response double-blind randomized placebo-controlled trial seemed to verify that at least in hypertensive populations, aged garlic extract reduces blood pressure over a 12-week period.

Evidence for an effect of garlic on platelet aggregation is conflicting. Although some *in vitro* studies have indicated an antithrombotic effect of garlic, this result has not been consistently observed in clinical trials. Several clinical studies conducted prior to 2002 reported inhibition of platelet aggregation, whereas more recent studies have reported no effect of consumption of garlic or garlic supplements. Raw garlic, boiled garlic, garlic powder, garlic oil, and aged garlic extract have been tested. When the equivalent of 8 g of raw garlic was provided daily for 4 weeks, platelet-rich plasma aggregation decreased less than 10% but collagen-induced whole blood aggregation increased 10–13%. Given that this dose of garlic is unusually high, the investigators who conducted this study concluded that normal levels of garlic likely would not have an

antithrombotic effect in healthy individuals. Additional carefully designed randomized controlled trials may be needed to clarify whether garlic or garlic formulations affect humans and particularly what subpopulations may be most likely to respond to the intervention.

Antiglycemic Effects

Several animal studies indicate that garlic preparations improve oral glucose tolerance. Nevertheless, evidence for antiglycemic effects in humans is scant. Most human studies conducted prior to 2000 do not support a role for garlic or garlic formulations in glucose control. Two recent studies report a positive effect of garlic supplements. A randomized double-blind placebo-controlled trial was conducted in 60 type 2 diabetic patients to test the antiglycemic effects of a 300 mg time-released garlic powder tablet taken twice daily. After 1 week, fasting blood glucose was lower compared to baseline in the garlic group, but this effect did not occur at 2, 3, or 4 weeks. Serum fructosamine, a measure of long-term compensation of metabolic control, was decreased compared to baseline and the placebo group at 3 and 4 weeks. In an open-label, prospective, comparative study conducted in a different population of 60 type 2 diabetic patients all receiving metformin for glucose regulation, an uncharacterized garlic supplement was provided to half of the patients. Fasting blood glucose and postprandial blood glucose were reduced at weeks 2, 4, 6, 8, and 12 in the garlic supplement group compared to the control group. In light of the inconsistent data, additional large-scale randomized controlled trials with well-characterized interventions are required to determine whether garlic or garlic preparations are useful as antidiabetic agents.

Conclusions

Garlic has been used as a food and as a medicinal herb for thousands of years. Based on current trends, garlic use can be expected to continue to expand. The unique flavor and aroma of garlic have inspired countless culinary dishes and remedies for treating numerous medical conditions. Garlic's sensory attributes are based on its remarkable sulfur chemistry, a biochemical system that is activated upon cellular disruption and that offers an expanding array of sulfur compounds with further processing. With the advent of modern science, the utility of garlic and garlic preparations for treating human diseases is under investigation. Based on preclinical studies, there is reason to be hopeful that garlic compounds may ameliorate certain human conditions, particularly as related to cancer and cardiovascular disease. Epidemiological studies and randomized controlled trials have lent mixed evidence for the efficacy of garlic and garlic preparations in humans. The inconsistent findings likely are related to the use of different garlic treatments, doses, durations of testing, subject populations, and outcome variables. Our understanding of the roles of garlic and garlic preparations in human health will benefit from additional well-designed human studies that carefully characterize the garlic interventions used and that investigate possible differential effects in various human subpopulations.

See also: Cancer: Diet in Cancer Prevention; Cholesterol: Factors Determining Blood Cholesterol Levels; Spices and Flavoring Crops: Uses and Health Effects.

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Gelatin

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Gelatin

Gelatin or gelatine, which mainly composed of 50.5% carbon, followed by 6.8% hydrogen, 17% nitrogen, and 25.2% oxygen, is a type of insoluble protein produced by hydrolysis of collagen extracted from a variety of animal sources such as the skin, bones, and connective tissues. These sources are normally generated as a by-product following animal slaughter by the muscle food processing industry (meat, poultry, and seafood). Collagen is part of the basic structure of animal bodies (including humans) and constitutes the skin, tendon, bone, membrane, and connective tissues. It consists of rigid bar-like molecules arranged in fibers and linked by covalent bonds. The molecules have three polypeptide chains stabilized by hydrogen and hydrophobic bonds and arranged in a triple helix structure due to the repeating sequence glycine–proline–hydroxyproline (Gly-X-Y).

There are a number of collagen types that can be classified based on their distribution within the tissues. However, most collagen consists of types I, II, and III. Type I collagen is distributed as loose or dense connective tissue and can be found in the skin, bones, tendons, and blood vessels. Type II collagen consists of tissues derived from the cartilage and vitreous humor and type III collagen is found in loose connective tissue and also in the muscle, where it has an association with type I collagen. These collagen types are also different in terms of molecular composition.

The amino acid content of gelatin is almost identical in composition with the original collagen from which it was derived. It also has repeating sequences of Gly-X-Y, with Gly representing proline and Y representing hydroxyproline. Gelatin possesses all the essential amino acids, with the exception of tryptophan. It contains a high amount of glycine, proline, and hydroxyproline but is low in cysteine, methionine, and tyrosine. Proline and hydroxyproline are very important with respect to gelling properties.

Properties Associated with Gelatin

Gelatin is translucent, almost tasteless and odorless, brittle, easy to digest, and a nonallergenic protein. Dry gelatin has a moisture content of about 10%. Generally, gelatin is processed into powder or granulated form. The color of the powder depends on the gelatin source. For example, gelatins derived from cows and pigs possess a yellowish color compared with gelatins derived from fish. Gelatin derived from pork skin also has less color than gelatin derived from bone or hide. However, color generally does not influence the properties of gelatin.

Gelatin is a relatively low-priced food ingredient since it is normally generated as waste. It is abundant (from a wide variety of land and marine sources) and has great functional properties. The properties of gelatin are dependent on a number of factors, such as collagen type, gelatin source, age of the animal at slaughter, and processes used to generate gelatin. Differences in amino acid composition, such as content of α-, β-, or γ-chain components and degree of molecular weight, can be affected by these factors.

Gelatin is pure, natural, and not chemically modified. It has a high amino acid content. Unlike collagen, gelatin is soluble in hot water. It is unique since it is a thermoreversible gel, possesses a unique melt-in-the-mouth property (melts at lower temperature compared to other forms of hydrocolloids), and has a wide application range within the food industry. Gelatin has also been widely used in pharmaceutical, cosmetic, and photographic industries.

Protein Structure of Gelatin

Proteins (from Greek 'prota,' which means 'of primary importance') are polymers that are composed of many amino acids joined together, as a linear chain, by amide bonds. Such proteins are called polypeptides. Proteins contain more than 100 amino acid residues from 20 proteinogenic amino acids. Amino acid residues occur when amino acids combine together (e.g., glycine and alanine) and release a water molecule as a consequence of this interaction. Proteins can differ in terms of form and function and the differences observed can be explained by overall protein structure, origin of proteins, and the amino acid composition comprising the protein.

There are four basic protein structures: primary, secondary, tertiary, and quaternary forms. A primary protein structure is one where the amino acid sequence is arranged linearly. Meanwhile, a secondary protein structure is one where the shape of the protein molecules exists in either α-helix or β-sheet (folding and coiling), which is caused by hydrogen bonding between –C═O and –NH groups within the chain and the primary amino acid building blocks. The tertiary protein structure results when folding and bending of the protein molecules occur due to interactions with the R groups on individual amino acids. This interaction may occur as a result of hydrogen bonding, dipole–dipole interactions, covalent bonding, or ionic bonding (salt bridges) depending on the polarity of the R groups. Meanwhile, a quaternary protein structure results as a consequence of noncovalent interactions that bind multiple polypeptides into a large macromolecular complex.

A basic amino acid contains a central carbon (α-carbon) bonded to hydrogen, an amino group (NH_2), a carboxyl group (COOH) and R group, and a unique amino acid side chain that distinguishes one amino acid from another. The 20 amino acids available in nature are listed in **Table 1**. Amino acids can be divided into nonpolar (hydrophobic), polar (hydrophilic), and electrically charged (positive or negative hydrophilic) groupings. Polar amino acids may be involved in hydrogen bonds due to its tendency to contact with water,

Table 1 20 different of amino acids

Amino acid	*Three-letter code*	*Properties*
Glycine	Gly	Hydrophobic
Alanine	Ala	Hydrophobic
Valine	Val	Hydrophobic
Leucine	Leu	Hydrophobic
Isoleucine	Ile	Hydrophobic
Methionine	Met	Hydrophobic
Phenylalanine	Phe	Hydrophobic
Tryptophan	Trp	Hydrophobic
Proline	Pro	Hydrophobic
Serine	Ser	Hydrophilic
Threonine	Thr	Hydrophilic
Cysteine	Cys	Hydrophilic
Tyrosine	Tyr	Hydrophilic
Asparagine	Asn	Hydrophilic
Glutamine	Gln	Hydrophilic
Aspartic acid	Asp	Negatively charged (hydrophilic)
Glutamic acid	Glu	Negatively charged (hydrophilic)
Lysine	Lys	Positively charged (hydrophilic)
Arginine	Arg	Positively charged (hydrophilic)
Histidine	His	Positively charged (hydrophilic)

whereas hydrophobic amino acids are normally located inside the core of protein. Meanwhile, an imino acid is a molecule that contains both imino (CNH) and carboxyl functional groups and, consequently, is related to amino acids. Proline is formally classified as an imino acid, although it is sometimes called an amino acid.

Gelatin is a mixture of different polypeptide chains, α-chains, β-chains (dimers of α-chain), and γ-chains (trimmers of α-chain). An α-chain has one peptide chain, a β-chain consists of two peptide chains that are still connected, and a γ-chain is composed of three connected peptide chains. Gelatin that contains higher quantities of α-chains exhibits higher gel strengths.

Types and Bloom of Gelatin

Gelatin can be classified into two types and this is determined by gelatin pretreatment during the gelatin manufacturing process. Type A gelatin with an isoionic point of 6–9 is obtained from acid-treated collagen, whereas type B gelatin (isoionic point of 5) is derived from an alkali-treated precursor. Gelatin derived from pig skin is normally referred to as type A gelatin and gelatin derived from beef skin is referred to as type B gelatin.

Gel strength and viscosity are the most important physical properties of gelatin. Gel strength, also known as 'bloom' value, is a measure of the strength and stiffness of the gelatin, reflecting the average molecular weight of its constituents, and is usually between 30 and 300 bloom (<150 is considered to be a low bloom, 150–220 a medium bloom, and 220–300 a high bloom). Higher bloom value indicates greater gelatin strength. Based on the type of product required and its function, a different bloom value for gelatin is applied.

Gelatin Manufacturing

Gelatin is commercially manufactured from collagen using a series of processing steps, including conditioning, extraction, filtration or purification, concentration, drying, and grinding. However, the process used may vary depending on the type of collagen source used, porcine or bovine sources, and skin or bone or hide origin. During the conditioning process, the material is cut and washed to cleanse and degrease the collagen before it is used to manufacture gelatin using either a type A (acid) or type B (alkaline) process. This is followed by the extraction process, whereby the material is mixed with clean and warm water before producing gelatin solution. The next process is filtration or purification in which few processes like centrifuging, ion exchange, and ultrafiltration may be applied to remove any salts or inorganic material. Concentration is the process of removing water from liquor gelatin by vacuum evaporators in a gentle process. At this stage, gelatin is highly viscous. The solution is filtered again through polishing filters to ensure the finest particles are removed. After gelatin solution is heat-sterilized, the solution is then dried on stainless steel conveyor belt using warm and dry air steam. Finally, after some physical, chemical, and bacteriological testing, gelatin will be ground, milled, sieved, and blended according to customer specifications. Some manufacturers set gelatin for quality assurance testing before delivering the products to their customers.

Sources of Gelatin

As previously stated, commercial gelatins have traditionally been obtained from pigs (porcine) and cattle (bovine) sources and derived from the skin, bone, and hide. However, gelatin derived from these sources are limited in some respects as they cannot be used or consumed by Muslims, Jews, or Hindus on various grounds.

Alternatively, gelatin is derived from marine sources. Gelatins from fish sources are obtained as a by-product from fish processing or from fish waste. About 30% of fish waste is in the form of bone and skin, which consist of a high collagen content. These types of gelatins have never been associated with prions, are religiously acceptable, and may possess unique functional properties. Nowadays, research and development of gelatin sources derived from fish are rapidly growing. This includes gelatin obtained from skins, bones, fins, heads, scales, or swim bladder.

There are a few factors that need to be considered when choosing gelatin from fish such as water-type species, fish types, and fish components used. The origin of fish, either warm-water (e.g., tuna, catfish, tilapia, Nile perch, shark, and megrim) fish or cold-water (e.g., cod, hake, Alaska pollock, and salmon) fish, may affect the physical properties of gelatin. Skin gelatins from warm-water fish have similar melting temperatures with skin gelatin obtained from warm-blooded animals. However, skin gelatins derived from warm-water fish have lower melting temperatures due to differences of imino acid content and degree of proline hydroxylation. Different extraction processes, temperatures, preservation methods, etc., also

influence the properties of fish gelatin. Despite there being an issue with allergic reactions related to fish gelatin, the wide variations of fish gelatin sources bring high prospect as important commercial gelatin sources in the future as commercial demand for gelatin continues to increase.

Apart from fish, the development of gelatin from other marine sources is ongoing, for example, from squid. Squid skins have a high degree of cross-linking and are obtained from a few species such as splendid squid (*Loligo formosana*) and giant squid (*Dosidicus gigas*). They are discarded as protein waste with low value and normally used as animal feed. However, the hurdle for gelatin extraction from squid skin is that color problems ensue due to pigments present in their skins. To overcome this problem, some researchers have used H_2O_2 as a bleaching agent to improve whiteness or brightness in extracted gelatin.

Recently, poultry sources like chicken skin, feet, meat residue, and bones are also being developed to obtain gelatin. Gel strength of chicken-derived gelatins may give higher bloom values than that of beef gelatins, the differences again being attributed to intrinsic characteristics such as amino acid content (higher imino acid, which is essential for gelling effect), molecular weight distribution, type of extraction treatment, and properties of collagen. The market for poultry gelatin is still niche and the global demand is not overwhelming as of yet. This is due to the higher price of poultry gelatin sources, lower production of raw materials compared to mammalian gelatins, and uncertainty on quality of gelatin.

In certain countries, gelatin derived from insects is also being considered as additional sources of gelatin. Melon bugs (*Aspongopus viduatus*) and sorghum bugs (*Agonoscelis pubescens*) are processed to produce oil that is utilized for medicine and cooking purposes. Gelatin can also be extracted. This type of gelatin has all of the essential amino acids, yet the quantity of the insect protein produced is only in the moderate range. Potential sources of collagen from alligator bones and giant red sea cucumber are also being studied. Alligator bones are identified as possessing collagen type I with imino acid contents, which are very similar to subtropical fish species and lower than that obtained from bovine skin. Figure 1 illustrates the sources of gelatin.

Gelatin Applications

Gelatin has been introduced in pharmaceuticals since early nineteenth century. Due to its film-forming, adhesive, and thermoreversible gelling properties, it is utilized as capsule in either two-piece hard capsules or soft elastic gelatin capsules, tablet coating, and encapsulation. It is also has been applied in surgical sponges, ointments, emulsions, syrups, wound dressing, health supplements, etc.

In terms of cosmetic application, this material is being used in lipsticks and hair care products (shampoos and conditioners) and as 'hydrolyzed animal protein.' Today, many cosmetic products such as creams, lotions, and masks utilize collagen as a moisture retainer as it has been proven to increase hydration and suitable for all skin types.

Gelatin has been used in photography since 1871 for many applications. Gelatin has been used by this industry as a coating layer, subcoating layer, noncurl layer, and antihalogen layer in a host of photographic products. Today, gelatin is widely used as the shell material in the outdoor paintball game as it is safe and nontoxic. Gelatin derived from pork skins and beef bones/hides is used in the manufacture of the paintball shell, a capsule with different size and shape that contains water-soluble dye. The broad applications of gelatin in the food industry will be discussed subsequently.

Application of Gelatin in Food

Gelatin is generally recognized as safe food ingredient and easy to use. Therefore, this material is utilized in many food products owing to the versatility of the ingredient, which can deliver the following functions: gelation, binding, water binding, emulsifying, foaming, film forming, elasticity, and viscosity. Adding gelatin to food products does not affect the flavor of original food products because it has a neutral flavor and odor. The variation of applications and its functions are influenced by the processing condition, bloom value, type of gelatin, viscosity, and percentage used. For example, texture and hardness of low-fat products can be altered by adjusting the gel

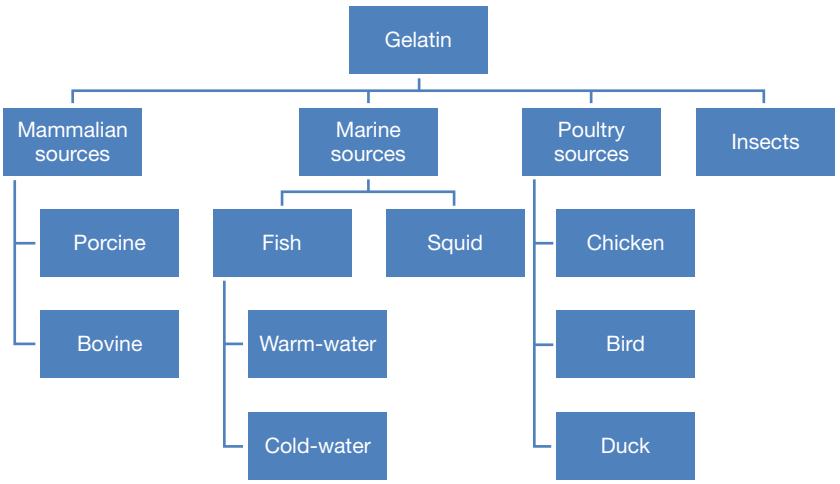

Figure 1 Gelatin sources.

Table 2 Some gelatin applications in foods

Product category	*Function*	*Food products*
Meat	Binding and glazing agent, casings, coatings	Canned meats, jellied meats, sausages, meat
Bakery and confectionary	Stabilizer, binding and gelling agent, emulsifier, elasticity and chewability improver, coating	Icing, glazes, creamy fillings, marshmallows, toppings, mousses, chiffons, gummy bears, gelatin and wine gums, toffees, chewing gums, compressed tablets, nougats, licorice, chocolate, and sugar-filling products
Dairy	Stabilizer, emulsifier, texture improver, water binder, increasing viscosity, fat replacement	Yogurts, creams, buttermilks, margarines, cheeses
Desserts	Stabilizer, gelling agent, coating	Pudding, custard, ice creams, milk ices, syrups, gravies, fruits
Wines and juices	Clarifying agent	Wines, beers, fruit juices, vinegar

strength or changing the concentration of gelatins. Table 2 summarizes some gelatin applications in foods.

Meat Products

Gelatin has been used as a binder and glazing agent in meat products and used to help set meat shape in canning applications. High bloom strength gelatin has also been used to gel the fluids of jellied meats allowing the pieces of meat to form certain shape. For sausage manufacture, collagen/gelatin has been used as edible casings as collagen/gelatin offers good uniformity of appearance and strength.

Bakery and Confectionary Products

Gelatin can also act as a stabilizer and can help to maintain crystal structure of sugar for bakery products such as icing, glazes, creamy fillings, and marshmallows. Additionally, because of its whipping and stabilizing properties, gelatin has been added to topping, mousse, and chiffon-type products. Gelatin is used in confectionary for its gummy properties, for example, in candy such as gummy bears and wine gums. Depending on the gelatin type, bloom strength, and concentration, the texture of gummy products can vary from soft to hard or from short to long. Gelatin is also compatible with other materials or gelling agents such as starch, pectin, and gum Arabic. Therefore, they can be mixed together and increase texture ranges in foods. Other confectionary products that may contain gelatin are toffees and chewing gums (improving elasticity), compressed tablets (as binder), nougat (enhancing chewability), and licorice (improving moisture retention, extending shelf life, and for binding properties). For chocolate or sugar-filled products, gelatin is used as a coating material before filling processes commence in order to prevent oil and moisture migration occurring from inside the product to the external product surfaces.

Dairy Products

In dairy products, gelatin is widely used due to its compatibility with milk proteins. It acts as an excellent stabilizer due to its gelling and water-binding actions, particularly for yogurts (including low and nonfat yogurts). It helps to improve yogurt texture by assisting in the development of smooth, creamy, and delicate mouthfeel properties while at the same time prolonging product shelf life. These properties also led gelatin to be used in concentrated cream or thickened cream by increasing cream viscosity. Small amounts of gelatin are frequently added to buttermilk and margarine to avoid separation and again promote smooth product textures. Gelatin also helps to stabilize the emulsion during spreading and storage. Additionally, high demands for low and nonfat dairy products give rise to the opportunity for gelatin use since it has unique 'fat-like melting characteristics' that mimic full-fat products. For cheese products like cottage cheese and cream cheese, gelatin acts as water binder and fat replacer. It also assists in the enhancement of smooth and creamy textures due to its unique melting point (close to dairy fats).

Dessert Products

Gelatin also has been used as one of the main ingredients in desserts, such as puddings, custards, ice cream, and milk ices as again it provides a unique texture, is a good stabilizer, possesses excellent gelling properties, and provides melt-in-the-mouth and unique mouthfeel properties.

Wine and Juice Products

Gelatin is being used in beverage products to clarify wines, beers, unfermented juices, and vinegar. These beverages normally contain insoluble matter that creates a haze or cloudiness and is impractical to remove. Therefore, gelatin has been applied for the clarification process or fining.

Basics of Gelatin Usage as Packaging Film Material

Gelatin also has high potential as biodegradable and/or edible films particularly for food packaging. Gelatin as packaging materials has high resistant to solvents and has excellent levels of flexibility. The uniqueness of gelatin as films in their physical properties is also influenced by intrinsic factors and physical parameters including sources of gelatin, concentration, gel strength or bloom value, film manufacturing process, temperature, drying time, and plasticizer type associated with molecular weight distribution and amino acid composition.

Gelatin is also being developed as active packaging material by incorporating antimicrobial and antioxidant agents to obtain films with good antioxidant and/or antimicrobial properties. These agents are normally derived from natural sources (plant extracts) such as essential oils (lemon, lime, kaffir lime, bergamot, ginger, turmeric, and plai), green tea extract (GTE), grape seed extract, ginger extract, and gingko leaf extract. There are also claims stating that gelatin itself also possess antioxidant activity. However, this finding is still not clearly demonstrated and still underway.

To date, gelatin (as either coatings or films) has been applied as 'food wrapper' mainly in meat-based products such as sea bass slices, rainbow trout, and cod fillets. Chicken meat powder and crackers are some dry food products that utilize gelatin as packaging materials. The application of this hydrocolloid as food packaging material is still constraint, but the research is evolving.

Conclusions

Higher demand of gelatin nowadays led to the exploration of alternative gelatins. Gelatin sources, ages of animals, processing conditions, etc., caused gelatin to exhibit diverse properties and characteristics attributed to differences of molecular weight distribution and amino acid contents. This scenario caused gelatin to have multifunctional properties and therefore catering for a broad range of applications. Numerous researches are ongoing to develop high yield and better quality of gelatin for commercialization purposes.

See also: Biofilms; Proteins: Chemistry, Characterization, and Quality.

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Genetically Modified Foods

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What Is GM Food?

Genetically modified (GM) food is manufactured from ingredients derived from a plant, animal, or microbe (fungus, bacterium, or yeast) in which a segment of nonnative DNA has been introduced using recombinant DNA technology. An alternative term used for these products is 'bioengineered' or 'genetically engineered' food. Currently, several GM crops have entered the human food chain either directly as food or indirectly via animal feed. Recombinant DNA technology (see Box 1), originally designed for manipulating bacterial DNA, has been adjusted to allow the incorporation of a gene(s) encoding a specific trait in a manner that is in principle highly predictable. Unlike conventional plant breeding, GM technology offers the possibility of producing phenotypes, which could never occur in nature, since the species origin of the introduced gene(s) (termed 'transgene') may well be far away from the recipient plant species. In a variant of the GM approach, the intention is not to introduce an exotic gene, but rather to suppress or enhance the expression of a native one. So far, the major investment in GM crops has been focussed on improving levels of resistance to insect damage, inducing resistance to herbicide, enhancing the nutritional quality of the end product, promoting the expression of resistance to pathogenic viruses, and prolonging the shelf life of the product. The next wave of GM crops is expected to widen the search to achieve the production of biomedical and industrial products, such as monoclonal antibodies, vaccines, plastics, and biofuels.

Three Generations of GM Crops: Purposes and Commonly Modified Traits

According to the FAO, global demand for food is predicted to increase by 40% by 2030. Whether this demand can be met without the deployment of GM crops is controversial. To successfully market a GM food, there must be some perceived advantage to either the producer or the consumer. The advantage to the former translates as a lower production cost and to the latter as a lower product price and/or improved end-use quality. The first generation of GM crops was targeted largely to producer traits, such as tolerance to environmental stresses (drought, salinity, etc.), enhanced disease/pest resistance, and tolerance to herbicide application. The second generation has begun to address consumer traits such as improved nutritional content, while the third-generation GM crops are largely directed at nonfood/feed applications, for example, the production *in planta* of pharmaceutically active molecules. The major GM food crops, their purposes, and potential benefits are discussed in the succeeding text.

Increasing Food Production

GM crops currently on the market have been designed to enhance crop production by bolstering the plants' resistance against disease, by protecting them against insect damage, or by introducing herbicide tolerance in order to control weeds more effectively. Such crops, initially focussed on maize and soybean in the United States, have been widely cultivated, and have arguably enhanced the profit margins of large-scale producers in the Americas, but are increasingly now being also deployed in smaller-scale production situations in Asia and Africa as a means of both increasing farmers' profitability and reducing exposure to pesticides.

Herbicide tolerance

Many of the first-generation herbicidal agents were nonselective and environmentally damaging. More recent products have addressed both of these drawbacks. Glyphosate, for example, is quite rapidly degraded by soil biota and so avoids the problem of persistence in the soil. Modern herbicides target plant-specific enzymes and so are generally nontoxic to animals (including humans) or insects. Two approaches have been taken to create herbicide-tolerant (HT) crops via genetic modification: one is to reduce the sensitivity of a host enzyme and the other is to engineer a herbicide detoxification pathway into the host. An example of the former approach is a gene isolated from a herbicide-resistant strain of *Agrobacterium tumefaciens*; it encodes an EPSPS enzyme not compromised by glyphosate. The gene was incorporated by Monsanto first into soybean and subsequently into a range of other crops. Transgenic plants tolerant to the herbicide acifluorfen, which inhibits chlorophyll synthesis, have been produced by overexpressing an enzyme involved in this process. In contrast, resistance to the herbicides glufosinate and bromoxynil is based on the latter approach, specifically by introducing a transgene that directs the conversion of the herbicide into a nontoxic product. In the case of glufosinate, the key bar gene is *Streptomyces hygroscopicus*, while for glyphosate, the gene, obtained from *Ochrobactrum anthropi*, encodes glyphosate oxidase. The availability of transgenic HT crops has promoted a significant switch from conventional to zero or low-tillage production systems, which implies energy savings and a reduction in both CO_2 emission and soil erosion. Herbicide tolerance has been commercialized in soybean, maize, canola (oilseed rape), and cotton, has been approved for sugar beet and alfalfa in several countries, and has been incorporated (but not yet approved) in both rice and wheat.

Insecticidal activity and disease resistance

The most widely exploited transgene conferring insecticidal activity is *Bt*, isolated from the bacterium *Bacillus thuringiensis*. GM-derived virus resistance crop has also been deployed. At the laboratory level, resistance has been engineered to several bacterial and fungal plant pathogens. The incorporation of *Bt* has been one of the most successful applications of GM technology. The Bt toxin is a potent natural crystal protein endotoxin, and *Bacillus thuringiensis* spores and preparations of

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Box 1 Plant Transformation Techniques: Established Methods and New Frontiers

Two established methods for plant transformation are recognized. The first exploits the natural ability of the soil bacterium *A. tumefaciens* to introduce a segment of its DNA into the host's genome; the second relies on the mechanical delivery of purified DNA into a temporarily receptive host cell. Both methods result in a rather random mode of insertion, which can have unpredictable effects. The cutting edge of plant transformation is predicated on achieving multigene transfer, site-specific transgene integration, and gene transfer between sexually compatible species.

Agrobacterium-*Mediated Transformation*

Agrobacterium-mediated transformation is the plant transformation method in most common use. Its cornerstone is the bacterium's transferred DNA (or T-DNA for short), which is carried on a plant tumor-inducing plasmid and is transferred upon infection into a host cell. In a successful infection, the T-DNA is integrated into the host's genome, leading to the host cell expressing certain bacterial genes. The T-DNA sequence has been adapted for genetic modification by replacing the bacterium's genes with the desired transgene(s), maintaining the left and right border sequences, which are needed to effect transfer and incorporation. Although the natural hosts for *Agrobacterium* are dicotyledonous species, it can be induced under laboratory conditions to effectively transform a wide range of higher plant species, including the monocotyledonous cereals.

Direct Gene Transfer

Particle bombardment (biolistic transfer): The earliest successes in plant transformation relied on a process whereby tiny tungsten or gold particles were coated with the transgene DNA sequence and then were propelled into cultured plant tissue by means of an electric discharge or a pressurized stream of helium gas. It remains effective as a transgene delivery method, especially in species that are easily not infectible by *Agrobacterium*.

Polyethylene glycol (PEG)-mediated transformation: In this method, the cell walls need first to be stripped from the host cells to leave a porous protoplast. When exposed to a mixture of polyethylene glycol and certain divalent cations, the protoplasts readily absorb DNA from the surrounding medium in a similar way to bacterial cells. Once within the protoplasts, some of this DNA becomes stably integrated into the plant chromosomes.

Electroporation: Originally developed to transform bacterial cells, this technique is also able to deliver DNA into plant protoplasts. The protoplasts are rendered porous by subjecting them to electrical pulses. DNA present in the surrounding medium can then migrate into the protoplast, where it can be incorporated as in the PEG method in the preceding text.

Silicon carbide whisker-mediated transformation: Silicon carbide whiskers are fine enough to physically puncture intact plant cells. The technique involves the violent stirring of a suspension of plant cells in the presence of the transgene DNA and the silicon carbide whiskers. The DNA enters the plant cells through the ensuing punctures, after which it can be incorporated as in the preceding text.

Microinjection: The concept is based on delivering the DNA in solution directly into a plant cell by means of a glass microcapillary injection pipette, in the same way as human in vitro fertilization is carried out. Few examples of the successful transformation of plants have ever been reported.

Bioactive beads-mediated gene transfer: The vector of the transgene DNA is represented in this application by a bead made of calcium alginate. The beads are introduced into protoplasts either via electroporation or via polyethylene glycol treatment.

Organelle-Mediated Transformation

Homologous recombination-mediated organelle transformation offers the possibility of transferring the transgene into a preselected position in the host genome. Chloroplast transformation vectors exploit two plastid sequences, which flank the transgene; the function of the flanking DNA is to promote homologous recombination at a predetermined locus in the chloroplast genome, once the vector has been delivered into the host protoplast either via particle bombardment or following PEG treatment. Plastid-transformed plants have been shown to express transgenes at a high level thanks to the high copy number of plastids present in the plant cells.

Cisgenesis and Intragenesis

The term 'cisgenesis' refers to the transfer of one of more genes originating either from the host species itself or from a close relative. A typical cisgene comprises the target gene itself along with its native promoter and terminator region. 'Intragenesis' refers to where the target gene (again isolated from the host species or a close relative) is attached to a host (or close relative) regulatory element, but not necessarily derived (as is the case for a cisgene) from the same locus as the gene sequence itself was.

Genome Editing with Sequence-Specific Nucleases

Genome editing using site-specific integration, deletion, and mutation is expected to have a profound impact on plant transformation in the near future. The technology is based on the use of chimeric nucleases, which induce targeted DNA double-strand breaks (DSBs). DSBs in nature are repaired either by homologous recombination or by error-prone nonhomologous end joining. The target genomic site can be repaired by inserting a transgene into the DSB. A number of engineered nucleases can in principle be designed to generate DSBs at almost any chosen genomic location.

Zinc-finger nucleases (ZFNs) are synthetic restriction endonucleases that combine a zinc-finger domain recognizing a specific DNA sequence with a nuclease domain (usually based on the *FokI* restriction enzyme). The sequence of the zinc-finger domain directs the ZFN to a specific genomic site.

TALENs are artificial endonucleases built by fusing the DNA binding domain present in a TAL protein to the *Fok*I cleavage domain. The DNA binding domain comprises a repetitive array of 33–35 amino acid residues, which determines the specificity of the TALEN's binding.

Homing endonucleases (or meganucleases) are highly specific DNA cleaving enzymes, ubiquitously found in microbes and in eukaryotic mitochondria and chloroplasts. They are encoded by genes that display genetic mobility, such as introns and inteins. A number of these enzymes have been completely redesigned for them to serve as tools for genome engineering. They are typically homodimeric but can be developed to form heterodimers of differently engineered monomers, targeting a large genomic recognition site (20–30 bp).

CRISPR/Cas, which represents 'clustered regularly interspaced short palindromic repeat-associated nucleases,' has recently emerged as a potential tool for genome editing in plants. The system is based on a Cas9 nuclease and an engineered single-guide RNA (sgRNA), which specifies the target nucleic acid sequence. sgRNA is a chimera created by fusing two short molecules called CRISPR RNA (crRNA) and transactivating crRNA (tracrRNA). The canonical length of the guide RNA is 20 nt.

crystalline Bt toxin have been used to control insect pests since the 1920s. This remains one of the few insecticides acceptable to organic or 'ecological' agricultural systems. The first Bt toxin gene was cloned in 1981, and the first field trial of transgenic tobacco expressing a Bt toxin gene was performed in 1986. The first GM rice involved *Bt* as the transgene (produced in 1988). Current *Bt* maize cultivars, which have been commercialized in the United States, contain several *Bt* genes to give a range of insect specificities, often in combination with herbicide tolerance. Other insecticidal proteins have in the meantime

been discovered, including lectins, protease inhibitors, antibodies, wasp and spider toxins, microbial insecticides, and insect peptide hormones.

China was the first country to commercialize virus-resistant GM crops in the form of virus-resistant tobacco in 1992, and this was followed by virus-resistant tomato, zucchini, squash, and watermelon. Commercially available transgenic papaya germplasms engineered to express the coat protein of papaya ringspot virus have shown a high level of resistance: 80% of the Hawaiian papaya crop is currently GM.

Abiotic stress tolerance

Abiotic stress, particularly soil salinity, drought, and extreme temperature, is a leading cause of crop loss. As water resources decline and desertification intensifies in response to climate change, such losses are likely to worsen. The expected increase in the area and extent of arable land salinization will require new technologies to ensure crop survival. Despite an enormous and continuing research effort, few genes able to enhance abiotic stress tolerance have been convincingly demonstrated as able to perform as promised under field conditions. The sequestration of solutes into the vacuole is a physiological strategy commonly adopted by plants as a response to drought and salinity stress. Enhancing the production of osmolytes such as mannitol and proline has been attempted in various plant species as a means of improving the level of abiotic stress tolerance. Upping the level of expression of the gene encoding choline oxidase (*codA*) is known to enhance the production of glycine betaine, a small molecule used by plants to achieve osmotic adjustment. In rice, expressing *codA* in the chloroplast has been shown to represent an effective means of enhancing the plant's tolerance against abiotic stress. The most remarkable example to date of a successfully commercialized GM crop improved with respect to abiotic stress tolerance is represented by Monsanto's DroughtGard™ maize, released in 2013 in the United States. The transgene introduced into this maize encodes cold shock protein B (CSPB) and was isolated from the bacterium *Bacillus subtilis*. CSPB acts as an RNA chaperone, helping to maintain physiological performance during a stress episode by binding to and then unfolding RNA molecules in a way that allows them to function normally. DroughtGard™ materials have been shown to maintain grain yield better than wild-type maize under a regime of water stress.

Improvement in Product Quality

Product quality is taken to include nutritional value, organoleptic quality, the prevention of, or at least the delay in, the postharvest degradation or even loss of nutrients; and the removal of compounds having a deleterious effect. This second generation of GM plants has yet to live up to expectations, and no commercial crops having these properties are currently on the market.

Improved shelf life

The Flavr Savr™ tomato was the first GM crop and whole food approved by the US government Food and Drug Administration. The product reached the market in 1994. It was engineered to exhibit prolonged shelf life, which was achieved by delaying its postharvest ripening, softening, and rotting. The technology involved was the introduction in the antisense orientation, of a gene encoding polygalacturonase. The Flavr Savr™ tomato was abandoned in 1999, in part due to consumer resistance to GM technology and in part to the fact that the trait had been introduced into a background that prevented the crop from performing well in a commercial situation. Delayed ripening is a trait that should be useful for a number of soft fruits, in particular raspberry and strawberry. Extending a product's shelf life not only benefits the producer and retailer but also enables the consumer to keep the product longer before it spoils.

Improved nutritional content

While in developed economies, the regular inclusion of fresh vegetables and fruit in the diet avoids micronutrient deficiency; this is more difficult in situations where the diet relies over heavily on cereals that lack key vitamins, minerals, and essential amino acids. Rice and maize, for example, are both low in lysine, vitamin A, iron, zinc, and selenium, all of which are essential for normal growth and metabolism. GM technology can in principle engineer cereals to produce higher amounts of these micronutrients in the grain. A leading example is 'golden rice,' the history of which illustrates not only how GM technology could help alleviate malnutrition in developing countries but also how intellectual property rights and patents owned by multinational corporations can complicate matters, even when the humanitarian case is strong. Vitamin A deficiency is widespread in the developing world; it is estimated to account for the deaths of some two million children per year and is a major cause of blindness. Humans can synthesize vitamin A from its precursor β-carotene, a common compound in plants but absent from the cereal grain. The golden rice concept succeeded in engineering the rice endosperm to produce β-carotene by introducing a set of three transgenes obtained from *Narcissus* and *Erwinia*. Some of the transgenic lines were able to accumulate enough provitamin A to impart a distinct yellow color to the endosperm (hence the name 'golden' rice). Further development has led to the production of 'Golden Rice 2,' in which the *psy* gene from maize has been combined with the *Erwinia uredovora* carotene desaturase gene *crtI*. Compared with the original golden rice, this version produces 23 times the amount of carotenoid (a maximum 37 $\mu g\, g^{-1}$) and an improved accumulation of β-carotene. It has been estimated that 72 g of Golden Rice 2 grain should provide 50% of the recommended daily allowance of vitamin A for a 1–3-year-old child. The intention of the golden rice project was to provide the technology free of charge to the developing world, which required the negotiation of more than 100 intellectual and technical property licenses. For more information, including technical, historical, and humanitarian aspects, the reader is referred to the web page of the golden rice project.

Rice is also a poor source of vitamin B9 (folate). The fortification of rice grain with folate has been achieved by heterologously expressing various genes from *Arabidopsis thaliana*, which are involved in the folate pathway. The best of the transgenic lines currently available accumulate 100-fold the wild-type level of folate, such that a 100 g portion of transgenic grain contains as much as four times the adult daily folate requirement. Iron deficiency, which leads to the development of anemia, is also being tackled by the biofortification of rice; the strategy being followed here focuses on a gene for ferritin,

driven by an endosperm-specific promoter. Similar successes have been recorded for increasing the lysine content of canola, soybean, and maize.

Fatty acids represent another potential target for GM-based improvement crop, because certain of these molecules have important effects on human metabolism and health. One of the most promising approaches has targeted the transgenic production of very-long-chain polyunsaturated fatty acids. The production of stearidonic acid by the removal of Δ^{12} desaturase activity has attracted much attention from various biotechnology companies, and a transgenic soybean, which produces 15% stearidonic acid and 5–8% GLA (gamma-linolenic acid), is reportedly close to commercialization. Increasing the accumulation of secondary metabolites in tomatoes is being attempted by inducing the overexpression of certain key structural or regulatory genes; for example, manipulating the expression of a certain transcription factor has proved to be particularly effective in enhancing the accumulation of anthocyanin up to a level of 3 mg g^{-1} fresh weight, of flavonols up to a level of 10 mg g^{-1} fresh weight, and of resveratrol. Importantly, a pilot experiment has shown that when cancer-susceptible mice were provided with a diet supplemented with high-anthocyanin tomatoes, their life span was significantly extended.

Production of Pharmaceuticals

A third generation of transgenic crops is represented by the engineering of plants to produce high-value products such as vaccines or industrially important molecules. The use of plants for this purpose (sometimes referred to as 'pharming') has some advantages over microbial platforms, particularly concerning scalability, production cost, and the use of a eukaryotic organism. A range of plant species have been experimented with as carriers. Potato, tomato, and carrot have all been transformed to express certain vaccines, with the potato and banana in particular seen as favorable as a source of orally administered vaccine. Lettuce, cabbage, and cauliflower have tended to be relatively inefficient as expressors of vaccines. Tobacco, as a nonfood crop, represents an ideal choice for the production of vaccine antigens. The species is very simple to transform (it was the first plant species to be transformed) and has been extensively bred to produce high quantities of biomass, and its management as a commercial crop is well developed. Grain crops have the advantage that proteins accumulated in the seed tend to be stable at ambient temperatures.

Currently, three pharmaceutically attractive proteins have been approved for manufacture, namely, an antibody to combat caries; a vaccine against the Newcastle virus (a pathogen of poultry) glucocerebrosidase, used for the treatment of Gaucher's disease; and anti-Ebola agent ZMapp produced in tobacco.

Food Derived from GM Crops

Most current GM crops involve a species in the human food chain, where the product is consumed either directly as food (e.g., rice) or indirectly as the raw material for a processed product (e.g., canola). The major nonfood GM crop is cotton. Table 1 lists examples of GM foods. GM food has been categorized for regulatory purposes into (i) GM food (potato, tomato, soya, maize, sunflower, rice, pumpkin, melon, etc.), (ii) food containing ingredients derived from GM plants (starch, oil, sugar, amino acids, vitamins, etc.), and (iii) food containing GM organisms (such as yogurts made with transgenic *Lactobacillus*).

Table 1 Examples of GM foods obtained from GM crops

GM crops	*Appear on ingredient list as*	*Examples of food*
Maize	Glucose, glucose syrup, dextrose, fructose, maltodextrin, starch modifier/thickener	Cakes, biscuits, muffins, muesli bars, breakfast cereals, simmer sauces, snacks, peanut butter
Soybean	Soy oil/vegetable oil, soy protein/vegetable protein, soy lecithin/ emulsifier	Mayonnaise, bread, cakes, biscuits, snack foods, chocolate, margarine-type spreads, sauces
Canola	Vegetable oil, canola oil	Cooking oil, margarine-type spreads, mayonnaise, bread, cakes, biscuits, snacks (such as potato chips)
Cotton	Vegetable oil/cottonseed oil	Cooking oil, margarine-type spreads, mayonnaise, snacks (such as potato chips), simmer sauces

Maize Products

Ground and dried maize constitutes a staple food in many parts of the developing world. GM versions of maize have been commercialized carrying transgenes endowing herbicide tolerance and insecticidal activity. In the developed world, a large proportion of the maize crop is used as livestock feed, while most of the rest is processed into high-fructose syrup and starch or cooking oil or is converted into biofuel (ethanol). A small proportion enters the human food chain directly in the form of grits, meal, and flour. Maize oil is sold directly without further processing as cooking oil or is used to make margarine.

Soybean Products

In addition to HT and *Bt* soybean, GM technology has also been used to alter the composition of its oil. The fatty acid profile of wild-type oil encourages its rapid oxidation, and the rancid flavor this generates has limited its usefulness to the food industry. GM versions of soybean have been derived in which the amount of oleic and stearic acids has been boosted, while that of linolenic acid has been reduced. Oil represents about 20% of soybean seed dry matter, with the remainder being used for the manufacture of a wide range of food and beverage products. A substantial proportion of the harvested seed supplies the poultry and animal feed market.

Fruits and Vegetables

GM versions of a number of fruit and vegetable species have been generated; the following lists just some of the more

significant ones. Papaya has been engineered to resist ringspot virus, which in the early 1990s was a major priority for Hawaiian growers. The resistance effectively saved the state's papaya industry. The NewLeaf™ potato, which harbors the *Bt* gene, was intended to provide protection from damage caused by the Colorado potato beetle. The product was released by Monsanto in the late 1990s but was withdrawn in 2001 due to resistance from the large fast-food retailers. Currently, no transgenic potatoes are on the market, although there is an application from BASF for approval to release 'Fortuna potato,' a cultivar resistant to late blight thanks to the presence of the two genes *blb1* and *blb2*, isolated from the wild related species *Solanum bulbocastanum*. A second GM potato produced by BASF was 'Amflora,' the starch content of which had been manipulated; this cultivar was grown in Germany and Sweden in 2011, but was no longer commercially available once BASF had decided to cease its involvement in GM crops in the EU in 2012. GM zucchini selected to be resistant against three viruses are currently grown in North America. The 'nonbrowning Arctic' apple, created by Okanagan Specialty Fruits, is currently being marketed in the United States and is expected to be released shortly in Canada, pending regulatory approval. Browning is caused by the action of polyphenol oxidase (PPO) enzymes, so a nonbrowning type requires the silencing of all of the active *PPO* genes present. Bangladesh (but not India) has approved the commercial cultivation of eggplant carrying Bt. Following a human trial of provitamin A-enriched banana in the United States, the aim is to growing the crop commercially in Uganda by 2020. The flesh of this GM banana is orange (rather than the normal cream color) as a direct result of the accumulation of provitamin A.

Processed Derivatives

Vegetable oil enters the human food chain in the form of both cooking oil and margarine and is used in the manufacture of many prepared foods. The GM crops contributing currently to commercial vegetable oil are predominantly not only maize and soybean but also canola and cotton. The refining process removes nearly all nontriglyceride ingredients, but both protein and DNA residues can still be detected in the oil. Canola is the source of the third most widely consumed vegetable oil; the product also has a range of nonfood uses, for example, in the cosmetics industry. Oil derived from cottonseed is largely used for culinary purposes. Both maize and soybean oil are used as a source of lecithin, a compound widely used as an emulsifier by the food processing industry. Glyphosate-resistant sugar beet has been extensively adopted in the United States and has been approved for cultivation in many countries. Sugar beet represents a major source of refined sugar and molasses, and the residues are used as an ingredient of animal feed. The refining process is highly effective, and there are no confirmed reports of the presence of residual DNA or protein in commercially produced sugar.

Global GM Production

Commercialized GM Crops

The first transgenic food to be commercialized was the Flavr Savr™ tomato, which was released in 1994, and other GM crops followed shortly thereafter. By 1996, 1.66 Mha of land was under cultivation by GM crops, largely maize and soybean. In spite of a widening international acceptance of GM technology, the portfolio of crop–trait combinations remains rather limited. By 2012, HT soybean accounted for 47% of the global area sown to GM crops and 79% of the traded volume in soybean. The second most widely exploited GM crop is maize, accounting for 35% of the global area sown to GM crops and 32% of the traded volume (2012 figures). The transgenes present in GM maize confer either HT- or *Bt*-mediated insecticidal activity; increasingly, these two traits are being combined into a single cultivar. The other two widely grown GM crops are cotton and canola. The resistance against bollworm and budworm conferred to cotton by the *Bt* gene is very attractive to developing country producers who often lack the infrastructure and financial resources to resort to control based on chemical insecticides; in the United States, combined Bt and HT cotton is commonly grown. HT canola is currently restricted to Canada, the United States, and Australia. The remaining GM crops (HT alfalfa and sugar beet, virus-resistant papaya, squash, eggplant, and zucchini) cover only relatively small areas.

An example of a multitransgene ('stacked') GM crop is SmartStax maize, produced by Monsanto and Dow AgroSciences. The line carries a total of eight transgenes (including *Bt*). The outcome of the GM intervention is to provide protection against both surface insects, such as the corn earworm (*Helicoverpa zea*), the European corn borer (*Ostrinia nubilalis*), and the southwestern corn borer (*Diatraea grandiosella*), and subsurface pests, such as western, northern, and Mexican corn rootworms (*Diabrotica virgifera virgifera*, *Diabrotica virgifera zeae*, and *Diabrotica barberi*), while incorporating HT crops to facilitate broad-spectrum weed control. The variety was approved for cultivation in the United States (and elsewhere) in 2009. A more recent innovation is Genuity® DroughtGard™ maize, which is the first GM intervention to address drought tolerance.

The Geographic Spread of GM Crop Production

The area and production of GM crops are documented by the International Service for the Acquisition of Agri-biotech Applications (Figure 1). By November 2013, regulatory approval for the cultivation of GM crops had been granted by 26 countries. Since 1996, the global area under cultivation by GM crops has increased 100-fold: no other agricultural technology has spread so quickly and on such a large scale. The 2013 area was 175.2 Mha, representing an increase over the 2012 area of 3% (5 Mha). The estimated value of the GM seed market reached US$14.84 billion in 2012, with maize taking 56% and soybean 32% of the sales. GM crops are grown throughout the Americas and a large part of Asia but to only a limited extent in Europe and Africa (Figure 1). The number of countries growing GM crops increased from six in 1996 to 12 in 1999, 17 in 2004, 21 in 2005, 25 in 2009, and 27 in 2013. More than half of the world's population live in the 27 countries currently growing GM crops; 19 of these are developing countries and contribute over half of the global production. The acreage in the developing world exceeded that in the industrialized countries for the first time in 2012. An estimated

Figure 1 World map showing which countries and mega-countries* grew GM crops in 2013. ISAAA Report 2013.

18 million farmers grow GM crops, 90% of whom are small and relatively resource-poor farmers in India, China, South Africa, and the Philippines. The most prominent GM crop in the developing world is *Bt* cotton, which cultivated by over seven million farmers in each of China and India. Some 400,000 Filipino farmers have chosen to grow GM maize. The expectation is that further diffusion of the technology will be more rapid in the developing than in the developed world, fueled by regulatory acceptance by more countries and the development of GM versions of crops, which are concentrated in developing countries, such as rice, cassava, and cowpea.

The United States remains the lead producer of GM crops; the level of adoption of GM maize is 88%, of GM soybean 93%, and of GM cotton 94%. Brazil is the second largest grower of GM crops (23% of the global acreage); its area sown reached 3.7 Mha in 2013, a 10% increase over the 2012 area, consisting largely of soybean, maize, and cotton – 40.3 out of the 46.2 Mha planted to these three crops is covered by GM cultivars. The third largest producer is Argentina, where 14% of the cropping area is sown to GM crops, mainly soybean. India ranks fourth (11 Mha); there, the leading GM crop is *Bt* cotton, which covers 95% of the total cotton acreage – an area that has increased 220-fold since 2002 – and involves an estimated 45 million small farmers. Canada grew 10.8 Mha of GM crops in 2013, with a 96% adoption rate of HT and/or *Bt* canola. China is the sixth largest producer: 4.2 Mha of GM crops, most of which is represented by *Bt* cotton, which enjoys a 90% adoption rate. In Europe, the only significant site of GM crop production is in Spain, where about 0.14 Mha of *Bt* maize was grown during 2013 (up to 15% from 2012). There remains a strong level of consumer resistance to GM technology in Europe. In Africa, currently, three countries have given regulatory approval for particular GM crops – these are South Africa, Sudan, and Burkina Faso – and the current area of GM crop production is estimated to be around 3.5 Mha. Seven other countries (Cameroon, Egypt, Ghana, Kenya, Malawi, Nigeria, and Uganda) have already, or are in the midst of conducting confined field trials, the penultimate step prior to regulatory approval. The public–private partnership 'Water Efficient Maize for Africa' aims to develop drought-tolerant and insect-protected maize using conventional breeding, marker-assisted breeding, and GM technology, with the goal of making such varieties available royalty-free to smallholder farmers in Sub-Saharan Africa; a time line of 2017 is expected for the first varietal releases. The African Agricultural Technology Foundation (AATF) is working towards making pod borer-resistant cowpea and bacterial wilt-resistant banana available to small farmers in Africa. The International Rice Research Institute has successfully bred the golden rice trait into its widely adapted breeding line IR64 and other Asian varieties to test whether the agronomic performance of the new varieties will be acceptable to farmers.

Public and Scientific Concerns Surrounding GM Crop Production and Consumption

Antibiotic Resistance Genes

Since the successful transformation of a plant cell is a rather low-frequency event, a means of positively selecting for transformed cells is highly desirable. Among the most common such selective agents are antibiotics, incorporated into the cell culture medium in order to kill cells (those that have not been transformed) that have not taken up the antibiotic resistance gene associated with the transgene. The deployment of antibiotic resistance genes for this purpose has proved to be a major barrier to the consumer acceptance of GM crops. The fear is that the incorporation of such resistance genes in a transgenic plant risks its horizontal transfer to pathogenic gut microorganisms when GM food is consumed. This scenario is possible, but highly unlikely. Firstly, the antibiotic resistance genes used to create GM plants originate from natural populations of bacteria, so there is as much, if not more, chance of horizontal transfer between natural bacteria in the environment and gut flora, especially as the gene is typically present in bacteria in a highly mobile structure called a plasmid. Secondly, the antibiotic resistance genes used in plant transformation confer resistance to antibiotics, which are longer used in human or animal medicine, because they have long since lost their effectiveness after many years of intensive use. Some progress has been made to sidestep the requirement for antibiotic resistance genes by adjusting the form of sugar made available to the in vitro cultured plant cells. Other more recently developed strategies have sought to exploit site-specific recombination systems (see **Box 1**).

The Problem of Super Weeds

A potential problem associated with the use of HT crops is the selection intensity, in which their use puts on weed populations to develop resistance, whereby rendering the weeds difficult to control. A way in which such resistance could readily develop is the transfer of the trait from the crop to a wild weedy relative through cross-pollination. Such an event has been documented already, as 24 glyphosate-resistant weed species have been identified over the period since the introduction in 1996 of Roundup Ready GM crops. Glyphosate-resistant weed species have now been found in 18 countries worldwide, with a significant impact on the economics of crop production in Brazil, Australia, and Argentina. It needs to be borne in mind, however, that herbicide tolerance can arise spontaneously by mutation among weed populations subjected to herbicide use quite independently of the GM situation, since 64 weed species have been shown to have developed resistance to atrazine, even though no atrazine resistance transgene has ever been deployed. The transfer of herbicide resistance to weeds is a live issue nonetheless, although at present it is regarded more as an issue for agricultural management than as a public hazard. The development of local populations of HT weeds needs to be dealt with by the use of alternative herbicides and/or a directed program of mechanical weed removal. Over the next few years, it is likely that GM products will be developed incorporating herbicide tolerance based on herbicides other than glyphosate, to give farmers more flexibility in managing weeds; one such product already available is the Bayer CropScience Liberty series of GM cotton, soybean, canola, and maize, which all carry resistance to the herbicide glufosinate.

'Genetic Pollution': Gene Flow from GM Crops into Landraces

In 2000, an attempt by a group of farmers in Oaxaca (Mexico) to gain organic certification for their maize landrace failed because it carried both the *Bt* and HT genes used in Monsanto GM material. Since GM crops were at the time not approved for commercial production in Mexico, it was assumed that the transgenes must have been transferred via cross-pollination from GM plants. Since this time, a number of scientifically rigorous studies have been made to characterize the extent of transgene flow into Mexican maize; some of these have failed to show any introgression of transgenes. The scientific community remains divided over whether transgenes have really entered the gene pool of Mexican landrace materials; some critics worry that the diffusion of these GM traits into local material may compromise plant fitness.

Health Risks Associated with the Consumption of GM Food

The possible health risks associated with the consumption of GM food center on the introduction of toxins and allergens. The argument is that the insertion of a transgene(s) can lead to unforeseen pleiotropic effects when it is expressed or that the insertion process per se causes changes to native genes. The risk that food may contain allergens is not confined to that produced from GM crops, since, for example, according to the US NIH National Institute of Allergy and Infectious Diseases, the prevalence rate of peanut allergy is 0.6%, while a substantial number of people cannot tolerate wheat gluten. Even conventional plant breeding can sometimes throw up unexpected toxicities. There are examples of GM products that were discarded prior to release because of this problem: An example is the attempt to improve the resistance of the pea to pea weevil attack by inserting a gene isolated from the kidney bean; the product was never marketed because test feeding to mice identified a potential negative immune response. A second, celebrated example involved a soybean GM bred as a nutrition-enhanced poultry feed that was discontinued when the transgenic protein tested positive in a serum assay. The industry has had to learn to pay due attention when foods may contain plant ingredients derived from GM crops engineered with genes encoding known allergenic materials, such as milk, eggs, nuts, wheat, legumes, fish, mollusks, and crustaceans. Part of the regulatory process for GM crops involves a demonstration that no unforeseen products are present ('substantial equivalence') in the comestible part of the proposed crop cultivar.

A 2012 publication, which attained wide notoriety, described a two-year feeding study of rats given grain of a GM HT maize cultivar. The study claimed that the GM material induced deleterious effects on the health of the rats. After a year-long investigation, the paper was withdrawn by the journal on the grounds that it was not sufficiently scientifically rigorous. The European Food Safety Authority later issued a statement confirming that the study's design and methodology

were both defective and that there was insufficient evidence to reopen the safety evaluation of this GM maize cultivar. Despite a nearly 20-year exposure of the US population to massive quantities of GM maize in their diet, there is still no convincing evidence that GM technology per se is associated with any specific health risk.

Business Related to GM Crops

Estimates of the economic benefits flowing from crop biotechnology lie in the billion dollar range, shared between consumers, producers, and the companies that have developed the GM crop varieties. Some concerns have been voiced regarding the risks of monopoly control of GM crops by a handful of powerful multinational corporations. Current regulatory requirements to bring a GM crop variety to the market are both time-consuming and expensive and constitute a major barrier to market entry and exclude public sector involvement. Nevertheless, the present generation of GM crops has rested largely on discoveries made in the public sector, so the public perception that crop biotechnology is the exclusive domain of multinational corporations is illusory. The so-called terminator technology has been promoted in some quarters as an example of big business imposing its wishes on farmers and consumers. This GM application attracted adverse publicity because cultivars engineered in this way would produce sterile offspring and thus prohibit the use of farm-saved seed. At the same time, the opponents of the technology have chosen to ignore a potential benefit of the technology in preventing the escape of transgenes into wild plants. Terminator technology has not been used in any GM crops to date.

See also: Genetically Modified Foods: Risk Assessment, Legislation, Consumer Behavior, and Ethics.

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Genetically Modified Foods: Risk Assessment, Legislation, Consumer Behavior, and Ethics

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Introduction

Genetically modified (GM) foods have been the main focus of academic and management practitioner's debate for the past 20 years. Consumer concerns over the safety of GM food products and the cultivation of GM crops are channeled through applied research results. The current economic turbulence and the food crisis, during which food prices are increasing, while income and consumer confidence plummets, bring the consumer audience over the hard choice to cut spending, and in the center of this debate, GM food is found to be promoted as both a solution and a problem to the current consumer food needs and wants. With the current unveiled scandal of horse meat contamination in products labeled as beef in Europe, consumers are losing trust to the food production chain, and the impact of this mistrust escapes the realms of the United Kingdom affecting the continental markets. Despite the scandalous turbulence in the media in both the United Kingdom and the EU, the rising prices on food are bringing GM food back in the center of the debate.

During the 2007–08 global food crisis, the alleged agribiotechnology solution was purely a resourceful spin of the agribiotechnology companies trying to exploit the media outburst on the food prices during that period. This trend has been pointed out by many studies. The debate in the area of the food crisis and GM food seems to circulate around the allegations that GM food is 'forced' into the food market or that the food crisis inevitably leads to the GM food adoption. This high polarization finds its roots in the global sentiment on international resources and the cost of living around the world midst one of the hardest economic downturns that the developed world has faced so far.

In the area of political discussion, scholars have been equally evolving the debate further. It has been observed that there is a political myth construct around GM food and genetically modified organisms (GMOs), attempting to present GM produce as 'Frankenfoods,' drawing an image to the public in this way of the mad scientist developing catastrophic recipes that will ultimately bring the Armageddon on earth. It sounds as an overstretched remark, and someone has to look twice to notice that these points are raised by an academic and not a reporter.

Many scholars focus on the EU reluctance in introducing or adopting GM food. This part of the debate appears to be split into two equal divisions: those that favor the introduction of GM foods in the EU and those that strongly oppose it.

Those that oppose it refer to possible risks of environmentally damaging effects, and others refer to ethical concerns on corporations setting patents and prohibiting farmers to reuse GM seeds, while the most prominent argument appears to be the insufficient test trials and the absence of evidence on long-term health effects through trials. On the other hand, there is an abundance of authors in support of the introduction of GM food, bringing forward the benefits of GM food technology and debating the prospect of GM food being an important remedy on world hunger and the current food crisis while insisting on the applications of GM food technology on a variety of commercial sectors that mostly help improve the human effect on the environment. These views have been portrayed as a form of 'anti-GMF' hysteria, and popular concerns on the issue have been discussed, offering evidence against these concerns.

GMOs can be defined as organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally. GM foods are derived from GMOs.

Currently, ~50 GM plants and derived products are approved for commercial use in the EU (http://ec.europa.eu/food/dyna/gm_register/index_en.cfm).

GM plant applications cover seven crops; mostly maize, followed by cotton and soybean, and – to a lesser extent – oilseed rape, potato, sugar beet, and rice.

The predominant traits include not only resistance to insect pests and tolerance against certain herbicidal active substances but also modified composition (e.g., altered fatty acid profile), tolerance to drought, and reduced amylose content. Many GM plant applications deal with 'stacked' events where two or more single transformation events have been combined by conventional crossing to introduce several traits into one plant.

In the economic arena, the arguments for the GM food outweigh those that are against. The positive impact of GM crops in developing countries on income, poverty, and nutrition has been defended by many authors. The positive developments of the applications of GM crops in China and India, with reference to Bt cotton and beta-carotene golden rice, have been discussed.

As the discussion on GM food continues, evidence suggests that there is a great rift in the production and adoption of GMCs (GM crops). It has been indicated that the 11 countries that grew one or more million hectares in 2012 are led by the United States that grew 69.5 million hectares, Brazil with 36.6 million hectares, Argentina with 23.9 million hectares, Canada with 11.6 million hectares, India with 10.8 million hectares, and China with 4.0 million hectares. Of the top 11 countries, each growing 1.0 million hectares or more of biotech crops, the majority are industrialized countries. Over 1 million hectares are grown also in Pakistan and Uruguay and about one million in Bolivia.

Legislation Issues

According to the Council Directive 90/220/EEC, "'genetically modified organism' (GMO) means an organism, with the exception of human beings, in which the genetic material has

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been altered in a way that does not occur naturally by mating and/or natural recombination." In the Council Directive 98/81/EC, "'microorganism' shall mean any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture." Also in the Council Directive 98/81/EC, "'genetically modified micro-organism' (GMM) shall mean a micro-organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination." "*Biotechnology* is the use of living systems and organisms to develop or make useful products, or 'any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use.'"

The Regulation (EC 1829/2003, Articles 12 and 13) imposes the labeling of GMOs in the words 'GM' and 'produced from GM (name of the ingredient)' in the following cases:

- In case of an accidental or technically unavoidable quantity of approved GMOs in conventional foods, the lowest detection concentration is specified at 0.9% for every isolated food ingredient included.
- In case there is an accidental or technically unavoidable quantity of GMOs in foods or cattle feed and there has been a positive scientific evaluation by the official European Community authorities, but its circulation has not yet been typically approved, the percentage is specified at 0.5%.

Moreover, Regulation (EC) No. 1830/2003 "concerns the traceability and labeling of genetically modified organism and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC." According to the previously mentioned community legislations, the process of assessment of environmental hazards from GMOs aims at determining and evaluating, in every case separately, the potentially unfavorable consequences (direct, indirect, short-term, and long-term) that their release may have on people's health. In parallel, the cumulative consequences are taken into account over a long time span.

The strict regulations that the EU imposes for the presence of GMOs in food and feed have been discussed thoroughly. The strict labeling laws allow the EU to effectively filter GM food imports and accept only those products that contain GM food ingredients that are in line with the EU food safety regulations (currently representing 0.9% of the total GMF varieties).

Moreover, it is discussed that where the EU is reliant on imports (particularly soybean for animal feed) from North and South America, these imports are allowed to enter the EU market despite the ban of those products that could contain unsafe GM foods or GM ingredients.

The US Food and Drug Administration (FDA) does not currently require genetically engineered organisms (GEOs) to be identified because it has determined that genetically engineered foods are substantially equivalent to conventional crops and as such they are safe.

Genetically engineered foods would require labeling only if modifications altered food composition significantly, changed the nutritional value, or introduced an allergen.

According to the Genetically Engineered Food Safety Act (2003), the Congress defines 'genetically engineered organism' as (A)

> "an organism that has been altered at the molecular or cellular level by means that are not possible under natural conditions or processes (including but not limited to recombinant DNA and RNA techniques, cell fusion, micro- and macroencapsulation, gene deletion and doubling, introducing a foreign gene, and changing the positions of genes), other than a means consisting exclusively of breeding, conjugation, fermentation, hybridization, *in vitro* fertilization, tissue culture or mutagenesis, and (B) an organism made through sexual or asexual reproduction (or both) involving an organism described in subparagraph (A), if possessing any of the altered molecular or cellular characteristics of the organism so described."

According to the Genetically Engineered Pharmaceutical and Industrial Crop Safety Act (2003), "'pharmaceutical crop' means a genetically engineered plant that is designed to produce medical products, including human and veterinary drugs and biologics. The term includes a crop intentionally treated with genetically engineered material that, in turn, produces a medical substance." "Pharmaceutical crops and industrial crops also pose substantial liability and other economic risks to farmers, grain handlers, food companies, and other persons in the food and feed supply chain."

Canada and the United States opted to regulate all plants or products with new traits developed through either genetic engineering or any other plant breeding techniques under the same, yet existing, regulatory system. The transformation techniques were not considered inherently risky. Therefore, the focus of product-based regulatory systems is on the risks of products and new traits or attributes introduced into a plant, rather than the method of production.

Argentina, Brazil, the EU, and many other countries have put new process-based regulatory systems in place to regulate the use of GMOs, as the techniques used for their production were thought to raise specific safety concerns. In these jurisdictions, a GMO is mainly characterized by the transformation techniques used in its production. "Techniques of genetic engineering/modification include, but are not limited to: recombinant DNA, cell fusion, micro- and macro injection, encapsulation, gene deletion and doubling. Genetically engineered organisms will not include organisms resulting from techniques such as conjugation, transduction and hybridization."

Moratoriums on GMO release and impacts on investment in GMO research are the cases for Australia. Technology assessment (TA) is a process that considers the societal implications of technological change in order to influence policy to improve technology governance. TA formally emerged with the US Office of TA, which began in 1974 to serve the US Congress. A formal TA agency is due to be formed in Australia, which would build capacity in TA to deal proactively with technology controversies and contribute to more socially aware technological development and provide the foresight, oversight, and community dialogue that informs technology governance in other countries, notably in Europe.

Risk Assessment of GM Food/Feed

According to WHO (1995), risk assessment is the scientific evaluation of known and potential adverse health effects resulting from human exposure to foodborne hazards.

Recently, the EU Standing Committee on the Food Chain and Animal Health (SCoFCAH) adopted the implementation of a Commission Regulation on the applications for authorization of GM food and feed in the European Union. The Regulation follows guidance developed by the European Food Safety Authority (EFSA) for the safety assessment of the foods/feed derived from GM plants. The new regulation demands a 90-day feeding trial in rodents for every single transformation event and, in specific cases, the same trial for plants containing transformation events stacked by conventional crossing. EFSA guidance recommends this type of experimentation only under certain conditions.

The risk assessment strategy for GM plants and derived food and feed is focussed on the comparison between GM plants and derived food and feed and their respective comparators. This comparative approach is based on the fact that traditionally cultivated crops have gained a history of safe use for consumers and/or domesticated animals. The concept of substantial equivalence applies here.

Risk assessment includes "(i) the molecular characterization, (ii) comparative agronomic, phenotypic and compositional analysis of the GM plant and derived food/feed and its traditional counterpart, (iii) toxicological assessment of newly expressed compounds and of constituent compounds the levels of which may have changed as a result of the genetic modification, (iv) the assessment of potential allergenicity of the novel protein(s) as well as of the whole food derived from the GM plant and (v) the nutritional assessment of the food/feed derived from a GM plant."

Toxicological testing must be proportionate to the potential source(s) of new hazards in order to be robust and efficacious. These hazards for GM products may be derived from the transformation process, that is, from newly expressed substance(s) or via some alteration of the plant composition.

Specific toxicity tests such as repeated dose–toxicity studies using laboratory test animals may be needed for the characterization of single substances (e.g., newly expressed compounds including proteins) and for the assessment of the consequences of any compositional alterations (e.g., in the content of micro- and macronutrients and natural toxins/antinutrients).

"Given the limitations of animal feeding trials with whole GM food/feed, these experiments should only be performed where necessary and EFSA (2011a) has indicated examples of conditions where such studies may be useful. These include (i) when the genetic modification has resulted in multiple and complex compositional alterations, (ii) when there is uncertainty and safety concerns on the possible occurrence of unintended (off target) effects (e.g., where new open reading frames (ORFs) are produced with similarities to toxins/allergens, or where metabolic pathways of the plant have been perturbed) or (iii) when there are indications for interactions between single events that have been stacked. This list of conditions is not exhaustive, and decisions should be made on a case-by-case basis, taking into account developments in our understanding of the potential impacts of genomic perturbations."

The updated guidelines for the risk assessment of GM plants and derived food and feed were used by the European Commission (EC) and EU member states as a basis for the preparation of the *Commission Implementing Regulation (EU) on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Regulations (EC) No 641/2004 and (EC) No 1981/2006*. This Regulation has been adopted in February 2013 by EU member states and came into force 6 months after its publication.

The landscape of GMOs is changing quickly including the development of GM plants targeted toward major compositional changes such as the development of crops with modified metabolism and physiology to provide improved quality and enhanced nutritional profiles.

However, a comprehensive safety assessment of the GM plant per se is advocated in cases where appropriate comparators are not available. GM plants with stress-tolerant phenotypes are now being widely tested in field trials around the world, with the first and most advanced drought-tolerant maize being launched commercially in the United States in 2013. These plants may raise specific issues for the environmental risk assessment that will be more challenging to resolve compared with the first generation of GM plants.

Besides new traits, the next generation of GMOs will consist of new types of organisms that have not been previously assessed by EFSA, such as GM trees, GM animals, and GM algae. Regarding GM trees, some risk assessment guidelines are already available from international discussions (OECD).

The EFSA GMO Panel concluded that cisgenic and conventionally bred plants represent similar hazards, whereas intragenic and transgenic plants could raise new hazards. The EFSA GMO Panel considered that its existing risk assessment guidelines for plants and products developed through transgenesis would generally apply to cisgenic and intragenic plants but that it would require less event-specific data, depending on the specific case. In addition, the EC has commissioned several reports on specific aspects of new biotechnological plant breeding techniques. In the United States, the Environmental Protection Agency has opened up a debate on a draft rule that would exempt certain cisgenic organisms from registration as GMOs. Another challenge is the development of regulatory frameworks for novel plant products that allow international harmonization.

The US Department of Agriculture, for example, announced that it does not have the authority to oversee cisgenic plants created without the help of a plant pathogen, whereas the Australian Office of the Gene Technology Regulator considered that certain cisgenic plants might not be regulated. Finally, the need to avoid disparities in risk assessment practices between products with equal potential to cause harm should be addressed. Several authors have argued that process-based regulatory approaches lack consistency because conventionally bred products can raise safety concerns similar to those for their transgenic counterparts. The European Policy Evaluation Consortium, commissioned by the EC to evaluate the EU GMO legislation, came to a similar conclusion. Only the Canadian legislative approach enables consistent evaluation of plants with similar new traits, irrespective of the techniques used.

The assessment of unintended effects caused by the genetic modification plays a key role in the safety/nutritional evaluation of GM foods/feed, which may lead to compositional alterations with potential toxicological effects on humans/animals. Perturbations in the plant's physiology and biochemistry will likely be detected by phenotypic, agronomic, or targeted compositional analyses.

The unintentional contamination can be due to accidental factors, such as cross contamination over the transfer of raw materials/ingredients in containers that have remains of GMOs. The unintentional contamination is mainly caused by the supply of ingredient or raw material contaminated with GMOs or any of its derivatives.

The unintentional contamination can be dealt with the application of preventive or corrective actions, after the risk for unintentional contamination in every ingredient or raw material has been determined. A promising and robust tool for the implementation of risk assessment is the Failure Mode and Effects Analysis, which requires a good knowledge of the system and its elements.

Prevalent Trends Toward GM Foods in Consumer Behavior Studies to Date

The disparity and uneven balance skewed toward a negative stance for GMF in the EU and the United Kingdom have been heavily analyzed by the academia. After 15 years of academic debate, still the future of GM food technology is continuously disputed and by no means concluded. In the field of consumer behavior, GM food has been equally debated.

Some authors have described GM food debate as a worldwide issue that has concluded negatively. The generally positive perception toward GMFs in developing nations stems from more urgent needs in terms of food availability and nutritional content.

By looking in the literature to date, there are three main inquiry paths in the field of GM food and consumer behavior research:

- Attitudinal surveys
- 'Willingness to pay (WTP)'
- Experimental auction markets

The aforementioned trends are linking each research path, with the chosen data collection and analysis approach, and they are indicative of their relationship with the marketing mix (price, place, promotion, and product).

Attitudinal Surveys

There are many in-depth attitudinal studies carried out by various academics, government agencies, and organizations and up-to-the-minute polls carried out by the media. Usually employed with a cross-sectional time horizon, they investigate popular opinions on GM food in various times (relevant to the time each survey was conducted). As a result, the opinions expressed vary considerably and the results rely on how the questions are structured and designed, the demographic design of the sample investigated (e.g., size, demographics, location, and cultural bias), and the time the survey was conducted.

Results show that Europeans have been seeing GM food as a negative approach to food production relative to other biotechnology developments, for example, medical or ecological ones. Equally, the public attitudes toward GM foods are also appearing to be more negative in Europe than in North America and elsewhere. Within the European market, attitudes have varied from country to country and over time.

A good example of the variation over time and by country is that of southern European countries before and after 2008, and earlier before and after 1997. For instance, southern European countries were more accepting GM foods than northern countries up until 1997. After 1999, Italy and Greece revealed the most negative attitudes toward GM foods in Europe. The responses changed again with the 2008 financial crisis, when southern European countries were seeking a more cost-effective approach toward agriculture, thus tending to be more interested in what GM food technology had to offer. According to some forum discussions, Greek farmers are now happier to discuss a possible introduction for GM crops, if that means that their production costs will be reduced.

Equally, consumers would be more willing to consider the benefits that GM food technology has to offer, if GM foods would be introduced as a cheaper and more nutritious solution. Evidently, the current economic hurdles of southern Europe had an impact on consumer attitudes and the recent trends are evident on other countries of southern Europe, such as Spain, Italy, Malta, and Portugal. So this particular category of studies can capture the 'public feeling' toward GM foods and biotechnologies and find trends of such sentiment and other associations with regard to GM food technology. Attitude surveys are relatively easy to run, a 'cheaper alternative' when compared to other inquiry tools and are generalizable to a population. While they can quantify and discuss briefly consumer views and sentiments toward 'softer' variables such as consumer preferences and acceptance, they are counterproductive when used to study price and income effects on consumer-stated preferences. One of the most important criticisms of the attitudinal surveys is their 'hypothetical structure' limitations. Attitude surveys have the capacity to view their subjects as citizens rather than consumers, allowing in this way a survey to be both a public opinion inquiry and a consumer attitude survey. Respondents may consider information and beliefs to reveal their views to the survey.

However, consumer attitudes may or may not be effective proxies of consumer market behavior. Attitude surveys many times appear to present significant biases. The structure of questions, how the information is presented to each subject, and the degree of knowledge and understanding of the respondent on the issue are only some of the areas where bias may form during the design and data collection stage. Another issue in this category of studies is the factor of comparability. Attitudes are known to be variable. Thus, differences in food culture, for example, could make results and trends coming out of a study as nontransferable and therefore not comparable. In any case, biases can be minimized but not eliminated through careful design and statistical analysis.

'WTP' Studies

As majority of the studies focussing on GM food attitudes employ attitudinal surveys, some authors have demonstrated that the model of 'WTP' can successfully encapsulate consumer response toward GM foods, by challenging respondents with realistic food choices. Studies have shown that consumers may be willing to pay more for GM foods, when considering

desirable properties of the food product, or alternatively to pay more to avoid them. These studies employ contingent valuation, which to date is the most popular 'WTP' method. This model develops measures that are projected from consumer responses to a set of hypothetical questions. A hypothetical scenario is presented to the subject who in turn has to make a choice based on the hypothetical attributes presented. Socioeconomic data about the respondents can be collected by using socioeconomic information from each subject, insert additional altitudinal questions, or employ a chain of questions linked with one or more variables in order to confirm that the subject understood the scenario offered.

Using WTP surveys, some authors concluded that many European consumers would need a discount to consume foods with GM ingredients. Some other authors also identified a mean price that consumers are willing to pay for GM food and also linked this price to socioeconomic characteristics.

Evidently, WTP surveys can offer important clues about individual consumer-stated preferences and offer an insight to consumer decision-making on GM food products. The model allows for sociodemographic data to be associated with consumer attitudes. One of the major limitations of WTP surveys is their theoretical design, which does not allow an in-depth insight to GM food products.

The roots of this criticism are founded on the notion that a theoretical question can only receive a theoretical answer. Equally, responses on theoretical scenarios could result in unreliable results to be given. This weakness in particular is a very important event, especially when in GM food attitudinal study, there are high levels of unaware respondents on the issues inquired for the technology. Therefore, the level of information of the participant on the issue is an important factor that combined with the hypothetical nature of WTP survey results into the noted weakness.

This method of inquiry is also vulnerable to two other types of bias: strategic bias and hypothetical bias. Strategic bias can be defined as the event when a respondent overestimates a price, for example, if they intend to affect the outcome of the inquiry. The hypothetical bias can be described as the event when a respondent is unable to accurately define their WTP. This may happen if the respondent was not exposed or was not informed about GM food (or in this case GM vs. non-GM). The nonexistence of real actual purchasing choices reinforces the aforementioned weakness of the WTP model approach. Finally, some studies have shown that even the order in which questions are asked can affect WTP measures by a significant magnitude.

Factors Affecting GMF Attitudes

When it comes to food, attitudes present a unique pattern. Consumers' views on what they consume appear to be sensitive, and sensitivity toward GM food has been reported to vary greatly from country to country. Different markets present different views and information needs. It has been shown that Canadian consumers responded similarly to US consumers, in a forwarded survey. Japanese and Chinese consumers' attitudes differed more from each other than those between Japanese and Norwegian consumers. The Chinese consumers were the most favorable toward GM foods. It has been argued that cultural attitudes including valuing tradition and skepticism of science are the prevailing factors when marketing GM products. It has been attempted in a study to analyze how public attitudes had accustomed the acceptance of GM food in the European Union in general and specifically in Spain and the United Kingdom. The authors found important information reliance and wide-ranging diversity in attitudes toward the uses of biotechnology.

The authors also highlighted the importance of information gateways in showing that consumer participation should precede the adoption of communication policies, as there is a need to align to the local cultural constructs and characteristics of each country. Thus, culture is central to adoption, acceptance, and shaping in general of consumer attitudes toward GM food. In the case of United Kingdom, which is well known for its opposition in introducing GMO cultivation and GMF in their markets, there is a historical trend in sensitivity when it comes to food products.

With a rich history of food scares, the United Kingdom presents a good example on how certain cultural sensitivities on food can affect consumer preferences and, in many cases, create a feeling of fear toward food. Good examples of UK food scares are those of Bovine spongiform encephalopathy or mad cow disease as formed in cattle and Creutzfeldt–Jacob Disease, the foot and mouth disease outbreak, the avian flu, swine flu, and more recently the horse meat scandal. GM food was brought to the spotlights creating, in a similar way, a controversy that resulted in the removal of GM ingredients and products from supermarkets in the United Kingdom or the strict labeling reformed laws that were applied by the EU, which was driven by consumer concern and media coverage. Clearly, there is a well-recorded spillover effect of such issues toward GM food debate; hence, the opposition to this important biotechnology development can be characterized as persistent. More recently (June 2013), the British Government has started a campaign for GM food. According to BBC (2013), Owen William Paterson, the Secretary of State for Environment, Food and Rural Affairs, said

> The EU chief scientist Anne Glover has said it pretty bluntly – there is no substantiated case of any adverse impact on human health on animal health or on environmental health.

This change of UK government policy toward GM food could find its roots on the current food crisis and the search for cost-effective agricultural developments, but considering the long period of resistance to GMO and GM food technologies would present a greater challenge to shift public opinion, attitudes, and perceptions toward GM food.

Perceptual Issues with GM Food: Risks and Benefits

It is essential for consumers to decide on whether they will consume or not GM food, and for this decision to be made accurately, as previously discussed, it is important for the consumers to have access to up-to-date information on GMF and GMO technologies. The term information currently refers to all the external and internal stimuli that a person receives from his or her environment enabling the person to form an

experience. Perception is defined as the organization, identification, and interpretation of sensory information in order to represent and understand the environment. Perception is not the passive receipt of these signals, but can be shaped by learning, memory, and expectation. Perception involves these 'top-down' effects and the 'bottom-up' process of processing sensory input. 'Bottom-up' information refers to low-level neural information (sensory stimuli), and 'top-down' information refers to more complex constructs such as memory, knowledge, and experience.

One factor that directly affects perceptions or attitudes is fear. Fear is an emotion that is often expressed in food-related issues. As discussed previously, in the case of the United Kingdom, several food scares have dominated the market for many years, and research shows that these food scares have a direct impact on consumer behavior. In the case of GMOs, food-related fears affect the behavior of the consumer.

The frequent appearance of fear messages in the media plays a significant role in promoting widespread fear of GMF ('Frankenfood') in the global market for food. Some authors have validated a scale to measure consumers' fear of GMF and found that Dutch consumers feel significantly more fearful of GMF than of other new food types. The results of the Dutch study did not find a relationship between demographics and fear toward GMF, assuming that GMF fear is consistent across the society.

A niche category among the different types of fear related to GMF is that of neophobia. Humans naturally display some degree of aversion to new foods, a trait that has been termed food neophobia. It is reported that food neophobia is widespread in omnivores and has been detected in warblers, rats, and chimpanzees, among other species. It is argued that beliefs about risks and benefits of the production and introduction of GMF in the food market are key in defining consumer acceptance. Moreover, the associated fears along with the current price sensitivity due to the squeezed consumer income are all considered to affect attitudes toward technology. This is proof that nonirrational factors could be influencing the outcome, and this is a trend supported by many studies that refer to 'superstition,' 'religious fears,' and 'magical beliefs,' factors that have been linked with negative attitudes toward GM foods.

Consumers are reluctant in seeing the benefits in European countries and the United Kingdom, for example, as they have accumulated experience of years of food scares as discussed previously or because they simply do not trust the corporations to 'manage' their food resources. It has been reported that plant-related or 'universal' uses were more acceptable than animal-related applications. Moreover, risk perceptions were greater in Europe than North America and Asia and the opposite was evident for benefit perceptions. Moral fears are higher in North America and Asia and both benefit and risk perceptions were found to change over time. On the other hand, in India, perceptions about the consumption of GM food are often distorted. Moreover, communication of information in India regarding GM food was leaning toward the benefits misrepresenting the risks, in order to promote to both farmers and consumers and accelerate the adoption of the GM food products. Four main factors of concerns on GM food products have been identified in India:

- Attitudes toward GM food
- Concern for health
- Knowledge for food
- Information about GM food

One of the major concerns of the general public about transgenic crops relates to the mixing of genetic materials between species that cannot hybridize by natural means. Two new concepts have been recently developed – cisgenesis and intragenesis – which imply that plants must be transformed with genetic material derived from the species itself or from closely related species. Several surveys in the United States and Europe clearly show that both intragenic and cisgenic crops are acceptable to a greater number of people than transgenic crops. At present, in most countries, the release of cisgenic or intragenic crops currently falls under the same regulatory guidelines as transgenic crops. However, the regulation of intragenic/cisgenic crops is presently under evaluation in the EU, and in the United States, regulators are considering if a subgroup of these crops should be exempted from regulation.

Different scientific committees concluded that cisgenic crops are as safe as traditionally bred varieties. So, cisgenesis is a powerful new tool for plant breeding with traditional traits.

Intragenesis in particular permits the introduction of new gene mixtures through *in vitro* rearrangements of functional genetic elements. Recent studies conducted surveys to investigate public perception of this more 'natural' approach to genetic modification and found higher public acceptance of intragenic/cisgenic when contrasted with persistent public negative views of transgenic-based techniques. In Denmark, a study that surveyed a random sample of 2000 Danish consumers investigated the impact of knowledge in the probability that a person would differentiate his or her views on medical and agricultural applications, and they found that this was true, with an exception on consumer opinion differentiation over the cisgenic and transgenic cereals. Presently, intragenic/cisgenic plants are labeled as transgenic plants worldwide despite their difference in production techniques. However, the aforementioned techniques are closer to traditional breeding techniques; thus, in the future, less strict regulatory measures can be anticipated. 'Cisgenic and intragenic' techniques are new and have been introduced only 9 and 7 years ago; thus, it is too early to judge consumer acceptance and more research has to be conducted to study more comprehensively any arising consumer attitudes toward intragenic/cisgenic crops against their more 'hated' transgenic cousins. Moreover, this is a proof of the importance of the two of the four concern factors (knowledge of the issue and quality and clarity of information), which ultimately enables the consumer to have a clear position on important issues in the area of food technology.

The Role of Ethics

Ethics is a field widely published with regard to GM food products and GMOs. It is undoubtedly the most debated field and one of the inconclusive chapters of the GM food issue. The ethics or moral dimensions of the GM issue usually are presented in recent literature as a medley of religious constructs

('playing god'), or moral dilemmas, sometimes mixed with ecological concerns. The field is equally skewed between those authors that are pro-GM and those that are against, leaving the subject without a constructive agreement but with many unresolved arguments. Popular themes involve the domination of multinational corporations, environmental risks, the fear of playing god, and the unfairness on offering GM food as a food resource solution to poorer regions of the globe.

The pluralism of arguments and rifts within this field as said before renders many times the arguments as inconclusive as subjects such as morals and ethics usually include personal bias. Often, scientists, those behind the engineering process and in the core of GM developments, are those found unable to communicate effectively to the public a conclusive answer to the consumer questions on the issue. There are a number of studies that attempt to summarize and conceptualize the field in order to disseminate information in a simpler way and enable the academic debate to move further faster. GM food ethical considerations have been summarized in three main propositions used to yield a positive response to GM crops:

- The rights of people in various countries to choose to adopt GM technology
- The balance of likely benefits over harms to consumers and the environment from GM technology
- The wisdom of encouraging discovery, innovation, and careful regulation

Key findings have summarized moral concerns in three main types:

1. The consequences of GM food–ecological/risk concerns (84% of the studies)
2. The value of naturalness, the value of biotic entities, and conceptual reductionism (57% of the studies)
3. Handling of risks and application of GM crops (43% of the studies)

Five main ethical concerns against GM crops have been raised: the prospective damage to human health, the prospective harm done to the environment, the harmful influence on traditional farming practice, the unwarranted corporate domination, and the 'unnaturalness' of the technology.

Taking under consideration the findings of the studies discussed, it is essential to note that the core concerns are those that link in with the perceived risks and benefits. When these concerns will be answered with unbiased information dissemination, then the consumer will be in a better position to decide, whether they wish to adopt/buy the technology or not buy and reject GM food products altogether.

See also: Genetically Modified Microorganisms.

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Genetically Modified Microorganisms

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Introduction

Microorganisms have a long history of use in food applications. Various types of fermented foods such as wine, beer, fermented milks, cereals, vegetables, meat and fish products, and cheeses have been manufactured for millennia using bacteria, yeasts, and filamentous fungi. While traditional processes, still used in many parts of the world, rely on spontaneous fermentations controlled mainly by external factors like water activity, temperature, and pH, defined starter cultures are nowadays commonly used in industrial-scale production. A more recent application is functional foods, in which health-promoting viable bacteria or yeasts are introduced into the food. In addition to their direct use in food, microorganisms are harnessed in the mass production of enzymes, pigments, sweeteners, antioxidants, vitamins, acidity regulators, flavor enhancers, thickeners, and preservatives for use as food additives or processing aids to make bread, sausages, dairy products, juices, ice cream, and other goods.

Industrial microorganisms have been gradually improved for a long time, often without detailed knowledge about the changes that have occurred in their genetic makeup. Genetic alterations can emerge spontaneously or can be induced in the laboratory by mutagenic chemicals, the cells with desired characteristics being then selected from the pool of genetic variants. 'Genetically modified microorganism' (GMM) has, however, a special meaning in the EU legislation (Directive 2009/41/EC). It is defined as "micro-organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination." Usually, it means the construction of new DNA sequence combinations in vector systems, followed by incorporation into host organisms. Mutagenesis and natural processes such as conjugation, transduction, and transformation are not considered as genetic modification.

The optimization of GMMs includes factors like host selection, central metabolism of the cell and its relationship with product biosynthesis, degradation, and by-product formation. The benefits that optimized GMMs can provide compared to other production systems are that they are efficient and robust, are easy and rapid to cultivate using safe raw materials, and do not produce toxic or otherwise undesirable compounds. With these properties, GMMs compete with solvent extraction from natural sources, or with chemical synthesis, which sometimes requires high temperatures and pressures and strong chemicals. Many of the produced compounds can thus originate from different sources, and the GMM-derived products are often indistinguishable from those produced by other means. In the absence of positive lists and labeling of the GMM-derived products, no exact figures can be given about their usage.

In this article, we review the development of GMMs for their possible use as live microorganisms in food and as production platforms for compounds utilized by the food processing industry. Health and safety aspects are discussed briefly.

Food Microorganisms and Their Genetic Modification

Although hundreds of bacterial species and thousands of bacterial strains are used in food production as either starters, probiotics, protective cultures, or production platforms, the vast majority are lactic acid bacteria (LAB), propionic acid bacteria, brevibacteria, staphylococci, micrococci, and bacilli. In the production of amino acids, *Escherichia coli* (mainly derivatives of *E. coli* K-12) and *Corynebacterium glutamicum* (also known as *Brevibacterium lactofermentum*) are important species. The eukaryotic microorganisms used in food processes include some yeasts such as *Saccharomyces cerevisiae*, *Komagataella* (previously *Pichia*) *pastoris*, and *Kluyveromyces lactis* and the filamentous fungi *Aspergillus niger*, *A. oryzae*, and *Trichoderma reesei/longibrachiatum*. It is possible that in the future, photosynthetic microorganisms (cyanobacteria, purple photosynthetic bacteria, and microalgae) are used increasingly, as they have the specific advantage of using economic resources (light, water, and carbon dioxide) for growth.

One of the main reasons for the use of limited selection of microbial species in food applications is that they have a long history of use in large-scale industrial processes. The strains have been optimized for growth on inexpensive nutrient sources and at high density and are easy to handle. Often, these microorganisms are also generally recognized as safe (GRAS) or qualified presumption of safety, which is similar to GRAS in concept and purpose but is modified by the European Food Safety Authority to take account of the different regulatory practices in Europe compared to the United States. One microorganism is not optimal in all cases and the choice of host organism is an important step in the strain development process.

The genes used for engineering can originate from diverse sources but can also be (semi)synthetic or tailored for the specific purpose. The genes can be expressed from plasmids or native or artificial chromosomes. The host genome can be edited in a site-specific manner by taking advantage of its natural mechanism, which repairs nuclease-mediated double-strand breaks in the DNA. Such nucleases used in editing are, for example, zinc finger nucleases, TALE nucleases, and RNA-linked CRISPR–Cas9 nuclease system. Gene expression levels can be optimized with promoters of suitable strength. For fine-tuning the expression, predicting and modeling tools are available for promoters and their regulatory elements.

In any case, in order to introduce, integrate, and express (foreign) genes in the host organism as a result of genetic modification, or to carry out gene editing, suitable DNA constructs and efficient transformation systems are needed. In the

following, specific features of common vector and transformation systems in different types of microorganisms are briefly discussed.

Genetic Modification of Bacteria

A majority of food fermentations around the world are based on LAB, either spontaneously grown or intentionally added in food matrices. The LAB, natural inhabitants of gastrointestinal tract, have been a focus of intensive genetic research since late 1970s, and currently, approximately 40 complete genomic sequences of LAB belonging to the main genera are in the public domain. Many important LAB species were originally rather recalcitrant to genetic modification, which was based on transformation of protoplast ('naked' cells). Electroporation or permeabilization of the cell walls by a short electric shock has subsequently made most of the species transformable. Many LAB contain natural plasmids, which can be used for the construction of transformation vectors. Based on these plasmids, a wide range of expression and integration vectors, the latter ones often utilizing phage attachment sites, have been developed. Various systems to control the expression of cloned genes have been introduced, such as NICE (based on autoregulation of the biosynthesis of the bacteriocin nisin) and SICE (based on stress-specific promoters). Because of the potential use of genetically modified LAB either in food or for therapeutic purposes, special attention has been paid to the selectable markers, as antibiotic resistance genes are not considered appropriate. Typical food-grade selectable markers include bacteriocin or heavy metal resistance or systems based on metabolic complementation.

Compared to LAB, no systematic genetic research has been carried out on other food bacteria such as *Propionibacteria*, *Brevibacteria*, and *Kocuria*. A notable exception is the bifidobacteria because of their potential uses as probiotics. Several bifidobacterial genomes are already in public domain, and the genetic modification systems are under development.

Metabolic engineering efforts for the production of various compounds have focussed for some time in *E. coli*, which can be modified using standard vectors and DNA transfer techniques. Genetic modification of the amino acid-producing species *C. glutamicum* has been more challenging. As its name implies, it was originally isolated and utilized as an efficient producer of L-glutamic acid. *C. glutamicum* is not naturally transformable, and protoplast transformation has not been very effective. Furthermore, it has restriction systems that impede the introduction of foreign DNA. However, electroporation combined with various cell wall-weakening treatments has enabled the genetic modification. Endogenous plasmids have provided a basis for vector construction, and the fact that *E. coli* promoters are functional in *C. glutamicum* has further enhanced the genetic modification possibilities.

Bacillus species are important producers of industrial enzymes. Bacilli, especially *B. subtilis*, are common experimental organisms in molecular biology, their genomes are well characterized, and genetic modification techniques are established. Bacilli are naturally transformable, both protoplast and electroporation systems being used in transformation. Small endogenous plasmids have provided the basis for vector construction. Accordingly, both integration and expression vectors, including efficient secretion systems to allow the harvesting of expressed proteins from the growth medium, have been developed.

Genetic Modification of Yeasts and Fungi

In contrast to the prokaryotic bacteria, yeasts and filamentous fungi are eukaryotic organisms. Their genomic organization, gene regulation systems, and secretion machineries are more related to those in animal and plant cells. On the other hand, they share with bacteria the ability to grow fast on relatively simple substrates and media, making them attractive for biotechnological purposes.

The yeasts are fungi characterized by nonfilamentous growth. Baker's yeast, *S. cerevisiae*, is the best-known species due to its many traditional uses in baking, brewing, and winemaking and to its extensive use in molecular biological research. It was the first eukaryotic organism that was successfully transformed by foreign DNA and was the first eukaryote to have its total genome fully sequenced in 1996. It is one of the few fungi that contain natural plasmids, the 2 μm circular plasmids that were used in the early vector constructs, and the yeast episomal plasmids.

Transformation methods applied in *S. cerevisiae* include protoplast (spheroplast) techniques, divalent cation-mediated transformation, and electroporation. Standard cloning vectors currently in use are shuttle vectors between *E. coli* and *S. cerevisiae*. Typical vectors consist of a bacterial replication region and a selectable marker (antibiotic resistance gene) combined with a yeast selectable marker (usually a gene that complements a defect in host amino acid biosynthesis, such as *LEU2*), yeast origin of replication (*ARS*), yeast centromere, and yeast telomeres. These vectors enable the cloning of large DNA fragments, which, when linearized, function as artificial chromosomes and are stably maintained in the GM yeast. Yeast integrative plasmids are shuttle vectors without the yeast origin of replication. After transformation, homologous recombination occurs between the selectable marker in the plasmid and the corresponding mutated host gene, integrating the plasmid into the chromosome and restoring the defective function.

Besides *S. cerevisiae*, a methanol-utilizing yeast *K. pastoris* has gained popularity as an alternative eukaryotic host in the production of recombinant proteins. Integration vectors utilizing the strong inducible *K. pastoris* alcohol oxygenase promoter *AOX1* have been designed to allow for optimized and easily controlled expression of the inserted genes.

Compared to yeasts, genetic modification of filamentous fungi represents a much greater technical challenge. Filamentous fungi apparently lack natural plasmids, and their chitinous cell walls resist transformation. The genomes are much larger, and the regulatory systems more complex. However, filamentous fungi, particularly *Aspergillus* and *Trichoderma*, are traditional producers of enzymes and organic acids like citrate, isocitrate, succinate, and fumarate, and there has been a strong incentive to apply genetic techniques also to these organisms.

Techniques applicable to filamentous fungi include protoplast transformation, electroporation, biolistics (bombardment of cells with DNA-coated particles), and *Agrobacterium tumefaciens*-mediated transformation normally used for plants. Approaches using protoplasts are technically challenging and

tend to yield multiple DNA insertions in the recipient genome. In contrast, *A. tumefaciens*-mediated transformation typically results in fewer and more targeted insertions. Thus, the selection of transformation techniques may depend case by case on the desired outcome.

The lack of natural plasmids from filamentous fungi led to the experiments with artificial plasmid constructs. These proved to be unstable, leaving chromosomal integration as the only reasonable alternative. However, in contrast to yeasts in which homologous recombination can be routinely achieved, the integration of transformed DNA in filamentous fungi occurs often in nonhomologous sites. In the selection of promoters, one also has to take into account the rather complicated induction/repression patterns typical to these organisms. Despite these difficulties, GM fungi are successfully used in industrial scale as enzyme producers.

Strain Improvement by Genetic Engineering

Microorganisms used as food cultures can be modified for fermentation profiles or enzymatic activities to improve the flavor, texture, keeping properties, and nutritional value of fermented foodstuffs. Although numerous GMM starter strains have been developed, including phage-resistant and bacteriocin-producing LAB, they have not been introduced into the market, mainly due to uncertainties related to consumer reactions. Recently, two GM wine yeasts have been approved for commercial use in the United States and Canada. One of the GM yeasts (ML01) has enhanced malolactic fermentation properties as a result of the introduction of a malolactic enzyme-encoding gene from LAB and a malate transporter-encoding gene from another yeast species, *Schizosaccharomyces pombe*. The other GM yeast (522^{ec-}) has been modified to improve the degradation of urea by placing the responsible gene under a constitutive promoter, thus preventing the formation of carcinogenic ethyl carbamate during wine fermentation. These two cases might represent the first signs of the introduction of viable GMMs into the food chain.

Much less controversial are GMMs used for the production of valuable compounds such as enzymes, amino acids, organic acids, and flavorings for food use. These microbial strains are under continuous optimization to make the processes economically more viable by increasing the yield or by improving other cellular functions or product quality or safety.

There are a number of ways to improve the production microorganism. Considering general performance, the GMM can be made capable of utilizing a mixture of different types of sugars, like galactose, xylose, and mannose in the presence of glucose, which in *E. coli* and in *S. cerevisiae* inhibits the use of other sugars. On the other hand, *E. coli* has been modified to enhance tolerance to high concentrations of glucose and sucrose. Robustness, that is, stability and resistance of enzymes to fluctuations in fermentation process conditions like medium composition and oxygen concentration, can be increased by introducing genetic switches that control cellular metabolism. Resource efficiency can be improved by using energetically preferable alternative reactions. As cofactors are important in transferring energy to the cell, one target is to increase the availability of redox carriers (NADH and NADPH) and energy metabolites (ATP). Increases in lysine production were achieved in *C. glutamicum* by overexpressing NAD kinase or replacing an NAD-dependent enzyme by NADP-dependent enzyme from *Streptococcus mutans*. To increase consumer safety, pathways that generate toxic or other harmful compounds can be deleted or interrupted, thus minimizing their accumulation in the production organism or culture medium.

The goals are increasingly achieved by the application and integration of the latest tools and strategies in systems biology, protein engineering, process engineering, high-throughput technologies for cultivation, screening, and analytics. Integrating comprehensive omics (fluxomics, metabolomics, proteomics, transcriptomics, and genomics) data with computational prediction algorithms can lead to the optimization of GMMs and to new designs with increasing speed and success. To replace traditional recombinant DNA methods, rapid pathway engineering has been developed, which combines enzymatic *in vitro* assembly and *in vivo* homologous recombination. However, strain design is still a major undertaking. The future scenario is to create highly efficient synthetic microbial minifactories, but this requires a full understanding of the minimal genome requirements of a microbe.

Many examples of how all of these approaches have been successfully employed exist, but it is unclear how extensively they have been used in the GMMs currently employed for the industrial production of compounds for food use.

Production of Industrial Enzymes

Enzymes isolated from GMMs are widely used today (Table 1). In the simplest case, a desired gene is introduced in the host to express the enzyme. Yield can be improved by increasing gene copy number in the plasmid or chromosome. Performance of the enzyme in food matrix can be improved by taking into account the conditions, in which the catalysis is expected to take place, for example, pH optimum, temperature or salt concentration, and also cofactor requirement. Selectivity of the enzyme can be improved by optimizing natural enzymes for novel substrates or for entirely new catalytic activities. In some cases, this can also decrease unwanted side reactions and products.

Major improvements in catalytic efficiency and substrate specificity have been achieved by enzyme engineering. The method combines different experimental and analytic tools (random mutations, error-prone PCR, DNA shuffling, saturation mutagenesis, and site-directed mutagenesis) with computational design tools (structural analysis, phylogenetic analysis, and *de novo* engineering). For example, to obtain cellulase with greatly enhanced thermostability, a computational structure-guided recombination method was used to inform the design of libraries of synthetic variants, which were then screened for the desired trait.

Production of Small Compounds

Only a few compounds such as amino acids, organic acids, and vitamins are produced on a large scale using industrial microorganisms. One reason is that engineering microorganisms to produce small compounds is highly complex. To achieve high production rates, high yields, and purer products, enzymes in metabolic pathways usually have to be improved for catalytic

Table 1 GMMs as platforms for food enzyme production

Enzyme	*Function in food; examples of possible applications*	*Production organism*
Acetolactate decarboxylase	Conversion of acetolactate to acetoin; reduction of butter aroma in beer	Ba, Bsu, Ss
α-Amylase family: α-amylase, branching glycosyltransferase, cyclodextrin glucanotransferase, glucanotransferase, glucoamylase, α-glucosidase, maltodextrin α-D-glucosyltransferase, maltogenic amylase, maltotetraohydrolase, pullulanase	Starch degradation and modification; baking, cereals, sweeteners, beverages	An, Ba, Bl, Bst, Bsu, Sv, Tr or Tl
Asparaginase	Asparagine (precursor of acrylamide) breakdown; bakery	An, Ao
Cellulose-degrading enzymes: cellobiose dehydrogenase, cellulase, endo-1,3(4)-beta-glucanase	Cellulose degradation; beverages, baking, diet products	Ao, Ba, Bsu, Fv, Tr or Tl
Chitinolytic enzymes: acetylhexosaminidase, chitinase	Chitin degradation; preservation (bakery)	Sv
Cross-linking enzymes: laccase, peroxidase, sulfhydryl oxidase	Produce covalent bonds between food polymers; cereal, dairy, meat, fish	An, Ao, Bsu
α-Galactosidase	Liberates terminal α-galactosyl groups, for example, from glyco-oligosaccharides; legumes (flatulence), sugar beet	Sc
Endo-1,3-β-glucanase	Breakdown of certain carbohydrate polymers; brewing	Sv
Glucose isomerase	Conversion of glucose to fructose; fructose syrup, low-calorie products	Sr
Hemicellulases: endo-1,3(4)-β-glucanase, hemicellulase, pentosanase, xylanase	Breakdown of plant cell walls; baking, beverages	An, Ao, Ba, Bsu, Sv, Tr or Tl
Hydrogen peroxide producing and decomposing enzymes: catalase (CAT), glucose oxidase (GOx), hexose oxidase (HOx)	CAT: hydrogen peroxide breakdown; GOx, HOx: hydrogen peroxide production; used alone or in combination: egg processing, baking	CAT: An; GOx: An, Ao; HOx: Pa or Hp
Lactase (beta-galactosidase)	Lactose hydrolysis; dairy products, ice cream, chocolates	Ao, Kl
Lipases: acyltransferase, monoacylglycerol lipase, triacylglycerol lipase, phospholipases A2, B, C, and D	Fat splitting and modification; cheese, spices, aromas, baking, noodles	An, Ao, Bl, Pc, Pa or Hp, Pf, Pp, Sv, Tr or Tl
Lysozyme	Bacterial cell wall rupture; antibacterial, cheese	Sv
Pectinases: pectin lyase, pectin methylesterase, polygalacturonase, and pectinase	Pectin degradation; beverages, fruits, vegetables	An, Ao, Tr or Tl
Phytase	Phosphate release from phytic acid; cereals, legumes	An, Ao, Tr or Tl
Proteases: aminopeptidase, serine carboxypeptidase, chymosin, other proteinases	Protein degradation and modification; dairy (milk-clotting), cheese, meat, baking, fish, beverages, baby food	An, Ao, Ba, Bl, Bsu, Cp or Ep, Fv, Kl, Sv, Tr or Tl

An, *Aspergillus niger*; Ao, *Aspergillus oryzae*; Ba, *Bacillus amyloliquefaciens*; Bl, *Bacillus licheniformis*; Bst, *Bacillus stearothermophilus*; Bsu, *Bacillus subtilis*; Cp, *Cryphonectria parasitica*; Ep, *Endothia parasitica*; Fv, *Fusarium venenatum*; Hp, *Hansenula polymorpha*; Kl, *Kluyveromyces lactis*; Pa, *Pichia angusta*; Pc, *Penicillium camemberti*; Pf, *Pseudomonas fluorescens*; Pp, *Pichia pastoris*; Sc, *Saccharomyces cerevisiae*; Sr, *Streptomyces rubiginosus*; Sv, *Streptomyces violaceoruber*; Tr, *Trichoderma reesei*; Tl, *Trichoderma longibrachiatum*.

Source: amfep

efficiency and perhaps also for selectivity. Optimal process should include robust microbial strain that provides high titer ($g\,l^{-1}$ product), yield (g product g substrate^{-1}), and productivity ($g\,l^{-1}\,h^{-1}$) and has acceptable by-product profile. A complicated factor is that while high titer facilitates product purification, the levels can be inhibitory to enzymes or toxic to the organism. The yield can be limited by low or inefficiently directed pathway flux, as well as nonoptimal energetics and NAD(P)H balance. Bottleneck enzymes can limit the productivity.

To improve the production organisms, several approaches can be taken, usually in combination. For optimal pathway flux (production rate or yield), carbon end energy resources can be redirected from central metabolism (biomass production) to the pathway leading to the desired product. Competing pathways from relevant endogenous intermediates can be deleted. Pathway bottlenecks caused by, for example, poor precursor (substrate or cofactor) availability or inefficient enzyme can be identified using metabolic flux analysis and protein design.

Fine-tuning of gene expression is important to attain proper (usually not maximal) level of each enzyme and thus to achieve optimal flow of metabolites through the pathway. Natural promoter can be tuned by mutagenesis, or a synthetic promoter can be created through shuffling of multiple promoters. Native regulation (regulatory genes, promoters, or small RNAs) can be replaced by a new, perhaps inducible system. RNA-based synthetic control modules can be used to obtain predictable fine-tuning of expression levels. Several genes that function in a common process can be introduced into a single bacterial operon. Rate of protein synthesis can be controlled using designer ribosomal binding sites. The optimization of codon usage can facilitate effective expression (translation) in a different host and to remove cryptic regulatory elements.

The titer is primarily dictated by the inhibition of some enzymes in the pathway or by end product toxicity to the production organism. An example is the amino acid L-valine, which at high concentrations is toxic to *E. coli*. Structure of

bottleneck enzymes can be improved to eliminate unwanted allosteric regulation in case the natural enzyme suffers from inhibition by the end product or a key metabolite. Toxic end products can be moved in safe storage compartments or exported out of the cell by introducing specific pumps. Spatial targeting of enzymes may increase yield by reducing the loss of intermediates via side reactions but can also decrease effects of possibly toxic intermediates. For example, in yeast, the subcellular compartmentalization allows for pathway isolation and increased fluxes.

Examples of amino acids are L-lysine, L-valine, and L-threonine in *C. glutamicum*. The production strains have been developed through classical mutagenesis, genetic modification, and metabolic engineering. All this would not have been possible without detailed knowledge of the interconnections between the glycolytic and biosynthetic pathways.

In the production of organic acids such as citric acid, lactic acid, malic acid, and succinic acid, yeasts have been attractive hosts because of their good tolerance to low pH. In glucose-grown batch cultures, an engineered *S. cerevisiae* strain produced malic acid at high concentrations. Yeast and *E. coli* have been successfully engineered to achieve near-theoretical yield of succinic acid.

Riboflavin (vitamin B2) can be derived from the pentose phosphate pathway intermediate ribulose 5-phosphate. Unconventional yeasts *Ashbya gossypii* and *Candida famata* have been used as hosts for riboflavin production. Coenzyme Q10 is a well-known vitamin-like ubiquinone involved in cellular electron transport. For instance, expression of genes from other bacteria enabled the production of coenzyme Q10 in *E. coli*. Total biosynthesis of vitamin C (ascorbate) from glucose has been reported in metabolically engineered yeast.

Potential to produce entirely new products in microorganisms is huge, as a whole biosynthetic gene cluster can be introduced into the new host by metabolic engineering. By mixing enzymes or their domains from different donors and pathways and altering enzyme specificities, even further diversity can be achieved. With the advent of metabolic engineering tools, the successful reconstitution of heterologous pathways in *E. coli* and other microorganisms provides an alternative to meet the increasing demand of, for example, natural antioxidants.

Sugar alcohols are calorie-free versions of sugars that can be used as sweeteners or flavoring agents. In *S. cerevisiae* strain, engineering has improved xylitol and ribitol titers. In *E. coli*, xylitol production increased by engineering an enzyme that converts NADH into NADPH. In the yeast *Yarrowia lipolytica*, high production of erythritol and mannitol was achieved from glycerol.

Plant secondary metabolites such as flavonoids and isoprenoids (β-carotene and lycopene) are used as food colorants and flavors. Extraction of pure compounds from plants is often problematic because of their low quantity and a multitude of structurally very similar compounds. Due to these reasons, synthetic compounds have gained increasing importance in the food industry. Metabolic engineering of microorganisms is a powerful approach to gain access to the bioactive compounds. Advances in the knowledge of the biosynthetic pathways in plants have driven the rational development of high-producing microbial strains.

Carotenoids have been subject to extensive metabolic engineering efforts for production in *S. cerevisiae*, *K. pastoris*, and, in particular, *E. coli*. One success story is the development of lycopene production in *E. coli* through knockout and overexpression of multiple gene targets. The approach was to divert carbon flux with an artificial regulatory circuit to lycopene pathway, inactivate competing pathways, and balance the supply of central metabolism. The tools used included systematic model-based search combined with random transposon mutagenesis, screening of shotgun library, and use of high-throughput method for automated genome engineering to create targeted genomic diversity and to program and expedite evolution of lycopene-producing cells. Heterologous production of other carotenoids (e.g., zeaxanthin, astaxanthin, and lutein) was achieved by extending the established lycopene and β-carotene production platforms.

Antioxidant activity of many phenolic compounds, such as flavonoids, has raised significant interest in the health-protecting effects of these compounds. Polyphenols have been produced in *E. coli* and *S. cerevisiae* by pathway reconstruction and optimization via metabolic engineering approaches. Phenolic acids represent simple polyphenols that include the hydroxybenzoic (e.g., gallic and vanillic acid) and hydroxycinnamic acids (e.g., *p*-coumaric, caffeic, ferulic, and chlorogenic acid). Biosynthesis of vanillin has been established in *S. pombe* and *S. cerevisiae* through metabolic engineering. Microbial platform for caffeic acid production has been developed in *E. coli*. Flavonoids and anthocyanins have been synthesized in *E. coli* after extensive pathway engineering. An economic process for the production of flavonoid naringenin from glucose has been developed in *E. coli* through the expression of four plant enzymes in the pathway and other modifications to metabolic fluxes.

Safety and Legislation

Depending on the type of GMM or product in question, that is, whether it is crystallized amino acid, food enzyme preparation, or live starter culture, the safety issues vary. Such issues include toxicity or pathogenicity of the organism, fermentation by-products and impurities, allergenicity, and effects on gut microbiota. International standards for the safety assessment of GMOs, including GMMs, have been developed under Codex Alimentarius Commission and OECD. Many countries have also developed specific GMO legislations. European Union has one of the most comprehensive systems for GMO safety evaluation, which follows the main internationally agreed principles. The EU is in a process of preparing a list of food enzymes based on their established safety and technological need.

Health Aspects

While the focus of the legislation on GMMs and their use is on the prevention of their potential harmful effects on the health and environment, GMMs can actually contribute positively to human health and well-being. By providing cheap, highly purified food ingredients without the need to use potentially hazardous traditional techniques, such as organic extractions

or poorly characterized raw materials, GMMs can improve the safety, nutritional quality, and sensory properties of food.

GMMs could, in the future, also be directly used to improve both intestinal health and general health. Already documented positive health effects of probiotics could be further enhanced through engineering metabolic pathways involved in the positive interactions with the host. An example of this approach is the elimination in *Lactobacillus acidophilus* of a gene involved in the synthesis of lipoteichoic acids (apparently inflammatory components of the cell wall of Gram-positive bacteria). In animal experiments, this GMM strain downregulated the synthesis of proinflammatory cytokines and markedly alleviated the symptoms of induced colitis.

Delivery of therapeutic molecules, such as vaccines, by genetically modified probiotics, which involves cloning and expression of antigens or in the probiotic hosts, has been a much-studied area during the past 20 years. In animal experiments, the results have been promising, but their eventual application to humans would require both an absolute certainty of safety and general consumer acceptance. This type of products would fall into a gray area between food and medicine legislations.

Future Prospects

Advances in understanding metabolic pathways and their regulation make the microorganisms increasingly attractive for metabolic engineering. While there is much research on the potential of LAB and Bacilli, the great metabolic diversity of yeasts and especially of filamentous fungi makes them particularly promising candidates for future metabolic engineering and even for generating entirely novel applications and products. Engineering microorganisms for plant natural product synthesis is expected to be an increasing focus as the beneficial compounds can be present in plants in very low amounts as mixtures with potentially harmful compounds.

The construction of genome-minimized hosts seems to be a feasible approach. The efforts thus far have resulted in *E. coli* strains with a genome size reduced by up to 15%. Together with the advances in synthetic biology, future metabolic engineering efforts of microorganisms will greatly expand the number of economically viable products.

See also: Genetically Modified Foods: Risk Assessment, Legislation, Consumer Behavior, and Ethics; Genetically Modified Foods; Probiotics.

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Ghee

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Introduction

Ghee (synonyms: ghritam, ghrit, havi, maslea, samna, samn, and rognezard), also known as clarified butter or anhydrous milk fat, is a fat-rich dairy product originally produced in India. It can be prepared from cow milk (CM), buffalo milk (BM), or mixed milk. Milk is separated into cream that may be directly converted into ghee or that can be first converted into butter and then into ghee. Ghee is prepared by heating butter or cream to evaporate nearly all the moisture content and to produce the characteristic flavor and then filtering out the precipitated milk solids. Heating destroys most of the microorganisms and enzymes. Low water activity hampers the growth of organisms that survive heat treatment or those contaminating the product. The origin of technology for ghee making is recorded in Indian epics and Vedas, which relate it to the third century BC. Ghee is the most widely used milk product in the Indian subcontinent. It is considered as the supreme cooking or frying medium.

Milk is utilized for ghee production due to the concentration of milkshed areas in rural localities, which are far away from the nearest urban-consuming centers. It is also used for shallow frying and deep-frying of traditional Indian foods. BM is preferred over CM to prepare ghee due to its higher fat content. Milk fat has commercial significance as payment to milk producers is based on the level of fat percentage. Ghee production is an important activity of the organized sector of the dairy industry, which employs mechanized systems for this purpose. Ghee is incidental to the product mix in the dairy plants, which manufacture dairy products.

In the Indian context, ghee is a product of the sacred cow and hence pure and confers purity to other foods when used in making them. Almost the entire range of Indian sweets, prepared with admixtures of milk, cereals, fruits, vegetables, and nuts, are preferably cooked using ghee as the medium. It is one of the basic elements in the Indian cooking. Sweets and meals cooked in ghee are recognized for their distinguished flavor attributes. Meals for special occasions are cooked in ghee. Ghee is also marketed in many African and Asian countries, the United Kingdom, Australia, Armenia, Belgium, the Netherlands, New Zealand, and the United States.

Technology of Ghee Manufacture

The method of ghee manufacture varies according to the base material used (milk, cream, or butter), intermediate treatment of raw materials, and handling of the semifinished or fully formed ghee. Because of its commercial importance, considerable refinement and mechanization have taken place in the manufacturing processes of ghee. In India, a considerable amount of ghee production is manufactured according to traditional methods used for cottage-level production. Some processes, developed for ghee production, are described later in the text.

Indigenous Milk–Butter Process

The indigenous methods of ghee making usually involve direct churning of raw milk or lactic acid fermentation of heat-treated milk followed by churning. Hand-driven wooden beaters are usually employed for separating the butter. The butter is melted in a metal pan or ceramic vessel on an open fire until almost all moisture is removed. The extent of frothing may be used as an index to judge when to terminate heating. After heating, the contents are left undisturbed. When the curd particles have settled, the clear fat is carefully decanted off into ghee storage vessels. The traditional ghee-making practice contributes about 90% of the total ghee production in India. This method leaves behind a large quantity of buttermilk of varying quality and also leads to low fat recoveries (75–80%).

Direct Cream Method

The small dairies use a technologically improved method for ghee making, which involves the separation of cream from milk by centrifugation. This process omits the need of production of butter because cream is directly converted into ghee. The fresh cream, cultured cream, or washed cream is heated to 115 °C in a stainless steel, jacketed ghee kettle fitted with an agitator, steam control valve, and pressure and temperature gauges. Heating is discontinued as soon as the color of the ghee residue turns to golden yellow or light brown. One of the limitations of this method is that it requires a long heating time to remove the moisture. A high content of serum solids in the cream may also produce a highly caramelized flavor in the ghee and lead to about 4–6% loss of butterfat in the ghee residue or during handling operations, depending upon the fat percentage in the cream. Economically, this method is less labor-intensive as compared to the creamery butter method. Lower recovery of fat and slightly greasy texture in the ghee obtained by this method are the demerits.

Creamery Butter Method

Most dairies use unsalted creamery butter or white butter as a raw material for ghee making. A typical plant assembly for this method comprises a cream separator; a butter churn; butter-melting outfits; steam-jacketed ghee kettle with agitator and process controls; ghee filtration devices; storage tanks for cream, butter, and ghee; pumps and pipelines interconnecting these facilities; crystallization tanks; and product filling and packaging lines.

In brief, the process involves melting of butter mass at 60 °C, which is pumped into the ghee boiler set at 90 °C. This temperature remains constant as long as the moisture is being driven off. The scum that collects on the top surface of the product is removed from time to time with the help of a perforated ladle. The temperature gradually rises and the heating at the last stage is carefully controlled. The end point shows the disappearance of effervescence, appearance of finer air bubbles on the surface of fat, and browning of the curd particles. At this stage, the typical ghee aroma is also produced. The final temperature of clarification is adjusted to 110 °C. The ghee is then pumped through an oil filter/clarifier into settling tanks, which are cooled by circulating water at 60 °C. After cooling and sedimentation, the ghee is filtered through a muslin cloth so as to separate it from the sediment – known as ghee – residue (consisting mostly of burned casein). It then goes on for granulation and packaging. The merit of this method is saving in labor costs, physical exertion, and exposure to uncomfortably high temperatures and humidity during actual ghee making compared to the direct-cream heating method.

Prestratification Method

This method takes advantage of gravity settling for removing most of the moisture from butter and saves the large thermal energy required for removing moisture from butter. In this method, cream is first churned into butter, and it is melted in the butter-melting vat. It is then pumped into a vertical storage tank and left undisturbed for 30 min. When butter is left undisturbed at a temperature of 80–85 °C, it stratifies, that is, separates into distinct layers, viz., a top layer of floating denatured particles of curd, a middle layer of fat, and a bottom layer of buttermilk. This separation into layers has been called prestratification. The bottom layer of buttermilk contains 60–70% of solids-not-fat (SNF) and also over 80% of the moisture originally present in the butter. The buttermilk is mechanically removed without disturbing the top and middle layers. The temperature of the remaining two upper layers is raised to the usual clarifying temperature of 110–120 °C. This process is essential to promote the development of a more characteristic aroma. This method offers the advantages of economy in fuel consumption up to 35–50%, saving in time and labor up to 45%, and production of ghee with lower free fatty acid (FFA) levels. The provisions of pressure gauge, safety valves, temperature regulators, and condensate outlet pipe make ghee of better quality and amenable to process controls. Economy in fuel consumption as compared to direct clarification, production of ghee with a lower acidity, and longer shelf life are the advantages of this method.

Continuous Ghee Making

To overcome the problems of batch methods of ghee making like limitations of scale of operation and excessive exposure of the plant operators to the stress of heat and humidity, the dairy plants have evolved continuous ghee-making system to suit their requirements. The process uses three successive stages of scraped surface heat exchangers, followed by flashing into vertical vapor separators. Molten butter is pumped through the successive stages. Ghee leaving the final stage is passed through a centrifugal clarifier to remove residue and clarified ghee is stored in a tank for final packing. A continuous ghee plant has been developed, the details of which are shown in Figure 1. In this method, the concentration of fat and breaking of fat-in-water emulsion are done mechanically with the help of centrifugal force in the clarifixator and concentrator, and the use of heat is limited to development of flavor and removal of traces of moisture. The plant requires low energy input and can be adapted for much larger volumes. The process also results in lower SNF losses and better fat recovery. The product compares well in flavor and other chemical characteristics with ghee prepared by the batch process. To produce 100 kg of ghee, the plant utilizes 32 kg of steam and 0.45 KWh of electrical energy.

Chemical Composition of Ghee

Ghee is largely made up of triglycerides (98%) and other minor constituents, such as diglycerides (1–2%), monoglycerides (0.1–0.2%), FFAs (1–10 mg/l00 g), phospholipids (0–80 mg/l00 g), cholesterol (0.25–0.4%), fat-soluble vitamins,

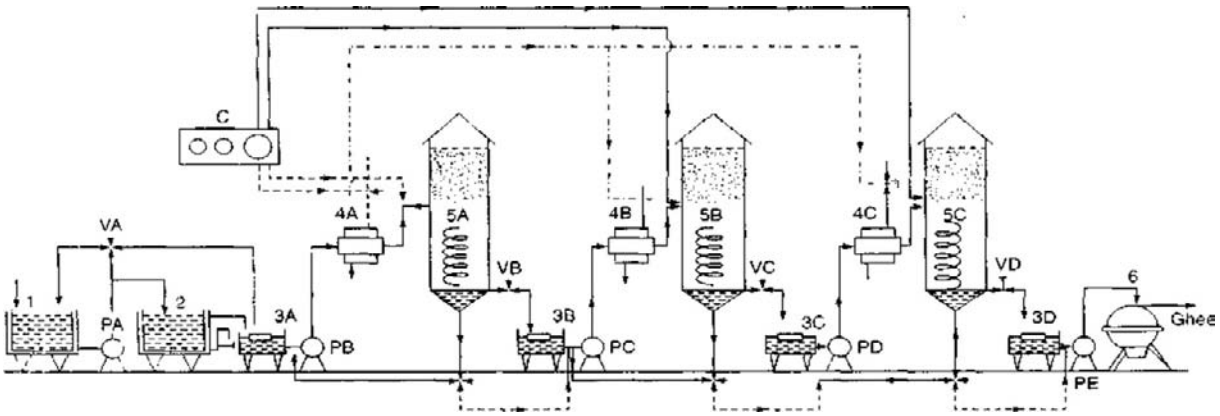

Figure 1 Industrial process for continuous production of ghee, with butter as starting material. 1, Butter Melting Vat; P-PA/B/C/D/E, positive displacement pumps (five); 2, gravity separator; 3A/B/C/D, balance tanks (four); 4A/B/C, scraped surface heat exchangers (three); 5A/B/C, vapor separators (three); 6, clarifier; V-A/B/C/D, valves (four); P, pump; C, control.

Table 1 Chemical composition of ghee[a]

Constituents	*Ghee*
Fat (%)	99–99.5
Moisture (%)	<0.5
Vitamin A (μg/100 g)	315–376
Cholesterol (mg/100 g)	252–284
Tocopherol (mg/100 g)	Mean 500
Free fatty acid (%)	<2.8

[a]Values are ranges.
Source: Compiled from various sources.

carbonyls (traces), glyceryl ethers (0.8 μmol g^{-1}), and alcohol (1.8–2.3 μmol g^{-1}). The chemical composition of ghee is shown in Table 1.

The majority of fatty acids in milk possess even-numbered carbon chains $C_{4-18.}$ The total proportion of polyunsaturated fatty acids is only 3–4% in ghee. Ghee prepared from BM is richer in butyric, palmitic, stearic, tetraenoic, and pentanoic acids than that of CM. The levels of other short-chain fatty acids (caproic to myristic) are higher in CM ghee than those in BM ghee.

At the temperature employed for clarification of ghee during its manufacture, the phospholipid content of ghee also increases progressively due to enzymatic actions.

Derived lipids are the fatty acids originated as a result of fat decomposition by enzymes or other fat splitting conditions, alcohols such as glycerol, and cholesterol and organic bases such as choline (associated with lecithin).

Dehydrocholesterol occurs in close association with fat as far as the physical properties are concerned. When its compound is irradiated with UV light, it is converted to vitamin D. It is insoluble in water but soluble in fat. Its content in milk is around 12 mg/100 g or 0.3–0.4% of fat, equivalent to ~400 mg in 100 g of fat.

Other compounds of significance from nutritive and health point of view present in milk fat are cephalin, sphingomyelin, and glycolipids. Associated flavor compounds of lipid nature are diacetyl and acetyl methyl carbinol. These compounds play an important part in the flavor development of butter. Diacetyl in low concentration has a butter-like aroma. Diacetyl ranges from 0.0002% to 0.004% in butter and its increasing amounts are said to increase flavor. This compound is frequently added to oils and fats to give a butter aroma. Considerable amounts of diacetyl have been found in starter culture and ways have been studied to increase its concentration. Starter distillates are concentrated sources of diacetyl. Accordingly, butter and ghee made from cultured milk/dahi have more pronounced aroma and flavor.

Carotene is the chief coloring material in milk fat, although other related carotenoids (xanthophylls) are present in small amounts. Carotene is derived from green plant sources in the feed of the lactating cow, which is the only mammal to transfer such a color to milk fat. The BM fat has no color because carotene in it has already been converted to colorless vitamin A. It is normally stable to oxidative agents, but in fat, it may be oxidized and bleached under unfavorable storage conditions. Carotene can be converted into two molecules of vitamin A by reacting with two molecules of water. The type of feed and breed of the cow affect the amount of carotene in milk fat. Winter stall-feeding results in 0.2–0.3 mg of carotene/100 g of fat, whereas summer pasture feeding increases it to 0.7–0.9 mg/100 g of fat.

The fatty acid composition greatly influences the fat constants and rheological properties, such as melting and crystallization behavior, solubility, surface activity, and ability to form emulsions. As per the Indian legislation, following are some of the important analytic constants or standards of mixed ghee produced under standard conditions:

- Melting and solidifying points: The melting point varies from 28 to 44 °C, while the solidifying point varies from 28 to 15 °C. (Being a mixture of glycerides, ghee does not have sharp melting or solidifying points.)
- Specific gravity: This varies from 0.93 to 0.94.
- Refractive index: The butyro refractometer reading (at 40 °C) varies from 40 to 45.
- Reichert–Meissl value: This should normally be not <28 (except for ghee from cottonseed feeding areas, where the limit is 20).
- Polenske value: This should normally be not more than 2 (except for cottonseed feeding areas, where the limit is 1.5).
- Saponification value: This should normally be not <220.
- Iodine value: This should normally vary from 26 to 38.

Comparative Appraisal of CM and BM Ghee

The ghee obtained from CM and BM has different physicochemical characteristics due to significant difference in their fatty acid composition. Fresh BM fat is white, unlike the light yellow color of CM fat due to carotene. Table 2 depicts the fatty acid composition of BM and CM fats. Preponderance of long-chain, saturated fatty acids, like palmitic and stearic acid, and substantially high amounts of high-melting triglycerides make BM fat harder than CM fat. This property contributes to the development of granular texture in ghee. Carbonyl compounds, besides FFAs and other fractions, are known to be important contributors to flavor. The BM ghee contains higher amounts of total carbonyls (saturated and unsaturated) and free monocarbonyls (saturated, alk-2-enals, and alk-2, 4-dienals) than CM ghee. Milk fat hydrolyzes faster in liquid state than in solid state. Because of the more solid fat in BM, its rate

Table 2 Fatty acid composition of buffalo and cow milk fat[a]

Fatty acids	*Buffalo milk fat%*	*Cow milk fat%*
Butyric	4.2–4.6	3.1–3.3
Caproic	1.4–1.6	2.0–2.2
Caprylic	0.7–0.9	1.1–1.3
Capric	1.2–1.4	2.5–2.7
Lauric	1.7–1.9	2.7–2.9
Myristic	10.4–10.12	11.8–12.0
Palmitic	33.0–33.2	30.5–30.7
Stearic	11.9–12.1	10.0–10.2
Oleic	27.1–27.3	27.3–27.5
Linoleic	1.49–1.51	1.48–1.52
Linolenic	0.49–0.51	0.59–0.61

[a]Values are ranges.
Source: Compiled from various sources.

of fat hydrolysis is slower than CM. This is why the CM ghee is more prone to developing rancid flavor during storage. The constituents of ghee tend to vary with the method of its manufacture. The cholesterol content of CM ghee made by the traditional method from dahi, cream, and butter displays average values of 292, 306, and 324 mg/100 g, respectively. The corresponding values for BM ghee have been observed to be 263, 275, and 294 mg/100 g, respectively. Generally, the composition of various constituents in ghee does vary.

Ghee Flavor

Aroma and taste constitute the flavor of ghee. A perfect ghee flavor is characterized by a multitude of sensory perceptions, which are pleasant, enjoyable, and lingering in the mouth. This is the most important characteristic looked for in ghee by the trade. The flavor is ascertained by rubbing a small quantity of the sample briskly on the back of the palm and inhaling it. Normally, a well-prepared sample of ghee has a pleasant, cooked, and rich flavor. The taste is usually sweet and characteristic of the milk fat, although a slight acidic flavor is preferred. Consumers always resist any change in the flavor of ghee, as this is one characteristic that predominantly determines acceptability. There are regional preferences for flavor in ghee. The preferred ghee flavors range from 'slightly curdy' to 'pronounced curdy,' cooked to caramelized and, at times, slightly oxidized in some quarters of the population. Technologies have been developed to produce ghee with desired flavors of both 'curdy' and 'cooked' types.

The flavor of ghee is mainly contributed by the heat interaction products formed between the unfermented serum portion, comprising the native carbohydrate and protein system, and by metabolic products of the starter culture when ripened cream is used for ghee making. The most important flavor components of ghee are FFAs (6–12 mg g^{-1}), methyl ketones (6–12 ppm), and a complex mixture of 44 lactones, both 5-lactones and y-lactones (29–43 ppm), aldehydes, ketones, and alcohols. About 34 monocarbonyls were found in the flavor volatiles of ghee.

Ghee Texture

Granulation of ghee is an important criterion for its selection. Consumers appreciate a good grainy texture. Milk fat has the unique property of forming grain/crystals because of a wide variety of complex triglyceride mixtures with varying melting points. The texture of ghee depends on source of the fat (animal species), method of preparation, temperature of clarification, rate of cooling, amount of FFAs, rate of seeding, and storage temperature. Seeding with grains of ghee at the rate of 2% by weight of ghee improves grain/crystal formation. The grain shape becomes needle-like in contrast to the spherical shapes obtained without seeding. The large number of fatty acid residues present in ghee results in a wide variety of crystallization patterns. The maximum amount of solid fraction (about 74%) is obtained at 28 °C in 20–24 h from BM ghee closely followed by CM ghee (39.5%) and a distinct low in goat milk ghee (30%). There can be significant differences in the melting curves of fat from the milk of buffalo, cows, and goat. The changes in the conditions of cooling have a pronounced effect on ghee texture. If ghee is cooled rapidly, a larger number of very fine crystals will be formed, all consisting of a mixture of high- and low-melting fats, leading to smooth, grease-like character. Slow cooling of ghee from a temperature higher than the melting point will lead to the formation of few crystals with a high melting point. As cooling proceeds, more and more fat solidifies, forming a mass of large crystals suspended in liquid fat.

Ghee Color

BM ghee appears whitish in color owing to the absence of carotene, which imparts a yellow color to CM ghee. In the village method of ghee making, the development of a greenish-yellow tinge in BM ghee is caused by the action of lactic acid bacteria. Ghee produced by the direct-cream method has a brownish color compared to that prepared by the creamery butter process. Stratification results in a lighter color. A more intense heating in the presence of a high SNF content will result in a darker color, especially if the raw material has been fermented.

Ghee Residue

This refers to the charred (burned) light to dark brown residue, which is obtained on the strainer after the ghee is filtered. It is a useful by-product of the ghee industry. It is largely made up of the SNF present in butter, cream, or butter appearing in the form of small particles during the manufacture of ghee. It contains considerable amounts of milk fat, protein, and minerals. Taking an average yield of ghee residue as one-tenth the quantity of ghee produced, at present level of about 3 383 000 metric tons, the bulk of ghee residue produced per annum works out to about 338 300 metric tons. Its composition is influenced by the composition of the nonfatty constituents butter or cream, the technique adopted for the production of ghee. Essentially, it contains heat-denatured milk proteins, caramelized lactose, and varying amounts of entrapped fat, besides some mineral and water. Chemical composition of ghee residue is depicted in Table 3.

Packaging of Ghee

Ghee is susceptible to deterioration from exposure to light, air, and metals. Therefore, it is packed in tin-coated (nontoxic and nontainting) containers. Food-grade plastic containers and polyethylene pouches are also used. Ideally, ghee should be stored in a cool and dry place for long shelf life. Ghee packaged in plastic film laminates should not be exposed to light to minimize the development of oxidative rancidity. Due to large institutional (military/temples) consumption of ghee, about 60% of the country's production is packed in 15 kg tin containers. The remainder is packed in consumer size tin containers (100 ml, 200 ml, 500 ml, and 1, 2, and 5 l packs), paper/plastic stand-up packs (100 ml, 200 ml, 500 ml, and 1 l), and pillow pouches of metalized polyester and plastic polylaminated film (500 ml and 1 l size).

Table 3 Chemical composition of ghee residue[a]

Source of ghee residue	Average % fat of source	Chemical composition (%)				
		Moisture	Fat	Protein	Lactose	Ash
Desi butter (from buffalo milk)	77.0	13.4	33.4	32.8	15.4	5.2
Creamery butter (unsalted)	85.0	5.7	65.0	25.5	Trace	3.8
Sweet cream	67.0	4.1	63.2	18.0	12.3	2.4
Sour cream	67.0	8.0	38.8	41.6	7.3	4.3
Washed sweet cream	71.0	1.7	80.8	16.2	Trace	1.3

[a]Values are the means.

Source: Compiled from various sources.

Adulteration of Ghee

Ghee has an important place in Indian diet because of its good flavor, pleasant aroma, high calorific value, fat-soluble vitamins, and essential fatty acids. However, its supply falls short of demand in the lean season. Therefore, to extend the available supplies and to earn extra profits, ghee is adulterated by unscrupulous traders with cheaper oils/fats which leads to health hazards. The problem of adulteration is further complicated by the fact that the composition of ghee varies with the species, season, and the diet given to the animals.

See also: Buffalo Milk; Butter: Manufacture; Cream: Types of Cream; Dahi; Dairy Products: Dietary and Medical Importance; Fatty Acids: Essential Fatty Acids; Fatty Acids: Metabolism; Fatty Acids: *Trans* Fatty Acids; Fermented Foods: Fermented Milks; Food–Herbal Medicine Interface; Triacylglycerols: Characterization and Determination.

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Gin

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Introduction

Spirit-based beverages account for a total of 39 million hectoliters in the European Union (EU), of which 8% are jenever (genièvre) and gin. The economic importance of gin and related beverages is evidenced in the extensive exportation from certain member states (notably the United Kingdom, France, and Germany) to the rest of the world. The United States is by far the most important import market.

Gin is an alcohol-based flavored beverage in which the predominant flavor is derived from juniper. It began its evolution in Holland around 1550 when Franciscus de la Boe, a professor of medicine, tried to create a cure for stomach complaints, based on the diuretic properties of juniper berries. Today, it is a colorless beverage containing at least 37.5% alcohol. It is consumed usually mixed with tonic water, ice, and lemon, or with other beverages, such as vermouth in preparing cocktails, notably dry martinis.

According to EU legislation, flavored spirit-based beverages are divided into four categories: juniper-flavored spirit drinks, gin, distilled gin, and London dry gin. In all types, juniper must be the predominant flavor. The following article focuses on the production, raw materials, and consumption of gin worldwide.

Gin: Legislation and Definitions

Flavored spirits are usually sold between 35% and 40% alcoholic and are colorless beverages with a wide range of formulations. According to European legislation, only juniper-flavored beverages may have a lower percentage of alcohol (30%). The latter is ethyl alcohol (of agricultural origin) flavored with juniper, whereas distilled and London gins are derived from distilling ethanol (96% vol.), in the presence of juniper berries (*Juniperus communis* L.) and other botanicals, such as coriander seed, angelica root, sweet and bitter orange peel, aniseed, and licorice root to add complexity. It is necessary that these botanical materials be of high quality, despite the predominant flavor being donated by juniper. The specific mixture involved in each brand is a proprietary secret.

According to European regulations, a geographical indication is a designation that identifies a spirit-based beverage originating in a territory or country, or a region or locality within a specific country, where the given quality, reputation, or other characteristics of that beverage is essentially attributable to its geographical origin. Table 1 notes the geographical indications registered by juniper-flavored beverages. Apart from the requirements specified in European legislation, only organoleptically neutral alcohol can be used in gin production.

To date, the scientific literature on gin has focused on identifying its key aroma constituents, its sensory profile, and investigation of newer distillation technologies.

Gin Production

There are two principal gin production methodologies: those with or without distillation. Distilled gins are considered of higher quality, particularly London dry gin. The latter is considered a separate category by the EU, to assist in maintaining its high standards and protect it from counterfeit versions. Gin distillation uses a batch process involving traditional copper stills employed uniquely for each brand. In addition, the mixture of botanicals and their quantities are specific to each brand. Usually, London dry gins, produced in the UK, are exported in stainless steel tanks and bottled locally to reduce cost.

At the onset of production, water, alcohol, and botanicals are added to traditional stills (usually of 20 000 l) before heating. Botanicals could be added either loose, or in a clean cotton bag, suspended in the headspace above the liquid. Usually these are added as whole ingredients, but may also be added as a powder, or prior maceration for 24 h in alcohol. The botanicals, the quantities used, and the method of distillation are specific to each brand of gin. The rate of heating is carefully controlled (up to 70–80 °C) and the alcoholic strength closely monitored. The first and the last fractions of the distillate (head and tail, respectively) have poor quality and are combined and redistilled to recover neutral-flavored alcohol. The head may contain impurities, such as other more volatile alcohols (notably methanol) as well as small amounts of flavorants because the degree of extraction is low. On the other hand, at the end of the distillation, the alcoholic strength of the distillate tail drops, and the character changes because less volatile botanical congenerers start to dominate the sensory characteristics of this fraction. The main fraction of the distillate is appropriate for use in the generation of high-quality gin. Recent investigation has studied the value of using vacuum distillation instead of traditional heat-based distillation. The benefits of vacuum distillation include higher yields, with more monoterpene oxides possessing greater stability than other related hydrocarbons. In addition, studies on lower distillation temperature suggest improved recovery rates, and thereby economic savings.

To ensure an appropriate quality in the distillate, both chemical and sensory aspects are investigated. Because the distillate is of high alcoholic strength, it must be reduced to the required strength by dilution with water. In the United States, the final alcohol strength can be as high as 40%, whereas within the EU 37.5% is legislated.

Alternatively, and simpler, is the maceration of botanicals in flavorless alcohol for a period, followed by separation of the botanical and reduction of alcohol strength to the desired value.

Ingredients

As noted, the principal ingredients of gin are water, neutral-flavored alcohol, and botanicals. All must be of appropriate quality to produce a commercial beverage.

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Table 1 Geographic appellations of gin according to European legislation 1576/89 (2000)

Appellation	*Country of origin*
Genièvre/Jenever/Genever	Belgium, The Netherlands, France, Germany
Genièvre de grains, Graanjenever, Graangenever	Belgium, The Netherlands, France
Jonge jenever, jonge genever	Belgium, The Netherlands
Oude jenever, oude genever	Belgium, The Netherlands
Hasseltse jenever/Hasselt	Belgium
Balegemse jenever	Belgium
O' de Flander-Oost-Vlaamse Graanjenever	Belgium
Peket-Pekêt/Pèket-Pèkèt de Wallonie	Belgium
Genièvre Flandres Artois	France
Ostfriesischer Korngenever	Germany
Steinhäger	Germany
Plymouth gin	United Kingdom
Gin de Mahón	Spain
Vilniaus Džinas/Vilnius gin	Lithuania
Spišská borovička	Slovakia
Slovenská borovička Juniperus	Slovakia
Slovenská borovička	Slovakia
Inovecká borovička	Slovakia
Liptovská borovička	Slovakia

Table 2 Characteristics of neutral ethyl alcohol (of agricultural origin) as designated by European legislation

Minimum alcoholic strength	96%
Acidity (maxim)	1.5 g acetic acid/HL of 100% alcohol
Esters	1.3 g ethyl acetate/HL of 100% alcohol
Aldehydes	0.5 g acetaldehyde/HL of 100% alcohol
Higher alcohols	0.5 g methyl-2-propanol/HL of 100% alcohol
Methanol	30 g/HL of 100% alcohol
Dry extract	1.5 g/HL of 100% alcohol
Volatile bases containing nitrogen	0.1 g nitrogen/HL of 100% alcohol
Furfural	Not detectable

Neutral alcohol is the main essential ingredient in any spirit-based beverage. It must be of agriculture origin. It is usually produced by a company unconnected with beverage production. Depending on the carbohydrate source, production may involve various stages, such as cooking and mashing, as well as the obligate fermentation and distillation. The characteristics of a neutral alcohol, as defined by European legislation, are listed in Table 2.

The second, and principal, ingredient is water. It may be distilled, demineralized, softened, or permuted and must comply with all directives related to water intended for human consumption. Water is added to reduce the alcoholic strength to the legislated value. Historically, distilleries were located near a good source of quality water. With modern means of purifying water this is no longer essential.

The third essential ingredient in the gin production is natural botanicals. Of these, *Juniperus communis* is the most important. *J. communis* is a member of Cupressaceae and a native evergreen shrub found in the Northern Hemisphere, notably in Europe and North America. Juniper 'berries' are the female seed cones produced by the various juniper species. They are not true berries, but the cone of a conifer with unusually fleshy and merged scales. This property gives them a berry-like appearance. The plant grows from western Alaska, throughout Canada and the northern United States, throughout Europe, and into northern Asia and Japan, normally between 500 and 1600 m. Although it can develop into a small tree of up to 7 m, it is usually found in bush form of less than 2 m in height. The plant's more characteristic features are its needle-like leaves, with a white band on the upper face, encircling the stem in groups of three. The fruit matures in the autumn after a 2-year development period on the plant. They are a low maintenance plant to grow, and although they prefer an acidic pH soil, they can grow on nonacidic soils. Shaped like irregularly sided spheres, the berries are green at first, but ripen after 18 months to a dark, blue-purple color – ready for harvesting and use in gin production. Juniper berries are primarily used dried, as opposed to fresh, but their flavor and odor are at their strongest immediately after harvest and declines during the drying process and subsequent storage.

Juniperus berries have been associated with medicinal properties, including diuretic, hypoglycemic, anti-inflammatory, antibacterial, and antifungal attributes. Recently, it has also been shown to prevent cell division in one type of cloned human cancer lines. The fragrant and spicy aroma of juniper oil has also been used as an ingredient in perfumes and to flavor meat and other alcohol-based beverages, for example, grappa. The essential oil content of juniper berries, varying between 0.2 and 3.5%, is comprised mostly of monoterpenes (58–85%), sesquiterpenes (2–10%), and their oxidized terpenes. Of some 74 terpenes, sesquiterpenes, and oxides isolated and quantified in the headspace of juniper berries, more than 80% have been detected in brands of London dry gin. The compounds found in the highest concentrations in juniper berries are terpinen-4-ol, *p*-cymene, β-myrcene, γ- and δ-cadinene, and α- and γ-muurolene. Despite this, the aromatic profiles of separate brands differ significantly.

Coriander seed is the second most commonly used plant material in gin production. It is a member of the Umbelliferae family and is typical grown in Mediterranean countries. *Coriander sativum* L. has been used since ancient Greek times as a medicine for digestive problems and diarrhea, as well as an aphrodisiac. It also possesses antioxidant, diuretic, antidiabetic, sedative, antimicrobial, anticonvulsant, hypnotic, anthelmintic, and antimutagenic properties. Coriander seed oil is one of the major essential oils found in consumer markets because of its stability and agreeable odor. Coriander seed contains less than 1% essential oil. Its main constituent is linalool (between 50% and 80%). Its concentration is highly dependent on the growth temperature, degree of sun exposure during fruit development, and the water supply. Other constituents in coriander seed oil are geraniol, terpinen-4-ol, α-terpineol, and hydrocarbons. The presence of linalool as the main monoterpenol in some gin brands has been interpreted as suggesting the use of coriander seed in their production.

Other ingredients potentially added as supplemental sources of flavor include cardamom, angelica root, and/or

citrus peel. Cardamom (*Elettaria cardamomum*) is usually obtained from India or Central America, where it grown as a major spice crop. Its fruit and seeds are commonly used in the food and cosmetic industry as a flavoring agent. Its essential oil has also been used as a hepatoprotective and anti-inflammatory, among other medicinal uses. Another essential oil often used in flavoring gin is angelica root (*Archangelica officinalis*). The essential oil was often used for its antispasmodic, carminative, depurative, diaphoretic, digestive, diuretic, expectorant, febrifuge, nervine, stimulant, stomachic, and tonic actions. In addition its pharmaceutical uses, the essential oil has been used due to its green-spicy 'top-note' and a pleasant musky 'dry-out.' The spicy notes are attributable to its terpene hydrocarbons (notably α-pinene, β- and α-phellandrene, limonene, p-cymene, and δ-3-carene). The musty odor is attributed to the macrolides, 13-tridecanolide, 15-pentadecanolide, 16-hexadecanolide, and 17-heptadecanolide. Finally, among other possible botanicals used, citrus peel is often used to flavor gin. Terpenes are the main compounds detected in citrus oil, with limonene being the principal component.

Analytic Methods Used in Quality Control

The principal objectives of gin analysis are quality control, brand authentication, and the nature of its raw materials. The analytic methods used in distilleries are highly diverse. The sensory and chemical analyses involve primarily its three principal ingredients (alcohol, water, and botanicals). European regulation recommends specific methods for analyzing spirit-based beverages. Alcohol strength is usually determined by pycnometry, refractometry, electronic densimetry, or densimetry using a hydrostatic balance. Total dry extract is determined by gravimetry, whereas more specific analyses usually involve chromatography. The ethanol used must comply with certain requirements relative to total acidity and congeners, such as aldehyde, higher alcohol, and ethyl acetate content. The method of choice is gas chromatography (GC), coupled with detectors such as flame ionization (FID) or mass spectrometry (MSD). To measure the presence of higher alcohols, methanol, ethyl acetate, and other congeners, direct injection is used. Before chromatographic analysis was developed, extraction was needed for minor constituents, based on their volatility (headspace techniques), solubility (solvent extraction), or both.

In the other hand, analysis of the dilutent water used in gin production involves conductivity measurements and sensory analysis to ensure the absence of any odors. The presence of trace minerals, such as sodium, potassium, calcium, and magnesium, is assessed by absorption spectrophotometry.

Because traditional methods are time consuming and expensive, alternative techniques that are both faster and more economical are under investigation. These include near-infrared spectroscopy (NIR), nuclear magnetic resonance (NMR), and Fourier transform infrared spectroscopy (FTIR).

Precise characterization of gin's aromatic constituents is complicated due to their multiple and diverse chemical nature. Minor volatile compounds are usually assessed using gas chromatography. Although it may be coupled with various detectors, the most common is a mass spectrometer. They have been frequently used to identify the key aroma compounds that characterize gin. The older literature, based on liquid–liquid extraction, identified the main six terpenes and sesquiterpenes found in gin aroma. Subsequently, different terpenic markers for different brands of gin have been identified, based on α-pinene, sabinene, myrcene, limonene, and linalool contents. Recently, headspace solid-phase microextraction has been used to compare the volatile composition of several London dry gins (Figure 1). Such studies have also been used, in combination with sensory profiles, to establish their chemical origins and to suggest the types of botanicals used in gin flavoring. For example the citric attribute has been associated with gins rich in linalool and γ-terpinene. Equally, a spicy note was correlated with a model gin containing sabinene, δ-3-carene, and p-cymene.

Sensory Profile and Gas Chromatography-Olfactometry

Despite advances in instrumental and analytic procedures, the flavor sensations perceived by the consumer can be assessed only by sensory analysis. Thus, sensory analysis remains an essential tool for assessing the properties of gin. In addition to gin's predominant alcohol and juniper attributes, the botanicals used in production donate much of each brand's distinctiveness. The individual contribution of each botanical depends of its source, concentration, and interaction with other ingredients. For sensory analysis to be used effectively as a quality control tool, a clear definition of gin's quality specifications must be established and adequate methods developed to adequately evaluate these specifications.

Spirit profile is usually assessed using a small number of attributes determined by a panel of tasters. Depending on the purpose of the analysis, a wide variety of sensory test methods may be employed: discrimination tests, descriptive methods, time-intensity scaling, and consumer tests. Discrimination tests often involve the triangle test, where two products are assessed on whether they are significantly different from a third. In contrast, consumer tests are often used in early stage product development to estimate whether it is likely to be commercially successful. Nevertheless, descriptive analysis is usually the sensory method preferred in quality control assessments and used with a specifically trained panel. Descriptive analysis has the potential to provide intensity rating for a variety of attributes considered to characterize a product.

In previous studies, the number of descriptors considered adequate to characterize gin have varied from 2 to 21. Nonetheless, most researchers have confirmed the need for a more refined vocabulary and the development of reference standards for each term. In addition, the techniques used in panel training have often been inconsistent. More recently, panel members have been trained specifically for intensity ranking, using hydro-alcohol extracts of botanicals and in their precise description and recognition. Vocabulary generation sessions have been performed. Statistical methods, such as principal components analysis (PCA) and one-way ANOVA, have been used to reduce the number of terms to those that are the most discriminatory. These include juniper, citrus, aniseed, licorice, and spice. Subsequent correlation of sensory analysis with

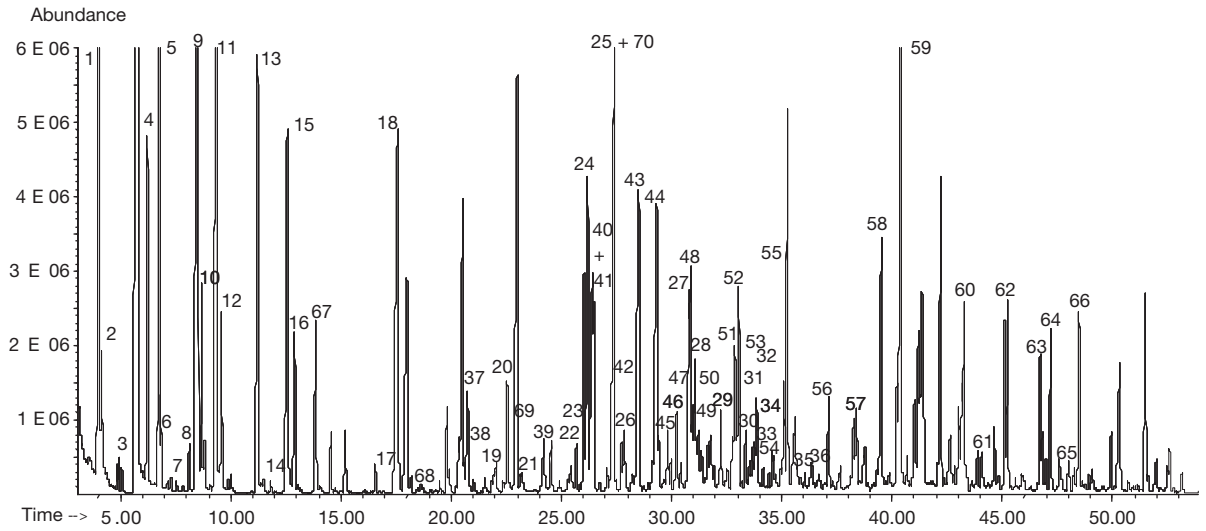

Figure 1 Volatile profile of London dry gin. 1, α-pinene; 2, α-thujene; 3, camphene; 4, β-pinene; 5, sabinene; 6, verbenene; 7, δ-3-carene; 8, l-phellandrene; 9, β-myrcene; 10, α-terpinene; 11, DL-limonene; 12, β-phellandrene; 13, γ-terpinene; 14, *t*-ocimene; 15, *p*-cymene; 16, α-terpinolene; 17, *c*-rose oxide; 18, verbenyl ethyl ether; 19, citronellal; 20, campholenal; 21, camphor; 22, linalool; 23, *c*-sabinene hydrate; 24, bornyl acetate; 25, l-4-terpineol; 26, myrtenal; 27, α-terpineol; 28, terpenyl acetate; 29, neryl acetate; 30, geranyl acetate; 31, cuminal; 32, β-citronellol; 33, myrtenol; 34, nerol; 35, *t*-carveol; 36, *t*-geraniol; 37, α-cubebene; 38, α-copaene; 39, β-cubebene; 40, *t*-β-caryophyllene; 41, β-elemene; 42, sesquiterpene; 43, γ-elemene; 44, α-humulene; 45, *t*-β-farnesene; 46, γ-muurolene; 47, sesquiterpene; 48, germacrene D; 49, α-selinene; 50, α-muurolene; 51, δ-cadinene; 52, γ-cadinene; 53, cadina-1,4-diene; 54, sesquiterpene; 55, germacrene B; 56, sesquiterpene; 57, α-calacorene; 58, sesquiterpene; 59, caryophyllene oxide; 60, torreyol; 61, elemol; 62, spathulenol; 63, *t*-cadinol; 64, *t*-muurolol; 65, eudesmol; 66, α-cadinol; 67, non identified; 68, nonanal; 69, benzaldehyde; 70, 2-undecanone.

volatile composition has shown the citrus note to be associated with γ-terpinene, linalool, and limonene, whereas α-pinene seems to be important to a juniper note.

The correlation between chemical constituents and sensory perception has to be facilitated by gas chromatography-olfactometry. Volatile compounds are separated by conventional gas chromatography with a human detecting the sensory attributes as individual compounds are released. The technique has also been used to develop a new odor classification wheel. One such wheel contains 12 super-categories: vegetable, unpleasant, spice, pungent, nut, microbiological, fruity, floral, earthy, chemical, caramelized, and woody, whereas another uses terms such as juniper, floral, fruity, spicy, licorice, vegetable, woody, and chemical.

Patterns of Consumption

The European Union is the area in the world where alcohol consumption is the highest. This may be associated with its extensive recreational use for at least 7000 years. Nonetheless, alcohol consumption and cultural preferences differ widely among individual states. Additional sources of variation include regional factors, age, gender, socioeconomic status, religion, and occupation. Average consumption is 11 l per year (based on alcohol equivalency of the various beverages). Of this, 44% is beer, 34% wine, and 23% spirits. Concern has recently been focused on the increased consumption by underage youth. Promotional campaigns to increase awareness of responsible consumption, accentuating sensory quality, and judicious social relationships are attempting to reverse inappropriate consumption of alcohol-based beverages.

The main spirits consumed in the EU and exported, amounts to about 4.6 million hectoliters (assessed by pure alcohol equivalency). Of exports, whiskey constitutes about 45%, vodka and brandy about 21% and 13%, respectively, whereas gin and jenever amount to about 8%. An indicator of the economic importance of gin is noted in the annual use of more than 79 000 t of cereal grains in gin production, being the equivalent of more than 10 million euros. The principal markets for gin consumption in the EU are the United Kingdom, Spain, the Netherlands, the Czech Republic, and Latvia, with considerable growth in Greece. Emerging markets in terms of spirit consumption include China, India, Turkey, Vietnam, Brazil, the Philippines, and Korea.

Availability, Absorption, and Metabolism – Health Effects and Clinical Studies

The main ingredient in all alcoholic beverages, including gin, after water, is ethanol. According to the scientific community 'moderate drinking' is defined as no more than 3–4 standard drinks per drinking episode, and no more than nine drinks per week for women and 12–14 for men. Epidemiological data suggest that moderate alcohol consumption is associated with reduced risk of fatal and nonfatal coronary heart disease as well as other cardiovascular disease, independent of the type of alcoholic beverage consumed. Moderate alcohol consumption is also related to a decreased risk of developing type-2 diabetes and may also reduce the likelihood of developing Alzheimer dementia. However, other researchers attribute most of the health benefits of alcohol-based beverages to their phenolic content, or their joint influences. Few studies have specifically

looked at gin in relation to health issues. Furthermore, limitations in clinical studies make it difficult to reach definitive conclusions relative to any specific beneficial effects related to gin consumption.

In contrast to the health benefits in moderate alcohol-based beverage consumption, some European countries have a serious alcohol problem. This situation is linked with enhanced morbidity and premature mortality. Excess alcohol intake is a contributing factor in about 60 diseases and conditions, including several cancers, liver disease, cardiovascular ailments, gastrointestinal problems, immunological disorders, lung disease, skeletal and muscular issues, reproductive disorders, and prenatal harm, including increased risk of prematurity and low birth weight.

See also: Alcohol: Properties and Determination.

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Glucose: Glucose Intolerance

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Introduction

Diabetes mellitus and obesity have reached epidemic proportions globally with the adoption of a Westernized lifestyle characterized by excess energy intake and inadequate physical activity. In the United States, 21.1 million people have diabetes, an increase of about 3 million over the last 2 years. Moreover, at least a quarter of people with diabetes are thought to be undiagnosed. As the prevalence of diabetes has increased, diabetes-related morbidity and mortality have become major public health issues. The 2025 worldwide projection for people with impaired glucose tolerance (IGT) is 418 million (8.1% of the adult population). Apart from the social aspects of the disease, the economic aspects are also significant: Annual diabetes-related costs in the United States are $174 billion (about 15% of national health costs), and costs related directly complications and medical care are $116 billion, while indirect costs (e.g., disability and premature mortality) amount to $58 billion.

Patients with diabetes are prone to multiple and complex complications. These complications include both macrovascular diseases (e.g., heart disease, stroke, and peripheral vascular disease) and microvascular diseases (e.g., retinopathy, neuropathy, and microalbuminuria). Epidemiological data from the last 5 years have shown complications begin early in the path from normal glucose tolerance (NGT) to diabetes. There is a time in glycemic control, between NGT and diabetes, when blood glucose levels are the harbinger of future diabetes and its complications. Thus, early detection and therapeutic intervention are crucial in reducing or delaying progression to diabetes and limiting the occurrence of related macro- and microvascular complications.

Prediabetes and Preventing Type 2 Diabetes Mellitus (T2DM)

Prediabetes is characterized by either impaired fasting glucose (IFG) with fasting blood glucose between 100 and 125 mg dl^{-1} (5.6–7 mmol l^{-1}) or IGT with fasting blood glucose within normal limits but between 140 and 199 mg dl^{-1} (~7–11 mmol l^{-1}) 2 h after an oral glucose tolerance test. These conditions may occur separately or coexist. According to a recent consensus statement of the American Diabetes Association (ADA), diagnosis of diabetes is appropriate when

- fasting blood glucose average of two measurements is >126 mg dl^{-1} (7 mmol l^{-1}),
- fasting blood glucose is >200 mg dl^{-1} (11 mmol l^{-1}) two hours after glucose load,
- blood glucose is >200 mg dl^{-1} (11 mmol l^{-1}) in random measurement, or
- HbA1c (glycated hemoglobin) is greater or equal to 6.5%.

Prediabetes constitutes an earlier stage in the evolution of type 2 diabetes, but this metabolic disorder, even in the absence of clinical symptoms, needs to be detected and treated early.

Prediabetes Check

The issue of prediabetes is of major importance, and based on diabetic standards, it is set to assume global epidemic dimensions. Worldwide, the number of people with prediabetes is about 314 million, but it is predicted that in 2025, numbers will reach 418 million. It is known that prediabetes increases short-term risk of type 2 diabetes mellitus (T2DM) from three to 10 times and some populations are at higher risk. Screening for prediabetes should be performed in populations with increased risk of diabetes such as those

- with a family history of DM,
- with cardiovascular disease,
- who are obese or have a sedentary lifestyle,
- who are hypertensive or hyperlipidemic,
- who take antipsychotic medicines,
- women with a history of gestational diabetes or polycystic ovary syndrome.

Pathophysiology of Prediabetes

Significant epidemiological differences between IFG and IGT indicate that different pathophysiological mechanisms contribute to the development of disorders in glucose homeostasis. While both conditions are associated with insulin resistance, they differ in the manifestation of insulin resistance at the tissue level. Those who have only IFG demonstrate hepatic insulin resistance and normal muscle insulin sensitivity, while those with IGT demonstrate normal or slightly reduced hepatic insulin sensitivity and moderate or severe muscle insulin resistance.

Therapeutic Intervention

Promotion of a healthy lifestyle is the option of choice for reducing incidences of pre- and type 2 diabetes, but it is challenging. Any dietary intervention must address both the quality and quantities of food consumed in all age groups and include personalized adjustments. However, recent studies have shown that meaningful intervention should also include exercise and weight loss since the benefits, especially in glucose tolerance, are limited if only one aspect is addressed. Apart from any debate about the treatment of prediabetes, according to the ADA recommendation, metformin (biguanide class antihyperglycemic drug) is recommended only when prediabetes

(IFG and IGT) coexists with an additional risk factor, such as age <60 years, BMI >30 kg m^{-2}, family history of diabetes in first-degree relatives, elevated triglycerides, reduced HDL, hypertension, or HbA1c $>6\%$.

Monitoring and Evaluating the Development of Prediabetes

The need for monitoring depends on overall risk but should include annual fasting glucose and HbA1c and oral glucose tolerance tests in those who have deteriorated. Microalbuminuria, fasting lipid concentration, and blood pressure should also be measured at least once every 12 months. In the future, biomarkers, genetic factors, and tools to measure risk of diabetes might be used to assess relative probabilities and allow more targeted monitoring and management of those with prediabetes.

In individuals with IGT, the main site of insulin resistance is muscle with only minor changes to insulin sensitivity in the liver and impaired early and late (60–120 min) insulin response during the course of the oral glucose tolerance test. Although the fasting blood glucose is not increased, there is a progressive prolonged increase in glucose during the oral glucose tolerance test, and the glucose values are higher than fasting levels after 2 h. This is supported by the finding that total glucose disposal may deteriorate gradually from NGT to IFG, IGT, and, finally, T2DM. Despite this, the clinical significance of these pathophysiological differences has not yet been established. Dysfunction of pancreatic β-cells is observed in subjects with IFG and IGT. Those who have only IFG exhibit a severely impaired early insulin response during oral glucose tolerance test, but insulin levels improve during the second phase of the test. Conversely, people who have only IGT exhibit reduced insulin secretion throughout the test.

Epidemiology

People with elevated blood glucose concentrations can be observed in both developed and developing countries. Aggregated data from 2.7 million adults, who participated in surveys for health and epidemiological investigations, suggest that mean fasting blood glucose corrected for age in 2008 was 99 mg dl^{-1} (5.5 mmol l^{-1}) for men and 97 mg dl^{-1} (5.3 mmol l^{-1}) for women. Compared with those in 1980, these values have increased by 1.8 mg dl^{-1} (0.1 mmol l^{-1}). People living in Oceania have the highest mean fasting blood glucose (110 mg dl^{-1} or 6.1 mmol for men and women). Elevated fasting blood glucose increases the prevalence of prediabetes although, in some populations, numbers with IGT have not increased despite the increase in T2DM. This might be because rates of obesity have increased, affecting fasting blood glucose (IFG) rather than values 2 h after glucose administration (IGT). The NHANES (National Health and Nutrition Examination Survey), during the period 2005–2008, assessed fasting blood glucose and HbA1c and showed that 35% of US adults older than 20 years and half of those older than 65 years were prediabetic. Extrapolating these results to the entire US population in 2010 led to the estimation that 79 million US adults are prediabetic.

The prevalence of IFG and IGT varies between different ethnic groups. Both are more prevalent in people over 40 years of age. Moreover, IFG is more common in men than in women, although the reasons behind this are not fully understood. The number of prediabetic adults (IGT) is expected to grow worldwide reaching 472 million people by 2030. Areas where the largest increases are expected are Southeast Asia and the western region of the Pacific Ocean.

It has been estimated prediabetes occurs in about 5–10% of people annually, but the incidence varies depending on the population and the diagnostic criteria. According to the ADA, up to 70% of people who are prediabetic may eventually develop diabetes. A meta-analysis of prospective studies published between 1997 and 2004 showed that the annual incidence of diabetes for those with IGT (4–6%) or IFG (6–9%) was lower than in individuals where IFG and IGT (15–19%) coexisted.

Healthy Lifestyle

Change in lifestyles, especially diet and exercise, has been shown to prevent or delay the onset of T2DM and its complications. However, proposed change in lifestyle should be personalized to strengthen implementation and compliance in the long term. Dietary management during the prediabetic stage involves significant changes in planning meals, selection and preparation of food, portion control, and eating away from home, which means most people struggle to comply. Common modifications suggested by health professionals relate to glycemic control including frequency of meals (small frequent meals), time of consumption (fixed hours), and the allocation of carbohydrates. The idea behind the smaller-sized, frequent meals at fixed hours is to help appetite control, possibly by reducing overconsumption of food during the main meal. However, the few studies investigating prediabetics, which have been published, present conflicting results.

Overall Metabolic Health

Recommended treatments to improve metabolic health include changes in diet and physical activity, which promote adipose tissue loss, enhance metabolically active skeletal muscle mass, and hence improve metabolic control. Energy-restricted diets are widely recommended for weight loss in overweight or obese individuals with poor metabolic health. However, about quarter of weight loss can be attributed to decreased skeletal muscle mass, which is undesirable because muscle is essential for mobility, maintaining and changing posture, activities of daily living, glycogen storage, etc. In addition, skeletal muscle has a major role in glycemic control accounting for up to 75% of tissue glucose uptake. Mitochondrial oxidative capacity is decreased in obese skeletal muscle as well as T2DM, possibly due to underlying physical inactivity.

Advancing age and a sedentary lifestyle are also risk factors for gradual loss of skeletal muscle mass, function, and, in turn,

muscle strength. The problem is further compounded by increased adipose tissue accumulation and myocellular lipid infiltration, which provide the basis of sarcopenic (accelerated muscle loss) obesity, and may lead to insulin resistance and increase metabolic risk. Intramyocellular lipid accumulation in obese individuals is difficult to reverse through weight loss. Activation of skeletal muscle protein anabolism is blunted in both obese and elderly people, although this may be associated with common underlying physical inactivity and insulin resistance.

Meal Frequency

There are several claims in the literature that support a correlation between eating frequency and glucose/insulin blood levels. Several mechanisms have been proposed to support an inverse relationship between frequency of meals and risk of diabetes. These are

a. reduced glycemic load resulting from the allocation of nutrients during the day;
b. suppression of free fatty acid secretion from adipose tissue, which promotes glucose disposal;
c. possible inverse correlation of insulinotropic polypeptide, which is glucose-dependent, with higher frequency of meals, whereby high meal frequency leads to less insulin secretion; and
d. potentially slower stomach emptying, fewer meals, and reduced distension of the stomach, which result in delayed movement of nutrients in the gut, so less insulin is needed to regulate blood glucose levels.

It is unclear whether long-term adherence to dietary pattern, namely, prandial high frequency, ultimately will improve glucose tolerance. Some correlation can be explained in terms of the frequency of meals and risk of diabetes. It is likely a longer period than most studies allow is needed for participants to adapt to a higher meal frequency compared with the time needed to observe changes in glucose and insulin. Moreover, the metabolic benefits of higher prandial frequency can be mitigated during weight loss, which also reduces glycemic load. More frequent meals may be beneficial to people with NGT at the start of the intervention, especially in comparison with T2DM. Furthermore, a greater number of meals (up to 16 per day) may be needed to achieve a metabolic advantage, while a lack of conformity in prandial frequency may explain in part the lack of benefits observed in epidemiological studies.

Insulin resistance was not examined in any of these studies. The effects of eating frequency on insulin resistance in T2DM or prediabetics, either in the retention phase or during weight loss, are largely unknown. There may be no effect since there are very few data suggesting a relationship between the frequency of meals and concentrations of glucose/insulin.

In two studies performed in healthy populations, observed glucose and insulin levels were flatter in the group consuming nine meals per day than those consuming three meals. However, the area under the curve (AUC) did not differ significantly between the two interventions (2 weeks apart). Moreover, a study with healthy individuals showed no significant effect of meal frequency (three meals per day against one meal per day) on glucose concentration or insulin indicators for T2DM risk. On the other hand, studies with prediabetic and diabetic individuals are largely inconclusive. Prediabetic (IGT) and type 2 diabetics consumed isocaloric diets with three and nine meals per day, respectively, for 4 weeks each. The results of this study could not confirm potential benefits of increased meal frequency in either group. Other studies that included only type 2 diabetics showed more frequent meals (between 3 and 17 meals per day) could reduce pronounced changes of glucose and insulin and improve glycemic control.

Lifestyle modification, including nutrition, is vital to manage the prediabetic state. Macro- and micronutrients, fiber, and other components of the diet modulate meal-induced insulin secretion through changes in gastrointestinal transit time and nutrient absorption rates.

Evidence to date suggests the frequency of meals has limited use as a strategy for weight management and physical health. Results from long-term randomized clinical trials are required to determine whether meal frequency should be integrated into recommendations for health promotion and disease management. Until then, it is necessary to estimate dietary habits and provide personalized diet programs. However, it is also important to note that no adverse effects have been observed from eating six meals per day. Thus, meal frequency for prediabetics could be left to personal choice provided energy balance is maintained.

High and Low Glycemic Index Diets

Glycemic index (GI) quantifies the response stimulated by carbohydrates in different foods and glycemic load (GL) is the mathematical product of the GI and carbohydrate content. GL was proposed as an indicator that reflects the response of glucose and insulin initiated by a portion of food.

Carbohydrates, as the primary component of food affecting blood glucose, by both volume and type, have a direct effect on postprandial blood glucose and insulin concentrations. There have been several studies examining difference between high GI (HGI) and low GI (LGI) diets in terms of insulin response, postprandial hyperglycemia, time challenge saturation, etc. Data obtained from short-term interventions in humans indicate that LGI meals have no significant positive effect on satiety compared with HGI meals. Generally, however, there are limited data in humans showing slow digestion of starch (i.e., LGI diet) may mitigate the postprandial blood glucose and insulin response leading to prolonged saturation compared with rapid digestion of starch from a HGI diet. However, LGI diets rich in dietary fiber (LGI-HF) are advantageous compared with HGI counterparts. The primary benefits are related to glucose metabolism after a meal and the corresponding postprandial hyperglycemia and hyperinsulinemia, especially in diabetics, compared with HGI diets. This is due to reduced regulation of glucose metabolism, particularly at the postprandial period.

HGL diets require increased insulin secretion for blood glucose to be regulated. However, there is a time point beyond which pancreatic β-cells are unable to complete this operation because of increasing insulin resistance, which can result in increased postprandial glucose and postprandial

hyperglycemia. Thus, LGI diets rich in fiber can help prevent diabetes and reduce insulin resistance, which predisposes a large group of people to diabetes.

Almonds

Frequent consumption of nuts is in alignment with the concept of an LGI diet. In addition, the content of one meal has the potential to affect insulin sensitivity at a second by altering circulating nonesterified free fatty acid concentrations and daylong insulin demands. Including almonds at breakfast decreased blood glucose concentrations and increased satiety acutely in IGT adults even after a second meal. Almonds are high in fiber and unsaturated fat and low in carbohydrate content. There is an inverse relationship between nut consumption and risk of T2DM. In addition, almond consumption increases satiety, reduces cardiovascular disease risk, and decreases postprandial glycemia and reduces oxidative damage. The lipid component of almonds appears to be responsible for the immediate postconsumption response, although it cannot account for the second-meal response. Daylong glucose, insulin, and nonesterified free fatty acid concentrations were attenuated with consumption of whole almond and almond oil, eliciting an improved hormonal profile. Importantly, the absolute magnitude of the blood glucose-lowering response equaled that achieved with acute administration of acarbose (a starch blocker used to treat T2DM and prediabetes) in IGT individuals, suggesting the findings are physiologically relevant.

Vitamin D

Over the last decades, there has been considerable scientific interest in the role of vitamin D in glucose homeostasis. Emerging evidence suggests that vitamin D (25-hydroxyvitamin D_3; calcidiol) has a role in the etiology of T2DM. Vitamin D levels are lower in people with T2DM and IGT compared with NGT. The bioavailability of cholecalciferol (unhydroxylated form of vitamin D) decreases with increasing proportion of body fat, due to preferential accumulation in the adipose tissue. In turn, imbalanced vitamin D status may reflect an increased risk of T2DM in the general population. The mechanism through which vitamin D acts on glucose metabolism is not clear, but possible mechanisms include direct effects on insulin synthesis and release or increased insulin sensitivity. A possible link between vitamin D and glucose metabolism could be the lipoprotein lipase, which is upregulated by vitamin D. Nevertheless, there is a great debate on the potential effects of vitamin D status on insulin sensitivity, as studies have variously found the correlation between vitamin D and insulin to be only modest to highly significant.

Obese patients with IGT tend to cluster more frequently with altered vitamin D status, after controlling for potential confounding factors. Data from adults showed vitamin D insufficiency is correlated with the development of IGT and an inverse correlation has been described between vitamin D concentrations and the risk of developing T2DM in a 22-year Finnish cohort study. Prospective studies revealed an inverse association between $25(OH)D_3$ levels and future glycemia and insulin resistance. Vitamin D supplementation in prediabetic obese patients has been shown to increase in $25(OH)D_3$ concentrations in association with increased insulin secretion and C-peptide concentration (C-peptide assesses insulin secretion even if insulin is injected).

Further studies are required to better define the impact of vitamin D status on changes in glucose metabolism. Furthermore, there are no data on the effect of vitamin D supplementation on glycated hemoglobin. Recently, however, poor vitamin D status has been inversely associated with fasting insulin and HOMA-IR (a method used to quantify insulin resistance and β-cell function) in obese adolescents, but the effect on HbA1c levels was not investigated.

Herbal Treatments

Recently published data suggest that some herbal medicines alone or in conjunction with current therapies may offer alternatives for preventing diabetes. In particular, a combination of ten Chinese medicinal herbs in a capsule reduced progression to T2DM in people with IGT in a randomized placebo-controlled trial in China. The key herb in the combination was Huang Lian (Coptidis Rhizoma). The critical component in this herb is berberine, which has been reported to exert potent antidiabetic effects. Huang Lian has been used traditionally for treating diabetic symptoms. Although no direct comparisons were made, the results were comparable with those of antidiabetic prescription drugs. The data also showed that, after a termination of the herbal treatment, the preventive effects on T2DM development remained significant. Given that the safety profile of this herbal medicine is satisfactory, without obvious adverse effects, this option seems promising if additional future data from large clinical trials verify these findings. However, an issue with herbal supplements remains their variation in potency. Herbal preparations are highly variable because amounts and potency of the active ingredient differ from plant to plant or even in different parts of the same plant. Thus, one capsule may not be equivalent to another, and there are currently no regulatory bodies supervising quality or distribution.

Intermittent Fasting

Several large studies have investigated lifestyle changes in prediabetics with a view to slowing or preventing progression to T2DM. However, there is considerable debate regarding the most effective manner lifestyle changes, such as whether diet and/or exercise should be implemented. Currently, intermittent fasting is generating particular interest.

Intermittent fasting can be undertaken in several ways, but the basic format alternates periods of normal caloric intake with days when caloric consumption is severely restricted. This can be done on an alternating daily basis or, more recently, on a 5:2 strategy where 2 days each week are assigned as fasting days (<600 cal for men, <500 cal for women). Importantly, this type of intermittent fasting has been shown to be as effective as or more effective than continuous modest

caloric restriction for weight loss, improved insulin sensitivity, and other biomarkers of health.

Those who undertake this form of diet are required to limit their calorie intake for 2 days each week. The calorie limit is approximately 25% of total daily energy expenditure (600 cal for men and 500 for women). On nonfasting days, individuals can eat normally up to approximately 2500 for men and 2000 for women. The majority of studies show positive effects on markers of metabolic health and body composition, in part due to the impact on metabolic tissues. In addition, caloric restriction studies undertaken in animals and humans have suggested fuel selection is altered and metabolic efficiency improved, while oxidative stress is reduced.

Research has identified several biological effects of intermittent fasting and/or caloric restriction on tissues that are central to metabolic and cardiovascular health. It is possible that short periods of fasting mobilize ectopic triacylglycerides (TAG) in nonadipose depots, reducing the detrimental effects of intramyocellular and intrahepatic TAG deposition and redistributing TAG into adipose tissue.

Original treatment regimens were based on intermittent fasting as opposed to restricted intake, a harsh regimen that challenges adherence. Despite the seemingly strict nature of fasting, intermittent fasting has, generally, a good adherence record and can cause significant reductions in body weight especially in obese individuals, suggesting this is a clinically relevant therapeutic approach. Intermittent fasting can reduce the incidence of diabetes among experimental animals, and there is evidence that fasting may also slow the progression of T2DM in obese individuals.

Early data reported a reversal of T2DM with daily caloric restriction, improved pancreatic function, and reduced occult TGA deposition. The diet limited intake to 600 cal daily, which may be too severe for many T2DM patients. Intermittent fasting may be more acceptable and still improve metabolic parameters, glucose levels, and insulin sensitivity. Indeed, intermittent fasting has been shown to achieve much of benefits seen with bariatric (weight loss) surgery, without the costs, restriction on numbers, and risks associated with surgical intervention. Whether intermittent fasting can be used as a tool to prevent diabetes, in those with IGT or IFG, or limit progression in those recently diagnosed with T2DM remains to be verified.

Fructose Intake

Chronic administration of fructose or sucrose to animals not only causes insulin resistance but also may result in T2DM. The major sources of fructose in the Western diet are soft and fruit drinks, which account for approximately 7% of caloric intake in adults and upward of 15% in adolescents. Intakes of sugar and soft drinks are higher in populations at increased risk for insulin resistance and T2DM, including African Americans, Hispanics, Native Americans, and individuals with lower incomes. A meta-analysis in 2010 concluded the intake of soft drinks is an independent predictor for the development of metabolic syndrome and/or T2DM.

Fructose increases intracellular and circulating uric acid levels due to increased nucleotide turnover and nucleotide synthesis. Elevated serum uric acid is one of the best independent predictors of T2DM and commonly precedes the development of both insulin resistance and T2DM. Furthermore, fructose increases body weight, and the mechanism of action is likely to be stimulation of hunger and blocking satiety responses.

The ability of fructose (and sucrose, which contains fructose) to stimulate food intake and reduce metabolic activity provides a mechanism for how high fructose intake may enhance weight gain and visceral fat accumulation. However, fructose and sucrose also alter fat stores and metabolism independent of excess energy intake. Although weight gain is largely controlled by overall energy intake, other aspects of metabolic syndrome can occur independent of weight gain. For example, rats fed with fructose develop fatty liver, hypertriglyceridemia, and insulin resistance when compared with rats fed with isocaloric glucose or starch-enriched diets. Indeed, hypertriglyceridemia, fatty liver, and T2DM can be induced in metabolic syndrome-prone rats with caloric restriction, provided the diet is high (40%) in sucrose. Epidemiological analysis in humans also found an association between prevalence of T2DM and sugar availability that was independent of total energy intake. The observation that uric acid can induce mitochondrial oxidative stress and fatty liver may explain how fructose induces insulin resistance.

Uric Acid

Uric acid may contribute to insulin resistance in the liver by inducing mitochondrial oxidative stress and steatosis (adipose degeneration). Uric acid also blocks the ability of insulin to stimulate vasodilatation, which is important for the delivery of glucose to the skeletal muscle. Uric acid also induces inflammation in the adipose tissue with a reduction in the production of adiponectin (hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid oxidation). Finally, uric acid may also have direct effects on the pancreatic islet cells leading to local oxidative stress and islet dysfunction.

Physical Activity

Regular physical activity and adoption of a healthy lifestyle may prevent or delay the onset of T2DM. The prediabetic state is characterized by IFG and/or IGT and is associated with a substantially elevated risk of T2DM. Thus, prevention efforts through lifestyle modification are particularly important for those exhibiting prediabetic indicators. Several large-scale prospective trials have examined the role of physical activity as part of more comprehensive lifestyle interventions in prediabetic individuals to delay or prevent transition to T2DM. The Da Qing Diabetes Prevention trial found exercise with or without dietary intervention was more effective than dietary changes alone in preventing T2DM. Other trials using multifactorial lifestyle interventions, with exercise combined with diet and/or weight loss programs, found that T2DM can be delayed or prevented effectively in the majority of prediabetic individuals. Data from several trials indicated that

lifestyle interventions are at least as effective as pharmaceuticals for most prediabetic individuals for prevention of T2DM.

In one study, a favorable effect of moderate-intensity aerobic training in older individuals was observed, driven by a subset of IGT individuals at baseline.

In a 2011 study, it was shown that metabolic adaptations to endurance exercise training were greater in magnitude among prediabetics than in normoglycemic individuals. However, traditional endurance exercise training without weight loss did not reverse completely IGT and insulin resistance in all prediabetic individuals.

The literature is mixed regarding possible relationships between changes in dietary habits and/or body composition and glucose metabolism in prediabetic individuals. Several large-scale trials have targeted IGT patients with the objective of reducing progression to T2DM. Generally, these trials have concluded the degree of weight loss with lifestyle intervention – a mixed intervention aiming to increasing energy expenditure through physical activity while reducing caloric intake – is associated with progression to T2DM. However, one of these large-scale trials (Da Qing study) compared the effects of exercise or diet alone and exercise with diet on the risk of developing T2DM and found exercise, with or without changes in dietary habits, was more effective than diet alone in preventing T2DM. Thus, the relative contributions of diet and exercise in the prevention of T2DM are not yet clear.

Recent data proposed that high-intensity interval training programs would have greater insulin-sensitizing magnitude for prediabetic individuals compared with traditional moderate-intensity programs. Whether high-intensity interval training is practical for widespread use in this population is, however, debatable.

Undoubtedly, the increasing prevalence of T2DM and obesity in recent decades is associated with increased comorbidities, including atherosclerotic macrovascular disease and premature mortality. Individuals with prediabetic hyperglycemia, such as IGT and IFG, are also at increased risk of premature cardiovascular disease, emphasizing the importance of interventions to improve glucose homeostasis in prediabetic and diabetic individuals.

See also: Fructose: Sources, Metabolism, and Health; Nuts: Health Effects; Obesity: Epidemiology of; Obesity: The Role of Diet.

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Glucose: Metabolism and Regulation

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Introduction

Glucose is the main product of carbon fixation by the photosynthetic organisms. It can be found as a monomer or polymer, including glycogen, cellulose, starch, and other complex molecules. Glucose is a vital metabolic substrate since it is an intermediate in the synthesis of many other molecules. Of pivotal significance, glucose is the first fuel used by most organisms, serving as substrate for ATP production (also under anaerobic conditions) through glycolysis and subsequent metabolic pathways (e.g., tricarboxylic acid (TCA) cycle and oxidative phosphorylation).

Breakdown of endogenous liver glycogen stores (glycogenolysis) delivers glucose to the muscles and whole-body energy stores are replenished from dietary glucose. In higher organisms, glucose comes directly from the diet or indirectly when disaccharides and polysaccharides are hydrolyzed. Glucose is taken up when it reaches target cells, stored, or further metabolized. The first step is transferred from the lumen of the small intestine to gut epithelium cells followed by transport to each organ, tissue, and cell via the circulation. These processes require that glucose cross the phospholipid bilayer cell membranes, which is mediated by specialized integral transporter proteins classified – based on functional and phylogenetic data – as sodium–glucose linked transporters (SGLTs) or facilitative glucose transporters (GLUTs).

Homeostasis aims to keep blood glucose concentrations constant within a narrow range. This is maintained by a dynamic equilibrium between glucose utilization and endogenous glucose production, which are controlled by the coordinated regulation of three processes:

1. Glucose absorption via the small intestine
2. Glucose production in the liver
3. Consumption of glucose by nearly all tissues

Hormones, metabolites including glucose, free fatty acids (FFAs), and carbon-3 compounds, such as lactate, alanine, and glycerol, regulate these processes. Insulin and glucagon are the most important hormones involved in the regulation of glucose. Most notably, insulin reduces blood glucose by increasing transport into insulin-sensitive cells (e.g., the muscles) and facilitating conversion to storage compounds, via glycogenesis and lipogenesis. Glucagon is produced in response to low glucose levels or hypoglycemia and aims to increase blood glucose by promoting glycogenolysis and gluconeogenesis. However, following a glucose-rich meal, glucagon is inhibited by hyperinsulinemia, which suppresses hepatic glucose production and helps maintain normal postprandial glucose tolerance. The bulk of glucose uptake in peripheral tissues occurs in skeletal muscles, where glucose can be used directly for energy production or stored as glycogen.

Muscles are insulin-dependent and, therefore, require insulin to activate glycogen synthase (GS), which is crucial in regulating glycogen formation. While the adipose tissue is responsible for a limited amount of peripheral glucose uptake, it has a significant contribution in total glucose homeostasis through the regulation of FFA release (which increases gluconeogenesis) from stored triglycerides, influencing insulin sensitivity in the liver and muscles. The kidneys also have an important role in glucose homeostasis, via the release of glucose into the circulation (gluconeogenesis), uptake of glucose from the circulation to cover renal energy demands, and reabsorption of glucose at the proximal tubule. The kidneys also contribute in the elimination of excess glucose through excretion in urine. While the liver does not require insulin to facilitate glucose uptake, it does need insulin to regulate glucose output. Consequently, hepatic glucose output increases when insulin levels are low. Furthermore, insulin aids storage of the majority of glucose absorbed from the diet in the form of glycogen in the liver.

In a well-fed individual, glucose is largely the only fuel for the brain. During starvation, ketone bodies (acetone, acetoacetic acid, and β-hydroxybutyric acid) are the predominant fuel. Insulin signals the fed state, stimulating the formation of glycogen, triglycerides, and proteins. Conversely, glucagon signals low blood glucose and induces degradation of glycogen and gluconeogenesis in the liver and hydrolysis of triglycerides in the adipose tissue. After a meal, the rise in blood glucose leads to increased insulin and reduced glucagon, resulting in glycogen synthesis in the muscles and liver. These phenomena are reversed when the levels of blood glucose fall, and glucose is produced by degradation of glycogen and the gluconeogenetic pathway, while fatty acids are released by hydrolysis of triglycerides. The liver and muscles use fatty acids instead of glucose to meet their energy needs, ensuring sufficient glucose is available for the brain. Metabolic changes during starvation are designed to reduce degradation of proteins. Large amounts of ketone bodies are produced in the liver from fatty acids and released into the bloodstream within a few days. After several weeks of starvation, ketone bodies are the main fuel for the brain. The reduced need for glucose decreases the degradation of muscle proteins, and thus, the probability of survival is increased.

Glycolysis and Glycogenesis

Glycolysis is a conserved metabolic pathway that converts glucose to pyruvate with a net gain of two ATP and two NADH molecules per glucose molecule. It takes place in the cytoplasm of cells in virtually all tissues. This pathway is unique in the sense that it can take place under aerobic and anaerobic conditions. Glycolysis is regulated by three enzymes:

(i) glucokinase (GCK), which provides glucose-6-phosphate (G6P); (ii) phosphofructokinase, which is inhibited by fructose-1,6-bisphosphate; and (iii) pyruvate kinase (PK), the final enzyme in this ten-step metabolic pathway.

PK is activated by its substrate and inhibited by ATP. Insulin, epinephrine/adrenaline, and glucagon also regulate PK via the phosphatidylinositol 3-kinase (PI3K) pathway, and carbohydrate-responsive element-binding protein (ChREBP) induces PK transcription in the presence of glucose. Pyruvate is further decarboxylized to acetyl-CoA and processed through the TCA cycle or utilized for *de novo* lipogenesis (DNL). The pentose phosphate pathway is an alternative for the degradation of G6P in hepatocytes, which provides cells with reducing equivalents, in the form of NADPH, an important antioxidant and cosubstrate for DNL, cholesterol, nucleotide, and aromatic amino acid syntheses. GS catalyzes the synthesis of glycogen after conversion of G6P to UDP-glucose; GS is regulated by the allosteric effector G6P and inactivated by phosphorylation. Glycogen synthase kinase 3 (GSK3) phosphorylates GS and is a downstream target for protein kinase Akt/PI3K and, thus, insulin signaling. GSK3 is a multifunctional kinase involved in cell senescence, apoptosis, and lipid metabolism via the phosphorylation of sterol regulatory element-binding protein-1c. Other protein kinases, which phosphorylate GS, are AMP-activated protein kinase (AMPK) and protein kinase A (PKA). Insulin activates glycogenesis via repression of PKA. The growing glycogen polymer is further branched by a specific glycosyltransferase (branching enzyme).

Glycogenolysis (Degradation of Glycogen to Glucose) and Gluconeogenesis (Glucose Formation)

In the fasting state, the liver supplies the body with glucose by glycogenolysis and, following prolonged fasting, gluconeogenesis. Glycogenolysis is catalyzed by glycogen phosphorylase, which cleaves glucose from the glycogen polymer to produce glucose-1-phosphate, which is converted to G6P by phosphoglucomutase. A debranching enzyme with glycosyltransferase and glucosidase activity cleaves the last four glucose monomers. Allosteric activation by AMP and phosphorylation by PKA, which is inhibited by insulin, regulate glycogen phosphorylase. Hepatic gluconeogenesis occurs during prolonged fasting episodes and begins in the mitochondria with the induction of pyruvate carboxylase in response to an abundance of acetyl-CoA. Gluconeogenesis is further regulated by allosteric and transcriptional activation of phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase, and glucose-6-phosphatase (G6Pase). Overexpression of PEPCK promotes insulin resistance in mice. Insulin inhibits PEPCK expression via Akt-mediated FoxO1 (Forkhead box protein O1) phosphorylation. This pathway is suppressed by the activation of peroxisome proliferator-activated receptor-α (PPAR-α) signaling-mediated induction of the pseudokinase tribbles homologue 3 (TRB3); TRB3 expression is associated with human insulin resistance. Other nuclear receptors (PPAR-γ coactivator-1α (PGC-1α) and farnesoid X receptor), glucagon, and glucocorticoids also affect the expression of PEPCK and G6Pase. PGC1a, HNF4a, CREBP, and PPAR-α directly and indirectly activate FoxO1, a transcriptional activator of G6Pase and PEPCK. Accordingly, PEPCK-knockout mice exhibit not only reduced gluconeogenesis but also more significantly decreased removal of TCA anions, which causes the accumulation of hepatic triglycerides and steatosis (fatty degeneration).

Role of Key Organs in Glucose Metabolism and Regulation

Brain

The brain is the largest consumer of glucose in the fasting or 'postabsorptive' state. In fact, glucose is the only fuel for the human brain, excluding periods of prolonged starvation. The brain does not store fuels and, thus, needs a continuous supply of glucose. The brain receives a constant amount of glucose, and glucose metabolism in the brain is not altered by mental activity, although local increases occur with specific activities. Glucose transport to the brain cells is mediated mainly through the glucose transporter GLUT3.

When blood glucose falls below approximately 40 mg dl^{-1} (2.2 mmol l^{-1}), glycolysis is reduced to ensure sufficient glucose for the brain. Fatty acids cannot be used by the brain for fuel: FFAs are bound to plasma albumin and, therefore, unable to cross the blood–brain barrier. During starvation, ketone bodies, produced in the liver, partially substitute glucose as fuel for the brain.

Muscle

The main fuels for muscles are glucose, fatty acids, and ketone bodies. The skeletal muscle is the major store and consumer of fatty acids as well as glucose. Glucose enters muscle cells via the GLUT4 transporter. During reduced oxygen availability or energy compromise, aerobic metabolism of glucose and fatty acids cannot proceed, and muscles rely on anaerobic metabolism of glucose to restore cellular energy status. Increased glucose uptake by the muscle is a key response to stimuli requiring rapid energy supply. Approximately three-quarters of the total glycogen in the body is stored in the muscles. Glycogen is rapidly converted to G6P for the muscle to use. Muscles, like the brain, lack G6P phosphatase and, thus, are unable to extract glucose. Instead, muscles retain glucose for use during periods of intense activity.

In an active contracting muscle, the rate of glycolysis greatly exceeds that of the TCA cycle. Pyruvate, produced under these conditions, is reduced to lactate, some of which goes to the liver where it is converted into glucose. These interconversions (known as Cori cycle) shift part of the metabolic load of the muscle to the liver. Furthermore, active muscles produce large amounts of alanine via transamination of pyruvate. Alanine, like lactate, can be converted to glucose in the liver. Muscles can absorb and transaminate branched-chain amino acids, but cannot form urea. Thus, nitrogen is released into the blood as alanine. The liver absorbs alanine, removes nitrogen, which is excreted as urea, and converts the resulting pyruvate into glucose or fatty acids. Resting muscle metabolism is quite different: the main fuel is fatty acids, which cover most energy demands.

Unlike the skeletal muscle, the cardiac muscle works almost exclusively under aerobic metabolism, as evidenced by its high number of mitochondria. Fatty acids are the main fuel for the

heart, although ketone bodies and lactate may also provide energy for the heart. The cardiac muscle has almost no glycogen reserves and will consume acetoacetate in preference to glucose.

Adipose Tissue

Triglycerides stored in the adipose tissue form a huge reservoir of metabolic fuels. The adipose tissue specializes in the esterification of fatty acids and their release from triglycerides. Adipose cells need glucose for triglyceride synthesis. Triglycerides in the liver are not employed directly by fat cells, but first hydrolyzed by an extracellular lipoprotein lipase. Processes triggered by insulin stimulate this lipase. Triglycerides are hydrolyzed to fatty acids and glycerol by intracellular lipases. Triglycerides in adipose cells are hydrolyzed and resynthesized constantly. The liver extracts glycerol, produced by hydrolysis, from the circulation. Most fatty acids produced by hydrolysis of triglycerides are reesterified in the presence of abundant glycerol-3-phosphate. Conversely, fatty acids are released into the blood if glycerol-3-phosphate is scarce, due to the lack of glucose. Thus, levels of glucose in lipocytes are a major factor in determining when fatty acids are released into the bloodstream.

Kidneys

The main function of the kidney is urine production, which acts as a vehicle for excretion of metabolic waste while maintaining the osmolality of body fluids. Daily, approximately 1–2 l of urine is produced. Water-soluble plasma components (e.g., glucose) and water are reabsorbed to prevent unnecessary and (metabolically) costly loss. The maximum capacity for glucose reabsorption (tubular maximum for glucose) corresponds to a blood glucose concentration of approximately 200 mg dl^{-1} (11 mmol l^{-1}). Glycosuria occurs once this threshold is reached and transporters are unable to reabsorb all the glucose (as in type 2 diabetes mellitus (T2DM)). Much of the reabsorbed glucose is transferred to kidney cells by the SGLT. Of the various GLUT proteins expressed in the kidneys, GLUT2 is the major transporter, releasing glucose into the circulation from that reabsorbed by SGLT proteins in the proximal tubular cells. In the case of T2DM, abnormal glucose metabolism in the liver and kidneys is observed; up to a threefold increase in renal glucose release has been measured in diabetics. Over time, the glomeruli are damaged and unable to filter blood efficiently. Thus, glomerular membranes leak protein (more than half of which is albumin) into the urine. In diabetics, the kidneys may be particularly susceptible to hyperglycemia, as these cells are unable to decrease glucose transport rates to prevent intracellular hyperglycemia in the presence of increased glucose concentrations. Importantly, during starvation and acidosis, the kidneys are an important site of gluconeogenesis and can generate up to half-blood-glucose concentrations.

Liver

Metabolic activities of the liver are necessary to supply fuel for the brain, muscles, and other peripheral organs. Most of the compounds absorbed by the intestine pass first through the liver. This enables it to regulate the levels of several metabolites in the bloodstream (e.g., glucose and folate). The liver metabolizes carbohydrates in a complex manner; it absorbs two-thirds of blood glucose and all the other monosaccharides. Other tissues use the small portion of remaining glucose. In the liver, glucose is converted to G6P by hexokinase and hepatic glucokinase. G6P has many metabolic possibilities, although the liver uses a small amount to meet its needs. Most of G6P is converted to glycogen. Excess G6P is metabolized to acetyl-CoA, which is used for fatty acids, cholesterol, and bile salts. The liver can release glucose into blood circulation by the degradation of glycogen and gluconeogenesis. The main precursors for gluconeogenesis are lactate and alanine from muscles, glycerol from adipose tissue, and glycogenic amino acids from foods.

The Three States of Glucose Homeostasis

The night starvation-feeding cycle has three phases:

1. Postabsorptive state after a meal
2. Initial period of overnight fasting
3. Refeeding state after breakfast

A major objective of the many biochemical changes during these phases is to maintain glucose homeostasis (i.e., constant appropriate level of glucose in the bloodstream).

Postabsorptive State (Well-Fed or Postabsorptive State)

After consuming and digesting the final meal of the day, glucose and amino acids are transported from the intestine into the bloodstream. Lipids are packed into chylomicrons (lipoprotein particles that consist of triglycerides, phospholipids, cholesterol, and proteins) and transported into the bloodstream by the lymph system. This results in postprandial insulin secretion from β-cells in the pancreatic islets stimulated by glucose and the parasympathetic nervous system. Essentially, insulin signals 'adequacy' and stimulates storage of fuel and synthesis of proteins. Thus, insulin stimulates glycogen synthesis in the liver and muscles and suppresses gluconeogenesis in the liver. Insulin also accelerates glycolysis in the liver, which increases the synthesis of fatty acids.

The liver also helps to limit the amount of glucose in the blood during periods of abundance, by saving it as glycogen, to be released during periods of starvation. Excess glucose is removed by the action of insulin, which accelerates uptake by the liver via GLUT2. Consequently, the liver produces G6P faster when blood glucose is increasing. The combination of increased G6P and insulin leads to increased storage of glycogen. High levels of insulin after a meal also promote uptake of glucose by the muscle and adipose tissue. Insulin stimulates glycogen synthesis in both the muscle and the liver and glucose provides glycerol 3-phosphate for triglyceride synthesis in the adipose tissue.

The Initial Period of Fasting (Early Fasting State)

The levels of glucose in the blood decrease after several hours, leading to a reduction in insulin secretion and increased

glucagon secretion. Glucagon is secreted from the alpha cells of pancreatic islets in response to low levels of blood glucose. Just as insulin signals the fed state, glucagon signals 'starvation.' It serves to mobilize glycogen reserves when there is no glucose uptake from food. The main target organ of glucagon is the liver. Glucagon induces glycogen degradation and inhibits glycogen synthesis. Glucagon also inhibits synthesis of fatty acids. Moreover, glucagon stimulates gluconeogenesis in the liver and inhibits glycolysis. The mobilization of glycogen stores, for glucose production by glucagon, is very rapid. The liver releases large amounts of glucose, formed by the G6P hydrolysis of glycogen, into the circulation. Glucose uptake by the muscle and adipose tissue is reduced in response to decreased blood insulin levels. Reduced utilization of glucose by the muscle and adipose tissue also helps maintain blood glucose levels. The net result of these actions is significantly increased glucose release into the blood from the liver.

Both the muscle and liver use fatty acids as fuel when blood glucose decreases. Thus, blood glucose remains at or above 80 mg dl^{-1} (4.4 mmol l^{-1}), due to

1. mobilization of glycogen to glucose production and release of the latter by the liver,
2. release of fatty acids from the adipose tissue,
3. switching from glucose into fatty acids for fuel by the muscle and the liver.

Depletion of glycogen stores in the liver results in gluconeogenesis using lactate and alanine sourced from peripheral tissues. Furthermore, the brain oxidizes glucose completely to CO_2 and H_2O. Thus, in order to have net glucose synthesis, another carbon source is required. Glycerol released from adipose tissue lipolysis provides a small number of carbon atoms, but the rest of the carbon required is provided via muscle proteolysis.

Refeeding State

When a hearty breakfast is consumed, fat metabolism continues as normal. However, this is not true for glucose metabolism: the liver does not initially absorb glucose from the blood, but leaves it for uptake by peripheral tissues. Furthermore, the liver continues with gluconeogenesis, but newly synthesized glucose is used to replenish glycogen reserves. While levels of blood glucose continue to increase, the liver continues to replenish diminished glycogen reserves and starts synthesizing fatty acids (lipogenesis) from excess glucose.

Prolonged Starvation

In case of prolonged starvation, fuel stores are sufficient to meet caloric needs for 1–3 months. However, carbohydrate reserves can be depleted in one day. Even under conditions of starvation, blood glucose is maintained above 40 mg dl^{-1} (2.2 mmol l^{-1}). The first priority for the metabolism during starvation is to provide sufficient glucose for the brain and other tissues (e.g., erythrocytes) that depend entirely on it for fuel. However, precursor molecules for glucose are not abundant. Most energy is stored in the triglycerides of fatty acid. The second priority for the metabolism during starvation is preservation of proteins, which is achieved by changing the predominant fuel from glucose to fatty acids and ketone bodies.

Metabolic changes on the first day of starvation are similar to those after an overnight fast. Low blood sugar leads to diminished insulin and increased glucagon secretion. The dominant metabolic processes are mobilization of triglycerides from adipose tissue and gluconeogenesis in the liver. The liver acquires energy for its needs by the oxidation of fatty acids released from adipose tissue. Glycolysis is suppressed while glucose uptake by the muscle is drastically reduced, because insulin is low, while fatty acids enter the muscle freely. Accordingly, the muscle changes almost completely from glucose for fuel to fatty acids. Pyruvate, lactate, and alanine are exported to the liver for glucose conversion. Glycerol, derived from the cleavage of triglycerides, is another resource for glucose synthesis in the liver. During starvation, degraded proteins are not replenished but serve as a carbon source for the synthesis of glucose. Primary protein sources are those recycled quickly, such as those of the intestinal epithelium and pancreatic secretions. Proteolysis of muscle proteins provides part of three carbon chains needed for synthesis of glucose.

After approximately three days of starvation, the liver synthesizes large amounts of ketone bodies and the brain begins to consume significant quantities of acetoacetate instead of glucose. About one-third of the energy needs of the brain are covered by ketone bodies. The heart also uses ketone bodies as an energy source. After several weeks of starvation, ketone bodies become the main source of fuel for the brain. Essentially, ketone bodies are the equivalent of the fatty acids, which can pass the blood–brain barrier. Efficient conversion of fatty acids to ketone bodies, by the liver, and their use by the brain reduce substantially the need for glucose. Survival time of an individual in a state of starvation is determined by the size of triglyceride reserves. Once triglyceride stocks have been exhausted, the only remaining fuel is protein. Protein degradation is accelerated and death, inevitably, occurs because of malfunction of the heart, liver, or kidney.

Regulation

Insulin is released by pancreatic β-cells prior to an increase in blood glucose concentrations. This so-called cephalic phase reflex is induced upon activation of taste receptors (and subsequently afferent projections) in the oral cavity. In addition, macronutrients in the intestine trigger duodenal K-cells and ileal L-cells to secrete incretins glucose-induced insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GUM), respectively. Both hormones stimulate insulin secretion. Besides its direct effects on glucose metabolism, insulin suppresses the secretion of glucagon by the pancreatic α-cells, thereby affecting hepatic glucose production indirectly.

Glucose thresholds for counterregulatory responses in humans are approximately 80 mg dl^{-1} (4.4 mmol l^{-1}) for glucagon, less than 68 mg dl^{-1} (3.8 mmol l^{-1}) for adrenaline and noradrenaline, and less than 66 mg dl^{-1} (3.7 mmol l^{-1}) for corticosterone and growth hormone. Glucagon secretion is stimulated during hypoglycemia and stimulates glucose production by the liver. Noradrenaline, released from

sympathetic nerve endings, and adrenaline, released from the adrenal glands, stimulate lipolysis. Glycogenolysis decreases insulin secretion and reduces insulin sensitivity.

The liver is the exclusive site of glucose production in humans during the postabsorptive state. Although animal and studies *in vitro* have documented that the kidney is capable of gluconeogenesis, glucose production by the human kidney has been regarded as negligible. This is based on net balance measurements across the kidney. However, recent studies combining isotopic and balance techniques have demonstrated that the human kidney is involved in the regulation of glucose homeostasis, making glucose via gluconeogenesis, taking up glucose from the circulation, and reabsorbing glucose from the glomerular filtrate. The human liver and kidneys release approximately equal amounts of glucose, via gluconeogenesis, in the postabsorptive state. In the postprandial state, although overall endogenous glucose release decreases substantially, renal gluconeogenesis increases by approximately twofold. Following ingestion of a meal, kidney glucose utilization increases.

Increased glucose uptake by the kidney may be implicated in diabetic nephropathy. Normally each day, the kidneys filter approximately 180 g of glucose, almost all of which is reabsorbed by means of sodium–glucose linked transporter 2 (SGLT2) expressed in the proximal tubules. However, the capacity of SGLT2 to reabsorb glucose from the renal tubules is limited and, when blood glucose exceeds the threshold, glucose appears in the urine. Renal glucose release is stimulated by adrenaline and is inhibited by insulin. Glucose handling in the kidney is altered in T2DM. Renal gluconeogenesis and glucose uptake are increased in both the postabsorptive and postprandial states, and glucose reabsorption is increased. Since renal glucose release is almost exclusively due to gluconeogenesis, it seems likely that the kidney is as important a gluconeogenic organ as the liver.

Although all cells require glucose, the brain consumes most in the fasting or 'postabsorptive' phase, accounting for approximately half the glucose uptake. A quarter of glucose consumption occurs in the splanchnic area (the liver and gastrointestinal tissue), and the remainder (25%) takes place in insulin-dependent tissues, including the muscle and adipose tissue. Approximately 85% of endogenous glucose production is derived from the liver, with glycogenolysis and gluconeogenesis contributing equally to the basal rate of hepatic glucose production. The remaining approximately 15% of glucose is produced by the kidneys.

In healthy adults, blood glucose is tightly regulated within a range of 70–99 mg dl^{-1} (3.9–5.5 mmol l^{-1}) and maintained by specific hormones (e.g., insulin, glucagon, and incretins), as well as the central and peripheral nervous system, to meet metabolic requirements. Various cells and tissues (in the brain, muscle, gastrointestinal tract, liver, kidney, and adipose tissue) are also involved in blood glucose regulation by means of uptake, metabolism, storage, and excretion.

This highly controlled process is particularly evident during the postprandial period, during which, under normal physiological circumstances, glucose levels rarely rise above 140 mg dl^{-1} (7.8 mmol l^{-1}), even after consumption of a meal rich in carbohydrates.

Amylin (islet amyloid polypeptide (IAPP)) is a 37-amino-acid peptide hormone that is cosecreted with insulin from the pancreatic β-cells in a ratio of approximately 100:1. It acts synergistically with insulin in glycemic regulation by slowing down gastric emptying and inhibiting digestive secretion (gastric acid, pancreatic enzymes, and bile ejection) and, as a result of reduced food intake, prevents postprandial spikes in blood glucose. Amylin is regulated by factors similar to insulin since they share a common regulatory promoter motif. The amylin promoter is also activated by stimuli that do not affect insulin, such as tumor necrosis factor alpha and fatty acids.

Incretins, GIP and GLP-1 (both glucose-dependent hormones secreted only when blood glucose rises above normal fasting blood glucose), are also involved in the regulation of blood glucose, in part by their affects on insulin and glucagon. Normally, these hormones are released in response to meals and stimulate insulin secretion by activating certain receptors on pancreatic β-cells. When glucose levels are low, however, GLP-1 and GIP (and their affects on insulin secretion) are diminished.

The human GLUT protein family consists of at least 14 members, indicating glucose delivery to cells is highly complex. GLUT4 is the major transporter for muscle, adipose, and cardiac tissues, whereas GLUTs 1, 2, 3, and 8 facilitate glucose entry into other organs (e.g., the brain and liver). Activation of GLUT4, and, in turn, facilitated glucose diffusion into muscle and adipose tissue, is dependent on the presence of insulin, whereas the function of other GLUTs is more independent of insulin.

While adipose tissue is responsible for much smaller amounts of peripheral glucose uptake (2–5%), it has an important role in maintaining total body glucose homeostasis by regulating release of FFA (which increase gluconeogenesis) from stored triglycerides and influencing insulin sensitivity in the muscle and liver. While the liver does not require insulin to facilitate glucose uptake, it does need insulin to regulate glucose output.

The pancreas has a remarkable capacity to adapt to conditions of increased insulin demand (e.g., in obesity, pregnancy, and cortisol excess) to maintain normoglycemia. Compensatory hyperinsulinemia also maintains glucose homeostasis. However, hyperglycemia occurs when β-cell secretion of insulin becomes inadequate for the glucose load. Progressive deterioration in β-cell function and mass over times is well known in T2DM, and the resultant state of impaired insulin secretion is found uniformly in T2DM of all ethnic backgrounds. Based on analyses from the UK Prospective Diabetes Study, a direct correlation exists between progressive loss in β-cell function and poor glycemic control (as measured by glycated hemoglobin (HbA1c)).

The exact cause of insulin resistance in any given individual is complex but may include defects in insulin-mediated cell signaling pathways, reduced insulin-stimulated muscle glycogen synthesis, or perhaps potentially fewer insulin receptors (IRs) (particularly in the muscle, liver, and adipose tissue in obese subjects).

The relative contributions of insulin secretion and insulin resistance to the development of hyperglycemia may differ. Under most circumstances, insulin resistance is the earliest detectable defect in prediabetes. Initially, enhanced insulin secretion may compensate for the insulin resistance, but early-phase insulin secretion is impaired. In the transition

from normal glucose tolerance to impaired glucose tolerance and diabetes, insulin sensitivity deteriorates by about 40%, whereas insulin secretion deteriorates three- to fivefold. In T2DM, chronic hyperglycemia may result in further deterioration of insulin sensitivity and secretion (glucotoxicity), which is worsened by elevated FFAs (lipotoxicity).

Considerable evidence implicates deranged metabolism and altered disposition of fat in the pathogenesis of glucose intolerance in T2DM. Because fat cells are resistant to the antilipolytic effect of insulin, the resulting chronic elevated plasma FFA levels stimulate gluconeogenesis, induce hepatic/muscle insulin resistance, and impair insulin secretion. These FFA-induced disturbances are referred to as lipotoxicity. Beyond this phenomenon, dysfunctional fat cells also develop insulin resistance, inducing inflammatory and atherosclerotic-provoking cytokines, and fail to secrete normal amounts of insulin-sensitizing adipocytokines (adiponectin).

The development of glucose intolerance involves multiple organs including the muscle, liver, pancreas (β-cells, insulin production, and α-cell, hyperglucagonemia), adipose tissue (accelerated lipolysis), gastrointestinal tract (incretin deficiency/resistance), kidney (increased glucose reabsorption), and brain (insulin resistance).

CNS and Glucose Regulation

The arcuate nucleus (ARC) of the hypothalamus contains two sets of neurons that express either the neuropeptide pro-opiomelanocortin (POMC) or coexpress agouti-related protein (AGRP) and neuropeptide Y (NPY). Both sets of neurons express leptin receptors (OBRs) and IRs. The actions of leptin and insulin on POMC neurons have an additive effect on the regulation of hepatic glucose production, possibly because they act on different POMC populations within the ARC. In addition, the action of insulin on neuronal populations of unknown phenotype results in the activation of ATP-sensitive potassium (KATP) channels, mammalian target of rapamycin (mTOR), and peroxisome proliferator-activated receptor-γ (PPAR-γ) to regulate hepatic glucose production. Leptin acts on PPAR-γ to regulate food intake, but it is not known whether this pathway also regulates glucose homeostasis. Thus, leptin and insulin signaling in the ARC use divergent and overlapping circuits and mechanisms. The hypothalamic ARC melanocortin system, consisting of POMC and NPY-AGRP neurons, has a key role in regulating both energy and glucose homeostases. There are also neurons within the paraventricular nucleus that are important for both energy and glucose homeostases. Within the ARC fuel-sensing systems, both mTOR and AMPK are also important for regulating energy and glucose homeostasis, whereas KATP channel activation is only important in regulating glucose homeostasis. Various molecular machineries sense the bioavailability of glucose, amino acids, and lipids as well as ATP stores. Metabolism of glucose and amino acids (leucine) via glycolytic and TCA cycle pathways results in events associated with the regulation of hepatic glucose production. In general, as nutrients become bioavailable, overall hepatic glucose production decreases.

Essentially, multiple mechanisms and hormones in many tissues interact to mediate glucose metabolism and homeostasis under different conditions. Knowledge of the fundamentals of normal glucose homeostasis is vital to understanding pathophysiological derangements that result from glucose imbalance disorders. Conditions such as insulin resistance, type 2 diabetes, and metabolic syndrome are characterized by an imbalance in glucose handling. A better comprehension of these processes should help researchers and clinicians to better manage these disorders and ameliorate clinical outcomes.

See also: Beverage: Health Effects; Carbohydrate: Digestion, Absorption and Metabolism; Cereals: Dietary Importance; Energy Metabolism; Glucose: Glucose Intolerance; Ketone Bodies.

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Glucose: Properties and Analysis

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Introduction

D-Glucose, the most abundant carbohydrate and the most abundant organic compound (if all its combined forms are considered), belongs to the class of carbohydrates called monosaccharides. Monosaccharides are carbohydrate molecules that cannot be broken down to simpler carbohydrate molecules by hydrolysis, so they are sometimes referred to as simple sugars. They can be joined together to form larger structures, namely, oligosaccharides and polysaccharides, that can be converted into monosaccharides by hydrolysis.

Glucose, also called dextrose, belongs to a group of carbohydrates known as simple sugars (monosaccharides). Glucose has the molecular formula $C_6H_{12}O_6$. It is found in fruits and honey and is the major free sugar circulating in the blood of higher animals. It is the source of energy in cell function, and the regulation of its metabolism is of great importance (gluconeogenesis). Molecules of starch, the major energy-reserve carbohydrate of plants, consist of thousands of glucose units, as do those of cellulose. Also composed of glucose is glycogen, the reserve carbohydrate in most vertebrate and invertebrate animal cells, as well as those of numerous fungi and protozoans.

Nomenclature of Glucose and Related Polymers

Glucose

Glucose was first isolated in 1747 from raisins by Andreas Marggraf. The name glucose was coined in 1838 by Jean Dumas, from the Greek word *gleucos*, which means 'sweet' or 'sugar,' and the structure was discovered by Emil Fischer around the turn of the century. The compound D-(+)-glucose or dextrose is 2,3,4,5,6-pentahydroxyhexaldehyde, more conventionally expressed as $C_6H_{12}O_6$, with a molecular weight of 180.16 kDa. Glucose is readily soluble in water in a powder form. Below 50 °C, α-D-glucose hydrate is the stable form; at 50 °C, the anhydrous form is obtained; and at higher temperatures, α-D-glucose is obtained. Glucose is also present in the diet as part of the disaccharides sucrose (glucose and fructose), lactose (glucose and galactose), and maltose (glucose).

D-Glucose is both a polyalcohol and an aldehyde. It is classified as an aldose, a designation for sugars containing an aldehyde group. The ending *-ose* signifies a sugar and- *ald* signifies an aldehyde group. Glucose has four chiral carbon atoms: C-2, C-3, C-4, and C-5. Naturally occurring glucose is designated as the D form, specifically D-glucose. It has a molecular mirror image, termed the L form, specifically L-glucose. Since each chiral carbon atom has a mirror image, there are 2^n arrangements for these atoms. Therefore, in a six-carbon aldose, there are 2^4 or 16 different arrangements of the carbon atoms containing secondary hydroxyl groups, allowing formation of 16 different six-carbon sugars with an aldehyde end. Eight of these belong to the D-series; eight are their mirror images and belong to the L-series.

All sugars that have the hydroxyl group on the highest numbered chiral carbon atom (C-5 in case of glucose) positioned on the right-hand side are arbitrarily called D-sugars, and all with a left-hand-positioned hydroxyl group on the highest numbered chiral carbon atom are designated as L-sugars. Two structures of D-glucose in its open-chain, acyclic form (called the Fischer projection) with the carbon atoms numbered in the conventional manner are given in Figure 1. In this convention, each horizontal bond projects outward from the plane of the page and each vertical bond projects into or below the plane of the page. It is customary to omit the horizontal lines for covalent chemical bonds to the hydrogen atoms and hydroxyl groups as in the structure on the right. Because the lowermost carbon atom is nonchiral, it is meaningless to designate the relative positions of the atoms and groups attached to it. Thus, it is usually written as $-CH_2OH$. D-Glucose and all other sugars containing six carbon atoms are called hexoses, the most common group of aldoses. The categorical names are often combined, with a six-carbon atom aldehyde sugar being termed an aldohexose.

L-Sugars are less numerous and less abundant in nature than are the D forms but nevertheless have important biochemical roles. Two L-sugars found in foods are L-arabinose and L-galactose, both of which occur as units in carbohydrate polymers (polysaccharides).

Glucose Oligosaccharides

Oligosaccharides (Greek *oligo*, 'few') are sugar polymers; the term usually refers to compounds containing 2–9 units but may include polymers containing up to 19 units. The dimer, trimer, and tetramer forms in which glucose molecules are joined by (1–4) linkages are referred to as maltose, maltotriose, and maltotetraose, respectively, since these substances are the products of starch digestion in the malting process. Sucrose, maltose, and lactose are common dietary disaccharides.

Starches

Starches are large-molecular-weight, α-linked polymers of glucose $(C_6H_{10}O_5)_n$. Most starches show a mixture of α(1–4) and α(1–6) linkages. The α(1–4)-linked polymer forms a linear structure that allows for hydrogen bonding between polymer chains and a more compact starch structure. Introduction of (1–6) linkages results in branch points and a more open structure that allows the (1–4)-linked backbone with the hemiacetal bond in the alpha configuration to coil like a spring into a helical form. Branched starches with the (1–6) linkage are more readily hydrated and digested compared to the (1–4)-linked linear starch. The (1–4)-linked starches are referred to as

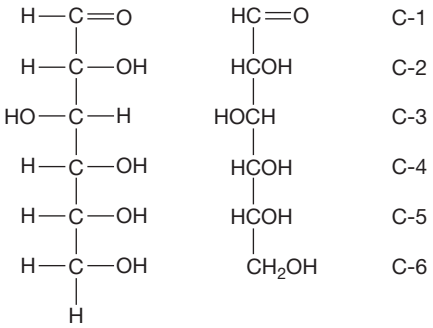

Figure 1 Structure of D-glucose (open-chain structure).

amylose starch, and (1–6)-linked starches are amylopectin starches.

Resistant Starch

Resistant starches are defined by their resistance to digestion in the human upper gastrointestinal tract. As with the term 'dietary fiber,' the definition is largely physiological. One proposed classification divides resistant starches into three classes: RS_1, RS_2, and RS_3. The first class, RS_1, is starch that escapes small intestinal digestion owing to the food form and incomplete enzymatic attack (e.g., large particle size or compact nature of food or starch entrapment by dietary fiber). The second, RS_2, includes the more crystalline starches that resist digestion (e.g., high-amylose starches that resist gelatinization). The RS_3 starches are retrograded starches (e.g., high-amylose starches that upon cooling after cooking form a compact, hydrogen-bonded crystalline structure that excludes water).

Cellulose

Like starch, cellulose is a (1–4)-linked glucose polymer $(C_6H_{10}O_5)_n$, but in this instance, the glucose molecules are β-linked, allowing the development of a linear polymer with strong intrachain hydrogen bonding. Cellulose polymers may consist of as many as 10 000 glucose monomer units. Cellulose is both resistant to small intestinal digestion and insoluble in cold or hot water and most dilute acids and alkali. It is partially degraded by colonic bacteria; the proportion degraded is dependent on the source, with cellulose from vegetables broken down to a greater extent than cellulose from cereals such as wheat.

β-Glucans

In many ways, these predominantly (1–4)-linked glucose polymers are the cellulose equivalent of the starch amylopectin. Here, it is the (1–3) linkages interspersed throughout the polymer that prevent the compact structure achieved with the cellulose polymer where only the (1–4) linkages exist. As a result of the more open molecular structure of the β-glucan, unlike cellulose, it is readily hydrated and soluble in water, forming a solution of high viscosity. The viscosity, in turn, is dependent on the molecular weight and the presence of the (1–3) linkages. The greater the molecular weight, the greater the viscosity. Thus, reduction of molecular weight by acid or enzymatic hydrolysis, which may also occur during food processing, may greatly reduce viscosity. The common feature shared by cellulose and the β-glucans is that both are resistant to digestion by small intestinal enzymes. However, whereas cellulose is only partially fermented by the colonic bacteria, β-glucans are completely fermented.

Hemicellulose

The term 'hemicellulose' should not be taken to imply a class of (1–4)-linked glucose polymers. The similarity with cellulose lies not in the chemical structure but in the fact that hemicellulose is also insoluble in hot or cold water or hot dilute acid. It is, however, soluble in dilute alkali. The polymeric structure is heterosaccharitic with two or more sugars (e.g., arabinoxylans found in cereals), with a relatively small molecular size (50–200 saccharide units).

Occurrence

Glucose is the primary carbohydrate energy source of vertebrates. In healthy humans, fasting blood glucose levels are approximately 3.5–5.5 mmol l^{-1} (depending on the laboratory) and increase postprandially to values considerably < 10 mmol l^{-1} (the renal threshold for complete reabsorption, above which glucose 'spills' over into the urine). Blood levels higher than 7.8 mmol l^{-1} 2 h after a 75 g glucose load are one of the diagnostic criteria for diabetes. Glucose is stored as glycogen, an α-linked polymer, predominantly in the liver and muscles ('animal starch'). On average, a 70 kg man may store 500 g of glycogen. Glucose can also be synthesized *de novo* by gluconeogenesis from the gluconeogenic amino acids lactate, glycerol, and pyruvate. Breakdown of glycogen into glucose is known as glycogenolysis.

Erythrocytes, renal tissue, and nervous tissue require glucose as an energy source. In erythrocytes and renal tissue, the glucose is not oxidized but is returned to the liver as part of the Cori cycle for glucose synthesis. The brain oxidizes glucose and requires 140 g day^{-1}. Despite this requirement, carbohydrate is still recommended to comprise between 45% and 65% of dietary calories. Glucose is present in fruit and vegetables, and although less sweet on a per gram basis than fructose or sucrose, it is responsible together with fructose and sucrose for the sweet taste of vegetables and fruit. With the exception of fruit such as green banana, seeds (grain and dried legumes), and tubers, in which starch is the major carbohydrate form, foods containing glucose, fructose, and sucrose in various ratios comprise the major available (i.e., absorbable in the small intestine) carbohydrate sources.

Physical Properties of Glucose

Glucose contains alcohol, ester (in the ring form), and aldehyde (in the linear form) as the functional groups, which are responsible for the majority of the chemistry that glucose undergoes. Some of the physical characteristics of glucose, such as melting point, solubility, and density, are listed in Table 1.

Structure

Glucose ($C_6H_{12}O_6$) contains six carbon atoms and an aldehyde group and is therefore referred to as an aldohexose. The glucose molecule can exist in an open-chain (acyclic) and ring (cyclic) form (in equilibrium), the latter being the result of an intramolecular reaction between the aldehyde C atom and the C-5 hydroxyl group to form an intramolecular hemiacetal. As the ring contains five carbon atoms and one oxygen atom, which resembles the structure of pyran, the cyclic form of glucose is also referred to as glucopyranose. In this ring, each carbon is linked to a hydroxyl side group with the exception of the fifth atom, which links to a sixth carbon atom outside the ring, forming a CH_2OH group. The formation of pyranose and furanose rings (cyclic hemiacetals) of glucose has been presented in Figure 2.

Table 1 Physical properties of glucose

Physical properties	*Characteristics*
Appearance	White, crystalline
Molecular weight	180.16 g mol^{-1}
Melting point	150 °C
Density	1.5620 g cm^{-3} (at 18 °C)
Solubility in:	
Water	Very soluble
Ethanol	Slightly soluble
Ethyl ether	Insoluble
Pyrimidine	Soluble

Solubility

One of the important physical properties of glucose is its ability to dissolve in aqueous, that is, water-based, solution. Since the blood, the extracellular fluid, and the fluid inside cells are all water-based, for glucose to dissolve in the bloodstream and be able to pass into cells, it must also dissolve well in water. Compounds consisting of only carbon and hydrogen show very poor water solubility. Elements such as oxygen and nitrogen, which are common in organic and bioorganic molecules, help increase water solubility. Glucose's six oxygen atoms increase its ability to dissolve in water, In particular, since five of those oxygen atoms are found in the form of alcohol groups, which allows it to readily form hydrogen bonds with water molecules and makes it very water-soluble, glucose dissolves quite readily in water.

All forms of glucose are colorless and easily soluble in water, acetic acid, and several other solvents. They are only sparingly soluble in methanol and ethanol. Table 2 shows the change in solubility with changing temperature for glucose in water.

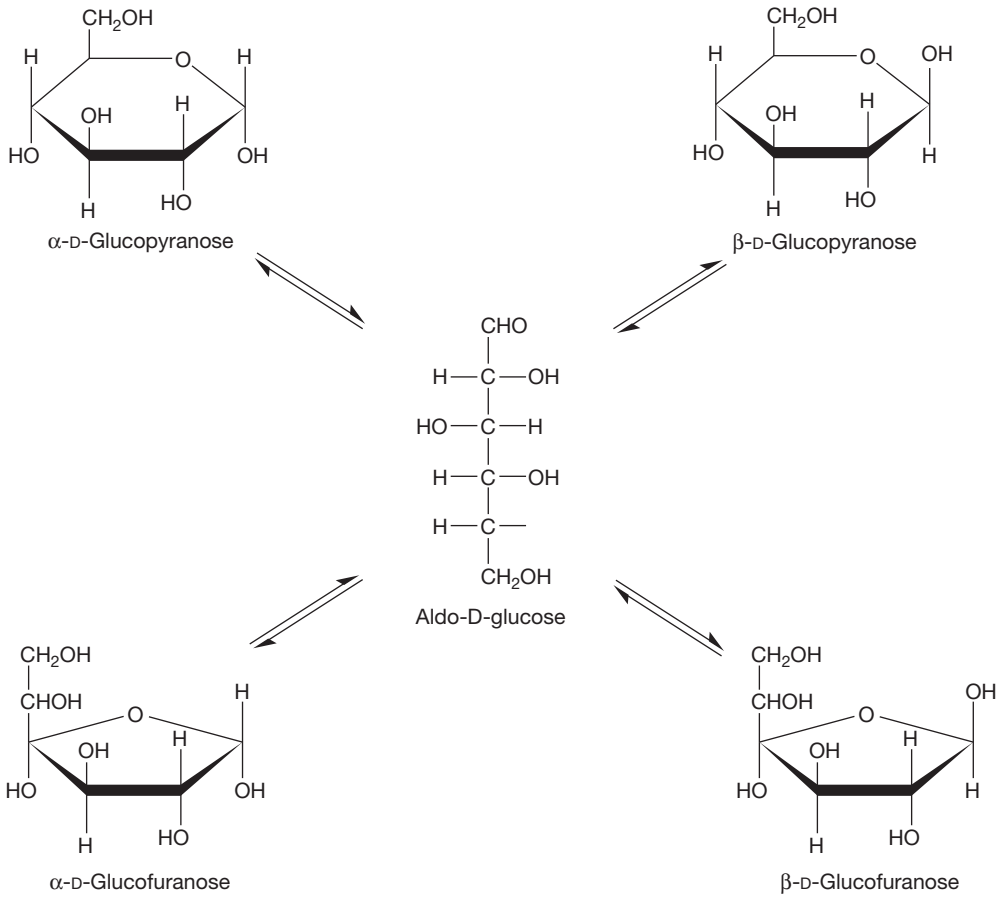

Figure 2 Pyranose and furanose rings (cyclic hemiacetals) of glucose.

Solid State

Depending on conditions, three major solid forms of glucose can be crystallized from water solutions: α-glucopyranose, β-glucopyranose, and β-glucopyranose hydrate.

Mutarotation

The open-chain form is thermodynamically unstable, and it spontaneously isomerizes to the cyclic forms. When sugar molecules are dissolved in aqueous solutions, a series of reactions, involving molecular rearrangements around the C-1, take place. These rearrangements are associated with the change in optical rotation and lead to the formation of a mixture of products that are in equilibrium. This process, first observed for D-glucose, is called mutarotation.

If one dissolves α-D-glucopyranose ($[\alpha]_D$ +112°) or β-D-glucopyranose ($[\alpha]_D$ +19°) in water, an equilibrium is formed with the $[\alpha]_D$ of the resultant solution being +52.7°. Theoretically, the mixture contains five different structural forms of glucose: α-D-glucopyranose, β-D-glucopyranose, α-D-glucofuranose, β-D-glucofuranose, and open-chain free aldehyde (Figure 2). The four-ring structures are transformed into each other via the open-chain form. The process will take place if the starting material represents any of the five forms. The mutarotation process is slow (it may take several hours to reach equilibrium) if conducted in water at 20 °C. The rate of mutarotation increases, however, 1.5–3 times with each 10 °C increase in the temperature. Both acids and bases increase the rate of mutarotation. Certain enzymes, such as mutarotase, will also catalyze the mutarotation reactions. The rate and the relative amount of products are also affected by the polarity of the solvent, with less polar solvents decreasing the rate of mutarotation. The reaction begins upon dissolution of sugar molecule and an attack, by either acid or base, on the cyclic sugar. It involves the transfer of a proton from an acid catalyst to the sugar or the transfer of proton from the sugar to a base catalyst as shown in Figure 3.

Table 2 Solubility of glucose

Temperature (°C)	*Solubility in grams of glucose per 100 ml of water*
25	91
30	125
50	244
70	357
90	556

Enolization and Isomerization

In the presence of alkali, sugars are relatively easily interconverted. The transformation involves epimerization of both aldoses and ketoses as well as aldose–ketose isomerization. The mechanism of the reaction is shown in Figure 4. The enolization reaction is a general reaction of a carbonyl compound having an α-hydrogen atom. Starting with *aldehydo*-D-glucose, the 1,2 enediol is first formed, which can be converted into another aldose (with opposite configuration at C-2) and the corresponding ketose. Therefore, by enolization and isomerization, D-glucose, D-mannose, and D-fructose can be easily interconverted. Either a base or an enzyme catalyzes isomerization, and it will also occur under acid or neutral conditions, although at a much slower rate.

Optical Activity

Whether in solution or in the solid form, D-glucose is dextrorotatory, meaning it will rotate the direction of polarized light clockwise. The effect is due to the chirality of the molecules, and indeed, the mirror-image isomer, L-glucose, is levorotatory (rotates polarized light counterclockwise) by the same amount.

The D- prefix does not refer directly to the optical properties of the compound. It indicates that the C-2 chiral center has the same handedness as that of D-glyceraldehyde (which was so labeled because it is dextrorotatory). The fact that D-glucose is dextrorotatory is a combined effect of its four chiral centers,

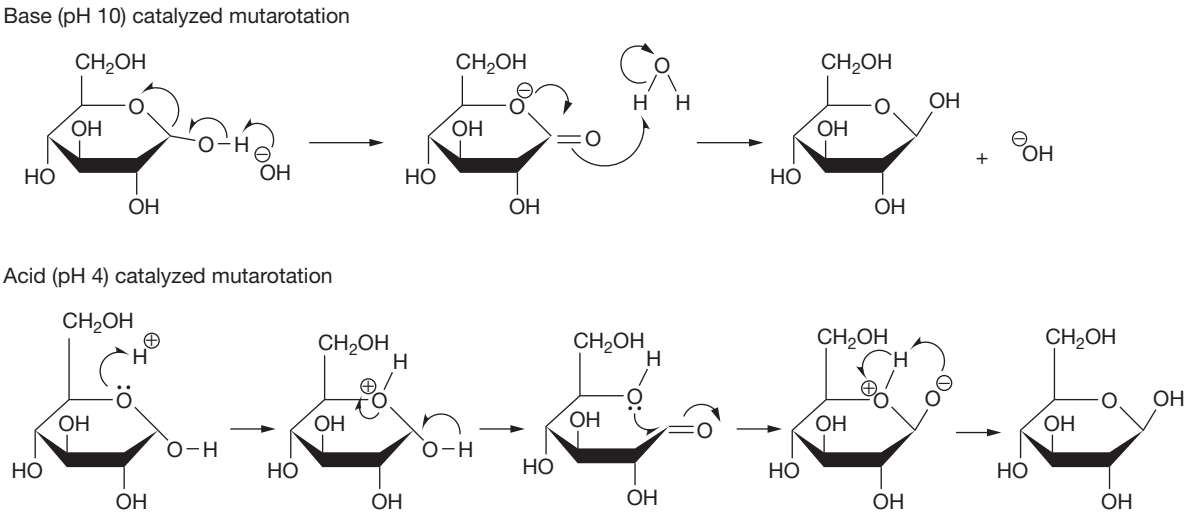

Figure 3 Mechanisms of base- and acid-catalyzed mutarotation reactions.

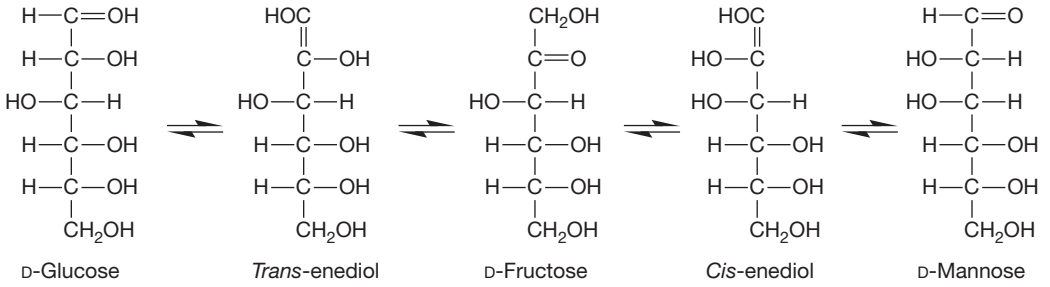

Figure 4 Enolization and isomerization reactions.

not just of C-2; and indeed, some of the other D-aldohexoses are levorotatory.

Chemical Properties of Glucose

All carbohydrate molecules have hydroxyl groups available for reaction. Glucose and most other low-molecular-weight carbohydrate molecules also have carbonyl groups available for reaction.

Oxidation of Glucose to Produce Sugar Acids

One of the earliest methods for quantitative measurement of glucose employed Fehling solution. Fehling solution is an alkaline solution of copper(II) that oxidizes an aldose to an aldonate and in the process is reduced to copper(I), which precipitates as brick-red Cu_2O. Variations, the Nelson–Somogyi and Benedict reagents, are still used for determining amounts of reducing sugars in foods and other biological materials.

Glucose is known as a reducing sugar, because in the process of oxidizing the aldehyde group of glucose to the salt of a carboxylic acid group, the oxidizing agent is reduced.

Ketoses are also termed as reducing sugars because, under the alkaline conditions of the Fehling test, ketoses are isomerized to aldoses. Benedict reagent, which is not alkaline, will react with aldoses but not with ketoses.

Glucose, when oxidized under different conditions, forms (i) gluconic acid, (ii) glucaric acid, and (iii) glucuronic acid as indicated in Figure 5.

Gluconic acid

Glucose is readily oxidized to gluconic acid. The reaction is commonly used for quantitative determination of glucose. Oxidation of glucose with Br_2–water converts the aldehyde (–CHO) group to a -COOH group. Br_2 reacts with water to form hypobromous acid, which acts as an oxidizing agent:

$$\text{D-Glucose} \xrightarrow[H_2O]{Br_2} \text{D-gluconic acid}$$

Another simple and specific method for quantitative oxidation of D-glucose to D-gluconic acid uses the enzyme glucose oxidase, with the initial product being the 1,5-lactone (an intramolecular ester) of the acid (Figure 6). The reaction is commonly employed to measure the amount of D-glucose in foods and other biological materials, including the D-glucose level of blood. D-Gluconic acid is a natural constituent of fruit juices and honey. The reaction given in Figure 3 is also used for the manufacture of commercial D-gluconic acid and its lactone. D-Glucono-delta-lactone (GDL), D-glucono-1,5-lactone according to systematic nomenclature, hydrolyzes to completion in water in about 3 h at room temperature, effecting a decrease in pH. Its slow hydrolysis, slow acidification, and mild taste set GDL apart from other food acidulants. It is used in meats and dairy products but particularly in baked goods as a component of chemical leavening agents for preleavened products.

Glucaric acid

Oxidation of glucose with conc. HNO_3 under proper conditions converts both aldehyde and primary alcohol groups to –COOH groups, forming a dibasic sugar acid, saccharic or glucaric acid:

$$\text{D-Glucose} \xrightarrow{\text{conc. } HNO_3} \text{D-Glucaric acid}$$

D-Glucaric acid is a naturally occurring aldaric acid, typically found in small amounts in a variety of fruits and vegetables. Potential commercial applications for D-glucaric acid include use as a metal sequestering agent, retarding agent for metallic mordants in the dyeing of textiles, and corrosion inhibitor for metals. A current commercial use of a salt form of D-glucaric acid as a dietary supplement claimed to help maintain healthy cholesterol levels and prevent cancer. As a dietary supplement, D-glucaric acid functions as a precursor to the β-glucuronidase inhibitor and D-glucaro-1,4-lactone. In addition to these end uses, D-glucaric acid is the starting material for the synthesis of other chemicals, particularly as a diacid monomer used to make a number of polyhydroxypolyamides.

Glucuronic acid

The terminal carbon atom (at the other end of the carbon chain from the aldehyde group) of a monosaccharide unit of an oligo- or polysaccharide may occur in an oxidized (carboxylic acid) form. Such an aldohexose with C-6 in the form of a carboxylic acid group is called as uronic acid.

When glucose is oxidized in such a way that the primary alcohol group is converted to –COOH group, without oxidation of aldehyde group, a glucuronic acid is formed:

$$\text{D-Glucose} \rightarrow \text{D-Glucuronic acid}$$

In the body, D-glucuronic acid is formed from glucose in the liver by uronic acid pathway, an alternative pathway for glucose oxidation. It occurs as a constituent of certain mucopolysaccharides. In addition, it conjugates toxic substances,

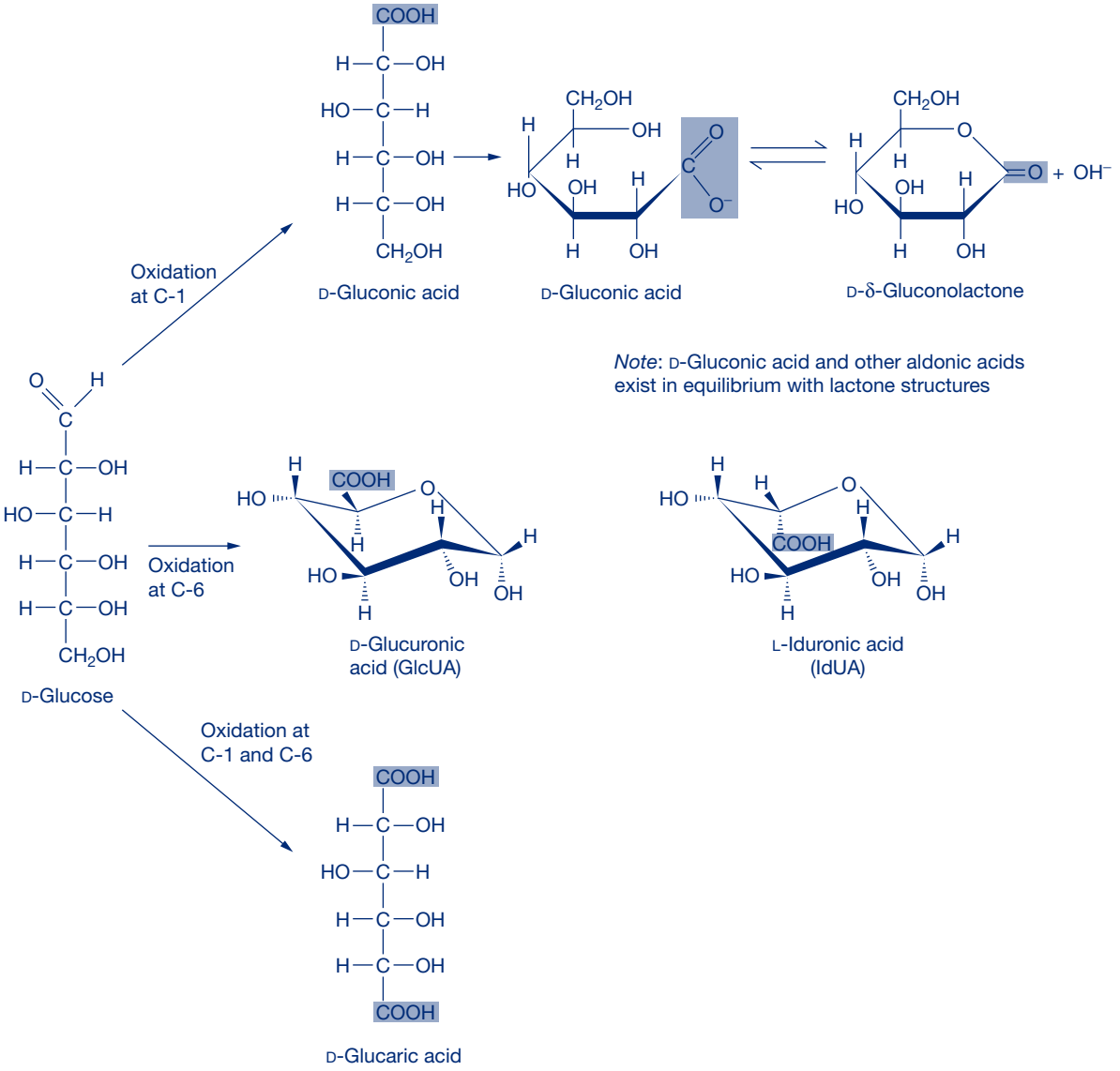

Figure 5 Oxidation of D-glucose to produce sugar acids.

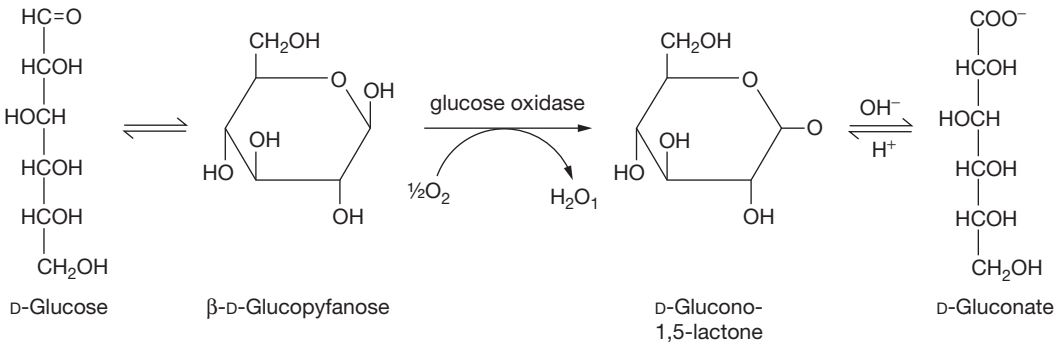

Figure 6 Oxidation of D-glucose catalyzed by glucose oxidase.

drugs, hormones, and even bilirubin (a breakdown product of Hb) and converts them to a nontoxic substance, glucuronide, which is excreted in urine.

Reduction of Glucose to Produce Sugar Alcohols

Hydrogenation is the addition of hydrogen to a double bond. When applied to carbohydrates, it most often entails addition of hydrogen to the double bond between the oxygen atom and the carbon atom of the carbonyl group of an aldose or ketose. Hydrogenation of D-glucose is easily accomplished with hydrogen gas under pressure in the presence of Raney nickel. The product is D-glucitol, commonly known as sorbitol, where the -itol suffix denotes a sugar alcohol (an alditol) (Figure 7). Alditols are also known as polyhydroxy alcohols and as polyols. Because it is derived from a hexose, D-glucitol (sorbitol) is specifically a hexitol. It is found widely distributed throughout the plant world, ranging from algae to higher orders where it is found in fruits and berries, but the amounts present are generally small. It is 50% as sweet as sucrose, is sold both as syrup and as crystals, and is used as a general humectant.

Formation of Hydroxyl Group Esters

The hydroxyl groups of carbohydrates, like the hydroxyl groups of simple alcohols, form esters with organic and some inorganic acids. Reaction of hydroxyl groups with a carboxylic acid anhydride or chloride (an acyl chloride) in the presence of a suitable base produces an ester.

Acetates, succinate half-esters, and other carboxylic acid esters of carbohydrates occur in nature. They are found especially as components of polysaccharides. Sugar phosphates are common metabolic intermediates (Figure 8). Monoesters of phosphoric acid are also found as constituents of polysaccharides. For example, potato starch contains a small percentage of phosphate ester groups. Cornstarch contains even less. In producing modified food starch, cornstarch is often derivatized with one or the other or both mono- and distarch ester groups. Other esters of starch, most notably the acetate, succinate, and substituted succinate half-esters, and distarch adipates are in the class of modified food starches. Sucrose fatty acid esters are produced commercially as emulsifiers. Members of the family of red seaweed polysaccharides, which includes the carrageenans, contain sulfate groups (half-esters of sulfuric acid, R-OSO_3^-).

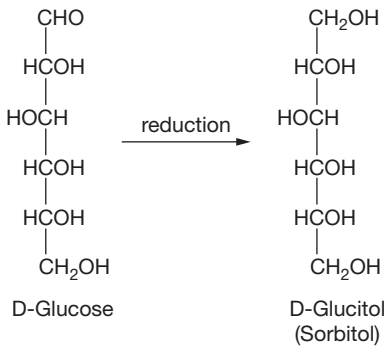

Figure 7 Reduction of D-glucose.

Formation of Hydroxyl Group Ethers

The hydroxyl groups of carbohydrates, like the hydroxyl groups of simple alcohols, can from ethers as well as esters. Ethers of carbohydrates are not as common in nature as are esters. However, polysaccharides are etherified commercially to modify their properties and make them more useful. Examples are the production of methyl (–O–CH_3), sodium carboxymethyl (–O–CH_2–$CO_2^-Na^+$), and hydroxypropyl (–O–CH_2–CHOH–CH_3) ethers of cellulose and hydroxypropyl ethers of starch, all of which are approved for food use. A special type of ether, an internal ether formed between carbon atoms 3 and 6 of a D-galactosyl unit, is found in the red seaweed polysaccharides, specifically agar, furcellaran, kappa-carrageenan, and iota-carrageenan. Such an internal ether is known as a 3,6-anhydro ring, whose name derives from the fact that the elements of water (HOH) are removed during its formation.

A series of nonionic surfactants based on sorbitol (D-glucitol) are used in foods as water-in-oil emulsifiers and as defoamers. They are produced by esterification of sorbitol with fatty acids. Cyclic dehydration accompanies esterification (primarily at a primary hydroxyl group, i.e., C-1 or C-6) so that the carbohydrate (hydrophilic) portion is not only sorbitol but also its mono- and dianhydrides (cyclic ethers). The products are known as sorbitan esters (Figure 9). The product called sorbitan monostearate is actually a mixture of partial stearic ($C_{18:0}$) and palmitic ($C_{16:0}$) acid esters of sorbitol (D-glucitol), 1,5-anydro-D-glucitol (1,5-sorbitan), 1,4-anhydro-D-glucitol (1,4-sorbitan), both internal (cyclic) ethers, and 1,4:3,6-dianhydro-D-glucitol (isosorbide), an internal dicyclic ether. Sorbitan fatty acid esters, such as sorbitan monostearate, sorbitan monolaurate, and sorbitan monooleate, are sometimes modified by reaction with ethylene oxide to produce so-called ethoxylated sorbitan esters, nonionic detergents for food use.

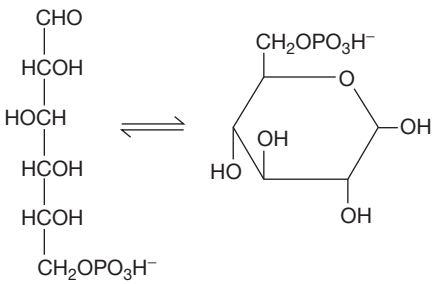

Figure 8 Example of sugar phosphate metabolic intermediate (D-glucose 6-phosphate).

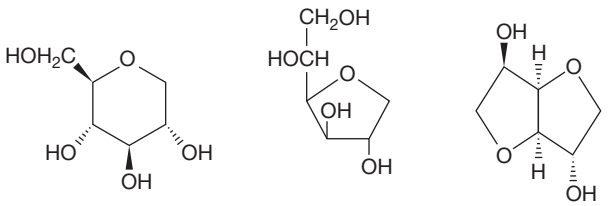

Figure 9 Anhydro-D-glucitols (sorbitans).

Nonenzymatic Browning

Under some conditions, reducing sugars produce brown colors that are desirable and important in some foods. Other brown colors obtained upon heating or during long-term storage of foods containing reducing sugars are undesirable. Common browning of foods on heating or on storage is usually due to a chemical reaction between reducing sugars, mainly D-glucose, and a free amino acid or a free amino group of an amino acid that is part of a protein chain. This reaction is called the Maillard reaction. It is also called nonenzymatic browning to differentiate it from the often rapid, enzyme-catalyzed browning commonly observed in freshly cut fruits and vegetables, such as apples and potatoes.

When aldoses or ketoses are heated in solution with amines, a variety of reactions ensue, producing numerous compounds, some of which are flavors, aromas, and dark-colored polymeric materials, but both reactants disappear only slowly. The flavors, aromas, and colors may be either desirable or undesirable. They may be produced by frying, roasting, baking, or storage.

The reducing sugar, D-glucose, reacts reversibly with the amine to produce a glycosylamine, as illustrated in Figure 10. This undergoes a reaction called the Amadori rearrangement to give a derivative of 1-amino-1-deoxy-D-fructose. Reaction continues especially at pH 5.0 or lower, to give an intermediate that dehydrates. Eventually, a furan derivative is formed, which is 5-hydroxymethyl-2-furaldehyde (HMF) (Figure 11). Under less acidic conditions (higher than pH 5.0), the reactive cyclic compounds (HMF and others) polymerize quickly to a dark-colored, insoluble material containing nitrogen.

Maillard browning products, including soluble and insoluble polymers, are found where reducing sugars and amino acids, proteins, and/or other nitrogen-containing compounds are heated together, such as in soy sauce and bread crusts. Maillard reaction products are important contributors to the flavor of milk chocolate. The Maillard reaction is also important in the production of caramels, toffees, and fudges, during which reducing sugars also react with milk proteins. D-Glucose undergoes the browning reaction faster than does D-fructose. Application of heat is generally required for nonenzymatic browning.

While Maillard reactions are useful, they also have a negative side. The reaction of reducing sugars with amino acids destroys the amino acid. This is of particular importance with L-lysine, an essential amino acid whose ε-amino group can react when the amino acid is part of a protein molecule. Also, a relationship has been found between the formation of mutagenic compounds and cooking of protein-rich foods. Mutagenic heterocyclic amines have been isolated from broiled and fried meat and fish and from beef extracts.

Analysis of Glucose

The analysis of glucose may involve chemical, enzymatic, electrochemical, and high-performance liquid chromatography (HPLC) systems. Prior to the introduction of enzyme-based analyses, chemical techniques were based on the reducing ability of glucose, and techniques employing copper sulfate were popular. Such techniques were influenced by other reducing sugars and reducing substances, including uric acid and vitamin C. With the introduction of the more specific glucose oxidase-based tests, the chemical tests were abandoned, although there was debate over the potential carcinogenicity of the early chromogens, *O*-dianisidine and *O*-toluidine. Later, more specific, hexokinase-based enzyme assays were introduced. Current methods for rapid determination of blood

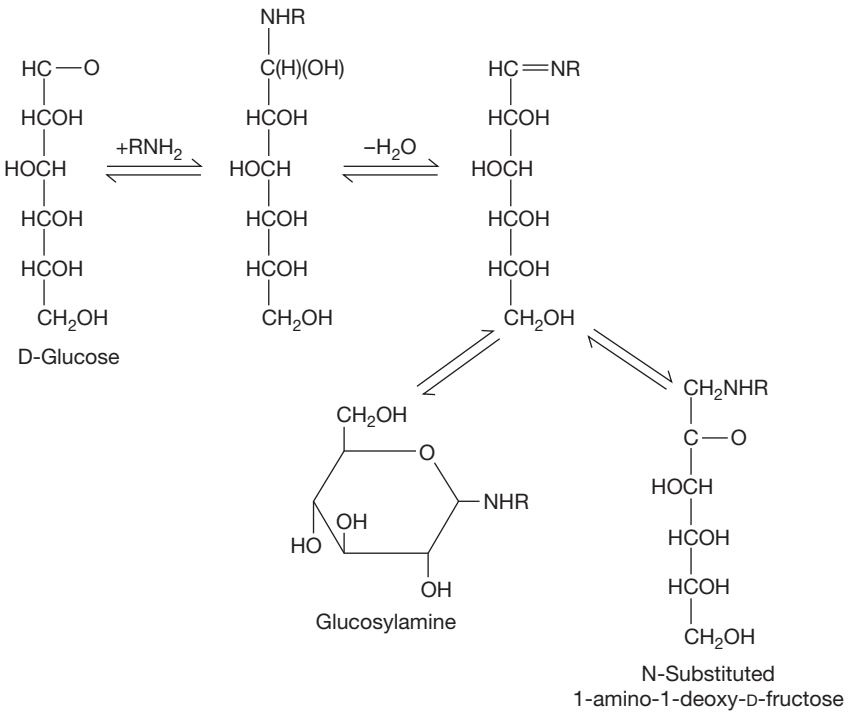

Figure 10 Products of reaction of D-glucose with an amine (RNH_2).

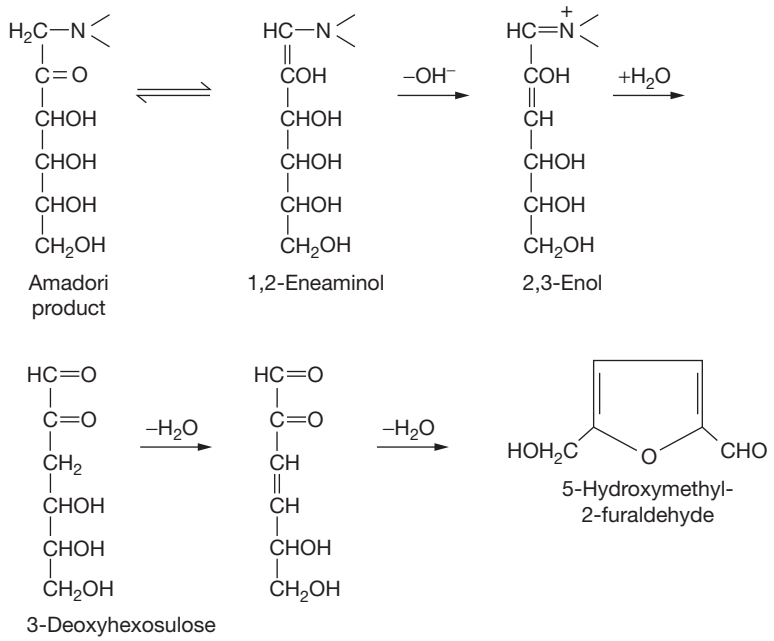

Figure 11 Conversion of the Amadori product into HMF.

glucose, which no longer require prior precipitation of plasma proteins, involve electrochemical detection. These methods rely on silver electrodes to detect electrons generated by the oxidation of glucose by glucose oxidase contained in membranes on the surface of the electrodes. For determination of glucose and α-limit dextrins resulting from starch digestion, HPLC techniques have proved useful.

Much attention has been given to the analysis of the glucose polymers – starches, resistant starches, cellulose, and β-glucans – in the context of dietary fiber. The ultimate assessment depends on the use of specific enzymes or enzyme systems to break the macromolecules down to their component glucose and other sugars when mixtures containing other polymers (dietary fibers) are being analyzed. These are then assessed by gas chromatography or HPLC and the ratios of the sugars determined. More routine assessment may involve a variety of chemical techniques combined with enzymatic digestion and, in the case of a popular Association of Official Analytical Chemists (AOAC)-approved technique for dietary fiber analysis, with a gravimetric determination. However, there is debate as to whether the resistant starch, which in the gravimetric AOAC technique is analyzed as dietary fiber, should be included as fiber or whether it is physiologically distinct. It is also debated whether a determination of β-glucan is sufficient without knowledge of its viscosity and molecular weight – factors that determine its physiological effect.

See also: Carbohydrate: Digestion, Absorption and Metabolism; Condensed Milk; Dahi; Food Intolerance: Lactose Intolerance; Glucose: Metabolism and Regulation; Lactic Acid Bacteria; Lactose; Milk: Role in the Diet; Milk: Sources and Composition; Sucrose: Dietary Importance; Whey and Whey Powders: Fermentation of Whey.

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Glucosinolates from the *Brassica* Vegetables and Their Health Effects

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Background

Glucosinolates impart a sharp, somewhat fiery character to many edible plants. Efforts to understand the chemical origin of these pungent flavors led to the first observations on the unique properties of glucosinolates when, in 1831, French chemists Pierre-Jean Robiquet and Antoine François Boutron-Charlard isolated the glucosinolate sinalbin from white mustard (*Sinapis alba*). Formerly referred to as mustard oil glycosides, the glucosinolates comprise a relatively small but chemically diverse group of defensive plant secondary metabolites. Plants employ intact glucosinolates as host recognition cues (as both deterrents and attractants) for specialist insect and generalist insect oviposition. Glucosinolates further serve as potent biopesticides, participating in a binary chemical defense system that protects plants from environmental stressors such as insect herbivory and microbial pathogen attacks. Glucosinolate-producing plants are of major economic and nutritional importance, demonstrating versatility in a wide range of applications that encompass industrial, agricultural, and phytopharmacological usages. In particular, glucosinolate degradation products have been identified as potent chemoprotective agents in humans against the pathogenesis of many chronic diseases such as cancer, cardiovascular disease, and neurodegenerative disease, among others. The diminutive, glucosinolate-rich thale cress (or, mouse-ear cress, *Arabidopsis thaliana*), a member of the Brassicaceae family, is highly valued as a model organism for plant biology and genetics research.

Sources and Production

The majority of glucosinolate-producing plants are members of the Brassicales order (formerly Capparales), which includes the cruciferous families Brassicaceae, Capparaceae, and Caricaceae. The Brassicales alone comprise more than 350 genera and 3000 species. Of the many hundreds of cruciferous species investigated, all are able to synthesize glucosinolates. Glucosinolates have also been identified in more than 500 species of diverse noncruciferous dicotyledonous angiosperms; of note are the singular glucosinolate-producing genus *Drypetes* (syn. *Putranjiva*) of the Euphorbiaceae family (no other members of this vast plant family have been shown to contain them) and the monogeneric family Moringaceae, which contains 13 species that all produce chemically unique glucosinolates.

Glucosinolates are decidedly stable compounds with little biological activity. Conversely, some glucosinolate degradation products, such as the isothiocyanates, are unstable electrophilic compounds that react spontaneously with biological nucleophiles. Glucosinolates and their degradation products lend a pungent aroma and sharp flavor (sometimes described as bitter) to edible plants and are responsible for the 'heat' most notably associated with wasabi and horseradish.

Glucosinolate distribution in plant structures varies widely between roots, leaves, stems, and seeds and is strongly influenced by plant age. As such, the highest concentrations of glucosinolates are usually found in the reproductive organs of plants, with substantially higher concentrations present in young plants versus mature plants. Following *de novo* synthesis in siliques, glucosinolates are transported from mature to developing plant structures where they are stored as inert, hydrophilic precursors to toxic isothiocyanates and other biologically active compounds. Glucosinolates coexist with but are compartmentally segregated from plant myrosinases, an endogenous family of β-thioglucosidase enzymes (EC 3.2.1.147), whose sole known substrates are glucosinolates. These enzymes are strategically sequestered throughout plants in specialized idioblast, or myrosin, cells of plant leaves, stems, and flower clusters in a species-specific manner; multiple forms of myrosinase can exist within the same plant.

Upon plant injury and subsequent loss of cellular integrity due to insect herbivory, human mastication, or food processing techniques, myrosinase hydrolyzes the neighboring glucosinolates to yield glucose, hydrogen sulfate, and unstable aglycones that spontaneously rearrange to form a diverse group of bioactive compounds that vary depending on chemical structure, environmental conditions (such as pH and presence of ferrous ions), and the coexistence of other factors present in the plant, such as epithiospecifier proteins (ESP) or epithiospecifier modifier proteins (ESM). Whereas the presence of ESP favors epithionitrile formation (primarily from aliphatic glucosinolates), ESM promotes isothiocyanate formation. Myrosinases have also been identified, but little characterized, in fungi and bacteria including many bacteria commonly associated with human and animal gut microflora. Myrosinases are activated to various degrees by ascorbic acid, and in some instances, the enzyme is almost inactive in its absence. Activation is not dependent on the redox reactivity of ascorbate, rather, it has been suggested that ascorbate provides a nucleophilic catalytic group to the reaction.

In humans, myrosinase-producing intestinal microflora convert unhydrolyzed glucosinolates to their cognate isothiocyanates. Microbial-induced conversion is highly variable and subject to interindividual differences in commensal microbiota population. As such, conversion is distinguished by high converters (individuals with high elimination profiles) and low converters (individuals with low elimination profiles). Antimicrobial agents or mechanical bowel cleansing abrogates microbial conversion (see **Figure 1**).

Chemical Structure

The defining characteristic of glucosinolate structure is a β-D-glucopyranose moiety, sulfur-linked to a β-thioglucoside-*N*-hydroxysulfate and an amino acid-derived side chain (R).

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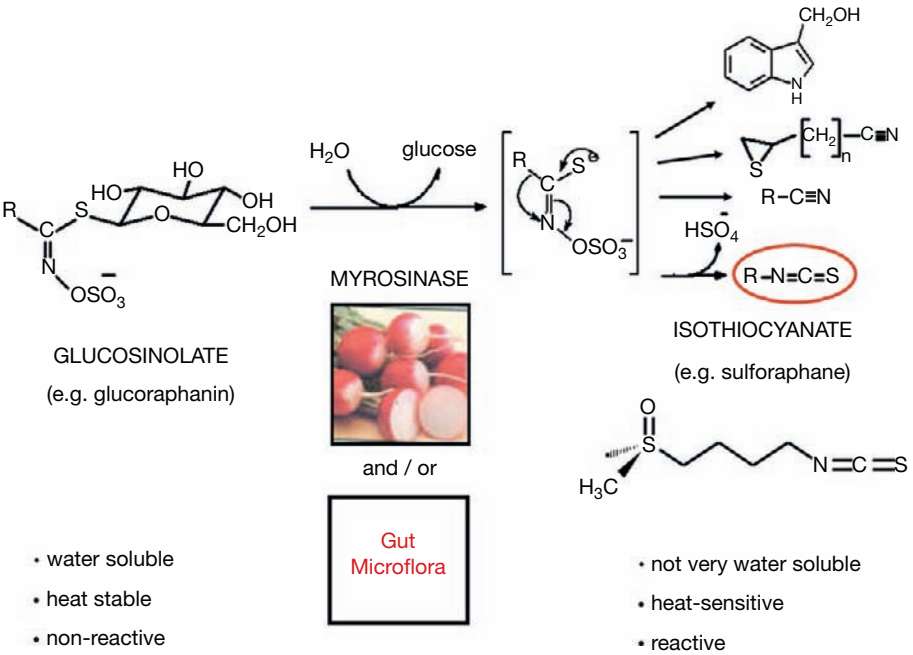

Figure 1 Glucosinolates are hydrolyzed to isothiocyanates by the enzyme myrosinase, which is physically segregated within the plant from its substrate. Myrosinase-like activity is also present in the gut microflora but is highly variable between persons.

During glucosinolate biosynthesis, the precursor side chains undergo extensive modification and may contain straight or branched carbon chains and aromatic or indole moieties. Further side-chain modifications include glycosylation, desaturation, or functionalization with hydroxyl groups, carbonyl groups, or sulfur linkages in various oxidation states, the latter occurring in more than one-third of all nonsynthetic glucosinolates. About 120 distinct glucosinolate structures have been identified and are broadly classified as aliphatic, aromatic, or heterocyclic (or indole), based upon structural similarities of their amino acid precursor and the extent to which their side chain is modified. Aliphatic glucosinolates are derived from alanine, isoleucine, leucine, methionine, or valine; aromatic glucosinolates are derived from phenylalanine or tyrosine; indole glucosinolates are derived from tryptophan. Methionine, in particular, undergoes the most extensive transformation and gives rise to the large family of methylthioalkyl-, methylsulfinyl-, and methylsulfonyl-glucosinolates. Some aromatic benzyl glucosinolates have an additional sugar moiety (rhamnose or arabinose) in glycosidic linkage to the aromatic ring. The implication of these sugars' presence has only been speculated upon to date, but their occurrence in two families of plants (Moringaceae and Resedaceae) whose genera are widely exploited for their pharmacological properties is significant.

Biosynthesis

The biosynthetic pathway of glucosinolates shares homology with that of the cyanogenic glycosides and likely occurs sequentially in the cytoplasm, endoplasmic reticulum, and chloroplast. The universally accepted model of this pathway transpires in a stepwise fashion involving side-chain elongation, core (glycone) biosynthesis, and side-chain modification. Side-chain elongation proceeds by *N*-hydroxylation of select precursor amino acids followed by decarboxylation. Multifunctional cytochrome P450 enzymes (CYP79s and CYP83s), glucosyltransferases, and sulfotransferases mediate biosynthesis of the core glycone structure, with aldoximes, aci-nitro or nitrile oxides, S-alkyl -thiohydroximates, thiohydroximic acids, and desulfoglucosinolates serving as transitory intermediates. The precise mechanism of side-chain modification, the terminal step of glucosinolate biosynthesis, has been the source of much speculation and little experimental work; however, during side-chain modification, oxidations, hydroxylations, and desaturations give rise to the wide range of structural diversity present in glucosinolates. This diversity further influences reactivity, solubility, and human biological activity of not only the glucosinolates but also the resultant products (isothiocyanates). For example, whereas initial oxidation of the side chain sulfur of methionine and its chain-elongated homologues likely gives rise to the large family of methylsulfinyl- and methylsulfonyl-glucosinolates, conjugation of a hydroxyalkyl glucosinolate with benzoic acid is expected to give rise to the benzoyloxyalkyl glucosinolates. Furthermore, oxidation of glucoerucin, the major glucosinolate present in arugula, gives rise to glucoraphanin, the precursor to the potent anticarcinogenic isothiocyanate sulforaphane. However, other metabolic modifications of glucoerucin may give rise to the goitrogenic glucosinolate progoitrin, with no demonstrated anticarcinogenic activity, but with the potential to cause dietary goiter (hence its name) if consumed at high levels. It is noteworthy that at least ten of the aromatic and indole glucosinolates, which exhibit high bioactivity, are singly or multiply methoxylated.

Hydrolysis of aliphatic and aromatic glucosinolates generally yields isothiocyanates while indole hydrolysis yields nitriles and unstable isothiocyanates that rapidly form indole-3-carbinol (I3C), indoleacetonitrile, thiocyanate ions, and 3,3′-diindolylmethane (DIM). I3C may then spontaneously condense under

the acid conditions of the stomach to form compounds that closely resemble 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) in structure, toxicity, and carcinogenicity. Despite this toxicity, indole glucosinolate metabolites, in particular, I3C and DIM, have been investigated for their potential as cancer chemoprotective agents.

Glucosinolate biosynthesis within individual cruciferous vegetables is both constitutive and inducible and varies considerably in response to genetic factors and environmental influences, such as jasmonate and salicylic acid signaling, pathogenic attack, insect herbivory, and soil nitrogen or sulfur content. As many as 25 glucosinolates may be present in a single plant (*Arabidopsis* spp.), although a census in the range of five to ten is far more typical; total content in plants can range from 1% of dry weight in vegetative tissues of many *Brassica* vegetables to as much as 10% in the seeds of some plants.

Patterns of Consumption

Cruciferous vegetables from the *Brassica* genus are the primary dietary source of glucosinolates in humans and include broccoli, bok choy, Brussels sprouts, cabbage, cauliflower, collard, kale, kohlrabi, mustard, rutabaga, and turnips. In addition, edible plants from other genera including arugula, capers, cress, moringa, papaya, radish, wasabi, and watercress contribute substantially to glucosinolate intake. (See Figure 2 and Table 1.)

Regional and cultural dietary patterns strongly influence glucosinolate consumption. Food disappearance data indicate Asian populations consume markedly higher quantities of cruciferous vegetables, many of which are consumed rarely in Western cultures. For example, whereas daikon, or Japanese white radish, is the most commonly consumed cruciferous vegetable in Japan (20 kg year^{-1}), broccoli ranks the highest in the United States (2.5 kg year^{-1}). Total crucifer intake varies considerably by country but average intake is estimated at 7.6 kg year^{-1}; the highest total intake is observed in China (37 kg year^{-1}) and the lowest in Greece (2.1 kg year^{-1}). Although selective breeding to increase glucosinolate content is advocated for health reasons, efforts to decrease glucosinolate content have been promoted as means to reduce bitterness, a quality perceived (by some) as an obstacle to palatability. (See Box 1.)

Accurate assessment of dietary glucosinolate intake has proved problematic. Traditional dietary assessment methods such as food frequency questionnaires and dietary recalls may be susceptible to reporting bias and fail to consider differences in glucosinolate content in plants, which demonstrates remarkable variability across species and cultivars. Environmental factors such as soil and growing conditions, cultivar, harvest time, and postharvest storage further modulate glucosinolate levels in foods. In addition, food processing and preparation methods may reduce exposure of humans to glucosinolate degradation products by 60–90%. For example, glucosinolates are water-soluble and may leach into cooking water; alternatively, heat-labile myrosinases are readily inactivated at most cooking temperatures. Furthermore, in light of the complex interactions between gut microbiota and host metabolism, circadian rhythms may influence exposure as well: Recent work suggests that whereas conversion of intact glucosinolates to bioactive metabolites predominates during the day, metabolism of isothiocyanates is more efficient during the night.

Cruciferous vegetable intake can serve as a proxy for glucosinolate intake but biomarkers such as urinary isothiocyanate metabolites may provide more definitive, complementary measures of habitual intake as well as metabolite-specific

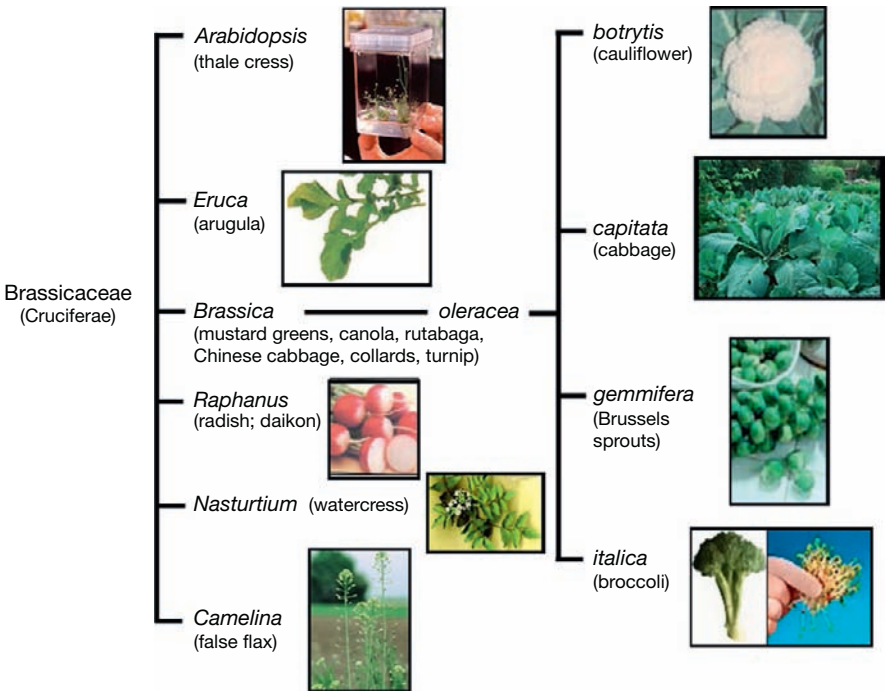

Figure 2 Commonly consumed vegetables in the family Brassicaceae (Cruciferae).

Table 1 Common dietary sources of glucosinolates

Dietary vegetable	*Latin binomial*
Aspiration™ (Chinese kale x broccoli)	–
Arugula (rocket)	*Eruca sativa*
Broccoli (Calabrese; sprouting broccoli)	*Brassica oleracea* var. *italica*
Broccoli raab (rapini; brocoletto; taitcat)	*Brassica rapa* var. *rapa*
Broccolini™ (Chinese kale x broccoli)	–
Broccoflower™ (bright green cauliflower)	*Brassica oleracea* var. *botrytis*
Brussels sprouts	*Brassica oleracea* var. *gemmifera*
Cabbage	*Brassica oleracea* var. *capitata*
Capers	*Capparis spinosa*
Cauliflower	*Brassica oleracea* var. *botrytis*
Charlock (kaber)	*Brassica kaber; Brassica arvensis; Sinapis arvensis*
Chinese cabbage	*Brassica rapa* var. *pekinensis*
Chinese kale (gai lan; gai lon)	*Brassica oleracea* var. *alboglabra*
Chinese mustards (including bok choy, pak choi, choy sum, and celery mustard)	*Brassica rapa* ssp. *chinensis*
Choy sum (mock pak choi; cai tai; saishin)	*Brassica rapa* ssp. *parachinensis*
Collards (collard greens)	*Brassica oleracea* var. *sabellica*
Colza	*Brassica napus* ssp. *napus*
Cress	*Lepidium sativum*
Daikon (mooli; Chinese radish; Korean radish; lo bok)	*Raphanus sativus* var. *longipinnatus*
Horseradish	*Armoracia rusticana*
Kale	*Brassica oleracea* var. *acephala*
Branching bush kale	*(also Brassica oleracea* var. *fruticosa)*
Borecole	*(also Brassica oleracea* var. *selenesia)*
Galega kale (couve galega)	
Marrow stem kale	*(also Brassica oleracea* var. *medullosa)*
Siberian kale (Hanover salad)	*(also Brassica napus* var. *pabularia)*
Thousand-headed kale	*(also Brassica oleracea* var. *ramosa; B. oleracea* var. *millecapitata)*
Kohlrabi (knol khol; turnip cabbage)	*Brassica oleracea* var. *gongylodes*
Maca	*Lepidium meyenii*
Mashua	*Tropaeolum tuberosum*
Mizuna (curled mustard; Japanese greens)	*Brassica rapa* ssp. *japonica*
Mustard (greens; brown mustard)	*Brassica juncea* varieties (many)
Mustard seed	
White mustard	*Sinapis alba*
Yellow mustard	*Sinapis hirta*
Chinese mustard	*Brassica juncea*
Black mustard	*Brassica nigra*
Field mustard	*Brassica campestris*
Ethiopian mustard	*Brassica carinata*
Papaya (pawpaw)	*Carica papaya*
Radish	*Raphanus sativus*
Swede (rutabaga)	*Brassica napus* var. *napobrassica*
Tendergreen (spinach mustard; komatsuna)	*Brassica rapa* var. *perviridis*
Texsel greens	*Brassica carinata*
Tronchuda cabbage (Portuguese cabbage; couve tronchuda; Galician cabbage; braganza; sea-kale cabbage)	*Brassica oleracea* var. *costata*
Turnip rape	*Brassica campestris* var. *oleifera*
Turnips	*Brassica rapa* var. *rapifera*
Wasabi	*Wasabia japonica*
Watercress	*Nasturtium officinale*

exposure levels. However, human exposure to nondietary sources of synthetic analogs of isothiocyanate metabolites such as those used extensively in agricultural and industrial applications can further distort quantification of exposure. Examples include the dithiocarbamates, which are used as pesticides against insects, fungi, and bacteria; accelerators in the vulcanization of natural rubber and synthetic rubber; precursors in the synthesis of sulfide materials and nanoparticles; and flocculants for the remediation of heavy metal-contaminated wastewater.

Availability, Absorption, and Metabolism

The chemical structure and properties of intact glucosinolates preclude absorption, and little, if any, of the parent

Box 1 Glucosinolates as Modulators of Bitter Taste Receptors

Glucosinolates have been described alternately as sharp, pungent, acrid, astringent, or lacrimatory, but they are most widely regarded – in some cases inaccurately – as bitter. Whereas the glucosinolates sinigrin and gluconapin (prominent in Brussels sprouts, cabbage, cauliflower, turnip, rutabaga, and kale) are associated with strong bitterness, glucobrassicin and neoglucobrassicin (prominent in mature broccoli) confer moderate bitterness. The glucosinolates glucoerucin, glucoiberverin, and glucoraphanin do not contribute appreciably to bitterness.

Isothiocyanates typically are not associated with bitterness but they do contribute to overall flavor. One notable exception is goitrin, the hydrolytic degradation product of the nonbitter glucosinolate progoitrin. Goitrin contributes to the bitterness of rutabaga, Brussels sprouts, cabbage, turnip, and cauliflower and presents a significant barrier to consumer acceptance.

Humans' innate aversive reaction to bitterness is a highly conserved protective measure against ingestion of toxic substances. Oral perception of bitterness entails the binding of hydrophilic molecules to G protein-coupled receptors encoded by the TAS2R family of taste receptor genes and ensues via subsequent initiation of attendant downstream signaling pathways. This activation of oral chemosensors elicits a cascade of events that induces local epithelial changes or alterations in autonomic responses, such as excessive salivation or increased gut motility, respectively. TAS2R receptors are expressed in humans in oral taste receptor cells and extraoral sites including tissues of the gut, airways, and testes, where they exhibit variable ligand specificity. Although their functions in these tissues have not been clearly identified, TAS2Rs have been implicated in a diverse portfolio of activities that mediate reflexive actions to protect humans (or their reproductive capacity), including neuroendocrine signaling, airway patency, and spermatogenesis. Whether bitter-tasting compounds such as some glucosinolates modulate cell signaling pathways regulated by TAS2Rs to elicit beneficial (or deleterious) health effects remains to be seen.

glucosinolates is recovered in urine or feces. As such, nearly all glucosinolate bioactivity in humans is attributable to their respective hydrolytic degradation products, of which the isothiocyanates are prominent examples. Only a small fraction of the 120 or so known glucosinolates, and even fewer isothiocyanates, occur frequently in the diet, however.

Isothiocyanates, as lipophilic nonnutrients, encounter the same biotransformative events as other xenobiotic compounds and are subject to a vigorous metabolic response in the small intestine and liver. Nevertheless, isothiocyanates exhibit variable absorption kinetics, high levels of metabolic disposition, strong modulatory effects on ATP-binding cassette (ABC) transporters, and an expansive range of bioavailability. The liver is the predominant site of isothiocyanate metabolism due to high hepatocellular expression of glutathione S-transferases (GST), phase 2 cytoprotective enzymes central to isothiocyanate biotransformation; however, the rapid rate of isothiocyanate clearance – within hours of ingestion – implicates other tissues in metabolism, including the oral, gastric, and intestinal epithelia. The small intestine, in particular, demonstrates uniform and abundant expression of GST and actively participates in isothiocyanate metabolism. Although traditional models of xenobiotic metabolism rarely consider extraintestinal uptake, sublingual and buccal tissues in the oral cavity demonstrate moderate permeability and absorption of other lipophilic compounds. Furthermore, they express the requisite enzymes and efflux transporters involved in isothiocyanate metabolism, raising the question regarding the location of these activities.

Although the sites wherein isothiocyanate biotransformation occurs have not been fully characterized, the processes that mediate the compounds' metabolic disposition likely transpire in this fashion: Following passive diffusion into cells, CYP450 enzymes may oxidize isothiocyanates to their cognate isocyanates, unstable intermediates of amines, carbamates, or benzene derivatives. More frequently, however, isothiocyanates undergo spontaneous or enzyme-enhanced conjugation to glutathione and subsequent cellular efflux via ABC transporters. The isothiocyanates are exceptionally avid substrates of multidrug resistance proteins (MRP2); as such, isothiocyanate conjugates are readily deconjugated and may be shuttled in and out of cells multiple times via MRP2, providing repeated exposures to cells. Intact glucosinolates, isothiocyanates, and their glutathione conjugates may enter the enterohepatic circulation, the bidirectional cycling of xenobiotic compounds and their metabolites between the intestine and liver via bile transporters. Enterohepatic circulation subjects these compounds to repeated bouts of recycling and may prolong the compounds' half-life; alternatively, they may be transported to the distal gut where they are subject to microbial hydrolysis and subsequent absorption or elimination in feces.

Absorbed isothiocyanate species are metabolized primarily by the mercapturic acid pathway, during which nonenzymatic and enzymatic conversions yield the dithiocarbamates cysteinylglycine, cysteine, and N-acetylcysteine conjugates. These metabolites are preferentially eliminated in urine, likely due to their low molecular weight, which is generally less than 500–600 Da. Generally speaking, glucosinolates, isothiocyanates, and their metabolites are cleared from urine within 72 h of ingestion. Over half of ingested glucosinolates, which require initial degradation to isothiocyanates by myrosinase, are cleared in the urine within 24 h, and they are fully cleared within 72 h. Over half of ingested isothiocyanates are cleared in the urine within 8 h, and they are fully cleared within 24–48 h. As such, N-acetylcysteine conjugates in urine serve as a biomarker of isothiocyanate (and glucosinolate) uptake and metabolism. However, interindividual differences in cellular metabolic competence, gut microbiota population, bowel transit time, and the presence of other dietary components may influence elimination rates.

The collective processes of MRP2 transport, enterohepatic circulation, and mercapturic metabolism likely play a key role in isothiocyanate bioavailability. Indeed, isothiocyanates demonstrate biphasic serum concentrations and urinary output rates, which may have implications for health effects as well as extrapolation of data across individuals. These redundant, iterative processes speak to the toxicity of isothiocyanates yet ultimately they may be responsible for the protective cellular responses induced by exposure to these compounds.

Health Effects

The health effects associated with the consumption of glucosinolates are incumbent upon conversion to their bioactive form, the isothiocyanates. Postabsorptive metabolism of isothiocyanates may be responsible for producing therapeutic moieties that mediate these beneficial effects; however, toxicities have been reported from consumption of some

non-isothiocyanate metabolites of certain glucosinolates not commonly consumed. Nevertheless, compelling evidence links increased consumption of fruits and vegetables, especially cruciferous vegetables, to reduced incidence of many chronic diseases, including cancer, cardiovascular disease, neurodegenerative disease, metabolic dysregulation and its pathological sequelae, and autoimmune dysfunction. A variety of clinical trials addressing the role of isothiocyanates in the prevention and management of these and other chronic diseases are under way as this article goes to press, with new data promising further insight into the mechanistic actions of this unique class of bioactive plant compounds.

Anti-Inflammatory and Immunomodulatory Agents

Inflammation and altered immune function are prominent features in the pathogenesis of many chronic diseases. The anti-inflammatory and immunomodulatory activities of isothiocyanates likely contribute to the putative protective effects associated with these compounds. For example, in rodents, sulforaphane, one of the most extensively studied isothiocyanates, has been shown to inhibit endothelial cell response to the vascular endothelial growth factor and downregulate the expression of cyclooxygenase-2 and proinflammatory cytokines such as interleukin (IL)-1β, IL-6, granulocyte macrophage colony-stimulating factor, and tumor necrosis factor-α (TNF-α). Sulforaphane also inhibits activation of the transcription factor NFκB, thus suppressing gene expression of proinflammatory mediators and reducing the number of activated macrophages in rodent models. Recent evidence suggests that through direct covalent binding to the N-terminal proline, isothiocyanates potently inhibit the tautomerase activity of the macrophage migration inhibitory factor, a proinflammatory cytokine implicated in the pathogenesis of inflammatory and autoimmune diseases.

Cancer

Isothiocyanates have been studied extensively for their role in preventing carcinogenesis. Ingestion of approximately two servings per day of cruciferous vegetables may reduce the relative risk for cancer by as much as 50% at certain sites. At least some, and perhaps most, of the cancer chemoprotective activity of these vegetables is believed to be due to their content of minor dietary components such as glucosinolates, a role supported by *in vitro* animal and human data. In particular, epidemiological data indicate that aliphatic and indolyl glucosinolate intakes are inversely associated with risk of some types of cancer; however, genetic polymorphisms in isothiocyanate metabolism and elimination may mitigate the protective effects. In rodent models of both spontaneous and chemically induced cancers, isothiocyanates confer protection at multiple sites including the lungs, esophagus, stomach, colon, mammary glands, bladder, pancreas, and skin. In addition, the isothiocyanates inhibit growth of human tumor cells in xenograft models of prostate, colorectal, esophageal, and breast cancer. Multiple mechanisms are likely at play including altered carcinogen metabolism due to modifications in the activities of drug-metabolizing enzymes, induction of cell cycle arrest and apoptosis, inhibition of angiogenesis and metastasis, altered histone acetylation status, and antioxidant, anti-inflammatory, and immunomodulatory activities. Critically, it is this ability of a single agent to exert such diverse effects on events that are intimately involved in the pathogenesis of cancer that makes the isothiocyanates such efficient protective agents. Early discoveries that isothiocyanates were capable of reducing the activation of procarcinogens by inhibiting phase 1 drug-metabolizing enzymes and inducing the transcription of cytoprotective phase 2 enzymes, ultimately decreasing the levels of the carcinogens available to mutate DNA, provided the impetus for the development of a quantitative bioassay for screening induction capabilities based on increased transcription of NAD(P)H: quinone oxidoreductase 1 (NQO1). Induction of this widely distributed NAD(P)H-dependent flavoprotein, which promotes obligatory 2-electron reductions of quinones to hydroquinones, is a salient feature of the phase 2 response and is of central importance in protecting cells against oxidative stress.

Broccoli extracts demonstrate the greatest capacity for NQO1 induction of any glucosinolate-producing plants evaluated thus far, and sulforaphane has been isolated as the principal active compound. In fact, no other phytochemicals in broccoli extracts have been shown to contribute to this activity. In addition, induction of other cytoprotective proteins, including both subunits of glutamate–cysteine ligase, the rate-limiting enzyme of glutathione biosynthesis, heme oxygenase-1, and GSTs, has been observed. Importantly, induction has long-lasting protective effects because the cytoprotective proteins typically have long half-lives of several days. Global gene expression profiling has confirmed that isothiocyanates serve as broad-spectrum chemopreventive agents and modulate extensive networks of cytoprotective genes and signaling pathways.

Many of the genes encoding cytoprotective proteins share a common transcriptional regulation through the Keap1–Nrf2–ARE pathway, a key mediator of cytoprotective responses to oxidative stress and electrophilic stress. Kelch-like ECH-associated protein 1 (Keap1) serves as the ubiquitin ligase substrate adaptor that presents the nuclear factor-erythroid 2 p45-related factor-2 (Nrf2) for ubiquitination and subsequent proteasomal degradation. Isothiocyanates react with specific cysteine residues of Keap1, eliminating Keap1's ability to target the Nrf2 for degradation. Consequently, the Nrf2 accumulates and translocates to the nucleus where it binds to antioxidant response elements (AREs), specific DNA sequences in the upstream regulatory regions of cytoprotective genes, and activates transcription. Nrf2 activation induces the coordinate transcription of a diverse group of cytoprotective enzymes, altering the metabolism and elimination of environmental procarcinogens in humans and affording protection against the damaging effects of environmental toxins by enhancing their detoxification. Sulforaphane is the most potent naturally occurring small-molecule inducer of cytoprotective proteins. In effect, isothiocyanates such as sulforaphane expedite their own metabolism and elimination through upregulation of phase 2 enzymes and subsequent formation of readily excretable metabolites. Isothiocyanates further reduce cancer risk through modulation of epigenetic marks via inhibition of histone deacetylase,

promotion of global histone acetylation, and the induction of cell cycle arrest and apoptosis of cancer cells, thus reducing tumor growth. Further, sulforaphane induces perturbations in several signaling pathways, such as transforming growth factor-β receptor, endothelial growth factor receptor, and insulin signaling. Because these pathways are associated with carcinogenesis and inflammation, it is reasonable to assume that the net effect of their modulation will be reduction in cell proliferation and maintenance of tissue homeostasis.

Cardiovascular Disease

Similarly, isothiocyanates' ability to induce cytoprotective proteins and prevent chronic inflammation reduces oxidative stress in cardiovascular tissues, as evidenced by increased glutathione, glutathione reductase, and glutathione peroxidase levels and decreased protein nitrosation. In rodent models of hypertension and stroke, endothelial-dependent relaxation of the aorta is improved, the number of infiltrating activated macrophages is reduced, and blood pressure is decreased. Similarly, in rodent models of cardiovascular ischemia and reperfusion, oral delivery of isothiocyanate-containing extracts improves postischemic ventricular function, reduces myocardial infarct size, and decreases cardiomyocyte apoptosis.

Nervous Tissue Injury and Neurodegenerative Disease

Oxidative stress and chronic inflammation are also central to the pathogenesis of diseases of the central nervous system, and the protective effects of isothiocyanates are evident in models of nervous tissue injury and neurodegeneration. In models of spinal cord injury, sulforaphane administration decreases infarct size, brain (or spinal cord) edema, and cortical apoptosis, reduces inflammatory markers and tissue damage, attenuates loss of endothelial cell markers and tight junction proteins, preserves blood–brain barrier function, and decreases the presence of neurological deficits. In addition, sulforaphane decreases both microglial activation and the upregulation of inflammatory markers following endotoxin injection. Sulforaphane also demonstrates a protective effect in rodent models of brain ischemia and reperfusion, traumatic brain and spinal cord injury, intracerebral hemorrhage, and contusion.

In the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease, sulforaphane protects nigral dopaminergic neurons against cell death and reduces astrogliosis, microgliosis, and the release of proinflammatory mediators in the basal ganglia. Intrathecal administration of sulforaphane reduces oxidative stress and proinflammatory cytokine expression caused by spinal nerve transection and inhibits the development of neuropathic pain. Following exposure to methylmercury, decreased mercury accumulation was observed in the brain of sulforaphane-treated mice, suggesting the potential of isothiocyanates to accelerate the metabolism and elimination of neurotoxins. In most cases, the neuroprotective effects of sulforaphane are accompanied by activation of the transcription factor Nrf2 and upregulation of its target genes. Furthermore, mice that are deficient in Nrf2 (knockout mutants) are more sensitive to damage and cannot be protected by sulforaphane, strongly suggesting that the transcriptional program orchestrated by Nrf2, and not the isothiocyanate itself, is largely responsible for the protective effects of sulforaphane.

Metabolic Dysregulation and Pathological Sequelae

Diabetes is a metabolic disorder characterized by a chronic, subacute state of inflammation. As such, anti-inflammatory therapeutic approaches are critical in the prevention and management of its pathological sequelae. Sulforaphane has been shown to reduce the serum C-reactive protein, IL-6, and TNF-α, common biomarkers of inflammation observed in individuals with type 2 diabetes. In addition, sulforaphane improves renal performance and minimizes pathological changes in the glomerulus while improving motor nerve conduction velocity and blood flow and reducing pain. These very recent findings encourage the potential development of dietary isothiocyanates as therapeutic agents to alleviate metabolic disorders and protect against renal damage and pain associated with diabetes. Interestingly, intervention in humans with diets rich in glucosinolates causes a decrease in the acylcarnitine levels in plasma and urine, indicating enhanced integration of fatty acid oxidation with the activity of the tricarboxylic acid cycle, which may have implications for metabolic derangements, as well as cancer and neurodegeneration.

Antimicrobial Activity

The antimicrobial activities of isothiocyanates against human pathogens such as *Escherichia coli*, *Salmonella typhimurium*, and *Candida* spp. are well documented. For example, the long history of use of cruciferous vegetables such as cabbage and mustard as wound poultices and antitumor agents likely is attributable to isothiocyanates. Sulforaphane, in particular, is bactericidal and bacteriostatic to *Helicobacter pylori*, and it decreases markers of colonization and inflammation associated with *H. pylori* infection in humans and rodents, which is strongly associated with the development of gastric cancer. In addition, isothiocyanate activity against a range of soil-borne fungal, bacterial, and nematode plant pathogens is profound and has been extensively characterized. It underpins the burgeoning use of cruciferous plants as green manure and soil biofumigation to exploit the fungicidal and nematicidal activities of isothiocyanates.

Antinutritional Characteristics

Some antinutritional effects of isothiocyanates have been reported. In particular, hydrolysis of ß-hydroxyalkenyl glucosinolates (e.g., progoitrin and epi-progoitrin) gives rise to ß-hydroxyalkenyl isothiocyanates, which further cyclize to oxazolidine-2-thiones. These compounds may elicit goitrogenic effects in mammals, a phenomenon first observed in rabbits and described as 'cabbage' goiter. Efforts to suppress the goitrogenicity of rapeseed (*Brassica napus*), an oilseed crop of significant economic and agricultural importance, have led to the highly successful development of the oilseed crop canola in Canada and the 00-rapeseed in northern Europe. The

goitrogenicity of dietary glucosinolates and isothiocyanates in humans is much less clear in the context of normal dietary intake or even enthusiastic consumption.

Conclusion

Glucosinolates are amino acid-derived plant secondary metabolites whose role in plant defense has been well characterized. A wealth of evidence supports the assertion that glucosinolates and their isothiocyanate metabolites provide myriad beneficial health effects in humans as well. In the human dietary context, it is not abundantly clear whether delivery of the glucosinolate (which might be viewed as a prodrug in pharmaceutical terminology) leads to a very different outcome, but clearly, tissue exposure is different with ingestion of isothiocyanate versus glucosinolate. The induction of cytoprotective enzymes, inhibition of inflammatory processes, and modulation of signaling pathways are among the many diverse pharmacological outcomes ascribed to isothiocyanate exposure. Unlike most small-molecule pharmacological agents that affect single targets, the intracellular targets of isothiocyanates are multiple. The activation of transcription factor Nrf2 alone, a major outcome of isothiocyanate exposure, leads to an orchestrated regulation of a vast network of genes comprising approximately 2% of the human genome, with cytoprotective, antioxidant, and anti-inflammatory functions. It is this ability to induce versatile and long-lasting responses, which ultimately protect against oxidative stress, electrophilic stress, and chronic inflammation (the fundamental underlying causes of most chronic diseases), that makes these unique phytochemicals exceedingly efficient protective agents. Although cell culture and animal studies strongly support the beneficial health effects of glucosinolates and isothiocyanates, it is challenging yet critical to translate the findings to human populations.

It is noteworthy that many glucosinolate-containing genera comprise plants that have been used for food or medicinal purposes by various cultures for many centuries. In addition, many tropical and subtropical species have such a compelling ethnopharmacology and such manifold food and medicinal uses that future, more rigorous investigations of their glucosinolates' properties seem promising. Undoubtedly, research in the area of functional genomics will serve to enhance our knowledge and understanding of the diverse biological roles of these unique compounds.

See also: Aflatoxin: A Global Public Health Problem; Antinutritional Factors in Legume Seeds: Characteristics and Determination; Antioxidants: Role on Health and Prevention; Brassica: Characteristics and Properties; Cancer: Diet in Cancer Prevention; Mustard; Papayas; Pesticides and Herbicides: Residue Determination; Pesticides and Herbicides: Types of Pesticide; Pesticides and Herbicides: Types, Uses, and Determination of Herbicides; Pesticides and Herbicides; Rapeseed Oil/Canola.

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Goat: Milk

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Introduction

Milk has been part of our staple diet since the agricultural revolution, so eliminating its consumption has nutritional consequences. Milk supplies an economical source of nutrients and confers numerous health benefits: it plays a critical role in nutrition and health. In recent years, cow milk (CM) and its derived products have suffered poor public perception. People believe it to be high in fat and energy, with consequent negative health effects. In addition, heightened awareness of intolerant and allergic symptoms arising from CM consumption has led those affected to look for alternatives. The goat milk (GM) with its unique composition could be a valuable alternative.

A number of recent scientific studies have examined differences between CM and GM. Disparities in their fat, protein, and sugar composition may explain why an increasing number of GM consumers report significant health benefits including improved digestion and asthma and reduced catarrh and eczema. GM production ranks third after bovine and buffalo milk but it is the milk of choice in most of the world. Nutritionally, it is comparable to CM as it contains similar levels of calcium, potassium, phosphorus, and many other nutrients that confer health benefits.

Dairy goat farming plays an important role in the diet and economy of many developing countries. It serves as one of the principal sources of animal protein, calcium, and phosphorus in many regions, such as the Middle East, southern Asia, and some tropical countries. It is often recommended by medical professionals in situations where bovine milk may cause allergic responses in consumers. Although GM, like CM and human milk, contains lactose, many people with lactose intolerance can drink GM because of its lower amount of αs1-casein. It is a nutritious dairy option for many patients of different age groups and lifestyle needs. Young children and seniors can be especially sensitive to CM and so can certain ethnic groups, including Asians, Hispanics, African-Americans, and Native Americans.

GM is an excellent option for any patient who is CM- or soy milk-sensitive and is necessarily concerned with obtaining adequate calcium from a natural dietary source. It is also an excellent source of dietary calcium important in the prevention of high blood pressure, osteoporosis, and other bone-related problems. For menopausal women, GM provides 13% more calcium than CM and can be consumed comfortably even by those women with milk sensitivity. While it is often recommended that children who have problems digesting CM change to vegetable protein soy-based milk, that is not always the answer. An estimated 20–50% of children with CM protein intolerance will react adversely to soy proteins. GM is a natural milk that children like and can consume comfortably, even if they are sensitive to CM and/or soy milk. The nutrient composition of GM is very different from that of CM containing 13% more calcium, 25% more vitamin B_6, 47% more vitamin A, 134% more potassium, and 350% more niacin. GM is also higher in chloride, copper, and manganese and contains 27% more of the essential nutrient selenium.

GM contains none of the controversial bovine growth hormones. It is available nationwide in evaporated and powdered forms and in fresh one-quart, refrigerated cartons (whole and 1% low fat), as well as aseptic quarts with an unopened 8-month shelf life. Nevertheless, GM products are considered speciality foods and nowadays becoming more popular in developed countries. The GM industry will continue to grow in the near future for the well-being of hundreds of millions of people worldwide and may become an important part of the economy in many countries.

Composition

Research in the past decades has extended knowledge on the basic and unique features of the composition and properties of GM. Composition varies according to several factors, such as animal, breed, feed, age of lactation, stage of lactation, and environment.

Lipid Content of GM

Fat is present in milk as droplets of triglycerides surrounded by a complex membrane derived from the mammary epithelial cell called milk fat globule membrane. The percentage of total fat in goat and CM is quite similar, and the fatty acid composition depends to a large extent on the diet composition in both species. Fat globules of GM resemble those of CM in lipid composition and properties of the globule membrane, but GM lacks 'agglutinin' that causes fat globules of CM to cluster when cooled. The GM fat is composed of several hundred fatty acids, the share of which in the total FA pool is considerably differentiated. The share of five of those acids (C10:0, C14:0, C16:0, C18:0, and C:18-1 cis) comprises over 75% of total FA in milk. Five principal proteins of GM, α-lactalbumin, β-lactoglobulin, κ-casein, β-casein, and αs2-casein, closely resemble their homologue in CM. GM lacks a homologue of bovine βs1-casein, the most abundant protein in CM.

Casein micelles of GM contain more calcium and inorganic phosphorus, are less solvated and less heat-stable, and lose β-casein more readily than bovine micelles. Average GM fat differs in the contents of its fatty acids significantly from average CM fat, being much higher in butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), and linoleic (C18:2) acids, but

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lower in stearic (C18:0) and oleic (C18:1) acids. A characteristic trait distinguishing GM from CM is the relation between the lauric acid (C12:0) and capric acid (C10:0) (0.46% vs. 1.16% of sum of acids). This is an important indicator, as it may be used to detect falsifications of GM with CM.

In the fat of GM, saturated fatty acids are the dominating group and their share in the milk fat ranges from about 67% to about 75%. The high concentration of the acid in the GM fat is not a characteristic of this animal species, but of a majority of mammals. In turn, the share of monounsaturated fatty acids (MUFAs) in the total FA pool of the GM fat may range from about 20% to about 32%. Among the MUFAs, oleic acid (C18:1) is characterized by the highest content, which is typical for a majority of mammals. Three of the medium-chain triglycerides (MCTs) (C6–C14) have actually been named after goats, because of their predominance in GM. Capric acid, caprylic acid, and MCT have become established medical treatments for an array of clinical disorders, including malabsorption syndromes, chyluria, steatorrhea, hyperlipoproteinemia, premature infant feeding, children with failure to thrive, infant malnutrition, epilepsy, cystic fibrosis, and gallstones. They are recommended after intestinal resection and coronary bypass because of (a) their unique metabolic ability to provide direct energy instead of being deposited in adipose tissues, (b) their actions of lowering serum cholesterol, and (c) inhibiting and limiting cholesterol deposition.

GM exceeds CM in MUFAs, PUFAs, and MCTs, which all are known to be beneficial for human health, especially for cardiovascular conditions. This biomedical superiority has not been promoted much in marketing GM, goat yogurt, and goat cheeses, but has great potential in justifying the uniqueness of GM in human nutrition and medicine for treating the various gastrointestinal disorders and diseases, besides its value in alleviating CM allergies. Fatty acid composition of GM fat can also be changed toward even more of the beneficial fatty acids by different regimes of feed supplementation to goats including changes of forage-to-concentrate ratios.

Protein Content of GM

Dairy products are a reliable source of high-quality proteins, which are well balanced in amino acids. The variation of total protein content is now well known. For GM, it depends on genetic polymorphism of αs1-casein. CM and GM do not differ significantly as far as the protein percentage is concerned, and, in contrast to milk fat, the protein content in both species is less amenable to dietary manipulation. GM proteins differ in genetic polymorphisms and their frequencies in goat populations. The presence of the αs1-casein trait has been studied much in recent years, when it was discovered that it has six different types, A, B, C, E, F, and 'null' in GM. In CM, αs1-casein is the major αs-casein. The 'null' type or absence in some GM means that in different goats, the major αs-casein is the αs2-casein variant, which has different digestibility and cheese-making properties.

The differences in genetic types are due to amino acid substitutions in the protein chains, which in turn are responsible for the differences in digestibility, cheese-making properties, and flavors of GM products, but the amino acid substitutions also enable the detection of even small amounts of adulteration with CM. Peptides formed from GM casein by proteases tasted much less bitter than those from CM casein. Casein micelles, the form of casein molecule suspended in GM, also differ markedly from those of CM: they have less complete sedimentation rate, greater β-casein solubilization, smaller size of micelle, more calcium and phosphorus, less salvation, and low heat stability.

Carbohydrate Content of GM

Lactose is the main carbohydrate in GM comprising about 4.4%. Its concentration does not vary excessively. However, GM lactose content is often largely increased by dietary plant oil supplementation in contrast to CM. Lactose is a valuable nutrient, because it favors intestinal absorption of calcium, magnesium, and phosphorus and the utilization of vitamin D. Lactose is a disaccharide made up of a glucose and a galactose molecule, which may also be present in small free amounts. Lactose is of major importance for maintaining osmotic equilibrium between the blood stream and the alveolar cells of the mammary gland during milk synthesis. Lactose content of GM is about 0.2–0.5% less than that of CM. Carbohydrates other than lactose found in GM are oligosaccharides, glycopeptides, glycol–proteins, and nucleotide sugars in small amounts. Milk oligosaccharides have considerable antigenic properties and are valuable in the growth promotion of the intestinal flora of the newborn. Nucleotide sugars in milk are of particular interest, since they are the glycosyl donors for glycosyltransferase in milk and the mammary gland and are the precursors of glycoproteins, glycolipids, and oligosaccharides in the biosynthesis of milk.

Mineral Content of GM

Mineral content in GM is much higher than cow and human milk. It varies from 0.70% to 0.85%. GM is distinguished by its high chloride and potassium content. Partition of calcium, phosphorus, and magnesium between the soluble and colloidal phases of milk is similar for CM and GM. Iron bioavailability is higher in GM than in CM due to higher nucleotide content contributing to better absorption in the gut. Bioavailability for zinc was found higher in the case of human milk, lower values for sheep milk, and average values for GM and CM.

Vitamin Content of GM

As goats convert all β-carotene into vitamin A in the milk, GM has higher amounts of vitamin A than CM. Caprine milk is whiter than bovine milk. GM supplies adequate amounts of vitamin A and niacin and excesses of thiamin, riboflavin, and pantothenate for a human infant. The vitamin content of GM is similar to that of cow and human milk. Nevertheless, GM is poor in folic acid and vitamin E. GM lacks β-carotene, which is entirely converted into retinol.

Nutritional Properties of GM

Milk is a complex biological fluid produced by the mammary gland that has three main functions: (i) nutritional, (ii)

immunologic, and (iii) physiological. Traditionally, GM and CM have been considered a fundamental food in the diets of many cultures. Milk provides an easily accessible matrix, rich in a large variety of essential nutrients like minerals, vitamins, and easily digestible proteins with balanced amino acid profiles, important in supporting most body functions. Both human and animal studies indicate that GM fat is utilized more efficiently than CM fat. This feature of GM most likely relates to its unique enrichment in medium-chain fatty acids.

It has been demonstrated that the GM powder prevented the loss of the intestinal barrier function that follows heat stress in a rat model, which may relate to the peculiar lipid profile and the relatively higher content of oligosaccharides. Feeding GM also increased the biliary secretion of cholesterol and decreased plasma cholesterol levels in rats. Growing piglets fed with GM generally had higher bone mineral density, less body fat, and less fat mass than those fed with bovine milk. The greater bone density in the GM-fed piglets may have been due to the greater concentrations of plasma minerals, reflecting a better uptake of minerals. Taurine in goat and sheep milk derived from sulfur-containing amino acids has important metabolic functions as does carnitine, which is a valuable nutrient for the human neonate. Mineral and vitamin contents of goat and sheep milk are mostly higher than those of CM.

Growth Factors in GM

GM is an excellent source of physiologically active compounds known as growth factors. Human milk contains physiological levels of growth factors, while bovine milk has much lower growth factor activity (GFA). Colostrum of most mammals usually contains rich levels of growth factors and other bioactive compounds, whereas the levels of these molecules decrease rapidly during the first 3 days of lactation. Epidermal growth factor (EGF) was identified using antibody neutralization assays along with several other growth factors in human milk, including hepatocyte growth factor and vascular endothelial growth factor.

Growth factors in milk have been shown to be involved in the development of the neonatal gastrointestinal tract. The EGF enhances the establishment of the epithelial barrier in the gastrointestinal tract and protects the gastroduodenal mucosa by activating ornithine decarboxylase in polyamine synthesis. Growth factors can withstand the harsh conditions of gastric acid exposure and can be absorbed through the GI tract to act on other tissues. It was suggested that milk-derived growth factors may be useful as an orally administered treatment for a wide variety of gastrointestinal disorders. GM has a much higher level of GFA than that of CM, whereby GM may be a feasible nutraceutical for gastrointestinal disorders.

The presence of EGF in caprine milk was observed using a human EGF (hEGF) polyclonal antibody. Caprine milk also has been shown to possess the ability to reduce heat-induced gastrointestinal hyperpermeability. It was found that Nubian GM contained the highest GFA among the five breeds of dairy goats tested. It was showed that Nubian milk contained higher total protein and nonprotein nitrogen contents than the Alpine breed, which might be associated with the highest growth factor activity. It was also found that the milk of pregnant goats does have a significantly higher GFA than those of postpartum goats. The GFA decreased during the first 8 weeks of lactation, fluctuated thereafter, and then increased dramatically after natural mating. The GFA during weeks 1 to 8 was inversely correlated with milk yield and week of lactation, while no correlation was found during weeks 9 to 29. The GFA in the milk after natural mating of goats correlated significantly with somatic cell count and conductivity, but was inversely correlated with milk yield. By using dialysis and ultrafiltration with 50, 30, and 3 kDa cutoff membranes, it was reported that more than 90% GFA was present in the 50 kDa fraction, in contrast to the 6 kDa molecular weight of EGF (hEGF). The GFA of GM was around pH 6.3 fraction, which differed from the pH 4.6 of EGF. It proves that the major growth factor of the molecular fraction of GM was different from that of human milk.

GM Products

GM and its processed products are greatly useful as functional foods, maintaining nourishment and health for the young and elderly, especially those who have CM allergy. GM has also been known for its beneficial and therapeutic effects on the people who have CM allergy. These nutritional, health, and therapeutic benefits enlighten the potentials and values of GM and its specialty products. The chemical characteristics of GM can be used to manufacture a wide variety of products, including beverages (low fat, fortified, or flavored) and ultrahigh-temperature milk, fermented products (such as cheese, buttermilk, and yogurt), frozen products (such as ice cream or frozen yogurt), butter, condensed/dried products, sweets, and candies.

Nevertheless, high-quality products can only be produced from good quality GM. The quality milk should have the potential to tolerate technological treatments and be transformed into a product that satisfies the expectations of consumers, in terms of nutritional, hygienic, and sensory attributes. Taste is the main criteria used by consumers to make decisions to purchase and consume GM and its products. Typical goat taste is considered as a quality component in certain goat cheese products.

Cheeses from GM

Cheese may have been one of the first manufactured foods consumed by humans. History records its usage more than 4000 years ago, but no one really knows when the first GM was made into cheese. Goat cheese originated in Mesopotamia. The milk was probably made into soft cheese, and then hard, ripened goat cheeses were later developed in the Mediterranean Basin countries. There are goat cheeses made from raw and pasteurized milk. In many countries, the manufacture of goat cheese from raw milk is prohibited due to food safety issues (e.g., brucellosis). Numerous varieties of goat cheeses are produced worldwide. Maturation or ripening of cheeses from GM and milk from other species is governed by many different factors. Besides, manufacturing techniques include wide variations in amount and species of organism used in culturing, incubation procedures, and forming or pressing techniques. Variations in aging time and conditions play the most

important part in determining the flavor, body, and texture of cheese. The type of milk used significantly influences the finished cheese. Southern European countries are known for the production of most caprine and ovine milks worldwide. Almost all such raw milk is converted into cheeses. The cheesemaker often relies on adventitious lactic acid bacteria and nonstarter microflora to lower pH and bring about ripening. Artisanal production of regional cheeses is a part of the cultural heritage of many countries in Europe (e.g., Portugal and Spain).

Most artisanal cheeses obtained from ovine and caprine milks are seasonal, and peak production is in spring and lowest production is in fall. Increased logistic difficulties associated with artisanal production (e.g., exodus of farmers toward urban areas and suboptimal exploitation of complementary agriculture activities) have led to a drastic reduction in the numbers of persons associated with some artisanal cheese varieties. It is reported that the production of artisanal cheeses in the European Union countries has increased to 65% (on average) from 1984 to 1996, with an extremely high increase (223%) in Spain and a moderate increase (19%) in Portugal. The large number of varieties of cheese made from GM has resulted in a great diversity in the nature of the products.

GM whey cheese is especially popular in Norway, the process where caramelized lactose in concentrated whey is combined with fat and whey proteins to make Gjetost cheese. Ricotta is a kind of cheese that may be made from goat cheese whey; cheeses of a similar nature have other names when made in other countries. Brocciu is a whey cheese made by combining GM with the whey from goat cheese. Many traditional goat cheeses are made in developing countries.

Fermented Milks from GM

The proportion of GM processed into cheeses and yogurts is higher in comparison to CM. Since 5000 BC, the nutritional and health values of fermented milk and beverages were described. As a process, fermentation consists of the transformation of simple raw materials into a range of value-added products by utilizing the phenomenon of growth of microorganisms and/or their activities on milk. Kefir is a viscous, refreshing drink, with high acidity and varying amounts of alcohol and carbon dioxide.

Commercial production of kefir occurs in many countries, particularly in eastern Europe. It is also manufactured under a variety of names including kephir, kiaphur, kefer, knapon, kepi, and kippi, with homemade production occurring in countries as widespread as Argentina, Taiwan, Portugal, Turkey, and France. Kefir is still most familiar to consumers in eastern Europe. Kefir has had a long history of health benefits in eastern European countries, where it is associated with general well-being and often the first weaning food received by babies. Stimulation of the immune system, inhibition of tumor growth, antimicrobial properties, gastrointestinal tract benefits, and positive effects on cholesterol metabolism are among the health benefits of kefir. Dahi, an Indian fermented milk product, is also popular because of its nutritional and therapeutic value. Many traditional fermented GMs are made in developing countries.

Ice Cream and Desserts

Ice cream made from GM is an attractive alternative for children and other consumers due to its nutritional and antiallergenic properties and creamy organoleptic properties. Ice cream and frozen yogurt manufactured from GM are usually flavored. Vanilla, chocolate, and premium white chocolate mix are the most commonly used flavors. GM produces the most acceptable ice cream followed by CM with a softer texture and specific melting characteristics.

Chakka is a concentrated product obtained after draining the whey from Dahi (fermented milk product). Mixing of chakka with sugar and other condiments results in shrikhand, a very popular and traditional dessert in India.

Fat-Rich Dairy Products from GM

Cream is produced by mechanical agitation of the whole milk to separate the fat globules. The fat globules are aggregated to form a semisolid mass with 80–85% fat, transforming into butter. Cream and butter are manufactured from GM, but are almost entirely consumed by those seeking to avoid cow dairy products for health reasons. Butter milk is usually made from skim GM ($<0.5\%$ fat) using a by-product from butter processing. Traditional Turkish 'yayik' butter made from GM had the most acceptable organoleptic characteristics. Ghee or heat clarified butter fat or butter oil is a fat-rich product, mostly used as a cooking medium in India and other tropical countries. It is manufactured by fermenting whole milk into curd and churning out butter, followed by heat clarification at 105–145 °C. GM is not suitable for the manufacture of ghee because of the relatively small fat globules, which may present problems during cream separation.

Whey Products from GM

GM whey has higher levels of α-lactalbumin, but is often discarded or given to animals as nutritional supplement, and little information on it is available. However, presently, there are many products made from GM whey; among them are GM whey-flavored beverage, tablets (chewable), whey protein concentrate, and athletic supplements.

Other Food Usages of GM

In some countries, customers can find breakfast cereals with GM and baby food and sweet and salty pies made from GM and cheese. These products are increasing in variety and quantity. Industrialized whole GM is also used for pet consumption.

Conclusion

GM has a high nutritional content. It is a rich source of calcium, phosphorous, chlorine, and vitamins. It is highly digestible and has a mild laxative effect. It is a good source of vitamin B1, which is useful in relieving symptoms of stress (neurotic indigestion, constipation, and insomnia). It may be used to replace CM for those suffering from allergy. It has a higher fat content than CM and low cholesterol levels than CM, hence suitable for people with high blood pressure. It is low in sugar than CM, hence good

for those suffering from diabetes. GM is closer to human milk and is therefore easily accepted especially by those young or frail. It has an alkaline reaction like mother's milk. It does not form mucous (phlegm) and is therefore better tolerated by asthmatics and those with allergies.

See also: Buffalo Milk; Cheese: Processing and Sensory Properties; Dairy Products: Dietary and Medical Importance; Food Allergies; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Sheep: Milk.

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Grapes

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Sources and Production

The grapevine is the most valuable fruit crop in the world and is grown widely for consumption as fresh fruit and processed products. A majority of the grapes produced worldwide are used for wine production followed by fresh consumption and other processed products such as jams, jelly, and juice.

The grapevine is believed to have originated and domesticated in areas of the Middle East and the Caucasus Mountains from where it spread to other regions of the world. The earliest chemical evidence of wine was obtained in the form of tartaric acid, an important component of wine, in jars believed to have been produced in 5400–5000 BC. Paintings in Egyptian civilizations depict grapevine cultivation and production of wine, which point to evidence of viticulture and wine production during this period. The grapevine was spread throughout Europe by the Romans and Greeks and introduced to the Americas in the sixteenth century. Grape is currently grown in subtropical and temperate regions of several countries with China, the United States, Italy, France, and Spain being the top five producers. Worldwide acreage under grapevine is currently estimated at 6.7 million ha with an annual production of 67 million tons.

Grape belongs to the *Vitaceae* family comprising 17 genera and more than 1000 species. The genus *Vitis* is of economic importance comprising approximately 60 species that are native to North America, Asia, and Europe. Although more than 3000 varieties, predominantly belonging to the *Vitis vinifera* group, are grown worldwide, approximately 35 elite varieties constitute more than 50% of the total acreage. 'Thompson Seedless' is the most popular grapevine cultivar worldwide for fresh consumption, while 'Chardonnay,' 'Cabernet Sauvignon,' 'Merlot,' and 'Shiraz' are popular wine cultivars.

A number of North American *Vitis* species have been used as parents in hybridization programs with *V. vinifera* to develop cultivars (commonly known as bunch grapes) with specific characteristics. For instance, *V. aestivalis* has been used as a source to develop grape cultivars with Pierce's disease resistance, a bacterial disease that limits the cultivation of pure *V. vinifera* cultivars in the southern United States. Popular grape cultivars developed from interspecific hybridization of *V. vinifera* and *V. labrusca* are used exclusively for the production of juice, jam, and jelly. A number of cold-hardy grapevine cultivars suitable for production in colder regions have been developed through breeding programs involving *V. riparia*. Several desirable traits such as drought, salt, and nematode tolerance have been exploited from North American species including *V. berlandieri*, *V. rupestris*, and *V. champinii* to develop rootstocks, which are extensively used to impart specific advantages under less than optimum growing conditions. *V. rotundifolia*, commonly known as the muscadine grape, is native to the Southeastern United States where it is commercially grown on more than 5000 acres for juice, jelly, and wine production. The muscadine grape exhibits greater tolerance to insects and diseases and performs better under hot and humid conditions where *V. vinifera* cannot be grown. A number of improved muscadine grape cultivars have been developed through conventional breeding; however, traits such as seedlessness and thin skin present in *V. vinifera* cultivars cannot be transferred to muscadine grape germplasm due to marginal sexual compatibility between the two species.

Vitis species are creeping vines that are supported by trellis systems in commercial production. Elite grapevine cultivars have an ancient pedigree and are exclusively propagated by clonal means. Hardwood cuttings obtained from 1-year-old growth are used for establishment of new vineyards. Vines established from hardwood cuttings can flower and bear fruit in the first year of planting itself, but commercial production can only be realized after 2–3 years. Flowering occurs from compound buds that develop in the current season to produce a crop for the subsequent season. Most commercial cultivars have perfect flowers; however, native species can be dioecious with male and female flowers borne on separate plants. Some muscadine grape cultivars have female flowers, which necessitates the planting of cultivars with perfect flowers to facilitate pollination and ensure sustainable yields. Bunch grape cultivars have fruit clusters comprising of 40–200 berries with varying size and color. Berries of muscadine grape are borne in small clusters of 3–10 berries with individual berries being much larger compared with those of bunch grapes. Berry development in grapevine can be separated into two phases. The first phase occurs from bloom to 60 days during which the berry is formed and increases in volume. Berry growth during this period coincides with the accumulation of acids (tartaric, malic, and hydroxycinnamic acids) and tannins in addition to other compounds, which contribute to the final wine quality. During the second developmental phase, the berry doubles in size leading to a decline in organic acids produced during the first phase. Color development in berries (veraison) is accompanied by accumulation of sugars and flavor and aroma compounds. Grape is classified as a nonclimacteric fruit, and harvesting occurs following completion of ripening. Grapes grown for wine or juice production are harvested mechanically, while table grapes destined for fresh consumption are harvested manually, graded, and then packed for ensuring high quality.

Patterns of Grape Consumption

Grape is either consumed fresh; processed into beverages, jam, and jelly; or dried to produce raisins. Grapes used for fresh consumption mainly include *V. vinifera* seedless grape cultivars, which are characterized by large-sized berries, high sugar content, mild flavor, and thin skin. A number of green and colored table grape cultivars developed from public and private

breeding programs are currently grown for fresh consumption. The muscadine grape is also used for fresh consumption in the Southeastern United States. Muscadine grape cultivars are characterized by a typical flavor and aroma, thick skin, and the presence of seeds. They differ significantly in nutritional composition compared with seedless table grapes and are considered to have higher levels of health beneficial compounds.

A majority of the grapes produced worldwide are crushed for wine production. Red wine is consumed in larger quantities than white wine. The United States is the largest wine consumer in the world followed by France and other European countries. While a majority of the wine produced and consumed contains alcohol, nonalcoholic wine is being increasingly consumed as the health benefits of various compounds present in wine are being identified. Grapes are also used for the production of other alcoholic beverages following distillation of fermented juice. Wine is extensively used for cooking purposes in addition to being consumed as a beverage.

A number of seeded and seedless grape cultivars are grown worldwide for the production of raisins. Raisin production consists of drying ripened grapes under natural conditions for 2–4 weeks until a change in color and significant decrease in moisture content occur. Drying emulsions are used in some cases to accelerate the drying process. Sulfur dioxide is also used during raisin production to prevent browning and preserve natural flavor and health beneficial compounds. 'Thompson Seedless' is most widely used for raisin production followed by other cultivars including 'Fiesta,' 'Zante currant,' 'DOVine,' and 'Muscat of Alexandria.'

Grape juice is largely produced from *V. labrusca*-based interspecific hybrid cultivars such as 'Concord,' 'Niagara,' and 'Delaware' with smaller quantities made from the muscadine grapes. Grape juice quality is governed by several factors including cultivar, management and harvesting procedures, and postharvest handling and processing. Processing of grapes to produce juice involves a hot-press or cold-press method where the crushed grapes are heated to 60–63 °C or used at room temperature for treating with pectinolytic enzymes to break down the pectins. Crushed and treated juice, and pulp, undergo further processing for obtaining the final product. Differences in yield and quality are observed between the two processing techniques. Grape juice concentrate is made by evaporating and concentrating grape juice to achieve 55–68% total soluble solids (TSS). Grape juice concentrate is used in fruit cocktails and as a sweetener in other food products. A substantial amount of juice concentrate is made from 'Thompson Seedless' in addition to the aforementioned cultivars. Other processed products include jam, jelly, and fruit spreads. Grape pomace left over following crushing and fermentation of berries accounts for 20–25% of the grape crushed for wine production. Grape pomace mainly consists of the skin, seed, and stems and has potential applications in the food industry. Grape pomace powder/flour is an optimum source of antioxidant dietary fiber when added to yogurt, salad dressings, and baked goods such as bread, muffins, and cookies. Components such as pectin, celluloses, and sugars are present in wine grape pomace and make it an ideal material for formation of edible films and coating to store fruits, vegetables, and food products. Additionally, the pomace can be ground into flour and used as a health supplement in the form of capsules. Grape pomace also has suitable applications as animal feed for ruminants and nonruminants. Grape seed extract and oil are increasingly being used as a health supplement and in the cosmetic industry. Grape seed extract is rich in a number of phenolic antioxidants and available as a dietary supplement in the form of capsules and liquid extract. Grape seed contains 13–19% oil that is rich in linoleic acid and vitamin E content and low in cholesterol, and its consumption may be beneficial in alleviating cardiovascular and circulatory disorders. Phenolic compounds, which are rich in grape seed, are found in low quantities in the oil; such compounds are lost during the extraction process. Grape leaves are widely used for cooking in Middle Eastern and Mediterranean cuisines. Tender leaves can be used fresh or canned in brine and used for cooking.

Availability, Absorption, and Metabolism

Grape and its processed products are a rich source of health beneficial compounds (Table 1). A number of phytochemicals are present in addition to water, sugars, acids, amino acids, and mineral elements. Glucose and fructose are the predominant sugars that contribute to its sweetness and converted to alcohol during the fermentation process. Thus, the amount of alcohol occurring in wine can be correlated to the amount of sugar initially present in the juice. Sugars predominantly accumulate during the later stages of fruit ripening. The final sugar content in berries varies widely with grape species and cultivars. Grape berries accumulate a significant amount of organic acids that include tartaric acid and those such as malic acid produced during respiratory catabolism of sugars during the early stages of berry development. The acid components are responsible for imparting the fresh and crisp taste of juice and wine. Grapes are also an important source of mineral elements and vitamins while having no sodium and cholesterol. Raisins produced by drying grapes are a rich source of energy, minerals, and vitamins. Grape and its products contain a number of phenolic compounds that mainly occur in the skin and seed of berries. Such compounds can be classified into simple phenolics, flavonoids, and stilbenoids. Simple phenolics are products of hydroxycinnamic and hydroxybenzoic acids that accumulate in berries during the early stages of development. A number of health beneficial flavonoids including flavones, flavonols, and anthocyanidins are found at varying levels in red and white grape cultivars. Stilbenoids are essentially located in the skin of grape berries and are predominantly found in red wine where the skin is retained during the fermentation process. Resveratrol is an important stilbenoid found in red wine. Wide variations are observed in the concentrations of phytochemicals among different *Vitis* species and cultivars. Total phenolic levels in muscadine grape are up to five times higher than those observed in bunch grapes, with greater levels found in the seed and skin compared to the pulp. Muscadine grape leaves exhibit up to 15 times greater antioxidant capacity compared to the skin, seed, and pulp of berries and have great potential for use in nutraceutical products. Another potential source of dietary supplements is the muscadine pomace that is discarded during the production of juice, jelly, and wine.

Wine is widely known for its health benefits and ranks among the top five beverages consumed worldwide. A number

Table 1 Nutritional composition of grape and grape products

Component	Vitis vinifera *(European bunch grapes)*[a]	Vitis rotundifolia *(muscadine grape)*[a]	Vitis labrusca *(North American) hybrids*[a]	*Raisins*	*Juice*	*White wine*	*Red wine*	*Leaves*
Water (g)	80.5	84.3	81.3	14.5	84.5	86.9	86.5	73.2
Energy (kcal)	69	57.0	67.0	302	60	82	85	93.0
Protein (g)	0.72	0.81	0.63	3.4	0.37	0.07	0.07	5.6
Total fat (g)	0.16	0.47	0.35	0.46	0.13	0.0	0.0	2.12
Carbohydrate (g)	18.1	13.93	17.15	79.5	14.8	2.6	2.6	17.31
Fiber (g)	0.9	3.9	0.9	4.0	0.2	0.0	0.0	11.0
Total sugars (g)	15.5	n/a	16.25	59.1	14.2	0.96	0.62	6.3
Calcium (mg)	10.0	37.0	14.0	53.0	11.0	9.0	8.0	363.0
Iron (mg)	0.36	0.26	0.29	1.8	0.25	0.27	0.46	2.63
Magnesium (mg)	7.0	14.0	5.0	35.0	10.0	10	12.0	95.0
Phosphorus (mg)	20.0	24.0	10.0	115.0	14.0	18.0	23.0	91.0
Potassium (mg)	191.0	203.0	191.0	746.0	104.0	71.0	127	272.0
Sodium (mg)	2.0	1.0	2.0	12.0	5.0	5.0	4.0	9.0
Zinc (mg)	0.07	0.11	0.04	0.32	0.07	0.12	0.14	0.67
Vitamin A (IU)	66.0	67.0	100	0.0	0.0	0.0	0.0	27 521
Vitamin B1 (mg)	0.07	n/a	0.09	0.008	0.017	0.005	0.005	0.04
Vitamin B2 (mg)	0.07	1.5	0.06	0.19	0.015	0.015	0.031	0.354
Vitamin B3 (mg)	0.19	n/a	0.3	1.14	0.13	0.108	0.224	2.36
Vitamin B6 (mg)	0.09	n/a	0.11	0.32	0.03	0.05	0.057	0.4
Vitamin B12 (μg)	0.0	n/a	0.0	0.0	0.0	0.0	0.0	0.0
Folic acid (μg)	2.0	n/a	4.0	3.0	0.0	1.0	1.0	83.0
Vitamin C (mg)	3.2	6.5	4.0	3.2	0.1	0.0	0.0	11.1
Vitamin E (mg)	0.19	n/a	0.19	0.12	0.0	0.0	0.0	2.0

[a]Values are based on per 100 g product.

Source: USDA national nutrient database, http://ndb.nal.usda.gov/ndb/.

of chemical constituents produced in berries accumulate in their concentrated forms during the production of wine. The chemical composition of wine has thus far revealed the presence of more than 500 compounds. Ethanol is the most prevalent alcohol in wine and ranges from 10 to 13% depending on the cultivar, the growing conditions, and the vinification process. Fortified wines may contain higher levels of alcohol. Anthocyanins are pigments responsible for wine color. Anthocyanins combine with other phenolic compounds to impart color stability to wine during the aging process. Tannins in wine are responsible for the astringency and bitterness occurring in wine. Levels of phytochemical compounds in wine vary widely with cultivar, soil, climate in which grapes are grown, and cultural operations adopted for grapevine production.

The beneficial effects of grape consumption depend on the availability of polyphenols present. A number of polyphenols present in red and white wines including anthocyanins and stilbenes are reported to be absorbed and metabolized. Absorption rates are influenced by chemical structure of such compounds, the amount ingested, and dietary sources. A few studies indicate that beneficial effects of wine are enhanced due to the presence of the alcohol component and an increase bioavailability can result from the fermentation process that occurs during the production of wine.

Health Effects

Grape and its products including juice, wine, and grape-derived dietary supplements are known to possess an array of health beneficial compounds. Wine is one of the oldest medicines in the history of mankind with potential applications for a number of aliments and disorders. Beneficial properties of grape products have been a subject of intense study for the past three decades since the formulation of the 'French paradox' concept in the 1980s by French epidemiologists. Studies indicated that the French had significantly lower levels of coronary

heart disease despite the consumption of food rich in fats and a poor lifestyle, which was attributed to the regular consumption of red wine. Studies also estimated that individuals who consumed no wine had a three to five times risk of mortality from heart disease compared with those who occasionally consumed wine. Critics of the 'French paradox' have put forth numerous aspects to contradict the findings including better socioeconomic class of wine consumers leading to a better lifestyle, inaccurate reporting of cardiovascular health disease by the French physicians, and an inability to separate the effects of alcohol and phytochemicals on improved health. A majority of the health benefits have been attributed to phytochemicals present in wine, which act in a variety of mechanisms to prevent or minimize inflammatory responses, thereby providing cardio-, neuro-, and chemoprotective properties. In general, greater health benefits are observed with red wine compared with white wine, and general recommendation for wine consumption includes a daily intake of 150 ml (one drink) for women and 300 ml (two drinks) for men. Such recommendations may however vary with age, genetics, body type, and daily medication/supplement use.

Benefits of cardiovascular health have been most widely reported in studies involving wine consumption. The cardioprotective properties of wine have been attributed to the alcoholic and nonalcoholic portions that contain antioxidants. Wine, rich in flavonoids and nonflavonoid polyphenols with potent antioxidant properties, may contribute to preventing atherosclerosis by inhibiting the oxidation of low-density lipoprotein and its subsequent buildup as plaques in arteries, which leads to clogging of arteries. Various phenolic compounds exhibit differential antioxidant capacities; thus, the antioxidant activity of wine is directly correlated with the content and level of polyphenols present. Such antioxidants may act early by preventing lipid oxidation of foods in the digestive system, suggesting that the best protection may be offered when wine is consumed along with a meal. Regular moderate consumption of wine has been associated with an increase in beneficial lipids, which impart cardioprotective properties. Wine consumption has also been correlated with decreased platelet activity and clumping, a reduction in blood pressure levels in case of hypertension, and decreased reactive oxygen molecules in the blood.

A number of phytochemicals present in wine and grape juice exhibit anticancer properties. Resveratrol is an important anti-inflammatory stilbenoid that has been intensively studied for its health beneficial properties in grape products. Resveratrol is the final product of the stilbene synthase pathway in grapevines and occurs at varying levels depending on the cultivar and vineyard management practices. Resveratrol has been shown to increase in concentration in response to pathogen infection and UV radiation of grapevines. Resveratrol exhibits antimutagenic and antiproliferative properties that contribute to prevention of tumor formation in a number of tissues. It also disrupts a number of inflammation-related pathways, thereby reducing overall inflammation and associated disorders including the development of cancer. The anticancer properties of resveratrol and other polyphenols are attributed to their ability to initiate changes in gene expression resulting in apoptosis or necrosis of cancer cells that ultimately decrease tumor initiation, promotion, and progression. Resveratrol has also been demonstrated to enhance the chemosensitivity of several types of cancers to chemotherapy due to its involvement in a number of stress signaling molecules. There are contradicting reports on the effect of resveratrol on breast cancer. Resveratrol has been suggested to exhibit either estrogenic or antiestrogenic properties in a dose-dependent manner; however, the affinity of these components towards estrogen receptor is extremely low, and their ability to compete with estrogen will be minimal. While higher concentrations can inhibit breast cancer, lower levels have shown to exhibit mammary tumor growth and metastasis in some animal models. Polyphenols from wine, and specifically merlot grape juice, were able to inhibit growth and tumor development of estrogen receptor-negative MDA-MB-231 cells in athymic nude mice. In other studies, muscadine grape skin extract was shown to inhibit prostate cancer cell growth and induce apoptosis without affecting growth of normal cells. Grape phytochemicals are known to exhibit antiproliferative properties with higher effects seen in colored berried cultivars containing greater polyphenols than white cultivars. Other chemicals including ellagic acid that occurs in muscadine grape, but not in wine grapes, are also known to exhibit anticancer properties. Antioxidants and fiber present in grape-derived dietary supplements may contribute towards a reduction in the prevalence of colon cancer.

Phytochemicals present in wine and other grape products have been linked to improved cognition and decreased risk of cognitive decline. Moderate wine consumption can impart protection against neurodegenerative diseases. Resveratrol may act as an antioxidant in neuronal cells, reduce oxidative stress, and prevent neural cell dysfunction and death, ultimately providing neuroprotection. It has also been shown to increase expression of genes associated with longevity. Other phytochemicals such as catechin and epicatechin gallate may suppress neuroinflammation and provide protection against Parkinson's disease, which occurs from brain injuries due to the production of endogenous neurotoxins. Anthocyanins may also improve cognitive brain function and provide neuroprotective abilities by reducing age-associated oxidative stress. Daily consumption of 'Concord' grape juice has been shown to provide cognitive and learning benefits probably from anti-inflammatory compounds present in the juice.

Other health benefits obtained from consuming grape products include a better control in blood sugar balance, insulin regulation, and increased insulin sensitivity; all leading to better management of diabetes. Grapes are classified as a low-glycemic index food and can be consumed by people who have difficulties in maintaining blood sugar balance. Regular consumption of dark-colored grape juice can improve immunity by significantly improving circulation of immune surveillance cells that are responsible for pathogen detection and serve as the immediate line of defense in pathogen attack. Grape seed extract that is used in a number of dietary supplements is known to exhibit antibacterial properties. Growth of several Gram-positive and Gram-negative bacteria was inhibited at varying concentrations of seed extracts, which was attributed to the presence of gallic acid in the extracts.

See also: Alcohol: Metabolism and Health Effects; Berries and Related Fruits; Cancer: Diet in Cancer Prevention; Wines: Champagne and

Sparkling Wines – Production and Effervescence; Wines: Madeira, Port and Sherry Fortified Wines – The *Sui Generis* and Notable Peculiarities. Major Differences and Chemical Patterns; Wines: Types of Table Wines; Wines: Wine and Health; Wines: Wine Production.

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Growth Promoters: Characteristics and Determination

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Introduction

Growth promoters are mainly used in farm animals with the purpose to promote growth, to improve the distribution of fat and protein, and to increase the feed-to-muscle conversion rate. However, some residues may remain in the tissues of the treated animals several days after its administration at toxicologically unacceptable concentrations. Other promoters like β-agonists affect consumers with certain phenomena like gross tremors of the extremities, tachycardia, nausea, headaches, and dizziness. This makes it necessary to provide accurate control rules and inspection services and analytic methodologies for safety assurance purposes.

Most growth promoters are orally active and can be administered either in the feed or in the drinking water, while other active hormones can be administered in the form of small implants into the subcutaneous tissue of the ears. This allows for slow release that may last for several weeks or even months and the ears are discarded at the slaughter. Growth promoters contribute to a better efficiency in the feed conversion rate. They allow for an increased protein deposition, partly due to muscle protease inhibition. The resulting meat is leaner and tougher due to the production of connective tissue and collagen cross-linking. Some fraudulent practices consist of the use of mixtures of several substances like β-agonists and corticosteroids at very low amounts in order to obtain a synergistic effect for a similar growth promotion but reducing the probability of detection by official control laboratories. This type of practice makes its detection rather difficult and justifies the need for advance analytic instrumentation.

Control

International and national legal bodies have established what is defined as maximum residue limits (MRLs) for substances allowed for veterinary use. These MRLs are derived from their respective acceptable daily intakes (ADIs) from foods of animal origin. In this way, the MRLs ensure that even at very high consumption patterns, there is no risk for consumers toward the intake of residues from such growth promoter. An MRL is set for each relevant food, like meat or liver, and for each relevant species. The reason is that the MRL is linked not only to the amount of the active substance remaining in the animal tissue at the end of the medical treatment but also to the amount of the daily consumption of the food. The MRLs may vary from country to country depending on the consumption patterns, the control services, and analytic methodology available. The withdrawal period or time period required for the active substance to decrease below the MRL is determined through depletion studies. Such withdrawal period guarantees that the foodstuff does not contain the given residues in quantities over its MRL. However, no MRLs are established for banned drugs, including carcinogenic substances, because there is no ADI.

Some outbreaks of residue-contaminated foods of animal origin have scared the consumers and increased the public's awareness of the use of growth promoters in farm animals. The possibility that some of such substances might exert carcinogenic, genotoxic, or other undesirable effects on health prompted the health authorities to establish accurate measures in order to ensure consumers' safety. Some growth promoters like estradiol, progesterone, and testosterone are allowed in the United States, but they are applied under strict measures, considering acceptable withdrawal periods. The use of certain growth promoters is also allowed in other countries like Canada, Mexico, Australia, and New Zealand. However, the use of growth promoters is officially banned since 1988 in the European Union where the monitoring of residues of substances having hormonal or thyreostatic action and β-agonists is regulated through the Council Directive 96/23/EC. **Table 1** lists the main veterinary drugs and substances with anabolic effect as defined in such directive. Group A includes unauthorized substances with anabolic effect, while group B includes veterinary drugs, some of them also exerting some growth-promoting effect and having established MRLs.

Commission Decision 2002/657/EC (EC, 2002) implemented the Council Directive 96/23/EC (EC, 1996) and is in force since 1 September 2004. This decision provides rules for the analytic methods to be used in the testing of official samples and specific common criteria for the interpretation of the analytic results of official control laboratories for such samples.

Characteristics

Growth-promoting agents are mainly based on anabolic agents added to improve feed conversion efficiency by increasing the lean-to-fat ratio. The main groups of substances are steroid hormones (androgens, gestagens, and estrogens) and other substances having hormonal action (testosterone, progesterone, trenbolone acetate, zeranol, and melengestrol acetate), resorcylic acid lactones, stilbenes (diethylstilbestrol), antithyroid agents, β-agonists (clenbuterol, mabuterol, cimaterol, salbutamol, etc.), and glucocorticoids (dexamethasone and corticosterone).

Other substances that exert a growth-promoting effect through an indirect action are antimicrobial agents. These substances might exert its growth promotion by making more nutrients available to the animal and not to the gut bacteria. However, this practice increases the abuse of antibiotics that has raised the concern of health authorities due to its contribution to the development of bacteria resistance to antibiotics. Some of these substances are β-lactams (penicillins,

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Table 1 List of substances having anabolic effect (group A) and veterinary drugs (group B) in accordance with the Council Directive 96/23/EC (EC, 1996)

Group A: Substances having anabolic effect	*Group B: Veterinary drugs and contaminants*
1. Stilbenes	1. Antibacterial substances
2. Antithyroid agents	Sulfonamides and quinolones
3. Steroids	2. Other veterinary drugs
Androgens	(a) Anthelmintics
Gestagens	(b) Anticoccidials, including nitroimidazoles
Estrogens	(c) Carbamates and pyrethroids
4. Resorcylic acid lactones	(d) Sedatives
5. β-Agonists	(e) Nonsteroidal anti-inflammatory drugs
6. Other compounds	(f) Other pharmacologically active substances

β-lactamase inhibitors, and cephalosporins), tetracyclines (tetracycline, oxytetracycline, and chlortetracycline), carbadox, and olaquindox, among others.

Sampling for Determination

Sampling

The choice of the target tissue or matrix is very important. It is advisable to choose the sample where the analyte is expected to accumulate (e.g., in the liver, kidney, and urine) because the concentrations are low and need very sensitive techniques for their detection. Of course, larger concentrations may be detected near the injection site. The sample must be chosen in a way that the result reflects the residue status of the laboratory sample and thus that of the sampled tissue or body fluid. A recommended practice is to portion the laboratory sample, either at the sample collection site or in the laboratory, for further analysis in case of doubtful results or any other analytic problem. This is usually done for legal inspection purposes where part of the sample is under custody for future contradictory analysis in case of need.

Sample Storage

The storage of samples is also very important because some time may elapse between the sample collection and analysis. The storage must be under correct freezing conditions ($-20\,^{\circ}C$) because not only some physicochemical reactions (like oxidation, proteolysis, and precipitation, even being covalently linked to proteins) but also microbiological or enzymatic reactions may occur.

Sample Extraction

The extraction must be focussed on the target analyte, considering not only the parent molecule but also its metabolites. The analyte may be extracted with aqueous buffers or organic solvents. However, some caution must be taken because most growth promoters may be conjugated as a way of detoxification, usually excreted through the urine and thus be present in the sample as conjugate forms (glucuronide or sulfate). It is necessary to hydrolyze the sample under mild conditions, typically achieved by incubating with *Helix pomatia* juice, which is a mixture of arylsulfatases and β-glucuronidases or any other hydrolysis procedure.

Solid matrices, including fat, kidney, liver, retina, and skin, are usually extracted by homogenization of the sample in the presence of solvents. Waring blenders, ULTRA-TURRAX, glass grinders, and stomachers can be used for homogenization; of course, heating of the sample must be avoided in order to preserve the integrity of the analyte. Sometimes, the sample can be ground in liquid nitrogen. Extraction is followed by cleanup in order to remove any matrix components that might be coextracted. The solid-phase extraction (SPE) is extensively used because the analyte can be isolated and concentrated in a single step with minimal sample and solvent consumption compared with the classic liquid–liquid extraction. SPE cartridges have evolved into miniaturized columns integrated in microwell assay plates and into disks consisting of membranes with high reversed-phase and ion exchange phase capacity. Immunoaffinity cartridges contain a support with linked antibodies for the specific target analyte that facilitates the affinity extraction with a high degree of specificity. An enormous concentration can be reached in a single step. Molecularly imprinted polymers (MIPs) are another alternative. MIPs can be synthesized from highly cross-linked polymers in the presence of the print molecule (a variant of the analyte). Removal of the print molecule leaves an imprinted sorbent for highly selective extraction of the analyte.

Other advanced extraction procedures are matrix solid-phase dispersion (simultaneous homogenization and extraction), microwave-assisted extraction (rapid heating of the solvent and accelerated extraction by reducing the needed time and consuming less solvent), supercritical fluid extraction (solvent extraction for lipophilic substances with carbon dioxide), and pressurized liquid extraction (fast and efficient extraction of the analyte). Once the analyte is extracted, the solution is concentrated by removing the excess of solvent by either evaporation or sublimation.

Analytical Determination

Analytic strategies are very important considering the wide variety of residues to be analyzed and the large number of samples to be analyzed in a short time. The initial phase usually consists of using low-cost screening methods having a high throughput and an acceptable false-positive incidence rate and a very low false-negative rate. Those noncompliant samples are then subject to further confirmatory methods that require expensive instrumental techniques, almost exclusively based on mass spectrometry (MS) detection, and highly skilled operators.

Those samples containing a residue at a concentration above the detection limit, or the CCα within the EU, are considered as noncompliant (positive). A sample containing a substance, which is above its MRL, will be also considered as noncompliant.

Screening Methods

Different types of screening methods are available and can be selected depending on the type of residues. The main types of screening techniques are listed in Table 2. So, residues of antimicrobial drugs can be screened with microbiological growth-inhibition assays like the EU Four-Plate Test, the New Dutch Kidney Test, and the Delvotest SP. The availability of relatively large amounts of immunoglobulins has also contributed to the development of a range of immunoassays (IAs) for many types of growth promoters and antimicrobials, including radioimmunoassays, enzyme IAs, enzyme-linked immunosorbent assays (ELISAs), and strip-based and dipstick-based IAs. Some of these assays are coupled to commercial instrumentation to facilitate its use and rapid obtention of the result. Other screening methods are based on the detection of an interaction between the biomolecule and analyte, like the surface plasmon resonance biosensors. Finally, high-performance liquid chromatography and more recently ultrahigh-pressure liquid chromatography (UHPLC) are also used for the screening of multiple residues in a sample in a relatively short time.

Confirmatory Methods

These methods are used as a next step for noncompliant samples. An internal standard is used to allow continuous assessment of the performance of the designed assay and to facilitate calibration in quantitative determinations. MS is the most reliable detection system. Hyphenated techniques, such as gas chromatography–MS (GC–MS), liquid chromatography (LC)–MS, LC–nuclear magnetic resonance (NMR), and GC–Fourier transfrom-infrared (FTIR), have been introduced as very powerful tools for the separation and molecular identification of residues. The main techniques are listed in Table 2, and the major advantages and disadvantages of confirmatory methods are summarized in Table 3.

In GC analysis, the universality and discriminative power of the MS using electron ionization as a universal source have led to this being the method of choice for some residues like steroids. In LC analysis, the atmospheric pressure ionization techniques (atmospheric pressure chemical ionization and electrospray ionization) coupled to triple quadrupole or linear ion traps have made MS the standard equipment in a residue analysis laboratory. Furthermore, the evolution of high-resolution MS (Fourier transform ion cyclotron resonance, magnetic sectors, orbitrap, and time of flight) is opening new prospects within the identification even though this does not always reach the required sensitivity.

Table 2 List of the main analytic techniques available for screening and confirmatory purposes

Screening methods	*Confirmatory methods*
Microbiological plate bioassays	Gas chromatography coupled to MS, MS–MS, or MS^n
Dipsticks or ELISA test kits	Liquid chromatography coupled to MS, MS–MS, or MS^n
Radioimmunoassay	UPLC coupled to MS, MS–MS, or MS^n
Multiarray biosensors	Liquid chromatography or UHPLC coupled to time of flight
High-performance thin-layer chromatography with full-scan UV/vis	Liquid chromatography or gas chromatography coupled to infrared spectrometry detection
Gas chromatography coupled to electron capture detector	
High-performance liquid chromatography (HPLC) coupled to UV/vis, DAD, or fluorescence detector	
Ultrahigh-performance liquid chromatography (UPLC) coupled to UV/vis, DAD, or fluorescence detector	

Source: Toldrá, F. and Reig, M. (2014). Residue analysis. In: Devine, C. and Dikemann, M. (eds.) *Encyclopedia of meat sciences* (2nd ed.), vol. 1, pp. 217–221, London: Elsevier Science.

Validation

Regulatory action can be taken only after unequivocal identification of the residue or its metabolite. When testing for the presence of a given prohibited substance or its metabolite(s), the control laboratories must comply with the minimum required performance level, which is an analytic parameter of technical performance based on the metabolism, stability, pharmacokinetics, and pharmacodynamics of the substance. The European Commission released the Commission Decision 2002/657/EC that describes the required quality performance criteria and guidelines for the interpretation of analytic results and validation of the methods. The Commission Decision 2002/657/EC also includes the decision limit (CCα), which is defined as the limit at and above that can be concluded with an error probability of α that a sample is noncompliant and the detection capability (CCβ) that is defined as the smallest content of the substance that may be detected, identified, and/or quantified in a sample with an error probability of β.

Topics that have to be assessed are trueness and precision of the result and specificity and selectivity of the method and its ruggedness. The validation of analytic methods is largely based on the fortification of control samples with a known quantity of the analyte. Finally, the Commission Decision 2002/657/EC also establishes the need to accomplish a number of the so-called identification points (IPs) for the correct identification

Table 3 Major advantages and disadvantages of current confirmatory methods

Advantages	*Disadvantages*
High sensitivity	Need of sample preparation (analyte extraction and purification)
High specificity	High initial investment (equipment)
Lots of information about the analyte	High maintenance costs
Short time (few minutes/sample) to obtain the results	Expertise required
Screening and confirmation in one injection	

Source: Toldrá, F. and Reig, M. (2014). Residue analysis. In: Devine, C. and Dikemann, M. (eds.) *Encyclopedia of meat sciences* (2nd ed.), vol. 1, pp. 217–221. London: Elsevier Science.

Table 4 Some examples of the number of identification points earned for different analytic techniques

Technique	*Number of ions*	*Identification points*
GC–MS electron ionization (EI) and chemical ionization (CI)	Two (EI) and two (CI)	4
GC–MS EI or CI	N	N
LC–MS	N	N
GC–MS–MS	One precursor and two daughters	4
LC–MS–MS	One precursor and two daughters	4
GC–MS–MS	Two precursor ions each with one daughter	5
LC–MS–MS–MS	One precursor, one daughter and two granddaughters	5.5

Source: EC (2002) Commission Decision 2002/657/EC of 17 August 2002 implementing Council Directive 96/23/EC concerning the performance of the analytical methods and the interpretation of results. *Official Journal of the European Community*, L 221, 8.

of the residue substance. Examples of the number of IPs earned when using different techniques are shown in **Table 4**. At least three IPs should be obtained for a satisfactory detection of a substance with MRL, whereas four IPs are needed for the positive identification of banned substances.

In summary, sensitivity and selectivity of the analytic methods will continue to improve in the next years thanks to the rapid developments in analytic instrumentation, especially in computerization and miniaturization so that the levels of detection will drop to very low ultratrace levels. The trends toward high-throughput techniques will continue so that more multiresidue methods will be developed with more analytes per sample to be analyzed per unit of time. New advanced biosensor systems, with microchips containing antibodies against many different analytes in one single chip, are also expected.

See also: Antibiotics and Drugs: Residue Determination; Mass Spectrometry: Applications; Meat: Eating Quality and Preservation; Pork Meat Quality, Production and Processing on.

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Guavas

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Scientific Classification

Kingdom: Plantae	Division: Magnoliophyta	Class: Magnoliopsida
Subclass: Rosidae	Order: Myrtales	Family: Myrtaceae
Subfamily: Myrtoideae	Genus: *Psidium* L.	

Introduction

Guava (*Psidium guajava* L.), the '*apple of the tropics*,' is considered to be one of the most exquisite, nutritionally valuable, and remunerative fruit crops of tropical and subtropical regions of the world. Guava fruits are relished world over for fresh consumption and processed into other delicacies. It outshines other tropical fruits in productivity, hardiness, adaptability, and nutritional value and ensures higher economic returns to the grower with minimal inputs. Guava cultivation is not seriously hampered by unfavorable weather patterns, namely, high temperature, low rainfall, marginal soils, waterlogging, and lack of quality inputs such as irrigation water and fertilizers. However, precise management is required to produce a profitable crop of good quality.

Guava originated in tropical America stretching from Mexico to Peru, but due to its wide adaptability, it is now grown throughout tropical and subtropical areas of the world. The English name 'guava' probably came from the Haitian name 'guajaba.' The Spaniards brought the crop across the Pacific to the Philippines and the Portuguese disseminated it to India. It spread rapidly and easily throughout the tropics by virtue of its abundant and highly viable seeds. Guava became naturalized to the extent that people in different regions consider that it is native to their own land. Today, the fruit can been found growing in more than 60 countries including some of the Mediterranean regions.

Guava cultivation became a commercial preposition in recent years, due to increasing international demand, for fresh and processed products with well-established market in more than 60 countries. India is the world's largest producer of guava followed by China, Kenya, Thailand, Indonesia, Pakistan, Mexico, Brazil, Bangladesh, Nigeria, the Philippines, Vietnam, and Egypt.

Botany

Guava is an important member of the family Myrtaceae. Some commercially important plants like cloves, eucalyptus, nutmeg, cinnamon, and java plum also belong to it. The genus *Psidium* consists of 150 species, but a few of them are of commercial importance and outgrow the others. The commercially cultivated guavas mostly belong to the *P. guajava* species. The characteristics of other important species of *Psidium* are as follows:

- *P. cattleianum* Sabine: It is a bushy ornamental shrub that may grow up to 7 m high. The bark is smooth and gray-brown in color and the young branchlets are cylindrical. The leaves are elliptic to obovate in outline, 5–7 cm in length, thick and leathery in texture, and deep green in color. The flowers are white, solitarily produced on axillary peduncles, and nearly about 2.5 cm in width. The calyx is lobed; the corolla is composed of four orbicular petals. The fruit is small, obovate to round in form, 2–3 cm in diameter, and purplish red in color, with a thin skin; the soft flesh is white toward the center and contains numerous hard seeds. The seeds are small and white in color and can be roasted as a substitute for coffee. The flavor is sweet and aromatic, suggesting that of strawberry. This species is more tolerant to low temperature than *P. guajava* L.
- *P. cattleianum* f. *lucidum*: This species is found in Hawaii. The tree height (up to 12 m) of this species is comparatively higher than that of Cattley or strawberry guava. Generally, it is propagated through seeds. The fruit is yellow in color and suitable for jelly making.
- *P. guineense* Swartz: It is a small tree or shrub of slender habit. The young branchlets are compressed, cylindrical, and finely hairy. The leaves are long-oval, acute or obtuse, and 8–12 cm long, with the lower surface pubescent. Red hairs are found on the midveins. Generally, 8–10 pairs of primary veins are found in one leaf. The flowers are borne upon a single peduncle, resembling that of *P. guajava*. The corolla is of creamy color with 5–7 petals and 150–185 stamens, and stigma is medium in size with 3–8 chambers in the ovary. Flowering occurs throughout the year. The fruit is round or nearly so, 2–3 cm in diameter, greenish-yellow in color, and rather hard when ripe, with whitish flesh containing numerous small seeds. The flavor is subacid and not as musky as that of *P. guajava*.
- *P. friedrichsthalianum* Berg: It is a shrub or small tree with square branchlets. Branches are thin and slender, with smooth brown to dark brown bark. Leaves are smooth, deep green, and oval or oblong–oval, with a prominent midrib below, pointed apex, and narrow base. Flowers appear axillary on young branchlets and solitary, with five petals and five locules in the ovary. It bears sulfur yellow, small-sized fruits. The fruits are globose in shape with high acid content. It can be used for jam and jelly making. Plants are tolerant to guava wilt.
- *P. montanum* Swartz: It is also called mountain guava found in the mountains of Jamaica. Its plants are similar to that of a shrub and attain a height of about 1.5 m, with flat-spreading branches; the branchlets of this species are square-angled; the leaves are oblong to oval and glabrous; apex is acuminate and spiny; and peduncles are with many flowers. The fruits are round in shape. The flesh is white with numerous seeds. Fruits are also poor in quality.

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- *P. molle* Bertd: The tree is small, similar to the size of a shrub. The leaves are green and obviate; the apex of the leaf is pointed. The lower surface of leaves is reddish and velvety. Red hairs are found on the central veins. Flowers occur in clusters of threes in leaf axils, with 5–11 petals and 196–239 stamens; stigma is long with a big ovary of 3–5 chambers. Its fruits are pale yellow in color and with an acidic white flesh. The seeds are numerous. Its fruits are small in size with an average weight of 13 g. It contains vitamin C at about 70 mg/100 g pulp.
- *P. pumilum*: The tree is pyramidal in shape. The leaves are small in size, light in color, and nonpubescent having 13–17 pairs of primary veins. There are seven petals, smooth and creamy color that drop immediately after anthesis. There are 252–327 stamens and small stigma with a medium-sized ovary having 4–5 chambers. It flowers twice in a year. It takes about 130 days before attaining the maturity of fruits. The average fruit weight is about 19 g and average vitamin C content is 171 mg/100 g of pulp.
- *P. cujavilis*: The growth characters and flowering habit of the plant are just like those of *P. guineense*. The fruit is small to medium, with an average weight of 30–50 g, and is sour in taste.
- *P. policarpum*: The growth characters are similar to those of *P. guajava* except the shape of the fruits is pyriform. The average fruit weight is about 200–250 g. The flavonoid pattern shows the close affinity between *P. guajava* and *P. molle*. However, in spite of the morphological similarities with *P. molle and P. guineense*, they show minute differences in flavonoid pattern.
- *P. araca* Raddi: It is found plenty in the uplands of Brazil. It is usually a large shrub, with young growth highly marked with hairs; its leaves are large oblong–oval with soft velvety pubescence above and dense pubescence below. The veins are conspicuous and reticulate. The flowers are axillary and formed in clusters of 1–3. The fruit is ovoid or oblong, is yellow in color, and is sweet when ripe.

Guava (*P. guajava* L.)

The Plant

The guava is a small tree or big shrub, which may grow up to 10 m. The plant grows into a symmetrical dome shape, with spreading and low branching canopy (Figure 1). It is a shallow-rooted small tree, branching close to the ground, and often heavily suckering from the base of the trunk. The trunk is slender with greenish brown scaly bark, which peels off in thin flakes from older branches and trunk. Its young twigs are quadrangular in shape. Its leaves are simple, opposite, 7–15 cm long, and 3–5 cm wide. They are oval to oblong–elliptic in shape, with short petioles, smooth, and light green in color. The leaves are finely pubescent below, with the venation conspicuously impressed on the upper surface. Depending on the climatic conditions, the flowering period can differ from one location to another. However, in the mild tropical climate region, the guava trees can bloom and bear fruit throughout the whole year, as long as the humidity and temperature do not become limiting factors. The flowers are perfect, epigynous, 25–30 mm in diameter with four incurved white petals, and a large tuft of white stamens with yellowish anthers borne solitary or in clusters of 2–3 in leaf axils on new growth from mature wood (Figure 2). Self-pollination is conspicuous (60–75%) since even isolated trees produce good crop; however, the distribution of cross-pollination by insects is about 35%.

Figure 1 A guava plant in bearing.

Figure 2 A flower of guava.

The Fruit

Botanically, the fruit of guava is a berry; its shape may vary from round to oblong, ovate, or pear. It may have rough to smooth skin and with small, hard or semihard seeds embedded in the center of the pulp. The skin color of ripe fruits ranges from pale green to bright yellow skin with or without a red blush. The flesh color varies from white, deep pink, to salmon red depending on the cultivar (Figure 3). The fruits of guava possess a strong, unique, and alluring flavor and aroma due to esters and terpenes. The predominant aroma volatile compounds in mature fruit consists of the esters (*Z*-3-hexenyl acetate, *E*-3-hexenyl acetate, ethyl hexanoate, and ethyl butanoate), 1,8-cineole, monoterpenes (myrcene and limonene), and sesquiterpenes (caryophyllene, α-humulene, and

Figure 3 Diversity in shapes and color among guava cultivars.

Figure 4 Fruits of guava 'Allahabad Safeda'.

β-bisabolene). However, the aroma compound profiles may vary with the stage of maturity and cultivar.

Cultivars

The cultivars of guava are classified into two broad categories, based on the fruit shape, namely, the pyriferum (the pear-shaped guava) and the pomiferum (the round-shaped guava), often called as pear guava and apple guava, respectively. They may be further grouped into red-fleshed or white-fleshed based on the flesh color of the ripe fruit. However, both types of cultivars are being grown commercially for fresh fruit and for processing purposes. There are about 400 cultivars of guava, but only a few of them under are commercial cultivation worldwide. 'Allahabad Safeda' (Figure 4) seems to be the world's most cultivated cultivar and is highly popular in India. It has a medium- to large-sized fruit, characterized by its thin, smooth skin; dense, white flesh; and only a few seeds, which makes it suitable for canning. The commercially important guava cultivars of different countries are listed in Table 1.

Table 1 Commercial cultivars of guava grown in various countries

Country	*Cultivars*
Australia	Indonesian Seedless, Allahabad Safeda, Lucknow-49, Beaumont, Ka Hua Kula, GA-11
Bangladesh	Swarupkathi, Mukundapuri, Kanchannagar, Kazi
Brazil	Paluma, Rica, Pedro Sato, Kumagai, Ogawa, Sassaoka, Yamamoto, Século XXI
Columbia	Puerto Rico, Rojo Africano, Extranjero, Trujillo Costa Rica, Tai-kuo-bar
Cuba	Enana Roja Cubana, EEA 1–23
Ecuador	Roja, Blanca, Crema
Egypt	Bassateen El Sabahia, Bassateen Edfina, Allahabad Safeda
India	Allahabad Safeda, Lucknow-49 (Sardar), Banarsi Surkha, Apple Color, Chittidar, Nasik, Dholka, Dharwar, Habshi, Seedless, Red-Fleshed, Behat Coconut
Indonesia	Indonesian Seedless, Indonesian White
Israel	Ben-Dov
Ivory Coast	Supreme, Elisabeth
Malaysia	Kampuchea, Jambu Kapri, Hong Kong Pink, Jambu biji, Putih; Maha 65, Bentong Seedless, Taiwan Pear
Mexico	Media China, Regional de Calvillo, China, la Labor, Acaponeta, Coyame, Kumagai, Paluma, Rica, White Ogawa, Red Ogawa
Pakistan	Safeda, Allahabad, Red-Fleshed, Seedless, Karela, Apple Color
Puerto Rico	Corozal Mixta, Corriente; Seedling 57-6-79 South Africa, Fan Retief, Frank Malherbe, TS-G2
Taiwan	Tai-kuo-bar, Chung-Shan, Shih-Chi, Li-Tzy, Red, Jen-Ju, Shuei-Jing
Thailand	Glom Sali, Glom Toon Klau, Khao Boon Soom
Trinidad	Centeno Prolific, Cayenne, Elisabeth
The United States (Hawaii)	Beaumont, Ka Hua Kula, Hong Kong Pink, Indonesian Seedless
The United States (Mainland)	Redland, Supreme, Red Indian, Ruby X, Miami Red, Miami White, Blitch, Patillo, Webber, Rolfs, Hart, Detwiler, Turnbull
Vietnam	Xa Ly Nghe, Ruot Hong Da Lang, Xa Ly Don

Source: Pommer, C. V., Murakami, K. R. N., and Watlington, F. (2006). A goiaba no mundo. *O Agronômico* **58**, 22–26; Singh, S. P. (2011). Guava (*Psidium guajava* L.). In: Yahia, E. M. (ed.) *Post-harvest biology and technology of tropical and subtropical fruits*. vol. 3. pp. 213–45. Cambridge, UK: Woodhead Publishing Limited

Climate and Soil Requirement

Guava can be successfully cultivated in tropical and subtropical climates. It can be grown in plains and submountainous regions provided with sufficient care and shelter against frost and cold winds during early stages of growth. The optimum temperature for guava production ranges between 23 °C and 28 °C. However, best quality guava fruit is obtained in the regions where low night temperature (10 °C) prevails in the winter months. Guava is fairly tolerant to high temperature and drought conditions but susceptible to severe frost as it may even kill the young plants. Annual rainfall ranging from 1000

to 2000 mm is ideal for guava cultivation, provided it should not coincide with the flowering and harvesting seasons.

Guava grows well in any type of soil ranging from alluvial to laterite, except severely waterlogged soils. However, for sustained commercial cultivation, it is better to grow these trees on fairly well-drained soils. It can tolerate pH ranging from 5.5 to 7.5. Shallow soils or those containing compacted layers may have adverse effect on growth and production. Ideally, guava prefers light sandy to clay loam soils; best quality guavas are produced in the river basins, which are known for their fertile top soil. Guava is one of the few tropical fruits that have some degree of tolerance to salinity.

Fruit Developmental Physiology

Guava is a climacteric fruit and exhibits a typical increase in respiration and ethylene production during the ripening period. Thus, it is a highly perishable fruit that completes its ripening process in just a few days after harvest at room temperature. Depending on the cultivar, guava fruits exhibit a change in skin color from green to yellow due to chlorophyll degradation during ripening. The flesh of the mature fruit changes color from white to creamy white, yellowish pink, deep pink, or salmon red depending on the presence of carotenoids, lycopene, and β-carotene. During different phases of guava maturation, ascorbic acid decreases while pectin methylesterase (PME)-specific activity increases with maturation.

A lot of physicochemical changes take place during the development of guava fruit. There is a gradual increase in fruit weight and volume up to the first 50 days after flowering, but the plant grows rapidly up to 100 days and then, the rate declines thereafter until maturity. Sugars build up in guava during ripening. Fructose content rises rapidly, but the glucose builds up slowly. The pectin content increases during fruit development but declines in overripe fruits mainly due to hydrolyzing action of pectin methylesterase. The extent of loss of total pectin content between cultivars correlates well with the differences in the rate of softening. The activities of the softening enzymes polygalacturonase (PG), PME, β-galactosidase, and cellulase increase with ripening.

Nutritional Value

Guava is highly nutritious, moderately good source of calcium and iron, and fairly rich in phosphorus. Its fruits contain high amounts of vitamins A, B_1 (thiamine), B_2 (riboflavin), and C (ascorbic acid). Vitamin C content of guava fruit is 2–5 times more than that of citrus. High concentration of pectin in guava fruit may play a significant role in the reduction of cholesterol and thereby decreases the risk of cardiovascular disease. Guava is an excellent source of dietary antioxidants; the skin is richer in ascorbic acid and phenols than the flesh. White-fleshed cultivars contain higher concentrations of ascorbic acid, phenols, and sugars compared with the red-fleshed cultivars. Red-fleshed guava is shown to be a richer source of lycopene, ascorbic acid, provitamin A, sulfur aroma compounds, and anthocyanins. Total phenol content is higher in seedless than seeded cultivars, while ascorbic acid was found higher in seeded cultivar.

Table 2 Nutrient composition of guava fruit

Nutritional value per 100 g	
Water	80.80 g
Energy	68 kcal
Protein	2.55 g
Total lipids (fat)	0.95 g
Carbohydrates	14.32 g
Dietary Fiber	5.4 g
Sugars	8.92 g
Minerals	
Calcium	18 mg
Iron	0.26 mg
Magnesium	22 mg
Manganese	0.15 mg
Phosphorus	40 mg
Potassium	417 mg
Sodium	2 mg
Zinc	0.23 mg
Vitamins	
Vitamin C	228.3 mg
Thiamine (B_1)	0.067 mg
Riboflavin (B_2)	0.040 mg
Niacin (B_3)	1.084 mg
Vitamin B_6	0.110 mg
Folate (B_9)	49 μg
Vitamin A (β-carotene)	624 IU
Vitamin E	0.73 mg
Vitamin K	2.6 μg
Others	
Lycopene	5204 μg

Source: USDA (United States Dept. of Agriculture) (2014). National Nutrient Database for Standard. Release 27

Guava fruit is composed of 20% peel, 50% flesh, and 30% seed core. Guava fruit has a low caloric value (68 kcal/100 g) and protein (2.55 g) (Table 2). Mainly, the citric, malic, glycolic, tartaric, and lactic acids contribute toward the acidity of guava fruit. Guava cultivars differ in their sugar contents; fructose, glucose, and sucrose have been reported to be the predominant sugars in guava. Phenolic compounds decrease with firmness, more rapidly initially in white-fleshed than in pink-fleshed guava, but the content remains consistently higher than those of the latter. Guava fruit is one of the richest sources of pectin that is affected by variety, stage of maturity, and crop season. Upon hydrolysis, guava pectin dissociates into D-galacturonic acid (72%), D-galactose (12%), and L-arabinose (4%). Guava is a good source of antioxidant carotenoids (cryptoxanthin, lycopene, and β-carotene), and it contains about 140 μg retinol equivalents/100 g of provitamin A. Pulp and peel fractions are known to contain high contents of dietary fiber and extractable polyphenols. Frequent use of guava has been shown to have hypoglycemic effect. Therefore, guava can be ranked among the most nutritious and healthy fruits of the tropics and subtropics of the world.

Medicinal Uses of Guava

In addition to its use for fresh consumption, guava is also extensively used in folk and traditional medicine system

around the world. The plant is recognized for its antioxidant, hepatoprotective, antiallergic, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, antispasmolytic, antidiabetic, anti-inflammatory, and antinociceptive activities, supporting its traditional uses worldwide. Different pharmacological experiments have identified many medicinally important phytoconstituents, with useful biological properties. These metabolites are mostly phenolic, flavonoid, carotenoid, terpenoid. and triterpenoid compounds. Extracts containing these metabolites, particularly those from leaves, bark, and fruits, possess useful medicinal properties (Table 3). The aqueous extracts of guava leaves are used in folk medicine for the treatment of various types of gastrointestinal disturbances such as vomiting, diarrhea, inhibition of the peristaltic reflex, gastroenteritis, spasmolytic activity, dysentery, abdominal distention, flatulence, and gastric pain. Guava plant finds its use in traditional medicine used as skin tonic and for the treatment of female reproductive problems like dysmenorrhea, miscarriages, uterine bleeding, and premature labor. The important active constituents are essential oils, flavonoids, carotenoids, polyphenolic compounds, pentacyclic triterpenoids, esters, and aldehydes. Leaf extract of guava possess strong antibacterial activity by virtue of its flavonoid glycosides, morin-3-*O*-α-L-lyxopyranoside, morin-3-*O*-α-L-arabinopyranoside, and quercetine-3-*O*-α-L-arabinopyranoside. Antidiarrhea effect is also contributed due to the content of quercetin. Among other major effects of the plant extract are antibacterial and trypanocidal activities, which may be attributed mainly to the broad antimicrobial property of the flavonoids and iron-chelating property of tannins. Traditionally, it is used to treat various conditions. Every part of the plant like leaves, roots, bark, and fruits is used for the medicinal use. The traditional uses are explained in the succeeding text according to the plant parts.

Root

In Fiji and Senegal, the roots of plant are used in the treatment of diarrhea, coughs, stomachache, dysentery, toothaches, indigestion, and constipation. The aqueous extract of root is used in the treatment of diarrhea and dysentery. The roots of the plant are used for their astringent property at a dose of 30–40 g for 1 l or 4–5 cups a day. In the Philippines, the

Table 3 The bioactive ingredient in different parts of guava plant and their medical uses

Part	*Bioactive constituents*	*Medical uses*
Fruit	Vitamin C; vitamin A; iron; calcium; manganese; phosphoric, oxalic, and malic acids; saponin combined with oleanolic acid Morin-3-*O*-α-L-lyxopyranoside and morin-3-*O*-α-L-arabinopyranoside, flavonoids, guaijavarin, quercetin Essential oil contains hexanal, 2,4-hexadienal, 3-hexenal, 2-hexenal, 3-hexenyl acetate, β-caryophyllene, nerolidol, 3-phenylpropyl acetate, caryophyllene oxide, pentane-2-thiol, 3-penten-2-ol, 2-butenyl acetate, 3-hydroxy-2-butanone, 3-methyl-1-butanol, 2,3-butanediol, 3-methylbutanoic acid, (*Z*)-3-hexen-1-ol, 6-methyl-5-hepten-2-one, limonene, octanol, ethyl octanoate (pink guava fruit)	Raw fruits: As astringent and treatment for diarrhea, ulcers, and wounds Ripe fruits: Treatment for anorexia, cholera, diarrhea, digestive problems, dysentery, gastric insufficiency, inflamed mucous membranes, laryngitis, swelling mouth, skin problems, sore throat, ulcers, vaginal discharge
Leaves	α-Pinene; β-pinene; limonene; menthol; terpinyl acetate; isopropyl alcohol; longicyclene; caryophyllene; β-bisabolene; caryophyllene oxide; β-copanene; farnesene; humulene; selinene; cardinene and curcumene; nerolidol; β-sitosterol; ursolic, crategolic, and guayavolic acids; cineol; quercetin; 3-L-4-4-arabinofuranoside (avicularin) and its 3-L-4-pyranoside; tannins; eugenol; caryophyllene; guajavolide and guavenoic acid (2 α-3 β-6 β-2,3-tetrahydroxy-12,20(30)-dien-28-oic acid); triterpenes such as oleanolic acid, triterpenoids; flavinone-2, 2′-ene; prenol; dihydrobenzophenanthridine; and cryptonine	Used as antiamoebic, anti-inflammatory, antispasmodic astringent, blood cleanser, febrifuge, gargle for sore throats and laryngitis, and treatment for bacterial infections, convulsions, cough, diabetes mellitus, diarrhea, dysentery, gastroenteritis, hypertension, itchy rashes caused by scabies, rheumatic pain, skin ulcers, vaginal irritation and discharge, sores, boils, sprains toothache, ulcers, and wounds
Bark	Polyphenols, resin, and crystals of calcium oxalate	Used as febrifuge, to expel the placenta after childbirth, as respiratory disturbance astringent, as a bath to treat skin ailments, and as treatment for colds, cough, hypoglycemia, affections of the skin, vaginal hemorrhage, wounds, dehydration
Root	Tannin, leucocyanidins, sterols, gallic acid, carbohydrates, salts, tannic acid	Used as skin tonic, treatment for painful menstruation, miscarriages, uterine bleeding, premature labor in women, wounds, diarrhea, coughs, stomachache, dysentery, toothaches, indigestion, constipation
Seed	Proteins, starch, oils, phenolics, flavonoid compounds, flavonol glycosides, quercetin-3-*O*-β-D-(2″-*O*-galloyglucoside)-4′-*O*-vinylpropionate	

Source: Joseph, B. and Mini, P. (2011). Review on nutritional, medicinal and pharmacological properties of guava (*Psidium guajava* Linn.). *International Journal of Pharmacy and Bioscience* **2**, 53–69; Gutiérrez, R. M. P., Mitchell, S. and Solis, R. V. (2008). *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology* **117**, 1–27

decoction and poultice of the root are used for the treatment of ulcers, wounds, and diarrhea.

Bark

The bark of the guava plant is extensively and anciently been used as astringent, febrifuge (antifever), and antiseptic properties. The decoction made from bark is used in the treatment of ulcers. The decoction and poultice form of the plant is used in Tahiti and Samoa to expel the placenta after childbirth and in the infection of the skin, caries, vaginal hemorrhage, wounds, and respiratory disturbances. In Kinshasa, the decoction form is also used to treat diarrhea and amoebic dysentery.

Leaf

The leaves of the plant have been known for the management of diarrhea, wounds, ulcer, toothache, stomachache, and diabetes. The infusion and decoction formulations of leaves are anciently used for the treatment of leucorrhea-like condition. Decoction of the leaves are used as gargles for sore throats, swelling of the mouth, laryngitis, external ulcer on the skin, and vaginal irritations in the Latin America, Central and West Africa, and Southeast Asia. Guava leaves possess anti-inflammatory properties and are also used in various lung problems. In addition to this, leaves are used in various bacterial infections, diarrhea, and blood cleansing. Leaf extract has also been reported to have the strong antimicrobial properties.

Fruit

Various evidences indicate that the ripe fruit of guava has laxative action, whereas the unripe fruit decoction is used as astringent and an antidiarrheic and has medicinal uses. It has been shown that fruit of *P. guajava* is used for the treatment of anorexia, cholera, diarrhea, digestive problems, dysentery, gastric insufficiency, inflamed mucous membranes, laryngitis, swelling of the mouth, skin problems, sore throat, ulcers, and vaginal discharge.

Harvesting and Postharvest Handling

Maturity of the fruits at the time of harvest determines its quality and storage life. The maturity of fruits is usually assessed on the basis of color, specific gravity, total soluble solids, acidity, etc. Fruits generally take 4–5 months after flowering to reach harvesting maturity, characterized by change in color from green to pale green. At the time of fruit harvest, the specific gravity of fruits becomes less than 1, that is, the mature fruits float in water. Various studies have reported that the fruits with specific gravity from 1.00 to 1.02 have better shelf life and tolerate long distance transport. Guava fruits are commonly picked by hand and carefully kept in containers under shade before transporting to grading and packing facility. Fruits stored in corrugated fiberboard boxes with ventilation and paper lining can be stored at 6–8 ° C with 85–90% relative humidity for 20 days.

Processing

Being a highly perishable fruit, guava is processed into a number of products to extend its availability throughout the year. Ascorbic acid content in these processed products is generally less than the fresh fruits as the losses during processing are inevitable. Ascorbic acid is highly heat-labile and the most critical factors in determining its stability in fruits are the storage temperature and duration. Guava can be either processed into juice, jam, jelly, squash, concentrate, toffee, etc., or preserved as pulp, canned as slices and dehydrated products, etc. The selection of guava cultivar for processing depends upon its quality characters like pulp, sugar/acid ratio, pectin, tannins, and vitamin C content. Some important processed products of guava are briefly discussed later in the text.

Puree

Guava puree is preferably made from slightly overripe guava fruits having yellow skin, soft flesh, and intense musky aroma. Both seriously spoiled and underripe fruits are sorted out from the lot meant for making puree. The underripened fruits are kept aside for further ripening. Fruits that meet the standards are washed and processed in a paddle pulper fitted with 0.008–0.11 cm screens. This pulp is then passed through a finisher fitted with 0.05 cm screens to remove stone cells. The fine puree thus obtained is deaerated and pasteurized for 60 s at 90 °C. This end product can be canned, preserved by freezing to −29 °C and storing at −18 °C, or aseptically packaged or dehydrated.

Juice

Guava juice is recognized for its refreshing taste and thirst-quenching and high nutritive value. Guava juice can be produced either from fresh fruits or from puree. Fully ripe fruits are cut into small pieces and supplemented with 0.2 g citric acid and 250 ml water/kg of fruit. The mix is cooked while stirring constantly and strained through a muslin cloth. The juice/extract thus obtained is collected and packed aseptically in bottles. Juice yield can be increased by using pectic enzymes. Similarly, juice can be prepared from the puree/pulp by diluting it with water followed by filtration. Guava juice is reported to have 11% TSS, 6.64% total sugars, 6.02% reducing sugars, 0.76% acidity, 3.85 pH, 243 mg/100 g ascorbic acid, and 1.02% pectin. Guava juice can be further processed and utilized for producing concentrates, beverages, jelly, powder, and other products.

Concentrate

It is convenient to make concentrate from guava puree or juice for facilitating overseas shipment and long-term storage. The equipments required for this purpose include a falling film evaporator, a rising film evaporator, and a centrifugal evaporator. Guava puree is treated with pectic enzymes before concentration to reduce its viscosity. This treated puree can then be easily concentrated to 34°Brix, whereas the clarified juice can be concentrated to 66°Brix as it remains flowable in an evaporator. The loss of flavor during evaporation is compensated by cutting back the product to a lower concentration or by diluting it with single-strength fresh-pasteurized puree or clarified juice.

Nectar

Guava nectar is a popular beverage that can be prepared from puree, clarified juice, or concentrate with sugar syrup, citric acid, and other flavoring additives. It is prepared using pulp, soluble solids, and acidity at a rate of 15%, 14%, and 0.25%, respectively. The nectar can be supplemented with vitamin C (100 mg/100 g). Guava nectar can also be diluted four times with water to prepare quality-bottled ready to serve (RTS) beverage. Guava nectar needs homogenization for maintaining consistency during storage. Then, it is either pasteurized or commercially sterilized for better shelf life. Under ambient storage conditions, canned guava nectar has a shelf life of 6 months.

Canned guava

It is another important product of guava that is quite popular in India, Pakistan, and Indonesia. The fruits are preserved by canning as halves or quarters. Fully ripe but firm guava fruits are either peeled with a knife or lye-peeled (by dipping for 15 s in boiling solution of 2.5% sodium hydroxide). After rinsing with water, it is dipped in 0.5% solution of citric acid to neutralize the remaining alkali. The fruit is cut into halves or quarters and the seed core is removed to obtain the shells. The cut pieces are dipped for 5 min in 2% brine solution and then canned in 40°Brix sugar syrup containing 0.25% citric acid. The cans are sealed, sterilized in boiling water for 20–25 min, and then cooled to room temperature.

Jelly

Guava jelly is a major product prepared from the guava fruits. Slightly underripe guava fruits are ideal for making jelly. The fruits are crushed and juice is extracted as explained earlier in the text. The juice is decanted and boiled with sugar for jelly making, whereas the pulp is used for the preparation of guava cheese. The quantity of sugar needed for making jelly depends on the pectin content of juice, which generally varies from 0.75 kg sugar/kg of pectin-rich juice to 0.5 kg sugar/kg of low-pectin juice. Boiling is continued until it forms a sheet when a small portion is cooled off in a spoon. This hot jelly is then filled into glass jars and sealed.

Guava fruit leather (or cheese)

Better quality cheese is prepared from fresh fruits rather than leftover pulp from jelly making. Firm ripe fruits are washed, sliced, and cooked in equal amounts of water for about 45 min. The pulp thus obtained is screened through a muslin cloth or filtered to remove the skins and the seeds. This pulp is supplemented with 1.25–1.50 kg sugar, 2.2–3.3 g citric acid, and 56 g butter per kilogram of fruit pulp. The mixture is cooked to a thick paste and small amounts of permitted color and common salt are added to improve the appearance of the final product. The hot cheese is spread on a greasy tray and is allowed to set. After cooling, it is cut into small pieces and wrapped in moisture-proof paper. This cheese can be stored at 4 °C for about 4 months without the loss of sensory quality.

Dehydrated guava products

Guava slices or chunks can be dehydrated by air-drying or osmotic dehydration. In air-drying technique, the guava slices are blanched in boiling water for 4 min, sulfured for 20 min, and then air-dried at 70 °C until final moisture level of 6–7% is achieved. Glazed guava slices are prepared by osmotic dehydration technique. Guava slices are heated in an equal amount of 70°Brix sugar syrup containing 0.1% KMS at 90 °C for 3 min. After cooling to room temperature, this mixture is allowed to stay overnight. Then, slices are drained and spread in glycerin-coated trays for drying at 80 °C for 1 h. The air temperature is then lowered to 65–70 °C for the next 7–8 h. The end product is packed in air-tight containers.

Future Development and Uses

Most of the nutritional and medicinal uses of guava are based on the traditional knowledge and very little is known about its bioactive ingredients. A detailed research is needed to identify and enhance these beneficial biomolecules so that it may be efficiently used to cure health problems. A few things that are needed for its further development and use are as follows:

- Dedicated breeding operations for developing the varieties for specific and dedicated use, namely, fresh consumption, processing, and medicinal uses.
- Colored varieties of guava are known to be more nutritionally rich, which calls for development of such cultivars packed with antioxidants.
- Identification, extraction, and isolation of the specific and medicinally important biomolecules need to be done from all parts of the plant.
- Biotechnological interventions are required to further enhance the content of medicinal and nutritional components in the plant.

See also: Antioxidants: Role on Health and Prevention; Apples; Ascorbic Acid: Physiology and Health Effects; Carotenoids: Occurrence, Properties and Determination; Diarrheal Diseases; Fruits of Tropical Climates: Biodiversity and Dietary Importance; Pectin and Health.

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Gums: Dietary Importance

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Background

The dietary importance of gums is related to their effects at various sites in the gastrointestinal tract from the stomach to the large intestine. Most of their actions in the upper gut have been related to the development of a high viscosity in the gut lumen. However, gums in natural foods may not be released in sufficient quantities to increase luminal viscosity. Isolated gums or foods fortified with gums, however, can have very significant effects on nutrient absorption and postprandial plasma nutrient levels. Other important effects relate to the fermentation of the gums by the microflora in the large intestine. A high intake of particular gums may help in the treatment of constipation and diabetes and in the prevention of colon cancer. However, only the benefits for treating constipation have been well established, and more research is needed in their use for other conditions.

Physiochemical Properties and Physiological Action

In this context, the term 'gum' covers a wide range of polysaccharide molecules that have varied structures, physical properties, and physiological actions. The term, for the most part, could be interchanged with 'soluble nonstarch polysaccharide' or soluble dietary fiber. There are many different dietary sources of gums. They are found naturally in plant cell walls (pectins and hemicelluloses) and as plant exudates (gum arabic) or mucilages (ispaghula[1]) and are produced by some bacteria (xylan) and algae (alginates). The effects of gums on human physiology will depend on whether the gum is ingested as part of a plant cell wall, as part of a food complex, or as an isolated polysaccharide.

Most, but not all, gums produce viscous solutions in their isolated form, and some (such as pectin) have cation-exchange properties. The extent of the viscosity or cation exchange depends on the length of the molecule and the constituent sugars. The physiological action of such gums in the diet can therefore vary enormously and may differ from one dietary source to another. If the gum is ingested within the framework of a plant cell wall, as with pectin in carrots, the pectin may not be released in sufficient amounts to have a significant action on small intestinal function but may still be available for fermentation in the large intestine. In addition, the cation-exchange properties of an isolated pectin may be greater than pectin contained in a food because the potential binding sites may already be taken up by minerals or other charged molecules found in the food matrix.

[1]The terms 'ispaghula' and 'psyllium' (used later) are interchangeable.

Another important consideration is the amount of gum ingested. The amount in foods, or added as stabilizers, emulsifiers, and fat substitutes, is small, and most of the effects described in the succeeding text require a substantial intake of isolated gum. For example, the standard dose of ispaghula for the treatment of constipation is 3–11 g per day. The dose recommended for the action of guar gum on small intestinal absorption is 15 g per day, whereas the amount of pectin in carrots is 1.5 g per 100 g, and there is only a trace of gums in ice cream.

The physical characteristics of each gum are determined by sugar composition as well as the order of sugars and degree of modification, e.g., the degree of methylation of pectin. Viscosity results from the entangling of free carbohydrate chains. This requires a disorganized network, and so the viscosity of gums is determined by their molecular weight and the degree of irregular versus regular sugar patterns, the latter allowing more ordered cross-linkage between chains.

The gums have a range of physiological and nutritional effects (Table 1) mediated mostly through effects on transit through the stomach, small intestine, and colon, by reducing mixing actions in the gut; by their actions on the interaction between nutrients, enzymes, and mucosal cells; and finally as a result of their fermentation by the colonic microflora.

Effects in the Stomach and Small Intestine

The effects of isolated gums on gastric emptying and digestion and absorption have been well studied. The effects of the same gums in foods are less well established. Viscous gums eaten as part of a mainly liquid meal can slow gastric emptying by

Table 1 Properties of gums that affect human physiology and health

Property	*Consequence*	*Main examples*
Viscosity	Slower absorption Lower blood glucose Reduced cholesterol Increased satiety	Guar gum, pectin, ispaghula (psyllium), oat gum
Cation exchange	Binding of minerals Binding bile acids Reduced cholesterol	Pectin
Fermentability	SCFA production Low colonic pH Altered microflora	Oat gum, pectin, guar gum
Butyrate production	Colonic fuel Colonic health Possibly anticancer	Oat gum
Stool bulking	Reduced constipation Dilution of carcinogens	Ispaghula (psyllium), gellan

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making the liquid meal of a more solid consistency. Guar gum, which has one of the highest viscosities, can delay gastric emptying half time by 60%. However, it has also been shown that increased viscosity can facilitate the emptying of discrete solids. In this case, they may act as lubricants and ease passage through the small 2 mm opening of the pyloric sphincter.

In the small intestine, the main impact of increased viscosity is to resist the movement of the intestinal contractions, reducing the mixing of the digestive enzymes with their substrates and inhibiting the movement of the nutrients to the absorptive epithelium. This can give the impression of an increase in the thickness of the unstirred water layer adjacent to the intestinal mucosa, through which nutrients must pass by diffusion. These two effects, along with slowed gastric emptying, can delay the process of digestion and absorption, resulting in decreased postprandial glycemia and potentially reducing fat absorption.

In addition, the viscous matrix of a gum solution can trap endogenous molecules such as bile acids. Bile acids are essential for the emulsification of fats to allow digestion and for the formation of micelles to facilitate movement of the products of fat digestion to the mucosa for absorption. A decrease in bile acid concentration will therefore disrupt and delay fat digestion. If cation-exchange properties persist, then the bile acids may be bound to the charged gum, and this will have an additional impact in fat digestion. The trapped bile acids, if not released in the large intestine during fermentation, are excreted in feces, which will result in reduced plasma cholesterol as replacement bile acids are synthesized from this precursor.

There are, however, some problems with this simplistic explanation of the effects of viscous gums on postprandial glucose and fat absorption. The viscosity shown in *in vitro* models to delay digestion and reduce mobility of nutrients may not be achieved in the part of the small intestine where most digestion and absorption occur.

Most digestion occurs in the duodenum and jejunum. When foods enter the duodenum, pancreatic secretion of large amounts of bicarbonate- and enzyme-rich juice is stimulated. This relatively large volume of fluid will dilute the small intestinal contents and considerably reduce the concentration and therefore the viscosity of the food gum. The relationship between concentration and viscosity is not linear but exponential, and changes in concentration can have a major impact on the viscosity maintained. Experiments in rats have failed to find any large increases in upper small intestinal viscosity after ingestion of large amounts of guar gum. The gums are not digested, however, so the viscosity is regained as the nutrients and fluid are absorbed. Thus, the viscosity in the ileum where bile acids are reabsorbed, as part of the enterohepatic circulation, will be much higher. It is well established, however, that postprandial blood glucose is reduced considerably after ingestion of gums. Bread containing lower-molecular-weight guar still has the action of reducing postprandial glycemia, again suggesting that viscosity may not be the critical factor.

Blood Glucose and Diabetes Mellitus

In the past, the use of gums such as isolated solutions of guar gum to reduce the rise in postprandial blood glucose was thought to be of some therapeutic use in diabetes mellitus. Although the effects of guar gum reducing postprandial hyperglycemia and insulinemia are well established, this approach in treating diabetics was not very successful, as the gum solutions used had poor organoleptic properties with an undesirable mouthfeel and aftertaste. Since then, the incorporation of gums such as guar and oat gum in bread products has been explored. It has been found that very palatable breads with proved effects on intestinal absorption rate can be produced, but their use in the diabetic diet has still to be established. There has also been much recent interest in the production and action of cereal products containing oat β-glucans, ispaghula (psyllium), and konjac mannan.

Therapeutic Enteral Diets

Sometimes, patients, including children, have a problem in dealing with normal foods and are fed with a simplified synthetic fluid diet of easily assimilated nutrients. These diets often have to be liquid to allow administration through tubes directly into the gastrointestinal tract. Until recently, these elemental or polymeric diets have contained no source of dietary fiber. This can result in constipation and, after prolonged use, produce atrophy of the colonic mucosa. Some of these liquid diets have now been formulated with soluble gums as a source of dietary fiber to prevent these symptoms and to maintain a healthy microflora. The gums used include soy polysaccharide, pectin, and carboxymethylcellulose.

Effect of Gums on Plasma Cholesterol

Heart disease is one of the major killers of the developed world, and one of the main risk factors is high plasma low-density lipoprotein (LDL) cholesterol coupled with low high-density lipoprotein (HDL) cholesterol. HDL cholesterol is associated with clearance of sterol from the peripheral tissues to the liver. Many of the viscous food gums (oat, pectin, guar, psyllium, and others) have been shown to have a significant and long-term effect in reducing LDL cholesterol while having little effect on, or increasing, HDL cholesterol. The effect of the gums is modest but may be of significance in reducing the risk of heart disease.

It is not clear by which mechanisms the gums act (Table 2). They could reduce fat digestion either directly or by interfering

Table 2 Possible mechanisms for the effect of gums on serum cholesterol

Inhibition of fat digestion
Viscosity inhibits mixing of enzymes and lipids
Viscosity/cation exchange traps/binds bile acids
Viscosity inhibits the formation of micelles
Viscosity inhibits the movement of micelles
Increased bile acid synthesis from cholesterol
Binding of bile acids by cation exchange, viscosity
Increased fecal bile acid losses
Increased fecal cholesterol losses
Inhibited cholesterol absorption
Increased intestinal cell losses
Decreased cholesterol synthesis
Inhibition of hepatic cholesterol synthesis by propionic acid (probably only a pharmacological effect)

with micelle formation. Although there is likely to be only a small increase in total fat losses, the delaying of fat absorption can result in a change in the type of chylomicra or lipoproteins produced. For those gums like pectin with cation-exchange properties, the bile acids may be bound to the carbohydrate molecule, which are then excreted in the feces. Viscous fibers without cation exchange may also trap bile acids within their matrix. Several gums, including guar, pectin, and ispaghula (psyllium), have been shown to increase fecal bile acid losses, and this has been associated with reduced cholesterol. However, if the fiber is fermented, the bile acid would be released in the large intestine where they could be converted to secondary bile acids and returned to the bile acid pool. Moreover, low-molecular-weight (and therefore low-viscosity) guar gum in bread still reduced cholesterol, and not all studies demonstrating an effect on cholesterol show an increase in fecal bile acid. If fecal bile acid losses are increased, cholesterol in the liver is used to replace them, and hence, plasma cholesterol falls.

Cholesterol levels could also be decreased due to the higher fecal losses of cholesterol from increased intestinal cell loss seen with viscous fibers, and there has been much discussion about the potential actions of propionic acid produced from the fermentation of the gums. However, the inhibitory effects of propionic acid on hepatic cholesterol synthesis appear to require pharmacological levels of propionate rather than those that are produced from the fermentation of food gums under normal conditions. Acetate is a precursor of fat synthesis and lactulose, a highly fermentable saccharide, increased serum LDL cholesterol when fed to healthy volunteers. This was associated with higher levels of serum acetate in some subjects. It is evident that the actions of gums on serum cholesterol are mediated by different mechanisms for individual gums and for the same gums under different circumstances.

Satiety and Obesity

If gums had a major effect on satiety, they could be a useful adjunct to the prevention and treatment of the increasing public health problem of obesity. However, although highly viscous gums such as guar have been shown to increase satiety and even promote some weight loss in short-term studies, the unpalatability of such gums and the moderate effects they have on weight make them of unlikely benefit in weight-loss or weight-maintenance programs.

Gums and Mineral Bioavailability

Some food gums with cation-exchange properties have been shown *in vitro* to bind minerals such as calcium. Even those gums without direct binding sites may still entrap these minerals and delay or inhibit their absorption. *In vivo* human studies have shown an increase in fecal mineral output, but the overall mineral balance is not affected in adults. The effect in young children is unknown and is one of the reasons why a high dietary fiber intake has not been encouraged in the under-fives. However, there is no evidence of significant mineral imbalance in children on high-fiber diets, and the effects are unlikely to be great. Moreover, there are several studies now suggesting increased calcium absorption in the large intestine, perhaps encouraged by the reduced pH from the bacterial fermentation of soluble fiber.

Effects of Gums in the Large Intestine

Most food gums are readily fermented in the large intestine of man by the diverse and dense microflora. The products and consequences of this fermentation may have a major influence on human health, and these effects may outweigh the impact of the food gums in the small intestine.

Colonic Fermentation

The human colonic microflora is thought to contain over 400 different species (perhaps even more as new molecular techniques, which measure bacterial DNA and rRNA, are discovering that the majority of bacterial groups have never been cultured). The mainly anaerobic bacteria use the carbohydrates to produce short-chain fatty acids (SCFA), mostly acetic, propionic, and butyric acids, and the gases CO_2, H_2, CH_4, and H_2S. Colonic pH is also determined by the balance between the rate of fermentation and the buffering of the SCFA by bicarbonate exchanged when the SCFA are absorbed.

The fermentability of each gum depends on its structure and water solubility. The presence of bacteria with the required enzymes for each step of gum degradation is also essential. The degradation and fermentation of a complex carbohydrate structure may be the result of a synergy between several bacterial species, and some of the enzymes may take time to be expressed either by induction of enzymes, in originally predominant bacteria, or by an increase in dominance by new groups. It has been shown in rats that it can take many weeks before the colonic microflora is truly adapted to the introduction of a new gum into the diet.

The extent and rate of fermentation of food gums are important. The rate of fermentation may determine the site of SCFA production. Most colonic disease occurs in the distal colon, and yet, most fermentation occurs in the proximal colon. Therefore, food gums that are slowly fermented may encourage prolonged fermentation and SCFA at more distal sites.

The pattern of SCFA produced from each gum also differs. Pectin increases the proportion of acetic acid produced, guar gum and ispaghula tend to promote the production of propionic acid, and oat gum increases the production of butyric acid.

Health Impact of Increased Colonic Fermentation

The colonic fermentation of carbohydrates including food gums can result in a substantial fall in colonic luminal pH. There are several benefits of reduced colonic pH, which include the inhibition of undesirable bacterial activities such as 7α-dehydroxylase, which forms secondary bile acids that can help to promote cancer. Reduced colonic pH can also lead to precipitation of fatty acids, sterols, and other molecules, which have been implicated as procarcinogens. Low pH also reduces the rate of colonic cell proliferation, thought to be a risk factor for colon cancer. However, the SCFA produced during

fermentation can stimulate cell proliferation, and so under normal conditions, the effects of pH here may be canceled out. It has been noted, however, that populations in South Africa with a low risk of colonic cancer also have a lower colonic and fecal pH than those from high-risk populations.

The side effects of increased colonic fermentation and of ingestion of large amounts of food gums include flatulence and abdominal distention. These are usually associated with eating large amounts of fermentable material but may reduce over time as the colonic microflora adapts to the new carbohydrate substrate supply and adopts a more efficient fermentation process. This may take over a week.

The SCFA have many potential actions that are generally beneficial for health. They promote the absorption of water and electrolytes and inhibit the growth of pathogens, reducing the risk of diarrhea. They all stimulate cell proliferation throughout the gut, even though they are produced in the large intestine, and this may be important for wound healing after gut surgery or after gastrointestinal disease. In addition to these general effects, each fatty acid has its own idiosyncratic actions.

Actions of Individual SCFA

Acetic Acid

Acetic acid is always the main SCFA produced, usually making up 50–70% of the SCFA. Acetic acid is the only SCFA to reach the systemic circulation in significant amounts. It can provide energy for muscles and other tissues and is noninsulinogenic. Acetate has less effect on the colonic enterocytes in terms of proliferation and motility than the other SCFA. Pectin has been shown to increase the proportion of acetate when fermented by fecal bacteria *in vitro*. Acetate produced during pectin ingestion may also beneficially affect fibrin network architecture in patients with hypercholesterolemia, reducing the risk of heart disease.

Propionic Acid

Propionic acid is usually the second most abundant SCFA produced during fermentation. Propionic acid is absorbed into the portal vein but is mainly removed by the liver. It is gluconeogenic, unlike the other SCFA, and was previously thought to inhibit cholesterol synthesis by the liver. However, although this effect has been well demonstrated under experimental conditions, it is now thought that not enough propionic acid is formed in the colon and absorbed into the portal vain to have this effect. However, it is worth noting that those gums that are associated with cholesterol-lowering effects are often those that promote propionate production.

Butyrate

Butyric acid is the preferred fuel for the colonic enterocyte. It has also been associated with several potential anticancer properties. An increase in the rate of colonic cell proliferation is believed to be a risk factor for cancer. However, all cells in the colon are programmed with a time to die by a controlled process called apoptosis. It is probably the balance between the levels of proliferation and apoptosis that determine whether a cancer will or will not develop. Although butyrate has been shown to stimulate cell proliferation of normal colonic cells, it has been shown to stimulate apoptosis in cancer cells *in vitro*. It may also affect other important stages in carcinogenesis such as the promotion of cell differentiation and inhibition of histone deacetylase, which may promote DNA repair.

Gums as Prebiotics

There has been much recent interest in the beneficial health effects of certain strains of lactic acid bacteria or probiotics believed to have various health benefits. These include reducing the duration of rotavirus diarrhea, reducing eczema in atopic infants, and potentially having protective actions against intestinal diseases, including colitis and cancer. These probiotics are usually bifidobacteria, lactobacilli, or streptococci but may include certain strains of *E. coli*. The survival of these bacteria in the gut can be enhanced by the presence of certain fermentable carbohydrates. These are called 'prebiotics,' but to fulfill the definition, carbohydrates must selectively increase the populations of the probiotic organisms. The most accepted prebiotics are fructooligosaccharides and resistant starch, but some gums may also increase bacterial populations.

Calories from Fermentation of Gums

SCFA produced from fermentation can be used by the colonic cells and other body tissues for fuel. It is now recognized that dietary fibers, including gums, contribute energy, but the exact amount depends on the fermentability and any increased fecal nitrogen losses. After absorption, the SCFA have to be acylated before entering the major metabolic pathways, and this also has an energy cost. It is now estimated that, on average, fermentation provides 8.4 kJ (2 kcal) per gram of carbohydrate.

Constipation

If a dietary gum is poorly fermented, it may have sufficient residual water-holding capacity to increase stool output. Ispaghula (psyllium), which has a water-holding capacity of approximately $7\ g\ g^{-1}$, has been used successfully to treat mild to moderate constipation for many years. Gellan, a bacterially produced gum, has also been shown to be a potent stool bulker but is not used therapeutically. Other gums such as karaya and xanthan, which are slowly fermented, may increase stool wet weight and fecal SCFA without increasing stool solids.

See also: Colon: Diseases and Disorders; Dietary Fiber: Determination; Pectin and Health; Pectin: Properties Determination and Uses; Probiotics.

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Gums: Properties and Uses

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Introduction

The term 'gums' refers to a range of natural polymers, mainly polysaccharides, that are widely used in the food industry to control the rheological and organoleptic properties of food products. They are employed to perform a number of functions including the thickening and gelling of products; the stabilization of foams, emulsions, and dispersions; the inhibition of ice and sugar crystal formation; and the controlled release of active compounds such as flavors and antioxidants. An area of considerable current interest is in the application of gums as edible films, which can be used to coat fruit and vegetables and hence increase their shelf life. Their film-forming properties are also facilitating their application in biodegradable packaging. The commercially important gums are listed in Table 1 together with their source, function, and market size. It is evident that the market is dominated by starch and gelatin.

The international numbering system (INS) for food additives has been developed so that additives approved for food use can be identified on food labels as an alternative to listing their names. In the European Union, they are referred to as E numbers, and where both INS and E numbers are available, they are interchangeable. The list of INS numbers are given in Tables 2 and 3.

Although gums have primarily been used in foods to control texture, organoleptic properties, and stability, consumers are now being made increasingly aware of their nutritional and health benefits. Many gums, including locust bean gum, guar gum, konjac mannan, gum arabic, xanthan gum, and pectin, have been shown to reduce blood cholesterol levels. In addition, the nonstarch polysaccharide gums are resistant to human digestive enzymes and pass through the stomach and small intestine without being metabolized. They are fermented in the large bowel to yield short-chain fatty acids, thus lowering the risk of colon cancer. Gums are nowadays widely used for fat replacement in a wide range of low-calorie products. They may be added in their natural form or as specifically fabricated microparticulates and serve to enhance the rheological properties of low-fat products.

Functional Characteristics

Gums will dissolve or swell in water, although in many cases, high temperature and vigorous agitation are required before complete dissolution is achieved. The solutions formed are usually thick and viscous even at very low concentrations (1%). There are, however, notable exceptions, for example, gum arabic solutions only become viscous at concentrations of ~50%. The viscosity of polymer solutions generally shows a marked increase at a critical polymer concentration, commonly referred to as C*, which corresponds to the concentration at which the polymer molecules start to touch and interpenetrate due to molecular crowding. C* decreases with increasing hydrodynamic volume of the gum molecules, which in turn is influenced by their molecular mass, shape, and charge density. Hence, high-molecular-mass, linear molecules possessing ionizable groups tend to be good thickeners.

Gum solutions normally exhibit Newtonian behavior at concentrations well below C*, that is, their viscosity is independent of the rate of shear. However, above C*, non-Newtonian behavior is usually observed, and the viscosity–shear rate profiles show three distinct regions, that is, a high-viscosity, low-shear Newtonian plateau (at shear rates typically $<1\ s^{-1}$), a shear-thinning region ($\sim 1–1000\ s^{-1}$), and a low-viscosity high-shear Newtonian plateau (typically $>1000\ s^{-1}$).

Some gums, notably xanthan gum, have a tendency to undergo weak intermolecular association in solution leading to the formation of a three-dimensional network structure, thus giving rise to a very high viscosity. The junction zones formed can be readily disrupted even at very low shear rates resulting in a dramatic drop in viscosity. Other polysaccharides self-associate and form stable junction zones, and as a consequence, strong gel structures are produced. The gels are referred to as 'physical gels' because the junction zones are formed through physical interaction, for example, by hydrogen bonding, hydrophobic association, and cation-mediated cross-linking, and differ from synthetic polymer gels, which normally consist of covalently cross-linked polymer chains.

Some gums form thermoreversible gels and examples exist where gelation occurs on cooling or heating. Some form non-thermoreversible gels. In such cases, gelation may be induced by cross-linking polymer chains with divalent cations. Gels may be optically clear or turbid and a range of textures can be obtained. Gel formation occurs above a critical minimum concentration, which is specific for each gum. Agarose, for example, will form gels at concentrations as low as 0.2%, while for acid-thinned starch, a concentration of ~15% is required. Typically, however, concentrations of less than 1% are required. Gel strength increases with increasing concentration.

Mixtures of gums are commonly used to impart novel textural characteristics to food products, and an added incentive is a reduction in costs. Classic examples include the addition of locust bean gum to kappa carrageenan to yield softer more transparent gels and also the addition of locust bean gum to xanthan gum to induce gel formation.

Gums are effective at either inducing or preventing flocculation in particulate dispersions. If insufficient gum is present to coat all the particles fully, aggregation occurs owing to polymer adsorbing onto two or more particles simultaneously, thus inducing bridging. If the particles are fully coated, particle aggregation is prevented by 'steric repulsion,' which arises as a result of interaction between the adsorbed polymer layers. Although nonadsorbed gum is generally considered to enhance the stability of dispersions (and emulsions) by increasing the viscosity of the aqueous phase, it can have the opposite effect and give rise to 'depletion flocculation.' This

Table 1 The main commercial gums

Gum	*Source*	*Principal function*	*Global market value $ million*[a]
Agar	Algae	Gelling agent	219
Alginate, (sodium)	Algae	Gelling agent	272
Propylene glycol alginate		Emulsifier and foam stabilizer	
Gum arabic	Tree gum exudate	Emulsifier	147
Carrageenan	Algae	Gelling agent	561
Processed *Eucheuma* seaweed		Gelling agent	
Carboxymethyl cellulose	Trees, cotton	Thickener	169
Hydroxypropyl cellulose		Thickener and emulsifier	80
Methyl cellulose		Thickener, emulsifier, and gelling agent	
Cellulose (microcrystalline)	Trees	Thickener and gelling agent	91
Gelatin	A protein obtained from the skins and bones of cattle and pigs and skins of fish	Gelling agent	1030
Gellan gum	Bacterial cell wall	Gelling agent	
Guar gum	Plant seeds	Thickener	94
Karaya	Tree gum exudate	Thickener	
Konjac mannan	Tuber	Thickener and gelling agent	
Locust bean gum	Plant seeds	Thickener	85
Pectin	Plant cell wall	Gelling agent	658
Amidated pectin		Gelling agent	
Starch (modified)	Plant seeds, roots, tubers, stem piths	Thickener and gelling agent	1275
Tragacanth	Tree gum exudate	Thickener	
Xanthan gum	Bacterial cell wall	Thickener	277

[a]Dennis Seisun in Gums and Stabilisers for the Food Industry, Williams PA and Phillips GO eds RSC Publication 335 pp3 (2012).

phenomenon occurs as a consequence of polymer molecules being excluded from the space between approaching particles. The difference in osmotic pressure between the depleted region and the bulk solution results in weak interparticle attractive forces, which induce aggregation.

Some gums, such as gum arabic, methylcellulose, and propylene glycol alginate, show amphiphilic character that has led to their use in the stabilization of emulsions and foams owing to their affinity to adsorb at the oil–water or air–water interface.

In systems containing sugar or ice crystals, gums can retard crystal growth either by adsorbing onto the crystals or by competing for available water molecules.

Properties and Uses of Individual Gums

Tree Gum Exudates

The four main tree gum exudates are gum arabic, gum karaya, gum tragacanth, and gum ghatti, with gum arabic being, by far, the most important commercially.

Gum arabic occurs as a sticky liquid that oozes from the stems and branches of acacia trees (*Acacia senegal* and *Acacia seyal*), which grow across the Sahelian belt of Africa, principally Sudan. The liquid dries in the sun to form glassy nodules, which are collected by hand. The gum from *Acacia senegal*, traditionally the main source of gum arabic, is a complex polysaccharide consisting of galactopyranose (~44%), arabinopyranose, arabinofuranose (~25%), rhamnopyranose (~14%), glucuropyranosyl uronic acid (~15.5%), and 4-O methyl glucuropyranosyl uronic acid (~1.5%). It also contains a small amount (~2%) of protein as an integral part of the structure. Analysis of the carbohydrate structure has shown that it consists of a core of (1,3)-β-D-galactose units with extensive branching at the C6 position. The branches consist of galactose and arabinose and terminate with rhamnose and glucuronic acid. As a consequence of the highly branched structure, viscous solutions are only formed at high gum concentrations (~50%w/w). The gum consists of three fractions that have been shown to differ principally in their molecular mass and protein contents. Most of the gum (~90%) contains very little protein and has a molecular mass of ~250 000 Da. A second fraction (~10% of the total) contains ~10% protein and has a molecular mass of ~1–2 MDa and has been shown to have a 'wattle blossom'-type structure where blocks of carbohydrates of molecular mass ~40 000 Da are connected to a common polypeptide chain. The third fraction (~1% of the total) contains up to 50% protein and has a molecular mass of ~250 000 Da. Fraction 2 has been shown to be responsible for the gum's excellent ability to stabilize oil-in-water emulsions. It has been proposed that the hydrophobic polypeptide chains adsorb onto the oil droplets and anchor the molecules to the surface, while the hydrophilic carbohydrate blocks protrude out into solution and prevent droplet aggregation and coalescence due to electrosteric repulsions. Its ability to act as an emulsifier has led to its use in stabilizing flavor oil emulsion concentrates for the soft drink

Table 2 INS numbers for polysaccharide gum additives

Polysaccharide	*INS number*
Alginic acid	400
Sodium alginate	401
Potassium alginate	402
Ammonium alginate	403
Calcium alginate	404
Propylene glycol alginate (propane-1,2-diol alginate)	405
Agar	406
Carrageenan (including furcellaran)	407
Processed *Eucheuma* seaweed	407a
Bakers yeast glycan	408
Arabinogalactan	409
Locust bean gum	410
Oat gum	411
Guar gum	412
Gum tragacanth	413
Gum arabic (acacia gum)	414
Xanthan gum	415
Gum karaya	416
Tara gum	417
Gellan gum	418
Gum ghatti	419
Curdlan gum	424
Konjac flour	425
Soybean hemicellulose	426
Pectins	440
Cellulose	460
Microcrystalline cellulose	460(i)
Powdered cellulose	460(ii)
Methyl cellulose	461
Ethyl cellulose	462
Hydroxypropyl cellulose	463
Hydroxypropyl methylcellulose	464
Methyl ethyl cellulose	465
Sodium carboxymethyl cellulose	466
Ethyl hydroxyethyl cellulose	467
Cross-linked sodium carboxymethyl cellulose	468
Sodium carboxymethyl cellulose, enzymatically hydrolyzed	469

Certain gums not included either are classed as food ingredients (e.g., gelatin) or have yet to complete the regulatory process.

Table 3 INS numbers for modified starches

*Modified starch**	*INS number*
Dextrin (roasted starch)	1400
Acid-treated starch	1401
Alkali-treated starch	1402
Bleached starch	1403
Oxidized starch	1404
Monostarch phosphate	1410
Distarch phosphate	1412
Phosphated distarch phosphate	1413
Acetylated starch	1414
Starch acetate	1420
Acetylated distarch adipate	1422
Hydroxypropyl starch	1440
Hydroxypropyl distarch phosphate	1442
Starch sodium octenyl succinate	1450
Starch, enzyme-treated	1405

*The Codex General Standard for the Labelling of Prepackaged Foods specifies that modified starches may be declared as such in a list of ingredients. However, certain countries require specific identification and use these numbers.

industry and also in the production of spray-dried encapsulated flavors for use in dry packaged products such as soup and cake mixes. In the latter case, the gum forms a film around the flavor particle, preventing oxidation and evaporation, and the gum's high solubility facilitates rapid flavor release. The ability of gum arabic to form concentrated solutions of low viscosity has led to its widespread use in confectionery products, particularly those with high sugar content such as pastilles where it functions by reducing sugar crystallization.

Gum tragacanth is obtained from shrubs of the *Astragalus* species notably *A. gummifer* and *A. microcephalus*, which are found in the dry mountainous areas in Iran and Turkey. It consists of a water-swellable fraction called tragacanthic acid (or bassorin) (60–70%) and a water-soluble fraction called tragacanthin. The former consists of a main chain of (1,4)-α-D-galactopyranosyl uronic acid residues with branches linked through (1,3)-β-D-xylose units terminating in L-fucopyranose. The latter is a highly branched arabinogalactan with a main chain of D-galactopyranosyl units either (1,6)- or (1,3)-linked with side chains consisting mainly of L-arabinofuranose but with a small proportion of D-galacturonic acid and L-rhamnose. Gum tragacanth gives rise to high-viscosity solutions even at 1% concentration. The viscosity decreases irreversibly on heating. The gum solution is stable under acid conditions and shows good emulsification characteristics, which has led to its use in salad dressings and sauces. Its cost and availability, however, have meant that it has been largely replaced in these products by other gums, notably xanthan gum. Gum tragacanth is still widely used in decorative icings for cakes.

Gum karaya is a gum obtained from *Sterculia urens* trees found in the highlands of central and northern India. It is a heavily acetylated polysaccharide composed of chains of α-D-galacturonic acid and α-L-rhamnose. The acid groups are glycosylated with β-D-galactose or β-D-glucuronic acid residues, whereas about half of the rhamnose groups carry β-D-galactose units as side chains. Gum karaya swells in water to yield highly viscous solutions; however, the solutions show a permanent loss in viscosity on heating. The gum finds some application in salad dressings.

Gum ghatti is obtained from *Anogeissus latifolia*, which is a large deciduous tree that grows in India. It has a main chain of alternating (1,4)-β-D-glucopyranosyl uronic acid and (1,2)-α-D-mannopyranose units and contains numerous side chains of L-arabinose, D-galactose, and D-glucuronic acid. It contains a water-soluble (>80%) and water-swellable fraction and was originally developed at the turn of the century as a replacement for gum arabic since it forms solutions of low viscosity at high concentrations and is also a good emulsifier.

Cellulosics

Cellulose is the most abundant of all the polysaccharides being the structural component of land plants. It is composed of linear chains of (1,4)-β-D-glucopyranose units that are

associated with hydrogen bonding giving rise to a number of crystalline forms. Although it is insoluble in water, it can be chemically modified to form a number of water-soluble derivatives with valuable functional properties. Derivatization usually involves etherification of the reactive hydroxyl groups on the glucose residues. The most common water-soluble cellulose derivatives used in food applications are carboxymethyl cellulose (CMC), methyl cellulose (MC), and hydroxypropyl methylcellulose (HPMC). Derivatization is carried out following the conversion of the cellulose to the sodium form by treatment with concentrated alkali. This process destroys the crystalline structure and yields alkali cellulose. The alkali cellulose is then reacted with the appropriate reagent, namely, sodium monochloroacetate for CMC, methyl chloride for MC, and methyl chloride/propylene oxide for HPMC. Since the reactions are heterogeneous, substitution can be very irregular.

CMC is the most widely used of the cellulose ethers and can be obtained in various grades, which vary in molecular mass and degree of substitution (DS). The minimum DS is ~0.4 (i.e., 4 hydroxyls substituted per 10 glucose residues) since below this, CMC is insoluble. Above this, CMC dissolves readily in water to form viscous solutions, but, since it is anionic, the viscosity is reduced upon the addition of electrolyte and at low pH due to compaction of the polymer chains. A synergistic increase in viscosity occurs in blends with guar gum and locust bean gum. CMC finds widespread use in ice cream and other frozen desserts and salad dressings, sauces, and gravies.

The nonionic cellulose ethers, MC and HPMC, are also supplied in a range of molecular sizes and DS. For HPMC, since the substituted moieties contain hydroxyl groups, they may also participate in the reaction. Hence, a molar substitution (MS) is also quoted to characterize the polymers. MC and HPMC dissolve readily in water to produce viscous solutions that on heating form thermoreversible gels. The gelation temperature is dependent on the degree of substitution. For MC containing 30% methoxyl groups, gelation occurs at ~50–55 °C, whereas for HPMC containing 20% methoxyl and 8% hydroxypropyl groups, gelation occurs at ~85 °C. Gelation is believed to be a consequence of molecular association through the hydrophobic methyl and hydroxypropylmethyl substituents. This functional characteristic has led to their use in combination with starch in soups and gravies to prevent undesirable loss of viscosity on heating. A further application is their use in fried foods, where gelation on heating helps retain the structural integrity of the product and additionally serves to prevent moisture loss and oil absorption. Since MC and HPMC are also surface-active, they are used in baked goods to aid uniform gas cell formation and in salad dressings to stabilize emulsion droplets.

Plant Extracts

Starch is obtained commercially principally from corn and potato and to a lesser extent from waxy corn, wheat, tapioca, rice, cassava, sorghum, pea, and sago. It is obtained in the form of granules that have varying degrees of structural order and consists of two polysaccharides, namely, amylose and amylopectin. The proportions of each depend on the source, for example, corn and potato starches contain ~27% and 21% amylose, respectively, while waxy corn contains <2% amylose.

Amylose consists of linear (1,4)-α-D-glucopyranose chains with very little branching (10 branch points per molecule) and has a molecular mass of typically 250 000–2 MDa. Amylopectin also contains sequences of (1,4)-α-glucopyranose units; however, it has extensive branching via (1,6) linkages and has a molecular mass of ~50 M–400 MDa. The starch granules are insoluble in water, but on heating, they swell and burst, releasing the polysaccharides and forming a viscous paste. The actual temperature at which the granules burst is very characteristic of the source of the starch and is (perhaps inappropriately) referred to as the gelatinization temperature. The typical values for corn and potato starches are 67 °C and 60 °C, respectively. On cooling, the amylose molecules readily self-associate, a process known as retrogradation, and it is then that a gel is formed. Natural starches form turbid gels that are prone to syneresis. Hence, most of the commercial starches used are derivatives that have a lesser tendency for retrogradation. Derivatives include hydroxypropyl starches, starch phosphates, oxidized starch, and acid- or enzyme-degraded starches. Starch derivatives are very widely used in food products including gravies, soups, sauces, fruit preparations, dairy products, baked goods, batters, and coatings.

Pectin is the general term for a group of polyuronans that occur as the structural components of plants. Commercial pectins are obtained from the pomace of apples or the peel of citrus fruits following hydrolysis, which renders the pectin water-soluble. Pectin molecules consist of linear chains of (1,4)-α-D-galacturonic acid residues, up to 80% of which occur as the methyl ester. The hydroxyls in the C2 and C3 positions may be acetylated. The chains also contain up to 4% (1,2)-α-rhamnopyranose units, which give rise to kinks. L-Arabinose, D-galactose, and D-xylose (10-15%) are linked to the rhamnose units forming ramified side chains, which are referred to as 'hairy regions' along the otherwise smooth galacturonan backbone. If the degree of esterification (DE) is >50%, it is referred to as high-methoxyl (HM) pectin. Deesterified pectin with DE < 50% is produced by mild acid or alkali treatment and is referred to as low-methoxyl (LM) pectin. If the alkali used is ammonia, amidated pectin is formed. Pectin is soluble in water and is most stable at pH 3–4. At lower pH, the glycosidic bonds are hydrolyzed. In alkaline conditions, the glycosidic and ester linkages are cleaved, yielding the free acid. Both HM and LM pectins form gels. For HM pectin (DE 60–75%), gelation occurs at high soluble solids content (typically 50–75% sugar) and at pH <3.5 over a period of time. The gels are not thermoreversible. Junction zone formation is believed to be a consequence of hydrophobic association between ester groups coupled with intermolecular hydrogen bonding between hydroxyl groups on the galacturonan backbone. For LM pectin (DE typically 20–40%), gelation is brought about by the addition of divalent cations that cross-link the galacturonic acid chains, and a high solids content and low pH are not a prerequisite. The gelation mechanism is referred to as the 'egg-box' model and is discussed further in the succeeding text.

Pectins are mainly used for the production of jams and jellies. High-ester pectins find widespread use in normal jams, whereas low-ester pectins are preferred for low-sugar jams. LM amidated pectin is used to stabilize fruit pulp, which is incorporated into products such as yogurt.

Konjac mannan is the soluble extract of konjac flour obtained by pulverizing the dried tuber of *Amorphophallus konjac*. It is a glucomannan and consists of a main chain of (1,4)-β-D-mannopyranosyl and D-glucopyranosyl units with (1,3)-linked branches approximately every 10 sugar residues. The ratio of mannose to glucose is 1.6:1. Approximately 1 in 19 sugar residues is acetylated. Konjac mannan forms highly viscous solutions on dissolution in water following heating. In the presence of alkali, thermally irreversible gels are formed as a consequence of deacetylation and aggregation of the glucomannan chains. In Japan, konjac mannan is made into noodles. The process involves pumping a solution of the gum through a spinnerette into a hot alkaline bath (e.g., calcium hydroxide solution), which results in the formation of long gel threads. The gum is also sold in blocks of gel, known as konnyaku, for use in traditional Japanese dishes and also as a dessert jelly. Konjac mannan is now finding application as a thickener and gelling agent. It forms thermoreversible gels with xanthan gum and also increases the gel strength, elasticity, and clarity of kappa carrageenan gels. For both mixtures, it has been argued that the synergistic behavior is due to the association of carrageenan and xanthan helices with the glucomannan chains.

Galactomannan Seed Gums

Locust bean (also known as carob), tara, and guar gums are storage polysaccharides obtained from the endosperms of leguminous seeds of *Ceratonia siliqua*, *Caesalpinia spinosa, and Cyamopsis tetragonoloba*, respectively. They have a molecular mass of the order of 1 MDa and consist of a linear main chain of β-(1,4)-linked D-mannopyranosyl units with galactopyranosyl units linked α-(1,6) to varying degrees. The mannose-to-galactose ratio, M/G, is approximately 4.5:1, 3:1, and 2:1 for locust bean, tara, and guar gums, respectively. The galactose residues are distributed nonuniformly along the mannan chain. The presence of galactose tends to inhibit intermolecular association between the galactomannan chains; hence, whereas guar gum is readily soluble in cold water, tara and locust bean gums have to be heated to high temperatures to disrupt the aggregation and achieve complete dissolution. Once dissolved, all three yield highly viscous solutions even at 1% concentration, and as a consequence, their main application is as thickeners.

Locust bean gum will self-associate in solution and will form thermally irreversible gels on freezing. It is commonly used in combination with other polysaccharides, particularly kappa carrageenan, since it leads to the formation of stronger, more elastic gels that have improved transparency and are less prone to undergo syneresis. Locust bean gum also forms strong thermoreversible gels with xanthan gum. It is believed that interaction occurs between the xanthan or carrageenan helices and mannose sequences along the locust bean gum backbone, which are devoid of galactose residues. This model also explains why guar gum is not as effective at forming gels.

Galactomannans are widely used as thickeners in a range of applications including convenience foods, dairy products, sauces, and bakery products.

Algal polysaccharides

The carrageenans are a family of sulfated galactans obtained from red seaweeds (*Rhodophyceae*) where they have a key structural function. The traditional method of extraction is by treatment of the seaweed with hot alkali for 10–30 h, followed by precipitation with alcohol and then drying. The three major types are kappa, iota, and lambda carrageenans. Kappa is obtained from a species of seaweed called *Eucheuma cottonii* and occurs together with lambda carrageenan in *Chondrus crispus*. Iota carrageenan is obtained from *Eucheuma spinosum*. They differ essentially in their degree of sulfation. The idealized structure for kappa carrageenan is a disaccharide repeat unit consisting of (1,3)-β-galactopyranose-4-sulfate and (1,4)-α-3,6-anhydrogalactopyranose residues. Iota carrageenan differs only in that the latter residue is sulfated at the C2 position. Lambda carrageenan is further sulfated and consists of (1,4)-α-galactopyranose 2,6 disulfate and (1,3)-β-linked galactopyranose, which is 70% substituted at the C2 position. The carrageenans are all soluble in water, but whereas lambda forms viscous solutions, kappa and iota form thermoreversible gels. In solution, the molecules undergo a thermoreversible coil-to-helix transition, and the helices self-associate giving rise to a three-dimensional gel structure (Figure 1). The temperature of gelation increases with increasing electrolyte concentration. It has been shown that potassium, rubidium, and cesium ions specifically bind to the helical structure of kappa carrageenan and hence promote helix formation and gelation at much lower concentrations than other electrolytes. As a consequence, kappa carrageenan gels are much stronger in the presence of potassium chloride compared with, say, sodium chloride. This ion specificity is not observed for iota carrageenan, which forms weaker more elastic gels compared with kappa. This is probably due to the fact that the increased charge on the iota carrageenan chains reduces the extent of helix self-association. Self-association results in the melting temperature being greater than the gelation temperature, and this hysteresis is more pronounced in kappa carrageenan.

Kappa carrageenan gels are more brittle and turbid than iota carrageenan gels and are more prone to syneresis. As discussed in the preceding text, the incorporation of locust bean gum or konjac mannan into kappa carrageenan gels

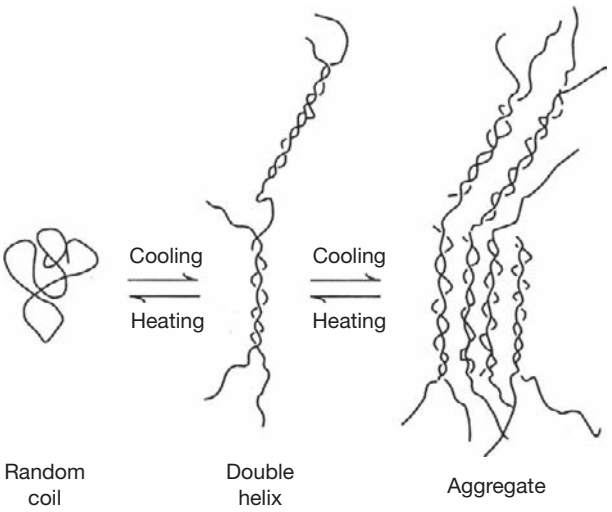

Figure 1 Schematic illustration of the coil–helix transition of carrageenan in solution.

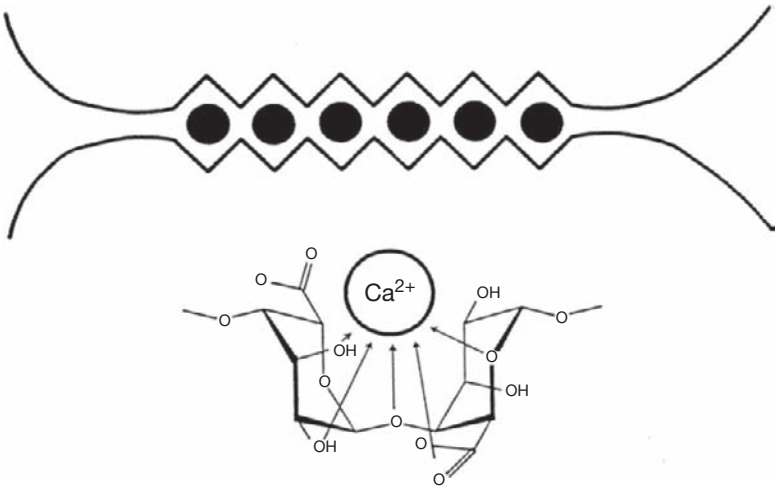

Figure 2 The egg-box model illustrating the cross-linking of alginate chains, which leads to gel formation.

reduces turbidity, brittleness, and syneresis and also increases gel strength.

Kappa carrageenan is widely used in dairy products because it interacts synergistically with kappa casein and prevents wheying off. Carrageenans are also used extensively in meat products such as cooked hams, poultry products, and sausages because of their ability to bind water and control the texture and structural integrity.

Agarose (the major component of agar) is also obtained from red seaweeds notably *Gelidium* and *Gracilaria* species. It is a linear neutral polysaccharide and has a similar structure to the carrageenans consisting of alternating (1,3)-β-D-galactopyranose and (1,4)-linked 3,6-anhydro-α-L-galactopyranose units. It dissolves in near-boiling water and gels on cooling to $\sim < 40^{\circ}$ C. The gelation mechanism is as described for the carrageenans, but, since the agarose chains are nonionic, extensive helix aggregation occurs resulting in the formation of very strong gels. The gels show pronounced hysteresis and melt only on heating to 80–90° C. Agar is used in the manufacture of a range of products including jams, jellies, pie fillings, icings, and gelled meats, but its use is far more restricted commercially than that of carrageenan owing to availability and cost considerations.

Alginate is obtained from brown seaweeds (*Phaeophyceae*) and is a linear (1,4)-linked polyuronan consisting of β-D-mannuronic and α-L-guluronic acids. The acids are present as blocks of separate or mixed sequences along the chain depending on the seaweed source. *Macrocystis pyrifera* and *Ascophyllum nodosum* have a high mannuronic acid content (61% and 65%, respectively), whereas *Laminaria hyperborea* has a high guluronic acid content (69%). Sodium alginate dissolves readily in water to form viscous solutions and forms thermally irreversible gels in the presence of divalent cations (notably calcium). It is the guluronic acid residues that are mainly responsible for gel formation, and their proportion and distribution along the polyuronan chain have a major influence on the properties of the gels produced. Adjacent diaxially linked guluronic acid residues form a cavity that acts as a binding site for cations, which interact with the carboxyl and hydroxyl groups. Intermolecular cross-linking of sequences results in the formation of junction zones and a three-dimensional gel network. This mechanism is often referred to as the 'egg-box model' and is illustrated in Figure 2.

Sodium alginate can be used to produce fruit analogs for pie fillings utilizing the so-called internal setting procedure. In this process, a sodium alginate solution containing a sparingly soluble calcium salt (e.g., calcium phosphate) is combined with a fruit puree, which contains a sequestrant (e.g., sodium citrate) and acid (citric acid). On mixing, the insoluble calcium salt begins to dissolve in the presence of the acid, releasing calcium ions that initially interact with the alginate, inducing gelation. The uniform gel formed is then cut, and a thickened fruit syrup is added to give the finished pie filling. Sodium alginate can also be used to prepare structured fruits using a coextrusion technique, and this is particularly appropriate for fruits such as black currants, which have an outer skin and liquid center. In the process, a sodium alginate solution and fruit puree mix are fed separately through nozzles consisting of coaxial tubes positioned above a bath containing a solution of calcium salt (e.g., calcium lactate). The flow of alginate solution is kept constant while that of the fruit puree is intermittent. As each drop of puree breaks away from the nozzle, it is coated with a film of the alginate solution, which gels instantly, forming a hardened skin upon hitting the calcium bath below. The fruit gels formed are then collected, and a thickened syrup is added to produce the pie filling.

Microbial Gums

Xanthan gum was first discovered in the 1950s and is now finding extensive application in the food industry since its introduction in the early 1970s. The gum is obtained from the genus *Xanthomonas*, notably *X. campestris*, by aerobic fermentation. The xanthan molecules have a (1,4)-β-D-glucopyranose backbone as in cellulose but in addition have a trisaccharide side chain on every other glucose residue linked through the C3 position. The side chain consists of two mannopyranosyl residues linked on either side to a glucuropyranosyl uronic acid group. The inner mannose residue connected to the backbone may be acetylated, while the terminal mannose residue may be pyruvated. The molecular mass of the xanthan molecules is very

high (>3 MDa), and the gum dissolves in water to yield highly viscous solutions. The xanthan molecules undergo a thermoreversible coil–helix transition in solution, which is shifted to higher temperatures by the addition of electrolyte. In the disordered coil form, the side chains are envisaged as protruding away from the backbone into solution, while in the ordered form, the molecules form a stiff fivefold helical structure with the side chains folded in and associated with the backbone. Most workers now believe that the helix is double-stranded. The stiff xanthan chains tend to associate in solution giving rise to a very high low-shear viscosity, which is sufficient to prevent particles from settling or oil drops from creaming. The associations are readily broken upon applying shear, giving rise to a dramatic drop in viscosity, and hence, the solutions readily flow. Unlike other polyelectrolytes, the viscosity of xanthan solutions is relatively insensitive to the addition of electrolyte since the electrolyte will promote helix formation. The helices are also stable over a broad range of pH. The high apparent yield stress and pronounced shear-thinning characteristics have led to a large increase in the use of this gum, which now finds application in a broad range of food products such as sauces, salad dressings and mayonnaises, and frozen and chilled dairy products.

Gellan gum is produced from *Pseudomonas elodea* by aerobic fermentation and consists of a linear tetrasaccharide repeat unit of (1,3)-β-D-glucopyranose, (1,4)-β-D-glucopyranose uronic acid, (1,4)-β-D-glucopyranose, and (1,4)-α-L-rhamnopyranose. In the native form, the (1,3)-linked glucose residues contain glycerate and acetate moieties. x-Ray fiber diffraction studies indicate that the gellan molecules form a 3-fold double helical structure and in solution undergo a thermoreversible coil–helix transition. The transition shifts to higher temperatures in the presence of electrolyte. Once formed, the helices tend to self-associate leading to the formation of a gel, as is the situation for agarose and carrageenan. Gels formed in the presence of monovalent ions are usually thermoreversible, although the melting temperature is normally much greater than the gelation temperature, a consequence of the extensive aggregation. If gels are formed by the addition of divalent ions, then they can be thermally irreversible. The native form produces soft elastic gels, whereas the deacetylated material, which is sold commercially, forms hard brittle gels. Gellan gum is used as a gelling agent for fruit fillings for pies and cakes, in dairy products such as milk beverages and yogurts, and in sugar confectionery.

See also: Bread: Dough Mixing and Testing Operations; Cellulose; Dietary Fiber: Determination; Emulsifiers: Types and Uses; Fat Replacer; Food Additives: Classification, Uses and Regulation; Gelatin; Gums: Dietary Importance; Ice Cream: Composition and Health Effects; Mayonnaise; Pectin and Health; Pectin: Properties Determination and Uses; Prebiotics; Probiotics; Satiety; Stabilizers: Types and Function; Starch: Modified Starches; Starch: Sources and Processing; Starch: Structure, Property, and Determination; Starch; Whey and Whey Powders: Production and Uses; Yogurt: The Product and its Manufacture; Aerated Foods.

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Gut Hormones

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Introduction

The gastrointestinal tract is the major endocrine organ in the body producing over 20 hormones from specialized enteroendocrine cells located throughout its epithelial lining. These hormones act both locally, for example, on the stomach and pancreas and distally, for example, on the hypothalamus and brain stem and are believed to play a part in mediating digestion, energy homeostasis, appetite, and satiety. They have an array of actions including smooth muscle contraction, further hormone release, and neuronal signaling, and many gut hormones act synergistically providing additional levels of control. Gut hormones are released in response to neural signals and/or receptors that sense nutrients including glucose, lipids, amino acids, bile salts, and short-chain fatty acids.

The role of gut hormones in patients with obesity, anorexia nervosa, and type 2 diabetes mellitus, among many other pathologies, is under investigation, as is the potential for gut hormones to be used therapeutically. Already, glucagon-like peptide-1 (GLP-1) agonists are used for the treatment of type 2 diabetes mellitus and obesity.

Ghrelin

Ghrelin was discovered as the endogenous ligand for the orphan growth hormone secretagogue receptor in 1999 by Kojima and colleagues. The human ghrelin gene is localized on the chromosome 3p25–26 and comprises five exons that are processed to give a 28-amino acid peptide. Ghrelin is the only circulating gut hormone known to have an orexigenic effect, that is, stimulating food intake and hunger. It is released from specific X/A-like enteroendocrine cells in the mucosal layer of the stomach in response to fasting, and secretion is suppressed by feeding. An octanoyl group on the serine residue in position 3, added by GOAT (ghrelin-*O*-acyltransferase), is required for binding to its receptor, the growth hormone secretagogue receptor.

Central or peripheral administration of ghrelin in rats increases food intake and stimulates growth hormone release, which chronically leads to weight gain. Human studies have also confirmed that the infusion of ghrelin causes increased food intake and feelings of hunger, and there is growth hormone release from the anterior pituitary, which increases adiposity and blood glucose. The plasma ghrelin concentration is particularly decreased by a high-fat meal. As well as controlling appetite, ghrelin also stimulates gastric acid secretion, gastric motility, and turnover of gastric and intestinal mucosae. Ghrelin concentrations are very high in anorexia nervosa and bulimia nervosa and are suppressed in obesity.

Gastrin

Gastrin's existence was first postulated in 1905 when British physiologist John Edkins noted in cats that the injection of a pyloric mucous membrane extract caused the release of gastric acid from the stomach. This was confirmed in 1938 and the structure elucidated in 1964. The human gastrin gene is localized on the chromosome 17q21 and consists of three exons of which two code for the precursor protein preprogastrin. Preprogastrin is cleaved to give progastrin, which is then further cleaved to give *C*-terminal glycine-extended gastrin-17gly and gastrin-34gly, which are then converted to *C*-terminal amidated gastrin-34 and gastrin-17. Gastrin acts on the cholecystokinin (CCK) 2 receptor, as it has an identical *C*-terminal pentapeptide sequence to CCK.

Gastrin is released from enteroendocrine G cells in the stomach in response to neuronal signaling, calcium, aromatic amino acids, and alcohol and is inhibited by somatostatin, whose release is controlled by intragastric pH. Gastrin stimulates acid secretion from the gastric parietal cell, as does histamine and neural stimulation. Gastrin also has a trophic effect causing gastric epithelial cell proliferation. Zollinger–Ellison syndrome is caused by gastrin-secreting tumors, and symptoms include multiple gastric and duodenal ulcers. Proton pump inhibitor therapy irreversibly blocks gastric acid secretion by the gastric parietal cell and hence causes increased gastrin release from G cells, due to the lack of negative feedback.

Cholecystokinin

CCK was previously called pancreozymin, as the stimulation of pancreatic exocrine secretion was the first of its functions to be discovered. The gene is located on chromosome 3, and CCK is really a family of peptides, of varying lengths, produced from the posttranslational modification of preprocholecystokinin. CCK usually has a sulfate group attached to tyrosine in position 7 in the *C*-terminus, which is essential for the activation of the CCK1 receptor.

CCK is released postprandially from enteroendocrine I cells in the small intestine and acts via CCK1 receptors on the vagal nerve to reduce food intake by inducing satiety. It is an acute signal; CCK has a half-life of 1–2 min, and secretion is activated by receptors on the I cells responding to aromatic amino acids and long-chain fatty acids in the gut lumen. It is particularly stimulated by fatty meals. CCK also acts in a paracrine way on the pancreas and gallbladder to stimulate pancreatic exocrine secretion and gallbladder contraction, allowing the digestion of the food intake. It also inhibits gastric emptying, which slows down transit and improves digestion and absorption, and stimulates feelings of satiety. The response to CCK is decreased in obese individuals. Experimentally, although CCK infusion reduces the amount of calories consumed at a meal, in the long term, subjects tend toward having more meals, and hence, there is no overall decrease in calories consumed. There is also a rapid development of tolerance and an increased risk of acute pancreatitis/pancreatic cancer.

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Secretin

Secretin was the first hormone ever discovered, by Bayliss and Starling in 1902. It is produced by enteroendocrine S cells, mainly in the duodenum, in response to the low pH of the gastric contents and the presence of products of protein digestion. It binds to the G protein-coupled secretin receptor. It is a 27-amino acid peptide. It inhibits gastric acid secretion and motility via the vagal nerve and stimulates pancreatic bicarbonate secretion, thus controlling the pH within the small duodenum.

Motilin

Motilin is a 22-amino acid peptide produced by enteroendocrine M cells in the duodenojejunum. During fasting, it has a cyclic release that helps to generate short bursts of peristalsis, which are thought to clear debris and bacteria from the stomach and small intestine. This stops after feeding. Motilin is similar in structure to ghrelin, and its receptor is part of the same family as the ghrelin receptor.

Glucose-Dependent Insulinotropic Polypeptide

Glucose-dependent insulinotropic polypeptide (GIP) is a 42-amino acid peptide hormone that is secreted from enteroendocrine K cells in response to nutrients especially glucose or fat. These cells are primarily located within the duodenum and proximal jejunum. It was initially known as gastric inhibitory peptide due to its ability to stop gastric acid secretion in dogs; however, this inhibitory effect only occurs at pharmacological doses. At physiological doses, it is an incretin, that is, it is secreted in response to enteral glucose to augment insulin secretion, more so than when the same amount of glucose is given intravenously. GIP also promotes pancreatic β cell growth and inhibits apoptosis. In adipose tissue, adipogenesis is stimulated. GIP is degraded by dipeptidyl peptidase-4 (DPP-4), which cleaves GIP(1–42) to form inactive GIP(3–42). GIP concentrations are normal to increased in type 2 diabetes mellitus, but the incretin activity of GIP is diminished.

Preproglucagon

The proglucagon gene is located on the long arm of chromosome 2. The precursor peptide is produced in the brain, gut, and pancreas. In the brain and gut, it is posttranslationally processed to give glicentin, GLP-1, and glucagon-like peptide-2 (GLP-2) by proprotein convertase subtilisin/kexin (PCSK) type 1. Glicentin is further processed to give oxyntomodulin and glicentin-related pancreatic polypeptide (PP). Preproglucagon is processed differently in the pancreas by PCSK type 2 to give glicentin-related PP, glucagon, and major proglucagon fragment (see Figure 1).

Glucagon-Like Peptide-1

The most common circulating active form of GLP-1 is the 7–36 amide, although 7–37 is also present and active. GLP-1 is degraded by DPP-4, which cleaves off two amino acids from the *N*-terminal forming inactive GLP-1 9–36 amide or GLP-1 9–37. GLP-1 exerts its actions through the GLP-1 receptor, which can also be activated by oxyntomodulin, although oxyntomodulin is a lower-affinity agonist by two orders of magnitude. GLP-1 is released 5–30 min after meals, from enteroendocrine L cells in the intestine, which are mainly distributed in the distal intestine, ileum, and colon. These L cells can also secrete oxyntomodulin and peptide YY (PYY), and there is sometimes cosecretion of GIP, which is more usually produced from K cells. The L cells sense nutrients both directly and indirectly, as GLP-1 levels rise before nutrients are present in the L cell-rich areas. Nutrients triggering GLP-1 release include sugars, free fatty acids, amino acids, bile acids, and short-chain fatty acids from bacterial fermentation of dietary fiber. There is also neural and hormonal regulation, with insulin, GIP, and leptin enhancing GLP-1 secretion and somatostatin inhibiting it. The amount of GLP-1 produced is in proportion to the calorie content of the meal. GLP-1 inhibits gastric acid secretion, gastric emptying, and intestinal motility to slow down digestion, improve absorption, and reduce food intake.

GLP-1 is a potent incretin like GIP. It also upregulates insulin gene expression, decreases pancreatic β cell apoptosis, and potentiates all steps of insulin biosynthesis. This is true even in patients with type 2 diabetes mellitus. GLP-1 also inhibits glucagon release from pancreatic α cells in a glucose-dependent manner that contributes to a lower plasma glucose concentration. It is important to note that because GLP-1 only augments the insulin response and inhibits the glucagon response, in the presence of an elevated glucose level, there is little risk of hypoglycemia developing. Human studies have

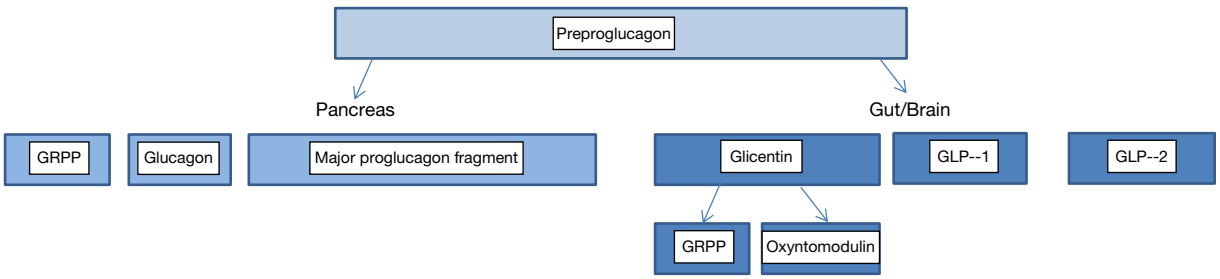

Figure 1 The posttranslational processing of preproglucagon in the pancreas and gut/brain. GRPP, glicentin-related peptide; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2.

shown a reduction in gluconeogenesis upon infusion of GLP-1, consistent with its glucagon inhibitory effect.

GLP-1 also acts on the central nervous system to modulate feeding behavior. It is an anorexigenic hormone, that is, it reduces appetite and induces satiety. GLP-1 appears to modulate appetite through two different routes: directly via diffusion across the incomplete blood–brain barrier in the median eminence to activate GLP-1 receptors in the hypothalamus and indirectly via vagal afferents, which innervate the portal circulation and signal back to the dorsal vagal complex in the brain stem and further on to the hypothalamus. Energy expenditure is, controversially, increased, an observation that has been made in animal studies but has not been observed in studies in humans. There is reduced secretion of GLP-1 in obese individuals, although response is maintained, and infusion of GLP-1 reduces food intake in a dose-dependent way.

Glucagon-Like Peptide-2

GLP-2 is secreted from enteroendocrine L cells in the intestine in response to nutrient, neural, and hormonal signals as per GLP-1. There is also a strong GLP-2 response to intestinal injury. GLP-2(1–33) is degraded by DPP-4 to GLP-2(3–33) and then renally cleared.

The effects of GLP-2 are increased proliferation of the intestinal mucosa in both the small and large intestines, enhanced intestinal blood flow, decreased apoptosis of intestinal cells, and increased intestinal barrier function. This crypt cell proliferation and enhanced blood flow increase intestinal absorption, and GLP-2 analogs such as teduglutide have found a role in the treatment of short bowel syndrome by reducing intestinal losses and improving the absorption of nutrients.

Oxyntomodulin

Oxyntomodulin is secreted 5–30 min after food ingestion, especially if the food contains fatty acids, from the enteroendocrine L cells in the intestine. It has the same structure as glucagon with a short *C*-terminal extension (see Figure 1). The amount secreted is proportional to the calorie content of the meal. It inhibits gastric acid secretion and motility along with GLP-1 and seems to signal via the GLP-1 receptor. Its affinity for the GLP-1 receptor is approximately two orders of magnitude weaker than that of GLP-1, but despite this, it exerts a comparable effect on food intake, speculatively via an alternative oxyntomodulin receptor. Oxyntomodulin acts to reduce appetite both via a direct effect on the CNS (similar to GLP-1) and by inhibiting ghrelin release. Oxyntomodulin infusion reduces food intake and increases energy expenditure in lean and obese individuals.

Pancreatic Polypeptide

PP was first extracted from chicken pancreas in the early 1970s. It is mainly synthesized and released by the PP cells of the pancreatic islets. Its structure contains a PP-fold, a tertiary structure of an alpha helix and polyproline helix connected by a beta turn, resulting in a characteristic U-shaped peptide. Other hormones with a PP-fold structure include neuropeptide Y and PYY. The PP-fold family of peptides characteristically have 36 amino acids, require amidation at the COOH terminal for biological activity, and bind and activate neuropeptide Y receptors. In turn, the seven transmembrane domain Y receptors are coupled to G_i resulting in inhibition of adenylate cyclase and reductions in intracellular cAMP. PP's cognate Y receptor is the Y4 receptor.

PP release is controlled by feeding and an intrinsic circadian rhythm. In fasting individuals, the PP concentration is at its lowest at 0200 and highest at 2100. Postprandial release is vagally controlled. PP's main effects are to reduce appetite, delay gastric emptying, and increase energy expenditure. The quantity secreted is proportional to calorie load but is also increased by gastric distension. Secretion is biphasic, and the profile of release is dependent on the timing of the meal, with the first meal of the day having a reduced first-phase response.

Peptide YY

PYY was first isolated from porcine intestines in 1980 by Tatemoto. PYY is, like PP, a PP-fold peptide; it is produced by the enteroendocrine L cells in the gut, with higher concentrations found distally. The full-length PYY(1–36) binds to all Y receptor subtypes. The most common circulating form is the amino-terminally truncated form PYY(3–36), which preferentially binds to Y2 and Y5 receptors. DPP-4 is thought to mediate this conversion between 1–36 and 3–36. PYY(3–36) can freely cross the blood–brain barrier unlike PP, due to its different *N*-terminus.

The PYY concentration increases after a meal, but this increased secretion starts 15 min postfeeding, before the nutrients have time to reach L cells, and is therefore likely to be vagally controlled. Concentrations peak at 1–2 h postfeeding. Caloric content, macronutrient constituents, and consistency affect the profile and peak of PYY release, with a greater release seen for fat-rich meals.

CCK, gastric acid, and bile acids also cause PYY secretion, but unlike PP, it is not released by gastric distension. The principal effect of PYY is to suppress appetite. Obese people have lower fasting levels of PYY and attenuated postprandial PYY release associated with decreased satiety. However, they do not have resistance to the hormone as infusion does reduce food intake. Fasting PYY concentrations correlate negatively with the BMI, waist circumference, and 15 h resting metabolic rate. Postprandial PYY concentrations correlate negatively with changes in body weight over 6 months and 24 h respiratory quotient.

The Role of Gut Hormones in Metabolism

Digestion

There is a distal-to-proximal feedback mechanism, called the ileal brake, which slows digestion and causes feeling of satiety on food entering the ileum. The purpose of this is to control digestion, allowing adequate time and resources for

degradation and absorption of the nutrients and preventing hyperalimentation. The presence of not only fat but also protein and/or carbohydrate in the ileum leads to increased transit time in the duodenum and jejunum and delayed gastric emptying by decreasing the frequency of peristalsis and increasing the pyloric sphincter pressure. There is also a reduction in pancreatic exocrine secretion, gastric acid secretion, and gallbladder contraction. PYY, oxyntomodulin, and GLP-1 are postulated to be involved in this feedback, possibly acting via neural signaling of the vagal nerve.

Energy Homeostasis

Food intake is controlled by gut mechanoreceptors, plasma composition, gut hormones, pancreatic hormones, the central nervous system especially the hypothalamus, societal norms, and leptin signaling from adipose tissue. The role for gut hormones was suggested in 1937 when Maclagan showed that a crude preparation of ground-up intestines given to rabbits decreased food intake by almost 30%, suggesting a possible agent(s) present in the intestine modulated appetite.

Gut hormones act directly on the brain neurons and also indirectly via the vagal nerve. Central nervous system centers responsible for energy homeostasis are in the hypothalamus (especially arcuate and paraventricular nuclei) and brain stem. Removal of the lateral hypothalamus causes hypophagia, and removal of the ventromedial hypothalamus causes hyperphagia, again suggesting that the hypothalamus has a key role to play in controlling appetite. PYY(3–36), oxyntomodulin, and GLP-1 acting both directly and indirectly all cause anorectic effects. Ghrelin acting directly and indirectly stimulates food intake. The system is modulated by additive effects of multiple gut hormones and feedback loops involving the higher brain centers, sympathetic nervous system, and thyroid axis.

Bariatric Surgery

Obesity is a growing epidemic and is associated with increased morbidity and mortality. Affected individuals are at greater risk of type 2 diabetes mellitus, cardiovascular disease, and musculoskeletal pathologies.

The only current effective treatment for obesity is bariatric surgery. Initially designed to reduce food intake and gut surface area available for absorption, its effect on the gut hormones profile may be a better explanation for its success.

Post Roux-en-Y gastric bypass, there is higher meal-stimulated GLP-1 and PYY release. These higher concentrations of the satiety hormones GLP-1, PYY, and also oxyntomodulin cause not only decreased food intake but also change in types of food eaten with lower fat and sugar intake long term. Poor responders to Roux-en-Y bypass can be identified by suboptimal postprandial satiety gut hormone concentrations. Post gastric sleeve, as well as Roux-en-Y gastric bypass, there is reduced ghrelin, likely due to decreased ghrelin-producing cells, as it is also seen in short bowel syndrome. Sleeve gastrectomy also causes increased GLP-1 secretion but gastric banding does not.

Therapeutic Agents

The therapeutic use of gut hormones is limited by their short half-life and peptide structure, which requires injected routes for administration. Their development has been along the lines of making therapeutic agents resistant to DPP-4 cleavage, renal clearance, and investigating the possibility of oral administration.

Exendin-4 is a 39-amino acid peptide, which is a naturally occurring GLP-1 receptor agonist found in the venom of the *Heloderma suspectum* lizard and is resistant to DPP-4 degradation. A synthetic version, exenatide, has been marketed as a therapy for type 2 diabetes mellitus. Subsequently, GLP-1 analogs have been manipulated in other ways to prolong their half-life, and other analogs are now available as diabetes treatments. GLP-1 agonists reduce mealtime-related glycemic excursion and glucagon concentration and increase insulin concentration. This leads to a lower HbA1c, weight loss, and reduced free fatty acid levels, although there is inhibition of gastric emptying and nausea. Nevertheless, GLP-1 analogs are now successfully and routinely used for the treatment of diabetes. Even in nondiabetic patients, GLP-1 analogs appear to be a potential treatment for obesity and may be approved for this purpose in the near future.

DPP-4 inhibitors have also been investigated as therapies for type 2 diabetes mellitus given the role of DPP-4 in degrading GIP and GLP-1. Trials of the DPP-4 inhibitors vildagliptin and sitagliptin have shown increased levels of GLP-1 and GIP, associated with increased insulin release postprandially and improved glycemic control. They are weight neutral, unlike the GLP-1 analogs. This may be due to other hormones affected by the inhibition of DPP-4, for example, by inhibiting the conversion of PYY(1–36) to PYY(3–36) and therefore inhibiting the anorexigenic feedback effect of PYY(3–36) after eating.

Ghrelin antagonists would theoretically be a potential treatment for obesity, but this has yet to be shown in human studies. Studies in growth hormone secretagogue receptor knockout mice and ghrelin-deficient mice show resistance to diet-induced obesity and altered metabolic fuel preference with a tendency to a high-fat diet. Ghrelin agonists have been used in patients with anorexia secondary to cancer or renal dialysis and have been shown to increase appetite. There is also a therapeutic benefit in diabetic patients with gastroparesis from giving a ghrelin agonist.

However, postprandially, multiple anorexigenic gut hormones are secreted, leading to coordinated reductions in appetite and activation of metabolic pathways. It should also be noted that gastric bypass, one of the proved and effective treatments for obesity, also induces a rise in the secretion of multiple gut hormones as noted in the preceding text. Therefore, the effective treatment of obesity may require multiple gut hormones administered in combination, and this is being actively researched.

Conclusion

Gut hormones working independently and synergistically play an important role in preparing the digestive system for food,

matching digestion and absorption to the amount of food taken, causing feelings of satiety, and controlling energy homeostasis and hunger. Harnessing their potential could allow us to reduce the burden of obesity, stimulate hunger in malnourished patients, and improve glycemic control in type 2 diabetes mellitus patients.

See also: Appetite Control in Humans: A Psychobiological Approach; Obesity: The Role of Diet; Satiety.

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H

HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto

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Introduction

Food safety nowadays is an international challenge requiring close cooperation between countries in agreeing standards and systems. European consumers not only require much higher dietary quality and hygiene and health standards in the products they purchase but also look for certification and protected origin products (national or geographic) and processing methods.

The hazard analysis and critical control points (HACCP) system is a science-based system created to identify specific hazards and actions to control them in order to ensure food safety and quality. It can be considered as an efficient tool for both the food industry and health authorities in preventing food-borne diseases. 'Hazard' is "a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect" according to Codex Alimentarius.

The HACCP system addresses and controls all significant hazards associated with a particular food.

Physical hazards include glass, metal, stones, wood, plastic, rubber, pests (typically larger pests), and sand. They commonly result from accidental contamination and poor food handling practices throughout the food chain from farm to fork.

Chemical hazards include cleaning chemicals, pesticides, fungicides, insecticides, fertilizers, allergens, toxic metals, nitrites and nitrates (when added to the product), plasticizers and packaging migration, veterinary residues, antibiotics, chemical additives, lubricants, paints, coatings, naturally occurring toxic substances and mycotoxins derived from fungi, drugs in water and milk, estrogens, phytoestrogens, and waterborne pesticides, for example, atrazine.

Biological hazards include food-poisoning bacteria such as *Salmonella*, *E. coli*, *Bacillus cereus*, *Campylobacter* spp., *Listeria* spp., and *Clostridium botulinum*, which are hazardous.

The adoption of a completely new system of managing food safety compared to HACCP led to the development of ISO 22000, which is the new international generic standard for food safety management systems (FSMS). It defines a set of general food safety requirements that apply to all organizations in the food chain. These requirements are listed in sections 4, 5, 6, 7, and 8 of ISO 22000. ISO 22000 controls food safety hazards through prerequisite programs (PRPs) and HACCP plans.

Risk Assessment

Risk assessment may be summarized as the evaluation of the likelihood of an adverse effect(s)/event(s) occurring under a defined set of conditions, together with a value judgment on the results.

Risk assessment is best addressed in four stages, namely, hazard identification, hazard characterization, exposure assessment, and risk characterization.

The term *hazard* is associated with the potential of an agent or situation to cause an adverse effect(s)/event(s).

Risk is widely recognized as a function of the probability and severity of an adverse effect/event occurring to man or the environment following exposure, under defined conditions, to a hazard.

Risk management is the process of weighing policy alternatives in the light of the result of a risk assessment(s) and of other relevant evaluations and, if required, of selecting and implementing appropriate control options (including, where appropriate, monitoring/surveillance activities).

Risk communication is the interactive exchange of information and opinions throughout the risk analysis process concerning risk. It should involve not only risk assessors and risk managers but also consumers and a wide range of other actual or potential stakeholders.

The elements of a structured approach to risk management are risk evaluation, risk management option assessment, implementation of management decision, and monitoring and review.

The microbiological safety of foods is principally assured by control at the source, product design, and process control and the application of good hygienic practices (GHPs) during production, processing (including labeling), handling, distribution, storage, sale, preparation, and use, preferably in conjunction with the application of the HACCP system. This 'preventive' system offers more control than end product testing, because the effectiveness of microbiological examination in assessing the safety of food is limited.

Control of *Listeria monocytogenes* in foods provides an example of the need to consider a structured risk management approach. Establishment of tolerable low levels of *Listeria* in specific foods may be one food safety objective established by risk managers after a rigorous and transparent risk analysis.

The Seven Principles of HACCP

The HACCP system consists of seven principles. These principles make up the Codex standard, which has become the reference for international food safety. They are as follows:

Principle 1: Conduct a hazard analysis.

Principle 2: Identify the critical control points (CCPs) in the process. CCPs are steps at which control can be applied and a food safety hazard can be prevented, eliminated, or reduced to acceptable levels.

Principle 3: Establish critical limits (CLs) for preventive measures associated with each identified CCP.

Principle 4: Establish CCP monitoring requirements and procedures for using monitoring results to adjust processes and maintain control.

Principle 5: Establish corrective actions to be taken when monitoring indicates that a particular CCP is not under control.

Principle 6: Establish procedures for verification to confirm that the HACCP system is working effectively.

Principle 7: Establish documentation concerning all procedures and records appropriate to these principles and their application.

HACCP Team Formation

A trained HACCP team is essential. The HACCP team has to provide the production-specific expertise and experience, which are necessary for the development of the HACCP plan. Responsibilities of the HACCP team include the organization and documentation of HACCP study, the review of deviations from CLs, the organization of internal audit of HACCP plans, and the communication, education, and training of employees in the operation of HACCP system.

Product Description

Information should be provided regarding ingredients, processing methods, retail, packaging and storage conditions, and their associated hazards. Moreover, information should be given on product shelf life, type of food packaging, intended use with preparation instructions focussing on specific population groups (infants, immunocompromised individuals, the elderly, etc.), labeling, storage, and distribution conditions.

Flow Diagram Construction

The flow diagram should be constructed by the HACCP team and should identify all processing steps including steps prior to and after processing in the plant.

On-Site Verification of Flow Diagram

The HACCP team should confirm the processing operation against the flow diagram during all stages and hours of operation and amend the flow diagram where appropriate. Modifications should be made to the flow diagram as necessary and documented. After these five preliminary tasks have been completed, the seven principles of HACCP are applied.

Prerequisite Programs

PrPs are normally in place before the HACCP plan is developed. They include personal hygiene, good manufacturing practices (GMPs), GHPs, supplier quality assurance, maintenance schedules, and training. These PrPs should be properly implemented before assessing HACCP implementation.

Good Manufacturing Practices

GMPs provide general rules for the manufacture, handling, and preparation of various kinds of food products. They aim at safeguarding good hygienic and sensory quality traits, which are essential for safe food production. In the United Kingdom, the Institute of Food Science and Technology publishes guides to GMP.

Industries that have adopted the GMPs manufacture safer and of better quality products; decrease consumer complaints; have a better, cleaner, and safer working environment; and present a greater employee motivation and productivity and improved psychological conditions.

GMPs focus on three areas: personnel, buildings, and facilities as well as production and process control.

Personnel should have adequate personal cleanliness and be responsible for disease control by testing themselves for sexually transmitted diseases; wash their hands thoroughly before starting work and after using the toilet; wear gloves and protective clothing, suitable footwear, and hair coverings; avoid smoking, eating, chewing, spitting, sneezing, or coughing in the production area; and remove all unsecured jewelry and other objects that might fall into food. Personnel should also be aware of the infectious nature and transmission routes of food-borne pathogens.

Buildings and Facilities Equipment

They should be located away from environmentally polluted areas or areas subject to flooding or areas prone to infestations of pests; they should have adequate supply of potable water, natural gas, electricity, fuel, waste disposal systems, ventilation system to minimize odors and vapors, air-conditioning, and dust control.

Floors should have non-slip surface and be made of materials that are impervious; durable; resistant to grease, cleaning agents, and biochemical and microbial attack; free from cracks and crevices; and easy to clean. Doors should open automatically or have heavy-duty plastic strips permitting easy access by personnel and forklift trucks.

Roofing is normally flat or slightly pitched and is supported by trusses or beams and should provide adequate natural light.

Finally, pest control should apply focussing on insects, flies, cockroaches, moths, beetles, and rodents.

Equipment should be readily disassembled for inspection and manual cleaning, sanitized with approved sanitizer, and rinsed with potable water if required, equipped with rounded corners and edges. All surfaces in contact with food should be smooth, not porous, inert, visible for inspection, accessible for manual cleaning, made of nontoxic material, and corrosion-resistant.

Production and Process Controls

No raw material or ingredients should be accepted if it is known to contain parasites, undesirable microorganisms, pesticides, veterinary drugs or toxic, and decomposed or extraneous substances.

Quality control of raw materials should take place to decide if deliveries are accepted or rejected. Moreover, inspection of the overall condition of the trucks used to transport low-moisture raw materials or frozen raw materials should take place.

Storage rooms for raw materials should be clean and have adequate space for inspection. They should also have good air circulation, correct temperature, and proper humidity. Raw materials should not be placed on the floor.

Packaging materials should be hygienic, odorless, and not reacting with either the contained food or the surrounding atmosphere. Finished products should be labeled and placed for checks to see their conformity with product specifications. Defective products should be placed on a site waiting for a decision on rework or disposal. Final products approved for release should be taken to the warehouses.

Control Measures

Control measures are grouped into PRPs that manage the basic conditions and activities, operational PRPs that manage control measures that the hazard analysis identifies as necessary to control identified hazards to acceptable levels, and an HACCP plan to manage control measures that the hazard analysis identifies as necessary to ensure control of identified hazards to acceptable levels (CCPs).

Operational prerequisite programs (OPRPs) are PrPs that are essential. They are essential because a hazard analysis has shown that they are necessary in order to control specific food safety hazards. OPRPs are used to reduce the likelihood that products will be exposed to hazards, that they will be contaminated, and that hazards will proliferate. OPRPs are also used to reduce the likelihood that the processing environment will be contaminated and that hazards might occur in that environment.

Critical Control Points

A CCP is a point in a step or procedure at which a control is to be applied to prevent or eliminate a hazard or reduce it to an acceptable level.

CCPs may be located at any point in the food production plant where hazards need to be prevented, eliminated, or reduced to acceptable levels. The identification of CCPs should ensure appropriate control measures that are effectively designed and implemented. In particular, if a hazard has been identified at a step where control is necessary for product safety and no control measure exists at that step, then the product or process should be modified at that step or at an earlier or later stage, to include a control measure. Moreover, a monitoring system per critical point should be established and implemented.

CCPs should be carefully identified and documented. They should be used only for purposes of product safety or their use should be justified by the critical nature of the CCP. CCPs should not be confused with control points that do not control safety but refer to quality issues.

A tree diagram can be used to detect CCPs or PrPs in a food processing plant as shown in Table 1.

OPRPs are PrPs that are essential. They are essential because they are necessary to control specific food safety hazards.

Critical Limits

The maximum or minimum value to which a physical, biological, or chemical hazard should be controlled at a CCP to prevent, eliminate, or reduce to an acceptable level the occurrence of the identified food safety hazard.

The establishment of CLs takes place in parallel or follows the identification of all factors associated with CCPs. CL levels should be determined scientifically and should be related to unacceptable consumer health risks.

HACCP Plan

The HACCP coordinator and HACCP team are responsible for the development of the HACCP plan or plans for specific food products having identified the highest risks to food safety within hazard analysis. The HACCP plan should include the different food safety hazards to be controlled at each CCP, the control of measures, CLs, monitoring of procedures, corrections and corrective actions if CLs are not in control, responsibilities and authorities, and monitoring records.

Table 1 Tree diagram for CCP and OPRP detection in food processing

Procedure step	*Q1 Do preventative control measures exist? (yes/no)*	*Q2 Is the step specifically designed to eliminate or reduce the likely occurrence of hazard to an acceptable level? (yes/no)*	*Q3 Could contamination with identified hazard (s) occur or could this increase to unacceptable levels? (yes/no)*	*Q4 Will a subsequent step eliminate identified hazard(s) or reduce likely occurrence to acceptable levels? (yes/no)*	*Is this step a critical control point? (yes/no)*
Receiving of raw materials	Y	N	N	–	OPRP
Storage of raw materials	Y	N	Y	N	CCP1 (M)

Verification Planning

Verification activities should confirm that PRPs and OPRPs are properly implemented, continually update the hazard analysis, and ensure that the HACCP plan is implemented effectively, hazard levels are within acceptable levels, and FSMS are performing effectively on a daily basis. The food safety team is involved in result evaluation.

Elements of verification include validation, CCP verification activities (calibration of monitoring devices, calibration record review, targeted sampling and testing, and CCP record review), HACCP system verification (observations and reviews and microbiological end product testing), and review by regulatory agencies.

Establishment of a Product Traceability System

A traceability system is mandatory in ISO 22000 but happens to be a common process in the food industry, often as a result of legislation. The auditor should check the batch and/or lot identification in records maintained throughout the process from material receipt to end product dispatch. The organization defines a retention period for traceability records and considers implications for disposition of potentially unsafe products and product withdrawal.

Corrective Actions

Initiation of corrective actions starts when nonconformities have been identified. A documented procedure defines requirements for nonconformities review (including customer complaints), determination of the causes of nonconformities, implementation of corrective action required, recording results of action taken, and review of corrective action taken.

Validation of Food Safety Control Measure Combinations

Validation of control measures relates to the control measures taken to manage hazards. These control measures need to be validated before being implemented. Steps prior to validation include identification of the food safety hazards to be controlled, identification of the necessary control measures, and prioritization of control measures to be validated.

The organization should validate that the selected control measures are capable of achieving the intended control of food safety hazards and that the control measures are effective and capable of ensuring control of the identified food safety hazards to obtain end products that meet the defined acceptable levels.

Validation is the element of verification focussed on collecting and evaluating scientific and technical information to determine if the HACCP plan, when properly implemented, will effectively control the hazards. It involves a scientific and technical review of the rationale behind each part of the HACCP plan from hazard analysis to each CCP verification strategy.

Example of a validation activity for cooked hamburgers is the collection of data to establish that CLs for maximum patty thickness, maximum belt speed, and minimum oven temperature will ensure that the internal temperature of every patty reaches the minimum requirement.

Continual Improvement

Continual improvement is an integral part of every step in environmental management and takes place by conducting routine internal audits and monitoring. Hence, policy, objectives, targets, and plans will have to be modified when nonconformities are identified. Actions for improvement include analysis and identification of areas for improvement; search and evaluation of possible solutions to achieve the objectives for improvement; implementation of the selected solution; measurement, verification, and analysis of implementation results to determine that the objectives have been met; and formalization of changes.

Management should ensure that the organization continually improves the effectiveness of the FSMS through communication, management review, internal audit, evaluation of verification results, analysis of results of verification activities, validation of control measures, corrective actions, and FSMS updating.

The New Standard ISO 22000

ISO 22000 is the new international standard for FSMSs, harmonizing key requirements and overcoming the difficulties of various food safety standards by region, country, activity, organization, and food type. ISO 22000 will help the organization to establish, implement, improve, and update an FSMS in the food plant. This standard promotes conformity of products and services to international standards by providing assurance about quality, safety, and reliability. It was created to gain universal acceptance and recognition by all segments in the food chain.

ISO 22000:2005 includes the following documents:

- ISO/TS 22003, FSMS – Condition for organizations that make certification and inspection of FSMS gives harmonized guidance for the accreditation (approval) of ISO 22000 certification bodies and defines the rules for auditing an FSMS as conforming to the standard. Published in the first quarter of 2006.
- ISO/TS 22004, FSMS – Guide-related practicing of ISO 22000:2005 gives harmonized guidance for the approval of ISO 22000 certification bodies and defines the rules for auditing an FSMS as conforming to the standard. Published in November 2005.
- ISO 22005, monitoring in bait and food chain – General principles and guide for system preparing and design.

The standard has three parts:

- Requirements for GMPs or prerequisite programs
- Requirements for HACCP principles of the Codex Alimentarius
- Requirements for management system

The standard aims at food safety enhancement, consumer protection, consumer confidence strengthening, cost efficiency

improvement throughout the food supply chain, compliance with Codex HACCP principles, alignment with ISO 9001:2008, and provision of auditing guidelines for internal audits and third-party audits.

Relation of ISO 22000 to HACCP

ISO 22000 dynamically combines HACCP principles as described by the Codex Alimentarius Commission with PrPs and HACCP plan using hazard analysis. The adoption of a solid PrP is important with regard to improving the economic viability for HACCP implementation. Representative PrPs are water supply, premises/equipment, transport and storage, sanitation and pest control, personnel hygiene, recall program, supplier evaluation, and approval. The ISO 22000 analysis worksheet for determination of the PrPs for food processing is shown in Table 2. Receiving of raw materials is a PrP, whereas pasteurization is not a PrP, that is, it is a CCP. This analysis worksheet can be used in conjunction with the decision tree or tree diagram shown in Table 1.

Failure Mode and Effect Analysis

In failure mode and effect analysis (FMEA), risk of contamination and its presence at hazardous fraction in the final product are expressed with the risk priority number (RPN), which is defined as follows:

$$\mathrm{RPN} = \mathrm{S} \times \mathrm{O} \times \mathrm{D} \qquad [1]$$

where S is the severity of contamination risk, O the occurrence of contaminated ingredient, and D is the detection probability of contaminated ingredient. S, O, and D are given values out of 10.

Corrective action is carried out when RPN is greater than 130.

The classification of hazardous elements occurs according to the RPN assessment, and corrective actions are proposed per identified hazard. Following calculation of the new RPN (the RPN′ after undertaking corrective actions), a new classification of hazardous elements is shown (Table 3).

Analysis is made based on both best expert opinion and product history (epidemiological studies) for similar items of all the ways that each component or subsystem might fail to comply with its intended function.

Table 2 ISO 22000 analysis worksheet for determination of the prerequisite programs for food processing

Processing step	*Are the technical infrastructure and the preventative maintenance program adequate?*	*Is it feasible to evaluate them?*	*Do they contribute in the control of recognizable food safety hazards?*	*Does the effectiveness of the remaining control measures depend on them?*	*Is it a prerequisite program?*
Receiving of raw materials	Yes	Yes	No	Yes	Yes
Pasteurization	Yes	Yes	No	No	No

Table 3 FMEA table of hazardous processing methods for food products

Defective products											*Estimated corrective actions result*
Production step	*Hazards*	*Causes*	*S*	*O*	*D*	*RPN*	*Corrective actions*	*S*	*O*	*D*	*RPN*
1. Receiving of raw materials: $T_r = < 6\ ^\circ C$ refrigeration, $T_r = < -18\ ^\circ C$ freezer, $T_r = < 18\ ^\circ C$ environment	Pathogens, chemical residues, foreign matter	Wrong handling	7	4	4	112	Not required	–	–	–	–
2. Storage of raw materials: $T_r = < 6\ ^\circ C$ refrigeration, $T_r = < -18\ ^\circ C$ freezer, $T_r = < 18\ ^\circ C$ environment	Growth of pathogens due to inadequate temperature conditions, chemical residues, foreign matter	Inadequate functioning of temperature during storage, improper sanitation	9	5	5	225	Extra control of storage temperature Storage place should be separate from the rest of the production line	9	2	2	36

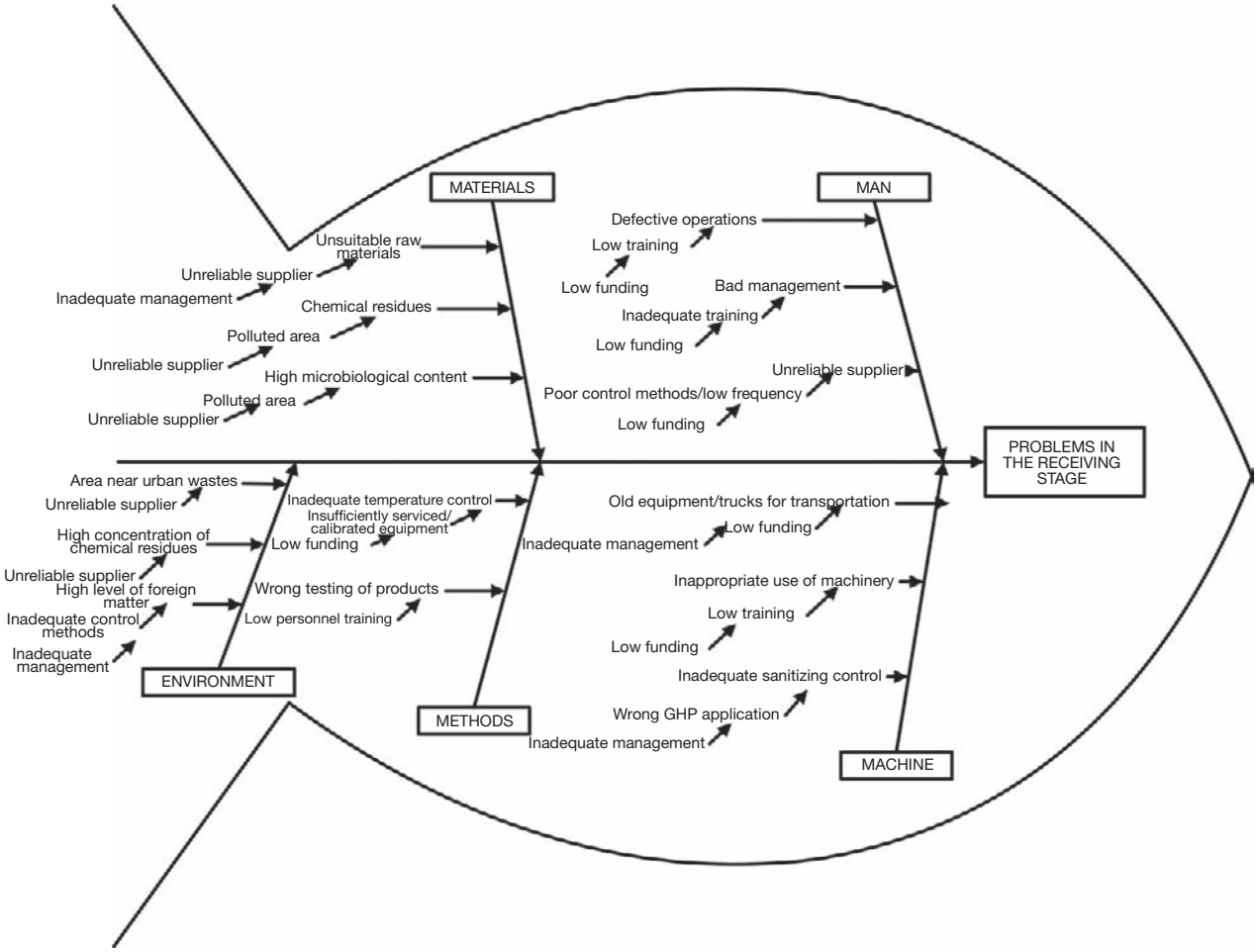

Figure 1 Application of Ishikawa diagram to food processing (CCP receiving of raw materials).

Ishikawa Diagrams

Ishikawa or fishbone diagrams analyze all the dangers at all processing stages where CCPs are incorporated. These diagrams consist of five basic axes: man, machine, materials, methods, and environment. In these axes, the danger is described.

The Ishikawa diagram or fishbone diagram was invented by Ishikawa, a Japanese quality control statistician. Due to its function, it is referred to as a cause and effect diagram, and due to its resemblance to fish skeleton, it is also referred to as a fishbone diagram. This diagram is an analysis tool that provides a systematic way of investigation of causes and effects. Causes create or contribute to those effects.

An implementation of Ishikawa diagram for the CCP of raw materials receiving in any food processing is shown in Figure 1.

Pareto Diagrams

A Pareto diagram is usually constructed aiming at the determination and display of particularly high-risk processing steps and their corresponding corrective actions. Then, a second Pareto diagram is drawn focussing on spinach pie processing aiming at the determination of new risk situation (following the suggested corrective actions) (Figures 2 and 3).

In a Pareto diagram, corrective actions for the 20% of causes of failures with the highest RPN are recommended. Then, further corrective actions are suggested for the remaining causes of failures.

Conclusion

HACCP and ISO 22000 are FSMSs that can be used effectively and proactively by the food industries along with GMPs and GHPs to enhance food safety and respond to food scares. Other food safety tools can be used in conjunction with HACCP and ISO 22000 such as FMEA, Ishikawa diagrams, and Pareto diagrams.

See also: Codex Alimentarius Commission: Role in International Food Standards Setting; Foodborne Pathogens; Risk Assessment of Foods and Chemicals in Foods.

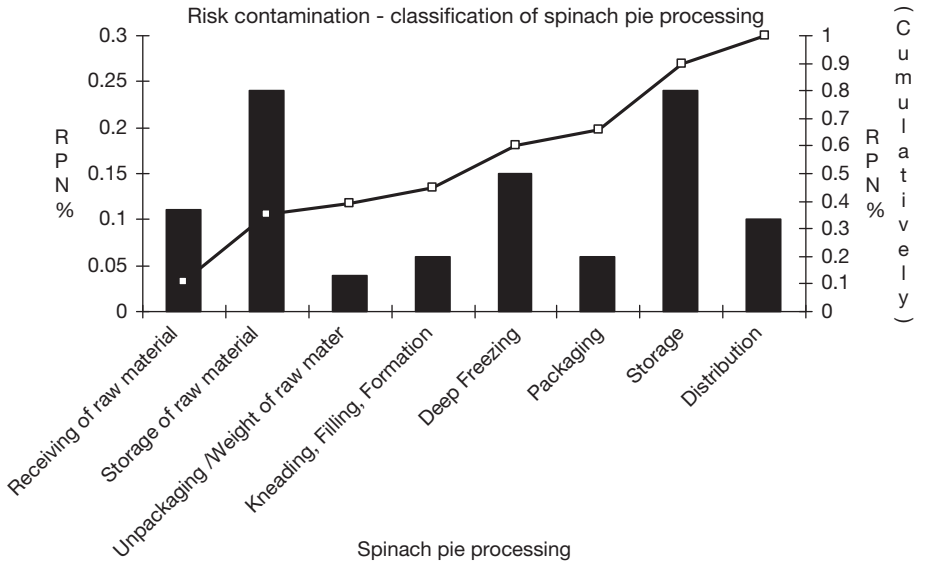

Figure 2 Pareto diagram prior to corrections for spinach pie processing.

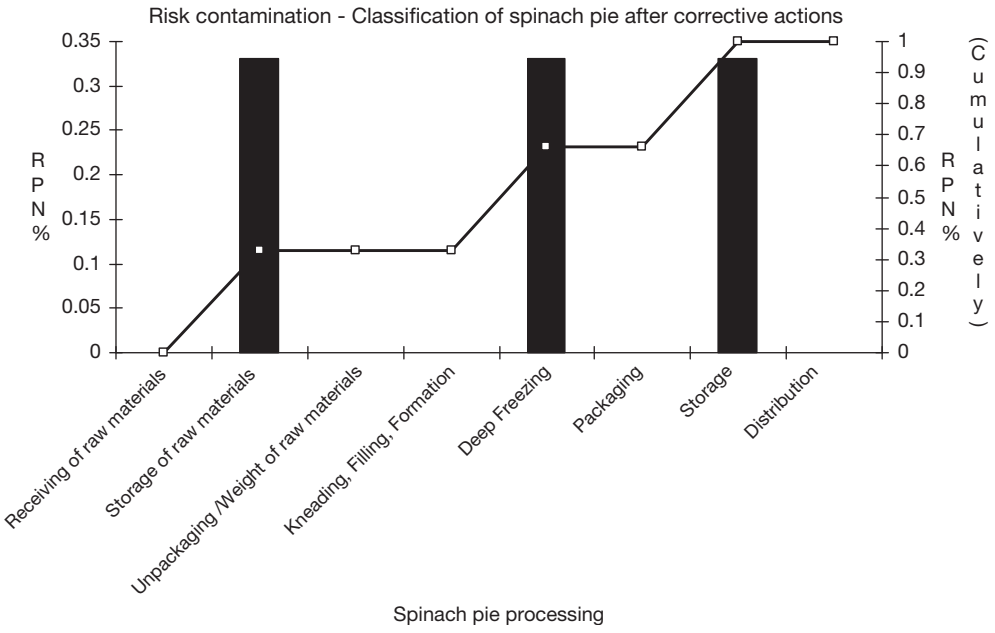

Figure 3 Pareto diagram for spinach pie processing after corrective actions.

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Ham: Cooked Ham

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Introduction

Cooked ham is typically a cured piece of pork derived from the hind legs of swine, which is further preserved through processes like cooking and, optionally, smoking. Cooked ham is also known as gammon in the United States. The processing of cooked ham involves the use of brine or curing solutions that are either injected or soaked, followed by the application of thermal treatment. The piece of ham may be intact with bones in or deboned before either curing or cooking. Once the final ham product is manufactured, it can be sold as a piece for slicing by retailers or sliced and packaged under various forms of vacuum or under modified atmosphere for its direct sale to consumers at retail outlets.

There are many variables at play during the manufacture of a cooked ham, and these all impact on the final quality of the final product, but the quality of raw materials and an adequate processing regime, based on minimum temperature–time treatments, are the most important to achieve a product possessing good organoleptic quality. Initially, special care must be taken with respect to the selection and quality of the meat cut chosen, the quality and level of ingredients used in cure formulation, the injected volume of brine or cure used, the rate and extent of tumbling employed, and the cooking time and temperature selected. Cooked ham is sold in many formats: from chilled and sliced formats lasting for several weeks to canned formats lasting for months to years. The consumption of cooked ham is quite relevant in many countries worldwide. The processing technologies employed for the production of cooked ham may change depending on the raw materials and the processing conditions adopted by ham manufacturers in different regions of the world, and these will be discussed in this article.

Types of Cooked Ham

The best cooked hams are achieved when low injection curing volumes are used and no addition of any ingredient, with the exception of salt and nitrite, is utilized in the cure formulation. In general, if excessive levels of water are injected into the ham, then the resulting product quality becomes poor. Water retention in ham products can be facilitated by the addition of processing aids like polyphosphates, carrageenans, alginates, and starches.

There are many types of products that depend on the raw materials used and the type of processing technology employed. Ham products are no exception. Some traditional hams have received the name of the region where they were originally produced, like British Wiltshire and York hams, French jambon de Bourgogne and jambon de Reims, and the Italian prosciutto cotto. For instance, Wiltshire ham is produced according to traditional recipes originating back to the eighteenth century using a wet cure, within which the meat is soaked for 4–5 days and which then could be smoked using oak whisky barrels.

If claims are made for any specific procedure like hickory-smoked ham, then hickory wood must be used for smoking. If the smoke flavor is injected, then the term added smoke flavor must be included on the label. Honey-glazed hams or honey-cured hams can be labeled if honey constituted at least 50% of the sweetener used and comprised at least 3% of the formula and has a discernible effect on flavor. Other sweet cure hams may be produced by using less salt and including sugar in the brine.

Processing Technology

The processing of cooked ham includes some key manufacturing stages, which are receipt and intake of hams, brine injection, tumbling and massaging, cooking, and cooling as shown in Figure 1.

Receipt and Intake

Meat for ham manufacture may be preserved through refrigeration or kept under frozen storage. Once received, they are controlled before processing. The microbiological condition of the hams must meet the microbiological standards and not exceed 5 °C upon arrival and be stored at −2 °C to +4 °C until processing commences. Previously frozen meat for ham manufacture must be completely thawed, taking care that the outer surface of the meat does not exceed +7 °C during the defrosting period. The high pH 6.0–6.5 of dark, firm, and dry leg meat is not a problem for cooked ham manufacture because any microbial risk is eliminated by the heat treatment, and its typically high pH assists in increasing water retention. The fat and its composition in fatty acids in meat are very important for flavor development in the resulting ham. Of course, this strongly depends on the pig crossbreeds used for meat supply, the production systems operated, and the type of feeds received by the pigs. In most cases, hams are boned to facilitate cure injection, cure diffusion, meat molding, and, later, slicing.

Brine Injection

The main ingredients in the brine or cure are salt and sodium or potassium nitrite. The amount of salt varies depending on the type of product to be produced. The salt in the brine or cure assists in the binding of muscles through myofibrillar protein solubilization, and this also results in the enhancement of product yield and final weight. Some carbohydrates, like sucrose, dextrose, and corn syrup, may be added to the brine or cure to impart a pleasant mild taste. Nitrite is added to assure the generation of nitric oxide for preservation purposes, and it also contributes to the typical cooked pink color brought about by

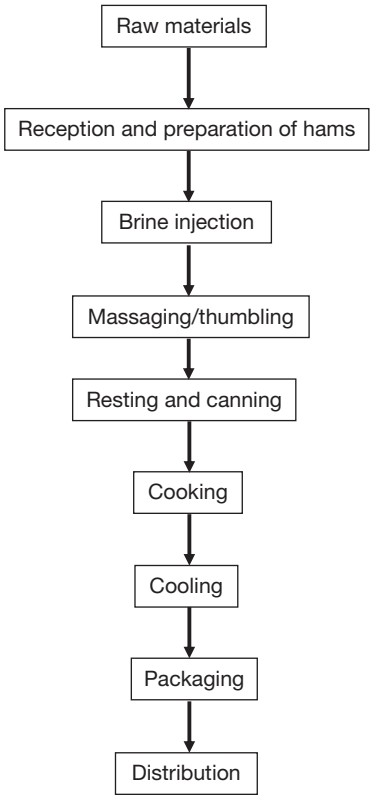

Figure 1 Flow diagram for a typical processing of cooked ham.

the formation of nitrosyl hemochrome. Sodium ascorbate or sodium erythorbate may be added as cure accelerators in order to speed up the conversion of nitrite to nitrous oxide, thus resulting in the disappearance of nitrite and lowering the generation of nitrosamines. Other additives may be added to the brine or curing solution, but this is mostly used for the production of low-quality hams where more water uptake is required to make such products more palatable. Consequently, additives such as alkaline phosphates, polyphosphates, and pyrophosphates elevate pH values to around 6.0–6.5, thereby shifting the pH far from the isoelectric point of muscle proteins (which lies around a pH value of 4.5), and consequently, the water holding capacity is increased and more water is retained within the ham product. Phosphates also enhance the ionic strength within hams and this also results in protein solubilization. The amount of phosphates that is permitted in the curing solution for cooked hams varies from country to country and from product to product, but in general, added phosphate content in cooked ham does not exceed 0.3–05% (as P_20_5). Levels certainly do not exceed 0.6% as higher usage levels will result in the generation of bitter aftertastes, which consumers can easily perceive.

The brine or cure is injected into the ham through a multi-needle system. Pumping speed and volume of injection may be controlled. The injection machines may pump brine into boneless hams or also into bone-in hams. Care must be taken when injecting bone-in meat in order to avoid needle breakage as this would pose a major health risk. When an immersion brine is used to cure hams, it is continually reused, and its composition, temperature, and pH are monitored between uses. A current practice, after brine injection, consists of holding the hams for a brief period of time for resting. This practice assures a full diffusion of salt and additives through the entire piece.

Massaging and/or Tumbling

This is a common operation also performed under refrigeration. Tumbling or massaging hams typically takes a few hours and is carried out in rotational tumblers or mixers under mild agitation to avoid severe physical disruptions or damages. Often, the tumbling process occurs in cycles of starts and stops (i.e., tumbling for 20 min and resting for 10 min, before the cycle starts again). It is very important to maintain a low speed so that the paddles or baffles contained within the tumbler do not destructively damage the muscles as they collide with these structures. The tumbling process is often accompanied by applying a vacuum within the tumbler to remove air and air bubbles within the meat, and this reduces undesirable oxidation reactions. Pulsed vacuum application can be applied to tumbling ham, and the serial application and removal of the vacuum pressure cause the ham to sequentially close and open the meat structure, thereby allowing processing salts to become more interactive with meat myofibrillar proteins.

Cooking

Following the tumbling process, the meat pieces and extracted exudate are often stuffed into metal molds, polymer-based bags, or elasticated polymeric netting and made ready for cooking. The purpose of cooking, apart from achieving an acceptable flavor and texture, is to assist in setting the three-dimensional gel matrix that helps bind meat pieces together and reduces the microbial load on the ham to a level that renders it safe. Endogenous enzymes are also inactivated. The cooking process used for ham manufacture is such that the time/temperature achieved in all parts of the ham is equivalent to 2 min at 72 °C. The actual temperature achieved in the center of the ham may vary between 68 °C and 75 °C, but an internal temperature of 72 °C is targeted typically. Heat treatment is calculated and applied to inactivate pathogens and spoilage microorganisms and requires a rigorous control of time and temperature to achieve the final desired effect and ensure product wholesomeness during the anticipated shelf life. Canned hams are contained in special metallic molds that produce the final product shape or optionally packaged in ham-shaped plastic (zero water loss). Cooking can be considered as a pasteurization process since the internal temperature in the ham can reach around 72 °C after 30–60 min of heating. The way heat is distributed within hams follows two heat transfer mechanisms. The first one is convection by transferring the heat from the heating medium to the ham surface, and the second is by conduction of the heat from the ham surface to the inner part of the ham. The rate of temperature increase during cooking is important. Cooking can be operated in three ways: (1) heating at a fixed temperature, which is a less used approach, (2) heating until a determined temperature inside the ham (usually 68 °C) is reached, and (3) heating through stages so that consistent increases in temperature within the ham are stepwise, not exceeding 25–30 °C during each sequential step, thereby avoiding excessive heating of the ham. The sensory

Figure 2 Example of a cooked ham slice.

characteristics of hams are developed during cooking as a consequence of multiple enzymatic and chemical reactions.

Cooling

This stage must be performed carefully to ensure the wholesomeness and safety of the hams. Ham molds are immersed in cold water or cooled by using cold water showers in order to reduce the temperature to below 5 °C inside the ham. The most hazardous period in the cooling process is the temperature transit between 50 °C and 12 °C where bacteria might grow during the time involved to achieve this, and consequently, this is why it is recommended not to extend the temperature reduction period for more than 10 h.

Final Product

Hams are removed from the molds and may be smoked at the end of the process. The smoking of ham is usually performed by hanging over burning wood chips in a smokehouse, through the use of friction smokers providing smoke to the smokehouse or by spraying atomized liquid smoke on its surface. In all cases, the final product appearance is equivalent. Once finished, the hams are packaged, sometimes with a previous surface heat treatment to avoid any recontamination. Cooked hams can be commercialized as a full piece to retailers or as ham slices packaged under vacuum or modified atmosphere ready to be consumed. For ease and consistency of slicing (thereby preventing ham slices from breaking apart), hams can be placed in a freezer for up to 12 h (usually overnight) to harden the ham surfaces. A typical slice of cooked ham is shown in Figure 2.

Quality Attributes of Ham

Color

During the heating process, the color of ham changes from the initial red pork meat color to the final pink color, typical of cooked ham. This is due to nitrosyl hemochrome, which results from the transformation of nitrosyl myoglobin by heating. The final color of the cooked ham depends primarily on the initial content of myoglobin present in the starting muscles used and, consequently, is dependent upon the biochemical muscle type (whether muscles are more oxidative or glycolytic in nature), age of the animal at slaughter (higher in older animals), and degree of exercise afforded to the pigs during production.

Texture

The extent of heating and the enzymatic proteolysis on myofibrillar and connective tissue proteins will affect the structural breakdown and, thus, its texture. The moisture content and the amount of intramuscular fat also exert a positive influence on some textural and appearance traits. When hams are manufactured using hydrocolloids as part of the curing solution, the final ham products may have distinctive textures as a result of containing more bound water and possessing unique mouthfeel properties.

Flavor

Several biochemical changes, mainly relating to enzymatic reactions (proteolysis and lipolysis) by endogenous muscle peptidases and lipases, contribute to the generation of free amino acids and fatty acids, all substances that influence the taste and aroma of the final product. The high water activity and relatively low salt favor these enzymatic reactions, even though muscle peptidases become rapidly inactivated by heating, especially at temperatures above 50 °C. The release of free amino acids and free fatty acids may increase in hams during a 1–2-day chilled resting period before processing. Such released amino acids and fatty acids will be subject to further chemical reactions (i.e., Strecker reactions) that can generate volatile aromatic compounds. Further chemical reactions, like Maillard reactions, can also proceed during cooking and contribute to the generation of aromatic volatile compounds. So, the final flavor will depend on the time and intensity of heating.

Nutritional Composition

As for other meat products, the nutritional content of cooked ham is variable and is dependent on the initial composition of the raw ham (which is affected by pig physiology, animal production practices, and type and composition of feed) and the type of processing conditions used (brine injected, amount of injected water, other ingredients added, and heating conditions). Therefore, all of these factors will directly impact upon both the macro- and microcomposition of the final ham products.

Lipids, Fatty Acids, and Cholesterol

The fat content in cooked ham varies (5–20%) largely depending on animal genetics (pig crossbreeds used) and the type of feed offered to pigs during production. Triacylglycerols, which are particularly rich in monounsaturated fatty acids (45–50%) and contain a moderate level of saturated fatty acids (SFA) (35–40%), are mainly located in fat cells, while phospholipids, which contain high amounts of polyunsaturated fatty acids (PUFAs) and some cholesterol, are located in cell and organelle

membrane lipids. Free fatty acids (9–20% of total lipids) can also be found in ham products. The PUFA/SFA ratios may range from 0.17 to 0.35. Cholesterol levels in cooked hams are in the range of 50–65 mg/100 g, so that consumption of 100 g of cooked ham would contribute nearly 20% of the maximum recommended daily intake of cholesterol (300 mg).

Proteins, Peptides, and Amino Acids

Cooked ham contains about 25–30 g of protein per 100 g ham. These proteins are considered of high biological value due to their high content of all essential amino acids. Animal proteins in the diet are also known to favor the absorption of minerals like heme iron and trace elements. There are also histidine-based dipeptides, carnosine, and anserine, compounds that all possess antioxidant activity and that are quite resistant to heat treatment, thereby functioning to prevent oxidation-based reactions in cooked ham products. However, their antioxidant properties may only be secondary to those provided by the presence of nitrite in these products.

Minerals

Cooked ham constitutes a good source of highly bioavailable heme iron, zinc, phosphorus, potassium, magnesium, and selenium.

Vitamins

Cooked ham constitutes an excellent source of B-group vitamins like thiamine (B_1), riboflavin (B_2), niacin, vitamin B_6, and vitamin B_{12}, but, as for other meat products, its content in major vitamins A, C, D, and K is rather poor. Vitamin E may be largely increased in hams if pigs have been supplemented with the vitamin in the feed prior to slaughter.

See also: Ham: Dry-cured Ham; Meat: Eating Quality and Preservation; Pork Meat Quality, Production and Processing on.

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Ham: Dry-cured Ham

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Introduction

Dry-cured ham constitutes a type of meat product that has been produced for centuries. These products exhibit a wide variety of flavors and textures. Today, these products still constitute an important part of any local economy, culture, gastronomic heritage, and tradition. Even though the technology used for ham manufacture has evolved and new technologies are introduced, the basic processing procedures still rely on traditional manufacturing processes that are mostly based on salting, drying, and ripening. The dry-cured ham products develop an intense and characteristic texture and flavor, especially during the ripening and drying phases, where endogenous muscle enzymes play an important role in the final character of the product. The drying of dry-cured ham products is a key and common practice in product manufacture and adopted to great effect in the Mediterranean region due to the privileged climatic conditions that exist there. Not surprisingly, this is a region where dry-cured meat products have typically been produced. The primary product types produced and their specific characteristics are described in the succeeding text.

Types of Dry-Cured Ham

Dry-cured ham constitutes one of the main and representative products in Mediterranean countries. Some of the most well-known European products include Spanish jamón ibérico and jamón serrano; French Jambon de Bayonne; Italian prosciutto di Parma, prosciutto San Daniele, and prosciutto Toscano; Belgian jambon d´Ardenne; Portuguese presunto; and Croatian Istrian dry-cured ham. The production of these hams is based on a careful selection of the raw materials (back legs), control of time and processing conditions, and, of course, quality control at the end of the process, especially for color, texture, and flavor attributes (see Figure 1). Minimum processing time for an acceptable quality of those nonsmoked products is usually >9 months, but for the highest-quality products like Iberian hams, the processing time may last up to 24–30 months. The result is a ham with extremely rich flavors that are generated through enzymatic reactions. Dry-cured hams are usually consumed raw and cut into thin slices (see Figure 2), without further smoking or cooking.

Culatello is a derived product from dry-cured ham. It is produced in Italy from the large part of the ham (underside). It is packaged with the pig's bladder and tied using slip knots in order to keep its nice round shape. Then, culatello is salted and dried like hams. The processing is usually between 10 and 14 months. Culatello di Zibello is produced in Parma (Italy).

Certain dry-cured hams have been protected with European Union certifications like the protected designation of origin (PDO), the protected geographical indication (PGI), or the traditional speciality guaranteed (TSG). There are many PDO-classified products in the Mediterranean area. In the case of Spain, there are three for Iberian hams: Guijuelo, Dehesa de Extremadura, and Huelva, and one for non-Iberian hams that is denominated Teruel. Serrano ham has TSG classification; French Bayonne ham has PGI classification, as does Spanish Trévelez ham. The accomplishment of the established requirements is controlled by consortia or local entities that take care of the control of raw materials, processing conditions, and quality of the finished products. For instance, Serrano ham, a TSG ham produced in Spain, is stamped once approved by the Serrano Foundation. In a similar way, the PDO prosciutto di Parma is stamped by the Parma consortium once it has been produced in a state of control and has met all of the requirements and quality attributes expected for the product by the consortium.

Hams in Northern Europe are processed for shorter times, than those in Southern Europe, and manufacture is followed by smoking and cooking before consumption. Some examples are the Norwegian Spekeskinke ham that is ripened for up to 12 or more months, Fenalâr ham produced from lamb or mutton, German Westphalian ham, the cold-smoked Katenschinken ham, and the Finnish 'sauna' ham.

The American country-style ham or country ham is salted, postsalted, and aged for about 1 month. It is usually smoked and cooked before consumption (pan frying, in the oven or roasting). Most relevant country hams are produced in Kentucky, Virginia, Tennessee, Missouri, and North Carolina (see Figure 3).

China has also produced dry-cured hams for centuries. Some of the most important are the Jinhua ham, produced in the Jinhua district; Xuanwei ham, produced in the Yunnan province in the southwest of China; and Rugao ham, produced in the Jiangsu province.

Production of Dry-Cured Ham

The traditional processing of hams was extensively used in ancient times, but today, they are limited in production and restricted to rural regions, which are located in mountainous areas where cool and dry winds are available most of the year. Basically, and focussing on the Mediterranean area, the process consists of rearing pigs at home for about 1 year. These pigs are slaughtered by the end of November to coincide with late autumn or early winter months. Hams are salted by rubbing salt on the surface of the hams, and these are left hanging for a few weeks. Salt diffuses through the whole hams over a period of 3 months, and then, they are hung in large rooms in the uppermost part of buildings for the ripening and drying processes to proceed, which progress during the spring and summer months. Effective drying can be achieved by correct employment of natural air circulation, which is achieved by manual opening of windows. The decision is based on

Figure 1 Image of Spanish dry-cured ham during ripening/drying.

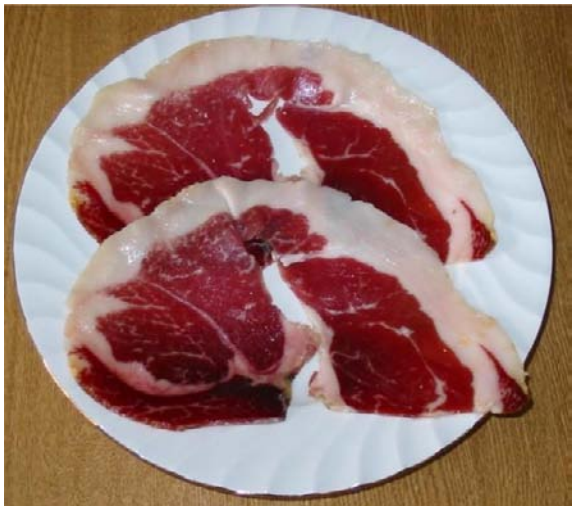

Figure 2 Slices of Iberian dry-cured ham showing typical marbling.

subjective evaluation of ham status. Total processing time takes nearly 1 year to complete.

The current processing technology is based on traditional steps but using modern technology. There is a careful selection of the raw meat materials (back legs from specific crossbreed pigs reared under controlled production regimes). The first stage in the manufacturing process involves meat reception, where hams are weighed and rubbed with curing salt (which is a mixture of sodium chloride and potassium nitrate) on the external surface of the ham. In some ham types, nitrate is not added. Immediately, hams are covered by salt during the salting stage. The main purpose of salting is to allow the

Figure 3 Image of a smoked American country-style ham.

penetration of salt and other curing agents into the meat. After 11–15 days, hams are hung to allow for diffusion of salt and curing agents through the entire meat piece and allow them to reach the inner part of the ham; this postsalting period lasts up to 3 months. Then, hams are ripened and dried to encourage flavor development and water loss for a period of time ranging anywhere from 1 to 18 months or more. Hams may be further ripened in cellars at mild temperatures for long periods of time; up to 24 months at temperatures between 10 and 20 °C in order to assist in the development of exquisite and intense flavors generated through further chemical and enzymatic reactions. The application of smoking is optional and more typically applied in Northern European countries and in the United States for country hams. Weight losses may reach up to 35%. Hams are rigorously controlled at the end of the production process for the quality attributes of color, texture, and flavor.

Traditional commercialization of hams is either as an entire piece (including the bone and foot) or as boned and molded pieces that facilitates its slicing at the point of retailing or directly by consumers at home. In the last few decades, the commercial distribution and retail display of ham slices have been through the employment of vacuum or modified atmosphere packaging systems.

Sensory Characteristics

Dry-cured ham has a typical bright red cured color, which is due to the formation of nitrosomyoglobin; a compound generated through the reaction of nitric oxide with myoglobin. This color is more intense in those oxidative muscles that contain larger amounts of myoglobin and in those hams derived from older animals.

The texture of hams depends on the extent of proteolytic breakdown of myofibrillar proteins and connective tissue and the extent of drying and the content in intramuscular fat, which also increases the juiciness and acceptability of hams.

Flavor of dry-cured ham is very intense, pleasant, and much appreciated. The taste is mainly associated to nonvolatile compounds like free amino acids and small peptides, which are generated in large amounts through proteolytic mechanisms

that take place throughout the entire ripening and drying process, thereby leading to the generation of numerous aromatic volatile compounds. These compounds are generated through different routes like enzymatic proteolysis and lipolysis and chemical reactions like amino acids degradation by Maillard and Strecker reactions.

Nutritional Composition of Dry-Cured Ham

Nutrient composition for dry-cured ham may be quite variable depending on not only factors intrinsic to the raw material, like pig breeding, animal production practices, type and composition of feed, age, and sex, but also factors related to meat processing like salting, drying, and ripening. So, some relevant differences in qualitative and quantitative data may be found in relation to not only macrocompounds, such as water, protein, fat, and salt content, but also microcompounds, like minerals and vitamins. All of these compounds are relevant to the nutritional characteristics of hams and to their potential implications for human health. The primary composition values for dry-cured hams are reported in the succeeding text.

Lipids, Fatty Acids, and Cholesterol

Fat content in dry-cured ham is extremely variable due to the influence of genetics (pig breeds used) and the differences in the type of feed offered to pigs prior to slaughter. For example, Iberian hams are known to contain large amounts of intramuscular fat (>9.5%), while those produced from usual crossbreeds contain much lower amounts: about 4.8% for Serrano hams, 3.6% for Parma hams, and 2.6% for Bayonne hams.

Triacylglycerols, which are rich in monounsaturated fatty acids (MUFA) and, to a lesser extent, in saturated fatty acids (SFA), are mainly located in fat cells, while phospholipids, which contain high amount of polyunsaturated fatty acids (PUFA) and some cholesterol, are located in membrane lipids. There are also free fatty acids that can range from 9% to 20% of total lipids. The main typical ranges for the fatty acid composition of lipids in dry-cured hams from white pigs are within 35–40% of SFA, 45–50% of MUFA, and 10–15% of PUFA. In special cases, and due to the use of particular pig breeds or due to feeds offered, these ranges may increase to 54–58% of MUFA. The most abundant SFAs are palmitic acid (25%), stearic acid (12%), and myristic acid (1.5%). The PUFA/SFA ratios may range from 0.17 to 0.35, which are typical ratios found in hams from white pigs such as Serrano or Parma hams. However, the $n-6/n-3$ ratio in dry-cured hams tends to be too high (reported values from 9 to 30) in relation to the recommended ratio of 6. However, these PUFA/SFA and $n-6/n-3$ ratios may be improved through adequate manipulation of pig genetics and feeding strategies. Cholesterol levels in dry-cured hams have been reported within the range 65–70 mg/100 g for Serrano and Parma hams. This means that consumption of 100 g of dry-cured ham would contribute nearly 25% of the maximum recommended daily intake of cholesterol (300 mg). Some oxidation of unsaturated fatty acids may be observed during the processing of dry-cured hams. This can be followed by determining oxidation-derived volatile compounds like hexanal. In the case of cholesterol, its oxidation products are found at very low concentrations (<1 ppm).

Proteins, Amino Acids, and Peptides

Dry-cured ham contains about 30 g of protein per 100 g ham, but this figure may change substantially depending on the extent of drying and final product fat content. These proteins are considered of high biological value due to their high content and due to their rich possession of all of the essential amino acids. A large proportion of these amino acids are readily available as a result of the extensive proteolysis, which takes place during dry-cured ham manufacture. Some additional for the nervous system from other amino acids like taurine (80 mg/100 g) and glutamine (5 mg/100 g) may be achieved. Animal proteins in the diet are also known to favor the absorption of minerals like heme iron and other trace elements. There are also histidine-based dipeptides, carnosine and anserine, which possess antioxidant activities that can control oxidation through the prevention of lipid oxidation by inactivating catalysts and/or free radicals in the cytosol. In any case, proteins experience some oxidation during processing and storage of dry-cured ham, but the determination of protein carbonyls shows intermediate oxidation when compared to the oxidation in other dry-cured and cooked meat products.

Recently, many peptides resulting from myofibrillar and sarcoplasmic protein degradation have been identified through the use of proteomic tools. Extracts of dry-cured ham and some of the identified peptides, with sequences containing between 2 and 10 amino acid residues, have shown a relevant inhibitory activity against the angiotensin I-converting enzyme (ACE). This enzyme plays an important role in the regulation of blood pressure by converting an inactive angiotensin I to angiotensin II, a potent vasoconstrictor. So, ACE inhibition can reduce arterial contraction and decreased blood pressure. The generation of bioactive peptides has been attributed to the action of muscle dipeptidyl peptidase enzymes, which remain active and stable during the complete processing cycle.

Minerals

Dry-cured ham constitutes a good source of iron (1.8–3.3 mg/100 g), which is highly bioavailable and, thus, provides a high nutritional contribution upon dietary intake. This is an important contribution because iron deficiency produces anemia, one of the main public health concerns that affect a substantial part of the population. In addition, heme iron favors absorption of nonheminic iron from other foods. Other relevant minerals found in dry-cured meat products are zinc, phosphorus, potassium, magnesium, and selenium (see Table 1). Zinc bioavailability also increases during the consumption of animal proteins. Recommended zinc intake levels are not being reached in many countries, and its deficiency considerably affects morbidity, mortality, growth, and development.

The main health concern with the consumption of dry-cured ham is related to its high salt content. Sodium concentrations up to 1200 mg/100 g may be reached in certain hams, but they are too high in relation to the recommended sodium intake that is < 2000 mg/100 g. This large amount of salt limits

ham consumption for a certain section of the population, which has a predisposition toward high blood pressure.

Vitamins

Dry-cured ham constitutes an excellent source of vitamins, especially the group B vitamins like thiamine (B_1), riboflavin (B_2), niacin, vitamin B_6, and vitamin B_{12} (see Table 1). The amount of thiamine that acts as a cofactor in various enzymatic reactions affecting appetite and the nervous system is very high (0.57–0.84 mg/100 g). Folate levels up to 13.5 μg/100 g are found in ham, which can help to reduce homocysteine levels. Levels of α-tocopherol in dry-cured ham usually range from 0.08 to 0.67 mg/100 g. However, its content in major vitamins such as A, C, D, or K is rather poor. Vitamin E may be largely increased in meat products if pigs are supplemented with this vitamin through the diet for several weeks prior to slaughter.

According to EU legislation (Directive 90/496/EEC, 2008), dry-cured ham can be considered a natural source of iron, zinc, phosphorus, selenium, thiamine, riboflavin, niacin, and vitamins B6 and B12, since it contains significant amounts (>15% RDA in 100 g) of these micronutrients (Table 1).

Dry-Cured Ham and Health

Some studies have been carried out in an attempt to relate the influence of the nutritional content of foods upon various pathologies, such as cardiovascular disorders, high blood pressure, various types of cancer, diabetes, and obesity. Few studies are available that assess the effects that dry-cured ham in the diet has on health, but a few studies have reported some beneficial effects of consuming dry-cured ham on atherogenic risk factors like a better lipid profile and a maintained blood pressure in spite of the high salt content.

In summary, dry-cured ham is a well-known product still produced through traditional recipes even in modern factories, giving particular sensory characteristics and a balanced nutritional profile.

Table 1 Minerals and vitamins in dry-cured ham and its contribution to recommended daily allowances (RDA)

Micronutrients	*Amount per 100 g*[a]	*RDA*[b]	*% of RDA*
Ca (mg)	12–35	800	1.5–4.4
Fe (mg)	1.8–3.3	14	13–23
Zn (mg)	2.2–3.0	10	22–30
Mg (mg)	17–18	375	4.5–4.8
K (mg)	153–160	2.000	7.6–8.0
P (mg)	157–180	700	22–26
Se (μg)	29	55	52
Na (mg)	1.100–1.500	–	–
Thiamine (B_1) (mg)	0.57–0.84	1.1	51–76
Riboflavin (B_2) (mg)	0.20–0.25	1.4	14–18
Niacin (equiv.)	4.5–11.8	16	28–73
Vitamin B_6 (mg)	0.22–0.42	1.4	16–30
Folic acid (μg)	Tr-13.49	200	6.7
Vitamin B_{12} (μg)	Tr-15.68	2.5	627
Vitamin E (mg)	0.08–1.5	12	0.7–12

[a]From Moreiras et al. (2006), MSC (1995), Mataix et al. (2003), Ventanas (2006).
[b]Directive 90/496/EEC (2008).
Source: Jiménez-Colmenero, F., Ventanas, J. and Toldrá, F. (2010). Nutritional composition of dry-cured ham and its role in a healthy diet. *Meat Science* **84**, 585–593, with permission from Elsevier.

See also: Cured Foods: Health Effects; Drying: Effect on Nutrients, Composition and Health; Meat: Eating Quality and Preservation; Meat: Structure; Nitrites and Nitrates; Pork Meat Quality, Production and Processing on; Vitamins: Overview.

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Heat Treatment: Effect on Microbiological Changes and Shelf Life

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Introduction

Foods are complex ecosystems constituted by the environment and the microorganisms that live in it. The food environment consists of intrinsic factors such as pH, water activity, and nutritional components and extrinsic factors such as temperature and gaseous composition, among others. By manipulating these, factors microbial growth can be limited and enzymatic degradation can be avoided, achieving the food preservation.

Thermal treatment is one of the most important physical methods for increasing the shelf life of foods. It is a versatile and economical system that can be adapted to almost any foodstuff and package. It can also be combined with other physical or chemical stress factors to achieve the microbiological stability of foods, causing the least possible destruction of quality. Traditionally, foods have been sterilized in hermetically sealed tinplate cans or glass jars. The intensity of the heat treatment applied depends on the type of foodstuff, quality of raw materials employed, pH, transportation and storage conditions, and geographic retail area. If the treatment complies with 'commercial sterilization' standards and no recontamination is allowed, it is clear that foods preserved by sterilization are stable and safe from a microbiological point of view during their shelf life. To avoid recontamination, sterilization relies on the existence of a hermetic package and, in some cases, such as with aseptic packaging, on very strict additional hygiene measures.

Preservation of foods by heat is a relatively old technology that started in 1810 with Nicolas Appert's work. In the development of this technology, there were various steps or milestones. The science application to food preservation by heat really started 85 years after Appert published his work. During those years, millions of canned foods were produced but no significant scientific advance was reported. Until well into the twentieth century, the main form of preservation was heat treatment, although salting, drying, fermentation, cold smoking, and other preservation methods were also used.

The evolution of thermal processes depended mainly on the equipment development and also on knowledge of the biological mechanisms involved in those processes. Isaac Solomon, a canner from Baltimore, modified the technology in 1860. He added calcium chloride to the cooking water, increasing the boiling point to 115 °C, which made it possible to increase the rate of production and also increase the bactericidal efficacy of the process. But the canning industry was revolutionized by A. K. Shriver in 1874, when he invented the pressure cooker. In 1910, Peter Durand patented a process that was, in essence, Appert's procedure but using a metal container instead of glass jars. During the twentieth century, the evolution of thermal processes permitted the development of the aseptic packaging technology, hydrostatic sterilization, and high-temperature short-time (HTST) procedures. These and other developments were, in principle, engineering improvements aimed at more efficient heating of canned products.

At the same time, there were advances on the scientific side related to process calculations. The first mathematical equations for process calculations appeared in 1920 and 1923 and published by Bigelow and Ball, respectively. More recently, the increase of HTST applications has been related to the development of time–temperature integrators for thermal process control and evaluation.

Heat treatment impact on the safety and quality of a product depends on the rate of the heat-induced reactions, and the process time treatment can have a strong and negative impact on the quality of processed foods, which is not desirable, especially nowadays, when the consumer trend is to demand minimally processed products. For preservation purposes, measuring the impact of heat in terms of safety and quality is of the greatest importance in the design, control, evaluation, and optimization of the sterilization process.

Effect of Heat on Microorganisms

Heat treatment is the most efficient and widely used method for destroying microorganisms. However, resistance to heat depends on the type of microorganism. A major problem in discussing mechanisms of heat inactivation and injury is that heat brings about many changes in biological material and therefore identification of 'the event' that causes death or injury is difficult. Moreover, there are differences among strains of the same species, among species, and between spores and vegetative cells.

Effect of Heat on Vegetative Cells

Most vegetative cells are killed at the sterilization temperatures normally used in the food industry, over 110 °C. The most important vegetative cells related to the safety of heat-treated foods are *Salmonella*, *Listeria*, *Campylobacter*, and *E. coli*, microorganisms that can contaminate various foods including meat, milk, vegetables, fish, and eggs and that are a great problem if the products are not properly heat-treated and stored.

Heat resistance to pasteurization temperatures depends on the species and strain and is usually expressed in terms of *D*-values (the time needed to reduce microbial concentration by one $\log_{10}$). Microorganisms are more resistant to dry heat than to wet heat, although, in view of food composition, much attention should be paid to wet heat in food microbiology. Physiological state is also a factor that affects microorganisms' resistance, cells in stationary growth phase being more resistant than cells in exponential phase. Other factors, such as low pH, modification of a_w, and presence of organic acids, also affect microorganism thermal inactivation.

In some cases, microorganisms can be sublethally injured by heat, making them more sensitive to adverse recovery conditions, such as organic acids, low pH, and low water activity. However, under these conditions, some microorganisms activate stress response mechanisms, and as a result, cross-resistance is often seen, leading, for instance, to greater heat resistance among the microbial population

Effect of Heat on Bacterial Endospores

To counteract unfavorable conditions, for instance, nutrient restriction, some microorganism species, especially *Bacillus* and *Clostridium*, activate a specific set of genes involved in sporulation. The bacterial endospore consists of a spore core with a membrane that will later form the vegetative cell. This core is surrounded by a cortex with a peptidoglycan structure and spore coat layers consisting of keratin on the outside. This morphology and the very low water content of the spore core play a crucial role in the high resistance (to heat and other stresses) of the bacterial endospore. When conditions become favorable, the spore starts to geminate and eventually develops into a new vegetative cell.

Since 1986, the year in which Cohn and Koch discovered the bacterial spore, its mechanisms of heat resistance and death have been studied in depth in an attempt to control inactivation processes. The high thermal resistance of spores has been attributed to the unequal distribution of water in the protoplast, providing stabilization to critical molecules such as DNA or RNA. However, this unequal distribution does not account for the extreme resistance of spores of some microorganisms, such as *Bacillus stearothermophilus*; consequently, it appears that there are other additional factors related to the high resistance of spores.

Heat can cause bacterial spore activation or induce deactivation and latency, damage, mutations, or complete inactivation. When the temperature is increased over the optimum for growth, the microorganism is inactivated or suffers some kind of deterioration, inability to initialize germination, or inability to duplicate critical molecules. It is possible that high temperatures affect the germination mechanism by damaging the trigger mechanism or cortex lytic enzymes. It has also been observed that some bacterial spores injured by heat are sensitive to antibiotics that under normal conditions would not affect them. This might suggest that heat affects the membrane, favoring the surface activity of antibiotics. Recent studies have indicated that physical damage of DNA does not take place, which means that in spores, the DNA is protected against heat and that the mechanism of thermal inactivation is something other than damage to those molecules.

There are many factors that can influence the heat resistance of bacterial endospores. The main factors affecting microorganisms can be grouped into four categories:

- Those affecting microorganisms during sporulation
- Those that affect microorganisms during storage of the spores that are produced
- Those that are a consequence of treatments given before, during, or after the treatment and also the nature of the medium
- Those affecting microorganisms during the recovery of survivors

Factors affecting microorganisms during the sporulation process determine the heat resistance of the spores. The presence of metal ions (Ca^{2+}, Mg^{2+}, or Mn^{2+}) in the sporulation medium is the main extrinsic factor contributing to an increase in the heat resistance of spores. If the presence of those ions decreases, the thermal resistance of the spores also decreases. A diminution of certain fatty acids during sporulation can increase the heat resistance of spores, and lack of glucose in the sporulation medium can reduce their heat resistance. The presence or absence of some amino acids makes the heat resistance of spores vary. One of the most important factors affecting the heat resistance of microorganisms during the thermal process is pH. Acidic pH values during the heating process reduce the heat resistance of spores, while at pH values close to neutrality, the thermal resistance does not vary substantially. There is an important interaction between pH and temperature; some studies have shown that the effect of pH diminishes as treatment temperature increases. Other factors that can influence the heat resistance of spores during the heating process are NaCl concentration, water activity (a_w), the presence of sugar or some germinant such as lysozyme that can help to increase the apparent heat resistance, and sublethal heating of spores prior to the thermal process.

Shelf Life

Shelf life is the time after manufacturing, processing, and packaging that a product remains acceptable under defined environmental conditions. It is a function of product characteristics such as composition, pH, water activity and fat content, the package, and the environment in which the product is transported, stored, and sold. It is established primarily not only in terms of microbial stability but also in terms of sensory acceptability.

Shelf life studies should be carried out in the following circumstances:

- Development of new products, processes or packaging, or reformulation or modification of existing ones
- Change of place of production or production team
- When there are no previous studies of shelf life

The starting point for determining shelf life is to obtain the necessary information about the food studied (chemical and physical characteristics, type of packaging, and storage, among other things) to determine the most important pathogenic microorganism that can grow in the product and to examine the relevant scientific literature. If the results of these studies guarantee that the pathogenic microorganism cannot grow in the food, it is not necessary to continue further studies. However, if the results are not clear on the possibility of growth of the organism, then it is necessary to conduct a series of additional studies that may include one or more of the following:

- Historical data for products manufactured
- Predictive microbiology
- Studies of specific laboratory life (durability studies and challenge tests)

Durability Studies

Durability studies assess the growth of a microorganism in a food during storage under reasonably foreseeable conditions without artificial inoculation, considering only natural contamination of the foodstuff. They are realistic and the evolution of the natural product contamination is studied. However, interpretation may be difficult because of the low prevalence of units contaminated with a certain microorganism, the low concentration of microorganisms present in the product under study, and/or the heterogeneity of the microbial distribution in the food. This would imply the need to conduct further studies and perform challenge tests.

Challenge Tests

In general, these tests are used only when other methods for assessing the food safety or its stability cannot be carried out or are not sufficiently clear about the possibility of pathogen growth. They provide information about the behavior of microorganisms inoculated in the food studied under certain conditions in a laboratory environment before storage. International committees have established protocols for conducting challenge tests. They consist essentially in inoculating the food with a defined concentration of the pathogenic microorganism and measuring the changes in the concentration levels during storage time, considering the worst case. This type of test should take into account the variability in food (using different batches) and specific food contamination (inoculation of isolates of food). However, it is difficult to imitate the level and heterogeneity of contamination and the physiological state of the bacteria.

Challenge tests can be performed with two different objectives:

(1) Assessment of growth potential (δ):

The growth potential (δ) is the difference between the $\log_{10}$ cfu g^{-1} at the end of the test and the $\log_{10}$ cfu g^{-1} at the beginning of the test. The experimental results may show a wide dispersion, notably because the lag phase is included. They depend on factors such as the strain(s) inoculated, the physiological state of the strain(s), the intrinsic properties of the food (pH, NaCl content, a_w, nutritional content, associated microflora, antimicrobial constituents, etc.), and the extrinsic properties (time–temperature profile, gas atmosphere, etc.) The main advantages of this method are (i) that it is relatively simple to implement and (ii) that the results can be used directly. Its drawback is the lack of flexibility in the interpretation: the results are only valid for the food studied in the conditions studied, so new experiments have to be performed each time there is a change.

(2) Estimation of growth parameters (μ_{max}):

The drawbacks of the previous approach can be solved by combining predictive microbiology models and challenge tests assessing μ_{max} (growth rates). This approach consists of a laboratory-based study that measures the growth rate of the pathogenic microorganism involved in an artificially contaminated food stored at an appropriate temperature. The temperature used for the experiment is not (necessarily) the one used for predictions since it is possible to predict growth at a temperature other than the one tested or in a time–temperature profile chosen to be representative of the foreseeable conditions of transportation, distribution, and storage. Once the test has been performed, the maximum growth rate is calculated from the growth curve. In the exponential growth phase, plotting the natural logarithm of the number of cells against time produces a straight line, and the slope of this line is μ_{max}. With this test, it is possible to estimate the microorganism concentration on a given day of the shelf life study if the initial concentration is known and/or to estimate the maximum allowable concentration of the microorganism that may be present in a food on the day of production in order to comply with legal limits at the end of its shelf life.

Thermal Process Calculation

Thermal process calculation is a fundamental step in ensuring the safety of thermally processed foods during their shelf life and their optimization. The calculation has two main components: (a) microorganism inactivation kinetics and (b) heat transfer mechanisms in food.

The inactivation kinetic of microorganisms is determined by using mathematical models that describe the rate of inactivation at a specific temperature and the dependence of the rate kinetic parameter on temperature. It has generally been accepted that when a homogeneous microbial suspension of microorganisms (spores or vegetative cells) is subjected to a constant temperature, microorganisms die at a constant rate. This means that, at a defined time, the same percentages of live microorganisms will die in the next time unit. According to these ideas, microbial inactivation by heat (spores and vegetative cells) follows two laws: (a) survivor law and (b) thermal destruction (TD) law.

Survivor Law

This law is based on the assumption that the destruction by wet heat of a pure bacterial culture at a constant temperature follows an exponential negative kinetic. This means that the number of viable cells is exponentially reduced during the time of exposure to a lethal temperature (Figure 1).

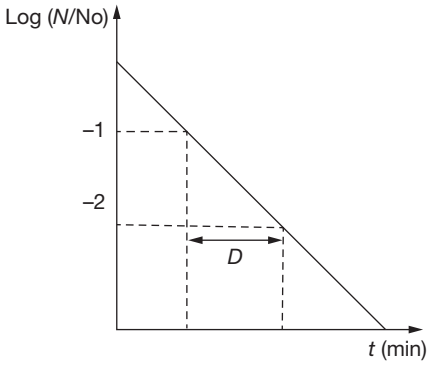

Figure 1 Survivor curve at constant temperature.

The parameter defining the destruction rate is named D_T, and it is defined as the treatment time, usually minutes or seconds, at a specific temperature that is required to reduce the bacterial population by 90%. This parameter is the best one for comparing the thermal resistance of different microorganisms, of the same or different species, at a given temperature, and of the same strain at different temperatures. It is also useful for evaluating the effect that environmental conditions have on the thermal resistance of microorganisms.

However, genetic or phenotypic heterogeneity of the cells in a population, mild or low temperature processes applied in food preservation, or any other stresses that may be applied to a microorganism population may result in nonlinearity. In these cases, shoulders appear, that is, the existence of a critical time before the microorganism is inactivated, or tails, showing a resistant population, or sigmoid curves, which are a combination of these two cases. A number of nonlinear models have been described in the literature, Weibull-like models, the biphasic model, normal distribution models, the logistic model, and Gompertz models being the ones most commonly used.

TD Law

When calculating sterilization or pasteurization processes, it is important to have knowledge of the variation of the rate constant with temperature.

The variation of D_T values with temperature was empirically modeled by Bigelow in 1921. This pioneer of thermal process calculations observed that by representing the logarithm of the rate constant versus temperature, a linear relationship was obtained for the temperature range studied. This curve was named the TD curve (**Figure 2**). The parameter that defines this TD relationship was called 'z,' and it indicated the change in treatment temperature (°C) required to achieve a tenfold increase or decrease in the D_T value.

Heat Penetration Curves and Process Calculation

The response of the product temperature to the temperature of the heating medium, for example, the steam retort temperature applied to a can, is governed by the physical laws of heat transfer and can also be expressed mathematically. Basically, there are two forms of temperature transfer: by conduction and by convection. The mechanism depends on the food; liquid food is usually heated by convection, while solid food is heated by conduction. The evolution of the temperature in the food can be measured by thermocouples installed in the package or in pipes containing the food, or mathematically by a computer model. The heat transport in foods is influenced by various factors including nature of the food, package, retort temperature, and the way in which the heat is applied. Heat penetration curves are obtained by plotting the evolution of food temperature versus treatment time. The parameters defining the heat penetration rate are deduced from heat penetration curves. These parameters, in addition to the microbial inactivation parameters, are used to calculate the thermal process properly.

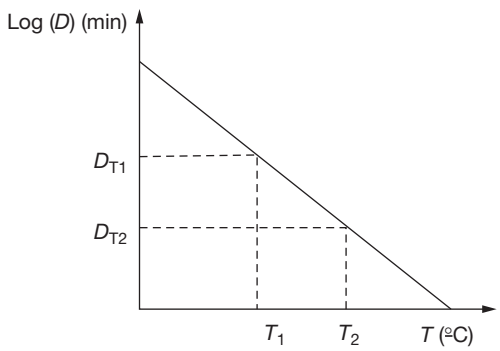

Figure 2 Thermal destruction curve.

There are two traditional ways to calculate the thermal process, the general method and the formula method first used by Ball in 1923 and improved in 1957 by Ball and Olson, known as Ball's formula method. The general method is more versatile and can be applied to almost any type of thermal process situation. However, this method is time-consuming and it soon gave way to the more convenient Ball's formula method. Ball's formula is very practical and rapid for estimating the sterilizing values of the process, although the accuracy of the calculation depends on how heat penetration parameters comply with certain restrictions established in the model.

Heat penetration curves and parameters can also be calculated mathematically, thus avoiding carrying out new experimental heat penetration assays when the retort or the size of the can is changed, for example. The models for carrying out these calculations are known as deterministic models, and they make use of a numerical solution by finite differences in the two-dimensional partial differential equation that describes conduction heat transfer in a finite cylinder.

The evolution of heat treatment procedures encouraged research on new models and tools to develop and validate thermal processes, not only from the point of view of safety but also from the point of view of retention of nutrients and sensory properties. Thus, the main objective of thermal process optimization is to maximize product quality while minimizing undesirable changes and cost. Optimization theory makes use of the different temperature sensitivities of microbial and quality factor destruction rates.

See also: *Clostridium botulinum*; *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; *Clostridium*: Occurrence and Detection of *Clostridium perfringens*; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; *Escherichia coli* and Other *Enterobacteriaceae*: Occurrence and Detection; Foodborne Pathogens; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Heat Treatment: Principles and Techniques; Irradiation of Foods: Processing Technology and Effects on Nutrients: Effect of Ionizing Radiation on Food Components; *Listeria*: Detection; *Listeria*: Properties and Occurrences; Packaging: Aseptic Filling; Pasteurization: Effect on Sensory Quality and Nutrient Composition; Pasteurization: Principles and Applications; Preservation of Foods; Pulsed Electric Fields; *Salmonella*: Detection; *Salmonella*: Properties and Occurrence; Shigella; Spoilage: Bacterial Spoilage; Spoilage: Yeast Spoilage of Food and Beverages; *Staphylococcus*:

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Further Reading

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Heat Treatment: Principles and Techniques

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Introduction

Given its practical implementation in both laboratory and industrial scales, heat treatment is one of the most preferred methods used for the destruction of spoilage and pathogenic bacteria. It is usually assumed that, at constant temperature, thermal death of bacteria is exponential with time, and thermal process calculations used in the food processing industry are based on this assumption. Despite the widespread use of the approach, it has been largely recognized that there are situations in which survival curves of many bacteria and other microorganisms after thermal and nonthermal treatments do not follow the exponential relationship.

Survival curves are represented as the decimal logarithm of the ratio between the number of microorganisms at a particular time, $N(t)$, and the initial number of microorganism, N_0, as a function of time t. As early as 1912, four types of survival curves have been recognized; they are illustrated in Figure 1. Since, in a thermal treatment, the number of microorganisms decreases with time, the ratio $N(t)/N_0$ is smaller than 1, so the decimal logarithmic values of that ratio are always negative.

Curve A is the survival curve most typically found and shows an initial lag in the bacterial death followed by an exponential decay (linear logarithmic decay), whereas curve C is similar but it includes a tail, which is hypothesized to be due to the presence of highly resistant populations. Survival curves as that represented by curve D indicate the presence of populations having different heat resistances.

The Linear Case (Curve B)

Curve B is the linear case that is traditionally used for most thermal and nonthermal processing calculations. To mathematically model thermal treatments practiced by the food industry, the thermal death of bacteria at a constant sterilizing temperature is assumed to follow a first-order kinetics expressed by the following equation:

$$\frac{dN(t)}{dt} = -k(T)\cdot N(t) \qquad [1]$$

where T is the temperature and k is a temperature-dependent kinetic constant. For practical reasons, it is convenient to express eqn [1] in terms of a change in the bacterial count after a certain time is elapsed. That is obtained by simply integrating eqn [1] from an initial time arbitrarily chosen to be zero in which the bacterial count is N_0 to a certain time t in which the number of surviving organisms is $N(t)$. If the heating process is carried out under isothermal conditions, integration of eqn [1] yields

$$\log\frac{N(t)}{N_0} = -\frac{k(T)}{2.303}\cdot t \qquad [2]$$

For application in thermal processes, the equation has been rearranged as

$$\log\frac{N(t)}{N_0} = -\frac{1}{D(T)}\cdot t \qquad [3]$$

where $D(T) = \frac{2.303}{k(T)}$ is the decimal reduction time; it has units of time (e.g., seconds or minutes) and represents the time necessary to treat a given food, at constant temperature T, in order to reduce its microbial count by 90% (i.e., when $N(t)/N_0$ is a factor 1/10). Thus, microorganisms with higher $D(T)$ values are more resistant to heat treatments than microorganisms with lower $D(T)$ values. Equation [3] clearly shows that by fitting the experimental data, obtained from a thermal treatment at constant temperature, the value $D(T)$ can be estimated from the slope of the straight line (Figure 2).

For calculation purposes, it is necessary to describe the relationship between $D(T)$ and temperature, which is usually done assuming that the death rate constant $k(T)$ varies with temperature following an Arrhenius-type relationship:

$$k(T) = \frac{2.303}{D(T)} = ae^{-E/RT} \qquad [4]$$

which after rearrangement in terms of $D(T)$ yields

$$D(T) = \frac{2.303}{a}e^{E/RT} = Ce^{E/RT} \qquad [5]$$

where C is an empirical constant. Equation [5] clearly shows that a plot of log $D(T)$ as a function of $1/T$ (where T is expressed as an absolute temperature) is a straight line. However, in some cases, experimental data plotted as log $D(T)$ versus T (either Fahrenheit or Celsius) over a small range of temperature yield a straight line. Figure 3 illustrates a typical log $D(T)$ versus T plot, from which a kinetic parameter known as z-value can be obtained from the slope of the line. From that relationship, an equation to determine the value of the decimal reduction time $D(T)$ at a given temperature T is obtained:

$$D(T) = D_{121}\cdot 10^{\frac{T-121}{z}} \qquad [6]$$

In eqn [6], T is expressed in degree Celsius (or Fahrenheit), as well as the z-value, and $D_{121.1}$ is decimal reduction time at the reference temperature considered to be 121.1 °C (250 °F). The decimal reduction at the reference temperature and z-values for some spore-forming microorganisms are given in Table 1.

A similar kinetics has been used to describe the destruction of nutrients and enzymes, some of which are given in Table 2. It is important to note that the values given in Tables 1 and 2 depend on the food and conditions such as concentration, pH, and processing temperatures.

For specific situations, the values of D and z including other reference temperatures can be found in several references. By combining eqns [3] and [6], an equation to estimate the

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00371-8

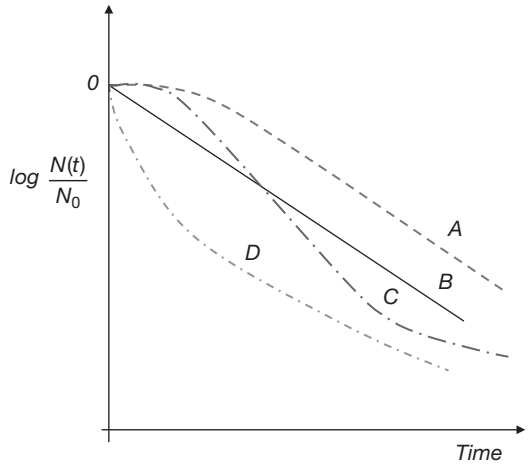

Figure 1 Different survival curves obtained in an isothermal heat treatment.

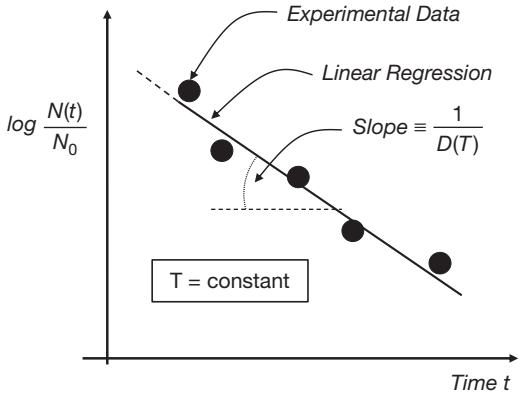

Figure 2 Linear survival curve and the determination of the relevant microbiological kinetic parameters.

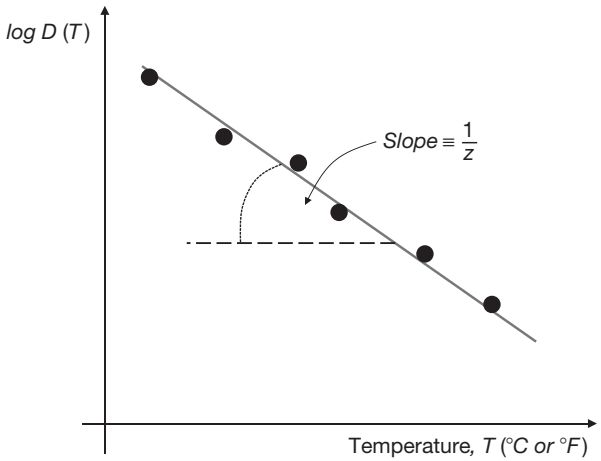

Figure 3 Typical plot of decimal reduction time as a function of temperature (in °C or °F) in a semilogarithmic scale.

Table 1 Thermal resistance characteristics of spore-forming microorganisms

Microorganism	$D_{121.1}$ *(min)*	z-*Value (°C)*
Clostridium botulinum	0.21	9.9
Bacillus cereus	0.0065	9.7
Bacillus stearothermophilus	4.0	7.0
Clostridium sporogenes	0.15	13.0
Clostridium thermosaccharolyticum	16–22	1.7–2.2

Adapted from Ahmed, J. and Rahman, M. S. (2012). *Handbook of food process design*. John Wiley & Sons Inc.

Table 2 Thermal resistance characteristics of enzymes and quality attributes of selected foods

Enzyme or quality attribute	$D_{121.1}$ *(min)*	z-*Value (°C)*
Anthocyanin (in grape juice)	17.8	23.2
Thiamine (in whole peas)	164	26.1
Thiamine (in peas in brine)	226.7	27.2
Lysine (in soybean meal)	786	21.1
Chlorophyll a (in spinach, pH 6.5)	16–22	1.7–2.2
Peroxidase (in peas)	3	37.2
Color (in peas)	25	39.4
Organoleptic quality (in peas)	2.3	28.3
Texture (in peas)	1.4	32.2
Overall quality (in peas)	2.5	32.2
Color (in green beans)	21	38.9
Color (in asparagus)	17	41.7

Adapted from Ahmed, J. and Rahman, M. S. (2012). *Handbook of food process design*. John Wiley & Sons Inc.

processing time t to achieve a given level of microorganism inactivation in the product (defined by the ratio $N(t)/N_0$) at a constant temperature T is obtained:

$$t = -D_{121.1} \cdot 10^{-\frac{T-121.1}{z}} \log \frac{N(t)}{N_0} \qquad [7]$$

A similar equation can be used to relate processing time with destruction of food nutrients, and for that case, the number of microorganism is replaced by concentrations of the respective nutrients or other components such as enzymes. By comparing z-values for microorganisms and food nutrients, it can be noted that the latter are significantly larger, which in a log t versus T plot yields straight lines with small negative slopes, which is schematically illustrated in Figure 4. For a given temperature, times on or over the respective lines indicate either sterility or oversterility. Concerning the food nutrients' destruction line, times on or above the line would indicate only nutrient or overnutrient destruction. Since the current trends and goals in thermal processing are to reduce the level of microbial contamination in the food while preserving its nutrients, flavors, appearance, and overall quality, Figure 4 clearly shows that high temperatures and short processing times are required to achieve these objectives.

Peleg et al. discussed and challenged the suitability of the classic Arrhenius and other relationships (e.g., the William-Landel-Ferry (WLF) equation, also known as the WLF model) used to describe rate–temperature relations in food and biological systems. Although these models have had some

applicability on chemical kinetics, food and biological systems exhibit a particular behavior as microbial inactivation of cells and spores is not done at ambient temperatures so the equal weight to rate deviations at low- and high-temperature conditions provided by the Arrhenius-type model is not appropriate to describe food and biomaterial-related kinetics. More details are discussed in the next section.

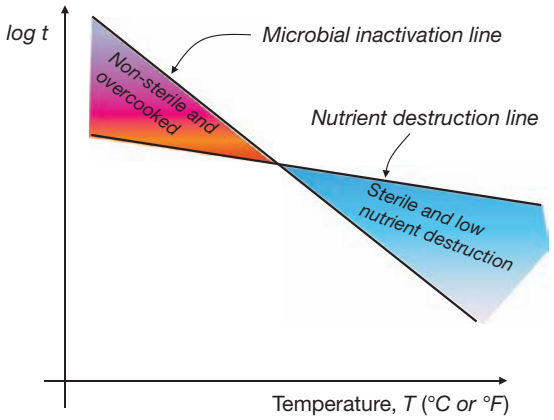

Figure 4 Schematic diagram illustrating the logarithm of the processing time as a function of temperature to achieve a given level of microbial inactivation and nutrient destruction.

The Nonlinear Case

Different mathematical models have been employed to describe nonlinear survival curves as those illustrated in Figure 1. Among them, the Weibull model has been extensively used. Alternative models that do not require the isothermal survival curves' log-linearity assumption have been also used and described in the literature.

The Weibull model can be written as

$$\log_{10}\frac{N(t)}{N_0} = -b(T)t^{n(T)} \qquad [8]$$

where $b(T)$ and $n(T)$ are temperature-dependent coefficients. Unlike the log-linear survival model, eqn [8] entails that even under isothermal conditions, the exponential inactivation rate can be time-dependent ($n \neq 1$) and the log-linear model is just a special case of eqn [8] when $n = 1$. Thus, the Weibull model can be applied to the traditional log-linear model but not vice versa.

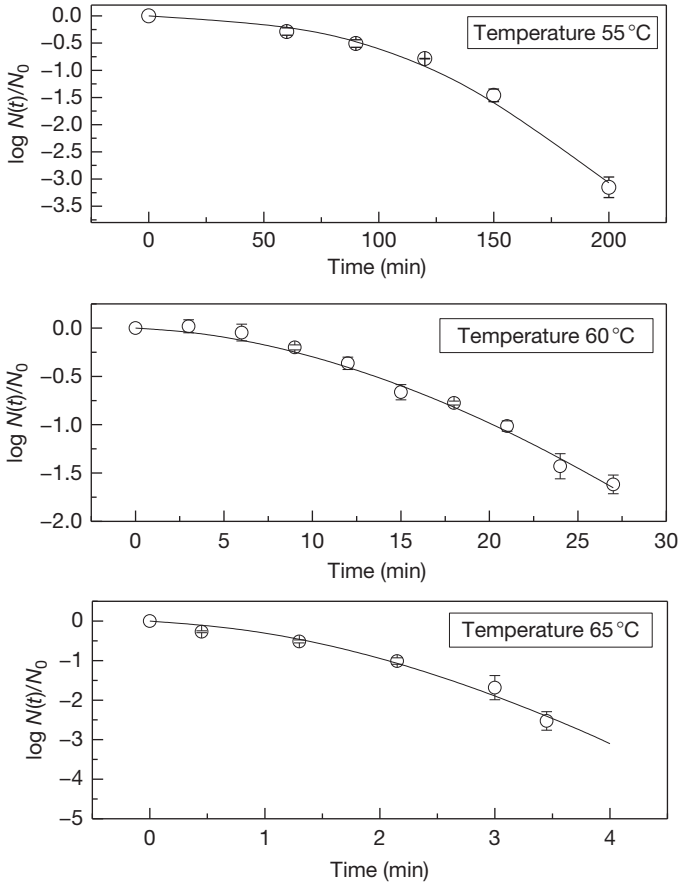

Figure 5 Experimental survival curves of *Listeria monocytogenes* inoculated on ready-to-eat meat after postpackage pasteurization at 55 °C, 60 °C, and 65 °C. Symbols are experimental points and lines are the fit of the Weibull model. Weibull parameters $b(T)$ and $n(T)$ can be obtained from fitting the experimental values, which for this particular case are plotted as a function of temperature and represented in Figure 6. Data extracted from Selby, T. L., Berzins, A., Gerrard, D. E., Corvalan, C. M., Grant, A. L. and Linton, R. H. (2006). Microbial heat resistance of *Listeria monocytogenes* and the impact on ready-to-eat meat quality after post-package pasteurization. *Meat Science* **74**, 425–434.

Figure 5 shows the heat inactivation of *Listeria monocytogenes* inoculated on ready-to-eat meat after postpackage pasteurization in a temperature range of 55–65 °C. It is clear from the figure that for all temperatures, the survival curves do not follow a first-order kinetics and the Weibull model provides a good description of the experimental data.

As shown in Figure 6, the parameter $b(T)$ is very small and becomes significant only when the postpasteurization temperature reaches a value that induces mortality to the bacterial population. The parameter $n(T)$ however has a very weak dependence on temperature (Figure 6(b)). In many instances, the coefficient n is practically constant over a considerable temperature range, that is, $n(T) = n$, further simplifying the use of eqn [8]. It has been shown that eqn [8] also applies to food components degradation.

Regardless of whether $n(T)$ is assumed to be constant constant or not, Figure 6 shows that there is a strong dependence of the parameter $b(T)$ with temperature. Campanella and Peleg found that for several bacterial cells and spores, *Clostridium botulinum* included, the dependence of $b(T)$ with temperature could be described by the log-logistic model given by the following equation:

$$b(T) = \ln\left(1 + \exp\left(m(T - T_c)\right)\right) \quad [9]$$

where T_c is a threshold temperature beyond which the inactivation becomes significant and m is a measure of the acceleration rate above T_c. It has been shown that eqn [9] is a better description of the effect of temperature on the parameter $b(T)$ than the traditional Arrhenius model because it provides a qualitative difference between the effects of temperature on microbial inactivation (or nutrient degradation) at low- and high-temperature ranges, which is defined by the value of T_c. To compare this approach with the traditional Arrhenius model given by eqn [6], the effects of temperature on the parameter $b(T)$ could be rewritten as

$$b(T) = Ae^{B \cdot T} \quad [10]$$

where A and B are empirical parameters and, in this case, T is the absolute temperature. After applying logarithm, eqn [10] yields

$$\ln b(T) = \ln A + B \cdot T \quad [11]$$

so a linear regression on $\ln b(T)$ versus T data will yield values of the parameters A and B. Figure 7(a) shows a comparison between the two approaches used to describe the effect of temperature on $b(T)$, whereas in Figure 7(b), the linear regression of the data expressed as eqn [11] is presented.

Determination of Survival Curve Kinetic Parameters

In order to design a thermal treatment, it is important to know how the kinetic parameters describing the survival curve change with temperature: specifically the parameters $D(T)$ and z if the traditional approach is used and the parameters $b(T)$ and $n(T)$ if the survival curve is better described by the Weibull model. Regardless of the model utilized to describe the survival curves, the traditional approach to determine these parameters is schematically described in Figure 8, which for the traditional approach is known as the thermal death time (TDT) method. As illustrated in Figure 8, one of the requirements of the procedure is that the thermal process be isothermal. That is practically achieved using small quantities of the sample inoculated with the microorganism under study using a variety of methods.

The use of small samples brings practical problems since the sample may not be representative of the whole food, in particular when the food consist of solids of different sizes suspended in a liquid phase (e.g., in a soup). The requirement of a constant temperature during the procedure to estimate the

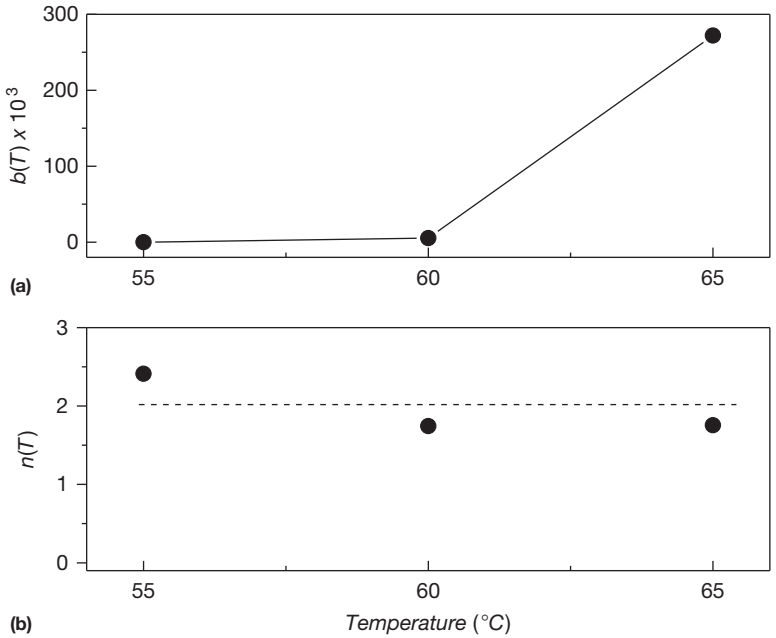

Figure 6 Weibull parameters $b(T)$ and $n(T)$ as a function of temperature derived from fitting the experimental data illustrated in Figure 5. (a) $b(T)$ versus T data and (b) $n(T)$ versus T data.

microbial resistance parameters $D(T)$ is also practically challenging as, in many situations, it becomes difficult to achieve a true isothermal process. That concern also holds when nonthermal treatments are used. For instance, for a chemical treatment, it is necessary to avoid dissipation of the chemical agent, and for high-pressure treatments, the come-up time is one of the main problems that may affect the determination of the microorganism resistance to the treatment. A methodology to estimate survival parameters under nonisothermal conditions has been proposed by Peleg et al. for the linear case, whereas for the nonlinear case, it was proposed by Peleg and Normand. For inactivation processes that are not isothermal, Peleg and Penchina demonstrated that the local slope of nonisothermal curve is a function of the momentary temperature $T(t)$. Thus, the corresponding survival of the microbial population at a given time t can be determined by integrating the following differential equation:

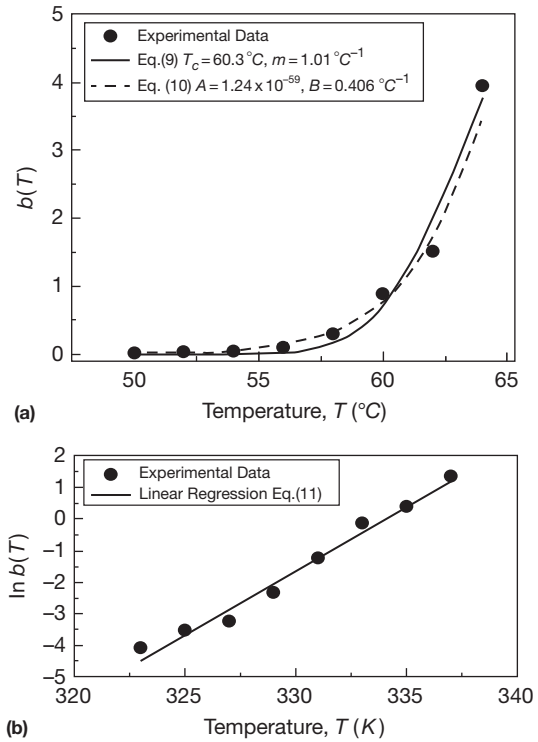

Figure 7 (a) Effect of temperature on the parameter $b(T)$ using the log-logistic equation and an Arrhenius-type equation. (b) Arrhenius equation expressed in logarithm form linear regression of experimental data. Experimental data from Stephens, P. J., Cole, M. B. and Jones, M. V. (1994). Effect of heating rate on the thermal inactivation of Listeria monocytogenes. *Journal of Applied Bacteriology* **77**, 702–708.

$$\begin{cases} \dfrac{\mathrm{d}\left[\log\left(\dfrac{N(t)}{N_0}\right)\right]}{\mathrm{d}t} = -b[T(t)]\cdot n[T(t)]\cdot\left[\dfrac{-\log\dfrac{N(t)}{N_0}}{b[T(t)]}\right]^{\dfrac{n[T(t)]-1}{n[T(t)]}} \\ \log\dfrac{N(t)}{N_0} = 0 \quad \text{at} \quad t = 0 \end{cases} \qquad [12]$$

The solution of eqn [12] can be obtained from a numerical integration algorithm using specialized software such as Mathematica® and Mathcad or by programming a numerical integration routine in a computing language such as MATLAB or other languages. The solution of that equation can also be implemented in a spreadsheet such as Microsoft Excel.

Online integration of eqn [12] using either Mathematica® or Microsoft Excel is also posted in Professor Peleg's websites, http://people.umass.edu/aew2000/ExcelLinks.html and http://people.umass.edu/aew2000/WolframDemoLinks.html.

In order to perform the integration of eqn [12], the relationship between the parameters $b(T)$ and $n(T)$ and temperature must be known as well as the temperature evolution in the thermal process indicated as $T(t)$. Although, as shown in previous section, the relationships between the kinetic parameter $b(T)$ and temperature can be expressed as either an Arrhenius-type equation or log-logistic model expressed by eqn [9], the experimental data shown in **Figure 6** clearly show that a model including differences between sublethal and lethal temperature ranges is preferred. Furthermore, Peleg et al. showed that a more simplified equation to describe the relationship between $b(T)$ and temperature could be used:

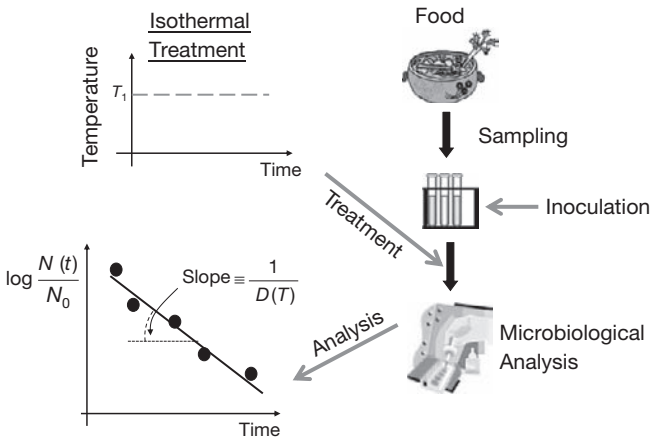

Figure 8 Schematic of the typical experimental procedure TDT to estimate the decimal reduction time at a constant temperature T_1.

$$\begin{cases} b(T) = 0 \quad \text{If } T < T_c \\ b(T) = m(T - T_c) \quad \text{If } T > T_c \end{cases} \qquad [13]$$

The relationship indicates that when processing temperatures are lower than the threshold temperature T_c (sublethal temperatures), there is no inactivation of the microbial population, whereas microbial inactivation is observed when processing temperatures are larger than T_c (lethal temperatures). After T_c, the dependence of $b(T)$ with temperature is linear (see Figure 6). By assuming that n is independent of temperature and substituting eqn [13] into eqn [12], the following equation is obtained:

$$\begin{cases} \dfrac{d\left(\log \dfrac{N(t)}{N_0}\right)}{dt} = 0 \quad \text{for } T < T_c \\ \dfrac{d\left(\log \dfrac{N(t)}{N_0}\right)}{dt} = -m[T(t) - T_c] \cdot n \cdot \left[\dfrac{-\log \dfrac{N(t)}{N_0}}{m[T(t) - T_c]}\right]^{\frac{n-1}{n}} \quad \text{for } > T_c \\ \log \dfrac{N(t)}{N_0} = 0 \quad \text{at } t = 0 \quad \text{initial condition} \end{cases} \qquad [14]$$

Equation [14] shows that provided the Weibull parameter n, the survival parameters T_c and m, and the temperature evolution are known, microbial inactivation at any given time can be estimated by integration of eqn [14].

Determination of Kinetic Parameters m and T_c

In a direct approach, integration of eqn [14] results in the survival curve corresponding to the parameters m and T_c associated to the specific microbial population and the food being treated as well as the temperature evolution of the thermal treatment $T(t)$. However, an inverse approach can also be used to estimate the parameters m and T_c provided the nonisothermal treatment and the resulting survival curve are known; Figure 9 illustrates a schematic of the steps needed in each approach.

The inverse approach mainly consists in solving a nonlinear regression algorithm in which the fitting parameters are T_c and m and the model to be fitted is the survival curve, which is mathematically described by the differential equation given by eqn [14]. The solution of the problem involves the use of a nonlinear regression approach to search for the parameters T_c and m that minimize the error between the experimental data (survival curve) and the model given by eqn [14]. Thus, every time the model is evaluated, eqn [14] must be integrated. Although a priori it could be thought that the approach involves a formidable computational task, current software is able to deal with this algorithm without major complications. Software like Mathematica®, used to introduce the approach, Mathcad, and MATLAB incorporate numerical solutions of differential equations and nonlinear regression algorithm that can be effortlessly integrated to estimate the fitting parameters T_c and m provided the temperature evolution of the nonisothermal thermal treatment and the resulting survival curve are known (see Figure 9 – inverse problem). Spreadsheets like Microsoft Excel that has minimization functions like 'Solver' could be also used if the numerical solution of an ordinary differential is incorporated in the spreadsheet. Thus, the function *Solver* is used to minimize the sum of squared errors (SSE) defined as the square of the difference between the experimental data (survival curve) and the model function (numerical solution of eqn [14]). The incorporation of numerical methods is an active area of research and teaching, and there is an extensive collection of articles and books dealing with this subject. Applications on the use of the Visual Basic language

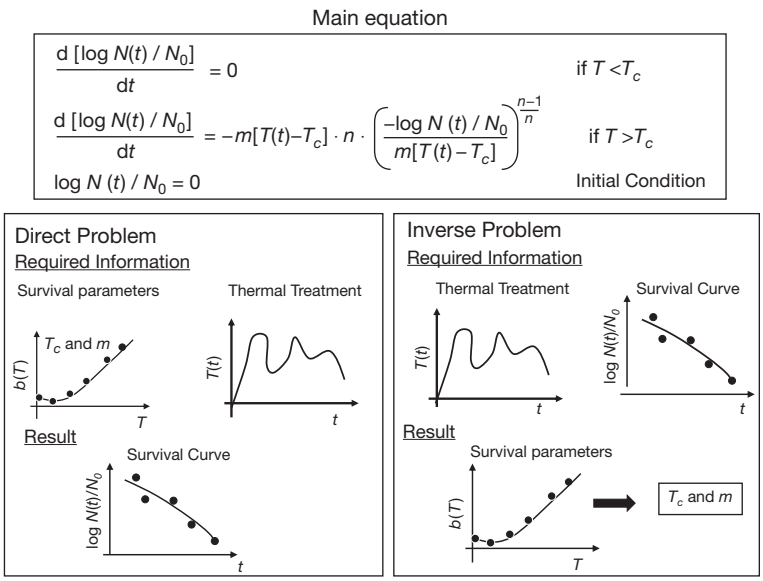

Figure 9 Schematic of the approach followed to determine the survival curve provided the parameters m and T_c and the nonisothermal temperature evolution are known (direct problem). In the inverse approach, the parameters m and T_c are determined from information of the survival curve obtained from a known thermal evolution.

to integrate numerical methods with minimization functions like *Solver* or *Goal Seek* can be also found in the web.

Testing the approach: The linear case

For the linear case, $n=1$, so eqn [14] simplifies to

$$\begin{cases} \dfrac{\mathrm{d}\left(\log \dfrac{N(t)}{N_0}\right)}{\mathrm{d}t} = 0 \quad \text{for } T < T_c \\ \dfrac{\mathrm{d}\left(\log \dfrac{N(t)}{N_0}\right)}{\mathrm{d}t} = -m[T(t) - T_c] \quad \text{for } T > T_c \\ \log \dfrac{N(t)}{N_0} = 0 \quad \text{at } t = 0 \quad \text{initial condition} \end{cases} \qquad [15]$$

which after integration yields

$$\begin{cases} N(t) = N_0 \quad \text{for } T < T_c \\ \log \dfrac{N(t)}{N_0} = -\displaystyle\int_0^t m[T(t) - T_c] \quad \text{for } T > T_c \end{cases} \qquad [16]$$

Equation [16] is the model that contains the parameters m and T_c to be determined from the nonlinear regression applied to the experimental survival curve. The question that immediately arises is whether the proposed method can be used to predict, or estimate, inactivation parameters of organisms on the basis of experimental survival data originating from nonisothermal heat treatments that have significant scattering data.

Since it was not possible to find that kind of experimental information in the literature, to test the practicality of the approach, the recovery of survival parameters was done from data generated with the proposed model on which a random noise was superimposed. Using *L. monocytogenes*' survival parameters, $T_c = 60.3\ ^\circ\mathrm{C}$ and $m = 1.01\ ^\circ\mathrm{C}^{-1}$ (see Figure 7) survival curves, with a superimposed random noise of 50%, corresponding to different nonisothermal heating processes were generated with eqn [16]. This generated survival data along the corresponding nonisothermal temperature profile were then employed to retrieve the original survival parameters T_c and m. Figure 10 shows that for a linear heating profile, the parameters T_c and m obtained were reasonable estimates of the generation parameters despite the considerable scattered data used. These results indicate that nonisothermal survival curves can be used to estimate an organism's survival parameters.

Testing the approach: The general case with n constant but different than 1

It was demonstrated in the previous section that the survival parameter n can be considered constant for *L. monocytogenes* and other microorganisms, whereas the parameter b depends strongly on temperature; it is almost zero at sublethal temperatures (identified with the parameter T_c) and then rises sharply with a rate defined by the parameter m when the temperature passes the lethal level. As shown earlier in the text, such a temperature dependence can be described by the log-logistic model defined by eqn [9]. A simplified version of eqn [9] was used in the previous section to demonstrate the applicability of the inverse approach to solve the linear problem. The method for nonlinear survival curves and n values is independent of T, whereas dependence of the parameter b with temperature described by eqn [9] can be extended and the differential equation to be integrated is

$$\begin{cases} \dfrac{\mathrm{d}\left(\log \dfrac{N(t)}{N_0}\right)}{\mathrm{d}t} = 0 \quad T < T_c \\ \dfrac{\mathrm{d}\left(\log \dfrac{N(t)}{N_0}\right)}{\mathrm{d}t} = -\ln\left[1 + \exp\left(m(T(t) - T_c)\right)\right] \cdot n \cdot \left[\dfrac{-\log \dfrac{N(t)}{N_0}}{\ln\left[1 + \exp\left(m(T(t) - T_c)\right)\right]}\right]^{\frac{n-1}{n}} \quad T > T_c \log \dfrac{N(t)}{N_0} \\ = 0 \quad \text{at } t = 0 \quad \text{initial condition} \end{cases} \qquad [17]$$

The model defined by eqn [17] containing the survival parameters T_c and m and the temperature profile of the nonisothermal treatment, $T(t)$, was numerically integrated for a set of guess initial parameters T_c and m, which were adjusted to minimize the SSE defined in the preceding text. The minimum of SSE, was obtained using the simplex algorithm, which can be easily implemented in software such as Mathematica®. Experimental survival curves from nonisothermal treatments of broth samples inoculated with *Salmonella* were used to estimate the survival parameters. Figure 11 illustrates the nonisothermal heating profiles used in the heat treatment as well as the resulting experimental survival curves (symbols) and the fitting of the data (lines) with the model defined by eqn [17].

The values of the survival parameters obtained from the approach are given in Table 3. As noted, they do not differ significantly from the different applied thermal treatment. The results also show that the Weibull parameter n was not affected by the temperature profile.

Estimation of Processing Time

Isothermal Conditions

If $T = 121.1\ ^\circ\mathrm{C}$ (250 °F) is substituted in eqn [7], the processing time is defined as the F_0 value for the process, which represents the value of a processing time that will produce the same degree of inactivation as the given process at its temperature T. Thus,

$$F_0 = -D_{121.1} \cdot \log \frac{N(t)}{N_0} \qquad [18]$$

By combining eqns [7] and [8], the following is obtained:

$$F_0 = t \cdot 10^{\left(\frac{T-121.1}{z}\right)} \qquad [19]$$

Equation [19] is used to estimate the value of F_0 of the thermal process at a given constant temperature T (°C) and a given processing time t.

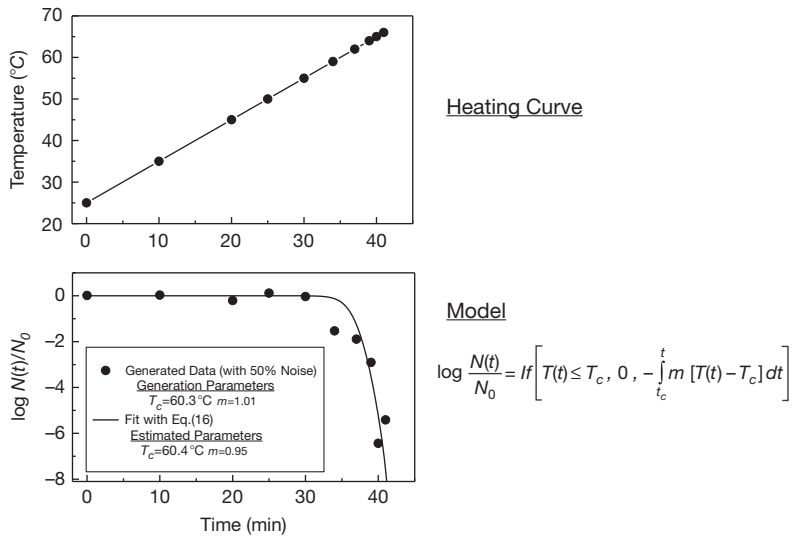

Figure 10 Determination of the survival parameters T_c and m from a known nonisothermal heat treatment and the resulting survival curve using the inverse approach.

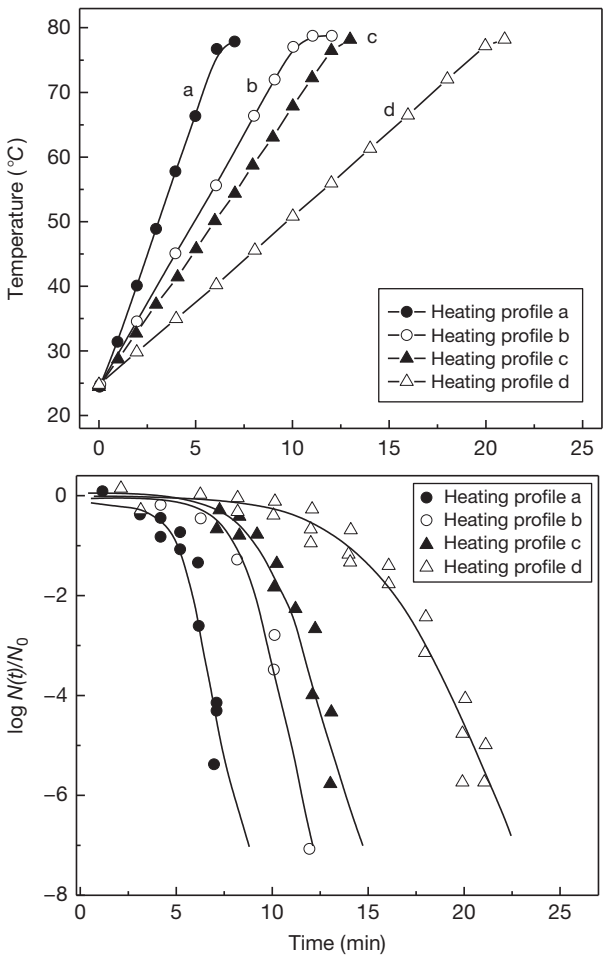

Figure 11 Experimental survival curves of *Salmonella* heat treated in a broth under four temperature profiles (top graph). In the bottom graph, symbols represent experimental data and lines, the model given by eqn [17] for the corresponding survival parameters T_c and m. The averaged survival parameters obtained from the different heating profiles are listed in **Table 3**. Adapted from Peleg, M. and Normand, M. D. (2004). Calculating microbial survival parameters and predicting survival curves from non-isothermal inactivation data. *Critical Reviews in Food Science and Nutrition* **44**, 409–418.

Table 3 Survival parameters for broth inoculated with *Salmonella* obtained from the inverse approach developed by Peleg and Normand for nonisothermal heat treatments

Medium	*Profile*	T_c (°C)	m ($°C^{-1}$)	*Reference*
Broth	a	61	0.20	
	b	63	0.22	
	c	61	0.17	Mattick et al.
	d	62	0.15	
	Mean	62	0.19	

Adapted from Peleg, M. and Normand, M. D. (2004). Calculating microbial survival parameters and predicting survival curves from nonisothermal inactivation data. *Critical Reviews in Food Science and Nutrition* **44**, 409–418

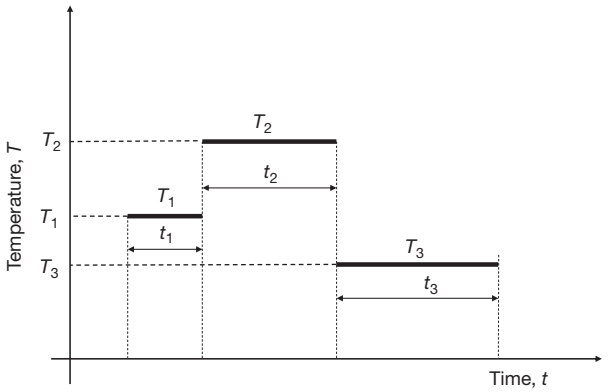

Figure 12 Hypothetical thermal process consisting of successive isothermal heat treatments.

Nonisothermal Conditions

Figure 12 schematically illustrates a thermal treatment consisting of successive processes having different processing times and different temperatures. By assuming that these successive thermal processes are additives, the F_0 of the entire treatment could be calculated by the following equation:

$$F_0 = t_1 \cdot 10^{\left(\frac{T_1 - 121.1}{z}\right)} + t_2 \cdot 10^{\left(\frac{T_2 - 121.1}{z}\right)} + t_3 \cdot 10^{\left(\frac{T_3 - 121.1}{z}\right)} \quad [20]$$

When the food material is being thermally processed with a processing time of duration t_f, the temperature at different locations of the product varies continually during the process (see Figure 13). As indicated in the figure for foods subjected to external heating and cooling using, for example, steam and cooling water, respectively, temperatures at locations close to the surface (identified as $T_s(t)$) respond faster to temperature changes in the heating/cooling media than temperatures close to the center of the food (identified as $T_c(t)$).

The value of the thermal process in a given location of the product for this general case can be calculated as

$$F_0 = \int_{t=0}^{t=t_f} 10^{\left(\frac{T(t) - 121.1}{z}\right)} dt \quad [21]$$

By calculating the F_0 value for a thermal process, two aspects concerning the temperature profile in the processed material must be taken into account. First, the complete temperature evolution, $T(t)$, at each location of the processed material must be known in the entire process. Second, different locations in the product receive different thermal treatments. This is important especially when the heat is transferred to the product by conduction because the slowest rate of this heat transfer mode increases temperature differences between the center and the surface of the product leading to significant differences in the product heat treatment, leading to serious quality concerns. By using a finite-difference algorithm, the heat conduction equation for a cylindrical can of finite length containing a homogeneous solid food with a constant thermal diffusivity α, which is given by the following equation, can be solved:

$$\frac{\partial T}{\partial t} = \alpha \left[\frac{\partial^2 T}{\partial r^2} + \frac{1}{r} \frac{\partial T}{\partial T} + \frac{\partial^2 T}{\partial h^2} \right] \quad [22]$$

where T is the local momentary temperature at a given location, t is the time, and r and h are the radial and vertical coordinates of a location in the material, respectively. Given suitable boundary conditions, for example, the temperature of the retort temperature, the solution of eqn [22] yields temperature–time relationships at any given point (r, h) within or on the surface of the cylindrical-shaped food.

Although experimental measurements and simulations clearly show significant temperature gradients during conductive thermal treatments, the calculated process' lethality (traditionally defined by the F_0 value) is based on the product's coldest point. However, if the temperatures at all locations of the product are known, an 'overall lethality' of the entire food could be calculated by summing up local lethalities. To compare the calculated 'coldest point' and the 'overall' lethalities for linear and nonlinear inactivation kinetics, simulated processes using parameters that match realistic process conditions used in the food canning industry were performed. The processing time was adjusted to achieve 12 decades' reduction (F_0) at the coldest point of the can as judged by the traditional criteria of thermal processing. Figure 14(a) shows a simulated retort processing temperature and the corresponding temperature profile at the product's coldest point. Temperatures at other locations were also calculated (for the sake of clarity, it is not shown in the figure). The corresponding survival curves, calculated with the linear and the Weibull models, are shown in

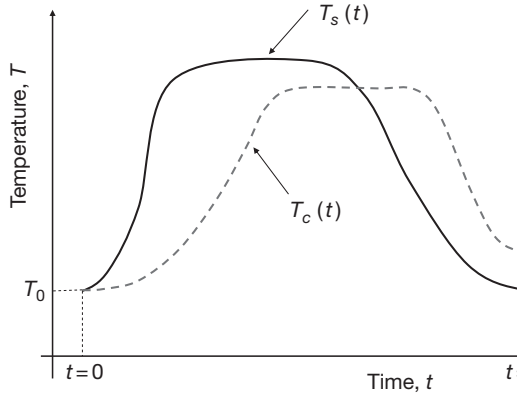

Figure 13 Typical temperature profile in different locations of a food product subjected to a heating/cooling process. $T_s(t)$ indicates temperature at locations close to the surface, whereas $T_c(t)$ indicates temperatures close to the center of the food.

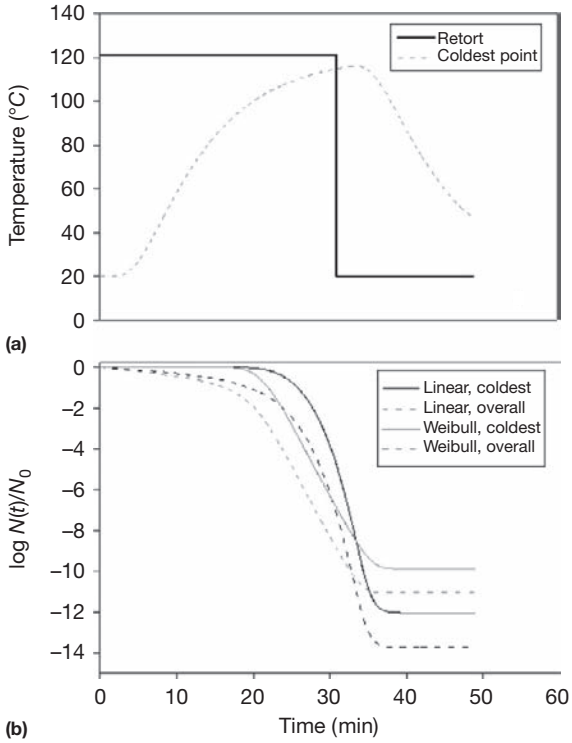

Figure 14 (a) Coldest temperature profile and (b) comparison between the lethality calculated at the coldest point of the product and the overall process calculated for both the traditional linear and the Weibull models. Conditions and parameters used for the simulations were as follows: can dimensions $H = D = 76.2$ mm, thermal diffusivity of the product $\alpha = 3.3 \times 10^{-7}$ m^2 s^{-1}, product's initial temperature $T_i = 20$ °C, retort temperature $T_p = 121.1$ °C, and cooling water temperature $T_w = 20$ °C. Survival parameters used in the simulation are reported in Table 4. Adapted from Chen, G., Campanella, O. H. and Peleg, M. (2011). Calculation of the total lethality of conductive heat in cylindrical cans sterilization using linear and nonlinear survival kinetic models. *Food Research International* **44**, 1012–1022.

Table 4 Thermal inactivation parameters for *C. botulinum* spores used to estimate survival curves (**Figure 14**) and lethality (**Figure 15**)

Model	*Linear*			*Weibull*	
Parameters	T_{ref} *(°C)*	D_{ref} *(min)*	z *(°C)*	b(T)	n(T)
Reference	121.1 USDA	0.2	10	$\ln\left(1+e^{0.30[T(t)-102.3]}\right)$ Campanella and Peleg	$0.325+\frac{0.425}{1+e^{(T-101)/10.057}}$

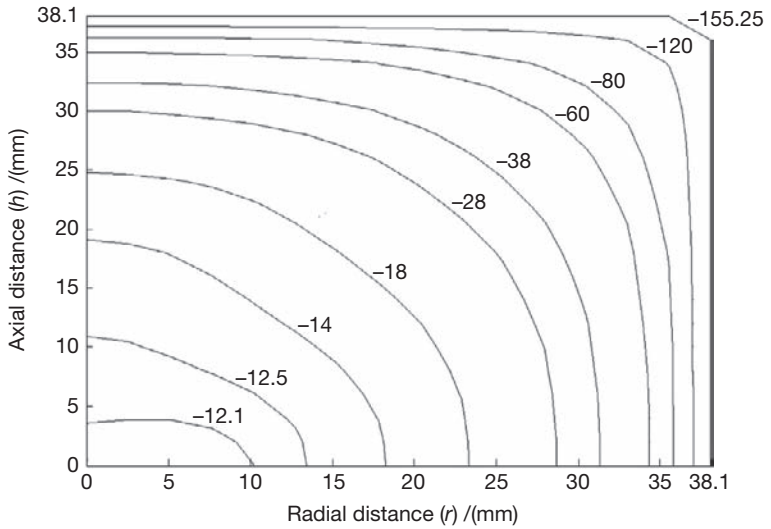

Figure 15 Process lethality calculated using the traditional approach expressed by eqn [21] as a function of location in one-fourth of the container's cross section at the end of the process illustrated in **Figure 14(a)**. Adapted from Chen, G., Campanella, O. H. and Peleg, M. (2011). Calculation of the total lethality of conductive heat in cylindrical cans sterilization using linear and nonlinear survival kinetic models. *Food Research International* **44**, 1012–1022.

Figure 14(b). Thermal inactivation parameters used in the simulations were those for *C. botulinum* spores, which are reported in **Table 4**.

Simulations involving the coupling of the numerical solution of the heat transfer equation (eqn [22]) with the rate model (eqn [17]) are able to generate a number of relationships that can be used to analyze many *what-if* scenarios. For instance, **Figure 15** shows the lethality distribution throughout one-fourth of the can, calculated by eqn [21] at the end of the thermal process depicted in **Figure 14**. Values show that 50–150 decades of reduction in microbial population warrants that sterility is accomplished in the outer regions of the can despite that in the center, the lethality achieved is just the recommended 12-log reduction. However, the potential value of such plots is not only to assess microbial survival but also to monitor the effects of heat treatments on the product's quality, for example, survival of nutrients and pigments or the accumulation of harmful and toxic compounds generated by excessive heat treatments.

Other 'what-if' scenarios discussed in the simulations involve effects of container shape, initial temperatures, or initial temperature distribution in the can (for the case that the product comes from a preheating or precooling process) and practical situations implying deviations in the retort temperature and during cooling stages. The particular importance for conductive heat transfer is the effect of the thermal diffusivity (α) on both temperature profile and survival curves because it provides a quantitative estimation of process requirements for different products and in addition sets margins for products having variable or nonuniform composition. Examples of simulated temperature profile and corresponding survival curves of *C. botulinum* spores are shown in **Figure 16** for three different values of thermal diffusivity α, selected to highlight the effect of the product's physical properties on the length of the process needed for its sterilization.

Industrial Aspects of Thermal Treatments

Thermal processing applications at the industrial scale have relied for many years on batch processing, and although the basic principles of the process have not changed significantly, improvements have been brought mainly through changes on automation and control of the operation. Innovations on the technology have included more flexibility in handling lighter-weight containers, better options for process optimization, and the introduction of fully automated retort operations. Due to important implications of food safety on public health, processors must

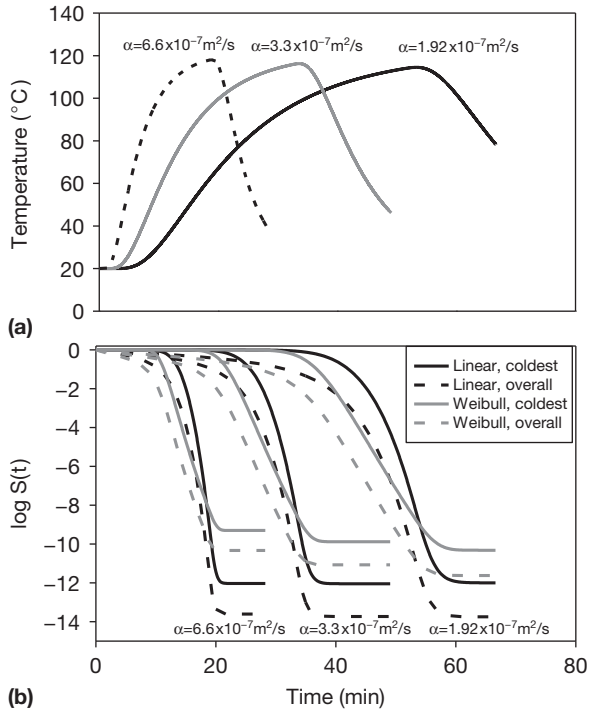

Figure 16 (a) Effects of thermal diffusivity α on the coldest temperature profiles and (b) microbial inactivation at the coldest and the overall inactivation calculated for the traditional and the Weibull approaches. Values of thermal diffusivity are indicated in the figure. Other parameters used in the simulations were as follows: $H = D = 76.2$ mm, product's initial temperature $T_i = 20$ °C, retort temperature $T_p = 121.1$ °C, and cooling water temperature $T_w = 20$ °C. Adapted from Chen, G., Campanella, O. H. and Peleg, M. (2011). Calculation of the total lethality of conductive heat in cylindrical cans sterilization using linear and nonlinear survival kinetic models. *Food Research International* **44**, 1012–1022.

operate in strict compliance with food regulations in particular in regard to low-acid canned foods. In United States, those regulations are controlled by the Food and Drug Administration who are requesting food processors to keep documentation and records of all critical control points during the sterilization of foods. Thus, a typical batch retort system included the following components: a loader/unloader product tracking and systems including control strategies for control points such as the initial temperature of the product, retort temperature, retort pressure, processing time, water flow, water level, and rotation speed, the latter to increase convective heat transfer during the processing of liquid products or products that contain particles, for example, soups. Mathematical models describing convective heat transfer and experimental and predictive models have been developed for liquid products containing particles, but the physics of the problem is complex and may result in less accurate prediction so process optimization for these products still strongly relies on experimental approaches.

Continuous processing including aseptic packaging is finding more application and uses, mainly focusing on the increase of productivity and improvement on product safety and quality while reducing operating cost and safety risks. Some of most widely used systems are nonagitating and agitating continuous retorts that include hydrostatics (e.g., JBT FoodTech Hydrostat) and rotary retorts (e.g., JBT FoodTech Sterilmatic).

Deviations in temperatures and pressures of the steam supply during thermal treatment in continuous processes are often unavoidable and present a challenge to food processors and equipment manufacturers. When a deviation from the temperature–time prescribed process schedule occurs and is not handled properly, it may not only compromise the sterility of the canned food but also lead to subsequent spoilage. Although considerable efforts have been directed toward the development of 'intelligent' online batch retort control systems capable of rapid evaluation, online correction, and printed documentation of any process deviations occurring during a thermal process, these types of algorithms have not been applied to continuous retorts due to the lack of suitable methodologies capable of providing accurate and fast evaluations of product temperature and lethality in response to process deviations. However, methodologies that would allow food processors and equipment manufacturers to employ residence time control through the use of variable conveyor speed in order to accommodate thermal process deviations in continuous retorts have been developed.

See also: Canning: Process of Canning; *Clostridium botulinum*; *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; *Clostridium*: Occurrence and Detection of *Clostridium perfringens*; Enzymes: Analysis and Food Processing; Foodborne Pathogens; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Heat Treatment: Effect on Microbiological Changes and Shelf Life; *Listeria*: Detection; *Listeria*: Listeriosis; *Listeria*: Properties and Occurrences; Pasteurization: Effect on Sensory Quality and Nutrient Composition; Pasteurization: Principles and Applications; Preservation of Foods; *Staphylococcus*: Detection; *Staphylococcus*: Food Poisoning; Sterilization of Foods.

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Heavy Metal Toxicology

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This article will address a few of the more clinically relevant heavy metals, mercury, cadmium, and arsenic, with regard to exposure via food intake as a source of toxicity. Most other metals pose a lower toxicity risk via food exposure and will not be discussed.

Mercury

Mercury, as inorganic mercury (elemental mercury oxidation state is 0; mercuric cation oxidation state is 2+), originates from the Earth's crust by a natural process of degassing into the atmosphere. Human actions also contribute to atmospheric mercury, for example, coal-fired power plant facilities. Mercury in food, mostly as organic methylmercury, originates from the aquatic food chain including plankton, fish, and sea mammals. The bioconcentration of fish tissue methylmercury can be as much as one thousand times or more the surrounding water mercury concentration. Fish eliminate methylmercury very slowly with a halftime of a few hundred days. In fish tissue muscle, methylmercury occurs as a water-soluble protein-bound form that does not degrade by cooking. Methylmercury in the past has been used as seed dressing (e.g., wheat) as a fungicide. The food content of inorganic mercury is much less than the methylmercury content. Mercury exposure through drinking water typically is quite low. Ionized mercury forms covalent bonds with sulfhydryl groups; this is considered one of the major biochemical mechanisms for mercury toxicity.

Methylmercury is a human teratogen. Health advisories for pregnant women regarding avoidance of certain seafoods (e.g., predatory fish such as pike, tuna and swordfish, shark, tilefish, and king mackerel) due to methylmercury content are based on both animal data that the mammalian fetal brain is adversely affected at low levels of methylmercury exposure and human cases of severe fetal brain malformations and severe fetal brain malfunction (Minamata disease; Minamata Bay disaster) (mental retardation, spasticity, blindness, visual field constriction, dysphagia, nystagmus, strabismus, dysmetria, chorea, athetosis, limb deformities, and seizure disorders and autopsy findings of brain cortical atrophy, cerebellar atrophy, corpus callosum hypoplasia, and demyelination of the pyramidal tract). Other seafood are not considered a risk by the FDA, that is, less than one kg per week of shrimp, catfish, salmon, flatfish, pollack, scallops, cod, clams, crabs, and tuna (nonalbacore). Mammalian animal fetal tissue concentrations of alkyl mercury compounds are about twice that of maternal tissue with fetal erythrocytes (greater fetal hemoglobin affinity for methylmercury) containing about one-third more alkyl mercury compared with maternal erythrocytes. Human (breast) milk contains methylmercury, ranging 5–30% of the maternal blood concentration; so, breast-feeding is not recommended for women with high methylmercury exposure. An estimate of methylmercury exposure in adult humans to cause the lowest observed adverse health effect (paresthesias) is 300 μg per day (adult) (about 4 $\mu g\,kg^{-1}$ per day), resulting in a blood concentration of about 200 $\mu g\,l^{-1}$ (nonexposed persons' background blood mercury concentration is less than 10 $\mu g\,l^{-1}$) (most blood methylmercury is in the erythrocytes). A group of adult persons self-reporting high seafood consumption had blood mercury concentrations of approximately 20–50 $\mu g\,l^{-1}$; they had no increased cognitive complaints. The human fetus is considered to be at risk at lower exposure doses compared with adults. The lower limits of tissue or blood concentrations of methylmercury causing specific human fetal adverse effects are not known.

Methylmercury human gastrointestinal absorption is about 90% or more, with about 90% of methylmercury excretion occurring via the feces. Once absorbed into the tissue, methylmercury is slowly enzymatically cleaved to its inorganic mercuric (divalent cation) form; this enzymatic cleavage is much more rapid and complete for long-chain alkyl and aryl organic mercury compounds compared with methylmercury. The human biologic halftime (half-life) of methylmercury is about 6–10 weeks (about 1% per day of body burden). A greater portion of methylmercury compared with inorganic mercury distributes to the brain, that is, about 10% of body burden of methylmercury distributes to the brain. Adverse neurocognitive and encephalopathic toxicity is a major risk and concern for excessive organic mercury exposure, both acute and chronic. Chronic oral doses of 10 $\mu g\,kg^{-1}$ per day may produce such adverse effects. US government advisories include less than 0.1 $\mu g\,kg^{-1}$ per day of methylmercury intake from fish (EPA reference dose) and less than 0.3 $mg\,kg^{-1}$ of fish tissue (FDA). Also, an FDA action level of one ppm (part per million) of mercury in fish was set to limit methylmercury intake to less than one-tenth of blood concentrations found in persons with adverse toxic effects. Within cells, mercuric (divalent cation) mercury becomes localized in lysosomes where it may persist for long periods of time. Inorganic mercury preferentially binds covalently to sulfhydryl groups. Methylmercury in the liver combines with glutathione in soluble complexes that undergo enterohepatic cycling. A cysteine–methylmercury complex crosses the blood–brain barrier via an amino acid transporter.

Neurotoxic adverse effects of methylmercury in humans may include (1) paresthesias, for example, the fingers, toes, lips, and mouth; (2) ataxia, dysarthria, and impaired swallowing; (3) impaired vision and/or hearing; (4) neurasthenia; (5) tremor and/or spasticity; (6) and even coma and death. Adverse cognitive neuropsychological effects were found in children in the Faroe Islands (whale meat mercury 1.6 $\mu g\,kg^{-1}$) and New Zealand (shark meat mercury 2.2 $\mu g\,kg^{-1}$), but not in Seychelles (fish mercury 0.3 $\mu g\,kg^{-1}$); perhaps, the threshold for chronic adverse neuropsychological effects may be somewhere in this range 0.3 $mcg\,kg^{-1}$ to 1.6 or 2.2 $mcg\,kg^{-1}$. The adverse neuropsychological effects persisted in children tested again at age 14 years in the Faroe Islands.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00373-1

Therapy of methylmercury poisoning (converted in the body to inorganic mercuric mercury), which is usually chronic exposure, is limited due to the lack of a large treatment effect (low effect size). Chelation with succimer (an oral hydrophilic analog of BAL) (succimer is the most superior treatment in decreasing mouse brain mercury after methylmercury administration), DMPS (dimercaptopropanesulfonate), or a nonabsorbable polythiol resin may be tried. British anti-Lewisite (BAL) or dimercaprol should not be tried due to some data showing redistribution of mercury into the brain, that is, increasing brain mercury concentration after administration of dimercaprol.

Cadmium

Cadmium (the most common oxidation state is 2+) exposure via food intake is a lower dose of chronic exposure typically compared with industrial exposure, the latter more often being exposure by inhalation. However, the major source of cadmium exposure for the general population is via food. Higher daily intakes of cadmium exposure via food and water occur in areas of cadmium contamination. Such areas most often are near mining sites; cadmium is a common constituent in many ores being mined. An estimate of daily oral intake of cadmium in the general population (food, water, and air) is approximately 10–50 μg per day. Only approximately an estimated 5% (some estimates of 5–20%) of oral cadmium is absorbed, although greater absorption may occur with iron-deficiency anemia or low-calcium diets or low-protein diets. Plants take up cadmium when grown in soils that have been exposed to cadmium-containing fertilizers, cadmium-containing water, or deposition from the air. Human waste sludge is another source of cadmium. Cadmium content varies – sludge up to 1500 mg cadmium per kg dry weight; shellfish (mussels, scallops, and oysters) 100–1000 μg kg^{-1}; meat, fish, and fruit 1–50 μg kg^{-1}; and grains 10–150 μg kg^{-1}. In the past, acidic contents, such as fruit juices packaged in cadmium-plated containers, were a source of higher-dose oral cadmium exposure.

Cadmium human kinetics reveal a long body halftime of 20–30 years. Absorbed cadmium is distributed rapidly and taken up mostly by the liver and kidneys. An estimated 50–75% of body burden of cadmium is in the liver and kidneys. Over time, there is a progressive accumulation in the human kidneys up to approximately age 50–60 years, after which there is a slow decline in tissue concentrations. A renal cortex cadmium concentration of 200 μg g^{-1} of cortex results in kidney tubular dysfunction in 10% of adults and 300 mcg g^{-1} tubular dysfunction in 50% of adults, as measured by increased urine excretion of low molecular proteins such as beta-2 microglobulin, that is, tubular proteinuria. Whole blood cadmium concentrations in nonexposed adults typically are less than 10 μg l^{-1}, and 24 h urine cadmium concentrations typically are less than 3 μg g^{-1} of urine creatinine. About 95% of adults in the United States have a urine cadmium concentration of less than 2 μg l^{-1} of urine and less than 1% of adults excreting 4 μg l^{-1} of cadmium in their urine; most urine cadmium is in the form of the cadmium–metallothionein complex.

Metallothionein is a protein that forms a low-molecular-weight complex with cadmium; the affinity of cadmium for metallothionein is very high. The cadmium–metallothionein complex accumulates with time in the kidneys in proportion to cadmium concentration. This complex appears to be nontoxic and somewhat protective until high cadmium tissue levels are reached and then kidney toxicity occurs.

The adverse toxic effects of cadmium (chronic exposure via oral intake) include renal toxicity (usually tubular dysfunction but may include glomerular dysfunction), skeletal toxicity including bone pain (itai-itai or ouch-ouch disease), osteomalacia and/or osteoporosis; carcinogenicity; and hypertension/cardiovascular disease. Carcinogenicity and hypertension/cardiovascular disease have been implicated from inhalation exposure, primarily from animal studies, with human data being weak. However, human renal and bone diseases are generally accepted as risks from chronic cadmium toxicity. Human skeletal disease with excessive oral cadmium exposure is associated with low serum 1,25-dihydroxyvitamin D concentrations perhaps due to inhibition of formation by cadmium. The result is decreased calcium absorption and decreased bone mineralization producing osteomalacia.

Chelation therapy for cadmium typically is not effective. This appears to be related to the rapid increase in metallothionein and binding of cadmium to form an intracellular cadmium–metallothionein complex resulting in cadmium not being available for chelation. Some experimental dithiocarbamates have been shown in animal studies of chronic cadmium exposure to reduce total body cadmium burden; however, these compounds are lipid-soluble and redistribute cadmium to the brain, an undesirable and unacceptable drug action.

Arsenic

Arsenic (trivalent arsenite cation oxidation state is 3+; pentavalent arsenate cation oxidation state is 5+) occurs widely in the environment. Natural sources include arsenic-containing mineral ores and groundwater. Pentavalent arsenic exists as the predominant form in the environment. Pentavalent inorganic arsenic is less toxic than trivalent inorganic arsenic. Industrial arsenic processes encompass smelting and refining of ores and metals, semiconductor manufacturing, metallurgy, and burning of wood treated with arsenic preservatives. Commercial products may include herbicides, insecticides, fungicides, animal feed additives, and wood preservatives. Past use of arsenic-containing chemical warfare agents illustrates the degree of widespread use of arsenic compounds. Food sources of arsenic vary such as seafood, kelp, and wine. Water, food, and soil contaminations are the main sources of the general population's exposure to arsenic. Foods grown in arsenic-contaminated soils, for example, rice and produce, may pose a risk of arsenic toxicity, but generally, food contains low concentrations of inorganic arsenic. Organic arsenic compounds include methylarsonic acid, dimethylarsinic acid (cacodylic acid), arsanilic acid, and arsenobetaine. Marine sources of arsenic include algae, fish, and shellfish. Seafood contains relatively large amounts of nontoxic organic arsenic in the form of arsenobetaine (a trimethylated compound), arsenocholine, and arsenosugars.

Inorganic arsenic (both 3+ and 5+ cations) is metabolized by the liver in humans to monomethyl arsenic (MMA) and

dimethyl arsenic (DMA). These methylated arsenic metabolites until recently were considered to be less toxic than inorganic arsenic; however, trivalent methylated arsenicals are more toxic than inorganic arsenite due to an increased affinity for sulfhydryl groups. Ionized arsenic compounds form a covalent bond with sulfhydryl groups. Methylated arsenic compounds are excreted to a greater extent in the urine compared with inorganic arsenic. Ingested pentavalent inorganic arsenic is converted to a significant extent in the human body to trivalent arsenic that then undergoes methylation. Ingested inorganic arsenic is well absorbed in the gastrointestinal tract, about 60–90% absorption. Ingested inorganic arsenic's biologic halftime is reported to range from about 10 h to 4 days, with the latter figure likely more representative of the true beta-phase halftime; some estimates are that by about 3 days, approximately 50–80% is excreted after a single-dose ingestion. Methylated arsenic's halftime approximates 30 h. Arsenobetaine (alpha phase about 7–11 h; beta phase about 75 h) and arsenocholine are largely cleared in about 2 days, being excreted unchanged in the urine. Because of the relatively large amount of essentially nontoxic organic arsenic forms in seafood, urine arsenic concentrations (measuring only elemental arsenic by atomic absorption spectroscopy or similar methods) transiently may be highly elevated after consuming seafood, for example, urine arsenic concentrations exceeding 1000 $\mu g\,l^{-1}$ of seafood nontoxic organic arsenic compounds. For comparison, unexposed (no seafood intake) persons' background urine inorganic arsenic concentrations typically are in the range of 5–50 $\mu g\,l^{-1}$. The diagnosis of increased inorganic arsenic exposure can be made with a 24 h urine collection with an inorganic urine arsenic concentration of 50 $\mu g\,l^{-1}$ or more, 100 μg per gram of urine creatinine or more, or 100 μg of total urine inorganic arsenic or more. Arsenic whole blood concentrations are elevated only for a short time after ingestion due to the short halftime of arsenic compounds in the blood; thus, whole blood concentrations (unexposed persons less than 10 μg of arsenic per l whole blood) typically are not useful in the assessment of arsenic exposure. Hair and nail arsenic concentrations have limited interpretive utility due to possible external contamination.

Seafood contains considerably more arsenic (nontoxic organic arsenic) compared with other foods. Market basket studies by the FDA in the 1990s in the United States showed a daily intake of arsenic from food of less than 10 μg per day. Drinking water estimates are that another 10 μg per day of arsenic is consumed, but this assumes 2 liters per day of drinking water per adult containing 5 μg arsenic per liter of drinking water. The majority of persons in the United States consume drinking water that contains less than 5 $\mu g\,l^{-1}$ of arsenic. In the early 1990s, it was estimated that about 350 000 persons in the United States drank water containing more than 50 $\mu g\,l^{-1}$ of arsenic (mostly in the states of California, Arizona, and Nevada). EPA mathematical modeling shows that 50 $\mu g\,l^{-1}$ of arsenic in drinking water is associated with an increased lifetime risk of lung and bladder cancers. It has been estimated that the daily inorganic arsenic intake in the diet ranges from about 8 to 14 μg per day (adults). Examples of food-related arsenic toxicity include episodes of multiple person exposures, for example, more than 12 000 Japanese children reported in 1972 consumed arsenic-contaminated dry milk with 130 deaths; nearly 400 persons in Hong Kong in 1857 became ill following consumption of arsenic-contaminated bread; approximately 6000 beer drinkers in England in 1900 were ill, and 70 died after drinking beer made with sugar that utilized arsenic-contaminated sulfuric acid; more than 60 persons in Japan in 1998 became ill after eating arsenic-contaminated curry; and 15 persons in the United States in 2003 were hospitalized, and one additional person died after consumption of arsenic-contaminated coffee.

The adverse toxicological effects of arsenic are many and varied. Target organs include the bone marrow, skin, and nervous system after either acute or chronic excessive exposure. Malignant and nonmalignant skin adverse effects, peripheral vascular disease (e.g., blackfoot disease (gangrene of extremities) in Taiwan), diabetes mellitus, hypertension, lung cancer, bladder cancer, and liver cancer may occur with chronic exposure to drinking water with elevated arsenic concentrations. Aplastic anemia, agranulocytosis, and acute myelogenous leukemia may occur after chronic exposure. In Bangladesh, where there is extensive excessive well-water arsenic chronic exposure, cohort studies show a small but statistically significant increased risk of birth defects and fetal demise. Cross-sectional evidence shows an association of persistent neurocognitive deficits in children following *in utero* excessive arsenic exposure or early childhood excessive arsenic exposure. These latter data need to be confirmed by long-term prospective randomized cohort studies.

For chelation therapy of subacute or chronic arsenic toxicity, the drug of choice that is available and marketed in the United States is oral succimer (only the oral formulation is available). DMPS (dimercaptopropanesulfonate) (not marketed in the United States) also is an effective arsenic chelator. DMPS is distributed both intracellularly and extracellularly, a theoretical advantage for effectiveness; DMPS also binds/chelates MMA. A monoisoamyl ester of succimer has increased intracellular distribution compared with succimer and increases survival in arsenic-poisoned laboratory rats.

In summary, food (and water) sources of mercury, cadmium, and arsenic remain a potential route of exposure and possible poisoning by these heavy metals. Poisoning by these metals via food and water has been lessened because of increased regulations, increased awareness, and actions to mitigate such exposure. Continued international efforts will be needed to further decrease and maintain lowered exposure to these metals via food and water consumption.

See also: Arsenic: Toxicology and Health Effects; Cadmium: Toxicology; Cobalt: Toxicology; Water Management, Treatment and Environmental Impact.

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Herbs: Composition and Dietary Importance

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Introduction

Herbs are the natural food additives that contribute immensely to the taste and flavor of our foods. These mysterious herbs are used for thousands of years in different foods and have been recognized to possess beneficial health properties and effectively used in the indigenous systems of medicine. Generally, the leaves used in cooking may be referred to as culinary herbs, for example, the green leaves of rosemary, oregano, coriander, basil, and parsley are used in Western and Continental cuisines for their aromatic flavors. The other parts like dried fruits (pepper), bark (cinnamon), and rhizomes (turmeric) are the important spices in Indian kitchen. Herbs are important for their disease prevention and health promotion properties, with an emphasis on diet. Regular use of culinary herbs in diet causes beneficial health effects, such as rosemary for jaundice, arthritis, and high blood pressure; oregano for stomach and respiratory ailments; turmeric as alternative to antibiotics; coriander as antioxidant, diuretic, expectorant, laxative, antibacterial, and antipyretic; basil to protect the heart; parsley as antioxidant; pepper for inflammation; and cinnamon to stimulate circulation. Dietary herbs could supply reasonable quantities of nutrients as well. The various aspects studied on these herbs can include safety of consumption, digestive stimulant action, influence on lipid metabolism, fat absorption, and antioxidant potential. Since each of the herbs possesses more than one beneficial health properties and there is a possibility of synergy among them in their action, a herbal diet is likely to make life not only more 'spicy' but also more healthy.

Cinnamomum zeylanicum

Cinnamomum zeylanicum (family Lauraceae), popularly known as cinnamon, is indigenous to Sri Lanka and southern parts of India and is used as an important spice in Indian kitchen in almost every delicious dish. Cinnamon has gained more importance in the Ayurvedic and folklore medicine in India, as remedy for the treatment of respiratory, digestive, and gynecologic ailments. A preclinical and clinical study confirmed anti-inflammatory and antimicrobial properties of cinnamon, which also reduces cardiovascular diseases and risk of colonic cancer, improves cognitive function, and reduces hypertension. Every part of the cinnamon tree has medicinal use including the leaves, bark, flowers, fruits, and roots. In addition, the *C. zeylanicum* is used for the treatment of dyspeptic conditions, flatulence, loss of appetite, abdominal pain, diarrhea, inflammation of the eye, leukorrhea, vaginitis, rheumatism, neuralgia, wounds, and toothache. *In vitro* studies evaluating antimicrobial properties of *C. zeylanicum* confirm its effectiveness against a wide variety of bacteria (Gram-positive and Gram-negative), fungi, and human rotavirus.

The volatile oils obtained from the different parts of the plant possess the same array of hydrocarbons in varying proportions with constituents such as cinnamaldehyde (bark), eugenol (leaf), and camphor (root). Three of the main bark oil components, *trans*-cinnamaldehyde, eugenol, and linalool, represent near 85% of the oil total composition. The *C. zeylanicum* leaf oil is rich in eugenol and the root bark oil in camphor, while the fruit oil is known to have varying major oil constituents depending on its origin (α- and β-pinenes, β-caryophyllene, *trans*-cinnamyl acetate, etc.). The essential oils obtained from the bark and important oil constituent, eugenol, express very powerful antioxidant activities by decreasing 3-nitrotyrosine formation and inhibiting the peroxynitrite-induced lipid peroxidation *in vitro* assays.

In vitro studies demonstrate various antidiabetic effects of *C. zeylanicum* extracts achieved in the following mechanisms: (1) reducing postprandial intestinal glucose absorption by inhibiting the activity of enzymes involved in carbohydrate metabolism (pancreatic α-amylase and α-glucosidase), (2) stimulating cellular glucose uptake by membrane translocation of GLUT-4, (3) stimulating glucose metabolism and glycogen synthesis, (4) inhibiting gluconeogenesis by effects on key regulatory enzymes, and (5) stimulating insulin release and potentiating insulin receptor activity. *In vivo* beneficial antidiabetic effects of *C. zeylanicum* include (1) attenuation of associated weight loss, (2) reduction of fasting blood glucose, (3) reduction of LDL and increase of HDL cholesterol, (4) reduction of HbA1c, and (5) increase in circulating insulin levels. In addition, this plant species also possesses valuable effects against diabetic neuropathy and nephropathy.

C. zeylanicum extract is known to have antisecretagogue and antigastric ulcer effects, as it effectively inhibits gastric hemorrhagic lesions induced by ethanol, NaOH, and NaCl. It has induced a dose-dependent analgesic protective effect against both thermal stimuli and the writhing syndrome, in addition to the evidence that it also shows an anti-inflammatory effect against chronic inflammation induced by cotton pellet granuloma indicating antiproliferative effects.

Piper longum

Piper longum L. (family Piperaceae), commonly known as 'long pepper,' is a climbing shrub, widely distributed in the tropical and subtropical regions of the world, throughout the Indian subcontinent, Sri Lanka, Middle Eastern countries, and the Americas. It is used as an important culinary spice, and although it is a very rare ingredient in European cuisines, it can be found in Indian vegetable pickles, some North African spice mixtures, and Indonesian and Malaysian dishes. In addition, it has gained importance in traditional system of medicine, such as Ayurveda, as an alternative to tonic.

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Traditionally, unripe fruits and roots of the plant are used for the treatment of bronchitis, cough, and cold; as antidote in snake biting and scorpion sting; and as contraceptive. In the northwestern Himalayan regions, it is used as a root decoction in ethnoveterinary medicine, for swellings of the joints of cattle. The fruits and the roots of *P. longum* contain a number of alkaloids and related compounds, the most abundant one being piperine, followed by methyl piperine, iperonaline, piperettine, asarinine, pellitorine, piperundecalidine, piperlongumine, piperlonguminine, refractomide A, pregumidiene, brachystamide, brachystamide A, brachystine, pipercide, piperderidine, longamide, tetrahydropiperine, terahydropiperlongumine, dehydropipernonaline, piperidine, piperine, terahydropiperlongumine, trimethoxycinnamoylpiperidine, and piperlongumine. Essential oil extracted from the fruits of *P. longum* is a complex mixture, excluding the volatile piperine; the three components of the oil are β-caryophyllene, pentadecane, and bisabolene. Apart from these, sesamin, pulvuatilol, fargesin, thujone, terpinolene, zingiberene, *para*-cymene, *p*-methoxyacetophenone, and dihydrocarveol are also present in the fruit oil of *P. longum*. The plant contains essential oil, which consists of sesquiterpene hydrocarbons and ethers (bisabolene, β-caryophyllene, β-caryophyllene oxide, and α-zingiberene) and saturated aliphatic hydrocarbons such as pentadecane, tridecane, and heptadecane. With respect to its medicinal importance, *P. longum* is well investigated pharmacognostically, chemically, and pharmacologically.

Various studies have revealed that the root has a weak opioid but potent NSAID type of analgesic activity, in addition to cardioprotective, antiarthritic, anticonvulsant, immunomodulatory, and anti-inflammatory activities. Various *in vitro* and *in vivo* models demonstrate effects of the plant extracts on angiogenesis by regulating the cytokines and growth factors in angiogenesis-induced animals. *P. longum* extract demonstrates anticancer properties due to antioxidant and its inhibitory action on inflammatory mediators like cytokines and TNF-α. At a higher dose, the extract of *P. longum* also shows hepatoprotective and antifibrotic activities. Alcoholic extract of the plant fruits and its constituent piperine is studied for possible immunomodulatory and antitumor activities; following their administration, bone marrow cellularity and esterase positive cells proved to be increased. Guineensine, isolated from chloroform extract, inhibited activity of acyl CoA:cholesterol O-acyltransferase in a dose-dependent manner, and dehydropipernonaline demonstrates vasorelaxant activity. Pharmacological inhibition of acyl CoA/diacylglycerol acyltransferase by alkamides isolated from *P. longum* emerged as a potential therapy for the treatment of obesity and type 2 diabetes.

Curcuma longa

Curcuma longa L. (family Zingiberaceae), known as turmeric, is the rhizomatous herbaceous perennial plant, native to tropical South Asia, and now widely cultivated in the tropical and subtropical regions of the world. The rhizome, which is yellow in color, is commonly used as a spice in many Indian dishes and as a medicine in traditional system of medicine in India. In Ayurvedic medicine, turmeric is primarily used for the treatment of inflammatory diseases such as arthritis and cancer, while in traditional Chinese medicine, it is used as stimulant, aspirant, carminative, cordial, emmenagogue, astringent, detergent, and diuretic; also for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic; or as an ointment to treat many skin diseases. The main components of turmeric include curcumin (curcumin I), demethoxycurcumin (curcumin II), and bisdemethoxycurcumin (curcumin III), together referred to as curcuminoids. Curcumin is a low-molecular-weight polyphenol responsible for the bright yellow color of turmeric; it is the active principle of turmeric that comprises 2–8% of most turmeric preparations. Preclinical and clinical studies confirm that curcuminoids achieve anti-inflammatory activity by inhibiting LOX, COX, phospholipases, leukotrienes, prostaglandins, thromboxane, nitric oxide elastase, hyaluronidase, collagenase, monocyte chemoattractant protein-1, interferon inducible protein, TNF, and interleukin-12. They also decrease prostaglandin formation and inhibit leukotriene biosynthesis via the lipoxygenase pathway. Water- and fat-soluble extracts of turmeric and its constituent curcumin exhibit strong antioxidant activity. Curcumin is found to inhibit cyclosporine A-resistant phorbol myristate acetate anti-CD28 pathway of T-cell proliferation. In addition, it reduces the testicular damage caused by exposure to di-*n*-butylphthalate (DBP), by increasing glutathione (GSH), testosterone levels, and glucose-6-phosphate dehydrogenase (G6PD) activity and decreasing malondialdehyde (MDA) levels. These properties are probably due to intrinsic curcumin's antioxidative abilities.

Animal research demonstrates that this compound inhibits all three stages of carcinogenesis, namely, initiation, promotion, and progression. During initiation and promotion, curcumin modulates transcription factors controlling phase I and II detoxification of carcinogens; downregulates proinflammatory cytokines, free radical-activated transcription factors, and arachidonic acid metabolism via COX and LOX pathways; and scavenges free radicals, and in progression stage, curcumin inhibits papilloma production, induces apoptosis of cancer cells, and inhibits angiogenesis.

Curcuminoids also prove the antidiabetic effects by stimulating following mechanisms: (1) cellular glucose uptake by GLUT-4, (2) glucose metabolism and glycogen synthesis, and (3) insulin release and insulin receptor activity potentiation.

In vitro and *in vivo* studies also demonstrate that turmeric extract and *C. longa* essential oil are active against various microorganisms and inhibit the growth of a variety of pathogenic bacteria, parasites, and fungi. Chronic administration of curcumin modulates the dysfunction of the hypothalamic–pituitary–adrenal axis, through selective increase in brain-derived neurotrophic factor in the frontal cortex and the hippocampus of the animals, showing its activity against anxiety and depression. Turmeric's protective properties of the cardiovascular system include lowering cholesterol and triglyceride levels, decreasing susceptibility of low-density lipoprotein to lipid peroxidation, and inhibiting platelet aggregation.

Coriandrum sativum

Coriandrum sativum L. (family Apiaceae) is herb indigenous to southern Europe and cultivated for its edible seeds and leaves. *C. sativum* seeds are known for over 5000 years and were found in the tombs of Egyptian pharaoh Ramses II and used in ancient Egypt as 'secret of happiness' (an aphrodisiac), and in history of Hebrews, the seed was considered as a gift from God

and was added to drinks during Passover and was used by Hippocrates as a healing herb.

C. sativum leaves, which are larger at the base and smaller on the top, have distinct powerful aroma, and the seeds, flowers, and roots are used in flavoring of rice, sausages, soups, stews, and ratatouille, in pickling, and in baked foods and are an important ingredient of the famous Belgian wheat beer. The coriander leaves have high contents of vitamin C, vitamin A, vitamin K, iron, manganese, thiamine, zinc, and dietary fiber. The fresh juice of the *C. sativum* leaves is helpful in curing deficiencies related to vitamins and iron. The seeds are very rich source of potassium, calcium, phosphorus, magnesium, sodium, and zinc. The finest flavor of *C. sativum* is associated to the plants grown in England, while those cultivated in Russia and Germany proved to be the richest in essential oil.

Fruits and leaves of *C. sativum* are traditionally used for digestive and gastric complaints, coughs, chest pains, bladder complaints, fever, dysentery, headaches (externally), oral and pharyngeal disorders, halitosis, and many other complains. Its crushed seeds can be applied externally for rheumatism and painful joints. *C. sativum* fruits demonstrate antibilious, antispasmodic, aphrodisiac, appetizer, aromatic, carminative, diaphoretic, diuretic, refrigerant, antirheumatic, stimulant, stomachic, and tonic properties; show antihypertensive potential and sedative, hypolipidemic, and vermifuge activities; and also influence loss of appetite, convulsion, and insomnia.

Essential oil from the *C. sativum* fruits contains high percentage of linalool, camphor, α-pinene, γ-terpinene, and limonene. Due to the high linalool content in the essential oil, coriander seeds demonstrate the analgesic activity through an opiate-mediated mechanism. The oil extracted from *C. sativum* leaves demonstrates strong antifungal, antiadherent, and antiproteolytic activities against *Candida* spp. through the synergistic effect of its mono- and sesquiterpene hydrocarbons and modulation of gene expression in chemokine and mitogen-activated protein kinase pathways of *Candida* spp. and expression of adhesion proteins.

C. sativum leaves, which have high content of carotenoids, show higher hydroxyl radical scavenging potential in comparison with its seeds. The ethyl acetate extract of the plant roots inhibits activity in MCF-7 cells by affecting antioxidant enzymes and cell cycle arrest at the G2/M phase and apoptotic cell death by the death receptor and mitochondrial apoptotic pathways. *C. sativum* is also widely used for the management of CNS disorders and demonstrates a high curing potential for Alzheimer's disease in the rat experimental model, through protection and improvement of functions in pyramidal cells in the cerebral cortex.

Ocimum basilicum

Ocimum basilicum L. (family Lamiaceae) is a commonly and widely cultivated European and Asian plant with intense distinctive herbal spicy and sweet aroma (from the Greek 'basileus king,' royal fragrance) and has its origins from the Greek mythology. In ancient Egypt, the so-called Odium basilicum has been used in combination with other essences and oils, during religious ceremonies and for mummification. *O. basilicum* was introduced in England in the sixteenth century. *O. basilicum* is widely used in medicine and for culinary and perfumery purposes all over the Europe, particularly in France. The leaves and the essential oil distilled from the flowering plants are used as powerful flavoring agents. The main *O. basilicum* exporters for the European market are France, Italy, Morocco, Egypt, and the United States (California).

In traditional medicine, *O. basilicum* has been intensively used for the treatment of gastric and respiratory diseases, anxiety, headaches and migraines, inflammation, cough, cold, digestive disorders, chest and lung complaints, fever, insect bites, menstrual cramps, and sinusitis and demonstrates carminative, stimulant, diaphoretic, diuretic, antiseptic, anesthetic, antispasmodic, anthelmintic, antidiarrheal, analgesic, and antitussive properties.

The *O. basilicum* essential oil has several chemotypes (linalool, methyl chavicol (estragole), eugenol, etc.). Basic chemical composition of the linalool chemotype of the *O. basilicum oil* is linalool, epi-α-cadinol, c-cadinene, germacrene D, and camphor. Methyl-chavicol chemotype of *O. basilicum* essential oils contains high percentage of methyl chavicol, 1,8-cineole, *trans*-ocimene, etc. Due to its richness in linalool (oxygenated monoterpene), the *O. basilicum* essential oil exhibits potent antimicrobial activity that is comparable to amoxicillin and flumequine. Phenolic components are the main contributors to the oil antimicrobial properties, causing the leakage of intracellular ATP and potassium ions of bacterial cells, which leads to the death of bacterial cell. Significant antibacterial activity of the oil is documented against Gram-positive and Gram-negative bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pasteurella multocida*, *Salmonella enteritidis*, *and Proteus vulgaris*), though some Gram-negative bacteria proved to be resistant, probably due to their hydrophilic outer membrane, which protects penetration of hydrophobic compounds into the cell.

The methyl chavicol, which is typical for *O. basilicum* methyl-chavicol essential oil, shows a broad spectrum of antifungal activities (*Aspergillus niger*, *Mucor mucedo*, *Fusarium solani*, *Botryodiplodia theobromae*, *Rhizopus solani*, *Candida albicans*, *and Microsporum gypseum*), which are achieved by disruption of fungal membrane. Methyl chavicol (estragole), one of the major *O. basilicum* oil constituents, is extensively used as a flavoring, antimicrobial, antioxidant, antiedematogenic, and anti-inflammatory agent. It has been shown to block neuronal excitability by direct inhibition of Na^+ channel conductance activation in peripheral nerves due to a local anesthetic mechanism.

The ursolic acid presented in the aqueous extract of sweet basil interferes with postinfectious replication mechanism of CVB1 group of Coxsackie virus. Phenolic compounds of *O. basilicum* plant essential oils and extracts also play an important role in stabilizing lipids against peroxidation and inhibiting various types of oxidizing enzymes. The hydroalcoholic extract of *O. basilicum* contains a range of potent oxygen scavengers (terpenoids, flavonoids, and tannins), which could be useful clinically in the improvement and retrieval of memory via reducing brain cells oxidative damage. Caffeic acid found in the leaf of the *O. basilicum* demonstrates antioxidant, anti-inflammatory, and cancer chemopreventive properties.

Aqueous leaf extract of *O. basilicum* demonstrates cytotoxicity against nasopharyngeal cancer cell line (KB), human liver hepatocellular carcinoma cell lines HeLa and HEp-2, and NIH 3T3 mouse embryonic fibroblasts. *O. basilicum* aqueous extract

has been shown to protect the testis against toxicity induced by one of the most known toxic heavy metals cadmium (Cd).

Petroselinum crispum

Petroselinum crispum (Mill.) Nym. (family Apiaceae) is a vegetable, a medicinal herb, and a spice, widely cultivated for more than 2000 years in the southeastern Europe and western Asia. Its name *Petroselinum* derives from the Greek term referring to the plant's habit to grow in rocky places. *P. crispum* is native to the central Mediterranean region and is cultivated all over the Europe. As a great flavoring to any salad and meet dish, the plant was introduced from Sardinia to England in the sixteenth century.

There are three different varieties of *P. crispum*: var. *crispum* (curles parsley) with a sharp peppergrass-like taste and cultivated in warmer climate; var. *neapolitanum* (flat leaf purslay) with a sweeter and smoother flavor and grown in a colder conditions characterized by dryness and snow (Italian and eastern European parsley); and the less known var. *tuberosum* (Humburg parsley), primarily grown for its roots.

P. crispum has bright green, fragrant leaves, it adds color and flavor to any food we eat, and it is used to soothe various digestive complaints, such as indigestion, flatulence, and loss of appetite, and may prevent stomach ulcers. Fresh *P. crispum* herb is widely used in Middle Eastern, European, Brazilian, and American cooking. French *persillade* (a sauce made of mixture of chopped garlic and parsley) is well known all over the world, and Italian and Spanish cuisines use the herb in delicious *salsa verde*.

In addition to its culinary use, *P. crispum* possesses many medicinal properties, first mentioned by the ancient Greeks. *P. crispum* plant is rich in ascorbic acid, carotenoids, flavonoids, apiole, terpenoic compounds, coumarin, phenylpropanoids, phthalides, tocopherol, and furanocoumarins. The leaves hold high content of vitamins (A, C, and K), β-carotene, and also lutein, zeaxanthin, folate, choline, niacin, and pantothenic acid. Together with leaves, the plant roots are wonderful source of a range of minerals (Ca, K, Mg, B, P, Fe, Na, Zn, F, Mn, and Se). High concentration of boron and fluoride in parsley leaves might help against bone thinning and osteoporosis, and its great combination of calcium and fluorine supports teeth health.

P. crispum essential oils and extracts demonstrate antioxidant, anti-inflammatory, calcium channel-blocking (in the intestine and uterus muscle), cancer preventive, laxative, and diuretic properties. Due to its essential oil content, *P. crispum* has great insect repellent potential and is always a good companion plant, repelling insects from nearby plants. The major constituent of the *P. crispum* leaf oil is *p*-1,3,8-menthatriene, followed by β-phellandrene, myristicin, and β-myrcene, while the major root oil constituent is apiole, followed by myristicin and terpinolene. In addition, the *P. crispum* leaf oil has immunomodulatory effect on mitogen-activated splenocytes and peritoneal macrophages apart that the seed oil is used as a fragrance in soaps, cosmetics, and perfumes and demonstrates strong diuretic activity. However, it is important to remember that *P. crispum* essential oils can also be phototoxic and abortifacient and should never be consumed or applied to the skin without caution.

The methanol extracts of *P. crispum* contain coumarins and express great antimicrobial activity against *B. subtilis*, *P. aeruginosa*, *S. epidermidis*, *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, and *Saccharomyces cerevisiae*, while hydroalcoholic extract of the same plant demonstrates a significant inhibition of the standard *P. aeruginosa strain*. The aqueous extracts of parsley had bactericidal effects against *Helicobacter pylori* and inhibited adhesion of the bacteria to animal stomach section. Daily consumption of *P. crispum* in salad significantly reduces the blood glucose potentially via inhibition of gluconeogenesis and direct stimulation of glycolysis.

In general, fresh *P. crispum* plant and its oils and extracts are widely used as antiaging, anticarcinogenic, antifungal, anti-inflammatory, digestive aid, laxative, diuretic, and aphrodisiac; help soothe arthritis pain; and are used for bronchitis, bruises, burns, itching, common cold, itching, menstrual cramps, bad breath, goiter, edema, swollen glands, bed-wetting, fluid retention, jaundice, asthma, fever, gas, swollen breasts, snakebites, dropsy, worms, obesity, and expulsion of the gravel and kidney stone.

Rosmarinus officinalis

Rosmarinus officinalis (family Lamiaceae) is one of the oldest Mediterranean shrubs with powerful pungent aroma and dark green elongated leaves and white or purple flowers. The rosemary herb was used as a food seasoning and natural medicine for over a million years. The name *Rosmarinus* derives from the Latin term that translates to 'dew of the sea.' Rosemary is a symbol of remembrance, since ancient Greece and Rome. Greeks used the herb to help their memory, to expel negativity by placing the sacred herb under the pillow, and to prevent nightmares. Dioscorides, Theophanus and Conrad Gesner, and ancient healers recognized the beneficial effects of rosemary in dealing with brain, liver, heart, and eye problems. According to legend, when Mary, Joseph, and the baby Jesus were fleeing to Egypt, Mary laid her cloak on a rosemary bush, and since that time, rosemary bush was called the 'Rose of Mary' in honor of the Virgin Mary. Rosemary plants were placed in the tombs of Pharos in ancient Egypt to remember the dead and were used to embalm them. During Medieval times in France, rosemary was burned for church incense and used to purify the air in the hospitals. Famous Napoleon's Eau de Cologne was made with rosemary oil.

R. officinalis leaves added to vinegar (marinade) make a great flavoring component in preparation of meat, shellfish, and vegetables. Rosemary plant is rich in vitamin A, vitamin C, thiamin, riboflavin, vitamin B_6, and folate, as well as such important minerals like Ca, Mg, iron, phosphorus, potassium, and copper. Carnosic acid and carnosol (rosemary polyphenol) pose chemopreventive, antioxidant, and anticancer activities against prostate, skin, breast, leukemia, lung, urinary bladder, and colon cancers through genes that encode antioxidant phase II enzymes. As anti-inflammatory, carnosol has ability to reduce leukotrienes, inhibit 5-lipoxygenase, antagonize the intracellular Ca^{2+} mobilization, and inhibit the secretion of leukocyte elastase. Triterpenoid compound ursolic acid has been shown to have anti-inflammatory and even antidepressant effects.

R. officinalis and its components were reported to possess chemopreventive properties in skin and breast cancers *in vivo*

by inhibiting genotoxicity (7,12-dimethylbenz(a)anthracene (DMBA)–DNA adduct formation). Polyphenolic preparation from R. officinalis in combination with low calcemic vitamin D_3 analogs demonstrates antileukemic activity *in vivo* in six- to eight-week-old female Balb/c mice.

R. officinalis leaf essential oil is composed mainly of α-pinene, 1,8-cineole, and camphor and when used in massage increases blood pressure and respiratory rates and has stimulatory effect on the autonomic nervous system through sympathetic activity. *R. officinalis* essential oil exhibits free radical scavenging activity and excellent hepatoprotective properties by limiting the lipid peroxidation. The 1,8-cineole from *R. officinalis* essential oil is a lipid-soluble compound that facilitates passage across the blood–brain barrier, producing neuronal effect on the receptor sites or impacting enzyme activity of neurons in brushtail possum.

R. officinalis essential oil has excellent antioxidant and antimicrobial activity and is capable to extend the shelf life of food and skin care products during the storage as natural preservative.

Origanum vulgare

Origanum vulgare, also called wild marjoram (family Lamiaceae), whose name comes from Greek *origanon*, is typical to North America, originating from Mediterranean, and have a long history of use as food and a medicinal plant. Oregano is a perennial herb (up to 80 cm in height), with dark oval fragrant leaves and white, pink, or purple flowers formed in spikes. In ancient times, oregano symbolized happiness, is used to combat sadness and bring luck, and is thought to be precious to the Greek goddess Aphrodite. In ancient Egypt, oregano was used as both a preservative and an antidote to poison, and Greeks used *O. vulgare* herb internally and externally as a fomentation to treat skin irritations and infections, dropsy, and convulsions and as an excellent poison antidote. Traditional Chinese healers have also used oregano for generations to treat a variety of complaints. In Shakespearean times, oregano was used for just about anything. It is said to encourage good luck and good health. Oregano gives a slightly bitter flavor to poultry, fish, sauces, eggs, and other dishes. This dried herb is also used in processed foods (alcohol beverages, snack foods, meat, and milk products). The charcoal-roasted lamb *souvlaki* is usually sprinkled with oregano. All the varieties of oregano are interchangeable in food preparation soups, herb bread, and tomato dishes. *O. vulgare* is also used as a fragrance component in perfumes, detergents, cosmetics, flavorings, soap, and pharmaceuticals.

The major constituents of *O. vulgare* leaves essential oil are carvacrol, p-cymene, c-terpinene, limonene, terpinene, ocimene, caryophyllene, β-bisabolene, linalool, and 4-terpineol. Oregano oil has antibacterial, antifungal, antiparasitic, antimicrobial, and antioxidant properties. The antifungal activity of *O. vulgare* essential oil against *A. parasiticus*, *A. niger*, *A. flavus*, and *A. ochraceus* through inhibition of growth and aflatoxin production is well documented. *O. vulgare* essential oil and extract are highly effective against *C. albicans*, *S. aureus*, *and Pseudomonas aeruginosa*.

The bioactive constituents of *O. vulgare* leaves extract are rosmarinic acid, linalool, thymol, carvacrol, tannins, flavonoids, triterpenes, phenol carvacrol, and thymol. Oregano also contains iron, vitamins, calcium, copper, niacin, magnesium, and thiamine. Oregano has been used in the treatment of cancer, muscular degeneration, and heart disease. Oregano taken as a tea or used as a salve inhibits bacterial growth and parasitic microorganisms. The herbal tea also helps to alleviate headaches, urinary problems, diarrhea, nausea, lung disorders, vomiting, and jaundice and is used as a mouthwash to help with tooth infections and sore throat. Oregano is a great cold and flu remedy and aids in the treatment of indigestion, bloating, flatulence, and menstrual symptoms.

Conclusion

As interest in the potential use of herbs in diets for health benefits increases, it will become more important to monitor the progress of the clinical findings and to communicate these in literatures. Modern research on the impact of the bioactive properties of culinary herbs has shed some light on their potential health benefits. However, advance research is required to fully understand the health-promoting mechanism of such herbs from different diets. Furthermore, there is very little knowledge about the impact of combining herbs in different cuisines and their bioactivity. Ultimately, it is determined that the dietary herbs possess true health benefits because of the presence of nutrients, vitamins, and polyphenols.

See also: Essential Oils: Isolation, Production and Uses.

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Histidine-containing Dipeptides: Properties and Occurrence in Foods

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Introduction

Histidine-containing dipeptides carnosine, anserine, and balenine (also known as ophidine) are endogenous dipeptides in mammalians, which are described to have important bioactive functions. The main properties of these dipeptides are based on their chemical structure consisting in an imidazole group belonging to the amino acid histidine, 1-methylhistidine, or 3-methylhistidine, respectively, linked by a peptidic bond to the amino acid β-alanine. These dipeptides are widely distributed in the animal body being especially abundant in the skeletal muscle where they exert their main action consisting of buffering effect for contributing to the maintenance of the acid–base balance. This allows rapid recovery of muscle that can continue working after intense exercise. Other locations are the central nervous system, brain, cardiac muscle, liver, kidney, and sense organs like eyes where they exert a combination of antioxidant and cell protective activities for those considered as 'antiaging' peptides. For all these reasons, they are used to prevent and treat diseases such as diabetes, neurodegenerative diseases, cancer, and diseases of the sense organs like cataracts. Carnosine has been demonstrated to have some effect in cell heart contraction via calcium-dependent mechanisms, thus opening a door for discovering alternative ways for treating heart failure. Also, a role as preventive agents, acting as a barrier against influenza virus infection, has been described.

The pool of carnosine in the body comes from endogenous and exogenous sources. Carnosine is synthesized in the organism by carnosine synthase from their constituent amino acids, β-alanine and histidine, when they are available, but it can also be directly ingested in the diet. Indeed, higher carnosine levels are found in people consuming diets rich in meat in comparison to vegetarians. The level of carnosine in tissues is controlled by a number of enzymes that reversibly transform carnosine into other carnosine-related compounds, such as anserine or balenine by methylation, carcinine (β-alanylhistamine) by decarboxylation, or *N*-acetylcarnosine by acetylation. Apart from these, carnosinase, a dipeptidase (2 isoforms) present in the cerebrospinal fluid, serum, and kidney, is able to cleave carnosine into histidine and β-alanine. Carnosinase preferably acts against carnosine, while anserine and balenine are hydrolyzed much more slowly (by 20%) and *N*-acetylcarnosine is not hydrolyzed at all. Another carnosine derivative also present in the animal body, which is homocarnosine (γ-aminobutyryl-histidine), has a strong, competitive inhibitory effect on carnosine turnover mediated by carnosinase. Therefore, the presence of homocarnosine or the metabolic transformation of carnosine to their derivatives can protect it against enzymatic hydrolysis as a spare way. Indeed, *N*-acetylcarnosine has been used as a substitute of carnosine in the treatment of cataracts, because of its same beneficial properties, while showing resistance against hydrolytic action of serum carnosinase.

The histidine-containing dipeptides content in the body is also dependent on age and gender. The activity of carnosinase tends to increase with age, leading to lower levels of carnosine in older people; higher carnosine content has been reported in males than in females.

Presence in Animal Species and Types of Muscle

The presence of histidine-containing dipeptides in muscle mainly depends on animal species and type of muscle. These dipeptides are more abundant in white muscles than in red ones that seem to be due to a kind of adaptation to the requirements of the muscles. Type IIB muscle fibers are fast-twitch glycolytic fibers prone to rapid responses. In fact, it has been observed that sprinter animals like thoroughbred horses or greyhound dogs or animals that need to escape from predators have a higher percentage of fast-twitch glycolytic fibers, which are richer in dipeptides that exert the buffering effect.

Figure 1 shows the content of dipeptides in some common animal muscle foods. It is interesting to highlight the big differences among them. Carnosine is predominant in terrestrial animals, except in those moving by jumping like kangaroo, rabbit, deer, goat, and, also, sheep that have more anserine or equal amounts of anserine and carnosine (see the contents in lamb shown in Figure 1). On the other hand, anserine is predominant in poultry with a very high content in chicken breast.

Migratory fish and birds have muscles rich in anserine to maintain posture and instantaneous power. As occur in birds, anserine is the predominant histidine-containing dipeptide, but, as in the case of fish, carnosine is almost negligible in comparison to the anserine content. Fish muscle contains two functionally and spatially distinct fiber types, red (dark) and white. In general, fish white muscle contains much higher (four to ten times more) amounts of anserine than red one, especially in migratory marine fishes such as mackerel, tuna, and bonito that use anserine to overcome fatigue. On the contrary, deep-sea and demersal fishes, like sole, halibut, code, and hake, contain less anserine. Nevertheless, there is a high variability in the amounts of histidine-containing dipeptides in fish due to the large number of fish species, and they can also vary even for a same species living in different climatic or environmental conditions.

Stability

The availability of these dipeptides through the diet is possible thanks to their good stability during processing (e.g., cooking, fermentation, and drying) and storage. Furthermore, the

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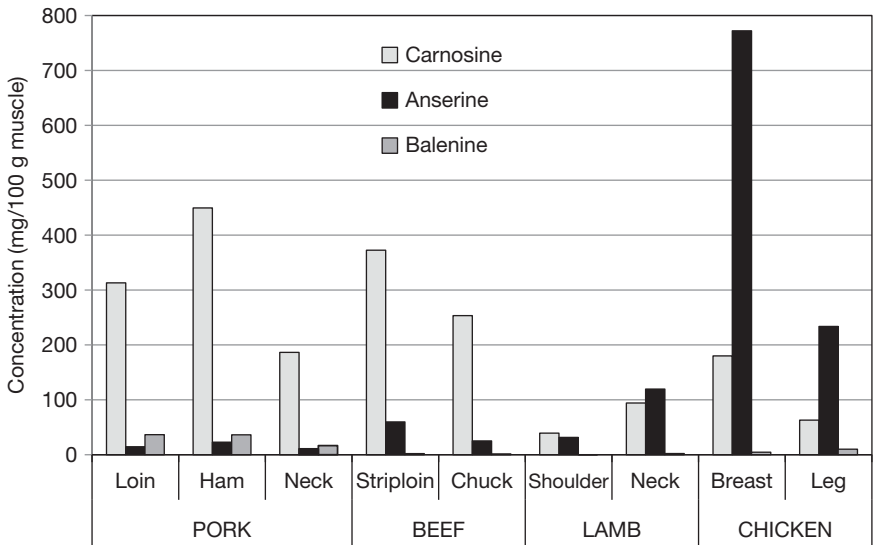

Figure 1 Histidine-containing dipeptide content in muscle foods from different animal species. Aristoy and Toldrá, unpublished data.

Table 1 Effect of different heat treatments (temperature and time) on the carnosine, anserine, and balenine contents (mg/100 g muscle, w/w) in pork loin

	Carnosine Time (min)			*Anserine Time (min)*			*Balenine Time (min)*		
Temperature (°C)	*30*	*60*	*120*	*30*	*60*	*120*	*30*	*60*	*120*
60	563.9	637.4	550.7	26.3	28.3	23.9	37.0	42.3	35.2
75	727.6	589.5	523.9	31.5	28.5	23.6	44.3	41.2	35.0
95	717.7	481.8	365.3	33.2	25.6	22.0	44.5	39.2	29.4
105	520.0	468.0	378.0	25.0	20.1	18.0	36.4	35.0	32.0

Source: Aristoy, M.-C., Soler, C. and Toldrá, F. (2004). A simple, fast, and reliable methodology for the analysis of histidine dipeptides as markers of the presence of animal origin proteins in feeds for ruminants. *Food Chemistry* **84**, 485–491.

ingested histidine-containing dipeptides are absorbed intact because of its resistance to the digestive tract peptidases.

Stability Against Heat Treatments

Different cooking methods for meat have different effects on its histidine-containing dipeptide content. Carnosine losses when using methods like boiling seem to be related to its solubilization in the water boiling medium more than to its degradation. Further, with higher-temperature treatments like those used for broiling meats, less degradation of carnosine was reported. Even higher-temperature broiling (200 °C) led to less reduction in the carnosine content than medium temperature (140 °C), probably due to the higher crusting when using high temperature and thus preventing drip losses and consequently carnosine depletion. Microwave cooking methods were reported to preserve the highest amounts of carnosine in beef meat (losses near 10%) and the total amount of anserine in turkey. These facts indicate that these dipeptides are quite heat-resistant, and therefore, high content of histidine-containing dipeptides may exist in commercial cooked meat products. Table 1 shows the content of dipeptides found in pork loin after different heating treatments. In this case, samples were packaged into a plastic bag and then submitted to different heat treatments. The drip loss was homogenized together with the meat sample to avoid losses in the drippings. At temperatures up to 75 °C, losses were, if any, less than 5% for the three dipeptides at any time of treatment (see Figure 2). Maximum losses were between 20% and 30% of both, carnosine submitted to 95 °C and anserine to 105 °C, after 120 min treatment.

Resistance to Muscle Endogenous Proteases

Endogenous proteases in the living muscle are important in the regulation of some physiological and cellular processes as well as in the prevention of the accumulation of unwanted or abnormal proteins in cells. After slaughter, peptidases start to degrade all proteins in the livestock or poultry muscles in a sequential chain in which endo- and exopeptidases like aminopeptidases are involved. Nevertheless, during meat aging under cold storage (14 days for beef or 7 days for pork), carnosine and anserine contents do not change. Different processes like dry-curing or fermentation and ripening are used for

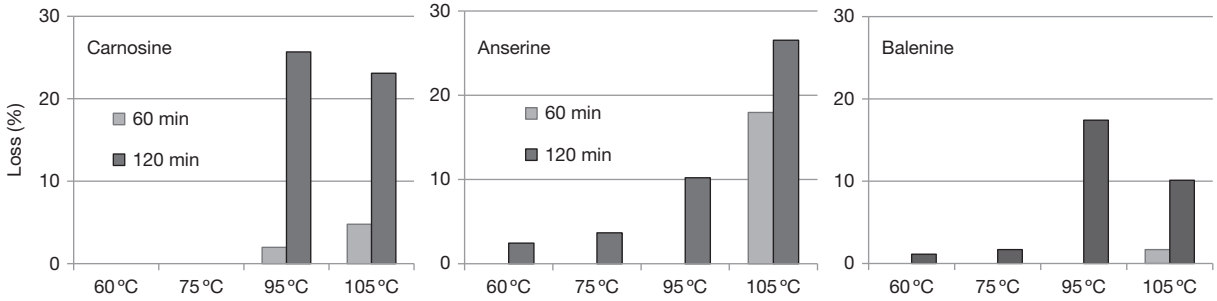

Figure 2 Losses, expressed as %, of carnosine, anserine, and balenine in pork loin after different heat treatments (temperature and time). Raw loin content (in mg/100 g muscle): carnosine, 491.0; anserine, 24.5; and balenine, 35.6. Pieces of loins were vacuum-packaged in plastic bags and submitted to different heat treatments for different times. Afterward, samples were homogenized together with the exudates and analyzed. Adapted from Aristoy, M.-C., Soler, C. and Toldrá, F. (2004). A simple, fast, and reliable methodology for the analysis of histidine dipeptides as markers of the presence of animal origin proteins in feeds for ruminants. *Food Chemistry* **84**, 485–491.

long processing times. Proteolytic enzymes are then the main responsible for the development of relevant sensory characteristics like texture and flavor.

High levels of the histidine-containing dipeptides have been reported in fermented meat products. Proteolysis in fermented sausages is mainly conducted by bacterial peptidases, while in dry-cured ham, muscle endogenous peptidases are responsible for these reactions. This explains the differences found in dipeptide content in both types of products. Muscle endogenous peptidases are not able to hydrolyze the peptidic bond of these dipeptides, while some bacteria (*Lactobacillus*, *Escherichia*, or *Salmonella*) have been described to contain carnosinase, an enzyme with the ability to hydrolyze this bond. So, different concentrations of carnosine and anserine are found in fermented meats depending on the bacterial flora. This flora is composed by the native microbiota together with different starter strains with more or less carnosinase activity and thus different abilities to metabolize the histidine-containing dipeptides. This factor will influence dipeptides amount to a larger extent than processing conditions. Nevertheless, high amounts of carnosine, about 1/3 and 1/2 of the initial concentration, remain intact in all the studied processes.

Figure 3 shows the evolution of carnosine in two types of fermented sausages: two from Spanish sausages and two from Italian salamis.

The very large amount of the histidine-containing dipeptides in dry-cured ham, similar to that from the raw pork ham, indicates a very reduced hydrolysis of the peptidic bond in the dipeptides. Further, carnosinase is not present in muscle. Table 2 shows the carnosine and anserine contents in several dry-cured hams from Spain, France, and Italy. Differences in breed, sex, or age of the animals should explain the differences among them, besides that in processing conditions (temperature, ripening length, etc.).

Figure 4 shows the evolution of carnosine content during a typical Spanish serrano ham process. The slight decrease of carnosine between 3.5 and 5 months of processing is in coincidence with a slight increase of β-alanine and anserine occurring in the same time and may be due to both mechanisms: a hydrolysis of carnosine into the constituent amino acids and a partial carnosine conversion into anserine by methylation.

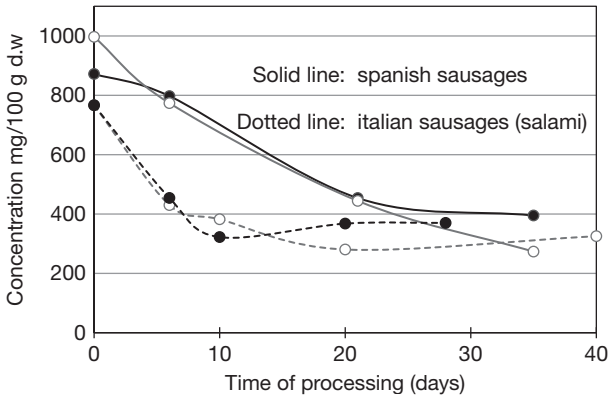

Figure 3 Evolution of the carnosine content in Spanish and Italian dry-fermented sausage processing. Adapted from Cassaburi, A., Aristoy, M. C., Cavella, S., Di Monaco, R., Ercolini, D., Toldrá, F. and Villani, F. (2007). Biochemical and sensory characteristics of traditional fermented sausages of Vallo di Diano (Southern Italy) as affected by the use of starter cultures. *Meat Science* **76**, 295–307; Durá, M. A., Flores, M. and Toldrá, F. (2004). Effect of Debaryomyces spp. on proteolysis of dry-fermented sausages. *Meat Science* **68**, 319–328.

Resistance to Digestive Peptidases

The resistance of these peptides to peptidase digestion has been assessed by analyzing them in plasma and urine after ingesting beef, breast chicken, and breast chicken broth. Anserine increased in plasma after chicken or chicken broth ingestion, while the presence of carnosine in plasma depended on the carnosinase, present in the small intestine and in the human plasma, which acts preferably on carnosine. The cleavage of carnosine can be detected in urine by analyzing its constituent amino acids. In any case, these findings confirm the intact absorption of both peptides.

Analysis of Histidine-Containing Dipeptides

The analysis of these dipeptides is relatively easy. They are soluble molecules and thus can be easily extracted from the

Table 2 Carnosine and anserine contents (expressed as mg/100 g d.w.) in different European dry-cured hams

	Ibérico[1]	*Serrano*[1]	*Corsican*[2]	*Bayonne*[2]	*Italian country style*[3]	*Parma*[3]
Carnosine	881.0	941.6	1043.8	1248.2	1141.5	1080.2
Anserine	125.0	77.9	112.7	89.7	81.5	95.7

[1]Spain.
[2]France.
[3]Italy.

Source: Virgili, R., Parolari, G., Bordini, C. S., Schivazappa, C., Cornet, M. and Monin, G. (1999). Free amino acids and dipeptides in dry-cured ham. *Journal of Muscle Foods* **10**, 119–130.

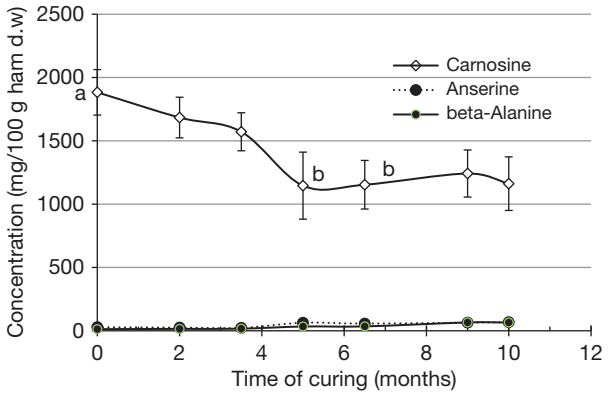

Figure 4 Evolution of carnosine, anserine, and β-alanine contents during a traditional dry-curing ham processing. Adapted from Marušić, N., Aristoy, M. C. and Toldrá, F. (2013). Nutritional pork meat compounds as affected by ham dry-curing. *Meat Science* **93**, 53–60.

matrix with either water or aqueous solvents. Afterward, proteins from the matrix should be eliminated by adding strong acids (perchloric or trichloroacetic acids) or organic solvents (acetonitrile, methanol, etc.) and, after centrifugation to eliminate the precipitated protein, sample is ready for separation by chromatography or micellar capillary electrophoresis.

Chromatography is the most used technique to analyze the histidine-containing dipeptides. These dipeptides are polar and ionic at physiological pH, and thus, they can be separated based on their charge by cation exchange chromatography (CEC) or by hydrophilic interaction chromatography (HILIC). Another interesting alternative is to increase the hydrophobicity of the histidine-containing dipeptides by making them react (derivatization) with a hydrophobic molecule (o-phthalaldehyde, OPA; phenyl isothiocyanate, PITC; dansyl chloride; or 2,4-dinitrofluorobenzene, DNFB, are the most used) that should also confer ultraviolet or fluorescent-absorbing properties. Derivatized dipeptides will then be separated by reversed-phase chromatography (RP-HPLC), which is a more efficient chromatography technique.

Several methods to detect the separated peptides are used. The UV absorption of the peptidic bond at 214 nm is used when no derivatization has been performed, which is the case of CEC or HILIC separations. Nevertheless, when derivatizing the molecule with a chromophore like PITC, OPA, dansyl chloride, or DNFB, more sensible and selective detection at higher UV wavelength or by fluorescence can be used. The derivatization can be achieved before separation by RP-HPLC method or after CEC or HILIC separations by means of a postcolumn reaction. This last method requires the derivatization reaction to be very rapid in order to avoid any spoiled separation. In this sense, OPA would be a very good choice. The use of mass spectrometry as a detection method to analyze histidine-containing dipeptides has become more popular in recent years. This detection does not need any previous derivatization and works very well after HILIC separations because of the good compatibility of both techniques with regard to the solvents used as mobile phases. When using the dansyl chloride derivatization and RP-HPLC coupled to single quadrupole mass spectrometry for the detection, very complex samples of nitrogen-based compounds (amino acids, nucleosides, carnosine, etc.) can be analyzed. Nevertheless, the high cost of this technique may not always be justified for the exclusive analysis of histidine-containing dipeptides because they are found in high concentration in foods.

See also: Bioavailability of Nutrients; Ham: Dry-cured Ham; Pork Meat Quality, Production and Processing on; Proteins: Chemistry, Characterization, and Quality.

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HIV Disease and Nutrition

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Introduction

Human immunodeficiency virus (HIV) infection is a worldwide problem of great magnitude. The World Health Organization (WHO) estimates that about 35 million adults and children were living with HIV in 2013, the majority of whom are in developing nations. In 2013, HIV and its related complications were the cause of death in 1.5 million people worldwide. However, the number of individuals infected with and dying from HIV has been steadily declining in the last 15 years. This trend reflects the expansion of services to prevent the transmission of HIV from mother to child, the wider use of antiretroviral therapy (ART), and the improved prophylactic regimens worldwide. In developed nations, HIV has become a chronic illness with highly active antiretroviral therapy or now what is currently called combination antiretroviral therapy (cART) as the mainstay of treatment. With more individuals living with HIV, the necessity of appropriate supportive care is paramount.

Clinicians who care for those with HIV should be aware of potential nutritional and metabolic problems and their consequences. In the past, and currently in some subpopulations, wasting syndrome is the primary nutritional concern for HIV-infected individuals. Malnutrition can have additive destructive effects on the immune system and host defense mechanisms. T cell-mediated immune responses are targeted primarily and have been termed the nutritionally acquired immune deficiency syndrome. It has been long thought that aggressive nutritional rehabilitation can combat and potentially reverse these effects. However, as we approach 20 years since the advent of cART, expanding cardiometabolic and related nutritional issues often prevails that can also alter immune responses and produce additional comorbidities. Knowledge and implementation of effective nutritional therapies are important to improve medical outcomes and quality of life. The problem of malnutrition in patients with the acquired immunodeficiency syndrome (AIDS) is multifactorial, and proper nutritional support will replenish body cell mass in these patients, which is associated with functional improvement. With appropriate combinations of ART, nutritional and other supportive care, many HIV-infected individuals are now able to lead relatively normal lives.

HIV and Gastrointestinal Tract: Nutrient Absorption

Gastrointestinal malabsorption can lead to malnutrition and a dysfunctional gastrointestinal tract can produce clinical symptoms that contribute to both morbidity and mortality in those with HIV-1 infection. The etiology of gastrointestinal malabsorption is multifactorial and includes gastrointestinal mucosal abnormalities, which in turn can lead to macro- and micronutrient malabsorption. These mucosal changes can be due to local HIV infection of the gut or secondary enteric infections. Villous atrophy and gastrointestinal tract dysfunction are coincident with high levels of HIV-1 viral load in the gut. There is evidence that certain gastrointestinal epithelial cells bind and selectively transfer HIV from the cells' apical to basolateral surface, where viral translocation across the epithelium encounters lamina propria macrophages and T cells.

The gastrointestinal tract has substantial CD4 T-cell depletion in all stages of HIV disease that is discordant with systemic CD4 T-cell repletion with effective ART. This immune derangement may contribute to altered epithelial permeability thereby permitting translocation of microbes and generalized immune activation leading to localized cytokine production and further replication of HIV. High systemic levels of lipopolysaccharides (LPS) as a result of bacterial translocation are associated with marked systemic immune activation that increases metabolic rate and sustains HIV infection. ART decreases the levels of LPS and promotes CD4 T-cell reconstitution and may subsequently decrease the systemic immune activation, although not completely.

As a result of either local HIV infection with associated bacterial translocation (which induces proinflammatory changes in the gastrointestinal mucosa and systemically) or the injury caused by a secondary infection (this is most typical when CD4 T cells drop below 200 cells per mm^3), mucosal function can be compromised resulting in gastrointestinal malabsorption, diarrhea, and malnutrition that may eventually contribute to excess morbidity and mortality, especially in developing nations.

Body Composition and Energy Expenditure

The interest in body composition of HIV-infected individuals grew as early as the onset of the HIV epidemic. The importance stems from the physiological and metabolic changes that have been attributed to HIV. HIV was initially described as a wasting disease, and since 1987, the wasting syndrome is considered a case definition of AIDS. The Centers for Disease Control and Prevention defines AIDS wasting syndrome as an involuntary weight loss of greater than 10% baseline body weight during the previous 12 months or a 5% loss of weight during the previous 6 months. However, establishing a single definition of wasting has been difficult for individuals of all ages. Even in the era of cART, wasting continues to be prevalent. Some have used the term cachexia as a more relevant description of body composition changes in HIV. Wasting is associated with weight loss while cachexia is related to loss of lean body mass. Some studies found the prevalence of wasting in patients who are cART-naive to be 48.4%.

The use of cART has become a standard practice in developed and, now with increasing frequency, developing nations and is the first line of care for many with HIV infection.

However, cART has been linked to body composition changes. Lipodystrophy, the redistribution of body fat characterized by the combination of lipoatrophy (loss of fat tissue) in the extremities and lipohypertrophy (excess accumulation of fat, usually in the trunk, dorsocervical region, and breasts), is associated with cART and in many situations specifically with protease inhibitor (PI) use. Fat can also accumulate in the abdominal viscera (usually the liver and mesentery) and heart. The prevalence of lipodystrophy is reported to occur in sometimes over 50% of cohorts with over 5 years of ARV exposure. However, with the advent of newer classes of antiretrovirals, fat redistribution for many individuals with HIV is less prevalent.

Mortality, accelerated disease progression, and impairment of functional status have all been associated with wasting. In one study, every 1% weight loss resulted in an 11% increase in the risk of death. Additionally, when weight loss exceeded 10% from baseline, there was a sixfold increase in relative risk for mortality. This highlights the importance of preventative measures needed to obviate the potential effects of wasting in HIV. Recent studies have assessed the effects of nutritional supplementation, physical activity (specifically resistance training), and the use of pharmacological methods on wasting and weight loss.

Chronic viral infections can influence energy utilization and potentially alter nutritional status. These infections can increase or shunt the effective use of energy substrates from normal, healthy anabolic patterns to energy pathways associated with inflammatory processes. The chronic viral activity of HIV is likely no different. Small studies of energy expenditure have shown few differences in resting energy expenditure (REE) or total energy expenditure (TEE) between HIV-infected children with growth failure and those with normal rates of growth. However, adults with HIV-1 infection show REE increases with severity of illness, especially with secondary infection and more advanced HIV disease, yet TEE is similar, suggesting a compensatory response with reduced physical activity. As HIV-infected individuals are treated with cART, previously noted increases in protein catabolism decrease to more normal rates, suggesting that chronic viral activity leads to increased protein catabolism and can be brought to more normal levels with successful viral suppression.

Macronutrients

Although the WHO has played a prominent role in efforts to fully integrate food and nutrition in all aspects of prevention, care, and treatment of HIV and AIDS, nutrition-specific recommendations for this patient group are limited. Knowledge about the nutritional needs of people infected with HIV over and above those required by uninfected people remains limited. This section discusses existing macronutrients recommendations for people living with HIV.

Patient groups vary in terms of their response to the virus, stage of the disease, susceptibility and exposure to opportunistic infections, nutritional status, and individual response to the various treatments received. Although compelling evidence exists for the direct association between BMI < 18 kg m^{-2} and accelerated disease progression, limited data exist regarding the role of macro- and micronutrients in disease progression.

Existing energy and macronutrient recommendations for this population based on HIV disease progression are summarized in Table 1. In addition to nutrient-specific recommendations, the nutritional interventions for the amelioration of growth and weight gain and for the prevention or management of metabolic complications of HIV disease for all age groups are discussed in the subsequent sections.

The energy and protein needs of HIV-infected individuals depend on their age, nutritional status, degree of gastrointestinal dysfunction, and the long-term effects of HIV disease such as acute/chronic infection or other complications. Some nutrient-specific recommendations for people living with HIV have been established for all age groups and pregnant and lactating women.

Current knowledge with regard to energy supports the existing WHO recommendations (i.e., calories increased by 10% for asymptomatic children, adolescents, and adults). The additional energy helps children to maintain normal growth, development, activity, and bodily functions. In terms of specific recommendations for HIV-infected adolescents, overall dietary goals are consistent with those in uninfected adolescents and include meeting the needs of normal growth. Most adults maintain body weight through a reduction in physical activity and/or an increase in caloric intake, so energy intake above the 110% of that recommended for uninfected population has not been advised. In those with chronic malnutrition, caloric needs will be greater, depending on the degree of malnutrition and other factors such as demands for physical activity.

In resource-rich settings, asymptomatic HIV infection is unlikely to significantly affect the energy requirements of pregnancy and lactation. Recommendations with regard to energy intake for pregnant women should be population-based because of differences in body size. To achieve optimal pregnancy outcomes, energy needs for women with healthy baseline body weight are no higher than the Estimated Energy Requirement for nonpregnant women until the second trimester; thereafter, the extra energy need per day is 340 and 452 kcal in the second and third trimesters, respectively. The 10% increase in REE currently recommended (above those of nonpregnant adult females) is likely reasonable for asymptomatic HIV-positive pregnant and lactating women. For pregnant and lactating women in resource-limited settings, some recommendations have been established, and they vary according to condition (pregnant and breastfeeding), HIV stage (stratified by CD4 T-cell count), age (14–20, >20–30, and >30–50 years), weight or BMI, food security score, micronutrient status including hematologic status (presence of anemia), and treatment status (ART or other drugs).

For all age groups, macronutrient (protein, carbohydrate, and fat) should be consumed at recommended amounts, with additional adjustments made that are consistent with the energy needs (i.e., if calories are increased by 10%, the macronutrient content should be increased proportionately). Although some studies in adults have suggested that protein requirements may be higher with HIV infection, as yet there is no consistent evidence that increasing protein intake above the required amounts for uninfected populations is beneficial.

During periods of acute/chronic infection, during weight loss, or when metabolic complications become more evident consequent to prolonged ART exposure, energy and nutrient

Table 1 Energy and macronutrients needs in HIV infection

Nutrient	*HIV infection factors* *Clinical situations*	*Nutrition recommendations*
Energy	When secondary infections are absent and growth is adequate in children and weight is stable in adults	*Children, adolescents, and adults*: target energy intake of 110% of that recommended for uninfected population (based on actual weight rather than on expected weight for age) (WHO 2009) *Pregnant and lactating women*: the 10% increase in REE currently recommended is likely reasonable for asymptomatic HIV-positive pregnant and lactating women. Energy requirements may be higher for pregnant adolescents (Raiten et al., 2011) Standard methods of assessing energy needs – calorie levels based on the Estimated Energy Requirements (EERs) and activity levels from the Institute of Medicine Dietary Reference Intakes (DRIs) (Macronutrients Report, 2002)
	During illness, decreased appetite, poor growth, weight loss	*Children*: energy requirements can increase by up to 20–30% during infections and recovery (WHO, 2009). Equations for estimating catchup growth requirements for both calories and protein can be used *Adults*: increase energy intake by 600–960 kcal day^{-1} in adults with weight loss may increase body weight (Grobler et al., 2013), which is better achieved by providing a balanced nutritional supplement *Pregnancy and lactation*: the energy needs may be even greater than 10% above those of nonpregnant adult females for women with inadequate rate of weight gain during pregnancy, excessive weight loss postpartum, or other ongoing health issues (Raiten et al., 2011)
	Advanced disease	Up to 50–100% additional energy to recover and regain weight (WHO 2003) For critically ill patients: energy requirements may be calculated by predictive equations or measured by indirect calorimetry (Singer, 2009), with special attention to the prevention of complications related to under- or overfeeding (Fraipont and Preiser, 2013) This is best achieved through enteral or parenteral (if enteral has failed) feeding
	Overweight or obesity	*Weight management*: Counseling on changing eating habits and patterns Regular exercise: physical activity counseling or physical activity program participation
Protein	During periods of well-being	As in asymptomatic children, protein should contribute about 10–15% of the energy intake and there is no need for additional protein other than what is present in a balanced diet (WHO, 2009) Acceptable Macronutrient Distribution Ranges (AMDRs) can be used to ensure sufficient intakes of essential nutrients (Food and Nutrition Board, 2005)
	Period of catchup growth	Use equation for estimating catchup growth requirements
Fat	During periods of well-being	Provide anticipatory guidance regarding the importance of a low-fat, low-saturated-fat, low-cholesterol diet for all otherwise healthy HIV-infected patients older than 2 years and their families due to increased risk of CVD in HIV-infected populations AMDR: Food and Nutrition Board (2005) 1–3 years: 30–40% of total calories 4–18 years: 25–35% of total calories *Pregnancy and lactation*: essential fatty acid needs are 30% higher than those of nonpregnant women, and adequate fatty acid intake should be ensured in a manner consistent with recommendations for uninfected women (Raiten et al., 2011)
	Fat malabsorption (due to HIV GI enteropathy or secondary infections)	In these cases, dietary modification of fat may be required Supplemental MCT and enteral formulas containing MCT can be used to supplement caloric intake
	Dyslipidemia	Dietary intervention with NCEP Therapeutic Lifestyle Changes (NCEP 2002) and exercise have been shown to reduce triglycerides in both HIV-infected (Stradling et al., 2012) and HIV-uninfected (Kelley et al., 2011) populations and to reduce LDL cholesterol in the general population (Yu-Poth et al., 1999) Counseling on changing eating habits and patterns per AHA/AAP
Carbohydrate	During periods of well-being	AMDR: Food and Nutrition Board (2005), 45–65% of total calories Added sugars should comprise no more than 10% of total calories consumed
Fiber		A reasonable intake is 0.5 g kg^{-1} day^{-1} to a maximum of 35 g daily

BMR, basal metabolic rate; EER, Estimated Energy Requirement; FTT, failure to thrive; DRI, dietary reference intake.
Acceptable Macronutrient Distribution Range (AMDR) is the range of intake for a particular energy source that is associated with reduced risk of chronic disease while providing intakes of essential nutrients.

needs are greater than that recommended for uninfected population. As for uninfected individuals, the estimation of energy requirements for critically ill patients may be calculated by predictive equations or measured by indirect calorimetry, with special attention to the increased risk of overfeeding during the early phase of acute illness and the increased risk of underfeeding during the late phase. Once the secondary infections are successfully treated and energy intake is increased sufficiently, patients are able to regain weight and remain weight-stable.

Micronutrients

In addition to macronutrient intake, careful attention to micronutrient status (vitamins and minerals) is a critical component in the nutritional treatment and care of people living with HIV infection. The WHO has long taken the lead in the establishment and dissemination of specific requirements for micronutrient consumption among people living with HIV throughout all stages of the life cycle. The importance of the role specific micronutrients serve in the maintenance of immune system function and in the reduction of morbidity and mortality from disease- and treatment-related symptoms has been well established. However, a general consensus of the long-term effects, optimal composition, and dosing of both single and multiple micronutrient (MMN) supplements has yet to be reached. This section provides an overview of current micronutrient guidelines for people living with HIV. Table 2 provides a summary of micronutrient guidelines for HIV-infected populations throughout the life cycle.

Table 2 Micronutrient recommendations in HIV infection

Population	*Current micronutrient recommendations*	*Comments*
Children and adolescents	*Maintain current WHO recommendations*: ● Ensure one Recommended Dietary Allowance (RDA) for all essential nutrients, preferably through a well-balanced diet ● When dietary intakes are insufficient, the use of micronutrient supplements at RDA levels is supported *Vitamin D intake*: ● Children on antiretroviral treatment (ART) should be given two to three times more vitamin D than the RDA for their age group	– Currently, there exists insufficient evidence to support a general policy encouraging the use of daily oral nutrient supplements – Careful attention to dietary quality and adequacy of micronutrients is critical; important factors to consider include high food insecurity or poor dietary diversity – Additional attention may be justified for specific nutrients (e.g., vitamin A, vitamin D, calcium, and iron) either in terms of dietary intake and poor exposure or in the context of potential treatment interactions
Adults	● Insufficient evidence to support a change from 2003 recommendations of one RDA for essential micronutrients, preferably from dietary intake ● Adults on ART should be given two to three times more vitamin D than the RDA for their age group	– Given the vast diversity of variables and study design throughout various studies investigating micronutrient supplementation in this population, it is not possible to recommend anything other than the current RDA or a routine multivitamin. However, there still remain safety concerns for potential harm from higher doses of selected micronutrients (e.g., vitamin A and multiple micronutrient (MMN) supplements, which may promote increased viral replication and increased shedding of virus in genital secretions, thus potentially increasing risk of transmission of HIV). Further research into optimal composition and dosing is warranted
Pregnant and lactating women	● Supplementation of one RDA amount of micronutrients for all HIV-positive pregnant and lactating women is recommended due to the high likelihood of food insecurity, the fact that micronutrient needs are higher during pregnancy and lactation and that deficiencies appear to be more common in HIV-infected women ● Supplements should contain the 14 core micronutrients provided in the UNICEF UNIMMAP (UN international multiple micronutrient preparation) supplement. These micronutrients include vitamins A, D, and E; thiamine; riboflavin; niacin; folic acid; vitamins B_6, B_{12}, and C; and minerals including zinc, iron, copper, selenium, and iodine ● In areas of endemic vitamin A deficiency, WHO recommends that a single high dose of vitamin A (200 000 IU) be given to women as soon as possible after delivery, but no later than 6 weeks after delivery ● Pregnant and lactating women require at least 600 IU day^{-1} of vitamin D; at least 1500–2000 IU day^{-1} of vitamin D may be needed to prevent/treat deficiency	– Supplementation at amounts above the RDA is not advised at this time as some studies have shown that they may in fact be harmful (e.g., vitamin A, vitamin D (25), and iron) – Iron supplementation is recommended at the RDA amount for HIV-positive pregnant women – Iron supplementation is not recommended in lactating HIV-positive women unless the presence of iron deficiency is clinically confirmed

Source: Raiten, D. J., Mulligan, K., Papathakis, P. and Wanke, C. (2011). Executive summary – nutritional care of HIV-infected adolescents and adults, including pregnant and lactating women: what do we know, what can we do, and where do we go from here? *The American Journal of Clinical Nutrition* **94**(6): 1667S–1676S. http://dx.doi.org/10.3945/ajcn.111.019711; Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., et al. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* **96**(7): 1911–1930. http://dx.doi.org/10.1210/jc.2011-0385; Irlam, J. H., Siegfried, N., Visser, M. E. and Rollins, N. C. (2013). Micronutrient supplementation for children with HIV infection. *Cochrane Database of Systematic Reviews* **10**: CD010666. http://dx.doi.org/10.1002/14651858.CD010666; Irlam, J. H., Visser, M. M., Rollins, N. N. and Siegfried, N. (2010). Micronutrient supplementation in children and adults with HIV infection. *Cochrane Database of Systematic Reviews* (12): CD003650. http://dx.doi.org/10.1002/14651858.CD003650.pub3; Roberfroid, D., Huybregts, L., Lanou, H., et al. (2012). Impact of prenatal multiple micronutrients on survival and growth during infancy: a randomized controlled trial. *American Journal of Clinical Nutrition* **95**(4): 916–924. http://dx.doi.org/10.3945/ajcn.111.029033.

Micronutrient deficiencies are often observed in conjunction with a wide range of impaired immune responses and clinical manifestations including increased susceptibility to infection, inadequate intake, malabsorption, diarrhea, and subsequently increased morbidity and mortality among various groups of people living with HIV. Although malnutrition likely plays a role in disease outcomes, current data regarding the role of micronutrients in disease progression remain limited. In conjunction with cART, inexpensive micronutrient supplements could provide several cellular and clinical benefits, ranging from the prevention of mitochondrial toxicity and oxidative stress to facilitation of immune reconstitution. Per the established WHO guidelines, HIV-infected children and adults should consume diets that provide micronutrient intakes at the Recommended Dietary Allowance (RDA). These recommendations state that in cases where dietary intakes are insufficient, the use of micronutrient supplements at the RDA in HIV-infected individuals of all ages is supported.

Low levels of vitamins, minerals, and other micronutrients are often observed among HIV-infected children. This may frequently be the result of inadequate nutritional intake from their diet or their bodies may demand increased micronutrient levels for the purpose of fighting the HIV infection itself or opportunistic infections. Vitamin A supplements, as in children without HIV infection, reduce diarrheal morbidity and mortality especially in young children. As stated in the 2003 WHO guidelines, HIV-infected 6–59-month-old children living in resource-limited settings should be given periodic (every 4–6 months) vitamin A supplements (100 000 IU for infants 6–12 months and 200 000 IU for children >12 months) in order to reduce all-cause mortality and diarrhea morbidity. This level is consistent with WHO recommendations for the prevention of vitamin A deficiency in children. Furthermore, studies have shown that vitamin A supplements are beneficial and safe and can half mortality overall in an analysis of three trials in different African countries.

Micronutrient supplements in adults are also shown to reduce the metabolic and cellular complications of cART. A greater intake of vitamin E and vitamin A in those on cART was associated with fewer metabolic complications, dyslipidemia, and insulin resistance. In addition, a daily supplement of vitamins C and E for 3 months reduced oxidative stress baseline CD4 counts <100 cell per μl.

Zinc is another important nutritional factor for HIV-infected individuals. Zinc supplements have been shown to help HIV-infected children recover from diarrheal illnesses, are safe, and appear to have similar benefits on diarrheal morbidity in children with HIV as in children without HIV infection. Long-term (18-month) zinc supplementation potentially reduces the likelihood of opportunistic infection in adults and children and improves CD4 counts in adults.

There has been considerable growth in clinical interest and research with respect to vitamin D deficiency and its effects on the progression of HIV infection. With mounting evidence that vitamin D deficiency can exacerbate immune dysfunction, contribute to skeletal fractures, and increase cardiovascular disease (CVD) and metabolic risk, there has been a great focus on how this nutrient contributes to disease progression in HIV. A number of clinical studies among populations in both developed and developing nations have associated vitamin D deficiency with a variety of poor health outcomes such as atherosclerotic CVD, increased viral load, and immune suppression. Up to a third of perinatally HIV-infected children can be vitamin D-deficient. As a result of the current data, the National Endocrine Society recognizes that ART increases the catabolism of 25-hydroxy vitamin D and 1,25-dihydroxy vitamin D levels, therefore recommending that children on ART be given two to three times more vitamin D for their age group.

As recommended for children and adolescents, HIV-infected adults should also consume well-balanced diets in order to reach micronutrient intakes of one RDA for essential micronutrients. In the event that RDA amounts of micronutrient intakes cannot be reached through dietary sources, evidence from current studies on this topic taken collectively indicates that there is a role for multivitamin supplements at the level of the RDA in most HIV populations, adults included. Furthermore, for adults on ART, it is recommended they be given two to three times more vitamin D than the RDA for their age group to treat deficiency and potentially mitigate the effects of ART on calcium balance and bone metabolism.

A well-balanced diet certainly remains the gold standard for micronutrient intake for all HIV-infected populations. However, the high prevalence of food insecurity, the increased metabolic and micronutrient demands of pregnancy and lactation, and the observation that deficiencies appear to be more common in HIV-infected women necessitate micronutrient supplementation at one RDA for all HIV-positive pregnant and lactating women. In general, supplements should provide the 14 core micronutrients that comprise the UNICEF UNIMMAP (UN international multiple micronutrient preparation) supplement: vitamin A, vitamin D, vitamin E, thiamine, riboflavin, niacin, folic acid, vitamin B_6, vitamin B_{12}, vitamin C, zinc, iron, copper, selenium, and iodine.

Since nutrient deficits are often observed during pregnancy and lactation and specifically among HIV-infected pregnant women, current guidelines continue to address specific instances of common vitamin and mineral deficiency states. In the case of anemia prevention, the WHO recommends daily iron–folate supplementation (400 μg of folate and 60 mg of iron) during 6 months of pregnancy and, in the case of clinically confirmed severe anemia, twice-daily supplements. Hence, these levels of iron and folate are incorporated into the UNICEF UNIMMAP supplement. In accordance with the 2003 WHO guidelines, vitamin A intake by HIV-infected women during pregnancy and lactation should not exceed the RDA levels as it may be harmful in some cases; in areas of endemic vitamin A deficiency, a single high dose of vitamin A should be given to women as soon as possible after delivery, but no later than 6 weeks after delivery. As for vitamin D, the National Endocrine Society suggests that pregnant and lactating women require at least 600 IU day^{-1} of vitamin D; at least 1500–2000 IU day^{-1} of vitamin D may be needed to prevent/treat deficiency. When taken collectively, available evidence suggests that the approach to caring for HIV-infected women is the same as that for uninfected women with special attention to HIV-specific factors such as viral load, CD4 T-cell count, and ART status.

Although there have been some advances in the study of MMN and positive clinical outcomes in HIV, the impact of specific micronutrient supplements as determined through

randomized, controlled trials remains few and less clear. Additional research is warranted to determine the long-term clinical advantages, potential adverse effects, and optimal composition of micronutrient supplementation among more diverse groups of HIV-positive research participants.

Cardiometabolic Complications

Obesity and Cardiometabolic Disease

Obesity is an emerging health problem among adolescents and adults living with HIV/AIDS. With the institution of cART, the immune and disease status of HIV-infected individuals has improved and eating patterns (healthy or not) often parallel those in healthy uninfected individuals. Poverty and food insecurity are among other factors that can contribute to childhood obesity that influence both HIV-infected and HIV-uninfected populations equally. Immigrant families are particularly vulnerable. As the prevalence of obesity increases among patients with HIV, the cumulative risk for CVD also increases.

Fat Redistribution

Coincident with the introduction of cART, a clinical syndrome of body fat redistribution and metabolic changes was first described initially in adults, but is now commonly reported among children. HIV-infected patients receiving cART regimens have developed a syndrome of peripheral insulin resistance, hyperlipidemia, and lipodystrophy (truncal obesity, dorsocervical fat pad, and extremity and facial wasting). Clinical and biochemical abnormalities associated with the fat redistribution syndrome or commonly termed, lipodystrophy syndrome, are shown in Table 3. Risk factors associated with developing the fat redistribution syndrome in adults include female gender, increasing age, family history of premature CVD, higher pretherapy body weight, and behavioral patterns such as tobacco and alcohol use. Complications associated with the fat redistribution syndrome include higher rates of diabetes mellitus and premature CVD. Lipodystrophy is almost exclusive to patients receiving ART and can improve after PI and NRTI switching. Medical compliance with drug therapy may be poorer owing to the cosmetic side effects of therapy.

Table 3 Clinical and biochemical abnormalities of the fat redistribution syndrome

Clinical features	*Laboratory features*
Increased abdominal (visceral) fat	Hyperlipidemia
Increased waist-to-hip ratio (more reliable in adults)	Increased triglycerides Increased total cholesterol
Buffalo hump	Increased LDL-C
Fat atrophy	Decreased HDL-C
Wasting of extremities	Insulin resistance
Wasting of buttocks	Normal to increased serum glucose
Loss or thinning of facial fat, prominence of nasolabial fold	Increased insulin Increased C-peptide
No change to increased weight	
Fatigue and weakness	

Lipodystrophy is described in children, as well. Early studies showed a beneficial nutritional effect of cART in both adults and children. Early initiation of cART is associated with more rapid restoration of growth in children of developing nations. Growth hormone levels are significantly lower in lipodystrophic HIV-infected adults with visceral adiposity. In addition to the positive improvement in growth and lean body mass, however, cART is also associated with abnormalities in fat distribution though some studies report similar lean mass in HIV-infected and HIV-uninfected individuals. Both peripheral lipoatrophy and central obesity occur in both HIV-infected adults and children. Fat redistribution can develop fairly rapidly after initiating a cART regimen and changes can progress over time. Metabolic abnormalities are associated with specific classes of drugs with many NRTIs and PIs implicated. Newer agents often have less impact on fat redistribution and are the preferred therapy if the virus is sensitive to the therapy.

Cardiometabolic Risk

Atherosclerotic CVD is a leading comorbidity and cause of mortality among HIV-infected adults. Several studies show that HIV-infected children, compared to healthy peers, have higher rates of CVD risk factors, including dyslipidemia, insulin resistance, obesity, and central adiposity. HIV infection also results in prolonged chronic inflammation, thereby increasing CVD risk. In adults with cART-related fat redistribution, several studies have suggested an increase in the risk of myocardial infarction relating to the level of viral control (increased inflammation) or to ARV exposures (including PIs and certain NRTIs). Exogenous obesity, common among all individuals with HIV, can also contribute to CVD risk. Children, compared to adults with HIV, may be at even greater risk of developing premature CVD as the lifetime exposures to factors associated with CVD risk are longer in children.

Dyslipidemia

Prior to the introduction of any ART, elevated triglycerides and LDL cholesterol and lower HDL cholesterol were reported and associated with HIV infection. This profile persists in the current era and suggests that chronic immune activation can influence lipid metabolism. Proinflammatory cytokines (as a response to the chronic viral infection) may affect lipid pathways. After PI therapy became widespread, several investigators in the United States and abroad reported a 20–50% rise in lipid levels of HIV-infected individuals. In hepatocyte cell lines, PIs have been shown to interfere with cholesterol synthesis by the inhibition of the proteasome, a potent regulator of apolipoprotein B production, among other mechanisms. Other factors associated with dyslipidemia include successful viral suppression, better CD4 T-cell counts, and demographic factors. The impact of PIs on lipid levels, independent of HIV, is effectively described in HIV-seronegative volunteers who developed dyslipidemia following PI treatment.

Insulin resistance and type 2 diabetes mellitus

Abnormal glucose homeostasis was documented in HIV-infected adults with lipodystrophy well before it was reported in children. The etiology of insulin resistance is multifactorial and has been linked with PI, NRTI, or nonnucleoside reverse

transcriptase inhibitor (NNRTI) use both singly and in combination, although exact mechanisms have not been well defined. HIV-uninfected adults treated with indinavir (a PI) showed a rapid onset of insulin resistance but not hyperlipidemia or body shape changes. Leptin, a hormone secreted by adipocytes, is low in congenital and HIV lipoatrophies. These patients also tend to have increased intramyocellular lipid in direct correlation to their degree of insulin resistance. A possible mechanism by which cART causes insulin resistance is by direct inhibition of the transport function of glucose transporter type 4 (GLUT4), which is responsible for insulin-stimulated glucose uptake into the muscle and fat. Other potential causes of insulin resistance include NRTI or NNRTI therapy associated with mitochondrial DNA (mtDNA) mutations or depletions. Although there are limited data in adults, there are striking similarities between rare mitochondrial disorders, such as multiple symmetric lipomatosis (MSL), a condition that is phenotypically very similar to cART-associated lipodystrophy. These mtDNA mutations in MSL lead to an impaired function of cytochrome c oxidase and a subsequent decrease in fat turnover. Inflammatory cytokines have been linked to both insulin resistance and diminished adiponectin, which affects insulin signaling and glucose homeostasis. The adipose tissue is a major determinant of insulin sensitivity and changes associated with lipodystrophy can alter the secretion of adiponectin. In one study, patients with PI-induced lipodystrophy had increased levels of circulating inflammatory cytokines.

Vascular inflammation and atherosclerosis

Vascular endothelial dysfunction, a process that is associated with atherosclerosis, may occur as a result of chronic inflammation and injury in the endothelium. Oxidative stress due to cART and direct cytopathic effect of the virus are likely causative agents in HIV. In addition, dyslipidemia, insulin resistance, and chronic inflammation due to ongoing immune activation in HIV-infected individuals also contribute to endothelial damage. The levels of proinflammatory cytokines are higher in HIV-infected individuals compared to contemporary controls. These vascular inflammatory biomarkers are positively correlated with clinical markers including higher viral load, lipids, and waist circumference. Furthermore, Wolf found that not only were levels of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and von Willebrand factor higher in untreated patients with HIV infection, but also they decreased significantly after treatment with a regimen containing either one PI or one NNRTI.

Preatherosclerotic lesions can be defined by imaging studies of the arterial structures. Common ways of evaluating CVD risk are through measurements of the carotid intima, brachial artery reactivity, or pulse wave velocity. Both adults and children with HIV have evidence of increased CVD risk when their measures are compared with controls.

Nutritional Interventions

People living with HIV are faced with many obstacles that challenge their ability to sustain proper nutritional status. Decreased energy intake, increased energy expenditure, and accelerated protein turnover are common in people with HIV. Promoting and supporting adequate nutrition and food security are critical for this population. Presently, there are a number of different strategies to improve nutritional outcomes for people living with HIV. Regardless, nutritional interventions in HIV should be designed under the premise that (1) ARVs are essential to prolong life and to halt the spread of HIV and (2) food and sound nutrition are essential to human health. Table 4 shows the gastrointestinal and nutritional side effects of current ARVs.

Overall dietary goals for HIV-infected individuals are consistent with those for non-HIV-infected and include meeting the needs of normal growth of children and adolescents, meeting the needs of adequate weight gain of pregnant women, consumption of a high-quality, nutrient-dense diet, building good eating habits, avoiding obesity through limiting intake of energy-dense but nutritionally poor foods, engaging in physical activity, and avoiding cigarettes, alcohol, and drugs. The diet plan should be individualized based on the patient's nutritional status, age, symptoms, and ability to meet nutrient requirements. Regular nutritional assessments are necessary in order to determine the appropriateness and effectiveness of the interventions. This assessment should include a review of the medical and dietary history, nutrient analysis of usual intake, anthropometric measurements, body composition, and measurement of biochemical values. In order to guarantee optimal nutrition care, the team should be multidisciplinary and be composed of a physician, nurse specialist, social worker, and dietician.

Consumption of a well-balanced diet remains the optimal choice for achieving adequate nutritional status for all HIV-infected individuals regardless of age for overall health and wellness, as well as growth and development in pediatric and pregnant populations. However, the increased metabolic demands of HIV infection and symptomatic HIV often present significant challenges for achieving or maintaining healthy body weight and immune system function. In circumstances where malnutrition and food insecurity are present, supplemental food sources and/or enteral nutrition supplements may be necessary to achieve positive health outcomes and are often effective when given in the appropriate amounts. Some data suggest that body weight and lean body mass tend to increase with nutritional support. Food-based interventions can treat malnourished HIV-infected patients and are necessary in order to address both the individual needs of the patients and the food security of their households. Ready-to-use therapeutic foods effectively treat malnutrition of HIV-infected individuals in developing nations.

Incentives provided through food supplements can be a helpful method to improve clinical adherence. There is limited evidence regarding the use of balanced nutritional supplements (consisting of 50–60% carbohydrate, 15–30% protein, and 20–30% fat) for improving energy intake and protein among HIV-infected adults with weight loss, but no effects on anthropometric or immunologic parameters. It is likely that decreased energy intake, not changes in energy expenditure, plays a major role in the weight loss experienced by those with HIV and secondary infection. Therefore, with appropriate treatment and nutritional resources, most patients with HIV-related weight loss will gradually increase ad libitum caloric intake and gain weight.

Table 4 HIV-related medications and common gastrointestinal and metabolic side effects

Medication	*Action*	*Side effects*
Abacavir	NRTI	Nausea, vomiting, diarrhea, abdominal pain, pancreatitis, abnormal liver function
Aciclovir	Antiviral	Nausea, abdominal pain, diarrhea, abnormal liver function
Atazanavir	PI	Nausea, diarrhea, abdominal pain, hyperbilirubinemia
Atripla (tenofovir, emtricitabine, and efavirenz)	Combination	Nausea, vomiting, diarrhea, abdominal pain, hepatitis, bone loss, pancreatitis, lactic acidosis
Azithromycin	Antibacterial	Nausea, vomiting, melena, jaundice
Ciprofloxacin	Antibacterial	Ileus, jaundice, bleeding, diarrhea, anorexia, oral ulcers, hepatitis, pancreatitis, vomiting, abdominal pain
Clarithromycin	Antibacterial	Nausea, diarrhea, abdominal pain, abnormal taste
Combivir (zidovudine–lamivudine)	Combination	Nausea, vomiting, abdominal pain, abnormal liver function, pancreatitis, lactic acidosis
Complera (rilpivirine, tenofovir, emtricitabine)	Combination	Nausea, vomiting, abnormal liver function, lactic acidosis
Darunavir	PI	Nausea, vomiting, diarrhea, abdominal pain, pancreatitis, hepatitis, constipation
Didanosine (ddI)	NRTI	Nausea, vomiting, abdominal pain, pancreatitis, abnormal liver function
Edurant	NNRTI	Abnormal liver function, lipodystrophy
Efavirenz	NNRTI	Nausea, vomiting, abnormal liver function, hyperlipidemia
Emtricitabine	NRTI	Lactic acidosis, hepatomegaly, diarrhea, nausea
Epzicom (zidovudine, abacavir)	Combination	Nausea, vomiting, abdominal pain, abnormal liver function, lactic acidosis, pancreatitis
Erythromycin	Antibacterial	Nausea, vomiting, abdominal pain
Etravirine	NNRTI	Nausea, vomiting, diarrhea, abdominal pain, hepatitis
Fosamprenavir	PI	Nausea, diarrhea, vomiting, abdominal pain
Enfuvirtide	FI	Nausea, diarrhea, abdominal pain, hepatitis, pancreatitis, dry mouth, anorexia
Ganciclovir	Antiviral	Nausea, vomiting, diarrhea, anorexia, abnormal liver function
Indinavir	PI	Nausea, vomiting, abdominal pain, diarrhea, changes in taste, jaundice, abnormal liver function
Ketoconazole	Antifungal	Hepatotoxicity
Lamivudine	NRTI	Nausea, diarrhea, decreased appetite, vomiting, abdominal pain, pancreatitis, abnormal liver function
Lopinavir/ritonavir	PI	Diarrhea, nausea, vomiting, abdominal pain, lipodystrophy, hyperlipidemia
Maraviroc	EI	Nausea, constipation, diarrhea, flatulence, abdominal pain, hepatitis, dysgeusia, stomatitis
Nelfinavir	PI	Nausea, diarrhea, fatigue, abnormal liver function, exacerbation of chronic liver disease
Nevirapine	NNRTI	Stomatitis, nausea, abdominal pain, raised gamma-glutamyl transpeptidase level, hepatotoxicity
Pentamidine	Antiparasitic	Abdominal pain, bleeding, hepatitis, pancreatitis, nausea, vomiting
Raltegravir	II	Gastritis, hepatitis, nausea, vomiting, abdominal pain, diarrhea, hyperbilirubinemia
Rifampin	Antibacterial	Abdominal pain, nausea, vomiting, diarrhea, jaundice
Rilpivirine	NNRTI	Lipodystrophy
Ritonavir	PI	Nausea, vomiting, diarrhea, abdominal pain, anorexia, pancreatitis, abnormal liver function
Saquinavir	PI	Mouth ulcers, nausea, abdominal pain, diarrhea, pancreatitis, abnormal liver function, exacerbation of chronic liver disease
Stavudine	NRTI	Nausea, vomiting, abdominal pain, diarrhea, pancreatitis, abnormal liver function, hepatic failure, lipodystrophy, hyperlipidemia, insulin resistance
Stribild (elvitegravir, cobicistat, emtricitabine, tenofovir)	Combination	Lactic acidosis, severe hepatomegaly with steatosis, and posttreatment acute exacerbation of hepatitis B
Sulfonamides	Antibacterial	Hepatitis, pancreatitis, stomatitis, nausea, vomiting, abdominal pain
Tenofovir	NRTI	Nausea, vomiting, diarrhea, abdominal pain, hepatitis, bone loss, pancreatitis, flatulence
Tipranavir	PI	Hyperlipidemia, nausea, vomiting, diarrhea, abdominal pain, pancreatitis, hepatitis
Tivicay	II	Gastritis, hepatitis, nausea, vomiting, abdominal pain, diarrhea, hyperbilirubinemia
Trizivir (abacavir–lamivudine–zidovudine)	Combination	Nausea, vomiting, abdominal pain, pancreatitis, abnormal liver function
Triumeq (abacavir, dolutegravir, lamivudine)	Combination	Lactic acidosis, abnormal liver function
Truvada (emtricitabine, tenofovir)	Combination	Lactic acidosis, nausea, vomiting, diarrhea, abdominal pain, hepatitis, bone loss, pancreatitis
Tybost	PE	Nausea, jaundice
Vitekta	II	Diarrhea
Zidovudine (ZDV)	NRTI	Nausea, vomiting, abdominal pain, abnormal liver function

HBV, hepatitis B virus; FI, fusion inhibitor; II, integrase inhibitor; EE, entry inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PE, pharmacokinetic enhancer of PIs.

Since the advent of cART, much of the wasting seen since the pre-cART era has been diminished in developed countries. HIV-infected patients are now faced with metabolic consequences of their ART regimen. Obesity, dyslipidemia, lipodystrophy, insulin resistance, and central adiposity are common cART-related complications due to increases in fat rather than lean mass.

HIV infection also results in prolonged chronic inflammation, thereby increasing CVD risk. Even though the mechanisms for these effects are likely multifactorial, care must be taken to avoid unwarranted weight gain and many nutritionally related components should be targeted. However, there are no specific guidelines that address lipid abnormalities or insulin resistance specific to HIV-infected patients. A recent meta-analysis has demonstrated a comparable clinical benefit of dietary intervention or omega-3 supplementation in reducing triglycerides, but no effects on other lipids in patients without concurrent lipid-lowering medication. Dietary intervention with the National Cholesterol Education Program (NCEP) Therapeutic Lifestyle Changes and exercise have been shown to reduce triglycerides in both HIV-infected and HIV-uninfected populations and to reduce LDL cholesterol in the general population. Treatment of these complications should, therefore, follow nutrition and lifestyle interventions guidelines recommended for the general population. Higher-intensity dietary interventions may also be required in the HIV population for LDL cholesterol reduction. Mediterranean-style diet plans, which promote the importance of healthy fats generally found in nuts, seeds, and olive and canola oils, have recently been shown to be effective in improving the lipid profiles and CVD risk factors in HIV-infected and HIV-uninfected adults. These diet plans are similar to other heart healthy eating regimens, emphasizing the consumption of fruits, vegetables, and whole grains.

It is well known that HIV and its treatment impact bone mineral density, so bone health must be considered when addressing the nutritional needs of HIV-infected individuals. Baseline bone assessments are recommended (typically evaluated with dual-energy x-ray absorptiometry), with periodic follow-up. It is important to ensure adequate intake of vitamin D and calcium and to emphasize the importance of weight-bearing exercise and avoidance of alcohol and tobacco, especially during adolescence, when peak bone mass is achieved.

Addressing food security is essential in the prevention, care, and treatment of HIV and AIDS. Food insecurity has been associated with worse ART adherence in low- and middle-income settings as a consequence of negative feedback (e.g., ART leads to improved appetite, which, in the absence of food, leads to poor adherence), worse virological and immunologic outcomes, worse physical and mental health status, and increased risk of mortality. Thus, interventions to ensure provision of food with assessment of food security and appropriate referrals to food assistance programs should be well integrated with HIV care programs. Health providers should be familiar with locally available resources that can be offered to this nutritionally vulnerable population.

For HIV-infected individuals who suffer from malnutrition and food insecurity, nutritional supplements can be effective if administered in adequate amounts. HIV populations at higher risk include those with increased metabolic demands such as pregnant and lactating women and children and those with active infections. Otherwise, with appropriate treatment and nutritional resources, most patients with HIV-related weight loss will gradually increase ad libitum caloric intake and gain weight. Future intervention efforts should quantify HIV-specific requirements across all ages/sexes so that supplements can be dosed in adequate amounts or administered by meal replacements. In developing nations, where malnutrition is of great concern, research should focus on locally based and sustainable foods. Food supplements that are economically feasible and efficient have the potential to improve nutritional intake, curtail weight loss, and alleviate HIV-associated symptoms. Future research efforts should analyze the long-term effects of nutritional interventions on LDL and HDL cholesterol and triglycerides in children and adults.

In addition to nutritional interventions involving diet, per se, other strategies to improve the nutritional status of HIV-infected individuals include progressive resistance training and hormone therapy. Exercise is a valuable option to increase lean tissue mass. Several studies in children/adolescents and adults have shown an increase in lean muscle mass as well as strength and cardiorespiratory fitness. Pharmacological treatments have also shown some benefit. Growth hormone-releasing hormone can improve central adiposity, while metformin has been reported to improve glucose homeostasis.

Summary

The multidimensional nature of nutritional problems associated with HIV infection is a microcosm for most nutritional disorders that span a vast array of both acute and chronic disease states in humans. With improved therapies and survival of HIV-infected individuals, the state of nutrition will continue to evolve. Future research and treatments in this field will focus on using nutrition to augment and optimize the clinical care of those chronically infected with HIV.

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Horse Meat

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Introduction

Meat is normally regarded as the edible part (muscle and offal) of livestock animals. Meat consumption practices vary based on geographic disposition, availability of animals, religious practices, and rituals and customs of the population. Conventional food of one community may not be same for the other. For example, global attitudes to the consumption of flesh derived from dog, elephant, hippopotamus, rhino, seal, whale, frog, and horse are extremely varied. Several works have demonstrated that horses from heavy breeds are well adapted to meat production. In some countries like Kazakhstan and Kyrgyzstan, horse meat (HM) is considered as an alternative to beef. HM has been historically obtained from animals that were slaughtered at the end of their working life. Horse carcasses have high dressing percentages and meat yields. HM is regarded as more digestible than that from sheep or cattle. Horse bones have also been used as a traditional medicine for treatment of bone fracture and arthritis in Korea. The consumption of HM is known as hippophagia and is very common in countries, such as Denmark, Belgium, Holland, and Germany. The top eight HM-consuming countries consume about 4.7 million horses a year. HM has been described as slightly sweet, tender, and low in fat. The slaughter weight of male and female horses is about 504.8 kg and dressing percentage is ~70%. The most important difference between male and female horses is in the degree of fatness, which is highest in females. Horses today are slaughtered at ages ranging from 16 to 24 months.

Pattern of HM Consumption

HM gained widespread acceptance in French cuisine during the later years of the Second French Empire. The high cost of living in Paris prevented many working-class citizens from buying meat such as pork or beef. In 1866, the French government legalized the eating of HM, and the first butcher's shop specializing in HM opened in eastern Paris, providing quality meat at lower prices. During the Siege of Paris (1870–71), HM was eaten by anyone who could afford it, partly because of a shortage of fresh meat in the blockaded city and also because horses were eating grain, which was needed by the human population. Many Parisians gained a taste for HM during the siege, and after the war ended, it remained popular. Likewise, in other places and times of siege or starvation, horses were viewed as a food source of last resort. Despite the general Anglophone taboo, horse and donkey meats were eaten in Britain, especially in Yorkshire, until the 1930s, and in times of postwar food shortage, HM consumption surged in popularity in the United States and was considered for use in hospitals. Horses are poor converters of meat; hence, they are not generally raised for meat production.

As indicated previously, the consumption pattern of HM is common in Europe and Central Asia. Some of the reasons for increased interest in HM consumption have arisen due to concerns pertaining to the occurrence of dioxins, bovine encephalopathy, and foot-and-mouth disease problems in conventional meat-producing farm animals. Therefore, consumers began to look for alternative red meats from other nontraditional species. The killing of horses for human consumption is widely opposed in countries such as the United States, the United Kingdom, Ireland, and Australia. In these countries, horses are generally considered to be companion and sporting animals only. HM consumption is forbidden by Romanian and Jewish cultures because horses do not have hoofs and do not chew cud (owing to the fact that horses are not ruminant animals). HM is generally not widely consumed in Spain (with the exception being that in the north of the country), although the country exports horses both 'on the hoof and on the hook' for the French and Italian markets.

HM is consumed in some North American and Latin American countries, but deemed illegal for consumption in some countries. For example, the Food Standards Code of Australia and New Zealand's definition of 'meat' does not include horse. Under Islamic law, the consumption of HM is forbidden. In north Africa, HM has been occasionally consumed, but almost exclusively by the Christian Copts and the Hanafi. Nowadays, consumption of HM is common in Iceland, wherein horses are mainly raised for this purpose. HM is generally considered by ethnic Russians to be a low-quality meat with poor taste, and it is rarely found in stores. It is popular among nomadic peoples of Eastern Russia such as Tatars and Yakuts and Central Asia such as Kyrgyzs and Kazakhs.

Production of HM

The Republic of Croatia is modest in production and consumption of HM. Geographic position, underutilization of great pasture grounds, and breeding structure of horse population in Croatia give good chances for a profitable production of HM with the possible export orientation. In Kazakhstan, HM is a large part of the diet, due mainly to the nomadic roots of the population. In Japanese cuisine, raw HM is called *sakura* or *sakuraniku*. In 2013, HM and traces of horse DNA were found in some food products where horse was not labeled as an ingredient, sparking the 2013 meat adulteration scandal across

Table 1 Major horse meat-producing countries

Country	*HM production (metric tons)*
Mexico	78 000
Argentina	57 000
Kazakhstan	55 000
Mongolia	38 000
Kyrgyzstan	25 000
Australia	24 000
Brazil	21 000
Canada	18 000
Poland	18 000
Italy	16 000
Romania	14 000
Chile	10 000
France	7500
Uruguay	8000
Senegal	9500
Colombia	6000
Spain	5000

Source: British Agriculture Industry Website (2009).

Europe. The major HM-producing countries and their production are depicted in Table 1.

Physical Characteristics of HM

HM has a soft consistency and is dark red in color. Following exposure to light, it becomes brown or black with a bluish tinge due to light-induced changes. Generally, horse fat is yellow in color and soft and greasy in consistency. Intermuscular fat is absent, and also, the large fatty adipose tissue is absent. Low level of adiposity appears in subcutaneous fat. Fat depots of flank and kidney are relatively lean. Horse is a lean and muscular animal as the whole body contains on average 69.6% muscle, 10.4% saturated fat, and 17.4% bone. It is high and at least ten times higher when compared to other muscle-based foods. Therefore, HM is a suitable meat source for those suffering from anemia or hypercholesterolemia, dieters, or those seeking an alternative to beef. Myoglobin content in HM is at a maximum (0.7%) compared to other food animals. The refractive index for horse fat is 53.5, which in pork fat is <50 and for beef fat is <40. HM has the highest iodine number (70–85) of all meats. For the lard, iodine number is 50–70 and ox and sheep fat 35–46. Horse fat contains 1–2% linoleic acid, whereas all other animal-derived fats contain $<0.1\%$.

Changes Occurring after Slaughter of Horse

There are a series of reactions that take place during the transformation of muscle to meat. Factors such as preslaughter care, mode and duration of transport, lairage conditions, and injury and stress of any other form on the animal awaiting slaughter all impact significantly this transformation. As biophysical and biochemical changes continue to take place in HM following slaughter and exsanguinations for several hours to several days, the conversion of muscle to meat is a complex process.

Palatability Characteristic of HM

Aroma and Flavor Development

The basic flavor of meat is primarily due to the presence of inosinic acid, and HM is no exception. In the process of heating, inosinic acid reacts with glycoprotein containing alanine. The inosinic acid reacts on heating with a glycoprotein containing alanine and glucose producing a basic meat flavor and odor. The breakdown of proteins and fats during aging also contributes to flavor by producing H_2S, acetaldehyde, ammonia, acetone, and diacetyl, but at the same time, prolonged aging is associated with loss of flavor.

Color

In living condition, horse muscle with sufficient oxygen becomes bright red in color. After slaughter, oxygen is used up by the muscle, and the tissue becomes dark purplish red in color. In the living animal, muscles with sufficient O_2 supply have a bright red appearance. In the postmortem muscles, as the O_2 is used up, the muscles become dark purplish red in color.

Firmness

Living muscles maintain certain amount of tone. Since they are attached at both ends directly or indirectly to some part of the skeleton, they are relatively firm. After rigor mortis, the muscle becomes firm and stiff; due to denaturation of protein and enzymatic degradation, it becomes more tender. This is same as in case of other meats.

Water-Holding Capacity

Moisture content of HM muscle is 65–80%. The capacity of meat to retain its own water or added water after the application of several forces is called as water-holding capacity of meat. It gives juiciness to the meat. HM is less juicy as compared to beef and carabeef.

Meat Composition

The intramuscular fat content in mutton is the highest (13%) and in buffalo meat the least (1.0%). Beef contains 2.5%, goat meat 3.5%, and pork 4.5%. Vitamin A content is significant in beef and mutton but highly insignificant in buffalo meat, chevon, and pork. The glycogen content is high in HM, by at least ten times compared with other food animals. Myoglobin content in HM is maximum (0.7%) compared with other food animals.

Body Fat Composition

The refractive index value for horse fat is 53.5, while in pork fat, it is <50, and in beef fat, it is <40.

Iodine Number

The iodine number of horse fat (70–85) is the highest compared to lard (50–70), ox, and sheep fat (35–46).

Linoleic Acid Content

Horse fat contains 1–2% linoleic acid, whereas all other fats contain $<0.1\%$.

Nutritive Value

Fatty acid composition of different game animals is given in Table 2. The fatty acid profile of HM is positive from a nutritional point of view and in line with health recommendations. However, health-conscious consumers tend to avoid red meat because of the public perception of the adverse effects of saturated fatty acids that have been linked to coronary heart disease. Among red meat varieties, HM is considered more dietetic because it contains less fat and cholesterol. The $N-6/N-3$ ratio is more favorable in HM than from other ruminant-derived meats. Among red meats, HM is known to contain high levels of unsaturated fatty acids such as α-linolenic (18:3 $n-3$), monounsaturated fatty acids (MUFAs), vitamin B_{12}, and heme iron (4 mg/100 g), thereby making HM one of the healthiest forms of red meat available for consumption.

Palmitic, stearic, and oleic acids are predominant in HM and contribute for 3–18% of total MUFA levels. The wide range is attributed to the feeding practices adapted. HM is considered an important source of minerals, especially iron, magnesium, and copper. Regarding Ca content, equine meat has relatively low values. The Mg content in HM is 21.00–43.31 mg/100 g. It is low in cholesterol. Glycogen content is ten times higher as compared with other foods. Myoglobin content is at maximum (0.7%) compared with other food animals. The refractive index is 53.5, while for pork fat, it is <50, and for beef fat, it is <40.

The HM is known to contain high levels of unsaturated fatty acids like α-linolenic (18:3 $n-3$) and MUFAs, indicating that it is more beneficial for health than beef. Retinol content is the highest, riboflavin is similar as in beef, but thiamine is higher than beef. Regular consumption of HM may contribute to reduced LDL and cholesterol and improve omega-3 index and docosahexaenoic acid (C22:3$n-6$) and iron status. Energy value of HM of medium fatness is 472 KJ, that is, 110 kcal per 100 g. The proteins from HM are better than the proteins from veal, pork, or beef by their quantity and quality (because of a smaller content of connective tissue, it is easier to digest).

Examples of Specific HM Products Found Internationally

Kazy and Shuzhuk

Kazy and shuzhuk are types of sausage found in Kazakhstan and are made from HM and the intestinal lining used as a natural casing. Another product known as *Zhaya* is made from hip meat, which is smoked and boiled. *Zhal* is made from neck fat, which is again smoked and boiled, while *Karta* is made from a section of the rectum, which, similarly, is smoked and boiled. Sur-yet is derived from HM and is produced as a dried meat.

Horse Satay

In Indonesia, chunk cuts of grilled HM are served with spicy sauce known as horse satay. More details about satay are not available in the literature.

Sakura

In Japanese cuisine, raw HM is called *sakura* or *sakuraniku* (sakura means cherry blossom and *niku* means meat) because of its pink color. It can be served raw as sashimi in thin slices dipped in soy sauce, often with ginger and onions added. In this case, it is called *basashi*, which is popular in some regions of Japan. Fat, typically from the neck, is also found as *basashi*, though it is white, not pink. HM is also sometimes found on menus for *yakiniku* (a type of barbecue), where it is called *baniku* literally 'HM' or *bagushi* ('skewered horse'); thin slices of raw HM are sometimes served wrapped in a shiso leaf.

Table 2 Fatty acid (%) content of intramuscular fat of final dry-cured products

Fatty acids	*Deer*	*Wild boar*	*Horse*	*Bovine*	*Goat*
14:0	2.44 ± 0.26	1.22 ± 0.06	1.87 ± 0.36	2.56 ± 0.06	2.50 ± 0.21
16:0	21.4 ± 0.89	23.1 ± 0.82	24.4 ± 1.08	31.4 ± 0.79	22.8 ± 0.64
16:1 $n-7$	4.71 ± 0.95	2.92 ± 0.15	3.63 ± 0.81	3.14 ± 0.17	1.95 ± 0.22
18:0	21.1 ± 1.66	11.2 ± 0.68	9.3 ± 1.25	13.9 ± 0.58	19.2 ± 1.52
18:1 $n-9$	20.6 ± 1.24	38.5 ± 1.32	19.0 ± 2.34	38.6 ± 0.56	36.5 ± 1.53
18:1 $n-7$	5.0 ± 0.55	4.20 ± 0.17	3.43 ± 0.07	1.85 ± 0.14	2.37 ± 0.22
18:2 $n-6$	8.3 ± 0.63	12.6 ± 0.97	23.0 ± 1.82	4.61 ± 0.47	7.5 ± 1.37
18:3 $n-3$	5.3 ± 0.43	1.22 ± 0.37	4.50 ± 0.93	0.42 ± 0.03	0.33 ± 0.04
20:4 $n-6$	2.95 ± 0.59	1.94 ± 0.26	4.84 ± 1.55	1.27 ± 0.17	3.01 ± 0.50
20:5 $n-3$	2.25 ± 0.26	0.38 ± 0.17	0.51 ± 0.11	0.19 ± 0.02	0.22 ± 0.04
20:6 $n-3$	0.78 ± 0.16	0.10 ± 0.02	0.48 ± 0.14	–	0.12 ± 0.03
Total SFA	44.9 ± 1.81	35.5 ± 1.54	35.6 ± 0.34	47.8 ± 1.00	44.5 ± 2.08
Total MUFA	30.3 ± 2.01	45.7 ± 1.32	26.0 ± 3.13	43.6 ± 0.56	40.9 ± 1.70
Total PUFA	19.6 ± 1.99	16.2 ± 0.80	33.3 ± 2.67	6.5 ± 0.64	11.2 ± 1.87

Source: Paleari, M. A., Moretti, V. M., Beretta, G., Mentasti, T. and Bersani, C. (2003). Cured products from different animal species. *Meat Science* **63**, 485–489.

Kumamoto, Nagano, and Ōita are famous for *basashi*, and it is common in the Tohoku region as well. Some types of canned 'corned meat' in Japan include HM as one of the ingredients. There is also a dessert made from HM called *basashi* ice cream. The company that makes it is known for its unusual ice cream flavors, many of which have limited popularity.

Kazy

It is a type of salted sausage that is produced as a regional delicacy by the Mongolian Kazakhs. In modern times, they prefer beef and mutton, though during the extremely cold winter, however, many people prefer HM due to its low cholesterol. It is kept nonfrozen, and traditionally, people believe that HM helps generate body heat. Other Asian nations import processed HM from Mongolia.

Tonga

In Tonga, HM is much more than just a delicacy. Consumption of HM is generally only reserved for special occasions. These special occasions may include the death of an important family member or community member or celebration during the birthday of an important family member or perhaps the visitation of someone important like the king of Tonga.

In Tonga, a horse is one of the most valuable animals a family can own because of its use as a beast of burden. Therefore, the slaughter of one's horse for the purpose of consumption becomes a moment of immense homage to the person or event the horse was slain for. Despite a diaspora into Western countries like Australia, United States, and New Zealand where consumption of horse meat is generally tabooed, Tongans still practice the consumption of HM perhaps even more so because it is more readily available and more affordable.

Lukba or Tapang Kabayo or Kabayo

In the Philippines, HM is commonly sold in wet local markets. HM is a famous delicacy. The method of preparation is very common, which includes marinating the meat with lemons, toyo (soy sauce), and patis (fish sauce). It is then fried and served. It is usually dipped into vinegar to give the meat a sour taste. It is known throughout the country as 'lukba,' 'tapang kabayo,' or just simply 'kabayo.'

Conclusion

The HM is soft, delicious, easily digestible, and favorable to health-conscious consumers. Its nutritional compositions, like proteins, fat, glycogen, essential amino acids, and macro- and microelements, rate better than beef. HM's high content of proteins, vitamin B_{12}, and iron and its low content of cholesterol make it the best and cheapest alternative for red meat.

See also: Fish: Fish in the Human Diet; Ham: Dry-cured Ham; Meat: Conversion of Muscle into Meat; Meat: Role in the Diet; Meat: Structure; Obesity: The Role of Diet; Pork Meat Quality, Production and Processing on; Poultry: Processing; Sheep: Meat.

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Human Milk: Composition and Nutritional Value

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Introduction

Human milk (HM) is a complex and dynamic fluid that has evolved to meet the nutritional needs of the infant. As such, the nutritional composition of HM changes continuously with the infant's development, which differs markedly from infant formula that is designed to be appropriate for the first year of life and does not change in composition. Unlike infant formula, HM contains a variety of bioactive proteins, lipids, oligosaccharides, and immunomodulatory components that facilitate the infant's transition to extrauterine life. This article will review the content and composition of HM, with a particular focus on the bioactive components in HM and their potential role in infant development. The factors that influence HM nutrient composition, such as maternal genetics, diet, stage (e.g., prematurity), and route of delivery, will be highlighted.

Breastfeeding

Health Benefits

HM is the optimal form of nutrition for the infant. Epidemiological studies have shown that compared with formula feeding, breastfeeding protects against common infectious diseases in infancy (otitis media, respiratory syncytial virus, and urinary tract infection), necrotizing enterocolitis (NEC) in preterm infants, and immune-mediated disorders in later childhood, including allergy, asthma, atopic dermatitis, inflammatory bowel disease, celiac disease, type 1 and type 2 diabetes mellitus, and leukemia. The reduced risk of immune-mediated diseases may be due in part to differences in the gut microbiota of breast-fed (BF) and formula-fed (FF) infants. Additional evidence support improvements in cognitive development and reduced risk of childhood obesity.

Breastfeeding Recommendations and Rates

Due to these health benefits, exclusive breastfeeding is recommended for the first year of life, with exclusive breastfeeding in the first 6 months, by the World Health Organization and leading pediatric societies, including the American Academy of Pediatrics (AAP). However, the rates of breastfeeding vary widely throughout the world, and only 14% of infants are exclusively BF for 12 months in the United States. Thus, most infants receive formula either exclusively, or in combination with breastfeeding, during the first year of life. For this reason, HM and infant formula composition will be compared throughout the article, where appropriate.

HM Composition

HM is initially synthesized as colostrum, which persists for ~72 h, followed by transitional and then mature HM. Colostrum contains low concentrations of lactose and fat, but is rich in immunoprotective and trophic compounds. After 72 h, milk production increases considerably and transitional milk composition changes, including increases in lactose and fat, to support the rapid growth and motor and cognitive development of the infant. By 15 days postpartum, milk is considered mature and its composition remains relatively stable until weaning. In addition to changes with duration of lactation, HM composition varies within feeding and between breasts in a few selected milk components. For instance, the lipid content of hind milk is twice that of milk secreted at the beginning of the feeding (foremilk). Similarly, vitamin A and E contents are higher in hind milk than foremilk. Differences in HM composition between breasts have been observed, although these differences tend to be more sporadic. More recent studies suggest that infant gender affects nutrient composition, with higher lipid content in milk of women breastfeeding male infants.

Nutrients in HM are derived from three major pathways: (1) secretion of components endogenously synthesized in the mammary gland, such as lactose, milk proteins, lipids, and oligosaccharides; (2) transport of substances from the maternal circulation to milk, such as immunoglobulins, other immune factors, and hormones; and (3) nonspecific entry of serum and interstitial components into HM during the process of milk synthesis, including urea, free amino acids, and other low-molecular-weight compounds. The nutrients in HM are compartmentalized based on their chemical structures. Water-soluble components are found in the whey (aqueous phase of milk), while the major protein components, such as casein, are found in the colloidal phase. Fat and hydrophobic components are emulsified in droplets surrounded by the milk-fat globule membrane (MFGM).

Colostrum and mature milk are approximately 88% water and have an osmolality of 286–319 mOsm l^{-1}, compared with 185–374 mOsm l^{-1} for infant formulas. Milk comprises 120 and 128 g l^{-1} solids in mature milk and colostrum, respectively. Milk solids are composed of 70 g l^{-1} carbohydrate, 40 g l^{-1} fat, 10 g l^{-1} protein, and 2 g l^{-1} mineral ash. The compositions of colostrum and mature milk are shown in Table 1. Colostrum has higher protein and lower fat and carbohydrate concentrations relative to mature milk. Despite the differences in protein and lipid content between colostrum and mature milk, they have similar energy content, which is less than that of formula (670 kcal l^{-1}).

Carbohydrates

The predominant carbohydrates in milk are lactose and HM oligosaccharides (HMO). Lactose is the least variable HM macronutrient throughout lactation. In addition, lactose is the major osmol in HM, and its concentration is highly correlated to milk production. Lactose is digested almost entirely by

Table 1 Energy and macronutrient composition of colostrum and mature human milk[1]

Constituent (l^{-1})	*Colostrum*	*Mature milk*
Energy (kcal)	536	577
Total carbohydrate (g)	50–70	70–85
Lactose (g)	30–50	67–70
Oligosaccharides (g)	22–24	5–15
Glucose (g)	0.2–1.0	0.2–0.3
Total lipid (g)	15–20	35–48
Triglyceride (g)	14.5–19.5	34–47
Fatty acids (g)	13–17	30–42
Cholesterol (g)	0.2–0.3	0.1–0.2
Cholesteryl esters (g)	0.05	0.01
Phospholipids and sphingolipids (g)	N.R.[2]	0.25–0.30
Total nitrogen (g)	3.0	1.9
Nonprotein nitrogen (g)	0.5	0.45
Protein nitrogen (g)	2.5	1.45
Total protein (g)	15–20	8–11
Casein (g)	3.8	3–5
β-Casein (g)	2.6	3–4
κ-Casein (g)	1.2	1–2
Whey proteins (g)	11–15	5–6
α-Lactalbumin (g)	3.6	2–3
IgM (g)	0.02	0.01
IgG (g)	0.01	0.05
Lactoferrin (g)	3.5	1–3
Lysozyme (g)	0.1–0.2	0.1
Secretory IgA (g)	2.0	0.5–1.0
Serum albumin (g)	0.4	0.3

[1]Mean values adapted from references: Agostoni, C., Marangoni, F., Lammardo, A. M., Galli, C., Giovannini, M. and Riva, E. (2001). Long-chain polyunsaturated fatty acid concentrations in human hindmilk are constant throughout twelve months of lactation. *Advances in Experimental Medicine and Biology* **501**, 157–161; Hester, S. N., Hustead, D. S., Mackey, A. D., Singhal, A. and Marriage, B. J. (2012). Is the macronutrient intake of formula-fed infants greater than breast-fed infants in early infancy? *Journal of Nutrition and Metabolism* **2012**, 891201. DOI: 10.1155/2012/891201; Kunz, C., Rodriguez-Palmero, M., Koletzko, B. and Jensen, R. (1999). Nutritional and biochemical properties of human milk, part I: general aspects, proteins, and carbohydrates. *Clinics in Perinatology* **26**, 307–333; Kunz, C., Rudloff, S., Baier, W., Klein, N. and Strobel, S. (2000). Oligosaccharides in human milk: Structural, functional, and metabolic aspects. *Annual Review of Nutrition* **20**, 699–722; Picciano, M. F. (2001). Nutrient composition of human milk. *Pediatric Clinics of North America* **48**, 53–67.
[2]Data not reported.

intestinal lactase, although a small proportion of undigested lactose may contribute to softer stools and increased absorption of minerals. HMOs are the third most abundant component in milk and are found in higher concentration in colostrum than mature milk (Table 1). HM is unique compared with that of other species in its high content and composition of HMO. Up to 200 structures of HMO have been identified in HM, of which ~70% are fucosylated. In contrast, oligosaccharide concentrations in cow's milk and cow's milk-based infant formulas are predominantly sialylated, and their concentration is negligible compared with HM.

Proteins

The HM protein content is lower than that of cow's milk (35 g l^{-1}) or cow's milk- (13–15 g l^{-1}) or soy-based infant formula (18–20 g l^{-1}). These proteins provide essential amino acids to support infant growth; however, many HM proteins are relatively resistant to digestion and are involved in immune protection, improvement of nutrient digestion (e.g., enzymes), and enhancement micronutrient availability (e.g., mineral binding proteins).

Milk proteins are broadly classified into casein and whey fractions. Colostrum has a whey-to-casein ratio of 90:10 compared with 60:40 in mature milk. Whey is composed of over 150 proteins. The most abundant whey protein is alpha-lactalbumin (α-LAC), which contains higher levels of tryptophan, lysine, and cysteine than other proteins in HM. α-LAC serves a critical functional role as a component of the lactose synthase complex. In addition, peptides released during α-LAC digestion have antimicrobial and prebiotic activities that stimulate beneficial bacterial colonization of the gut, while inhibiting the growth of pathogenic bacteria. Other HM whey proteins are lactoferrin, immunoglobulins, and lysozyme, which are related to the immune protection of the neonate and will be discussed later in this article.

The HM casein fraction differs from bovine milk with lower casein concentration in HM (3–5 vs. 26 g l^{-1}, respectively), and it is composed of β- and κ-casein, while bovine milk is predominantly composed of α-casein. Glycosylation of casein, particularly of κ-casein, is associated with protection against infection and increased micronutrient absorption.

Fats

Fat is the second most abundant macronutrient in HM and is an important energy source for the infant. Approximately 98% of the fat in HM is in the form of triglycerides, which are synthesized in the mammary alveolar cells from fatty acids derived from maternal diet or adipose tissue, or *de novo* synthesis. Of the fatty acids in mature HM, 32–52% are saturated, 30–50% are *cis*-monounsaturated, and 2.5–13.8% are *trans*-monounsaturated. The remaining are polyunsaturated fatty acids (PUFA). Compared with bovine milk, HM has a lower percentage of short-chain fatty acids and considerably more long-chain fatty acids in the form of arachidonic acid (AA) and docosahexaenoic acid (DHA) than bovine milk. The HM AA content is relatively constant, whereas HM DHA content is sensitive to maternal diet. Dietary DHA and AA benefit infant visual and cognitive development, which has led to their supplementation into infant formula.

The fat in HM is surrounded by the bipolar MFGM, which originates from the mammary apical plasma and mature Golgi vesicle membranes. The MFGM also contain a diverse group of proteins, including mucin and lactadherin, which act as antimicrobial agents. Other molecules present in MFGM that may be involved in brain development and myelination are sphingomyelin, gangliosides, sialic acid, and cholesterol.

Vitamins

In most cases, HM contains all essential vitamins in adequate concentrations to meet the nutritional requirements of the growing infant. Their content is, however, dependent on maternal nutritional status and dietary habits. Vitamin supplementation during lactation is recommended to compensate for

any dietary insufficiency. The content of fat-soluble and water-soluble vitamins in HM is summarized in Table 2.

The fat-soluble vitamins in HM are contained within the MFGM, with the exception of vitamin D, which is present in whey attached to vitamin D-binding protein. Vitamin A includes retinol, β-carotene, and carotenoids, which have roles in vision, growth, healing, reproduction, cell differentiation, and immune function. HM is enriched with retinol esters and contains 1.8 μmol l^{-1} carotenoids, with α-and β-carotenoids and cryptoxanthin accounting for 50% total carotenoids. The vitamin A content of HM is sensitive to maternal diet and decreases from colostrum to mature milk (Table 2).

Table 2 Vitamin composition of human milk[1]

Constituent (l^{-1})	*Colostrum*	*Mature milk*
Fat-soluble vitamins		
Vitamin A and carotenoids		
Retinol esters (μmol)	1.45–2.0	1.22
Total carotenoids (μmol)	5.35	1.8–3.0
α-Carotene (μmol)	0.17	0.02–0.03
β-Carotene (μmol)	0.42	0.04–0.07
β-Cryptoxanthin (μmol)	0.23	0.03–0.06
Lutein/zeaxanthin (μmol)	0.2	0.04–0.10
Lycopene (μmol)	0.51	0.02–0.06
Vitamin D_3 (cholecalciferol) (μg)	0.1–0.3	0.5–2.0
Vitamin E		
α-Tocopherol (mg)	15	3–5
γ-Tocopherol (mg)	1.5	1.0
Vitamin K		
Phylloquinone, K_1 (μg)	0.64–3.7	0.86–3.9
Menaquinone, K_2 (μg)	1.8–2.6	1.2–2.0
Water-soluble vitamins		
Ascorbic acid (mg)	80–100	40–100
Biotin (μg)	4.5	5–9
Vitamin B_{12} (μg)	1.2–1.5	0.5–0.8
Folate (μg)	5–10	50–100
Niacin (mg)	0.5	1.5–2.0
Pantothenic acid (mg)	2.5	2.0–2.5
Pyridoxine, B_6 (μg)	20–40	90–300
Riboflavin, B_2 (μg)	500	300–400
Thiamin, B_1 (μg)	20	160–210

[1]Mean values adapted from references: Canfield, L. M., Clandinin, M. T., Davies, D. P., et al. (2003). Multinational study of major breast milk carotenoids of healthy mothers. *European Journal of Nutrition* **42**, 133–141; Dawodu, A. and Akinbi, H. (2013). Vitamin D nutrition in pregnancy: current opinion. *International Journal of Women's Health* **5**, 333–343; Greer, F. R. (2001). Do breastfed infants need supplemental vitamins? *Pediatric Clinics of North America* **48**, 415–423; Kamao, M., Tsugawa, N., Suhara, Y., et al. (2007). Quantification of fat-soluble vitamins in human breast milk by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography B* **859**, 192–200; Korchazhkina, O., Jones, E., Czauderna, M. and Spencer, S. A. (2006). Effects of exclusive formula or breast milk feeding on oxidative stress in healthy preterm infants. *Archives of Disease in Childhood* **91**, 327–329; Macias, C. and Schweigert, F. J. (2001). Changes in the concentration of carotenoids, vitamin A, alpha-tocopherol and total lipids in human milk throughout early lactation. *Annals of Nutrition and Metabolism* **45**, 82–85; Mock, D. M., Mock, N. I. and Dankle, J. A. (1992). Secretory patterns of biotin in human milk. *Journal of Nutrition* **122**, 546–552; Picciano, M. F. (2001). Nutrient composition of human milk. *Pediatric Clinics of North America* **48**, 53–67; Song, W. O., Chan, G. M., Wyse, B. W. and Hansen, R. G. (1984). Effect of pantothenic acid status on the content of the vitamin in human milk. *American Journal of Clinical Nutrition* **40**, 317–324.

Vitamin D is present in its hydroxylated form or as 25-hydroxyvitamin D (25(OH)D) in HM, and its content is dependent on maternal vitamin D status, diet, and sun exposure. Vitamin D is involved in calcium and phosphorus metabolism, bone formation, and modulation of the immune system toward a more tolerogenic behavior. The HM vitamin D content (20–80 UI l^{-1} or 0.5–2 μg l^{-1}) is not sufficient to meet the AAP recommendation of 400 IU (100 μg) per day in exclusively BF infants who have limited exposure to sun. Infant formula is fortified to 270–670 UI l^{-1}. Maternal supplementation with cholecalciferol increases HM 25(OH)D to levels that are sufficient to avoid deficiency in BF infants. Vitamin D can also be directly supplemented to exclusively BF infants.

Vitamin E refers to a family of eight tocopherols that function as antioxidants in cell membranes and the development of the nervous system, skeletal muscle, and retina. The alpha-tocopherol form is the most abundant in HM, and it is higher in colostrum than mature milk and four times higher in hind milk compared with foremilk.

Vitamin K is important for blood coagulation, cell cycle regulation, and cell adhesion and bone metabolism. HM contains both forms of vitamin K: phylloquinone (K_1) and menaquinone (K_2) with an additional form, melaquinone-4 (MK-4), that is synthesized in the body from dietary phylloquinone by bacteria. Vitamin K is not readily transported through the placenta and is low in HM. Therefore, the AAP currently recommends a single intramuscular injection of vitamin K (0.5–1.0 mg) immediately after birth.

The other major class of vitamins is the water-soluble vitamins, which include vitamin C and eight B vitamins: thiamin (B_1), riboflavin (B_2) niacin (B_3), pyridoxine (B_6), biotin, pantothenic acid, folic acid, and cyanocobalamin (B_{12}). The concentration of the water-soluble vitamins is dependent on maternal diet and vitamin supplement use.

Vitamin C is critical in collagen synthesis and synthesis and activation of neuropeptides and is an important antioxidant. The concentration of vitamin C in milk does not vary greatly from colostrum to mature milk and ranges from 40 to 100 mg l^{-1}. The B vitamins are important components or cofactors in intermediary metabolism. The concentration of B vitamins in HM increases from colostrum to mature milk with the exception of B_{12} and riboflavin, whose content decreases as lactation progresses (Table 2). HM concentration of B vitamins is adequate to fulfill the needs of exclusively BF infants; however, concentrations of folate, B12, and B6 in HM may be compromised if maternal intake of these vitamins is inadequate.

Minerals

The mineral and trace element concentrations in HM change over the course of lactation and are higher in colostrum than mature milk (Table 3). The transport of minerals and trace elements is highly regulated, and there is typically no association between maternal serum mineral concentration or dietary intake and HM content, with the exception of selenium, fluorine, and iodine. Many minerals in HM are bound to proteins, which increases their bioavailability. Minerals in infant formula, whether cow's milk- or soy-based, are found in higher concentration than HM to compensate for lower availability and absorption rates.

Table 3 Macromineral and micromineral composition of human milk[1]

Constituent (l^{-1})	*Colostrum*	*Mature milk*
Macrominerals		
Calcium (mg)	250	200–250
Chloride (mg)	600–800	400–450
Magnesium (mg)	30–35	30–35
Phosphorus (mg)	120–160	120–140
Potassium (mg)	600–700	400–550
Sodium (mg)	300–400	150–250
Microminerals		
Boron (μg)	30	28
Chromium (μg)	17	25–75
Copper (mg)	0.5–0.8	0.1–0.3
Fluoride (μg)	5–20	4–15
Iodine (μg)	40–50	140–150
Iron (mg)	0.5–1.0	0.3–0.75
Manganese (μg)	5–12	3–4
Selenium (μg)	25–32	10–25
Zinc (mg)	5–12	1–3

[1]Mean values adapted from references: Atkinson, S., Alston-Mills, B., Lönnerdal, B. and Neville, M. C. (1995). Major minerals and ionic constituents of human and bovine milks. In: Jensen, R. G. (eds.) *Handbook of Milk Composition*, pp. 593–622. San Diego, CA: Academic Press; Casey, C. E., Smith, A. and Zhang, P. (1995). Microminerals in human and animal milks. In: Jensen, R. G. (eds) *Handbook of Milk Composition*, pp. 622–674. San Diego, CA: Academic Press; Esala, S., Vuori, E. and Helle, A. (1982). Effect of maternal fluorine intake on breast milk fluorine content. *British Journal of Nutrition* **48**, 201–204; Fransson, G. B. and Lönnerdal, B. (1983). Distribution of trace elements and minerals in human and cow's milk. *Pediatric Research* **17**, 912–915; Krachler, M., Li, F. S., Rossipal, E. and Irgolic, K. J. (1998). Changes in the concentrations of trace elements in human milk during lactation. *Journal of Trace Elements in Medicine and Biology* **12**, 159–176; Lönnerdal, B. (1998). Copper nutrition during infancy and childhood. *American Journal of Clinical Nutrition* **67.** 1046S–1053S; Parr, R. M., DeMaeyer, E. M., Iyengar, V. G., et al. (1991). Minor and trace elements in human milk from Guatemala, Hungary, Nigeria, Philippines, Sweden, and Zaire. Results from a WHO/IAEA joint project. *Biological Trace Element Research* **29**, 51–75; Picciano, M. F. (2001). Nutrient composition of human milk. *Pediatric Clinics of North America* **48**, 53–67.

The major HM macrominerals are calcium, phosphorus, magnesium, sodium, potassium, and chloride. Calcium concentration in HM is markedly lower than that of bovine milk and formula (250 vs. 1250 and 500 mg l^{-1}, respectively). Most HM calcium is found in whey, while 15% is bound with phosphorus in casein micelles. The linkage between calcium and phosphorus occurs in a 2:1 ratio resulting in high calcium availability. The concentration of sodium, potassium, and chloride in milk is mostly determined by electrochemical gradient, as HM should be iso-osmolar with plasma. Sodium levels have been reported to vary significantly between women in the early phase of lactation. This variation decreases by 50 days postpartum. Magnesium content of HM is relatively stable due to tight regulation of serum concentration.

Trace elements in HM include iron, zinc, manganese, selenium, copper, chromium, boron, fluorine, and iodine (Table 3). The concentration of iron in HM is low and averages 0.5–1.0 mg l^{-1} in colostrum and 0.3–0.7 mg l^{-1} in mature milk. However, exclusively BF infants rarely develop iron deficiency or anemia in the first 6 months of life, most likely due to high iron bioavailability. Lactoferrin and vitamin C may facilitate the uptake of these elements from HM. Iron in infant formula has a bioavailability of 4–7%; thus, most formulas are iron-fortified. By 6 months, BF infants should be gradually introduced to iron-fortified foods, such as cereals to prevent iron deficiency. Zinc and manganese in HM follow similar patterns as iron, as both decrease after the first days of lactation but present higher availability than those found in infant formula.

Maternal iron, zinc, and manganese intake does not affect HM concentration even when mineral supplementation is taken. On the other hand, minerals such as selenium, fluorine, and iodine in milk are associated with maternal intake and vary with geographic location and supply of mineral-rich foods. Milk selenium tends to be lower in areas with limited grass and cereal such as Finland and New Zealand compared with other areas. Likewise, milk fluorine varies greatly geographically, with higher levels in areas where fluorine is added to the water supply (4.5–50.7 vs. 4.3–14 μg l^{-1}, respectively). APP recommends no fluorine supplementation to BF infants in the first 6 months of life, but suggests fluorine supplementation from ages 6 months to 3 years in areas where water supply has limited concentration. Iodine concentration in milk is affected by maternal intake and has a strong correlation with maternal milk intake since cow's milk is a rich source of iodine. In addition, salt iodization increases milk iodine; the milk iodine levels rose from 70 to110 μg l^{-1} between 1950s to 1985 in the United States.

Bioactive Components of HM

HM contains a multitude of biologically active compounds that are likely to have a key role in the health benefits of HM. Many of these components are proteins and are thought to modulate immune function, response to infection, intestinal bacterial colonization, and development. In addition, the HMOs, which are not digested by the infant, act as prebiotics and stimulate gut colonization and have antiadhesive properties against pathogenic bacteria. Infant formulas do not contain many of these bioactive components, and it may explain differences between disease incidence between BF and FF.

Hormones and Growth Factors

An array of hormones and growth factors are present in HM. Although their role for the newborn is still unclear, many may contribute to the healthy development of the immune and neurological systems and maturation of the gastrointestinal (GI) tract. Hormones in HM include adrenal gland hormones (e.g., corticosteroids, glucocorticoids, cortisol, and corticosteroid), gonadal hormones (e.g., estrogen and progesterone), brain–gut hormones (e.g., gonadotropin-releasing hormone, somatostatin, growth hormone-releasing hormone, and thyrotropin-releasing hormone), insulin-like growth factors (e.g., IGF-I and IGF-II), epidermal growth factors, transforming growth factors (α, β1, and β2), growth hormones (e.g., insulin, relaxin, growth hormone, and prolactin), neurotrophic factors (e.g., brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and ciliary neurotrophic factors), and hormones that regulate metabolism (e.g., leptin, adiponectin, resistin, ghrelin, apelin, nesfatin-1, copeptin, and adropin). Hormone and growth factor concentrations vary by

the stage of lactation. For instance, colostral IGF-I, vascular endothelial growth factor, adiponectin, and insulin contents are higher in transitional than mature HM.

Adipokines are cytokines that are released from adipocytes with hormone-like activities. Adipokines in HM include leptin, adiponectin, resistin, ghrelin, and apelin. They are purported to regulate energy homeostasis, glucose homeostasis, food intake, and energy balance, but the bioactivity of these factors in HM has not been directly tested. Other proteins involved in energy metabolism that are present in HM include nesfatin-1, copeptin, adropin, irisin, and obestatin. It is hypothesized that adipokines and hormones in HM may contribute to the differences in body composition seen in BF versus FF infants in early life.

Among the most studied bioactive components in HM are insulin, IGF-I, and EGF. Animal studies have demonstrated that all three proteins given orally are most likely not absorbed, but act locally within the GI tract, resulting in small intestine maturation and decreased gut permeability. While the insulin concentration in HM remains constant throughout lactation, IGF-I and EGF are higher in colostrum than mature HM.

Transforming growth factor β (TGF-β) family constitutes the major cytokines in HM, with TGF-β2 being the most abundant. TGF-β2 is proposed to stimulate the maturation of the infant's intestinal immune system. Recent studies suggest that TGF-β1 and TGF-β2 survive the low pH of the infant's stomach and are absorbed by the infant's intestine. TGF-β may influence the immunity beyond modulating the GI tract.

Lastly, neurotrophic factors such as BDNF and GDNF may reduce the risk of NEC by modulating the function and integrity of the infant's gut. Specifically, BDNF helps with enteric nervous system development, enhances peristalsis, and protects against intestinal infections. GDNF is involved with the development of the enteric nervous system and maintenance of the intestinal epithelial barrier integrity.

Immunological Factors

HM contains factors that stimulate the development of the infant's immune system, including immunoglobulins, cytokines, maternal mammary epithelial cells, maternal immune cells (e.g., macrophages, neutrophils, natural killer cells, T cells, B cells, and progenitor cells), nucleotides, chemokines, long-chain PUFA, and compounds that promote microbial intestinal colonization. Secretory IgA is the most abundant and biologically active immunoglobulin found in HM. It resists proteolytic digestion in the stomach, and its abundance may compensate for the infant's inability to produce sufficient levels of sIgA in the first month postpartum. Specifically, sIgA binds to microbial antigens, blocks adhesion of pathogens to epithelial cells, enhances phagocytosis, and modulates local immune function. Other immunoglobulins such as IgG and IgM are present in much smaller quantities in breast milk.

Cytokines are multifunctional peptides or glycoproteins that bind to receptors on immune and nonimmune cells to produce various actions in the immune system, including stimulating the development of the infant's immune and GI systems. Many cytokines are present in HM, including IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, TGF-β, INF-γ, TNF-α, and granulocyte-colony stimulating factor, with IL-6, IL-8, IL-10, TGF-β, and TNF-α being the most abundant. Evidence suggests that cytokines are produced in the mammary gland. Osteopontin is a multifunctional protein with cytokine-like activity that has been shown to regulate the development and maintenance of the immune system and enhance host resistance to infection.

Antimicrobial Peptides

Among the antimicrobial peptides detected in HM are cathelicidin (LL-37), human β-defensin 1 and 2, lactoferrin, sIgA, lactoperoxidase, and lysozymes. The presence of antimicrobial peptides in HM has been associated with protection against mastitis in the mother while simultaneously acting as immunomodulators for the infant. Antimicrobial peptides from the cathelicidin and defensin families have been shown to protect the epithelial surfaces of the infants' intestine, lung, and skin from bacterial colonization and modulate the potency of neutrophils, macrophages, and mast cells.

Lysozyme and lactoferrin are antimicrobial whey proteins that also give rise to potent antimicrobial peptides during the process of digestion by the infant. Lysozyme is secreted by polymorphonuclear leukocytes and degrades the peptidoglycan cell walls associated with most bacteria. The high concentration of lysozyme found in HM ($0.3–0.5\ g\ l^{-1}$) may play a role in local protection of the infant's GI tract. Lactoferrin is an iron-binding antimicrobial protein that produces a potent antimicrobial peptide lactoferricin after being hydrolyzed by pepsin in the stomach. Lactoferricin's antimicrobial property is associated with its high affinity for iron. Other mechanisms of bactericidal activity include increased permeability of the cell membrane, thereby impairing the function of the cell, and inhibition of DNA, RNA, and protein synthesis after incorporation of lactoferricin into the bacterial cytoplasm. Lactoferrin-derived peptides share common characteristics with antimicrobial peptides such as the positively charged region of the peptide that contribute to its antimicrobial effects.

Conclusions

Health organizations worldwide support breastfeeding as the optimal form of nutrition for the human infant. HM is a living fluid that meets the nutritional needs and contains many bioactive components that likely foster early postnatal development and contribute to the demonstrated benefits in health outcomes associated with breastfeeding.

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Hunger

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Glossary

Hedonic hunger A drive to consume palatable food, which is driven by pleasure and not a need for energy.

Homeostatic hunger The drive to restore the body to a state of homeostatic equilibrium. That is, when the body is in energy-deficit (more energy has been expended than energy that has been taken in), the body will respond by creating a strong motivational drive for the individual to seek and consume food.

Hunger A motivational force to find and consume food, which often (but not necessarily) precedes an eating episode.

Satiation The process that develops during eating that generates negative feedback to end the eating episode. This determines meal size (energy, weight, or volume).

Satiety A state of noneating, characterized by the absence of hunger, that follows at the end of a meal and that arises from the consequences of food ingestion. This determines the length of time between eating episodes.

Definition

The term 'hunger' is used in many different ways. It can be used figuratively as a motivational driver (e.g., 'She's hungry for success'), to refer to cravings for specific foods (e.g., something sweet), and to refer to malnutrition and starvation (i.e., 'world hunger'). In a scientific sense, hunger has a more precise definition. Here, we refer to hunger as the motivational force that drives people to seek and consume food. There are two forms of hunger: firstly, homeostatic hunger, which is the body's drive to increase energy intake in response to an energy deficit, in order to restore energy balance or equilibrium (this is an important biological expression of the body's need for energy to sustain growth and life) and secondly, hedonic hunger, a powerful psychological driver to eat foods known to be pleasurable and rewarding. These expressions of hunger are not always distinct and it is not always possible to identify which type of hunger a person may be experiencing. For example, a person who has not eaten for several hours and whose upper digestive tract is empty may experience not only homeostatic hunger but also hedonic hunger triggered by others nearby eating palatable foods.

Hunger can be inferred in two distinct ways within nutritional science. Firstly, it can be inferred through directly observable behavior, such as the willingness to work for food or through observable events such as a long period of food deprivation (this is the method used in animal studies). In this sense, hunger becomes a mediating concept or an intervening variable. Secondly, subjective feelings or sensations of hunger can be measured through rating scales and other measurement devices. The subjective perception of hunger is often accompanied by easily identifiable bodily sensations in the stomach, mouth, throat, and head, for example, increased saliva production, stomach rumbling, and preoccupation with food-related thoughts.

It is worth noting at this point that eating food ordinarily decreases the sensation of hunger and increases the sensation of fullness. However, although hunger and fullness are related concepts, they are not opposite dimensions of the same concept. For example, fullness can be increased via gastric distention (e.g., by inflating a balloon in the stomach or through ingesting large quantities of water) but these methods only marginally reduce hunger. This suggests that physical sensations of emptiness within the gastric tract are only one aspect of hunger sensations.

Measurement of Hunger

Hunger is an internal subjective state and there is not an objective 'standard' by which internal experiences can be calibrated. There are therefore several difficulties in attempting to objectively quantify and measure hunger. Firstly, there is an inherent mistrust of subjective reports of appetite, which are often varied, and therefore, the accuracy of which is often doubted. Another difficulty is that the term 'hunger' is not only a scientific concept but also a commonly used colloquial word and people may have their own individual interpretations of what the word refers to. These difficulties are somewhat illustrated by the large amount of between-subject variation in hunger reports.

Measurement Scales

The two most common methods for quantifying hunger are fixed-point rating scales and visual analog scales (Figure 1). Fixed-point rating scales are quick and simple to use and provide numeric data, which are easy to analyze. These scales can be affected by the word choice and the number of points chosen. Furthermore, past examples of these scales show that they vary considerably in complexity. In considering the appropriate number of points to be included in this type of scale, the freedom to make a range of possible responses must be balanced against the precision and reliability of the device. Research indicates that scales with an insufficient number of fixed points can be insensitive to subtle changes in subjective experience. In addition, the fixed points affect how people interpret the scales and distribute their ratings. One way of overcoming some of these failings is to remove these points.

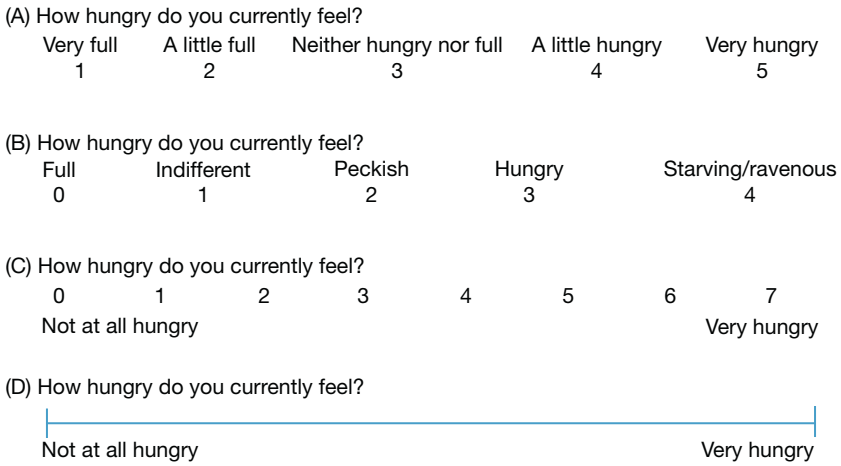

Figure 1 Examples of different types of scales used to determine hunger: fixed-point scales with points defined (a and b), fixed-point Likert scale (c), and visual analog scale (d).

Visual analog scales are normally 100 mm horizontal lines, which allows data to be easily converted into percentage scores (they can be longer to allow more freedom of responses). Participants are asked to mark the line at the point that most accurately reflects their current feelings of subjective hunger. The researcher then measures the distance from the negative end of the scale (not hungry) to the participant's mark, yielding a score of 0–100 (in the case of 100 mm lines). The scales are unbroken and unmarked, which has the advantage of making it difficult for participants to remember exactly where they may have marked the line in previous hunger questions or assessments. This helps to reduce a cross-checking response bias, whereby participants amend some question answers to be consistent with previously answered questions, which can overly inflate the reliability of some scales.

Scales can be computerized or presented via pen and paper. Computerized scales remove human error in recording the data and can ensure that scales are completed at precise time points in the day. By removing verbal labels (except the end anchors), visual analog scales retain the advantages of fixed-point rating scales while avoiding many of the problems with uneven response distributions and are therefore often the preferred scale in many experiments.

It is important to interpret these scales' results properly. For example, it cannot be assumed that the difference between 20 and 30 mm on a visual analog scale is perceptually the same as the difference between 80 and 90 mm. Nor can a hunger rating of 80 mm be said to represent a feeling of hunger that is twice the intensity of one rated as 40 mm. The variation of scores on these scales represents fluctuations in hunger perception and not absolute change of some physical commodity. Individuals will use these scales idiosyncratically (each individual may interpret the scale and gauge their response differently). For example, a minority of subjects are reluctant to make ratings away from the upper or the lower end points of the scale, despite clear instructions. This leads to the problem of 'end effects.' However, participants are generally consistent on repeated occasions (participants will use their own parameters, producing a reliable record of alterations in an individual's appetite). Despite their limitations, data from such scales are often analyzed using parametric statistics, such as analysis of variance, and in general, this appears to be satisfactory.

Hunger and Eating Behavior

The subjective experience of hunger is a biologically useful indicator of a depleted nutritional state and often precedes an eating episode. However, hunger and eating behaviors, although closely linked under many circumstances, are not always entirely dependent upon each other. Hunger is neither a necessary nor sufficient condition for eating to occur. There are many examples of people who are hungry but do not eat, for example, those who fast for political or moral reasons, those who deliberately restrict their energy intake for weight loss, or even those who do not want to spoil their appetite before a planned evening meal. Hunger is therefore not a sufficient condition to cause food intake, even when food is readily available. People can, and regularly do, resist the drive to consume. Hunger is also not a necessary condition to cause food intake. Often, people will overeat at special occasions such as weddings and Christmas, will eat a desert despite having just consumed a filling meal, and finish a large meal in the spirit of economic prudence.

Laboratory-based research notes that increasing the palatability and variety of food can encourage eating beyond the limits of normal satiety. It takes longer to achieve sensory-specific satiety (where one becomes bored of a particular flavor) with highly palatable and energy-dense foods. It is also easy to overconsume energy-dense foods because they can be eaten more quickly. As satiety signals are not instantaneous, eating quickly makes it more likely that a person will overconsume. Therefore, it is misplaced to criticize the validity of hunger ratings due to a discrepancy with recorded food intake, as the previous examples show that in certain circumstances, the two can be disengaged.

Proximity to food cues can have dramatic, instantaneous effects on appetite expression. For some individuals,

responsiveness to food cues overwhelms normal appetite control and the normal rhythm of hunger. For others, eating in the 'absence of hunger' is a component of their natural eating repertoire. It is clear that hunger ratings cannot be used simply as a proxy measure for food intake. Equally, when such factors are taken into account, there is good evidence that self-report ratings of hunger correlate statistically and meaningfully with eating.

By questioning the relationship between hunger and eating, we are also forced to place the action of hunger within a broader context of social and psychological variables that moderate food choice and eating behavior. Eating patterns and food intake are affected by habits, attitudes, and opinions about the value and suitability of foods and an overall liking for them. Food can be avoided, even when an individual is hungry, for health, moral, or political reasons: for example, reducing sugar intake to protect against diabetes, eating a vegetarian diet in support of animal rights, and avoiding palm oil to reduce the impact of deforestation. These factors, derived from the cultural ethos, largely determine the range of foods that people consume and sometimes the timing of consumption. In addition, recent research suggests that social modeling and mimicry can have an important impact upon food intake. Laboratory research suggests that a participant's *ad libitum* food intake can be manipulated by how much a stooge eats and that even food-deprived participants will eat less if a stooge only eats a little. Fluxes in hunger are therefore only one aspect of the range of eating determinants.

Hunger, Satiation, and Satiety

Hunger is a motivational driver to seek and consume food. When a meal is initiated, the process of satiation will begin, which generates negative feedback to end the eating episode. Hunger declines as satiation develops and usually reaches its lowest point at the end of a meal. Satiation therefore determines meal size (energy, weight, or volume). Satiation can be measured via eating rate within a meal. Satiety is defined as a state of noneating, characterized by the absence of hunger, which follows at the end of a meal and arises from the consequences of food ingestion. This determines the length of time between eating episodes. The intensity of satiety can be measured by the duration of time until the next eating episode, by the amount consumed at the next meal, or by how long hunger is suppressed.

Satiety does not refer to a single event or process, but rather an interconnected series of sensory, cognitive, postingestive, and postabsorptive signals. By definition, satiety is not an instantaneous event but occurs over a considerable time period. In the very short term, hunger is inhibited by sensory effects generated through the smell, taste, temperature, and texture of food. Hunger is inhibited for a little longer by cognitive beliefs about the properties of foods and appropriate portion sizes. Hunger is increasingly suppressed by postingestive processes such as gastric distension, rate of gastric emptying, and the release of hormones such as cholecystokinin and peptide YY. These factors will inhibit the rebound of hunger during the early postprandial period. In the postabsorptive phase of satiety, metabolites are absorbed from the intestine into the bloodstream. These signals will continue to suppress hunger late into the postprandial period.

Hunger and satiety are affected by the nutritional composition and structure of foods; therefore, some foods have a greater capacity to maintain suppression over hunger than others. This will depend on the food's sensory impact, physical characteristics, energy density, and macronutrient composition. The satiating potential of foods is often tested via a preload design, whereby isocaloric 'preloads' (usually soup or milk shakes) are consumed before an *ad libitum* meal and their effects on intake are assessed.

Research suggests that per unit of energy proteins are the most satiating macronutrient, then carbohydrate, and then fat. The satiating effect of protein may depend on the preabsorptive factors of elevated cholecystokinin and glucagon-like peptide-1 release and the postabsorption of circulating amino acids. The satiating effects of protein are prevalent in short-term studies in both lean and obese participants. Emerging evidence suggests that high-protein diets can have medium-term effects on satiety. Current research is investigating the long-term satiating effects of high-protein diets. Fats are the most energy-dense of the macronutrients at 9.5 kcal per gram and high-fat diets (in comparison with low-fat and high-carbohydrate diets) have a disproportionately weak action on satiety.

Dietary fiber is highly satiating for several reasons: It takes longer to chew (sensory satiety), soluble fiber binds to water and swells causing bulk (mechanical satiety), it slows down gastric emptying and remains in the stomach longer (mechanical satiety), and soluble fiber prolongs the intestinal phase of nutrient digestion and absorption (thereby prolonging the postabsorptive satiety signal). Fiber-rich foods can therefore suppress hunger for longer. Hunger is also reduced more by solid foodstuffs than energy-loaded drinks. Sugar-sweetened beverages provide energy without many of the cues for satiety (such as mastication, bulk, and cognitive beliefs about the drink being filling).

Hunger: Physiological Determinants

Hunger often begins with signals from the environment, such as the sight and smell of food and learned contextual cues such as location and time of day. Environmental cues of highly palatable foods can instigate hedonic hunger, which is a drive to consume for pleasure and not energy. Hedonic hunger is underscored by the endogenous opioid system, such as beta-endorphins released in the hypothalamus. Research suggests that opioid antagonists, such as naltrexone, reduce food intake by lowering the perceived pleasantness of foods. It should therefore be noted that the biological mechanisms critical to the expression of hunger are not independent of psychological ones. Indeed, the sensory and cognitive cues that stimulate hunger produce physiological changes that anticipate the ingestion and metabolism of energy and subsequently aid these processes. This gives rise to the psychological factors critical in the expression of appetite.

The ability for external stimuli to incite an anticipatory hunger is known as the cephalic response, which prepares the body for digestion. The cephalic response is expressed in several parts of the gastrointestinal tract and acts to anticipate food ingestion and optimize nutrient absorption. Cephalic

responses include increased release of saliva, stomach acid, and digestive enzymes. Upon food intake, stomach distension and the detection of macronutrients within the gut act as powerful satiety cues, bringing a meal to an end and temporarily inhibiting further consumption. Eventually, hunger again prevails and food intake follows. The flux between hunger and satiety is episodic and underpins the expression of our eating behavior throughout the day.

In addition to episodic hunger signals, tonic signals such as a reduction in blood glucose levels (glucoprivation or hypoglycemia) or low levels of the hormone leptin (produced by the adipose tissue) indicate a deficit in available energy and stored energy reserves and therefore promote feelings of hunger. Other tonic factors that indicate the body's energy status, such as adiponectin, cytokines, and gonadal hormones, appear to act on energy regulator centers within the brain, particularly the hypothalamus, mainly to suppress hunger. Again, their absence serves to promote appetite.

However, not all physiological signals, episodic or tonic, inhibit hunger. The peptide hormone ghrelin is a potent stimulator of food intake. It is released by the stomach and the brain and is detected by receptors in the upper intestine, the mesolimbic system, and various orexigenic circuits in the hypothalamus. Blood levels of ghrelin increase with fasting, reduce after meals, and are correlated with self-reported hunger levels. Further studies demonstrate that ghrelin infusions increase food intake. It may be due to this hormone's role in appetite that most people have a three meal per day macrostructure of food intake, as an individual's preprandial ghrelin levels tend to be similar before breakfast, lunch, and dinner, suggesting that this hormone is a major instigator of meals. The rare disorder Prader–Willi syndrome is a genetically determined conditioned characterized by chronically high ghrelin levels and insatiable hunger. Most people with the disorder are obese and their excessive levels of food intake are associated with both a delayed reduction in hunger while eating and a more rapid return to premeal states when eating has finished. However, mice with a knockout of the ghrelin gene eat almost normally, suggesting that appetite is too important to entrust to only one mechanism.

Other hunger signals from the digestive tract include the lack of satiety hormones (such as cholecystokinin, peptide YY, amylin, and glucagon-like peptide-1). Finally, in further support of the assertion that the body's energy regulatory system is far too important to be entrusted to any singular mechanism, many neurotransmitters and neuropeptides have been identified that stimulate appetite, including neuropeptide Y, agouti-related peptide, melanin-concentrating hormone, orexin A, melanocortin-concentrating hormone, glucocorticoids, endocannabinoids, and galanin. It should be noted that the hypothalamus is a key location for the release of such appetite-promoting neuropeptides (particularly the lateral hypothalamus and the arcuate nucleus of the hypothalamus) and the ventrolateral medulla is important for receiving hunger signals transmitted to the brain via the vagus nerve from the rest of the body.

Hunger: Cognitive and Affective Consequences

It is a common observation among the lay public that hunger affects concentration, attention, and memory. Recent research supports these observations. Systematic reviews indicate that children who eat breakfast have superior cognitive function and academic performance in comparison with those who do not eat breakfast. Such research has led to health and well-being initiatives introducing breakfasts in schools.

Furthermore, bodily sensations of hunger often accompany diffuse feelings of restlessness and excitability. The consumption of food changes both the pattern of physical sensations and the accompanying emotional feelings. Classic drive reduction theory suggests that hunger is associated with unpleasant feelings, and food consumption eases these. Thus, for example, after eating, an aching stomach becomes relaxed and feelings of excitement and irritability become contented. People frequently make judgments regarding their own hunger state using these sensations. However, there is much within- and between-subject variability. In other words, not every person sitting down to eat nor every act of eating by an individual will necessarily be accompanied by bodily sensations of hunger or aversive emotional states.

Conditioned Hunger

As the classical work of Pavlov demonstrates, hunger responses can be easily entrained to specific stimuli. Omnivores, like carnivores, are meal eaters (in contrast to grazers); however, their dietary decisions are more complex. One of the essentials for an omnivore faced with a variety of new and different foods is the capacity to learn. It is not possible for an innate preference or aversion to guide the choice of every possible food. Therefore, we learn which foods are beneficial (and which are not) through experience.

Emerging research suggests that some food preferences can be acquired prenatally and during lactation. This is because flavonoids from the pregnant woman's food are passed to the fetus via the amniotic fluid and to infants via breast milk. Babies are often averse to bitter-tasting vegetables (which is an evolutionary advantage, as many bitter alkaloid plants are poisonous to humans). However, babies who are exposed to high levels of bitter vegetables in the womb are more likely to find vegetable juices pleasant in comparison with babies who were exposed to lower levels. This emphasizes how exposure to different flavors is essential to developing our taste preferences. This principle extends throughout our lifetimes.

Through our experiences, we learn that ingesting food creates pleasant postingestive sensations of satiety that serve as a positive experience, leading to learned preferences, as studied by Birch. Alternatively, if consumption leads to an association between a food and negative gastrointestinal consequences such as nausea, then a conditioned aversion to that food (or even other similar tastes and flavors) is likely to develop. The learning process involves the association between the sensory and the postabsorptive characteristics of foods. In this way, the sensory characteristics of foods act as cues and come to predict the impact that foods will later have. Consequently, these cues should suppress hunger according to their relationship with subsequent physiological events. The potency of taste aversions following vomiting or illness indicates the strength of cue–consequence learning. Food intake depends upon the stability and reliability of the

relationship between the taste (sensory cues) and physiological effect (metabolic consequences) of food.

It is possible to demonstrate experimentally how human beings adapt their eating to a food's energy content. A distinctively flavored food that contains 'extra' hidden energy, presented on several occasions, will result in a change in eating and in preference. When deprived of food, the subjects' preference for the taste increases with gained experience. If presented when satiated, preference for the taste decreases. This process is also observable in young children, who eat smaller meals following a taste previously associated with a high-energy snack and larger meals following a taste previously associated with a low-energy snack. Our hunger can thereby be potently reduced by the learned associations of satiety. This relates to the cognitive factors discussed earlier regarding a person's beliefs about foods and their satiating potential.

In environments where food sources remain limited and dietary variety is controlled, learned responses may effectively control subjective feelings of appetite. However, in wealthy countries where there is an abundance of food, there is distortion, variation, and extreme complexity in the relationship between sensory characteristics and nutritional properties. The conditioned control of hunger is therefore weakened or lost in such rich environments. In such a scenario, hunger may become less controllable. The variety of foods available has many different sensory characteristics. Furthermore, some ingredients preserve sensory qualities while altering the nutritive value (such as diet products that substitute sugar for sweeteners or that remove certain ingredients and replace them with artificial bulking agents). Learned hunger therefore is a relatively less important factor when the food supply contains many food items with identical tastes but differing metabolic properties.

In addition to learning associations between the sensory properties of foods and their satiating potential, people also learn association between food cues and future food intake. Food cues are plentiful and researchers have found that food intake increases after television viewing when food adverts are shown and also after playing advert games (online games often designed and produced by food manufacturers, where food products are highly prominent). Some individuals are more receptive to the effects of food cues than others and demonstrate an attentional bias toward them (their attention is more easily 'grabbed' and maintained by food-related stimuli). Reactivity to food cues emphasizes the importance of conditioned hunger and how associating cues with the naturally rewarding properties of energy intake serves to promote hunger and eating behaviors.

The naturally rewarding properties of food consist of two distinct psychological components, wanting and liking, that are controlled by separate neurobiological systems. Wanting is primarily mediated by dopamine, whereas liking is mediated by opioids in hedonic hot spots. Some research has noted that obese individuals are more sensitive to the rewarding properties of food than lean individuals, promoting greater hunger. While other research suggests that obese individuals have greater hunger to compensate for reward deficiency due to reduced dopamine function; more research is needed to resolve this debate.

Disorders of Hunger

The clinical eating disorders anorexia nervosa and bulimia nervosa are commonly believed to encompass major disturbances of hunger. Yet the role that hunger may play is not entirely clear. Contrary to the literal meaning of the term, 'anorexia' is not experienced as a loss of appetite. Rather, clinicians recognize that anorexics may endure intense periods of hunger during their self-restricted eating. For some, their strength in resisting intense episodes of hunger provides a feeling of self-mastery and control that is absent in other areas of their lives. Research suggests that restricting anorexics (compared with those who binge) have the greatest blunting of hunger responses and that this disturbance in hunger is not a product of other areas of perceptual confusion.

There is evidence that under conditions of total starvation, hunger may become temporarily diminished. This circumstance is extremely rare and obviously relatively brief. Once eating is recommenced, hunger returns rapidly and with extreme intensity. The accounts of the male volunteers who submitted to a 6-month period of semistarvation during World War II (the 'Minnesota Starvation Experiment') are a testament to the extreme power of hunger. Referred to as semistarvation neurosis, the behavior of these men was shaped by their need for food, and their hunger experience was extreme. Nearly two-thirds reported feeling hungry all the time and a similar proportion experienced physical discomfort due to hunger. Participants described a marked increase in what was referred to as 'hunger pain.' For some, this was mildly discomforting and vaguely localized in the abdomen. For others, it was extremely painful. This account is especially useful in reminding us why energy-reduced diets aimed at achieving weight loss are often difficult to maintain and easy to abandon.

Bulimia literally means 'ox hunger'; however, the term is imprecise. Close analysis of the precursors of binge episodes shows hunger to be lower than it is before a typical meal. In addition, although the urge to eat may be strong during a binge, the large amount of food consumed implies some defect in satiation rather than in hunger. Moreover, binging is often a well-practiced behavior that develops and changes with time. Similarly, binge-eating disorder (binging without the purging behaviors that are present in bulimia) is characterized by binging even in the absence of hunger and continuing until uncomfortably full. For all three of these disorders, it is likely that a stable eating pattern is necessary in order to normalize the experience of hunger, a process that may take a long time to establish. The question of whether obesity reflects a disorder of hunger is now regarded as largely redundant. Obesity is strictly a disorder of energy balance and partitioning. There is limited evidence that heightened hunger contributes to excessive energy input.

See also: Appetite Control in Humans: A Psychobiological Approach; Eating Disorders; Energy: Intake and Energy Requirements; Food Environment; Gut Hormones; Obesity Management; Obesity: The Role of Diet; Pituitary Gland: Pituitary Hormones; Satiety; Snack Foods: Role in Diet.

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Hypertension and Diet

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Why Do We Care About Hypertension and Diet?

Hypertension is a chronic medical condition in which the blood pressure (BP) in the arteries is elevated. Untreated, hypertension increases the risk for coronary heart disease (CHD) and stroke (Figure 1).

After poor diet and tobacco use, hypertension is the third leading risk factor for premature death in the United States, and approximately one in three US adults (78 million) suffer from this condition. It affected ~972 million people worldwide in 2000, and global prevalence is projected to almost double, to 1.56 billion, by 2025. Hypertension is responsible for ~450 000 deaths annually and contributes ~8% to disability-adjusted life years (DALYs). Worldwide, hypertension is responsible for an estimated 9.4 million premature deaths and accounted for 7% of DALYs in 2010. These estimates are based on the World Health Organization (WHO)'s global burden of disease study, which compares a theoretical minimum risk exposure distribution (TMRED) to estimates of the actual population distribution. For example, the TMRED for tobacco use would be the distribution of the population that has never smoked in the past. For hypertension, the TMRED is defined as a systolic BP (SBP) distribution curve with a mean of 110–115 mmHg and a standard deviation of 6 mmHg.

A 2011 study by the American Heart Association (AHA) estimated that the direct medical costs of hypertension in the United States would triple from ~$70 billion in 2010 to $200 billion in 2030 and indirect costs associated with lost productivity would increase from $23.6 billion to $39.8 billion. A 2009 study by Gaziano and colleagues estimated global health-care costs attributed to hypertension among adults to be $370 billion in 2001, about 10% of all health-care expenditures. The authors estimated that over a 10-year period, hypertension could cost nearly $1 trillion globally in increased health spending and $3.6 trillion annually in indirect costs. Each year, excess sodium consumption, a significant risk factor for hypertension, is estimated to kill between 40 000 and 90 000 Americans and cause up to $20 billion in medical costs.

Hypertension is a significant public health priority, because it is very common and largely preventable. Early detection and effective treatment can reduce morbidity and mortality significantly. Poor nutrition, especially high salt consumption, is a significant contributor to high BP, and a low-sodium diet has been shown to reduce a person's risk of developing hypertension. Only 82% of US adults with hypertension are aware of their condition, and three-quarters are using antihypertensive drugs; however, only about half of these individuals are controlling their condition to target BP levels.

Definition

Although there is no evidence of a BP threshold that separates healthy people from people with hypertension, guidelines have been established to diagnose and treat hypertension in clinical practice. In the United States, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) defined BP as comprising four categories ranging from 'normal' to 'stage 2 hypertension' (Table 1).

In December 2013, the Eighth Joint National Committee (JNC8) published their '2014 Evidence-Based Guideline for the Management of High Blood Pressure in Adults.' The controversial guideline proposed less restrictive BP targets for adults 60 years of age and older and for people with diabetes and chronic kidney disease. JNC8 set goals for SBP and diastolic BP (DBP) at $<$150/90 mmHg, a sizable increase from the JNC7 goal of $<$140/90 mmHg. The recommendations were based on a different methodology from that used for JNC7. For example, unlike JNC7, JNC8 restricted the scope of evidentiary review to published randomized controlled trials.

Contrary to previous practice, the US National Heart, Lung, and Blood Institute (NHLBI) did not publish the JNC8 guideline; rather, it declined to endorse the new recommendations. Furthermore, none of the national scientific organizations, including the AHA, the American College of Cardiology (ACC), and the American Society of Hypertension, have accepted all of the JNC8 recommendations, particularly the less restrictive goals for BP treatment and control for older adults. The AHA and ACC issued a statement endorsing JNC7 as the national standard, and proposals are under way to develop a new AHA/ACC guideline. An independent study by Navar-Boggan and colleagues found that compared with JNC7, JNC8 was associated with a reduction in the proportion of US adults recommended for antihypertensive treatment and a substantial increase in the number of adults, primarily older adults, considered to have achieved goal BP.

The somewhat arbitrary nature of BP classification is also demonstrated by the fact that the European Society of Hypertension and the European Society of Cardiology define BP differently than does the US system. 'Optimal' BP is SBP $<$120 mmHg and DBP $<$80 mmHg, whereas 'normal' BP is defined as 120–129 mmHg SBP and/or 80–84 mmHg DBP. The European classification does not have a category for prehypertension and defines three grades of hypertension.

For children, hypertension is not defined by absolute levels but on the relative rank of a child within the BP distribution. Reference distributions for sex, age, and height are used to determine the 90th, 95th, and 99th percentiles (Table 2). The

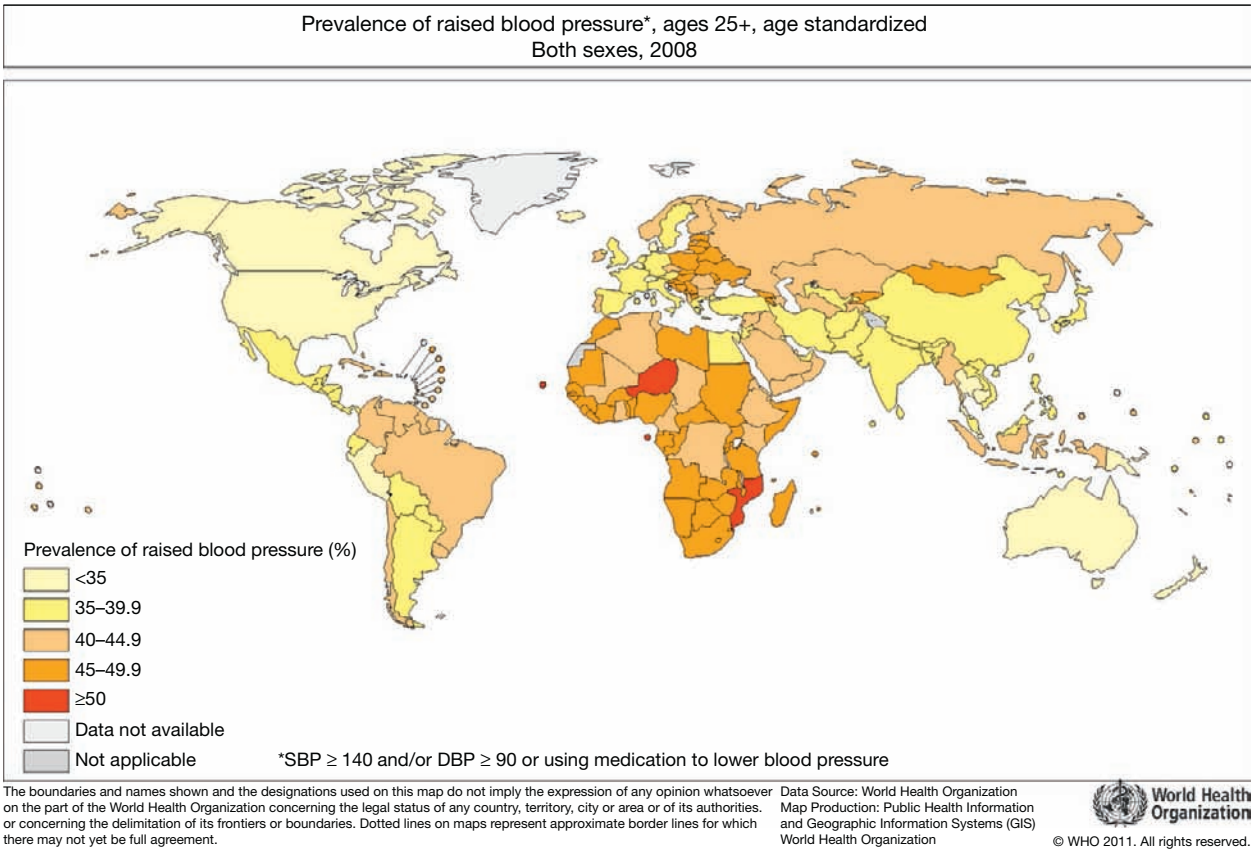

Figure 1 Prevalence of raised blood pressure ages 25+, age standardized. Both sexes 2008.

Table 1 Classification of blood pressure in adults (JNC7)

Classification	*SBP (mmHg)*	*DBP (mmHg)*	*Treatment*
Normal	<120	and <80	Encourage lifestyle modification to keep to goal No antihypertensive drug therapy unless for compelling indications such as diabetes
Prehypertension	120–139	or 80–89	As earlier, but initiate lifestyle modifications
Stage 1 hypertension	140–159	or 90–99	Lifestyle modifications Thiazide-type diuretics for most patients Also consider ACEI, ARB, BB, CCB, or combination For compelling indications, drug therapy as needed
Stage 2 hypertension	≥160	or ≥100	Lifestyle modifications Two-drug combination for most (usually thiazide-type diuretic + ACEI, ARB, BB, or CCB) For compelling indications, drug therapy as needed

SBP, systolic blood pressure; DBP, diastolic blood pressure; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BB, beta blocker; CCB, calcium channel blocker.

Source: Chobanian, A. V., Bakris, G. L., Black, H. R., et al. (2003). Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Journal of Hypertension* **42**, 1206–1252.

NHLBI released an updated evidence review through 2008 for high BP among children and adolescents, including an algorithm and flow diagram to help clinicians identify hypertension. From a population-based, health-promotion perspective, the AHA defines cardiovascular health as the product of seven science-based risk factors (Life's Simple Seven™), which include ideal BP defined as <120/80 mmHg in adults and below the 90th percentile in children.

Determinants of Hypertension and the Role of Diet

Although demographic factors such as age, gender, and race/ethnicity, as well as genetics and family history, are associated with BP, diet plays an important role in the development of hypertension. Studies comparing BP among Japanese people living in Hawaii with that of Japanese people living in Japan found higher BP among those living in Japan despite lower

Table 2 Classification of hypertension in children and adolescents

Classification	*SBP or DBP percentile*	*Treatment targets*
Normal	<90th	Recheck at next scheduled physical exam Encourage healthy diet, sleep, physical activity
Prehypertension	90th to <95th or if BP exceeds 120/80 mmHg even if <90th percentile up to <95th	Recheck in 6 months Weight-management counseling if overweight Introduce physical activity and diet management No pharmacotherapy unless compelling indications such as diabetes, chronic kidney disease, or heart failure
Stage 1 hypertension	95th to 99th + 5 mmHg	Recheck in 1–2 weeks if patient is symptomatic If persistently elevated, evaluate or refer within 1 month Weight-management counseling if overweight Introduce physical activity and diet management Initiate pharmacotherapy if needed or if compelling indications as previously mentioned
Stage 2 hypertension	>99th + 5 mmHg	Evaluate or refer within 1 week or immediately if patient is symptomatic Weight-management counseling if overweight Introduce physical activity and diet management Initiate pharmacotherapy

SBP, systolic blood pressure; DBP, diastolic blood pressure.

Source: National Heart, Lung, and Blood Institute. *The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents*. http://www.nhlbi.nih.gov/health-pro/guidelines/current/hypertension-pediatric-jnc-4; Labarthe, D. R. (2011). *Epidemiology and prevention of cardiovascular diseases: a global challenge* (3rd ed.). Sudbury, MA: Jones and Bartlett Learning.

body weight, which may be related to higher salt and lower potassium intake. The Japanese are at higher risk of stroke and have higher rates of hypertension than American and European populations. Ueshima and colleagues concluded that this may be explained by higher sodium and lower calcium and animal protein in the diet and higher alcohol intake among men in the Japanese population than in the Western population. Studies conducted in South Africa in the 1980s showed significant differences between the prevalence of hypertension in urban Zulu populations and that in rural Zulu populations: 25% versus 9.4%, respectively. The age-associated increase in hypertension seen in the urban population was not observed among rural Zulus. This suggests that environmental and dietary patterns may affect BP more than genetic or age-related factors. Increased physical activity and more traditional dietary patterns in the rural Zulu population may have affected BP favorably. Lower levels of obesity among rural Zulus and less exposure to the stresses of urban life, including detribalization, are factors thought to contribute to these differences. Similar rural–urban differences in hypertension rates were observed among the Xhosas of South Africa and the Haryana population of India.

Within the United States, non-Hispanic blacks are at higher risk than are other groups. Younger women also tend to have lower levels of hypertension than do older women or men. These patterns among adults have been observed across the world. Among children and adolescents, statistically significant differences between sexes, ages, and races have been observed. By age 12, boys have higher SBP than girls, and black boys have higher DBP than nonblack girls. The underlying causes of these differences are not yet well understood.

Family history is another important risk factor for hypertension. In one study of Utah students, adults 20–49 years old who had at least one first-degree relative with high BP at any age had a relative risk of high BP 2.3 times higher than that among students with no family history of hypertension. Several genotypes have been identified that influence renal salt handling via the renin–angiotensin–aldosterone axis.

Dietary Factors Affecting BP

High intake of fruits and vegetables and certain diets such as the DASH (Dietary Approaches to Stop Hypertension) diet have strong evidence of antihypertensive effects and are recommended by the Academy of Nutrition and Dietetics and the AHA. A systematic review of dietary factors associated with hypertension reports fair evidence for the antihypertensive effects of potassium, calcium, magnesium, and polyunsaturated fatty acids. The Nurse's Health Study of 41 541 US women found that age, body weight, and alcohol intake were significant predictors of hypertension, whereas calcium, dietary fiber, potassium, and magnesium were inversely associated with BP. These results suggest that diet contributes to BP regulation and a diet rich in fruits, vegetables, whole-grain bread, and rice may reduce BP.

Salt

There is a direct dose–response relationship between salt consumption (sodium or sodium chloride) and high BP: the greater the amount of salt in one's diet, the higher one's risk of hypertension. There is strong evidence from experimental studies that adopting a diet low in sodium lowers BP and reduces the risk of hypertension. A review by He and colleagues found that a reduction in salt intake of 6 g day^{-1} predicts a BP decrease of 7.11/3.88 mmHg in individuals with hypertension and 3.57/1.66 mmHg in those without hypertension. Reducing sodium intake to reduce BP is recommended by the Dietary

Guidelines for Americans, the WHO, the World Heart Federation, and other international groups.

Salt increases water retention, raising BP and affecting the kidneys, arteries, heart, and brain. High salt intake reduces the ability of the kidneys to remove excess water effectively. Over time, this additional stress can cause kidney disease, which hampers the kidneys in removing waste products from the body. As BP increases, artery walls become thicker to handle the strain, which increases BP further. Over time, the arteries can rupture or become blocked, preventing blood flow to the heart and other tissues and causing angina. Arterial damage can lead to reduced blood flow to the brain, resulting in vascular dementia.

Recommendations for sodium intake include <2300 mg day^{-1} (one teaspoon of salt) in the US Dietary Guidelines and <2000 mg day^{-1} for adults by the WHO. All national guidelines recommend reducing sodium consumption. In 2005–06, US men consumed an average of 10.4 g day^{-1} of salt, and women consumed 7.3 g day^{-1}. The majority of the sodium in the American diet – 75% – comes from processed foods. Only 6% is added at the table and 5% during cooking; the remaining 12% occurs naturally in foods. Bread can contain more than 200 mg sodium per slice, and deli turkey can contain more than 1000 mg per 3 oz. In China, 76% of sodium is added in home cooking, whereas 63% of sodium intake in Japan comes from soy sauce. In the United Kingdom, an estimated 95% of sodium comes from processed foods.

Weight

Maintaining a healthy weight helps normalize BP levels. High BP can be controlled with a weight loss of 5–10 pounds. Overweight and obesity are associated directly and indirectly with hypertension. Weight loss reduces BP in individuals with and without hypertension. In addition, hypertension predicts overweight. The Framingham Heart Study found that the relative risk for developing obesity was 1.62 among men with hypertension and 0.79 among men with normal BP. Among women, the relative risk for developing obesity was 1.45 for those with hypertension and 0.75 for those with normal BP. A maximum body mass index of 25 kg m^{-2} is recommended.

Alcohol

Excessive alcohol intake can increase BP. The risk for high BP increases for people who have more than two alcoholic drinks per day, and this relationship is independent of other risk factors such as age, obesity, and salt intake. The AHA recommends a maximum of two drinks per day for men and one per day for women, as well as at least two alcohol-free days per week. Hypertension risk increases by 57% among men and 81% among women who consume more than 3.5 standard drinks per day and by 147% among men and 181% among women who consume more than seven standard drinks per day. A standard drink is defined as 12 oz of beer, 5 oz of wine, and 1.5 oz of spirits.

Potassium

Potassium lowers BP through its vasodilating action and its natriuretic effects: the more potassium consumed, the higher the volume of sodium excreted in urine. Dietary potassium exerts a dose-dependent inhibitory effect on sodium sensitivity. The AHA recommends 4.7 mg day^{-1} of potassium to help control BP, and several guidelines recommend food sources of potassium (beans, dark leafy greens such as spinach, yogurt and milk, bananas, and winter squash) rather than supplements. Systematic evidence reviews suggest that potassium is associated with lower BP among individuals with hypertension, but estimates of the BP-lowering effect for individuals with BP in the normal range are mixed.

Fish oil

Omega-3s and fish oil are included in several national recommendations to address hypertension. Meta-analyses of the effect of fish oil intake on hypertension have been conducted, and consistent evidence suggests that fish oil supplements reduce BP in individuals with hypertension, although fairly high doses (more than 3 g) are generally required.

DASH diet

In addition to the specific issues discussed earlier, research on whole dietary patterns has shown promising results in the management of hypertension. The DASH trial was a multicenter randomized trial that tested the effects of different dietary patterns on BP. The study focused on three patterns – a control diet with nutrient composition typical of the American diet, a diet high in fruits and vegetables, and a 'combination' diet that emphasized fruits, vegetables, low-fat dairy products, whole grains, poultry, fish, and nuts and limited red meat, sweets, and sugar-containing beverages. It was also lower in saturated fat and cholesterol than the typical Western diet and provided higher amounts of calcium, potassium, magnesium, fiber, and protein. The sodium content was kept constant in all three diets, with an intake of 3 g day^{-1}.

The study showed that the DASH diet was the most effective in reducing BP among both normotensive and hypertensive individuals. The DASH diet reduced SBP and DBP by 3.5 (5.3–1.6) mmHg and 2.1 (3.6–0.5) mmHg, respectively, in normotensive people. In hypertensive individuals, the diet reduced SBP by 11.4 (15.9–6.9) mmHg and DBP by 5.5 (8.2–2.7) mmHg.

Reductions in BP were seen within 2 weeks of diet initiation and maintained for 6 weeks following cessation of the trial. Dietary adherence rates were high, with low participant dropout rates, suggesting that DASH is a feasible long-term diet.

The DASH diet is recommended in guidelines as a key lifestyle choice for the management of hypertension. The reduction in BP observed among people with normal BP also suggests that following the DASH diet might be helpful in preventing hypertension. The DASH diet recommends 8–10 servings of fruits and vegetables per day, far exceeding the recommended 5–7 servings per day for the general population. Recommendations for dairy products are consistent with those for the general population: 2–3 servings per day of predominantly low-fat dairy foods. The DASH diet is nutritionally balanced and can be adopted by the general population. However, because of its relatively high potassium, phosphorus, and protein content, it is not recommended for individuals with stage 3 or 4 chronic kidney disease.

The exact mechanism by which the DASH diet lowers BP is unknown. It is likely the combination of beneficial nutrients it provides rather than the effect of individual factors. Because of the high proportion of fruits, vegetables, and low-fat dairy products, the DASH diet is low in sodium and high in potassium and calcium. Some benefits may be due in part to its

more close resemblance to a vegetarian diet, which has a positive impact on BP, than a traditional Western diet. Further studies have shown that when combined with a low sodium intake, DASH results in even further reductions in SBP and DBP. The DASH sodium study observed stepwise decreases in BP with successive decreases in sodium intake of 150, 100, and 50 mmol day^{-1}. Reductions in BP were of similar magnitude to those yielded by antihypertensive drug therapy for people with mild hypertension. For this population, following the DASH diet is a feasible alternative to medication, and doing so may delay the need to begin using medication.

Observations of older populations suggest that the DASH diet in combination with reduced sodium intake may lessen the effects of age-related increases in BP. Greater reductions in BP due to the DASH diet have been seen among adults more than 45 years of age than among younger people. The DASH diet has also been shown to benefit African Americans by 1.5- to 2-fold more than it does white Americans. African Americans are a high-risk group who experience higher rates of hypertension than do white Americans. Wide adoption of the DASH diet at a population level could reduce the occurrence of cardiovascular disease (CVD) caused by hypertension. It is estimated that a population-wide reduction in BP through the intake of a DASH-like diet would decrease CHD incidence by 15% and stroke incidence by 27%. Consumption of a DASH-like diet is also associated with lower all-cause mortality and mortality from stroke among adults with hypertension. In addition, because it is consistent with dietary patterns recommended for the prevention and management of other cardiovascular risk factors such as hyperlipidemia and type 2 diabetes, the DASH diet may well be effective in preventing CVD caused by factors unrelated to hypertension.

Behavioral Strategies to Address Hypertension

Behavioral strategies that address lifestyle modifications have long been recommended as first-line or adjunctive treatment for hypertension. Following a healthy diet, reducing sodium intake, moderating alcohol intake, and exercising regularly are some of the lifestyle modifications that have been shown to lower BP. However, achieving and sustaining the necessary diet and lifestyle changes to manage hypertension can be challenging.

A number of studies have used behavioral strategies to encourage adherence to diet and lifestyle recommendations. These strategies have been based on a variety of behavioral theories and models such as social cognitive theory, the transtheoretical model, behavioral self-management theory, and the relapse-prevention model. Typically, the strategies are incorporated into interventions that emphasize skills training, motivational interviewing, self-monitoring, and self-regulation. The type of intervention implemented and its efficacy depend heavily on the target population and the chosen setting.

The social cognitive theory-based 'DASH-dinner with Your Nutritionist' trial showed significant improvements in BP management. Participants were African American adults from low-income areas who had poorly controlled BP. The intervention was a joint university and neighborhood initiative conducted at local health-care centers with the aim of promoting adoption of the DASH diet. Participants were divided into groups of 12–15 that met for 1–2 h weekly for 8 weeks. The courses were based on building knowledge and self-efficacy for following the DASH diet and included a range of behavioral strategies such as incentives, role-playing, and engaging social support. The sessions began with a nurse measuring BP and weight; this was followed by a brief nutrition education presentation from a nutritionist, meal service and 'table talk,' and recipe demonstrations and taste testing. BP was significantly lowered among participants who attended at least six of the eight sessions. This style of intervention could be applied to other at-risk groups as an effective community-based technique for achieving behavior change.

A culturally tailored behavioral intervention for improving hypertension-related health behaviors among Hispanic adults conducted at a community health center in North Carolina consisted of six weekly group sessions based on principles derived from social cognitive theory and techniques of behavioral self-management. It emphasized the importance of the individual's ability to regulate behavior by setting goals and attaining the skills necessary to achieve them. Motivational interviewing was used to help participants move through different stages of change. Participants were encouraged to monitor their daily intake of fruits, vegetables, dairy, and fat and to record physical activity; were provided with education on the DASH diet, food preparation, and weight loss; and took part in exercise sessions and cooking demonstrations. Cultural adaptations included conducting the study in a familiar place (a community health center), using a Spanish-speaking interventionist, and incorporating culturally appropriate food choices and physical activity. At the end of the intervention, all physiological, diet, and exercise outcomes were more favorable, with the exception of fat intake.

Home-based behavioral interventions, delivered via telephone or over the Internet, offer effective strategies for managing hypertension for those in remote areas or with difficulty accessing health-care facilities. Combined BP monitoring and a tailored behavioral telephone intervention were shown to improve BP over a 24-month period compared with standard clinical care involving antihypertensive medication. The telephone intervention centered on self-management strategies including adhering to a DASH-style diet, moderating alcohol intake, stopping smoking, and increasing physical activity.

Overall, evidence suggests that behavioral interventions are effective in managing hypertension in the short term, usually up to 6 months. A review of three common education-based behavioral interventions for managing hypertension – counseling, a structured training course, and self-monitoring of BP – showed that counseling about diet and lifestyle modifications was the most effective and offered significant improvements in BP compared with standard medical care. Long-term effectiveness has not yet been demonstrated. Despite this, behavioral interventions should be part of a comprehensive approach to managing hypertension that includes addressing environmental and cultural factors that encourage poor dietary and lifestyle habits.

Lifestyle Versus Medication for Hypertension

According to the AHA, reducing hypertension requires adopting seven key lifestyle modifications: eating a healthy diet, engaging

in regular physical activity, maintaining a healthy weight, managing stress, not smoking, adhering to prescription medication regimens, and limiting alcohol intake. Improving diet means eating higher nutrient quality foods and decreasing portion sizes if weight loss is needed. There are many options for medications to treat hypertension, including diuretics, antiadrenergics, direct-acting vasodilators, calcium channel blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers. The most common hypertension drugs act on the renin–angiotensin–aldosterone system, which affects blood volume, arterial pressure, and heart function.

The major challenge to pharmacotherapy is poor adherence. Nonadherence was observed among 28% of US patients with hypertension, with similar rates for hyperlipidemia and diabetes. Developing effective strategies to improve adherence is critical to preventing stroke and death. A Finnish study found that the relative risk for fatal and nonfatal strokes was 4.5 for patients with adherence rates of 30% or below compared with patients with adherence rates of 80% or above.

Innovations to improve adherence can be patient-focused or integrated into the health-care system. Data to support the use of electronic medication packaging devices are limited and variable. Devices with digital displays and audible alarms were found to be most useful in improving adherence; in addition, interventions integrated into the health-care system with greater complexity were often associated with improved adherence. For example, an Internet-connected packaging and feedback intervention called Glowcaps has been found to improve medication adherence by 27%. Lottery-based financial incentives have also shown promise. Volpp and colleagues found that the proportion of incorrect pills taken over 3 months decreased to 2.3% (with a \$5 day^{-1} expected lottery value) and 1.6% (with a \$3 day^{-1} expected value) compared with a historic mean of 22%.

Innovations to Address Hypertension

Individual-level interventions such as education campaigns that promote the DASH diet or drug therapy can reduce the prevalence of hypertension; however, only population-based strategies that target the underlying causes of high BP have the potential to reduce incidence and shift the distribution of hypertension. The WHO recommends adopting comprehensive national evidence-based policies to combat hypertension such as lifestyle modification, reductions in alcohol and sodium consumption, and increased physical activity and including more systems-based approaches such as food policy, voluntary reformulation of processed foods by industry, regulation, and improvement of the quality and consistency of medical care for patients. Over the last few decades, there have been many examples of communities' adopting comprehensive strategies to reduce heart disease and stroke and improve BP control.

The North Karelia Project

The North Karelia Project in Finland is perhaps the most widely cited example, serving as a model that has been replicated in communities throughout the world. In the 1960s, CHD mortality among Finnish men was the highest in the world. In 1972, a pilot program was launched in North Karelia province to reduce the prevalence of risk factors such as smoking, high serum cholesterol, and BP. Several interventions were implemented in collaboration with multiple stakeholders such as health service providers, nongovernmental organizations, businesses, and the media. After 5-year results showed a significant decline in risk factors in North Karelia compared with a control province, a national prevention strategy was gradually implemented based on the same principles. Recent analysis of 35-year trends in cardiovascular risk factors in Finland have shown an 80% decline in coronary mortality since the 1960s and continued reductions in most risk factors including BP, although obesity rates are on the increase.

Million Hearts™

Million Hearts™ is a national US campaign launched in September 2011 with the goal of preventing 1 million heart attacks and strokes by 2017. More specifically, Million Hearts™ sets targets for improving BP control (by 65% from a baseline of 45%) and reducing sodium intake (a 20% reduction from a baseline of 3.5 g day^{-1}). The campaign is an innovative public–private partnership created by the Centers for Disease Control and Prevention and the Centers for Medicare and Medicaid Services in partnership with more than 100 private sector and nonprofit organizations to focus, coordinate, and enhance CVD prevention activities. Partner organizations include the AHA, the American Pharmacists Association, the YMCA of the United States, the retail pharmacy group Walgreens, the insurance company UnitedHealthCare, and many others. The strategic approach involves both primary CVD prevention (empowering Americans to make more healthy lifestyle choices by reducing tobacco use and sodium and trans fat consumption) and secondary CVD prevention by emphasizing the widespread and consistent use of the *ABCs* (*a*spirin for people at risk, *b*lood pressure control, *c*holesterol management, and *s*moking cessation). Tactically, Million Hearts™ targets both clinical and community prevention services and systems by

- implementing education campaigns to increase awareness of modifiable risk factors;
- using health information technology to increase access to and delivery of timely, evidence-based care;
- promoting community efforts to discourage smoking and reduce sodium in the food supply.

Examples of Innovative Partnerships to Implement These Tactics

Sodium reduction

The California Department of Public Health and eight independent restaurants in northern California worked together to reduce sodium content in meals, achieving an average 26% reduction across 15 meals offered at the restaurants.

BP risk reduction

Measure Up/Pressure Down is a partnership with the American Medical Group Foundation to support hypertension control in

medical groups and health systems. To date, 30 participating medical groups have helped 30 000 people reduce their risk for heart attack and stroke.

Heart360

Heart360 is a home BP-monitoring program implemented by pharmacists in which participants upload their home BP measurements three to four times per week to the AHA's Heart360 Web site. Clinical pharmacy specialists monitor the patients' BP readings and adjust medication as needed. This program has led to improved BP control, increased reductions in BP, and greater patient satisfaction with care.

Government salt regulation

Because community efforts to reduce hypertension have existed for decades and prevalence remains high, public health officials and academics have increased their requests that governments use tools such as regulation to improve population health. The US Food and Drug Administration does not regulate salt, because it is currently classified as 'generally recognized as safe.' Sodium content is, however, one of those that manufacturers of packaged and processed foods are required to disclose on the Nutrition Facts panel. Given persistent high levels of sodium consumption and the fact that ~75% of consumed salt comes from packaged, processed, and restaurant food, interventions such as regulation aim to reduce the availability of sodium in the food chain and, thus, reduce consumption; improve public health; and reduce health-care costs. Several countries, including the United Kingdom, Finland, Portugal, and Japan, have reduced population-wide salt consumption successfully through a combination of consumer education and government regulation.

He and colleagues estimated the effect of a salt reduction program in the United Kingdom initiated in 2003–04 and found that sodium consumption decreased by 1.4 g day^{-1} between 2003 and 2011. During the same period, deaths from heart attack declined by 40% and deaths from stroke by 42%. Although other factors could account for these improvements, such as increased fruit and vegetable consumption and greater BP control, the study still found a significant decrease in BP among untreated individuals (i.e., people not on antihypertensive medication) even after adjustment for several factors, including fruit and vegetable consumption and body mass index. This suggests that salt intake reduction played a significant role. According to the UK government, the program saved an estimated £1.5 billion year^{-1} in health-care-related costs. Despite this positive outcome, a sobering finding of the study is that average salt intake in England was still 35% higher than the recommended daily level.

A study by Bibbins-Domingo and colleagues estimates that a 3 g day^{-1} reduction of salt in the United States would reduce annual deaths from all causes of CVD by between 44 000 and 92 000 and save $10 billion to $24 billion each year in avoided health-care costs. All segments of the population would benefit, and the regulatory intervention would be cost saving even at a more modest salt reduction of 1 g day^{-1}. Despite the public health need, estimated health-care cost savings, and calls from the Institute of Medicine for the Food and Drug Administration to regulate salt, there is political resistance to government regulation of the food industry.

Australia has proposed a stepwise reduction in salt based on a responsive regulatory approach. Food manufacturers are challenged to meet 'legislative scaffolds' in reducing salt via product reformulation. This model supports industry collaboration and innovation. Phase 1 allows food manufacturers to negotiate reformulation targets. Phase 2 requires companies that have failed to reformulate products to include high-salt warning labels on these products; this would occur concurrently with media and marketing campaigns encouraging reduced salt intake and release of company compliance data. Finally, phase 3 imposes court-enforced penalties on companies that continue to fail to reduce salt. This approach encompasses several components that predict success: industry collaboration, stepwise reductions that allow companies to reformulate and consumer taste to adjust, and incentives for compliance.

Industry efforts to reduce sodium

Voluntary commitments have been made by eight packaged-food companies that collaborate in the International Food and Beverage Alliance. These companies met or exceeded their sodium reduction targets in 2011 (Table 3). Large-chain restaurants have also made efforts to reduce sodium. McDonald's

Table 3 Sodium reduction targets

Company	*Territory*	*Target*
General Mills	United States	−20% across top 10 product categories (2015)
Grupo Bimbo	United States/ Mexico	−30% across bread portfolio and −15% in snacks key brands (2015)
Kellogg	Global	Continue responsible sodium reduction globally
Kraft Foods	Global	Continue sodium reduction efforts, in addition to region-specific goals
	Latin America	−10% across cheese and biscuit portfolio (by end 2013)
	North America	−10% across product portfolio (by end 2012)
Mars	Global	−15% across entire global food portfolio (2015)
Nestlé	Global	−25% in products with a sodium content >100 mg/100 kcal (2005–10). Continue reducing sodium levels by an average of 10% in products that can best contribute to reducing sodium intake (e.g., soups, recipes, and pizza portfolio) (2012–15)
PepsiCo	Global	−25% in key global brands in key countries (2015)
Unilever	Global	The first milestone was to reduce salt levels to an interim target of 6 g day^{-1} by the end of 2010. This required reductions of up to 25%. Unilever aims to reduce levels by a further 15–20%, on average, to meet the target of 5 g of salt per day. The company's goal is to help consumers meet the recommended 5 g of salt per day based on globally recognized dietary guidelines

Source: https://www.ifballiance.org/sites/default/files/IFBA%20Progress%20Report%202011%20%28FINAL%2029%204%202012%29.pdf (p.7).

has pledged to cut sodium by 15% across US menu items by 2015 and already has reduced salt in fries by 20% in Europe.

Internationally, especially in developing countries where informal food sector calorie intake is high, large companies are challenged with maintaining the taste profile of products that adhere to regulation, while informal sector products that fall below regulation continue to have high salt content. Since regulations are only as strong as their enforcement, which is weak in many countries, the government must consider alternative ways of encouraging industry from reducing sodium in products aside from only regulation. Companies reducing sodium often do not market this change due to consumer bias against products labeled low sodium. Rather, companies often reduce sodium without changing their marketing – known as stealth reduction – and consumers must compare the old and new Nutrition Facts panel to know the change.

Conclusion

Hypertension is a significant burden worldwide, increasing the risk for CHD and stroke. Poor diet is a leading contributor to hypertension risk, and improving diet is a key factor for primary and secondary prevention of hypertension. Several dietary factors contribute to hypertension risk, including sodium consumption and alcohol intake, whereas intake of potassium and fish oil reduces hypertension. Overall, the DASH diet has strong antihypertensive effects. Medications exist to support hypertension control, although poor adherence is a challenge. Behavioral strategies that address lifestyle modifications are recommended to treat hypertension. Innovations have been developed to address hypertension through comprehensive community-level strategies. In addition, the food industry has made significant progress in reducing sodium in packaged foods.

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See also: Adolescent Nutrition; Alcohol: Metabolism and Health Effects; Dairy Products: Dietary and Medical Importance; Dietary Practices; Dietary References: US; Fish: Dietary Importance and Health Effects; Fish Oils: Composition and Health Effects; Food Allergies; Low-salt Foods: Types and Manufacture; Meat: Role in the Diet; Milk: Role in the Diet; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Nuts: Health Effects; Obesity Management; Potassium: Physiology; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Salad Crops: Dietary Importance; Snack Foods: Role in Diet; Sodium: Properties and Determination; Vitamins: Overview; Yogurt: Dietary Importance.

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Hypovitaminosis A

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Introduction

Hypovitaminosis A is the deficiency of vitamin A, a fat-soluble vitamin discovered simultaneously in 1913 by two groups of North American scientists, McCollum and Davis in Wisconsin and Osborne and Mendel in Connecticut. It has been endemic in most parts of the world at one or another time in history, and its complications and consequences can greatly increase the risk of mortality, especially in childhood. For this reason, it has been considered one of the most prioritized public health problems to be addressed by the World Health Organization and health authorities of countries in which hypovitaminosis A is endemic. A WHO map of nations with endemic vitamin A deficiency is shown in Figure 1.

The Vitamin

Vitamin A belongs to a class of compounds known as retinoids. The circulating form in blood is as the alcohol form: retinol. Retinol has a molecular weight of 286.5 kDa. In biological systems, however, it is more often found in other chemical forms. In foods of animal origin, the vitamin is part of a retinyl ester, that is, with the retinoid bound to a fatty acid. This is also the form of the vitamin in storage in human tissues. In the retina of the eye, where it participates in both daylight vision and nocturnal vision, it is the retinaldehyde (retinal) form that is active in the visual signal of the retinal rods and cones. The most important among the vitamin's physiological functions is its regulation of transcription at the level of the cell nucleus. The chemical form of the retinoic acid (RA) participates with specialized nuclear receptor sites that act as transcription factors.

Because of its size as an organic molecule with only a limited number of exposed oxygen groups, retinoids are better soluble in lipids (fat soluble) and less soluble in water. Retinol and its derivatives are relatively stable to heat but are degraded by exposure to white light.

Vitamin A Transport

Vitamin A can be transported to and from the different sites in the body by various ways. One involves the transport of the recently absorbed vitamin from the intestine to the liver. This involves transport within the lipoproteins formed in the intestinal cells to export newly absorbed lipids (chylomicrons). These travel from the lymph into the bloodstream and are transformed into increasingly smaller sizes until they are completely cleared by the liver. Once in the liver, vitamin A is stored for a short term in the hepatocytes and for a longer term in special stellate fat-storage cells of the liver. To be mobilized for transport to the peripheral tissues, vitamin A in the hepatocyte is attached to a molecule of a specialized transport protein, retinol-binding protein (RBP) (molecular weight = 21.1 kDa). This, in turn, couples with transthyretin (prealbumin, molecular weight = 55.0 kDa) for transport in the systemic circulation.

The exact mechanism of transfer of vitamin A across the placenta from the mother to the developing fetus is not known. What is established is that, as a fat-soluble vitamin, the movement is much slower than that of water-soluble vitamins. This has implications for preterm infants, who will be born before having the chance to accumulate appropriate body stores of the vitamin. Once born, the infant ideally will be fed with breast milk exclusively for the first 6 months of life. The transport and delivery of vitamin A to human milk appear to be more closely dependent on recently ingested vitamin A than on the maternal liver stores. Rather than moving to the mammary glands from the liver on RBP, it is taken up directly from the chylomicrons for its incorporation into milk.

Consequences of Vitamin A Deficiency

The essence of a discussion of hypovitaminosis A revolves around the consequences of vitamin A deficiency. These include metabolic and functional consequences that may not express themselves in a clinical manner. For the practitioner, however, the clinical signs and symptoms of more advanced deficiency are an important concern.

Metabolic and Functional Role of Vitamin A

Vision and the eye

Vitamin A is instrumental in vision both in its neurosensory mechanism and for the structural integrity of the ocular structure. Vitamin A is part of the classical visual cycle, which involves not only the rod structures of the eye's retina for vision in dim light but also the cone structures for daylight color vision. 11-*cis*-Retinal is linked to an opsin protein. Exposure of this unit to a photon of light produces a decoupling accompanied by the transduction of a signal via the optic nerve interpreted as vision. The all-*trans*-retinal released is converted to all-*trans*-retinol and then regenerated to the 11-*cis* form for recoupling to the opsin to await another cycle. Vitamin A is also important for vision, supporting the anatomical integrity of the eye. Ocular mucins, a secretory variety from the goblet cells of the conjunctival cells of the eye, and another membrane-spanning mucin from both conjunctival and corneal lining cells are exquisitely sensitive to vitamin A deficiency. It is extrapolated, moreover, that there is probably similar mucin-regulatory process at play within the oropharyngeal, gastrointestinal, and urinary tracts as well. It is assumed that the transcription of the peptide skeleton of the mucin glycoprotein is suppressed by the absence of or deficiency in nuclear RA.

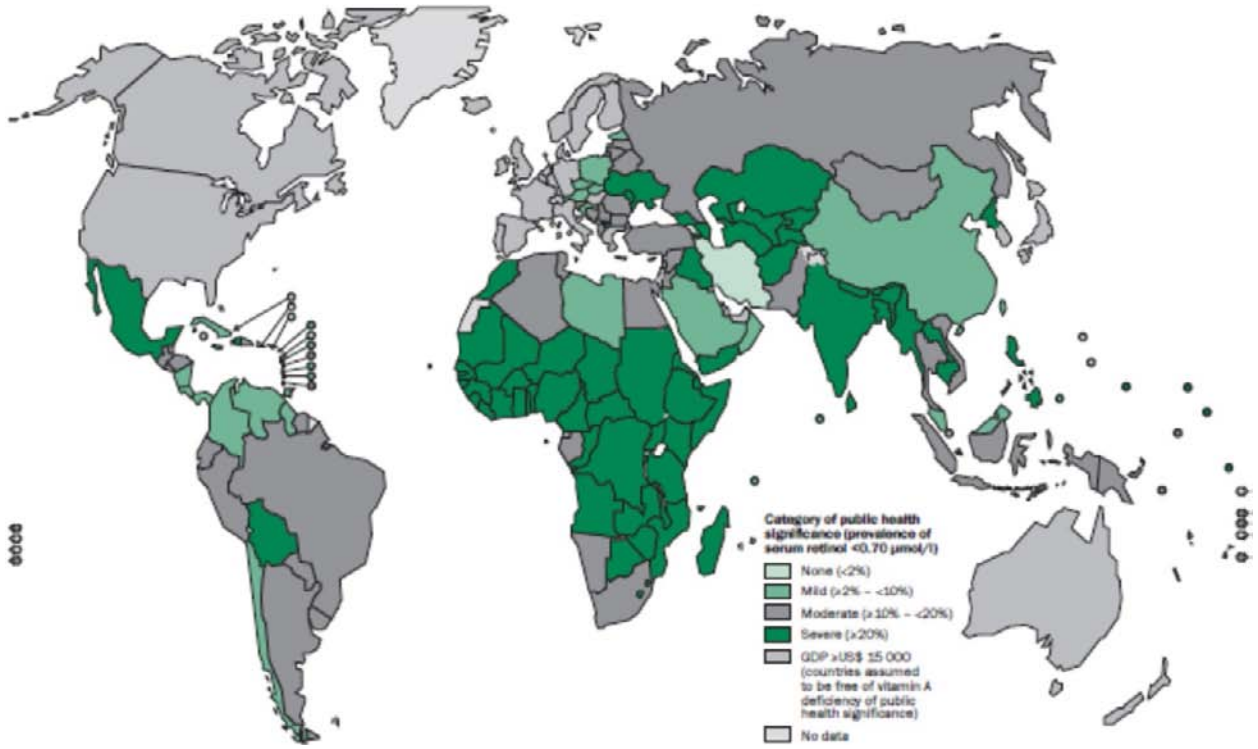

Figure 1 Countries and areas with survey data and regression-based estimates: preschool-age children. Reproduced from the WHO Summary tables and maps on global prevalence of vitamin A deficiency 1995–2005.

The cell cycle

RA, derived from vitamin A, is a pivotal signaling molecule, interacting with RA receptors in the nuclei of all cells. It can either enhance or inhibit transcription depending on the configuration of the nuclear receptor. As such, it is instrumental in regulating all aspects of the cell cycle from maturation and differentiation to proliferation and programmed cell death (apoptosis). RA is instrumental in the expression of the delta-5 desaturase enzyme, working with elongating enzymes to form the long-chain essential fatty acids of both the omega-3 and omega-6 series. In one aspect, they participate with RA in the transcriptional regulation. Yet another function is in modulating inflammation.

Immunity and host defense

The immune response is a final function worthy of mention. Recently, several lines of evidence have converged to show that RA, a major oxidative metabolite of vitamin A, plays a key role in the differentiation of T-cell subsets, the migration of T cells into tissues, and the proper development of T cell-dependent antibody responses.

Clinical Manifestation of Human Vitamin A Deficiency

Night blindness

This symptom manifests as the inability to adapt to vision under dim light. Sufferers may stumble into objects at night. It relates to the disruption of the visual cycle by depriving the retinal rods of the precursor of 11-*cis* retinal.

Xerophthalmia and nutritional blindness

These manifestations are not related to the biochemistry in the retina, but rather to the decline in mucus production across the surfaces of the eye. Xerophthalmia literally derives from ancient Greek for *dry eye*. It evolves in stages from dryness of the conjunctiva, to the formation of foamy deposits of debris on the lateral aspect of the conjunctiva (Bitot's spots), to drying of the cornea itself. Depending on the extent of corneal damage, degrees of visual impairment range from scarring to complete perforation into the ocular globe. The latter produces full blindness.

Skin changes

A number of skin changes have been described in vitamin A deficiency, but the thickening to mounds around the hair follicles (follicular keratosis), probably due to cell-cycle dysregulation, is the most common.

Gastrointestinal dysfunction

Dry mouth, swallowing difficulties, and diarrhea have been described in vitamin A-deficient individuals. The specific interference with the production and secretion of mucin for the normal maintenance of the mucus membranes is the putative origin of clinical manifestations, perhaps in conjunction with altered renewal of the epithelial lining.

Decreased infectious disease resistance and resilience

Vitamin A supplementation has been shown to retard the emergence of a recurrent childhood infection following the resolution of pneumonia or diarrhea. This may be a

pharmacological effect, however, as the epidemiology does not recognize dramatically increased incidence rates in the context of hypovitaminosis A.

Assessment of Vitamin A Status

Most nutrient deficiencies pass from a milder, subclinical phase on the way to a more severe, clinically evident stage, in which signs, symptoms, or both become manifest to the patient, the family, or the health practitioner. The consequence is that deficiency in the subclinical stage can only be revealed by some form of laboratory or functional test. This is notably true for vitamin A. Moreover, the assessment of vitamin A status can proceed for two qualitatively distinct goals. One is to determine the status of a given *individual* in the clinical setting; the other is to assess the risk of a *population* for endemic hypovitaminosis A.

Assessment of Individual Status

In a conceptual manner, in order to identify vitamin A deficiency in an individual, one would ideally need standards of what constitutes an adequate amount of vitamin A in a body of a given size or the concentration of vitamin A in the human liver, which constitutes the main reserve. Due to its invasive nature, liver biopsy can only be used in exceptional circumstances (e.g., when primary liver disease is suspected) or in the postmortem state.

Because circulating levels of retinol are homeostatically regulated, serum or plasma retinol is a poor indicator for an individual. In an experimental setting, such as evaluating an intervention, total body dilution studies with stable isotope-labeled vitamin A can give a quantitative estimate of body stores.

In a clinical setting, quantitative dark-adaptation testing can be used to confirm indications from clinical chemistry. Of course, with advancing deficiency, the symptom of night blindness and the ocular manifestations of xerophthalmia provide diagnostic evidence.

Collective Epidemiological Assessment of Status

Population-level public health assessment addresses one primary question: is there an indication of an endemic hypovitaminosis A that would warrant a public health intervention? In an official manner sanctioned by the WHO, the prevalence of retinol concentrations is below 20 dl^{-1} in a nationally or regionally representative sample of children aging 6–59 months. Additionally, the symptom of night blindness in the same population sample is an alternative indicator of population risk.

As discussed, the circulating retinol level is problematic due to its homeostatic regulation. It is also susceptible to distortion by the presence of inflammation or infection, which decreases circulating concentrations. A functional test of hepatic handling of newly absorbed vitamin called the modified relative dose response (MRDR) has been shown to classify low liver stores from normal ones; the MRDR technology, however, is expensive and confined to a very limited number of centers around the world, and it cannot detect elevated stores. Interestingly, given the growing concern for excessive vitamin A exposure as the result of confluent interventions, in Guatemala, the isotope dilution approach is being introduced into the epidemiological domain.

Dietary Sources of Vitamin A

Two diet-related determinants are imposed upon the public health occurrence of hypovitaminosis A as well as the geographic distribution of its endemicity (see Figure 1). One is the variety and abundance of vitamin A-rich foods and the other is the economic, cultural, and physiological issues relating to their accessibility and acceptability of these sources. The most active and available form of the vitamin, preformed vitamin A in a retinyl ester form, is only found in foods of animal origin; these are expensive and often consumed only selectively for cultural or religious regions. As discussed, plants are a source of a less biologically available form of provitamin A carotenoids. Even in this domain, the richer (orange-colored) sources may be more expensive than the less bioconvertible forms in dark-green leafy vegetables (DGLVs) and herbs. The primary, but not exclusive, contributor to hypovitaminosis A is the chronic consumption of a diet that does not fulfill on an average daily basis the recommended intakes to satisfy requirements. The recommended amounts, arrayed by age, sex, and physiological condition, are provided in Table 1.

Expression of Vitamin A Content in Foods and in the Diet

The expression of vitamin A in foods and in pharmaceutical preparations has evolved over the years. The options are international units (IUs, original), retinol equivalent (RE, 1967), and retinol activity equivalent (RAE, 2001). They are not entirely mutually interconvertible, which is a source of considerable confusion.

International units

The initial system was the IU. It was an expression for food analysis, but for dietary purposes, it has fallen into disuse. It persists today only in the pharmaceutical domain. Medicinal supplements are expressed in IU. One IU vitamin A is approximately the biological equivalent of 0.3 μg retinol, or of 0.6 μg beta-carotene. The IU system cannot account for provitamin A sources other than beta-carotene.

Retinol equivalents

This system dates back to 1967, but it is still the standard of the UN system: WHO and FAO. One retinol equivalent is equal to

- 1 μg of retinol,
- 6 μg of all-*trans* beta-carotene,
- 12 μg of other provitamin A carotenoids.

Retinol activity equivalents

As it was recognized that provitamin A carotenoids were more bioconvertible when dispersed in oil but less convertible when consumed from foods than what is considered in the RE, the

Table 1 Recommendations for daily intake of vitamin A as derived from the United Nations system (WHO/FAO) and the US–Canada consultation (Institute of Medicine)

	United Nations system (WHO/FAO)		*US and Canada dietary reference intakes*	
	Estimated average requirements[a]	*Recommended nutrient intakes*	*Estimated average requirements*	*Recommended dietary allowances*
Population	*RE per day*		*REA per day*	
0–6 month	–	375	–	400 AI[b]
7–12 month	–	400	–	500 AI
0–1 year	290	400	–	–
1–3 year	320	450	210	300
4–6 year	320	450	–	–
4–8 year	–	–	275	400
7–9 year	360	500	–	–
9–13 year	–	–	445[c],420[d]	600
10–18 year	430	600	–	–
14–18-year-old males	–	–	630	900
14–18-year-old females	–	–	483	700
Adult males	430	600	625	900
Adult females	360	500	500	750
Pregnant	570	800	550	770
Lactating	610	850	900	1300

[a]EAR retrofitted a posteriori from RNI values after Allen et al. (2006) and rounded off to zero.
[b]AI, adequate intake.
[c]Males.
[d]Females.

Dietary Reference Intakes (DRIs) for the United States and Canada reformed the expression to RAE. One RAE is equal to

- 1 μg of retinol,
- 2 μg of all-*trans* beta-carotene,
- 12 μg of all-*trans* beta-carotene,
- 24 μg of other provitamin A carotenoids.

If the vitamin A source in question is entirely preformed vitamin A, then the three expressions do become mutually interconvertible; otherwise, they diverge substantially.

Dietary Vitamin A of Animal Source Origin

Cod-liver oil, goose liver pate, beef liver, and chicken liver would rank among the four richest sources commonly consumed. Per 100 g portion, these would range from 1000 to 30 000 RAE, primarily as retinyl esters. Eggs and dairy items are also dietary sources, at lower specific contents. It should also be noted that provitamin A carotenoids also form part of flesh foods and milk. Intact carotenes are the pigment that gives the yellow tinge to animal fat; as such, this can supply vitamin A to the consumer. Beef liver also has provitamin A carotenoids as a minor storage component.

Vitamin A in human milk is primarily preformed retinyl esters, with a variable amount of provitamin A, depending on the carotene content of the maternal diet. Human milk should contain from 300 to 700 $\mu g\,l^{-1}$ to be both nourishing and safe for the exclusively breast-fed infant. This compares with 450 $\mu g\,l^{-1}$ in unfortified bovine milk and 900 $\mu g\,l^{-1}$ in fortified commercial milk.

Dietary Vitamin A of Plant Source Origin

The other major domain of vitamin A intake generally constitutes from 40% of total vitamin A intake in industrialized countries and up to 100% in societies that are totally vegetarian by religious practice or economic necessity. Exotic and regional sources of provitamin A include unrefined red palm oil (*Elaeis* spp.) and oil from the buriti fruit (*Mauritia vinifera*) from Brazil, and other parts of Latin America were reported to have ~6000 and ~50 000 RE per 100 g of oil, by using the 1967 conversion factors. As oil suspensions, the newer conversion-efficiency norms of the DRI would revalue these amounts to ~18 000 and 150 000 RAE per 100 g.

For Western societies, dehydrated forms of orange vegetables, such as carrot or sweet potato chips, can provide over 1000 RAE per 100 g. Carrots, sweet potato, and pumpkins as well as kale and other DGLVs can exceed 700 RAE in a 100 g portion when cooked and prepared. Most other edible DGLVs have important quantities of provitamin A as do other orange fruits and tubers.

Provitamin A carotenoids and their conversion to active vitamin A

A series of plant pigments, including beta-carotene, alpha-carotene, and alpha-cryptoxanthin, among others, can be converted into active (retinoid) vitamin A by enzymatic activity in the small intestine. The responsible enzyme uses molecular oxygen to cleave the carotenoid molecule in the center of the chain, yielding either two (in the case of beta-carotene) or one (with other provitamin A carotenoids). The enzyme has been termed carotene-15,15′-monooxygenase (CMO I), and it is

located inside the intestinal cells. A certain amount of fat is required in a meal containing provitamin A to evoke the intestinal mechanisms of fat digestion and absorption and make its intestinal handling most efficient.

Genetic traits govern the efficiency of action of the carotene oxygenase enzyme in the intestine. Conversion efficiency for plant precursors to the active vitamin has a genetic component with different assortment of alleles conveying distinct extents of bioconversion. Ethnic groups with historically more vegetarian-type diets seem to have conserved the more efficient cleavage constitution. An important caveat is the fact that the conversion of provitamin A to the active form in the intestine is homeostatically regulated and self-limited.

There is no example of developing *hyper*vitaminosis A from excessive intake of provitamin A sources. The only consequence is a yellowish discoloration of the skin (carotenodermia), which can be unsightly but is harmless to health and reversible upon reduction of carotene intake.

Nondietary Mechanisms for Vitamin A Deficiency

Dr. Victor Herbert sagely pointed out that, beyond an insufficient intake of a nutrient, mechanisms that impaired its intestinal uptake, interfered with its internal utilization, or accelerated its excretion would also contribute to poor nutritional status. With respect to vitamin A, we can identify important instances of these factors. Cystic fibrosis and other diseases of the liver or pancreas that impair fat digestion will impair vitamin A uptake, as will small intestinal diseases such as celiac disease, persistent diarrhea, or environmental enteric dysfunction. Since its mobilization is dependent on a hepatic transport protein (RBP), protein deficiency and certain liver diseases will impair the utilization. During fever, excessive amounts of vitamin A are lost in the urine. Acute or chronic febrile illness, therefore, can reduce or deplete vitamin A reserves. Finally, certain internal threadworms (filarial) have a high specific requirement for vitamin A. Patients with high body burdens of these parasites in their tissues might suffer from the competition if dietary sources are also marginal.

Prevention of Hypovitaminosis A

There is a generally accepted hierarchy of behaviors and measures that can be applied, alone or in combination, to prevent hypovitaminosis A in vulnerable populations. These include dietary diversification, food fortification and periodic oral supplementation, and general health measures. They differ with respect to the time frame for onset of effect, popular acceptability and long-term sustainability, economic cost, and risk of adverse consequences. On the dietary side, they would include consumption on a daily basis of the amounts of vitamin A recommended on an age-specific basis. The recommendations from two authoritative sources are provided in Table 1.

Dietary Diversification for the Prevention of Hypovitaminosis A

Dietary diversification is a somewhat euphemistic term for a strategy to improve the nutritional quality of a population by enabling them to substitute foods of lower micronutrient density for foods of higher nutrient content. In the present context, it would mean increasing the percentage of the required energy from the rich sources of preformed vitamin A or provitamin A than are currently in the diet. One example would be the substitution of red palm oil for the refined variety; the red staining of traditional dishes, however, generally deters its adoption.

Fortification for the Prevention of Hypovitaminosis A

Several countries with background endemic conditions for hypovitaminosis A have opted to mandate the fortification of one or another staple food that is consumed widely throughout a population. Table sugar and edible oils with added retinyl esters are the prime examples. Wheat and maize flours have also been vitamin A-fortified.

Fortification of commercial products

More and more, commercial products, such as ready-to-eat cereals or precooked gruels. This category of fortification has been termed 'market-driven' fortification. Problems of overconsumption may arise when this combines with publically mandated programs; this has been documented in preschool and school children in Guatemala.

Biofortification of plants

The amount of provitamin A in plants can be enhanced either by crossbreeding of high-carotenoid plants (i.e., hybridization) or by genetic modification, that is, the insertion of carotene-regulating genes from another organism. Orange-fleshed sweet potatoes are an example of the former. Golden rice is an example of the latter.

Home fortification for complementary food

A mode of adding vitamin A, along with a multiple host of companion micronutrients, directly to complementary foods to enrich the dietary intake of infants and toddlers during the weaning process has arisen. It is in response to the generally low micronutrient density of traditional paps and gruels of natural cereal grains. Foodlet, a crushable tablet, was developed during the first the International Research on Infant Supplementation meeting in 1999, with weekly periodicity. Sprinkles® was the first in the series of currently expanding microencapsulated micronutrients in a sachet. The major thrust has been combating iron deficiency anemia, but it contains 300 RAE per sachet. Lipid nutrient spreads such as Nutributter®, derived from Plumpy'Nut®, generally supply 400 RAE per 20 g sachet.

Supplementation for the Prevention of Hypovitaminosis A

Periodic supplementation is recommended for children in the appropriate settings aged 6–59 months. The periodicity is once every 4–6 months; the latter is the standard. The dosage is 100 000 IU between 6 and 11 months and 200 000 from 12 months onward. The trigger public health condition for implementing the program is a prevalence of night blindness in children of this age range of 1% or greater or a prevalence of 20% low serum or plasma retinol concentration ($<20\ \mu g\ dl^{-1}$)

in a representative survey of under-fives. When such survey data are unavailable, severe poverty or unsanitary conditions often instigate the adoption of the program *under an abundance of caution* motivation. Poor sustainability and low coverage rates among the most rural populations have been documented as common operational problems in high-dose supplementation. The official WHO recommendations for supplementation, revised in 2011, are shown in Table 2.

General Health Measures for the Prevention of Hypovitaminosis A

The failure to absorb vitamin A has the equivalent negative effect on vitamin A status as the failure to have consumed it in the first place. Treatable small bowel diseases such as celiac disease and Crohn's jejunoileitis will respond to remission therapy with normalization of vitamin A uptake. In irreversible pancreatic insufficiency, such as in cystic fibrosis, meals accompanied by oral pancreatic enzymes will improve absorption of lipids including fat-soluble vitamins. In the tropics, recurrent and persistent diarrhea, intestinal protozoan infections, helminthes, and environmental enteric dysfunction are treatable small bowel afflictions that can interfere with uptake of dietary vitamin A. Immunizations to prevent enteric illness contribute to gut resistance. Decreased exposure to parasites through environmental sanitation, appropriate hygienic practices, and periodic deparasitization can reduce the rates of protozoan and helminthic parasitosis, which have been associated with lower vitamin A status.

Febrile illnesses lead to urinary wasting of vitamin A. Vaccinations are available from many severe infectious. Malaria and dengue fever can be prevented in part with the use of insecticide-impregnated bed nets. Appropriate treatment with antimicrobials and antipyretic medicines can limit the ravages of fever and reduce vitamin A loss.

Treatment of Hypovitaminosis A

With the exception of clinical situations, the prophylactic regimens discussed earlier in the text would restore – at least transiently – a normal vitamin A status in a child with depleted vitamin A stores. The clinical situations of note, both considered to be a medical emergency, are (1) acute xerophthalmia and (2) measles with a medical complication.

Any child reported to have night blindness or having ocular manifestations of xerophthalmia should immediately receive an oral dose of 200 000 IU of vitamin A and an equal dose on the following calendar day. If the conjunctival and corneal lesions are severe, direct care for the integrity of the eye should accompany vitamin A dosing.

The eradication of measles has been retarded in recent years by resistance in certain populations to vaccination. It has become recrudescent in many areas of the world. When hospitalization for a complication of measles such as pneumonia or encephalitis occurs, even in an area not certified as endemic for hypovitaminosis A, it is prudent to provide high-dose oral vitamin A in the schedule described in the preceding text: 200 000 IU immediately, repeated on the following day.

Hypervitaminosis A as a Consequence of Combating Hypovitaminosis A

One can have too much of a good thing and this is becoming potentially and actually true in current efforts to eradicate and prevent endemic hypovitaminosis A. Concerted efforts to increase the intake of vitamin A – both mandated and voluntary – can tip the pendulum of dietary exposure to the vitamin from deficit to excess. Under certain circumstances, and in certain dosages and forms, too much vitamin A can be as bad as or worse than an insufficiency.

Table 2 The 2011 World Health Organization guidelines on vitamin A supplementation

Population category	*Guideline for vitamin A supplementation*
Neonates	The WHO does not recommend vitamin A supplementation for neonates as a public health intervention
Infants 1–5 months of age	The WHO does not recommend vitamin A supplementation for infants 1–5 months of age as a public health intervention. Mothers should continue to be encouraged to exclusively breast-feed infants for the first 6 months to achieve optimal growth, health, and development
Infants and children 6–59 months of age	The WHO does recommend high-dose vitamin A supplementation every 4–6 months for infants and children 6–59 months of age, as a public health intervention to reduce child morbidity and mortality
Pregnant women	The WHO does not recommend vitamin A supplementation during pregnancy as part of routine antenatal care. However, in areas where there is a severe public health problem of vitamin A deficiency, the WHO recommends giving vitamin A supplementation during pregnancy for the prevention of night blindness
Postpartum women	The WHO does not recommend vitamin A supplementation for postpartum women as a public health intervention for the prevention of maternal and infant morbidity and mortality
During pregnancy for reducing the risk of mother-to-child transmission of HIV	Currently, the WHO does not recommend vitamin A supplementation in HIV-positive women as a public health intervention for the prevention of mother-to-child transmission of HIV

The WHO standard for 'high-dose' vitamin A supplementation refers to an oral dose of 100 000 IU (30 303 RE) for 6- to 12-month-old infants and 200 000 IU (60 606 RE) from 1 year onward, including adults. These are also the age-specific dosages for specific therapeutic indications for oral vitamin A supplementation.
Modified from the guidelines for routine immunizations of the World Health Organization. Available at: http://www.who.int/nutrition/publications/micronutrients/guidelines/en/index.html.

Acute exposure to massive amounts of preformed vitamin A can produce a series of consequences leading to death. These include blurring of vision; headaches and coma, due to increased intracranial pressure; and acute liver failure due to hepatotoxicity. Various skin rashes can also occur. The visceral organs of marine mammals, such as seals, walruses, narwhals, and polar bears, have enormous concentration of retinyl esters. The food culture of traditional inhabitants of arctic climes evolved to recognize the hazards of the organ meats of these species, and they were consumed in moderation. Arctic explorers were unaware of the danger and avidly consumed whole polar bear liver, which contains upward of 350 000 IU per 100 g, succumbing to the aforementioned syndrome and alerting the medical community of acute hypervitaminosis A. In lower chronic dosages, that is, above 8000 μg daily, usually due to self-prescribed oral retinol supplements or daily cod-liver oil, a more indolent presentation of hypervitaminosis A can occur. This includes headaches and visual disturbances, fibrosis of the liver leading to cirrhosis, and softening and weakening of the bones.

The periodic supplementation of retinyl palmitate involves dosage forms of 200 000 IU (~60 000 RAE), generally spaced at 4- to 6-monthly intervals in children between 12 and 59 months of age. For children between 6 and 11 months, a single dose of 100 000 IU (~30 000 RAE) is recommended (see Table 2). At this dosing, the exposure is generally safe. In the medical emergencies of xerophthalmia and complicated measles, the standard dose is repeated twice to a dosage of 400 000 IU within 48 h for therapeutic purposes. The threat from the primary diseases far outweighs any consideration of vitamin A toxicity in these sight- and life-threatening circumstances. Lower doses of both 50 000 and 100 000 IU have been used in trials and public health practice in infants below 6 months of age. These children still have not sealed the seams of the cranial bones. Administration of these doses in the first semester of life produces an overt increase in intracranial pressure, manifested by transient bulging of the fontanels of the scalp. The very existence of this anatomical 'escape valve' and the failure to document sequelae convinced the public health community of the safety of the practice. Administration in this age group is currently not recommended.

In periodic-supplementation setting, however, in which multiple agencies address the same population of children without common clinic-visit controls, eligible children can be dosed more frequently than the prescribed intervals. Moreover, a notorious instance of alleged overdosing comes from Assam, India, in 2001, where the local preparation of a supplement syrup was used, rather than the imported capsules. Through miscommunication to field workers, five times the intended volume of syrup was administered in a regional campaign, and deaths attributed to acute overdose with retinyl esters were alleged. Measures were instituted to prevent subsequent occurrences, but the reputation of high-dose supplementation remains damaged in the Indian community.

Conclusion

Of all the micronutrient deficiencies of public health interest, that of vitamin A has had both the most intensive investigation and the most aggressive investment. The role of periodic high-dose supplementation as a measure against child mortality – but not as a means of sustained adequate vitamin status – has confused the discourse. Because vitamin A deficiency tracks with the extremes of poverty, the most needy are often not reached by the attempts to reduce the deficiency. Biofortification of plant sources holds promise to improve sustainability in a fortification form that does not risk excessive superimposition of sources of provitamin A. The lesson from contemporary Guatemala should be a cautionary tale regarding the uncoordinated offering preformed vitamin A across the population. More effort, therefore, is needed to develop sustainable formats to keep the pendulum of vitamin A status away from the extreme of either severe deficiency or inadvertent overload.

See also: Bioavailability of Nutrients; Carotenoids: Occurrence, Properties and Determination; Carotenoids: Physiology; Chlorophyll; Dietary Practices; Food and Agriculture Organization of the United Nations; Food Environment; Nutrition and Infection; Papayas; Peppers and Chilies; Retinol: Physiology; Retinol: Properties and Determination; Solanaceous Fruits Including Tomato, Eggplant, and Peppers; Vegetables of Temperate Climates: Carrot, Parsnip, and Beetroot; Vitamins: Overview; World Health Organization.

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Ice Cream: Composition and Health Effects

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Introduction

Ice cream is a frozen dairy product made by freezing the ice cream mix with agitation. It is composed of a mixture of food ingredients like milk products, sweetening materials, stabilizers, colors, flavors, and egg products. Ice cream mix is the unfrozen mixture of the ingredients, consisting of all the ingredients of ice cream with the exception of air and flavoring materials. The composition of ice cream is usually expressed as a percentage of its constituents, for example, the percentage of milk fat, milk solids not fat, sugar, egg solids, stabilizers (which are the compounds added in very small quantities to strongly influence the formation and growth of ice crystal in the ice cream so as to render the product with desired body and texture), and the total solids. Ice cream and related products are classified as frozen desserts, which include ice cream, frozen custard, frozen confections, ice milk, sherbets, water ices, and mellowing products.

Ice cream had its origins in Europe and was introduced later in the United States where it developed into an industry. It is widely believed that ice cream evolved from iced beverages and water ices. Ice cream probably came to the United States with the early English colonists. In 1851, the first wholesale ice cream industry in the United States was established in Baltimore, Maryland. Ice cream plants were also established in New York, Saint Louis, Chicago, Washington, and Cincinnati. The development of condensed and dried milks and the introduction of the pasteurizer and homogenizer, improved freezers, and other preserving equipments accompanied a slow growth in the industry only after 1900. The ice cream soda was introduced in 1879, and ice cream cone and Eskimo Pie were introduced in 1904 and 1921, respectively.

Drivers of Extensive Development of the Ice Cream Industry

It was around 1920 that the value of ice cream as an essential food was recognized and the product was immensely popular since then. The following developments have made ice cream widely available to the consumer:

- Improved manufacturing technologies and development of automated operating systems, refrigeration, and transportation
- Ingredients of better quality and enhanced knowledge of their usage
- Establishment of product standards
- Quality assurance in the ice cream industry
- Widespread academic programs, research, and extension activities in ice cream technology
- Attractive packaging and efficient distribution network
- Extensive advertising and merchandising programs

In India, an initiative was taken by the Ministry of Food Processing Industries (Goverment of India) by recommending the Cooperative and Public Sector milk plants to produce ice cream. It enabled the production of a range of low-cost ice creams for those who cannot afford it and frozen novelties that dominate the market. At the same time, this step widened the product mix of the cooperative dairy plants and improved their efficiency and viability. Already, encouraging results have been reported from field trials of ice cream production by the Cooperative Sector. The entry of the Cooperative Sector resulted into a boom in the production and sales of ice cream.

Composition of Ice Cream

Ice cream is frequently considered as a 'fun food,' which is undeserving consideration, and even was considered as a 'junk' food. These depreciatory statements have affected industrial development virtually from its inception. In reality, ice cream is a relatively well-balanced, wholesome, easily digestible, and delicious food. It is because ice cream is a nutritious element of the diet that is frequently used as a meal component for hospital patients. The energy value and nutrient content of ice cream depends upon the food value of the products from which it is made. Ice cream contains about four times as much carbohydrates as milk. The milk products that go into the ice cream mix convey the constituents of milk, but in different amounts. In developing a formulation of satisfactory composition, some of the factors that need to be considered are personal preference of company management or customer demands for flavor, body and texture, and color characteristics of the finished product, that is, natural or fortified flavor with artificial flavoring; chewy to heavy, higher overrun; or more cooling body and texture characteristics. Composition standards are also influenced by demands of customers, volume

Table 1 Composition of plain ice cream (per 100 g edible portion)

Constituents	Good/average ice cream	For different fat (%)			Ice milk	Ice cream cones	Water ices
		~10	~12	~16			
Water (%)	61.7	63.2	62.1	62.8	66.7	8.9	66.9
Food energy (Cal)	196.7	193.0	207.0	222.0	152.0	377.0	78.0
Protein (%)	4.1	4.5	4.0	2.6	4.8	10.0	0.4
Fat (%)	12.0	10.6	12.5	16.1	5.1	2.4	Trace
Total carbohydrates (%)	20.7	20.8	20.6	18.0	22.4	77.9	32.6
Wt./100 cal portion (g)	50.8	51.7	48.3	45.0	65.6	26.5	128.4

Source: Arbuckle, W. S. (1986). *Ice cream* (4th ed.). New York: AVI Publishing

Table 2 Nutritive value of commercial ice cream and related products

Product	Weight (g)	Fat (g)	Protein(g)	Carbohydrate (g)	Total solids (g)	Calories
Vanilla ice cream	100	12	4.0	20.7	38.3	204
Mango ice cream	100	10	3.5	21.2	36.3	188
Strawberry ice cream	100	8	3.6	21.2	40.8	194
Chocolate ice cream	100	13.1	3.6	25.8	42.1	221
Saffron pistachio	100	13.9	4.8	21.8	40.8	225
Ice milk	100	4.0	4.1	20.5	33.5	144
Low-calorie ice milk	100	2.0	4.2	23.5	28.9	91
Diabetic ice cream	100	9.0	4.3	20.6	28.6	152
Orange sherbet	100	1.5	1.3	13.2	30.5	124
Lemon sherbet	100	–	–	27.3	29.1	113
Ice lolly	60	–	–	27.6	14.5	54
Chocolate coated ice cream bar	60	10.4	1.8	14.1	23.1	142
Vanilla sandwich ice cream	50	3.7	3.5	10.7	24.0	115
Milk shake	100	3	3.9	16.6	24.5	104
Frozen yogurt	100	3.5	4.0	21.7	31.1	133

Source: Bhandari, V. (2001). *Ice cream*: *manufacture and technology*, pp. 6–18. New Delhi, India: Tata McGraw-Hill Publishing Company

of operation, quality of ingredients, and ingredient costs. Detailed composition of ice cream is given in Table 1.

Ice cream contains three- to fourfold more fat than milk and about 15% more protein than milk. It also contains other food products such as fruits, nuts, eggs, dry fruits, and sugar, which boost its nutritive value. The milk solids in ice cream are usually subjected to higher heat treatments than those of pasteurized milk, they are also subjected to lower temperatures in the freezing process, and they are stored longer before consumption. It is an excellent source of food energy due to its enhanced fat content than that of milk, and 50% of its total solids content is sugar, including lactose, sucrose, and corn syrup solids. The fact that these constituents are almost completely assimilated makes ice cream an especially desirable food for growing children and persons who need to put on weight. For the same reason, its controlled use finds a place in the diet of persons who need to reduce or who do not wish to gain weight. Like milk, ice cream is not a good source of iron and some of the trace elements. The nutritive value of commercial ice cream and related products is depicted in Table 2.

Proteins in Ice Cream

The milk proteins contained in ice cream are of excellent biological value, because they contain all the essential amino acids. Milk proteins are important sources of essential amino acids like tryptophan and lysine. Proteins in the diet supply the amino acids required for the growth of infants and children and for the maintenance of tissues in adults. Not only are milk proteins known to be complete, but also the assimilation of ingested milk proteins is 5–6% more complete than other proteins in general. Milk proteins also have high chemical quality and high net protein utilization (NPU) values. The chemical quality of a protein is determined by comparing its amino acid content with that of hen's egg protein, which is recommended as a reference protein. It is difficult to obtain NPU values using man as a test animal, but relatively easy using the rat. The values for protein are calculated from determinations of the nitrogen content in the food. Early analysis of proteins showed that they have close to 16% nitrogen. The general practice then was to multiply the nitrogen content by 6.25, the conversion factor for the protein content. For milk proteins, the conversion factor is 6.38. The glycosylated proteins make the ice cream more compact and smooth and tend to prevent a weak body and coarse texture. However, excessive amounts result in a salty or cooked flavor and soggy or sandy body and texture defect.

Carbohydrates in Ice Cream

Carbohydrates provide a significant amount of energy in all human diets. These are synthesized by plants from carbon

dioxide and water with the liberation of oxygen under the influence of sunlight. The chief products are the sugars, which are a convenient source of energy for the cells. These include starch, dextrin, cellulose, pectin, gums, and related substances. Sugars of several kinds may be used in the manufacture of ice cream. The commonly used sugar is sucrose. It may come from either cane or beet, as these are identical in composition. Corn sugar, now used extensively, is predominantly glucose or is converted to maltose or fructose. Invert sugar, a mixture of equal amounts of the monosaccharide fructose and glucose, is used at times. Lactose constitutes over one-third of the solid matter in milk and ~20% of the carbohydrates in ice cream, which makes this product suitable for lactose-intolerant people. Furthermore, it is readily hydrolyzed into glucose and galactose. It enhances the utilization of calcium and phosphorus in the human body. Additionally, ingestion of higher amounts of lactose is conducive to the growth of lactose-fermenting *Lactobacillus acidophilus*, which produces acidic condition in the intestine, thereby creating unfavorable conditions for the growth of putrefactive bacteria. A sandy body and texture defect is caused by a high concentration of lactose.

Fat in Ice Cream

Fats are a rich source of energy. It is of major importance in ice cream. It contributes a rich flavor and is a good carrier for added flavor compounds and promotes desirable texture qualities. It is essential to use the correct percentage of milk fat to balance the mix properly as also to satisfy legal standards. Milk fat is in the form of an emulsion; hence, it does not lower the freezing point as it is not a true aqueous solution. It tends to retard the rate of whipping. High fat content limits consumption, will increase the cost, and increase calorific value. Generally, the fat content of a good average ice cream is considered to be 12%. The best source of milk fat is fresh cream. Other sources are frozen cream, plastic cream, butter, butter oil, and condensed milk blends. Fats are present in foodstuffs and storage depots of most animals, mainly in the form of triglycerides. Milk fats have both saponifiable matter and nonsaponifiable matter. The saponifiable fraction includes the glycerides, phospholipids, and other esters, such as those of cholesterol and minor acid components. Milk fat contains at least 60 fatty acids. Milk fat contributes significantly to the nutritive value of ice cream. It is a rich source of energy, serves as a carrier of fat-soluble vitamins A, D, E, and K, and contains a significant amount of essential fatty acids, for example, linoleic and arachidonic acids. The amount of saturated and unsaturated fatty acids and cholesterol in ice cream, ice cream cones, and ice milk is given in Table 3.

Minerals in Ice Cream

A variety of inorganic elements are essential for growth and performance. Those needed in substantial amounts, such as calcium, phosphorus, magnesium, sodium, potassium, and sulfur, are termed as major minerals or macronutrients. Those needed in small amounts, such as copper, cobalt, iodine, manganese, zinc, fluorine, molybdenum, and selenium, are termed as trace elements. The inorganic nutrients are interrelated and should be in particular proportions in the diet. Calcium and phosphorus are of vital concern, since they are very closely related. Milk and products like ice cream are the richest sources of calcium, phosphorus, and other minerals essential in adequate nutrition. It has been proved by research workers that additional amounts of lactose in the diet favor the assimilation of calcium. As ice cream is a rich source of lactose, it favors the assimilation of greater quantities of the calcium content of diet, which is needed by growing children and some adults. The calcium contents of milk and ice cream are 0.118 and 0.132 g/100 g, respectively; the corresponding phosphorus contents are 0.093 and 0.105 g/100 g, respectively. The minerals impart a slightly salty taste, which rounds out the flavor of the finished ice cream.

Vitamins in Ice Cream

Vitamins are organic substances, which are required in small amounts by the body for its metabolism and cannot be synthesized in sufficient quantities by the body. These do not contribute appreciable amounts of energy. Vitamins are divided into two groups: fat-soluble and water-soluble vitamins. The fat-soluble vitamins include A, D, E, and K; the water-soluble vitamins include B_1 or thiamine, B_2 or riboflavin, B_6, and B_{12}. Like milk, ice cream is a rich source of many of the essential vitamins without which normal growth and health cannot be maintained. A brief description of the better-known vitamins may help to emphasize the importance of milk and ice cream in the diet.

Fat-soluble vitamins in ice cream

Vitamin A: Ice cream is an excellent source of this anti-infective vitamin (492 IU 100 g^{-1}). It is the principal milk fat vitamin. It is essential for growth and normal functioning of the retina. Its deficiency results in night blindness and follicular keratosis, a skin disorder.

Table 3 Selected fatty acids and cholesterol in ice cream and related products (per 100 g edible portions)

	Fatty acids				
Item	*Total (g)*	*Total unsaturated (g)*	*Unsaturated oleic (g)*	*Linoleic (g)*	*Cholesterol (mg)*
Ice cream	12.5	7.0	4.0	Trace	45.0
Ice cream cones	2.4	1.0	1.0	Trace	0.0
Ice milk	5.1	3.0	2.0	Trace	21.6

Source: Arbuckle, W. S. (1986). *Ice cream* (4th ed.). New York: AVI Publishing

Vitamin D: Ice cream contains little quantities of this antirachitic vitamin (4 IU 100 g^{-1}). Its deficiency results in decreased growth rate and lowered calcium and inorganic phosphorus levels in the blood. Vitamin D-enriched ice creams can be prepared from fortified milk.

Vitamin E: Ice cream is a fair source of this antisterility vitamin, containing about 3 mg kg^{-1}. This vitamin helps to maintain normal health and reproductive organs in laboratory animals. This vitamin is found in all cell membranes. It acts as an antioxidant for polyunsaturated fatty acids and prevents a large number of degenerative disorders.

Vitamin K: This vitamin has been found necessary for the formation of prothrombin, which prevents bleeding by clotting the blood. The concentration of this vitamin is very low in milk, and pasteurization and evaporation destroy it.

Water-soluble vitamins

Vitamin B_1 (thiamine): Ice cream contains an average of 0.48 mg kg^{-1} of this vitamin, with a range of 0.38–0.65 mg kg^{-1}, which is essential for proper metabolism and health. Its deficiency causes beriberi, which is manifested by loss of appetite, anxiety, exhaustion, and irritability. In some cases, it can cause nervous disorders or dilation of the heart.

Vitamin B_2 (riboflavin): Riboflavin is a dietary essential vitamin for humans. Ice cream is a fairly good source of riboflavin, containing an average of 2.3 mg kg^{-1}, with a range of 2.0–2.6 mg kg^{-1}. Its deficiency causes lesions of the eye and mouth and reddening of the lips.

Vitamin B_6 (pyridoxine): It is an important coenzyme in the metabolism of amino acids. Its deficiency can cause anemia, convulsions, decreased growth rate, and dermatitis. Ice cream contains an average of 0.0047 mg kg^{-1} of this vitamin, with a range of 0.0026–0.0078 mg kg^{-1}.

Vitamin B_{12} (cyanocobalamin): It is one of the anemia-preventing vitamins and is unique among vitamins, as it is not found in any plants. This vitamin has the most complex structure of all vitamins. It is an essential metabolite for a wide variety of organisms. Ice cream contains an average of 0.0047 mg kg^{-1} of this vitamin, with a range of 0.0026–0.0078 mg kg^{-1}. Its deficiency can produce anemia and degeneration of the spinal cord.

Vitamin C (ascorbic acid): It is widely distributed in the tissues of all plants and animals. It is abundantly found in fruits, fruit juices, and green leafy vegetables. These vitamins act as an antiscorbutic factor in the prevention of scurvy. Fruit ice creams are excellent sources of this vitamin. On an average, ice cream contains 3 mg kg^{-1} of this vitamin, with a range of 0–11 mg kg^{-1}.

Stabilizers

Stabilizers are used to prevent the formation of objectionable large ice crystals in ice cream. They have high water-holding capacity; in addition, they prevent ice crystal formation in storage, give uniformity of product, give desired resistance to melting, and improve handling properties. The effect of stabilizers on flavor is indirect. They increase viscosity, have no effect on the freezing point, and generally decrease whipping ability. The amount of stabilizers to use varies with its properties, with the solids content of the mix, with the type of processing equipment, and other factors.

Generally, stabilizers are added at the rate of 0.2–0.3% of the mix. Stabilizers commonly used are sodium alginate, sodium carboxymethyl cellulose (CMC), guar gum, locust bean gum, carrageenan, gelatin, and pectin. It is not necessary to age the mix when alginates are used. CMC produces a chewy characteristic in the finished product. Gelatin produces a thin mix and requires an aging period. Pectin is used alone or in combination with gums as a sherbet or ice stabilizer. The addition of excessive amounts of stabilizers results in soggy or heavy body and high resistance to melting in the finished product.

Emulsifiers

Emulsifiers are used to produce ice cream with a smoother body and texture, to impart dryness, and to improve whipping ability of the mix. Emulsifiers extensively used are monoglycerides or diglycerides, sorbates, and polysorbates and are added at the rate of 0.1–0.4% of the finished product. Egg yolk solids are also used as emulsifiers. Excessive amounts of emulsifiers produce slow melting characteristics and body and texture defects in the product.

Flavors

Flavor is generally considered as one of the most important characteristics of ice cream. The kind of flavoring material influences the quality of the ice cream mix since slight off-flavor in it can obscure the delicate flavor of the flavoring material to be added. Local preference of the consumers determines the type and intensity of flavor to be added. Natural and synthetic flavor substances are available for the flavoring of ice cream.

Colors

Ice cream should have a delicate, attractive color that can be readily associated with the flavor. Most colors are of chemical origin. Colors are available in liquid or powder form. Most ice cream manufacturers prefer to purchase dry colors since these are more economical and can be dissolved in boiling water as needed.

Digestibility and Palatability of Ice Cream

Ice cream is considered as an ideal food for people suffering from stomach or throat ailments when other foods cannot be taken. There are several reasons to support this viewpoint. Its sweet, pleasant flavor, smooth texture, and characteristic coolness make it a highly palatable food. Its high palatability stimulates the flow of digestive juices, which enhances digestion. Moreover, homogenization employed in its manufacture facilitates digestion as it happens with homogenized milk, as the digestive juices readily act upon the fat globules broken into small globules. Many experiments show that homogenized milk is more digestible than milk not so treated. The same holds true for the fat in ice cream mix when it is forced through the homogenizer, as is now the practice in commercial ice cream plants.

Health Effects of Ice Cream

To combat osteoporosis

Dairy products like ice cream contain 0.122 g calcium per 100 g. It is beneficial for strong and healthy bones. According to the Office of Dietary Supplements (ODS), 99% of the body's calcium can be found in the bones and teeth where it is used to help function and structure. When the body is not receiving a sufficient amount of calcium daily, it can take calcium from where it is stored. Regular calcium intake from ice cream and other dairy products can also reduce the risk of osteoporosis, a disease related to an increase in bone fractures. Not only is calcium good for the bones and teeth, but also it plays a part in weight loss. Studies have shown a correlation between reduced weight and weight gain prevention with an adequate daily intake of calcium. When the body is not receiving an adequate amount of calcium, it causes fat cells to enlarge by storing fat. The lack of calcium is correlated to the creation of fat-producing hormones and a slowing effect of fat breakdown leading to weight gain.

In women's reproductive health

It is very strongly advocated by the researchers that ice cream is helpful in maintaining the reproductive health of women. A recent investigation comprising 18 000 women of 24–42 years suggests eating ice cream increases chances of ovulating. The study looked at the eating habits of the women under the feeding trials. After accounting for other factors, like smoking and drinking, the researchers found that women who ate full-fat ice cream two more times a week had a 38% lower risk of ovulation-related infertility. That is compared to women who had full-fat ice cream less than once a week. So what about low-fat foods? The study found that women who ate at least two servings of low-fat milk products a day were 85% more likely to have problems ovulating than women who only ate one serving. The researchers suggest women trying to get pregnant keep a careful eye on calories, but eat full-fat until conception.

Improvement of immunity

As ice cream is rich in lactoferrin and cytokines, it improves the immunity against various diseases, including influenza. Ironically, there is a misapprehension that ice cream is regarded as a cause of colds and coughs. In fact, when we eat ice cream, melting ice cream that goes into the mouth is affected by body temperature, so at that point, the temperature of ice cream is not so low any longer.

Ice cream as a delivery medium for probiotics

Probiotics are distinct live microorganisms that, when administered in sufficient amounts, exert a health benefit on the host. In recent years, probiotic bacteria have increasingly been incorporated into dairy foods as dietary adjuncts. This is an ideal way to reestablish the balance of intestinal microbiota. Probiotic ice cream can be produced by the incorporation of probiotic bacteria in both fermented and unfermented mixes. Ice cream is an ideal vehicle for the delivery of these organisms in the human diet. *Lactobacillus* and *Bifidobacterium* are the most common species of lactic acid bacteria used as probiotics for this purpose. Among the frozen dairy products with live probiotics, probiotic ice cream is also gaining popularity for its neutral pH. The high total solids level in ice cream including the fat and milk solids provides protection for the probiotic bacteria. Because the efficiency of added probiotic bacteria depends on dose level, type of dairy foods, presence of air, and low temperature, their viability must be maintained throughout the product's shelf life and they must survive the gut environment. Live probiotic bacteria are more than unviable cells; therefore, the International Dairy Federation (IDF) recommends that a minimum of 10^7 probiotic bacterial cells should be alive at the time of consumption per gram of product. Studies indicate, however, the bacteria may not survive in high enough numbers when incorporated into frozen dairy products unless a suitable method is used against freeze injury, and oxygen toxicity represents factors affecting probiotic survival in ice cream.

Another way is to adjust the conditions of production and storage for more survival rates. The physical protection of probiotics by microencapsulation is a new method for increasing the survival of probiotics. Encapsulation helps to isolate the bacterial cells from the adverse environment of the product and gastrointestinal tract, thus potentially reducing cell loss. Selecting suitable probiotic strains depends on their ability to survive simulated conditions of ice cream (high sucrose concentrations, high oxygen, freezing, and storage temperatures), acidic (to simulate gastric conditions) and alkaline conditions (to simulate intestinal conditions). Microencapsulation of probiotics can further protect these bacteria from the mentioned conditions.

During the last two decades, several studies have shown that ice cream has a good ability as a medium for delivery of probiotics into the human gut. Frozen dairy products create ideal conditions for probiotic bacteria to survive for a long term of production, distribution, and storage. Ice cream provides good conditions for probiotic growth in large numbers and their survival during storage. Low-fat ice cream in comparison with regular ones provides better conditions for the survival of probiotics. Incorporation of probiotic bacteria in ice cream does not create any problem because ice cream pH (6.6–6.5) is ideal for survival of these microbes.

It could be concluded from the ongoing discussion that ice cream is particularly valuable for its content of high-quality protein and easily assimilated calcium. It provides many nutrients and can make a useful and enjoyable contribution to the daily intake of energy in a healthy and balanced diet. The future success of functional probiotic ice creams in marketplace depends on its consumer acceptance. The development of probiotic ice cream is a key research priority for food design and a challenge for both industry and science sectors. Especially, ice cream is a good vehicle to transfer probiotics to the human intestinal tract. An additional way to keep the levels up of the probiotic cells in the gut is to consume this frozen dessert on a regular basis. More studies are needed to further investigate the probiotic survival in harsh conditions of ice cream formulation and manufacturing.

See also: Carbohydrate: Digestion, Absorption and Metabolism; Ice Cream: Uses and Method of Manufacture; Milk: Role in the Diet; Nutrition and Infection; Osteoporosis; Probiotics; Protein: Digestion, Absorption and Metabolism; Vitamins: Overview.

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Ice Cream: Uses and Method of Manufacture

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Introduction

Ice cream is a frozen dairy product made by freezing the ice cream mix with agitation. It is composed of a mixture of food ingredients like milk products, sweetening materials, stabilizers, colors, flavors, and egg products. Ice cream had its origins in Europe and was introduced later to the United States where it developed into an industry. It is widely believed that ice cream evolved from iced beverages and water ices. Ice cream probably came to the United States with the early English settlers. In 1851, the first wholesale ice cream industry in the United States was established in Baltimore, Maryland. Ice cream plants were then established in New York, St. Louis, Chicago, Washington, and Cincinnati. The development of condensed and dried milks and the introduction of the pasteurizer and homogenizer, improved freezers, and other preserving equipments accompanied the growth of this industry after 1900. The ice cream soda was introduced in 1879, and ice cream cone and Eskimo Pie were introduced in 1904 and 1921, respectively.

Global *per capita* consumption of ice cream and related products is presented in Table 1. The US ice cream market in 2007 was $23 billion. The global value of the ice cream industry is US$59 billion, growing on an average by 4% per annum with a growth rate of 7–9%. More than 90% of American households purchase ice cream. Global market share, according to point of manufacture/consumption, take-home, impulse, or artisanal products, is shown in Table 2. The take-home category is defined as grocery store purchases and home consumption, the impulse category is categorized by hand-held, single-serving eat-on-the-spot products (sometimes referred to as novelty products), and the artisanal category is defined as being manufactured at the site of purchase and consumption or sold directly by the manufacturer to the consumer (e.g., ice cream parlors and street vendors). There are very large differences globally, impacted in large part by the presence of home freezers.

Ice creams are unique frozen food because they are consumed in the frozen state, usually as a scooped product or as a single-serving item, sometimes on a stick and often with other confectionery items. These products rely on a concomitant freezing and whipping process to establish the desired structure and texture. The manufacturing process for most of these products is similar. It involves the preparation of a liquid mix, which is prepared by blending the desired ingredients, followed by pasteurization, homogenization, and aging. It is followed by whipping and freezing this mix dynamically under high shear to soft, semi-frozen slurry. The flavoring ingredients are then incorporated in this partially frozen mix. It is then placed in molds for desired shaping and packaging the product. It is further subject to freezing (hardening) under static, quiescent conditions. Swept-surface freezers are used for the first freezing step, while forced convection freezers, such as air blast tunnels or rooms, or plate-type conduction freezers are used for the second freezing step.

A wide variety of ingredients are used to produce ice creams. Even any one kind of ice cream can be made by combining the ingredients in any of several different combinations of ingredients. Ice cream mix is the unfrozen mixture of the ingredients, consisting of all the components of ice cream with the exception of air and flavoring materials. The composition of ice cream is usually expressed as a percentage of its constituents, for example, percentage of milk fat, milk solids-not-fat, sugar, egg solids, stabilizers, and the total solids.

Ice Cream Mix

Ice cream mix comprises milk fat, milk solids-not-fat (MSNF), sweeteners, stabilizers, colors, emulsifiers, and water. Dairy and other ingredients used to supply these components are chosen on the basis of their cost, availability, and quality of the finished product. An account of composition of various frozen dairy desserts is presented in Table 3. The source of fat that is derived from milk ingredients (e.g., cream and butter) is common in North America and many other parts of the world, while fat derived from nondairy sources (e.g., coconut oil and palm kernel oil) is more common in parts of Europe and Asia. The triglycerides in milk fat have a wide melting range of 40 to −40 °C. Consequently, at refrigeration temperatures, there is always a combination of liquid and crystalline fat within the globules. The resulting solid–liquid ratio at freezer barrel temperatures is important for formation of ice cream structure, as crystalline fat is required for partial coalescence. Nondairy fat sources must also be chosen to provide a suitable solid fat content.

The Milk Solids-Not-Fat

It comprises lactose, casein, whey proteins, minerals, vitamins, and other minor components of the milk or milk products from which they were derived (e.g., skim milk powder, condensed milk, and whey protein concentrate ingredients). Proteins contribute much to the development of the structure of ice cream, including emulsification, whipping, and water-holding capacity. Whipping of proteins in ice cream contributes to the formation of the initial air bubbles in the mix. Emulsification of proteins in the mix arises from their adsorption to fat globules due to homogenization. Viscosity of mix is enhanced due to water-holding capacity of proteins, which imparts a desired body to the ice cream, increases the meltdown time of ice cream, and contributes to reduced iciness.

Table 1 *Per capita* consumption of ice cream and related products in selected countries (2002, unless otherwise noted)

Country	*Consumption*
New Zealand	22–23 (2006)
The United States	18.3 (2007)
Australia	18 (2006)
Finland	14 (2006)
Sweden	12
Italy	9.2
Ireland	9.0
Denmark	8.7
Canada	8.7 (2007)
The United Kingdom	6 (2007)
Chile	5.6
Malaysia	2.0
China	1.9 (2006)

Source: Goff, H. D. (2011). Ice cream and frozen desserts: product types. In: *Encyclopedia of dairy science*, 2nd ed., pp. 893–898. London: Elsevier.

Table 2 Global market share characterized by point of manufacture/consumption (2006)

	Take-home	*Impulse*	*Artisanal*
Global, by volume	41	50	8
Global, by monetary value	26	52	21
Regional, by monetary value			
North America	43	34	21
Western Europe	21	45	34
Australasia	39	49	19
Asia Pacific	10	86	4
Eastern Europe	28	67	5
Latin America	24	63	13

Source: Goff, H. D. (2011). Ice cream and frozen desserts: product types. In: *Encyclopedia of dairy science*, 2nd ed., pp. 893–898. London: Elsevier.

Sweeteners

Sweeteners improve the texture and palatability of the ice cream, impart sweet taste, and enhance flavors. They also lower the freezing point of the mixture, which imparts a measure of control over the temperature–hardness relationship. In determining the proper blend of sweeteners for an ice cream mix, the total solids required from the sweeteners, the sweetness factor of each sugar, and the combined freezing point depression of all sugars in solution (including lactose from the dairy ingredients) must be calculated to achieve the proper solids content, the appropriate sweetness level, and a satisfactory degree of hardness. The most common sweetening agent used is sucrose. It provides sweetness, depresses freezing point, affects freezing performance, affects body and texture, enhances flavor, and contributes bulk or total solids and impacts on economics. Generally, the equivalent of 15% sucrose is considered optimal sweetness in ice cream. Substitute sweeteners derived from starch hydrolysate syrup (glucose syrup) for all or a portion of the sucrose can also be used as it is relatively cheaper. Glucose syrups impart greater smoothness by contributing to a firmer and chewy body, provide better meltdown characteristics, bring out enhanced fruit flavors, and reduce heat shock potential that improves the shelf life of the finished product. Nonnutritive sweeteners, which do not provide any significant calories can also be used for diabetics. They include sucralose, aspartame, saccharin, cyclamates, acesulfame-K, and many others. As little as 0.07% aspartame can provide the sweetness equivalent to 15% sucrose.

Stabilizers

Apart from sweeteners, stabilizers are also important nondairy ingredients. The term stabilizer is used for a group of substances that help stabilize the structure of ice cream. The commonly used stabilizers in ice cream mix are a group of ingredients (polysaccharides) such as guar, locust bean gum, carboxymethylcellulose, and xanthan. Stabilizers increase the

Table 3 Approximate composition (%) of commercial frozen desserts by formulation category

Group	*Milk fat*	*Milk solids-notfat*	*Sweeteners*[a]	*Stabilizers*[b]	*Total solids*
Nonfat ice cream	<0.5	12–14	18–22	1.0	28–32
Low-fat ice cream	2–5	12–14	18–21	0.8	28–32
Light ice cream	5–7	11–12	18–20	0.5	30–35
Reduced-fat ice cream	7–9	10–12	18–19	0.4	32–36
Economy ice cream	10	10–11	15–17	0.4	35–36
Standard ice cream	10–12	9–10	14–17	0.2–0.4	36–38
Premium ice cream	12–14	8–10	13–16	0.2–0.4	38–40
Super premium ice cream	14–18	5–8	14–17	0–0.2	40–42
Frozen yogurt: regular	3–6	9–13	15–17	0.5	30–36
Frozen yogurt: nonfat	<0.5	9–14	15–17	0.6	28–32
Sherbet	1.2	1–3	22–28	0.4–0.5	28–34

[a]Includes sucrose, glucose, corn syrup solids, maltodextrins, polydextrose, and other bulking agents, some of which contribute little sweetness.
[b]Includes ingredients such as locust bean gum, guar gum, carrageenan, cellulose gum, and cellulose gel, as stabilizers, and also mono- and diglycerides and polysorbate 80, as emulsifiers.
Source: Goff, H. D. (2011). Ice cream and frozen desserts: product types. In: *Encyclopedia of dairy science*, 2nd ed., pp. 893–898. London: Elsevier.

viscosity of the mix; produce smoothness in body and texture; retard or reduce ice and lactose crystal growth during storage, especially during periods of temperature fluctuation, known as heat shock; and hold flavorings in uniform suspension. The mechanism of action of stabilizers in enhancing frozen stability is related primarily to its effect on the ice and unfrozen serum phases. Stabilized ice cream has smaller ice crystals than unstabilized ice cream after storage at fluctuating temperatures. Usually, stabilizers are used at 0.1–0.5% levels in the mix, but the actual amount depends on the type and strength of the stabilizer, total solids and fat level of the mix, duration and temperature of storage of ice cream, and the method of pasteurization. High-fat and high-total solid mixes require lower levels of stabilizers. More stabilizer is needed for ice cream that is stored for a long period of time or if the temperature fluctuation during storage is frequent.

Emulsifiers

Emulsifiers are sometimes integrated with the stabilizers in proprietary blends, but their function and action are very different from the stabilizers. They exert their action on the fat phase of ice cream. These are surface-active agents. Emulsifiers facilitate the mixing of fat and water because these molecules have two domains, one that likes water (hydrophilic) and another that likes fat (hydrophobic). When the hydrophobic part of a surfactant interacts with the fat, the water-loving part of the molecule can interact with water, thus facilitating the suspension of fat in water. Generally, mono- and diglycerides and ethoxylated esters of sorbitol (polysorbates) are the commonly used emulsifiers. Mono- and diglycerides are derived from fatty acids and glycerol. Therefore, emulsifiers are fatty substances. They also show fatlike properties such as melting point and crystallinity, and they can be composed of saturated or unsaturated fatty acids. The presence of emulsifiers in ice cream leads to smoother texture and better shape retention while improving the ability of the mix to incorporate air. The mode of action of emulsifiers is related to their activity at the air–serum and fat–serum interfaces. At the fat–serum interface, they displace proteins from the surface of the fat globules, rendering the fat globules more susceptible to partial coalescence and structure formation during the freezing and whipping process.

Figuring the Mix

Ice cream processing operations can be divided into two distinct stages: figuring the mix and freezing operations. Once a formula has been finalized, a recipe has to be created. Formulas specify composition of the desired mix in terms of percentages of fat, MSNF, sweeteners, stabilizers, and emulsifiers. A recipe calculates the weight and/or volumes of ingredients needed to meet the formula requirements. These calculations are called mix calculations. Mix calculations are essential for manufacturing consistent quality finished products. When composition of raw materials varies or the economics of ingredients changes, the recipe for making an ice cream mix has to change. Further, this change has to occur in a manner that the finished product composition is not altered. In some instances, changing regulatory definitions and health claims may necessitate manufacture of products to carefully defined specifications.

Mix calculations are important in standardizing mixes prior to freezing. Ice cream plants now use computer software programs to calculate the amounts of various ingredients to conform to required specifications of composition of ice cream mix. However, for basic understanding, the fundamentals of the mix calculations are discussed here. Mix calculations can be performed by Pearson's square, algebraic methods, and arithmetic methods. Pearson's square is of limited utility; algebraic methods are complicated and involve the use of simultaneous equations and matrices. Arithmetic calculations are simpler and require fewer computations than algebraic methods.

Figuring the mix consists of the following unit operations: combination and blending of ingredients, batch or continuous pasteurization, homogenization, and mix aging. Ingredients are usually preblended prior to pasteurization, regardless of the type of pasteurization system used. Blending of ingredients is relatively simple if all ingredients are in the liquid form, as automated metering pumps or tanks on load cells can be used. When dry ingredients are used, powders are added either through a pumping system under high velocity or through a liquefier that chops all ingredients as they are mixed with the liquid.

Pasteurization

It is designed to destruct pathogenic bacteria, if any are present. In addition, it serves a useful role in reducing the total bacterial load and in solubilizing some of the components (proteins and stabilizers). Both batch and continuous (high-temperature short-time (HTST)) systems are in common use. In a batch pasteurization system, blending of the ingredients is done in large jacketed vats equipped with some means of heating, usually saturated steam or hot water. The product is then heated in the vat to at least 69 °C and held for 30 min to satisfy legal requirements for pasteurization. This is necessary for the destruction of pathogenic bacteria. Various time–temperature combinations can be used, depending on the legal jurisdiction. Continuous pasteurization is usually performed in an HTST heat exchanger following the blending of ingredients in a large, insulated feed tank. Regulations concerning time–temperature combinations for continuous pasteurization usually specify a minimum temperature of 80 °C for at least 25 s.

Homogenization

Following pasteurization, the mix is homogenized using high pressures. It is responsible for the formation of the fat emulsion by forcing the hot mix through a small orifice under pressures of 15.5–18.9 MPa, depending on the mix composition. The resulting eight- to tenfold increase in the surface area of the fat globules is responsible in part for the formation of the fat globule membrane, composed of adsorbing materials attempting to lower the interfacial free energy of the fat globules. With single-stage homogenizers, fat globules tend to cluster as bare

fat surfaces come together or adsorbed molecules are shared, especially in ice cream mix. Therefore, a second homogenizing valve is frequently placed immediately after the first with applied back pressures of 3.4 MPa, allowing more time for surface adsorption to occur. The net effects of homogenization are in the production of smaller, more uniform fat droplet size, resulting in a greater stability of fat droplets during aging, a better whipping ability, and a smoother, more uniform final product with a greater apparent richness.

Aging of the Mix

The time of 4 h or greater at 2–4 °C is recommended for aging following mix processing prior to freezing. Aging is performed in insulated or refrigerated storage tanks, silos, etc. or in single-walled tanks in chilled rooms, where valves and pipeline can also be kept cold. Aging allows hydration of milk proteins and stabilizers (some viscosity increase occurs during the aging period), crystallization of the fat globules, and a membrane rearrangement to produce a smoother texture and better-quality product. Nonaged mix exhibits a low viscosity, is very wet at extrusion from the dynamic freezer, and exhibits variable whipping abilities. The appropriate ratio of solid–liquid fat must be attained at this stage, a function of temperature and the triglyceride composition of the fat used, as a partially crystalline emulsion is needed for partial coalescence in the whipping and freezing step. Emulsifiers generally displace milk proteins from the fat surface during the aging period. The whipping qualities of the mix are usually improved with aging. Mix temperatures should be maintained as low as possible without freezing.

Flavorings

Flavor is an important attribute of a food. It is a sensory response that has three components, olfactory (odor/smell), gustatory (taste), and tactual (mouthfeel). Ice cream is cold, creamy, refreshing, and sweet and releases aroma upon melting in the mouth. When the word 'flavor' is used in everyday parlance, we imply taste and olfaction. Taste compounds are sweet, salty, bitter, and sour. Generally, compounds imparting these tastes can be detected at levels of 0.01–0.5. Olfactory compounds (smelly stuff) are volatile and have thresholds in the parts per million to parts per trillion range. Threshold is defined as the minimum concentration that at least 50% of the population can detect. Threshold of aroma compounds are 10–10 million times less than taste compounds.

Perception of aroma is affected by the composition, physical structure, and temperature of the food. Undesirable flavors are called off-flavors. Off-flavors affect the overall flavor qualities of the food. Deteriorative reactions are time-dependent and cumulative. Therefore, the length and conditions of storage have a profound influence on the perception of overall flavor. These deteriorative reactions occur in ingredients used in ice cream manufacture. Therefore, careful attention should be paid to the quality of ingredients used in ice cream manufacture.

Glass Transition Temperature

Temperature stability of ice cream has focused on carbohydrate glass formation as a function of temperature. A glass is characterized as a metastable solid with a high viscosity. At the glass transition temperature, polymeric materials change from a viscoelastic liquid to an amorphous solid (glass) with an associated increase in viscosity. For ice creams, this temperature is defined as the glass transition temperature of a maximally freeze-concentrated solution. The interest in glassy state in ice creams has increased steadily since the last 15 years. It is now well established that the glassy-state temperature influences the storage stability as above these temperatures, the solutions are unstable and reactive. Ice crystallization can also occur in this phase. Below the glass transient temperature, the materials become glossy and no molecular motion or chemical reaction occurs. The ice crystallization is prevented and the shelf life of the product is prolonged. Glass transition temperature for ice cream is −32 °C. Storage of ice cream at this low temperature to maintain the glassy state for enhanced stability is not practical.

Freezing Process

The process of freezing of ice cream in simple words is the creation of ice from water in the mix. Therefore, the only constituent of the mix being frozen is water. During the freezing process, the equilibrium between water and ice is altered. Freezing is facilitated by the removal of heat from a substance. In the old salt and ice machine, used prior to mechanical refrigeration, ice served as the refrigerant and addition of salt lowered the freezing point of water. The brine extracts heat from the mix. The mix temperature is lowered and the brine temperature increases. Brine is not a good refrigerant. With the advent of mechanical refrigeration, the use of ice and salt for freezing ice cream was relegated to a hobby status.

Modern ice cream freezing consists of two distinct stages: (1) passing the mix through a swept-surface heat exchanger under high-shear conditions to promote extensive ice crystal nucleation and air incorporation and (2) freezing the packaged ice cream under conditions that promote rapid freezing and formation of small ice crystals. The freezing and whipping process is one of the most important unit operations for the development of quality, palatability, and yield of finished product. Due to the incorporation of air, foam is created. It leads to the formation of the ice phase and the partial destabilization of the fat emulsion. The objective of ice cream manufacturers is to produce ice crystals that are below the threshold of sensory detection at the time of consumption. This threshold has been suggested to be between 40 and 50 μm. Therefore, the freezing steps of the manufacturing process and the temperature profile throughout the distribution system are critical factors in meeting this objective. Flavoring and colorants are added as desired to the mix prior to passing through the barrel freezer, and particulate flavoring ingredients, such as nuts, fruits, candy pieces, and ripple sauces, can be added to the semifrozen product at the exit from the barrel freezer prior to packaging and hardening.

Continuous Freezers

Continuous freezers are commonly used in larger ice cream manufacturing plants where more than 500 gal (1875 l) of ice cream per day may be manufactured. These freezers have larger capacities and can be operated continuously, ingredients can be added in-line, and packaging can be also automated. Also, continuous freezers make it possible to produce ice cream of different shapes through extrusion devices. Novelty extrusions, such as sandwiches, prefilled cones and cups, and cakes, are possible through the use of continuous freezers. The ice cream from a continuous freezer is smoother and creamier than a product from a batch freezer. This is because the ice crystals formed in a continuous freezer are smaller and the air cells may also be more uniform. The ice cream exiting a continuous freezer is also generally colder than that coming out of a batch freezer. There are a number of different types of continuous ice cream freezers; some are vertical freezers, especially for smaller-scale operations; others are horizontal ice cream freezers. Regardless of whether the freezing cylinder is horizontal or vertical, all continuous freezers have a set of blades for scrapping the walls of the freezers. In a continuous freezer, a mixture of air and the mix is introduced at one end and is progressively frozen until ice cream is discharged at the other end. The conveyance of the mixture of air and the mix and the discharge of the ice cream may be facilitated by coordinated pumps in some models. Also, the newer models of freezers are equipped with microprocessor controls that monitor and control the discharge temperature of the ice cream, the viscosity of the ice cream, and the overrun of the ice cream. Further, these microprocessors can work in tandem with other downstream equipment such as ingredient feeders and packaging lines.

These freezers dominate the commercial ice cream industry. In this type of process, ice cream mix is drawn from the flavoring tank into a scraped-surface heat exchanger, which is jacketed with a liquid, boiling refrigerant (usually ammonia; a chlorinated, fluorinated hydrocarbon (CFC) such as R-12, R-22, or R-502; or one of the newly developed CFC substitutes).

A continuous freezer is usually operated under pressure, to minimize the volume of air in the ice cream and thus maximize heat transfer. Air bubbles expand rapidly when the ice cream is drawn through the outlet valve to atmospheric pressure. The introduction of low-temperature extrusion equipment has been a recent development in ice cream freezing technology. This is a continuous-flow heat exchanger in the form of a screw extruder that reduces the temperature of ice cream from the draw temperature of the barrel freezer, typically $-5\,^{\circ}C$, to a range of -10 to $-14\,^{\circ}C$. The ice cream passes at low shear through a twin-screw or single-screw design with a residence time of 1–2 min. Ice cream that results from such processing shows much smaller ice cream and air bubble sizes, and the fat is agglomerated into a structure that provides a creamy texture.

When the product emerges from the low-temperature extruder, it is still pliable, so some particulates can be added to it, and it can be cut and packaged, but it sets up very quickly thereafter to a very firm consistency. Hence, the claim is made that low-temperature extrusion can replace the need for conventional hardening of ice cream.

Batch Freezers

Batch freezers are commonly used by small ice cream units that make ice cream on the premises. In batch freezers, a predetermined amount of mix is charged into the freezing chamber; refrigeration is turned on as is the agitation. Generally, the mix will occupy half of the barrel. The mix is agitated and whipped while being cooled. After some time, the mix begins to freeze, and when it achieves a certain consistency, it begins to incorporate air. Incorporation of air in conjunction with the freezing stiffens the ice cream. At this point, the refrigeration should be turned off and agitation continued for some additional period of time. When the desired overrun is achieved, the ice cream is discharged from the barrel with the agitator mechanism still on.

Just prior to discharge of the ice cream, fruits and nuts can be added to the barrel, but the preferred method of addition of particulate inclusions is to fold them into the ice cream as they are being discharged from the barrel. Once this process is complete, the next batch of mix can be charged into the freezer barrel and the process repeated.

The batch freezer differs considerably from the continuous systems described in the preceding text. The barrel of a batch swept-surface heat exchanger is jacketed with refrigerant and contains a set of dashers and scraper blades inside the barrel. It is filled to about one-half volume with the liquid mix. Barrel volumes usually range from 2 to 12 l. The freezing unit and agitators are then activated, and the product remains in the barrel for sufficient time to achieve the desired degree of overrun and stiffness. Whipping increases with time and cannot exceed the amount that will fill the barrel with the product (i.e., 100% overrun when starting half full). Batch freezers are used in smaller operations where it is desirable to run individual flavored mixes on a small scale or to retain an element of the 'homemade'-style manufacturing process.

The formation of the ice phase during the continuous freezing stage is responsible in part for the structure and texture of the final product. The crystallization of water to ice involves two major steps: nucleation and crystal growth. Nucleation occurs at the wall of the heat exchanger during start-up. After start-up, the continual scraping action of the blades acts as a seeding mechanism, by providing a source of tiny crystals into the bulk where they grow.

Overrun in Ice Cream

Incorporation of air into ice cream, termed the overrun, occurs at this stage. Some overrun is necessary to produce desirable body and texture. Two main types of air incorporation systems are used in continuous freezers. In older systems, the pump configuration resulted in a vacuum either at the pump itself or on the mix line entering the pump. Air was then incorporated through a spring-loaded, controllable needle valve. Newer types of freezers use compressed air, which is injected into the mix. This type of air handling system allows for air filtration (0.65 μm micropore filter) prior to admission into the mix.

Following aeration, the water in the mix is partially frozen as the mix and air combination passes through the barrel of the heat exchanger. Ice forms on the inside wall of the heat exchanger from the water in the mix, resulting in a freeze

concentration of the dissolved solutes. Rotating knife blades are responsible for continually scraping this frozen mix off the surface of the heat exchanger wall and mixing it into the bulk flow of freeze-concentrated liquid mix, where these tiny ice crystals grow. The dasher keeps the product agitated inside the barrel and provides a more homogeneous mixture of ice and freeze-concentrated liquid. Residence time for mix through the annulus of the freezer varies from 0.4 to 2 min, although some products may remain for much longer times; especially with open cage dashers, freezing rates can vary from 5 to 27 °C min^{-1}, and a draw temperature of −6 °C can easily be achieved.

Hardening of Ice Cream

After freezing, the flavors, nuts, chocolates, etc. are added, and the product is packed and immediately transferred to a hardening chamber (−30 °C or colder, either forced convection or plate-type conduction freezers) where the majority of the remaining water freezes. Rapid initial freezing is essential to set up as many crystal nuclei as possible so that during the maturation or growth stage, their size stays small. In the same context, rapid hardening is also necessary to keep ice crystal sizes small.

Further temperature reduction during hardening accounts for continued growth of the preformed crystals. As water freezes out of solution in a relatively pure form, the formation of ice acts to concentrate the dissolved sugars, lactose, milk proteins, salts, and hydrocolloids in an ever-decreasing amount of water. Water and its dissolved components are referred to as the serum or matrix of the mix. Because the freezing point of the serum is a function of the concentration of dissolved solids, formation of more ice concentrates the serum and results in an ever-decreasing freezing temperature for the remaining serum. Therefore, at temperatures several degrees below the initial freezing temperature, there is always an unfrozen phase present. Ice cream hardness is a function of temperature due to its effect on this conversion from unfrozen water to ice and further concentration of the serum phase surrounding the ice crystals, which helps to give ice cream its ability to be scooped and chewed at freezer temperatures.

Structure Formation During Manufacture

The texture of ice cream is perhaps one of its most important quality attributes. It is the sensory manifestation of structure. Hence, establishment of optimal ice cream structure is critical to maximal textural quality. The structure of ice cream begins with the mix as a simple emulsion, with a discrete phase of partially crystalline fat globules surrounded by an interfacial layer composed of proteins and surfactants. Ice cream is a complex food colloid in that the mix emulsion is subsequently foamed, creating a dispersed phase of air bubbles, and is frozen, forming another dispersed phase of ice crystals. Air bubbles and ice crystals are usually in the range of 20–50 μm. The serum phase is freeze-concentrated. In addition, the partially crystalline fat phase at refrigerated temperatures undergoes partial coalescence during the concomitant whipping and freezing process, resulting in a network of agglomerated fat, which partially surrounds the air bubbles and gives rise to a solid-like structure.

Packaging of Ice Cream

A good package must contain the product, protect it, provide convenience, and provide information on the product to the consumer. Food packages provide protection against physical, chemical, and biological damages. It also provides information useful to the consumer, for example, ingredient label, nutritional label, net contents, serving suggestion, and methods of preparing the product. Besides these attributes, a good food package keeps the food at nearly the same quality as when it was manufactured. During distribution, packages are subjected to physical abuses such as shocks, vibrations, compression, and, in the case of ice cream and frozen desserts, heat shock.

For frozen dessert packaging, three main factors have to be considered. First, the package has to protect the product against temperature fluctuations, photooxidation, dehydration, and odor transmittance. Second, it has to take into consideration distribution-related factors such as package integrity, thermal shock, and cube efficiency. Third, municipal solid waste management factors have also to be considered.

Storage and Distribution

Frozen and hardened product is stored and often distributed prior to the enjoyment by the end consumer. The intermediate steps involved in storage vary depending upon the scale of manufacture, market share, point of sale, and consumer preferences. In the simplest case of a retail ice cream manufacturer, the product is made fresh in the store and sold very soon after manufacture, and this requires relatively few controls. Ice cream is unique in that it is the only product that is consumed in the frozen state. Therefore, once it is manufactured, it has to be stored, transported, distributed, and sold in the frozen state. In the United States, frozen foods are distributed in a separate chain than ice cream is because the cold chain for frozen foods is −18 °C (0 °F) and is inadequate for ice cream. Ice cream cold chain maintains −23 °C (−10 °F). The distribution chain is called the cold chain and varies from manufacturer to manufacturer. Regardless of variations, the cold chain is imperfect. This imperfection affects the quality of the product at the point of purchase and impacts consumer satisfaction. Factors affecting the shelf life of ice cream are manufacturing procedures, warehouse equipment, warehouse handling practices, transportation, storage at retail premises, retail display equipment, and retail handling practices.

Uses of Ice Cream

Ice cream is such a frozen dessert that is liked by all age groups. It is an energy-rich product and may be a part of countless recipes and gastronomic specialties. It can be used as a most promising carrier medium for probiotic organisms.

See also: Ice Cream: Composition and Health Effects.

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Immunoassays: Principles

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Introduction

Immunoassays encompass a wide range of antibody-based testing platforms that have successfully been applied by food manufacturers and regulatory agencies to ensure the safety of the food supply. Because of the diversity in the platforms, suitable antibody detection assays are available for use at any stage during pathogen testing including enrichment screening, presumptive isolate confirmation, and subspecies characterization. Additionally, immunocapture assays may in some cases replace or minimize enrichment times or be used as an additional means to increase the overall sensitivity of a bacterial isolation procedure. Immunoassays exploit the high binding affinity and the stringent binding selectivity of antibodies to identify target foodborne pathogens or other targets of interest (e.g., bacterial toxins, fungal toxins, and allergenic proteins). The popularity of immunoassays for food safety testing is due in large part to the availability of a wide variety of diagnostic platforms that allows them to be applied at any step of the sample analysis, which is both time- and labor-intensive. Their popularity is also due in part to the commercial availability of highly specific antibodies for the majority of the recognized foodborne pathogens and many of their toxic metabolites. There are many benefits to including immunoassay detection in a food testing program. One of the biggest hurdles in foodborne pathogen surveillance programs is the number of samples that can be effectively processed within a given time period. The use of immunoassays decreases the time required for detection and the time needed to confirm the identity of foodborne pathogens. By speeding up analysis time, more samples can then be tested within the same time frame. Immunoassays can also provide subspecies-level information about the target pathogen that cannot be obtained from biochemical testing alone. This is an important initial step taken by regulatory agencies for linking foodborne and clinical strains during a food-associated illness outbreak.

Antibody Modifications

Antibodies

Immunoassays rely on antibodies to capture and/or detect the target of interest. The antibodies used in immunoassays are broadly classified as either polyclonal or monoclonal. Polyclonal antibodies are a population of antibody molecules that target the same antigen (e.g., bacterial cell wall) but at different locations on the antigen (i.e., epitopes). Polyclonal antibodies are purified from the serums of animals that have been inoculated with the target antigen; commercially available antibodies are frequently obtained from goats, rabbits, or mice. In contrast, monoclonal antibodies recognize the same epitope on the target antigen. The production of monoclonal antibodies is more complicated than the production of polyclonal antibodies. The host animal is inoculated with the target antigen, and after an appropriate incubation period, B cells (antibody-producing cells) are isolated from the host's spleen. Individual B cells are fused with myeloma cells (cancerous B cells), forming a hybridoma and allowing the antibody-producing B cell to be propagated *in vitro* without the need for an animal host and, with proper maintenance, allowing for the production of the antibody for an indefinite period of time. There are several excellent references at the end of this article that provide a more in-depth description of antibody production.

Both polyclonal- and monoclonal-based immunoassays are frequently used for food safety applications, and each type has its own benefits. Polyclonal antibodies are usually far less expensive to produce than monoclonal antibodies, and so, immunoassays using the former tend to be more popular and more widely available. Recognizing multiple epitopes can be an added benefit for polyclonal antibody-based assays when the entire genus or species is pathogenic. A good example of this situation is the genus *Salmonella*. There are two recognized species of *Salmonella*, *S. bongori* and *S. enterica*, and both are considered pathogenic. There is considerable variation associated with the cell wall of *S. enterica*. This variation can limit which strains of the organism can be captured and/or detected by the assay depending on the presence or absence of the specific epitope recognized by the antibody. The use of a monoclonal antibody-based assay for the capture and/or detection of *Salmonella* may limit its sensitivity (i.e., inclusivity) by only being able to recognize a fraction of the strains associated with foods. In order to ensure that the assay recognizes as many strains as possible, a polyclonal antibody assay would be more appropriate; sometimes, antibodies are used in combination with other ligands such as recombinant phage proteins to further increase assay sensitivity. For other foodborne pathogens, it is more desirable to use a monoclonal antibody-based immunoassay for capture or detection. A good example is *Escherichia coli*, which has both pathogenic and nonpathogenic strains within the same species and can frequently be found in food environments; the most widely recognized pathogenic subtype is *E. coli* O157:H7, which gets its designation based upon its reaction with specific monoclonal antibodies or antiserums. When only a fraction of the species is pathogenic, it is desirable to use a monoclonal

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antibody-based assay that targets the pathogenic strains with little to no cross-reactivity with the nonpathogenic strains.

Another beneficial utility of monoclonal antibody-based immunoassays is the ability to exploit their narrow epitope recognition sensitivity for classifying bacteria at the subspecies level. Subspecies classification is commonly used to establish relatedness between strains, to distinguish between pathogenic and nonpathogenic strains within the same species, and to provide an initial link between clinical and foodborne isolates during a foodborne outbreak of disease. At the subspecies level, there are numerous subtypes of *S. enterica* based upon monoclonal antibody typing; these are referred to as serotypes or serovars and are based on the original classification by Kauffman and White. Although there are only two species of *Salmonella*, there are over 2400 serotypes of *S. enterica*.

Several advancements have been made that enhance the performance or extend the utility of antibodies in terms of assay design and function. One such advancement is the use of antibody fragments as opposed to the entire antibody. The use of antigen-recognizing fragments of the antibody for either capture-based or detection-based assays can result in increased sensitivity. For capture-based assays, this is due primarily because more antibody fragments can occupy the same surface area compared with intact antibodies. For detection-based assays, steric hindrance will limit the number of intact antibodies that can bind to a target (e.g., bacterial cell). Antibody fragments, because of their smaller size, may be able to occupy more of those available binding sites on the target.

Attachment Chemistries

Although antibodies are the 'backbone' of immunoassays, the ability to modify antibodies in order to maximize their utility is almost as equally important. Modification of antibodies allows them to be tethered to solid supports such as columns, beads, or plates, giving rise to several different types of antigen capture platforms. Immobilized antibodies are useful in recovering low levels of target pathogens directly from foods, aiding in removing potential matrix-specific interferences, which hinder subsequent detection steps, and isolating pathogenic species/subtypes from enrichment broths containing other closely related species/subtypes. Additionally, antibody attachment chemistries allow for the addition of specific signal-generating chemistries such as fluorescent molecules or enzymes that yield colored signals upon the addition of substrate.

Perhaps, the most widely recognized and utilized attachment chemistry for modifying antibodies is based on the avidin–biotin interaction. Avidin is a commercially available biotin-binding protein typically isolated from the egg whites of hens. Biotin is also known as vitamin B_7 and is a water-soluble B vitamin that is an important cofactor for many carboxylase enzymes. The biotin molecule has a reactive carboxylic acid group to which a spacer molecule can be attached. The spacer molecule separates the biotin from the antibody, thus minimizing steric hindrance during avidin binding. Spacer groups are typically simple alkyl groups (6-aminohexanoic acid) or polyethylene glycol groups linked together. At the end of the spacer, there is another reactive group that allows the biotin to be covalently attached to the antibody. A succinimidyl ester is the most commonly used reactive group, and it reacts with primary amine groups (i.e., ε-amino group of lysine) on the surface of the folded protein. In addition to amine-reactive chemistries, there are reactive chemistries that target sulfhydryl groups of proteins (e.g., maleimide and iodoacetyl) and reactive chemistries that target carboxyl groups of proteins (i.e., amine).

Signal/Detection Chemistries

Another important antibody modification that has greatly expanded the number of immunoassay platform types is the ability to label with a reporter group (e.g., fluorescent molecule or enzyme). The antibody recognizing the target antigen (i.e., the primary antibody) can be directly labeled with a reporter group. However, more frequently, a secondary antibody is labeled with the reporter group; a secondary antibody does not directly recognize the target antigen but binds to the primary antibody, which recognizes the target. The purpose of using a labeled secondary antibody is to amplify the signal since the target antigen may be present in low quantities and because multiple-labeled secondary antibodies can bind to a single primary antibody. An added benefit to using labeled secondary antibodies is that they are commercially available with a wide variety of reporter groups. Because secondary antibodies are species-specific in their epitope recognition and are produced from just a few animal species (e.g., anti-goat and anti-rabbit), they can be used in a wide range of applications, making it economical for manufacturers to supply them.

Fluorescein is perhaps the most widely recognized fluorescence-based reporter group used for immunoassay detection. Fluorescein is a synthetic dye that absorbs light maximally at a wavelength of ~494 nm and fluoresces maximally at a wavelength of ~544 nm; the exact wavelengths vary slightly depending on the fluorescein derivative. There are several important fluorescein derivatives useful for antibody labeling. Fluorescein isothiocyanate (frequently abbreviated as FITC) and the succinimidyl esters of fluorescein or carboxyfluorescein are amine-reactive and can be used to label the ε-amino group of lysine on the surface of the antibody or label the antibody's amino-terminus. Similar to succinimidyl esters of biotin, these fluorescein derivatives are available with a variety of spacer atoms between the fluorescein and the succinimidyl ester. Carboxyfluorescein is frequently abbreviated as FAM (fluorescein amidite), which actually refers to the phosphoramidite derivatives used to label nucleic acids. There are many other commercially available fluorescent dyes such as the Molecular Probes' Alexa Fluor dye series (Life Technologies, Inc.) that are useful for antibody labeling. A thorough description of these is outside the scope of this article, and the reader is referred to some of the supplemental readings listed at the end of the article.

Enzymes are another highly versatile type of reporter group that can be used to label antibodies. Enzyme-conjugated antibodies yield a signal upon the addition of the appropriate substrate. Three types of signals can be obtained depending on the choice of substrate and are chromogenic, fluorescent, and chemiluminescent. Two of the more common enzymes used to label antibodies are alkaline phosphatase and horseradish peroxidase. Alkaline phosphatase catalyzes the dephosphorylation of nucleic acids and proteins in cells. Its

ability to recognize synthetic substrates makes it a valuable reporter group. The substrate *p*-nitrophenyl phosphate is hydrolyzed by this enzyme into *p*-nitrophenyl and inorganic phosphate; the former is chromogenic with an absorption maximum at a wavelength of 405 nm. Another synthetic substrate, AMPPD (3-(2′-spiroadamantane)-4-methoxy-4-(3″-phosphoryloxy) phenyl-1,2-dioxetane), gives a chemiluminescent signal, and 4-methylumbelliferyl phosphate (4-MUP) gives a fluorescent signal in the presence of alkaline phosphatase. Upon dephosphorylation of AMPPD, the remaining AMPD is unstable and splits, resulting in the production of a phenolate ion, which is in an excited state resulting in the emittance of light. Dephosphorylation of 4-MUP yields the fluorescent methylumbelliferone molecule. Horseradish peroxidase is a heme-containing enzyme that uses hydrogen peroxide to catalyze oxidation reactions on a variety of substrates in the plant cells. Similar to alkaline phosphatase, this enzyme also recognizes several synthetic substrates that are exploited for use in immunoassays. The three synthetic substrates 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), luminol, and 3-*p*-hydroxyphenylpropionic acid (HPPA) can be used to obtain chromogenic, chemiluminescent, and fluorescent signals, respectively.

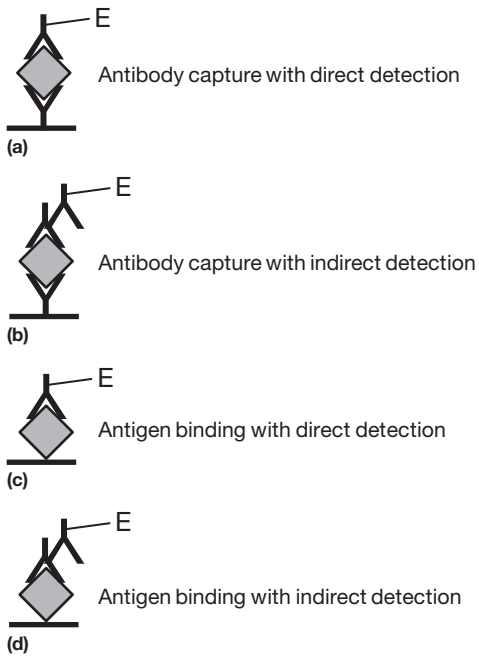

Figure 1 Depiction illustrating various ELISA formats (E, enzyme attached to either primary or secondary detection antibody; diamond represents the target antigen).

Immunoassay Platforms

Enzyme-Linked Immunosorbent Assay

The enzyme-linked immunosorbent assay (ELISA) is a widely used immunoassay platform for the detection of pathogens, their toxic metabolites, or allergens in foods. In an ELISA, signal detection is achieved by enzymatic activity on an appropriate substrate as detailed in section '**Signal/Detection Chemistries**.' The enzyme is covalently attached (i.e., linked) to the antibody. The ELISA is most commonly performed using a 96-well microplate; however, other styles of microplates (e.g., 48-well, 24-well, and 384-well, among others) are also popular. Nonmodified antibodies are added to wells of the microplate where they bind nonspecifically; these are referred to as capture antibodies. Because many antigens can also bind nonspecifically and because the test sample may contain multiple highly similar targets, it is important that any remaining sites in the wells not occupied by antibodies be blocked by the addition of nonantigenic proteins such as bovine serum albumin, which will bind nonspecifically to those sites. Once the wells have been blocked and the excess blocking solution removed, the test sample can be added. If the target antigen is present, it will bind to the capture antibodies. Any nonbound test sample is removed by washing the wells with a high-stringency buffer or mild detergent solution. The presence of the target antigen is revealed by the addition of labeled detection antibodies. This type of ELISA is known as an antibody capture ELISA because the antibody that is bound to the plate captures the target antigen (**Figure 1(a)** and **1(b)**).

If the target antigen is capable of binding to the wells of the microplate, then the ELISA can also be performed by first adding the test sample. The target antigen binds nonspecifically to the wells of the plate. The nonbound test sample is removed by washing, and the wells of the plate are blocked using a blocking solution. The purpose of blocking is to prevent detection antibodies from binding nonspecifically, resulting in false-positive signals. The presence of the target antigen is revealed by the addition of a labeled detection antibody. This type of assay is known as a direct antigen-binding ELISA or sometimes antigen capture ELISA (**Figure 1(c)** and **1(d)**). It should be noted that depending on the composition of the test sample, there may be other biological molecules competing for binding sites within the microplate well. This could lower the levels of antigen binding, resulting in an overall decrease in the sensitivity of the assay.

Once the target antigen has been captured in the well of the microplate, it has to be detected. The detection step typically uses either one or two antibodies; if two antibodies are used, only one is labeled. When a single enzyme-labeled primary antibody is used, the type of detection is referred to as direct detection (**Figure 1(a)** and **1(c)**). When a nonlabeled primary antibody followed by an enzyme-labeled secondary antibody is used, the type of detection is referred to as indirect detection (**Figure 1(b)** and **1(d)**). The benefits of using a labeled secondary antibody are described in section '**Signal/Detection Chemistries**.'

The two different binding arrangements (i.e., antibody capture and direct antigen binding) and the two different detection types (i.e., direct antibody detection and indirect antibody detection) can be used in various combinations, yielding several different ELISA formats. Each format can be used with chromogenic, chemiluminescent, or fluorescent substrates; when fluorescent substrates are used, the assay is often referred to as an enzyme-linked fluorescence assay (ELFA). The ELISA/ELFA format using antibody capture and either direct or indirect detection is commonly referred to as a 'sandwich ELISA' because the target antigen is sandwiched between two antibodies.

Immunofluorescence Detection Assay

The ELISA, which utilizes fluorogenic substrates (i.e., ELFA) described in section 'Enzyme-Linked Immunosorbent Assay,' is also considered an immunofluorescence assay. In addition to this type of assay, others that use antibodies directly labeled with fluorescent reporter molecules and that do not rely on enzyme activity for signal production are also immunofluorescence assays. Simple immunofluorescence detection assays share many similarities with ELISAs/ELFAs. These assays can be used with both antibody capture (Figure 2(a) and 2(b)) and direct antigen-binding arrangements (Figure 2(c) and 2(d)). Also, these assays can be used with either direct antibody detection (Figure 2(a) and 2(c)) or indirect antibody detection (Figure 2(b) and 2(d)).

Immunofluorescence assays are not limited to the microplate format. The use of flow cytometry has recently gained interest, driven by the development of fluorescently colored microspheres that allow for multiplex-type analysis. For multiplex analysis, multiple microspheres, each with a unique fluorescence signature, are individually labeled with antibodies recognizing target antigens. For instance, microspheres of one fluorescence color might be labeled with anti-*Salmonella* antibodies, microspheres of a second fluorescence color might be labeled with anti-*Listeria monocytogenes* antibodies, and microspheres of a third fluorescence color might be labeled with anti-*E. coli* O157:H7 antibodies. The labeled microspheres are pooled and then used for applications such as screening enrichments for multiple pathogens or identifying unknown purified colonies. Antibody-labeled microspheres can also be used for identifying pathogenic variants within the same species of bacteria. For example, various fluorescently colored microspheres can be labeled with specific antibodies for identifying various enterohemorrhagic *E. coli* serotypes or *S. enterica* serotypes. The fluorescence signature of the microsphere identifies the target antigen (e.g., bacterial cell or microbial toxin), and the attached antibody serves as a capture antibody similar to an ELISA. The formation of a signal is needed for the analyst to determine if the target antigen is present. For signal detection, antigen-specific detection antibodies directly labeled with a fluorescent reporter are used. Because it is the fluorescence signature of the microsphere that identifies the target, the same fluorescent reporter can be used to label all of the detection antibodies in the multiplex analysis. During the analysis, fluorescence signals resulting from the microsphere and the detection antibody indicate the presence of the target.

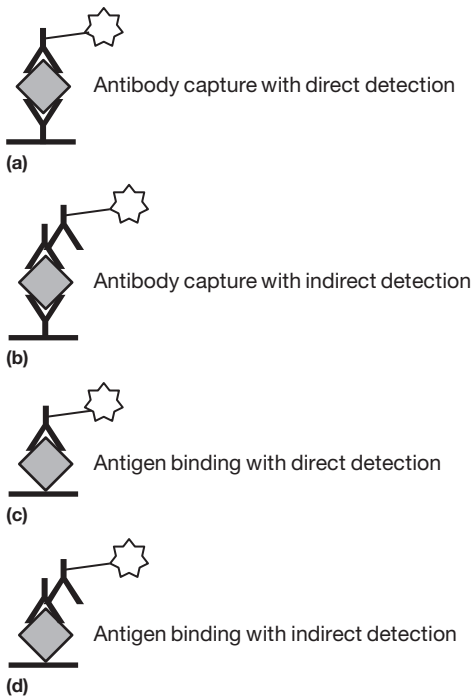

Figure 2 Depiction illustrating various simple immunofluorescence detection formats (star, fluorescence reporter attached to either primary or secondary detection antibody; diamond represents the target antigen).

Immunochromatographic Assay

Immunochromatographic assays are another type of immunoassay that has become extremely popular for food safety testing because of the relative ease of use and ease of interpretation. These assays use a solid support strip (e.g., nitrocellulose) capable of capillary action that has three distinct functional zones. At one end of the support strip is an application site where the prepared sample is added or the support strip is immersed in the sample (for this reason, these assays are sometimes referred to as 'dipsticks'). Commonly used immunochromatographic assays for food safety applications typically only require minimum sample preparation such as boiling of a small aliquot from an overnight enrichment. Once the application site is saturated, the sample flows through capillary action into the first zone where target-specific antibodies conjugated to colloidal gold or colored latex particles are present. If the target antigen is present in the sample, it will be labeled, forming an antibody–antigen complex, as it passes through the first zone. As the sample continues to flow through the test strip, it enters the second zone and encounters nonlabeled target-specific antibodies that are immobilized to the solid support of the test strip. The target antigen, labeled in zone 1, is held in place by the immobilized antibodies in zone 2, resulting in their increased localized concentration and forming a visible line. Because of their mode of action, these assays are sometimes referred to as immunoprecipitation assays. As the sample continues to flow through the chromatographic matrix, it enters the third zone where it encounters immobilized particle-specific antibodies that bind to the remaining colloidal gold or latex particles, resulting in their increased localized concentration and forming a visible control line that indicates that the assay performed properly. Because these assays rely heavily on the flow of the sample for functionality, they are often referred to as lateral flow devices. If the target antigen is present in the test sample, then two lines are observed, one indicating a positive reaction and the other, the control line (Figure 3(a)). If the target antigen is not present, then only the control line is observed (Figure 3(b)).

Antibody Magnetic Capture Assay

In some of the immunoassays previously mentioned, one of the functions of the antibodies was to capture the target antigen (e.g., bacterial cell or bacterial toxin) so that it could be more readily detected. This capturing ability has been further

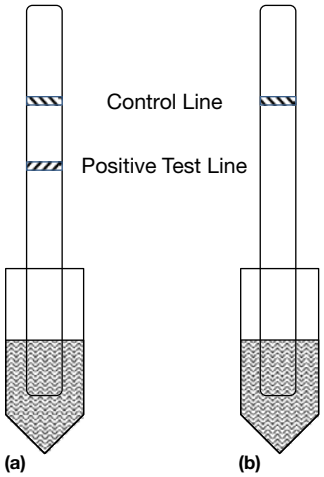

Figure 3 Immunochromatographic assay depicting a positive (a) and negative (b) test result. In both instances, the presence of the control line indicates successful assay functionality.

exploited by tethering target-specific antibodies to paramagnetic beads (microspheres). Paramagnetic beads become magnetized in the presence of magnetic field but lose their magnetic property when the external magnetic field is removed. This is an important property because if the beads maintained their magnetic properties, they would be attracted to each other (i.e., clump) and could not be easily dispersed, making them difficult to work with and lowering their capturing efficiency.

The antibody-labeled paramagnetic beads are simply combined with the sample, or sample blended in an appropriate buffered solution, which is then thoroughly mixed. This can be done in batch using a beaker or a disposable 50 ml conical tube (Figure 4(a)). After mixing, an external magnetic field is applied to one side of the container using a simple magnet. The target–antibody–paramagnetic bead complex is attracted to the magnet, holding it in place and allowing the remaining sample to be removed (Figure 4(b)). While the magnetic field is still applied, the captured cells can be washed to remove any potential matrix interferences that might hinder subsequent detection. There are commercially available magnetic stands designed specifically to use with 15 and 50 ml conical tubes. Additionally, there are commercially available sample recirculation systems that continually circulate the blended sample over antibody-labeled paramagnetic beads, which are held in place by a magnetic field. After a period of sample recirculation, the sample can be replaced with a buffer solution for washing the captured cells, or the cell–antibody–bead complex can be recovered and washed in batch using a conical tube. Immunomagnetic cell capture can be used to prepare samples for any number of detection technologies including polymerase chain reaction (PCR), immunoassays, and even plating onto microbiological recovery media.

Latex Agglutination Assay

Latex agglutination (LA) assays use antibody-labeled latex beads to capture target bacterial cells and rely on multiple binding interactions for cell–antibody–bead complex formation, resulting in clumping, which is evident by visible

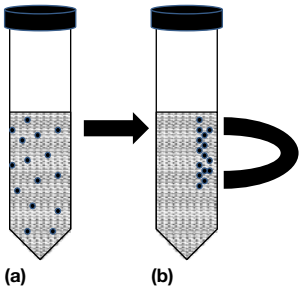

Figure 4 Illustration of an immunomagnetic capture assay. After thorough mixing, the antibody-labeled paramagnetic beads are well dispersed in the test sample (a). When exposed to a magnetic force, the beads become magnetized and are attracted to the magnet where they are fixed in place (b).

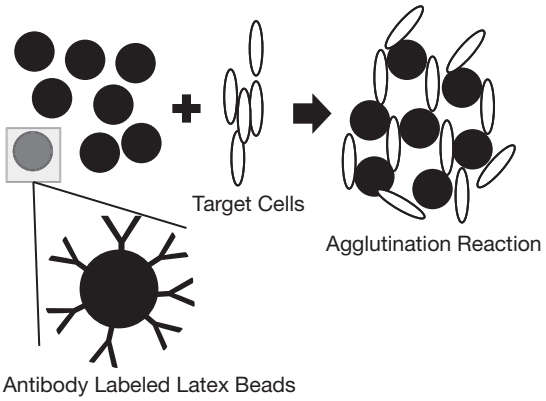

Figure 5 Latex agglutination assay. Target-specific antibody-labeled latex beads are mixed with the cells. Multiple interactions between the cells and beads result in the formation of a visible precipitate.

turbidity (Figure 5). LA assays are quick and simple to perform and do not require specialized instrumentation for signal measurements. Because LA assays rely on visible turbidity for determining positive results, these assays are not well suited for sample screening as food matrix material will regularly interfere with the determination of the results. However, these assays are frequently used, particularly by regulatory agencies, for serotype determination of foodborne pathogens and as way of determining pathogenic from nonpathogenic subtypes as in the case of *E. coli* O157:H7. For these uses, a small drop of the antibody-labeled latex beads in solution is placed on the surface of a glass microscope slide or petri plate lid. A single pure colony is then thoroughly mixed with the beads, and the slide is rocked back and forth and observed for granular clumping. The surface of the slide or lid should be well cleaned so that dust particles are not mistaken for clumping, a sign of agglutination. Additionally, the cells must initially be thoroughly mixed with the antibody–bead solution or clumps of the original colony can be mistaken for agglutination.

Applications of Immunoassays in Food Safety Testing

The isolation and identification of foodborne pathogens typically follow a sequential procedure. Because pathogen contamination is typically low and frequently < 1 cell per gram, the

test sample is manipulated in a manner that allows for the growth of the target pathogen so that it can reach detectable levels. During this pathogen enrichment period, selective agents (e.g., antibiotics, dyes, and salts) are typically added that allow the target organism to grow with little to no hindrance and that also minimize the growth of other microorganisms that are present in the test sample. Although not applicable to every type of food matrix, antibody capture methods have been successfully used to effectively concentrate pathogen levels from foods, thus eliminating or greatly reducing the time required for selective enrichment. Following enrichment, the target pathogen must be isolated; this is typically accomplished by 'steak plating' an aliquot of the enrichment onto a selective/differential agar plate. This is another step where antibody-based detection assays have become extremely popular particularly with analytic microbiology laboratories that frequently process many samples. Immunoassays such as lateral flow devices, ELISAs, and ELFAs have become increasingly relied upon for screening selective enrichments. Enrichments that test negative during the immunoassay screen are culled, and testing efforts are focused on the remaining enrichments that yielded positive screening results. Presumptive colonies (colonies that appear typical of the target pathogen for a particular medium) that form on the selective/differential medium must still be confirmed. This task has historically been done using the results of series of biochemical reactions. Colony confirmation is another area in which antibody-based assays (e.g., LA, ELISAs, and ELFAs) are becoming more popular. This is especially true when antibody reaction-based nomenclature is inherently tied to the organism's identification such as the serological classification of *S. enterica* or is used to identify nonpathogens from pathogens such as *E. coli* and *E. coli* O157:H7. Although other types of technologies have been and are being applied and developed (e.g., PCR and mass spectrometry) for pathogen detection, the reliability of immunoassays ensures their continued place in food safety testing programs.

See also: Foodborne Pathogens; *Listeria*: Detection; *Salmonella*: Detection.

Further Reading

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Infants: Feeding Problems

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Introduction

Nutrition is of great importance in infancy. Infants are entirely dependent on others for their nutrition and carers may have difficulty recognizing their needs. This dependence and infants' high nutritional requirements for growth and development make malnutrition a relatively common problem. Prolonged malnutrition can impair both somatic growth and brain development.

Failure to Thrive

Failure to thrive (Figure 1) is failure to gain in height and weight at the expected rates (determined from recognized growth data). The causes are numerous (Table 1), but if the precipitating causes can be successfully treated, failure to thrive is followed by accelerated or 'catch-up' growth (Figure 1(a)), provided extra food meets the nutritional needs of accelerated growth. Without extra nutrients, catch-up growth is likely to be incomplete. Affected infants may then resume normal growth rates, but along lower growth centiles than previously (Figure 1(b)).

Some children with growth below the third centile or ≈−2sd weight-for-age or below (outside the recognized 'normal' range) may be labeled as failure to thrive although growing at more or less normal rates for age. They may have been low weight for gestational age at birth and always small (Figure 1(c)). These constitutionally small children may show delayed bone age, delayed adolescence, and sometimes late catch-up to more normal adult size.

Careful dietary histories should indicate which infants are failing to thrive because of inadequate intakes, inappropriately constituted feeds, or inappropriate weaning practices. Average milk or formula intakes are 150 ml kg^{-1} day^{-1} (441 kJ kg^{-1} day^{-1}) for full-term normal infants. Around 6–8 weeks of age, intakes often stabilize despite continuing increases in weight and intakes per kilogram per day thus decrease.

It may be difficult to assess the adequacy of milk intake in breast-fed infants. Normal growth velocities and general contentment after feeding suggest intakes are adequate. If there is concern, weighing infants in clean diapers prior to breast-feeding and then weighing them again after feeding without diaper change provides some indication of the weight, and thus the volume, of milk consumed. This test weighing must be for all feeds over 24 h since infants may consume large feeds early in the morning and in the evening, but relatively small amounts at other times of the day.

When breast milk output seems insufficient for an infant's needs, improving maternal nutrition and increasing the frequency of suckling may improve milk secretion. Current recommendations are that infants should be exclusively breast-fed until at least 4 months of age and preferably until 6 months of age. Usually, breast-feeding failure arises because of inappropriate feeding practices (e.g., infrequent suckling, especially in early lactation, or incorrect positioning and failure by the infant to latch on). Advice on feeding technique may resolve the problems. However, mothers who are sick or very malnourished may sometimes fail to produce sufficient milk to support normal infant growth. The decision whether to continue struggling to increase breast milk production, to introduce infant formula feeds, or to introduce weaning foods early is determined by individual circumstances such as the age of the child and cause of breast-feeding failure. Failure to thrive without evidence of nutritional inadequacy, or secondary to persistent anorexia and vomiting, requires urgent medical investigation.

Occasionally, failure to thrive occurs without evidence of clinical disease or nutritional inadequacy. Children with such nonorganic failure to thrive may eat well, sometimes excessively, but grow poorly. Typically, they are apathetic, showing little social interaction, although some are restless and inattentive. Developmental delay, particularly in social and cognitive skills, is common. These children come from home environments of social and/or emotional deprivation. Environmental stress and lack of love inhibit normal growth. Changing the home environment without necessarily changing the diet can induce accelerated growth giving a dramatically improved effect – confirming the diagnosis.

Vomiting

Most normal infants vomit occasionally and some vomit frequently. Probably the most common cause of persistent vomiting in infancy is laxity of the gastroesophageal sphincter. The sphincter is the spiral muscular area, 2–3 cm in length, at the lower end of the esophagus. Sphincter competence is related to the length of intra-abdominal sphincter (which increases with age) and to intragastric pressure. Competence tends to be poor at birth but improves after the first 2 weeks of life, regardless of gestational age. The fluid diets of young infants and the high volumes of liquid needed to meet nutritional requirements produce gastric distension, high intra-abdominal tension, and easy regurgitation. Thickening feeds to increase resistance to regurgitation, feeding smaller feeds more frequently, and raising the head end of the cot help reduce gastric distension and regurgitation. As infants grow, consume more solid food, and adopt an upright posture, reflux ceases to be a problem.

Feeding problems, including gastroesophageal reflux, are common in children with neurological handicap. Failure to

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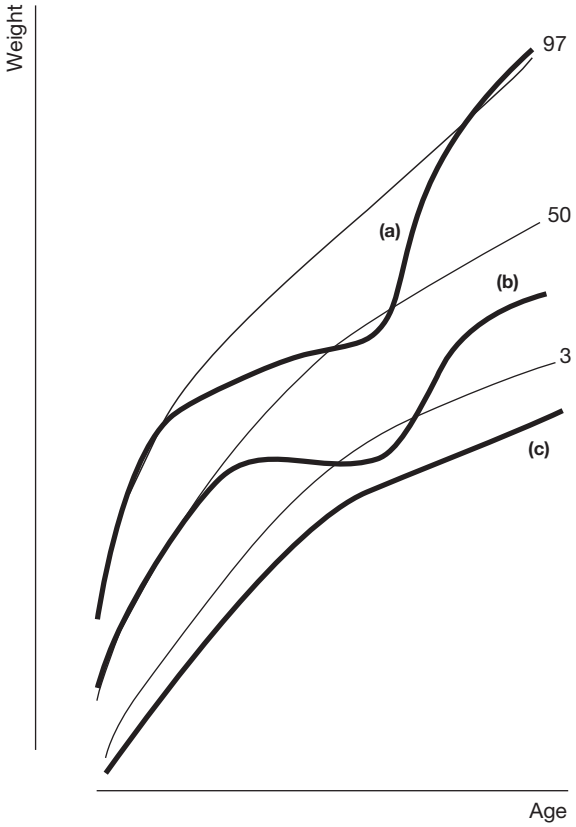

Figure 1 Weight of children with (a) failure to thrive and complete catch-up growth; (b) failure to thrive and failure of complete catch-up growth; and (c) constitutional small size plotted against the 3rd, 50th, and 97th weight-for-age centiles.

Table 1 Classification of nutritional problems associated with failure to thrive

Basic problem	*Examples*
Inadequate intake	Poverty; famine; ignorance of nutrient needs
	Vomiting: all causes
	Feeding problems, including neurological damage
	Anorexia
Increased losses	Protein-losing enteropathy
	Glycosuria; proteinuria
	Exfoliative skin conditions
	Suppuration
Failure to absorb food	Cystic fibrosis
	Gluten-sensitive enteropathy
	Giardiasis
	Lactose intolerance
Failure to utilize food	Inborn errors of metabolism
	Endocrine problems
	Micronutrient deficiencies, for example, zinc
	Chronic renal failure
	Emotional deprivation
Increased requirements	Small-for-dates and premature infants
	Catch-up growth
	Congenital heart disease: left-to-right shunt

thrive can be severe and may improve with intragastric feeding through gastrostomy, but this exacerbates gastroesophageal reflux. Surgical fundoplication is usually performed, prior to gastrostomy, to control reflux.

Vomiting can be a symptom of serious gastrointestinal or parenteral disorder. When infants vomit and fail to thrive, vomit bile or blood, or vomit to the extent of causing dehydration, medical investigation is required urgently.

Diarrhea

Diarrhea is an increase in total stool output, usually from increase in water content, volume, and frequency of stools. The color, consistency, and frequency of stools vary widely both between different infants and in the same infant on different occasions. Exclusively breast-fed infants tend to have frequent watery yellow stools. Formula-fed infants have less frequent, greenish, drier-looking stools.

In infancy, diarrhea is commonly part of either a specific gastrointestinal or a generalized (usually viral) infection. Infectious diarrhea has an acute onset but is usually short-lived and associated with other signs of infection: pyrexia, vomiting, anorexia, and general malaise. Weight loss from anorexia and dehydration is common. Weight recovery is generally rapid and complete. Acute diarrhea has little effect on long-term weight for age and even less impact on long-term height for age. However, chronic diarrhea and significant growth faltering are common with some gastrointestinal infections (especially rotavirus, *Giardia lamblia*, and *Entamoeba histolytica*).

Malnutrition causes profound changes to gastrointestinal mucosal structure and function. This predisposes malnourished children to severe diarrheal infection and high mortality from diarrheal illness.

All diarrhea should be managed with extra fluids, usually in the form of oral rehydration solution (ORS). ORS suitable for most infant diarrhea has lower sodium and chloride content than that intended for rehydration in adults.

Diarrhea, which is persistent and associated with failure to thrive, dehydration, bleeding, or other obvious illnesses, requires medical investigation.

Toddler Diarrhea

Some young children persistently pass watery stools or have bouts of loose watery stools alternating with episodes of constipation without evidence of infection, malnutrition, or other ill health. The diarrhea stools are typically voluminous and watery with bits of undigested food in them, indicating the visible bean husks, tomato skins, and other vegetable matter present, although less obviously so, in normally formed stools. Children with toddler diarrhea are active and thriving but their parents are usually very concerned.

Toddler diarrhea has no recognized specific pathology and probably has many causes. Whole-gut transit time is reduced but there is no evidence of abnormal duodenal or jejunal secretion, nor of malabsorption. Minor infection may set off bouts of diarrhea, and in some children, there is a family history of response to stress with diarrhea. Poor hygiene and low-grade infection associated with constant pacifier (dummy) sucking and prolonged bottle-feeding may contribute to the problem.

Many affected children have high intakes of sweetened fruit juices and/or low intakes of dietary fat. High fluid intakes with high simple carbohydrate content encourage rapid intestinal transit, which overwhelms small intestinal function, so sugars reach the large intestine unabsorbed. These and their fermentation products in the colon stimulate osmotic diarrhea. Reducing fluid intakes, particularly of sweetened juices and carbonated drinks, reduces the symptoms in some toddlers. Changing to more adult diets with higher concentrations of complex carbohydrates and fats can also reduce diarrhea. Lipids in the ileum slow duodenal and jejunal transit time (the ileal brake) and may modulate mucosal contact time and the absorption of intestinal luminal contents. Graduating to cup-feeding usually results in less excessive fluid intakes and, particularly if pacifiers are avoided, encourages more hygienic feeding practices.

Toddler diarrhea usually resolves as children move on to more mature diets and develop continence. It is unusual after the age of 6 years.

Micronutrient Deficiencies

Significant micronutrient deficiencies, other than for iron, are rare in normal-weight, full-term healthy infants in developed countries. Vitamin D deficiency rickets can be a problem in low-birth-weight (LBW) infants and non-Caucasian infants. In developing countries, low levels of vitamin A are associated with high morbidity and mortality from diarrheal diseases, respiratory infections, and measles. Iodine deficiency is also common. Many countries now have supplementation programs to prevent vitamin A and iodine deficiency.

Iron Deficiency

The human fetus has high hemoglobin levels to accommodate low oxygen tension *in utero*. After birth, in response to the high oxygen tension in the extrauterine environment, the bone marrow becomes relatively quiescent and hemoglobin levels decline. Eventually (usually at 4–6 weeks of age), the bone marrow becomes more active again, but by then, infants have grown and their blood volumes have expanded. Hemoglobin levels remain below adult levels, at 11–12 g dl^{-1}, until late childhood.

Where infants are small and growing very rapidly (e.g., premature and small-for-dates (SFD) infants), with initially low total blood volume and low total body iron, the need for iron for new blood formation may soon exceed the iron stored, so anemia results. Once body weight has doubled, all infants are at risk of iron deficiency since, without good external sources of iron, iron stores of the body will be distributed over twice the blood volume and hemoglobin levels will have halved. However, iron supplementation should probably be avoided in exclusively breast-fed infants unless they show clinical evidence of deficiency, since lactoferrin is a major immunologic component of breast milk. It binds iron in the small intestine, speeding absorption and inhibiting multiplication of iron-dependent bacterial pathogens. Iron fortification of infant formulas reduces the prevalence of severe iron deficiency in non-breast-fed infants.

Table 2 Classification of problems predisposing to iron deficiency in childhood

Basic nutritional problem	*Example*
Inadequate intake	Early introduction to cows' milk
	Prolonged exclusive breast-feeding
	Poor quality of weaning diet
	Strict vegetarian diet
Increased losses	Bleeding disorders
	Reflux esophagitis
Failure to absorb	Celiac disease
	Pyrexia
	Iron in ferric form
	High-phytate diet
Failure to utilize	Chronic infection
	Hypothyroidism
	Riboflavin deficiency
Increased requirements	Rapid growth: low-birth-weight infants
	Catch-up growth

Iron sufficiency is important. Table 2 lists some factors predisposing to iron-deficiency anemia. The frequency of iron-deficiency problems is an indication of the small margin of safety between requirements for iron and the quantity of iron young children are likely to ingest and absorb from their diets. Low-grade iron deficiency can impair growth rates, intellectual function, and the immunologic response to infection. In countries where malaria is common, anemia resulting from iron deficiency puts infants at great risk of life-threatening severe malarial anemia.

Vitamin D Deficiency: Rickets

Rickets used to be common in weanlings. The Clean Air Act (1956) probably did as much to reduce rickets in the United Kingdom as any medical intervention, since vitamin D metabolites are predominantly derived from irradiation of 7-dehydrocholesterol in the skin by sunlight of wavelength around 300 nm.

Rickets is still seen in the United Kingdom in newborn Asian infants and mothers. The mothers have both inadequate absorption of dietary vitamin D and inadequate skin synthesis of endogenous vitamin D because of indoor habits and clothing cover when out of doors. Low calcium absorption due to low dietary intake and the high phytate content of traditional flours exacerbates the need for vitamin D to optimize calcium absorption from the small intestine. Skin pigmentation increases the need for sunlight exposure when the levels of ultraviolet light of appropriate wavelength are low (e.g., winter in northern latitudes).

Infants born to vitamin D-deficient mothers may present with hypocalcemic tetany and convulsions soon after birth or with rickets in the weaning period. The incidence of rickets in Asian children is diminishing in the United Kingdom as Asian women in this country adopt lifestyles more suited to northern latitudes and their need for vitamin D supplementation is better recognized. However, signs of vitamin D deficiency in mothers and children are widely reported from the Middle East where there is plenty of sunshine of vitamin D

synthesizing wavelength, but little social opportunity for women and children to expose their bodies to the sunlight.

Vegetarian Diets in Infancy

Infants weaned on to vegetarian diets may become deficient in a variety of nutrients. If diets include eggs and milk, it is not difficult to achieve dietary requirements, particularly if breast milk or infant formula is continued as a milk source beyond the first year of life. Milk usually provides a substantial proportion of riboflavin, calcium, and total energy intake in weanling children. Infant formula can provide a fairly absorbable dietary source of iron, which may otherwise be deficient.

Vegetarian diets tend to be of low energy density because of high-fiber and low-fat content. Children should be fed frequently (at least four times a day) to enable them to consume the volumes of food necessary to meet nutrient needs. No vegetable protein contains the full range of essential amino acids so diets should include a variety of protein sources (e.g., beans, lentils, cereals, dark green leaves, soya, and homogenized nut products).

It is difficult to achieve adequate nutrition in very young children with vegan, macrobiotic, and other strict vegetarian diets. These diets are not recommended for infants unless there is close pediatric dietetic supervision.

LBW Infants

The problems of both premature and LBW for gestational age (SFD) infants are similar, although the greater immaturity of the premature infants' gastrointestinal tracts and of other organs makes the nutrition of the former more difficult than that of the latter. In both groups, there is a need for more nutrients per unit of body weight to cope with the rapid growth to parallel intrauterine growth in premature infants and catch-up growth in SFD infants. The small stomach volumes of LBW infants restrict the volumes that can be fed at any one time without causing apnea, vomiting, or necrotizing enterocolitis. Respiratory problems in premature infants may also restrict the amount of enteral food tolerated. Thus, there is a conflict between increased (proportionate to body weight) nutrient needs, especially for protein, calcium, phosphate, fluid, and energy, and the amounts of food that can be administered safely. Even when LBW infants are fed parenterally, there are restrictions on the fluid volumes, and thus the quantity of nutrients, that can be given safely. The high demands for growth and development, particularly for the brain, lead to deficiencies of some nutrients that are usually synthesized by the body. This critical nutrient status applies to the long-chain polyunsaturated fatty acids, docosahexaenoic and arachidonic acids, and possibly also carnitine, nucleotides, and other nutrients. For this reason, breast milk, which has high levels of these nutrients, may have advantages over infant formulas for the nutrition of LBW infants, if supplemented with energy to meet the high requirements of these infants. However, manufacturers, aware of the physiological needs of LBW infants, are gradually developing formulas that aim to meet these special needs.

Table 3 Nutritional problems of low-birth-weight infants

Problem	*Precipitating factors*
Immediate hypoglycemia	Limited liver glycogen Delay in mobilizing fat stores Problems with supplying nutrition
Immediate hypocalcemia	Loss of high calcium flux across the placenta Immaturity of calcium homeostasis Occasionally maternal vitamin D deficiency
Later poor growth	Low energy density of breast milk Poor tolerance of other energy/nutrient-dense feeds Poor fat absorption by immature infants Increased needs due to bronchopulmonary dysplasia
Bone disease of prematurity	Substrate deficiency, especially phosphate Renal calcium loss from acidosis and/or diuretics Vitamin D, protein, or copper deficiency
Anemia	Vitamin E deficiency increasing early hemolysis Folate deficiency Iron deficiency

Table 3 lists the problems of LBW infants, which are discussed elsewhere. LBW infants should be supplemented at least with vitamins A, C, and D and folate over the first year of life. Iron supplementation in the neonatal period risks encouraging bacterial infection and damage by free iron radicals, since iron stores are replete following temporary bone marrow inactivity associated with high hemoglobin levels at birth. However, iron supplementation is advisable in formula-fed infants after the first 4 weeks. Iron supplements should probably not be given at all to wholly breast-fed infants unless they show hematologic evidence of iron deficiency, since free iron in the gastrointestinal tract can counteract the protective effects of lactoferrin.

Allergy

Cows' Milk Protein Intolerance

Food allergies, particularly cows' milk protein intolerance (CMPI), are frequently diagnosed in infants. True CMPI affects between 1.5% and 5% of the population of western Europe. In families with a strong atopic history, the incidence may be much higher. CMPI most commonly develops in children under 2 months of age, although it may develop at a later age after gastrointestinal surgery, malnutrition, or gastrointestinal infection.

Children with diarrhea and/or blood loss in association with CMPI may show villous atrophy and increased inflammatory cell infiltrations on jejunal biopsy or acute colitis on sigmoidoscopy. Those with respiratory symptoms may present with pulmonary hemosiderosis, which resolves on removal of all cows' milk-containing products from the diet. However, for most children, manifestations of CMPI are varied and nonspecific.

Infants, who vomit, have diarrhea, have a rash, or cry a lot, are liable to be diagnosed as CMPI. They are put on to commercial

cows' milk-free formulas and may seem to improve. Improvements may have been coincidental or as a result of mothers feeling reassured that 'something was being done.'

Commercial cows' milk protein-free formulas are also lactose-free. Excluding formula and cows' milk products from the diet does not distinguish between CMPI and lactose intolerance. Children with lactose intolerance commonly have a preceding history of gastrointestinal infection, surgery, or malnutrition. They have loose stools, weight loss, and abdominal distension. Stools are acid and contain reducing substances. Thus, if infants' stools show pH < 5.1 and have no reducing substances in them and symptoms resolve on cows' milk protein-free diet but recur when milk protein is reintroduced, CMPI is likely.

Where the child's symptoms have been dramatic (e.g., angioneurotic edema and profuse life-threatening diarrhea), challenging the diagnosis of CMPI by reintroduction of milk and milk products can be dangerous. Children should only be challenged with milk under hospital supervision and even then with great care.

The particular elements of the cows' milk protein causing allergenic responses vary. At least 18 components that can induce antibody formation have been identified. When purified protein fractions are used as challenges, β-lactoglobulin levels and casein produce symptoms in over 60% of affected individuals. Antibodies to cows' milk protein are widespread and neither their presence nor their levels in the blood correlate well with reactions to cows' milk.

Breast-fed infants often show circulating cows' milk protein antibodies. Changing from breast-feeding to cows' milk formula feeding can, very rarely, precipitate dramatic anaphylactic symptoms of angioneurotic edema with acute and very dangerous swelling of tissues around the neck and airway. Relevant proteins from the cows' milk consumed by the mother are thought to be transferred to affected infants either across the placenta or in breast milk, causing sensitization. The infants are then desensitized by the small amounts of antigen in maternal milk but are overwhelmed when confronted with a large load of antigen from formula feeding.

The management of CMPI involves the removal of all cows' milk protein-containing products from the diet. Pediatric dietetic advice is advisable since milk and milk products continue to contribute significant amounts of energy and other nutrients to normal children's diets, even after the start of mixed feeding. Difficulties in meeting nutritional needs on milk-free weaning diets can lead to undernutrition. Soya-based infant formulas can be helpful but infants with CMPI may also develop soya protein intolerance. Hydrolyzed cows' milk protein formulas are more suitable for the treatment of CMPI but are rather unpalatable (to mothers anyway), not available in supermarkets in the United Kingdom, and costly.

Tolerance to cows' milk protein usually develops as children move into the second or third year of life.

Eczema

The extent to which food intolerance is relevant to the development of infantile eczema remains controversial. Some infants with atopic eczema show significant improvement in their skin problem when all milk products are withdrawn from the diet. Others only improve when put on very restricted lists of foods. Studies of infants with familial high risk of atopic eczema suggest that eczema may be prevented in some infants by exclusive breast-feeding for the first 6 months of life. Moreover, exclusively breast-fed infants who do develop eczema may have become sensitized to foreign proteins transferred through the breast milk.

Other Food Allergens

Other food allergies are quite common in infancy, especially in those with an atopic family history. Common precipitants are egg protein, citrus fruits, nuts (not advised for young children anyway because of the risks of inhalation), and the food colorants tartrazine and quinoline yellow. Diarrhea, vomiting, rashes, wheezing, and – rarely – behavioral disturbances may occur. (Most behavioral problems are disciplinary rather than allergic.) The pattern of response in each child is usually repeated on subsequent contact with the allergen but there is great variation in the pattern of individual responses.

Colic

Around 3 months of age, healthy infants often develop bouts of fractiousness and inconsolable crying, usually in the early evening. Their abdomens become tight and distended, probably because of the air swallowed while crying. Infants tend to flex their legs when crying and this may be interpreted as abdominal pain. 'Three-month colic' is an ill-defined problem with a wide range of symptomatology. In the long term, colic resolves without specific treatment. It may be a behavioral rather than a medical problem. Nevertheless, it causes great distress to the child and parents. Removal of cows' milk formula and substitution with nonallergenic formula or soya protein formula have sometimes been associated with improvement. However, it is difficult to know if this has anything to do with resolution of symptoms in a benign self-limiting condition.

Cot Death: Sudden Unexpected Infant Death Syndrome

Sudden unexpected infant death (SUID) affects about 1 in 500 infants in the United Kingdom. The condition is of mixed etiology, since detailed history and autopsy reveal the cause of death or evidence of illness in many of the infants. There remains a group of SUID infants for whom detailed investigation shows no adequate explanation for death. These are the cases of true 'sudden infant death syndrome' (SIDS). In the past, anaphylactic shock from acute CMPI was sometimes cited as possible etiology for SIDS since SUID is more common in formula-fed than in breast-fed infants. Some infants have postmortem evidence of milk/formula in the respiratory tract, perhaps as a terminal event. However, true SIDS is probably not more common in formula-fed than in breast-fed infants. It now seems likely that a major component of death in SIDS relates to impaired cardiorespiratory arousal. Putting infants to sleep supine, rather than prone or on their sides, has reduced SIDS by about 35% in many countries.

Another nutritional explanation proffered for some forms of SUID is undiagnosed inborn errors of metabolism involving inhibition of breakdown of long-chain fatty acids. Children with inborn errors of this kind are unable to metabolize fats to cope with the stress of fasting and die of hypoglycemia. Postmortem examination reveals acute fatty degeneration of the liver. In such very rare cases, SUID may be familial since inborn errors of metabolism involving fatty acid breakdown are inherited, usually by autosomal-recessive genes.

Teething Problems

Teeth are erupting more or less continuously between 6 and 30 months. Teething children may show excessive salivation, gingival swelling, and general irritability. These symptoms may exacerbate existing illness but there is no evidence that teething causes fever or diarrhea. Rubbing the gums with cool cloths or giving infants firm objects to bite may reduce teething discomfort.

Most children develop their first teeth around 6 months of age but some do not develop teeth until around 2 years. Late tooth erupters usually cope well with chewing and should be encouraged to progress in the weaning process in the normal way.

Nursing Bottle Syndrome: Rampant Caries in Infancy

Nursing bottle syndrome is the rampant carious destruction of the upper incisors in young children. Although associated with offering bottles of juice/milk or pacifiers sweetened with sugary solutions to infants when they are ready to sleep, rampant caries in this age group probably also reflect underlying enamel hypoplasia in the primary teeth, which is secondary to prematurity or to maternal nutritional deficiencies in pregnancy.

Constant bathing of the upper incisors with sugary fluids, together with reduced salivary secretion and reduced mouth movements at night, encourages the development of plaque. *Streptococcus mutans*, which survives well at low pH, collects under plaque and adheres to tooth enamel. Enamel is eroded, allowing the opportunity for further tooth infection by other bacteria. Excessive vomiting, by creating an acid environment in the mouth, may contribute to the risk. Teeth in the front of the upper jaw are reduced to carious discolored stumps. Children may become faddy eaters due to the discomfort of chewing meat and biting vegetables and fruit.

Fluoridation of water supplies with one part per million of fluoride protects against dental caries. If water is not fluoridated, children can be supplemented with fluoride 1.25 mg day^{-1} in infancy and 1.5 mg day^{-1} between 1 and 3 years. Regular, at least daily, tooth brushing should be taught from the first appearance of teeth. Adults should supervise loading children's toothbrushes with small pea-sized lumps of toothpaste since small children may eat toothpaste rather than spit it out after cleaning their teeth. As much as 1.66 mg of fluoride could be consumed by small children brushing their teeth inexpertly twice a day with fluoridated toothpaste.

All food, and particularly sweetened drinks, should be discouraged after tooth brushing at night.

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Cholecalciferol: Properties and Determination; Malnutrition: Concept, Classification and Magnitude; Malnutrition: Prevention and Management; Tocopherols: Properties and Determination; Vegetarian Diets.

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Infants: Nutritional Requirements

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Introduction

The beginning of the twentieth century was characterized by periods of low access to food, thus restraining optimum growth and leading to nutrition-related diseases such as pellagra, rickets, and goiter. In the last decades, however, the scene has changed dramatically in the developed world. Economic growth and high access to food in developed countries have led to food overconsumption, which in turn resulted in increasing prevalence of obesity, high blood pressure, and metabolic syndrome in all layers of the population, including children. This shift in observed health risks has led the scientific community to develop new recommendations for macro- and micronutrients, which are both adequate and balanced to help individuals optimize their health, preventing diseases and avoiding excessive consumption of a specific nutrient.

Infancy and childhood are both critical periods of rapid physical growth and cognitive and emotional development. It is also well recognized that nutrition in these age groups is not only an important factor for the normal development but also fundamental for future health status. It has been shown that the right nutrition in the first 1000 days (i.e., *in utero* and during the first 2 years of life) builds the foundation for a child's ability to grow, learn, and thrive. Nutrient supply, in terms of both quantity and quality, during these early days is instrumental in differentiation of tissues and organs and has both short- and long-term health effects. Breastfeeding is the preferred and recommended form of nutrition for healthy infants for the first 6 months of life. Breast milk not only contains the necessary nutrients for a healthy growth and development but also serves as the first immunization against illness and disease. After the sixth month, complementary feeding is introduced, and thus, the assessment of the adequacy of infants' nutrient intake becomes more important.

Physiological Considerations in Setting Nutritional Requirements for Infants

Infants are considered a vulnerable group because they have a relatively high requirement of nutrients per unit body weight during a sensitive period of rapid growth and development. In addition, recent research has shown that infancy is a critical period, setting the foundation of long-term health and reduced risk for chronic diseases such as cardiovascular disease, dyslipidemia, obesity, and osteoporosis. Some of the main physiological considerations include weaning, increased growth rate, occurring brain development, and the still immature function of kidneys and the gastrointestinal system.

Weaning

Breastfeeding benefits for the health and well-being of infants have been well documented and recognized. According to the World Health Organization (WHO), "adequate nutrition during infancy is essential for lifelong health and wellbeing. Infants should preferably be exclusively breastfed for the first six months of life to achieve optimal growth, development and health. Thereafter, to meet their evolving nutritional requirements, infants should receive nutritionally adequate and safe complementary foods, while ideally continuing to breastfeed for up to two years or more."

The WHO recommends that infants start receiving complementary foods at 6 months of age in addition to breast milk, initially 2–3 times a day between 6 and 8 months, increasing to 3–4 times daily between 9–11 months and 12–24 months with additional nutritious snacks offered 1–2 times per day, as desired.

The estimated intakes of fully breastfed infants are used to derive nutrient requirements for infants up to 6 months old. For older infants (7–12 months), the estimated intakes from complementary foods are also used for reference in addition to that of human milk. It should be noted though that it is difficult to determine the actual intakes of breastfed infants due to not only the variability of available milk volume and composition between women but also the changing composition of the milk during the course of lactation, during the day or even during a single feeding.

Increased Growth Rate

Growth rate during infancy is faster compared to the other life stages; it represents ~33% of daily energy needs in the first 4 months of age, decreasing to 7.4% between the ages of 4 and 12 months. In general, most healthy infants double their birth weight by 6 months of age and triple it by 12 months of age.

The critical processes of physical, mental, and psychomotor development of the infant imply increased demand for energy and nutrients, such as essential fatty acids, iron (as a component of hemoglobin and several enzymes), and zinc (since infants do not have any stocks since intrauterine life). Breast milk ideally meets these needs until the sixth month; after that, complementary feeding is necessary to meet the required intakes for iron and zinc. Especially for zinc, intake should be as close as possible to the recommendation since higher amounts are associated with lower copper levels and suppression of the immune system.

Brain Development

Approximately 20% of the dry weight of the brain is composed of essential fatty acids (incorporated into phospholipids), which

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are critical for the structural integrity of neuronal membranes. About 1/3 of these fatty acids are omega-3 fatty acids. The optimal development of the nervous system is ensured by the intake of omega-3 fatty acids, as they reduce cholesterol levels in neuronal membranes, keeping their liquidity constant. Elevated cholesterol levels would reduce the liquidity of the membranes making it difficult for the cell to carry out its normal functions and increasing the potential for injury or cell death. Inadequate intakes of essential fatty acids in infancy are associated with impaired memory and learning ability, impaired vision, and, in general, reduced functionality of the sensory organs, possibly due to a delay in the myelination process. Infants can receive essential fatty acids through breast milk, and it is therefore important that the mother's diet contains a good supply of omega-3 fatty acids. Breast milk provides $\sim 5.6\ g\ l^{-1}$ of linoleic acid and $0.63\ g\ l^{-1}$ of omega-3 fatty acids (including alpha-linolenic acid and docosahexaenoic acid). Manufacturers of infant formulas usually add blends of vegetable oils, which are high in linoleic acid, to improve essential fatty acid content. Food sources of essential lipids in the older infant's diet, other than breast milk and infant formula, include fatty fish, vegetable oils, nuts, meats, and eggs.

Kidney Function

Despite the fact that nephrogenesis is complete by the 34–36th week of gestation, the kidneys continue to mature through the postnatal period. The immature kidneys of very young infants have difficulty handling high-protein foods due to their high renal solute load. Breast milk has a lower renal solute load compared to infant formulas and as such is better handled by infants' immature kidneys. A balanced dietary intake is particularly important in the case of protein, which should on the one hand provide the necessary amino acids for the support of anabolic processes but on the other hand should not be extremely high so that it does not burden infants' immature kidneys and liver.

Gastrointestinal System

Due to the immaturity of the digestive system and the increased risk of infections, the intake of antioxidant vitamins and prebiotic fiber is rather important to ensure the healthy development of the intestinal flora. There are some naturally occurring oligosaccharides in breast milk that function similarly to prebiotics, stimulating the growth of highly specific strains of bifidobacteria and thereby selectively altering the infant's microbiota. The use of prebiotic-supplemented formula to healthy infants does not raise safety concerns with regard to growth and adverse effects although more research is needed on the long-term effects of its use. Similarly, due to the insufficient intestinal colonization by bacteria, the intestinal synthesis of vitamin K is low, and therefore, the adequate dietary vitamin K intake should be ensured.

Nutritional Recommendations for Infants

Different countries and committees have set micronutrient reference values for infancy, which vary considerably among them. The variability is caused by various factors, for example, disparities exist between underlying concepts, the terminologies used, and the methodologies and assumptions that have been made in the definition of micronutrient requirements and derivation of reference values for intake.

Ideally, an average requirement (referred to as AR (term used by, i.e., European Commission, Nordic countries, and the Netherlands), average nutrient requirement (term used by, i.e., France and UNU/FAO/WHO), or estimated average requirement (term used by, i.e., the United Kingdom and the United States) is established, referring to the level of (nutrient) intake that is adequate for half of the people in a population group, given a normal distribution of requirement. When an AR can be established, then it is also possible to establish the level of (nutrient) intake that is adequate for virtually all people in a population group (usually corresponding to two SDs above the AR). This value is referred to as recommended dietary allowance (term used by the United States), population reference intake (term used by European Commission), recommended nutrient intake (term used by the United Kingdom), or individual nutrient level 97.5 (INL97.5) (term used by UNU/FAO/WHO). When however the establishment of the AR is not possible due to the lack of scientific information, then the adequate intake (AI), that is, the average observed daily level of intake by a population group (or groups) of apparently healthy individuals that is assumed to be adequate, is used for the purposes of dietary assessment. Upper levels have also been set for some of the nutrients and refer to the highest level of a nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in the general population. As intake increases above the set upper level, the risk of adverse effects increases. Figure 1 provides a graphic representation of the risks of nutrient inadequacy and nutrient excess as we move from a low intake of a nutrient to a high intake.

AIs for young infants (ages 0–6 months) are generally estimated to be the average intakes of full-term healthy infants who are exclusively fed with human milk. Deriving dietary intake data for exclusively breastfed infants is already a challenge due to uncertainties pertaining to the variation in breast milk volume and composition. The introduction of complementary food introduces a further number of issues, some of which are the bioavailability of different forms of the nutrient in different formula types and the non-identification of the milk source in most intake surveys.

In the first 6 months of life, the AI is usually based on the average concentration of the nutrient in human milk using an average volume of milk intake of about $0.75\ l\ day^{-1}$. For older infants aged 6–12 months, extrapolation has been used in some cases to establish ARs or AIs. Values were either extrapolated down from those for young adults or extrapolated up from those of young infants; in both cases, adjustment for metabolic or total body size and growth was used. In this age category, the AI is derived based on the content of the nutrient provided by the average volume of $0.6–0.65\ l\ day^{-1}$ of human milk and the content of the nutrient provided by the usual intakes of complementary weaning foods consumed by infants in this age category.

The following sections include information on the food sources, functions, and concerns regarding major nutrients

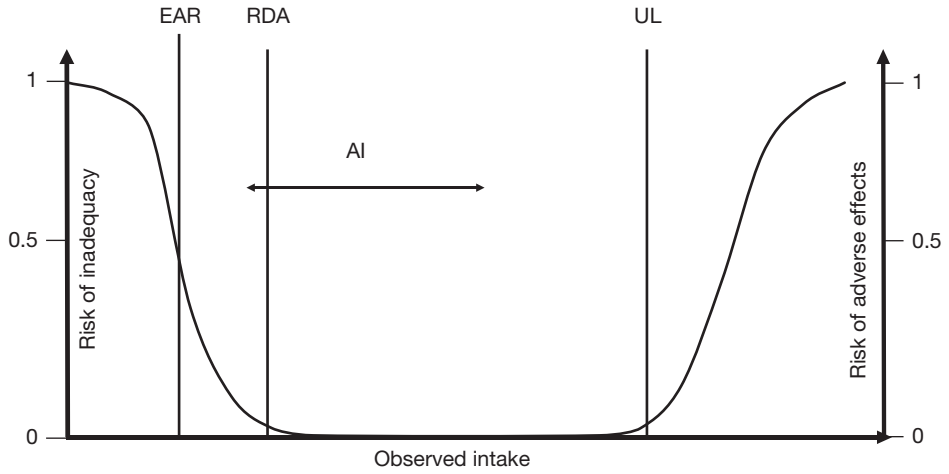

Figure 1 Graphic representation of the risks of nutrient inadequacy and excess when moving from a low intake of a nutrient to a high one. The terminology used by the Institute of Medicine has been used in this graph. EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; UL, upper level.

and nutrients considered to be of public health significance to infants. A review of existing recommendations for infants can be found in Tables 1–5.

Energy and Macronutrients

The energy requirements of infants and young children should balance energy expenditure for achieving normal development and allowing for growth of tissues. Satisfactory growth is a sensitive indicator of whether energy needs are being met. The brain, liver, heart, and kidney account for most of the basal metabolism of infants. The contribution of the brain to basal metabolic rate is exceptionally high in the neonatal period and throughout the first years of life.

The determination of the AI for protein for infants is based on data from infants fed with human milk as the principal source of nutrients during the period 0–6 months of age. For older infants, the protein recommendations are set to cover the estimated requirements for maintenance (nitrogen intake and nitrogen balance), protein deposition, and miscellaneous losses.

For carbohydrates, most bodies suggest a percentage of consumed energy that ranges between 45% and 60%, while the American recommendations have set an AI of 60 g day^{-1} for infants up to 6 months of age and 95 g day^{-1} for older infants.

Lipids are the main energy source in the infant diet and are therefore necessary for normal growth and physical activity. Fats normally provide around half of the energy in human milk (and in most artificial formulas). Special consideration is given to the essential fatty acids, that is, alpha-linolenic and linoleic acids, which are important for the development of the nervous system, while some bodies have set recommended values for long-chain polyunsaturated fatty acids as well. Some of the nutrient recommendation bodies also provide values for saturated fatty acids and monounsaturated fatty acids based mainly on their link to the development of coronary heart disease or risk factors for coronary heart disease (such as blood concentrations of total cholesterol and LDL cholesterol).

Vitamins and Minerals

Tables 4 and 5 provide an overview of the available recommendations for vitamins, minerals, and electrolytes. Some detailed information can be found in the succeeding text for a selected number of vitamins and minerals particularly of interest for this age group.

Infants constitute a population at risk for vitamin D deficiency because of relatively large vitamin D needs brought about by their high rate of skeletal growth. At birth, infants have acquired *in utero* the vitamin D stores that must carry them through the first months of life. Breastfed infants are particularly at risk because of the low concentrations of vitamin D in human milk. This problem might be further reinforced by a restriction in exposure to sun for seasonal, regional, cultural, or social reasons. Most nutrient recommendation setting bodies suggest the use of a vitamin D supplement of 10 μg day^{-1}.

In infants up to the age of 6 months, vitamin K deficiency, although rare, represents a significant public health problem throughout the world and can cause the so-called vitamin K deficiency bleeding. As there is no screening test, it is common pediatric practice to give oral or intravenously injected vitamin K in the immediate perinatal period.

The iron endowment is directly proportional to the lean tissue mass and blood volume of the baby, both functions of its intrauterine growth. Most full-term infants are born with adequate iron stores to sustain its hematopoiesis for the first 6 months of life and even beyond, because breast milk is physiologically low in iron. In comparison, low-birth-weight and preterm infants (including twins) have lower iron stores at birth due to their lower lean tissue mass and blood volume, and, because of their rapid growth rate, their iron stores may get depleted by 2–3 months of age. For these first months, as the mobilization of body iron stores is very high, the requirement for exogenous iron is virtually zero. The interaction

Table 1 Review of recommendations for energy requirements of infants

Body	Units	Age (months)	Estimated energy requirements	
			Male	Female
Europe[a]	kJ day^{-1}	1	1900	1900
		3	2500	2300
		6	3200	3000
		9	3600	3400
		12	4000	3800
France[b]	kcal day^{-1}	0–6	450–500	
		7–12	700–850	
Germany, Austria, and Switzerland[c]	kcal day^{-1}	0–4	500	450
		4–12	700	
International[d]	kcal kg^{-1} day^{-1}	1–3	104	101
		4–6	81	82
		7–9	79	78
		10–12	80	79
The Netherlands[e]	MJ kg^{-1} day^{-1}	0–2	0.39	
		3–5	0.35	
		6–11	0.35	
Nordic countries[f]	MJ kg^{-1}day^{-1}	0–1	1.3	1.4
		3	2.1	2.2
		6	2.6	2.7
		12	3.4	3.7
The United Kingdom[g]	kcal day^{-1}	1–2	574	502
		3–4	598	550
		5–6	622	574
		7–12	718	646
The United States[h]	kcal day^{-1}	1	472	438
		2	567	500
		3	572	521
		4	548	508
		5	596	553
		6	645	593
		7	668	608
		8	710	643
		9	746	678
		10	793	717
		11	817	742

[a]Commission of the European Communities (1993). *Nutrient and energy intakes for the European Community*. Reports of the Scientific Committee for Food. Luxembourg: Office for Official Publications of the European Communities.
[b]AFSSA (Agence Française de Sécurité Sanitaire des Aliments) (2001). *Apports nutritionnels conseillés pour la population française*. Paris: Editions Tec&Doc.
[c]D-A-CH (Deutsche Gesellschaft für Ernährung – Österreichische Gesellschaft für Ernährung – Schweizerische Gesellschaft für Ernährungsforschung – Schweizerische Vereinigung für Ernährung) (2008). *Referenzwerte für die Nährstoffzufuhr*. Frankfurt am Main: Umschau Braus Verlag.
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[h]Institute of Medicine (IoM) (2005). *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*. Washington DC: National Academies Press.

Table 2 Review of protein intake recommendations for infants

Body	Age (months)	Protein intake		
		g kg^{-1}	g day^{-1}	%E
Europe[a]	4–6	1.86	14	–
	7–9	1.65	15	–
	10–12	1.48	15	–
France[b]	0–6	2.10	–	–
	7–12	1.65	–	–
Germany, Austria, and Switzerland[c]	0–1	2.7	12	–
	1–2	2.0	10	–
	2–4	1.5	10	–
	4–6	1.3	10	–
	6–12	1.1	10	–
International[d]	6	1.31	10.2/9.4 (m/f)	–
	12	1.14	14/10.8 (m/f)	–
The Netherlands[e]	0–2	–	9/8 (m/f)	8
	3–5	–	10/9 (m/f)	7/6 (m/f)
	6–11	–	10	6
Nordic countries[f]	6–11	–	–	7–15
The United Kingdom[g]	0–3	–	12.5	–
	4–6	–	12.7	–
	7–9	–	13.7	–
	10–12	–	14.9	–
The United States[h]	0–6	–	9.1	–
	7–12	–	11	–

[a]Commission of the European Communities (1993). *Nutrient and energy intakes for the European Community*. Reports of the Scientific Committee for Food. Luxembourg: Office for Official Publications of the European Communities.
[b]AFSSA (Agence Française de Sécurité Sanitaire des Aliments) (2001). *Apports nutritionnels conseillés pour la population française*. Paris: Editions Tec&Doc.
[c]D-A-CH (Deutsche Gesellschaft für Ernährung – Österreichische Gesellschaft für Ernährung – Schweizerische Gesellschaft für Ernährungsforschung – Schweizerische Vereinigung für Ernährung) (2008). *Referenzwerte für die Nährstoffzufuhr*. Frankfurt am Main: Umschau Braus Verlag.
[d]WHO/FAO/UNU (2007). *Protein and amino acid requirements in human nutrition*. Report of a Joint WHO/FAO/UNU Expert Consultation. FAO Food and Nutrition Technical Report Series 935. Geneva: World Health Organization.
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[f]Norden (2004). *Nordic Nutrition Recommendations 2004. Integrating nutrition and physical activity*. Copenhagen: Nordic Council of Ministers.
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between iron, zinc, and copper should be noted as they act as antagonists. Iron supplements may inhibit intestinal zinc and copper absorption because these elements may compete for binding to a transporter molecule located in the small intestine (divalent metal transporter 1). Zinc causes an elevation of metallothioneins (small cysteine-rich proteins that bind to heavy metal ions, protecting cells from metal toxicity), which can bind both zinc and copper but have higher affinity for the latter, thus making copper unavailable.

Table 3 Review of fat intake recommendations for infants

			PUFA						
Body	*Age (months)*	*Total fat (%E)*	*Total (%E)*	*LA (g day^{-1})*	*AA (g kg^{-1})*	*ALA (g day^{-1})*	*DHA (g kg^{-1})*	*SFA (%E)*	*MUFA (%E)*
Europe[a]	6–11	–	–	4	–	0.5	–	–	–
France[b]	0–6	45–50	–	2.7 (%E)	0.5 (% total fat)	0.45 (%E)	0.32 (% total fat)	–	–
	7–12	45–50	–	2.7 (%E)	–	0.45 (%E)	70 (mg day^{-1})	–	–
Germany, Austria, and Switzerland[c]	0–4	40–45	–	4	–	0.5	–	–	–
	4–12	35–45	–	3.5	–	0.5	–	–	–
International[d]	3–6	40–60		–	0.2–0.3 (%E)	0.2–0.3 (%E)	0.1–0.18 (%E)		
	6–12	~35		3–4.5 (%E)	–	0.4–0.6 (%E)	–	–	–
The Netherlands[e]	0–5	45–50		0.6 (g kg^{-1})	0.04	0.08 (g kg^{-1})	0.02	25	–
	6–11	40		2	–	1	–	–	–
Nordic countries[f]	6–11	30–45	5–10	4	–	1	–	<10	10–25
The United Kingdom[g]		–	–	–	–	–	–	–	–
The United States[h]	0–6	31 (g day^{-1})		4.4	–	0.5	–	–	–
	7–12	30 (g day^{-1})		4.6	–	0.5	–	–	–

AA, arachidonic acid; ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; LA, linoleic acid; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids.

[a]Commission of the European Communities (1993). *Nutrient and energy intakes for the European Community*. Reports of the Scientific Committee for Food. Luxembourg: Office for Official Publications of the European Communities.

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Table 4 Review of vitamin intake recommendations for infants

Body	*Age (months)*	*Vit A (mcg day^{-1})*	*Vit C (mg day^{-1})*	*Vit D (mcg day^{-1})*	*Vit E (mg day^{-1})*	*Vit K (mcg day^{-1})*
Europe[a]	6–11	350	20	10–25	–	–
France[b]	0–12	350	50	20–25	4	5–10
Germany, Austria, Switzerland[c]	0–4	500	50	10	3	4
	4–12	600	55	10	4	10
International[d]	6–12	375	25	5	2.7	5
	7–12	400	30	5	2.7	10
The Netherlands[e–g]	0–5	450	35	10	0.8 mg (g MUFA)$^{-1}$	–
	6–11	400	35	10	0.67 mg (g MUFA)$^{-1}$	–
Nordic countries[h]	6–11	300	20	10	3	–
The United Kingdom[i]	0–6	350	25	8.5	–	–
	7–12	350	25	7	–	–
The United States[j–l]	0–6	400	40	10	4	2
	7–12	500	50	10	5	2.5

(*Continued*)

Table 4 (Continued)

Body	Age (months)	Thiamin (mg day^{-1})	Riboflavin (mg day^{-1})	Niacin (mg day^{-1})	Vit B_6 (mg day^{-1})	Folate (mcg day^{-1})	Vit B_{12} (mcg day^{-1})	Biotin (mcg day^{-1})
Europe[a]	6–11	0.3	0.4	5	0.4	50	0.5	–
France[b]	0–12	0.2	0.4	3	0.3	70	0.5	6
Germany, Austria, Switzerland[c]	0–4	0.2	0.3	2	0.1	60	0.4	5
	4–12	0.4	0.4	5	0.3	85	0.8	5–10
International[d]	0–6	0.2	0.3	2	0.1	80	0.4	5
	7–12	0.3	0.4	4	0.3	80	0.7	6
The Netherlands[e–g]	0–5	0.2	0.4	2	0.12/0.20[m]	50	0.4	4
	6–11	0.2	0.4	2	0.2	60	0.5	–
Nordic countries[h]	6–11	0.4	0.5	5	0.4	50	0.5	–
The United Kingdom[i]	0–6	0.2	0.4	3	0.2	50	0.3	–
	7–9	0.2	0.4	4	0.3	50	0.4	–
	10–12	0.3	0.4	5	0.4	50	0.4	–
The United States[n]	0–6	0.2	0.3	2	0.1	65	0.4	5
	7–12	0.3	0.4	4	0.3	80	0.5	6

[a]Commission of the European Communities (1993). *Nutrient and energy intakes for the European Community*. Reports of the Scientific Committee for Food. Luxembourg: Office for Official Publications of the European Communities.
[b]AFSSA (Agence Française de Sécurité Sanitaire des Aliments) (2001). *Apports nutritionnels conseillés pour la population française*. Paris: Editions Tec&Doc.
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[j]Institute of Medicine (IoM) (2000). *Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*. Washington, DC: National Academies Press.
[k]Institute of Medicine (IoM) (2001). *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*. Washington DC: National Academies Press.
[l]Institute of Medicine (IoM) (2011). *Dietary reference intakes for vitamin D and calcium*. Washington DC: National Academies Press.
[m]Breastfed/formula-fed.
[n]Institute of Medicine (IoM) (1998). *Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin and choline*. Washington DC: National Academies Press.

Table 5 Review of recommendations for the intake of minerals and electrolytes in infants

Body	Age (months)	Calcium (mg day^{-1})	Chromium (mcg day^{-1})	Copper (mg day^{-1})	Fluoride (mg day^{-1})	Iodine (mcg day^{-1})	Iron (mg day^{-1})	Manganese (mg day^{-1})	Magnesium (mg day^{-1})
Europe[a]	6–11	400	–	0.3	–	50	6	–	80
France[b]	0–6	400	0.1–1	0.4–0.8	0.1	40	6–10	–	30
	7–12	500	0.1–1	0.6	0.2	50	6–10	–	75
Germany, Austria, Switzerland[c]	0–4	220	1–10	0.2–0.6	0.25	40[d]	0.5	–	24
	4–12	400	20–40	0.6–0.7	0.5	80[d]	8	0.6–1.0	60
International[e]	0–6	300[f]/400[g]	–	–	–	90	–	–	26/36
	7–12	400	–	–	–	90	7.7[h]	–	54
The Netherlands[i,j]	0–5	210	–	0.3–0.5	–	–	5	–	35–60
	6–11	450	–	0.3–0.5	–	–	7	–	35–60
Nordic countries[k]	6–11	540	–	0.3	–	50	8	–	80
The United Kingdom[l]	0–3	525	–	0.2	–	50	1.7	–	55
	4–6	525	–	0.3	–	60	4.3	–	60
	7–9	525	–	0.3	–	60	7.8	–	75
	10–12	525	–	0.3	–	60	7.8	–	80
The United States[m–o]	0–6	200	0.2	200	0.01	110	0.27	0.003	30
	7–12	260	5.5	220	0.5	130	11	0.6	75

(*Continued*)

Table 5 (Continued)

Body	Age (months)	Phosphorus (mg day^{-1})	Selenium (mcg day^{-1})	Zinc (mg day^{-1})	Sodium (mg day^{-1})	Potassium (mg day^{-1})	Chloride (mg day^{-1})
Europe[a]	6–11	300	8	4	–	800	–
France[b]	0–6	100	15	5	–	–	–
	7–12	275	20	5	–	–	–
Germany, Austria, Switzerland[c]	0–4	120	5–15	1	100	400	200
	4–12	300	7–30	2	180	650	270
International[e]	0–6	–	6	2.8[p]	–	–	–
	7–12	–	10	4.1[p]	–	–	–
The Netherlands[i,j]	0–5	35–50[g]	10–20	3–4	–	–	–
	6–11	400	10–20	3–4	–	–	–
Nordic countries[k]	6–11	420	–	5	–	1100	–
The United Kingdom[l]	0–3	400	10	4	210	800	320
	4–6	400	13	4	280	850	400
	7–9	400	10	5	320	700	500
	10–12	400	10	5	350	700	500
The United States[m,n,q]	0–6	100	15	2	120	400	180
	7–12	275	20	3	370	700	570

[a]Commission of the European Communities (1993). *Nutrient and energy intakes for the European Community*. Reports of the Scientific Committee for Food. Luxembourg: Office for Official Publications of the European Communities.
[b]AFSSA (Agence Française de Sécurité Sanitaire des Aliments) (2001). *Apports nutritionnels conseillés pour la population française*. Paris: Editions Tec&Doc.
[c]D-A-CH (Deutsche Gesellschaft für Ernährung – Österreichische Gesellschaft für Ernährung – Schweizerische Gesellschaft für Ernährungsforschung – Schweizerische Vereinigung für Ernährung) (2008). *Referenzwerte für die Nährstoffzufuhr*. Frankfurt am Main: Umschau Braus Verlag.
[d]Switzerland has adopted the 1996 WHO recommendations of 50 mcg day^{-1}.
[e]FAO/WHO (2002). *Human vitamin and mineral requirements*. Report of a Joint FAO/WHO Expert Consultation. FAO/WHO non-series publication. Rome: Food and Agriculture Organization.
[f]For exclusively breastfed infants.
[g]For formula-fed or nonexclusively breastfed infants.
[h]Values are based on iron bioavailability of 12%.
[i]Nutrition Council (1989). *Dutch dietary reference values [Nederlandse Voedingsnormen]*. The Hague: Information Office for Nutrition.
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[p]Values are based on moderate zinc bioavailability.
[q]Institute of Medicine (IoM) (2004). *Dietary reference intakes for water, potassium, sodium, chloride, and sulfate*. Washington, DC: National Academies Press.

Recommendations and Priorities for Future Research

Although the nutrition-related health problems have changed over the last decades, the need to monitor children's health and diet-related indices is still emerging. It was previously considered that malnutrition takes only the form of undernutrition, which is characterized by a diet that does not provide adequate calories, protein for growth and maintenance, and micronutrients. Thus, the focus was on the quantity of food in order to continuously meet dietary energy requirements. Nowadays, malnutrition also takes the form of hidden hunger, that is, chronic inadequate supply with essential micronutrients through the diet. A person may have access to a sufficient amount of calories but lack adequate micronutrients, and thus, the symptoms of hidden hunger are not always obvious. As the right nutrition (in terms of both quantity and quality) in the first 1000 days is crucial for child survival, growth, and development, the continuous recording of food and nutrient intakes is a necessary practice, because it can provide guidelines for the health professionals and help in forming legislation on food fortification.

See also: Amino Acids: Metabolism; Ascorbic Acid: Physiology and Health Effects; Calcium: Physiology; Energy: Intake and Energy Requirements; Energy Metabolism; Growth promoters: Characteristics and Determination; Human Milk: Composition and Nutritional Value; Infants: Feeding Problems; Infants: Weaning; Iron: Physiology of Iron; Protein: Requirements.

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http://www.fao.org/ag/humannutrition/nutrition/en – World Health Organization (WHO)/Food and Agriculture Organization (FAO). Nutritional Requirements.

http://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes – Institute of Medicine (IoM). Dietary Reference Intakes.

http://www.who.int/topics/infant_nutrition/en – World Health Organization – Infant nutrition.

Infants: Weaning

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Introduction

The term 'weaning' comes from the word 'wenian,' which means 'to accustom.' It is a gradual process through which an infant moves from total dependence on milk to eating normal family meals. The most widely used definition is the process of introducing semisolid food into the infant's diet in addition to milk. Definitions of weaning vary from country to country and include 'the process of expanding the diet to include food and drinks other than breast or infant formula,' 'cease to be suckled,' or 'complete discontinuation of breast-feeding.' As many of the foods initially introduced are not truly solid, the German word 'beiokost' is sometimes used to refer to these foods as a group.

History of Weaning

In ancient Egyptian and Babylonian times, the common age of weaning was 3 years. In the sixteenth and seventeenth centuries, pap (mixtures of flour, water, and sometimes milk) and panada (a broth of bread, flour, meat or lentils, and sometimes milk) were described as infant foods in the literature. Weaning was quite a dangerous time when started in early infancy, and Queen Anne is said to have lost 18 children from spoon-feeding and wet-nursing. The infant food industry started in 1867. By the latter part of the nineteenth century, there was a more cautious approach to solid-food introduction. It was widely recommended that vegetables, particularly green, were not given to infants before 2–3 years of age, although cereals and custards were commonly introduced by 4 months. The situation changed again in the early part of the twentieth century with the trend toward earlier weaning in developed countries. This continued in the United Kingdom until the 1970s and 1980s, although the latest Office of National Statistics (ONS) report would suggest that more parents are delaying the introduction of solids until at least 3 months of age.

Why Wean?

There are four important reasons for weaning. These are as follows:

- If an infant is still unweaned by 6 months, the intake of energy is likely to be inadequate. Infants double their birth weight in the first 4–5 months of life, and breast or formula milk can no longer meet energy needs. Late weaning is associated with a failure to thrive in infants.
- Breast milk provides little iron and vitamin D. In the later stages of lactation, zinc and copper concentrations also begin to fall. However, the bioavailability of nutrients in breast milk is good, and no other foods are usually needed until the age of 4 months, provided the mother is taking a good mixed diet. In addition, for the first 4 months, the full-term infant relies on iron stores to help meet requirements. Sooner or later, these stores will be depleted, and iron-deficiency anemia will occur unless there is a supply from the weaning diet. Formula milk contains higher quantities of iron, vitamin D, zinc, and copper, but with a different bioavailability than breast milk, but for other reasons, it is still recommended that solids be introduced by 6 months.
- There are important developmental reasons for introducing solid food. The neuromuscular coordination needed to eat solid foods develops during the early months of life. At 4 months, most infants can put objects to their mouths, take soft foods from a spoon, form a bolus, and swallow (Table 1). From about 5 months, teeth begin to erupt, and infants are able to chew. Feeding behavior therefore proceeds from sucking to biting and chewing. Chewing improves the mouth and tongue coordination, which is important for speech development. The introduction of different tastes and textures promotes biting and chewing skills. However, if introduction of solids is delayed beyond 6 months, the weaning process becomes more difficult. Infants are less receptive to new tastes, and it is more difficult to teach older infants to chew. From 7 months of age, infants learn to shut their mouths, turn their heads, and refuse to feed.
- The fourth reason is to provide a variety of tastes and flavors. This will enable the infant to eventually accept and enjoy normal family meals.

When to Wean?

The ideal time to introduce solids will vary between infants and differ between cultures. In traditional societies, it is still a practice for infants to be almost entirely breast-fed for the first year of life. In many Western countries, particularly Europe and North America, it is recommended that 'the majority of infants should not be given solids before the age of four months but a mixed diet should be offered by the age of six months.' Early weaning is discouraged for a number of reasons:

- Poor muscular coordination. Before 3 months of age, infants have poor head control, and back support is weak, so it is difficult to hold infants in a position where they can be fed and can swallow semisolid food.
- The kidney is immature and may not be able to cope with a higher renal solute load, and this could lead to higher

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Table 1 Developmental changes relevant to weaning

4 Months
Can maintain posture if supported in a chair
5 Months
Can take soft, pureed food from a spoon, form a bolus, and swallow it
Can hold objects and put them to their mouths to suck
6–8 Months
Teeth begin to erupt
Sits without support
Can chew
Can hand-feed items like biscuits
Learns to shut mouth, turn head, and indicate feed refusal
9–12 Months
Can sit easily
Attempts to use spoon
Indicates and understands 'no'
Pincer grasp of small objects

plasma concentrations of sodium and urea if there is insufficient water available for their excretion.

- The gastrointestinal tract is immature, the intestinal and pancreatic enzyme production is not fully developed, and the gut is more permeable to some of the foreign proteins introduced with food, thus increasing the risk of allergic reactions.
- Early weaning in the developing countries has been associated with a poorer growth velocity than in infants who are exclusively breast-fed. This is probably associated with the lower energy density of the cereal-based gruel offered, resulting from the addition of extra water to produce a food of suitable consistency. Also, iron bioavailability from cereal-based gruel is low. Another major problem is that contaminated weaning foods causes infection and diarrhea. This, in turn, increases nutritional requirements but often reduces an infant's appetite and overall nutritional intake.
- Solid feeding before 15 weeks is associated with increased wheeze during the first 7 years of life. There is also evidence that early exposure to solids is associated with an increased risk of eczema. In a group of children studied until the age of 10 years, it has been reported that infants given solids during the first 4 months were 1.6 times more likely to develop eczema than infants not given solids before 4 months.
- There is evidence that early introduction of solids is associated with increased body fat and weight in childhood.
- There is some suggestion that early introduction of gluten in solids may contribute to a higher incidence of celiac disease. In Glasgow, for example, records indicate that increased numbers of children were admitted to hospital with celiac disease and were diagnosed earlier during 1958–65 and 1966–73 than during 1950–57. Cereals containing gluten were also introduced earlier in Glasgow during 1958–63, and 91% of infants were given gluten by 3 months of age in 1965.

In Western countries, there is still a trend toward early weaning. The ONS survey reported that 55% of infants had been given solids by 3 months and 91% by 4 months. Babies of Asian origin in the United Kingdom were given solids slightly later than white babies, but 90% still had solids by 4 months.

Table 2 Estimated average requirements for energy during weaning

Age (months)	*Energy requirement (kcal kg^{-1} day^{-1})*
3	100
6	95
9	95
12	95

Source: Department of Health (1991). *Dietary reference values for food energy and nutrients. Report on Health and Social Subjects No. 41*. London: HMSO.

Nutritional Requirements of the Weaning Infant

In the early months of infancy, the nutrient intake is derived almost totally from human or infant formula, but in later months, the diet becomes more diverse with the introduction of solid foods. In ensuring that the provision of all nutrients is met for infants, a careful selection of solid foods given in the appropriate amount is required.

Energy

Energy should be provided in adequate quantities throughout infancy to provide fuel for the basal metabolic rate (BMR), growth, and physical activity. The amount of energy required per kilogram of body weight declines gradually, owing to a decreasing growth rate and BMR, even though the energy requirements for activity will increase as the infant becomes older.

The UK estimated average requirements for energy are given in Table 2. These are based on the results of studies using the doubly labeled water technique of measurement that reflects the total energy expenditure in free living infants and children with additions made for the energy value of new tissue deposited during normal growth.

Despite the introduction of solids, milk still needs to provide a significant proportion of the total energy intake throughout the first year of life. Fat contributes 50% of the energy in breast and infant formula milk and is the main source of energy in infants <6 months of age. It still continues to be important in childhood, and infants over 6 months of age will still need 500–600 ml of milk daily. From studies estimating the nutrient intake of older infants, solids contributed 52%, 57%, and 64% of the total energy intake at between 7 and 8 months, 9 and 10 months, and 11 and 12 months, respectively, while milk made up the rest of the energy deficit.

However, adequate energy from weaning foods should be assured, and the energy density of weaning foods is important. If it is high in carbohydrate and low in fat, the food will be very bulky, and it may be difficult for the infant to eat sufficient quantities to meet their energy needs. Failure to thrive, which is a common problem in developing countries, may result.

Protein

Protein is a crucial determinant of linear growth; it provides nitrogen and essential amino acids for synthesis of body tissue. The DOH recommends an adequate intake of protein with a proper balance of essential amino acids during weaning

(Table 3). The protein/energy ratio is also important, and it is recommended that infants between 6 and 9 months have an intake of not <1.74 g of protein per 100 kcal. In developed countries, there should be few problems in achieving an adequate energy/protein ratio, but in developing countries, if weaning foods such as rice and maize are supplemented with oil to improve energy density, this would significantly reduce the energy/protein ratio below the minimum safe ratio.

Vitamins and Minerals

Vitamin A

This is required for growth, normal development, and differentiation of tissues. The 1988 FAO/WHO expert group considered that a daily intake of 350 μg retinol equivalents would meet the needs of all healthy infants and allow the building and maintaining of sufficient liver stores (Table 4).

Vitamin D

This has an important metabolic role in the control of calcium homeostasis and deposition of calcium in the bones and teeth. There are two main forms of vitamin D: cholecalciferol, formed by the action of sunlight on 7-dehydrocholesterol, which occurs naturally in the skin of man, and ergocalciferol, a synthetic source of vitamin D. Weaning infants are vulnerable to poor vitamin D status. Weaning diets are generally low in vitamin D, and breast milk contains little vitamin D. Infant formula is fortified with vitamin D, and provided adequate amounts are consumed, infants fed with these products are unlikely to become depleted. In the United Kingdom, it is recommended that vitamin supplements including vitamin D be given to breast-fed infants from the age of 6 months and to formula-fed infants when the volume of milk decreases below 500 ml daily.

Table 3 Reference nutrient intake for protein during weaning

Age (months)	*Protein ($g^{-1}kg^{-1}day^{-1}$)*
4–6	1.6
7–9	1.6
10–12	1.5

Source: Department of Health (1991). *Dietary reference values for food energy and nutrients. Report on Health and Social Subjects No. 41*. London: HMSO.

Table 4 Reference nutrient intake for vitamins and minerals during weaning

	Age		
Nutrient	*0–3 Months*	*4–6 Months*	*7–12 Months*
Vitamin A (μg)	350	350	350
Vitamin D (μg)	9	9	7
Vitamin C (mg)	25	25	25
Calcium (mg)	525	525	525
Iron (mg)	1.7	4.3	7.8
Zinc (mg)	4.0	4.0	5.0

Source: Department of Health (1991). *Dietary reference values for food energy and nutrients. Report on Health and Social Subjects No. 41*. London: HMSO.

Iron

This is an important nutrient during weaning, which is necessary for the structure of the heme part of hemoglobin, as well a component of myoglobin and many enzymes. For breast-fed infants, after the age of 6 months, the amount of iron from breast milk is insufficient to meet increasing needs. Therefore, adequate intakes of iron, from heme (meat) and nonheme sources (cereals, pulses, and vegetables), should be encouraged with foods rich in vitamin C to aid absorption. Furthermore, the absorption of iron from breast milk is greater if breast-feeds are given separately from solid food as inhibitors in the food bind the iron from breast milk in unabsorbable complexes. Formula milks in the United Kingdom are fortified with iron, and provided infants are taking over 500 ml daily, they are at less risk of iron-deficiency anemia. However, it is still important that iron-rich weaning foods be encouraged.

Zinc

This is a component of several mammalian enzymes and is active in many metabolic pathways. Zinc deficiency may limit growth and may be a vulnerable nutrient during the weaning period. In the United Kingdom, there is evidence that the average daily intake of zinc is below the reference nutrient intake. Although meat and meat products are the richest sources of zinc, these foods provided only 10% of the dietary zinc intake.

Which Foods Should Be Given?

Initial foods should be semisolid and have a smooth consistency and bland taste, and suitable first foods are cereal, such as baby rice or pureed home-cooked rice; mashed potato; custard; plain (unsweetened) yogurt; and pureed fruit and vegetables. This should then progress to pureed meat, pulses, and a wider variety of cereals. In many parts of the world, the first food to be introduced is traditionally a cereal. In western European cultures, a commercially prepared infant cereal containing added vitamins and minerals is commonly the first food. In developing countries, the local staple cereal is more likely to be given, and this may include cereals such as corn, barley, oatmeal, millet, sweet potato, cassava, rice, plantain, yam, arrowroot, potato, and sago. These are mixed to a porridge or paste with milk or water.

Between the ages of 6 and 12 months, the texture of foods given should gradually progress from semisolid to soft family foods. From the age of 6 months, minced or mashed food with soft finger foods can be encouraged. Meat should be coarsely pureed; whole wheat cereals or instant oat cereal can be given in addition to well-cooked egg. Suitable finger foods include soft fruits, for example, banana and melon; cheese fingers; soft cooked carrot; and fingers of toast, chapati, and pita bread.

From the age of 10 months, foods such as meat should be minced or finely chopped. Cooked vegetables need only be chopped, and pasta dishes with small-shaped pasta can be given. Finger foods are particularly important at every meal to encourage the infant to participate in self-feeding.

Commercial Versus Homemade Family Weaning Foods

Homemade family weaning foods

The use of appropriately homemade foods is encouraged, and in the United Kingdom, about 40% of infants are given some homemade weaning foods. They have several advantages: (1) they accustom an infant to the taste of adult-based food; (2) they are cheap and easy to prepare; (3) wastage is low; and (4) meat-based halal weaning foods can be prepared for Muslim infants. Unfortunately, analysis of homemade weaning foods from the United Kingdom has revealed that some meals contain low amounts of energy, iron, and zinc but high amounts of nonstarch polysaccharides, protein, and sodium. Intakes of iron are also lower in infants receiving mainly family foods. Typical homemade foods given to a group of 6- to 8-month-old infants from a deprived inner city area in the United Kingdom were mashed potato, tinned spaghetti, and baked beans. However, breast or infant formula milk or cereal can be added to homemade weaning foods to the improve energy and nutrient density.

Commercial baby foods

In the developed world, commercial weaning foods (tins, jars, and packet foods) are popular, especially cereals, ready meals, and desserts. They may be fortified with extra vitamins and minerals and contain no added salt. Even with commercial baby foods, there are some cultural differences concerning manufactured foods given to infants. The Italians prefer individual constituents, that is, a meat dish to which pasta can be added, whereas the British prefer composite sweet and savory dishes.

The Codex Alimentarius Commission (WHO/FAO) has set compositional standards for baby foods. In 1996, the European Commission issued a directive on processed cereal-based foods and baby foods for infants and young children (96/5/EC); it was amended in 1999. The regulations prescribe manufacturing, compositional, and labeling requirements and prohibit the sale of any processed cereal-based food or baby food, unless it complies with the regulations. The UK government has also published an expert report on additives in food especially prepared for infants and young children, which recommended that additives should only be used when there is a strong justification on technological grounds.

Although not essential, most mothers in developed countries use commercially produced weaning foods at some stage because they are so convenient. In the early stages of weaning, dried products can be made up in small quantities, so avoiding waste. However, commercial weaning foods are relatively expensive, there is a limited range of vegetarian foods, and there is a tendency for some parents to keep infants on strained varieties for prolonged periods of time, which may contribute to the delayed acceptance of more lumpy food.

Foods Avoided During Weaning

Salt

In the West, it is widely recommended that salt should not be added either to weaning foods during the cooking process or to the food at the table, as the developing kidney is unable to excrete large sodium intakes. Excessive sodium intake in young infants is associated with hypernatremia. In addition, there is some concern that a high salt intake in infancy and childhood may cause hypertension in later life; the evidence for this is unconvincing.

Sugar

It is recommended in North America and Europe that sugar should be kept to a minimum and should not be added to foods during preparation. The preference for sweet foods and drinks has been demonstrated even in preterm infants. To avoid encouraging the taste for sweet foods and to reduce the risk of dental caries, sugar should be avoided as much as possible. Even so, in the United Kingdom, it is common to introduce sugar-based foods early in the diet. In the MAFF survey looking at infant-feeding practices between 6 and 12 months, 62% had been given sweets and chocolate, 70% received biscuits, and 45% of mothers gave milk-based dishes sweetened with sugar.

Gluten

There are many disputes about the best time to introduce gluten into the diet. Delayed introduction is associated with an increased age of onset, although there are those who maintain that it decreases the incidence. Challacombe et al. suggested that later introduction of gluten, increased use of gluten-free foods for weaning, and increased incidence of breast-feeding are associated with a decreasing incidence of celiac disease and transient gluten intolerance. However, in Sweden, a doubling of the gluten content of commercial baby foods was associated with an increased incidence of celiac disease from 1.7 children per 100 live births during 1978–82 to 3.5 per 1000 thereafter. This occurred in spite of an increased prevalence and duration of breast-feeding and the delay in the introduction of dietary gluten from 4 to 6 months. Gluten-sensitive individuals who are breast-fed and who are introduced to gluten relatively late may not present clinically during weaning, although the disorder is still thought to manifest at an older age, and the cumulative prevalence is unaffected. Therefore, to prevent celiac disease developing at least during the early weaning period, when the infant is particularly vulnerable nutritionally, it is recommended by most health professionals that cereals given to infants under 6 months should be gluten-free.

Food Allergens

Infants are most vulnerable to the initiation of food allergy in the first months of life, and the risk of food allergy is greatly increased by family history of atopic disease such as eczema and asthma. When there is a strong family history of allergy, mothers should be encouraged to breast-feed for 6 months or longer. Weaning should be particularly discouraged before 4 months, and foods traditionally regarded as allergenic (e.g., cows' milk, soya protein, gluten, eggs, fish, and nuts) should not be introduced until 6 months of age at the earliest. In the United Kingdom, a working group for the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment recommended that peanuts or peanut products

should be avoided until 3 years of age in all children with a parent or sibling with an atopic disease.

Suitable Milks During Weaning

Breast milk

This should be encouraged, but from 6 months of age, breast milk cannot supply the requirements for several micronutrients, including iron, copper, zinc, and vitamin D. Dietary sources should be given either as part of the weaning diet or as specific supplements, for example, vitamin D supplements.

Infant formula

Providing it is iron-fortified, it should be continued until the age of 1 year. It is a particularly good source of vitamin D, and infant formula sold in the United Kingdom is also rich in iron. Provided that a volume of 500 ml daily is taken, there should be no need to give extra vitamin supplements.

Follow-on formula

This is intended for older infants over the age of 6 months. It is based on modified cows' milk. It contains less protein, calcium, and phosphorus than cows' milk but more than standard infant formula. Most follow-on formula contains almost double the iron and 45% more vitamin C than standard infant formula. It can provide a useful drink for older infants if breast-feeding has been stopped or the volume of standard infant formula is <400 ml daily. However, follow-on formula should not replace breast milk or standard infant formula if the volume of either is adequate.

Whole cow's milk

This is not suitable for infants under 1 year as the main drink. It is low in iron, vitamin C, and vitamin D. It may be used in small quantities in the preparation of solid foods.

Goat's and sheep's milks

These are low in iron, vitamin D, and folic acid and should not be given to infants under 1 year of age. Although these milks may be perceived as less allergenic or else providing special nourishment, none of these claims have been substantiated.

Problems Associated with Weaning

Iron-Deficiency Anemia

Iron-deficiency anemia in later infancy and the toddler years is common throughout the world, and simple dietary iron deficiency is the most common cause. In the United Kingdom, it is particularly frequent in ethnic and socioeconomically deprived toddlers, and 4–28% of older infants have been reported to have iron-deficiency anemia. It can cause psychomotor delay, apathy, irritability, reduced attention span, pallor, decreased resistance to infection, reduced exercise capacity, and poor appetite. It is associated with prolonged use of breast-feeding, early introduction of whole cow's milk, and inadequate dietary iron during weaning. There have been many initiatives to try and reduce the prevalence of iron-deficiency anemia in the Western world. These include screening, health education, iron fortification of food, and extension of the use of iron-rich infant formula. Although large-scale dietary education has failed to reduce iron-deficiency anemia in the United Kingdom, programs to provide supplemented food containing iron to target groups have been shown to be successful in the United States. In addition, in the United Kingdom, a project extending the use of iron-fortified follow-on formula beyond 1 year significantly reduced the prevalence of iron-deficiency anemia.

Rickets

Prolonged deficiency of vitamin D can result in rickets. The main symptoms include skeletal deformity, delayed dentition, bone pain, muscle weakness, hypocalcemia, misery, and failure to thrive or short stature. Children under 3 years are particularly vulnerable to a poor vitamin D status because of demands resulting from the high rate at which calcium is being laid down in the bone. Although it is uncommon in Europe and North America, new cases are still being reported particularly in northern climates and immigrant children. In the United Kingdom, low-serum 25-hydroxycholecalciferol concentrations have been noted in 20% of Bangladeshi, 34% of Pakistani, and 25% of Indian 2-year-old children living in England. Low 25-hydroxy vitamin D levels have been noted in the winter in British children not taking vitamin D supplements.

Rickets is commonly reported in some hot climates. In a recent Nigerian study, overt rickets was found in 2.4% of households. Factors in hot climates such as increasing urbanization and the tendency of mothers and their young children to avoid exposure to sunlight, either by remaining indoors or in the shade or by covering the skin, must all contribute to a decreased endogenous synthesis of vitamin D, in combination with a poor vitamin D or calcium intake. In Western countries, prolonged breast-feeding by mothers of poor vitamin D status, early introduction of whole cows' milk, poor weaning sources of vitamin D, little exposure to sunlight, and even the use of sunscreen are contributing factors.

Vitamin A Deficiency

In the developing world, vitamin A deficiency is one of the most common causes of blindness in children, particularly in the Middle East, the Indian subcontinent, and Southeast Asia. It causes xerophthalmia, keratomalacia, and eventually blindness. This is due to reduced infant stores of vitamin A due to maternal deficiency, low vitamin A-containing breast milk, and weaning diets low in preformed retinol from animal food. Vitamin A deficiency is often accompanied by protein-energy malnutrition. Vitamin A supplements, even in the absence of clinical overt deficiency, reduce child mortality.

Weaning Practices in Developing Countries

The weaning infant is potentially at risk in developing countries, and many nutritional problems arise with the introduction of solids. The need to introduce nonmilk food sources with only the availability of rather crude preservation processes, poor hygiene, sanitation, and inadequate water supplies

introduces the risks of gastrointestinal and parasitic infection because of the heavy contamination of foodstuffs with infecting organisms. In rural Bangladesh, a significant association has been found between the frequency of isolation of *Escherichia coli* in the weaning foods and *E. coli* diarrhea in the infants consuming them, and there can be little doubt that food plays an important role in the epidemiology of diarrhea in young children. The effects of infection lead to anorexia and pyrexia with increased metabolic requirements, and this can only serve to exacerbate any effects of a poor nutritional intake. In addition, many infants have been breast-fed for prolonged periods of time without weaning solids, and when they are introduced, it is probably a low-energy-density, cereal-based gruel that may be given only once or twice a day. The continued effects of deteriorating nutritional status and infection may lead to protein-energy malnutrition.

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Carotenoids: Occurrence, Properties and Determination; Cholecalciferol: Properties and Determination; Infants: Nutritional Requirements; Iron: Properties and Determination; Protein: Requirements; Retinol: Properties and Determination; Zinc: Properties and Determination.

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Infrared Spectroscopy: Applications

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Introduction

Infrared spectroscopy (IRS) is one of the most important analytic techniques available to food scientists and industry nowadays. IRS provides qualitative and quantitative information in a fast, cost-effective and nondestructive way, does not require the use of polluting chemicals, and can be carried out even by minimally trained personnel. This makes IRS a potent tool for the quality control/assurance in a variety of different (industrial) settings and suitable for applications under steady process conditions, that is, on-line. IRS has also widened the horizons for the detection of adulteration and the authentication of food products. The first IRS instrumentations became available in the late 1940s. The earliest applications of IRS were reported in the 1950s, but it was not until the 1960s that IRS was used for the analysis of food samples. The most significant advancement in IRS has resulted, however, from the introduction of Fourier transform (FT) instruments that has made IRS even more rapid, reproducible, and sensitive.

IR Spectra and Spectral Analysis

In IRS, a test sample is irradiated with infrared radiation and the transmitted or reflected radiation is measured. Infrared spectra are obtained by plotting the measured absorbance against the corresponding wavelengths. IR radiation triggers transitions in the vibrational states of the sample molecules. Each functional group of a molecule shows characteristic IR absorption at specific wavelengths. The IR region of the electromagnetic spectrum can be divided into three portions: The far IR (FIR, 400–10 cm^{-1}) has the lower energy and induces rotational transitions in the molecules. It has very limited applications in the food domain. The mid-IR (MIR, 4000–400 cm^{-1}) induces fundamental vibrational transitions in the molecules. The near-IR region (NIR, 14 000–4000 cm^{-1}) induces transitions over two (first overtone), three (second overtone), or higher energy levels. A typical spectrum in the NIR region is shown in Figure 1. In general, the absorption bands in the MIR region are more intense than those in the NIR region. Besides, the NIR spectra mainly arise from the superposition of combination modes and overtones, which makes NIR spectra more complex and difficult to interpret than MIR spectra. Besides the chemical composition of the test sample, the IR spectral characteristics also depend on the test sample light scattering properties and thus to its microstructure. Chemical information can be extracted from an IR spectrum from the bands position, intensity, and shape. In pure component systems or very simple mixtures, the band position can be easily related to the molecular structure of a component in the mixture, whereas the band intensity is related to its concentrations through the law of Lambert–Beer. However, foods are very complicated and heterogeneous systems, constituted of countless chemical compounds, which gave rise to complex IR spectra with overlapping bands. In such a situation, when the effect of all the functional groups is taken together, the whole spectrum represents a molecular fingerprint that can be used to verify the nature of the sample. This means that the whole information contained in the IR spectrum (or a selected part of it) is used to infer about the properties of the system under study. To extract relevant information from this complex spectra, however, a special statistical tool is necessary, that is, chemometrics.

Multivariate Statistical Analysis of IR Data

Chemometrics can be defined as the science of extracting chemically relevant information from multivariate datasets by using appropriate statistical techniques. Chemometrics are used to reduce the dimensionality of the data, to reveal relationships between samples, and to detect characteristic patterns that can be used to identify a certain material. As a first step, unsupervised pattern recognition techniques (so-called because they do not require any prior knowledge of the sample properties) are applied to investigate the natural relations between the samples. Examples are hierarchical cluster analysis, cluster analysis, and principal component analysis (PCA). As a further step, supervised pattern recognition techniques may be applied to either classify a certain product or quantify a certain property of the product. Classification models aim to classify new unknown samples in one of the known classes on the basis of its pattern of measurements. The techniques most widely used are k-nearest neighbors, soft independent modeling of class analogy, partial least square regression discriminant analysis (PLS-DA), linear discriminant analysis, support vector machine, and artificial neural network. Regression models establish a functional relationship between a quantitative sample property and a set of independent variables, and the model is used to predict the property of interest in the unknown sample. Multivariate regression methods are used such as principal component regression and partial least square regression.

The building of a classification (or regression) model based on IR spectra normally comprises the following steps: Spectral pretreatment (1) is necessary to exclude all the irrelevant information contained in the spectra (noise) before further modeling. Centering of the data (subtracting the average from each variable) is often the first preprocessing procedure. It may be followed by spectral standardization (dividing every wavelength by the standard deviation of the spectrum at that wavelength), which is not always advisable in IRS because of the amplification effect it may have on spectral noise. Normalization procedures are used to compensate for additive (baseline shift) and multiplicative (tilt) effects in the spectral data induced by physical effects, such as the nonuniform scattering throughout the spectrum (the degree of scattering depends on the wavelength of the radiation) or the particle size. The standard normal variate (SNV), multiplicative scatter correction, and wavelet transforms are typical normalization procedures

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in IRS. Derivation (calculation of first and/or second derivatives) is sometimes applied to remove baseline shifts and correct additive or multiplicative effects. One of the effects of derivation is to enhance the spectrum resolution and the importance of small peaks and shoulders compared to bigger peaks. Other manipulative techniques such as smoothing (reduction of the spectrum 'resolution' and hence of the associated noise) and deconvolution may help in improving the interpretation of IR spectra. The following step is the selection of a training set (2), which consist of objects of known class membership (or known quantitative values of a sample property) for which the IR spectra are measured. In the variable selection step (3), the wavelengths that contain information for the target classification/regression are retained, whereas those with no discriminating power are eliminated. Then, a model is built using the training set (4). A mathematical model is derived between the selected wavelengths measured on the training set and their known categories (or quantitative values of the sample property). Finally, the model is validated (5) to test the reliability of the classification achieved using either an independent set of samples (external validation) or the training set (cross validation). The accuracy of classification models is usually expressed as sensitivity (% of samples of class 1 correctly allocated to their actual class) and specificity (% of samples of class 2 correctly allocated to their actual class). The accuracy of regression models is estimated by the root-mean-square error of calibration, cross validation, and prediction, which measure the difference between predicted values and those measured by the standard reference method on samples of the calibration, training, and test set, respectively.

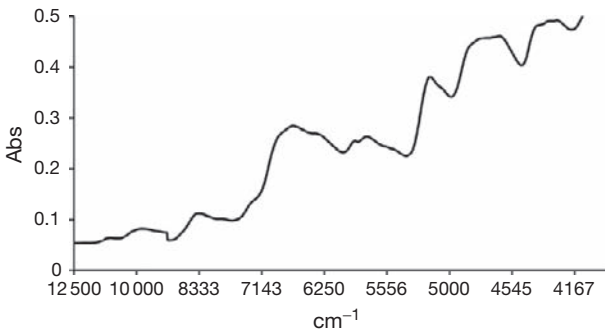

Figure 1 Typical NIR spectrum of a milk powder sample.

Instrumentation and Measurement Setup

Conventional IR instruments consist of a light source, accessory for the sample preparation, a monochromator, a detector, and optical components (lens, collimators, beam splitters, etc.). A schematic description of a typical dispersive IR instrument with double-beam operation is given in Figure 2. Radiation produced by a broadband source is split into two by a beam splitter. One radiation is sent to the sample and the other to a reference. The radiation transmitted (or reflected) by the sample and the reference is then dispersed by a monochromator into component frequencies and collected by the detector, which compares the two radiations and generates an electrical signal, which is converted to an IR spectrum. The radiation source is commonly an inert solid electrically heated to 1000–1800 °C. Based on the type of monochromator, IR spectrophotometers can be classified as filter instruments, scanning monochromator instruments (a grating or prism is used to separate the single wavelengths), or FT spectrophotometers. In a FT spectrophotometer, conventional prisms or grating monochromators are replaced by an interferometer. FTIR utilizes interferometric modulation of radiation to measure multiple frequencies simultaneously, producing an interferogram that is converted to the original spectrum using the Fourier mathematical transform. The most common IR detectors may be either thermal-sensitive (thermocouples, thermistors, and pneumatic devices) or photon-sensitive (based on the interaction of IR radiation and a semiconductor material).

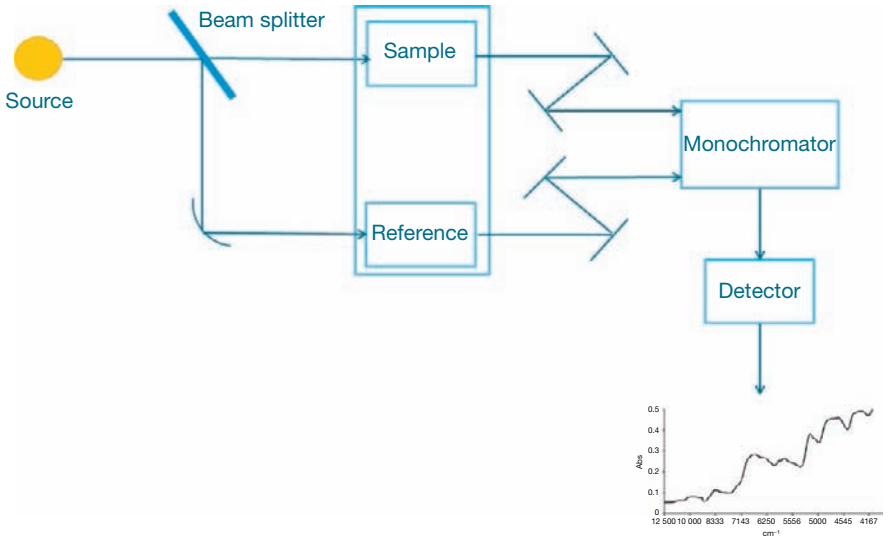

Figure 2 Schematic representation of a typical conventional dispersive IR instrument in transmittance mode and double-beam operation.

Three different acquisition modes are possible according to the arrangement between the sample, the radiation source, and the detector. In the (diffuse) reflectance mode, the IR radiation that is reflected by the test sample surface is acquired by the detector, which is positioned on the same side as the source. The radiation penetrates in the samples to a depth depending on the geometry of the optical probe and the scattering properties of the sample before being reflected so that information on sample surface and, partially, interior is obtained. Under specific conditions, light can undergo internal reflection, which occurs when a beam of radiation enters from a more dense medium (with a higher refractive index) to a less dense medium and which forms the basis for the attenuated total reflection (ATR) technique. In internal reflectance, information on the sample surface is obtained. The reflectance mode is well suited for opaque or strongly absorbing materials. In the transmittance mode, the IR radiation passes through the sample and is collected by the detector that is positioned opposite to the light source. The transmittance mode is more suited for translucent or weak absorbing media. In the interactance mode, the light is at the same time absorbed and reflected by the test sample and collected by the detector that is positioned in a way that prevents specular reflection to reach the monochromator. Sample preparation and presentation as well as the acquisition mode differs according to the instrumentation setting used, the localization of the compound(s) to be measured, and the mode of measurement (off-line, on-line, or at-line). Solid samples can be measured as such (intact material) or after grounding to reduce particle size and related scattering and, for heterogeneous solids, to ensure that surface is representative of the sample thickness. Normally, an amount of sample variable from 0.1 g to few grams is needed depending on the food materials and measurement mode. Thicker samples will usually require more energetic radiation (shorter wavelengths) than thin samples to obtain useful information.

IRS has also been combined with imaging techniques and microscopy to yield hyperspectral imaging (HSI) technologies and IR microspectroscopy (IRMS), respectively. In HSI, spectral and spatial description of a test sample is obtained, which is contained in hyperspectral images or hypercubes, that is, three-dimensional datasets, which contain spatial information on the X,Y plane and spectral information along the *Z*-axis. Thousands of spectra can be obtained each from a specific spot on a sample surface that may give information, for example, on the distribution of chemical compounds in a sample. Hyperspectral camera makes use of the same essential parts of conventional IR spectrophotometers with the addition of an area detector, that is, a device that is able to scan and acquire spectral information from the whole of the object area. In IRMS, the IR spectrophotometer is combined with an optic microscope. In IRMS, the spectra can be acquired from a sample area measuring only few micron, which allows to obtain spectral information from a small amount of sample or from small regions within a sample.

Applications of IRS

The chemical and physical information contained in an IR spectrum can be used for several purposes. Currently, applications of IR spectroscopy in the food domain have been reported in the following fields: (1) chemical characterization of a food sample, (2) detection of spoilage and analysis of foodborne pathogenic bacteria, (3) prediction of food quality, and (4) authentication and detection of adulteration. The selection of the most suitable IR technique, instrumentation, and acquisition mode will depend on the problem at stake, the property to be measured, and the micro- and macrostructure of the test food. In practice, NIR spectroscopy (NIRS) requires less sample preparation and is thus more suited for on-line or *in situ* analysis but is less sensitive (especially toward minor components), selective, and reproducible than MIR spectroscopy (MIRS). On the other hand, NIRS provides more complex structural information that is advantageous for fingerprinting approaches and is able to probe food at higher depth and through NIRS transparent packaging materials. Finally, NIRS is less sensitive to water but it is more sensitive to the diffusion of light. Most of the reported applications have been carried out in a laboratory environment, but several implementations on-line or in-line in industrial settings have been reported as well. An overview (not exhaustive) of the applications of IRS in the food domain is reported in Table 1.

Chemical Characterization of Food Products

One of the major applications of IRS for food quality control is the rapid and nondestructive quantitative analysis of food constituents. Both major (water, protein, fat, and carbohydrates) and minor components can be quantified as well as groups of chemically or functionally similar compounds. For quantitative purposes, a multivariate regression model has to be developed based on a specific set of wavelengths of the IR spectra. Conventional IRS provides spot measures of the concentration of the target compound. This information can be extended to the whole test sample if the constituent has a homogeneous distribution throughout the sample or across its surface. Several foods are, in fact, heterogeneous in composition. HSI has the potential to provide a detailed description of the spatial distribution of compounds and their concentration gradients.

The advantage of IRS is that several food constituents can be quantified at once from the same spectrum. For instance, MIRS is applied for the quantitative analysis of major milk components (proteins, fat, and lactose) since decades and is extensively employed for routine compositional quality control analysis in the dairy industry. For quantification purposes, the absorption bands at approximately 1175 cm^{-1} (corresponding to the triglyceride ester bond, C–O), 1750 cm^{-1} (corresponding to the carbonyl group of fatty acids (FA)), and 2800–3000 cm^{-1} (corresponding to the C–H bonds in FA acyl chain) are used to quantify fat. The bands at 1653 cm^{-1} (amide I) and 1567 cm^{-1} (amide II) are used for the quantification of proteins and give information on protein secondary structure. The region between 1100 and 1000 cm^{-1} (arising from C–O and C–H stretch) is used to quantify lactose. NIRS has also been successfully applied to the quantification of major food constituents. Indeed, an NIRS-based method has been approved by AOAC INTERNATIONAL as official method for the analysis of moisture, fat, and protein in meat and meat products (AOAC 2007.04). The quantification is carried out by

Table 1 Overview (not exhaustive) of the reported applications of IRS in food analysis

Food category	*Chemical characterization*	*Microbiological quality*	*Quality grade and sensorial/ technological attributes*	*Authentication and detection of adulterations*
Dairy	Proximate composition	Total viable count in milk	Sensorial attributes in cheeses	Geographic origin of cheese
	Dry matter	Detection and quantification of pathogens	Freezing point depression of milk	Pasture grazing and animal diet for cows' milk
	Water-soluble nitrogen			Adulteration of sheep/goat milk with cow milk
	Urea			Adulteration of milk, milk powders, and infant formula with melamine
	Free FAs			
Meat and meat products	Proximate composition	Total viable count	Quality grade	Adulteration with cheaper meat
	Collagen	Level of spoilage	Tenderness	Discrimination between fresh and frozen/thawed meat
	Individual FAs		Juiciness	Farming system
			Texture	Organic meat
			Chewiness	Animal diet
			Water holding capacity	Adulteration with pork meat
			Press and cook loss	Storage conditions
			2-day loss postmortem	Animal breed
			Drip loss	
			Warner–Bratzler shear force	
			Fecal contamination of carcasses	
Edible oils	Peroxide value		Antioxidant stability	Adulteration of EVOO
	Free fatty acids		Freshness	Adulteration of animal fat
			p-Anisidine value	Botanical origin
				Geographic origin
Fish and fish products	Proximate composition		Freshness	Farming system
	Dimethylamine		End-point temperature	Authentication of wild fish
				Discrimination between fresh and frozen-then-thawed fish
Grain and cereals	Proximate composition		Mixogram midline point	Varieties
	Trans FAs		Digestibility by cellulase	Organic farming
	Amylose			
	Ash			
	Protein secondary structure			
	Starch gelatinization/ Retrogradation			
	Dry matter			
	Starch			
	Dietary fiber			
Fruits and vegetables	SSC	Total viable count	Sensorial attributes	Geographic origin
	°Brix		Firmness	Adulteration of jam, fruit juices
	Dry matter		Stiffness	Adulteration of maple syrup
	Sugars		Internal defects and diseases	Varieties
	Carotenoids and antioxidants		Ripeness and maturity	
	Starch		Mechanical damages	
	Acidity			
	Essential oil			
	Cell wall pectins			
Honey				Authentication of specialty honey
				Geographic origin
				Botanical origin
				Adulteration with cheaper honeys/ components

measuring the absorbance of O–H bonds at 6895 and 5140 cm^{-1} (water), of the N–H bonds at 6370–6850 cm^{-1} and 4590–5000 cm^{-1} (proteins), and of the C–H bonds of FA at 7150–9000, 5900, and 4160–4500 cm^{-1} (fat). Similarly, grain proximate composition can be accurately predicted by NIRS. These methods have already been adopted in inspection programs by the Canadian Grain Commission and the Grain Inspection, Packers and Stockyards Administration in United States for testing protein content in wheat since early 1980s and have been adopted by the American Association of Cereal Chemists and AOAC for grain analysis. Nowadays, NIRS field instruments that can analyze grain composition directly on grain elevators or harvesting machines are available.

Minor food constituent can also be predicted with acceptable accuracy by NIRS and MIRS. Normally, the prediction of food constituents that occur in lower concentrations MIRS is more advantageous compared to NIRS. Some examples are urea and free FA in milk (by MIRS), single FA in milk and beef (by NIRS and MIRS), total fibers in grains and cereal-based goods (NIRS), soluble sugars and starch in a variety of food products (NIRS and MIRS), alcohol content in wine and spirits (NIRS), and many more. Applications of IRS for the quantification of toxins and other harmful compounds have been also reported. Two ATR-FTIR methods have been recently adopted as official standards for the quantification of *trans* FA content of food (AOCS Cd 14d-99 and AOAC method 2000.10). These methods are based on the IR band at 966 cm^{-1} corresponding to out-of-plane deformation of the C–H bond. Applications are also reported for mycotoxins, tetracyclines, and pesticides.

The applicability of IRS for the quantification of minerals and ash is limited because IR radiation does not interact with minerals or inorganic compounds in their ionic forms. However, information on the mineral composition can be obtained from the modification of the IR spectra induced by the associations with organic acids, organic ions, and chelating agents.

Besides the quantitative determination of single food constituents, IRS can be used to monitor complex chemical and physical changes during food processing. Such changes would reflect in changes in the IR spectrum that may therefore be used to gain insight in the advancement of a process (thermal treatments, fermentation, curd formation in cheese-making, and fruit ripening) or on structural (and functional) changes of its constituents (changes in the protein secondary structure during dough mixing or compositional changes during the gelatinization and retrogradation of starch).

Detection of Spoilage and Analysis of Foodborne Pathogenic Bacteria

One of the most promising applications of IRS in food is the rapid and cost-effective quantification of microbial contamination, the identification of microbial species or strains, and the monitoring of food spoilage. This is especially important because the traditional microbiological methods for the quantification and identification of bacteria or fungi in food are extremely time-consuming and expensive and require highly trained personnel. Spectra can be acquired indirectly from cultured microorganisms or directly from the surface of the food.

The principle behind the application of IRS for the detection and the monitoring of food spoilage is that spoilage is accompanied by chemical modification of the substrate due to the microbial enzymatic activity that is reflected in changes of the IR spectra of the test food sample. Applications have been reported for meat, minced beef, fish, milk, and cabbage, most employing FT-MIRS. The enumeration of spoilage bacteria in milk, for instance, is possible with acceptable accuracy with bacterial loads as low as 10^3 cfu ml^{-1} for whole milk and 4×10^2 cfu ml^{-1} for semiskimmed and skimmed milk. Microbial load from the surface of comminuted chicken breast can be also predicted with acceptable accuracy (RMSE = 0.27 log units in independent test set external validation) by FT-MIRS and to a less extent in rump steak or minced beef. In meaty matrices, the changes in the MIRS spectra involve in particular the fingerprinting region and especially the wave numbers corresponding to the vibration of C–N bonds in amides and amines, which is consistent with the fact that the main biological event occurring during spoilage of meat is proteolysis. Where microbial load cannot be predicted with acceptable accuracy, classification models can be developed in order to discriminate well-defined sensory groups or groups characterized by different levels of spoilage (for instance, fresh, semi-fresh, and spoiled) as reported, for example, on beef fillets and minced pork meat.

Another IRS application is the identification and quantification of selected microorganisms (bacteria and fungi) in food systems. Each bacterial or fungal species possesses a distinctive molecular structure that depends on the protein, lipid, nucleic acid, and carbohydrate composition of its cell wall/membrane and cytoplasm. As a result, each species or strain has its own 'whole organism' spectral fingerprint that can thus be used for species/strain identification. Applications have been reported by both NIRS and MIRS as well as by IR microspectroscopy. When the MIRS region of the spectrum is used, the fingerprinting region (900–1500 cm^{-1}) is especially important for the discrimination of bacteria at strain level. The results reported in the scientific literature do not allow general conclusions to be drawn. In some studies and for certain bacteria, the identification is highly accurate at the species and even the strain level, whereas in other cases, accurate prediction could be obtained only at the genus level. The identification and quantification of pathogenic microorganism are clearly of major interest in food systems. This is more conveniently achieved by first extracting and isolating the microorganism from the food product and then comparing the IR spectra with a spectral library of known bacteria. Several pathogens can be, for instance, detected and quantified directly in apple juice, whereas there are few reports on detection of pathogens from solid food by FTIR. The limit of detection of pathogens in food is reported ranging from 10^3 to 10^6 CFU g^{-1} depending on bacteria and food.

Another interesting application of IRS is the discrimination between dead and injured bacterial cells in a food product. The presence of injured cells poses a serious health threat to the food industry if they revive at a later step. Investigations have been reported for several bacterial species (*L. monocytogenes*, *S. typhimurium E. coli*, etc.) injured with a variety of methods (heat, denaturants, sonication, and radicals). Normally, the differences between live and injured cells are minimal, which

requires special data processing of the IR spectra, for example, derivation, for those small spectral differences to be amplified and mainly concern the absorption bands for proteins and nucleic acids that are most sensitive to denaturation.

Determination of Quality Grade, Technological, and Sensorial Attributes

IRS can be used for the assessment of the quality grade or qualitative properties of food. This is especially important because this might require extremely time-consuming and expensive analyses such as sensory analysis by trained panelists. For instance, FT-MIRS has been found to predict with acceptable accuracy the sensorial attributes (chewiness, creaminess, meltability, etc.) of cheddar cheese or the instrumental texture and meltability attributes of processed cheese. Some quality attributes are correlated with major chemical food constituents and may be therefore predicted with accuracy. For instance, fruit maturity is correlated, even though not exclusively, to sugar content and can be therefore successfully predicted by NIRS. Another example is the antioxidant capacity of fruits and vegetables, which is obviously correlated to the content in antioxidant compounds and can be predicted with acceptable accuracy by MIRS. Reasonable results have been achieved by NIRS for the texture attributes of fruits and vegetables that are related to the scattering properties of their tissues. Technological attributes can also be predicted by NIRS or MIRS with varying degree of success. Several efforts have been made to predict important technological attributes of meat such as texture, water holding capacity, drip, cook and press loss, and shear force, generally with unsatisfactory results. On the other hand, NIRS can accurately predict meat quality grade and sensory tenderness. Other examples include the assessment of fish freshness and the detection of internal browning in apples. Finally, HSI is naturally suited for the (1) monitoring of fruit surface quality attributes such as bruises and other mechanical damages, (2) the detection of surface or internal defects and diseases in fruits and vegetables, and (3) detection of contamination with fecal matter or parasites in chicken carcasses and fish.

Food Authentication and Detection of Adulteration

In recent years, countless IRS applications have been reported in the scientific literature for the authentication of food and detection of adulterations. Those applications span from the verification of the geographic origin and traditional production to the genetic origin of raw ingredients, from the verification of farming management system (organic, biodynamic) to processing (irradiation, freezing/thawing, and heat treatment), and from the verification of sustainable productions. For the authentication of food products, IRS can be used quantitatively, that is, to detect and quantify the amount of a target adulterant, or qualitatively, that is, by discriminating and predicting the real nature of an unknown sample (e.g., organic food). There are two major advantages in using IRS for food authentication. The first advantage relies on the fingerprinting nature of IRS. Indeed, some quality attributes such as the geographic origin or the farming management systems (organic, free range, etc.) cannot be easily verified by traditional approaches based on target analysis of one single marker. The second advantage is that traditional quality control strategies are not designed to look for all the potential adulterants so that an adulteration can be detected only if the adulterant is known beforehand and explicitly searched for by the analyst. IRS may allow the detection of yet unknown adulterants by highlighting untargeted differences between the test material and a genuine reference standard.

One of the most relevant examples of application of IRS is the detection of melamine adulteration in dairy products. Melamine (2,4,6-triamino-1,3,5-triazine) is a nitrogen-rich compound (N makes up approximately 60% of the melamine molecule) that may be fraudulently added to milk to increase the apparent protein content when this is measured by Kjeldahl method, which measures the total nitrogen content as indicator of protein level. Reference methods for the detection of melamine in dairy products are sensitive and specific but are time-consuming and labor-intensive. Both MIRS and NIRS can be successfully applied to detect melamine in dairy products (infant formula, milk powder, and liquid milk) with a limit of detection below 1 ppm, which is the threshold limit set, by FDA in infant formulas. The spectral regions 6900–6780 cm^{-1} (attributed to aromatic amine structures) and 12 497–6098 and 5450–4249 cm^{-1} (attribute to the overtone absorbance of NH_2 stretching and bending) are the most important for the discrimination between genuine and adulterated samples.

Another example of successful application of IRS for detection of adulteration concerns oils and fats adulteration. Olive oil is probably the most popular vegetal oil consumed. The extra virgin olive oil (EVOO) represents the highest quality and most expensive olive oil category. EVOO is frequently adulterated with recycled oils, refined olive oil, or cheaper edible oils. NIRS and MIRS have been both investigated for the detection of such adulteration. The adulteration of EVOO with a wide range of oils may be detected by FT-MIRS at a level that depends on the type of oil and may be as low as 1% w/w (palm oil). The region of the MIRS spectra that contains the most valuable information for the authentication of oils is the fingerprinting region (1500–900 cm^{-1}), in particular the bands associated with bending of the C–H bonds of FAs. NIRS is reported to be as successful as MIRS for the detection of adulteration of EVOO with cheaper edible oils such as sunflower oil, at a level as low as 1% (w/w). IRS has also been employed for the authentication of lard and other animal fats. For instance, the adulteration of shortening with lard can be detected at levels ranging from 0 to 100% and a standard error of calibration of 1.75% by using the regions 1.117–1.097 cm^{-1} and 990–950 cm^{-1} of the NIRS spectrum. The standard of identity for butter requires that no vegetable oil nor margarine is added to the product. The presence of margarine can be detected by NIRS coupled with PLS with a standard error of cross validation $<1.2\%$.

IRS can also be proficiently used for the authentication of food products, for example, specialty honey. Manuka honey is a high-quality monofloral honey produced exclusively in New Zealand and Australia from the nectar of the manuka tree. It is classified as a therapeutic good in Australia because of its proved antibacterial properties related to the high content in methylglyoxal. Manuka honey can be successfully discriminated from non-manuka honey by NIRS. In Figure 3(a), the raw IRS

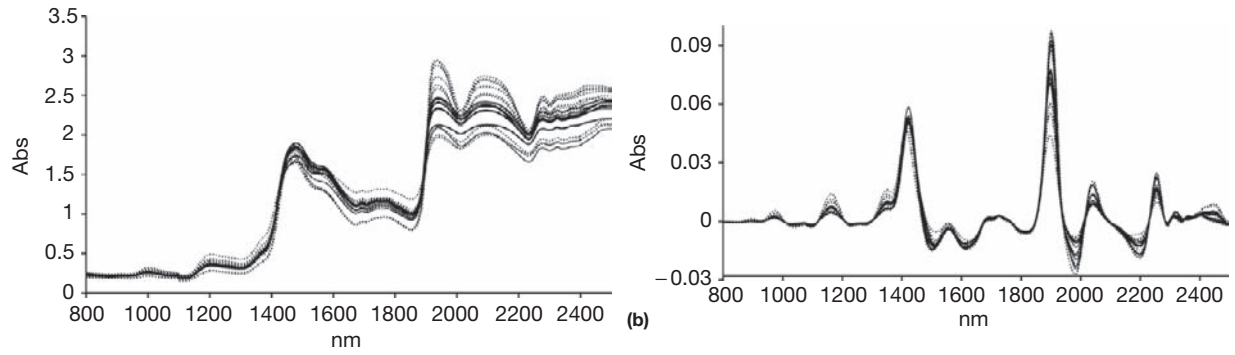

Figure 3 NIR spectra of conventional (full line) and manuka (dotted line) honey before (panel a) and after preprocessing (SNV and first derivative, panel b).

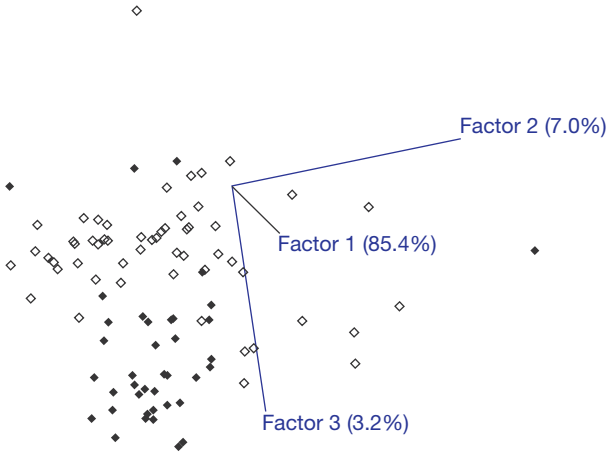

Figure 4 PCA score plot of the first three principal components of the preprocessed (mean centering, SNV, and first derivative) NIR spectra. Black diamonds = manuka honey; white diamonds = non-manuka honey.

reflectance spectra obtained from manuka honey and non-manuka honey samples are compared. The differences in the scattering properties of the honey samples caused a drift in the baseline, which made it mandatory the preprocessing of the spectra to normalize the physical effect. In Figure 3(b), the preprocessed spectra (mean centering, SNV, and first derivative) are illustrated. At this stage, some differences between the two classes are visible at several wavelengths, but to detect the distinctive patterns for the two groups, a chemometric treatment of the data is necessary. In Figure 4, a PCA score plot of the first three principal components of the NIRS data is depicted. In the PCA score plot, a discrimination between the manuka honey samples and the conventional honey samples appears. The two groups form relatively distinct and separate clusters. A PLS-DA model developed on the preprocessed NIR spectra correctly classified 8 out of the 10 manuka honey samples and 12 out of the 14 conventional samples in external validation.

Further examples of IRS applications in the authentication field are (i) verification of fresh grass feeding/pasture grazing for cows' milk; (ii) assessment of the ripening time in cheese; (iii) discrimination between wild and farmed fish and verification of the fish rearing system (extensive, intensive, cage, pond, etc.); (iv) discrimination between fresh and frozen/thawed fish and meat (related to modification of the myofibrillar proteins and the corresponding change in their water holding capacity); (v) verification of the geographic origin of cheeses, strawberry, saffron, honey, tea, wines, and orange; and (vi) authentication of organic feeds and coffee varieties (arabica and robusta).

Advantages and Limitations of IRS

One of the great advantages of IRS is that virtually any sample in any state may be studied with the choice of the proper sampling technique, acquisition mode (transmittance, interactance, and reflectance), and instrumentations. Moreover, since all the molecules adsorb radiations in the IR region to undergo transitions in the vibrational state, IRS has a wider applicability compared to other spectroscopic techniques such as fluorescence spectroscopy, which relies on the presence of fluorescent compounds or visible spectroscopy.

Another advantage of IRS (as well as of other spectroscopic techniques) over the traditional wet analytic techniques is that IRS is incomparably faster and has more high throughput. For instance, the determination of the protein content in a food samples by IRS requires few seconds (the time needed to acquire the IR spectrum, if a reliable calibration curve has already been developed), whereas the reference Kjeldahl method would require several hours. This advantage is even greater if one considers that several parameters (not only chemical properties but also physical properties, quality attributes, and the genuine/adulterated nature of a food sample) can be estimated simultaneously from the same IR spectra. Moreover, the information contained in an IR spectrum can be stored for a formally indefinite time to be used in the future.

Another remarkable advantage of IRS is that it is a non-destructive analysis, which makes it suited for the in-line quality control in a variety of different industrial settings. The food and beverage industry has indeed long used IRS for the quality control of raw materials and intermediate and final products and for the process monitoring. The assembling of an IRS equipment on the production line allows a monitoring of the process at any desired time and the early detection of failure or deviations. From this standpoint, the appearance of fiber-optic probes has significantly improved the ability of IRS to monitor and control processes remotely. However, this mainly holds true

for NIRS, whereas traditional MIRS instruments normally require a certain level of sample preparation before analysis. The introduction of the ATR technique has partially overcome this problem making it possible the analysis of food samples by MIRS regardless of their physical state with little or no sample preparation.

Finally, a big advantage of IRS (as well as other spectroscopic techniques where no sample preparation is needed) is that this technique is more environmental friendly and more respectful of users' health because it does not require the use of solvents and chemicals and does not produce any waste and the measurements of potentially hazardous compounds can be carried out remotely. Finally, the routine use of an IRS instruments from the sample preparation for analysis (where needed) to the spectrum acquisition is extremely simple and does not require specialized but only minimally trained personnel.

However, there are also some limitations in the use of IRS. First, even though the collection of the spectrum is fast, the model building phase can be laborious and time-consuming. Robust calibration/classification models may require hundreds or even thousands of samples to be analyzed by IRS. Indeed, it is essential that in the model building phase, most of the potential variability in the parameter to be estimated is included in the experimental plan. It would also stress the importance to rely on an independent test set (samples that originate from a batch different to that used for the model-building phase). However, in many cases, regression or classification models are extremely product-specific so they need to be frequently updated to accommodate changes in the sample matrix (e.g., in a product recipe), even for the same type of sample and analyte. The model building phase also requires time and proper expertise in multivariate statistics. Furthermore, when used for quantification purposes, a conventional, well-accepted reference method must be used to supply the analytic results required for the calibration of IR spectral data against the concentration of the target analyte (this, of course, would not hold true when IR spectra are used as fingerprints of the test samples for authentication purposes). This means that in the calibration phase, a large amount of samples have to be analyzed by the reference method as well. The fact that the calibration models are developed against a reference method also implies that total predictive error is higher compared to the reference method because the uncertainty in the IRS estimate will sum up to the uncertainty of the reference method measurement. Finally, the classification or regression models are theoretically valid only on the instruments with which the calibration has been carried out. This is because different spectrophotometers may differ in wavelength calibration and photometric response. The commercial implementation of IRS requires that calibration models can be transferred to an instrument to another that requires the use of dedicated calibration transfer techniques.

Conclusions and Future Perspectives

IRS represents a valuable tool for fast and nondestructive qualitative and quantitative analysis of food products and is already a common instrumental platform available in most QA laboratories. IR spectra can provide information on the chemical composition of food, as well as on its nutritional, sensorial, and technological quality in either static or dynamic conditions (on-line analysis). IRS has also showed its potential for the authentication of food products and the detection of fraud. Its advantages include the possibility of authentication of products for which target analysis based on specific markers is not yet available and detection of adulterations based on yet unknown adulterants. However, despite the numerous successes claimed in the scientific literature, it is questionable whether many of the applications reported in this area so far have the necessary robustness to ensure a consistent and accurate prediction. Most of the studies have been carried out on a limited number of samples, which is unlikely to include all the potential variability in the categories of interest and/or lack a proper external validation. The combination of IR with microscopy and imaging techniques is regarded as the natural expansion of conventional IRS and is likely to broaden its applicability in the food domain, provided that low-cost equipment for dedicated applications will be available in the future and, in the case of HSI, proper processing algorithms for handling the huge crop of data obtained are developed. Finally, it is reasonable to assume that with further advancements in IR technology and computational power, IRS may become a promising diagnostic tool for routine investigation and detection of food spoilage and foodborne bacteria.

See also: Authenticity of Food; Food Fraud; Quality Control in Food Processing; Spectroscopy: Types.

Further Reading

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Insect Pests

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Introduction

There are more than six million identified insect species and an estimated ten million that yet have not been described. Of these, probably no more than about 0.5% interact directly or indirectly with man. Approximately 3000 species constitute pests that in conjunction with others organisms cause significant damage to agricultural products and goods. Historically, farmers have had to compete with insect pests for crop products grown for human foods and animal feed consumption. In addition to direct losses caused by insects to plants, fruits, and seeds, these biotic agents cause indirect losses because they leave behind important contaminants such as body parts or exoskeletons, weblike material, insect eggs, feces, urine, and off-odors. Some types also have the ability to attack and damage packaging materials and the structure of grain elevators and transportation facilities.

Insect Biology

Arthropods, the group of joint-limbed animals to which insects belong, have been evolving for about 350 million years. Insects have been such successful inhabitants of the Earth because of their tremendous reproductive potential, survival capacity, small size, and diversity. They have come to occupy most of the ecological niches, and in many instances, insects are also beneficial to human activity especially in food production through their role in plant pollination and as agents of biological control and other activities.

In general, adult insects are characterized by having an external skeleton and a main body divided into three anatomical parts: head, thorax, and abdomen. The head is composed of antennae, eyes, and mouth parts, which are adapted for chewing, piercing–sucking, and chewing–lapping, among other functions. The thorax is divided into three segments, each of which usually possesses a pair of jointed legs; winged forms have pairs of wings on the second and third segments. Among some flying insects, the pair of wings on the third segment of the thorax is modified to serve as a balancing organ. The external reproductive organs are carried on the abdomen.

Insects generally pass through metamorphosis of four developmental stages: egg, larva or nymph, pupa, and adult. In the immature stages (larva or nymph), insects do not have wings, and some may lack legs and other typical structures, giving them an appearance totally different from that of the pupa and adult forms. The primitive wingless insects are an exception. Metamorphosis process is gradual (as in the development of nymphs into adult grasshoppers, leafhoppers, and bugs) and only in some groups is it complete (as with the moths, beetles, and flies, which pass through all stages from larva to adult). In a few cases (e.g., aphids), the mature parthenogenesis female (one that reproduces without fertilization) gives birth to living nymphs, though it may also eventually produce eggs.

Main crops are a substrate for more than 3000 insect species although only 20–30 of these are important pests. Temperate/cold environments inhibit insect activity, whereas tropical and subtropical areas are the preferred habitat for these insects. The tropical zones have all the essential elements for their survival and proliferation: food, favorable conditions of relative humidity, and warm and adequate temperatures throughout the year. Most insects have the capacity to hibernate and cause important damage during the seasons that favor their biology.

Food moisture and environment humidity are the most relevant factors that affect insect growth and development. A high moisture content and adequate temperature conditions favor a rapid growth in the insect population. In general terms, from 9% to 100% moisture content favors insect reproduction, which translates into important overpopulation. Temperature is the most important environmental factor affecting insect development and reproduction. Most insects do not reproduce at temperatures lower than 12 °C or higher than 34 °C. The optimum temperature for reproduction is about 26 °C. However, if a gradual temperature increase is present, this will increase insect metabolism and reproduction rate until achieving a point in which the excess temperature will inhibit or even kill insects.

Insect Damage

Insects are the main biotic stress responsible for crop losses in the world. The direct losses are related to the amount of dry matter consumed and the indirect losses due to contamination of damaged kernels and processed products. Total losses of all food crops in the world are estimated at 30–40% despite an annual expenditure of more than $30 000 million on insecticides. Insect pests are highly adaptable to man-made manipulated habitats where crops are selected for their large size, high yield, and nutritious value and clustered in confined areas. This provides a highly conducive environment for herbivorous insects. In the process of artificially selecting suitable crops for human consumption, highly susceptible plants for insect infestation are also selected. Insect pests are also capable of evolving to biotypes that can adapt to new challenges such as host plant resistance, insecticides, or climatic changes.

Among crops, the total global potential loss due to pests varies from about 50% in wheat to more than 80% in cotton production. The responses are estimated as losses of 26–29% for soybean, wheat, and cotton and 31%, 37%, and 40% for maize, rice, and potatoes, respectively. Overall, weeds produced the highest potential loss (34%), with animal pests and pathogens being less important (losses of 18 and 16%). As an example, global losses in maize production associated with biotic stresses are ~10.9% due to diseases, 14.5% due to

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insects, and 13.1% due to weeds. An additional 10% postharvest loss brings the total estimated loss to 48.5%. A summary of current global losses due to various categories of pests in major crops is presented in Figure 1.

Insects are classified, as primary or secondary pests, according to their habits and characteristics. Primary insects are more harmful because they have the capacity to damage intact plant and crop derivatives. They usually perforate the plant and grain for feeding and reproductive purposes. These insects mainly consume plant tissues (root, shoot, fruit, and kernel) and use them as the ideal site for oviposition and the future growth and development of the larvae. Secondary insects are opportunistic because they attack plant tissues that have already been damaged by primary insects or processed products such as flours, grits, or end products. Both primary and secondary insects use the plant tissues as the ideal place to complete their life and reproductive cycle. In addition, they obtain all the substrate and food and the microenvironmental ideal conditions of temperature and relative humidity. It is noteworthy to mention that both types of insects lower field yield, food value, grain viability, and grain quality as raw material for mills and terminal food industries.

In general, the group of insects represented by moth, which includes cutworms, armyworms, earworms, borers, and grain moths, are the most damaging to crops worldwide followed by the beetles (rootworms, wireworms, grubs, grain borers, and weevils). Next in importance is the group of insects that serve as carriers (vectors) for disease agents (viruses, mycoplasma, bacteria, and fungi) among which the sap-sucking bugs (leafhoppers and aphids) are the greatest problem. In the taxonomic point of view, the primary pests are located in the Orthoptera, Lepidoptera, Coleoptera, and Hymenoptera orders. According to their feeding habits, insects are classified as monophages or polyphages. The first type only feeds from one crop and grain, whereas the second type is adapted to consume various types of plant tissues and grains. The main recognized group of insect pest that cause major losses are summarized in Table 1.

An example situation of insect pests associated with crops can be given by maize. Maize is grown over a wide range of agroecologies, ranging from the tropical lowland at sea level to temperate environments slightly above 50° N/S latitude. For each distinct environment, a different suite of biotic stresses is encountered from the time of seed germination (soil pests, diseases, and weeds) to grain storage (storage insects and diseases). Since all

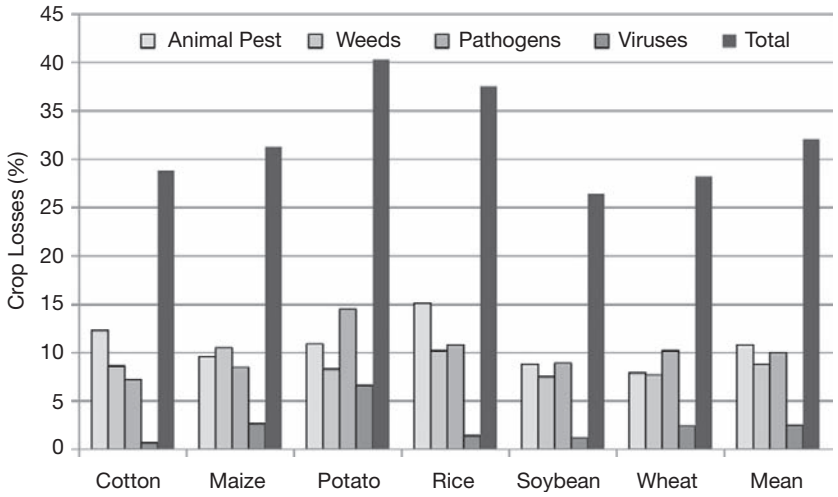

Figure 1 Insect pest global losses in main major crops (with data of Oerke, 2006).

Table 1 Current serious global insect pests due to changes in habitats and climate changes

Insect pest	*Genera/species*	*Plants attacked*
Fruit fly	*Bactrocera* spp.	Fruits and vegetables
American bollworm	*Helicoverpa armigera* (Hubner)	Field crops, ornamentals, and vegetables
Serpentine leaf miner	*Liriomyza trifolii* (Burgess)	Cotton, vegetables
Wheat aphid	*Macrosiphum miscanthi* (Takahashi)	Crops, wheat, barley, oat, maize
Brown plant hopper	*Nilaparvata lugens* (Stal)	Rice
Diamondback month	*Plutella xylostella* (Linnaeus)	Roots
Pyrilla	*Pyrilla perpusilla* (Walker)	Sugarcane and rice
Pink stem borer	*Sesamia inferens* (Walker)	Crops, wheat, barley, oat, maize
Gall midge	Several species	Fruit and crops
Mealy bugs	Several species	Horticulture crops
Thrips and pomegranate	Several species	Ornamental, filed crops, vegetables
Postharvest pest	*Sitophilus* spp.	Crops and pulses
Polyphagous pests: termites, white grubs, hairy caterpillars	Several species	Several species many agroecologies

Table 2 Insect pest associated with crop production in different agroecologies in the world

Insect pest	*Highlands*	*Upper wet MA*	*Lower wet MA*	*Dry MA*	*Wet lowlands*	*Dry lowlands*	*Global*[a]	*Importance*	*Hot spots*
Busseola fusca	1.5	1.5	2.8	3.8	5	4.8	3.2	M	Highlands
Chilo partellus	4.5	3.5	2.6	2.8	1.8	2.1	2.9	M	Africa, India
Cutworm	4.7	4.7	4.7	3.9	3.7	3.8	4.3	L	
Diabrotica spp.	4.5	4.3	2.4	2.9	3.5	3.8	3.5	L	Central America
Diatraea spp.	3.4	3.1	3.4	2.8	2.7	2.8	3.0	M	Central America
Eldana saccharina	.	4.0	5.0	5.0	2.5	3.5	4.0	L	Africa
Sesamia spp.	4.0	.	4.0	3.0	3.0	3.0	3.4	L	Africa, Middle East
Ostrinia furnacalis	–	3.0	2.0	.	2.0	2.0	2.3	H	Asia
Heliothis spp.	2.0	3.0	4.0	4.0	4.0	4.0	3.5	L	highlands of America
Spodoptera spp.	4.5	3.8	3.8	3.8	2.0	2.6	3.4	L	South America
Aphids	4.0	4.8	4.8	4.7	4.5	4.3	4.5	L	
Leaf/plant hoppers	4.7	3.3	3.3	2.9	3.3	3.3	3.5	L	Central America
Grasshoppers	5.0	5.0	5.0	3.3	4.2	3.3	4.3	L	Africa
Flea beetles	3.7	4.8	4.8	4.9	5	4.7	4.6	L	
Spider, mites	4.7	4.8	4.7	2.8	4.8	3.7	4.2	L	Arid environments
Termites	4	4	3.5	2.8	4	2	3.4	L	Africa
Phyllophaga spp.	3.9	3.9	3	3.9	3	3	3.5	L	
Wireworms	3.9	3.9	2.9	3.9	3.2	3.2	3.5	L	Central America
Sitotroga cerealella	4.9	5	5	4	5	5	4.8	L	Central America
Plodia interpunctella	4.9	5	5	4	5	5	4.8	L	
Prostephanus truncatus	3.8	2.3	1.8	2.3	1.6	2	2.3	H	Central America, Africa
Sitophilus spp.	3.3	2.3	1.8	3.1	1.7	2.4	2.4	H	Humid environment

[a]Ranking on a 1–5 scale with 1 being very important and 5 being of low importance at a global level (with data of Bergvinson et al., 2006).

pests are poikilotherms, their development is largely regulated by temperature, making tropical environments ideal for rapid population growth and subsequent crop damage. In order to manage this diversity of agroecosystems and associated pests, maize production areas have classified the major pests and abiotic stresses of maize for the different agroecologies, namely, highlands, upper wet midaltitude, lower wet midaltitude, dry midaltitude, and wet lowlands. The major pests are summarized in Table 2. Using this approach, we are better able to identify the stress traits required for yield stability in a given agroecology, thereby focussing our regional breeding efforts to address the production constraints faced by maize producers over the wide range of environments where maize is produced.

Insects in Stored Food

Storage of cereal food is critical in terms of food security for the more than 7.3 billion people inhabiting the world. The main purpose of grain and food storage is to equilibrate supply and demand, and this postharvest operation is a key step in the complex logistics of moving grain from producers to processors and grain products from processors to consumers.

The FAO has estimated that grain storage losses in most developing countries, mainly located in tropical and subtropical areas of the globe, are much higher compared to developed counterparts due to lower investments in proper infrastructure and a climate that favors grain respiration and growth of insects, molds, and rodents. Therefore, there is a great opportunity to upgrade storage systems in order to diminish losses and assure food for the most needed. If new and better storage facilities are built, the world can save at least 15% of the total cereal production estimated in 2013 in more than 2.78 billion tons.

Most cereal grains have to be stored on farms or commercial grain elevators because they are harvested in specific seasons of the year and are gradually utilized by the various industry segments. Generally, imported grains are also stored for significant periods of time because they are usually acquired in large quantities in order to keep low costs and large inventories. The main objective of storage is to provide wholesome grains, free of insect and mold damage, mycotoxins and pesticides, and insect fragments and rodent filth. Furthermore, the storage of cereals also allows the standardization, classification, and selection of grains for trading purposes. The final target is to manage stored grain wisely with insignificant losses while maintaining its nutritional and functional qualities.

There are only 20–30 insect species that attack cereal grains during storage. The warm temperatures of tropical and subtropical areas are the preferred habitat for these insects, whereas cold weather inhibits insect activity. Insects are the main factor responsible for grain losses throughout the world.

Primarily, pests of stored food cause most of the insect damage in storage and shipment. These are the granary, rice, and maize weevils; the large and lesser grain borer; and the Angoumois grain moth. In dry subtropical areas around the

world, the larger grain borer is of economic importance. Insects that specialize in attacking stored grains belong to the Coleoptera or Lepidoptera orders. Most insects belong to the Coleoptera order that includes beetles, grain borers, and weevils. The Lepidoptera order that includes all butterflies or moths has three important cosmopolitan species that attack stored grains: the Angoumois grain, Indian meal, and Mediterranean flour moths. Primary pests that cause most grain damage are the granary, rice, and maize weevils; the lesser grain borer; and the Angoumois grain moth. The large grain borer is of economic importance in tropical areas around the globe.

Temperature is the most important environmental factor affecting insect development and reproduction. Most insects do not reproduce at temperatures lower than 12 °C or higher than 34 °C. The optimum temperature for reproduction is about 26 °C. Other relevant factors are grain moisture and water activity. The presence of foreign material, dockage, and damaged kernels enhances insect development, growth, and reproduction. Kernels containing more than 14% moisture will have a higher respiration rate and will produce heat spots and enhance insect and mold growth. These interactive effects will increase the stored grain temperature and moisture making the grain more prone to further damage. Insect damaged kernels will lose 25–60% weight and nutrients and leave important contaminants such as body parts, cocoons, feces, and eggs. Some insects such as the confused flour beetle and red beetle usually feed from flours and processed products, secreting odoriferous compounds and irritants. These substances also produce a pinkish coloration to white flours. Other insects such as the worms or larvae of the Indian moth and Mediterranean flour moth leave silken threads and webs that ruin the appearance of flours.

Pest Control

The objective of pest control under the concept of integrated pest management is to achieve a crop production system in a way that optimizes the use of natural resources, protects the environment, and maximizes output in a sustainable way. Control of pests assumes a prominent position, especially in developing countries where insect and disease pressures tend to be severe due to the subtropical and tropical climates found there. Insect control strategies emphasize the reduction and/or use of selective pesticides and include the use of natural control and cultural control. Natural control involves the reduction of insect pest populations by the use of insect natural enemies, while cultural control consists in the deliberate alteration of the production system to reduce pest populations or avoid pest injury to crops.

Chemical

There are four types of chemical insecticides, which protect against insect infestations: organophosphate, carbamates, organochlorine, and pyrethroid. Organophosphate and carbamate insecticides affect the nervous system by disrupting the enzyme that regulates acetylcholine, a neurotransmitter, and usually are not persistent in the environment. Organochlorine insecticides were commonly used in the past, but many have been removed from the market in some parts of the planet due to their health and environmental effects and their persistence (e.g., Dicloro Difenil Tricloroetano (DDT) and chlordane). Parathyroids were developed as a synthetic version of the naturally occurring pesticide pyrethrin, which is found in chrysanthemums. Some synthetic pyrethroids are toxic to the nervous system.

One of the most effective organophosphate insecticides is malathion due to its relative high insect toxicity and low human toxicity. In addition, it is effective against all insects and has a long-lasting effect or good residual power that makes it ideally suited to treat surface areas of grain elevators and machinery. Pyrethrins such as permethrin and bioresmethrin are popular insecticides due to their low toxicity to mammals and safe use. Other insecticides such as methoxychlor and ethylene dichloride are used to protect contact surfaces and storage equipment. Piretrolones are fast-acting insecticides and are particularly effective against adults. Although pyrethrins rapidly lose toxicity, they maintain their repellent power for a long time. Fumigants are chemicals that produce gas at ambient temperature and therefore can reach practically all areas. Due to their excellent distribution, they generally kill all insect population due to the toxicological effect on the insect nervous system. Examples of fumigants are methyl bromide (CH3Br, highly toxic and efficient against all adult insects and larvae), phosphine gas (PH3, tablets that gradually release phosphine, carbon dioxide, and ammonia), and carbon tetrachloride (CCl4). Insecticides have several advantages over fumigants: Their effect is long-lasting, and they are easier to apply, are less dangerous, and can be applied both as preventive agents or to kill established insect populations.

Botanical

Botanical insecticides are naturally occurring chemicals extracted from plants. Natural insecticides are available as an alternative to chemical control. Some of the most deadly, fast-acting toxins and potent carcinogens occur naturally. More than 3500 compounds in ~400 plant families have been described to have toxic insect effects. Some botanical insecticides are similar in toxicity to humans. Botanical insecticide effects are not as efficient as synthetic ones.

Citrus oils (limonene and linalool), which are extracted from citrus peels, have been combined with soaps as contact poisons against aphids and mites. Nicotine derived from tobacco is commonly sold as a 40% nicotine sulfate concentrate. Nicotine is a fast-acting contact killer for soft-bodied insects. Pyrethrin, which is found in chrysanthemums, is the most popular botanical insecticide that acts by contact as a poison. Pyrethrin is effective on most insects, but does not control mites. It rapidly breaks down in sunlight, air, and water. Rotenone is derived from the roots of over 68 plant species. It is toxic to animals because direct contact may cause skin and mucous membrane irritation. It has a broad-spectrum poison mainly used to control leaf-eating caterpillars and beetles. Sabadilla is derived from the seeds of South American lilies with a broad-spectrum contact poison, but it is most effective against true bugs such as harlequin and squash bugs. Sabadilla degrades rapidly in air and sunlight and has little residual toxicity with low toxicity for humans. Neem (*Azadirachta indica*) is derived from the tropical neem tree. Neem extracts has effective control for over 200 types of insects, mites, and nematodes. The solution is effective for only

8 h after mixing. Neem is most effective under humid conditions or when the insect and plants are damp. It has a low toxicity to mammals.

Biological

The use of beneficial insects, pheromones, and growth regulators has been studied as a possible alternative for insect control. Among the most promising biological control techniques are the use of predators and the release of sterile insects. Sterile insects treated with mutating agents can considerably lower grain infestations especially when insects belong to the Lepidoptera order. The main disadvantage of the use of beneficial insects is that they leave behind body casts, exoskeletons, and other insect residues. Pheromones are chemical agents secreted by insects as sexual attractors. These chemicals are used to attract insects and predict populations. Pheromone traps are used to catch insects especially when the number of insects infesting is low. This gives the opportunity to detect and control insect populations before they cause economic damage. The use of pheromones when insect populations are reduced also lowers their reproduction rate because these chemicals confuse insects. The use of growth agents and hormones that are essential for metamorphosis has potential in insect control programs. These chemical compounds inhibit important physiological processes and have a very low toxicity for mammals.

See also: Cereals: Storage; Food and Agriculture Organization of the United Nations; Maize; Mycotoxins: Classification; Pesticides and Herbicides: Types of Pesticide; Pesticides and Herbicides; Potatoes and Related Crops: Role in the Diet; Rice: Types and Composition; Soy Beans: The Crop; Wheat: The Crop.

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Iodine: Iodine Deficiency Disorders (IDD)

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Introduction

Iodine (I) is an essential anionic trace element and occurs naturally in the environment as iodide found in seawater and soils. The distribution of iodine varies widely in the Earth's crust. The soils of mountainous and volcanic areas have low iodine content. The soil iodine content directly determines the iodine content of foods and water and indirectly determines the iodine status of human and animals in the region. Humans derive iodine through their diets, ~90% via food and ~10% from water. Rich sources of dietary iodine include seafood and seaweeds due to high concentrations of iodine in the ocean, good sources include eggs and dairy products, while trace amounts of iodine are present in grains and vegetables. Additionally, foods such as breads, fish sauce, salt, and water can be fortified with iodine. The use of iodized salt is widely recommended for populations inhabiting endemically iodine-deficient regions. Dietary iodide in food is absorbed in the stomach and duodenum and enters the circulation where it is cleared by the thyroid and kidney. The thyroidal uptake of iodine in the blood depends on iodine status, with 10% in iodine-sufficient and >80% uptake in iodine-deficient individuals.

The adult human body contains 15–50 mg of iodine with two-thirds found in the thyroid gland and the remaining one-third in the circulating blood, primarily contained in the thyroid hormones (TH). Iodine is an essential component of the TH, which include the prohormone, thyroxine (T_4), and the active hormone, 3,5,3′-tri-iodothyronine (T_3). T_4 contains four iodine atoms with a half-life of 5 days, while the bioactive and more potent T_3 contains three iodine atoms and has a half-life of 1.5–3 days. TH regulate a wide variety of physiological processes such as protein synthesis and enzymatic activities; cell differentiation, growth, and development of different tissues, especially of the central nervous system; maturation; and energy production and cellular respiration, which influences the metabolic rate of the body. The main organs influenced by TH are the developing brain, muscle, heart, pituitary, and kidney. The human body regulates TH concentrations through thyroid-stimulating hormone (TSH) secreted by the pituitary gland via a negative auto-feedback mechanism. In addition, TSH influences iodine metabolism through uptake of iodine by the thyroid and synthesis and secretion of TH.

Iodine requirements vary throughout the life cycle. The World Health Organization (WHO)-, United Nations Children's Fund (UNICEF)-, and the International Council for Iodine Deficiency Disorders (ICCIDD)-, now known as the Iodine Global Network (IGN)-recommended iodine intakes for different stages of the life cycle are outlined in Table 1. The requirement for iodine is highest in pregnant and lactating women. In pregnancy, iodine requirements escalate due to increased production of TH and transfer of iodine to the fetus and, in lactating women, to support the excretion of iodine in breast milk.

A constant low daily intake of iodine below the requirement leads to chronic iodine deficiency (ID), which decreases TH secretion and, eventually, impairs normal thyroid function; this is the most important cause of ID. Low iodine content in the diet is caused by environmental factors such as glaciation, floods, and erosion resulting in the depletion of soil iodine content. Consequently, locally grown foods in these regions contain insufficient iodine for human beings and animals. Also, some foods contain a variety of substances or goitrogens that interfere with thyroidal iodine uptake (Table 2). Industrial pollutants such as perchlorate are competitive inhibitors of thyroidal iodine uptake. Cigarette smoking increases serum thiocyanate levels that compete with iodine for uptake into both the thyroid and the secretory epithelium of the lactating breast. When iodine intakes are adequate, goitrogens are normally not harmful; however, in the presence of ID, goitrogens might exacerbate the clinical effects of ID. Further, coexisting deficiencies of selenium, iron, and vitamin A aggravate the effects of ID either via impairing activities of mineral-dependent enzymes such as selenium-dependent deiodinases and iron-dependent thyroperoxidase or by vitamin A-mediated suppression of the pituitary TSHβ gene. Thus, chronic insufficient intakes of iodine eventually precipitate ID in human beings.

Epidemiology

ID is the most common cause of preventable mental impairment and affects about 2 billion people worldwide; it is a major public health challenge. ID is widespread in both developed and developing countries. According to ICCIDD, 29.8% of school-age children worldwide are iodine-insufficient and more than a half of these children reside in two regions: 78 million in Southeast Asia and 58 million in Africa. The proportion of school-age children with insufficient iodine intake is highest in Europe (43.9%; 30.5 million), followed by Africa (39.5%; 58.1 million). There has been a decline in the global prevalence of ID in school-age children from 36.5% in 2003 to 29.8% in 2011, the overall progress being marginal in Africa. In 2011, ICCIDD classified 32 countries with ID (9 moderately with ID and 23 with mild ID), 69 countries as iodine adequate, 36 as more than adequate, and 11 as excessive. Globally, there is a dearth of nationally representative prevalence estimates of ID in pregnant and lactating women.

Iodine Deficiency Disorders

ID has several adverse effects on human beings. As coined by Hetzel in 1983, the term iodine deficiency disorders (IDDs) collectively refers to the adverse effects of ID on growth and development in human and animal populations (Table 3). Chronic insufficient iodine intake stimulates the production

Table 1 Recommended iodine intake

Age or population group	*Recommended iodine intake ($\mu g\ day^{-1}$)*
Children 0–5 years	90
Children 6–12 years	120
Children >12 years and adults	150
Pregnant women	250
Lactating women	250

Source: WHO/UNICEF/ICCIDD (2007). *Assessment of iodine deficiency disorders and monitoring their elimination*. Report of a joint WHO/UNICEF/ICCIDD consultation. Geneva: WHO

Table 2 Goitrogens and their mechanisms

Goitrogens	*Mechanism of action*
Foods	
Cassava, lima beans, linseed, sorghum, sweet potato	Contain cyanogenic glucosides; they are metabolized to thiocyanates that compete with iodine for thyroidal uptake
Cruciferous vegetables such as cabbage, kale, cauliflower, broccoli, turnips, rapeseed	Contain glucosinolates; metabolites compete with iodine for thyroidal uptake
Soy, millet	Flavonoids impair thyroid peroxidase activity
Industrial pollutants	
Perchlorate	Competitive inhibitor of the sodium/iodine symporter, decreasing iodine transport into the thyroid
Others (e.g., disulfides from coal processes)	Reduce thyroidal iodine uptake
Smoking	An important goitrogen; smoking during breastfeeding is associated with reduced iodine concentrations in breast milk; high serum concentration of thiocyanate due to smoking might compete with iodine for active transport into the secretory epithelium of the lactating breast
Nutrients	
Selenium deficiency	Accumulated peroxides might damage the thyroid, and deiodinase deficiency impairs thyroid hormone synthesis
Iron deficiency	Reduces heme-dependent thyroperoxidase activity in the thyroid and might blunt the efficacy of iodine prophylaxis
Vitamin A deficiency	Increases TSH stimulation and goiter through decreased vitamin A-mediated suppression of the pituitary TSHβ gene

TSH, thyroid-stimulating hormone

Source: Zimmermann, M. B., Jooste, P. L., and Pandav, C. S. (2008) Iodine-deficiency disorders. *Lancet*. **372**: 1251–1262.

Table 3 Spectrum of iodine deficiency disorders

Age groups	*Health consequences of iodine deficiency*
All ages	Goiter, including toxic nodular goiter Increased occurrence of hypothyroidism in moderate-to-severe iodine deficiency; decreased occurrence of hypothyroidism in mild-to-moderate iodine deficiency Increased susceptibility of the thyroid gland to damage and thyroid cancer from iodine radio isotopes (e.g., from radioactive fallout)
Fetus	Abortion Stillbirth Congenital abnormalities Perinatal mortality Psychomotor defects
Neonate	Infant mortality Endemic cretinism Impaired physical growth Neonatal hypothyroidism
Child and adolescent	Impaired mental function Delayed physical development
Adults	Impaired mental function Lower intelligence quotient and cognitive index Iodine-induced hyperthyroidism, goiter Overall, moderate-to-severe iodine deficiency causes subtle but widespread adverse effects in a population secondary to hypothyroidism, including decreased educability, apathy, and reduced work productivity, resulting in impaired social and economic development
Pregnant women	Subclinical hypothyroidism Poor pregnancy outcomes Premature delivery

Source: WHO/UNICEF/ICCIDD (2007). *Assessment of iodine deficiency disorders and monitoring their elimination*. Report of a joint WHO/UNICEF/ICCIDD consultation. Geneva: WHO.

of TSH from the pituitary gland. A series of TSH-mediated adaptations occur in the thyroid gland before the onset of goiter. TSH stimulates thyroidal uptake of iodide, enhances the breakdown of thyroglobulin (Tg), and promotes secretion of T_3 and conversion of T_4 to T_3 in peripheral tissues to conserve the number of iodine atoms used. The serum Tg concentration increases due to TSH stimulation. Increased iodide uptake by the thyroid leads to decreased renal clearance, thus a fall in the urinary iodine concentration (UIC). Further, the hyperplasia (i.e., increase in the number of cells) and hypertrophy (i.e., increase in the size of cells) of the thyroid cells enhance the use of available iodine efficiently to synthesize TH and underpin the enlargement of the thyroid gland or goiter. Goiter is the classic and most visible effect of IDD. In chronic ID, the initial diffused homogeneous enlargement of the thyroid gland gradually results in the formation of thyroid nodules. Women over 50 years with chronic ID have a high prevalence of multinodular goiter. IDD affects all ages of the population in manifold ways. Goiter, physical sluggishness, growth retardation, and decreased productivity are the well-known adverse outcomes of ID.

WHO/UNICEF/ICCIDD recommends the use of UIC to assess the iodine nutrition of children >2 years old, adults,

pregnant women, and lactating women. The use of UIC centers on the fact that ~90% of ingested iodine is excreted in the urine. Although useful to estimate recent dietary intake, the collection of 24 h urine samples is cumbersome in the field. Thus, to facilitate the process of sample collection, WHO/UNICEF/ICCIDD recommends the use of nonfasting casual or spot urine samples from a representative population. The median UIC, expressed as $\mu g\,l^{-1}$, determined in spot urine samples are only useful as a population estimate and cannot be used to assess iodine status in individuals (Table 4). IDD is considered as a public health problem when a population has median UIC $<100\ \mu g\,l^{-1}$ in a representative sample of school-age children; the median UIC of the sample can also be used to categorize the degree of ID, which can be mild, moderate, or severe as shown in Table 4. UIC has only been validated in school children although it is used for adults. Given the higher dietary iodine requirements of pregnant women, a median UIC between 150 and 250 $\mu g\,l^{-1}$ is used to indicate adequate iodine status in this group.

Recently, the use of Tg concentrations in school-age children to assess iodine status has been proposed to assess iodine status in a population. A median Tg concentration $>13\ \mu g\,l^{-1}$ and/or >3% of Tg values $>40\ \mu g\,l^{-1}$ indicates ID in the population. In pregnant women, TH and TSH are used to determine the optimum thyroid function, which may be used as a surrogate marker for ID; however, the normal reference ranges of TH and TSH are wide; thus, these measures are considered insensitive in areas of moderate-to-mild ID. The epidemiological criteria for the assessment of severity of ID based on the TSH concentration in neonates and total goiter rate in school-age children are extensively used in some countries (Table 5).

Table 4 WHO epidemiological criteria for assessment of iodine nutrition in a population based on median or range of UIC

Median UIC ($\mu g\,l^{-1}$)	*Iodine intake*	*Iodine nutrition*
School-aged children		
<20	Insufficient	Severe iodine deficiency
20–49	Insufficient	Moderate iodine deficiency
50–99	Insufficient	Mild iodine deficiency
100–199	Adequate	Optimum
200–299	More than adequate	Risk of iodine-induced hyperthyroidism in susceptible groups
>300	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)
Pregnant women		
<150	Insufficient	
150–249	Adequate	
250–499	More than adequate	
≥500[a]	Excessive	
Lactating women[b]		
<100	Insufficient	
≥100	Adequate	
Children <2 years of age		
<100	Insufficient	
≥100	Adequate	

[a]The term excessive means in excess of the amount needed to prevent and control iodine deficiency.

[b]In lactating women, the numbers for median urinary iodine are lower than the iodine requirements, because of the iodine excreted in breast milk.

Source: WHO/UNICEF/ICCIDD (2007). *Assessment of iodine deficiency disorders and monitoring their elimination*. Report of a joint WHO/UNICEF/ICCIDD consultation. Geneva: WHO.

IDD in Pregnancy, Lactation, and Infancy

ID during pregnancy has a deleterious impact on the fetus and the mother. Healthy women from iodine-sufficient areas have about 15–20 mg of iodine stores in the thyroid. There is a 50% increase in iodine requirements in pregnant women to produce adequate TH that meets maternal and fetal needs. Additional iodine is needed to maintain maternal TH concentration and transfer TH to the fetus before the fetal thyroid begins to function in the second trimester (around 16–20 weeks of gestation), transfer iodine to the fetus during late gestation, and maintain iodine levels in response to increased renal iodine clearance by the mother. Until the second semester, maternal T_4 serves as the only source of TH for the developing fetus. Pregnant women in chronic ID areas have little iodine stored in their thyroid. In such situations, poor iodine intake may lead to the onset of ID in the mother, and subsequently the fetus. Despite mild-to-moderate maternal ID during the first trimester of pregnancy, maternal TH concentrations typically remain within the normal reference range due to suppression of TSH secretion by the human chorionic gonadotrophin in response to an increase in maternal T_4 (i.e., maternal euthyroidism or normal thyroid function) and the physiological adaptations of the thyroid gland in response to ID. Regardless of the maternal euthyroidism, the fetus is exposed to hypothyroxinemia (i.e., low T_4 in the blood). Consequently, specific parts of the developing fetal brain suffer from a hypothyroxinemic insult, which impairs neurodevelopment. In the second trimester, the fetal thyroid begins to function but is not fully matured and cannot autoregulate the mechanisms needed to normalize TH secretion. This may lead to a surge in fetal TSH production and subsequently fetal hypothyroidism.

Normal concentrations of maternal TH are essential for fetal brain growth. TH influence two phases of brain growth;

Table 5 WHO epidemiological criteria for assessment of severity of iodine deficiency in populations

Criteria	*None*	*Mild*	*Moderate*	*Severe*
Total goiter rate (%)	0–4.9	5–19.9	20–29.9	>30
Neonatal TSH >5 mIU ml^{-1} (%)	<3	3–20	20–40	>40
Classification of goiter by palpation				
Grade 0	No visible or palpable goiter			
Grade 1	A goiter that is palpable but not visible when the neck is in the normal position			
Grade 2	A palpable, visible, and enlarged thyroid when the neck is in the normal position			

TSH, thyroid-stimulating hormone

Source: WHO/UNICEF/ICCIDD (2007). *Assessment of iodine deficiency disorders and monitoring their elimination*. Report of a joint WHO/UNICEF/ICCIDD consultation. Geneva: WHO

the first phase takes place between the third and fifth months of gestation when neuronal multiplication, migration, and organization occur, while the second phase takes place from the third trimester to the second and 3rd years after birth and involves glial cell multiplication, migration, and myelinization. Evidence from animal studies indicates that TH deficiency may impair intelligence, language function, and visuospatial and memory functions or result in autism. Also, TH insufficiency at different stages of pregnancy affects different regions of the brain. For instance, TH insufficiency in early gestation influences basal ganglia while in late gestation impairs cerebellar and hippocampal development. Early maternal TH deficiency can impair visual attention, visual processing, and gross motor skills, while in later gestation, it can influence visual skills, processing speeds, and fine motor skills. Studies in Dutch pregnant women showed that maternal hypothyroxinemia impaired psychomotor development of infants at 10 months of age and that psychomotor damage persisted even at 1 and 2 years of age. Additionally, a recent Dutch study reported that maternal hypothyroxinemia increased risk of children's verbal language and nonverbal cognitive delays and a low maternal UIC in early gestation was associated with inhibition problems and executive functioning impairments in children at 4 years of age. There is increasing concern that even a mild decrease in maternal TH production may cause cognitive delays in offspring. Collectively, these studies highlight the important roles of TH in normal brain development.

Severe ID *in utero* results in gross mental defects along with varying degrees of physical impairments including short stature, deaf-mutism, and spasticity referred to as cretinism. Cretinism is the most severe consequence of ID (**Figure 1**). The two types of cretinism observed are neurological and myxoedematous cretinism; however, the presentation of neurological abnormalities is similar in both types. These include severe mental retardation, intellectual impairment, deafness, motor spasticity, and squint. Nevertheless, myxoedematous cretinism may be differentiated based on postnatal manifestations of progressive thyroid atrophy in neonates, with clinical manifestations of growth retardation, sexual immaturity, and persistent hypothyroidism. Evidence suggests that the clinical characteristics of endemic cretinism occur as result of severe ID at different times in the development of the fetus and neonate; the first occurs *in utero* due to a lack of TH that manifests as neurological impairment in the fetus resulting in neurological cretinism, while the second reflects the progressive thyroid atrophy and hypothyroidism in the postnatal life of neonates resulting in myxoedematous cretinism. In addition to overt cretinism, severe ID *in utero* leads to 10–20% lower developmental scores in young children, lower birth weights, and increased risk of infant mortality. In pregnant women, severe ID is associated with poor pregnancy outcomes such as abortions, stillbirths, prematurity, congenital abnormalities, and increased perinatal mortality.

Conversely, the effects of *in utero* mild-to-moderate ID on psychomotor development of offspring are less clear. Recent studies have shown an association between mild-to-moderate ID in pregnancy and cognitive outcomes such as verbal intelligence quotient (IQ), reading accuracy, and reading comprehension in 8–9-year-old children born to mild-to-moderately deficient mothers compared with iodine-sufficient mothers. A meta-analysis of controlled and observational studies from areas of mild-to-moderate ID reported that adequate iodine intake in pregnancy resulted in normal thyroid volume in both the mother and their newborns and serum Tg concentrations compared with women of lower iodine intake. Further, adequate maternal iodine intake prevented a rise in the maternal TSH concentration with little impact on maternal free or total TH concentrations. More information is needed on the influence of mild-to-moderate ID on the neuromotor development and cognition in infants and children. Additionally, little is known about the adverse clinical outcomes of maternal mild-to-moderate ID in pregnancy.

Figure 1 Myxedematous endemic cretinism. Republished from version 1 August 2014 of www.endotext.org, the FREE online comprehensive endocrinology web-based textbook, with permission.

IDD in Children

The prevalence of ID in school-age children is widely studied as a part of regular health surveillance systems. Cretinism in children is now nonexistent even in areas where severe ID had been common over 30 years ago. Evidence from several observational studies suggests that ID during childhood may impair psychomotor skills and cognition. A few systematic reviews and meta-analyses have assessed the effects of ID on cognition in school-age children by comparing children from iodine-deficient and iodine-sufficient areas. A meta-analysis of 21 observational or experimental studies in children, adults,

and infants with 2214 participants investigated the influence of iodine status on IQ and reported a 13.5 point deficit in intelligence in participants with moderate-to-severe ID. A Cochrane review of 26 longitudinal trials suggested that increased iodine intake in children was associated with reduced goiter rates; however, the results for cognition and psychomotor development were mixed with a few studies showing no improvement. The previously mentioned evidence collectively suggests the beneficial effects of adequate iodine intake in children from moderate-to-severely iodine-deficient areas; however, there is limited information available on the effects in mild-to-moderate ID in children.

The association between mild-to-moderate ID in school-age children and cognition has only been investigated in two studies using randomized controlled trials. The first trial tested the effect of improving the iodine status of moderately ID Albanian children aged 10–12 years using a battery of tests for cognition and motor performance. Children who were given a bolus dose of iodine became iodine-sufficient after 6 months with resulting improvements in four out of seven tests for cognition and motor performance compared with children who remained iodine-deficient. In the second trial of mildly ID New Zealand children aged 10–13 years, daily iodine supplementation for 28 weeks resulted in adequate iodine status and better performance on two out of four subtests of cognition, compared with children who took a placebo containing no iodine. These findings are important because poor school performance adversely affects the productivity and economic growth of countries with mild-to-moderate IDD. However, more randomized control trials are required to elucidate and establish the benefits of improving the iodine intakes of mild-to-moderate ID populations.

The effects of ID on growth have been explored in many observational and cross-sectional studies. Iodine status may influence growth via TH; TH stimulates the secretion of growth hormone, insulin-like growth factor, and insulin-like growth factor-binding protein. In hypothyroidism, lower than normal concentrations of growth hormone, insulin-like growth factor, and insulin-like growth factor-binding protein have been reported. Three randomized control trials have evaluated the implications of iodine supplementation using iodized salt or oral iodized oil on growth of school-age children from severe, moderate, and mildly ID areas. The results showed that children from severe and moderately ID areas supplemented with iodized salt and oral iodized oil, respectively, had significantly increased TH, insulin-like growth factor, insulin-like growth factor-binding protein concentrations, weight-for-age z-scores, and height-for-age z-scores compared with children receiving a placebo; there was no effect on these indexes in supplemented children from the mildly iodine-deficient area.

IDD in Adults and the Elderly

The majority of the studies investigating the effects of ID on physical and mental growth, development, and cognition have focussed on reproductive-age women, neonates, and children. Limited information is available on the implications of ID in adults and the elderly. An 11-year follow-up study evaluated changes in thyroid volume in adults pre- and post-mandatory iodine fortification of salt in two Danish areas, one with mild ID and the other with moderate ID. The results indicated a significant increase in the thyroid volume in adults from the mild ID area, while the adults from moderate ID showed a slight decrease in thyroid volume, post iodine fortification. Additionally, a recent meta-analysis investigated the dose–response relationship between iodine intake and biomarkers of iodine nutrition such as UIC, TSH, and Tg. In adults and elderly populations, it was found that doubling iodine intake increased UIC by 57%; a single dose of iodized oil (200–500 mg of iodine) decreased TSH by 5%; and 200–1500 μg of iodine as potassium iodate resulted in a slight decrease in Tg.

An abrupt increase in iodine intake causes excessive production of TSH that results in hyperthyroidism or iodine-induced thyrotoxicosis. The hyperthyroidism is characterized by high metabolic rates, weight loss, irritability, tachycardia, nervousness, and elevation of body temperature. The condition initially develops as simple goiter that subsequently progresses to toxic nodular goiter. Elderly adults who have lived in areas of ID for long periods of their life are particularly susceptible to iodine-induced thyrotoxicosis. The National Bureau of Economic Research (NBER) has recently evaluated the effect of the salt iodization program in the United States implemented in the first half of the twentieth century. Between 1910 and 1960, the United States witnessed a rise in death rates due to iodine-induced hyperthyroidism post implementation of salt iodization; this was highest among the elderly population and was attributed to longer exposure of suboptimal iodine intakes in the elderly (compared with other age groups), followed by a sudden exposure to increased iodine intake from salt iodization. A New Zealand study reported that short-term high doses of iodine (i.e., >50 mg of iodine for 8 weeks) also resulted in hypothyroidism in some elderly adults and hyperthyroidism in others; however, this resolved for most cases 4 weeks' normalization of iodine intakes. Evidence from different parts of China, with varying degrees of ID, showed that more than adequate or excessive iodine intake not only results in hypothyroidism but also may cause autoimmune thyroiditis. Excessive iodine intakes are believed to have an inhibitory effect on the thyroid or induce autoimmunity in genetically susceptible individuals. Although these studies indicate an increase in the proportion of thyroid disease post salt iodization program, this is outweighed by the benefits of a universal salt iodization program to combat IDD and its potential consequences, especially those on the developing brain.

IDD Control Programs

The ICCIDD, UNICEF, and WHO in association with the Network for Sustained Elimination of ID have been instrumental in the implementation of the IDD control program in various countries. These programs aim to reduce the incidence of IDD and to improve the iodine status of the populations. Different food-based vehicles have been used to provide iodine, including iodine in the form of iodized oil, iodinated water, iodized salt, and foods fortified with iodized salt, and control IDD in different countries.

The WHO recommends universal iodization of salt (USI) for human and animal consumption to improve the iodine

nutrition of populations worldwide. Iodized salt was first introduced in the United States and Switzerland in the mid-1920s. The use of salt as a vehicle for iodine has proved to be a cost-effective and successful strategy to combat IDD. Salt was preferred over other foods for a number of reasons including the widespread daily use of salt, the quantities of salt used remaining constant throughout the year regardless of season and socioeconomic status, the ability of salt to be integrated easily into animal feeds, and the simple and straightforward technology used to produce iodized salt. Additionally, the aesthetic and culinary properties of salt are unaffected, and the iodine levels in the salt can be adjusted to suit the needs of the local population. The factors considered while determining the amount of iodine used to fortify salt take into account the degree of ID, daily salt intake of the local population, and the losses incurred during transportation and storage. The recommended iodine content per kilogram of salt typically varies between 15 and 40 ppm of iodine and added as potassium iodate. Many countries including the United States, United Kingdom, the Netherlands, Switzerland, Australia, and New Zealand practice voluntary fortification of salt, while in China, India, and Denmark, fortification of salt is mandatory. Presently, about two-thirds of the world population is covered by adequately iodized salt (i.e., 15–40 ppm of iodine). The implementation of USI needs frequent monitoring and evaluation to ensure that iodine intakes do not become excessive. Excessive intakes of iodine may result in iodine-induced thyrotoxicosis or autoimmune thyroid disease as discussed earlier.

Monitoring the iodine intake of special groups such as pregnant and lactating women is important, as additional iodine in the form of supplements may be warranted to meet their higher iodine requirements. For instance, a study in Thailand observed that pregnant women were ID despite school-age children being adequate in iodine. There are different strategies used to improve the iodine status of pregnant and lactating women in some countries. For example, in order to meet the additional iodine requirement of pregnant and lactating women, especially from regions of moderate-to-severe ID, the WHO/UNICEF/ICCIDD recommends either a daily dose of 250 μg day^{-1} as potassium iodide or one annual depot dose of 400 mg as oral iodized oil and exclusive breastfeeding of the infant. Iodized oil is used in some areas where the distribution of iodized salt to remote areas is difficult and not a practical measure. Such periodic use of iodized oil was observed in some of the severe iodine-deficient areas in Papua New Guinea, Indonesia, Zaire, and some parts of China, as a provisional measure before the implementation of salt iodization programs in 1970s, especially among young women of childbearing age and children. Iodized oil was administered either as intramuscular injection or as oral drops. In Australia and New Zealand, regions with a long history of mild ID due to low soil iodine, and where only bread is fortified with iodized salt, the daily use of an iodine supplement with 150 μg of iodine for pregnant and lactating women is recommended. In many countries, despite regional or smaller studies indicating mild-to-moderate ID in pregnant and lactating women, strategies to combat ID in these special groups are yet to be implemented. A recent study in lactating Moroccan women found that giving mothers a single dose of oral iodized oil (e.g., 400 mg of iodine) immediately after delivery was more effective at ensuring adequate iodine status of infants in the first 6 months of life than giving iodine supplements directly to the infants. The benefits of maternal iodine supplementation are twofold, by maintaining adequate iodine status of the lactating women and by increasing iodine content of breast milk and consequently the iodine status of the infant.

Other food-based vehicles of iodine include food items such as bread and processed foods that are made with iodized salt; iodine can be directly added to sugar, fish sauce, or water. Iodized bread has been used in Australia and New Zealand, Tasmania, Russia, and the Netherlands with reasonable success to control IDD. In Malaysia, water supplied to schools and communities was iodinated. Concerted activities by government and nongovernment organizations through the implementation of locally suited IDD control measures have been instrumental in the eradication of severe forms of IDD; however, mild-to-moderate IDD remains unabated in many parts of the world and has even seen a reemergence in some countries due to changing food patterns and farming practices.

IDD control programs including USI are beneficial in two ways. Firstly, they have improved the iodine status of the populations and thereby reduced the adverse outcomes of IDD. These programs have been highly effective in eradicating severe ID and endemic cretinism globally while improving iodine status of populations in the mild-to-moderate ID regions. Secondly, the improvements in neuropsychomotor growth and development will result in increased economic growth and productivity. An economic cost–benefit analysis from the United States of universal salt iodization in developing countries showed an approximate gain of 35 billion US dollars. Further, correcting mild ID through adequate dietary iodine intakes may have added developmental gains and reduced health-care costs.

See also: Food Composition Databases; Food Fortification: Rationale and Methods; Infants: Nutritional Requirements; Iodine: Physiology; Pregnancy: Metabolic Adaptations and Nutritional Requirements; Trace Minerals and Trace Elements.

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Iodine: Physiology

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Iodine is an essential nutrient for thyroid hormone production. It exists in oxidation status −1 to 7 (atomic mass weight 126.9 Da) and occurs widely in trace amounts, with local concentration depending on geologic and water condition. Most iodide ($IO3^-$ anion, oxidation state +5) is found in the oceans (50–60 $\mu g\, l^{-1}$) and the concentration in freshwater is 1–10 $\mu g\, l^{-1}$. Iodide ions in seawater oxidize to form elemental iodine, which is volatile and evaporates into the atmosphere, then dissolves into water droplets that fall as rain. However, the cycle of iodine in many regions is slow and incomplete, leaching due to glaciation, frequent flooding, and erosion and depletes surface soils iodine (and soils). Consequently, groundwater easily becomes deficient in this element. Crops grown in these soils may be tenfold lower in iodine content (as low as 10 $\mu g\, kg^{-1}$ of dry weight) than those from iodine sufficient region (about 1 $mg\, kg^{-1}$ of dry weight in plants). The iodides in the sea accumulate in marine organisms, whereas on land, small amounts of iodine are taken up by plants that are subsequently ingested by herbivores. The only stable isotope of iodine is ^{127}I.

Iodine is essential for the synthesis of the thyroid hormones, thyroxine or 3,5,3,5-tetraiodothyronine (T4), and the biologically active form 3, 5, 3′-triiodthyronine (T3), which maintain the body's basal metabolic rate by controlling energy production and oxygen consumption in cells. Reverse T3 (rT3) is an inactive form of T3 that is produced in the body, particularly during periods of stress. Under normal conditions T4 will convert to both T3 and rT3 continually, and the body eliminates rT3 quickly. The synthesis of thyroid hormones requires a normally developed thyroid gland, an adequate nutritional intake of iodide, and a series of sequential biochemical steps. Thyroid hormone (T4 and T3) has pleiotropic effects, affecting multiple biological processes. These iodide-containing thyroid hormones are essential for normal growth and maturation along with the development of the central nervous system and regulation of numerous metabolic pathways. The physiological function of T4 and T3 encompasses the regulation of mitochondrial energy metabolism as well as cellular oxidation, thermoregulation, intermediate metabolism, carbohydrate, lipid and protein metabolism, and nitrogen retention. Iodine is primarily obtained through the diet but it is also a component of some medications. Various mechanisms can lead to thyroid disorders, and hypo- and hyperthyroid status can be observed in cases of both insufficient and excessive iodine intakes.

Intestinal Absorption

Iodine is ingested in a range of forms that are readily reduced to iodide (I^-) in the gut prior to absorption. The amount of iodide absorbed is largely dependent on the level of dietary iodine intake, rather than on its chemical form or the composition of the diet. Absorption is extremely efficient and takes place mainly in the stomach and upper small intestine, that is, the duodenum. Iodide is almost completely absorbed, whereas the absorption and metabolism of iodized oils is not entirely known. Under normal conditions, the absorption of dietary iodine is greater than 90%. The iodate ($IO3^-$) from supplements is reduced in the gut to iodide, but most dietary iodine is an inorganic salt form of iodide. Organic iodine is usually ingested only in the form of thyroid hormone T4 supplements and about 75% of an oral dose of the T4 is absorbed intact. For iodine and iodate from salt and supplements, absorption in the gut is high and not modified by other food components. However, iodine bioavailability may be influenced when encapsulation methods are used for iodine supplements, for example, tablets versus gelatin capsules, different coating substances, or the amount of pressure used to form the tablets, but only limited data is available The Institute of Medicine (IOM) in 2001 brought the consideration an absorption efficiency of 92% (Figure 1).

Iodide is absorbed by an active transport protein called the sodium-iodide (Na^+/I^-) symporter present in the brush border of the membrane and mediates active iodine accumulation. As its name indicates, the sodium–iodide symporter (NIS) simultaneously transports both Na^+ and I^- ions from extracellular fluid (i.e., blood) into the thyroid epithelial cells. NIS cotransports two sodium ions along with one iodide ion, with the sodium gradient serving as the driving force. The energy required to produce the sodium gradient is provided by the ouabain-sensitive Na^+/K^+-ATPase. NIS is expressed on the enteral apical surfaces of enterocytes and in numerous organs of the body including the thyroid gland, stomach, breast, placenta, salivary glands, and eyes. The genetic expression of the NIS is negatively regulated, depending on the concentration of iodine. That is, high concentrations of iodine in the plasma directly decrease NIS expression. However, NIS expression in many parts of the body remains to be completely understood.

Some foods contain goitrogens, that is, substances that interfere with thyroid hormone production or utilization. It should be recognized that goitrogens are usually active only if iodine supply is limited and/or goitrogen intake is of long duration. Goitrogens in some foods (thiocyanates from cassava, glucosinolates from cruciferous vegetables like cabbage, broccoli, cassava, and lima beans), presence of humic acids in drinking water, and nitrates, fluorides, calcium, magnesium, and iron in food and water, and a deficiency of other micronutrients, such as selenium or iron, interfere with thyroid metabolism and can aggravate the effect of iodine deficiency (Table 1). Foods containing goitrogenic cyanoglycosides such as sweet potato release thiocyanates that compete with iodine for absorption, blocking its uptake by the thyroid. Iodine absorption efficiency from water is influenced by the content and nature of humic substances in water. There has been

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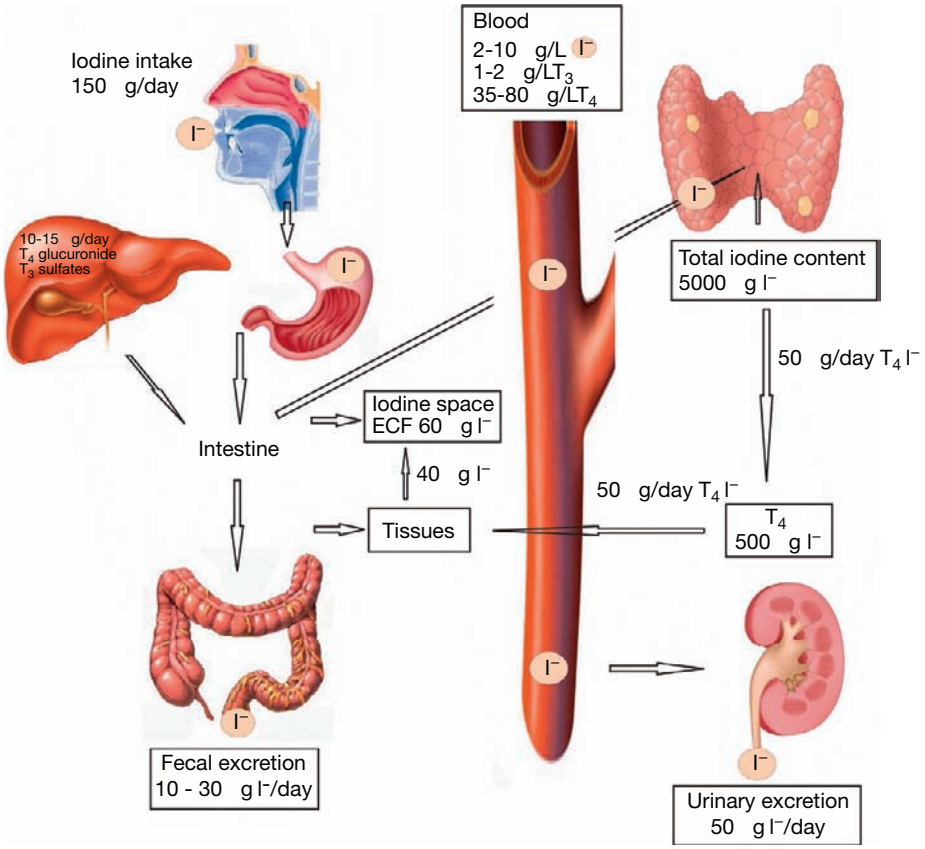

Figure 1 Iodine physiology.

Table 1 Goitrogens in food, industrial pollutants, and micronutrient deficiency and their mechanisms that interfere with thyroid hormone production or utilization

Foods	*Mechanism of action*
Cassava, lima beans, linseed, sorghum, sweet potato	Contain cyanogenic glucosides (they are metabolized to thiocyanates that compete with iodine for thyroidal uptake)
Cruciferous vegetables such as cabbage, kale, cauliflower, broccoli, turnips, rapeseed	Contain glucosinolates (metabolites compete with iodine for thyroidal uptake)
Soy, millet	Contain flavonoids (impair thyroid peroxidase activity)
Industrial pollutants	
Perchlorate	Competitive inhibitor of the Na/I symporter (decrease iodine transport into the thyroid)
Others (e.g., disulphides from coal processes)	Reduce thyroidal iodine uptake
Smoking	Smoking during breastfeeding (reduced iodine concentrations in breast milk) High serum concentration of thiocyanate (compete with iodine for active transport into the secretory epithelium of the lactating breast)
Nutrients	
Selenium deficiency	Accumulated peroxides (might damage the thyroid, and deiodinase deficiency impairs thyroid hormone synthesis)
Iron deficiency	Reduces heme-dependent TPO activity in the thyroid
Vitamin A deficiency	Increases TSH stimulation and goiter (through decreased vitamin A-mediated suppression of the pituitary TSHβ gene)

Source: Zimmermann, M. B. (2009). Iodine deficiency. *Endocrinology Reviews* 30, 376–408; Vanderpas, J. (2006). Nutritional epidemiology and thyroid hormone metabolism. *Annual Review of Nutrition* 26, 293–322.

concern about the presence of perchlorate in foods and drinking water because perchlorate inhibits the uptake of iodine in the thyroid gland.

Transport in the Blood

Total plasma iodine concentrations in euthyroid subjects (persons with adequate thyroid hormone status) range from 40 to 80 $\mu g\,l^{-1}$. Under normal circumstances, absorbed iodine has a half-life of approximately 10 h in plasma. This half-life value may be shortened in patients with iodine deficiency due to a more rapid thyroid intake. In blood plasma, the concentration of inorganic iodide is proportional to dietary intake and ranges from 2 to 6 $\mu g\,l^{-1}$ for average intakes below 200 $\mu g\,day^{-1}$. Concentrations between 80 and 250 $\mu g\,l^{-1}$ are generally associated with hyperthyroidism. Most organic iodine is present as T4, which accounts for more than 10 times the iodine content of T3, reverse T3 (rT3), mono-iodinated tyrosine (MIT), and di-iodinated tyrosine (DIT). Less than 1% of T3 and T4 is free in plasma. T4 occurs either free (fT4) or bound to proteins, mainly to thyroxine-binding globulin (TBG) and to a lower extent to transthyretin (or prealbumin) and albumin.

Distribution to Tissues

Fifteen percent of ingested iodine is actively taken up by the basal membrane of the thyroidal follicular cells using NIS, within 24 h of ingestion. The specific activity of NIS in thyroid cells is three to four times greater in thyroid tissue than in other tissue in body. Goitrogens including bromine (from fruit fumigants and processed bakery products), chlorine (chloramine by-product from drinking water chlorination), ammonium perchlorate (rocket fuel found in tap water), fluorine (naturally occurring in well water plus drinking water fluoridation), and thiocyanate (from cigarette smoke) can bind to the NIS receptor and damage it preventing iodine from entering the cell. The basal membrane of the thyroid cell has the specific ability to pump iodine into the interior of the thyroid cell. This is called *iodide trapping*. Once the iodide is inside the thyroid follicular epithelial cells, it is then transferred across the apical membrane to the follicle lumen by pendrin, a sodium independent iodide/chloride transport protein. In the thyroid, the follicular cells synthesize intracellular glycoprotein thyroglobulin (Tg), which is then stored in the central intrafollicular colloidal lumen. Thyreoperoxidaze (TPO), an iron-containing enzyme, is then catalyzed by the process of *organification*, in which iodide is oxidized and attached to the phenyl ring of Tg tyrosyl residues either in the form of MIT or DIT. T4 is formed by the coupling of two DIT molecules, whereas T3 is formed by one MIT and one DIT molecule. Soy isoflavones and goitrogens such as sulfur-containing thionamides present in vegetables of the brassica family impair the building of iodine to Tg and prevent oxidation of iodine by TPO. Depending on the overall iodine status, the thyroid intracellular colloid space stores enough covalently bound iodine in Tg for the synthesis of hormones for several weeks. The synthesis and release of T3 and T4 is regulated by a thyroid-stimulating hormone (TSH, or 'thyrotropin') secreted by the anterior pituitary gland. This regulation is subject to feedback inhibition deficiency. Increased TSH stimulates thyroglobulin peroxidase and subsequently releases thyroid hormones into circulation by endosomal and lysosomal cellular proteases. When the presence of thyroid hormone is low, thyrotropin-releasing hormone (TRH) is secreted by the hypothalamus and stimulates the secretion of thyrotropin. TRH secretion is also stimulated by noradrenergic impulses and inhibited by dopaminergic impulses. TSH is increased resulting in the positive regulation of NIS. Within 15–20 min, TSH stimulates the secretion of thyroid hormones and causes an increased iodide uptake by the thyroid and an increased Tg breakdown. To release thyroid hormones, thyroglobulin is engulfed by pinocytosis, digested in lysosomes, and then secreted into the bloodstream at the basolateral membrane. The iodined tyrosines are removed from the Tg by proteolitic enzymes, and T4 is released into circulation. Circulating T4 and T3 are bound to various iodine-binding transport proteins including albumin, TBG, and transthyretin. Once delivered to the relevant target tissues T4 is de-iodinated to the metabolically active form, T3. De-iodination requires selenocystein as the active form of selenium in the iodothyronine deiodinases. About one-third of iodine is present in the form of T3 and T4; the rest is present as MIT and DIT. Iodine consists of 65% and 59% of the weights of T4 and T3, respectively. Released MIT and DIT are de-iodinated by an iodotyrosine deiodinase providing an efficient intrathyroidal recycling of iodide. The turnover is slow: the half-life of T4 is about 5 days and for T3, 1.5–3 days. The released iodine is returned to the serum iodine pool where it can be taken up by the thyroid or excreted by the kidney. Other tissue sites for iodide uptake from plasma are the salivary glands, the choroid plexus, the mammary gland, the kidneys and the gastric mucosa. Within the range of usual intakes, the iodine flux in saliva is up to 10 $\mu g\,h^{-1}$, depending on the level of intake.

Elimination

Extrarenal clearance of iodide (range 3–40 ml of serum/min) in euthyroid subjects is assumed to represent thyroid clearance, corresponding to the capture of iodine by the thyroid gland, There is an inverse relationship between thyroid uptake and dietary iodine intake or urinary excretion. The percentage of capture by the thyroid can change within 12–14 weeks, and sometimes faster, in response to changes in iodine intake in euthyroid subjects. More than 90% of ingested iodine is ultimately excreted in the urine. Clearance of iodine by the kidneys remains relatively constant as a percentage of filtered iodide in plasma, depending on the glomerular filtration rate and physiologic rate. In adults with adequate iodine intake, excess iodine not taken up by the thyroid is excreted in urine, with partial reabsorption occurring in the renal tubules. Renal iodide clearance significantly decreases both in patients with impaired renal function or with myxedema. In comparison with habitual fluid intakes, an increase in fluid volume intake can lead to additional iodine losses: for an increase in urinary volume of one liter, there are additional iodine losses of around 15 $\mu g\,day^{-1}$.

Feces losses vary, but are generally low (10–30 $\mu g\,day^{-1}$). Small amounts are also lost via the skin. Compared with urinary

iodine (UI) concentrations, iodine concentrations in sweat appeared to be relatively constant at around 35–40 $\mu g\,l^{-1}$ over a large range of iodine intakes (UI from 8 to 280 $\mu g\,l^{-1}$), and in various climatic conditions, meaning that, in contrast to urinary excretion, sweat excretion is less adaptive. There is evidence that the mean correlation coefficient (r) between iodine elimination for blood/saliva was 0.99; for blood/urine, 0.95; and for saliva/urine, 0.97. The absolute value of iodine concentrations in urine revealed marked variability, which was corrected by adjusting for creatinine levels. Biliary conjunction of T4 (enterohepatic circulation) may occur with concomitant fecal elimination almost entirely as conjugate thyroid hormone. The expression of the NIS is positively regulated in the lactating mammary gland, and the uptake of iodine and secretion in breast milk, with the concentration in milk being 20–50 times higher than in plasma. More than 80% of iodine in breast milk is in the form of inorganic iodide, whereas T4 is $<2\,\mu g\,l^{-1}$, and T3 is $<0.05\,\mu g\,l^{-1}$. The iodine concentration of breast milk varies between 10 and 300 $\mu g\,l^{-1}$ (interindividual variability from 25–87%). Assuming an average dietary reference value (DRV) for iodine concentration in breast milk was 75 $\mu g\,l^{-1}$ and with average breast milk secretion of 0.8 $l\,day^{-1}$, calculated mean iodine loss was of 60 $\mu g\,day^{-1}$. Maternal T4 crosses the placenta before the onset of fetal thyroid function at 10–12 weeks and represents up to 20–40% of T4 measured in cord blood at birth. In colostrum, the iodine concentration ranges from 200 to 400 $\mu g\,l^{-1}$.

Storage

The thyroid gland accumulates iodine depending on the body's iodine and thyroid hormone status. The body of a healthy adult contains approximately 10–20 mg of iodine, of which 70–80% is in the thyroid, which is on average 8–15 mg of iodine. The main function of the thyroid gland is to store iodide and make it available for the biosynthesis of thyroid hormones. In chronic iodine deficiency, the iodine content of the thyroid might drop to less than 20 μg. In iodine-sufficient areas, the adult thyroid traps about 60 μg of iodine per day to balance losses and maintain the synthesis of thyroid hormones. The iodide cycle consists of a series of transport, oxidation, and coupling steps in thyroid follicular cells to produce thyroid hormones. NIS transports iodide into the thyrocyte at a concentration gradient 20–50 times that of plasma. TSH regulates NIS transcription and biosynthesis but also mediates NIS activity by posttranscriptional mechanisms. In the presence of TSH, NIS is active and inserted in the basolateral membrane of thyrocytes. Upon TSH withdrawal, NIS protein's half-life decreases from 5 to 3 days, and it translocates from the plasma membrane to intracellular compartments. Iodide is another factor that can regulate its own accumulation in the thyroid. It has been shown that high doses of iodide block iodide organification in a rat's thyroid; this phenomenon, known as the acute *Wolff-Chaikoff effect*, is a reversible process, because iodide organification resumes when the iodide concentration in the serum decreases. The mechanisms underlying the *Wolff-Chaikoff effect* are complex and involve acute regulation of several key genes and proteins within the thyrocytes. In the lactating mammary gland, NIS plays an important role by concentrating iodide in the milk, thereby supplying newborns with iodide for thyroid hormone syntheses. The thyroid iodine content at birth is around 100–300 μg and increases progressively to around 0.8–374 mg at 4–6 years of age. At the same time, the thyroid hormone requirements of a developing infant are at their highest per kilogram body weight compared with subsequent growth periods and is also at a time when adequate thyroid hormone is critical for ongoing neurodevelopment. Uptake and storage by the placenta are dependent on maternal iodine intake. The placenta has an iodine content of 30 $ng\,g^{-1}$ compared with the content of below 2 $ng\,g^{-1}$ for the myometrium. The concentration of iodine in amniotic fluid (around 15 $\mu g\,l^{-1}$) is weakly correlated with maternal UI excretion, and it can be independent of the ingestion of iodine supplements.

Iodine Metabolism During Pregnancy

Healthy women maintain iodine stores of 15–20 mg in the thyroid, and during pregnancy, the stored iodine can be used to help meet the approximate 50% increase in maternal iodine requirement. Iodine requirements increase during pregnancy due to a variety of physiological changes. Beginning in early gestation, maternal thyroid hormone production normally increases by approximately 50% in response to increased levels of serum TBG (resulting from the increase in estrogen levels) and because of the stimulation of TSH receptors by ß-human chorionic gonadotropin. For pregnant women, endogenous T4 production is estimated to be increased by a mean of 37 $\mu g\,day^{-1}$, corresponding to an additional iodine demand of 25 $\mu g\,day^{-1}$ for hormone synthesis in the thyroid. The glomerular filtration rate increases, resulting in increased maternal UI excretion. The glomerular filtration rate of iodide increases in early pregnancy by 30–50%, resulting in increased maternal UI excretion and decreasing the circulating pool of plasma iodine. The placenta is a rich source of the type 3 inner ring deiodinase, which enhances the degradation of T4 to biologically inactive T3. As human chorionic gonadotropin concentrations fall after week 10 of gestation, TSH concentration tends to rise. Thus, there is a normal physiological rise in TSH and a drop in FT4 from the beginning to the end of pregnancy that needs to be considered in assessing thyroid function. Fetal thyroid hormone production does not start until 14–16 weeks of pregnancy; before that time, the fetus is reliant on the transplacental passage of small amounts of maternal TSH. The additional requirements due to the development of the fetus, placenta, and amniotic fluid are equivalent to a net transfer of 1 μg iodine per day. Iodine needed for fetal synthesis of thyroid hormones leads to a total additional iodine intake rounded to 50 $\mu g\,day^{-1}$. Fetal thyroid hormone production increases during the second half of pregnancy, further contributing to increased maternal iodine requirements because iodide readily crosses the placenta. Thus, thyroid hormone demand increases, which requires an adequate iodine supply that is obtained primarily from the diet and/or as supplemental iodine.

Interactions with Other Nutrients

Selenium and iron deficiencies may impair iodine/thyroid metabolism. Deficiencies of iodine and selenium intake often show up together, with selenium status influencing the outcome of iodine deficiency. Glutathione peroxidase and the deiodinases, the selenium-dependent enzymes, are impaired in selenium deficiency. This leads to accumulation of peroxidases, which can damage the thyroid, whereas deiodinase deficiency impairs thyroid hormone synthesis. In anemic pregnant women, endemic goiter may provoke anemia. Iron deficiency anemia during pregnancy can result in both higher TSH and lower T4 concentrations. Chronic iodine deficiency favors subclinical hypothyroidism, and the risk of hypothyroidism is increased in anemic goiter patients. Iron deficiency reduces heme-dependent TPO activity in the thyroid, resulting in impaired production of thyroid hormone. Iron supplementation use with iodized salt affects both anemia and goiter in children. Also, intervention trials in iodine and iron-deficient populations have shown that the addition of both iron and iodine results in greater improvements in thyroid function and volume. Iron supplementation improves the efficacy of iodized oil and iodized salt. Also, deficiencies of vitamin A, and possibly zinc, may interact with iodine nutrition and status and thyroid function. Vitamin A supplementation given alone or in combination with iodized salt significantly improved thyroid function and thyroid size. Vitamin A deficiency can worsen goiter in populations with iodine deficiency, through decreased vitamin A-mediated suppression of the pituitary TSHβ gene and is not relevant in the European context (Asia and Africa). However, further research is needed to establish a conclusive relationship between iodine and other micronutrients.

Effect on Genotype

Iodine is responsible for gene expression and the synthesis of xenobiotic-metabolizing enzymes in the liver. Excess iodine intake also altered mRNA and protein levels of numerous thyroid genes involved in lipid metabolism and inflammatory activities. Alterations in the expression of these genes may contribute to the progression of iodine-induced autoimmune thyroid disease. Genotypes that predict differences in requirements have not yet been identified as important at the population level, although genotype may influence the risk of thyroid autoimmune disease in iodine repletion studies.

Dietary Sources and Intake Data

Iodine is found in food and water mainly as iodide. The main dietary sources of iodine are iodized salt, saltwater fish, seaweed, and grains (if grown in iodine-repleted soils). The native iodine content of most foods and beverages is low (Table 2). Most commonly consumed foods provide 3–80 μg of iodine per serving. Saltwater fish, shellfish, and seaweed are food sources with the highest natural iodine content. Because seaweeds are not a component part of the Western diet, ocean fish are the only good sources of iodine. Iodine content in fish and shellfish varies from less than 5 to more than 200 μg/100 g (haddock, cod fish, and sole contain > 140 μg/100 g, freshwater fish contain only 2 μg/100 g). The mean iodine content in commercial fish feed was reported to be 4.6 mg kg^{-1} and ranged between 1.2 and 10.5 mg kg^{-1}. One cup of low-fat plain yogurt and milk have 75 and 56 μg iodine, respectively (50 and 37% DRV). Fish sticks, 3 oz, have 54 μg iodine (36% DRV); tuna canned in oil, drained, 3 oz, 17 μg iodine (11% DRV); ice cream, chocolate, ½ cup, 30 μg iodine (20% DRV); large egg 24 μg iodine (16% DRV); apple juice, 1 cup 7 μg iodine (5% DRV); and medium banana 3% (2% DRV).

Table 2 Iodine content in food

Food type	*Content*[a]	*Food type*	*Content*
Marine fish	1456 μg kg^{-1}	Meat	68 μg kg^{-1}
Freshwater fish	103 μg kg^{-1}	Cereals	56 μg kg^{-1}
Nuts	89 μg kg^{-1}	Oils and fats	36 μg kg^{-1}
Leafy vegetables	89 μg kg^{-1}	Fresh fruits	31 μg kg^{-1}
Other vegetables	80 μg kg^{-1}	Bread	17 μg kg^{-1}
Dairy	84 μg kg^{-1}	Water	6.4 μg kg^{-1}

[a]Presented as geometric mean concentration.

Sources: Fordyce, F. M. (2003). Database of the iodine content of food and diets populated with data from published literature. Nottingham, UK, British Geological Survey Commissioned Report, CR/03/84 N. 50 pp.

Foods of marine origin have higher iodine content because of a high concentrated amount of iodine in seawater. Japanese diets in the coastal regions of Japan contain large amounts of seaweed, and they have remarkably high iodine intakes ranging from 50 to 80 mg day^{-1}. A bowl of common soup in the Japanese diet can contain 5 mg of iodide. Major dietary sources of iodine in the United States and Europe are dairy products and whole grains. Mean iodine content in milk in northern Europe is at the level of 15–20 μg/100 g. The iodine content in cow's milk depends on the amount of iodine given to the animal in mineral mixture, and iodophors (preparation containing iodine complexed with a surfactant or povidone resulting as a water-soluble material that releases free iodine when in solution) used for cleaning milk cans and teats in the dairy industry can increase the native iodine content of dairy products. Also, iodine content in milk shows seasonal variation, with the highest content during winter and the lowest content during summer. Fifty years ago, bread was a major source of dietary iodine, with a content as high as 150 μg/slice. Traditionally, iodate is used in bread making as a conditioner for dough, but today it is being replaced by non-iodine-containing conditioners. The decreased use of iodate bread conditioners is thought to have contributed to the reduction in dietary iodine levels.

Salt iodization is a term used to describe the iodization of salt for human consumption (food industry and household) and for livestock feed. In many countries, the use of iodized salt in households for cooking and at the table provides additional iodine. Boiling, baking, and canning of foods containing iodated salt causes only small losses (≤10%) of iodine content. Iodine can be added to salt as potassium iodide (KI), potassium iodate (KIO_3) or less frequently, sodium iodide. North America and some European countries use KI as a form for fortification, whereas most tropical countries use KIO_3, which

is more stable in the presence of salt impurities, humidity, and porous packaging. To meet the iodine requirements of a population, it is recommended to add 20–40 mg iodine/kg salt, depending on local salt intake. According to the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD), iodine fortification of salt has been implemented in 40 European countries and is mandatory in 13. The amount of iodine added varies from 10 to 75 mg kg^{-1} salt.

The iodine concentration in drinking water is highly variable within different regions. Denmark covers a relatively small geographic area; however, its iodine content in drinking water varies from 2 to 100 μg l^{-1}. In Germany, a variation from 0.2 to 15.5 μg l^{-1} (median 2.6 μg l^{-1}) is shown when traveling from the north to south.

Results of dietary studies show the following general order of percentage of daily iodine intake from the main food groups in Western countries: dairy (50%), cereals (20%), fish (9%), meat (8%), vegetables (7%), sweets (5%), and fruits (1%). On the basis of these reports, the average (geometric mean) iodine content of 494 foods is 87 μg kg^{-1} (Table 2). Without adventitious sources of iodine or a marine foods component, most diets would fail to provide the recommended daily intake of 150 μg day^{-1}. Based on data from the Third National Health and Nutrition Examination Survey, 12% of men and 15% of nonpregnant women took a supplement that contained iodine, and the median intake of iodine from supplements was approximately 140 g day^{-1} for adults. Supplements vary in iodine content and form and may be available as KI tablets, prenatal multivitamin preparations, or iodized oil. Iodine readily binds to the double bonds in fatty acids, allowing the preparation of iodized oils that are used for supplementation outside the EU. Iodized oil, intramuscular, or oral administration, provides significant and prolonged iodine supplementation without inducing any clinical or laboratory adverse effects. Supplements with iodized oil give effective prophylaxis in iodine-deficient subjects. The intramuscular route has a longer duration of action than does oral administration. Other sources of iodine include water purification tablets, radiographic contrast media, and medicine (e.g., a 200 mg tablet of amiodarone, an antiarrhythmic drug, contains 75 mg), and skin iodine-based disinfectants are commonly used in clinical settings. (e.g., povidone iodine contains approximately 10 mg ml^{-1}).

Assessment of Iodine Status

To date, expert bodies have used physiological or mechanistic (balance and turnover) iodine studies to derive DRVs for iodine. Clearly, the shape of the intake–status relationship has an important bearing on the predicted adequate nutrient requirement estimates using iodine status biomarkers. In populations, median UI concentrations of 100–200 μg l^{-1} indicate adequate iodine intake and optimal iodine nutrition (Table 3). As an indicator of iodine intake, median UI concentration does not provide direct information about thyroid function and longer-term iodine exposure.

In a systematic review, the available evidence, with regard to the usefulness of iodine status biomarkers, was assessed. UI, serum Tg, serum T4, and TSH were found to be useful biomarkers of iodine status depending on age and population group and their iodine status. The model of dose–response quantification used to describe the relationship between iodine intake and biomarkers of iodine status indicated that the doubling of dietary iodine intake increased UI concentrations by 14% in children and adolescents, 57% in adults and the elderly, and 81% in pregnant women. The dose–response relationship between iodine intake and biomarkers of iodine status indicated a 12% decrease in thyroid-stimulating hormone and a 31% decrease in thyroglobulin in pregnant women. Serum TSH and Tg concentrations primarily indicate thyroid function, which is largely dependent on iodine intake, as a means of detecting functional iodine deficiency.

Serum Tg is easier to assess than thyroid volume measurement, and is a useful biomarker of iodine status in children and adolescents. Methods for Tg determination on dried blood spots have been developed. Serum Tg represents a very sensitive index of thyroid hyperstimulation and reflects iodine deficiency. In iodine adequacy, the concentration of Tg is below 10 μg l^{-1}.

Serum TSH is a suitable biomarker of iodine status in neonates and possibly in pregnant and lactating women. Measurements can be performed on dried blood spots on paper. The normal serum TSH range is 0.1–5 mIU l^{-1}. An important caveat is that

Table 3 Assessment of iodine nutrition in a population based on UI concentrations

UI concentration (cutoff values in μg l^{-1})	*Iodine intake*	*Iodine nutrition*
Children <2 years of age		
<100	Insufficient	
>100	Adequate	
School-aged children		
<20	Insufficient	Severe ID
20–49	Insufficient	Moderate deficiency
50–99	Insufficient	Mild deficiency
100–199	Adequate	Optimum
200–299	More than adequate	Risk of iodine-induced hypothyroidism
>300	Excessive	Risk of adverse health
Pregnant women		
<150	Insufficient	
150–249	Adequate	
250–499	More than adequate	
>500	Excessive	
Lactating women		
<100	Insufficient	
>100	Adequate	

Sources: World Health Organization/International Council for the Control of the Iodine Deficiency Disorders/United Nations Children's Fund (WHO/ICCIDD/UNICEF). Assessment of the iodine deficiency disorders and monitoring their elimination. WHO/NHD/01.1. Geneva: World Health Organization, 2001; Andersson M, de Benoist B, Delange F, Zupan J. Prevention and control of iodine deficiency in pregnant and lactating women and in children less than 2 years old: conclusions and recommendations of the Technical Consultation. WHO Secretariat. Public Health Nutr 2007; 10: 1606–11.

newborn testing must be performed >48 h after birth to be valid because there is a normal physiological surge in infant TSH concentrations after birth. Serum concentrations of T4 and T3 are generally less sensitive for estimating iodine status. The normal serum concentrations are around 100 nmol l^{-1} (80 μg l^{-1}) for total T4 and around 1.8 nmol l^{-1} 569 (1.2 μg l^{-1}) for total T3; concentrations of free T4 forms are in the ranges of 11–18 pmol l^{-1} and free T3 3.5–570 pmol l^{-1}, respectively.

Goiter prevalence and thyroid volume are useful long-term clinical indicators of iodine status. Thyroid size can be determined by the palpitation method, expressed in percentages of the different stages at population level and by thyroid ultrasonography with the thyroid volume given as ml. Thyroid volume is best determined from ultrasound measurements rather than by palpation, which is less sensitive in the case of mild or moderate iodine deficiency. In an adult population, thyroid enlargements, defined as a thyroid volume >18 ml for adult women and >25 ml in adult men, are generally considered to represent goiter.

Dietary Requirements

Optimal dietary iodine intake is between 100 and 200 μg day^{-1}. With respect to adequate iodine intake, the cutoff values are set at the levels that should be optimal for general health and the prevention of deficiency. Alternatively, other endpoints used to assess adequacy are adequate iodine concentration in the thyroid gland and iodine turnover (the Nordic countries, the DACH-countries, WHO/FAO, 2002), or more generally, a proper thyroid function (Belgium, the UK, WHO/FAO, and EC) and iodine balance (EC). For setting recommendations, there are two parts of the factorial approach: the calculation of basic requirements (needs for growth, maintenance, losses etc.) and the application of a bioavailability factor. For iodine, bioavailability can be defined as the combination of absorption of dietary iodine and of thyroid uptake and the utilization of iodine. It is based on the definition that bioavailability should be sufficiently broad to cover changes in absorption or/and excretion attributable to physiologic regulatory mechanisms that maintain and restore optimal homeostasis.

Iodine intake is difficult to determine by dietary assessment because the iodine content of foods is variable, and reliable values are not always available in food composition tables. There are only a few food composition databases containing information on iodine-rich foods. Food composition databases generally contain information on the salt content of foods, but they rarely specify if the salt used in processed foods is iodized or not. Dietary assessment methods FFQ, food diaries, or 24-h food intake recall are not reliable quantitative methods for assessing the total iodine intake. The lack of accuracy in measuring iodine intake from iodized salt is thus a major limitation of dietary assessment. Dietary data can be used to identify the most important food sources of iodine but do not accurately quantify the usual iodine intake, The FFQ method captures iodine-rich sources that are irregularly consumed and day-to-day variation in the overall consumption patterns such as frequency and portion sizes of iodine-containing foods. Food diaries or 24-h recalls measure short-term intakes, and often underestimate salt consumption.

Adequate dietary iodine intake is essential for the production of thyroid hormones. The recommended daily allowances for iodine intake vary by age. A prevalence of goiter below 5% in school-age children was almost systematically observed in all the areas of the study for a UI concentration above 100 μg l^{-1} (corresponds to an approximate iodine intake of 150 μg day^{-1} in adults). The European Commission, the EFSA Panel for iodine DRV concluded that an adequate intake (AI) for iodine for adult men and women can be set at 150 μg day^{-1} (Table 3). For infants ages 7–11 months and for children and taking into account age-specific urinary volumes and body weights, AIs of 70 μg day^{-1} to 130 μg day^{-1} were derived. According to the EPSA panel the same AI is proposed for lactating as for pregnant women, that is, 200 μg day^{-1}.

The WHO established recommended nutrient intakes (RNIs) that cover the needs of nearly all healthy individuals for specific life-stage groups. WHO has set criteria for the assessment of school-age children and pregnant and lactating women based on UI concentrations. WHO/ UNICEF/ ICCIDD recommend daily iodine intakes of 90 μg day^{-1} for preschool children and 150 μg day^{-1} for adults, reaching 250 μg day^{-1} for pregnant/lactating women (Table 4). For the United States

Table 4 Recommendations for iodine intake

Age	*EFSA (μg day^{-1})*	*Age*	*U.S. Institute of Medicine (μg day^{-1})*	*Age*	*WHO (μg day^{-1})*
7–11 months	70	0–12 months	110–130		
1–3 years	90	1–8 years	90	0–5 years	90
4–6 years	90	9–13 years	120	6–12 years	120
7–10 years	90		150		
11–14 years	120				
15–17 years	130	>14 years + adults	150	>12 years + adults	150
≥18 years	150				
Pregnancy	200	Pregnancy	220	Pregnancy	250
Lactation	200	Lactation	290	Lactation	250

Sources: EFSA NDA Panel (EFSA Panel on Dietetic Products Nutrition and Allergies), (2014). Scientific Opinion on Dietary Reference Values for iodine. *EFSA Journal* 12 (5), 3660, 57 pp. doi:10.2903/j.efsa.2014.3660 www.efsa.europa.eu/efsajournal; World Health Organization/International Council for the Control of the Iodine Deficiency Disorders/United Nations Children's Fund (WHO/ICCIDD/UNICEF) (2001). Assessment of the iodine deficiency disorders and monitoring their elimination. WHO/NHD/01.1. Geneva: World Health Organization; Institute of Medicine, Academy of Sciences (2001). Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington: National Academy Press.

Table 5 Tolerable upper intake levels for iodine

Age group	*European Commission/ Scientific Committee on Food*	*U.S. Institute of Medicine*
1–3 years	200	200
4–6 years	250	300
7–10 years	250	600
11–14 years	300	–
15–17 years	450	900
Adult	500	1100
Pregnant and lactating women	600	1100

Sources: Scientific Committee on Food; Health and Consumer Protection Directorate-General (2002). Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Iodine. SCF/CS/NUT/UPPLEV/26 Final. Brussels: European Commission; Institute of Medicine; Academy of Sciences (2001). Dietary Reference Intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington: National Academy Press.

and Canada, the IOM established dietary reference intakes for iodine, and specifically an AI for infants and an estimated average requirement and recommended dietary allowance for children, adolescents, and adults. The following simplified equation to calculate daily iodine intake has been proposed by the IOM: UI ($\mu g\ l^{-1}$) $\times$ 0.0235 $\times$ body weight (kg). European and United States expert committees have recommended tolerable upper intake levels for iodine (**Table 5**). The tolerable level for iodine intake is set at 1.2 mg day^{-1} for adults.

See also: Adolescent Nutrition; Codex Alimentarius Commission: Role in International Food Standards Setting; Dietary Exposure Assessment; Dietary References: US; Food Fortification: Rationale and Methods; Food Fraud; Infants: Nutritional Requirements; Iodine: Iodine Deficiency Disorders (IDD); Nutritional Epidemiology; Pregnancy: Dietary Guidance for Pregnancy; Pregnancy: Metabolic Adaptations and Nutritional Requirements; World Health Organization.

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Iron: Biosynthesis and Significance of Heme

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Importance and Functions of Heme in Metabolism

Heme or iron protoporphyrin IX is a unique and ubiquitous tetrapyrrole that has various and versatile functions in living organisms. This heterocyclic macromolecule consists of four pyrrole rings, which are joined together by methene bridges. Furthermore, the pyrrole rings are coordinated axially by four nitrogen atoms to ferrous ion (Fe^{2+}) in heme or ferric ion (Fe^{3+}) in hemin. Moreover, the central Fe ion can exchange or transfer electrons as it exists in more than one oxidation state and can also axially coordinate one or two additional ligands to histidine, cysteine, or methionine (sometimes tyrosine or lysine) of proteins. The tetrapyrrole ring structure has eight side chains that interact with coordinating proteins – 4 methyl, two vinyl, and two propionate (see Figure 1). There are two common ligation motifs, namely, a 5-coordinate monohistidine (e.g., in hemoglobin or myoglobin) and a 6-coordinate bis-histidine as in cytochrome *c*. Heme is covalently ligated to holoproteins by heme lyase. Some heme-binding proteins have the unique sequence motif CXXCH (C: cysteine, X: any amino acid, and H: histidine) in which the two cysteine units are linked to protoporphyrin and the histidine is coordinated to Fe. Heme can also be reversibly attached to gases such as oxygen, nitric oxide (NO), and carbon monoxide (CO). Consequently, the structural components of heme enable a repertoire of interactions, coordination and binding with diverse molecules to exert a broad array of metabolic reactions, and physiological functions that sustain life. Moreover, complexities in this diverse and divergent array of functions are induced by chemical modifications and substitution of side chains of the pyrrole rings, hydrophobic interactions, and electrostatic interaction such as salt bridges with numerous hemeproteins. Consequently, the binding versatilities of heme favor conformational variations in protein scaffolds and architecture that culminate in vastly disparate functional capabilities. There are five types of heme ligands (a, b, c, d, and d_1), in general, based on positions of and differences among side chains and the types of bonds to various apoproteins. Heme b (Figure 1) bears the basic structure from which other types are formed. The heme variants function as cofactors or prosthetic group of hemeproteins apart from serving as effector molecule involved in signal transduction, cell growth and differentiation, drug and steroid metabolism, circadian rhythm, microRNA processing, and gene regulation among others. A summary of examples of functions of hemeproteins are listed in the succeeding text.

Functions of Heme in Systemic Metabolism

Heme as cofactor or prosthetic group of hemeproteins:

- Archetypal and quintessential functions of hemeproteins arise from reversible binding of small molecules and conformational changes that facilitate cellular transport of gaseous molecules for exchange or storage and gaseous ligand binding that senses diatomic gases, for example, hemoglobin (Hb), myoglobin, nitrite reductase, and NO regulator.
- Cytochromes are heme proteins that are involved in electron transfer reactions based on reversible oxidation–reduction of heme Fe in the mitochondria. For example, cytochrome reductase catalyzes electron transfer from ubiquinol to cytochrome *c* and is also involved in proton pumping across mitochondrial membrane. Cytochrome *c* complex catalyzes electron transfer from reduced cytochrome *c* to molecular oxygen on the mitochondria inner membranes.
- Some other hemeproteins such as cytochrome P450, peroxidases, NO synthase, nitrite reductase, and soluble guanylate cyclase are involved in both electron transfer and catalytic functions. Cytochrome P450 monooxygenase enzymes are involved in phase I drug metabolism and xenobiotic reactions. They catalyze such reactions as deamination, hydroxylation, and epoxidation among others to catabolize hydrophobic toxicants into polar forms for excretion and detoxification.
- Cystathionine β-synthase (CBS) is a unique heme-dependent enzyme that catalyzes the condensation of serine and homocysteine into cystathionine using pyridoxal 5-phosphate as a cofactor. Deficiency of CBS results in homocystinuria, an inherited disease that is characterized by high levels of toxic homocysteine in the serum. Enzymatic activity of CBS is influenced by the reduction or replacement of Fe by CO in the noncovalently bond heme ligand.

Heme as a regulator of transcription:

- Heme is a transcriptional regulator of the mammalian repressor BtbAndCnc homology 1 (Bach1), a basic leucine zipper protein. When intracellular heme level is low, genes containing the Maf recognition element (MARE) sequence in the promoter region, for example, globin, erythroid δ-(aminolevulinic acid) ALAS, and HO-1, are bound by Bach1–Maf dimer and are transcriptionally repressed in the nucleus. However, when heme levels are high, heme binds to the heme response motif (HRM) on Bach1 causing dissociation of Bach1-Maf hererodimer from MARE. A leader protein, exportin 1 (Crm1), binds Bach1 and facilitates its export from the nucleus into the cytoplasm for subsequent degradation and ubiquitination and degradation by HOIL-1. Heme nullifies the repressor function of Bach1. Dissociation of Bach1 from MARE DNA sequence, its nuclear translocation, and its cytoplasmic degradation all require heme binding to HRMs to enhance the transcription of genes such as erythroid δ-ALAS and HO-1.
- Heme stabilizes the nuclear factor erythroid-2-[NF-E2]-related factor 2 (Nrf2) that binds small MAF proteins.

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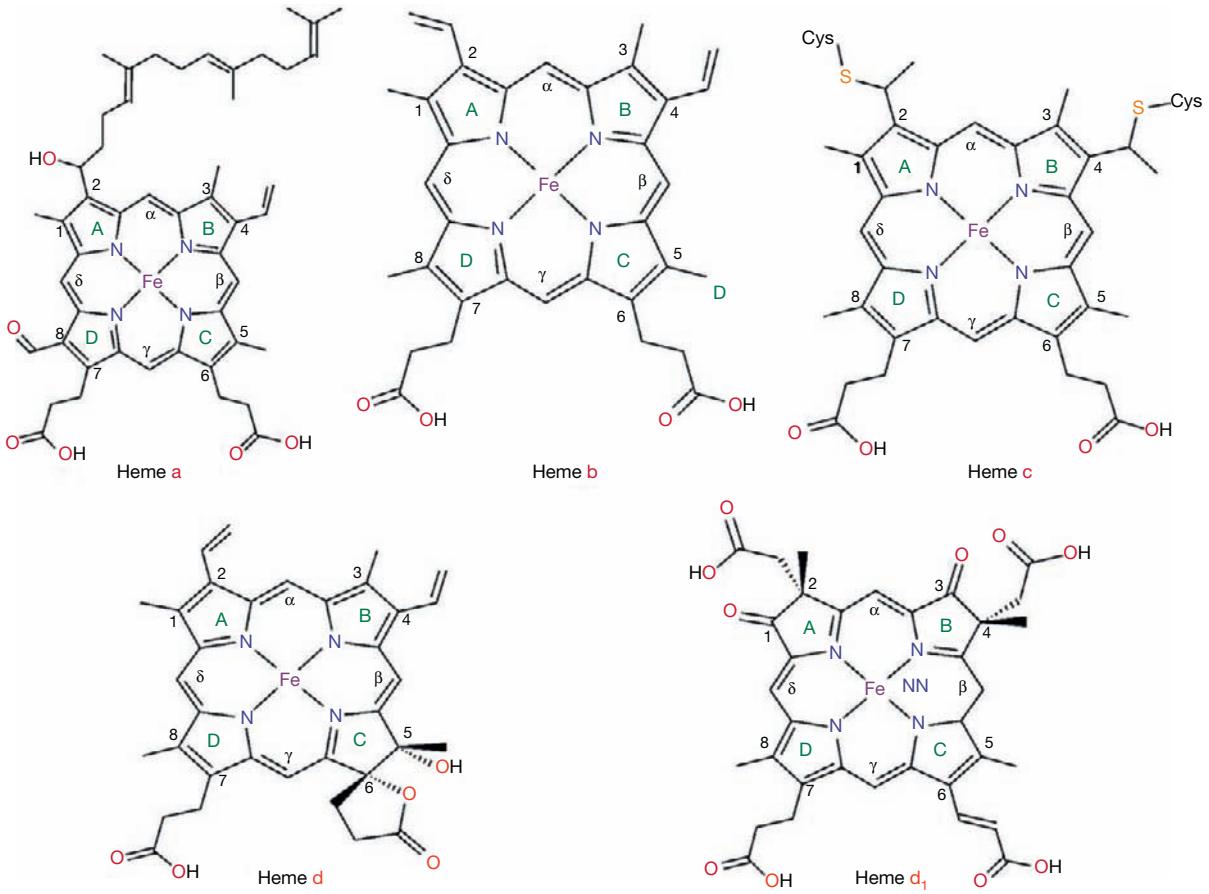

Figure 1 Structure of naturally occurring hemes a, b, c, d, and d1. http://metallo.scripps.edu/PROMISE/HAEMMAIN.html.

Unlike Bach1, Nrf2–Maf dimers activate MARE-containing genes. When heme level is low, Nrf2 binds to Kelch-like ECH-associated protein 1 (KEAP1) in the cytoplasm. However, when heme levels are high, heme binds to KEAP1, freeing Nrf2, and Nrf2–Maf binds to the promoter region to activate gene transcription via antioxidants response element-mediated gene expression, for example, antioxidant genes.

Heme regulates translation:

- Heme regulates translation through a heme-regulated elF2α kinase inhibitor (HRI). Translation initiation factor elF2α is phosphorylated by the kinase to inhibit protein synthesis when heme level is low. When heme levels are high, however, heme binds to the HRI kinase to inhibit phosphorylation, thereby suppressing its activity. Consequently, elF2α initiates translation of α and β globin chains. HRI senses heme levels to coordinate and regulate heme and globin synthesis during erythropoiesis.

Heme regulates cell cycle progression:

- Heme may modulate cell cycle indirectly through the activity of zinc protoporphyrin (ZnPP) when iron is deficient. Zn is incorporated into protoporphyrin IX during iron deficiency. ZnPP subsequently inactivates transcription factors such as Egr1 and Sp1 to inhibit cell cycle progression. Hemin is a regulator of the growth and differentiation of erythroid and nonerythroid cells.

Heme in microRNA processing:

- DiGeorge syndrome critical region gene 8 (DGCR8), an RNA-binding protein, forms a heterodimer with the nuclease, RNase Drosha, and the complex recognizes and cleaves primary transcripts of microRNAs (pri-miRNAs) in the processing of mammalian microRNAs (miRNAs). Heme binds to DGCR8 to regulate its activity in the processing and maturation of pri-miRNA to microRNA. The heme-binding motif on DGCR8, IPCL (isoleucine, proline, cysteine, and leucine), shares a close similarity with HRM.

Heme in circadian transcriptional regulation of genes:

- Heme plays a pivotal role in circadian-coupled transcriptional regulation of genes. Evidence suggests the regulation of circadian-linked transcription by availability of intracellular heme and heme-based sensing of CO and possibly NO. Transcription of several circadian genes is activated by binding of the NPAS2-Bmal1 or clock-Bmal1 heterodimer to the promoter region. These genes can be regulated by a feedback mechanism where the gene products bind to NPAS2-Bmal1 or clock-Bmal1 and inhibit binding to DNA. Furthermore, binding of heme and CO to NPAS2 or clock induces dissociation of the heterodimer, thereby switching off

transcription of circadian genes. In addition, NPAS2-Bmal1 or clock-Bmal1 transcription is also regulated in another heme-dependent mode. Rev-ErbA, a nuclear hormone receptor, binds to heme and forms a heterodimer with nuclear receptor (NR) corepressor (NCoR) in partnership to repress transcription of circadian genes. It cannot inhibit transcription in the absence of heme.

Heme Biosynthesis

Heme may be synthesized *de novo* in all human cells as required, but the major sites of biosynthesis are in the bone marrow by erythrocytes for incorporation into hemoglobin and the liver in humans. Most of the heme produced in the liver is for the synthesis of cytochrome P450 for the detoxification of xenobiotics. Heme biosynthesis in erythroid cells in humans occurs in eight sequential enzymatic reactions (Shemin pathway) of which the first and the last three steps occur in the mitochondria, while the intermediate steps occur in the cytosol (Figure 2).

Formation of the Pyrrole

The first step in pyrrole biosynthesis is the condensation of glycine with succinyl-CoA in the mitochondria to produce δ-ALA by the catalytic action of δ-aminolevulinic acid synthase (ALAS2). This is the rate-limiting step, which is regulated by the availability of iron and heme. ALAS1 is the ubiquitous housekeeping, nonerythroid isoform that is expressed in most tissues and, in particular, in the liver. ALAS1 is regulated by heme, and this could involve a number of transcriptional factors, ubiquitination of the protein, energy metabolism transcription coactivators, nuclear receptors, chemicals, and drugs. ALAS1 regulation is therefore a therapeutic target for the treatment of porphyrias. Solute carrier family 25, SLC25A38, and ABCB10 have been shown to be transporters of ALA from the mitochondria to the cytosol. The second step involves the condensation of two molecules of ALA by a zinc-dependent ALA dehydratase (ALAD) enzyme in the presence of pyridoxal phosphate to form porphobilinogen (PBG). This step and the subsequent four enzymatic reactions take place in the cytosol. ALAD is inhibited by lead (Pb).

Formation of Tetrapyrrole

Four molecules of PBG are condensed (oligomerization) in a head-to-tail fashion to form a linear tetrapyrrole hydroxymethylbilane (HMB), in a reaction catalyzed by PBG deaminase (PBGD, also called HMB synthase). Next, HMB undergoes intramolecular rearrangement and ring closure to form a tetrapyrrole uroporphyrinogen III (URO III) molecule, with constituent eight carboxylic (four acetyl and four propionyl) side chains. This reaction is catalyzed by URO III synthase.

Modification of Carboxylic Side Chains

Four acetyl groups on URO III are subsequently decarboxylated to methyl groups by URO decarboxylase (UROD) to form coproporphyrinogen III (Copro III), which is transported

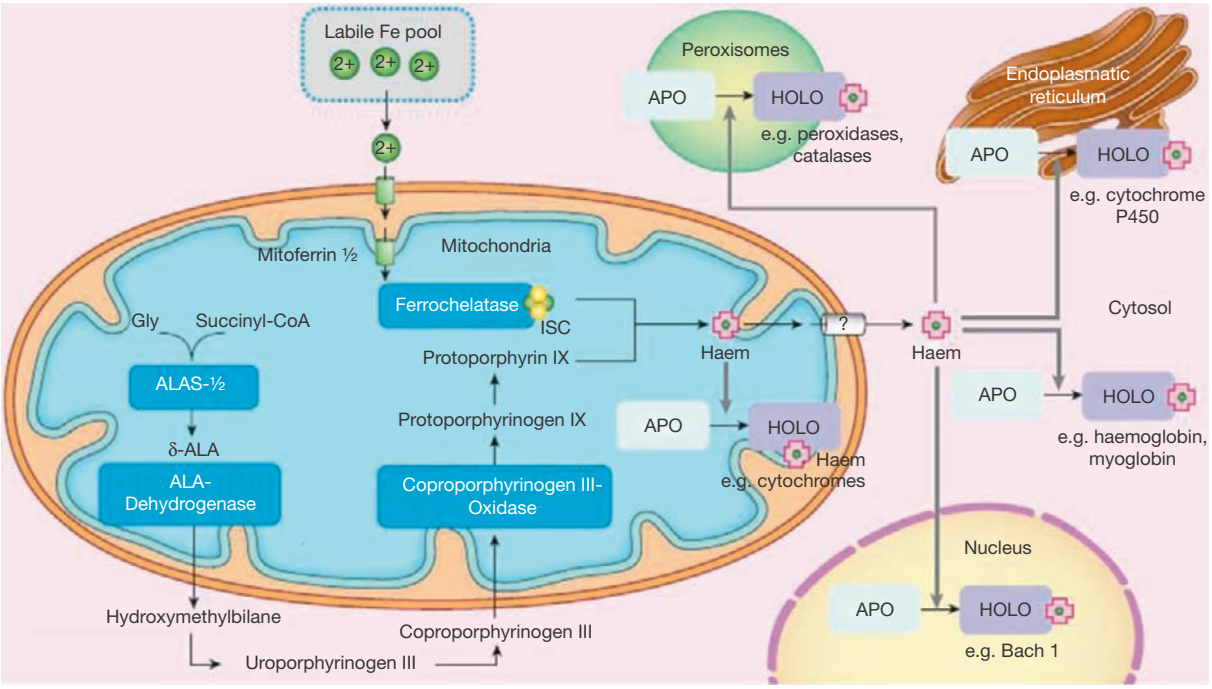

Figure 2 Heme biosynthesis. The rate-limiting reaction of heme biosynthesis is the generation of delta-aminolevulinate (d-ALA) from succinyl-CoA and glycine, assisted by d-ALA-synthase (ALAS). Synthesis of the porphyrin ring is then achieved in a multistep process, taking place in both the mitochondria and cytosol. Iron is loaded onto protoporphyrin IX by the iron–sulfur cluster-containing enzyme ferrochelatase. Heme is then transported to apoproteins by yet unknown pathways. Reproduced with permission from Evstatiev, R. and Gasche, C. (2012). Iron sensing and signalling. *Gut* **61**(6), 933–952.

into the mitochondria and further decarboxylated and oxidized by coproporphyrinogen oxidase to produce protoporphyrin IX in the mitochondria in an oxidative decarboxylation step. In this reaction, two propionate groups are converted into vinyl groups and the methylene bridges of the pyrrole rings become methane. The putative transporter of Copro III from the cytosol into the mitochondria is ABCB6. Protoporphyrin oxidase catalyzes the enzymatic oxidation of methylene to methane bridges in protoporphyrin IX.

Insertion of Iron into Protoporphyrin IX

The final step in heme biosynthesis is the insertion of ferrous ion Fe^{2+} by ferrochelatase (FECH), also known as proheme ferrolyase, into the center of protoporphyrin IX to form heme in the mitochondria. Heme is presumed to be exported out of the mitochondria by feline leukemia virus subgroup 1b, (FLVCR1b), catalysis by FECH is also a rate-limiting enzyme and it is transcriptionally regulated by GATA factors, Sp1 and NF-E2. In general, the posttranscriptional regulation of heme synthesis in erythroid cells is by iron–sulfur clusters (ALAD2 and FECH), whereas heme regulates biosynthesis in nonerythroid tissues instead. Iron is transported by FECH into the mitochondria by mitoferrin1 (MFRN1) and mitoferrin2 (MFRN2), respectively, in erythroid and nonerythroid tissues. Alternate splice variants of the enzymes ALAD, PBGD, and UROS are employed in reactions in heme biosynthesis in nonerythroid tissues. ALAS exist in two forms, ALAS1 and ALAS2, coded for by distinct genes, ALAS1 on chromosome 3 and ALAS2 on the X chromosome. While ALAS1 is regulated by heme, ALAS2 levels are modulated by erythroid-specific cis-acting transcriptional factor GATA-1 and by iron translationally via iron-response element and iron-response protein (IRE-IRP) regulatory system. Mutations or defects in ALAS2 and the subsequent seven enzymes in heme biosynthetic pathway cause chromosome X-linked sideroblastic anemia (acquired or inherited) and porphyrias, respectively (Table 1). It is of major significance that some key intermediates of the heme biosynthetic pathway, namely, delta-ALA (5-ALA), and its esters have clinical application in photodynamic therapy of cancer malignancies. Delta-ALA is administered to induce the production of protoporphyrin IX (PpIX), a potent photosensitizer, which when activated by light and oxygen produces reactive oxygen species (ROS) that destroy tumor cells. Photodynamic therapy is manipulated for low ROS toxicity, tissue selectivity, and specificity.

Erythropoiesis

A significant amount of heme is synthesized by normoblasts (also known as erythroblasts) in the bone marrow during erythropoiesis. In mammals, erythropoiesis also takes place in the mesoderm of the yolk sac of the early fetus and in the liver of midterm fetuses. Extramedullary erythropoiesis may take place in the spleen. Pluripotent stem cells generate hematopoietic progenitors that differentiate along various lineages. In the erythroid lineage, the detectable precursor is called burst-forming unit-erythroid (BFU-E). BFU-E cells proliferate and differentiate to form colony-forming unit-erythroid (CFU-E), the progenies of which divide and progress through proerythroblasts, basophilic erythroblasts, polychromatic erythroblasts, orthochromatic erythroblasts, and finally reticulocytes that are released into the circulation. These stages are accompanied by chromatin condensation, increasing hemoglobinization, enucleation and culminates in erythrocytes, which are devoid of organelles. Iron required for erythropoiesis is delivered by transferrin receptor 1 (TfR1) in an endocytic cycle comprising DMT1 (a six-transmembrane epithelial antigen of the prostate 3 (STEAP3)) and SEC15 (an exocyst complex component 6). Mice lacking TfR1 die *in utero*, while the haploinsufficiency of this receptor or of any other component of the endocytic cycle causes microcytic anemia. Conversely, excess iron is effluxed from erythroblasts by ferroportin 1b, an isoform that lacks the 5′ IRE, while excess heme is extruded principally by feline leukemia virus subgroup C receptor (FLVCR1). Thus, FLVCR1 is therefore involved in the heme detoxification process during erythroid differentiation and erythrophagocytosis. Excess heme that is not effluxed in mutant FLCVR mice causes cellular apoptosis, maturation arrest, and macrocytic anemia. The iron requirement for erythropoiesis is from dietary absorption of the nutrient, even though significant amounts of iron are available from the reticuloendothelial recirculation by macrophages of degraded erythrocytes principally in the spleen and, to some extent, in the liver. This iron source

Table 1 Disorders associated with heme biosynthetic pathway

Diseases	*Gene mutation*	*Features/symptoms*
Sideroblastic anemias		
X-linked sideroblastic anemia (XLSA)	ALAS-2	Ringed sideroblasts and mitochondria iron accumulation, microcytic anemia
X-linked sideroblastic anemia with ataxia (XLSA/A)	ABCG7	Spinal ataxia and cerebrum hypoplasia, mild microcytic anemia
Glutaredoxin 5 (GLRX5)-related sideroblastic anemia	Glutaredoxin	Hypochromic, microcytic anemia, and iron overload
Erythropoietic porphyrias		
Congenital erythropoietic porphyria (CEP)	UROS	Photosensitivity, hemolytic anemia
Erythropoietic protoporphyria (EPP)	FECH	Photosensitivity, microcytic anemia, and liver malfunction
Hepatic porphyrias		
ALA dehydratase deficiency porphyria (ALAP)	ALAD	Acute neurological disorder
Acute intermittent porphyria (AIP)	HMBS	Acute neurological disorder
Hereditary coproporphyria (HCP)	CPOX	Photosensitivity, acute neurological disorder, abdominal pain
Variegate porphyria (VP)	PPOX	Photosensitivity, neurological disorder, abdominal pain
Porphyria cutanea tarda (PCT)	UROD	Photosensitivity, skin lesions, and liver malfunction

contributes significantly to the maintenance of plasma iron homeostasis and is regulated by hepcidin, the liver-expressed peptide hormone that controls iron efflux functionality of ferroportin. Hepcidin binds ferroportin to cause internalization, ubiquitination, and degradation of the protein to inhibit iron efflux from macrophages, enterocytes, and hepatocytes. In the event of stress enhanced erythropoiesis, however, erythroferrone represses hepcidin expression to promote enhanced iron absorption and release from stores by ferroportin activity. Iron deficiency due to poor diets, dysfunctional absorption machinery, or sequestration in tissues could inhibit heme biosynthesis and result in anemia. Iron deficiency anemia is a public health disorder affecting vulnerable population groups globally. It is often associated with poor dietary iron and low bioavailability of iron from foods. Anemias are characterized by increased gastrointestinal iron absorption and enhanced iron release into the circulation from macrophages and hepatocytes due to low levels of hepcidin expression. Other forms of anemia include hemolytic (increased RBC destruction), pernicious (vitamin B12 deficiency), and aplastic (bone marrow stem cell malfunction). Sickle cell anemia, congenital dyserythropoietic anemia, polycythemia vera, and thalassemias (α and β) are examples of anemia and blood disorders arising from specific gene defects.

Heme Degradation

The RBC reaches senescence after a 120 day life span to become engulfed at extravascular sites of the reticuloendothelial system particularly in the spleen. Hb within the RBC is consequently metabolized to globin and the four heme groups incorporated into 2α and 2β globin chains. Heme is catabolized into one molecule of iron, bilirubin IXα, and CO by sequential actions of a NADPH-cytochrome P450 reductase (CR), heme oxygenase (HO), and biliverdin reductase (BR). The initial reaction is an electrophilic attack on the ferrous ion by CR to form a ferric–heme complex in the presence oxygen and NADPH. Next, HO catalyzes the specific cleavage of the α-methene bridge of the porphyrin ring to yield iron, biliverdin IXα, and CO. Three isozymes of HO, namely, HO-1, HO-2, and HO-3 are known, of which the inducible HO-1 plays the major function of heme degradation. HO-1 is inducible by heme, heavy metals, hypoxia, ischemia perfusion, and oxidative stress factors among other influences. Tissue-specific regulation of HO-1 and its general regulation have wider implications in various metabolic disorders. Consequently, a new paradigm has emerged to describe the induction of HO-1 in response to oxidative and inflammatory stimulus as being protective against tissue damage. These stimuli modulate HO-1 gene expression through transcription factors such as AP1, Bach1, and Nrf2. Biliverdin IXα is reduced to the major bile pigment, bilirubin IXα, by BR and, once released into the circulation, binds to serum albumin and is cleared by the liver. Bilirubin, before it enters the biliary circulation, is rendered water-soluble by conjugation with glucuronic acid in a reaction catalyzed by the bilirubin UDP-glucuronyl transferase. First, heme degradation by HO-1 therefore permits the reuse of iron from heme, as this porphyrin molecule is not recyclable due to its potential toxicity in the circulation. Second, degradation eliminates equally toxic porphyrin compounds, and third, CO produced in the degradation of heme has been shown to be a second messenger neuromodulator, akin to NO in the nervous system. CO induces vasodilation and a number of anti-inflammatory reactions. In addition, CO regulates p38 mitogen-activating protein kinase signaling pathway. Lastly, biliverdin, bilirubin, and their glucuronides are potent antioxidants, which confer protection against oxidative tissue damage. HO-1 deficiency in humans is characterized by the condition asplenia characterized by arteriogenesis, intravascular hemolysis, inflammation, and heme-induced endothelial dysfunction. Asplenia culminates, ultimately, in intracranial hemorrhage and fatality. Remarkably, however, hemin by its propensity to induce HO-1 has been shown to protect cells from drug-induced cytotoxicity, oxidative stress, apoptosis, and tissue injury. Other defects in heme degradation are manifest in forms of hyperbilirubinemia arising from bilirubin accumulation in the skin and resulting in jaundice. Neonatal jaundice is caused by low amounts of bilirubin UDP-glucuronyl transferase in the first few days of life post parturition. Hyperbilirubinemia is also caused by disorders that are associated with increased hemolysis such as sickle cell anemia or hereditary spherocytosis. Other forms of hyperbilirubinemia are the Dubin–Johnson, Rotor, Gilbert, and Crigler–Najjar syndromes, which are caused by the partial or complete loss of UDP-glucuronosyltransferase activity, which contributes to inhibition of bilirubin degradation. Consequently, bilirubin levels are high in the blood for these syndromes, but differences arise in each type according to the pattern of inheritance, manifestation of either conjugated or unconjugated bilirubinemia, degree of molecular defects, and clinical symptoms.

Intercellular and Intracellular Transport of Heme

Heme is hydrophobic and highly insoluble in water and readily forms aggregates in aqueous solution. Sources of free labile heme include impaired heme coupling to apoproteins, increased heme protein oxidative catabolism, and deregulated HO activity. Free heme attaches to proteins and membranes to induce peroxidation and generate toxic ROS. The lipophilic property of heme favors its intercalation into phospholipid bilayer where it causes oxidation and peroxidation of membranes. Moreover, free heme is a repository of high redox-active iron that can generate toxic hydroxyl radicals in the Fenton reactions. Recent compelling evidence has shown that chemotaxis of heme to the RBC membrane causes hemolysis and membrane rupture resulting in the activation of pro-inflammatory signal transduction. Thus, heme directly induces inflammatory responses, increases cytokine production, and promotes platelet aggregation and endothelial activation, which consequently culminate in vascular and immune dysfunction. Moreover, Hb in RBC in extravascular space could either lyse or oxidize spontaneously or be induced by NO to form methemoglobin (MetHb). Peroxidation of MetHb produces FerrylHb, which could also act directly as an inflammatory agent. FerrylHb is a complex mixture of globin, porphyrin, and heme radicals, which directly stimulates synthesis of adhesion molecules and induces actin polymerization in endothelial cells. The ramifications of the ensuing cascade of signaling events are evident in vascular inflammation in hemolytic and

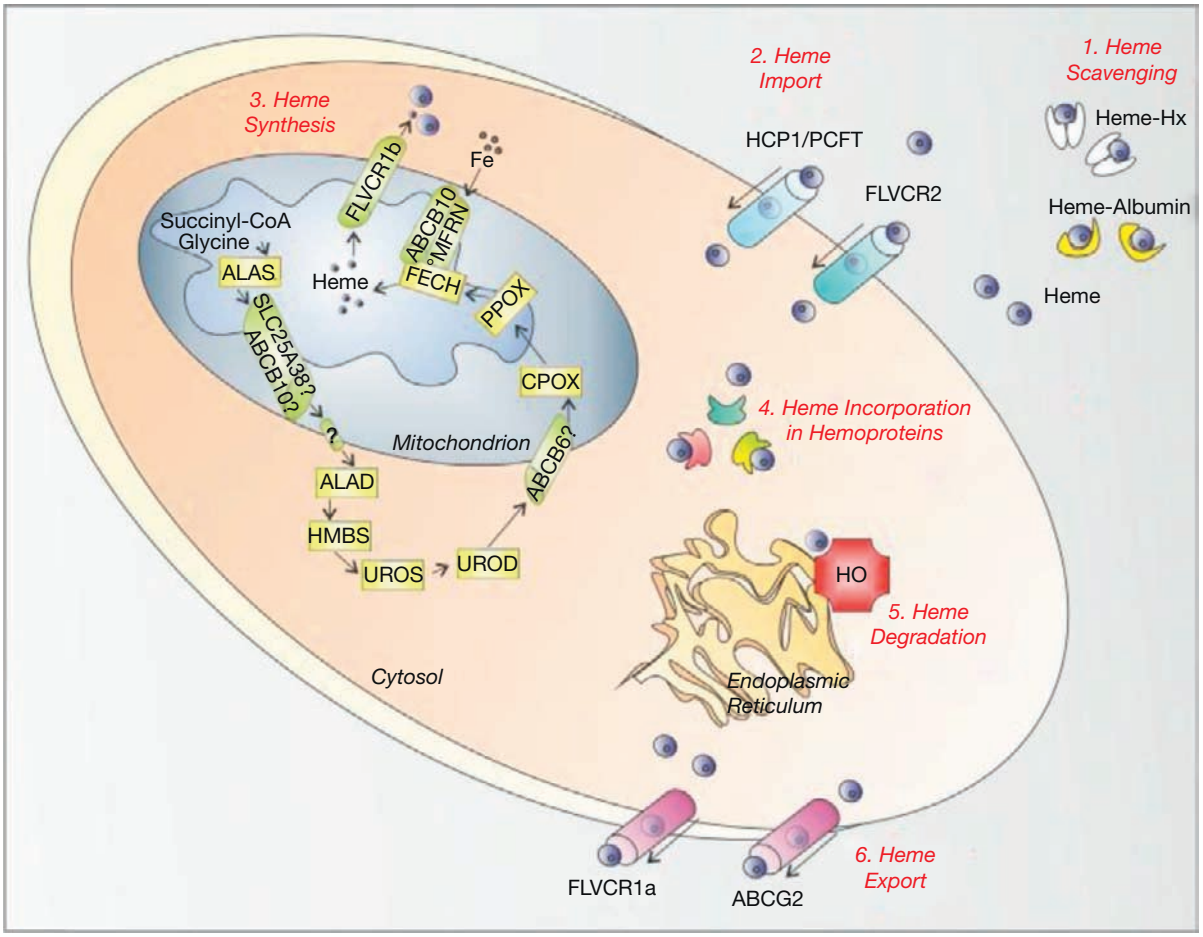

Figure 3 Inter- and intracellular heme metabolism. The main mechanisms involved in the inter- and intracellular control of heme are depicted. (1) Heme scavenging: Circulating free heme toxicity is averted by the action of the scavenging proteins Hx and albumin. (2) Heme import: Heme might be imported into the cell via the putative heme importers HCP1/PCFT and FLVCR2. (3) Heme synthesis: Starts by the formation of ALA from succinyl-CoA and glycine in the mitochondrion, traversing through the cytosol before the final step of Fe incorporation into PPOX to form heme in the mitochondrion. Heme export out of the mitochondrion to the cytosol is by heme exporter FLVCR1b. (4) Heme incorporation in hemeproteins: In the cytosol, heme is inserted in apo-hemeproteins to form functional hemeproteins. (5) Heme degradation: In the endoplasmic reticulum, the heme-degrading enzyme HO catabolizes heme into iron (Fe), carbon monoxide, and biliverdin. (6) Heme export: The heme exporters FLVCR1a and ABCG2 regulate heme export out of the cell across the plasma membrane. ALAS, aminolevulinic acid synthase; SLC25A38, solute carrier family 25 member 38; ABCB10, ATP-binding cassette subfamily B member 10; ALAD, amino levulinic acid dehydratase; HMBS, hydroxymethylbilane synthase; UROS, uroporphyrinogen III synthase; UROD, uroporphyrinogen decarboxylase; ABCB6, ATP-binding cassette subfamily B member 6; CPOX, coproporphyrinogen oxidase; PPOX, protoporphyrinogen oxidase; FECH, ferrochelatase; MFRN, mitoferrin; FLVCR, feline leukemia virus subgroup C receptor; HCP1/PCFT, heme carrier protein 1/proton-coupled folate transporter; ABCG2, ATP-binding cassette subfamily G member 2; HO, heme oxygenase; Hx, hemopexin. Reproduced with permission from Chiabrando, D., Vinchi, F., Fiorito, V., Mercurio, S. and Tolosano, E. (2014). Heme in pathophysiology: a matter of scavenging, metabolism and trafficking across cell membranes. *Frontiers in Pharmacology* **5**, 61.

hemorrhagic disorders as sickle cell disease, thalassemia, malaria, and infectious processes. Moreover, these pathological disorders could release significant amounts of free heme into the circulation. Consequently, labile free heme is maintained at very low concentration ($<10^{-9}$ M) in systemic metabolism. Intercellular regulation ensures the availability of sufficient heme for its functions in the mitochondria, nucleus, and cytosol where spatial and temporal dynamics favor coordinated channeling of substrates, reaction intermediates, and products across membranes of these organelles. A number of intercellular heme transport proteins in particular in the mitochondria and lysosomes have been characterized (Figure 3). While the transit of heme by passive diffusion is a possibility, a number of cytosolic heme-binding proteins for targeted transport have also been characterized. These include Z-protein or fatty acid-binding protein, ligandin or glutathione transferases, heme-binding protein 23 (HBP23), HBP22 SOUL, and more recently identified HeBP1 and HeBP2 that are possibly involved in heme detoxification during erythroid differentiation. Homologues of HBP23 (peroxiredoxins) in some species such as yeast and mouse exhibit peroxidase and antioxidant properties rather than heme binding. These buffering proteins bind free heme, thereby neutralizing reactivity and toxicity in cells. Other potent detoxifying mechanisms are heme degradation by HO-1

(described earlier) and the coupling of heme catabolism with ferritin sequestration of the iron released.

There are specific adaptive responses that prevent and avert the pro-oxidant and cytotoxic consequences of intracellular free heme in the circulation. Principal among these are soluble acute-phase proteins, namely, haptoglobin (Hp) and hemopexin (Hx), that scavenge and sequester, respectively, free hemoglobin (Hb) and heme. Both of these acute-phase serum proteins have very high affinities for heme with dissociation constants Kd $\sim 10^{-13}$ and Kd 10^{-8} for heme and Hb, respectively. It is presumed that a transient first-order heme buffering sink is provided by low- and high-density serum lipoproteins (LDL and HDL) before the subsequent transfer to Hp or albumin. Hp–Hb complex is engulfed and binds to receptor CD163 on macrophages of the reticuloendothelial system, while heme–Hpx complex is internalized by LDL receptor-related protein 1 or CD91 on hepatocytes for subsequent catabolism to release inorganic iron. Hemopexin, akin to transferrin, is recycled for reutilization, while haptoglobin is degraded in the lysosome. Consequently, these acute-phase buffers, namely, haptoglobin, hemopexin, and serum albumin (Kd $\sim 10^{-8}$), ensure reutilization of Hb- and heme-bound iron and protect cells from potent free heme- and Hb-induced oxidative and from free radical damage. Moreover, hemopexin is an exogenous antioxidant that is capable of binding heme extracellularly to inhibit influx of free heme into cells and consequently confers cytoprotection against intracellular toxicity and tissue damage. It is significant to note, however, that both innate and adaptive infectious responses could induce Hb lysis and degradation in tissues and organs. Resultant heme produced from Hb could lead, via the c-Jun N-terminal kinase signaling transduction pathway, to program cell death and tissue damage. Moreover, during chronic intravascular hemolysis, megalin and cubilin complexes in the kidney endocytose reabsorb free Hb. Megalin and cubilin possess broad substrate specificities and capacity for reabsorption and intracellular trafficking of free Hb during chronic hemolysis and are involved in the transport of other nutrients and metabolites as well.

Extracellular transmembrane heme transport also encompasses apical intestinal heme absorption and putative basolateral heme efflux. While the absorption of inorganic iron is obligatory, that of heme is dispensable to virtually all organisms because of the inherent capacity for *de novo* synthesis. Paradoxically, however, heme iron sources are quantitatively more highly bioavailable than nonheme iron. The unique porphyrin structure of heme also confers a nutritional advantage to the mode and mechanism of its absorption and transport in the gastrointestinal tract (GIT). Consequently, dietary heme is of significant importance in iron nutrition. However, the molecular mechanisms of the vectorial apical transit of heme in the gastrointestinal tract still remain enigmatic and unresolved. Heme carrier protein 1 (HCP1), a putative heme transporter, has also been shown to be a higher-affinity proton-coupled folate transporter (PCFT). Nevertheless, heme, as an intact metalloporphyrin, protected from luminal dietary inhibitors (except calcium), is highly absorbed in the proximal section of the GIT. Internalized heme is possibly trafficked from the cytoplasm into lysosome vesicles by intracellular heme-responsive gene 1 (HRG1) protein where it is catabolized by an inducible heme oxygenase (HO-1) to yield ferrous iron, CO, and biliverdin that is reduced to bilirubin (as described earlier). HGR1 has also been implicated in phagolysosomal heme recycling. Fe that is liberated enters the inorganic iron pool of the enterocytes and is effluxed by ferroportin. Although ^{59}Fe nonheme was found at the basolateral side of the duodenum after absorption of ^{59}Fe heme, studies carried out in guinea pigs provided evidence that some heme effluxed from the enterocytes into the circulation occurred as an intact metalloporphyrin. FLVCR1 and ABCG2 could be putative basolateral heme efflux proteins pumping intact heme into the circulation. ABCG2 furthermore averts protoporphyrin accumulation in the GIT. These areas of heme research remain subjects of intensive investigations. Another facet of heme iron nutrition is the positive correlation between high meat consumption and the incidence of metabolic disorders. A large body of data from epidemiological studies has demonstrated an association of high heme intake, particularly from red or processed meat, with increased risk in humans of various cancers and metabolic diseases. The GIT modulates iron absorption according to physiological requirements. However, dysregulation may arise from mutations in the genes involved in the iron transport machinery and in the components of the systemic signal transduction regulatory pathway for iron. There are different types of hereditary hemochromatosis (HH), and those with other iron overload disorders are caused by mutations in iron metabolism and in genes regulating hepcidin expression. Consequently, enhanced iron absorption and that of the highly bioavailable heme leads to tissue iron overload in HH, β-thalassemia, and the other forms of iron loading conditions. Heme that is released from hemeproteins contributes significantly to toxic free iron pool and generates ROS, which damages DNA and proteins and causes peroxidation of lipids. Moreover, nitrites and nitrosamine in processed meats have been shown to be cytotoxic and damaging to specific cells of the colon, pancreatic, and liver hepatocytes. The resultant effects have implications in the etiology of diverse cancers and metabolic diseases such as type 2 diabetes, obesity, coronary heart disease, alcoholic steatohepatitis, and some neurodegenerative diseases.

Heme Homeostasis

Heme metabolism in mammals is composed of biosynthesis of heme for varied vital hemeproteins and for erythrocytes in a circuit that recirculates iron from heme catabolism. In essence, biosynthesis and biodegradation (erythrophagocytosis) of heme must be balanced and regulated to maintain homeostasis. Three essential substrates, iron, porphyrin, and heme, are potentially toxic if permitted to accumulate. Iron, heme, and photosensitized porphyrin are pro-oxidants, which can cause damage to DNA, proteins, and cell membrane systems. The exogenous supply of iron, a limiting nutrient, and the *de novo* synthesis of heme and porphyrin are therefore strictly coordinated and channeled to avert the accumulation of excess toxic metabolites. Consequently, inter- and intracellular transport of heme requires specific membrane proteins that are possibly responsive and adaptive to demands in the respective habitat, niche, or site locale of and within the organism. Advances in

Table 2 Some heme transport genes in mammals

Genes	*Functions*	*Features/symptoms*
HCP1/PCFT	High-affinity folate transport, low-affinity heme transporter	Hereditary folate malabsorption syndrome (HFM), macrocytic normochromic anemia
ABCG2	Porphyrin efflux in erythroblast, intestine	Photosensitization, PPIX accumulation
FLVCR1a	Heme efflux in erythroblast, red cell aplasia, erythrophagocytosis	Embryonic lethality; impaired erythropoiesis
FLVCR1b	Heme efflux from mitochondria into cytosol	Hepatic heme and iron loading; reduced heme synthesis and cytochrome P450 expression/activity in hepatocytes
FLVCR2	Extracellular endocytosis of heme	Decreased Zn mesoporphyrin import
ABCB10	ALA efflux from mitochondria into cytosol	Increased oxidative stress, erythrocyte apoptosis and embryonic fatality
ABCB6	Coproporphyrinogen III transport from cytosol into mitochondria	Abnormal erythropoiesis, abnormal mitochondrial physiology, decreased physiological sensitivity to xenobiotic
ABCB7	Fe–S cluster transport from Mitochondria into cytosol	Refractory anemia with ring sideroblasts, mitochondrial ferritin accumulation
Mitoferrin1 (MFRN1) and mitoferrin2 (MFRN2)	Iron import from cytosol into mitochondria	Embryonic lethality. Severe anemia, protoporphyria, cholestasis
HRG-1	Heme transport from cytosol into endosomes/lysosomes	Erythropoietic defects and accumulation of heme analogs

heme metabolism research particularly with the use of mouse knockout models have revealed a number of heme transport proteins in organelles and in inter- and intracellular membranes of tissues and organs. Table 2 shows some heme transport genes and their functions.

Heme (iron protoporphyrin IX) is an essential molecule for diverse biological processes. Heme is found as a prosthetic group in numerous hemeproteins such as respiratory cytochromes, cytochrome P450, catalases, peroxidases, NO synthases, and guanyl cyclases, among others. Heme is therefore involved in the transport, sensing and storage of oxygen, electron transfer reactions, xenobiotics detoxification, signal transduction, and transcriptional, translational, and microRNA gene regulation. Heme biosynthesis comprises eight enzymatic reactions, the initial reaction of which commences in the mitochondria and proceeds in the cytosol and the last three steps conclude in the mitochondria. Consequently, metabolites of the biosynthetic pathway and heme product must be shuttled in and out of the mitochondria by specific transporters. Porphyrias are manifestations of loss of function mutations of the genes coding for the heme biosynthetic enzymes of which phenotypes could be either the cutaneous damage or the exhibition of neurological symptoms. ALAS2 gene mutation, however, leads to a gain of function X chromosome-linked sideroblastic anemia. Heme catabolism by HO-1 recycles iron and produces CO and bilirubin. Excess heme is potent and toxic and has the propensity to activate oxidative stress and inflammatory processes. Maintenance of heme at levels for optimal beneficial functions that avert toxicity is ensured by a number of mechanisms. These include the regulation of anabolism, storage, catabolism, and inter- and transcellular transit of heme across membranes and into the blood circulation. Disequilibrium of heme homeostasis occurs under disorders and diseases such as hemolysis, hemorrhage, and ischemia reperfusion. Consequently, cytosolic heme-binding proteins operate as buffers, while acute-phase proteins, haptoglobin and hemopexin, respectively, scavenge extracellular Hb and heme. HO-1 and ferritin also acute-phase partners are rapidly induced to degrade heme and mop up the resultant toxic free iron. In essence, the biogenesis, biodegradation, and inter- and intracellular trafficking of heme are regulated and coordinated to maintain optimal heme levels for metabolic processes in the whole organism.

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Iron: Physiology of Iron.

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Iron: Physiology of Iron

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Introduction

Paradoxically, although iron is the fourth most common element in the Earth's crust, iron deficiency is one of the most prevalent nutritional deficiencies. Iron is essential for life but can also be toxic; mammals have evolved many mechanisms to keep free iron levels as low as possible. Iron exists in two stable oxidation states, ferrous iron (Fe^{2+}) or ferric iron (Fe^{3+}), which confers the ability to combine with or donate an electron. This means that iron is involved in oxidation/reduction (redox) reactions in the body such as in the mitochondrial respiratory chain, but it can also be a prooxidant and generate hydroxyl radicals via the Haber–Weiss and Fenton reactions.

Intracellular iron catalyzes the formation of hydroxyl radicals ($HO\bullet$), which have unpaired electrons and are highly reactive:

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \quad (\text{Haber} - \text{Weiss reaction})$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^{\bullet} \quad (\text{Fenton reaction})$$

The combination of these two reactions leads to the formation of highly reactive hydroxyl radicals and can be summarized as

$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + HO^- + HO^{\bullet}$$

Hydroxyl radicals can cause damage to membrane lipids, proteins, and DNA and are implicated in a wide range of pathologies and in the aging process itself.

Physiological Roles for Iron

Iron is essential in the body for many processes including the transport of oxygen to tissues, the production of mRNA and proteins from DNA, and the regulation of energy metabolism. All cells require iron but some cells have distinctive roles (see Table 1): enterocytes that are involved in the absorption of dietary iron; the erythroid cells ('erythron') of the bone marrow that produce red blood cells and have the highest iron requirements; hepatocytes that store iron; and macrophages, predominantly in the spleen, that salvage and recycle iron from senescent red blood cells.

Approximately 70% of body iron is located in red blood cells where, as part of the heme cofactor, it is essential for the oxygen-carrying capability of hemoglobin. Heme is also present in myoglobin where it provides a short-term oxygen store in muscle and contains ~10% of body iron. Numerous iron-containing metalloproteins are involved in essential cellular processes including the cytochromes and dehydrogenases of mitochondrial electron transport, which provide the vast majority of energy for all cellular processes in all but a few cell types. Numerous other iron-containing enzymes are essential in many metabolic pathways including the TCA cycle (aconitase), protein and DNA metabolism, liver detoxification (cytochrome p450), neurotransmitter synthesis, myelin formation in oligodendrocytes, and antioxidant protection.

Iron Balance and Sources of Iron

Most of the daily iron requirements are for erythropoiesis in the bone marrow where ~200 billion new red blood cells are produced by the body each day requiring about 20 mg of iron. Most of this iron comes from the recycling of senescent red blood cells; abut 5–10% of daily iron requirement comes from the diet. Essentially, the dietary contribution is to replace obligatory iron losses, which are primarily due to cell loss. Cell loss is associated with the continuous loss of the short-lived enterocytes into the gut lumen, skin desquamation, and blood loss. Blood loss may be physiological such as that lost in menstruation (approximates to an average of 1 mg day^{-1}) or associated with pregnancy, or it may be pathological due to gut bleeding, excessive menstrual loss (menorrhagia), surgery, injury, or loss in the urine (hematuria; usually associated with renal stones or kidney failure). Blood loss is one of the biggest variables in iron balance. Iron balance is controlled by gut absorption and storage; there is no regulated route of iron excretion and no mechanisms to up- or downregulate iron loss.

Dietary Iron Absorption

About 1–2 mg of iron is absorbed per day corresponding to about 10% of dietary iron from a typical intake of about 12–30 mg in food. Women absorb more iron, to replenish their losses that exceed those of men during their reproductive life. The proportion of dietary iron absorbed depends on physiological state and the quality of a person's diet. The presence of dietary inhibitors and enhancers of iron absorption affects the bioavailability of iron. Dietary iron is in excess so that most of it either is not absorbed or remains in enterocytes and is shed into gut as cells are lost.

Dietary iron is predominantly in the form of heme or nonheme iron, and absorption occurs within the duodenum. A small amount of nonheme iron may also be absorbed within the large intestine. The mechanisms of brush border iron transport are different for heme iron and nonheme iron. All of these mechanisms require iron to be in a soluble form and to be accessible to the brush border membrane. Once absorbed, both heme iron and nonheme iron join a common iron pool. Iron is stored within the enterocyte and/or exported across the basolateral membrane for distribution to extra-intestinal tissues. All processes of iron transport across cell membranes, storage, and distribution are tightly regulated.

In acidic conditions such as the stomach, dietary nonheme iron is reduced from the relatively insoluble ferric (Fe^{3+}) form to the soluble ferrous (Fe^{2+}) form. As the acidic chyme moves

Table 1 Characteristics of key cells involved in iron metabolism

Cell	*Enterocytes*	*Erythroid cells*	*Hepatocytes*	*Macrophages*
Primary location	Duodenum and small intestine	Bone marrow	Liver	Spleen
Main role in iron metabolism	Dietary iron absorption	Synthesis of red blood cells	Iron storage	Recycling/phagocytosis of red blood cells (erythrophagocytosis) Maintain plasma iron
Predominant source of iron	Fe^{2+} via DMT1 Heme Dietary ferritin	$(Fe^{3+})_2$-Tf (holotransferrin)	Holotransferrin plus NTBI	Heme from degraded red blood cells
Iron stores	Ferritin	Ferritin	Ferritin	Ferritin
Iron export	Via ferroportin (in cooperation with hephaestin)	Probably no export mechanism (all iron is used by erythron and incorporated into hemoglobin)	Via ferroportin (in cooperation with ceruloplasmin)	Via ferroportin or sequestered
Notes	Iron may be sequestered and lost when enterocytes are sloughed off from tip of villus	These cells have the highest iron requirements	Hepatic iron storage is higher in males and increases in women postmenopause	Retention of iron in macrophages is an important mechanism to restrict iron available to pathogens

Fe^{2+}, ferrous iron (divalent); DMT1, divalent metal transporter 1; $(Fe^{3+})_2$-Tf, diferric transferrin; NTBI, nontransferrin-bound iron.

from the stomach to the duodenum, pancreatic bicarbonate oxidizes Fe^{2+} back to Fe^{3+}, the majority of which aggregates into long insoluble polymers, which cannot access the brush border membrane and so cannot be absorbed. Iron solubility within the small intestine can be significantly altered by the presence of specific dietary components. Phytates and tannins have a high affinity for Fe^{2+} and chelate nonheme iron within the stomach. However, like nonheme iron, phytates and tannins are also insoluble under oxidizing conditions and further promote the precipitation of iron into insoluble polymers within the small intestine. Conversely, vitamin C and components within meat, fish, pork, chicken, and shellfish have a high affinity for Fe^{2+} and so maintain the solubility and, therefore, the bioavailability of nonheme iron within the small intestine.

Heme Iron Absorption

Heme iron accounts for over half of the iron absorbed from Western diets in which red meat is consumed but contributes much less in other diets with low red meat intake. Unlike nonheme iron, heme iron is soluble under oxidizing conditions. This is because the porphyrin ring prevents heme iron from forming insoluble polymers in the oxidizing environment of the small intestine. Additionally, heme iron transport across the brush border membrane is not inhibited by the presence of divalent cations.

Heme iron is cleaved from globin in the intestine by mechanisms that are not well characterized. Heme iron can be transported across the brush border membrane by two independent mechanisms:

1. Facilitated transport through the folate transporter PCFT/HCP1 where heme iron enters the cytosol
2. Receptor-mediated endocytosis where heme iron enters the enterocyte bound within an endosome

Once within the enterocyte, iron is released from the porphyrin ring by the enzyme heme oxygenase. The activity of heme oxygenase is different depending on the route of heme iron entry and appears to be the rate-limiting step for heme iron absorption. Once released from the porphyrin ring via degradation of the heme molecule, iron is either stored in ferritin or transported across the basolateral membrane as ferrous iron via ferroportin (FPN). This ferrous iron is oxidized by the multicopper oxidase, hephaestin, so that exported Fe^{2+} is oxidized to Fe^{3+}, which can be bound by transferrin (Tf) and transported in plasma. FPN is important in many other cell types such as hepatocytes and macrophages. In these cells, an alternative multicopper oxidase to hephaestin, ceruloplasmin, oxidizes exported Fe^{2+} to Fe^{3+}, which can be transported via plasma Tf. The porphyrin ring is exported by the transporter feline leukemia virus subgroup C. This process is illustrated in Figure 1.

Nonheme Iron Absorption

Nonheme iron is transported from the intestinal lumen across the enterocyte brush border membrane into the cytosol by the divalent metal transporter 1 (DMT1). The transport of iron by DMT1 is actively driven by the concurrent flow of hydrogen ions traveling into the cytosol down their own concentration gradient. This hydrogen ion gradient is maintained by a sodium/hydrogen exchanger also present within the brush border membrane (Figure 2).

DMT1 transports Fe^{2+}, but not Fe^{3+}. For this reason, any soluble Fe^{3+} must be reduced to Fe^{2+} prior to transport. Fe^{3+} is reduced to Fe^{2+} by duodenal cytochrome B (DcytB), a brush border membrane protein in close vicinity to DMT1. DcytB utilizes the cofactor ascorbic acid to provide the electrons for this reduction of ferric to ferrous iron. Once iron has been transported across the brush border membrane into the

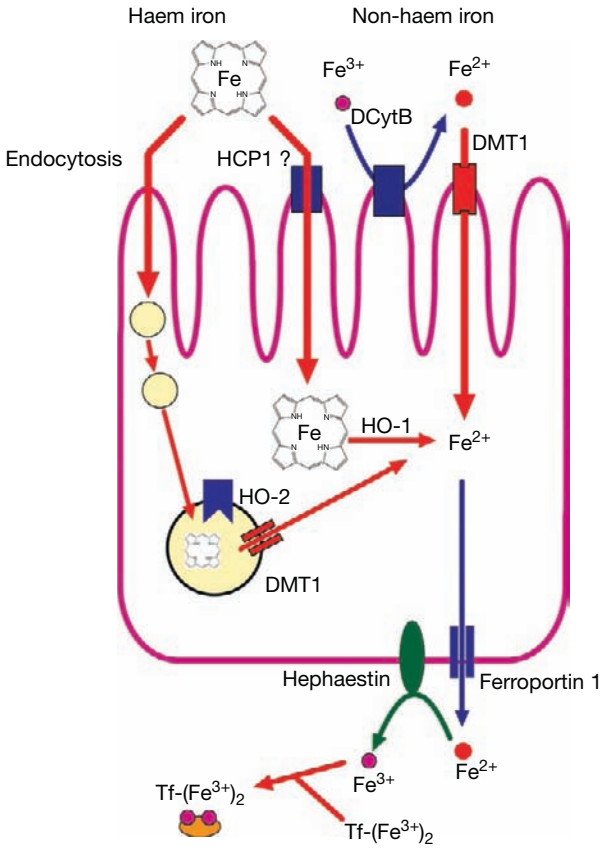

Figure 1 Nonheme iron uptake and the proposed pathway for heme iron absorption. Dietary iron is absorbed across the gut wall as heme iron (from animal food sources) and nonheme iron (from animal and plant food sources). Heme iron is absorbed directly and this has been suggested to be via heme carrier protein 1 (HCP1) though this protein has been recently shown to be a proton-coupled folate transporter. Once transported across the apical membrane, cytosolic heme is catabolized by HO-1 and released into the labile iron pool. Heme iron may also be absorbed by receptor-mediated endocytosis and processed in endosomes to release Fe^{2+}, which is transported to the cytosol via DMT1. Fe^{2+} derived from both sources is released across the basolateral membrane via ferroportin1 and oxidized by hephaestin to Fe^{3+} so that it can be transported in plasma by transferrin. The mechanism of heme iron efflux from the absorptive enterocyte is unknown but may include the transporter FLVCR.

enterocyte cytosol, it joins the cytosolic labile iron pool. Fe^{3+} reduction and DMT1 transport are illustrated in Figure 1.

DMT1 is not specific for nonheme iron and can transport other divalent cations including copper, manganese, cobalt, cadmium, zinc, nickel, and lead. When present together within the intestinal lumen, these cations directly compete for transport by DMT1. Indirect competitive inhibition can also occur in the presence of calcium. Calcium inhibits the flow of hydrogen ions through DMT1 (which are required to drive divalent cation transport) and stimulates the removal of DMT1 from the brush border membrane. The presence of direct and indirect competitors of DMT1 iron transport within the diet will, therefore, reduce the bioavailability of iron from the diet.

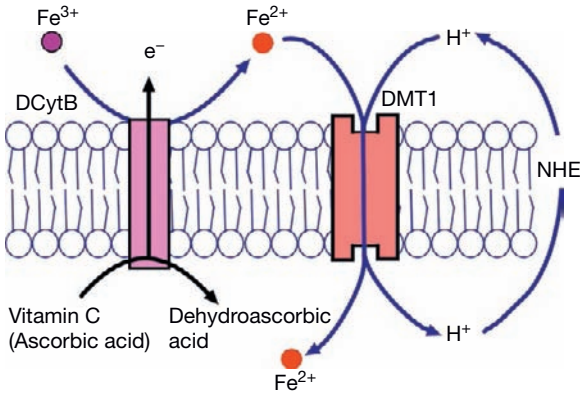

Figure 2 Iron transport across the brush border membrane. Soluble ferric iron is reduced to ferrous iron by DcytB, a process facilitated by the oxidation of cytosolic ascorbic acid to dehydroascorbic acid. Ferrous iron is transported across the brush border membrane by DMT1, a symport transporter driven by the proton gradient, which results from the entry of gastric acid into the duodenum and is maintained by the Na–H exchanger (NHE).

Alternative Mechanisms of Iron Absorption

Other mechanisms of nonheme iron transport across the brush border membrane have been proposed including the integrin/mobilferrin/paraferritin pathway and the Zrt- and Irt-like protein pathway. However, genetic mutation studies suggest that DMT1 is by far the most important brush border nonheme iron transporter and the possible significance of these alternative pathways has not been established.

Destination of Absorbed Iron

There are several possible destinations for iron absorbed in the small intestine:

1. Iron may be directly used by the absorptive enterocyte.
2. All cell types have a requirement for iron for a range of cell functions. Mitochondria have dedicated mitochondrial iron transporters and use iron for the synthesis of iron–sulfur clusters and heme proteins. In addition, iron metalloproteins are crucial in many aspects of intracellular metabolism.
3. Iron may be donated to specific target proteins within the enterocyte.
4. Absorbed iron may be stored within the enterocyte in ferritin and exported from the enterocyte via FPN with subsequent transport in the circulation by Tf.

Ferritin

Ferritin is a self-assembling nanocage that functions as a cell storage protein able to sequester ~2000 iron atoms per ferritin cage under normal conditions. Ferritin prevents free iron concentrations from increasing in the cytosolic and nuclear compartments. The highest intracellular concentrations of ferritin occur in hepatocytes that are involved in iron storage, in the spleen reticuloendothelial macrophages that recycle red blood

cells, and in the bone marrow where most erythropoiesis occurs. During intracellular iron overload, excessive iron deposition in ferritin (>3000 iron atoms/nanocage) can cause ferritin degradation to hemosiderin, which forms iron-rich deposits that cannot be mobilized.

Although most ferritin is intracellular, there is some release of ferritin into the blood. Serum ferritin levels reflect total intracellular iron and hence body stores and iron status. If body iron stores are low, serum ferritin concentration will be low and dietary iron absorption will usually be promoted. Conversely, high iron stores are associated with high serum ferritin concentrations and decreased dietary iron absorption. However, while low serum ferritin levels always indicate low iron status, high levels need to be interpreted carefully. Serum ferritin increases with iron overload and is also an acute-phase reactant, which increases in chronic inflammatory conditions such as obesity and high alcohol consumption and in the presence of infections, tumors, and renal disease. This is a protective mechanism; mammals have evolved mechanisms to sequester iron within cells to limit its availability for pathogenic microorganisms. This is effective for acute challenges such as infections, but modern conditions associated with inflammation can be chronic and lead to anemia of chronic disease, which is refractory to oral iron therapy. Thus, serum ferritin is a useful indicator of iron status and more sensitive than the measurement of hemoglobin concentration in populations unlikely to be affected by infections, but markers of infection and inflammation such as C-reactive peptide need to be measured concurrently to exclude coexisting infection.

Ferroportin

The only known transporter of Fe^{2+} from the cell is FPN. All cell types express FPN, but it has been characterized in macrophages and on the basolateral membrane of duodenal enterocytes. Export of iron from cells is controlled by hepcidin and FPN is the hepcidin receptor. When FPN transports Fe^{2+} out of the cell, it is immediately oxidized to Fe^{3+} by multicopper oxidases such as hephaestin and ceruloplasmin. This is the reason why copper deficiency impairs iron export and results in iron deficiency anemia.

The magnitude of iron release from enterocytes, hepatocytes, and macrophages is regulated by the hepatic bactericidal protein hepcidin (see Figure 3). Hepcidin is a small, highly conserved peptide hormone (a defensin) with antimicrobial activity, which is expressed in the liver in order to regulate the concentration of utilizable iron within the circulatory system. Hepcidin is expressed in response to systemic iron loading and in response to infection and inflammation. Hepcidin expression is reduced by the hormone erythropoietin in response to erythropoiesis (during training, acclimatization to altitude, pregnancy, growth, or blood loss) and by the expression of hypoxia-inducible factor (HIF) proteins during hypoxia. Increased hepcidin levels are associated with a decreased release of iron from enterocytes, hepatocytes, and macrophages, whereas low levels of hepcidin have the opposite effect.

Hepcidin expression can be stimulated by bone morphogenetic proteins in response to intestinal and hepatic iron loading or by holo-Tf (iron-saturated Tf) in response to high concentrations of utilizable iron within the circulatory system. Hepcidin expression can also be stimulated by proinflammatory cytokines released during infection or inflammation, in order to restrict the amount of iron available for utilization by pathogens as part of the innate immune response.

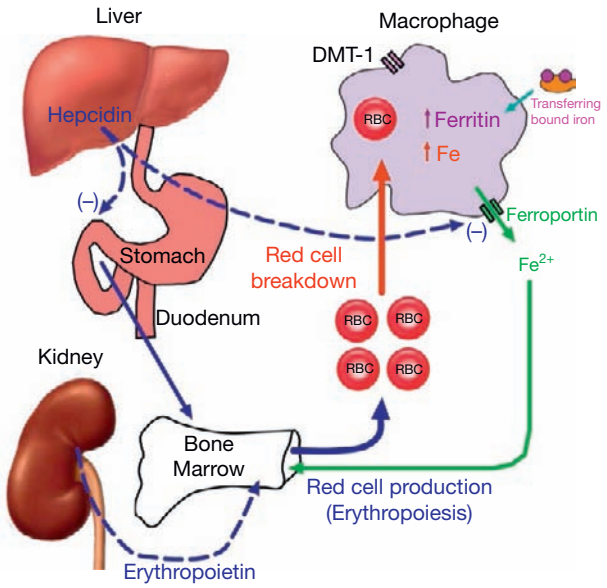

Figure 3 Control of iron export by hepcidin. Iron is absorbed predominantly in the proximal small intestine (duodenum) and its major requirement is for red cell production (erythropoiesis) in the bone marrow, a process that is regulated by erythropoietin secreted by the kidney. The main regulator of iron status is hepcidin, which is produced by the liver and inhibits the release of iron into the circulation from absorptive enterocytes and from heme breakdown from red cells scavenged by macrophages. Infection and inflammatory conditions increase hepatic hepcidin production via proinflammatory cytokines. Hepcidin binding causes endocytosis of ferroportin in enterocytes, macrophages, and hepatocytes, resulting in sequestration of iron in intracellular stores. This not only decreases iron availability for pathogens but also compromises iron availability for erythropoiesis that can cause functional anemia, which is refractory to oral iron therapy.

Once released into the circulatory system, hepcidin binds to receptors present on the plasma membrane of intestinal enterocytes, hepatocytes, and macrophages. Binding of hepcidin to its receptor stimulates the endocytosis and lysosomal degradation of FPN, and, because FPN is the only iron-efflux transporter, its removal inhibits iron export from these cells. Any dietary iron stored within intestinal enterocytes is lost in feces when the cells are shed from the tip of the villus. Any iron stored within hepatocytes or macrophages must be retained until required by the body for assimilation into body tissues.

Control of Iron

There is very little free iron in the body; most iron is tightly bound to proteins. This limits iron availability for pathogens and nontransferrin-bound (free) iron (NTBI), which could be involved in the production of damaging free radicals.

Iron Transport

Iron is transported in the circulation in red blood cells and bound to Tf. The iron component of hemoglobin cannot be exchanged or released from circulating red blood cells.

In the plasma, 95% of the iron is in the form of Fe^{3+} bound to Tf. Absorbed iron is loaded onto apotransferrin (apo-Tf) to give Tf-$(Fe^{3+})_2$, which is referred to as holotransferrin (holo-Tf). Transferrin is an iron-binding glycoprotein with high affinity for iron, which is mostly produced by the liver although other tissues such as the brain also contribute. It transports Fe^{3+}, mostly from macrophages, enterocytes, and hepatocytes, around the body and delivers it to cells requiring iron, the major target being the erythroid cells of the bone marrow. Transferrin has two lobes each with a single Fe^{3+}-binding site; binding requires the presence of an anion (HCO_3^-). Iron is released from transferrin if the pH falls below 5.5.

Transferrin can be used as a marker of iron status; Tf production increases if the body is iron-depleted, whereas iron repletion and overload result in decreased Tf production, but serum transferrin also decreases in acute reactions. Transferrin is usually about 30% (range is 25–50%) saturated with iron but, in deficiency states, it can be <5% saturated, which means there will be insufficient iron delivery for erythropoiesis. If transferrin saturation exceeds 60%, there starts to be measurable amounts of NTBI.

Uptake of iron from transferrin is determined by the number of specific Tf receptors (TfR) for the diferric-transferrin complex. There are two types of Tf receptors expressed on cell membranes; TfR1 is expressed by all dividing cells (such as the erythron) and TfR2 is expressed primarily by hepatocytes, which are involved in iron storage. TfR expression on cells increases with increased iron demand so that iron stores are diminished.

When the diferric transferrin binds to the transferrin receptor, the iron–transferrin receptor complex is internalized by receptor-mediated endocytosis via the formation of clathrin-coated pits, which form endosomes (Figure 4). The ATP-dependent proton pump inserted in the endosome membrane reduces the pH to below pH 5.5, which means the Fe^{3+} is released from holo-Tf. A ferrireductase (STEAP3) in the endosomal membrane reduces Fe^{3+} to Fe^{2+}, which is then released into the cell cytosol by cotransport with hydrogen ions via DMT1. The transferrin receptor bound to apo-Tf is returned to the cell surface, which has a neutral pH so apo-Tf dissociates from TfR. The TfR remains inserted in the cell membrane and apo-Tf reenters the circulation to bind and deliver more exported iron (Figure 4).

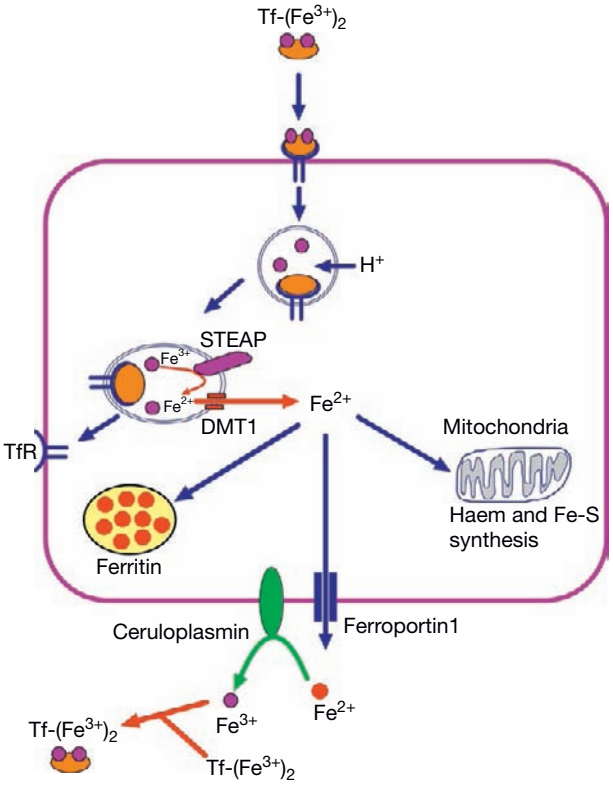

Figure 4 Cellular iron uptake from holotransferrin. Holotransferrin with two ferric atoms binds to the transferrin receptor (TfR) on the cell membrane, and TfR–Fe^{3+} complex is endocytosed via a clathrin-coated pit into the cell. The vesicular membrane proton pump lowers endosomal pH to liberate free ferric iron, which is then reduced to ferrous iron by STEAP. Ferrous iron leaves the endosome to enter the cytosol via DMT1. The apotransferrin receptor complex is recycled to the cell membrane, liberating transferrin at the near neutral pH.

Regulation of Dietary Iron Absorption

The human body has no mechanism to excrete excess iron. For this reason, iron homeostasis must be maintained by regulating the amount of iron that is absorbed from the diet by the absorptive intestinal cells (enterocytes) and the amount of iron that may be utilized or stored by extraintestinal tissues. Iron transport across the enterocyte is regulated by its own iron status, the iron status of the liver, the concentration of utilizable iron within the circulatory system, hypoxia, erythropoiesis, and the presence of inflammatory cytokines.

Iron absorption is increased if dietary iron bioavailability is low, when body iron stores are low, or if there is a drive for increased erythropoiesis, for example, because of altitude, pregnancy, low hemoglobin concentration, or hypoxia. Conversely, absorption of dietary iron is decreased in iron repletion and in the presence of systemic inflammation or infection, which promotes hepcidin production. Low iron status leads to an increased expression of DcytB, DMT1, and FPN, which results in increased iron absorption. Transferrin expression and the expression of its receptor (TfR) on cells can also increase under these conditions. Furthermore, exposure of enterocytes to high concentrations of iron such as dosing regimens used to treat low iron status downregulates DMT1. This has led to studies investigating the efficacy of intermittent iron supplementation.

Iron Regulatory Proteins

The amount of iron that can be transported or stored by the enterocyte is regulated by the concentration of iron and oxygen within the cytosol. If the enterocyte iron concentration is too high, the expression of brush border iron transporters will be reduced and the expression of storage proteins enhanced. If the

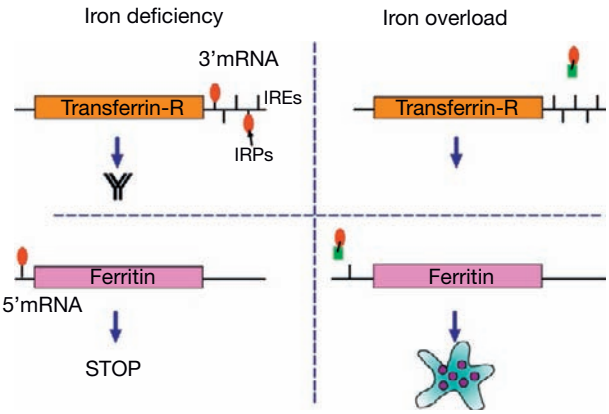

Figure 5 Changes in transferrin receptor and ferritin mRNA under low or high iron concentrations. When cytosolic iron concentrations are low, IRP has a high affinity for IRE and will stabilize 3′ mRNA strands promoting the expression of iron transport proteins (e.g., transferrin receptor) and block 5′ mRNA strands, inhibiting the translation of iron storage proteins (e.g., ferritin). When cytosolic iron concentrations are elevated, IRP will lose its affinity for IRE; therefore, transport protein mRNA translation is reduced and ferritin mRNA translation is increased.

enterocyte iron or oxygen concentration is too low, the expression of iron transporters is enhanced and the expression of storage proteins is reduced by transcriptional and post-transcriptional mechanisms.

The expression of iron transporters in response to cytosolic iron concentrations is regulated by the iron regulatory element/iron regulatory protein (IRE/IRP) system. The iron response element (IRE) is a conserved mRNA sequence on the 5′ end of ferritin mRNA and the 3′ end of DMT1, Dcytb, and TfR mRNA. IRPs are a family of cytosolic iron-binding proteins that bind specifically to IREs. IRPs will only bind to IREs when the cytosolic iron concentration is low. When cytosolic iron concentrations increase, IRPs undergo a conformational change and lose their affinity for IREs (see Figure 5).

Binding of IRPs to IREs can either inhibit or enhance mRNA translation depending on the specific location of the IRE. If the IRP binds to the 5′ IRE, the IRP blocks mRNA from being translated. If the IRP binds to the 3′ IRE, the mRNA is stabilized and the protein is translated. Two examples of this process are illustrated in Figure 5.

In addition to the IRE/IRP system, the HIF system augments the transcription of TfR, FPN, and HO-1 genes within the enterocyte during hypoxia. Low cytosolic oxygen concentrations promote the expression of HIF proteins, a family of gene regulators that collectively promote the transcription of iron transporter genes in order to facilitate systemic iron distribution. When cytosolic oxygen concentrations are normalized, HIF expression is significantly reduced and iron transporter gene transcription is no longer enhanced.

Iron Pathologies

Iron Deficiency Disorders

Iron deficiency can result in anemia and compromised oxygen transport. Anemia can be a result of dietary deficiency but can also be genetic or acquired. Iron deficiency anemia is the most common form, but anemia can be due to either insufficient intake or excessive loss of iron. The ability to upregulate the iron acquisition mechanisms is limited, so excessive blood loss may not be matched by increased absorption of dietary iron. In developing countries, blood loss due to parasitic infection is a significant cause of iron deficiency anemia. In developed countries, causes of excessive blood loss include heavy menstrual loss, frequent blood donation, and gastrointestinal bleeding. Because copper is required for the oxidation of iron exported from the enterocyte, dietary copper deficiency can result in secondary iron deficiency. Genetic anemias are due to mutations of genes for proteins involved in iron acquisition.

Acquired anemias can be a consequence of inflammation or infection or be anemia of chronic disease. Conditions, such as obesity and cancer, which result in raised hepcidin, cause iron retention by enterocytes; this promotes hypoferremia, which deprives potentially pathogenic bacteria of iron. The sequestration of iron in cells makes it unavailable, not only for bacteria but also for the synthesis of hemoglobin by erythroid cells, so if hepcidin is raised for a prolonged period, the consequence will be anemia, which is refractory to oral iron therapy. Hepcidin mRNA transcription is induced by cytokines such as interleukin 6. Some tumors may also produce hepcidin and promote inflammatory responses that promote hepatic production of hepcidin. In older age, cytokine production can be dysregulated resulting in raised hepcidin causing anemia of old age. This may be compounded by age-related compromised gastric acid production, which abrogates the conversion of Fe^{3+} to Fe^{2+} with a negative impact on iron absorption in the duodenum.

There is a hierarchy of iron requirements as iron becomes deficient. Hemoglobin production is protected at the expense of other tissue iron requirements, so iron required for brain use, for example, may be compromised even though hemoglobin concentrations are within the normal range. This state is known as iron deficiency without anemia and is indicated in subjects by normal hemoglobin concentrations but low iron stores (reflected by low serum ferritin concentrations). Iron deficiency without anemia is of concern because it affects cognition and endurance and may have negative consequences for immune function.

Iron Overload Disorders

In iron overload disorders, plasma iron exceeds the binding capacity of transferrin so free iron levels are raised, which can generate dangerous free radicals. This is usually because iron absorption is not controlled. In hereditary hemochromatosis, there is inadequate hepcidin production or mutations in FPN, so iron uptake from the gut is high. The parenchymal tissues such as the liver, heart, pancreas, and skin accumulate the excess iron, which can lead to tissue damage and fibrosis. Hemochromatosis can lead to liver cirrhosis, 'bronze' diabetes, and discoloration of the skin as iron is deposited in the tissues.

Intravascular Hemolysis

Hemolysis (breakdown) of red blood cells in the circulation results in the contents of the cells including iron being released into circulation. This can occur as a consequence of

autoimmune disorders, hemolytic anemias, and viral and bacterial infections and as a side effect of drug use. As with the iron overload disorders, there is a consequent risk of damage due to iron compounds generating hydroxyl radicals in the Fenton reaction. Free hemoglobin from hemolyzed red blood cells binds to haptoglobins, which are taken up and metabolized by the liver. Free heme from the oxidation of the free hemoglobin binds to hemopexin or albumin, which is also taken up by the liver. The liver metabolizes heme into bilirubin, which is excreted via feces and urine. Any heme passing through kidney is resorbed.

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Ascorbic Acid: Physiology and Health Effects; Iron: Biosynthesis and Significance of Heme; Iron: Properties and Determination; Meat: Role in the Diet.

Further Reading

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http://www.who.int/nutrition/topics/ida/en/ – World health Organization: Micronutrient deficiencies - iron deficiency anemia.

Iron: Properties and Determination

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Sources of Iron in the Earth

Iron originates in the large stars where it is formed by alpha nucleosynthesis, originating from silicon. During evolution, iron was present as soluble ferrous iron in the oceans, but with the evolution of photosynthetic life and the release of oxygen as a by-product, such ferrous iron was oxidized and became insoluble banded iron formations, magnetite (Fe_3O_4) or hematite (Fe_2O_3), deposited within sedimentary rock during the pre-Cambrian age, between 2400 and 1800 million years ago. Such formations consisted of repeated, thin layers of silver to black iron oxides, alternating with bands of iron-poor shales and cherts, which were often red in color. Today, these rocks are an important commercial source of iron ore, for example, in Western Australia, the Pilbara region, and in Minnesota, the United States, the Animikie Group.

Physical and Chemical Properties

Iron, element 26 in the periodic table, is the second most abundant metal after aluminum and the fourth most abundant element in the Earth's crust. Iron, a transition element, is redox-active, is a good Lewis acid, and is capable of forming bonds with electronegative elements (oxygen, nitrogen, and sulfur). The biological importance of iron resides to a great extent in its capacity to exist in several oxidation states, the principal ones being ferrous (Fe^{2+} or Fe(II)) and ferric (Fe^{3+} or Fe(III)), although higher-valent Fe^{4+} or Fe^{5+} is generated during the catalytic cycle of a number of enzymes such as catalases, peroxidases, and cytochromes P450. However, iron is potentially toxic since it can react with oxygen to generate reactive oxygen species, which may trigger cell damage.

Sources of Iron in Food

Iron is present in food that is derived from both animals and plants and exists in two forms: heme iron and nonheme iron (Figure 1).

Heme Compounds

Heme iron, derived from hemoglobin and myoglobin, is present in meat and fish while seafood and poultry contain both heme and nonheme irons (Table 1). In meat, 30–70% of the total iron content is present as heme iron, of which 15–35% is absorbed. Heme iron is highly bioavailable and absorbed to a much greater extent than nonheme sources, predominantly in the alkaline environment within the lumen of the small intestine, its absorption not being affected by other dietary components. However, cooking will decrease heme iron, the severity of the heme losses being dependent on the cooking method.

Nonheme Iron

Nonheme inorganic iron is the predominant form of iron in dietary components of plant origin and accounts for 80–90% of iron in a standard diet although a very small amount of this iron is absorbed, <10%. The richest sources of nonheme iron are shown in Table 1. The absorption of nonheme iron is influenced by a wide number of constituents within food, which either enhance or decrease iron absorption (Table 2). For example, vitamin C, present in fruits and vegetables, could enhance the potential availability of iron for mucosal uptake. Excessive alcohol consumption may increase iron status in susceptible individuals. This is attributed to increased rates of iron absorption from the intestine, possibly reflecting alcohol-induced downregulation of hepcidin expression in the liver. Other constituents may diminish the absorption of iron (Table 3). Calcium and phytates, present in cereals and vegetables, and phenolic compounds, in tea, coffee, and other beverages, could bind iron and restrict its availability for absorption.

In some developing countries, contamination of iron from mechanical or chemical reactions may occur during food production and preparation and be an important source of iron. In the early studies of the Bantu tribe in South Africa, the high intake of iron in this tribe was caused by the consumption of beer that had been brewed in nongalvanized steel drums. Iron contamination is not considered to be a significant source of iron in the United Kingdom and developed countries since the use of cast-iron cookware is not widespread.

Iron Fortification of Food

Food fortification is an attractive public health strategy and has the advantage of reaching wider at-risk population groups through existing food delivery systems, without requiring major changes in generalized consumption patterns. Various approaches can be utilized, such as mass fortification in foods that are widely consumed and fortification of target foods consumed by specific age groups, for example, infant complementary foods.

In the 1930s, fortification of flour with iron and B complexes was introduced in a number of developed countries and, as can be seen in Table 4, is now widespread in many products. Iron deficiency remains the commonest nutritional deficiency worldwide (affecting approximately 20% of the world's population), which could be effectively ameliorated by promoting nutritious foods and fortifying food with iron.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00403-7

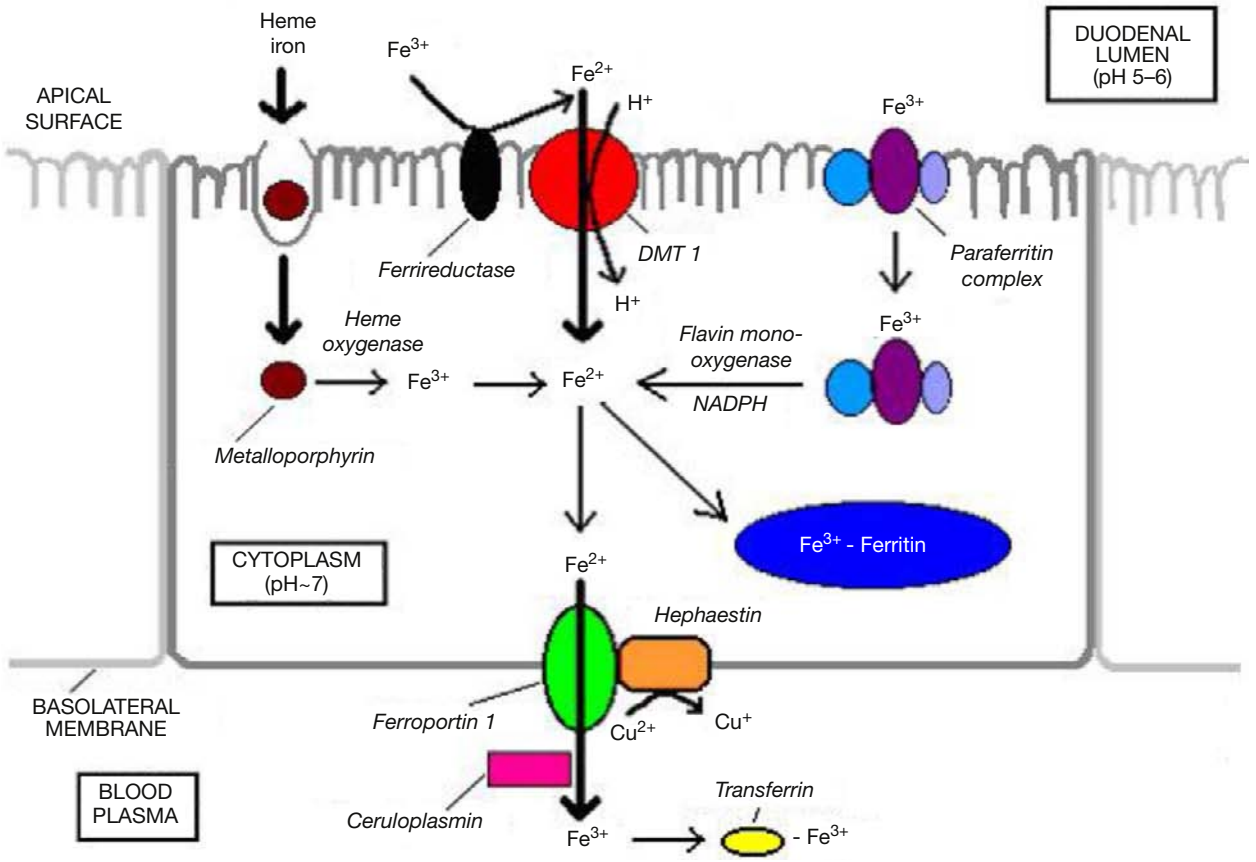

Figure 1 Possible pathways of heme and nonheme iron uptake and transport in enterocytes. From info@medchemtherapeutics.co.uk.

However, these strategies are not easy to implement and practice, especially for poor families with limited financial resources. Vegetarians may be at particular risk from iron deficiency due to the low levels of poorly bioavailable iron in plants. Iron deficiency could be mitigated by increasing the iron content and bioavailability of food grains, such as wheat and rice. Recently, three approaches were combined to increase the iron content of rice seeds: (i) enhancing iron storage in grains *via* the expression of the Fe-storage protein, ferritin; (ii) enhancing Fe translocation from roots to seeds through overproduction of the iron chelator, nicotinamide; and (iii) enhancing Fe flux into the endosperm through overexpression of *OsYSL2*, the gene responsible for long-distance translocation of NA-chelated Fe into sink tissues, including leaves and grains. The iron concentration of polished seeds of field-grown rice increased fourfold in comparison to that of wild-type plants. It is apparent from the figures shown in Table 4 that there are vast differences in the amounts of iron added to the different foods throughout the world and there appears to be no clear consensus as to what a safe amount of iron might be. However, since such iron fortification regimes rely on the fact that iron uptake, storage, and utilization are meticulously coordinated to prevent excessive amounts of iron being absorbed (only 1–2 mg of iron is needed to replace daily losses), it is assumed that excessive amounts of iron will not be absorbed. However, in Northern Europe, hereditary hemochromatosis has a prevalence of 1 in 200 in the population where a gene defect may enhance iron loading. Indeed, in Norway, where there is an even high prevalence of hereditary hemochromatosis, it has been argued successfully that iron supplementation is an unacceptable risk for this population. Excess iron stores may promote cancer and increase the cardiovascular risk. Furthermore, increasing the iron status in the population of developing countries by such fortification may also impose risk, since there could be an increased morbidity, due to pathogenic bacteria competing effectively for iron in the circulation, resulting in an exacerbation of existing infections.

Iron Salts and Complexes Used for Food Fortification

It is necessary to protect the supplemented iron in the food from potential inhibitors of iron absorption. Different forms of iron have been utilized: (a) freely water-soluble (ferrous sulfate, ferrous gluconate, ferrous lactate, and ferric ammonium citrate), (b) poorly water-soluble, but soluble in dilute acids such as gastric juice (ferrous fumarate, ferrous succinate, and ferric saccharate), (c) water-insoluble and poorly soluble in diluted acid (ferric orthophosphate, ferric ammonium orthophosphate, ferric pyrophosphate, and elemental Fe powders), and (d) protected iron compounds (Fe–EDTA and hemoglobin) (Table 4). In addition, new forms of iron administration are under development, for example, ferrous sulfate,

Table 1 Iron content of different foods

	Iron content in 100 mg	*Heme iron in 100 mg*
Heme iron		
Chicken, breast leg, thigh	0.4–0.7	0.12–0.29
Turkey, breast leg, thigh	0.5–1.0	0.14–0.49
Beef, sirloin, fillet, roast beef	2.0–2.4	1.72–1.77
Veal	0.85	0.71
Lamb	2.23	1.68
Horse	2.21	1.75
Ostrich	2.43	1.76
Rabbit	0.45	0.25
Pork	0.36	0.20
Liver (pork, chicken, turkey, lamb, beef)	3.7	
Mollusks		
Clams, mussels, oysters	28	
Fish		
Sardines	2.6	
Tuna	1.6	
Nonheme iron		
Egg yolk	2.7	
Vegetables		
Spinach	3.6	
Fresh parsley	6	
Palm hearts	3	
Lentil sprouts	3	
Swiss chard	2.3	
Dried fruit		
Sun-dried tomatoes	9	
Dried apricots	6	
Dried coconut	3.3	
Dried Zante currants and raisins	3	
Beans and pulses		
Beans, lentils, chickpeas	3.7	
Squash and pumpkins seeds	15	
Whole grains, fortified cereals, and bran		
Quinoa	1.5	
Nuts		
Cashew, pine hazelnut, almond	6.1	
Olives	3.3	
Dark chocolate and cocoa powder	17	
Tofu	2.7	

Source: www.healthaliciousness.com; www.healthlinkbc.ca; Lombard-Boccia et al., 2006.

Table 2 Dietary constituents that enhance nonheme iron absorption

Food	*Active substance*
Guava, pawpaw	Ascorbic and citric acids
Beef, lamb, pork	Cysteine-containing peptides
Liver, chicken, fish	
Orange, pear, apple, pineapple juices	Ascorbic and citric acids
Plum, rhubarb, banana	Ascorbic and citric acids
Mango, pear, cantaloupe, carrot, potato, beetroot	Citric, malic, and tartaric acids
Pumpkin, broccoli, cauliflower, tomato, cabbage, turnip	
Cauliflower	Ascorbic acid
Salad, lettuce, tomato	Ascorbic acid
Green pepper, cucumber	
Beer	Ethanol, lactic acid
Sauerkraut	Lactic acid
Wine	Ethanol
Rice miso	Fermentation products
Soy sauce	Fermentation products
	Cysteine
	Glutathione

Adapted from *Iron: Nutritional and Physiological Significance*.

Table 3 Dietary constituents that inhibit nonheme iron absorption

Food	*Active substance*
Wheat bran	Phytate
Tea	Polyphenols
	Tannins
Nuts	Phytates, polyphenols
	Tannins
Legumes	Phytates, polyphenols
Soya proteins	Phytate
Oats	Phytate
Oregano	Polyphenols
Leafy vegetables	Polyphenols
Coffee	Polyphenols
Maize	Phytate
Milk chocolate	Phytate, calcium, polyphenols
Milk, cheese	Calcium + phosphate
Rice	Phytate
Eggs	Phosphoprotein, albumin
Spinach	Polyphenols
	Oxalic acid
	EDTA
Carbonated soft drinks	Phosphates
Food additives	EDTA
Drugs	
Antacids, H2 blockers, proton-pump inhibitors	
Cholestyramine	
Calcium carbonate	
Tetracycline, quinolones, cholesterol	

Adapted from iron, nutritional and physiological significance, The report of the British Foundation's Task Force. (1995) Publ Chapman and Hall 1–186.

stabilized and microencapsulated. Ferrous sulfate is commonly used as an iron supplement (Table 4), while other compounds, such as ferrous lactate, have the advantage of being white and more stable and are unaffected by high humidity. $NaFe^{3+}EDTA$ remains stable during processing and storage and causes fewer organoleptic problems than other water-soluble iron compounds. Hemoglobin is also a form of food although it has low iron content (0.34%) and an intense red-brown color. Lastly, some problems may occur with iron fortification, since various adverse effects with the iron compounds utilized, such as poor solubility, metallic taste, discoloration, stability, speckling development of off-flavors, and fat oxidation, may arise.

Iron Salts and Complexes Used in Medications for Iron Deficiency

Oral Iron Therapy

Table 5 shows the different iron formulations used for (a) oral, (b) intravenous, and (c) intramuscular iron administration.

Table 4 Iron fortification of food stuffs

Product	*Iron form supplement*	*Concentration*
(*A*) *Bakery products and cereal*		
Bread	Ferrous sulfate	10, 20, 30, 40 mg
	Fe–EDTA	Fe per 100 g flour
Wheat bread	Ferrous sulfate heptahydrate	
Cookies	Bovine hemoglobin concentrate	6%
Wheat rolls (100 g flour)	$FeSO_4$, $NaFe^{3+}EDTA$	5 mg per 100 g per day
Whole maize	Ferrous sulfate	
	Ferrous bisglycinate	
	Ferric trisglycinate	
Flour and breakfast cereal	Ferrous iron	1 mg
Stimulated rice	Ferrous sulfate heptahydrate	1:100, 1:200
Weaning cereal	Ferrous sulfate	200–500 mg kg^{-1}
White wheat flour		65 mg kg^{-1}
Milk infant cereal		12 mg l^{-1}
Infant cereal	Ferric pyrophosphate	
	Ferrous fumarate	
Maize	Ferrous sulfate	30 or 60 mg kg^{-1}
	Ferrous bisglycinate	
	Ferric trisglycinate	
	$NaFe^{3+}EDTA$	
(*B*) *Dairy products*		
Infant formula	Ferrous sulfate, microencapsulated[a]	6% Ferrous sulfate + 15% GMS
Milk and dairy products	Ferrous sulfate, microencapsulated[b]	
Cheese	Iron–casein and ferric chloride	
Ras cheese	Iron citrate	40, 80, and 120 mg Fe per kg
	Iron chloride	
	Iron gluconate	
Mozzarella cheese	Casein-chelated iron	25, 50 mg kg^{-1}
	Whey prochelated Fe	
White soft cheese	Electrolytic iron	40, 60, 80 mg Fe per kg
	Ferric chloride	
	Ferrous sulfate	
Havarti-style cheese	Ferrous sulfate	4 mg oz^{-1}
	Ferrous sulfite	
	Ferrous sulfate + ascorbic acid	
Cheddar cheese	Ferric chloride	40 mg kg^{-1}
	Iron–casein	
	Iron–whey protein complexes	
Yogurt	$FeCl_3$	10 mg Fe per 100 ml
	Fe complexed with casein	
	Fe complexed with whey protein	
Flavored yogurt		10, 20, 40 mg kg^{-1} yogurt
Edam cheese	Ferrous chloride, ferrous citrate	150 mg kg^{-1}
	Ferrous gluconate	
Milk	Ferrous sulfate	15 mg l^{-1}
(*a*) *Other products*		
Whole wheat peanuts		16.5%
Cereal products, salt, sugar	$NaFe^{3+}EDTA$	5 g per 100 g
Chocolate drink	Ferrous sulfate	6.3 mg
Turkey meat	Ferrous sulfate	100, 250, 500 mg kg^{-1}
Soy sauce	Sodium feredetate	
	$NaFe^{3+}EDTA$	

[a]Spray-drying using glycerol monostearate as wall material.
[b]Spray-drying using lecithin.

Ferrous forms of iron (sulfate, glutamate, fumarate, and succinate) are frequently administered orally to iron-deficient patients, while ferric compounds (ferric–hydroxide polymaltose complex) and carbonyl iron powder (prepared by chemical decomposition of purified iron pentacarbonyl) are less frequently utilized. Such iron will be absorbed in the duodenum and proximal jejunum although only one-tenth of the dose (100–200 mg per iron per day) is absorbed under normal conditions and, during inflammation, even less. Bioavailability is also reduced when other drugs are coadministered.

Table 5 Oral, intramuscular, and intravenous iron complexes utilized for iron supplementation

(a) *Oral iron medication*			
Iron salt	*Generic name*	*Dosage*	*Elemental iron content*
Ferrous salts	Ferrous sulfate	Tablet 300 mg	60 mg
	Ferrous fumarate	Tablet 300 mg	90 mg
	Ferrous gluconate	Tablet 300 mg	35 mg
	Ferrous succinate	Tablet	92.5 mg
	Ferrous glycine sulfate		
	Ferroglycine sulfate		
Ferric compounds	Nonionic ferric–hydroxide polymaltose complex[a]	Tablet	100 mg
Iron powder	Carbonyl iron	Tablet	50 mg
(b) *Iron formulations for intravenous use*			
Preparation	*Molecular mass* (*kDa*)		*Carbohydrate*
Iron sucrose	43		Sucrose
LMW iron dextran	80–103		Dextran
HMW iron dextran	265		Dextran
Sodium ferric gluconate in sucrose solution	38		Gluconate
Iron carboxymaltose	150		Carboxymaltose
Ferumoxytol	185		Polyglucose sorbitol carboxymethylether
Iron isomaltoside	69		Isomaltoside
(c) *Intramuscular iron preparations*			
Iron sorbitol complex with citric acid			
Iron dextran	100–150		Dextran

[a]Slow-release formulations.

Therefore, administration 1–2 h before meals of any drugs is recommended for such therapy. Care should be taken in that excessive doses of iron are not administered since iron could generate oxidative damage with local and systemic lesions in the digestive tract. Various side effects occur with oral iron supplementation such as nausea, heartburn, epigastric pains, bloating, and constipation or diarrhea. Therefore, slow-release ferrous preparations have been developed to alleviate such symptoms.

Parenteral Iron Therapy

Such iron therapy (intravenously or intramuscularly) is given when (a) oral therapy has failed, (b) oral absorption is seriously compromised, (c) benefit from oral therapy cannot be expected, or (d) fast improvement is required. For intravenous injections, colloidal solutions of spherical or ellipsoidal nanoparticles, composed of an iron core of polynuclear ferric–oxyhydroxide complexes with various carbohydrates, are utilized (Table 5). The carbohydrate capsule stabilizes the gel and retards the liberation of bioactive iron from the core to the plasma and sustains the nanoparticle in colloidal solution. The chemical form and structure of such iron compounds are comparable with those of ferritin, which explains their low toxicity. After parenteral administration, the nanoparticles are phagocytosed, mainly by macrophages of the reticuloendothelial system (Figures 2 and 3).

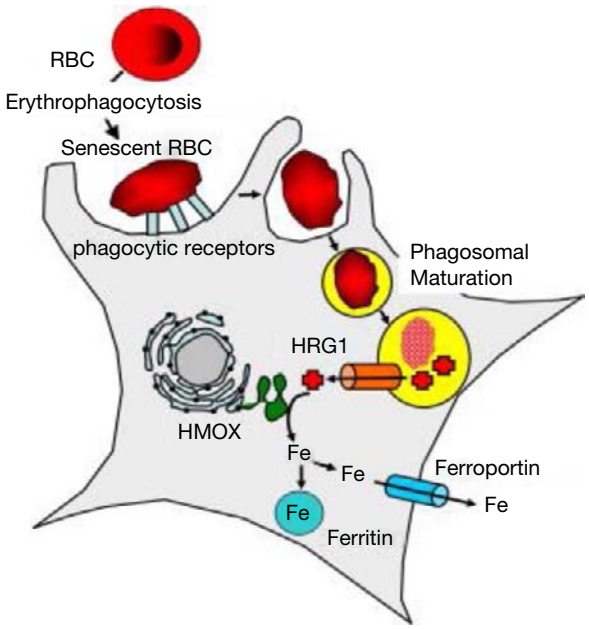

Figure 2 Macrophage heme iron recycling.

Determination of Iron in Food

In order to determine the iron content of food, it is necessary to remove organic matter by ashing techniques to leave only noncombustible ash, which is then analyzed for its elemental composition. Two major techniques are utilized, wet ashing and dry ashing.

Wet Digestion

Samples need to be digested with strong acids, such as nitric, perchloric, or sulfuric acid, in a Kjeldahl flask for many hours.

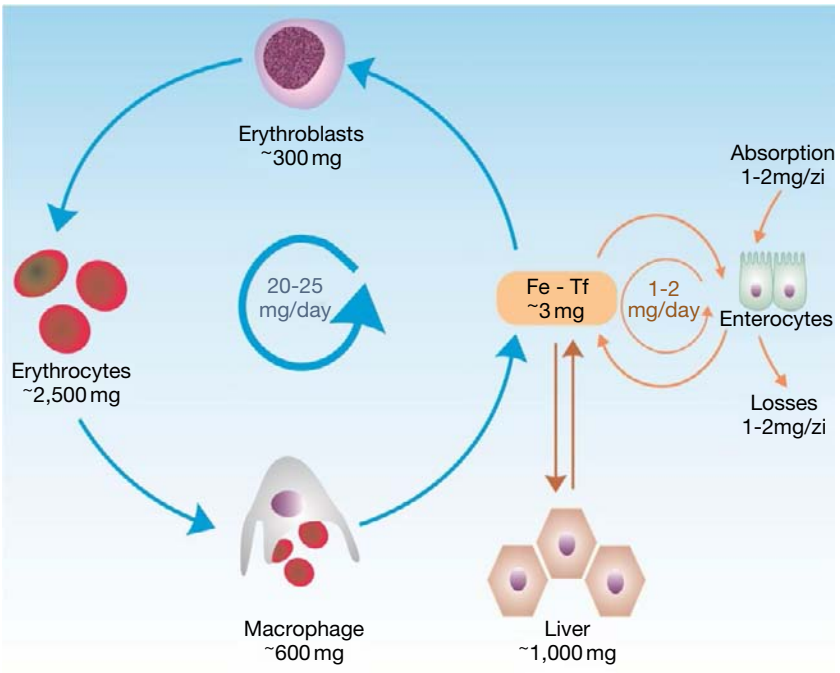

Figure 3 The cycle and distribution of iron in individuals with a balanced iron metabolism. Most of the iron is in the circulating erythrocytes (2150 mg). Daily 20–25 mg iron follows the blue circuit: macrophages, which catabolize heme iron to Fe^{2+}, and erythroblasts, which incorporate iron into newly formed red blood cells. A smaller amount of iron, 1–2 mg per day, follows the orange circuit, losses, and absorption of iron. The balance between the two circuits is maintained by iron stores in the liver, 1000 mg, and in the macrophages, 500 mg, through the small transport pool, 3 mg.

Organic matter will be oxidized to water and carbon dioxide with the iron remaining in an uncomplexed form.

Dry Ashing

Dry ashing is usually performed by placing the sample in an open inert vessel and destroying the combustible (organic) portion of the sample by thermal decomposition using a muffle furnace. Typical ashing temperatures are 450–550 °C. Magnesium nitrate is commonly used as an ashing aid. Charring the sample prior to muffling is preferred, which is accomplished by an open flame.

Analysis: Spectroscopic and Electrochemical Techniques

Spectroscopic Methods

UV/visible absorbance

Both Fe^{2+} and Fe^{3+} form colored complexes. After ashing, the iron will be present as Fe^{3+}. The addition of thiocyanate (SCN^-) will react with Fe^{3+} ions to form a blood red-colored complex

$$Fe^{3+}(aq) + SCN^-(aq) \rightarrow [FeSCN]^{2+}(aq)$$

the intensity of which is measured at 490 nm.

If iron is present as Fe^{3+}, it can be reduced to Fe^{2+} through reaction with hydroxylamine hydrochloride:

$$2Fe^{3+}(aq) + 2NH_2OH(aq) + 2OH^-(aq) = 2Fe^{2+}(aq) + N_2(g) + 4H_2O$$

The presence of Fe^{2+} can be detected in solution after reaction with various compounds such as 1,10-phenanthroline and ferrozine.

Atomic absorption spectroscopy

Both flame atomic absorption spectroscopy (FAAS) and electrothermal atomic absorption spectroscopy (EAAS) can be utilized to analyze iron. For FAAS, the solution containing the iron complexes is introduced into the flame as an aerosol where the atomization occurs. In EAAS, the sample can be placed directly into the small graphite or pyrolytic tube where a sequence of drying, ashing, and atomization is carried out. Free atoms released after the atomization will absorb light at a specific wavelength, which is characteristic of iron.

Inductively coupled plasma atomic absorption spectroscopy solutions containing the solubilized iron complexes are introduced into the plasma as an aerosol and the emissions specific for iron are assayed in a spectrophotometer.

Iron in Biological Systems

There are a considerable number of techniques available for the study of iron in biological systems, some of which are highly specific and others with more general applicability. Such data will give information on the coordination chemistry of the metal ion in relationship to other elements (Table 6).

Table 6 Spectroscopic methods in the study of iron in biological systems

Magnetic susceptibility	*Number of unpaired electrons/ground spin state*
Mössbauer spectroscopy	For Fe57 sites, oxidation and spin state: chemical environment
Electron paramagnetic resonance (EPR)	Usually for odd electron metal sites
Electron-nuclear double resonance	Combines sensitivity of EPR and NMR to probe ligand superhyperfine interaction
Nuclear magnetic resonance (NMR)	For paramagnetic proteins giving structural information
Vibrational spectroscopy (Raman and IR)	Identification of ligands coordinated to a metal center
Electrothermal atomic absorption	Concentration of metal in biological tissue
Electronic absorption spectroscopy (ABS)	Direct probe of ligand field and charge transfer excited states
Magnetic circular dichroism	Greater sensitivity than ABS
Circular dichroism	Allows detection of transitions not readily observable in absorption
Resonance Raman spectroscopy	Study of chromophoric active sites in biological molecule
Extended X-ray absorption fine structure (EXAFS)	Identification of ligand atoms, their distance from metal: number of scattering ligands of a given type
X-ray diffraction	Identification of ligands to metal center more precise that EXAFS

Table 7 Laboratory analysis for the assessment of iron status in a healthy individual

Measurement	*Reference range*
Functional iron	
Hemoglobin	
12–16 g l^{-1} female	
13–18 g l^{-1} male	
Red cell indexes	
MCV	80–94 fl
MCH	27–32 pg
Tissue iron supply	
Serum iron	10–30 μmol l^{-1}
Transferrin saturation	16–60%
Red cell zinc protoporphyrin	<80 μmol mol^{-1} Hb
Red cell ferritin	3–40 ag per cell
Serum transferrin receptor	2.8–8.5 mg l^{-1}
Iron stores	
Tissue biopsy iron	3–33 μmol g^{-1} dry wet
Serum ferritin	15–300 μg l^{-1}
Urine chelatable iron	>2 mg $24\ h^{-1}$
Serum TIBC/transferrin	47–70 μmol l^{-1}

Table 7 shows the various laboratory tests to ascertain iron status in an individual, with respect to functional iron, for example, hemoglobin; tissue iron supply, for example, transferrin saturation; and storage iron, for example, ferritin (Figures 4 and 5).

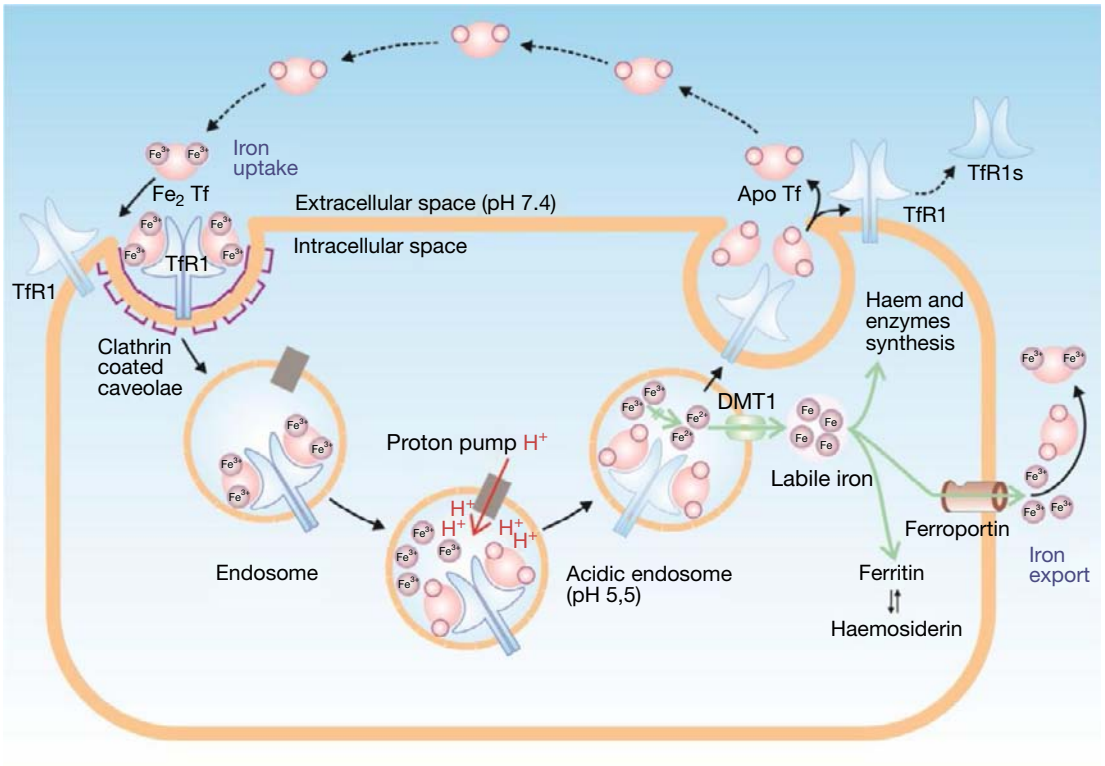

Figure 4 The iron access to the cells via the transferrin (Tf)–transferrin receptor 1 (TfR1) complex. DMT1, divalent metal transporter 1; STEAP, STEAP family of metalloreductase.

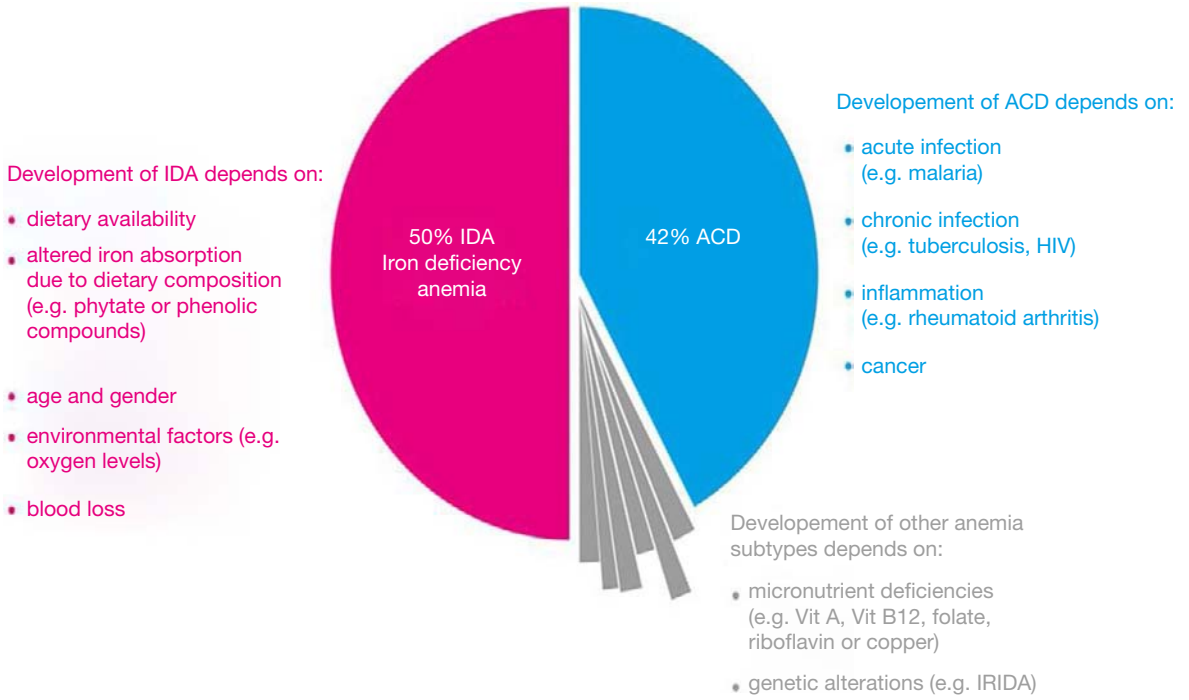

Figure 5 Prevalence of anemia in the population worldwide.

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Irradiation of Foods: Processing Technology and Effects on Nutrients: Effect of Ionizing Radiation on Food Components

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Introduction

Irradiation of Food

The world food needs require more advanced processing techniques and storage since there are increasing losses caused by the absence of appropriate technologies. The process of food irradiation is able to facilitate this demand because it preserves the food's chemical, physical, and sensory components.

The use of ionizing radiation for food preservation arose after the discovery of radioactivity in 1895. In 1905, patents were filed in the United States and Britain for the use of irradiation in foods. World War II contributed to the development of technologies related to irradiation area. The first commercial use of ionizing radiation in food occurred in Germany in 1957, in spices, with the use of irradiation with electrons in condiments. In 1980, the Food and Agriculture Organization, the International Atomic Energy Agency and the World Health Organization (FAO/IAEA/WHO) published 'food irradiated with doses up to 10 kGy had no toxicological effects or nutritional changes.'

Ionizing radiation includes gamma rays (from radioactive isotopes of Co and Cs), beta rays generated by electron accelerators, and X-rays. These sources do not have sufficient energy to induce radioactivity in food, but have enough energy to remove electrons from atoms and form ions or free radicals. These free radicals interact with other chemical bonds in different molecules present in the food, including microbial DNA and insects, promoting the impossibility of survival and reproduction. The radiation dose is the quantity of absorbed ionizing radiation, which the measurement unit is Gray (Gy). A Gray is equivalent to a $J\,kg^{-1}$ or 100 and 1 rad is equal to 100 erg g^{-1}.

The main advantage of ionizing radiation use in food is a total absence of direct use of chemical compounds that may leave residues in treated food, making the food unsafe for consumption. Therefore, ionizing radiation use in food attracted interest around the world from various organizations such as the IAEA, FAO, Organization for Economic Cooperation and Development (OECD), and WHO along with the participation of 24 countries in studies to unravel the changes that are caused in the food with the use of radiation.

Since 1988, the WHO recommends the use of this process, and in the 90s, following experiments with food irradiation, concluded that few foods tolerate doses above 10 kGy without loss of sensory quality. Studies with animals fed with irradiated diet, however, showed no adverse effect to health was observed, even at high doses of 70 kGy.

Radiolytic Effects on Foods

Basic Considerations

The aim of the food irradiation process is comparable with other preservation methods. The main purpose is the destruction of microorganisms and to create a longer shelf life for the food. The process is capable of sterilizing meat or meat products (high dose, 10–50 kGy); of extending the useful life of fresh fish and chilled chicken, fruits, and vegetables (mean doses, 1–10 kGy); of inhibiting sprouting bulbs and tubers; of delaying the ripening of fruits; and of killing insects in grains and cereals (low doses, below 1 kGy).

As with heat treatments, chemical changes are induced in foods as a result of treatment with the irradiation process.

The energy of radiation can cause chemical reactions. If the irradiated material is a food, some chemical changes are expected, and the extension of these increases with increasing the radiation dose.

When energetic electrons pass through a food they cause:

Primary Chemical Effects

Excitation of Molecules

There are two mechanisms for the production of excited molecules by ionizing radiation:

a. Direct: $A \rightarrow A^*$
b. By neutralization of ions: an excited ion $(A+)^*$ can be formed at the time of ionization: $A \rightarrow (A^+)^* + e^-$

Neutralization can occur subsequently and produce the excited molecule A*: $(A^+)^* + e^- \rightarrow A^*$

An excited molecule can retain its extra energy for a period of about 8–10 s. The loss of excitation energy can occur in many ways and the reactions can produce new products.

The new products formed may be stable molecules, such as molecules or reactive entities like free radicals.

A *Free radical* is an atom or molecule that has one or more unpaired electrons that are available to form a chemical bond; are designated with a point $\rightarrow \bullet(AH)$ or $\bullet E$.

Ionization

Normally, an ion reacts with another of opposite sign to produce a neutral entity $\rightarrow A^+ + B^- \rightarrow C$.

$$A^+ + e^- \rightarrow A$$

Ions can exist as a transitory ion–molecule complex, which under neutralization can form new compounds.

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Secondary Chemical Effects

The net result of the primary chemical effects is the formation of free radicals or excited molecules.

The new molecules formed in the primary effect can react with themselves or with other food molecules as well as free radicals.

Because of the high reactivity of the free radicals produced in the primary effect, secondary chemical effects can occur. These effects result from several types of reactions of free radicals, including: adding, combining, dissociation, recombination, disproportionation (dismutation), and electron capture.

The complete process that leads to stable final products as a result of irradiation is called radiolysis, and the products of the primary and secondary effects are the radiolytic products. This process occurs in fractions of microseconds. Because some final products are not stable, post-irradiation effects may occur in some systems for days or months.

Free radicals do not only occur in irradiated products. Several biochemical reactions in plants and animals, as well as in drying and heating, produced free radicals.

The instrument that detects free radicals is the electron resonance spectrometer.

Effects of Ionizing Radiation on Water

Water is present in nearly all foods. These proportions are roughly 90% in vegetables; 80% in fruits; 60% in meat; and 40% in breads. Dried products contain some water: wheat flour 13%; dried vegetables around 10%; and nuts 5%. The water radiolysis is therefore of particular interest in food irradiation.

When pure water is irradiated, the radiolytic products formed are:

●OH radical hydroxyl; e^-_{aq} aqueous electron; ●H hydrogen atom; H_2 hydrogen; H_2O_2 hydrogen peroxide; H_3O^+ proton hydrated.

The electron e^- and H^+ hydrogen ion cannot exist freely in water. They are associated with water molecules.

Final Products of Water Radiolysis

While ●OH, e^-_{aq} and ●H are highly reactive transient species, hydrogen and hydrogen peroxide are the only stable end products of water radiolysis, because of the reactions.

$$H_2O_2 + e^- \rightarrow \bullet OH + OH^- H_2 + \bullet OH + H_2O + \bullet H$$

Hydrogen and hydrogen peroxide are widely consumed. They are therefore produced in low yield, even when irradiated with high doses. When the water is saturated with oxygen, it can greatly enhance the production of hydrogen peroxide.

The formation of hydrogen peroxide has great significance in irradiated foods. Like all foods contain substances that can oxidize or reduce, many reactions can occur when foods containing water are irradiated.

Influence of oxygen

The presence or absence of oxygen during the irradiation process may have an important influence on the radiolysis course. The water, when in equilibrium with the oxygen in the air, contains a low concentration of oxygen (about 0.27 mM at room temperature). Hydrogen atoms can reduce the oxygen to a hydroperoxyl radical: $\bullet H + O_2 \rightarrow \bullet HO_2$ which is an oxidizing agent. This is in equilibrium with a superoxide anion: $\bullet HO_2 \rightarrow H^+ + \bullet O^-_2$.

Both hydroperoxyl radicals and superoxide can form hydrogen peroxide:

$$2 \bullet HO_2 \rightarrow H_2O_2 + O_2 \rightarrow \bullet O^-_2 + HO_2 + H^+ \rightarrow H_2O_2 + O_2$$

The hydrogen peroxide formation in irradiated aqueous systems depends on the presence of oxygen. Very little H_2O_2 is produced when oxygen is excluded.

Several other radicals are produced when foods are irradiated. Oxygen can form some of these radicals, known as peroxides.

$$\bullet R + O_2 \rightarrow \bullet RO_2$$

To prevent the formation of these radicals may irradiate the products under vacuum, in nitrogen, or other shielding gases.

The extent the exclusion of oxygen will benefit an irradiated food varies according to its chemical composition, particularly its fat content, but also according to other factors, such as temperature at the radiation process and the time of storage after irradiation. Foods containing lipids are particularly affected by O_2 during irradiation and can develop strange and rancid flavors. The presence of oxygen is very important in the production of damage during irradiated foods storage: their exclusion preserves nutrients.

The retention of vitamin E during storage is considerably improved by the exclusion of oxygen, an effect that is more pronounced in irradiated foods than non-irradiated.

Effect of temperature

The temperature during irradiation process also influences the extent of radiolytic changes. The cold can have a big effect on protection. Generally, damage is greater when irradiating at room temperature. In the frozen state, the rates of diffusion of free radicals and the reactivity of gatherers are drastically reduced. A notable increase in protection against radiation damage becomes evident near −20 °C.

Little benefit is obtained by radiating at low temperatures when the aim is pasteurization (doses lower than 10 kGy) where vegetative cells are the primary targets of treatment. The improvement in quality obtained by irradiation in the frozen state is lost by the high doses required to inactivate bacteria, but bacterial spores are more resistant to radiation and are usually not very protected by the freeze. So, for sterilization by irradiation, where the aim is to kill all microorganisms, including spores, there is a considerable benefit that is obtained by irradiating frozen foods.

These effects are advantageous both organoleptic and nutritionally.

Influence of pH

The pH of aqueous systems is another factor that can influence the outcome of radiation because the equilibrium reactions are

pH dependent. An acidic environment may favor the disappearance of e^-_{aq} for the first reaction,

$$e^-_{aq} + H^+ \rightarrow \bullet H$$

while a basic environment can favor the formation of the second e^-_{aq}

$$\bullet H + OH- \leftrightarrow e^-_{aq}$$

Effect of dilution

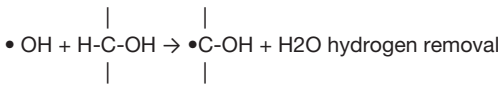
When dilute solutions are irradiated, the extent of degradation of the solute depends on the number of reactive radicals which will react with the molecules of the solute. The most crucial differences between the radiation chemistry of pure substances and complex food is rather quantitative than qualitative. While diluted aqueous solutions of amino acids, enzymes, sugars, and vitamins are commonly quite sensitive to radiation, these same compounds are much more stable when irradiated as a constituent food.

Effects of Radiation on Ionizing Other Food Components

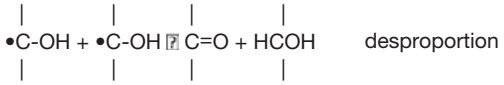
Besides water, the major components of food are carbohydrates, proteins, and lipids.

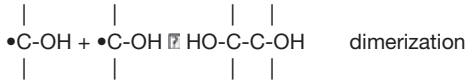
Carbohydrates

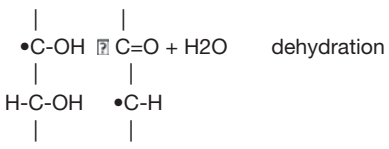
In the presence of water, ●OH radicals especially attack carbohydrates. The ●OH radicals predominantly abstract hydrogen from C–H bonds, forming water:



The resulting radicals react through various mechanisms:







Depending on the C═O molecular position formed by disproportion or dehydration, the resulting product can be an acid, a ketone, or an aldehyde. Thus, removing H of C − 1 of the glucose can induce to gluconic acid.

Since ●OH radicals can remove hydrogen from all six carbon atoms of glucose, a variety of products can be formed.

When disaccharides or polysaccharides are irradiated, the reactions observed with monosaccharides may also occur. Additionally, the glycosidic bonds that connect the monosaccharide units can be broken. Dextrin, maltose, and glucose are produced. This reduction in the degree of polymerization reduces the viscosity of the polysaccharide solutions. The solubility increases with increasing irradiation dose. Quite different results may be obtained irradiating granulated sugar instead of an aqueous sugar solution. The pharmaceutical industry uses crystalline sugar as a carrier of drugs. This sugar is sterilized. When carbohydrates are irradiated as components of food, they are much less sensitive to radiation than in pure form. The protein has a protective action on carbohydrates.

Proteins

Proteins consist of chains of amino acids connected by peptide bonds. The discussion of the effect of radiation in proteins is based on the study of the radiation chemistry of amino acids. Initiated reactions with hydrated electrons are the major route in the radiolysis of amino acids and proteins. When proteins are irradiated in the presence of water, all of the reactions that are possible with amino acids are also possible with proteins containing these amino acids. With 20 constituent amino acids and proteins with three reactive species of water radiolysis, many complex interactions are possible. Additional effects are exerted by the spatial configuration of the protein chain, determined by hydrogen bonds, disulfide bonds, hydrophobic bonds, and ionic bonds. Alone, amino acids, which are susceptible to attack by radicals when irradiated, are less sensible when they are part of the protein structure and are more or less inaccessible to reactions with radicals. Another factor that likely contributes to the increased strength compared with the protein isolated amino acid sequence is, due to a greater or lesser rigidity of the spatial structure of the protein, radicals formed as a result of irradiation molecule are safe in the position and have a high chance of recombination.

A large proportion of radiant energy deposited in irradiated proteins apparently advances to denaturation, changes in secondary and tertiary chains, before the destruction of the amino acid constituents. This denaturation is much less extensive than that caused by heat. This is because sterilizing radiation in food for long time storage combines with heat treatment. Enzymes are more sensitive to heat.

Lipids

In the presence of oxygen, irradiation can also accelerate the autoxidation of fats. This may be because the irradiation accelerates one or more of the following reactions: free radical formation, which can combine with oxygen to form hydroperoxides; breaking hydroperoxides, giving action to various decomposition products, particularly carboxyl compounds; and destruction of antioxidants.

Lipids are usually exposed to irradiation as food constituents coexisting with others to a greater or lesser amount. The

radiolytic products obtained in isolated lipids are found when they are part of food, but in smaller proportions, and the effect of radiation is divided among all the constituents of the food.

It has been reported that when triglycerides were irradiated with very high doses 250 kGy, volatile and non-volatile products formed were similar to those obtained by heating and frying 180 °C for 1 h. Irradiation results in partial destruction of fat soluble vitamins, and tocopherol is particularly sensitive.

Main Types of Food Irradiation

Radiopasteurization

Related to decontamination and destruction of bacteria and parasites, this term refers to the treatment of foods by applying a dose of ionizing radiation in the range from 2 to 8 kGy sufficient to reduce the number of viable pathogenic non-sporulating bacteria at such a level that none is detectable in the treated food when it is examined by any known bacteriological method.

Radurization

When applied to food at a dose of ionizing radiation around 0.4–10 kGy it is enough to reduce the number of pathogenic microorganisms and contaminants and to promote the decontamination of harmful insects to the food, thereby increasing their quality and prolonging the storage period without affecting the sensory characteristics. This process is considered ideal for use in products that are in nature and can also be used to preserve seafood, dried fruit, and some meat products.

Radapertization

This term corresponds to food characterized as 'sterile commercially,' because the radiation within a range of dose above 10 kGy, considered high by experts, is applied in order to reduce the number of activity of pathogenic microorganisms, such that few or none, with the exception of viruses, are detectable by the methods of analysis recommended, independent of time and under what conditions food is stored, provided that it is properly packed.

The doses of radapertization that are submitted to foods like fish, raw and cooked meats, vegetables, dehydrated foods, salt, sugar, herbs, and spices, among others, are high doses above 10 kGy, which sterilizes food for a variety of uses, such as feeding astronauts during space travel, hospitalized immunocompromised patients, hikers, boaters, and other sportsmen.

The Safety of Irradiated Food

Food irradiation has been the subject of intense research for more than 40 years. International organizations such as FAO and the WHO reviewed this research and concluded that food irradiation is safe and beneficial. Similarly, the nutritional value of irradiated foods was compared with the food processed by other methods, with favorable results.

In 1983, the Codex Alimentarius Commission, a group of the United Nations, that develops international food standards, concluded that foods irradiated below 10 kGy do not show toxicological risk. Currently, treatment levels within this range are used throughout the world. Neither gamma energy, nor international levels established for electron beams can cause food to become radioactive. The radiation processing does not make food radioactive in the same way that X-rays used for security at airports do not make radioactive baggage.

Storage Post-Irradiation

Changes in composition during storage deserve special attention in the case of fresh fruits and vegetables. Irradiated or not, plant metabolism materials are constantly changing, which can be accelerated by factors such as mechanical damage, low temperature storage, or modified atmosphere. Depending on the applied dose and the product to be considered, the irradiation can accelerate or decelerate the metabolic changes. At low radiation doses, tolerated by fresh fruits and vegetables – usually below 2 kGy and in some cases below 1 kGy – direct chemical effects are minimal in vitamins.

Heating

Another factor is the possibility of loss of vitamins during the cooking. In oat bran, the losses of vitamin B1 (thiamine) caused by heating for 10 min in oven at 100 °C, were always higher than in the irradiated samples. After a storage period of about 6 months unheated, the sample had 50% loss of B1 vitamin, smaller than the non-irradiated control, whereas after heat treatment, irradiated samples had lost about 70% less the non-irradiated control.

Low Temperatures

Radio-sensitive vitamins cannot be protected only by the exclusion of oxygen, but also by irradiation at low temperatures. Although the losses of vitamins increase with the increasing of radiation dose, food irradiated with high doses always requires processing conditions which minimize unpleasant sensory effects. Oxygen must be excluded, that is, the vacuum packaging and irradiation is conducted at cryogenic temperatures. Under these circumstances, even vitamins usually considered radio-sensitive can be well protected.

Dose Rate

There are reports of better retention of vitamins when irradiating by electron beam than with gamma radiation. Research using 60 kGy at −45 °C found retention of 60% in electron beam, while gamma radiation was 35%. It was explained that the best retention was in meat irradiated by electrons as a result of the higher rate of dose released by the electron beam, which produces a situation of free oxygen in the irradiated sample.

Liposoluble Vitamins

Vitamin A (retinol) and pro vitamin A (carotenoids)

In early studies of the effects on food vitamins, researchers observed a loss of vitamin A in different products irradiated

at a dose of 4.8 kGy: milk 70%; butter 68%; cream cheese 54%; cheddar 47%; and margarine 14%. When they treated porcine liver with 5 kGy at 0 °C, it contained 4% less retinol than the non-irradiated control after 1 week and 13% less after 4 weeks.

The liposoluble vitamins in decreasing order of radiation sensitivity are: Vitamin E > carotene > Vitamin A > Vitamin D > Vitamin K.

Vitamin E (alpha tocopherol)

The radiation sensitivity of this vitamin depends on the presence of oxygen. Researchers found no loss of vitamin E in meat irradiated with 30 kGy and nitrogen, but observed a loss of 37% when the meat was irradiated in the presence of air. High temperatures also influence the losses of this vitamin.

The main sources of vitamin E in human nutrition are butter, margarine, vegetable fats, and oils. None of these foods are indicated to irradiation. Therefore, despite the radiation sensitivity of this vitamin, it is difficult to imagine a situation where irradiated foods would supply any deficiency of vitamin E.

Vitamin K

Many species of bacteria synthesize vitamin K. This also occurs in the human gut (except for in newborn children and patients treated with antibiotics), so they are independent of the diet for the supply of vitamin K. Vitamin K is present in fresh green leafy vegetables. Liver contains comparable concentrations. Most other food sources are poor.

Studies have shown that vitamin K is more resistant to radiation of fat-soluble vitamins. Broccoli, kale, spinach, and other vegetables irradiated with between 28 and 56 kGy, and stored for 9–15 months at room temperature, showed no loss of activity of vitamin K.

Water Soluble Vitamins

Vitamin B1 (thiamine)

This water-soluble vitamin is more sensitive to radiation. A research showed an increase of losses of this vitamin with increasing of the radiation dose, and losses are smaller after irradiation at low temperatures. Similar loss of thiamine can be observed in various types of meat. The losses of thiamine in irradiated foods become more apparent after storage and cooking. The level of this vitamin loss is directly proportional to the radiation dose applied into the sample. Even so, their losses are minimum when compared the other processes of conservation of foods. Those losses do not get to take the tianina deficiency to arrive to the clinical condition of a cerebral lesion potentially irreversible, or even to take to the lack condition that can cause some physiological and morphological alterations.

Vitamin B2

The combination of irradiation and cooking can occasionally result in improvement in retention of the vitamin. The time required for cooking dried vegetables can be considerably shortened by irradiation. Breakfast products are the largest source of vitamin B2 in human nutrition, and are generally considered unsuitable for irradiation because they develop a bad flavor. In order to have breakfast products for patients in immunosuppressive therapy, radiation techniques, such as keeping these products in a nitrogen atmosphere at −78° C, have been developed, providing a dose of 40 kGy in the breakfast dietary for these patients.

Vitamin B12

This vitamin seems to be pretty stable for irradiation. No loss was observed in irradiated haddock filets at 25 kGy, and several species of fish irradiated with doses of 30 kGy.

Folic acid

No loss of folic acid in radiation sterilized meat has been presented by some researchers, as well as with poultry meat.

Vitamin C

The major sources of vitamin C in human nutrition are fruits and vegetables. The total activity of vitamin C is the sum of ascorbic acid (AA) and dehydroascorbic acid (DHAA). In the human body, DHAA has the same vitamin activity of AA. Fresh agricultural products contain many AA and researchers analyzed only AA as a measure of vitamin C. However, both AA and DHAA and AA and DHAA together can change dramatically during storage and processing.

The sensitivity of the vitamins varied depending on the process conditions in which irradiate the food. Vitamins C and B1, the most sensitive and water soluble; vitamins E and A are the most sensitive among the fat soluble vitamins. However, during irradiation, these changes can be minimized simply by changing process conditions, such as the use of controlled atmosphere packaging.

Vitamin C (AA) in solution is very sensitive to irradiation, but in fruits and vegetables it is already more stable at low doses. AA by irradiation becomes DHAA, which is active, such as vitamins.

Effect of Enzymes

Existing enzymes in foods must be inhibited before they undergo radiation, because these are resistant. This inactivation must be realized through heat treatment, this form being stronger than that used for microorganisms, because enzymes are stronger than microorganisms. Irradiated foods are unstable during storage due to their sensitivity to enzymatic attack.

The high resistance to irradiation of enzymes is demonstrated by the milk phosphatase, which is not destroyed by the irradiation doses used for the sterility of the milk. Enzymes are affected by the indirect effects of free radicals formed in the solvent phase effects. Thus, the diluted solutions of enzymes are relatively more sensitive to radiation than concentrated solutions. In addition, enzymes in their natural environments, such as in foods, are relatively resistant. The enzyme activity is affected in doses used for food conservation, and thus extend the shelf life of fruits and vegetables.

Conclusion

Irradiation has been the subject of intense and rigorous studies for decades. It is a safe and effective method of processing. The products have good quality, in particular, the level of food security and conservation. The nutritional value, compared

with other foods that have undergone such a process, reveals favorable results. Conservation methods, such as heating, can cause much greater reductions on food's nutritional value.

The lack of information about this technology and its benefits has led to many controversies. Correct information must be guaranteed, scientifically based, so that consumers can make informed choices. The labeling of irradiated products provides the decision to buy them or not.

The market for irradiated products is still small, including in countries where this process of conservation is allowed. The legal and safety aspects of the process, the factors of equipment, costs, and consumer acceptance contribute to their low marketing.

In summary, the macronutrients – proteins, fats, and carbohydrates – are not significantly altered by irradiation in terms of nutritional value and digestibility. Micronutrient and some of the vitamins are susceptible to irradiation, which greatly depend on the composition of the food as well as its processing and storage. Whether or not the losses of vitamins in irradiated food are of great importance will depend on the circumstances, such as the contribution that food makes the total diet.

Taking these factors into consideration, it seems that thiamine is the only vitamin that may require special attention, because it is highly sensitive to radiation, and because foods that contribute significantly to supply this vitamin, such as pork meat, are candidates for irradiation process in a large scale. However, even in the case of thiamine, there is no reason to assume greater nutritional consequences of irradiation.

We can also say that potential nutrient losses in irradiated foods are not basically different from losses in food undergoing other treatment processes. Rather, heating, drying, and using other traditional methods can cause loss of nutritional value higher than with the use of irradiation.

See also: *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; Fumigants; Pasteurization: Principles and Applications.

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Ketone Bodies

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Introduction

The ketone bodies comprise three small, water-soluble compounds produced by the liver in response to reduced glucose availability. Their formation may signify prolonged fasting or a pathophysiological state, such as type I diabetes (insulin-dependent diabetes mellitus). As their synthesis is linked to the metabolism of fatty acids, certain dietary regimes can induce ketone body formation, either as a means of weight loss or to control certain neurological conditions such as refractory epilepsy. In a more contemporary context, novel compounds have been developed, which enhance the formation of ketone bodies *in vivo*, and have been suggested to improve athletic performance.

Brief Review of Metabolism

In the normal, well-fed state, glucose is the primary substrate for energy production, achieved through the three well-known pathways glycolysis, tricarboxylic acid (TCA) cycle, and electron transport chain. Following digestion, the liver receives the first pass of dietary glucose, initially used not only for ATP production but also for synthesis of storage forms of glucose such as glycogen. As humans have the capacity to consume food at a faster rate than is required for survival, the excess glucose is also converted to palmitic acid, which is esterified to triacylglycerol and packaged as nascent very low-density lipoproteins, which are secreted into the circulation. This is distinct from dietary fat, which is packaged in the intestines as chylomicrons; both lipoproteins, however, have the same eventual metabolic fate – they circulate in the blood until they are acted upon by lipoprotein lipase, an enzyme particularly abundant in adipose tissue, which hydrolyzes triacylglycerols to free fatty acids, which are taken up by adipocytes and reesterified for storage.

When blood glucose levels are low, for example, during fasting, lipolysis of triacylglycerols stored in the adipose tissue yields free fatty acids, which are used in preference to glucose by many tissues, most notably in the heart and muscle where fatty acids inhibit glycolysis. In the liver, fatty acids are oxidized through β-oxidation to produce acetyl-CoA, a thioester that plays a central role in metabolism. The acetyl-CoA produced by hepatic β-oxidation provides most of the ATP required for gluconeogenesis; however, there is a large surplus of acetyl-CoA that is converted to ketone bodies in the mitochondria of perivenous hepatocytes. Ketone bodies represent a usable form of lipid-derived energy, as they can penetrate the blood–brain barrier and supply the brain with a source of energy (fatty acids cannot pass the blood–brain barrier) (Figure 1).

Ketone Body Metabolism

General Features

The three endogenous ketone bodies, acetoacetic acid, (*R*)-3-hydroxybutyric acid, and acetone, are structurally simple compounds derived from acetyl-CoA (Table 1). Ketones as a whole are more acidic than the corresponding alkane owing to the resonance stabilization of the enolate ion (Figure 2). Therefore, we see from the values of their pK_a that both acetoacetic acid and (*R*)-3-hydroxybutyric acid are present in their conjugate base forms at physiological pH, that is, acetoacetate and (*R*)-3-hydroxybutyrate. As acetoacetate is a β-keto acid, it undergoes spontaneous decarboxylation to give the more stable keto isomer, in this case acetone. When the levels of acetoacetate are particularly high, the enzyme acetoacetate decarboxylase can also mediate this reaction. As acetone has a relatively high vapor pressure (boiling point 56.5 °C), it is released on exhalation. This accounts for the often fruity smell on the breath of individuals who are in a state of ketosis.

As outlined briefly in the previous section, under hypoglycemic conditions, when the levels of acetyl-CoA are high due to increased β-oxidation, acetyl-CoA is converted to acetoacetyl-CoA by the ubiquitous enzyme 3-ketothiolase (also known as β-ketothiolase or 2-methylacetoacetyl-coenzyme A thiolase). This enzyme catalyzes the Claisen condensation of two moles of acetyl-CoA, forming acetoacetyl-CoA and free CoA (Figure 3):

$$2\text{Acetyl-CoA} \rightleftharpoons \text{Acetoacetyl-CoA} + \text{CoA}$$

$$K_{eq} = \frac{[\text{Acetoacetyl-CoA}][\text{CoA}]}{[\text{Acetyl-CoA}]^2} = 6.0 \times 10^{-5}$$

The equilibrium constant for this reaction is lower than expected ($\Delta G^o \approx 24$ kJ mol^{-1}), so to shift the position of equilibrium to favor the products, the 3-ketothiolase is bound to the second enzyme in ketogenesis, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase, which converts the recently formed acetoacetyl-CoA to HMG-CoA virtually instantaneously. As this

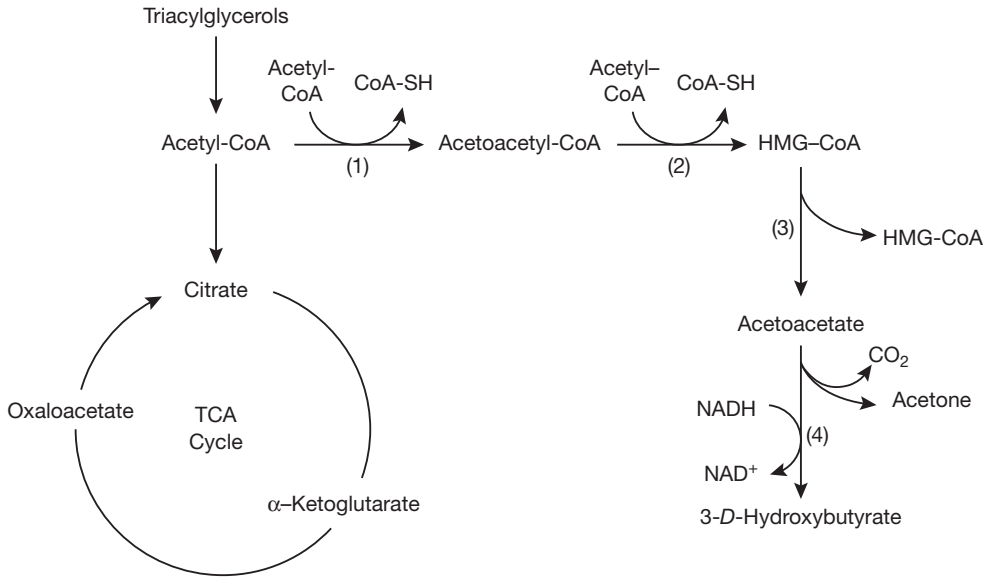
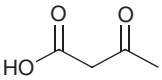
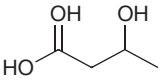


Figure 1 Overview of metabolism. Acetyl-CoA plays a crucial role in ketone body synthesis. It initially undergoes conversion to acetoacetyl-CoA through the action of 3-ketothiolase (1), which is then converted to HMG-CoA, mediated by 3-hydroxy-3-methylglutaryl-CoA synthase (2). The HMG-CoA undergoes cleavage by HMG-CoA lyase (3) to give acetoacetate, which may spontaneously decarboxylate to (*R*)-3-hydroxybutyrate or be converted by acetoacetyl-CoA deacetylase (4).

Table 1 Physicochemical properties of the ketone bodies

	Acetoacetic acid	*(*R*)-3-hydroxybutyric acid*	*Acetone*
IUPAC name	Oxobutanoic acid	3-Hydroxybutyric acid	Propanone
Structural formula			
Relative formula mass (g mol^{-1})	102.88	104.10	58.08
pK_a	3.58	4.70	19.2
Reference range (blood) (mM)	<4	<4	<0.01

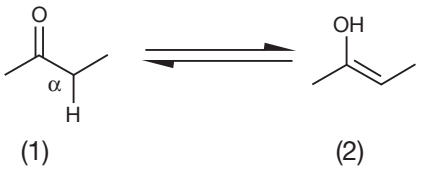


Figure 2 Keto–enol tautomerism. The keto form (1) exists in equilibrium with the enol form (2) such that the α-hydrogen of the former is lost, causing a rearrangement of bonds, leading to the enol tautomer.

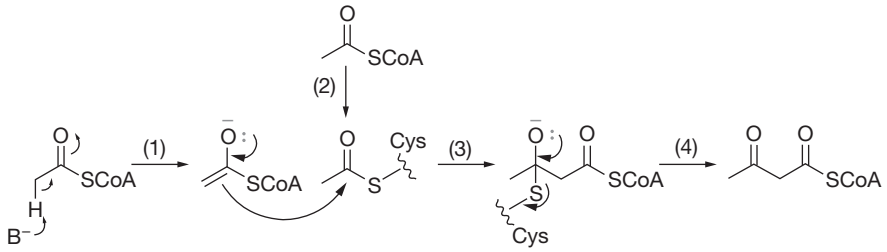


Figure 3 Mechanism of acetoacetyl-CoA formation. The formation of acetoacetyl-CoA involves a so-called ping-pong mechanism within the active site of 3-ketothiolase. Initially, one molecule of acetyl-CoA is deprotonated, forming an enolate (1). This is then combined with a second molecule of acetyl-CoA (2) in a nucleophilic attack mediated by a catalytic cysteine residue (3). The tetrahedral intermediate then rapidly collapses, expelling the acetoacetyl-CoA.

latter reaction is associated with the hydrolysis of a high-energy thioester linkage, the overall reaction has a much higher equilibrium constant, further compensating for the unfavorable equilibrium in the earlier step ($\Delta G^{o} \approx -0.7$ kJ mol^{-1}):

$$3\text{Acetyl-CoA} + H_2O \rightleftharpoons \text{HMG-CoA} + 2\text{CoA}$$

$$K_{eq} = \frac{[\text{HMG-CoA}][\text{CoA}]^2}{[\text{Acetyl-CoA}]^3[H_2O]} = 1.33\,\text{dm}^3\,\text{mol}^{-1}$$

HMG-CoA has two potential metabolic fates: the first is to undergo reduction to mevalonate by HMG-CoA reductase, the rate-limiting step in isoprenoid biosynthesis, and the second is to undergo cleavage by HMG-CoA lyase to form acetoacetate and acetyl-CoA. If this reaction occurs in the cytosol, the first pathway is favored; if it occurs in the mitochondria, the second pathway occurs. This level of control originates from a slight difference in the structure of the HMG-CoA synthase found within the mitochondria. The gene encoding the mitochondrial form of HMG-CoA synthase (HMGCS2) has three steroid-responsive elements, such that fatty acids increase its transcription. This ensures that HMG-CoA produced during hypoglycemia is directed toward ketogenesis rather than mevalonate synthesis. The cleavage of HMG-CoA to acetoacetate and acetyl-CoA is the primary route for synthesis of acetoacetate; however, acetoacetyl-CoA can alternatively be directly deacetylated to acetoacetate by acetoacetyl-CoA deacetylase. This pathway accounts for a very small proportion of the acetoacetate produced as the K_m of the acetoacetyl-CoA deacetylase is high in comparison to the concentration of acetoacetyl-CoA found in hepatocytes. Acetoacetate is converted to (*R*)-3-hydroxybutyrate by the action of (*R*)-3-hydroxybutyrate dehydrogenase. The relative proportions of acetoacetate and (*R*)-3-hydroxybutyrate can be anywhere in the region of 1:1–1:10 depending on the redox state of the mitochondria (i.e., the ratio of NADH to NAD$^+$).

Many tissues can utilize ketone bodies as metabolic fuel and their small molecular mass ensures that they can cross the blood–brain barrier. The breakdown of ketone bodies (ketolysis) is initiated by the conversion of acetoacetate back into acetoacetyl-CoA by succinyl-CoA-oxoacid transferase (SCOT), the rate-limiting step in this process (Figure 4). The acetoacetyl-CoA so produced is then cleaved by methylacetoacetyl-CoA thiolase to produce acetyl-CoA, which is funneled into the TCA cycle for ATP production. The shift in the equilibrium mediated by (*R*)-3-hydroxybutyrate dehydrogenase as a consequence of the consumption of acetoacetate leads to the conversion of (*R*)-3-hydroxybutyrate to acetoacetate, which is then metabolized as outlined earlier.

Energetics of Ketone Body Metabolism

Experimental models suggest that ketone body metabolism operates at higher efficiency than glucose metabolism. In isolated rat perfusion models, ketone bodies increased the efficiency of cardiac muscle by ~28% when compared to glucose as fuel. We can rationalize this observation by considering the fundamental thermochemistry of the two principal substrates for energy production: pyruvate and (*R*)-3-hydroxybutyrate. The enthalpy of combustion (ΔH^{o}) for pyruvate is -278.5 kcal mol^{-1}, while that of (*R*)-3-hydroxybutyrate is -487.2 kcal mol^{-1}. The greater amount of energy released on combustion of (*R*)-3-hydroxybutyrate is a consequence of the greater number of C–H bonds. Since (*R*)-3-hydroxybutyrate is more reduced than pyruvate, it has the potential to increase the difference in redox potential between the mitochondrial NAD couple and the coenzyme Q couple. The greater this difference, the greater the ATP production. In essence, ketone bodies act as a 'superfuel' for key metabolic processes.

Role of Ketogenesis During Starvation

The importance of ketone bodies was truly highlighted in the 1960s by George Cahill and colleagues working at Harvard. Their work focused on metabolic adaptations to starvation and essentially proved that ketone bodies are normal fuels of

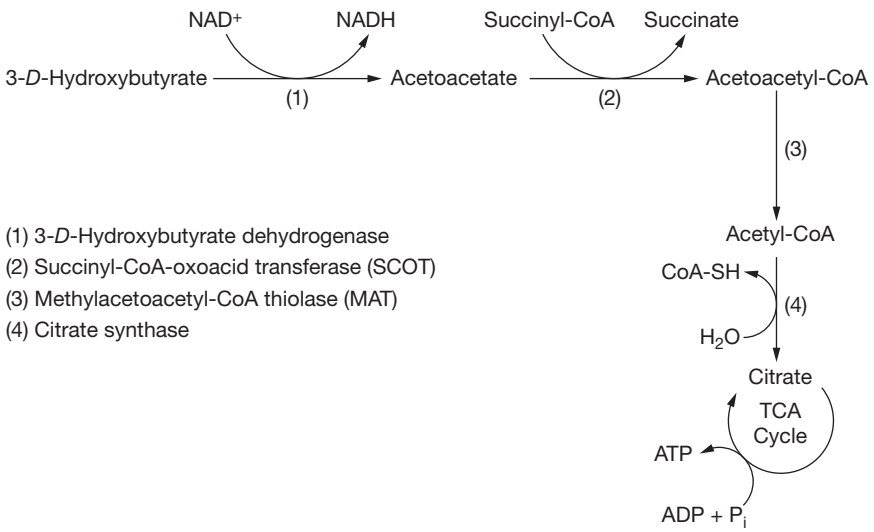


Figure 4 Ketolysis. Ketone bodies are broken down in a series of reactions in essentially the reverse of ketogenesis.

respiration and that they represent a substantial source of energy. Of course, mammals have evolved to utilize glucose as their primary metabolic fuel. However, in the event that glucose availability drops, a series of metabolic events occur to ensure survival. The linchpins of metabolism at this level are the hormones glucagon and insulin, both produced by the pancreas but in histologically distinct structures within the islet of Langerhans named the α and β cells, respectively. Careful glucose homeostasis is of vital importance for long-term survival during starvation conditions, not least because the brain and red blood cells are dependent on glucose as fuel. As the data in Table 2 demonstrate, an average 70 kg man has fuel stores that should ensure survival for about 3 months (assuming a basal energy expenditure of 1600 kcal day^{-1}). The utilization of these reserves was studied by Cahill and coworkers, who divided glucose homeostasis into five stages, charting the levels of glucose over a period of 40 days.

Phase I is the fed stage when glucose is plentiful, the ratio of insulin to glucagon is high, and glucose is directed toward the glycolytic pathway, producing ATP and a range of intermediates, including malonyl-CoA. This latter intermediate controls the fate of excess glucose; malonyl-CoA inhibits the passage of fatty acids to the mitochondrial matrix, the site of ketogenesis. Instead, the excess glucose is converted to fatty acids and ultimately stored as triacylglycerols in the adipose tissues. In phase II, the early fasted stage, glucose levels begin to decline and the ratio of insulin to glucagon decreases, initiating glycogenolysis. The release of glucagon also prevents the absorption of glucose by muscle and adipose tissue, allowing the glucose released to supply higher-priority tissues (viz., the brain and red blood cells). The liver gains energy through β-oxidation, and as the levels of acetyl-CoA and citrate increase, hepatic glycolysis is stopped. Similarly, muscle tissue gains energy through β-oxidation, and the acetyl-CoA formed causes accumulation of pyruvate and lactate, which are exported to the liver for conversion to glucose.

After ~24 h (phase III), glucose requirements are substantially met by gluconeogenesis. As glucagon also activates hormone-sensitive lipase in adipose tissues, lipolysis occurs, liberating 3-monoacylglycerol and fatty acids. The 3-monoacylglycerol acts as the main substrate for gluconeogenesis. The fatty acids liberated by the same process cannot generally be used for gluconeogenesis due to an inability to produce oxaloacetate independent of glycolysis (an exception to this is the metabolism of odd-chain fatty acids, which yield propionyl-CoA, a precursor of succinyl-CoA). As the levels of malonyl-CoA will be low, the fatty acids released can cross the inner mitochondrial membrane and enter the β-oxidation pathway. The acetyl-CoA produced can feed into the TCA cycle and supplement the ATP required for gluconeogenesis. However, as outlined earlier, there is a large surplus of acetyl-CoA, which is then used to synthesize ketone bodies. At phase IV, the levels of ketone bodies are high enough for them to cross the blood–brain barrier and meet some of its energy requirements. After about 3 days' starvation, one-third of the brain's energy demands are met by ketone bodies and cardiac muscle begins to consume ketone bodies for fuel. If starvation continues to phase V, the role of ketone bodies becomes even more significant, until the fat stores are depleted. At this stage, proteolysis supplies further ATP through liberation of glucogenic and ketogenic amino acids.

Table 2 Approximate energy stores in an average 70 kg man

	Available energy (kcal)		
	Glucose or glycogen	*Triglycerides*	*Proteins*
Blood	60	45	0
Liver	400	450	400
Brain	8	0	0
Muscle	1200	450	24 000
Adipose tissue	80	135 000	40

The switch from the well-fed state, when the liver is engaged in glycogenesis and lipogenesis, to the fasting state, when the liver is glycogenolytic, gluconeogenic, and ketogenic, is obviously tightly controlled. The first level of control, particularly for ketogenesis, is the availability of substrate; fat is only stored in the adipose tissue when glucose is in excess of the calorie demands of the individual. Therefore, the ability to synthesize ketone bodies is directly related to earlier excess calorie intake. This is a very coarse level of control but does give some basis for the adage 'fat burns in the flame of sugar.' The first example of fine control of ketogenesis comes from the passage of fatty acids across the inner mitochondrial membrane. In order for this to occur, they must be esterified to carnitine by carnitine acyltransferase I (CAT I), an enzyme only present on the outer surface of the mitochondrial membrane and inhibited by malonyl-CoA. Therefore, when glucose is in excess and fatty acids are being synthesized, the levels of malonyl-CoA will be high and CAT I will be inhibited. In the fasting state, the levels of malonyl-CoA will be low, CAT I will be active, and the molecules of fatty acyl carnitine can traverse the mitochondrial membrane to the mitochondrial matrix where they are broken down by β-oxidation and the acetyl-CoA converted to ketone bodies. Greater control is afforded through acetyl-CoA carboxylase (ACC), which modulates the flow of malonyl-CoA through two metabolic pathways. Citrate, produced by the TCA cycle, partially activates ACC, producing malonyl-CoA at a slow rate, which reduces CAT I activity and promotes lipogenesis. Insulin causes dephosphorylation of ACC, fully activating it toward malonyl-CoA formation, whereas glucagon promotes phosphorylation, inactivating the enzyme. Interestingly, in the long term, it appears that a high-carbohydrate diet increases the synthesis of ACC, while a high-fat diet decreases its synthesis.

Pathological Ketogenesis

Type I Diabetes Mellitus

Type I diabetes mellitus is by far the most studied and most prevalent cause of pathological ketogenesis. In type I diabetes, the pancreatic β cells have been destroyed by an autoimmune reaction, abolishing insulin production, thereby impairing glucose uptake by cells. Glucagon production occurs at higher than normal rates, causing metabolism in type I diabetes to shift to starvation mode. The liver responds by breaking down glycogen and synthesizing glucose through gluconeogenesis. Hence, not only will blood glucose be high due to exogenous glucose, but also this is supplemented by endogenously

produced glucose. As this glucose cannot be absorbed by cells (due to the absence of insulin), glucose is excreted in the urine, leading to dehydration. As the liver is engaged in gluconeogenesis, the supply of oxaloacetate is soon exhausted, and the TCA cycle stops producing citrate, which reduces the supply of malonyl-CoA. Fatty acids are then free to enter the mitochondrial matrix and undergo conversion to acetyl-CoA, which is in turn converted to ketone bodies. As insulin is not produced, there is no regulation of lipolysis, effectively meaning that ketogenesis is similarly unregulated. The resultant hyperketonemia continues until a threshold of about 7 mM, at which point the kidneys excrete excess ketone bodies into the urine.

Urinary excretion of ketone bodies results in the most serious complication of hyperketonemia – diabetic ketoacidosis (DKA). Recall that acetoacetate and (*R*)-3-hydroxybutyrate are relatively strong organic acids, dissociating into H^+ and the corresponding anion in the blood plasma. Initially, the H^+ ions are effectively buffered by bicarbonate anion, producing carbonic acid. The carbonic acid breaks down to give carbon dioxide and water, with the carbon dioxide stimulating the respiratory center in the brain, expelling it through exhalation. The sodium salts of the ketone bodies are then excreted in the urine. If the production of ketone bodies exceeds the clearance rate, a high anion gap metabolic acidosis occurs. When this is coupled to the dehydration associated with the osmotic diuresis caused by high blood glucose levels, hypotension and death can follow.

Ethanol Intoxication

Ethanol cannot be excreted directly and is therefore metabolized by one of two pathways. The first pathway involves two reactions: the conversion of ethanol to acetaldehyde mediated by alcohol dehydrogenase and the conversion of acetaldehyde to acetate using aldehyde dehydrogenase:

$$CH_3CH_2OH + NAD^+ \rightarrow CH_3CHO + NADH + H^+$$

$$CH_3CHO + NAD^+ + H_2O \rightarrow CH_3COO^- + NADH + H^+$$

From the equations, we see that metabolism of ethanol leads to an accumulation of NADH, which among other things prevents the conversion of lactate to pyruvate, causing modest lactic acidosis. The acetate produced is converted to acetyl-CoA, which cannot enter the TCA cycle as NADH also inhibits two of the enzymes involved in this cycle. Therefore, the acetyl-CoA is directed toward ketogenesis, further decreasing the pH of the blood plasma and causing significant metabolic acidosis.

Salicylate Overdose

Acetylsalicylate acid (aspirin) is hydrolyzed to salicylate, which has a pK_a of 2.7. It therefore dissociates in plasma to give a mild metabolic acidosis. As the pH of the blood decreases, the undissociated salicylate becomes increasingly lipid-soluble and can pass through the mitochondrial membrane where it inhibits the enzymes of the TCA cycle, specifically succinate dehydrogenase and α-ketoglutarate dehydrogenase. The accumulated pyruvate can have two main metabolic fates: the first is to undergo conversion to lactate (provided that NADH is in plentiful supply) and the second is to undergo oxidative decarboxylation and coupling to CoA, forming acetyl-CoA. The acetyl-CoA then acts as a substrate for 3-ketothiolase, forming acetoacetyl-CoA and proceeds toward ketone body formation.

Inborn Errors of Ketone Body Metabolism

Inborn errors of metabolism are broadly regarded as a group of rare inherited disorders in which a single gene defect causes a clinically significant effect. Newborns are screened within 7 days from birth for common inborn errors of metabolism, including HMG-CoA lyase deficiency and β-ketothiolase deficiency, both of which affect ketone body metabolism. Two further conditions, HMG-CoA synthase deficiency and SCOT deficiency, also affect ketone body metabolism.

When we consider the production of ketone bodies, the rate-limiting step is the formation of HMG-CoA, which is mediated by mitochondrial HMG-CoA synthase. The autosomal recessive condition, HMG-CoA synthase deficiency, is the only disorder that exclusively affects hepatic ketogenesis, essentially preventing *de novo* synthesis of ketone bodies. Subsequently, when an affected individual is in a fasting (hypoglycemic) state, ketone bodies are not produced to compensate, and pronounced hypoketonemia is present. This is an extremely rare disorder, with the first case only being recognized in 1997. The next stage in ketogenesis, conversion of HMG-CoA to acetyl-CoA and acetoacetate, can be associated with a deficiency in HMG-CoA lyase, first reported in 1976. This disorder has a similar clinical presentation to HMG-CoA synthase deficiency in that it is characterized by fasting hypoketonemia.

Deficiency in the enzymes of ketolysis, namely, SCOT and β-ketothiolase, appears to be marginally more prevalent in the population and is associated with significant hyperketonemia, often leading to ketoacidosis. SCOT deficiency, first recognized in 1972, is characterized by supraphysiological levels of acetoacetate and (*R*)-3-hydroxybutyrate and is a differential diagnosis of DKA. The second step in ketolysis can be affected by β-ketothiolase deficiency, which, as in the previous case, leads to an accumulation of acetoacetate and (*R*)-3-hydroxybutyrate. Both these disorders have a favorable prognosis if detected early and appropriate dietary observance can result in a more or less normal life. For females, pregnancy can carry a higher than normal risk, as pregnancy is associated with mild ketosis, which when combined with nausea and vomiting common in the first trimester can lead to significant hyperketonemia.

Detection and Measurement of Ketone Bodies

In light of the role of ketone bodies in various pathophysiological states, monitoring the levels of ketone bodies in various patient groups is a significant task in modern clinical chemistry laboratories. Urinalysis is the most commonly employed procedure, especially for near-patient testing or home monitoring. The simplest assay is based on the Rothera reaction, in which alkaline sodium nitroprusside reacts with methyl ketones (such as acetoacetate) to form a red adduct:

$$Na_2[Fe(CN)_5NO] + CH_3COCH_2COOH + 2NaOH \rightarrow Na_4[Fe(CN)_5NCHCOCH_2COOH] + H_2O$$

This reaction forms the basis of many commercially available ketone body test kits. Some versions include glycine, which undergoes Schiff base formation with acetone, leading to detection of acetoacetate and acetone. However, this test will not detect (*R*)-3-hydroxybutyrate, and assays based upon it should only be used for initial diagnosis or as an adjunct to more comprehensive analysis.

More reliable and accurate analysis of blood ketones is typically achieved by enzymatic assays, although methods employing gas chromatography or capillary electrophoresis have also been reported. In the enzymatic method, (*R*)-3-hydroxybutyrate dehydrogenase is used to convert (*R*)-3-hydroxybutyrate to acetoacetate in the presence of NAD^+. The associated reduction of NAD^+ to NADH can be followed spectrophotometrically by the action of diaphorase; this latter enzyme reoxidizes the NADH, which in turn reduces a formazan dye (typically WST-1) to produce a red/purple adduct.

Ketogenic Diets

Ketogenic diets are characterized by a high fat to carbohydrate ratio and have been employed in various guises since the early 1920s. The classic ketogenic diet was developed by Russell Wilder following his initial observations that such diets had an anticonvulsant effect in children with refractory epilepsy. Wilder's original diet consisted of the consumption of fats and carbohydrates in the ratio of 4:1, with the fat content comprising mainly of long-chain, saturated fatty acids. Since such a diet was associated with ketosis, Wilder attributed an anticonvulsant effect to ketone bodies. In fact, the Greek physician Hippocrates noted in the Hippocratic Corpus that "epilepsy is cured as quickly as it had appeared through complete abstinence of food and drink." It is now believed that although ketone bodies may have a direct anticonvulsant effect, their major action in refractory epilepsy is to promote the biosynthesis of γ-aminobutyric acid (GABA), a key inhibitory neurotransmitter in mammalian brains. This would seem to be linked to increased TCA cycle activity, such that the increased synthesis of α-ketoglutarate leads to increased GABA production, which dampens the hyperexcitability indicative of epilepsy.

In an evolution of Wilder's original 4-to-1 ketogenic diet, the medium-chain triglyceride diet was developed in the late 1960s by Peter Huttenlocher. In this diet, medium-chain triglycerides (6–12 carbon atoms in length) are found to be significantly more ketogenic than long-chain triglycerides. The real benefit of this diet was that it permitted higher proportions of protein and carbohydrate, increasing the palatability of the diet. Despite various gastrointestinal side effects, the medium-chain triglyceride diet remains in clinical use throughout the world, although the medium-chain triglycerides are significantly more expensive than those obtained from animal fats.

One of the most recent ketogenic diets that became popular in the 1990s is the Atkins diet. In this regimen, the majority of the energy requirements are met by fat metabolism, which, as previously outlined, will be associated with significant ketogenesis. However, as this ketogenesis is driven by dietary fat, rather than stored fat, there is some uncertainty as to the true origin of the weight loss observed in the Atkins diet. More recent investigations of the mechanism of weight loss seem to suggest that it is the restrictive food choices that contributed most significantly to weight loss, rather than increased fat metabolism. In addition, concerns have been raised about the long-term effects on cardiovascular health with a diet high in fat. In this regard, evidence is conflicting, and it seems that, somewhat paradoxically, the Atkins diet decreases total cholesterol and triglycerides. This may be due to increased consumption of polyunsaturated fatty acids, which are known to decrease plasma low-density lipoprotein ('bad cholesterol') and increase high-density lipoprotein ('good cholesterol'). It may also be due to reduced availability of acetyl-CoA for isoprenoid (cholesterol) synthesis.

In the main, ketogenic diets are effective for moderate weight loss. Initial (rapid) weight loss is undoubtedly due to diuresis as excretion of ketone bodies in the urine (ketonuria) increases renal sodium and hence water loss. Furthermore, glycogenolysis, another hallmark of ketogenesis, is associated with water loss (2 g of water released per 1 g of glycogen broken down). The mechanism for later weight loss is much harder to explain. While it is true that the reduced insulin secretion found in high-fat diets increases lipolysis, it is unlikely that this would contribute significantly to overall weight loss. The general consensus is that it is the restrictive food selection that promotes weight loss, which, when coupled with the other factors discussed, leads to an overall moderate reduction in weight. Of course, since many of these diets are not sustainable in the long term, individuals often gain weight after the dietary intervention. A compromise has been suggested to include 30% fat, 50% carbohydrate (with a low glycemic index), and 20% protein.

Applications of Ketosis in Humans

Given the fairly central role of ketone bodies in human physiology, it is unsurprising that they have been the focus of research for many years. For example, consider the application of exogenous ketone bodies to improve athletic performance. We know from empirical evidence that ketone bodies increase the hydraulic output of the heart by ~25%. This would have obvious advantages in terms of increasing the perfusion of oxygenated blood. In a similar sense, the use of ketone body supplements may prove useful in patients following ischemic stroke, where oxygen supply is reduced. As ketone bodies can cross the blood–brain barrier, they may have the effect of reducing collateral damage to neighboring neuronal tissue.

An emerging area of research is in the use of ketosis to reduce the progression of free radical-mediated pathologies, such as Parkinson's disease and Alzheimer's disease. In the former, dopaminergic neurons are destroyed by the various actions of hydroxyl radicals, $HO^{\bullet}$. The underlying mechanisms of free radical generation are based on well-known reactions. Initially, superoxide anion, $OO^{\bullet -}$, is produced from the reaction of molecular oxygen with the partially reduced semiquinone moiety of coenzyme Q:

$$QH^{\bullet -} + O_2 \rightarrow OO^{\bullet -} + H^+$$

The superoxide produced is in itself a free radical and capable of mediating one-electron oxidation of biological molecules, as well as reduction of ferric iron to ferrous iron through the Haber–Weiss reaction:

$$OO^{\bullet -} + Fe^{3+} \rightarrow Fe^{2+} + O_2$$

The ferrous iron then participates in the formation of the highly toxic hydroxyl radical through the Fenton reaction:

$$H_2O_2 + Fe^{2+} \rightarrow HO^{\bullet} + HO^- + Fe^{3+}$$

From this sequence of reactions, it is clear that the initial event is the regulated production of reduced coenzyme Q in dopaminergic neurons. From earlier sections, we know that metabolism of ketone bodies oxidizes coenzyme Q, lowers the intracellular levels of the reduced form, and thus decreases oxidative stress. Similarly, ketone bodies restore the $NADP^+$/NADPH balance, replenishing endogenous antioxidants such as glutathione.

The etiology of Alzheimer's disease is complex, with some six different genes being implicated in its development. However, the formation of β-amyloid is a hallmark of Alzheimer's disease, which is believed to form as a result of an inflammatory response within neuronal tissue. In murine models of Alzheimer's disease, a ketogenic diet was found to reduce the deposition of β-amyloid, in a mechanism believed to involve the transporter protein monocarboxylic acid transporter-1, which maintains a positive energy balance within the brain. Ketone bodies may also have a direct antioxidant effect within cells. Acetoacetate and (*R*)-3-hydroxybutyrate both act as Brønsted–Lowry acids and are therefore capable of acting as proton-donating antioxidants. Also, the standard reduction potential for the reaction

$$\text{Acetoacetate} + 2e^- \rightleftharpoons (R)\text{-3-hydroxybutyrate}$$

has $E^{o'} = -0.346$ V, which is certainly sufficient to bring about the reduction of some radical species *in vivo*.

Given that a mild state of ketosis appears to have beneficial effects, a reasonable question would be 'how do we bring about a ketogenic state.' Obviously, fasting or a diet high in fat would bring about a ketogenic state, but this is not sustainable in the long term. Another approach is to use oral administration of sodium (*R*)(*S*)-3-hydroxybutyrate at a dose of ~ 900 mg kg^{-1} day^{-1}. This is sufficient to give a peak plasma level of around 0.36 mM; however, given the adverse side effects associated with the administration of large amounts of sodium salts, this approach is unsustainable in the long term. An interesting alternative is to use poly-(*R*)-3-hydroxybutyrate (Figure 5), a naturally occurring ester found in certain microbial species. This is extracted in large quantities to serve as a feedstock for polyethylene production, and hydrolysis of the ester into smaller units may serve as a useful source of therapeutic ketone bodies. A derivative of the oligomeric form, (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate (Figure 5), has recently been synthesized and tested in animal models with a view to use in human subjects.

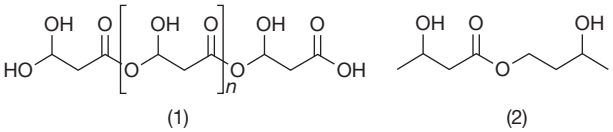


Figure 5 Novel sources of ketone bodies. Poly-(*R*)-3-hydroxybutyrate (1) and (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate (2).

See also: Energy Metabolism; Fatty Acids: Metabolism; Lipoproteins; Obesity: The Role of Diet; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Renal Function and Disorders; Satiety.

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Kiwifruit

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Introduction

'Kiwifruit' are the edible fruits produced by plants in the genus *Actinidia* Lindl. Kiwi plants originated from China where they grow wild, and they were brought to New Zealand in the early twentieth century by missionaries, where they were eventually domesticated and cultivated. In China, this fruit is known as 'mihoutao' (monkey peach) and it became known as 'Chinese gooseberry' in New Zealand. Its older name 'Chinese gooseberry' was considered unsuitable, and in 1959, the New Zealand fruit handling firm Turners & Growers Ltd. renamed this fruit as 'kiwifruit' to get a better marketing response when they first exported *Actinidia deliciosa* fruit to the United States. Within 10 years, kiwifruit had replaced 'Chinese gooseberry' as the common name for the fruit. *A. chinensis* and *A. deliciosa* are the most important *Actinidia* species under commercial cultivation accounting nearly for all international trade. In china, kiwifruit orchards are diverse with many different cultivars grown. *A. deliciosa*, the fuzzy kiwifruit, is the most widely available species and at present is under cultivation in many countries such as the United States, Italy, Chile, France, Greece, Brazil, and Japan. There is some commercial production in British Columbia (Canada).

Source and Production

Kiwifruit belongs to the family Actinidiaceae and is composed of more than 75 species and cultivars with a variety of sensory attributes and characteristics. Most *Actinidia* species are native to China. All *Actinidia* species are woody climbing vines. Vines are deciduous and dioecious. Male and female flowers are borne on different plants, and to ensure cross-pollination and fruit set, both sexes have to be planted in close proximity in a vineyard at the ratio of eight females to one male. Bees are necessary for pollination. Generally, vines do not flower until 3 or 4 years old. But *A. chinensis* is more precocious than *A. deliciosa*. Important *A. chinensis* cultivars in China are tetraploid, but 'Hort16A' is diploid. All cultivars of *A. deliciosa* are hexaploid ($2n = 6 \times = 164$). *Actinidia* species vary in their climatic requirements. But *A. chinensis* is more susceptible to winter cold and spring frosts. Kiwi plants flower about 2 months after bud break and fruit can be harvested 5–6 months after flowering. Generally, plants are propagated through vegetative or clonal methods. One preferred method of propagation is grafting varieties onto seedling rootstock. Occasionally, plants are also propagated by rooted cuttings of the preferred variety. Micropropagation through tissue culture has also been reported.

Cultivation of Kiwifruit

Commercial orchards of *A. deliciosa* were first established in New Zealand around 1930. Almost all currently cultivated kiwifruit cultivars are either derived from a few generations, removed from the wild, or selected directly from the wild. The most popular variety in New Zealand is 'Hayward'. Hayward was a selection of *A. deliciosa* made by Hayward Wright and subsequently named after him. It descended from a small number of seedlings derived from a single seed introduced into New Zealand from China at the beginning of the twentieth century. 'Hayward' has many favorable attributes such as large fruit, good flavor, and long shelf life. Hayward needs a growing season of about 225 to 240 frost-free days. For many years, Hayward was the only kiwifruit cultivar grown in all countries except China. Hayward and its associated males still account for at least 60% of the kiwifruit grown throughout the world.

The first *A. chinensis* cultivar established for commercial production was 'Hort16A' and the fruit was marketed as Zespri® Gold. 'Hort16A' is the result of a cross between a female (the product of open pollination of seedlings from a wild accession) and a male raised from a seed accession from the wild. It has bright yellow flesh and sweet flavor. 'Jintao' is the only other cultivar of *A. chinensis* becoming established commercially outside China, and its fruit is marketed as Jingold®. Both 'Hort16A' and 'Jintao' are protected cultivars. The Chinese kiwifruit industry produces more kiwifruit than any other country, but almost all are sold within China. In the past decade, other cultivars of *A. deliciosa* and other *Actinidia* species such as *A. arguta* also appeared in the market.

Recently, other *Actinidia* species such as *Actinidia arguta*, *Actinidia kolomikta*, and *Actinidia purpurea* are also gaining interest because of their frost resistance and capability to grow in cooler regions. *A. arguta* is hardy to −10 to −25 °C under most conditions. *A. arguta* is grown in Oregon in the United States, Europe, New Zealand, and Chile commercially and also by hobbyists. *A. arguta* fruits are smaller than the other *Actinidia*, but they can be readily consumed fresh as the fruit skin is edible being smooth and hairless. *A. arguta* or hardy kiwi plants are very vigorous and produce good quality, highly aromatic fruit that is quite different from the fruit of *A. deliciosa*. But their shelf life is limited and harvesting is labor-intensive. *A. kolomikta* is also small and sweet with high vitamin C content and is grown in colder areas. Kiwifruit are firm at harvest and soften during storage. Mature fruit can be stored for long periods. Kiwifruit are stored at or close to 0 °C. 'Hort16A' fruits are softer and have to be handled carefully, since they are more prone to damage. 'Hayward' can be stored for up to 6 months under refrigeration. But other *A. deliciosa* cultivars have short shelf life.

Fruit Morphology

Botanically, *Actinidia* fruits are berries with numerous black seeds embedded in a juicy pericarp. *Actinidia deliciosa*

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(A. Chev.) 'Hayward' ('green' kiwifruit), a green fleshed kiwifruit, is the most common commercial variety with superior flavor. It is an oval berry about the size of a hen's egg with a light brown hairy skin and emerald-green flesh with numerous tiny black seeds embedded in a juicy pericarp. *Actinidia deliciosa* fruits have persistent long, hard, bristlelike hairs, which are partially removed during grading and packaging. The green color of the flesh of *A. deliciosa* is due to the presence of chlorophyll, which is retained during fruit maturation and ripening. The flesh has a combination of tangy, sweet, and sour flavors. The most common yellow-fleshed kiwifruit is *A. chinensis*, 'Hort16A'. *A. chinensis* fruits are usually covered with soft, downy hairs that are shed early in fruit development. Fruits of *A. chinensis* have a characteristic 'beak,' which is a protrusion of stylar end. Fruit flesh of *A. chinensis* is yellowish due to partial or complete loss of chlorophyll.

Patterns of Consumption

Ripe kiwifruit are generally eaten fresh. Fruits are served as appetizers as slices or chunks in fruit salads, salsas, yogurt, and ice cream. They are also used for making jelly, jams, chutney, and pickles. Kiwifruit are also freeze-dried and used as health food. Ripe fruits are used for making smoothies, sherbets, and various beverages. Kiwifruit are also processed into purees, sauces, drinks, mixed juices, soft drinks, wines, and spirits. Most processed products lack the attractive attributes of fresh fruit because processing results in changes to color, aroma, texture, and taste. Processing of kiwifruit is common in China than in other countries. Yellow-colored and sweet *A. chinensis* fruits are preferred for processing in China.

Nutritional Composition

The nutritional composition of fresh *A. deliciosa* and *A. chinensis* fruit per 100 g was presented in Table 1 (US Department of Agriculture, 2011). Kiwifruit are high in vitamin C, dietary fiber, and other numerous health-benefiting phytochemicals. It is also high in potassium and magnesium. Ripe *A. deliciosa* contain chlorophylls a and b and the carotenoids such as β-carotene, lutein, violaxanthin, and neoxanthin. In addition, kiwifruit also contain unusually high levels of myoinositol, a cyclic sugar alcohol found in many foods. Myoinositol level was high during the early stages of fruit development and falls to 1–2% with fruit ripening. Kiwifruit is not a significant source of energy.

Fibers

Kiwifruit contain approximately 2–3% (fresh wt) of nonstarch polysaccharide. Recent studies have shown that the dietary fiber of both green and gold kiwifruit constitutes about one-third of soluble fiber and two-thirds of insoluble fiber. However, gold kiwifruit contain less dietary fiber than green. The soluble fraction of fibers is composed of pectic polysaccharides, while the insoluble fiber is mostly cellulose and hemicellulose with small amounts of pectin. Kiwifruit can supply 10% of the recommended daily dietary fiber requirement.

Sugars

Glucose, fructose, and sucrose are the predominant sugars contained in *Actinidia*. The content and ratios of sugars vary with stages of maturity and variety of kiwifruit. A total sugar level of 8.4 g 100 g^{-1} fresh wt was reported in *A. deliciosa* Hayward. Total sugar content ranging from 7.7 to 10.8 g 100 g^{-1} fresh wt was also reported (New Zealand Institute for Plant & Food Research). The glycemic index for kiwifruit is relatively low and its sugar content has a beneficial effect on the blood sugar level.

Protein

Kiwifruit is not a significant source of protein. Kiwifruit contain actinidin, which is a cysteine protease known for its protein hydrolytic properties. Actinidin is the predominant soluble protein in green kiwifruit accounting for 40% of soluble protein. The other proteins identified include kiwellin and a thaumatin-like protein. Scientific reports on the role of actinidin in aiding digestion and digestive mobility are available.

Vitamin E

Kiwifruit contain fairly high levels of vitamin E and the main α-tocopherol form of vitamin E is in the flesh and therefore potentially bioavailable. A new form of vitamin E, δ-tocomonoenol, was reported in kiwifruit. The lipid content in kiwifruit is entirely confined to the seeds. Three vitamin E forms, β-sitosterol, stigmasterol, and its Δ isomer were identified from the crude pulp extracts of Hayward.

Carotenoids

Kiwifruit contain a variety of carotenoid compounds such as β-carotene, lutein, violaxanthin, and neoxanthin. Lutein and β-carotene are the most abundant carotenoids. Among the various commonly consumed fruits, kiwifruit contains the highest level of lutein ranging from 0.09 to 1.08 ± 0.17 mg 100 g^{-1} fresh wt. *A. arguta* and *A. rufa* have the highest lutein content followed by *A. deliciosa* and *A. chinensis*. *A. chinensis* and *A. deliciosa* have similar β-carotene content (0.07 ± 0.01 to 0.15 ± 0.04 mg 100 g^{-1} fresh wt), and *A. arguta* has the highest level of 0.29 ± 0.04 mg 100 g^{-1} fresh wt. In general, green varieties have higher total carotenoid content than the gold/yellow varieties.

Organic Acids

Citric, malic, and quinic acids, together with much smaller amounts of glucuronic and galacturonic acids, form the major acids in kiwifruit. Quinic acid occurs in exceptionally high concentration in kiwifruit. Generally, other fruits contain quinic acid in small amounts. Ascorbic acid also contributes to the acidity of kiwifruit.

Phytochemicals

Phytochemical analysis of crude extracts of kiwifruit pulp from 'Hayward' led to the identification of two caffeic acid glucosyl

Table 1 Nutritional composition of kiwifruit in 100 g edible portion[a]

Nutrient	*Unit*	Actinidia chinensis *Yellow kiwifruit (Hort16A)*	Actinidia deliciosa *Green kiwifruit*
Proximates			
Water	g	83.22	83.07
Energy	kcal	60	61
Protein	g	1.23	1.14
Total lipid	g	0.56	0.52
Carbohydrate	g	14.23	14.66
Total fiber	g	2.0	3.0
Total sugars	g	10.98	8.99
Minerals			
Calcium, Ca	mg	20	34
Iron, Fe	mg	0.29	0.31
Magnesium, Mg	mg	14	17
Phosphorus, P	mg	29	34
Potassium, K	mg	316	312
Sodium, Na	mg	3	3
Zinc, Zn	mg	0.10	0.14
Vitamins			
Vitamin C	mg	105.4	92.7
Thiamin	mg	0.024	0.027
Riboflavin	mg	0.046	0.025
Niacin	mg	0.280	0.341
Vitamin B6	mg	0.057	0.063
Folate, DFE	µg	34	25
Vitamin A, RAE	µg	4	4
Vitamin A, IU	IU	72	87
Vitamin E	mg	1.49	1.46
Vitamin K	µg	5.5	40.3
Choline	mg	5.0	7.8
Lipids			
Total saturated fatty acids	g	0.149	0.029
Total monounsaturated fatty acids	g	0.036	0.047
Total polyunsaturated fatty acids	g	0.207	0.287
Cholesterol	g	0	0

[a]Source: The USDA National Nutrient Database for Standard Reference.

derivatives, 2 coumarin glucosides, campesterol, chlorogenic acid, and some flavone and flavanol compounds. Phenolic compounds were separated and identified in kiwifruit juice. The level of phenolic compounds in juice was low. The major strongly acidic phenolic acid compounds identified include coumaric and caffeic acid derivatives including chlorogenic acid, protocatechuic acid, and a derivative of 3,4-dihydroxybenzoic acid. Catechin, epicatechin, procyanidin dimers, and oligomers are the weekly acidic phenolic acid in clarified juice. Glycosides of quercetin (glucoside, rhamnoside, and rutinoside) and kaempferol (rhamnoside and rutinoside) are the flavanols present. The total anthocyanin content in kiwifruit is lower than in many other berries, not significantly contributing to the antioxidant capacity of the fruit. In *A. deliciosa*, anthocyanins are found in a ring around the central core of the fruit. The major anthocyanin identified in the *A. chinensis* was cyanidin-3-O-xylo-galactoside, with smaller amounts of cyanidin-3-O-galactoside. In *A. deliciosa*, cyanidin 3-O-galactoside and cyanidin 3-O-glucoside are the major anthocyanins detected, and no cyanidin-3-O-xylo-galactoside was identified.

Health Effects

Antioxidant Capacity

Dietary antioxidants protect the cells from the oxidative reactive oxygen species generated during various cellular processes. Evidence from various studies supports the role of diets rich in fruits and vegetables in helping to reduce the risk from degenerative diseases, including cardiovascular diseases and cancer. Vitamin C, vitamin E, carotenoids, and phenolic compounds contribute to the antioxidant capacity of fruits. Generally, kiwifruit has high antioxidant capacity that appears to be largely influenced by vitamin C and polyphenol levels. The antioxidant capacity of *Actinidia* is strongly influenced by species and cultivars. Kiwifruit contain an array of antioxidants such as vitamin C, vitamin E, lutein, zeaxanthin, and other phytochemicals. Fruits of 8 *Actinidia* genotypes were evaluated for their antioxidant capacity by various assays (DPPH, ABTS, ORAC, FRAP, SASR, and MCC) by Du and coworkers in 2009. Significant genotype differences were noticed in the total antioxidant capacity as well as in the total phenolics and vitamin C contents of fruit. The antioxidant capacity of various kiwifruit cultivars also correlated well with their total polyphenol and vitamin C content. The wild species *A. eriantha* and *A. latifolia* have significantly higher antioxidant capacity (about 3.3–8.7-fold higher) than the other genotypes and ranked highest in antioxidant capacity. Vitamin C content generally varies from 50 to 430 mg 100 g^{-1} fresh wt in the 3 main species of *Actinidia*. *Actinidia chinensis* has higher vitamin C level than *A. deliciosa*. Also, the phytonutrient composition varies with the growing conditions, fruit maturity, and other postharvest conditions.

In a study conducted using Jurkat T cells, Hayward juice concentrate protected cells from cell death induced by hydrogen peroxide-mediated oxidative stress. Additionally, aqueous extracts from 4 *Actinidia* species remarkably suppressed intracellular peroxyl radical generation in Caco-2 colonic epithelial cells. Similarly, ingestion of kiwifruit juice improves free radical scavenging in human plasma. Regular consumption of green or yellow kiwifruit also increased vitamin C, vitamin E, and lutein/zeaxanthin levels in plasma. Consumption of green kiwifruit was associated with increased antioxidant capacity postprandially and thus may play a role in preventing postprandial oxidative stress. Studies also showed increased resistance towards oxidative DNA damage in human lymphocytes with the daily consumption of 1–3 green or gold kiwifruit. Reduced lipid peroxidation was also noticed with the consumption of kiwifruit.

Gut Health and Metabolic Function

Kiwifruit are high in both soluble and insoluble fibers containing about 3.4 g fiber per 100 g fruit. Kiwifruit contain high lignin content, which contributes to their insoluble dietary fiber content. Kiwifruit have also been known for its beneficial effect on gut health and also for its strong laxative properties. Evidence suggests that increased solubilization of polymers

occurs during ripening within the cell walls of kiwifruit, resulting in an enhanced ability to hold water. Several studies have demonstrated the usefulness of kiwifruit consumption in the treatment of constipation in healthy elderly persons. Actinidin, the proteolytic enzyme in kiwifruit, also enhances protein digestion in both the stomach and the small intestine in concert with the gastric and intestinal proteases. In another study, kiwifruit consumption significantly reduced the colon transit time in patients with irritable bowel syndrome. Kiwifruit has also been shown to be effective in treating iron deficiency. Significant improvement in iron status of young women with mild iron deficiency was noticed after consumption of gold kiwifruit along with an iron-fortified breakfast cereal. In a placebo-controlled crossover trial with 25 athletes, kiwifruit drink supplementation during athletic training was beneficial in maintaining blood glucose and mineral levels, with no significant effect on plasma insulin. Improvement in serum ferritin (nearly 50%) and hemoglobin concentrations was also noticed in women who consumed kiwifruit along with an iron-fortified breakfast. Aqueous extract of Gold kiwifruit also showed β-glucuronidase-inhibiting and β-glucosidase-promoting activity. Also, both gold and green kiwifruit extracts could promote the growth of fecal lactic acid bacteria and reduced the growth of *E. coli*.

Immune Functions

Kiwifruit is a good source of various phytonutrients and chemicals associated with healthy immune system. A small number of animal and human cell-based studies have investigated the immunomodulatory effects associated with kiwifruit consumption. Gold kiwifruit extract significantly enhanced innate and adaptive immune cell function of human blood cells *ex vivo*. Studies conducted in older adults and preschool children investigated the effects of regular consumption of gold kiwifruit on the incidence and symptoms of upper respiratory tract infections. These studies demonstrated that consumption of gold kiwifruit reduced symptoms of cold and flu and their severity in the study groups. Increases in plasma vitamin C, tocopherol, lutein/zeaxanthin, and erythrocyte folate concentration were also noticed in the older adults who consumed gold kiwifruit.

Cardiovascular Diseases

Consumption of 2 green kiwifruit daily for 4 weeks favorably affected plasma HDL-C, TC/HDL-C ratio, and apolipoprotein B/apolipoprotein A1 ratio in hypercholesterolemic man compared with a healthy control diet. Some other studies have also showed that the consumption of 1–3 green or gold kiwifruit per day for 4–8 weeks increased HDL-C, decreased TC/HDL-C ratio, and decreased triglycerides compared with baseline concentrations. In male smokers, consumption of 3 green kiwifruit per day for 8 weeks significantly reduced diastolic and systolic pressure compared with the control treatment. Further, a reduction in angiotensin-converting enzyme activity was also noticed.

Several studies investigated on the effect of *Actinidia* on processes and factors associated with the development of cardiovascular diseases. Plasma samples from healthy human subjects who consumed 2–3 kiwifruit per day for two 28-day periods showed a significant decrease in *in vitro* ADP-induced platelet aggregation, compared with control. Antioxidant and vitamin C levels of plasma were increased compared with control. Persons with hyperlipidemia who consumed kiwifruit for 8 weeks showed an increase in HDL cholesterol concentration. Additionally, blood antioxidant status improved and markers of lipid peroxidation was reduced. In another study, consumption of kiwifruit for 3 weeks had no effect on glucose or insulin levels or on lipid profiles. Studies have shown that kiwifruit is effective in the prevention of atherosclerosis as noticed in a study in which kiwifruit consumption resulted in a reduction in the levels of triglycerides in the blood by 15% compared with control.

Anticarcinogenic Activity

Two of the 12 phenolic compounds isolated from the roots of *A. chinensis* showed remarkable cytotoxic activity against P-388 (leukemia) and A-549 (carcinomic human alveolar basal epithelial cells) cell lines. In another study, a polysaccharide compound isolated from the root of *A. chinensis* also demonstrated antitumorous activity in mice. Various extracts from gold kiwifruit showed selective cytotoxicity against human oral tumor cell lines. Two triterpenoids isolated from *A. chinensis* roots also showed cytotoxic actions against human adenocarcinoma and human liver carcinoma cell lines. Extracts from *A. arguta* strongly inhibited the growth of HepG2 and HT-29 cells. In *in vitro* systems, extracts or juices from kiwifruit showed antimutagenic effect towards chemical carcinogens including the heterocyclic amines, such as 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP).

Other Uses

In ancient China, *Actinidia* plants were used for symptom relief of numerous disorders, such as digestive problems, rheumatism, dyspepsia, and hemorrhoids, as well as a therapy for various cancers. Extracts from various plant parts of *A. chinensis* (leaves, fruit, and stem) showed antibacterial activity against Gram-positive and Gram-negative bacterial strains. The antibiotic activity of fruit was contained in the seeds, and in general, antibacterial activity of fruit extracts was more than the vegetative plant parts.

Kiwifruit Allergy

Kiwifruit also contain allergens that can result in allergenic reactions in susceptible individuals. Allergy to kiwifruit was first reported in 1981 in a 53-year old woman who developed urticaria, wheeze, and laryngeal edema on handling the fruit. Since then, there have been an increasing number of reported kiwifruit allergies as consumption of this fruit continues to expand worldwide. Kiwifruit allergy can take several different forms. Symptoms of kiwifruit allergy are often manifested as oral allergy syndrome and urticaria. Oral allergy syndrome consists of itching of mouth, lips, and throat; swelling of lips and throat; and formation of small blisters in the oral mucosa. Other reported symptoms are indigestion, nausea, vomiting,

wheeze, rashes on skin, and other severe allergic reactions such as anaphylaxis. Some individuals develop allergy as a result of eating kiwifruit, while in others, allergy is associated with pollen and latex allergies. This is due to the similarity between the allergens in kiwifruit and natural rubber latex, a condition known as the latex-fruit syndrome.

Certain varieties of Kiwifruit, gold kiwifruit, contain less allergen than the green variety. However, people allergic to green kiwi fruit can also react to gold or yellow variety. There are also cross-reactions between kiwi and several other fruits such as avocado, banana, fig, chestnut, melon, sesame seeds, poppy seeds, hazelnuts, and rye grain. In the last few decades, occurrence of kiwi fruit allergy in some European countries is increasing. Several components in kiwifruit have been tentatively identified as potential allergenic agents. Eleven allergens from kiwifruit, *A. deliciosa* 'Hayward', have been registered in the Allergen Nomenclature Database including 30 kd cysteine protease actinidin, 24 kd thaumatin-like protein, 26 kd cell wall protein kiwellin, and cysteine protease inhibitor cystatin. Actinidin is the most recognized major kiwifruit allergen. A study conducted among school children in France reported that kiwifruit allergy is as common as egg and peanut allergies. Three allergens from *A. chinensis* were officially recognized.

Kiwifruit are also relatively high in oxalate content and is mainly concentrated in the skin, inner pericarp, and seed. The presence of calcium oxalate may cause oral irritation. Evidence suggests that oxalate may interfere with nutrient absorption and are also involved in kidney stone formation if oxalate-rich foods are consumed in excess. In green kiwifruit, the oxalate content varies between 18 and 45 mg 100 g^{-1} of fresh wt and is concentrated in the skin, inner pericarp, and seed. Oxalate content varies with species and fruit maturation. The oxalate levels vary between species reaching a maximum during fruit development and reducing during fruit maturation and storage.

See also: Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Ascorbic Acid: Properties, Determination and Uses; Fruits of Tropical Climates: Biodiversity and Dietary Importance; Fruits of Tropical Climates: Dietary Importance and Health Benefits.

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Laboratory Management: Microbiological Safety

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Introduction

Whenever human pathogens are cultured, a risk exists to human health. This risk is both for the workers in the laboratory and to anybody who might be exposed to the pathogens that are inadvertently transmitted to people outside of the laboratory. There is a moral, economic, and legal responsibility to control that risk as far as is reasonably possible.

To help managers of food microbiology laboratories, guidance exists in the form of the World Health Organization (WHO) Laboratory Biosafety Manual, 3rd Edition.

Beyond guidance in most jurisdictions, a legal framework exists describing the requirements for managing laboratory safety. In some parts of the world, laboratories operate outside the main framework to managing occupational safety and health, but in the EU, for example, laboratories are treated as just another workplace and are subject to the same sort of controls that any other workplace is subjected to. In the EU, the Biological Agents Directive (Council Directive 90/679/EEC of 26 November 1990 on the protection of workers from risks related to exposure to biological agents at work, OJ No L 374, p. 1) sets out how member states of the EU must approach workers' safety in relation to work with biological agents.

All of these guidelines rely on particular concepts including

- classification of biosafety levels relating to the types of pathogens that are being examined in a particular laboratory or are likely to occur in such a laboratory (even though these might not be the organisms being examined),
- assessing the risks that these organisms pose and then adopting controls that are appropriate and suitable for these organisms.

This article will go into more detail describing these classifications and then describe how to apply a risk assessment in a food microbiological laboratory.

Hazards

In occupational health and safety, a hazard is defined as something that poses a level of threat to life, health, or the environment. The hazard has the potential to cause harm, with only a theoretical risk of actual harm; however, once a hazard becomes 'manifest,' it can create an actual risk. If a risk is identified, it must be controlled.

The level of risk is often described as follows:

$$\text{Level of risk} = \text{severity of the hazard} \times \text{likelihood of it becoming manifest}$$

Identification of hazard severity is the first step in performing a risk assessment.

Under the WHO system, the severity (i.e., the potential for harm) of the various pathogens is classified using the criteria in Table 1.

This process should be carried out whenever work with pathogens is being planned. In many jurisdictions, government occupational health agencies, such as the Health and Safety Executive (HSE) in the United Kingdom, publish guidance notes that classify pathogens in accordance with this approach.

The aim is to assign pathogens to one of 4 biosafety groups (or hazard groups).

The classifications are shown in Table 2.

The HSE guidance is published as 'The Approved List of biological agents. HSE – Advisory Committee on Dangerous Pathogens. Misc208(rev2).'

Foodborne organisms will fall mainly into risk group 2 with some being classed as being risk group 3. No foodborne organisms fall into risk group 4.

Table 1 Criteria for classifying human pathogens

1. Pathogenicity of the agent and infectious dose
2. Potential outcome of exposure
3. Natural route of infection
4. Other routes of infection, resulting from laboratory manipulations (parenteral, airborne, ingestion)
5. Stability of the agent in the environment
6. Concentration of the agent and volume of concentrated material to be manipulated
7. Presence of a suitable host (human or animal)
8. Information available from animal studies and reports of laboratory-acquired infections or clinical reports
9. Laboratory activities planned (sonication, aerosolization, centrifugation, etc.)
10. Any genetic manipulation of the organism that may extend the host range of the agent or alter the agent's sensitivity to known, effective treatment regimens
11. Local availability of effective prophylaxis or therapeutic interventions

Table 2 Biosafety risk groups

Risk group	*Description*
Risk group 1 (no or low individual and community risk)	A microorganism that is unlikely to cause human or animal disease
Risk group 2 (moderate individual risk, low community risk)	A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited
Risk group 3 (high individual risk, low community risk)	A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available
Risk group 4 (high individual and community risk)	A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available

Although a laboratory may be designated for handling a particular risk group, as a laboratory cannot completely control the samples it receives, it might find itself inadvertently handling organisms of a higher risk group than anticipated. There should be a procedure for dealing with these sorts of situation in the individual laboratory's code of practice (CoP).

If a sample is receiving samples of unknown provenance, then it should be assumed that organisms in risk group 2 are present unless there is any evidence to suggest that organisms of a higher risk group are present.

Risk Assessment

When assessing the risk from biological agents in a laboratory, one needs to look at the following:

(a) Biosafety group classification of any organisms being cultured or tested for in the laboratory.
(b) The type of work being carried out, in particular if it gives rise to aerosols such as centrifugation or vortexing.
(c) The concentration of the cultures. The higher the concentration, the higher the risk.
(d) The sort of controls that the laboratory has adopted such as the use of microbiological safety cabinets (MSCs).
(e) The results of relevant health surveillance; is there any evidence of laboratory-acquired infections among the staff?
(f) The results of monitoring by, for example, the use of air plates or culturing swabs taken from work surfaces.
(g) Individual factors such as the physical capability of the staff working in the laboratory must also be taken into account. Particular care must be taken with pregnant staff. They are both physically at risk and directly at risk from certain pathogens such as *Listeria monocytogenes*. This infection during pregnancy can lead to miscarriage, stillbirth, premature delivery, or life-threatening infection of the newborn. In such cases, pregnant women should not be allowed access to the laboratory.

Once a risk assessment has been carried out, it must be recorded and the findings circulated to the relevant stakeholders including the staff.

The risk assessment must be regularly reviewed to make sure that it still valid. In particular, it must be checked to make sure that there has not been any 'method drift.' This occurs when an established method gradually changes over the years and the risk assessment is not updated alongside of this.

The person carrying the risk assessment must be competent to do so. He or she must

- understand local legal requirements in relation to work with human pathogens,
- understand the hazards of working with human pathogen,
- understand the nature of the work taking place within the laboratory and details of the work taking place there,
- be in a position to recommend what the controls might be suitable and to see them carried out.

It might be necessary for the risk assessment to be carried out collectively with several people working together to gather the information necessary and to assess the likelihood of exposure to human pathogens. One individual should coordinate, consult, compile, assure quality, record, communicate, and implement the risk management measures and monitor their effectiveness, as well as consider the need for reviewing the assessment.

Controls

Having created the risk assessment, it is necessary to adopt control measures that will make the risk of infection in the staff as low as reasonably practicable. The biosafety level requirements are compiled in Table 3.

The four key control areas are

- the people working in the laboratory,
- the equipment in the laboratory,
- the methods being used in the laboratory,
- the laboratory environment.

People

No system or level of equipment can create a safe working laboratory unless the laboratory has competent staff. Competency is defined as the ability for an employee to do a job safely without creating risks to themselves, their colleagues, or the wider community. It is a combination of practical and theoretical knowledge, cognitive skills, behavior, and values used to maintain safety. It can also be described as being suitably qualified, having the ability to perform a specific role. Competency can be summarized by saying it demonstrates

Table 3 Summary of biosafety level requirements

	Biosafety level			
	1	*2*	*3*	*4*
Isolation of laboratory[a]	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation				
Inward airflow	No	Desirable	Yes	Yes
Controlled ventilating system	No	Desirable	Yes	Yes
HEPA-filtered air exhaust	No	No	Yes/no[b]	Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Anteroom	No	No	No	–
Anteroom with shower	No	No	Yes/no[c]	No
Effluent treatment	No	No	Yes/no[c]	Yes
Autoclave				
On site	No	Desirable	Yes	Yes
In laboratory room	No	No	Desirable	Yes
Double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personnel safety monitoring	No	No	Desirable	Yes
View of working laboratory[d]	Not required	Desirable	Yes	Yes

[a]Laboratory should be separated from general traffic routes with limited or controlled access.
[b]Depends on location of exhaust outlet.
[c]Depends on agents being used in the laboratory.
[d]For example, a window, viewing panel, or closed circuit television showing the working interior of the laboratory.

- workers with the correct skills,
- workers with the correct knowledge,
- workers with a suitable attitude,
- workers with appropriate training,
- workers with relevant experience.

All staff working in a food microbiology laboratory should be trained in good microbiological techniques.

They should understand the equipment that they are working with. Manuals should be kept in the laboratory and be available to the staff.

Young staff tend to be more inexperienced than older staff and as such at higher risk. In some jurisdictions, statutory limits are placed on the age at which people are allowed to work in a microbiological laboratory.

The laboratory needs to be managed. There should be a competent individual chosen to act as the biosafety manager. They should have the authority to oversee all of the work that is being carried out in the laboratory and if necessary stop any work that they believe poses an unacceptable risk.

Human factors such as staff attitudes and morale are key for safe management in a laboratory. Well-motivated staff are generally safer in a laboratory and less likely to have accidents. They are also more likely to be open with management and be willing to discuss any issues including safety issues.

Equipment

General

The equipment in a laboratory should be designed so that it can be easily disinfected.

Figure 1 Typical laboratory hand basin incorporating an eyewash facility.

Ideally, it should be movable or portable so that it can be moved so that floors and bench tops underneath it can be routinely disinfected.

In laboratories working at biosafety level 3 and above, the equipment will need to be able to resist severe decontamination methods such as fumigation with formaldehyde.

The laboratory should have adequate washing facilities. This should consist of a hand basin (equipped with soap), which is separate from any other laboratory sinks.

Some form of eye wash is also required. Ideally, this should be an eyewash connected to the laboratory water supply, but if that is not practicable, then a supply of sterile eyewash bottles might suffice. The risk assessment will inform this decision (Figure 1).

The laboratory should have sufficient benching for the work to be carried out safely. It should be stable and easy to clean. It should be resistant to routine laboratory chemicals, in particular to any disinfectants used in the laboratory.

It should be possible to clean under the bench to prevent the buildup of any debris.

There should be sufficient seating for the staff to work comfortably at the bench where there should be space underneath the benching for them to fit their legs while sitting.

There should be suitable storage within the laboratory; typically, this will consist of shelving, which should be strong enough to support the weight of any items that will be stored on it. The role of such shelving is to clear as much space as possible on the benching, which should be used as far as possible for actual work.

It might be necessary to create special storage facilities for things like toxic reagents and drugs. These need to be secure and might have to be kept under lock and key (often, these matters are regulated under local regulations). The key will need to be kept with a responsible person.

Most laboratories will include freezers and fridges. These might also need to be kept locked if they contain microbiology culture collections.

Incubators need to be kept clean and should be regularly disinfected.

Autoclaves

The WHO Laboratory Biosafety Manual recommends that the laboratory have access to an autoclave for the disposal of contaminated equipment, media, and consumables. This will have to be disposed of in accordance with local laws.

The requirements for the provision of an autoclave in a laboratory are as follows:

1. The autoclave must be capable of maintaining a temperature capable of sterilizing a load safely. Typical temperatures would be 121 °C for 15 min. The risk assessment process will identify what would be the appropriate temperature and duration of sterilization for a particular laboratory and the sort of loads that it needs to deal with.
2. Before the heating cycle takes place, the autoclave must reduce the air pressure in the chamber to ensure that the superheated water vapor penetrates the load completely.
3. There must be a way to confirm that the load has actually achieved this temperature during serialization. This is usually done with an internal temperature probe installed in the autoclave, inserted into a test load, typically a container of water in the middle of the load.
4. There must be a way to test that sterilization has actually occurred. This is usually done using test stripes containing *Bacillus subtilis* spores, which are then cultured.
5. The autoclave must include a pressure relief mechanism that will prevent the chamber from becoming overpressurized.
6. There should be a thermal lock that prevents the chamber from being opened while the internal temperature of the sterilizing chamber is above 80 °C. An override key should not be kept in the machine, but kept securely by an authorized person.
7. The machine and in particular its pressure chamber will need to be checked periodically by a suitably qualified person. There may be local laws dealing with this matter.
8. Care must be taken when loading the autoclave and in particular the staff should avoid placing chemicals (including disinfectants) in any load. This might create vapors containing hazardous chemicals.

If loads of contaminated material are to be taken out of the laboratory (e.g., to be autoclaved), they should be transported in suitable containers with watertight lids, which will not spill their contents if they are accidently dropped or turned on their sides.

Microbiological safety cabinets

MSCs are a form of local exhaust ventilation. They are designed to contain any harmful aerosols from tasks that might be carried out at a bench, for example, centrifuging and macerating.

They should not be confused with fume cupboards, which, as their name suggests, are designed to control volatile fumes. The MSC consists of an enclosed work area, which is under negative pressure (it draws air in) to the room it is in. This prevents aerosols from affecting the operator. Air is exhausted from the MSC through a high-efficiency particulate absorbent (HEPA) filter, which prevents dispersal of any pathogens to the room.

MSCs should not be confused with laminar flow cabinets. The latter are not intended to provide operator protection. Their sole function is product protection; as such, they must never be used for any pathogen manipulation.

There are three types of cabinet available:

- Class I
- Class II
- Class III

Class I cabinets

These types of cabinet draw air from the laboratory through the opening at the front and force it out through a HEPA filter. By maintaining a high negative pressure relative to the laboratory, they ensure that no aerosols produced in the cabinet can escape into the laboratory.

The main disadvantage of these types of cabinet is they confer no aseptic protection on work being carried out in the cabinet.

Class II cabinets

These types of cabinet draw air from the laboratory through a filter. Then the airflow is split. Part of it forms an 'air curtain' at the front opening of the cabinet, and the rest is blown through the cabinet and then out through a HEPA filter. They maintain a negative air pressure relative to the laboratory, but particles from the laboratory should not be able to enter the cabinet directly. This means that a Class II cabinet does provide aseptic protection for work inside it. These cabinets are rather complicated, and it is very easy to disrupt the air curtain in front of the cabinet, reducing or even eliminating user protection.

Class III cabinets

These are in some ways the simplest of cabinet designs. Air is drawn into the cabinet through a HEPA filter and discharged through a HEPA filter. To work in the cabinet requires operators to use long gauntlets attached to ports at the front of the cabinet. These are very restrictive of movement but provide the highest level of operator protection. These are used for work with organisms in biosafety groups 3 and 4.

MSCs should be carefully positioned so that they work at maximum efficiency.

They must be kept as free of clutter as possible (especially class II cabinets).

They should be routinely tested to make sure that they are working correctly by an external engineer or technician. Such testing is often legally mandated and complies with a set standard such as BS EN 12469:2000 'Biotechnology – Performance criteria for microbiological safety cabinets.'

Methods

All people working in a food microbiology laboratory need to be trained in the methods and work procedures that take place there.

All methods and policies and procedures should be written up, and these documents need to be managed in such a way that everybody has current copies of these documents available.

All the methods used should be covered by a risk assessment.

Code of practice

There should be a local CoP that will describe the overall system of work that is to be carried out in the laboratory. It will define the following:

1. What work can take place in the laboratory, in particular what biosafety group organisms will be cultured and tested for in the laboratory.
2. The level of competence of the people working in the laboratory.
3. The level of supervision in the laboratory, identifying those staff who can work alone and those that can only work under supervision. This might depend on what sort of work individuals are actually doing.
4. The code should also describe the sort of training that the staff might need. This might be formal training supplied by a teaching establishment or provider. It might be informal 'on-the-job' training.
5. As well as routine procedures, there must be a procedure for dealing with any sort of emergency, for example, a spillage of a liquid culture. All staff must be aware of what is required of them to be able to deal with the emergency without putting themselves at risk. It would be unacceptable to expect untrained cleaning staff, for example, to clear up microbiological contamination. Such staff should only be used if the area has been rendered safe (by, e.g., disinfection).

General hygiene and disinfection

Food samples once brought into the laboratory must be treated as if they were contaminated. The staff must never be allowed to take food samples home even if they have not been purposefully contaminated or analyzed and found uncontaminated. It should be assumed that any food in the laboratory is contaminated, and eating and drinking are not permitted in any laboratory.

Mouth pipetting of liquids should not be permitted in any laboratory. Liquids should be dispensed using a mechanical or electric pipetting device.

There should be a standard procedure for disinfection, which is available for all staff to follow.

It must describe which sort of disinfectants to use in relation to which sort of materials. There is no such thing as a universal disinfectant. Each disinfectant has its own specific range of activity. Manufacturers should be able to define this range.

A disinfectant cannot act instantaneously but requires a minimum period of contact time for it to produce its microcidal effect. Once applied, a disinfectant must be left for this minimum period before it is cleaned up. Again, the manufacturer's guidance should define this minimum contact time.

Usually, when the efficacy of a disinfectant is established, it is done on a clean surface. This does not always produce a realistic estimation of its actual effectiveness. Disinfectants can be inhibited by substances like blood, soil, and similar biological substances.

Many disinfectants have a limited shelf life especially those that are made up in a solution prior to use. Care should be taken to make sure that only 'in date' disinfectants are used.

There is no such thing as a totally safe disinfectant; all disinfectants pose some risk to users. All staff working in the laboratory must be trained in how to use the disinfectants correctly and safely. In particular, they need to be told when not to mix disinfectants together.

Laboratory Environment

The laboratory environment must be maintained as a comfortable place to work. It should be temperature-controlled and suitably ventilated. This will probably require some sort of forced air ventilation. If this is installed, then the laboratory must be kept at a negative air pressure in relation to the outside of the laboratory. The air exhaust from the laboratory should be discharged in a place where it does not pose a risk to people in the location. There might be local laws defining how and where such air can be exhausted. They might also require that the air be filtered in some way.

The laboratory should have sufficient space for the work being carried out. There should be space to move about safely and to enable people to work at the benches without them colliding with each other. The laboratory should not be used as a thoroughfare between different rooms.

It might be necessary to install some form of access control to the laboratory, for example, a card swipe or combination lock.

There should be sufficient light for the work taking place, so that the staff can see what they are doing. This will involve artificial lighting, and it might be necessary to provide local task lights in some parts of the laboratory.

The floors should be easy to clean and resistant to routine laboratory chemicals and also have a seamless seal between floors and walls, especially in C3 labs.

Table 4 Requirements for the safe management of a food microbiology laboratory

1. A suitable and sufficient risk assessment for the work that is to be carried out in the laboratory
2. Appointment of competent staff to operate the laboratory including a biosafety manager
3. Establishing a code of practice to set out the basic rules for working in this laboratory
4. Equipping the laboratory with all of the necessary equipment and arranging for this equipment to be maintained while in use in the laboratory
5. Proving sufficient training, information, instruction, and supervision to all staff working in the laboratory so that they can carry out their work safely
6. Monitoring the work in the laboratory to ensure that it is being carried in accordance with the requirements of the code of practice
7. Periodically reviewing the code, the policies and procedures, and the methods in use to ensure that they are still fit for purpose and pose the minimum risk to the staff working in the laboratory

All waste should be routinely removed from the laboratory and not allowed to accumulate. It should be disposed of in accordance with local laws. Contaminated material should be autoclaved or sent for incineration.

The lab and especially the benches should be routinely disinfected, usually at the end of the working day.

Summary

The requirements for the efficient safe management for a food microbiology laboratory are summarized in Table 4.

Conclusion

Work with any sort of human pathogens including foodborne pathogens poses a risk to the staff working in the laboratory. This risk can be identified through a process of risk assessment and then mitigated by applying the types of controls described in this article.

There is no reason why people in a food microbiology lab should be exposed to any significant risks to their health through their work.

Further Reading

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Lactic Acid Bacteria

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Introduction

Taxonomy and Metabolism of Lactic Acid Bacteria

Lactic acid bacteria (LAB) are a heterogeneous group of phylogenetically closely related microorganisms that produce lactic acid as the major or sole product of carbohydrate fermentation. LAB are Gram positive, nonsporulating, catalase-negative, acid tolerant, nonrespiring but aerotolerant, usually nonmotile cocci or rods with low Guanidine + Citosine (G + C) content. Except for a few species belonging to the *Streptococcus*, *Enterococcus*, *Lactococcus*, and *Carnobacterium* genera, LAB are nonpathogenic with a generally recognized as safe (GRAS) or food-grade status.

LAB have complex nutritional requirements for amino acids, peptides, nucleotide bases, vitamins, minerals, fatty acids, and carbohydrates, components that are usually present in the natural niches they inhabit. These microorganisms are found in a plethora of environments such as plant materials and dairy-, meat-, and cereal-based (fermented) foods, the animal gastrointestinal tract and vagina, and in soil and water. Their ability to inhabit different niches is trait of their metabolic diversity and adaptability.

The monograph on LAB written by Orla-Jensen in 1919 greatly affected the systematics of these microorganisms; LAB were classified based on their morphology, mode of glucose fermentation, ability to grow at different temperatures, and use of sugars as carbon substrates. Other features, such as configuration of lactic acid produced and tolerance to salt, acid, and alkali, were also considered. According to the current taxonomic classification LAB belong to the phylum Firmicutes, class Bacilli, order *Lactobacillales*. The families include Aerococcaceae, Carnobacteriaceae, Enterococcaceae, Lactobacillaceae, Leuconostocaceae, and Streptococcaceae. Four main genera of LAB, namely *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*, were originally described; however, recent taxonomic revisions have proposed the following new genera: *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Globicatella*, *Lactococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weisella*. Of all the genera mentioned, the lactobacilli and carnobacteria are rods; the remaining genera are cocci, except for *Weisella* species, which may be either rods or cocci. Researchers classified some of the newly described genera using fatty acid composition and motility features.

With the availability of improved molecular methodologies such as 16S rRNA gene sequencing and studies on the microbial diversity of different environments, many species have been discovered and identified. Thus, the heterogeneous LAB group consists of about 450 species distributed as follows: *Aerococcus*, 7; *Alloiococcus*, 1; *Carnobacterium*, 12; *Dolosigranulum*, 1; *Enterococcus*, 49; *Globicatella*, 2; *Lactobacillus*, 189; *Lactococcus*, 7; *Leuconostoc*, 23; *Pediococcus*, 15; *Oenococcus*, 2; *Streptococcus*, 101; *Tetragenococcus*, 5; *Vagococcus*, 8; and *Weisella*, 18.

LAB are defined as chemotrophic microbes as they generate energy (ATP) by substrate-level phosphorylation. Although these bacteria ferment sugars when they grow under anaerobic conditions, they can also grow in the presence of oxygen. Despite their well-established fermentative metabolism, several LAB species can switch from fermentation to aerobic respiration metabolism when heme or heme and menaquinone groups are provided. Electron transport chains and evidence of cytochromes in heme-grown cultures were reported first in lactococci, leuconostocs, and some enterococci. Interestingly, it has been observed that, in *Lactococcus lactis*, respiration has a positive effect as bacterial biomass increases together with oxygen resistance and survival.

Sugar metabolism

Sugars are the primary carbon and energy sources for LAB grown on substrates used for fermented foods and feed production, as well as in laboratory media. According to the way LAB ferment carbohydrates, they are classified as homofermentative or heterofermentative. Homofermentative LAB (*Lactococcus*, *Streptococcus*, *Pediococcus*, *Enterococcus*, and some *Lactobacillus* species) ferment sugars by the Embden–Meyerhoff–Parnas pathway to pyruvate, which is converted into lactic acid by lactate dehydrogenase (LDH). Two types of lactate isomers, L and D, can be produced by stereospecific nicotinamide adenine dinucleotide (NAD)-dependent enzymes, L-LDH and D-LDH. Under certain growing conditions such as carbon limitation, the homolactic metabolism can be shifted to a mixed-acid metabolism where formate, acetate, ethanol, and/or CO_2 besides lactate, are produced (Figure 1).

Heterofermentative LAB such as *Leuconostoc*, *Oenococcus*, and certain *Lactobacillus* species ferment sugars generally by the phosphoketolase pathway (PKP, also known as the pentose phosphoketolase shunt or the 6-phosphogluconate pathway). Fermentation of pentoses such as xylose and ribose leads to the formation of pyruvate and acetyl-P and their subsequent conversion to lactate and acetate, respectively. Hexoses (i.e., glucose, fructose, and mannose) can be converted to lactate, CO_2, and ethanol. CO_2 is a product of 6-P-gluconate degradation, which occurs during conversion of hexoses to pentoses. The specific enzyme of the heterofermentative pathway is D-xylulose-5P phosphoketolase, which catalyzes the conversion of xylulose-5P into glyceraldehyde-3-phosphate (GAP) and acetyl-P.

The fermentation type (homolactic or heterolactic) is an important taxonomic criterion. The genera *Leuconostoc*, *Oenococcus*, and *Weisella* as well as certain *Lactobacillus* species such as *Lactobacillus buchneri*, *Lactobacillus brevis*, *Lactobacillus fermentum*, and *Lactobacillus reuteri* are obligate heterofermentative. Other lactobacilli, including *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, *Lactobacillus helveticus*, and *Lactobacillus salivarius*, are obligate homofermentative and not able to metabolize pentoses. Finally, *Lactobacillus casei*, *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Lactobacillus sakei*, and most LAB can homofermentatively ferment hexoses and pentoses and are known as *facultatively heterofermentative*.

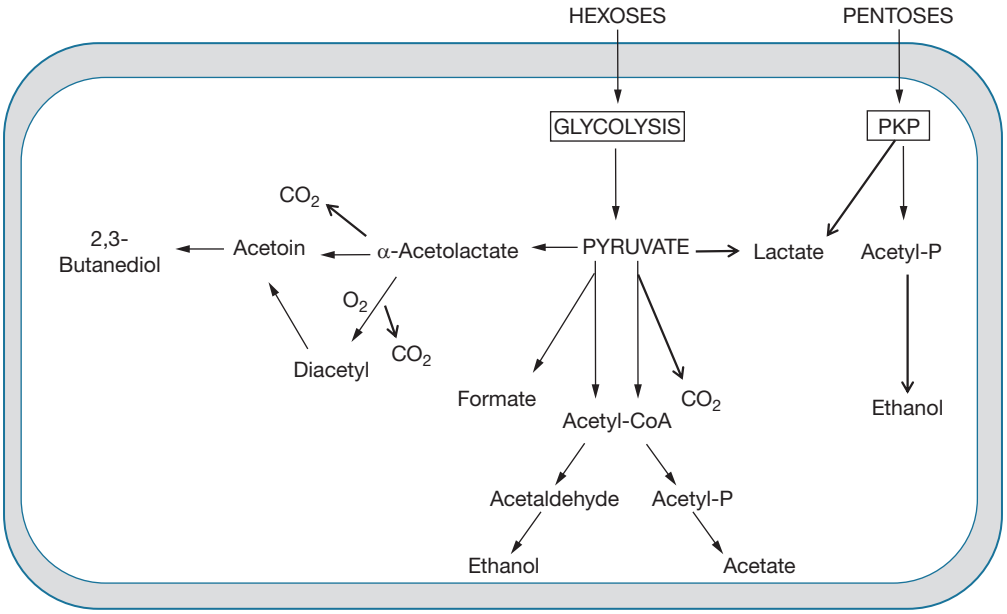

Figure 1 Sugar metabolism in LAB.

Pyruvate has a key role in the fermentation pathways; it usually acts as an electron acceptor to form lactic acid and to help maintain the intracellular redox balance. Nevertheless, and depending on the LAB species, pyruvate can be metabolized by different enzymes leading to the synthesis of different metabolites such as diacetyl/acetoin, formate, and acetyl-coA, which can subsequently yield ethanol or ATP and acetate. Also, oxygen and fructose may be used as alternative electron acceptors rather than pyruvate.

In addition to being capable of fermenting several monosaccharides, LAB can also use disaccharides as energy substrates. In this regard, lactose metabolism has been extensively studied, especially in those species commonly used in dairy applications. In this case, lactose can enter the cell through a lactose-specific permease or a lactose-specific phosphoenolpyruvate–phosphotransferase (PEP–PTS) system or both. In the first system, lactose is cleaved into glucose and galactose by a beta-galactosidase enzyme, which in turn can be fermented through the main fermentation pathways. If lactose enters the cells by a PEP–PTS system, lactose-P is split into glucose and galactose-6-P by the enzyme phospho-beta-galactosidase. On the other hand, the disaccharides maltose (present in barley) and sucrose (present in vegetables and fruits) can also be used by certain LAB members.

Nitrogen metabolism

Many LAB have a very limited capacity to synthesize amino acids from inorganic nitrogen sources so they depend on the availability of amino acids present in the environment or growth medium. Dairy LAB highly depend on their proteolytic system, which in turn can degrade external proteins and transport the resulting peptides and amino acids into the cell. The proteolytic system of LAB has been intensively studied, especially in model organisms such as dairy lactococci but also in many lactobacilli such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii* subsp. *lactis*.

Degradation of casein, the major milk protein, is dependent on a cell wall-associated proteinase; this protease hydrolyzes casein into oligopeptides of varied sizes that in turn enter the cell by different transport systems. Inside, peptides are further degraded into amino acids for microbial metabolism and growth.

Applications of LAB

Microbial fermentation increases the shelf-life of perishable raw materials and imparts a characteristic flavor to the fermented products. In addition, fermentation by beneficial microorganisms often introduces or increases health benefits.

Since ancient times, LAB have been empirically used in the preservation and production of fermented foods of plant or animal origin. Since the 1930s and 1940s, LAB have been used as lactic starter cultures in the fermented food industry. Later, because of increased knowledge in the immunology field, these microorganisms started to be used as probiotics, while much more recently efforts have been made to use LAB as microbial cell factories for the production of interesting metabolites. These applications are discussed later in the section 'LAB as Microbial Cell Factories'.

LAB as Starter Cultures in the Food Industry

LAB, the so-called powerhouse or workhorse microbes of the fermented food industry, are intensively employed in food and feed fermentation as well as in food biotechnology.

The ability of LAB to produce lactic acid from various sugars plays an important role in food fermentation. As lactic acid derives from pyruvate (a glycolysis end product), a fast and desirable lactic acid production rate requires a high glycolytic flux. In addition to lactic acid, other alternative end products such as ethanol, acetic acid, and formic acid are formed by many species. LAB have the ability to ferment a variety of

substrates from dairy, meat, vegetables, and cereals, transforming them into tasty products with unique textures and improved (longer) shelf lives. Depending on the raw material, specific, niche-adapted LAB genera and species are employed to carry out fermentation to produce a variety of fermented foods and beverages such as yogurts, fermented milks, buttermilk, kefir, cheeses, fermented sausages, fermented fish, sourdough, pickles, olives, sauerkraut, and other fermented vegetables and fruits. Examples of such fermented foods and beverages and the LAB involved are shown in **Table 1**.

Despite the desirable role of LAB in food fermentation, some psychrotrophic species (i.e., *Leuconostoc mesenteroides*, *Leuconostoc gasicomitatum*, *Lactobacillus curvatus*, and *Lactobacillus sakei*) have been reported to cause detrimental effects in packaged (air, vacuum, and modified-atmosphere) foods. Souring, strange flavors, gas formation, discoloration, slime production, and decreased pH are some defects caused by the presence of this type of bacteria in ready-to-eat vegetable and fruit salads, fresh raw meat, cooked meat products, and composite foods.

Lactic starter cultures are defined as actively growing cultures of LAB that are used to start and control the fermentation process; in addition, these microorganisms contribute to the sensory characteristics of the fermented products and to their safety. Even today, the most relevant industrial application of LAB is their use as starter cultures in food fermentation. Because the starter industry relies on the use of selected strains of certain species with known metabolic properties, the use of starter cultures has undoubtedly improved the commercial and hygienic quality of the fermented final products and the standardization of the entire process. In contrast, the limited number of available strains with high technological performance and the risk of bacteriophage attack results in the search for new strains to diversify products. In this respect, artisanal or homemade fermentations represent a rich source of indigenous LAB for commercial use. Currently, defined, multi-, or mono-strain cultures prepared as frozen or freeze-dried cell concentrates are commercially available.

During the past few decades because of the huge worldwide industrial market for lactic fermentation (estimated as more

Table 1 Examples of fermented foods and beverages using LAB

Fermented foods and beverages	*LAB species*
Dairy products	
Yogurt	*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*
Fermented milks	*Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, *Lactobacillus reuteri*
Kefir	*Lactobacillus kefir*, *Lactobacillus kefiranofaciens*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus kefiri*
Buttermilk	*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis* var. *diacetylactis*
Cheeses	*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, *Streptococcus thermophilus*
Meat products	
Fermented sausages	*Lactobacillus sakei*, *Lactobacillus curvatus*, *Pediococcus acidilactici*
Fish products	
Fermented fish	*Lactobacillus alimentarius*, *Carnobacterium piscicola*
Vegetable products	
Olives	*Pediococcus acidilactici*, *Pediococcus parvulus*, *Leuconostoc mesenteroides*, *Leuconostoc pseudomesenteroides*, *Lactobacillus plantarum*, *Lactobacillus pentosus*
Sauerkraut	*Pediococcus pentosus*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*
Leeks	*Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus sakei*
Eggplant, mustard, beets, peppers, tomatoes, carrots, capers, cabbage	*Lactobacillus fermentum*, *Lactobacillus pentosus*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Lactobacillus brevis*, *Lactobacillus paracasei*, *Lactobacillus pantheris*, *Pediococcus acidilactici*, *Lactobacillus curvatus*, *Weisella consufa*, *Weisella soli*, *Enterococcus faecium*
Soybeans	*Leuconostocs*, *Lactococcus lactis*
Fermented fruits	
Pineapples	*Lactobacillus plantarum*, *Lactobacillus rossiae*
Kiwis	*Lactobacillus plantarum*
Papayas	*Lactobacillus plantarum*, *Lactobacillus pentosus*, *Weisella confusa*, *Weisella cibaria*
Cherries	*Lactobacillus plantarum*, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Pediococcus pentosaceus*
Alcoholic beverages	
Wine	*Oenococcus oeni*
Rice wine	*Lactobacillus sakei*, *Pediococcus acidilactici*
Cereal-based products	
Sourdough	*Lactobacillus sanfranciscensis*, *Lactobacillus farciminis*, *Lactobacillus fermentum*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus amylovorus*, *Lactobacillus reuteri*, *Lactobacillus pontis*, *Lactobacillus panis*, *Lactobacillus alimentarius*, *Pediococcus pentosaceus*, *Weisella cibaria*
Fermented maize products	*Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, *Lactococcus lactis* subsp. *cremoris*

than 100 billion euros in 2011), especially in Western countries, many efforts have been made to improve the performance of LAB as starter cultures or to better understand their physiology for the production of industrially interesting metabolites. As a result, innovation is leading to the commercialization of more stress-tolerant, phage-resistant strains as well as starter cultures that lead to improved organoleptic properties. In this context, some metabolic traits of lactic starter cultures of technological interest, such as production of aroma compounds, bacteriocins, exopolysaccharides (EPS), low-calorie sugars, and so on, have been the targets of research and will be discussed later in this article.

LAB metabolism results in both decreased carbohydrate content in the raw material they ferment and decreased pH caused by lactic acid production. As mentioned earlier, this rapid acidification process is one of the most desirable effects of LAB growth, enabling the inhibition of detrimental microorganisms including several common human pathogens, and thus prolonging the shelf lives of fermented products. While lactic acid imparts a distinctive, desirable, and fresh acid taste to fermented products, the fermentation process must be controlled to avoid excessive acid concentration, which may be rejected by consumers and may mask more delicate flavors such as diacetyl. In cheese making, lactic acid production is also responsible for milk coagulation and texture properties. The pH decrease indirectly affects flavor by controlling the proteolytic activity of both coagulant and natural milk proteinases and by influencing the biochemical reactions involved in the formation of other aroma compounds. In this respect, LAB may enhance the organoleptic properties of the fermented product by imparting a characteristic flavor, which may derive from the fermentation of lactose or other sugars, citrate, the degradation of milk proteins and fat, and the metabolism of amino acids and free fatty acids. The flavor of the final product depends on several factors such as composition of the starter culture and raw material, type of fermentation, and ripening and storage conditions.

Besides the contribution of LAB to the flavor of fermented foods, some strains may contribute to the texture and nutritional quality of the final product. In addition, some LAB secrete natural food preservatives because of the production of antifungal and antibacterial compounds such as additional carboxylic acids (i.e., phenyl-lactic acid and acetic acid), fatty acids, ethanol, carbon dioxide, hydrogen peroxide, and bacteriocins.

Bacteriocin production is a desirable feature because they help control microbial populations in fermented foods and, thus, extend product shelf-life and safety. Bacteriocins produced by LAB are a diverse group of ribosomally synthesized antimicrobial peptides, which may be classified into different groups depending on the presence of posttranslational modifications and if they are active as single- or two-peptide bacteriocins. Nisin is a broad spectrum bacteriocin, which inhibits Gram-positive foodborne pathogens and spoilage microbes as well as Gram-negative bacteria if combined with additional preventatives. Nisin has been the paradigm, the most studied and commercially applied bacteriocin to date. Narrow spectrum bacteriocins, such as lactococcin A, can also be of value as these bacteriocins have lytic effect on sensitive lactococci and may release key enzymes that can accelerate cheese ripening and enhance the development of important organoleptic properties. In food, bacteriocins may be present via in situ production by bacterial fermentation, by the addition of purified or semipurified preparations, or as an ingredient based on a fermentate of a bacteriocin-producing strain.

Certain LAB strains are able to produce EPS, which are polymers of carbohydrates. Depending on their chemical compositions, EPS can be classified as homopolysaccharides, which contain a single type of monosaccharide, and heteropolysaccharides, which are formed from several repeating units of different monosaccharides. The ability to synthesize EPS is widespread among LAB species; however, a wide diversity in respect to EPS structures and to the amount of polysaccharides produced exists among the LAB producer strains. The inclusion of EPS-producing strains in starter cultures is desirable as these biopolymers naturally confer suitable technological characteristics to dairy products, such as improvement of rheological properties and reduction of syneresis of fermented milks as well as better consistency of curd in low-fat ripened cheeses. In addition, health-promoting effects such as a possible role as prebiotics, serum cholesterol-lowering ability, and immunomodulatory and anticarcinogenic activities have been ascribed to EPS produced by LAB. Conversely, the presence of EPS in wine and beer is detrimental.

LAB as Microbial Cell Factories

The central role of glycolysis in LAB has been at the basis of numerous studies to identify potential bottlenecks of the flux control needed not only for optimizing food fermentation processes but also for novel applications of LAB, such as their use as cell factories for the production of food ingredients, green fuels, and chemicals. In this respect, LAB are being used as 'cell factories' for the production of certain metabolites either to be used as purified compounds or to be produced in situ in fermented foods; examples include the production of bacteriocins, vitamins, amino acids, and low-calorie sugars. The use of LAB as cell factories is mainly because of their food-grade status and beneficial effects in the gastrointestinal tract, the availability of genetic tools for strain improvement, the possibility of using cheap substrates for growth, and direct application of their metabolites in food products. It can be affirmed that LAB applications have currently moved beyond the traditional food fermentation processes to the use of LAB as delivery vehicles for drugs, as vaccines, and as microbial cell factories for industrially relevant metabolites.

Metabolic engineering approaches in LAB were first reported in 1995 in an attempt to deviate pyruvate formation in the production of the buttery aroma compound diacetyl by *Lactococcus lactis*. Gene manipulation techniques together with some features of LAB such as small genomes (1.8–3.4 Mbp), fast growth, high sugar update rates, and a relatively simple metabolism, allowed the generation of efficient microbial cell factories. In the past few years, *Lactococcus lactis* and certain *Lactobacillus* species have been manipulated to synthesize metabolites such as lactate, 1,3-propanediol (1,3-PDO), biofuels, food flavors, sweeteners, and EPS. More recently, efforts are being directed to the production of commodity compounds and chemicals with applications in medicine (Table 2).

Table 2 Use of LAB as microbial cell factories for the synthesis of industrially interesting metabolites

Type of metabolite	*Compounds*	*Applications*
High added-value compounds	Lactic acid	Food additive and food preservative; used in food and plastic industries
	2,3-Butanediol (2, 3-BDO)	Antifreeze in plastic and other industrial processes
	1,3-Propanediol (1, 3-PDO)	Polymer, solvent, and cosmetic industries
	Ethanol	Biofuel alternative to gasoline
	Butanol	Solvent; alternative biofuel
Food ingredients	Alanine	Low-calorie sweetener in food
	Diacetyl	Aroma compound in food
	Acetaldehyde	Aroma compound in food
	Vanillin	Flavoring compound in food
Nutrients	Polyols (mannitol, sorbitol, erythritol, etc.)	Low-calorie sugars and sugar replacers in food industry; in medicine, used as a potent osmotic diuretic
	Vitamins (B_{12}, folate, riboflavin)	Essential nutrients in food
	Short-chain fatty acids (SCFAs)	Beneficial health effects
	Conjugated linoleic acids (CLAs)	Beneficial health effects
	Bioactive peptides	Antihypertensive, antioxidant, antimutagenic, etc.
	Gamma-aminobutyric acid (GABA)	Relaxing effect on gut muscles

High added-value compounds

Recently, the production of high added-value chemicals by LAB has been reported, including lactic acid, 2,3-butanediol (2,3-BDO), 1,3-PDO, bioethanol, and butanol.

Lactic acid has been used in the food industry as a flavor additive and food preservative; however, its main use in the market is in the creation of biodegradable plastics (as a precursor of poly-lactic acid) and pharmaceuticals. Traditionally *Lactobacillus casei* has been used to produce lactic acid because of its ability to convert more than 95% of the available sugar into this organic acid. However, by means of metabolic engineering processes and the use of renewable resources such as agro-industrial wastes as substrate, other species such as *Lactobacillus helveticus* and *Lactobacillus plantarum* have been able to efficiently form lactic acid either by achieving the deletion of the *L-ldh* (lactate dehydrogenase) gene or by introducing the *xyl*AB operon of *Lactobacillus pentosus* in the *Lactobacillus plantarum* genome. By these means, production of pure D-lactic acid with a 0.8 g g^{-1} yield was achieved.

Another important chemical compound is 2,3-BDO which is used as an antifreeze in the plastic, chemical, pharmaceutical, and cosmetic industries. Moreover, acetoin and diacetyl, important buttery flavors in some food products, can be formed by dehydrogenation of 2,3-BDO. Biotechnological processes have produced 2,3-BDO using *Klebsiella* strains; however, the pathogenic status of this bacterium has encouraged its synthesis by LAB members because many species possess the required biosynthetic pathway. In this respect engineered strains of *Lactobacillus plantarum* and *Lactococcus lactis* have been shown to efficiently produce 2,3-BDO from inexpensive substrates such as whey permeate.

1,3-PDO is another compound with multiple applications in the production of polymers, solvents, resins, detergents, and cosmetics. To date, this diol is industrially produced by chemical synthesis; however, its microbial production from glycerol, a byproduct of the biodiesel industry, has been intensively studied. Again, the food-grade status of LAB makes it the preferred option for synthesis of 1,3-PDO, rather than *Klebsiella* and *Clostridium* strains, which are considered opportunistic pathogens and thus are not applicable for food and biomedical products. Among the naturally lactobacilli producers of 1,3-PDO, *Lactobacillus reuteri* has been reported to be the best one so far.

Biotechnological production of ethanol may be one of the most important sources of renewable energy because this compound is used as an alternative biofuel to gasoline. In the past few years, to overcome the natural restrictions of crude oil use, researchers have become interested in using LAB to produce ethanol from renewable biomass such as lignocellulose, given that these microorganisms can metabolize different sugars. So far, the most promising results have been reported for an LDH-negative strain of *Lactobacillus plantarum*, that expresses pyruvate decarboxylase from a strain of the Gram-positive *Sarcina ventriculi*.

Also, butanol is a desirable solvent that may be an attractive fuel alternative. While butanol-producing strains cannot tolerate amounts higher than 2%, researchers may apply genetic engineering strategies to certain LAB members to produce butanol in the presence of 3% butanol.

Food ingredients/additives

Several compounds formed by LAB, such as alanine, diacetyl, and acetaldehyde, are currently employed as food ingredients.

Alanine is an amino acid with a natural sweet taste that is used as a food and pharmaceutical additive. It is produced through pyruvate reduction by alanine dehydrogenase in the presence of ammonium. Homoalanine fermentation is achieved by a metabolic engineering strategy through the heterologous overexpression of alanine dehydrogenase from *Bacillus sphaericus* in an LDH-deficient *Lactococcus lactis* strain.

Diacetyl is an important flavor compound that has a buttery aroma and is typically used for dairy products; diacetyl is naturally produced by LAB from citrate, which limits its production because citrate availability in raw materials is usually low. To surpass this limitation, researchers have created some

metabolic engineering strategies to produce diacetyl from lactose or glucose.

Acetaldehyde is an important aroma compound, especially in dairy products; for instance, it is the main aroma compound in yogurt. Because acetaldehyde is an intermediate metabolite from ethanol metabolism, efforts to increase diacetyl production are usually connected with the ethanol pathway. So far, the best engineered strategy for this purpose consists of the heterologous overexpression of pyruvate decarboxylase from *Zymomonas mobilis* with native NADH oxidase in *Lactococcus lactis*.

Vanillin is one of the most used flavoring compounds worldwide; as the annual world market demand of vanillin cannot be met by natural extraction, chemical synthesis or tissue culture biotechnological approaches have been considered. Biovanillin synthesis using GRAS LAB and metabolically engineered microorganisms is currently being studied.

Low-calorie sugars

Polyols, or low-calorie sugars, are sugar alcohols that naturally occur in nature; among them, mannitol and erythritol are the only ones naturally produced by LAB. These two sugar alcohols, together with sorbitol and xylitol, are widely used in the food and pharmaceutical industries and in medicine because of their interesting physicochemical properties. Moreover, as these low-energy, nonmetabolizable sweeteners do not affect insulin levels, they are employed in dietetic and diabetic food products.

In the past decade, biotechnological production of polyols by LAB has been sought as an alternative to their current chemical production. While heterofermentative LAB may naturally produce mannitol and erythritol under certain culture conditions, sorbitol and xylitol have been synthesized only through metabolic engineering processes. Although strains belonging to the *Oenococcus*, *Leuconostoc*, and *Weisella* genera have been reported to form mannitol, the best mannitol-producing microbes reported so far are heterofermentative lactobacilli such as *Lactobacillus fermentum* and *Lactobacillus reuteri*. Controlled culture conditions using selected LAB strains have yielded amounts of mannitol that are similar to those of current industrial chemical processes.

Sorbitol production by *Lactobacillus casei* and *Lactobacillus plantarum* strains has been achieved by reverting the sorbitol catabolic pathway, taking advantage of the reversibility of the enzyme sorbitol 6-P dehydrogenase, which is responsible for the interconversion of sorbitol 6-P into fructose 6-P.

While metabolic engineering strategies using homologous genes have been employed for sorbitol synthesis by LAB, xylitol production has been achieved only by heterologous expression of D-xylose reductase and xylose transporter genes from the yeast *Pichia stipitis* CBS 5773 and the strain *Lactobacillus brevis* ATCC 8287, respectively.

Nutrients

A wide range of compounds with nutritional relevance such as vitamins, polysaccharides, short-chain fatty acids (SCFAs), conjugated linoleic acids (CLAs), and bioactive peptides are synthesized by LAB.

Vitamins are essential micronutrients for the metabolism of living organisms. Because of insufficient food intake or unbalanced diets, human vitamin deficiencies are still very common. As an attractive alternative to chemical vitamin synthesis, biotechnological production of vitamins using LAB had been under study in the 2000's. Furthermore, fermentation with food-grade LAB offers unique opportunities to improve the nutritional value of food products and the development of novel functional foods with enhanced vitamin content. As a result of secondary metabolism, certain LAB are able to synthesize some group B vitamins such as B_{12} (cobalamin), B_{11} (folate), and B_2 (riboflavin). In this respect, several industrially important LAB such as *Lactococcus lactis* and *Streptococcus thermophilus* are capable of synthesizing folate as has been observed in fermented milks with high vitamin levels. Riboflavin's biosynthesis has been identified in several species, and some applications of riboflavin-producing LAB in dairy and cereal-based products have also been reported. Cobalamin, a complex corrin compound, was found to be produced for the first time by *Lactobacillus reuteri*, a well-recognized probiotic species. In general, vitamin production by LAB varies considerably because it is a species-specific or strain-dependent trait. This feature is generally related to the partial or complete interruption of the genetic information for vitamin biosynthesis. The use of vitamin overproducer LAB strains may be exploited for their in situ production of fermented products.

SCFAs are not produced anabolically in LAB but rather as the final catabolites of the energy metabolism. The most studied SCFA are butyrate, propionate, and acetate. Besides the SCFA acidifying desirable properties, acetate may be metabolized by muscles and other tissues when entering the peripheral circulation while propionate is taken up by the liver. Both acids can lower glycemia, improving insulin sensitivity. Conversely, butyrate has been claimed to have detrimental effects on body weight and obesity because it represents an additional energy source. In contrast, butyrate may control oxidative and metabolic stress at the molecular level by enhancing repair responses and inhibiting the synthesis of pro-inflammatory (IL12, TNF, gamma IF) cytokines.

CLAs are a novel type of biologically beneficial functional lipids. Human metabolism can also be affected by these compounds as some CLA isomers may reduce cancer cell viability and ameliorate insulin resistance while others may lower the HDL/LDL ratio and modify eicosanoid production, with detrimental health effects. So far, the best CLA-producing strains belong to the genera *Lactobacillus* and *Streptococcus*. The most frequently found isomers of linoleic acid have the unsaturation site at positions 9, 11 or 10, or 12, namely, Δtrans 9-, cis11-octa decadienoic acid, and, in lower amounts, Δcis10-, trans12-linoleic acid. CLAs could have health beneficial effects on inflammation, cancer, metabolic disorders, and cardiovascular diseases. However, the beneficial effects of CLAs depend on the isomers and doses.

Bioactive peptides are hidden peptides, which are encrypted in the latent state within the primary sequence of proteins that require enzymatic proteolysis for their release. Bioactive peptides can be produced during gastrointestinal digestion or food processing. LAB that have reduced amino acid biosynthetic abilities harbor a sophisticated proteolytic system, which may contribute to the release of bioactive peptides or their precursors. Thus, proteolytic LAB strains used as starter cultures in the manufacture of different foods (e.g., yogurts, fermented milks, and cheeses) may have a positive effect in this sense. Diverse

health benefits have been ascribed to bioactive peptides derived from LAB, including antimicrobial, immunomodulatory, antithrombotic, opioid, and antioxidant effects as well as enhancement of mineral absorption and/or bioavailability and blood pressure-lowering activities. Today some milk-derived peptides are commercially produced and used as dietary supplements in functional foods, personal care products, or drugs.

Among other compounds, some LAB strains biosynthesize gamma-aminobutyric acid (GABA), which has a relaxing effect on gut smooth muscles, and beta-phenylethylamine, which contributes to the control of satiety and mood.

LAB as Probiotics

The gut microbiota is a highly diverse and relative stable ecosystem that is being increasingly recognized for its impact on human health. Deviation from its normal composition is usually associated with localized and systemic diseases. Modulation of the gut microbiota may help to improve health and can be achieved by different nutritional concepts, varying from specific food ingredients to complex diets or by the ingestion of particular live microorganisms such as probiotics.

Probiotics have been defined as "live microorganisms that when administered in appropriate dose, they confer a health benefit to the host" (FAO/WHO 2001). Probiotics have been empirically used for centuries, but only in recent decades has their contribution to the modulation of immunological, respiratory, and gastrointestinal functions started to be fully appreciated and scientifically evaluated. To date, the majority of the available commercial probiotics are LAB, mainly of the *Lactobacillus* genera but also bifidobacteria (a group of bacteria with a high G+C content included in the phylum Actinobacteria that also produce lactic acid but always in combination with acetic acid). A much smaller number of leuconostocs, pediococci, lactococci, enterococci, and streptococci are also used as probiotics.

Probiotic strains are usually isolated from traditional fermented products and the gut, feces, and breast milk of human subjects. The identification of microorganisms is the first step in the selection of potential probiotics. The ability to examine fully sequenced genomes has accelerated the application of genetic approaches to elucidate the functional roles of probiotics.

Fermented foods and beverages possess various nutritional and therapeutic properties; in this sense LAB play a major role in the positive health effects of fermented milks and related products. Such foods, beverages, and powders are highly acceptable to consumers because of their flavor and high nutritional value. Potential health benefits of fermented milks include antimutagenic and antitumor activity (associated with the cell wall of starter bacteria), prevention of gastrointestinal infections, and reduction of serum cholesterol levels. Although probiotics are mainly used for gastrointestinal benefits, from which the best-demonstrated clinical benefits of probiotics are the prevention and treatment of antibiotic-associated diarrhea, their use can also extend to skin, oral, and vaginal health, as well as the treatment of allergies, liver disorders, and metabolic diseases (Table 3). Recently, investigation of the gut-brain connection suggested that the gut microbiota play a key role in mood and anxiety disorders, supporting the inclusion of probiotics in animal and human products. It has been suggested that an improvement of the intestinal barrier function through microbiota modification can decrease lipopolysaccharide infiltration, reduce inflammatory parameters in the fat tissue, and, consequently, reduce the chances of developing obesity and insulin resistance. Probiotics may also regulate host intestinal microbiota, improve gut immune barrier function, and achieve a balance between pro-inflammatory and anti-inflammatory cytokines.

Table 3 Examples of beneficial health properties of LAB used as probiotics

Beneficial health effect	*Associated LAB species*
Antidiarrheal	*Lactobacillus acidophilus*
Antitumor activity	*Lactobacillus reuteri*
Antimutagenic activity	*Lactobacillus casei*
Anticarcinogenic effects	*Lactobacillus paracasei*
Serum cholesterol reduction	*Lactobacillus rhamnosus*
Prevention of gastrointestinal infections	*Lactobacillus kefir*
	Lactobacillus plantarum
Improvement of the gut immune barrier	*Lactobacillus helveticus*
	Lactobacillus delbrueckii subsp. *lactics*
Modulation of the immune response	*Lactobacillus delbrueckii* subsp. *bulgaricus*
Treatment of allergy disorders	*Lactobacillus gasseri*
Regulation of the gut and vagina microbiota	*Lactobacillus johnsonni*
	Lactococcus lactis
	Lactobacillus salivarius
	Lactobacillus pentosus
	Streptococcus thermophilus
	Enterococcus faecalis
	Enterococcus faecium
	Pediococcus acidilactici
	Pediococcus pentosaceus

Probiotic bacteria typically colonize the intestinal tract first and then reinforce the host defense systems by inducing generalized mucosal immune responses, including modulation of dendritic cell/natural killer interaction, a balanced T-helper cell response, self-limited inflammatory response, and the secretion of polymeric Immunoglobulin A. Many reports have demonstrated that LAB, mainly *Lactobacillus* as well as *Bifidobacterium* and their fermented products, may improve innate and adaptive immunity, prevent gastric mucosal lesions, alleviate allergies, and create a defense against intestinal pathogen infection. Also, probiotic LAB have been included in the production of functional foods with specific properties in order to potentially prevent or treat a range of intestinal maladies including inflammatory bowel disease, constipation, and colon cancer.

It has been shown that fermented animal feed using LAB has been beneficial in pig nutrition and the nutrition of other farm animals as a tool to reduce gut microbial disorders, to increase antibacterial activity, and to enhance immunity. Colonization of the digestive tract in animals begins immediately after birth because adult microbiota dramatically change during the host's life. In farm animals, the role of the gut microbiota, which is similar to that in humans, does not differ much among animal species. To ensure the animals optimal growth, production, and health, the microbiota of the animal

gastrointestinal tract can be manipulated by probiotic bacteria. Probiotics have already been given to different farm animals such as cattle, chicks, piglets, lambs, and small ruminants. In this respect, LAB have been proposed as protective bacteria to reduce *Escherichia coli* O157 in cattle and *Salmonella* in poultry, as well as to prevent diarrheal disease in piglets.

Conclusions

Here I have presented information about the versatility of the heterogeneous, broad LAB group, including findings about their metabolism, food and industrial applications, and health benefits. Their traditional and empirical use in food preservation from the time of the earliest written words has evolved beyond imagination along with scientific progress. This long and challenging journey has allowed not only the development of rational and novel applications of LAB but also a deeper understanding of the involved mechanisms to improve human health and well-being.

See also: Fermented Foods: Composition and Health Effects; Fermented Foods: Fermented Meat Products; Fermented Foods: Fermented Milks; Fermented Foods: Fermented Vegetables and Other Products; Fermented Foods: Origins and Applications; Fermented Foods: Use of Starter Cultures; Functional Foods; Probiotics; Yogurt: Yogurt Based Products

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Lactose

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Introduction

The disaccharide lactose occurs in milk, mainly free with a small extent as a component of higher oligosaccharides. Its concentration in milk varies with the mammalian source from 2.0% to 8.5%. Cow and goat milks contain 4.5–4.8% and human milk about 7%. It is the primary carbohydrate for developing mammals. In humans, lactose constitutes 40% of the energy consumed during nursing. The utilization of lactose for energy must be preceded by hydrolysis to the constituent monosaccharides, D-glucose and D-galactose. Milk also contains 0.3–0.6% of lactose-containing oligosaccharides, many of which are important as energy sources for growth of a specific variant of *Lactobacillus bifidus*, which is the predominant component of the intestinal microbiota of breast-fed infants.

Lactose is ingested through milk and other unfermented dairy products, such as ice cream. Fermented dairy products, such as most yogurt, dahi, and cheeses, contain less lactose because during fermentation, some of it is converted into lactic acid. It stimulates intestinal adsorption and retention of calcium. Lactose is not digested until it reaches the small intestine, where the hydrolytic enzyme lactase is located. Lactase (β-galactosidase) is a membrane-bound enzyme located in the brush border epithelial cells of the small intestine. Lactase catalyzes the hydrolysis of lactose into its constituent monosaccharides. Only monosaccharides among the carbohydrates are absorbed from the intestines. Both D-glucose and D-galactose are rapidly absorbed and enter the bloodstream.

Status of Lactose

Lactose is the most prominent carbohydrate of milks of most species. It is virtually unique to milk, having been found elsewhere only in fruits of certain members of the Sapotaceae. The presence of lactose is observed in *Forsythia* flowers and ripe fruits of *Achras sapota* (Sapotaceae). Less than 0.1% of lactose is present in the milk of *Echidna* and *Platypus*, while highest concentration of lactose is present in human milk, that is, 7.0%. Its concentration is lowest in the blood and urine especially during pregnancy and lactation. The presence of lactose in urine is known as lactosuria. It is the principal carbohydrate present in milk of all mammals.

It could be seen from the content of Table 1 that the concentration of lactose in milk varies widely between species. Its concentration decreases progressively and significantly during lactation. Mastitis causes an increased level of NaCl in milk and depresses the secretion of lactose. Lactose, along with sodium, potassium, and chloride ions, plays a major role in maintaining the osmotic pressure in the mammary system. Thus, any increase or decrease in lactose content (a secreted constituent, i.e., formed within the mammary gland) is compensated for by an increase or decrease in the soluble salt (excreted) constituents. This osmotic relationship partly explains why certain milks with high lactose content have low ash content and vice versa.

Structure of Lactose

Lactose is a disaccharide consisting of galactose and glucose, linked by a β-L-4 glycosidic bond (Figure 1). Its systematic name is β-0-D-galactopyranosyl-(1–4)-α-D-glucopyranose (α-lactose) or β-0-D-galactopyranosyl-(1–4)-β-D-glucopyranose (β-lactose). The hemiacetal group of the glucose moiety is potentially free (i.e., lactose is a reducing sugar) and may exist as an α- or β-anomer. In the structural formula of the α-form, the hydroxyl group on the C_1, of glucose, is cis to the hydroxyl group at C_2 (oriented downward).

Physical Forms of Lactose

In milk, lactose is normally found as either α-lactose or β-lactose or amorphous glass form (mixture of α- and

Table 1 Concentration of lactose in milk of selected species

Species	*Lactose (%)*
California sea lion	0.0
Hooded seal	0.0
Black bear	0.4
Dolphin	0.6
Echidna	0.9
Blue whale	1.3
Rabbit	2.1
Red deer	2.6
Gray seal	2.6
Rat (Norwegian)	2.6
Mouse (house)	3.0
Guinea pig	3.0
Dog (domestic)	3.1
Sika deer	3.4
Goat	4.1
Elephant (Indian)	4.7
Cow	4.8
Sheep	4.8
Water buffalo	4.8
Cat (domestic)	4.8
Pig	5.5
Horse	6.2
Chimpanzee	7.0
Rhesus monkey	7.0
Human	7.0
Donkey	7.4
Zebra	7.4
Green monkey	10.2

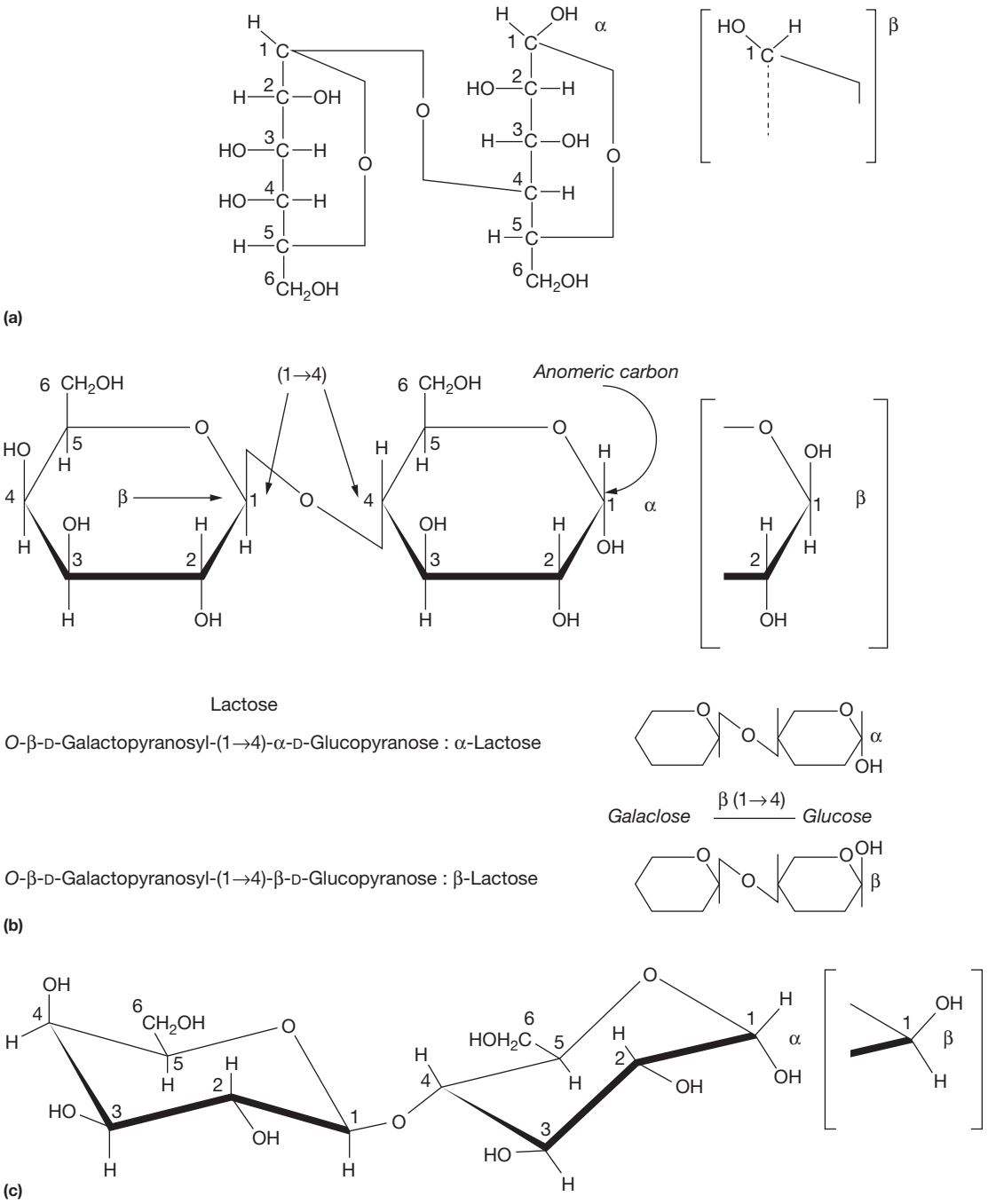

Figure 1 Structures of α-lactose and β-lactose. (a) Fischer projection, (b) Haworth projection, and (c) conformational formula.

β-forms). Several other forms can be prepared under special condition as given in the following:

α-Lactose Monohydrate ($C_{12}H_{22}O_{11}.H_2O$)

Any form of lactose crystallizes below 93.5 °C to α-monohydrate form (if allowed). In milk, α-anhydrous form is not prepared. Supersaturate solution of lactose when allowed to crystallize at 93.5 °C or below will give α-monohydrate form. Above 93.5 °C, it will give β-anhydrous. It contains 5% water of crystallization. It has solubility of 70 g l^{-1} water at 20 °C, specific rotation +89.4°, and melting point of 202 °C. It forms a number of crystal shapes, depending on the conditions of crystallization; the most common type when fully developed is tomahawk-shaped. Crystals are hard and dissolve slowly. In the mouth, crystals less than 10 μm are undetectable, but above 16 μm, they feel gritty or sandy, and at 30 μm, a definite gritty texture is perceptible. The term sandy or sandiness is used to describe the defect in condensed milk, ice cream, or processed cheese spreads where, due to poor manufacturing techniques, large lactose crystals are formed.

Anhydrous α-Lactose

Regular (unstable) anhydrous α-lactose

Anhydrous α-lactose may be prepared by dehydrating α-hydrate in vacuum at temperatures between 65 and 93.5 °C; it is stable only in the absence of moisture. During the reaction, moisture loss is negligible up to 85 °C, whereas it is significant at 90 °C and rapid at 120–125 °C.

Anhydrous α-lactose is stable in dry air and is highly hygroscopic. In the presence of moisture, it forms α-hydrate before dissolving. In a solution that is saturated with α-hydrate lactose, it will not dissolve; this behavior suggests that no change has occurred in its properties.

Stable anhydrous α-lactose

Stable form of α-lactose is prepared by dehydrating α-hydrate in vacuum of 6–80 mm Hg at temperatures between 100 and 190 °C and crystallizing it with suitable solvent especially dry methanol or 95% absolute ethanol.

New crystalline structure reduces tendency to absorb water so this anhydrous form remains stable even in an environment of 50% relative humidity. Product containing 99% anhydrous lactose can be prepared by refluxing α-hydrate crystals with methanol in the ratio of 1:10 for 1 h. At room temperature, reaction is slower. The rate decreases with increasing proportion of α-hydrate in dry methanol. Anhydrous α-lactose is stable, has high density, and is nonhygroscopic. It dissolves in water forming hydrate. It dissolves readily in solution and that is saturated with α-lactose hydrate. It is more soluble in water than either α-hydrate or β-anhydride form.

β-Anhydride Form

Since β-lactose is less soluble than the α-isomer above 93.5 °C, the crystals formed from aqueous solutions at temperatures above 93.5 °C C are β-lactose; these are anhydrous and have a specific rotation of +35° and melting point of 252 °C. β-Lactose is sweeter than α-lactose, but is not appreciably sweeter than the equilibrium mixture of α- and β-lactose normally found in solution. β-Lactose dissolves much faster than α-lactose hydrate at room temperature, as its solubility is about ten times higher and the crystals are usually smaller with a larger surface area.

α- and β-Anhydrous Forms (Lactose Glass)

It is also called as amorphous noncrystalline glass. When a lactose solution is dried rapidly, its viscosity increases so quickly that crystallization cannot take place. The dry lactose is essentially in the same condition as it was in solution, except for removal of the water. This is spoken of as 'concentrated syrup' or an 'amorphous' (noncrystalline) glass. Lactose glass is stable if protected from moisture, but since it is very hygroscopic, it rapidly takes up moisture from the air and becomes sticky. When the moisture content reaches about 8% or a relative vapor pressure near 0.5, the lactose achieves a maximum weight; a discontinuity is observed in the sorption isotherm, and water is desorbed from the lactose.

Lactose in spray-dried milk exists as a concentrated syrup or amorphous glass that is stable if protected from air but is very hygroscopic and absorbs water rapidly from the atmosphere, becoming sticky.

Biosynthesis of Lactose

Lactose is essentially unique to mammary secretions. Lactose is synthesized in the epithelial mammary cells from two molecules of glucose absorbed from the blood. One molecule of glucose is converted (epimerized) to UDP-galactose (uridine diphosphate-galactose) via the four-enzyme Leloir pathway (Figure 2), which is widespread in animal tissues and bacterial cells. The UDP-galactose is then phosphorylated and condensed with a second molecule of glucose through the action of a unique two-component enzyme, lactose synthase. One component, that is, component A, is a nonspecific UDP-galactosyl transferase (EC 2.4.1.22), which transfers galactose from UDP-galactose to any of several acceptor molecules in the biosynthesis of glycoproteins and glycolipids. The specificity of the transferase is controlled and modified by component B (α-lactalbumin – one of the principal milk proteins). The α-lactalbumin reduces the KM for glucose 1000-fold, and in its presence, the transferase

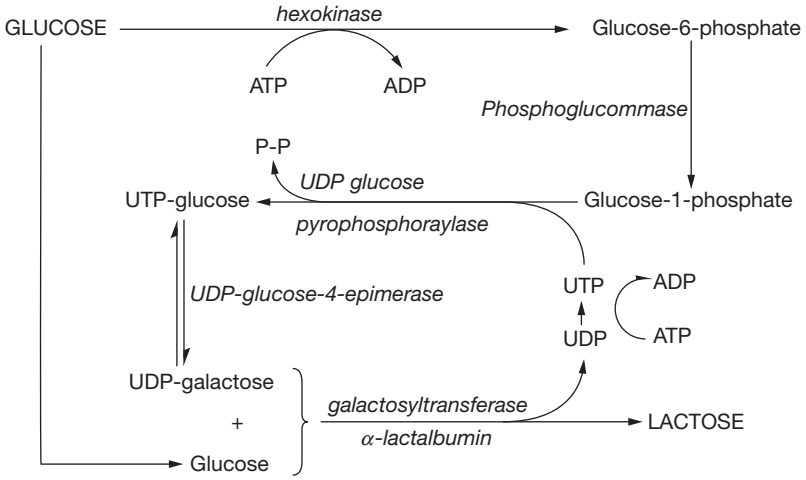

Figure 2 Leloir pathway of lactose biosynthesis.

becomes highly specific for glucose and most of the galactose is transferred to glucose, leading to the synthesis of lactose. Thus, α-lactalbumin is an enzyme modifier and its concentration in the milk of several species is directly related to the concentration of lactose in those milks; the milks of some marine mammals contain neither α-lactalbumin nor lactose (e.g., the milk of the California sea lion or the hooded seal, which contains no lactose and also lacks α-lactalbumin).

Properties of Lactose

The properties of lactose are generally similar to those of other sugars but it differs in some technologically important respects. Some important characteristics of lactose are as follows:

- Lactose is a reducing sugar, that is, it has a free, or potentially free, carbonyl group (an aldehyde group in the case of lactose).
- Like other reducing sugars, lactose exists partially as an open-chain form with an aldehyde group that can form a hemiacetal and, thus, a ring structure. The formation of a hemiacetal creates a new chiral center (asymmetrical carbon), which may exist as two isomers (enantiomorphs), α and β. By alternatively opening and forming the ring structure, the molecule can interchange between α- and β-anomers, a process referred to as mutarotation.
- The α- and β-anomers of lactose have very different properties, the most important of which are specific rotation, $[\alpha]_D^{20}$ (+89° and +35° for α- and β-lactose, respectively), and solubility (70 and 500 g l^{-1} for α- and β-lactose, respectively).
- Like all reducing sugars, lactose can participate in the Maillard (nonenzymatic browning) reaction, resulting in the production of (off-)flavor compounds and brown polymers. The Maillard reaction contributes positively to the flavor and color of many foods, that is, crust of bread, toast, and deep-fried products, but the effects in dairy products are usually negative and must be avoided.
- Redox titration using alkaline $CuSO_4$ (Fehling's solution) or chloramine-T is the principal standard method for the quantitative determination of lactose, although in large laboratories, it is now usually determined by infrared spectrophotometry. It may also be determined by polarimetry, spectrophotometry at a visible wavelength after reaction with phenol or anthrone in strongly acid solution, or high-performance liquid chromatography or enzymatically (using an enzyme assay kit).
- Among sugars, lactose, especially the α-enantiomorph, has low solubility in water, but when in solution, it is difficult to crystallize, which may cause problems in lactose-rich dairy products, that is, skimmed milk powder and whey powder, unless precautions are taken to induce and control crystallization.
- α- and β-lactose are soluble in water to the extent of about 70 and 500 g l^{-1}, respectively, at 20 °C; at equilibrium, the ratio of α:β is about 37:63, giving a total solubility of about 180 g l^{-1} at 20 °C. The solubility of α-lactose is more temperature-dependent than that of the β-anomer and it is more soluble at >93.5 °C (Figure 3). Hence, α-lactose is the form of lactose that crystallizes at <93.5 °C and is the usual commercial form of lactose; β-lactose may be prepared by crystallization at >93.5 °C.
- α-Lactose crystallizes as a monohydrate, while β-lactose forms anhydrous crystals; thus, the yield of α-lactose is approx. 5% higher than that of β-lactose.
- When milk or whey is spray-dried, any lactose that has not been precrystallized forms an amorphous glass that is stable if the moisture content of the powder is maintained low. However, if the moisture content increases to >6%, the lactose crystallizes as α-hydrate, the crystals of which form interlocking masses and clumps that may render the powder unusable if very extensive, that is, inadequately, crystallized powder is hygroscopic. The problem can be avoided by adequate crystallization of lactose before drying or by using effective packaging.

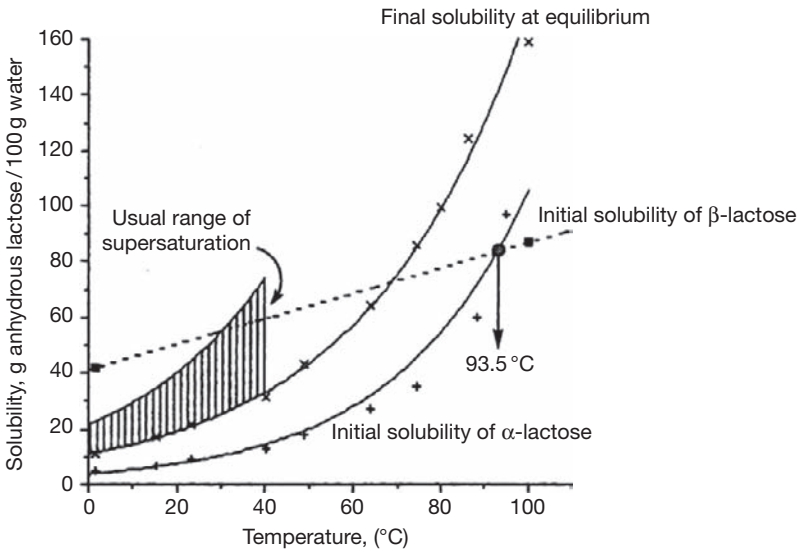

Figure 3 Solubility of α- and β-lactose as a function of temperature.

- Interestingly, crystalline lactose has very low hygroscopicity and is used in icing sugar blends.
- The densities of the various lactose crystals differ slightly from each other. The density of α-hydrate form is 1.540, anhydrous β is 1.589, anhydrous α formed by dehydration under vacuum is 1.544, and anhydrous α-lactose crystallized from alcohol is 1.575. Densities of lactose solutions are not linear functions of concentration.
- Among sugars, lactose has a low level of sweetness; it is only about 16% as sweet as sucrose at 1% in solution and hence has limited value as a sweetening agent, the principal application of sugars in foods. However, it is a useful bulking agent when excessive sweetness is undesirable.
- Lactose is important in the manufacture of fermented dairy products where it serves as a carbon source for lactic acid bacteria (LAB) that produce lactic acid.

Derivatives of Lactose

Like other sugars, lactose has reactive functional groups and can be converted to several more valuable food-grade derivatives.

β-Galacto-oligosaccharides (GOSs) are generally synthesized from lactose by transgalactosylation of lactose by the enzyme β-galactosidase. In contrast, lactosucrose is prepared by a different enzymatic route. Here, lactose, in the presence of sucrose, acts as the acceptor of fructose in a transfructosylation reaction catalyzed by the enzyme β-fructofuranosidase. Fructooligosaccharides are also synthesized from sucrose by similar enzymatic routes.

Lactulose and lactitol are prepared by two different chemical syntheses from lactose. Lactulose is prepared by alkaline isomerization of lactose, while lactitol is synthesized by catalytic hydrogenation of lactose. Lactulose can also be prepared by an enzymatic route, though this is not used commercially. Lactobionic acid is prepared by dehydrogenation of lactose at high pH using a metal catalyst. However, it can also be prepared in high yield by microbial bioconversion and by enzymic oxidation of lactose. Tagatose was prepared initially from galactose by alkaline isomerization in a manner parallel to the preparation of the disaccharide, lactulose from lactose, but it is now manufactured by an enzymatic route from lactose. The basis of the preparation of GOS, lacto-sucrose, lactulose, lactitol, lactobionic acid, and tagatose from lactose is shown in Figure 4.

Properties of Lactose Derivatives

Properties of oligosaccharides

Food-grade oligosaccharides are not pure products, but are mixtures containing oligosaccharides of different degrees of polymerization (dp), the parent polysaccharide or disaccharide and monomer sugars. A typical commercial product

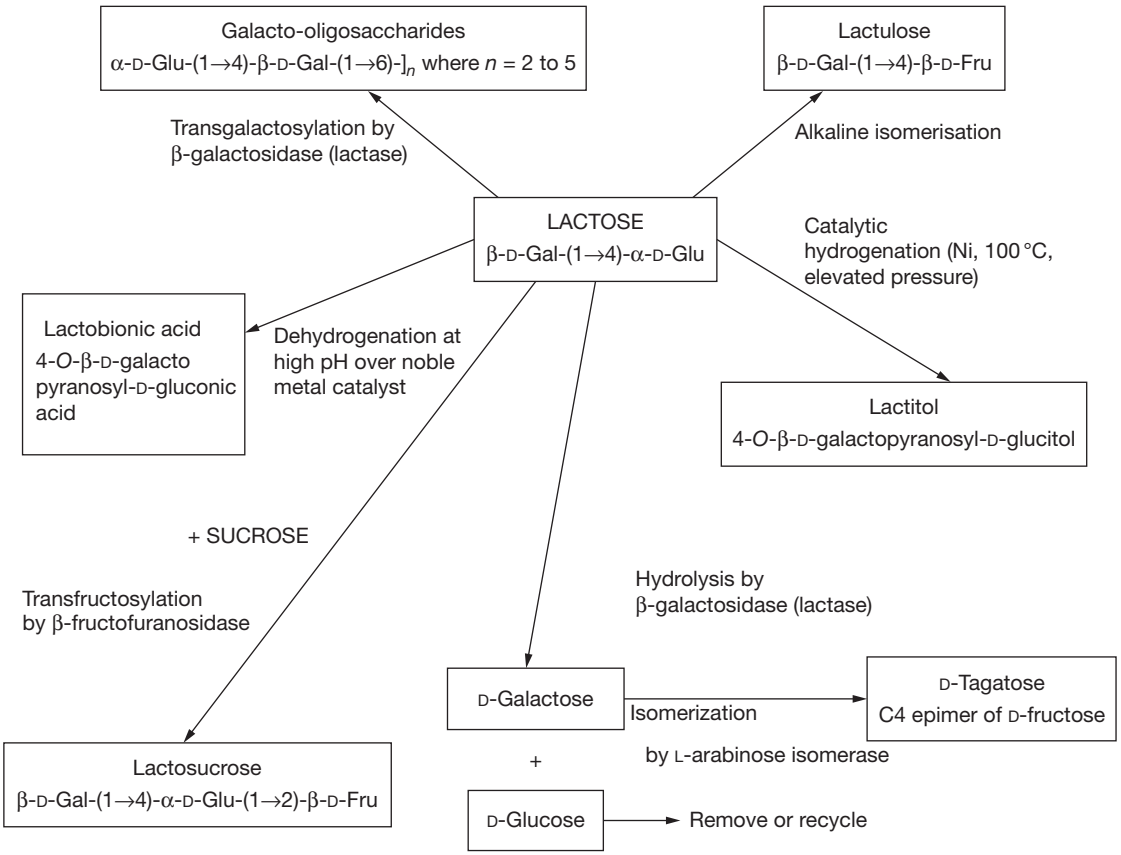

Figure 4 Derivatives of lactose (the preparation of galacto-oligosaccharide, lacto-sucrose, lactulose, lactitol, lactobionic acid, and tagatose from lactose).

composition for an unpurified GOS may comprise glucose and galactose 10%, disaccharides (lactose) 70%, trisaccharides 18.7%, tetrasaccharides 1.2%, and pentasaccharides 0.06%. More advanced manufacturing processes result in much higher concentrations of GOSs in the product. For example, Oligomate 50 produced by the Yakult Company contains 50–52% GOSs, 10–13% lactose, and 36–39% monosaccharides. Purified products are available from many manufacturers, but costs increase considerably because of the extra processing steps required. Purified products usually contain between 85 and 99% GOSs. It is obvious that there are important differences in the properties and actions of food-grade oligosaccharide mixtures depending on the mixture purchased. The applications of oligosaccharides as components in foods will therefore vary considerably. Most producers also provide safety and toxicological data for acute, subacute, and chronic toxicities. Data are also commonly provided to demonstrate changes in colonic microbial flora following ingestion of oligosaccharides. Usually, substantial increases in bifidobacteria are demonstrated relative to the other bacterial classes measured. The physicochemical properties of oligosaccharides are largely dependent on the molecular weight of the particular oligosaccharide product.

General properties of GOSs

Appearance	Translucent/colorless
Sweetness	Typically 0.3–0.6 times that of sucrose
Calorific value	Lower than sucrose
Water activity	Similar to that of sucrose
Viscosity	Similar to that of high-fructose glucose syrup
Heat stability	Stable to 160 °C for 10 min at pH 7.0 and to 100 °C for 10 min at pH 2.0
Acid stability	Stable to pH 2
Indigestibility	Resistant to salivary and pancreatic α-amylases and other carbohydrases and gastric juices and thus reducing the glycemic index of foods
Energy value	About 50% of that of sucrose
Freezing point	Reduces the freezing point of foods
Bacteriostatic property	Through competition for mucosal binding sites and adhesion by pathogens. Some oligosaccharides are also able to absorb lectin-like toxins, for example, VTEC and ETEC
Bifidogenic ability	Able to increase the population of most species of bifidobacteria in mixed populations of bacteria in both *in vitro* and *in vivo* studies in humans and animals
Cariogenicity	Acts as an anticaries agent (anticariogenic)

Physicochemical properties of oligosaccharides

Taste	Sweetness decreases as molecular size increases
Mouthfeel	Larger-sized oligosaccharides have applications as fat replacers
Viscosity	Increases with increasing molecular size
Solubility	Decreases with increasing molecular weight
Hygroscopicity	Oligosaccharides can be used to control the moisture level in foods
Color reactions	Decreases as molecular weight increases
Freezing point	Depression of freezing point is inversely proportional to molecular weight
Osmolality	Decreases as molecular weight increases

The susceptibility to hydrolysis of oligosaccharides during passage through the gastrointestinal tract is an important characteristic. This has been determined in a range of *in vitro* model systems of the stomach and small intestine of humans. Different types of oligosaccharides have been subjected to regimes such as (1) hydrolysis by human saliva (50%, v/v, saliva, 30 min exposure, pH 7.0, 37 °C), (2) hydrolysis by gastric acid (4 h exposure, pH 2.0, 37 °C in acid/pepsin), and (3) hydrolysis by pancreatic and brush border carbohydrases (17%, v/v, porcine pancreatic and duodenal homogenates for 1 h exposure, pH 7.0, 37 °C). Carbohydrate analysis before and after exposure by HPLC then determines the degree of hydrolysis. Tests such as these have shown that xylo-oligosaccharide mixtures, palatinose condensates, commercial GOS mixtures, and lactulose are very resistant to hydrolysis, whereas lactosucrose and gentio-oligosaccharides, soybean oligosaccharides, fructooligosaccharide mixtures, and inulin oligosaccharides are hydrolyzed slightly by such conditions.

Properties of lactulose

Lactulose solution is yellowish, odorless clear syrup with a sweet taste. Dry lactulose is a white, odorless crystalline powder. It is soluble in water, but poorly soluble in methanol and insoluble in ether. Its solubility in water is 76.4% (w/w) at 30 °C, rising to 86% at 90 °C. The melting point is 168.5–170.0 °C. Its sweetness is classified as 0.48–0.62 times that of sucrose. It is 1.5 times sweeter than lactose. Acid hydrolysis of lactulose yields galactose and fructose. Unlike lactose, lactulose cannot be hydrolyzed by human intestinal enzymes. Lactulose can be fermented by some human colonic bacteria and acts as a prebiotic ingredient. Heat sterilization is a common step in food manufacture. Lactulose is stable and little decomposed when heated to 130 °C for 10 min at low pH. This relatively high stability makes it a suitable ingredient for normal food processing.

Properties of lactosucrose

Lactosucrose is very soluble in water (up to 3670 g l^{-1} at 25 °C). It has sweetness relative to sucrose of 0.3. It is stable for 1 h at pH 4.5 and 120 °C. It is most stable at neutral pH and remains fairly stable at pH 3 at 80 °C. As a powder, it has a high moisture-retaining capacity and is highly hygroscopic. It acts as a prebiotic carbohydrate and is not readily digested by intestinal enzymes in the stomach and small intestine. It is selectively fermented by *Bifidobacterium* in the human colon.

Properties of lactitol

Lactitol is an odorless, colorless, sweet, nonhygroscopic, and stable sugar alcohol. As it lacks a reactive carbonyl group, which is characteristic of sugar molecules, it does not participate in Maillard browning reactions with amino groups. It occurs in both monohydrate and dihydrate crystalline forms as pure products with good flowability. Lactitol is stable under both alkaline and acid conditions and at high temperatures likely to be encountered during food processing. Its melting

point is 146 °C and it is very soluble in water. The sweetening power of lactitol is about 40% of sucrose and the calorific contribution of lactitol after it is metabolized by colonic bacteria is less than 2.4 cal g^{-1} (10 kJ) compared to 4.0 cal g^{-1} (17 kJ) for sucrose. Lactitol is not hydrolyzed or absorbed in the small intestine and passes through to the colon. It also acts as a prebiotic carbohydrate and enhances the growth of certain groups of bacteria (e.g., bifidobacteria) in the colon.

Properties of lactobionic acid

Lactobionic acid is best described as a natural polyol acid with a formula weight of 358.30 Da and a pKa of 3.8. It is soluble in water as a free acid or as a salt. The molecule possesses multifunctional groups and thus acts as a metal ion chelator and can sequester calcium. Lactobionic acid inhibits the production of hydroxyl radicals as a result of its iron-chelating capacity and it functions as an antioxidant in tissues. It has a sweet taste despite being a weak acid. Lactobionic acid can be dehydrated to a lactone. Lactobionic acid is hygroscopic and forms a gel containing about 14% water with atmospheric moisture. Concentrated solutions will form gels and films. Its superior water-retention ability is valuable in cosmetic applications. Its main uses derive from its important chelating and emulsifying properties.

Properties of D-tagatose

D-Tagatose is a monosaccharide with a formula weight of 180.16 Da; it is a white solid with a melting point of 133–135 °C. It has a similar texture to sucrose and is 92% as sweet, but its calorific value (1.5 kcal g^{-1}) is only 38% that of sucrose since it is metabolized differently from sucrose. Its indigestibility in the stomach and small intestine results in a minimal effect on blood glucose levels in humans. Values for the absorption of tagatose in the small intestine vary widely from 20% to about 80%. The unabsorbed fraction is then completely fermented in the colon by some intestinal microorganisms. Those genera able to ferment tagatose included *Enterococcus* and *Lactobacillus*, but not *Bifidobacterium*. Many dairy LAB, such as lactococci and streptococci, fermented tagatose readily. It is claimed that its fermentation in the colon results in an increased production of butyrate relative to that of acetate and propionate. It has a glycemic index of only 3 compared to 100 for glucose. Its glycemic index is markedly lower than those for other natural sugars and most sugar alcohols. It is claimed to have prebiotic properties, but its inability to be fermented by *Bifidobacterium* species limits its application as a prebiotic. However, it is noncariogenic and non-plaque-forming.

Uses of Lactose

Lactose has many uses in the food and pharmaceutical industries. In the food industry, its uses are based on its relative sweetness and being a source of energy. It is less sweet than sucrose, with up to 3.3 times the concentration of lactose being required to give the same level of sweetness as sucrose. This means that more lactose can be used without making the product too sweet. Lactose maintains the crystallized sugar texture without causing the food to become too sweet.

Lactose also has a number of low-volume, special applications in the food industry, for example, as a free-flowing or agglomerating agent, to accentuate/enhance the flavor of some foods, to improve the functionality of shortenings, and as a diluent for pigments, flavors, or enzymes.

Lactose is used in the confectionary industry to produce caramel flavors through the Maillard reaction, usually with milk proteins, often added with the lactose in the form of sweetened condensed milk. The Maillard reaction is also important for its use in the bakery industry where it is used to promote crust browning as the yeast used during the rising process cannot utilize lactose, leaving it as a reducing sugar available to undergo the browning reaction. Lactose can also adsorb food dyes and flavors and it finds uses in confectionery where this property is exploited.

In the pharmaceutical industry, lactose is used as the main carrier (about 70% of tablets contain lactose) for drugs because it is not sweet, it is safe, it is available in highly refined form, and it makes good quality tablets. It has found uses in the industry in a number of different product forms. The main one is α-lactose monohydrate, which can be used as a tablet excipient, but it can also be finely milled to produce inhaler-grade lactose. Here, the lactose acts as a carrier for micronized drug materials to reach the lungs. Both anhydrous lactose (β-lactose) and spray-dried lactose are also used to make tablets. The form of the lactose is critical for consistent tablet formulations, and much emphasis is placed on the reproducibility between batches of the particle properties that are required to produce consistent tablets, with an even spread of the active drug dispersed within the lactose powder being used as an excipient.

Nutritional Problems Associated with Lactose

Lactose Intolerance

Mammals cannot absorb disaccharides from the intestine as they are initially hydrolyzed in the small intestine to monosaccharides followed by which they are absorbed. Lactose is hydrolyzed by β-galactosidase (β-gal; also referred to as lactase), which is secreted by cells in the brush border of the small intestine. The young of most mammalian species secrete adequate amount of β-gal, but as the animal ages, its secretion declines and becomes inadequate to hydrolyze ingested lactose, which enters the large intestine. If the ingested lactose is only partially hydrolyzed or is not hydrolyzed at all, a clinical syndrome called lactose intolerance results. If there is a deficiency of lactase, some lactose remains in the lumen of the small intestine. The presence of lactose tends to draw fluid into the lumen by osmosis. This fluid produces abdominal distention and cramps. From the small intestine, the lactose passes into the large intestine (colon) where it undergoes anaerobic fermentation to lactic acid (present as the lactate anion) and other short-chain acids (Figure 5). The increase in the concentration of molecules results in still greater retention of fluid. In addition, the acidic product of fermentation leads to lower pH and irritates the lining of the colon, leading to an increased movement of the contents causing diarrhea. The gaseous products of fermentation cause bloating and cramping.

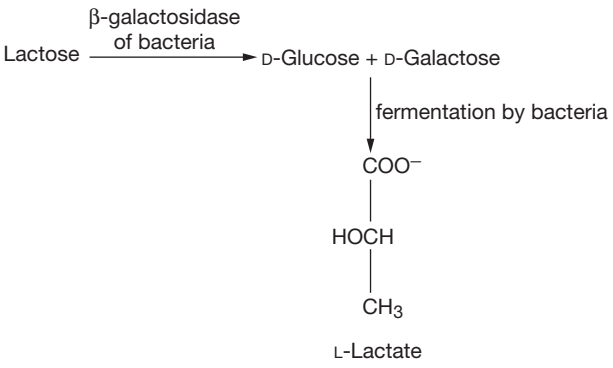

Figure 5 The fate of lactose in the large intestine of persons with lactase deficiency.

Lactose intolerance is not usually seen in children until about 6 years of age. At this point, the incidence of lactose-intolerant individuals begins to rise and increases throughout the life span, with the greatest incidence in the elderly. Both the incidence and the degree of lactose intolerance vary by ethnic group, indicating that the presence or absence of lactase is under genetic control.

Lactase deficiency can be overcome by the removal of lactose by fermentation or by producing reduced-lactose milk. However, both products of hydrolysis, D-glucose and D-galactose, are sweeter than lactose, and at about 80% hydrolysis, the taste change becomes too evident. Therefore, most reduced-lactose milk has the lactose reduced as close as possible to the 70% limit. In a technology under development, live yogurt cultures are added to refrigerated milk. The bacteria remain dormant in the cold and do not change the flavor of the milk but, upon reaching the small intestine, release lactase.

Galactosemia

It is caused due to inability to metabolize galactose on account of a hereditary deficiency of galactokinase or galactose-1-phosphate (Gal-1-P)/uridyl transferase. The lack of the former enzyme leads to the accumulation of galactose, which is metabolized via other pathways, leading, among other products, to galactitol, which accumulates in the lens of the eye, causing cataract in 10–20 years (in humans) if consumption of galactose-containing foods (milk and legumes) is continued. The incidence is about 1: 40 000. The lack of Gal-1-P/uridyl transferase leads to the accumulation of Gal and Gal-1-P. The latter interferes with the synthesis of glycoproteins and glycolipids (important for membranes, e.g., in the brain) and results in irreversible mental retardation within 2–3 months if the consumption of galactose-containing foods is continued. The incidence of this disease, often called 'classical galactosemia,' is about 1 in 60 000. The ability to metabolize galactose decreases on aging (after 70 years), leading to cataract.

See also: Calcium: Physiology; Carbohydrate: Digestion, Absorption and Metabolism; Condensed Milk; Dahi; Dairy Products: Dietary and Medical Importance; Food Intolerance: Lactose Intolerance; Glucose: Metabolism and Regulation; Lactic Acid Bacteria; Maillard Reaction; Milk: Role in the Diet; Milk: Sources and Composition; Sucrose: Dietary Importance; Whey and Whey Powders: Fermentation of Whey.

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Lauric Oils

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Introduction

Lauric oils have in common that their main fatty acid is lauric acid, 12:0, or dodecanoic acid. In addition, they contain larger amounts of 8:0 (caprylic acid or octanoic acid), 10:0 (capric or decanoic acid), and especially 14:0 (myristic or tetradecanoic acid) than the common seed oils like soybean oil, rapeseed oil, and sunflower seed oil. Historically, the main lauric oil is coconut oil, an oil produced from copra, the meat inside a coconut. Annual production has been around 3 million tons for some decades but has now increased to 3.5 million tons. However, with the growing production of palm oil, which is now the predominant vegetable oil at more than 60 million tons *per annum*, more and more palm kernels have become available. Palm kernels contain a lauric oil, and the annual production of palm kernel oil is now close to 6 million tons and thus exceeds the annual coconut oil production. In addition to these two major lauric oils, there are also several South American palm kernel oils in which lauric acid is the main fatty acid. Table 1 summarizes their fatty acid compositions and compares these with those of coconut oil and palm kernel oil and Laurical®, a genetically modified rapeseed plant.

Table 1 gives the overall fatty acid composition, irrespective of the position of the fatty acid on the glycerol backbone. When lauric oils are analyzed stereospecifically, it becomes clear that the fatty acid composition of the 2-position differs from that of the outer *sn*-1,3-positions. This is illustrated for coconut oil and palm kernel oil in Table 2.

The fatty acid values in Table 2 are slightly different from those in Table 1 because they originate from different sources. The fatty acid composition of the 1,3-positions has been calculated from the values in the two previous columns. They show that palmitic acid (16:0) and stearic acid (18:0) have a strong preference for the outer, 1,3-positions. This feature is common to most other vegetable oils. It is also valid for myristic acid (14:0), but lauric acid (12:0) on the other hand has a strong preference for the middle position of the glycerol backbone. The preference for the 2-position of linoleic acid (18:2) that is common with seed oils is not present in the lauric oils.

Given the fatty acid compositions of the 1,3-positions and the 2-position, it is possible to calculate a triglyceride composition according to the 1,3-random, 2-random theory. According to this theory, the molar concentration of a certain triglyceride in an oil equals the product of the mole fractions of the three fatty acids concerned. So, if the molar fraction of palmitic acid (P) at the 1,3-positions were to be 40% and the molar fraction of linoleic acid at the 2-position were to be 50%, the triglyceride PLP would have a concentration of $0.4 \times 0.5 \times 0.4 \times 100 = 8\%$. This holds for symmetric triglycerides. If the fatty acid at the 1-position were to differ from the fatty acid at the 3-position, there would be two positional isomers that can be listed separately or together and shown as a group. In the latter case, the calculated percentage has to be multiplied by a factor of 2.

Table 3 shows that coconut oil has relatively more triglycerides with a lower carbon number, whereas in palm kernel oil triglycerides with carbon number >46 are still relatively abundant. This is caused by the higher oleic acid content of palm kernel oil, which is also reflected by the higher iodine value of this oil: about 19 g iodine per 100 g oil for palm kernel oil and half that value for coconut oil.

Because lauric oils are so rich in lauric acid and myristic acid, their saponification values are much higher than those of the common seed oils: 245–265 vs. 185–195 mg KOH per g oil. The free fatty acid content of these seed oils is usually expressed as wt% oleic acid, but in the case of lauric oils, it is more common to express it as wt% lauric acid by assuming a molar mass of 200 instead of 282 for oleic acid.

The low unsaturated fatty acid content of the lauric oils is reflected by their low iodine values. It also retards the formation of rancid off-flavors. However, the presence of medium-chain fatty acids makes the oil more susceptible to hydrolysis, resulting in the formation of free fatty acids and a soapy taste.

The bottom row of Table 1 gives the fatty acid composition of Laurical®, the oil of a genetically modified rapeseed developed by Calgene in 1996. The aim of this development was to provide a cheap and reliable source of lauric acid, away from the Philippines, where typhoons frequently damage crops.

Table 1 Fatty acid composition of several lauric oils

Oil	*6:0*	*8:0*	*10:0*	*12:0*	*14:0*	*16:0*	*18:0*	*18:1*	*18:2*
Coconut oil	0.4	7.3	6.6	47.8	18.1	8.9	2.7	6.4	1.6
Palm kernel oil	0.2	3.3	3.5	47.8	16.3	8.5	2.4	15.4	2.5
Babassu oil		6	4	45	17	9	3	13	3
Cohune oil		8	7	46	16	9	3	10	1
Murumuru oil		1	1	42	37	5	2	11	1
Ouricury oil	2	10	8	46	9	8	2	13	2
Tucum oil		1	4	49	22	6	2	13	3
Laurical®				38.8	4.1	2.7	1.6	32.8	11.2

Source: Table 3.8 in Gunstone, F. D., Harwood, J. L. and Padley, F. B. (eds.) *The lipid handbook* (2nd ed.). London: Chapman & Hall.

Table 2 Stereospecific fatty acid analysis of coconut oil and palm kernel oil

	Coconut oil			*Palm kernel oil*		
Fatty acid	*TAG*	*2-Position*	*1,3-Positions*	*TAG*	*2-Position*	*1,3-Positions*
6:0	0.6	–	0.9	0.4	–	0.6
8:0	9.4	0.3	13.9	5.7	2.7	7.2
10:0	7.0	3.0	9.0	4.7	2.5	5.8
12:0	50.0	80.4	34.8	52.8	62.7	47.9
14:0	17.4	8.6	21.8	16.2	15.8	16.4
16:0	7.5	1.6	10.4	7.6	2.9	9.9
18:0	2.0	0.6	2.7	2.2	0.3	3.1
18:1	4.7	3.5	5.3	9.2	11.8	7.9
18;2	1.3	1.5	1.2	1.2	1.3	1.2

Source: Table 3.162 in Gunstone, F. D., Harwood, J. L. and Padley, F. B. (eds.) *The lipid handbook* (2nd ed.). London: Chapman & Hall.

Table 3 Molar triglyceride composition in percentage of major triglycerides for coconut oil and palm kernel oil

	Mol% in oil			*Mol% in oil*[a]	
Carbon number	*Coconut*	*Palm kernel*	*Major glyceride type*	*Coconut*	*Palm kernel*
30	4.2	1.3	6,12,12	1.6	
			8,10,12	1.8	
32	15.8	8.6	6,12,14	1.1	
			8,12,12	11.9	6.4
34	19.0	9.8	6,12,16	1.3	
			8,12,14	9.1	3.6
			10,12,12	5.7	4.7
36	20.3	25.7	8,12,16	2.4	
			8,14,14	2.4	19.8
			10,10,16	2.0	
			10,12,14	1.5	
			12,12,12	10.6	
38	17.2	16.8	8,12,18	2.6	
			8,14,16	1.2	
			10,12,16	1.7	
			12,12,14	10.8	14.1
40	9.6	9.5	10,12,18	1.3	
			12,12,16	3.8	3.5
			12,14,14	3.1	3.6
42	6.4	7.8	12,12,18	2.8	3.3
			12,14,16	2.7	2.7
44	3.2	5.8	8,18,18		1.0
			12,14.18	1.6	3.5
46	3.5	2.5	12,16,18	1.0	2.7
48	1.0	1.5	12,18,18		2.9
			14,16,18		1.2
50	0.5	2.2	14,18,18		1.2
52	0.2	2.2	16,18,18		1.9
54		1.8	18,18,18		1.3

[a]Values below 1% have not been listed.

Source: Table 3.92 in Gunstone, F. D., Harwood, J. L. and Padley, F. B. (eds.) *The lipid handbook* (2nd ed.). London: Chapman & Hall.

However, the growth of palm oil production increased the availability of the lauric palm kernel oil so that there was less need for alternative sources. Consequently, the entire stock of this oil was sold for use as fuel in 2001.

The oils listed in Table 1 will now be discussed in more detail in alphabetical order.

Babassu Oil *(Orbignya oleifera)*

The babassu palm is native to Brazil. It is a solitary stemmed palm, with stem diameter varying from 20 to 50 cm and attaining some 30 m in height. The crown contains 10–25 erectly arching pinnate leaves. The leaf sheath varies from 40 to

120 cm in length, the petiole from 10 to 40 cm, and the leaf rachis from 550 to 850 cm. There are 300–400 leaflets along the rachis, each 20–185 cm long by 1–6 cm wide depending upon position and tree health and age.

The inflorescences are androdioecious (either exclusively staminate (male) or androgynous (staminate and pistillate (female) together)), one arising from the axil of each leaf, although occasional abortions occur. Before anthesis, the inflorescence is covered by a woody bract that splits open along the lower side to release the inflorescence. The peduncle and the main bunch rachis are 50–180 cm long. In staminate inflorescences, there are 270–400 rachillae, each bearing 15–100 staminate flowers. In androgynous inflorescences, there are 320470 rachillae, each bearing 1–2 (rarely 3) pistillate flowers and one to several staminate flowers that are frequently aborted.

The fruits are broadly elliptic to oblong, 6–13 cm long, 4–10 cm wide, and weighing 40–440 g (dry weight). The epicarp is fibrous and 1–4 mm thick. The mesocarp is mealy, dry, and 2–12 mm thick. The endocarp is woody and 35–75 mm in diameter and contains 3–6 ovate to elliptic seeds, each 3–6 cm long, with an oily white endosperm. The endosperm is used locally to make charcoal.

In comparison with the related species *O. phalerata*, *O. oleifera* has a higher sex ratio (more female inflorescences), resulting in greater fruit yields ($\approx$5 MT ha^{-1} year^{-1} vs. $\approx$1.5 MT ha^{-1} year^{-1} for *O. phalerata*), and a higher kernel to fruit ratio (10–22% vs. 7%), resulting in significantly higher estimated oil yields (0.5–1.1 MT ha^{-1} year^{-1} vs. 0.1 MT ha^{-1} year^{-1}). Oil extraction is mainly artisanal and annual production is around 100000 tons. The oil is used locally in food, soap, and cosmetics and exported when there is a coconut oil shortage after typhoon damage in the Philippines. Outside Brazil, the oil is mainly used in cosmetics.

Coconut Oil *(Cocos nucifera)*

Coconut palms grow in coastal regions between 20° north and 20° south of the equator. Indonesia has now overtaken the Philippines as the main producer; both countries produce close to 20 million tons of nuts per annum. India also produces more than 10 million tons of nuts per annum and is followed by Brazil and Sri Lanka at <3 million tons. Total world production in 2013 was 62 million tons. Because its production is limited to the tropics, more than 50% of the oil is exported either as oil or as copra, the dried meat lining the coconut shell. Copra contains 65–70 wt% oil.

Botanically, the coconut fruit is a drupe, a fleshy fruit with a thin skin and a central stone containing the seed, just like a coffee cherry or a plum. Coconuts sold in Europe have the exocarp removed. Under this skin is a thick layer of fibers, the so-called coir, that have many applications in rope and coarse fabrics used, for instance, in flooring and mats. The actual nut is inside this coir. The very hard shell has three germination pores that are clearly visible after the husk has been removed.

The coconut palm grows to a height exceeding 20 m, but dwarf varieties have also been introduced; the latter have a shorter productive life. The hard shell of the fruit has a diameter of 15–30 cm. Harvesting can be by collecting fallen fruits or by picking manually or mechanically. Many nuts are used locally, and the excess is used to produce copra and oil. Copra is produced by splitting the outer shell and drying the endosperm meat. The yield of dry copra is about 12% of the original nuts.

This meat can be isolated before or after drying. The drying itself can be in the sun or in an oven or kiln. Direct contact of the meat with flue gases causes oil-soluble flue gas components such as polyaromatic hydrocarbons (PAHs) to be absorbed by the meat. They remain dissolved in the oil during extraction so that the PAH content of crude coconut oil has to be closely monitored. Low-boiling PAHs will be removed from the oil during its deodorization/steam refining. High-boiling PAHs need an adsorptive treatment with activated carbon.

Coconut oil is produced by expelling copra using a screw press, followed by solvent extraction of the expeller cake using *n*-hexane. The meal can be used in animal feed. Crude coconut oil has a phosphatide content of only 0.02–0.05 wt%, corresponding to 10–20 ppm phosphorus, which is very much lower than the 2–4 wt% of soybean oil or the 1.5–3.5 wt% of crude rapeseed oil. Its free fatty acid content is at least 3%, which is much higher than the 0.5–1.0 wt% of soybean oil. Consequently, crude coconut oil is very well suited to be physically refined after having been degummed/bleached in the so-called dry degumming process.

In this process, an amount of 0.05–0.15% of relatively strong phosphoric acid is mixed into the crude oil and allowed to react with the phosphatides. Then, bleaching earth is added, and after this has been in contact with the oil for about half an hour, the bleaching earth is removed by filtration. Subsequently, the oil is heated to the deodorization temperature of 220–240 °C and stripped with an amount of 0.3–1.5 wt% of steam under a vacuum of 2–4 m bar. Because coconut oil has a melting point of 23–26 °C, heating is required during storage and transport. During transport, the minimum and maximum temperatures recommended by the IASC (International Association of Seed Crushers) are 27 and 32 °C, respectively. On discharge, they are 40 and 45 °C, respectively.

In comparison with other vegetable oils, coconut oil contains very small amounts of unsaponifiables. These are the compounds that are not hydrolyzed by caustic soda and can be extracted from the soaps with an organic solvent. Major components of the unsaponifiable are the phytosterols and the tocopherols. Vegetable oils may also contain unsaponifiable compounds that are particular to that particular oil such as gossypol in cottonseed oil, oryzanol in rice bran oil, and sesamol in sesame seed oil.

The phytosterol content of coconut oil is only some 700 ppm. This is very low in comparison with soybean oil (3 000 ppm), sunflower seed oil (3 800 ppm), rapeseed oil (8 200 ppm), and especially corn oil (9 200 ppm). Of the major vegetable oils, only palm oil has a similarly low phytosterol content (500–700 ppm). The sterol composition of coconut oil is similar to that of other vegetable oils with β-sitosterol accounting for about 70%, stigmasterol for almost 20%, and campesterol for the remainder. As with most other vegetable oils, less than half the sterols (about 40%) are esterified with a fatty acid.

In comparison with other vegetable oils, the tocopherol and tocotrienol contents of coconut oil are even lower. It

contains 30 ppm tocotrienols and the tocopherol content is only 11 ppm: 5 ppm α-tocopherol and 6 ppm γ-tocopherol. Compare this with 650 ppm tocopherols and 530 ppm tocotrienols in palm oil, about 1000 ppm tocopherols in soybean oil, and almost 3000 ppm tocopherols in sesame seed oil.

Cohune Oil *(Attalea cohune)*

The cohune palm is indigenous to Belize. The tree can reach 30 m in height and produces bunches of nuts that are apparently very hard to crack. Although the meat contains some 70% oil, the oil yield on nuts is only 10% or thereabouts. Oil is produced in an artisanal manner, and attempts to exploit the ample source of cohune nut oil more professionally seem to have failed.

Murumuru Oil *(Astrocaryum murumuru)*

The murumuru palm is confined to Amazonia. It can grow to heights of 30 m and bears yellow fruits, the flesh of which is eaten by animals and man alike. The nuts are indigestible and spread by these animals. The tree is full of black spines that are very sharp; they can reach a length of half a meter. Fruits are therefore harvested by picking them up from the ground. Early last century, small enterprises harvested and processed the nuts that contain 40% of oil and used the lauric oil for food applications or soap, but the oil could not compete with other cheaper oils. Nowadays, some murumuru oil is used in cosmetics.

Ouricury Oil *(Syagrus coronata)*

The ouricury palm is a tree commonly found in *cerrado*, or dry, bush vegetation in many parts of Brazil. In some small communities, especially in the northeast of the country, the fruit is exploited as an oil source. The oil is used for cooking, frying, and soap production. The oil has in common with other, low-tonnage oils that it is often listed in patents as one of a series of lauric oils in an attempt to prevent the competition from bypassing the patent.

Palm Kernel Oil *(Elaeis guineensis)*

Palm kernels are a coproduct of palm oil production, and as opposed to palm fruit, the kernels can be stored after they have been dried. This means that palm oil has to be produced at or near the plantation and that palm kernel oil production can be centralized.

When the fresh fruit bunch (FFB) reaches the oil plant, it is sterilized with steam. The loosened fruits are stripped from the bunches in a threshing machine and passed to a digester with slowly moving arms where a mash is formed. This mash is passed to a screw press where it is separated into a liquid comprising the oil and fruit juice and a press cake that contains the palm nuts that amount to 15% of the FFB weight.

After the screw press, the nuts and the fiber traverse a heated breaker/conveyor that removes moisture from the fiber and causes the fiber and nuts to separate. The fiber and nuts then pass into a pneumatic, vertical separating column, called the 'winnowing column or depericarper.' The fiber is blown into a cyclone close to the boiler and the nuts pass down a polishing drum that removes pieces of stalks and any dirt or fibers attached.

The nuts are dried and conditioned before being cracked in, for instance, ripple mills. Cracking results in a mixture of kernels and bits of shell that are separated via a winnowing system followed by a cyclone or a clay bath. Suspending clay in water causes its specific gravity to increase, and by choosing a value between the specific gravity of the kernel ($1.07\ g\ ml^{-1}$) and that of the shell ($1.17\ g\ ml^{-1}$), the kernels will float and the bits of shell will sink. The clay bath achieves a better separation but consumes about 4.5 kg clay per ton of FFB and consumes more energy to dry the kernels to a residual moisture content of $<7\%$.

The oil content of the palm kernel is about 50%, so the most economical way to extract this oil is by prepressing followed by solvent extraction, just as rapeseed and sunflower seeds are processed. However, this way is only economical on fairly large scale because the investment requirements of a solvent extraction plant benefit strongly from an economy of scale. Consequently, there are also plants that only press the kernels and thus produce a meal that still contains some fat. The expeller oil invariably contains meal particles, so it is allowed to settle. The top oil is decanted and the foots are recycled to the expeller press.

Most oil seeds are passed through a flaker to open their cells and thus facilitate squeezing the oil out (screw press) or washing it out (solvent extractor). So, the kernels are also comminuted in a hammer mill to pieces of about 2 mm, and then, they are conditioned at 110–120 °C to break the cell walls. This conditioning also reduces the moisture content of the kernel pieces to about 2.5%.

Because the phosphatide content of the crude palm kernel oil is low, the oil can be physically refined after having been degummed and bleached in a single operation, the so-called dry degumming process. These processes are very similar to those used for coconut oil; further details have been given under the appropriate heading. The temperatures recommended for transport and discharge are the same as for coconut oil: 27–32 °C during the voyage and 40–45 °C on discharge. For the stearin and olein fractions, some different temperatures are recommended: for olein, the temperature range of 25–30 °C is recommended during the voyage and 30–35 °C on discharge, whereas for stearin, a range of 32–38 °C is recommended during the voyage and the unchanged range of 40–45 °C on discharge.

The sterol content of palm kernel oil has been reported to be some 875 ppm with a composition of 68% β-sitosterol, 14% stigmasterol, and 9% campesterol. Literature values for the tocopherol/tocotrienol content of palm kernel oil are 13 ppm α-tocopherol and 21 ppm α-tocotrienol. These latter values are somewhat higher than the corresponding values for coconut oil but still low in comparison with most other oils.

An important modification process for palm kernel oil is the dry fractionation process. It is a partial (fractional)

crystallization followed by filtration. The filtrate is called the olein fraction and the filter cake is the stearin fraction. In the dry fractionation process, two crystallization systems are used. The main system is the so-called suspension crystallization in which the oil to be cooled is fed into a large crystallization vessel that comprises cooling elements and an agitator. Suspension crystallization is used, for instance, for palm oil and its fractions, anhydrous milk fat, and beef tallow.

The other system of crystallization is the stationary or stagnant crystallization system in which the cooling melt is not agitated but allowed to crystallize in a tray or crystallization chamber to form a block of partially crystallized fat. Originally, this block was wrapped in filter cloth and placed in a hydraulic press in which the olein was squeezed out of the blocks. These had then to be unwrapped to obtain the stearin filter cake. This was a very labor-intensive process of doubtful hygienic standards. The stationary crystallization system is used for oils and fats that do not form a slurry of separate crystals on partial crystallization but tend to form crystal networks and aggregates; palm kernel oil is such an oil.

To eliminate the manual handling of the blocks, several more automated processes have been developed. In an early development, the molten palm kernel oil was poured into trays that were positioned on a moving belt that passed through a cooling tunnel. At the end of the tunnel, the trays were emptied by turning them upside down so that the partially crystallized blocks fell out. The blocks were then worked mechanically into a pumpable paste that was fed into a membrane filter press.

In a more recent development, cooled but still liquid palm kernel oil is fed into crystallization chambers in a piece of equipment (called Statoliser®) that are similar to the chambers in a membrane filter press except that there is no filter cloth and that the space behind the membrane is now fed with cooling medium. After the equipment has been filled, the temperature of the cooling medium is controlled to ensure the partial crystallization of the palm kernel oil. When the desired degree of crystallization has been reached, the press is opened, and the partially crystallized blocks fall down to be crushed and fed into a membrane filter press.

Fats intended for confectionery products that are similar to chocolate can be divided into three main groups. There are the cocoa butter equivalents (CBEs) that are chemically and thus physically very similar to cocoa butter. They have a high content of symmetric monounsaturated triglycerides. CBEs are produced from naturally occurring fats like palm oil, shea butter, and illipe butter. They can be mixed with cocoa butter in any proportion without seriously affecting the physical properties such as solid fat content (SFC) profile and snap on breaking.

Another group is formed by the cocoa butter replacers (CBRs). Their physical properties are very similar to those of cocoa butter, but chemically, they are quite different. CBRs are prepared by fractionating partially hydrogenated blends of vegetable oils, for example, rapeseed oil and palm olein. They have a high *trans* isomer content and can be mixed with cocoa butter without affecting its physical properties when not too much of either component is present. The third group is formed by the cocoa butter substitutes (CBSs) that have similar physical properties to cocoa butter but are totally incompatible with the latter.

Palm kernel oil itself cannot act as a CBS because its SFC is far too low to be of any use: 0.2% at 30 °C; 13.3% at 25 °C, and 37.3% at 20 °C. It is possible to increase the SFC by hydrogenating the palm kernel oil, but this oil contains 8 wt% of triglycerides with two unsaturated fatty acids that on hydrogenation form triglycerides with two stearate groups. Their melting points would be very high and give the hydrogenated product a poor mouthfeel. So, the answer to this problem is to use palm kernel stearin (PKS) since on fractionation, the triglycerides with one or two unsaturated fatty acids are concentrated in the olein because their low melting points prevent them from crystallizing. On hydrogenation, the SFC at 35 °C increases to only 5% so that it also melts at this temperature.

As shown in Figure 1, a single-stage fractionation of palm kernel oil leads to a yield of 40% stearin with IV = 7, which fraction has to be hydrogenated to lower its IV and increase its

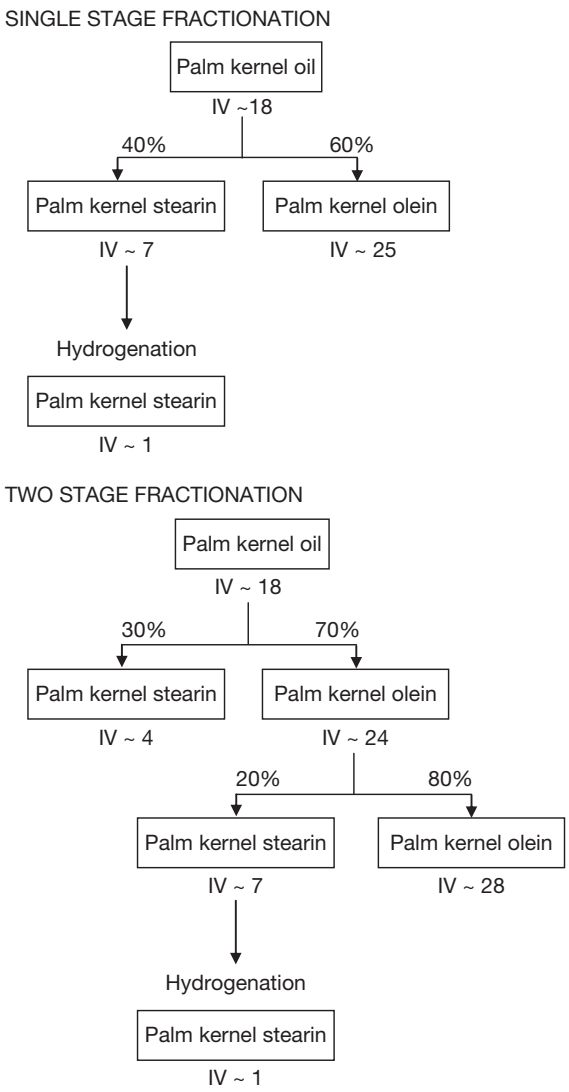

Figure 1 Fractionation of palm kernel oil. Adapted from Figure 12.15 in Chapter 12, Gibon, V. Palm oil and palm kernel oil refining and fractionation technology. In: Lai, O.-M., Tan, C.-P. and Akoh, C. C. (eds.) *Palm oil*, *production*, *processing*, *characterization*, *and uses*. Urbana IL: AOCS Press.

Table 4 Physical properties of lauric oils and derived products in comparison with cocoa butter

		SFC (%) at temperature (°C)					
Product	*Iodine value*	*20*	*25*	*30*	*35*	*40*	*Melting point*
Palm kernel oil (PK)	18	44	20	0			28
PK stearin (PKS)	7	82	68	29	0		32
Hydrogenated PKS	4	87	70	24	1	0	31
	0.4	95	90	50	5	1	35
Cocoa butter	34.5	76	70	45	0		34
Palm kernel olein (PKO)	17.5	44	20	0			
Hydrogenated PKO	1	73		27	14	6	41
Coconut oil (CN)	8.5	36	0				24
CN stearin (CNS)	4	84	53	2	0		30
Hydrogenated CNS	1.5	92	57	8	2		32

Source: Table 4.20 in Gunstone, F. D., Harwood, J. L. and Padley, F. B. (eds.) (2007). *The lipid handbook* (3rd ed.). Boca Raton: CRC Press; Hamm, W., Hamilton, R. J. and Calliauw, G. H. (eds.) (2013). *Edible oil processing*. Chichester: Wiley-Blackwell; Wambeck, N. (1999). Oil palm process synopsis. In: *Oil palm mill, systems and process* (vol. 1). Kuala Lumpur.

SFC. To maximize the CBS yield, a double fractionation process has been developed in which a first fractionation yields 30% stearin with an IV = 4, which can be used as a CBS without hydrogenation, and a second fraction of the olein yields a further 14% stearin with an IV of 7, which fraction has to be hydrogenated.

Table 4 shows that the SFC profile of the hydrogenated PKS is close to that of cocoa butter but that trying the same with coconut oil does not lead to a suitable CBS.

Tucum Oil *(Astrocaryum vulgare)*

The tucum palm is native to the Amazonian region, possibly to the state of Pará, where its center of distribution is located, and is also found in French Guiana and Surinam. It is a characteristic palm of terra firma, low vegetation cover, or even open fields. The tree is 10–15 m in height and regenerates easily by growing multiple stems. The tucum palm is considered a pioneer plant of aggressive growth, has the ability to grow new shoots after a fire, and mainly inhabits secondary forests and pastures. According to some sources, its resistance to diseases and high productivity make this species an alternative for the production of biodiesel, since the operating costs of an orderly plantation are much lower than those of an oil palm plantation.

The tree trunk is covered with spines and even the inflorescences are covered by a thorny spathe. Fruit bunches can reach >3 m and weigh almost 50 kg. The yellow-orange fruit is about the size of a chicken egg. Like the fruit of the oil palm, both the flesh and the seed/nut contain oil; whereby, the fruit oil contains some 60% of oleic acid (18:1) and 30% palmitic acid (16:0). It is therefore slightly more unsaturated than palm oil and therefore has a higher iodine value of 64 as against 53 g iodine per 100 g oil. Tucum kernel oil is a lauric oil as shown in Table 1.

On average, the fruit weighs 30 g; 34% of this weight corresponds to the external pulp that contains 14–16% of the oil when it is raw. A mature tree can produce up to 50 kg of fruits per year (25 kg per tree on average), which corresponds to 2.5 kg of pulp oil and 1.5 kg of seed oil. This ratio differs from the ratio observed for palm oil, where it is close to 9–1. On 1 ha, 400 palm clusters can be planted, with each cluster having three trunks. In total, this equals to 1200 palm trunks and will result in 4.8 tons of fatty material per hectare. It forms clusters that do not need to be replanted.

See also: Cocoa: Production, Chemistry, and Use; Enzymes: Analysis and Food Processing; Fatty Acids: Fatty Acids; Fatty Acids: *Trans* Fatty Acids; Fruits of Tropical Climates: Biodiversity and Dietary Importance; Heat Treatment: Principles and Techniques; Nuts: Brazil Nuts; Palm Oil; Phospholipids: Properties and Occurrence; Tocopherols: Properties and Determination; Triacylglycerols: Characterization and Determination; Triacylglycerols: Structures and Properties.

Further Reading

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Leavening Agents

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Introduction

Most baked products are leavened by the expansion of the batter or dough. Leavening results in baked products with a larger volume with an open, porous texture. The three major leavening gases are air, steam, and carbon dioxide gas. Other leavening agents include ethanol and ammonia gas. Ethanol, which is a by-product of yeast fermentation, has a leavening effect in yeast-leavened products such as bread and rolls when it vaporizes during baking in the oven. Ammonia gas is produced by the decomposition of ammonium bicarbonate, or ammonium phosphate during heating and is used to leaven some dry cookies and crackers.

Air

Air is present in all baked products and is essential for leavening to occur. The expansion of the air itself during baking can provide some leavening, but it is not enough to get the desired volume. The most important function of the air bubbles in leavening is that they serve as nuclei for the other leavening gases, which diffuse into them and cause them to expand. Air must be incorporated into the dough during mixing. Yeast and chemical leaveners cannot create new bubbles in the dough or batter. Bubble mechanics show that the pressure (P) inside of an air bubble is related to the radius of the bubble (r) and the interfacial tension (γ) as follows: $P=2\gamma/r$. This means that in a dough or batter where the interfacial tension is constant, if the radius is zero, then the pressure required to form a new bubble is infinitely large. Therefore, no new bubbles are formed. The composition of air (~78% nitrogen and 20% oxygen) makes it an ideal gas for leavening. Nitrogen is insoluble in water so it remains as a gas in the dough or batter. The oxygen is soluble in water and will dissolve in the aqueous phase. Carbon dioxide gas, which is produced by yeast fermentation or chemical leavening reactions, is also water-soluble; however, it will quickly saturate the aqueous phase of the dough or batter and then will remain as a gas. The number and size of air bubbles incorporated into the dough or batter are important to the texture of the final product. A low number of large-sized air bubbles will produce a final product with an open, coarse crumb grain, while a large number of small-sized bubbles will produce a fine, uniform crumb grain. Uniform air bubble size is also important. If a small bubble and a large bubble are positioned near one another in the batter or dough, the gas will diffuse from the smaller one into the larger one causing the larger one to expand and the smaller one to disappear. This phenomenon is called disproportionation or the Ostwald ripening. Adjacent bubbles can also coalesce when the film between them ruptures. The result of these instabilities is a lower number of larger-sized air bubbles.

Steam

All baked products contain water. Steam is produced as the water in the dough or batter is heated. As the temperature increases, the vapor pressure of water increases and expands the gas bubbles in the batter or dough. The usefulness of steam as a leavening agent in most doughs and batters is low compared to that of other leavening gases because of the high boiling point of water. The temperature of most baked products reaches the boiling point of the water too late in the baking cycle for a large increase in the size of the product to occur. Steam plays a large role as a leavening agent in products that are heated at a very fast rate such as saltine crackers, in extruded products such as breakfast cereals and snacks, and in products where the temperature exceeds 100 °C such as some types of cookies, crackers, and snacks. Water present in roll-in margarine used in puff pastry and laminated products vaporizes during baking to help provide lift between the layers of dough.

Carbon Dioxide

Carbon dioxide gas is produced by yeast during fermentation and also by chemical leavening agents. In the chemical leavening reaction, a food acid and a leavening base react in the presence of water to form carbon dioxide gas.

Yeast

The majority of bread-type baked products are leavened by yeast. *Saccharomyces cerevisiae*, also known as baker's yeast, is the species of yeast that is most often used. Other species of yeast are used in sourdough bread and in some frozen doughs. Yeast is a living organism that is put into a dormant state by drying or by storage at low temperatures. When the yeast is incorporated into a dough system, the conditions are suitable for it to become active. Once activated, the yeast will quickly consume the air in the dough; thus, dough fermentation is an anaerobic process. During anaerobic fermentation, the yeast organisms do not grow or reproduce, but rather, they metabolize sugar to produce carbon dioxide, alcohol, acids, flavor compounds, and water:

$$\underset{\text{(glucose)}}{C_6H_{12}O_6} + \text{Yeast} \rightarrow \underset{\text{(ethanol)}}{2C_2H_5OH} + \underset{\text{(carbon dioxide)}}{2CO_2}$$

During fermentation, the pH of the dough will decrease from about 6.0 to about 5.0. The decrease is the result of the aqueous phase of the dough becoming saturated with carbon dioxide gas and also by the production of organic acids by bacterial fermentation. Commercial yeast always contains some bacteria. As a result of the pH drop, most of the leavening

gas present in the dough at the end of fermentation will be as carbon dioxide gas with only a small quantity of bicarbonate or carbonate. The carbon dioxide gas produced during the leavening reaction diffuses into the air bubbles that were incorporated into the dough during mixing and makes them larger. These large bubbles are then subdivided into smaller bubbles during the sheeting steps in the dough making process. This results in a larger number of smaller-sized air cells, which are more stable and which also produce a finer, more uniform crumb grain in the final baked product. In addition to subdividing the large air cells, the sheeting steps in the bread production process serve the function of bringing fermentable carbohydrates into contact with the yeast cells to sustain fermentation. The yeast is active in the dough during fermentation, proofing, and the early stages of baking until it becomes inactivated by the heat in the oven. During fermentation and proofing, the yeast functions to produce carbon dioxide gas that diffuses into the air cells and expands them, thus leavening the dough. In the oven, the size of the loaf dramatically increases during the first few minutes of baking due to a phenomenon called oven spring. Oven spring occurs because the increased temperatures cause the yeast to become more active, which results in the fast production of carbon dioxide until the yeast is killed at about 55 °C. Carbon dioxide in the aqueous phase becomes less soluble at the higher temperatures and moves into the air bubbles. The carbon dioxide in the air bubbles expands due to the higher temperature. Finally, the water–ethanol mixture vaporizes.

Chemical Leavening

Essentially all soft wheat products are chemically leavened. Most chemical leavening systems consist of a carbon dioxide source (leavening base) and one or more food acids. Carbon dioxide sources in chemical leavening systems include ammonium bicarbonate, potassium bicarbonate, sodium carbonate, and sodium bicarbonate.

Leavening Bases

Leavening bases are the carbon dioxide sources in chemical leavening systems and include ammonium bicarbonate, potassium bicarbonate, and sodium bicarbonate. Release of the carbon dioxide from the leavening base occurs by two mechanisms: decomposition and chemical neutralization. In decomposition, the leavening base is broken down by moisture and heat to release gases, which can then leaven the product. This is the case for ammonium bicarbonate. Potassium bicarbonate and sodium bicarbonate release carbon dioxide by chemical neutralization where a leavening acid is added, which reacts with the leavening base to produce the carbon dioxide gas.

Ammonium bicarbonate

Ammonium bicarbonate decomposes during baking to produce ammonia gas, carbon dioxide gas, and water. All three of these products act as leaveners. Because ammonia gas dissolves in water, it can be retained in moist products, giving them a sharp, pungent odor and rendering them inedible. For this reason, it is only used as a leavening agent in some dry cookies and crackers that have a final moisture content of less than 5%. Ammonium bicarbonate decomposes completely with heat, so a leavening acid is not needed. One advantage of ammonia bicarbonate is that it does not produce salts, which have an adverse effect on dough rheology:

$$\underset{\text{(ammonium bicarbonate)}}{NH_4HCO_3} \xrightarrow{\text{heat}} \underset{\text{(ammonia)}}{NH_3} + \underset{\text{(carbon dioxide)}}{CO_2} + \underset{\text{(water)}}{H_2O}$$

Sodium bicarbonate

Sodium bicarbonate, also known as baking soda, is the most commonly used leavening base. It is soluble in water and dissolves quickly during mixing. It only partially decomposes during heating, releasing less than half of the available carbon dioxide. It can be used alone in some cookies and snack crackers. However, in products that require higher carbon dioxide gas levels (i.e., cakes and other batter products), a leavening acid is needed to produce complete release of the carbon dioxide. Sodium bicarbonate is inexpensive, easy to handle, nontoxic, has high purity, and gives no aftertaste to the final product. The drawback is that the neutral salts produced in the reaction have a negative effect on the rheological properties of the dough, making it stiffer and less elastic, and some impact the flavor, color, odor, and crumb appearance of the final product:

$$\underset{\text{(sodium bicarbonate)}}{NaHCO_3} + \underset{\text{(acid salt)}}{H^+} \rightarrow \underset{\text{(neutral salt)}}{Na^+} + \underset{\text{(carbon dioxide)}}{CO_2} + \underset{\text{(water)}}{H_2O}$$

Potassium bicarbonate

Potassium bicarbonate can be used as a substitute for sodium bicarbonate to lower the sodium content of the finished product. It is soluble in water and partly decomposes upon heating to give partial release of the available carbon dioxide gas, thus requiring a leavening acid in order to yield complete release of the carbon dioxide. Potassium bicarbonate is seldom used because it has a bitter aftertaste and is highly hygroscopic, making it difficult to store:

$$\underset{\text{(potassium bicarbonate)}}{KHCO_3} + \underset{\text{(acid salt)}}{H^+} \rightarrow \underset{\text{(neutral salt)}}{K^+} + \underset{\text{(carbon dioxide)}}{CO_2} + \underset{\text{(water)}}{H_2O}$$

Leavening Acids

Only a small portion of the carbon dioxide is released when sodium bicarbonate is dissolved in water. The sodium bicarbonate decomposes into carbon dioxide, sodium carbonate, and water. The sodium carbonate raises the pH of the system and inhibits the reaction. The pH must be lowered in order for the remaining carbon dioxide to be released. This is done by adding an acid. The acid may be present in formula ingredients or added in the form of organic acids, food-grade phosphates, or acidic salts. Leavening acids are categorized by the time and rate at which they become soluble and able to react with the sodium bicarbonate to release carbon dioxide gas. This allows for the selection of the appropriate leavening acid to coordinate carbon dioxide release with the setting of the product. The reaction rate is critical to the quality of the final product. If the carbon dioxide gas is produced too soon, most of it escapes before the structure is set, while carbon dioxide gas produced

too late after the product has set may cause blowouts inside the product due to the high pressure. Ingredients in the formula such as calcium, sugar concentration, starch, hydrocolloids, and water level can affect the reaction rate. Leavening acids that solubilize immediately are fast-acting, while those that solubilize slowly or with heating are slow-acting.

Many acidic food ingredients such as buttermilk, sour milk, vinegar, fruit, fruit juice, sour cream, yogurt, honey, cocoa, and molasses are found in bakery product formulas. These foods do decrease the pH and allow the sodium bicarbonate to release more carbon dioxide gas. Drawbacks of these food ingredients are that they do not contain uniform acid content, impart distinct colors and flavors that may not be desirable in some products, and are all fast-acting. Commercial leavening acids (acidic salts) are more reliable and more consistent. Most common leavening systems utilize the reaction of sodium bicarbonate with an acid phosphate salt to produce carbon dioxide gas. The three broad categories of leavening acids are nucleating agents, time-release agents, and heat-release agents.

Nucleating agents solubilize quickly in the dough or batter and are referred to as fast-acting. Organic acids (citric, tartaric, fumaric, adipic, lactic, and malic), cream of tartar, monocalcium phosphate (MCP), and anhydrous monocalcium phosphate (AMCP) are in this category. During mixing, air is incorporated into the dough. Carbon dioxide gas generated in the chemical leavening reaction during the mixing step diffuses into the air cells and expands them. The large air bubbles are then subdivided into smaller-sized air bubbles by the mixer. The result is a larger number of smaller-sized air cells, which are more stable and which also produce a finer, more uniform crumb grain in the final baked product. Fast-acting leavening acids are generally not used alone because much of the carbon dioxide gas released during mixing gets lost during production.

Time-release leavening acids solubilize more slowly to react during a given period of time. Sodium aluminum pyrophosphate (SALP) and glucono-delta-lactone (GDL) are in this category. Both products have a slightly bitter aftertaste, which can be masked with calcium, sugar, and flavoring agents. These leavening acids find use in specific products such as cake doughnuts and refrigerated canned biscuit dough. In cake doughnuts, the carbon dioxide gas should be released in the fryer in order to raise the doughnut up out of the oil and expand it rapidly before it sets. In canned biscuit dough, the biscuits need to leaven inside the can to completely fill it and exclude all of the air from the can but not overexpand and explode the can.

Heat-release leavening acids react only with heating in the oven. Sodium aluminum sulfate (SAS), sodium aluminum phosphate (SALP), dimagnesium phosphate (DMP), and dicalcium phosphate dihydrate (DCPD) are in this category. The temperature at which they solubilize varies. The most commonly used leavening acids are listed in Table 1.

Monopotassium tartrate (cream of tartar) [$KHC_4H_4O_6$]

Cream of tartar was the first leavening acid to be used in baking. However, it is not highly used today. It is expensive and has such as fast reaction rate, generating about 70% of the available carbon dioxide gas during mixing; it is difficult to produce consistent products. It finds use in products such as angel food cakes where it is used not as a leavening acid but to lower the pH to improve the whipping of the egg whites.

Monocalcium phosphate [$Ca(H_2PO_4)_2{\cdot}H_2O$]

MCP is a nucleating or fast-acting leavening acid. It reacts quickly in the mixer, causing 60–70% of the available carbon dioxide gas to be released. There are several granulations available. The granulations do not impact the reaction rate but rather provide more stability during storage. Larger particles are more stable. However, if the particle size is too large and prevents the particle from completely solubilizing during mixing, black spots may occur on the final product. MCP is typically used in combination with other leavening acids. It is often the fast-acting leavening acid in double-acting baking powder.

Anhydrous monocalcium phosphate [$Ca(H_2PO_4)_2$]

AMCP is MCP that is coated with aluminum and potassium phosphates. The coating delays dissolution by a few minutes, causing release of about 15% of the available carbon dioxide gas in the mixer and 35% during the next 10–15 min of bench time. It is still a fast-acting leavening acid and acts as a nucleating agent. The coating basically increases stabilization in storage. AMCP may be used alone in corn-based products like corn bread or corn muffins or used in combination with other leavening acids in other baked products.

Table 1 Properties of common leavening acids

Leavening acid	*Acronym*	*Formula*	*NV*[a,b,c]
Monopotassium tartrate	Cream of tartar	KHC_4H_4O	45
Monocalcium phosphate	MCP	$Ca(H_2PO_4)_2{\cdot}H_2O$	80
Anhydrous monocalcium phosphate	AMCP	$Ca(H_2PO_4)_2$	84
Sodium acid pyrophosphate	SAPP	$Na_2H_2P_2O_7$	72
Sodium aluminum phosphate	SALP	$Na_3H_{15}Al_2(PO_4)_8$	100
Sodium aluminum sulfate	SAS	$Al_2(SO_4)_3{\cdot}Na_2SO_4$	100
Dimagnesium phosphate	DMP	$MgHPO_4$	40
Dicalcium phosphate dihydrate	DCPD	$CaHPO_4{\cdot}2H_2O$	33
Glucono-delta-lactone	GDL	$C_6H_{10}O_6$	50

[a]Neutralizing value (NV) is the parts of sodium bicarbonate that 100 parts of leavening acid neutralizes.
[b]NV for potassium bicarbonate is calculated by multiplying the listed NV by 1.19.
[c]NV for ammonium bicarbonate is calculated by multiplying the listed NV by 0.94.

Sodium acid pyrophosphate [$Na_2H_2P_2O_7$]

Sodium acid pyrophosphate (SAPP) is a time-release leavening acid, which reacts over time. There are several grades of SAPP (SAPP 21, SAPP 26, SAPP 28, SAPP 37, SAPP 40, SAPP 43, and SAPP 45), each with different reaction rates, which are controlled by the manufacturing process. The higher the number, the faster the reaction rate. Typically, the fastest SAPP that the product can tolerate is used to ensure a complete reaction. SAPP 21 and SAPP 26 have the slowest rate within the SAPP products and are commonly used in refrigerated canned biscuits and cake mixes and for products made using long production cycles. SAPP 28 is commonly used in commercial baking powder intended for all-purpose and institutional baking done in large batches that have long holding or bench times. SAPP 37, SAPP 40, SAPP 43, and SAPP 45 have the fastest reaction rates within the SAPP products and are commonly used in cake and cake doughnut production. SAPP is known to impart a characteristic off-flavor termed 'pyro' to the final product. This flavor can be masked with sugar, calcium, and flavoring agents.

Sodium aluminum phosphate [$NaH_{14}Al_3(PO_4)_8{\cdot}4H_2O$]

SALP is a heat-triggered leavening acid, which reacts at 38–41 °C. It is stable at low temperatures and generally does not react with other formula ingredients, so it maintains batter viscosity during holding or bench time. It has a bland, neutral flavor. It imparts a strong structure and gives resiliency to the final product. It is commonly used as the slow-acting leavening acid in double-acting baking powders. SALP is often used in combination with MCP or AMCP in self-rising flour, cakes, pancakes, muffins, dough made using long bench times, refrigerated dough, refrigerated batters, frozen dough, and frozen batters.

Dimagnesium phosphate [$MgHPO_4$]

DMP is heat-activated and reacts at 41–44 °C. It is stable at low temperatures and maintains batter viscosity during holding or bench time. It has a bland, neutral flavor. It imparts a strong structure and gives resiliency to the final product. It contains no sodium so it finds use in low-sodium products.

Dicalcium phosphate dihydrate [$CaHPO_4{\cdot}2H_2O$]

DCP is not an acidic salt and so not a true leavening acid, but it is grouped with the leavening acids. DCP slowly solubilizes over time and thereby lowers the pH and allows the release of carbon dioxide gas from the sodium bicarbonate. DCP does disproportionate to form phosphoric acid, MCP, and tricalcium phosphate, which can react with the sodium bicarbonate. However, this occurs at temperatures of 57–60 °C, which is too high to be effective in most products. DCP is used in combination with other leavening acids to provide continuous carbon dioxide gas evolution late in the bake cycle of products like cakes and muffins and also as a means to adjust the pH of the final baked product.

Sodium aluminum sulfate [$Al_2(SO_4)_3{\cdot}Na_2SO_4$]

SAS is a slow-acting, heat-activated leavening acid. It reacts too slowly to give good oven spring during baking so it not widely used. It has no reaction at room temperature and so is not used alone, but is used often in combination with MCP. SAS has a weakening effect on the crumb texture and a slightly astringent or metallic aftertaste. It is most commonly used as the slow-acting leavening acid in double-acting household-use baking powder. Commercially, it is used in chocolate cake for color adjustment and to produce the characteristic large holes in the crumb of English muffins.

Glucono-delta-lactone [$C_6H_{10}O_6$]

GDL is also not a true leavening acid but is categorized with them. GDL slowly hydrolyzes to gluconic acid, which results in a slow, continuous release of carbon dioxide gas over time. It works well as a yeast replacement in refrigerated and frozen dough products. A drawback is that it has a slightly bitter aftertaste.

Baking Powder

Baking powder provides a complete leavening system in one product. It is composed of sodium bicarbonate, one or more leavening acids, and a diluent. The diluent, typically starch or calcium carbonate, acts as a standardizing agent to dilute the active ingredients and to extend the shelf life of the baking powder by preventing the sodium bicarbonate and leavening acid from reacting in the container.

The proper balance of leavening acid and sodium bicarbonate is critical. In general, there should be little or no unreacted sodium bicarbonate or leavening acid in the final product. If the blend contains too much leavening acid relative to the sodium bicarbonate, the final product will be too acidic and have a tart or bitter flavor, an off color, and a pH that is too low (acidic). On the other hand, blends with too much sodium bicarbonate will produce final products with a soapy taste, yellow color, and alkaline pH.

In order to determine the appropriate amount of leavening acid to add, the neutralizing value (NV) is considered. NV is the number of parts by weight of sodium bicarbonate that can be neutralized (cause to release all available carbon dioxide gas) by 100 parts by weight of the leavening acid. NV is determined in the laboratory using titration. NVs of the common leavening acids for sodium bicarbonate are listed in Table 1. The NV of the leavening acid is different for ammonium bicarbonate and potassium bicarbonate. NV for these bases is calculated by multiplying the NV for sodium bicarbonate by 0.9375 for ammonium bicarbonate and by 1.1875 for potassium bicarbonate.

To calculate the leavening composition, first, the level of the sodium bicarbonate (or other leavening bases) is set based on the amount of leavening that is needed in the product. The level of acid needed to neutralize the sodium bicarbonate is then calculated by multiplying the level of sodium bicarbonate by 100 and dividing by the NV of the leavening acid. If multiple leavening acids are being used, the percentage of each acid is determined; then, the addition level of each is calculated. An example is shown in Figure 1. The calculated values provide a starting level, which may need to be adjusted depending on the product being made. Some products may contain other food acids in the formula, which may neutralize some of the sodium bicarbonate, thus reducing the level of leavening acid that is

Leavening system in formula:

0.75% sodium bicarbonate
10% of CO_2 generated in mixer with MCP (NV = 80)
90% of CO_2 generated in oven with SAPP (NV = 72)

Leavening acid level equation:

$$\text{amount of leavening acid} = \frac{\text{sodium bicarbonate \% x leavening acid \% x 100}}{\text{NV of acid}}$$

Amount of MCP to add:

$$\frac{0.75 \times 0.10 \times 100}{80} = 0.094\% \text{ MCP}$$

Amount of SAPP to add:

$$\frac{0.75 \times 0.90 \times 100}{72} = 0.900\% \text{ SAPP}$$

Figure 1 Sample leavening calculation.

needed. Also, it may be desired to have excess sodium bicarbonate or leavening acid in the formula in order to adjust the pH of the final product. Product pH is important for flavor and color. If a sharper, more astringent flavor is desired, the pH can be decreased (more acidic) by adding excess leavening acid. On the other hand, a more bland-flavored product may require less leavening acid to leave an excess of the sodium bicarbonate to increase the pH (more alkaline). Excess acid can produce undesirable tartness, while excess sodium bicarbonate can produce an undesirable soapy flavor. Product color is also impacted by pH. For example, a slight excess of sodium bicarbonate may be desired in a chocolate cake or muffin in order to provide the desired brown color.

By law, baking powder must release 12% carbon dioxide. Manufacturers often formulate baking powder to release 18% carbon dioxide to account for the loss of activity due to storage time and improper storage conditions to ensure that the final product releases 12% carbon dioxide. Baking powders are classified by type of release (single-acting or double-acting) and market segment (household or commercial). Single-acting baking powders contain only one leavening acid, which may be fast-acting and react in the mixer or may be slow-acting and react in the oven. Double-acting baking powders contain both fast-acting and slow-acting leavening acids. An ideal double-acting baking powder will release a small amount (~20%) of carbon dioxide gas in the mixer and the remainder in the oven. Most baking powders are double-acting. Household baking powders are formulated as 'all-purpose' products to be used in a variety of baked goods. They do not need to be bench-stable as the consumer typically mixes the batter and bakes it immediately. The most common leavening acids used in household baking powder are SAS and MCP. Household baking powders typically have a 1-year shelf life. Commercial baking powders are formulated for slow release as many commercially made or institutionally made products are done in batch operations. MCP and SAPP 28 are the most common leavening acids in commercial baking powders. Shelf life is 6 months.

Baking powder is convenient and reduces the number of minor ingredients that must be weighed out, and the leavening formulation has been properly calculated and balanced. Disadvantages are that the activity varies from 12% to 18% carbon dioxide release depending on the age of the baking powder and the leavening acids are general use and not tailored to specific products. For these reasons, many commercial bakers make their own leavening blends for consistency, economy, and correct timing of carbon dioxide gas generation.

Adjustments for High Altitude

As altitude increases, the atmospheric pressure decreases. This results in (1) greater expansion of leavening gases, (2) lower boiling temperature of water, and (3) faster evaporation of moisture. Alterations to the formula and process to account for these changes should be considered at elevations higher than 2500 ft above sea level.

The greater expansion of the leavening gases means batters and doughs will rise more quickly at higher altitudes. The effect is less pronounced in yeast-leavened products and is easily controlled by reducing the yeast level and increasing the flour level in the formula to make the dough stiffer. Another alternative is to keep yeast level constant but reduce fermentation and proof times. In the case of sweet yeast-raised dough, egg is added or the level of egg is increased to provide more structure.

In chemically leavened products such as cakes and cookies, the levels of sodium bicarbonate and leavening acids should be reduced. The amount of reduction is dependent on the altitude and varies from 25% reduction at 2000 ft to 75% reduction at 10 000 ft. Other suggested formula alterations include decreasing sugar level and increasing the levels of liquid, flour, and eggs.

The lower boiling temperature and faster evaporation of water make it necessary to increase baking time and/or baking temperature to ensure that the product is completely baked and the structure is fully set.

See also: Acids: Natural Acids and Acidulants; Aerated Foods; pH: Principles and Measurement; Yeasts.

Further Reading

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Relevant Websites

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www.aibonline.org – American Institute of Baking International (AIB).

Legal Requirements for Food Hygiene

RH Dwinger, Netherlands Food and Consumer Products Safety Authority, Utrecht, The Netherlands
K De Smet, European Commission, Health and Food Safety Directorate-General (DG SANTE), Brussels, Belgium

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Introduction

'Hygiene' refers to conditions and practices that help to maintain health and prevent the spread of diseases. It therefore includes a specific set of practices associated with the preservation of health. Implementing hygiene in the entire food chain forms an essential element to prevent major foodborne illnesses such as caused by *Salmonella* or *Listeria* species in humans. For example, the number of *Salmonella* cases in humans reported by all member states of the European Union (EU) has been decreasing due to the introduction of EU-wide control programs, focussing on primary production (farms), and the food hygiene legislation preventing contamination at all stages of the production chain. The number of confirmed *Salmonellosis* cases in humans was 192 703 per year in 25 member states in 2004 as compared to 92 916 in 27 member states in 2012. Since the beginning of the twenty-first century, food hygiene legislation introduced some important new elements, such as that the food producer is held responsible for producing safe food, that all food producers should implement a auto-control system based on the hazard analysis and critical control points (HACCP) principles (except primary producers), and that decisions by regulatory authorities should be based on a risk analysis (consisting of risk assessment, risk management, and risk communication). Additionally, there was an extension of a harmonized set of microbiological criteria for food safety and the introduction of such criteria for process hygiene, which had to be implemented by the food business operators. By requiring strict rules at farm level, at processing and distribution levels, and during retail, hygiene levels of the final products for sale to the end user were further improved. Currently, the food hygiene legislation in Europe has been strengthened to provide a high level of consumer protection and a harmonized set of rules to create a single market and a level playing field. At the same time, the legislation provides flexibility to preserve traditional food products or local processing traditions and to facilitate small- and medium-sized enterprises. The various aspects of the food hygiene legislation in the EU will be explained in detail and related legislative aspects will be mentioned briefly. The European structure will also be compared with some distinguishing elements in the food hygiene legislation of some of the major trading partners.

Food Hygiene in the EU

Food hygiene as part of veterinary health rules has been laid down in the EU legislation almost since its creation. The veterinary health rules were already introduced as early as 1964 in the framework of the Common Agricultural Policy launched in 1962. The aim was to ensure a harmonized approach in order to ensure the safe movement of animals and animal products within the EU market. Such legislation evolved into a set of vertical rules on veterinary public health (and animal health) covering each sector (meat and meat products, milk and dairy products, fishery products, etc.).

In the 1990s, consumer confidence was severely undermined due to a number of events involving food, such as the occurrence of bovine spongiform encephalopathy (BSE) in cattle initially in the United Kingdom and later in many other countries and the detection of polychlorinated biphenyls and dioxins in animal feed in Belgium. As a result, the European Commission (EC) adopted the White Paper on Food Safety in 2000. The white paper outlined a plan for strengthening food safety policy intended to actualize the existing legislation to changing needs and establish a coherent and transparent set of rules, introducing the 'farm to fork' approach and the risk analysis methodology, with the objective to guarantee a high level of human health and consumer protection.

This ambitious program contained a number of recommendations aimed at increasing food safety, improving the traceability of food products, and ensuring consumer confidence in the food industry. To this end, a set of new legislation on food and feed was adopted between 2002 and 2006 with the following characteristics: to complete and update the legislation, improve official controls, and ensure their efficient implementation. The package consists of a number of elements: the general food law, the hygiene rules for food business operators, a comprehensive set of requirements for the official controls by the competent authorities, and a number of related pieces of legislation.

Around 10 years later, experiences gained with this package were evaluated. As a result of this evaluation, adaptations to the legislation were prepared and revisions considered (see section '**Ongoing Legislative Work**').

General Food Law

The objective of the general food law (Regulation (EC) No 178/2002) is to create a framework ensuring consistency between all food- and feed-related legislations. It lays down guiding principles and establishes common definitions, such as for 'primary production,' 'food business operator,' 'retail,' 'risk,' and 'hazard.' It aims at bringing together various related aspects of EU legislation by including not only safety aspects but also protection of consumer interests, by covering all foods and by including action at both national and EU levels. Furthermore, the regulation puts the overall responsibility for producing safe food on the food business operator. It requires the food business operator to have a system in place enabling them to identify the supplier(s) of the raw materials and the immediate customer(s) of their products in order to ensure

traceability. Other issues that are covered within this regulation are the principles of risk analysis, withdrawal of food from the market by the food business operator if safety is at stake, and the precautionary principle, which enables the adoption of provisional risk management measures as long as scientific uncertainty persists. Finally, it lays down the principles and requirements for the rapid alert system for food and feed (RASFF) and for the establishment of the European Food Safety Authority (EFSA).

The RASFF is a network involving member states and the EC. It is part of a global communication network on foodborne hazards (Infosan). Whenever a member of the network has any information relating to the existence of a serious direct or indirect risk to human health, this information is immediately notified to the EC following the RASFF procedures. The EC in turn transmits the information instantly to the other members of the network, which should take corrective measures, if applicable.

EFSA (see section '**European Food Safety Authority**'), based in Parma, Italy, is in charge of risk assessment and related risk communication. Starting from the risk assessment, the legislators and decision makers within the EC and the member states develop and implement risk management measures and communicate these measures accordingly.

Legislation on Food Hygiene Addressed to Food Business Operators

The 'hygiene package' consists of a total of four basic legislative parts, described in this section and in the following one, and of implementing acts described in section '**Transitional and Implementing Measures of the Hygiene Package**' (see **Figure 1**). It became applicable with effect from 1 January 2006. The hygiene package further clarifies the responsibility of the food business operator for producing safe food, while the competent authority of the member state verifies correct implementation of the rules. Production should be based on good hygienic practice and procedures based on the HACCP principles including requirements concerning microbiological criteria and temperature limits. The legislative texts deal with a variety of food types and cover the entire food chain ('from stable to table'). Two basic regulations apply to the competent authorities (see section '**Legislation on Official Controls**'), while the two others apply directly to food business operators:

- Regulation (EC) No 852/2004 on the hygiene of foodstuffs
- Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin

Regulation (EC) No 852/2004

This regulation lays down general hygiene requirements to be respected by all food business operators at all stages of the food chain including primary production (farms) for any kind of food.

The regulation requires all food business operators to put in place, implement, and maintain general hygiene requirements (such as concerning infrastructure, layout and equipment, (pre)operational hygiene measures, pest control, water quality, and personal hygiene). The general hygiene requirements (called prerequisite programs in North American legislation) provide the foundation for effective HACCP implementation and should be in place before an HACCP-based procedure is established. The details of the general hygiene requirements are laid down in Annexes to the Regulation. In addition, all food

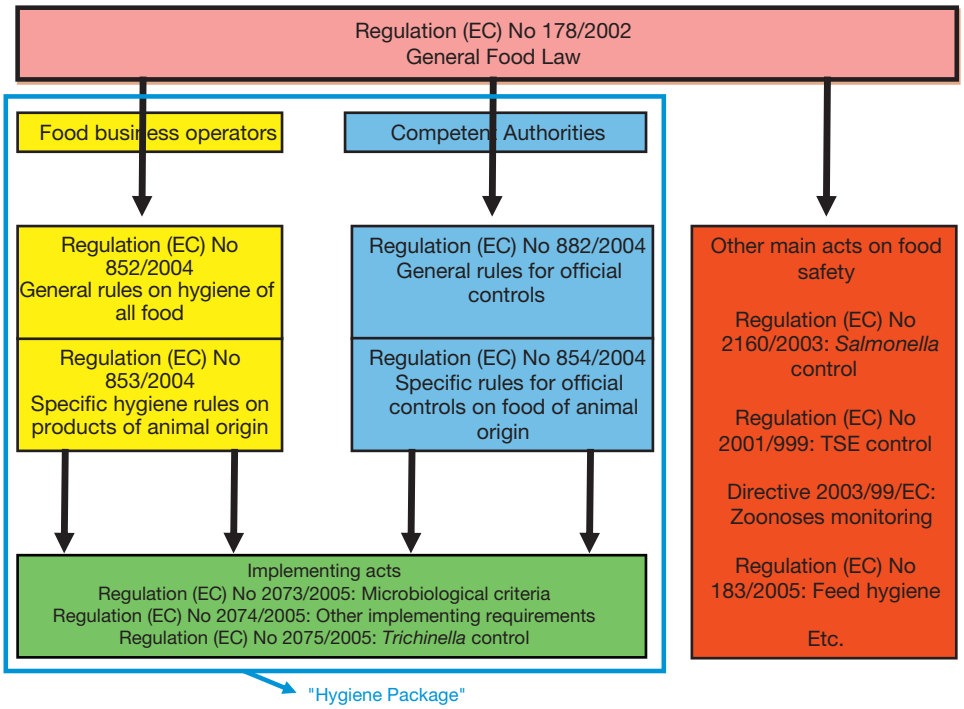

Figure 1 Overview of EU legislation on food safety.

business operators need to implement a program based on the HACCP principles (with the exception of those involved in primary production). The regulation allows the HACCP-based procedures to be implemented with flexibility so as to ensure that they can be applied in all situations including in small businesses. Guides to good practice have been developed by the food business sectors themselves, either at national level or at EU level, to help food business operators to implement general hygiene requirements and HACCP-based procedures tailored to the characteristics of their production.

In addition, the regulation requires food businesses to be at least registered to the competent authority. Such registration is a simple procedure whereby the competent authority is informed about the address of the establishment and the activities carried out. Already existing registration systems used for other purposes (environmental, animal health, or other administrative purposes) can be used. Apart from food business operators producing food of animal origin (see section '**Regulation (EC) No 853/2004**'), certain high-risk establishments may have to be approved before starting production (e.g., farms producing sprouts).

The regulation does not apply to small quantities of primary products supplied directly by the producer to the final consumer or to local retail establishments directly supplying the final consumer. National rules must however be adopted ensuring the achievement of the objectives of Regulation (EC) No 852/2004 but allowing at the same time a proportionate approach to the risk and the local situation. Examples of such products are vegetables, fruits, eggs, and raw milk, directly obtained from the farm, or products collected in the wild such as mushrooms and berries. All beekeeping activities are also considered as primary production. However, fresh meat is not a primary product since it is obtained after slaughter.

Regulation (EC) No 853/2004

This regulation is more specific than the previous one by laying down more detailed hygiene requirements to be respected by food business operators handling food of animal origin such as meat, live bivalve mollusks, fishery products, raw milk and dairy products, eggs and egg products, frogs' legs and snails, collagen, and gelatin at all stages of the food chain. The regulation mostly does not apply to retail, which for food hygiene purposes means all activities involving direct sale or supply of food of animal origin to the final consumer. In such cases, Regulation (EC) No 852/2004 does apply. Establishments handling food of animal origin for which the regulation lays down requirements in an Annex must be approved. Those establishments carrying out only primary production, transport operations, or storage of products not requiring temperature-controlled storage conditions are exempted from the approval procedure. Approval procedures involve an on-site visit by the competent authority to verify if the establishment fulfills all the requirements concerning infrastructure, layout, equipment, and hygiene. One of the requirements for approval is that the HACCP program has been validated and implemented correctly. By granting a conditional approval for 3 months (renewable for up to a maximum of 6 months), the competent authority will be able to assess the correct implementation of the HACPP program in the case of a newly started business under working conditions.

Flexibility Available Within the Legislation

In order to preserve the European heritage in product diversity and to ensure a proportionate approach for certain food businesses with a low throughput, legislation provides several opportunities for flexibility. Flexibility is available with regard to equipment and infrastructure requirements for the production of foods with traditional characteristics. Flexibility is also available to accommodate the needs of food businesses with a low throughput or situated in regions that are subject to special geographic constraints and for the continued use of traditional methods of production (at any stage of production, processing, or distribution of food). Flexibility for businesses with a low throughput is reflected in a reduction of frequency of auto-controls (e.g., for microbiological criteria) or even exclusion of certain provisions (e.g., primary production from procedures based on the HACCP principles and most retailers from the specific requirements in Regulation (EC) No 853/2004).

Finally, in certain cases, the legislation provides for the competent authority to authorize flexibility. These situations are usually introduced by wording, such as 'if the competent authority so permits,' 'unless otherwise authorized by the competent authority,' 'with authorization of the competent authority,' etc.

Apart from establishment-specific assessments mentioned in the previous paragraph, member states have to prepare national measures laying down and detailing the flexibility and need to notify them providing sufficient information to the EC and the other member states. This procedure is needed in order to ensure full transparency and fair trade since food may be distributed throughout the entire EU.

Legislation on Official Controls

This legislation is directed at the competent authorities and lays down the general principles to be respected and checks to be carried out for ensuring the official controls are objective and efficient. Furthermore, the legislation has been designed to promote a more risk-based approach for official controls.

Regulation (EC) No 882/2004 (Official Feed and Food Controls)

Regulation (EC) No 882/2004 on official controls was the result of a review of various EU rules on the subject, which were adopted separately for the feed (animal nutrition), food, and veterinary sector. The regulation covers the basic principles and the entire range of activities dealing with feed and food law, including animal health, animal welfare, and in certain aspects plant health. By means of this regulation, the EU approach to official controls was harmonized.

As a consequence of this regulation, the member states had to reorganize their official controls systems so as to integrate controls at all stages of production and in all the concerned sectors, using the 'farm to fork' principles. They had to submit a multiannual national control plan for the implementation of feed and food legislation and to report annually on the implementation of that plan. The control plans and reports need to take into account guidelines published by the commission as Decision 2007/363/EC.

The regulation provides for a set of general rules applicable to the official controls of all feed and food, whether produced within the EU or exported to or imported from third countries. In addition to the general rules, there are specific control measures to maintain a high level of protection. This is, for example, the case for the specific veterinary control rules on imports of animals and food of animal origin or for the specific control rules for organic products. The regulation also provides the possibility to draw up a list of feed and food of nonanimal origin, which shall be subject to an increased level of official controls at the point of entry. This list can be found in Regulation (EC) No 669/2009 and is regularly updated by adding (or removing) products and countries of origin or by increasing (or decreasing) the percentage of imported products to be retained for analyses. Therefore, it is important to check the latest amendment of Regulation (EC) No 669/2009 or to search for the consolidated version (see section '**How to Find the Legislation**').

Regulation (EC) No 882/2004 also lays down European reference laboratories (EURLs) to be appointed to assist the EC (follow-up and validation of new laboratory methods for the detection of foodborne infections, technical support to specific legislation, etc.). If a EURL has been established, each member state must appoint a national reference laboratory (NRL). Each EURL regularly evaluates the performances of the NRLs by organizing proficiency tests. Annex VII of Regulation (EC) No 882/2004 provides a list of the by now more than 40 EURLs dealing with different subjects (*Salmonella*, *Listeria*, and nine others in the area of food hygiene) with their exact addresses in the various member states.

The Food and Veterinary Office (FVO), a directorate of the Directorate General for Health and Consumers (DG SANTE) of the EC, is based in Grange, Ireland. The role of the FVO is mainly auditing (national) competent authorities with the purpose of verifying the efficiency of the control systems in the member states and legislation and control systems in third countries for compliance or equivalence with EU rules. The requirement for all member states to submit a multiannual national control plan facilitates the carrying out of these audits. The inspectors of the FVO visit all member states and third countries that (wish to) export to the EU. Every inspection results in a report, which is published on the DG SANTE Internet site. With regard to food hygiene, a large number of inspection reports are available not only on individual countries but also an overview on a specific theme of a series of inspections, carried out in member states or third countries. In addition, FVO has compiled individual country profiles, which give an overview of the organization of food safety, animal health, animal welfare, and plant health control systems in each member state.

Currently, a major revision (under ordinary procedure – see section '**Revision by Ordinary Procedure**') of Regulation (EC) No 882/2004 is being undertaken as part of a package of five interrelated texts dealing with animal health, protective measures against pests of plants, plant reproductive materials, official controls, and food and feed expenditure. These texts have been published by the EC as proposals for discussion by the European Parliament and the Council. It usually takes 2 years before the final versions of the texts can be adopted and another couple of years (by way of a transitional period) before the requirements need to be applied by the member states.

Regulation (EC) No 854/2004

Regulation (EC) No 854/2004 supplements the more general Regulation (EC) No 882/2004, with detailed requirements for independent, official controls on animals sent for slaughter, official controls with regard to processing and placing on the market of fresh meat, fishery products, raw milk, and dairy products, and with control procedures concerning imports. Slaughterhouses provide an opportunity for inspection and evaluation of fitness for human consumption as it allows checking the live animals on arrival (antemortem inspection) as well as the carcasses and other parts such as organs of slaughtered animals (postmortem inspection). Meat inspection at the slaughterhouse is therefore a very important part of Regulation (EC) No 854/2004. Meat inspection should be risk-based. Meat inspection can be facilitated by imposing stricter hygiene measures at the farm level and by requiring the farm operator to send animals for slaughter in a clean state together with relevant management and health information, called food chain information. These principles were introduced in the regulation. Currently, a number of initiatives are ongoing with the main purpose to better adapt meat inspection to current and emerging risks (see section '**Risk-Based Meat Inspection**'). In addition, the revision of Regulation (EC) No 882/2004 (see section '**Regulation (EC) No 882/2004 (Official Feed and Food Controls)**') repeals Regulation (EC) No 854/2004 but takes over its major requirements such as the distribution of tasks in meat inspection, taking into account the primary responsibility of the food business operator for his product, but maintaining an independent, risk-based, and cost-efficient supervision by competent authorities.

Transitional and Implementing Measures of the Hygiene Package

Transitional Arrangements

Transitional arrangements have the purpose to facilitate a smooth transition from previous rules to new ones allowing adaptation without causing interruption of trade. Remaining transitional measures are laid down in Regulation (EU) No 702/2013 and Regulation (EU) No 1079/2013. They include a temporary derogation for the accreditation of laboratories located in a slaughterhouse or a game handling establishment, carrying out official testing for the presence of *Trichinella* parasites in meat and import conditions for certain food of animal origin and for composite products (products containing both products of nonanimal origin and processed products of animal origin, e.g., pizza with ham and cheese). Within the ongoing revisions under ordinary procedure, final provisions should be decided on (see sections '**Regulation (EC) No 854/2004**' and '**Revision by Ordinary Procedure**').

Implementing Rules on the Examination of Meat for *Trichinella* Parasites

Detailed legislation has been in place for many years to specify the examination for *Trichinella* of carcasses of swine, horses, and other susceptible animal species. Regulation (EC) No 2075/2005 contains details on the sampling procedure, the

various examination techniques in the laboratory, and the derogations granted. The regulation introduced a number of elements to increase food safety for the consumer and simplify the sampling procedure for those establishments where the parasite has not been encountered for a long time. A substantial review of the rules for *Trichinella* testing in pig meat applies from 1 June 2014 (see section '**Risk-Based Meat Inspection**'). In line with amended standards of the World Organisation for Animal Health (OIE (2014)) and with the 'Guidelines for control of specific zoonotic parasites in meat: *Trichinella* species' intended to be adopted by the Codex Alimentarius Commission in July 2015 and in line with evidence observed at surveillance during the last decennia, systematic testing of slaughter pigs kept under controlled housing conditions is no longer mandatory.

Regulation on Microbiological Criteria for Foodstuffs

By the adoption of Regulation (EC) No 2073/2005, previously existing microbiological criteria were reviewed and some new ones added, taking into account recent developments in food microbiology and updated scientific advice. The main objectives of this regulation are to ensure a high level of consumer protection with regard to food safety and to harmonize the microbiological criteria in the member states. National food safety criteria can only be imposed exceptionally upon agreement after notification to the EC and other member states. Therefore, this harmonization also substantially facilitated trade between member states, who might have applied different national criteria before 2005.

The purpose of the regulation is to reduce the number of specific foodborne diseases in humans. Two different types of criteria for foodstuffs are used, which need to be complied with by the food business operator: food safety and process hygiene criteria.

Food safety criteria have been laid down for agents that commonly cause serious foodborne diseases in humans, such as certain *Salmonella*, *Listeria monocytogenes*, *Enterobacter sakazakii*, staphylococcal enterotoxins, and histamine. If limits of food safety criteria are exceeded, the batch has to be withdrawn from the market. Examples of food safety criteria are the following combinations of food category/microorganism: a *L. monocytogenes* criterion for all ready-to-eat foods (e.g., cheese, ham, and smoked fish) and a *Salmonella* criterion for certain ready-to-eat foods (e.g., ice cream), minced meat, meat preparations, and certain meat products.

In addition, the regulation includes process hygiene criteria, such as *Enterobacteriaceae* and *Salmonella* in carcasses of slaughtered animals, *Staphylococci* in certain types of cheese, and *Escherichia coli* in precut fruit and vegetables. These criteria are indicators of the correct functioning of the manufacturing process and are not related to a direct major public health risk. Noncompliance, therefore, does not result in the withdrawal of the affected batch but must result in measures to improve the hygiene of the production process.

The sampling frequency is stipulated for a few criteria, for example, for *Salmonella* in minced meat and carcasses. In other cases, the food business operators have to decide on the sampling frequency on a case-by-case basis taking into account the risk related to their products and integrated in the procedures based on the HACCP principles. Although the regulation is directed at food business operators, competent authorities must, for various reasons, take samples to verify that the criteria laid down in the regulation are met (a guidance document has been prepared – see section '**Guidance Documents for the Legislation on Food Hygiene**'). It is important to consult the consolidated version (see section '**How to Find the Legislation**') of Regulation (EC) No 2073/2005 since this piece of legislation is continuously evolving due to the addition of new criteria and/or new limits or procedures as scientific insights progress. For example, new food safety criteria were introduced, for example, for *Salmonella enteritidis* and *S. typhimurium* in fresh poultry meat and for certain verotoxin-producing *Escherichia coli* (VTEC) on sprouts as a result of an outbreak implicating sprouted seeds in Germany in May 2011.

In the absence of EU food safety criteria, the evaluation of the food must be done in accordance with Article 14 of Regulation (EC) No 178/2002, which provides that unsafe food must not be placed on the market. On the basis of this article, competent authorities can take corrective measures as long as the measures are proportionate and based on a case-by-case risk assessment.

Other Implementing Measures

A wide range of other implementing measures have been laid down in Regulation (EC) No 2074/2005 including detailed provisions concerning food chain information, fishery products, recognized testing methods for detecting marine biotoxins, calcium content of mechanically separated meat, lists of establishments, and a derogation for foods with traditional characteristics.

Guidance Documents for the Legislation on Food Hygiene

A number of documents have been prepared to give guidance on the implementation of the food hygiene requirements and related subjects. The documents aim to assist the food business operators and the competent authorities of the member states. The guidance documents are not formal acts of legislation, but the EC will defend where necessary the consensus laid down in these documents. Some examples are given in the succeeding text (those with an * have been placed on the DG SANTE Internet site):

- Guidance document on the implementation of the main General Food Law requirements*
- Guidance document on Regulation (EC) No 852/2004*
- Guidance document on Regulation (EC) No 853/2004*
- Guidance document on the implementation of HACCP and facilitation of the implementation of the HACCP principles in certain food businesses*
- Guidance document on community guides to good practice*
- Practical guide on food contact materials*
- Guidance documents on import requirements*
- Guidance document on the preparation of multiannual control plans as laid down in Regulation (EC) No 882/2004 (published as Decision (EC) No 2007/363/EC)

- Guidance document laying down criteria for the conduct of audits (published as Decision (EC) No 2006/677/EC)
- Guidance document on official controls, under Regulation (EC) No 882/2004, concerning microbiological sampling and testing of foodstuffs*
- Document concerning the flexibility available within the various hygiene regulations, namely, two different parts: commission staff working document on the understanding of certain provisions on flexibility provided in the Hygiene Package and Frequently Asked Questions: guidelines for food business operators*
- Commission staff working document on the understanding of certain provisions on flexibility provided in the Hygiene Package and Frequently Asked Questions: guidelines for the competent authorities*

Related Food Safety Legislation

Food hygiene rules set the major framework for safe food, avoiding foodborne infections. When considered appropriate, these rules are supplemented by measures for the control and monitoring of certain specific pathogens (foodborne zoonoses such as *Salmonella*, BSE). In addition, food hygiene is further enhanced and complemented by hygiene rules at preceding steps (feed hygiene) and safe disposal of by-products (not intended for human consumption) of the production of food of animal origin. Finally, although the presence and cross contamination of all hazards, including chemical ones, form part of the hygiene package, specific rules on chemical hazards must also be implemented. These additional rules will be briefly specified.

Provisions on the Control of Specific Foodborne Zoonotic Infections and Monitoring of Zoonoses

Regulation (EC) No 2160/2003 is a specific application of hygiene rules focussing on specific foodborne infections (mainly biosecurity measures at farm level). Implementing rules address *Salmonella* control in poultry but can be extended to other species (e.g., pigs) or pathogens (e.g., *Campylobacter*). Targets for reduction of disease prevalence rates and trade restrictions on infected flocks/herds must ensure that fewer animals affected by a foodborne pathogen are introduced in the food chain.

Directive 2003/99/EC and Decision 2119/98/EC oblige member states to monitor foodborne (and other zoonotic or infectious diseases such as rabies, influenza, vector-borne diseases) in food/animals and humans, respectively. This monitoring is essential to evaluate the effect of measures taken on food hygiene, to assess trends, and to identify emerging risks. The results are published annually in a joint report prepared by EFSA and the European Centre for Disease Prevention and Control (ECDC). This continuous monitoring has triggered a revision of measures for *Salmonella* and *Trichinella* controls.

Legislation on Transmissible Spongiform Encephalopathies (Including BSE)

The recognition of the causal link between BSE and the human variant Creutzfeldt–Jakob disease led to one of the major crises that ever affected the feed and food sectors. The key piece of legislation to protect human and animal health from the risk of BSE and other transmissible spongiform encephalopathies (TSEs) is Regulation (EC) No 999/2001 (the TSE Regulation). The TSE regulation provides measures targeting all animal and public health risks resulting from all animal TSE and governing the entire chain of production and placing on the market of live animals and products of animal origin. It includes rules for the monitoring of TSE in bovine, ovine, and caprine animals, restrictions concerning the use of animal proteins in animal feeding, and the removal of the so-called specified risk material. Specified risk materials are animal tissues being most at risk of harboring the TSE agent (e.g., brain and spinal cord). In order to prevent any recycling of possible TSE agent, these tissues are collected and completely destroyed through incineration. Recently, the commission produced a road map on the TSE strategy, which outlines possible amendments of certain measures in the short, medium, and long terms without endangering the health of the consumer or the policy of eradicating BSE. Due to the stringent and extensive measures taken, the number of cases tested positive in the EU for BSE has been decreasing each year, and the number of countries recognized by OIE as having a negligible BSE risk in accordance with its strict rules as laid down in the Terrestrial Code is increasing.

Feed Hygiene

Regulation (EC) No 183/2005 ensures that hygiene is considered at all stages that may have an impact on feed safety and, consequently, food safety, including during primary production (at farm level). It requires registration of all feed business operators and the approval of some of them using more sensitive feed additives in premixtures or compound feedingstuffs. The regulation requires operators to follow harmonized hygiene requirements and implement HACCP-based procedures. The development of EU and national guides to good practice in feed production has been successfully encouraged.

Legislation on Animal By-Products

Regulations (EC) No 1069/2009 and Regulation (EU) No 142/2011 lay down health rules for the collection, transport, storage, handling, processing, and use or disposal of all animal by-products (ABPs) not intended for human consumption. The regulations complete the hygiene rules by providing a framework for the safe disposal of products that may present a risk to animal or public health directly through (unintended) consumption or indirectly, for example, by contaminating the environment. To that end, the legislation distinguishes three different categories of ABPs, based on risk. Category 1 material has the highest risk and is usually incinerated. Category 2 is less risky material and can not only be incinerated but also be composted or used for biogas production. Category 3 material can be used for animal feed under certain conditions. The last category includes parts of slaughtered animals that have been found fit for human consumption, but are not for one reason or another intended for human consumption. ABPs include among others bones and other parts of slaughtered animals but not intended for human consumption (food), fallen stock, animal feed (e.g., based on fishmeal and processed animal

protein), organic fertilizers and soil improvers (e.g., manure, guano, and products on the basis of processed animal protein), technical products (e.g., pet food, hides and skins for leather, wool, and blood for producing diagnostic tools). In the EU, more than 20 million tons of by-products emerges annually from slaughterhouses, plants producing food for human consumption, and dairies and as fallen stock from farms.

Legislation on Residues

Residues are substances that can occur in foodstuffs as a side effect of using veterinary medicines or phytosanitary products. They are unwanted traces of medicines or plant protection products or derivatives thereof that remain in the final product. Member states need to adopt and implement every year a plan to monitor live animals and products thereof, including meat, for residues of prohibited substances (e.g., hormonal substances for fattening purposes) or for substances permitted below a certain threshold, the so-called maximum residue limit. The latter group of substances includes veterinary medicinal products, pesticides, and environmental contaminants. Details of the substances involved and of the residue monitoring plan can be found in Directives 96/22/EC and 96/23/EC and their amendments. The aim of the national residue monitoring plan is to ensure that permitted levels are not exceeded and that forbidden substances are not present in food. A useful manual on how to design a national residue monitoring plan is available.

Legislation on Contaminants

Contaminants are substances that can unintentionally enter food during the various stages of its production, packaging, transport, or holding or as a result of environmental contamination. Regulation (EEC) No 315/93 lays down the basic principles to minimize contaminants in food, while Regulation (EC) No 1881/2006 sets maximum levels for certain contaminants in foodstuffs. EU measures have been taken for the following contaminants: mycotoxins, heavy metals, dioxins and PCBs, polycyclic aromatic hydrocarbons, 3-MCPD (3-monochloro-propane-1,2-diol is a by-product in soy sauce and can be also present as a contaminant in some food additives and may be present in drinking water as flocculants or coagulant aids), and nitrates.

Other Legislation

Additional legislation may apply, such as rules on food additives (substances added intentionally to foodstuffs to perform certain technological functions), biocides, food contact materials, and labeling requirements.

Animal health requirements apply to the live animals that are going to be slaughtered, while welfare requirements apply to the management of animals on the farm, the transport of animals to the slaughterhouse, and the killing of animals at the slaughterhouse. Additional legislation to take into consideration regarding the production of fruits and vegetables concerns the use of plant protection products (e.g., pesticides), food contact materials, genetically modified organisms (GMOs), etc.

Ongoing Legislative Work

The commission prepared a report to the European Parliament and the Council in 2009, reviewing the experience gained with the application of the hygiene package and recommended changes, amendments, or improvements to facilitate implementation of the hygiene package. Most recommendations have already been effected. Others are still under discussion since amendments can only be made following the (quite heavy) ordinary procedure (see section '**Revision by Ordinary Procedure**') and/or scientific advice from EFSA and comprehensive consultation of all stakeholders involved including member states.

Revision by Ordinary Procedure

Basic provisions of the hygiene Regulation Nos 852/2004, 853/2004, and 854/2004 can only be amended following the ordinary procedure, including consultations and the preparation of an impact assessment before sending an EC proposal to the European Parliament and the Council for discussion and adoption. Two main issues are intended to be addressed in the EC proposal:

- Definitive requirements for import of composite products using a risk-based and proportionate approach
- Simplification of the notification procedure for adaptations of requirements, needed to allow a proportionate application of the hygiene rules by small- and medium-sized enterprises (see flexibility under section '**Flexibility Available Within the Legislation**').

The proposal of the EC may be forwarded to the European Parliament and the Council in 2015. It would be a starting point for discussion and additional possible amendments may be put on the agenda (e.g., revision of certain definitions).

Treatment to Remove Surface Contamination

Regulation (EC) No 853/2004 permits substances other than potable water to remove surface contamination from products of animal origin, subject to approval by the EC.

Treatment to remove surface contamination might constitute an additional element in further reducing the number of pathogens in food, especially *Salmonella*, VTEC, and *Campylobacter*, provided that an integrated control strategy is applied throughout the entire food chain. Surface decontamination may never replace good hygiene practices or procedures based on the HACCP principles.

Before approving, a file must be submitted and assessed by EFSA containing information on the efficacy, safety (including effect on antimicrobial resistance of pathogens), and environmental effects. Following a favorable scientific opinion by EFSA, so far, only the use of lactic acid to reduce microbiological surface contamination on bovine carcasses, half-carcasses, or quarters in the slaughterhouse has been authorized provided that an HACCP-based management system is in place and sampling for compliance with the microbiological criteria has taken place beforehand (Regulation (EU) No 101/2013).

Risk-Based Meat Inspection

Meat inspection has focussed traditionally on the detection of zoonotic diseases, such as tuberculosis and brucellosis, which have been largely eradicated from herds kept under modern management conditions or do not occur in the majority of the very young and generally healthy animals slaughtered nowadays. Other hazards such as *Salmonella* and *Campylobacter* have become the most important foodborne infections, and cross contamination by these pathogens may even be enhanced by incisions and palpations as part of current meat inspection procedures. Therefore, a reorientation of meat inspection toward these current risks is ongoing. EFSA recently adopted a number of opinions, setting priorities for meat inspection in different animal species and making recommendations for the adaptation of official controls. The EC has agreed with the member states on amending inspection of slaughtered fattening pigs, for example, by making visual inspection the general rule, limiting palpation and incisions to cases where needed (Commission Regulation (EU) No 218/2014), simplifying testing procedures for *Trichinella* parasites (Commission Regulation (EU) No 216/2014), and enhancing *Salmonella* monitoring (Commission Regulation (EU) No 217/2014). Step by step, meat inspection in other animal species will be reconsidered and legislation will be revised.

How to Find the Legislation

All the legislative texts can be recovered in the 24 official languages of the EU from the Internet site EUR-Lex, the gateway to the law of the EU. By way of the button 'simple search' and thereafter 'reference number document,' one can type the year of publication and the number of the required legislative text. The entire text can then be read in the desired language. Some legal texts have been amended a number of times following their publication. In such a case, it is advisable to request the 'consolidated version' (instead of the 'reference number document'). When amendments have been made to the original act, they are as quickly as possible inserted and properly indicated in the consolidated version.

European Food Safety Authority

EFSA deals with risk assessment and related risk communication issues on a European scale. EFSA provides scientific advice and recommendations on the control of existing and emerging risks and is independent from the risk managers. Its remit covers food and feed safety, nutrition, animal health and welfare, plant protection, and plant health. In addition, EFSA performs environmental risk assessments of genetically modified crops, pesticides, feed additives, and plant pests. EFSA often collaborates with ECDC, the EU agency aiming to strengthen and develop continent-wide human disease surveillance and early warning systems across Europe. EFSA and ECDC jointly prepare every year a summary report on the occurrence of zoonoses (see section '**Provisions on the Control of Specific Foodborne Zoonotic Infections and Monitoring of Zoonoses**').

Food Hygiene Legislation Outside of the EU

The legislation in the EU can be considered as rather progressive due to its emphasis on placing the responsibility for producing safe food on the food business operators, the efforts to lay down rules concerning the entire food chain from start to finish and due to the great emphasis on risk assessment, risk communication, and risk management. Moreover, various key elements form distinguishing features of the European food hygiene legislation, such as the requirement to implement HACCP in all food-producing establishments (except in primary production), the emphasis on risk-based inspections, separating risk assessment from risk management, etc.

However, for trade purposes, it is necessary to fulfill food hygiene requirements of the importing country (e.g., at import in the EU, third countries need to comply with EU rules), and therefore, it is worthwhile to examine briefly some of the distinguishing features of the legislation in other major trading partners and/or some useful guidance documents.

International Aspects

Where international standards exist, they have been taken into consideration in the development or adaptation of the European food safety legislation. This applies to standards developed by the Codex Alimentarius Commission, which has been created by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) to develop food standards, guidelines, and related texts such as codes of practice. It also applies to standards related to hygiene rules at animal rearing, animal health, and animal welfare developed by the OIE. Similarly, ISO (International Organization for Standardization) and CEN (European Committee for Standardization) standards have been incorporated in the EU legislation as analytic reference methods as far as possible (as can be seen, e.g., in Regulation (EC) No 2073/2005).

The United States

In the United States, the work regarding food safety is divided between the Food Safety and Inspection Service (FSIS), a public health agency in the US Department of Agriculture responsible for ensuring the safety of the nation's commercial supply of meat, poultry, and egg products, and the Food and Drug Administration (FDA), which regulates foods other than meat, poultry, and egg products. The FDA is also responsible for the safety of drugs, medical devices, biologics, animal feed and drugs, cosmetics, and radiation-emitting devices. Differences exist in inspection approach and procedures between FSIS and FDA. This has an effect on the traceability of food, the implementation of HACCP in some food establishments (e.g., voluntary for dairy processing establishments), and requirements for food business operators. Differences also exist between US and EU legislative requirements regarding *Salmonella* and *Listeria* criteria for food products. In order to facilitate trade in live animals and animal products between the two partners, a veterinary equivalency agreement was signed in 1999. The annexes to the agreement were amended by Decision 2003/833/EC on sanitary measures to protect

public and animal health. On 14 June 2013, member states gave the EC the green light to start trade and investment talks with the United States. The negotiations aim to establish a bilateral free trade agreement focussing initially on cutting barriers to trade and harmonizing regulations across a large range of economic activities including food safety. The current negotiations in the Transatlantic Trade and Investment Partnership will have to resolve some differences in approach between the United States and the EU concerning food safety. Subjects that are likely to be discussed range from differences in microbiological criteria and decontamination in the slaughterhouse to approval for use of GMOs and the use of growth promoters in the animal industry.

Canada

The EU has a veterinary agreement with Canada, while discussions concerning a comprehensive free trade agreement have been quite successful and are in its final stages. Some examples of the issues still under discussion concern an organics equivalence agreement, which aims to recognize each other's organic production standards, trade in products derived from animal cloning, the EU approval system for GMOs, use of growth hormones in cattle and pigs, and decontamination of meat in slaughterhouses.

New Zealand

The New Zealand Food Safety Authority has implemented a new approach to food safety since its inception in 2002. The approach is based on a system of performance-based inspections across the entire food chain and provides for a full cost recovery of inspections. Food businesses manufacturing or processing animal products (all primary processors and certain secondary processors) are required to adopt a risk management program. Those food businesses selling food need to implement a food safety program, which is a risk-based approach to food safety. Detailed guidance is provided by the competent authority and codes of practice are available reflecting acceptable industry-agreed practice.

China

Approximately ten government departments and ministries under the State Council monitor food safety in China. The China Food and Drug Administration has been established to oversee all aspects of food safety regulations and unify food safety controls. However, law enforcement is facing severe challenges due to Chinese food market characteristics: scattered and small production units without quality and safety priorities, lack of consumer awareness of food safety concerns, and overlapping competencies between central and provincial governments. The General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ) is responsible for the supervision of safety concerning imports and exports. The EU SME Centre has published a useful guideline to assist food businesses with understanding the rules for exporting food to China. The Netherlands and China recently agreed to facilitate import of dairy products through electronic certification. The Food Safety Law is currently being revised.

Southeast Asia

A very useful HACCP manual has been developed in 2005 for food business operators in Southeast Asia, while FAO and WHO have issued a year later guidance to governments on the application of HACCP in small and/or less-developed food businesses.

Acknowledgments

We wish to acknowledge the critical comments from J. Ordeig-Vila from DG AGRI and T. Chalus from DG Santé and the helpful information provided by D. Beutener, Canadian Embassy in the Netherlands and N. Runia, Dutch agricultural counselor in Beijing.

See also: Authenticity of Food; Beef; *Clostridium*: Food Poisoning by *Clostridium perfringens*; Codex Alimentarius Commission: Role in International Food Standards Setting; Codex Alimentarius; Consumer Protection Legislation; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; Food Additives: Classification, Uses and Regulation; Food Fraud; Foodborne Pathogens; Genetically Modified Foods: Risk Assessment, Legislation, Consumer Behavior, and Ethics; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Horse Meat; Laboratory Management: Microbiological Safety; Poultry: Processing; Quality Control in Food Processing; Risk Assessment of Foods and Chemicals in Foods; Sanitization; Sheep: Meat; *Staphylococcus*: Food Poisoning; Traditional Foods; Zoonoses.

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Legumes in the Diet

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Introduction

Many diets in developing countries are based on legume and cereal products. Legumes are a good source of proteins, carbohydrates, dietary fibers, and micronutrients such as vitamins, minerals, and fatty acids. There is an increasing interest in other minor components – or phytochemicals – that may provide additional health benefits. Multiple experimental and clinical studies support the role of phytochemicals and functional foods in the prevention and treatment of diseases. The consumption of these compounds has been associated with the reduced risk of cancer, cardiovascular disease, diabetes, chronic inflammation, neural degeneration, and other chronic ailments and illnesses. Legumes are a good source of raw materials for the production of ingredients for nutraceuticals and functional foods.

Most of the phytochemicals, including dietary fibers and polyphenols, are found in the germ and seed coat. Therefore, in general, food products made from whole grains or legumes are preferable. During processing, grains and legumes often have the germ and the seed coat removed; thus, important dietary fibers, vitamins, minerals, and phytochemicals are lost. The processing of beans and other legumes to produce both nutritional and nutraceuticals food-based products represents a major opportunity to the health-conscious market.

This article focuses on the nutritional value, phytochemical composition, the properties of the potential health benefits of legumes, and how these can be used to produce food ingredients in addition to their traditional consumption.

Economic Importance of Legumes

The *Leguminosae* (*Fabaceae*) family (also called legumes) is the third largest family of flowering plants, comprising 751 genera and 19 500 species. Agricultural purposes of legumes include grain and blooms production, medicinal and industrial uses, pasture, forage, fallow, green manure, and timber. It is the seeds (or grains) that are the primary portion of the plant used for human consumption.

Alfalfa (*Medicago sativa*) and clover (*Trifolium sp*) are important legume crops used mostly for silage and forage. Their production is significantly higher than that reported for other legumes (Figure 1). But, it is important to consider that around 80% of their weight is water; which is much higher than the moisture content of other harvested legumes (around 15%), such as soybeans. The United States of America, Egypt, Argentina, and México are among the top producing countries, delivering from 28 to 228 million tons in a 10-year period (2003–13).

Soybeans (*Glycine max*) and peanuts, or groundnuts, (*Arachis hypogaea*) are legumes used for oil extraction or harvested green for food. The top producing countries include USA, Brazil, Argentina, China, and India (Figure 1). Soybean and pulses cover the most harvested area among legumes. By 2012, 105 million hectares worldwide were utilized for soybean cultivation and 80 million were utilized for pulses. Groundnuts, alfalfa, and clover reached a harvested area of 25, 15, and 3 million hectares respectively (Figure 2).

Pulses are another sub-category of legumes used for human consumption. According to the FAO (Food and Agricultural Organization), 11 primary crops of dry seeds are considered pulses: Dry beans, dry broad beans, dry peas, chickpeas, dry cowpeas, pigeon peas, lentils, Bambara beans, vetches, lupins, and minor pulses. Globally, consumption of pulses is 19 g per person per day. Asian countries are among the major consumers and producers. Between 3 and 15 million tons were produced in a 10-year period (2003–13) from the top producing countries, which includes India, China, Canada, Myanmar, and Brazil (Figure 3).

Legumes are used for rotation farming with cereals. They supply nitrogen to the cereal crops. Depending on the crop, plant nitrogen derived from N_2 fixation can range from 36% on common beans to 65% for pulses like the chickpea, lentil, pea, cowpea, mung bean, or pigeon pea. Although much of the N_2 fixed by the legumes is usually removed at harvest, it is well known that legume inclusion in a cropping sequence improves the productivity of the following crops and may replace a significant quantity of N fertilizer.

Nutritional Value of Legumes

Legumes contain 58–63% complex carbohydrates. Compared to cereals, they contain a higher amount of protein. Among legumes, pulses are an inexpensive source of proteins that have long been one of the main – or staple – sources of protein in many parts of the world. They have been used as a substitute for meat in more affluent societies, and play an important role in alleviating protein–energy malnutrition in less affluent societies. In many low-income countries, where they are part of staple diets, pulses provide a significant portion of total protein consumed (Figure 4).

Dietary fiber is another exceptional feature of dry beans; their content is relatively high (10.6–25.0%) compared to rice (1.3–3.5%), corn (7.3–8.5%), and wheat (10.5–12.7%). Dietary fiber intake from legumes has also been associated with the reduction of prostate and colorectal cancer risk.

Micronutrients are involved in all aspects of the human body, and their deficiencies can cause an increased risk of death by infectious and chronic diseases. The most important minerals for human health are iron, calcium, and zinc; their deficiencies in the human diet contribute to high rates of mortality in developing countries. A portion of dry beans (1 cup = 85 g) may contribute up to 22%, 54%, and 29% of the recommended daily intake of calcium, iron, and zinc. Also,

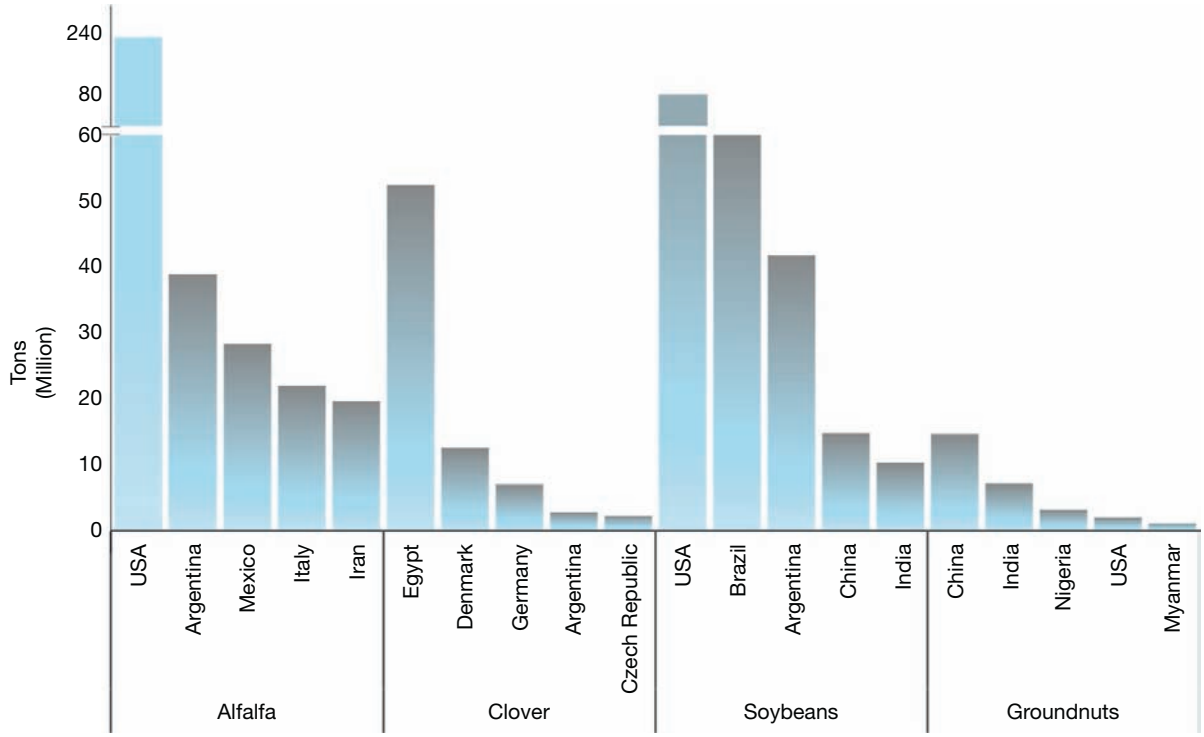

Figure 1 Average production (millions of tons) of the top five producing countries of alfalfa, clover, soybeans, and groundnuts on a 10-year period (2003–13). Data obtained from FAOSTAT (2015).

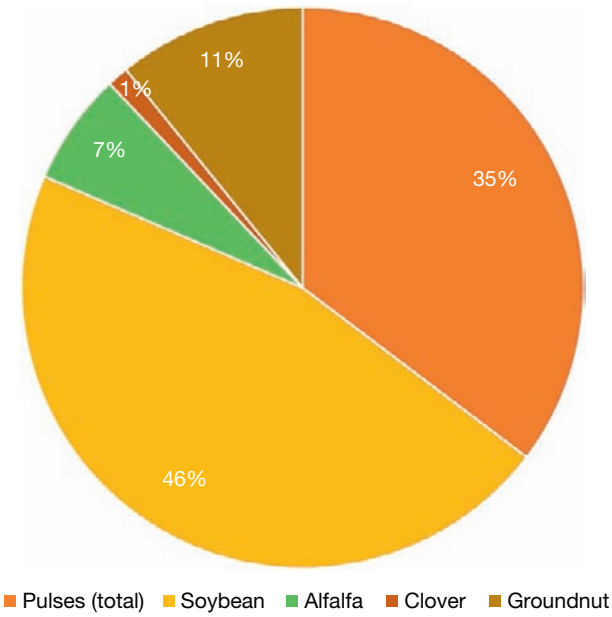

Figure 2 Comparison of the world harvested area in 2012 for: soybean, pulses, groundnuts, clover, and alfalfa. Data obtained from FAOSTAT (2015).

dry beans are an excellent source of Cu, P, Mn, and Mg. Furthermore, legume seeds are an excellent source of water soluble vitamins. A cup of dry beans can satisfy 45–107% of thiamine, 20–27% of riboflavin, and 11–27% of niacin daily requirements according to the United States Department of Agriculture (2015).

Nutraceuticals Found in Legumes

Beyond the relevant nutritional contribution of legumes to the diet, epidemiological studies have shown that the intake of legumes is associated with a reduction in the incidence of various cancers, HDL cholesterol, type-2 diabetes, metabolic syndrome, and heart diseases. Increasing legume consumption, such as dry beans, peas, peanuts and peanut butter, from 1 to 4 times or more per week was associated with a 22% lower risk of coronary heart disease and an 11% lower risk of cardiovascular disease. Incorporation of legumes as part of a low glycemic index diet improves both glycemic control and reduced coronary heart disease risk.

The inverse association between legume intake (including soy), and the risk of metabolic syndrome has been studied – regarding their fiber content. These benefits are also related to legumes' diverse bioactive proteins and peptides, as well as their bioactive micro-constituents (also known as phytochemicals), like phytoesterols, tocopherols, polyphenols, and triterpenic acids.

Bioactive Proteins and Peptides

Most of the protein found in legume seeds is composed of storage proteins, which, depending on the source, can reach up to 80% of the total protein content. The rest of the protein

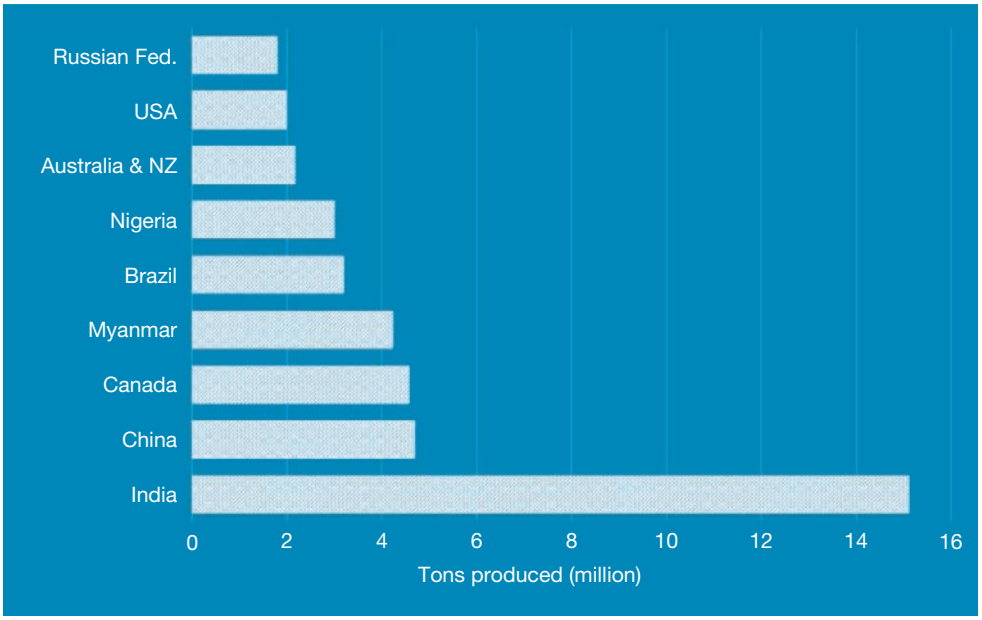

Figure 3 Average production (millions of tons) of the top ten producing countries on a 10-year period (2003–13). Abbreviations: Russian Fed., Russian Federation; USA, United States of America; Australia and NZ, Australia and New Zeland. Data obtained from FAOSTAT (2015).

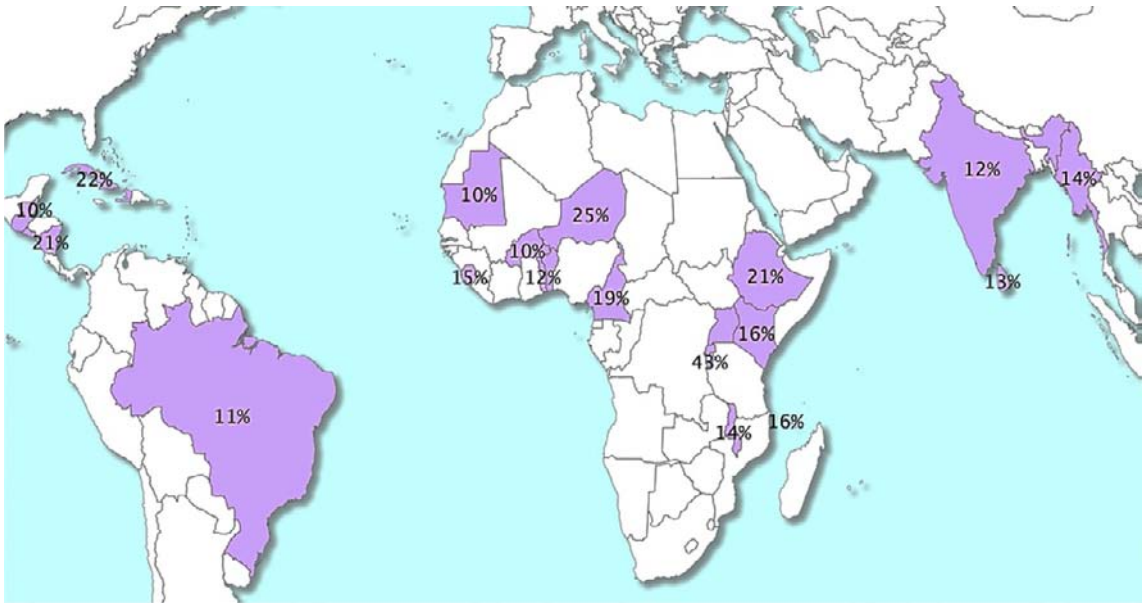

Figure 4 Map showing developing countries with high consumption of pulses and corresponding contribution as percentage to total per capita protein intake.

in pulse seeds include enzymes, as well as two classes of macromolecules typically referred to as 'antinutritional' – protease inhibitors and lectins. Lectins are carbohydrate-binding proteins found in a wide variety of important crop plants. Lectins are commonly found in unprocessed seeds like pea, chickpea, lentil, and other pulse crops; but, once legumes are cooked the lectins are destroyed.

When raw seeds, or their flour, are consumed by humans and livestock, lectins can cause deleterious effects like diarrhea, bloating, vomiting, and red cell agglutination. Nevertheless, it has also been found that lectins may play a key role in preventing certain cancers, and the activation of certain innate defense mechanisms. They have also been evaluated as therapeutic agents for preventing or controlling obesity.

Similar to lectins, protease inhibitors are considered antinutritional compounds since they reduce digestibility and the bioavailability of nutrients. However, some protease inhibitors, such as the Bowman-Birk inhibitor (BBI), have been

shown to reverse the initiation of carcinogenesis according to epidemiological, *in vitro* and *in vivo* studies. BBI has been evaluated as a chemopreventive agent against oral, head, and neck cancer without detectable clinical or laboratory toxicity even at high doses. Common beans contain lower levels of protease inhibitors than soybean. The difference in levels of protease inhibitors relates more to the cultivar (genetics) than to the site of cultivation (environment).

Bioactive peptides generated during proteolysis (protein hydrolysates) have been commercialized as nutraceuticals or functional ingredients. For example, lunasin is a soybean derived bioactive peptide currently commercialized for its anti-cholesterolemic and anti-diabetic properties. Protein hydrolysates and BPs from legumes have been reported to possess antioxidant, hypocholesterolemic and angiotensin converting enzyme inhibitory activities.

Phenolic Compounds of Legumes

Legume phytochemical profiles vary widely, and their survival through the stages of food processing is still under study. Soaking prior to cooking, and germination results in the lixiviation (and loss) of many phytochemicals.

Most phenolics and antioxidants in legumes are concentrated in the hulls or seed coats. These antioxidants have free radical scavenging activity as well as cytotoxic effects against cancer cells. Flavonoids are responsible, in large part, for seed color. For example, anthocyanins in black beans may account for 2.5% of the seed coat, a level comparable to that reported in fruits such as berries.

Some flavonoids have been isolated, specifically from black bean hulls, to be tested against certain human cancer cells *in vitro*, and also to evaluate their antioxidant activity. Particularly, non-glycosylated flavonols such as myricetin, quercetin, and kaempferol have been attributed to the antioxidant capacity of germinated black beans.

Another important group of bioactive flavonoids are the isoflavones, which possess estrogenic and other activities. Particularly, genistein and daidzein have been associated with the prevention of cancer, cardiovascular disease, and osteoporosis. Additionally, supplementation with soybean isoflavones has been used to reduce postmenopausal symptoms.

Controversy surrounds the understanding of the risks and benefits of exposure to isoflavones, especially during early life exposure. Some studies have demonstrated that children consume an even greater amount of phytoestrogens than adults. Most of the beneficial effects of isoflavones consumption have been proven for adults, but some *in vivo* models have demonstrated adverse effects derived from early exposure to these phytochemicals, particularly related to neurological diseases and the early onset of puberty. The Center for the Evaluation of Risks to Human Reproduction of the United States of America expressed a minimal concern for adverse developmental effects related to the consumption of soy infant formula. Additionally, the American Academy of Pediatrics states that "There is no conclusive evidence from animal, adult human, or infant populations that dietary soy isoflavones may adversely affect human development, reproduction, or endocrine function."

Saponins in Legumes

Legumes, particularly soybeans (*Glycine max*), are a rich source of saponins. A wide variety of triterpenoid saponins known as the soyasaponins, have been isolated from soybeans. Other legumes, such as peas (*Pisum sativum*), peanuts (*Arachis hypogaea*), faba beans (*Vicia faba*), and common beans (*Phaseolus vulgaris*) also contain saponins. Saponins in legumes vary depending on the amount and type of sugar bonded to the oleanene structure.

Soyasaponins affect the activity of several enzymes in plants, mammalians, insects, and fungi. They inhibit cholesterol micellar solubility; have anti-inflammatory, anticarcinogenic, antimicrobial and antimutagenic activity. They also exhibit antiviral, cardioprotective, and hepatoprotective effects, as well as interact with biliary acids, and improve immune response.

Legumes, Dietary Fiber, and Resistant Starch

Legumes are also high in fiber, which is now commonly perceived to be a food component that can lead to reduced cholesterol levels and reduced risk of ischemic heart disease. Diets rich in bean fiber enhanced the cholecystokinin response to a meal, and prolonged the presence of apolipoprotein B48 in plasma. These proteins are related to the digestion and absorption of a meal, and small intestine transit time.

Fiber from legumes (common, black, and navy beans) has also been correlated with the reduction of colon cancer by about 50% in animal tumor models, and also with inhibiting the formation of colonic aberrant crypt foci (a pre-cancerous model system) in rats with azoxymethane induced colon cancer.

Other Phytochemicals in Legumes

Phytic acid has long been cited as a phytochemical that reduces bioavailability of minerals, but it is also known as an antioxidant for its ability to reduce blood glucose levels, exert a protective action against colon cancer, and act as a prebiotic due to its ability to bind enzymes. A large number of studies have been published in the last 30 years relating phytic acid to cancer. The studies span effects from therapeutic uses, to prevention, to promotion effects. A great majority of the studies were done using animals, and showed that phytic acid had anti-neoplastic properties in breast, colon, liver, leukemia, prostate, sarcomas, and skin cancer.

Phytosterols are another group of phytochemicals that have potential as nutraceuticals. In particular, phytosterols extracted from black beans showed hypercholesterolemic effects not only by the reduction of cholesterol absorption, but also in the regulation of genes involved in hepatic lipogenesis.

Legumes as a Raw Material and Source of Functional Ingredients

Heat treatments, traditionally used to improve the nutritional value of legumes, have a deleterious effect on many compounds with nutraceutical properties. Considering the

potential of many of the phytochemicals contained in legumes, commercial interest in the exploitation of the use and application of extracts is increasing. For example, some phytochemicals could be recovered from an aqueous extract obtained as a byproduct of canned bean processing and used for the treatment or prevention of cancer, heart strokes, elevated levels of cholesterol, hypertension, obesity, and inflammatory disorders in humans. Black bean seed coat extracts have also been used to purify anticancer compounds. In fact, several efforts are being made to optimize the dehulling process and obtain seed coats to be used as nutraceuticals and cotyledon flours with high protein content and good functionality.

Pulse proteins might be used in different products in a similar way as soy or whey. Besides nutritional functionality, pulse proteins possess functional properties that have been understudied. Furthermore, the addition of legume flour is a good alternative for improving the nutritional value of gluten free bakery products.

Additionally, other biotechnological alternatives have been evaluated to reduce antinutritional components of legumes. Particularly, germination, fermentation, or the addition of phytase has been proven to reduce or remove considerable amounts of phytate that chelates important micronutrients such as iron and zinc.

See also: Antinutritional Factors in Legume Seeds: Characteristics and Determination; Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Bioactive Peptides in Foods; Bioavailability of Nutrients; Cancer: Diet in Cancer Prevention; Cholesterol: Factors Determining Blood Cholesterol Levels; Colon: Diseases and Disorders; Colon: Structure and Function; Dietary Fiber: Bran; Dietary Fiber: Determination; Ethnic Foods; Extrusion Cooking: Principles and Practice; Fats: Classification and Analysis; Fermented Foods: Composition and Health Effects; Functional Foods; Obesity: The Role of Diet; Peanuts; Peas and Lentils; Phenolic Compounds: Bioavailability and Health Effects; Phenolic Compounds: Occurrence, Classes, and Analysis; Phytic Acid: Properties, Uses, and Determination; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Proteins: Chemistry, Characterization, and Quality; Pulsed Electric Fields; Saponins; Soy Beans: Dietary Importance; Soy Beans: Processing; Soy Beans: Properties and Analysis; Soy Beans: The Crop; Soybean Oil; Starch; Whey and Whey Powders: Protein Concentrates and Fractions.

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Lipoproteins

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Introduction

Most lipids are not soluble in aqueous environments. Therefore, organisms have developed an array of strategies for the transport of exogenous and endogenous lipids toward different tissues, where they are employed or stored. In all cases, this transport involves macromolecular protein-containing complexes: lipoproteins. This article will deal with the composition of these aggregates, their structures, and the changes suffered during metabolism and also with their effect on human health because of their great clinical importance. The first part of this article will mainly describe the plasma lipoproteins that play a major role in the transport of lipids in vertebrates, with special attention to human beings. Other lipoproteins and other lipid–protein complexes will be described in the last part of the article.

Plasma lipoproteins are synthesized mainly in the liver and intestines and circulate as soluble particles in body fluids of vertebrates. They are used to facilitate the delivery and clearance of lipids and lipophilic molecules throughout the body. These aggregates are in constant change of composition and physical structure as the peripheral tissues take up components before the remnants come back to the liver. In addition, there are interchanges of proteins, enzymes, and lipid constituents among the heterogeneous mix of all these lipoprotein fractions.

Plasma Lipoprotein Composition and Structure

The general structure of lipoprotein particles includes a hydrophobic core surrounded by a hydrophilic shell (Figure 1). The core comprises the hydrophobic components, mainly triacylglycerols and cholesterol esters, and the hydrophilic shell includes the hydrophilic components, mainly phospholipids (phosphatidylcholine and sphingomyelin especially), unesterified free cholesterol, and various proteins (apolipoproteins), which play a role in lipoprotein assembly and mediate interactions with a variety of other molecules including enzymes, transporters, and receptors through a dynamic process.

Lipoproteins can be classified according to their size, density, electrophoretic mobility, and composition or to the apolipoprotein or apolipoproteins that they contain. However, the practical procedure employed to separate the different lipoprotein classes (their density) is what has determined the commonly employed nomenclature. Thus, lipoproteins are classified as chylomicrons (CMs), very-low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs) depending on their relative densities on ultracentrifugation. In addition, these classes can also be divided into several subclasses. Thus, intermediate-density lipoproteins (IDLs) and subdivisions of the HDL (such as HDL_1, HDL_2, and HDL_3) are often defined. The density of these particles is a consequence of the relative concentrations of triacylglycerols and proteins and of their diameters. These particles are mostly spherical and diameters vary from about 6000 Å in CMs to 100 Å or less in the smallest HDL. Some compositional details are listed in Table 1.

Plasma Lipoprotein Classes

Chylomicrons

Triacylglycerols are the predominant fats in the diet, contributing to 90–95% of the total energy derived from dietary lipids. In the intestine, triacylglycerols are decomposed by pancreatic lipase into 2-monoacylglycerols and free fatty acids and absorbed from the intestinal lumen into the enterocyte. Both monoacylglycerols and free fatty acids are utilized to resynthesize triacylglycerols in the endoplasmic reticulum. The produced triacylglycerols are incorporated together with dietary cholesterol and a small amount of cholesterol esters into CMs with a surface layer of phospholipids and a single molecule of a truncated form of apoB (apoB-48), which is a characteristic of triacylglycerol-rich lipoproteins of intestinal origin. In addition, CMs also contain some apoA-I, which is synthesized either in the intestines (about 30% of circulating apoA-I) or in the liver (about 70% of circulating apoA-I).

Produced CMs are transported via the intestinal lymphatic system and enter the blood stream at the left subclavian vein. A schematic of the metabolism of CMs and other lipoproteins is shown in Figure 2. As soon as CMs reach circulation, apoA-I is transferred to HDL particles, and apoE and apoC-II are transferred from the HDLs to the surface of the CMs. Triacylglycerols are removed by the peripheral tissues by means of endothelial-bound lipoprotein lipase. The produced fatty acids enter into the muscle for energy production and into the adipocytes for storage. At the end, the depleted, or 'remnant' CMs, mainly containing the dietary cholesterol, apoB-48, and apoE (apoC-II can be transferred back to HDL), reach the liver where they are cleared from circulation by a receptor-mediated process that requires the presence of apoE.

Very-Low-Density Lipoproteins

Triacylglycerols of the remnant CMs, newly synthesized triacylglycerols from the free fatty acids that reached the liver from the adipose tissue, cholesterol, cholesterol esters, and phospholipids are secreted by the liver into the circulation in the form of VLDLs. These particles also contain one molecule of the full-length form of apoB (apoB-100). As occurred with CMs, if VLDLs contain some apoA-I, this apolipoprotein is rapidly transferred to the HDLs at the same time that VLDLs take apoC-II and apoE from HDLs.

Once again, triacylglycerols are hydrolyzed by lipoprotein lipase into free fatty acids and 2-monoacylglycerols. In

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00422-0

addition, this enzyme is also involved in the nonhydrolytic uptake of esters of cholesterol and retinol. As the transport of VLDLs progresses, the core of triacylglycerols is reduced and proteins and phospholipids on the surface are transferred away to the HDLs. VLDL remnants (or intermediate-density lipoproteins (IDLs)) with apoB-100 and apoE as the remaining proteins can be either absorbed by the liver or converted to LDLs with further loss of triacylglycerols.

Low-Density Lipoproteins

LDLs, and also IDLs, are stable particle configurations that are reached sequentially during the course of intravascular catabolism of VLDLs. The transformations from VLDLs to IDLs and LDLs are allowed by the presence of sufficient triacylglycerols to sustain lipolysis. Therefore, LDLs are poor in triacylglycerols (10% lipids vs. 55% lipids in VLDLs) and rich in cholesterol and cholesterol esters (65% lipids vs. 25% lipids in VLDLs). Consequently, LDLs are the main carriers of cholesterol to the adrenals and adipose tissue, where there are receptors requiring apoB-100 that are able to take in the LDLs. Within these tissues, the cholesterol esters are hydrolyzed to yield free cholesterol, which is incorporated into plasma membranes as required. Additionally, LDLs can be taken up by the liver by a process of endocytosis and catabolized. Nevertheless, the liver is able to scavenge CM remnants much more rapidly than LDL particles.

Cholesterol is of vital importance for the human body. It is a constituent for most biological membranes, it is needed for the formation of bile salts, and it is the precursor for steroid hormones and vitamin D. However, excess of cholesterol in the cells might be harmful. Thus, the accumulation of cholesterol in arteries can lead to atherosclerosis. Therefore, a route for cholesterol disposal, which has been termed 'reverse cholesterol transport,' is needed.

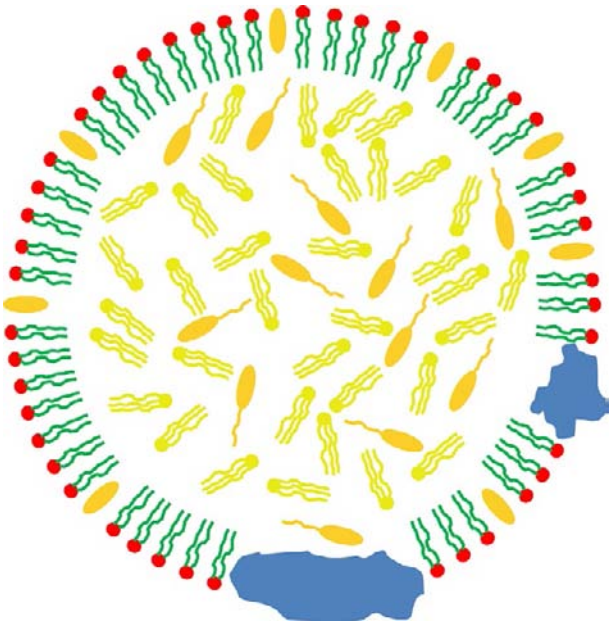

Figure 1 Lipoprotein structure. Lipoproteins usually have a spherical form with phospholipids, cholesterol, and apolipoproteins in the outer layer. The hydrophobic core comprises triacylglycerols and cholesterol esters. Courtesy of Francisco J. Hidalgo-Zamora.

High-Density Lipoproteins

The 'reverse cholesterol transport' is carried out by HDLs. HDLs are a complex group of diverse lipoproteins that can contain many different protein constituents and carry out diverse functions related to the return of cholesterol: secretion of cholesterol from the cells, esterification of cholesterol in the plasma, transfer of cholesterol to other lipoproteins, and return of cholesterol from the peripheral tissues to the liver for excretion in the bile. In addition, these lipoproteins also participate in triacylglycerol transport by facilitating the activation of lipoprotein lipase, in the transfer of triacylglycerols between lipoprotein classes, and in the removal of CM remnants and VLDLs enriched in triacylglycerols.

ApoA-I, either synthesized in the liver or spontaneously released by CMs, is the major protein component of HDL in the plasma and determines most of its functions. In addition to apoA-I, plasma HDLs also contain many other apolipoproteins, including apoC-II and apoE.

HDL biogenesis starts with the formation of the nascent discoidal HDL through apoA-I and ABCA1, a specific transporter molecule that facilitates the transfer of phospholipids and cholesterol to apoA-I. In the plasma, apoA-I activates the

Table 1 Density and average composition of plasma lipoproteins

	Plasma lipoprotein				
	CM	*VLDL*	*IDL*	*LDL*	*HDL*
Density	<0.95	0.95–1.006	1.006–1.019	1.019–1.063	1.063–1.21
Lipid (%)	99	91	85	80	44
Triacylglycerols	85	55	37	10	6
Cholesterol esters	3	18	28	50	40
Cholesterol	2	7	8	11	7
Phospholipids	8	20	27	29	46
Protein (%)	1	9	15	20	56
Main apolipoproteins	ApoB-48, apoA-I, apoC-II, apoE	ApoB-100, apoC-II, apoE	ApoB-100, apoC-II, apoE	ApoB-100	ApoA-I, apoC-II, apoD, apoE

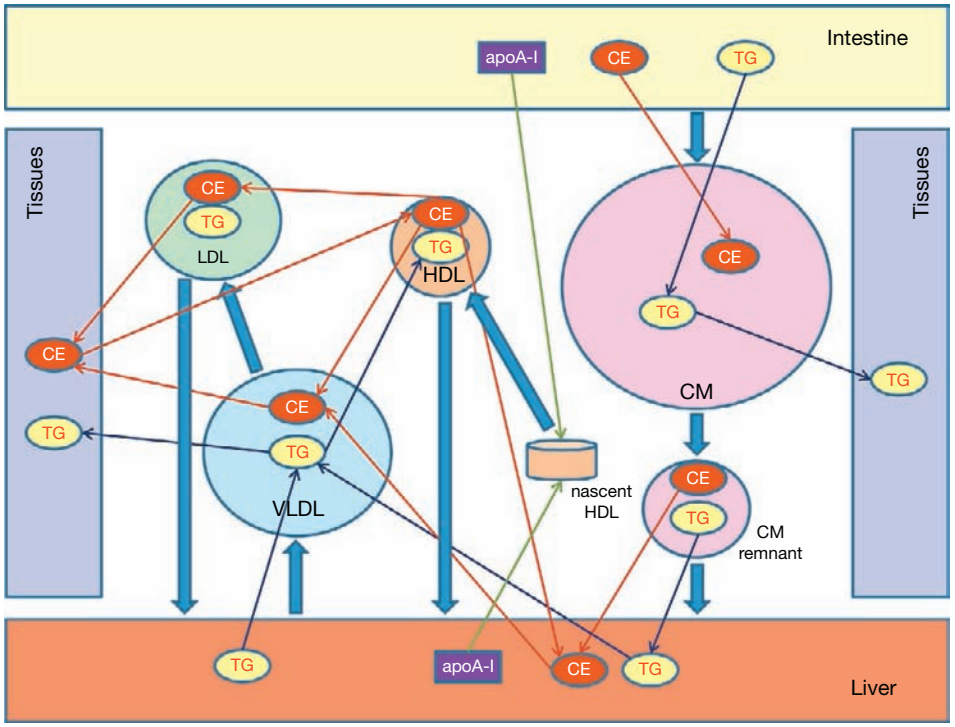

Figure 2 Schematic lipoprotein metabolism. Lipoproteins are in constant change of composition and physical structure as the peripheral tissues take up components before the remnants come back to the liver. In addition, there are interchanges of proteins, enzymes, and lipid constituents among the heterogeneous mix of all these lipoprotein fractions. Only interchanges of triacylglycerols (TGs) and cholesterol/cholesterol esters (CEs) are shown.

enzyme lecithin–cholesterol acyltransferase, which converts discoidal HDL to mature, spherical, cholesteryl ester-rich HDL particles by drawing free cholesterol and phospholipids from IDLs and LDLs until spherical HDL particles are formed with a surface coat of phospholipid, free cholesterol, and apolipoproteins.

Cholesterol is incorporated from cell surface membranes to the spherical HDLs. As a result, the levels of intracellular cholesterol are reduced because the cholesterol stored in the cells in the form of cholesterol esters is mobilized to replace that removed from the plasma membrane. The removal of excess cholesterol from macrophage-derived foam cells present in atherosclerotic plaques is particularly important.

Lipid-rich HDLs can enter the hepatocytes through an apoA-I receptor, where it can transfer cholesterol and cholesterol esters to distinct pools within the cell. The modified HDLs are then secreted back into the circulation where they can acquire further cholesterol before returning to the liver. In a second less-efficient pathway, HDLs can be taken up by the liver analogously to the earlier described for LDLs.

Plasma Lipoproteins and Health

Epidemiological and clinical studies and meta-analyses have shown a relationship between low plasma levels of HDLs and increased risk of major coronary events. In addition, observational studies have found inverse associations between HDLs and risks of ischemic stroke, type 2 diabetes mellitus, and various cancers. Furthermore, HDLs seem to exert many effects *in vitro* and *in vivo* that protect the organism from chemical and biological harm and may interfere with the pathogenesis of diverse diseases. Moreover, in several animal models, overexpression or exogenous application of apoA-I decreased or prevented the development of atherosclerosis, glucose intolerance, or tissue damage induced by ischemia or mechanical injury. This section will review some of the present knowledge of an area that is, at present, under active investigation.

Coronary Heart Disease

Alterations of lipid/lipoprotein metabolism and inflammatory events contribute to the formation of the atherosclerotic plaque, which is characterized by the accumulation of abnormal amounts of cholesterol and macrophages in the artery wall. Therefore, the decrease of cholesterol level by means of the reverse cholesterol transport described in the preceding text may counteract the events that produce the formation and development of atheroma. In fact, *in vivo* studies have shown an inverse relationship between the efficiency of the reverse cholesterol transport and atherosclerotic cardiovascular diseases. HDLs are key lipoproteins in all stages of reverse cholesterol transport: They remove excess cholesterol from cells and they release cholesterol to the liver for final disposal. Therefore, the assessment of HDL function, evaluated as their capacity of promoting cell cholesterol efflux, is considered a strong predictor of atherosclerotic extent in humans and may represent a useful biomarker of predictor of cardiovascular risk.

Type 2 Diabetes Mellitus and Metabolic Syndrome

The cluster of pathologies that constitutes metabolic syndrome (insulin resistance, central obesity, high blood pressure, and hypertriglyceridemia) and type 2 diabetes mellitus is associated with low levels of HDL cholesterol and the presence of dysfunctional HDLs. Recent evidences suggest that HDL modulates insulin sensitivity, insulin-independent glucose uptake, insulin secretion, and beta-cell survival. Thus, HDLs are believed to enhance glucose-stimulated insulin secretion and to reverse the proapoptotic effects of native and oxidized LDLs. In addition, HDL and apoA-I promote glucose uptake and activate AMP-kinase in primary skeletal muscle cells and differentiated adipocytes by an insulin-independent way and also enhance oxidative metabolism in the skeletal muscle. A dysfunction in these actions could play a role in the pathogenesis of these diseases.

Acute and Chronic Inflammatory Pathophysiological Conditions

HDLs have been shown to play a protective and anti-inflammatory role in autoimmune and inflammatory diseases. HDLs are believed to be part of both innate and adaptive immune systems, helping mammals to fight against invading pathogens. HDL activities rely not only on the ability to modulate cholesterol availability in immune cells (the activity of these cells is modulated upon changes in the lipid raft composition and structure) but also on the role of specific molecules shuttled by HDLs. Nevertheless, both acute and chronic inflammatory conditions induce posttranslational modifications of HDL, which impairs both its lipid homeostasis-regulating and anti-inflammatory properties, and can even turn HDLs into proinflammatory molecules.

Infectious Diseases and Sepsis

Significant alterations in lipid metabolism and lipoprotein composition occur during infection. Triacylglycerols and VLDL cholesterol levels increase, while reduced HDL cholesterol and LDL cholesterol levels are observed. Low HDL cholesterol levels inversely correlate with the severity of septic disease and associate with an exaggerated systemic inflammatory response. In addition, HDL particles undergo changes that dramatically alter their composition and function. On the contrary, an increase of HDL cholesterol is associated with an attenuation of gram-negative bacterial lipopolysaccharide-induced inflammatory responses.

Neurodegenerative Disorders

Cognition is a complex brain function that includes all aspects of perception, thought, and memory. This function often declines during aging and neurodegenerative disorders appear, including Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis. Emerging evidence suggests that HDLs may play an important role in preserving cognitive function under normal and pathological conditions. However, the mechanism of action for HDLs to modulate cognitive function remains to be elucidated.

Other Lipoproteins

In addition to the previously described lipoproteins, other lipoproteins are also known. Some of them are described in this section.

Insect Lipoproteins

The circulatory fluid in insects (hemolymph) contains a single multifunctional lipoprotein that transports lipids to wherever they are required. This lipoprotein is termed lipophorin. Lipophorins can be classified as low-density, high-density, and very-high-density lipophorins, depending on their lipid loading. The main lipid components are 1,2-diacylglycerols together with hydrocarbons, phospholipids, and sterols. It has also two apolipoproteins: apolipoprotein I and apolipoprotein II. Lipophorin functions as a reusable lipid shuttle, which delivers lipids to storage or peripheral tissues before returning for another cycle of loading and unloading.

Lipoproteins in Other Invertebrates

Lipoproteins have been studied in insects with preference to other invertebrates because of the high lipid mobilization rates during flight. However, these complexes also exist in these animals and some differences appear. Thus, for example, in crustaceans, the predominant lipid species in lipoproteins are phospholipids (especially phosphatidylcholine) and, to a lesser extent, diacylglycerols. On the other hand, in mollusks, the predominating lipids are also phospholipids and to a lesser extent sterols and triacylglycerols.

Bacterial Lipoproteins

Bacterial lipoproteins are a set of membrane proteins that have been modified through the covalent attachment of a diacylglycerol moiety to the thiol group on the side chain of a cysteine residue. This modification is catalyzed by the enzyme lipoprotein diacylglyceryl transferase, and the diacylglycerol is linked by a thioether bond to the protein. Additionally, the amino group of the terminal cysteine residue can also be esterified producing a triacyl derivative. Therefore, different from the other lipoproteins discussed in this article, lipid and protein are covalently bonded in bacterial lipoproteins. This modification facilitates the anchoring of hydrophilic proteins to the cell wall phospholipids by hydrophobic interaction among all acyl groups. Bacterial lipoproteins have been shown to perform various roles, including nutrient uptake, signal transduction, adhesion, conjugation, and sporulation, and participate in antibiotic resistance, transport, and extracytoplasmic folding of proteins.

Egg Yolk Lipoproteins

Egg yolk is a complex system that can be fractionated into two: plasma and granules. Granules represent 19–23% of yolk dry matter, accounting for about 50% of yolk proteins and less than 10% of yolk lipids. They are mainly constituted by HDLs (70%) and phosvitin (16%) linked by phosphocalcic bridges.

Plasma corresponds to about 77–81% of yolk dry matter and is composed of 85% LDLs and 15% livetins. It accounts for about 90% of yolk lipids (including nearly all the carotenoids) and 50% of yolk proteins.

Egg yolk LDLs are spherical nanoparticles (17–60 nm) with a lipid core of triacylglycerols and cholesterol esters surrounded by a monofilm of phospholipids and proteins. Some cholesterol is also included in the phospholipid layer, increasing its rigidity. LDLs are composed of 11–17% protein and 83–89% lipid, of which 69% are triacylglycerols, 27% phospholipids, and 4% cholesterol and cholesterol esters. LDL particles comprise at least six different apolipoproteins, which have about 40% hydrophobic amino acid residues.

Lipid–Protein Complexes Other than Lipoproteins

The existence of lipid–protein complexes other than lipoproteins with important biological functions has also been widely described. Some few examples are discussed in this section.

Lipid–Protein Complexes in Cell Membranes

Different studies have shown that the folding, structure, and function of membrane proteins are influenced by their lipid environments and that lipids can bind to specific sites. In fact, resistance to membrane protein unfolding correlates with specific-binding events, enabling to distinguish between lipids that merely bind from those that modulate membrane protein structure and/or function.

Fatty Acid Transport Proteins

Because of their low solubility in aqueous solutions such as blood plasma and interstitial fluid, fatty acids need binding proteins to increase their concentration in vascular and interstitial compartments. Albumin acts as the main fatty acid-binding protein in extracellular fluids. Plasma albumin possesses about seven binding sites for fatty acids with moderate to high affinity. On the other hand, cellular fatty acid uptake is carried out by several protein groups including plasma membrane fatty acid-binding protein, the scavenger receptor CD36, and the six fatty acid transport proteins, also called carrier family 27A1-6.

Fat Globules in Milk

Although the fat content of mammalian milks varies enormously, all species, including humans, secrete milk lipids in similar, highly specialized fat globules that are unique to milk. These globules are composed of a central core containing mostly triacylglycerols surrounded by three layers: an inner monolayer of proteins and polar lipids with the fatty acid tails facing the globule core and a bilayer of polar lipids, proteins, glycoproteins, and cholesterol. In addition, there is an entrained layer of cytoplasm about 10–20 nm wide between the inner single membrane and outer bilayer. This membrane is only found in milk and functions to stabilize the milk fat globule as an emulsion in the aqueous milk environment. More importantly, it provides important components that have nutritional and immunologic roles for the infant and the intestinal microbiome, with growing evidence that the lipids are packaged in such a way to contribute to digestive efficiency and preserve biological activity.

The Complex HAMLET (Human α-Lactalbumin Made Lethal to Tumor Cells) in Human Milk

The protein–lipid complex HAMLET was purified from the casein fraction of human milk and was found to consist of α-lactalbumin in a partially unfolded conformation that is stabilized under physiological conditions by the oleic and linoleic acids contained in the human milk. This protein–lipid complex has a potent and specific bactericidal effect against the respiratory tract pathogen *Streptococcus pneumoniae*. In addition, sublethal concentrations of HAMLET potentiate the effect of common antibiotics (penicillins, macrolides, and aminoglycosides) against pneumococci.

Lipid Droplets in Cells

Lipid droplets, also termed lipid bodies or adiposomes, are lipid-rich organelles present in virtually all organisms, from bacteria to mammals. They are characterized by their unique, highly conserved, and simple architecture. It consists of a neutral lipid core containing triacylglycerols and sterol esters encased within a monolayer of phospholipids, which is in turn associated with a unique proteome. This proteome is highly specialized and contains from 40 to 300 different proteins depending on the species. Lipid droplets are involved in a myriad of cellular processes, which are at present under active investigation. Thus, for example, lipid droplets in leukocytes have been related to cell signaling and activation, regulation of lipid metabolism, membrane trafficking, and control of the synthesis and secretion of inflammatory mediators.

Lipoproteins as Additives

Emulsifying Properties of Egg Yolk LDLs

The emulsifying properties of egg yolk are mainly attributed to its LDLs because LDLs form a film at the interface between oil and water, and this film contributes to the stabilization of emulsions made with yolk. The structure of the LDLs seems essential to ensure their interfacial properties because LDLs serve as a vector of surfactants, like proteins and phospholipids, up to the interface. Once at the interface, the LDL particle spreads and the proteins and phospholipids are absorb at the interface. The produced interfacial film assures both the decrease of interfacial tension and the resistance to the rupture and permits the formation and the stability of food emulsions made with yolk.

Cryoprotective Effect of Egg Yolk LDLs in Extenders on Bull Spermatozoa

Semen extenders are liquid diluents added to semen to preserve its fertilizing ability. Egg yolk LDLs have been proposed to be used in extenders with cryoprotective effects on frozen-thawed bull spermatozoa. The addition of egg yolk LDL has

been shown to produce better results (higher sperm motility and better movement characteristics) than the addition of egg yolk. The cryoprotective effect of egg yolk LDLs seems to be related to the ability of the lipoproteins to bind the binder of sperm proteins contained in the seminal plasma of many mammalian ungulates. These proteins modify the sperm membrane by removing cholesterol and phospholipids, which adversely affect the ability of sperm to be preserved. The binding between these proteins and LDL is rapid, specific, and stable even after freeze-thawing of semen.

Functional and Nutritive Properties of Milk Fat Globule Membrane

Because of their amphiphilic nature and original function in stabilizing the fat globules in whole milk, milk fat globule membrane fragments are considered to be efficient, natural emulsifiers. In addition, they have been used to produce liposomes. The obtained liposomes are more stable than analogous liposomes obtained from soya lecithins in a range of pH conditions, at a variety of storage and processing temperatures, and in the presence of mono- and divalent cations. Furthermore, milk fat globule membrane includes many components with attractive properties for the health, including sphingolipids, proteins with bioactive properties, and antigens.

See also: Buffalo Milk; Cholesterol: Absorption, Function and Metabolism; Cholesterol: Factors Determining Blood Cholesterol Levels; Cholesterol: Properties, Processing Effects, and Determination; Condensed Milk; Eggs: Composition and Health Effects; Eggs: Use in the Food Industry; Goat: Milk; Human Milk: Composition and Nutritional Value; Milk Powder; Milk: Processing of Milk; Milk: Role in the Diet; Milk: Sources and Composition; Phospholipids: Physiology; Phospholipids: Properties and Occurrence; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Proteins: Chemistry, Characterization, and Quality; Sheep: Milk; Triacylglycerols: Characterization and Determination; Triacylglycerols: Structures and Properties.

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Liqueurs: Cream Liqueurs

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Liqueurs

A liqueur is an alcoholic beverage made from a distilled spirit that has been flavored with fruit, cream, herbs, spices, flowers, or nuts and bottled with added sugar or other sweeteners (such as high-fructose corn syrup). Liqueurs are typically quite sweet; they are usually not aged for long after the ingredients are mixed, but may have resting periods during their production to allow flavors to marry. A typical production process involves slicing and steeping plants in alcohol and allowing the resulting extract to mature, yielding alcoholic beverages. Other liqueurs are fermented from grains such as rice, millet, and wheat; anthograin liqueurs, for example, are produced through fermentation of anthograins such as purple wheat.

Liqueurs were historically herbal medicines; they were made in Italy as early as the thirteenth century and were often prepared by monks. Nowadays, liqueurs are made worldwide and are served in many ways: by themselves, poured over ice, served with coffee, or mixed with cream or other mixers to create cocktails. They are often served with or after a dessert and are also used in cooking. Examples of liqueurs include berry liqueurs, chocolate liqueurs, coffee liqueurs, cream liqueurs, crème liqueurs, flower liqueurs, fruit liqueurs, herbal liqueurs, anise-flavored liqueurs, honey liqueurs, nut-flavored liqueurs, and whiskey liqueurs (list extracted from en.wikipedia.org/wiki/List_of_liqueurs).

Fruit liqueurs are alcoholic beverages, the main natural components of which are fruits or fruit extracts. In their preparation, the whole fruit is used, and it needs to be ripe in order to give the maximum flavor to the liqueur. Fruit liqueurs are prepared without fermentation. The manufacturing process may involve alcoholic maceration of fruits or distillation of macerates aromatized with the base fruit. Other techniques are also used, such as alcoholic dissolutions of different fruit aroma compounds; these beverages are usually colored with glucose or sucrose caramel, and they are highly sweetened with grape sugar, honey, glucose, and/or sucrose syrups. Acidity regulators and, occasionally, stabilizers, antioxidants, and other technological aids may also be added. Fruits used for liqueurs are known for their beneficial impact on human health because they are a good source of phenolic compounds endowed with antioxidant activity.

Walnut is a traditionally important and widely spread deciduous tree in many parts of the world. Its ripe fruit has been used for fresh consumption and in confectionery, while unripe fruit can be used to make liqueurs. Walnut liqueur is a dark-brown, bitter, and tasteful beverage and is often served as an aperitif or sometimes taken to treat stomachache. Nocino, a similar after-dinner walnut liqueur of Celtic origin, is very well known and appreciated in Italy and other countries. Walnut fruits and many walnut products, such as walnut liqueur, are rich in phenolic compounds.

Limoncello is a very popular homemade Italian liqueur, traditionally based on an infusion of the flavedo (the outer, yellow skin of lemons). Honey liqueur is an alcoholic drink derived from honey and strong fruit brandy, traditionally made in Serbia and other Balkan countries. Tobacco plants have also been used as a base for making liqueurs.

One particular type of liqueur, first described in an ancient Scottish recipe, is a cream-based alcoholic beverage prepared by steeping oatmeal in whiskey overnight and filtering the following morning. The oatmeal imparted a delicious nutty flavor to the whiskey. Cream and honey were added to the filtered whiskey and mixed well to give Atholl Brose. The modern cream liqueur was first marketed in the United Kingdom in 1974 as Baileys Original Irish Cream Liqueur and is now sold worldwide. The remainder of this article will focus on the manufacture and properties of cream liqueurs.

Composition of Cream Liqueurs

The leading commercial brands of cream liqueurs are reported to contain around 39–40% (w/w) solids, including 15–16% (w/w) butterfat, 3% (w/w) sodium caseinate, 19% (w/w) added sugar, and 1.5% (w/w) milk solids nonfat. The alcohol content is usually 12.7% (w/w) (or 17% v/v). Commercial cream liqueurs may contain small proportions of low-molecular-weight (LMW) surfactants such as glycerol monostearate (GMS). Aspects of the composition, processing, and quality characterization of cream liqueurs were first described in detail in the scientific literature by Banks, Muir, and colleagues in the 1980s.

Cream and milk components

Double cream (48% fat) is normally used for the manufacture of cream liqueurs. Cream is an o/w emulsion comprising a dispersion of fat globules in a continuous phase containing proteins (caseins and whey proteins), lactose, mineral salts, vitamins, and enzymes (milk serum phase).

Milk fat globules are coated by a natural membrane termed the milk fat globule membrane, consisting of amphiphilic molecules with both hydrophilic and hydrophobic sections. Milk fat globules vary in diameter from about 0.2 to 15 μm, with a mean diameter of ~4 μm, and are characterized by a wide size distribution. The density of milk fat at 20 °C is 918 kg m^{-3}, and milk fat is considered to be fully liquid at 40 °C.

There are two categories of proteins in milk: caseins, which are insoluble at pH 4.6 (their isoelectric point) at 20 °C, and whey proteins, which are soluble under these conditions. The caseins, which comprise 80% (w/w) of the milk proteins, exist in a unique colloidal complex known as the casein micelle. The whey proteins, which comprise 20% (w/w) of the milk proteins, exist in their native state as compact, globular proteins that are highly susceptible to heat-induced denaturation and irreversible polymerization via intermolecular disulfide bond formation.

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Lactose is the major carbohydrate in milk. Bovine milk contains ~4.5–5% (w/w) lactose, and it is present in cream liqueurs at a concentration of ~1% (w/w). Lactose is a disaccharide of glucose and galactose. Heating lactose in the presence of amino groups results in the Maillard reaction, which involves the reaction of the lactose with the amino group of proteins. In milk, amino groups are mainly lysine residues; heating milk results in a brown color and a cooked flavor, which are important characteristics of many heated dairy products.

Use of sodium caseinate and other emulsifiers

The first step in the isolation of the casein fraction from milk is the removal of the fat by centrifugation to yield skim milk. Following destabilization of the casein by adjusting the pH to 4.6, the insoluble casein is separated from the soluble whey proteins, lactose, and salts and is washed and dried. Sodium caseinate, the water-soluble form of casein most commonly used in food products, is usually prepared by solubilizing acid casein with NaOH.

In the manufacture of cream liqueurs, the milk protein in the cream is usually complemented with sodium caseinate. Sodium caseinate is regarded as a partially aggregated mixture of four individual caseins, α_{s1}-, α_{s2}-, β-, and κ-casein, in an approximate ratio of 4:1:4:1. Proteins make good emulsifiers by virtue of their amphipathic nature and ability to spread at interfaces. Sodium caseinate is the preferred emulsifier for cream liqueur manufacture because of its excellent functional and organoleptic properties. In addition to stabilizing the fat droplets, sodium caseinate also provides a significant improvement in the perceived creaminess and allows the spirit source to impart its characteristic organoleptic properties. No other protein has been found to equal the functional and organoleptic properties of sodium caseinate in cream liqueurs.

When mixed with carbonated or citrus drinks, precipitation may occur in cream liqueurs containing sodium caseinate if the isoelectric point is reached. Calcium in cream liqueurs comes mainly from the cream source, and, even though the calcium concentration in a cream liqueur is only ~16% of that found in milk, this may be sufficient to cause calcium-induced aggregation, with eventual gelation of the product. Calcium-induced aggregation is accelerated when cream liqueurs are stored at high temperatures.

The addition of calcium-sequestering agents such as trisodium citrate to prevent calcium-induced aggregation of cream liqueurs increases the shelf life of these products. The effects of removing calcium (e.g., by the use of anhydrous butter oil or washed cream) and adding citrate on the stability of cream liqueurs have been studied and have been found to have a significant positive influence on shelf life at 45 °C. As washing cream is an expensive process, most manufacturers add trisodium citrate to cream liqueurs to chelate ionic calcium.

In addition to a protein emulsifier, low-molecular-weight (LMW) surfactants such as GMS and sodium stearoyl lactylate (SSL) can be used in cream liqueurs. Studies have been conducted on the effect of LMW surfactants on the stability of cream liqueurs. The presence of glycerol monostearate (GMS) or SSL substantially reduces creaming at ambient temperature and prolongs shelf life. The improved creaming stability appears to be associated with a change in bulk rheological properties of the emulsion, probably arising from complex formation between LMW surfactants and caseinate, both in the bulk aqueous phase of the emulsion and at the surface of the fat droplets.

The role of sugar

The sugar in a cream liqueur can be in the form of sucrose, maltose, dextrose, lactose, corn syrup, invert sugar, high-fructose corn syrup, sorbitol, or glucose. Sucrose is, however, normally used as the sugar source for cream liqueurs. Sucrose, which is a disaccharide of fructose and glucose, primarily functions as a sweetener and bacteriostatic agent while also contributing to viscosity. For ease of blending, sucrose is usually introduced in the form of an aqueous syrup. Alternative sweeteners may be used without changing the color, smell, or viscosity, although the perception of alcohol flavor may be enhanced due to the fact that sucrose, through its sweetness, is very effective at masking this perception.

Ethanol

Most cream liqueurs contain neutral spirit along with spirit from other sources (e.g., whiskey). Spirit with a neutral pH is necessary if sodium caseinate is used as an emulsifier. At concentrations up to 20% (w/w), the presence of ethanol during emulsification leads to fat globules with smaller diameters due to a lowering of interfacial tension. However, at higher ethanol concentrations, the stability of the liqueur decreases due to poor solvent quality, which induces protein aggregation and precipitation. Cream liqueurs with higher alcohol contents may be manufactured by washing the cream to remove serum phase components and proceeding with the normal manufacturing process.

Manufacture of Cream Liqueurs

Conventional processes for the manufacture of a stable cream liqueur involve the preparation of a caseinate–trisodium citrate blend at 85 °C (with or without sugar). This is followed by the addition of cream with continuous high-speed mixing at 55 °C to give a cream base. LMW surfactants are usually incorporated in molten form into the cream base. A major source of variation in the processing of cream liqueur is the stage of alcohol addition.

Figure 1 outlines a typical cream liqueur manufacturing process. In a one-step manufacturing process, alcohol is added to the cream base before the product is homogenized. In this case, an aqueous-alcohol solution (with or without sugar) is added to the cream base and mixed thoroughly. Colors and flavors are usually incorporated in the alcohol. The blended pre-emulsion is normally homogenized twice at 55 °C at a pressure in the range of 20–30 MPa, cooled to 20 °C, and bottled in opaque bottles to prevent light-induced oxidation.

In two-step manufacturing processes, a concentrated alcohol-free cream base is first homogenized; this homogenized mix is then cooled and an alcohol solution is added. The alcohol can also be added as a solution with the sugar. A combination of one-step and two-step processes is also possible, whereby the non-alcohol base is first homogenized, the alcohol is then added, and the product is rehomogenized. The addition of alcohol to a cream base before homogenization

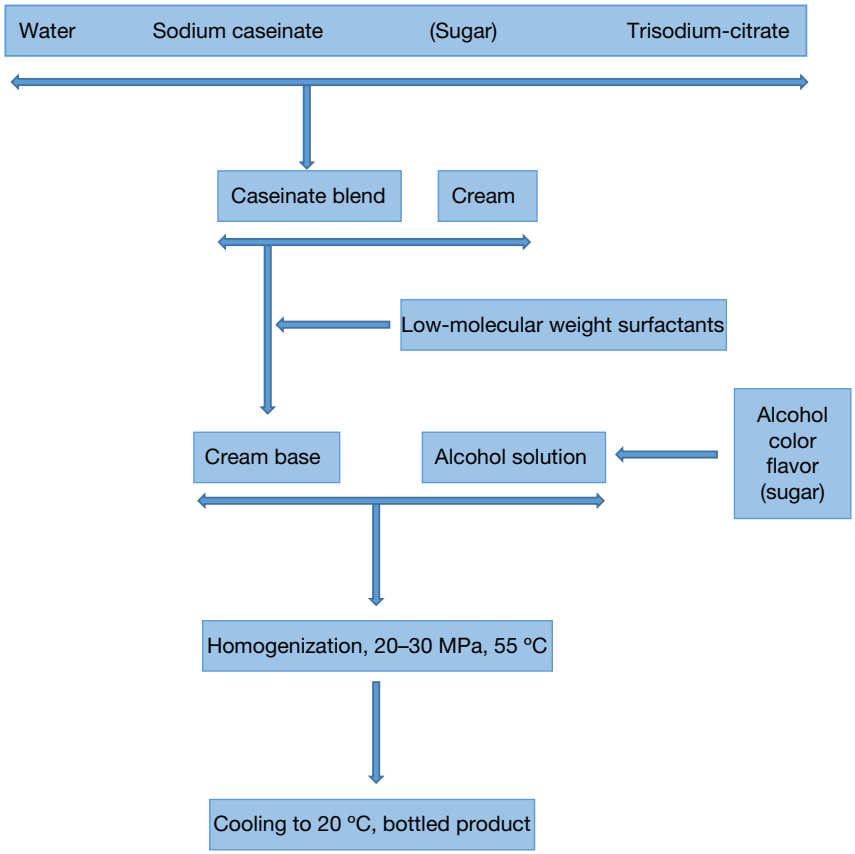

Figure 1 Schematic diagram illustrating a one-step cream liqueur manufacturing process.

results in a cream liqueur with fat droplets of smaller diameters, due to reduced viscosity and interfacial tension during homogenization; the resulting liqueurs are reported to have an increased shelf life.

Homogenization is the most important step in the manufacture of cream liqueurs and is necessary to produce a finely dispersed emulsion with long-term physical stability. During homogenization, the size of the fat globules is decreased and sodium caseinate transfers from the serum phase to the newly exposed fat globule surface to stabilize the product. Cream liqueur manufacturers employ a high protein/fat ratio (~0.19–0.21) in their products to ensure efficient coverage of the fat droplets and to ensure that small droplets are formed during homogenization. The effectiveness of homogenization in reducing fat globule size depends on feed temperature and homogenization pressure; higher homogenization pressures and feed temperatures used in processing are claimed to result in more stable products.

Novel technologies for the manufacture of cream liqueurs

Homogenization technologies for dairy products in general have evolved in recent years, driven in part by demands for longer shelf life products with better stability. Progress in high-pressure technology has led to new opportunities for alternative homogenization technologies for cream liqueurs; these were studied in detail by Heffernan and colleagues at the University College Cork in Ireland (the authors of this article). For example, cream liqueurs with finely dispersed droplets were produced on single-pass homogenization using a high-pressure homogenizer. Standard radial diffuser homogenizer requires multiple homogenization passes to produce a stable cream liqueur. In addition, high-pressure homogenization markedly increased cream liqueur temperature, due to pressure buildup and shear effects associated with the conversion of kinetic energy into heat. Cream liqueurs with small mean fat droplet diameters, homogenized using the high-pressure homogenizer, displayed a slower rate of increase in droplet diameter and in viscosity during storage at 45°C than those produced using a low-pressure homogenization process. Figure 2 illustrates the volume frequency distribution of fat droplets in cream liqueurs homogenized in a high-pressure homogenizer and in a standard homogenizer.

A number of high-pressure homogenizers, including a counter-jet-disperser (Microfluidizer) homogenizer, an orifice nozzle homogenizer, a radial diffuser homogenizer, and an ultrasonic system, have been evaluated and compared in terms of their efficiency in model cream liqueur emulsification. The jet-disperser (Microfluidizer) homogenizer produced cream liqueurs with fat droplets of the smallest diameters, while homogenization by an orifice nozzle or a radial diffuser homogenizer produced cream liqueurs with very wide volume frequency distributions of fat droplet diameters. For all high-pressure homogenization processes, cream liqueurs with fat droplets of smaller mean droplet diameters were produced at higher pressures. Lower fat droplet diameters resulted in cream liqueurs with improved storage stability. Ultrasonic

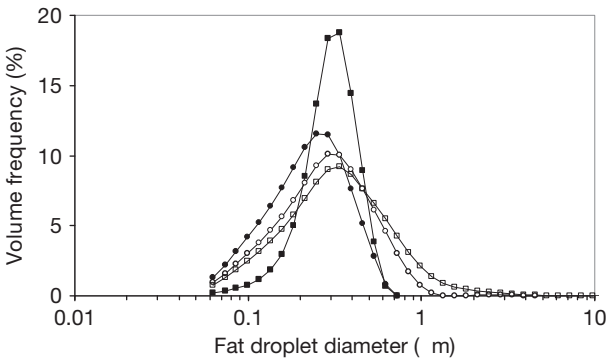

Figure 2 Volume frequency distributions of fat droplet diameters in model cream liqueurs: homogenized once at 20 MPa in a high-pressure homogenizer (○) and in a standard homogenizer (□); homogenized for five passes at 20 MPa in a high-pressure homogenizer (●) and in a standard homogenizer (▪). Adapted from Heffernan, S.P., Kelly, A.L., and Mulvihill, D.M. (2009). High-pressure-homogenized cream liqueurs: emulsification and stabilization efficiency. *Journal of Food Engineering* **95**, 525–531.

homogenization produced a cream liqueur with finely dispersed fat droplets; however, problems during shelf life appeared for highly processed samples.

Defects of cream liqueurs

Cream liqueurs are bacteriostatic; that is, they contain sufficient alcohol and sugar to inhibit bacterial growth. Thus, physical instability is the main defect during storage of cream liqueurs.

Age thickening–age gelation

Age thickening or gelation is one of the most serious problems affecting cream liqueurs and other long-life emulsions. Gelation is a problem in countries with high ambient temperatures. Liqueurs in which skim-milk powder (SMP) was used as an emulsifier were particularly prone to age gelation and exhibited aggregation after a short period of storage at ambient temperature. Flocculation in these systems can lead to full gelation, often followed by syneresis. In the early stages of gelation, the liqueur appears thick and aggregated; this process rapidly progresses to gelation, with subsequent syneresis producing a clear liquid at the bottom. It has been proposed that age gelation is due to calcium-induced aggregation, where the ionic calcium from the cream interacts with the protein and forms a three-dimensional network that gels and syneresis. Replacement of the SMP by sodium caseinate leads to a significant increase in liqueur shelf life. At ambient temperature, the shelf life of cream liqueurs stabilized with sodium caseinate can be increased from months to years by the inclusion of trisodium citrate in the formulation.

Neck plugs

Inefficient homogenization conditions can result in the formation of a distinct creamed layer at the top of the cream liqueur, but this is usually readily dispersed by shaking. On occasion, neck plugs, which are semisolid lumps of fat, 2–3 cm in thickness, occur in the tops of bottles. These structures, which are not readily dispersed by shaking, are considered to be inverted o/w emulsions. Inadequate homogenization (e.g., due to valve wear) and insufficient emulsifier level are the causes of neck plug in cream liqueurs. It has also been reported that there may be a link between neck plugging and excessively severe homogenization. Neck plugs are more likely to occur in cream liqueurs of low pH and high calcium content and in liqueurs that were inadequately homogenized and subject to temperature fluctuations and agitation during storage. Control of pH during the manufacture of cream liqueurs is one of the most important parameters influencing stability.

Granular precipitation

A crystalline precipitate often forms on prolonged storage of cream liqueurs, usually composed of calcium and citrate. Storage at high temperature accelerates the formation of the deposit, corresponding to the decreased solubility of calcium citrate at high temperatures. Lowering the level of trisodium citrate and adding it as late as possible in the manufacturing process were reported to reduce the problem.

Free-fat flecks

Another common observation in cream liqueurs is the presence of a small number of flecks at the surface of the liqueur. These are thought to be composed of protein or protein plus fat and may aggregate at the surface of the liqueur, forming an unappealing ring in the neck of the bottle. This type of instability does not pose a major problem because it is readily reversible by shaking. The presence of free fat may arise in cream liqueurs that are stored at sufficiently high temperatures. Droplets of free oil and flecks that form at the surface of the liqueur may not be noticed in a sufficiently opaque container.

Appearance and color

As discussed in the preceding text, droplet size is one of the most important factors that influence the stability of cream liqueurs, with the aim of emulsification of products, including cream liqueurs, being to produce droplets that are as small as possible, in order to ensure a long shelf life. However, this may have repercussions in terms of the sensory properties, as the droplet size distribution influences the color of emulsions. Cream liqueur lightness decreases with decreasing mean fat droplet diameter (0.40–0.08 μm) and decreasing fat droplet concentration (12.5–6.3%, w/w). Table 1 outlines the effect of mean fat droplet diameter on the instrumental color values and visual appearance of model cream liqueurs.

Table 1 Influence of mean fat droplet diameter ($d_{3,2}$, as measured using a Coulter LS) on the L^* and C values and on the visual appearance of model cream liqueurs

Homogenizer	*Pressure (MPa)*	$d_{3,2Coulter}$ *(μm)*	L* *value*	C *value*	*Visual appearance*
Pre-emulsion		3.340 ± 0.100	50.3 ± 1.90	29.5 ± 1.45	
APV homogenizer	20	0.404 ± 0.400	63.3 ± 1.20	25.1 ± 0.58	
Microfluidizer®	20	0.094 ± 0.002	55.1 ± 1.50	16.4 ± 0.46	
Microfluidizer®	100	0.079 ± 0.002	41.4 ± 0.50	9.6	

Adapted from Heffernan, S. P. (2009). Development of novel process emulsification technology for the manufacture of cream liqueur. PhD Thesis, National University of Ireland, Cork.

Characterization and Testing of Cream Liqueurs

Freshly produced cream liqueurs should be characterized to establish a baseline against which to measure subsequent long-term stability. Important parameters include viscosity and droplet size distribution.

Viscosity

The viscosity of a fluid emulsion is a very important characteristic, since it influences such aspects as the rate of creaming, the physical shelf life of the product, and the organoleptic properties of the product. Viscosity is defined as the ratio of the shear stress to the shear rate in liquids undergoing laminar flow or, simply, the resistance to flow. Emulsions with flocculated droplets have higher viscosities than those containing the same concentration of unflocculated droplets, because the effective volume of the flocculated droplets is greater than the effective volume of the unflocculated droplets, due to the presence of continuous phase trapped within the flocs.

Droplet size distribution

One of the most important characteristics of an emulsion is its droplet size distribution, since subsequent stability and other properties such as emulsifying characteristics of ingredients are influenced by this variable. Various methods are available for the characterization of the droplet size distribution of dairy emulsions; these include microscopic observation, light scattering methods, ultrasound spectrometry, electrical conductivity, and nuclear magnetic resonance (NMR). The most commonly used technique for the characterization of dairy emulsions, including cream liqueurs, is laser light scattering. Using laser diffraction analyzers (e.g., Coulter LS, Malvern Mastersizer), the shape of the particle size distribution is determined after the measurement of scattering of laser light by a diluted emulsion at different diffraction angles; up to 100 detectors capture data from various angles, and these are processed under a suitable model such as the Mie theory of interaction of light with matter.

Shelf life testing

The amount of visible creaming is usually measured after a given time and expressed as a percentage of the original volume (height of the emulsion). Centrifugal methods may be applied to speed up creaming; however, acceleration of creaming may result in incorrect assumptions as it may not reflect the complexity of changes occurring during the storage period. Various authors have measured the type and amount of creaming in cream liqueurs by incubating samples at elevated temperatures.

Viscosity of cream liqueurs increases over time, and, thus, viscosity can be used as one important index of shelf life; eventually, excessively high viscosity will become a limiting factor to consumer acceptability. Accelerated shelf life testing of cream liqueurs is typically carried out at 30 °C and 45 °C and typically involves measurement of changes in viscosity as an index of physical stability.

Sensory aspects

The flavor of cream liqueurs involves a complex interaction of volatile and nonvolatile favor compounds. The core background flavor originates from the liqueur components such as cream, cocoa, vanilla, and whiskey. The polarity of these flavor compounds determines how they partition in the continuous phase of the cream liqueur emulsion, within the fat globule, or across the globule–aqueous phase interface. Flavor partitioning affects flavor release and ultimately the flavor profile of the liqueur, with the more polar flavors being perceived first and the more nonpolar perceived last perhaps or even as after-taste sensations.

Ethyl esters also contribute to the overall flavor balance of cream liqueurs. However, at high concentrations, they can impart a very fruity flavor, which can become the dominant flavor in the liqueur. Ethyl ester level increases in concentration as cream liqueurs age, and thus, the level of fruitiness may become a limiting factor to consumer acceptance in aged liqueurs. Ethyl esters form via the reaction of the alcohol and fatty acids, and the presence of excessive levels of short-chain fatty acids can result in the formation of ethyl esters such as ethyl acetate, ethylbutanoate, and ethyl hexanoate, which are the principal causes of this fruity defect. The level of ethyl esters may be used as an indicator of the ability of the interfacial protein membrane to maintain compartmentalization of the fat and aqueous/ethanol phases. It has been found that high-pressure homogenized cream liqueurs had lower levels of ethyl decanoate following storage than liqueurs produced using a lower-pressure homogenization process, indicating that high-pressure homogenized liqueurs had a greater ability to maintain fat–aqueous phase compartmentalization during storage.

The levels of these ethyl esters can be monitored and quantified using gas chromatography (GC) or gas chromatography–mass spectrometry (GC-MS) typically following extraction of methylated esters, for example, using solid-phase microextraction (SPME). Cream liqueur manufacturers perform regular sensory profiling of their products over the course of their real-time shelf life. Panels are trained and sensory changes are tracked over time. Producers may also employ triangle tests to differentiate a product at a point in its shelf life from a fresh or gold standard product.

Conclusion

This article gives an overview of liqueurs in general while focussing specifically on cream liqueurs. The main ingredients used in cream liqueur manufacture are described, that is, sodium caseinate, cream, sugar, and ethanol. A typical process for cream liqueur manufacture is outlined, and the critical role of homogenization in the manufacture of cream liqueurs is discussed, as well as the latest research on alternative emulsification technologies. Physical instability, the main problem facing cream liqueurs, is described, and finally parameters, including viscosity and droplet size distribution, used to characterize and test subsequent long-term stability are outlined.

See also: Alcohol: Properties and Determination; Milk Powder; Milk: Processing of Milk; Milk: Sources and Composition; Pregnancy: Metabolic Adaptations and Nutritional Requirements.

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Listeria: Detection

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Introduction

Listeria genus contains ten species, namely, *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. seeligeri*, *L. welshimeri*, *L. grayi*, *L. rocourtiae*, *L. marthii*, *L. fleischmannii*, and *L. weihenstephanensis*, of which *L. monocytogenes* and *L. ivanovii* are considered pathogenic microorganisms for humans and animals, respectively. *Listeria* spp. are gram-positive, facultatively anaerobic, rod-shaped, and motile at temperatures below 30 °C. They are ubiquitous and widely diffused in the environment. Among the species belonging to the *Listeria* genus, *L. monocytogenes* is a major concern for food producers. Due to its physiological characteristics, such as resistance to acidic stress, ability to grow at low temperature, and possibility to form biofilms, it can persist and/or recontaminate food products, thereby representing an important risk for the safety of the consumers. The term '*Listeria* hysteria' was coined toward the end of 1980s following a series of listeriosis outbreaks due to the consumption of soft cheese and ready-to-eat (RTE) meats in the United Kingdom. The constant occurrence of listeriosis, as reported in the community summary reports on foodborne outbreaks in the European Union, warns for the need for special attention to this foodborne pathogen in order to combat its presence in foodstuffs. *L. monocytogenes* infection presents a high mortality rate, up to 20%, particularly among vulnerable groups such as the elderly. Listeriosis is also very threatening for pregnant women as it can cause fetal infections, miscarriages, and stillbirths. The foods most frequently associated with the outbreaks have been identified as RTE foods, particularly smoked fish and other fishery products, followed by meat products and cheese. Since *L. monocytogenes* is widely diffused in the environment, the route of contamination is often characterized by a postprocessing cross contamination.

Authorities have approached the risk of *L. monocytogenes* in food following different ways, and there are mainly two approaches that have been used worldwide: the zero tolerance and the 100 cfu g^{-1} or ml limit. RTE foods are most often considered in the legislations, and when the 100 cfu g^{-1} or ml limit is accepted, they are usually divided into categories that support or not the growth of the pathogen. For example, in the case of the European Commission Regulation 2073/2005, amended by the 1441/2007, RTE foods are divided first into two categories based on the final consumer: foods intended for risk categories (e.g., hospitalized, elderly, newborns, and pregnant women) and foods intended for the general population. In the first case, the absence of *L. monocytogenes* in 25 g or ml for 10 sampling units is requested, while for the second category, a further differentiation is made, based on the capability of the food product to support or not the growth of *L. monocytogenes*. If the food is not able to permit growth, the limit is below 100 cfu g^{-1} or ml until the expiration date, while if *L. monocytogenes* can grow and multiply in the product, then it must be absent in 25 g or ml, immediately after production, before it is placed on the market or the food business operator has to guarantee that the 100 cfu g^{-1} or ml limit will not be exceeded before the expiration date.

In the last 30 years, microbiologists have put a lot of efforts in the development of methods able to detect, quantify, and identify *Listeria* spp. and *L. monocytogenes*. Due to its low number in foods, the strategy that has been proposed is similar to the one used for the detection of *Salmonella* spp., and it is based on a series of enrichments able to support the selective growth of *Listeria* spp. while inhibiting the other members of food microbiota. After this step, selective and differential agars are used to streak the enriched broth and to allow the formation of colonies with specific color and morphology typical for *Listeria* spp. Recently, a new chromogenic agar, named agar *Listeria* according to Ottaviani and Agosti (ALOA), allowed the specific detection and enumeration of *L. monocytogenes* due to the characteristic green-blue color of the colony surrounded by an opaque halo.

Culture-based methods for the detection of *Listeria* spp. and *L. monocytogenes* in the last years have been criticized. Several authors, in fact, have reported an underestimation of *L. monocytogenes* incidence due to the masking effect of other *listeriae* during the enrichment step, which are able to grow faster (e.g., *L. innocua*). Moreover, it has been previously proven that *L. monocytogenes* undergoing stresses enters a viable but nonculturable (VBNC) state that makes its recovery by culture-dependent methods impossible. These arguments have promoted the development of alternative methods for the detection of this pathogenic microorganism in food and the approaches that most frequently have been used are molecular biology-based. Conventional and quantitative PCR (qPCR) protocols have been developed for the detection, identification, and quantification of *L. monocytogenes*, while less frequently, the other members of the genus have been considered.

In this chapter, a description of the principal detection methods for *Listeria* spp. and *L. monocytogenes* is reported, with an emphasis on the differentiation between culture-dependent and culture-independent methods.

Culture-Based Methods

As outlined earlier, the procedure used for the detection of *Listeria* spp. and *L. monocytogenes* in foods is based on a first step of enrichment in selective broths, followed by growth in selective agar plates, where colonies with a typical aspect are then isolated and further confirmed as belonging to this genus and species. A list of *Listeria* enrichment broths and selective isolation agars is described in Table 1. The list contains media specified by regulatory bodies, such as the US Food and Drug Administration (FDA), the US Department of Agriculture (USDA), and the International Organization for Standardization (ISO). Regarding the enrichment, most

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Table 1 *Listeria* enrichment broths (LEBs) and selective agars for the detection of *Listeria* spp. and *L. monocytogenes*

Media	*Selective agents (mg l^{-1})*	*Other additions*
Enrichment broths		
Primary LEB		
Half Fraser	Acriflavine (12.5) Nalidixic acid (10)	NaCl LiCl
IDF	Acriflavine (10) Nalidixic acid (40)	NaCl, cycloheximide
UVM I	Acriflavine (12) Nalidixic acid (20)	NaCl
Buffered peptone water (PHLS method)		NaCl
Secondary LEB		
Fraser	Acriflavine (25) Nalidixic acid (20)	NaCl LiCl
UVM II	Acriflavine (25) Nalidixic acid (20)	NaCl
Lovett (PHLS method)	Acriflavine (15) Nalidixic acid (40)	
Combined LEB		
FDA	Acriflavine (10) Nalidixic acid (40)	NaCl, cycloheximide, sodium pyruvate
Listeria repair broth (LRB)	Acriflavine (12[a]/15[b]) Nalidixic acid (20[a]/40[b])	NaCl, cycloheximide, glucose, yeast extract $MgSO_4$, $FeSO_4$, pyruvate
Selective agars		
Harlequin listeria medium	LiCl (15) Colistin (10) Acriflavine (2.5)	Fosfomycin, cefotetan, cycloheximide
LPM	LiCl (5)	Moxalactam, glycine anhydride, phenylethanol
Oxford	LiCl (15) Colistin (20) Acriflavine (5)	Fosfomycin, cefotetan, cycloheximide
PALCAM	LiCl (10) Polymyxin (10) Acriflavine (5)	Ceftazidime
ALOA[c]	LiCl (10)	Cycloheximide, nalidixic acid
LMPM[c]	Composition not published	
RAPID'*L. mono*[c]	Composition not published	

[a]USDA concentrations.
[b]FDA concentrations.
[c]Agars specific for the detection of pathogenic *Listeria*.
Source: Beumer, R. R. and Curtis, G. D. W. (2012). Culture media and methods for the isolation of *Listeria monocytogenes*. In: Corry, J. E. L., Curtis, G. D. W. and Baird R. M. (eds.) *Handbook of culture media for food and water microbiology* (3rd ed.), pp. 115–129. UK: The Royal Society of Chemistry.

standards foresee a double step, in which two different broths are used. Usually, primary enrichment broths contain a reduced amount of the selective agent (e.g., Half Fraser and UVM I) in order to allow the recovery of cells that are stressed, injured, or in a sublethal state. The selective agents that are most frequently used in enrichment broths are acriflavine, nalidixic acid, and cycloheximide. While the first two are specifically used to inhibit the RNA and DNA synthesis of gram-positive and gram-negative bacteria, respectively, cycloheximide is able to avoid the growth of yeast and molds, since it prevents protein synthesis in eukaryotic cells. As indicated earlier, during enrichment, nonpathogenic *Listeria* may be able to overgrow *L. monocytogenes*, making its recovery on the agar plate very complicated. It should be pointed out, however, that since *L. innocua* and *L. monocytogenes* share the same environmental niches, the detection and identification of *L. innocua* should be considered as an indication of a probable presence of *L. monocytogenes* as well. Moreover, during the enrichment step, different serotypes of *L. monocytogenes* may behave differently in terms of growth speed; thereby, the intraspecies ecology in the sample can be altered. This aspect has a great impact in terms of the epidemiology of the different serotypes involved in food outbreaks, since only those that are favored by the enrichment medium will be isolated on the agar plates.

Considering the selective isolation agars, lots of efforts have been dedicated to the optimization of media, which are highly selective for *Listeria* spp. and especially *L. monocytogenes*. Most of the agars differentiate *Listeria* spp. based on the hydrolysis of the esculin, which in the presence of iron is able to form colonies with a black surrounding. This principle does not allow the differentiation of *L. monocytogenes* from other *listeriae*, and for this reason, when selective media such as Oxford or PALCAM are used, it is common that isolation and identification of at least five presumptive *Listeria* colonies are performed in order to assess the presence of *L. monocytogenes*. This obstacle was partially circumvented by the development of blood-containing

media that allow the separation of the hemolytic species (*L. monocytogenes*, *L. ivanovii*, and *L. seeligeri*) from the nonhemolytic ones (*L. innocua*, *L. welshimeri*, and *L. gray*). However, the determination of this character is not always easy to perform and misidentifications can be reached. The development and introduction of chromogenic agars selective for *L. monocytogenes* have improved the detection of this pathogen in food products. The phosphatidylinositol-specific phospholipase C (PI-PLC), which hydrolyzes a specific substrate producing an opaque halo around the colony, is the basis for the differentiation of *L. monocytogenes* in several chromogenic agars, such as the ALOA, *L. monocytogenes* selective/differential plating medium (LMPM), and the RAPID'*L. mono*. Available data in the literature suggest that the use of these chromogenic agars allows for a better understanding of the prevalence of *L. monocytogenes* in foods. However, it should be emphasized that further improvement of detection of contaminated samples can be reached by the combined use of several selective agars.

Molecular Biology Methods

The exploitation of molecular biology in food microbiology has allowed the development of approaches, which do not rely on the capability of the target microorganism to grow on synthetic media. Molecular techniques are based on the detection of genetic material (nucleic acids, i.e., DNA or RNA) that is unique to the target organism, and for this reason, they are characterized by a high specificity. In this context, the polymerase chain reaction (PCR) is one of the most important rapid methods for the sensitive and specific detection of pathogenic microorganisms. The introduction of qPCR enables the quantification of the number of pathogens in foods.

The PCR technique, first described by Kary Mullis in the mid-1980s, is based on a three-step cyclic *in vitro* procedure, in which the DNA polymerase copies a strand of DNA. By using two primers, the sequence in between is amplified exponentially with each cycle making the technique very sensitive. Theoretically, even one molecule of DNA can be amplified reaching billions of copies. The amplicons of PCR are traditionally (conventional PCR) visualized by agarose gel electrophoresis, where the identification is based on the molecular weight. Further confirmation can be obtained by sequencing.

In the early 1990s, the 'second'-generation PCR technology was introduced exploiting intercalating dyes, for example, SYBR Green, or DNA probes, for example, molecular beacons, TaqMan probes, or Scorpions, enabling real-time detection. qPCR offers the opportunity to quantify a nucleotide sequence since the fluorescence emitted by the reported dye is continuously measured. In addition, qPCR consists of a closed-tube format and does not need postamplification processing of the PCR product (e.g., gel electrophoresis), reducing the risk of contamination. Lastly, qPCR offers a higher sensitivity and more rapid results in comparison with conventional PCR.

Since the introduction of the PCR approaches in the field of food microbiology, the detection of foodborne pathogens has evolved enormously. The optimization of the DNA polymerase enzyme, the PCR master mixes, and PCR equipment has improved the sensitivity and robustness of the technique significantly, pushing the PCR to its theoretical detection limits, that is, almost single DNA copies per PCR tube.

As for other foodborne pathogens, also for *L. monocytogenes*, the literature is rich in papers describing the development, optimization, and application of PCR (both conventional and quantitative) for detection, identification, and quantification in different foods. One of the most common targets for PCR amplification is the *hly* gene; however, others such as *inlA*, *inlB*, intergenic spacer regions, *iap*, *plcA*, and *actA* have been used as well. The food matrices, in which *L. monocytogenes* has been detected by means of molecular methods, are represented by meat and meat products, milk and dairy products, fruits and vegetables, fish (e.g., salmon), and RTE and deli products. The limit of detection of the available protocols is usually 100 cfu g^{-1} or ml, while in the case of qPCR, the limit of quantification is often above 10^3 cfu g^{-1} or ml. These limits are still too high for practical application in the food sector, and for this reason, PCR amplification is often preceded by an enrichment step. In the case of qPCR, it should be pointed out that after enrichment, the method from quantitative becomes qualitative, since no correlation between the number of cells at the end of the enrichment and those originally contained in the sample can be calculated. Some examples of conventional and quantitative amplification protocols for *L. monocytogenes* in food are reported in Table 2.

An inherent drawback with DNA amplification by PCR is the inability of the method to distinguish between live and dead cells. Since DNA is a very stable molecule, it persists long after the death of the microorganism, and it can be amplified. This consideration is particularly relevant for foodborne pathogens, since the presence of live or dead pathogenic microorganism in foods has a totally different significance. Considering also that food processing exploits bactericidal treatments (e.g., pasteurization and sterilization), it is common to find in foods DNA molecules generated from pathogen cell lysis. For this reason, it is important to correctly interpret the results obtained by DNA-based PCR. The possibility to differentiate live from dead cells in PCR protocols has been addressed by the researchers mainly in two ways: the use as target in the amplification of the RNA and the pretreatment of the sample with ethidium monoazide (EMA) or propidium monoazide (PMA). In the first case, since RNAs are molecules that are degraded immediately after cell death, a positive amplification correlates well with the presence of alive populations of the pathogenic bacteria in the food sample. In the second case, EMA and PMA are able to penetrate into damaged and dead cells, they bind to the DNA, and after a photosensibilization, they are able to provoke the hydrolysis of the molecule, which will not be amplified. These solutions are valuable alternatives to the classic DNA-based PCR approach; however, they are not frequently applied. Nevertheless, it should be pointed out that if the PCR amplification is able to detect specific targets of the pathogenic microorganism, independently from the fact that this is DNA from live or dead cells or RNA, it means that during the food chain, a contamination has occurred and this has to be taken into consideration and corrected.

Lastly, especially when environmental conditions influence the fitness and behavior of the pathogens, such as in the

Table 2 Examples of PCR protocols (conventional and quantitative) for the detection and/or quantification of *L. monocytogenes* in foodstuffs

PCR method applied	*Primers and probes (5′–3′)*	*Target gene*	*Enrichment*[a]	*Matrix*	*Limit of detection/ quantification*
Conventional	MAR 1, GGGCTTTATCCATAAAATA MAR 2, TTGGAAGAACCTTGATTA	*iap* gene	N	Several	30 cfu g^{-1} or ml^{-1} (LOD)
Conventional	CGGAGGTTCCGCAAAAGATG CCTCCAGAGTGATCGATGTT	*hly* gene	Y (24 + 24)	Several	2×10^3 cfu ml^{-1} of enrichment (LOD)
	ACAAGCTGCACCTGTTGCAG TGACAGCGTGTGTAGTAGCA	*iap* gene			
Quantitative with TaqMan probe	LMrt3F, CAAAGCGAGAATGTGGCTATAAATGA LMrt3R, TAATTTCCGCTGCGCTATCCG listP, FAM-ACC CTG GAT GAC GACGCT CCA CT-TAMRA	*actA* gene	Y (24 + 5, 6 or 25)	Several foods	1 cell per 25 g or ml (LOD)
Quantitative with TaqMan probe	IGS 1, GGCCTATAGCTCAGCTGGTTA IGS 2, GCTGAGCTAAGGCCCCGTAAA Probe IGS, FAM-ATAAGAAATACAAATAATCAT-TAMRA	16S–23S intergenic region	N	Several foods	10^3–10^4 cfu g^{-1} or ml^{-1} (LOQ)
Quantitative with SYBR Green	L-1, CACGTGCTACAATGGATAG L-2, GATTAGGGTATTTTGATAAGA	16S rRNA gene	Y (4 + 44)	Leafy vegetables	10 cfu g^{-1} (LOD)
Quantitative with FRET probes	LssrA F, GCATCGCCCATGTGCTAC LssrA R, TCTACGAGCGTAGTCACCG Hybprobe 1, CCATTCAGCTAGTCTGATTAAGCTCT-fluorescein Hybprobe 2, LC Red 640-CTATTTAACCCCAGACGGAGA-phosphate	*ssrA* gene	Y (22 + 4)	Several foods	1–5 cfu 25 g^{-1} or ml^{-1} (LOD)
Quantitative with TaqMan probe	hlyQF, CATGGCACCACCAGCATCT hlyQR, ATCCGCGTGTTTCTTTTCGA hlyQP, FAM-CGCCTGCAAGTCCTAAGACGCCA-TAMRA	*hly* gene	N	Salmon	10^3 cfu g^{-1} (LOQ)

[a]N, not applied; Y, applied (duration of primary + secondary enrichment in hours).

presence of salt, or when the cells have to respond to several stresses such as disinfectants, starvation, and dry conditions, molecular methods represent good tools to properly detect them. As outlined earlier, it has been previously proven that *L. monocytogenes* undergoing stresses enters a VBNC state, which makes impossible its recovery by culture-dependent methods. The application of molecular methods can result in a better comprehension of the spread of a specific pathogen in the food chain, thereby allowing the implementation of corrective actions to eliminate or decrease the risk associated with its presence in the final product.

PCR is the most common technique used for *Listeria* spp. and *L. monocytogenes* nucleic acid amplification. Nevertheless, other techniques such as nucleic acid sequence-based amplification and the loop-mediated isothermal amplification have been developed. Both methods have the advantage of being carried out at a constant temperature, thereby avoiding the purchase of expensive equipment for the thermal cycling in PCR amplification.

The use of PCR-based methods in official routine analysis requires a validation. Generally, this includes a comparison with the current reference method using artificially and naturally contaminated food samples and an interlaboratory study. Validated protocols of PCR-based methods for the detection of *L. monocytogenes* in foods, such as raw milk, are available in the literature. Lastly, many commercial PCR kits available on the market are now validated according to certification bodies, such as AOAC, AFNOR, or NordVal.

Immuno-Based Methods

The high specificity of the antigen–antibody interaction has been exploited in food microbiology to develop specific methodologies for the detection and identification of foodborne pathogens. One of the main drawbacks of these methods is the low sensitivity, since a microbial load of about 10^5 cells per g or ml is necessary to produce a measurable signal. This

number is extremely high considering pathogenic microorganisms of food relevance, and it does not stand well against the limits of detection of the plate count (about 10^4 cells per g or ml) or molecular methods (10^2–10^3 cells per g or ml). Moreover, for some microorganisms, it is very complicated to find specific antibodies, without cross-reactions with other closely related species.

The antigen–antibody interaction in the field of food microbiology for the detection of *Listeria* spp. and *L. monocytogenes* has been used for the specific separation (and immunoconcentration) of the microorganism from enriched samples, to be then identified by molecular methods, or in the development of enzyme-linked immunosorbent assays (ELISAs), in which two specific antibodies are used and one of them is conjugated with an enzyme (often a peroxidase).

The Dynabeads are beads with a metal core, with *Listeria*-specific antibodies embedded in the polystyrene surface. As indicated earlier, Dynabeads can be used in enriched samples, in order to capture *Listeria* spp. After the separation, the beads can be streaked on a selective medium or subjected to DNA extraction followed by specific identification.

Regarding the ELISA, anti-*Listeria* antibodies are usually immobilized in a microtiter plate. The antibody blocks the target inside the well, allowing washes to remove non-specifically bound microorganisms, and it is used in combination with a second antibody that is linked to an enzyme. The addition of a chromogenic substrate, through the activity of the enzyme, is producing color, which can be evaluated visually or with a spectrophotometer. ELISA technique is characterized by a low sensitivity, as all the immuno-based methods, and positive signals are recorded above a threshold concentration of about 10^5–10^6 cells per g or ml. For this reason, an enrichment step is necessary that renders the assay qualitative.

Conclusions and Future Perspectives

Listeria spp. and *L. monocytogenes* detection has benefited from the implementation of molecular methods applied in food microbiology. Due to its low contamination level, methods of detection still rely on the use of enrichment broths, able to favor the growth of this microorganism and at the same time inhibit the microbiota associated to the specific food sample processed. The combination of culture-dependent and culture-independent methods has increased the possibility to detect this important foodborne pathogen, creating the prerequisites for better control in the food chain.

The 'omics' approaches can be considered as a valuable tool in order to better understand the physiology of the microorganism and as a consequence improve its control. Genomics, and more specifically metagenomics, can be used, for example, to detect specific signatures of *L. monocytogenes* in a food sample with a higher sensitivity compared to PCR, due to the number of sequences that the approach can read, while metatranscriptomics appears to be a powerful approach to understand the behavior of the pathogenic microorganism in terms of stress response, virulence, and survival during food processing and even when it is entering the human body. The information collected through the application of these modern approaches will have to be integrated in risk assessment models that have to properly take into account both intrastrain variability and differences in the microbial behavior.

See also: Foodborne Pathogens; *Listeria*: Listeriosis; *Listeria*: Properties and Occurrences; Zoonoses.

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Listeria: Listeriosis

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Listeria Species and Subtyping

The Gram-positive *Listeria* genus of bacteria comprises motile, facultative, anaerobic, coccobacillary, non-spore-forming rods that are up to 2 μm in length. They are psychrotrophic or 'cold-tolerant' and are widely disseminated in the environment in relatively low numbers where they live as saprophytes in soil and vegetation. Currently, the *Listeria* genus is identified and designated into 15 species, and two of these, *Listeria monocytogenes* and *L. ivanovii*, are pathogenic primarily to humans and animals, respectively. Of these, *L. monocytogenes* is the most important pathogen that is a facultative intracellular organism and has attracted extensive research attention, not least because of its serious potential risk to human health. *L. ivanovii* shares facultative pathogen status, and while it is an important cause of disease in ruminants, there are only occasional reports of it infecting debilitated humans as an opportunistic pathogen. The other 13 described nonpathogenic species are *L. seeligeri*, *L. innocua*, *L. welshimeri*, *L. grayi*, *L. fleischmannii*, *L. marthii*, *L. rocourtiae*, *L. weihenstephanensis*, *L. floridensis*, *L. aquatica*, *L. cornellensis*, *L. riparia*, and *L. grandensis*.

The most studied member is *L. monocytogenes*, which is the most important pathogenic species. *L. monocytogenes* is remarkably adaptable and resilient. Other habitats identified for it include water, sewage, animal feeds (especially silage), farm, and food processing environments. Growth occurs over the range 4–45 °C but optimally at 30–37 °C, and biochemically, it is catalase-positive and oxidase-negative. Colonies on blood agar media after incubation for 24 h are small, smooth, and transparent and usually surrounded by a narrow zone of complete beta-hemolysis.

Commercially available biochemical test kits, Matrix assisted laser desorption/ionization - time-of-flight (MALDI-TOF) mass spectrometry, and various polymerase chain reaction (PCR) assays can be used to distinguish the *Listeria* species. Strain typing is performed using methods from molecular biology and involves, on the one hand, digestion of genome nucleic acid with restriction endonucleases or amplification of fragments followed by electrophoretic separation. These methods permit subtyping by generating fragment banding patterns by pulsed-field gel electrophoresis, ribotyping, amplified fragment length polymorphism, random amplified polymorphic DNA analysis, and repetitive extragenic palindromic sequence PCR. On the other hand, sequence-based subtyping, like multilocus sequence-based typing, multilocus variable tandem repeat analysis, multi-virulence-locus sequence typing, and microarrays, now plays an important role in determining the source of listeriosis outbreaks and is critical for the understanding of the natural history and transmission of the organism. Subtyping tests are now standardized internationally by the World Health Organization and the PulseNet International network.

Classification of *L. monocytogenes* has also benefited from comparative genomics and the organism now ranks among the most documented pathogens. Nevertheless, knowledge about subtype, natural history, and association with disease does not provide information about the virulence potential of *L. monocytogenes* isolates.

Seventeen serotypes of *L. monocytogenes* have been recognized based on somatic (O) and flagellar (H) antigens. These are identified alphanumerically. Whereas serotypes 1/2a, 1/2b, and 1/2c are the types most frequently isolated from food or the food production environment, more than 95% of human infections are associated with serotypes 1/2a, 1/2b, and 4b. Serotype 4b has been linked to international outbreaks, for example, those that have implicated coleslaw, soft cheese, pâté, and pork tongue (see in the succeeding text); serotype 1/2b has been linked to contaminated milk, rice salad, and imitation crabmeat products; and serotype 1/2a has frequently been linked to outbreaks stemming from food processing plants. Other serotypes have also been occasionally linked to sporadic cases, for example, 1/2c (Switzerland 2011–13, Spain 2009, Italy 2000–10).

L. monocytogenes has at least four distinct phylogenetic, evolutionary lineages. The lineages have different, but overlapping, ecological niches. Taken together, serotypes 1/2b, 3b, 4b, 4d, and 4e form a distinct evolutionary lineage, now recognized as lineage I. Lineage II comprises serotypes 1/2a, 1/2c, 3a, and 3c. Lineages III and IV comprise serotypes 4a and 4c. However, the latter ones are rare and predominantly identified in animals. Interestingly, the latter lineage serotypes are rarely isolated from foods or human patients. Characterization of *L. monocytogenes* isolates from listeriosis outbreaks has led to the suggestion that many of these outbreaks were caused by a small number of epidemic clones. The global transmission pathways and the true reservoirs of the *Listeria* species have not been yet defined.

Human Listeriosis

Listeriosis, infection with *L. monocytogenes*, is a relatively rare disease with an incidence rate of 0.26–0.32 reported infections per 100 000 habitants. However, it is one of the most serious foodborne diseases in humans. Listeriosis is associated with mild or severe, localized or generalized infections in most domestic animals and humans, particularly in temperate climates. *L. monocytogenes* was initially associated with septicemia and monocytosis in laboratory animals in 1926 following an epidemic infection among rabbits and guinea pigs. The name reflected the monocytosis observed. Meningitis in a human patient was first described in 1929.

However, it was not until the early 1980s that reports of *Listeria* isolates increased in the literature and a series of epidemic outbreaks in humans in North America and Europe thereafter established listeriosis as an important foodborne

infection. Until that time, listeriosis was considered a rare and sporadic disease. Since then, multiple large outbreaks have been reported internationally. Most cases of human listeriosis in adults are sporadic and likely go unreported, and this makes estimates of their incidence difficult. Besides sporadic illnesses, human listeriosis can also take the form of disease outbreaks following consumption of contaminated food, which is usually a particular food item.

In Europe and the United States, the hospitalization rate is estimated at over 95%. Once clinical infection is established, the overt form of listeriosis in humans is associated with a fatality rate of 25–30%. Outbreaks in humans often occur in urban environments in summer, and epidemics have been traced to foods of animal origin or fecally contaminated plant products, postprocess contamination of meats, or selective, prolonged growth of *L. monocytogenes* in refrigerated foods. Ready-to-eat foods are especially implicated. Preexisting gastrointestinal disease may be a risk factor for infection with *L. monocytogenes*.

Sourcing of epidemics is complicated by the relatively low incidence of *L. monocytogenes* and the long incubation period for the invasive forms of the infection, which has been cited to be up to 70 days, with a median of 8 days. A longer median incubation period of 27.5 days has been cited for pregnant women. While the infectious dose of the organism is not known, it may be as high as 1 million colony-forming units per gram of contaminated food. It may be considerably lower for susceptible individuals such as infants, pregnant women, the elderly, and immunocompromised people. Other likely variables influencing the infectious dose are strain virulence, susceptibility of the host, and the nature of the particular food matrix.

Original contaminated food sources are difficult to retrieve due to the perishable nature of food and the significant clinical incubation period. The actual level of original contamination is often never determined. However, exceptionally, an outbreak in central Europe occurred between June 2009 and February 2010, when 34 people became ill and eight of them died. The source of contamination was successfully traced epidemiologically and, by the concerted efforts of multidisciplinary specialists, to patient food purchase receipts from a Quargel cheese production site in Austria. Molecular typing was key to the identification. The outbreak could be traced to two specific *L. monocytogenes* serotype 1/2a clones, and for the first time, it has been possible to determine the contamination level of *L. monocytogenes* in the very cheese lots that caused the outbreak.

As an environmental organism, *L. monocytogenes* is capable of transition into a pathogen eliciting overt clinical disease following ingestion especially by susceptible consumers. These are typically infants, pregnant women, the elderly, debilitated and immunocompromised people, and particularly those with impaired T cell-mediated immunity. The latter include patients receiving immunosuppressive drug therapies, those undergoing hemodialysis, those with transplants, diabetics, human immunodeficiency virus (HIV) carriers, and drug abusers. This list may even be extended to include those receiving antacid drugs in that gastric acid may confer a degree of protection from ingested bacteria.

Infection with *L. monocytogenes* in humans causes a spectrum of illness, ranging from febrile gastroenteritis to outright life-threatening invasive disease, including bacteremia, sepsis, and meningoencephalitis.

Noninvasive Listeriosis in Adults

Self-limiting skin lesions in the form of papules on the hands and arms are occasionally observed in veterinarians and farmers following contact with ruminants infected with *L. monocytogenes*. Individuals not in high-risk groups may experience gastrointestinal discomfort, diarrhea, and fever with resolution of symptoms in 24 h following ingestion of the bacteria. This form of infection was described from an outbreak of gastroenteritis and fever due to consumption of infected chocolate milk at a picnic in Illinois, the United States, in 1997. None of the victims of the outbreak were in high-risk groups or subsequently showed signs of invasive listeriosis. Due to its transient nature, noninfectious listeriosis is likely to go underdiagnosed and unreported.

Invasive Listeriosis in Adults

Hematogenous spread of *L. monocytogenes* following oral ingestion can result in focal infections, which commonly involve the peritoneum, joints, endocardium, or eyes. This form of infection with *L. monocytogenes* is an important cause of bacterial sepsis, meningitis, or meningoencephalitis. The case fatality rate is ~30%.

Listeriosis during Pregnancy

Most cases of listeriosis during pregnancy occur in otherwise healthy women. Infection of pregnant women can lead to abortion, fetal death, or neonatal morbidity in the form of septicemia and meningitis. Pregnant women may be asymptomatic or present with nonspecific clinical symptoms. However, clinical cases can be life-threatening and typically present as septicemic or meningoencephalitis syndromes. Frequently, patients present with fever or flu-like illnesses lasting ~6 days. Pregnant women may be susceptible to infection with *L. monocytogenes* as their cellular immunity decreases secondary to increased progesterone secretion and the bacteria have a tropism for the uterus and placenta. The fetus becomes infected by transplacental transmission following maternal bacteremia. Women with multiple fetuses may be at increased risk of infection compared with singleton pregnancies, and listeriosis cases affecting pregnant women most frequently occur during the third trimester.

Neonatal Listeriosis

While maternal listeriosis often takes the form of a mild illness, neonatal infection is invariably serious and frequently fatal. Neonatal listeriosis can take early-onset or late-onset forms.

The early-onset form of neonatal listeriosis is acquired *in utero* and appears to result from aspiration of infected amniotic fluid, most probably as a result of transplacental infection. It results in illness at the time of birth or within the first week of life. Symptoms include septicemia, respiratory distress, and less frequently meningitis and *granulomatosis infantiseptica*, which is associated with disseminated abscesses or granulomas in various internal organs. The mortality rate for infected infants is ~20%, and this can increase to 50% when abortion and still births are taken into account.

Late-onset neonatal listeriosis frequently occurs during delivery of a full-term baby and can occur up to several weeks after birth. The route of infection is not well understood. Late-onset neonatal listeriosis can progress to meningoencephalitis and sepsis. The mortality rate has been cited at 10%, and a high number of surviving neonates develop severe and chronic neurological complications and blindness.

Diagnosis and Treatment

In all cases, diagnosis of human listeriosis infection with *L. monocytogenes* necessitates recovery of the bacteria from infected tissues, the blood, or the cerebrospinal fluid. There are no reliable serological tests. Fecal cultures are not informative as healthy individuals can be silent carriers of the bacteria.

Isolates of *L. monocytogenes* are susceptible to penicillins, aminoglycosides, trimethoprim, tetracycline, macrolides, and vancomycin, with reduced susceptibility or resistance to sulfamethoxazole, cephalosporins, and early quinolones. Aggressive antibiotic treatment is normally continued for at least 2 weeks. Longer treatment periods are recommended for neonatal infections and for immunocompromised patients with meningitis and bacteremia.

Pathogenesis

Emergence of *L. monocytogenes* as a foodborne pathogen in the 1980s also prompted substantial molecular biology research underlying its pathogenesis. Following ingestion of *L. monocytogenes* in contaminated food, passage into the gastrointestinal tract, and survival of gastric acid, translocation can occur directly by invasion of intestinal epithelial cells or indirectly by active penetration of M cells overlying Peyer's patches. Systemic spread occurs via the lymph and blood. From the intestines, it is disseminated to the mesenteric lymph nodes, spleen, and liver, and from there, *L. monocytogenes* can reach the brain and placenta.

Demonstration in the early 1960s that *L. monocytogenes* can survive and multiply in macrophages prompted its use in immunological research as a prototype intracellular parasite.

L. monocytogenes invades host cells, phagocytes, and nonphagocytes and replicates in the cytosol after phagosomal escape. In this way, *L. monocytogenes* is a classic example of a 'cytosol-adapted pathogen' that can readily escape from these macrophage phagosomes and replicate rapidly in the cytosol. Virulent strains are able to survive within cells by escaping from phagosomes before maturation to phagolysosomes.

The pathogenicities of *L. monocytogenes* and *L. ivanovii* are enabled by an ~9 kb virulence gene cluster that is also present in a modified form in *L. seeligeri*. This gene cluster appears to mediate the saprophyte-to-cytosolic parasite transition of *L. monocytogenes* through careful modulation of the activity of the transcriptional activator protein *prf*A.

Discovery of *L. monocytogenes*' hemolysin (listeriolysin) gene *hly* and elucidation of the key role that hemolysin plays in escape from destruction inside phagosomes, a prerequisite for intracellular proliferation, helped piece together its virulence strategies. Listeriolysin has also been proposed as a virulence factor that promotes encystment and supports survival of *L. monocytogenes* within ciliated protozoa.

Once within the cytoplasm, motility of the bacteria is facilitated by production of *act*A, an actin-polymerizing protein that promotes formation of taillike structures from host cellular microfilaments. These motile forms can then induce pseudopod-like projections on the cytoplasmic membrane, which enables them to be taken up by adjacent cells. By forming plasma membrane protrusions, *L. monocytogenes* can continuously avoid host defense mechanisms, and dissemination can occur via macrophages and the bloodstream. Due to this cycle, *L. monocytogenes* can move between host cells without being present in the extracellular environment. In this way, it escapes from the human T cell immune response. Since populations of *L. monocytogenes* can replicate in macrophage vacuoles, this has been anticipated as being important for the establishment of chronic infections.

Listeriosis and Food

It is now recognized that nearly all cases of human listeriosis are foodborne. Poor hygiene practices most likely increase the risk of *L. monocytogenes* persistence and survival in food processing environments and the risk of contamination from outside. Knowledge that *L. monocytogenes* is far more commonly associated with the digestive tracts of animals, especially silage-fed ruminants, than humans led to the association of food contamination with animal feces. Correspondingly, typical sources of ingestion are currently considered to be raw foods closely associated with their originating environments, such as unpasteurized milk and dairy products, fresh meat, smoked fish and other seafoods, and raw fruits and vegetables. Together with this association and the extraordinary viability of *L. monocytogenes* within wide temperature (−1.5 up to 45 °C) and pH ranges (4.4–9.4) and its ability to survive at high salt concentrations (up to 14%) and to form persistent biofilms, it poses a significant and ongoing challenge to the food industry. Due to these characteristics, *L. monocytogenes* is able to survive under adverse environmental conditions for long periods, which include persistence on food processing surfaces and equipment. Thus, food processing environments have emerged as one of the major, direct sources of *L. monocytogenes* found in human food.

High-risk foods generally have the potential for contamination and the potential to support growth of the bacteria to high numbers, require refrigeration, have an extended shelf life, and contain high numbers of bacteria at the time of consumption. This applies to ready-to-eat seafood, prepared raw vegetables, refrigerated cold meat, and dairy products.

Pasteurization and adequate cooking do kill *L. monocytogenes*. However, contamination may occur after cooking and before packaging. In conjunction with the long clinical incubation period, contaminated perishable food items are not likely to remain for investigation in the event of a listeriosis outbreak. Furthermore, the ease with which contaminated foods can now be transported internationally and the size of large-scale modern food production, combined with complex transnational retailing systems and consumer

expectations for food items with a long shelf life, add to the impediments to outbreak investigation.

Ready-to-eat meats and cheeses made from unpasteurized milk have been the most frequently identified vehicles implicated in outbreaks. Again, it is worth stressing that ready-to-eat foods, such as hotdogs and delicatessen meats, can be contaminated after cooking, before the packaging step.

Fruit and Vegetables

Raw vegetables can be vehicles for infection of consumers. A particular example of such contamination was seen in 1981 in Nova Scotia when the infection of 42 people was linked to the consumption of a coleslaw product. The cabbage used for the coleslaw was harvested from fields fertilized with sheep manure from a farm with a history of ovine listeriosis. There were 34 pregnancy-associated and 7 non-pregnancy-associated adult cases. Of infants born alive, 27% later died. This outbreak provided the first convincing evidence that listeriosis could be a foodborne disease and highlighted the potential for uncooked vegetables to be a source of infection.

More recently, in 2011, 147 people in 28 US federal states became ill after eating contaminated cantaloupes contaminated with *L. monocytogenes*, 33 people died. Whole cantaloupes produced by a single Colorado farm were linked to the outbreak. Unsanitary conditions identified in the processing facility operated at the farm are thought to have resulted in contamination of the cantaloupes with *L. monocytogenes*. This outbreak revealed that fresh produce could be associated with an outbreak.

Dairy Products

Pasteurized milk was implicated as the most likely vehicle for an outbreak in Massachusetts, the United States, in 1983, where 49 cases of listeriosis occurred and the case fatality reached 29%. However, most episodes of infection with dairy products have involved cheese. An outbreak in California in 1985 was associated with Mexican-style cheese; there were 142 cases and 48 fatalities. In Switzerland, between 1983 and 1987, 34 people died out of 122 cases of listeriosis associated with Vacherin Mont d'Or cheese. A blue mold cheese in Denmark between 1989 and 1990 was responsible for 26 cases and seven deaths. Contaminated Mexican-style cheese in the year 2000 in the US state of North Carolina resulted in 13 cases of listeriosis and five deaths. Butter was the vehicle in 25 cases in Finland in 1999 when six people died. In Quebec, Canada, in 2002, contaminated cheese made from unpasteurized milk resulted in 17 cases, but no reported deaths. In 2003, in Texas, the United States, Mexican-style cheese was again the vehicle associated with 12 cases. Finally, a large listeriosis outbreak occurred in Austria, Germany, and Czech Republic in 2009 and 2010. The outbreak was traced back to a traditional Austrian curd cheese called 'Quargel.' This outbreak accounted for 34 clinical cases and eight deaths.

Fish and Seafood

Ingestion of contaminated smoked fish and other seafoods has resulted in human listeriosis. While the prevalence of *L. monocytogenes* in raw fish is relatively low, ranging from 0% to 10%, significant contamination can arise when these products are handled, processed, or stored in conditions that favor growth of the pathogen. This has resulted in isolated cases of listeriosis and food recalls, associated with vacuum-packed graved and/or cold-smoked rainbow trout (Finland 1999–2000), imitation crab meat (Canada 1996), herring in oil (Germany 2010), and different smoked products like salmon or mussels.

Meat Products

In the United Kingdom, between 1987 and 1989, there were 366 cases of listeriosis associated with pâté; the exact number of deaths attributable to this outbreak is unknown. An outbreak in France in 1992 resulted in 279 cases and 85 deaths, which was associated with pork tongue in jelly. Also in France, in 1993, there were 38 cases and ten deaths associated with rilletts, a meat specialty similar to pâté. Hotdogs were implicated in a multistate outbreak in the United States between 1998 and 1999 involving 108 cases and 14 deaths. Again in France, between 1999 and 2000, rilletts were the vehicle for another outbreak of ten cases and three deaths. During the same period in France, 32 cases and ten deaths were associated again with pork tongue. In 2000, in the United States, contaminated ready-to-eat delicatessen turkey meat resulted in 30 cases and seven deaths. In 2002, a multistate outbreak in the United States, again attributed to ready-to-eat delicatessen turkey meat, resulted in 54 cases and eight deaths.

Food and Food Premise Monitoring

Minimizing cases of human listeriosis depends on improving our understanding of how to limit contamination of food with *L. monocytogenes*, which since the 1980s has become a major public health concern. The potential seriousness of listeriosis means that the presence of *L. monocytogenes* in foods has become intensively regulated by national and international authorities. The bacteria can enter the food chain at nearly every stage of production, and most incidences of food contamination can be traced to contamination of food processing facilities. Longitudinal studies have revealed that different types of facilities have a great diversity of strains in different foods. However, it is seldom clear how these strains come to contaminate premises.

Consequently, biosurveillance, early warning monitoring of foodstuffs, and the food processing environment for the presence of *L. monocytogenes* are of great importance. The monitoring involves numerous steps, which include frequent microbiological sampling strategies and incubation in enrichment broths, such as half and full Fraser broths, for various times, usually 24–48 h, followed by plating on a variety of selective and chromogenic agar media, such as Oxford, Palcam, and ALOA agars. These qualitative and quantitative microbiological methods are included in the standard method ISO 11290.

The accuracy of the quantification is very dependent on the food matrix involved. Some foods such as fat-rich cheeses are less amenable to dissolution in growth media than, for

example, lean meat samples, and plate counts can greatly underestimate actual *Listeria* counts in a food. New techniques to free viable bacteria from the food matrix of a variety of food types are undergoing development, and these are proving to make enumeration more specific and precise. The key word in this respect is sample preparation. The aim is to reduce the volume of the matrix, thereby conserving the target organisms. One such approach is *matrix lysis*, which reduces the 6.25–12.5 g of various foodstuffs to < 100 µl of bacterial pellet. Methods like this have the further advantage that the cells are alive and therefore suitable for both microbiological enrichment and molecular methods such as PCR.

PCR is particularly advantageous as it has the potential to detect nonviable organisms or viable but not culturable (VBNC) forms. However, a caveat is that PCR cannot distinguish between live and dead organisms. On the other hand, legislation dictates that detection is linked to growth of the bacteria as only living bacteria are considered as a risk. This undisputed the premise for official testing. Nevertheless, it is worth thinking about alternative methods. First, the whole microbiological identification process from sampling to confirmation can take several days; PCR is faster; second, VBNC forms are high risk as viability cannot be estimated; and third, one can argue that each *Listeria*, which is found in a food production plant, is an indication for a problem. Moreover, the detection limit of qPCR is as low as one DNA molecule per sample. This advantage together with the information, which is given by microbiological methods, indicates the combined use as preferable. This will generate the most output in terms of available information.

The widespread and resilient nature of *L. monocytogenes* in the food environment is particularly challenging from a public health perspective, and it may not even be realistic to expect its complete eradication from food processing establishments. While food producers are responsible for the safety of their products, to attain this goal, they must establish food safety programs. However, translating from a regulation to industrial practice can pose a huge burden on food business operators, who often run small enterprises lacking risk management capacities. The elements of an in-house monitoring system (sampling points and sample frequency) are not widely harmonized, and the extent of measures executed in many situations is limited by the financial resources available for quality assurance.

Control strategies include prevention of survival/growth of *L. monocytogenes* in niches from where foods can be contaminated, implementation of sampling programs to monitor the effectiveness of control measures, rapid response facility procedures for dealing with contamination, follow-up measures to ensure contamination has been removed, and ongoing short- and long-term monitoring practices to determine contamination trends.

Awareness of *L. monocytogenes*, monitoring practices, consumer education, and legislation have contributed to decreased incidences of listeriosis in most, but not all, industrialized countries. This includes implementation of hazard analysis and critical control point (HACCP) programs in food production and legislation requiring zero tolerance for *L. monocytogenes* in most countries for ready-to-eat foods that could support growth. Control measures now expected from public health agencies include development and maintenance of surveillance programs, rapid follow-up of listeriosis outbreaks, and enforcement of existing legislation. Consumers themselves are particularly advised to cook raw meats thoroughly; wash raw vegetables before eating; separate uncooked meats from vegetables, cooked foods, and ready-to-eat foods; consume only pasteurized dairy produce; and maintain clean hands and kitchen utensils following preparation of uncooked food.

Neglected Routes of Transmission

While studies suggest that the soil environment and farm animal produce remain key sources of contamination, growing awareness that wild and domestic animals, humans, and insects can be silent carriers of *L. monocytogenes* is necessitating a rethink as to how best to protect human health. True reservoirs of infection are elusive. In comparison to farm and rural environments, the urban environment has been poorly studied. However, there are suggestions that this can be influenced by both human silent carriers and transport of soil detritus on footwear by travelers.

Concluding Remarks

Since the relatively recent recognition of the human pathogenic potential of *L. monocytogenes*, it continues to emerge globally as a serious threat to human health. Despite the implementation of food processing hygiene measures, education programs, and legislation, its occasional presence in food at alarming levels remains an ongoing challenge to those tasked with the processing and preparation of foodstuffs. Possible explanations for the emergence of recent epidemics of listeriosis since the 1980s reflect major changes in food production, processing, and distribution, increased reliance on refrigeration, and changes in eating habits to reflect desires for ready-to-eat processed foods and changes in population dynamics reflecting aging populations and chronic diseases associated with immunopathies or the long-term use of certain medications. Improved techniques for continuous biosurveillance for the presence of *L. monocytogenes* in food processing facilities offer a certain reassurance to consumers and food businesses. However, intermittent outbreaks of human listeriosis continue to surprise and evoke legitimate public concern.

See also: Biofilms; Cheese: Chemistry and Microbiology; Cheese: Types of Cheeses – Soft; Food Environment; Food Poisoning: Classification; Food Poisoning: Epidemiology; Food Poisoning: Tracing Origins and Testing; Foodborne Pathogens; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; *Listeria*: Detection; *Listeria*: Properties and Occurrences; Meat: Role in the Diet; Milk: Role in the Diet; Pregnancy: Dietary Guidance for Pregnancy; Zoonoses.

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Listeria: Properties and Occurrences

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The Genus *Listeria*: General Features

Listeria is a gram-positive, rod-shaped, non-spore-forming bacterium that is widely distributed in the environment and is isolated primarily from soil, silage, water, foods, and food processing environments. It appears to maintain itself as saprophyte in decomposing plant materials, considered to be the natural habitat of *Listeria*. All known *Listeria* species grow in the presence of ambient oxygen, and those that have been characterized extensively have been shown to be facultative anaerobes (able to grow in the absence of oxygen) as well as facultative microaerophiles (preferring, but not requiring, reduced oxygen levels). The genus *Listeria* includes 15 species, nine of which have been only recently described (since 2009). Phylogenetic analysis suggests four distinct clades within the genus *Listeria*: clade I (*Listeria sensu stricto*) includes *L. monocytogenes* and five other species (the previously known species *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. ivanovii* and the recently identified *L. marthii*); clade II consists of three recently described species (*L. fleischmannii*, *L. aquatica* sp. nov., and *L. floridensis* sp. nov.); clade III consists of five species, all recently identified (*L. rocourtiae*, *L. weihenstephanensis*, *L. cornellensis* sp. nov., *L. grandensis* sp. nov., and *L. riparia* sp. nov.); and clade IV contains the previously known species *L. grayi*. *Listeria* spp. from foods and food-related environments tend to be *L. monocytogenes*, *L. welshimeri*, *L. innocua*, and *L. seeligeri*, while the majority of the novel species recently identified were from other ecosystems (soils, water, and plants). Such trends suggest that additional novel species of *Listeria* will be identified as isolations of the organism from diverse ecosystems are more extensively pursued.

Within the genus *Listeria*, *L. monocytogenes* is the only species that is pathogenic to both humans and other vertebrates, while *L. ivanovii* is pathogenic only to ruminants.

L. Monocytogenes as Foodborne Pathogen

General Attributes of *L. Monocytogenes*

L. monocytogenes was first identified in 1924 by Murray, Webb, and Swann and named as *Bacterium monocytogenes* due to pronounced monocytosis observed in infected rabbits and guinea pigs. In 1929, *L. monocytogenes* was isolated for the first time from humans that had mononucleosis-like disease.

L. monocytogenes is a facultative intracellular pathogen with the ability to grow between 1 and 45 °C. Its ability to grow at low temperature qualifies it as a psychrotolerant and has important food implications. On blood agar, *L. monocytogenes* typically exhibits narrow beta-hemolytic zones, while pronounced hemolytic zones are produced by *L. ivanovii*. With the exception of *L. seeligeri*, nonpathogenic *Listeria* species are typically nonhemolytic (although occasional exceptions involving atypical strains have been reported). *L. monocytogenes* is motile via peritrichous flagella at low temperatures (up to ~28 °C) with motility repressed at 37 °C. Cold enrichment was earlier employed for isolation of *Listeria* from environmental samples. However, food safety interest in *L. monocytogenes* has promoted development of novel selective enrichment approaches and chromogenic media, greatly improving the sensitivity and speed of isolation of the pathogen.

Symptoms and Epidemiology of Listeriosis

L. monocytogenes is responsible for severe, systemic illness (listeriosis) with symptoms such as meningitis and meningoencephalitis with neurological symptoms, septicemia, abortions, and stillbirths. Although relatively uncommon, listeriosis remains a leading cause of death and hospitalizations due to foodborne illness in the United States. At high risk are pregnant women and their fetuses, neonates, the elderly, and immunocompromised patients. Mortality can be high (~21%, even under use of antibiotics). In addition to invasive listeriosis, febrile gastroenteritis without accompanying mortalities can occur in individuals without obvious risk factors. As in humans, in other vertebrates, invasive listeriosis is typically accompanied by neurological symptoms and stillbirths.

Even though 13 serotypes of *L. monocytogenes* are known, >96% of human listeriosis cases involve serotype 1/2a, 1/2b, and 4b. Two major genomic divisions (lineages) are represented among strains implicated in most human listeriosis: lineage I (serotypes 1/2b and 4b) and lineage II (serotype 1/2a). A small number of genetically related strains (epidemic clones) have been repeatedly involved in human listeriosis outbreaks. Animal listeriosis involves not only serotypes 1/2a, 1/2b, and 4b (also observed in human disease) but also strains of serotypes 4a and 4c (lineage III); the latter are uncommon in human listeriosis.

Foodborne Transmission of *L. Monocytogenes*

Although nosocomial infection of neonates and transmission through direct contact with animals has been reported, the primary transmission route of *L. monocytogenes* is foodborne. Processed, cold-stored, ready-to-eat foods are typically implicated in listeriosis with most outbreaks involving deli meats, soft (especially Mexican-style) cheese, and fresh produce. Soft cheese (especially Mexican-style) and fresh produce were the primary food categories associated with *L. monocytogenes* cases in the United States between 2009 and 2011.

Foods are typically contaminated via transfer of the pathogen from contaminated environmental sources and equipment. *L. monocytogenes* is killed following cooking or other thermal treatments. Risks occur with foods that are untreated or minimally treated (e.g., fresh produce), inadequately treated (e.g., soft cheeses made from raw milk), or subject to post-processing contamination and consumed without further

treatment. Risk levels depend on several factors, including temperature and duration of food storage, ability of *Listeria* to grow in or on a specific food (food composition, ingredients, other microbiota, etc.), and the virulence of the contaminating strain(s). In the United States, *L. monocytogenes* is considered an adulterant for ready-to-eat foods (to be consumed without further cooking or reheating), and 'zero tolerance' levels are enforced for such foods. In certain other countries, the presence of *L. monocytogenes* is permitted up to a certain threshold (not to exceed 100 viable cells per gram) in ready-to-eat foods that do not permit growth of the pathogen.

Survival and Growth in Foods

A number of stress tolerance attributes of *L. monocytogenes* provide advantages for its survival in foods. These include salt and acid tolerance and commonly involve regulation by the alternative sigma factor sigma B. Of special food safety relevance is the ability of *L. monocytogenes* to grow at refrigeration temperatures, as foods implicated in listeriosis commonly include cold-stored products (deli meats, soft cheeses, fresh produce, etc.). This has led to extensive research on the cold tolerance and cold shock response mechanisms of *L. monocytogenes*. One of the characterized cold survival mechanisms involves changes in cell membrane fatty acid composition. *L. monocytogenes* adapts to low temperatures by having shortened fatty acid chains and differentiation of the branching of the fatty acids from iso to anteiso, promoting cell membrane fluidity at low temperature. Another mechanism for survival at low temperatures is uptake of compatible solutes, such as glycine betaine and carnitine. *L. monocytogenes* accumulates glycine betaine and carnitine by acquiring these compounds from the growth media during its growth at low temperatures, and low-temperature growth is enhanced in the presence of these compatible solutes. RNA helicases critical for prevention of RNA misfolding at low temperature and for transcription initiation are also critical for the ability of *L. monocytogenes* to grow in the cold. In addition to low temperature, certain RNA helicases also impact responses to heat, alkali, oxidative stress, and ethanol.

Persistence in Food-Related Environments

L. monocytogenes strains of certain serotypes, for example, 1/2a, 1/2b, and 1/2c, tend to be more commonly isolated from food processing environments. Molecular subtyping has repeatedly revealed strains that persist in the same processing plant over many years, sometimes decades. It has been proposed that, depending on their access to specific harborage sites in the processing plants, all *L. monocytogenes* strains are capable of persistence. However, strains differ in a number of attributes that may impact their ability to persist in the environment, including biofilm forming potential, resistance to disinfectants, cold tolerance, and resistance to *Listeria*-specific viruses. For instance, strains of a major epidemic clone implicated in several outbreaks in the United States and elsewhere were resistant to all tested *Listeria*-specific viruses when grown at temperatures below 30 °C. The molecular ecology of *L. monocytogenes* in the food processing ecosystem remains poorly understood.

Prevention and Control of Food Contamination by *L. Monocytogenes*

To prevent contamination of foods by *L. monocytogenes* at the processing plants, effective cleaning and sanitation procedures must be employed. Food contact surfaces should be regularly tested for *L. monocytogenes*, and identification of positive samples should be followed by enhanced cleaning and sanitation. *Listeria* spp.-positive samples may suggest the simultaneous presence of *L. monocytogenes*.

Several food-level interventions can also inactivate *Listeria* and reduce or prevent contamination. Adequate heating of the food product will inactivate *Listeria* that may be present, and numerous other postprocessing treatments (high pressure, irradiation, etc.) have shown potential to reduce levels of viable *Listeria* in the food. Pasteurization of raw milk will inactivate *Listeria*, suggesting the need to pasteurize milk for soft cheese production. Refrigeration will reduce the rate of growth and freezing will prevent growth though *Listeria* may remain viable and grow further after thawing. Certain combinations of acid and salt will prevent growth, suggesting the need for modified formulations in manufacture of ready-to-eat meat products. Substantial research is investigating uses of GRAS antimicrobials such as nisin and essential oils against *Listeria*, alone or in combination with other ingredients and hurdles. Biological controls include *Listeria*-specific viruses (phages), which have been approved for use in ready-to-eat foods and other microbes with competitive exclusion potential against *Listeria*.

Virulence and Pathogenicity

Several key virulence factors including listeriolysin O (LLO), phospholipases, and ActA (mediating intracellular motility and actin polymerization) are clustered in one pathogenicity island (*Listeria* pathogenicity island 1 (LIPI-1)) on the chromosome of *L. monocytogenes*. These virulence genes are regulated by the regulatory protein, PrfA, encoded by a gene also harbored on LIPI-1. The genes are thermoregulated, being expressed at 37 °C but not below 30 °C. LLO is a cholesterol-dependent cytolysin and a pore-forming toxin. LLO binds to the cholesterol of the host cell membrane and causes the formation of pore. Several signals (such as pH and temperature) are important for the activity of LLO, restricting it to avoid lysis of the host cells. This is essential for the intracellular life cycle of *L. monocytogenes*, as damage to the host cell would cause cell death and *L. monocytogenes* would be exposed to the components of host immune system. LLO plays a crucial role in virulence of *L. monocytogenes* as it enables escape of the organism from phagosomal compartment of the host organism to its cytoplasm, where it multiplies (Figure 1). In contrast, LLO-deficient mutants are known to be nonhemolytic and avirulent in murine models. Additionally, two membrane-active phospholipase C enzymes (PI-PLC and PC-PLC) encoded by LIPI-1 genes are involved in escape of *L. monocytogenes* from the phagosome of the host cell to the host cytosol. The actin polymerization protein, ActA, of *L. monocytogenes* is another essential virulence factor as it facilitates intracellular motility of the organism upon escape from the phagosome and

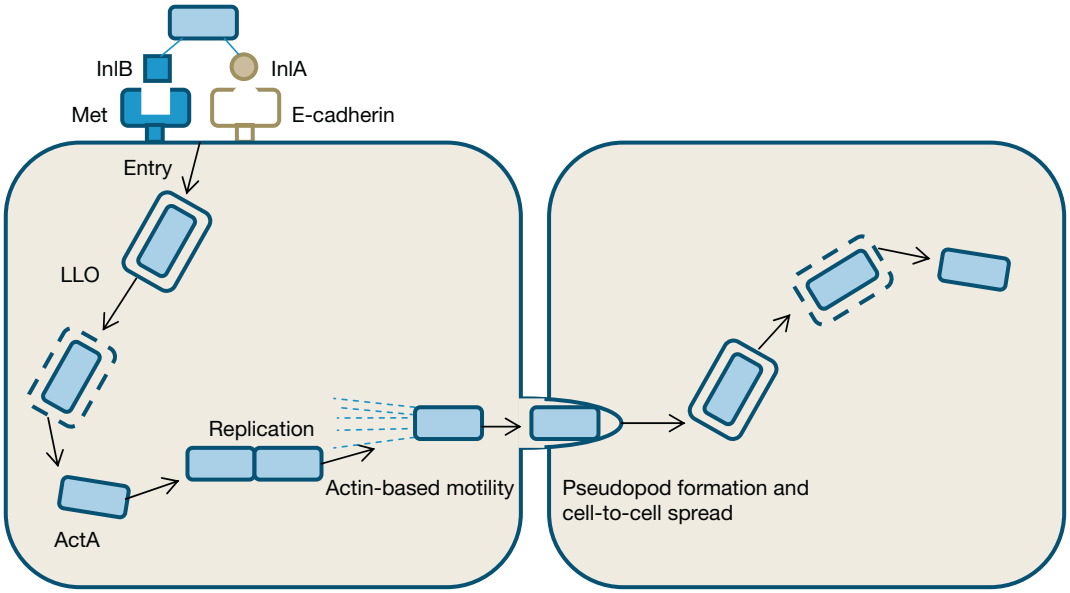

Figure 1 Invasion and cell-to-cell spread of *L. monocytogenes*. Reproduced from Tilney, L. G. and Portnoy, D. A. (1989). Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*. *Journal of Cell Biology* **109**, 1597–1608; Travier, L. and Lecuit, M. (2014). *Listeria monocytogenes* ActA: a new function for a 'classic' virulence factor. *Current Opinion in Microbiology* **17C**, 53–60.

cell-to-cell spread via membrane protrusions (pseudopods) that become engulfed by neighboring host cells, forming double-membrane *Listeria* phagosomes harboring the bacteria (Figure 1). Direct cell-to-cell spread is essential for survival of *L. monocytogenes* in the host as it minimizes recognition of the organism by the immune response components of the host. ActA allows intracellular motility of *L. monocytogenes* even without involvement of other bacterial factors such as flagella. ActA has recently been shown to have intriguing roles in aggregation of *Listeria* in the intestinal lumen and biofilm formation.

Numerous additional factors of *L. monocytogenes* with possible roles in virulence have been identified, encoded by genes outside of LIPI-1 but in certain cases also under PrfA control. Extensively characterized are invasion-associated proteins such as members of the internalin (Inl) family, especially InlA and InlB (Figure 1). InlA is anchored into the cell wall and is essential for invasion of intestinal epithelial cells. It is recognized by E-cadherin in cells from certain hosts (e.g., humans, guinea pigs, and gerbils), while E-cadherin from mouse cells fails to bind InlA due to a single amino acid substitution (glutamic acid instead of proline). Interestingly, a large fraction (~50%) of food and environmental strains of serotypes 1/2a, 1/2b, and 1/2c harbor premature stop codons in InlA, resulting in truncated InlA that fails to become anchored into the cell wall and in virulence attenuation in animal models; such truncated InlA is rare among clinical isolates.

Animal and Cell Culture Models Used for *L. Monocytogenes* Virulence Determination

Numerous cell culture models have been employed for analysis of *Listeria* invasion and cell-to-cell spread. As *L. monocytogenes* has the capability to pass the gastrointestinal barrier and reach the placenta and the central nervous system, it is especially important to characterize its virulence and persistence in animal models. Therefore, a variety of model systems have been used to study virulence of *L. monocytogenes*, including mice, guinea pigs, chick embryos, and zebrafish.

Murine models

Murine models have been most commonly employed for the characterization of virulence mechanisms of *L. monocytogenes*. Intravenous inoculations have been extensively used to compare virulence based on multiplication of *L. monocytogenes* in the liver and spleen. However, *L. monocytogenes* infections are typically foodborne and there is need for oral and intragastric infection models. Persistence and spread (gallbladder and brain of the host) of *L. monocytogenes* following oral infection via feeding of inoculated food samples in murine models have been reported. However, oral infections in murine models require high numbers of *L. monocytogenes* as inoculum and are poorly reproducible in terms of lethality to the host or breach of the gastrointestinal barrier. Murine models are effective for the characterization of T-cell response to intravenous infection of *L. monocytogenes* but cannot be readily used for the assessment of the role of internalin (InlA) as the murine E-cadherin (receptor for InlA) is unable to bind InlA due to a single amino acid substitution (proline in the E-cadherin of species that recognize InlA, e.g., humans and guinea pigs, vs. glutamic acid in the mouse). Mouse strains expressing either the human E-cadherin or a modified murine E-cadherin that recognizes InlA have been developed and extensively employed.

Guinea pig and gerbil models

In contrast to mice or rats, guinea pigs carry a functional E-cadherin receptor for InlA. This characteristic of guinea pigs makes them favorable to use as a model system for *in vivo* characterization of virulence of *L. monocytogenes*. Thus,

L. monocytogenes can be isolated from maternal and fetal tissues of pregnant guinea pigs that were inoculated by *L. monocytogenes* through the oral route as early as 2 days following inoculation. Yet, strain-specific differences in virulence of *L. monocytogenes* may be found using the intragastric guinea pig infection model system. Also, guinea pigs (as mice and rabbits) lack the receptor for another major invasion-associated determinant, InlB. Of the different rodent species tested, gerbils, which are also a natural host for *L. monocytogenes*, were the only species with receptors for both InlA and InlB and are being extensively employed as model systems.

Other animal models

The chick embryo model has been used for virulence assessments of different *L. monocytogenes* strains, with virulence corresponding to embryo mortality. Zebrafish larvae have been employed for real-time observations of interactions between macrophages and *L. monocytogenes*, administered into the larvae intravenously. *Galleria mellonella*, an insect model system, has been employed with *L. monocytogenes*. It is especially attractive as inoculated *Galleria mellonella* can survive at 37 °C, a temperature that permits expression of key virulence genes of *L. monocytogenes*. This model is also attractive due to low costs, ease of use, and the absence of animal care restrictions associated with vertebrate animals in research.

Conclusions

In this article, an overview of occurrence and key features of *Listeria* is presented. Numerous adaptive features of *Listeria*, including its ability to grow at low temperatures, to tolerate several other types of environmental stress, and to persist in biofilms, mediate its persistence in food processing environments and its capacity to contaminate foods. At the same time, *L. monocytogenes* has evolved numerous sophisticated adaptations mediating its ability to function as a facultative intracellular pathogen. Upon consumption of contaminated foods without further processing, *L. monocytogenes* can cause listeriosis, a serious, foodborne disease with symptoms such as stillbirths, septicemia, and meningitis and with high fatality rate. Complete genome sequencing and advances in other omics tools have markedly enhanced our understanding of *Listeria*'s environmental adaptations, virulence, and epidemiology.

See also: Chilled Foods: Effects on Shelf-life and Sensory Quality; Codex Alimentarius Commission: Role in International Food Standards Setting; Food Environment; Foodborne Pathogens; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; *Listeria*: Detection; *Listeria*: Listeriosis; Risk Assessment of Foods and Chemicals in Foods; Sterilization of Foods.

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Low-fat Foods: Types and Manufacture

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Introduction

Organizations like the World Health Organization (WHO) are driving measures to reduce saturated fat content in foods by raising the consumers' awareness and setting guidelines around ingredient usage for companies. The prevalence of excess levels of adipose tissue buildup in the human body is widely recognized as a leading world health problem and is referred to as an obesity epidemic. The WHO found in 2008 that more than 1.4 billion adults, aged 20 and older, were overweight. Out of these overweight adults, 200 million men and nearly 300 million women were classified as obese. The WHO also found that more than 40 million children under the age of 5 were overweight in 2010.

Fat Replacers

Fat levels are very much on the agenda for the consumer when purchasing food, and food processors have endeavored to tackle the issue by the production of fat-reduced foods. However, fat also has some important sensory properties and contributes greatly to texture, mouthfeel, and satiety and assists in the sensation of lubricity in foods and overall flavor. It is thus very challenging for food processors to compensate for these qualities in fat-reduced food variants.

There are several ways in which reduced-fat foods may be produced. The first and simplest method is the partial reduction of fat through the use of technologies or alternative ingredients. This might include using baking instead of frying in the production of snack foods or the use of skimmed milk in yogurt production or leaner meat in a sausage formulation. The second method involves the replacement of fat with carbohydrate-, protein-, or lipid-based fat replacers that can be classified into either fat substitutes, fat mimetics, or bulking agents. Bulking agents are ingredients that increase the bulk of a food with minimal effects on sensory properties. Many of the carbohydrate fat mimetics are also bulking agents and include starch and cellulose-based components, as well as the polysaccharide mimetic polydextrose.

Fat Substitutes

Fat substitutes are materials that simulate the chemical and physical properties of fats and oils and can directly replace fat on a weight to weight basis. These materials can be produced by enzyme-modified oils and fats and can also be synthesized chemically. Fat substitutes quite often take on board the processing characteristics of conventional fats and can be used in baking and frying. Examples of common fat substitutes include fat-based sucrose polyesters such as olestra (Olean™), esterified propoxylated glycerol, fatty acid esters of sorbitol, and sorbitol anhydrides (Sorbestrin) (Table 1). One of the principal drawbacks of using olestra is that product labels must contain the warning 'This product contains olestra.' Olestra may cause abdominal cramping and loose stools. Olestra inhibits the absorption of some vitamins and other nutrients.

Fat Mimetics

Fat mimetics are compounds that simulate the sensory properties of fat and are often protein- or carbohydrate-based. Generally, they have a much lower calorific value compared to conventional fats and oils as they can bind relatively large volumes of water. For this reason, they can limit the processing characteristics of the reduced-fat foods they are incorporated into and, generally, cannot be used in products that are fried and carry water-soluble flavors. This issue can be partly resolved by the incorporation of emulsifying agents and more nonpolar or lipophilic flavoring compounds. Examples of common fat mimetics with generally recognized as safe status include carbohydrate-based compounds such as cellulose (Avicel™), starches (modified and dextrin), hydrocolloid gums (Slendid™), pectins, β-glucan, and inulin- or protein-based materials such as Simplesse® or Dairy-Lo® (Table 1). Simplesse® is a spray-dried, microparticulate protein powder used as a texturizer in many different foods such as dairy products (cheese, yogurt, and frozen desserts), margarine, salad dressings, sauces, and soups. Dairy-Lo® is a modified whey protein concentrate that can form a set matrix gel with heat and can hold the gel structure during shelf life and is quite often used as a fat replacer in cheese-based products.

Fluid gels offer a wide range of applications in reduced-fat food formulations. Gel-forming polysaccharides such as k-carrageenan, which form gels in the presence of water, can be used as fat replacers for emulsion-based, semisolid foods such as mayonnaise, margarine, custards, gravies, and pourable dressings. Hydrocolloids can form molecular gels through association of parts of the molecules forming 'junction zones.' Gel properties assist in the flavor release from gelled foods. Other common hydrocolloid food gelling agents include gelatin, starch, pectin, carrageenan, alginate, agar, celluloses, gellan gum, konjac, milk proteins, and soy proteins. Blends of the hydrocolloid κ-carrageenan and starch have also been widely adopted in the food industry as a texture enhancer for dairy products.

Meat Products

Researchers have been working on strategies to reduce animal fat usage in meat, and thus, this area has received considerable recent research focus on products such as frankfurters, breakfast sausage, beef patties, pork patties, bologna, and black and

Table 1 Fat substitutes and mimetics

	Fat mimetic				
		Carbohydrate			
Fat substitute	*Protein*	*Starch*	*Cellulose*	*Gums*	*Polysaccharide*
Salatrim Acyl triglyceride molecule Energy density: 5 kcal g^{-1} *Functional properties*: emulsify, stabilize, texturize, lubricate, flavor carrier *Application*: cheese, dips, sauces, dairy desserts, confectionary	*Simplesse* Whey protein or white egg protein Energy density: 4 kcal g^{-1} *Functional properties*: stabilize, texturize *Application*: cheese, yogurt, dips, mayo, dairy spreads, desserts	*Starches*: corn, potato, tapioca, rice, maize, waxy maize Energy density: 4 kcal g^{-1} *Functional properties*: texturizer, water holding, thickening, gelling *Application*: processed meats, margarines, sauces, dressings, baked foods	*Methylcellulose* Energy density: 0 kcal g^{-1} *Functional properties*: texturizer *Application*: frozen desserts, powdered soups/sauces	*Guar*, *locust bean*, *xanthan* Energy density: 0 kcal g^{-1} *Functional properties*: retard staling in baked goods, retain moisture *Application*: bakery goods	*Maltodextrin* Hydrolyzed starch Energy density: 4 kcal g^{-1} *Functional properties*: water binding, imparts mouthfeel, body, viscosity *Application*: processed meats, dairy spreads, salad dressings, baked foods, frozen desserts
Caprenin Caprocaprylobehenic triglyceride molecule Energy density: 5 kcal g^{-1} *Functional properties*: emulsify, stabilize, texturize, lubricate, flavor carrier *Application*: candy, confectionary	*Trailblazer* White egg protein and xanthan gum Energy density: 4 kcal g^{-1} *Functional properties*: stabilize, texturize *Application*: dairy, soups, sauces, baked foods		*Microcrystalline cellulose* Energy density: 0 kcal g^{-1} *Functional properties*: stabilizer, texturizer, imparts mouthfeel *Application*: dairy, salad dressing, sauces desserts	*Carrageenan* Energy density: 0 kcal g^{-1} *Functional properties*: texturizer, imparts mouthfeel, viscosity *Application*: processed meats, yogurts, salad dressings, desserts, ice cream, chocolate	*Polydextrose* Energy density: 1 kcal g^{-1} *Functional properties*: texturizer, bulking agent *Application*: confectionary, spreads, salad dressings, baked foods, frozen desserts, sauces, toppings
Olestra (*Olean*) Sucrose polyester Energy density: 0 kcal g^{-1} *Functional properties*: texturize, lubricate, flavor carrier *Application*: reduced-fat snacks, potato chips				*Pectin* Energy density: 0 kcal g^{-1} *Functional properties*: texturizer, imparts mouthfeel, viscosity *Application*: sauces	*β-Glucan* Energy density: 1 kcal g^{-1} *Functional properties*: texturizer *Application*: baked foods
				Gum arabic Energy density: 0 kcal g^{-1} *Functional properties*: texturizer, imparts mouthfeel, viscosity *Application*: dressings	

white puddings. Overconsumption of meat and meat products has been linked with obesity, cancer, and cardiovascular diseases primarily due to the high amounts of sodium chloride and saturated fat present in processed products. However, fats and oils play vital functional and sensory roles in various food products and are important in processed meat for flavor development and satiety, having a large impact on eating quality as they interact with other ingredients and assist in developing texture and mouthfeel and provide a lubricating effect.

For meat, there are several methods to produce fat-reduced products such as using leaner meat, less fat and salt, and more

water, replacing parts of animal fat with plant oil, and the application of fat replacers.

One of the first methods of fat reduction in processed meat systems is the reduction of fat in formulations without replacement. This involves the sequential reduction of salt and fat in processed meats and has been demonstrated for a number of such products including beef patties, frankfurters, breakfast sausages, and black and white puddings. Sensory methods are used to identify the optimal formulations with different meat systems varying in their respective optimization criteria. Both salt reduction and fat reduction have been shown not to negatively impact on consumer acceptability in patties and sausages, while only fat reduction could be achieved in frankfurters without negative assessor feedback.

By replacing animal fat in processed meats with vegetable oils, the fatty acid profile can be nutritionally optimized as well as producing a lower-fat- and/or cholesterol-containing products. Unsaturated oils such as vegetable or fish oils have been used as a partial substitute to animal fat. Palm oil, palm stearin, cottonseed oil, hazelnut oil, and mixes of these oils have been used to increase the ratio of unsaturated to saturated fatty acids and maintain product quality in processed meats. Similarly, olive oil, linseed oil, fish oil, and konjac oil have also been used to produce products scored as acceptable in consumer trials.

As explained earlier in this article, fat replacers are categorized into fat substitutes and fat mimetics. Fat substitutes display a chemical structure close to fats, have similar physicochemical properties, and are either low in calorie or indigestible such as olestra, caprenin, and salatrim. By contrast, fat mimetics show distinctly different chemical structures from fat, are usually protein- and/or carbohydrate-based, and can be used to simulate some of the physicochemical attributes and eating qualities of fat such as mouthfeel, appearance, and viscosity. The use of fat replacers can drastically change the sensory profile of a meat product as over half the volatile compounds found in meat originate from the lipid fraction. The use of fat mimetics is, in general, more common, for example, a fat reduction of 46% can be achieved in salami, 60–83% in frankfurters, and 55% in beef patties using tapioca starch, oat fiber, maltodextrin, or milk protein.

Numerous studies have highlighted the benefits to human diets of high plant fiber intake on overall and digestive health through regular bowel movement. These include reduced risk of cancers, coronary heart disease, obesity, and diabetes. Essentially, fiber is a complex carbohydrate that can be incorporated into processed meat formulations as partial fat replacers and positively affect physicochemical properties of the resulting product by potentially improving water binding, texture, cooking loss, and texture quality of processed meats such as sausages, puddings, bologna, and meatballs. Hydrocolloids including polysaccharides and proteins can also be used as fat replacers in processed meat products to increase water binding and texture properties. Hydrocolloids, which thicken, gel, bind, and stabilize pH and emulsions are generally animal-based proteins such as gelatin, blood proteins, casein, or whey. Several researchers have reported the effectiveness of fat reduction using whey protein, soy protein, and surimi. As with oil incorporation, textural differences were detected in the finished product, but these changes did not have a negative impact on overall sensory acceptability.

Stabilizers, emulsifiers, or thickeners are added to meat to help maintain a uniform texture and a diverse range of polysaccharides are available on the market. These include celluloses (carboxymethyl cellulose), gums (guar, alginate, pectin, and locust bean), fibers (β-glucan), starches (corn, wheat, maize, potato, and tapioca, pea), and chitosan and xanthan derived from microorganisms. Starch can be added with either κ-carrageenan or locust bean gum to produce similar results in terms of texture as full-fat products. Cold-set binders are important to the meat industry as they can optimize the textural properties of a meat system, without the application of heat, thus keeping the meat product in its natural raw uncooked state. They also have applications in the replacement of salt and fat and the production of healthier processed meats. Examples of cold-set binders include nonthermal gelatin, alginate, and fibrin. Commercially available cold-set binding systems include Fibrimex™, alginate, and Activa™.

Dairy Products

Fat has an important sensory role in dairy products, contributing to both flavor and texture, and thus, technological difficulties can be encountered when levels are reduced. Cheeses like cheddar and mozzarella become firmer and more rubbery on removal of fat because of increasing protein content. Flavor is particularly affected as the fat/protein matrix changes and affects flavor perception deleteriously. Several studies have investigated the sensory optimization of reduced-fat cheese through the use of curd washing and incorporating adjunct cultures in cheddar cheese. The adjunct *Lactobacillus helveticus* in combination with *Leuconostoc cremoris*, *Lactococcus lactis* var. *diacetylactis*, and *Streptococcus thermophilus* can be used to produce 50% fat-reduced cheddar cheese with a higher preference score than for a similarly produced cheese manufactured without the use of the adjunct culture. However, the flavor profile of the end product is different from regular cheddar primarily because of the presence of burnt off-flavor notes. Fat mimetics have also been investigated in mozzarella cheese using two protein-based fat replacers (Simplesse® D100 and Dairy-Lo®) and two carbohydrate-based fat replacers (Stellar™ 100X and Novagel™ RCN-15).

Flavor optimization of reduced-fat cheddar cheese can also be achieved by removing the fat from normal-aged cheddar once the flavor had fully developed. According to sensory studies, this approach managed to retain the same flavor intensity as regular cheese, although the texture was quite different. It is also reported that reduced-fat emmental cheese made using a preprocessing routine involving homogenization of milk improved the sensory aspects and market position of these reduced-fat cheeses compared with full-fat emmental cheese made with the same process.

The protein-based fat replacers Dairy-Lo® and Simplesse® D-100 and the carbohydrate-based fat replacers Perfectamylgel MB and Satiagel ME4 have been investigated to determine the effects on the chemical, physical, and sensory attributes of low-fat, white-pickled cheese. These low-fat cheeses, produced by adding Dairy-Lo®, Perfectamylgel MB, and Satiagel ME4, were highly acceptable compared to the low-fat cheese manufactured without fat replacers. Also, differences between cheeses

made with Simplesse® D-100 and full-fat cheese or low-fat cheese manufactured without fat replacer, as found by the taste panel, were not detectable.

Fat is integral to the formation of the microstructure of ice cream, particularly aeration, as it stabilizes the air phase and freezing and is important as a carrier of lipophilic flavors and in their release during consumption. It is thus a difficult challenge for food processors to reduce the fat content of ice cream and maintain these fundamental qualities. Fat also increases the creamy flavor notes and mouth coating, in full-fat ice creams compared with low-fat ice cream. Generally, low-fat ice creams have lower flavor and texture ratings when measured by sensory analysis and can display textural defects like coarseness, iciness, crumbliness, shrinkage on storage, and reduced flavor release. Nonetheless, ice cream manufacturers have attempted to simulate the texture and flavor of fat-containing ice cream by adding bulking agents and concentrated milk components to fat-free formulations. Carbohydrate bulking agents, such as maltodextrin and polydextrose, are currently used in low-fat formulations because they produce minimal negative effects on ice cream production, shelf life, and price.

Inulin can also be used as a fat replacer in ice cream as it increases the viscosity and decreases the freezing point and produces a product with acceptable sensory properties. The protein-based fat replacers, Dairy-Lo® and Simplesse®, have been used to make both low-fat and nonfat ice creams. Simplesse® has been observed to be more similar to milk fat than Dairy-Lo® with respect to textural stability but was less similar in terms of thickness and mouth coating.

Confectionary Products

Fat and sugar cannot be easily replaced, especially in a complex food system such as biscuits. The fat mimetics, Oatrim and Z-Trim (both derived from oats), have been used in cookies and produced acceptable sensory characteristics at 50% fat replacement. The fat substitutes derived from pectin, gums, and oat bran (Slendid, Kel-Lire BK, and Trimchoice-5) have also been used to replace the fat in biscuit manufacture with the result that tenderness decreased as substitute use increased when biscuits were rated by the sensory panel. In a consumer location test, assessors rated the biscuits as moderately acceptable. Inulin (Raftiline, Orafti Active Food Ingredients, Oreye, France), Simplesse®, C*deLight (Cerestar, Brussels, Belgium), and polydextrose (Litesse (Pfizer, Inc., New York, NY, USA)) have been used for partial fat replacement and lactitol, sorbitol, and maltitol, for sugar replacement in low-fat, sugar-free cookies. Cookies prepared with Simplesse® had the least acceptable flavor, while cookies prepared with C*deLight were rated as the most acceptable by a sensory panel. Simplesse® has been used as an oil replacer in high-fiber muffins, which produced products with better stability toward batter refrigeration than full-fat muffins. These reduced-fat muffins were different from the freshly baked, full-fat control muffins, but they were still of comparable sensory quality. It has also been observed that Simplesse® does not affect full-fat taste when it replaced fat up to 93% in cakes, muffins, and brownies. Polydextrose (Litesse), acid-treated cornstarch (Stellar, A.E Staley, Decatur, IL), Slendid™, mono- and diglycerides, Simplesse®, and potato maltodextrin have been observed to replace 50% fat or over in chocolate chip cookies, but products had different flavor characteristics compared to full-fat control products.

Consumer studies have revealed that fat replacement with up to 15 g/100 g of inulin or hydroxypropyl methylcellulose provided acceptable biscuits, but higher replacement decreased overall acceptability. The replacement of up to 25% of shortening in crisp oatmeal cookies can be also achieved using polydextrose (Litesse) while maintaining physicochemical and sensory properties.

Salad Dressing and Sauces

Xanthan gum, as it forms weak gels, is often used in salad dressings. Polysaccharides (carrageenan, xanthan, pectins, etc.) are also incorporated into commercial salad dressing products, particularly those of low oil content as they stabilize the oil droplets and prevent creaming. Fat mimetics such as Simplesse® can be used as fat replacers and texturizers in margarines, salad dressings, sauces, and soups. Trailblazer can also be used as a texturizer in sauces and soups. Cooked starch may be incorporated into these products since starch gelatinization results in the release of polysaccharides, which act as emulsion stabilizers and thickeners. Corn, waxy maize, wheat, potato, tapioca, rice, and waxy rice starches can be used as fat mimetics in dressings, sauces, margarines, and spreads. Microcrystalline cellulose is used in reduced-fat salad dressings and maltodextrin has found applications in low-fat margarines, salad dressings, and sauces.

See also: Cakes: Types of Cakes; Fats: Classification and Analysis; Low-salt Foods: Types and Manufacture; Triacylglycerols: Characterization and Determination; Triacylglycerols: Structures and Properties.

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Low-salt Foods: Types and Manufacture

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Introduction

There is a worldwide concern for the high dietary sodium intake, which, in most cases, considerably exceeds nutritional recommendations. High sodium intakes may have relevant consequences for consumers' health.

The main concerns as regards NaCl consumption is that high levels of sodium intake may be crucial for the development of hypertension and the age-related rise of blood pressure typically observed in modern society. It has been observed that NaCl is an important factor in the blood pressure regulation, which is usually associated to a type of lifestyle including also overweight and physical inactivity. There is also an increase in the risk of cardiovascular diseases (CVD) such as strokes, heart attacks, and heart failure. Elevated blood pressure has a high incidence in population of Western societies. For instance, it affects approximately one in three US adults, but another 78 million adults suffer from slightly elevated blood pressure, which can in turn lead to high blood pressure.

Apart from the adverse influence of high salt consumption on blood pressure, there are other evidences of diseases that can be negatively affected by salt. Such is the case of renal disease, hepatic affections, water retention, stomach cancer, and osteoporosis. An increasing evidence of the relationship between salt intake and obesity has also been reported.

All those negative effects of salt on health leads to the fact that salt reduction is very cost-effective apart from the evident health advantages.

The main source of sodium in the diet is in the form of sodium chloride, typically referred to as table salt, and its daily intake in Western societies is estimated to be within the range of 8–12 g day^{-1}, which is considered very high and, consequently, needs to be substantially reduced.

On a popular basis, it has been established that the consumption of more than 6 g NaCl/day/person is associated with an increase in blood pressure with age. Therefore, limitation of dietary sodium intake should be achieved by restricting daily salt (sodium chloride) intake to <5 g day^{-1}. Such recommendations are addressed to the general public. It is, however, generally recognized that those individuals, who are genetically salt-susceptible or suffering from hypertension, will particularly benefit from low-sodium diets. In such cases, the salt content should range between 1 and 3 g day^{-1}.

It has been observed that a reduction in the NaCl intake of 3 g day^{-1} results in blood pressure reduction of between 3.6–5.6 and 1.9–3.2 mmHg (systolic/diastolic) in persons with hypertension. Taking into account those values, it has been estimated that a reduction of 13% in cerebrovascular accident and 10% in CVDs could be achieved in the overall population with a NaCl reduction of 3 g day^{-1}. Further reductions could be achieved by further decreasing the NaCl intake.

Health authorities in most industrialized countries are trying to progressively reduce the salt content in foods by either instructing industry to add less salt to foods during processing or recommending consumers to add less salt during cooking.

This task is not easy to achieve due to the technological relevance of salt during processing and its effects in the final product quality, especially with respect to flavor enhancement. In fact, the effects of NaCl are so pronounced that its contribution to flavor, lower water activity, higher microbial stability, activation/inhibition of enzyme activity, increased water-holding capacity, protein solubility, and texture are difficult to substitute or replace. There are different approaches used in the reduction of salt in foods, and these are summarized in this manuscript.

Manufacture Strategies for Low-Salt Foods

Health institutions and agencies have assisted the food industry in selecting target foods where salt reduction is more of a priority on account of the reduction needed to achieve the desired salt intake reduction. Most dietary sodium comes from processed foods, prepackaged foods, and restaurant foods. Some of these targeted foods include meat products like bacon, ham, sausages, and meat pies; dairy products like cheese and butter; different types of breads, biscuits, crisps and snacks; sauces and dressings; and canned foods. The contribution of added salt during home cooking is quite low, and the natural occurrence of salt in foods is around 12%. It is also important to understand the mechanisms for taste perception and physiological controls, especially how the salt in the product interacts with and is perceived by the tongue. For instance, the use of salts with different sizes or shapes may maximize saltiness perception while using less salt. So, the strategies followed for salt reduction in foods are as follows:

Sodium Reduction

The easiest way to reduce the salt content in food products is simply through the reduction of added salt during processing. This means that ingredient formulations are modified to contain less salt. If brine is used in the manufacturing process, either the amount of salt in the injected brine or the duration of brining may be reduced. If salt is incorporated by rubbing on the outer surface of the food, as in the case of some muscle foods like dry-cured ham, the reduction may be achieved by reducing the salting time as less salt penetrates into the product. However, one limitation to using this approach, in addition to the shelf-life of the food, is that you must have a clear understanding of what the consumer will and will not accept in terms of salt reduction for each specific product. Therefore, attempting to provide a single salt concentration reduction strategy will not work in reality.

The main concern in reducing salt in processed food products is related to the preservation action of salt and consequently to food safety. When salt is reduced, the water activity of food increases if the moisture content is maintained

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at original levels in the product formulation, and thus, an increase in the rate of spoilage reactions occurs. Consequently, alternative preserving techniques may be necessary to ensure the safety of food, such as the following:

- Use of other solutes that can contribute to reduce the water activity of food, such as polyols or sugars
- Use of nonthermal preserving techniques, such as high pressure or electric pulses
- Addition of preservatives that enables the control of microbial growth
- Use of hurdle technology (combination of packaging, low or high temperatures, modifying acidity, and redox potential, among other factors)

As highlighted previously, the influence of salt reduction on the sensory perception of food is also an issue of major concern, and the final product could achieve sensory properties that preclude it from consumption by consumers. Sometimes, it is possible to add products that can hinder the taste and flavor changes as a consequence of salt reduction, such as the addition of natural acids, spices, flavorings, and yeast hydrolysates. Some products that have been used in this manner are pepper, onion, garlic, tomato, sweet pepper, basil, parsley, thyme, celery, lime, chili, nettle, rosemary, smoke flavoring, curry, coriander, and lemon.

There are other aspects that are influenced due to the reduction of sodium content, such as enzyme activity, water-holding capacity, protein solubility, and texture that can be influenced to such an extent that it becomes impossible to further reduce salt in products.

For example, salt reduction in dry-cured ham results in an increased enzymatic activity, mainly through the presence of endopeptidases, which can result in an excessive breakdown of myofibrillar proteins, thereby leading to an excessive softness.

Sodium Replacement

When direct NaCl reduction is not a feasible alternative due to technological or sensory constraints, sodium replacement can constitute a valid alternative. So, NaCl can be partially or totally replaced by other edible salts that do not contain sodium. The most commonly used salts in this strategy are potassium chloride and potassium lactate. Other salt forms that have been examined for suitability of use are magnesium chloride, calcium chloride, potassium citrate, and potassium acetate, among others. Those salts can be used alone or in combination.

The use of salts targeted at replacing sodium in food has some factors that must be taken into account. The first factor that must be considered is the different solubility of each salt, which makes the control of the salting process difficult; the formed brine on food surface, when using dry salting, can have a different concentration than the used salt mixture. Additionally, the pH of the brine can change dramatically depending on the salt mixture, which can in turn affect the water-holding capacity and other quality and safety aspects of the product. In the same sense, it has to be considered that the salt diffusion inside the food product depends on each ion type present. Some studies have demonstrated that the diffusion rate is higher in monovalent ions than with divalent ions. This implies that the salt composition inside the salted food can differ to a great extent from the salt composition present in dry salt or brine.

Another effect related with sodium replacement is the capability of each salt type used to reduce water activity, which, as pointed out previously, can affect the safety status of the salted product. This is another major issue of public health concern that has to be considered.

Apart from those effects, the use of other salt mixtures can influence food texture and color, flavor, enzyme activity, or antimicrobial activity. This implies that the processing conditions must be adapted to the new salt formulation and/or other compounds must be added to the food in order to compensate for the new effects generated by the use of these salts. One of the most undesirable effects related with the use of non-sodium-containing salts are the off-flavors associated with their use, and these have been described as unpleasant in taste, with bitter or metallic aftertaste, especially when potassium, magnesium or calcium chlorides are used. Therefore, masking agents can be used to reduce these unpleasant tastes, such as pepper, onion, garlic, tomato, sweet pepper, basil, parsley, thyme, celery, lime, chili, nettle, rosemary, smoke flavoring, curry, coriander, and lemon.

National Plans for Salt Reduction in Foods

The National Salt Reduction Initiative (NSRI) was launched by New York City, and its main aim was to encourage food manufacturers and chain restaurants to voluntarily achieve effective sodium reductions in the United States. It was planned to reduce 25% sodium in the food supply by 2018 and reduce population sodium intake by 20% within the same time period.

In most countries, national health authorities are recommending that the food industry in the respective jurisdictions reduce the amount of salt formulated during food processing. To date, Finland has achieved a 30% reduction in salt intake, while in the United Kingdom, the Consensus Action on Salt and Health (CASH) and the UK Department of Health reached an agreement to achieve 20–30% reduction in the salt content of processed foods that can be bought in supermarkets. Similar actions are being taken in other European countries.

Current Regulations on Labeling of Foods with Reduced/Low Salt Content

There are regulations about the terminology that can be applied to packaging labels when claiming salt reduction or low salt content in foods. In the European Union, Regulation EC 1924/2006 on nutrition and health claims on foods described the terms that may be used in claims for foods with low salt content. So, this regulation distinguishes the following types of claims depending on the degree of sodium content: low in sodium/salt for <0.12 g of sodium, or the equivalent value for salt, per 100 g or per 100 ml; very low in sodium/salt for <0.04 g of sodium, or the equivalent value for salt, per 100 g or per 100 ml; sodium/salt-free for <0.005 g of sodium, or the equivalent value for salt, per 100 g; and reduced

sodium/salt for at least 25% less sodium, or the equivalent value for salt, than in the original food product.

In the United States, the Nutrition Facts Label lists the percent daily value (%DV) of sodium in one serving of a food, which is based on 100% of the recommended amount of sodium, which is < 2400 mg day^{-1}.

The FDA *Food Labeling Guide* stipulates the terms in respect of sodium that can be labeled in the package: salt/sodium-free for < 5 mg of sodium per serving, very low sodium for 35 mg of sodium or less per serving, low sodium for 140 mg of sodium or less per serving, reduced sodium for at least 25% less sodium than in the original product, light in sodium or lightly salted for at least 50% less sodium than the regular product, and no-salt-added or unsalted when no salt is added during processing, but this does not mean necessarily sodium-free making necessary to check the Nutrition Facts Label to be sure. When the food exceeds 480 mg of sodium per serving but other health claims are made (e.g., low in fat), then a disclosure statement is required.

See also: Hypertension and Diet; Potassium: Physiology; Sodium: Properties and Determination.

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Lupine

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Introduction

Functional foods are attracting more interest at the consumer level due to the potential to provide health benefits. Proteins sourced from plants are considered to be valuable ingredients in the preparation of functional foods by food industry. In recent times, lupine (*Lupinus* spp.) a legume crop has been investigated for its health benefits in relation to its high protein and fiber contents. Lupine-enriched foods increase satiety and reduce energy intake, decrease blood pressure, and decrease blood glucose and cholesterol levels. The lupine seed proteins have been considered to be the main contributor to these claimed health benefits. The other reported bioactivities of lupine protein include plasma cholesterol- and triglyceride-lowering effects, antihypertensive properties, and angiotensin-converting enzyme inhibitory activity.

Lupine has been used as a green manure since early Greek times and as a cultivated crop plant for more than 3000 years. There is evidence of lupine consumption in human food since early Egyptian times. The Mediterranean region and South America are considered the ancient homes of this crop; it was gradually introduced into Europe to improve soil health.

Evolution of *Lupinus*

In the family Fabaceae, *Lupinus* L. is a large genus consisting of herbaceous, soft-woody shrubs and small tree species. Herbaceous lupines include 200–500 annual and perennial species that are mainly used as grain crops. The diversified species of lupine are grouped as Old World and New World species. The New World species compose >90% of the total species that are distributed from Alaska to South Argentina and Chile. The Old World species include 13 lupine species from the Mediterranean and African regions, which are annual, herbaceous, and large-seeded. Three Old World species (*L. albus* L. (white), *L. angustifolius* L. (narrow-leafed lupine/Australian sweet lupine), and *L. luteus* L. (yellow)) and one New World species (*L. mutabilis* (bitter)) are cultivated as crops for human consumption (Figure 1). Some other species are used as green manure, forage, and ornamentals and for land stabilization.

Lupines represent a group of species characterized by a diverse geographic distribution and morphological and chromosomal polymorphisms, which potentially have large variation in seed protein levels. During the last 50 years, systematic and taxonomic relationship studies among lupine species were with particular emphasis on Old World species mainly because of the limited number of species and economic interest as fodder, food, and N-fixing crops. Based on seed coat texture, Old World species had been categorized as either smooth- or rough-seeded species, which generally agrees with chemotaxonomic and morphological relationships among the species. The five smooth-seeded species are further categorized in four sections: *Albi*, *Micranthi*, *Angustifoli*, and *Lutei*. Six or seven species have been identified

Figure 1 Commonly cultivated lupine species: *Lupinus angustifolius* (narrow-leafed lupine or blue lupine or Australian sweet lupine), *L. albus* (white lupine), *L. luteus* (yellow lupine), and *L. mutabilis* (pearl lupine).

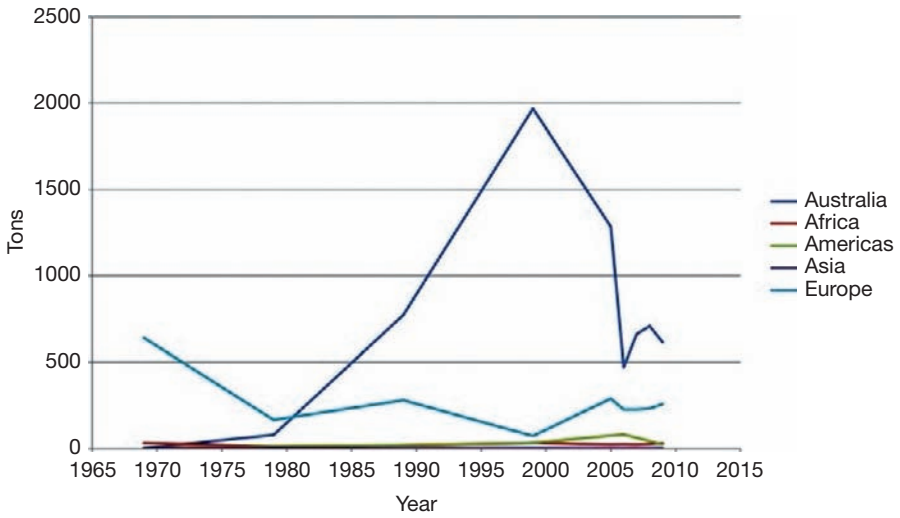

Figure 2 Production of lupine in various years from 1969 to 2009 by continent. Prepared based on the data obtained from FAOSTAT database (2011). FAO in http://faostat.fao.org/site/567/DesktopDefault.aspx.

as rough-seeded with higher interspecies resemblance and typical scabrous, tuberculate testa, which is unique to the genus.

In contrast, New World lupines comprise hundreds of annual and perennial species, which makes phylogenetic relationships very complex within this genus. New World lupine species are mainly distributed in South and North America. For North American species, 18 groups have been categorized comprising ~200 species, although >1700 names have been proposed for New World *Lupinus*. New World lupines are reported as largely polymorphic species by floristic investigations. The complexity of this genus may come from its high morphological, breeding, and ecogeographic diversity and the lack of clear diagnostic characters to distinguish species. Although a number of groups and complexes are already recognized, some groups are roughly circumscribed, which may be valuable for further modern analyses.

Current Production

Lupine occupied 2.7% of world pulse production (tonnes) in 2009. In the last three decades, Australia has been the largest lupine producer, capturing approximately 70% of the world's production (Figure 2). The other major producers are European countries. Blue lupine (*L. angustifolius*) is mostly cultivated in Australia, while white (*L. albus*) and yellow (*L. luteus*) lupines are traditionally cultivated in Europe and South America. In African and Mediterranean countries such as Morocco, South Africa, Syria, and Egypt, mainly *L. albus* and *L. luteus* are grown. Bitter *L. mutabilis* is still cultivated in some parts of Ecuador, Peru, and Bolivia.

In Australia, the state of Western Australia produces roughly 70% of the country's total followed by South Australia, New South Wales (NSW), and Victoria. The lupine industries in Western Australia and South Australia are dominated by *L. angustifolius*. The world's largest lupine-breeding program has been based in Western Australia since the 1960s and includes a goal to improve cultivars suitable for human consumption.

Consumption

A major part of the global lupine production is used by stock-feed manufacturers. Consumption by ruminants (cows and sheep) is the most significant, followed by pigs and poultry. Lupine has been increasingly used as a feed source in the aquaculture industry in recent years.

Although lupine is largely used as animal and fish feed, it has been used as a human food since ancient times. Different communities in the world have traditional methods of producing lupine-enriched food. Most commonly, lupine seeds are first soaked in water to remove most of the alkaloids, followed by cooking or toasting. With the improvement of nutritional science and equipment in food industries, lupine is now being considered for many food preparations such as bread, cookies, cake, pasta, noodles, muffins, and drinks. Although about 4% of global production is currently consumed as human food, it has been reported that ~500 000 tonnes of lupine-enriched food product is consumed annually in the EU, mainly through inclusion of lupine flour in wheat-based baked goods.

Composition of Lupine Grain

Nutritional Value of Lupine

Oilseeds and legumes are the most commonly consumed crops due to their high protein, mineral, and vitamin contents. Among the legumes, soybean is one of the most important for its nutritional properties and desirable functionality. However, lupine is an excellent alternative to soybean or any other legume due to its high protein content, low starch and lipid contents, and high dietary fiber (nonstarch polysaccharide). Analyses of nutritional values of sweet lupine (Figure 3) have shown that the bioavailability of the constituents is comparable to those of processed soybeans.

Amino Acid Composition of Proteins

Lupine seeds contain approximately 40% protein (by weight). Seed proteins occupy a key position in the interests of nutritionists, not only for their nutritional role but also for a

number of other beneficial effects for the human body like cholesterol-lowering effect and enzymatic activities.

Lupine represents an important source of proteins for human beings (Table 1), with a biological value of 91% compared to egg proteins. Storage globulins, which make up the bulk of the protein of legume seeds, contain more than the adequate levels of most of the essential amino acids and make this group a convenient protein source. For example, lupine proteins contain high levels of lysine, an amino acid that is underrepresented in cereals generally.

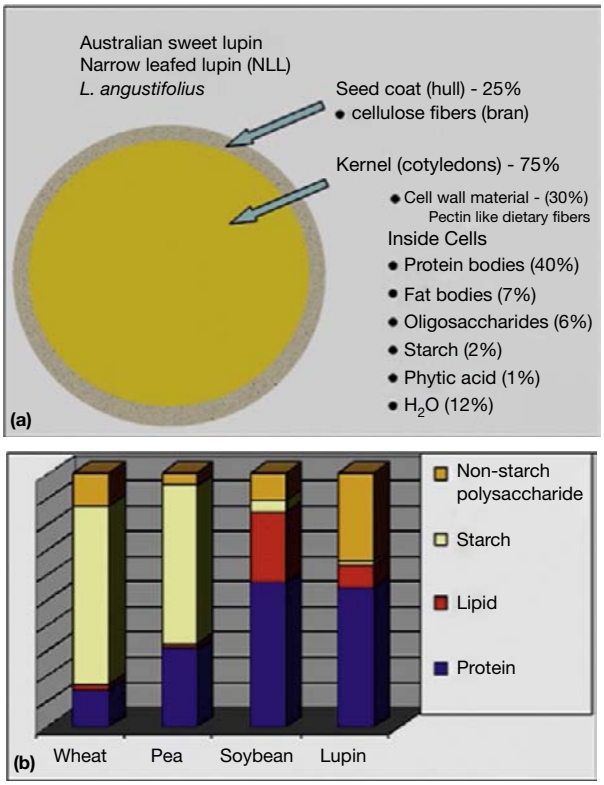

Figure 3 Comparison of major nutritional components of lupine (a) and a comparison among wheat, pea, soybean, and lupine (b). *Source*: Pulse Western Australia (http://www.lupins.org/feed/). Layman D, Anthony TG, Rasmussen BB, Adams SH, Lynch CJ, et al. (2015) Defining meal requirements for protein to optimize metabolic roles of amino acids. *American* 101(Suppl):1330S–1338S; Leidy HJ, Clifton PM, Astrup A, Wycherley TP, Westerterp-Plantenga MS, Luscombe-Marsh ND, et al. (2015) The role of protein in weight loss and maintenance. *American Journal of Clinical Nutrition* 101 (Suppl):1320S–1329S.

Fiber

A significant nutritional component of lupine seed is its high fiber content, which has health benefits for human consumption. Lupine kernel fiber (LKF) is a novel insoluble nondigestible carbohydrate that also has value as a fat replacer. It is beneficial for bowel health and the digestive system. LKF is primarily insoluble but is similar to pectin, a soluble fiber, in its chemical structure. LKF also appears to have an unusually high water-binding capacity and viscosity compared with other insoluble fibers, indicating the potential to induce physiological effects more characteristic of soluble fibers, such as lowering glycemic response.

Other than the direct consumption of LKF in lupine flour, kernel fiber can also be manufactured from dehulled lupine seeds after extraction of protein, lipid, and soluble carbohydrate fractions. This fiber, in the form of a pale-colored powder with bland flavor, can be used to boost the dietary fiber content of baked goods while maintaining high levels of consumer acceptability.

Minerals

See Table 2.

Antinutritional Factors

Like some other grain legumes, lupine has antinutritional components (Table 3). Some of these, such as alkaloids, are generally toxic to humans and have an adverse effect on taste. In the developed/modern varieties of lupine, these components have been reduced to tolerable levels. For example, Australian sweet lupine is considered suitable for human consumption since it has low levels of bitter-tasting and potentially toxic alkaloids compared with wild species. The risk level of alkaloid content of lupine for human consumption is >0.012 on a dry weight basis, whereas most of the cultivars of narrow-leafed lupine have alkaloid between 0.004% and 0.02%. Furthermore, lectins and protease inhibitors, which can reduce protein digestibility,

Table 1 Essential amino acid content of narrow-leafed lupine protein in comparison with wheat and egg proteins (g/16 g N)

	Narrow-leafed lupine protein			
Amino acids	*Mean (min–max)*	*Number of samples tested*	*Wheat protein (whole grain)*	*Egg protein (whole egg)*
Arginine	11.62 (10.6–12.9)	116	3.98	6.4
Histidine	2.57 (2.12–2.86)	118	2.21	2.1
Isoleucine	3.91 (3.57–4.41)	118	3.82	8.0
Leucine	6.90 (5.91–7.40)	118	6.72	9.2
Lysine	4.75 (4.22–5.19)	118	2.32	7.2
Methionine	0.66 (0.49–0.82)	113	1.68	4.1
Phenylalanine	3.85 (3.17–4.17)	113	4.75	6.3
Threonine	3.54 (3.00–3.82)	118	2.77	4.9
Tryptophan	1.00 (0.56–1.16)	17	1.53	1.5
Valine	3.78 (3.17–4.23)	118	4.46	7.3
Cys + Met	2.01 (1.50–2.45)	113	4.27	6.5

Source: DAFWA (Department of Agriculture and Food Western Australia) and 'Amino Acid Composition and Biological Value of Cereal Proteins.'

Table 2 Mineral content of narrow-leafed lupine (per kg)

Mineral	*Mean (minimum–maximum)*	*Number of samples tested*
Calcium (g)	2.20 (1.5–2.9)	623
Magnesium (g)	1.60 (1.1–2.0)	524
Phosphorus (g)	3.00 (2.1–4.3)	846
Potassium (g)	8.00 (6.6–9.1)	614
Sodium (g)	0.40 (0.3–1.1)	422
Sulfur (g)	2.30 (1.5–2.9)	611
Copper (mg)	4.70 (2.5–6.8)	547
Iron (mg)	68.50 (31–150)	535
Manganese (mg)	19.00 (6.7–76)	584
Molybdenum (mg)	1.60 (0.7–2.9)	41
Zinc (mg)	34.10 (24.7–45)	551
Cobalt (g)	78.00 (10–260)	56
Selenium (g)	89.00 (18–240)	138

Source: DAFWA (Department of Agriculture and Food Western Australia). http://www.agric.wa.gov.au/PC_92144.html#pro.

Table 3 Antinutritional factors in narrow-leafed lupine

Factor	*Units*	*Mean (minimum–maximum)*	*Number of samples tested*
Alkaloids	%	0.02 (<0.01–0.04)	1316
Oligosaccharides[a]	%	4.07 (2.9–5.2)	202
Phytate	%	0.50 (0.32–0.84)	254
Saponins	mg kg^{-1}	573.00 (442–740)	11
Tannins (total)	%	0.29 (0.19–0.51)	208
Tannins (cond)	%	0.01 (n.d.–0.11)	236
Trypsin inhibitor activity (TIA)	mg kg^{-1}	0.12 (0.05–0.24)	148
Chymotrypsin inhibitor activity (CTIA)	mg kg^{-1}	0.08 (n.d.–0.59)	8
Lectins	Dilute	n.d.	28

n.d., not detected.

[a]Sum of raffinose, stachyose, and verbascose.

Source: DAFWA (Department of Agriculture and Food Western Australia). http://www.agric.wa.gov.au/PC_92144.html#pro.

are found at lower levels in lupine than in many other legumes. However, some of the protease inhibitors of lupine have now been positively reevaluated considering the impending utilization of their biological behavior in pharmacological and medical applications. For example, an inhibitor, belonging to the Bowman–Birk inhibitor family, has been isolated from *Lupinus albus* L. having the ability to prevent or suppress carcinogen-induced transformation.

Protein Diversity in Lupine

Generally, the proteins of legume seeds are the storage type, mostly located in storage vacuoles of cotyledonary tissues, and their main role is to supply nitrogen and carbon to the emerging plantlet. With the advancement of proteomic and food technologies, seed proteins are considered significant components for the human diet for their nutritional value and health benefits including hypolipidemic, hypoglycemic, hypotensive, anticarcinogenic, and antiobesity effects. At the same time, they have been investigated for adverse effects on human health such as food intolerance, allergies, and inhibition of endogenous hydrolytic enzymes.

Types of Lupine Seed Proteins

Lupine seeds have two types of proteins, namely, albumin and globulins. Their quantitative ratio is approximately 1:9. Other minor fractions of proteins include prolamins. Albumins are the functional proteins of lupine seed, which are largely metabolic enzymes, linked to the storage role of the seed cotyledon. Globulins comprise a number of protein molecules, which are the salt-soluble storage proteins of seeds.

The first separation of lupine seed protein was carried out in 1975 that differentiated lupine proteins into four main fractions, namely, α-, β-, γ-, and δ-conglutins, on the basis of their electrophoretic mobility. Further separations of lupine protein showed the extreme heterogeneity of the different protein fractions. The α-conglutin fit into the 'legumin-like' or 11S globulin family. This protein group consists of hexamers of disulfide-linked subunits. The β-conglutins are referred to as 'vicilin-like' or 7S globulins. These are trimeric proteins with monomers. In addition to these main protein types, lupine seed proteins contain considerable amounts (5–6%) of γ-conglutin, a basic 7S protein consisting of two heterogeneous disulfide-linked subunits, and a monomeric low-molecular-weight protein similar to other 2S albumins, named δ-conglutin.

Genetic and Proteomic Variations at Species Level

Differences in basic genetic components among lupine species were demonstrated by several cytological studies. The New World lupines' predominant chromosome number is $2n = 48$ with a basic chromosome number of $x = 6$. In contrast, Old World lupines have a series of basic chromosome numbers from $x = 5$, 6 (or 9), 7, 8, 13 at different ploidy levels. Intense selection/evolution in changeable environments is considered as the reason for recent changes in chromosome number of lupine species. Among the smooth-seeded Old World lupines, *L. angustifolius* has lower chromosome numbers ($2n = 40$) compared with *L. albus* ($2n = 50$), *L. micranthus*, *L. luteus*, and *L. hispanicus* ($2n = 52$).

DNA-based analyses have revealed extensive genetic diversity among different lupine species clearly separating the Old World from the New World species. Seed protein studies also reported substantial variation among Old World lupine species and found that heterogeneous smooth-seeded lupines were distantly related to rather homogeneous rough-seeded lupines. The phylogenic analysis based on matrix-assisted laser desorption/ionization time-of-flight (MALDI/TOF) clustered the Old World smooth- and rough-seeded and New World lupine species separately, in broad agreement with clustering based on morphological characters, life history, and cytogenetic and DNA polymorphism. Interestingly, two New

World lupine species *L. mutabilis* Lindl. and *L. succulentus* were grouped into the smooth-seeded Old World group.

Genetic and Proteomic Variations at Cultivar Level

There has been considerable effort to increase the genetic diversity of lupines by crossing breeding lines with diverse wild germplasm. An obvious, substantial genetic diversity among different accessions/cultivars within the same species has been developed, and considerable proteomic differences have been revealed accordingly. The genetic diversity of *L. angustifolius* appears to be larger than *L. luteus* and *L. albus* due to the domestication of this crop in places far from the naturally distributed areas. Characterization of morphological and molecular diversity of *L. angustifolius* revealed considerable genetic differences among cultivars. For example, DNA-based fingerprinting found substantial genetic variation among the cultivars of *L. angustifolius*. Two-dimensional gel electrophoresis revealed seed globulin differences among three varieties each of *L. albus* and *L. angustifolius*. Variation in grain protein content among wild accessions has been reported from under 32% (dry matter basis) to almost 43%. In contrast, grain protein content of cultivars ranged from 32% ('Belara') to 35% ('Coromup'). Thus, the lupine-breeding program at the Department of Agriculture and Food Western Australia (DAFWA) has set the target to increase protein content by initially crossing wild accessions with current varieties. DAFWA has released 25 cultivars of *L. angustifolius* since the 1960s. Recently, a qualitative variation of proteins among 25 Australian cultivars of *L. angustifolius* using MALDI/TOF showed that a pairwise distance between the cultivars ranged 15–50% indicating considerable proteomic variation across the cultivars.

Allergenicity of Lupine Proteins

Considering the increasing and potential use of lupine in food preparation, allergenicity of this grain is a significant issue that needs to be addressed. Since many legume crops, particularly peanut and soybean, have some allergic effect, it is not unexpected that this might also be the case for lupine. A small proportion (<1%) of the world population is allergic to lupine proteins. Allergic reactions to lupine have been reported either as primary lupine allergy or as a result of cross-reactivity to other legumes, especially peanut. Furthermore, lupine flour is a cause of occupational IgE-mediated allergy. A number of studies have reported on lupine globulins that comprise the bulk of lupine proteins as IgE-binding molecules. The overall pattern of lupine allergenicity is similar to that of soybean, peanut, and other edible legume seeds. Like other legumes, the allergic reaction to lupine is of concern, particularly with regard to healthy lupine-enriched bread.

Although the proteins responsible for lupine allergic reaction have not been fully characterized, the basic subunits of α-conglutin (legumin-like, 11S), β-conglutin (vicilin-like, 7S), γ-conglutin (7S), and δ-conglutin (2S-albumin) appear to be responsible for the allergic reactivity. Characterization of the lupine allergic proteins may elucidate their role as cross-reactive and unique lupine allergens. β-Conglutin was reported as the major allergen of *L. angustifolius* named as 'Lup an 1,' whereas α-conglutin was reported as the predominant allergen in *L. albus*. On the other hand, γ-conglutin was suggested as the IgE-binding component of lupine protein in both *L. angustifolius* and *L. albus*. These differences in lupine allergic reaction might be due to the genotypic variation of proteins. Differential expression of some allergic proteins has been identified among four cultivars of *L. angustifolius*.

More defined information on lupine seed protein allergenicity is crucial for the continued adoption of this legume grain in the food industry.

Use of Lupine Proteins in Food

As lupine has been used in different communities as a human food for a long time, there are unique processing and food preparation methods for different product purposes. In modern food technologies, the most common use of lupine in human food is as flour in confectionary and baked products such as bread, crackers, biscuits, noodles, and pasta.

In addition to its direct use as flour, lupine seed derivatives including protein isolates and protein concentrates are used for various food preparations such as drinks, yogurt, sauces, and infant formula. To remove the bitter-tasting alkaloids, which limit the use of lupine in the human diet, protein isolates or concentrates may be an alternative choice for food preparation since alkaloids are water-soluble and removed during the preparation of protein isolates. The use of plant protein isolates in foods as functional ingredients, to improve the nutritional quality of products or for economic reasons, is increasing in modern food production. Lupine protein isolate is useful as a food ingredient for changing the emulsifying capacity and improving the texture of food. However, due to incomplete recovery of proteins by isoelectric precipitation, a technology commonly used in industry, the protein quality of isoelectrically precipitated lupine protein isolate is lower than that of lupine flour from which it was extracted.

It is worth noting that, for bread preparation, gluten (normally present in wheat) plays a vital role in baking by forming long chains of gluten networks, which help to retain gas. Lupine does not contain gluten, and for this reason, lupine is mixed with wheat when utilized in the baking process.

Effect of Adding Lupine Flour to Wheat on Food Properties

The nutritional attributes of food in relation to the impact on biochemical and physiological processes of the human body are now considered key quality features of grains. For example, wheat flour has moderate protein content (11.4 g/100 g) and is low in lysine, an essential amino acid (2.4 g/100 g).

Physical properties

Physical properties of dough and bread were noticeably different when a considerable amount of wheat flour was substituted with lupine flour. The addition of lupine flour tends to improve baked products' flavor and often color and also increases its shelf life. Bread firmness, product volume, and water absorption significantly increased with the addition of lupine flour, which can be considered a positive change for bread quality. Although sensory value decreased after the addition of lupine flour, the products are considered

acceptable. On a negative note, dough development time increased and dough strength decreased, as proteins from lupine obstruct the interconnection of the wheat gluten matrix and ultimately affect the bread texture.

The particular breadmaking properties of wheat flour are recognized mainly by the ability of its gluten proteins to provide viscoelasticity by forming a gluten network when mixed with water. When a portion of wheat flour is supplemented by other flours rich in proteins (e.g., lupine), the added protein molecules interact with the gluten network in both direct and indirect ways: directly by cross-linking with gluten proteins and indirectly through the water availability of wheat gluten proteins. Reduction of gluten networks leads to weakening of dough elasticity. However, an optimum proportion of lupine flour (between 5% and 10%) permits dough elasticity to remain in an acceptable range.

Enriched health attributes

The high fiber content of lupine seed makes it particularly significant for the human diet. Lupine–wheat bread is rich in fiber, improving bowel health and keeping the digestive system smooth. It also helps to reduce fat and energy intake.

The inhibitory effect of foods on appetite – the satiating power of foods – has recently been acknowledged by nutritionists to be vital for planning low-energy/weight-loss diets. High-protein diets are more satiating than high-carbohydrate diets and high-fiber diets are more satiating than low-fiber diets. As a result, foods enriched in protein, fiber, or both have the potential to increase satiety and reduce energy intake. From this point of view, lupine-enriched bread is attracting attention due to the increased protein and fiber levels and the associated positive contribution to reducing obesity.

Available evidence also suggests that substitution of refined carbohydrates in the diet with protein and fiber may benefit blood pressure. This means that lupine-enriched bread has the potential to control blood pressure and cardiovascular disease. Lupine-enriched bread also decreases blood glucose and cholesterol levels, which benefits controlling diabetes. Proteins play key roles in the health benefits, for example, glucose-lowering effect of lupine γ-conglutin.

Wheat Seed Proteins and Interaction with Lupine Proteins in Mixture

The direct way of interaction between lupine and wheat protein (while a portion of wheat flour is supplemented by lupine) is cross-linking between lupine and gluten proteins. During dough preparation, different ingredients such as oxidizing agents, salts, and water are added that promote and affect the formation of protein cross-links.

Research on lupine–wheat flour interaction is limited to physical and chemical properties and sensory evaluation of baked bread. However, it has been reported recently that the two major subgroups of conglutins showed very different solubilities after the baking process indicating different levels of interaction with gluten protein. Most of the α-conglutins were readily extracted from the lupine–wheat bread even at low-salt and nonreducing/nondenaturing extraction conditions. In contrast, most of the β-conglutins lost extractability suggesting that they were trapped in the bread gluten matrix. On the other hand, a number of wheat proteins observed in wheat bread became unextractable from lupine–wheat bread. The higher thermal stability of α-conglutins compared with β-conglutins is speculated to account for this difference.

Conclusion

Lupine-enriched food is a relatively new health-food source, in which consumer awareness and acceptance are still low. An efficient way to bring lupine-associated health benefits to consumers is to add lupine flour to a widely consumed food product such as bread.

See also: Alkaloids: Properties and Determination; Alkaloids: Toxicology and Health Effects; Allergies: Public health; Amino Acids: Determination; Antinutritional Factors in Legume Seeds: Characteristics and Determination; Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Appetite Control in Humans: A Psychobiological Approach; Bioactive Peptides in Foods; Biscuits, Cookies and Crackers: Chemistry and Manufacture; Bread: Breadmaking Processes; Bread: Chemistry of Baking; Bread: Dough Mixing and Testing Operations; Carbohydrate: Digestion, Absorption and Metabolism; Cereals: Types and Composition; Cholesterol: Properties, Processing Effects, and Determination; Chromatography: High-Performance Liquid Chromatography; Dietary Fiber: Bran; Dietary Fiber: Determination; Enzymes: Analysis and Food Processing; Enzymes: Functions and Characteristics; Fats: Classification and Analysis; Food Additives: Classification, Uses and Regulation; Food Allergies: Occurrence and Analysis; Food Allergies; Food and Agriculture Organization of the United Nations; Functional Foods; Glucose: Properties and Analysis; Legumes in the Diet; Mass Spectrometry: Principles and Instrumentation; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Proteins: Chemistry, Characterization, and Quality; Proteomics: Contribution of Proteomics Techniques to Understanding the Interrelationship between Food and Health; Pulsed Electric Fields; Quality Control in Food Processing; Satiety; Sensory Evaluation: Sensory Rating and Scoring Methods; Soy Beans: Dietary Importance; Soy Beans: Processing; Soy Beans: Properties and Analysis; Soy Beans: The Crop; Wheat: The Crop.

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Magnesium

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Magnesium – An Essential Mineral

Magnesium Functions

Magnesium (Mg^{2+}) is an essential mineral because it cannot be synthesized and is essential for a vast number of body functions and cellular processes. Hence, it must be obtained from dietary sources. Magnesium is the second most abundant intracellular cation after potassium. It is fundamental to the function of adenosine triphosphate, acts as cofactor of more than 300 enzymes, stabilizes proteins and membranes, is necessary for nucleic acid synthesis, and interacts with some cell receptors, like *N*-methyl-D-aspartate receptor. Magnesium involvement in ion fluxes regulation is mandatory for extra/intracellular ion balance. Indeed, magnesium is central for the control of sodium and potassium transport across cell membranes. In addition, because of its antagonistic action on calcium fluxes, magnesium is considered as 'nature's calcium channel blocker.' Therefore, alterations of magnesium concentration lead to a variety of disturbances at the cellular level that may translate to severe dysfunctions and diseases.

Magnesium in the Body and Its Homeostasis

In an adult male, the total body magnesium is approximately 24 g. More than 60% resides in bones, while about 25% is in the muscles where magnesium plays an important role in contraction. Magnesium is predominantly intracellular. Indeed, only 1% of magnesium is in the blood, 55% of which is ionized and biologically active, while 30% is bound to proteins and 15% is complexed to citrate, bicarbonate, ATP, and phosphate. Magnesium is fundamental for cell functions and, consequently, its homeostasis is tightly regulated. The main contributors to whole body magnesium homeostasis are intestinal absorption, kidney reabsorption, and bone stores of magnesium.

Magnesium is absorbed in all parts of the intestine. However, its absorption is maximal in the distal jejunum and ileum. There are two modes of magnesium absorption: passive paracellular – unsaturable mechanism, which is driven by an electrochemical gradient, and active transcellular – saturable absorption mediated by transporters. In healthy subjects, 30–50% of the ingested magnesium is usually absorbed. Because the major part of magnesium in the intestine is absorbed via the paracellular pathway, the amount of magnesium in foods is the main determinant of the quantity absorbed. Active absorption appears to play an important role when magnesium intake is low or in the case of deficiency. Hence, lower magnesium supply results in higher efficacy of its absorption (higher fractional absorption).

Systemic magnesium homeostasis is largely based on its renal handling via filtration–reabsorption processes. Of the 2000–2500 mg of magnesium filtered daily by the glomeruli, about 95% is reabsorbed in the kidney, mainly in the thick ascending limb of the loop of Henle. Magnesium reabsorption depends upon its concentration in the blood. Under conditions of magnesium deficiency, reabsorption in the loop of Henle is significantly increased, while, when magnesium is in excess, its urinary excretion increases. This is the reason why high magnesium intake does not usually result in a marked hypermagnesemia.

Bones are an important reserve of magnesium. About 35% of skeletal Mg is located in the cortical bone either on the surface of apatite or in the hydration shell around the crystals. This is exchangeable magnesium useful to maintain physiological extracellular concentrations. The rest of bone Mg is incorporated into bone crystals and released upon bone resorption. Most magnesium deficiencies are acquired and may result from insufficient intake, altered absorption, and gastrointestinal or renal losses. Several rare monogenic disorders with marked abnormalities in magnesium homeostasis have been identified (including Gitelman, Bartter, and hypomagnesemia syndromes) and permitted to identify several genes involved in magnesium metabolism. However, at present, the data on the impact of gene polymorphism on magnesium status in the general population is limited.

Magnesium Intake

Dietary Recommendations

Several guidelines of recommendations for magnesium intake have been emitted by various countries or organizations (France, Germany, Germany, UK, Nordic countries, WHO/FAO). As an example, Table 1 presents recommended dietary allowances (RDA) provided by Food and Nutrition Board Institute of Medicine National Academy of Sciences U.S. The term of RDA is used to represent the average daily dietary intake that is sufficient to meet the nutrient requirements of

Table 1 Recommended dietary allowances (RDAs) for magnesium (mg) provided in the dietary reference intakes (DRIs) developed by the Food and Nutrition Board at the Institute of Medicine of the National Academies

Age	*Male*	*Female*	*Pregnancy*	*Lactation*
Birth to 6 months	30[a]	30[a]		
7–12 months	75[a]	75[a]		
1–3 years	80	80		
4–8 years	130	130		
9–13 years	240	240		
14–18 years	410	360	400	360
19–30 years	400	310	350	310
31–50 years	420	320	360	320
51+ years	420	320		

[a]Adequate intake (AI).

Source: Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, and Institute of Medicine National Academy of Sciences (1997). Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: National Academy Press.

nearly all (97–98%) individuals in a life stage and gender group. In fact, RDAs are the estimated average requirements (EAR) + 2 SD. When evidence is insufficient to develop an RDA an adequate intake (AI) is established at a level assumed to ensure nutritional adequacy. The RDA values vary by age, sex, and physiological condition (such as pregnancy, lactation, etc.) and are provided for each of these specific situations. Similarities, but also discrepancies, exist between the various guidelines on RDA (EAR, reference nutrient intakes (RNI)) for magnesium. This is due to the scarcity of studies permitting to precisely estimate allowances. As an example, the RDA for 31–50 years old men and women are 429 mg (EAR 350 mg) and 320 mg (EAR 265 mg) of magnesium/day, respectively. French guidelines (ANC – Les apports nutritionnels conseillés pour la population française; AFSSA, 2000) with 6 mg of magnesium/kg body weight/day are similar to U.S. RDA. RNIs in the United Kingdom for adult men and women are 300 and 270 mg of magnesium/day, respectively. However, FAO/WHO guidelines (2001) consider these values overestimated, and then RNIs for the adults are 4 mg of magnesium/kg body weight/day.

Several dietary surveys point to the inadequate magnesium intake in large parts of the populations of western countries. National Health and Nutrition Examination Survey (NHANES) of 2005–06 found that nearly one-half of Americans (aged 1 year and over) had inadequate intakes of magnesium. This inadequacy was more important in 14–18 year olds and in adults over 71 years. More recently, published results from a prospective cohort study on 73 684 postmenopausal women enrolled in the Women's Health Initiative observational study also indicate an important proportion of women consuming less magnesium than RDA. In France, magnesium intake in subjects from the SU.VI.MAX cohort (35–60-year-old women and 45–60-year-old men) published in 1997, was lower than RDA in 77% of women and 72% of men; 23% of women and 18% of men consumed less than two-thirds of these RDA.

Table 2 Magnesium content in selected foods

Food	*Magnesium (mg/100 g)*
Bread (with cereals and grains)	181
Rye bread	110
Bread (standard baguette)	20
Rice (complete, cooked)	50
Rice (white, cooked)	11
Almonds (with skin)	231
Hazelnuts	88
Spinach (cooked)	53
Bean (white, cooked)	60
Lentils (cooked)	35
Split peas (cooked)	20
Potatoes (cooked)	17
Chicken (roasted)	25
Fish (cooked)	27
Pizza (cheese)	22
Milk	11
Yogurt	12
Cheese (white)	11
Banana	32
Orange	12
Apple	6
Lettuce	12
Broccoli	11
Cauliflower	12

Source: Data from French food composition table Ciqual (https://pro.anses.fr/TableCIQUAL/).

Dietary Sources of Magnesium

Food and water

Magnesium is fairly ubiquitous in commonly available foods. Nevertheless, some foods are of particular interest for magnesium supply because of their abundance in magnesium. Green vegetables have a high content of magnesium because magnesium is the central atom of chlorophyll's porphyrin ring. Also, grains, nuts, beans, peas, seeds, cocoa, and connected products are rich in magnesium. From the nutritional point of view, not only magnesium content of specific foods should be considered, but also their processing, their matrix (presence of other components), and the eating habits of consumers (such as Western diet, severe dietary restriction, some athletes' diets), all factors that may affect magnesium content and intake. The refining of the wheat grain, leading to remove magnesium-rich bran and germ from the grain, results in important loss of magnesium. Consequently, white refined flour is poor in magnesium. As an example, there is a marked difference between magnesium content in wheat white bread and whole-wheat bread. Similarly, other refined products such as sugar and oil are totally depleted of magnesium. Food processing, industrial as well as household, may result in significant losses of magnesium. This is particularly important when vegetables are cooked in boiling water and cooking water is discarded. The U.S. Department of Agriculture's (USDA's) Nutrient Database and the French food composition table CIQUAL (ANSES) provide a comprehensive list of foods and their content in magnesium. Table 2 illustrates magnesium content in selected foods.

In nutrition, not only the content of magnesium in foods should be considered, but also food servings and caloric density of foods. Vegetables are of high nutritional interest with regard to magnesium, especially in Western nutrition, because they are abundant in magnesium and low in calories.

Water, either tap or bottled, can be an important source of magnesium. In general, water with adequate magnesium content is an interesting magnesium source because of the zero calories supply and good magnesium absorption. However, magnesium concentration depends upon the water sources. Some waters are rich but many others are poor in magnesium, to the point of approaching the composition of distilled water. Different studies have shown that variations in magnesium levels in drinking water between different areas correlate with the incidence of various diseases. Following, are a few examples. A large epidemiological study in Taiwan has shown the significant protective effect of magnesium from drinking water on the risk of cerebrovascular disease. Research performed in Sweden has demonstrated that magnesium in drinking water is an important protective factor for death from acute myocardial infarction. Recently, magnesium levels in drinking water have been linked to the incidence of hip fracture for both men and women. It is therefore important for the consumers to be worried about mineral content of the consumed water.

Effect of dietary components on magnesium absorption and status

Dietary components may affect magnesium absorption and/or magnesium excretion, thus influencing body magnesium status. The quantity of ingested magnesium and its solubility are major determinants of the quantity of the absorbed magnesium in the intestine. Therefore, diet components positively or negatively affecting its solubility may favor or reduce its absorption.

Because of the phytate content, the negative role of fibers and fiber-rich diets on magnesium absorption was extensively debated. However, in the standard western diet, this negative effect seems overemphasized. In fact, the whole diet rich in vegetables as a source of fibers, is also a substantial source of magnesium. In addition, fiber fermentation in the large bowel improves magnesium absorption in this part of the digestive tract. Within nutritional conditions, an increase of calcium and of vitamin D intakes does not significantly affect magnesium absorption.

High protein intake may affect both magnesium absorption and excretion. Low protein intake may negatively affect magnesium absorption while high protein intake may increase its urinary excretion. This last effect is of particular interest with regard to the Western diet, which, *per se*, is rather deficient of magnesium. In fact, the Western diet, which is usually rich in animal proteins and low in fruits and green vegetables, leads to a chronic, low-grade metabolic acidosis. This metabolic alteration considerably affects magnesium homeostasis. In particular, renal magnesium reabsorption is reduced, thereby resulting in their increased urinary excretion. It could be anticipated that this effect on urinary magnesium losses may be amplified in elderly subjects and in diabetics whose renal function is impaired.

It is well established that chronic alcohol consumption affects whole body magnesium homeostasis. Indeed, magnesium deficiency is frequent in chronic alcohol abusers. This is due to general malnutrition, intestinal malabsorption and renal dysfunction leading to magnesium urinary loss, all typical features of people with alcohol dependence. In addition, ethanol impairs magnesium transport and homeostasis in brain, brain vessels, liver, skeletal muscle, and heart.

Dietary supplements

A large variety of magnesium supplements is available. The most customary are magnesium chloride, oxide, and several organic salts (citrate, aspartate, pidolate, gluconate, lactate, etc.). The main determinant of the absorption of these different forms is their solubility. Frequently, in supplements, magnesium is associated with vitamin B6. Routinely, within nutritionally relevant doses, magnesium supplementation does not provoke significant adverse effect in healthy subjects. This is due to the renal intervention in regulating magnesemia so that magnesium in excess is excreted in the urine. Therefore, magnesium supplementation should be restricted in subjects with kidney dysfunctions characterized by an excess of magnesium excretion. Most commonly, magnesium supplementation with high doses may have laxative effects because of the osmotic activity of unabsorbed salts in the intestine and the stimulation of gastric motility. A tolerable Upper Intake level of magnesium for adults from supplements (without magnesium present in food and beverages) has been fixed by the U.S. Food and Nutrition Board at 350 mg/day and the EU Scientific Committee for Food at 250 mg/day.

Magnesium Status Assessment

The assessment of magnesium status is still a challenge for the physicians and for clinical laboratories. Only a marginal part, less than 1%, of magnesium is in the blood, and therefore it is not representative of overall magnesium levels. Thus, it is comprehensible that laboratory assessment of magnesium status is problematic because it is difficult to perform measures reflecting the tissue/cell content of magnesium. The conciliation between an easy obtained sample, rapid and robust laboratory test, and the parameter representative for intracellular magnesium is extremely difficult to reach. Current clinical laboratory tests are almost limited to total serum (or plasma), ionized serum (or plasma) magnesium concentration, and, seldom, 24 h urinary excretion of magnesium (Table 3).

Table 3 Laboratory approaches to assess magnesium status

Clinical laboratory available assessments
– Total serum magnesium concentration
– Serum ionized magnesium concentration
– Twenty-four hour excretion of Mg in urine
Assessments used for research purposes
– Total and free red blood cell magnesium concentration
– Leucocyte, platelets, buccal cell magnesium
– Magnesium retention test (intravenous or oral magnesium challenge)
– Tissue magnesium (muscle, bone)
– Electron probe for total magnesium in tissue (x-ray spectrometry)
– NMR for magnesium in tissue
– Stable Isotope studies (balance, body pool size and in vitro blood load test)
– Fluorescent probes (flow cytometry and fluorescence spectroscopy)

Total serum (or plasma) magnesium concentration is so far the most widely measured parameter for magnesium status assessment, because it is rather easy to perform and inexpensive. NHANES I study defines the reference interval value for U.S. adult population between (central 95th percentile) 0.75 mmol l^{-1} and 0.955 mmol l^{-1} with a mean concentration of 0.85 mmol $^{-1}$. Even if it is admitted that magnesium serum concentration reflects magnesium status, the current laboratory assessment of deficiency is difficult due to the heterogeneity of serum magnesium values. Therefore, serum magnesium may reflect a severe magnesium deficiency, but it is not a sufficient discriminant of latent chronic deficiency. In addition, serum and erythrocyte magnesium concentrations have been shown to have a heritable component with heritability estimates of ~30%. Recent advances in molecular genetics permitted to identify a variety of genes implicated in magnesium transport mechanism. However, the potential impact of genetics in the context of low magnesium status in the general population is still poorly known.

Apart from the above discussed markers, other various approaches have been used to determine magnesium status, but they are mostly restricted to research purposes. The aim of these techniques is to obtain information as accurate as possible about the amounts of intracellular magnesium which better reflect body stores. Therefore, a range of studies has evaluated blood cell magnesium, mainly in erythrocytes, and to a lesser extent, in leukocytes and platelets. Only a few reports concerned the determination of tissue magnesium (bone, muscle).

An important challenge in the assessment of magnesium status is to evaluate whole body magnesium stores. Currently, this measurement could be achieved through the magnesium retention test, which evaluates urinary magnesium excretion after intravenous or oral magnesium loading. This test is presently considered the gold test for magnesium evaluation. An advent of stable isotopes of magnesium permits to evaluate the exchangeable magnesium pool size. However, at present the number of performed studies is scarce. It should be stressed that, at the moment, there is no clearly validated candidate to serve as indirect biomarker of magnesium (not related to direct magnesium determination).

Magnesium in Health and Disease

Magnesium deficiency may lead to characteristic neuromuscular and cardiac clinical symptoms. However, severe magnesium deficiency is rare, and subclinical latent deficiency mostly occurs with nonspecific symptoms (muscle contractions, weakness, fatigue, digestive troubles). This subclinical, chronic deficiency may contribute to various dysfunctions and diseases (see below). As we discussed, it is difficult to make a diagnosis of this deficiency by the assessment of serum magnesium. However, population studies have shown an important occurrence of hypomagnesemia (values below 0.75 mmol l^{-1}) in Western countries. For example, in Germany, the study of 16 000 individuals from an unselected population indicates that, with the lower limit set of magnesemia at 0.76 mmol l^{-1}, the frequency of hypomagnesemia was estimated about 14.5% of the population. Several studies suggest high frequency of hypomagnesaemia in latent magnesium deficiency prone populations, especially, as discussed earlier, those with metabolic syndrome and type II diabetes. Other population groups are also prone to magnesium deficiency due to lower intake or to magnesium losses, that is, chronic alcohol abusers, individuals with gastrointestinal and kidney diseases, diabetics, elderly, and people treated with some medicines like diuretics, aminoglycosides, and cisplatin, among others.

Epidemiological studies stress the need to pay more attention to magnesium intake, often low in healthy populations. Magnesium intake below RDA does not mean that subjects are magnesium deficient, but supports increased risk of chronic latent magnesium deficiency in large numbers of people.

It is by controlling many cellular and molecular functions that magnesium participates in the maintenance of tissue and systemic homeostasis, from neuromuscular conduction to bone integrity, from the regulation of blood pressure to glucose and lipid metabolism. Consequently, it is unsurprising that chronic latent magnesium deficiency is a contributing factor to several common chronic diseases which exact a high toll in terms of morbidity and mortality, such as diabetes, metabolic syndrome, osteoporosis, hypertension, Alzheimer's, and cardiovascular diseases. Magnesium deficiency promotes inflammation and oxidative stress, known to act as partners in crime in the pathogenesis of all these diseases. It is relevant to note that a low magnesium status associates with increased chronic inflammatory stress, partly generated by excess free radicals, which is alleviated by augmenting magnesium intake. An issue that has been overlooked until now is relative to the altered ratio magnesium/calcium. Since Mg^{2+} is an antagonist of Ca^{2+}, when deficient it cannot properly counterbalance Ca^{2+} signaling, and this impairs the tightly tuned complex mechanisms regulating cell and tissue homeostasis, thus leading to disease.

Let us begin our overview through data linking magnesium deficiency to insulin resistance and type II diabetes. Magnesium participates to the regulation of glucose metabolism since it is important for pancreatic β-cells proliferation and function; it is required for tyrosine kinase activity and for insulin signaling. Magnesium also directly affects the activity of GLUT4 which uptakes glucose in peripheral tissues. Another relevant issue is that inflammatory cytokines, which are produced in excess when magnesium is deficient, reduce the sensitivity to insulin. Several studies have associated type II diabetes with hypomagnesemia. In particular, magnesium deficiency is linked to the development and severity of the disease. Supplemental magnesium improves glucose and insulin metabolism both in healthy and in diabetic adults, and higher magnesium intake lowers the risk of progression to diabetes in insulin resistant individuals. Insulin resistance is one of the hallmarks of the metabolic syndrome, a disease of modern times which is defined also by the presence of hypertension, dyslipidemia, and obesity. Hypomagnesemia is often detected in patients with metabolic syndrome and a high intake of magnesium decreases the risk of this syndrome in a dose-dependent fashion. To this purpose, it is noteworthy that hypomagnesemia is linked also to hypertension and dyslipidemia, thus suggesting that hypomagnesemia is a master contributor to this disorder.

With regard to the regulation of blood pressure, magnesium regulates vascular tone mainly by antagonizing calcium channels in smooth muscle cells, thus reducing vascular resistance.

Most patients with hypertension are hypomagnesemic and magnesium supplementation determines a clinically significant reduction of diastolic and systolic blood pressure. In particular, it is dietary magnesium that shows the strongest inverse correlation with blood pressure.

Magnesium deficiency is implicated in promoting atherosclerosis, an inflammatory disease which is initiated by and progresses in the context of hypercholesterolemia. In experimental models, magnesium deprivation activates inflammation and causes hyperlipemia, thus accelerating and exacerbating atherogenesis. In humans, an inverse association exists between magnesium intake and C-reactive protein level, a marker of systemic inflammation and a known risk factor for cardiovascular diseases. In addition, magnesium deficiency induces a pro-atherogenic lipid profile because it decreases HDL and increases total serum cholesterol, LDL and triglycerides. In the light of epidemiological and experimental studies, magnesium deficiency is also emerging as an inducer of endothelial dysfunction, an early and pivotal event in atherogenesis. Accordingly, an inverse relationship between serum magnesium and development of coronary artery disease has been described. Hypomagnesemia has also been linked to increased intima-media thickness and the risk of carotid plaques. Oral magnesium supplementation exerts favorable effects by increasing HDL, decreasing markers of systemic inflammation, and reverting endothelial dysfunction in patients with ischemic heart disease. It is possible to conclude that correcting and maintaining magnesium homeostasis prevents and retards atherogenesis directly by acting on the endothelium and indirectly by controlling major risk factors such as hypertension, dyslipidemia, and diabetes.

Since magnesium is fundamental in controlling synapse density and plasticity, it is noteworthy that magnesium levels are reduced in the serum and in the brain of patients with moderate to severe Alzheimer's disease. Magnesium supplementation facilitates learning and ameliorates cognitive impairment. These beneficial effects might be explained in the light of studies in an Alzheimer's disease mouse model showing that magnesium reduces Aβ plaques and prevents synapse loss and memory decline.

Mainly the kidneys but also the intestines are protagonists in maintaining magnesium homeostasis by acting through a complex network of transporters. Bone is a co-star, because it stores about 60% of total magnesium and functions as a reservoir of exchangeable magnesium. Indeed, the levels of bone surface magnesium are related to serum magnesium. When deficient, magnesium is mobilized from the surface of the bone to buffer hypomagnesemia, thus leading, in the long run, to osteopenia and osteoporosis. Mg is also important for bone integrity since it acts directly on bone cells by regulating the interplay between osteoclasts and osteoblasts, and indirectly by affecting the homeostasis of the two master regulators of calcium homeostasis, that is, parathyroid hormone and 1,25 (OH)2-vitamin D. Indeed, lower magnesium intake is associated with lower bone mass density of the hip and whole body, and a correct magnesium intake can ameliorate this condition. Since a winning strategy to prevent osteoporosis is to build healthy bones starting early, from childhood, it is relevant to recall that pre-adolescent dietary intake of magnesium positively relates to bone mass density in Caucasian girls.

Memory decline, Alzheimer's disease, atherosclerosis, insulin resistance and osteoporosis are all age-related disorders. A link between low magnesium and aging has been described and, in turn, aging itself is a risk factor for developing a magnesium deficit, thus creating a vicious circle that drives the clinical patterns observed in aging. Aging is an important medical, social, and economic topic of interest in industrialized countries where the number of elderly citizens is rapidly growing. Therefore, correcting nutritional intakes of magnesium might be an easy solution for healthier aging and for reducing the impact of age-related diseases.

A last issue is about magnesium intake and tumors. Epidemiological studies indicate magnesium deficiency as a risk factor for some types of neoplasia, among which breast, colon, prostate, liver, esophageal, and ovarian cancers. It is therefore feasible to propose a contribution of altered magnesium homeostasis to tumorigenesis, partly because magnesium deficiency promotes inflammation and oxidative stress, both involved in cancer. It should also be noted that hypomagnesemia frequently occurs in oncologic patients. This seems to be due to the fact that tumor cells are avid for the cation and tend to accumulate magnesium. To further complicate the picture, radiotherapy and chemotherapy influence magnesium homeostasis through different mechanisms and cause magnesium deficiency. Although it is still debated whether or not to supplement magnesium in oncologic patients, magnesium is emerging as a chemopreventive agent that might contribute to the fight against cancer.

Conclusions

A tight control of magnesium homeostasis is fundamental for health. However, diet is not the only determinant since differences in magnesium handling on a genetic basis have been reported. In the last years, hereditary single-gene mutations resulting in hypomagnesemia have been described, thus leading to the identification of several molecular players participating to magnesium homeostasis. New hints about the relevance of magnesium to maintain health and prevent diseases will derive from nutrigenomics and nutrigenetics. Recently, magnesium supplementation has been shown to differently modulate gene expression and proteomic profile in overweight healthy individuals. The possibility of individuating the effects of nutrients on gene expression will lead to personalized food-based recommendations on the basis of the genotype. Nutrigenetics evaluates the effects of genetic variation on the relation between nutrients and health. In the case of magnesium, by single nucleotide polymorphisms, six different genomic regions were individuated that contain variants reproducibly associated with hypomagnesemia.

These results will change our outlook on the complex mechanisms involved in magnesium absorption and distribution and will allow new interpretations of interventional studies.

See also: Bioavailability of Nutrients; Dietary References: US; Legumes in the Diet; Mineral Water: Types of Mineral Water; Nuts: Health Effects; Obesity: The Role of Diet; Renal Function and Disorders; Wheat: Grain Structure of Wheat and Wheat-based Products.

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Maillard Reaction

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Introduction

In the last 150 years, heat treatment became as a common practice in the food industry. Treatments such as sterilization or baking improve the organoleptic and health properties of heat-treated foods. Many different chemical reactions take place during heating and storage of foods, giving rise to the formation of new compounds that affect the final characteristics of foodstuffs and consumer acceptability. Some of these transformations include a complex set of reactions known as non-enzymatic browning (NEB), leading to the formation of brown colors without the intervention of enzymes. Among them, the Maillard reaction (MR) is the most ubiquitous due to its ability to develop in a large range of temperatures, water activity, presence–absence of oxygen, etc. Therefore, the MR development is desirable in foods like coffee or bread, where specific color and aroma are expected. Contrary, in foods like milk or fruit juices, the MR is avoided due to the formation of strange colors and aromas as well as nutritional decrease. To do so, the food industry establishes different processing standards and control measures aiming at decrease the extension of the MR.

The Chemistry Behind the MR

General Pathways

The MR is a complex net of chain chemical reactions responsible for the aroma, color, and taste of many thermally treated foods. It is favored under the absence of oxygen – although its presence does not avoid its development – neutral–basic pH, low/intermediate water activity, and mild heating conditions. The MR was first described in 1912 by the French chemist Louis Camille Maillard, who described the reaction between glycine and glucose, producing a yellow-pale color in water solution. In 1953, Hodge proposed a scheme (Figure 1) currently considered the most appropriate to describe this reaction.

(a) The first step of the MR consists of the nucleophilic condensation of a carbonyl group with an amino group and, after dehydration, an unstable *Schiff base* is formed, which is transformed rapidly into an N-substituted-glycosylamine or imine known as Schiff base for either glucose or fructose (Figure 2). This reaction is reversible because in a strong acidic medium, the sugar and the amino acid can be regenerated. The amino group can be a free amino acid, the side chain of an amino acid (like lysine) incorporated in a protein or the amino group of the last amino acid in each protein. In the case of the carbonyl groups, they are usually reducing sugars, although they can be also carbonyl compounds from the intermediate stages of the MR and/or lipid oxidation.

(b) The next step consists of the irreversible rearrangement of the N-substituted glycosylamine. As a result of the hydroxyl group present in the α-position, the imines (Schiff base) can rearrange via the 1,2-eneaminols corresponding to the 1,2-enediol (Figure 3). When the molecule is an N-substituted-aldosylamine, by means of the *Amadori rearrangement*, the 1-amino-1-deoxy-2-ketose is formed (the Amadori compound). However, when the starting product is an N-substituted-ketosylamine, a 2-amino-2-deoxy-2-ketose is formed by means of the *Heyns rearrangement* (the Heyns compound). The Amadori products are more stable than the parent Schiff base, being the first MR compounds that can be measured in foods. The reason for such partial stability compared with imines is explained by their cyclic molecular structure: imines can be converted to the cyclic hemiaminal, which is unstable because they mutarotate very easily. However, the Amadori rearrangement leads to a furanose, which, as a hemiacetal, has a stability to mutarotation comparable with that of carbohydrates.

(c) The Amadori and Heyns products are decomposed, depending on the pH and temperature of the medium, giving rise to the formation of different intermediate compounds responsible for the final aroma and color of heated foods. Different osuloses – named *1-, 3-, or 4-deoxyosone* and *reductones* – are formed, which again decompose and react in the intermediate steps of the MR. The formation of deoxyosones is accomplished at pH ranging from 4 to 7. The Amarodi product 1-amino-1-deoxy-2-ketose can be converted to 2,3-eneminol as well as 1,2-eneaminol, which decomposes to form the subsequent deoxyosone (Figure 4). Water elimination from the 1,2-eneaminol and hydrolysis of the imine cation gives 3-deoxyosone. The 2,3-eneaminol has two different β-elimination options: the elimination of the amino acid by a retro-Michael reaction with formation of 1-deoxyosone and water elimination at carbon 4 of the 2,3-eneminol with the formation of 4-deoxyosone.

Further decomposition of 3-deoxyosones at low pH, through 1,2-enolization, gives rise to the final formation of *furanic compounds* like 5-hydroxymethylfurfural (HMF) and furfural from hexoses and pentoses, respectively. A 2,3-enolization takes place at basic pH, generating pyrroles and pyranones. The reductones so formed can be dehydrated again to form dehydroreductones, which form polymers by reacting with amino groups at the advanced stages of the MR.

(d) The Amadori compounds can be split into different *fission products*, such as acetal or acetaldehyde.

(e) The interaction of amino acids with dicarbonyl compounds, like dehydroreductones or fission products, is known as the *Strecker degradation* and implies the loss of amino acids in foods. As a result of this degradation pathway, new aldehydes with one carbon atom less – lost as

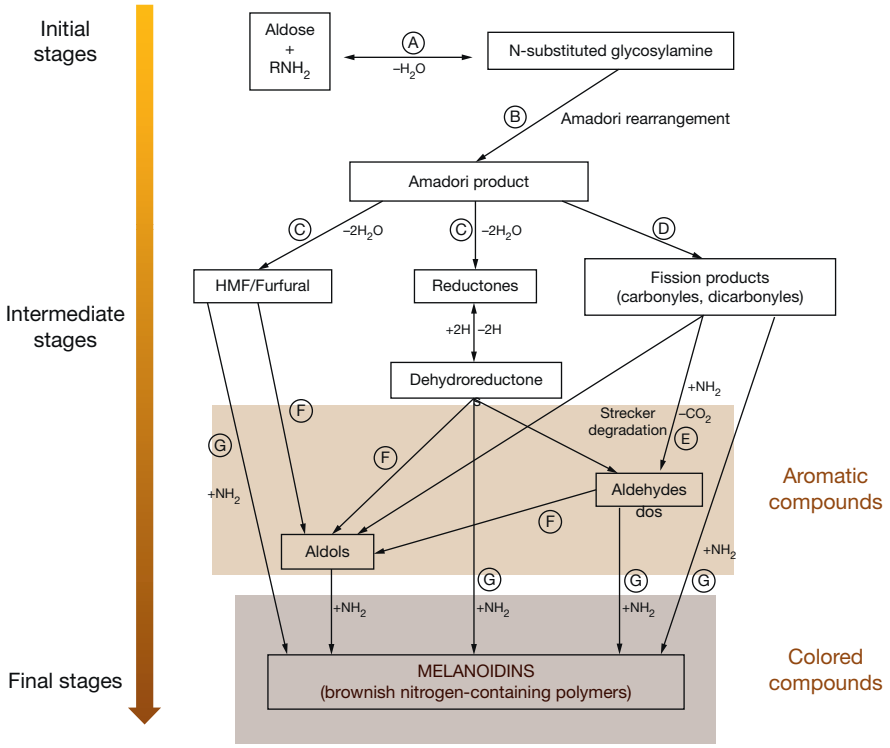

Figure 1 The Maillard reaction.

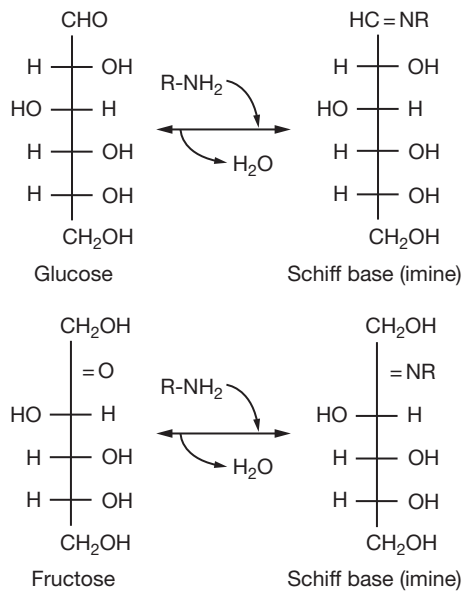

Figure 2 The formation of Schiff base from glucose and fructose.

CO_2 – are formed. It occurs in foods at higher concentrations of free amino acids and under more drastic reaction conditions (high temperature or under pressure). The aldehydes so formed (i.e., methional, phenylacetaldehyde, and 3-methylbutanal) have a very high aroma potential, depending on the amino acid degraded.

The next steps (f and g) are known as the advanced steps of the MR, where two different classes of compounds are formed: melanoidins and volatile aromatic compounds.

(f) *Volatile aromatic compounds* are formed directly from the Amadori compounds and do not need the mediation of free amino groups. However, a 1% of the total volatile compounds produced are formed by the reaction of 2-deoxyglucose with amino acids. As in the case of the Strecker degradation, many different chemical odorants are formed, like pyrazines, pyrroles, thiazoles, and thiophenes. For example, alkylpyrazines are the main flavors of roasted meat, while boiled meat gives rise to other products like 2,4,5-trimethyl-3-thiazoline.

(g) *Melanoidins* are brown polymeric anionic compounds produced by means of the condensation of aminated products of the intermediate stages of the MR such as N-substituted pyrroles, N-substituted 2-formylpyrroles, and 2-furaldehyde. Melanoidins have a wide molecular weight and different absorbance spectra. When formed from a carbohydrate backbone (coffee, balsamic vinegar, etc.), they are known as melanosaccharides, but if the melanoidin core is formed by a protein (i.e., bakery products), they are known as melanoproteins.

The MR has an initial induction period necessary to obtain enough the Amadori and Heyns products to start the intermediate stages of the reaction. The cascade of complex reactions that follows the formation of these initial intermediates means that the MR is not a classical chemical reaction. Rather, it represents a series of consecutive and parallel reactions that occur in the same reaction pool to produce a complex matrix of reactions. The propagation of the MR is best described by the formation and interaction of the so-called chemical pools

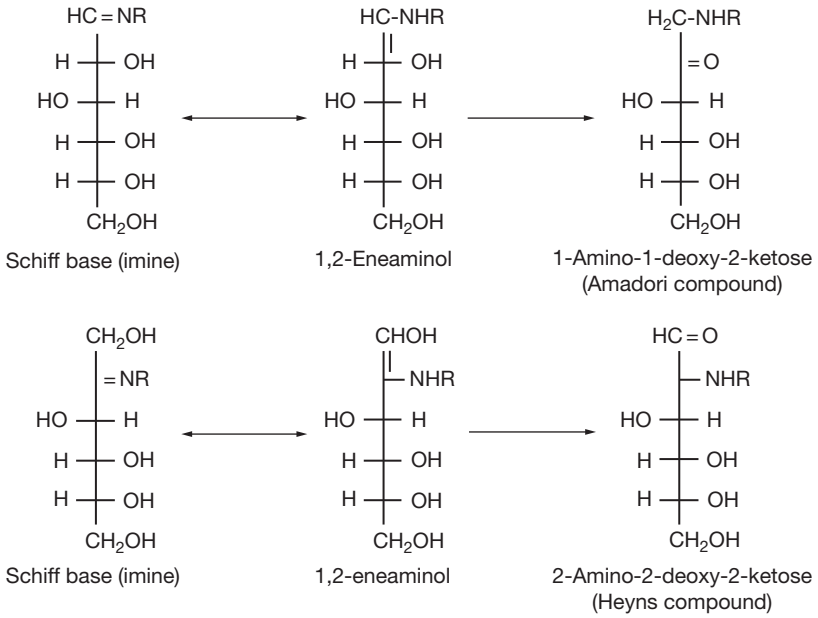

Figure 3 The formation of the Amadori and Heyns compounds.

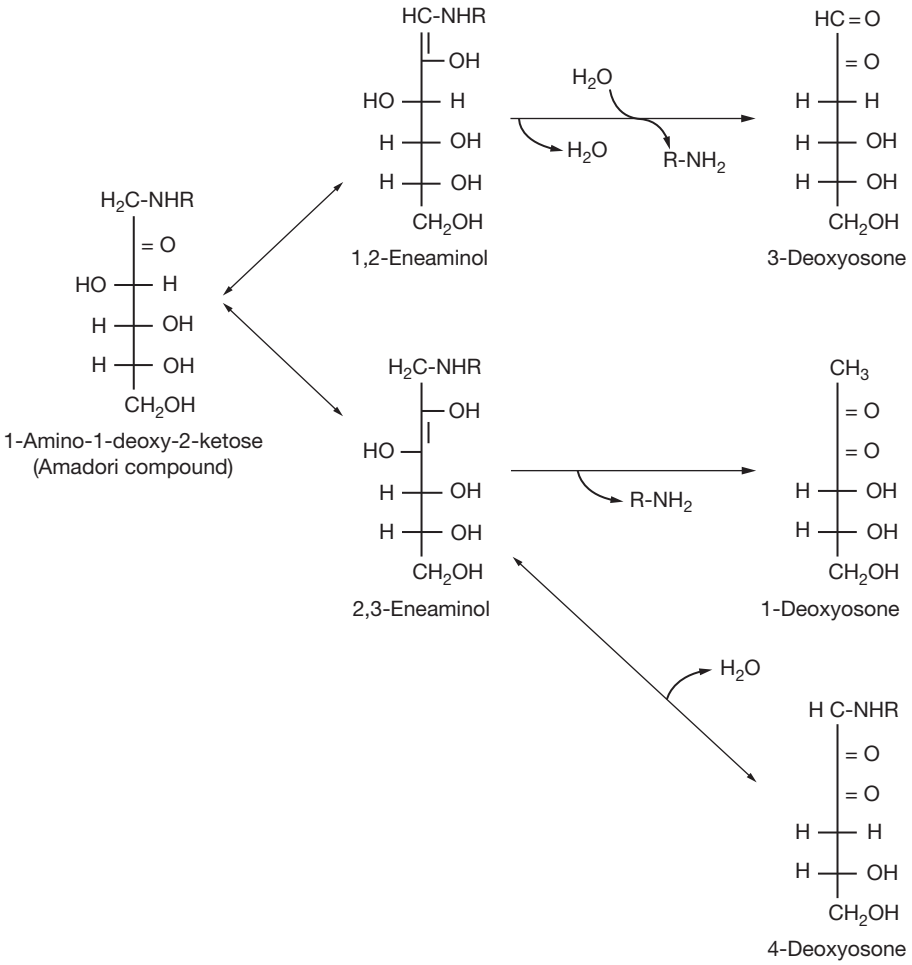

Figure 4 The formation of deoxyosones.

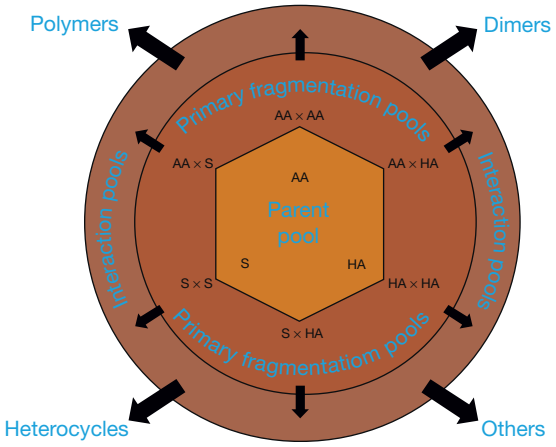

Figure 5 The pool theory of the Maillard reaction.

generated from specific precursors, theory stated in 1997 by Prof. Yaylayan. The intermediates produced during the initial phase of the MR arise from three well-defined main precursors: sugars (S), amino acids (AA), and the Heyns or Amadori products (HA) (Figure 5). This parent pool ($S \times AA \times HA$) will determine the pathway of the MR under specific conditions. In addition, each principal precursor can generate its own specific fragmentation products, being the collection of products arising from a single precursor or from interacting different precursors termed as pool:

- Amino acid fragmentation pool (AA): amino acids, amines, carboxylic acids, alkanes, etc.
- Sugar fragmentation pool (S): C_1 fragments (formaldehyde, formic acid, etc.), C_2 fragments (glyoxal, acetic acid, etc.), C_3 fragments (glyceraldehyde, hydroxyacetone, etc.), C_4 fragments (tetroses, 2,3-butanedione, 2-hydroxybutanal, etc.), C_5 fragments (pentoses, pentuloses, furans, etc.), and C_6 fragments (hexoses, pyranones, glucosones, deoxyglucosones, etc.)
- The Heyns and Amadori fragmentation pool (HA): amino acid–propanone, amino acid–propanal, amino acid–butanones, amino acid–pentadiuloses, amino acid–hexadiuloses, etc.

Each pool consists of either stable products and reactive intermediates that can rapidly undergo interactions either within a pool or with components of other pools or polymerize, resulting in 'interaction pools' (intermediate MR products (MRPs)) that give rise to 'final' compounds such as polymers, heterocycles, or other compounds.

Connection with Other Chemical Reactions

As stated earlier, the MR comprises different well-defined pathways, although most of them share different substrates and compounds formed with other common chemical reactions found in foods such as ascorbic acid degradation, caramelization, and lipid oxidation. Therefore, the development of one of these chemical reactions may lead to a higher development of another one.

In the case of the *degradation of ascorbic acid*, different compounds like furfural or reductones are formed, which take part in the Strecker degradation. In the case of *caramelization*, the enolization reactions can produce more aldoses to increase the development of the MR, as the hydrolysis of oligosaccharides to produce monosaccharides. In addition, the formation of osuloses or heterocyclic compounds – such as HMF – also increases the speed of the MR.

Lipid oxidation products are able to modify the MR by promoting the reaction or by reacting with some intermediates and producing compounds different to those commonly formed in the absence of lipids, for example, the development of aroma in cooked meat mediated not only by Strecker degradation aromatic compounds but also by different aldehydes produced during lipid oxidation and the reaction of the amino group of the polar head of phospholipids with carbohydrates or other carbonyl compounds. There are also common intermediates in both lipid oxidation and MR pathways. Both reactions produce carbonyl compounds, some of which are identical when starting from both lipids and carbohydrates. Thus, two dicarbonyl compounds, namely, glyoxal and methylglyoxal, are produced from both oxidized lipids and carbohydrates. These compounds have a special interest due to their high reactivity, promoting the development of the MR. In addition to carbonyl compounds, many other volatile (pyrroles) and nonvolatile (N-substituted hydroxyalkylpyrroles) compounds are also produced in both reactions. The formation of N-substituted hydroxyalkylpyrroles has a special interest because they are key compounds in the development of browning due to the MR and lipid oxidation.

Variables of the MR

The development of the MR depends on many different physical (temperature and heating time) and chemical parameters (pH, water activity, and substrates) that should be kept under control.

Substrates

The substrates involved in the MR are carbonyl groups – particularly from reducing sugars – and amino groups from amino acids and proteins. Carbonyl groups coming from fat oxidation also contribute, although to a lesser extent. Although the MR is an isomolecular reaction taking place between a sugar and an amino acid, the loss of sugars is always greater than that of amino acids due to the coexisting caramelization reactions.

Low-molecular-weight (LMW) *carbohydrates* are more reactive than higher-molecular-weight (HMW) ones due to reduced steric hindrance. Thus, standardized concentrations of sugars produce lysine losses coming from 16% for disaccharides, 42% for hexoses, and 60% for pentoses. The reactivity of sugars is also proportional to the amount of open form. In isomers, the stereochemical configuration determines the reactivity. Therefore, the general degree of browning of sugars decreases in the following order:

1. Pentoses: ribose > xylose > arabinose
2. Hexoses: galactose > mannose and glucose > fructose
3. Disaccharides: maltose ≥ lactose

However, it should be taken into account that the reactivity of sugars is not absolute and depends also on the state and nature

of the proteins with which they react. For example, raffinose destroys six times more lysine with lactalbumin than with globulins, while pentoses and hexoses have a similar reactivity with both proteins.

Other molecules with carbonyl groups formed by the Strecker degradation (formaldehyde, acetal, and propional) or those produced during the degradation of the Amadori compounds (furfural and hydroxymethylfurfural) can react with the amino groups, and the reaction starts again. Thus, in foods like cheese or fruit juices, whose composition includes such kind of MRPs, the MR can develop without the involvement of sugars.

All *proteins*, except the insoluble keratin, can react with sugars. The α-amino acids, which form the peptide bond with the subsequent carbonyl group, are inaccessible during heat treatments, unlike the terminal amino acids and those having a second amino group, such as lysine. In casein, the amount of free ε-amino groups is 50 times higher than the amount of the free α-amino group, so that the loss of lysine via the ε-group is the most serious nutritional consequence. For example, when a mixture of casein and glucose is heated, the destruction of histidine accounts for 17%, arginine for 22%, and lysine for 46%. Browning intensity is proportional to the concentration of lysine in feed protein. Thus, milk proteins turn brown faster than soy proteins and these faster than cereals proteins.

In the case of free basic *amino acids*, they react with sugars at a rate markedly higher than other amino acids but are the stereochemical configurations that determine their behavior. Thus, it has been established a classification of amino acids according to the degree of browning they produce:

- Amino acids producing high browning: lysine, glycine, tryptophan, and tyrosine
- Amino acids producing an intermediate degree of browning: proline, leucine, isoleucine, alanine, hydroxyproline, phenylalanine, methionine, valine, glutamic acid, and asparagine
- Amino acids producing a low degree of browning: histidine, threonine, aspartic acid, arginine, glutamic acid, and cysteine

pH

The effects of pH are complex, as each stage of the MR has an optimum pH: at pH <3, the browning is low and increases with increasing pH to a maximum of 10. At pH greater than 10, the decrease of protons required for catalysis of the Amadori and Heyns restructuring may be the cause of the decline of this reaction. The reactivity of the amino group is higher when the amino acid is in anionic form and this value depends on the acid–base character of the amino acid: For acidic amino acids (aspartic and glutamic acids), the anionic form occurs at pH 3, but for basic amino acids (lysine and arginine), a pH of 10 is required.

Taking into account this behavior, the initial pH of the product and the buffer capacity of the system influence the type and intensity of the MR. Then, foods can be divided in three groups depending on their pH and reactivity:

- *Foods with a pH between 6 and 8* (milk, eggs, cereals, and meat) where conditions are favorable to the MR: a decrease in pH can reduce browning during dehydration but adversely modifies the organoleptic characteristics.
- *Intermediate pH foods* (canned vegetables, fermented foods, honey, sauces, and soups) in which the MR and degradation of ascorbic acid can occur simultaneously.
- *Foods with a pH between 2.5 and 3.5* (acidic juices and fruit concentrates): these products are poor in amino acids and the MR occurs very weakly. Ascorbic acid degradation and caramelization of sugars are responsible for browning.

Water Activity

The intensity of the MR depends on the amount of water present in the foodstuff. The maximum activity is obtained at 50–70% relative humidity (10–15% water in the product or water activity of 0.67–0.70). Higher moisture decreases the rate of the MR and it is believed to not exist in the absence of water. Such kinetic is explained as follows: At very low water content, the diffusion of chemical species is decreased; the addition or increase of water content facilitates the diffusion of the reactants and increases the rate of browning. However, with higher amounts of water, the concentrations of reactive substances in solution decrease and the rate of reaction decreases. Typically, it is during the dehydration process when the risk of browning is higher, particularly at the stage of the process in which the water content is below 20% and the temperature is high, as in the final stages of milk dehydration.

Temperature and Time

The MR occurs at both room temperature (storage of many products) and high temperature (sterilization processes) and gives rise to the loss of amino groups due to the time/temperature pairing. Thus, equivalent losses are obtained during a high-intensity short-time heating than during a weak treatment for a long time period. For example, the same lysine loss is observed in a mixture of α-lactalbumin and lactose after 4 h of heating at 96 °C than after 4 days at 55 °C.

The activation energy values of the MR range from 10 to 160 kJ mol^{-1}. Depending on such activation energy and the energy supplied during heat treatment, one step of the MR or others are favored. As an example, Table 1 shows how different amino acids react with the same single sugar to produce different aromas depending of the heating temperature.

Nutritional Consequences of the MR

The MR have a high impact not only on the sensory properties of foods but also on their final composition, due to the decrease of nutritionally relevant substrates (such as essential amino

Table 1 Effect of temperature over the development of aromas in the MR

Amino acid	*100 °C*	*120 °C*	*180 °C*
Leucine	Sweet chocolate	Bread crust	Burnt cheese
Threonine	Chocolate	Wheat	Burnt
Valine	Rye bread	Bread crust	Chocolate-penetrating

acids), inhibition of various enzymes needed for the digestion of food, and the development of new compounds with both healthy (antioxidant activity) and harmful (acrylamide, heterocyclic amines, etc.) properties. It is evident that most of the effects refer to isolated foods, but little is known about the total intake of these compounds in the population or the overall nutritional and physiological effects of their consumption. This situation should be investigated for a better understanding of the positive and negative aspects of the intake of MRPs, especially because the current eating habits of young people comprise the intake of large amounts of snacks and fast food, which are foods plenty of MRPs.

Negative Effects

Loss of essential amino acids

The MR reduces the nutritional value of foods by decreasing the bioavailability of the essential amino acids. This reduction occurs even if no changes are observed in the color and flavor of food and is a consequence of amino acid consumption as the MR develops. Lysine is the most affected amino acid, although others like sulfur amino acids, tryptophan, and histidine are also decreased. The Schiff base formed in the first stage of the reaction is still nutritionally available as amino acid, while the Amadori compound is not. Only 5–15% of the *N*-ε-fructose-lysine can be used as a source of lysine, while the remaining 90% is metabolized by the gut microbiota thanks to the presence of an amadoriase enzyme.

Effect on digestive enzymes

The MR produces changes in the protein structure that prevent the action of digestive peptidases in the proximity of the cross-links. In this sense, *in vivo* assays on glycated proteins show a decrease in their digestibility, while *in vitro* digestions show a larger release of small peptides than free amino acids. Besides, it has been observed also that some components of the MR can inhibit the activity of certain digestive *proteases*. LMW compounds formed during the MR can inhibit the activity of trypsin, carboxypeptidases A and B, aminopeptidase N, and the glycine–leucine cytosolic dipeptidase, while dipeptidyl dipeptidase IV is not affected. In fact, it has been demonstrated that certain MRPs can even decrease nitrogen uptake from non-glycated proteins. Intestinal *disaccharidases* may also be affected. LMW MRP products are *in vitro* inhibitors of lactase, invertase, maltase, and trehalase at concentrations of 5–10 mg ml^{-1}. Other HMW compounds cause also inhibition at concentrations of 1.4–2.8 mg ml^{-1}. Quantitatively only the latter appear to be important in the reduction of enzyme activity.

Effect on vitamins

The influence exerted of MRPs on vitamins is not fully understood. Some MRPs can react with and destroy vitamins. As an example, losses of thiamine and pyridoxal have been demonstrated during the storage of milk powder for 9 weeks at 60 °C. Thiamine has an amino group and vitamin B6 an aldehyde group that can theoretically participate in the MR. Other vitamins affected are B1, B6, B12, and pantothenic acid, while nicotinic acid and biotin are relatively insensitive.

The destruction of the ascorbic acid in foods is closely related to the MR (see section '**Connection with Other Chemical Reactions**'). Dehydroascorbic acid reacts via the MR with the amino compounds of foods to form red and brown pigments. However, the nutritional loss of vitamin C by the MR is negligible; in the usual conditions of storage and processing, most of the ascorbic acid is destroyed before the MR became predominant.

Effect on minerals

The products of the MR also interfere with the absorption and metabolism of minerals. Melanoidins, the final polymeric brown pigments formed in the MR, have usually a net negative charge, responsible for the chelation of mineral cations such as Fe^{3+} and Cu^{2+}, leading to a final decrease on mineral absorption. It has been proposed that minerals are chelated through replacement of H^{+} ions within the melanoidin structure. Thus, the rank order of affinity for complexing the different minerals could be as follows:

$$Mg > Ca = Cu > Zn$$

The presence of MRPs decreases not only the absorption of minerals but also their excretion rate and deposition within the human body. The consumption of diets rich in MRPs gives rise to an increased renal excretion of calcium, iron, and zinc. In addition, there is a depletion on calcium content in bone and its deposition in other nonusual organs such as the bladder. In addition, the metal complexing capacity of MRPs could explain some effects such as inhibition of metalloenzymes (carboxypeptidases A and B) as stated earlier.

Development of substances with mutagenic and/or carcinogenic activity

Heat treatment is very common as a source of new compounds with pleasant colors and flavors. However, there is a concomitant formation of different compounds with toxic properties, reducing the biological value of food. These chemicals are known as processing contaminants, compounds that were not present in the fresh food, and its genesis is directly related to the technological process. Most of these compounds are associated with mutagenic, teratogenic, or carcinogenic activities in living organisms and therefore should be evaluated and, if necessary, controlled by the appropriate food safety agencies. As examples, heterocyclic amines, polycyclic aromatic hydrocarbons (e.g., benzopyrene), *N*-nitrosamines, monochloropropanediols (e.g., 3-MCPD), furan, acrylamide, and hydroxymethylfurfural are important processing contaminants, which are detailed in other chapters.

Positive Effects

Antioxidant activity

One of the positive features of the MR is its antioxidant action, as some MRPs, like melanoidins, have antioxidant properties. Its antioxidant activity is particularly interesting since they are formed naturally in the food during processing or storage. The mechanism of the antioxidant activity of these MRPs is not well understood, but is believed to be based on the ability to trap positively charged electrophilic species, elimination of oxygen radicals, and/or chelation of metals to form inactive complexes. *In vivo* antioxidant activity has been found to be exerted

in cell cultures, animal models, and human beings. For example, digested coffee melanoidins protected human hepatocytes HepG2 under oxidative stress. In human beings, coffee melanoidins increase the antioxidant activity of plasma up to a 7%, shortly after consumption, and protects human lymphocytes against DNA damage induced by ROS.

Antimutagenic effect

Reference should also be made to the antimutagenic effect of Maillard compounds, either by chemical or enzymatic modification or by inducing its suppressive effect on DNA alterations stimulated by mutagenic agents. For example, melanoidins can modify the activity of phase I and phase II enzymes at the liver, which performs a chemopreventive activity due to the processing of xenobiotics in order to be excreted in urine. In addition, *N*-methylpyridinium, a known MRP produced during coffee roasting, has been identified as the key compound in coffee brews modulating phase II glutathione *S*-transferase and induction of Nrf2 gene transcription, a transcription factor in the nucleus responsible for the antioxidant and detoxifying enzymes. Other types of enzymatic systems modulated by melanoidins are matrix metalloproteases, endopeptidases that allow tumor growth and metastasis. In addition, melanoidins can bind LMW harmful compounds like acrylamide. The nucleophilic amino groups of amino acids from the protein core of melanoidins react with acrylamide by a Michael addition reaction.

Antimicrobial action

Certain products of the MR exert an antimicrobial action. The first assays showed that 20 different bacterial genera diminishing or inhibiting their growth in the presence of MRPs originated in different ways. In subsequent trials, it has been found that melanoidins present in foods such as beer, cookies, and coffee have bacteriostatic and bactericidal actions, depending on their concentration in the foodstuff. This antimicrobial activity is shown with different types of bacteria, either Gram+ or Gram− such as the species *Staphylococcus aureus* and *Escherichia coli*. The mechanism of action by which melanoidins exert bactericidal action is related to the ability of melanoidins to chelate the Mg^{2+} ions present in the outer bacterial membrane, which is finally disrupted. The bacteriostatic activity is performed by iron chelation, which is an essential cofactor for the biochemical metabolism in bacteria.

Antiallergic character

Some intermediates of the MR have antiallergic action, such as the Schiff base formed during the first step of the reaction. This is supported by some experiments that showed a reduction on antigenicity and allergenic response of those foods rich in protein and carbohydrates heated under mild conditions. However, it should also be noted that such reactions are able to introduce new antigenic determinants in dietary proteins.

Food Processing and Prevention of the MR

Processes such as roasting, baking, and frying rely on favorable effects of the MR such as the development of pleasant colors and flavors. However, during pasteurization, sterilization, or drying, the occurrence of the MR is unfavorable. The main challenge in the food industry is therefore the specific design, optimization, and control of the aforementioned processes for the production of food with the desired quality and stability. All the parameters listed in the section of variables of the MR can be manipulated in the daily laboratory work with model systems in order to make the reaction rate controllable. However, given the chemical complexity presented by foods in a real environment, it is only possible to moderately modify the rate of the MR. Reducing the pH, temperature, and water activity significantly inhibits reaction, but sometimes, it is technically and economically impossible to achieve that.

One of the most common ways to control browning reactions consists of the addition of sulfite, metabisulfite, or sulfur dioxide. These compounds react with carbonyl compounds and Schiff bases to give highly stable sulfonates. Then, sulfites fix many reactive intermediates of the MR, extending the induction period and delaying the onset of browning. However, changes in the laws of different countries, caused by the adverse effects of sulfites, have hampered the use of sulfites, causing the search for alternatives. Sulfur amino acids can prevent browning in various food products like fruit juices, being *N*-acetyl-L-cysteine and others – SH-containing compounds – as effective as sodium bisulfite in decreasing the rate of browning. The addition of glutamic or aspartic acid also causes a decrease in browning. This effect may be associated with the stability of the Amadori compounds so formed with glutamic or aspartic acids, as the rate of the MR is lower with such Amadori compounds.

Another way to control the development of the MR is the removal of substrates from food. For example, during the preparation of dried eggs, glucose is usually oxidized to gluconic acid by the enzyme glucose oxidase to gluconic acid or removed by fermentation. In the case of potatoes, storage for 2 weeks at a 20 °C decreases the amount of reducing sugars, giving rise to a lower production of acrylamide during potato frying. Furthermore, the conversion of the amino group in the amide function by the activity of transglutaminase enzymes can preclude the first stages of the MR.

For those foods where the inactivation of pathogenic and spoilage microorganisms and enzymes is developed by means of thermal processes, a minimal treatment temperature and a corresponding holding time are required. Thus, a suitable method of control the extension of the MR is to reduce the thermal load to which a product is exposed during processing. As a general rule, it is desirable to work with low-intensity thermal treatments and to store foods at moderate temperatures in order to not to exceed the MR activation energy. The reduction of processing times for heating to a final temperature and cooling is possible by improving the heat transfer or by applying alternative thermal technologies such as direct steam injection and ohmic heating. These new alternatives avoid temperature gradients and heat transfer limitations. In addition, preservation of food can be achieved also by nonthermal based on alternative inactivation mechanisms. Pulsed electric field processing and high hydrostatic pressure treatments work at reduced treatment temperatures, avoiding the occurrence of heat-induced product changes.

Contrary, the occurrence of the MR is desired to a certain extent in foods like bread of baked meat where the formation

of color and flavor is expected on the surface of the food. As an example, the application of emerging technologies such as infrared heating allows the achievement of a pleasant surface color and flavor while reducing the total thermal process intensity. Compounds such as malonate, phenylacetone, tertiary amine salts, acetic acid, and boron trifluoride can also be added in order to catalyze the MR and Amadori rearrangement. Another possibility is the addition of MR substrates such as milk proteins or beaten egg to the surface of baked dough, a common practice in the bakery industry.

Conclusions

The MR is a ubiquitous reaction taking place in heat-treated foods and also those foods stored during long time at room temperature. The development of the MR is desired in products where a brown color and a special aroma are expected, such as coffee, bread, and meat. Contrary, many efforts have been done by the food industry in order to limit the extension of the MR in foods like milk or fruit juices, where brown colors denote a low-quality product. In addition to this, the MR is responsible for a decreased nutritional value in many foods and the development of harmful compounds as well. Furthermore, the MR shares different biochemical pathways with other common reactions such as lipid oxidation, caramelization, and ascorbic acid degradation. Therefore, a fine-tuning of the MR alone is complicate under a technological point of view. Currently, the knowledge of the shared chemical pathways of these reactions constitutes the basis for obtaining new foods with healthier and tasteful properties.

See also: Acrylamide; Ascorbic Acid: Properties, Determination and Uses; Biscuits, Cookies, and Crackers: Chemistry and Manufacture; Bread: Chemistry of Baking; Browning: Non-enzymatic browning; Caramel: Methods of Manufacture; Coffee: Types and Production; Cooking: Domestic Techniques; Drying: Effect on Nutrients, Composition and Health; Extrusion Cooking: Chemical and Nutritional Changes; Milk: Processing of Milk; Oxidation of Food Components; Pasteurization: Effect on Sensory Quality and Nutrient Composition; Polycyclic Aromatic Hydrocarbons; Proteins: Chemistry, Characterization, and Quality.

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Relevant Websites

www.imars.org/online/ – International Maillard reaction Society.

Maize

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Introduction

Maize (*Zea mays* L.) is the leading grain in the world with an annual production of more than 1 billion tons in 2013, and it constitutes staple foods for large groups of people in Latin America, Africa, and Asia. Maize production has increased the most because of its greater adaptation to different ecological systems and strong demand for bioethanol and animal feed and for the production of sweeteners and other nonfood industrial products (i.e., biodegradable packaging materials). The genetic modification of maize (*Bt* maize) is partially responsible for record productions observed in the past 10 years. According to the FAO, 54%, 19%, 13.8%, and 14% were used for feed, food manufacture, direct food, and bioethanol, respectively. Maize is used for the production of numerous indigenous foods, maize meals, flours, grits, starches, sweeteners, cooking oils, breads, tortillas, breakfast foods, snacks, bioethanol, and alcoholic beverages. Processed maize products are manufactured from raw materials obtained from three major industries: dry milling, wet milling, and nixtamalization (Figure 1). Dry millers produce an array of products widely used by the breakfast cereal, snack, baking, and brewing industries (Figure 2). Most of the prime starch obtained from wet millers (Figure 3) is enzymatically converted into maltodextrin, maltose, glucose, and high-fructose syrups (Figure 4). The nixtamalization industry generates fresh or dry masa flours, which are further converted into table tortillas and lime-cooked snacks such as corn and tortilla chips.

The Americas and particularly Mexico are the cradle of maize, the most adapted cereal to different ecosystems. The most accepted position of the maize origin is that teosinte is the ancestor because of the free and frequent hybridization of teosinte and maize in nature; they own the same chromosomic number ($2n = 20$), and cultivars shared several anatomical traits including analogous morphological pollen features. The Mesoamericans created maize by transforming a tiny two-rowed ear of teosinte of about 5 cm long into the more than 300 races known today. After the Spaniards conquered Mexico, maize was taken to Europe and then quickly disseminated into Asia and Africa. Today, maize is planted in all continents under different climatic conditions and agrosystems. Open-pollinated varieties are generally planted in developing countries while high yielding and *Bt* hybrids in developed countries. Among commercial cereals, maize is the only one that has the male or staminate and female or pistillate flowers separated.

Maize is a crop with a remarkable genetic variability, and it is classed according to the form of the caryopses into dent and flint or corneous and subclassed according to color into white or yellow. Most of the maize planted worldwide is yellow dent (Table 1), which is the preferred by the feed, bioethanol, breakfast cereal, snack, and starch processors. The use of white dent maize is growing because it is preferred for tortilla and related snack production. Nutritionally, maize is considered as an excellent source of calories due to its high starch content (between 65% and 70% starch) and an adequate source of insoluble dietary fiber. Most maizes contain between 8.5% and 11% protein, which is considered intermediate in terms of essential amino acid balance. The maize proteins lack sufficient levels of lysine and tryptophan to sustain infant growth. The supplementation of maize foods with legumes, eggs, and other animal proteins greatly enhances protein quality and levels of micronutrients. Nutrient composition of maize is reported in Table 3.

However, many specialty genotypes such as popcorn, waxy, high-amylose or amylomaize, sweet, blue and other pigmented, Cuzco, and quality protein are planted for specific uses (Table 1).

Traditional Foods

Regular and specialty types are widely consumed in numerous forms in Latin America, Asia, Africa, and the Balkans. White dent maize is generally preferred for foods because it favors the final color of processed foods, but yellow types are preferred in China, Brazil, Argentina, Italy, and some other countries. Most traditional foods are produced from kernels that are milled into whole wheat or refined grits, meals, or flours.

The use of immature maize on the cob is practiced worldwide. Maize cobs with or without husks are boiled in water or cooked over a fire and then flavored with salt, cream, butter, margarine, and other sauces. In most areas, regular maize is used as green maize. In Latin America, maize is additionally processed into tortillas, arepas, couscous, polenta, porridges, and gruels. The traditional method to convert maize into tortillas was developed by ancient Mesoamericans. In the traditional process, maize is lime-cooked in clay pots over a fire followed by overnight steeping. The alkaline-cooked kernels named *nixtamal* are ground into a dough or *masa* with a stone grinder. Pieces of *masa* are hand-molded into disks, which are baked on a hot griddle to yield tortillas. Industrially produced tortillas are mainly used as wraps to prepare tacos that hold different types of fillings (beans, various meats, cheeses, eggs, and vegetables). Tacos are the symbol of the Mexican cuisine and are known and consumed worldwide. Many other nixtamalized foods such as *tamales*, *atole*, and *pozol* are produced from *masa*.

Arepas, the national maize bread of Venezuela and Colombia, are traditionally produced from white grits or meals that are moistened, cooked in water, and ground to a dough. The dough is hand-shaped into flat disks that are baked on each side on a griddle. Arepas are stuffed with various types of meats, cheeses, butter, jellies, and other fillings. Stuffed arepas are also fried to produce *hallaquitas* or *empanadas*. Large Venezuelan plants commercially manufacture instant arepa flours that require only hot water to produce the dough. Briefly, grits

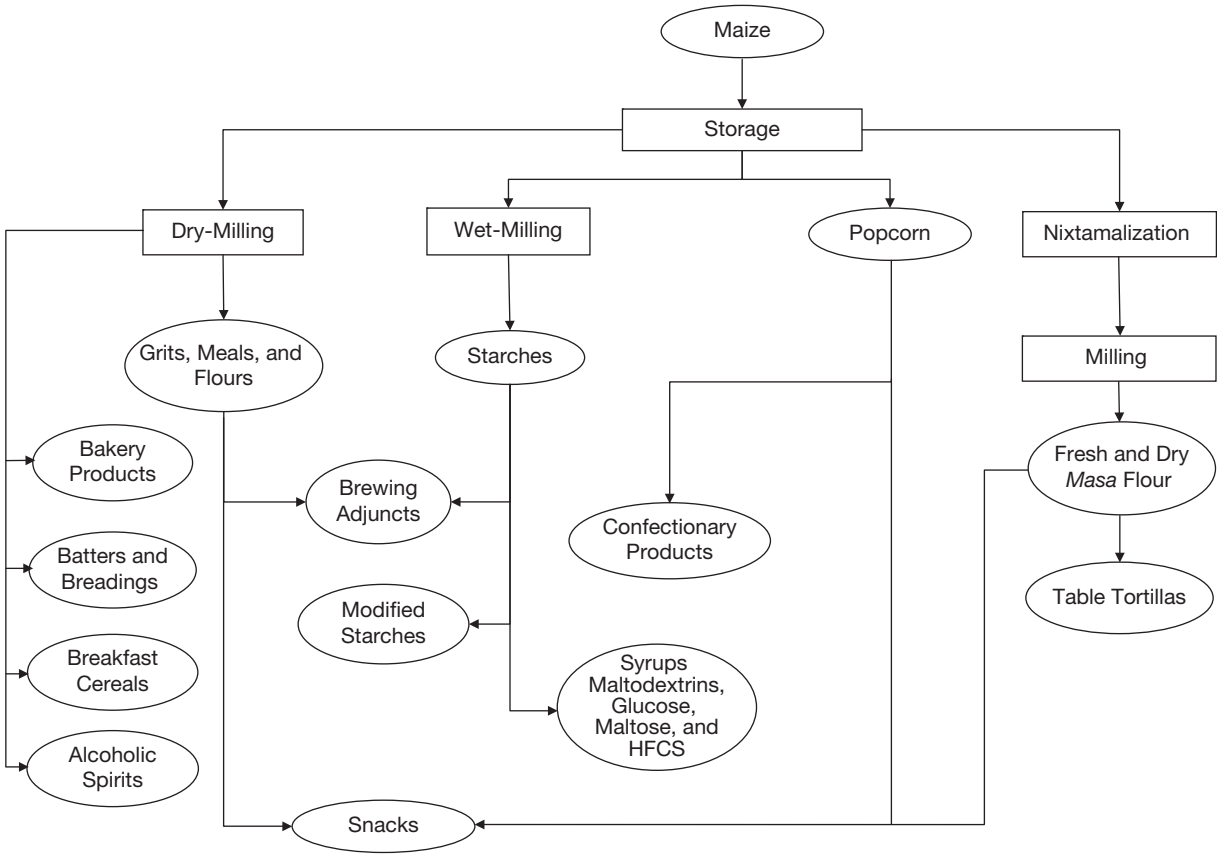

Figure 1 General flowchart of the postharvest management and processing of maize for human foods.

Nixtamalized products from maize.

are cooked and then passed through flaking rolls to gelatinize the starch. The flakes are dried and ground into flour to meet a certain particle size distribution. With precooked flour, arepas can be prepared in approximately 30 min instead of 12–24 h required by the traditional process.

Maize is also used for the production of many traditional beverages still widely consumed worldwide. Chicha is a prehispanic sacred fermented beverage prepared in Peru and Bolivia in which ground maize is first treated with amylases from germinated maize or saliva and then naturally fermented. Other alcoholic beverages such as tesguino, pito, talla, and other related opaque beers are still traditionally consumed in Africa and Mexico.

In Africa and Asia, maize is generally dry-milled into grits, meals, and flours for the production of flat breads, unfermented and fermented porridges, steamed foods (couscous), and alcoholic or nonalcoholic beverages. Many of the traditional foods are produced from fermented or sprouted kernels. Tô, pap, nshima, and sadza are popular maize porridges widely consumed in Africa.

Industrial Processing of Maize for Foods

Most of the maize is first industrially processed by dry milling, wet milling, or nixtamalization. Dry-milling manufactures refined endosperm fractions of varying particle sizes (grits, meal, and flour), germ for oil, and sometimes dietary fiber ingredients (Figure 2). Starch and refined dry-milled products are mainly used for the production of breakfast cereals, snacks, sweeteners, lager beers, and fuel or potable ethanol. Wet millers (Figure 3) produce relatively pure starch, protein, fiber, and germ, whereas the nixtamalization industry produces dry masa flours, tortillas, and related snacks that are increasing rapidly in popularity in many countries around the globe.

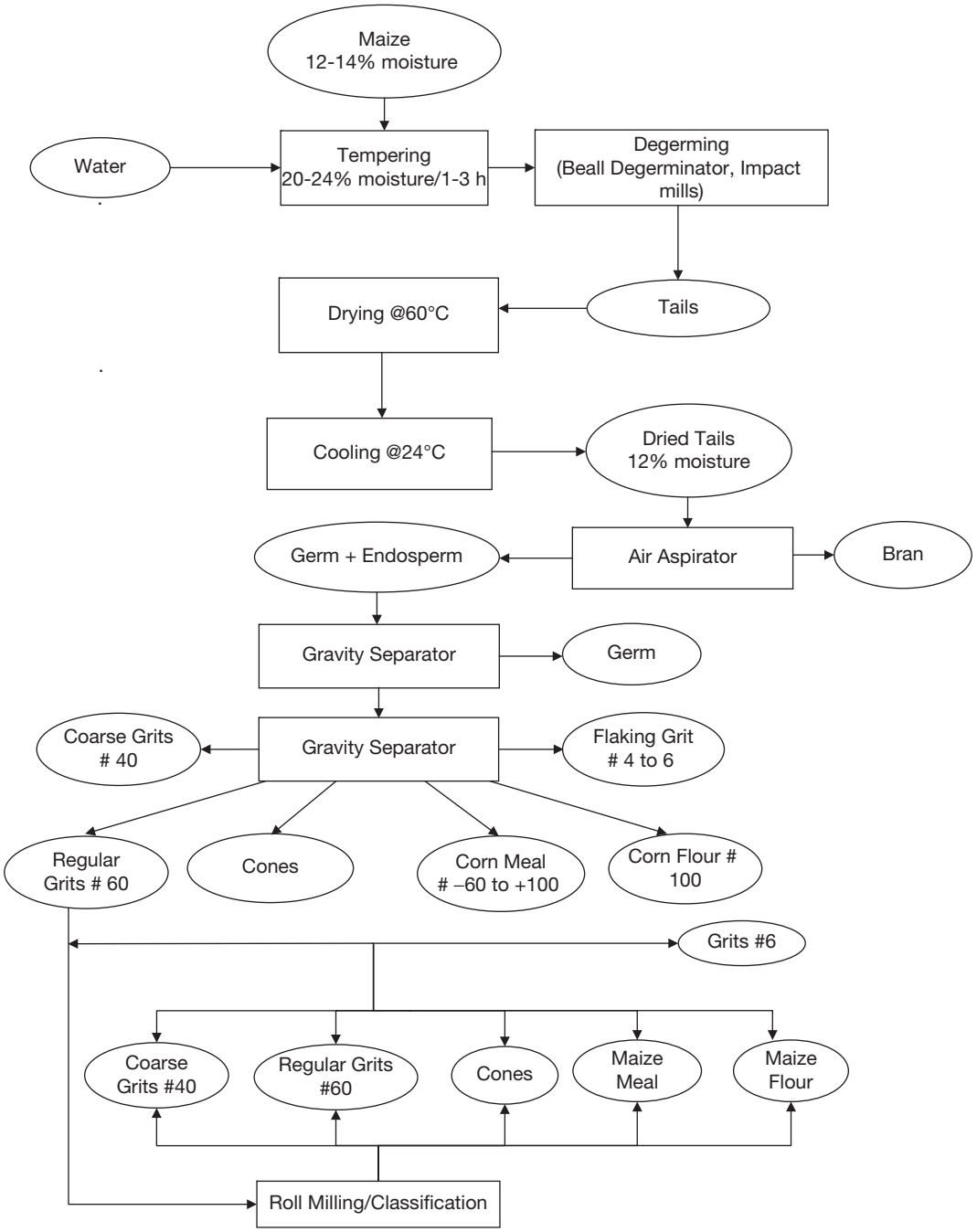

Figure 2 Flowchart of the degerming–tempering dry-milling process of maize.

Food Uses of Dry-Milled Fractions

Endosperm products from dry milling, ranging from large grits to flour, are widely used by brewers and snack food and breakfast cereal processors (Table 2). Flaking grits are large (the United States, 3.5–6 mesh sieves) endosperm particles obtained after degerming. These large pieces of endosperm are used to produce corn flakes still considered the most popular ready-to-eat breakfast cereal worldwide. The traditional cornflake process starts when yellow flaking grits are pressure-cooked (1 kg cm^{-2}) in rotary cookers for 1–2 h with syrup, sugar, nondiastatic malt, salt, and water. The cooked grits are delumped and partially dehydrated to about 20% moisture. Then, the grits are equilibrated, flaked through a pair of counterrotating rollers, and toasted in order to develop the crisp texture, brown-golden color, and the characteristic flavor. Sugar-coated flakes are made by the same process, but the sweetener is sprayed onto the flakes immediately after toasting. Cornflakes can also be made from meal or grits that are extruded into third-generation half products or pellets.

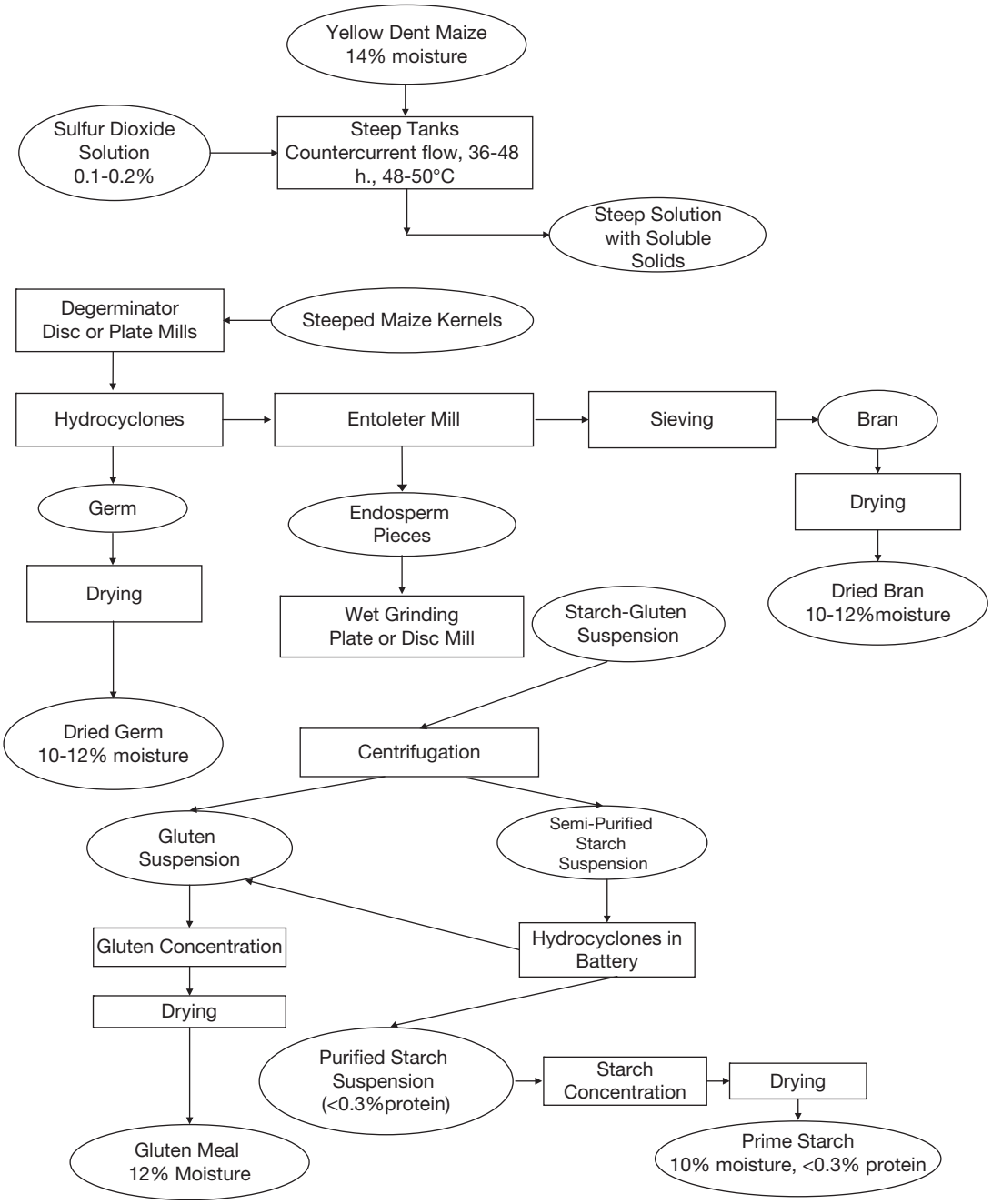

Figure 3 Flowchart of the sulfur dioxide wet-milling process for the production of maize starch.

Corn grits are low in fiber and contain less than 1% oil (Table 3). Grits of various granulations are extensively utilized by the snack, breakfast cereal, and brewing industries. In many countries around the globe (the United States, Italy, and Argentina), cooked white or yellow maize grits are consumed as a side dish. Instant or precooked maize grits, which require only 5 min cooking, are popular.

Traditionally, the brewing industry has been the largest user of grits because they are a cheap source of fermentable carbohydrates. Whole ground or refined grits are also utilized to produce alcoholic beverages. Straight bourbon whiskey is obtained from distillation of a fermented mash containing at least 51% maize in the United States.

Refined milled fractions from maize alone or in combination with other cereals and ingredients are often used in ready-to-eat breakfast and snack foods. Grits, meals, or flours are cooked to gelatinize the starch, denature the protein, and produce a pregelatinized dough, which can be formed into flakes, shreds, puffs, or collets. Extruded gun-puffed cereals are still one of the most popular types of ready-to-eat breakfast cereals. The puffing gun is charged with equilibrated collets, sealed, and heated to increase the pressure to 10.5–14 kg cm^{-2}.

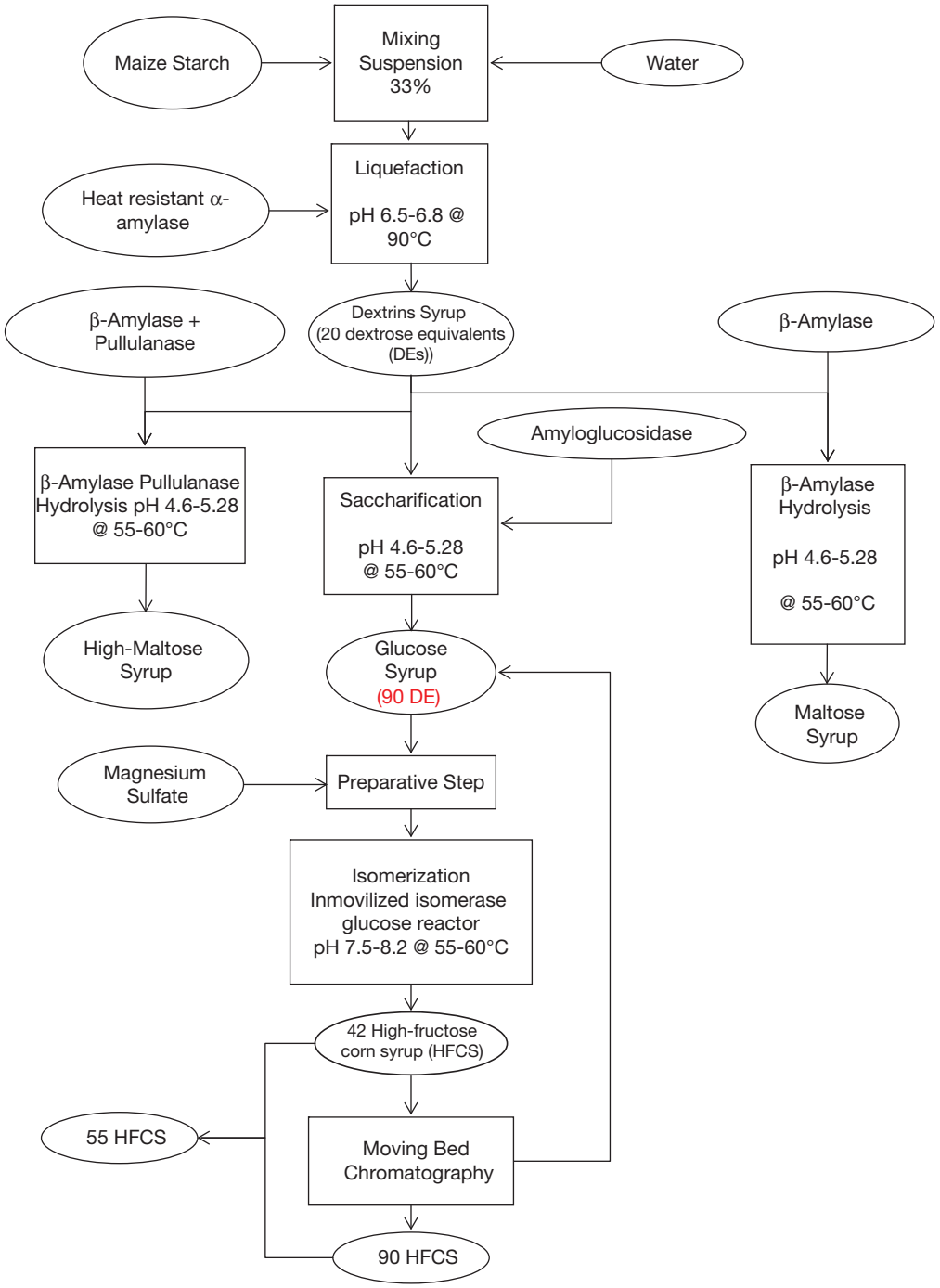

Figure 4 General scheme followed for the production of maize syrups.

The pressure is suddenly released and the material puffs and explodes from the gun. The degree of puffing varies according to temperature and pressure and commonly varies from 10 to 16 times. Products that require less expansion are generally oven-puffed. This process yields products with a three- to fourfold expansion.

Corn meal or grits are processed through extrusion cooking and puffing to produce snacks such as curls, puffs, and balls. Extrudate expansion is closely related to product texture and is affected by the viscoelastic nature of the material and the amount of moisture in the material flowing through the extruder die assembly. The extrudates are baked or fried, flavored, and packaged to produce the final ready-to-eat item. Pellets or half products are usually manufactured using a combination of two extruders. The first extruder cooks the raw ingredients while the second cools, forms, and sizes the extrudate into dense pellets, which are dried, stored, and then baked or fried into the third-generation snacks. Baked collets are crisp

Table 1 Characteristics and food uses of different types of maizes

Type of maize	*Food uses*
Feed grade yellow maizes	These are the most popular types grown in the world. The high yielding yellow dent maizes with soft-textured endosperms are widely used as feed and for the production of first-generation bioethanol and refined starch
Food grade yellow and white maizes	These types have improved processing efficiency in dry milling, nixtamalization, and snack food processing plants. These maizes have high test weight and density
Quality protein maize (QPM)	QPM has the opaque-2 gene that is combined with modifier genes that significantly improve the hardness and agronomic performance of the crop. These types contain about twice the amount of lysine and tryptophan compared to their regular counterparts. Both yellow and white QPM open-pollinated varieties and hybrids are being used in Brazil, Mexico, Ghana, and other countries. Harder QPMs are suited for dry milling and alkaline cooking
Popcorn	Special kind of flint or corneous maize. Hybrids or varieties with high proportions of translucent or vitreous endosperm give higher expansion rates. Expansion volume is the most critical quality factor for popcorns. Most commercial popcorn has a 35- to 40-fold expansion. The pericarp or bran of the kernel contributes directly in the popping mechanism by serving as a pressure vessel enclosing the endosperm. Popped corn with a spherical shape is called mushroom or ball type and is preferred in the confection industry. The butterfly-type popcorn has a higher expansion, lower apparent bulk density, and better mouthfeel and therefore is preferred for on premises popping
Blue maize	Most blue maizes have a soft or floury endosperm texture that generally grow in long ears (8–12 rows). The aleurone layer contains the anthocyanins that impart the blue coloration. Blue corn is used to produce traditional foods in Mexico and other parts of America and especially for the production of organic flours and foods such as tortillas and chips. These colored genotypes contain higher levels of flavonoids that are an excellent source of antioxidants
Sweet maize	Sweet maize types have recessive genes (sugary 1 or 2 and su1 or 2) that cause an alteration in the endosperm that results in higher levels of soluble sugars and reduced levels of starch in the kernel. Sweet-corn hybrids have been developed specifically for the canning industry or for consumption on the cob
Baby maize	Special varieties that are grown and shucked immediately after pollination when the ears are about 5 cm long. These small ears are used as pickles and other tasty snacks in salad bars. Most of the baby corn used is produced in Thailand and exported to Europe and North America
Waxy maize	Waxy maize is named for the somewhat waxy appearance of the kernel. Waxy maize starch is composed entirely of amylopectin. It is utilized mainly by the wet-milling industry. Waxy starch has a higher hot viscosity and produces softer, more stable, and clearer gels due to its lower retrogradation. It also has a higher freeze–thaw stability
High-amylose maize	Also named amylomaize. Expresses high quantities of linear amylose because of the recessive *ae* gene located in chromosome 5. Most genotypes contain amylose from 37% to 65%. The amylomaize starch has potential for the paper, textile, corrugating, and adhesive industries. The high-amylose starch produces rigid opaque gels with potential for the confectionery industry and as a thickener in various puddings and processed foods
Cuzco/cacahuacintle maize	Cuzco maizes come from eight-rowed ears that produce the largest known dent kernels. Cuzco corn grows at high altitudes and produces white kernels with soft endosperm texture and bland flavor. Cuzco kernels are mainly used to manufacture Cornnuts™ and Cacahuacintle kernels for hominy and *pozole* production

Maize dry-milled fractions. Clockwise: maize kernels, bran or pericarp, corn flour, regular grits, flaking grits, and germ.

Maize starch and syrup.

with a light and crunchy texture. Deep-fat-fried collets are crisp with smoother texture due to the uptake of the oil.

Corn meals and flours are commonly used to produce an assortment of chemically leavened baked and fried products such as corn bread, muffins, pancakes, sticks, fritters, and hush puppies (Table 2). Most maize bread formulations contain wheat flour, chemical leavening agents, sugar, salt, milk powder, and other ingredients. Maize does not have functional gluten so wheat flour is included to give the dough more

Corn flakes, the most popular breakfast cereal sold worldwide.

Popcorn, one of the most popular snack foods worldwide.

elasticity and hence produce a more aerated product. Composite flours of maize and wheat are also frequently used to produce batter and breadings.

Food Uses of Starch

More than 90% of the commercial starch is from maize due to the low cost of the grain, high starch content, and high value of the coproducts. The industrial wet-milling process of maize is detailed in Figure 3. In this process, yellow dent maize kernels are steeped in a sulfur dioxide solution for about 2 days in order to disrupt the protein matrix that entraps starch granules. Then, the wet kernels are degermed and ground to free the starch, which is separated from the gluten via centrifugation and use of hydrocyclones. The resulting dehydrated prime starch contains less than 0.3% protein. The refined starch is channeled to various food industries to be used as additive or enzymatically hydrolyzed into various types of syrups. The main types of products obtained are dextrins, maltose, glucose, and fructose syrups (Figure 4). In addition, the by-products are highly utilized by the feed and oil-crushing industries. The bran is generally used to feed domestic animals, although some is finely ground to produce high dietary fiber foods. The germ is demanded by oil mills to produce the refined oil and defatted protein meal. Corn oil is widely used as cooking oil because of its stability and high levels of polyunsaturated fatty acids.

Corn Syrups

In 2014, nearly 20 million metric tons of maize was converted into syrups in the United States. About 64% of this production was transformed into high-fructose syrups (HFCSs). The production and utilization of syrups have increased during the past decades because the soft drink industries prefer to use sweeteners instead of crystallized sugar. Maize sweeteners are preferred over sucrose because they quickly dissolve in water and are easier to incorporate into soft drinks. The HFCSs containing 90% fructose impart 1.7 times more sweetness than sucrose at equivalent concentrations.

The various types of syrups are manufactured from acid hydrolysis or preferably after enzymatic conversions. The industrial production of amylolytic enzymes allowed the production of better-quality low-dextrose-equivalent (DE) syrups and the production of glucose syrups with high DE or sweetness. The industry evolved with the commercial availability of heat-stable α-amylase and amyloglucosidase that could transform practically all the starch into glucose. The greatest technological developments were the utilization of immobilized glucose isomerase and the continuous separation of glucose and fructose by moving bed chromatography (Figure 4).

Low-DE Syrups (Maltodextrins)

The low-DE or maltodextrin-rich syrups are industrially produced by acid hydrolysis or by α-amylase conversion. These syrups are the easiest to manufacture and are the first step for the production of maltose, glucose, and HFCS. The acid hydrolysis is performed in 35–40% starch suspensions using a 0.02–0.2 N hydrochloric solution in a pressurized reactor. This technology is now limited because it can yield undesirable compounds such as methylfurfural, formic acid, and/or off-flavors and off-colors. The most popular way to convert starch into dextrins is via the utilization of α-amylases. Nowadays, heat-stable α-amylases are used because they require shorter incubation. Their optimum activity is achieved at pH of 6.5 and temperature of 90–100 °C. These syrups with 10–20 DE are very viscous, rich in dextrins, and low in sweetness; therefore, they are mainly used as thickeners. Dextrin syrups are alternatively used as substrate for cyclodextrin production. These cyclic oligosaccharides are composed of six, seven, or eight glucose units linked by α-(1–4) linkages to form α-, β-, or γ-cyclodextrins, respectively. Cyclodextrins are used to enhance chemical stability and reduce volatility of complexed molecules and mask undesirable odors and flavors.

Table 2 Principal food uses of refined maize dry-milled fractions

Type of dry-milled fraction	*Particle size of US mesh sieve*	*Major food uses*
Flaking grits	3.5–6	Used for the production of breakfast cereals such as corn flakes and corn pops. In Brazil, flaking grits are used to produce a traditional food named *canjica*
Coarse grits	10–14	Used for the production of extruded breakfast cereals (i.e., extruded flakes and puffs) and second-generation (i.e., puffs) and third-generation (i.e., pellets) snacks
Medium grits	14–28	Used as brewing adjuncts and for the production of extruded breakfast cereals (i.e., extruded flakes and puffs) and second-generation (i.e., puffs) and third-generation (i.e., pellets) snacks
Fine grits	50–75	Used as brewing adjuncts and raw material for the production of maize porridges and for the production of extruded breakfast cereals (i.e., extruded flakes and puffs) and second-generation (i.e., puffs) and third-generation (pellets) snacks
Cones	50–80	Used as raw material for the production of corn bread, extruded, breakfast cereals (i.e., Cheerios™) and second-generation (i.e., puffs) and third-generation (i.e., pellets) snacks
Meal	50–75	Used for the production of breadings and batters and maize-based bakery items (pancakes, muffins, biscuits, waffles, and corn bread)
Flour	−75	Used for the production of breadings and batters, thickening agents, baby foods, meat extenders, and maize-based bakery items (pancakes, muffins, biscuits, waffles, and corn bread)

Table 3 Nutrient composition of whole maize and its dry-milled fractions

		Refined dry-milled fractions			
	Whole maize	*White grits*	*Yellow grits*	*Hominy grits*	*Degermed meal*
Moisture (g)	10.37	10.00	10.00	10.48	11.59
Energy (kcal)	365	371	371	347	366
Protein (g)	9.42	8.80	8.80	8.53	8.48
Lipids (g)	4.74	1.20	1.20	1.36	1.65
Saturated (g)	0.667	0.155	0.155	0.260	0.225
Monounsaturated (g)	1.251	0.300	0.300	0.200	0.412
Polyunsaturated (g)	2.163	0.516	0.516	0.680	0.710
Carbohydrates (g)	74.26	79.60	79.60	79.16	77.68
Dietary fiber (g)	7.3	1.6	1.6	4.8	7.4
Calcium (mg)	7	2	2	4	5
Iron (mg)	2.71	1	1	3.52	1.10
Sodium (mg)	35	1	1	2	3
Thiamine (mg)	0.385	0.130	0.130	0.580	0.140
Riboflavin (mg)	0.201	0.040	0.040	0.330	0.050
Niacin (mg)	3.627	1.200	1.200	4.810	1.000
Folate (mcg)	19	–	–	N/A	48

USDA (2014) – http://ndb.nal.usda.gov/.

Maltose Syrups

Regular and high-maltose corn syrups are manufactured starting with a low-DE syrup that is treated with β-amylase or a combination of pullulanase and β-amylase (Figure 3). β-Amylase activity is optimized at pH of 5 and temperature of 55 °C. The utilization of only β-amylase yields syrups with 50–55% maltose, while the use of pullulanase and β-amylase results in syrups with about 80% maltose. Maltose syrups are extensively used as flavorings for breakfast cereals, beverages, and ice creams.

Glucose Syrups

For the production of 90 DE glucose syrups, low-DE syrups are treated with amyloglucosidase or saccharifying enzyme at pH of 4.6–5.2 and a temperature of 55–60 °C (Figure 4). The syrup is refined and clarified through columns of activated carbon and ionic resins with the aim of removing minerals, pigments, soluble protein, fat, and enzyme-resistant starch. Glucose syrups are utilized as sweeteners for soft drinks and baking formulations and as a source of fermentable carbohydrates for light beers, alcohol production, and yeast-leavened bakery items. Crystallized dextrose can be produced by concentrating the syrup to 75% solids, adding glucose, and gradually cooling to drop the temperature to 20–30 °C for several days.

High-Fructose Corn Syrups

HFCSs are manufactured starting from 90 DE glucose syrup that is further treated with immobilized glucose isomerase (Figure 4). The refined glucose syrup is deaerated and treated with magnesium sulfate so as to assure oxygen removal and the

sequestration of calcium that lowers enzyme activity. There are three major types of HFCS: 42, 55, and 90. The 55 and 90 HFCSs are produced from the 42 HFCS. The 42 HFCS is industrially produced by passing glucose syrup through a reactor with glucose isomerase. The optimum operating conditions are pH of 7.5–8.2 and temperature of 55–60 °C. The glucose syrup flow rate is controlled to convert 42–45% of the glucose into fructose. The 90 HFCS is obtained after separating fructose and glucose in a moving bed chromatography system that produces two syrups with about 90% of each type of sugar (Figure 4). The 55 HFCS, which is analogous to crystallized sugar, is manufactured by mixing 42 and 90 HFCSs. The 90 HFCS has approximately 1.7 times more sweetness than crystallized sugar. HFCSs are used as substitutes of table sugar especially in soft drinks.

Food Uses of Nixtamalized Products

Three basic types of products are industrially produced from lime-cooked maize: table tortillas, corn chips, and tortilla chips. Corn and tortilla chips are primarily produced and consumed in developed countries, where they have an important share within the salted snack food market.

Nixtamalized traditional foods.

Table Tortillas

Tortillas and masa products constitute the staple food for large populations in Mexico and Central America. Tortillas are produced using traditional and industrial processes. The industrial production of tortillas is labor-intensive and requires considerable equipment. Frying has expanded the market for *masa*-based foods because the final product has excellent organoleptic properties and a long shelf life. The two most popular snacks, tortilla and corn chips, are usually made from coarsely ground fresh *masa* or *masa* flours. Corn chips are produced directly from *masa* and contain more oil than tortilla chips. Tortilla chips are baked similarly to tortillas before frying. Corn and tortilla chips are often salted and flavored immediately after frying. Most popular flavorings include nacho cheese, hot/spicy, barbecue, lemon salt, and jalapeño. Special processes to produce baked low-fat tortilla chips that combine air impingement, infrared, and microwaves have been developed.

See also: Beer: Fermentation; Beer: Raw Materials and Wort Production; Cereals: Dietary Importance; Cereals: Types and Composition; Extrusion Cooking: Principles and Practice; Fructose and High-Fructose Corn Syrup; Functional Foods; Snack Foods: Types and Composition; Starch: Sources and Processing; Sweeteners: Classification, Sensory and Health Effects; Tea: Analysis and Tasting; Tortillas; Vegetable Oils: Oil Production and Processing; Vegetable Oils: Types and Properties; Whisky, Whiskey, and Bourbon: Products and Manufacture.

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Malnutrition: Concept, Classification and Magnitude

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Abbreviations

BMI	Body mass index
FAO	Food and Agriculture Organization of the United Nations
MAM	Moderate acute malnutrition
MDGs	Millennium Development Goals
NCD	Noncommunicable disease
PEM	Protein–energy malnutrition
SAM	Severe acute malnutrition
SGA	Small for gestational age
SUN	Scaling Up Nutrition
UNICEF	United Nations Children's Fund
WHA	World Health Assembly
WHO	World Health Organization

Introduction

Malnutrition does not always imply hunger or famine, which is the first association that typically comes to mind. It is manifest in many forms, ranging from hunger to obesity, and can be defined in terms of undernutrition, overnutrition, and micronutrient deficiencies. Simply put, malnutrition is caused by excessive intakes, insufficient food consumption, and inadequate utilization of nutrients provided by foods ingested. On the one hand, insufficient intakes often result from precarious circumstances and inequalities existing at the level of economic resources, education, access to food and safe drinking water, adequate health care, and sanitary living conditions. On the other hand, excessive intake of foods high in saturated fats and sugars and industrially processed and refined foods accompanied by decreased physical activity has led to increased obesity and cardiovascular diseases.

The face of malnutrition is changing the manner in which it presents in some form or another in nearly all countries. There is currently a global pandemic of overweight and obesity. Simultaneously, the burden of disease in low- and middle-income countries is rapidly changing from communicable to noncommunicable illnesses. Yet micronutrient deficiencies are still an issue as seen in the rates of anemia and wasting, which have not changed substantially for more than 20 years. The World Health Organization (WHO) estimates that at least one-third of the world's population is affected by micronutrient malnutrition and that, in 2008, more than 1.4 billion adults over the age of 20 were overweight. In effect, no country is free of malnutrition, and many households, communities, and regions struggle with all of these forms of malnutrition at the same time. Countries are confronted with the burden of complex, overlapping, and interrelated malnutrition problems, with a combination of stunting in children under 5 years of age, anemia in women of reproductive age, and adult overweight and obesity being the most common. Less than 20 countries have only one of these manifestations of malnutrition at problematic levels in nationally representative survey data. These different conditions are connected not only at a physiological level but also at a resource and political level. As obesity is dealt with in other articles, this article will focus on malnutrition in terms of undernutrition and micronutrient deficiencies.

Definitions for the Discussion of Malnutrition

For clarity, several definitions are presented so as to better understand the context of malnutrition and how to distinguish its various forms.

Malnutrition

Malnutrition is defined as an abnormal physiological condition caused by inadequate, unbalanced, or excessive consumption of macronutrients, micronutrients, or both. Malnutrition includes undernutrition and overnutrition as well as micronutrient deficiencies.

Overnourishment

Overnourishment is defined as food and beverage intake that is continuously in excess of dietary energy requirements.

Overnutrition

Overnutrition is a result of excessive food intake relative to dietary nutrient requirements.

Overweight and Obesity

These comprise body weight that is above normal for height as a result of an excessive accumulation of fat. It is usually a manifestation of overnourishment and/or sedentary lifestyles and a lack of physical activity. Overweight is defined as a body mass index (BMI) of more than 25 kg m^{-2}, but <30 kg m^{-2}. Obesity is defined as a BMI of 30 kg m^{-2} or more.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00439-6

Protein–Energy Malnutrition

Protein–energy malnutrition (PEM) is a classic medical terminology referring to a group of related disorders that include marasmus, kwashiorkor, and intermediate states of marasmus–kwashiorkor. This name implied that malnutrition is a deficiency of protein or energy and it is, as for other specific deficiencies, suggested that treatment should consist of proteins or energy. Textbooks usually went on to say that marasmus (extreme thinness) was due to a lack of energy and kwashiorkor (edematous malnutrition) was the result of a lack of proteins. This has now been proved incorrect due to the role of deficient intakes of essential micronutrients needed for growth and tissue renovation. Marasmus can occur with excess energy if no nutrients are ingested and kwashiorkor can become manifest even with excess protein in the diet.

Stunting

Stunting is also known as linear growth retardation, and it is defined as low height for age, reflecting a past episode or episodes of chronic undernutrition.

Undernourishment

Undernourishment is a state, lasting for at least 1 year, of inability to acquire enough food, defined as a level of food intake insufficient to meet dietary energy requirements.

Undernutrition

Undernutrition is the outcome of undernourishment, poor absorption, and poor biological use (bioavailability) of nutrients consumed as a result of repeated infectious/parasitic diseases, alone or in combination. It includes being underweight for one's age, too short for one's age (stunted), dangerously thin for one's height (wasted), and deficient in vitamins and minerals (micronutrient malnutrition).

Underweight

Underweight is defined as low weight for age in children and a BMI of <18.5 kg m^{-2} in adults, reflecting a current condition resulting from inadequate food intake, past episodes of undernutrition, or poor health conditions.

Wasting

Wasting is defined as low weight for height and is generally the result of weight loss associated with a recent period of acute hunger/starvation or disease.

Malnutrition and Mortality

The recent 2013 *Lancet* Series on Maternal and Child Nutrition highlights that undernutrition, including fetal growth restriction, suboptimum breastfeeding, stunting, wasting, and deficiencies of vitamin A, iodine, and zinc, causes 3.1 million deaths annually in children younger than 5 years. This represented a staggering 45% of the total child deaths in 2011. As part of this total, the combined distribution of suboptimum breastfeeding and fetal growth restriction in the neonatal period resulted in 1.3 million deaths or 19% of all deaths of children younger than 5 years. There is clear evidence about the importance of undernutrition as a predisposing factor of an increased risk of becoming ill or dying and as an antecedent condition of structural and functional limitation. During the acute stage of severe undernutrition, the greater susceptibility of the child to infectious disease is undoubtedly of major clinical significance and has important consequences for child mortality.

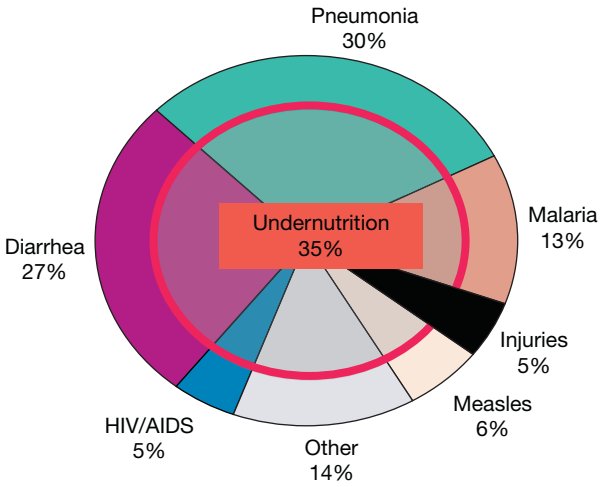


Figure 1 Undernutrition-related risk of death associated with specific diseases. UNICEF emergency nutrition network training course adapting *Lancet* 2008 data.

Figure 1 shows the contribution of different childhood illnesses on child mortality based on data from the 2008 *Lancet* Series on Maternal and Child Nutrition. As depicted, pneumonia (30%) and diarrhea (27%) comprise over 50% of childhood deaths. The area within the red circle represents the percentage of deaths due to the child being undernourished. Children with severe, moderate, or even mild undernutrition have a higher risk of mortality from these childhood illnesses than a well-nourished child. As such, undernutrition constitutes an underlying cause for most childhood deaths associated with severe infections.

Nutrition and Sustainable Development

The first International Conference on Nutrition held in Rome in 1992 called for a global commitment to address the problem of malnutrition. The Millennium Development Goals (MDGs) established in 2000 included the target of reducing the prevalence of underweight children and thus established an accountability mechanism to ensure global action. With the publication of the 2008 *Lancet* Series Maternal and Child Nutrition, the evidence base for effective actions to address malnutrition in these target populations was provided. However, in spite of the advances made to decrease hunger and achieve poverty goals and targets, much remains to be done.

Table 1 How investing in nutrition is critical to achieving the MDGs

Goal	*Nutrition effect*
Goal 1: Eradicate extreme poverty and hunger	Undernutrition erodes human capital through irreversible and intergenerational effects on cognitive and physical development
Goal 2: Achieve universal primary education	Undernutrition affects the chances that a child will go to school, stay in school, and perform well
Goal 3: Promote gender equality and empower women	Anti-female biases in access to food, health, and care resources may result in undernutrition, possibly reducing women's access to assets Addressing undernutrition empowers women more than men
Goal 4: Reduce child mortality	Undernutrition is directly or indirectly associated with most child deaths, and it is the main contributor to the burden of disease in the developing world
Goal 5: Improve maternal health	Maternal health is compromised by undernutrition, which is associated with most major risk factors for maternal mortality. Maternal stunting and iron and iodine deficiencies are common problems
Goal 6: Combat HIV/AIDS, malaria, and other diseases	Undernutrition may increase risk of HIV transmission, compromise antiretroviral therapy, and hasten the onset of full-blown AIDS and premature death. It increases the chances of tuberculosis infection, resulting in disease, and it also reduces malarial survival rates

Source: World Bank. (2006). *Repositioning nutrition as central to development. A strategy for large-scale action*. Washington, DC: The World Bank.

Across the globe, 805 million people are still suffering from chronic dietary energy insufficiency, 162 million children are stunted, 51 million children are wasted, and there are over two billion people who suffer from one or more micronutrient deficiencies.

Now that the end point of the MDGs is nearing, sustainable development has taken center stage as the prevailing priority post-2015. The role of nutrition emerges as the ideal example of a sustainable development objective. Table 1 shows the impact that undernutrition has on six of the eight MDGs.

Human and Economic Costs

Improving nutrition contributes to productivity, economic development, and poverty reduction by improving physical work capacity, cognitive development, school performance, and health by reducing disease and mortality. As demonstrated in the Copenhagen Consensus Center, who works with the world's top economists to research and publish the best solutions to global challenges, investing in nutrition is critical, as poor nutrition perpetuates the cycle of poverty and malnutrition through three main courses: (1) direct losses in productivity from poor physical status and losses caused by disease linked with malnutrition, (2) indirect losses from poor cognitive development and losses in schooling, and (3) losses caused by increased health-care costs. The economic costs of malnutrition are steep, amounting to several billion dollars a year in terms of lost gross domestic product.

Since early childhood offers a special window of opportunity to improve nutrition, the bulk of the investment needs to be targeted between prepregnancy and two years of age. Economic studies show that every dollar spent on nutrition in the first 1000 days of a child's life (fetus to age two) can give an average savings of $45 and in some cases as much as $166 (Figure 2).

SUN framework

In April 2010, a policy brief – Scaling Up Nutrition: A Framework for Action (SUN) – was released at joint meetings of the World Bank and the International Monetary Fund. It was a collective effort stimulated by the publication in January 2008 of the aforementioned *Lancet* Series on Maternal and Child Nutrition. The series encouraged an emphasis on the 1000-day window from the start of pregnancy to a child's second birthday, with interventions that both were cost-effective and yielded high returns for cognitive development, individual adult income, and economic growth.

The SUN Movement, which started in September 2010, is the most important symbol of the increased interest in nutrition. By 2014, the movement has grown to include more than 50 countries that are committed to the scale-up of direct nutrition interventions and the advancement of nutrition-sensitive development, including 21 of the 34 highest burden countries, in which 41% of the global burden of child stunting is located.

Undernutrition

Types of Malnutrition

Undernutrition is the outcome of undernourishment, poor absorption, and infectious/parasitic diseases, alone or in combination.

Undernutrition is a physical condition characterized by a nutritional disorder resulting from insufficient food intake (undernourishment) and/or poor absorption and poor biological use (bioavailability) of nutrients consumed as a result of repeated infectious disease, alone or in combination. It encompasses being underweight for one's age, too short for one's age (stunted), dangerously thin for one's height (wasted), and deficient (status and function) in vitamins and minerals (micronutrient malnutrition). Although the terms malnutrition and undernutrition are often used interchangeably, the first is more extensive in scope. In this article, both are used, but not interchangeably. Indeed, malnutrition is a nutritional disorder that can manifest itself in distinct forms:

(a) *Overnutrition*: a result of excessive food intake relative to dietary nutrient requirements. Previously more prevalent in developed countries, but now also appearing in countries with emerging economies.
(b) *Undernutrition*: as previously defined in the text. It is worth noting that different organizations have distinct definitions for this term. Some refer to it as an insufficient intake of nutrients to sustain a state in which the physical

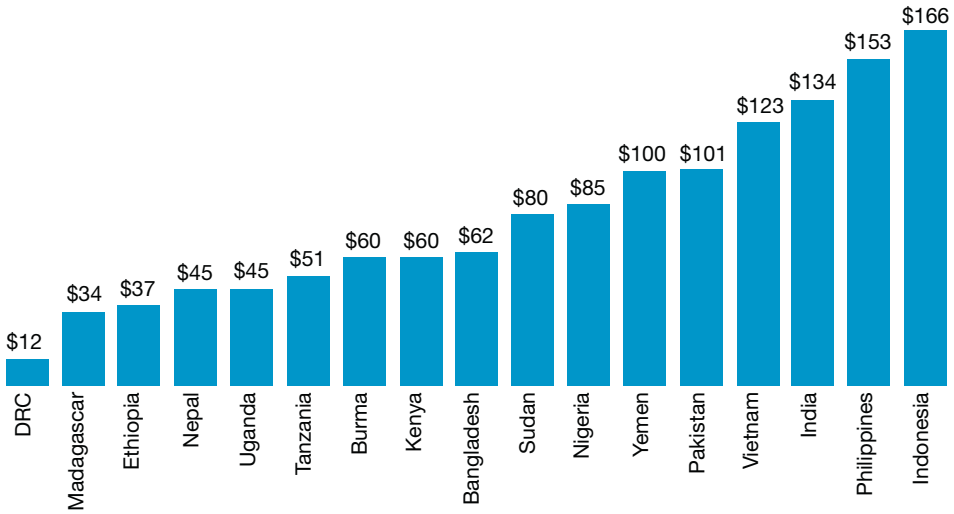


Figure 2 Reducing stunting: benefit for every dollar spent by country (discount rate = 3%, final working age = 50). Copenhagen Consensus Data.

function of an individual is impaired to the point where he or she can no longer maintain adequate bodily performance processes such as growth, pregnancy, lactation, physical work, and resisting and recovering from disease.

(c) *Secondary undernutrition*: not caused by diet but rather by diseases or pathological conditions (diarrhea, infections, measles, intestinal parasites, etc.) that prevent the body from absorbing ingested nutrients.

(d) *Micronutrient deficiency*: when essential micronutrients such as vitamins, minerals, or both are not present in adequate amounts in the diet.

The last three are common in developing countries in which varieties of undernutrition, also known as moderate acute malnutrition (MAM) or severe acute malnutrition (SAM), are the most relevant. These three situations, and especially undernutrition, are often referred to colloquially as hunger. In a given place and time, it undergoes a process of deterioration that is accompanied by other factors (impoverishment, epidemics, and often an increase in mortality), the situation turns into a famine. Undernutrition affects all age groups, but some are particularly vulnerable, mainly children (especially those between 6 months and five years, after weaning), the elderly, and pregnant and lactating women.

Currently, undernutrition is a problem that affects developing and industrialized countries, and in both cases, the most economically disadvantaged are those that are most affected. In developed countries, obesity is rapidly extending, especially among the poorest segments of the population, bringing with it an epidemic of nutrition-related noncommunicable diseases (NCDs) such as diabetes and cardiovascular disease, which increase health-care costs and reduce productivity. In developing countries, although undernutrition and micronutrient deficiencies continue to be very widespread, the swiftly spreading problem of obesity is also a burden. Currently, it is common to find underweight children and obese adults within the same household, in both developed and developing countries.

Manipulating market dynamics and prices fail to address the problem of undernutrition when families have no money to buy enough food or pay for health-care expenses. Advocacy linked to human rights and equity should be considered, as well as other initiatives related to income generation, thus justifying government intervention to support those families in need. However, undernutrition also occurs in many families with adequate economic resources, as people do not always know which foods or feeding practices are best for themselves or for their children, in which inadequate marketing practices from industry play a part. It may also be difficult for parents to know whether their children are starting to suffer from undernutrition as growth deficits and micronutrient deficiencies are not easily detected without previous training or education on the topic.

Table 2 shows the main problems of undernutrition throughout the life cycle.

Causes of undernutrition

The fundamental causes of undernutrition and mortality quite often fall outside the nutrition sector and are associated with each country's levels of potential resources (e.g., energy, geography, and climate), as well as with all the factors that condition their use, such as management of the population density in relation to the resources available, poverty, social inequalities, the side effects of growth-oriented macroeconomic policies on structural adjustment, and the migration to cities.

The classification of these causal chains can be simplified into three broad categories:

(a) *Food insecurity*: This includes problems of food production or supply at the national, regional, or household level, as well as problems of families and communities in accessing sufficient amounts of safe and nutritious food for normal growth and development and an active and healthy life. It may be caused by the unavailability of food, insufficient purchasing power, inappropriate distribution, or inadequate use of food at the household level. Food insecurity may be chronic, seasonal, or transitory and may be induced by a myriad of factors including agricultural and trade policies, wars, famine, and climate change, among others.

(b) *Poor conditions of health and sanitation*: The aspects of environmental hygiene include the supply of safe drinking

Table 2 Undernutrition throughout the life cycle, according to disorder and main consequences

Life cycle stage	*Nutrition disorder*	*Main consequences*
Embryo/fetus	• Intrauterine growth retardation • Iodine deficiency disorders • Lack of folate	• Low birth weight • Prematurity • Incomplete nervous system development • Stillbirth
Neonate (newborn)	• Low birth weight • Iodine deficiency disorders	• Growth retardation/failure to thrive/deafness • Delayed cognitive development • Anemia
Infants and children	• Protein energy malnutrition • Iodine deficiency disorders • Vitamin A deficiency • Iron deficiency anemia	• Chronic undernutrition • Delayed physical and cognitive development • Increased risk of infections • Blindness • Anemia
Adolescents	• Protein energy malnutrition • Iodine deficiency disorders • Vitamin A deficiency • Iron deficiency anemia	• Delayed growth spurt • Stunting • Delayed intellectual development • Goiter • Increased risk of infections • Blindness • Anemia • Inadequate bone mineralization
Pregnant and breastfeeding women	• Protein energy malnutrition • Iodine deficiency disorders • Vitamin A deficiency • Iron deficiency anemia • Folate deficiency • Calcium deficiency	• Inadequate prenatal weight gain • Maternal anemia • Increased maternal mortality risk • Increased risk of infection • Night blindness • Increased risk of fetal mortality • Low birth weight
Adults	• Protein energy malnutrition • Iodine deficiency disorders • Obesity • Nutrition-related diseases	• Thinness • Permanent fatigue • Obesity • Cardiac disease • Diabetes • Cancer • Arterial hypertension/myocardial infarct • Anemia
Elderly	• Protein energy malnutrition • Iodine deficiency disorders • Obesity • Osteoporosis • Nutrition-related diseases	• Obesity • Vertebral and hip fractures • Cardiovascular disease • Diabetes • Cancer • Cognitive impairment

Source: WFP and CDC. (2007). *A manual: measuring and interpreting malnutrition and mortality*. Rome: World Food Program.

water and food products, sanitation of the environment in all its forms, and hygienic practices of the population. Health aspects address the areas of infectious and parasitic diseases, on the one hand, and the systems and utilization of health care, on the other.

(c) *Inappropriate care and feeding practices*: The concept of the provision of care and feeding practices refers both to care at the household level and to the broader aspects of solidarity and social protection at the community or national level. Thus, it encompasses the full range of maternal and child care, with mothers and children constituting the principal at-risk groups. In general, it refers to the attitudes and practices of the members of the household or community toward the most vulnerable members in their social sphere (i.e., available time, food distribution, and emotional and material support), as well as the educational level of the caregivers (Figure 3).

The United Nations Children's Fund (UNICEF) conceptual framework, which the Nutrition Community has been using for programming over the past three decades, identifies three levels of causes of undernutrition: immediate, underlying, and basic. It schematically illustrates the multifactorial and interconnected causes that usually form the conceptual basis of 'nutrition security,' in other words, achieving good nutritional status through the control of the different determinants of undernutrition, with particular attention to the underlying causes. This analysis must be, however, more specifically tailored to each local situation, as well as the selection of

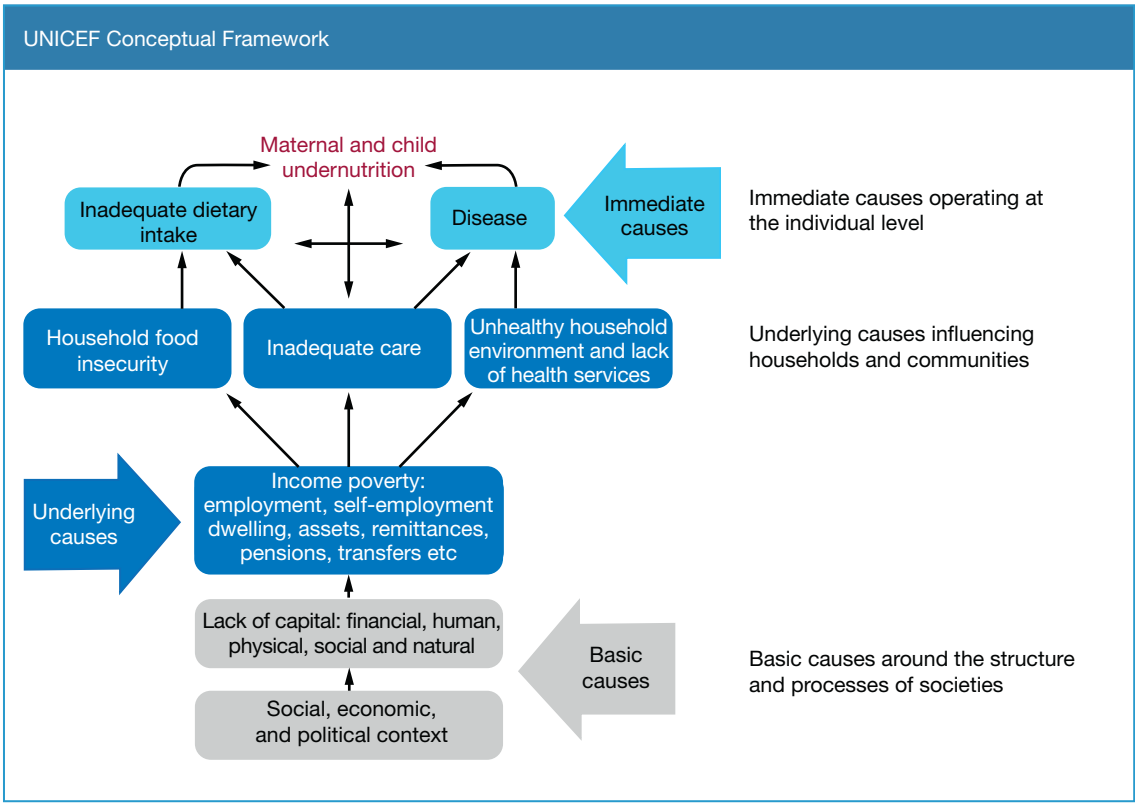


Figure 3 Conceptual framework of the causes of undernutrition. UNICEF (2011).

Box 1 Why does undernutrition occur in food-secure households?

- Pregnant and breastfeeding women have inadequate intakes of calories and protein, and infections – such as sexually transmitted diseases – if left untreated can cause low birth weight and insufficient rest.
- Mothers lack sufficient time to care for small children or for themselves.
- Mothers of newborns discard their colostrum, or first milk, which contains substances that strengthen the immune system of the child.
- In many cases, instead of exclusively breastfeeding, mothers of babies younger than 6 months give them foods other than breast milk, which constitutes the best source of nutrients and protection against infectious and chronic diseases.
- Caregivers wait too long to start giving weaning foods.
- Caregivers give children under two too little food or foods with low-energy content.
- Although foods are available, there is inadequate distribution within the household, usually at the expense of women and young children whose diets are often times insufficient in proteins or micronutrients.
- Children's caregivers do not follow recommendations for feeding when the child has diarrhea or fever, or shortly thereafter.
- Due to a lack of hygiene on the part of caregivers, foods become contaminated with bacteria or parasites.

Source: World Bank 2006

appropriate indicators corresponding to the distinct causal levels and to each determinant.

As we have seen and contrary to popular belief, undernutrition/malnutrition is not simply a result of food insecurity. Many children from environments that are food secure and from families with economic resources are below the normal weight or have short stature (stunting), due to inappropriate infant feeding and care, poor access to health-care services, or inadequate sanitation. As shown in Box 1, the World Bank in 2006 has outlined a scenario for the occurrence of undernutrition even in households that do not meet the criteria of food-insecure.

In many countries where undernutrition is widespread, food production is not the limiting factor, with the exception of famine cases. The most important factors are as follows: firstly, insufficient knowledge about the benefits of exclusive breastfeeding, complementary feeding practices, and the function of micronutrients and, secondly, the lack of time a woman has to adequately care for their babies or for themselves during pregnancy. The most pernicious effects of undernutrition occur during pregnancy and the first 2 years of life, and the consequences of such early damage to health, brain development, intelligence, and possibilities of benefiting from education and productivity are largely irreversible. There is evidence suggesting that obesity and NCDs such as cardiovascular disease and diabetes may also have their origins in early childhood. As such, some have criticized the classification of malnutrition into undernutrition and overnutrition as being simplistic due to the interrelationship between the two.

Causal Determinants

Biological and Dietary Factors

Maternal undernutrition can result in intrauterine undernutrition and low birth weight, among others. Insufficient food intake to allow for compensatory growth that could offset this situation brings about the onset of SAM in early life stages.

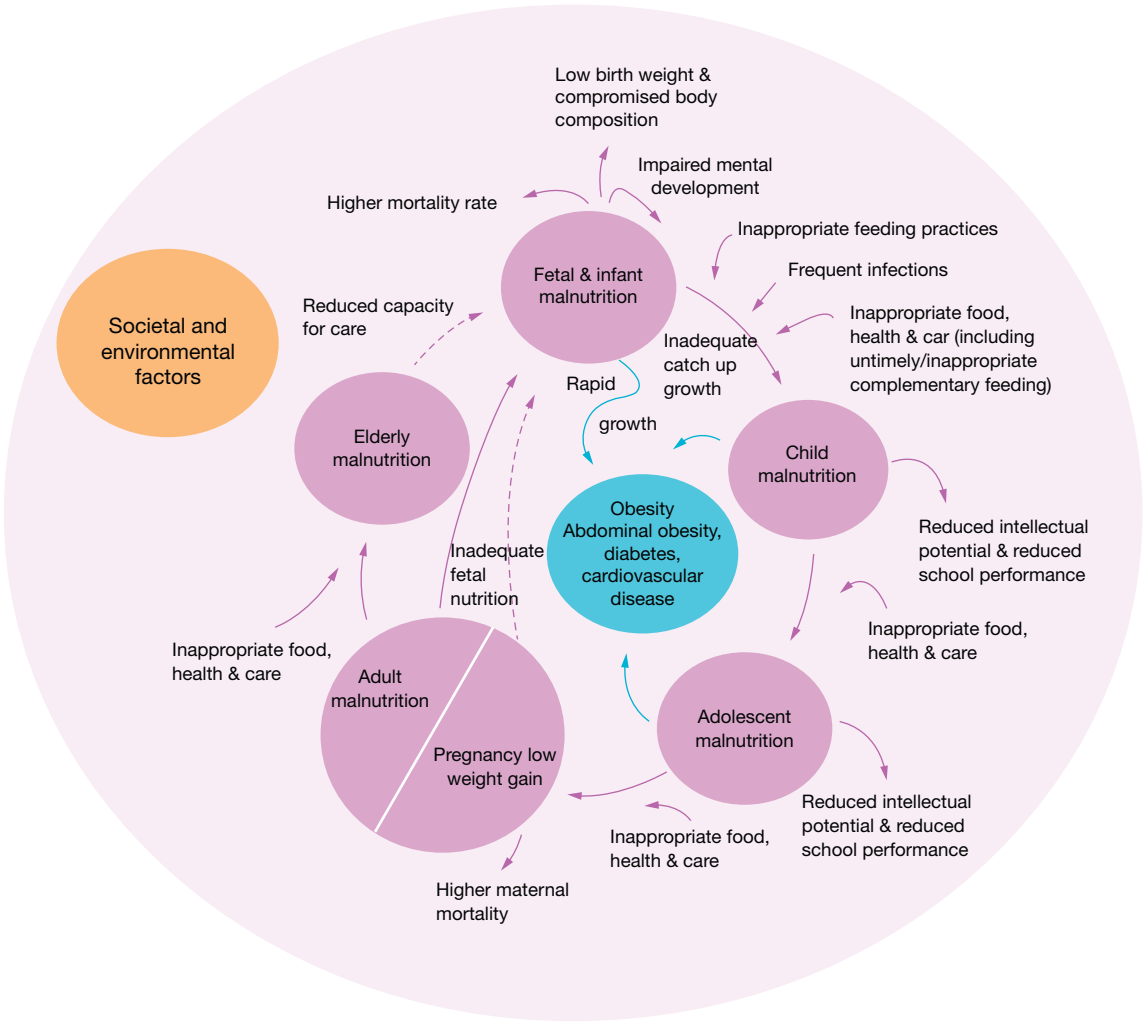


Figure 4 Life course: The proposed causal links of malnutrition. Reproduced from Darnton-Hill, I., Nishida, C. and James, W. P. T. (2004). A life course approach to diet, nutrition and the prevention of chronic diseases. *Public Health Nutrition* **7**(1A), 101–121.

Figure 4 shows the vicious circle of undernutrition. When a woman who is undernourished during pregnancy does not improve her nutritional status, the newborn presents with low birth weight. If that child is inadequately fed, it grows up being undernourished; and if the poor diet continues, the child will become an undernourished adult. If female, when this undernourished woman becomes pregnant, she will give birth to an undernourished infant, perhaps with difficulty through a narrowed pelvis, and the cycle is perpetuated.

Socioeconomic Factors

Poverty is often associated with undernutrition due to reduced access to food, unsanitary living conditions, overcrowding, and inadequate child care. Lack of knowledge leads to poor child care practices, misconceptions about the use of various foods, inadequate feeding practices during illness, and inadequate distribution of food among family members. The reduction in the initiation and duration of breastfeeding, combined with inadequate weaning practices, is also associated with high rates of childhood undernutrition.

Social problems, such as the physical and emotional abuse of children, maternal deprivation, neglect of the elderly, alcoholism, and drug addiction, can lead to the onset of undernutrition. Moreover, cultural and social practices that impose taboos and prohibit given foods, certain diet fads, and the precarious conditions that characterize the migration from traditional rural areas to urban poverty zones also contribute to the development of undernutrition (Figure 5).

Environmental Factors

Overcrowded housing and poor sanitation favor the emergence of frequent infections and infectious disease outbreaks such as Ebola. Natural disasters, such as droughts or floods, and human-induced disasters, such as wars and forced migration, produce sudden, prolonged, or cyclical food shortages and can produce undernutrition in large population groups. New studies have brought to light the impact of global climate change, which is projected to reduce future crop yields and thus threaten food security and eliminate much of the improvement in child undernutrition levels

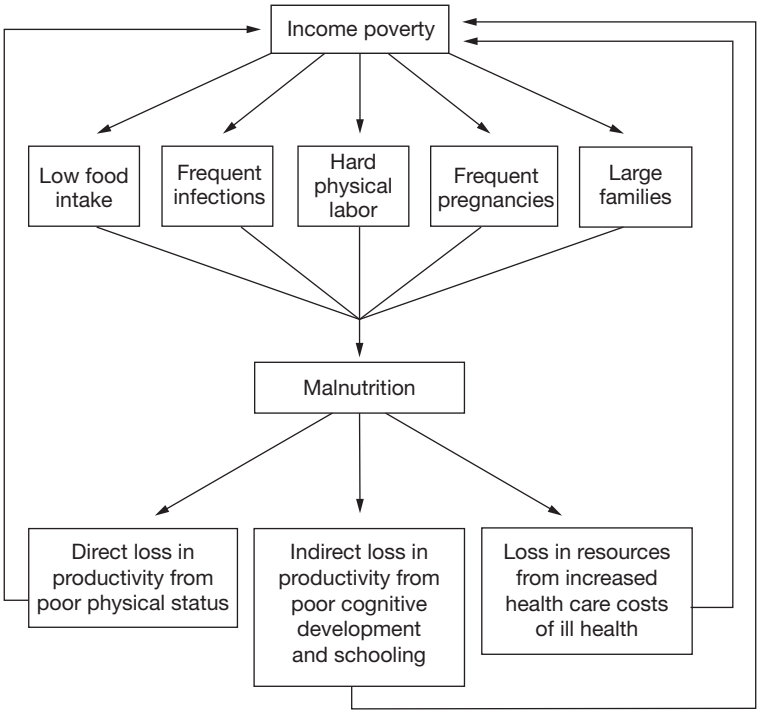


Figure 5 Vicious cycle of poverty and malnutrition. Reproduced from World Bank (2006). *Repositioning nutrition as central to development. A strategy for large-scale action*. Washington, DC: The World Bank.

that would occur with no climate change. Simon Lloyd's research estimated that climate change would lead to a relative increase in moderate stunting of 1–29% in 2050 compared with a future without climate change. This study also predicted that climate change would have a greater impact on severe stunting rates, projected to increase from 23% (central sub-Saharan Africa) to 62% (South Asia). Moreover, losses would be exacerbated by the additional loss of food postharvest due to bad storage conditions as well as inadequate systems of food distribution, thus also contributing to the development of undernutrition, even after periods of agricultural abundance.

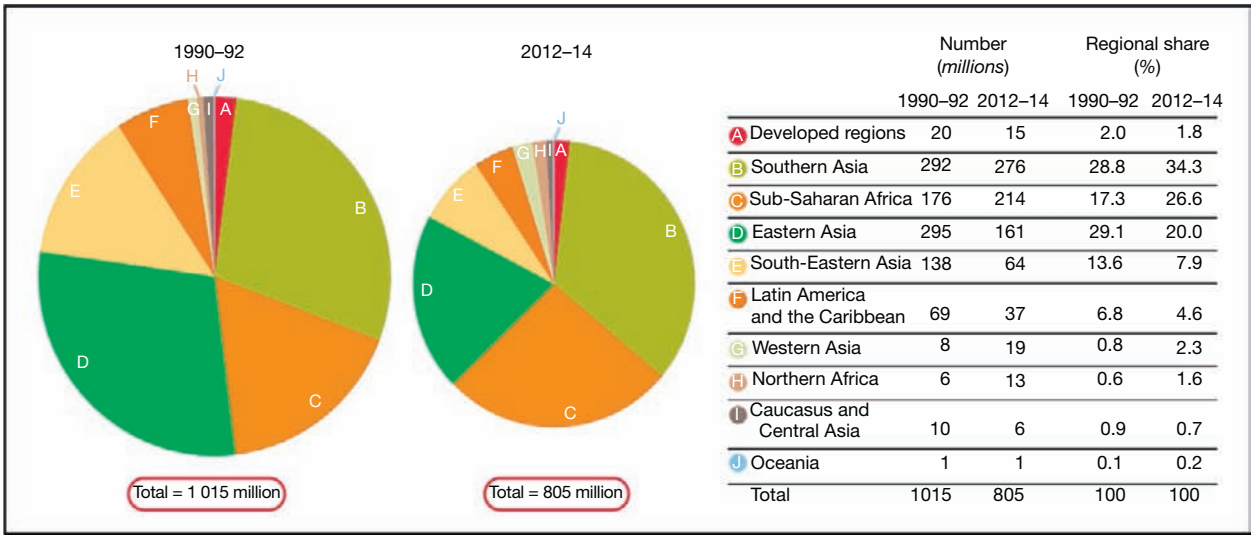
Epidemiology

The 2014 FAO estimates indicate that global undernutrition rates continue their downward trend. About 805 million people were estimated to be chronically undernourished in 2012–14, a reduction of more than 100 million over the last decade and of 209 million since 1990–92.

Nonetheless, worldwide, approximately one in every nine people still suffers from insufficient food intakes to maintain an active and healthy life. The majority of these undernourished people live in developing countries, where an estimated 791 million were chronically hungry (undernourished) in 2012–14. Although developing countries have also shown the most improvements in the last two decades – reducing the overall number of undernourished people by 203 million since 1990–92 – around one of eight people in these regions (13.5% of the overall population) remains chronically underfed. Figure 6 compares the numbers and proportion of undernourished people by region. The MDG hunger target aims to halve the *proportion* of undernourished people, whereas that of the World Food Summit (WFS) was more ambitious – to halve the *number* of undernourished people by 2015. Meeting the MDG hunger target seems to be within reach, but not that of the WFS.

As depicted in Figure 6, despite the progress in developing regions as a whole, significant differences are seen across regions. East Asia and Southeast Asia have already met the MDG hunger target. The same is true for Latin America and the Caribbean, whereas the Caucasus and Central Asia are projected to reach the MDG objective by the end of 2015. Latin America and the Caribbean are also expected to reach the more ambitious WFS goal. In contrast, sub-Saharan Africa and South and West Asia have not shown sufficient progress to achieve the MDG target. Sub-Saharan Africa has become home to more than a quarter of the world's undernourished people, owing to an increase of 38 million in the number of hungry people since 1990–92.

According to UNICEF data, worldwide, 99 million children under 5 years old were underweight in 2013, two-thirds of which lived in Asia and around one-third in Africa. Underweight prevalence continues to decline, but at a slow pace. Between 1990 and 2013, it decreased from 25% to 15% of the under-five population worldwide. If current trends continue, the MDG 1 target (halving the 1990 underweight prevalence by 2015 at the global level) will not be met. Three regions have already met or exceeded the target: East Asia and the Pacific, Latin America and the Caribbean, and Central and eastern Europe and the Commonwealth of Independent States (CEE/CIS). Africa has experienced the smallest relative decrease, with underweight prevalence of 17% in 2013, down from 23% in 1990; Asia reduced underweight prevalence during the same period from 32% to 18% and in Latin America and the Caribbean from 8% to 3%.



	Number (*millions*)		Regional share (%)	
	1990–92	2012–14	1990–92	2012–14
A Developed regions	20	15	2.0	1.8
B Southern Asia	292	276	28.8	34.3
C Sub-Saharan Africa	176	214	17.3	26.6
D Eastern Asia	295	161	29.1	20.0
E South-Eastern Asia	138	64	13.6	7.9
F Latin America and the Caribbean	69	37	6.8	4.6
G Western Asia	8	19	0.8	2.3
H Northern Africa	6	13	0.6	1.6
I Caucasus and Central Asia	10	6	0.9	0.7
J Oceania	1	1	0.1	0.2
Total	1015	805	100	100

Figure 6 The changing distribution of hunger in the world: numbers and shares of undernourished people by region, 1990–92 and 2012–14. Reproduced from FAO (2014).

In children under 5 years of age, 15% were classified as being underweight, and an additional 8% suffered from SAM/undernutrition. Sixty percent of all cases with acute undernutrition are found in just ten countries. A total of 32 countries account for 10% or more of children under 5 years with SAM of the type that requires urgent treatment and for which WHO has developed community- and clinic-based protocols.

UNICEF data show that, in early childhood, there are no substantial differences in nutritional status based on gender. However, this changes dramatically during adolescence, in which a greater propensity of females presents with undernutrition and anemia. In this light, India, Bangladesh, Niger, and Senegal show the highest rates of adolescent females with a BMI falling below the recommended levels. Additionally, the majority of girls marry before the age of 18, which exposes them to high-risk pregnancies and the risk of increased maternal and infant mortality rates.

Severe Acute Malnutrition

SAM was previously known as PEM, or protein–calorie malnutrition, and is an important nutritional condition in developing countries due to its high prevalence and its association with high rates of infant mortality, changes in growth and development, and decreased ability to work, altogether leading to inadequate social and economic development.

SAM occurs when the diet does not provide sufficient protein, energy substrates (calories), or both, to satisfy the body's nutritional needs. It is usually also associated with a deficiency of vitamins and minerals and essential micronutrients needed for growth and tissue renovation. The term SAM includes severe clinical syndromes of kwashiorkor (edema), marasmus (without edema), marasmic–kwashiorkor (edema and the combination of chronic energy deficiency and chronic or acute protein deficiency), and mild and moderate cases that are much more numerous than severe forms. The term 'malnutrition' is generally used in common vernacular to refer to SAM.

SAM may be of primary or secondary origin. Primary SAM is caused by inadequate nutrient intake. Secondary SAM results from disorders or drugs that interfere with nutrient use, such as cases of diseases accompanied by low food intake, inadequate nutrient absorption or utilization, increased nutritional requirements, increased nutrient losses, or any or all of these factors. Although SAM is mainly an issue in early childhood, it may also occur in older children and adults, particularly the elderly. This article focuses on primary SAM of gradual onset, with predominance of metabolic disorders and clinical features of protein and energy deficiency.

Physiopathology and Reductive Adaptation

The body tends to maintain a dynamic balance in energy intake through a series of physiological mechanisms. When there are long periods of energy and/or protein restriction, the body gradually adapts to this restriction in order to maintain an adequate functional state within the margin that the limited nutrient supply allows.

Nutrient reductive adaptation represents the phenomenon that the body undertakes in order to survive two synergistic assaults, the lack of nutrients and frequent infections. It does so by modifying normal biological patterns and creating new homeostatic conditions. SAM progresses gradually and thus allows for the following series of metabolic adjustments that result in a reduction in nutrient requirements and a nutritional balance compatible with lower availability of cellular nutrients. If at this point the nutrient supply continues to be increasingly lower than that accepted by the body in its adaptive state, death ensues. However, although in the majority of cases, this nutrient supply is low, it is not sufficient to cause death and the individual is capable of living in an adaptive state with decreased intake.

Mobilization and Energy Expenditure

Energy expenditure falls rapidly after the reduction of caloric intake; this explains the diminished periods of play and physical activity observed, as well as longer periods of rest and less physical work. When the decrease in energy expenditure cannot compensate for insufficient intake, body fat reserves are mobilized and the adipose tissue and body weight decrease. Lean body mass is reduced at a lower rate as a result of muscle protein catabolism, which increases the amino acid pool. As the energy deficiency becomes more serious, subcutaneous fat is significantly reduced and the catabolism of proteins leads to muscle wasting. Visceral proteins are preserved for a longer period of time.

Protein Metabolism

The lack of dietary proteins reduces protein synthesis. Reductive adaptations allow for preserving structural proteins and maintaining essential functions that depend on proteins. This leads to enzymatic changes that favor the degradation of muscle protein, the reduction of hepatic protein synthesis, and the mobilization of energy substrates from fat deposits. When the nonessential tissue proteins are depleted, visceral organ proteins are employed and death may be imminent if nutritional therapy is not initiated.

Protein half-life increases, the rate of albumin synthesis decreases, and albumin is transferred from extravascular to intravascular reserves to maintain adequate levels of circulating albumin in light of their reduced synthesis. Adaptive mechanisms fail when protein depletion is very severe and their concentration in serum decreases. Secondary reduction of intravascular oncotic pressure and leakage of fluid into the extravascular space contribute to the formation of edema in kwashiorkor.

Endocrine Changes

There is a reduction in the activity of hormones involved in the increase of body reserves, elevation of metabolism, and nonvital functions related to growth, such as insulin, somatomedin (or insulin-like growth factor), thyroid hormones, and gonadotropins.

The activity of glucocorticoids is normal or elevated, which favors the functional catabolism of the hypothalamus–pituitary axis, whereas the adrenal medulla is kept normal, which allows for adequate endocrine and metabolic responses under conditions of stress. Some researchers have postulated that the evolution of MAM into kwashiorkor or marasmus (SAM) may be due, in part, to differences in the adrenocortical response. A better response would make it possible to more efficiently maintain visceral protein integrity, thus resulting in marasmus, which is associated with improved adaptation.

Hematology and Oxygen Transport

The decrease in hemoglobin concentration and red blood cells that is observed in almost all serious cases of SAM constitutes an adaptive phenomenon related, at least in part, to tissue oxygen requirements. The decrease in lean body mass and lower physical activity levels in patients with undernutrition also lessen the demand for oxygen. The simultaneous decrease of amino acids provided in the diet results in a decrease in hematopoietic activity, as amino acids are reserved for the synthesis of other more necessary proteins. As tissues receive enough oxygen, this response should be considered as an adaptive mechanism and not as a 'functional' form of anemia. When the synthesis of the tissue, lean body mass, and physical activity improve with diet therapy, the demand for oxygen increases and it becomes necessary to accelerate hematopoiesis. If there is a lack of sufficient iron, folic acid, and vitamin B_2, functional anemia with secondary tissue hypoxia will appear.

Other Physiological and Metabolic Changes

Not all of the pathophysiological changes in SAM lead to beneficial life-preserving reductive adjustments. Certain functions are affected and some nutrient reserves decrease, making it more likely that the undernourished patient will undergo alterations with serious repercussions.

Cardiovascular and renal functions

Cardiac output, heart rate, and blood pressure decrease and central circulation takes on greater importance than peripheral circulation. Cardiovascular reflexes are altered and cause postural hypotension and decrease in venous return. The main form of hemodynamic compensation is tachycardia without increasing the ejection volume rate. Both the renal plasma flow and glomerular filtration rate may decrease because of the reduced cardiac output, while apparently, the ability to excrete liquids and concentrate and acidify urine remains intact.

Immune system

The main effects observed in SAM seem to affect T lymphocytes and the complement system. The number of lymphocytes that originate in the thymus gland is drastically reduced and the gland atrophies. Furthermore, the depletion of T lymphocytes in the spleen and lymph nodes is observed. In SAM, the production of various complement components decreases. These deficiencies can account for the great propensity to sepsis by gram-negative bacteria. As a consequence, such changes lead to increased susceptibility to infections and to severe complications. There is a well-known link between undernutrition and increased risk of infection as depicted in Figure 7.

Electrolytes

Total body potassium decreases due to the reduction in muscle proteins and the loss of potassium from the intracellular compartment. The low action of insulin and the decreased energy within the cell reduce the availability of ATP and phosphocreatine. This leads to the entrance of sodium and water into the cell, with consequent intracellular overhydration.

Gastrointestinal functions

The absorption of lipids and disaccharides may become altered and the velocity of glucose absorption decreases in severe protein deficiency. Moreover, reduced production of gastric, pancreatic, and biliary substances occurs. These changes further affect altered absorptive function brought on by diarrhea and perhaps also by irregular bowel motility and

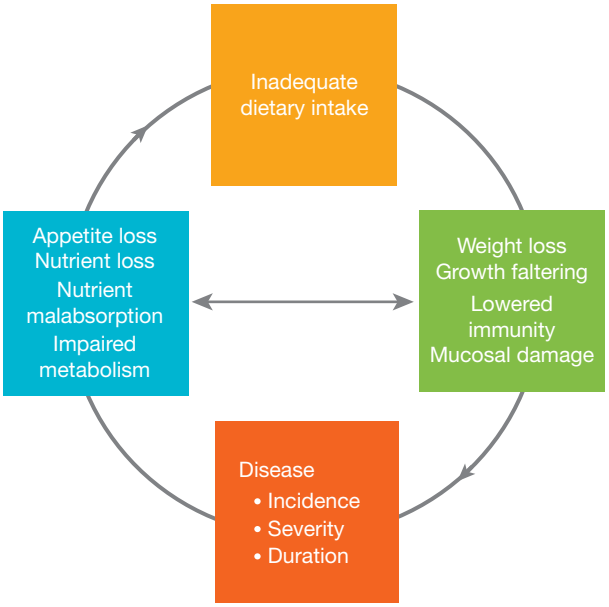


Figure 7 Relationship between undernutrition and infection. Reproduced from East, Central, and Southern African Health Community (ECSA-HC), Food and Nutrition Technical Assistance Project (FANTA), and LINKAGES Project (2008). *Nutrition and HIV/AIDS: a training manual for nurses and midwives*. Arusha, Tanzania: ECSA-HC.

gastrointestinal bacterial overgrowth. Diarrhea increases malabsorption and can further aggravate compromised nutritional status.

Central and peripheral nervous system

Patients who present with SAM at an early age may have decreased cerebral growth, nerve myelination, neurotransmitter production, and nerve conduction velocity. It is still not clear what the long-term functional implications are of these alterations and they have not been associated with subsequent behavior or level of intelligence.

Rupture of Adaptive Responses

When the cells and tissues of affected patients fail to obtain sufficient energy for proper functioning, a series of compensatory mechanisms are produced including hypoglycemia, hypothermia, altered circulatory and renal functions, acidosis, coma, and death. These events can take place rapidly, within a matter of hours.

Infections may cause serious metabolic disorders due to an increase in protein catabolism and loss of nitrogen, mobilization of nitrogen metabolism to acute-phase protein synthesis, alterations in energy metabolism, and/or production of free radicals.

Clinical Manifestations and Classification

Mild and MAM

Its main clinical feature is weight loss with notable thinning accompanied by a reduction in subcutaneous adipose tissue. WHO/UNICEF defines MAM as a weight for age between −3 and −2 *z*-scores below the median of the WHO Child Growth Standards (see section on '**Anthropometric Indicators for Measuring Malnutrition**' in this article). The criteria for mid-upper arm circumference (MUAC) measurements are having an MUAC from 115 to <125 mm. MAM can be due to a low weight for height (wasting) or a low height for age (stunting) or to a combination of both. Similarly, moderate wasting and stunting are defined as a weight for height and height for age, respectively, between −3 and −2 *z*-scores.

MAM affects many children in poor countries. Children with moderate malnutrition are at increased risk of mortality and MAM is associated with a high number of nutrition-related deaths. If some of these moderately malnourished children do not receive adequate support, they may progress toward SAM (severe wasting and/or edema) or severe stunting (height for age less than −3 *z*-scores), which are both life-threatening conditions.

Children with mild to moderate malnutrition tend to be more sedentary and participate less frequently in games and activities that require some degree of physical exertion. Older children and adolescents may show a reduction in their ability to perform strenuous physical work for prolonged periods. Other nonspecific manifestations include pallor, apathy, shortened attention span (particularly in school settings), and frequent episodes of diarrhea. Clinical lab tests do not contribute to the diagnosis of mild or moderate malnutrition.

Severe Acute Malnutrition

In addition to clinical manifestations, the dietary and clinical histories of the patient are important for diagnosis. Marasmus is usually associated with food shortages, prolonged semi-starvation, premature weaning, and extended time intervals between the administration of foods to infants and young children. Kwashiorkor is often associated with low-protein intake, late weaning with inadequate complementary feeding practices, and current or recent episodes of diarrhea or measles. Various bouts of infectious diseases and chronic or recurrent diarrhea occur frequently. WHO/UNICEF classification of SAM is based on presenting with weight for height (W/H) < −3 SD or MUAC <115 mm or edema, any of which is accompanied by complications such as lack of appetite, fever ≥38.5 °C, hypothermia ≤35.5 °C, vomiting, or severe dehydration.

Wasting refers to children that are too thin for their height and is a common manifestation of SAM. The WHO global nutrition target on wasting is to reach or maintain levels below 5% by 2025. Weighted quartiles (in populations of stunting in children under 5 years of age) based on 119 countries with data between 2005 and 2012 show that 9 countries with wasting rates ≥15.7% (3rd quartile) total more than 55% of the global estimated number of wasted children (Bangladesh, Chad, Djibouti, India, Niger, Papua New Guinea, South Sudan, Sudan, and Timor-Leste). Given that wasting rates can change rapidly, the composition of the list countries with very high wasting levels is also extremely variable.

Marasmus

Marked muscle wasting and the extreme reduction of subcutaneous fat in cases with severe nonedematous SAM present with

Figure 8 Nutritional marasmus with severe wasting. Reproduced from Latham, M. C. (2002) Human nutrition in developing countries. FAO (ed.), *Food and nutrition series — number 29*. Rome: United Nations Food and Agriculture Organization.

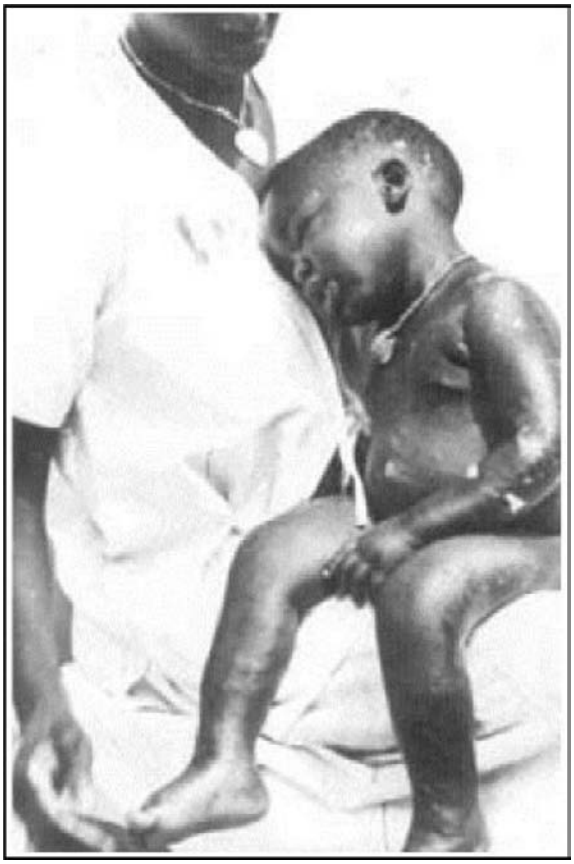


Figure 9 Kwashiorkor. Reproduced from Latham, M. C. (2002) Human nutrition in developing countries. FAO (ed.), *Food and nutrition series — number 29*. Rome: United Nations Food and Agriculture Organization.

a 'skin and bones' appearance. Children with marasmus frequently have 60% or less of the expected weight for height and suffer from short stature. Their hair tends to be dry, thin, and dull and at times lacking in quantity (Figure 8).

Children with marasmus often show complications such as acute gastroenteritis, dehydration, respiratory infections, and eye lesions due to hypovitaminosis A. Systemic infections can lead to septic shock or disseminated intravascular coagulation syndrome with high mortality rates. Obtaining a medical history plays an important role for the differential diagnosis of SAM secondary to AIDS, tuberculosis, cancer, and other debilitating diseases.

Kwashiorkor

The main feature is mild edema, being palpable without pain and usually presenting in the feet, ankles, and legs but, in severe cases, possibly extending to the perineum, upper extremities, and the face. Many patients have pellagroid skin lesions at the edema sites and in areas of pressure or frequent irritation. The skin can be erythematous and shiny in the regions of edema, alternating with areas showing dry, hyperkeratosis, and hyperpigmentation that tend to converge. Where the epidermis becomes dark and dry, it splits open when stretched to reveal pale areas between the cracks ('lacquered flaky paint' and 'crazy pavement dermatosis'), leaving exposed tissues that become infected easily. Subcutaneous fat tends to be preserved, and there may be some degree of muscle wasting. Weight loss, after correcting for edema, is usually not as severe as in marasmus. Children may have short or normal stature, depending on the length of the current episode and their nutritional history (Figure 9).

The hair is dry, brittle, and dull and can be pulled out easily without causing pain. Curly hair becomes straight and pigmentation changes usually acquiring a brownish, reddish, or yellow-white tone. Patients who alternately undergo periods of very poor and relatively good intakes of protein may present with alternating bands of depigmented and normal hair.

Patients have the same complications as described for marasmus cases, but diarrhea and respiratory and skin infections are more frequent and severe. Apart from diarrhea, they frequently present with anorexia and postprandial vomiting. These general conditions improve as nutrition therapy progresses, without specific treatment for gastrointestinal disorders. Kwashiorkor cases may have infections without fever, tachycardia, shortness of breath, or leukocytosis, making diagnosis difficult. The most frequent causes of death are pulmonary edema with bronchopneumonia, gastroenteritis, septicemia, and electrolyte disturbances. A differential diagnosis of other conditions that cause hypoproteinemia and edema should be carried out, as well as secondary undernutrition due to alterations in metabolism and protein absorption.

Marasmic–Kwashiorkor

This form of edematous SAM combines the clinical features of marasmus and kwashiorkor. The major manifestations are the edema of kwashiorkor with or without skin lesions accompanied by the muscle wasting and subcutaneous fat reduction characteristic of marasmus. In the initial stages of treatment, once the edema is resolved, the patient acquires an appearance that is very similar to the child with marasmus. The patient's biochemical profile is characteristic of kwashiorkor and marasmus but the alterations due to severe protein deficiency are those that predominate.

Long-Term Consequences

Studies assessing cognitive development indicate that there is a close interrelationship between undernutrition and intellectual development. In recent years, progress has been made for improved recovery of neurological development in severely undernourished infants when they are systematically stimulated as part of their treatment. Augmented deterioration observed in severely undernourished children is mostly due to electrolyte imbalance, gastrointestinal disorders, cardiovascular or kidney insufficiency, and compromised immune function to combat infections. The psychological alterations can also be profound. Severe anorexia, apathy, and irritability make the child difficult to feed and handle, making prognosis more precarious.

In general, death is secondary to bronchopneumonia, gram-negative septicemia, severe infections, and acute cardiovascular, hepatic, and renal failure. The signs and symptoms of severe vitamin and mineral deficiencies may also be of critical importance and may become manifest during early rehabilitation if an adequate supply of these nutrients is not provided. The clinical picture may be complicated by a severe folate, thiamin, and niacin deficiency with or without associated acute deficiency of potassium, sodium, and magnesium as well as chronic deficiency of iron, zinc, copper, and chromium.

Chronic Undernutrition

A child who suffers from chronic undernutrition experiences delay in his or her growth. This growth retardation is measured by comparing the height of the child with the standard recommended for their age. It indicates a lack of the necessary nutrients over an extended period of time and, thus, the increased risk of becoming ill and the subsequent effects of these events on the physical and intellectual development of the child. Chronic undernutrition, although a problem of greater magnitude in terms of the number of children affected, has up until recently received less attention than more acute forms of undernutrition.

Stunting

Chronic undernutrition is reflected in stunting, the indicator measuring length/height for age and is defined as having a length-/height-for-age *z*-score that is more than two standard deviations (SD) below the age–sex median for a well-nourished reference population. Stunting is a largely irreversible outcome of chronic inadequate nutrition and repeated bouts of infection during the first 1000 days of a child's life. Almost all stunting takes place in the first 1000 days after conception.

Specifically, factors that impact on stunted growth and development include poor maternal nutrition and health, inadequate infant and young child feeding practices (non-exclusive breastfeeding and complementary feeding that is limited in quantity, quality, and variety), and infection. Special importance is placed on maternal nutritional and health status before, during, and after pregnancy, which determines a child's early growth and development, beginning in the womb. Maternal contributors to stunting include inadequate nutrition during pregnancy leading to low birth weight, maternal short stature, short birth spacing, and adolescent pregnancy. The latter impairs nutrient availability to the fetus due to the competing demands of the adolescent mother's own growth.

Stunting has long-term effects on individuals and societies, including diminished cognitive and physical development, reduced productive capacity and poor health, and an increased risk of degenerative diseases such as diabetes. In fact, stunted children who experience rapid weight gain after 2 years of age show an increased risk of becoming overweight or obese later in life, thus increasing the risk of coronary heart disease, stroke, hypertension, and type 2 diabetes onsets. As for the economic impact, the World Bank estimates show that a 1% loss in adult height due to childhood stunting is associated with a 1.4% loss in economic productivity.

The Role of Fetal Growth Restriction

The most common definition of intrauterine growth restriction is a fetal weight that is below the 10th percentile for gestational age as determined through an ultrasound. This can also be called small for gestational age (SGA) or fetal growth restriction. New findings in the 2013 *Lancet* series highlight the impact of SGA on attributable neonatal deaths. Thirty-two million babies were born SGA, and about 800 000 neonatal deaths and 400 000 postneonatal infant deaths could be attributed to the increased risk associated with having fetal growth restriction. These new data contradict the widespread assumption that SGA infants, as compared to preterm babies, are not at a substantially increased risk of mortality. Additionally, infants who are SGA have an increased risk of growth faltering in the first 2 years of life. Causes of childhood growth faltering are multifactorial, but fetal growth restriction might be an important contributor to stunting and wasting in children. The 2013 *Lancet* Series on Maternal and Child Nutrition demonstrated that about 20% of childhood stunting could have its origins in the fetal period, as shown by being born SGA. As such, there is now impetus to focus attention on preventing SGA by scaling up prenatal nutrition interventions. In 2010, 32.4 million babies were born SGA, with 27% of all births occurring in low- and middle-income countries (LMIC). Figure 10 shows the prevalence of SGA by UN LMIC regions with higher rates occurring in Asia and Africa.

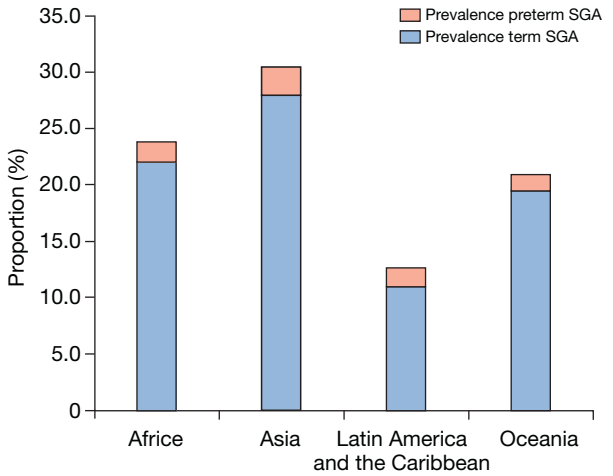


Figure 10 Prevalence of small-for-gestational-age births, by UN regions. Reproduced from Black, R. E., Victora, C. G., Walker, S. P., et al. (2013). Maternal and child undernutrition and overweight in low-income and middle-income countries. *The Lancet* **382**, 427–451.

Epidemiology

Stunting remains widespread in LMIC. Robert Black's analysis estimated that in 2011, 165 million children in LMIC were stunted. East and West Africa and south-central Asia have the highest prevalence estimates in UN subregions with 42% (East Africa) and 36% (West Africa and south-central Asia). There has been a dramatic reduction in the prevalence of stunting in Asia, from 49% in 1990 to 28% in 2010, nearly halving the number of stunted children from 190 to 100 million. Nevertheless, the majority of those stunted remain in Asia, with India being the country having the largest number of stunted children under 5 years of age – about 61.7 million. The stunting prevalence in Africa has stagnated at about 40% since 1990 and accounts for the second largest regional share of world stunting, a share that is increasing.

Eighty percent of stunted children live in 24 countries. Six of the 10 countries (India, China, Nigeria, Pakistan, Indonesia, Bangladesh, Ethiopia, the Democratic Republic of the Congo, the Philippines, and the United Republic of Tanzania – from highest to lowest number of cases) that contribute most to the total figure are in Asia – Bangladesh, China, the Philippines, India, Indonesia, and Pakistan – partly due to the high population reported for these countries. To summarize, Figure 11 depicts the regional trends of malnutrition in children under 5 years of age, showing data for stunting, underweight, wasting, and overweight.

Anthropometric Indicators for Measuring Malnutrition

Several measures and anthropometric indices are used to diagnose and classify malnutrition. The choice of a measure or index of choice will depend on its simplicity, accuracy, and sensitivity, as well as the availability of instruments to make measurements and the existence of reference standards for interpreting the results. In clinical practice, the utilization of reference growth charts for weight, height, and BMI for age and weight for height is common in the pediatric population. Growth charts consist of a series of percentile curves that illustrate the distribution of selected body measurements in children. The reference standards for anthropometric measurements presented in this article are those used in 2010 WHO's Nutrition Landscape Information System (NLiS), a comprehensive data collection system that compiles nutrition-related indicators in standardized form.

Malnutrition in Children

The measurement of weight and height is used for the calculation of indicators such as 'weight for age,' 'height for age,' 'weight for height,' and BMI (BMI = weight in kg/height squared in meters). Other anthropometric measures employed are the measurements of arm circumference and head circumference. In statistical terms, it is more appropriate to use SD ('*z*-scores') or percentiles to evaluate differences between the patient and the reference standard. This is the preferred expression for anthropometric indicators in surveys. It is the difference between the value for an individual and the median value of the reference population for the same age or height, divided by the SD of the reference population. In other words, by applying *z*-scores, one can describe how far a child's weight is from the median weight of a child at the same height in the reference value.

Underweight, stunting, and wasting

These indicators are used to measure nutritional disorders resulting in undernutrition (assessed from underweight, wasting, and stunting) and overweight. Child growth is globally acknowledged as an important indicator of nutritional status and health in populations. The percentage of children with a low height for age (stunting) reflects the accumulated effects of undernutrition and infections since and even preceding birth. Thus, it can be interpreted as an indication of poor environmental conditions or long-term restriction of a child's potential to grow. The percentage of children who have low weight for age (underweight) can reflect 'wasting' (i.e., low weight for height), indicating acute weight loss, 'stunting,' or both. As such, 'underweight' is a composite indicator and may therefore be difficult to interpret.

Definitions used

- Underweight (thinness): weight for age less than −2 SD of the WHO Child Growth Standards median (severe underweight: less than −3 SD)
- Stunting (short for age): height for age less than −2 SD of the WHO Child Growth Standards median (severely stunted: less than −3 SD for length/height for age) (children under 2 are usually measured lying down (recumbent length), and those over 2, standing height is taken)
- Wasting: weight for length/weight for height less than −2 SD of the WHO Child Growth Standards median (severe wasting less than −3 SD for weight for length/weight for height or BMI for age)
- Overweight: weight for height greater than +2 SD of the WHO Child Growth Standards median

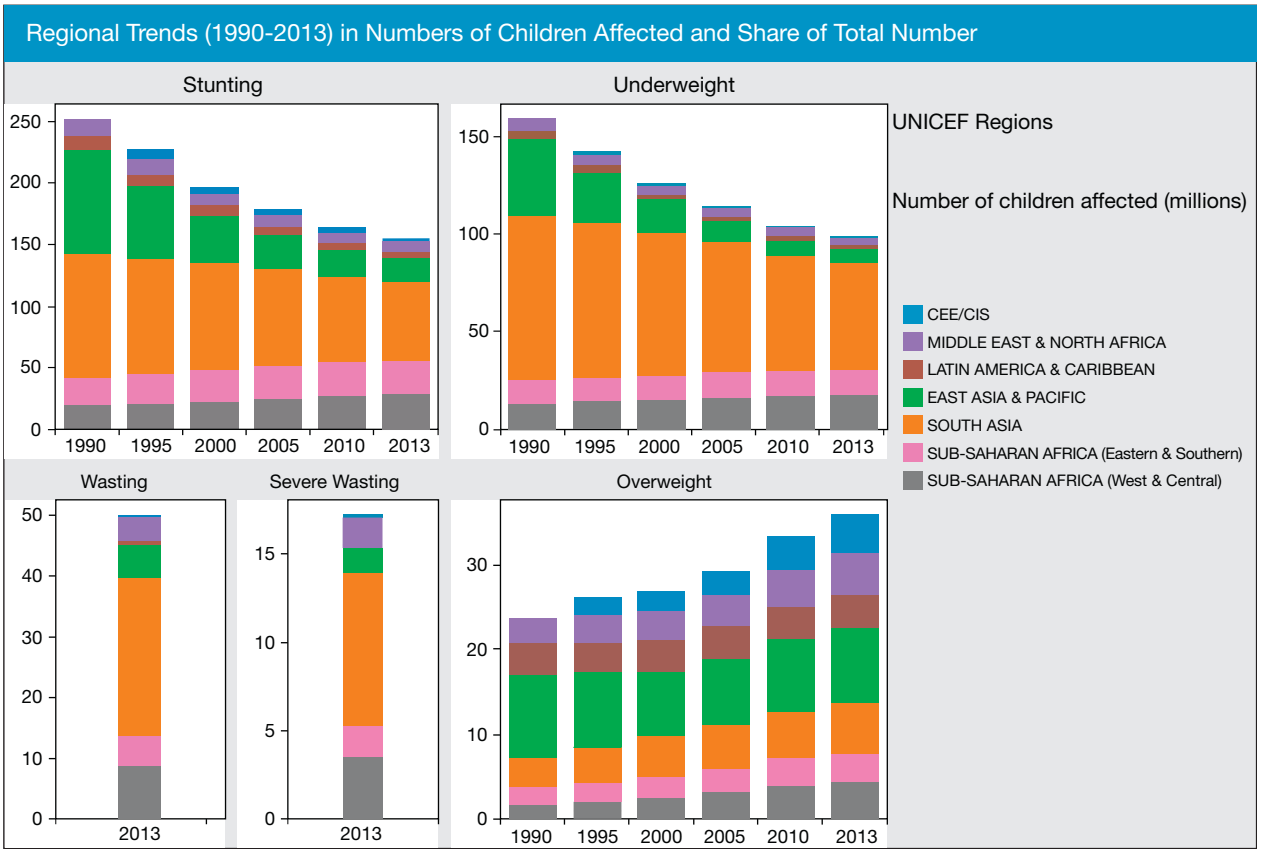


Figure 11 Regional trends (1990–2013) of malnutrition in children under 5 years old. The UNICEF-WHO-World Bank Child Nutrition Interactive Dashboard.

Interpretation

Underweight: As weight is easy to assess, this has been the most widely collected indicator. Evidence has shown that the mortality risk of children who are even mildly underweight has increased, and severely underweight children are at even greater risk.

Stunting: Children who suffer from growth retardation as a result of poor diets or recurrent infections tend to be at greater risk for illness and death. Stunting is the result of long-term nutritional deprivation and often results in delayed mental development, poor school performance, and reduced intellectual capacity. This in turn affects economic productivity at the national level. Women of short stature are at greater risk for obstetric complications because of a smaller pelvis. Small women are at greater risk of delivering an infant with low birth weight, contributing to the intergenerational cycle of malnutrition, as infants of low birth weight or retarded intrauterine growth tend be smaller as adults.

Wasting: Wasting in children reflects acute undernutrition, usually as a consequence of insufficient food intake or a high incidence of infectious and parasitic diseases, especially diarrhea. In addition, it is associated with an impaired immune system functioning and can lead to increased susceptibility, severity, and duration of infectious diseases as well as increased mortality risk. In the WHO protocols for SAM management, MUAC is also assessed, along with the presence or absence of edema.

Overweight/obesity: Childhood obesity is linked with a higher probability of obesity as an adult, which can result in a variety of disabilities and pathologies, such as diabetes and cardiovascular diseases.

Malnutrition in Adults

The values for BMI are age-independent for adult populations and are similar for both males and females. However, differences in BMI may not correspond to the same degree of fatness in different populations as there may be variations in body proportions.

Moderate and severe thinness, underweight, overweight, and obesity

- BMI <17.0 kg m^{-2} indicates moderate and severe thinness
- BMI <18.5 kg m^{-2} indicates underweight
- BMI 18.5–24.9 kg m^{-2} indicates normal weight
- BMI ≥ 25.0 kg m^{-2} indicates overweight
- BMI ≥ 30.0 kg m^{-2} indicates obesity

Moderate and severe thinness: A BMI <17.0 kg m^{-2} indicates moderate and severe thinness in adult populations. This has been associated with increases in illness in adults studied in three continents, and this is considered a reasonable further limit to choose as a cutoff point for moderate risk. A BMI <16.0 kg m^{-2} is associated with a markedly increased risk for ill health, poor physical performance, lethargy, and even death; as such, this cutoff point is considered a valid extreme limit.

Underweight: The cutoff point of 18.5 kg m^{-2} for underweight in both genders has less scientific validity as a cutoff point for moderate and severe thinness but is considered a reasonable value for use pending further comprehensive studies. The proportion of the population with a low BMI that is considered a public health problem is closely linked to the resources available for correcting the problem, the stability of the environment, and government priorities. Approximately 3–5% of a healthy adult population have BMIs <18.5 kg m^{-2}.

Micronutrient Malnutrition

Micronutrient Deficiencies

More than 2 billion people are deficient in micronutrients such as vitamin A, iodine, iron, and zinc. Malnutrition due to the lack of vitamins and minerals (micronutrients) can make its appearance in a variety of ways. Fatigue, reduced learning capacity, and impaired immunity are just a few examples. These nutrients are called micronutrients since the body requires them in small amounts for growth, development, and health maintenance. The combination of different ecological factors such as climate; low production, availability, and consumption of certain micronutrient-rich foods; cultural patterns that limit the consumption of given foods; and poverty-induced food restrictions contributes to mapping a specific region's particular micronutrient and health profile. In regions with a high prevalence of undernutrition, associated micronutrient deficiencies occur, which contribute to the symptoms that appear. Or they may remain in an asymptomatic subclinical phase, without apparent clinical expression, thus giving rise to the expression 'hidden hunger.' This expresses the phenomenon of a chronic lack of vitamins and minerals that often have no visible warning signs, such that those affected by it may not even be aware of it. Yet it nevertheless brings about significant negative health consequences.

Unlike many other impediments to social and economic development, micronutrient deficiencies can be reduced with relatively inexpensive investments in public health, agriculture, and education. The technology exists to solve many of these deficiency states. However, they persist for various reasons, including the lack of awareness among policy-makers on the importance of resolving the issues, as well as the lack of knowledge by program planners about their consequences and the strategies available to combat them.

In recent decades, the research community, governments, development agencies, and nongovernmental organizations (NGOs) have made considerable progress in identifying the population groups that have the greatest risk of micronutrient deficiencies and in developing programs for short-term improvement of given deficiencies. These strategies focus on improving access, availability, and consumption of foods rich in vitamins and minerals. The benefits of these food-based strategies are that they not only address improving the intake of certain nutrients but also enhance the overall situation in terms of food and health. Since 1985, FAO has actively participated in the definition and implementation of programs to reduce micronutrient deficiency diseases. FAO strongly supports the promotion of producing and consuming micronutrient-rich foods as a sustainable solution to the problem of micronutrient deficits. Globally, the three micronutrients having the greatest repercussions for public health are vitamin A, iron, and iodine, which will be the focus of this article. Other deficiencies of relevance also exist, such as of folate, zinc, and calcium, and the reader is referred to the supplementary materials for more information on these topics.

Vitamin A Deficiency

Vitamin A deficiency (VAD) most frequently occurs in young children. A child who lacks this vitamin is more susceptible to the negative effects of infections, which will be more severe and will increase the risk of mortality. Deficient intakes increase the risk of blindness. It also leads to alterations in the skin, mouth, stomach, and the respiratory system. Vitamin A supplementation reduces the risk of death from measles. It is estimated that in populations with this problem, administering vitamin A can reduce infant mortality due to measles by 50% and mortality from diarrhea by 40%. The overall risk of infant mortality can be reduced by 23%. Interventions that provided the basis for these data included programs of food enrichment and oral supplementation.

As seen in Figure 12, WHO shows regional data on the proportions of preschool-age children with biochemical VAD, as indicated by a serum retinol concentration <0.70 μmol l^{-1}. In all developing countries, an estimated 163 million children have VAD (by low serum retinol), with a prevalence of around 30%. South-central Asia (which includes India) has the highest prevalence with the greatest number of children (two-thirds of affected children) and pregnant women and, along with Central and West Africa, has a prevalence of more than 40%. South and Central America and the Caribbean have the lowest prevalences, near 10%.

Vitamin A (also called retinol, for one of its chemical forms) is essential for normal vision, growth and development, the maintenance of epithelial integrity (the layer of cells that make up the lining of internal and external body surfaces), immunity, and reproduction. VAD occurs when organic reserves of the micronutrient drop below the level that allows for the maintenance of proper physiological functioning, generally as a result of insufficient intake of foods containing this vitamin. The integrity of the epithelial barriers (part of natural immunity and physical barriers against microorganisms such as the skin and mucous membranes) and immune response, itself, is the first to be affected, followed by visual functioning. As a result of the body's compromised defenses, the frequency and severity of infections increase and there is a greater risk of mortality, especially in children. Moreover, anemia and the dryness of the skin (hyperkeratosis) constitute other extraocular manifestations of VAD.

The eye lesions caused by the lack of vitamin A are known as xerophthalmia and are characterized by progressive dryness, opacity, and weakening of the cornea. The first manifestation is the appearance of night blindness (hemeralopia), which is evident in children when they stop playing or start tripping over objects within the home at sundown. Ocular lesions in the conjunctiva and cornea are easily visible when Bitot's spots appear (small grayish foamy triangular deposits on the conjunctiva). At this stage, the eye disorder is still reversible, but without treatment, it progresses to corneal necrosis (disintegration), which results in permanent blindness.

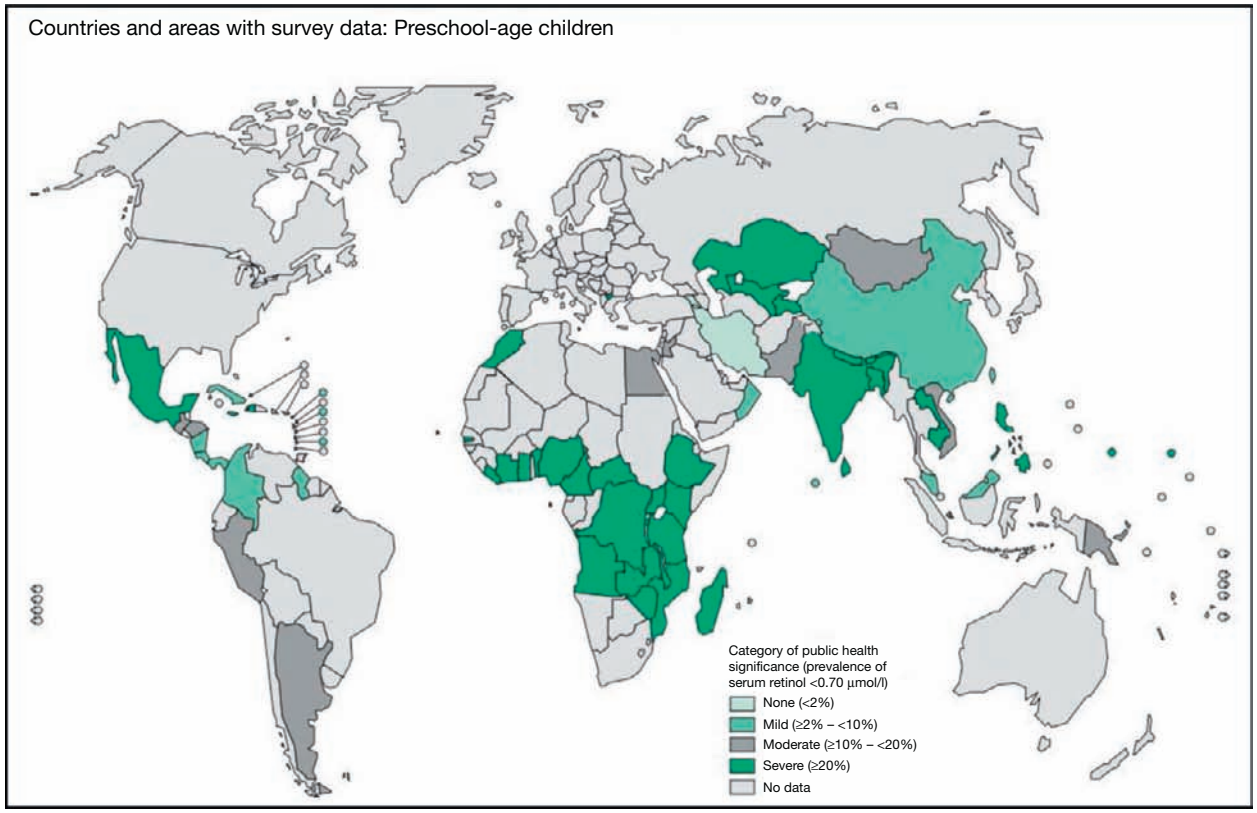


Figure 12 Global prevalence of preschool-age children with biochemical VAD. Reproduced from WHO (2009). *Global prevalence of vitamin A deficiency in populations at risk 1995–2005. WHO global database on vitamin A deficiency*. Geneva: World Health Organization.

Table 3 Global anemia prevalence and number of individuals affected

	Prevalence of anemia		*Population affected*	
Population group	*Percent*	*95% CI*	*Number (millions)*	*95% CI*
Preschool-age children	47.4	45.7–49.1	293	283–303
School-age children	25.4	19.9–30.9	305	238–371
Pregnant women	41.8	39.9–43.8	56	54–59
Nonpregnant women	30.2	28.7–31.6	468	446–491
Men	12.7	8.6–16.9	260	175–345
Elderly	23.9	18.3–29.4	164	126–202
Total population	24.8	22.9–26.7	1620	1500–1740

Source: de Benoist, B., et al. (eds.) (2008). *Worldwide prevalence of anaemia 1993–2005. WHO global database on anaemia*. Geneva: World Health Organization.

Different strategies exist to prevent VAD. The three main ones consist of the administration of vitamin A (normally in capsule form), food fortification with the vitamin, and food-based enhancement by providing access to foods that contain this nutrient. Each strategy has its advantages and disadvantages, but a combination of approaches may result in even greater efficacy for program interventions.

Iron-Deficiency Anemia

The lack of iron is the most common nutrient deficiency in the world and mainly affects children and women of reproductive age. The resulting anemia significantly contributes to maternal and neonatal mortality. Anemia reduces resistance to diseases and weakens children's learning capacity and physical vigor while it significantly increases the risk of maternal mortality during childbirth. As seen in Table 3, globally, anemia affects 1.62 billion people, which corresponds to around 24.8% of the population worldwide. The highest prevalence is in preschool-age children (47.4%), and the lowest prevalence is seen in men (12.7%). However, the population group with the greatest number of individuals affected is that of nonpregnant women (468.4 million).

Anemia is a disease caused by the decrease in the amount of hemoglobin in the red cells of the blood. Hemoglobin is composed of a protein attached to an iron molecule and is the

component of red blood cells that carries oxygen from the lungs to the tissues. There are different causes of anemia, the most common of which is iron-deficiency anemia. The lack of other nutrients such as folate (or folic acid), protein, and vitamin B_{12} can also contribute to the onset of anemia. Ascorbic acid (vitamin C), vitamin E, copper, and pyridoxine (vitamin B_6) are also necessary for the production of red blood cells. It is worth noting that VAD is also associated with anemia.

Unlike other micronutrient deficiency diseases, iron deficiency anemia is also common in industrialized countries, although it is of a greater magnitude in developing countries. Food sources of iron are principally of animal origin (known as heme iron), such as liver, red meat, fish, and meat products. The plant sources of iron (called nonheme iron) are found in legumes, dark-green leafy vegetables, nuts, and seeds, but the body has greater difficulty in assimilating this type of iron. The total amount of iron in the diet is not the only factor that determines the development of anemia. Nonheme iron from plant foods is better absorbed when taken with certain foods (vitamin C-rich fruits and vegetables) to favor its absorption. Yet other foods (such as raw egg yolk and tea) impede its absorption. In addition, iron losses can result from a variety of conditions, of which one stands out in many tropical countries – parasitic infections, caused by intestinal helminths. Schistosomiasis, a parasitic disease that affects genitourinary or intestinal blood vessels, is also an important cause of blood loss, and consequently iron, via urine or fecal matter. Moreover, malaria is also a major cause of anemia, although in this case, it is not related to the loss of iron, but to the destruction of red blood cells by the disease process.

As the body manages iron very effectively, the daily nutritional requirement of this mineral in healthy men and postmenopausal women is quite low. However, women of childbearing age must replace iron lost during menstruation and at childbirth and should meet their increased need for iron arising from pregnancy and lactation. Children have relatively high iron requirements, not only derived from an increase in body size but also augmented blood volume. Anemia in children is associated with reduced development of cognitive capacity, increased learning problems, and difficulties in concentrating in and out of school, as well as decreased ability to perform physical work and a reduction in appetite and growth.

Anemia is particularly hazardous for pregnant women, as it significantly increases the risk of death during childbirth as a result of hemorrhages. In addition, anemia is associated with preterm births and low-birth-weight infants. The principal clinical manifestations common to almost all types of anemia are tiredness, weakness, fatigue, shortness of breath after exertion, dizziness, headaches, palpitations, and pale mucous membranes. Reduced resistance to infection is another sign of anemia. Severe anemia, which is rare, can progress to heart failure and in some cases death. The signs and symptoms, at least in the early stages of anemia, are discrete and not at all severe, which often results in the disease being ignored or neglected.

Iron-deficiency anemia is relatively easy and inexpensive to treat via the administration of oral iron supplements, which usually also incorporate folate. People with iron-deficiency anemia due to poor diets should be advised to eat more fresh fruits and vegetables at meal times. These foods contain vitamin C, which facilitates the absorption of iron content coming from cereals, tubers, legumes, nuts, and seeds. In addition, fruits and vegetables contain folate and a variety of vitamins and minerals. To the extent possible, the anemic patient should be encouraged to consume, even in very small quantities, foods rich in heme iron, such as meat, especially liver or kidneys. The iron in breast milk is well absorbed, especially when compared to that found in cow's milk, infant formula, or common milk powder. As such, the protection, support, and promotion of breastfeeding are strategies for preventing iron deficiency in infants during exclusive breastfeeding and for maintaining adequate iron intake when complementary foods are introduced. Moreover, as breastfeeding delays the onset of menstruation, the nursing mother is protected from blood loss, thus enhancing iron status.

Iodine-Deficiency Disorders

Iodine-deficient diets can result in various diseases, including endemic goiter and cretinism. Iodine is an essential component for the synthesis of thyroid hormones, which have an important role in the regulation of metabolism, sustaining normal growth and intellectual development. The synthesis of these hormones occurs in the thyroid gland, located at the bottom of the neck and invisible under normal conditions. The most common discernible manifestation of the lack of iodine in the diet is endemic goiter. The neck swells in response to the substantial enlarged size of the thyroid gland as it attempts to increase hormone production.

On the one hand, a less important cause of goiter is the consumption of foods considered to be goitrogenic (suppress the function of the thyroid gland by interfering with iodine uptake), rapeseed, cabbage, mustard seeds, and especially cassava, which is the staple food in different regions of Africa. On the other hand, cretinism, which in its acute form includes mental retardation, deaf–muteness, short stature, and other defects, is due to a diet devoid of iodine consumed by the mother during pregnancy.

Endemic goiter and cretinism are the obvious components of iodine-deficiency disorders (IDDs). These abnormalities can be diagnosed with relative ease by health professionals without the use of laboratory or other medical tests. However, other harmful consequences caused by iodine deficiency are not easily detected. These include less visible enlargement of the thyroid gland, different degrees of mental retardation, poor growth in children, and a greater incidence of spontaneous abortions and stillbirths. In areas with a high prevalence of iodine deficiency, many children are unable to fully develop their intellectual potential due to poor school performance and to a lower IQ as compared to similar groups without iodine deficiency. This is detrimental not only to the affected children but also to the development of the country insofar as human resource potential and productivity are diminished (**Figure 13**).

Unlike nutrients such as iron, calcium, and vitamins, iodine is a nutrient that is not naturally occurring in given foods. Rather, it is present in the soil and is ingested through foods that have been cultivated in it. The food produced in endemic iodine-deficient areas will never be able to provide enough iodine for the population and livestock that consume it. Iodine

deficiency is due to unequal distribution of this micronutrient in the soil covering the land's surface area.

Ocean water contains adequate amounts of iodine, and therefore, people living close to the sea and those who eat saltwater fish and marine products tend to have sufficient iodine. However, these foods are not accessible to all and the mere fact of living in coastal regions does not necessarily guarantee sufficient intake levels of this nutrient. Soil in the mountain ranges of the Himalayas, the Alps, and the Andes are prone to be deficient in iodine content. Many other parts of the world also have soils with scarce iodine levels, such as vast regions of Central Africa. Figure 14 shows the global impact of IDDs.

Figure 13 Children with goiter in Tanzania's Ukinga mountains. Reproduced from Latham, M. C. (2002) Human nutrition in developing countries. FAO (ed.), *Food and nutrition series — number 29*. Rome: United Nations Food and Agriculture Organization.

Of the three key micronutrients with most relevance for malnutrition (iodine, iron, and vitamin A), IDDs are the easiest to control. Iodine is a vital nutrient that is required in small quantities. An adult does not need more than 100–200 μg of iodine per day. The main measure to control IDD has been via the iodization (iodine fortification) of salt (or other basic staples such as bread), which is a simple and relatively cheap method that does not change salt's appearance or taste.

Malnutrition and Sustainable Diets

In 2012, FAO defined sustainable diets as those diets with low environmental impacts that contribute to food and nutrition security and to healthy life for present and future generations. Sustainable diets are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair, and affordable, as well as nutritionally adequate, safe, and healthy, while optimizing natural and human resources. This same organization estimated that of a total of 300 000 flowering-plant species, 10 000 plant species have been used

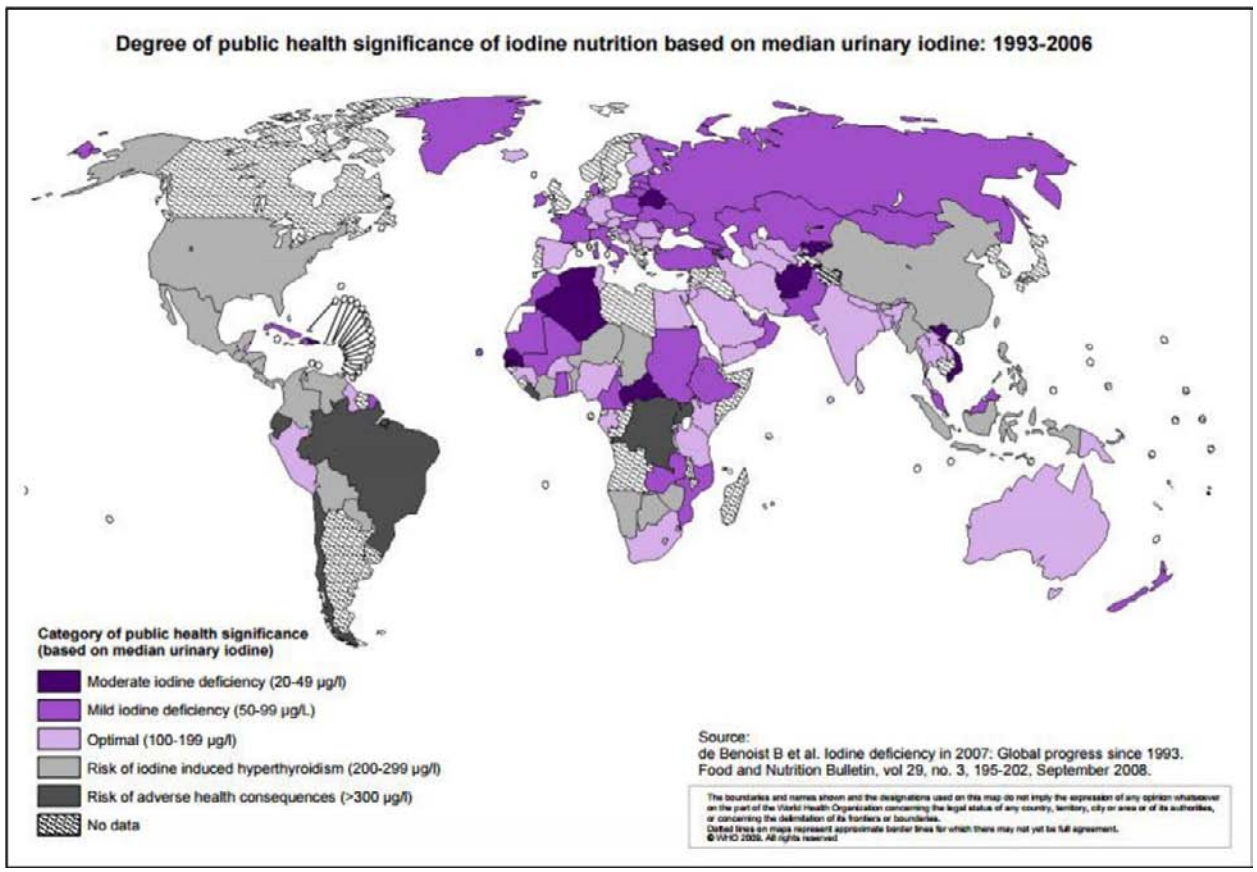


Figure 14 Global iodine public health significance.

for human food since the origin of agriculture. Out of these, only a mere 150–200 species have been commercially cultivated, with rice, wheat, maize, and potato flour supplying 50% of the world's food energy needs and with 30 crops providing 90% of the world's food energy intake.

The intensification of agricultural systems, poverty, population demands, urbanization, and changes in lifestyle have altered food production and consumption in ways that profoundly affect diets, resulting in an overall reduction in seasonality and concentration of staple food sources. What is more, the disturbing speed of biodiversity loss and ecosystem degradation accompanied by climate change and resource extraction, and the negative impact of this on farming systems, livelihoods, and health, make a convincing case for re-examining food systems from an environmentally sustainable and public health perspective.

Helping people and families to follow a sustainable diet is also a major objective of the Cooperative for Childhood Nutrition (CONIN). CONIN, founded in Chile in 1975, has worked for the recovery of children from 0 to 3 years who present with primary or secondary undernutrition. Since 1993, this project has been extended to the province of Mendoza, Argentina, as the CONIN Foundation, where this initiative was consolidated and extended by creating Undernutrition Prevention Centres in many Latin American countries. In these centers, health prevention and promotion strategies are developed with a focus on sustainability and empowerment. The challenge continues to develop preventive programs that involve educating and impelling the family to modify its lifestyle in order to improve its health and its education. CONIN serves as an example of how investing in human capital early in life will optimize and sustain the growth and socioeconomic development of children, families, and communities, thus contributing to break the intergenerational transmission of poverty and undernutrition.

The Centre for Nutrition Recovery and Education (CREN), administered by the Nutrition Without Borders (NWB), an NGO, in Gambia, provides another example of integrated sustainable actions carried out at the community level. Following the WHO, UNICEF, and NaNA (the Gambian National Nutrition Agency) malnutrition protocols, NWB regularly conducts community screenings supported by local counterparts (NaNA) to detect severe cases of undernutrition (SAM), which are referred to CRENs for clinic-based treatment. The moderate cases of undernutrition are treated in the community, where actions to improve dietary quality and quantity are reinforced by the implantation of NWB-supervised community-run vegetable gardens, which form part of NWB's strategy to reduce undernutrition. NWB has implemented a complementary agricultural project to support the promotion of sustainable farming, encouraging and training the inhabitants of selected villages in the catchment area ($\sim$50 000 individuals) about the cultivation and consumption of local plants with higher nutritional content (such as *Moringa* and millet). CRENs also have an on-site vegetable garden where mothers of children under treatment also participate and learn about food preparation, conservation, and hygiene as part of the strategy to prevent recurrent cases of undernutrition in the community.

The UN's Zero Hunger Challenge aims for all food systems to be sustainable. Current food production and consumption patterns contribute to biodiversity loss, put pressure on natural resources, and provoke greenhouse gas emissions and excessive agrochemical inputs, particularly in developed countries. Industrialized regions are living beyond the Earth's capacity, yet their food systems are too often considered as role models for developing countries. Food systems – in both developed and developing countries – need to be restructured and take into account social, environmental, and public health impacts. Agriculture policies and programs have concentrated so far on commodities and value chains. There is a need for policy-makers to move beyond the current focus on commodity goods and instead implement actions centered on whole diets and food systems. Biodiversity offers a wealth of untapped potential for livelihoods, health, nutrition, and environments. Interdisciplinary collaboration is needed if global efforts are to tackle malnutrition, NCDs, and the impact of food on climate change, water, and biodiversity. In order to be sustainable, it is essential that diets be healthy, compatible with sustainable management of natural resources and social equity. As has been done with nutrition, the time has come to advocate for making sustainable food production central to development.

Conclusions

In the last two decades, the world has undergone profound changes. Over 800 million are hungry and 2 billion lack essential micronutrients, while 2 billion are overweight and prone to diet-related diseases. The burden of malnutrition presents itself in multiple, complex, and connected ways and countries need concentrated action at the policy, health-system, and community levels. For this reason, in 2012, the World Health Assembly Resolution 65.6 endorsed a comprehensive implementation plan on maternal, infant, and young child nutrition, which specified a set of six global interrelated nutrition targets for the year 2025 with the aim to (1) achieve a 40% reduction in the number of children under 5 years of age who are stunted, (2) achieve a 50% reduction of anemia in women of reproductive age, (3) achieve a 30% reduction in low birth weight, (4) ensure that there is no increase in childhood overweight, (5) increase the rate of exclusive breastfeeding in the first 6 months up to at least 50%, and (6) reduce and maintain childhood wasting to $<$5%. It is evident that although there are clear measurable comprehensive targets to help us focus our efforts in combating malnutrition, the remaining challenges are many. For this purpose, it is necessary to strive for sustained political commitment and cooperation among the many stakeholders concerned, both public and private, in and out of the field of nutrition. Governments, international partners, civil society, NGOs, and the private sector have a key role in the creation of healthy environments and conditions for affordable, accessible, and sustainable healthy dietary choices for communities and especially for those most at risk of malnutrition in all its forms.

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Diarrheal Diseases; Energy: Intake and Energy Requirements; Energy Metabolism; Famine, Hunger, and

Undernourishment; Food and Agriculture Organization of the United Nations; Food Fortification: Rationale and Methods; Foodborne Pathogens; HIV Disease and Nutrition; Hypovitaminosis A; Iodine: Iodine Deficiency Disorders (IDD); Iron: Physiology of Iron; Malnutrition: Prevention and Management; Mycotoxins: Toxicology; Nutrition and Infection; Obesity: Causes and Prevalence; Obesity: Epidemiology of; Obesity: The Role of Diet; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; World Health Organization.

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Malnutrition: Prevention and Management

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Introduction

In theory, deficiencies and excesses occur with respect to strictly essential, conditionally essential, and beneficial nutrients. For the purposes of this article, the concept of deficiency or excess will be restricted to the setting of status; this relates to the nutrients within the body. One cannot rely on dietary intake to be a reliable proxy for nutrient status. Aside from the vagaries of evaluating habitual intake of nutrients, consumption of a nutrient above or below the recommended levels is a poor predictor of the nutrient status within the body. It is recognized, however, that assessment of status is not readily or validly performed for all nutrients. Individuals can often adapt to intakes below the recommendations by augmenting uptake efficiency or reducing turnover. Conversely, in the face of an excessive dietary offering, adaptations to reduce absorption or increase excretion can allow the individual to maintain normative status.

The Spectrum of Malnutrition

Defining and characterizing 'malnutrition' is not straightforward. The most important may be that it is varied and spans a spectrum from deficits below the normative level to excesses above the norm. An important paradigm shift emphasized in the writings of Prof Ricardo Uauy is to be concerned with 'malnutrition in all of its forms'; that means a focus on both *under-* and *over*nutrition in individuals, within households, and across societies. It has specificity for the macro- or micronutrient nutrients in question, and the total dietary energy (derived for a collective of the macronutrients of carbohydrate, fat, protein, and at times alcohol) is to be included in the malnutrition spectrum. There is also a gradient of degree. This can be partitioned as *subclinical* (diagnosable by way of laboratory testing, but not clinically evident) or *clinical* (presenting with detectable signs and/or symptoms). Within the latter category, there is further differentiation into mild, moderate, and severe for deficits in weight and stature. For micronutrients, this gradation often takes on the language of 'marginal' or 'deficient' in the parlance of vitamin B complex or 'insufficient' or 'deficient,' as for vitamin D.

The Principles of Assessment of Malnutrition

The public health or clinical address of malnutrition in all of its forms would presuppose the availability of diagnostic tools to determine when a deficit or excess of nutrients exists. One could begin with an analogy to banking. How much is a satisfactory reserve of assets and what is a precarious deficit from borrowing? Information could easily be assessed by banking statement sheets. As with all other aspects of nutrition, this objective is fraught with caveats and difficulties, and the analogy of financial assets and liabilities quickly breaks down in practice. This is nutrient-specific and can be further seen in a perusal of the relevant chapters in this encyclopedia.

Clinical malnutrition, by definition, has its associated signs, symptoms, measurements, and imaging. A skilled practitioner, familiar with the symptoms and physical manifestations, should be able to recognize manifestations consistent with under- or overnutrition states. Few manifestations are pathognomonic for a nutritional origin, however. When clinical deficiency of protein, essential fatty acids, or micronutrient is suspected, further laboratory testing or a therapeutic trial with the nutrient in question may be needed for conclusive diagnosis.

Subclinical deficiencies and excesses are far more elusive to detection. They require a high level of suspicion, from history or circumstances, to invoke an investigation. Thereafter, they must rely on diagnostic tests based on amounts of the nutrient in the body or functional responses to nutrient deficiency that are in no way completely sensitive or specific.

Assessment is relevant to both individuals, to trigger individual therapeutic action, and to populations, to trigger public health action. A limited number of routine biomarkers are reliable for defining the nutritional status of an individual across the entire spectrum from deficient, to depleted, to normative, to excessive, to toxic. One such marker is serum ferritin as an indicator of total-body iron reserves, a caveat, however, that concurrent inflammation must not be present, or this will distort the assessment. Circulating retinol concentrations will not discriminate the same gradient for vitamin A status in an individual, as there is homeostatic regulation of levels across a wide range of total-body vitamin A stores. As a screening tool for the assessment of a population, the standard of >20% of the under-5-year-old segment having a circulating retinol concentration $<20\ \mu g\ dl^{-1}$ can be used as an indication of hypovitaminosis A risk and a trigger for public health action, but it does not confirm the adequacy or inadequacy of any given subject in the representative national sample. To assess an individual with any degree of graded reflection of actual vitamin A reserves generally requires total-body dilution studies with exogenous, stable isotope-labeled tracer retinol; this test is too expensive and cumbersome for wide-scale use but can be used in monitoring of public health interventions.

Furthermore, not all nutrients have a pool or storage reserve of nutrients that can be called upon in the absence of a dietary supply. Clearly, iron and vitamin A, the previous, do have storage in tissues. Along with some other vitamins and minerals, these storable nutrients are called by Golden as type I nutrients (Table 1) and lend themselves to assessment through biomarkers of body reserves. For another series of nutrients outlined in Table 1, however, there are no tissue-storage reserves, so-called type II nutrients. The stress of underconsumption leads to a regulatory reaction by increased retention, shutting down to the degree possible the excretion of

Table 1 The concept of type I and type II nutrients as derived from the formulation of Michael Golden

Type I nutrients
All of the 13 vitamins, calcium, choline, chromium, copper, essential fatty acids, fluoride, iodine, iron, manganese, molybdenum, selenium
Comments: Have accessible storage pools/reserves in specific tissues. Humans continue to grow despite deficiency, often further depleting stores. Diagnostic biomarkers of circulating concentrations or tissue levels are often responsive to status and potentially useful in status assessment
Type II nutrients
Nitrogen, essential amino acids, magnesium, potassium, phosphorus, sodium, sulfur, zinc
Comments: Do not have accessible storage pools/reserves in specific tissues. Cessation of growth is an early response to deficiency, as one mechanism for retention. Diagnostic biomarkers of circulating concentrations or tissue levels are often homeostatically regulated and are relatively unresponsive to change in status

the nutrient. Zinc is a classic member of this group, and circulating zinc is a relatively poor biomarker of status. In young individuals, part of the conservation mechanism is a loss of appetite (acute anorexia) and a cessation of growth (growth arrest). Both are clear manifestations in human zinc deficiency and in the depletion of the companion type II nutrients. Evidence for reduced excretion can often be used as a diagnostic test for this class of nutrients. However, because of the absence of a storage pool, the quantity of the nutrient in urine, stool, or saliva is really an indicator of the level of recent dietary intake.

In both clinical medicine and public health epidemiology and programs, it is prudent to support the actions with efforts to assess the initial and posttreatment or postintervention status of the patient or population, respectively. This must be done, however, with a consciousness of the challenges and limitations in the reliability of biomarkers and diagnostic tests to reflect the true nutritional status with respect to the nutrient(s) of interest.

Prevention of Malnutrition

Although not the sole predictor and determinant, nutritional status depends heavily on the supply of nutrients; therefore, the first principle of preventing malnutrition begins with the assurance of an adequate diet. In the absence of disease, dysfunction, or adverse environmental conditions, habitually consuming at or above the recommendations for one's sex, age, and physiological (reproductive) condition (e.g., pregnancy or lactation) should be a safeguard against deficiency. Conversely, restricting nutrient intakes to below the upper tolerable intake limits (UL) should generally prevent excessive deposition of or exposure to those nutrients that can be harmful in excess. This framework for prevention, based on the assumption of good health, however, is fraught with caveats.

The first consideration is ignorance in the populace regarding exactly how close their intakes are to the upper or lower boundaries of recommended intake. Few people know how much a person of their age and sex should be consuming of the total dietary energy or the gamut of nutrients. Even if this information was generalized and socialized, however, knowing how many nutrients are in the food makes selecting an adequate intake a virtual impossibility. Commercially processed foods provide some indication of their nutrient content on the labels; but this only relates to nutrients providing a certain percentage of the 'daily value' needs; moreover, it is not presented in an expression useful to the average consumer. When it comes to unprocessed or lightly processed items such as foods of animal origin (meat, foul, fish, eggs, and dairy), produce (fruits, vegetables, roots and tubers, and herbs), and nonpackaged bakery goods, only a food-composition table can provide reliable nutrient-content information.

The most useful datum would be the total dietary energy. In Western industrialized countries, the concern would be for an *excess* of calories. In situations in the low-income world of natural disasters, war and social turmoil, and/or climate change and famine, a *deficit* of calories is the concern. See chapters on obesity and famine. This would be followed in priority by knowledge on the consumption of sodium (salt and monosodium glutamate) and successively on sugars, cholesterol, and saturated fats.

For micronutrients (vitamins and minerals) and other beneficial nutrients (essential fatty acids, fiber, and polyphenols), the popular adage of 'consume a balanced diet' makes a positive contribution. The connotation of 'balanced,' as well as an operational definition, however, is critically important for program and policy discussion. To prevent malnutrition, a diet maximizing food variety, which is having a large number of different food and beverage items consumed, should be followed. It should likewise be of high dietary diversity, which is with balanced representation of all of the principle food groups (meat and eggs; milk and dairy products; fruits; legumes and vegetables; and cereals and tubers). There are a number of challenges to these broad, preventive recommendations, especially for the less affluent segments of any society. These include the year-round availability, affordability, and quality of the items in the food supply.

No matter how balanced the diet, an adequate intake of iodine and fluoride cannot be assured. This is likely to depend on the iodization and fluoridation of salt or the iodine fortification of bread or the enhanced fluoride content of the drinking water.

Micronutrient epidemiology tells us that insufficient intakes of bioavailable forms of specific nutrients will predispose to endemic deficiencies. Among the candidate nutrients for inadequacy in low- and middle-income societies and among the poor of affluent societies are vitamins D and A and folate, along with iron and zinc. Problems with vitamin D and iron, moreover, will not be unknown even among the affluent. Omega-3 fatty acids, vitamins B_{12} and E, and selenium are micronutrients with greater geographic specificity in their deficiencies. In free-living populations, however, nutritional deficiencies or excesses of vitamin K, thiamine, niacin, vitamin B_6, pantothenic acid, biotin, copper, manganese, and chromium are unlikely to occur. Specific diseases, surgeries, medications, or chronic parenteral feeding can predispose to occasional occurrence of manifest deficiencies of the latter group of nutrients.

On the side of overnutrition, a series of actions aimed at consumer education, improving access to healthful foods, suppressing consumption of items of higher energy density, and

stimulating exercise in recreational activities, are available. Further detail is presented in other chapters.

Food Fortification for Prevention of Undernutrition

When dietary intakes are shown to be low by survey evidence or when nutrient deficiencies are endemic, food fortification policies and programs can be initiated. The conventional approach is to find a staple food item that is widely consumed across all social strata and age groups in a precarious population. Salt, table sugar, cooking oil, and flours are the most commonly identified staples. Based on an assessment of the dietary gap and the level of habitual consumption across the society, a specific addition of the nutrient is made during processing. Among the advantages of fortification are the lack of need for any change in behavior and the relative low cost. The concerns of fortification are problems at both ends of the spectrum: on the one hand, insufficient intake or nutrient bioavailability, which may lead to poor effectiveness in its impact on nutrient deficiency and, on the other hand, the risk of excessive intake for the segment of the population with the highest consumption of the fortified foods.

Two other elements of fortification are worthy of mention. Food manufacturers have voluntarily added fortificant nutrients to their products as a value-added gesture; this is known as 'market-driven' fortification. Folic acid, iron, zinc, and vitamin C are most commonly added. When combined with a mandatory national fortification programs, excess intakes in the high-consumer sectors can be exacerbated. Biofortification is a novel approach in which the intrinsic content of nutrients in foods can be increased by genetic approaches, either hybridization or genetic modification. This extends the vehicles of micronutrients from grains to legumes, tubers, fruits, and vegetables.

Periodic Supplementation for Prevention of Undernutrition

Periodic supplementation is an approach much more targeted to specific groups within an at-risk population than fortification. The target groups often considered most vulnerable to undernutrition, namely, children under 5 years old, pregnant and lactating mothers, and women of reproductive age, are often the groups included in national nutrition surveys.

The threshold for initiation of a supplementation program is generally the documentation of a certain prevalence of deficient biomarkers, for example, >20% for low retinol or >40% for anemia. Alternatively, folic acid supplementation can be targeted only to fertile-age women at risk of pregnancy to prevent neural tube defects, whereas a 10-day course of oral zinc is triggered by a prior episode of diarrhea or pneumonia. Interestingly, with the exception of iron and folic acid for anemia, the outcomes are not really supporting nutritional status. Rather, they seek to prevent specific conditions: child mortality with vitamin A, birth defects with folic acid, and recurrent infections with zinc.

Periodic supplementation is restricted to a specific period of the life span (vitamin A and folic acid) or a certain specific time interval for the recipient (iron and zinc). If the dosage is correct, hazards of excessive exposure are minimal. Overdosage with a vitamin A syrup has been documented in India, and high-dose iron supplements represent a hazard for poisoning if inadvertently consumed by young children. The disadvantages include the need for a conscious behavior change and for the logistics for distribution, which can require a substantial investment of public funds. Combinations of supplementation and fortification programs occur in some settings, notably for vitamin A and folic acid, which increases the chances for excessive exposure in dual recipients.

Health and Sanitation Interventions for Prevention of Undernutrition

Nutrients are poorly absorbed, poorly retained, and underutilized in the presence of intestinal or systemic diseases caused by microbes and parasites endemic to low-income countries. Indeed, just lack of general sanitation, safe potable water, and organic and inorganic contaminants in the environment can adversely affect nutrient metabolism, even with an adequate dietary intake. These programs of immunizations, periodic deparasitizing, environmental hygiene, pure drinking water, hand washing, household ventilation, suppression of mycotoxin, etc., can lead to better absorption, retention, and utilization of nutrients in the diet, thereby preventing malnutrition.

Breast Feeding in Preventing Malnutrition

The World Health Organization recommends exclusive breast feeding for the first six months of an infant's life. This permits the exceptions of medicinal or nutrient drops and oral rehydration therapy (ORT). As a lesser option, WHO defines predominant breast feeding (PBF) as the aforementioned exceptions along with pure water, juices, and ritual fluids. Mixed feeding, which is the offering of infant formula or any semisolid or solid food, before the seventh month of age is dissuaded in early child feeding recommendations. Once complementary feeding is introduced after 6 months, partial breast feeding is to be encouraged for as long as it is mutually desirable to both elements of the dyad.

EBF, and to a lesser extent PBF, can be considered as an important – if not consummate – measure for the prevention of both undernutrition in the short term (the first half of infancy) and overnutrition during infancy and potentially throughout the course of life. Human milk has the energy density to satisfy both caloric and hydration needs as governed by the hunger and thirst of the infant. Prevention of most forms of undernutrition is virtually assured in EBF as breast milk is close to being nutritionally complete. Commercial breast-milk substitutes may – or may not – be as nutritionally complete, and bottle formulas present the risk of overfeeding and nutrient imbalance. EBF has two admitted vulnerabilities in covering infant nutrient requirements: iron and vitamin D. Iron drops are often recommended as part of EBF. The vitamin D problem is particularly notable in darkly pigmented mothers; vitamin D supplement to the mother or in drops for the infant can cover the latter situation.

Breast milk can protect infant nutrition in additional ways. As secreted from the breast, it can be hygienic as no other beverage offered to the young infant; this avoids nutrient wasting diarrheal episodes. Human milk has immunologic properties that support the resistance to pathogen of the infant. Its high lactose content and blend of oligosaccharides support a

fecal microbial flora that supports colonic health and appropriate stool consistency and regularity.

Energy balance as 'not too much, not too little,' that is, appropriate growth velocity for length and proportional weight gain, is easily achieved with EBF and PBF as the modes of early infant feeding. Although the studies are not all totally consistent, for the longer term into childhood and later life, a history of extensive breast feeding is associated with lower risk of overweight and obesity. Geographic variation in the outcomes of such studies could be related to the overall background rates of excess weight.

Management of Malnutrition

When prevention efforts have been absent or incompletely responsive to societal or individual conditions, cases of malnutrition – of deficit or excess – are destined to occur. Whether they are epidemic or sporadic determines the overall strategy and the tactical address for the deficiencies and excesses. When multiple nutrient problems are society-wide, the concepts of therapy and prevention will blur over the long term.

Principles of Nutritional Therapy

When a deficiency is detected in the clinical setting, its reversal will almost inevitably require the application of supplementary nutrients, as either nutrient supplements, fortified foods, or nutrient-rich food sources. Historically, foods were the source of therapeutic nutrients. Scurvy has been cured with citric fruits, beriberi with brewer's yeast, and the vitamin B_{12} deficiency of pernicious anemia with beef liver. Cod liver oil has been the treatment for both vitamin D-deficiency rickets and vitamin A-deficiency night blindness for decades. Insofar as pharmaceutical chemistry provides pure forms of most organic micronutrients through extraction or synthesis, fortification or supplementation interventions have emerged as the modalities of choice.

For overnutrition and nutrient overload, the first principle is to adjust the diet or identify the exposure that is introducing more nutrient into the body than can be removed at a comparable rate. In the case of micronutrients, it might involve suspending a supplementation regimen or reduce the intake of heavily fortified foods. When overnutrition relates to energy, therapy involves reduction in total caloric intake combined with regimens of enhanced physical activity that enhanced expenditure of energy. In severe cases, medicines (which block macronutrient absorption, enhance urinary glucose excretion, or accelerate oxidative metabolism) and surgery (which restricts the gastric volume) can be important adjuncts in the treatment of obesity.

Some important caveats exist regarding when to treat malnutrition, how aggressively to treat, or even when to defer intervention. At one end of the spectrum is the situation of ocular xerophthalmia in severe vitamin A deficiency. Since this poses a risk of visual impairment or blindness, multiple high doses of vitamin A are warranted immediately upon diagnosis. A specific manifestation of severe thiamine deficiency, Wernicke's encephalopathy, is also a medical emergency risking death, and it is to be treated by immediate injection of high-dose thiamine. At the other end of the spectrum are situations in which aggressive therapy is contraindicated and repletion with the nutrient in question should be graded or even deferred. It was observed that feeding high amounts of energy and protein to survivors of World War II concentration camps had a high mortality. The lesson was relearned in the instances of kwashiorkor (edematous protein–energy malnutrition) and anorexia nervosa. Acute compartment shifts in circulating volume and in cellular fluxes of potassium and phosphate can lead to fatal consequences from vascular collapse or cardiac arrhythmias. Iron supplementation in malaria-endemic areas may do more harm than good until measures against malaria transmission are assured.

Nutritional deficiencies have been somewhat arbitrarily divided into the categories of primary and secondary. The concept from the late Prof. Victor Herbet can provide guidance in this regard (Table 2). The first cause of insufficient intake constitutes the primary form, and the remaining causes generally comprise the secondary forms.

Treatment of Primary Deficiency

If a nutritional deficiency is due simply to a lack of intake or of low dietary bioavailability, therapy with a supplemental dose is indicated for rapid restoration. Primary undernutrition is most commonly the result of adverse social, economic, or environmental conditions. It is common in famine, drought, warfare, and refugee situations. Voluntary resistance, such as hunger striking, is a cause of primary deficiency. Iatrogenic causes of primary deficiency cannot be overlooked. Physicians and dieticians can recommend a dietary regimen that, when adhered to, produces deficient intakes of protein or certain micronutrients. Early in the era of intravenous (parenteral) nutrition, primary deficiencies of trace elements were produced due to the lack of recognition of their essentiality.

The pockets of chronically poor and food fads and taboos have a role in primary undernutrition in high-income countries. Without the income to purchase a quality diet, food selection may include enough food to supply needed calories but with 'empty calories' in terms of essential nutrients. Macrobiotic and fruitarian diets have been associated with protein and micronutrient deficiencies as have strict vegan regimens. Vitamins D and B_{12} and omega-3 essential fatty acids are among the commonly deficient nutrients with fads or taboos.

A plan to prevent recurrence for the patient should be developed and implemented even as acute treatment of primary undernutrition is under way. If dietary imbalance is the underlying cause of low intake, adding a rich food source is the prophylactic action of choice. Food culture, prohibitive pricing, or scarce availability may thwart any food-based

Table 2 The six possible causes of nutrient deficiency (modified after the concepts of Victor Herbert)

Reduced consumption
Impaired absorption
Increased excretion
Impaired utilization
Enhanced destruction
Increased requirements

route with natural foods. An appropriately fortified food might be the next best food-based solution, providing that the same impediments do not apply. Finally, daily or weekly supplementation with the problem nutrient(s) would be the recourse.

De facto primary undernutrition can occur in the context of adequate energy intake when the intrinsic bioavailability of a nutrient is affected. A curiosity example of this was for people who consumed high quantities of raw eggs, which contain an antinutritional protein called avidin. Avidin tightly binds biotin from the diet and could theoretically reduce biotin nutriture. A more practical concern, however, is that of the bioavailability of iron and zinc from unrefined grains, seeds, and legumes. These are the bases for the maize-based diets of Meso-America and the whole-grain wheat-flour diets of the Middle East. In absence of sufficient fat in the meals, active vitamin A cannot be converted from provitamin A carotenes. Thus, persons with plant-based diets can develop hypovitaminosis A despite the orange and green fruits and vegetables in their customary fare.

Finally, in suspected 'primary' nutrient deficiency, practitioners need to consider – and rule out – occult secondary causes to the extent that diagnostic resources permit. For example, a young woman may be anemic and have a proved deficit of bioavailable iron in her diet. However, her excessive menstrual bleeding, representing a secondary aggravating condition and a treatable condition, may be overlooked in the evaluation.

Treatment and Management of Secondary Deficiency

A primary lack of food intake can also be secondary to pathological conditions. Anorexia nervosa and bulimia, or even psychic depression or adolescents' 'fear of fatness,' are psychological origins of diminished oral intake. Hyperemesis gravidarum, the excessive nausea and vomiting of pregnancy, would also fit this category. Thereafter, the framework of Herbert (Table 2) provides guidance. Obviously, pathological conditions from the mouth through the small intestine in the alimentary tract can compromise the digestion and/or absorption of nutrients. Among these are certain intestinal parasites. Metabolic and renal conditions, as well as fever, are the primary factors that reduce retention of nutrients and lead to their excessive wastage in the urine. In certain acute and chronic intestinal diseases, however, excessive loss of nutrients can occur via the bile or intestinal secretions. Excessive destruction of vitamin C occurs in tobacco users, and poorly absorbed antibiotics can interfere with recovery of vitamin K and biotin from our own intestines. Inflammatory conditions and lead exposure can interfere with the utilization of iron to make red blood cells.

When nutritional deficiency is clearly secondary to a primary medical condition, attempts should be made to palliate or eradicate the underlying problem. Even though it is possible, with sufficiently intense supplementation, to overcome iron deficiency anemia in hookworm-infected individuals, without eradicating the parasites, treatment with antiparasitic medications and prevention of recurrence are worth pursuing as well. This can reduce the iron dosage and make the iron treatment self-limited. With a condition such as cystic fibrosis, however, supplementation with fat-soluble vitamins will not only be needed to treat the initial deficiency; maintenance therapy will be required throughout the patient's lifetime. Zinc therapy will correct deficiency symptoms in acrodermatitis enteropathica, a zinc-absorption disorder; thereafter, continued treatment will permanently suppress all clinical manifestations of the disease.

Admonitions for Nutritional Therapy

Just as fortification of staple foods can lead to excess accumulation of nutrients, as in the case of vitamin A fortification of sugar in Central America, the chronic use of nutrient supplements runs a similar risk. Correspondingly, weight-loss diets, medications, and surgery are all associated with potential adverse consequences. Monitoring the body weight and nutritional status of any patient undergoing nutritional therapy is essential, and their regimens should be continuously adjusted accordingly. In situations of primary deficiency, the goal is weaning from overt therapy to a diet-based maintenance of good nutritional health. With secondary deficiencies, the need for supplements may fluctuate with remissions or exacerbations of the primary affliction conditioning the nutritional deficiency.

Conclusions

The prevention of malnutrition should begin with a prudent, balanced, and nutritionally adequate diet; measures to protect against nutrient wasting, recurrent illnesses, and environmental stress; and a personal regimen of modest physical activity. Programs of food fortification or periodic supplementation may be required when dietary and/or environmental issues cannot be resolved. Primary deficiency states should be addressed by nutritional supplementation and the recovery consolidated by dietary and social measures. Secondary deficiencies require a judicious combination of attention to ameliorating the inciting condition and restoring nutritional status of the patient.

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Cancer: Diet in Cancer Prevention; Coenzymes and Cofactors; Colon: Diseases and Disorders; Cystic Fibrosis, Nutrition in; Diarrheal Diseases; Eating Disorders; Enteral Feeding; Famine, Hunger, and Undernourishment; HIV Disease and Nutrition; Hunger; Nutrition and Infection; Nutritional Epidemiology; Obesity: Causes and Prevalence; Obesity: Epidemiology of; Parasites in Food: Illness and Treatment; Parenteral Nutrition; Shigella; Vitamins: Overview; Zoonoses

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Manganese

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Introduction

Manganese (Mn) is a hard, silvery white metal, brittle, easy to oxidize, and difficult to fuse, with a melting point of 1244 °C. It is found in soil, water, and plants, being the 12th most abundant element in the Earth's crust. Mn is mainly mined in Australia and South Africa and is commonly used in the metallurgical industry, especially iron, aluminum, and steel production. It is also used in the oil industry as an octane enhancer and engine antiknock agent, as well as in the battery industry.

Mn is abundant in the Earth's crust and is absorbed by plants and deposited in oceans, and rivers. The highest levels of Mn in food products are found in seafood, and these levels are region-dependent. Some plants, such as nuts, pumpkins, beans, and cloves, contain relatively high levels of Mn. Mn is common in the human diet and is absorbed by the gut and distributed to all organs and metabolized and excreted predominately in the bile and feces. Intracellularly, Mn serves as a cofactor for multiple enzymes and is incorporated into numerous proteins. Excessive consumption of dietary Mn may lead to its increased accumulation, and within the central nervous system (CNS), it may target dopaminergic (DAergic) neurons.

In the general population, enteral intake of Mn is <5 mg Mn/kg (with a range of 0.9–10 mg Mn/day). Human studies defined the no observed adverse effects levels in adult subjects at 11 mg day^{-1} from food sources. The National Academy of Sciences (2001) established an adequate intake (AI) in adult men and women at 2.3 and 1.8 mg Mn/day, respectively. AIs for newborn (<6 months of age) infants are approximately 3 μg day^{-1}, while AIs increase to 600 μg day^{-1} in children 7–12 months of age. Children between 1–3 and 4–8 years of age require a daily intake of ~1.2 and 1.5 mg Mn/day, respectively. There are multiple sources of Mn in the diet as discussed later.

Manganese in Food Sources

Numerous studies have focused on nutritional contents in food products, including their metal content. Mn is found in different food products, and its consumption may be altered by dietary components. Major sources of Mn intake include seafood, grains, rice, and nuts. Meat products from pork, chicken, lamb, and beef contain small and variable amounts of Mn. Higher levels of Mn intake are more frequently encountered in vegetarian and ovo-lacto vegetarian adults. Mn content may considerably vary in relation to the vegetable species, Mn levels, and pH in the soil, to name a few.

As mentioned earlier, Mn levels are relatively high in cereals; however, in other food staples, such as soy-made tofu, seafood, and meat, Mn may be present at even higher concentrations (see Table 1). The *açaí* berry, a Brazilian fruit, can contain ~6 mg Mn/100 g. This represents a high level of Mn compared to other sources, such as cooked rice (0.46 mg/100 g). Furthermore, a popular tea consumed in the southern Brazil and surrounding countries, such as Paraguay, Argentina, and Uruguay, named 'chimarrao' (*Ilex paraguariensis*), contains Mn levels that vary between 2.3 and 1069 mg l^{-1}, dependent upon the location of the harvested plants.

Assessment of Manganese Status

A range of techniques have been used for metallic specie determination including inductively coupled plasma optical emission spectrometry, inductively coupled plasma mass spectrometry, and flame atomic absorption spectrometry (FAAS). However, the Mn levels and sample properties should dictate the choice for the appropriate method. Low-Mn-concentration issues may be suppressed by using a separation or preconcentration procedure before the detection. While FAAS is extensively used for Mn quantification in food products, this is not the best method in wine samples, given alcohol's content of ethanol, sugars, and other organic compounds.

Mn Availability, Absorption, and Metabolism

Mn Absorption

Mn absorption occurs predominantly through the pulmonary or gastrointestinal tract. Oral Mn absorption is affected by several factors, such as the quantity of carbohydrate or iron (Fe) consumed. Mn absorption by the lung occurs largely in occupational cohorts exposed to this metal, such as welders, miners, alloy, and battery production workers.

In relation to Mn absorption from food products and drinking water, according to the World Health Organization's (WHO) Environmental Health Criteria, healthy adults should consume a daily average of 3.6 mg Mn, considering a gastrointestinal absorption rate approximating 3–5%. This equates to systemic Mn absorption of approximately 180 μg Mn/day.

Mn homeostasis is governed predominantly by excretion, which is rapid and efficient. In a normal adult, oral Mn absorption is ~3–5% of an ingested dose. When dietary Mn levels are high, adaptive changes include reduced GI absorption, enhanced Mn liver metabolism, and increased biliary and pancreatic excretion of Mn. Additional factors such as Fe, phytate, ascorbic acid, and other minerals can influence Mn

Table 1 Mn content in different food products

Food product	*Amount of Mn in 100 g portion (mg)*
Mussels	6.8
Açaí berry	6.16
Hazelnuts	5.6
Pumpkin seeds	4.5
Whole-wheat bread	2.1
Bovine meat	1.5
Tofu	1.2
Lima beans	1.3
Spinach	0.9
Damascus (fruit)	21

uptake and retention. Dietary Fe ion competes with Mn, decreasing GI absorption. Conversely, low Fe levels represent a risk for increased GI Mn absorption.

Mn body distribution and elimination

The total Mn content of a 70 kg man is ~12–20 mg, and it is distributed in all tissues throughout the body (Table 2). The highest Mn concentrations are found in the liver; Mn is mostly eliminated via the bile in the feces (>90%). Very little Mn is excreted in urine and sweat.

Blood–Brain Barrier and Cellular Mn Uptake

Mn in the environment exists in various chemical forms including 11 oxidation states (Mn^{2+}, Mn^{3+}, Mn^{4+}, Mn^{6+}, Mn^{7+}, and others), salts (sulfate and gluconate), and chelates (aspartate, fumarate, and succinate). However, within the human body, Mn largely exists only in two Mn oxidation states (Mn^{2+} and Mn^{3+}).

Mn^{2+} transporters

As mentioned earlier, absorption and accumulation of Mn and Fe are mediated via shared transporters. Fe has a strong influence on Mn homeostasis. Both metals can be transported by the divalent metal transporter-1 (DMT-1) including across the blood–brain barrier (BBB). DMT-1 functions as a hydrogen ion symporter, transporting one hydrogen ion and one divalent cation in the same direction. DMT-1 belongs to a large family of metal transporters, which are responsible for the transport of other divalent metals ions, including Fe, zinc (Zn), cobalt (Co), copper (Cu), and cadmium (Cd). Deletion of the DMT-1 gene results in reduced tissue levels of both Mn and Fe. Notably, DMT-1 is widespread in the brain and highly concentrated in DAergic neurons and the nasal epithelium.

Other important Mn^{2+} carriers are the zinc transporters, ZIP-8 and ZIP-14. These transporters function as Mn-bicarbonate symporters, carrying one HCO_3^- ion and one divalent Mn cation across the membrane. Although ZIP-8 and ZIP-14 are expressed in brain capillaries, it has yet to be proved that they are functional at the BBB.

Mn^{2+} can also be carried by Mn-citrate transporters; however, the relevance of these carriers has not yet been fully elucidated. Hip14 and the product of the park9 gene are novel putative Mn transporters. The choline transporter also has high affinity for Mn^{2+} relative to the other metals (Cd and Al) and may potentially function as a Mn transporter.

The cytoplasmic Fe exporter, ferroportin (Fpn), and SLC30A10 (solute carrier family 30, member 10) function as the predominant Mn exporters.

Table 2 Mn distribution in the human organs

Human tissues	*Approximate values of Mn (mg kg^{-1} wet weight)*
Liver	2.05
Pancreas	1.18
Kidneys	0.9
Intestine	0.35
Heart	0.32
Brain	0.3
Lung	0.22

Mn^{3+} transporters

The one known transporter of trivalent Mn (Mn^{3+}) is the transferrin receptor (TfR): TfR is the major cellular receptor for Tf-bound Fe^{3+}, but since Tf can also bind trivalent Mn, TfR can also mediate its transport. This protein carries Mn^{3+} into the cell with subsequent endocytic reduction of Mn^{3+} to Mn^{2+} and ensuing transport via DMT-1 into the cytosol.

Mn Uptake in Various Cellular Organelles

Mitochondria, the Golgi apparatus, and nuclei store the majority of intracellular Mn. Mitochondria in the basal ganglia accumulate the highest Mn concentration, predominantly via the Ca^{2+} uniporter channel. Efflux of mitochondrial Mn^{2+} is mediated preferentially through an active, but slow, Na^+-independent mechanism. This slow Mn efflux accounts for the net accumulation of Mn in the mitochondria and its protracted half-life. In the Golgi apparatus, a P-type pump, referred to as SPCA1, serves as a Mn/Ca pump. GPP130, another Golgi apparatus-related protein, rapidly traffics from the Golgi apparatus to lysosomes where it is degraded. The degradation of GPP130 occurs in response to increased intra-Golgi Mn levels.

Other mechanisms that regulate Mn homeostasis and detoxification remain largely unknown. It is likely that optimal tissue Mn levels are maintained by multiple Mn transporters, both importers and exporters.

Effects of Mn on Human Health

Mn Deficiency

The requirement for Mn varies throughout the life span and based on gender. Mn levels are higher in women of childbearing age than in men and rise during pregnancy, in both the mother and the fetus. Cellular energy, immune function, digestion, reproduction, bone growth, and blood clotting require optimal Mn concentrations. Mn plays an important role as a cofactor of numerous enzymatic reactions, controlling optimal levels of amino acids, lipids, proteins, and carbohydrates. Mn is inherent to antioxidant enzymes, such as superoxide

dismutase, and it activates glycosyltransferase, an essential step in bone and connective tissue growth.

Mn deficiency, though rare, causes poor bone formation and skeletal abnormalities, compromised growth, decreased fertility and birth defects, abnormal glucose tolerance, and altered lipid and carbohydrate metabolism. Suboptimal Mn status may occur in humans with osteoporosis or exocrine pancreatic insufficiency and children with Perthes' disease or phenylketonuria. Mn deficiency has also been associated with reduction in body and liver weights, total plasma protein, high density lipoprotein (HDL) cholesterol, and apolipoprotein E protein (present in HDL). Mn deficiency also leads to decreased triglyceride synthesis, reinforcing the role of Mn in lipid, protein, and carbohydrate metabolism.

Mn Toxicity

Mn may cause adverse hematologic, endocrine, male reproductive, nephrotoxic, or hepatotoxic effects, but its predominant target is the CNS. Mn neurotoxicity has been primarily observed in occupational cohorts, where the potential for chronic exposure exists. The predominant route of exposure in occupational settings is via inhalation of Mn-laden dust derived from welding, mining, smelting, or battery manufacturing. The neurological effects of Mn result in muscle weakness, limb tremor, whispering speech, salivation, and postural changes, all of which are collectively referred as 'manganism.' These effects are commonly preceded by neuropsychiatric disturbances.

The cellular and molecular mechanism(s) underlying the neurotoxicity of Mn remains to be fully understood. Mn impacts many biological functions, dependent upon dosage, chemical form (oxidation state), routes and duration of exposure, and the sex and age of the exposed individual.

Mechanisms of Mn toxicity

Alzheimer type II astrocytosis

Astrocytes are specialized glial cell present in the CNS. These cells play many critical physiological roles, all of which are essential for optimal neuronal activity. Astrocytes have the ability to concentrate Mn to levels 50-fold higher than the surrounding media, representing the major homeostatic regulator and storage site for Mn in the brain. Such preferential accumulation renders astrocytes potentially the most vulnerable neural cell to Mn toxicity. Primate models of Mn toxicity have been associated with pathological alterations in astrocytes, known as the *Alzheimer type II astrocytosis*. Moreover, Mn accumulation in astrocytes has been shown to alter glutamate (Glu) homeostasis and to elicit excitatory neurotoxicity, as astrocytes are critical for the removal of synaptic Glu.

Mitochondrial dysfunction

Mn accumulation in mitochondria can inhibit the electron transfer chain. Such effects result in a diminished ATP production, leading to increased leakage of electrons and increased superoxide ($O_2^{\bullet -}$) production, thereby causing cellular collapse. Excessive Mn uptake in astrocytes and subsequent mitochondrial accumulation of this metal may compromise energy metabolism, augment oxidative damage, and impair astrocytic–neuronal communication, thus leading to alterations in excitatory and inhibitory control and contributing to the subsequent development of secondary Glu-mediated toxicity.

Once inside the mitochondria, Mn can inhibit the enzymatic complexes of the mitochondrial electron transport chain, notably complexes I and II. Remarkably, mitochondrial complex II is a key mediator of Mn^{2+}-induced hydrogen peroxide production. In addition, it has been shown that Mn^{2+} can bind to ATP, reducing its availability.

Mn has also been reported to alter Ca^{2+} homeostasis in the mitochondria by inhibiting its efflux, thus increasing its levels in this organelle. As a consequence, oxidative stress generated by high Mn concentrations leads to the induction of mitochondrial permeability transition (MPT), a Ca^{2+}-dependent process, characterized by the opening of the MPT pore in the inner mitochondrial membrane. Ca^{2+}-mediated MPT opening leads to the activation of the Bcl-2 family of proteins, especially Bax/Bac, culminating with the release of *cytochrome c* (*Cyt c*). *Cyt c*, via the ERK pathway, activates the cysteine protease, caspase-3, an executioner protein, which is responsible for mediating apoptosis, chromatin condensation, and DNA fragmentation.

Endoplasmic reticulum stress

Endoplasmic reticulum (ER) stress is caused as a result of the accumulation of excessive unfolded protein response, leading to apoptosis. The ER is important for the synthesis, proper folding, posttranslational modification, and transport of nascent proteins. Thus, the presence of a protein overload in the ER results in ER stress. ER stress is inherent to Mn-induced neurotoxicity. Mn induces ER stress via activation of pancreatic ER kinase RNA signaling pathway. Mn exposure induces the ER stress genes glucose-regulated protein 94 and C/EBP homologous protein and increases the phosphorylation of the eukaryotic translation initiation factor 2a. These effects are accompanied by the activation of the ER-resident enzyme caspase-12 and caspase-3. Notably, the Mn-induced responses are enhanced by Fe depletion.

Mn-induced alterations in neurotransmitter systems

DAergic system Excessive Mn accumulation may cause symptoms similar to those observed in Parkinson's disease; thus, special attention has been directed to its effects on the DAergic system. Chronic exposure to Mn is associated with nigrostriatal DAergic neurodegeneration. Nevertheless, the effect of Mn on striatal dopamine (DA) levels remains controversial.

Both Mn^{2+} and Mn^{3+} react with DA via the Fenton reaction, catalyzing its autooxidation and generating reactive species, thereby contributing to oxidative damage. However, it is noteworthy that neither Mn^{2+} nor Mn^{3+} can directly generate hydroxyl radicals from hydrogen peroxide and/or superoxide via the Fenton-type or Haber–Weiss-type reactions. Hence, Mn-induced DA autooxidation is a complex process involving several steps in which semiquinone and aminochrome, L-cysteine, or copper (Cu) and NAD(P)H are implicated. Possible mechanisms underlying semiquinone- and aminochrome-induced damage in Mn-induced neurodegenerative process may therefore include NADH or NADPH depletion, inactivation of enzymes by oxidizing thiol groups or

essential amino acids, formation of reactive oxygen species, and lipid peroxidation.

Effects of Mn on glutamatergic system Mn accumulation produces symptoms consistent with an excitotoxic mechanism. Glu is the most abundant excitatory neurotransmitter, and it plays several major roles in normal brain function. Glu excitotoxicity is associated with an excessive amount of Glu in the extracellular space, which can lead to a cascade of downstream degenerative processes. Excess Glu is removed by cellular uptake, which is mediated by Glu transport proteins, such as glutamate–aspartate transporter (GLAST) and the glutamate 1 transporter (GLT1); both are particularly abundant in astrocytes.

Mn exposure can decrease the ability of astrocytes to remove synaptic Glu, thereby increasing its excitotoxicity. Decrease in GLAST and GLT1 expression and activity has been documented in Mn-exposed astrocytes. In addition, the distribution of Glu is highly sensitive to changes in energy supply. Thus, mitochondrial dysfunction must be considered as a key mechanism in mediating Mn-induced Glu dyshomeostasis. Furthermore, Mn-induced reactive oxygen species generation may directly inhibit Glu uptake, effectively increasing extracellular Glu concentrations.

Effects of Mn on the GABAergic system GABA (γ-aminobutyric acid) is the main inhibitory neurotransmitter. Previous data have been pointed that the GABAergic system is affected by exposure to Mn, and, there is a temporal factor that contributes to these effects. Mn augments GABA content in the caudate nucleus and increases its release. In cell culture models, striatal GABAergic neurons are more vulnerable to Mn relative to mesencephalic DAergic neurons. Other studies have shown decreased pallidal GABA concentration in response to Mn exposure. It has yet to be determined which is the primary effect of Mn on GABAergic systems.

Effects of Mn on the cholinergic system The cholinergic system, which is involved in emotions, cognition, and/or locomotion, consists of acetylcholine (Ach)-secreting neurons and associated glia. Neurotoxicants targeting the cholinergic system lead to excessive extracellular Ach levels due to hyperproduction or limited degradation, consequently leading to hyperresponsiveness to Ach, oxidative stress, and cell death.

A number of cholinergic synaptic mechanisms are putative targets for Mn activity, such as presynaptic choline uptake, quantal release of Ach into the synaptic cleft, postsynaptic binding of Ach to receptors, and its synaptic degradation by acetylcholinesterase. Moreover, Mn influences astrocytic choline transport and astrocytic Ach-binding proteins. Thus, the symptoms associated with the early psychotic phase of manganism, also called 'manganese madness,' may be attributed to an impairment of the septohippocampal cholinergic system, which is involved in both the physiological and the behavioral responses to stress.

Conclusions

The ubiquitous presence of Mn in food products and drinking water assures its optimal absorption and function in regulating cellular metabolism and homeostasis. Mn is important for optimal development, growth, and antioxidant system and energetic metabolic pathways. However, the intake of this essential metal must be tightly regulated, since high levels of intracellular Mn may cause mitochondrial dysfunction, lipid peroxidation, and alterations in neurotransmitter systems, just to name a few.

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See also: Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Bioavailability of Nutrients; Iron: Physiology of Iron; Nuts: Health Effects; Parenteral Nutrition.

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Mangoes

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Introduction

Mango (*Mangifera indica* L.) is one of the most relished and important tropical fruits consumed in fresh or processed form globally. Mango products such as canned slices, puree, nectar, pickles, and chutney experienced worldwide popularity and also gained an increasing importance in US and European markets. Mango has been listed at the top of the super fruit list because of their health-promoting properties. India is the world's largest mango producer, followed by China, Thailand, Indonesia, Pakistan, Mexico, and Brazil. Commercial processing of mango into juice, nectar, pulp, puree, fruit leather, and jam produces 35–60% waste consisting of peel, kernel, and cull fruit. Most mangoes are consumed uncooked as a dessert fruit, the rest of them being processed into various products, such as nectar, juice powder, dried mangoes, canned mango slices in syrup, chutneys, and pickles, among others. One of its most processed forms is pulp, which is further used for manufacturing finished products such as juice, nectar, fruit drink, jam, and squash.

There are over one thousand different cultivars of mangoes growing worldwide, and mangoes are considered to be one of the most widely eaten fruits. Mango is known to be an excellent source of nutrients and vitamins such as ascorbic acid, thiamine, riboflavin, niacin, and ß-carotene. Mangoes are also high in phytochemicals. Epidemiological evidences suggest that a diet rich in fruits and vegetables provide health benefits by preventing the development of chronic diseases such as cardiovascular diseases and cancer. These health beneficial effects are due to the presence of various bioactive compounds that are present in mango fruits, and these compounds are shown to exhibit antioxidant, antiallergenic, antiatherogenic, anti-inflammatory, antimicrobial, and antithrombotic effects. Several studies have reported that *Mangifera* species are rich in dietary fiber and phytochemicals in all their parts, which play an important role as antioxidants.

Mango as a Fruit

The common English term 'mango' and the botanical name '*Mangifera indica* L.' originate from the ancient Tamil name 'manga.' The *Mangifera indica* belongs to the family Anacardiaceae that consists of 64 genera, mostly of trees and shrubs, some of which are poisonous. Mango is one of the oldest tropical fruits and has been cultivated for over 4000 years, originating apparently in the Indo-Burma region. Mango fruit is one of the most popular, nutritionally rich fruits with unique flavor, fragrance, taste, and health-promoting qualities, often labeled as a 'super fruit.' Mango is a tree cultivated in many tropical regions, and now, its farming is distributed widely across the world in many continents.

Mango comes in different shapes and sizes depending upon cultivar types. Internally, its flesh is juicy and orange–yellow in color with numerous soft fibrils radiating from its centrally placed nearly flat, oval-shaped stone (enveloping a single large kidney-shaped seed). Its flavor is pleasant and rich, and it tastes sweet with mild tartness. Mango fruit is a large fleshy drupe containing edible mesocarp of varying thickness. It varies in size, shape, color, and flavor. The exocarp (peel) is thick and glandular. The mesocarp (pulp) can be fibrous or fiber-free with flavor ranging from turpentine to sweet. The mesocarp provides edible pulp, which is firm containing a sweet, well-flavored juice. The endocarp is woody, thick, and fibrous, forming a thick, tough, leathery glandular covering of the seed, termed as husk. The seed is exalbuminous. It is solitary, large and flat, and ovoid oblong and is surrounded by the endocarp at maturity. The testa is thin and papery. The outer skin is smooth and is green in unripe mangoes but turns into golden yellow, crimson red, yellow, or orange–red when ripened depending upon the cultivar type.

There are around a thousand varieties of mango available all over the world, which includes Ataulfo, Haden, Keitt, Kent, Edward, Palmer, Manila, Kesar, and many more exotic delicacies. Apart from numerous seedling varieties, more than a thousand vegetatively propagated mango cultivars have been reported. Most of these have originated as chance seedlings selected earlier and further maintained asexually. Majority of these cultivars is of Indian origin, and representation from other parts of the world is limited in number.

Mango: Production Scenario

Mango is one of the most important tropical fruit crops in the world and is ranked fifth in production among major fruit crops. Mangoes are produced in over 90 countries worldwide. FAO estimates that the mango harvest will be around 28 million tons in 2014, that is, 35% of the production of the world's tropical fruit. Asia accounts for approximately 77% of global mango production, and the Americas and Africa account for approximately 13% and 9%, respectively. Mango has a long history of cultivation, and its cultivation has recently expanded to countries in temperate zones by use of protected cultivation. Most of the major mango-producing countries like India, China, Thailand, Indonesia, Pakistan, the Philippines, and Bangladesh are located in Asia; however, the cultivation has spread to countries such as Mexico, Brazil, Madagascar, Tanzania, Dominican Republic, Haiti, Brazil, and Australia.

Currently, only 3% of the world production of mango is traded globally. Trade centers for mango in the world can be divided into three regional areas: Asia, which trades mainly with the Middle East; Africa, which exports to the European market; and Latin America, which supplies to the American and European markets. The two major import markets are the United States and Europe. The mango market has grown considerably over the last 30 years. Latin American countries have made the most of this dynamism by increasing their supply. In

the last 10 years, it has grown in volume by 38% in the United States and doubled in Europe. The highest levels of consumption are in Asia, followed by Latin America, Africa, and Australia. In Asia, unripe mangoes are especially popular, while in the West, ripe mangoes are mainly eaten. The United States and the European Union have increased their import share.

The most popular export mango cultivars are Kent, Tommy Atkins, Haden, and Keitt, which have fruits with a red blush and that are fiberless, firmer, and more suited for long-distance transportation than other types of cultivars. The green cultivars, such as Ataulfo and Amélie, are now widely accepted in the international market. Other cultivars gaining popularity in the international market are Alphonso, Dudhpeda, Kesar, Sindhu, Pairi, Desi, Chaunsa, Langra, and Katchamita. There is also evidence that the processed mango fruit market is increasing. Processed fruit products include mango juice, pickled mangoes, mango chutney, mango pulp, mango paste, mango puree, dried mango fruit, mango slices in brine, and mango flour. India is the main exporter of processed mangoes, followed by Pakistan, Brazil, and Zimbabwe. Major importers include the United Arab Emirates, Saudi Arabia, Kuwait, the United States, the United Kingdom, and Canada.

Mango: Consumption Pattern

Mango is an important component of the diet in many less developed countries in the subtropics and tropics. Mango fruit is valued because of its excellent flavor, attractive fragrance, delicious taste, and health-giving properties. Before 1970, mangoes were not famous to consumers outside the tropics, and the trade involving fresh fruit was nonexistent. In the subsequent years, a rapid expansion of mango production into nontraditional areas resulted in the mango trade becoming well established. Mango industry has provided livelihood opportunities to its growers and those involved in its marketing channel. Creation of essential infrastructure for preservation, cold storage, refrigerated transportation, rapid transit, grading, processing, packaging, and quality control is the important aspect, which needs more attention. The international market is becoming more competitive, and other mango-producing countries are also entering the market.

As mango is a seasonal fruit, mango fruit is processed and used at almost every stage of its growth. The range of products includes products derived from both raw and ripe fruit. Mango and mango products are experiencing worldwide popularity and also gained increasing importance in the market. Therefore, processing and product development are an important factor in mango marketing. Mangoes are processed at two stages of maturity. Green fruit is used to make chutney, pickles, curries, and dehydrated products. Ripe mangoes are processed as canned and frozen slices, puree, juices, nectar, and various dried products. Mango juice, nectar, and squash are some of the important beverages prepared on a commercial scale. The pulp was extracted from fully ripe fruits, canned and used for preparing nectars and juice. Mango-based carbonated beverages and wines are also available in the market. Production of fruit juice powder has important role in food processing industry in recent years because of several advantages. Dried mango powder is a good adjunct in ice cream, bakery, and confectionery industry. The pulp of the sour and seedling varieties is traditionally sun-dried for use as flavoring ingredients especially in Indian food preparations.

The mango processing industry faces many problems related with its industrialization and market expansion. The high number of varieties with their various attributes affects the quality and uniformity of processed products. A lack of simple, reliable methods for determining the stage of maturity of varieties for processing also affects the quality of the finished products. A significant problem in developing mechanized equipment is the large number of varieties available and their different sizes and shapes. There is, however, a considerable export potential to developed countries, but in these countries, the processed mango products must compete with the market for established processed fruits.

Nutritional Value of Mango

The mango fruit is an important part of human nutrition in several parts of the world. Mango is used as food in all stages of its development. During the ripening process, the fruit is initially acidic, astringent, and rich in ascorbic acid. Mango fruit contains amino acids, carbohydrates, fatty acids, minerals, organic acids, proteins, and vitamins (Table 1). The fruit flesh of ripe mango contains about 15% sugar. Sugars present in mango comprise sucrose, glucose, fructose, and maltose. Sucrose is the principal sugar in ripe mango. Sucrose content of ripe fruit of three important cultivars, namely, Alphonso, Pairie, and Totapuri, ranges from 11% to 20%. Free sugars including glucose, fructose, and sucrose generally increase during ripening. Mango fruit contains 0.5–1.0% protein on a fresh weight basis. The presence of amino acids including the essential ones such as lysine, leucine, cysteine, valine, arginine, phenylalanine, and methionine in fruits of various cultivars has been recorded. Total lipid content in seven commercial mango cultivars ranged from 0.26% to 0.67% at harvest.

Mango fruit is particularly a rich source of vitamin C. Raw mango contains more vitamin C than that of ripe or fully ripe mango. Literature reports indicate great variation in ascorbic acid contents, ranging from 9.79 to 186 mg per 100 g of mango flesh. Daily consumption of mango fruits by population groups of all life stages can meet the recommended dietary

Table 1 Food value of mango (100 g fresh fruit)

Component	*Content*
Calories	62.10–63.70
Moisture	78.90–82.80 g
Protein	0.36–0.40 g
Fat	0.30–0.53 g
Carbohydrates	16.20–17.18 g
Fiber	0.85–1.06 g
Ash	0.34–0.52 g
Calcium	6.10–12.80 g
Phosphorus	5.50–17.90 g
Iron	0.20–0.63 g

Source: Morton, J. (1987). Mango. In: Julia F. Morton (eds.) *Fruits of warm climates*. Miami, FL: Florida Flair Books, pp. 221–239.

requirements of vitamins A and C. Mangoes differ in their ascorbic acid content because of genotype variations, climatic factors, agricultural practices, and ripening stage.

Fruit acidity is mainly due to the presence of malic and citric acids. The presence of folic acid (3.6 mg/100 g), vitamin B1 (35–63 mg/100 g), and vitamin B2 (37–73 mg/100 g) in mango has been reported. Mango also contains the B vitamins thiamine and riboflavin (0.08 mg) and niacin (0.09 mg) per 100 g of ripe mango. The vitamin A equivalent in mango ranges between 1000 and 6000 IU. β-Carotene was found to account for 60% of total carotenoids in mango. It was found that mevalonic acid, a precursor of carotenoids, increased progressively during the ripening of mangoes. Mango consumption is very important for some populations in tropical regions, where the deficiency of vitamin A constitutes a public health problem. A study using an *in vitro* model indicated that there are varietal differences in the content and bioavailability of β-carotene from mango. Ash content decreased during fruit development with some increase near maturity, while crude fiber remained constant. Studies on mango flesh showed that a high proportion of the fiber fraction consists of pectin, and its content in the peel is also quite high. The US Department of Agriculture (USDA) Nutrient Database indicates α-tocopherol content of 1.12 mg/100 g. Alpha-tocopherol is the only detectable tocopherol in seven mango cultivars examined. 'Haden' and 'Tommy Atkins' mangoes had the highest amounts (380 and 470 μg/100 g, respectively), with 200–250 μg/l00 g in the other cultivars.

Mango is known for its medicinal properties at both raw and ripe stages. The unripe fruit is astringent and a stimulant tonic. The acid content in the green mango increases the secretion of bile and has a role in intestinal absorption. Ripe mangoes are highly beneficial in the treatment of night blindness. Ripe mango is antiscorbutic, diuretic, laxative, and mildly astringent. In Ayurvedic system, mango is considered to increase seven body 'elements' called *dhatus*, namely, food, juice, blood, flesh, fat, bone marrow, and semen.

Bioactive Compounds in Mango and Its Health Benefits

Mangoes are found to be rich in bioactive compounds such as polyphenolics, carotenoids, vitamins, and dietary fibers and have many of the pharmacological properties attributed due to the presence of these bioactive compounds in them. The presence of gallic acid, *m*-digallic acid, *m*-trigallic acid, gallotannin, quercetin, isoquercetin, mangiferin, and ellagic acid was reported in mango fruit. Digallic acid seemed to undergo a small degree of polymerization, whereas quercetin and isoquercetin disappeared during ripening. Another type of polyphenols found in mango fruit is alkylresorcinols, which are predominantly found in unripe fruit and decrease in quantity during ripening. Reports suggest that the phytochemical content of mango pulp consists of gallic acid, mangiferin, quercetin glycosides, and many identified and uncharacterized hydrolyzable tannins. Other compounds in smaller concentrations include *p*-OH-benzoic acid, *m*-coumaric acid, *p*-coumaric acid, and ferulic acid. In most mango varieties, free gallic acid, 3,4,5-trihydroxybenzoic acid, is the predominant compound present and has been shown to possess a high antioxidant capacity with numerous implications to overall human health.

The major polyphenols in mango in terms of antioxidative capacity and/or quantity are mangiferin, catechins, quercetin, kaempferol, rhamnetin, anthocyanins, gallic and ellagic acids, propyl and methyl gallate, benzoic acid, and protocatechuic acid. Nutraceutical and pharmaceutical significance of mangiferin, which is a special polyphenol in mango, has been extensively demonstrated and continues to attract much attention especially in its potential to combat degenerative diseases such as cardiovascular diseases and cancer. The amount of the different polyphenolic compounds in mango varies from part to part. Mango polyphenols acts mainly as antioxidants, preventing DNA damage and many degenerative diseases. Bioactive compounds found in mangoes, just as in other plants and herbs, have been shown to have possible health benefits with antioxidative, anticarcinogenic, antiatherosclerotic, antimutagenic, and angiogenesis-inhibitory activities.

Polyphenolic Compounds

Polyphenols are a large family of natural compounds that are secondary metabolites and are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plant. Phenolic compounds exhibit a wide range of beneficial properties to health, such as antiallergenic, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, cardioprotective, and vasodilatory effects. The main classes of polyphenols are defined according to the nature of their carbon skeleton, and they are phenolic acids, flavonoids, stilbenes, and lignans.

Mangiferin, gallic acids (*m*-digallic and *m*-trigallic acids), gallotannins, quercetin, isoquercetin, ellagic acid, and β-glucogallin are among the polyphenolic compounds already identified in the mango pulp. Gallic acid (6.9 mg kg^{-1}) and mangiferin (4.4 mg kg^{-1}) have been identified as the major polyphenol present in mango fruits and pulp, followed by *p*-OH-benzoic acid, *m*-coumaric acid, *p*-coumaric acid, and ferulic acid. In a polyphenol screening of 20 mango varieties, gallotannins represent the major components of unripe fruits and seeds. Gallic acid is the substrate of polyphenol oxidase in the fruit pulp, whereas ellagic acid is the predominant substrate in mango peel.

Mangiferin (C 2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone) is a xanthone, and xanthones are some of the most potent antioxidants known and sometimes unofficially referred to as 'super antioxidants.' Mangiferin, generally called C-glucosyl xanthone, is widely distributed in higher plants, and it is a pharmacologically active phytochemical and a natural polyphenolic antioxidant present in the bark, fruits, roots, and leaves of *M. indica* Linn. It has been found to exhibit a wide range of pharmacological effects including antioxidant, anticancer, antimicrobial, antiatherosclerotic, antiallergenic, anti-inflammatory, analgesic, and immunomodulatory, among many others. Mangiferin also inhibited body weight gain in experimental rats and can be used in food products for special dietary needs for obese people. Mangiferin protects erythrocytes and RBCs from reactive oxygen species production, thus contributing to integrity and functionality of these cells. It was reported that mangiferin inhibits the glucosidase enzymes sucrase, isomaltase, and maltase from rats, which are involved in the digestion of carbohydrates into simple sugars in the gut leading to delay or inhibition of carbohydrate breakdown and

subsequent slower glucose absorption from the intestine. Mangiferin was found to significantly reduce plasma total cholesterol, triglycerides, and LDL-C associated with a concomitant increase in HDL-C levels and a decrease in atherogenic index in diabetic rats indicating its potential antihyperlipidemic and antiatherogenic activity.

Flavonoids can be divided into several classes according to the degree of oxidation and substitution of the benzopyran ring, including flavones, flavanones, flavonols, flavanols, isoflavones, anthocyanins, and proanthocyanidins. Mangoes are a rich source of flavonoids and xanthones such as mangiferin, catechins, quercetin, kaempferol, rhamnetin, flavonol, and xanthone. Quercetin and isoquercetin are reported in mango pulp, and quercetin, kaempferol, rhamnetin, mangiferin, mangiferin gallate, isomangiferin, and isomangiferin gallate can be found in peels. Other flavonoids found in mango include fisetin and astragalin (kaempferol-3-glucoside). The skin of mango variety Haden is reported to contain peonidin-3-galactoside. Catechins is a group of flavonoid including (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, and (+)-gallocatechin. Several epidemiological and *in vitro* studies suggest that catechins have beneficial effects on human health due to their free radical-scavenging and antioxidant activities. The major flavonoids identified in mangoes are quercetin and related glycosides in mango pulps, with the predominant flavonol glycoside, namely, quercetin 3-galactoside (22.1 mg kg^{-1}), followed by quercetin 3-glucoside (16.0 mg kg^{-1}), quercetin 3-arabinoside (5.0 mg kg^{-1}), and quercetin aglycone (3.5 mg kg^{-1}). Peels of the cultivar Tommy Atkins contained a large number of flavonol O-glycosides and xanthone C-glycosides including isomangiferin, mangiferin gallate, isomangiferin gallate, quercetin 3-O-diglycoside, quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-xyloside, quercetin 3-O-arabinopyranoside, quercetin 3-O-arabinofuranoside, quercetin 3-O-rhamnoside, kaempferol 3-O-glucoside, rhamnetin 3-O-galactoside, rhamnetin 3-O-glucoside, quercetin (aglycone), mangiferin, isomangiferin, mangiferin gallate, and isomangiferin gallate.

A wide range of biological activities, including antibacterial, antithrombotic, vasodilatory, anti-inflammatory, and anticarcinogenic effects mediated by different mechanisms, are associated with flavonoids. Studies have shown biological effects of quercetin on inhibition of protein kinases, DNA topoisomerases, and regulation of gene expression. There is consistent evidence from these studies that quercetin may reduce the risk of cancer. Quercetin may also provide a promising approach for treatment of Alzheimer's disease and other oxidative stress-related neurodegenerative diseases. A population study showed that higher intakes of kaempferol tended to lower ischemic heart disease mortality and the incidence of cerebrovascular disease.

Phenolic acids such as caffeic acid and gallic acid are the most well-absorbed polyphenolics, followed by catechins, flavanones, and quercetin glucosides. Mango gallotannins most likely are not absorbed in the small intestine and might reach the colon where they are transformed into low-molecular-weight galloyl glycosides derivatives by bacterial action. Polyphenolics such as the proanthocyanidins, the galloylated tea catechins, and the anthocyanins are not absorbed well in the GIT. These polyphenolics are extensively metabolized by the colonic microflora into a wide array of low-molecular-weight polyphenolic breakdown products.

Carotenoids in Mango

The mango fruit is a rich source of carotenoids, and some of them are identified as β-carotene (all-*trans*), β-cryptoxthanin (all-*trans* and *cis*), zeaxanthin (all-*trans*), luteoxanthin isomers, violaxanthin (all-*trans* and *cis*), and neoxanthin (all-*trans* and *cis*). Carotenoids are responsible for the yellow–orange color of mango mesocarp. Beta-carotene is generally the predominant carotenoid in mango pulps, comprising 48–84% of the total carotenoids. During mango fruit ripening, carotenoid levels increase, and ripe fruits are tenfold richer in carotene than partially ripe fruit, while unripe green mangoes contain only trace amounts. Mevalonic acid, a precursor of carotenoids, increases progressively during mango ripening. Variation in carotenoid content in mango is mainly due to several factors such as cultivar, geography, climate, storage/processing conditions, and analytic procedures employed.

The absorption of these carotenoids is influenced by many factors. In the case of carotenoids, they are absorbed at the level of the mucous of the duodenum by the mechanism of passive diffusion. The principal factor limiting the bioavailability of carotenoids in foods is its liberation from the food matrices where they are enclosed and dissolved in lipid droplets. In fruits, carotenoids are found in the chromoplast, hence limiting their liberation during digestion. It is better to transform the fruits (juice or fresh slices) before consumption. The quantity and quality of food lipids constitute a primordial factor affecting bioavailability of carotenoids. Transportation of carotenoids and their products of hydrolysis from the intestines to the blood vessels and liver is assured by lipoproteins rich in triglycerides.

Conclusions

Mango is a fruit with high nutritional value providing human diet with calories, fiber, vitamins, and minerals. Mangoes could also serve as a rich source of bioactive compounds such as polyphenols and carotenoids (Table 2). Bioactive compounds present in fruits and vegetables are receiving increased attention due to their health-promoting benefits including protection against cardiovascular diseases and cancer. The major polyphenols in the mango in terms of antioxidative capacity and/or quantity are mangiferin, catechins, quercetin, kaempferol, rhamnetin, anthocyanins, gallic and ellagic acids, propyl and methyl gallate, benzoic acid, and protocatechuic acid. The nutraceutical and pharmaceutical significance of mangiferin, which is a special polyphenol in the mango, has been drawing much attention especially in its potential to combat degenerative diseases such as heart diseases and cancer. The amounts of the different polyphenolic compounds in mango vary from part to part (pulp, peel, seed, bark, leaf, and flower) with most polyphenols being found in all the parts. Being a very popular plant, especially within the tropics and owing to its uniqueness in taste and flavor, mango could be a readily available source of dietary polyphenols with great antioxidative potential that will help to manage degenerative diseases such as cancer, atherosclerosis, diabetes, and obesity.

Table 2 Bioactive compounds in mango

Bioactive compounds in mango
Phenolic acids
Gallic acid (*m*-digallic and *m*-trigallic acids), benzoic acid, 3,4-dihydroxybenzoic acid, *p*-OH-benzoic acid, *m*-coumaric acid, *p*-coumaric acid, ferulic acid, ellagic acid
Flavanoids
Mangiferin, mangiferin gallate, isomangiferin, isomangiferin gallate
Quercetin, isoquercetin, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-arabinoside, quercetin aglycone, quercetin 3-O-diglycoside, quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-xyloside, quercetin 3-O-arabinopyranoside, quercetin 3-O-arabinofuranoside, quercetin 3-O-rhamnoside
Kaempferol, kaempferol-3-glucoside
Rhamnetin, rhamnetin 3-O-galactoside, rhamnetin 3-O-glucoside
Catechin, epicatechin
Carotenoids
β-Carotene (all-*trans*), β-cryptoxthanin (all-*trans* and *cis*), zeaxanthin (all-*trans*), luteoxanthin isomers, Violaxanthin (all-*trans* and *cis*), neoxanthin (all-*trans* and *cis*)

See also: Cancer: Diet in Cancer Prevention; Fruits of Tropical Climates: Biodiversity and Dietary Importance.

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Margarine: Composition and Analysis

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Introduction

Since its development as a butter substitute by the end of the nineteenth century, margarine has become a highly versatile product due to the advances in technology and the great variation in composition that it can exhibit. Margarine manufacture technology enables the production of very varied products that can satisfy the needs and requirements of different segments of the population. This has contributed to an increase in margarine consumer demand over time. Thus, in 2012, the world production of margarine amounted to 9.4 million tons.

Definition and Types of Margarines

Strictly speaking, margarine is a water-in-oil (W/O) emulsion derived from vegetable and/or animal fats, with a fat content of between 80% and 90% and a milk fat content of no more than 3%. The emulsion remains solid at 20 °C. Other W/O emulsions with a fat content of 39–41% may be designated as minarine or halvarine, according to the Codex Stan 256-2007. In addition to these two kinds of emulsions, there are others with fat contents outside the limits mentioned, which could be described as fat spreads. In spite of these clear differences in relation to the fat content of the different kinds of products and in order to simplify matters, this article will use the term 'margarine' in a general way, regardless of fat content. Table 1 shows the different kinds of margarines and related products and their denominations according to their fat content. Moreover, depending on their composition and texture, they have been described as hard, soft, and semisoft margarines.

Margarine Manufacture Process

Margarine manufacture involves the blend of a lipidic and an aqueous phase in order to obtain a W/O emulsion with a fat percentage of at least 80%. Apart from 80% of fat, the rest is usually skim milk, whey, or a water solution containing salt and flavors.

Preparation of the fatty raw material

One of the main requirements of the margarine lipidic fraction is that it provides an appropriate texture to the final product; this will depend both on the fat composition and on its physical structure. The type of fats used for margarine manufacture can be very varied, including vegetable oils with different unsaturation degrees, marine oils, solid fats of either vegetable or animal origin, and their mixtures. Some of these fats are usually subjected to specific treatments aimed at achieving the desired characteristics in the final product.

In the beginning, partial hydrogenation of liquid vegetable oils was the technology used for margarine production. This process, which involves the addition of hydrogen gas in the presence of a suitable catalyst, raises the melting point of the oil by saturation of double bonds, providing a firmer consistency. However, partial hydrogenation can produce *trans* isomers of fatty acids (FAs), which have been related to harmful effects on human health. In an attempt to reduce the levels of *trans* isomers in margarine, other strategies and technologies, such as the introduction of some modifications in the hydrogenation process, have been developed. These include hydrogenation in critical fluids, pressure-controlled hydrogenation, the use of novel catalysts, and conditions known to suppress *trans* FA formation including low temperatures, high pressures, and increased catalyst loadings. Even so, it could be said that the most widely used technology to avoid the occurrence of *trans* isomers in margarine is interesterification, which results in an exchange of acyl groups in the glycerol backbone either of the same triglyceride (TG) or of different TGs. This does not change the unsaturation degree of the fat but causes a modification in the fat physical properties that enables the obtention of fat with suitable melting point and crystallization behavior. The reaction can be catalyzed by sodium methoxide (chemical interesterification) or by lipases (enzymatic interesterification), each process having specific technological conditions. Carrying out this type of reactions usually requires the use of fully hydrogenated oil or of another type of solid fat such as palm stearin together with an unsaturated liquid oil.

Another possibility to produce the lipid-based stock for margarine manufacture is to blend different oils (hydrogenated or not), each with specific melting characteristics. However, it is sometimes necessary to include in the mixture harder components, like stearin.

Description of the process

Margarine production includes the following steps:

- Blending of the suitable fats with other oil-soluble components such as emulsifiers (lecithin, mono- and diglycerides, or a combination of them), oil-soluble vitamins, and colorants (β-carotene and annatto).
- Preparation of the aqueous fraction by mixing different water-soluble components. These include milk or water with salt and flavors such as diacetyl or starter distillate.
- Mixing of both the lipidic and the aqueous phases to form the W/O emulsion at temperatures around 50–60 °C.
- Pasteurization of the emulsion at temperatures around 70–86 °C.
- Chilling to 15–25 °C in a scraped-surface heat exchanger while undergoing vigorous agitation and kneading (plasticizing). This makes an extremely fine dispersion of the water phase in the fat phase possible.
- Solidification of the margarine before extrusion to the packaging line.

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Table 1 Kinds of margarines and related products according to their fat content (Council Regulation (EC) No. 1234/2007)

Denomination	*Fat content (%)*
Margarine	≥80 to <90
Fat spreads X %	>62 to <80
Three-quarter fat margarine	≥60 to ≤62
Fat spreads X %	>41 to <60
Half-fat margarine[a]	≥39 to ≤41
Fat spreads X %	<39

[a]Corresponding to minarine or halvarine in Codex Stan 256-2007.

Margarine Composition

Factors Influencing Margarine Composition

Two main factors influence margarine composition: the raw material from which it comes from and the conditions under which its processing takes place.

The raw material can vary a great deal. Vegetable oils such as soybean, sunflower, canola, palm kernel, palm, corn, cottonseed, safflower, coconut, and olive, among others, have been used for margarine production. They can be either totally or partially hydrogenated or not hydrogenated at all. This wide range of raw materials is one of the reasons for the great variability observed in margarine composition.

The processing method in margarine manufacture has a decisive influence on its composition, especially on its unsaturation degree and on its content in unsaturated acyl groups with *trans* configuration. However, when the processing involves enzymatic interesterification instead of partial hydrogenation, the *trans* isomer content is considerably reduced and may even reach zero.

Margarine Components

Triglycerides

These are the main margarine components. The nature of their acyl groups and the position of these in the glyceryl backbone define the properties and physical characteristics of margarine, such as melting and crystallization temperatures, which in turn affect its textural properties. TG composition also influences margarine behavior during processing and storage, as well as its effects on nutrition and health.

Many different acyl groups have been found in margarine TG, with a chain length usually ranging from eight to twenty four carbon atoms and with different unsaturation degrees. Among these, *cis* isomers predominate over *trans* isomers while acyl groups having an even number of carbon atoms predominate over odd chain acyl groups.

Over the years, different compositional parameters have been described as being important for margarine quality and safety. Factors taken into account have included polyunsaturated (P), linoleic (L), saturated (S), and *trans* (*t*) acyl groups content, as well as such ratios as P/S, P/(S+*t*), L/*t*, and omega-6/omega-3 ($\omega-6/\omega-3$). The values considered optimal for each one of these parameters have changed over time, as knowledge of their importance has advanced.

Table 2 shows the ranges in percentage by weight of each one of the FAs in different studies. A wide range of acyl group compositions in margarines can be observed from this table. This reflects the variety of fat sources and processing methods used for their production.

Palmitic acyl groups (C16) are the most abundant of the S groups, whereas the major monounsaturated (M) groups are those with 18 carbon atoms. Of the P acyl groups, the diunsaturated are found in the highest proportions, with triunsaturated acyl group content being much lower. The widest diversity can be found in the S group, with acyl group chain lengths between 14 and 22 carbon atoms. In addition, acyl groups with fewer than 14 carbon atoms and with 24 carbon atoms have also been found in some margarines. The presence of long-chain acyl groups has been related to the use of fish oils for margarine production.

As Table 2 shows, most *trans* unsaturation is found in the monoene fraction, especially in octadecenoic acyl groups. In general, higher *trans* contents have been reported for hard-type margarines than for soft margarines. Broadly speaking, a decrease in the *trans* content of margarine has been observed over the years.

Minor margarine components

Apart from TG, other minor components can be found in margarine. Some of them are naturally present in the fats used for margarine production but their concentrations can get bigger by external addition. Others can be exclusively added during manufacture. The Codex Stan 256-2007 establishes which type of substances can be added and their maximum use level. Vitamins A, D, and E constitute an exception, since these are regulated by national legislations. The most common are the following:

- Mono- and diglycerides

  These components can be present in margarine derived either from the vegetable oils used in its manufacture or as a consequence of hydrolytic reactions during processing and/or storage. They can also be added to margarine due to their emulsifying properties.
- Free fatty acids (FFAs)

  Although there is very little information about the occurrence of FFA in margarine, mean values below 0.5% have been found in margarines prepared both from hydrogenated fats and from fractionated or interesterified fats. These values are considered comparable to those usually found in refined vegetable oils.
- Carotenes and vitamin A (retinol)

  Both carotenes and vitamin A are present in many of the fats used in margarine manufacture. Moreover, in some cases, they are added for their coloring, antioxidant, nutritional, and healthy properties. Vitamin A is usually added as palmitate or acetate esters.

  Although there is little information about carotene and/or vitamin A content of margarine, concentrations of carotene ranging from 0.1 to 1.3 mg $100\,g^{-1}$ have been reported. In margarines declared as being colored with β-carotene or carotene, the concentration of total carotenes ranges between 0.3 and 0.9 mg $100\,g^{-1}$. However, this value can decrease to 0.2 mg $100\,g^{-1}$ in margarines colored with carrot extract and in those with no colorant declared.

Table 2 Ranges, in percentage in weight of each one of the several kinds of fatty acids, found in margarine in different studies

	S (%)					*M (%)*			*P (%)*		trans *(%)*	
Country	*C14*	*C16*	*C18*	*C20*	*C22*	*C16:1*	*C18:1*	*C20:1*	*C18:2*	*C18:3*	*C18:1* t	*C18:2* t
Canada/the United States (1976)[a]												
Bar (hard) margarines ($n=27$)	0.7–1.4	2.0–17.8	5.5–11.5	0.5–1.1		11.7	32.8–81.2	2.1–6.9	1.5–51.8	0.5–6.1		
Tub (soft) margarines ($n=58$)	0.6–4.9	1.5–20.9	3.4–12.0	0.8–6.1	0.5–2.1		18.8–80.2	1.2–12.9	5.1–66.8	0.5–6.9		
The United States (1985)[b] ($n=83$)		8.5–24.3	5.0–15.2				21.6–41.7		6.1–46.4	0.2–3.8	3.9–30.1	
Canada/the United States (1991)[c] ($n=16$)		4.1–12.2	4.9–9.4				17.7–68.6		12.0–57.0	0.5–6.9	0.6–14.1 (total *trans*)	
Denmark (1996)[d]												
1992 ($n=40$)	0.0–7.6	7.7–34.2	3.7–16.5	0.0–2.9	0.0–2.8	0.0–2.2	13.3–42.2	0.0–2.5	7.3–61.1	0.0–6.0	0.0–22.3	
1995 ($n=34$)	0.0–8.4	5.6–33.7	2.9–20.8	0.0–4.0	0.0–2.3	0.0–1.7	14.8–52.9	0.0–2.2	4.2–53.8	0.0–7.3	0.0–8.2	
1998[e] ($n=59$)	0.0–6.9	5.1–37.1	2.3–23.1	0.0–4.7	0.0–5.2	0.0–1.1	15.3–58.4	0.0–3.2	3.8–61.2	0.0–9.1	0.0–14.2	
Germany (2000)[f]												
1994 ($n=11$)	0.2–0.4	6.9–9.4	8.4–16.4	0.1–0.4	0.2–0.7	0.1–0.3	32.5–49.2	0.1–0.4	27.0–40.8	0.2–0.6	12.9–25.9	0.4–2.0
1999 ($n=9$)	0.2–2.6	6.7–27.2	6.1–19.4	0.3–0.3	0.3–0.7	0.1–0.2	20.6–33.4	0.2–0.2	21.7–54.5	0.2–0.5	1.8–5.6	0.1–0.6
Portugal (2002)[g] ($n=16$)	0.5–4.3	11.9–37.6	4.0–12.3				18.2–41.2		10.6–54.0	0.1–5.2	0.1–8.2	0.1–2.2
Bulgaria (2002)[h] ($n=68$)	0.0–2.1	8.5–34.7	2.9–8.9				20.7–49.9		9.3–50.5	0.0–6.4	0.0–27.2	
Spain (2003)[i] ($n=12$)	0.1–1.8	7.1–21.7	3.2–15.5	0.3–0.5	0.2–0.7	0.0–0.1	20.3–37.0		26.1–49.5	0.2–6.1	0.1–18.6	0.0-tr
Pakistan (2006)[j] ($n=10$)	0.0–3.3	24.4–40.0	6.2–12.8				21.9–35.8		7.0–21.0	0.0–1.5	2.5–19.1	0.0–2.0
2008[k] ($n=10$)	0.2–8.7	16.9–33.8	6.1–19.0	0.1–1.3	0.0–0.8	0.0–2.3	5.7–34.8	0.0–0.7	3.8–35.4	0.0–2.0	2.2–34.7	
Greece (2011)[l] ($n=31$)	0.1–3.6	5.0–42.1	2.7–6.9	0.3–0.7	0.1–1.3	0.1–0.6	22.8–59.7	0.0-tr	11.5–51.2		0.0–0.4	0.1–0.8
Saudi Arabia (2013)[m] ($n=4$)	0.1–1.7	10.2–24.0	4.1–7.8	0.4–0.8		0.1	26.6–29.8	0.3–0.5	37.0–40.6	0.5–4.6	0.0–6.9	0.1–0.9

S, M, and P: saturated, monounsaturated, and polyunsaturated, respectively.

tr, traces.

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- Tocopherols and tocotrienols

  Tocopherols are well-known natural antioxidants and they are present in margarine coming from the vegetable oils used in its manufacture. Of them, α-tocopherol deserves special attention from the nutritional point of view. Tocopherols can also be added externally with the aim of improving both the nutritional properties and the oxidative stability of margarine.

  The tocopherol content of margarine can vary widely due to the very different compositions of the fats/oils used in its manufacture. As an example, margarines produced from corn, sunflower, and cottonseed oils have been reported to have higher α-tocopherol levels than those obtained from soybean oil. Moreover, the vegetable source has also an influence on the relative proportions of the different tocopherol isomers. α-Tocopherol concentrations with values between 0.3 and 44.62 mg 100 g^{-1} have been found in margarines from different countries.

  Tocotrienols, also important due to their nutritional value and to their antioxidant activity, are always in lower concentrations than tocopherols. It is worth noting that margarines made from palm oil can be expected to be among the richest in tocotrienols, since this oil is especially rich in this type of components.
- Vitamin D

  There is very little information concerning the occurrence of vitamin D in margarine. The presence of this vitamin results from its addition in the form of ergocalciferol (D_2) or cholecalciferol (D_3). The values reported for vitamin D_3 content range from 1.08 to 3.9 IU (international units; 1 IU = 25 ng) g^{-1}, exceeding the labeling claims in some cases.
- Vitamin K_1 (phylloquinone)

  Some oils used for margarine manufacture, such as soybean or canola oil, contain significant amounts of vitamin K_1. For this reason, it is present in certain margarines whose consumption could contribute to the dietary intake of this vitamin. Different contents of vitamin K_1 have been found depending on the type of margarine, with a concentration ranging from 4 to 161 μg 100 g^{-1}. In general, hard margarines have been found to contain lower levels of vitamin K_1 than soft ones, when comparing products with similar fat levels and similar oil sources.
- Phytosterols

  The phytosterols that are usually present in margarines come from the vegetable oils used in their manufacture, although in low concentrations. However, certain oils such as rapeseed or corn oils are especially rich in sterols; in fact, higher sterol contents have been observed in margarines obtained from these sources.

  These compounds can also be added in significant proportions (7.5–8%) to some margarines, because of their interest as functional food ingredients, which are able to lower blood cholesterol levels. In this case, they are added in the form of FA phytosteryl or phytostanyl esters, to improve their solubility.

  As in the case of tocopherols, the amounts of sterols in margarines can be very variable. Of these, β-sitosterol has been found to be among the most abundant, in concentrations ranging from 5.4 to 412.9 mg 100 g^{-1}, followed by campesterol (2.0–106.3 mg 100 g^{-1}) and stigmasterol (1.6–60.9 mg 100 g^{-1}).
- Minerals

  There is very little information about the mineral content of margarine. Sodium and potassium appear to be the most abundant, in concentrations ranging between 13.2 and 9870 μg g^{-1} and between 58.2 and 1140 μg g^{-1}, respectively, followed by calcium, magnesium, and iron. Some of these are important from the sensory point of view. They include sodium, responsible for a salty taste, or potassium, related to bitter notes. Others, such as iron, can favor oxidation and thus the formation of compounds responsible for both unpleasant flavors and toxic effects. Other elements such as zinc and manganese are in lower concentrations, with values between 0.38 and 2.71 μg g^{-1} for the former and between 0.12 and 0.59 μg g^{-1} for the latter.

  With regard to the elements that can be toxic in high concentrations, the small amount of existing data reveals that cadmium seems to be the most abundant, with concentrations ranging between 0.02 and 3.66 μg g^{-1}, whereas the mean contents of lead and arsenic do not exceed the maximum of 0.1 mg kg^{-1} established for them in the Codex Alimentarius. Variable concentrations of nickel, used as catalyst in margarine manufacture, have also been observed in a few margarines, up to 1.70 μg g^{-1}.
- Gelatin

  This is sometimes used in margarines with a low fat content, to improve the stability of the emulsion. It has been observed that the addition of gelatin to the aqueous phase of margarine at 0.02% increases the firmness of the product. This enables the addition of 25% more water, when compared to margarine with no gelatin.
- Other components

  In addition to the earlier-mentioned, other components such as sugars, proteins, and different types of additives can also be found in margarine. Apart from the additives already mentioned in the text (carotenes, tocopherols, and emulsifiers such as mono- and diglycerides), acidity regulators, antifoaming agents, synthetic antioxidants such as BHA and BHT, coloring agents such as bixin-based annatto extracts, preservatives such as sorbates, stabilizers, thickeners, and flavorings can also be used. Among these latter, lactones can be cited, as they have been added to some margarines in order to give them a buttery flavor.

Composition of Margarines and Effects on Health

A high content in polyunsaturated *cis* acyl groups and a content of both saturated and *trans* acyl groups as low as possible have been considered desirable from the health point of view for a long time. In fact, S acyl groups in dietary lipids have been held responsible for certain cardiovascular diseases. However, more recent studies have shown that there was insufficient scientific evidence to support this negative opinion of these groups.

As far as *trans* groups are concerned, nutritional studies have suggested a direct relationship between this type of acyl groups and the risk of heart disease, since they increase low-density lipoprotein (LDL) and reduce high-density lipoprotein (HDL) cholesterol levels. In spite of this, nowadays, there are no regulations to control *trans* group content in margarine in a general way, although some organisms such as the World Health Organization (WHO) or the International Margarine Association of the Countries of Europe (IMACE) have given

recommendations aiming at reducing the occurrence of *trans* isomers in margarine. Moreover, some countries have established legal limits for this type of acyl groups, resulting in an effective decrease in the *trans* group content of margarine.

In relation to polyunsaturated acyl groups, certain values of the $\omega-6/\omega-3$ ratio have been considered optimal from the point of view of health, and great efforts have been made to produce margarines with such an acyl group profile. However, this optimal value has changed over time. It should be pointed out that there has been a recent tendency to reduce the $\omega-6$ acyl group content in fat foods and to increase the content in $\omega-3$ because of the beneficial effects attributed to the latter.

As a consequence of the current interest in the development of functional foods whose goal is to improve consumer health, there is a growing trend toward enriching margarine with bioactive compounds.

Therefore, margarines enriched in $\omega-3$ acyl groups are available on the market due to the beneficial cardiovascular effects attributed to these groups. As a result, fish oils and vegetable oils rich in $\omega-3$ acyl groups, such as camelina oil, have been used to this aim. Likewise, the enrichment of margarines with specific acyl groups considered bioactive, like pinolenic, a tri-unsaturated acyl group present in pine nut oil, has also been carried out. However, it should be noted that margarines with high levels of P acyl groups are highly susceptible to oxidation; therefore, special care should be taken in their use when thermal treatments are involved, because toxic compounds can be easily formed upon the degradation of this type of acyl groups.

The effects of phytosterol and phytostanol consumption on lowering cholesterol levels seem to be well established; for this reason, margarines with significant levels of these compounds have received wide consumer acceptance. Phytostanols could have an advantage over phytosterols in view of their lesser tendency toward to oxidation.

In an attempt to help prevent iodine deficiency, the possibility of obtaining iodine-fortified margarine, with a content of 1 μg iodine g^{-1} product, from iodine-fortified sunflower oil has been studied. This could contribute in part to eradicating this deficiency, which can affect both physical development and intellectual development.

Analysis of Margarine

Many efforts have been dedicated to the analysis of margarine components, especially to the nature and content of acyl groups. The methodologies and analytic techniques used have evolved over the years alongside technological improvements and the development of new and more accurate techniques.

Analysis of the TGs

The technique used for the determination of the TG profile of margarine is high-performance liquid chromatography (HPLC) coupled either to an evaporative light-scattering detector or to an atmospheric pressure chemical ionization mass spectrometer (APCI-MS) detector. Before HPLC analysis, the TG fraction can be isolated by column chromatography on silica gel using a mixture of petroleum ether and diethyl ether as eluent.

In addition to HPLC, ^{13}C nuclear magnetic resonance (^{13}C NMR) has also been used for the study of the TG composition of margarines and the distribution of acyl groups in the glycerol structure.

Determination of the Different Types of Acyl Groups

The most usual way to study margarine acyl groups is to perform an alkaline TG hydrolysis in methanol, either directly on the margarine sample or after solvent extraction of the lipidic fraction, followed by the transformation of FAs in the corresponding methyl esters (FAME).

Once the FAME are obtained, they can be analyzed directly by gas chromatography using either flame ionization detector (GC-FID) or mass spectrometry (GC-MS) detector. Over the years, several kinds of columns have been used for this technique with the aim to achieve an optimal separation of *cis* and *trans* isomers; the best results have been obtained by using high-polar columns of great length (100 m). However, with this methodology, the separation of *trans* and *cis* C18:1 isomers cannot be totally obtained, especially when the concentration of the *trans* isomers is higher than the 5%.

Other methodologies involve an additional isolation step by means of thin-layer chromatography (TLC) with silica gel containing silver nitrate (Ag-TLC) before the GC analysis. This technique enables the separation of FAME into fractions differing in the number and geometric configuration of the double bonds. The subsequent GC analysis of each of the fractions makes identification of the different geometric and positional isomers present in the margarine fat possible. However, this methodology is lengthy and not suitable for routine analysis.

As an alternative to the combined procedure of Ag-TLC and GC, a method based on GC-FID and infrared spectrophotometry has also been used, leading to equivalent results. This latter method was recommended for samples with >5% *trans* unsaturation.

HPLC has also been used by some authors for the determination of acyl groups in margarine. However, although some advantages over GC have been identified, HPLC has been little employed for margarine acyl group analysis.

In addition to the previously mentioned techniques, ^{13}C NMR provides information regarding the composition and nature of the acyl chains present in margarine TG. Likewise, ^{1}H NMR has also been proved to be very useful in determining the composition in acyl groups of margarine samples, in only a few minutes and with minimal or no sample pretreatment. The spectral signals from which the different types of acyl groups can be determined are shown in Figure 1.

Determination of the Unsaturation Degree

The total content of double bonds in margarines has also been subject of analysis. Total double bonds can be determined by chemical methods by means of the so-called iodine index or by easier methods involving spectroscopic techniques such as Fourier transform infrared spectroscopy (FTIR) or ^{1}H NMR.

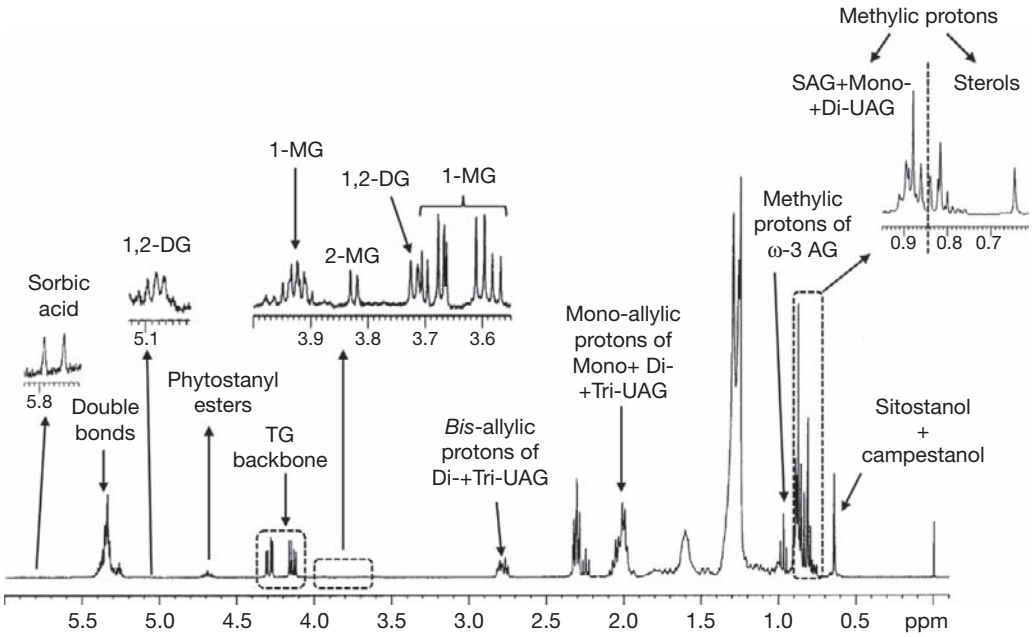

Figure 1 ^{1}H NMR spectrum of the lipidic fraction of a margarine enriched in phytostanyl esters. AGs, acyl groups; UAGs, unsaturated acyl groups; SAGs, saturated acyl groups; MGs, monoglycerides; DGs, diglycerides.

Specific Determination of *trans* Unsaturation

The most common methodology for the determination of the *trans* unsaturation degree of margarines is based on FTIR, it being possible either to use attenuated total reflectance or not. The advantage of this technique in relation to chromatography is that it does not require either fractionation or special sample preparation or chemical modifications of the sample. The band of *trans* double bonds appears near 966 cm^{-1}. This determination has also been carried out by means of optothermal window.

Another spectroscopic technique used for the determination of the *trans* unsaturation in margarine is ^{13}C NMR.

Mono- and Diglycerides

The analysis of mono- and diglycerides in margarine has received very little attention. One of the techniques used for this purpose is HPLC. Monoglyceride determination can be carried out after derivatization with 3,5-dinitrobenzoyl chloride using a ultraviolet (UV) detector or without any sample pretreatment by using a glyceride-selective postcolumn reactor detector.

Both mono- and diglycerides can also be determined by ^{1}H NMR. This technique allows the detection and even quantification of 1- and 2-monoglycerides, as well as of 1,2- and 1,3-diglycerides, simultaneously with the determination of the different kinds of acyl groups. The spectral signals from which the different types of mono- and diglycerides can be determined are shown in Figure 1.

Fatty Acids

This is usually carried out by the methods used to determine this kind of compounds in other fat-containing foods such as vegetable edible oils.

Carotenes and Vitamin A

The analysis of these compounds has been carried out by means of different methodologies and techniques. UV spectrophotometry has been used to determine carotenes and vitamin A after previous extraction of the unsaponifiable matter, separation, isolation, and purification of both kinds of compounds. As saponification can degrade these compounds, there has been a tendency in recent years to avoid this step and carry out the determination directly in a hexane extract of the margarine sample. Carotene absorption occurs near 450 nm and that of vitamin A near 325 nm.

Another method used to determine these compounds is HPLC with two UV detectors connected in series to detect both carotenes and vitamin A simultaneously. This method is faster than the previously mentioned since the isolation and purification steps are carried out by this technique automatically. With HPLC, the determination of these compounds can also be achieved directly from a margarine hexane extract, avoiding the saponification stage.

The isolation step can also be carried out by gel permeation chromatography, directly on the margarine sample dissolved in methylene chloride, followed by nonaqueous reverse-phase (RP) HPLC.

More recently, and with the same aim of avoiding saponification, photoacoustic spectroscopy and optothermal window, two rapid and extremely simple techniques, have also been applied to the analysis of total carotenes in margarines with satisfactory results.

It should be taken into account that, with the exception of HPLC methods, these methodologies without either saponification or an isolation step only give information about total carotenes.

Tocopherols and Tocotrienols

The determination of this type of components usually involves a first step of alkaline saponification. Next, the unsaponifiable material is extracted with an appropriate organic solvent such as hexane. The analysis of tocopherols can be performed either by GC, after derivatization to trimethylsilyl ethers, or by HPLC. This latter technique is the most commonly used to this aim, being the fluorescence (FL) (excitation wavelength set at 290 nm and emission wavelength at 330 nm) preferable to the UV detector.

Given that tocopherols are very prone to oxidative degradation, it is recommended to perform saponification in darkness and in a nitrogen atmosphere or in the presence of an antioxidant such as ascorbic acid or pyrogallol. In order to avoid the saponification step, the determination of tocopherols has also been achieved directly from a margarine hexane extract by HPLC with FL detector.

The direct determination of tocopherols from the margarine hexane extract has been also carried out by using an online coupling of both chromatographic techniques HPLC-FL and GC-FID in order to avoid interfering compounds.

In addition to tocopherols, the determination of tocotrienols may also be of interest. The determination of these latter can be carried out by using some of the earlier-mentioned techniques simultaneously with that of tocopherols.

Vitamin D

As in the case of other liposoluble vitamins, the methodology for vitamin D determination includes an initial alkaline saponification of the margarine sample and extraction of the unsaponifiable material with an organic solvent like hexane. HPLC is usually used for the subsequent isolation of vitamin D fraction, which is finally injected to another RP-HPLC system for the separation and quantification of vitamins D_2 and D_3 with a UV detector at 264/265 nm.

An automatic method to simultaneously determine liposoluble vitamins (A, D_3, and E) has been also used. The margarine samples were dissolved in an aqueous micellar medium and directly subjected to an online sample treatment that included alkaline hydrolysis and solid-phase extraction (SPE). This system was coupled to liquid chromatography with amperometric detection.

Vitamin K_1

The methodology employed in the determination of this vitamin is based on HPLC analysis. This can be carried out after enzymatic digestion or after direct extraction with hexane and subsequent purification either by semipreparative HPLC or by SPE on a silica column. Quantification is performed by RP-HPLC equipped with a dual-electrode electrochemical detector or with FL.

Phytosterols and Phytostanols and/or Their Esters

Margarine can contain phytosterols, phytostanols, and esters of these compounds. The total determination of all of them requires alkaline hydrolysis, extraction with an organic solvent like diethyl ether, and a cleanup step using SPE or TLC. The analysis of the isolated compounds is done by GC previous derivatization to trimethylsilyl ethers. Although FID has been sometimes used, MS detectors are the most commonly used nowadays.

Recently, analytic methodologies have been developed to determine phytosteryl and phytostanyl esters in enriched margarines. They are based on RP-HPLC/MS analysis with different types of ionization, such as APCI and electrospray ionization. The sample preparation steps differ from some methodologies to others, ranging from a minimal sample pretreatment to the use of SPE cartridges for the isolation of steryl esters from a hexane extract of the margarine sample.

Some of the sterols present in margarine can also be determined by ^{1}H NMR. Among these, brassicasterol, β-sitosterol+campesterol, stigmastanol, or Δ7-avenasterol can be mentioned. It is worth noting that this determination can be carried out simultaneously with that of the several kinds of acyl groups, mono- and diglycerides. Some of the spectral signals corresponding to phytosterols and/or phytostanols are shown in Figure 1.

Minerals

The first step in determining minerals in margarine involves the release of the several elements from the fat matrix, which can be achieved by several methods. Afterward, the determination of each element can be carried out by different techniques, some of which involve sample emulsification. The most frequently used are atomic absorption spectrometry and inductively coupled plasma optical emission spectrometry. With the first technique, flame atomic absorption spectrometry is usually used to determine those elements that are in greater concentrations, whereas graphite furnace atomic absorption spectrometry is chosen for those elements whose concentrations are expected to be much lower.

The determination of the isolated elements can also be achieved by ion chromatography (IC), either with a conductivity detector or with a variable-wavelength UV–vis detector, depending on the element. In addition, electrochemical methods such as differential pulse anodic (or cathodic) stripping voltammetry and galvanostatic stripping chronopotentiometry have also been used for the analysis of minerals in margarine; these have shown a good agreement with the results obtained by IC. Finally, it is worth commenting on that instrumental neutron activation analysis also enables the measurement of a large number of trace elements in margarine, with minimal sample handling and processing.

Other Components

The analysis of some of the additives used in margarine manufacture, such as benzoic or sorbic acid, together with acids such as formic, lactic, citric, acetic, and butyric can be carried out by means of ^{1}H NMR, from the margarine polar fraction. The spectral signals corresponding to sorbic acid are shown in Figure 1.

See also: Carotenoids: Occurrence, Properties and Determination; Cholecalciferol: Properties and Determination; Fats: Classification and Analysis; Fatty Acids: Determination and Requirements; Fatty Acids: Fatty Acids; Fatty Acids: *Trans* Fatty Acids; Functional Foods; Retinol: Properties and Determination; Tocopherols: Properties and Determination; Triacylglycerols: Characterization and Determination; Vegetable Oils: Composition and Analysis; Vitamin K: Properties and Determination.

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Mass Spectrometry: Applications

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Introduction

Food science concerns the physical–chemical nature of foodstuff and its evolution due to storage, handling, and processing. In a broader meaning, food science also embraces all those aspects that are related to nutrition, including the knowledge of how human body transforms foods and how foods impact on human health.

The definition of the parameters that concur to the global perspective of food science is an extremely challenging task. From an analytic point of view, foods are heterogeneous mixtures of very different classes of compounds and reflect the complexity of the animal or vegetal organism(s) from which they derive. Therefore, the enormous heterogeneity of almost all food matrices, which often derives from complex biochemical systems (for instance, milk or fermented foods), complicates the detection of all the components that occur in concentrations spanned over several orders of magnitude. Generally speaking, the impact of the various constituents on human health is not necessarily correlated to their amounts, considering that a bacterial or an algal toxin can be harmful to health even at ppb–ppt concentrations. Also, the variety of technological practices applied to process raw materials in order to get the final product multiplies the variables for the analytic definition of food quality. A food product is the result of concomitant and/or consecutive physical and chemical processes: thermal treatments or cooking, spray drying, extrusion, gel or dough formation, chemical or enzymatic hydrolysis, cross-linking, and oxidation, just to mention a few, all of which induce deep and different structural changes to the food constituents.

The development of 'omic' sciences (from genomics to transcriptomics, proteomics, glycomics, lipidomics, and metabolomics) has introduced integrated high-throughput approaches to address the understanding of the biochemical systems and of their dynamic evolution. When applied to the field of food and health, 'omic' sciences are delineating a global approach, which is inclusively referred to as foodomics. In the development of the advanced foodomic platforms, mass spectrometry (MS) techniques have assumed an unquestionable 'core' position because of their potential of providing comprehensive biomolecular profiles, or at least multiplexed snapshots for the various classes of biomolecules.

The ultimate aim of foodomics is the sustainable production of high-quality and safe foods for improving human health and well-being. Here, we critically survey the achievements and the potentiality of MS applied to foodomics through selected recent research outcomes.

MS: Platform and Instruments

MS has its roots in the chemical and physical studies regarding the nature of the matter at the end of the nineteenth century, in particular in the pioneering work of Sir J. J. Thomson (1856–1940). Long remained a pure tool of physicists, MS has gradually emerged as an invaluable analytic technology that offers an elevated informative level, unparalleled sensitivity and specificity, high-throughput, and extreme versatility. Rather than a discipline positioned between physics and chemistry, MS is nowadays considered a self-standing branch of science and technology, whose application field is the production, study, and characterization of gas-phase ions. The evolution of MS and the technological advances have supported the building of progressively more sophisticated mass spectrometers.

MS data are recorded as 'mass spectra,' which are two-dimensional (2-D) graphs displaying ion intensity versus the mass-over-charge (m/z) ratio. The accurate measurement of the m/z enables the access to a unique characteristic of the molecules, that is, their molecular weight. Often combined with data generated by the fragmentation of selected molecules ('hard' ionization techniques or MS/MS experiments), this information allows to identify or structurally characterize (or contributes to this aim) the components of a mixture. Today, MS embraces any array or analytic tool able to analyze the m/z of ions produced or introduced within a mass spectrometer. The basic parts of a mass spectrometer are the ion source (in which ions are generated), the ion analyzer (where ions are separated and their m/z ratio determined with high accuracy), and the ion detector.

Since their introduction, more than 50 years ago, commercial MS instruments, substantially electron impact ionization (EI) and chemical ionization (CI) ion sources with quadrupole (Q) analyzers, have been successfully applied to the analysis of small organic molecules such as, in the case of foods, hazardous contaminants, including pesticides, halogenated hydrocarbons, dioxins, bisphenol, and bacterial metabolites in food matrices such as vegetables, milk, and fish. More importantly, the quite easy combination of EI and CI with high-resolution separation techniques such as gas chromatography-mass spectrometry (GC-MS) soon provided a handful of analytic tools to face qualitative and quantitative aspects of food composition, safety, and nutritional quality. Also, in different configurations, for example, EI and CI ion sources coupled to Q, ion trap (IT), time-of-flight (TOF) analyzers, hyphenated GC-MS remains one of the most powerful approaches in food metabolomics and metabonomics, allowing fast and accurate tracking of metabolite patterns indicative of food quality and preservation over time. One recent example is the detection of the distinctive volatile signature of meat spoilage bacteria.

However, at that stage, determining the largest part of the constituents of a food was still precluded, because GC-MS methods were only able to analyze volatile compounds with a mass not exceeding 1 kDa. This gap was bridged in the early 1980s, by the introduction of 'soft' ionization techniques, such as fast atom bombardment, and then by the electrospray

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ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), which allowed to measure nonvolatile, high-molecular-mass molecules. In 2002, John Fenn and Koichi Tanaka were awarded the Nobel Prize in Chemistry for the pioneering application of ESI and MALDI, respectively, to measure the molecular weight of large proteins and (bio)polymers. For the first time, it was possible to measure with high accuracy the molecular weight of intact (poly)peptides, proteins, polysaccharides, and complex lipids, covering the whole range of the food constituents. ESI and MALDI are the two techniques most commonly used for MS. Thanks to the availability of these techniques, MS-based food and nutrition proteomics is now capable to address a wide range of analytic questions, which include issues related to food quality and safety, certification and traceability of (typical) products, and definition of the structure/function relationship of food proteins and peptides.

As for GC-MS, the easy combination of ESI with liquid chromatography (LC) separation allowed the analysis of complex mixtures, such as those occurring in most food matrices. This high-throughput approach to molecular characterization also relies on the high resolving power of the LC-MS/MS systems, which include several configurations of quadrupole time-of-flight (Q-TOF) hybrid analyzers, TOF/TOF, Fourier transform ion cyclotron resonance (FT-ICR), and the most recent Orbitrap instruments. Besides the introduction of the soft ionization sources, other key advances have contributed to set the stage for the success of MS applied to the omic sciences: (i) the development of microscale capillary reversed-phase LC, (ii) the introduction of nanoelectrospray sources enabling the continuous interfacing of capillary chromatography with high-sensitivity mass spectrometers, and (iii) the development of computer algorithms to correlate MS and MS/MS data to database entries.

Biological MS in combination with separation methods and with statistical and bioinformatics tools is today the key analytic technique that the recently established 'omic' technologies such as proteomics ad metabolomics rely on.

For quantitative purposes, an essential issue in food analysis, the isotope dilution technique, relying on the use of standards labeled with heavy stable isotopes (^{2}H, ^{13}C, etc.) allows accurate measurement of trace analytes even in the presence of complex matrices. The addition of a stable isotope-labeled internal standard at the beginning of the extraction enables the control of possible variation (for instance, partial loss of the compound of interest) that can occur during sample preparation. An internal standard should ideally be an isotope-labeled analog that displays similar behavior (extraction, chromatography, ionization, and ion suppression/enhancement) as the monitored analyte. These approaches require specific methods to be developed and validated for each analyte or class of compounds, as well as analytic instruments/analyzers able to provide the necessary stability and reproducibility among chromatographic analysis. The most widely used systems are triple quadrupole (QqQ) instruments, which exhibit a relatively ample linear dynamic range, and IT, whereas the recently introduced triple TOF is expected to probably ensure maximum performance in quantitative proteomics. In foodomic studies, for instance, in the detection of harmful protein components (allergens, toxins from pathogens, and antinutritionals) or for adulterated foods, monitoring of a whole target protein is surrogated by the analysis of short peptides contained within the protein sequence ('proteotypic peptides'). These peptides are more easily detected than the parent protein. The targeted monitoring of mass and transitions of selected 'proteotypic peptides' overcomes the bias due the presence of a large number of dominant components. According to the absolute quantification (AQUA) workflow, once proteotypic peptides are chosen, it is possible to synthetize copies of them, which are labeled with isotopes to one or more amino acid positions in order to shift molecular mass, without influencing retention time and ionization properties. Protein samples after tryptic hydrolysis are spiked with known amounts of standard AQUA peptides and both native and surrogate peptides are monitored by LC-MS operating in multiple reaction monitoring (MRM) mode. The absolute amount of peptide(s) is determined by the ratio of the ion intensities of AQUA peptide and its native cognate.

MS to Food and Health

Application of MS to food science has a long and outstanding history. From the beginning, MS-based techniques have rapidly evolved for the determination of quality, authenticity, functionality, and safety issues.

Food Proteomics

In the proteomic approach, the entire protein/peptide repertory of a food product can be explored by using MS-based methodologies following a high-resolution electrophoretic or chromatographic separation step.

The MS-based proteomic workflows can be performed through two fundamental analytic strategies: the bottom-up or the top-down approaches. In the bottom-up workflow, the protein identity is inferred by the MS/MS sequencing of proteolytically generated peptides (peptide-centric proteomics). In top-down proteomics, intact protein ions undergo gas-phase fragmentation for MS analysis. Proteins are identified or even partly sequenced through the assignment of fragmentation patterns that are generally predictable. The separation requirements and the type of MS instrumentation differ between the two methodologies. The original bottom-up strategy is accomplished by the combination of 2-D gel electrophoresis (2-DE) separation combined with MALDI-TOF MS or LC tandem MS (MS/MS) of peptides arisen from in-gel proteolytic digestion (e.g., with trypsin) and database search (peptide mass fingerprinting, PMF). The 2-DE approach allows to resolve very complex proteomes, but it is time-consuming and suffers from several pitfalls, primarily including the limited dynamic range.

An advanced bottom-up approach referred to as shotgun or multidimensional protein identification technology is increasingly adopted. This methodology relies on the mono or 2-D HPLC separation and online ESI-MS/MS analysis of the peptides generated from the proteolysis of the whole protein fraction, and it is performed with high resolving power instruments. In spite of the enhanced sample complexity due to a multiplying of the components in the hydrolyzate (each

protein produces a variable number of peptides), peptides are separated and analyzed by MS at much higher sensitivity and accuracy than proteins. Improved peptide-centric pipelines for deep expression proteomics can be exploited to characterize posttranslational and process-induced modifications of proteins too. Overcoming many of the 2-DE gel-based analysis, the shotgun approach allowed, for instance, to characterize hundreds of minor proteins in human milk, which are potentially implied in numerous biological functions. Although the cutting-edge shotgun techniques are not broadly available yet, time seems mature for achieving unbiased, complete, and ubiquitous characterization of whole proteomes. In the near future, these techniques will be applied to deliver deeper knowledge of food systems, their changes, and their influence on the physiology of human body.

One of the major drawbacks of the gel-free approach is that the information on the intact protein is lost, which implicates that information on the protein integrity is usually not available. This limitation can be important in the case of fermented foods (yogurt, cheese, and sausage), where proteins undergo extensive proteolysis. In cases such as these, unhydrolyzed proteins and peptides require a previous prefractionating step and separate analysis workflows.

Food Metabolomics

Similar to proteomics, the metabolomics workflow implies the combined use of advanced analytic techniques (mainly omic tools) and bioinformatics methods to recover relevant information.

Foods are constituted by an enormous variety of compounds, both intrinsically present and generated by technological processes, which are responsible for the quality (texture, flavor, odor, color, and shelf life) and nutritional value (vitamins, antioxidants, and nutrients) of the final product. Therefore, a food and its nutritional quality can be fully defined in terms of the metabolic profile of the material concerned at a particular time (metabolome).

Metabolomics aims to the characterization of the repertory of small (<1500 Da) molecules of a biological system. The asset of metabolites in a food is the result of the specific metabolic pathways of the organism(s) and of the modifications induced by processing or by interaction with other components. Well beyond the cataloging of the food components, metabolomics, combined with *in vitro* assays, cell models, or *in vivo* interventional studies, is also increasingly used to assess, under a molecular profile, the impact of specific dietary components on human health (metabonomics). Shifting nutritional status assessments to an 'omic-oriented' nutritional phenotyped perspective is providing insights into the relationship between diet and health (nutrigenomics).

In food technology, metabolomics and metabolome profiling is increasingly used for many purposes, among which are (i) tracing the evolution of a product from 'the farm to fork,' (ii) evaluating the microbial action (both desired and detrimental) on food quality, (iii) monitoring the evolution of functional and bioactive compounds, and (iv) assessing the impact of technological treatments (physical, chemical, enzymatic) on the development of rheological and sensory characteristics.

Analytic tools of metabolomics are based on traditional GC-MS and LC-MS, aside from different approaches based on the nuclear magnetic resonance (NMR). GC-MS analysis, also in its 2-D version (GC × GC-MS), has been extensively used in metabolomics because of its high separation efficiency and the easy interfacing of GC with MS. It can be used to analyze a wide range of volatile and chemically derivatized semivolatile compounds. However, sensitive and specific LC-MS methods, which do not require derivatization of polar/nonvolatile metabolites, are often replacing GC-MS. A recent introduction into the field of metabonomics is ultraperformance liquid chromatography (UPLC) systems, with narrow columns either monolithic or packed with 'core-shell' or small diameter particles, coupled to MS/MS instruments. Compared with HPLC-MS system, the UPLC-MS system has very short run time, higher sensitivity and resolution, and signal-to-noise ratio that could be increased by fivefold.

NMR and MS are considered complementary in metabolomics. Although MS often requires cleanup or prefractionation steps, it is more sensitive than NMR, enabling the detection of much less abundant components. Mass measurement with high-accuracy MS (e.g., Orbitrap or FT-ICR MS), especially when coupled with downstream MS/MS or MS^n data, and database interrogation is successful in most of cases for the identification of the common metabolites. Several authors have proposed the use of direct infusion of nonfractionated extracts in very high-accuracy mass spectrometers (typically <1–2 ppm) to obtain metabolic profiles without previous chromatographic step.

A present limitation of food metabolomics relates to our current inadequacy to structurally characterize the vast majority of metabolites in a complex foodstuff. The lack of available reference standards for many of the food metabolites (particularly the myriad of plant secondary metabolites) is a challenge that can be somehow tackled with the MS-based 'untargeted' metabolomic approach. Untargeted metabolomic comparison between wild-type and genetically modified soybeans has been recently exploited to assess the perturbation in the metabolic asset induced by gene modifications with the aim of supporting the safety and the bioequivalence with respect to conventional crops. Targeted modes of data acquisition (generally relying on the single/multiple ion monitoring, SRM/MRM) are designed to monitor by MS preselected compounds with higher sensitivity and specificity. Targeted experiments are conveniently carried out in second instance, after the untargeted 'discovery-driven' profiling. The general metabolomic approaches and strategies that also apply to foods are schematized in **Figure 1**. Among several others, the website www.metabolomicssociety.org provides a compendium of metabolomic online resources and tools.

Lipidomics and glycomics concern the definition of the global array of specific metabolite classes, that is, oligosaccharides and lipids, respectively. In consideration of the relevance of these compounds in biological systems, lipidomics and glycomics are emerging as standing alone 'omics' from the wider melting pot of metabolomics. Food lipidomics and glycomics are now relevant issues in food science and nutritional research, and several examples taken from the recent literature exemplify the nontrivial MS-assisted transition from component-oriented traditional analysis of lipids or sugars to the respective omic perspective.

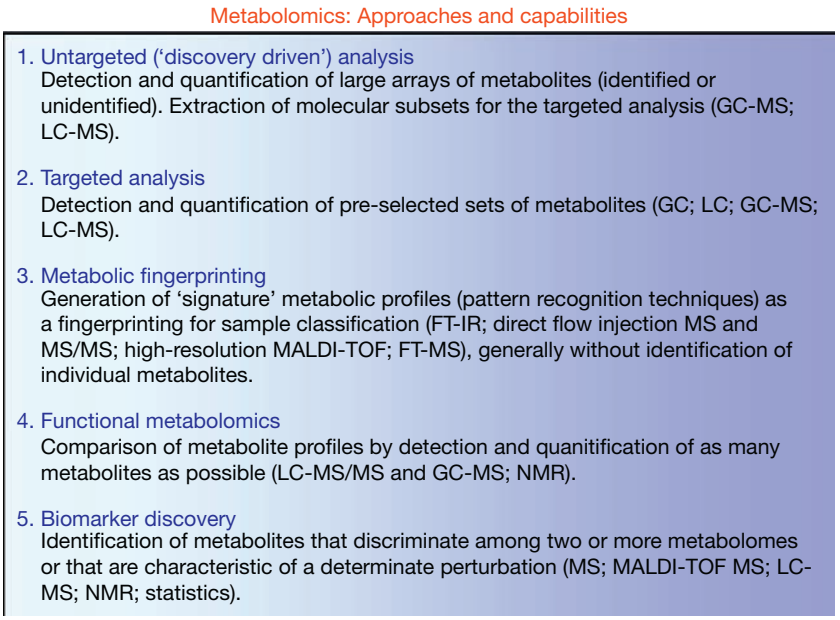

Figure 1 List of metabolomics that also applies to food analysis. The most commonly used analytic strategies are indicated for the various approaches.

Due to a relatively less heterogeneous class of compounds, if compared to the whole metabolome, lipidomic analyses have been often performed by direct infusion into MS instruments (shotgun lipidomics). For instance, a very fast shotgun lipidomic strategy has been applied to the characterization of the lipids in fisheries, finalized to the species differentiation. The determination of the acylglycerol fraction is particularly relevant for foods. Detailed composition and fingerprinting profiles have been obtained for several vegetable oils by LC-ESI-MS. Because of the minimal sample handling required and short analysis time, MALDI-TOF-MS is increasingly used to profile food phospholipids and neutral lipids. For instance, phospholipids and triacylglycerols (TAGs) from hen egg yolk have recently been profiled by MALDI-TOF-MS. When tested for assessing cocoa butter authenticity, MALDI-MS delivered information comparable to GC even in quantitative terms. Very complex lipid mixtures, such as those of TAGs and modified acylglycerols in thermo-oxidized vegetable oils, have been profiled by MALDI-TOF-MS with emphasis on the detection of potentially harmful neoformed compounds.

Regarding glycomics, carbohydrates are present in foods in very different forms, ranging from free saccharides to polymers or to glycoconjugates. In consideration of the large spectrum of physiological actions of dietary oligosaccharides, food glycomics is gaining a rising interest. Particularly interesting is the characterization of bioactive oligosaccharides from both human and bovine milk. In foods, both N- and O-linked glycoproteins can affect not only the technological properties of proteins but also the digestibility and the potential allergenicity. Multistage MS has become one of the most powerful and versatile techniques for the structural characterization of the glycan moieties of glycoproteins and of glycoconjugates in general. Due the complexity of the glycan fractions, direct MS analysis is sometimes not sufficient for a complete profiling. Thus, high-resolution strategies based on hydrophilic interaction or porous graphitized carbon LC are crucial for carrying out accurate online MS analysis of oligosaccharides.

Also in the case of metabolomics, and hence lipidomics and glycomics, the use of robust bioinformatic tools is crucial to convert the MS-generated datasets in useful information. Several lists of lipidomic and glycomic resources are available in the Internet (e.g., www.lipidmaps.org; www.functionalglycomics.org).

Food Composition and Safety

MS in food science is aimed to define the entire and detailed composition of raw materials and ingredients, as well as their modification throughout the production process, to correctly evaluate food quality and safety aspects. The frontier of analytic chemistry for food safety is represented by the development of multiclass, multiresidual techniques to simultaneously monitor the presence of substances belonging to different chemical groups in a sample.

Among contaminants along the food chain, algal toxins produced by cyanobacteria in water reservoirs are a special concern. The occurrence of toxin-producing blooms in freshwaters has been implicated in several animal and human poisoning outbreaks worldwide. The analysis of algal toxins is therefore of growing interest for water surveillance authorities. MALDI-TOF-MS and ESI-Q-TOF-MS/MS have been useful for fast screening and quantitative determination, as well as for structural characterization of known and unknown toxin structures (untargeted analysis). MS analysis has been then extended to the setup of a method for quantitative determination of microcystins in food integrators for human and animal nutrition, as well as to the analysis of biological fluids in patients to diagnose toxin poisoning. In this way, qualitative

and quantitative information can be obtained to monitor rivers, lakes, and water reservoirs and to improve current knowledge on algal contamination in the food, feed, and drinking water chain and also to detect protein marker to discover the use of unsafe water in fish and seafood aquaculture.

Also, CE-MS has been proposed as a methodology to study proteins (phycobiliproteins) from the edible microalga like *Spirulina platensis*, which is a natural source of functional ingredients. Reliable and reproducible CE-MS analysis of the main proteins found in this microalga has been reported. It was demonstrated that both analyzers, CE-IT-MS and CE-TOF-MS, allowed the characterization of these proteins with coherent results.

One of the most urgent issues in food science is the fast and accurate detection of ingredients used in food preparations. Although most of them are not harmful for the great majority of consumers, they can be highly toxic for allergic or sensitive subjects. MS analysis has also provided important outcomes in the field of food authentication, in accordance with the comprehensive system of food and feed traceability established by the European Food Safety Authority in order to ensure food safety. This issue, particularly pressing in the case of protected denomination of origin (PDO) products, also requires efficient traceability systems, to ensure the authenticity of the raw materials and the correct application of the procedures throughout the productive processes and during distribution. For many food matrices, markers can be found in the protein fraction, which closely reflects the nature and the structural changes induced by technological processes of production.

Several MS-based procedures have been developed to authenticate ingredients in dairy products. A case example is represented by milk powders, widely used as ingredients by dairy, bakery, and meat industry to improve nutritional and rheological properties and for animal feedings. The EU subsides the use of milk powder in compound feeding stuffs. Recent reports of adulterated milk powders by the addition of lower value plant proteins (wheat, soy, pea, and other legumes) caused alarm as their presence is not revealed by official reference methods. For these reasons, a sensitive identification method was developed based on LC-ESI-Q-TOF-MS/MS analysis of tryptic proteotypic peptides specific for the storage proteins of each vegetal matrix (soy glycinin and conglycinin, pea legumin, and vicilin).

Several MS strategies, relying on both MALDI- and ESI-based monitoring of species-specific target proteins of derived peptides, also enable the detection of adulterating cheaper bovine milk to other ruminants' milk used in the manufacture of PDO cheese. Another exemplary case is the analysis of gluten residues in declared gluten-free foods and beverages, exploited by several studies. In the assessment of meat and fish genuineness and in foodborne bacterial identification, MS-based profiling of strain-specific gene products has the capability to substitute or complement other available strategies, such as the multiplex PCR assay.

A food product is in general the result of a series of physical and chemical processes including heat treatments, all of which induce deep structural changes in the food constituents. Food components, especially proteins, encrypt information distinctive of the identity and history of a product. Modifications induced by thermal treatments include deamidation of glutamine residues, formation of nonnatural amino acids, and nonenzymatic glycosylation caused by heat treatments. Several markers of heat treatments have been identified and used to set up procedures either to monitor the correctness of the treatment on food matrices (milk, soy, juices, and jams) or to detect the fraudulent addition of prohibited ingredients in PDO products. For instance, lysinoalanine, an unnatural amino acid, has been proposed as a LC-MS quantifiable marker to demonstrate either excessive heat treatments or the addition of heated milks/milk powders to fresh milks destined to cheesemaking.

Heat treatment is applied not only to ensure microbiological food safety but also to inactivate antinutritional components such as lectin phytohemagglutinin (PHA) present in raw legumes, including bean, pea, and soy, where lectin level is 1–10 g kg^{-1}, implying a possible risk for health in processed food. The most common methods of inactivation use heat treatment in boiling water or by extrusion. Inaccurate heat treatments of legumes have caused severe poisoning outbreaks in restaurants or refectories. Furthermore, commercial protein concentrates of common beans (*Phaseolus vulgaris*) are also the most used ingredients for production of dietary supplements to control body overweight in obesity (starch blockers and weight blockers). MS-based strategies have been developed to set up novel proteomic and glycomic methods for lectin structural and functional definition and to monitor the structural changes (proteolysis, oxidation, and sugar changes) in raw and in industrially treated products. In this way, possibly combined with the enrichment with affinity chromatography, they constitute the basis to allow for either the structural or quantitative analysis of PHAs in food samples.

Food Allergy and Digestion

Food allergy is an increasingly important issue for food safety, as the prevalence of the immune-mediated reaction to foods is steadily increasing. This is making the detection of allergens of primary importance for both food industry and consumers. MS strategies for *de novo* sequencing combined with the serum IgE recognition of allergens from animal (milk, fish, and crustaceans) or plant (cereals, legumes, and tree nuts) foods are leading to the identification of several previously uncharacterized 'type I' food allergens, even in cases when the genome of the injuring species is not fully sequenced.

Due to the concerns related to the food allergy in infants, to a relatively high incidence of cow's milk allergy, and to the very large use as ingredients in a countless variety of food preparations, one of the main targets of investigation is milk proteins (either casein or whey proteins). MS procedures to detect milk proteins in biscuits and baked goods have been proposed, using casein peptides as markers. In comparison with ELISA, the reference screening method showed good agreements between the two approaches.

These data demonstrate that the MS approach is promising in complementing or even replacing the ELISA methods currently used to discover the occurrence of trace allergens in foods and also in beverage. A recent study performed on a significant number of beers produced with different brewing systems compared quantitative ELISA results with those obtained by nano-LC-ESI-QqQ-MS/MS analysis of target

peptides. The analytic response was superior for the latter approach, in terms of either sensitivity or, most importantly, specificity. Indeed, the MS approach does not depend on the antibody affinity, and it is only marginally affected by matrix effects. Furthermore, while ELISA is targeted for a single allergen, the MS-based approach is potentially multiplexed, allowing the multiallergen detection in a single run.

The shotgun proteomic approach also expands the dynamic range of the detectable allergens. This could be not a particular advantage for the characterization of allergens, because these are generally proteins abundant in the food matrix. However, it is the most effective strategy to monitor 'hidden' allergens or cross contaminating species that occur in traces. Hidden allergens are those proteins whose presence is not immediately suspected in a food. This is the case of milk, soy, and gluten proteins used in wine as clarifying agents. MS analysis after selective enrichment by use of combinatorial peptide ligands, as well as shotgun MS analysis, revealed the unsuspected occurrence of allergenic proteins, which could be quantified by selection of target 'probe' peptides.

These encouraging results are now pushing the development and diffusion of automated allergen detection and quantification methods at low-ppm levels fully based on MS analysis and commercialized by MS instrument producers to this purpose. Initial sample preparation stages have been simplified for rapid and easy handling. Reliable allergen quantification (1 ppm) is based on the monitoring of the MRM transition of proteotypic peptides using the MRM-initiated detection. The derived method for allergen detection is already finding applications in food control companies and agencies in the analysis of major allergens in processed foods as it is advantageous on ELISA tests. Importantly, MS has been entrusted the task of challenging the issue of (food) allergen standardization that is related to diagnosis and treatment of allergies besides the analytic aspect of the identification and quantification of allergens.

With rare exceptions, 'type I' food allergens are proteins particularly stable to gastrointestinal digestion. Protein stability is dramatically affected by processing; thus, the digestibility of food proteins requires to be evaluated case by case. The MS-based procedures appear at the moment as those exclusively practicable for a suitable characterization of the 'digestome,' that is the pool of proteins/peptides surviving *in vitro* or *in vivo* digestion models of dietary proteins, in order to predict their immunogenic potential. MALDI-TOF and HPLC-ESI-Q-TOF-MS/MS analyses have allowed to identify the digestion-stable domains of wheat prolamins that elicit celiac disease and the potential epitopes of β-lactoglobulin that survive all the digestion compartments of a complete *in vitro* model and are able to cross a cell model of the intestinal epithelium.

The full elucidation of the 'kinetics' and 'dynamics' of nutrients in human body is one of the next goals of food science, and MS-based technologies will undoubtedly play a pivotal role for its achievement.

Conclusion

Food and nutrition play a crucial role in health as well as in the development of several diseases. MS-based omics are already assisting multiple aspects of the food chain (agriculture, food production, food safety, and quality assurance), are delivering economic benefits and improving aspects of human nutrition and health. By dissecting the food proteome and metabolome in their constituents, using the new technologies, specific markers are being identified to trace the complete productive processes from raw materials to end products, and structure/function links can be clarified for the design of novel functional foods. Quantitative analysis of food components and contaminants provided by MS is ensuring control of crucial food quality and safety aspects.

In analogy with the recently introduced concept of systems biology, according to which a biological system has to be regarded in its entirety considering the spatiotemporal integration of the metabolic networks, the global approach of 'foodomics' relocates the subject of the investigation in food sciences in order to access to a higher information level. Foods are in general complex dynamic biomolecular systems; the interactions among the constituents are a key aspect for determining its technological, sensory, and functional properties. Furthermore, the impact of foods on human physiology is often the result of synergistic (or on the contrary antagonistic) activity due to more than a single component. The intake of active ingredients elicits in turn complex biological responses that for many traits remain to be elucidated. It is clear, in such a scenario, that integrated multi-omic approaches will be the next step forward for progressing in food science, for understanding what foods do to human body, and for designing novel health-promoting food products. The parallel pillars of the individual -omic datasets have to sustain an orthogonal (and transversal) superior block of knowledge that is represented by foodomics (Figure 2). The new 'meta-omic' platform defined as 'nutrigenomics' aims to acquire an integrated viewpoint of the -omic datasets from interacting nutrients, host, and microorganisms, with the ultimate objective of a

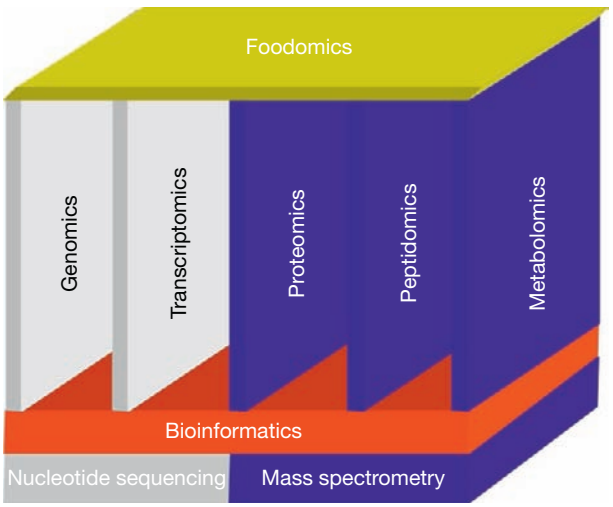

Figure 2 Foodomics, regarded as a transversal superior level of information in food science, is sustained by the '-omic' platforms, many of which relying on mass spectrometry. The foodomic block further integrates with the dataset acquired from food effects on host and from interaction with microbiota, in the 'meta-omic' perspective of 'nutrigenomics.'

functional personalized nutrition. At the status, it seems impossible to imagine meta- or multi-omic achievements lacking the 'core' role of MS, because several among the 'omic pillars' firmly reside on the MS ground.

See also: Authenticity of Food; Bioactive Peptides in Foods; Casein and Caseinate: Methods of Manufacture; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Food Allergies; Food Allergies: Occurrence and Analysis; Food Composition Databases.

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Mass Spectrometry: Principles and Instrumentation

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Introduction to Mass Spectrometry

Mass spectrometry (MS) is a powerful analytic technique used to quantify known materials, to identify unknown compounds within a sample, and to elucidate the structure and chemical properties of different molecules. The complete process involves the conversion of the sample into gaseous ions, with or without fragmentation, which are then characterized by their mass-to-charge (m/z) ratios and relative abundances. MS depends upon chemical reactions in the gas phase where sample molecules form ionic and neutral species.

A mass spectrometer generates multiple ions from the sample under investigation; it then separates them according to their specific m/z ratio and then records the relative abundance of each ion type. The instrument consists of three major components: the ion source for producing gaseous ions from the substance being studied, the mass analyzer for resolving the ions into their characteristic mass components according to their m/z ratio, and the detector system for detecting the ions and recording the relative abundance of each of the resolved ionic species (conversion dynode with secondary electron multiplier, multichannel plate). A general scheme of a mass spectrometer is shown in Figure 1. A sample introduction system is necessary to admit the samples to the ion source while maintaining the high vacuum requirements of the technique. Mass spectrometers are instruments of high complexity and are operated by special software that controls the instrument and acquires, manipulates, and reports data.

MS represents the biggest share in the analytic industry in annual investment and sector growth and lately also on publications and citations. This is also reflected in the analytic applications in food sciences as shown in Figure 2 where the numbers of publications are plotted for different analytic techniques. The prominence of MS is evident in view of its faster growth in contrast to other techniques.

Ionization Methods

Many ionization methods are available and each has its own advantages and limitations. The ionization method to be used depends on the type of sample under investigation and the mass spectrometer available. The most common modes of ionization are electron impact (EI), chemical ionization (CI), fast atom bombardment (FAB), matrix-assisted laser desorption ionization (MALDI), electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI).

EI is widely used for relatively volatile samples that are insensitive to heat and have relatively low molecular weight. A beam of electrons passes through a gas-phase sample and collides with neutral analyte molecules (M) to produce a positively charged ion or a fragment ion. Generally, electrons with energies of 70 eV are used. The spectra, usually containing many fragment-ion peaks, are useful for structural characterization and identification. CI is applied to similar samples and analytes and is used to enhance the abundance of the molecular ion that permits the calculation of the molecular mass of a compound. Ionization begins when a reagent gas is ionized by EI and then subsequently reacts with analyte molecules to produce analyte ions. CI is a lower-energy alternative to EI that provides simpler mass spectra with reduced fragmentation. These ionization techniques are widely applied to gas chromatography separation techniques coupled to mass spectrometry (GC–MS).

FAB is a softer ionization method than EI. The target is bombarded with a fast atom beam (4–10 KeV argon or xenon) that desorb molecular-like ions and fragments from the analyte. The spectrum often contains peaks from the matrix, a few fragments, and a peak for a protonated or deprotonated sample molecule. FAB-MS is used to obtain the molecular weight of sensitive, nonvolatile compounds but is prone to suppression effects due to the high chemical background.

In MALDI, the analyte is dissolved in a solution containing an excess of a matrix that has a chromophore that absorbs at laser wavelength. A small amount of this solution is placed on the laser target. The matrix absorbs energy from the laser pulse and produces plasma that results in vaporization and ionization of analyte molecules. MALDI is used to quantify or determine the molecular weight of peptides, proteins, oligonucleotides, and other compounds of biological or synthetic origin. The amount of sample needed is very low (pmoles or less). MALDI is ideally coupled to time-of-flight (TOF) mass analyzers. Lately, MALDI is also coupled to orbitrap analyzers with applications (among others) on MS imaging.

The most common ionization methods utilized with liquid chromatography–mass spectrometry (LC–MS) include ESI, APCI, and APPI. In ESI, a solution is nebulized under atmospheric pressure and exposed to a high electric field (3–5 kV), which creates a charge on the surface of the sample droplets. Droplets rapidly become smaller through solvent vaporization and produce molecular ions. ESI is extremely useful for accurate mass measurement and for producing multiple-charged ions to high-molecular-mass substances (i.e., proteins, nucleotides, and polymers). In APCI, a corona discharge electrode is used to ionize the analyte in the atmospheric pressure region. The gas-phase ionization in APCI is more effective than ESI for analyzing less-polar species. ESI and APCI can be considered as complementary methods. The ionization process in APPI is initiated by photons emitted by a discharge lamp (typically krypton, 10 eV), which ionizes nonpolar analyte molecules, while ionization is rather selective. The utilization of combined ESI/APCI or ESI/APPI ion sources facilitates

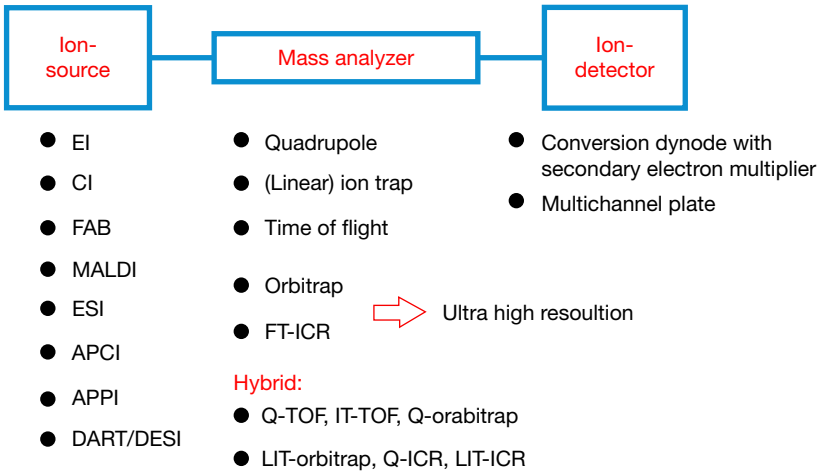

Figure 1 General scheme of a mass spectrometer.

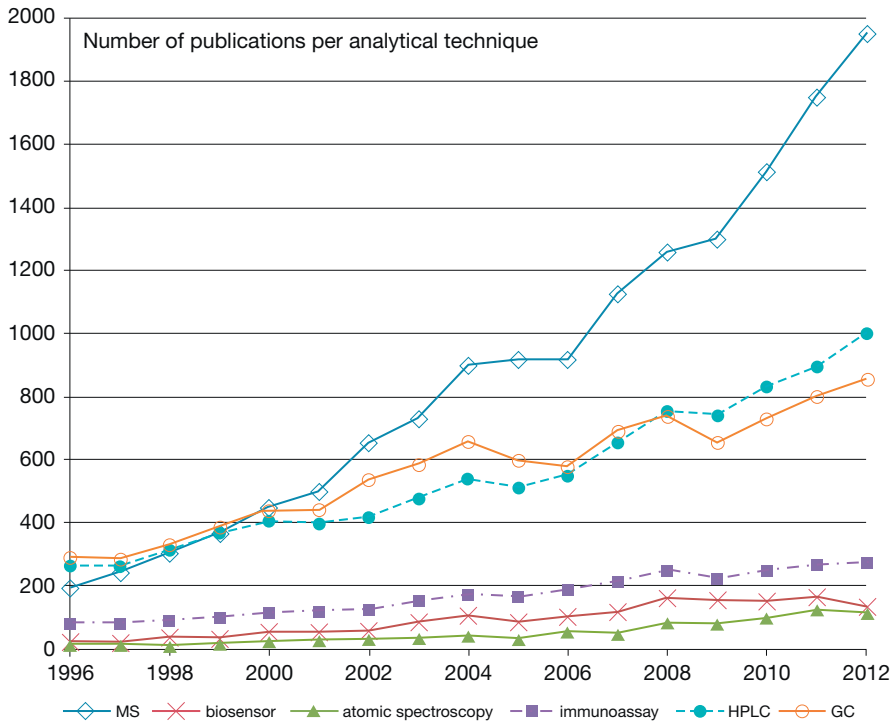

Figure 2 Plot of the numbers of publications per year and analytic technique. Search was made in Scopus in July 2013 using the terms food and the analytic technique depicted (gas chromatography, mass spectrometry, and so forth).

simultaneous detection of both polar and nonpolar analytes, which is useful for high-throughput biological screening to increase the analytic coverage of the method. Figure 3 represents the relative use of individual atmospheric pressure ionization techniques (ESI, APCI, and APPI) in LC–MS papers based on the Web of Science search from 2012.

Mass Analyzers

The main function of the mass analyzer is to separate, or resolve, the ions formed in the ionization source according to their m/z ratios. There are a number of mass analyzers currently available, the better known of which include quadrupoles (Qs), TOF analyzers, ion traps (ITs), Fourier transform ion cyclotron resonance (FT-ICR), and orbitrap configurations. These mass analyzers are characterized by different features, including the m/z range that can be covered, the mass accuracy, and the achievable mass resolution. The compatibility of different analyzers with different ionization methods varies. Table 1 gives an outlook of some of the most fundamental characteristics of the common mass analyzers.

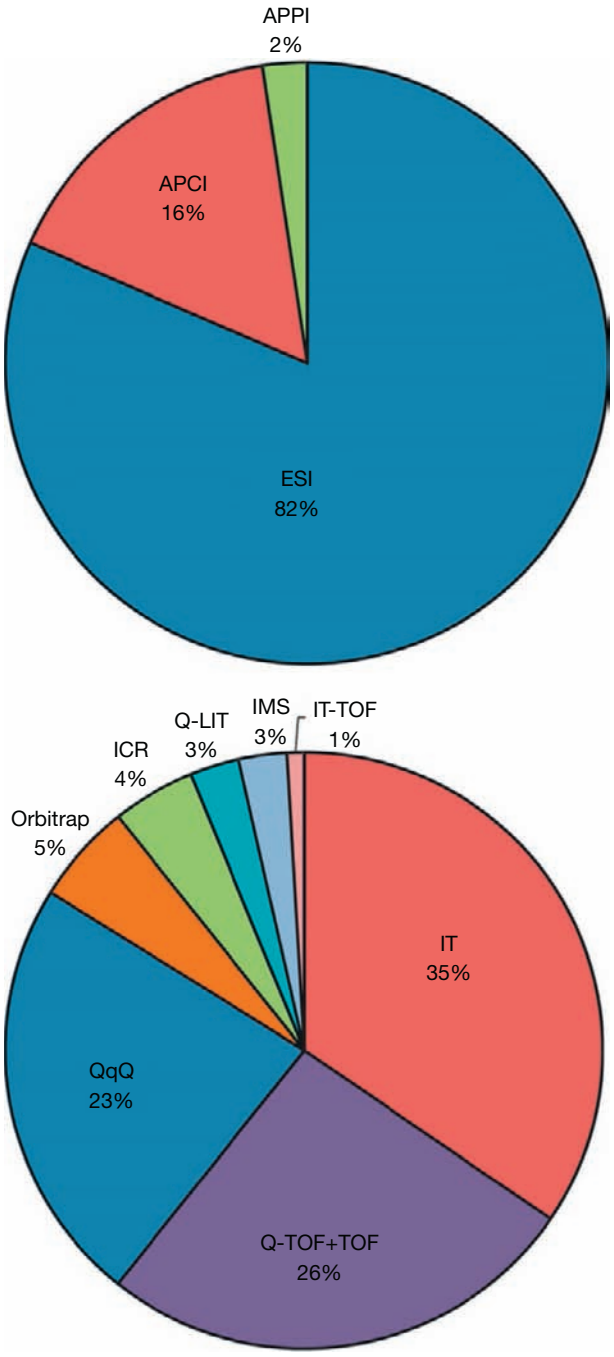

Figure 3 Relative use of individual atmospheric pressure ionization techniques and types of mass analyzers in LC–MS papers based on the Web of Science search from 2012. Reproduced from Holcapek, M., Jirásko, R. and Lísa, M. (2012). Recent developments in liquid chromatography–mass spectrometry and related techniques. *Journal of Chromatography A* **1259**, 3–15, with permission from Elsevier.

Quadrupole

The quadrupole mass analyzer is the simplest, most affordable, and robust mass spectrometer. The limitations of quadrupole analyzers are the low resolution (<3000), poor mass accuracy (>100 ppm), and limited mass range (typically up to 3000 amu). Quadrupoles are easily coupled to GC–MS or LC–MS due to their rather low vacuum requirements and high scanning speeds. As a result, quadrupoles are the most frequently used mass analyzers. Quadrupoles (also termed Q analyzer) consist of four parallel rods that function as electrodes: positive and negative terminals of a direct current (DC) source. Variable radiofrequency alternating current (AC) potentials are applied to each pair of electrodes. Ions accelerated between the rods are resolved by modulating the AC/DC potentials. Quadrupole MS are the workhorse of life sciences and the instruments of choice for a huge number of analyses. Most manufactures offer a number of different configurations that in the case of triple quadrupole MS/MS instruments offer increasing sensitivities (typically up to an order of magnitude) but with the corresponding economic cost.

Ion trap

ITs have distinct advantages over the quadrupole, as it is capable of tandem mass spectrometric experiments up to MS^n, thus offering unparalleled potential for the identification of unknowns and *de novo* structure elucidation. Upon introduction into the IT, ions are trapped by the AC voltage applied to the ring electrode. Then, they are cooled to the middle of the trap due to the presence of helium as dampening gas. Then, selective instability scanning is performed, manipulating the voltages so that ions are ejected toward the detector in the order of increasing m/z ratio. Linear ion traps (LITs) have a higher ion capacity, resulting in increased linear dynamic range in quantitative analysis. The resolution of ITs is slightly higher in comparison with quadrupoles.

Time of flight

TOF-MS is based on the fact that ions with the same energy but different masses travel with different velocities. Basically, ions formed by a short ionization event are accelerated by an electrostatic field obtaining the same kinetic energy and then travel over a drift path to the detector. The lighter ions arrive before the heavier ones and a mass spectrum is recorded. Measuring the flight time for each ion allows for the determination of their m/z ratios. Advantages of TOF mass analyzers include their simplicity, ruggedness, high scan speed, a virtually unlimited mass range, and a higher resolution compared with Q and IT mass analyzers. However, TOF mass analyzers suffer from limitations in resolution, related to the relatively large distribution in flight times among identical ions.

Orbitrap-MS and FT-ICR-MS

Fourier transform instruments such as ICR or orbitrap provide much higher resolution and mass accuracy compared with other mass analyzers. Orbitrap is basically an electrostatic IT: Ions are trapped and then they travel in circular motion before being ejected according to their m/z values. The orbitrap technology has rapidly emerged in the last years, improving performance and reaching mass resolutions higher than 240 000, with mass accuracies lower than 1 ppm (internal calibration). Orbitrap-based mass spectrometers are widely used in all major applications of life science such as proteomics, metabolomics, and environmental, food, and safety analysis. Orbitraps are interfaced not only to LC but also to ambient real-time ionization methods.

Table 1 Characteristics of the most common mass analyzers

	Quadrupole	*Ion trap*	*Time of flight*	*Orbitrap*	*FT-ICR*
m/z range (amu)	Up to 3000	Up to 20 000	Virtually no limit	Up to 6000	Up to 30 000
Mass accuracy	>100 ppm	<30 ppm	5–100 ppm	<1–2 ppm	<0.6 ppm
Mass resolution (FWHM)	<3000	<5000	5000–40 000	>240 000	2.5×10^6
Scanning rate	0.5–4 Hz	1–10 Hz	20–200 Hz	0.3 Hz	1 Hz
Quantification	Good–very good	Poor	Medium–good	Medium	Medium
Handling	Easy	Easy	Easy–medium	Medium	Demanding
Cost (k€)	<150	<150	<250	<500	<1000

The FT-ICR mass analyzer is an extraordinary instrument, considering its extremely high price, large size, extremely high vacuum needs, resolution (which could be higher than 10^6), and mass accuracy (routinely <1 ppm with external calibration or even lower with internal calibration). Recently, a Q-ICR was introduced achieving a mass resolution up to 2.5×10^6 with a mass accuracy < 0.6 ppm. Ions are placed in a very strong magnetic field (5–14 T), which causes them to move in circular paths. Ions are trapped by the magnetic field, and next, wide-band frequency excitation is performed. The ion motion induces an AC image current. The image current is recorded and is Fourier-transformed to produce mass spectra. FT-MS instruments offer the strongest potential for the identification of unknown analytes. FT-MS can be coupled to LC, but it should be kept in mind that such devices are typically operated only by highly trained personnel.

Tandem Mass Spectrometry

Tandem mass spectrometers (MS/MS) are instruments that have more than one analyzer and so can be used for structural and sequencing studies. In MS/MS, the ion of interest is selected in the first analyzer and collided with inert gas atoms in a collision cell, and the produced fragments are separated by a second analyzer. Two, three, and four analyzers can be incorporated into commercially available tandem instruments. Analyzers are not (necessarily) of the same type, in which case the instrument is called a hybrid mass spectrometer. Common tandem mass spectrometers include those of the triple-stage quadrupole (QqQ) ITs. Popular hybrid configurations include combinations of TOF, orbitrap, and ICR with Q or LIT as the first mass analyzer (Q-TOF, IT-TOF, Q-orbitrap, LIT-orbitrap, Q-ICR, and LIT-ICR). The common parameters that evaluate the overall performance of such mass spectrometers are the resolving power, mass accuracy, sensitivity, acquisition speed, linear dynamic range, and price. QqQ provides higher sensitivity compared to other mass analyzers and better linear dynamic ranges, followed by the IT and TOF mass analyzer configurations. High resolving power is obtained by TOF mass analyzers and ultrahigh resolving power by orbitrap and ICR mass analyzers, but the latest suffer from limitations in the dynamic range. As one of the most important tasks in analytical chemistry is sample throughput, high-speed separation techniques are applied to increase efficiency. The use of small particle size at ultrahigh pressures in LC requires high acquisition speed for these mass spectrometers. The fastest mass analyzer within common speeds between 10 and 50 Hz is the TOF mass analyzer, with the Q-TOF combination achieving up to 100 Hz.

Real-Time Analysis Mass Spectrometry

Direct analysis without sample preparation is an ultimate goal in analytical chemistry. In the last years, several approaches have been introduced for direct analysis to MS with limited sample preparation and without the use of chromatographic separation. Real-time analysis mass spectrometry techniques allow a direct, online measure of volatile organic compounds (VOCs) or semivolatile organic compounds (SVOCs). These configurations increase sample throughput but may still suffer from ion suppression, caused by matrix interferences, thus hindering analyte quantitation.

Popular ambient ionization techniques include direct analysis in real time (DART) and desorption electrospray ionization (DESI).

DART/DESI Source

DART ion source can analyze gases, liquids, solids, and materials on surfaces. DART is an APCI-like ionization technique. Helium or nitrogen is used for producing ionizing plasma. The gas flows through a chamber where an electric discharge (corona discharge) produces ions, electrons, and excited-state (metastable) atoms and molecules that generate protonated gaseous water clusters in ambient air. The water clusters exchange protons with the sample, giving rise to positive ions. In negative ion mode, oxygen water clusters are deemed responsible for ionization. DART has been used to analyze a wide range of analytes including drugs, pesticides, toxic materials, inks, dyes, spices, beverages, and fatty acids.

In DESI, a mixture of liquid similar to LC mobile phases is used to provide a spray. DESI provides soft ionization desorbing ions directly from animal or plant tissue or biological materials. Desorbed ions can be analyzed by atmospheric pressure ionization mass spectrometers. The working species in DESI appears to be the charged droplets or desolvated solvent ions generated in the electrospray process. DESI generates secondary ions directly from the surface of the material of interest.

Proton Transfer Reaction-Mass Spectrometry

Proton transfer reaction-mass spectrometry (PTR-MS) is based on efficient implementation of soft CI combined to the swarm technique of the flow tube and flow drift tube. The generation of precursor reagent ions and CI of VOCs are individually controlled and spatially separated processes. This leads to constant and well-defined conditions, enabling quantitative determinations without the use of gas standards. Briefly, the sample

gas is continuously introduced into a drift tube where it is mixed with a very intense pure (>99.8%) beam of H_3O^+ ions. VOCs with proton affinities higher than water are ionized by proton transfer from H_3O^+, pass through the mass analyzer (a Q in the early version), and are detected as ion counts per second. Since most of the constituents of normal air (i.e., O_2, N_2, Ar, and CO_2) have proton affinity lower than water, normal air is used as buffer without interferences. Recent introduction of TOF-MS brought significant improvements in mass accuracy and dynamic range. The first applications of PTR-TOF-MS in food science focussed on the study of milk storage conditions on the VOCs profile of Trentingrana cheese and the monitoring of VOC evolution during lactic acid fermentation of milk. PTR-TOF-MS was recently proposed as a novel tool for fruit metabolomics analysis due to its high-throughput capabilities. PTR-MS facilitates direct online monitoring of fast processes that cannot be followed by other techniques (e.g., GC), for example, monitoring of flavor release during eating. Analytic capabilities of the technique have been, now, extended by the coupling with a switchable reagent ion system (H_3O^+, O_2^+, NO^+, Kr^+, or Xe^+) that allows for virtually all volatile substances to be detected in real time.

Hyphenated Technologies

GC–MS in Food Analysis

GC–MS combines the features of GC with those of MS. GC can separate compounds and MS can provide specific, sensitive detection and structural information. As a result, GC–MS is considered a 'gold standard' for substance identification and the method of choice, the method required by the regulatory authorities for a wide number of analyses of high significance such as the analysis of pharmaceuticals/drugs of abuse, steroids and hormones, contaminants, and other analytes.

A major advantage of GC over other separation methods (e.g., LC) is its simplicity and higher separation power.

Food products are usually complex matrices made of organic or inorganic substances that consist of essential body nutrients, such as carbohydrates, fats, proteins, vitamins, or minerals. Furthermore, different sources of contaminants such as pesticides, environmental pollutants, natural toxins, veterinary drugs, and packaging materials can taint foodstuffs. GC–MS is extensively used for the analysis of such complex matrices. GC–MS is the method of choice for the VOC analysis and for routine qualitative and/or quantitative determination of components such as fatty acids, sterols, alcohols, oils, and low-mass carbohydrates and for the detection and quantification of a large number of contaminants. MS is ideally coupled to GC, for example, GC is the 'classical' method for the determination of fatty acid composition, but only the coupling with MS, allowing the identification of the number of double bonds, can provide peak assignment and compound identification.

Depending on the scope of the analysis, different GC–MS solutions are nowadays available. GC–MS following derivatization is used in the quantification of several food constituents such as carbohydrates or phenolic acids in fruits and vegetables. For the detection and quantification of target compounds in complex matrices at trace levels, GC coupled to triple quadrupole MS is a very powerful instrument providing greater selectivity and sensitivity than single quad rupole MS. The system provided higher selectivity, resulting to less interference from coeluting compounds and matrix and consequently a better signal-to-noise (S/N) ratio, allowing quantitation with lower limits. Furthermore, MS/MS instruments facilitate the development of multiresidue analysis of pesticides. Nowadays, in a single GC run, it is possible to analyze hundreds of trace compounds in food products such as environmental contaminants or pesticides.

Comprehensive two-dimensional GC (GC × GC) coupled to TOF-MS facilitates the analysis of very complex matrix, that is, components of essential oils, residual pesticides, or contaminants in food products. In GC × GC, compounds are separated on two columns of different selectivities connected in series by a modulation device. The modulator continuously collects small fractions of the effluent from the first column that are quickly transferred to the second column. GC x GC provides much higher peak capacity, better separation power, and enhanced sensitivity. TOF-MS provides high data acquisition speed suitable to GC x GC even though high-speed Qs are nowadays available. Recent applications in food-related topics include the investigation of the impact of malolactic fermentation on the volatile composition of Pinotage wines and the analysis of VOCs in cacao beans and its quality control.

LC–MS/MS in Food Analysis

The combination of LC with MS/MS is currently the most popular technique applied for the analysis of contaminants (veterinary drugs, growth promoters, mycotoxins, organic contaminants, and pesticides) and nutrients (vitamins, flavonoids, amino acids, terpenoids, and saponins) in food.

The most common MS/MS mass analyzers are QqQ, Q-TOF, and QqLIT using APCI, ESI, and APPI ionization techniques, covering a very wide applicability range (molecules of different properties such as polarity or molecular weight). APPI ionization overcame the difficulty in analyzing nonpolar compounds such as vitamins (carotenoids) that represent essential ingredients. Despite their broad applications, the quantification of some contaminants can present limitations in the selection of an adequate number of transition ions (at least 2 for the unambiguous confirmation of the analyte) and in the number of analytes detected (often reaching numbers than 150 compounds). High-resolution mass spectrometers (HRMSs) (QqTOF and LIT-orbitrap) gave the opportunity to overcome such restrictions, providing unlimited number of analytes acquired with fragmentation patterns, together with accurate mass measurements of product ions. Figure 3 shows the relative use of types of mass analyzers in LC–MS papers based on the Web of Science search from 2012. LC-HRMS full-scan modes allowed the development of multigeneric extraction procedures in the determination of hundreds of pesticides, pharmaceuticals, veterinary drugs, and growth promoters in various animal foods and feeding stuff. Figure 4 shows a representative extracted ion chromatogram of a spiked pig feed sample containing veterinary drugs with LC-ESI-orbitrap technology. HRMS data (full-scan mode) offer also the potential for revisiting data at later stages to evaluate the presence of new analytes not known at the time of

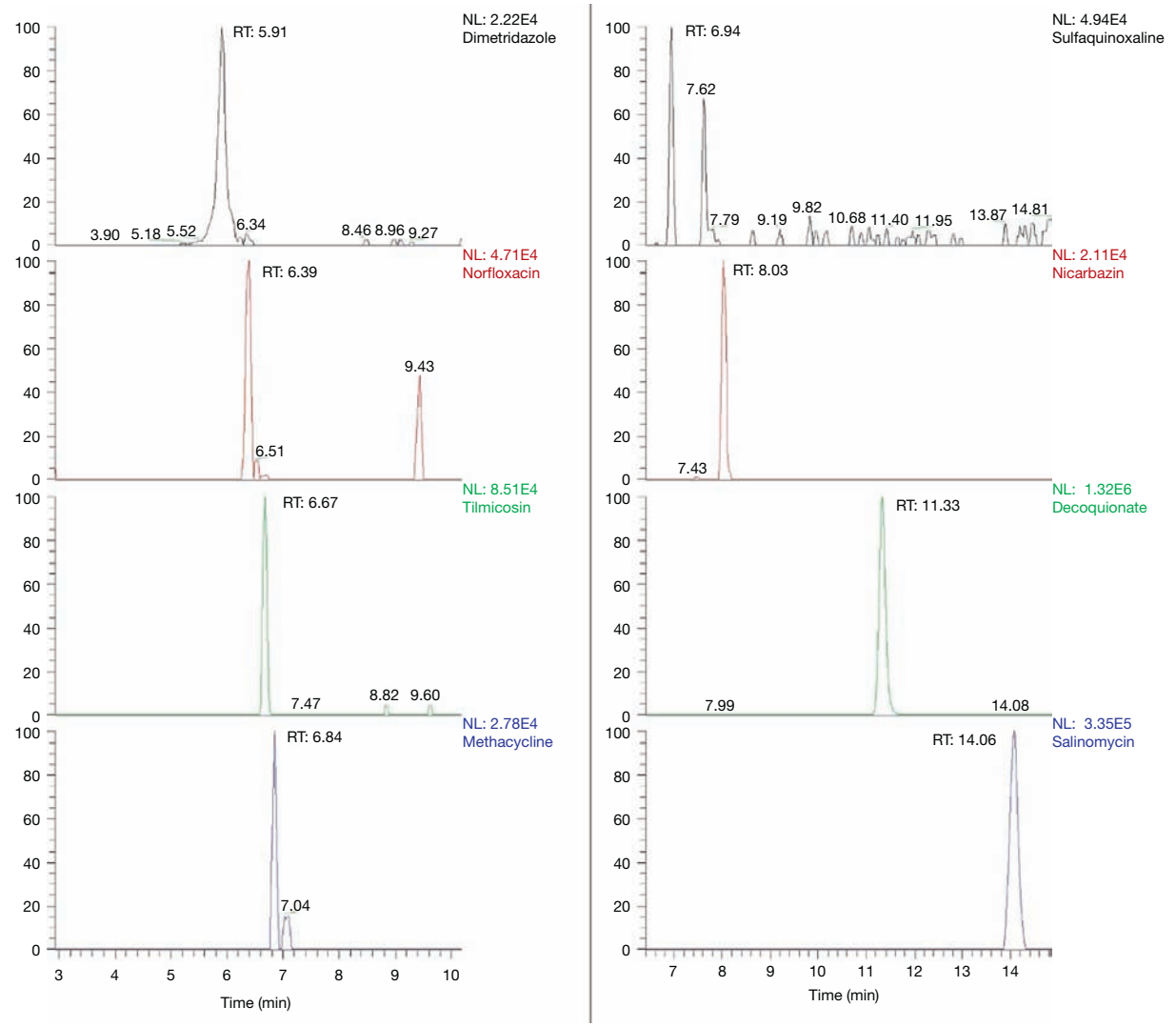

Figure 4 Representative extracted ion chromatogram of a spiked pig feed sample containing dimetridazole (RT: 5.91), norfloxacin (RT: 6.39), tilmicosin (RT: 6.67), methacycline (RT: 6.84), sulfaquinoxaline (RT: 6.94), nicarbazin (RT: 8.03), decoquinate (RT: 11.33), and salinomycin (RT: 14.06) at a concentration of 50 μg kg^{-1} (LC-ESI-orbitrap, mass extraction window of 5 ppm). Reproduced from Kaklamanos, G., Vincent, U. and von Holst, C. (2013). Analysis of antimicrobial agents in pig feed by liquid chromatography coupled to orbitrap mass spectrometry. *Journal of Chromatography A* **1293**, 60–74, with permission from Elsevier.

data acquisition, and its use is now advocated in fields such as sports antidoping control.

Technological improvements in LC promote fast separations without compromising the resolution and the separation efficiency (sub-2 μm particle-packed columns, monolithic LC columns, and fused-core columns). Such improvements in combination with improvements in fast acquisition rates of MS (QqTOF) have broadened the application field to more complex matrices. The improved resolution of ultrahigh-pressure LC can facilitate the separation of isomers, such as growth promoters, mycotoxins, and industrial contaminants in food and biological samples. Monolithic LC columns offer the potential of using high flow rates in conventional column lengths without increase of the back pressure. Monolithic LC columns have found application in food analysis, such as aflatoxins, methylxanthines, and antibiotics in foodstuffs. Core–shell materials result to faster mass transfer and less peak broadening with 'conventional' back pressure. The technology is new, but applications have been developed for the determination of organophosphorous compounds, bisphenol A, and chlorinated compounds in foodstuff.

Multidimensional LC (LC × LC) has given solutions for the separation of complex mixtures (such as natural phenols, peptides, proteins, and oligosaccharides) in food and beverages. Online LC × LC analysis is achieved by automatic transfer of the selected fraction of the analytes from the first column to a second column, which functions in a different separation mechanism. Multidimensional LC can be implemented in a number of ways and can be tailored to the requirements of particular sample types, achieving unparalleled separation power.

A topic of high significance for the health of food consumers is seen in the analysis of food allergens such as milk,

egg, wheat, tree nuts, soybeans, peanuts, fish, and shellfish food products. The topic was till recently dominated by immunoassays. MS offers high selectivity, sensitivity, and unambiguous analysis of proteins and peptides. The high resolving power and mass accuracy of LC-HRMS spectrometry (QqTOF, LIT-orbitrap, and Q-orbitrap) have opened the way for the explanation of the mechanism of action of allergenic proteins and peptides, capturing fragmentation patterns, facilitating their structural elucidation.

In the same line, powerful MS analytic platforms have also boosted the field of natural products and metabolite identification. Medical plants contain hundreds of chemical constituents. The identification and determination of the bioactive constituents are necessary in order to successfully evaluate the efficacy, safety, and quality of phytomedicines and traditional medicaments.

Nontargeted Analysis: Metabolomics Analysis

The metabolome can be described as the total content of small molecules in the analyzed sample: a biofluid, a cell, or an organ. Metabolomics include analysis toward the identification and quantitative determination of all small molecules present in the sample. The potential of metabolomics is on the correlation of concentration patterns with different pathophysiological conditions, genetic variation, or other factors or properties of the analyzed samples.

Metabolomics require the application of the highest level of analytical chemistry, advanced statistical analysis (typically multivariate statistics), solid knowledge of the biochemistry of the system under investigation, and finally expertise in the specific applied sciences of interest: agricultural, environmental, life, or other sciences. Metabolomics utilize information-rich spectroscopic techniques: NMR spectroscopy and MS coupled to chromatographic separations (LC or GC). The following section describes (in short) the application of MS technologies in food sciences.

MS-Based Metabolomics Studies in Food Sciences

In food sciences, metabolomics have found application toward various aims from gathering new biochemical knowledge to more applied/practical endeavors such as the traceability and determination of origin, breeding, and species improvement and the development of advanced methods for controlling and monitoring sensory properties. Furthermore, metabolomics hold great promise in nutritional studies either independently or as part of greater nutriomics studies where metabolomics are combined with genomics or transcriptomics findings.

MS has become the major analytic technology in metabolomics and in omics research in general. MS provides the necessary high sensitivity and high resolving power in order to detect the myriads of molecules present in the sample.

GC–MS-based food metabolomics provided the ground for the first applications of metabolomics. GC–MS has reached a high maturity state already for several years and, despite its limitations (i.e., restricted to small molecular weight $<$350 amu), is a very powerful technique. The advantages of GC–MS are its high sensitivity, wide dynamic linearity ranges, and high resolving power and the fact that it can provide analyte identities due to the existence of high-quality spectral databases, for example, NIST and Agilent Fiehn. GC–MS is typically applied following a dual derivatization scheme that first applies reaction with methoxyamine to cover carbonyl moieties and then applies silylation to cover the active hydrogen atoms (OH, NH_2, and COOH moieties). Derivatization is a dynamic organic reaction so preparations and parameter control should be thorough.

Data treatment in GC–MS metabolomics can follow two routes: The first route is to extract data with bespoke software that can perform deconvolution, analyte identification, signal normalization, peak alignment, and a number of related functions that would lead to the formation of peak table. This peak table is imported for multivariate statistical analysis that will reveal significant signals. Then, efforts are put to identify the compounds that generate the signals. In the second route, analytes are first identified by comparing retention times and EI spectra with data from libraries, and then statistical analysis is performed in a very 'light' peak table aiming to identify biomarkers.

LC–MS is the analytic method with the widest applicability and the biggest increase in annual budget (instruments and consumables). The applicability and potential of MS-based metabolomics in food sciences are immense. MS metabolic profiling can provide solid trustworthy results on a single instrumental configuration (from one injection or from one analyst) on a huge variety of substances of high importance such as biogenic amines, amino acids, organic acids, sugars, and secondary metabolites (carotenoids, flavonoids, terpenes, and so forth). This practically means that instead of resorting to ten or more different instruments, analytic conditions, and software and reporting schemes to cover, for example, 160 analytes of interest, one can now obtain this information from a single injection in one instrument. Such evolutions not only improve productivity but also improve the quality of the results, eliminating potential sources of error while augmenting the potential for standardization and protocol harmonization.

Conclusions and Perspectives

MS is now the 'biggest player' in the arena of analytic technologies and is expected to maintain its dominance for many years. The number of practitioners and installed instruments increases globally. The great versatility and utility of the technique is seen in the various different tasks that are undertaken and resolved using MS. MS can be as simple so that it can be considered as undemanding as a typical chromatography detector (in fact, new MS instruments occupy the same laboratory space with a UV detector); at the same time, ultrahigh-resolution hybrid instruments cost close to 10^7€ require very highly trained personnel and provide gigabytes of data and unparalleled wealth of information. Instrument developers invest heavily on the improvement of their products' performance and introduce new instruments with a pace unprecedented for other analytic techniques in order to cover the needs of an ever-expanding market. MS is now applied by a variety of scientists from analytical chemists to biologists, biochemists,

toxicologists, pharmacologist, forensic scientists, and many others. Especially in the field of food and nutritional sciences, MS is increasingly applied as an indispensable method for (among others) quality and safety assurance, determination of nutrients, or monitoring of sensory properties and product origin. Developments in miniaturization, quality of reporting, and data management software are also needed to help the analyst in handling data and improving productivity. Apart from developments in ion physics/chemistry and hardware, developments in the software could be seen as the crucial factor for the growth of the sector.

See also: Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: High-Performance Liquid Chromatography; Mass Spectrometry: Applications.

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Mayonnaise

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Introduction and Origins

Mayonnaise is an emulsion of oil droplets in water, a so-called oil-in-water (O/W) emulsion. Such emulsions are characterized by the presence of a dispersed phase of oil and oil-based ingredients, an emulsifier or combination of emulsifiers, and a continuous phase of water and water-based ingredients. The oil-based ingredients may be soluble or suspended in oil; likewise, the water-based ingredients may be soluble or dispersed in water. In mayonnaise, the emulsifier is typically egg-based, and the water phase typically contains vinegar.

O/W emulsions are prepared by simply blending an oil phase into a water phase in the presence of an emulsifier. Mayonnaise typically has a thick and spoonable texture that is derived from the viscosity of the water phase and/or the high dispersed oil content. The oil droplets in mayonnaise are typically of the order of 5 μm in diameter, resulting from a combination of the egg-based emulsifier and the manufacturing equipment. A light micrograph of a mayonnaise product is given in Figure 1, clearly showing a multiplicity of dispersed oil droplets (image courtesy K. Groves, Leatherhead Food Research).

Mayonnaise is believed to have originated in the mid-eighteenth century when the original concept was brought back to France in 1756 from Mahón, a city on Minorca. It was created for a victory banquet to celebrate the capture of Mahón by forces under Louis François Armand de Vignerot du Plessis, duc de Richelieu (1696–1788), a marshal of France and a grandnephew of Cardinal Richelieu. The new sauce, made with olive oil instead of cream, was named Mahónnaise in honor of the victory. The word evolved to mayonnaise, probably deriving from the old French words for egg yolk and to stir or blend, *moyen* and *manier*. Later on, the French chef Marie Antoine Carême (1784–1833) lightened the original recipe by blending the vegetable oil and egg yolk into an emulsion. This product, presumably containing water as part of the emulsion, became the basis for the mayonnaise product that we know today.

Perhaps, the first and most well-known commercial product was introduced by Richard Hellmann in the early twentieth century. Hellmann emigrated to New York City from Germany in 1903, and in 1905, he opened a delicatessen, selling salads that contained his wife's recipe for mayonnaise. Customer demand was such that Hellmann began selling the mayonnaise itself, in the 'wooden boats' that were used for weighing butter. The mayonnaise ultimately made it into jars, and in 1912, the label incorporated a 'blue ribbon' design to identify the preferred recipe. The blue ribbon survives to this day. Hellmann's mayonnaise is now owned by Unilever and is produced and consumed worldwide.

Types

Compositional Standards

Products sold as 'mayonnaise' are manufactured, distributed, and sold worldwide. The compositional standards vary widely, but there appears to be common agreement regarding the key components, namely, vegetable oil, acidifying agent, and egg components. These ingredient types will be described in section 'Key Ingredients.' The compositional standards tend to refer to a minimum content of fat or vegetable oil, with occasional references to the levels of the other key components.

In Europe, the Codex standard 168 for mayonnaise from 1989 quotes a minimum total fat content of 78.5% and a minimum 'technically pure' egg yolk content of 6%. However, this does not appear in the 2014 list of Codex standards but may still be considered to be a reference point for what tends to be known as 'real' mayonnaise; see succeeding text.

In the United States, the FDA 'Requirements for Specific Standardized Food Dressings and Flavorings,' Sec. 169.140 from 2013, quotes a minimum vegetable oil content of 65%, one or both of acetic acid (minimum 2.5%, from vinegar) and citric acid (minimum 2.5%, from lemon and/or lime juice), and egg yolk-containing ingredients.

In South Africa, Government Notice No. R. 92 of 1986 quotes a minimum vegetable oil content of 52%, together with an acidifying agent and 'egg or modified milk protein.'

In addition to the vegetable oil, acidifying agent, and egg components, there are a number of optional ingredients in all of the compositional standards, some of which are described in section 'Key Ingredients.'

Real Mayonnaise

Real mayonnaise tends to comply with the Codex compositional standard for mayonnaise, specifically the minimum fat and egg contents of 78.5% and 6%, respectively. For example, in the United Kingdom, Hellmann's Real Mayonnaise contains 79.1% fat (77% vegetable oil) and 8% egg and egg yolk.

Full-Fat Mayonnaise

Full-fat mayonnaise typically contains in the region 65–75% fat, although fat contents outside of this range are known. For example, Sainsbury's Mayonnaise in the United Kingdom contains 78% rapeseed oil, and Calvé Mayonaise in the Netherlands contains 70 g fat per 100 ml (approximately 66% w/w). The products with the lower fat contents in this category tend to include one or more thickening agents in the formulation in order to provide the desired thick and spoonable texture. The Calvé product, for example, contains xanthan gum.

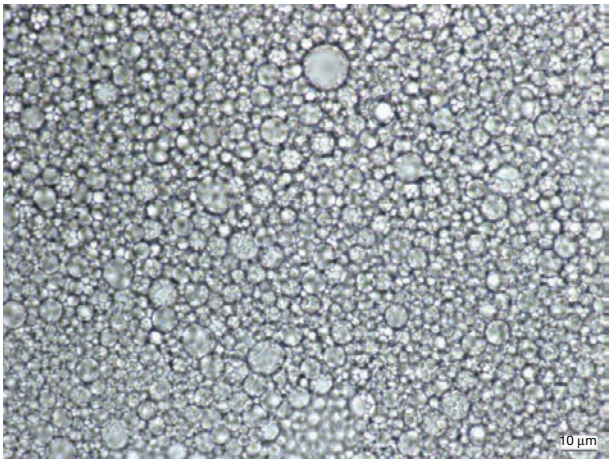

Figure 1 Light micrograph of full-fat mayonnaise.

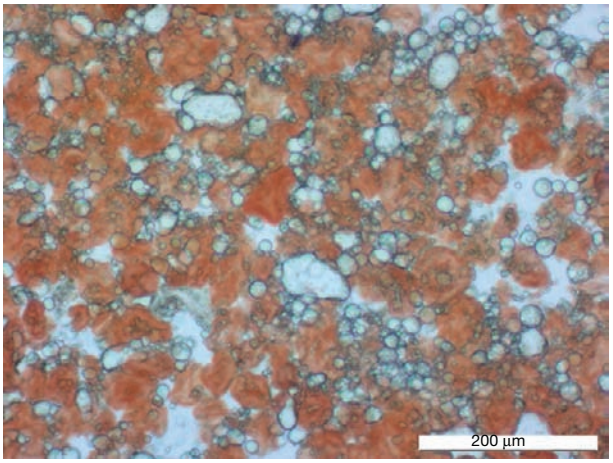

Figure 2 Light micrograph of light mayonnaise.

Confusingly, the 'Basics' mayonnaise from Sainsbury's in the United Kingdom contains only 25% rapeseed oil, presumably in an attempt to provide a low-cost option by replacing much of the oil with water. In this case, the texture has been maintained by the addition of modified maize starch, xanthan gum, and guar gum.

Light Mayonnaise

There are a multiplicity of 'reduced-fat' and 'light' mayonnaise products on the market, and they typically contain in the region 20–30% fat, although fat contents outside of this range are known. For example, in the United Kingdom, Hellmann's Light Mayonnaise contains 28% vegetable oil, and Sainsbury's Reduced Fat Mayonnaise contains 26% rapeseed oil. Both products contain modified maize starch and xanthan gum (also guar gum in Hellmann's) in order to provide the desired thick and spoonable texture. This combination of thickening agents is very common in dressing products generally.

Because of the reduced levels of fat, light mayonnaise requires lower levels of egg-based ingredients for emulsification. The Hellmann's and Sainsbury's products contain 4.2% egg and egg yolk and 3% egg yolk, respectively.

The microstructure of such products comprises a combination of an O/W emulsion as for full-fat and 'real' mayonnaise and a modified starch gel. It is likely that the starch gel dominates the texture with the emulsified oil droplets merely filling the structure. A light micrograph of a light mayonnaise is given in Figure 2, showing swollen starch granules (stained brown in iodine solution) and dispersed oil droplets (image courtesy K. Groves, Leatherhead Food Research).

Extra Light Mayonnaise

Extra light mayonnaise typically contains less than 10% fat. In the United Kingdom, Hellmann's Extra Light Mayonnaise contains 3% fat, with citrus fibers in addition to modified maize starch and xanthan gum being used to provide the desired thick and spoonable texture. Although hardly required for emulsification, the product contains 4% egg and egg yolk. The microstructure of extra light mayonnaise comprises a thickened starch-based water phase with a small amount of emulsified oil droplets.

Other Mayonnaise Types

Apart from the main types of mayonnaise products described earlier, there are a number of flavor variants, including garlic, lemon, and chili, as well more specialized types such as organic and egg-free. Egg-free mayonnaise contains alternative emulsification agents such as soya or pea protein.

Key Ingredients

Oils

The main types of vegetable oils that are used in mayonnaise products are rapeseed, sunflower, and soybean. These oils are all liquid at room temperature and can easily be emulsified at ambient temperature or above, depending on the required processing methodology. They tend to support omega-3 claims for the mayonnaise, depending on the levels applied. Sunflower oil can form waxes at chill temperatures, leading to cloudiness and possibly emulsion instability. It is therefore often 'winterized' to remove the waxes for use in mayonnaise.

Olive oil is applied at relatively low levels in olive oil mayonnaise. At higher levels, it can tend to impart a bitter taste, is relatively expensive, and can form waxes at lower temperatures, especially in the case of the extra-virgin grades. For example, in the United Kingdom, extra-virgin olive oil mayonnaise from Waitrose contains 5% extra-virgin olive oil.

Egg Ingredients

Egg-based ingredients are used in all mayonnaise products primarily for the emulsification of the vegetable oils. The phospholipids and to some degree the proteins impart the emulsification ability of yolk. Egg yolk can also contribute color and flavor at the appropriate levels. The egg-based ingredients are generally egg yolk or a blend of whole egg and egg yolk. They are pasteurized and supplied in either liquid or frozen form.

The ingredients contain salt as part of the preservation system, for example, egg yolk '92-8' comprises 92% egg yolk and 8% salt. An alternative system is '92-7-1,' which comprises 92% egg yolk, 7% salt, and 1% potassium sorbate. Egg yolk powder is also used in some products.

Whole egg tends to be applied in products for which the egg white component contributes to the structuring of the water phase, resulting in a more spoonable-type texture. For the viscous products, egg yolk alone is used.

Modified egg yolk is applied in many products. In this case, the egg yolk has been treated with the phospholipase A2 enzyme to improve the emulsification capability and generally result in a firmer mayonnaise. Crucially, the enzyme-treated egg yolk has improved temperature stability and so can be applied in 'hot' manufacturing processes without 'scrambling.'

In mayonnaise manufacture, it is generally considered preferable to keep the egg and acids apart for as long as possible. This is due to acid damage, which can reduce the emulsification capability of the egg yolk and result in a thinner product. For this reason, the vinegar tends to be added as late as possible during the manufacturing process.

The application level for egg ingredients is determined by compositional standards legislation in some markets as described in section 'Types.' In practical terms, the appropriate ratio of egg to oil for acceptable emulsification is generally 1:10 for liquid egg ingredients or 1:20 for egg yolk powder.

Vinegars and Acetic Acid

Acetic acid, in the form of vinegar, is the main acidulant applied in mayonnaise products. Acetic acid is a weak acid and the undissociated form is especially suitable as it contributes significantly to the preservation and is a key factor in stability models such as CIMSCEE (Comité des Industries des Mayonnaises et Sauces Condimentaires de la Communauté Économique Européenne). Acetic acid is mostly undissociated at the pH values normally associated with mayonnaise products, typically in the range 3.5–4.5 and ideally less than pH 4.

The dissociated acid contributes to the reduction in pH, another key factor in stability models such as CIMSCEE.

Spirit vinegar is used widely in mayonnaise products because it is colorless and contributes little to the flavor (apart from the acid taste). It is typically supplied at a strength of 12% acetic acid in water but both 10% and 14% are common. The higher levels are preferable for transportation cost reasons.

Wine vinegars, both red and white, are also extensively used, as is balsamic vinegar in specialized products. In the latter case, the strength is usually 5% acetic acid in water, but this is generally not an issue as lower levels are used.

Occasionally, chemically synthesized, food-grade acetic acid is used in addition to the vinegar. This is likely to be for cost and manufacturing efficiency reasons.

Thickeners

A wide range of water-based gelling and thickening agents are used in mayonnaise products with the most common being modified starch, xanthan gum, and combinations of these. Guar gum, locust bean gum, carrageenan, and gelatin are also applied. The modified starch may be a preswelled cold-soluble version for products that are manufactured and filled under ambient conditions or a standard cook-up version for pasteurized products. Of the range of modified starches, the most useful tend to be those that are the most resistant to shear damage through the colloid mill emulsification devices. Accordingly, they require significant heat and shear energy in order to fully swell the granules and achieve full functionality.

The new generation of 'clean-label' starches and flours does not yet appear to be widely used in mayonnaise products, presumably because they are not sufficiently robust for the typical equipment and manufacturing processes.

Gelling and thickening agents are primarily applied in order to compensate for the loss of texture due to fat reduction at total fat levels of around 70% and below. Accordingly, there has been significant research to develop specific blends in order to match the product quality of the full-fat versions. The emergence of citrus fiber as a 'clean-label' thickening agent is an example of innovation in ingredients in this area.

Other Ingredients

Apart from the obvious flavoring ingredients such as garlic and chili, there are a huge number of other ingredients that are widely used in mayonnaise products. This section is not exhaustive in describing these.

Apart from vinegar and acetic acid, citric acid in the form of lemon or lime juice is used both for the flavor contribution and for pH reduction. Citric acid does not have the preservation capability of acetic acid. Then, there are around 23 or so acids that are approved according to EU legislation as given in Table 1. These acids are added in order to achieve a reduction in pH, and accordingly, strong acids that readily dissociate are used. Phosphoric acid is an example of such an acid. Such acids however are relatively rare in comparison with citric acid and especially acetic acid.

Mustard is applied in some products mainly for flavoring reasons. The proteins and phospholipids in mustard contribute to emulsification but are much less effective in this regard than egg yolk. The mustard may be added as the liquid product itself or as mustard flour. Some mayonnaise products do not contain mustard but instead contain a mustard flavoring in the form of AITC (allyl isothiocyanate), the pungent component of mustard, dissolved in oil.

Table 1 Acids permitted for use in foods in Europe

Sorbic acid	E200	Phosphoric acid	E338
Benzoic acid	E210	Metatartaric acid	E353
Acetic acid	E260	Adipic acid	E355
Lactic acid	E270	Succinic acid	E363
Propionic acid	E280	Alginic acid	E400
Boric acid	E284	Hydrochloric acid	E507
Malic acid	E296	Sulfuric acid	E513
Fumaric acid	E297	Gluconic acid	E574
Ascorbic acid	E300	Glutamic acid	E620
Erythorbic acid	E315	Guanylic acid	E626
Citric acid	E330	Inosinic acid	E630
Tartaric acid	E334		

Salt is another component of mayonnaise that plays a role in stability models due to its functionality as a preservative. It is added as a component of the egg ingredient and may also be added directly as a separate ingredient. Salt also makes an important contribution to the taste of many mayonnaise products. A key challenge in the current environment of salt reduction is achieving the necessary preservation at the lower fat levels. As the water content is increased, so the ingredients in the water phase contributing to the microbiological safety and stability have to increase too. This includes both vinegar and salt. Developing lower fat mayonnaise products that do not taste too sour or contain too much salt in comparison with the full-fat equivalents can be very difficult. This topic will be discussed further in section '**Health Effects of Mayonnaise**.'

Sugar (sucrose) is applied in some mayonnaise products albeit at relatively low levels. It plays a role in preservation and contributes to the texture as well as the sweetness. Other sugars such as glucose and fructose, especially glucose/fructose syrup, are also used. Often, this is in combination with sugar for cost and manufacturing efficiency reasons. The current trend for sugar reduction may not be as significant for mayonnaise as for other food and beverage products because the starting levels are generally much lower and the major calorific contribution is from the fat.

Milk powder is sometimes added at low levels to impart some dairy-type flavoring notes. The small amount of protein added may make a small contribution to the emulsification of the oil phase.

The main types of coloring agents used in mayonnaise products are paprika extract, beta-carotene, and titanium dioxide. Most products are pale yellow or off-white in color and contain one of these coloring agents in order to compensate for differences in oil droplet size and color intensity of the main ingredients such as egg yolk. Titanium dioxide tends to be used for products with a relatively inefficient emulsification process, lower fat content, or larger oil droplet size, and that would otherwise result in a dull white appearance.

In addition to the key elements for preservation, namely, acetic acid, pH, salt, and pasteurization, chemical preservatives are occasionally used. The main preservative applied in mayonnaise products tends to be potassium sorbate, specifically to control the growth of spoilage organisms. These are often considered to be introduced during packaging; therefore, hot filling is another effective decontamination mechanism. Accordingly, potassium sorbate is mostly applied in products that are filled under ambient conditions.

The usage of unsaturated oils means that mayonnaise is prone to the development of rancidity through oxidation. Indeed, this is the critical quality deterioration mechanism and the main determinant of shelf life. Steps are usually taken during manufacture and filling to reduce the inclusion of oxygen, with vacuum emulsification and steam capping, for example, and the consumer advice to store the product in the fridge after opening also prolongs the life from a quality as well as a spoilage perspective. Even with these actions, rancidity can develop relatively quickly and the use of antioxidants is recommended. The antioxidant that tends to be applied in mayonnaise products is calcium disodium ethylenediaminetetraacetic acid.

Sources and Production of Mayonnaise

Introduction

Mayonnaise is manufactured worldwide in factories ranging from a few kilograms per day to several tonnes per hour. In its simplest form, the equipment is fairly straightforward, comprising blending, emulsification, and filling. The higher-fat products do not always require pasteurization as the vinegar and salt levels can control the microbiological status of the water phase, thus simplifying the manufacturing further. Accordingly, there are many artisanal producers of specialty mayonnaise products, selling their products through local outlets.

Emulsification

The colloid mill is the method of choice for the manufacture of mayonnaise, and many types are available, including both batch and continuous systems. They work on the rotor and stator principle with high shear forces generated that can effectively reduce the oil droplet size of even the most viscous products. The pumping action ensures that the shear forces are distributed evenly throughout the sample, but care has to be taken that particulates and other sensitive ingredients are not damaged too much. Pilot-scale colloid mills are available and have the advantage over many other unit operations in that they tend to be reasonably predictable in scale-up from pilot to factory production.

Colloid mills are especially suitable for mayonnaise due to the high viscosities that are required, but a number of other emulsification devices may be applicable in specific cases. The key task is to reduce the mean oil droplet diameter to ideally around 3–5 μm, and this can also be achieved using high-pressure homogenizers, high-shear mixers, membrane devices such as those supplied by Micropore Technologies, and the recently reported magnetohydrodynamic dispersion device. As in all manufacturing operations, the key task is to select the most appropriate equipment for the desired product quality and production efficiency reasons. This may require a combination of unit operations, including storage tanks for intermediate and finished products.

Emulsification processes tend to be more predicable when a preemulsification step is included. Working on the 'rubbish in, rubbish out' principle, an initial high-shear mixing step to produce a crude emulsion with a consistent mean oil droplet diameter means that a single pass through the colloid mill may be sufficient to achieve the required consistency. As part of a rigorous quality assurance scheme, monitoring the properties of the preemulsion, with appropriate control measures, is essential.

In section '**Key Ingredients**,' the challenges in selecting the most appropriate starch ingredient for the application were described. The colloid mill step can be especially damaging to the integrity of swollen starch granules; therefore, highly robust modified starches are mostly applied. These not only will be more stable to the high-shear emulsification step but also are more difficult to cook up in the first place, requiring more heat and shear energy than other grades. It is therefore essential to monitor the status of the starch granules in the

finished product and to ensure compatibility between the starch and the process, especially if a change is made. Pregelled starches are also available but tend to have more limited use in mayonnaise products due to the generally reduced robustness when compared to the cook-up versions.

Batch and Continuous Processing

Mayonnaise products are manufacturing using three basic types of process, namely, batch, continuous, and batch–continuous.

Batch processing is probably the simplest and involves the manufacture of individual batches of product in a suitable vessel and subsequent direct filling. The batch vessel ranges in size from 50 to 500 kg or more, incorporates ingredient addition and product evacuation, and operates under a vacuum to reduce the incorporation of air. A high-shear impeller generates a suitable preemulsion and the product circulates through a colloid mill device at the bottom of the vessel via a return loop back into the vessel. The mayonnaise is circulated and emulsified until the appropriate product quality has been achieved. The vessel is temperature-controlled via a jacket for either high- or low-temperature processing steps. Batch processing requires careful control and consistency of the ingredient addition and key timings in order to achieve acceptable product quality for each of the batches.

Continuous processing takes the liquid ingredient streams and combines them in a continuous manner immediately before emulsification. There may be four or six different streams and the challenge is to ensure a constant flow rate of each such that the final mayonnaise has the correct composition. In practice, this can often be difficult as the quantities of the ingredient streams may differ widely, and they may have differing viscosities. For example, in the case of full-fat mayonnaise, the quantity of oil will be much greater than any of the other ingredient streams, and for light mayonnaise, the starch/water stream will have the highest viscosity. Particular difficulties are likely to be encountered with extra light mayonnaise with a very low oil content and a very viscous starch/water stream.

Batch–continuous processing combines the two approaches in order to achieve a consistent quality product at a high throughput. In this case, relatively small batches, say, 250 kg, of preemulsion are produced and then pumped into intermediate storage tanks. This achieves a consistent feed for the subsequent colloid mill final emulsification step. The combined preemulsion batches are then processed on a continuous basis through the colloid mill and then to the filler. This combination of consistent preemulsion and long run time for the colloid mill means that the final mayonnaise product is optimized for quality and manufacturing efficiency. This methodology can be applied to all kind of products with varying levels and viscosities of the ingredient streams.

Ingredient Streams

As with all products, mayonnaise manufacture requires the addition of the ingredients in a consistent and predictable manner. In the batch process, this will involve the simple and direct addition of the ingredients in their standard liquid or powder form. However, even in this case, and certainly in the continuous and batch–continuous processes, the preparation of liquid ingredients streams may be appropriate. These are based on solutions or dispersions in oil or water and will include one or more of the following.

The starch may be added either as a cold water slurry or as a gelatinized dispersion. In the latter case, the starch/water mixture will have been heated to swell the starch granules and then maintained at a high temperature or cooled prior to adding as an ingredient stream. Other minor components such as salt, sugar, and preservative may also be added at this stage.

The remaining aqueous ingredients may be added as a separate stream. This includes any other thickening agents such as xanthan gum, salt, and sugar if not added with the starch, preservative, and antioxidant. Again, this could be a slurry, dispersion, or solution of the ingredients in water. The lemon juice, mustard, and any acids, apart from the vinegar, may be added at this stage.

The bulk oil or oils will be added as an ingredient stream. This may be simply the rapeseed oil or sunflower oil, or a blend if this is applied, or with a small amount of olive oil for this type of product. The bulk oils will generally be stored in bulk tanks and may be chilled or have nitrogen blankets in order to reduce the amount of oxidation.

The oil ingredient stream includes all of the minor oil-soluble ingredients such as the colors and flavors, together with an appropriate amount of the bulk oil in order to balance the quantities and ensure consistent addition rates of these components.

The vinegar or blend of vinegars is added as a separate ingredient stream. As stated previously, the vinegar is often added as the last ingredient stream in order to reduce the impact on the functionality of the egg ingredients for emulsification. In some manufacturing processes, the addition of the vinegar assists in the cooling of the water phase streams that have been heated for pasteurization or starch cooking.

The egg ingredients are added as a separate stream at an early part of the process in order to achieve efficient emulsification before many of the other ingredients, such as the vinegar, are added. The egg ingredients tend to be supplied in bulk containers under chilled conditions, can separate somewhat during storage, and have a limited shelf life. As stated previously, they are also always pasteurized and contain salt.

Hot and Ambient Processing and Filling

Depending on the microbiological status of the ingredients and of the finished product, the mayonnaise product may be processed under ambient conditions or with heat, and ambient- or hot-filled.

Ambient processing is reserved for those products in which the microbiology is controlled by the formulation itself. The high-fat products tend to have a small number of ingredients that are considered to be a relatively low microbiological risk, such as salt and sugar, meaning that heat treatment for decontamination is not necessary.

Hot processing is applied to those products in which the formulation cannot control the microbiology without further interventions. There are likely to be a larger number of ingredients that pose a higher level of microbiological risk, and

pasteurization is required in order to achieve the appropriate microbiological status. Hot processing is also required in order to activate the cook-up starches that are used in most mayonnaise products containing 70% fat and below.

Ambient filling is reserved for those products in which the packaging does not pose an enhanced microbiological risk. Either the packaging has been decontaminated in a previous step or the microbiology is controlled by the product. The product itself may have been ambient- or hot-processed according to the criteria described earlier. In addition, the products are likely to contain potassium sorbate as a preservative.

Hot filling is primarily applied as a mechanism for decontaminating the packaging and ensuring acceptable microbiological status. It is most often conveniently combined with hot processing, thus avoiding the need to cool the product before filling.

Patterns of Consumption of Mayonnaise

Sauce Market

Mayonnaise may be considered to be part of the global sauce category although the various market reports tend to differ in the subdivision below this level, making comparisons difficult. For example, sauces may be divided into 'cold' and 'hot,' mainly according to the host food, so cold sauces include mayonnaise, ketchup, barbecue sauces, salad dressings, and mustard and are characterized by a long open shelf life of at least 4 weeks under chilled conditions. Hot sauces then include cooking sauces for casseroles and stir-fries, with a relatively short open shelf life of a few days under chilled conditions.

A global food markets report on sauces from Leatherhead Food Research in 2010 defined sauces as follows:

- Cooking sauces (wet and dried (including soy sauce in China))
- Accompaniment sauces (e.g., ketchup)
- Mayonnaise and salad dressings
- Other sauces (e.g., mustard, vinegar, soy (excluding China), and other sauces)

In this context, the global sauce market amounted to more than 30.2 million tonnes in volume terms in 2008, with value sales in excess of USD 67.4 billion. The mayonnaise and salad dressing sector was the second largest, comprising around 26% global market value.

Mayonnaise Consumption

The global leader in the consumption of mayonnaise is Russia at about 5.1 kg per capita in 2013, over three times the amount for the United Kingdom (1.6 kg) and five times the amount for Germany (1.0 kg). Overall, the top 10 is dominated by eastern European countries as shown in Table 2.

The United States and Canada do not make the top 10 but nevertheless consume 1.9 kg per capita. This amounts to around USD 2 billion worth of mayonnaise in the United States. Within this, low-fat mayonnaise has doubled in size in the United States since 2005 and is now worth over USD 500 million.

Table 2 Global league table of mayonnaise consumption

1	*Russia*
2	Lithuania
3	Ukraine
4	Belarus
5	Belgium
6	Estonia
7	Latvia
8	Chile
9	The Netherlands
10	Poland

Availability, Absorption, and Metabolism

The nutritional characteristics of mayonnaise are dominated by those of the component oils, which are present at relatively high levels, from 20% in light or reduced-fat mayonnaise to 80% in full-fat and 'real' mayonnaise. The component oil in a mayonnaise product is usually rapeseed oil, sunflower oil, or soybean oil, as discussed in section '**Key Ingredients**,' with the occasional use of olive oil in appropriate products.

The bioavailability, absorption, and metabolism of the common vegetable oils used in mayonnaise products are described in the relevant chapters of this encyclopedia. There are no known aspects that apply to mayonnaise over and above those of the component oils. It is possible however that future nutritional studies may take into account the water-continuous basis of this product type, and the presence of other components such as salt and acid, to determine whether the bioavailability, absorption, and metabolism of the oil may be enhanced.

Health Effects of Mayonnaise

Introduction

The impact of mayonnaise products on dietary health extends beyond the bioavailability, absorption, and metabolism of the component oils as described in section '**Availability, Absorption, and Metabolism**.' Of particular concern is the fat content that can be as high as 80% and is generally around 30% even for the light and reduced-fat versions. Therefore, the potential solutions for reducing the fat level while maintaining product quality are of interest. In a similar vein, there are moves to reduce the salt and sugar levels, although in these cases, the challenge is around preservation as well as product quality. And finally, the presence of common allergens means that the 'free-from' agenda is relevant too.

Omega-3

Mayonnaise products contain omega-3 and omega-6 fatty acids from the component oils. As stated in section '**Key Ingredients**,' the oil component is usually rapeseed oil, sunflower oil, or soybean oil, and the fatty acid compositions of these oils are described in the relevant chapter of this encyclopedia. In the United Kingdom, Hellmann's Real Mayonnaise and Hellmann's Light Mayonnaise both claim to be 'a good natural

source of omega-3,' containing 8.4 g/100 g and 2.6 g/100 g, respectively, of omega-3 fatty acids 'from plant sources.' They also contain 16.8 g/100 g and 6.1 g/100 g, respectively, of omega-6 fatty acids.

Other mayonnaise products such as the one from Sainsbury's in the United Kingdom give a breakdown of the saturates, monounsaturates, and polyunsaturates, but do not quote the levels of omega-3 or omega-6 fatty acids.

Allergens

The nature and composition of mayonnaise products are such that they tend to contain some of the components that require labeling as allergens. Chief among these is egg, the emulsifying agent present in all except the egg-free versions.

Mustard is the next most popular allergen with many mayonnaise products containing this as an ingredient mainly for flavoring reasons. Some products such as Hellmann's mayonnaise in the United Kingdom contain mustard flavoring but not mustard itself. The mustard flavoring tends to be AITC in oil, as described in section 'Key Ingredients,' and as such is not protein-based as are all allergens apart from sulfite/sulfur dioxide. However, as a precaution and to guard against adverse reactions to AITC, mustard allergen labeling tends to be carried out.

Other allergens may be present depending on the mayonnaise formulation, for example, dairy when milk powders are used for flavoring reasons.

Fat Reduction

Full-fat and 'real' mayonnaise can contain 75–80% fat and may therefore be considered to be unhealthy as part of the obesity agenda. A single portion or tablespoon of mayonnaise (15 ml/14 g) can contain 11 g fat, and for this reason, the light and reduced-fat versions are important for some sectors of the population.

As described in sections 'Types' and 'Key Ingredients,' fat reduction in mayonnaise products has been successfully achieved using a range of gelling and thickening agents such as modified starch and the emerging ingredients such as citrus fiber. The extra light products containing as little as 3% fat have demonstrated that acceptable thick and spoonable textures can be achieved using these ingredients. However, such compositions, especially the highly viscous water phases, can present significant problems during manufacturing. An alternative approach that is especially suitable for liquid, water-based systems is that of water-in-oil-in-water (WOW) double emulsions.

WOW emulsions have been studied for many years for their ability to reduce the fat contents of water-continuous emulsions by 'hiding' some additional water inside the oil droplets as a second emulsion. Figure 3 is a light micrograph of a WOW emulsion, clearly showing the internal water droplets (image courtesy K. Groves, Leatherhead Food Research). WOW emulsions are prepared by firstly emulsifying the internal water phase into the oil using a highly lipophilic emulsifier. Polyglycerol polyricinoleate, PGPR (E476), is especially suitable for this purpose although lecithins have also been used. This water-in-oil emulsion is then emulsified into the external water phase using an egg ingredient such as egg yolk as the emulsifier. In this way, an emulsion has been created in which the internal water phase is considered to be part of the oil, increasing the effective oil content.

Various studies have been carried out to demonstrate the fat-reducing capability of WOW double emulsions. A project at Leatherhead Food Research involved the reduction of the fat content of a mayonnaise-type sauce by 20% using WOW double emulsion technology. The resulting emulsions were characterized using rheology and sensory analysis and found to be similar to the full-fat equivalent products prepared as standard O/W emulsions. Figures 4 and 5 clearly demonstrate these effects. WOW double emulsion technology therefore appears to be a potential alternative to the use of gelling and thickening agents for mayonnaise products containing around 70% fat or less. The potential challenges to this appear to be the requirement to use PGPR as an additional emulsifier and the requirement for two emulsification steps.

Salt and Sugar Reduction

Salt and sugar reduction activities in foods and beverages have mainly focused on gradual reductions and the use of alternative ingredients. In the case of salt, this has mostly been potassium chloride, and for sugar, a combination of ingredients for sweetness and texture. There are many options available

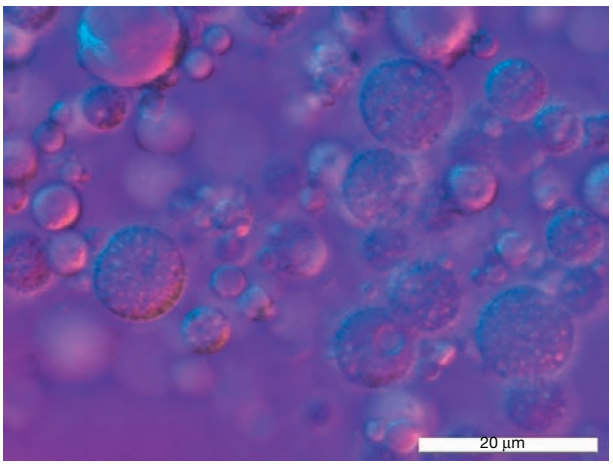

Figure 3 Light micrograph of WOW emulsion.

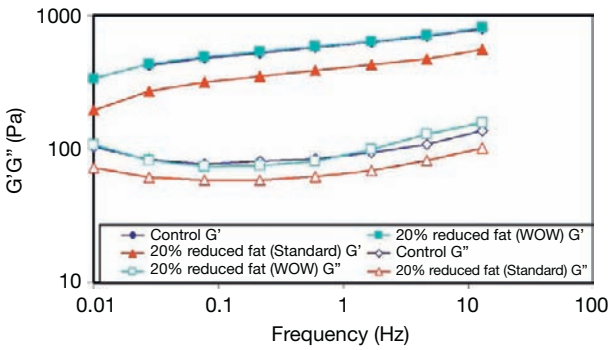

Figure 4 Rheology of emulsions.

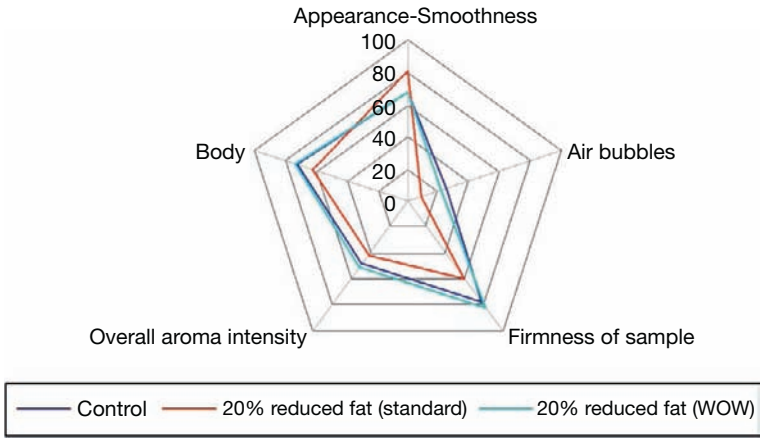

Figure 5 Sensory properties of emulsions.

although in general terms, these have not been applied significantly in mayonnaise products. As stated previously, salt is important for preservation, so care must be taken in reduction or replacement exercises. Sugar, when used, is generally at relatively low levels and contributes little to the calorific value in comparison with the fat.

WOW double emulsions have also been studied for the capability to reduce salt levels in thickened water-based foods. In this case, the technology relies on the internal and external water phases being of differing compositions, specifically the majority, if not all of the salt being present in the external water. The hypothesis is that the internal water is swallowed without being detected in the mouth; therefore, it does not need to contain any salt. A study reported in 2010 incorporated all of the salt in the external phase of a thickened soup-type WOW emulsion, with sensory assessments of the resulting saltiness. It was demonstrated that a salt reduction of 10% could be achieved with acceptable sensory performance. A potential challenge in the application of this technology in mayonnaise products appears to be the preservation of the internal water phase, although a balancing of the acetic acid and pH levels between the two water phases would assist in this.

As for salt reduction, it is possible that WOW emulsion technology could be used for sugar reduction, although there do not appear to be any reported studies. This is a potential area for future research.

See also: Emulsifiers: Types and Uses; Food Colloids and Emulsions; Stabilizers: Types and Function.

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Meat: Conversion of Muscle into Meat

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Introduction

Transformation of muscle to meat involves biochemical and metabolic processes induced by the animals' tissues in an attempt to retain homeostatic control. The size, extent, and timing of these responses postharvest can significantly affect the development of meat quality. In order to understand the influence of postharvest biochemistry on the structure of the muscle and consequent effects on quality, a brief review of muscle structure is required. Also, in order to understand postharvest muscle biochemistry, an appreciation of the importance of the contribution of energy management is required. Postharvest biochemistry of muscle has profound influences on muscle structure, with consequent effects on consumer acceptability and quality. Aberrations in meat quality are usually a result of a divergence from normal muscle biochemistry, due to variations in the genetics of the animal, stress preharvest, or chilling and freezing of the muscle. These aberrations in meat quality are discussed.

Muscle Structure and Fibers

Skeletal muscle is the largest tissue of most animal species and is composed of whole muscles, usually attached to bone by a tough inextensible connective tissue called epimysium. Muscles are subdivided into muscle bundles, which are surrounded by a connective tissue called perimysium (see Figure 1). Muscle bundles contain a number of muscle cells (also called muscle fibers), which are surrounded by a connective tissue called endomysium. Endomysium, perimysium, and epimysium are composed primarily of the protein collagen.

Muscle cells, when viewed longitudinally in the microscope, have a striped appearance due to alternating dark and light bands, which arise from the arrangement of myofibrils within the muscle cell. Individual myofibrils have these alternating dark and light bands and all myofibrils are in register and aligned within a muscle cell; hence, the striated appearance is evident at the level of the muscle cell and the myofibril and also when the myofilaments are viewed under electron micrographs (Figure 2). Thick and thin filaments, which comprise the myofilaments, are composed primarily of the proteins myosin and actin, respectively, and the light band of the sarcomere is the portion where only thin filaments are present and the dark band is where both thick and thin filaments are present. Other proteins associated with the thick and thin filaments include titin, nebulin, tropomyosin and troponin and are structural or regulatory in function. The thick and thin filaments are bounded by a z-line at each end, which is composed of the protein α-actinin, and a sarcomere is composed of two z-lines, with a pale band of thin filaments at either end, next to the z-line, or a dark band of thick and thin filaments in the middle. The unit of contraction is the sarcomere, and *in vivo* and postharvest contractions are a result of thick and thin filaments sliding past each other and attaching, which is termed the 'sliding filament theory' of contraction. *In vivo*, the presence of adenosine triphosphate (ATP) allows the myosin head on the thick filament to detach and relax from its bound state to actin. Postharvest, contraction, and shortening can occur in the sarcomeres and myofibrils, but once ATP is depleted, 'myosins' bond to actin remains as actomyosin, and rigor mortis is the result, with stiff and inextensible muscles.

An important point to make is that all muscles are not homogeneous. Muscles differ in their ratio of fiber types (slow, red, oxidative vs. fast, white, and glycolytic), their length, their width, and also their biochemistry and chemical composition. They also go into rigor at different times, and within a muscle, fibers can vary in the rate at which they go into rigor mortis. The effect of temperature on metabolic processes and on the rate of conversion of muscle to meat is also very important. Postharvest, carcasses are usually transferred into coolers within 30–60 min. But the rate of chilling can vary and the depth of the muscle and the weight of the carcass influence the rate of temperature decline in any muscle. This is exemplified in the difference in the temperature at different depths in muscles postharvest, as shown in Figure 3.

Process of Rigor Mortis

The process of rigor mortis is one of the recognizable signs of death as a result of the chemical changes in the muscles, causing the musculature to lose extensibility and become stiff. Figure 4 shows the changes that occur postharvest, using a pig carcass as an example. Synthesis of ATP is initially by creatine phosphate (CP) but later by glycogenolysis and glycolysis. CP usually drops to low levels within 1–2 h postharvest. Once CP levels drop to 75% of initial levels, ATP starts to decline. As CP disappears, Pi increases in the cell postharvest, and lactate accumulates concomitantly with increasing hydrogen ion (H^+) concentration. Once ATP reaches low levels, the extensibility of the muscles starts to approach 0% of its original, the pH approaches the final pH, and lactic acid reaches its final concentration of 5–6 μmol g^{-1} of tissue (in a well-fed, rested animal). Rigor mortis is defined by the decline of ATP to zero, 0% extensibility, an ultimate pH that is reached, and the production of lactic acid that has plateaued.

Metabolism and Biochemistry

The energy balance and biochemical reactions in muscle fibers that ensue postharvest, and end in rigor mortis, are complex and still being studied. The excellent reviews given in the suggested reading list give comprehensive information on this important topic.

In the living animal, ATP is formed through glycolysis and in the oxidative phosphorylation pathway and oxygen is

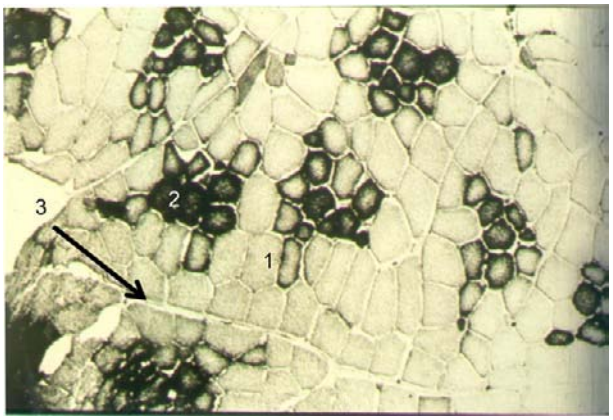

Figure 1 A cross section through a porcine longissimus muscle, with staining for succinate dehydrogenase, which differentiates between oxidative and nonoxidative fibers. 1 = fast twitch glycolytic or oxidative fiber, 2 = slow twitch oxidative fiber, 3 = a gap between muscle bundles where perimysial connective tissue exists. This cross section shows the characteristic groupings of the fiber types in porcine muscle, with oxidative fibers clustered together in a muscle bundle, surrounded by glycolytic fibers. The glycolytic and oxidative fibers are about 100 and 50 μm in diameter, respectively.

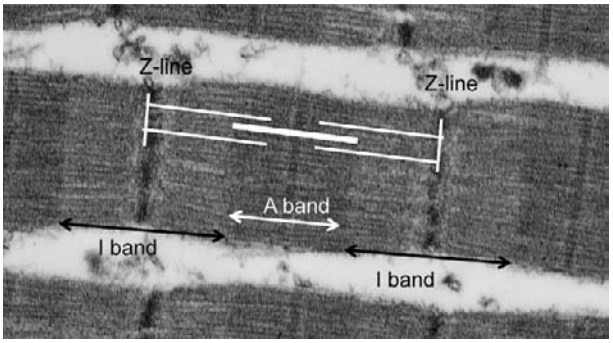

Figure 2 Transmission electron micrograph of a longitudinal view of muscle. The banding pattern is explained by the position of the thick and thin filaments. The I-band contains thin filaments and is bisected by the z-line. The A-band contains thick and thin filaments. The white lines show the thick and thin filaments and z-line, stylized to assist in understanding the structure. The sarcomere spans from one z-line to the next and is ~1.8 μm.

available to cells via the bloodstream. Energy-rich compounds, such as ATP, are used to maintain homeostasis in cells including steady state of reactions to produce heat and keep body temperature constant. Energy is stored in the cells as glycogen, lipid, and CP. After harvesting of an animal, the blood supply stops, leading to anoxia throughout the organs and tissues. Anoxia of skeletal muscle results in dramatic changes in the metabolism and biochemistry both *in vivo* and postharvest. Furthermore, the loss of blood supply postharvest means that the muscles can no longer maintain body temperature and the temperature drops. These changes in skeletal muscle postharvest involve the conversion of muscle into meat and are associated with biochemical and structural changes, which determine the meat quality parameters of tenderness, juiciness, flavor, and shelf life.

The postharvest muscle cell attempts to maintain its ATP levels, as ATP is required for metabolic reactions and ion transport. ATP is split at about 0.5–0.65 μmol g^{-1} of muscle/min at 38 °C and the enzyme with the greatest demand for ATP is the myosin ATPase. In resting muscle, myosin is not bound to actin and ATP splitting is inhibited by millimolar concentrations of Mg^{2+}. A slow hydrolysis of ATP to ADP and Pi occurs and as myosin is 20–30% of the total protein in muscle, myosin ATPase accounts for the majority of ATP splitting in resting and postharvest muscle. The effect of temperature on ATP turnover is very similar to the effect of temperature on myosin ATPase activity, giving evidence for their dependence. When cytosolic Ca^{2+} increases from $<10^{-8}$ M to 10^{-6}–10^{-5} M, myosin ATPase activity is stimulated 300–500-fold. This increase in Ca^{2+} occurs not only during contraction but also during rigor mortis development. The Ca^{2+}-ATPase pumps Ca^{2+} back into the sarcoplasmic reticulum, and as membranes become leaky postharvest, ATP is broken down to support this and other active ion transport pumps such as the mitochondrial ATPase, the sarcolemmal Na^+, K^+-ATPase, and sarcolemmal Ca^{2+}-ATPase.

ATP is not only generated during glycolysis but also consumed, as shown in Figure 5. Thus, ADP is required in order for glycolysis to continue. Glycogen is the main storage unit of carbohydrate in muscle and is equivalent to starch in plant cells. Glucose-6-phosphate can be formed from glucose, a reaction catalyzed by hexokinase (see Figure 5), but very little of this reaction occurs in postharvest muscle. Glycogen is hydrolyzed to glucose-1-P by glycogen phosphorylase and the compounds are then sequentially broken down to lactate, in postharvest anaerobic muscle. The accumulation of H^+ from lactate and ATP hydrolysis account for the pH decline in muscle postharvest. The sequence of enzymatic steps in glycolysis postharvest is shown in Figure 5, and ATP is generated in two steps after fructose 1,6-bisphosphate, generating 4 ATP per glycogen–glucose molecule (as one 6-carbon molecule is split into two 3-carbon molecules). Also and importantly, ATP is consumed in two reactions prior to fructose-1,6-bisphosphate. Thus, only three ATP are generated from the conversion of a glucose-1-P molecule to lactate. This process is called anaerobic glycolysis and, in the living animal, is recognized to be very inefficient and can only be used in short bursts when demand for energy is high, such as sprinting. In the living animal, the lactate produced from anaerobic glycolysis is transported out of the muscle, via the blood to the liver, where it is reconverted to glucose. Postharvest, in the absence of circulating blood, it remains as a waste product and causes the decline in pH. The hydrolysis of nucleotides (ATP, ADP, AMP, and IMP) also generates hydrogen ions (and Pi) (see Figure 6); thus, recent literature has pointed out that the pH decline postharvest is due to hydrogen ions accumulating not only from lactic acid but also from nucleotide hydrolysis. In order for glycolysis to proceed, ADP is required by the steps involving the enzymes phosphoglycerate kinase and pyruvate kinase.

The postharvest contribution of creatine kinase- and adenylate kinase-catalyzed reactions to ATP production is shown in Figure 6, as well as the interconversion and degradation of high-energy phosphates.

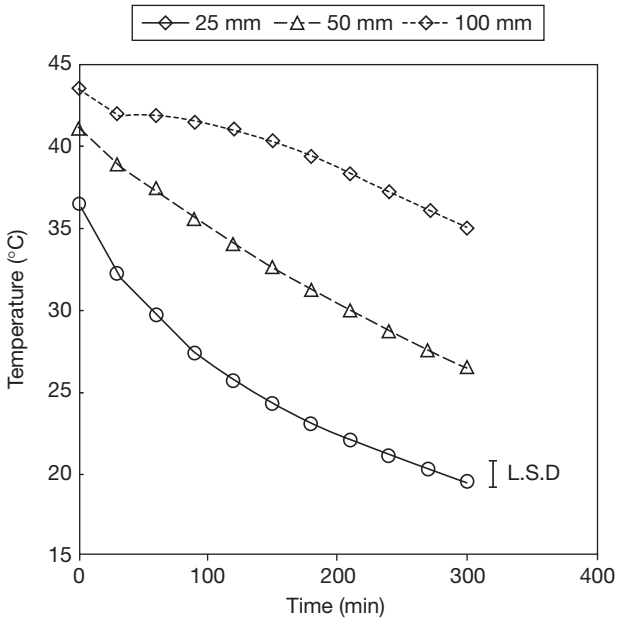

Figure 3 The temperature (°C) of the fascial seam between the *M. semimembranosus* and *M. semitendinosus*, in a beef carcass, at depths of 25, 50, and 100 mm from the surface. (Values are means. LSD represents the average least significant difference ($P < 0.05$) for comparing different means for different depths and different times. Differences between means that are greater than the LSD are significantly different.) Reproduced from Jacob, R. H., Beatty, D. T. and Warner, R. D. (2014). A preliminary study into the use of heat pipes to prevent high rigor temperature in beef carcasses by increasing cooling rate. *Animal Production Science* **54**, 504–509.

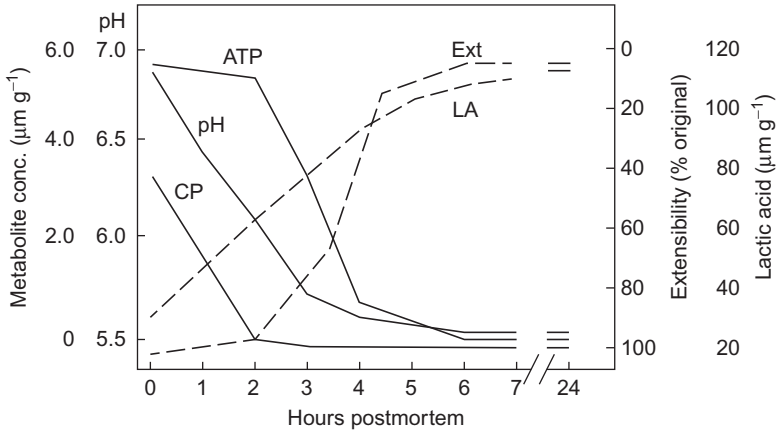

Figure 4 Chemical and physical changes in muscle postharvest. The pattern shown is typical for pig muscle undergoing normal postharvest metabolism. Abbreviations: ATP, adenosine triphosphate; CP, creatine phosphate; LA, lactic acid; Ext, extensibility. Reproduced from Greaser, M. L. (2001). Postmortem muscle chemistry. In: Hui, Y. H., Nip, W.-K., Rogers, R. W. and Young, O. A. (eds.) *Meat science and applications*, pp. 21–37. New York: Marcel Dekker.

Before glycolysis starts postharvest, creatine phosphate (CP) maintains the ATP concentration according to the reaction

$$CP + ADP + H^+ \rightarrow ATP + \text{creatine}$$

Thus, creatine phosphate acts as a short-term ATP buffer and amounts to 20–30 mmol kg^{-1} in resting muscle. Thus, in the initial period 0–30 min postslaughter, there is no fall in pH and ATP levels remain constant, as CP is supplying ATP (see Figures 4 and 6). CP is quickly exhausted, and as the demand for ATP continues, glycolysis proceeds and the reactions shown in Figure 5 ensue.

The net equation can be simplified as

$$\text{Glucose (from glycogen)} + 3\ ADP + 3Pi + 3H^+ \rightarrow 3\ ATP + 2\ \text{lactate} + 3H^+$$

As can be seen in Figure 5, this is an oversimplification as it is a complex series of steps with enzymes catalyzing each reaction. The enzyme phosphofructokinase is seen as a key regulator of the rate of glycolysis, and it has recently been

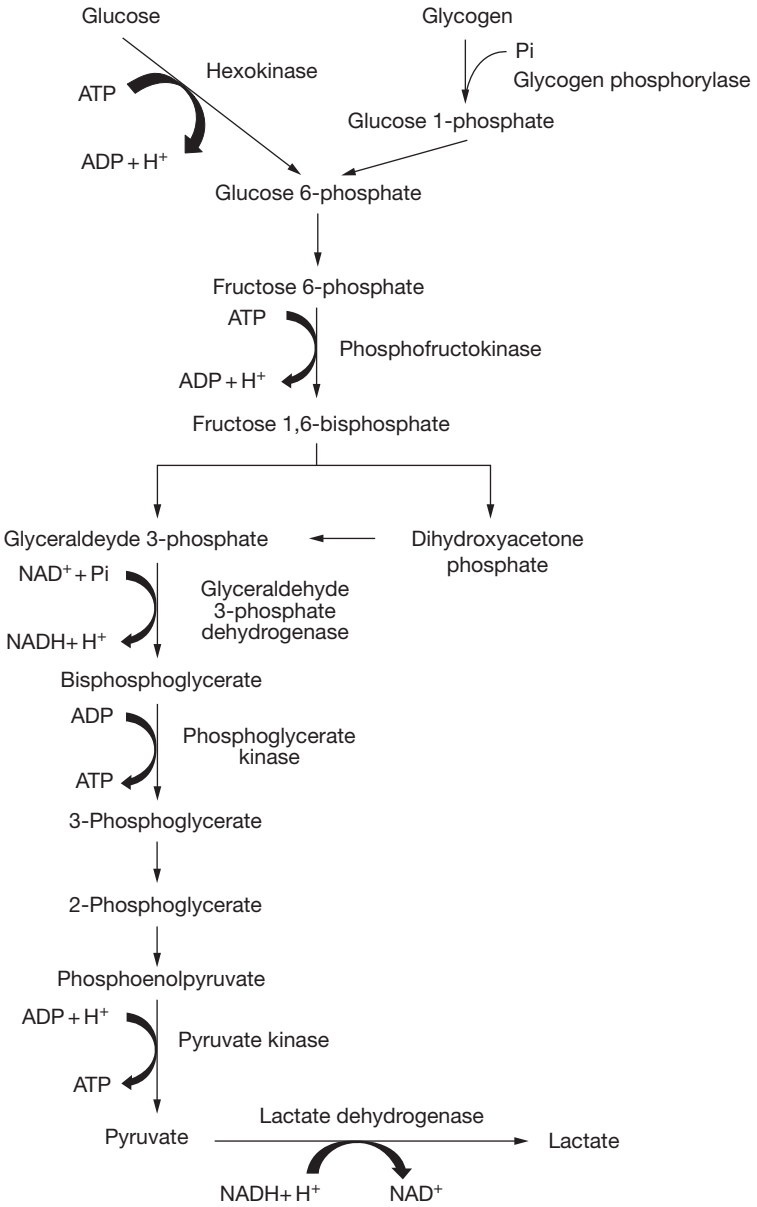

Figure 5 The glycogenolytic/glycolytic pathway. Glucose or glycogen is metabolized via glycolysis. Under anaerobic conditions, the final product, pyruvate, is converted to lactate. H^+ are released during the early steps when ATP is 'invested' as well as in the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase; H^+ are consumed during the ATP-harvesting step catalyzed by pyruvate kinase and in the production of lactate. Reproduced from Scheffler, T. L., Park, S. and Gerrard, D. E. (2011). Lessons to learn about postmortem metabolism using the AMPK [gamma] 3R200Q mutation in the pig. *Meat Science* **89**, 244–250.

conclusively shown that this enzyme fails below pH 5.5, thus causing the cessation of glycolysis. Previously, it was thought that the loss of adenosine dinucleotides to the IMP form was the cause of glycolysis cessation at pH < 5.5.

Finally, glycolysis is driven by the demand by ATPases for ATP (Figure 6). The main ATPase-consuming ATP postharvest is the myosin ATPase, but the ATPases in the sarcolemma and mitochondria also contribute. The hydrolysis of ATP by these ATPases generates H^+ ions as shown in Figures 6 and 7. The consumption of ATP by mitochondria postharvest has recently been discussed as 'mitochondrial treason' and is considered to contribute to postmortem pH decline and biochemistry.

Changes in Muscle Structure That Impact on Meat Quality

The extent and rate of pH fall are influenced by biochemical events pre- and postharvest, which have their action on structural components in muscle cells and the associated connective tissue. The influence of rate and extent of pH fall on protein denaturation, myofilament lattice spacing, and the shrinkage in muscle cells is fundamental in determining the quality of raw meat. Furthermore, the influence of the extent of pH fall (ultimate pH) on muscle structure is a fundamental mechanism behind many genes associated with quality.

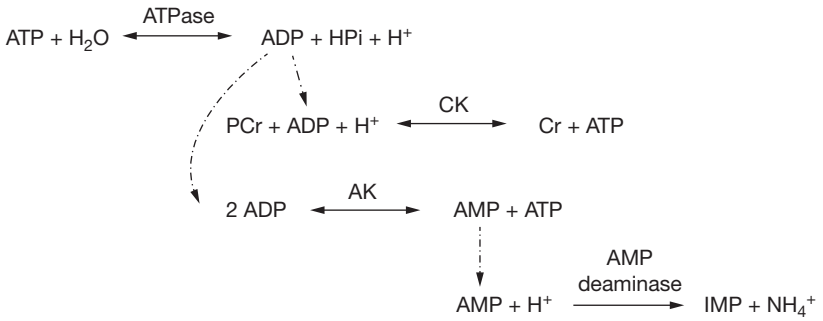

Figure 6 Contribution of creatine kinase (CK)- and adenylate kinase (AK)-catalyzed reactions to ATP production. Creatine phosphate is labeled as PCr (phosphocreatine) in this diagram. AMP is deaminated to inosine monophosphate (IMP) to preserve high ATP/ADP and free energy of ATP hydrolysis. H^+ are released by ATP hydrolysis, and H^+ are consumed by the CK and AMP deaminase reactions. Reproduced from Scheffler, T. L., Park, S. and Gerrard, D. E. (2011). Lessons to learn about postmortem metabolism using the AMPK [gamma] 3R200Q mutation in the pig. *Meat Science* **89**, 244–250.

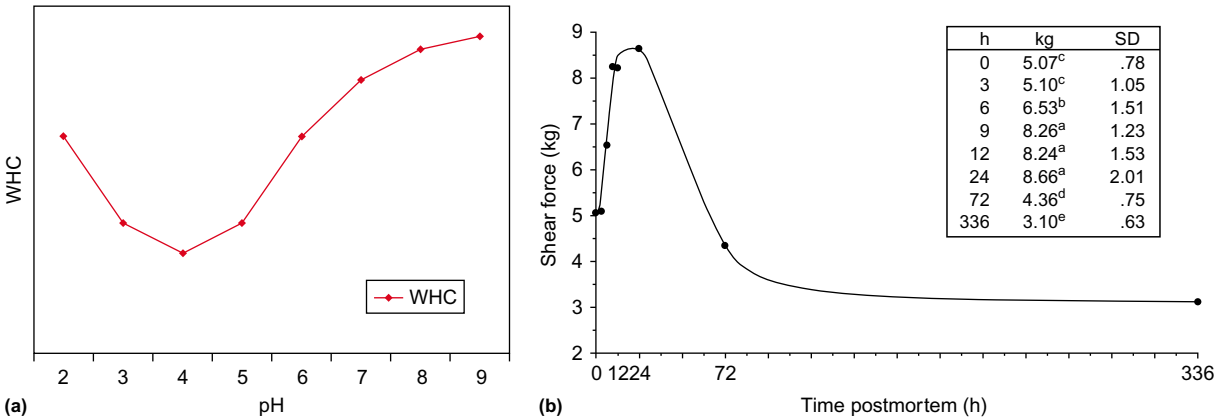

Figure 7 (a) Influence of pH on the water-holding capacity (WHC) of muscle homogenates. Reproduced from Brewer, M. S. (2014). Chemical and physical characteristics of meat|water-holding capacity. In: Dikeman, M. and Devine, C. (eds.) *Encyclopedia of meat sciences* (2nd ed.), pp. 274–282. Oxford: Academic Press. (b) Warner–Bratzler shear force of lamb *longissimus thoracis et lumborum* at specific times postharvest. Mean separation and SD are shown in the inset. Means without a common superscript differ ($P < 0.05$). Reproduced from Wheeler, T. L. and Koohmaraie, M. (1994). Prerigor and postrigor changes in tenderness of ovine longissimus muscle. *Journal of Animal Science* **72**(5), 1232–1238.

Water-Holding Capacity

Postrigor muscle is about 75% water, with up to 87% of the volume of the muscle cell being myofibrils, which contain the majority of the water. Hence, any changes in the length or the diameter of the myofibril impact on water loss from the myofibril, and hence the muscle cell, and eventually the meat. The decline in muscle pH postharvest associated with the glycolysis and onset of rigor mortis results in a fall in pH from about 7.0 to about 5.5, in a muscle of 'normal' quality. As the pH drops, it comes closer to the isoelectric point (the point at which the molecules carry a net zero charge) of the muscle proteins (~5.0–5.1), resulting in a reduction in negative charge between the myofilaments, and these myofilaments can then approach each other more closely. As the myofilaments approach each other, with a consequent shrinkage in the diameter of the myofibril, less water is held in the myofibrillar structure and the water moves to the intracellular space, and subsequently to the extracellular space, and then is lost from the muscle structure as drip, with associated reduction in water-holding capacity (Figure 7(a)). In addition, if myosin is denatured postharvest, due to conditions of fast pH fall at high muscle temperatures (e.g., pale, soft and exudative (PSE), or heat shortening; see in the succeeding text), the myosin head shrinks and draws the thin filament in more closely, causing a shrinkage in the myofibril diameter and further water loss. Finally, if the sarcomeres within the myofibril shorten, causing a reduction in the length of the myofibril, as may occur due to heat shortening, rigor shortening, or cold shortening (see in the succeeding text), this will also cause loss of water from the structure and reduced water-holding capacity of the muscle.

Tenderness

In the prerigor period, muscle is quite tender, due to a lack of cold, heat or rigor shortening. As rigor commences (see 'Rigor Shortening' section), the sarcomeres shorten and transverse shrinkage of the myofibril occurs, and this is associated with an increase in toughness (Figure 7(b)). The reduction in myofilament lattice spacing in the myofibrils increases the interdigitation between actin and myosin, increases the protein in a defined area, and thus impacts on measured tenderness. It is also likely that there is reduced access of proteases to the

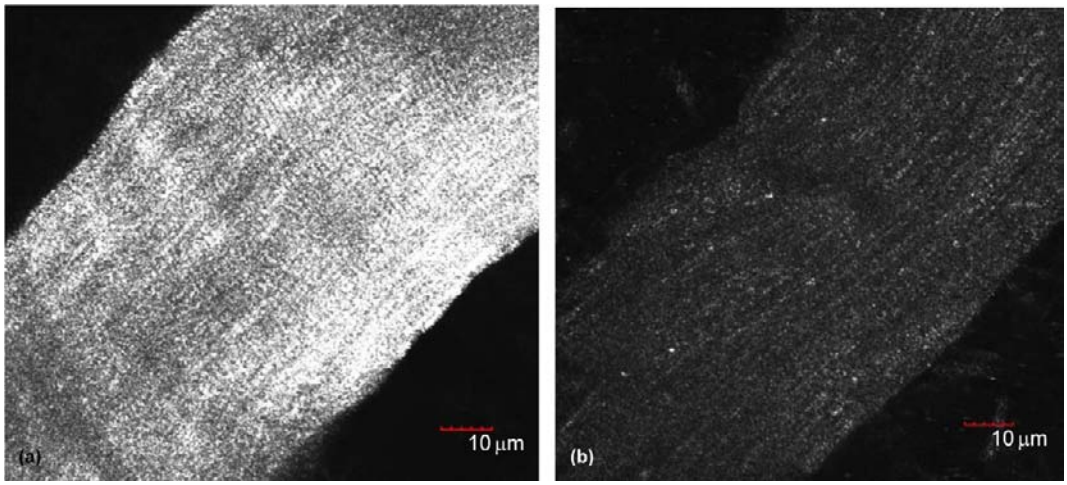

Figure 8 Reflection confocal scanning microscopy images of a muscle fiber from beef *longissimus lumborum* from (a) a low ultimate pH muscle, pH = 5.4 and (b) a high ultimate pH muscle, pH = 6.4. Reproduced from Hughes, J. M., Oiseth, S. K., Purslow, P. P. and Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity, and tenderness. *Meat Science* **98**(3), 520–532.

myofibrillar proteins, due to the reduced spacing. Proteases are the enzymes that are associated with the degradation of myofibrillar proteins and associated improved tenderness. Thus, the toughness in meat postharvest due to rigor shortening is most likely due to increased protein density and reduced access of proteases to meat protein substrate.

Color

As the pH declines from 7.0 to 5.5, there is an increase in light scattering within the structure of a muscle cell, which can be seen when viewed under confocal microscopy (Figure 8). This increase in scattering is associated with an increase in paleness of the meat surface and is one explanation for why the meat surface appears paler, as the pH declines from 7.0 to 5.4. Thus, the meat surface is pale in a low ultimate pH muscle (pH = 5.4–5.7) and dark in a high ultimate pH muscle (pH > 5.8) (Figure 8). Thus, prerigor meat will also appear dark until the pH has fallen from 7.0 to 5.5, as a consequence of glycolysis. Apart from scattering, the other reason that the meat surface appears dark in a muscle of high pH is because the pigment myoglobin, which is the main pigment in meat, is predominantly in the dark purple-red reduced deoxymyoglobin state. In meat of low pH, the predominant form of myoglobin in the surface is the bright-red oxymyoglobin, which has an O_2 bound to the iron atom in the center of the protein.

Aberrations in Meat Quality

Final meat quality is in part a result of the change in muscle temperature and pH in the prerigor period, prior to the onset of rigor mortis. Quality traits that are influenced by the rate and extent of decline in temperature and pH are tenderness, water-holding capacity, shelf life, bacterial growth, and color.

PSE or weepy, pale meat is a consequence of increased rate of early postharvest glycolysis and is associated with elevated muscle temperature and fast pH decline. This phenomenon is similar to what some describe as heat shortening or high rigor temperature meat. The occurrence of high prerigor temperature accompanied by a faster than normal pH decline in muscle causes denaturation of sarcoplasmic and myofibrillar proteins, with consequent negative effects on all quality traits. These negative effects include the occurrence of unacceptably pale meat, high water loss, variable tenderness, and increased susceptibility to oxidation and consequent reduced shelf life. The cause of PSE is attributed to stress at slaughter, particularly in stress-susceptible pigs, such as those carrying the halothane gene or RYR1 gene. Electrical stunning of pigs, or the application of electric prodders to animals, can also cause an increased occurrence of PSE in pigs. PSE also occurs in turkey and poultry muscles. Recently, pale, weepy (PSE-like) meat has been found in the muscles of beef and sheep carcases and, in cattle, is attributed to grain feeding beyond 70 days and the use of electrical stimulation at the immobilizer, hide puller, and bleeding points on the processing floor. For sheep carcases, the limited data suggest it is due to the excessive use of electrical stimulation on the carcass. In pigs, poultry, and turkey, PSE is defined by a muscle pH at 45 min postslaughter <5.8–6.0. In cattle and sheep, PSE-like is defined by a muscle that has a temperature $>35\,^{\circ}C$ when the pH drops below 6.0 (Figure 9).

High ultimate pH meat is called dark firm dry or dark-cutting and is defined by the ultimate pH of the muscle being >5.8–6.0. The high ultimate pH is high due to a low concentration of muscle glycogen at slaughter; thus, insufficient glycolysis occurs to generate lactic acid and hydrogen ions. The critical level of muscle glycogen in order to achieve an ultimate pH <5.8 is about 40–60 $\mu mol\,g^{-1}$ of tissue. There is usually about 80–150 μmol of glycogen per gram of tissue in the muscles of a healthy well-fed animal. The excess glycogen remains in the muscle as residual glycogen, once glycolysis ceases. Muscle glycogen levels are usually high if the animals are on an increasing weight trajectory prior to dispatch for slaughter. Time-off feed in pigs and stress encountered preslaughter, by any animals, can deplete muscle glycogen stores and result in high ultimate pH meat. Meat of a high ultimate pH is dark in color and has variable tenderness and reduced shelf life, as it tends to exhibit 'greening' in the vacuum bag.

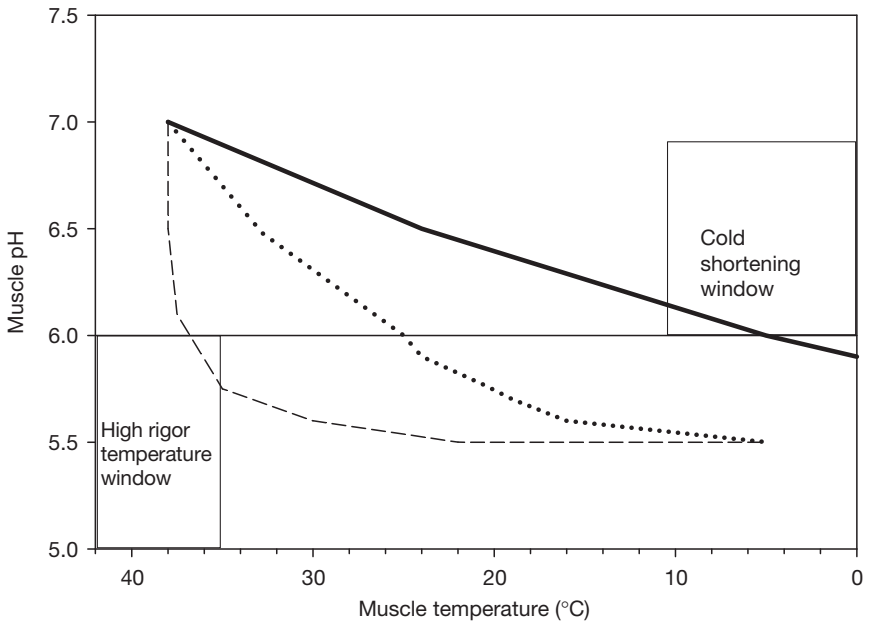

Figure 9 The pH temperature window used by Meat Standards Australia to optimize the decline in muscle pH relative to the temperature of the muscle in beef. The dotted line represents an optimal rate of decline, the solid line a cold shortening, and the dashed line, a high rigor temperature scenario. The regions to avoid for assurance of quality meat are the cold shortening region and the high rigor temperature region. Reproduced from Gutzke, D. A., Franks, P., Hopkins, D. L. and Warner, R. D. (2014). Why is muscle metabolism important for red meat quality? An industry perspective. *Animal Production Science* **54**, iii–iv.

Cold Shortening

Cold shortening is dependent on the cooling rate of a muscle and generally occurs if the muscle temperature drops below 10 °C while the muscle is still in the prerigor state (pH > 6.0; Figure 9). The underlying mechanism appears to be the inability of the sarcoplasmic reticulum and mitochondria to bind and sequester calcium at temperatures <10 °C, which causes an excess of calcium in the intracellular space, causing contraction. The maximum amount of shortening occurs close to the freezing point of meat. Adequate ATP levels need to be present for cold shortening to occur. The result of cold shortening is shorter sarcomeres and tough meat. In hot-boned muscles free of the physical restraint of the carcass, excess shortening >40% can occur, which produces meat that is more tender. This appears to be due to the myofilaments driving through the z-line and severely disrupting the muscle structure, increasing the tenderness. Cold shortening can be prevented by the application of electrical stimulation to the carcass. Care must be taken for beef and sheep carcasses as excessive stimulation can lead to the quality problem of pale, weepy meat (see in the preceding text). Electrical stimulation is not recommended for pig carcasses due to the likelihood of inducing PSE.

Thaw Rigor

This occurs if muscle has been frozen while still in the prerigor state. Thaw rigor or thaw contracture occurs upon thawing of the muscle. It is characterized by massive shortening, excessive drip loss from the muscle structure, and very tough meat. The mechanism is similar to cold shortening except that massive levels of calcium flood the intracellular space, causing very rapid glycolysis and pH drop. Because of the phenomenon of thaw shortening, it is not recommended to freeze prerigor meat.

Rigor Shortening

Unrestrained muscle generally shortens by up to 30% as it goes into rigor mortis. This shortening is temperature-dependent and is higher at 40 °C than at 15 °C. If muscle is restrained from shortening during rigor mortis, it develops weak tension, which can be measured using a rigorometer (measures the development in tension during the onset of rigor mortis). The force that develops is either due to a rise in cytoplasmic calcium, as ATP disappears (and can no longer pump calcium back into organelles), or because of the activation of contraction when the myosin heads bind to actin. Rigor shortening can be prevented by the application prerigor of a stretch to muscles. 'Tenderstretch' or 'aitch-bone' hanging has been implemented in some processing plants around the world, as this method stretches most muscles in the hindquarter and also has some effects on the loin muscle. The disadvantages of utilizing these methods include the time and labor involved in rehanging the carcass, the increased space required in chillers, and the change in the shape of the primal cuts.

See also: Beef; Pork Meat Quality, Production and Processing on; Sheep: Meat; Skeletal Muscle.

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Meat: Eating Quality and Preservation

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Introduction

Meat and meat products are an important group of nutritionally dense foods, which are widely consumed all over the world and as such are of great environmental and socioeconomic importance. Modern consumers are increasingly demanding meats that are safe, are of good eating quality, are nutritious, and are produced through sustainable farming practices. Thus, it is an ongoing challenge for the meat industry to produce high-value table cuts of consistent good quality, and so eating quality and preservation are major issues in that respect. This article provides basic information on the main factors determining the eating quality of meat and offers a review of the technologies used to preserve meat.

Eating Quality of Meat

Meat quality is a multidimensional concept that plays a major role in product marketability. The way in which it is perceived by consumers, and hence their preferences and the degree of acceptability, is influenced by various factors (Figure 1), mainly relating to (a) the intrinsic properties of the product, (b) consumer characteristics, and (c) the sociocultural environment. The sensory perspective is an essential part of meat quality, including appearance aspects considered at the point of sale (color, marbling, drip, etc.) and those considered at the table, which come under the general heading of eating quality. Eating quality combines sensory aspects like appearance, tenderness, juiciness, flavor, and overall palatability, and they are the most important characteristics by which consumers judge meat quality. Of these, tenderness is arguably the most important factor, determining eating satisfaction, and, hence, poses the greatest challenge in terms of acceptability of meat purchased by consumers. A thorough understanding of the most important factors influencing meat eating quality is imperative in order to produce a consistent product that meets consumer expectations. This knowledge can be used in the industry to produce a more consistent product in terms of enhanced eating quality.

Factors Affecting Meat Eating Quality

Eating quality (especially tenderness and flavor) at the consumer level is of paramount importance for the industry in order to remain competitive in the marketplace. The eating quality of meat is a result of complex interactions between the biological traits of the live animal and biochemical processes that occur *postmortem* as muscle is converted to meat (Figure 2). The variability in eating meat quality stems from several factors that arise throughout the production chain, and so in order to guarantee a particular and consistent meat eating quality (limiting this variability), global programs have been pursued to assess the effectiveness of meat production systems and develop a tool (quality assurance strategies) conducive to a detailed understanding of the factors that interact to determine (and assure) the eating quality of meat. These factors are mainly related to animal type, preslaughter handling, *postmortem* processing (including storage), and consumer intervention (cooking) (Figure 2).

Biological and preslaughter factors

There are major preslaughter factors that are known to influence the eating quality of meat (Figure 2). Firstly, there are what might be called 'intrinsic muscle characteristics,' which refer to a set of particular attributes associated with the animal in question and which will affect not only the quality of the meat but also how suitable it is for processing; these are determined by biological factors relating to species, breed, sex, etc., physiological aspects (genetic background, stress responses, etc.), and production practice (feeding, finishing weight, age at slaughter, etc.). These characteristics may be classified as (a) anatomical and histological, for example, the fiber type (red and white, with mechanical, biochemical, structural, and functional differences) with varying degrees of susceptibility to cold shortening, presence of fat cover affecting chilling rates and weight loss, or muscle location, again affecting chilling rates and the degree of sarcomere shortening; (b) chemical composition (proteins, lipids, glucogen, etc.), influencing, inter alia, postmortem biochemical processes; and (c) physical properties, relevant in thermal processes (cooling and heating), and affecting technological processes (e.g., preservation methods).

Preslaughter treatments, such as those involved in transport, reception, lairage, etc. produce a number of changes (stress response and glucogen depletion), which alters metabolic response and causes various types of glycolysis (ultimate pH). Some of these have undesirable effects, for instance, the kind of rapid, intense glycolysis that results in pale, soft, exudative (PSE) muscle or slow and limited, which results in a dark, firm, dry (DFD) muscle. The effect of preslaughter treatments on *postmortem* events has major impacts on various quality attributes, such as color, texture, and shelf life. Regardless of the biological and physiological aspects of the animals, both these situations can be avoided or restricted by good management practices in farming, transport, and lairage.

Postmortem factors and treatments

Following slaughter, the carcass undergoes a number of physiological, biophysical, and biochemical changes through which muscle becomes meat. These processes go through various stages, which have considerable impact on the quality, technological suitability, and stability of the meat (Figure 2). The consequences of *postmortem* treatments are generally more serious than those deriving from livestock rearing. When the animal dies, the blood flow ceases, oxygen ceases to reach the muscle, and glycolysis becomes anaerobic. At the same

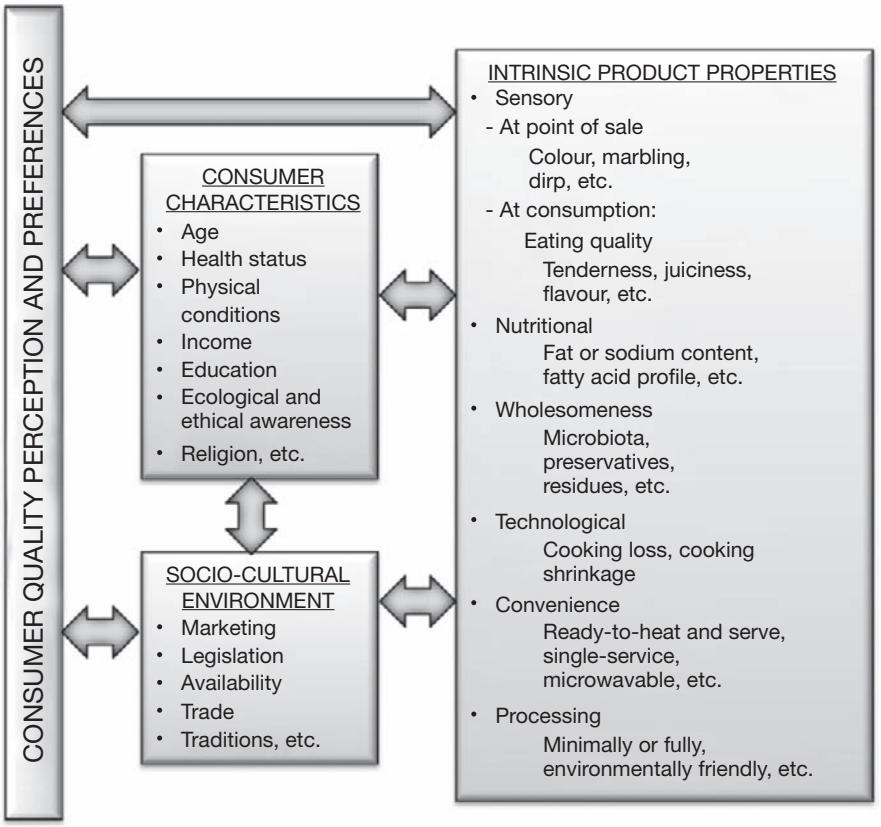

Figure 1 Eating quality in the context of factors affecting consumer meat quality perception and preferences.

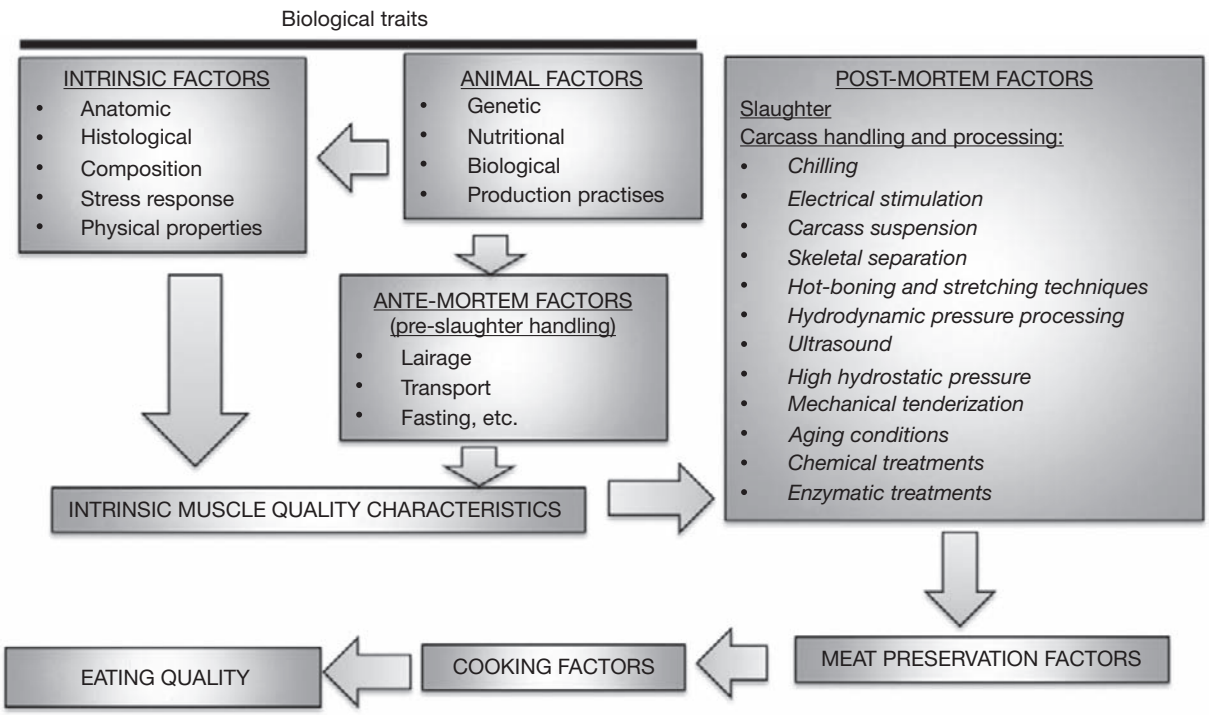

Figure 2 Production, processing, and consumer factors, which affect the eating quality of meat.

time, there are a buildup of lactic acid and a drop in pH (until the energy store is depleted or until the glycolysis enzyme is inactivated). As levels of adenosine triphosphate (ATP) decline, actin–myosin bonds form, producing actomyosin and causing shortening of the sarcomere, so that *rigor mortis* gradually sets in. The rate of these processes is influenced by intrinsic factors, which vary from one animal to another and in some cases from one muscle to another in the same animal, and also by extrinsic factors including the tension on the muscle and above all the temperature at which *rigor* sets in. The extent of shortening of the muscle fiber during the onset of *rigor* is influenced by the temperature at which the *postmortem* processes take place. This means that a poor adjustment may cause cold shortening and thaw rigor, resulting in a considerable decline in the eating quality of meat, which will be tough even after cooking. Meat quality is also considerably affected by the type of glycolysis, which can range from moderate, characterized by a gradual drop in pH, to types that result in PSE or DFD muscle (given the link between ultimate pH and tenderness).

Various *postmortem* treatments have been assayed to improve the eating quality of meat by influencing biochemical and structural events (**Figure 2**). These are based on strategies to (a) optimize the temperature/biochemistry postmortem relationship, (b) increase the tension on the muscle during the onset of rigor mortis, (c) promote processes conducive to maturation, and (d) induce structural damage in certain muscle components (contractile system and connective tissue). Based on their mode of action, these *postmortem* interventions can be classified in three main categories (physical, chemical, and enzymatic).

Carcass chilling conditions

Postslaughter chilling conditions are crucial for meat eating quality and safety. For purposes of shelf life and food safety, carcasses should be chilled as quickly as possible; however, chilling conditions can influence *postmortem* biochemical processes (*rigor*) and hence eating quality. Since cold shortening occurs when the muscle temperature drops below 10 °C while the muscles still contain sufficient energy (at pH > 6.0) to allow muscle contraction and the degree of sarcomere shortening is lower when rigor takes place at temperatures over 10 °C (generally lowest between 14 °C and 20 °C), 'high-temperature conditioning (slow or delayed chilling rates)' has been proposed to improve eating qualities (especially tenderness and flavor). However, depending on how it is carried out, this procedure can pose problems in terms of both economy (high weight loss, need for more storage space, etc.) and health/hygiene (greater microbial growth). 'Very fast chilling,' consisting of reducing the temperature to −1 °C after 5 h *postmortem* throughout its mass, has many potential benefits, including the production of tender meat, which can be attributed to two mechanisms: (a) the outer surface of the muscle may be frozen and act as a straitjacket to prevent cold shortening and (b) the intense cold releases bound calcium so that tenderizing enzymes are stimulated. However, this approach has some limitations: The carcass must be intact and a high degree of control and automation is required.

Electrical stimulation

This consists in passing an electric current through the muscle mass of the freshly slaughtered animal, producing a large number of muscle contractions in a short space of time. This speeds up postmortem glycolysis (rapid decline in pH), so that higher chilling rates can immediately be applied to reduce the risk of cold shortening and thaw rigor. Electrical stimulation (ES) generally makes the meat more tender by preventing excessive muscle shortening during rigor and enhancing proteolysis (release of Ca ions) and physical disruption of muscle structures. Moreover, rapid chilling reduces weight loss and microbial growth while allowing added savings in space and time. There are many forms of ES applications, ranging in magnitude of voltage, current, frequency of pulses, and waveforms. Low-voltage (<150 V) stimulation is performed with simple, low-cost equipment and requires little in the way of safety measures, although it must be done immediately after slaughter. High voltage (300–1000 V) is applied at the end of the deskinning chain and requires more safety precautions. While ES of beef and lamb carcasses is widely used to prevent cold shortening, with beneficial effects on tenderness, in the case of pigs, ES accelerates the rate of pH decline, resulting in increased frequency of PSE.

Carcass suspension

One means of avoiding toughening due to muscle shortening during rigor is to hang the carcass in such a way that there is maximum tension on the muscles and they cannot shorten during rigor, thus increasing the sarcomere length and tenderness. One method that has been proposed for this purpose is tenderstretching. This new technique entails a change from the normal ways of hanging carcasses to stretch the higher commercial cuts (tenderizing effects depending on the muscle location on the carcass); the carcass is hung from the pelvic bone (obturator foramen or aitchbone) rather than from the Achilles tendon in the traditional way. The backbone in a tenderstretch carcass is straight, and so there is tension on the loin muscle, which prevents it from shortening during *rigor*. In addition, the tenderstretch leg hangs in a more natural position and there is tension on most muscles so that shortening cannot occur.

Skeletal separation

Severance of the vertebral column prior to chilling can increase carcass and sarcomere lengths and improve the tenderness of beef meat cuts. In view of this, a process known as Tendercut™ has been developed, consisting of making cuts (prerigor carcass) in the skeleton at specific locations on the m. longissimus in order to achieve stretching without the need to alter the carcass suspension. In addition to breaking the vertebrae at the 12/13th rib junction, all tissues surrounding the loin are cut, so that it is the only the dorsal component that is holding the forequarter to the hindquarter. This method has proved effective in improving tenderness in the loin and the round muscle.

Hot-boning muscle/stretching techniques

Hot boning of meat consists in separating the muscles from the carcass when in prerigor. This technique has many economic advantages including reduced chiller space requirements,

quicker meat turnover in the abattoir, lower chilling costs, and reduced weight loss. However, it also has a major limitation in that removing the muscle from its skeletal anchors before *rigor* increases muscle toughness as compared with cold-boned muscles (*postrigor* carcass removing). Various different techniques for artificial restraint of hot-boned muscles have been developed; among them are the Tenderbound (Pi-Vac Elasto-Pack) system and SmartStretch™ technology, which wraps hot-boned *prerigor* muscles to prevent muscle contraction and shortening during *rigor*, thus producing a tenderizing effect. The main advantage of these systems is that they are very flexible and can be applied to any targeted muscle.

Hydrodynamic pressure processing

Hydrodynamic pressure, also known as hydrodyne, is a meat tenderizing technology that involves underwater detonation of a high-energy explosive in a containment vessel to generate a shockwave pressure front at velocities exceeding the speed of sound. A sudden increase in pressure is exerted on the meat, disrupting the structural integrity of the muscle and connective tissue. Although significant tenderization has been documented, this approach has a number of technological limitations. Subsequently, alternative methods of producing shockwaves have been developed. One such is the TenderClass Process in which a shock wave is electrically generated by creating a high-voltage arc across two electrodes immersed in non-compressible fluid, preferably water.

Ultrasound

Ultrasound treatment has been proposed as an alternative technology for modifying properties of meat and meat products, including tenderization. Although ultrasound has been shown to induce some structural changes in muscle and activation of proteolytic systems, reports on the effects on meat tenderness have been inconsistent. This may be related to differences in the application conditions (acoustic parameters, ultrasound systems, type of muscle, etc.). Generally speaking, only high-intensity treatments seem to be capable of producing a significant tenderizing effect.

High-pressure processing (HPP)

High pressure is useful for food processing and preservation thanks to its effects on food constituents. HPP has been used to tenderize meat, but the effect depends on various factors; among them are the pressure level applied, temperature, processing time, and rigor state of the meat. High pressure (<200 MPa) can tenderize meat when applied in prerigor, but it has no marked effect on postrigor meat at low temperature (<30 °C). HPP treatment of postrigor meat has been found to improve tenderness, but only when applied at higher temperatures (40–60 °C). The effect of pressure may be the result of dissociation of myofibrillar components through denaturation and/or the release of various cathepsins from lysosomal structures leading to an increase in proteolytic activity. Although undesirable meat color changes (browning) are a limitation (>300 MPa), there are ongoing studies aimed at preventing color changes in the pressurization of frozen meat. Other possible advantages of HHP treatment of frozen hot-boned meat include prevention of shortening caused by thaw rigor and enhancement of overall tenderness.

Mechanical tenderization

This technique consists in inserting (manually or by means of an automated system) a set of needles or blades (spaced at a given intervals of distance) in *postrigor* meat cuts. Penetration produces partial destruction of connective tissue and severance of muscle fibers, which weakens the meat structure and, thereby, enhances the tenderness of the meat. This method, which can be applied more than once depending on the type of cut and the level of tenderization required, has the advantage of achieving instant tenderization without the need of holding time, exposure to relatively high temperatures, or the addition of nonmeat components.

Aging conditions

After rigor, the muscle undergoes a series of gradual, complex changes, which tend to improve eating quality. The mechanism through which this occurs is mainly associated with the breakdown of muscle structural proteins by endogenous enzymes. Since the aging process depends on many factors (type of muscle, pH, chilling conditions, aging temperature and time, etc.), different conditions such as wet (boned vacuum-packed) or dry (boned non-vacuum-packed) meat aging have been assayed to optimize its impact on eating quality of meat. Aging, for appropriate periods of time and under appropriate storage conditions, greatly improves meat tenderness. Besides effective tenderizing conditions, economic factors and the risk of meat spoilage are also factors to be taken into account.

Chemical treatments

Infusion, marination, and injection with salts of calcium (e.g., chloride, lactate, ascorbate, or carbonate), sodium (e.g., chloride, acetate, citrate, or ascorbate), phosphate, or some commercial preparations have all been used to improve the tenderization of meat. The effect is achieved through the physical weakening of muscle structures (myofibers and perimysial connective tissue) or through the activation of proteolysis. However, these effects are frequently accompanied by undesirable changes in other eating quality factors (particularly flavor).

Enzymatic treatments

Exogenous proteases from tropical plants (e.g., ficin, bromelain, and papain) or of bacterial, fungal (e.g., collagenase or aspartic protease), or animal (porcine pancreatin) origin have been used (by marination or injection) to tenderize meat, although with some effect on flavor. These enzymes act on myofibrillar proteins and connective tissues to various degrees to produce a more fragmented and disintegrated structure.

Cooking

Most meat and meat-based products are cooked before being eaten. The cooking process not only destroys microorganisms but also develops sensory properties, which are specific to the cooked product. The rise in temperature during cooking produces dramatic changes in the meat structure, the most striking being protein denaturation/coagulation, muscle fiber shrinkage, water loss (expelled from the meat matrix by protein denaturation and shrinkage of muscle structures), changes in

color, a warmed-over flavor, etc. The nature and extent of these changes, which considerably influence the final eating quality of meat (Figure 2), depend on factors – the temperature on the surface of the meat, the temperature profile through the meat, and the mode of heat transfer – associated with common cooking techniques (roasting, grilling, frying, boiling, and microwaving). Meat characteristics (e.g., type of meat, cuts, and composition) also affect the consequence of the cooking process.

Cooking causes denaturation of the myofibrillar components, which results in toughening. Conversely, it can also promote structural alterations and solubilization of collagen, which makes the meat more tender. Optimum meat tenderness occurs between 55 and 65 °C, although this varies with a number of factors, among them being collagen content. Muscles with high collagen content require higher temperatures (>75 °C) to bring the collagen to a soluble, gelatinized state and, thus, achieve softening. Color is influenced not only by meat composition (e.g., hemoprotein content) but also by the heating process and end point temperature (browning with increasing temperature). Thermal treatment induces changes in various components of meat (e.g., amino acids, carbohydrates, and fats), producing various types of compounds; among them are Maillard reaction products, which contributes to the flavor of cooked meat. Loss of juices is one of the key factors influencing the quality of cooked meat. If water loss is too high, the meat will become tough and fibrous, while keeping water loss to a minimum will produce juicier cooked meat. An increase in the end point temperature will cause a decline in juiciness.

Also, cooking has a considerable effect on the nutritional properties of meat (loss of fats, water-soluble compounds, and temperature-sensitive components), improving digestibility while at the same time favoring reactions between compounds inherent in meat, yielding genotoxic substances (e.g., polycyclic aromatic hydrocarbons and heterocyclic aromatic amines).

Preservation

Because of its composition, meat is a highly perishable product, and therefore, to preserve it, a number of measures need to be applied to delay or prevent microbiological, chemical, and physical alterations that adversely affect its quality (including eating quality; Figure 2), shorten its shelf life, and render it unfit for consumption. Of all the types of deterioration undergone by fresh meat, microbial spoilage is the most important in that it normally precedes all the other types and can render the meat harmful to health in the event that the microorganisms include pathogenic species or compounds have formed that are toxic to humans. There are a variety of procedures (physical, chemical, and microbial) that can be used to preserve meat (Table 1). While some methods (e.g., chilling, irradiation, and high hydrostatic pressure) serve to retain the original characteristics of the product, others (curing, drying, fermentation, etc.) cause desirable changes in quality attributes, contributing to products' diversification.

Table 1 Meat preservation methods

Physical methods
Low temperature: chilling, superchilling, freezing/thawing; high temperature: heating, canning; drying; irradiation; high pressure; luminic pulses; packaging: overwrap, modified and controlled atmosphere, active and intelligent systems
Chemical methods
Curing; smoking; chemical preservatives: ozone, organic acids, sulfites, etc.
Microbiological methods
Fermentation; biopreservation: natural or controlled microflora
Hurdle technologies
Superimposing of effects by combination of hurdles (e.g., temperature, a_w, acidity, redox potential, preservatives, pressure, radiation, or competitive flora)

Physical Methods

Refrigeration

Refrigeration of meat and meat products serves two basic purposes: to preserve the initial quality at the time of consumption and to keep them at temperatures that propitiate certain processes, some of which play a decisive role in the characteristic eating quality attributes of the product (texture, appearance, flavor, odor, etc.). Among these, for example, are early *postmortem* events, the maturation of sausages, and the curing of ham. The preservative effect of low temperatures essentially derives from the fact that they slow down the chemical and enzymatic reactions that cause spoilage, and they prevent microbial growth. The quality of refrigerated meat is highly dependent on maintenance of the cold chain, meaning the series of successive steps in the low-temperature retailing of meat. This includes processes in the abattoir, transport, cold storage, sale and display cabinets, etc., and also domestic refrigerators. The refrigeration process may consist of either chilling (short-term) or freezing (medium- and long-term) depending on the desired storage life.

Chilling

Chilling generally means a process embracing the reduction of the carcass temperature immediately after slaughter to appropriate temperatures for storage, without the formation of ice crystals in its structure, and also maintenance of the temperature (and other parameters) at an appropriate level throughout storage. Rapid carcass chilling reduces microbial growth, increases product yield (reduces weight loss from evaporation and also pulverization of water during air spray chilling), and reduces infrastructure requirements (less space and less chilling facilities needed). It is essential to properly control early and fast chilling and early postmortem events; otherwise, the eating quality of the meat may be adversely affected (e.g., by cold shortening). Chilling takes place in various kinds of facilities (cold rooms, cells, or tunnels) basically using air as the chilling medium (water immersion or spraying is also used for poultry).

Once it is chilled, the meat must be kept in optimum storage conditions (controlling factors such as temperature and relative humidity of chilling cabinets) to preserve quality and improve stability. The meat inevitably undergoes changes

of various kinds depending on the raw material, the processing, and the conditions of chilling treatment and storage. Some of these factors improve eating quality (aging process), while others – physical (weight loss by evaporation and exudation), chemical (color changes), and microbiological – promote spoilage. This is generally the key limiting factor in chilling storage of meat. Various chilling adjunct strategies have been used to prolong storage life.

Superchilling has been suggested for preserving meats. With this technology, the temperature of the product is lowered to 1 or 2 °C below the initial freezing point. This has several major advantages: maintenance of food freshness, maintenance of high food quality, inhibition or suppression of most microbial activity, and increased shelf life.

Freezing/thawing

Freezing is an excellent means of preserving the original characteristics of the meats practically intact for a considerable period of time. The effect derives both from the reduction of temperature and from the reduction of water activity, as most of the water is turned to ice. The effectiveness of freezing in preserving meat and meat products quality and prolonging its useful storage life depends on a number of factors: (a) product characteristics (inherent characteristics, *rigor* state, prior chilling process, use of packaging, etc.), (b) freezing process and storage (moment and rate of freezing, storage temperature and duration, etc.), and (c) thawing conditions. The method most commonly used to freeze carcasses is air blast. Plate contact or cryogenic fluid freezing methods are not used so much for meat and then generally only for technological and/or financial reasons. The freezing rate and the procedure selected depend on the processing conditions (rate and air temperature) and also on the product characteristics (size, shape, fattiness, thermal properties, etc.).

Freezing prolongs the shelf life of meat, but a number of cumulative and irreversible changes take place as storage time progresses, and these compromise the final quality. The most significant changes that can occur arise from processes whose origin may be physical (e.g., ice recrystallization and sublimation), biochemical (ATP degradation), or chemical (e.g., lipid oxidation, protein denaturation, and color changes), and they may cause not only weight loss but also undesirable alterations to the sensory characteristics and technological suitability of the meat.

Thawing in inappropriate conditions can promote more spoilage than in the freezing stage, as well as being slower and harder to control. Meat is generally air-thawed, but thawing can also be performed in water, under vacuum or high pressure, or electrically (resistance, dielectric, microwave, etc.), depending on the characteristics (shape, size, electrical properties, type of packaging, etc.) of the product, the target market, and the economic considerations. In some cases, the meat only requires tempering, which entails raising the temperature to a point (below freezing) suitable for a given processing step.

Heat processing methods

There are two broad types of heat treatment: preconsumption cooking to promote a desirable texture, flavor, and color and render meat products more palatable and appetizing for the consumers and production of pasteurized (heating) or commercially sterile (canning) products by reducing the microbial content to prolong shelf life and eliminate potential food poisoning agents. Thermal treatment consists in placing the products in cooking vessels, which contain hot water, steam, or a mixture of both. Radiant (e.g., dielectric or ohmic) heating offers interesting possibilities, although these are limited in the case of meat-based applications and generally have to be combined with other techniques. Pasteurized products generally reach an internal temperature of 71 °C (kills most microorganisms and the product must be refrigerated), while sterile meats are heated to 121 °C so that all microorganisms are killed and products are shelf-stable. After thermal treatment, the product must be chilled as quickly as possible to avoid overcooking. The intensity of thermal treatment can be defined in terms of a value of F_0 (between 1 and 10 for most canned meats). Important to note is the time–temperature relationship in the impact on inactivation of microorganisms and on the sensory quality of the product. Sous-vide cooking of vacuum-packed meats at relatively low temperatures and long cooking times have emerged as popular techniques to provide foods of superior sensory quality with a longer shelf life as compared with conventional technologies.

Drying

Drying is probably one of the oldest methods of food preservation. It is a process in which water is removed (generally by evaporation), lowering the water activity (a_w) in the meat and thus inhibiting microbial growth. Meat may be dried for the sole purpose of dehydrating fresh meat for longer storage, but it may also be one of the various processing steps during the manufacture of specific meat products (curing or fermentation). Meat drying is a simple but efficient procedure. Dried meat can be stored at ambient temperatures for many months. Drying causes numerous physical (e.g., shrinkage, shape alteration, and texture modification) and chemical (e.g., increased concentrations of protein, fat, etc. and greater susceptibility to oxidation of lipids) changes that affect the eating quality of meat.

Irradiation

Irradiation is one of the most efficient intervention strategies to reduce or eliminate pathogens in meat products. It is a physical process that exposes meat to an ionizing radiation (accelerated electrons and γ- and x-rays), which create ions or free radicals from atoms. These molecular species can fragment or destroy the DNA of microorganisms, killing the pathogens present in a meat matrix. International organizations (e.g., FAO and WHO) recommend a maximum dosage of 10 kGy. This technology enables the meat industry to obtain non-heat-treated products essentially free of pathogenic food-borne bacteria, which can be processed in the package due to the high penetrative ability of γ-rays, avoiding further contamination and with lower energy consumption. The adverse effect of irradiation on the wholesomeness and quality of meat products is very low compared to some other preservation methods; however, it does have some drawbacks such as color changes and/or production of off-flavors.

High-pressure processing

In addition to meat tenderization (previously described), HPP (100–1000 MPa) has been used for preservation. HHP renders

food more stable and safer due to its ability to reduce the number of spoilage and pathogenic microorganisms and to inactivate certain food enzymes. The effectiveness of HHP for microbial control depends on factors such as the process parameters, pressure level, temperature, and exposure time, as well as intrinsic factors in the food itself, such as pH, strain and growth stage of microorganisms, and the meat matrix. The undesirable effect of HHP on fresh meat color can adversely affect consumer preference at the point of sale, whereas it is not perceptible when the product is served after cooking. There are numerous meat products where HHP is used to prolong shelf life (e.g., cured products) to which this limitation does not apply.

Pulsed light

This is a nonthermal technology for food preservation, which consists in the application of short- (10^{-3}–10^{2} ms) broad-spectrum (200–1000 nm) light pulses. Its antimicrobial effect derives mainly from the UV-C component (200–290 nm) emitted in each pulse, which prevents cell replication. In solid foods, decontamination takes place on the surface because little light penetrates, and so the technology has been assayed on ready-to-eat meat products.

Packaging

Packaging consists in surrounding or wrapping meat and meat products with suitable material to protect them against deteriorative effects, including physical, chemical, sensory, and microbiological (pathogenicity) changes. Variables that influence shelf life properties of packaged meat and meat products are product type, gas mixture, package and headspace, packaging equipment, storage temperature, and additives. There are currently many meat packaging systems, each with different attributes and applications. These systems range from overwrap packaging for short-term chilled storage and/or retail display to a diversity of protective atmospheres such as specific modified atmosphere, controlled atmosphere packaging systems, and vacuum packaging for longer-term chilled storage and/or display. The last few years have seen the introduction of new approaches such as active and intelligent packaging systems for use in meat and meat products. Active packaging refers to the incorporation of additives in packaging systems in order to maintain or enhance meat product quality and shelf life. Active packaging systems may include oxygen scavengers, carbon dioxide scavengers and emitters, moisture control agents, and antimicrobial packaging technologies. Intelligent packaging systems monitor the condition of packaged foods to give information regarding the quality of the packaged food during transport and storage.

Chemical Methods

Curing

Meat curing practice involves the addition of sodium and/or potassium chloride, sodium nitrite, and other ingredients (e.g., erythorbate, polyphosphates, and seasoning) to raw meats. Curing is used in the manufacture of meat products in varying conditions of structural breakdown (from comminuted meat to whole pieces) and thermal treatment (raw and cooked), following various strategies (dry, brine, tumbling, and injection). In the past, when refrigeration was not commonly available, curing was mainly used to prolong the storage life of whole pieces of muscle utilizing the preservative effects of common salt (removal of moisture and reduction of water activity) and sodium nitrite. Today, this aspect is not so important as there are more efficient meat preservation methods and curing is now mainly used to achieve characteristic eating quality and to inhibit growth of *Clostridium botulinum*. Cured-raw meats need to be kept in controlled conditions throughout processing, when fermentation and ripening take place simultaneously with drying in order to achieve the desired palatability. In the case of cured-cooked meats, after the curing process, a specific heat treatment is applied (>60 °C) to induce stability and achieve the desired palatability. One new procedure for cured meat products that has been attracting interest is indirect addition of nitrate or nitrite, in the form of natural or alternative curing (e.g., nitrate-rich ingredients).

Smoking

Smoking is a technique whereby meat is exposed directly to wood smoke (or more recently liquid smoke) for purposes of preservation, appearance, and flavor. These are the effects of a combination of heat, drying (decrease of a_w), and the antibacterial and antioxidant action of components of smoke particles. All of them act to prolong the shelf life of the product, although the preservative effect varies with the intensity of the smoking. This procedure is used in combination with others (drying and curing) and in low-temperature conditions.

Chemical preservatives and natural extracts

Various different chemical compounds have been used in meat processing to inhibit, retard, or prevent the activity and growth of spoilage and pathogenic microorganisms. Their chief purposes are to prolong the shelf life of foodstuffs by protecting them against deterioration caused by microorganisms and to enhance their safety. Various chemical preservatives and application conditions (e.g., additives and carcass rinses) have been used depending on the type of product. These include ozone, carbon dioxide, organic acids (e.g., lactic, acetic, propionic, citric, sorbic, and benzoic acids and the methyl and ethyl esters of 4-hydroxybenzoic acid – parabens – or their salts and benzoic acid), sulfur dioxide and sulfites, and nitrates and nitrites (see section 'Curing'). Antioxidants (synthetic or natural) are used to retard lipid oxidation. Recently, natural compounds such as plant extracts, essential oils, chitosan, and some proteins (lysozyme and lactoferrin), with antimicrobial or antioxidant properties, have been used.

Microbiological Methods

Fermentation

Fermented meats have traditionally been produced all around the world. They are generally made with chopped or minced meat and fat, mixed with salt, seasoning, and spices, stuffed into a casing, and then ripened and dried, or in some cases smoked. There are many types of sausages responding to various factors (geographic, environmental, technological, etc.). These may be classified according to various criteria, such as acidity, particle size, diameter and type of casing, degree of drying, and type of starter cultures. Their main

characteristics – shelf life and eating quality (specific flavor, texture, and color) – are determined by a combination of acidulation (generation of lactic acid by lactic acid bacteria from natural contamination or well-defined starter cultures) and the lowering of a_w by the action of salt and drying. The interrelated microbiological, biochemical, and physicochemical changes that take place during meat fermentation are affected by the ingredients used, the processing technology, and the microbiota. While dry sausages take a long time to ripen, depending on the intensity of dry, semidry sausages ferment rapidly at high temperatures, and the drying step may even be omitted.

Biopreservation

Biopreservation is based on the use of natural or controlled microbiota (such as lactic acid bacteria) or antimicrobial natural products (such as lactic acid and bacteriocins) to extend the shelf life and enhance the safety of food products by reducing or eliminating microbial spoilage. Bacteriocins (nisin is the best known) are a heterogeneous group of antibacterial proteins that vary in spectrum of activity, mode of action, molecular weight, genetic origin, and biochemical properties.

Hurdle Technologies

As has been stressed throughout this article, generally, most meat preservation strategies (although analyzed separately) combine various technologies, factors, and/or agents in order to maximize the preservative effect. This superimposing of effects, known as the 'hurdle concept,' consists in an appropriate combination of barriers or hurdles (e.g., temperature, a_w, acidity, redox potential, preservatives, pressure, radiation, or competitive flora) to improve the microbial stability and the sensory quality of meat and meat products.

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See also: Beef; Canning: Process of Canning; Chilled Foods: Effects on Shelf-life and Sensory Quality; Chilled Foods: Modified Atmosphere Packaging; Chilled Foods: Packaging Under Vacuum; Cooking: Domestic Techniques; Cured Foods: Health Effects; Drying: Effect on Nutrients, Composition and Health; Drying: Principles and Types; Fermented Foods: Composition and Health Effects; Fermented Foods: Fermented Meat Products; Fermented Foods: Origins and Applications; Freezing Theory; Ham: Dry-cured Ham; Heat Treatment: Effect on Microbiological Changes and Shelf Life; Horse Meat; Irradiation of Foods: Processing Technology and Effects on Nutrients: Effect of Ionizing Radiation on Food Components; Meat: Conversion of Muscle into Meat; Meat: Role in the Diet; Nitrites and Nitrates; Pasteurization: Effect on Sensory Quality and Nutrient Composition; Pasteurization: Principles and Applications; Poultry: Processing; Preservation of Foods; Preservatives: Food Use; Sausages and Comminuted Products: Cooked Sausages; Sausages and Comminuted Products: Dry Fermented Products; Sheep: Meat; Skeletal Muscle; Spoilage: Bacterial Spoilage; Spoilage: Yeast Spoilage of Food and Beverages; Sterilization of Foods; Storage Stability: Mechanisms of Degradation; Storage Stability: Shelf Life Testing.

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http://www.teagasc.ie/research/reports/foodprocessing/4191/eopr-4191_new.pdf – Very fast chilling in beef.

Meat: Role in the Diet

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Introduction

The consumption of meat from wild and domesticated animals is evident throughout human history. Archaeological evidence supports the consumption of meat from wild animals beginning about 500 000 BC and from domesticated animals since about 7000 BC. It is thought that sheep were among the first domesticated animals (*c.*7000 BC), followed by cattle (*c.*5000 BC) and pigs (*c.*2500 BC). Progression of domestication from free breeding to breeding in captivity and, lastly, selective breeding to promote desirable characteristics has resulted in the meat consumed today. Developments in refrigeration, processing, packaging, integration, and transportation as well as increased relative wealth have allowed for the worldwide availability and consumption of meat. The efficient production of meat enhances the nutritional quality of the food supply and promotes global food security.

Sources and Production

Meat sources vary throughout the world. Pork, beef including veal, poultry, lamb/mutton, and goat meats are the most commonly consumed; however, regional variations exist. According to availability or local custom, rabbit, deer (venison), horse, camel, and other mammals often serve as sources of meat. Currently, pig stocks lead the world's meat production with China and the United States producing more than half (55%) of the world's pork (Tables 1 and 2). The United States is the largest producer of beef cattle, accounting for 19% of global production, followed by Brazil with 14% of global production. The United States, China, and Brazil are also the top producers of chicken, representing 19%, 14%, and 13% of global production, respectively. The United States is the largest producer of turkey, providing almost half (48%) the global production.

The production of livestock for food is increasing driven by a shift toward the inclusion of more animal products in the diet. In Asia, where the bulk of increase in the world population has occurred over the last decade, consumption of meat has increased an estimated 3% annually. The rapid growth in meat production has been primarily the result of increased demand for poultry with beef, buffalo, and pork production remaining relatively stable as shown in Figure 1. According to the Food and Agricultural Association (FAO) Statistical Yearbook 2013, a change in the composition of livestock production has been occurring since the 1960s. Growth of global beef production has gradually declined from almost 2% per year in the 1960s to $<1\%$ per year in the early 2000s. Pig production has undergone a greater decline, from a growth rate of 4% per year to 0.8% per year during the same time period, while poultry stocks continue to grow at a rate of $\sim 3\%$ per year. However, China and Oceania are exceptions to the trend in red meat production elsewhere. Pig stocks in China have shown a significant increase from 36.8 million tons in 2000 to 49.4 million tons in 2011, representing an increase of over 34% during the 11-year period. In Oceania countries, the percentage of total livestock produced – specifically cattle and buffalo – has increased from 11.8% in 2000 to 14.4% in 2011 (Figure 2).

Several production systems contribute to the availability of meat around the world. No single production system best fits all livestock or the availability and security needs for food across different world regions and communities. Productions systems vary on a continuum from those that rely on livestock grazing and consumption of feed sources not readily consumed by humans to those occupying limited land, which rely, at least in part, on feed sources common to the human diet. Over 60% of the world's poultry meat and eggs are produced in so-called 'landless' production systems, which is not surprising, as poultry require limited space due to their size. As monogastric animals, poultry consume a diet containing food sources that also contribute nutrients to the human diet. In contrast, over 70% of the world's beef was produced using a mixed system including grazing and, as ruminant animals, consumption of crop residues and concentrates not otherwise edible for humans.

Patterns of Consumption and Related Nutrient Intake

Consumption Patterns

Globally, estimates of meat consumption often rely on FAO balance sheets or similar. These data are not derived from direct intake/consumption measurements, but rather are based on production and disappearance of available food from the food supply. As such, loss due to trimming, cooking, waste, and spoilage is not captured and can inflate the estimate of meat consumption on a per capita basis. Conversion factors aimed at limiting the inflation caused by these inedible aspects have resulted in as much as a 100 g difference between adjusted and unadjusted daily intake estimates for meat consumption. Food consumption estimates based on disappearance-type data are, therefore, best described as 'average apparent consumption.' Based on FAO food balance data for developed countries, the average apparent consumption of meat ranges from 225 to 330 g day^{-1} including ~ 110 g red meat. Recommended levels for total meat intake range from 65 to 250 g daily of meat and meat alternatives. As such, red meat consumption appears to be within recommended levels in most developed countries. In contrast, average apparent meat consumption in the least developed nations is roughly 40 g per capita/day, the bulk of it as poultry and beef. Interestingly, in the decade spanning 2001–11, average estimated meat intake in the least

Table 1 Top meats produced worldwide (2011)

Meat source	*Production (metric tons)*
Pig	108 055 178.57
Chicken	90 143 993.68
Cattle	62 941 735.68
Sheep	8 348 110.64
Turkey	5 462 721.70
Goat	5 262 747.23
Meat (total)	297 479 837.24

Source: FAO, Statistics Division (FAOSTAT), http://faostat.fao.org/

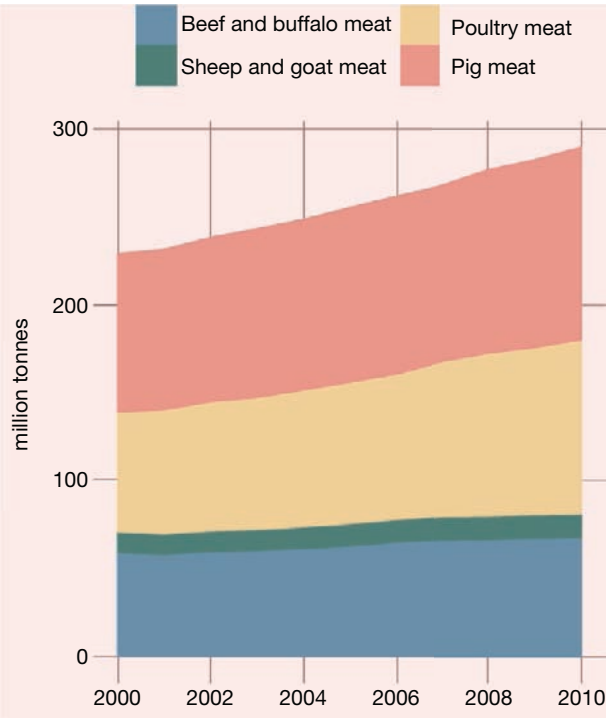
Table 2 Top meat-producing countries (2011)

Meat source	*Country*	*Production (metric tons)*
Cattle	The United States	11 983 308.00
	Brazil	9 030 000.00
	China	6 182 155.00
	Argentina	2 497 250.00
Chicken	The United States	17 111 240.00
	China	12 170 062.00
	Brazil	11 421 730.00
	Russian Federation	2 895 489.00
Goat	China	1 889 602.00
	India	596 600.00
	Nigeria	292 100.00
	Pakistan	285 000.00
Pig	China	49 396 351.00
	The United States	10 330 808.00
	Germany	5 616 074.00
	Spain	3 469 345.00
Sheep	China	2 050 000.23
	Australia	512 235.00
	New Zealand	465 318.00
	Sudan (former)	324 000.00
Turkey	The United States	2 626 531.00
	Brazil	489 000.00
	Germany	467 354.00
	France	398 082.00
	Italy	309 483.00

Source: FAO, Statistics Division (FAOSTAT), http://faostat.fao.org/

developed nations grew by 10 g daily. A global trend in increased meat consumption is predicted with estimates suggesting that production will need to increase to 470 million metric tons by 2050 to meet worldwide demand.

In addition to estimations of meat consumption based on balance sheet data, large national dietary surveys are also important to the understanding of meat consumption. In the United States, for example, the National Health and Nutrition Examination Survey (NHANES) has collected nutrition-related data since 1971 and contributes to the understanding of dietary intake patterns. NHANES examines how meat is consumed (main dish or as a component of a recipe) and collects demographic data allowing consumption patterns to be examined based on age, income, ethnicity, education, and geographic location, providing detailed information rather than balance sheets. However, these survey data may be collected using food frequency questionnaires or food diaries over



FAO Statistical Yearbook 2013 (FAOStat); FAO endorsement is not implied

Figure 1 World meat production (2000–10).

a limited period of time or even a single day. Such cross-sectional data, which often rely on recall, provide only a snapshot of evidence making assumptions regarding continuous intake difficult. While it is interesting to understand the use of meat in various composite foods, it is also difficult to disaggregate such data to obtain a clear picture of meat consumption. Thus, determining meat consumption patterns is complicated, and observations made regarding population consumption patterns must be made with caution to avoid conclusions that meat consumption is insufficient or in excess.

Research publications, particularly observational studies of dietary patterns, often group meats into broad categories including 'meat,' 'red meat,' 'processed meat,' 'red and processed meat,' 'poultry,' and 'white meat.' The term 'meat' is most commonly used to describe all muscle meat excluding fish, but this is far from standardized. Generally, the terms 'red meat' and 'red and processed meat' represent meat from beef including veal, pork, and lamb regardless of whether it is fresh or processed, but often, these terms are undefined by the researchers reporting them. The broad and inconsistent grouping of meat within dietary patterns complicates our understanding of how meat is commonly consumed and limits our understanding of nutrients contributed to the food supply by individual types of meat. The term 'processed meat' often reflects a combination of red and poultry-based products but the type of processed meat is rarely, if ever, reported. Processing of meat for products such as sausage, bacon, and deli meats adds sodium, spices/flavorings, and preservatives that can change the nutrient profile. Reformulations of processed meat products over the past decade to include a lower sodium and fat content makes earlier observational data regarding

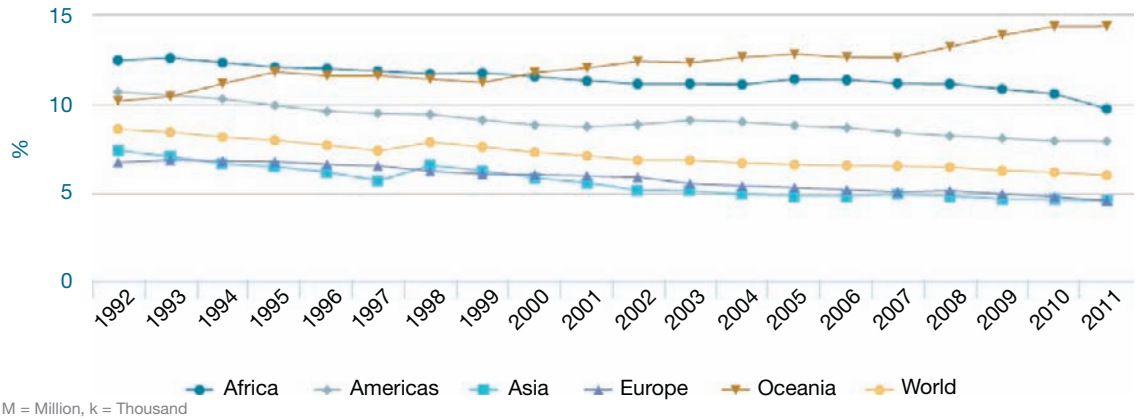


Figure 2 Trend of cattle and buffaloes as percent of total livestock produced 1992–2011. FAO, Statistics Division (FAOSTAT), http://faostat.fao.org.

nutrient intake and related health effects of processed meat of questionable relevance today.

Lipids

Worldwide data indicate that more than half (55%) of total dietary fat comes from vegetable products although the distribution of fat from plant- versus animal-based products varies from region to region. Global disappearance data suggest that, in the meat category, pork is the biggest contributor to total fat in the diet followed by poultry and beef. Change in production practices over several decades has led to a leaner supply of red meat. Driven largely by changes in breeding and retail trimming of external fat, it is estimated that in the United States, for example, the estimated amount of total fat available in the food supply from beef has been reduced by 44% (from 13% to 7%) since the 1970s. A similar experience is reported in the production of red meat in the United Kingdom with just over 20 years of change in animal husbandry resulting in 30% less carcass fat in pork, 15% in beef, and 10% in lamb.

Regardless of origin, meat fat predominantly contributes monounsaturated fatty acids (MUFA) to the diet (Table 3). MUFA are also derived from vegetable oils, nuts, and seeds. Olive oil, rich in MUFA, is the cooking oil used primarily in the Mediterranean dietary pattern, which is associated with decreased risk of many noncommunicable diseases. Oleic acid is the most common MUFA in meat.

Saturated fat (SFA) is the second most predominant fatty acid class supplied by meat. While SFA has traditionally been viewed as a major dietary contributor to coronary heart disease (CHD), not all SFAs are created equal with some contributing more than others to increased blood lipids associated with CHD risk. Also worth noting is, over the last decade, a growing body of research that has shown that blood lipids may be influenced as much by dietary carbohydrate as it is SFA. Generally speaking, shorter-chain SFAs (4–12 carbon chains), often found in high-fat dairy products, and refined carbohydrate-based food and snacks with added fats and sugars, have a more pronounced effect on blood lipids than longer-chain SFAs. In fact, stearic acid (18:0), as demonstrated by decades of data from randomized controlled trials (RCTs) and observational studies, is neutral in regard to its effect on total and low-density lipoprotein (LDL) cholesterol, and it appears to promote higher levels of high-density lipoprotein (HDL) cholesterol. Compared to vegetable oils, grains, nuts, and seeds, meat fat is low in the essential polyunsaturated fatty acids (PUFAs), linoleic acid (LA; 18:2 $n-6$), and alpha-linolenic acid (ALA; 18:3 $n-3$), although both lean and dark meat poultry contain higher PUFA content than red meat (Table 3). PUFAs supply essential fatty acids that cannot be made by the human body and, thus, viewed as healthy fatty acids often being recommended as replacements for SFA in the diet. Whereas meat contributes a relatively small amount of PUFA to the diet, meat is a good source of the long-chain omega-6 PUFA, arachidonic acid (ARA). ARA along with the long-chain omega-3 fatty acid ($n-3$ LCPUFA) docosahexaenoic acid, found mainly in fish, is important in the diet of infants and young children to promote growth and nervous system development.

Interest in manipulation of the PUFA content of meat has been a popular area of research. Generally speaking, the ability to modify the PUFA content of meat through changes in animal feeding practices is far more successful in monogastric animals than ruminant animals. Dietary PUFAs ingested by poultry and swine (monogastric) are reflected directly in the resulting nutritional content of meat and eggs. Dietary PUFAs ingested by ruminant animals, however, are transformed by rumen microflora in a process called biohydrogenation. Biohydrogenation saturates dietary PUFA resulting in very little 'bypass' of dietary PUFA for deposition in the resulting meat. Particular interest in increasing the $n-3$ LCPUFA content of meat and eggs stems from the limited supply of these fatty acids in populations that are landlocked or do not commonly consume fish. Grass/pasture feeding of cattle is often promoted as a means to increase $n-3$ LCPUFA in beef, but plants contain no preformed $n-3$ LCPUFA. Little of the $n-3$ LCPUFA precursor found in plants, ALA, escapes rumen biohydrogenation to be further metabolized to $n-3$ LCPUFA. Research to encapsulate or enable dietary $n-3$ LCPUFA to bypass the rumen has been successful but makes only modest PUFA contributions to the diet compared with naturally PUFA-rich foods. While beef from grass-finished cattle tends to have a slighter higher level of total $n-3$ PUFA, research has shown that beef from both grain- or grass-finished cattle can make a modest

Table 3 Select lipid contributions of meat

	100 g cooked portion (lean, skinless, and boneless)							
Lipid type	*Beef*	*Pork*	*Lamb*	*Chicken breast*	*Chicken thigh*	*Turkey breast*	*Turkey thigh*	*Lipid characteristics and functions*
Total fat (g)	9.28	9.21	9.52	3.17	8.15	2.08	6.04	Concentrated energy source important for health: supplies essential fatty acids; spares protein; carries fat-soluble vitamins; insulates tissues and organs for protection and regulation of body temperature. Enhances food flavor, texture, and color
Saturated fat (g)	3.54	3.09	3.40	0.99	2.31	0.59	1.81	Fatty acids containing no double bonds; generally solid at room temperature. Some saturated fatty acids increase total and LDL cholesterol when consumed in excess
Palmitic acid	2.11	1.92	1.83	0.70	1.75	0.37	1.18	
Stearic acid	1.14	1.00	1.17	0.24	0.48	0.18	0.51	
Monounsaturated fat (g)	3.90	3.91	4.17	1.21	3.36	0.63	2.06	Fatty acids containing one double bond; type of fat also found in olive and avocado oils; generally liquid at room temperature. Reduces LDL and total cholesterol when used to replace excess saturated fat
Oleic acid	3.55[a]	1.44[b]	3.86[a]	1.03[b]	2.79[b]	0.54[b]	1.77[b]	
Polyunsaturated fat (g)	0.32	0.86	0.62	0.76	1.70	0.53	1.66	Fatty acids containing two or more double bonds; type of fat also found in vegetable seed oils; liquid at room temperature. Provides omega-6 and omega-3 fatty acids essential for health. Reduces LDL and total cholesterol when used to replace excess saturated fat
Cholesterol (mg)	86	84	92	104	133	80	128	Critical component of cell membranes in all animal tissues; not present in plant products. Precursor to steroid hormones and vitamin D; essential in brain cell function

Source nutrient information: USDA Nutrient Database for Standard Reference, Release 27. Nutrient Data Laboratory Home Page. http://ndb.nal.usda.gov/ndb/. Accessed 10-27-14 (NDB#13364 beef composite, 10093 pork composite, 17004 lamb composite, 05747 chicken breast, 05098 chicken thigh, 05220 turkey breast, 05741 turkey thigh).

[a]Reported as 18:1 undifferentiated.

[b]Reported as 18:1 *c*.

Source for lipid characteristics and functions: Shils, M. E., Olson, J. A., Shike, M. and Ross, A. C. (eds.) (1999). *Modern nutrition in health and disease* (9th ed.). Philadelphia, PA: Lippincott Williams & Wilkins

contribution to $n-3$ LCPUFA intake goals. Conjugated linoleic acid (CLA) is a metabolic end product of the rumen biohydrogenation of LA and accumulates in the fat and muscle of ruminant animals. Health benefits attributed to CLA include reduced biomarkers of CHD and changes in various cancer-related outcomes in animal models and cell lines. However, clinical trials with CLA have shown that intakes to achieve these benefits are typically associated with supplemental levels, averaging 5 g daily; a 100 g serving of meat may provide only 20 mg.

Finally, like all animal products, meat is a source of dietary cholesterol. While cholesterol is often viewed as responsible for increased risk of CHD, cholesterol is actually a critical component of cell membranes and essential for brain function. Regardless of meat source, cholesterol averages 80–130 mg per 100 g serving. One large chicken egg provides almost all the daily reference value for cholesterol, while several servings of meat must be consumed to accomplish the same.

Protein

It is estimated that ~40% of the world's protein intake (79 g/capita/day) is derived from animal products (31 g/capita/day). Protein intake worldwide has increased by roughly 15% since 1992, but only 5% of this increase has been from animal products. Of the worldwide total protein derived specifically from meat, dairy, and eggs, ~45% (14.2 g/capita/day) is contributed by meat. More specifically, in the most developed nations, it is estimated that 35 g of daily available protein is derived from meat, while in the least developed countries, about 6 g of available daily protein is sourced from meat.

The proportion and profile of the essential amino acids, and the true ileal digestibility of each essential amino acid, determine the quality of protein. Animal proteins contribute a near complete profile of dietary essential amino acids and are classified as of high biological value, while those lacking in essential amino acids, such as most plant-based proteins, are classified as incomplete or of low biological value. The protein digestibility-corrected amino acid score (PDCAAS) has long been used to determine the value of various proteins in the human diet. The PDCAAS score considers both the ability of a protein to supply essential amino acids and the ability of humans to digest that protein. Proteins are given a score on a continuum from 0 to 1.0 with protein from whole wheat and wheat gluten considered of poor value (0.25 and 0.42 PDCAAS

scores, respectively) and milk, egg, and soy protein providing the highest value with a score of 1.0. It is acknowledged, however, that PDCAAS often overestimates dietary protein quality, particularly for lower-quality proteins. PDCAAS relies on fecal digestibility, evaluating amino acids remaining at the end of the colon from various food sources. Overestimation occurs because, once present in the colon, amino acids are no longer available for absorption. PDCAAS also underestimates the quality of animal proteins because values are truncated at 1 and most animal proteins would be expected to score well over 1. Consequently, FAO has endorsed a newer scoring method, digestible indispensable amino acid score (DIAAS). DIAAS gives a more accurate assessment of digestion and absorption based on ileal digestibility (i.e., amino acids available from the small intestine). So, while milk and soy score 1.0 for protein quality using PDCAAS, based on DIAAS methodology, they would score 139 and 102, respectively. The amino acid content of meat varies depending on collagen content, with those cuts containing more collagen containing less essential amino acids. DIAAS data for all meat sources or cuts are not yet available, but a recent estimate indicates a DIAAS score of 131 for beef.

Protein/Energy Ratio

Caloric deficit in developing countries contributes to diseases of malnutrition. Caloric excess in developed countries contributes to weight gain and obesity and the various chronic diseases caused by excess body weight. It is estimated that about 10% of the world's caloric intake comes from protein, whereas more than 20% comes from more calorically dense fat. Globally, consumption of meat and offals contributes approximately 17% of the dietary energy supply or about 225 kcal/capita/day. Because plant-based proteins are often low in SFA, they are promoted as an alternative to animal-based proteins. However, the caloric cost of about 1 day's intake of protein from a range of plant-based sources is five times higher than if derived from lean meat. Studies designed to promote and maintain weight loss in overweight and obese adults indicate that proteins derived from lean red meat, poultry, or fish all support healthy body weight.

Current research further suggests that protein-based foods are more satiating compared to carbohydrate-based foods. This evidence indicates that shifting the macronutrient mix to favor a higher percentage of calories from protein (doubling on average) is associated with higher satiation and preservation of lean mass. For overweight or obese individuals, higher protein weight loss diets have also been shown to sustain weight loss more effectively than carbohydrate-rich diets.

Micronutrients

Meat not only has a favorable protein to energy ratio but also is nutrient-dense. A serving of meat (100 g) is a concentrated source of the trace minerals iron, zinc, and selenium. Red meat or dark poultry meat supplies up to 20% of adult daily requirements for iron. Beef is the richest source of zinc followed by lamb with beef providing up to 45% of zinc requirements (Table 4). All meat provides at least 30% of average selenium requirements with pork being the most significant, providing over half the recommended daily intake. The trace minerals available from meat are highly bioavailable compared with those from plants. While plant foods can supply similar quantities of iron per 100 g serving (Table 4), the bioavailability of iron from these foods is limited. Heme iron, present in meat, is more bioavailable than nonheme iron, present in plants. The absorption rate of iron from a meat-containing meal averages approximately 25% but can range from 10% to 40% depending on the iron status of an individual, the calcium content of the meal, and how the food was prepared with higher cooking temperatures decreasing heme iron absorption. In contrast, the absorption of nonheme iron is at most half that of heme iron ranging from 5% to 12% and influenced by the presence of pro- and antinutrient factors, such as ascorbic acid and phytates, respectively, as well as the iron status of the individual. Like iron, zinc is abundant in the diets of those regularly consuming meat, but limited in largely plant-based diets. In grains, the majority of zinc is located in the germ and bran portion, and up to 80% of zinc is lost during milling. Furthermore, while plants can contain similar amounts of zinc per serving as lean poultry, the presence of phytates limits its absorption. Selenium content of foods varies largely across geographic locations and is related to environmental and agricultural factors such as selenium content of soil and fertilizer. These factors influence directly the content of cereal grains, animal feeds, and resulting meat products. Generally speaking, however, meat is a more significant source of selenium than plant-based foods.

Meat is also a key source of B vitamins. Vitamin B_{12} is only found in animal products, and thus, individuals adhering to a diet free from animal products are at risk for developing a deficiency. Beef liver is one of the best sources of B_{12} providing more than ten times recommended intakes in a 100 g serving. Meat from cattle and sheep can provide up to half the recommended intake per serving (Table 4). Pork is notable as a particularly rich source of thiamine, second only to the yeast extract spread vegemite. Unrefined grains are also a source of thiamine, but processing removes a considerable amount, meaning reliance on polished rice as a dietary staple can contribute to the development of thiamin deficiency disease (i.e., beriberi). Poultry meat and pork are also leading meat sources of vitamin B_6 and niacin.

Health Effects

Growth and Development

The transitional stage from 6 to 12 months, when infants are introduced to complementary foods (solid or liquid), is viewed as a particularly vulnerable and dangerous period for development of diseases of malnutrition, including stunting and wasting, starvation, and a predisposition to chronic noncommunicable disease later in life. Iron deficiency anemia is the most common nutrient deficiency in the world, affecting growth and development of infants and young children as well as pregnancy outcomes. Zinc deficiency is widespread in developing countries and related to increased risk of morbidity and mortality from diarrhea, pneumonia, and malaria, particularly among infants and children. First weaning foods in most countries are low-fat, carbohydrate-rich grain-based products

Table 4 Select nutrient contributions of meat

		100 g cooked portion (lean, skinless, boneless)								
Nutrient	*NRV-R*[a]	*Beef*	*Pork*	*Lamb*	*Chicken breast*	*Chicken thigh*	*Turkey breast*	*Turkey thigh*	*Pinto beans*	*Nutrient function*
Macronutrient										
Protein (g) (%NRV-R)	50	29.88 (59.8)	27.51 (55.0)	28.22 (56.4)	30.54 (61.1)	24.76 (49.5)	30.13 (60.3)	27.71 (55.4)	9.01 (18.0)	Supplies essential amino acids required for: normal growth, immune function; and, tissue protein synthesis including immunoglobulins, enzymes, hormones. Maintains fluid and pH balance; provides energy
Minerals										
Iron (mg) (%NRV-R)	14	2.99 (21.4)	1.00 (7.1)	2.05 (14.6)	0.45 (3.2)	1.13 (8.1)	0.71 (5.1)	1.43 (10.2)	2.09 (14.9)	Component of: hemoglobin and myoglobin to carry oxygen; hormones and enzymes including those needed for cell respiration
Phosphorus (mg)	NE[b]	231	231	210	258	230	230	212	147	Component of: bones and teeth; nucleic acids and phospholipids; enzymes involved in energy metabolism. Intracellular buffer
Potassium (mg)	NE[b]	355	357	344	391	269	249	227	436	Participates in the regulation of acid–base balance and osmotic pressure of body fluids. Impacts blood pressure and muscle contraction, especially cardiac muscle
Selenium (μg)	NE[b]	21.3	44.2	26.1	28.4	27.1	30.2	31.4	6.2	Component of antioxidant systems, which aid vitamin E and fatty acid metabolism and tissue respiration
Zinc (mg) (%NRV-R)	15	6.78 (45.2)	2.90 (19.3)	5.27 (35.1)	0.90 (6.0)	1.92 (12.8)	1.72 (11.5)	3.51 (23.4)	0.98 (6.5)	Component of antioxidant enzyme systems. Essential for insulin formation and function
Vitamins										
Thiamin (mg) (% NRV-R)	1.2	0.090 (7.5)	0.672 (56.0)	0.100 (8.3)	0.107 (8.9)	0.096 (8.0)	0.035 (2.9)	0.060 (5.0)	0.193 (16.1)	Component of enzyme required for metabolism of carbohydrates, fat, and branched-chain amino acids
Riboflavin (mg) (% NRV-R)	1.2	0.240 (20.0)	0.311 (25.9)	0.280 (23.3)	0.213 (17.75)	0.218 (18.2)	0.205 (17.1)	0.375 (31.3)	0.062 (5.2)	Component of enzymes involved in tissue respiration and hydrogen ion transport
Niacin (mg) (%NRV-R)	15[c]	4.000 (26.7)	6.503 (43.4)	6.320 (42.1)	12.133 (80.9)	6.208 (41.4)	11.750 (78.3)	6.685 (44.6)	0.318 (2.1)	Component of enzyme required for oxidation of carbohydrate and fat. Required for glycogen and fatty acid production
Vitamin B_6 (mg) (% NRV-R)	1.3	0.350 (26.9)	0.577 (44.4)	0.160 (12.3)	1.157 (89.0)	0.462 (35.5)	0.807 (62.1)	0.438 (33.7)	0.229 (17.6)	Component of enzymes needed for: synthesis and breakdown of amino acids, glycogen, and unsaturated fatty acids; production of niacin from tryptophan
Vitamin B_{12} (μg) (% NRV-R)	2.4	2.64 (110.0)	0.68 (28.3)	2.61 (108.8)	0.21 (8.8)	0.42 (17.5)	0.39 (16.3)	1.65 (68.8)	0.000 (0.0)	Essential for biosynthesis of nucleic acids and nucleoproteins. Required for maturation of red blood cells. Closely linked with folate metabolism
Choline (mg)	NE[b]	114	65	NR[d]	NR[d]	72	84	96	35	Component of acetylcholine and phospholipids involved in cell communications and nutrient metabolism

USDA Nutrient Database for Standard Reference, Release 27. Nutrient Data Laboratory Home Page. http://ndb.nal.usda.gov/ndb/. Accessed 10-27-14 (NDB#13364 beef composite, 10093 pork composite, 17004 lamb composite, 05747 chicken breast, 05098 chicken thigh, 05220 turkey breast, 05741 turkey thigh, 16043 pinto beans cooked).

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[a]Nutrient reference values – requirements.

[b]Not established.

[c]Niacin equivalents.

[d]Not reported.

Source: Shils, M. E., Olson, J. A., Shike, M. and Ross, A. C. (eds.) (1999). *Modern nutrition in health and disease* (9th ed.). Philadelphia, PA: Lippincott Williams & Wilkins

that provide limited amounts of key micronutrients, fatty acids, or high-quality protein. In many developing regions, plant-based foods remain as staples throughout the first years of life at the expense of animal products and, thus, limit intake of key nutrients such as iron, zinc, and $n-6$ and $n-3$ LCPUFA. Because of the nutrient density of meat, and similar nutrient profile to human milk, small amounts of meat can dramatically improve the nutritional status of children during development. Consumption of meat 1–3 times a week among older infants has been shown to reduce the incidence of stunting by 36% and wasting by half. However, estimates suggest that consumption of meat on a regular basis may be as low as 10% for infants and 20% for young children in developing countries.

Sarcopenia

Middle-aged and older adults often experience a gradual loss of muscle mass and function known as sarcopenia. Stressors such as illness and injury resulting in bed rest or decreased activity often hasten its progression. While many factors contribute to the development of sarcopenia, diet, specifically protein intake, is an important consideration. The type, amount, and timing of protein intake throughout the day are all factors for dietary strategies to reduce the risk of sarcopenia. Evidence suggests that a range of protein intakes, often higher than commonly recommended (0.8 g kg^{-1} body weight), may be necessary to support optimal health. The protein intake of older adults is often skewed with most consumed as plant-based protein during the evening meal. Current research suggests that 25–30 g of high-quality protein at each meal improves postprandial muscle protein synthesis and performing exercise in proximity to protein intake further enhances muscle anabolism. Further, the high leucine content of meat and dairy supports muscle protein synthesis.

Cancer

It is estimated that about a third of cancer-related deaths may be due to lifestyle factors such as diet, physical activity, and obesity. While maintaining a healthy body weight through balanced nutrition and adequate physical activity consistently lowers cancer risk, evidence for individual dietary components, as related to cancer, is much less clear. For example, increased fruit and vegetable consumption is commonly advised to reduce cancer risk, but research since the mid-1990s has weakened the scientific evidence and is now described by expert panels to be somewhat less impressive. Similarly, research aiming to identify an independent effect of meat and/or red meat on cancer has also been inconsistent. Studies reporting positive associations between red meat and cancer are often confounded by an unhealthy lifestyle including smoking, higher body weights, and inadequate physical activity. Meat, particularly red meat, has often been associated with a 'Western' dietary pattern characterized by high intakes of animal products, refined sugars, and fats, which collectively are thought to contribute to an increased risk of various chronic diseases. Certain assumptions often underpin the conclusions drawn from dietary pattern data, in particular the assumption that all foods in a food group are created equal and, therefore, all equally contribute to health outcomes attributed to various patterns. Disentangling the independent effects of individual foods on health outcomes is a substantial challenge.

Heart Disease

Early research examining dietary patterns and risk of developing chronic disease determined a relationship between excess dietary fat intake and development of cardiovascular disease. High animal fat intake, particularly from red meat, was marked as a culprit in raised blood total and LDL cholesterol levels. Resulting nutrition recommendations often encouraged reduced consumption of red meat and/or consumption of lean meats as a means of controlling blood cholesterol levels and, thereby, reduced risk of disease. However, more recent data from high-quality RCTs have demonstrated that diets designed to reduce CHD risk are as successful at achieving target blood lipid levels (i.e., total cholesterol, LDL, HDL cholesterol, and triglycerides) and may be easier to adhere to long term, if they contain sufficient protein from lean red meat.

Conclusion

In developed counties, meat contributes significantly to daily protein and micronutrient intakes, but as a source of dietary fat, its positive role in the diet has been confounded by dietary patterns associated with caloric excess in developing countries. Meat consumption has been a fundamental component of the human diet throughout history. Today, global demand for meat continues to increase. A substantial contributor of high-quality protein and essential micronutrients, meat intake has an important role in nourishing humans.

See also: Beef; Fats: Production and Uses of Animal Fats; Meat: Conversion of Muscle into Meat; Meat: Eating Quality and Preservation; Poultry: Processing; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Vegetarian Diets.

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Meat: Structure

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Introduction

Meat is the mostly widely used term to describe the edible part of animal tissues and any processed products prepared from these tissues. The term 'meat' not only refers to the skeletal muscle, adjacent fat, and other tissues but also can be used to refer to other edible tissues such as offals. Meat is a highly prized food commodity that provides many nutrients essential for human health.

In general, meats are classified by the source of provenance (type/species of animal), as follows:

- Red meat corresponds to mammal meat.
- White meat corresponds to fowl meat.
- Seafood corresponds to fish and shellfish meat.
- Game represents the meat from animals that are not commonly domesticated.

In general, beef refers to the meat from cattle, veal from calves, pork from pigs, lamb from young sheep, and mutton from sheep older than 2 years.

Meat is a very complex food product that can be affected by both *antemortem* and *postmortem* factors. The structure, composition, and characteristics of meat are highly linked with the structure of the living muscle. Contractile proteins and connective tissue as components of the muscle can thus influence the quality of the final product. Knowledge of the structure and composition of the muscle, as well as the process of its conversion to meat, is very important for the characterization and appreciation of the quality of meat.

Meat: Structure

The muscle consists of contractile cells or fibers that are largely composed of contractile protein complexes (termed myofibrillar proteins). The connective tissue surrounds and supports the muscle cells. The muscle has three layers of connective tissue, as presented in Figure 1: endomysium, perimysium, and epimysium.

The connective tissue is involved in the 'background toughness' and the myofibrillar proteins are involved in the 'myofibrillar toughness.' The toughness of meat is highly related to the sarcomere length in terms of interaction between actin and myosin proteins from the muscle. Knowledge of the muscle function, force generation/transmission, and the associations between the structures is very important for the interpretation and understanding of the properties of meat.

The muscle cells in red meat include the following components:

- The sarcolemma, also called myolemma, plasmalemma, and plasma (cell) membrane, is the double-layered membrane inside the endomysium, the tissue that completely surrounds each fiber and defines the limits of the muscle cell. The sarcolemma displays the typical characteristics of eukaryotic cell membranes. The sarcolemma provides a barrier between extra- and intracellular components, whose properties are defined largely by the lipids (especially phospholipids) in its composition.
- The sarcoplasm is the cytoplasm of the muscle fiber, populated mostly by myofibrils, and contains organelles (mitochondrion, lysosomes, nuclei, Golgi apparatus, and ribosomes), enzymes, and other substances (glycogen and lipid droplets), which are essential components for normal cellular metabolic activity.
- The sarcoplasmic reticulum stores and releases available calcium and surrounds myofibrils. The sarcoplasmic reticulum and T-tubules as components of intracellular membrane form a system that transports a neural impulse to the inner part of the muscle cells controlling the processes of relaxation and contraction.
- The sarcolemmal cytoskeletal proteins can be classified into
  - myofibrillar proteins (α-actinin, nebulin, titin, and desmin) and
  - sarcolemmal proteins (vinculin, integrins, talin, paxillin, dystrophin, and glycoprotein complex).
- The myofibrillar proteins that form the contractile system represent ~80% of the muscle cell volume. From all the muscle cell components, myofibrillar proteins are the most important in the proteolytic process that involves changes in meat tenderization during *postmortem* storage.

Myofibrils are characterized by light bands, which are isotropic, named I-bands, and dark bands, which are anisotropic, called A-bands. I-bands have at their center dark lines, called Z-lines or Z-disks (the contractile system represents the component located between two adjacent Z-disks). At the center of the A-bands is a light zone – the H-zone, characterized by the H-line. The A-band consists of thick filaments (mainly myosin) and the I-band consists of thin filaments (mainly actin).

The Thick Filament. The most predominant myofibrillar protein of the thick filament is *myosin* (approximately 45% of myofibrillar protein). Myosin is a structural protein, an asymmetrical molecule, consisting of six polypeptide units, and has an elongated rod-shaped structure; at the end of the myosin is a thickened area, called the head region; the thin region, named the tail of the molecule, consists of two polypeptides in a coiled α-helix. Myosin presents two globular heads at one end (double-headed molecule). It also possesses an ATPase activity; the double-headed regions bind and hydrolyze ATP to ADP. The myosin filament has a cylindrical shape and the center of the A-band contains the rod region of myosin (free of the globular double heads) – this region is named the pseudo H-zone.

The Thin Filament is composed of three important myofibrillar proteins: actin, tropomyosin, and troponin. The thin filament (visible in the I-band) has two coiled strands of

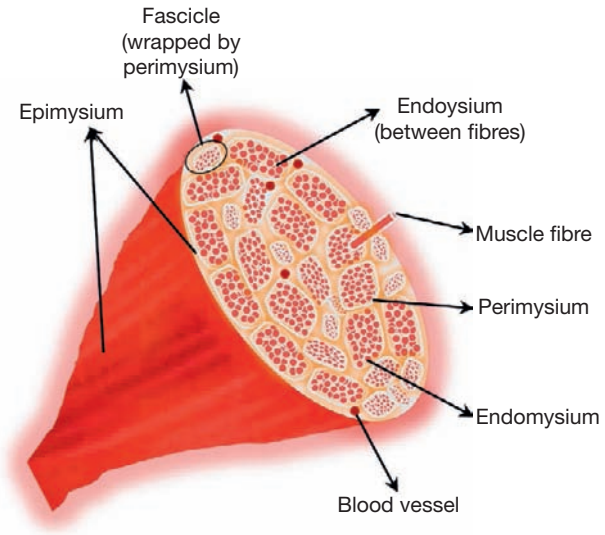


Figure 1 Structure of the skeletal muscle.

polymerized actin fibrous molecules (*F-actin*) that wrap around each other, creating a double-helix structure, a thin filament appearing like a four-stranded rope. Since actin is the protein with the highest concentration in thin filaments, it is usually referred to as actin filaments.

Actin molecules represent ~20% of the myofibrillar proteins. Tropomyosin consists of two polypeptide chains that associate to form a coiled helix (α-helical structure). It has been reported that tropomyosin is located in the groove of the coiled actin, characterized by two different units, having different molecular weights, called α-tropomyosin and β-tropomyosin.

Troponin is an asymmetrical protein and has three fractions:

- Troponin T is characterized by the fact that it is bound to troponin subunits C and I and links in the I-band the troponin and tropomyosin molecule.
- Troponin C binds to troponin I and troponin T, is responsible for the process of forming the complex troponin–tropomyosin–actin (calcium complex), and is the Ca^{2+} receptor that controls its regulation – in terms of muscle contraction.
- Troponin I represents the inhibitory fraction, interacting between actin and myosin in the relax stage, and also binds tightly to troponin C and actin (the presence of calcium ions represents a favorable situation) and binds slightly to tropomyosin/troponin T.
- Each *F-actin* filament is anchored in a Z-disk that consists mainly of actin. The Z-disk consists of thin filaments – Z-filaments –that are connected with actin filaments.

α-Actin is the major component of the Z-line being an integral part of Z-line. Desmin is another important constituent of the Z-disk and presents in the periphery of Z-disk. Since it connects many components of the cytoplasm, desmin has a very important role.

Muscle Contraction

Muscle contraction refers to the voluntary contraction of thick filaments (which contain myosin) and thin filaments (which contain actin). The process of hydrolysis of ATP is considered an energy provider; the movement of the actin filament over the myosin filament leads to the formation of the actomyosin protein complex, characteristic of muscle contraction (as presented in Figure 2).

The crossbridge can be classified as weak or strong, in terms of the nucleotide status of myosin. Strong crossbridges can be found in rigor muscles and contracting muscles (stiff/inflexible muscles), while weak crossbridges can be found in relaxed muscles (extensible muscles). Troponin and tropomyosin are involved in the regulation of the interaction between myosin and actin. Tropomyosin is a flexible protein, whose position fluctuates on the surface of the thin actin-containing filament. The contraction of a skeletal muscle cell starts with the activation in the motor cortex of the brain using a signal of a potential action in a motor neuron that leads to the release of acetylcholine. A membrane depolarization process then takes place, followed by calcium release into the cytosol creating a higher calcium concentration there. Once strong crossbridges are formed, the sarcomere shortens, the force is transmitted to the tendon and bone, and the crossbridge cycle is completed.

The relaxation process implies repolarization, that is, the removal of acetylcholine from synaptic cleft and the release of cytosolic calcium, which leads to a decrease of calcium concentration in the cytosol.

Voluntary muscular contractions can be classified according to the changes that can occur in their length or in terms of the force levels, as follows:

- Concentric contraction: The force generated is sufficient to overcome the resistance, being characterized by the process of muscle shortening.
- Eccentric contraction: The force generated is insufficient to overcome the external load on the muscle, being characterized by the process of lengthening of the muscle fibers.
- Isometric contraction: There are no changes in the length of the muscle.
- Isotonic contraction: This is characterized by the fact that despite a change in muscle length, the tension in the muscle remains constant.
- Isovelocity or isokinetic contraction: This is characterized by the fact that the muscle contraction velocity remains constant, while force is allowed to vary.

Theory of Contraction: Sliding Filaments

Figure 2 presents the formation of a crossbridge and the process of how the muscle uses energy to break the crossbridge:

- In the first stage, myosin attaches to actin; in this stage, the myosin head presents the highest energy configuration. ADP and P_i remain bound to myosin.
- The second stage is characterized by the orientation of the myosin head on the actin, sliding thorough the thin filament, and resumes the tightly bound rigor state; the power stroke is initiated by the release of P_i.
- During the third stage of crossbridge formation, a molecule of ATP is attached to the myosin head; the result of this is the detachment of the crossbridge.
- The fourth stage is characterized by the hydrolysis of ATP, the formation of ADP and P_i; as a result, the process of formation of another crossbridge can begin.

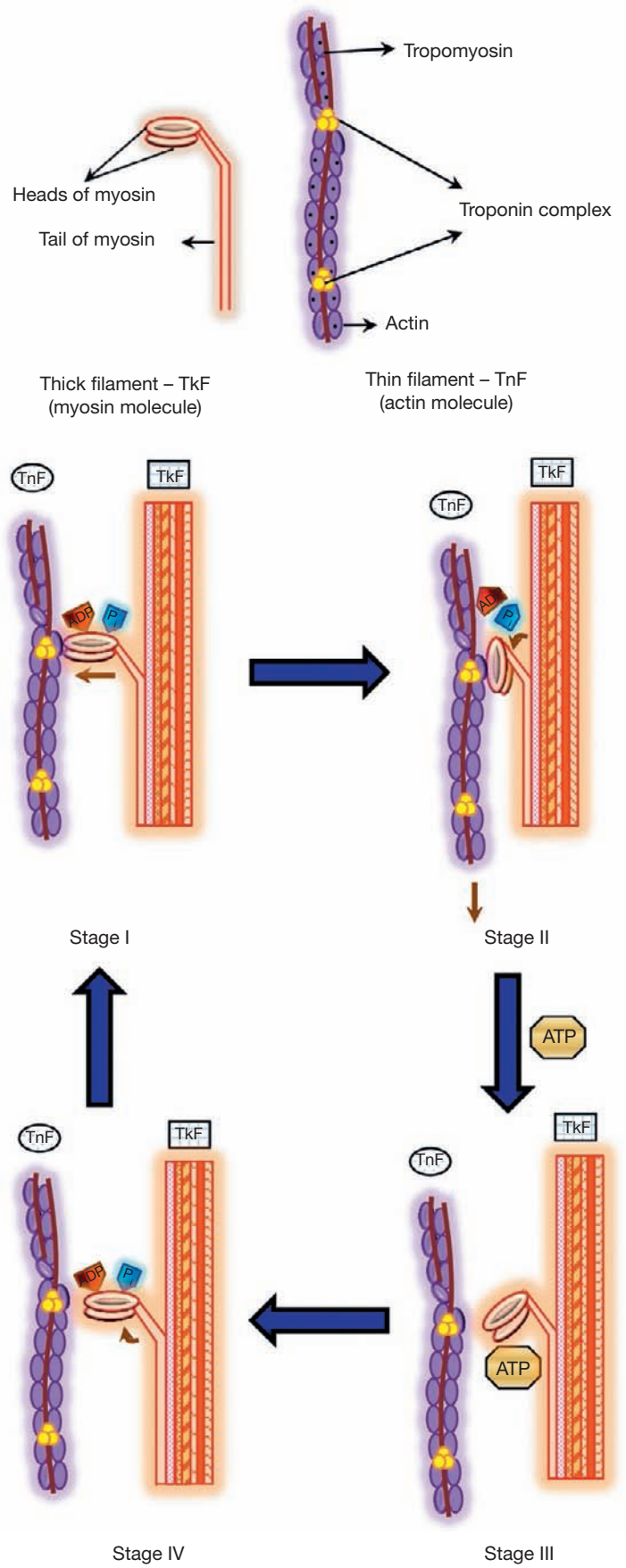


Figure 2 The stages of crossbridge cycle formation during muscle contraction.

Postmortem Changes in Meat: Conversion of Muscle to Meat

In the conversion of prerigor to postrigor muscle, the ATP concentration drops very low, the concentration of crossbridges becomes high and the calcium level becomes low – preventing the relaxation of the muscle. If no ATP is available, the thick and thin filaments remain bound together. The intrinsic metabolic process that is responsible for restoring ATP will stop functioning once the animal dies. The rigor state is characterized by the fixation of the actomyosin structure, which is very important in the process of conversion of muscle into meat (via glycolysis). During the process of conversion from muscle to meat, several chemical and structural changes occur that have a high impact on the quality and functionality of the meat. The most important changes that take place during slaughter and processing are the following:

- Declining pH due to the accumulation of lactic acid (meat is becoming more acidic leading to a reduced water-holding capacity)
- Increasing ionic strength (the sarcoplasmic reticulum loses the ability to sequester calcium)
- Changes in the redox potential
- Decreases of the energy levels
- Color changes
- The rigor bonds (crossbridges between actin and myosin cause rigor mortis process in the muscle or muscle rigidity, a process that reduces the tenderness of the meat)

To improve meat palatability in terms of flavor and tenderness, meat is stored for a period of time at refrigerated temperature – this period of time is named aging. During the aging process, meat tenderness increases – in terms of the myofibrillar component, for example, a decrease in the intensity of Z-lines can be observed in the meat structure under microscopy.

Meat tenderness increases slightly during the first 3–4 days of aging. Some of the *postmortem* changes occur due to some specific proteolytic enzymes from the lysosomes called cathepsins. Also involved in *postmortem* changes, especially in the tenderization that occurs during the aging process, is the calpain system. It has been reported that calpains degrade myofibrillar proteins including titin, nebulin, desmin, and TnT and greatly contribute to the process of *postmortem* tenderization. Collagen molecules also have a very important role in defining meat tenderness. The proteolysis of collagen *postmortem* is very low, and intermolecular crossbridges are not degraded. Collagen will hydrolyze to form gelatin, if heated in the presence of moisture. One important collagen crossbridge is pyridinoline, which is specific to older animals, is heat-stabile, and is nonreducible. The improvement of tenderness that occurs during *postmortem* changes is therefore due mainly to proteolytic degradation of the myofibrillar proteins and the toughness associated with collagen level and cross-linking in *postmortem* meat, termed 'background toughness.'

Meat flavor is strongly associated with the protein and fat content and their status. The breakdown of protein and fat (proteolysis and lipolysis) produces flavor compounds including acetaldehyde, hypoxanthine, hydrogen sulfide, and xanthine, which combine to define the complex flavor of aged meat.

Meat: Composition

Nutrients are chemical compounds or environmental substances that are used for energy and growth and for an organism's physiological functions, so they can be:

- energy providers (carbohydrates, proteins, and lipids),
- growth promoters (proteins, lipids, vitamins, minerals, and water),
- physiological function regulators (carbohydrate, proteins, lipids, vitamins, minerals, and water).

Depending on the amount needed, nutrients can be classified as macronutrients (proteins, carbohydrates, and lipids) and micronutrients (vitamins and minerals).

Each macronutrient provides energy, expressed in calories. In terms of energy, proteins and carbohydrates provide 4 calories per gram and lipids 9 calories per gram.

The main chemical components of meat tissue are water (moisture), proteins, lipids, carbohydrates, and inorganic matter. Factors that can have variable influence on the chemical composition of meat include species, breed, genetics, gender, nutrition, chronological and physiological maturity at harvest, weight at slaughter, muscle or cut of meat, preslaughter conditions, *perimortem* conditions, and *postmortem* processing. The composition of meats largely depends on the lean to fat ratio, in terms of providing energy and high nutritional value proteins.

Water/Moisture

The principal constituent of meat is water. In living muscle tissues, the water content can reach up to 80% of the total mass.

The water in the muscle can be present within and/or between the myofibrils, between the myofibrils and sarcolemma, between muscle cells, and between groups of muscle cells. In terms of location, water is present in muscles in three phases: bound water, immobilized water, and free water. In the process of conversion of muscle to meat, the formation of lactic acid leads to a reduction of the pH value in the meat. When the pH value is close to the pH value of muscle proteins, especially myosin, then the positively and negatively charged groups (components of the protein) will be attracted to each other. This means that the concentration of water attracted and bonded to muscle proteins will be reduced. Myofibrillar proteins therefore play an important role in defining how much moisture is retained by meat *postmortem*, termed the meat's water-holding capacity.

Protein

Proteins comprise long peptide chains of amino acids, encoded by DNA, and meats are very good sources of high-quality proteins. Muscles contain ~30% protein with a high biological value. Skeletal muscle tissue proteins can be generally classified according to their physiological function:

- Myofibrillar (contractile)
- Sarcoplasmic (metabolic)
- Stromal (connective or support)

Protein concentration in the muscle, and therefore meat, can vary with muscle fiber type, maturity, species, sex, and muscle type.

The color of meat is a very important parameter that can influence the purchasing decisions of consumers. Myoglobin is the pigment in meat largely responsible for its color and is composed of a protein moiety (globin) and a heme prosthetic group. Myoglobin is the most important iron- and oxygen-binding protein of the muscle.

The polypeptide structure of globin, consists of eight helical segments positioned around the heme moiety. Myoglobin contains an iron atom that can exist in two forms: oxidized and reduced, Fe^{2+} (ferrous form) and Fe^{3+} (ferric form), respectively.

In fresh meat, myoglobin can be found in four redox forms:

- Deoxymyoglobin (Fe^{2+}, when iron binds with water) – interior of raw meat
- Oxymyoglobin (Fe^{2+}, when iron binds with oxygen) – surface of meat in air
- Metmyoglobin (Fe^{3+}, when iron binds with hydroxide)
- Carboxymyoglobin (Fe^{2+}, when iron binds with carbon monoxide)

The formation of metmyoglobin – which causes a specific brown coloration in meat – is the result of the oxidation of Fe^{2+} (ferrous form) to Fe^{3+} (ferric form). Metmyoglobin is a physiologically inactive compound, incapable of binding oxygen.

On average, 40% of the amino acids in meat protein are termed 'essential' – these types of amino acids cannot be synthesized in the body and have to be supplied by the diet.

The essential amino acid composition of the proteins of the most important/principle types of meat is presented in Table 1.

Lipids/Fat/Triacylglycerols

The fat present between the bundles of muscle fibers within the meat is termed marbling and it contributes to the juiciness and flavor of meat. The degree of marbling in meat is a very important parameter that can also impact the firmness of meat.

Animal adipose tissue or simply fat is composed mainly of adipocytes (fat cells) and also contains stromal vascular fractions, connective and nerve tissues, and immune cells.

Fat can be present in meat as:

- intermuscular fat (the fat located between the muscles),
- intramuscular fat (the fat located within the muscles),
- subcutaneous fat (the fat located under the skin).

The main role of the adipose tissue is to store energy, through the lipid components. Due to the fact that it produces resistin and leptin hormones, the adipose tissue represents an endocrine organ that is a highly active component and is involved in coordinating various biological processes like the metabolism of energy and immune and neuroendocrine functions.

Lipids (fats) are energy-dense macronutrients, producing 2.25 times more energy compared to proteins or carbohydrates. The components of the animal adipose tissue are important in the solubilization and transportation processes for fat-soluble vitamins (A, D, E, and K).

Most of the fat is present as glycerol esters, but it also exists as cholesterol, phospholipids, and fatty acid esters. Neutral lipids represent >90%, 10% being represented by sterol esters (e.g., cholesterol) and phospholipids (phosphatidylethanolamine, or cephalin), phosphatidylserine, and phosphatidylcholine, or lecithin.

Table 2 presents the chemical structures for some representative compounds in the animal adipose tissue, with a high impact on human health. The chemical structure of phospholipid (as shown in Table 3) is very similar to the structure of triacylglycerol – phospholipids have a phosphoric acid inserted between the glycerol ester bond and the fatty acid that corresponds to position R3.

Triglycerides can be classified into:

- simple (all attached fatty acids are identical),
- mixed (two or more attached fatty acids are different).

Fatty acids can be classified as

- saturated (containing no double bonds between carbon atoms),
- monounsaturated (containing one double bond between two carbon atoms),
- polyunsaturated (containing two or more double bonds in the carbon chain).

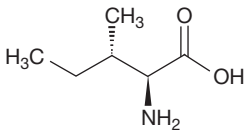
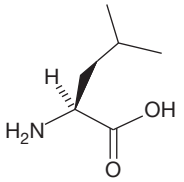
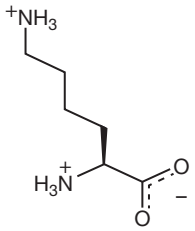
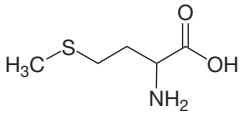
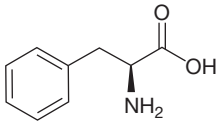
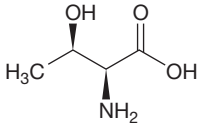
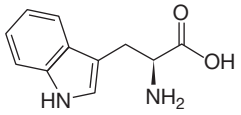
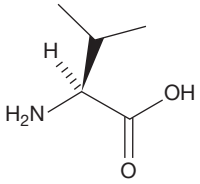
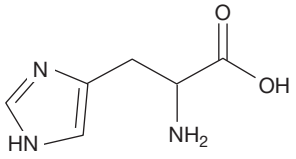
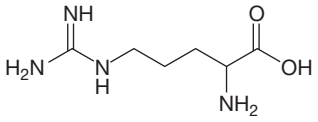
Fat is present in organisms around vital organs, subcutaneously, inter-/intramuscularly. The adipose tissue is dynamic and is constantly being stored or mobilized. Fatty acids can be synthesized by animals in the liver and adipose tissue starting from carbohydrates and proteins. It has been reported that *trans*-fatty acids have been involved and implicated in contributing to coronary heart disease, increasing low-density lipoprotein cholesterol, and in decreasing high-density lipoprotein cholesterol. Palmitic acid is the fatty acid with the highest concentration for meat samples (beef, pork, and lamb), as shown in Table 2.

Carbohydrates

Glycogen is the most predominant carbohydrate present in animal tissue; it is a multibranched polysaccharide, the principal storage form of glucose, formed by linear chains of α-D-glucose molecules linked by α-1,4- and α-1,6-glycosidic bonds. The amount of glycogen present in muscle tissues at slaughtering is very important due to the fact that it has a direct impact on the final color, texture, firmness, water holding capacity (WHC), emulsifying capacity, and shelf life of the meat. Glycolysis is the catalytic process of degradation of glucose; glycolysis means 'splitting sugars,' the process of converting glucose in two molecules of sugars containing three atoms of carbon each. The glycolytic process can occur with or without oxygen, and the components that result in the end of the process are two molecules of ATP (free energy), two molecules of pyruvic acid, and two molecules of NADH (high-energy electrons).

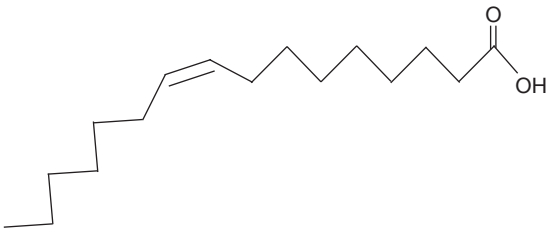
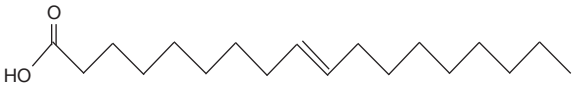
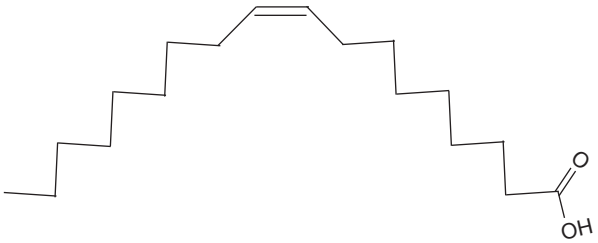
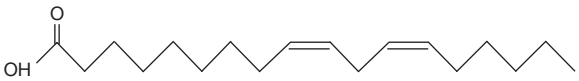
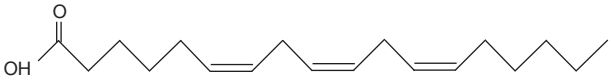
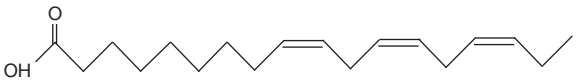
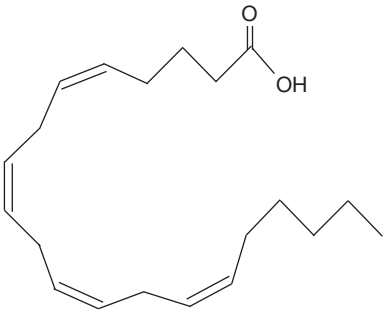
Since the content of glycogen available in the muscle is transformed into lactic acid *postmortem*, few carbohydrates are present in meat at the time of consumption. Once death occurs and circulation ceases, no oxygen is accessible to the muscle, ATP is not produced via the mitochondria, and this leads to a

Table 1 Essential amino acid composition of fresh meats from different species, expressed as % of crude protein

		Concentration of crude protein (%)		
Essential amino acid	*Chemical structure of amino acid*	*Pork*	*Beef*	*Lamb*
Isoleucine		7.9	5.1	4.8
Leucine		7.5	8.4	7.4
Lysine		7.8	8.4	7.6
Methionine		2.5	2.3	2.3
Phenylalanine		4.1	4.0	3.9
Threonine		5.1	4.0	4.9
Tryptophan		1.4	1.1	1.3
Valine		5.0	5.7	5.0
Histidine (essential for infants)		3.2	2.9	2.7
Arginine (essential for infants)		6.4	6.6	6.9

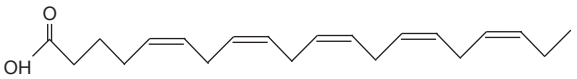
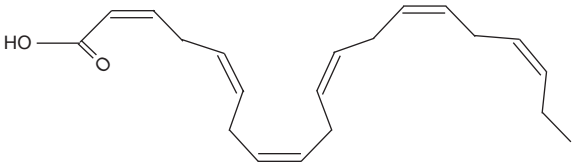
Source: Lawrie, R. A. (1998). *Lawrie's meat science* (6th ed.). Boca Raton, FL: CRC Press.

Table 2 Comparative fatty acid composition (mg/100 g muscle) of muscle from pork, beef, and lamb

Fatty acid	*Chemical–structural formula of the fatty acid*	*Fatty acid composition (mg/100 g muscle)*		
		Pork	*Beef*	*Lamb*
Saturated				
C12:0 Lauric acid		2.61	2.92	13.8
C14:0 Myristic acid		30	103	155
C16:0 Palmitic acid		526	962	1101
C18:0 Stearic acid		278	507	898
Monounsaturated				
C16:1 Palmitoleic acid		62	175	109
C18:1 ω-9 *trans*-elaidic acid		ND	104	231
C18:1 ω-9 *cis*-oleic acid		759	1395	1625
C18:1 ω-7 Vaccenic acid		92.3	91.6	71.7
Polyunsaturated				
C18:2 *n*−6 Linoleic acid		302	89	125
C18:3 *n*−6 γ-Linolenic acid		1.23	ND	ND
C18:3 *n*−3 α-Linolenic acid		20.6	26	65.8
C20:4 *n*−6 Arachidonic acid		46	22.3	29

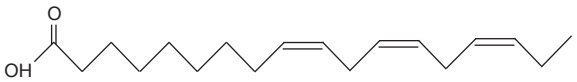
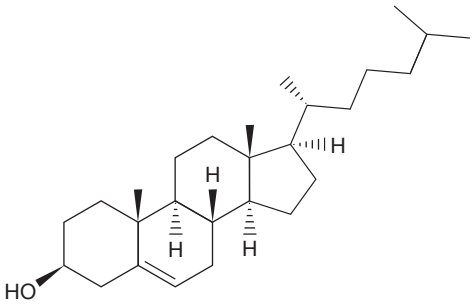
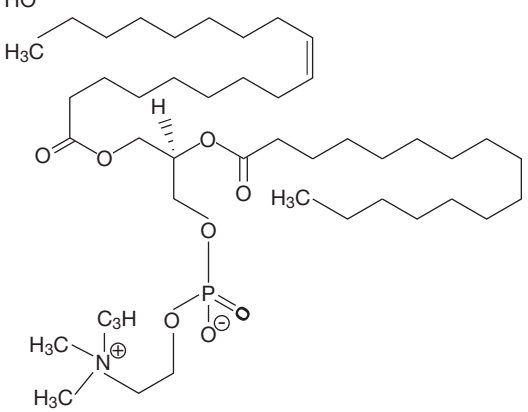
(*Continued*)

Table 2 (Continued)

Fatty acid	*Chemical–structural formula of the fatty acid*	*Fatty acid composition (mg/100 g muscle)*		
		Pork	*Beef*	*Lamb*
C20:5 *n*−3 Eicosapentaenoic acid		6.51	9.95	21
C22:6 ω-3 Docosahexaenoic acid		8.33	1.63	7.2

Source: Enser, M., Hallet, K., Hewitt, B., Fursey, G. A. and Wood, J. D. (1996). Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Science* **42**(4), 443–456.

Table 3 Chemical structure of some representative compounds for triacylglycerol, sterol esters, and phospholipids

Name	*Chemical structure*
Triacylglycerols (α-linolenic fatty acid)	
Sterol esters (cholesterol)	
Phospholipids (lecithin)	

switch in muscle metabolism to anaerobic glycolysis. Until the muscle enters rigor *postmortem*, the lactate concentration continually increases, while the glycogen concentration decreases, accompanied by a decline in the pH value. Where glycogen is depleted *premortem*, anaerobic glycolysis *postmortem* is limited, and this leads to meat that is dark, firm, and dry (DFD) with a poor shelf life. DFD is more common in beef, compared with pork. Pale, soft, and exudative (PSE) meat is more common in pork and represents the result of a rapid decrease in the pH value coincident with a high muscle temperature. PSE meat presents abnormal properties in terms of water-holding capacity, color, texture, and consistency, and as a result, meat becomes unappealing for consumers. Genetic conditions and stress level that could occur around the time of slaughter have an important impact on the PSE meat.

Apart from glycogen, there are two other important carbohydrates present in animal muscle tissue: glycosaminoglycans and proteoglycans.

Glycosaminoglycans are linear heteropolysaccharides, formed by repeating disaccharide unit that contain either of two modified sugars, *N*-acetylgalactosamine (GalNAc) or *N*-acetylglucosamine (GlcNAc), and a uronic acid (glucuronate or iduronate).

Proteoglycans are complex compounds that are formed by a core protein and one/more covalently attached chains of glycosaminoglycan.

Micronutrients in Meat

Minerals (inorganic matter)

Meats – without referring to any particular species – contain ~1% minerals. It is very well known that iron is the main nutrient that is associated with meat, but meat is a valuable source of many other micronutrients such as vitamin D, vitamin B complex, zinc, and selenium. Meats have a relatively high content of iron, zinc, phosphorous, and a low level of calcium, iodine, and magnesium.

Iron is present in meats in two forms: heme and nonheme. Heme iron represents ~50% of the total iron available in meat and it is readily absorbed because it is found naturally in the composition of hemoglobin and myoglobin (porphyrin ring). Liver contains twice as much iron as muscle meats.

Because iron is very important in the formation of hemoglobin in red blood cells, a deficiency caused by a lower bioavailability of iron can lead to anemia, defective psychomotor development in infants, impaired educational performance of children in school, adverse perinatal outcome in pregnancy, and generally a diminished work capacity, which are all associated with iron deficiency. Nonheme iron absorption is inhibited by phytate, oxalate, and other minerals (zinc, calcium, and copper) and is enhanced by vitamin C.

Meat is a valuable source of *zinc*. Meat/meat products can supply 30% of the total zinc intake, and it has a high bioavailability. Zinc from other food products is poorly absorbed. Zinc is an important cofactor in the functioning or composition of more than 200 enzymes and is involved in growth, healing, gene expression, brain maturation, cognitive development, and reproduction. Zinc deficiency can cause dwarfism.

Selenium is a micronutrient that acts as cofactor in the reduction of some specific antioxidant enzymes, such as glutathione peroxidases and thioredoxin reductase. Selenium is a component of glutathione peroxidase, a highly important enzyme that inactivates toxic hydrogen peroxide and hydroperoxides. Due to its role in this process, selenium can be considered an essential antioxidant nutrient.

Even though meat contains a relatively low concentration of selenium (as shown in Table 4), organ meats (e.g., kidney and liver) are considered valuable sources of selenium.

Selenium deficiency can cause various chronic diseases, such as cancer, diabetes, tuberculosis, and coronary heart disease.

Table 4 Mineral and vitamin composition of muscle meat (pork, beef, and lamb) (concentration expressed per 100 g of lean meat)

Micronutrient	*Pork*	*Beef*	*Lamb*	*RDI*[a]
Vitamin B_1 (mg)	0.48	0.04	0.12	1.5
Vitamin B_2 (mg)	0.18	0.18	0.23	1.7
Vitamin B_3 (mg)	6.61	5.0	5.2	20
Vitamin B_5 (mg)	0.69	0.35	0.74	10
Vitamin B_6 (μg)	0.68	0.52	0.10	2
Vitamin B_{12} (μg)	0.53	2.5	0.96	6
Iron (mg)	0.63	1.8	2.0	18
Calcium (mg)	19	4.5	7.2	1000
Zinc (mg)	1.77	4.6	4.5	15
Magnesium (mg)	25	25	28	400
Sodium (mg)	55	51	69	460–920
Potassium (mg)	343	363	344	2800–3800
Phosphorus	209	215	194	1000
Selenium (μg)	35.9	17	14	70

[a]Reference Daily Intake/Recommended Daily Intake (RDI) represents the daily intake concentration of a nutrient, considered to be sufficient to meet the requirements of 97–98% of healthy individuals. It has been used in the country where it has been developed, the United States, and in other countries.

Source: Williams, P. G. (2007). Nutritional composition of red meat. *Nutrition and Dietetics* **64**(4), 113–119; Patterson, K. Y., Trainer D., Holden, J. M. and Howe, J. C. (2009). USDA Nutrient Data Set for Fresh Pork (From SR), Release 2.0.; Council Responsible for Nutrition (www.crnusa.org).

Vitamin D (cholecalciferol)

Vitamin D is an essential component in the process of bone growth and tooth development, being involved in the process of absorption and metabolism of calcium and phosphorus. The main source of vitamin D is sunlight. The process of synthesis of vitamin D starts with the oxidation of cholesterol from food or bile to provitamin D_3 (7-dehydrocholesterol) that is transported to the skin, mainly the epidermis. UVB rays converts 7-dehydrocholesterol, which lies in the skin, to cholecalciferol.

The process continues with the hydroxylation of cholecalciferol to calcidiol (25-hydroxyvitamin D_3 ($25OHD_3$); liver) and to calcitriol (1,25-dihydroxyvitamin D_3 ($1,25OHD_3$); kidney), compounds that stimulate the process of calcium absorption. Research reports indicated that $25OHD_3$ (active metabolite synthesized in the liver) is five times more active than cholecalciferol, while $1,25OHD_3$ (active metabolite synthesized in the kidney) is 25 times more potent than vitamin D_3. So, in the calculation of concentration of vitamin D_3 in meat, the levels of $25OHD_3$ and $1,25OHD_3$ are also very important.

A serious deficiency of vitamin D can lead to the occurrence of osteomalacia, rickets, bone loss, and osteoporotic fractures. Rickets is characterized by imperfect calcification, softening, and distortion of the bones, typically resulting in bow legs.

Vitamin D is also protective against various diseases: diabetes, tuberculosis, muscle weakness, coronary heart disease, and different types of cancer.

Despite the fact that it can be synthesized in the body, many people in northern regions do not get enough sunlight exposure and thus are at risk of vitamin D deficiency. Research has indicated that lean red meat is a valuable source of vitamin D and is present in meat in a highly absorbable form. Because vitamin D is a fat-soluble vitamin, higher quantities are found in meat with high levels of fat, compared with lean meat.

Vitamin B complex

Meat is a very important and relevant source of vitamin B, which are water-soluble vitamins, so the lean meat contains

higher amounts than meat fat does. Because they are temperature-sensitive, some losses of vitamin B occur during thermal processing, depending on the cooking temperature and method.

Vitamin B_1 (*thiamine*) It is an antineuritic factor and is present in meats, particularly in pork. Pork is actually the richest meat sources of thiamine (0.91 mg/100 g), compared with beef (0.04 mg) or lamb (0.12 mg). Vitamin B_1 is a cofactor for enzymes involved in the processes of converting lipids and carbohydrates to energy. Vitamin B_1 regulates the appetite process and the functions of the nervous system, promotes growth, and is very important for normal functioning of nerve tissue.

Vitamin B_2 (*riboflavin*) It is usually known as vitamin G and is a cofactor for enzymes involved in citric acid cycle (Krebs cycle), which is the key pathway in which carbohydrates, fats, and protein are metabolized, supplying energy to the organism. A deficiency of riboflavin reduces appetite, can cause anemia, and also impairs the conversion of tryptophan to niacin.

Vitamin B_3 (*nicotinic acid*) It is an essential human nutrient that has two sources in the diet: nicotinic acid (vitamin) and tryptophan (amino acid). Meat is considered the richest food source of vitamin B_3, and more than half of the niacin available from meat is provided by tryptophan. A high deficiency of dietary nicotinic acid causes pellagra – a disease characterized by diarrhea, dermatitis, and dementia, as well as hyperpigmentation, thickening of the skin, inflammation of the mouth and tongue associated with digestive disturbances, delirium, etc.

Vitamin B_5 (*pantothenic acid*) It has an important role in the metabolism of fats, being involved in energy production and sustaining the synthesis of steroid hormones. Organisms require vitamin B_5 to synthesize and metabolize fats, carbohydrates, and proteins and to synthesize coenzyme A. Pantothenic acid is an anti-gray hair factor.

Vitamin B_6 (*pyridoxine*) Meat is a valuable source of pyridoxine. Even though vitamin B_6 is also present in plants, it cannot be absorbed from some vegetables because of the abundance of glycosides that act as inhibitors to absorption. Vitamin B_6 also plays a role in red blood cell formation and converts fat to energy, contributing to supplying the body with fuel.

Vitamin B_{12} (*cobalamin*) It is produced as a result of bacterial fermentation in the gut and is an important parameter in the process of red blood cell formation and also in maintaining a normal central nervous system. A deficiency of vitamin B can favor the occurrence of megaloblastic anemia. It is recommended that vegans and vegetarians take supplements of vitamin B_{12}, since it has an exclusively animal origin. Elderly people have a very low capacity to absorb dietary vitamin B_{12}.

See also: Beef; Lipoproteins; Meat: Conversion of Muscle into Meat; Meat: Role in the Diet; Pork Meat Quality, Production and Processing on; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Proteins: Chemistry, Characterization, and Quality; Skeletal Muscle.

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Mediterranean Diet

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Definition

The traditional Mediterranean diet (MD) is the diet prevailing until the early 1960s in the olive tree-growing areas of the Mediterranean basin. Until then and in spite of frequent violent disruptions, daily life and dietary habits in the Mediterranean region remained largely unchanged, and populations still applied simple and time-honored nutritional approaches. In the 1960s, however, the food industrial revolution, which was gradually expanding in Europe after the Second World War, finally reached the Southern European countries causing a serious disruption in the traditional dietary pattern of the Mediterranean populations.

The traditional MD is the heritage of millennia of exchanges of people, cultures, and foods of the countries around the Mediterranean basin. The succession of different dominant population groups introduced and/or contributed to the diffusion of different crops and foods. Some plants, like the olive tree, wheat, and the grapevine, have apparently been in this area since antiquity. Other plants, however, such as orange and lemon trees, as well as tomatoes, eggplants, corn, rice, and potatoes, were imported at different time periods. All of these, however, have found their way into the MD, which reflects a balanced ecosystem supported by the benevolent climate of this region.

The MD implies a common dietary pattern in Mediterranean countries; however, there are differences in the dietary patterns of the Mediterranean populations due to regional, cultural, and religious factors. However, in all of them, olive oil occupies a central position. It is therefore legitimate to consider these diets as variants of a single entity, the MD, which refers to dietary patterns found in olive-growing areas of the Mediterranean region until the 1960s. The traditional MD is characterized by high consumption of olive oil, vegetables, legumes, fruits, and unrefined cereals; regular but moderate wine intake, mostly during meals unless prohibited for religious purposes; moderate to high consumption of fish; low consumption of meat; and low to moderate intake of dairy products.

The word diet derives from the ancient Greek word 'diaita,' which implies equilibrium and lifestyle. Therefore, the traditional MD is more than just a diet; it is a whole lifestyle pattern with physical activity playing an integral role. This healthy lifestyle is also the consequence of the availability of local agricultural products, related to the geographic and climatic Mediterranean environment. A life-affirming aspect of the Mediterranean food culture is the central role of conviviality: the pleasure of sharing food with others and of celebrating communal culinary traditions and life at large. A notable point is wine consumption, which, except when prohibited on religious grounds, has been consumed in moderation in Mediterranean countries, almost always during meals and as a rule in the company of friends – the ancient Greek word 'symposium' means drinking in company, but with a connotation of intellectual interchange.

The MD was acknowledged by UNESCO in 2010 as an intangible cultural heritage with the following description:

> The Mediterranean diet constitutes a set of skills, knowledge, practices and traditions ranging from the landscape to the table, including the crops, harvesting, fishing, conservation, processing, preparation and, particularly, consumption of food. The Mediterranean diet is characterized by a nutritional model that has remained constant over time and space, consisting mainly of olive oil, cereals, fresh or dried fruit and vegetables, a moderate amount of fish, dairy and meat, and many condiments and spices, all accompanied by wine or infusions, always respecting beliefs of each community. However, the Mediterranean diet encompasses more than just food. It promotes social interaction, since communal meals are the cornerstone of social customs and festive events.

The traditional MD reflects as much the benign climate and the flora of the area, as the culture and the hardship of the people who were mostly responsible for the formulation of this diet.

Pictorial Presentation

The healthy traditional Mediterranean dietary pattern was popularized through a traditional Mediterranean diet pyramid (MDP) representation, which graphically highlights the foods to be consumed on a daily or weekly basis or less frequently. Throughout the years, various MDPs have been designed for different Mediterranean populations, tailored to their variable food habits.

After the recognition of the MD as an intangible cultural heritage of humanity, scientists have presented a consensual communication tool for the general public, health professionals, and other stakeholders. This is a pyramid with which scientists hope to contribute to a better adherence to this healthy dietary pattern and its way of life in the Mediterranean area countries and elsewhere. This graphic representation (Figure 1) provides guidance for the selection of foods, indicating the relative proportions and consumption frequency of servings of the various food groups.

Plant-origin foods are situated at the base of the pyramid. They provide key nutrients and fibers that contribute to general well-being, satiety, and the maintenance of a balanced diet and thus should be consumed in higher quantities and frequencies. This pyramid follows a standard pattern: at the base, foods that should sustain the diet and, at the upper levels, foods to be eaten in moderate to low amounts, such as those of animal origin and/or rich in sugars and saturated fats. Moreover, lifestyle elements characteristic of the Mediterranean way of life

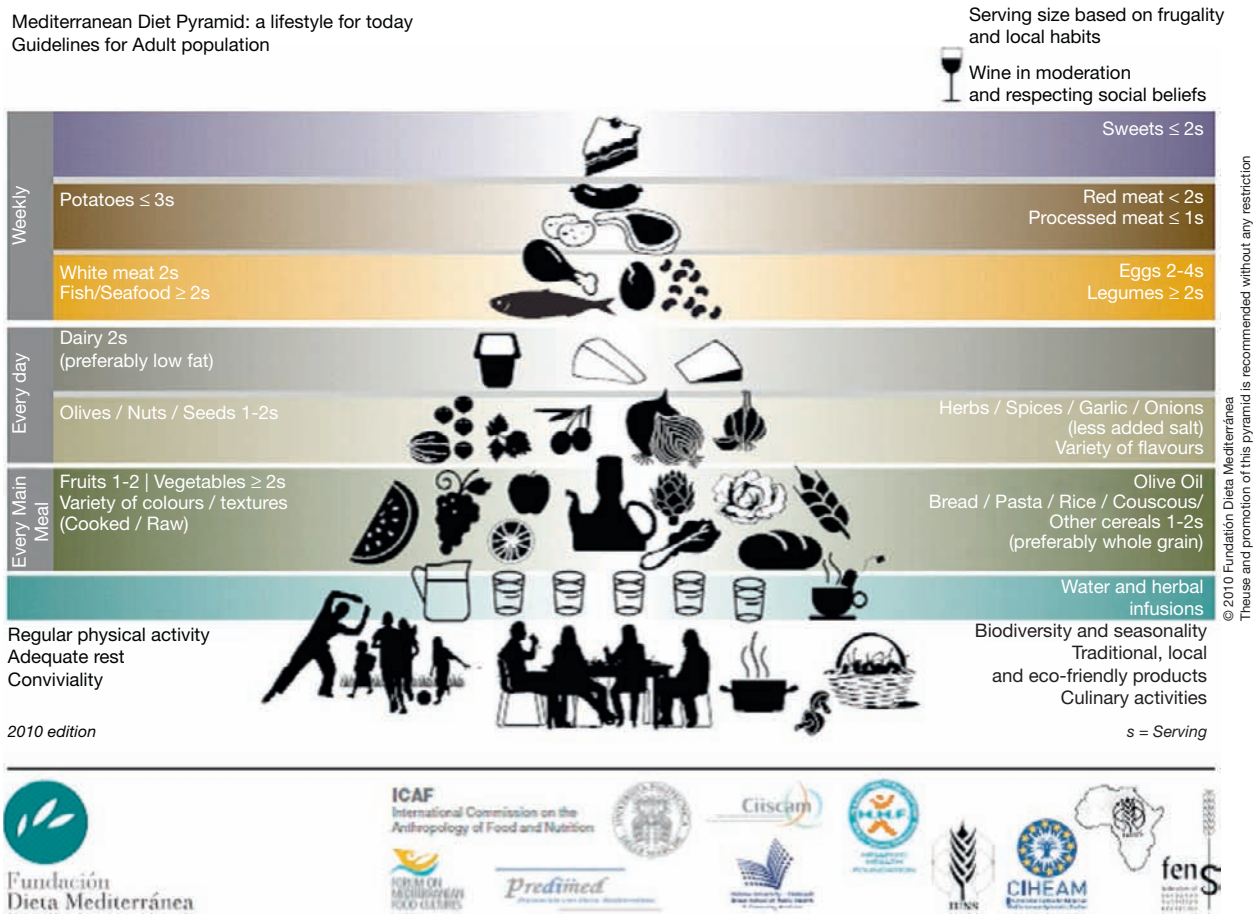

Figure 1 The new Mediterranean diet pyramid. Reproduced from Bach-Faig, A., Berry, M.E., Lairon, D., et al. (2011). Mediterranean diet pyramid today. Science and cultural updates. *Public Health Nutrition* **14**, 2274–2284.

are incorporated in the graphic design. So, it is about not only prioritizing some food groups from others but also paying attention to the way of selecting, cooking, and eating in addition to pointing to the composition and number of servings per meals. A balanced composition of the main meals should include vegetables and legumes (immersed on or cooked with olive oil) and fruits and whole-grain cereals, complemented with relatively lower intakes of dairy products and protein sources.

The MDP was conceived as a simplified main frame in order to be adapted to the different country-specific variations related to the various geographic, socioeconomic, and cultural contexts of the contemporary Mediterranean lifestyle. It addresses the healthy adult population and should be adapted to specific requirements in the case of children, pregnant women, and particular health circumstances.

In this graphic representation of the MD culture and lifestyle, elements are taken into account, such as conviviality, culinary activities, physical activity, and adequate rest, along with proportion and frequency recommendations of food consumption. These elements are stressed without omitting other items associated with the production, selection, processing, and consumption of foods, such as seasonality, biodiversity, and traditional, local, and eco-friendly products.

Health Effects

The MD addresses the paradox that although the people living in Mediterranean countries tend to consume relatively high amounts of total lipids, they have far lower rates of cardiovascular diseases than people in countries where similar levels of total lipid consumption are found. This implies that it may be the types of lipids consumed rather than the amount that adversely affect health, highlighting the beneficial role of extra virgin olive oil, generally consumed in Mediterranean countries. The MD was first considered in the pioneer Seven Countries Study as a low-saturated lipid diet that was conveying protection against coronary heart disease by lowering plasma cholesterol levels (the distinction between high-density lipoprotein cholesterol and low-density lipoprotein cholesterol was not known at that time). Over the years, however, the emphasis has shifted away from the low saturated lipid content of this diet towards its high content of olive oil and its overall composition. Moreover, the study of the MD expanded beyond its effects on coronary heart disease, to include possible effects on cancer occurrence and total mortality.

The MD is widely considered as a healthy dietary pattern, and a greater adherence to the MD has been associated with significant improvements in health and longevity. In 1995, a 9-fold

score was introduced to evaluate the adherence to the traditional MD, allowing the study of its possible health effects. The MD is characterized by high intake of (i) vegetables, (ii) legumes, (iii) fruits and nuts, (iv) minimally processed cereals, (v) moderately high intake of fish, (vi) high intake of monounsaturated lipids coupled with low intake of saturated fat, (vii) low-to-moderate intake of dairies, (viii) low intake of meat products, and (ix) regular but moderate intake of wine during meals. Adherence to a Mediterranean dietary pattern has been measured using this score with components reflecting intake of each dietary factor. The health beneficial components that are mostly consumed in the traditional MD (vegetables, legumes, fruits and nuts, wholegrain cereals, fish, and olive oil, as reflected in the ratio of monounsaturated-to-saturated lipids) were indexed positively, while the components less consumed and more typical of the Western-industrialized world (meat and dairy products) were negatively indexed. Several score variants have since been introduced and used in observational and some intervention studies to assess the relationship between conformity with the MD and various health outcomes, including total mortality, incidence of or mortality from coronary heart disease and stroke, and incidence of or mortality from cancer overall and from specific cancer sites. Indeed, numerous studies confirmed that closer adherence to the traditional MD is consistently associated with a markedly reduced risk of cardiovascular events and mortality, with a lower incidence of the metabolic syndrome and type 2 diabetes and certain types of cancer, and, eventually, increased longevity.

The diet that the Mediterranean populations developed many years ago, without any scientific input, appears to meet current dietary recommendations. Adherence to the MD may also provide the consumer with an adequate overall micronutrient supply including inorganic constituents, vitamins, and phytochemicals, thus meeting requirements for optimal health and functioning. Phytochemicals represent a common element in the typical foods of the MD, and an antioxidant action provides a plausible explanation for some of the health benefits. The variety and amount of phytochemicals taken with the consumption of primary and composite foods of the MD may provide better antiatherogenic properties than single phytochemicals.

Several studies, undertaken in both Mediterranean and other countries, have documented the superiority of the traditional MD, as an integral entity, in the prevention and supportive treatment of coronary heart disease, the prevention of certain malignant neoplasms, and, eventually, longevity and quality of life. The realization that the traditional MD is conducive to health and longevity went through several stages but reached a momentum during the last decade, following three scientific developments. In brief, these developments were the recognition that refined carbohydrates are inferior to unrefined ones, the documentation of the superiority of olive oil among the frequently used added lipids, and the successful operationalization of the traditional MD.

In conclusion, a diet that adheres to the principles of the traditional Mediterranean one is associated with better health and longer survival. Two elements distinguish the MD from other prudent diets. It stresses the pattern rather than individual components and provides no restriction on lipid intake, so long as lipids are not saturated and are preferably in the form of olive oil.

Sustainability and Mediterranean Traditional Foods

The MD has been acknowledged by the Food and Agriculture Organization of the United Nations (FAO) as an example of a sustainable diet in which nutrition, biodiversity, local food production, and local culture are closely interconnected.

> According to FAO:
>
> Sustainable Diets are those diets with low environmental impacts which contribute to food and nutrition security and to healthy life for present and future generations. Sustainable diets are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe and health; while optimizing natural and human resources.

The traditional MD is considered as sustainable because it is inextricably linked to biodiversity: It includes a variety of food practices and food preparation techniques; its main food groups are beneficial to health; it reflects an adherence to culture and traditions; it instigates respect for seasonality; it contains foods less demanding in primary energy and has limited environmental impact, due to low consumption of animal products; and it involves pastoralism, with a favorable impact on carbon sequestration, as well as meat quality.

It is important to preserve the cultural heritage of the MD as an outstanding resource of sustainable development as it contributes to promoting local production and consumption, encourages sustainable agriculture, and safeguards landscapes. Sustainable food combines environmental conservation and community development through social and economic processes.

Based on a variety of diverse local foods, which are integral to the Mediterranean environment, the MD is still an underexplored resource for biodiversity and nutrition, in the context of food and nutrition security in the region. More than just a dietary pattern, it is a potential model for effective sustainable development in the Mediterranean basin. Environmental sustainability and biodiversity may also benefit from the perpetuation of Mediterranean traditional foods, which are generally prepared with local products.

Traditional foods are footprints of the past in the contemporary life of many population groups. Traditional foods reflect cultural inheritance and impose their imprints on the respective dietary patterns. The longevity associated with the MD could be partly attributed to Mediterranean traditional foods, which this diet incorporates. 'Traditional' characterize foods that have long been used by smaller or broader local societal groups, and their recipes have been perpetuated from generation to generation by word of mouth or in writing.

Traditional foods, apart from being vehicles of our culture, may also possess health qualities, since tradition rarely honors foods that are not palatable and healthy. Beneficial effects can be attributed to traditional foods that incorporate the knowledge and wisdom of past generations who, living under difficult conditions, have learned how to optimize use of locally

available ingredients in order to produce palatable foods and recipes with potential to preserve and improve human health.

Traditional foods are generally perceived as foods with beneficial properties, due also to their naturalness and local preparation, implying lack of massive industrial processing. To consider food products as traditional in a more systematic context, it is important to look at the traditionality of the raw materials, the formulation in terms of ingredients, total composition, and methods of production and/or processing. In early 2005, the EuroFIR scientific working group on traditional foods recognizing the lack, at the time, of a definition for traditional foods established a definition of the term 'traditional' in order to classify traditional foods in European food composition databases. The EuroFIR definition of traditional foods was later acknowledged by FAO. According to the EuroFIR definition,

'*Traditional*' means conforming to established practice or specifications prior to the Second World War. '*Traditional food*' is a food of a specific feature or features, which distinguish it clearly from other similar products of the same category in terms of the use of '*traditional ingredients*' (raw materials or primary products) or '*traditional composition*' or '*traditional type of production and/or processing method*.'

The selected time limit 'prior to the Second World War' implies 'prior to the era of mass food production,' and it delineates the period when populations still applied simple and time-honored approaches. This is before the introduction of technological innovations has substantially altered the food production processes. This food industrial revolution appeared earlier in Northern European countries than Southern European countries. Under this perspective, the time limit of the EuroFIR definition could be expressed as 'prior to the 1960s' when referring exclusively to European Mediterranean countries.

Mediterranean traditional foods are often rich sources of nutrients and bioactive compounds. Many of them have a high potential of meeting micronutrient requirements, while a great number of Mediterranean traditional foods have a high potential for nutrient function claims that fall within the scope of the recent European Commission regulation on nutrition and health claims made on foods.

Traditional foods, notably those from the Mediterranean area, have earned a reputation for their nutritional quality and should, therefore, be protected and supported. The MD is currently appreciated for its low environmental impact. It is considered as a model among the world's diets. Indeed, the MD not only does offer considerable health benefits but also respects the environment.

See also: Cancer: Diet in Cancer Prevention; Olive Oil: Its Role in the Diet; Traditional Foods.

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Microscopy: Light Microscopy and Histochemical Methods

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Techniques of microscopy are frequently used to understand the structure of both healthy and sick tissues and organs. More particularly, they are used for studying the digestive tract and aid the understanding of its physiology. These methods are used in medical research and in clinical studies in order to appreciate diseases in different organs such as the liver and more especially in different parts of the digestive tract (esophagus, stomach, and intestine). Methods used in microscopy are diversified and their principles are based upon chemical and physical principles.

A Brief History of Microscopy

The anatomy of animals and plants has been known for a long time. Representations of animals with very precise details have been discovered on cave walls. During several centuries, the observations were done with the naked eye only. In the seventeenth century, the invention of the microscope introduced a revolution in the methods of observation and permitted the discovery of the smallest living beings. The inventor of the microscope is not precisely known. Galileo (1564–1642) wrote that he used a 'telescope' to observe the smallest particles. Janssen (1588–1631) probably used a microscope. Hooke (1635–1703) built a microscope and observed cavities on a piece of cork he called 'cells.' Anton van Leeuwenhoek (1632–1723) also built and improved microscopes with which he observed pieces of plants, sperm (called worms), and pond water in which he found small organisms.

Microscopy then became more and more successful. Histology developed with fixation and embedding and staining allowing the understanding of tissue organization. The cell theory was developed in 1839 by Schleiden (1804–81) and Schwann (1810–82) explaining that each living organism is constituted by cells, and the theory was completed in 1858 by Virchow (1821–1902) who said that each cell came from another cell.

After the use of dyes to differentiate the parts of tissues, giving a general view, the methods of histochemistry based upon biochemical methods were adapted to histology in the first part of the twentieth century. Later, immunohistology used the antigen–antibody reaction in order to detect the precise molecules directly on the tissues. To date, the use of *in situ* hybridization permits the appreciation of the structure of DNA or the expression of genes (mRNAs). Automatic methods allow image analysis and quantification with the help of a computer.

The Light Microscope

The principle of the light microscope is based upon the image obtained after a light is crossed through a thin sample and submitted to a system of lenses. So, a light microscope consists of an optical system with a tube with lenses on each tip: the 'ocular' near the eye and the 'objective' near the sample. Generally, several objectives are available to perform small (4 or 10×), medium (40 or 50×), or high (100×) magnifications. In this last case, it is necessary to dump the objective into special oil in order to obtain a refractive index compatible with high magnifications. To obtain total magnification, one must multiply the magnification of objective with the ocular's one (generally 10×); so with an ocular with a magnification of 10× and an objective with a magnification of 50×, the result is 500×. A system producing light is situated under the preparation. The sample is placed on a stage and the lenses can be adjusted in order to obtain a sharp image of the object. A condenser under the sample is used to modify the light intensity and the contrast of the image (Figure 1).

The magnification is directly linked to resolution or resolving power defined as the smallest distance between two points at which they can be separately observed. Resolution is given by the formula:

$$d = 0.61\,\lambda/n\sin\alpha$$

where d is the resolution, λ the wavelength of light source, n the refractory index of the medium (air or oil), and α the aperture of beam.

Light microscopes are often equipped with a camera that can be linked to a computer in order to obtain photography or to perform image analysis.

In fluorescent microscopes, a steam lamp of mercury is used. In epifluorescence microscopes, the illumination is done over the object, exciting fluorescent dyes on the sections; consequently, a fluorescent light is emitted. Filters are used to choose the good wavelength in order to obtain the fluorescent image.

General Principles of Histology

To perform a histological study, tissues and cells must be submitted to a series of operations: fixation, dehydration, clarification, impregnation, embedding, sectioning, adhesion of sections on a slide, staining, dehydration, and mounting. Each step differs according to the nature of the results expected, tissue studied, and the technique used.

Tissue Fixation

All biological material can be visualized: histological sections, smears, karyotypes, and monolayer or suspension cell cultures. Fixation allows tissues to conserve a state as close as possible to the living one. But fixation must also allow the visualization of components by histological or histochemical methods. So, this process must preserve the tissue morphology in a recognizable

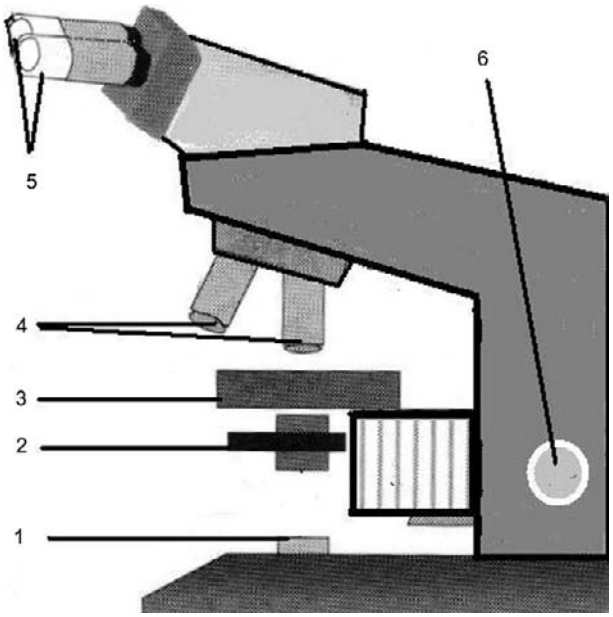

Figure 1 A light microscope; 1, light source; 2, condenser; 3, stage; 4, objective lenses; 5, ocular lenses; 6, screw used to focus.

form, without eliminating the components, and the fixative must not react with the atomic groups that will be used to visualize the structure.

Simple fixatives are constituted of only one molecule (i.e., ethanol or formalin). Composed fixatives are constituted by a mixture of simple fixatives. Some fixatives are denaturant of proteins, giving a reticulated aspect to the cytoplasm. Others are not denaturant and permit a homogeneous aspect of the cytoplasm. The effects of several simple fixatives are additive and fixative mixtures are most often used. The duration of fixation must be adapted to the thickness of the piece, and it is well variable: 24–48 h with Bouin's fluid, 4 h with Carnoy's fluid, and indefinitely with formalin.

Some methods are based on physical principles: fixation by cold between −20 °C and −40 °C and sections obtained with a freezing microtome (or cryotome). Cryodessication and freezing–dissolution combine several physical properties. It is often useful to store preserved tissues in holding fluids such as ethanol 70%, formalin, or butanol.

Embedding

Paraffin Embedding

Paraffin is hydrophobic, constituted of carbon and hydrogen with no functional group that can react with tissue molecules. Tissues are first dehydrated and immersed in a substance in which paraffin is soluble, such as toluene, xylene, or warm butanol. After this operation, tissue can become transparent. Tissue is then immersed for several hours in liquid paraffin maintained at a temperature between 50 and 65 °C, the fusion temperatures of paraffin used for histology. Then, it is immersed into liquid paraffin at room temperature in order to harden. When paraffin is solid, the organ can be cut with a microtome.

Other Embedding

Paraffin is not the alone means used for embedding. Paraplast, a mixture of paraffin and synthetic carbohydrates, is often used to obtain thinner sections than what are obtained with paraffin. Celloidin embedding has been widely used to study organs comprising both hard and smooth structures. Gelatin and resin embedding are also used.

Not only was resin embedding used primarily for electron microscopy, but also it can be used for light microscope. Blocks are particularly hard, allowing one to obtain semithin sections (0.5–1 μm) and to increase the precision of images. Epon and araldite are resins frequently used.

Histological study of calcified tissues can be done from decalcified preparations. Methyl methacrylate can be used to embed bones without decalcification, a useful method to appreciate osteoporosis.

Sections

Paraffin Sections

Paraffin blocks are put on the stage of a vertical microtome with a pair of clamps. The block is submitted to a vertical up-and-down motion. When it goes down, a section usually 5 μm thick is cut by a steel razor. The block then goes up behind the blade. Sections can be directly taken from the blade with a brush. Generally, the microtome movement is manipulated by hand but some microtomes are automatic. Horizontal microtomes can also be used.

Frozen Sections

Frozen sections are sometimes useful to detect lipids, for histoenzymology or immunohistology. They are obtained by the use of a cryotome, which is a vertical microtome installed in a cryostat that is a cooled chamber. The tissue is frozen and directly embedded in a wax that becomes solid at low temperatures. Sections are obtained like with a classic microtome for paraffin sections.

Adhesion of Sections

Several methods can be used to stick the paraffin sections on slides: sections can be stuck with water, glycerin, albumin, and gelatinous water or on gelatinized slides. Plastic wax sections are adhered with water. It is also possible to put the sections on distilled water and then water evaporates on a warming stage. Frozen sections are recovered directly from the blade by apposition of the slide, which has been refrigerated.

Staining

Before staining, paraffin must be eliminated from sections. Dewaxing is done with a solvent. But dyes being usually in water solution, sections must be rehydrated by putting slides in baths containing decreasing degrees of ethanol and finally water.

Dyes

Staining is a complex phenomenon concerned by chemical and physical factors, often not precisely known. Dyes are chemical substances able to permanently stain a tissue. Competition between dyes and blinding of one dye by another are also used.

A dye is a molecule with two chemical groups: the chromophore giving the color and the auxochrome required to fix the dye molecules on the tissue. Chromogens are colored because they possess a chromophore but they do not possess an auxochrome, and so, they cannot fix themselves on the tissues. If there is no auxochrome, a mordant, usually an aluminum salt, can be used to allow the fixation of the staining substance on the tissue. The tissue can be first submitted to the action of this molecule and then to the staining substance, or the mordant can be added to the staining solution. Lysochromes are dissolved into lipids that are stained.

In classic histology, there are acidic or basic dyes. But this terminology is not linked to the pH of the dye solution. Basic dyes are nuclear and acidic dyes are cytoplasmic (but cytoplasm can also be stained by acidic dye).

Dyes for Nucleus

Nuclear staining is performed with dyes having an animal or vegetal origin or being obtained by organic synthesis. They are not used in histochemistry for their mode of action is not well known and cannot be controlled.

Carmine, the only dye with an animal origin, was the first one used in histology. The staining molecule is carminic acid characterized by kinonic groups that are chromophores. Carmine is obtained by treating dry body extracts of a female Mexican cochineal with iron alum. Carmine is often replaced with azocarmine, a synthetic molecule.

Hematoxylin is extracted from *Haematoxylum campechianum*, a South American tree. It is not a dye by itself but hematein, its oxidation product with a kinonic group, presents chromophoric qualities. Therefore, hematoxylin must be oxidized before use. Hematein, which does not possess an auxochrome group, has no affinity for tissues and it is necessary to use a mordant, generally an aluminum salt, giving hemalum. Words 'hemalum,' 'hematein,' and 'hematoxylin' are often used interchangeably. Nuclear fast red is a synthetic dye.

Staining of Connective Tissue

The connective tissue is characterized by several cells synthesizing a matrix composed of a fundamental substance containing several molecules such as proteoglycans and several fibers such as collagen fibers; reticulin fibers, which are single fibers of collagen; and elastic fibers. In some organs , such as large arteries, elastic fibers are associated to form blades. Loose connective tissue, cartilage, and bone can be distinguished according to the nature of the fundamental substance, fibers, and cells.

In loose connective tissues, fibroblasts and fibrocytes synthesize both fundamental substance and fibers. Mastocytes synthesize some proteoglycans. Plasmocytes are characterized by the disposition of chromatin in a 'wheel-rays' fashion; they are migrating cells belonging to the immune system. Several macrophages and leucocytes are also found. Adipocytes stocking fats are grouped together but are separated from each other with fundamental substance and fibers. In the cartilage, chondroblasts and chondrocytes synthesize the fundamental substance containing chondroitin sulfuric acid and chondroitin sulfate salts. In the bone, fundamental substance contains calcium salts and more particularly hydroxyapatite giving the hardness to the bone. Osteoblasts and osteocytes are implicated in bone dynamics; osteoclasts are cells with several nuclei, devoted to the reduction of the bony matrix.

Methods used to visualize connective tissue concern essentially the fundamental substance and fibers. It is possible to stain fundamental substance with several dyes used in bichromic or trichromic reactions, giving a staining reaction with the connective tissue. In hemalum–eosin staining, the connective tissue is stained by eosin. Collagen is mainly composed of proteins and can be visualized in several manners. It is possible to use both acidophilic character and permeability of collagen fibers to visualize them on histological sections. In common methods such as hemalum–phloxine–saffron, saffron dissolved in ethanol gives a yellow coloration to collagen fibers (Figure 1). Methods using phosphomolybdic acid and/or aniline are based on fast green or aniline blue. In Masson's trichrome, azan staining, or still van Gieson's method, visualization is based upon the competition between two acidic dyes. Argyrophily (capacity to reduce silver salts) can give a staining of reticulin fibers, which are single fibers of collagen. Elastic fibers can be specifically visualized with orcein or paraldehyde fuchsin.

Stainings Often Used

With hemalum eosin, the nucleus is dark blue or brown-stained, while the acidophilic cytoplasm is pinkish. Some secretions such as the mucous of the stomach wall remain uncolored. Hemalum eosin staining yields quick results and it is very often used, but it is not sufficiently selective to visualize tissue components with a high precision.

Hemalum–phloxine–saffron method uses hemalum as a nuclear dye and phloxine as a cytoplasm one. Saffron is collagen-specific. The nuclei are blue-stained; the cytoplasm, muscle fibers, and red blood cells are red; and collagen is yellow. This staining is a standard method in pathological anatomy in several countries (Figure 2).

Trichrome

Masson's trichrome is a classical method of visualization with several variants. The nuclear dye can be Masson's hemalum or several hematoxylin solutions. The cytoplasmic dye is fuchsine. An aniline-derived dye allows the differentiation of collagen fibers (Figure 3). The nuclei are brownish blue; the cytoplasm is red; secretions are red or green; and the muscles and collagen fibers are green-stained if fast green or sulfo green (in Masson–Goldner's variant) is used and blue if aniline blue is used. This staining method yields good results for morphological studies because the different parts of the tissue are very well contrasted.

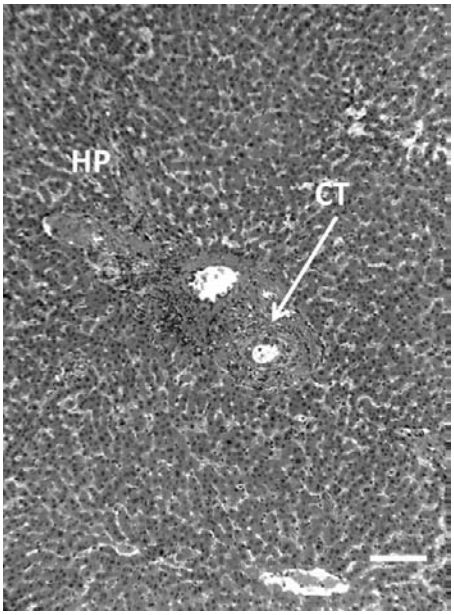

Figure 2 Section of liver stained with hematoxylin–phloxine–saffron; HP, hepatic parenchyma; CT, connective tissue. Bar = 100 μm.

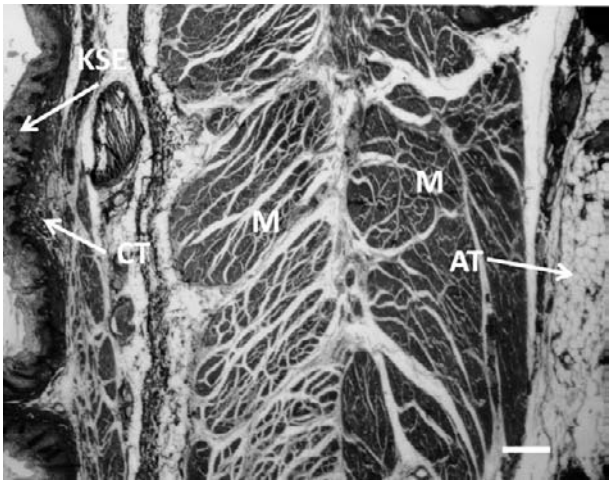

Figure 3 Section of amphibian skin stained with Masson's trichrome; KSE, keratinized stratified epithelium; CT, connective tissue; M, muscles; AT, adipose tissue. Bar = 100 μm.

Other trichromes are available, particularly Prenant's triple staining, Ramon y Cajal's trichrome, and Herlant's tetrachroma, or still one-time trichrome can be good methods for morphological studies. Cleveland and Wolfe's staining is useful to study the pituitary cells (Figure 4).

Staining with Azoic Dyes

Azocarmine and aniline are at the base of these types of staining. They allow several histological structures, particularly the chromatin repartition, to be visualized with great precision. After staining with Heidenhain's azan or Romeis's azan, the nuclei and certain cytoplasms are red-stained; other cytoplasms are yellow or gray. Collagen is blue-stained. Secretions can be different in function of their nature. Acid mucopolysaccharides are blue-stained. The nuclei are very well stained. If the protocol is well done, all details of chromatin can be observed.

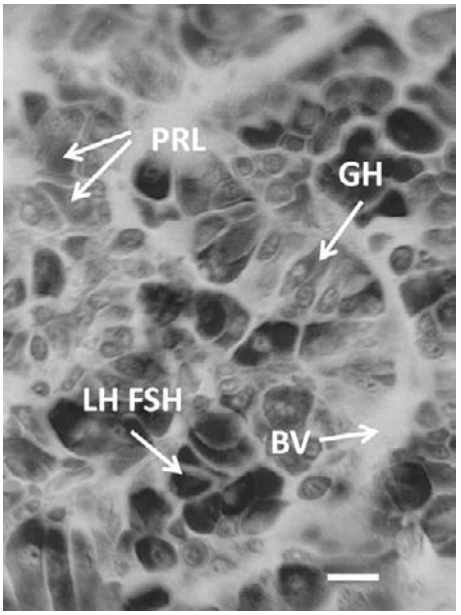

Figure 4 Staining of a section of amphibian pituitary with Cleveland and Wolfe's staining. BV, blood vessel; GH, somatotropic cells; LH–FSH, gonadotropic hormones; PRL, lactotropic cell. Bar = 5 μm.

Mounting Sections

After staining or histochemical reaction, sections are generally dehydrated and mounted with a hydrophobic medium. When staining can disappear after dehydration, a hydrophilic medium is directly used.

Histochemical Methods

The purpose of histochemistry is to visualize *in situ* the chemical components of cell and tissue structures. Sections are obtained according to the method previously described. Then, tissue components are visualized with several reactions based upon physical and histochemical properties. The reaction between the tissue or cell molecule and dye is known. The use of control methods allows to accurately measure if the staining obtained is specific.

Fixation

Fixatives used for histochemical investigations may be the classic fixatives described but it is indispensable to know if the fixative does not prevent the visualization of the researched molecule.

Characterization of the Chemical Nature of Structures

The visualization of the chemical nature of cell and tissue substances requires the use of chemical or physical methods. Two types of chemical methods can be used. The first allows visualization of a peculiar atom or atomic group. The second is used to visualize an atomic group that can belong to several different substances. Several methods based upon physical properties are also useful such as solubility, extraction, microincineration, and the use of polarized light.

Basophilic Staining

Basophilic components of tissues react with basic dyes. Acidophilic staining occurs when tissues react with an acidic dye. A basic dye is a salt, the cation of which is colored and not the anion. It is the contrary for an acidic dye. So, in basophilic staining, a colored cation reacts with the tissue, which becomes colored. In acidophilic staining, a colored anion reacts with the tissue, which becomes stained.

Metachromasy

A metachromatic dye visualizes a tissue by staining it with a color that is different from the color of the diluted dye solution. Variations of concentration or temperature modify the color of an aqueous solution of metachromatic dye. For example, solutions of toluidine blue are blue-colored at the concentrations usually used and become purple if concentration increases; the solution remains blue at high temperature but becomes red at low temperature. The color change is called metachromasy; the cell or tissue element is called the chromotrope. Chromotropic substances are mainly acidic mucopolysaccharides, nucleic acids, and silica particles.

Visualization or Reductive Groups

Several types of reactions based upon reductive properties of numerous substances are used for their detection. Reductive substances can act on ammonium silver nitrate to yield a black precipitate of reduced silver. In this argentaffin reaction, the tissue substance is the reagent. Other reactions are based on the visualization of an argentophilic tissue component by silver deposition. These reactions are silver impregnations, but they are not histochemical for the tissue does not react as a reagent. These silver impregnations are frequently used to visualize the nucleolus organizer (AgNOR method).Reductive groups can be visualized with other reducing reactions.

Visualization of Carbonyl Groups

Carbonyl groups are characteristic of aldehyde and ketone, and their visualization mainly groups several reactions used to visualize DNA with Feulgen and Rossenbeck's reaction or carbohydrates with periodic acid–Schiff (PAS) reaction.

Histochemistry of Carbohydrates

Carbohydrates are constituted with carbon, several hydroxyl chemical groups, and aldehyde or ketone groups. They are used for storage and distribution of energy. Fixation of tissues depends on the type of carbohydrate researched. Usually, classical fixators can be used.

Periodic Acid–Schiff

Carbohydrates are oxidized with periodic acid to give aldehyde functions that react with Schiff's reagent (sometimes called faded fuchsin) that gives a red staining to the sections (Figures 5 and 6). If glycogen is researched, its identification needs comparison between section with a normal reaction and sections that have been submitted to an enzymatic digestion with amylase. For other molecules, it is necessary to control the carbohydrate nature of PAS-positive substances and to submit sections to acetylation followed by saponification.

Acidic Carbohydrates Detection

These molecules, which are abundant in the intestine, possess carboxylic or sulfate acidic functions (Figure 7). They are metachromatic; they can also be visualized with alcian blue at different pH or with combined reactions such as alcian blue–PAS and alcian blue–alcian yellow at different pH.

Histochemistry of Proteins

For thirty years, immunohistochemical methods became the main means used to visualize proteins, so the classical methods were often abandoned. Nevertheless, some of these methods

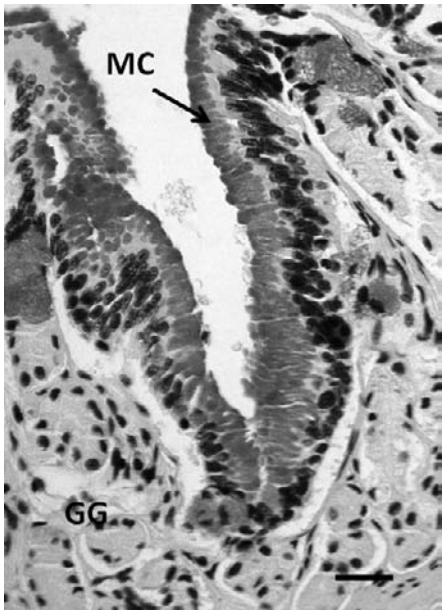

Figure 5 Visualization of mucous cells with PAS reaction on a section of amphibian stomach. MC, mucus cells; GG, gastric glands. Bar = 30 μm.

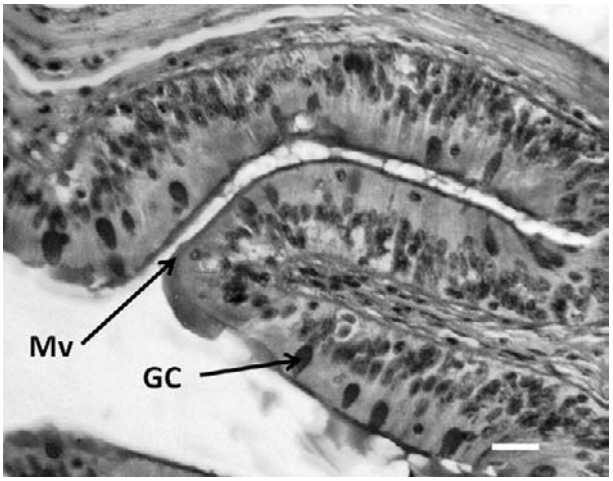

Figure 6 Visualization of PAS-positive goblet cells and mucus on the surface of amphibian intestine wall. GC, goblet cell; Mv, microvilli. Bar = 30 μm.

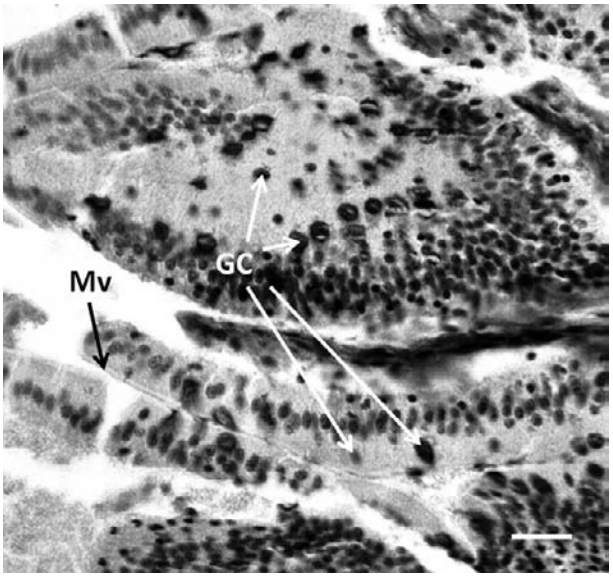

Figure 7 Visualization of goblet cells and mucus with alcian blue on the surface of amphibian intestine wall. GC, goblet cells; Mv, microvilli. Bar = 30 μm.

remain useful. All the current fixatives can be used to detect proteins, but it is always possible that the reactive functions of proteins can be modified with fixative action.

A lot of dyes used in classic histology, like eosin, phloxine, orange G, or Coomassie brilliant blue, can be considered as protein-specific. Danielli's tetrazoreaction is based on a double reaction. The first one consists of the combination of the tissue with a diazonium salt. The product is then combined with a naphthol (fast blue B or H acid), giving an intense brown color. Chloramine-T–Schiff method is based upon the oxidative deamination with the production of aldehydes that re-stained with Schiff's reagent. With ninhydrin–Schiff method, free amino acids are oxidized to give aldehydes that are visualized with Schiff's reagent.

Visualization of sulfhydryl proteins is based on the detection of thiols (R S H) and thioether (R S S R′). They can be useful in visualizing normal or pathological keratins, methionine, cysteine, and cystine particularly well distributed in the skin and rodent esophagus and cardia. Chevremont and Fredéric's method is based upon the reduction of ferricyanide with –SH groups giving a blue-colored ferric ferrocyanide also called 'Prussian blue.' With Barnett and Seligman's method, staining with D D D (2, 2′ dihydroxy-6, 6′ dinaphthyl disulfide) gives a blue staining of -SH chemical groups.

Histochemistry of Lipids

Lipids are fat components entering in the composition of cell membranes, they have storage and structural functions, and some of them are hormones. Lipids are stocked into adipocytes and they can be particularly abundant in hepatocytes in pathological liver.

Fixation and Tissue Preparation

Homophasic lipids are hydrophobic and physically isolated looking like droplets into the cytoplasm. Heterophasic lipids are associated with other components, giving them some hydrophilic properties.

A lot of lipids are soluble in organic solvents, so the use of organic and paraffin embedding is not possible. Consequently, if embedding is necessary, lipids must become insoluble with the use of chrome salt; frozen section can also be prepared. Contrarily, masked lipids are strongly bound to other molecules such as proteins or polysaccharides and must be demasked with the use of organic solvents, acids, proteolytic enzymes, and salt solutions.

Visualization

Physical methods such as polarization microscope can be used to study lipids. Staining methods for lipids with lysochromes such as red oil or Sudan black are often used. Nile blue sulfate allows the visualization of both neutral lipids that are pinkish-stained and acidic ones that are blue-stained. Osmium tetroxide can be used to visualize the unsaturated lipids.

Staining of Pigments

Some pigments, such as melanin, are observed in healthy and pathological tissues (the skin and liver); others are found in several pathological cases only. They can be visualized by direct observation or autofluorescence. Solubility in several solvents and whitening in oxidizing reagents are also used to characterize pigments. Several staining methods are also used. Melanin can be visualized by the reduction of a silver salt.

Staining of Biogenic Amines

Biogenic amines (adrenalin, noradrenalin, dopamine, serotonin, and histamine), implicated in various manners in the

organism, are secreted by endocrine cells such as enterochromaffin cells of the intestine or parietal cells of the stomach. Biogenic amines can also be used such as neurotransmitter. Staining method based on chromaffinity with the use of a mixture of potassium dichromate and potassium chromate gives a brown color to these cells.

Mineral Detection

Mineral elements are implicated in several ways: membrane polarization, nerve influx, intercellular cement, coenzyme factors, and contractility of the muscle. Minerals are important at low concentration, but an increased concentration can become lethal for the organism. So the presence of an increased amount of mineral elements can be the sign of a pathological state of the tissue.

General Principles of Mineral Visualization

Fixative must not bring mineral elements and not dissolve mineral elements out of sections. Microincineration is a technique consisting of burning the tissue, obtaining a spodogram on which only mineral elements are conserved such as ashes. With general staining methods, mineral elements can be visualized with colored reactions based on metal substitution, formation of colored lacquers, formation of complexes, and reactions of conversion. Several physical methods can be also used.

Visualization of Iron with Perls' Method

This method is used to detect iron in pathological organs such as the pancreas. The reaction is based upon a combination of potassium ferrocyanide, hydrochloric ferrocyanide, and iron of the tissue. Ferric iron appears blue-stained.

Visualization of Calcium: Stoelzner's Method

A heavy metal is substituted to calcium and then it precipitates to give a sulfide. Components containing calcium are black-stained. Results must be compared with decalcified sections.

Detection of Nucleic Acids

Structure of Nucleic Acids

DNA is characterized by a sugar, deoxyribose, associated to a nitrogenous base (a deoxynucleoside) and a molecule of phosphoric acid. Nucleoside and phosphate constitute a deoxyribonucleotide. Puric bases are adenine and guanine; pyrimidic bases are cytosine and thymine. The molecular structure of DNA is that of a double helix, composed of two molecular chains that are linked by nitrogenous bases. Adenine is always bound to thymine and cytosine to guanine. The histones, which are basic proteins, are bound to DNA contributing to its stability. RNA is characterized by a ribose associated with one nitrogenous base (ribonucleoside) and a phosphoric group. Ribonucleoside and phosphoric group constitute a ribonucleotide. Adenine is linked to uracil and guanine to cytosine.

Methods of Detection

Four types of methods can be performed to visualize nucleic acids. Detection of bases with ultraviolet spectrography (Caspersson's spectrophotometric method) and detection of pentose with tetrahydrofluorone are not frequently used. The detection of phosphoric acid can be performed with the use of basic dyes such as gallocyanin, methyl green for DNA, and pyronine for RNA or with Pappenheim–Unna's staining, that is, a combination of both these two stainings. Controls with RNase must be performed. Detection of the protein part can be performed with Danielli's tetrazoreaction after benzoylation or acetylation.

The Feulgen and Rossenbeck nuclear reaction consists of submitting DNA to an acidic hydrolysis that exclusively reacts with puric bases and deoxyribose binding, releasing aldehydes that are stained by Feulgen's reagent. The duration of the hydrolysis, depends on the nature of the fixative. Acidic fluids must be avoided for they induce the beginning of hydrolysis, and if hydrolysis is too strong, DNA degradation will continue, finishing with a total degradation of its molecule. It is possible to realize a Feulgen and Rossenbeck reaction on tissues preserved with very different fixatives, but it is necessary to modulate the duration of the hydrochloric acid action. Other methods based on the same principle can replace the original reaction. The difference is mainly linked to the methods used to visualize aldehydes such as thionine–SO_2 and azure A–SO_2, reacting like Schiff reagent. Silver salts can be reduced by the reducing group of aldehyde and metal silver deposits. The Feulgen and Rossenbeck method allows quantification.

Fluorescent Methods

Nucleic acids can be visualized by the use of fluorochromes. Intercalating fluorescent dyes such as orange acridine, ethidium chloride or bromide, and coriphosphine O are fixed between the two DNA strands or within a RNA loop. Feulgen–Schiff-like fluorescent dyes such as acriflavine or auromicine can be used on aldehyde functions obtained after DNA acidic hydrolysis. Fluorescent dyes react with specific base pairs. DAPI, DIPI, and Hoechst 33258 or Hoechst 33342 are intercalated between adenine and thymine. Chromomycin A3, mithramycin, or olivemycin intercalates between guanine and cytosine. Fluorescent staining is stoichiometric so quantification is possible. Visualization of X chromosome can be performed with quinacrine mustard on smears.

Enzyme Histochemistry or Histoenzymology

Enzyme histochemistry is used to demonstrate the activity of enzymes present on tissues. The visualization is based on the action of the enzyme on a specific substrate. Following this reaction, an insoluble product develops providing the location of enzyme. If the product is not stained, a metal precipitation technique or coupling with azoic dye and tetrazolium salt can be performed. Several types of enzymes can be visualized with these methods: alkaline phosphatase, acid phosphatase, ATPase, glucose-6-phosphatase, cholinesterase, acetyl cholinesterase, β-galactosidase, peroxidase, etc.

Preparation of the Tissue

Fixation

The choice of the fixative must be related to the enzyme searched. Activity of some enzymes such as phosphatases and hydrolytic enzymes can be correctly visualized after formaldehyde fixation. Yet an overfixation or exposition to high temperatures must be avoided. The enzyme histochemical reactions are often performed on frozen tissues avoiding inactivation by fixatives; the transfer in ethanol, which can alter the enzyme; and temperature used to melt paraffin, which can inhibit enzymatic activity. Nevertheless, for some enzymes, the paraffin with low melting point can be used.

Factors Influencing the Reaction

Enzymes can be inactivated by fixation or embedding and several factors could influence the success of the enzymatic reaction. The buffered medium must be at an optimal pH allowing the enzyme reaction; the substrate should be soluble in water or in the buffer solution.

Controls

Controls are necessary to monitor the success and the specificity of the reaction. Some mammal tissues can be used as positive controls.

Example: Alkaline Phosphatase

The activity of this enzyme can be visualized in the intestine by the use of Gomori's calcium method. Paraffin sections are incubated in a medium containing β-glycerophosphate salt; the product, phosphate, is captured with nitrate cobalt and then with ammonium sulfide. The presence of enzyme is finally visualized with a dark brownish precipitate. Alkaline phosphatase can also be visualized with sodium naphthyl phosphate and appears reddish.

Immunohistochemistry and *In Situ* Hybridization

The immunohistology is based upon both principles of immunology and histology. It is based upon a reaction between an antigen (corresponding to the researched molecule) and an antibody, a reagent prepared in an animal species. The tissue must be preserved in order to permit the reaction. Fixatives must not prevent the reaction, so they must not react with the functions used for the immunoreaction. In direct immunohistological method, a specific antibody of the researched component tagged with a fluorochrome or a molecule that can be stained is applied on the section. Finally, the researched molecule becomes labeled. In the indirect method, the first antibody prepared in the species 1 (e.g., a goat) is specific of the molecule researched. This antibody is then coupled to a second one, which can be visualized, prepared in a second species (e.g., a rabbit) directed against proteins of species in which the first one has been prepared (the goat). Finally, the molecule researched is visualized.

Visualization can be obtained with a fluorochrome, an enzyme whose activity has been revealed with a histoenzymology reaction. Several methods of amplification exist in order to ameliorate the visualization of the molecule researched.

Several cautions must be considered. It is essential to control specificity of the reaction and of the antibody.

In situ hybridization is a method used to visualize mRNAs in order to appreciate gene expression or to visualize the presence of genes in DNA to appreciate several displacements of genes in chromosomes. Methods of visualization resemble immunohistological ones.

See also: Adipose Tissue: Structure and Function of Brown Adipose Tissue; Adipose Tissue: White Adipose Tissue Structure and Function; Carbohydrate: Digestion, Absorption and Metabolism; Colon: Structure and Function; Colors: Properties and Determination of Natural Pigments; Enzymes: Functions and Characteristics; Fats: Classification and Analysis; Glucose: Properties and Analysis; Iron: Physiology of Iron; Lipoproteins; Nucleic Acids; Osteoporosis; Pituitary Gland: Pituitary Hormones; Proteins: Chemistry, Characterization, and Quality; Renal Function and Disorders.

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Milk Powder

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Introduction

Milk is a nutritious food. Raw milk from healthy cows is supposed to contain relatively few bacteria. Drinking of raw milk of healthy cows reduces the health risk but it is highly perishable. In history, mankind has made various attempts to preserve and concentrate milk so as to extend its shelf life. Marco Polo in the thirteenth century reported that soldiers of Kublai Khan carried a dried milk on excursions. The belief is that part of the fat was removed from the milk before drying and dehydration was accomplished by solar heating. The first usable commercial production of milk powder (MP) was invented by the Russian chemist M. Dirchoff in 1832. In 1850, Birdseye concentrated milk with added sugar until a solid was obtained. In 1855, T. S. Grimwade filed a patent on process of drying of milk, though William Newton had patented a vacuum-drying process as early as 1837. The real beginning of the concentrated and dried dairy product industry began in the nineteenth century when Nicolas Appert, a French inventor, described his procedure for concentrating and drying milk. In 1909, Nicolas Appert developed dried milk in tablet form by air-drying of milk solids concentrated to a 'dough' consistency. During the second half of the nineteenth century, attempts were made to produce a dried milk, which involved the addition of other dry products to concentrated milk. Sugar, cereal products, and sodas, singly or in combinations, were added.

The MP is a perfect solution to those who lack immediate access to adequate refrigeration facilities. MP results from extracting water content out of milk. The main purpose of converting milk into MP is to convert the liquid perishable raw material to a product that can be stored without substantial loss of quality, preferably for some years. The MP has various applications in confectionaries, bakeries, infant formulas, nutritional foods, etc.

Production of MP has become an increasingly important segment of the dairy industry, which is expected to grow further because of its features such as better keeping quality, less storage space, and lower transportation costs, which result in attractive economics and convenience during industrial and domestic formulation of composite foods. Nonfat dry milk serves the same purpose for milk solids-not-fat that traditionally butter has done for milk fat. The ultimate aim of the industry is to obtain dry products, which if recombined with water give little or no evidence of detrimental change compared to the original liquid product. Various names have been applied to the same dry milk product. For example, nonfat dry milk also has been called skim MP (SMP), dried skim milk, nonfat dry milk solids, and dehydrated skim milk. Dry whole milk, dry cream, dry buttermilk, and others frequently are called dried or powdered whole milk, according to their identities.

Today, MP is usually made by spray-drying. Alternatively, the milk can be dried by drum (roller)-drying and freeze-drying. Freeze-drying preserves almost all the nutrients in milk but it is very expensive.

China, the United States, and France are the major markets for dried milk products. New Zealand is the largest exporter of MP. Even though the United States, Australia, Argentina, and European regions are the largest producers of MP, they do not fall in to the category of largest exporters. The United Kingdom, Germany, the Netherlands, and Brazil are the major importers of the MP.

Definition and Composition

The internationally agreed definition of the MP is "milk products which can be obtained by the partial removal of water from milk. The fat and/or protein content of the milk may have been adjusted, only to comply with the compositional requirements in Section 3 of Codex Standard, by the addition and/or withdrawal of milk constituents in such a way as not to alter the whey protein to casein ratio of the milk being adjusted."

The standard specifies that only milk and cream are permitted as raw materials for MP. However, the protein content of the powder may be decreased by addition of lactose or increased by addition of skim milk or partially skim milk or by addition of the retentate or permeate obtained from the treatment of milk by ultrafiltration (UF). The standard also lists other permitted additives from within the following groups: stabilizers, emulsifiers, acidity regulators, anticaking agents, and antioxidants. No other additives are sanctioned. Standards are also provided for thresholds for contaminants, production hygiene, and product labeling. The composition of different types of powder is depicted in Table 1.

Technology of MP Manufacture

The MPs are dairy products from which the water has been removed to the greatest extent possible, thus creating hurdles in the growth of microorganisms. Production of MP consists of the processing steps like reception, clarification, cooling and storage, standardization, heat treatment, evaporation, homogenization, drying, and packaging as shown in Figure 1.

Raw milk plays an important role in deciding the quality of MP. Milk should be of high chemical, sensory, and bacteriological quality. At reception point, chemical and bacteriological tests are carried out before selection of raw milk. High microbial load in raw milk adversely affects the solubility of MP. After reception, the milk may be clarified or treated in

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Table 1 Composition of milk powders

No.	Constituents	Powder form		
		Whole milk powder	*Partly skim milk powder*	*Skim milk powder*
1.	Milk fat	Minimum 26% and less than 42% m/m	More than 1.5% and less than 26% m/m	1.5% m/m
2.	Minimum milk protein in milk solids-not-fat[a]	34% m/m	34% m/m	34% m/m
3.	Maximum water[a]	5% m/m	5% m/m	5% m/m

[a]The water content does not include water of crystallization of the lactose; the milk solids-not-fat content includes water of crystallization of the lactose.
Source: Codex Alimentarius Commission (2011). *Milk and milk products* (2nd ed.). FAO & WHO.

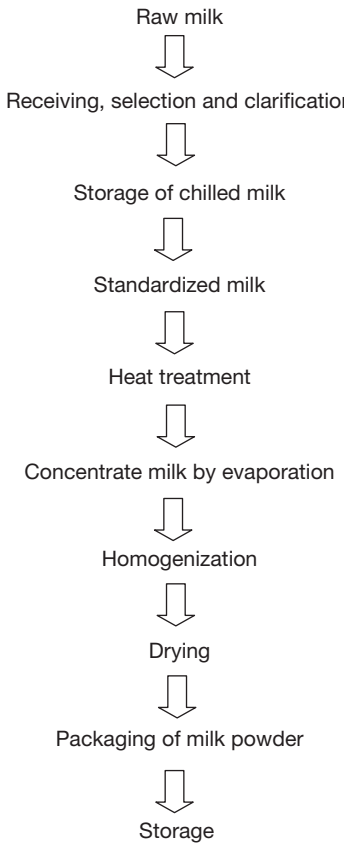

Figure 1 Generalized scheme for production of milk powder.

bactofuge to reduce the contamination by particulate matter and bacteria. Nowadays, microfiltration technique is used for manufacture of low-heat powders with very low bacterial loads. To avoid subsequent growth of psychotropic bacteria, clarified milk is stored under refrigeration until it ultimately heat-treated. Raw milk is standardized by adding calculated amount of skim milk or cream. Standardization of the protein content involves addition of either permeate from the UF of skim milk or retentate to reduce protein content, or retentate is added to increase the protein content.

The heat treatments eliminate all the pathogenic organisms. It also destroys saprophytic microorganisms, inactivates lipases, and activates SH groups of β-lactoglobulin, thus increasing resistance to autoxidation during storage. Milk is concentrated prior to drying; usually, a continuous multiple effect evaporator is used. The concentrate is not always homogenized, especially in the case of spray-dried powders. Homogenization of concentrated milk increases its viscosity, which leads to coarse droplets during atomization. The concentrated milk is generally dried by roller-drying or spray-drying method.

There have been attempts to develop completely new drying methods. However, only spray-drying and roller-drying methods are used for industrial production of MP. In the case of roller-drying, concentrated milk comes in direct contact with the hot drum surface, resulting in some irreversible changes such as caramelization of lactose, Maillard's browning, and denaturation of proteins, which decrease the solubility of the powder.

Spray-drying in the dairy industry dates back to around 1800, but it was not until 1850 that it became possible in major scale to dry milk. All processes, however, required addition of sugar, sulfuric acid, or alkali, so that the end product could not be considered pure. One of the first spray-drying patents was applied for in 1901 by the German Mr. Stauf, who sprayed the milk by nozzles into a chamber with warm air. The first real breakthrough, however, was in the United States in 1913, when the American Mr. Grey and the Dane Mr. Jensen developed a nozzle spray dryer and started to produce and sell drying installations on a commercial scale. The first rotary atomizer was developed by the German Mr. Kraus in 1912, but not until 1933, when the Danish engineer Mr. Nyrop filed his world patent, which was the real breakthrough of atomization. The basis for the modern MP industry, formed by these pioneers, has led to new developments, and the spray-drying equipment installed nowadays is in most cases very sophisticated and involves a highly technical and technological design. The feed is pumped from the product feed tank to the atomizing device, located in the air disperser at the top of the drying chamber. The drying air is drawn from the atmosphere via a filter by a supply fan and is passed through the air heater to the air disperser. The atomized droplets meet the hot air and the evaporation takes place, while cooling of the air happens simultaneously. After the spray is dried in the drying chamber, the majority of the dried product falls to the bottom of the chamber and enters a pneumatic conveying and cooling system. The fines, which are the particles with a small diameter, will remain in the air, and it is therefore necessary to pass the air through cyclones to separate them. The fines leave the cyclone at the bottom via a locking device and enter the

pneumatic system, too. The air passes from the cyclone to the atmosphere via the exhaust fan. The two fractions of powder are collected in the pneumatic system for conveying and cooling and are passed through a cyclone for separation, after which they are bagged off. The instrumentation comprises indication of the temperature of the inlet and outlet air, as well as automatic control of the inlet temperature by altering the steam pressure and amount of oil or gas to the air heater and automatic control of the outlet temperature by altering the amount of feed pumped to the atomizing device. The spray-drying process has become a highly acceptable method for drying milk. After drying, it is essential to cool the powder immediately to a temperature below the melting point of fat to maintain better flavor, body characteristics, and long shelf life.

The production of SMP slightly differs from the whole MP (WMP). Pasteurization is the most intensive heat treatment by low-temperature method to achieve the minimal detrimental heat-induced changes like serum protein denaturation. Homogenization is omitted and the milk can be concentrated to higher solid contents. Sometimes, vitamin A preparations are added by dry mixing. SMP is susceptible to humidity and heat-induced changes, which can intensify the adverse effect on the powder quality; therefore, it must be promptly cooled.

Powder should be packed in suitable containers that protect it from moisture, air, light, etc. Common wrappings are paper, multilayer boxes or bags with a polyethylene layer inside, metal barrels with polyethylene bags inside, or tins covered with aluminum foil at the contact surface. When planning the quantity of wrapping material, it is necessary to consider the bulk density of the product, because it is highly affected by processing parameters and techniques. When the product is intended for long storage, it is packaged in an atmosphere of inert gas, mostly nitrogen, or in a partial vacuum (4.0–5.3 KPa) to avoid oxidative changes of fat and other components. Properly produced and packed milk power, with low oxygen content, is stored at ambient temperature.

Instantization

Instant MP has better reconstitution properties than other MPs. The instantization process patented by Peebles significantly improved the quality and economical aspects of the drying technology. The principal purpose of instantizing is to improve the rate and completeness of reconstitutability of MP. The aim of spray-drying process is to produce particles with a big surface/mass ratio. The reconstitution of small particles of powder in water is, however, difficult and requires intensive mixing for their dispersion. One of the major objectives of new innovations in the area of spray- and fluid bed-drying is to produce instant powder by effecting agglomeration. Agglomeration provides the structure of porous aggregates, which disperse readily and quickly without vigorous agitation to produce stable reconstituted milk. The properties of dried products improved positively by the instantizing process include wettability, penetrability, sinkability, dispersibility, and dissolvability. The process permits a better equilibrium among these variables. An effective agglomeration is achieved by either rewet or straight-through methods: the rewet process, where the instantization is carried out after the powder is obtained in dry form, and the 'straight-through' process, where instantization is accomplished during drying.

Dry MP is the starting material for the rewetting procedure. The powder is dispersed in the wetting chamber and gains a water content of 5–10%, causing powder particle agglomeration. The agglomerated product is transferred to the vibrating fluid bed dryer and cooler, where it is redried in a hot air stream at 90–120 °C and immediately cooled to approximately 10 °C. The final product from the fluid bed dryer has a water content <4% (2–4%). Hot air, blown upward through the fine perforated plate of the fluid bed dryer, 'fluidizes' the powder and carries the smallest powder particles or fines to the cyclone, where separation takes place. The air is discharged into the atmosphere after heat and solids have been recovered and the fines are returned to the start of the process. Instantization process is complicated in the case of WMP as the surface of particles is covered with milk fat layer. Free fat forms a hydrophobic layer on the particle surfaces, impregnating them and decreasing their water-binding capability. In order to prevent this and improve the recombination properties, powder particles are lecithinated by adding lecithin mixed with butter oil during instantization. In this process, the powder is dried in two stages and the fines are recirculated to the wet zone. The lecithinated powder should be cooled and packed in a suitable material to protect the agglomerates from breaking down.

Properties of MP

The important properties of MP are particle size, shape and surface density, flowability, dissolution, insolubility, and whey protein nitrogen (WPN) index. These properties decide the quality and the suitability of MP in specific applications.

Particle Size

Particle size is an important physical property of MP and can relate to its appearance, reconstitution, and flow characteristics. Particle size in MP can affect powder reconstitution properties. Difference in particle size can lead to stratification of the powder with the higher solids concentrated at the top, which will affect reconstitution of the dry product. Wettability and dispersibility of MP can also be influenced by particle size. Dispersibility decreases as percentage of fine particles below 90 μm increases. Flowability also depends on particle size and shape. Large particles tend to flow more easily than smaller particles. Shape and size of the particles will affect the closeness of the particle pack, which in turn will affect the powder bulk density. The particle of MP generally consists of a continuous mass of amorphous lactose and other low-molar-mass components in which fat globules, casein micelles, and serum proteins are embedded. The particles also contain vacuoles of occluded air where particle surfaces are not in contact. The surface of spray-dried particles not only is usually smooth but also may be wrinkled. Conditions such as higher inlet air temperature and larger temperature differences between the hot air and powder particles may contribute to wrinkles. The presence of particles of different morphology in the same sample can be attributed to the different drying conditions to which the individual particles were exposed. Particle size can be

influenced by the milk characteristics, processing conditions, and the type of equipment used in the drying process. Atomization parameters influence product shape and size distribution of powder particles making it possible to control powder particle size. A spray-dried particle using centrifugal atomization will give a larger particle than the pressure nozzle atomization. Spray-dried powder particles usually are spherical with diameters in the range of 10–250 μm. Rapid dispersion requires a particle size of approximately 150–200 μm diameter. The particles in agglomerated MP are larger and irregular in shape and contain very few separate small particles. Agglomeration causes an increase in the amount of air incorporated between the powder particles. The air is replaced by water during reconstitution. Hence, the agglomerates are readily dispersed and dissolve quickly.

Density

Bulk density is a very complex product property for MP and is of great importance for economical and functional reasons. High bulk density is desirable for reducing shipping and packaging costs. On the other hand, low bulk density, as seen in agglomerated products, influences other powder properties such as flowability and instant characteristics. Bulk density is the weight of a volume unit of powder and is usually expressed in g/cm^3, kg/m^3, or g/100 ml. Bulk density is usually determined by measuring the volume of 100 g of powder in a 250 ml graduated cylinder after exposure to compaction by standardized tapping. The bulk density of nonfat dry milk has a wide range, from 0.18 to 1.25 $g\,ml^{-1}$. Regular spray-dried nonfat dry milk is approximately 0.50–0.60 $g\,ml^{-1}$, while roller-dried nonfat dry milk is 0.30–0.50 $g\,ml^{-1}$. The bulk density of the final powder is a result of particle density (occluded air and density of the solids) and the interstitial air. Bulk density can be influenced by many different factors, which include density of the solids, amount of air entrapped in the particles (occluded air) or the particle density, and amount of interstitial air (air between the particles). Occluded air is one of the most important factors for controlling bulk density.

Flowability

Flowability is the ability of a powder to flow freely without forming lumps or aggregates. It can be measured as the time necessary for a given volume of powder to leave a rotary drum. Flowability depends upon particle size and shape, density, and electrical charge. Larger particles flow more easily than smaller particles with a diameter of <90 μm. Flowability can considerably be improved by adding some inert very fine powders such as SiO_2, Na–Al–silicate, or $Ca_3\ (PO_4)_2$. Such additions are applied, for instance, in MP for coffee machines.

Dissolution

When dissolving MP in water, it is dispersed, implying that the powder particles will become fully wetted, which may take some time. Subsequently, the soluble components dissolve, and the colloidal particles (fat globules and casein micelles) become dispersed in the solution. This will take several minutes. Generally, a small part does not dissolve. After a solution is obtained, it may take about a day before its composition, especially the salt distribution, has more or less reached equilibrium. This process may be accelerated by heating the reconstituted milk to 50–60 °C and then cooling it again.

Insolubility

Insolubility can be estimated in various ways. The powder is dissolved in water under standardized conditions (concentration, temperature, and duration and intensity of stirring) and centrifuged. The supernatant is removed and replaced by water and is centrifuged again before reading the volume of insoluble residue. The result is called the insolubility index. In WMP, aggregates of coagulated protein with entrapped milk fat globules may float to the surface; the quantity involved usually is more than the sediment. Hence, the insolubility found closely depends on the method used.

Whey Protein Nitrogen Index

It measures the amount of heat treatment the powder has received during processing. Whey proteins are denatured or altered by heat. The test measures the amount of undenatured whey protein present in the powder. After application of low centrifugal forces under specified conditions, a high value indicates that the whey proteins are more soluble and product is therefore more soluble. WPN is expressed as milligram (mg) undenatured WPN per gram of nonfat MP with a moisture content of 3.16%. Casein and the heat-denatured whey proteins are removed by filtration after precipitation of the reconstituted milk with NaCl. The filtrate contains all undenatured whey proteins. By adding HCl, the proteins denature and develop turbidity depending on the concentration of the whey proteins. The turbidity is measured as percentage transmittance, in a spectrophotometer at a wavelength of 420 nm. By using the standard curve, this reading can be converted directly into milligram undenatured whey protein N/g powder (WPN).

Sensory Evaluation of MP

In order to determine the temperature sensitivity of a reaction, a Q10 model is often employed. This describes how much faster a reaction will go if the product is held at temperatures differing by 10 °C; Q10 models can be used to predict quality and nutrient loss, as well as shelf life of a product.

MPs, particularly SMP, are manufactured in large quantities in the flush season and subsequently used in lean period in reconstituted form to maintain milk supply. It has become an important export commodity. MP has been graded on the basis of bacterial, physicochemical, reconstitutional (i.e., when the powder is dispensed in water to prepare reconstituted milk), and organoleptic properties. These properties are basic elements of quality specifications. During drying, care should be taken to preserve the natural properties of the original raw milk. Evaluation of MP on the basis of its sensory characteristics plays an important role toward its consumer acceptance.

Desirable Attributes and Defects in MPs

Whole Milk Powder

Upon rehydration, WMP should have clean, rich, sweet, fresh, and pleasant flavor. Sensory defects may be due to use of either poor quality ingredients, manufacturing conditions, drying method, or abusive storage conditions. The development of storage-based defects in WMP is most difficult to control. This relatively rapid onset of off-flavors in WMP is primarily a function of the generation of lipid oxidation products. The product should be free-flowing to some extent with absence of lumps. The presence of hard lumps is a serious defect in WMP. The color of WMP depending on the source of fat varies from white to deep yellow. Tactile properties of WMP vary with the method of manufacture, degree of concentration, particle size, and porosity after drying. Spray-dried WMP may be extremely fine and uniform throughout, but two powder defects that may occasionally be noted are lumpy and caked. Some common quality defects encountered in WMP and their characteristics are the following.

Flavor defects

1. *Stale*: This is a characteristic age defect associated with protein. This defect closely resembles the musty flavor common to casein. This defect is observed in MP after storage for 9 months. When the defect is intense, it may be accompanied by a darkening of the product.
2. *Rancid*: Rancid WMP has bitter, soapy, unclean taste, which is persistent after the sample has been chewed up and spit out. The reason may be insufficient temperature used for fore warming and the lipase enzyme that remains active and causes rancidity.
3. *Oxidized/tallow*: It is the most troublesome flavor defect of WMP. Many factors are responsible for the development of this defect such as temperature, light, moisture, acidity, metallic salts, condensation, and type of packaging.
4. *Scorched*: This flavor is produced in products that have been subjected to excessive heat during the drying stage or have been permitted to remain in the drying chamber for too long period of time. It is usually accompanied by a large number of scorched specks in the product and sometimes by a dark discoloration typical of overheating.

Body and texture defects

1. *Lumpy*: A lumpy powder lacks homogeneity. This defect is found frequently in the spray process product. Lump formation is due to insufficient drying, dripping from spray nozzles, or exposure to moisture-laden air. Hard lumps ranging in size from a grain of wheat upward may be interspersed throughout.
2. *Caked*: Usually, this defect is not observed in WMP, but when it does occur, the product loses its powdery consistency and becomes a rock-like solid. When the mass is broken up, it remains in chunks, thus failing to return to the original powder state. This defect is serious since such milk solids have lost their sales value for human consumption.

Color defects

Normal dry whole milk is light yellow in color but varies reasonably with the color in the fat from a creamy white to a deep yellow:

1. *Browned or darkened*: When the defect is present, the normal creamy color has been replaced by a distinct brown. The defect is usually associated with an old, stale flavor.
2. *Scorched*: Discoloration due to browning of the milk solids is usually associated with the roller process. The powder may vary from light to dark brown.
3. *Lack of uniformity*: This defect may be due to either partial discoloration after packaging or partial scorching during the manufacturing.

Skim Milk Powder

The flavor of good quality SMP, when reconstituted, should be similar to that of fresh skim milk, that is, normally a flat taste because of practically no fat in it. Otherwise, the flavor is clean, sweet, and pleasant and may have a slightly cooked or heated perception. The common flavor defects of SMP are stale/storage, neutralizer, oxidized, and scorched. Instant SMP is usually quite granular, the product pours readily somewhat like that of corn meal. SMP prepared by spray-drying process is very fine, light, and dusty and has flow characteristics similar to the flow of corn meal. Scorched off-flavor is developed due to exposure of MP to abnormally high heat during processing. Cake formation is the most frequently occurring defect related to texture of SMP. Sometimes, the defect is so serious that the product looks as hard as a rock. The color of SMP should be uniform and showing complete absence of foreign specks, scorched particles, and browning. The product should have a creamy white or light yellow color.

See also: Condensed Milk; Dairy Products: Dietary and Medical Importance; Protein: Food Sources.

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Milk: Processing of Milk

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Introduction

Milk is nutritious, appetizing, and nature's perfect food (except vitamin C, copper, and iron) and is recommended by nutritionists for development of sound body and consumed by all sectors of people. People suffering from lactose intolerance and old age are unable to digest milk, but can easily digest fermented foods like yogurt (as lactic acid bacteria converts lactose into lactic acid). Demand for milk was greater in developed countries as compared to developing countries, but the gap has narrowed due to an increase in urbanization, population, and consumption. Total production of milk up to 20 July 2014 was 5339.4 million liters, which is exhibiting an increasing trend from the previous year. Pasteurized milk and ultra-high temperature (UHT) milk market share in 2014 increased to 83.7% and 6.9% (in volume), respectively, as compared to 81.9% and 5.2% in 2013. India is the largest producer of milk with a total volume of 121.8 million tons followed by the United States and China.

One of the disadvantages associated with heating of milk is destruction of nutrients like water-soluble vitamins, but technological advancement and research have made it possible to prepare fortified milk or milk with added supplements. Milk is a perishable commodity, and being rich in nutrients it acts as the perfect substrate for growth of microflora, and to reduce this, different thermal and nonthermal techniques are implemented. Thermal treatments are the common techniques used for extending the shelf life of milk, for example pasteurization, sterilization, and UHT, but loss of nutrients is a concern associated with these treatments. Nonthermal treatments like high-pressure processing (HPP), pulse electric field, ultrasonication, and irradiation are also explored to process milk to minimize the loss of nutrients as compared to thermal treatment. Postprocess contamination is a major factor that can affect the shelf life of milk, and to maintain the product, safe packaging plays an important role when the milk and milk products are stored at refrigeration or ambient temperature. Aseptic packaging of milk when used in combination with high temperature can considerably extend shelf life of milk for more than 6 months at room temperature.

Types of Products

With judicious preservation technique and by harnessing the potential of milk into different types of products, milk could be made available year-round in those sectors where it is not being produced or during the lean season. Different types of liquid milk include pasteurized milk, sterilized milk, homogenized milk, and special milk. Special milk includes whole milk, standardized milk, soft curd milk, flavored milk, vitaminized/irradiated milk, fermented milk (natural buttermilk, cultured buttermilk, acidophilus milk, kumis, and kefir), reconstituted/rehydrated milk, recombined milk, soya milk, toned, double toned milk, filled milk, and imitation milk that are generally pasteurized or UHT treated (Figure 1). Standardized milk, toned and double toned are those milks in which standardization of fat and solid not fat (SNF) content are done by addition or removal of cream. Fermented milks are categorized depending on inoculation of different cultures of bacteria for example *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Streptococcus lactis*. Flavored milk is prepared by incorporated flavors like chocolate, strawberry, mango, butterscotch, and vanilla along with fruit pulps, cocoa powder, sugar, permitted color, and stabilizers followed by sterilization/UHT treatment. Milk products available in the market include cream, butter, butter oil, ice cream, cheese, powdered milk, and condensed milk. Manufacturing of milk products varies worldwide as a consequence of traditional practices, different dietary habits, and social, cultural, and religious dissimilarities. Market milk is available in different packaging materials including glass containers, PET (polyethylene terephthalate) bottles, tetra packs, and high-density polyethylene (HDPE) laminate pouches. In the dairy market, liquid milk (54%) occupies the largest segment followed by ghee, yogurt, and whiteners 14%, 8%, and 4%, respectively.

Collection and Storage of Raw Milk

Previously milk was collected by small-scale dairies directly from nearby farms so there was no critical requirement of chilling centers. However, with an increase in distance between collection and processing, large dairy processing units required chilling centers, as on many occasions it took 3 days for transportation from distant places. Milk can be collected directly from producers, through cooperative societies, or by the chilling centers. If the processing plant or dairy is near a village/town, then farmers individually can contact processors on a daily basis. Sometimes a middleman is also involved for transporting raw milk from villagers to the dairy using cans on rickshaw, bike, and so on. A cooperative society is formed by a group of persons at the village level. They do not ensure to take milk on daily basis and can deny on various grounds.

The milk is procured either on a weight or a volume basis, and payment is made on the basis of percentage of fat, SNF, and other quality attributes. Generally cans, insulated tankers, trucks, or rail tankers are used as a means to transport milk to a processing plant. At a chilling center the milk is usually stored at a temperature below 5 °C in insulated tanks/silos, and the cooling of milk can be achieved by implementing techniques like can immersion, in-can cooling, rotor freeze, bulk milk cooler, plate chiller, tubular cooler, brine cooling, and ice cooling. Milk storage

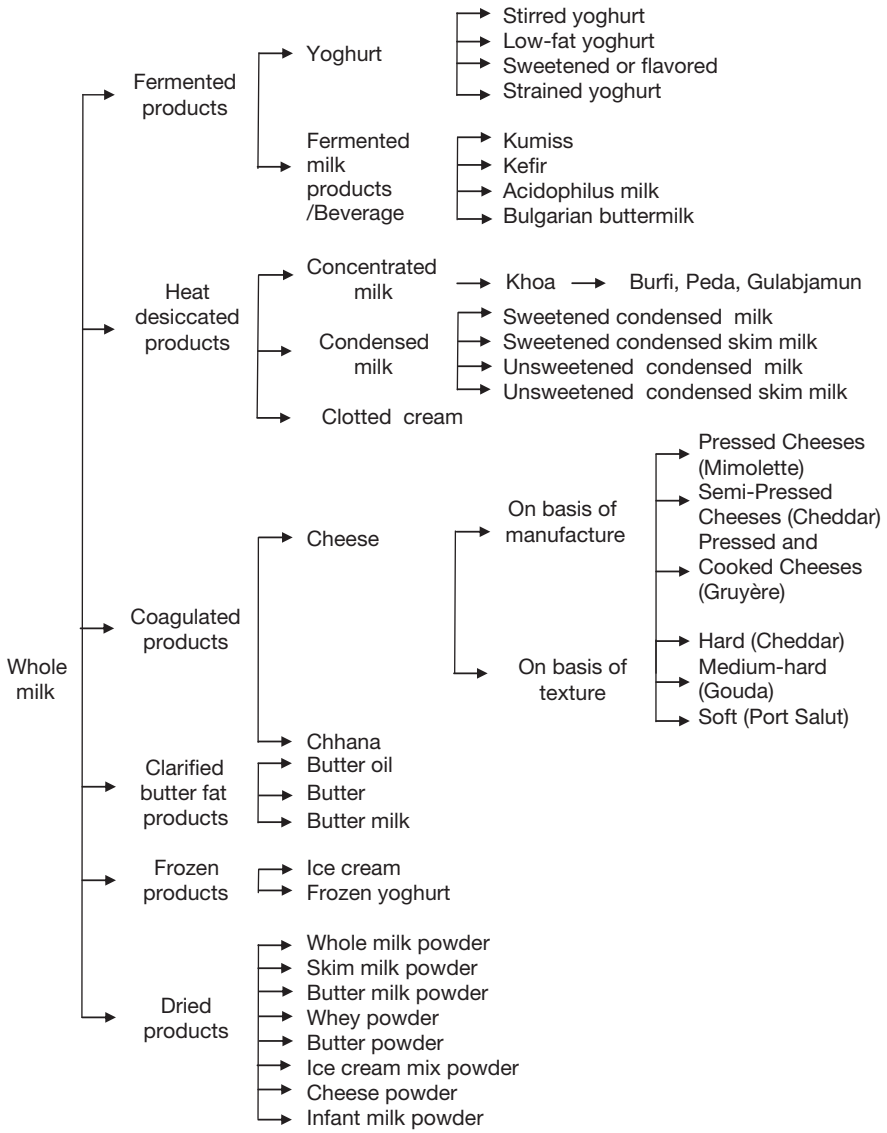

Figure 1 Milk and milk products.

temperature is generally kept low, as it will minimize the bacterial growth and thereby avoid milk spoilage, which is very high if the milk is stored at 30–40 °C, as it is the optimum growth temperature for most of the microorganisms present in milk.

Quality assessment is the foremost requirement as soon as the milk is received. It includes a platform test like organoleptic (taste, smell, and visual), temperature, clot on boiling (COB), sediment test, neutralizer test, and alcohol-alizarin test, which are performed on the dock itself and are indicators of the quality of the milk. Based on the results of platform tests, a decision is taken to accept or reject the milk. After acceptance of the milk, tests are further conducted in a laboratory that will include determination of fat, SNF, acidity, methylene blue reduction test (MBRT), microbial counts (direct microscopic, standard plate count, and presumptive coliform test), and tests for adulterants that ensure the quality of raw milk. Milk is further pumped into large storage insulated tanks (silos) in which milk is stored at temperature less than 4 °C until it is further pasteurized or sterilized, depending on the requirement.

Standardization

Standardization involves adjustment of the fat/SNF content of milk, or a milk product, by addition of cream or skim milk as appropriate to obtain a given fat/SNF content. Generally raw milk is standardized before processing, and it also depends on regulatory authorities, as standardization is not allowed in some countries. Depending on scale of automation in the dairy, either direct inline or batch standardization can be carried out. Milk products that are utilized for adjusting fat in milk include cream, butter oil, and whole milk powder, and for increasing the SNF content skim milk powder is often used.

Preservation Techniques

Because milk is a rich source of nutrients, it is susceptible to growth of microorganisms if not processed or stored at a low temperature. Second, to meet the demand of the urban population and to supply the milk in the lean season, milk needs to

be dried and stored in the flush season. With the growing demand for milk and milk products and changing lifestyles, it is necessary to increase the shelf life of the milk not only by days or months but up to years while maintaining the nutrients present. Extension of shelf life can be achieved by implementing one of these techniques: lowering the temperature (chilling, freezing), heat treatment (pasteurization, sterilization, UHT), removal of moisture by concentrating (evaporation, drying, sweetening, or salting), modified atmosphere (removal of oxygen), or microfluidization and cold pasteurization techniques (HPP, pulse electric field, oscillating magnetic field and irradiation; Figure 2).

Pasteurization

Louis Pasteur in 1860 demonstrated that heat treatment (50–60 °C) killed most of the spoilage microorganisms in fruit juices. In the case of milk, pasteurization means treating every particle of milk with a time–temperature combination effective to destroy bacteria (*Mycobacterium tuberculosis*, *Coxiellaburnetii* and *Listeria monocytogenes* as indicator) without altering nutritional status, as well as color and flavor. At the industrial level batch pasteurization or low temperature for longer time (LTLT) and high temperature for short time (HTST) are carried out at 63 °C for 30 min and 72 °C for 15 s, respectively, which is immediately followed by cooling to 5 °C or below. LTLT involves a water jacketed vat, waterspray, or a coil vat pasteurizer. LTLT has benefit of low equipment cost, but the energy and labor requirement is high compared to HTST. LTLT impairs creaming properties due to excessive agitation that leads to churning. The HTST pasteurizer provides a continuous flow of milk, occupies less floor area, uses automation (clean-in-place [CIP] cleaning), and requires low operating cost (effective regeneration). Other time–temperature combinations are 89, 90, 94, and100 °C for 1, 0.5, 0.1, and 0.01 s, respectively (US code of federal regulation). Regeneration allows heating of incoming milk and cooling of outgoing milk, where 80% of pasteurization temperature is achieved and 94–95% of heat from the outgoing milk is recovered.

In HTST, plates are generally used for exchanging the heat and are made of stainless steel (SS type 320) of 0.8–2.0 gauge with a gap of 2.5–4.0 mm between two consecutive plates. Apart from the fact that copper has an excellent heat transferring capacity of 218 BTU h^{-1} ft^{-1} F^{-1} as compared to stainless steel (10 BTU h^{-1} ft^{-1} F^{-1}), it is not used in fabrication of plates as it may produce off flavor by catalyzing the oxidation of milk fat. The heating medium used for heating of milk may be steam or hot water, and for cooling of milk, glycol, brine, water, or ice water can be used. Capacity of the dairy plant using plate heat exchanger can be as high as 1 00 000 L h^{-1}, which can be adjusted by increasing or decreasing the size of

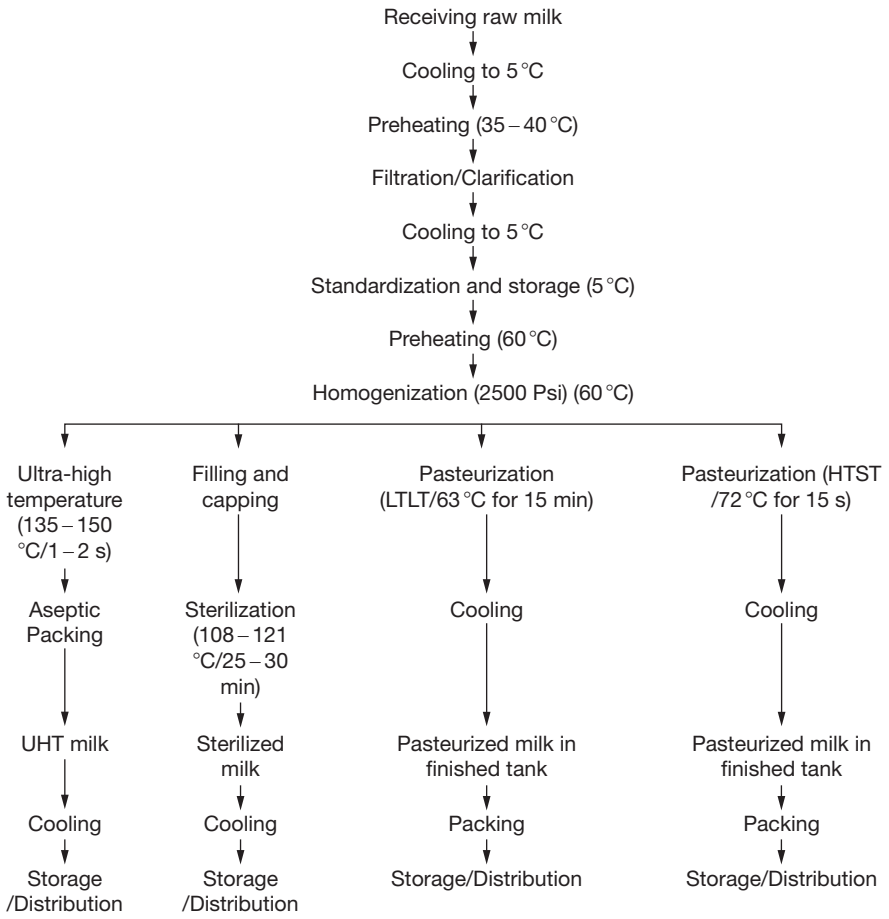

Figure 2 A flowsheet for the manufacture of pasteurized liquid milk, sterilized milk, and UHT milk.

the plate and the number of plates. In the plate heat exchanger, the heat of the pasteurized milk is utilized to warm the incoming cold milk that is simultaneously precooled. This in turn saves the cost of heating and refrigeration, and this capacity of the plate exchanger is called regenerative heat exchange or, more commonly, heat recovery. The regeneration section is divided into different subsections with the help of a block, and these blocks are further connected to processes like homogenization, clarification, microfiltration, and separation. Shell and tube heat exchangers are used for pasteurization of highly viscous products like cream, and an added advantage of shell and tube heat exchanger over plate heat exchanger is of low maintenance.

UHT

UHT processing of milk involves heating milk in a continuous process to temperatures higher than 135 °C for a few seconds, cooling rapidly, and aseptically packaging in the milk into sterile containers. UHT treatment must be sufficient to produce a commercially sterile product in which bacterial growth will not occur under normal storage conditions, but not severe enough to cause chemical changes that result in an unacceptable flavor, color, and nutritive loss. In general, the heating should be equivalent to a minimum of 9 log reduction of thermophilic spores (referred to as a B^* value, >1) and a maximum reduction of 3% in the level of thiamine (referred to as a C^* value, <1). UHT was invented in 1960, and UHT-treated milk was commercially available in 1970 with an objective to reduce nutritional loss caused by in-container sterilization. UHT-treated milk is generally packed in paperboard made of six different layers. A polyethylene layer is used for sealing in the milk and also acts as a moisture barrier; aluminum foil acts as an oxygen barrier and protects from off-flavors and light. UHT-treated and aseptically packed milk can have a long shelf life extending up to 6 or even 9 months without any preservatives at ambient temperature. The disadvantage associated with UHT treatment of milk is that in many instances the milk appears to have a cooked taste with a slight to dark brown color that may be due to Maillard browning. Development of sulfurous flavor is also associated with UHT milk, which is developed on thermal treatment as a result of unfolding of whey proteins. Milk with a high preprocessing microbial count is more susceptible to gel formation than milk with a low count. Microorganisms that produce heat-stable enzymes cause the most serious gelation problems. Longer refrigeration times prior to sterilization allow increased growth of psychotropic microorganisms and concomitant production of heat-stable enzymes, especially proteinases and lipases.

Two main types of UHT processes are used commercially: direct heating, in which milk comes into direct contact with the heating medium, that is, steam; and indirect heating, in which the heating medium, steam or superheated water, is separated from the milk by a stainless steel plate or wall of a tube. In direct systems, heating to the specified temperature and cooling from the high temperature is very fast due to transfer of the latent heats of condensation and evaporation, respectively, between the steam and liquid milk. A plate heat exchanger used in UHT processing is able to withstand high temperature and internal pressure as compared to a pasteurizer. A plate heat exchanger provides more surface area for heat transfer, low cost, and simple design. Tubular heat exchangers are more commonly used for thick liquids; do not need to acquire an aseptic homogenizer, as they can withstand 200–300 bar pressure required during homogenization; and by employing homogenizer valve will serve the purpose as a source of contamination in a homogenization pump thus eliminating the requirement of a pump. The rate of fouling of tubular heat exchangers is less as compared to a plate heat exchanger that has narrow channels for milk flow. Cooling to around 20 °C is achieved in a vacuum chamber. Direct heating requires sterilization temperatures 3–4 °C higher than indirect heating to achieve an equal sterilization effect because of the greater heat input during the heat-up phase of indirect heating. As compared to direct heating systems, indirect systems have a low heat transfer rate and a higher rate of deposition over the surface that requires frequent cleaning, but less processing and operational cost (less pumps and accessories, regeneration of energy) and require less controls. To exploit the benefits of direct as well as indirect processes, systems using a combination of direct and indirect heating are available commercially (e.g., the High-Heat Infusion system of APV88 and the Tetra Therm Aseptic Plus Two system of Tetra Pak). These achieve better energy economy than conventional direct systems (due to greater heat regeneration) and cause less chemical damage, especially flavor change, than conventional indirect UHT systems. Some of the adversely affected nutrient components in milk after UHT treatment are ascorbic acid, folacin, vitamin B_{12}, vitamin B_6, thiamine, and riboflavin up to an extent of 15–25%, 10–20%, 0–30%, 0–10%, <10–18%, 0–10%, respectively. Fat-soluble vitamins, proteins, and amino acids are affected to a much lesser extent.

Sterilization

In-can sterilization of milk is usually carried out above 100 °C with a time–temperature combination of 121 °C for 15 min at 15 psi. The sterilization process may be a batch (autoclaves) or continuous process (vertical hydrostatic towers or horizontal sterilizers) and can be carried out in either cans or glass bottles, which are prefilled and hermetically sealed.

Sterilization effect is achieved by using hot water or saturated steam, which transfers the heat to milk and milk products through the walls of the container. The main objective of sterilization is to kill vegetative cells and endospores or reduction of 12 log cycles (12 D reduction) of *Clostridium botulinum* by using a suitable time–temperature combination. The fact that sterilization takes place after bottling eliminates the need for aseptic handling, but on the other hand, heat-resistant packaging materials must be used. Sterilized milk usually has a creamy appearance and an extended shelf life of more than 6 months as compared to a few days for pasteurized milk. Rendering milk to high temperature with a long holding time often results in burnt flavor and brown color, which may be a result of the Maillard reaction. Popularity and consumption of sterilized milk declined after introduction of UHT processed milk. Nutrient loss is a major concern associated with sterilization, as it is reported by various scientists that almost 50% of vitamin C and 30–40% of thiamine and vitamin B_{12} are lost, but

components like vitamin A, riboflavin, carotene, and nicotinic acid remain unaffected.

Homogenization and Microfluidization

Homogenization of milk has become a standard process that is applied as a means of stabilizing the fat emulsion against gravity separation. Gaulin, who invented the process in 1899, described it in French as 'fixer la composition des liquides.' Homogenization is carried out by using a homogenizer that pumps the milk under pressure through an orifice of 0.1 nm to disintegrate the fat globules to less than 2 μm. The disintegration of the milk fat globules is achieved by a combination of contributing factors such as turbulence and cavitation that do not allow visible cream separation in milk and also increase the mouthfeel, color, and digestibility. Homogenizers used in the milk industry are either single-stage or two-stage, depending on the number of homogenizing devices. Single-stage homogenization is used to homogenize products with a high viscosity, whereas two-stage homogenization is applied for products with a high fat content or where high homogenization efficiency is desired. Homogenization temperatures normally applied are 60–70 °C, and homogenization pressure is between 10 and 25 MPa (100–250 bar), depending on the product. In a two-stage homogenization, pressure of 2000 psi and 500 psi is applied at the first stage and second stage, respectively. Pressure at the first stage reduces fat globules size and increases viscosity, whereas a second stage is used to prevent fat cluster reformation. The purpose of homogenization of milk is to stabilize the milk fat emulsion, as during storage and distribution a creamy layer appears at the top surface as a result of separation under gravitational force. Homogenized milk cannot be efficiently separated and also on many occasions it leads to development of sunlight flavor. Moreover, the homogenized milk is also reported to have low heat stability, and also the milk is not suitable for production of semihard and hard variants of cheese, as the coagulum formed will be too soft, which will make it vulnerable during dewatering. Homogenized milk is more sensitive to light and enzymatic activity of lipase, so pre-heat treatment is recommended before homogenization of milk to inactivate lipase.

Microfluidization is a novel technique, using high pressure up to 241 MPa by combining high velocity, shear, turbulence, and collision inside the chamber for a short duration of 5 s to obtain high-quality products by breaking down large particles. It is a continuous process that has a chamber for fluid flow, in which pressure stream is forcibly directed to flow into two different fine orifices. At the point of intersection, both splited microstreams clash into each other at high speed, resulting in the reduction of large-size particles to very small sizes. Breakdown into fine particles inside the interaction chamber occurs due to high speed, high shear, cavitation, air compression, and coerces of the microstreams. The milk obtained after microfluidization is more homogenized and viscous when compared with commercially available homogenized milk using a two-stage homogenizer. Microfluidized milk, when used for cheese preparation, can improve textural and melting characteristics and also lower the microbial population. Yogurt prepared from homogenized milk was comparable to low-fat (1.5%) microfluidized yogurt in terms of texture profile and water retention property. Some of the characteristics associated with microfluidization include chances of contamination, hard to scale up the production rate, loss of product, and encapsulated material structure due to high shear and striking coerces during microfluidization.

Novel Nonthermal Milk Processing Techniques

Consumers prefer products that are minimally processed; have natural aroma, fresh taste, and no additives; are stable; are convenient to use; and are microbiologically safe. Although thermal processing (heating or cooling operation) maintains safety of food, it adversely affects nutritional value. However irradiation, pulse electric field, and HPP are alternatives to traditional thermal processing. Technologies such as ohmic heating, dielectric heating (which includes microwave heating and radio-frequency heating), and inductive heating have been developed that can replace, at least partially, the traditional heating methods that rely essentially on conductive, convective, and radiative heat transfer. They all have a common feature: heat is generated directly inside the food and this has direct implications in terms of both energetic and heating efficiency. Among these nonthermal techniques, HPP of milk is well studied although other techniques has attracted little attention and commercial application but has considerable potent future.

- **High Pressure Processing**

HPP is a technique that involves the application of pressure in the range of 100–900 MPa, which is instantaneously and uniformly transmitted throughout the product irrespective of its size and shape. The principle of HPP is based on Le Chatelier's principle (with increase in pressure, decrease in volume occurs) and isostatic transfer (uniform transmission of pressure in all sides). Pressure of 300–600 MPa breaks ionic and electrostatic bonds but does not disrupt covalent bonds. HPP processing of milk usually has least effect on color, flavor, and nutritional value, but is effective enough to reduce microbial load as well to enhance rennet or acid coagulation of milk. Milk processed at a pressure of 600 MPa was found to enhance the shelf life of milk by reducing the microbial population, and factors that govern the microbial reduction are duration of exposure and target species, as well as composition. Microbial quality of HPP (400–600) treated raw milk was found to be comparable with pasteurized milk (72 °C for 15 s), and treatment at 400 MPa for 15 min and 600 MPa for 3 min is effective enough to increase the shelf life at 10 and 20°C, respectively, for 10 days.

- **Pulse Electric Field (PEF)**

PEF has proven to have a potential for increasing the milk shelf life, as it is highly applicable to liquids. PEF had shown minimal effects on the nutritional, flavor, and functional characteristics of milk and also has an ability to inactivate microorganisms. PEF is based on the application of pulses of high voltage (typically 20–80 kV cm^{-1}) delivered to the product placed between a set of electrodes that confine the treatment gap of the PEF chamber. The process can be conducted at ambient temperature for a fraction of second, and energy loss due to the heating of foods is minimized. Preservation of milk and fluid dairy products seems to be one of the main market

niches for PEF technology because it is mainly intended for preservation of pumpable fluid or semifluid foods. When self-inoculated (*Salmonella*) milk was treated with PEF (36.7 kV cm^{-1} and 40 pulses, 25 min) and stored at 7–9 °C for 8 days, the level of *Salmonella* was below detection limit. PEF-treated cheese milk has not shown any significant changes on physicochemical characteristics and quality of cheese. No adverse effect of PEF on water-soluble vitamins in milk (riboflavin, thiamin, and ascorbic acid) and fat-soluble vitamins (cholecalciferol and tocopherol), after applying treatments of up to 400 μs at field strengths from 18.3 to 27.1 kV cm^{-1}, was found except for ascorbic acid. As compared to LTLT- and HTST-treated milk, PEF-treated milk retained more ascorbic acid after a 400 μs treatment at 22.6 kV cm^{-1} (93.4%) than either a LTLT (49.7%) or HTST (86.7% retained).

- **Other Techniques**

Milk irradiation is a process of exposing milk to ionizing radiation (gamma rays, beta-particles), emitted by a source (cesium and cobalt) to destroy the spoilage-causing microorganisms. Treatment of milk with 2–5 kGy reduced bacterial population by around 99%, but to achieve complete sterilization of milk, a dosage of 10–20 kGy is required. A hurdle in irradiation of milk is development of off-flavor and color change. Irradiation can be effectively used for sterilization of packaging material used for packing of milk and milk products. Ultrasound waves are similar to sound waves but have a frequency that is above 16 kHz. It produces very rapid localized changes in pressure and temperature that cause shear disruption, 'cavitation' (creation of bubbles in liquid foods), thinning of cell membranes, localized heating, and free radical production that have a lethal effect on microorganisms. A combined heat and ultrasound treatment under pressure was termed 'manothermosonication' (MTS) and can be used for enhancing the shelf life of milk. Unfortunately, ultrasound does not inactivate alkaline phosphatase or lactoperoxidase enzymes and hence cannot be used to indicate a successful ultrasonic treatment. If ultrasonication is to be used as an alternative to thermal pasteurization, a need exists to find a quick and efficient method to indicate whether ultrasonication was sufficient in terms of ensuring a microbiologically safe product.

Packaging, Storage, and Distribution

Packaging is a medium between product manufacturers and consumers aiding the maintenance of original quality of the packaged products and providing information and characteristics of the packaged products to the consumers. The functions of packaging are to enable efficient food distribution, maintain product hygiene, protect nutrients and flavor, and reduce spoilage and waste. Immediately after heat treatment, milk products need to be packaged to avoid postprocess contamination that will affect the shelf life. In earlier days returnable glass bottles were used for packaging and distribution of milk, but with technological advancement plastic bottles, tetra packs, laminates, and sachet are now preferred over glass bottles. Before a packaging material is selected for milk and milk products it is important to know the type, composition, compatibility, conditions of storage, and transportation (light, temperature, humidity, mechanical stress during transportation, and storage). The ideal packaging material for milk should be an inert, nontoxic, safe, and hygienic design and should not directly or indirectly impart smell. Packaging material should be well labeled with manufacturing, expiration date, storage conditions, and ingredients. Single-use packages are used for packaging of fluid milk that is predominately plastic pouch (PE, LDPE, HDPE, polyvinylchloride) that has a good moisture barrier properties but poor recyclability and disposal complications. Cartons are generally used as a secondary storage of milk; paper and paperboards materials include laminates consisting of outer kraft paper with PE coatings and aluminum foil. The advantage of single-use packaging material is that they decrease the use of detergents, which is required for washing returnable glass bottles. Apart from sterilizing the milk and milk products and also the packaging material, sterilization of equipment, line, and filling area can enhance the shelf life of milk. Aseptic packaging is being utilized in developing countries for presterilized packages under sterile environment into sterile packaging material.

For storage and distribution of milk, insulated tanks, cans, bottles, tetrahedral package, paperboard cartons, and automatic vending machines are used. The most important factor to be considered during the storage and distribution of milk is the temperature of storage, which should be kept at 5 °C. Hygienic conditions must be maintained throughout the supply chain so that the milk products are not cross contaminated and are also free from defects like off flavor due to exposure to chemicals.

Quality Assurance and Quality Control

Quality assurance is an important element for safe milk and milk products from any of the contaminants that can render the product unsafe for consumers. Addition of water by deceitful middlemen is one of the challenges faced by the dairy industry in developing countries. To provide a quality milk and milk product it is of utmost importance to start the process by procuring good-quality raw milk and maintaining strict quality measures at different checkpoints (collection, transportation, receiving dock, after processing, and during dispatch), including transportation and storage. Quality testing includes a platform test at the receiving dock and physicochemical cum microbiological analysis in the processing (before–after) industry and should follow up the standards as laid out by regulatory agencies/bodies. To ensure the quality, dairy industries should follow good manufacturing policies (GMP), hazard analysis critical control points (HACCP), and ISO:22000 (2005) based programs, which will help in controlling the quality by identifying and reducing the hazards within a safe limit for consumers. Other key factors that control quality are hygiene, safety, adequate thermal treatment during manufacturing, packaging, and postprocess contamination.

Steps Where Preventive Measures Are Required to Maintain Quality

- Good and hygienic milking practices must be carried out at the farm level that can minimize the proliferation of microorganisms from environment, dirt, soil, feces, human beings, and equipment into milk.

- Utensils and equipment used for milking the animals must be well sanitized and preferably manufactured using stainless steel.
- Milk mulched from mulching animals suffering from mastitis and other diseases must not be mixed with a batch of good raw milk.
- Longer storage temperatures of raw milk must be avoided as it allows few psychrotrophic organisms (*L. monocytogenes* and *Pseudomonas* species) to grow that in return can secrete enzymes like proteases and lipases, which produces defects like sweet curdling in UHT-treated milk.
- Monitoring the milk for adulteration with adulterants including urea, sugar, neutralizers, and nondairy proteins.
- Routine monitoring must be done for critical control points (time–temperature) for pasteurization and sterilization that can be evaluated by conducting an alkaline phosphatase test (pasteurization) and turbidity test (sterilized).
- Monitoring the sanitation and CIP programs before processing and after processing to ensure that the residual milk in pipeline/equipment is removed and they are in sanitized condition, which will not support the further growth of spoilage bacteria.
- Hygiene conditions must be maintained during processing and also after packaging of milk to avoid postprocess contamination.
- Usage of unsterilized packaging material for packing of milk and milk products must be avoided.
- Temperature fluctuation during the storage and distribution of milk (collection center, transportation, storage distribution) must be avoided to maintain the product safety.

Good farming practice, animal health management, control of feed, hygienic milking operations, inspection, quality analysis, sanitation, and hygiene during manufacturing as well as packaging and effectively monitoring every parameter during processing will help in reducing microbial load to render products safe for end consumers.

See also: Acidophilus Milk; Cheese: Chemistry and Microbiology; Fermented Foods: Composition and Health Effects; Fermented Foods: Origins and Applications; Fermented Foods: Use of Starter Cultures; Functional Foods; Lactic Acid Bacteria.

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Milk: Role in the Diet

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Introduction

Milk has been a part of the human diet for millennia and is valued as a natural and traditional food. Milk and its products are considered to be one of the main food groups (dairy) important in a healthy balanced diet and, as such, features in the majority of national food-based dietary guidelines. The popularity of milk as a staple food over the centuries is owed to its versatility. Bovine (cows') milk is by far the most commonly consumed milk, dominating global milk production. However, in certain parts of the world and locally, milk from other species has a significant share in consumption; after bovine milk (85%), buffalo milk is consumed most (11%) followed by milk from other ruminants (3.9%).

In the context of current dietary advice, it is difficult for most individuals to meet national nutrition goals unless they are consuming milk and dairy products. Early humans discovered that milk could be churned to make butter or fermented to make yogurt preserving some or all of the nutrients in milk for consumption at a later date. Milk is one of the most nutritionally complete foods. Milk and milk products are widely recognized as important sources of nutrients.

Milk provides a wide range of essential nutrients, in particular protein, and a range of vitamins and minerals. It is, however, a poor source of iron and vitamin D and contains no starch or dietary fiber. As milk provides a substantial amount of vitamins and minerals in relation to its energy content, it is a nutrient-dense food. Scientific studies provide evidence that intake of milk, and milk products, is related to improved bone health and lower risk of cardiovascular disease, hypertension, and type 2 diabetes. It is because of these recognized benefits that individuals are encouraged to consume three cups per day of fat-free or low-fat milk (750 ml) or equivalent milk products. On the other hand, milk is also recognized as a source of both calories and saturated fat.

Milk is a rich source of calcium, but it is perhaps less commonly known that milk and milk products are also an important source of good quality protein, B vitamins (B2 and B12), and the minerals iodine, potassium, and phosphorus. Milk consumption has been called a marker for an overall healthy diet because of its association with increased nutrient intake.

Clinical Evidence of Dietary Importance of Milk

The Dietary Guidelines for Americans Advisory Committee report states that, without milk products, 19–50-year-old women achieve only 44% of calcium, 57% of magnesium, and 57% of potassium recommended intakes. Similarly, there are some data to show that nutrient intake is enhanced when dietary patterns include milk and dairy products. For example, in an 18-month randomized controlled trial, British girls in the intervention group were asked to drink extra milk, while the control group maintained their usual diet. Consuming an approximate additional glass (186 ml) of milk every day significantly increased intakes of protein, calcium, phosphorus, magnesium, zinc, and riboflavin, while milk consumption and nutrient intake were unchanged in the control group.

In a US study of milk drinkers, both flavored and plain, intakes of vitamin A, calcium, phosphorus, magnesium, and potassium were significantly higher than among non-milk drinkers.

Milk and dairy products are major contributors to calcium, riboflavin, phosphorus, and iodine intakes for people of all age groups in the United Kingdom. For young children, dairy products contribute significantly to protein, vitamin A, potassium, magnesium, and zinc intake. In countries such as the United States, where milk is fortified, dairy foods make an important contribution to vitamin D, but this is not the case in the UK.

Composition of Milk

Milk of all mammals contain proteins, carbohydrates, fats, vitamins, minerals, and water, but their percentage varies widely between ruminant milk and nonruminant milk. Milk composition differs considerably due to genetic, physiological, and nutritional factors as well as frequency of milking and environmental conditions. The greatest changes in composition occur during lactation. Colostrums have the highest concentration of proteins, especially immunoglobulins and fat, and a low concentration of lactose. Dairy animals on nutritional feed produce milk with a higher level of fat and protein than those on feed of lower quality.

Dietary Importance of Milk

Milk is the first food of newborn mammals, including human beings. It contains all the nutrients required for normal growth. Milk and milk products have had a key role throughout the development of human civilization. It supplies almost all the essential nutrients in significant amounts, more than any other single food. It is essential for the growth and development of infants. It is not just an important food during early childhood but, in one form or the other, continues to provide benefits throughout life. It is one of the most versatile animal-origin foods and is a component of diets globally in many forms, such as cheese, yogurt, ice cream, ghee, and milk powders. The calcium content in cows' milk is lower than

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buffalo milk, while buffalo milk is richer in fat. β-Carotene is absent in buffalo milk but is present in cows' milk.

Milk Proteins

The principal proteins found in milk and its products are casein, lactalbumin, and lactoglobulin. Milk has a high biological value as it contains all eight essential amino acids. In addition, milk can improve the overall protein quality of a meal when consumed with foods of lower protein quality, such as cereals and pulses. Milk proteins are complete proteins of high quality. The energy value of milk proteins is 17.2 J g^{-1}. The contribution of milk to nutrient intakes in UK is shown in Table 1. The proportion of bovine casein, which is micellar, depends on the temperature and gravitational force used to sediment casein micelles. The size of casein micelles in cows' milk ranges from 50 to 500 nm (mean 120–150 nm, volume 4.18 ml g^{-1}). Buffalo milk casein micelles contain more calcium and magnesium, but lower levels of sialic acid and hexose. The lactoferrin content of buffalo milk is 3.2 mg ml^{-1} compared to 0.15 mg ml^{-1} in cows' milk. Immunoglobulins, lactoferrin, lactoperoxidase, and lysozymes in milk make it a good dietary and health food.

Bovine milk contains about 3.5% protein of which 80% is casein and 20% whey proteins. Casein, the dominant protein in milk, can be fractionated into four major components: alpha-, beta-, gamma-, and kappa-caseins. Whey protein is composed predominantly of beta-lactoglobulin and alpha-lactalbumin, but other whey proteins include serum albumin, immunoglobulins (IgA, IgG, and IgM), protease peptones, lactoferrin, and transferrin. Colostrum contains minor bioactive components, such as peptides, hormones, and growth factors, which were associated with advantageous intestinal effects in rat studies. Buffalo milk has a slightly higher concentration of β-lactoglobulin than cows' milk, and it is the major whey protein. Human milk contains no β-lactoglobulin, but contains essential and branched-chain amino acids and a retinol-binding protein, which has the potential to modulate lymphatic responses. Milk can yield antibacterial peptides, which are released after proteolytic digestion with trypsin and are active against Gram-positive bacteria. Whey comprises a heterogeneous group of proteins that remain in the supernatant after precipitation of caseins. They are characterized by genetic polymorphisms that usually translate into replacements for one or more amino acid residues in their original peptide sequence.

Milk Fat

The fat content of milk varies depending on whether milk is whole, semiskimmed, or skimmed. Fat in milk is in the form of minute droplets, which rise to the top when milk is left to stand. The principal component of milk fat is triacylglycerol, three fatty acids joined to a glycerol backbone. All triacylglycerols contain mixtures of three families of fatty acids: saturated, monounsaturated, and polyunsaturated. Despite a misconception that milk is 'fattening,' even bovine whole milk contains only around 3.5% fat. Semiskimmed milk contains 1.7% fat and skimmed milk contains only 0.1% fat. Milk fat contributes unique characteristics to the appearance, texture, flavor, and satiability of dairy foods. Dairy fat is a source of energy, essential fatty acids, fat-soluble vitamins, and several other components, such as conjugated linoleic acid (CLA) and sphingolipids.

About a quarter of the fat in milk is monounsaturated. The remainder is mostly saturated, although some polyunsaturated fats and other minor fatty acids are also present. Palmitic, stearic, and myristic acids are the major saturated fats found in milk. It is comparatively rich in the short- and medium-chain saturated fats. There are a variety of saturated fatty acids in milk, many of which have no effect on plasma cholesterol. When milk is skimmed, it is the amount rather than the type of fat that changes, and so the fatty acid profile remains the same. Milk fat contains small amounts of two essential fatty acids, linoleic acid (1.4 g per 100 g fatty acids) and linolenic acid (1.5 g per 100 g fatty acids).

Table 1 Contribution of milk to nutrient intake (% total) in the UK diet

Milk constituents	*Age groups*			
	1.5–4.5 years	*4–18 years*	*19–64 years*	*65+ years*
Protein	24	11	9.7	13.6
Vitamin A	30.5	10.9	7.0	13.0
Riboflavin (B2)	37.5	24.8	23.3	26.7
Vitamin B6	22.5	9.4	8.3	14.0
Vitamin B12	39.7	36.6	29.1	24.7
Calcium	46.9	28.3	26.2	36.1
Iodine	39.6	33.7	28.5	25.5
Magnesium	22.2	10.3	8.1	13.5
Phosphorus	32.7	16.4	15.1	21.2
Potassium	25.6	13.0	10.8	15.7
Zinc	25.0	13.0	9.7	13.6

Source: Henderson L, Gregory J, and Swan G (2002a) The national diet and nutrition survey: adults aged 19 to 64 years and over. *Types and quantities of foods consumed*. vol. 1. London: TSO; Henderson L, Gregory J, Irving K, and Swan G (2002b) The national diet and nutrition survey: adults aged 19 to 64 years and over. *Energy, protein, carbohydrate, fat and alcohol intake*. vol. 2. London: TSO; Henderson L, Irving K, Gregory J, Bates CJ, Prentice A, Perks J, Swan G, and Farron M (2002c) The national diet and nutrition survey: adults aged 19 to 64 years and over. *Vitamin and mineral intake and urinary analytes*. vol. 3. London: TSO

The nutritional interest in buffalo milk products is higher than cows' milk because its fat-derived products are a good source of CLA, like other food products from ruminants. CLA are a group of polyunsaturated fatty acids that exist as positional and stereoisomers of conjugated dienoic acid (18:2). Synthetic mixtures of CLA can be purchased as nutritional supplements but are composed primarily of the *cis*-9 and *trans*-11 CLA as well as *trans*-10 and *cis*-12 isomers. Numerous potential physiological effects have been attributed to CLA including those related to its potential antiadipogenic, antidiabetogenic, anticarcinogenic, and antiatherosclerotic properties. In dairy products, CLA concentrations typically range from 2.90 to 8.92 mg g^{-1} fat. CLA content of cheeses typically ranges from 3.59 to 7.96 mg g^{-1} fat. CLA content of cows' milk ranges from 3.38 to 6.39 mg g^{-1} fat. Amounts of CLA found in dairy products are a direct reflection of the diet of the milch animals. CLA concentrations increase linearly when animals are pasture-fed and decrease when grass intake declines. Supplementing the diet with polyunsaturated oils, which contain either corn or sunflower oil, increases CLA content of milk fat substantially.

Milk Carbohydrates

The principal carbohydrate found in milk is lactose. Cows' milk contains about 4.5 g lactose per 100 g milk, and there is some evidence that lactose is the least cariogenic of common dietary sugars. It exists in two anomeric forms, α-D-lactose and β-D-lactose, in an equilibrium of about 37.3% α to 62.7% β at 20 °C, and in an inverse relationship with the chloride content, making it isoosmotic with blood. Various other components in milk have also been considered protective against dental caries.

Lactose is a disaccharide comprising a molecule of glucose and a molecule of galactose. This sugar is found naturally only in milk and is much less sweet than sucrose. In the small intestine, lactose is digested by the enzyme lactase (β-galactosidase) to its two component monosaccharides prior to their absorption.

Lactase is present in babies, young children, and most ethnic-European (Caucasian) adults. However, in some adults, enzymatic activity can decrease, making milk in quantity less well tolerated. Such individuals are lactose-intolerant. Most can tolerate small quantities of milk, and fermented milk products are better tolerated as the lactic acid bacteria present produce lactase. Cheese is also usually well tolerated as it contains only trace amounts of lactose. In the UK, lactose intolerance is relatively rare but is more common in those of Asian, Far Eastern, and African descent, particularly first-generation members of these ethnic groups.

Other milk carbohydrates, which are free or bound to lipids, proteins, or phosphate, include a small fraction of oligosaccharides. They are composed of galactose, fructose, *N*-acetylglucosamine, and/or *N*-acetylneuraminic acid (sialic acid) and include mostly a lactose unit at the reducing end. They modulate the growth of intestinal flora, influence gastrointestinal and inflammatory processes, and provide protection against bacterial and viral infections. In general, the oligosaccharide level is much lower in animal milk (0.25–0.30 g l^{-1} goat milk, >0.1 g l^{-1} buffalo milk, 0.03–0.09 g l^{-1} bovine milk, and 0.02–0.04 g l^{-1} sheep milk) compared with human milk (5–10 g l^{-1}).

Minerals in Milk

Milk contributes to virtually all the minerals and trace elements known to be essential for human health. These are often present in a form that is well absorbed and utilized by the body (high bioavailability). Milk is an excellent source of calcium, which is essential for the healthy growth and maintenance of teeth and bones. Calcium is also important for blood coagulation, energy metabolism, muscle and nerve function, digestive function, and regulation of cell division and differentiation. Calcium content of cows' milk is 120 mg/100 ml compared with 160 mg/100 ml buffalo milk. For most people in the Western world, milk and milk products are the primary source of calcium. Almost 60% of the calcium in the typical British diet, for example, is made up of milk and milk products (Table 2).

Milk and milk products are the main sources of calcium in preschool children and adolescents (4–18 years), adults (19–64 years), and the elderly (65 years and over). A 200 ml glass of semiskimmed milk can provide a 6-year-old child with over half (55%) of their calcium requirement and can provide an adult (19–64 years) with over a third (35%) of their daily calcium requirement. Calcium as a bioactive mineral occurs widely in a range of calcium-fortified foods, including fortified milk and other beverages. In the diets of developed countries, milk and dairy products provide approximately 70% of recommended daily intakes of calcium.

The role of calcium as a protective factor in the etiology of colon cancer has been well documented. It is also believed to be associated with binding and removal of carcinogenic agents along the gastrointestinal tract. Calcium is important for the development and maintenance of skeletal integrity and prevention of osteoporosis.

Zinc content in milk is greatest among the trace minerals. Zn in goat and cows' milk is greater than in human milk. Its deficiency results in skin lesions, disturbed immune function, growth retardation, and impaired wound healing. The contribution to zinc requirements made by milk is relatively low compared with meat (major contributor). However, the zinc in milk is highly bioavailable, and there is evidence that the combination of milk (or meat) with vegetables, in which zinc is less bioavailable, can enhance bioavailability of zinc from the whole meal.

In the UK, milk and milk products are the main sources of potassium for preschool children and the elderly (65 years and over) and the second largest contributor, after potatoes, for schoolchildren and adolescents (4–18 years). A 200 ml glass of semiskimmed milk will provide a 6-year-old child with 29% of their daily requirement for potassium and an adult (19–50 years) with 9%.

Milk is also a good source of iodine. Iodine is required for the production of the thyroid hormones and normal thyroid function and for normal energy yielding metabolism and contributes to the maintenance of normal skin.

Milk is naturally low in iron. Despite similar iron contents, bioavailability is greater from goat milk compared with cows'

Table 2 Ranked contribution of milk (and milk products) to vitamin and mineral intakes in the UK diet

	Preschool (1.5-4.5)	*School (4–18)*	*Adult (19–64)*	*Elderly (65+)*
Vitamin B2	37.5 (45.8)	24.8 (31.3)	23.3 (28.7)	26.7 (31.0)
	Largest	Largest	Largest	Largest
Vitamin B12	39.7 (47.8)	36.6 (41.9)	29.1 (36.5)	24.7 (28.1)
	Largest	Largest	Largest	2nd largest
Calcium	46.9 (58.5)	28.3 (40.6)	26.6 (39.2)	36.1 (45.7)
	Largest	Largest	Largest	Largest
Iodine	39.6 (49.7)	33.7 (42.0)	28.5 (34.3)	25.5 (31.1)
	Largest	Largest	Largest	Largest
Phosphorus	32.7 (41.3)	16.4 (24.1)	15.1 (22.5)	21.2 (26.7)
	Largest	Largest	Largest	Largest
Potassium	25.6 (29.2)	13.0 (15.4)	10.8 (12.7)	15.7 (17.0)
	Largest	2nd largest	4th largest	Largest

Source: Henderson L, Gregory J, and Swan G (2002a) The national diet and nutrition survey: adults aged 19 to 64 years and over. *Types and quantities of foods consumed*. vol. 1. London: TSO; Henderson L, Gregory J, Irving K, and Swan G (2002b) The national diet and nutrition survey: adults aged 19 to 64 years and over. *Energy, protein, carbohydrate, fat and alcohol intake*. vol. 2. London: TSO; Henderson L, Irving K, Gregory J, Bates CJ, Prentice A, Perks J, Swan G, and Farron M (2002c) The national diet and nutrition survey: adults aged 19 to 64 years and over. *Vitamin and mineral intake and urinary analytes*. vol. 3. London: TSO

milk. Goat milk has a higher share of nucleotides that contribute to enhance iron absorption from the intestine.

Phosphorus plays a vital role in the maintenance of healthy bones and teeth as well as cell membrane structure and regulation of energy metabolism. The main sources of phosphorus, in the UK diet, come from milk and milk products across all age groups. A 200 ml glass of semiskimmed milk will provide a 6-year-old child with 55% of their daily requirement for phosphorus and an adult (19–50 years) with 36%.

Vitamins in Milk

Vitamin content of milk is highly variable depending on feeding regimen of the dam. Levels of water-soluble vitamins are influenced most by feed. All of the known vitamins are found in whole milk (Table 1), although some are present in low quantities.

Milk and milk products make up a significant contribution to intakes of a number of vitamins. Buffalo milk is a rich source of most water- and fat-soluble vitamins. Fat-soluble vitamins – A, D, E, and K – are removed when milk is skimmed. Consequently, they are present only in trace amounts in skimmed milk and in reduced amounts in semiskimmed milk. Whole milk is a good source of vitamin A, a pint (568 ml) providing 47% of the adult male and 55% of the adult female UK reference nutrient intake. All of the three major types of cows' milk (whole, semiskimmed, and skimmed) are good sources of riboflavin (vitamin B2) and vitamin B12. Milk can also make an important contribution to intakes of thiamin (vitamin B1), niacin, and ascorbic acid (vitamin C), particularly where the overall diet is poor.

Vitamin A content is higher in buffalo milk (340 IU kg^{-1}) than cows' milk (230 IU kg^{-1}). However, due to the absence of carotenoids and the high fat content in buffalo milk, the total vitamin A potency/unit weight of fat is less than cows' milk.

Human infants fed solely on goat milk are oversupplied with protein, calcium, phosphorous, vitamin A, thiamin, riboflavin, niacin, and pantothenate per the FAO–WHO requirements.

The tocopherol (vitamin E) content of buffalo milk is 334 μg kg^{-1} and that of cows' milk 312 μg kg^{-1}. However, due to higher fat, buffalo milk is poor in tocopherol (26 μg g^{-1} fat compared with 35 μg g^{-1} fat in cows' milk).

Some vitamins are sensitive to heat and light. Over 90% of milk for liquid consumption is pasteurized, a mild heat treatment that causes vitamin C to be lost. In general, vitamin losses are less than 10% during pasteurization. However, while ultra-heat treatment (UHT) substantially increases the shelf life of milk, the higher temperatures used result in greater losses (10–20%) of some vitamins, particularly B6, B12, and folate as well as vitamin C. Sterilization has a greater effect still with about one-third of thiamin and half of the vitamin C, folate, and B12 destroyed. Some loss of vitamins is inevitable when milk is stored. The extent of these losses is dependent on translucency and permeability to light of the container and the length and conditions of storage.

Milk exposed to bright sunlight on the doorstep will readily lose its vitamin C. Loss of riboflavin is slower but, after 4 h, half will be lost and only a third remains after 6 h. Therefore, measures should be taken to limit such exposure. There will also be gradual losses of some vitamins from UHT and sterilized milks, even under ideal storage conditions, because of the small amounts of oxygen remaining in the pack or bottle. Boiling milk also reduces its vitamin content, ranging from a 5% reduction in vitamin B12 to a half the vitamin C.

Pigments in Milk

Biliverdin IX alpha, a latent blue-green pigment, occurs in fresh buffalo milk but is absent in cows' milk. The average concentration of biliverdin in buffalo skimmed milk is around 60 μg/100 ml. The concentration of this pigment varies significantly at different stages of lactation and lactation number. Biliverdin is primarily associated with α, β, and γ caseins and the proteose-peptone fraction of the milk. It is converted to bilirubin during storage and souring of the milk.

Nutritional Significance of Milk in the Diets of Children

As a child is weaned on to 'solids,' quantities of milk can increase, for example, cows' milk may be used to mix infants' cereals. However, cows' milk should not be introduced as a drink until a child is one-year-old. This is because, unlike infant formulas, cows' milk is relatively low in iron and vitamin D and there is growing concern about the iron status of older infants and preschool children. Whole milk is a good source of protein, calcium, zinc, and vitamins A, B2, and B12. It also makes a valuable contribution to intakes of iodine, niacin (vitamin B), and vitamin B6.

There is adequate evidence that calcium has a major role in the development and maintenance of skeletal strength. Sufficient milk helps to achieve peak bone density in early adulthood, thus protecting against osteoporosis in later life. Approximately 45% of minerals in the adult skeleton are laid down during adolescence. By the age of about 17 years (earlier in girls), more than 90% of bone mineral (e.g., calcium) that will be present in the skeleton is already there. Calcium requirements are, therefore, particularly high during adolescence. Dental experts recommend milk as the most suitable drinks for children to strengthen the dental health.

Nutritional Significance of Milk for Adults

During pregnancy, especially the final months, requirements for a number of nutrients increase. Milk is seen to have a major role in providing a broad range of nutrients. The needs for some nutrients, for example, calcium, rise dramatically after the baby is born in women who choose to breast-feed. Dietary requirements for calcium almost double, and consumption of several servings of dairy products daily is one of the few ways of easily achieving such intakes. Breast-feeding teenagers need even more calcium as their skeleton is still developing as well as that of the baby.

Milk and milk products remain a nutritional safeguard for other adults, especially those who restrict food choices because they are slimming or following a vegetarian diet. For elderly people, whose overall food intake and dietary variety can be limited, milk can be of particular importance in meeting nutrient needs, especially with the convenience of doorstep or other (e.g., online) delivery.

See also: Buffalo Milk; Cheese: Processing and Sensory Properties; Condensed Milk; Dairy Products: Dietary and Medical Importance; Fermented Foods: Fermented Milks; Food Allergies; Goat: Milk; Milk Powder; Milk: Sources and Composition; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Sheep: Milk.

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Milk: Sources and Composition

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Introduction

Historically, milk from livestock animals has been an important source of nutrients, especially protein for humans. Bovine milk is by far the most commonly consumed type of milk, dominating global milk production because of large cattle population. However, in certain parts of the world and local contexts, milk from other animal species has a significant share in milk consumption. Apart from bovine milk (85%), the buffalo milk is highest (11%), followed by ruminants (3.9%). There is paucity of world statistics on milk of nonruminant animal species, which contributes less than 0.1%. The horse and donkey milk seem to experience a revival in continental Europe with new horse dairies in Belgium, France, the Netherlands, and Norway. A number of therapeutic properties are attributed to these milks. Bovine milk is produced on industrial scale and as such mostly standardized with respect to fat and protein content; equine milk or milk from other species is usually produced on a local scale. The up-to-date information is necessary on how milk and dairy products can contribute to human nutrition and how dairy development can contribute to increasing food security and sustainable development and thereby alleviating poverty.

Composition of Milk

Milk is a complex biological fluid consisting of fats, proteins, minerals, vitamins, enzymes, and sugar. Milk of all mammals contains proteins, fats, carbohydrates, vitamins, minerals, and water, but their content varies widely between ruminant and nonruminant milk. The milk composition differs considerably due to genetic factors, physiological factors, nutritional factors, frequency of milking, and environmental conditions. The greatest changes in composition occur during lactation. Colostrum, the first milk after calving, has a high concentration of fat and protein, especially immunoglobulin, and a low concentration of lactose. The volume of milk produced increases during the early stages, which after peaking slowly declines toward the latter stages of lactation. The mastitis results in changed composition of milk with decreased levels of lactose and potassium and increased levels of sodium, chloride, and serum proteins. In addition, a large number of somatic cells and bacteria are present. Generally, the animals on nutritionally superior feed produce more milk with a higher level of fat and protein than animals on feed of lower nutritional quality. The fat content and protein content of milk are positively correlated within the population of dairy cattle; however, different breeds of cattle vary in average component levels. Gross composition of milk from various mammalian species is depicted in Table 1.

Milk Proteins

Milk proteins are divided into two broad categories: caseins and soluble proteins, which include enzymes. Caseins contribute to about 80% of the total milk proteins in bovine and sheep milk and about 50% of horse milk proteins and less than 50% of human milk proteins. Caseins are organized into macromolecular units called micelles. In general, a distinction is made between 'casein' milk (i.e., ruminant milk, which is relatively rich in casein) and 'albumin' milk (i.e., nonruminant milk, which has proportionally a higher whey protein content as indicated by the lower casein/whey ratio). The main caseins are designated as α_s1-caseins, α_s2-caseins, β-caseins, and κ-caseins with other minor caseins that are derived from these by proteolysis, glycosylation, or phosphorylation. The whey proteins consist of β-lactoglobulins, α-lactalbumins, immunoglobulins, and serum albumin in decreasing amounts.

The nutritional value of milk proteins depends to a great extent on the presence of essential amino acids. As to the thermal stability of milk proteins, differences between species are mainly due to differences in amino acid sequences and in milk environment. Whey proteins of bovine milk are less resistant to heat denaturation compared to those of buffalo milk, which are less heat-resistant than camel whey proteins. Advancement in scientific and commercial interest has been focussed on the biological properties of milk proteins. Intact protein molecules of both major milk protein groups, caseins and whey proteins, exert distinct physiological functions *in vivo*. Many of the bioactive whey proteins like immunoglobulins, lactoferrin, and growth factors occur in colostrum in much greater concentrations than in milk, thus reflecting their importance to the health of the newborn.

The Caseins

α-Casein has four variants, A, B, C, and D, of which B is the most common. Its polypeptide chain has three distinct regions that are strongly hydrophobic. It is highly negatively charged, while the rest of the molecule is essentially neutral. The three hydrophobic regions, 1–44, 90–113, and 132–199, of the B variant contain 21, 8, and 30 hydrophobic residues (Ala, Val, Leu, lie, Pro, Phe, Trp, and Met), respectively. Region 41–80 contains a cluster of phosphoseryl residues and this will be highly charged at pH 6.6. The molecule has little or no α-helical structure, a very small amount of β-structure, mainly around turns, and much random coil.

αs2-Caseins (variants A, B, C, and D) are the most hydrophilic of all the caseins because they contain 10–13 phosphoseryl residues. In the polypeptide chain of the A variant, those

Table 1 Gross composition of milk from different mammals

	Nonruminants			*Ruminants*							
Parameters	*Human*	*Horse*	*Donkey*	*Cow*	*Sheep*	*Goat*	*Buffalo*	*Camel*	*Llama*	*Yak*	*Reindeer*
Total solids%	10.7–12.9	9.3–11.6	8.8–11.7	11.8–13.0	18.1–20.0	11.9–16.30	15.7–17.2	11.9–15.0	12.8–13.4	13.5–18.4	20.1–27.1
Proteins%	0.9–1.9	1.4–3.2	1.4–2.0	3.0–3.9	4.5–7.0	3.0–5.2	2.7–4.7	2.4–4.2	3.4–4.3	4.2–5.9	7.5–13.0
Casein/whey ratio	0.4–0.5	1.1	1.28	4.7	3.1	3.5	4.6	2.7–3.2	3.1	4.5	4.5
Fat%	2.1–4.0	0.3–4.2	0.3–1.8	3.3–5.4	5.0–9.0	3.0–7.2	5.3–9.0	2.0–6.0	2.7–4.7	5.3–9.5	10.2–21.5
Lactose%	6.3–7.0	5.6–7.2	5.8–7.4	4.4–5.6	4.1–5.9	3.2–5.0	3.2–4.9	3.5–5.1	5.9–6.5	3.3–6.2	1.2–4.7
Ash%	0.2–0.3	0.3–0.5	0.3–0.5	0.7–0.8	0.8–1.0	0.7–0.9	0.8–0.9	0.6–0.9	0.5–0.9	0.4–1.0	1.2–2.7
Energy (kJ l^{-1})	2843	1936–2050	1607–1803	2709–2843	4038–4439	2802–2894	4244–4779	2410–3286	3286–3358	3811–4295	5541–8436

Source: Claeys, W. L., Verraes, C., Cardoen, S., De Block, J., Huyghebaert, A., Raes, K., Dewettinck, K. and Herman, L. (2014). Consumption of raw or heated milk from different species: An evaluation of the nutritional and potential health benefits. *Food Control* **42**, 188–201.

in residues 8–16, 56–61, and 129–133 are clustered. Hydrophobic regions occur at 90–120 and 160–207. The C-terminal is positively charged, containing either basic or neutral residues only and having a net charge of +9.5 at pH 6.6 compared with a net charge of −21 for the first 68 residues of the N-terminal. Thus, electrostatic interactions may be important for its structural stability.

β-Casein occurs in five genetic variants (A1, A2, and A3, B, C, D, and E) and is the most hydrophobic of the caseins. The 21 residues from the N-terminal have a charge of −12 at pH 6.6, while the hydrophobic region (from approximately 69–209) has no net charge. It has at least one cluster of phosphoseryl residues (15–19). As with αs1-casein, the hydrophobic region is a random coil.

κ-Casein is the smallest and occurs in two main variants, A and B. κ-Caseins surround αs-casein and β-caseins in the micelles. κ-Caseins have no phosphoseryl clusters, but have tetrasaccharide chains attached to threonyl residues 31, 33, 35, and 36 (variant A only). Its N-terminal is hydrophobic, while the other terminal is polar and has no Asp or Glu residues. At pH 6.6, the residues of the C-terminal plus the carbohydrate chains confer a net negative charge of −16 or −17. The disulfide form exists *in vivo* as there is sulfhydryl oxidase activity in raw milk. This molecule is thus highly amphoteric and plays a crucial role in the coagulation of milk catalyzed by chymosin.

The Whey Proteins

Whey proteins are well-ordered globular proteins. Bovine milk contains about 3% protein, of which 80% is caseins and 20% is whey proteins. Whey comprises a heterogeneous group of proteins that remain in the supernatant after precipitation of caseins; they are characterized by genetic polymorphisms that usually translate into replacement of one or more amino acid residues in their original peptide sequence. Two major types of proteinaceous material can be found in whey: (1) β-lactoglobulin (β-Lg) and α-lactalbumin (α-La) and (2) proteose peptone (derived from hydrolysis of β-casein (β-CN)), small amounts of proteins derived from blood (including bovine serum albumin (BSA) and immunoglobulins (Igs)), and low-molecular-weight peptides derived from casein hydrolysis. Whey proteins have a compact globular structure that accounts for their solubility. These proteins have amino acid profiles quite different from caseins. They have a smaller fraction of Glu and Pro, but a greater fraction of sulfur-containing amino acid residues (i.e., Cys and Met). These proteins are dephosphorylated, easily denatured by heat, insensitive to Ca^{++}, and susceptible to intramolecular bond formation via disulfide bridges between Cys sulfhydryl groups. A brief account of whey proteins is summarized in the succeeding text:

- β-Lactoglobulin (β-Lg) is found with at least four genetic variants, with A and B being the most common. It is the major whey protein in bovine milk and accounts for about 50% of the proteins in whey, but it is not found in human milk. It poses a variety of functional and nutritional characteristics that have made this protein a multifunctional ingredient for many food and biochemical applications. It has been proved an excellent source of peptides with a wide range of bioactivities, such as antihypertensive, antimicrobial, antioxidative, anticarcinogenic, immunomodulatory, opioid, hypocholesterolemic, and other metabolic effects.
- α-Lactalbumin (α-La) has three genetic variants A, B, and C, of which only B is found in cattle of Western breeds. It is a small molecule having four disulfide bonds but these are much less reactive than those of β-lactoglobulin. It has considerable homology with lysozyme: 47 of its 123 residues are identical and others are conservative replacements. α-Lactalbumin is not an enzyme but it acts as a modifier of galactosyltransferase, which synthesizes lactose in the mammary gland. α-La is the predominant whey protein in human milk and accounts for about 20% of the proteins in bovine whey. It is fully synthesized in the mammary gland where it acts as coenzyme for biosynthesis of lactose. It is a good source of the essential amino acids tryptophan and cysteine, which are precursors of serotonin and glutathione, respectively. It has been speculated that the oral administration of α-La could improve the ability to cope with stress. It also improves cognitive functions in stress-vulnerable subjects by increased brain tryptophan and serotonin activity. Based on its high degree of amino acid homology to human α-La, bovine α-La and its hydrolysates are well suited as an ingredient for infant formulas. A few α-La-enriched formulas have already been launched on the market.
- Immunoglobulins (Ig) and bovine serum albumin (BSA) are identical with the proteins found in blood serum and arise in milk by transferring from the blood in the mammary gland during milk production. It provides the neonates with passive immunity before its own immune system is fully functional. Igs are divided into different classes on the basis of their physicochemical structures and biological activities. The major classes in bovine and human lacteal secretions are IgG, IgM, and IgA. The basic structure of all Igs is similar and is composed of two identical light chains and two identical heavy chains. These four chains are joined with disulfide bonds. IgG content of colostrum varies from one animal to the other and also from one breed to the other. IgG accounts for up to 70–80% of the total protein in colostrum, whereas in milk, it accounts for only 1–2% of the total protein. Colostrum-based products have found a growing worldwide market as dietary supplements for humans. Igs link various parts of the cellular and humoral immune system. They are able to prevent the adhesion of microbes and inhibit bacterial metabolism.
- Lactotransferrin (LF) is similar to serum transferrin. It is active both in iron transport and in helping to protect against gut infections in the neonates. It is an iron-binding glycoprotein found in colostrum, milk, and other body secretions and cells of most mammalian species. It is known to confer many antimicrobial, antioxidative, anti-inflammatory, anticancer, and immunoregulatory properties. During the past three decades, it has become evident in many animal and human studies that oral administration of LF can exert several beneficial effects on the health of human beings.
- Lactoperoxidase (LP) is a glycoprotein that occurs naturally in colostrum, milk, and many other human and animal secretions. LP represents the most abundant enzyme in milk and can be recovered in substantial quantities from

whey using chromatographic techniques. It catalyzes peroxidation of thiocyanate anion and some halides in the presence of hydrogen peroxide source to generate short-lived oxidation products of SCN-, primarily hypothiocyanate (OSCN-), which kill or inhibit the growth of many species of microorganisms. In *in vitro* studies, the LP system has been shown to be active against a wide range of microorganisms, including bacteria, viruses, fungi, molds, and protozoa. It is bactericidal against Gram-negative pathogenic and spoilage bacteria and bacteriostatic against many Gram-positive bacteria. The LP system is considered to provide a natural method for preserving raw milk because milk contains naturally all necessary components to make the system functional.

- Enzymes have been identified in milk to a tune of 60. They are important because they are responsible for certain flavor and stability problems, may help protect the neonate against bacterial infection, and can be used to test the efficiency of pasteurization. In particular, there are several proteolytic and lipolytic enzymes that may cause serious degradation of milk components when milk is stored under less than ideal conditions. The enzyme plasmin, which is found in the milk fat globule membrane (MFGM), degrades caseins even at quite low temperatures. Homogenization of milk releases lipase from the MFGM, which can lead to the degradation of fats into fatty acids, giving rancid flavors to the milk.

Milk Lipids

The lipids in milk are secreted from the mammary gland in the form of globules surrounded by a membrane that derives from the plasma membrane of the gland itself. The fat within the membrane consists mainly of triacylglycerols with small amounts of di- and monoacylglycerols, free cholesterol and cholesterol esters, free fatty and acids, and phospholipids. Bovine milk fat is composed of more than 400 fatty acids with different chemical compositions. Most of these fatty acids are esterified to the glycerol molecule backbone and appear in milk fat mostly as triacylglycerols. There are 15 major fatty acids with chain lengths from C4:0 to C18:3; the rest are present at very low concentrations. They have a strong influence on flavor, especially via C4:0-C10:0 fatty acids. The fatty acids are derived either from the plasma lipids of the animal or by de novo synthesis in the mammary gland. The diet of the animal has considerable influence on the fatty acid profile. There is much variability in the triacylglycerols of bovine milk but certain general trends may be seen. The proportion of each species of fatty acid varies with diet and thus is influenced by the season of the year.

Conjugated linoleic acid (CLA) refers to a collection of positional and geometric isomers of cis-9, cis-12-octadecadienoic acid (C18:2) with a conjugated double bond system. The major CLA isomer in milk fat is 9-cis, 11-trans, also called rumenic acid. CLA is formed partially by bioconversion of PUFA in the rumen by anaerobic bacteria and primarily endogenously by Δ9-desaturation of vaccenic acid in the mammary gland of lactating cows. Milk fat is the richest natural source of CLA and contents range from 2 to 53.7 mg g^{-1} fat. A number of studies have confirmed that pasture feeding can increase significantly CLA concentrations as compared to indoor feeding. Also, the grass composition of pasture seems to affect the CLA levels. Feeding cows with meals containing plant oils and/or marine oils can effectively increase the CLA content. Feeding of concentrates rich in linoleic acid (rapeseed, soybean, and sunflower) has a better impact than other polyunsaturated plant oils (peanut and linseed), whereas the dietary fish oils and their combination with plant oils appear even more effective than plant oils alone.

Milk Sugars

Lactose is quantitatively the main milk sugar. It is a disaccharide. It exists in two anomeric forms (α-D-lactose and β-D-lactose) in an equilibrium of about 37.3% α to 62.7% β at 20 °C. It is in inverse relationship with the chloride in milk, making it isosmotic with blood. It may be that evolution has favored lactose over, say glucose, because a given weight of lactose exerts only half the osmotic pressure of the polysaccharide and therefore twice the energy value for a given osmotic pressure. Its concentration is similar in horse, donkey, and human milk, but lower in bovine or other ruminant milk. Other milk carbohydrates, which are free or bounded to lipids, proteins, or phosphate, include a small fraction of oligosaccharides. They are composed of galactose, fucose, *N*-acetylglucosamine, and/or *N*-acetylneuraminic acid (sialic acid) and contain mostly a lactose unit at their reducing end. They modulate the growth of intestinal flora, influence different gastrointestinal and inflammatory processes, and provide protection against bacterial and viral infections. In general, the oligosaccharide level is much lower in animal milk (0.25–0.30 g l^{-1} in goat milk, >0.1 g l^{-1} in buffalo milk, 0.03–0.09 g l^{-1} in bovine milk, and 0.02–0.04 g l^{-1} in sheep milk) compared to human milk (5–10 g l^{-1}).

Vitamins in Milk

Vitamins are physiological, biochemical, and metabolic bioactive compounds occurring in milk. All known vitamins are contained in milk and they have specific biological functions in the body. The vitamin content of milk is highly variable and depends on feeding regime of the dam. The level of water-soluble vitamins is mostly influenced by the feed than the level of fat-soluble vitamins. Table 2 depicts the vitamin content of milk from some nonruminant and ruminant species. Cow milk is the richest source of human dietary requirements of vitamins, particularly riboflavin (B2) and vitamin B12. Goat milk has higher amounts of vitamin A than cow milk. Goat milk supplies adequate amounts of vitamin A and niacin and excesses of thiamin, riboflavin, and pantothenic acid for a human infant. The human infants fed solely with goat milk are oversupplied with protein, Ca, P, vitamin A, thiamin, riboflavin, niacin, and pantothenic acid in relation to the FAO/WHO requirements. Goat milk, however, has a significant drawback of deficiencies in bioactive vitamins, folic acid, and vitamin B12 as compared to cow milk. Cow milk has five times more folate and vitamin B12 than goat milk. Folate is necessary for the synthesis of hemoglobin.

Table 2 Vitamin content (μg/100 ml) in milk of various nonruminants and ruminants

	Nonruminants			*Ruminants*				
Parameters	*Human*	*Horse*	*Donkey*	*Cow*	*Sheep*	*Goat*	*Buffalo*	*Camel*
Water-soluble vitamins								
Thiamine (B1)	14–17	20–40	21–60	28–90	28–80	40–68	40–50	10–60
Riboflavin (B2)	20–60	10–37	30–97	116–202	160–429	110–210	100–120	42–168
Niacin (B3)	147–178	70–140	57–90	50–120	300–500	187–370	80–171	400–770
Pantothenic acid (B5)	184–270	277–300	–	260–490	350–408	310	150–370	88–368
Pyridoxine (B6)	11–14	30	–	30–70	27–80	7–48	25–330	50–55
Biotin (B7)	0.4–0.6	–	–	2–4	0.9–9.3	1.5–3.9	11–13	–
Folic acid (B9)	5.2–16	0.13	–	1–18	0.24–5.6	0.24–1	0.6	0.4
Cobalamin (B12)	0.03–0.05	0.3	0.11	0.27–0.7	0.30–0.71	0.06–0.07	0.3–0.4	0.2
Ascorbic acid (C)	3500–10 000	1287–8100	2000	300–2300	425–6000	900–1500	1000–2540	2400–18 400
Fat-soluble vitamins								
Vitamin A	30–200	9.3–34	1.7	17–50	41–50	50–68	69	5.97
Cholecalciferol (D3)	0.04–0.1	0.32	–	0.3	0.18–1.18	0.25	–	0.3–1.6
α-Tocopherol (E)	300–800	26–11	5.1	20–184	120	–	190–200	21–150
Phylloquinone (K)	0.2–1.5	2.9	–	1.1–3.2	–	–	–	–

Source: Claeys, W. L., Verraes, C., Cardoen, S., De Block, J., Huyghebaert, A., Raes, K., Dewettinck, K. and Herman, L. (2014). Consumption of raw or heated milk from different species: An evaluation of the nutritional and potential health benefits. *Food Control* **42**, 188–201.

Table 3 Mineral contents (per 100 ml) in milk of various mammals

Minerals	*Human milk*	*Buffalo*	*Goat milk*	*Cow milk*	*Sheep milk*
Ca (mg)	33	166	134	122	196.9
P (mg)	43	190	121	119	152.3
Mg (mg)	4	20	16	12	20.2
K (mg)	55	138	181	152	128
Na (mg)	15	65	41	58	52.4
Cl (mg)	60	66	150	100	130
S (mg)	1.33	1.02	2.89	1.12	2.57
Fe (mg)	0.20	0.12	0.07	0.08	0.09
Cu (mg)	0.06	0.024	0.05	0.06	0.04
Mn (mg)	0.07	0.033	0.032	0.02	0.039
Zn (mg)	0.38	0.45	0.56	0.53	0.65
I (mg)	0.007	0.027	0.022	0.021	0.032
Se (μg)	1.52	1.16	1.33	0.96	1.58

Vitamin content of horse and donkey milk is on an average lower than that of ruminant milk. An exception is the level of vitamin C, which is relatively higher in horse milk. Camel milk is known to be a rich source of vitamin C. It is reported to be three to five times higher than that in bovine milk. Hence, raw and fermented camel milk could be a good source of vitamin C for the people living in the desert area where vegetables and fruits are not available. Sheep, goat, and buffalo milk have higher vitamin A content than cow milk. Their milk is whiter than other milk due to their ability to convert the yellow β-carotene to vitamin A, which is chalky white.

Due to differences in milk composition, the effect of heating on the vitamin content slightly differs from one milk type to another. Conventional sterilization causes modest losses in vitamins B6 (pyridoxine), B3 (niacin), B5 (pantothenic acid), B7 (biotin), A, D, and E content. As such, the heat lability of vitamin C is higher in camel than in bovine milk, and upon different heat treatments, higher vitamin losses are reported in bovine milk compared to buffalo milk. However, the effect of heating on milk vitamins seems to be insignificant from a nutritive point of view, since many vitamins are naturally found in relatively low levels in milk.

Minerals in Milk

Ash content is lower in nonruminant milk compared to ruminant milk. Although there are major differences in the mineral content of milk from different species, the concentration of most minerals is higher in donkey and horse milk than in human milk, but significantly lower than in ruminant milk. Milk is mainly a good source of calcium (Ca) and phosphorus (P), which are necessary for bone growth, development, metabolism, and maintenance, but is less important with respect to other minerals. The data on mineral content of milk from various species are presented in Table 3.

Ca is an important nutrient involved in the health of bone. The Ca as a bioactive mineral is demonstrated widely in a range of calcium-fortified foods, including modified milk and beverages. In the diets of developed countries, milk and dairy products provide approximately 70% of the recommended daily intake of Ca. Its role as a protective factor in the etiology of colon cancer has been well documented. It is also believed to be associated with binding and removal of carcinogenic agents along the gastrointestinal tract. The Ca is important for the development and maintenance of skeletal integrity and prevention of osteoporosis. Hypertension is another disease that is related to a low Ca intake. Ca supplementation reduces blood pressure in hypertensive patients. It is also showed that people who ingest diets low in sodium and high in potassium, magnesium, and calcium do not have hypertension and cardiovascular disease. Bovine milk contains about 50% more Ca and twice as much P and potassium (K) than horse and donkey milk, but horse and donkey milk contain about 2–3 times more Ca and P than human milk. Sheep, goat, buffalo, and reindeer milk contain on average more Ca and P than bovine milk, although these levels may vary greatly.

Goat milk contains about 121 mg P/100 g but human milk contains only its one-fourth. Phosphorus exerts several important metabolic functions in the body, including bone mineralization, energy metabolism, fat and carbohydrate metabolisms, body buffer system and formation, and transport of nucleic acids and phospholipids across cell membranes for body cell functioning. In underdeveloped countries, where meat consumption is very limited, goat milk is an important daily food source of P and Ca due to lack of availability of cow milk.

There are a large number of elements in milk at concentrations of less than 1 mg l^{-1}. They come from the foods eaten by the animal and some are essential for health. Deficiencies of trace elements in the soil may lead to dietary deficiencies and require the feeding of mineral supplements. Elements in milk that are important in human nutrition include chromium (glucose tolerance), cobalt (in vitamin B12), copper (enzyme cofactor and hemoglobin formation), iodine (in thyroid hormones), manganese (enzyme cofactor), molybdenum (in xanthine oxidase), selenium (vitamin E and enzyme component), and zinc (in insulin and enzyme cofactor).

Iron contents in goat and cow milk are lower than the iron content in human milk. Iron occurs in milk in combination with several proteins, such as lactoferrin, transferrin, and ferrilactin. Iron also occurs in blood as hemoglobin and transferrin in the plasma in the ratio of 1000:1, and a small quantity of ferritin is present in erythrocytes. Iron deficiency causes anemia and impaired growth and affects lipid metabolism. Goat milk has a greater hemoglobin regeneration efficiency and iron bioavailability than cow milk. Goat milk diet has greater iron and copper apparent digestibility coefficient and bioavailability in different animal organs.

Zinc content in milk is greatest among trace minerals. Zn in goat and cow milk is greater than that in human milk. Its deficiency would result in skin lesions, disturbed immune function, growth retardation, and impaired wound healing. In a feeding trial with rats, animals fed with a goat milk diet exhibited a greater bioavailability of zinc and selenium as compared to those fed with a cow milk diet. Goat and human milk contain higher concentrations of selenium than cow milk. Less than 3% of the total selenium is associated with the lipid fraction of milk. The selenium-dependent enzyme, glutathione peroxidase, is higher in goat milk than in human and cow milk. Goat milk total peroxidase activity is 65% as against 29% for human and 27% for cow milk. Goat and cow milk contain significantly greater levels of iodine than human milk, which may be important for human nutrition, since iodine and thyroid hormone are closely related to the metabolic rate of physiological body functions. There are several other trace minerals that exhibit active bioactive functions in the body metabolism, including Mo, Cr, Co, Mn, F, As, Sn, and V.

Comparison of raw bovine milk with UHT-treated and sterilized bovine milk shows essentially no differences in milk salt and trace element levels. Intestinal absorption and utilization, and thus the bioavailability of minerals, depend on their chemical form. Upon heating, the diffusible salt content decreases due to a shift to the colloidal form, the extent of which is proportional to the severity of the treatment. The majority of changes are reversible on cooling, but more severe heat treatments (>90 °C) may give rise to some irreversible modifications. Not only the chemical form or heating but also other milk components also affect the bioavailability of milk minerals. As such, lactose, lactulose, CLA, vitamin D, and casein phosphopeptides have been suggested as potential enhancers of calcium absorption.

Iron (Fe) is naturally low in milk. Despite a similar Fe content, the Fe bioavailability is higher in goat compared to bovine milk. Goat milk has a higher share of nucleotides that contribute to an enhanced Fe absorption in the intestine. On the other hand, calcium and casein that have on average higher levels in goat milk inhibit the absorption of dietary nonheme iron.

Contaminants in Milk

Some trace materials include pesticide residues, fluorine, silicon, and heavy metals. Most of these come from contamination of the feed or from water:

- Pesticide residues may be present in milk as a result of the cow eating contaminated food. Around 160 (out of 209 theoretically possible) polychlorinated biphenyls (PCBs) have been identified in milk. PCBs are highly lipophilic and accumulate in the food chain; hence, it is necessary to monitor their presence. Several chronic maladies are associated with pesticide residues in foods like testicular dysfunction (leading to infertility in human), immune dysfunction, delayed polyneuropathy, and deterioration of memory.
- Dioxins occur in milk by ingestion of contaminated food or by inhalation. They are considered to be some of the most toxic substances known. They are formed during the manufacture of certain pesticides and on burning at relatively low temperatures of a wide range of man-made materials, including PCBs.
- Radionuclides occur in milk by contamination of the food either naturally or from accidents such as at the nuclear reactors. The principal radionuclides found are caesium-137, strontium-89, strontium-90, iodine-131, and barium-140. Strontium is absorbed into the bone along with

calcium leading to highly localized concentrations of radioactivity. Iodine is adsorbed by the thyroid where it can cause significant radiation damage.

See also: Buffalo Milk; Cheese: Processing and Sensory Properties; Dairy Products: Dietary and Medical Importance; Food Allergies; Goat: Milk; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Sheep: Milk.

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Millets

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Introduction

The millets are small-grained cereals, varying in size between approximately 1/4 and 1/10 that of a wheat grain. Their centers of origin are Africa and Eurasia, and they are still very widely consumed as staple foods and traditional beverages, particularly in Africa, Central and southern Europe, India, and China. Today, millets are cultivated across the world, not only in Africa and Eurasia but also in North and South America and Australia. They are becoming popular in Western diets as components of multigrain foods, primarily because of their generally high content of phenolic phytochemicals and also because they are gluten-free. Millets as nonfood grain are primarily used as poultry and caged bird and game bird feed. Millets are also increasingly being grown as fodder crops.

There are some 13 cultivated millet species. By far, the most important in terms of production are, in descending order, pearl millet, foxtail millet, proso millet, finger millet, teff, and fonio (two species, white fonio and black fonio) (Table 1; Figure 1). Collectively, millets are quantitatively the sixth most important cereal type after maize, rice, wheat, barley, and sorghum. Data on millet production do not seem to be very accurate. According to FAOSTAT, world millet production in 2012 was approximately 30 Mt. However, this figure may well be an underestimate. Information from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), the responsible CGIAR organization, states that pearl millet accounts for 50% of the total millet crop and gives figures for the major producing areas that, in total, are in excess of 24 Mt.

Generally, in developing countries, millets are cultivated by traditional agriculture technologies. However, improved varieties and hybrid-type cultivars are being developed and are replacing the traditional varieties and landraces. The hybrids are much higher yielding if provided with optimum agricultural inputs such as fertilizer, as shown in the case of a hybrid foxtail millet from China (Figure 1(e)). Experimental genetically modified (GM) millet lines have been developed with improved disease resistance, for example, downy mildew-resistant pearl millet. However, at present (2014), all cultivated millets are non-GM.

Although millets account for only some 1–2% of the world's cereal production, pearl millet in particular provides a substantial proportion of nutrients in some of the most food insecure African countries, those encompassing the Sahel (Sahara desert margin) region. In 2009, the five countries that obtained the most energy (kJ/capita/day) from millets were Niger (4389), Mali (2184), Gambia (2176), Burkina Faso (1894), and Chad (1235), equivalent to between 14% and 50% of an adult's energy requirement. These five countries in 2009 were home to 1.9, 1.4, 0.2, 3.8, and 4.1 million malnourished individuals, respectively. Individuals in these countries also obtained a substantial proportion of their protein intake (g/capita/day) from millets: Niger (20.5), Mali (11.3), Gambia (14.7), Burkina Faso (11.6), and Chad (8.3), equivalent to between 15% and 40% of an adult's protein requirement.

Despite their importance, the millets are very inadequately researched and much of the available information is incomplete. For example, the USDA National Nutrient Database for Standard Reference lists four millet products. However, the names of the millet species are not given. This article attempts to provide accurate information, where known, about the structure, macronutrient, micronutrient, phytochemical, and antinutrient composition of the major millet species. Additionally, some of the more important traditional millet-based foods and beverages are examined, with a focus on the effects that processing has on their nutritional value. Lastly, this article looks at the future of millets as sources of essential nutrients and phytochemicals in the diets of people in the less developed and more developed world.

Structures of Millet Kernels

Although millet grains are small, the kernels of the various millet species vary greatly not only in size but also in weight and importantly in structure (Table 2). Pearl millet is by far the largest, with a 1000 kernel weight of approximately 8 g (20% of a wheat kernel), and teff and fonio are the smallest, 1000 kernel weight approximately 0.3–0.5 g. In general terms, the structure of millet kernels (Figure 2(a)) is the same as that of the major cereals, comprising three parts: the endosperm that contains the starch and the prolamin and glutelin storage proteins; the germ that is rich in lipids and albumin and globulin proteins, minerals, and tocopherols; and the bran that is rich in insoluble dietary fiber. Strictly speaking, the aleurone layer, which is rich in minerals and B vitamins, is part of the endosperm, but in practice, it is often removed when the grain is debranned.

Pearl millet and teff are naked kernels, which can be eaten in their entirety as 'whole grain.' Finger, foxtail, and proso millets are utricles, whereby the pericarp is loosely attached to the kernel and is removed during harvesting and processing. Fonio has a tightly attached hull (Figure 2(b)), which must be removed by dehulling prior to consumption. Unfortunately, millet compositional data rarely indicate whether the kernels analyzed were complete with pericarp/hull, or as would be consumed. Additionally, a fact often ignored in general articles and even in scientific literature is that the chemical composition of millet grains can differ greatly between species and even within species. Statements such as 'millet (*sic*) is very nutritious' should be treated with caution.

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Table 1 The major millet species

Generally used English name	*Other common and vernacular names*	*Taxonomy (tribe, genus, species, subspecies where applicable and authority(s))*	*Major producing regions/countries*	*Approximate percentage of world millet production*
Pearl millet	Bulrush millet Cattail millet Babala Bajra/bajira	Tribe Paniceae *Pennisetum glaucum* (L.) R. Br.	West and Central Africa (Niger, Mali, Burkina Faso, Nigeria), India, East and South Africa (Namibia, Botswana), the United States, Brazil	46
Foxtail millet	Italian millet Foxtail bristle grass German millet Hungarian millet	Tribe Paniceae *Setaria italica* (L.) P. Beauv. subsp. *italica*	Eurasia (southern Europe, tropical and subtropical Asia (China, India, Korea)), the United States, Australia	19
Proso millet	Common millet Broom millet Hog millet Panic millet	Tribe Paniceae *Panicum miliaceum* L. subsp. *miliaceum*	Eurasia (China, Kazakhstan, Afghanistan, India, Turkey, Romania), the United States, Australia	17
Finger millet	Ragi Wimbi	Tribe Eragrostideae *Eleusine coracana* L. Gaertn.	East and South Africa (Uganda, Kenya, Zimbabwe) and Asia (Near-East to Far-East) (India, Nepal, China)	13
Teff	Tef Teff grass Abyssinian lovegrass	Tribe Eragrostideae *Eragrostis tef* (Zuccagni) Trotter	Ethiopia, Eritrea, South Africa, the United States, Australia	4
White fonio	Fonio (Acha) Fonio millet Hungry rice	Tribe Paniceae *Digitaria exilis* (Kippist) Stapf	Africa – Sahel region (Guinea, Nigeria) (both species)	1 (both fonio species)
Black fonio	Black acha Hungry rice	Tribe Paniceae *Digitaria iburua* Stapf		

Figure 1 The major millets. (a) White fonio – Nigeria (courtesy of Dr. M. O. Edema (left in photo), Federal University of Technology, Nigeria). (b) Pearl millet – Kenya. (c) Proso millet (courtesy of Forestry Images – Dr. H. F. Schwartz, Colorado State University). (d) Finger millet – Kenya. (e) Teff – Ethiopia. (f) Foxtail millet – China (heads of hybrid foxtail millet around a head of a traditional variety).

Nutrition and Health-Promoting Aspects of the Different Millet Grains

Macronutrients

In millet grains, starch is the major macronutrient and source of energy for nutrition, as in all cereal grains. However, the energy density of the different millets varies slightly (1396–1522 kJ/100 g), with a 100 g portion of millets providing a possible 52–57% of the daily energy requirements of an average adult (Table 2). Finger millet and fonio have lower energy densities, probably due to their lower lipid and protein contents and high fiber contents.

Like other cereals, whole-grain millets contain high amounts of fiber (8–18 g/100 g), and according to most governmental organizations, foods with a fiber content of 6 g/100 g or higher can claim 'high fiber.' The high fiber contents

Table 2 Kernel weight, structure, and macronutrient contents (g/100 g db except where stated otherwise) of the major millet species

Millet species	*1000 kernel weight (g)*	*General kernel structure*	*Energy (kJ/100 g)*	*Protein*	*Lysine (g/100 g protein)*	*Carbohydrate*	*Dietary fiber*	*Total lipids*	*Palmitic acid (16:0)* (% of total fatty acids)	*Stearic acid (18:0)*	*Oleic acid (18:1)*	*Linoleic acid (18:2)*	*Linolenic acid (18:3)*
Pearl millet	8.0	Naked	1499 [56] (1442–1517)	12.4 [26] (6.9–20.9)	2.8 [63] (1.7–6.5)	70 (67–72)	13.8 [100] (8.5–15.3)	5.1 [28] (3.1–8.8)	14 (9–24)	3 (1–8)	25 (20–30)	54 [42] (37–64)	2 [8] (1–7)
Foxtail millet	5.0	Utricle	1467 [55] (1467)	9.6 [17] (6.0–17.0)	1.9 [41] (1.5–2.8)	69 (63–73)	9.4 [68] (9.4)	4.5 [19] (1.6–9.3)	8.6 (7–10)	3 (1–7)	14 (11–16)	69 [29] (65–72)	3 [5] (2–4)
Proso millet	5.0	Utricle	1522 [57] (1522)	12.6 [23] (6.4–16.0)	1.5 [33] (0.8–4.3)	71 (64–76)	13.1 [95] (13.1)	2.6 [24] (1.7–4.1)	9.8 (7–11)	2 (2–3)	21 (18–24)	65 [37] (62–70)	2 [7] (1–3)
Finger millet	2.5	Utricle	1396 [52] (1392–1405)	8.5 [15] (4.9–11.3)	2.9 [65] (2.5–5.5)	75 (73–82)	15.2 [110] (11.7–18.6)	1.4 [8] (1.3–1.6)	23 (23)	2 (2)	51 (51)	21 [12] (21)	4 [2] (4)
Teff	0.3–0.4	Naked	1441 [54] (1388–1536)	10.0 [18] (7.9–12.6)	2.7 [61] (2.0–4.0)	74 (73–77)	8.0 [58] (8.0)	2.1 [12] (2.0–2.4)	14 (14)	4 (4)	24 (24)	39 [18] (39)	6 [3] (6)
Fonio	0.5	Hulled	1409 [53] (1300–1529)	8.4 [15] (5.1–10.4)	2.2 [49] (1.9–2.5)	75 (75)	18.2 [132] (15.7–20.7)	2.6 [15] (1.8–4.5)	No data				

Values are the average (range) of reliable published data.

[ ], Percentage contribution a 100 g portion would make to the RDA for each nutrient (WHO recommendations) of an average-sized (65 kg) adult.

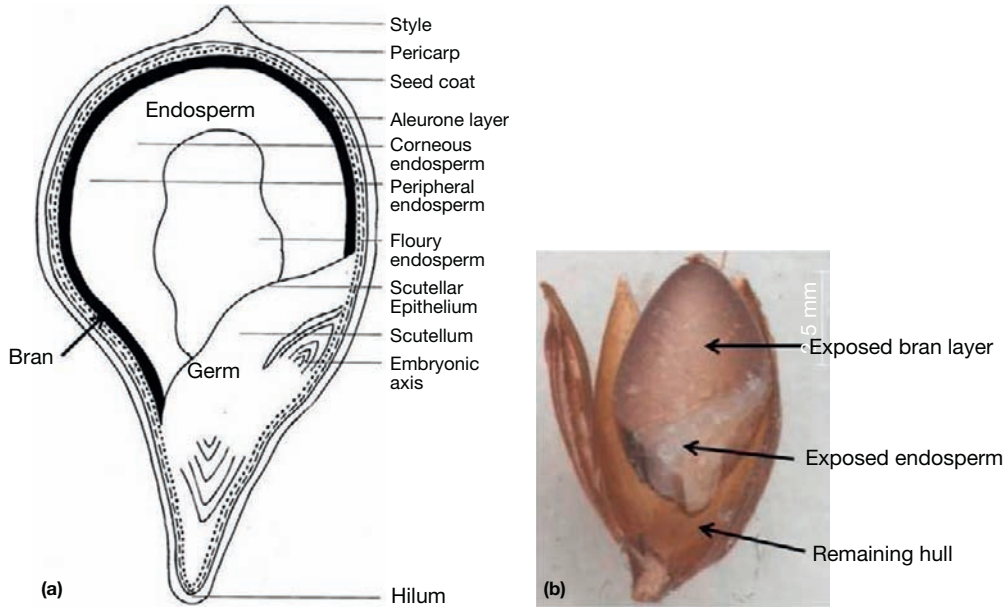

Figure 2 Structure of millet grains. (a) Schematic longitudinal section of pearl millet (courtesy of S. C. Barrion, University of Namibia). (b) Stereomicrograph of partially dehulled and decorticated white fonio (courtesy of Dr M.O. Edema, Federal University of Technology, Nigeria).

of whole-grain millets have also been proposed to contribute to them having a low glycemic index (GI). However, today, there is good evidence that whole-grain cereals in the flour form (as opposed to intact grains) do not have low GI. The millets with the higher fiber contents (pearl, proso, and finger millets and fonio) can, in a 100 g portion, provide between 55% and 66% of the prescribed daily fiber intake, which is associated with health benefits such as reduced risk of cardiovascular disease (CVD), diabetes, and certain cancers.

Millets, like all cereals, have low protein contents compared to legume and animal sources. However, pearl and proso millets have approximately 19–33% higher protein contents than the other millets. A 100 g portion of these two millets can provide approximately 23–26% of the recommended dietary allowance (RDA) for protein, compared to 15–18% of the other millets. This indicates that where millet is consumed as a staple (100 g 2–3 times/day), it could provide a substantial portion (30–80%) of protein RDA. However, millet proteins are not complete, providing only 30–65% of the required lysine, with lysine being the first limiting indispensable (essential) amino acid. The low protein contents of the millet together with the low lysine content of the protein result in millets providing approximately only 9% (foxtail, proso, and fonio millets) to 18% (pearl millet) of the 1.93 g lysine requirement in adults.

There has been an increase in documented cases of celiac disease in both developed and developing countries. This together with the 'fad' trend in Western countries that 'gluten is bad' has resulted in the increased demand for gluten-free food products. Millet grains can be recommended as a safe food for celiac disease patients. The millets are members of the Paniceae and Eragrostideae tribes (Table 1) and hence are only distantly related to the Triticeae cereals like wheat.

According to governmental organizations, most millets qualify to be labeled as 'low fat,' as they contain less than 3 g fat/100 g (1.3–2.0 g/100 g), except for pearl millet and foxtail millet, which can contain up to an average of 5 g/100 g. The lipids in millet are high in both mono- and polyunsaturated fatty acids. The lipids of all the millets (except finger millet) contain high levels of omega-6 fatty acids (linoleic acid), an essential fatty acid. A 100 g portion can provide 20–30% of the recommended intake of this fatty acid, associated with health benefits such as reduced risk of CVD.

Micronutrients

There is considerable variation in the mineral contents of the millets, both within and between species (Table 3). This is caused by inherent (e.g., cultivar and maturity) and environmental (e.g., rainfall, temperature, and soil mineral content) factors. Further, some published data on millet mineral content are suspects. This is related to the fact that as millet grains are so small, the effect of any soil mineral contamination is relatively greater than with the larger-grained cereals. While there are some variations in the average iron and zinc contents of the millets, there is no particular species that has an exceptionally high iron or zinc content. The content of iron and zinc in staple grains is very important as iron deficiency is the most prevalent nutrient deficiency in the world. Also, it is widely agreed that in developing countries, zinc deficiency is as prevalent as and as important as that of iron deficiency. Biofortified high iron and zinc pearl millet has been developed by ICRISAT using conventional breeding. It has a 2.5–3.5-fold increase in iron content and double the zinc content with the highest contents up to 12.4 and 8.4 mg/100 g, respectively. While the increased levels of iron were still within levels previously reported for normal pearl millet and other millets (Table 3), the zinc contents are substantially increased. The biofortified pearl millet has also been found to have improved iron and zinc bioavailability and iron absorption.

Most millets contain very little calcium (19–38 mg/100 g). However, teff has a much higher calcium content (161 mg/100 g), similar to that of the major cereals. Notably, finger

Table 3 Major dietary mineral, vitamin and antinutrient contents (per 100 g db) of the major millet species

Millet species	*Ash (g)*	*Fe (mg)*	*Zn*	*Ca*	*Mg*	*K*	*Na*	*P*	*Vitamin B_1*	*Vitamin B_2*	*Vitamin B_3*	*β-Carotene (μg RE[a])*	*Vitamin E (μg α-TE[b])*	*Phytate (mg)*	*Oxalate (mg)*
Pearl millet	2.8 (0.3–5.1)	9.5 [21] (7.5–11)	2.5 [4] (2.0–3.0)	31 [3] (10–42)	121 [50] (111–300)	468 [13] (440–495)	13 [1] (10–15)	341 (332–350)	0.35 [32] (0.30–0.38)	0.21 [17] (0.19–0.22)	2.7 [18] (2.5–2.8)	0.9 [0.3] (0.8–1.2)	1.1 (0.2–1.9)	585 (180–990)	No data
Foxtail millet	2.6 (1.5–4.3)	8.4 [19] (2.8–24)	1.9 [9] (2.1–5.1)	24 [2] (10–40)	115 [48] (70–140)	335 [10] (270–580)	45 [2] (10–130)	290 (70–360)	0.54 [49] (0.48–0.59)	0.12 [10] (0.11–0.12)	3.5 [23] (3.2–3.7)	No data	3.1 (3.1)	660 (650–700)	27 (25–28)
Proso millet	2.7 (0.8–8.8)	9.4 [21] (2.9–31)	2.1 [10] (1.7–6.8)	19 [2] (8–40)	130 [54] (60–180)	345 [10] (200–590)	35 [2] (10–100)	245 (210–340)	0.52 [47] (0.41–0.63)	0.25 [21] (0.22–0.28)	3.2 [21] (1.8–4.5)	No data	No data	1140 (500–1670)	23 (21–23)
Finger millet	2.7 (2.0–5.0)	8.7 [15] (3.9–18.2)	2.0 [3] (1.5–2)	343 [34] (240–410)	127 [53] (110–140)	438 [12] (314–580)	46 [2] (20–90)	243 (200–280)	0.38 [35] (0.24–0.48)	0.14 [12] (0.11–0.19)	1.1 [8] (1.0–1.3)	No data	2.2 (2.2)	590 (450–700)	29 (29–30)
Teff	2.6 (2.2–2.9)	11.6 [26] (1.5–30)	2.1 [3] (0.7–4.0)	161 [16] (157–180)	177 [74] (168–184)	378 [11] (310–427)	26 [1] (12–46)	384 (350–429)	0.38 [35] (0.30–0.45)	0.18 [15] (0.10–0.27)	2.6 [18] (2.0–3.4)	0.8 [0.3] (0.8)	0.08 (0.08)	682 (528–842)	No data
Fonio	3.4 (1.0–6.0)	11.4 [25] (1.0–55)	2.3 [4] (1.3–3.6)	38 [4] (30–44)	415 [173] (400–430)	160 [5] (160)	20 [1] (20)	195 (160–234)	0.24 [22] (0.08–0.30)	0.14 [12] (0.10–0.26)	2.0 [13] (0.9–3.0)	0.0 [0] (0.0)	No data	514 (413–5480)	No data

Values are the average (range) of reliable published data.

[ ], Percentage contribution a 100 g portion would make to the RDA for each nutrient (WHO recommendations) of an average sized (65 kg) adult.

[a]Retinol equivalence (RE), 1 μg β-carotene = 0.167 μg RE.

[b]α-Tocopherol equivalents.

millet has the highest calcium content (~343 g/100 g) of all cereals. This is important as calcium is thought to play a vital role in reducing the prevalence of rickets, which is still high in developing countries, despite sufficient UV radiation for vitamin D synthesis.

The magnesium contents of all the millets are high, where 100 g can provide more than 50% of the RDA. Importantly, fonio has been reported to have exceptionally high magnesium content (~415 mg/100 g), although this has not been thoroughly investigated. Magnesium is considered as an 'orphan nutrient' as it has been considerably less studied than other minerals such as calcium and iron. However, low magnesium intakes and blood levels (which have been found to be more common than expected) have been associated with type 2 diabetes, obesity, and CVD.

The K:Na ratios of the millets (7:1 and 36:1) are much higher than that of an average Western diet (~1:2). It has been found that increasing the K:Na ratio of foods consumed could decrease the risk of CVD.

The phosphorus in millets, as in all other grains, is largely stored in the form of phytate (*myo*-inositol hexaphosphate), which is an antinutrient. Phytate chelates, especially the divalent minerals (Fe, Zn, Ca, and Mg), which renders them (and the phosphorus in the phytate) insoluble and not bioavailable for absorption. In worst cases, the cause of mineral deficiencies may be actual insufficient dietary intake. However, more frequently, the cause is low bioavailability of nutrients, due to high levels of antinutrients, inhibitors of nutrient absorption. These are mostly found in plant foods and contribute substantially to these deficiencies. While whole-grain millets contain the most minerals, whole grain also contains much higher levels of phytate than refined flour and sometimes also tannins in the case of finger millet (Table 4), which further reduce the already low bioavailability of nonheme iron and zinc. It has also been reported that millets contain some oxalates, which decrease the bioavailability of calcium, iron, and magnesium. However, the documented levels of oxalate in millets are negligible (Table 3) compared with those in dark green leafy vegetables such as spinach and chard.

The millets contain appreciable amounts of some B vitamins, especially vitamin B_1 (thiamine), where a 100 g portion of millets can provide between 20% and 50% of the RDA. A 100 g of millet can also provide 10–15% of vitamin B_2 (riboflavin) and 8–23% of vitamin B_3 (niacin). Some of the millet species have been found to contain β-carotene, but at very low levels, not even contributing to 1% of the RDA. While yellow-endosperm pearl millet has been identified and proposed to have increased levels of β-carotene, very little research has been done. While the information is limited, the millets contain α-tocopherol. As there are no specific recommendations regarding vitamin E intake, it is difficult to comment on the quality of the millets as a source of vitamin E. The data on the α-TE in millets compare well to those of wheat (~1.0 α-TE/100 g).

The challenges in providing adequate macronutrients (protein) and micronutrients (most minerals and vitamins) from a millet-based staple cereal diet are made even worse when the increased nutrient requirements of children and pregnant and lactating women are considered. While the nutrient content of the different millet species differs somewhat, with the

Table 4 Phenolic phytochemicals and health-related aspects of the major millets

Millet species	*Presence of tannins*	*Flavonoids (μg g^{-1})*	*Phenolic acids (μg g^{-1})*	*Some significant phenolics*	*Health-related aspects*
Pearl millet	Probably absent in all varieties	Low levels Soluble – 62 Insoluble – 8	High levels Soluble – 581 Insoluble – 1003	*trans*-Ferulic acid C-Glycosylflavones (vitexin), apigenin, myricetin	Goitrogenic effects
Foxtail millet	Essentially none	Intermediate levels Soluble – 248 Insoluble – 2	High levels Soluble – 628 Insoluble – 1807	*p*-Coumaric acid, *trans*-ferulic acid Catechin, quercetin, apigenin, kaempferol	Antihyperglycemic and hypolipidemic effects
Finger millet	White and red – none Brown – some varieties	High levels Soluble – 2100 Insoluble – 13	Low levels Soluble – 167 Insoluble – 523	*trans*-Ferulic acid, protocatechuic acid Catechin, gallocatechin, epicatechin, epigallocatechin, taxifolin, vitexin, tricin, luteolin, myricetin, quercetin, kaempferol, naringenin, daidzein Procyanidins B_1 and B_2 (some brown varieties)	Antihyperglycemic and anti-inflammatory effects
Proso millet	Essentially none	Low levels Soluble – 54 Insoluble – 34	High levels Soluble – 498 Insoluble – 2082	*p*-Coumaric acid, *trans*-ferulic acid Kaempferol, apigenin, myricetin	Serum cholesterol improvement
Teff	None	No data Higher in brown and red varieties than white varieties	No data Total phenols – 66	*trans*-Ferulic acid, vanillic acid, *p*-hydroxybenzoic acid Quercetin, apigenin, luteolin	
Fonio	None	White fonio – total flavonoids <1 Black fonio – no data but should be higher	White fonio – low levels Soluble – 24 Insoluble – 38	*trans*-Ferulic acid, protocatechuic acid Luteolin, apigenin	

exception of the high calcium content of the finger millet, the millets provide similar levels of macro- and micronutrients compared to other major staple grains.

Bioactives/Phytochemicals

Generally, millet grains are rich sources of phenolic phytochemicals, mainly flavonoids and phenolic acids (Table 4). However, the levels differ considerably between species. Finger millet seems to contain the highest levels of flavonoids. However, the available data are very limited. It is probable that the levels of flavonoids vary considerably between varieties of millet species, particularly pearl millet, finger millet, and teff, with the red and brown pigmented types of these species containing high levels due to the anthocyanin flavonoid-type pigments. With regard to phenolic acids, it appears that pearl millet, foxtail millet, and proso millet contain the highest levels. However, a high proportion of the phenolic acids are insoluble as they are bound in the cell walls and as such may not be bioavailable. Concerning tannins (proanthocyanidins/procyanidins), despite some reports to the contrary, it is probable that finger millet is the only millet that contains tannins and that they are present only in some brown varieties.

Regarding phytochemical-related health-promoting properties of millets, by far, most research has been carried out on finger millet, mainly in India. Although a subject of ongoing research and discussion, there is evidence from human trials that consumption of finger millet foods alleviates type 2 diabetes and its associated conditions. Several reasons have been put forward. Finger millet tannins are powerful inhibitors of digestive amylase enzymes and hence lower glycemic response. The phenolics may also reduce protein glycation, the reactions between glucose and proteins that are associated with, for example, cataracts and kidney damage. Extracts from foxtail millet have also been shown in animal models to exert strong antihyperglycemic effects. Animal feeding experiments have also shown that extracts from finger millet and proso millet can lower serum triglyceride levels and in the case of finger millet also reduce inflammatory responses. The data suggest that consumption of these millets may be protective against CVD.

There is also evidence that phenolics in millets can have negative health implications. It has been found that condensed tannins, as present in some finger millet varieties, have an extremely high inhibitory effect on iron and zinc bioavailability. The inhibitory effect of the tannins can be so high that it completely negates the beneficial effect or reducing/totally removing the phytate from the grain. Consumption of pearl millet as a staple in certain regions of Sudan and India has been implicated as the cause of the high incidence of goiter in these populations. C-Glycosylflavone-type flavonoids, particularly the compound vitexin, a powerful goitrogen, have been proposed as the causal agent. The flavonoids luteolin and apigenin isolated from white fonio have also been shown to exert strong goitrogenic activity.

Millet Foods and Beverages and the Effects of These Processing Methods on the Nutritional and Health-Promoting Aspects

The millets are processed into a very wide range of traditional food and beverage products (Figure 3). These include whole-grain snacks, 'rice,' semolina, couscous, doughs and dumplings,

Figure 3 Traditional millet foods. (a) Pouring injera batter (teff) – Ethiopia. (b) Cooking uji (finger millet) – Kenya. (c) Pearl millet and sorghum couscous agglomeration – Senegal. (d) Foxtail millet and yak milk tea – China. (e) Finger millet porridge – Uganda. (f) Foxtail millet gruel – Taiwan. (g) White fonio rice and peanut sauce – Burkina Faso.

flatbreads, porridges and gruels (nonfermented, fermented, and alkali-cooked), lactic acid-fermented and malted nonalcoholic beverages, malt-based opaque and cloudy beers, and 'wine.' Today, in developing countries, as a consequence of rapid urbanization, an increasing variety of traditional-type millet food products are being manufactured for sale in supermarkets (Figure 4). Also, in Western countries, the market for and production of millets and millet-based products as gluten-free foods (Figure 4(c), 4(f), 4(h)) has expanded greatly over the past decade.

The processing of millets into foods and beverages can greatly change their nutritional value compared to that of the original grain, as is the case with processing any food commodity. Millets, like other cereal grains, are almost invariably debranned (the process being often referred to as dehulling or decortication) in order to improve the palatability of the food. However, debranning substantially reduces the content of insoluble dietary fiber and also reduces the levels of lipids, B vitamins, minerals, and phenolics somewhat. This is because these nutrients and phytochemicals are concentrated in the outer layers of the grain (pericarp, germ, and aleurone). For example, 21%, 12–65%, 72%, and 2% reductions in phenolic content have been reported for finger, foxtail, proso, and pearl millets, respectively, when these grains were 'dehulled.'

Millets are generally cooked by wet cooking (boiling or steaming) but sometimes by a dry cooking technology such as gun-puffing (Figure 4(f)). Both the method of cooking and subsequent cooling can dramatically influence starch digestibility. For example, noodles and biscuits made with finger millet blended with wheat were found to have lower GIs than the products made from wheat alone, whereas finger millet porridge was reported to have a much higher GI than wheat porridge.

In the Sahel region of North Africa, pearl millet flour is used to make a gel-like type of porridge called tô. A common method of preparation involves cooking under alkaline conditions through the addition of wood/millet stalk leachate or lime. Undoubtedly, this leads to a reduction in protein quality, since such conditions result in the formation of amino acid residues such as lysinoalanine and also to intra- and intermolecular covalent cross-links in polypeptides. The high pH could also have a negative effect on mineral bioavailability. Not only does phytate bind more minerals at a higher pH, but also even unbound minerals precipitate out of solution and are unavailable for absorption. Also, with alkaline cooking, there might be large losses of vitamins B_1 and B_2. The effects of cooking millets on phenolics have been observed to be very complex, as both the release of bound phenolics and depolymerization of polyphenols can take place.

The process of making many African traditional millet products such as injera (teff) flatbread, porridges, uji (finger millet-based) gruel, and oshikundu (pearl millet) beverage involves a lactic acid bacteria fermentation. This fermentation can somewhat improve starch and protein digestibility. It may also increase B vitamin content and very substantially reduces phytate content. However, most importantly, the low pH of these lactic acid-fermented products, pH <4, renders them microbiologically safe. This is particularly important as still today, many people in Africa do not have access to safe water. Some of the lactic acid bacteria responsible for these fermentations have been found to be probiotics, viable microorganisms that promote or support a beneficial balance of the microbial population in the gastrointestinal tract. However, currently, there is insufficient evidence as to whether the probiotic organisms confer special health-promoting properties to these traditional lactic acid-fermented products.

Figure 4 Commercial millet foods and beverages. (a) Pearl millet and sorghum couscous – Senegal. (b) Fermented mahangu flour (pearl millet) – Namibia. (c) Pearl millet semolina – Sahel. (d) Instant kunun tsamiya porridge (pearl millet and tamarind) – Nigeria. (e) Uji flour (finger millet) – Kenya. (f) Finger millet and soy flour – Tanzania. (g) Organics Nu-Vit hulled millet – Australia. (h) Puffed foxtail millet – USA. (i) Foxtail millet wine – Taiwan.

In Africa and India, the process of making many traditional millet foods and beverages involves the use of millet malt. Examples are the uji gruel from Kenya and the ragi malt drink in India, both based on finger millet malt. The malting (sprouting) process mobilizes endogenous amylases that liquefy porridges through hydrolysis of the starch to sugars. This improves carbohydrate availability and makes the products sweeter and hence more palatable. Also, importantly, the reduction in viscosity reduces porridge bulk. The high bulk (high water content and sticky paste texture) and consequently low nutrient density of cereal porridges are major factors contributing to infant malnutrition in Africa. Inclusion of malted millet or sorghum flour enables a porridge to be made that has much higher solids content (hence higher nutrient density) but has a sufficiently low viscosity that it can be consumed by infants. The use of malted millet/sorghum flour, referred as power flour or amylase-rich flour in weaning food preparation, has been widely promoted by nutritionists.

Malting also has other nutritional benefits. It improves protein quality, through the synthesis of lysine-rich proteins and making the protein more available for digestion. Additionally, the contents of B vitamins are increased through synthesis, and mineral bioavailability is improved by phytate reduction through the action of endogenous phytases. However, it is important to be aware that malting is a skilled process. Failure to properly control malting conditions such as temperature, moisture, and aeration can easily lead to the proliferation of toxigenic fungi.

Conclusions and Future Directions

The rapid urbanization that is taking place in developing countries, especially in Africa, provides a huge opportunity to develop local food processing industries to meet the needs of people who are now working and living in cities. As explained, with rapid urbanization, many traditional-type millet products are now being produced by small-scale entrepreneurs. The gluten-free market in Western countries also presents a great opportunity for small-holder millet farmers in developing countries. However, the small size of the millet grains remains a challenge, especially with regard to efficient and effective milling to produce high-quality flour for this latter market. In the Sahel region, innovative indigenous technologies have been developed for milling pearl millet and even for fonio, despite it being tiny. Notwithstanding this, there is still a way to go until millet industrial milling technology approaches the level of sophistication as that of the major cereals such as wheat, rice, and maize.

Our knowledge of the phenolic compounds present in millets is increasing rapidly, and it is clear that millets are generally much richer in these phytochemicals than the major cereals. However, the need is now to confirm that these phenolics actually function as nutraceuticals and to establish whether regular consumption of millet foods is preventative against the so-called lifestyle diseases such as type 2 diabetes, CVD, and colorectal cancer. Related to this, the indications that certain flavonoid compounds in millets, especially those in pearl millet, may exacerbate goiter in at-risk communities are serious causes for concern. Research is clearly needed to determine whether regular consumption of such millets actually poses a risk. If so, breeding will be required to substantially reduce the levels of these compounds.

Biofortification of millets, as exemplified by the high iron and zinc pearl millet developed by ICRISAT, is a promising way of improving the nutrition of those who are chronically malnourished and depend on millets as their staple food. However, malnutrition is rarely caused by deficiency of a single nutrient. Thus, there is need to develop millets that are biofortified with other micronutrients, particularly provitamin A, and possibly also to develop types with reduced levels of phytate so as to improve dietary mineral bioavailability.

However, probably the biggest challenge is that millet yields are generally much lower than those of the major cereals. Thus, in developing countries, particularly in India, there is a trend for farmers to switch from cultivating millets as they have done for hundreds years to growing cereals such as maize. An important reason for the lower yields of millets is that they have not been subject to such intensive breeding. The potential of what can be achieved through the development of hybrid lines is shown in the example of foxtail millet. Similar successes have been achieved in many countries with pearl millet. However, for cultivation of millets to expand in developing countries in order to feed their burgeoning populations, the breeding and on-farm implementation of improved open-pollinated varieties and hybrids need to be greatly intensified.

See also: Antinutritional Factors in Legume Seeds: Characteristics and Determination; Antioxidants: Role on Health and Prevention; Bioavailability of Nutrients; Cereals: Dietary Importance; Cereals: Types and Composition; Energy: Intake and Energy Requirements; Fatty Acids: Essential Fatty Acids; Fermented Foods: Composition and Health Effects; Food Allergies; Genetically Modified Foods: Risk Assessment, Legislation, Consumer Behavior, and Ethics; Phenolic Compounds: Bioavailability and Health Effects.

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Relevant Websites

www.fao.org – Food and Agriculture Organization.
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www.icrisat.org – International Crops Research Institute for the Semi-Arid Tropics.

Mineral Water: Sources and Storage

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Drinking water of good quality is essential for human health and sustainable development. One of the most desired kinds of water available for consumers worldwide is mineral water, which comes from springs. They naturally generate water containing minerals or other dissolved substances. These alter the taste or give it a purported therapeutic value. A spring is a place where water issues from the ground and flows or lies in pools that are continually replenished from below. Wholly artificial openings, such as artesian wells, are not regarded as springs. Numerous springs are found around the world and are of diverse characters, from small trickles of water into an old hollowed-out log to large pump lift systems into stainless steel tanks for municipal and industrial use and by the bottled water industry. The location of a given spring is unpredictable. However, the majority are located in mountainous regions and upland areas. The taste and properties of the water are closely connected to the type of soil or geologic structure in which the spring is located. Details of water available in various types of soil are summarized in Table 1.

Springs are classified according to several criteria. One system of classification is based on the magnitude of water flow or discharge. The amount of water that flows from a spring depends on several factors, including rainfall, the size of caverns within the rock, the water pressure in the aquifer, and the size of the spring's recharge basin. Human activities can also influence the volume of water that discharges from a spring. For example, increased groundwater withdrawals can reduce the hydraulic pressure in an aquifer, causing water levels to decline and the spring flow to decrease. Generally speaking, springs have dynamic flows dependent on the climate and hydrogeology, and a spring classified as being a certain magnitude at one time may not continue to flow at that rate at other times. However, it is expected that over an extended period, seasonal fluctuation in flow rate will generate a relatively stable discharge. Moreover, the groundwater table must be deep, with a sufficiently large basin. An example of springs classified by magnitude, used by the Florida Bureau of Geology, is summarized in Table 2. First-magnitude springs discharge the largest amount of water.

Springs may be divided according to temperature into thermal and nonthermal springs. Most nonthermal springs have temperatures that are approximately the same as the mean annual temperature of the air of the region in which they are found. Perennial or permanent springs flow throughout the year. Intermittent or temporary springs flow only during or after rains. Springs due to deep-seated water may be divided into two classes (volcanic and fissure springs), according to their geographic distribution, with respect to localities of volcanic or tectonic disturbance. Volcanic springs are associated with present or past volcanism, while fissure springs in general have a strong and relatively constant flow, not subjected to annual fluctuations. They are usually warm or hot, and many are highly mineralized. They appear to rise along deep fractures, extending far into the Earth's crust. Another classification of springs is those involving shallow water. Springs due to shallow water may be divided into four large groups, according to the character of the rock in which they occur:

- Springs in porous rock
- Springs in porous rock overlying impervious rock
- Springs in porous rock between impervious rock
- Springs in impervious rock

Due to the unique properties of a mineral water taken from a given spring, it is often bottled using a regional name that indicates its location and permits it to be recognizable by consumers, according to the characteristics of the water. Natural mineral waters should not be transported from the source to the bottling plant. In addition, transport from a source to the bottling plant must be in a closed pipeline, made of suitable material (i.e., that will not alter the characteristic properties of the water or cause the water to become contaminated). Equipment used in the filling system must ensure that there is no contamination or alteration of the water prior to closure.

The most common way to transport water-based drinks is in bottles. Bottled water is packaged in either glass or, more commonly, polymer bottles with metallic or plastic caps of a different material, shape, and color. Polymer bottles and containers are made of one or more monomers, involving several additives (e.g., accelerators, catalyzers, stabilizers, antioxidants, coupling agents, and plasticizers). Labels must show an abbreviation indicating the nature of the container. The list of plastics or packaging used for bottling and codes appropriate for recycling factories are summarized in Table 3.

The most common plastic material used for bottling mineral water, soda, and vinegar is polyethylene terephthalate (PET). Other plastics used in the food industry include high-density polyethylene (HDPE) for milk; polyvinyl chloride (PVC) for cooking oil and coffee cups; low-density polyethylene (LDPE) for wrapping films, grocery bags, and sandwich bags; polypropylene (PP) for yogurt cups; and polystyrene (PS) for coffee cups and hot beverage cups.

PET is the material most commonly used to make clear plastic bottles in which water is sold. PET is usually produced by the polymerization of petroleum monomers terephthalic acid and ethylene glycol, using antimony-, titanium-, or germanium-based catalysts. However, PET synthesis without added catalysts is possible. Manufacturers prefer PET because it is relatively safe, transparent, and versatile. Customers choose it for its safety, light weight, resealability, shatter resistance, and recyclability. Up to 100% of a PET package can be made from recycled PET and recycled multiple times.

PE is the most common plastic in commerce. It is a polymer of ethylene, being itself a pair of methylene groups connected together. Catalysts readily combine ethylene into a polymer. The most common catalysts consist of titanium(III) chloride (Ziegler–Natta catalysts) or Phillips catalyst prepared by

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Table 1 Details of water available in various types of soils

Type of soil	*Depth at which water may be available*	*Amount*	*Taste*
Clay	Near the surface	Scanty	Not good
Loose gravel	Lower down in the surface	Scanty	Unpleasant
Black earth		–	Excellent
Gravel		Small and uncertain	Unusually sweet
Coarse gravel, common sand, and red sand		More certain	Good
Red rock		Copious	Good
Flinty rock and foot of mountain		Copious	Cold and wholesome

Table 2 Spring classification by magnitude defined by the Florida Bureau of Geology

Magnitude	*Volume of flow (discharge)*
1	100 cfs or more
2	10–100 cfs
3	1–10 cfs
4	0.223 cfs (100 gpm) to 1 cfs
5	0.0233 cfs (10 gpm) to 0.223 cfs
6	0.0022 cfs (1 gpm) to 0.0233 cfs
7	0.00028 cfs (1 pint min^{-1}) to 0.0022 cfs
8	0.00028 cfs or less

Units: cfs, cubic feet per second; gpm, gallons per minute; pint min^{-1}, pints per minute.
Conversions: 100 cfs = 64.6 million gallons per day; 1 cfs = 646316.9 gal per day; 1 gal min^{-1} = 0.0022 cfs; 1 pint min^{-1} = 0.00028 cfs.

Table 3 The type of plastic or packaging used for mineral water bottling and appropriate codes for recycling factories

Abbreviation	*Name*	*Code*
VE	Glass	–
PET	Polyethylene terephthalate polycondensate	1
PE:	According to the IUPAC polyethylene or poly (methylene)	
HDPE	Polyethylene of high density	2
LDPE	Polyethylene of low density	4
PVC	Polyvinyl chloride	3
PP	Polypropylene	5
PS	Polystyrene	6
PC	Polycarbonate	7
CA	Polyethylene-coated board	7
FEP	Fluorinated ethylene propylene	7
PFA	Perfluoroalkoxy polymer	7

depositing chromium(VI) oxide on silica. Polyethylene is classified according to several categories. The most common forms used by manufacturers to produce bottles are polyethylenes of low and high density (LDPE and HDPE, respectively). HDPE is defined by a density of greater or equal to 0.941 g cm^{-3}, while LDPE is defined by a density range of 0.910–0.940 g cm^{-3}.

PVC is a synthetic resin made from the polymerization of vinyl chloride. Its estimated worldwide demand is second only to PE among plastics, relative to production and consumption. PVC is a relatively lightweight polymer and can be manufactured in both rigid and flexible 'plasticized' forms. Because of these features, the list of its uses is endless. It is found in a wide range of consumer products, such as packaging, cling films, bottles, credit cards, raincoats, shower curtains, and construction materials, such as window frames and indoor pipes. Regrettably, PVC products can leak harmful additives during use and disposal, when they are burn or buried.

PP is a thermoplastic of linear structure, based on the monomer C*n*H2*n*. It is synthesized from propylene gas, in the presence of a catalyst such as titanium chloride. A major advantage of PP, in comparison with HDPE, is PP's higher temperature resistance. This makes PP particularly suitable for items such as trays, funnels, pails, bottles, carboys, and instrument jars that have to be sterilized frequently for use in a clinical environment. PP is a translucent material with excellent mechanical properties and has gradually replaced polyethylenes for many purposes.

PS is a thermoplastic polymer made from styrene monomers. Because of its versatility, it can be rigid or foamed and can be made to possess a number of variant attributes. This is why the material is one of the most widely used plastic materials. Applications include housewares, containers, packaging, glazing and light diffusers, casings, cups, take-out containers, and cassette cases. Polystyrene is also widely used as a structural foam and expanded into beads for packaging and cushioning. Polystyrene plastic bottles are often offered by manufacturers since they are excellent moisture barriers for short shelf-life products, and it has extraordinary optical clarity (in its general-purpose form), significant stiffness in both foamed and rigid forms, low density, and thermal conductivity.

PCs are a group of long-chain, linear, thermoplastic polyesters that are easily worked, molded, and thermoformed. PCs receive their names because they contain a carbonate group in a polymer chain. The presence of the phenyl groups on the molecular chain and the two methyl side groups contributes to molecular stiffness in the polycarbonate. This stiffness has a large effect on the properties of polycarbonate. First, the attraction between the phenyl groups of different molecules contributes to a lack of mobility of the individual molecules. This results in good thermal resistance but relatively high viscosity (i.e., low melt flow) during processing. The inflexibility and lack of mobility prevent polycarbonate from developing a significant crystalline structure. The lack of a crystalline structure (the amorphous nature of the polymer) allows for light transparency. It can be injection molded, blow molded, or extruded and is an ideal engineering plastic with good electrical insulating properties. Thus, it finds applications in electric meter housings and covers, casket hardware, portable tool housings, safety helmets, computer parts, vandal-proof windows, light globes, and the food industry.

FEP is a copolymer of hexafluoropropylene and tetrafluoroethylene. It differs from the polytetrafluoroethylene (PTFE) resins in that it is melt-processable, using conventional injection molding and screw extrusion techniques. FEP can be used

in producing articles having a combination of mechanical, chemical, electrical, temperature, and friction-resisting properties, unmatched by any other material. It is essentially inert. Up to the highest use temperature (200 °C), very few chemicals are known to react chemically with these resins. Those that do include molten alkali metals (e.g., Li, Na, K, Rb, Cs, and Fr), fluorine, and a few fluorochemicals such as chlorine trifluoride (ClF_3) or oxygen difluoride (OF_2). Both readily liberate free fluorine at elevated temperatures. Applications include electronic items such as wire covering and FEP spaghetti tubing, FEP linings for pipes in the chemical industry, aircraft wiring, and glazing for solar energy collectors.

PFA is a fully fluorinated, translucent, slightly flexible polymer, with a low coefficient of friction and outstanding antistick properties. From a structural point of view, it is a copolymer of tetrafluoroethylene (C_2F_4) and perfluoroethers (CF_2F_3OR'), where R′ is a perfluorinated group such as trifluoromethyl (CF_3). This tough material resists stress cracking and attack by nearly all chemicals and solvents. PFA is also relatively temperature-resistant, making it ideal for use in both high- and low-temperature environments. PFA is chemically stable and has excellent dielectric strength.

Several additives may be present in plastic containers. Thus, compounds such as lead, zirconium, antimony, bisphenol A (BPA), and dioxins can be among important contaminants and may present serious health risks to humans. Contamination with metals, leaching from glass and plastic bottles, has been evaluated by many researchers under a variety of treatment conditions, for example, heating, cooling, and sunlight exposure. Glass bottles may contaminate bottled water with trace amounts of Pb (see Table 4), whereas many PET bottles contaminate the water with trace amounts of Sb.

Lead poisoning can be hard to detect. Even people who seem healthy can have high blood levels of lead since signs and symptoms usually do not appear until dangerous amounts have accumulated. Unfortunately, according to the Agency for Toxic Substances and Disease Registry and US Environmental Protection Agency (USEPA), there is no such thing as a 'safe' or 'dangerous' threshold level of lead, only levels that we are willing to accept as tolerable. The signs and symptoms of lead poisoning are highly dependent on individual sensitivity, and hence, specification about dangerous limits is difficult. Blood lead levels as low as 2 μg dl^{-1} may not cause distinctive symptoms but are associated with decreased intelligence and slower neurobehavioral development in the form of cognitive and language deficits. Other effects can begin to occur at these low blood levels. Lead present in drinking water consumed on a daily basis can cause a variety of adverse health effects. Consumption 'on a daily basis' is significant to potential toxicity, because lead not stored in the bones is slowly eliminated by the kidneys and gastrointestinal tract. Negligible amounts are lost via perspiration. The 'half-life' of lead in the body varies from about a month in the blood, 1–1.5 months in soft tissue, to about 25–30 years in bones. The USEPA has set drinking water standards with two levels of protection: the maximum contaminant level goal (MCLG) is 0 μg l^{-1}. This is the level determined to be safe by toxicological and biomedical consideration, independent of feasibility. This, therefore, is the goal for lead content in water. The other rule establishes an 'action' level of 15 μg l^{-1}, which is the maximum allowable content in drinking water. Because the concentration of lead caused by leaching from glass and PET is far lower than the 'action' level established by the USEPA (Table 4), consuming bottled water can be considered safe. About 99% of the lead absorbed by an adult is eliminated shortly thereafter, whereas about 32% is lost by a child in the same period. In babies and children, long-term exposure to lead in drinking water from all sources above the action level can result in delays in physical and mental development, along with learning difficulties, irritability, loss of appetite, weight loss, sluggishness and fatigue, abdominal pain, vomiting, constipation, hearing loss, and slowed growth. Recently, the Centers for Disease Control and Prevention (CDC) identified 10 μg lead per deciliter (dl) of blood, the level of concern in children. However, adverse effects can occur at lower levels than previously thought. This is why current experts use a reference level of 5 μg dl^{-1} to identify children with blood levels that are higher than normal. This reference value is based on the 97.5th percentile of the US National Health and Nutrition Examination Survey concerning blood lead distribution in children. Adults who drink water with elevated lead contents for many years could develop kidney problems, abdominal pain, constipation, joint pains, muscle pain, declines in mental functioning, high blood pressure, headache, memory loss, mood disorders, reduced sperm count, abnormal sperm, and miscarriage or premature birth in pregnant women.

The US Environmental Protection Agency and the Council of the European Communities classify Sb as a serious food contaminant. In cases of acute intoxication, the following symptoms can arise: stomachache, diarrhea, desiccation, muscle aches, shocks, anemic uremia, serious myocardial inflammation, shivering, and death-causing liver necrosis. Some research studies report that a single intake of lemonade, accidentally contaminated with antimony to a concentration of 30 μg ml^{-1} Sb, caused nausea, vomiting, and diarrhea in 150 children. Fortunately, most of the affected children recovered within a few hours, and such Sb concentrations do not occur in drinking

Table 4 Median concentration (μg l^{-1}) of some metals in the same water sold in glass and PET bottles and in the same water sold in green and clear glass bottles

	Median		*Median*	
Metal	*Glass*	*PET*	*Clear*	*Green*
Pb	0.139	0.0102	0.135	0.127
Sb	0.01555	0.326	0.0185	0.015
Ce	0.0178	0.000546	0.0117	0.0182
Al	6.09	0.863	5.17	5.35
Zr	0.05615	0.008515	0.036	0.061
Ti	0.16	0.0294	0.123	0.174
Hf	0.00116	<0.0005	0.00101	0.00109
Th	0.00201	0.000404	0.00132	0.00249
La	0.00843	0.00172	0.00455	0.00841
Pr	0.00173	0.000392	0.00102	0.00153
Fe	3.66	0.871	3.09	4.09
Zn	2.53	0.621	2.27	2.3
Nd	0.00553	0.00393	0.00437	0.00649
Sn	0.0112	0.00424	0.0107	0.0131
Cr	0.209	0.0871	0.138	1.01

water. The concentration of antimony in groundwater and surface water normally ranges from nondetectable to 0.2 $\mu g\,l^{-1}$. Antimony is not likely to occur at significantly higher concentrations in natural waters, except those areas affected by acid mine drainage. Risk to human health, when it occurs, is through ingestion (drinking, cooking, and teeth brushing). Because of the toxic effects of lead and antimony, the maximum allowable limits in mineral water established by the European Union community and selected European countries are 5 $\mu g\,l^{-1}$ and 10 $\mu g\,l^{-1}$ for Pb and Sb, respectively. Slightly higher permissible limits have been established by the USEPA and World Health Organization (WHO) for drinking water (6 and 20 $\mu g\,l^{-1}$ for Sb and 15 and 10 $\mu g\,l^{-1}$ for Pb). Well water with antimony levels higher than 6 $\mu g\,l^{-1}$ may safely be used for bathing, hand washing, and dishwashing. The daily oral uptake of antimony ranges from 10 to 70 μg; however, the total exposure from environmental sources (air and soil) and food/drinking water is low compared with exposure in the workplace. Assuming 20 μg of Sb as an average daily oral uptake of an adult, it is estimated that approximately 38% (about 7.4 μg Sb per day) would come from drinking water, especially when stored in PET bottles. Some scientists consider the leaching of Sb from PET bottles into mineral water could possibly represent a risk to human health. According to data presented in Table 4, the Sb concentration in PET bottles can be about thirty times higher than in glass bottles. However, it is still far below the allowable content established by organizations mentioned earlier. Basing on testing of more than 100 brands of bottled water from Japan, Canada, and several European countries, the observed Sb concentration increased over time and temperature during storage. While Sb leaching is negligibly low (it does not exceed a few % of the total amount of Sb present in the entire plastic bottle) below storage temperatures of 60 °C, above this temperature (i.e., inside automobiles and garages), antimony release can occur rapidly. Moreover, the leaching rate of Sb is generally higher in the case of sparkling mineral waters, compared with the still mineral waters, due to the lower pH. Fortunately, only a very small percentage of the antimony present in PET bottles leaches into the water. For example, when a plastic bottle contains around 250 mg Sb per kg, the leaching rate should not exceed 1 ng Sb per ml after 3 years. Hence, even then, it remains far below the allowable content of Sb in drinking water. Comparing to exposure of the typical urban population to antimony from air, estimated to be between 60 and 460 $ng\,day^{-1}$ per person, the daily intake of 2 l of bottled water, stored in darkness and at room temperature, is fully safe.

In general, water being sold in PET bottles shows a significantly higher concentration of Sb than the same water sold in glass bottles. Nonetheless, glass leaches out a longer list of elements, with trace amounts of Ce, Pb, Al, Zr, Ti, Hf, Th, La, Pr, Fe, Zn, Nd, Sn, and Cr. The majority of these elements have potential toxic effects on humans. However, most toxicity studies have been done on *Drosophila melanogaster*, rats, dogs, or rabbits, since performing the necessary toxicological studies on humans is unethical. Fortunately, the concentrations noted in Table 4 are far from toxic levels (i.e., the toxic dose of lead in pets is 10–15 $mg\,kg^{-1}$, when given as a single dose), and hence, the presence of trace amounts of Ce, Pb, Al, Zr, Ti, Hf, Th, La, Pr, Fe, Zn, Nd, Sn, and Cr in bottled water, according to current knowledge, has no known health significance.

A glass bottle, used as a container for mineral water, comes in five major colors, with clear and green being the prevailing colors. It was found that for several elements, especially for Cr, water stored in the green glass showed a significantly higher concentration than those sold in clear glass. Dark-colored bottles (especially dark green and brown) appear to leach more elements than bottles made of clear glass (see Table 4), due to the elements added to provide the color characteristics to the glass, that is, Fe, Cr, Sn, and REEs (rare earth elements). Iron(II) oxide together with Cr gives bottles a rich green color, while chromium in higher concentrations generates a black color. Chromium together with tin oxide yields emerald-green glass.

As mentioned earlier, inorganic compounds may be present as residues from the catalysts or additives used to produce PET. In addition, some organic compounds can migrate into the water. Formaldehyde and acetaldehyde are the most relevant carbonyl compounds migrating out from PET bottles. These compounds are generated in the thermomechanical and thermooxidative degradation of PET. Despite the fact that phthalates in food are subject to strict regulations, and are not thought to be used in the manufacture of PET, they have been found in PET bottled water.

Diisobutyl phthalate, di-*n*-butyl phthalate, benzyl butyl phthalate, and di(2-ethylhexyl) phthalate have been found in noncarbonated mineral water, with the following concentrations: $<3.0\ ng\,l^{-1}$–$0.2\ \mu g\,l^{-1}$, $6.6\ ng\,l^{-1}$–$0.8\ \mu g\,l^{-1}$, $<6.0\ ng\,l^{-1}$–$0.1\ \mu g\,l^{-1}$, and $16.0\ ng\,l^{-1}$–$1.7\ \mu g\,l^{-1}$, respectively. Up to now, no information is available on any acute and chronic effects in humans from inhalation or oral exposure to di-*n*-butyl phthalate. It has also been detected in some drinking water supplies at levels of 0.1–5 ppb. The MCLG for di(2-ethylhexyl) phthalate, suggested by the US Environmental Protection Agency, is 0 $mg\,l^{-1}$. The current maximum contaminant level (MCL) permitted is 0.006 $mg\,l^{-1}$ or 6 ppb. A person drinking water at or below 5–6 ppb of phthalates, whether briefly, occasionally, or daily for a lifetime, would experience little or no health risk. People who drink water highly contaminated by phthalates for many years may develop liver problems, experience reproductive difficulties, and/or have an increased risk of cancer. Polybrominated diphenyl ethers (PBDEs), in particular decabromodiphenyl oxide (BDE-209), are used as flame retardants in the preparation of both PET and PC plastics, whereas tetrabromobisphenol A (TBBPA) is also used in epoxy and PC resins. Analytic reports on the presence of certain PBDE congeners in plastics, such as BDE-153, BDE-183, BDE-196, BDE-197, BDE-203, BDE-206, BDE-207, and BDE-209, were qualitatively reported in HDPE, polystyrene (PS), PP, and BDE-47 in PET. Although controversial, PET bottles have been designated as a possible source of estrogenic substances.

BPA is released mainly from polycarbonate drinking bottles; however, trace leaching is also observed from PET bottles. It can migrate into water at a similar rate at room temperature from both new and used PC bottles. Exposure to elevated temperatures, above those typically used for washing by consumers, but not outside normal household practice (e.g., boiling to sterilize infant feeding bottles) or outdoor applications (additions of very hot or boiling water or beverages to drinking bottles), greatly elevates the rate of BPA migration. However, the volume of water stored in bottles made

Table 5 The time of storing bottled water in the pantry and in the refrigerator advised by the IBWA

	Pantry	*Refrigerator*
Unopened		
Bottled water lasts for	2+ years	2+ years
Flavored water lasts for	9 months	9 months
Vitamin water lasts for	9 months	9 months
Sparkling water lasts for	1+ years	1+ years
Opened		
Sparkling water lasts for	2–3 h	2–3 days
Bottled water lasts for	2–3 h	3–5 days

from polycarbonate plastic needs to be consumed to reach the tolerable daily intake (TDI) that is estimated at 124 l day^{-1}. In the case of bottles made from PET, the daily consumption required to reach a TDI would have to be even higher, since the migration of BPA from the plastic into water is smaller (does not exceed 24.2 μg l^{-1}).

Bottled drinking water is often stored under unpredictable conditions for prolonged periods before being sold and drunk. The storage time and its conditions will clearly have an influence on how much of an undesirable element or compound leaches into the water. It was found that high-temperature and long-term storage can yield antimony and lead concentrations that approach or exceed the MCLs. In contrast, within a pH range of 6–8, sunlight exposure had small effects on antimony leaching. This is why the International Bottled Water Association (IBWA) advises consumers to store bottled water at room temperature or cooler, away from solvents and chemicals such as gasoline, paint thinners, household cleaners, and dry cleaning chemicals and out of direct sunlight. When water (bottled or tap) is exposed for extended periods to direct sunlight or heat sources, algae or molds may grow in the water. The advised durations for storing bottled water at room temperature or in the refrigerator are noted in Table 5.

Labels on bottled mineral waters are regulated by legislative norms. According to European regulations, there are both mandatory and optional indications:

- *Mandatory indications*: Sales description, volume, the 'best before...' date, storage and use conditions, the name and address of the manufacturer and/or packager, the batch reference, the place where the spring was located, the name of the spring, and the analytic composition for natural mineral waters (mineral content, trace elements, and dry residue) and information on any treatments, among the few permitted (e.g., total or partial elimination of carbon dioxide).
- *Optional indications*: About the properties related to health. All indications attributing properties related to the prevention, treatment, or cure of human illnesses, associated with natural mineral water use, are prohibited, except for indications on a very specific list.

Some indications may be used only when some restrictive criteria are fulfilled. For example, water can only be labeled with the description 'spring water' if it originates from an underground water source, is bottled at the source, and satisfies most of the acquisition conditions, microbiological criteria, and some labeling requirements that apply to natural mineral waters. Similarly, only water recognized as a natural mineral water can be labeled and sold as 'natural mineral water.'

See also: Food Classification and Description; Mineral Water: Types of Mineral Water; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Trace Minerals and Trace Elements; Water Supplies: Water Analysis.

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Mineral Water: Types of Mineral Water

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Undoubtedly, water is one of the most important liquids on Earth. As stated by von Goethe (Faust II, 1833): "Everything comes from water! And everything is kept alive by water!" Water has extraordinary taste properties and provides essential elements such as magnesium, iron, and calcium, which have proven to have therapeutic properties. Because of this, both the production and consumption of various types of bottled water have increased in the majority of countries. For example, in the Netherlands, the nationwide consumption has increased from 5.5 to 15.9 l per person within the period 1980–97, while in Poland, consumption increased from 2.4×10^7 l in 1980 to about 20×10^7 l in 2005.

Bottlers should use standard identifiers, prescribed by regulations (e.g., the US Food and Drug Administration (FDA), the European Commission (European Union), and the Ministry of Health (Poland and Italy)), but their specific meaning may be different from country to country. These terms may refer to both the geographic sources of the water and the treatment methods applied. The most common phrase applied is 'mineral water'; however, in many cases, this phrase is colloquially used to mean any bottled water: artesian, sparkling, still, spring, table, etc.

'Mineral water' definitions are based on two general concepts:

- The concept of a protected source and more specifically to bacteriological quality
- The concept of minimal concentration of dissolved salts

According to the Council of the European Communities, natural mineral water is defined as "microbiologically wholesome water, originating in an underground water table or deposit, and emerging from a spring tapped at one or more natural or bored exits" (Directive 80/777/EEC and 96/79/EEC).

Natural mineral water can, in addition, be clearly distinguished from ordinary drinking water by

1. its nature, which is characterized by its mineral content, trace elements, or other constituents and, where appropriate, by certain effects;
2. its original state.

Both conditions have been preserved because of the underground origin of the water, being protected from the risk of pollution. The composition, temperature, and other essential characteristics of natural mineral water must remain stable at the source, within the limits of natural fluctuation. In particular, these properties must not be affected by possible variations in the flow rate. The chemical composition of mineral waters is determined by the composition of the rock from which it is abstracted. Depending on geochemical processes, however, similar types of rocks may lead to different characteristics in the mineral water.

In some countries (e.g., the United States), the definition of what is a 'mineral water' is based on a minimum concentration of dissolved salts. The FDA classifies mineral water as water containing at least 250 parts per million total dissolved solids (TDS), originating from a geologically and physically protected underground water source (21CFR165.110, 2014). According to European regulations (Directive 80/777/EEC), mineral water has to contain a minimum of 150 mg l^{-1} of minerals. However, local standards differ from country to country. In general, mineral water can be classified, according to its mineral salt content, as water of very low mineral content, low mineral content, and rich in mineral salts. According to Directive 2009/54/EC, some indications and their criteria are summarized in Table 1.

Mineral water is characterized by its constant levels and relative proportions of minerals and trace elements at the source. No minerals may be added to mineral water. The dissolved chemical ingredients of mineral waters are made up as presented in Table 2.

Natural mineral water, in its state at source, may not be the subject of any treatment other than (Directive 2009/54/EC):

1. the separation of its unstable elements, such as iron and sulphur compounds, by filtration or decanting, possibly preceded by oxygenation, in so far as this treatment does not alter the composition of the water as regards the essential constituents which give it its properties;
2. the separation of iron, manganese and sulphur compounds and arsenic from certain natural mineral waters by treatment with ozone-enriched air in so far as such treatment does not alter the composition of the water as regards the essential constituents which give it its properties. . .;
3. the separation of undesirable constituents other than those specified in 1 or 2, in so far as this treatment does not alter the composition of the water as regards the essential constituents which give it its properties. . .;
4. the total or partial elimination of free carbon dioxide by exclusively physical methods.

Mineral water can be put on the market and/or exploited for healing purposes only after the recognition by the appropriate official bodies (e.g., the Ministry of Health, Italy). Main authorization procedures valid in the majority of countries comprise:

- permission of research and regional mining claim;
- evaluation of hydrogeologic, chemical, physical, microbiological, chemico-pharmacological characteristics, and recognition by appropriate official bodies;
- operations authorized by the recognition decree;
- authorization for health claims;
- regional authorization for the use of the water in the manufacture of soft drinks;
- regional authorization for marketing.

Due to increased interest and nationwide consumption, the maximum allowable content of mineral elements in mineral water was established by the European Community and selected European Countries and in drinking water by the

Table 1 Mineral water indications and their criteria

Indications	*Criteria*
Very low mineral content	Mineral salt content, calculated as a fixed residue, not greater than 50 mg l^{-1}
Low mineral content	Mineral salt content, calculated as a fixed residue, not greater than 500 mg l^{-1}
Rich in mineral salts	Mineral salt content, calculated as a fixed residue, greater than 1500 mg l^{-1}
Contains bicarbonate	Bicarbonate content greater than 600 mg l^{-1}
Contains sulfate	Sulfate content greater than 200 mg l^{-1}
Contains chloride	Chloride content greater than 200 mg l^{-1}
Contains calcium	Calcium content greater than 150 mg l^{-1}
Contains magnesium	Magnesium content greater than 50 mg l^{-1}
Contains fluoride	Fluoride content greater than 1 mg l^{-1}
Contains iron	Bivalent iron content greater than 1 mg l^{-1}
Acidic	Free carbon dioxide content greater than 250 mg l^{-1}
Contains sodium	Sodium content greater than 200 mg l^{-1}
Suitable for the preparation of infant food	Sodium or chloride content not greater than 20 mg l^{-1}[a] Fluoride content not greater than 0.7 mg l^{-1}[a] Nitrate content not greater than 10 mg l^{-1}[a] Nitrite content not greater than 0.02 mg l^{-1}[a]
Suitable for a low-sodium diet	Sodium content less than 20 mg l^{-1}
May be laxative	–
May be diuretic	–

[a]Obligatory in Poland.

Table 2 Chemical ingredients present in various types of mineral waters

Common ingredients	
Acids	Carbonic, sulfuric, hydrochloric, silicic, etc., usually in combination
Aluminum	Oxide and sulfate
Calcium	Carbonate, chloride, sulfate, phosphate
Iron	Carbonate, bicarbonate, sulfate, oxide
Lithium	Carbonate, bicarbonate, sulfate, chloride
Magnesium	Sulfate, carbonate, bicarbonate, chloride
Potassium	Carbonate, bicarbonate, chloride, sulfate, phosphate
Silicon	Usually as silica or silicon dioxide, occasionally as the bicarbonate
Sodium	Chloride, carbonate, bicarbonate, sulfate
Gases	Carbonic anhydride or carbonic acid gas, carbon dioxide; hydrogen sulfide or sulfured hydrogen gas; oxygen, nitrogen
Rarer ingredients	
Acids	Crenic and apocrenic, usually as crenates
Ammonium	Nitrate, chloride, crenate
Antimony	Oxide and sulfate
Arsenic	Arsenate of sodium and potassium, arsenious acid
Barium	Baryta or barium oxide and the sulfate
Boron	Biborate of soda or borax
Bromine	Bromides of sodium
Cadmium	Sulfate
Cesium	Sulfate
Chlorine	Chlorides, rarely free
Cobalt	Very rare
Copper	Very rare
Fluorine	Fluorides
Iodine	Iodides of sodium and potassium

United States Environmental Protection Agency (US EPA) and World Health Organization (WHO) (Table 3).

Mineral waters may be gaseous (sparkling) or nongaseous (still). Very often, natural mineral water contains dissolved CO_2. Carbon dioxide may be released spontaneously in noticeable ways under conditions of normal temperature and pressure. Because of this feature, gaseous natural mineral waters are divided into three general categories:

- Natural mineral waters, naturally saturated with CO_2 – for identification of water type, in which the concentration of CO_2 in an underground water table or deposit is equal to the concentration of CO_2 after the separation of unstable elements and bottling; in the case of necessity, the water can be again saturated with CO_2 obtained from the same underground water source, to the same concentration as lost during the processes mentioned in the preceding text

Table 3 Maximum allowable content of mineral elements in mineral water established by the European Union (EU) Community and selected European countries and in drinking water by the United States Environmental Protection Agency (US EPA) and World Health Organization (WHO)

Parameter/component	*EU*[a]	*Italy*[b]	*Austria*[d]	*France*[e]	*Germany*[f]	*England*[g]	*US EPA*[h]	*WHO*[i]
pH	–	–	–	–	–	–	$\geq$6.5 to $\leq$8.5	–
Aluminum ($\mu g\ l^{-1}$)	–	–	–	–	–	–	–	200
Antimony ($\mu g\ l^{-1}$)	5	5	–	–	–	–	6	20
Arsenic ($\mu g\ l^{-1}$)	10	10	–	–	–	–	–	10
Barium ($\mu g\ l^{-1}$)	1000	–	1000	1000	1000	1000	2000	700
Beryllium ($\mu g\ l^{-1}$)	–	–	–	–	–	–	4	–
Boron ($\mu g\ l^{-1}$)	–	5000	–	–	–	–	–	2400
Cadmium ($\mu g\ l^{-1}$)	3	3	3	3	3	3	5	3
Chromium ($\mu g\ l^{-1}$)	50	50	–	–	–	–	100	50
Copper ($\mu g\ l^{-1}$)	1000	1000	1000	1000	1000	1000	1300	2000
Fluoride ($mg\ l^{-1}$)	5	5 (1.5[c])	5	5	5 since 2008	5	4	1.5
Iron ($\mu g\ l^{-1}$)	–	–	–	–	–	–	200	–
Lead ($\mu g\ l^{-1}$)	10	10	10	10	10	10	15	10
Manganese ($\mu g\ l^{-1}$)	500	500	500	500	500	500	–	–
Mercury ($\mu g\ l^{-1}$)	1	1	–	–	–	–	2	6
Nickel ($\mu g\ l^{-1}$)	20	20	20	20	50 20 since 2008	20	–	70
Nitrates ($mg\ l^{-1}$)	50	50 (10[c])	25	50	50	50	10	50
Nitrite ($mg\ l^{-1}$)	0.1	0.1	0.1	0.1	0.1	0.1	1	3
Selenium ($\mu g\ l^{-1}$)	10	10	10	10	10	10	50	40
Thallium ($\mu g\ l^{-1}$)	–	–	–	–	–	–	0.5–2	–
Uranium ($\mu g\ l^{-1}$)	–	–	–	–	–	–	30	30

[a]Directive 2003/40/EC.
[b]DM 29/12/2003.
[c]Legal limit for water intended for infant consumption.
[d]Mineralwasser- und Quellwasserverordnung.
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[h]US EPA (2009).
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Table 4 Classification of therapeutic groundwaters based on their pharmacodynamic factors in Poland

Total dissolved solids (TDS)	*Pharmacodynamic factors – specific components*		*Temperature*
$\geq$1 $g\ l^{-1}$	2 mg F^-	Fluoride water	>20 °C thermal water
	1 mg I^-	Iodide water	
	1 mg S(II)	Sulfide water	
	70 mg H_2SiO_3	Silica water	
<1 $g\ l^{-1}$	10 mg Fe(II)	Ferruginous water	<20 °C cold water
	74 Bq	Radon or radioactive water	
	250 mg free CO_2	Carbonated water	
	1000 mg free CO_2	CO_2-rich water	

- Natural mineral waters enriched with CO_2 from the source – for identification of water type, in which the concentration of CO_2, after the separation of unstable elements and bottling, is higher than determined in an underground water table or deposit
- Natural mineral waters artificially saturated with CO_2 – for identification of water types in which during bottling, water was saturated by CO_2 from an underground water table or deposit or with CO_2 of quality required for food

In the group of sparkling waters, several types are commonly recognized according to the concentration of CO_2:

- Low saturated – below 1500 $mg\ l^{-1}$ CO_2
- Averagely saturated – 1500–4000 $mg\ l^{-1}$ CO_2
- Highly saturated – above 4000 $mg\ l^{-1}$ CO_2

Both concepts used to define 'mineral waters' are applicable for therapeutic mineral waters. They are used in the treatment of disease, either by internal administration or by external application. They owe their virtue to their solid or gaseous constituents or to their elevated temperature. In Poland, several therapeutic groundwaters are defined according to their pharmacodynamic factors (Table 4). Specific criteria may differ per country.

See also: Food Classification and Description; Mineral Water: Sources and Storage; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Trace Minerals and Trace Elements; Water Supplies: Water Analysis.

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Introduction

In food preservation, an increase in the severity of the process usually decreases the quality of the treated food. Consumers are looking beyond the taste and texture of foods and beverages and are asking for safe, healthy, and high-quality foods. In addition, consumers and fast-food establishments require convenience products, for reasons of either cost or labor. Therefore, minimally processed (MP) foods are preferred. These must have characteristics of freshness or 'just been made' and must be microbiologically safe and stable. The minimal processing of foods has two purposes: to maintain and deliver fresh products conveniently without losing its nutritional quality and to ensure that the product has a sufficient shelf life to allow distribution to intended consumers. Moreover, many of these minimal processes aim at being energy saving and environmentally friendly.

MP products are a major growth segment in the local retail food sales. The increasing demand of these MP products poses a challenge for researchers and processors to make them stable and safe. The main initiatives focussed on by the research community and the industry to harmonize these demands without compromising safety include the optimization of traditional preservation techniques to enhance sensorial, nutritional, and microbiological quality of foods and to increase yield and energy efficiency; the development of mild techniques by novel combinations of traditional physical and chemical preserving factors, applied at a low intensity, to obtain products with quality attributes reminiscent of the fresh or native state of a given food but with longer shelf life; and the development of new techniques to obtain innovative foods with fresh quality attributes by using combinations of emerging preservation factors or combinations of emerging factors with traditional ones, all of them applied at low doses.

These new and improved intervention technologies to enhance food microbiological safety have their own set of limitations and advantages. In most cases, the trend for mildly preserved foods comes with the combined preservation/hurdle technology as the principle in designing the overall treatment, that is, the combination of several mild antimicrobial agents in multifactorial processes capable of delivering safe foods with unaltered quality and nutritional value.

The production and manufacture of MP foods nowadays continues being at a stage of innovative dynamics. The aim of this article is to revise the concept of MP foods and to give an overview of mild technologies and minimal processes, emphasizing preservation of freshly cut fruits and vegetables.

The Concept of Minimal Processing and Types of MP Foods

Currently, the MP concept has evolved to give a wider approach than the terms used earlier by Rolle and Chism, Shewfelt, Huxsoll and Bolin, Wiley, and Ohlsson. According to Rolle and Chism, the manipulation and basic preparation of foods, as well as life permanence in the biological tissue, were the elements that distinguish minimal processing from other processes. Shewfelt added to the definition the possible use of low-level irradiation and individual packaging as preservation factors that allow safety and high quality. Examples of MP products presented by Shewfelt included vegetable stick snacks, packaged tossed salads, chilled peach halves, peeled and cored whole pineapple, shelled fresh legumes, microwaveable fresh vegetable trays, and gourmet chilled dinners, among others. The use of refrigeration appears as a fundamental preservation factor for this type of foods. Huxsoll and Bolin included those foods whose tissues are no longer alive, but freshness should be kept as an important characteristic. Wiley presented the modification and control of atmospheres in food packages as other preservation factors to be employed during the MP food elaboration. Ohlsson and other authors mentioned, as an important element, the necessity that this processing should be enough and adequate to maintain the quality of the product during the storage period that goes from preservation to consumption. Manvell, more recently, defined that MP must be a safe balance between desired and adverse effects of preservation processes, and accordingly, a minimal process is 'the least possible treatment' to achieve a purpose. Thus, he considered as minimal processes the available traditional techniques that have been improved to obtain safe and high-quality products (i.e., aseptic packaging, ohmic heating, radio-frequency heating, and microfiltration) and techniques based on emergent preserving factors (i.e., high pressure, ultrasound, and pulsed electric fields). In the same trend, Ohlsson stated that improved thermal technologies can be considered 'minimal' when they minimized quality losses in foods compared to conventional thermal techniques. Lastly, and in partial agreement with these last two authors, Snyder divided MP foods into three groups according to the type and severity of treatment: foods that need no cooking (i.e., raw foods, nuts, grains, and eggs); foods with a mild disinfection or reduction by 10^{-2}–10^{-5} of pathogenic substances, chemicals, and microorganisms by blanching, washing without or with chemicals, etc. (i.e., freshly cut fruits and vegetables and berries); and

pasteurized foods with a 10^{-5}–10^{-7} reduction of pathogens by applying irradiation, heat, pulsed light, ultraviolet light, high pressure, etc. (i.e., *sous-vide* products, pasteurized and refrigerated products, tomato sauces, and salad dressings with preservatives), as well as foods that are formulated to be safe by fermentation, drying, salting, or acidification.

Presently, MP focuses on preservation while keeping food quality attributes, following the principle of 'as little as possible, but as much as necessary.' 'Minimal processing' includes a number of technologies, mostly nonthermal, that are applied to a food at sublethal levels, aiding in the retention of nutritional and sensory quality while guaranteeing safety. Therefore, the MP concept includes a series of products and processes that may be grouped in diverse food categories such as MP, with invisible processing, carefully processed, and partially processed. These terms are not exactly equivalent but reflect the great diversity of processing alternatives for high-quality foods.

The Hurdle Concept

Preservation procedures overcome, temporarily or permanently, the various homeostatic reactions that microorganisms have evolved in order to resist stress. The degree of change in environmental conditions will determine whether the microorganism will lose their viability, become injured, or express adaptive mechanisms that would allow them to survive or even to grow under stress. When stress is sensed by the microorganism, mechanisms are induced by signals to cope with the stressor. These mechanisms involve modifications in gene expression and protein activities. In foods preserved by combined methods (the so-called 'hurdle' technologies), the active homeostasis of vegetative microorganisms and the passive refractory homeostasis of spores are disturbed by a combination of sublethal antimicrobial factors or stressors at a number of sites ('targets') or in a cooperative manner. That is, low levels of different stresses are employed rather a single intensive stress allowing for a less severe preservation procedure and a higher quality in the product. For vegetative cells (where homeostasis is energy-dependent), the goal is to reduce the availability of energy (removing O_2, limiting nutrients, and reducing the temperature) and/or to increase the demand for energy (reducing water activity, reducing pH, and adding membrane-active compounds). For spores (where homeostasis is nonenergetic and depends on the structures of the organism), the goal is to damage key structures (by chemical, enzyme, or physical attack on coats, cortex, etc.) or to release spores from dormancy (initiating germination with natural germinants or with false triggers or applying high pressures).

The hurdle technology approach could be applicable to a wider spectrum of food preservation processes, not only the interference of homeostasis by additive or synergistic hurdles on the same microorganism but also the selective application of preservation factors that may be effective against a specific organism or group of organisms while not against others.

Nowadays, multiple-hurdle applications have aimed toward improving the quality and safety of food products at the farm level and in the industry. The type, number, and intensity of hurdles could be proposed according to the type of food, spoilage and pathogen microorganisms of concern, impact of hurdles on quality, required shelf life, technical compatibility of the selected factors, and/or available processing/storage infrastructure. Overall, multiple disturbances of microbial homeostasis have been used/suggested in food preservation in different arrangements: (a) using two or more stressors simultaneously to prevent the growth of spoilage and pathogenic microorganisms and (b) using one or more stressors (in simultaneity or in sequence) to inactivate/injure or physically remove some microorganisms and then, in sequential mode, one or more stressors to prevent survival/proliferation of remaining refractory or sublethally damaged cells (these last with greater sensitivity to adverse agents). The order in which sequential inactivation stressors are applied has been reported to affect the effectiveness of inactivation.

One of the most difficult aspects of hurdle technology design is the selection of stressors. The combination of antimicrobial factors should deliver not only the promised improvements in quality but also an equivalent or enhanced level of safety compared to traditional preservation methods. The terms 'additive,' 'synergistic,' and 'antagonistic' were traditionally used to describe possible stressor interactions. The current view of hurdle interactions in the growth domain is that their effects are not synergistic except where one hurdle modifies a target to make it more susceptible to a second hurdle. For inactivation of microorganisms, the bases that underlie synergic or additive effects have so far been hardly determined and a delivery of treatments with the same target would improve the chances of irreversible damage being caused.

Preservation Agents in Minimal Processing

Potential hurdles for use in combination in the minimal processing of foods can be divided into physical, physicochemical, microbial-derived, and miscellaneous hurdles. Some MP techniques utilize many of the classic agents to preserve foods, such as high or low temperature, reduced water activity (a_w), reduced redox potential (E_h), mild heat treatment, increased acidity, preservatives, and competitive flora, while others use what could be considered more novel approaches in food preservation, such as pulsed light, shortwave ultraviolet radiation, high hydrostatic pressures, high-intensity pulsed electric field, sound waves, gas absorbers or emitters, aseptic packaging, controlled/modified-atmosphere packaging, ozone, hydrogen peroxide, edible films, and natural antimicrobials. These stressors have diverse modes of action toward microorganisms: They can restrict the access of microorganisms to products, they can inactivate microorganisms, or they can slow down or prevent the growth of microorganisms. Each agent has pros and cons depending on the initial contamination, the nature and topography of food, the mitigation protocol, the type and dose of the stress factors employed in combination, and the order in which they are applied, among others.

Most of the modern agents cause physical or chemical inactivation of microorganisms at ambient or sublethal temperatures. These nonthermal factors are being encouraged for food preservation because, without the need for severe heating, they cause minimal damage to the flavor, texture, and nutritional quality of some foods. Most of them are effective in inactivating vegetative cells of most microorganisms but spores are far more tolerant. Thus, their applications are analogous to

Table 1 Selected nonthermal inactivation stressors for obtaining fresh-like fruit products

Factors and mechanisms of action	*Advantages*	*Limitations and drawbacks*	*Potential application/ products on the market*	*Hurdles in combination investigated*
High hydrostatic pressure (HHP): application of 100–800 MPa, below 0–100 °C, from seconds to about 20 min, instantaneously and uniformly throughout food, independent of size, shape, and food composition Mechanism: multitarget and dependent on pressure level: membrane damage, protein denaturation, leakage of cell contents, and dissociation of ribosomes	Inactivation of some enzymes according to HHP dose Little change in vitamins, pigments, flavor, and antioxidant activity, although effects depend on fruit matrix, pressure, and temperature	High cost of equipment, increased metal fatigue, long cycle times High resistance of browning enzymes and pectin methyl esterase to HHP Undesirable sensory changes at high doses (color, appearance, skin loss, structural/ texture changes)	Jam, jellies, fruit juices and purees, guacamole, fruit yogurts, dairy-based fruit smoothie, sauces (in use since 1990) Shelf-stable low-acid fruits (combined with moderate temperatures)	Low pH Natural and synthetic antimicrobials Temperatures below or above room temperature Refrigerated storage Vacuum packaging and refrigerated storage Mild heating
Shortwave ultraviolet (UV-C) light: radiation from the shortwave ultraviolet region of the electromagnetic spectrum (200–280 nm) Mechanism: damage to DNA, membranes, and enzyme activity induced by UV-C light absorption; hormetic effects	Moderate to low cost of equipment Little effect on color, vitamin C, and taste of fruit juices Little changes in tissue darkening, color, texture, and visual quality of cut fruits at low doses	Low penetration into solids and opaque juices, long treatment times in solids Enzymatic browning of cut fruit surfaces at high doses, more notorious as storage time increases	Pasteurization of apple cider and clear juices (in use since 2000). Surface decontamination of whole and cut fruit surfaces. Reduction of fruit decay and softening	Refrigerated storage MAP Mild thermal treatment US Sanitizers
Pulsed light (PL): few flashes applied in a fraction of a second of intense pulses of broad spectrum light (ultraviolet to the near-infrared region) Mechanism: damage to DNA and destruction of cellular components by the high peak power and the photothermal effects of visible and near-infrared portions of the flash spectrum	Very short treatment times ($\leq$60 s) Little effect on color, texture, and antioxidant and sensory properties at low doses	Low penetration into solids and opaque juices Engineering solutions needed for juice treatment Thermal damage of product at high doses Browning and dehydration of cut fruit surfaces, more notorious as storage time and PL dose increase	Reduction of microbial load on surfaces of whole and cut fruits and in clear juices	Refrigerated storage UV-C Mild thermal treatment
Pulsed electric fields (PEFs): application of oscillating, bipolar, exponentially decaying, or square-wave electric pulses of high voltage (20–70 KV cm^{-1}, pulse duration 1–30 s) Mechanism: perturbation of cell membrane and loss of membrane permeability	Little effect on food quality attributes Very short treatment times ($\leq$30 s)	Restricted to foods that can sustain high electric fields, have low electrical conductivity, and do not have/ provoke bubbles	Limited to pasteurization of fruit juices (in use since 2005) and fruit smoothies	Biopreservatives, essential oils, other antimicrobials Moderate temperatures UV-C PL
High-power ultrasound (US): energy generated by sound waves of 20 kHz or more and intensities higher than 1 W cm^{-2} Mechanism: disruption of cellular structures (wall, membranes, organelles, DNA) and cell lysis attributed to cavitation, shear, localized heating, and free radical formation	Inactivation of enzymes when US is combined with heat and pressure Little change in the color of juices and cut fruits	High energy consumption, intensity of industrial-scale equipment limited, long treatment times Heating of the product. Undesirable sensory changes and rupture of skin in berries at high doses	No commercial fruit products; suggested for juice pasteurization. Actual applications limited to product modification and process efficiency improvements (enhancement of mass and heat transfer, degassing of liquids, cleaning of surfaces)	Moderate temperature and pressure Sanitizers Natural antimicrobials UV-C PEFs

(*Continued*)

Table 1 (Continued)

Factors and mechanisms of action	*Advantages*	*Limitations and drawbacks*	*Potential application/ products on the market*	*Hurdles in combination investigated*
Hydrogen peroxide: application in aqueous solution or in vapor phase Mechanism: strong oxidant agent; intermediate in oxygen reduction to generate more reactive oxygen species; oxidative damage to bacterial proteins, DNA, lipids and cellular membranes; alterations in proteins of spore coats	Effect on a wide spectrum of bacteria, yeast, molds, viruses, and spore-forming organisms Rapid decomposition into water and oxygen No effect on color and surface fruit structure at low doses Inhibitory or lethal action on microorganisms depending on concentration	Activity very dependent on environmental conditions (pH, T, organic material)	Sanitization of fresh fruits (whole or cut)	pH Temperature Refrigerated storage
Ozone: application in gaseous or aqueous state Mechanism: powerful oxidant action attributed to the subsequent reaction of its decomposition products (hydroxyl radical): effect on intracellular enzymes, nuclear material, components of cell envelope, spore coats, and viral capsids	Effective against bacteria (including bacterial spores), viruses, parasites, and fungi at low concentrations Rapid decomposition to molecular oxygen Inactivation of pectin methylesterase, peroxidase, and polyphenoloxidase No color loss or other negative sensorial effect at low doses	Decreases in ascorbic acid and anthocyanin contents at high doses Difficult monitoring and control of concentration Reduced efficacy in presence of organic materials High capital costs Safety problems	Decontamination of fresh fruits (whole or cut), juices, dried fruits Commercial equipment available	Refrigerated storage Carbon dioxide Low pH $UV/H_2O_2/O_3$ UV/O_3 Hot water Blanching

Source: Bermúdez-Aguirre, D. and Barbosa-Cánovas, G. (2011). An update on high hydrostatic pressure, from the laboratory to industrial applications. *Food Engineering Reviews* **3**, 44–61; Gómez, P., Alzamora, S. M. and Welti-Chanes, J. (2011). Hurdle technology in fruit processing. *Annual Reviews in Food Science and Technology* **2**, 447–465.

thermal pasteurization. It is likely that combining nonthermal agents or traditional preservation factors with nonthermal agents in a multihurdle preservation approach will control spoilage and food-borne microorganisms while reducing treatment intensities, detrimental effects on product quality, and energy input.

This contribution does not pretend to cover the impressive number of studies existing in the application of intentional hurdle technology for obtaining MP foods, but focus on some selected examples related to MP fruits and vegetables.

Minimal Processing in Practice: Preservation Techniques for Freshly Cut Fruits and Vegetables

'Freshly cut' can be defined as any fruit or vegetable that has been physically modified from its original form, through several processes such as peeling, slicing, chopping, shredding, coring, trimming, and mashing and washing, to obtain an edible product that is subsequently packaged and stored under refrigeration. These products have been available in supermarkets for sale to consumers since 1930; however, the MP product industry was developed initially to supply hotels, restaurants, and other institutions, since they have a number of advantages, including reduced labor during food preparation, reduced waste and subsequent handling, and reduced preparation time of dishes, with specific shapes of fruits or vegetables.

Generally, MP fruits and vegetables deteriorate faster than their fresh/intact counterparts. In order to choose the right treatments, it is necessary to understand the physiological and microbiological changes that may occur during and after handling, processing, and storage, since these may reduce product quality or even jeopardize its safety. Regarding the deterioration of MP fruits and vegetables, distinctions can be made between physiological deterioration and microbial spoilage, but one definitely influences the other.

From the physiological point of view, minimal processing operations injure the living tissue, which begins a series of metabolic reactions that result in changes in texture, rapid ripening and senescence, development of unpleasant odors, discoloration, and other undesirable events that make products unmarketable. Handling and processing shorten shelf life because they favor a higher production of ethylene, a gaseous hormone involved in plant maturation, which is associated with changes in color and texture of various fruits and vegetables; it also increases the respiration rate, because sugars and acids are used as substrates. From a microbiological spoilage point of view, during peeling and cutting, the product surface is exposed to air and possible contamination from bacteria,

yeasts, and molds. The main sources of contamination are the tools used for processing. In particular, in the case of MP vegetables, most of which fall into the category of low acidity (pH 5.8–6.0), the high moisture and the number of cut surfaces can provide ideal conditions for microbial growth. Microbial growth is characterized by different processes, the production of enzymes and metabolites resulting in visual defects and texture changes, and the development of bad odors. Fruit and vegetable composition will determine the type of spoilage. Finally, peel-removal and sanitizing processes disrupt the normal microbial ecology and allow contamination by pathogenic microorganisms. Pathogens can be present in raw materials or due to cross contamination during processing. Typical sources of microorganisms in primary production are water, soil, or waste waters contaminated with fecal matter. Many pathogens (such as *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Shigella* spp., *Salmonella* spp., *Aeromonas hydrophila*, *Bacillus cereus*, *Yersinia enterocolitica*, and *Campylobacter* spp., parasites, and viruses) have been isolated from MP vegetables and fruits, which serve as carriers of several diseases. Multiple intervention methods that can be sequentially applied aiming to achieve decontamination in produce and the freshly cut produce industry. They include physical cleaning, temperature, chemical-based cleaning treatments, emerging inactivation factors, and modified atmospheres, among others. The efficiency of each intervention practice can be further improved in a combined approach. **Table 1** shows selected chemical and physical inactivation hurdles (already used industrially or still in development or testing) along with the information available about their mode of action on microorganisms, their advantages and disadvantages, and the combined processes in which they have been applied to preserve whole and freshly cut fruits as well as some fruit products.

Future Trends

There is no doubt that MP foods are a growing segment in the food retail industry. Key points for a widespread commercial use include a more in-depth knowledge of microbial behavior at molecular and physiological levels, the availability of systematic kinetic data on the impact on quality attributes (with special emphasis on dose response and the influence of critical process parameters), and the optimization of equipment.

See also: Consumer Protection Legislation; Controlled Atmosphere Storage: Applications for Bulk Storage of Foodstuffs; Controlled Atmosphere Storage: Effect on Fruit and Vegetables; Essential Oils: Isolation, Production and Uses; Food and Agriculture Organization of the United Nations; Pineapple; Plums and Related Fruits; Preservation of Foods; Preservatives: Food Use.

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ENCYCLOPEDIA OF FOOD AND HEALTH

EDITED BY Benjamin Caballero, Paul M. Finglas and Fidel Toldrá

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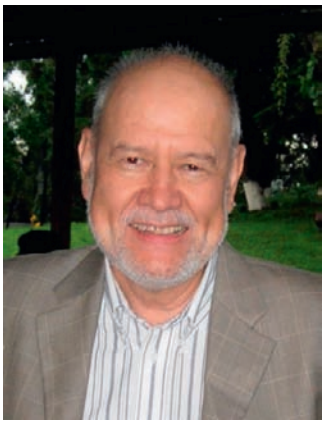

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Prof. Caballero has focused his research on child nutrition and health in developing countries. In particular, he has explored the combination of undernutrition and overweight that has become increasingly prevalent in low- and middle-income countries. He was a member of the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences, USA, and of a number of expert panels created by the Institute, including the Dietary Reference Intakes (DRI) Committee, the Expert Panel on Macronutrient Requirements, and the Childhood Obesity Task Force. He was also a member of the Dietary Guidelines for Americans Advisory Committee, of the Scientific Advisory Board of the Food and Drug Administration (FDA), and of a number of advisory committees of the National Institutes of Health (USA).

He is the editor-in-chief of the *Encyclopedia of Food Sciences and Nutrition*, a 10-volume work on food production, consumption and biological effects. He is also editor-in-chief of the *Encyclopedia of Human Nutrition*, which received the Book of the Year Award from the British Medical Association. His *Guide to Dietary Supplements* summarizes the current scientific basis for the use of mineral and vitamin supplements. His book *The Nutrition Transition: Diet and Disease in the Developing World* explored the impact of demographic and economic development on diet- and lifestyle-related diseases in developing countries. His book *Obesity in China* summarizes research conducted in rural and urban China to track the impact of socioeconomic development on health outcomes. He is also coeditor of a widely used textbook on human nutrition, *Modern Nutrition in Health and Disease*.

He is a member of the Spanish Academy of Nutritional Sciences, and a Fellow of the American Society for Nutrition and of the Royal Society of Medicine (UK). Recent awards include the Donald Medearis Lectureship from the Massachusetts General Hospital/Harvard Medical School, the Mataix Prize for lifetime achievements in nutrition science from the Spanish Academy of Nutritional Sciences, the Ancel Keys Prize for achievements in international public health, and the Thompson–Beaudette Lectureship from Rutgers University.

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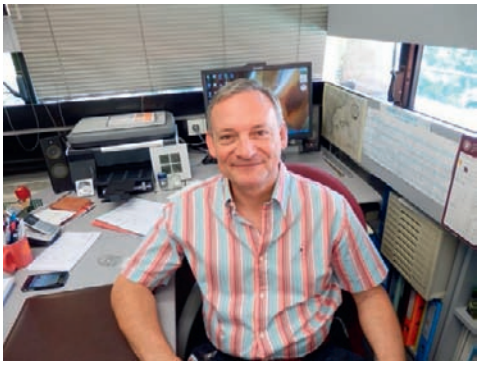

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Prof. Toldrá has focused his research on food biochemistry and its relationship with nutrition, quality and safety. He has filed 12 patents, directed 22 PhD thesis and published over 245 manuscripts in recognized scientific journals and more than 115 chapters of books. His h-index is 41. Prof. Toldrá has authored two books and edited/co-edited more than 30 books for major publishers like CRC Press, Wiley-Blackwell, Elsevier and Springer.

Prof. Toldrá is the European editor of *Trends in Food Science and Technology* (2005–) and associate editor of *Meat Science* (2014–); he was the editor-in-chief of *Current Nutrition & Food Science* (2005–2012), section editor of the *Journal of Muscle Foods* (2009–2010) and guest editor of 12 special journals issues. He is a member of the editorial boards of *Food Chemistry*, *Food Analytical Methods*, *Journal of Food Engineering*, *Journal of Food and Nutrition Research*, *The Open Nutrition Journal*, *The Open Enzyme Inhibition Journal*, *Recent Patents in Agriculture*, *Food and Nutrition*, *Food Science & Nutrition* and *Current Opinion in Food Science*.

He has been a member of the Scientific Panel on food additives, flavorings, processing aids and materials in contact with foods (periods 2003–2008) and the Scientific Panel on flavorings, enzymes, processing aids and materials in contact with foods (2008–2015) of the European Food Safety Authority (EFSA) acting as Chairman of the Working groups on Irradiation (2009–2010), Processing Aids (2011–2014) and Enzymes (2010–2015). He was a member of FAO/WHO group of experts to evaluate chlorine-based disinfectants in the processing of foods (2008–2009). He was a member of the Executive Committee of the European Federation of Food Science and Technology (EFFOST, 2002–2009). He is a Fellow of the International Academy of Food Science and Technology (IAFOST, 2008) and of the Institute of Food Technologists (IFT, 2009–). He received the Iber Award on Food and Cardiovascular Diseases (1992), the Institute Danone award in Food, Nutrition and Health (2001), the International Prize for Meat Science and Technology from the International Meat Secretariat (2002), GEA award on R+D activity from the Valencian Community (2002), and the Distinguished Research Award (2010) and Meat Processing Award (2014), both from the American Meat Science Association.

EDITORIAL ADVISORY BOARD

Siân Astley has worked extensively with individuals and organizations throughout Europe from a variety of disciplines including research, food and biotech industries, and the media. She is the author of more than 300 popular science articles for magazines and trade publications as well as 25 peer-reviewed papers, and she was awarded her Diploma in Science Communication in 2009 (Birkbeck University of London). After 14 years as a bench-scientist, Siân became communications manager for NuGO, one of the first FP6 networks of excellence, and was the European communications manager for the Institute of Food Research in Norwich (UK) until April 2012. Currently, she is the training and communications manager for the European Food Information Resource (EuroFIR AISBL) supporting training within EU-funded research projects and networks, and communication of research activities.

David J. Baer is a supervisory research physiologist with the US Department of Agriculture's Beltsville Human Nutrition Research Center located in Beltsville, Maryland. He serves as the research leader for the Center's Food Components and Health Laboratory and also serves as the director of the Center's Human Studies Facility.

Dr. Baer conducts controlled dietary intervention studies to investigate the relationship between diet and the risk for chronic degenerative diseases, especially cardiovascular disease, cancer, and diabetes in people. His research also includes studies on the health impacts of weight gain and determining the calorie content of foods. Some of the dietary interventions he has investigated include the effects of different types of protein, fats and fatty acids, fiber, margarine, butter, plant sterols, salad dressings, nuts, whole grains, berries, alcohol, and tea on overall nutrition and health. In addition to dietary intervention studies, Dr. Baer is involved in research studies to validate food survey methodologies and in developing new methods for dietary assessment.

Dr. Baer earned his bachelor's degree from the University of Illinois and his master's and doctorate in nutrition from Michigan State University.

Marina Carcea was awarded a master degree in agricultural sciences at the University of Pisa, Italy "cum laude" in 1980, and a PhD in food science also "cum laude."

She is currently a senior researcher in the Research Center on Food and Nutrition of the Council for research in agriculture and analysis of agricultural economy (CRA-NUT formerly INRAN National Research Institute on Food and Nutrition) and she was the director of the Cereals Research Programme in INRAN. CRA-NUT is a primary research institute in Italy under the egis of the Ministry of Agriculture. Dr. Carcea joined INRAN in 1989 after having worked in Italian and English universities (Queen Elizabeth College, King's College, and University of London) and after a two-year contract with the Food and Agriculture Organization (FAO) of the United Nations (UN), Rome.

She has a vast experience in the field of research on foods, cereals in particular. In recent years, her main research interests have been: chemical characterization and study of the functional properties of cereal components; study of the interactions between components and of the interrelationships between the biochemical properties of components and the technological properties of the raw material and derived products; development of new, cereal-based products; development of methods to assess technological parameters of the raw material; nutritional value of cereals; and developments of protocols for quality assurance of cereals, food authenticity.

She has taken part and/or co-ordinated several research projects within national or international programs (European Commission, FAO) involving several institutions. She is the author of more than 160 scientific publications, mostly in international journals, eight book chapters, and two scientific books. She delivered lectures on her research activity at about 150 national and international congresses and she seats in several national and international committees (Italian Ministry of Agriculture, Codex Alimentarius, and European Commission) regarding food and nutrition topics. She is also a member of the editorial board of scientific journals.

From 1994 to 2006, she has also been a lecturer of food science and technology at the University of Tor Vergata, Rome, Italy.

She is a founding member of AISTEC, the Italian Association of Cereal Science and Technology. Since 1996, she is an elected member in the Executive Committee of the same association and since 2009, president of the association.

Since 2000, she is the Italian National Delegate of the International Association for Cereal Science and Technology (ICC) and she was also the president of the same association for 2011–2012.

In 2004, she was the first woman to be awarded the International Harald Perten Prize for her excellent research achievements in the field of cereal science and technology.

She is also a member of the Georgofili Academy in Florence, Italy.

Lawrence J. Cheskin graduated from Dartmouth Medical School and completed a fellowship in gastroenterology at Yale–New Haven Hospital. He is an associate professor of health, behavior, and society at the Johns Hopkins Bloomberg School of Public Health, with a joint appointment in International Health–Human Nutrition, and in medicine (GI) at the Johns Hopkins University School of Medicine. Dr. Cheskin is also a founder and director of the Johns Hopkins Weight Management Center, a comprehensive treatment program for obesity.

In his research, Dr. Cheskin has studied the effects of medications on body weight, the gastrointestinal effects of olestra, how cigarette smoking relates to dieting and body weight, and the effectiveness of lifestyle and dietary changes in weight loss and weight maintenance.

He is also the author of four books: *Losing Weight for Good*, *New Hope for People with Weight Problems*, *Better Homes and Gardens' 3 Steps to Weight Loss*, and *Healing Heartburn*. Dr. Cheskin has appeared on television news programs and lectured to both professional and lay audiences on the topics of obesity and weight control.

Nigel Cook is a graduate of the University of Dundee. After postdoctoral research in the Universities of Aberdeen and Leicester, he moved to the Central Science Laboratory (now the Food and Environment Research Agency (FERA)) at the Food Science Laboratory, Torry, Aberdeen in September 1994, before relocating to new facilities in York. At FERA, he studies the transmission of pathogens, particularly enteric viruses, through foods and the environment. He has a visiting professorship at the Katholieke Universiteit Leuven in Belgium. He is a councilor of the International Association for Food and Environmental Virology. He is a project leader within the standardization working group ISO TC34 SC9 WG6, currently developing a standard for detection of *Cryptosporidium* and *Giardia* on berry fruits and leafy green vegetables. He was a coordinator of the European Framework 7 project "Integrated monitoring and control of foodborne viruses in European food supply chains (VITAL)," and a chair of COST Action 929 "A European Network for Environmental and Food Virology" from 2006 to 2010. Between 2009 and 2014, he was a member of various European Food Safety Authority's Working Groups preparing opinions on the risk of foodborne viruses, and represented the European Communities on the Codex Committee on Food Hygiene Working Group developing *Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food*. He was a member of the UK Advisory Committee on the Microbiological Safety of Food's Viral Infections Subgroup. He was the founding editor of the journal *Food and Environmental Virology*, published by Springer.

Luca Simone Cocolin graduated in 1994 in food science with a grade of 110/110 and remark followed by food biotechnology PhD studies from 1995 to 1998. In February 1999, he defended his thesis acquiring the title of PhD in food biotechnology. From 1998 to 2001, he received a scholarship from the Friuli Venezia Giulia region (Italy). From November 1, 2001, he was an assistant professor at the University of Udine, Faculty of Agriculture, Food Science Department, Italy, and in October 1, 2006, he became an associate professor at the University of Torino, Italy. In January 2014, he had the habilitation for full professor and from June 2015, he is the full professor in food microbiology at the University of Torino.

From September 2008, he is an executive board member of the International Committee on Food Microbiology and Hygiene (ICFM) part of the International Union of Microbiological Societies (IUMS) (http://www.icfmh.org/). From January 2008, he is the editor-in-chief of the *International Journal of Food Microbiology* and he is a member of the editorial board of *Applied and Environmental Microbiology*, *Food Analytical Methods*, *Frontiers in Food Microbiology*, and *Frontiers in Nutrition and Food Science Technology*. He regularly reviews paper for *Food Microbiology*, *Meat Science*, *Journal of Applied Microbiology*, and *Letters in Applied Microbiology*. He is a co-author of more than 180 papers on national and international journals and he attended national and international congresses with oral and poster presentations. On Scopus (www.scopus.com, consulted on March 2015) he has 172 documents reviewed, which were cited 3520 times, resulting in an h index of 33.

His scientific interests comprise:

development, optimization, and application of molecular methods for the detection, quantification, and characterization of foodborne pathogens;
study of the microbial ecology of fermented foods (mainly sausage, cheese, and wine) by using culture independent and dependent methods;
bioprotection: molecular characterization of bacteriocin production and its study *in vitro* and *in situ*;
selection of new putative probiotics from artisanal fermented foods; and
study of the human microbiome and its influence on human health.

Christopher Duggan, for the past 25 years, has been performing clinical trials in the fields of pediatric nutrition, gastroenterology, and global health. His early work centered on the management of diarrheal diseases in children, including trials that demonstrated the feasibility and efficacy of oral rehydration solutions (ORS) for diarrhea management in the United States and globally. In collaboration with colleagues at Harvard TS Chan School of Public Health and Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania, Dr. Duggan and colleagues are evaluating the efficacy of micronutrient supplementation in infants and young children born to women with or at risk of HIV infection. Recent studies include the development of new biomarkers of environmental enteric dysfunction as well as the evaluation of nutritional status on neurodevelopment. With colleagues at St John's Research Institute in Bangalore, India, he is evaluating the efficacy of maternal vitamin B12 supplementation on biochemical and clinical parameters during pregnancy and infancy. He is a course co-director of the Bangalore, Boston Nutrition Collaborative (http://bbnc.globalhealth.harvard.edu). Past and present research support has come from the National Institutes of Health, the Gates Foundation, and the World Health Organization.

In addition to his global health research interests, he is a pediatric gastroenterologist and a nutrition physician at Boston Children's Hospital where he directs the Center for Nutrition (http://www.childrenshospital.org/nutrition). He is a medical director of the Center for Advanced Intestinal Rehabilitation, one of the largest centers in the United States for the care of children with intestinal failure/chronic diarrhea syndromes (http://www.childrenshospital.org/cair). He is also the course co-director of an inaugural Harvard College course "Nutrition and Global Health" and mentors undergraduate, graduate, and postdoctoral students at HMS and HSPH (http://www.hsph.harvard.edu/nutrition-and-global-health/).

He is a professor of pediatrics at Harvard Medical School and a professor in the Department of Nutrition at the Harvard TS Chan School of Public Health (http://www.hsph.harvard.edu/faculty/christopher-duggan/).

Jed William Fahey's current research concerns elucidating the mechanisms of how plants protect themselves against unfavorable and stressful conditions, and how this understanding can be translated to chemoprotection of eukaryotic mammalian systems. This work draws on elements of natural product chemistry, enzymology, nutritional epidemiology, and clinical research in order with isothiocyanates (e.g., sulforaphane) and glucosinolates. His work led to the discovery that broccoli sprouts are an exceptionally rich and consistent source of phytochemicals that induce the detoxification of carcinogens, and to the development of methods for their detection and for assessing their metabolism in humans. He discovered that one of the inducers, sulforaphane, has potent antibiotic activity against *Helicobacter pylori*, a causative agent of peptic ulcer and stomach cancer, and followed up with trials in animals and in *H. pylori*-infected humans. Ongoing collaborations examine the effects of broccoli, Moringa, and the other plants and their phytochemicals against a range of chronic diseases. Dr. Fahey has for years taught courses in chronic disease prevention and nutrition at both medical and public health schools.

Manuel Franco is an associate professor at University of Alcalá in Madrid (Spain) where he leads the social and cardiovascular epidemiology research group (http://www3.uah.es/cardiosocialepi/). He is also an adjunct professor at Johns Hopkins University (Baltimore).

Prof. Franco's work focuses on the social determinants of cardiovascular diseases and its major risk factors as diet. His methodological interests include the measurement of the urban environment and large social and economic changes in relation to cardiovascular health. He is the lead investigator of the Heart Healthy Hoods, study funded by the European Research Council, that will study the urban environment in relation to cardiovascular health in Madrid (http://hhhproject.eu/). This longitudinal study will be collecting neighborhood level data (via audits, Google Street view, photovoice, and qualitative methods) and linking them to clinical outcomes collected from patients enrolled at the City of Madrid primary healthcare clinics. Prof. Franco trained in Spain and Germany to obtain his MD and obtained his PhD from Johns Hopkins Bloomberg School of Public Health working with Dr. Ana Diez-Roux in the MESA study on food environment and dietary patterns. He has published over 30 international high impact articles and collaborates with universities in the United States, Europe, and Latin America.

Maria Glibetic is a research director of Centre of Research Excellence in nutrition research, Institute for Medical Research in Belgrade, University of Belgrade, Serbia, and member of executive board of directors for food data association EuroFIR AISBL. She is an experienced basic and nutritional scientist with over 250 scientific publications and presentations. Maria has considerable experience in leading national and international projects and since 2006, she participated in nine EU funded projects including EuroFIR, EURRECA, BaseFOOD, CHANCE, BACCHUS, and ODIN. Maria and her team are responsible for the creation of the first online national food database, for designing food data management system, and for the development of different nutritional tools for intake analysis. She was a principal leader of many nutrition intervention studies evaluating the plant bioactive component effects on human cardiovascular health. She leads postgraduate department for integrated nutritional sciences at University of Belgrade, where she teaches two courses.

Linda Harvey obtained her PhD from the University of East Anglia, UK. She is currently the head of the Human Nutrition Unit at the Institute of Food Research, Norwich, UK. Her research interests include micronutrient requirements, bioavailability, and metabolism.

Ronald Jackson received his bachelor's and master's degrees from Queen's University and doctorate from the University of Toronto. His time in Vineland, Ontario, and subsequent sabbatical at Cornell University, redirected his interest in *Botrytis* toward viticulture and enology. As part of his teaching duties at Brandon University, he developed the first wine technology course in Canada. For many years he was a technical advisor to the Manitoba Liquor Control Commission, developing sensory tests to assess candidates of its sensory panel, and was a member of its external tasting panel. He is the author of *Wine Science: Principles and Applications, 4th edition* (2014), *Wine Tasting: A Professional Handbook, 2nd edition* (2009), *Conserve Water, Drink Wine*, and chapters and technical reviews in other multiple books and encyclopedia. He is retired in Bronte, Ontario, but remains active writing, cycling, doing yoga, and traveling, as well as being a fellow in the Cool Climate Viticulture and Oenology Institute, Brock University, St. Catharines, Ontario, Canada.

Joe P. Kerry is a senior college lecturer and the head of the food packaging research group in the School of Food and Nutritional Sciences at University College Cork (UCC). He received his doctorate in microbiology at University College Galway in 1995. Prof. Kerry is also a qualified member of the Institute of Packaging. He is very involved in national and international research projects both at fundamental and applied levels. Primary research interests address various aspects of food packaging, shelf-life stability, food composition, and numerous aspects of food quality, particularly in relation to muscle foods. He also has very strong links with industry and his research team assists companies in relation to many aspects of new food product development. He has over 220 publications in peer-reviewed international journals, over 300 presentations at major international conferences, along with several other significant publications. His expertise includes use and manipulation of modified atmosphere packaging systems for use with foods, use of extrusion technology for the manufacture of food products/packaging materials, and applications and sensor/new technology developments within the area of food packaging, especially in the area of smart packaging.

Frédéric Leroy, after studying bio-engineering at Ghent University, obtained a PhD in applied biological sciences at the Vrije Universiteit Brussel in 2002, where he continued his academic career at the research group of Industrial Microbiology and Food Biotechnology (faculty of sciences and bio-engineering sciences). As associate professor, his lecturing activities include courses in food science and technology (i.e., "Nutrition," "Technology of animal products," "Food microbiology and ecology," and "Quantitative and predictive microbiology"). Dr. Leroy's research primarily deals with the ecology and functional roles of bacterial communities in (fermented) foods, in particular with respect to the generation of quality, safety, and/or nutritional and health advantages. Focus is mostly on meat products, but other food systems are also being studied, including fermented milks and sourdough breads. In addition, his research interests relate to elements of tradition and innovation in foods, both from a technological and societal point of view.

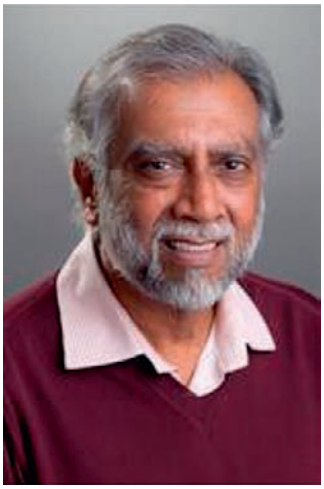
Catherine M. Logue completed her undergraduate and postgraduate degrees in Ireland and earned a PhD in meat microbiology from the University of Ulster, UK in 1996. Dr. Logue was a faculty member at North Dakota State University from 1999 to 2011 rising through the ranks of assistant to associate and full professor. In 2011, she re-located to Iowa State University's College of Veterinary Medicine and is a professor of veterinary microbiology and preventive medicine. Dr. Logue is also the director of faculty and staff advancement and equity for the college. Her research interests focus on foodborne pathogens of food animals and the contamination of meat and meat products destined for human consumption. Her research studies the detection, isolation, and characterization of a range of foodborne pathogens such as *Salmonella*, *Campylobacter*, *Listeria*, *Escherichia coli*, and methicillin-resistant *Staphylococcus aureus* (MRSA) in poultry, bovine, and swine. She also focuses her research on antimicrobial resistance in commensals and pathogens of production animals. She has been an author and a co-author on more than 90 peer-reviewed papers and book chapters as well as more than 150 abstracts and presentations at national and international meetings.

F. Xavier Malcata graduated in chemical engineering in 1986 from the University of Porto (Portugal), received a PhD in chemical engineering/food science from University of Wisconsin, Madison (USA) in 1991, and his habilitation in food science and engineering by Portuguese Catholic University in 2002. He was the dean of College of Biotechnology of Portuguese Catholic University, the chairman of Portuguese Society of Biotechnology, Portuguese representative at VI and VII European Union Framework Programs of research and development, expert for European Food Safety Agency, and a co-ordinator of Portuguese Engineering Accreditation Board in chemical engineering for Northern Region. He is currently a full professor at University of Porto.

His major research interests have focused on technological improvement of traditional Portuguese foods and upgrade of byproducts thereof, development of nutraceutical ingredients and functional foods, design and optimization of enzymatic reactors for edible oil processing, characterization of plant proteases toward cheese and whey cheese manufacture, production of starter and nonstarter cultures from adventitious microflora, and optimized application of unit operations to food processing.

With an academic career of independent research and teaching for more than two decades, Prof. Malcata published more than 450 papers in refereed journals worldwide, wrote 11 books, and prepared more than 45 chapters for edited books. Among many international distinctions, he was

recipient of Ralph H. Potts Memorial Award in 1991 by American Oil Chemists' Society (AOCS, USA), Foundation Scholar Award – Dairy Foods in 1998 by American Dairy Science Association (ADSA, USA), Young Scientist Research Award in 2001 by AOCS, Canadian/International Constituency Investigator Award in physical sciences and engineering in 2002 and 2004 by Sigma Xi (USA), Danisco International Dairy Science Award in 2007 by ADSA, Scientist of the Year Award in 2007 by European Federation of Food Science and Technology (the Netherlands), Samuel C. Prescott Award in 2008 by Institute of Food Technologists (IFT, USA), International Leadership Award in 2008 by International Association for Food Protection (IAFP, USA), Elmer Marth Educator Award in 2011 by IAFP, Distinguished Service Award in 2012 by ADSA, and William V. Cruess Award in 2014 by IFT. He has been elected for the honor societies of food science (Phi Tau Sigma, USA), scientific research (Sigma Xi, USA), and engineering (Tau Beta Pi, USA). He was also elected for fellow of IFT, ADSA, AOCS, and International Academy of Food Science and Technology.



Gopinadhan Paliyath is a professor at the Department of Plant Agriculture, University of Guelph, and the research program director for "Food for Health," under the UG/OMAFRA partnership. Dr. Paliyath is a biochemist and has an interest in various aspects of fruits and vegetables, specifically the nutraceutical components and their mechanism of action. He obtained his BSc Ed degree (botany and chemistry) from the University of Mysore, MSc degree (botany) from the University of Calicut, and PhD degree (biochemistry) from the Indian Institute of Science, Bangalore. Subsequently, he did postdoctoral work at Washington State University, University of Waterloo, and University of Guelph. Dr. Paliyath's research is focused on the biochemistry of plant senescence, specifically pertaining to postharvest biology and technology of fruits and vegetables. Investigations on the role of phospholipase D (PLD) in membrane homeostasis and signal transduction have led to advances in the understanding of the mechanism of membrane deterioration that occur during stress and senescence. Another aspect of his research is focused on understanding the mechanism of action of food components in disease prevention. The efficacy, bio-accessibility, bioavailability, and molecular mechanisms of action of nutraceuticals in fruits and processed products in relation to their cancer-preventive and anti-inflammatory actions are being investigated using mammalian cell lines, and animal model systems.

Dr. Paliyath has developed technologies and products for enhancing the shelf life and quality of fruits and vegetables based on PLD inhibition. R&D activities relevant to the industry sector include: (1) optimization of an enhanced freshness formulation for application to various fruits, vegetables, and flowers; (2) developing methods for nutraceutical carriers that would enhance the functional food quality and delivery (e.g., stabilizing lycopene in tomato juice, sauce, etc., for health beneficial effects); and (3) developing novel technologies to enhance the cancer-preventive ingredients in fruit products, etc. Patents awarded include: (1) # 6,514,914 (US) and 2,298,249 (Canada); (2) #7,198,811 (USA), 4141387-1 (Japan), 260738 (Mexico), 1469736 (Turkey), 028 284763 (China), and 223077 (India). The patents describe the use of nanoformulations based on hexanal and other generally regarded as safe (GRAS) ingredients for enhancing the shelf life and quality of fruits, vegetables, and flowers by pre or postharvest treatments. These technologies are currently being evaluated for extending shelf life and quality of mango in India and Sri Lanka with the assistance of the Canadian International Food Security Research fund. The collaboration involves researchers from Canada, India (Tamil Nadu Agricultural University), and Sri Lanka (Industrial Technology Institute).

Dr. Paliyath is also the research program director for the food and health theme-related activities under the OMAF/MRA/University of Guelph research partnership. He serves on the editorial board of several journals. He is a member of American Chemical Society and Canadian Society of Plant Biologists.

(Total-refereed publications in journals – 92; patents and intellectual properties – 2; disclosures – 4; chapters in books – 27; nonrefereed contributions – 10; research reports – 28; conference proceedings – 88; edited books – 9; book reviews – 6 (Google Scholar: h index – 31, i10 index – 68, citations – 4332; RG score – 35.63)).

Yolanda Picó is a full professor of nutrition and food science at the University of Valencia since 1998. She is currently the head of the research group on food and environmental safety of the University of Valencia. Her research interests are the development of new analytical methods to determine organic contaminants in food and the environment, identification of unknown compounds by liquid chromatography–mass spectrometry, micro-extraction separations, and environmental and food safety. To the date, she is the author of nearly 200 peer-reviewed papers, 170 scientific papers in journals of SCI, 25 book chapters, and editor of four books on food and environmental safety.

Vieno Piironen is a professor of food chemistry at the Department of Food and Environmental Sciences, University of Helsinki, Finland. She received her PhD in food chemistry at the University of Helsinki in 1987 and has approximately 35 years of experience in food research and education on bachelor, master, and PhD levels. She has participated actively in international research and education projects and networks. Her research has focused especially on chemical and nutritional properties, reactions and analysis of lipids, vitamins, and other bioactive compounds. Research on vitamins has been active from the beginning of 1980s. She has studied both lipid- and water-soluble vitamins; their chemical and nutritional properties and importance in foods and diets as well as factors influencing vitamin levels. In addition, development of analytical methods for different vitamers as well as validation and harmonization of the methods through international collaboration have been among the priorities. Recent collaboration projects have focused on enhancing vitamin contents in cereal-based foods by plant breeding, utilization of vitamin-rich grain fractions, and bioprocessing. Currently, the research focus lies on investigating microbial *in situ* synthesis of folate, vitamin B12, and other B vitamins in cereal and legume matrices as a means to improve nutritional quality of foods and to develop new food applications. In lipid research, different lipid classes and their chemical and enzymatic reactions in food matrices are studied. Diverse methods are used to study proceeding of oxidation from primary products to monomeric oxides, volatiles, and polymerization products and to study possibilities to control oxidation. Controlling enzymatic reactions leading to off-flavors in cereal and legume matrices is also among the interests. Phytosterols and their conjugates have been studied as natural food components belonging to the dietary fiber complex. On the other hand, questions related to sterol enrichment such as oxidation susceptibility and mechanisms as well as factors affecting oxidation reactions have been of interest. She has also studied nutrients and anti-nutrients in legumes and more recently started research on utilization of high value components in microalgae. She has approximately 160 papers in international journals and a number of other publications.

David Rodríguez-Lázaro is a doctor in veterinary medicine (DVM), specialized in food science (BSc and MSc) and molecular microbiology (PhD). He is a senior scientist at ITACyL and an assistant professor of microbiology at the University of Burgos. He has performed research stays in the Danish Institute for Food and Veterinary Research (Denmark), the University of Prague (Czech Republic), the Food and Environmental Research Agency (UK), and the University of Bristol (UK). He was a Leverhulme visiting professor in the Institute of Advanced Sciences in the University of Bristol during the years 2004 and 2005 and Marie Curie research fellow in the faculty of medical and veterinary sciences in the University of Bristol (UK) until 2007.

His research interest is focused on the establishment of reliable, quantitative molecular strategies for detection of important food-borne pathogens from environmental sources and various types of foodstuffs, the characterization of the prevalence of the main foodborne pathogens in food and food-related environments, and the development of emergent food preservation processes and their effects in the microbial virulence. He has participated in a number of coordinated EU-funded projects such as PROMISE, BASELINE, VITAL, FOOD-PCR, SACROHN, and MONI-QA, having established active links with the leading European research groups working in food safety.

He has published more than 100 international scientific papers and book or book chapters regarding food safety. He is currently a member of the editorial board of *Applied and Environmental Microbiology*, *International Journal of Food Microbiology*, *Food and Environmental Virology*, and *International Journal of Food Contamination* and the editor-in-chief of the journal *Food Analytical Methods*. He was awarded with the XV Jaime Ferrán Award in 2013 by the Spanish Society for Microbiology for his promising scientific career in microbiology.

Turid Rustad is a professor and the head of the food science group at the Department of Biotechnology, Norwegian University of Science and Technology. The main research focuses on the biochemistry of marine raw materials, the relationship between biochemistry and quality, and changes in raw material properties during processing. Studies of enzymatic activities in different raw materials have been linked to studies of changes in the biochemistry of these raw materials. She has worked with characterization of composition and enzymatic processes in a wide range of different raw materials, such as fish, fish by-products, and zooplankton in relation to different storage and processing methods such as chilling, heating, superchilling, and frozen storage.

Noel W. Solomons has lived and worked in Guatemala for 40 years. He was born and educated in Massachusetts in the United States. As a young child, he became an amateur naturalist and was a nature counselor at various summer camps; this would guide him to a career in science. In his young adulthood, he would participate in the civil rights and anti-war movements, only to become disillusioned by the intractable nature of the injustice elements in the fabric of American society. As a physician by graduate training, he performed his university studies at Harvard College and Harvard Medical School; it was during overseas electives in his medical training that he visited Peru and Colombia and committed to an expatriate life trajectory outside of his homeland. Clinical training included a residency in internal medicine and infectious diseases at the Hospital of the University of Pennsylvania and specialization in gastroenterology and clinical nutrition at the University of Chicago. He became a resident of Guatemala in 1974 as an affiliated investigator at the Institute of Nutrition of Central America and Panama. He would later commute for eight years to a faculty position in the Department of Nutrition and Food Science of the Massachusetts Institute of Technology. Assuming a full-time Guatemala commitment in 1985, he co-founded the Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM) where he remains its scientific director. Over 40 local university theses have been completed by Central American students in that institution as well as an equal number of master's degree research projects from international students from Europe, and North and South America. He has supervised doctoral dissertations for 12 PhD candidates from the United States, Canada, Germany, Spain, and the Netherlands through CeSSIAM.

Dr. Solomons has 332 publications indexed on Medline. In addition, he has edited two books and contributed over 100 articles, reviews, editorials, and commentaries in nonindexed venues and over 50 book chapters. These are dedicated to the scientific and academic interests of his career including: clinical nutrition; human growth and body composition; lactose maldigestion; dietary intake, nutritional status, intestinal absorption, and food fortification related to various micronutrients (vitamins, trace elements, and essential fatty acids); complementary feeding; nutrition in aging and chronic disease; and the interaction of malnutrition and infection.

Among the honors bestowed upon Dr. Solomons are the International Nutrition Prize of the International Union of Nutritional Sciences and the Kellogg Prize of the Society for International Nutrition Research. He is a fellow of the American Society of Nutrition. He is an academic member of the Guatemalan Academy of Medical, Physical and Natural Sciences and the Spanish Academy of Nutrition and Food Science. He was the awardee of the 2010 National Medal for Science and Technology for Guatemala.

He has been a visiting professor in university courses in Mexico, Peru, Brazil, Indonesia, and Spain. He currently holds adjunct professorial appointments at the Boston University School of Public Health, and the Friedman School of Nutrition Science and Policy and the Department of Community Medicine and Public Health, both at Tufts University. He is a founding board of directors, member of the Hildegard Grunow Foundation in Munich and the Essential Nutrient Foundation of Singapore. Finally, Dr. Solomons is a coordinator for Central America of the Nevin Scrimshaw International Nutrition Foundation in Boston, and an associate editor for the Foundation's *Food and Nutrition Bulletin*. He serves on editorial boards for ten scientific journals.

Maria Tsimidou is a professor of food chemistry and the head of the Laboratory of Chemistry and Technology in the School of Chemistry at the Aristotle University of Thessaloniki (AUTh), Greece. Her teaching is food chemistry, analysis, quality control, and food legislation. Research interests are related to virgin olive oil chemistry, quality and authenticity, saffron chemistry, authenticity and quality, antioxidant activity of plant extracts and constituents, new sources of targeted bioactive compounds (squalene, carotenoids, and phenols), and analytical procedures for their determination. She has published many research papers, review articles, and contributions to scientific books and encyclopedias on the above-mentioned topics. Currently, she is an associate editor in the *European Journal of Lipid Science and Technology* and chairs the COST ACTION FA1101 "Saffronomics."

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Peter J. Wilde graduated in biophysics at the University of East Anglia in 1985 and has been researching the colloidal and interfacial properties of food systems at the Institute of Food Research (IFR) for over 25 years. IFR is the only publicly funded UK research institute that focuses on the underlying science of food and health to address the global challenges of food security, diet, and health, healthy aging, and food waste. IFR is the one of eight institutes that receives strategic funding from the Biotechnology and Biological Sciences Research Council (BBSRC). It also receives funding from government agencies and departments, the EU, charities, and industry, from the UK and overseas.

Pete's research expertise is the interfacial behavior of proteins and other surface active components in food relevant systems. The aim is to determine how the molecular and interfacial processes control the functionality of foams and emulsions. Currently, the functional aspects of his research have focused on improving the dietary impact of emulsified foods. These include fundamental studies on how interfacial layers control emulsion rheology to develop novel fat reduction strategies; the design of interfacial structures to control lipid digestion to promote satiety or the delivery of fat-soluble nutrients and drugs; and to determine the physico-chemical role played by the salivary film in perceiving fat content in emulsions. The impact of this research will be to aid the rational design of foods with enhanced nutritional benefits to address the global challenges of obesity, type 2 diabetes, and other major diet-related conditions.

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HOW TO USE THE ENCYCLOPEDIA

All articles in the encyclopedia are arranged alphabetically as a series of entries.

1. Contents

Your first point of reference will likely be the contents. The complete contents list appears at the front of each volume providing volume and page numbers of the entry. We also display the article title in the running headers on each page so you are able to identify your location and browse the work in this manner.

2. Cross-references

The majority of articles within the encyclopedia have an extensive list of cross-references that appear at the end of each article, for example:

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Iron: Biosynthesis and Significance of Heme; Iron: Physiology of Iron.

3. Index

The index provides the volume and page number for where the material is located, and the index entries differentiate between material that is a whole article; is part of an article, part of a table, or in a figure.

4. Contributors

A full list of contributors appears at the end of volume 5.

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INTRODUCTION

Until a few decades ago, virtually all known health effects of foods were related to their content of essential nutrients. The clinical description of most diet-related illnesses mirrored the signs of essential nutrient deficiencies, such as pellagra, beriberi, and others. Consequently, the key public health concern regarding diet was ensuring that everyone consumed enough food. It was only in the past 50 years that large-scale epidemiological observations began to associate chronic diseases like diabetes and cardiovascular disease with nonessential diet constituents such as saturated fat, fiber, and cholesterol. Taking advantage of the emergence of digital informatics, these studies were able to manipulate increasingly large sets of data and provide, for the first time, a picture of the secular changes in the health of large populations and its association with what they ate regularly. These findings progressively shifted the concern from eating enough to avoiding excessive consumption of certain foods. Eating enough was replaced by eating well.

But it turned out that defining how to eat well is far more complex than defining minimum needs of essential nutrients. First, there is no single paradigm to study those relationships, given the wide variety of biological mechanisms and the long exposures involved. Second, many of the experimental models used to define essential nutrient needs are not applicable to the study of long-term effects of diets in free-living populations. And it is now clear that experiments with isolated dietary compounds do not reflect the actual effects of the complex food matrix we consume daily. Finally, while the discovery of essential nutrients and their role in health was the domain of a few specialties speaking a common language (primarily biochemists and physiologists), the study of the long-term effects of whole diets in humans must of necessity involve epidemiologists, social and behavioral scientists, food scientists, clinicians, policy experts, etc., making far more difficult the development of consensus and foundational concepts.

It is thus not surprising that today we have still not achieved a stable consensus on how to eat 'well.' Furthermore, while few nonscientists would care about the minimum requirement of a vitamin to sustain life, there are plenty of opinions among nonscientists on how to eat 'well.'

Our goal in preparing this encyclopedia has been to contribute to the understanding of that complex diet–health relationship by providing a multidisciplinary, integrative and accurate source of information. We aim to serve the needs not only of established and in-training scientists, but also of the increasingly important group of professionals who are key to disseminate and sustain the practice of science: journalists, science writers, science administrators, fund raisers, donors, and policymakers. In preparing this work, we had the enormous advantage of working with one of the publishers with the most extensive expertise in major reference works, Elsevier. This first edition builds on the impressive breadth of knowledge of over 922 authors and on the tireless work of our editorial advisory board. We are very grateful to all of them.

Benjamin Caballero
Paul Finglas
Fidel Toldrá

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Mushrooms and Truffles: Role in the Diet

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Introduction

Mushrooms are important constituents of minor forest produce; they grow on cellulose, the most abundant biomolecule. Macrofungi, in the form of mushrooms and truffles, have been used as food and medicine from ancient times. Geologically, mushrooms existed on the earth before man as evidenced by the fossil records from the lower cretaceous period. Originally, these organisms were reported to be associated with the Mediterranean region and are described in Egyptian temples in some poems. During the Greek and Roman eras, they were imported from Libya and sold in the southern part of Europe and, in Africa, the nomadic people of the Kalahari Desert have consumed truffles for millennia.

Mushrooms are visible to the naked eye as they grow above the ground; whereas truffles grow underground at depths between 5 and 10 cm (Figure 1).

Truffles are usually collected by specialists who have the skills and experience to locate this type of flora (Table 1). Sometimes, truffle collectors use trained animals, such as pigs and dogs, to discover this underground fungus. The animals do this using their high sensitivity to the characteristic truffle volatile compounds. Traditionally, mushrooms and truffles are treated as prized foods and may be consumed either raw or cooked. In addition, they have been used widely as components of folk medicine, because of their rich content of proteins and amino acids, fatty acids, fiber, minerals, vitamins, terpenoids, sterols, flavor compounds, and carbohydrates.

Mushrooms

Mushrooms are epigeous macrofungi and have been highly valued in many cultures because of their nutritional value and characteristic aroma and taste. Out of ~3000 edible species of mushrooms only 100 are cultivated commercially, and only ten are produced on an industrial scale. Their global economic value is, nevertheless, considerable. The prime reason for the upswing in consumption is increased health awareness. This has led to selection of foods with superior nutritional or (perceived) nutraceutical benefits.

The nutritional value of mushrooms is established; they are rich sources of complete proteins, containing all the essential amino acids and fiber and very little fat. Mushrooms also provide significant quantities of vitamins (B_1, B_2, B_{12}, C, D, and E) and bioactive compounds such as unsaturated fatty acids, phenolic compounds, tocopherols, and carotenoids.

Mushroom production has increased over the last two decades. As depicted in Figure 2, China has emerged as the biggest producer of mushrooms (~2.0 million tonnes). The species mostly cultivated worldwide is *Agaricus bisporus* followed by *Lentinula edodes*, *Pleurotus* spp., and *Flammulina*

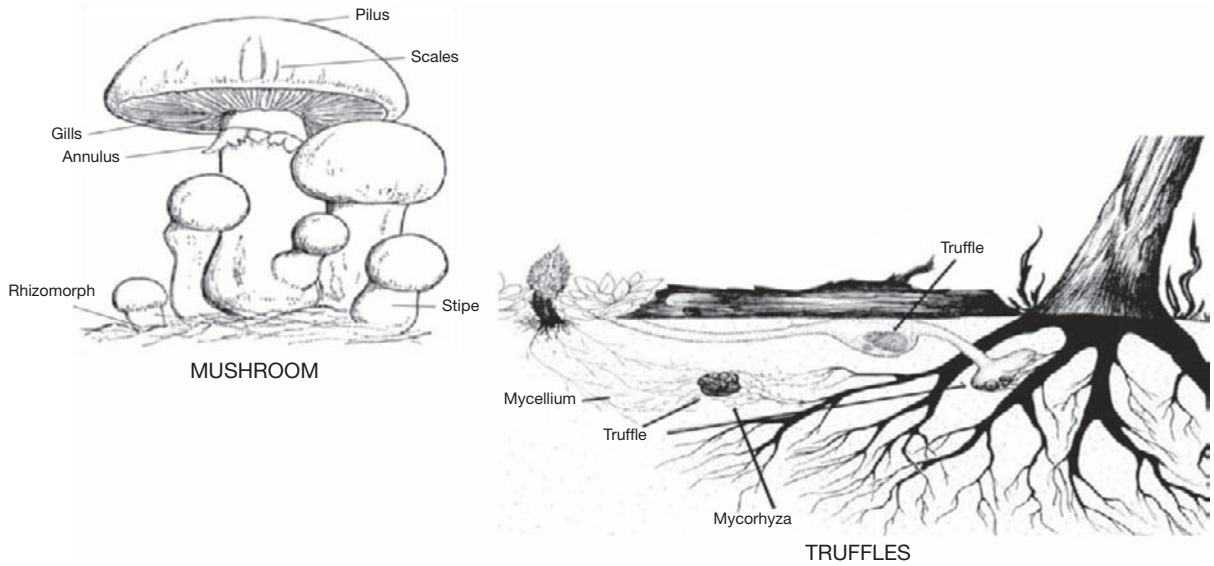

Figure 1 Morphology of mushrooms and truffles.

Table 1 Some illustrative truffle species and their properties

Truffle species	*Common name*	*Characteristics*	*Image*
Tuber aestivum	Summer truffle	The black summer truffle is found across Europe and is prized for its culinary value. Native to France, Italy, and Spain, the summer truffles are usually at their best in July, but can be found from May to October. They have a black exterior and off-white interior, and a relatively light scent	
Tuber uncinatum	Burgundy truffle	Burgundy truffles are harvested in autumn until December and have aromatic flesh of a darker color.	
Tuber gibbosum & *T. oregonense*	Oregon white truffle	These are firm and brittle, will have a beige to smokey-colored marbled interior, and a pleasant earthy odor. Mushroom eggs (and many truffle species that are only appetizing to squirrels) are generally squishier, spongy, or cartilaginous	
Kalapuya brunnea	Oregon brown truffle	It has a reddish-brown exterior and a grayish mottled interior. When mature, the odor can be quite garlicky	
Leucangiumcarthusianum	Oregon black truffle	Less common than Oregon white truffles, Oregon blacks are larger (golf-ball to baseball size), and are often much deeper in the soil than Oregon whites (commonly 4–10″ deep). They are very dark inside and out, and have a very pungent, earthy odor when ripe. Some equate the aroma to a strange mix of pineapple, port, mushrooms, rich soil, and chocolate	
Tuber melanosporum			

Table 1 (Continued)

Truffle species	*Common name*	*Characteristics*	*Image*
	French black (Perigord) truffle	Native to the oak forests of the Perigord region of central and southwest France, it is now widely cultivated in Spain, Australia, and the United States. It has a blue-black exterior when fresh, fading to brown-black with age, and a pungent, earthy odor	
Tuber magnatum	Italian white (Piedmont) truffle	Considered to be second best to the French black truffles, its cost can exceed that of the Perigord. The flesh is solid, light-colored, and very brittle; it is not unheard of for a fresh truffle to shatter if dropped on the floor. Large specimens can weigh as much as a pound, but most are the size of large walnuts. The white truffle is slightly more perishable than its darker cousins, and the flavor and aroma diminishes within a week or two after harvest. The white truffle has a distinctive pepper edge and is often eaten raw. The skin is a dirty beige when fresh, turning a darker brown with age	
Tuber borchii	Tuscan truffle	Similar to the Italian white truffle in appearance, having a chestnut to muddy tan exterior and a softish interior equally divided between chocolate brown and white. The flavor can be distinctly garlicky	
Tuber sinensis, *Tuber indicum*, and *Tuber himalayensis*	Chinese truffles	These are three distinct species found in southwest China, but pickers tend to lump them together as Chinese truffles. This is unfortunate because the flavor and quality vary from one species to another. *T. indicum* is recognizable by its brown interior and very fine white veins. *T. sinensis* has a dark brown interior with large ivory veins, and is said to be chewy and oily with a bitter aftertaste	
Terfezia boudieri, *Terfeziapfeilii*, *Terfezia claveryi*, and others	Desert truffles	Native to northern Africa and the Middle East, these truffles have been a staple for many nomadic tribes for millenia. Sometimes called the Lightning Truffle, they often fruit shortly after thunderstorms wet the desert	

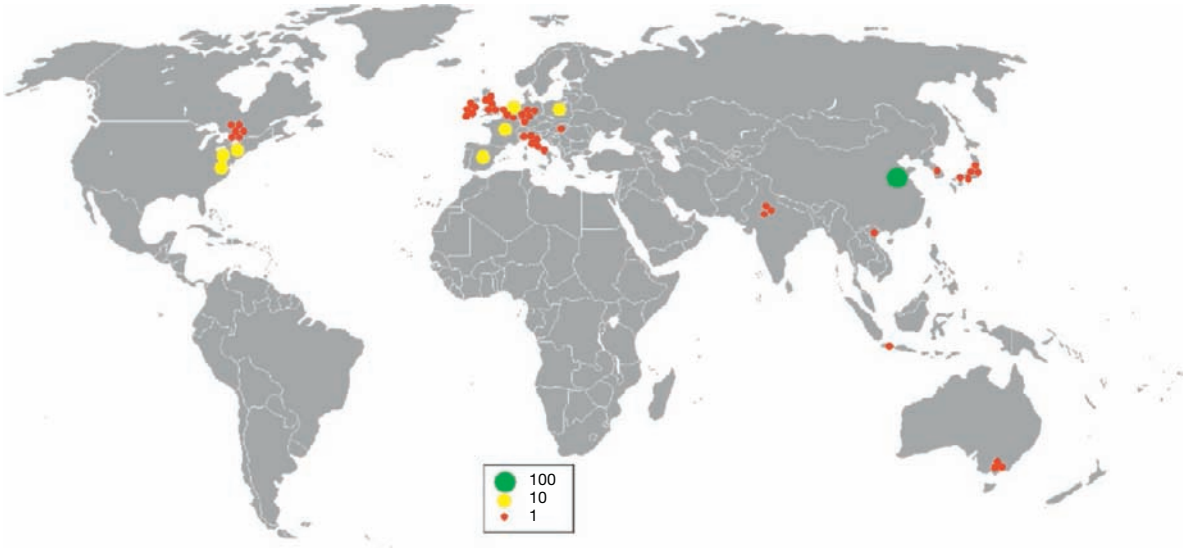

Figure 2 Mushroom and truffle growing – a global scenario on by percent.

velutipes. These species grow to market size in a short period compared with other edible mushrooms. As a result, by controlling some of the environmental parameters, these species can be cultivated in a simple and affordable way.

Truffles

The truffles are hypogenous mushrooms that grow exclusively underground and are recognized as ectomycorrhizal fungi. They usually occur at the interface between the organic litter and the mineral soil, about 2.5–15 cm deep, but can emerge at the surface or be found at more than 30 cm deep. A truffle is the fruiting body of a subterranean *Ascomycete fungus*, one of the many species of the genus *Tuber*. They rely on animals eating them (*mycophagy* from the Greek words 'mykes' meaning fungus and 'phagein' meaning to eat, fungivores) to distribute spores, instead of air dispersion like mushrooms. Truffle spore-bearing surfaces are enclosed, and they do not form a prominent stem, rather resembling small potatoes.

Truffles are made up of two parts: the fruiting body, which is edible, and the roots. The fruiting body appears during different seasons depending on the type of truffle. It is made of an exterior layer (peel) of variable thickness, which can be smooth or sculptured in appearance (with bumps or wrinkles) and light or dark in color, and an internal mass (pulp or gleba), which may range from white to brown, gray, pink, or black and is marked by criss-crossing 'veins.' This internal mass varies in thickness and the alveoli contain cells bearing spores (ascospore).

No specific environment is required for truffles and they can potentially be found almost anywhere trees grow. Truffles prefer woods that have mixed trees with a little undergrowth to maintain humidity and are often found along the banks of streams, but also by footpaths, and under pine trees or poplars. Each species of truffle needs different soil and climate conditions (Table 1). Only a few families of trees (e.g., maples and cedars) are not associated with truffle-forming fungi.

There are hundreds of different kinds of truffles, and while none are known to be poisonous, only a few of them are considered to be delicacies by humans. Truffles and mushrooms are the only fruits of the fungus; the main perennial fungal body exists as a web of filamentous hyphae in the soil.

All of the truffle fungi form a symbiotic (Mycorrhizae) (i.e., mutually benefiting) association with the roots of trees, and are essential for the tree to acquire some nutrients. The fungal hyphae not only permeate the soil, but also penetrate root cells of plants and facilitate nutrient exchange where the host plant acquires nutrients from the soil via the fungi, and the fungi (which cannot perform photosynthesis) obtains sugars from the host plant.

As most truffles never break the surface of the soil, they rely on animals to eat them and distribute spores. To ensure they are found, truffles have evolved to exude pungent, fruity, and sometimes unpleasant aromas when mature.

Role of Mushrooms in the Diet

Since antiquity, man has hunted for the wild mushrooms and higher fungi have been treated as a food due to their unique palatability and flavors. They are low in calories, but contain high amounts of vegetable protein, specifically chitin. Mushrooms consist of two main parts, the mycelium and the fruity body (sporocarp). The mycelium consists of a tree-like structure (hyphae), which remains buried under the soil or substrate. The mycelium absorbs nutrients while the hyphae form mycelia, which in turn forms the fruit (sporocarp) structure on the surface when atmospheric conditions, particularly humidity, are favorable. The spore-producing tissue is called the hymenium. Mushrooms vary in size, color, texture, and structure, which favors spore formation. The cap is called the cuticle and varies among mushroom species; it may be sticky or slimy. The stalk is the stem-like structure on which the cap is mounted and varies in length depending on the species.

Many species of mushrooms are edible. *Ganoderma* spp., *Omphalotus olearius* and *Hebeloma mesophaeum* are species commonly found in Africa and reported to be among the most edible. Some are medicinal such as *Auricularia* spp. and *Tremella fuciformis*, which are used for treating hemorrhoids and maintaining healthy lung tissue, respectively; others are poisonous including *Pholiota semarrasa* and *Amanita vaginata*. Mushrooms have assumed greater importance in the diets of both rural and urban dwellers. The success in utilizing plant proteins as ingredients depends largely on the beneficial qualities they impart to foods, which, by extension, depend on their functional properties. The functionality of plant protein has been reported to be dependent on the chemical characteristics inherent in the seed. Some mushroom extracts are used as a component for soups and teas to boost the immune system. Consumption is also associated with reduced risk of cancer and heart disease.

Mushrooms have begun to be treated as nutriceuticals and there is a body of research exploring their chemical composition, which suggests consumption may not only reduce risk of disease but also treat others. Traditional medicine has used mushrooms such as *Hydnum coralloides*, *Agaricus campestris*, *Helvella crispa*, *Hypoxylon vernicosum*, *Morchella esculenta*, and *Polyporus mylittae* as a health food for generations and 100–200 g daily (dry weight) are thought to maintain optimal nutritional balance in a man (60 kg). It has even been suggested the food value of mushrooms lies with vegetables and is comparable with meat, egg, and milk food sources.

In general, mushrooms contain 83–90% water and 10–16.5% dry matter (Table 2). Importantly, the protein content varies between 27% and 48%. Carbohydrates are $<60\%$ and lipids are between 2% and 8%. On average, a mushroom is about 16.5% dry matter of which 7.4% is crude fiber, 14.6% is crude protein, and 4.48% is fat and oil (Table 2). There is also a significant difference in the nutrient contents of the pileus versus the stalks.

Several thousand mushroom species are known worldwide, and around 3000 are considered edible.

Table 2 Nutritional facts about mushrooms

Nutrients	*Range*
Energy (kcal/100 g)	24–31
Moisture (g/100 g)	83–90
Protein (g/100 g)	1.94–3.00
Fat (g/100 g)	0.20–0.34
Ash (g/100 g)	0.79–0.91
Carbohydrate (g/100 g)	3.69–8.42
Dietary fiber (g/100 g)	1.45–2.70
β-Glucan (g/100 g)	0.21–0.79
Ergosterol (mg/100)	37–69
Calcium (mg/100 g)	1–4
Copper (mg/100 g)	0.11–0.30
Iron (mg/100 g)	0.22–1.15
Magnesium (mg/100 g)	10–16
Manganese (mg/100 g)	0.05–0.10
Phosphorus (mg/100 g)	74–105
Potassium (mg/100 g)	204–359
Sodium (mg/100 g)	1–15
Riboflavin (mg/100 g)	0.20–0.33
Niacin (mg/100 g)	2.80–7.03
Pantothenic acid (mg/100 g)	0.27–1.36
Vitamin B_6 (mg/100 g)	0.05–0.10
Folic acid (mg/100 g)	6–52

Proteins

Protein is an important constituent of mushroom dry matter with digestibility values as high as 72–83% and it contains all essential amino acids. The protein content of mushrooms depends on the composition of the substratum, size of the pileus, harvest time, and species, but ranges from 14% to 27% crude protein (dry weight). In terms of the amount of crude protein, mushrooms rank below meat, but above most other foods, including milk. In general, mushrooms are very useful for vegetarians because they contain essential amino acids that are usually obtained from animal proteins. The total nitrogen content of dry mushrooms is 79% compared with 100% for an ideal protein (Table 2).

Carbohydrates

The carbohydrate content of mushrooms represents the bulk of the fruiting bodies, accounting for 50–65% (dry weight), with free sugars amounting to about 11%. Mannitol, also called mushroom sugar, constitutes about 80% of the total free sugars, and a fresh mushroom contains 0.9% mannitol, 0.28% reducing sugar, 0.59% glycogen, and 0.91% hemicellulose. Water-soluble polysaccharides of mushrooms have been shown to have some antitumor activity, and mannitol is associated with improved brain function.

Fats

Mushrooms contain very little fat compared with carbohydrates and proteins, ranging from 1% to 5%. The fats present in mushroom fruiting bodies are dominated by unsaturated fatty acids rich in linolenic acid, which is an essential fatty acid (Table 2).

Vitamins

Mushrooms are an excellent source of some vitamins, particularly the B vitamins, and wild mushrooms contain much more vitamin D_2 than dark cultivated mushrooms. Mushrooms also contain a small amount of vitamin C.

Minerals in Mushrooms

The fruiting bodies of mushrooms contain high levels of minerals including potassium, phosphorus, sodium, calcium, and magnesium as well as copper, zinc, iron, molybdenum, and cadmium. K, P, Na, and Mg constitute $\sim$56–70% of the total ash content of mushrooms while potassium alone forms 45% of the total ash (Table 2). Mushrooms have been found to accumulate heavy metals including cadmium, lead, arsenic, copper, nickel, silver, chromium, and mercury. The mineral proportions vary according to the species, age, and the diameter of the fruiting body as well as the type of the substratum.

The mineral content of wild edible mushrooms has been found to be higher than cultivated mushrooms.

Therapeutic Value

First century Greek physician Dioscorides included the lurch polypore (*Fomitopsis officinalis*, *Laricifomes officinalis*) in his *De Materia medica* known then as *Agaricum* and later as the Quinine conk. It was used for the treatment of a disease now known as tuberculosis. Moreover, mushrooms as a medicine have been used in China since 100 AD, but these properties have only been investigated since ca. 1960. Mushrooms have been used in healthcare for treating age-old common diseases to present-day more complex conditions.

Mushrooms possess antiallergic, anticholesterol, antitumor, and anticancer properties. The antitumor effects of hot water extracts from several mushrooms has been observed where the main active components are polysaccharides, especially β-D-glucans such as *lentinan*. It is also reported that mushrooms 'cure' epilepsy, and help with wounds, skin diseases, heart ailments, rheumatoid arthritis, and cholera besides intermittent fevers, diaphoresis, diarrhea, dysentery, colds, anesthesia, liver disease, gall bladder diseases, and they are used as vermicides. Most of the treatments including mushrooms are now available in tablet form. Mannentake (*Ganoderma lucidum*) are known to lower blood pressure and serum cholesterol concentration whereas *Lentinus tigrinus* and *G. lucidum* have been shown to reduce cholesterol levels. *L. edodes* have been used to enhance vigor, libido, and energy as well as being an antiaging agent. Lentinan sulfate obtained from the *Lentinus* species inhibits human immunodeficiency virus (HIV).

Mushrooms are thought to act as biological response modifiers by promoting positive factors and eliminating negative factors from the human body. They are regarded as part of conventional cancer treatment in some countries; polysaccharide-K (PSK), an anticancer treatment based on the mushroom, *Coriolus versicolor*, accounted for a quarter of chemotherapeutic drug sales in Japan in 1987. Mushroom health supplements, marketed in the form of powders, capsules, or tablets, are made from dried fruiting bodies, extracts of mycelium, biomass, or extracts from liquid fermentation; *P. sajor-caju* has been reported to induce growth of probiotic bacteria. Waxy cap mushroom extracts (*Hygrocybe coccinea*) inhibit sarcoma and immunoceticals, isolated from more than 30 mushroom species, have been shown to have anticancer action in animals. Schizophyllan from *Schizophyllum commune* may also be effective in head and neck cancer.

Antioxidant Activity

Antioxidants protect cells and cell components from damage caused by unstable molecules including free radicals and reactive oxygen (ROS) and nitrogen species (RNS). Oxidants react with enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, but over production leads to oxidative stress meaning food antioxidants and bioactive compounds are an important defense against ROS/RNS. Mushrooms are rich sources of these compounds. Many species of mushrooms have also been found to be potent immune enhancers, enhancing the immune response to neoplastic changes.

Role of Truffles in Diet

Truffles are highly sought after because of their strong scent, which comes from the aromatic hydrocarbon compound bis(methylthio) methane. The nutritional value of truffles is very high and almost identical for the different species. It varies depending on the environmental conditions in which truffles are grown. Unlike mushrooms, no truffles are known to be poisonous.

Truffles are appreciated in French, Georgian, Greek, Italian, Middle Eastern, and Spanish cooking as well as in international *haute cuisine*. Truffles are rare and luxurious tubers, and one of the most sought after ingredients by top chefs internationally. They have been called the 'diamond of the kitchen,' and widely acknowledged as one of the most desirable gourmet foods. Truffles are used to season dishes or as an accompaniment in greater or lesser quantities. Thus, their contribution to human nutrition remains relatively low.

Truffles are different from other vegetables because of their flavors; small amounts can bring out the taste, flavor, and aroma of a dish. Because they are found rarely, truffles are expensive, but truffles are also noted for their medicinal value. Like mushrooms, truffles are also low in fat, cholesterol-free, and regular consumption may lower serum cholesterol.

Nutritional Facts and Health Benefits of Truffles

Truffles contain 72% water, very little fat (0.6%) and some protein (8.6%) (Table 3). Truffles are some of the most nutritious fungi available. Their proteins are intact and rich in methionine, cysteine, and lysine. Truffles are also rich in minerals, such as phosphorus, calcium, magnesium, potassium, sodium, iron, zinc, fiber, sulfur, chlorine, and silicone. They also contain fiber (7.6%), an important component to facilitate digestion and intestinal motion. Details of truffle nutritional composition are given in Tables 3 and 4. The calorific value of truffle per serving is about 62.1 kJ.

Truffles are a good source of oil, but cooking over 90 °C causes the oil to lose its flavor; it is only designed for salads and

Table 3 Nutritional composition of truffles

Composition	*By weight*	*Percent*
Fat	4.4 g	7
Saturated fat	2.7 g	14
Polyunsaturated fat	0.15 g	–
Monounsaturated fat	1.3 g	–
Cholesterol	6.6 mg	2
Protein	0.72 g	1
Carbohydrate	5.7 g	2
Dietary fiber	0.32 g	1
Protein	0.72 g	1
Sodium	9.0 mg	0
Potassium	38.8 mg	1

Table 4 Trace elements in truffles

Trace elements	*Percent*
Vitamin A	1
Calcium	2
Vitamin D	0
Thiamin	1
Niacin	0
Vitamin B_6	0
Phosphorus	2
Selenium	1
Vitamin C	0
Iron	1
Vitamin E	0
Riboflavin	2
Vitamin B_{12}	1
Manganese	0
Copper	2
Magnesium	1
Zinc	1

similar dishes. Black truffles have the strongest flavor and a pungent smell, and black truffle oil has an earthy smell. White truffles, on the other hand, have a tendency to take on the characteristics of the soil they are found in, meaning they have a more delicate taste and, often, a garlic-like aroma.

Handling and Storage of Fresh Truffles

After buying fresh truffles, they should be cleaned by rubbing gently with a small brush under running water. Truffles are highly perishable and need to be consumed immediately after harvesting: they tend to dehydrate (at room temperature they lose 1–2% of their weight every day) and lose their scent. Often, truffles are sold in containers filled with rice, which absorbs the flavor and aroma of the fungi and can be used for cooking. Truffles can be stored between uses in dry paper towel, which is changed daily, in an airtight glass jar at 2–5 °C for about 1–2 weeks.

Health Benefits: Mode of Action of Mushrooms and Truffles

Mechanisms through which mushrooms and truffles influence health remain an emerging area of research. Moreover, the chemical composition and total intake determine any potential health attributes.

Gut Microbiota

The relationship between host and gastrointestinal microbiota are increasingly recognized to be crucial for health. A healthy microbiota controls pathogens, promotes development and appropriate response of the immune system, and supports tolerance of food antigens and harmless microbes. Consuming 1% of the total dietary intake of white button mushrooms (equivalent to 75 g fresh weight) in a restricted diet increased gut bacterial diversity. In two different models of gastrointestinal injury (dextran sulfate sodium treatment and *Citrobacter rodentium* infection), resolution and recovery were improved by mushroom consumption, including weight gain and colonic length, and reduced hyperplasia and immune infiltrates in the colon histopathology. These findings highlight the potential of white button mushrooms to reduce risk of diseases where the microbiota has been shown to have an impact. White button mushrooms also influence gastrointestinal tract healing through the action of prebiotics, nondigestible food ingredients that stimulate the growth and/or activity of commensal bacteria. It has been hypothesized that the microbiota and host both use nutrients present in white button mushrooms, and the microflora make bioactive compounds from the polysaccharides in mushrooms.

Immunomodulatory Effect

Dietary mushrooms have been reported to improve the activity of natural killer cells, which are an important component of the innate immune system responsible for antiviral and antitumor defense. Increased natural killer cells activity may be mediated through increased production of interferon gamma and tumor necrosis factor alpha. Mushroom consumption appears to elicit a shift toward T-helper 1 response, and there is a trend for higher interleukin-2 production and lymphocyte proliferation. Mushroom supplementation *in vitro* has been shown to promote maturation and function of dendritic cells, which are the most important antigen-presenting cells bridging innate and adaptive immunity, suggesting an enhanced development of adaptive immunity. Dendritic cells maturation was enhanced by changes in dendritic cell function, including increased interleukin-2 production, reduced phagocytosis, and enhanced antigen presenting function, resulting in an elevated T-cell response to the specific antigen. These results suggest that mushrooms may have the potential to improve the development of adaptive immunity after initial exposure to an antigen or pathogen.

Poisonous Wild Mushrooms

Some mushrooms are poisonous and symptoms range from mild gastrointestinal discomfort to death. For example, a wild species of mushroom, *Amanita phalloides*, famously known as the 'death cap,' contains amatoxins that cause severe gastroenteritis and hepatic necrosis. The toxin of *Psilocybe semilanceata* (liberty cap) is a potent hallucinogen; anxiety and peripheral sympathomimetic symptoms may follow ingestion. Another species, *Amanita pantherina* (panther cap) may cause severe anxiety and agitation, hallucinations, and peripheral anticholinergic symptoms. The toxin in *Cortinarius speciosissimus* has an almost exclusively nephrotoxic action.

The most common reason for poisoning is misidentification or the close resemblance of edible mushroom species to toxic mushrooms. Thus, an expert should inspect mushrooms before consumption. Most importantly, there are no antidotes for mushroom poisoning and symptoms can only be treated in the hospital, and may still be fatal. It is worth noting that:

- Symptoms can appear after 12 h or longer following consumption.

- Toxins will remain in the system and continue to cause harm, meaning raw samples should be retained for (correct) identification.
- While casual handling of poisonous mushrooms is not problematic, these should not be mixed with edible mushrooms if harmful contamination of foods is to be avoided.
- Some people are allergic to edible mushrooms.

See also: Food and Agriculture Organization of the United Nations; Fungi; Single Cell Proteins; World Health Organization.

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Mustard

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Introduction

Mustard (*Brassica* spp.), a native to temperate regions of Europe, was one of the first domesticated crops. This crop's economic value resulted in its wide dispersal, and it has been grown as a herb in Asia, North Africa, and Europe for thousands of years. Ancient Greeks and Romans enjoyed mustard (*Sinapis*) seed as a paste and powder. In about 1300, the name 'mustard' was given to the condiment made by mixing mustum, which is the Latin word for unfermented grape juice, with ground mustard seeds.

Mustard has been a major specialty crop in North America since supplies from Western Europe were interrupted by World War II. California and Montana were the major production areas until the early 1950s. The production of mustard in the Upper Midwest began in the early 1960s. Mustard is currently grown on approximately 250 000 acres annually in the United States. Canada is the world's largest producer of pure mustard seed, accounting for an average 28% of the world crop (Table 1). Canadian mustard production increased for 20 years until it peaked in the mid-1980s. Alberta, Manitoba, and Saskatchewan currently grow a large share of the world's mustard crop. The French people are the largest consumers of mustard (1.5 lbs/person/year) and buy approximately 70% of the annual Canadian production. Three types of mustard, yellow, brown, and oriental, are grown in North America. Yellow mustard (*Brassica hirta*) comprises about 90% of the crop in the Upper Midwest. In Europe, yellow mustard is also known as white mustard (*Sinapis alba*) – an older botanical name.

Ignoring mustard/rapeseed blends that are grown on the Indian subcontinent, world mustard seed production averages just under 529 000 Mt year^{-1}. Most of this production is destined for the condiment and spice trade.

Mustard has been used as a spice for over 5000 years, with references appearing in ancient Sumerian and Sanskrit texts around 3000 BC, Egyptian texts around 2000 BC, and Chinese texts around 1000 BC. Its use as a condiment is thought to have started with the Romans. The production of Dijon mustard in France started around the tenth century. In recognition of the long tradition of making mustard, Dijon was granted an appellation d'origine contrôlée in 1937. The first use of mustard as a hot dog condiment was reported at the St. Louis World's Fair in 1904 when R. T. French Company introduced its mild prepared mustard, which is bright yellow due to turmeric.

General Description and Cultivation

Brassica juncea (L.) Czern. belongs to the Cruciferae (Brassicaceae) plant family, commonly known as the mustard family. The name crucifer is derived from the shape of the flowers that have four diagonally opposed petals in the form of a cross. *B. juncea* has pale green foliage, with a few hairs on the first leaves and leaf blades that terminate well up the petiole. Mature *B. juncea* plants grow to a height of 1–2 m. The lower leaves are deeply lobed, while the upper leaves are narrow and entire. *B. juncea* is distinct from its close relatives *B. napus* and *B. rapa* in that the upper leaves of *B. juncea* are not clasping. The inflorescence is an elongated raceme, and the flowers are pale yellow and open progressively upward from the base of the raceme. The seed pods are slightly appressed and 2.5–5 cm in length exclusive of the beak. The beak is 0.5–1 cm long. Seeds are round and can be yellow or brown.

There are both vegetable and oilseed varieties of *B. juncea* that possibly have different origins. Both types are considered to be natural amphidiploids (AABB genome, $2n=36$) of *B. rapa* (AA genome, $2n=20$) by *B. nigra* (BB genome, $2n=16$) crosses. It has been shown by molecular analysis that *B. juncea* contains conserved genomes of the progenitor species. *B. juncea* can be divided into four subspecies, with different morphologies, quality characteristics, and uses.

1. *B. juncea* ssp. *integrifolia*, used as a leaf vegetable in Asia
2. *B. juncea* ssp. *juncea*, cultivated mainly for its seed, occasionally as fodder
3. *B. juncea* ssp. *napiformis*, used as a root-tuber vegetable
4. *B. juncea* ssp. *taisai*, stalks and leaves being used as vegetables in China

For spice purposes, the appearance of the seed is particularly important, and therefore, the crop is grown in more southerly, drier regions where the risk of green seed in the harvested crop is reduced. Compared with the more widely grown canola species *B. napus* and *B. rapa*, *B. juncea* is more tolerant to heat and drought stress. The species does not shatter as readily as *B. napus* and so it can be straight cut or swathed and combined. Features of few cultivated species (Table 2) of mustard (Cruciferous) group of crops have been reported.

Crop Production and Nutrient Management

Cultivation of oilseeds that require high *S* is concentrated in rainfed regions. Maintenance of optimum organic matter level is imperative to avoid multiple nutrient deficiencies. Among micronutrient disorders, Zn deficiency in soils is the most common. In general, mustard is grown on coarse textured soils, very low in organic matter. Under such situations, organic manures can be exploited to boost the production of rapeseed–mustard and to improve fertilizer use efficiency. Balanced fertilization is all the more essential even at low levels of fertilizer usage for maintaining long-term fertility.

Mustard is reported to respond to zinc application at 10–20 kg ha^{-1} as basal spray or 0.5% as foliar spray. Next to

zinc, boron nutrition is important to mustard. Deficiency of boron could be corrected either through soil application or foliar application of borax. *Azotobacter* and *Azospirillum* are the two major microorganisms that are found to help rapeseed–mustard group of crops to fix atmospheric N. The associative and antagonistic actions of soil microflora and organic matter content of the soil greatly influence the growth of *Azotobacter* and its N-fixing ability. The lack of organic matter in mustard-growing areas is a limiting factor, and the beneficial effects of small amounts of humus on the growth of *Azotobacter* and its N fixation are well documented.

Technologies like resource conservation technology, crop contingency planning, precision farming, crop ecological zoning, and crop modeling and simulation are some of the key areas of innovative agrotechniques. Resource-conserving technologies improve the efficiency of the use of natural resources, including water, air, fossil fuels, soils, inputs, and people. Resource-conserving technologies have been successfully developed, tested, and deployed for rice–wheat cropping system. The development of suitable machinery, studies on residue management in oilseed crops, development and agronomic testing of varieties suitable for zero tillage conditions, impact of land leveling on rapeseed–mustard production, productivity and input use efficiency, etc., are some of the indicative areas of research gaps that need to be addressed.

Chemical plant growth regulators are being increasingly used as an aid to yield enhancement. Brassinolide is the most bioactive form of the growth-promoting plant steroid termed as brassinosteroids. Biologically active brassinosteroids show high growth-promoting as well as antistress activity besides other multiple effects on growth and development. NPK accumulation and yield were maximal when spraying of GA3 was done at 40 DAS.

Table 1 World's top 10 mustard seed traders (5-year average trading volume in metric tons)

Exporters	*Quantity*	*Importers*	*Quantity*
Canada	141 660	United States	64 812
Ukraine	26 977	Germany	45 973
Germany	15 224	Bangladesh	31 685
Czech Republic	14 412	France	23 796
Russia	13 949	The Netherlands	16 366
The Netherlands	11 075	Nepal	14 904
Belgium	7067	Poland	10 043
Hungary	3279	Belgium	9093
India	2598	Japan	5933
Romania	2552	The United Kingdom	3648

Source: United Nations Food and Agriculture Organization (FAO). The average annual trading volume for the period between 2006 and 2010

The Mustard Species

Black mustard plants are tall (up to 3 m) and sparsely branched, and they produce many short pods (sliques). As the pod matures, the highly pungent, small (1.5 g per 1000 seeds), round, dark brown seeds are shed, necessitating frequent hand harvesting or cutting and stacking immature plants on the threshing floor.

The characteristic seed shedding and seed dormancy of black mustard made it unsuited to monoculture and mechanized agriculture, and the spice trade turned to the production of a closely related species, *B. juncea* (L.) Czern and Coss. Plants of *B. juncea*, when compared to black mustard, are shorter (1–2 m) and have many upright, heavily podded branches with longer pods that retain their seeds when ripe. The seed is larger (3 g per 1000 seeds) but produces the same pungency. The seed color is either brown (brown mustard) or yellow (oriental mustard).

The species originated from a natural cross between *B. nigra* and *B. rapa* L. (turnip rape) followed by chromosome doubling to produce a vigorous and productive interspecific hybrid. This interspecific cross is believed to have occurred more than once where the two species occupied the same region. Possible centers of origin are believed to be North Africa, northern India and Pakistan, and western China.

The third condiment mustard species, *Sinapis alba* L., is called yellow or white mustard and produces a different pungency from both *B. nigra* and *B. juncea* seeds. From its Mediterranean center of origin, it has been widely disseminated throughout the temperate regions where day lengths (hours of sunshine) were sufficient to stimulate flowering. When moistened, the ripe seed will exude a mucilage from its yellow seed coat to form a whitish coating when dry. This may explain why white mustard is the common name in Europe, while in North America, it is called yellow mustard.

Plants of yellow mustard are shorter (0.6 m) than either *B. nigra* or *B. juncea* and have deeply lobed leaves. The short,

Table 2 Salient features of cultivated species of mustard (Cruciferous) group of crops

No.	*Common name*	*Botanical name*	*Days to maturity (days)*	*Yield potential (kg ha^{-1})*	*Oil (%)*
1	Indian mustard	*Brassica juncea*	105–160	1500–3000	38–42
2	Yellow mustard	*Brassica rapa* var. yellow sarson	120–155		41–47
3	Brown sarson	*Brassica campestris* syn. *B. rapa* var. brown sarson	100–235	900–2000	40–45
4	Black mustard	*Brassica nigra*	70–90	1000–1200	40-41
5	Karan rai	*Brassica carinata*	150–200		36–43
6	Toria	*Brassica rapa* var. *toria*	70–100	600–1800	36–44
7	Taramira	*Eruca sativa*	140–150	700–1400	34–38
8	Gobhi sarson	*Brassica napus*	145–180	1300–2700	37–45

Source: Shekhawat, K., Rathore, S. S., Premi, O. P., Kandpal, B. K., and Chauhan, J. S. (2012). Advances in agronomic management of Indian mustard (*Brassica juncea* (L.) Czernj. Cosson): an overview. *International Journal of Agronomy*, Article ID 408284, 14 pp, http://dx.doi.org/10.1155/2012/408284

hairy pods, with flat beaks, contain and retain 5–6 seeds when ripe. The seed is significantly larger than the other mustard species (6 g per 1000 seeds), which aids in rapid seedling establishment. In Europe, the crop is frequently sown and plowed under as a green manure crop.

Centers of Origin

The origins of *B. juncea* are unclear. *B. juncea* is an amphidiploid with a chromosome number of 18. It is believed to have derived from natural interspecific hybridization between *B. nigra* ($n=8$) and *B. rapa* ($n=10$). *B. juncea* likely originated where distributions of *B. nigra* and *B. rapa* overlap, such as in the Middle East and neighboring regions. The foliage types (Chinese) and oleiferous types (Indian) have suggested separate origins, a concept supported by the glucosinolate studies. The view that *B. juncea* probably evolved somewhere between Eastern Europe and China is also supported.

Reproductive Biology

B. juncea varieties are annuals, usually self-pollinated, pollinated by bees, at times outcrossing has been reported among closely located plants. The pollen is heavy and sticky. Outcrossing using a herbicide resistance marker in resistant *B. juncea* surrounded by nonresistant plants has been studies. Gene flow data for *B. napus* involving gene flow between commercial fields of cultivars with different herbicide resistance traits have been studied. Many variables affect outcrossing between fields, including size of fields, fertility of recipient plants, environmental conditions, and the presence of insect pollinators.

Crop Breeding and Improvement Programs

B. juncea breeders aim to make simultaneous improvement of agronomic performance, disease resistance, and quality traits. Agronomic traits include yield, lodging, maturity, herbicide tolerance, drought tolerance, shattering resistance, and seed size. Disease resistance breeding efforts may include blackleg, white rust, *Alternaria* black spot, and *Fusarium* wilt resistance. For mustard varieties, low oil content, high glucosinolate content, and a fatty acid profile with a moderate level of erucic acid are desired.

Most varieties of mustard are pure lines, developed through repeated cycles of inbreeding or through the use of doubled haploid technology. As with most inbred crops, developing a new variety begins with the creation of F1 hybrid seed by crossing two or more parents. The aim is to combine the desirable traits from the parents into a new variety. In pedigree breeding programs, F2 populations are created by selfing the F1. Selection of highly heritable traits may begin in the F2 generation. Selection will continue on either a single plant or family basis for several generations. Selection for complex traits such as yield will wait until the breeding line is reasonably uniform. Doubled haploid technology is also used, where immature pollen grains from F1 plants are grown through tissue culture techniques to produce haploid plants. A mitotic inhibitor such as colchicine is used to develop homozygous breeding lines. Molecular markers are sometimes employed to help select for traits that are difficult to measure and to help select parents or pollen donor plants.

The major crop breeding programs focus on

- improving oil and seed yield,
- hybrid development,
- stabilizing yield through insulation of cultivars against major biotic (white rust, *Alternaria* blight, sclerotium rot diseases, and aphid insect) and abiotic stresses (drought and salinity),
- improving oil (low erucic acid) and seed meal (low glucosinolate) quality,
- developing suitable varieties for nontraditional areas.

Improved Varieties

There are several varieties of yellow mustard with desirable qualities like earlier maturing, lower yielding, and shorter in height than brown or oriental varieties. Yield differences among the types of mustard are reflected usually in the prices offered by contracting firms. Some mustard varieties include the following:

Gisilba: yellow mustard; similar to ochre in field performance; originated in Germany; distributed by Northern Sales Co. Ltd., Winnipeg; licensed in 1974

Kirby: yellow mustard; released by Colman Foods, Norwich, England, in 1970; distributed by Minn-Dak Growers Association, Grand Forks, ND

Ochre: yellow mustard; released by Agriculture Canada, Saskatoon; licensed in 1981

Tilney: yellow mustard; similar to *Kirby* in field performance but has a high mucilage content desired by processors; released by Colman Foods, Norwich, England, in 1978; distributed by Minn-Dak Growers Association, Grand Forks, ND

Carrow 85: oriental mustard; undesirable small seed; released by Colman Foods, Norwich, England, in 1980

Domo: oriental mustard; released by Agriculture Canada, Saskatoon; licensed in 1977

Lethbridge 22A: oriental mustard; released by Agriculture Canada, Saskatoon, in 1967; licensed in 1974

Blaze: brown mustard; released by Agriculture Canada, Saskatoon; licensed in 1976

Improved Indian varieties with desirable traits like salinity, high temperature tolerance, high oil content, earliness, intercropping, frost tolerance, drought (rainfed) tolerance, low erucic acid/glucosinolate, and white rust resistance and varieties for nontraditional areas and late sowing have been developed and are listed in Table 3.

The Chemistry of Mustard

All three mustard species contain a significant amount of edible oil and high quality protein. On the Indian subcontinent, *B. juncea* seeds are the second most important source of edible

Table 3 Improved varieties of Indian mustard for specific environmental conditions

Stress/situation/ condition	*Varieties recommended*
Salinity	Indian mustard: CS-52, CS-54, CS 234-4, Narendra Rai-1
High-temperature-tolerant	Indian mustard: Kanti, Pusa Agrani, RGN-13, Urvashi
High oil content	Indian mustard: Narendra Swarna Rai 8
Earliness	Indian mustard: Kanti, Narendra Ageti Rai 4, Pusa Agrani, Pusa Mahak
Intercropping	Indian mustard: RH-30, RH781, Vardan
Nontraditional areas	Indian mustard: Pusa Agrani, Pusa Jaikisan, Gujarat Mustard 2
Late-sown	Indian mustard: Ashirwad, RLM 619, Swaran Jyoti, Vardan, Navgold
Frost-tolerant	RGN13, RH-781, Swaran Jyoti
Drought (rainfed)	Indian mustard: RH-819, RH-781, GM1, Pusa Bahar, Pusa Bold, Aravali Mustard, Sej-2, JD-6, Geeta, RGN-48, RL-99-27, Shivani, PBR-97
Low erucic acid/ glucosinolate	Indian mustard: Pusa Karishma, Pusa Mustard 21, LET-17, LET-18 Gobhi Sarson: Hyola 401, GSC 5, GSC 6, NUDB 26-11, Teri Uttam Jawahar
White rust resistance	Indian mustard: Basanti, JM 1, JM 2, Maya

Source: www.circ.org/fileadmin/documents/Bulletins/B25/B25_06.

vegetable oil, and the residual high protein meal, after it is soaked in water, is fed to cattle. However, for the spice trade, the important ingredient is the presence and concentration in the seeds of sulfur compounds called glucosinolates. Over 40 such compounds are known, and their presence and quantity determine the flavor and odor of the cole vegetables as well as the taste and heat of the mustards. The glucosinolate that imparts the pungency and flavor to black and *B. juncea* mustards is called 'sinigrin,' while in *Sinapis alba*, it is 'sinalbin.' When the seeds are stored whole and dry, they retain their quality for several years. However, when the cells of a mustard seed are broken and moisture is present, the enzyme myrosinase, also present in the seed, breaks down the glucosinolates to release sugar, sulfur, and the hot principles called isothiocyanates. Black and *B. juncea* mustard seeds release the pungent, volatile, biting allyl isothiocyanate found in powdered and Dijon mustards. Yellow mustard, on the other hand, releases the milder, nonvolatile *para*-hydroxybenzyl isothiocyanate.

Comparing protein and oil contents of yellow and brown varieties, it was observed that yellow mustard has higher protein content (36.73%) than brown mustard (32.48%), whereas the oil content was higher in brown mustard (36.32%) when compared with yellow mustard (31.78%).

The flavor of mustard seeds is derived from glucosinolates, which are isothiocyanate glycosides. Sinalbin is responsible for the flavor of white mustard seed, and sinigrin is responsible for the sharper taste associated with black and brown mustards. The pungency is due to glucosinolates, which are hydrolyzed by myrosinase enzyme to flavor active isothiocyanates. Allyl isothiocyanate (AITC) has the potential to be used as flavoring, antibacterial, antifungal, antifermentative, and antibrowning agent in food industry.

The aroma and pungent flavor of mustards come from the essential oil *sinalbin*. This compound releases isothiocyanate chemicals upon enzymatic reaction mediated by myrosinase: a glycoside (sinigrin) and an enzyme (myrosin); in contact with water, myrosin acts on sinigrin, setting free AITC (mustard oil) responsible for mustard's pungent smell.

Phytonutrients

Mustard seeds contain high amounts of phytonutrients, particularly glucosinolates, according to a 1990 study published in *Plant Science*. Glucosinolates are sugar-derived compounds that are found naturally in many flowering plants. A study published by the *International Journal for Vitamin and Nutrition Research* in 2002 found that glucosinolates found in plants may have a protective effect against certain types of cancer, especially lung cancer.

Healthy Minerals

Mustard seeds contain a variety of minerals, including iron, magnesium, zinc, calcium, and phosphorus. Getting adequate amounts of these minerals is essential for the normal function of many biological and biochemical processes within your body and can help prevent a mineral deficiency, which can have potentially debilitating side effects. According to USDA National Nutrient Database for Standard Reference, ground mustard seed contains 26.6% of your daily calcium, 51.2% of your daily iron, 92.5% of your daily magnesium, 40.5% of your daily zinc, and 82.8% of your daily phosphorus.

Omega-3 Fatty Acids

Mustard seed is acidic and a source of omega-3 fatty acids, according to the book, *Today's Herbal Health: The Essential Reference Guide*. The University of Maryland Medical Center states that omega-3 fatty acids found in some foods can benefit individuals with high cholesterol, high blood pressure, heart disease, diabetes, rheumatoid arthritis, systemic lupus erythematosus, osteoporosis, depression, bipolar disorder, schizophrenia, attention-deficit disorder, skin disorders, inflammatory bowel disease, asthma, macular degeneration, menstrual pain, and some cancers. However, omega-3 fatty acids are not intended to treat or prevent any disease. Consult with your physician before taking food items or supplements containing omega-3 fatty acids.

Crop Protection and Disease Management

This crop is vulnerable to several diseases, among which the most serious are sclerotium stalk rot (white mold), downy mildew, white rust, leaf spots, and mosaic virus. Good cultural practices are the most effective control measures for diseases. These practices should include keeping records of disease occurrence, compliance with the proper crop rotation, control of host plants for diseases in fallow fields and non-crop areas, and the use of seed treatments.

Sunflower, rapeseed, canola, safflower, soybeans, crambe, and dry beans should not be grown in rotation with mustard since they have similar disease problems. If these crops are produced in rotation with mustard, damage from these diseases can increase to economic levels. Numerous broadleaf weeds can also serve as hosts or sources of infection for these diseases. Wild mustard, pigweed, field pennycress, and shepherd's purse are examples of predominant hosts. One of the best methods to avoid serious disease problems in mustard (and leaf diseases of small grains) is to produce this crop in a small grain rotation. Mustard should be spaced four or more years apart in the crop rotation to avoid problems with soilborne diseases. After potato or flax crops, 1 year should pass before mustard is raised on the same field due to the presence of root rot or damping-off pathogens.

The following strategies are helpful for disease management:

- Choosing the sowing time is very important as it affects the disease incidence significantly.
- Deep summer plowing.
- The use of disease-resistant or disease-tolerant or early maturing disease-escaping cultivars.
- The use of good quality seeds.
- Seed treatment with biocontrol agents, namely, *Trichoderma viride* and *Gliocladium virens*, or botanicals like *Allium sativum* bulb extract (1% w/v) or carbendazim at 0.1% a.i. or mixture of carbendazim and Apron 35 SD (6 g kg^{-1}). There is a need for mixture of fungicides for avoiding resistance development in pathogens to fungicides. The use of biocontrol agents is advantageous as they are often effective against a wide range of soilborne pathogens. Moreover, they are eco-friendly and cost-effective and their use avoids the risk of development of resistance in the pathogen towards the control agent.
- Application of recommended doses of N, P, and K fertilizers with split application of N.
- Maintenance of optimum plant population with recommended spacing.
- Proper drainage to avoid water stagnation.

Insects

These crops are highly vulnerable to a large number of insect pests. About 50 insects are known to damage *Brassica* crops. Among these insects, mustard aphid (*Lipaphis erysimi*) is the key pest of the Brassica crops, while sawfly (*Athalia proxima*) and painted bug (*Bagrada cruciferarum*) cause yield losses. Yield losses due to aphid may be up to 97% and sawfly and painted bug up to 15% and 30%, respectively. The period of peak activity for aphids is January–February in most part of the country. The economic threshold level values for aphid in rapeseed–mustard for different states have also been worked out. The integrated insect management strategies are as follows:

- Crops should be sown at optimum time recommended for particular area.
- Overuse of fertilizers particularly nitrogen attracts more aphids; hence, the use of balanced dose of fertilizers is recommended.
- Plucking and destruction of infested twigs/plants at initial stage of attack are very useful.
- Plucking the infested leaves and burying them in soil help in the reduction of the pest.
- Plant extracts of *Azadirachta indica*, *Lantana camara*, and *Ipomoea carnea* proved toxic to mustard aphids.
- Microbial pesticides have been widely used in the management of insect pest and avoiding the development of resistance. The use of *Verticillium lecanii* against the mustard aphid and other aphids has been found encouraging.

With no available sources of durable resistance in the presently cultivated varieties of rapeseed–mustard, efforts are being made throughout the world to develop stable disease resistance in oilseed brassicas by utilizing the contemporary tools of biotechnology. The *Alternaria* blight pathogen and various technologies, namely, embryo rescue, somatic hybridization, somaclonal variations, genetic transformation, molecular markers, and signal transduction, being used for the incorporation of resistance against the pathogen in oil-yielding brassicas have been described as the studies on the diversity of sclerotium isolates.

Utilization of Biotechnological Tools

Biotechnological techniques like tissue culture and genetic transformation have revolutionized the search for economically promising disease-resistant crops, helping to combine the traits from distantly related *Brassica* sp. for the development of superior *Alternaria* blight-tolerant varieties of *Brassica*.

In vitro embryo rescue technique was proved to be very efficient for transferring *Alternaria* blight tolerance in oilseed brassicas. *In vitro* ovary culture was used to transfer the resistance against *Alternaria* blight from *B. tournefortii* to *B. juncea* 'RH 30'. Intergeneric hybrids of *B. campestris* and *B. spinenscens* have been developed and multiplied using somatic embryogenesis. The resistance trait from *Sinapis alba* 'Carine' was transferred to *B. napus* 'Brutor' using *in vitro* fertilized ovary culture. Intergeneric hybrids with *Alternaria* blight resistance were developed between *Erucastrum cardaminoides* and *B. oleracea* var. *alboglabra* using sequential ovary and ovule culture. Interspecific hybridization in combination with *in vitro* ovule culture was used to incorporate high tolerance to *Alternaria* blight and white rust.

Somatic hybridization has been utilized effectively in brassicas, but the studies are limited to *B. napus* and *B. oleracea*. Attempts to transfer *Alternaria* resistance trait from *B. carinata* to *B. juncea* have been made. Leaf mesophyll protoplasts from *Moricandia arvensis* and *B. napus* were hybridized to produce disease-resistant hybrid plants. *B. carinata* has been resynthesized by protoplast fusion between *B. nigra* and *B. oleracea*. The hybrids thus obtained were fertile and grew into robust plants.

Studies have shown the use of somaclonal variations for the incorporation of disease resistance/tolerance against *Alternaria* blight. Resistance to susceptible varieties of *B. juncea* was achieved by inducing variations through mutagenesis, by exposing the seeds to gamma radiations, and by treating the

microspores/microspore-derived embryos with chemical mutagens.

Transformation systems have been developed in almost all the economically important species of *Brassica* and *B. nigra*.

Uses

In general, three main varieties of mustard are grown worldwide for use:

- White mustard seeds (*Brassica alba*): The seeds are light straw yellow-colored and are slightly larger than the other two varieties. White seeds exhibit mild pungency.
- Black mustard seeds (*Brassica nigra*): The seeds are commonly seen in South Asia. The seeds are sharp and more pungent than other two varieties.
- Brown mustard seeds (*Brassica juncea*): The seeds are native to sub-Himalayan plains of northern India.

Nutritional Benefits of Mustard Seeds

Mustard seeds are indeed very rich in phytonutrients, minerals, vitamins, and antioxidants (Table 4) and are rubefacient, irritant, stimulant, diuretic, emetic, pungent, and laxative and aid in digestion. This well-known spice has its use in medicine as a stimulant when applied externally. The rubefacient action causes a mild irritation to the skin, stimulating the circulation in that area and relieving muscular and skeletal pain. Its stimulating, diaphoretic action can be utilized in the way that cayenne and ginger are. An infusion or poultice of mustard will aid in cases of bronchitis:

- Mustards are very high in calories; 100 g of seeds provides 508 cal, and the seeds are made of quality proteins, essential oils, vitamins, minerals, and dietary fiber.
- The seeds are high in essential oils as well as plant sterols. Some of important sterols include *brassicasterol*, *campesterol*, *sitosterol*, *avenasterol*, and *stigmasterol*. Some of glucosinolate and fatty acids in the seeds include *sinigrin*, *myrosin*, *erucic*, *eicosenoic*, *oleic*, and *palmitic acids*.
- Mustard seeds are an excellent source of essential vitamin B complex such as folate, niacin, thiamin, riboflavin, pyridoxine (vitamin B_6), and pantothenic acid. These vitamins are essential in the sense that the body requires them from external sources to replenish. These B complex groups of vitamins help in enzyme synthesis, nervous system function, and regulating body metabolism.
- 100 g of mustards provides 4.733 mg of niacin (vitamin B_3). Niacin is a part of nicotinamide coenzymes and helps lower blood cholesterol and triglyceride levels.
- Mustard seeds contain flavonoid and carotenoid antioxidants such as carotenes, zeaxanthin, and lutein. In addition, the seeds comprise a small amount of vitamin antioxidants such as vitamins A, C, and vitamin K.
- The seeds are a source of vitamin E and gamma-tocopherol and contain about 19.82 mg per 100 g. Vitamin E is a powerful lipid-soluble antioxidant, required for maintaining the integrity of cell membrane of mucus membranes and skin by protecting it from harmful oxygen-free radicals.
- Mustards are a rich source of health-benefiting minerals. Calcium, manganese, copper, iron, selenium, and zinc are some of the minerals especially concentrated in these seeds. Calcium helps build bone and teeth. Manganese is used by the body as a cofactor for the antioxidant enzyme *superoxide dismutase*. Copper is required in the production of red blood cells. Iron is required for the red blood cell formation and cellular metabolism.

Phytonutrient Compound

Like other *Brassicas*, mustard seeds contain plentiful amounts of phytonutrients called *glucosinolates*. The seeds also contain *myrosinase enzymes* that can break apart the glucosinolates into other phytonutrients called *isothiocyanates*. The isothiocyanates in mustard seed (and other *Brassicas*) have been repeatedly studied for their anticancer effects. In animal studies – and particularly in studies involving the gastrointestinal tract and colorectal cancer – intake of isothiocyanates has been shown to inhibit growth of existing cancer cells and to be protective against the formation of such cells.

Anti-inflammatory Effects from Selenium and Magnesium

Mustard seeds emerged from our food ranking system as an excellent source of selenium, a nutrient that has been shown to help reduce the severity of asthma, decrease some of the symptoms of rheumatoid arthritis, and help prevent cancer. They also qualified as a good source of magnesium. Like selenium, magnesium has been shown to help reduce the severity of asthma, to lower high blood pressure, to restore normal sleep patterns in women having difficulty with the symptoms of menopause, to reduce the frequency of migraine attacks, and to prevent heart attack in patients suffering from atherosclerosis or diabetic heart disease.

Mustard seeds also qualified as a very good source of omega-3 fatty acids and manganese as well as a good source of phosphorus, copper, and vitamin B_1.

Medicinal Uses

- Mustard seeds and its oil have traditionally been used to relieve muscle pain, rheumatism, and arthritic pain. In India, mustard oil is applied over the scalp and is believed to stimulate hair growth. Its ground seeds act as a laxative and stimulant to gastric mucosa and increase intestinal secretion. Mustards have been used in traditional folk medicine in Turkey, to treat a variety of ailments including peritonitis and neuralgia. They are used in mustard plasters to treat rheumatism, arthritis, chest congestion, aching back, and sore muscles by making a mustard plaster with equal parts of flour and powdered mustard and spreading it on a doubled piece of soft cloth and applying it to the affected area for about 15 min.
- *Anticarcinogenic properties*: Mustard seeds are suggested to prevent and slow the progress of cancers especially in the gastrointestinal tract. Evidences also suggest that mustard seeds may restrict the growth of already present cancer cells and prevent the formation of new cancers. AITC, which occurs in many common cruciferous vegetables, was

Table 4 Nutrient profile of mustard seeds

Basic macronutrients and calories		
Nutrient	*Amount*[a]	*DRI/DV (%)*
Protein	1.04 g	2.08
Carbohydrates	1.12 g	0.50
Fat – total	1.45 g	–
Dietary fiber	0.49 g	1.96
Calories	20.32	1.13
Macronutrient and calorie detail		
Carbohydrate		
Starch	–	
Total sugars	0.27 g	
Monosaccharides	0.12 g	
Fructose	0.00 g	
Glucose	0.12 g	
Galactose	0.01 g	
Disaccharides	0.15 g	
Lactose	0.00 g	
Maltose	0.00 g	
Sucrose	0.15 g	
Soluble fiber	–	
Insoluble fiber	–	
Other carbohydrates	0.36 g	
Fat		
Monounsaturated fat	0.90 g	
Polyunsaturated fat	0.40 g	
Saturated fat	0.08 g	
Trans fat	0.00 g	
Calories from fat	13.05	
Calories from saturated fat	0.72	
Calories from trans fat	0.00	
Cholesterol	0.00 mg	
Water	0.21 g	
Micronutrients		
Vitamins		
Water-soluble vitamins		
Vitamin B complex		
Vitamin B_1	0.03 mg	2.50
Vitamin B_2	0.01 mg	0.77
Vitamin B_3	0.19 mg	
Vitamin B_3 (niacin equivalents)	0.35 mg	
Vitamin B_6	0.02 mg	1.18
Vitamin B_{12}	0.00 mcg	0.00
Biotin	–	–
Choline	4.91 mg	1.16
Folate	6.48 mcg	1.62
Folate (DFE)	6.48 mcg	
Folate (food)	6.48 mcg	
Pantothenic acid	0.03 mg	0.60
Vitamin C	0.28 mg	0.37
Fat-soluble vitamins		
Vitamin A (retinoids and carotenoids)		
Vitamin A international units (IU)	1.24 IU	
Vitamin A (mcg) retinol activity equivalents (RAE)	0.06 mcg (RAE)	0.01
Vitamin A (mcg) retinol equivalents (RE)	0.12 mcg (RE)	
Retinol (mcg) retinol equivalents (RE)	0.00 mcg (RE)	
Carotenoid (mcg) retinol equivalents (RE)	0.12 mcg (RE)	
Alpha-carotene	0.00 mcg	
Beta-carotene	0.72 mcg	
Beta-carotene equivalents	0.72 mcg	
Cryptoxanthin	0.00 mcg	
Lutein and zeaxanthin	22.72 mcg	

(*Continued*)

Table 4 (Continued)

Basic macronutrients and calories

Nutrient	*Amount*[a]	*DRI/DV (%)*
Lycopene	0.00 mcg	
Vitamin D		
Vitamin D international units (IU)	0.00 IU	0.00
Vitamin D (mcg)	0.00 mcg	
Vitamin E		
Vitamin E (mg) alpha-tocopherol equivalents (ATE)	0.20 mg (ATE)	1.33
Vitamin E international units (IU)	0.30 IU	
Vitamin E (mg)	0.20 mg	
Vitamin K	0.22 mcg	0.24
Minerals		
Boron	–	
Calcium	10.64 mg	1.06
Chloride	–	
Chromium	–	–
Copper	0.03 mg	3.33
Fluoride	–	–
Iodine	–	–
Iron	0.37 mg	2.06
Magnesium	14.80 mg	3.70
Manganese	0.10 mg	5.00
Molybdenum	–	–
Phosphorus	33.12 mg	4.73
Potassium	29.52 mg	0.84
Selenium	8.32 mcg	15.13
Sodium	0.52 mg	0.03
Zinc	0.24 mg	2.18
Individual fatty acids		
Omega-3 fatty acids	0.15 g	6.25
Omega-6 fatty acids	0.24 g	
Monounsaturated fats		
14:1 Myristoleic	0.00 g	
15:1 Pentadecenoic	0.00 g	
16:1 Palmitoyl	0.00 g	
17:1 Heptadecenoic	0.00 g	
18:1 Oleic	0.34 g	
20:1 Eicosenoic	0.16 g	
22:1 Erucic	0.37 g	
24:1 Nervonic	0.03 g	
Polyunsaturated fatty acids		
18:2 Linoleic	0.24 g	
18:2 Conjugated linoleic (CLA)	–	
18:3 Linolenic	0.15 g	
18:4 Stearidonic	–	
20:3 Eicosatrienoic	–	
20:4 Arachidonic	–	
20:5 Eicosapentaenoic (EPA)	–	
22:5 Docosapentaenoic (DPA)	–	
22:6 Docosahexaenoic (DHA)	–	
Saturated fatty acids		
4:0 Butyric	0.00 g	
6:0 Caproic	0.00 g	
8:0 Caprylic	0.00 g	
10:0 Capric	0.00 g	
12:0 Lauric	0.00 g	
14:0 Myristic	0.00 g	
15:0 Pentadecanoic	0.00 g	
16:0 Palmitic	0.04 g	
17:0 Margaric	0.00 g	
18:0 Stearic	0.02 g	

Table 4 (Continued)

Basic macronutrients and calories		
Nutrient	*Amount*[a]	*DRI/DV (%)*
20:0 Arachidic	0.01 g	
22:0 Behenate	0.01 g	
24:0 Lignoceric	0.00 g	
Individual amino acids		
Alanine	0.04 g	
Arginine	0.07 g	
Aspartic acid	0.09 g	
Cystine	0.03 g	11.54
Glutamic acid	0.20 g	
Glycine	0.06 g	
Histidine	0.03 g	3.33
Isoleucine	0.04 g	3.20
Leucine	0.08 g	2.91
Lysine	0.07 g	2.75
Methionine	0.02 g	2.99
Phenylalanine	0.04 g	4.49
Proline	0.11 g	
Serine	0.03 g	
Threonine	0.03 g	3.09
Tryptophan	0.01 g	3.85
Tyrosine	0.03 g	4.11
Valine	0.06 g	3.57

[a]Amount present is indicated in approximately 4 g of seeds. The designation '–' represents those nutrients for which no value was included in this version of the database. (http://www.whfoods.com/genpage.php?tname=nutrientprofile).

Source: The nutrient profiles provided are derived from The Food Processor, Version 10.12.0, ESHA Research, Salem, Oregon, USA.

recently shown to be selectively delivered to bladder cancer tissues through urinary excretion and to inhibit bladder cancer development in rats.

- Sinigrin, a major glucosinolate present in Indian mustard (*Brassica juncea* L.) seeds and leaf tissue, is also reported to be a precursor of the anticancer compound AITC.
- *Antioxidant properties and antiaging action*: Mustard seeds and powder contain the amino acid, cysteine, which acts as an antioxidant in your body. Cysteine protects our body from free radicals, which are harmful compounds in your body that damage DNA and cell membranes, according to the University of Maryland Medical Center. Cysteine may also be useful in individuals suffering from angina, bronchitis, influenza, and other conditions. Mustard is a great source of carotenes, zeaxanthins, and lutein (also called flavonoid and carotenoid antioxidants) and vitamins A, C, and K. All these components put together make it rich in antioxidants, which in turn slows the aging process.

Apart from these, mustard usage has been suggested to

- *Relieve symptoms of asthma*: Mustard seeds are high in selenium and magnesium. Both the components give it unique anti-inflammatory property. Consumed regularly, it is known to control and keep the symptoms of asthma, cold, and chest congestion at bay.
- *Prevent obesity*: The wonder seeds are packed with vitamins B complex like folate, niacin, thiamine, and riboflavin. Apart from all the individual merits of these components, they are great in speeding up one's metabolism leading to weight loss.
- *Lower cholesterol levels*: Mustard contains high levels of niacin or vitamin B_3. Niacin has properties that help lower cholesterol levels and protect the arteries from atherosclerosis (plaque buildup). It also helps to regulate blood flow and protects the body from hypertension.
- *Relieve constipation and symptoms of piles and fissures*: The seeds contain mucilage, which is a thick slimy substance that is the key to relieving constipation. Apart from that, it is also high in fiber
- *Fight skin infections*: Packed with sulfur, mustard seeds are a great way to curb skin infections. Sulfur gives the seed antifungal and antibacterial properties that help fight common skin ailments.
- *Improve immunity*: Because mustard has a large number of elemental minerals like iron, manganese, and copper, it helps improve the body's ability to fight disease.
- *Act as preservative*: Mustard oil is rich in tocopherols and acts against rancidity. This contributes to longer shelf life of finished food products.

Culinary Uses

- Mustards are used extensively in Indian, Pakistani, Bangladesh, Mediterranean, and German cooking. Whole seeds, ground or powdered form; prepared pastes; sauces; and oil are all used in cooking.
- Mustard seeds are used in condiments from time immemorial by Romans, Greeks, and Indians. Pilani a physician of Rome writes: "It is so pungent in flavour that it burns like fire, though at the same time it is remarkably wholesome

for the body." Used internationally as an appetizer, flavoring agent, and food preservative, its presence in the food and pickles prevents the growth of *Aspergillus niger* (black mold).

- Raw mustard seeds have a face-warping bitter quality, which seems to render the medicinal properties accrued to their use.
- But when treated with heat, mustard's fragrance transforms to an acceptable pungency that renders all preparations an appetizing flavor, making it an indispensable topping in all Indian curries. Powdered or crushed mustard is used in pickles, and the whole seeds are used to flavor vegetables and pulses, where they are fried in hot oil to give the flavor.

Mixing ground mustard seeds with water causes a chemical reaction between the enzyme myrosinase and glucosinolates such as sinigrin, myrosin, and sinalbin. The myrosinase enzyme turns the glucosinolates into various isothiocyanate compounds known generally as mustard oil. The concentrations of different glucosinolates in mustard plant varieties and the different isothiocyanates that are produced make different flavors and intensities. AITC and 4-hydroxybenzyl isothiocyanate render the sharp hot pungent sensation in mustards by stimulating the heat and acidity-sensing pain receptors of both oral and nasal passages. The gradual chemical breakup of 4-hydroxybenzyl isothiocyanate leads to the dissipation of heat from prepared mustard, over a period of time. Thioglucoside is hydrolyzed during the processing of mustard seeds, releasing glucose, which supplements the natural reducing sugars.

Skin Care

- *As a natural sunscreen*: Due to its thick consistency and high levels of vitamin E, topical application of this oil protects your skin against the harsh ultraviolet rays and other pollutants, thus preventing skin cancer. Vitamin E prevents aging and wrinkles besides acting as a sunshield.
- *Stimulates sweat glands*: Whether consumed or applied topically, mustard oil stimulates the sweat glands and opens the pores of the skin. Thus, it helps in reducing the body temperature and removes unwanted toxins, water, and salts from the body.
- *Treats rashes and infections*: Due to its antibacterial and antifungal properties, mustard oil is effective in treating rashes and other skin infections, thus preventing your skin from dryness, dullness, and itching. A body massage with mustard oil rejuvenates and cleans skin by increasing blood circulation. Besides, due to its anti-inflammatory properties, it is effective in reducing skin inflammation and facilitates quick healing of cuts and wounds.

The functional properties of Brown mustard seed and its flavor have been reviewed. The whole mustard seed has no flavor but can provide a pungent taste after chewing. The heat experienced in yellow mustard is on the tongue, whereas in brown and black mustards, the heat is also felt in the nose and eyes. The severity of pungent aroma varies with different mustards. The white or yellow mustard seed has a less pungent aroma than brown mustard seeds, which have a very pungent aroma. Black mustard seeds have the highest pungency. In ground mustard, aroma does not persist. However, flavor and pungency are experienced when enzymatic action is triggered in the presence of water, which releases mustard's flavor or pungency. It is due to a variety of isothiocyanate compounds that exist in mustard tissue as glycosides.

Conclusion

Mustard crop offers immense scope for further yield enhancement. Yield enhancement possibilities exist with improvement in crop production and crop programs. The development of crop production and protection techniques that are eco-regional in scope and simultaneously incorporate the constraints imposed by the natural resource availability of the region is the need of the hour. With the growing demand for edible oil crops like brassicas and the immense losses caused by the fungal pathogens like *Alternaria*, there is an urgent need to curb this pressure and focus on the production of disease-free superior brassica. Biotechnology has revolutionized the classical breeding process and has enabled the combining of traits from distant species for developing promising hybrids. Studies on host-pathogen interactions would help identify the genes activated during pathogenesis and the defense-related proteins released in response to the *Alternaria*. Integrating conventional breeding methods with the modern tools will help in evolving a disease management strategy in brassicas with superior agronomic traits.

See also: Cancer: Diet in Cancer Prevention; Garlic; Herbs: Composition and Dietary Importance; Peppers and Chilies; Spices and Flavoring Crops: Fruits and Seeds; Spices and Flavoring Crops: Uses and Health Effects; Vegetable Oils: Composition and Analysis; Vegetable Oils: Dietary Importance; Vegetable Oils: Oil Production and Processing; Vegetable Oils: Types and Properties.

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Mutagens

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Background

Mutagens are chemical compounds or forms of radiation (such as ultraviolet (UV) light or x-rays) that cause irreversible and heritable changes (mutations) in the cellular genetic material, deoxyribonucleic acid (DNA). Mutagenic lesions persist when they escape detection by protective cellular DNA repair mechanisms, when mistakes occur in the repair process, or when repair mechanisms are overwhelmed by extensive damage. Upon subsequent cellular replication, these mutations become permanent (*fixed*) in the genome and are inherited by all daughter cells. In this way, mutagenesis becomes a cumulative process, stretching over the lifetime of an organism.

The biological consequences of a mutation depend upon many critical factors such as the target loci, size of the mutation, timing during the cell cycle, and compounding effects of preexisting mutations. Thus, a mutagenic event occurring in a nonfunctional area of DNA will have no effect (*silent mutation*), whereas a similar change in an actively transcribed region may profoundly affect gene expression and phenotype or even lead to cell death (*lethal mutation*). The influence of mutations in human health is underscored by several human disease states caused by mutations that disrupt regulatory regions or gene coding sequences, resulting in altered gene expression and protein function. For example, mutations in genes that promote or inhibit growth and cellular replication (*proto-oncogenes* and *tumor suppressor genes*, respectively) or code for components of DNA repair pathways are important contributors to the multistage development of cancer. In addition, the accumulation of mutations over time, leading to gradually less efficient cellular repair capabilities, has been linked to the aging process and associated degenerative diseases. While the health of a particular individual can be affected by mutations in somatic cells, mutagenic events in germ cells (*germ line mutations*) lead to the transmission of genetic diseases to subsequent generations. Genetic predispositions to certain breast and colon cancers, cystic fibrosis, and Huntington's disease are included among many examples of such inheritable diseases.

Although many dietary and environmental agents have been classified as mutagens, cells are constantly subjected to a barrage of spontaneous DNA damage. The spontaneous hydrolysis of DNA bases from the sugar–phosphate backbone, constant exposure of cellular DNA to oxygen-derived free radicals, and low rates of miscopying during DNA replication all contribute towards the mutational burden. In addition to these sources, significant exposure to mutagenic compounds can occur through food and water and through environmental and occupational sources. Many natural constituents of food are mutagenic and are produced by plants as defense agents. However, additional foodborne mutagens can be present as residues of compounds used during food production or leached from packaging materials. Mutagenic compounds can also be produced during food cooking and preparation. Foods also contain many compounds that can modulate the activity of mutagens. In order to minimize exposure to mutagens, the majority of developed countries have instituted regulatory protocols governing the introduction of new foods and food-associated chemicals before they are accepted into the marketplace. With advances in the understanding of mutagenic processes, existing foods and food contact items may also be reassessed for acceptability.

Molecular Mechanisms of Mutagenesis

DNA Damage

DNA is a double helical structure composed of paired heterocyclic nitrogenous purine and pyrimidine bases attached to a backbone of deoxyribose and phosphoric acid. The purine bases (guanine and adenine) and pyrimidine bases (cytosine and thymine) form specific base pairs (guanine/cytosine and adenine/thymine) stabilized by hydrogen bonding. The specificity of base pairing and sequence of bases along the DNA double helix forms the foundation of the genetic code, and any change can give rise to mutations, expressed as alterations in gene expression or in protein structure and function.

Spontaneous damage

Instability of various parts of the DNA molecule in aqueous solution leads to spontaneous DNA damage and mutations. In addition to base deamination, the bonds between purines or pyrimidines and deoxyribose can spontaneously hydrolyze, creating apurinic/apyrimidinic (AP) sites, leaving the sugar–phosphate backbone susceptible to strand breakage. Also, the bases can exist in a number of slightly different (*tautomeric*) forms, several of which lead to mispairing because of altered hydrogen bonding characteristics.

Chemical adducts

DNA also comes under assault from a number of dietary and environmental factors that interact with several relatively reactive sites within the structure of DNA, leading to mutagenic events. Many chemical mutagens are electrophiles that are reactive, electron-deficient species able to form covalent adducts with nucleophilic sites within DNA. Binding of chemical adducts to the purine/pyrimidine bases can stabilize alternative tautomeric structures or otherwise alter their structural and hydrogen bonding characteristics. These modifications not only alter the base pair coding properties and cause mistakes to be made during replication and transcription but also lead to increased base hydrolysis and AP site formation. In addition, some chemical agents produce inter- and intrastrand cross-linking of DNA, which prevents strand separation and causes particular difficulties for replication, transcription, and repair processes.

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Oxidative damage

Other mutagens can act through the induction of oxidative stress and cause oxygen or nitrogen radical formation. Radicals are highly reactive compounds that contain unpaired electrons and include hydroperoxide, hydroxyl, and superoxide radicals. Transition metal ions such as iron and copper can catalyze the formation of free radicals (*Fenton reaction*). The interaction of free radicals with DNA results in single- and double-strand breaks, hydroxylated derivatives of bases, and several other lesions.

DNA intercalation

Finally, some mutagens do not actually react with DNA but modify its structure by intercalating between the complementary strands of DNA, disrupting hydrogen bonding between base pairs. The presence of such compounds causes misreading during replication and transcription, leading to the generation of mutations.

Metabolic Activation

Some mutagenic compounds (*direct-acting* mutagens) are able to interact with DNA without any further modifications; however, many other compounds (*indirect-acting* mutagens) become mutagenic as unwanted by-products of transformations carried out by cellular detoxification pathways. Several hydroxylation (*phase I*) and conjugation (*phase II*) pathways are available for detoxification to increase the water solubility of chemical compounds and aid in their excretion. Although the enzyme systems are generally most highly expressed in the liver, other tissue types maintain these capabilities to varying degrees. The phase I reactions are carried out by the cytochrome P450 enzyme system, which consists of a large family of related enzymes in which each isozyme shows specificity for particular chemical structures. During phase II reactions, more polar groups can be added by glutathione *S*-transferase, glucuronide transferase, microsomal epoxide hydrase, or acetyltransferase. Generally, phase II reactions inactivate mutagens, but instances of the opposite outcome have been found. Several other enzymes, including flavin monooxygenase and prostaglandin H synthetase, are also known to activate mutagens.

Mutagens as Genotoxic versus Nongenotoxic Carcinogens

Chemicals or other treatments found to induce cancer (*carcinogens*) are further characterized as either *genotoxic* or *nongenotoxic*, according to their mechanisms of action. In general, genotoxic treatments result in alterations to the structure of DNA, while nongenotoxic carcinogens operate through receptor-based or indirect oxidative stress mechanisms, such as inflammation. Since mutagens result in heritable alterations to DNA, they are generally regarded as a subset of genotoxic carcinogens. However, conditions set in motion by nongenotoxic carcinogens, such as the release of oxidative species during an inflammatory response, may activate mutagens and thus produce a secondary genotoxic response.

DNA Repair

Several mechanisms have evolved to remove lesions in DNA and to restore the structural and coding properties of the base sequence in the damaged region.

Direct reversal

Direct reversal of damage is perhaps the simplest process, whereby a single gene product can repair a particular form of damage. In mammalian cells, mutagenic O^6-methylguanine residues are removed by a damage reversal mechanism in which the O^6-alkyl group is removed by an O^6-methylguanine transferase in a 'suicide reaction,' resulting in inactivation of the enzyme. A second form of damage reversal is observed in the repair of simple strand breaks, which can be repaired by ligation in a process catalyzed by DNA ligase. Finally, a DNA purine insertase activity has been described by which apurinic sites in DNA are repaired by the direct insertion of purines.

Excision repair

The major and most versatile mechanism used by mammalian cells to remove altered bases or nucleotides within DNA is known as *excision repair*. Excision repair proceeds in several successive steps in a multiple enzyme process. Three major excision repair pathways using different sets of enzymes have been described: base excision repair, nucleotide excision repair, and mismatch repair.

Base excision repair removes misincorporated bases such as uracil and most methylated base damage. The majority of base damage is repaired by the replacement of a single damaged nucleotide with its normal counterpart, but base excision repair can also result in the synthesis of 2–10 nucleotide 'repair patches.' Initially, the damaged base is recognized and removed by a damage-specific glycosylase leaving an AP site in the DNA. For single-nucleotide repair, the deoxyribose phosphate residue in the DNA backbone is removed and a replacement nucleotide inserted by activities associated with DNA polymerase β. Longer patches result when either polymerase β or another repair DNA polymerase synthesizes a stretch of DNA. In this process, a flap region of single-stranded DNA is displaced and is then degraded by a deoxyribonuclease activity. Both types of base excision repair are completed when DNA ligase seals the final nick remaining in the DNA backbone.

The *nucleotide excision repair* pathway involves 20–30 gene products and is responsible for removing damage caused by agents that introduce bulky adducts into DNA. The initial recognition of DNA damage by a recognition complex recruits the entire multicomponent repair enzyme complex (*excinuclease*) to the lesion. The excinuclease nicks the damaged strand on either side of the damage site, releasing an oligonucleotide of approximately 30 base pairs in length. After the gap is filled in by a DNA polymerase using the opposite strand as a template, the nick at the end of the repair patch is sealed by ligase. Nucleotide excision repair has also been shown to be closely linked with transcription (*transcription-coupled repair*) by which actively transcribed genes are preferentially repaired compared with silent regions of the genome. A component of the nucleotide excision repair complex (TFIID) is shared with

the complex involved in gene transcription and mediates the recruitment of DNA repair machinery to the damage site when RNA polymerase stalls at a lesion.

Mismatch repair is a form of excision repair that corrects mismatched base pairs resulting from replication errors and heteroduplexes formed during recombination. The interaction of various components involved in mismatch repair is less understood but results in repair patches of 100–1000 nucleotides. Mutations in mismatch repair genes have been linked to an increased risk for inherited forms of colon cancer.

Classes of mutations

Mutations can range in size from single base pair changes to the loss or duplication of entire chromosomes. Many factors, such as the mutagenic agent, particular region of DNA affected, preexisting damage, and timing during the cell cycle, can influence the magnitude of a mutation. Mutations can be classified into four broad groups: extragenic, gene, chromosomal, and genomic mutations.

Extragenic mutations

Extragenic mutations occur outside of the gene coding sequence and are usually silent. However, if the mutation occurs in an important regulatory sequence, gene expression can be affected.

Gene mutations

Gene mutations occur within the coding sequence of a gene. *Point mutations* involve the substitution of bases, causing either transitions (the substitution of a purine for purine or pyrimidine for pyrimidine) or transversions (the substitution of a purine for a pyrimidine or vice versa). If such a mutation occurs in the wobble position of a codon, the chance of a change occurring in the translated protein structure or function will be much reduced. However, a single base pair change elsewhere in the sequence can result in proteins with decreased or modified functions and can also affect protein synthesis and degradation. *Frameshift mutations* occur when bases are either lost or inserted, thus resulting in a shift of the reading frame by the transcriptional machinery. Frameshift mutations can result in the production of modified or inactive proteins and may be lethal to the cell. *Block mutations* occur when large sections of a gene are deleted, inverted, or duplicated and can lead to defective proteins being produced.

Chromosomal mutations

Compared with gene mutations, chromosomal mutations represent a class of mutations that affect larger regions of DNA within chromosomes and may involve the deletion, translocation, duplication, or further amplification of whole genes. Chromosomal mutations may lead to either increased or decreased gene expression and can also result in the abnormal expression of normally silent parts of the genome.

Genomic mutations

Genomic mutations involve the duplication or loss of entire chromosomes. Such mutations can have far-reaching effects on gene expression and survival. Typically, the progression of a cancer is marked by cells that have developed progressively altered complements of chromosomes.

Mutagenicity Assays

Unlike the typical genotyping of a human or other organism in which inherited mutations are generally common to all, or most, tissues and cells within the body, exposure to a mutagen, either environmentally or in a laboratory setting, will create a more-or-less random collection of mutations differing from cell to cell, making detection akin to finding a needle in a haystack. Thus, when testing for mutagens, the detection of a rare mutational event requires some form of selection procedure to amplify or isolate a particular mutated target before more exacting characterization of mutated sequences can take place.

Despite these limitations, many assay systems have been developed to examine mutagenicity and induction of DNA damage. In many instances, mutagenicity assays are used as shorter-term, more economical surrogates for the 2-year rat carcinogenesis bioassay. Although a high correlation exists between mutagenicity and carcinogenicity, neither this correlation nor that between results obtained with different mutagenicity tests is perfect. Therefore, test chemicals are generally subjected to a battery of three tests or more to gain a more balanced assessment. Widely used mutagenicity assays include both short-term bacterial, fungal, and mammalian cell-based (*in vitro*) and animal-based (*in vivo*) assay systems. Mutations within a target gene are detected as a loss of function (*forward mutations*) or as a reversal of the effect of a previous mutation (*reverse mutations*). To help meet regulatory needs, several mutagenicity assays have been validated and accepted internationally through the Organisation for Economic Co-operation and Development (OECD) and are available as *OECD Test Guidelines*. The test guidelines represent protocols and performance standards agreed upon by international panels of experts and provide standardized methods for industry, academia, and government testing laboratories, which are recognized by the majority of regulatory bodies.

These assays generally rely upon a change in phenotype as an end point and therefore may miss some silent mutations. *In vitro* systems are generally conducted in the presence and absence of a source of metabolic activation, which can include rat liver homogenate (S9 microsomes) or liver cells. The application of molecular biology and biotechnology to many of these systems has led to further refinement and increased molecular characterization of the mutations induced.

The Ames *Salmonella* test and the *E. coli* tryptophan assay are bacterial systems that measure reversions induced at genes responsible for the synthesis of histidine and tryptophan, respectively. Growth of the bacteria on minimal plates requires back mutations at the indicated loci, and therefore, test chemical mutagenicity is assessed according to colony formation. The Ames *Salmonella* test has been widely accepted and validated as a bacterial assay with different strains available that respond to base pair and frameshift mutagens and oxidative damage. Various refinements to the Ames *Salmonella* test have been developed, including a series of bacterial strains that are reverted at specific base pairs, allowing the spectrum of induced mutations to be assessed, as well as bacteria that

express various mammalian phase I and phase II genes to examine the effects of particular types of activation. Several selectable targets can be examined for the induction of forward mutations in mammalian cells, including gene loci encoding thymidine kinase in L51278Y mouse lymphoma cells, hypoxanthine-guanine phosphoribosyltransferase (*HGPRT*) in Chinese hamster ovaries or V79 cells, and Na^+/K^+-ATPase in V79 cells. Mutagenesis at the HGPRT locus has also been examined in human lymphocytes and fibroblasts. Plasmid shuttle vectors have also been developed, which can be exposed to the test chemical, introduced into mammalian cells for repair, and then recovered and reintroduced into an indicator strain of *E. coli* for scoring of mutations. If needed, the plasmids can be recovered again and sequenced.

More recently, faster (*higher-throughput*), fluorescence-based assays have been developed that use instrumental analysis (flow cytometry) to replace the time-consuming step of resistant colony formation. These assays are particularly useful for mammalian or human cell-based assays, which are slower than bacterial assays but better reflect the human situation. For instance, cell lines have been created, which contain the gene encoding the jellyfish green fluorescent protein (GFP) under transcriptional control of a promoter requiring the mutation or reversion of other gene sequences for expression. Thus, the fluorescence signal of GFP is used as a *transcriptional reporter* reflective of mutational events elsewhere in the genome. *In vivo* mutagen exposures can be evaluated by determining the loss of cell surface proteins requiring glycosylphosphatidylinositol (GPI) for attachment. Reduced fluorescence of peripheral blood cells stained with fluorescence-labeled antibodies specific for GPI cell surface proteins is indicative of mutations to the PIGA gene, the product of which is necessary for GPI synthesis.

Germ line mutations may be assessed in rodents using the specific locus test, dominant lethal assay, and heritable translocation test. Transgenic mice incorporating λ phage *lacI* or *lacZ* gene sequences have also been developed, which provide shorter-term, *in vivo* mutagenicity assays. DNA isolated from these animals is incorporated into λ phage, which is used to infect the proper *E. coli* host, forming either clear or colored plaques in the bacterial lawn. The transgenic assays allow mutations to be examined in all somatic tissues and germ line tissues and provide a well-characterized target for sequence analysis.

Chromosomal aberrations can be examined both *in vitro* using cell lines and *in vivo* using animal tissues, generally bone marrow. Stained metaphase chromosome spreads are examined microscopically for structural chromosomal aberrations. A newer variation of this technique, fluorescence *in situ* hybridization, uses fluorescent-labeled DNA probes specific for particular chromosomal sequences to test for aberrations. Another chromosomal aberration assay is the micronucleus test using bone marrow and peripheral blood erythrocytes or lymphocytes. Micronuclei are small particles containing acentric fragments of chromosomes or entire chromosomes that are left behind in the cytoplasm after mutagen treatment. Micronuclei formation may be enumerated by microscopy or flow cytometry.

Sister chromatid exchange uses differential labeling techniques to measure the crossing over between each half of a replicated chromosome. The biological significance of sister chromatid exchange is not known, as the phenomenon does not appear to be related to the mechanisms involved in chromosomal aberration formation and is not directly related to mutagenesis. However, most alkylating agents and compounds that create adducts and single-strand breakage increase sister chromatid exchange. Therefore, the assay tends to be used as a complement to mutagenesis and chromosomal aberration assays.

Quantitative Structure–Activity Relationship Modeling

Although high-throughput assays hold much promise, the sheer number of new and legacy chemicals requiring characterization places huge demands on time and laboratory resources. These challenges have led to a computer-aided informatics approach known as *quantitative structure–activity relationship* (*QSAR*) modeling. QSAR approaches have led to the development of a number of computer (*in silico*) models that scan the chemical structure for *structural alerts* indicative of mutagenic or other toxic potentials. Data from benchtop experimentation continue to strengthen these models such that structural features may, in the future, be sufficient to negate the need for *in vivo* testing and limit requirements to a minimum of *in vitro* confirmatory assays.

Food-Associated Mutagens

The investigation of mutagens in food is an important component of food safety research. Mutagens found in food can be divided into three groups: naturally occurring compounds, those formed by heating or processing, and additives and contaminants, including pesticides.

Naturally Occurring Mutagens

The natural components of food account for over 99% of the carcinogenic and toxic chemicals to which humans are exposed. Most of these natural mutagens are produced by plants as natural pesticides. Many of these intrinsic chemicals are almost impossible to regulate or remove, although guidelines for acceptable exposure limits of some natural contaminants such as mycotoxins are enforced.

Flavonoids form a major group of natural plant compounds, and in a survey of mutagenesis using the Ames *Salmonella* test, 33 out of 70 natural and synthetic flavonoids proved to be mutagenic, producing both base pair and frameshift mutations. Studies indicate that only the flavonol (3-hydroxyflavone) members of this group are mutagenic. Myricetin and quercetin are direct-acting mutagenic flavonoids, whereas kaempferol is indirect-acting. Norwogonin and sexangularetin are two other indirect-acting flavonoids. The *pyrrolizidine alkaloids* represent another large group of widespread mutagenic plant compounds found in some herbal teas and medicines and include compounds such as monocrotaline, dehydro monocrotaline, retrorsine, and isatidine. Pyrrolizidine alkaloids are indirect-acting mutagens, with some compounds inducing DNA strand breakage, whereas others form DNA and DNA–protein cross-links. Another class of mutagenic compounds is the *glucosinolates*, which are found in cruciferous vegetables. Glucosinolates are broken down into isothiocyanates or nitriles, including the mutagenic allyl isothiocyanate glucoside (sinigrin). The mutagen aquilide A

(ptaquiloside) from bracken fern (fiddleheads) is a related *glycoside* and can also be passed through cows' milk. *Catechol-type phenolics*, such as tannins and caffeic acid and its esters (chlorogenic and neochlorogenic acids), are more widespread than other natural pesticides and can induce chromosomal damage. Caffeic acid conjugates can be present in higher plants, with coffee, apple, and lettuce as major sources in the human diet. A class of chemicals known as *linear furanocoumarins*, found in parsley and celery, has several mutagenic members, including psoralen, 5-methoxypsoralen (bergapten), and 8-methoxypsoralen (xanthotoxin). Linear furanocoumarins intercalate into DNA and form mono- and diadducts in the presence of long-wave UV light. A number of mutagenic *hydrazine* compounds such as hydrazinobenzoate are found in 30 species of mushrooms. Estragole and safrole are mutagenic *alkenylbenzenes* that are found in a number of spices such as black pepper, basil, fennel, and nutmeg. Other food-related mutagens include ethyl acrylate from pineapples, sesamol from sesame seeds, and benzyl acetate from basil, jasmine tea, and honey.

Mycotoxins are an important source of mutagenic contamination resulting from mold growth in foodstuffs. Human exposure can occur through grain crops, meat products, milk and eggs, nuts and peanuts, fruits, and juice. *Aspergillus* spp. produce a family of aflatoxin mycotoxins (aflatoxins B1, B2, G1, and G2), of which aflatoxin B1 is the most potent. Food products produced from livestock fed with aflatoxin-contaminated feeds can also serve as a potential source of human exposure. Other mutagenic mycotoxins include fusarin C produced by *Fusarium* sp., luteoskyrin (*Penicillium* sp.), sterigmatocystin (*Aspergillus* sp.), ochratoxin A, and patulin (*Aspergillus* and *Penicillium* spp.). *Alternaria* spp., of which *A. alternata* is primarily responsible for black spots on tomatoes and blackheads on grain, produce a number of mutagenic toxins such as altertoxins I, II, and III and stemphyltoxin III (Figure 1).

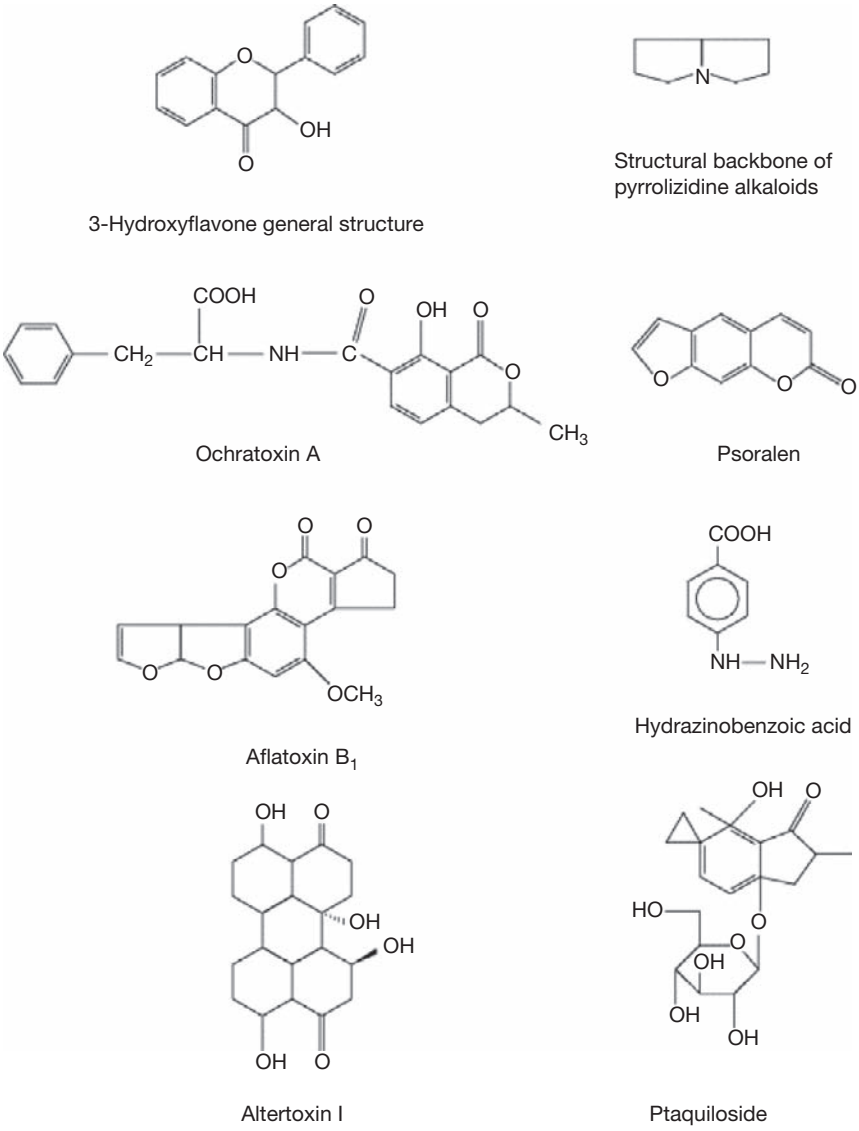

Figure 1 Structures of selected naturally occurring mutagens.

Mutagens Formed during Processing, Preparation, or Storage

Many very significant mutagens are formed during the processing and preparation of food. Mutagens that are formed as a result of heating and preparation are extremely difficult to avoid or regulate.

Grilling or charring of protein-rich foods has been shown to produce some of the most potent mutagens described to date. *Polycyclic heterocyclic amines* are indirect mutagens formed during frying or grilling of meat and fish. This group includes several related amino-imidazo-azaarene structures belonging to three major families: (1) imidazoquinolines (4-MeIQ), (2) imidazoquinoxalines (8-MeIQx and 4,8-DiMeIQx), and (3) imidazopyridines (PhIP). Grilling also produces a number of amino acid pyrolysis products (Trp-P-1, Trp-P-2, Lys-P-1, Phe-P-1, and IQx) and oxygen-containing heterocycles. Generally, PhIP is the predominant heterocyclic amine found and can be transferred to infants through breast milk.

Heterocyclic amines appear to be formed by a Maillard reaction between creatine or creatinine, an amino acid, and a sugar such as glucose or fructose. The Maillard reaction is responsible for browning, which provides the improved appearance, flavoring, and aroma of heated foods. The reaction starts as a condensation between reducing sugars and the amino groups of amino acids, peptides, or proteins. The initial products may proceed through a very complex series of transformations resulting in the formation of thousands of compounds, including both mutagens and nonmutagens. Two contaminants produced during heating are acrylamide and furan, which can be metabolized to the mutagens glycidamide and *cis*-2-butene-1,4-dial, respectively.

Polycyclic aromatic hydrocarbons are a second class of mutagenic compounds formed during the grilling or smoking of meat and fish. Polycyclic aromatic hydrocarbons are common pyrolysis products of most organic materials and include benzo[a]pyrene, 1-nitropyrene, and 1,6-dinitropyrene. The *furfurals*, another class of compounds, are produced during the heating of sugars. Hydroxymethylfurfural is a heat-induced decomposition product of hexoses, which becomes mutagenic after sulfate conjugation.

Other processes can give rise to mutagens. *Urethane* (ethyl carbamate) is found in fermented foods and in beverages treated with the fungicide pyrocarbonate. The use of acid hydrolysis during soy sauce production can produce chloropropanediols and their related fatty acid esters, of which 3-monochloropropanediol can be metabolized to the mutagen *glycidol*, an epoxide. *N-Nitrosodimethylamine* is a naturally occurring mutagen present in cheese, soybean oil, canned fruit, meat products, and alcoholic beverages but is also formed from the use of elastic rubber nettings in cured pork products. Most nitrosamines cross the placental barrier or pass into the mother's milk, thereby leading to possible *in utero* and infant exposure. Lipid peroxidation occurs with the storage of fats and oils, owing to the reaction of oxygen with sites of unsaturated bonds. The resulting lipid hydroperoxides, peroxy radicals, and hydroxyl radicals cause rancidity and are potential DNA-damaging agents. A variety of epoxides and aldehydes are also formed, including malondialdehyde, a potential mutagen (Figure 2).

Food Additives and Contaminants

Food additives and contaminants (including pesticide residues) are compounds that are added to food at some point during its production. Exposure to these substances can be controlled, and the introduction of new chemicals used in food manufacture falls under government regulation. Older additives or chemical contaminants are also reviewed periodically for exposure and health effects.

The use of nitrite, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) as food preservatives has been controversial. *Nitrite* enters the diet both through its use as a common food preservative and through gut microbe-mediated reduction of nitrate from ingested vegetables. Nitrite cycles from the gut to the saliva and can react at acidic pH with amines, amino acids, phenols, and mercaptans, frequently resulting in the formation of mutagenic (nitroso) compounds. Mutagens can also be found in the smoke of nitrite-treated meat during frying. The formation of 2-chloro-4-methylthiobutanoic acid, a mutagen in salted, pickled fish, has also been linked to nitrite use. BHA and BHT have been added to foods and packaging as synthetic antioxidants. Previous concerns over BHA's carcinogenic risk to humans appear unwarranted and related to the large doses used in rodent feeding studies. BHT increases the proliferation of pretumor liver cells in rodent models but has little effect on normal cells.

The artificial sweeteners saccharin and cyclamate have also been shown to have mutagenic properties, although saccharin-induced bladder carcinogenesis in rodents also appears to result from the large doses used. Also, the mutagenicity of some colors has led to their use being banned, although low levels of extractable mutagenic contaminants have been detected in others. Mutagenic heterocyclic amino acids have been found in some flavors.

Contaminants may occur in food as residues of pesticides and fungicides, solvents, or compounds, such as styrene leaching from packaging material. The persistent organochlorine pesticide toxaphene has been found in rural foods consumed by indigenous peoples in the Arctic region, having been carried and deposited through airborne transport from sites in the south.

Mutagens in Water

Drinking water provides another potential source of mutagenic compounds. Water disinfection by chlorination can produce many types of mutagenic chlorinated organic compounds, of which 3-chloro-4-(dichloromethyl)-5-hydroxy-2-(5H)-furanone (MX) is the most potent. Other mutagenic chlorinated butenoic acids, including EMX (the reduced form of MX), brominated trihalomethanes, and dichloroacetonitrile, are also produced. Dichloroacetonitrile is a direct-acting mutagen and induces DNA strand breaks in cultured human lymphoblastic cells. Other less potent mutagenic disinfection by-products include dichloroacetic acid, trichloroacetic acid, and chloral hydrate. Radioactive contamination can also contribute mutagenic activity in areas where water is contaminated with ^{40}K or uranium and thorium decay products (such as radon).

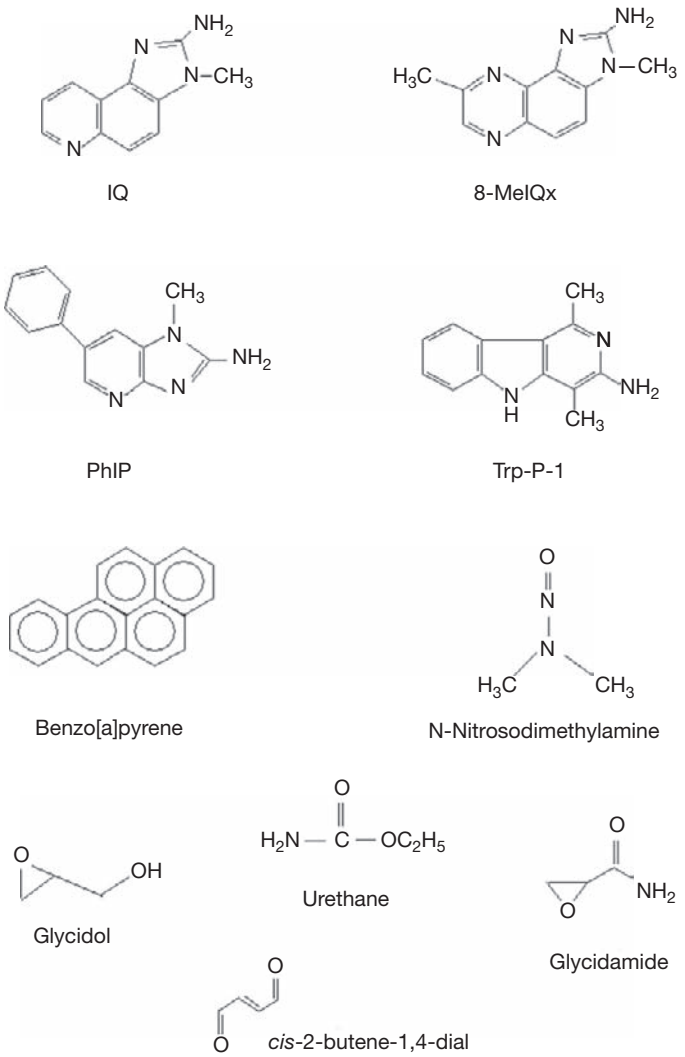

Figure 2 Structures of selected mutagens formed during grilling and processing.

Mutagens in Feces

Human feces have been found to contain a class of mutagenic compounds known as fecapentaenes, the major forms of which are fecapentaene-12 and fecapentaene-14. These compounds appear to be synthesized by intestinal bacteria and are lipid-soluble, direct-acting base pair and frameshift mutagens. Fecapentaene-12 also causes increased sister chromatid exchanges, *HGPRT* mutagenesis, and oxidative damage. Bile salts have been shown to augment the ability of fecapentaenes-12 and -14 to induce DNA repair in bacteria, underscoring their possible role in colon carcinogenesis associated with high-fat, low-fiber diets.

Prevention and Modification of Mutagen Formation

A well-balanced diet also provides many compounds that can decrease exposure to mutagens. Studies consistently show that a greater consumption of fruits and vegetables is associated with a lowering of cancer incidence. These compounds can act at all levels of mutagenesis by (1) preventing mutagen formation and acting as antioxidants, (2) deactivating mutagenic compounds, and (3) inducing detoxifying enzymes or inhibiting monooxygenase activity.

Several natural antioxidants, such as ascorbic acid, β-carotene, α-tocopherol, polyphenolic acids, flavonoids, and other plant phenolics, have a direct mutagen-scavenging activity. For example, in addition to neutralizing oxygen radicals, many of these compounds inhibit the formation of nitrosamines by competing with the amine group. Mutagenic agents may be inactivated through the formation of complexes with calcium, casein, chlorophyll, and carbonyl compounds. Some evidence suggests that calcium can inhibit familial colon carcinogenesis through inhibition of lipid peroxidation. Both iron deficiency and excess have been associated with oxidative DNA damage, and zinc deficiency causes chromosome breaks. The activity of glutathione-dependent detoxification systems is increased by selenium and diallyl disulfide, from garlic, cysteamine,

cysteine, and a variety of phenolic compounds. Monooxygenase activity and the formation of reactive compounds can be inhibited by phenolic acids, free fatty acids, flavonoids, and other polyphenols, methylxanthines, vitamins, and biogenic amines. Conjugated dienoic isomers of linoleic acid are antimutagenic compounds in extracts of fried ground beef and dairy products, which appear to act through signal transduction pathways and prostaglandin synthesis. Tannins or polyphenols in green tea have been found to be antimutagenic in some assays, but the effects appear to depend upon the dose and timing of exposure. Several other compounds have shown antimutagenic activity, including β-diketones, catechins, lignans, and isoflavones. Finally, dietary fiber can complex with mutagenic compounds and limit their uptake.

Mutagenic effects resulting from altered DNA metabolism can also be influenced by diet. Adequate intake of essential nutrients such as folate, vitamin B_{12}, and vitamin B_6 aids nucleotide synthesis and prevents DNA strand breakage. Niacin, methylxanthines, and some phenols have been attributed to the promotion of DNA damage repair (Figure 3).

Further Considerations

Much work remains to completely characterize the role of food-associated mutagens and antimutagens in human carcinogenesis. Despite the magnitude and frequency of exposure to these compounds, they remain underrepresented in mutagenicity studies relative to synthetic chemicals. Additional characterization is needed of the human consequences of exposure to many antioxidants that appear to inhibit mutagenesis at low concentrations but have the opposite effect at high concentrations. In addition, more emphasis should be placed on the effects of chemical mixtures to study the effects of combinations of compounds encountered in the diet.

Assay systems can be improved. Cell culture-based assays promise higher throughput and minimization of animal use, but many cell types have lost or possess unstable metabolic activities, limiting confidence in results. The effects of metabolic activation may be better studied by introducing expression vectors representing the genes of interest into cultured cells. Such an approach will also help to address cell-type specific mechanisms of mutagenesis and aid in developing increasingly realistic models. Although genomic, proteomic, and metabolomic analyses of perturbations in biochemical pathways hold much promise in delineating toxicological effects, much more characterization is needed to correlate these end points to mutagenesis per se. The influence of gut flora on mutagen availability, activation, and possible protective effects is an emerging field. In addition, the identification of the active mutagenic species, probably short-lived reactive intermediates identifiable by specialized spin trapping

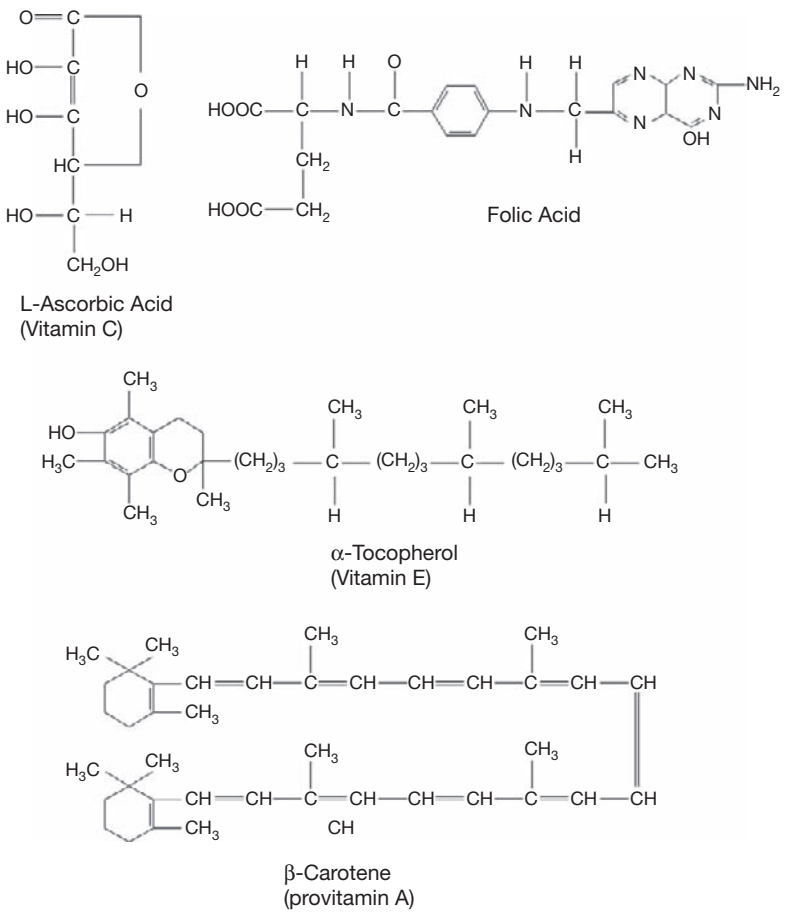

Figure 3 Structures of common dietary antioxidant antimutagens.

techniques, will greatly assist in mechanistic understanding and QSAR development.

Finally, the nature of the dose response exhibited by mutagens and the use of such data in human health risk assessments continue to evolve. Regulatory bodies have routinely applied an extra level of safety in considering the activity of mutagens, which has been thought to display a linear response with concentration, regardless of dose. However, assays usually present a *point of departure* from linearity at lower concentrations, which may be indicative of assay detection limits but, particularly *in vivo*, may also reflect the interplay of a number of factors, including absorption, excretion, bioactivation/detoxification, and inter-/intracellular transport. A better understanding of mutagenic activity in dose–response relationships will strengthen health risk assessments and the regulatory process.

See also: Biogenic Amines; Carcinogens: Identification of Carcinogens; Food Additives: Classification, Uses and Regulation; Mycotoxins: Classification; Mycotoxins: Occurrence and Determination; Mycotoxins: Toxicology; Polycyclic Aromatic Hydrocarbons; Saccharin – How Sweet It Is.

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Mycotoxins: Classification

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Introduction

Mycotoxins are natural, secondary fungal metabolites produced by molds, invading a wide variety of foods and feeds. The recognition of moldy grains was described in the Old Testament of the Bible and the potential adverse effects from moldy infected grains (ergots) were observed centuries ago in several human epidemics and termed 'holy fires' and 'Saint Anthony's Fire' associated with humans experiencing burning pain from gangrenous effects of ergot alkaloids. A majority of the fungi contaminating foods and feeds are *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and *Claviceps purpurea* associated with ergot production. The growth of molds and subsequent mycotoxin production can occur at several points: (1) on crops in the field, (2) during grain storage, (3) transportation of feeds from the farm to destinations of use, and (4) in food processing (such as malting and fermentation). The knowledge of toxic effect of moldy feeds in humans and animals was slow to be gained, with human epidemics of ergot poisoning documented since the Middle Ages. In the latter part of the twentieth century, moldy feed and adverse conditions in humans and animals were associated with chemical compounds produced by molds or mycotoxins. Mycotoxins are chemical compounds that, at sufficiently high concentrations, can cause adverse effects in humans and animals. Both adequate food availability and scientific awareness that mycotoxins in sufficiently high concentrations can limit food use need to be evaluated for the continuing health of human populations, particularly in developing countries. Several recent surveys of thousands of feed and feed raw materials worldwide indicate that over 70% of grains and feeds are contaminated with mycotoxins, although the mycotoxin concentrations are often below regulatory guidelines. The surveys also detected multiple mycotoxins contaminating feeds. Increasing mycotoxin surveillance programs in feeds, using adequate sampling protocols to sample suspected feeds and detect the 'hot spots' of mold growth, using appropriate testing of samples, and utilization and enforcement of regulatory guidelines aids in control and limiting the use of highly contaminated feeds in human and animal populations and safeguards health. A description of the toxicities of the more commonly detected mycotoxins in foods is discussed elsewhere in this encyclopedia. Hundreds of mycotoxins have been identified, but only a relatively few mycotoxins are well characterized and recognized in foods or feeds. This article describes the more commonly detected mycotoxins found in foods and the conditions of mold growth in feeds and foods.

Aflatoxins

Aflatoxins are an important group of structurally related difuranocoumarin compounds produced primarily by *A. flavus*, *A. parasiticus*, and *A. nomius*. These mycotoxins are found worldwide, but more commonly are produced in warm, subtropical, and tropical climates. Aflatoxins can occur preharvest on starchy cereal crops, cottonseeds, and peanuts or postharvest on stored commodities (Table 1). Strains of *A. flavus* typically invade insect- or drought-damaged or senescent plant tissue and mainly produce aflatoxin B_1. Aflatoxin B_1 is generally found in the highest concentration and considered the most toxic and carcinogenic of the aflatoxins. Strains of *A. parasiticus* can produce aflatoxins B_1 (AB1) and B_2, and G_1 and G_2 that fluoresce blue or green, respectively, under ultraviolet light. Aflatoxins M_1 and M_2 are 4-hydroxylated metabolites of aflatoxin B_1 and B_2, respectively, and can occur in aflatoxin-contaminated feed and more importantly in tissues, milk, and dairy products. In heavily infected grains, a yellowish-green pigmented mass of *Aspergillus* growth can be visible on the corn kernels (Figure 1). Invaded kernels can contain extremely high concentrations of aflatoxin (up to 400 $\mu g\ g^{-1}$), which illustrates the importance of adequate sampling of suspected commodities for testing. High concentrations of aflatoxins can occur in field crops with insect or bird damage and hot weather. Sweet corn is considered potentially more vulnerable for aflatoxin accumulation than is field corn because of the higher sugar content. Grain commodities stored on the farm outside on the ground or in inadequate structures that allow access to moisture and oxygen can undergo extensive mold invasion and aflatoxin production during the storage period. During an investigation of possible mycotoxin contamination of feedstuffs, a concern is that the grain or food consumed by the susceptible population prior to the investigation may be entirely consumed and none may be available for chemical testing and mycotoxin determination.

Mold growth and aflatoxin production in a field crop occurs during drought, but aflatoxins could potentially be reduced by the use of pesticides to diminish insect invasion and by using a crop variety that is more tolerant to high temperatures. To prevent aflatoxin production postharvest, storage moisture of $<12\%$ is recommended for cereals grains and <8–9% moisture for oilseeds over a wide range of temperatures. Storing grains and oil seeds in clean, weatherproof structures and cleaning grains to remove light weight or broken grains can reduce mycotoxins. Changing the grain source or avoiding incorporation of the contaminated commodity in the diet will prevent adverse effects, but in poor populations that depend on local grain sources for survival these may not be options. Several measures, including mold inhibitors, physical separation of damaged grain, fermentation, thermal treatment (roasting), irradiation, ammoniation, ozonation, and the use of 'mycotoxin adsorbents,' have been taken to reduce aflatoxin contamination of feeds. No method is without drawbacks, including cost, large-scale application, and only partial success. Numerous products are marketed to sequester or 'bind' mycotoxins and reduce absorption from an animal's gastrointestinal tract; in the United States, these agents are typically sold as anti-caking or free-flow agents. Agents tested include sodium calcium aluminosilicates, activated charcoal, bentonite and

Table 1 Summary table of fungal species and their mycotoxins and commodities affected

Fungal species	*Mycotoxin classification*	*Chemical structure*	*Mycotoxins identification*	*Commodity*
A. flavus, *A. parasiticus*	Aflatoxins	Difuranocoumarins	B1, B2, G1, G2 M1, M2	Corn/maize, peanuts, tree nuts, cottonseed, rice, copra, heat, spices, cocoa (chocolate products) Milk products
Fusarium sp. (*F. graminearum*, *F. poae*, *F. acuminatum*, *F. sporotrichiodes*, *F. equiseti*)	Trichothecenes	Sesquiterpenoids with a C12-13epoxy group	DON (deoxynivalenol) or vomitoxin, nivalenol, 3- and 15-acetylDON, T-2 toxin, HT-2 toxin, T-2 tetral, DAS (diacetoxyscirpenol), neosolaniol	Wheat, maize, hay
F. graminearum, *F. culmorum*, *F. equiseti*	Zearalenone	Macrocyclic lactone	Zearalenone	Wheat, maize/corn, hay
F. verticillioides (syn. *F. moniliforme*), *F. proliferatum*	Fumonisins	Aliphatic hydrocarbons	FB1, FB2, FB3	Corn Corn based foods
Claviceps sp. *C. purpurea* (*C. fusiformis*, *C. paspali*)	Ergot alkaloids	Indoles	Ergopeptine alkaloids: ergotamine, Ergocristine, ergosine, ergocryptine, ergovaline, Ergoline alkaloids: Lysergic acid, Lyergol, lysergic acid amide, ergonovine, Clavines	Rye, wheat, Cereals Grasses
A. alutaceus var. *alutaceus* (formerly *A. ochraceus*), *A. westerdijkiae* and *A. Steynii* and *Penicillium verrucosum* (formerly *P. viridicatum*)	Ochratoxins	Isocoumarin	Ochratoxin A	Cereal grains (wheat, barley, oats, corn), Dry beans, peanuts, grapes, raisins, cheese, Apple juice Swine tissue
Penicillium expansum, *Aspergillus* sp., *Byssochlamys fulva*, *B. nivea*.	Patulin	Cyclic γ-lactone	Patulin	Rotten Fruits, Apple juice, Applesauce

Figure 1 *Aspergillus* sp. mold growing on corn kernels in silage. (Photo: Dr. M. Mostrom, NDSU.)

various clays, yeast cultures, esterified glucomannan, and others. Several of these compounds show potential in partially binding aflatoxin B_1 and reducing aflatoxin M_1 contamination in milk. Positive responses in reducing aflatoxin M_1 in milk are not consistent for all these binders. The US Food and Drug Administration (FDA) has not licensed any product for use as a 'mycotoxin binder' in animal feeds.

Fumonisins

Fumonisins are a group of heat-stable, water-soluble mycotoxins primarily produced in white and yellow corn in the field by *Fuarsium verticillioides* (syn. *F. moniliforme*) and *F. proliferatum*. Fumonisin contamination of other commodities is very limited, although fumonisins can be concentrated

in coproducts of corn ethanol production in the distiller's grains. A period of drought during the growing season followed by wet, warm conditions during pollination of corn favors development of fumonisins. Insect or bird damage to corn provides a portal for mold invasion of the kernels, and often fumonisins are found in very high concentrations in corn screenings containing damaged kernels. Frequently, fumonisins are found to co-occur with other mycotoxins, particularly aflatoxin and tricothecenes, specifically deoxynivalenol (DON). Toxicity is associated with fumonisins B_1 and B_2, with fumonisin B_2 usually occurring at about 30% of fumonisin B_1 concentration. Fumonisins FB_3, FB_4, FA_1, and FA_2 occur at low concentrations and do not appear to be important toxicants at this time. Fumonisins are chemically characterized as an aliphatic hydrocarbon with a terminal amine group and tricarboxylic acid side chains and are structurally similar to sphingolipids in cellular membranes. Horses (and rabbits) are the sensitive species. Fumonisins inhibit ceramide synthase enzyme involved with the conversion of sphinganine to sphingosine, which can increase sphinganine tissue concentrations and interfere with cellular growth, differentiation, and cell communication resulting in toxicity and carcinogenicity. Liver toxicity is often involved across species. Tissue and milk residues are not considered to be a problem.

Ergot Alkaloids

Historically, ergot has been associated with gangrene (peripheral vascular constriction in extremities), central nervous system (hallucinations and giddiness) and gastrointestinal disturbances, and reproductive effects of abortion in humans and agalactia (poor milk production) in animals. Ergot bodies are *C. purpurea* mycelia and hardened exudates of 'honeydew' with fungal conidia that replace a grass or cereal grain ovary with a hardened, dark sclerotium. The sclerotium is harvested with the grain, and if not physically screened out of the grain, it can end up in feeds and foods (such as flour). Rye, triticale, wheat, barley, oats, and various grasses can be infected by *C. purpurea*. The US Standards for grain define ergoty wheat and barley at >0.05% and >0.3% ergot sclerotia, respectively. Ergot alkaloids can also be found within plant tissues, especially tall fescue grasses, produced by endophytic fungi. Due to the pharmacological actions of several ergot alkaloids, these compounds have been used therapeutically for vasoconstrictive action and several of the ergot alkaloids are biosynthesized for medical uses. The pharmacological effects are the result of the ergotalkaloids' effects of partial agonism or antagonism at adrenergic, dopaminergic, and serotonergic receptors. To complicate the pharmacological effect, the species, physiological and endocrinological state influence response to the ergot alkaloid effects.

Climatic condition in the spring involving cool damp weather delays crop pollination and allows *C. purpurea* germination and development in the field. No-till farming, shallow cultivation, and failure to rotate crops increase the potential for ergot germination. Sporadic cases of cutaneous and gangrenous ergotism occur when livestock are made to pasture grasses with infected seed heads. Ergot alkaloids are composed of primary toxicants or ergopeptine alkaloids (ergotamine, ergocristine, ergosine, ergocryptine, ergocornine, and ergovaline) and ergoline alkaloids (ergonovine, lysergic acid, lysergol, and lysergic acid amide).

Ochratoxins

Ochratoxins are phenylalanine–dihydroisocoumarin compounds produced primarily by *A. alutaceus* var. *alutaceus* (formerly *A. ochraceus*), *A. westerdijkiae*, and *A. steynii* and *P. verrucosum* (formerly *P. viridicatum*); *A. alutaceus* is a common fungi in the warmer, temperate or tropical climates and *P. verrucosum* is considered a postharvest contamination of grain in colder climates (<30 °C). *A. carbonarius* and *A. niger* have been recently recognized as ochratoxin producers, although *A. niger* is a very uncommon producer of ochratoxins. *A. alutaceus* and ochratoxins have been reported in dried and stored foods, including dried meats and fish, beans, nuts, cereals and cereal products, coffee beans, grapes, figs, and spices. A majority of the exposure to ochratoxins is from *Penicillium* infection of wheat and barley in Europe and to a lesser extent in Canada (Figure 2). Consumption of ochratoxin-contaminated grain by swine can lead to accumulation of ochratoxins in body tissues and residues in swine kidneys. The European Union has regulatory guidelines for ochratoxin in foods for human consumption and for feedstuffs for animals; the US FDA currently does not have guidelines for ochratoxin.

Trichothecenes and DON

Trichothecenes are produced by several genera of fungi, including *Fusarium*, *Stachybotrys*, *Myrothecium*, *Trichothecium*, *Trichoderma*, *Cephalosporium*, *Cylindrocarpon*, *Verticimonosporium*, and *Phomopsis*. Trichothecene mycotoxins have also been isolated from Brazilian plants, *Baccharis* sp.; however, all medically and economically important sources to date have been fungal, especially *Fusarium*. *Fusarium* is a major agricultural plant pathogen in temperate growing regions where it causes *Fusarium*

Figure 2 *Penicillium* and *Fusarium* spp. mold growing on grain stored in metal grain bins. (Photo courtesy of Dr. Barry Jacobsen, Plant Pathologist, Montana State University.)

head blight in wheat, barley, triticale, and other grains. *F. graminearum* has an optimum temperature range for growth of 26–28 °C at a water activity (a_W) >0.88. *F. culmorum* grows optimally at 21 °C when a_W > 0.87. While increased rainfall will increase *Fusarium* head blight, the incidence of blight is primarily affected by moisture at anthesis (flowering) when the temperature is in the optimum range.

Fusarium species vary in toxigenic potential by strain, which in turn varies with geographic location. Mycotoxin production by *Fusarium* fungi is heavily dependent on oxygen, environmental pH, osmotic tension, and sometimes temperature. For example, the trichothecene DON is produced under conditions of low oxygen tension, whereas production of the mycoestrogen zearalenone by the same *Fusarium* fungus requires oxygen saturation. Unusually cool weather conditions in late summer and early fall, coupled with heavy rainfall in the upper Mid-west of the United States, can result in widespread, severe *Fusarium* infestation and mycotoxin production in corn. Toxigenic *Fusarium*, such as *F. sporotrichioides*, *F. poae*, and *F. graminearum* can produce several trichothecenes under these conditions (DON, T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS), neosolaniol, and T-2 tetraol) and zearalenone. *Fusarium* molds are the most economically important source of trichothecene mycotoxins.

Trichothecenes are sesquiterpenoid compounds with an epoxy group at C12–13 that is considered essential for toxicity. While over a hundred trichothecene mycotoxins have been identified, typically a relative few are considered important in foods. Trichothecenes are stable compounds and can remain present at toxic concentrations in feed for years. Trichothecenes are chemically classified into four types based upon substitutions at five positions of the trichothecene skeleton. Type A trichothecenes include toxic trichothecenes, T-2 toxin, its deacetylated metabolite HT-2 toxin, and DAS or anguidine. These are most commonly produced by *F. sporotrichioides* and *F. poae*. Both of these *Fusarium* spp. produce toxic metabolites in light or darkness and at low temperatures (<−2 °C); however, sharp fluctuations in temperature increases the toxicity of extracts. Optimal production of T-2 toxin by *F. sporotrichioides* in grains occurs at 6–12 °C as compared with zearalenone production at an optimal 19–20 °C. The production of T-2 toxin by *F. sporotrichiodes* has been proposed as the causative agent of alimentary toxic aleukia in humans consuming wet, moldy grains in the former USSR. Type B trichothecenes are common field contaminants of grains and include DON or vomitoxin and acetylated mycotoxins 15- and 3-acetyl DON, nivalenol, and fusarenone-X (4-acetylnivalenol) produced by *F. culmorum* and *F. graminearum*, and other, closely related fungi. In North America, *F. graminearum* characteristically produces DON and 15-ADON; during cooler, wet conditions, production of 3-ADON and nivalenol can be detected in cereals. *F. graminearum* most commonly occurs in southern China, North America, and Eastern Europe, and *F. culmorum* more commonly occurs in Western Europe. Other strains of *F. graminearum*, mostly of Japanese origin, produce nivalenol and fusarenone X, zearalenone, and butenolide. *F. culmorum* strains from the Netherlands produced predominately DON and 3-ADON, while strains of *F. crookwellense*, collected from four continents, produced 4,15-diacetylnivalenol regardless of the source. The Type B trichothecenes are characterized by a keto group at C-8 and hydroxyl group at C-7; these are less toxic than Type A trichothecenes and the macrocyclic trichothecenes produced by *Stachybotrys*. Although not a trichothecene, the estrogenic mycotoxin zeralenone is often found together with DON in North America. More serious toxic effects could be underestimated in moldy corn if only DON and not acetylated DON or additional trichothecenes are assayed.

The Type C trichothecenes, such as crotocin (or Antibiotic T), have a second epoxide ring at C-7,8 and are not produced by *Fusarium* nor are these compounds associated with toxic effects. The Type D trichothecenes are potent, cytotoxic compounds with a macrocyclic ring linking C-4 and C-15 on the trichothecene skeleton. The genus *Myrothecium* can produce verrucarins and roridins that are diesters of the trichothecene verrucarol and are acutely toxic to mammalian cell lines *in vitro* and a wide range of animals with *in vivo* exposure. Naturally occurring toxicoses have rarely been attributed to the *Myrothecium* toxins. Although *Fusarium* does not produce type D trichothecenes, the fungus *S. alternans* Bonorden (synonyms *S. atra* Corda and *S. chartarum* (Ehrenberg ex Link) Hughes), which grows worldwide on cellulosic vegetation and mildewed wet straw and dry wall in wet areas of a building (i.e., bathrooms), produces macrocyclic trichothecene mycotoxins. These mycotoxins, including satratoxins G and H, verrucarin J, and roridin E, are stable and highly toxic. While horses are the most sensitive species to these compounds, stachybotryotoxicosis has been described in numerous animals in many countries of Europe, South Africa, and India.

In North America, the trichothecenes produced by *Fusarium* are the major contaminates in cereal crops. *Fusarium* contamination increases with delayed harvests under wet conditions on a variety of crops. *Fusarium* mycotoxins not only occur in the grain, but also in the vegetative part of the plant. Straw and hay contamination with DON may be as high as 100–150 mg DON per kg (dry weight basis) in wet periods. *Fusarium* infection of immature corn generally results in higher trichothecene and zearalenone concentrations in the cob, as compared with the mycotoxin concentrations in the kernels (**Figure 3**). Usually grain stored at moisture concentrations <0.70a_W or <14.5% moisture by weight is not susceptible to fungal growth or mycotoxin production. Both the European Union and the US FDA have regulatory guidelines for DON.

Zearalenone

Zearalenone, previously known as F-2, is a nonsteroidal estrogenic mycotoxin produced by *F. graminearum* (teleomorph *Gibberella zeae*). Additional *Fusarium* fungi capable of producing zearalenone include *F. culmorum*, *verticillioides* (*moniliforme*), *sporotrichioides*, *semitectum*, *equiseti*, and *oxysporum*. Zearalenone is chemically described as 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcyclic acid lactone. At least seven derivatives of zearalenone have been reported in natural occurrence in corn. Zearalenone and uterotrophic active derivatives were classified as estrogens in that they have an affinity for the estrogen reception and produce estrus or cornification of the

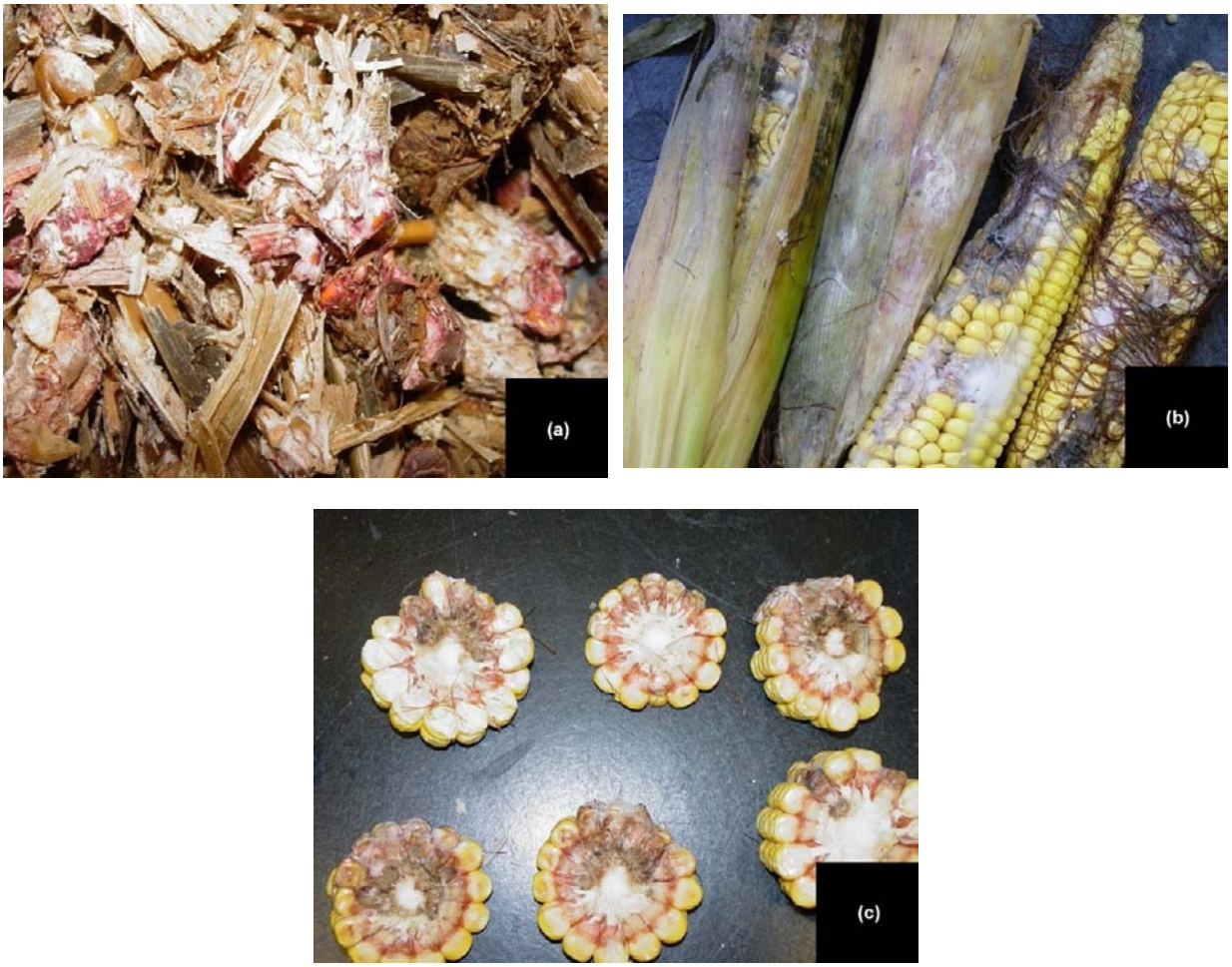

Figure 3 *Fusarium* sp. growing on corn silage with DON contamination (a), field corn with *Fusarium* sp. growth and T-2 toxin contamination (b), and Fusarium sp. invading corn kernels and cobs (c). (Photos: Dr. M. Mostrom, NDSU.)

vagina of adult mice. Zearalenone can be produced on numerous substrates, including wheat, barley, corn, corn silage, rice, sorghum, and occasionally in forages. Production in soybeans is uncommon. Moisture content and the presence of oxygen are critical factors for zearalenone production. In laboratory cultures, *Fusarium* growth occurs during a 3-week period at moisture levels over 20% and between 20 and 25 °C. If the fungus is stressed by cool temperatures of 8–15 °C for several weeks, zearalenone can be produced. Production of zearalenone in the field is similar to that in the laboratory, with alternating moderate and low temperature weather capable of stimulating zearalenone production. Very high concentrations of zearalenone, which can occur naturally in some field samples, generally result from improper storage at high moisture rather than production in the field. In addition to the co-occurrence of other estrogenic metabolites, such as α- and β-zearalenol, zearalenone is commonly detected in grains with another *Fusarium* mycotoxin DON. Zearalenone is heat stable, but can be partially destroyed during extrusion cooking of cereals.

Contamination of cereal grains by zearalenone has been reported worldwide, primarily in temperate climates. Typically, zearalenone concentrations are low in grain contaminated in the field, but increase under storage conditions with moisture > 30–40%. Zearalenone has major effects on reproduction that can lead to hyperestrogenism.

Mycotoxin Conjugates

The relatively recent finding of conjugated mycotoxins, including the 'masked mycotoxins' for soluble conjugates or 'bound mycotoxins' for insoluble conjugates, has complicated interpretation of mycotoxin concentrations and importance of these findings for toxic effects. Current thinking is that conjugated mycotoxins can originate from fungi, plants, food processing, and animals. Some of the fungal conjugated mycotoxins include 3-acetyl DON (3ADON) and 15-acetyl DON (15ADON) that are often detected in *Fusarium*-contaminated grains along with DON. These mycotoxins are unique compounds that appear to be produced under different conditions. Typically in North America, when higher DON concentrations (over 3–5 mg kg^{-1}) are detected, 15-ADON can be often found; usually *F. graminearum* is the contaminating fungus on grain. Not as frequently detected in North America is 3ADON, which is found with higher concentrations of DON

and can be associated with wetter climatic conditions. Zearalenone 4-sulfate is another conjugated mycotoxin produced as a natural *Fusarium* metabolite, and can be detected in fungal cultures and in maize.

Plants appear to have protective mechanisms to convert toxic compounds (such as mycotoxins) into more polar metabolites. The conjugated mycotoxin, DON 3-β-D-gluopyranoside (D3G) was initially found in maize cell suspension cultures and then in naturally contaminated maize, wheat, and barley. D3G has been reported in significant concentrations (1000 $\mu g\,kg^{-1}$) and can be detected at 70% or the DON concentration. The question of whether the glucosides are less toxic storage mycotoxins that are formed for plant self-protection and whether the release of toxic aglycones from the glucosides by herbivore ingestion can cause toxicity is yet to be determined. Food processing, such as heating and fermentation, can alter mycotoxin concentrations and mycotoxin structure. Higher concentrations of D3G have been detected in malt as compared to the DON plus D3G concentrations in the original grain used. It has been hypothesized that bound mycotoxins in the cell wall polymer of grains could be released enzymatically during malting. Excessive heating, such as baking, can alter the chemical structure of mycotoxins.

Mycotoxins can undergo metabolism in the mammalian liver and other organs and be excreted in urine and feces. Mycotoxins undergo conjugation with glucuronide (i.e., DON and zearalenone), and sulfates or react with proteins, glutathione, and cysteine (i.e., ochration A and patulin) with subsequent excretion. These conjugated mycotoxins could be used as biomarkers of exposure but are not likely to be found in food or feedstuffs and play a role in masked mycotoxins.

See also: Aflatoxin: A Global Public Health Problem; Mycotoxins: Occurrence and Determination.

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Mycotoxins: Occurrence and Determination

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Introduction

The term mycotoxin comes from the Greek words 'mykes' for fungus and 'toxicon' for poison. Mycotoxins are toxic secondary metabolites produced by a large number of fungal species potentially infesting food at all stages of production, processing, and storage. The main genera of mycotoxin-producing fungi are *Aspergillus*, *Fusarium*, and *Penicillium*. Although secondary metabolites are commonly formed by simple biosynthetic reactions from small molecules (acetates, pyruvates, etc.), mycotoxins are chemically and structurally diverse (Figure 1). More than 300 mycotoxins are known today, but only aflatoxins (aflatoxins B1, B2, G1, G2, and M1), fusarium toxins (deoxynivalenol, zearalenone, fumonisins B1 and B2, and T-2 and HT-2 toxins), patulin, and ochratoxin A are regulated by European Union (EU) legislation for different foods. Raw materials and products that are frequently contaminated include corn, sorghum, wheat, barley, rice, oats, and livestock such as milk and cheese by-products.

Mycotoxins are a potential threat to human and animal health through the intake of contaminated food. These compounds produce a wide range of adverse and toxic effects in animals: (i) aflatoxins are carcinogenic and cause acute hepatitis, and also reduce the immune response; (ii) citrinin has a nephrotoxic effect; (iii) T2 toxin produces a loss of weight and causes beak lesions in birds; (iv) fumonisins are carcinogenic, hepatotoxic agents, and in horses cause leukoencephalomalacia; (v) ochratoxins are also carcinogenic, nephrotoxic, teratogenic, and hepatotoxic; (vi) trichothecenes have activity as immunodepressants and cause gastrointestinal bleeding; and (vii) zearalenone has estrogenic activity. To give an idea of the interest in these compounds, in the last 3 years, the European Food Safety Authority (EFSA) has launched three calls for data on mycotoxins' occurrence to support the scientific opinions about their importance within the food and feed safety. Currently, the new scientific opinions issued in 2011 and 2012 cover a large number of traditional and emerging mycotoxins.

Methods for the analysis of mycotoxins in food number in the thousands. Conventional methods of analysis for mycotoxins have included enzyme-linked immunosorbent assay (ELISA), thin-layer chromatography (TLC), high-resolution liquid chromatography with fluorescence (HPLC-FL), high-resolution liquid chromatography with diode array (HPLC-DAD), high-resolution liquid chromatography coupled to mass spectrometry (HPLC-MS), and gas chromatography coupled to mass spectrometry (GC-MS). Most of these methods employ a cleanup step by solid phase extraction (SPE) or by immunoaffinity techniques to eliminate interference and improve the mycotoxins' determination. With these newer and higher throughput analytical tools, researchers are now able to get results more efficiently and cost effectively in a matter of hours to days. The purpose of this chapter is to briefly evaluate the extent of mycotoxins' contamination and their co-occurrence in food, and to discuss the current procedures to analyze them. For this, the importance of sampling and sample preparation is discussed, and the most-often-used methods to identify and quantify mycotoxins are described. Particular emphasis is given to recent developments and new techniques that have been used in the analysis and detection of mycotoxins.

Occurrence in Food

The Food and Agriculture Organization (FAO) estimates that 25% of the world's food crops are affected by mycotoxins. Contamination of mycotoxins can occur when food is a growing crop or during storage, processing, or handling, and after the finished food products have been prepared for sale or consumption. Thus, economical losses occur at all levels. Environmental stress conditions such as insect infestation, drought, cultivar susceptibility, mechanical damage, nutritional deficiencies, and unseasonable temperature, rainfall, or humidity can promote mycotoxin production in growing crops. Most mycotoxins are chemically stable during storage and processing, even at high temperatures. Consequently, mycotoxins may also be found in beer and wine resulting from the use of contaminated barley, other cereals, and grapes in their production. They can also enter the human food chain via meat or other animal products such as eggs, milk, and cheese as the result of livestock eating contaminated feed.

Mycotoxins can occur both in temperate and tropical regions of the world, depending on the species of fungi. For example, *Aspergillus* species find optimal conditions in tropical and subtropical areas, whereas *Fusarium* and *Penicillium* species are also adapted to Europe and US climates. However, globalization of the food and feed trade results in a worldwide distribution of mycotoxins. As an illustrative example, the Biomin Mycotoxin Survey Program deserves mention. In the more than 4000 samples analyzed, aflatoxins were present in 25%; zearalenone, 46%; deoxynivalenol, 64%; fumonisins, 56%; and ochratoxin A, 31% (Figure 2). The ability of mold species to produce several kinds of mycotoxins originates multiple contaminations in a food. Several types of mycotoxins may also be in the same food because they came from different contaminated ingredients or raw materials.

Mycotoxins comprise a wide variety of chemical compounds (more than 300, as mentioned), with very different molecular structures as a result of the numerous species of fungi responsible for their production. Major and minor classes of mycotoxins can be differentiated. Aflatoxins, ochratoxins, trichothecenes, fumonisins, patulin, zearalenone, and ergot alkaloids are the major classes, and also raise the most important concern for human and animal health.

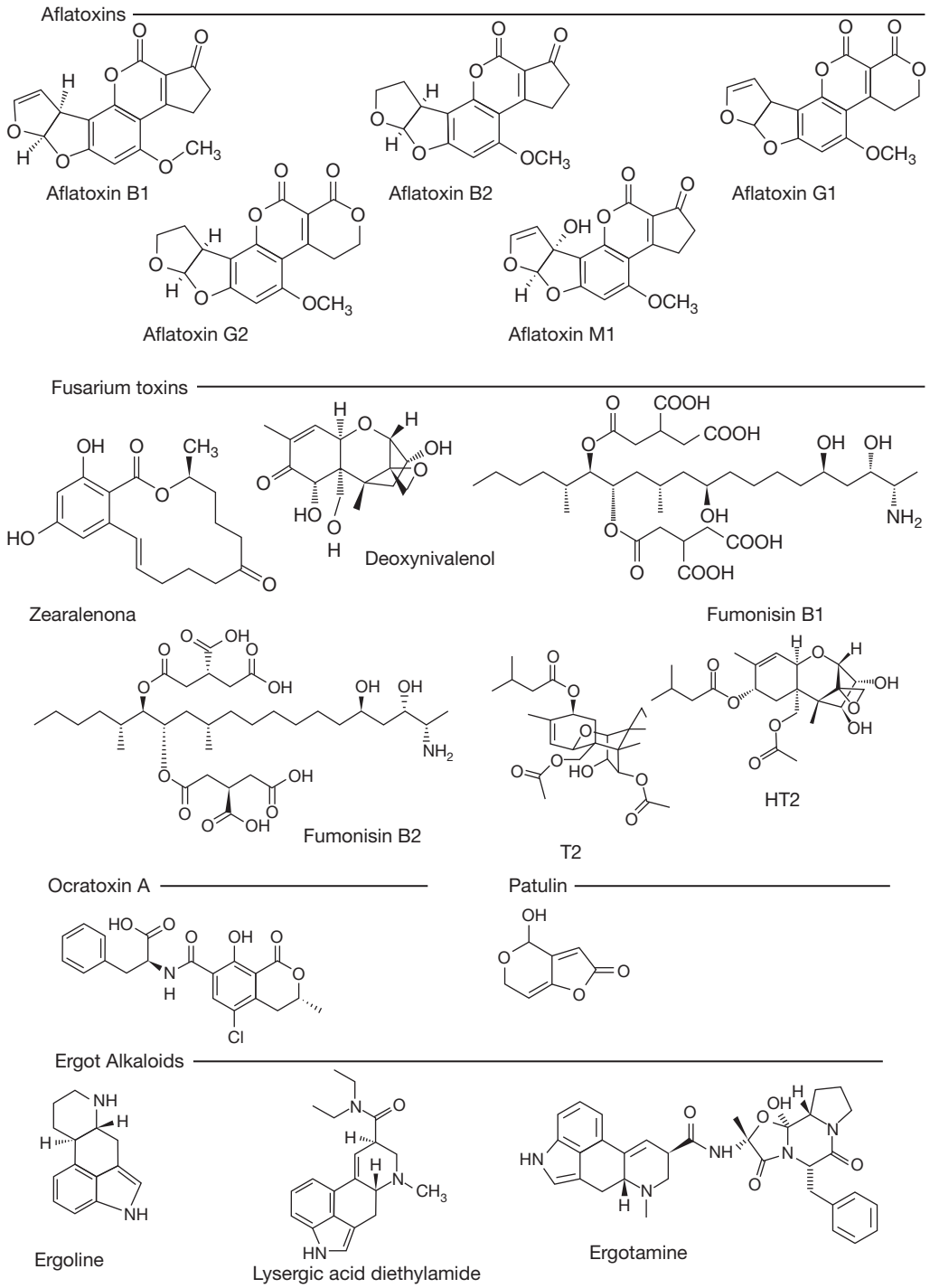

Figure 1 Examples of common mycotoxins, illustrating the structural diversity of these compounds.

Aflatoxins, produced primarily by *Aspergillus flavus* and *A. parasiticus*, are mycotoxins of major concern because they are carcinogenic. There are at least 14 different types of aflatoxins produced by these molds, but the four major aflatoxins are B1, B2, G1, and G2, plus two additional metabolic products, M1 and M2, that are of significance as direct contaminants of foods and feeds. The aflatoxins M1 and M2 were first isolated from the milk of lactating animals fed with aflatoxin-contaminated feed, hence the M designation. The B designation of aflatoxins B1 and B2 resulted from the exhibition of blue fluorescence under ultraviolet (UV) light, while the G designation refers to the yellow-green fluorescence under UV light. Aflatoxins are substances that are chemically related to difuranocoumarin and classified in two groups according to their chemical structure (Figure 1): the difurocoumarocyclopentenone series (including aflatoxins B1 and B2 and characterized by a

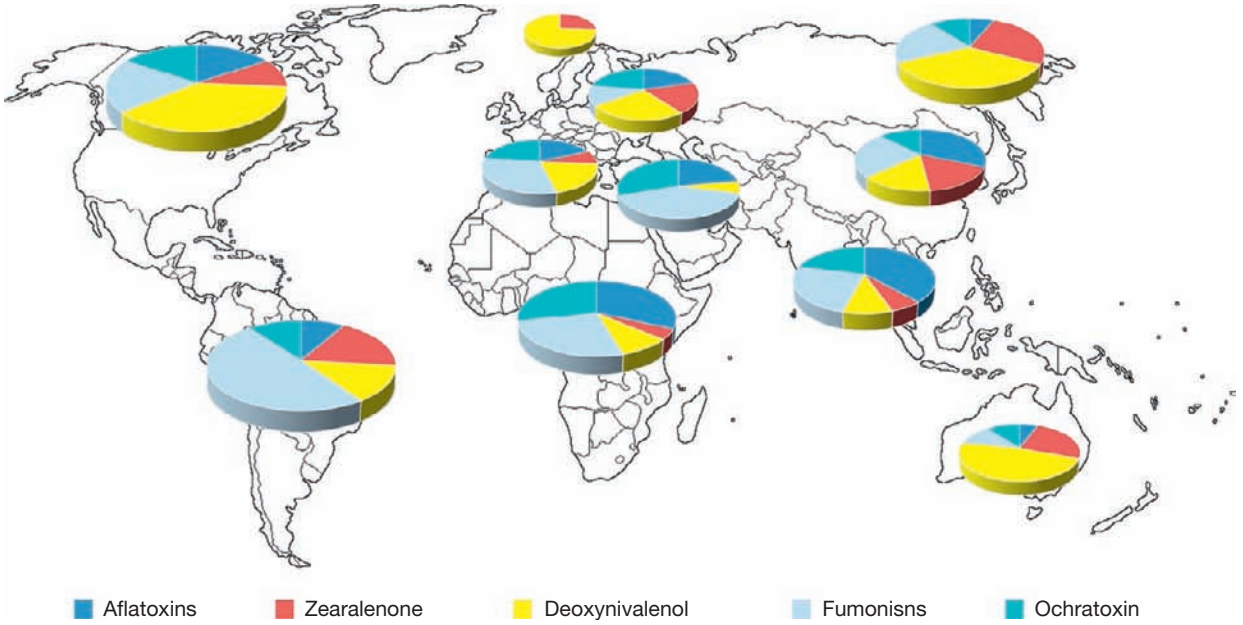

Figure 2 An overview of mycotoxin contamination worldwide (from data reported by BIOMIN Holding GmbH, 2012).

cyclopentenone ring) and the difurocoumarolactone series (including aflatoxins G1 and G2 that contain a D lactone ring). Aflatoxins have been detected in milk, cheese, corn, peanuts, cottonseed, nuts, almonds, figs, spices, and a variety of other foods and feeds. Milk, eggs, and meat products can be contaminated because of the animal consumption of aflatoxin-contaminated feed. However, the commodities with the highest risk of aflatoxin contamination are corn, peanuts, and cottonseed.

Trichothecenes are a group of about 150 mycotoxins produced by several fungi Fusarium — particularly *Fusarium graminearum* and *F. sporotrichioides* — *Trichoderma*, *Trichothecium*, and others. The most important structural features causing the biological activities of trichothecenes are 12, 13-epoxy ring, the presence of hydroxyl or acetyl groups, and the structure and position of the side chains (Figure 1). They can be distinguished into two groups: macrocyclic and nonmacrocyclic. The latter group is also subdivided into the Type A and Type B trichothecenes. Type A trichothecenes include T-2, HT-2, neosolaniol, and diacetoxyscirpenol, and Type B consists of deoxynivalenol, nivalenol, and 3-acetyldeoxynivalenol. Type A are of special interest because they are more toxic than the other foodborne mycotoxins. However, deoxynivalenol (Type B) is of concern, as it is the most prevalent trichothecene in Europe. The thricothecenes have been frequently detected in cereals, infant foods, fruits, vegetables, and even products of animal origin.

Fumonisins are a group of mycotoxins produced primarily by *F. verticillioides* and *F. proliferatum*, although a few other Fusarium species may also produce them. There are at least 28 different forms of fumonisins, most designated as A-series, B-series, C-series, and P-series. Fumonisin B1 is the most abundant, followed by B2. Chemically, fumonisin B1 is a diester of propane-1,2,3-tricarboxylic acid and 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxyeicosane (Figure 1). Fumonisin B2 is a deoxy-analog of the fumonisin B1. Fumonisin B1 typically accounts for 70–80% of the total fumonisins produced, while fumonisin B2 usually makes up 15–25%. Fumonisins are soluble in polar solvents because of the four free carboxyl groups, the hydroxyl groups, and the amino group. Their insolubility in many organic solvents such as chloroform and hexane commonly used in mycotoxin analysis partly explains the difficulty in their original identification. The natural occurrence of fumonisins in cereals, particularly maize and maize-based products, has been reported worldwide.

Zearalenone is a nonsteroidal, phenolic resorcyclic acid lactone estrogenic toxin produced mainly by *F. graminearum*, *F. culmorum*, and *F. sacchari*. It is commonly found in maize but can be found also in other crops such as wheat, barley, sorghum, and rye. Zearalenone is a white crystalline compound that exhibits blue-green fluorescence when excited by long wavelength UV light (360 nm) and a more intense green fluorescence when excited with short wavelength UV light (260 nm). Soluble in water and in aqueous alkali, it is slightly soluble in hexane and progressively more soluble in benzene, acetonitrile, methylene chloride, methanol, ethanol, and acetone.

Ochratoxin A, produced primarily by *Penicillium viridicatum*, but also by certain Aspergillus molds (e.g., *A. ochraceous*), is a compound with phenylalanine-containing dihydroisocoumarin and also a chlorine on the aromatic ring, which accounts for its toxicity. It is a colorless crystalline compound, exhibiting blue fluorescence under UV light. Its sodium salt is soluble in water; as an acid, it is moderately soluble in polar organic solvents such as chloroform and methanol and dissolves in dilute aqueous sodium bicarbonate. On acid hydrolysis, ochratoxin A yields phenylalanine and an optically active lactone acid, ochratoxin α. Reaction in methanol and chloridric acid yields the ochratoxin methyl ester, which can be used as a confirmatory. Ochratoxin A has been detected in various types of food, including cereal products, coffee, spices, beer, grape and its derivatives, and products of animal origin.

Patulin is a lactone produced by a variety of molds, in particular, *Aspergillus* and *Penicillium* and *Byssochlamys*. Most commonly found in rotting apples, in general the amount of patulin in apple products is viewed as a measure of the quality of the apples used in production. In addition, patulin has been found in other foods such as grains, fruits, and vegetables. Patulin is destroyed by alcoholic fermentation and thus is not found in either alcoholic fruit beverages or vinegars produced from fruit juices. Thermal processing causes only moderate reductions in patulin levels, thus patulin present in apple juice will survive the pasteurization processes. Patulin can be isolated as colorless to white crystals from ethereal extracts that have no optical activity. It melts at about 110 °C. It is soluble in water, methanol, ethanol, acetone, and ethyl or amyl acetate and less soluble in diethyl ether and benzene.

Ergot alkaloids are mycotoxins produced by several species of fungi in the genus Claviceps. In AD 944–945, in the Aquitane region of France, the symptoms of the disease called St. Anthony's fire – burning skin, insects crawling under the skin, and the loss of hands and feet – suggested ergot poisoning. Ergot poisoning in humans and domestic animals is known as ergotism. This disease may cause strange hallucinations, the feeling of itchy and burning skin, gangrene, loss of hands and feet, and even death. Ergotism is one of the oldest known human diseases caused by mycotoxins. The ergot alkaloids are indole compounds that are biosynthetically derived from L-tryptophan and represent the largest group of nitrogenous fungal metabolites found in nature. There are four main groups of ergot alkaloids: the clavines (agroclavine, elymoclavine, lysergol), the lysergic acids, the lysergic acid amides (ergine, ergonovine), and the ergopeptides (ergotamine, ergovaline, α-ergosine). More than 80 different ergot alkaloids have been isolated, mainly from various Claviceps species (more than 70 alkaloids), but also from other fungi and from higher plants. In addition to the naturally occurring ergonovine (used as an oxytocic) and ergotamine (an analgesic used to control migraines), synthetic derivatives of continuing importance today are the oxytocic methergine, the antimigraine drugs dihydroergotamine and methysergide, the antisenility nootropic (smart drug) Hydergine™, and bromocriptine, used for numerous purposes including treatment of Parkinson's disease. Selected members of these groups of compounds are involved in either nervous or gangrenous syndromes in humans and animals that consume grains or grain products contaminated with the sclerotia of the fungus.

In addition to this major class of mycotoxins, the minor class (citrinin, cyclopiazonic acid, sterigmatocystin, moniliformin, gliotoxin, citreoviridin, tremorgenic mycotoxins, penicillic acid, roquefortine, 3-nitropropionic acid, and fusaproliferin) is also noteworthy.

Determination of Mycotoxins

The classification of the analytical methods to determine mycotoxins can be performed according to different criteria. The more useful one is done according to the type of chemical/biological/physical principle used and to the time of analysis:

- Rapid methods (ELISA, lateral flow devices (LFD), dipsticks, biosensors, fluorescence polarization immunoassay (FPIA))
- Chromatographic methods (HPLC, GC, TLC, LC-MS/MS)

In addition to that, an appropriate sampling plan is mandatory in order to provide accurate and representative results, and proper sample extraction and cleanup is also required.

Sampling

Sampling is always an important step for any method aiming to quantify food contaminants due to the overall complexity of food matrices, but particularly in the case of mycotoxins, sampling is by far the largest contribution to the total error. In addition, the associated variability is largely dependent on the level of the toxin. Then, taking a representative sample from the lot under investigation presents almost unique characteristics because only a few units (0.1% approx. for a wide variety of agricultural commodities) are likely to be highly contaminated, resulting in an overall significant contamination of the lot. Appropriate sampling procedures are a prerequisite for obtaining reliable results because of the heterogeneous distribution of mycotoxins in grains and other food products. To reduce the variability of analytical results, the European Union has established a common sampling procedure according to Community Regulation No. 401/2006 for the official control of mycotoxin levels in foodstuffs (cereals and derived products, dried fruits, groundnuts, nuts, spices, milk and derived products, coffee and derived products, fruit juice, and solid apple products). The basic principle underlying the sampling plan is the sampling of an usually high number of incremental samples evenly distributed in the lot, the gathering of the collected incremental samples in a number of aggregate (generally one) samples, the grinding or slurring of the aggregate(s) samples, and the deriving of the laboratory samples from the aggregate sample(s). The number and weight of the incremental samples and of the aggregate sample(s) are dependent on the matrix and the size of the lot under consideration.

Sample Preparation

Although solid liquid extraction using solvents is the oldest sample preparation technique, it is still one of the most frequently used for mycotoxin extraction. A wide variety of solvents have been used, depending on the analyte to be extracted. Most mycotoxins are soluble in polar and slightly polar solvents, and insoluble in apolar solvents. Thus, mixtures of organic solvents, such as acetone, acetonitrile, chloroform, dichloromethane, ethyl acetate, or methanol, with small amounts of diluted acids or water are frequently used. The addition of water or acidified water solutions (i.e., with formic acid, acetic acid, and citric acid) usually improves the efficiency of extraction, because water increases penetration of the solvent into the material and an acid solution can help the extraction by breaking interactions between the toxins and other sample constituents like proteins or sugars. Sometimes, nonpolar solvents such as hexane and cyclohexane are used before or following the solvent procedure to remove lipophilic components. In addition to the type of solvent, sample/extractive solvent ratio, temperature, and time of extraction are other important parameters that should be carefully controlled to achieve accurate quantification. Following the solvent

extraction, a centrifugation or filtration of the extract is normally required before concentration and/or cleanup steps.

The development of multi-mycotoxin methods with one common sample preparation and a single final determination is highly desirable. In this regard, the most critical step is the development of an extraction that attains good recoveries for all mycotoxins under investigation in the food matrix. For multi-mycotoxin extraction, the mixtures of acetonitrile and water are the most common. In fact, these binary mixtures provide good yields for most of the analytes, with the exception of fumonisin, which needs acidification to obtain good recoveries. At a low pH, the four carboxylic groups of fumonisin are protonated (i.e., neutral), leading to less interaction and binding to the sample matrix.

Recently, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction procedure has been frequently employed as a sample preparation methodology for multiresidue mycotoxin analysis. The original QuEChERS method developed for pesticide residues consists of extraction with acetonitrile by salting out after the addition of adequately mixed salts (anhydrous magnesium sulfate and sodium chloride), after which the extract is subjected to dispersive solid-phase cleanup (d-SPE) with primary secondary amine and anhydrous magnesium sulfate. However, many modifications have been introduced, such as buffering the extraction medium with an acetate or citrate buffer, or changes in the dispersive sorbents.

The solid–liquid extraction required an energy input as mechanical shaking or ultrasounds to favor the extraction. Nonconventional extraction uses other types of energy inputs and includes pressurized liquid extraction, also known as accelerated solvent extraction; supercritical fluid extraction; and microwave-assisted extraction. They have the advantage of requiring smaller volumes of solvent, and they usually provide better extraction efficiencies (in terms of extraction yield and/or recovery) when compared with conventional solid liquid extraction.

Other miniaturized extraction techniques, such as matrix solid phase dispersion, solid-phase microextraction, and dispersive liquid–liquid microextraction, have been less employed to achieve extraction and cleanup during the determination of multiresidue and multiclass compounds in different matrices requiring less organic solvent, and in general yield satisfactory results.

Cleanup Methods

The most used cleanup procedures are liquid/liquid partition, SPE, and multifunctional solid phase extraction (Mycosep® SPE). SPE is currently applied for the extraction of mycotoxins from liquid samples or, in the case of solid matrices, as cleanup and/or concentration step after a previous extraction. In the simpler procedures, the sample is passed through a cartridge or a disc filled with a solid sorbent where the mycotoxins are absorbed and then eluted with an organic solvent. There is a wide range of column packings commercially available with different sorbents, so the choice depends on the food matrix, the analytes, and the interferences that could be present, and also when SPE is used as the cleanup step on the solvent in which the analytes were extracted. Silica gel cartridges, Florisil®, and cyano find widespread application in the determination of thricothecenes, while strong anion-exchange was used to isolate anionic compounds such as fumonisins.

Mycosep® and Multisep® columns are other well-established, non-specific SPE cleanup methods. These columns are filled by adsorbents such as charcoal, celite, ion-exchange resins, polymers, and other materials, packed into a plastic tube between two filter discs. In this process, interfering substances such as proteins, fats, and pigments are adsorbed in the solid phase, while the purified extract passes through the column. Many types of Mycosep® and Multisep® columns are available (Mycosep® and Multisep® 113, 216, 225, 226, 227, 229, etc.) with different adsorbent composition, the choice of which depends on the matrix and the mycotoxins. This cleanup procedure is very simple and quick, because there is no need for column preconditioning or rinsing. However, the columns are single-use only and do not allow any sample concentration, and purification of extracts is not always effective, depending on the matrix. Moreover, they are designed per analyte or a group of similar compounds.

SPE extraction is a safe, efficient, and reproducible technique. However, the procedure also presents some limitations, such as the impossibility of using a single cartridge for all mycotoxins, and the fact that method performance can be affected by several conditions, such as pH, type of solvent, and ionic strength of the sample.

Highly selective sorbents are also important tools that have gained popularity in recent years within the cleanup of mycotoxins. The most popular are based on antibodies (immunoaffinity columns, IAC) or molecularly imprinted polymers (MIPs), with IAC being the most used in mycotoxin analysis.

IAC consists of an activated solid-phase support, binded to specific antibodies for a given mycotoxin that binds it when the extract passes through the column, while impurities are removed by a rising step. Then, the mycotoxins are eluted with a miscible solvent or by antibody denaturation. If the sample is very complex, the combination of IAC with another extraction procedure, such as liquid–liquid extraction and SPE, could be necessary. The major advantage of IAC is the specific interaction between the antibody and the target analyte, as well as its rapidity. However, the procedure has some weaknesses, such as antibodies' denaturation in the presence of even low concentrations of organic solvents. The extract must be an aqueous solution containing little or no organic solvent. As with all antibody-based methods, there is also the possibility of nonspecific interactions due to cross-reactivities with other mycotoxins. Furthermore, immunoaffinity materials are expensive, and the columns only enable a single application. There are commercially available columns for thricothecenes, ocratoxins, aflatoxins, fumonisins, and others. Multifunctional columns for the simultaneous determination of different groups of mycotoxins are also available. Although there are some publications using IAC multi-mycotoxins, the use of IAC for multi-mycotoxin determination requires changes in development and format before its application to a large number of mycotoxins.

MIPs are prepared by polymerization of selected monomers around the template molecule, which is usually a structural analog of the compound of interest. The retention of the

analyte results from a shape recognition in artificial binding sites that recognize the target molecule. It is very promising and provides several advantages, such as high selectivity and great stability to heating and pH shifts. However, some mycotoxins are too toxic or too expensive to be used in MIPs preparation and they can pose some problems, such as inconsistent molecular recognition, polymer swelling in unfavorable solvents, slow binding kinetics, and potential sample contamination by template bleeding. Then, this cleanup method is less used.

Qualitative and Quantitative Analysis

Screening methods

Screening assays are very important tools for monitoring mycotoxins in food and feed. Most of the methods correspond to mere qualitative tests able to demonstrate the presence or absence of the toxin in question, although there are also a variety of rapid tests providing semi-quantitative or quantitative results.

Among the screening methods, immunochemical methods – which include ELISA, LFD, dipstick tests, FPIAs as well as, more recently, biosensors and noninvasive methods based on infrared and acoustic techniques – have shown great potential for mycotoxin analysis.

The ELISA method for determining mycotoxins has been available for more than 15 years. The technology is based on the ability of a specific antibody to distinguish the three-dimensional structure of a given mycotoxin. The direct competitive ELISA is used commonly in mycotoxin analysis. A microtiter ELISA plate consists of the balance of the antigen–antibody complex reaction. The majority of test kits available commercially to determine mycotoxins by ELISA are currently working on the base of the kinetics of antigen–antibody union before this union reaches the equilibrium, which reduces the incubation time from hours ($\sim$2 h) to minutes ($\sim$10 min). Although the reduction in the time of incubation may lead to some loss of sensitivity of the assay, the technique can provide accurate and reproducible results.

In addition, various immunostrips or immunodipsticks, also named lateral flow devices, have been developed for mycotoxin testing in the field, using either membranes with immobilized antibodies or dry reagents on the test strip, where the sample flows by capillary forces. The results provided by these tests are mostly based on a visual evaluation, giving a yes/no answer, or on portable photometric strip readers, providing quantitative or semi-quantitative results. Nevertheless, problems with reproducibility, reliability with different matrices, and sensitivity may sometimes limit their application, together with the lack of reliable method validation.

Several FPIA formats are available for mycotoxins. The sensitivity of FPIA was similar to ELISA; however, it is the least labor-intensive and requires the least amount of time to be performed. The FPIAs required less than 90 min to be performed, and no coating of plates was required.

Immunoassay methods, whatever type they are, were developed for detection of mycotoxins belonging to the same group. However, there is a trend in the more recent assays to detect simultaneously several mycotoxins from different groups.

Biosensors are composed of one biological component (nucleic acids, enzymes, antibodies, cells) that reacts selectively with the mycotoxin of interest, and by a transducing element responsible for converting the change of the physical variable produced by the interaction between the target molecule and the biological component into a measurable signal. The advantages of this technology may include more rapid assays, reusable and long-life sensor elements, the capacity for continuous monitoring, and industrially low-cost devices. The use of biosensors for the detection of mycotoxins is rapidly increasing, including disposable DNA electrochemical biosensors, handled immunoaffinity fluorimetric biosensors, and biosensors based on lipid films and liposomes. The most-used biosensors for the analysis of mycotoxin are those based on the interaction of a specific antibody, or an antibody fragment, and the mycotoxin. The transducing elements can be optical, piezoelectric, or electrochemical. Optical detectors can be based on surface plasmon resonance, fluorescence (fluorescence-based array biosensors, also called NRL-array biosensors), optical waveguide light mode spectroscopy, or total internal reflection ellipsometry, while electrochemical detectors are based on potentiometry with a carbon working electrode, differential pulse voltammetry, conductometry, and so on. Some of these sensors are designed for a single mycotoxin detection, while others are capable of multi-mycotoxin sensing.

Chromatographic methods

Nowadays, mycotoxins are typically determined using chromatographic methods, usually gas chromatography–mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). Mycotoxins are mostly polar compounds. Then, LC-MS is clearly preferred over GC-MS. GC analysis commonly requires a previous derivatization step, with silylation and acylation reactions being the most common approaches for mycotoxin analysis.

TLC-based methods have decreased steadily over the past few years, but, especially in developing countries, this analytical technique is still routinely used. TLC methods allow for a precise determination at levels not lower than 2 ng g^{-1}. Their disadvantages, however, include the use of solvents that are considered to be an ecological hazard. A large number of TLC-based methods were accepted by the AOAC as official methods, including the methods for the determination of deoxynivalenol in barley and wheat, for aflatoxins in peanuts and corn, for aflatoxin M1 in milk and cheeses, for ochratoxin A in barley and in green coffee, and for zearalenone in corn. A recent TLC improvement is the use of a microcomputer interfaced with a fluorodensitometer to simplify data handling.

Traditionally, mycotoxins have also been determined by HPLC-DAD, and those that are also fluorescent as fumonisins by HPLC-FLD. However, over the last few years, liquid chromatography–tandem mass spectrometry (LC-MS/MS) has become the most extensively used analytical method for mycotoxins belonging to different chemical families, enabling their efficient quantitative determination in several food commodities. HPLC separation exclusively relies on reversed-phase materials (RP-18) with acetonitrile/methanol/water, methanol/water, and acetonitrile/water mixtures as mobile phases, isocratic or in gradient. Acetic acid and ammonium acetate are predominantly applied as additives either to improve chromatographic separation or to force adduct formation for improved MS. APCI and ESI have been effectively applied in

positive and negative ionization mode for the analysis of mycotoxins. Multistage MS applications with triple quadrupole and/or linear ion trap instrumentation have been performed either to achieve accurate quantification down to the low ppb range working in multiple selected reaction monitoring mode, or for structural elucidation by enhanced precursor and product ion scans. Combining HPLC with tandem MS results in a powerful tool for characterization and identification, especially for detection of so-called conjugated mycotoxin, in which the toxin is usually bound to a more polar substance like glucose or other sugars. These conjugated mycotoxins are referred to as masked mycotoxins, as these substances escape routine detection methods but can release their toxic precursors after hydrolysis. Also with LC-MS, there is currently a strong trend toward multi-mycotoxin methods for simultaneous determination, particularly for mycotoxins belonging to different chemical families. This approach has the advantage of enabling the reduction or possibly the omission of sample cleanup. In addition to the multi-analyte requirement, multi-substrate methods are even more demanding. This approach, nevertheless, has the advantages of speed and ease of application with one method for multiple mycotoxin–matrix combinations over a broad range of mycotoxin classes, which is of special interest for high-throughput routine analysis. However, the latest trends in mycotoxin analysis have been to take advantage of ultra-high resolution mass spectrometry, which simultaneously

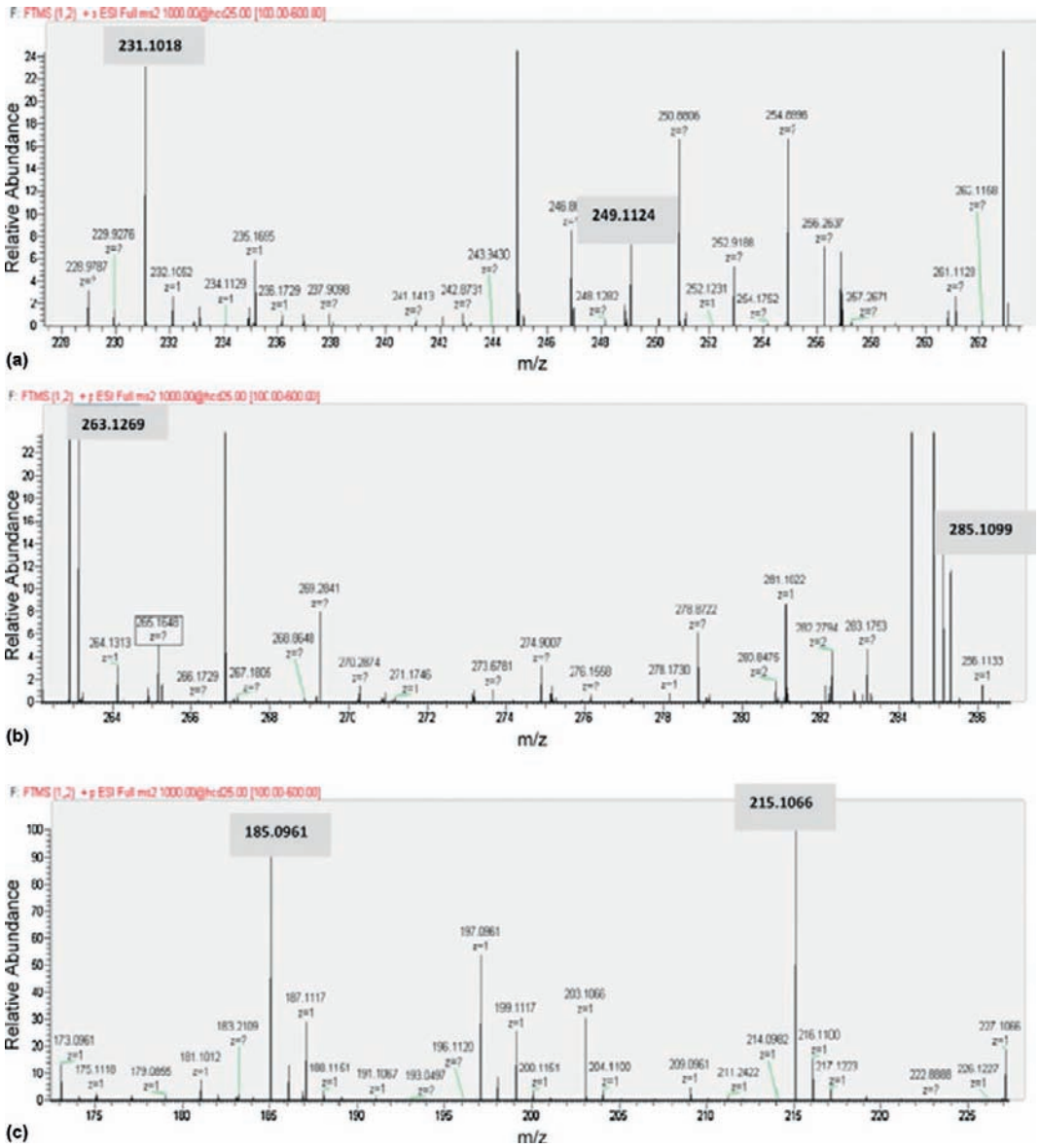

Figure 3 Examples of HCD-MS spectra of deoxynivalenol (a), HT-2 (b), and T-2 (c) obtained by analyzing a bread sample spiked with the three mycotoxins at 412.5 ng g^{-1}. Reproduced from Monaci, L., De Angelis, E. and Visconti, A. (2011). Determination of deoxynivalenol, T-2 and HT-2 toxins in a bread model food by liquid chromatography–high resolution-Orbitrap-mass spectrometry equipped with a high-energy collision dissociation cell. *Journal of Chromatography A* **1218**(48), 8646–8654, with permission from Elsevier.

achieves qualitative and quantitative analysis for target, nontarget, and unknown compounds. High-resolution mass spectrometry has been applied to mycotoxin analysis using orbitrap or quadrupole time-of-flight, and has been recently applied to detect mycotoxins in beer and human milk. An example of typical accurate spectra obtained for each mycotoxin by its fragmentation is depicted in Figure 3.

GC-based methods have a narrower scope, but they have been applied to determine thricothecenes to confirm patulin and to determine some mycotoxins. In particular, the analytical techniques employing GC and capillary columns are useful for the simultaneous determination of different trichothecenes. For the detection step, heptafluorobutyryl, trimethylsilyl, and trifluoroacetyl derivatives are frequently used, coupled with electron capture detection. However, MS is strongly recommended for peak confirmation. In the multistage tandem mass spectrometric analysis, triple quadrupole is used for accurate quantification and ion trap is selected for the fragmentation behavior studies acquiring information about the product ions, while GC-TOF is used as a novel technique for accurate mass measurements based on the extract masses. Limitations such as the need to derivatize the samples before analysis have led to a prevalence of LC-MS over GC-MS approaches.

In recent years, substantial developments have been made in the field of mass spectrometry, enabling introduction of novel ambient desorption ionization techniques such as direct analysis in real time, desorption electrospray ionization, surface desorption atmospheric-pressure chemical ionization, and atmospheric-pressure solid analysis probe. These ion sources achieve high-throughput of analyses, which can be carried out under ambient conditions without (chromatographic) separation of sample components prior to desorption/ionization or need for complicated and time-demanding sample pretreatment procedures. These interfaces have already shown proper prospects to be applied in the mycotoxin analysis.

Conclusions

Although there is a large range of methods for screening and quantification of mycotoxins in food, not all of them cover a broad range of mycotoxins. The current trend has been increasing through the development of multi-mycotoxin methods. One of the latest developments, based on the QuEChERS procedure and its variants, increased the scope of mycotoxins to be analyzed simultaneously when coupled to GC-MS or LC-MS/MS instruments. The application of MS in conjunction with other tools for decreasing limits of detection has been of increased interest in recent times. Future trends would focus on rapid assays and tools that would measure multiple toxins from a single matrix.

See also: Aflatoxin: A Global Public Health Problem; Alkaloids: Properties and Determination; Alkaloids: Toxicology and Health Effects; Mycotoxins: Classification; Mycotoxins: Toxicology.

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Mycotoxins: Toxicology

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Introduction

Mycotoxins or fungal metabolites are found in many of the staple foods worldwide. These natural chemical compounds can be produced by molds on crops in the field, during storage and transportation, and in food processing. If consumed in adequate quantities, mycotoxins can cause a variety of adverse effects in humans and animals termed mycotoxicosis. Often, the better-quality grains are used for export markets, which have more stringent regulatory guidelines and safe usage for grains and foods than use of contaminated grains within the country. This strategy results in the use of higher mycotoxin-contaminated foods in poorer, more vulnerable human populations, particularly in developing nations with little regulatory oversight of raw products.

Cereal grains are at their highest quality and have little risk of mold during storage when they are fully mature at harvest, are free of field mold damage, and have little mechanical damage to the outer seed coat and when harvest is not delayed by climate factors. Climatic events, particularly wetter weather during plant flowering and higher temperatures during field maturation and storage, increase the ability of molds to infect food supplies. The conditions for mold growth, including adequate growth matrices, source of oxygen, moisture level in grain or the water activity (a_w), and temperature, are critical to the success of mold invasion in grains and food. Subsequent conditions and mold stress can cause the mold to produce mycotoxins, which can be generated very rapidly in stressful conditions. A wide variety of molds can grow on field crops; these molds may or may not be mycotoxin producers. Successive mold populations can occur during storage by increasing temperature and moisture in the local environment of grains ('hot spots') and favor the growth of additional molds. Mold spores can deteriorate, but mycotoxins can remain remarkably stable for years in foods and survive many processing procedures.

Over the years, thousands of mycotoxins have been identified, but only a few mycotoxins are well recognized and documented in contaminated foods and feeds. Historically, the knowledge that certain foods can be associated with adverse effects has been observed in populations. Even prior to the Middle Ages, clinical symptoms of ergotism, including marked central nervous system (hallucinations) and gangrenous (peripheral vascular constriction of extremities) effects in humans, were noted in wetter years. The poorer populations consuming coarser grains, such as rye flour, were more prone to adverse effects. In the early to mid-1900s, human epidemics characterized by fever, hemorrhages, and immune suppression were reported in the former USSR. The syndrome was termed alimentary toxic aleukia and was linked with consumption of overwintered grains contaminated with mold, which was later associated with *Fusarium* and T-2 toxin. In animal populations, the use of corn screenings from drought years was often associated with blindness, ataxia, and death in horses, and the use of mold-contaminated corn in wet years was associated with estrogenic signs (swollen vulvae and enlarged mammary glands) in pigs, although no toxic compounds were identified in these syndromes until the latter half of the twentieth century. In the early 1960s, 'Turkey X disease' was identified in England and associated with mold-contaminated peanuts in poultry feed and death of turkeys. Aflatoxins were identified as the cause of marked liver or hepatic damage in the turkeys. Subsequently, a number of mycotoxins were identified in grains and food. Mycotoxins can cause a variety of acute and chronic effects in humans and animals. Some of these conditions are well characterized, and some are possible associations of cause–effect. This article will discuss the more common mycotoxins in food and adverse effects attributed to these toxins.

Aflatoxins

Aflatoxins are potent liver toxins, immunosuppressants, carcinogens, and mutagens and can cause important public health problems. Clinical symptoms of aflatoxicosis include vomiting and abdominal pain, liver necrosis with elevated hepatic enzymes and bilirubin, prolonged clotting times, decreased production of milk and eggs in animals, growth impairment in the young, and reduced immune competence (i.e., vaccine failure or poor antibiotic response in animals and lower populations of immune cells in human subjects). Several large outbreaks of acute aflatoxicosis involving homegrown maize causing liver necrosis and deaths have been reported in India and Kenya. Numerous governments regulate the allowable concentrations of aflatoxins in human foods, fluid milk, and animal feeds. Table 1 summarizes the fungi and associated mycotoxins found in various commodities and the clinical diseases observed in humans and animals.

Aflatoxin B_1 has been associated with hepatocellular carcinoma in humans and is included in group 1 as a human carcinogen by the International Agency for Research on Cancer (IARC). Aflatoxin M_1, a metabolite of aflatoxin B_1, has lower toxicity and carcinogenic activity than AB1. Sterigmatocystin, a mycotoxin produced by *Aspergillus versicolor* and other molds, is a precursor in the synthesis of aflatoxins, a hepatotoxin and carcinogen, and capable of toxicosis similar to, but less toxic than, aflatoxins.

Aflatoxins are low-molecular-weight, lipophilic compounds that are passively absorbed from the gastrointestinal tract. Aflatoxins are absorbed from the intestinal tract into the hepatic portal system. Young animals absorb aflatoxins more efficiently than older animals – therefore, the concern for exposure to aflatoxins in milk and baby foods. Aflatoxin B_1 can bind reversibly to albumin, with unbound aflatoxin B_1 passing from the circulation into tissues. Aflatoxins do not

Table 1 Summary table of fungi, associated mycotoxins, and clinical disease

Fungal species	*Mycotoxin classification*	*Mycotoxins identification*	*Commodity*	*Clinical signs/symptoms*
Aspergillus flavus, *Aspergillus parasiticus*	Aflatoxins	B1, B2, G1, G2 M1, M2	Corn/maize, peanuts, tree nuts, cottonseed, rice, copra, heat, spices, cocoa (chocolate products) Milk products and tissues	Liver toxicity, poor weight gains, immunosuppression, hemorrhages, cancer, death
Fusarium sp. *(F. graminearum, F. Poae, F. acuminatum, F. sporotrichiodes, F. equiseti)*	Trichothecenes	DON (deoxynivalenol) or vomitoxin, nivalenol, 3- and 15-acetylDON, T-2 toxin, HT-2 toxin, T-2 tetral, DAS (diacetoxyscirpenol), neosolaniol	Wheat, maize, hay, straw Overwintered grains	Gastroenteritis, vomiting, diarrhea, immunosuppression, poor growth
Fusarium graminearum, F. culmorum, F. equiseti	Zearalenone	Zearalenone	Wheat, maize/corn, hay	Hyperestrogenism, infertility
Fusarium verticillioides (syn. *F. moniliforme*), *F. proliferatum*	Fumonisins	FB1, FB2, FB3	Corn Corn-based foods	Mild liver toxicity, mild kidney lesions, leukoencephalomalacia (horses), pulmonary edema (swine), possible carcinogen in humans
Claviceps sp. *C. purpurea [C. fusiformis, C. paspali] [Neotyphodium coenophialum in tall fescue grasses]*	Ergot alkaloids	Ergopeptine alkaloids: ergotamine, ergocristine, ergosine, ergocryptine, ergovaline, ergoline alkaloids: lysergic acid, lyergol, lysergic acid amide, ergonovine, clavines	Rye, wheat, cereals, grasses	Vasoconstriction with loss of extremities (ears, feet, tails in livestock), agalactia (reduced milk production in animals), abortions (humans), gastroenteritis, hallucinations (humans)
Aspergillus alutaceus var. alutaceus (formerly *A.ochraceus*), *A. westerdijkiae* and *A. Steynii* and *Penicillium verrucosum* (formerly *P. viridicatum)*	Ochratoxins	Ochratoxin A	Cereal grains (wheat, barley, oats, corn), dry beans, peanuts, grapes, raisins, cheese, apple juice Swine tissue	Liver and kidney toxic, immunosuppressive
Penicillium expansum, *Aspergillus* sp., *Byssochlamys fulva*, *B. nivea*	Patulin	Patulin	Rotten fruits, apple juice, applesauce	Immunotoxicity, liver, spleen, and kidney damage

readily accumulate in tissues, but can be detected in the liver and milk. Repeated exposures can generate toxic effects in tissues. Aflatoxins may cross the placenta and damage fetal tissue; however, little work has focused on reproductive effects. Aflatoxin elimination is through the bile, feces, and urine and into the milk and eggs. Most species eliminate the toxin within 24 h after exposure.

Aflatoxin B_1 is biotransformed by the liver and to a lesser extent the kidney, intestinal tract, and other organs into several products, with the most important toxic product being aflatoxin B_1-8,9-epoxide. The covalent binding of aflatoxin 8,9-epoxide to DNA, RNA, and proteins results in tissue adducts and reduced synthesis of these products and disruption of cellular processes and organ function. The outcome results in cellular necrosis, immunosuppression (both humoral and cell-mediated immunities), mutagenesis, and neoplasms. Additional biotransformation products (e.g., AFM_1, AFM_2, AFQ_1, and aflatoxicol) are less toxic; conjugation of these products with glucuronide and sulfate is detoxification reactions. The metabolite aflatoxicol, which is a carcinogen to animals and possibly a carcinogen to human, can be oxidized back to aflatoxin B_1 and serve as a source for AFB_1 in the body. Aflatoxicol has been detected in milk, eggs, fermented dairy products, and tissues. Pasteurization and ultrapasteurization of milk with various level of fat content did not destroy aflatoxicol. Aflatoxin M_1 is the major excretion product in urine and milk and can be monitored for exposure; aflatoxin M_1 appears quickly in milk and is regulated with allowable concentrations in milk. The European Union maximum allowable concentration in milk is 50 ng l^{-1} (0.05 ppb) aflatoxin M_1, and the US Food and Drug Administration action (FDA) limit is 500 or 0.5 μg l^{-1} (0.5 ppb) in milk. The US FDA action limit for human food products (except milk) is 20 μg kg^{-1} (ppb); the European

Union regulations are stricter for aflatoxin contamination of human food.

All animals are susceptible to aflatoxins, but the sensitivity varies between species with young animals and monogastrics being more at risk for toxicosis. Numerous studies including case-control studies, cross-sectional surveys, and prospective cohort investigations in high-risk human populations have examined the relationship between aflatoxin exposure and human hepatocellular carcinoma, with epidemiological studies supporting animal experimental studies that aflatoxin dietary levels are correlated with human hepatocellular carcinoma. Exposure to aflatoxin and hepatitis B virus greatly increases the risk of liver carcinoma, a particular problem in developing countries. Many mammals and fish are susceptible to the toxic effects of aflatoxin; dogs are quite susceptible to lower aflatoxin concentration in pet foods, while sheep are considered the most resistant domesticated species to toxic effects of aflatoxins.

A diagnosis of aflatoxicosis is based on typical clinical signs and lesions, demonstrating toxic (not trace) concentrations in the ration. Positive fluorescence of grain under a 'black light' (blue-green color at 365 nm) suggests *Aspergillus* metabolites, but not specifically aflatoxins. Various analytic methods can be utilized to determine aflatoxins in feedstuffs; the most important aspect of analysis is to acquire a representative sample of the suspect feed or feeds. When sampling for aflatoxins in feed, the more random and larger the sampling, the better chances will be to detect aflatoxins. In a small sample, even one contaminated kernel can dramatically alter analytic results. Uneven distribution of mold and mycotoxins in a storage facility requires multiple samples at both the perimeter and center of the storage area. A more representative sample is obtained by periodic sampling from a flowing or moving stream of suspect feed to generate a large sample volume (5–10 kg), mixing the sample, and subsampling for analysis. No specific treatment is available for aflatoxicosis beyond quickly removing the contaminated ration and replacing with an uncontaminated food sources. Providing optimum dietary protein, vitamins, and trace elements may aid recovery.

Fumonisins

Fumonisins are produced by *Fusarium* molds primarily on corn and absorbed from the gastrointestinal tract and appear to be rapidly excreted unmetabolized from the gut. Several studies have linked fumonisin contamination of maize with esophageal cancer in humans in South Africa and China; however, a definitive link of fumonisin B1 to human cancer has not been determined. Fumonisin B1 is classified as possibly carcinogenic to humans (IARC group 2B) with inadequate evidence of carcinogenicity in humans but sufficient evidence for carcinogenicity in rodent livers. Fumonisin exposure and possible neural tube defects have been reported in animal studies and suggested to occur in human babies when mothers in early pregnancy consume fumonisin-contaminated maize.

Fumonisins are somewhat unique in that markedly different clinical signs occur in different species with toxicity, although liver damage appears to be common across species. Horses are very sensitive to fumonisins and develop liver toxicity and neurological signs of blindness, ataxia, and aimless circling that progresses to death; the lesion is leukoencephalomalacia or liquefactive necrosis of the white matter in cerebral hemispheres. At toxic levels of fumonisins, pigs develop open mouth breathing, cyanosis of the skin and mucous membrane, high respiratory rate, inactivity, and death that is related to pulmonary edema. Poultry and adult cattle are relatively resistant to fumonisins and may develop liver damage and reduced feed intake at quite high concentrations of fumonisins in feed. Both the US FDA and European Union have recommended guidance levels of fumonisins in animal feeds.

Trichothecenes

Of the trichothecenes, deoxynivalenol (DON) is probably the most commonly detected in cereal grains throughout the world. This mycotoxin is resistant to milling and processing and readily enters the animal feed and human food chains. Foodborne trichothecene contamination, in particular DON, has been linked to acute human adverse effects (gastroenteritis) or mycotoxicoses in China, India, and Japan, but little information is available regarding potential health effects from chronic exposure.

Field reports of trichothecene toxicity in livestock in North America typically involve DON contamination. Most trichothecene exposures are of a chronic to subchronic nature with low dietary DON concentrations associated with nutritional impairment, reduced production, and possible diminished immune responses. Swine and monogastrics are more sensitive to trichothecenes. DON is not associated with fatal disease.

Trichothecenes have multiple toxic effects on eukaryotic cells, including inhibition of protein, RNA, and DNA syntheses, alteration of membrane structure and mitochondrial function, stimulation of lipid peroxidation, induction of programmed cell death or apoptosis, and activation of cytokines and chemokines. Diarrhea is fairly common following trichothecene ingestion. Altered intestinal absorption of compounds and impaired permeability are caused by morphological and functional damage to intestinal mucosa. Significant metabolism of trichothecenes can occur prior to absorption. The hallmark clinical sign of trichothecene toxicosis in animals is feed refusal. Free DON and conjugated DON have been detected in cow's milk at low levels ($<4\ \text{ng ml}^{-1}$) with an estimated 0.0001% of administered dose excreted in milk.

Clinical signs of the more toxic trichothecenes (type A) in animals and humans include vomiting or emesis; feed refusal and weight loss; diarrhea; immunomodulation; coagulopathy and hemorrhage; cellular necrosis of mitotically active tissues such as intestinal mucosa, skin, bone marrow, spleen, testis, and ovary; and death. T-2 toxin can also cross the placenta and potentially affect a developing fetus. Poultry are sensitive to the type A trichothecenes and can develop yellow, caseous plaques on the beak and in the oral cavity along with a dramatic drop in egg production and weight at low concentrations of T-2 toxin. These very toxic trichothecenes are cytotoxic mycotoxins with clinical signs resembling radiation poisoning. T-2 and HT-2 toxin, often found together in plants, are some of the most toxic trichothecenes detected in feeds. Dramatic weather patterns have created wet, cooler conditions in central and

southern United States leading to *Fusarium* growth on wet pasture grasses and production of T-2 toxin, HT-2 toxin, and other mycotoxins in feedstuffs that normally would not be considered to be susceptible to these molds.

Due to the vague nature of toxic effects attributed to low concentrations of trichothecenes, a solid link between low-level exposure and a specific trichothecene(s) is difficult to establish. Mycotoxins may play a role in subtle adverse effects, but diagnostic consideration needs to move beyond just 'test the feed for toxins' and evaluate nutrition and coexisting diseases. Storage of grains at less than 13–14% moisture (<0.70 α_w) and that of hay/straw at less than 20% moisture are important in preventing trichothecene production. After the contaminated feed is removed from the diet and exposure stopped, animals generally have a good prognosis for recovery from type B trichothecenes, but a graver prognosis for recovery from type A trichothecene toxicosis. Table 1 provides a summary of the toxic effects of trichothecenes in animals.

Deoxynivalenol or Vomitoxin

The fungus usually involved in scabby grain blights was identified as *Gibberella zeae* (Schwabe) or *Fusarium graminearum*, its asexual or conidial phase. DON or vomitoxin, also known as Rd toxin, 4-DON, was demonstrated to be the *Fusarium* toxin responsible for feed refusal and emesis (vomiting) in monogastric animals fed with contaminated corn.

Of the trichothecenes, DON is regarded as the most commonly detected trichothecene in cereal grains throughout the world. This mycotoxin is resistant to milling and most processing and readily enters the human food and animal feed chains. Foodborne trichothecene contamination, in particular DON, has been linked to acute human toxicoses in China, India, and Japan, but little information is available regarding potential health effects from chronic exposure. The primary complaint was gastroenteritis.

After oral exposure, DON is rapidly and nearly completely absorbed in the stomach and upper gastrointestinal tract. Pigs dosed with DON had quick absorption of greater than 50% of the DON administered that was highly distributed, with an apparent volume of distribution (V_d) higher than total body water and serum elimination half-lives of 6.3 and 5.3 h in the chronic and acute DON-fed pigs, respectively. A majority of DON ingested from dietary exposure is eliminated in the urine and feces, with the urine being the main excretory route of DON in an unmetabolized form. Minor elimination of DON is in the bile.

In contrast with the poor metabolism of DON by swine, the rumen of cattle is capable of extensive metabolism of DON and other trichothecenes. The major metabolite of oral DON in ruminants is 3α,7α,15-trihyroxytrichothec-9,12-diene-8-one (deepoxydeoxynivalenol or DOM-1). Deepoxidation of DON to DOM-1 is considered a deactivation step resulting in a noncytotoxic compound; thus, ruminal metabolism serves a protective function. Because biotransformation occurs in the rumen, little parent compound is available for absorption.

Trichothecenes bind to ribosomes in eukaryotic cells, in particular to the 60S ribosomal subunits, and interfere with peptidyl transferase activity. Inhibition of protein synthesis requires an intact 9,10 double bond and the C-12,13 epoxide. Trichothecenes can be divided into two groups based on their site of action on protein synthesis, either preferential inhibition of initiation or inhibition of elongation or termination. The cytotoxicity of DON, a trichothecene with a keto group at C-8 and a hydroxyl group at C-7, results from protein synthesis inhibition at the ribosomal level during the elongation and termination step in mammalian cells. The US FDA and European Union have issues recommended levels of DON in human foods and animal feeds.

Zearalenone

Estrogenism in swine was reported in the mid 1920s in the Midwest. Much later in the century, the discovery of the *Fusarium* mycotoxin zearalenone was linked to hyperestrogenism. Typical clinical signs of hyperestrogenism in animals are swelling of the vulva, increase in uterine size and secretions, mammary gland hyperplasia and secretion, prolonged estrus, anestrus, increased incidence of pseudopregnancy, infertility, and decreased libido, and secondary complications are rectal and vaginal prolapses, stillbirths, and small litters. Prepubertal swine are the most sensitive species.

Following ingestion, zearalenone undergoes reduction of the 6′ ketone to a secondary alcohol, which leads to two diastereoisomeric zearalenols (α and β) that are naturally occurring fungal metabolites. The α-zearalenol metabolite is about three times more estrogenic than zeraralenone.

Zearalenone and metabolites can interact directly with the cytoplasmic receptor that binds 17β-estradiol and translocate receptor sites to the nucleus. In the nucleus, stimulation of RNA leads to protein synthesis and clinical signs of estrogenism. Within the resorcylic acids, α-zearalenol exhibited the greatest binding affinity for cytosolic estrogen receptors, while zearalenone and β-zearalenol displayed much lower binding affinities. The hydroxylation of zearalenone to α-zearalenol apparently is an activation process, whereas the production of β-zearalenol would be a deactivation process. Interspecies variations in sensitivity to zearalenone in the feed could be related to different metabolites produced and the relative binding affinities of zearalenone and metabolites formed.

While zearalenone primarily affects reproduction, it may have additional effects. Zearalenone can also act on the hypothalamic–hypophysial axis. Zearalenone acted as a ligand for human pregnane X receptor, which can activate a transcription factor regulating the expression of numerous hepatic drug-metabolizing enzymes, including expression of cytochrome P450 enzymes. This suggests a potential for zearalenone to induce metabolism of drugs. At natural contamination levels in food and feeds, zearalenone does not appear to impact the immune response.

Zearalenone is fairly, rapidly, and extensively absorbed with oral exposure. Following zearalenone administration, zearalenone can be localized in reproductive tissues (ovary and uterus), adipose tissue, and interstitial cells of the testes. The reported biological half-life of total plasma zearalenone radioactivity following the oral dosage in pigs is 86 h. Species differences in zearalenone susceptibility might be related to

hepatic biotransformation, with the highest amount of α-zearalenol produced by pig hepatic microsomes, whereas chicken microsomes produced the highest amounts of β-zearalenol.

Zearalenone can undergo rumen metabolism, with reduction to mostly α-zearalenol (increased estrogenic activity compared to zearalenone) and to a lower amount of β-zearalenol. Zearalenone undergoes extensive enterohepatic circulation and biliary excretion in most species. The major route of excretion for most species is through the feces, although rabbits primarily excrete zearalenone in the urine. No major retention of radio-labeled activity was found in edible muscle tissue, but lipophilic metabolite(s) was reported in egg yolk (at about 2 mg kg^{-1} concentration) 72 h postdosing.

Concern has focused on potential residue of zearalenone and its metabolites in milk, eggs, and foods and precocious development of sexual characteristics in young female girls, although no definitive link has been made. Zearalenone and α- and β-zearalenols can be transmitted into the milk of sheep, cows, and pigs administered high doses of zearalenone. Hyperestrogenism has been reported in lambs and pigs nursing dams dosed with zearalenone. This research indicated that minimal transmission of zearalenone occurs into milk and only for a short period of time after exposure to high concentrations of zearalenone. Zearalenone residues in edible tissue are considered minimal.

Zearalenone has low acute toxicity in most species. In most natural conditions, the concentrations of zearalenone are less than 20 mg kg^{-1} (ppm) and generally less than 5 mg zearalenone/kg feed. Abortions have been associated in field cases with natural *Fusarium* mold exposure, but have not been reproduced with experimental studies. The European Union has regulatory guidelines for zearalenone in animal feeds, whereas the US FDA has not issued any guidelines for zearalenone.

Ochratoxin

Ochratoxins are produced by the fungi *A. alutaceus* var. *alutaceus* (formerly *A. ochraceus*), *A. westerdijkiae*, and *A. steynii* and *Penicillium verrucosum* (formerly *P. viridicatum*). Ochratoxin contamination occurs in a wide variety of foods, including grapes, raisins, coffee, wheat, and other cereal crops. The most toxic of the ochratoxins is ochratoxin A (OTA). OTA is absorbed in the gastrointestinal tract and bound to plasma proteins and is the major compound found in blood. Metabolism can occur through cytochrome P450 enzymes and by cyclooxygenase, lipoxygenase, and epoxygenase enzymes to numerous metabolites, including ochratoxin α, which is considered nontoxic and excreted in urine. Ochratoxins are potent kidney toxicants, immunosuppressants, and possible human carcinogens. The role of OTA in potential carcinogenicity has been hypothesized to be from formation of DNA adducts, oxygen radical formation, and alteration in gene expression involved in cellular proliferation and cell death. OTA is predominately distributed to the kidneys and to a lesser extent to the liver, muscle, and fat. Lesions have been reported in the liver, heart, and gastrointestinal tract of various species exposed to OTA. They inhibit phenylalanine–tRNA ligase causing inhibition of protein, RNA, and DNA syntheses and deplete humoral factors, especially immunoglobulins, and decrease natural killer cell activity.

Descriptive reports associating OTA with cancer usually focused on the incidence of Balkan endemic nephropathy in humans and urinary tract tumors in Balkan countries. However, recent studies indicate that dietary exposure to aristolochic acid from plants is a significant risk factor for chronic kidney failure and the cause of Balkan endemic nephropathy. Aristolochic acid exposure was associated with DNA adducts in kidney tissues and mutations of A:T pairs of the p53 gene. There is evidence that OTA is carcinogenic in experimental animals, but the evidence is not adequate for an increased risk of cancer in humans. OTA is classified in IARC group 2B, possibly carcinogenic to humans

Ruminants are more resistant to ochratoxin, as compared to monogastrics, because rumen protozoal metabolism quickly converts OTA to the less toxic ochratoxin α. The elimination half-life in ruminants is short, about 17.3 h as compared to 100 h in pigs. Regulatory concerns for OTA are focused on potential residues in the kidneys of pigs and in ruminants for accumulation in tissues and milk residues. Another nephrotoxic mycotoxin often found with ochratoxins is citrinin. Citrinin is produced by *Penicillium* molds under similar conditions of ochratoxins, with optimal production at 30 °C. OTA and citrinin target the renal proximal tubule, causing dehydration, hypothermia, and death in animals. Histological lesions in the kidney were hyaline casts, dilated tubules, and fibrosis in the kidneys and fatty changes in the liver.

Ergot Alkaloids

Ergot can refer to the disease caused by *Claviceps* sp. invading the developing ovary of certain grasses and to the visible sclerotium or ergot body of *Claviceps purpurea* growing on rye, wheat, and other cereals and grasses.

Following ingestion of the ergopeptine alkaloids, the compounds are rapidly cleared from the blood by the liver and undergo primarily biliary excretion; ergot alkaloids are not thought to be excreted in milk. Ergopeptine alkaloids cause vasoconstriction through inhibition of the D_1 dopaminergic receptor and partial agonism of the α_1-adrenergic and serotonin receptors. Constriction of small arteries can lead to ischemia and necrosis of the distal portions of the limbs and ears and tails of animals; the burning sensation from vasoconstriction leads to the term used with ergot toxicity or 'holy fire.' Uterine contractions in late pregnancy are associated with stimulation of α_1-adrenergic receptors, but evidence for abortion is vague in animals, but described more frequently in human conditions. Decreased prolactin secretion by lactotropes in the anterior pituitary is associated with ergopeptine alkaloid stimulation of D2-dopamine receptors, which leads to clinical signs of poor milk production in animals. Table 1 summarizes the fungi, ergot alkaloids, and clinical disease associated with ergotism.

Susceptibility of livestock to ergotism appears to vary with species, breed, age, gender, and physiological state. Ruminants are considered susceptible because of the types of diets (both grains and grasses) and ruminal pH that can favor fermentation and enhanced extraction of ergot alkaloids from sclerotia. Numerous risk factors influence the development of clinical

signs, including extremes in environmental temperatures (too cold or hot), physiological state, and ergot alkaloid dose. The concentration of ergot alkaloids in *C. purpurea* sclerotia is highly variable, between 0 and almost 10 000 mg kg^{-1}, and the relative concentrations of individual ergopeptine alkaloids vary. The threshold concentrations of total ergot alkaloids or ergopeptine alkaloids capable of causing ergotism in animals have not been established; however, the threshold ergovaline concentrations that produce clinical fescue toxicosis related to ergot alkaloids are approximately 200–800 μg kg^{-1} in a total ration and likely approximate the threshold concentrations of ergopeptine alkaloids that produce ergotism in livestock. Dietary concentrations of 0.3–1% sclerotia have been associated with clinical signs of ergotism in cattle. Initial clinical signs of ergotism can appear as pain and lameness (e.g., stamping the feet) with affected areas feeling cool to the touch. With disease progression, a sharp line of demarcation appears between healthy and nonviable tissue, which can be followed by necrosis and sloughing of tissue. Additional clinical signs may affect the gastrointestinal tract (e.g., constipation, diarrhea, or internal bleeding). During hot, humid weather, clinical signs of ergotism may include hyperthermia and lower milk production and feed intake in animals. Hyperthermia is related to poor heat dissipation from the body surface, and reduced milk production is associated with hypoprolactinemia. To avoid adverse effects of ergot on livestock performance, the total dietary concentrations of ergot alkaloids should be less than 100–200 μg kg^{-1}.

Patulin

Patulin is a cyclic γ-lactone mycotoxin found in fruits, particularly moldy apples and apple juice. Several molds produce patulin including, *P. expansum*, *Aspergillus* sp., and *Byssochlamys fulva* and *B. nivea*. Patulin is a small, water-soluble polar compound that is absorbed quickly, metabolized, and excreted in urine. Characterized as an electrophilic molecule, patulin can cause adverse effects through covalent bonds to the thiol groups of proteins and glutathione. Acute symptoms of patulin exposure include immune toxicity, particularly inhibition of macrophage functions, neurotoxicity, and possible damage to the liver, spleen, and kidney.

See also: Aflatoxin: A Global Public Health Problem; Mycotoxins: Classification; Mycotoxins: Occurrence and Determination.

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Nanotechnology in Food Processing

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Abbreviations

GIT Gastrointestinal tract
O/W Oil-in-water
W/O Water-in-oil

Introduction

The considerable potential of nanotechnology for improving food safety, quality, and healthfulness is increasingly being recognized, as demonstrated by the major increase in the number of publications and patents on this topic. Some of the most important potential applications of nanotechnology in food are summarized in the succeeding text:

- *Encapsulation systems* for the protection, controlled release, and targeted delivery of bioactive, flavor, color, or antimicrobial molecules
- *Texture building structures* for improving food quality and novelty
- *Biosensors* for rapid detection of microbial or trace element contamination
- *Active packaging* strategies to improve food quality and safety

Food nanotechnology focuses on those structures that have at least one dimension smaller than around 1000 nm but larger than a single molecule and include nanoparticles, nanofibers, and nanofilms. All of these structures already find widespread application in food processing or storage. Their small dimensions result in unique, novel, and very attractive functionalities.

Nanoparticles can be produced by two different fabrication principles, that is, top-down and bottom-up techniques. In the top-down process, a large clump of material is broken down into nanosized particles by an external force (e.g., physical, chemical, or mechanical forces). In the bottom-up process, atoms, molecules, or small particles assemble into nanostructures. Bottom-up processes are preferable in terms of controllability, engineering, cost, and energy requirement and will therefore be the focus of this article. There are numerous examples of bottom-up nanostructure formation processes found in nature. Spontaneous self-assembly is one of the driving forces for bottom-up processes, which is mediated through relatively weak intermolecular interactions, such as van der Waals, hydrogen bonding, electrostatic, hydrophobic, and/or steric exclusion interactions. In this manuscript, covalent bonds will also be considered as these are often useful in the food industry to further stabilize the formed nanostructures by strengthening the bonds between the self-assembled molecules. Self-assembly processes usually take place under relatively mild conditions (i.e., temperature, pressure, and pH), and upscaling of these methods is typically relatively straightforward. Nanostructures to be used in the food production chain are mainly produced in a liquid phase to ensure the mobility, stability, and the interaction/reaction of the various components involved.

In this article, different nanostructure types will be described and the molecular self-assembly process will be discussed. The application of the different nanostructures in food products will also be highlighted, before concluding with some future perspectives and consumer perception toward nanotechnology.

Nanostructure Types

Different nanostructure types (Figure 1) can be produced from food-grade ingredients that have different compositions, structures, properties, and applications. The materials used to build these different structures for food applications should typically be natural, biocompatible, biodegradable, and food-grade. Biomolecules that already have found widespread use are polysaccharides (e.g., alginate, carrageenan, chitosan, dextran, pectin, and xanthan), proteins (e.g., casein, gliadin, soy protein, whey protein, and zein), lipids (e.g., long- and medium-chain triglycerides and flavor oils), and phospholipids (e.g., dairy, soy, or egg lecithin).

Nanoparticles

Different types of nanoparticles can be distinguished based on their composition and structure, for example, capsules, hydrogel

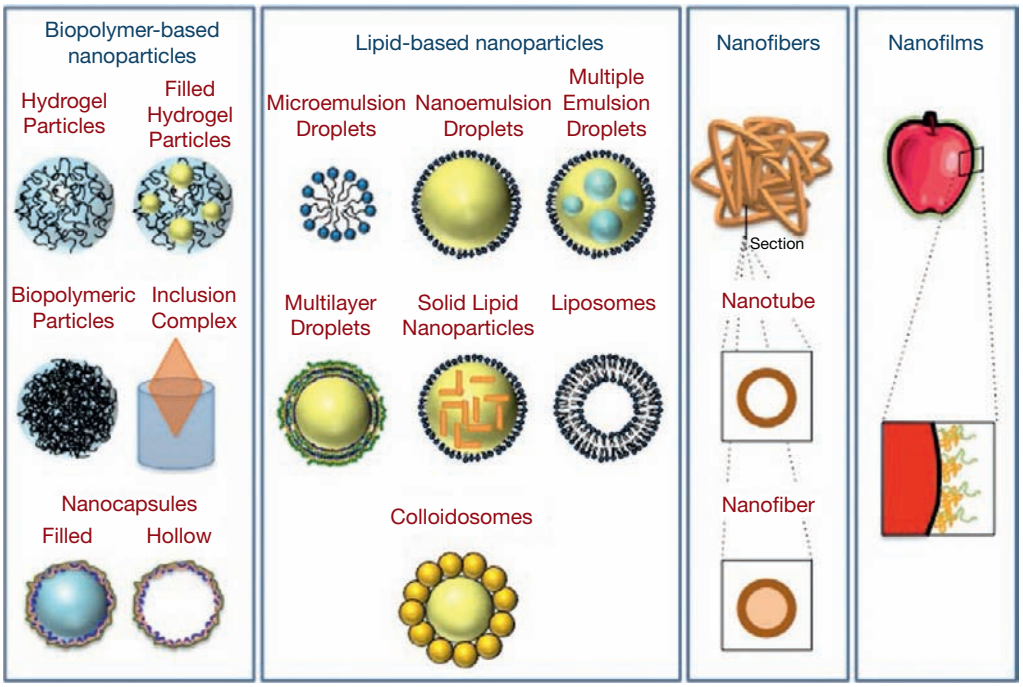

Figure 1 Examples of nanostructures that can be used in food processing (structures are not drawn to scale).

particles, biopolymeric particles, liposomes, nanoemulsions, molecular inclusion complexes, and solid lipid nanoparticles. Some of the most suitable nanoparticles for food applications will be briefly discussed in this article. For clarity, the particles are divided into two sections based on their main building material, that is, biopolymers and lipids. However, the boundary between these two groups is not always clear as lipid-based systems are often stabilized and coated by biopolymers, while biopolymeric nanoparticles can also contain lipid inclusions.

Biopolymer-based nanoparticles

Biopolymers, that is, polysaccharides and proteins, are excellent building molecules for nanoparticles. They cover a wide range of properties and functionalities due to the versatility of their basic units (monosaccharides or amino acids), arranged in a specific macromolecular structure. Different chemical, physical, or enzymatic methods can be used to modify their structures and therefore give rise to different macromolecular properties and functionalities. Some of the most important molecular properties of biopolymers that influence their ability to form nanoparticles with specific functional properties are their molecular weight, electrical charge characteristics, polarity, and chemical reactivity.

Nanosized hydrogels are composed of a cross-linked polymeric matrix that fills the entire volume of the particle and holds high amounts of water. Gelation can be induced by cross-linking agents (e.g., multivalent salts) or enzymes and pH or temperature changes (e.g., heating or cooling). Techniques that can be used to produce nanosized hydrogel particles are emulsion templating and gelation in nanosized molds. The hydrogel particles can also enclose smaller substructures such as lipid droplets. These more complex structures are denoted as filled hydrogel particles.

Biopolymeric nanoparticles are also fabricated from biopolymers but typically have a much denser internal core (lower water content) than hydrogel particles. These particles can be made by liquid antisolvent precipitation, thermal denaturation of globular proteins (e.g., soy, egg, or whey proteins), or self-assembly of flexible proteins (e.g., casein).

Inclusion complexes entrap a guest molecule in a host molecule by physicochemical forces. These resulting molecular complexes may increase the water dispersibility of the guest molecule, protect it from chemical degradation, and help mask undesirable flavor attributes (such as bitterness). The list of host molecules suitable for food systems is currently rather limited, with cyclodextrins, starch, and whey proteins being the most widely used in a food context.

Nanoparticles typically have a homogeneous structure throughout, whereas *nanocapsules* have an external shell that surrounds an internal core, which may be gaseous, liquid, or solid. Bioactive components are typically encapsulated within the core, while the shell is designed to protect them or to control their release. The shell may be constructed from a single layer or from multiple layers. Based on the application and the substance that makes up the core, the nanocapsules can be classified as filled (or loaded) or hollow (or unloaded).

Lipid-based nanoparticles

Lipids are typically immiscible with water, which leads to phase separation and the creation of an oil–water interface. The interfacial area for lipid-based nanoparticles is typically large and needs to be stabilized by amphiphilic surfactants, biopolymers, or particles.

Microemulsions are thermodynamically stable colloidal dispersions with particle diameters well below 100 nm. Oil-in-water (O/W) microemulsions are usually fabricated from

predominantly hydrophilic surfactants. The surfactant molecules arrange themselves so that the nonpolar tails form a hydrophobic core and the polar heads form a hydrophilic shell that is in contact with water. Conversely, water-in-oil (W/O) microemulsions are typically formed from hydrophobic surfactants. In this case, polar head groups form a hydrophilic core, while the nonpolar tails form a hydrophobic shell in contact with the surrounding oil. Microemulsions form spontaneously when certain combinations of surfactant, oil, and water are mixed together under appropriate environmental conditions (e.g., temperature).

Nanoemulsions are colloidal dispersions typically formed from emulsifiers, oil, and water. Like conventional emulsions, nanoemulsions are thermodynamically unstable systems; however, they have smaller droplet sizes (i.e., radius <100 nm). As a result, they have different physicochemical properties, such as higher optical clarity, higher stability to aggregation and creaming, and higher bioavailability. Two basic nanoemulsion types can be distinguished, that is, O/W and W/O nanoemulsions. O/W nanoemulsions consist of oil droplets dispersed into a continuous aqueous phase, while W/O nanoemulsions consist of water droplets dispersed into a continuous oil phase. Nanoemulsion production techniques can be categorized as low- or high-energy methods. Low-energy methods can be considered to be bottom-up methods and are based on the spontaneous formation of nanosized oil droplets when the composition and temperature of surfactant–oil–water systems are changed. These, hence, do not require high-energy input. Nanoemulsions can also be fabricated by high-energy methods that are a top-down approach that utilizes intense disruptive forces to break down larger droplets into smaller ones. Typical production methods involve high-pressure homogenization or sonication.

The simple emulsions discussed in the preceding text can be used to build more complex structured emulsions using nanotechnology principles. *Multiple emulsions* (such as W/O/W and O/W/O) are formed by homogenizing an emulsion with a liquid that has an opposite polarity to its continuous phase (e.g., a W/O emulsion with water). *Multilayer emulsions* are composed of lipid droplets coated by multiple layers of biopolymers or other materials. The formation of multiple layers can improve the physical or chemical stability of emulsions or be used to design controlled or targeted delivery systems. The multiple layers are typically formed by layerwise electrostatic deposition of oppositely charged biopolymers. *Solid lipid nanoparticles* are O/W nanoemulsions in which the lipid phase is either fully or partially solidified. These systems are usually prepared by preparing an O/W nanoemulsion at a temperature that is higher than the melting point of the lipid phase and then cooling down to initiate crystallization of the lipid phase.

Nanoliposomes are formed from phospholipids that tend to self-assemble into vesicle structures in aqueous media upon energy input. They may have single or multiple bilayers and may have diameters of as small as tens of nanometers. Their physicochemical and functional properties depend on the composition, preparation method, and environmental conditions.

Colloidosomes or *pickering emulsions* are emulsions stabilized by the adsorption of other particles to the oil–water interface. Once the particles are adsorbed at the interface, they prevent the aggregation and coalescence of the emulsion droplets. In foods, the stabilizing particles may be fat crystals or biopolymer biopolymer-based nanoparticles.

Nanofibers and Nanotubes

Nanofibers and nanotubes are being developed as potential structure building or encapsulation ingredients in foods. Nanofibers composed of biopolymers can be produced by electrospinning. Typically, the biopolymers are dissolved in an appropriate solvent and then forced through a nozzle using a high-voltage electrical field and collected on a grounded plate. In principle, nearly every soluble polymer with a high enough molecular weight can be electrospun. However, research to date has mainly focussed on proteins and it has often proved difficult to spin proteins under food-grade conditions. Furthermore, the complex three-dimensional structure and strong inter- and intramolecular interactions of proteins often need to be overcome by additives. Inclusion of gelatin or zein as carriers for other proteins during electrospinning has also been successfully explored. Nanofibers have the advantages that they are easily recoverable but still have a very large surface-to-volume ratio. Nanotubes that typically have a hollow core may be useful for delivery applications as they can be fairly easily loaded with large quantities of bioactive molecules.

Nanofilms

Nanofilms can be described as a thin layer of polymers attached to a food product or food packaging material. Nanofilms are very fragile due to their extremely thin nature; therefore, they are more likely to be used as coatings that are attached to food surfaces, rather than as self-standing films. Nanofilms are usually created using layer-by-layer deposition techniques. These structures have potential applications in the active food packaging field and for nanofiltration.

Intermolecular Interactions and Self-Assembly

All atoms, molecules, and particles experience forces between them, which will determine their tendency to self-assemble into specific structures (Figure 2). These interactions can be characterized by their sign (attractive or repulsive), strength (weak to strong), and range (short to long) (Table 1). Atoms, molecules, and particles typically experience a combination of forces. Several theories have been developed to model the total interaction energy between molecules and particles. One of the most popular theories to predict the interaction between charged particles is the DLVO theory. In this theory, the total interaction between molecules and nanostructures is calculated as the sum of the van der Waals attraction and electrostatic repulsion. The DLVO theory has proved useful for predicting the stability of particles against aggregation in various food systems. However, there are many limitations to the practical application of this theory due to its underlying assumptions: it ignores many important interactions (e.g., steric, hydrophobic, and depletion), it assumes the particles

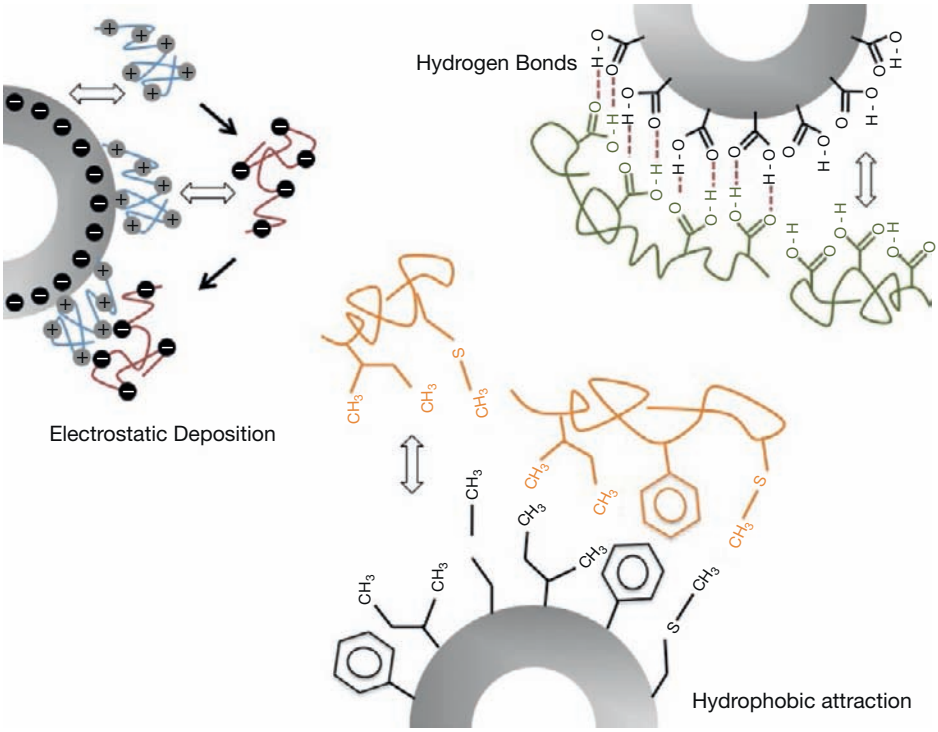

Figure 2 Schematic representation of the interaction between molecules and particle surface for electrostatic attraction, hydrogen bond formation, and hydrophobic attraction.

Table 1 Overview of the different intra- and intermolecular interactions and their characteristics

Interaction type	*Description*	*Attractive or repulsive*	*Other characteristics*
Electrostatic or Coulomb interaction	Interaction between charged entities	Attractive or repulsive	Inversely related to the square of the distance between the charge centers. Not direction-specific and quite strong interaction
Hydrogen bond	Interaction between a hydrogen atom in a molecule and an electronegative group (typically oxygen or nitrogen)	Attractive	Relatively short-lived, moderately strong, direction-specific
Hydrophobic interaction	Attraction between aliphatic chains and aromatic groups	Attractive	Relatively short-ranged, not direction-specific, moderately strong, but their magnitude increases with increasing temperature
van der Waals interaction	Interaction between (induced) dipoles	Attractive	Ubiquitous, relatively weak, very short-ranged (inversely related to the sixth power of the distance between the dipoles), additive
Covalent bond	Electrons are shared between the orbitals of more than one atom	Attractive	Direction-specific, atom-specific, short-ranged

are homogeneous spheres, and it assumes only two particles are involved in the interaction.

Spontaneous self-assembly is the organization of molecules into larger structures solely based on their intermolecular interactions and thermal energy effects, without the interference of external agents. This kind of self-assembly occurs during the formation of surfactant micelles. Directed self-assembly relies on the tendency for the molecules to spontaneously associate with each other, but the type of structures formed is controlled by manipulating external conditions. This kind of self-assembly occurs during multilayer formation. Both spontaneous and directed self-assembly of biopolymers and lipid-like molecules occur in nature during the creation of biological nanostructures, such as protein complexes, cell walls, or phospholipid membranes. Food scientists can use the knowledge of the molecular interactions between different molecules to assemble different structures in foods.

Electrostatic Attraction

Electrostatic attraction between oppositely charged groups is one of the most important interactions utilized to assemble specific structures and is widely used to form nanoparticles and to modify nanoscale surface properties.

Ionic gelation: Charged biopolymers are often made to gel by adding oppositely charged substances, such as mineral ions. For example, anionic polysaccharides (such as alginate, carrageenan, and pectin) can be gelled by adding cationic mineral ions (such as calcium). Ionic gelation is commonly used as a step in the formation of hydrogel particles, such as calcium alginate or pectin beads.

Coacervation: Coacervates are hydrogel particles that are typically formed when oppositely charged biopolymers associate with each other through electrostatic attraction. These particles are widely used in the food industry for encapsulation and release purposes.

Hydrogen Bonds

Hydrogen bonds are important in the formation and stabilization of biopolymer complexes and may play an important role in the formation of hydrogel particles, for example, gelatin beads. They have also been employed as the driving force to create and stabilize layer-by-layer films intended for food packaging. Hydrogen bonds typically weaken as the temperature is increased, which may lead to dissociation of gel-like structures. This phenomenon can be used for controlled release applications.

Hydrophobic Interactions

Hydrophobic interactions play an important role in the formation of many heat-induced gels. For example, the heating of a globular protein solution triggers the unfolding of the protein and the exposure of hydrophobic groups that are normally buried inside the protein interior. Interaction between the exposed hydrophobic patches on the protein surfaces results in the formation of an irreversible gel structure. Hydrophobic interactions also play an important role in the *liquid antisolvent process*, one of the techniques used to produce biopolymeric nanoparticles. By decreasing the solvent power of the solution in which a biopolymer/protein has been dissolved, the three-dimensional structure of the protein changes, and the protein partially denatures or unfolds by the exposure to the less favorable environmental conditions. As a result, the biopolymers prefer to associate with each other over the interaction with the 'low-quality' solvent. As several structures on the biopolymer are now exposed, which are usually buried inside the native biopolymer structure (e.g., hydrophobic patches in proteins), new interaction possibilities between the biopolymers are created. Although hydrogen bonds and electrostatic interactions will probably also play a role, the irreversible hydrophobic interactions are believed to be a significant driving force behind the formation of protein particles using this method. Hydrophobic attraction is also the driving force to create *inclusion complexes*. The center of the host molecules (such as cyclodextrins and amylose coils) is usually highly hydrophobic. In aqueous media, hydrophobic guest molecules will be attracted to the center of these structures, replacing part of the water inside. These complexes are therefore more thermodynamically stable.

Covalent Interactions

Covalent bonds are often used to stabilize the nanostructures in foods against changes in pH, temperature, and ionic strength. The formation of covalent bonds can be induced by heating a mixture of polysaccharides and proteins to induce Maillard reactions, by adding cross-linking chemical agents or enzymes (e.g., transglutaminase or laccase), or by UV light treatment.

Combined Interactions

In reality, the interaction between the different building blocks used to fabricate nanoparticles often involves more than one type of interaction, and therefore, these all have to be carefully controlled to obtain the desired structure and performance.

Surface Engineering

The surface properties of particles determine their behavior in the food matrix and in the gastrointestinal tract (GIT). The properties of nanostructures can be manipulated by controlling their surface properties, for example, by coating them with one or more layers of biopolymer or lipid. The attachment of biopolymers to the nanostructure surface is generally driven by electrostatic attraction. The interfacial layers can also be further stabilized, and their permeability can be reduced by (enzymatic) cross-linking. The electrostatic layer-by-layer deposition technique has been widely used to create multilayered particles or film surfaces using biopolymers. Every new coating layer reverses the charge on the particles, which ensures that only single layers of the molecules are deposited during each coating cycle, and that this method can be repeated for different cycles and can give rise to the formation of particles with multiple layers. Polyelectrolytes are generally preferred over small molecules as these ensure a good adhesion of a layer to a surface by having a certain number of ionic bonds. Salt can be used to smoothen the conformational structure of the multiple layers as they can break ionic bonds between the different layers, which, after removal of the salt, can reassemble in a more equilibrated conformation. Next to electrostatic interactions, hydrophobic attraction, hydrogen bonding, and/or specific molecular interaction can also be used to trigger the deposition of a new layer of molecules on a surface.

Furthermore, edible or nonedible nanofilms can also be engineered in such a way as to display a specific functionality, such as antimicrobial or chelating properties. The attachment of the bioactive molecules to the surface can be based on electrostatic or covalent interactions. As mentioned in section 'Covalent Interactions,' covalent bonds can be created by UV light irradiation or by the use of cross-linking agents. In the case of nonedible films, a wider selection of cross-linking agents can be used since they do not necessarily have to be food-grade because they are not directly applied on the food product. A common and promising practice in the food industry is the modification of a surface to make it more suitable for the attachment of bioactive compounds. Surface modification can be performed through a wide variety of techniques: wet chemical technique, silane monolayers, ionized gas treatment, and UV light irradiation.

Utilization in Food

In this section, we highlight a number of promising and emerging nanotechnology applications in the food industry.

Delivery Systems

Nanostructures are promising carriers for biological and functional compounds with regard to their protection, controlled release, and targeted delivery during food processing, storage, and/or after food ingestion. These systems can also be utilized to mask off-flavors, to stabilize the color of food products, to increase the shelf life of products by controlled release of antimicrobial agents, to increase the solubility of bioactives, or to decrease unwanted chemical reactions. Next to increasing the stability of the enclosed component by shielding it from environmental influences, sometimes, targeted delivery of the enclosed component in the GIT is envisaged. Size reduction often goes hand in hand with improved bioadhesive properties of nanosized particles, which leads to a prolonged residence time and, often, higher absorption in the GIT. Combined with a large surface area per volume, this leads to a higher bioavailability of the enclosed bioactive components. Next to size, the interaction of nanoparticles with the GIT is determined by nanoparticle morphology, charge, surface characteristics, and hydrophilicity. A decision scheme has been developed to predict the potential uptake of nanoparticles in the GIT. To study the behavior and suitability of a nanosystem for the delivery of bioactives in the human body, standardized laboratory protocols have been developed to mimic the conditions in the GIT. However, these methods have predominantly been applied to lipid-based nanoparticles. Only very few *in vitro* studies can be found that focus on the bioavailability of components enclosed in biopolymer-based nanoparticles.

Stabilizers of Food Texture

Nanostructures are promising materials to alter, improve, or mimic the structure and texture of food products. The quest for healthier food products often involves the reduction of one food ingredient (e.g., fat) or product (e.g., meat) in our diet. However, the reduction or removal of a food ingredient often has drastic effects on the overall food matrix properties. Although reducing the fat content will decrease the caloric content of the food product and can provide a solution for obesity, this often also leads to a decrease in the palatability of the food product. Therefore, the development and marketing of these products still prove challenging. The role of fat in food products is very diverse since it affects texture, appearance, flavor, etc., and so, the mere replacement of fat droplets by another component without losing fat functionality is not straightforward. A possible strategy that recently came into focus is controlled flocculation of fat droplets, which leads to increases in viscous or elastic properties. In this way, highly viscous and even gel-like textures typical for high-fat products can be created while using significantly lower fat contents. Flocculation can be induced by changing the system pH, by heating, or the addition of multivalent cations, dependent on the emulsifier used.

Nonmeat products have been developed that nevertheless have an appealing meat-like structure. One of the explored strategies comprises the use of protein nanofibers, produced by electrospinning. After spinning, the fibers also need to be aligned in order to mimic the meat structure.

Nanobiosensors

Nanobiosensors are built to recognize several target groups/molecules to detect pathogens or hazardous chemicals in a fast and reliable way. The overall goal is to produce nanobiosensors that are user-friendly and reduce the reagent volumes and detection time without compromising the test sensitivity. The incorporation of nanotechnology in the development of biosensors has drastically reduced the detection limits of the devices. The sensitivity of these sensors can even go as low as one pathogen cell. The improved sensitivity of the sensors relies on the size of the nanoparticles that control various electronic and optical features for detection and quantification.

Hydrogel nanoparticles can also be used to build nanobiosensors, since these structures can improve the retention of analytes and can be tailored to create specific responses to mechanical, optical, thermal, magnetic, and electrical stimulation. The signals that the nanobiosensors use to detect the analytes can be microgravimetrically, electrochemically, magnetically, and/or optically detected. Microfluidics are an important tool for designing and manufacturing these kinds of sensors to reduce sample volume and to make devices more portable and autonomous. Nanobiosensors can be used along the whole production chain from the field to the supermarket. They can be used to detect pathogens, harmful substances, and adulteration.

Food Packaging

Prolonging and ensuring the shelf life of a food product is a constant challenge for food scientists and technologists. The use of nanostructured or nanomodified materials is a promising way to achieve this goal. Edible food coatings can be applied directly on the product, while nonedible coatings often work as a protective container without being part of or being directly applied to the food product.

Edible nanofilms can be engineered as single or multilayer structures that function as a barrier for agents that are detrimental to stability or safety, such as oxygen, moisture, and/or microorganisms. Edible nanofilms can also work as delivery systems for antioxidant or antimicrobial agents like essential oils, which ideally should be released over time. Probiotic bacteria have also been introduced to edible films to create functional food products. The advantage of using edible films is that the probiotic can be added to minimally processed foods like fruits or vegetables or food products that have already been processed to ensure the viability of the enclosed probiotic.

The surfaces of nonedible films, such as plastics for food packaging, can also be engineered to serve as active packaging materials. This strategy often focuses on oxidation prevention or minimizing microbial spoilage of the contained food. Surface engineering can also be applied to surfaces that are in contact with foods during processing to avoid fouling, the formation of biofilms, or the loss of process efficiency.

Societal Perception and Future Trends

The application of nanostructures in the food industry raises questions on food safety and security. All materials, to some

extent, pose some threat to human health and environment, but, as described in the preceding text, the small dimensions of these structures further increase the reactivity and interaction with the GIT. Nanotoxicology evaluates the toxicity effects of nanoscale materials on the health of organisms. This research field will need to keep pace with the developments made in the food nanotechnology field. Next to assessing the risk, the public perception also needs to be taken into consideration. Thinking and talking about food nanotechnology in all openness with all partners involved is essential to the future and success of the application of nanotechnology in a food context.

One of the obvious short-term trends in the food nanotechnology field is pushing the limits of what is possible by combining materials, fabrication strategies, and intermolecular force manipulation. To date, however, nanostructures to be used in the food science field are often developed in an empirical way. This has led to the development of a lot of different systems but is simultaneously very time-consuming and expensive. A reverse engineering approach, which starts from the functionality that the particles must display to select appropriate ingredients and production methodologies, would tremendously speed up the fabrication of particles with tailor-made functionality. However, the absence of in-depth knowledge on the underlying intermolecular interactions determining the particle formation and stability is slowing down the applicability of the reverse engineering approach. Furthermore, the complexity and biological variability and, hence, unpredictable behavior of the majority of the ingredients used to produce these nanoparticles further hamper this approach. A thorough characterization and more reliable, constant supply of certain macromolecules with well-defined properties and specifications would greatly enhance a more evidence-based approach to the production of tailor-made particles to be used in the food context.

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See also: Antioxidants: Role on Health and Prevention; Bioavailability of Nutrients; Biosensors; Food Colloids and Emulsions; Maillard Reaction; Meat: Structure.

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Natamycin

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Introduction

Natamycin is a naturally occurring antimicrobial compound that is generally produced by the bacterium *Streptomyces natalensis*. Given a generally recognized as safe (GRAS) status, it is considered as a natural preservative by the European Union (EEC No. 235). Natamycin is usually applied at concentrations between 1 and 10 mg l^{-1}. It was discovered in the 1950s where it was described for the first time by Struyk as "a new crystalline antibiotic, pimaricin, has been isolated from the fermentation broth of culture of *Streptomyces* species, isolated from soil sample obtained near Pietermaritzburg, State of Natal, Union of South Africa. The organism which makes it has been named *Streptomyces natalensis*."

Natamycin also known as pimaricin or tennectin is a polyene macrolide antimycotic produced by submerged fermentation of strains of *Actinomycetes* such as *Streptomyces natalensis*, *Streptomyces chattanogenesis*, and other closely related species. Due to its inherent property of being safe to humans, it is widely used against yeast and molds. In a study, it was found to be effective in controlling the growth of *Aspergillus carbonarius* strains and the production of its mycotoxin under different environmental conditions. Diverse works have shown its activity against *Aspergillus flavus*, *Aspergillus carbonarius*, *Aspergillus niger*, *Aspergillus versicolor*, *Penicillium chrysogenum*, *P. glabrum*, *P. commune*, *P. verrucosum*, *Byssochlamys*, and other species of molds. Because of this selective action, major applications of natamycin as a biopreservative have been seen in bacterial fermented foods since it exhibits no antibacterial activity. Thus, it can be used on the surface of cheese and fermented meats or in yogurt, fermented creams, and similar products. It is effective at low concentrations, possesses broad-spectrum activity, lacks resistance development, has no negative effect on the quality of food products, and has prolonged working time. The amphoteric character of natamycin is responsible for its low solubility in most solvents.

At extreme pH values (high or low), natamycin is completely soluble, which can be used to optimize/control the availability of active natamycin. Under these conditions, natamycin possesses strong antimycotic activity but is also rapidly decomposed. Therefore, such solutions might be beneficial in the case of extreme contamination, but under normal hygienic conditions, there is no added value in this approach.

Commercial Preparations of Natamycin

Commercial preparations available are Natamax® (Danisco, Denmark), Delvocid® (DSM, Holland), Natacyn® (US and Canadian brand names), Miconacina (Mexican brand name), and Silver Elephant Natamycin (Zhejiang Silver Elephant Bioengineering Co., Ltd., China). Most of the preparations comprise 50% natamycin with the incipient being lactose, glucose, or salt. Preparations are also available that contain food-grade polymers that aid the adherence of natamycin for surface treatments of foods.

Chemical, Physical, and Biological Properties

Natamycin belongs to the group of polyene macrolide antimycotics characterized by its macrocyclic lactone ring with a number of conjugated carbon–carbon double bonds (Figure 1). Chemically, it is (1*R*,3*S*,5*R*,7*R*,12*R*,22*R*,24*S*,25*R*,26*S*)-22-{[(3*S*,4*S*,5*S*,6*R*)-4-amino-3,5-dihydroxy-6-methyloxan-2-yl] oxy}-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo [22.3.1.$0^{5,7}$]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid, used for a variety of fungal infections, mainly topically.

The structure of natamycin could be determined using proton and ^{13}C NMR (nuclear magnetic resonance) spectroscopy and 3-D structure by the latter. The molecular weight of natamycin is 665.75 with an empirical formula $C_{33}H_{47}NO_{13}$. Its CAS registry number is 7681-93-8. The isoelectric point of natamycin is 6.5. It is a white or creamy-white powder with almost no odor or taste. Solid natamycin and natamycin suspensions are quite stable to heat. Even heating for several hours at 100 °C causes only a slight decrease of its activity. Natamycin is soluble in polar organic solvents, like glacial acetic acid and dimethylformamide, slightly soluble in methanol, and practically insoluble in fatty and mineral oils and water.

Solubility of Natamycin

In aqueous solutions, its solubility is approximately 40 ppm. A suspension containing 0.5% natamycin at pH 6.5 hardly loses any activity at room temperature in the dark, which proves that neutral aqueous suspensions of natamycin are also very stable. Natamycin has low solubility in water (~40 μg ml^{-1}), but this low solubility is an advantage in the surface treatments of foods because it ensures that the preservative remains on the surface of the food where needed, rather than migrating into the foods. Increased solubility occurs with the range of solvents.

Degradation Products of Natamycin

Under the influence of ultraviolet light, gamma irradiations, or acid conditions, natamycin decomposes resulting in the loss of the tetraene structure yielding among others mycosamine, aponatamycin, and dinatamycinolidediol for degradation products. The acute toxicity of these products was investigated and is at an even higher dose (based on LD50 values) than natamycin itself. Natamycin is inactivated by chlorine, peroxides,

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00482-7

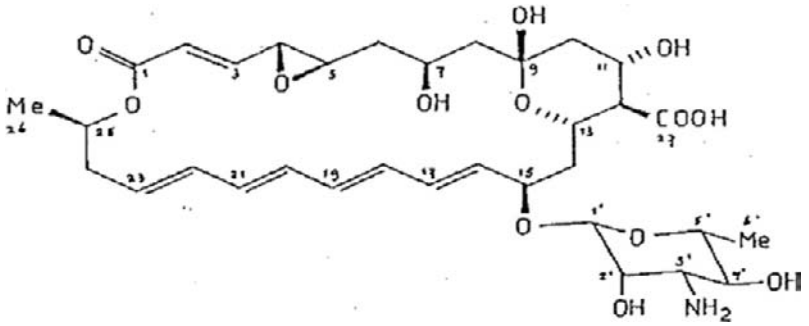

Figure 1 Structure of natamycin.

antioxidants, sulfites, and sodium formaldehyde sulfoxylate, which might be used as cleaning agents.

Anhydrous Form of Natamycin

It can be prepared by drying the trihydrate in vacuum at room temperature over phosphorus pentoxide. The anhydrate is unstable; it loses 15% of its activity when stored for 48 h under optimal conditions. However, when stored under nitrogen or at low temperatures, it is more stable.

Stability of Natamycin

The barium and calcium salts of natamycin and a new crystalline form, also a trihydrate, have also been studied. The stability of the new trihydrate is less than the stability of the regular type. The salts in their solid form are stable but, once in contact with water, they dissolve rapidly and are converted into the more stable regular trihydrate. In principle, this phenomenon could be used to obtain a highly active preparation of dissolved natamycin for a short period of time.

Production of Natamycin

Natamycin is produced by inoculating a pure culture of *Streptomyces natalensis* into a medium containing a carbon source (starch and molasses), nitrogen source (corn steep liquor, casein, and soybean meal), and antifoaming agents with continuous aeration and mechanical agitation, which aids the process with a temperature and pH range of 26–30 °C and 6–8, respectively (Figure 2). After completion of fermentation, insoluble natamycin is subjected to centrifugation where its crystals are separated from most of the residual mycelium and are isolated as a suspension. The suspension is further recrystallized, filtered, and washed, which is again resuspended and filtered. Finally, natamycin crystals are dried and milled to the final powder form and packed. The dry powder is in trihydrate form, which is sufficiently stable to be stored for years with few percent loss of activity. It has been established that the nitrogen source (inorganic and organic) exhibited a significant effect on the natamycin production.

The addition of short-chain fatty acids (viz., acetic, propionic, and butyric) not only increased the antibiotic yield but also decreased the production time from 96 h to only 84 h in shake flask cultures. The maximum volumetric production of 3.98 g l^{-1} was achieved after 84 h in carboxylic acid-supplemented culture (acetate and propionate in a ratio of 7:1). Under optimal cultivation conditions, high concentrations of natamycin can be obtained (3.94 g l^{-1}) with *Streptomyces gilvosporeus* LK-196 in pilot plants that could be further enhanced by chromosomal integration of the *Vitreoscilla* hemoglobin gene (vgb) responsible to reduce oxygen limitation, which is a critical factor in its production. Improved natamycin production by the engineering of phosphopantetheinyl transferases in *Streptomyces chattanoogensis* L10 has been observed. Also, the engineered *Streptomyces chattanoogensis* Dl, generated by inserting an extra copy of scnRII into the chromosome of strain L10, increased its natamycin production by 3.3-fold in a YSG medium and 4.6-fold in a YEME medium without sucrose.

Method of Assay

The methods used for the determination of natamycin are agar diffusion bioassay, high performance liquid chromatography (HPLC), ultraviolet spectrophotometry, and the enzyme immunoassay.

Regulatory Status for Use in Foods

Natamycin is allowed for use in many countries as a food additive and a natural preservative in a variety of foods including dairy foods to control the growth of fungi and yeast (Table 1). On 1 April 2014, the Food Standards Australia New Zealand (FSANZ) published Notification Circular 06-14, which includes Application A1095 to extend the use of natamycin as a preservative in fermented milk products. The primary food uses are in the surface treatment of cheeses and sausages. Several codex standards for cheese permit the presence of natamycin on the cheese surface/rind at a level of 2 mg dm^{-2} to a maximum depth of 5 mm (e.g., mold-ripened cheese like Brie, Camembert, Munster, and Port Salut). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the safety of natamycin in 1968, 1979, and 2001 and assigned an acceptable daily intake value of 0.3 mg kg^{-1} body weight per day.

Elsewhere, the permitted uses differ from country to country. In most non-European countries, natamycin is allowed for the surface treatment of cheeses and/or sausages or other meat products. Since regulations may change, the regulation in a country must be checked before applying natamycin in food products.

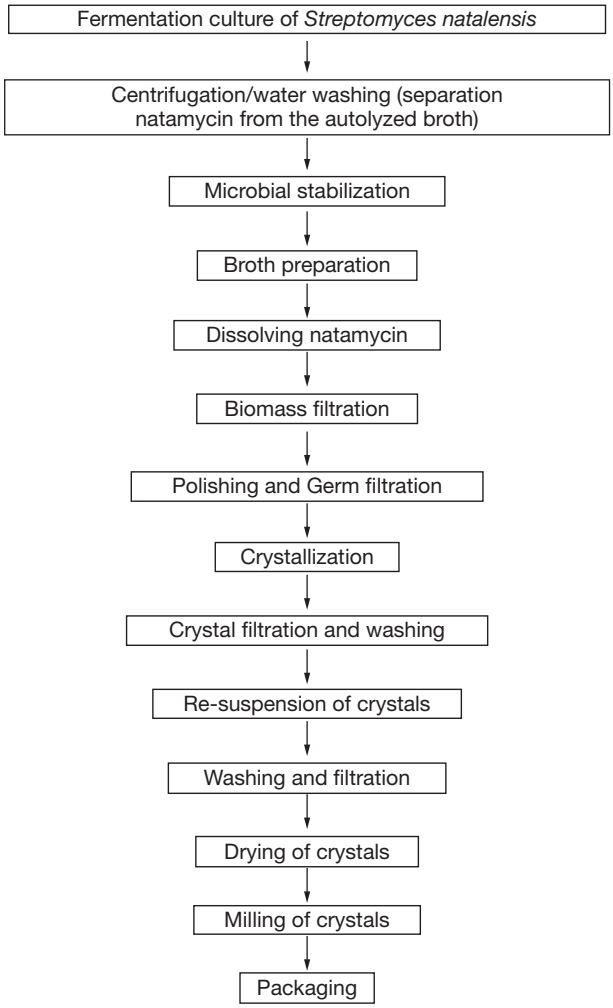

Figure 2 A flow chart of the natamycin production.

Table 1 Permitted levels of natamycin in different countries

Country	*Permitted level*	*Application*
The European Union	1 mg dm^{-2} surface Depth – >5 mm	Surface treatment of hard, semihard, and semisoft cheeses and dried and cured sausages
The United States	Not to exceed 20 g kg^{-1}	Applied on cheese
Canada	Up to 20 ppm	Cheeses
Canada	Up to 10 ppm	Shredded/grated cheese
Australia	15 mg kg^{-1}	Cheese and cheese products

Source: Stark, J. (2003). Natamycin: an effective fungicide for food and beverages. In Roller, S. (eds.) *Natural antimicrobials for the minimal processing of foods*, pp. 82–97. Cambridge: Woodhead Publishing Ltd.

Mode of Action of Natamycin

Natamycin appears to have an all-or-none effect, which destroys the cell membrane without noticeable prior damage. The eukaryotic cell membrane has lipids, phospholipids, proteins, and sterols (ergosterol) where sterols play an important role in the selective action of polyene antimycotics. Natamycin has a high affinity for ergosterol and binds irreversibly to the ergosterol in the fungal cell membrane. This results in the formation of the polyene–sterol complex, which leads to the rapid leakage of essential ions and small peptides by altering the permeability of the membrane (**Figure 3**). Although the activity against fungi is dose-related, natamycin is predominantly fungicidal. Natamycin is not effective *in vitro* against Gram-positive or Gram-negative bacteria.

Yeast sterol biosynthetic mutants revealed the importance of the double bonds in the B ring of ergosterol for the natamycin–ergosterol interaction and the consecutive block of fungal growth by not affecting the permeability of the yeast plasma membrane. As recently demonstrated, natamycin inhibits the growth of yeast and fungi via the immediate inhibition of amino acid and glucose transport across the plasma membrane. This is attributable to ergosterol-specific and reversible inhibition of membrane transport proteins. Vesicles of *Acholeplasma laidlawii* were prepared with either ergosterol or cholesterol as the membrane sterol. Studies showed that natamycin induced leakage of potassium ions in the vesicles of *Acholeplasma laidlawii* containing membrane sterol (ergosterol) equivalent to that of fungal cell membrane but caused no

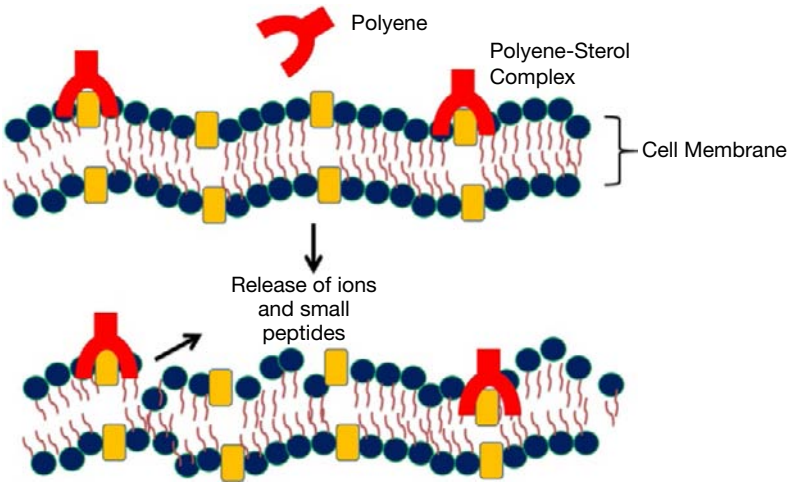

Figure 3 Mode of action of natamycin – a polyene.

damage when cholesterol was present in the vesicles. Also, natamycin inhibited the priming stage of vacuole fusion, which is known to be ergosterol-dependent. This led to the conclusion that ergosterol is the molecular target for natamycin on the fungal cell membrane.

The detailed mechanism of action of natamycin resembles that of another polyene antimycotic, amphotericin B, which forms chiral aggregates at the bilayer–water interface. In the absence of ergosterol, the aggregates remained at the surface of the bilayer, whereas in its presence, the aggregates get embedded in the phospholipid bilayers, which are the precursors of pore formation. Also, as the fungal culture ages, natamycin becomes less effective in its fungicidal action. The cell wall prevents direct interaction of natamycin with the older tissue in the lower part of the hyphae; therefore, natamycin stops the growth of the fungal mycelium only at the tip of the growing hypha. The older cells are not killed directly but are also unlikely to survive as natamycin inhibits the germination of fungal spores by an unknown mechanism. It was suggested that natamycin prevents fungal spoilage by the inhibition of early endocytosis in fungal cells.

Table 2 Sensitivity to natamycin of fungi isolated in Dutch cheese factories and warehouses

Microorganism	*MIC (ppm)*
Aspergillus flavus and *Aspergillus parasiticus*	10–20
Aspergillus fumigatus, *Aspergillus penicillioides*, and *Aspergillus versicolor*	<10
Cladosporium candidum	1–15
Debaryomyces hansenii	1–15
Eurotium herbariorum	1–15
Geotrichum candidum	1–15
Mucor racemosus	1–15
Penicillium brevicompactum, *P. camemberti*, *P. commune*, *P. corylophilum*, *P. glabrum*, *P. nalgiovense*, *P. roqueforti*, and *P. solitum*	1–15
Scopulariopsis brevicaulis and *Scopulariopsis fusca*	1–15
Syncephalastrum racemosum	1–15
Wallemia sebi	1–15
Penicillium discolor	20–30

Source: Stark, J. (2003). Natamycin: an effective fungicide for food and beverages. In Roller, S. (eds.) *Natural antimicrobials for the minimal processing of foods*, pp. 82–97. Cambridge: Woodhead Publishing Ltd.

Sensitivity of Molds and Yeast to Natamycin

Natamycin is active against most fungi and yeast at low concentrations. The minimum inhibitory concentration (MIC) for natamycin against all foodborne fungi and yeast is less than 20 ppm, while the solubility of natamycin in aqueous food systems is around 40 ppm. It has been demonstrated in practice that under acceptable hygienic conditions, this concentration of dissolved natamycin is sufficient to prevent fungal and yeast growth. The majority of yeast and molds were inhibited at concentrations of 1–15 ppm of natamycin in various dairy foods and beverages except a few listed in Tables 2–4.

All isolates of fungal flora obtained from ten farm-level cheese production plants in Spain had MICs of 10 ppm or less except for a *Geotrichum* species that had an MIC of 12.5 ppm. Similarly, in a survey of 16 Dutch factories producing dry sausages to isolate less sensitive yeast and molds, growth was observed on very few plates containing 2 ppm natamycin. In a German study, the sensitivity of 83 yeast to natamycin was examined where none of the yeast isolated from food products were able to grow at a concentration of 3 ppm of natamycin.

Resistance to Natamycin

After decades of continuous use, no resistant molds and yeast were reported against natamycin, which is quite remarkable as compared with other preservatives. In general, yeast are less resistant (MICs below 5 ppm) than molds (MICs above 10 ppm). The EFSA Panel on Food Additives and Nutrient Sources Added to Food has revised its use, concluding that natamycin is very poorly absorbed and therefore does not possess a toxic risk.

Table 3 Sensitivity to natamycin of fungi and yeast occurring on sausages

Microorganism	*MIC (ppm)*	*Source of microorganism*
Molds		
Alternaria alternata	10–20	Blood
Aspergillus flavus	<5	Air
Aspergillus niger	<5	Fruit
Aspergillus versicolor	<5	Soil
Cladosporium cladosporioides	<5	Meat stamp
Eurotium appendiculatum	<5	Smoked sausage
Eurotium herbariorum	<5	Board
Geotrichum candidum	5–10	Soil
Mucor racemosus	<5	Sausage
Penicillium chrysogenum	<5	Meat
Penicillium glabrum	5–10	Soil
Penicillium nalgiovense	<5	Sausage
Penicillium verrucosum	<5	Meat
Rhizopus stolonifer	5–10	Bread
Wallemia sebi	<5	Sea salt
Yeast		
Candida zeylanoides	<5	Sausage
Cryptococcus laurentii	<5	Air
Debaryomyces hansenii	<5	Sausage
Rhodotorula mucilaginosa	<5	Air
Trichosporon pullulans	<5	Frozen beef

Source: Stark, J. (2003). Natamycin: an effective fungicide for food and beverages. In Roller, S. (eds.) *Natural antimicrobials for the minimal processing of foods*, pp. 82–97. Cambridge: Woodhead Publishing Ltd.

The lack of increased resistance among fungi was due to the lethal activity of the compound. The possibility might arise in low-ergosterol or ergosterol-free mutants, but they are unlikely to survive in nature. Attempts to induce natamycin tolerance in molds were not a success story. To date, the maximum tolerance to natamycin is seen in *P. discolor*. Although it was proved that natamycin has no toxic effects even at high levels of ingestion, its application as food additive is restricted, because of the possible danger of resistance formation.

Applications of Natamycin

In Dairy Foods

Natamycin is used in the food industry as a hurdle to fungal outgrowth in dairy products, meats, and other foods where it remains on the surface of the product and hardly penetrates the product, which differentiates it from other preservatives. For the surface treatment of cheese, natamycin is usually applied as a coating and as a dipping or spraying agent or could be added to the brine as an alternative treatment. Successful application of natamycin is on almost every type of cheese. It was found effective in preserving soft cheeses with no harmful effects on ripening. Galotyri cheese (Greek traditional cheese), when treated with natamycin and nisin, not only inhibited the growth of molds and yeast but also enhanced the shelf life and product characteristics (>28 days) as compared with the control sample (14–15 days).

A study on the inactivation of *Saccharomyces cerevisiae* treated with different levels of natamycin and inoculum sizes in liquid cheese whey revealed that an apparent reduction of the yeast population occurred at concentrations of 12.5 mg l^{-1} and during storage. The surface treatment of blue cheese with natamycin was also responsible for alleviating the growth of spoilage organisms and enhanced the flavor of the cheese without affecting the development of blue mold in the punch holes. Similar effects were being observed in various sausages when applied in a concentration ranging from 500 to 2000 ppm. Moreover, 10–100 ppm of natamycin was responsible for enhancing the shelf life of berries. It was observed that the presence of natamycin in the dairy product labneh (Greek yogurt) prevented fungal and yeast spoilage, although it was not legally permitted. A study confirmed that there is no harm

Table 4 Sensitivity to natamycin of yeast isolated from beverages and fruit products

Microorganisms	*MIC (ppm)*	*Source of microorganisms*
Saccharomyces cerevisiae	1.5	Grape juice
Saccharomyces cerevisiae	1.5	Apple juice
Saccharomyces exiguous	<1.0	Soft drink
Saccharomyces carlsbergensis	<1.0	Brewer's yeast
Zygosaccharomyces microellipsoides	1.0	Apple juice
Zygosaccharomyces microellipsoides	2.5	Orange juice
Zygosaccharomyces bailii	1.0	Apple juice
Zygosaccharomyces bailii	1.0	Pear juice
Zygosaccharomyces rouxii	1.0	Strawberry juice
Schizosaccharomyces pombe	<1.0	Grape juice
Brettanomyces bruxellensis	2.5	Wine
Brettanomyces intermedius	<1.0	Wine
Dekkera anomala	2.5	Stout
Dekkera anomala	5.0	Soft drink
Candida etchellsii	<1.0	Concentrated lemon juice
Candida glabrata	4.0	Concentrated orange juice
Debaryomyces hansenii	1.0	White wine
Pichia membranifaciens	1.0	Lemonade
Pichia kluyveri	1.0	Fruit
Pichia angusta	<1.0	Orange juice
Lodderomyces elongisporus	2.0	Concentrated orange juice
Torulaspora delbrueckii	<1.0	Lemonade
Hanseniaspora osmophila	<1.0	Cider
Williopsis saturnus	1.0	Fruit juice
Rhodotorula rubra	<1.0	Yogurt
Molds		
Byssochlamys nivea	2.0	Fruit juice
Trichosporonoides nigrescens	5.0	Melon jam
Mucor circinelloides	2.0	Strawberries
Stemphiliomma valparadisiacum	4.0	Apple juice
Talaromyces macrosporus	2.0	Pineapple juice
Cladosporium tenuissimum	2.0	Fruit
Zygosporium mycophilum	2.0	Apple pulp
Gilbertella persicaria	10.0	Fruit

Source: Stark, J. (2003). Natamycin: an effective fungicide for food and beverages. In Roller, S. (eds.) *Natural antimicrobials for the minimal processing of foods*, pp. 82–97. Cambridge: Woodhead Publishing Ltd.

to health in the use of natamycin as a biopreservative in a doogh (Iranian yogurt drink) in spite of the national regulatory agencies not permitting the preservative use. The additions of natamycin at a concentration of 8 ppm in yogurt resulted in maximum inhibition of yeast counts and did not affect quality parameters before or after fermentation of the milk base as the bacteria involved in the fermentation are insensitive toward it. Preservation studies in yogurt showed the efficacy of natamycin at doses of 5–20 ppm, which prevented the growth of most relevant food spoilage molds.

In Fermented Foods

Natamycin could also help to control the spoilage of fresh packed or cured seasoned table olives caused by yeast. Studies showed that natamycin at low concentrations can efficiently inhibit the growth of three yeast species widely found in table olives in the presence of low and high concentrations of citric acid and sodium chloride, respectively. Similar observations were observed in black olives. Natamycin (160 mg l^{-1}) inhibited the growth of *Aspergillus flavus* on whole olives for 21 days at 15 °C and for 7 days in olive paste. Also, the addition of natamycin could retard the spoilage of acidified foods by *Saccharomyces cerevisiae* when combined with mild heat treatments.

In Antimicrobial Packaging

Packages with antimicrobial activity based on preservative releasing offer a promising form of active packaging systems applicable in food processing. In this context, polymer packaging films releasing nisin and natamycin inhibited the growth of indicator microorganisms (*Penicillium expansum*, *Fusarium culmorum*, *Lactobacillus helveticus*, and *Listeria ivanovii*) when applied on surface-ripened cheese, thus proving a potent antimicrobial agent. Edible films produced from whey protein isolate (WPI) and glycerol, including the incorporation of lactic acid and propionic acid (PRO), chitooligosaccharides (COS) with a nominal MW of 3 kDa, and natamycin as antimicrobial agents exhibited different degrees of effectiveness against several target microorganisms. Organic acids showed highest activity against Gram-positive microorganisms, COS against Gram-negative microorganisms, and natamycin against yeast. Based on this, the formulation for an active edible film (10%(w/w) WPI with 5%(w/w) glycerol (as base matrix), incorporated with 6 g l^{-1} lactic acid (LA) and 0.25 g l^{-1} natamycin (NA)) could be applied in dairy products, namely, cheese wrapping due to their good synergistic performance against microorganisms commonly found on cheese surfaces, that is, bacteria and yeast, without significantly compromising the tensile, barrier, and optical properties of the resulting WPI films.

The matrix films of whey proteins containing antimicrobial agents, namely, malic acid, nisin, and natamycin, were shown to be active against *Penicillium aeruginosa*, *Y. lipolytica*, *L. monocytogenes*, *Penicillium commune*, and *Penicillium chrysogenum*. Chitosan films containing natamycin showed antimicrobial activity against various emergent foodborne pathogens, proving to be a feasible and attractive method for food biopreservation. The physicochemical properties of alginate/chitosan composite films containing natamycin as an antimicrobial agent concluded that composite films added with natamycin exhibit suitable functional attributes and show good perspectives for acting as antimicrobial films intended for food applications. Low-density polyethylene films coated by commercially available polyvinyl chloride lacquer with the addition of nisin preparation Nisaplin® (5% w/w) and/or natamycin preparation Delvocid™ (10% w/w) significantly retarded the growth of harmful microorganisms causing hindrances in the storage of cheese and raw chicken meat.

A study showed that the incorporation of low concentrations of natamycin in a tapioca starch–glycerol matrix acted as an effective antimycotic against *Saccharomyces cerevisiae*, as high concentration of it affects the mechanical properties and color of the starch edible films. Natamycin was also incorporated into alginate films to produce antimicrobial packaging using three different approaches (conventional loading method, immersion procedure, and supercritical solvent impregnation (SSI) method) where the SSI (CO_2 + EtOH) process produced visually attractive and translucent films with antimicrobial packaging.

Other Food Commodities

Apart from a potent application as a food preservative, the potential of using natamycin as a biocontrol agent for plant fungal pathogens, such as *F. oxysporum*, *B. cinerea*, and *M. laxa*, has been suggested. Natamycin has been quite effective in controlling crop pests and was found to have no adverse effects on nontarget organisms or on the environment. The combined treatment of button mushrooms with natamycin and pure oxygen has the potential to improve the quality of button mushrooms and extend the shelf life. Even natamycin finds its use as a drug of choice in mouth dissolving tablets, a noninvasive drug delivery system, emerged as an alternative to conventional capsules and tablets mainly for the pediatric and geriatric patients finding difficulty in swallowing. Besides, it has been applied in medical therapy to handle various ocular problems like conjunctivitis and fungal keratitis, which is a major cause of blindness in developing countries of the world.

Conclusion

Economic losses due to spoilage and health risks associated with the growth of pathogenic or mycotoxin-producing molds are the important issues. Natamycin has a GRAS status and long history of safe use in preventing fungal growth on the surface of various foods and beverages. It is a potent antimycotic agent used to overcome the problem of fungal spoilage, which decreases the shelf life of foods. Therefore, it can offer a tremendous opportunity for advancing the field of food preservation and safety. In addition to this, it has found wide applications in medical therapies.

See also: Preservation of Foods; Preservatives: Classifications and Analysis; Preservatives: Food Use; Yeasts.

Further Reading

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Niacin

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History

Niacin also known as vitamin B_3, the third water-soluble vitamin discovered, plays a vital role in the overall development of the body, deficiency of which could result into problems associated with the skin (pellagra), digestion, and brain functions. Pellagra was first described in Spain by Casal in 1735 after the introduction of maize into Europe from the America. The history of pellagra, its recognition, and causation are listed in Table 1. In the 1920s, Goldberger in the United States reported that pellagra and black tongue in dogs responded to treatment with animal protein and also to boiled protein-free extracts of yeast. Niacin was first described by chemist Hugo Weidel in 1873 and extracted by Casimir Funk, but he thought it to be thiamine. Afterward, Conrad Elvehjem a biochemist from Wisconsin in 1937 extracted an active ingredient from livers that was referred as 'pellagra-preventing factor' and the 'anti-black tongue factor' called niacin. In general, it has been termed as 'vitamin PP,' 'vitamin P-P,' and 'PP factor.' As a memory jogger, one can recall that 'low B_3 = the 3 Ds of pellagra,' that is, dermatitis, diarrhea, and dementia. The name niacin comes from *nic*otinic *ac*id + vitam*in*, to distance itself from the word nicotine, which the public knows as a toxic chemical found in tobacco. Nicotinic acid and nicotinamide had been isolated from the coenzymes now known as nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) by 1934–35; hence, knowledge of their biochemical roles in electron-transfer reactions preceded the discovery of their nutritional significance. By 1946, the metabolism of dietary tryptophan to an active form of the vitamin had been demonstrated.

Chemistry

The term niacin is accepted as a broad descriptor of vitamers, nicotinic acid, nicotinamide, and a variety of pyridine nucleotide structures. By definition, niacin refers to nicotinic acid (pyridine-3-carboxylic acid), nicotinamide (pyridine-3-carboxamide), and derivatives that exhibit the biological activity of nicotinamide. Nicotinic acid and nicotinamide are not directly convertible to each other; however, both the compounds are precursors of coenzymes NAD and NADP stored in the cytoplasm, bound to protein. NAD converts to NADP by phosphorylation in the presence of the enzyme NAD^+ kinase. The structure of NAD can be envisaged as the adenosine diphosphate-ribose (ADP-ribose) moiety attached covalently to nicotinamide through a β-*N*-glycosidic linkage (Figure 1). This linkage constitutes a high-energy bond, the energy of which supplies the driving force for various ADP-ribosylation reactions catalyzed by NAD glycohydrolases. NAD is actively involved not only in the catabolism of fat, carbohydrate, protein, and alcohol but also in the cell signaling as well as DNA repair, whereas NADP is mostly involved in anabolism reactions such as fatty acid and cholesterol synthesis.

Physical and Chemical Properties of Niacin Vitamers

Niacin vitamers are categorized under water-soluble vitamin B complex where nicotinamide is more water-soluble than nicotinic acid. The molecular weight of nicotinic acid and nicotinamide is 123.11 and 122.12 g $mol^{-1,}$ respectively, with an empirical formula of $C_6H_5NO_2$ and $C_6H_6N_2O$. Nicotinic acid and nicotinamide have CAS registry numbers, namely, 59-67-6 and 98-92-0. Nicotinic acid is a white crystalline odorless powder with stability under ordinary conditions but should be protected from light at room temperature. Niacin vitamers are resistant to heat, oxidation, and alkalies, which makes niacin the most stable vitamin. Niacin is well absorbed when taken orally. Nicotinic acid sublimes at boiling point, 236 to −239 °C, and its melting point is 128–131 °C. It is slightly soluble in boiling water and boiling alcohol and does not dissolve in propanediol, chloroform, alkali solution, and lipid-soluble ether solvent with pH of 2.7. It has specific gravity of 1.473 and vapor density of 4.25. The acute toxicity of nicotinic acid is based on LD50 values when supplemented orally in rats (7000 mg kg^{-1}). It is generally recognized as safe substance, but washing with water is preferred immediately when it comes in contact with skin or eyes. Nicotinamide is a solid white crystalline powder. It sublimes at boiling point 224 °C and its melting point is 127–131 °C. It has density of 1.4 and vapor pressure of 31.4 hPa. Its solubility in water is high and is used to enrich various foods, drinks, and feeds.

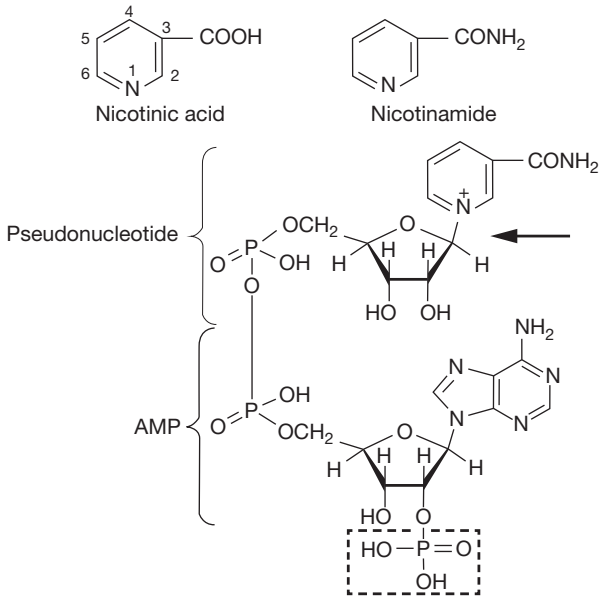
Deficiency Indicators and Outcomes

Niacin deficiency is sometimes seen in developed countries, and it is usually apparent in conditions of poverty, malnutrition, chronic alcoholism, psychosis, and some types of slow-growing tumors called carcinoid tumors. Studies revealed that in patients with alcoholic pellagra, niacin deficiency may be an important factor influencing both the onset and the severity of this condition. Patients with alcoholism typically experience increased intestinal permeability, leading to negative health outcomes. High-energy requirements (the brain) or high-turnover-rate organs (the gut and skin) are usually most susceptible to deficiency of niacin, which is usually found in the people with high intake of maize or millet in their diet, because the excess of leucine interferes in the conversion of tryptophan to niacin. Hartnup's disease is a hereditary metabolic disorder, affecting the absorption of tryptophan culminating to deficiency of niacin in the intestines and kidneys. The lack of

Table 1 History of pellagra and recognition of its causes in human populations

1	A poorly understood disease (dermatitis, gastrointestinal, and mental signs/symptoms) appeared in Europe in the eighteenth and nineteenth centuries and then in the southern United States in the first decade of the twentieth century and was named 'pellagra' (meaning raw/rough skin)
2	Favored causal hypotheses included infection, moldy grain, and insects
3	In 1914–16, Joseph Goldberger disproved the infection hypothesis: by self-experimentation and then producing pellagra in prisoners fed with mainly corn diets
4	In 1920s, Joseph Goldberger developed the 'black tongue' model of pellagra in dogs fed with corn diets
5	In 1930s, nicotinic acid was isolated as a pure water-soluble compound (vitamin) of known structure, from yeast and liver extracts, able to cure pellagra and black tongue
6	In the mid-twentieth century, unavailable niacin is bound in the complex niacytin in corn. Heating in an alkaline environment (as in Mexican tortillas) can release it
7	Niacin can be produced from tryptophan in the body. Pellagragenic diets are low in tryptophan as well as in niacin. The concept of 'niacin equivalents' (milligram niacin plus 160th of milligram tryptophan) in food developed. Human requirements estimated
8	The Indian cereal 'jowar' is shown to be pellagragenic. Some, but not all, studies have implicated its high leucine content
9	Niacin and riboflavin deficiencies often coexist; the signs and symptoms of pellagra-like disease are often attributable to multiple nutrient deficiencies
10	Certain inborn errors of metabolism (genetic defects), or iatrogenic effects of drugs, can mimic the signs, symptoms, and metabolic defects of pellagra

Source: Bates, C. J. (2013). *Niacin and pellagra. Encyclopedia of human nutrition* (3rd ed.). pp. 182–188. Oxford: Elsevier.



Source: Ball GF, 2008

Figure 1 Structures of niacin compounds and the nicotinamide nucleotides. Source: Ball, G. F. (2008). Niacin: nicotinic acid and nicotinamide. In: *Vitamins: their role in the human body*. pp. 301–309. Oxford: Blackwell Publishing Ltd.

Table 2 Signs and symptoms of niacin deficiency in humans and animals

Human deficiency
Loss of appetite and weight
Dermatosis (hyperpigmentation, hyperkeratosis, and desquamation of the epidermis, especially where frequently exposed to strong sunlight)
Anorexia
Achlorhydria
Angular stomatitis, cheilosis, magenta tongue
Diarrhea
Anemia
Neuropathy (headache, dizziness, tremor, neurosis, and apathy)
Death in severe and prolonged cases
Black tongue in dogs and cats
Pustules in mouth and excessive salivation, darkening, and necrosis of the tongue
Diarrhea
Pigs
Neurological lesions affecting ganglion cells; histopathology of nerves
Degeneration of intestinal mucosa and diarrhea
Anemia
Rats
Reduced growth rate
Alopecia (roughened skin)
Damage to peripheral nerves (cells and axons)
Birds (*e.g., chickens and ducks*)
Inflammation of the upper gastrointestinal tract
Dermatitis
Diarrhea

Source: Bates, C. J. (2013). *Niacin and pellagra. Encyclopedia of human nutrition* (3rd ed.), pp. 182–188. Oxford: Elsevier.

niacin may also be observed in pandemic deficiency disease, which is caused by a lack of five crucial vitamins (niacin, vitamin C, thiamine, vitamin D, and vitamin A).

Niacin deficiency can result in mild and severe effects (Table 2). Mild niacin deficiency has been shown to slow metabolism, causing decreased tolerance to cold. Severe deficiency results in pellagra, a chronic wasting (three Ds) disease, which consists of dermatitis (hyperpigmentation, hyperkeratosis, and desquamation – especially when exposed to the sun), diarrhea, and dementia (mental depression) as well as 'Casal's necklace' lesions on the lower neck. The dermatitis is symmetrical, bilateral, hyperpigmented, and photosensitive and begins with erythema resembling sunburned areas. It appears mostly on skin exposed to sun such as hands, legs, face, and neck. Gradually, these areas become reddish brown, rough,

scaly, and keratotic. Gastrointestinal disorders and mucosal abnormalities are also observed. Cerebral manifestations including headache, irritability, confusion, fatigue, apathy, and insomnia precede encephalopathy (inflammation of the brain). Mental changes consist of depression, irritability, and delirium. Other symptoms are nausea, loss of appetite, tiredness, weight loss, coated tongue and sores in the mouth, forgetfulness, anemia, and death if left untreated.

Dietary Sources

Niacin is not a true vitamin as it can be produced by the metabolism of essential amino acid tryptophan. Because of the contribution of tryptophan, foods containing balanced protein are important contributors to the total niacin equivalent intake. The term 'niacin equivalent' (NE) is used to describe the contribution to dietary intake of all forms of niacin that are available to the body and refers to preformed niacin (provides ~50% of the niacin supply in the diet) in addition to niacin formed from the conversion of tryptophan where 60 mg of tryptophan is equal to one milligram of niacin. Niacin is found in both plant and animal foods. Lean red meat, poultry, and liver contain high levels of both niacin and tryptophan and, together with legumes, are important sources of the vitamin. Peanut butter is an excellent source of niacin. Cheese and eggs are relatively poor sources of preformed niacin, but these high-protein foods contain ample amounts of tryptophan and therefore have a high niacin equivalent. Fruits and vegetables provide useful amounts, depending upon the dietary intake. Other useful sources are whole grain cereals, bread, tea, and coffee. Predominantly in maize and other cereals, vitamin occurs in an unabsorbable form called niacytin. The milling of cereals removes most of the niacin since it is concentrated in the aleurone and germ layers. Therefore, in the United Kingdom, it is compulsory by law to add niacin to white flour (mostly 70% extraction rate) at 16 mg kg^{-1}, and all flours other than whole meal (100% extraction) must be enriched. From Table 3, it can be seen that different foods differ considerably, not only in their contribution to NE but also in the ratio of the contribution from preformed niacin and from tryptophan.

Bioavailability

The majority of the bound nicotinic acid in mature cereal grains is biologically unavailable after conventional cooking. About 10% of the total niacin was released as free nicotinic acid after extraction of sorghum meal with 0.1 N HCl. This suggests that a small proportion of bound nicotinic acid can be hydrolyzed by gastric juice and made available. Pellagra, the disease caused by a deficiency of both niacin and tryptophan, has commonly been found in population groups having maize as their staple food. In 1951, it was found that niacin in maize is biologically unavailable and could only be released in very alkaline lime water of pH 11; this process is known as nixtamalization. Mexican and Central American peasants and Hopi Indians in Arizona rely upon maize as a staple food and yet do not experience pellagra. The explanation for this paradox lies in the way in which these people prepare the maize for bread making. In the traditional preparation of Mexican tortillas, the maize is soaked at alkaline pH in lime water before baking, and this process releases the nicotinic acid from its bound forms. In the making of piki bread, the Hopi Indians use wood ash, which is alkaline and also results in the liberation of nicotinic acid.

Recommended Daily Allowance

Requirement varies with the protein content of diet. The requirement for niacin increases in increased calorie intake or expenditure, in acute illness, after severe infection, during injury, etc. The recommended daily allowance (RDA) for niacin, revised in 1998, is based on the prevention of deficiency and given by the Food and Nutrition Board (FNB) of the US

Table 3 Niacin equivalents in mg/100 g of selected foodstuffs

Food	*Niacin equivalents from preformed niacin (mg/100 g)*	*Niacin equivalents from tryptophan (mg/100 g)*	*Total niacin equivalents (mg/100 g)/(mg MJ^{-1})*
Milk	0.2	0.6	0.8/2.9
Raw beef	5.0	4.7	9.7/18
Raw white fish	2.4	3.4	5.8/17
Raw eggs	0.1	3.7	3.8/6.1
Raw potatoes	0.6	0.5	1.1/3.4
Raw peas	2.5	1.1	3.6/10.5
Raw peanuts	13.8	2.9	19.3/8.2
White bread	1.6	2.0	3.6/2.5
Rice, uncooked	4.2	1.6	5.8/3.6
Maize (sweet corn)	2.0	0.5	2.5/5.3
Cornflakes (fortified)	15.0	0.9	15.9/10.0
Coffee	24.8	2.9	27.7/87

Note: Coffee is a good source of niacin, because it is released from the bound form, trigonelline, by roasting. Much of the niacin in maize and part of that in other cereals, including unfortified bread, are unavailable for use by the body, being bound in large molecular complexes. On average, 60 mg tryptophan yields 1 mg NE.
Source: Bates, C. J. (2013). *Niacin and pellagra. Encyclopedia of human nutrition* (3rd ed.). pp. 182–188. Oxford: Elsevier.

Table 4 Recommended dietary allowance (RDA) for niacin

Life stage	*Age*	*Males (mg NE[a]/day)*	*Females (mg NE/day)*
Infants	0–6 months	2 (AI)	2 (AI)
Infants	7–12 months	4 (AI)	4 (AI)
Children	1–3 years	6	6
Children	4–8 years	8	8
Children	9–13 years	12	12
Adolescents	14–18 years	16	14
Adults	19 years and older	16	14
Pregnancy	All ages	–	18
Breast-feeding	All ages	–	17

[a]NE, niacin equivalent: 1 mg NE = 60 mg of tryptophan = 1 mg niacin; AI, adequate intake.

Source: DRI report. (1998). Niacin. In: Food and Nutrition Board, Institute of Medicine (ed.) *Dietary references intakes for thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline*. Washington, DC: National Academy Press; chapter 6, pp. 123–149.

Institute of Medicine. Excretion metabolites of niacin were used as an indicator of niacin nutritional status rather than symptoms of pellagra. The RDA of niacin for different age groups is given in Table 4. The recommended therapeutic dose could be up to 300 mg daily for considerable time. For treating osteoarthritis and type 1 diabetes, the recommended doses of nicotinamide are 3 g and 25 mg day^{-1}, respectively. To prevent heart disease in the people with high cholesterol, recommended dose of niacin is 2–6 g daily.

Absorption and Metabolism

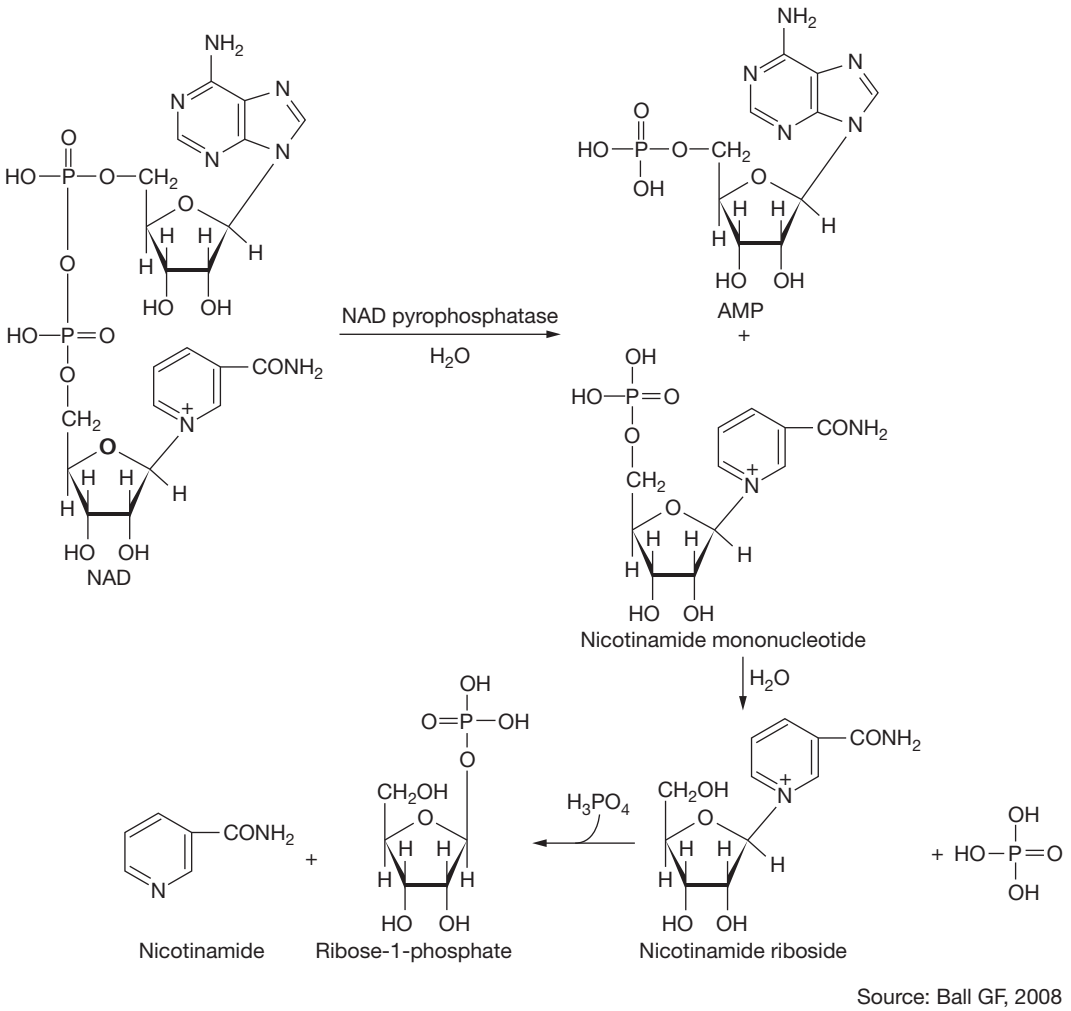
Niacin vitamers are rapidly absorbed in the small intestine. For intact nucleotides, pyrophosphatase activity in the upper small intestine metabolizes NAD(P) to yield nicotinamide mononucleotide (NMN), which is then quickly hydrolyzed to form nicotinamide riboside and eventually free nicotinamide. Absorption of both nicotinic acid and nicotinamide at low concentrations appears to be via sodium-dependent facilitated diffusion or by carrier-mediated transport making use of proton cotransporters and anion antiporter. Higher concentrations of both forms appear to be absorbed by passive diffusion. Once absorbed from the lumen into the enterocyte, nicotinamide may be converted to NAD or released into the portal circulation. The bulk of nicotinic acid taken up by the enterocyte is converted via the Preiss–Handler pathway to NAD, whereas some of nicotinic acid moves into the blood in its native form. As required, NAD glycohydrolases in the enterocytes release nicotinamide from NAD into the plasma as the principal circulating form of niacin.

Possible pathway involved in the degradation of NAD (or NADP) to nicotinamide is depicted in Figure 2. The enzyme present in intestinal secretion, NAD pyrophosphate (EC 3.6.1.22), breaks the phosphodiester bond of NAD to form NMN and adenosine monophosphate (AMP). NMN is rapidly hydrolyzed to nicotinamide riboside and inorganic phosphate followed by the reaction of the riboside with inorganic phosphate to form nicotinamide and ribose 1-phosphate. These reactions happen in the presence of intestinal cells, showing that enzyme responsible for catalyzing the reactions is intracellular or membrane-bound. Upon entry into the cells, the nicotinamide was immediately metabolized to NAD.

Synthesis of NAD from the dietary tryptophan is carried out via the kynurenine pathway (Figure 3). The first and rate-limiting enzyme, tryptophan dioxygenase, is sensitive to induction by both glucocorticoid hormones and glucagon. This is true induction of new mRNA and protein synthesis. The first step is catalyzed by the extrahepatic enzyme indoleamine 2, 3-dioxygenase (IDO), which is responsible for the oxidative cleavage of tryptophan. The chronic stimulation of tryptophan oxidation, mediated by an increased activity of IDO and/or inadequate niacin levels, is observed in a number of diseases, including human immunodeficiency virus infection. However, the synthesis of niacin from tryptophan is a fairly inefficient pathway that depends on enzymes requiring vitamin B_6 and riboflavin, as well as an enzyme containing heme (iron). A variety of nutrient deficiencies, toxins, genetic and metabolic abnormalities, etc., all influence the niacin status and requirements. Because the electron-transport functions of NAD frequently involve flavin coenzymes, and because both flavin coenzymes and vitamin B_6 coenzymes are involved in the conversion of tryptophan to niacin *in vivo*, there are important metabolic interactions between these B vitamins. Their clinical deficiency signs also converge, sometimes making it difficult to distinguish between deficiencies of different B vitamins.

Postabsorptive Metabolism

Rapid absorption of niacin occurs into the human upper ileum, peak levels appearing in blood plasma within 5–10 min. Absorption also occurs in the large intestine, making niacin from gut bacteria available to the intestinal cells. The liver is a major site of conversion of niacin to its functional products, that is, nicotinamide–nucleotide coenzymes. Nicotinamide can pass readily between the cerebrospinal fluid and the plasma, thus ensuring a sufficient supply to the brain and the spinal cord. The liver contains greater niacin coenzyme concentrations than most other tissues, but all metabolically active tissues contain these essential coenzymes. There is a continuous turnover of these nucleotides in the body and very little storage; the excess of niacin is converted to methylated derivatives in the liver, which are excreted into the urine.



Source: Ball GF, 2008

Figure 2 Pathway of NAD degradation to nicotinamide in *in-vivo*. Source: Ball, G. F. (2008). Niacin: nicotinic acid and nicotinamide. In: *Vitamins: their role in the human body*. pp. 301–309. Oxford: Blackwell Publishing Ltd.

Biochemical Functions

Coenzyme Role in Oxidation–Reduction Reactions

Niacin is a component of two coenzymes: NAD and NADP where their oxidized forms, that is, NAD^+ and $NADP^+$, play an essential role in many biochemical reactions, especially redox reactions in which oxidoreductase enzymes transfer hydrogen. Examples of typical reactions are listed in Table 5. The oxidation of a substrate catalyzed by an NAD^+- or $NADP^+$-dependent dehydrogenase is shown in (Figure 4). Two hydrogen atoms (comprising a total of two protons and two electrons) are removed from the substrate to be oxidized (AH2). The pyridine ring of the oxidized form of coenzyme accepts a hydride anion (H^-), consisting of the two electrons and one of the protons; the remaining proton is released to the medium. Reduction of the coenzyme is stereospecific with regard to the hydrogen on the carbon-4 of the pyridine ring. Some enzymes have stereospecificity for the A side, while others for the B side.

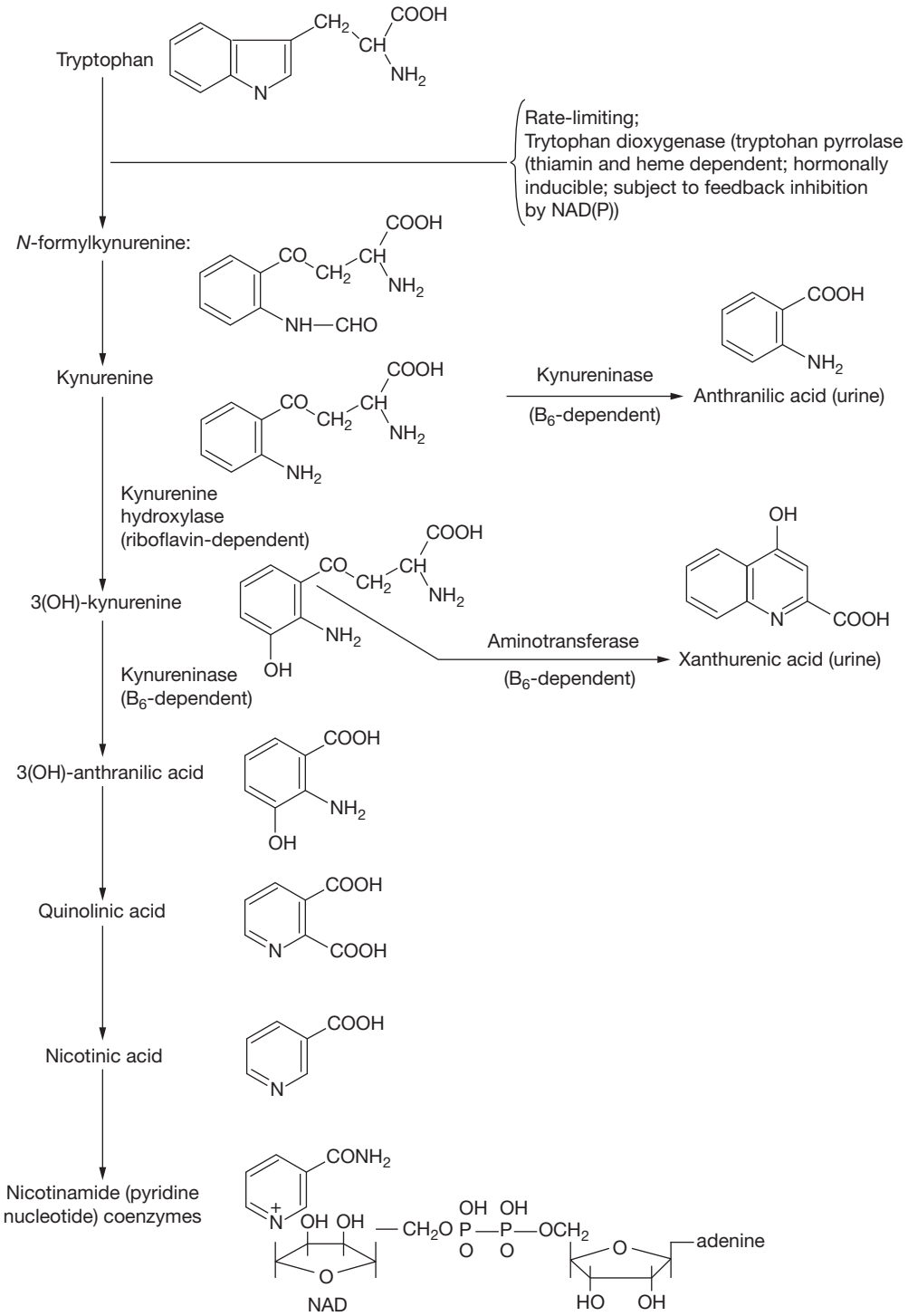
The nicotinamide coenzymes are not tightly bound to their apoenzymes and are not, therefore, prosthetic groups with an important exception of glyceraldehyde-3-phosphate dehydrogenase in mammalian muscle containing a binding site for NAD^+ in each of its four subunits.

Although NAD and NADP undergo similar reversible reduction at the molecular level, their metabolic functions are entirely different. The NADH produced during the tricarboxylic acid cycle is reoxidized by transferring its electrons to the electron-transport chain, thereby providing ATP by the coupled process of oxidative phosphorylation. NADH required for gluconeogenesis is generated via the oxidation of fatty acids and deamination of amino acids. NADP, on the other hand, participates in the pentose phosphate pathway, which is not primarily a provider of energy.

The resulting NADPH is used as a reducing agent to synthesize, among other materials, fatty acids, glutamate, cholesterol (precursor of steroid hormones), and deoxyribonucleotides (precursors of DNA).

Role in Nonredox Reactions

NAD functions in ADP-ribosylation, a reversible post-translational modification of proteins in which the ADP-ribose



Source: Bates CJ, 2013

Figure 3 *In vivo* conversion of tryptophan to nicotinic acid and NAD. *Source*: Bates, C. J. (2013). *Niacin and pellagra*. *Encyclopedia of human nutrition* (3rd ed.). pp. 182–188. Oxford: Elsevier.

Table 5 Enzymes that require NAD and NADP

Enzyme	*Function*
Alcohol dehydrogenase	Metabolizes alcohol
Glyceraldehyde phosphate dehydrogenase	Catalyzes important step in glycolysis
Lactate dehydrogenase	Catalyzes reactions in muscle and liver cells
Pyruvate dehydrogenase	Catalyzes reactions connecting glycolysis to the Krebs cycle
α-Ketoglutarate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase	Catalyze reactions in the Krebs cycle, aerobic metabolism
NADH dehydrogenase	Catalyzes oxidative phosphorylation reactions
Hydroxyacyl-CoA dehydrogenase	Important in fat catabolism
Glucose-6-phosphate dehydrogenase	Catalyzes reactions in the pentose phosphate pathway
β-Ketoacyl-ACP reductase, β-enoyl-ACP reductase	Catalyze reactions in fatty acid synthesis
Chloroplast glyceraldehyde-3-phosphate dehydrogenase	Catalyzes reactions in the Calvin cycle, glucose synthesis

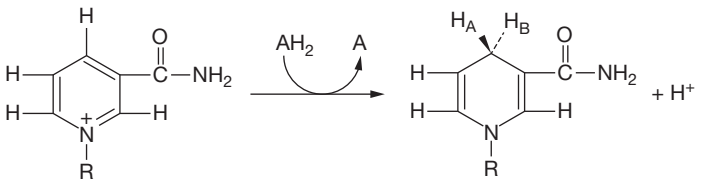


Figure 4 Oxidation reaction catalyzed by NAD^+- and $NADP^+$-dependent dehydrogenase. Reduction of the coenzyme is stereospecific with regard to the hydrogen on carbon-4.

ADP-ribose-nicotinamide + protein ⟶ ADP- ribose-protein + nicotinamide + H^+

Figure 5 In mono-ADP-ribosylation, a single unit of ADP-ribose is transferred to the target protein with the concomitant release of nicotinamide and a proton.

moiety of NAD is transferred to acceptor proteins, thereby altering their function. ADP-ribosylation reactions are classified into two major groups: mono-ADP-ribosylation and poly-ADP-ribosylation.

Mono-ADP-Ribosylation

The transfer of ADP-ribose to the acceptor protein is catalyzed by ADP-ribosyltransferase enzymes, which were first discovered in certain bacteria, where they mediate the action of toxins, such as cholera, diphtheria, and pertussis toxin as well as heat-labile enterotoxin. In mammalian cells, these enzymes transfer an ADP-ribose residue from NAD to a specific amino acid of a target protein, with the creation of an ADP-ribosylated protein and the release of nicotinamide (Figure 5). Since much information is present regarding ADP-ribosylation in bacterial toxins, therefore, they will be further discussed.

The cholera toxin and heat-labile enterotoxin are an oligomeric complex also denoted as AB_5. Subunit B binds while subunit A activates the G protein, which activates adenylate cyclase. Subunit A has two important segments. The A1 portion of the chain (CTA1) is a globular enzyme payload that ADP-ribosylates G protein, while the A2 chain (CTA2) forms an extended alpha helix, which sits snugly in the central pore of subunit B ring. The CTA1 fragment catalyzes ADP-ribosylation of the Gs alpha-subunit ($G_\alpha s$) proteins using NAD, which causes the $G_\alpha s$ subunit to lose its catalytic activity in hydrolyzing GTP to GDP + Pi, so it remains activated longer than normal. Increased $G_\alpha s$ activation leads to increased adenylate cyclase activity, which increases the intracellular concentration of cAMP to more than 100-fold over normal and overactivates cytosolic protein kinase A (PKA). These active PKAs then phosphorylate the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel proteins, which leads to ATP-mediated efflux of chloride ions and leads to the secretion of H_2O, Na^+, K^+, and HCO_3^- into the intestinal lumen. In addition, the entry of Na^+ and consequently the entry of water into enterocytes are diminished. The combined effects result in rapid fluid loss from the intestine.

Pertussis toxin ADP-ribosylates alpha-subunit of the inhibitory G protein (Gi). The modified G protein uncouples from the receptor, thereby maintaining the protein as its inactive heterotrimer. Because this inhibitory G protein is inactivated, inhibition of adenylyl cyclase is removed and the result is increased cyclase activity.

Two bacterial exotoxins, diphtheria toxin and *Pseudomonas aeruginosa* exotoxin A, catalyze the transfer of NAD^+ to a

diphthamide residue in eukaryotic elongation factor-2, inactivating this protein. It does so by ADP-ribosylating the unusual amino acid diphthamide. In this way, it acts as a RNA translational inhibitor and the uncontrolled action of these exotoxins results in the death of the host cells. The catalyzed reaction is as follows:

$$\mathrm{NAD^{+}peptide\ dipthamide} \leftrightarrow \mathrm{nicotinamide+}$$
$$\mathrm{peptide} - N - (\mathrm{ADP} - \mathrm{D} - \mathrm{ribosyl})\mathrm{dipthamide}$$

Poly-ADP-Ribosylation

Multiple groups of ADP-ribose moieties can also be transferred to proteins to form long branched chains, in a reaction called poly-ADP-ribosylation carried out by the poly(ADP-ribose) polymerases (PARPs), which are found in most eukaryotes, but not prokaryotes or yeast. The poly(ADP-ribose) structure is involved in the regulation of several cellular processes.

Role of NAD in Repairing DNA Nicks

The role of nicotinamide in DNA repair and maintenance of genomic stability is tightly related to its functions as a precursor and a substrate for PARP-1. PARP-1 is a nuclear enzyme, which detects DNA damage, binds to DNA single- or double-strand breaks, and then uses NAD^{+} as a substrate to form nicotinamide and ADP-ribose. Subsequent enzymatic reactions lead to the formation of branched ADP-ribose polymers on a nuclear acceptor protein. Poly-ADP-ribosylation of the acceptor protein has been hypothesized to function in DNA repair by modifying structural proteins proximal to DNA strand breaks, facilitating the opening of the condensed chromatin structure, which is required for the recruitment of DNA repair complexes. Poly(ADP-ribose) glycohydrolase is the main enzyme involved in the catabolism of poly(ADP-ribose), reversing the automodified state of poly(ADP-ribose) polymerase; this reactivates the polymerase and restores DNA binding.

PARP-1 itself is also known to be part of chromatin structure and involved in maintaining a compact chromatin structure, preventing inadvertent transcription from occurring. Unfolding of the compact chromatin structure allows DNA regulatory and repair processes access to the damaged sites as well as to replication and transcription initiation sites. PARP-1 has been reported to play a key role in the nucleotide excision repair (NER) pathway used to remove bulky DNA adducts and in the base excision repair (BER) pathway by interacting with BER protein XRCC1 (x-ray repair cross complementing 1). PARP-1 is involved in maintaining chromosomal integrity by protecting broken DNA from inappropriate homologous recombination during DNA repair and replication. PARP knockout mice exhibited dramatically increased sensitivity to ionizing radiation and alkylating agents and showed a two- to threefold increase in spontaneous sister chromatid exchange (SCE) and amplified SCE and micronuclei (MN) formation induced by carcinogens. PARP-null mice also showed extreme sensitivity to nitrosamine-induced carcinogenesis and had shorter telomeres and increased end-to-end chromosomal fusions, aneuploid cells, and chromosome fragments. Thus, nicotinamide is involved in the maintenance of genomic stability by providing a substrate for PARP-1, preserving a cellular energy reserve for ATP-dependent DNA repair, and enabling preservation of PARP-1 integrity.

Role of Niacin in Diseases

Pharmacological levels of niacin (2–6 g day^{-1}), which are in excess of the RDA, have been used in attempts to treat Hartnup's disease, carcinoid syndrome, poor glucose tolerance, atherosclerosis, hyperlipidemia, schizophrenia, insulin-dependent diabetes mellitus, and a variety of skin disorders.

Historically, nicotinic acid has been administered to patients with a variety of disorders, often more for the dramatic skin reaction than proved curative powers. Its most successful use is for the treatment of hyperlipidemia.

Nicotinamide does not share these effects of nicotinic acid on lipid metabolism or its associated toxicity. However, it has been shown to act as an inhibitor of poly(ADP-ribose) synthetase in pancreatic β cells in animal studies, thereby protecting them from chemically induced diabetes, albeit sometimes complicated by β-cell tumor formation. Studies on human diabetes have so far yielded inconsistent results. One preliminary study suggested that nicotinamide may improve arthritis symptoms, including increased joint mobility and reduced amount of nonsteroidal anti-inflammatory drugs needed. Moreover, researches are going on for studying topical forms of nicotinic acid to treat rosacea, aging, attention-deficit hyperactivity disorder, migraines, dizziness, depression, motion sickness, and alcohol dependence and prevent skin cancer. Nicotinamide is applied to the skin for treating a skin condition called inflammatory acne vulgaris. One among every four men in the United Kingdom reportedly suffers erectile dysfunction at some point in their lives. Niacin plays a crucial role in nourishing the scalp and hair cells. Niacin and other B vitamins help body to produce energy from the foods. Getting too little niacin may drain your energy, making it difficult to burn more calories than one eat, an essential component of successful weight loss. Furthermore, many niacin-rich foods help with weight loss because they provide other nutrients, such as protein and fiber, that aid in healthy weight management.

Mechanism of Action in Cardiovascular Disease

Pharmacological doses of nicotinic acid (but not nicotinamide) are used as a cholesterol-lowering drug. Nicotinic acid has been found to result in markedly increased HDL-cholesterol levels, as well as decreased serum Lp(a) lipoprotein concentrations, and a shift from small dense low-density lipoprotein (LDL) particles to large, buoyant LDL particles, all of which are considered cardioprotective changes in blood lipid profiles. The mechanisms of action of high-dose nicotinic acid are unrelated to the formation of NAD(P) and unrelated to the actions of nicotinamide. The main effect is a decrease in lipolysis in adipose tissue due to an inhibition of adenylate cyclase activity. The resulting drop in cAMP levels leads to the decreased mobilization of fatty acids, which is at least partially responsible for a drop in very low-density lipoprotein (VLDL) formation by the liver and a subsequent drop in LDL levels, although there may also be direct effects on liver lipid metabolism. In addition to the blood lipid effects, high-dose nicotinic acid causes a dramatic skin flush of the face and upper

trunk. All of these blood lipid effects appear to be due to the binding of nicotinic acid to a high-affinity receptor, which is in turn linked to an inhibitory G protein, leading to decreased cAMP levels and inhibition of hormone-sensitive lipase. This receptor is known as the niacin receptor or HM74A. It is termed as an orphan receptor, as nicotinic acid is not thought to be its natural ligand, given the unphysiologically high doses required to activate it. The skin flush, which is mediated by prostaglandin formation, also appears to be caused by the binding of nicotinic acid to HM74A, in this case, on the surface of macrophages.

Interactions of Niacin with Drugs

Niacin interacts with some of the drugs resulting into various side effects. Allopurinol (zyloprim), probenecid, and sulfinpyrazone (anturane) are used to treat gout. Taking large doses of niacin along with these drugs might worsen gout and decreases their effectiveness. Clonidine and niacin both lower blood pressure, and ingestion of niacin in high amount with clonidine might cause blood pressure to become too low. Long-term use of nicotinic acid and nicotinamide might increase blood sugar and thereby might decrease the effectiveness of diabetes medications. Niacin can adversely affect the muscles; it was observed that taking niacin with statins can increase the risk of muscle problems. Taking nicotinic acid and/or nicotinamide and using a nicotine patch can increase the possibility of becoming flushed and dizzy.

Toxicity

Niacin from foods is not known to cause adverse effects. However, sometimes administration of chronic high oral doses ($>$1200 mg day^{-1}) of nicotinic acid used to lower the LDL cholesterol and triglyceride levels and massive consumption of foods fortified with high levels of niacin can lead to hepatotoxicity as well as dermatologic manifestations. In high doses, nicotinic acid competes with uric acid for excretion, culminating to increase in the incidence of gouty arthritis. Extremely high doses of niacin can also cause niacin maculopathy, a thickening of the macula and retina, which leads to blurred vision and blindness, whereas the complication was observed to be reversible after decreasing the niacin intake. The common side effects of niacin include flushing, itching, and gastrointestinal disturbances, such as dyspepsia, nausea, and vomiting. Hepatotoxicity (liver cell damage), including elevated liver enzymes and jaundice, has been observed at intakes as low as 750 mg of niacin per day for less than 3 months. In contradiction, nicotinamide is generally better tolerated than nicotinic acid, and generally, it does not cause flushing; however, nausea, vomiting, and signs of liver toxicity have been observed at very high doses (3 g day^{-1}). High dose of niacin can result into more complications in the prevalence of certain diseases like allergy, irregular heartbeat, diabetes, gout, gall bladder disease, liver and kidney disease, and stomach or intestinal ulcers. The US Food and Nutrition Board proposed an upper limit (UL) of 35 mg day^{-1} for niacin. This value was also set for pregnant and lactating adult women. The UL was adjusted for children and adolescents on the basis of relative body weight, and it is applicable to individuals who are being treated with a nutrient under medical supervision, such as for lowering of cholesterol levels. The tolerable upper intake levels (UL) of niacin for various age groups adopted by the US Food and Nutrition Board are given in Table 6.

Table 6 Tolerable upper intake levels (UL) of niacin for various age groups

Age groups	*Niacin equivalents (mg day^{-1})*
Infants, 0–12 months	Not possible to establish
Children	
1–3 years	10
4–8 years	15
9–13 years	20
Adolescents, 14–18 years	30
Men, 19 years and older	35
Women, 19 years and older	35
Pregnant women	35
Lactating women	35

Source: DRI Report. (1998). Niacin. In: Food and Nutrition Board, Institute of Medicine (ed.) *Dietary references intakes for thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline*. Washington, DC: National Academy Press; chapter 6, pp. 123–149.

Assessment of Niacin

Since the development of blood-based status analysis for niacin has lagged behind that of the other components of the vitamin B complex, therefore, some studies suggested that the erythrocyte concentration of the niacin-derived coenzyme NAD may provide useful information about the niacin status of human subjects, that a reduction in the ratio of NAD to NADP to below 1.0 in red cells may provide an evidence of niacin deficiency, and that a decline in plasma tryptophan levels may indicate a more severe deficiency than a decline in red cell NAD levels. The niacin coenzymes can be quantitated either by enzyme-linked reactions or by making use of their natural fluorescence in alkaline solution. At present, niacin status is most commonly assessed by the assay of some of the breakdown products of niacin coenzymes in the urine. Among these, N1-methylnicotinamide (NMN) is the easiest to measure, because of a convenient *in vitro* conversion to a fluorescent product, which can then be quantitated. However, more definitive and reliable information can be obtained by the measurement of urinary NMN in conjunction with one or more of the urinary pyridone turnover products (N1-methyl-2-pyridone-5-carboxamide and N1-methyl-4-pyridone-3-carboxamide), which can be detected and quantitated by UV absorption following high-pressure liquid chromatography (Figure 6).

Conclusion

Niacin a water-soluble vitamin plays a vital role in human body, deficiency of which results into 3 Ds of pellagra, that is, dermatitis, diarrhea, and dementia. It is available in different forms, out of which nicotinic acid has cholesterol-lowering

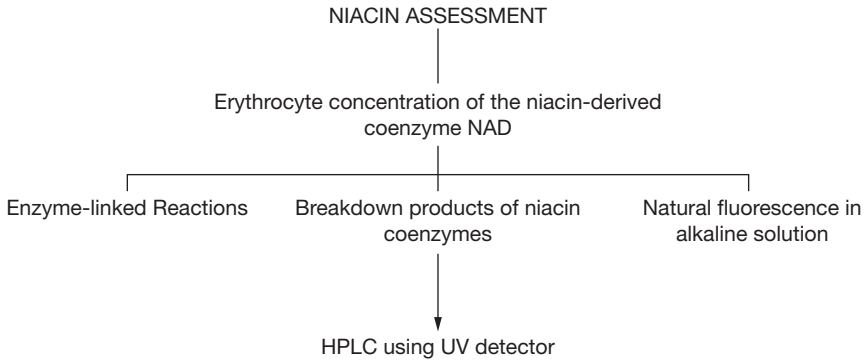


Figure 6 Methods for niacin assessment.

property. However, niacin overdose can also worsen the situation and might culminate to various health complications. Recent research has indicated that nicotinamide riboside uniquely supports neuronal NAD synthesis, thereby deserving future therapeutic studies. Niacin and some related compounds have many wide-ranging metabolic effects, and their medical significance has attracted renewed interest in recent years.

See also: Amino Acids: Metabolism; Bioavailability of Nutrients; Vitamins: Overview.

Further Reading

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Nitrites and Nitrates

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Sources and Production

All life requires nitrogen compounds. Nitrogen is the most abundant element in the atmosphere, making up 78% of the air we breathe. N_2 is chemically inert and must be converted to biologically active 'fixed' nitrogen. Through the atmospheric nitrogen cycle, elemental nitrogen (N_2) is fixed into usable forms for plants such as ammonia, nitrite, and nitrate by both lightning strikes and nitrifying bacteria. These nitrogen oxides participate in complex biological processes essential for agriculture and human biology. Any plants or vegetables grown in soil will accumulate nitrite and nitrate. As a result, when humans or other animals consume plants or vegetables, we are exposed to nitrite and nitrate. There are four primary sources of exposure to nitrite and nitrate: (1) endogenous production of nitric oxide (NO), (2) dietary exposure to nitrite and nitrates in food, (3) drinking water, and (4) swallowing saliva. The majority of exposure to nitrite and nitrate in most people is through dietary consumption of vegetables. Cured and processed meats contribute very little to the overall burden of exposure. Individual levels of exposure are dependent upon where and how one lives and works, what types of food one eats, endothelial function, and lifestyle habits. Adequate exposure to nitrite and nitrate from these sources poses little risk with known benefits.

Endogenous Production

Nitrite and nitrate are formed from the oxidation of endogenously produced NO. NO is produced by the enzyme nitric oxide synthase (eNOS) in the endothelial cells that line all blood vessels and lymphatic vessels throughout the body. NO is also produced within the nervous system (nNOS) and immune system (iNOS). NO is a potent vasodilator, inhibitor of platelet aggregation, and the active species of nitroglycerin. NO is synthesized by mammalian cells from L-arginine through a complex oxidation reaction catalyzed by the NOS enzymes. NOS catalyzes the NADPH- and oxygen-dependent oxidation of L-arginine to NO plus L-citrulline in a reaction that requires several cofactors in the presence of oxygen, including NADPH, FAD, FMN, tetrahydrobiopterin, heme, reduced glutathione, and calmodulin. The endogenous production of NO by NOS has been established as playing an important role in vascular homeostasis, neurotransmission, and host defense mechanisms. In fact, NO is one of the most important molecules produced within the human body. The major metabolism of NO is the stepwise oxidation to nitrite and nitrate. In plasma, NO is oxidized almost completely to nitrite, in which form it will remain stable for several hours. In contrast, NO and nitrite are rapidly oxidized to nitrate in whole blood. NO can also be enzymatically oxidized to nitrite by ceruloplasmin or other metal-containing proteins. The half-life of NO is about 1 s, whereas the half-life of nitrite in human blood is about 110 s when produced within the vascular compartment. Nitrite, when consumed orally, can have a half-life of greater than 1h. Nitrate, by contrast, has a circulating half-life of 5–8 h. During fasting conditions with a low previous intake of nitrite-/nitrate-rich foods, enzymatic NO formation accounts for the majority of nitrite and nitrate in the human body. It is now estimated that our body (average 70 kg) produces 1.68 mmole NO per day (based on 1μmole per kg h^{-1} NO production) in a healthy individual. The majority of this NO produced will end up as nitrite and nitrate. In the plasma, steady-state concentrations of nitrite are conserved across various mammalian species, including humans, in the range of 150–600 nM. It has been shown that up to 50–70% of plasma nitrite is derived from eNOS activity in fasted humans and other mammals. As much as 50% of NO activity/availability can be derived from eating nitrite- and nitrate-rich foods. This becomes extremely important when one realizes that you can combat conditions of low vascular NO production through consuming nitrite- and nitrate-rich foods.

Diet

Diet is considered the largest source of nitrite and nitrate exposure. Nitrite and nitrate have been used for centuries in curing and preserving meats and fish and in manufacturing certain cheeses. These two anions are also found naturally in certain foods, primarily green leafy vegetables. When added to foods, specifically meats for curing, nitrite has at least three functions. Firstly, it contributes to the flavor due to the inhibition of the development of rancidity. Secondly, it reacts with myoglobin to give mononitrosylhemochrome, which give the characteristic pink color of cured meat. Thirdly, it inhibits the growth of food spoilage bacteria, most importantly, *Clostridium botulinum*. *C. botulinum* thrives under anaerobic conditions and produces a neurotoxin, which is one of the most lethal natural products known. In this regard, nitrite is critical to the food and meat industries. Vegetables contribute over 85% of the daily dietary intake of nitrate and nitrite. We and others have estimated that approximately 80% of dietary nitrate and nitrite are derived from vegetable consumption. Therefore, the primary source of exposure to nitrate by humans is through eating vegetables. Most people would not argue that a diet rich in vegetables is healthy. Recent reports have shown that less than 5% of the ingested nitrite and nitrate are derived from cured meat sources with the remainder 10–15% coming from drinking water and our own saliva.

Historically, much of the quantified exposure of nitrite and nitrate has been based on the focus of dietary ingestion and endogenous formation of nitrosamines as a cause of human cancers. What is often not understood is that nitrite and nitrate are essential for growth and reproduction of all plant and animal life. Nitrogen is a basic constituent of proteins. The form of nitrogen within plants when consumed by animals,

including humans, has important effects on growth and reproduction. Several different groups of nitrogen-containing compounds may be found in plants. The amount of each form depends on plant species, maturity, and environmental conditions during growth, amount of sunlight, and time of harvest. These nitrogen compounds may be broadly classified as either protein or nonprotein compounds. Under normal growing conditions, plants use nitrogen to form plant proteins. When normal growth is affected, protein formation may be slowed, and the nitrogen content in the plant may then become nonprotein nitrogen. Nitrate, nitrite, amides, free amino acids, and small peptides make up most of the nonprotein nitrogen fraction. Nitrate is a natural material in soils. Adequate supply of nitrate is necessary for good plant growth. More than 90% of the nitrogen absorbed by plants is in the form of nitrate. Soils can be supplemented with nitrogen often in the form of ammonium nitrogen (NH_4^+), which is rapidly converted to nitrate (NO_3^-) in the soil by nitrifying bacteria. Chemical fertilizers may be composed of ammonium nitrate, ammonium phosphates, ammonium sulfate, alkaline metal nitrate salts, urea, and other organic forms of nitrogen. Microorganisms must change organic nitrogen to ammonium or nitrate ions before plants can use it. Usual release of available nitrogen from soil organic matter is 1–4% annually, depending on soil texture and weather conditions. Animal manure is an excellent source of nitrogen and can contribute significantly to soil improvement. Animal manure contains about 10 lb of nitrogen per ton, poultry manure 20 lb per ton, and legume residues 20–80 lb per ton. About half of this organic nitrogen may be converted to nitrate nitrogen and become available for plant use the year it is added to the soil. However, if it is low in phosphorus content; excessive manure applications can result in toxic levels of nitrate in forage crops the same as excessive use of chemical nitrogen fertilizer. Adding phosphate fertilizer to manure can reduce the nitrate content in the crop produced.

Nitrate content of plants is determined by their inherited metabolic pattern (genetics) and the available nitrate in the soil. Applying fertilizer in amounts beyond the ability of the vegetable crop to use them may result in an accumulation of nitrate, particularly if some other essential nutrient is not adequate, such as phosphorus. Leafy green vegetables and some root crops naturally contain nitrates, and there are wide variations between species and even variations between geographic regions in which they are grown. There are also seasonal differences. Nitrate content in spinach can be almost twice as high in autumn–winter harvest as in spring harvest. Disruption of normal plant growth increases the probability for nitrate accumulation in leaves, stems, and stalks. Factors favoring accumulation of nitrate in plants include drought, high temperatures (rapid transpiration of water), shading and cloudiness (reduced protein synthesis), deficiency of other plant growth nutrients (phosphorus, potassium, and calcium), excessive soil nitrogen (manures, legume, residues, effluents and solid wastes, and nitrogen fertilizer), damage from insects and certain weed control chemicals, and immaturity at harvest. As a result of all of these factors, nitrate content of vegetables is highly variable but still represents the main dietary source of nitrate. A survey of several vegetables from five different US cities reveals that the nitrite and nitrate content can vary by as much as 500-fold in the same vegetable purchase at retail in different cities. This creates a real problem when trying to get an average of nitrite and nitrate content for estimation of exposure across the United States.

Breast milk is nature's most perfect food and is the primary nutrient source for infants. Previously published studies in our lab reveal the presence of high concentrations of nitrite and nitrate in human breast milk. Early-postpartum breast milk from certain mothers contained the highest nitrite concentration of any food or beverage product tested (near 20 μM). This becomes extremely interesting in terms of levels of nitrite exposure determined from ingestion and based on body weight of an infant (near 1 mg kg^{-1}). Nitrite and nitrate not only are important for adult nutrition and diet but also are very important for infants. In fact, formula-fed babies do not receive any nitrite and nitrate from the formula and may be a possible mechanism for the health disparities between breast-fed and formula-fed babies.

Drinking Water

Even though drinking water usually contains very low levels of nitrate and nitrite, when large volumes are consumed, this can be a significant source for ingestion. The US Environmental Protection Agency regulates nitrate and nitrite contamination levels in approximately 160 000 different water supplies across the United States and does periodic monitoring. The regulatory maximum contaminant level (MCL) for nitrate is 10 mg l^{-1} nitrogen from nitrate, which equates to 44 mg nitrate ion per l. For nitrite, the MCL is 1 mg l^{-1} nitrogen or 3.3 mg l^{-1} nitrite ion. Using these maximum values as an estimate for intake may provide a crude estimate of an upper limit for the drinking water contribution to ingestion of nitrite and nitrate in the United States. Recommended dietary consumption of water and beverages is listed at 2.7 l per day for adult males and 2.2 l per day for females. Thus, as much as 60–119 mg per day nitrate ingestion from drinking water may occur in drinking water meeting legal standards. Likewise, as much as 4.6 to 8.9 mg per day of nitrite may also be ingested from drinking water.

The natural background levels of nitrate and nitrite are very low in water, generally < 10 mg l^{-1} for the former and rarely over 3 mg l^{-1} for the latter. The increased use of nitrogen fertilizers in agriculture during the second half of the twentieth century contributed to potential contamination of water supplies by nitrate-containing runoff from farms to watersheds that ultimately became sources of drinking water. Levels of nitrate can be significantly higher in shallow wells that are connected to agricultural runoff. Much less is known about private wells, which in the United States are usually required to be tested only when the well is constructed or when the property is sold.

Saliva

Similar to the environmental nitrogen cycle, there is now a recognized human nitrogen cycle (Figure 1), whereby nitrate is reduced to nitrite and to NO. Nitrate, after its absorption in the upper gastrointestinal tract, reaches the salivary glands via the blood circulation where it is secreted into the oral cavity and partially reduced to nitrite by the oral microflora. There is a

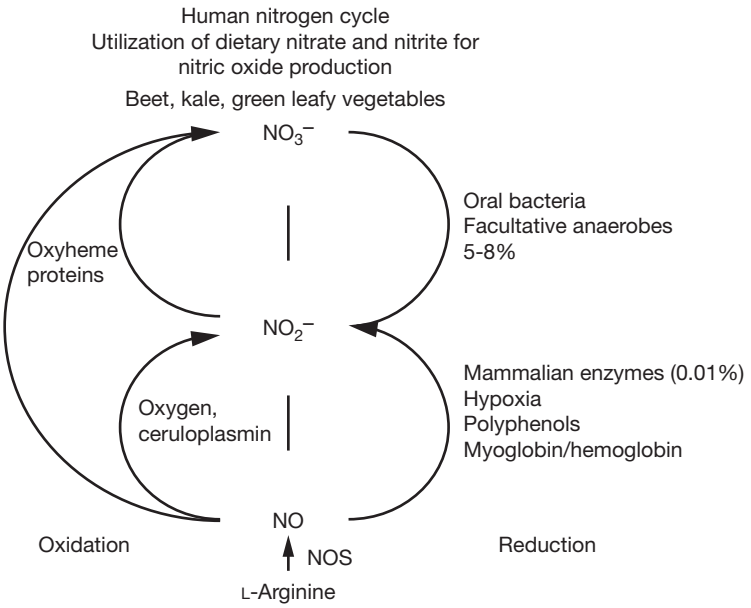

Figure 1 Nitrate: nitrite: nitric oxide recycling pathway.

linear relationship between the amounts of nitrate ingested and amounts of nitrate and nitrite found in saliva. The ability of the oral microflora to reduce nitrate to nitrite depends on the individual community of oral commensal bacteria. Age may also be a factor. Mean salivary nitrite was found to increase from well below 1 ppm in infants of up to 6 months to about 7 ppm or higher in adults, but levels over 500 mg l^{-1} have been reported to occur subsequent to consumption of nitrate-rich foods. Early work by Tannenbaum et al. reported that nitrite in saliva of healthy individuals is 6–10 mg l^{-1} and higher levels in the hundreds of mg l^{-1} could form under conditions of very high nitrate intake and active bacterial reduction in saliva. Recently, it was reported that salivary nitrite levels in fasting individuals were 1.9 mg l^{-1}, which were increased to near 100 mg l^{-1} after a standardized nitrate load of 4.6 mg kg^{-1} body weight was administered. It is extremely difficult to accurately quantify the contribution of saliva to nitrate and nitrite ingestion and exposure, but it must be considered to be a major source. The current consensus is that as much as 25% of dietary, water-solubilized, and endogenously generated nitrate is recycled in the body via the enterosalivary route and that up to 20% of that nitrate pool is reduced in the saliva by commensal bacteria. Based on an average 2.0 mg l^{-1} concentration of nitrite in saliva and secretion of 1.5 l per day, saliva can commonly be a source of as much 15 mg ingested nitrite per day, but under conditions of sustained nitrate intake from vegetables, this could go even higher.

Although it is not widely realized, potassium nitrate is used in many toothpaste formulations to reduce sensitivity. Potassium nitrate is the active antisensitivity ingredient that penetrates the exposed tubules (tiny holes) of the teeth to relieve the pain from sensitive nerves inside teeth. Additionally, it is one of the ADA-approved ingredients to treat tooth hypersensitivity. Toothpaste designed for sensitive teeth is formulated with up to 5% (5000 ppm) potassium nitrate, which is higher than the concentration found in vegetables. Since toothpaste is not commonly swallowed, the net contribution to nitrate and nitrite exposure cannot be accurately estimated. However, nitrate and nitrite as water-soluble ions are likely to be extracted from the toothpaste and mixed with saliva. The total exposure could therefore be equivalent to or greater than that contributed by water or cured processed meats.

Patterns of Consumption

The National Research Council report The Health Effects of Nitrate, Nitrite, and N-Nitroso Compounds (NRC 1981) reported estimates of nitrite and nitrate intake based on food consumption tables. They report that the average total nitrite and nitrate intakes in the United States were 0.77 and 76 mg, respectively, per day. The mean intake estimates for nitrate and nitrite in the United States and Europe vary by investigator but are consistent and comparable. International estimates of nitrate intakes from food are 31–185 mg per day in Europe and in the United States about 40–100 mg per day. The bioavailability of dietary nitrate is 100%. Nitrate intakes from sources other than vegetables, including drinking water and cured meats, have been estimated to average 35–44 mg per person per day for a 60 kg human. Nitrite intakes vary from 0 to 20 mg per day or up to 0.25 mg kg^{-1} body weight. Based upon a conservative recommendation to consume 400 g of different fruits and vegetables per day at median nitrate concentrations, dietary concentration of nitrate would be ~157 mg per day. In the EU population, where fruit consumption (nitrate concentration averaging <10 mg kg^{-1} FW) constitutes over half the 400 g intake recommendation, actual nitrate intakes would approximate 81–106 mg per day before additional nitrate losses are taken into consideration based on washing, peeling, and/or cooking. The Dietary Approaches to Stop Hypertension (DASH) studies found that diets rich in

vegetables (i.e., 8–10 servings) and low-fat dairy products can lower blood pressure to an extent similar to that achieved with single hypertensive medications. We have previously demonstrated that persons consuming the DASH (Dietary Approaches to Stop Hypertension) diet could consume upward of 1000 mg of nitrate per day or roughly 12.5 mg kg^{-1} per day and may explain the mechanism of action for the antihypertensive effects of the DASH diet.

The Mediterranean diet, which is known to be cardioprotective and confer anticancer properties, is inherently high in nitrate. Green leafy vegetables and cured meat are staples of the dietary patterns of the people in the Mediterranean regions. Multiple studies now reveal that the health benefits of the Mediterranean diet may be partly due to the nitrite and nitrate content of their diet. Similar studies have been reported for the Japanese diet. Dietary nitrate in Japanese traditional foods lowers diastolic blood pressure in healthy volunteers. Since the majority of nitrite and nitrate are derived from green leafy vegetables, any diet rich in vegetables will increase the daily exposure to nitrite and nitrate, particularly vegetarians. Most if not all of the epidemiological data to date reveal that a diet rich in green leafy vegetables is extremely healthy and can prevent many diseases. We may now have a mechanism that explains some or most of the health benefits from certain diets and dietary patterns through their nitrite and nitrate content.

Availability, Absorption, and Metabolism

Nitrate ingested from the diet is rapidly absorbed in the small intestine, mixes with the endogenous nitrate from oxidation of NO, and is readily distributed throughout the body. About 25% of an oral nitrate load is concentrated and excreted by salivary glands, so that salivary nitrate concentration is approximately 10 times higher than plasma nitrate. Approximately 20% of salivary nitrate is reduced to nitrite in the mouth by facultative anaerobic bacteria, which are found on the surface of the tongue, resulting in about a 5% reduction of total ingested nitrate to nitrite. Swallowed nitrite in the saliva then disproportionates with the formation of NO (nitrite $pK_a \sim 3.4$) after entering the acidic environment of the stomach, helping to reduce gastrointestinal tract infection and increase mucous barrier thickness and gastric blood flow. As a result, nitrate ingestion is the main source of nitrite exposure in humans through the bacteria reduction of nitrate to nitrite. This biochemistry has been demonstrated stoichiometrically in mice fed twenty times higher nitrate than nitrite. Humans, unlike prokaryotes, are thought to lack the enzymatic machinery to reduce nitrate back to nitrite, so this pathway is largely dependent upon oral commensal bacteria to reduce nitrate to its active form nitrite. Nitrate when consumed orally reaches a peak plasma concentration in about 1 h. Since nitrate is a relatively small anion and is not protein-bound, its pharmacokinetics and half-life suggest that it is reabsorbed in the renal tubules. Nitrate is excreted in the urine directly or after conversion to urea. Clearance of nitrate from the blood to urine approximates 20 ml min^{-1} in adults indicating considerable renal tubular reabsorption of the ion. It is estimated that 96% of the filtered nitrite and nitrate are reabsorbed in the renal tubules. Other studies in dogs suggest that approximately 80% of filtered nitrate is reabsorbed. The high concentration of nitrate and nitrite in saliva, continuous production from NO in the vascular compartment, and then reabsorption from renal tubules strongly suggest that nitrate and nitrite have a definite role in normal human physiology. They serve as important substrates for NO production.

Nitrite and nitrate have many functions in the skin, including the mediation of inflammation and antimicrobial defense, wound healing, regulation of keratinocyte homeostasis, and regulation of apoptosis following ultraviolet (UV) radiation via their metabolism to NO. Nitrate, nitrite, or nitrosothiols present on the skin are stable until cleaved by UV to release NO. This may explain in part the health benefits of moderate sunlight exposure. Nitrate and nitrite found in sweat are very important in the production of NO. The concentration of nitrite and nitrate in and on our body is controlled by dietary intake of these anions and endogenous production from the oxidation of NO and the presence of denitrifying bacteria.

Health Effects

The number of basic science reports and even clinical reports on the use of nitrite and nitrate has risen dramatically over the past decade. According to clinicaltrials.gov, there are now over 45 clinical trials using sodium nitrite and over 50 studies using sodium or potassium nitrate for specific human diseases. Some of the earliest studies on the cardioprotective and hepatoprotective effects of nitrite were conducted in mice. Nitrite has been shown to clearly protect the heart from injury from heart attack, suppress microvascular inflammation, prevent renal injury from hypertension, and reverse vascular endothelial dysfunction and large elastic artery stiffness with aging. A study administering a sodium nitrite capsule to diabetics found that a single administration of 80 mg sodium nitrite was well tolerated. The single dose caused a significant drop in systolic blood pressure with no effect on the diastolic pressure. Plasma nitrite levels rose between 3 and 4 μM. The dose used in that study is far greater than what could be consumed through a diet. Studies using a patented formulation (US patents 8 303 995, 8 298 589, and 8 435 570) using 15–20 mg of sodium nitrite in the form of an orally disintegrating tablet found that it could modify cardiovascular risk factors in patients over the age of 40, significantly reduce triglycerides, and reduce blood pressure. This same lozenge was used in a pediatric patient with argininosuccinic aciduria (ASA) and significantly reduced his blood pressure when prescription medications were ineffective. A more recent clinical trial using the nitrite lozenge reveals that a single lozenge can significantly reduce blood pressure, dilate blood vessels, and improve endothelial function and arterial compliance in hypertensive patients. Furthermore, in a study of prehypertensive patients (BP $>120/80<139/89$), administration of one lozenge twice daily leads to a significant reduction in blood pressure (12 mmHg systolic and 6 mmHg diastolic) after 30 days. These studies clearly demonstrate the safety and efficacy of low doses of nitrite in humans, either consumed as a therapy or consumed through the diet.

Nitrate itself is inactive and without effect. Nitrate must first be reduced by oral commensal bacteria to nitrite, and then,

nitrite has biological activity. As described in the preceding text, only about 5% of the total nitrate is reduced to nitrite. All biological effects of nitrate are abolished by antiseptic mouthwash that kills oral bacteria. There are now many studies showing health benefits in humans of dietary nitrate including blood pressure regulation and sports performance. Nitrate administered in the drinking water of mice deficient in the enzyme that makes NO can reverse features of metabolic syndrome. Nitrate studies have now been translated to humans. For the majority of studies in humans, beetroot juice has been the dietary nitrate source of choice. However, to obtain sufficient nitrate levels for improved performance, a minimum of 500 ml of beetroot juice needs to be provided at least 2.5 h prior to exercise in order to allow sufficient time for enterosalivary circulation and reduction to nitrite. Research indicates that raising the nitrate and nitrite levels in the body prior to exercise by consumption of dietary nitrate can increase NO production and lead to an increased oxygen efficiency and exercise performance. Consumption of 500 ml of beetroot juice, which is high in dietary nitrate (527 mg), 2.5 h before exercise improved the power output and performance of 9 club-level competitive male cyclists during a 4 km and 16.1 km cycling time trial compared to placebo. Additionally, in another study in older adults, dietary supplementation with beetroot juice, containing approximately 400 mg nitrate per dose twice per day (816 mg total), increased plasma nitrite concentration, reduced blood pressure, and positively influenced physiological responses to exercise. Nitrate supplementation at a dose of 6.62 mg kg^{-1} can also enhance exercise performance in patients with peripheral artery disease. Improvements in exercise performance following beetroot juice consumption have typically been found to range between 2% and 16% and demonstrated in such exercise modalities as running, cycling, rowing, and resistance exercise.

There are also several studies showing blood pressure-lowering effects of nitrate when consumed through beetroot juice. Investigators have administered 0.5 l of 15 mM (638 mg) nitrate to healthy adults and found that it lowered blood pressure 6 h later. Others have administered potassium nitrate capsules containing 700 mg or 1488 mg of nitrate or had patients drink beetroot juice that contained 468 mg of nitrate and found that all forms of nitrate led to a dose-dependent decrease in blood pressure beginning after 90 min and lasting for several hours. Consumption of 0.1 mmol kg^{-1} body weight nitrate (595 mg for a 70 kg adult) for 3 days can significantly reduce diastolic blood pressure. The diastolic blood pressure was on average 3.7 mmHg lower after nitrate supplementation than after placebo supplementation, and the mean arterial pressure was 3.2 mmHg lower. These authors state that this is the equivalent of nitrate found in 150–250 g of green leafy vegetables or beets. Similarly, other investigators demonstrate that drinking (0.5 l per day; 5.2 mmol of NO_3 per day) of beetroot juice (442 mg per day) acutely reduces blood pressure and the oxygen cost of submaximal exercise and that these effects are maintained for at least 15 days if supplementation is continued. Interestingly, in another study, supplementation of the diet with 7.5 mmol (638 mg) of nitrate per day for 2 weeks caused an increase in plasma nitrite and nitrate concentration, but did not lower blood pressure, improve endothelial function, or improve insulin sensitivity in individuals with type II diabetes. There appear to be patient populations that do not respond to nitrate. Administration of 9.3 mg kg^{-1} sodium nitrate for 4 weeks to older patients with increased cardiovascular risk profiles can reverse vascular dysfunction. There is a relatively large range of nitrite and nitrate dosing that has been studied in humans and mice that provide clear therapeutic benefit without any signs of toxicity.

Summary

As science advances and new discoveries and understanding are made apparent, it is important to be able to incorporate these new findings into meaningful guidelines that can enhance health, lengthen life, and reduce illness and disability. It is known that it takes approximately 17 years for new basic science discoveries to become standard of care or be fully implemented into public health. As Paracelsus exclaimed, "the dose makes the poison." There are clear and delineated doses of both nitrite and nitrate that provide indisputable evidence of promoting health and even treating serious medical conditions. Fortunately, these doses fall well below toxic and fatal doses. This provides a sufficient range that can safely be achieved from diet alone. At a time when the globe is faced with epidemics of heart disease, obesity, and metabolic syndrome, we can no longer ignore fundamental nutritional, biochemical, and physiological benefits of nutrients found in the most healthy and nutritious foods, nitrite and nitrate, especially since the etiology of the aforementioned diseases is based on poor diet and nutrition. As with any nutrient, it is time to consider the risk–benefit analysis of nitrite and nitrate. It is known that lowering blood pressure by just 5 mmHg reduces the risk of stroke by 35% and the risk of ischemic heart disease by 21%, the top two killers of people worldwide. There are now clear, indisputable blood pressure-lowering effects of dietary nitrite and nitrate by at least 5 mmHg. By recognizing these relatively new findings and implanting nutritional and dietary interventions with sufficient nitrite and nitrate, perhaps, 35% or roughly 6 million deaths could be prevented each year. I have referred to nitrite as a vitamin previously and even proclaimed it 'vitamin N,' but perhaps, it may fit the characteristics of a dietary mineral. A mineral is by definition a solid inorganic substance of natural occurrence. After all, sulfates and phosphates are recognized minerals, and nitrite and nitrate are similar in structure and composition except replacing the sulfur and phosphorus with nitrogen, respectively, with different oxidation states. How we classify nitrite and nitrate may not be important at this stage other than to finally recognize them for nutrients that they are. The underlying chemistry of these two anions must be controlled for to maximize the benefits while preventing any unwanted nitrosation chemistry causing *N*-nitrosamine formation. Nutritionists, physiologists, physicians, toxicologists, meat scientists, and dieticians from both academia and industry need to converge to recognize and establish nutrient guidelines for nitrite and nitrate similar to other well-recognized nutrients. The data and facts are now available for such an initiative. It is time to change the dialogue from one of avoiding nitrite and nitrate in the diet to one of including sufficient amounts to achieve the well-documented health benefits from them.

See also: Cured Foods: Health Effects; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; *Escherichia coli* and Other *Enterobacteriaceae*: Occurrence and Detection; Meat: Eating Quality and Preservation; Smoked Foods: Principles and Production; Water Supplies: Water Analysis.

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Novel Foods

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The term 'novel foods' is a food regulatory construct currently enacted in some form in the European Union, the United States, Canada, China, South Korea, Japan, Singapore, Australia, and New Zealand and other countries. In the United States, regulations related to novel foods fall under the category of 'new dietary ingredients' (NDIs), a part of the Dietary Supplement Health and Education Act (DSHEA). Novel foods include foods or food ingredients that have not been used to any significant extent in the particular country prior to the date of the regulation. Such regulations have been in place since the late 1990s and pertain to a number of different types of foods/ingredients (Table 1). The safety of novel foods is the driving concern for such regulations. The novel food must be shown to not cause harm when ingested. Harm may be due to toxicants present in the novel food. As an example, the introduction into soybeans of a gene coding for a known allergenic protein from Brazil nut created allergic responses in skin prick tests of individuals with a Brazil nut allergy. This finding halted further development of that variety of genetically modified (GM) soybeans for use as a food for humans and has remained an obvious, crucial cautionary example in the realms of GM technology and novel foods. But harm might also occur if the novel food is intended to be used to substitute for a conventional food, yet the novel food is lacking key nutrients present in the conventional food. For example, an orange-colored and orange-flavored drink that mimicked orange juice but did not contain substantially equivalent amounts of vitamin C and folic acid would be of concern for consumer safety by this criterion. Providing appropriate information to consumers through labeling of novel ingredients or ingredients that may pose ethical concerns for some consumers has also been a driving force for novel food regulations, especially in the European Union. Labeling of novel foods remains controversial, mostly in relation to GM foods. Such labeling is not mandated in the United States, but a number of states have attempted to enact 'right to know' labeling for GM foods, with Vermont recently passing such a law. A petition to US Food and Drug Administration (FDA) to require GM food labeling was submitted in 2011 by the Center for Food Safety, a nongovernmental organization with headquarters in Washington DC, but the petition has yet to be acted upon. US federal legislation has been introduced to block state 'right to know' laws, but this also remains to be acted upon.

Novel Food Categories and Examples

Most novel foods appearing in the novel food catalog of the European Union are in the category of plant species or products derived from plant species with no significant history of use in European countries. Novel foods generally fall into one or more of four subcategories:

- Plant species or components (e.g., leaf, bark, and root) used as alternative food ingredient sources
- Fruits and vegetables not native or unfamiliar to the country
- Plant species or components with medicinal uses but unfamiliar to the country
- New pure food ingredients derived from plant species

Many of the plant species listed by the EU as novel foods are sources of food ingredients. The extraction or other preparation of such ingredients may serve as alternatives to traditional ingredient sources. Examples include *Abies alba* (European silver fir) from which an essential oil for flavoring may be derived; *Agave sisalana*, a source of fructose syrup; and *Acacia nilotica* (prickly acacia or wattle) as a source of gum Arabic.

Tropical fruits and unusual vegetables are also included as novel foods. Breadfruit (*Artocarpus altilis*) and kiwi fruit (*Actinidia deliciosa*) are examples. Examples of novel vegetables include sea aster (*Aster tripolium*), a salt-tolerant plant that grows in marshes that may be eaten as a young plant, and *Atriplex hortensis*, orache, or mountain spinach. The acidic green or red leaves may be used in salads.

Examples of medicinal plants or plant components in the novel food catalog include root extract of *Astragalus membranaceus*, which is thought to benefit heart disease and suppress inflammatory responses, based on *in vitro* and animal model studies. *Angelica sinensis* or dong quai, a perennial in the carrot family, is a botanical listed among novel foods that may be marketed as relieving hot flushes during menopause, but very limited clinical trial data do not support that use.

New pure food ingredient examples include agmatine ((4-aminobutyl) guanidine sulfate), which had protective effects against Alzheimer's disease in a rat model, but no human trials have yet been published on this compound. Agmatine is found in plant foods, fish, and meat and is produced by bacteria. Fermented cabbage, soy, meats, and cheeses contain a few mg agmatine per kg versus much lower to nondetectable amounts in nonfermented similar foods, as reviewed by Galgano et al. Arabinogalactan is another novel ingredient, derived from larch wood and classified as 'generally recognized as safe' for use as a food gum.

NDIs: Novel Foods in the United States

In the United States, examples of NDIs from the inception of DSHEA through 2004 were well described in a review of this aspect of DSHEA by McGuffin and Young. The American Herbal Products Association (AHPA) maintains a NDI database that includes nearly 700 items to date. This database is accessible only by subscription. The regulation of NDIs is intended to keep drugs separate from foods. For example, FDA ruled that acamprosate (acetyl homotaurine, a synthetic amino acid used to help maintain sobriety in individuals who have abused ethanol) and idebenone (a synthetic analog of coenzyme Q_{10} that is being investigated for efficacy in treating

Table 1 Novel foods and food ingredients: categories and examples

Category	*Description and notes*	*Examples*[a]
Pure chemical	New food additives. Regulated under food additive laws	Carnosine, antioxidant found naturally in meats Lycopene, antioxidant found in tomatoes
Genetically modified plant, animal, or microorganism with a history of use as a food source or source of food ingredient	Organism modified by insertion of a new gene or genes, from either the same species or a different species (transgenic). Standard of 'substantial equivalence' may permit use, that is, that the genetic modification does not substantially change the food product composition (<10% difference in amount of food chemical component such as protein, starch, and vitamin)	Foods or food ingredients derived from genetically modified maize line NK 603 Foods or food ingredients derived from Roundup Ready maize line GA21
Genetically modified plant, animal or microorganism with *no* history of use as a food source or source of food ingredient	Organism modified by insertion of a new gene or genes, from either the same species or a different species (transgenic)	None approved to date
Complex novel food, not genetically modified	New food or food with novel ingredients from nongenetically modified sources	Dragon fruit (in countries outside Southeast Asia) Chia seed Margarine-type spread containing phytostanols
Food processed by a novel technology	New processing methods that have the potential to change nutritional composition of a food or introduce toxic substances into the food	High-pressure pasteurized fruit products (juices and pastes)
Cloned animals	Animals derived from cloning	None approved to date, proposed EU regulations to ban cloned animals as food sources were tabled in 2013

[a]Examples from the European Union Novel Food Catalogue (http://ec.europa.eu/food/food/biotechnology/novelfood/novel_food_catalogue_en.htm) or list of authorizations (http://ec.europa.eu/food/food/biotechnology/novelfood/authorisations_en.htm).

several neurological diseases) were not to be considered NDIs. Purified trans-resveratrol, while a component of grapes and some other plants, was also deemed by FDA not to qualify as a NDI. Such purified plant constituents, as opposed to plant extracts containing trans-resveratrol, would require investigational new drug applications to be approved by FDA for their further development for use by humans. The reason for this is that even if the constituent is to be used in amounts similar to that in a food, humans have not been exposed to the pure compound, which might act differently when completed and separated from its food matrix. Effective doses of purified food constituents for disease prevention or treatment may be many-fold greater than what would be possible to obtain from the food. For example, a trial of resveratrol in mice with diet-induced obesity showed that 22 mg resveratrol per kg body weight increase longevity and significantly suppressed adverse metabolic effects of obesity while not reducing body weight. This dose, on a surface area-equivalent basis, would be about 100 mg resveratrol per person. Resveratrol concentration in a type of red wine was about 2 mg l^{-1}. Needless to say, 50 l of wine per day would not be a feasible, effective resveratrol source. Thus, resveratrol in the context of preventing adverse effects of obesity would seemingly be needed in pharmacological rather than dietary amounts.

Investigational new drug (IND) applications must include animal pharmacology and toxicology studies across doses and time periods of intended use of the new drug; detailed information about the manufacturing, composition, and stability of the new drug; and information about the clinical trial protocols including investigator qualifications and risk assessment so that FDA can assure safety of the trial participants before the new drug is investigated. IND applications require considerable financial resources.

It is not uncommon for dietary constituents to have the ability to prevent, mitigate, or treat diseases and to help in the maintenance of health, either in general or for specific organs or functions of the body. Further, in the interest of separating drugs from foods, food labeling regulations require FDA approval for a 'health claim' to be made about a food or food ingredient/component. A 'health claim' is basically that a food or food ingredient acts similarly to a drug (prevents, mitigates, or treats a disease). For what is referred to as an 'unqualified' health claim to be permitted on a food label, vast amounts of human clinical trial data and other safety and efficacy data must be submitted by the petitioner, and an expert panel must agree that the preponderance of evidence supports the claim (Table 2). Lesser amounts of data may be submitted for varying levels of 'qualified' health claims (Table 2). Premarket notifications of an NDI for which a 'health claim' is not sought, but for which there is some reason to think a health benefit exists that does *not* involve prevention, mitigation, or treatment of a specific disease, are a way for food and food ingredient companies to introduce novel foods/ingredients that may benefit consumers' health, without meeting rigorous evidence standards or conditions required for marketing of disease-specific therapies, but permitting assurance of safety of the novel food/ingredient. Labels of products containing NDIs would not be permitted to bear health claims unless a

Table 2 Examples of food ingredients with varying levels of US FDA approved health claims

Food ingredient	*Health claim*	*Level of claim*
Whole oat-soluble fiber	Diets low in saturated fat and cholesterol that include soluble fiber from whole oats may reduce the risk of heart disease	Unqualified
Soy protein	Diets low in saturated fat and cholesterol that include 25 g of soy protein a day may reduce the risk of heart disease. One serving of (name of food) provides 6.25 g of soy protein	Unqualified
Plant sterol esters	Plant sterols: foods containing at least 0.65 g per serving of plant sterols, eating twice a day with meals for a daily total intake of at least 1.3 g as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of (name of food) supplies ( ) grams of vegetable oil sterol esters	Unqualified
Omega-3 fatty acids	Supportive but not conclusive research shows that consumption of EPA and DHA omega-3 fatty acids may reduce the risk of coronary heart disease. One serving of (name of the food) provides ( ) gram of EPA and DHA omega-3 fatty acids (see nutrition information for total fat, saturated fat, and cholesterol content)	Qualified – moderate level of scientific support
Monounsaturated fats from olive oil	Limited and not conclusive scientific evidence suggests that eating about two tablespoons (23 g) of olive oil daily may reduce the risk of coronary heart disease due to the monounsaturated fat in olive oil. To achieve this possible benefit, olive oil is to replace a similar amount of saturated fat and not increase the total number of calories you eat in a day. One serving of this product contains (x) grams of olive oil	Qualified – limited level of scientific support
Corn oil and corn oil-containing products	Very limited and preliminary scientific evidence suggests that eating about one tablespoon (16 g) of corn oil daily may reduce the risk of heart disease due to the unsaturated fat content in corn oil. FDA concludes that there is little scientific evidence supporting this claim. To achieve this possible benefit, corn oil is to replace a similar amount of saturated fat and not increase the total number of calories you eat in a day. One serving of this product contains (x) grams of corn oil	Qualified – lowest level of scientific support

separate health claim petition was approved. Products containing NDI may bear substantiated 'structure–function' claims that strictly avoid mention of efficacy against diseases, such as 'improves mental function,' 'improves bone health,' or 'helps with healthy aging.' FDA should be notified of, but does not approve, such claims. What constitutes substantiation of structure/function claims ranges from a history of use for certain functions to experimental evidence, but not necessarily involving human clinical trials.

From the review of the legal history of NDIs by McGuffin and Young, some patterns emerge. NDIs are almost entirely of botanical origins. They may take the form of a dietary ingredient, an extract, a source of a raw material, or a component of a dietary supplement. FDA may affirm, acknowledge, or reject premarket notifications of NDIs. For example, FDA affirmed that a dietary supplement containing 250 mg of the yeast *Phaffia rhodozyma* as a source of the carotenoid astaxanthin would reasonably be expected to be safe. FDA also affirmed conjugated linoleic acid as an NDI. FDA acknowledged an astaxanthin-containing extract of *Haematococcus pluvialis* with language indicating that the safety rationale presented was not inadequate, as it also did for a brand of krill oil. FDA rejected more than 25 NDI notices between 1994 and 2004. Examples include *Kalanchoe pinnata* leaf and *Sutherlandia frutescens* and *Hordeum vulgare* extract and *Echium plantagineum* as a seed oil source. *Kalanchoe pinnata* leaf may have been rejected because it contains cardiotoxic bufadienolides. Although *Sutherlandia frutescens* did not show toxicity in a limited number of animals and short-term human phase I trials, it contains ~2 mg canavanine per g leaf, a nonprotein amino acid shown to exert toxicity in animal studies. *Hordeum vulgare* (barley straw) extract was shown to have mutagenic activity, which presumably would have raised concern with FDA. *E. plantagineum* (also known as purple viper's bugloss) contains a mixture of fatty acids including stearidonic acid that may suppress inflammatory responses in patients with asthma, but this plant is also a good source of pyrrolizidine alkaloids that are hepatotoxic to humans and livestock, causing severe illness in some cases. These compounds are soluble in ethanol and would not be expected to carry over in high concentrations into oil extracted from the plant material, but this seems not to have been studied to date. Thus, FDA may have acted out of caution for potential toxicity to reject an NDI notification for this oil source. Recent examples of NDIs filed by the FDA are noted in Table 3; most are new sources or derivatives of essential nutrients.

Table 3 Recent examples of new dietary ingredient notifications filed by US Food and Drug Administration

Ingredient name	*Year*
Almega PL omega-3 oil from microalgae *Nannochloropsis oculata*	2014
PureQQ pyrroloquinoline quinone	2013
Quatrefolic®, (6S)-5-methyltetrahydrofolic acid glucosamine salt	2010
AlgaeCal, calcium carbonate, and magnesium carbonate derived from marine algae	2009
Diosmin, inulin, and inulin-type fructans	2008
Capsiate Natura and capsiate (capsinoid) supplement	2007

Safety Assessment: Best Practices for Novel Foods and NDIs

Safety of novel foods may well be assessed according to principles published in 2003 laid out by an expert group from

International Life Sciences Institute (ILSI) Europe (http://ilsi.org/Publications/14_ILSI-safetyNovelFood.pdf). Before toxicology testing, consideration of the chemical structure of the pure ingredient or key constituents of the novel food may predict the likelihood of uptake and bioavailability of the substance and toxicity. Large (molecular weight >500), nondigestible, and water-soluble substances would be much less likely to be absorbable or take up into body tissues. Mutagenicity, for example, is well characterized chemically, and predictive computer programs are available that may assist in ruling out this possibility for the new ingredient or novel food.

Assessing the intended and likely intake of novel foods or NDIs, including consideration of the intake by specific subpopulations intended to be marketed to, is a key step in evaluating safety, as the likely intake sets the boundaries for intake of any potential toxicants in the novel food. If the novel food is intended to take the place nutritionally of a conventional food, the nutritional compositional equivalence of the new food to its conventional counterpart is a crucial consideration and would almost certainly require fairly detailed analytic chemistry of nutrients in the novel food.

For nutritional analysis, for assessment of 'substantial equivalence,' or for adequate characterization of a novel food with no clear traditional equivalent, analytic chemistry will generally be a crucial challenge. Foods are complex mixtures. The forms of bioactive constituents of lower molecular weight (m.w. < 500) in foods are very often glucosides. Rarely are standards available for these glucosides or their aglucons. Often, an aglucon may have multiple glucosidic forms with additional substituents present, as for soybean isoflavones. Considerable effort will be needed to identify and purify key novel food constituents; advanced analytic chemistry expertise and instrumentation will be required, including NMR and mass spectrometry. Stability analysis of standards will also be needed. Some commercial analytic laboratories exist that may assist with this aspect of developing a novel food. Collaboration with university faculty and staff, especially with food analysis expertise, would be another likely route of assistance in adequately characterizing a novel food.

For GM foods or ingredients derived from GM species, assuring that the GM species has similar agronomic or production characteristics to the traditional version of the species is a key general approach that helps to avoid unintended effects of the GM process. Assurance of compositional equivalence (proximate analysis of lipid, protein, moisture, and ash and analysis of amino acids, vitamins, minerals, key toxicants, and antinutrients) of the GM food compared to the conventional version of the food should be done. Because GM foods contain modified genes, which code for specific proteins, allergenicity (which is due to reaction to specific proteins) is a major toxicological consideration. Avoiding the introduction of a gene for a major allergenic protein to a GM food is an obvious caution, as mentioned in the preceding text with the example of Brazil nut protein introduced into a GM soybean variety. Comparing the amino acid sequence of the protein expressed by the genetic modification with known allergenic proteins would be a key need in GM food safety evaluation.

In most cases of toxicological testing of novel foods or NDIs, the crucial tests would compare the new food/ingredient to a conventional counterpart that the new food is intended to replace. The key question is whether the new food is as safe as its traditional counterpart. Probably at a minimum, rodent subchronic (90-day) and short-term human trials would be important for such comparisons. Testing of whole foods is much more complex than testing of pure ingredients; well-controlled dietary formulations are crucial, but controlling for differences in multiple ingredients as would be highly likely with a whole food test is very challenging. The finding of a serious adverse effect from such a test would need to lead to additional tests to pinpoint the cause and to determine approaches for remediation and mitigation. FDA and its counterpart agencies in many countries specify types of testing required for new food additives, which are quite extensive. Based on analytic information on the novel food obtained before testing, at least some of the same types of tests used for assessing safety of newly proposed food additives would be recommended for novel foods. Specific standards for safety testing for novel foods would be very difficult to create, given the broad range of types of such foods. Postmarket assessment, including monitoring of adverse events, is important once a novel food or NDI is introduced.

From recent reports of the American Association of Poison Control Centers, in the general area of dietary supplements, major adverse events are quite rare (Figure 1). Deaths from vitamins, minerals, and botanical dietary supplements reported to US Poison Control Centers range from 0 to 4 per year in total in each of these three categories from 2003 to 2012 (http://www.aapcc.org/annual-reports/). Such data suggest that current regulatory frameworks for dietary supplements and NDIs may be adequate to protect public health. But as seen in Figure 1, the decline in adverse events from botanical dietary supplements after 2005 shows that hundreds of people suffered adverse effects before FDA was able to obtain a final ruling to ban ephedra and related substances. DSHEA permits FDA to ban a dietary supplement only after it can prove harm from the dietary supplement. The recent data trend (Figure 1) suggests that the US dietary supplement industry has taken the ephedra example as a caution. Hopefully, such cases may be avoided by voluntary action of this industry, the appropriate use of NDI notifications, and adequate pretesting of NDIs.

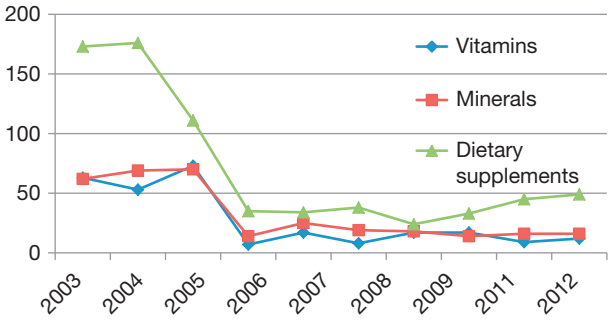

Figure 1 Major adverse events (requiring hospitalization) reported to US Poison Controls Centers from 2003 to 2012. Data were extracted from American Association of Poison Control Centers annual reports (http://www.aapcc.org/annual-reports/).

Conclusion

Although recent trends in the US suggest that dietary supplements including NDIs are rarely harmful, increasing trends for consumers to want 'natural' products, nondrug remedies, and foods that do not contain manufactured chemical additives indicate that consumers will need to be increasingly vigilant about novel foods and NDIs because their regulation is less stringent than for conventional food additives. Past examples illustrate that 'natural' sources of food or dietary supplement ingredients are not necessarily safer. For example, lead was present in the majority of 'natural' calcium supplement products tested and to lesser extent in refined calcium supplement products. The mineral dolomite as a calcium supplement tended to be more heavily lead-contaminated than other sources. Plant or phytotoxins are widely distributed in nature and vary greatly in chemical structure, and a history of use may not suffice as evidence of lack of adverse effects of a particular novel food source. Interactions between plant constituents and pharmaceuticals are common. Users of dietary supplements should disclose such use to their health care providers. Use of standardized dietary supplement products will help to limit variability in effects. Using products for which scientific data are available, especially human clinical placebo-controlled trial data on safety and efficacy under the conditions of intended use, would help to assure novel food safety for consumers. Maintaining skepticism when a novel food product is recommended by someone who stands to gain financially from the product sale is also a wise course of action. Novel food product information from government or university sources generally will be nonbiased and provide reliable help for safety assurance for consumers.

See also: Antibiotics and Drugs: Drug–Nutrient Interactions; Antinutritional Factors in Legume Seeds: Characteristics and Determination; Beverage: Health Effects; Bioavailability of Nutrients; Codex Alimentarius Commission: Role in International Food Standards Setting; Food Additives: Classification, Uses and Regulation; Food Composition Databases; Food-Herbal Medicine Interface; Fruits of Tropical Climates: Dietary Importance and Health Benefits; Functional Foods; Irradiation of Foods: Processing Technology and Effects on Nutrients.

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Nucleic Acids

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Introduction

The nucleic acids, vital constituents of all living cells, were discovered by Miescher in 1868, isolated from the nuclei of pus cells and spermatozoa of Rhine salmon. Some of the earliest observations of macromolecules within living cells were of nucleic acids in the form of chromosomes. Nucleic acids were named partly based on their chemical properties and partly on the observation that they represent a major constituent of the cell nucleus. The fact that they form the chemical basis for the transmission of genetic traits was not realized until 1941. When the fine thread-like nucleic acids were isolated from cells and analyzed biochemically, they were found to be composed of three relatively simple components: sugar, phosphate, and a base. For many years, this chemical composition, which is relatively simple compared to that of proteins, led to nucleic acids being overlooked as genetic macromolecules. There are two main types of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is found largely in the nucleus of the cell, but certain organelles also contain DNA, including mitochondria and plastids. RNA is also found in the nucleus, but it is also present in the cytoplasm in large amounts. DNA and RNA are responsible for storing and implementing the genetic information in the cell. DNA is a long polymer made from repeating units called nucleotides (adenine, guanine, thymine, and cytosine). As first discovered by James D. Watson and Francis Crick, the structure of the DNA of all species comprises two helical chains each coiled around the same axis, and each with a pitch of 34 Å (3.4 nm) and a radius of 10 Å (1.0 nm).

Physiology

Chemical Structure of Nucleic Acids

DNA is a polymeric molecule, which is composed of repeating nucleotide subunits. The order of nucleotide subunits contained in the linear sequence or primary structure of these polymers represents all of the genetic information carried by a cell. Each nucleotide is composed of a phosphate group; a pentose (5 carbons) sugar and a cyclic nitrogen-containing compound called a base. In DNA, the sugar moiety is 2-deoxyribose. Eukaryotic DNA is composed of four different bases: adenine, guanine, thymine, and cytosine. The bases are attached as side chains to the sugars; they differ from one 'letter' to the other. These bases are classified based on their chemical structure into two groups:

Adenine and guanine are double-ring structures termed purines, and thymine and cytosine are single-ring structures termed pyrimidines. The overall composition of DNA shows that the concentration of thymine is always equal to the concentration of adenine, and the concentration of cytosine is always equal to guanine. Thus, the total concentration of pyrimidines always equals the total concentration of purines. These monomeric units are linked together into the polymeric structure by 3′,5′-phosphodiester bonds (Figure 1). Natural DNAs display large size variations, depending on the source, with the relative molecular weight ranging from 1.6×10^6 Da for bacteriophage DNA to 1×10^{11} Da for a human chromosome.

The RNA molecule is chemically single-stranded, but double helical regions arise when stretches of complementary sequences allow hairpin loops to form. In addition, the base uracil (U) is found in RNA instead of thymine, and the pentose is ribose. Ribosomes are very large molecular systems, which translate genomic information from messenger RNAs (mRNAs) into proteins. Special attention has recently been given to certain small RNA strands that form specific 3D structures with catalytic activity: the ribozymes. Interest in these molecules is twofold: they may provide new perspectives on the question of what came first, proteins or DNA, and they may allow the construction of new types of enzymes.

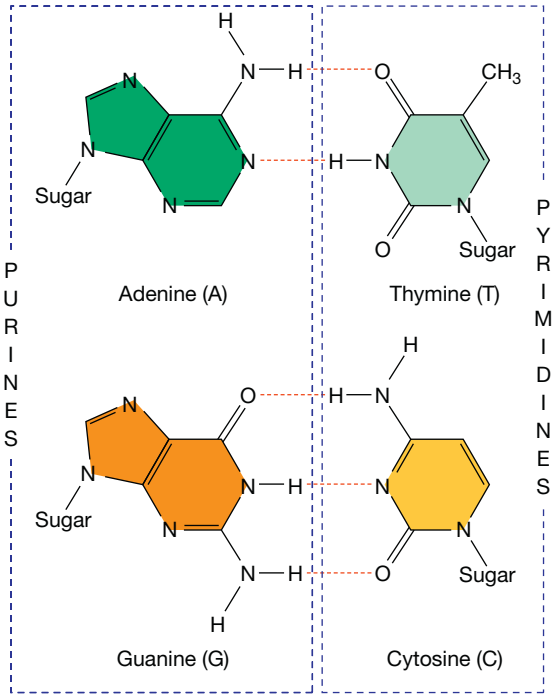

Figure 1 Chemical structure of purine and pyrimidine deoxyribonucleosides and their pairing pattern.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00487-6

Nucleic Acids and Genetic Information

Genetic information is stored in the sequence of bases along a nucleic acid chain. The bases have an additional special property: they form specific pairs with one another that are stabilized by hydrogen bonds. The base pairing results in the formation of a double helix, a helical structure consisting of two strands. These base pairs provide a mechanism for copying the genetic information in an existing nucleic acid chain to form a new chain. Although RNA probably functioned as the genetic material very early in evolutionary history, the genes of all modern cells and many viruses are made of DNA. DNA is replicated by the action of DNA polymerase enzymes. These exquisitely specific enzymes copy sequences from nucleic acid templates with an error rate of less than 1 in 100 million nucleotides.

Genes specify the kinds of proteins that are made by cells, but DNA is not the direct template for protein synthesis. Rather, the templates for protein synthesis are RNA molecules. In particular, a class of RNA molecules called mRNA are the information-carrying intermediates in protein synthesis. Other RNA molecules, such as transfer RNA (tRNA) and ribosomal RNA (rRNA), are part of the protein-synthesizing machinery. All forms of cellular RNA are synthesized by RNA polymerases that take instructions from DNA templates. This process of transcription is followed by translation, which is the synthesis of proteins according to instructions given by mRNA templates. Thus, the flow of genetic information, or gene expression, in normal cells is as follows:

$$\text{DNA} \xrightarrow{\text{transcription}} \text{RNA} \xrightarrow{\text{translation}} \text{Protein}$$

This flow of information is dependent on the genetic code, which defines the relation between the sequence of bases in DNA (or its mRNA transcript) and the sequence of amino acids in a protein. The code is nearly the same in all organisms: a sequence of three bases, called a codon, specifies an amino acid. Codons in mRNA are read sequentially by tRNA molecules, which serve as adaptors in protein synthesis. Protein synthesis takes place on ribosomes, which are complex assemblies of rRNAs and more than 50 kinds of proteins.

The hereditary material in the nucleus of human cells is packed into 23 chromosomes, and additional DNA is found in the mitochondria. The human genome is known to contain $\sim 3 \times 10^4$ coding sequences, or genes, and a substantial proportion of DNA has a regulatory role in the transcription of the genes. The sequence of the four bases and the capacity of DNA to be copied into two complementary strands underlie the genetic information of all living organisms. Interactions between DNA and the transcription factors determine the time and place in the body where genes are transcribed, causing development and metabolism to occur.

RNA molecules are synthesized initially on a DNA template by a DNA-dependent RNA polymerase in a process called transcription, in which ribonucleotides complementary to the bases of one strand of DNA are joined by 30–50 phosphodiester bonds. Cells contain three types of RNA, each of which is chemically modified after transcription from the DNA template. The three kinds of RNA together are important in the translation of the genetic message to synthesize proteins in the cell. Most RNA is in the cytoplasm, principally in the form of rRNA (80% of the total), which performs structural and catalytic roles in ribosomes, the site of the growing polypeptide chain in protein synthesis, whereas mRNA (5%) provides the template for protein synthesis. The amino acids are brought to the assembly site covalently bonded to tRNA (15%), with their order in the growing protein being specified by the order of the bases in mRNA.

Biosynthesis of Nucleic Acids in Humans

Formation of Deoxyribonucleotides

The first step in the synthesis of DNA and RNA in humans (and probably for all living beings) involves the formation of the purine and pyrimidine ribonucleotides. Two routes derive them endogenously: the energy-intensive multistep *de novo* route, using small molecules such as carbon dioxide, amino acids, and ribose sugars as precursors; and the energetically less expensive 'salvage' pathway (Figure 2).

The typical cell contains five to ten times as much RNA (mRNAs, rRNAs, and tRNAs) as DNA. Therefore, the majority of nucleotide biosynthesis has as its purpose the production of rNTPs. However, because proliferating cells need to replicate their genomes, the production of dNTPs is also necessary. This process begins with the reduction of rNDPs, followed by phosphorylation to yield the dNTPs. The phosphorylation of dNDPs to dNTPs is catalyzed by the same nucleoside diphosphate kinases (NDKs) that phosphorylate rNDPs to rNTPs, using ATP as the phosphate donor (Figure 3).

Given that the goal of nucleotide synthesis is to create eight distinct types of nucleotide, each containing three modular parts (sugar, base, and phosphate(s)), the process is fairly complex. As with any biochemical pathway, a metabolic map is helpful for conceptualizing the overall framework. Figure 3 depicts the assembly of each type of nucleotide at the general level. The total intracellular concentration of a given type of nucleotide (e.g., AMP + ADP + ATP) is tightly regulated and stays relatively constant, although there may be major changes in the individual concentrations, depending on the energy state of the cell (e.g., ATP increases while AMP decreases). The pathways for the *de novo* synthesis of purines and pyrimidines are primarily regulated by the concentrations of their own products, which inhibit further production. This form of

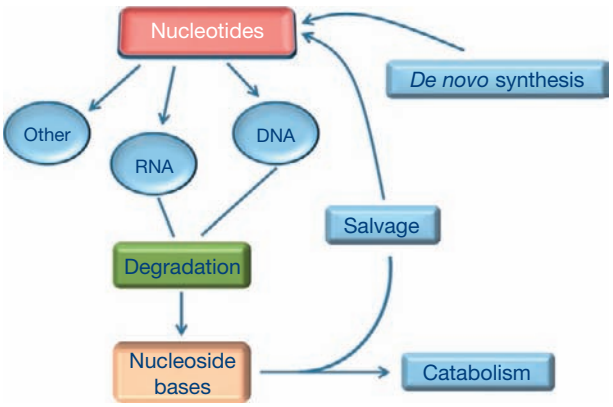

Figure 2 Overview of nucleotide metabolism.

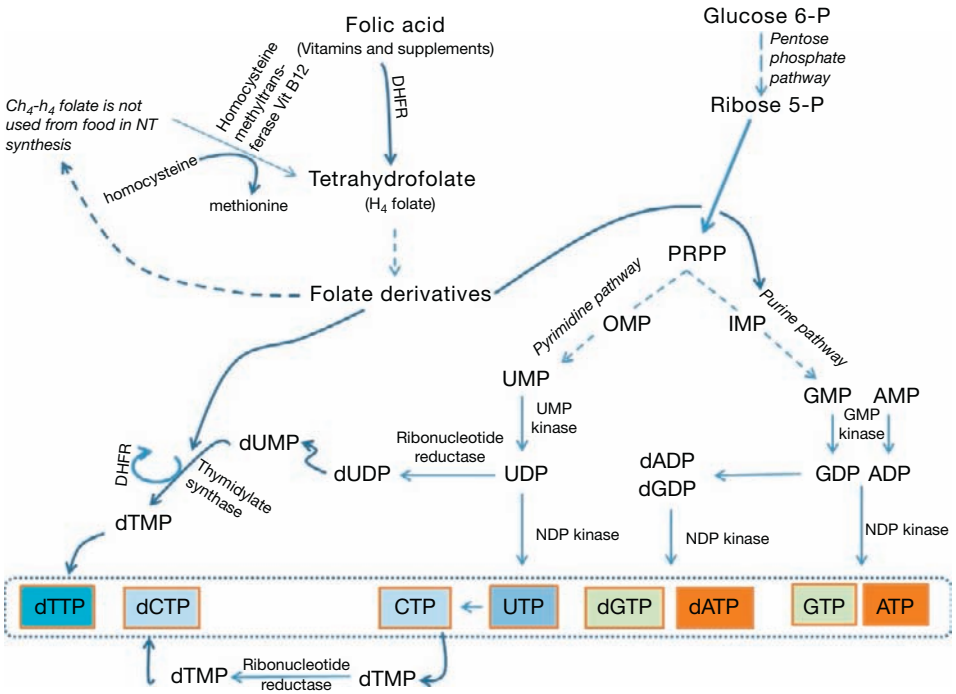

Figure 3 General plan of nucleotide synthesis.

regulation ensures an adequate supply of nucleotides while preventing their overproduction. The synthesis of the 5-carbon sugar ribose pathway gives a clear view about regulatory pathways in nucleotide synthesis.

The pentose phosphate pathway

Synthesis of nucleotides requires a source of ribose 5-phosphate. This compound is produced from glucose 6-phosphate via the pentose phosphate pathway (also called the hexose monophosphate shunt). In addition to producing ribose 5-phosphate, the pathway is a major source of NADPH, a coenzyme required for anabolism and the repair of oxidative damage. The pentose phosphate pathway occurs in the cytosol of cells. The pathway has oxidative and nonoxidative parts, which can operate more or less independently. The oxidative part of the pathway converts glucose 6-phosphate to ribose 5-phosphate and produces two NADPH (Figure 4).

Most of the steps in this part of the pathway are thermodynamically irreversible, thus ensuring that the cell maintains a high NADPH-to-NADP ratio, which is particularly important in cells that carry out NADPH-dependent processes such as fatty-acid biosynthesis (liver, mammary glands (lactating), and adipose), steroid-hormone synthesis (testes, ovaries, and adrenal cortex), and the reduction of glutathione (erythrocytes; a key reaction in protection from oxidative damage). Further utilization of ribulose 5-phosphate occurs via reversible nonoxidative reactions. In cells that have large needs for nucleotides, most of the ribulose 5-phosphate is converted to ribose 5-phosphate and used for nucleotide biosynthesis. In cells that need more NADPH than nucleotides, the excess ribulose 5-phosphate is converted to compounds that enter glycolysis in a series of reversible reactions.

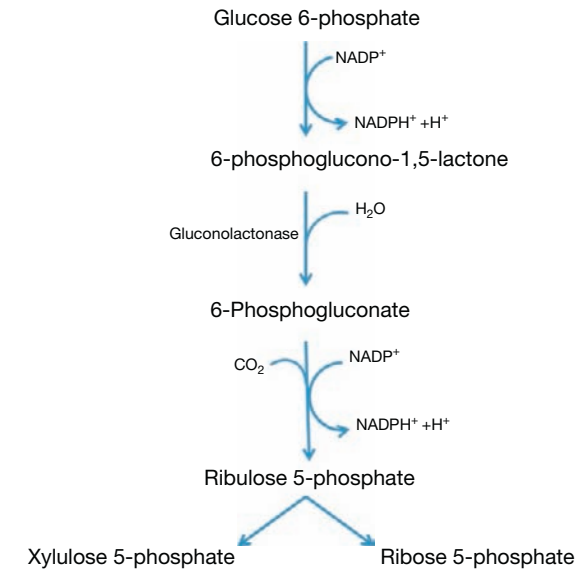

Figure 4 Pentose-phosphate synthesis pathway.

Glucose 6-Phosphate Dehydrogenase Deficiency

Interestingly, the key disorder associated with defects in the pentose phosphate pathway does not arise from a failure to synthesize nucleotides, but rather deficient NADPH production. The first NADPH-producing reaction of the pentose phosphate pathway is catalyzed by glucose 6-phosphate dehydrogenase (G6PD), an enzyme produced from a gene on the X chromosome. Unlike other cells, erythrocytes lack mitochondrial pathways that produce NADPH, and they rely solely on the pentose phosphate pathway for NADPH production.

Mutations that reduce G6PD activity result in a decreased ability to produce NADPH, which impairs the normal red blood cell response to oxidative damage. G6PD-deficient individuals are typically asymptomatic unless exposed to conditions that increase production of reactive oxidants (e.g., hydrogen peroxide) that damage hemoglobin, membrane lipids, and other cellular components. Such oxidative damage can lead to hemolytic anemia. Conditions that can precipitate hemolytic anemia in the setting of G6PD deficiency include infection, the use of certain drugs (including sulfa drugs and antimalarial drugs), and consumption of fava beans.

Role of NADPH in G6PD deficiency

Normally, hydrogen peroxide is eliminated by glutathione. Glutathione is a tripeptide made up of glutamate, cysteine, and glycine. Its sulfur-containing side chain can reduce hydrogen peroxide to water, and it is oxidized in the process (Figure 5). Glutathione reductase restores glutathione to its reduced form using NADPH as the source of reducing power. Ribonucleotide reductase (RR) is a multifunctional enzyme that contains redox-active thiol groups for the transfer of electrons during the reduction reactions. In the process of reducing the rNDP to a dNDP, RR becomes oxidized. RR is reduced, in turn, by either thioredoxin or glutaredoxin. The ultimate source of the electrons is NADPH. The electrons are shuttled through a complex series of steps involving the enzyme, thioredoxin reductase, that regenerates the reduced form of thioredoxin. Glutaredoxin gives up its electrons to glutathione, which is then reduced by glutathione reductase.

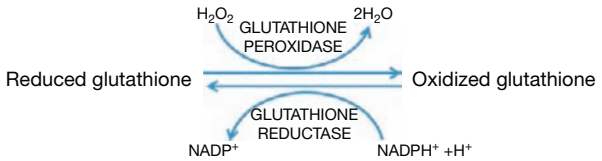

Figure 5 Restoring glutathione using NADPH.

Thus, mutations that decrease G6PD activity impair the red cell's ability to detoxify hydrogen peroxide. G6PD deficiency is very common; about 400 million people worldwide are affected. Frequencies are highest in tropical Africa, parts of the Middle East, and Southeast Asia (Figure 6). On the other hand, in the process of nucleic-acid synthesis, the folic acid obtained from vitamins and other food supplements coverts into tetrahydrofolate (THF), folate derivatives, which further enter into the purine pathway, as well as the dUMP-to-dTMP pathway (Figure 3). Thus, THF plays an important relevance in physiological processes for nucleic acid synthesis.

THF – Clinical Relevance

THF is regenerated from the dihydrofolate (DHF) product of the thymidylate synthase reaction by the action of dihydrofolate reductase (DHFR), an enzyme that requires NADPH (Figure 3). Cells that are unable to regenerate THF suffer defective DNA synthesis and eventually result in cell death. For this reason, as well as the fact that dTTP is utilized only in DNA, it is therapeutically possible to target rapidly proliferating cells over nonproliferating cells through the inhibition of thymidylate synthase. Many anticancer drugs act directly to inhibit thymidylate synthase, or indirectly, by inhibiting DHFR.

The class of molecules used to inhibit thymidylate synthase is called the suicide substrates because they irreversibly inhibit the enzyme. Molecules of this class include 5-fluorouracil and 5-fluorodeoxyuridine. Both are converted within cells to 5-fluorodeoxyuridylate, FdUMP. Many DHFR inhibitors have been synthesized, including methotrexate, aminopterin, and trimethoprim, which are an analog of folic acid.

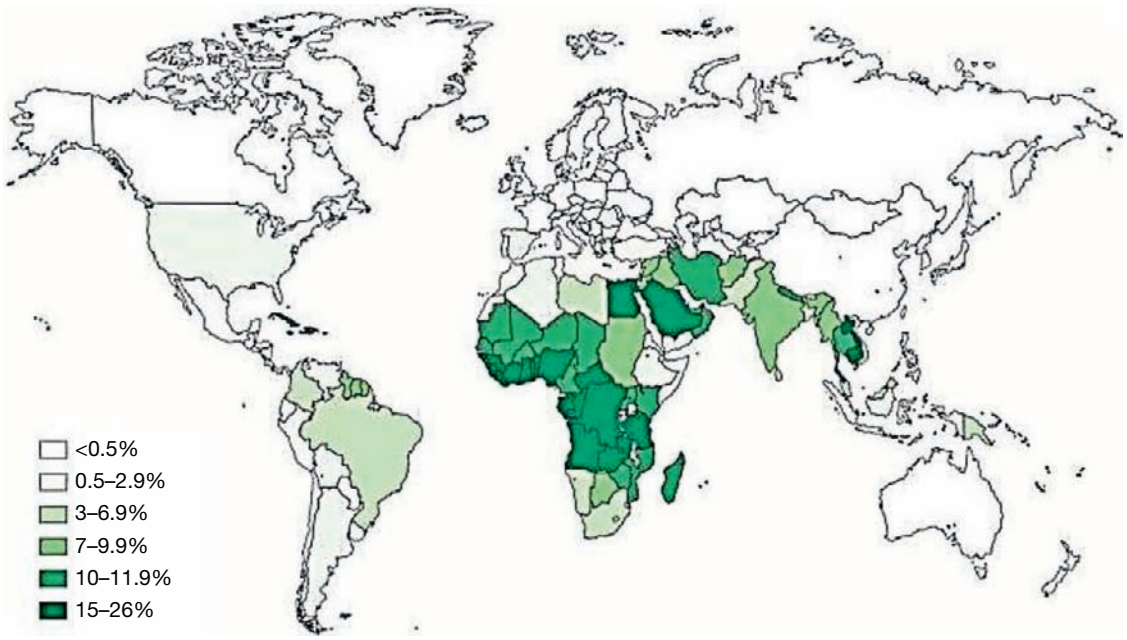

Figure 6 Global scenario of G6PD deficiency.

De novo *ribonucleotide synthesis*

Purine and pyrimidine nucleoside 5′-monophosphates can be synthesized *de novo* from 5-phosphoribosyl-1-pyrophosphate (PRPP) and various carbon and nitrogen donors. The raw materials for both types of nucleotide have a common origin. However, the pathways by which they are formed are separate and distinct in organization. In the pyrimidine pathway, the ring structure of the base is assembled first and then attached to the pentose sugar PRPP. In contrast, the purine pathway starts with the pentose sugar and builds the ring structure of the base upon it. Nucleoside 5′-monophosphates are phosphorylated to form the corresponding diphosphates by one of several nucleoside monophosphate (NMP) kinases, each of which is specific for the base component of the nucleotide (Figure 3). Nucleoside diphosphates (NDPs) can be converted to triphosphates by a nonspecific NDK. ATP is the phosphate donor in all of these phosphorylation reactions.

General Overview of Nucleotide Catabolism and Salvage

Nucleic acid turnover occurs continuously in most cells. mRNA, in particular, is actively synthesized and degraded. These degradative processes can lead to the release of free purines in the form of adenine, guanine, and hypoxanthine (the base in inosine 5′-monophosphate (IMP)). Ther breakdown of DNA and RNA releases nucleoside 5′-monophosphates, which can be hydrolyzed by 5′-nucleotidases to yield nucleosides. These substances represent a metabolic investment by cells. So-called salvage pathways exist to recover them in useful form. Salvage reactions involve the resynthesis of nucleotides from bases via phosphoribosyltransferases. Although both purine and pyrimidine nucleosides can be degraded to waste products that are excreted, the catabolic pathways have branch points in most cells at which the components of nucleotides can be salvaged (Figure 7).

Interconversion of the Nucleotides

During the catabolism of nucleic acids, nucleoside mono- and diphosphates are released. The nucleosides do not accumulate to any significant degree, owing to the action of nucleoside kinases. These include both NMP kinases and NDP kinases. The NMP kinases catalyze ATP-dependent reactions of the type

$$\text{dNMP} + \text{ATP} \leftrightarrow (\text{d})\text{NDP} + \text{ADP}$$

There are four classes of NMP kinases that catalyze, respectively, the phosphorylation of

(i) AMP and dAMP; this kinase is known as adenylate kinase
(ii) GMP and dGMP
(iii) CMP, UMP, and dCMP
(iv) dTMP

The enzyme adenylate kinase is important for ensuring adequate levels of energy in cells such as liver and muscle. The predominant reaction catalyzed by adenylate kinase is

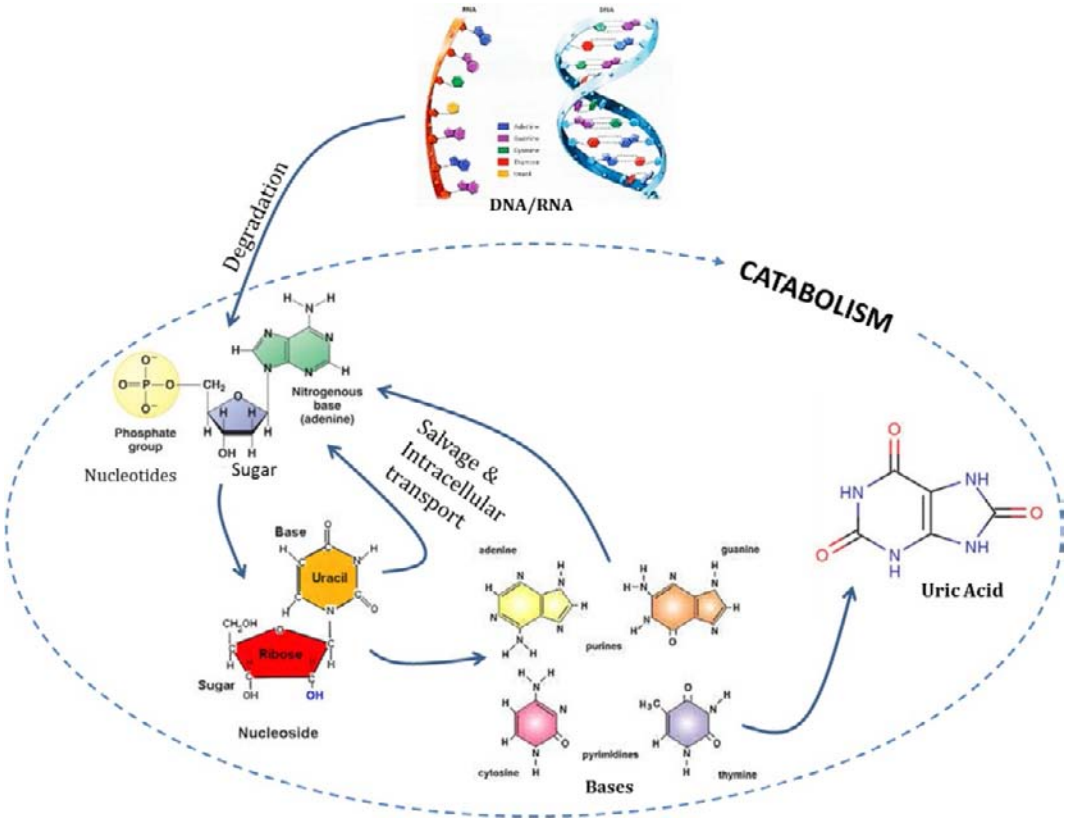

Figure 7 Overview of nucleotide salvage.

$$2ADP \leftrightarrow AMP + ATP$$

The NDP kinases catalyze a reaction of the type

$$N_1TP + N_2DP \leftrightarrow N_1DP + N_2TP$$

N_1 can represent a purine ribo- or deoxyribonucleotide, and N_2 can represent a pyrimidine ribo- or deoxyribonucleotide. The activity of the NDP kinases can range from 10 to 100 times higher than that of the NMP kinases. This difference in activity maintains a relatively high intracellular level of (d)NTPs relative to that of (d)NDPs. Unlike the substrate specificity seen for the NMP kinases, the NDP kinases recognize a wide spectrum of (d)NDPs and (d)NTPs.

The shared catabolism and salvage pathways saves metabolic energy on one hand while prevent nucleotide pools from reaching toxic levels on other side. Unless the flow of nucleotides into the shared salvage–catabolism pathway is greater than usual, normally more components are salvaged than are catabolized (intestinal epithelial cells are exceptions to this rule; they completely catabolize the components of dietary nucleotides rather than salvage them). This makes sense because, although nucleotide synthesis is energy-intensive, the complete breakdown yields very little energy. As with many metabolites, the liver is a 'way station' for nucleotides. The liver is a major site for both *de novo* nucleotide synthesis and degradation. Excess nucleosides and bases are supplied by hepatocytes to other tissues, such as the brain and muscle, which utilize salvage pathways to generate needed nucleotides. Importantly, nucleosides and bases cross membranes but nucleotides do not. The salvage pathways also make it possible for nucleotides and their components released by cells undergoing apoptosis to be reused. Many cell types contain 5′-nucleotidases on the outer surface of the plasma membrane and can therefore convert extracellular nucleotides to nucleosides, which can be taken up using Na^+/nucleoside symporters. Pyrimidine catabolism and salvage pathways are rarely associated with disease. Derangements of purine salvage–catabolism are more common. Purine salvage pathways are also important clinically for metabolism of certain drugs.

Clinical Considerations

A few important drugs inhibit key steps in *de novo* purine nucleotide synthesis and, as a result, kill rapidly dividing cells. Mercaptopurine and azathioprine (a prodrug that is converted in the body to mercaptopurine), both inhibitors of several steps in *de novo* purine synthesis, are cancer chemotherapeutic drugs that are also used as immune suppressants in the treatment of many diseases, including rheumatoid arthritis, lupus, and inflammatory bowel disease (IBD).

IMP dehydrogenase, an enzyme that carries out one of the steps in synthesis of GMP from IMP, is inhibited by the immunosuppressant drug mycophenolate mofetil, which is widely used to prevent solid organ transplant rejection. Lymphocytes are unique in being unable to utilize salvage pathways to generate GMP. Their dependence on *de novo* synthesis for GMP means the proliferation of these cells is selectively inhibited by this drug. Note the role of formyl-H_4folate, a form of reduced folate carrying a formyl group, in providing a 1-carbon unit for the synthesis of the inosine base.

Moreover, clinically important carbamoyl phosphate is an intermediate in another key metabolic pathway, and it is utilized as a substrate in the urea cycle by the enzyme ornithine transcarbamoylase. Inherited deficiency of this enzyme (the most common urea-cycle defect) is associated with hyperammonemia and associated problems, but it is also marked by elevated blood and urinary orotate, because excess carbamoyl phosphate shunts into pyrimidine nucleotide metabolism. Another inherited defect in pyrimidine nucleotide synthesis, orotic aciduria, arises from deficiency of enzymes that convert orotate to orotidine monophosphate (OMP) and uridine monophosphate (UMP). In addition to urinary excretion of orotate, this disease is associated with poor growth and megaloblastic anemia. Also importantly, a step in the synthesis of UMP from orotic acid is inhibited by the antirheumatic drug leflunomide.

Dietary Nucleic Acid Metabolism in Humans

The normal human diet is rich in both DNA and RNA because food is derived from once-living organisms. The metabolism of these exogenous nucleic acids follows a pattern similar to the intracellular process described previously, but the bacterial flora of the intestine are the first point of attack. This digestion is rapid. Some studies demonstrated that up to 50% of radiolabeled dietary purine was degraded and lost as carbon dioxide gas within 30 min, with the remaining 43% being recovered in the urine and 5% in the feces. It has been shown that dietary pyrimidine nucleotides, but not purines, are incorporated into RNA.

Humans thus have no apparent requirement for purines from the diet, and the intestinal mucosa provides an effective barrier to their uptake through a battery of enzymes that rapidly degrade purine nucleotides, nucleosides, and bases to the metabolic waste product, uric acid. This phenomenon may represent an important evolutionary development to protect the integrity of the cellular DNA or to ensure that levels of ATP do not fluctuate in concert with the dietary intake of purines. The potential toxicity of dietary nucleic acids to humans usually arises not from the nucleic acids but from their metabolic end-products (principally uric acid). Many investigators have shown that the fate of the dietary purine moiety depends on whether it is administered in the form of DNA, RNA, mononucleotides, nucleosides, or bases, with some being catabolized more readily than others. When normal subjects are fed RNA, the increase in the excretion of uric acid is dramatic alongside a modest increase in plasma-urate concentrations. The effect of RNA is also twice that of DNA when the increase in purines in the plasma is measured. On the other hand, pyrimidine mononucleotides and uridine, but not the base uracil, are absorbed readily from the intestine and utilized for nucleic-acid synthesis. This has been demonstrated by studies of humans with hereditary orotic aciduria, a rare defect in *de novo* pyrimidine synthesis at the fourth step involving the conversion of orotic acid to UMP. Such patients have a severe megaloblastic anemia that does not respond to the usual forms of treatment. They have been sustained for more than 40 years on oral uridine, indicating that the dietary pyrimidine is

absorbed intact and can compensate totally for lack of *de novo* synthesis in humans. Studies using radiolabeled purines and pyrimidines in mice have provided further evidence for the incorporation of dietary pyrimidine nucleosides, but not purine nucleosides, into hepatic RNA.

Gout: An Excess of Uric Acid

Gout is the clinical term describing the physiological consequences resulting from the derangement of uric-acid metabolism accompanying excessive uric-acid accumulation in body fluids. Uric acid and urate salts are rather insoluble in water and tend to precipitate from solution if produced in excess. Uric acid is the end product of purine metabolism in humans. At physiological pH, uric acid is 98% ionized and is present mainly as urate ions. In extracellular fluids, uric acid exists as a solution of monosodium urate, which has very low solubility and can precipitate very easily. The most common symptom of gout is arthritic pain in the joints as a result of urate deposition in cartilaginous tissue. The joint of the big toe is particularly susceptible.

Normal Uric-Acid Metabolism

Purine nucleotides are components of nucleic acids; they are also intimately involved in energy transformation and phosphorylation reactions and act as second messengers.

There are three sources of purines in humans:

(i) The diet
(ii) Degradation of endogenous nucleotides
(iii) *De novo* synthesis

The kidney and the intestine excrete urate. In the intestine, urate is metabolized to carbon dioxide and ammonia by bacterial action ('uricolysis').

Urate-handling by the kidney is complex (Figure 8). It is filtered by the glomeruli and then almost completely reabsorbed in the proximal tubules; in the distal tubules, both reabsorption and secretion occurs. Net urate clearance is only 10% of the filtered load. If the filtered load is increased in normal subjects, excretion is increased. In patients with renal failure, however, urate excretion decreases with a decrease in glomerular filtration rate, and the serum level rises. Dietary purines account only for 30% of excreted urates.

The metabolic pathways leading to endogenous uric-acid synthesis are mediated by several enzymes in a complex fashion. There is *de novo* synthesis from ribose-1-phosphate to inosine, as well as a secondary salvage pathway that leads to the production of uric acid by converting guanine and adenine bases into inosine, which is in the main pathway of xanthine production (Figure 9). Xanthine is oxidized by xanthine oxidase to uric acid. Uric acid is the last stage in purine degradation in humans, because all primates lack the enzyme (uricase) to convert uric acid into allantoin.

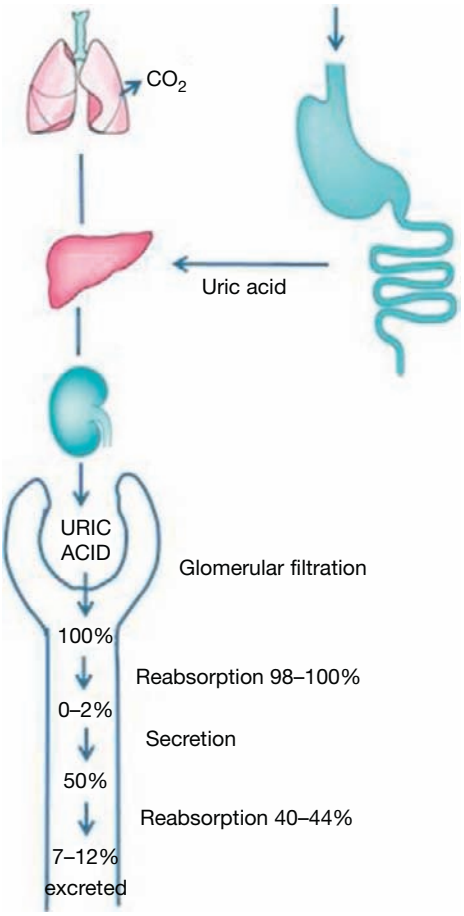

Figure 8 Uric-acid filtration mechanism.

Uric Acid in Serum

Serum concentrations of uric acid are higher in men than in women (gout is much more common in men), tend to rise with age, and tend to be higher in individuals of higher economic classes and in obese people. There is considerable variation in serum-urate concentrations between different ethnic groups.

Although the mechanism of gout has not been completely elucidated, the likelihood of gout increases with increased serum urate levels. However, only a small proportion of individuals with elevated uric acid levels develop gout (in the Western Hemisphere, it is estimated that 10% of the population has high uric acid levels, but only 0.5% develop gout). The solubility of urate decreases with decreasing temperature, explaining the increased incidence of gout in peripheral joints (which are cooler). Hyperuricemia can be caused by increased formation of uric acid, by reduced excretion by the kidneys, or by a combination of both mechanisms.

Increased formation of uric acid occurs with:

- High dietary intake
- Increased purine synthesis
  - Idiopathic
  - Inherited (several genetic mechanisms have been described)

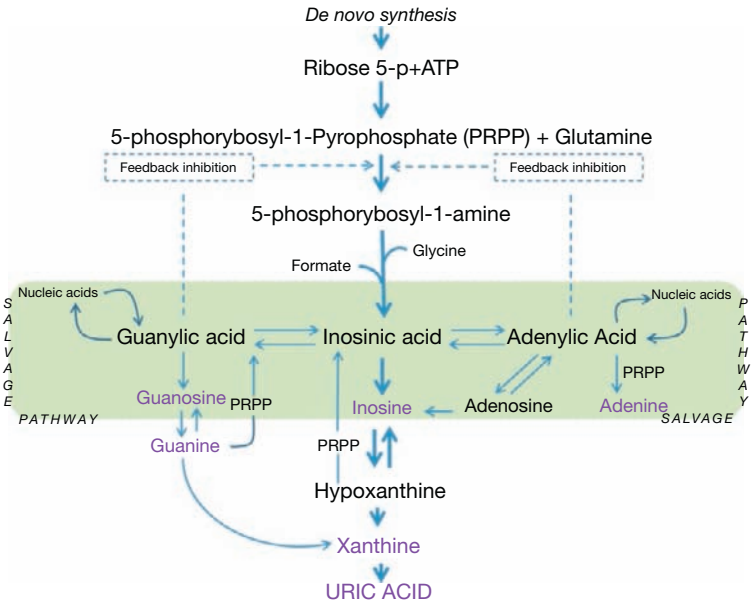

Figure 9 Endogenous uric-acid synthesis.

- Increased nucleic acid turnover
  - Myeloproliferative disorders (e.g., leukemias)
  - Psoriasis
  - Secondary polycythemia
  - Carcinomatosis
  - Chronic hemolytic anemias
  - Cytotoxic drugs

Reduced excretion of uric acid by the kidneys occurs with:

- Chronic renal disease
- Drugs (thiazides, salicylates)
- Poisons (lead)
- Increased organic-acid concentration in blood (lactic acid, acetoacetic acid, beta-hydroxybutyrate)
- Hyperparathyroidism

Dietary Sources of Nucleic Acids

Nucleic Acid–Rich Foods

Animal origin

- Wild or farmed game meats: venison, pheasant, rabbit, hare, deer, and pork
- Seafoods: sardines, sprats, herring, bloaters, anchovies, fish roe, caviar, taramasalata, trout or salmon, lobster, crab, and prawns

Plant origin

- Vegetables: asparagus, avocado pears, peas, spinach, mushrooms, broad beans, cauliflower, and eggplant
- Pulses and grains: legumes, pulses, and soya products such as bean curd, tofu, and Quorn
- Cereals: all bran, oat, rye, or wheat cereals and products; whole meal, rye, and brown breads
- Other: beer and yeast extracts/tablets; meat or vegetable extracts

Foods That Are Moderate or Low Sources of Purine

Animal origin

- Beef, lamb, pork (steak or chops), bacon, ham, sausages, some poultry, and tongue (all should be eaten in moderation)
- Fish: bass, bream, cod, crab, eel, kippers, lobster, lemon sole, plaice, and trout

Plant origin

- Carrots, parsnip, potatoes, lettuce, leeks, cabbage, sprouts, marrow, courgettes, peanuts, cashew nuts, white bread or flour, cakes, scones, biscuits, and cereals

Purine-Free Food Sources

Animal origin

- Milk, cheese, eggs, butter, margarine, cream, and ice cream

Plant origin

- Sugar, jam, marmalade, honey, sweets, cucumber, tomato, onions, pumpkin, fruits (fresh, cooked, or tinned), nuts, puddings, custards, yogurt, fruit juices, and soft drinks

See also: Ascorbic Acid: Physiology and Health Effects; Choline: Physiology; Choline: Properties and Determination; Enzymes: Functions and Characteristics.

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Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling

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Introduction

The WHO Global Action Plan for the Prevention and Control of NCDs (2013) highlights the importance of the diet and calls upon the private sector to improve the provision of affordable and accessible healthy options, which may be the result of reformulation or fortification and which meet a high standard of nutrition labeling. Together with other population lifestyle changes, WHO aims to achieve a 25% relative reduction in premature mortality from cardiovascular diseases, cancer, diabetes, and chronic respiratory conditions by 2025. There are three key voluntary targets for foods: to reduce the mean population salt intake by 30%, to lower blood pressure by 25%, and to halt the rise in obesity and diabetes.

The links between diet and health are well documented, including those highlighted in the preceding chapters of this book. The body of evidence continues to grow, from not only ongoing research in well-established areas of nutrition but also emerging science suggesting benefits from new research areas or innovative foods and ingredients. Whether they are called 'functional foods,' 'nutraceuticals,' 'bioactives,' or similar, they are all foods with health benefits, resulting from either naturally occurring food components or those that are added or removed during manufacturing. When referring to the promotion or advertising of foods, nutrition claims can highlight a nutritional property of the food, while health claims are used to describe a particular beneficial relationship between food and health.

The use of valid health messages and clear nutrition labeling are a crucial part of assisting consumers to make informed choices about their diet. When consumed in the recommended quantities, as part of a healthy diet and lifestyle, foods can offer a range of benefits, from cholesterol reduction to the maintenance of normal bone structure in adults; and normal visual development in young babies or normal cognitive development in children. Such messages have been the mainstay of public health nutrition campaigns and healthcare professionals in the past, but food manufacturers now also use them as a marketing tool to help increase the appeal of a product or brand range. Although nutrition and health claims are by no means a requirement to sell foods with health benefits, if companies do choose to use them, they will most likely need to comply with regulations to promote fair trade and protect consumers from misleading or stretched claims.

As to meaningfulness to the consumer and the effect of claims on purchasing choice and longer-term health outcomes, the jury is still out. Further research is needed to help inform industry, health practitioners, and policy makers. It has been found that health claims can influence expectations about both the sensory and health benefits of the product, but not always accurately. Furthermore, consumer understanding can be affected by a number of variables, which, together with the nature of controls around the world, will be covered further in this article.

Regulatory Control of Nutrition and Health Claims

A number of countries around the world have adopted, or are in the process of adopting, some form of regulation for the responsible use of nutrition and health claims. Although there are some key differences, these systems are grounded in similar principles that aim to provide a level playing field and help ensure that robust science is behind the claims to protect consumers. Some of the key differences relate to pre-market approval versus notification, legislation versus self-regulation, whether 'therapeutic' foods are included or covered by other legislation, or the ability to use alternative wording of authorized claims. Some countries such as the United States and Japan also allow for lower levels of substantiation to enable the use of 'qualified health claims', that is, claims with a disclaimer to inform the consumer that they are not based on consensus opinion. It has been argued that to support the global marketplace, from both the consumer perspective and industry perspective, greater harmonization is required on the substantiation and regulation of claims.

Some countries use the term 'nutrition claim' while others refer to 'nutrient content claims.' There is no global definition of 'health claim'; however, they are usually subcategorized along the lines of either 'health maintenance' (or structure/function) claims or 'disease risk reduction' claims. Product-specific claims are permitted in a few countries, while generic claims relating to food components tend to be more widely accepted. Each regulatory system can be highly detailed, and comprehensive reviews and comparisons of various countries have been published elsewhere.

To provide a flavor of the regulatory systems governing health messages used in a commercial context, an overview of the European Regulation on Nutrition and Health Claims (EC 1924/2006) follows as an example.

Regulation EC 1924/2006 ('the EC Regulation' hereafter) applies to all information in commercial communication relating to packaged or unpackaged food (including labels and all forms of promotion and advertising, such as TV, web, and social media) that could state, suggest, or imply a relationship with health such as messages about the general diet, natural foods, manufactured foods, ready meals, nutrients, dietary supplements, beverages, and any other food-related elements within wider health plans such as fitness and well-being programs or weight loss regimes.

As defined by the EC Regulation, a 'nutrition claim' infers that a food "has a particular beneficial nutritional property due to the presence or absence of energy or nutrients" (e.g., 'low in salt,' 'reduced energy,' and 'high in fiber'), whereas 'health claim' means "any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health" (e.g., "Biotin contributes to normal functioning of the nervous system"). The EC Regulation also

provides a specific definition for 'reduction of disease risk claim,' that is, "any health claim that states, suggests or implies that the consumption of a food category, a food or one of its constituents significantly reduces a risk factor in the development of a human disease" (e.g., "Supplemental folic acid intake increases maternal folate status. Low maternal folate status is a risk factor in the development of neural tube defects in the developing foetus").

In the EU only those claims that have been authorized by the European Commission are available for use with complying foods (as opposed to a notification system employed by some other countries). As of January 2015, there were some 256 authorized health claims; however, 222 of these were published as one list in 2012, nearly 2 years later than expected, highlighting the complexity of developing an EU-wide list of permitted claims.

Each authorized claim carries specific conditions for use for that claim, such as product compositional criteria and additional labeling requirements for that claim. Manufacturers must also meet all other general conditions set out in the regulation, such as providing full nutrition labeling, ensuring that claims are not ambiguous or misleading, ensuring that claims do not encourage or condone excess consumption of a food, and ensuring that they do not imply that a balanced diet cannot provide necessary nutrients. To this end, the European Commission and some individual EU member states have prepared guidance on the implementation of the regulation to help companies apply the claims appropriately.

The EC Regulation states that claims must "be well understood by the average consumer." The preamble to the regulation describes this to be someone who is "reasonably well-informed and reasonably observant and circumspect, taking into account social, cultural and linguistic factors" and goes on to say that the regulation "makes provision to prevent the exploitation of consumers whose characteristics make them particularly vulnerable to misleading claims."

Alternative wording of claims is permitted; however, this must not mislead or confuse consumers and must retain the meaning of the authorized wording. Ideally, claims with associated periphery text and imagery in their final context should be tested with consumers to ensure legal and scientific acceptability. In a bid to assist companies and enforcement in the application of this principle, the UK Department of Health has published specific guidance developed in partnership with other EU member states.

The European Food Safety Authority (EFSA) is the scientific body that assesses the evidence, submitted by individuals or organizations, and provides an opinion on whether or not a 'cause and effect' relationship has been established on which to base a claim. Following a positive opinion, the claim is adopted into EU law, unless there is a legal reason not to do so (two positive opinions, in relation to fats and sodium, were rejected on the basis that they "would convey a conflicting and confusing message to consumers, because it would encourage consumption of those nutrients for which, on the basis of generally accepted scientific advice, European, national and international authorities inform the consumer that their intake should be reduced" and it was considered that these two claims would breach the EC Regulation in terms of being ambiguous or misleading in light of wider dietary recommendations (Commission Regulation (EU) No. 432/2012)).

The regulation also allows for new claims to be added to the list, including product-specific claims. Applicants may also request 'proprietary data status', granted for 5 years, if it can be demonstrated that the claim could not be authorized otherwise.

The submission procedure requires that companies prepare a dossier of evidence, which must adhere to the scientific criteria and format set out in EFSA guidelines. This includes characterization of the food or food component and a systematic review, based on the totality of available pertinent data, to demonstrate that a specific beneficial physiological effect has been achieved in a study group that is representative of the target population for which the claim is intended.

The process of scientific assessment and legal authorization takes a minimum of 9–12 months; however, there is minimal opportunity to communicate with EFSA prior to or during the submission process when questions arise. EFSA agrees that dedicated submitter support would be beneficial to all concerned; however, at the time of writing, funding has not been sufficient to provide additional resources for this purpose.

In addition to the stringent authorization process, there are extra rules that limit certain types of claims or their application.

Blanket prohibitions exist for messages that could state or imply:

- that health could be affected by not consuming the food;
- the treatment, prevention, or cure of disease(s) as a result of eating, or not eating, certain foods;
- a specific rate or amount of weight loss;
- that an individual doctor or healthcare professional has endorsed the food or that they are made reference to within a claim;
- nutrition or health benefits of alcohol.

Vague, non-specific suggestions such as 'good for you' are permitted by the regulation, but only when anchored by a relevant, authorized claim to validate their use. The EC has published specific guidance on this topic, provided for under Article 10 of the EC Regulation (European Commission 2013/63/EU).

Mandatory statements are required with all health claims, as follows:

- A statement indicating the importance of a varied and balanced diet and a healthy lifestyle.
- The quantity of the food and pattern of consumption required to obtain the claimed beneficial effects.
- Where appropriate, a statement addressed to persons who should avoid using the food.
- An appropriate warning for products that are likely to present a health risk if consumed to excess.

Regarding the balanced diet and lifestyle statement, although the regulation does not stipulate where this information should appear in relation to the claim, research suggests it should be at least in the same field of vision so to reflect the importance of the overall diet and not to mislead consumers as to the potency of the product in isolation.

In terms of which foods can carry nutrition or health claims, the EC Regulation includes an article on 'nutrient profiles,' included to restrict the use of nutrition and health

claims to foods with an overall 'favorable' nutrient composition, with "the aim to avoid a situation where nutrition or health claims mask the overall nutritional status of a food product, which could mislead consumers when trying to make healthy choices in the context of a balanced diet."

In theory, 'less healthy' foods can fail one of the nutrient profile criteria and still make a nutrition claim, as long as the failing nutrient is stated clearly, for example, 'low in fat, high in salt.' However, no health claims will be permitted if the food fails any of the criteria.

EFSA has provided a scientific opinion on the setting of nutrition profiles (2008); however, due to the complex and political nature of agreeing thresholds to suit all interested parties, a system has not yet been agreed. Once in place, companies will have 2 years to comply by removing claims or reformulating their products to meet the nutrient thresholds.

In addition to the overarching EC Regulation and associated community and national guidance, some European member states are subject to further local rules. There is an argument that this is creating an inconsistency in local implementation of the regulation and does support a harmonized approach across Europe. For example, in the United Kingdom, companies must also comply with the self-regulatory codes on broadcast and non-broadcast advertising (also covering the Internet). Complaints about misleading claims are investigated and judged by the Advertising Standards Authority, and some consider that its approach is grounded in an overly stringent interpretation of the rules.

At the time of writing, there are a number of 'on-hold' health claims, which relate mostly to botanical ingredients. This is due to an overlap between the EC claims Regulation (foreseen to include botanical food products) and the existing legal framework for traditional herbal medicinal products, under Directive 2004/24/EC. Here a pharmacological effect is considered plausible based on traditional use only, as defined by the Directive. Given that medicinal claims are forbidden in relation to foods, differences in legal requirements between health claims and traditional herbal products could mean that the same substance is treated differently, depending on whether it is present in a food or in a medicine, which possibly leads to consumer confusion. Until such time as a decision is reached, national legislation will continue to apply. It is not currently known when a decision will be reached on these claims, but when they are, it is likely that companies will have 6 months to remove unauthorized claims.

The Consumer Perspective

Research into consumer attitudes to foods with health benefits and understanding of claims is important on a number of levels. In the first instance, testing can demonstrate that a company's claims are well understood by the average consumer and not misleading – a general principle under most jurisdictions. Research can also help predict whether claims are appealing to consumers and will encourage purchases and brand loyalty, which in turn helps to drive innovation. In the longer term, and from a public health perspective, research should help determine whether claims actually result in ongoing and wider good food choices and, ultimately, whether these food choices improve major health outcomes. However, the research into consumer attitudes and preferences is mixed.

The previously strong global growth in 'health and well-being' foods has declined in recent years, largely due to the economic recession influencing consumer food purchases and new regulations imposing stricter control of the use of nutrition and health claims. However, some claim areas are thought to be reinvigorating the market, such as those in relation to energy, mood enhancement, digestion, and heart benefits. Given that the use of nutrition and health claims is primarily a marketing choice and not a prerequisite for the sale of 'healthier' foods, it can be expected that claims will come and go over time, selected by food companies based on reported consumer trends. This variation may add to the challenge of mapping and understanding consumer acceptance of claims, given that food purchases (and consumption) are already governed by a complex set of dynamic interactions in addition to differences due to cultures, food types, and eating occasions.

The global aging population phenomenon has led to the UN predicting that by 2047, the number of older people in the world will, for the first time in history, exceed the number of young people. The associated increasing prevalence of non-communicable diseases means that studies relating to consumer preferences and understanding of nutrition and health claims in older subjects will be particularly useful. This was highlighted by a Canadian study, where the mean age was 70.8 years, which found that subjects were motivated to increase their understanding of foods with health benefits and that labels were the preferred source of information. Subjects reported that more information could be provided to assist them in their understanding of the health benefits, adding that this was likely to mean they would consume more of these products.

Yet an international study in the general population, related to salt reduction, where the mean age was 39.7 years, found that TV advertising, social network sites (e.g., blogs), and newspapers were the preferred means of communication rather than food labels and packaging. Thus, it is important that research captures the differences within and between age groups.

For claims to have any chance at success, attention must first be paid to getting the product fundamentals right, such as taste, price, and the general appeal of the product and packaging. It has also been found that for claims to be well received by consumers, they must already have a positive attitude towards healthy foods in general and, ideally, be familiar with the subject of the claim for it to be perceived as useful.

Claims can be helpful in facilitating choice and educating consumers about lesser known foods or ingredients and their beneficial effects as well as reinforcing the widespread public health messages and big campaigns. However, it has been noted in relation to the EU health claims system that there is some misalignment between authorized claims and some general dietary recommendations. Examples include a lack of claims for the benefits of fruit and vegetables or wholegrains, since the Regulation requires specific nutrients to be clearly defined (or 'characterized') rather than a food group being the subject of the claim. This could ultimately confuse some consumers and place an additional burden on healthcare professionals in order to explain the differences.

In some instances, claims have been authorized but have had low uptake by the industry in response to some research

findings, which could also help explain the previous point. For example, in Europe, 'health by stealth' and incremental reductions through reformulation have been highly successful approaches to reducing population salt intakes. Evidence suggests that nutrition or health claims announcing 'low' or 'reduced salt' may be off-putting to consumers who make assumptions that foods with less salt will not meet their taste expectations. Yet other researchers have reported that for some consumers, the impression of healthiness is linked to positive connotations such as increased 'freshness' and 'quality' and associated foods are therefore expected to taste better too. Further research is needed to build understanding on how certain nutrients or health messages are likely to be received by consumers.

Due to the number of variables and need for large-scale research, the European Community has provided funding under its Seventh Framework Programme and has begun work on 'CLYMBOL,' a project running from 2012 to 2016. The work aims to gain a better understanding of the effects of health claims and symbols on food labels and how this affects purchase and consumption behavior. Interim results in 2013 relating to five EU countries found that there was a low proportion of health claims in the market generally compared with nutrition claims, with only 10% prepacked foods carrying health claims and 20% bearing nutrition claims.

Future insights gained from research such as CLYMBOL will help both food companies and policy makers to understand how to optimize the use health messages as claims and, where appropriate, how best to align with public health messages delivered by healthcare professionals or through public health campaigns.

Other Types of Food Legislation and Nutrition Labeling

Food legislation is designed to protect consumers from foods that could be injurious to their health (laws on food safety) and from misleading information (laws on food labeling, advertising, and fair commercial practices). Food manufacturers must also adhere to any specific legislation concerning their particular category (e.g., 'natural spring waters,' 'novel foods,' and 'dietetic foods') and their marketing choices, such as nutrition and health claims. Compositional criteria may be stipulated (e.g., for foods marketed as 'meal replacements' and milk products) as well as specific advertising rules and mandatory statements (e.g., in relation to infant formulas, allergen labeling, and country of origin labeling). In addition to legislation, there may also be local rules, guidelines, and codes of practice to help interpret and implement the laws in a uniform manner.

Globally, food law is vast in its breadth and depth, and detailed requirements should be considered by experts in the field to ensure completeness and accuracy of application for the local market. In an attempt to promote consistency of all food standards work undertaken by international governmental and nongovernmental bodies, the Codex Alimentarius Commission was set up in 1963 by FAO and WHO to "develop harmonised international food standards, guidelines and codes of practice to protect the health of the consumers and ensure fair practices in the food trade." Its work includes, to name a few, a long and varied list of topics such as 'Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods'; 'Principles for the Risk Analysis of Foods Derived from Modern Biotechnology'; 'Guidelines on Substances used as Processing Aids'; 'Guidelines for Vitamin and Mineral Food Supplements'; 'Guidelines for the Use of Nutrition and Health Claims and Nutrition Labelling'; 'Standard for Fermented Milks'; 'Standard for Fat Spreads and Blended Spreads'; and 'Recommended Methods of Analysis and Sampling.'

Within its 'Guidelines on Nutrition Labelling,' the Codex Alimentarius Commission defines nutrition labeling as "a description intended to inform the consumer of nutritional properties of a food, which has two components: a nutrient declaration and supplementary nutrition information. Nutrient declaration means a standardized statement or listing of the nutrient content of a food and supplementary nutrition information. The content of supplementary nutrition information will vary from one country to another and within any country from one target population group to another according to the educational policy of the country and the needs of the target groups."

Nutrition labeling is most commonly found on the back of prepacked foods but can also be found on the front of pack (repeating some but not all of the details on the back in the form of icons, symbols, numbers, or text); on websites and in leaflets; in menus or on menu boards at eating establishments; and in relation to non-prepacked foods.

In many jurisdictions, the standard practice has been to provide this information in a numerical and tabular format; however, research found that this is not always understood well by consumers. Recent studies suggest that labels would be more effective if they were less numerically complicated and if they provided more interpretive elements about the nature of the product (such as color, symbols, and text) to aid consumer understanding of the information and to motivate healthier choices.

Each year, the European Food Information Council (EUFIC) and its partner food information organizations publish the 'Global Update on Nutrition Labelling,' providing a snapshot in research developments and approaches to nutrition labeling. The latest update (2015) highlights that although many countries now have some form of nutrition labeling in place, there is still disagreement about which format most effectively influences consumer behavior (14 countries have a voluntary scheme and around 50 countries operate mandatory labeling). More research is required to determine which labeling scheme is preferred by consumers and offers the best guidance to influence purchasing decisions and overall diets.

The World Cancer Research Fund (WCRF) has developed the NOURISHING framework, which aims to help identify the effectiveness of nutrition labeling and claims in relation to population health outcomes. This framework has been designed to help policy makers around the world to promote healthy diets and reduce obesity by identifying and monitoring areas for improvement, including tailoring actions to different populations. A key policy area within the framework is 'Nutrition label standards & regulations on the use of claims and implied claims on foods,' which is based on evidence that

nutrition labeling and claims can be a valuable tool to both consumers and food manufacturers. The WCRF publishes an updated list of international 'key action' examples with the aim of sharing knowledge and encouraging the use of clear nutrition information as appropriate around the globe.

Summary

The focus of this article has been limited to nutrition and health claims for food and the regulatory controls governing their use in the commercial context, together with a brief mention of nutrition labeling and other food-related laws. Given the relative newness of the subject, evidence is still building about whether the various systems around the world will achieve their aims to help protect and inform consumers; facilitate choice and encourage innovation, investment, and fair trade. Ongoing research is required in a number of areas including the influence of factors such as age, education, culture, and socioeconomic groups on food choice and consumer perception of claims. Research is also required to determine the relative differences of specific nutrients and their value as nutrition or health claims in different food applications.

For nutrition and health claims to be accepted and relevant to target populations, the research net must be cast far and wide to gain insight into the perceptions of consumers from all walks of life and all countries where claims are permitted. The findings and building a picture of key themes will have relevance to policy makers, health professionals and marketeers alike.

See also: Adolescent Nutrition; Anemia: Prevention and Dietary Strategies; Cancer: Diet in Cancer Prevention; Codex Alimentarius Commission: Role in International Food Standards Setting; Codex Alimentarius; Consumer Protection Legislation; Convenience Food; Dietary References: US; Dietary Surveys: National Food Intake; Elderly: Nutrition Requirements; Food Composition Databases; Food–Herbal Medicine Interface; Functional Foods; Genetically Modified Foods: Risk Assessment, Legislation, Consumer Behavior, and Ethics; Genetically Modified Foods; Hypertension and Diet; Infants: Nutritional Requirements; Low-fat Foods: Types and Manufacture; Low-salt Foods: Types and Manufacture; Nanotechnology in Food Processing; Novel Foods; Nutritional Epidemiology; Obesity Management; Obesity: The Role of Diet; Osteoporosis; Risk Assessment of Foods and Chemicals in Foods; Sports Nutrition; Traditional Foods; Vegetarian Diets; World Health Organization.

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Nutrition and Infection

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Introduction

Nutrition is a fundamental aspect of life, and it is linked to multiple components, systems, and processes, including those occurring during diseases, such as infection. In this context, nutrition has a close relationship with infection. For example, malnutrition is considered to be an important cause of immunodeficiency worldwide, predisposing the malnourished to multiple infections. All forms of immunodeficiency predispose to malnutrition, particularly those opportunistic, related to those altered states of immunity. Malnutrition is a condition that, by itself, increases the host's susceptibility to infectious diseases, and these infections, in turn, have negative repercussions on the metabolism of the host, worsening the nutritional state. In other words, malnutrition alters the host's response to infections, and infections increase malnutrition. This malnutrition–infection complex is associated with significant morbidity and mortality worldwide, particularly in developing countries, where food insecurity and undernutrition primarily affect children. Accordingly, children constitute one of the most vulnerable populations, and infectious diseases cause over half of the total deaths in children under 5 years of age. As stated, the relationship between malnutrition and infection is bidirectional, because infectious diseases can be associated with malnutrition (e.g., giardiasis can compromise intestinal absorption). In regard to parasitoses, many epidemiological studies have found significant connections between helminthic diseases (e.g., ascariasis, trichuriasis, hookworm, and strongyloidiasis, etc.) and protozoan infections (e.g., giardiasis, amebiasis, etc.).

Micronutrient deficiencies and infections are intricately linked, with deficiencies being related to poor growth, impaired intellectual performance, and predisposition to infections (mainly bacterial, viral, and parasitic). In fact, poor nutrition may drive the enormous worldwide impact of parasitic infections. Foodborne infection, in particular, represents a major burden on public health and even the political and economic development of many countries and regions, and this phenomenon should be the subject of ever more focused investigation.

This article describes the relationship between nutrition and infection, with a focus on how malnutrition affects susceptibility to infection and the burden that infections, especially foodborne infections, place on public health.

Food and Infections

Foodborne diseases are considered to be a major public health problem, being an important cause of morbidity and mortality worldwide, with the highest rates in low- and middle-income countries. Foodborne infections are related to unsafe food, and their global burden and economic costs are considered to be a threat to public health and development. According to the U.S. Centers of Disease Control and Prevention (CDC), around 76 million cases of foodborne illnesses occur in each year in the United States, leading to 325 000 hospitalizations and 5000 deaths. The resulting economic burden is estimated to be $10–83 billion USD per year. Australia and New Zealand have estimated costs up to $86 million per year, and across Europe, the annual cost has been estimated to be $71 million. However, there are no comprehensive global assessments of foodborne disease, and, as a result, the real total burden remains unclear, especially in the tropical, developing countries where these infections are most prevalent.

A foodborne disease occurs as the result of the ingestion of contaminated food products, and it includes a huge number of illnesses caused by different pathogens (e.g., bacteria, fungus, viruses, parasite, and even prions). Chemicals in foods can also produce illness, but this type of illness is not infectious in nature, and it will not be considered here. The World Health Organization (WHO), United Nations Food and Agriculture Organization (FAO), and CDC, as well as other international health organization, have identified risk factors crucial to foodborne outbreaks, including the use of unsafe water sources, inadequate cooking processes, contaminated equipment, and poor personal hygiene, especially as it applies to the food handler. Recently, the FAO and WHO investigated the ten foodborne parasites with the greatest global impact, developing a joint report, 'Multicriteria-based ranking for risk management of food-borne parasites,' based on the parasites' burden on human health and other factors, including the geographical ranges of the organisms. According to the report, the top ten parasites are as follows:

1. *Taenia solium* (pork tapeworm): in pork
2. *Echinococcus granulosus* (hydatid worm or dog tapeworm): in fresh produce
3. *Echinococcus multilocularis* (a type of tapeworm): in fresh produce
4. *Toxoplasma gondii* (protozoa): in meat from small ruminants, pork, beef, game meat (red meat and organs)
5. *Cryptosporidium* spp.(protozoa): in fresh produce, fruit juice, milk
6. *Entamoeba histolytica* (protozoa): in fresh produce
7. *Trichinella spiralis* (pork worm): in pork
8. *Opisthorchiidae* (family of flatworms): in freshwater fish
9. *Ascaris* spp. (small intestinal roundworms): in fresh produce
10. *Trypanosoma cruzi* (protozoa): in fruit juices

Although the report looked at the problem of parasites on a global scale, individual parasites can have greater relevance to

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different countries and regions. For instance, *T. cruzi* is commonly encountered in rural Brazil, where *Opisthorchiidae* is unknown, while the latter is prevalent in Southeast Asia, which has no presence of the former.

The different pathogens can be transmitted to food by many ways. *T. cruzi*, which causes Chagas disease, is most commonly associated with household insect vectors (bed bugs), but it can also be orally transmitted. However, hand hygiene plays the most important role as a vehicle for transfering pathogens to food. That is why the food handlers, defined as the persons who manipulate the food in process, are considered to be crucial as vectors of foodborne infections. All of these factors remain of utmost importance, leading to the necessity for strict hygiene practices among food handlers, in order to prevent foodborne illness. Fecal–oral transmission tends to be to most common cause of food contamination, and it involves the fecally contaminated hands of food workers. Bacterial, viral, and parasitic infections have been connected to this way of transmission, including *Salmonella* spp., hepatitis A viris, *Giardia intestinalis*, and *Yersinia* spp., among many others. The only effective strategy for preventing of fecal–oral transmission is strict personal hygiene. Another important point to consider is that the presence of infected skin lesions in the food handler has been closely related with staphylococcal and streptococcal infections. Regarding the different types of food implicated in foodborne diseases, there appears to be a relationship between hand contact time during the preparation of the food and the transmission of pathogens, with sandwiches, salads, and cooked meals being the most important vehicles of transmission.

In addition to hands, nasopharyngeal secretions can cause the transmission of a pathogen into the food supply. Foodborne illness via staphylococcal and streptococcal infections has also been related to nasopharyngeal secretions, as shown by the development of streptococcal pharyngitis and its connection to the presence of the bacteria in the pharynx of the food handler.

Aerosolization, fomites (infectious media adhering to solid objects), and insects are also different ways for pathogens to be transmitted, outside of the contamination of food. Aerosolization transmits the norovirus. Fomites can also spread the norovirus, as well as hepatitis A. Finally, insects, such as flies, cockroaches, and triatomine bugs, are the vectors for assorted bacteria and protozoans.

Incidence and Trends

The Foodborne Diseases Active Surveillance Network (FoodNet) has been describing the incidence and trends of foodborne disease in the United States since 1996, and the 2012 FoodNet report shows that there was a total of 19 531 infections, leading to 4563 hospitalizations and 68 deaths (for a overall case fatality rate of 3.4‰). Another two recent reports from FoodNet show that *Salmonella*, *Campylobacter*, *Shigella*, *Cryptosporidium*, and Shiga toxin *Escherichia coli* (STEC) remain the main causes of laboratory-confirmed foodborne diseases in the United States.

Age is an important factor in foodborne illnes, with most cases in children under 5 years of age. In people older than 65 years, there has also been a high incidence, including hospitalizations and fatalities. Comparing in these populations for the period 2006–08 with the year 2012, infection rates increased for *Campylobacter* (by 14%) and *Vibrio* (by 43%). *Vibrio* infection was also related to a high percentage of mortality in persons over 65 years.

For different infections, the incidence rates, per 100 000 people, were as follows: *Salmonella*: 7800; *Campylobacter*: 6793; *Shigella*: 2138; *Cryptosporidium*: 1234; non-Shiga toxin-producing *E. coli* (nSTEC): 551; STEC: 531; *Vibrio*: 193; *Yersinia*: 155; *Listeria*: 121; and *Cyclospora*: 15. In children under 5 years of age, there was a high incidence of *Cryptosporidium*, and in adults over 65 years, there were more cases of *Listeria* and *Vibrio* infection.

Malnutrition and the Infection Cycle

The complex interaction between infection and malnutrition creates a hostile environment that perpetuates a vicious circle that leads to the two entities benefiting from each other. Some of the phenomenon involved during different parts of the cycle include decreases in the activity of macrophages, diminishment of the inflammatory response, and a reduction in the capacity to create specific antibodies. However, there are two effects that can occur in the presence of both malnutrition and infection. The first one is the synergistic effect, and it happens when an infection worsens the malnutrition or when the malnutrition contributes to decreasing the immune response to infection. The second effect is an antagonist mechanism, which occurs less frequently than the synergistic effect. The antagonistic mechanism happens when malnutrition decreases the multiplication of the agent.

Malnutrition can make a person more susceptible to infection, and infection contributes to malnutrition, which causes a vicious cycle (Figure 1).

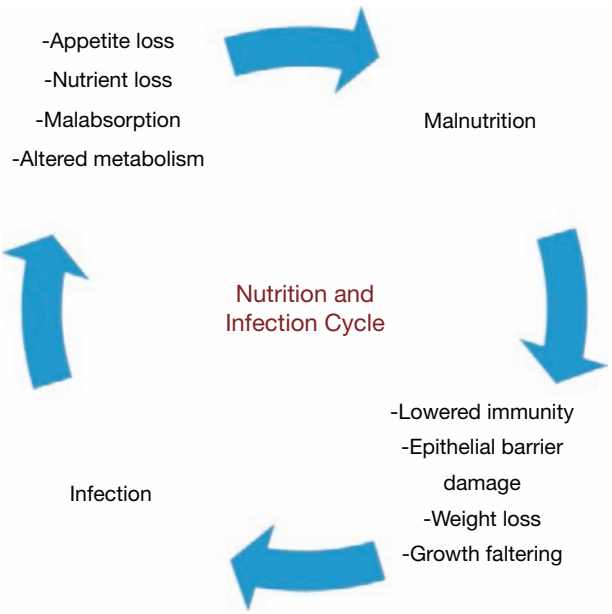

Figure 1 Nutrition and infection cycle.

Worldwide, severe malnutrition is the most prevalent cause of immunodeficiency, affecting as much as 50% of the population in some impoverished communities. Severe protein energy malnutrition (PEM) in children is clinically defined as a weight-to-height ratio below 70% and/or the appearance of pitting edema on both feet, and it is categorized as either marasmus, a chronic wasting condition, or kwashiorkor, which is characterized by edema and anemia. PEM causes susceptibility to the infections described in the following section.

During childhood, severe malnutrition affects thymic development, which, in turn, compromises immunity in children through a long-term reduction of peripheral lymphocyte counts, causing susceptibility to infections and nutritionally acquired immunodeficiency syndrome. Thymus atrophy has been observed in children with malnutrition, and the patent atrophic condition appears to result from a decrease in T-cell proliferation and increased depletion by apoptosis, affecting mainly immature T CD4+ and T CD8+ cells. This has been, at least partially, attributed to lower leptin levels during starvation or malnutrition.

Malnutrition clearly affects hematopoiesis, determining anemia, leucopenia, and severe reduction in bone marrow. The production of IL-6 and TNF-α by bone marrow cells is also significantly lower in malnourished animals. The capacity of malnourished hematopoietic stroma to support the growth of hematopoietic stem cells (CD34+) in vitro is also decreased. This finding relates to disease formation, because CD34+ cells are able to generate multiple lymphohematopoietic lineages, including myeloid, erythroid, and lymphoid (B and T) types.

In malnourished children, both acquired and innate immunity is affected, making them more susceptible to opportunistic pathogens such as *Pneumocystis jirovecii*. In addition, PEM impairs the linear growth of children; it has been suggested that acute-phase response and proinflammatory cytokines directly affect the bone remodeling required for longitudinal growth.

Specific Immunological Alterations in Undernutrition

A series of specific immune responses and their alteration during undernutrition have been described and characterized.

Cell-mediated immune response alterations

As previously mentioned, severe malnutrition in newborns and small children causes atrophy of the thymus, with reduced cell numbers and, subsequently, ill-developed peripheral lymphoid organs (e.g., lymph nodes and spleen). This leads to long-lasting immune defects characterized by leucopenia, depressed serum complement activity, decreased CD4 to CD8 ratio, and increased numbers of CD4/CD8 double-negative T cells, contributing to the appearance of immature T cells in the periphery. The biological function of different cell types (macrophages and Kupffer cells) is clearly decreased during nutritional deficiencies. There is also impaired activation of T cells, which has been clearly associated with deficits of cytokine production, which are the main molecular mediators of immunity. This was evident from a study of malnourished children who showed reduced production of type 1 cytokines (IL-2 and IFN-γ).

Epithelial barrier alterations

Immune defense at the epithelial barrier of the undernourished host is compromised because of the altered architecture of the gut mucosa, with structural effects including the flattening of the hypotrophic microvilli, reductions in lymphocyte counts in Peyer's patches, and reductions in immunoglobulin A (IgA) secretion, all of which are characteristics of environmental enteric dysfunction.

Complement alterations

The availability of complement components and phagocyte function are compromised during malnutrition, directly affecting pathogen elimination. This happens because the complement system by itself can destroy bacteria or viruses or because complement receptors present on the phagocyte surface mediate the capture of pathogens. Significantly lower levels of complement, especially C3, which is the main opsonic component, were described by Sakamoto and coworkers. In addition, the ability of phagocytes to ingest and kill pathogens was also reduced. The restriction of complement components affects the capacity of professional phagocytes to engulf and eliminate pathogens.

Antibody alterations

In regard to antibody production, malnourished children are deficient in IgE antibodies that protect against *Ascaris lumbricoides*, even though they have high concentrations of total IgE; the IgE antibodies that these children do have are neither worm-specific nor protective, and their memory T cells do not recognize helminthes antigens.

Malnutrition Increases Risk of Infection

Malnourished children suffer more frequently from respiratory infections, infectious diarrhea, measles, and malaria, with each condition characterized by a protracted course and exacerbated disease. Tuberculosis is an infection that is particularly influenced by undernutrition, and it is a major cause of morbidity and mortality in developing countries. Approximately one-third of the world's population is infected with *Mycobacterium tuberculosis*, generally in a subclinical form. Similarly, undernutrition may also affect the clinical outcome of tuberculosis. A recent meta-analysis suggested that low serum vitamin D levels are associated with higher risk of tuberculosis activation. It has also been suggested that malnutrition may contribute to the appearance of *M. tuberculosis* strains that are multiply resistant to antituberculosis medications.

Another disease that is associated with malnutrition is measles; even though effective vaccines for measles exist, it continues to cause severe disease and death in children worldwide. Among other factors, malnutrition and vitamin A deficiency increase complication rates. There is experimental evidence that vitamin A supplementation in children is associated with an average reduction of 23% in mortality risk, as well as an attenuation in disease severity. For this reason, the WHO recommends the administration of two oral doses of 200 000 IU of vitamin A to children with measles who live in vitamin A deficiency areas.

Malnutrition and intestinal parasitism share a similar geographic distributions, with the same individuals experiencing

both diseases simultaneously. The coexistence of undernutrition and nematode infection involves two causal pathways: (1) malnutrition augments susceptibility to infection, and (2) the infection itself leads to a more accentuated undernutrition. Parasites that clearly affect the nutritional status are soil-transmitted helminths, *G. intestinalis*, *E. histolytica*, coccidian protozoans, and *Schistosoma* spp.

Noma is an opportunistic infection promoted by extreme poverty, which evolves rapidly from a gingival inflammation to mutilating orofacial gangrene. Even though it can be observed worldwide, noma is much more common in sub-Saharan Africa. It results from very complex interactions between malnutrition, infection, and compromised immunity, and it is very frequently preceded by malaria, measles, and severe necrotizing ulcerative gingivitis.

As mentioned previously, undernourished hosts are more susceptible to infections and to developing a severe course of infection due to impaired immune function. However, current works suggest that nutritional status can also increase the pathogenicity of the infecting agent or even change an avirulent virus into a virulent one. Animal models, particularly mice models, have provided the foundation of this theory. In a mouse model with selenium and vitamin E deficiency, a benign strain of Coxsackie virus B3 became virulent and caused myocarditis, as the result of a mutation in the viral genome caused by a decrease in dietary nutritional antioxidants; in other words, nutritional deficiencies changed an avirulent virus into a virulent one. Additionally, deficiencies of lycopene, vitamin E, and the B group vitamins have been serologically related to both CVB4 and CVA9, which were responsible for an epidemic of optic and peripheral neuropathy in Cuba in the early 1990s. These studies suggest that host nutritional status has influence beyond the host itself, altering the genome of viruses present in the host community.

Micronutrients in the Prevention of Infections

Ensuring adequate micronutrient status is important for the prevention of certain infections. Several micronutrients, such as vitamin A, beta-carotene, folic acid, vitamin B12, vitamin C, riboflavin, iron, zinc, and selenium, have immunomodulating functions and thus influence susceptibility to infections, as well as the course and outcome of the illness. The effects of micronutrient deficiency have been evaluated by several experimental models (Table 1).

Vitamin A has been termed the 'anti-infective nutrient,' although there is little evidence that it prevents the incidence of new infections. The one area in which the administration of this vitamin has actually been effective is in the prevention of neonatal infections. It has also been demonstrated that children with infectious diseases such as pneumonia and rheumatic fever tend to have low levels of vitamin A. In addition, adequate vitamin A stores are related to faster growth and better appetite.

Similarly, although iron supports components of the host-defense system, such as phagocytosis, there is no evidence that iron itself prevents infections. In fact, rich stores of iron and the high-dose administration of the nutrient have been associated with increased susceptibility to or morbidity from certain intracellular pathogens such as the plasmodium of malaria and the protozoa *E. histolytica*, among others.

By contrast, zinc is emerging as a nutrient that can reduce the risk of infections and reinfection through supplementation. Recent trials of zinc supplementation have shown substantial benefits to children at risk of deficiency. Zinc supplementation also appears to reduce the duration and severity of acute diarrhea in India, as well as to improve outcomes in subsets of patients with persistent diarrhea in Peru. Zinc supplementation may not be safe and advantageous for everyone, however. In children with severe PEM, a three-armed trial in Bangladesh comparing a placebo to two doses of zinc supplementation found a higher mortality rate among those receiving a very high zinc dose. Finally, vitamin D has recently emerged as a nutrient of interest with promise for prophylactic application in epidemic viral diseases such as influenza and diverse microbial infections.

Obesity and Infection

Obesity is an increasing problem in public health, clearly associated with a high risk of multiple pathological conditions,

Table 1 Effects of malnutrition on the immune system in some experimental models

Experimental model	*Diet restriction*	*Effect*
Human reconstituted SCID mice	Total vitamin A restriction; 7-day gestation period	Impaired antibody production after tetanus toxoid immunization
SD rats	Total Zn restriction; 34 weeks	Thymus atrophy, oligospermia, testicular atrophy, and loss of sperm cells and spermatocytes
BALB/c mice	Total vitamin A restriction; 2–5 weeks	Increased Th2 and T regulatory cells, decreased Th1
C57BL/6 mice	Total dietary restriction (70%); 52 weeks	Decreased humoral response to hepatitis B virus

Source: Molrine, D. C., Polk, D. B., Ciamarra, A., Phillips, N. and Ambrosino, D. M. (1995). Impaired human responses to tetanus toxoid in vitamin A-deficient SCID mice reconstituted with human peripheral blood lymphocytes. *Infection and Immunity* **63**(8), 2867–2872; Anstead, G. M., Chandrasekar, B., Zhao, W., Yang, J., Perez, L. E. and Melby, P. C. (2001). Malnutrition alters the innate immune response and increases early visceralization following Leishmania donovani infection. *Infection and Immunity* **69**(8), 4709–4718; Rivadeneira, D. E., Grobmyer, S. R., Naama, H. A., Mackrell, P. J., Mestre, J. R., Stapleton, P. P., et al. (2001). Malnutrition-induced macrophage apoptosis. *Surgery* **129**, 617–625; Nodera, M., Yanagisawa, H. and Wada, O. (2001). Increased apoptosis in a variety of tissues of zinc-deficient rats. *Life Sciences* **69**, 1639–1649; Anstead, G. M., Chandrasekar, B., Zhang, Q. and Melby, P. C. (2003). Multinutrient undernutrition dysregulates the resident macrophage proinflammatory cytokine network, nuclear factor-kappa-B activation, and nitric oxide production. *Journal of Leukocyte Biology* **74**(6), 982–991; Sakai, T., Mitsuya, K., Kogiso, M., Ono, K., Komatsu, T. and Yamamoto, S. (2006). Protein deficiency impairs DNA vaccine-induced antigen-specific T cell but not B cell response in C57BL/6 mice. *Journal of Nutritional Science and Vitaminology* **52**(5), 376–382.

including cardiovascular and metabolic diseases. Likewise, obesity affects different organs and systems, generating an environment susceptible to an increased risk of infection, and there is evidence of immune system impairment, indicated by alteration in cell differentiation, cytokine production, and thus chemotaxis during the immune response. In terms of cardiorespiratory function, overweight states produce mechanical ventilation disorders, causing pulmonary restriction and affecting the immune response at this level; this is analogous to infections at the skin level, because of altered tissue perfusion.

There has been developing research interest on the relationship between obesity and infection, with a focus on the bidirectional interaction between the two: obesity has been linked to increased risk of infection with different pathogens, and some pathogens have been associated with an increased risk for developing obesity. For example, *Taenia* infection has been associated with decreased levels of leptin, a hormone associated with signaling satiety after food intake. Also, an association has been found between seropositivity for *T. gondii* and obesity, although the causal mechanism of this interaction has not been identified. As a matter of speculation, it may be mediated by complex mechanisms through which *T. gondii* influences different neurological centers involved in motivation and survival.

Finally, it has been shown that obesity is an independent risk factor for the development of *Clostridium difficile* infection, but the mechanisms underlying this relationship are still unknown. Given this evidence, future research should be directed toward clarifying the mechanisms involved in the relationship between obesity and the risk of infection, as well as the clinical significance of this interaction, in order to define the relevance and potential benefit of early therapeutic interventions for patients predisposed to acquiring potentially dangerous pathogens.

From Infection to Malnutrition

Multiple mechanisms have been associated with nutritional impairment during infection, including factors related to the decreased intake of nutrients and increased nutrient loss. Although initially representing a host-defense strategy, some of these mechanisms can ultimately have a negative impact on nutritional status.

Many infectious diseases that compromise the nutritional status directly affect the gastrointestinal tract. Hyporexia, poor appetite, or frank anorexia is frequent when the food intake is associated with vomiting or diarrhea. The symptomatic manifestations can evolve from a variety of associated mechanisms, however; these include the decreased intake of solids due to xerostomia (dry mouth) secondary to diarrheal dehydration; changes in the absorption surface of the gastrointestinal lumen, with destruction of the intestinal villi, induced by bacterial toxins; and nutrient loss through hypersecretion across the mucosa. These mechanisms directly affect the absorption or retention capacity of a nutrient. Not only the nutrients acquired through diet are lost, though. Endogenous nutrients that are generated during the normal cell turnover of the gastrointestinal epithelium can also be lost, when, under normal conditions, they would be largely reabsorbed.

Similarly, respiratory diseases can affect undernutrition. Delay in growth has been documented in children with recurrent lower respiratory infections, as compared to unaffected children. Although the mechanisms behind this relationship have not been clearly elucidated, it has been suggested that fever and hyporexia are the main associated factors.

There are also chronic infectious diseases whose characteristics of chronicity lead to a state of cachexia, as occurs with tuberculosis and HIV infection. In fact, in children with HIV, alterations in nutritional status can often represent the first clinical sign of the condition, and wasting syndrome is considered to be a feature of AIDS. In HIV patients with more severely disrupted nutritional status, the CD4 lymphocyte count is general substantially reduced, emphasizing the important role of immunological alterations in the pathogenesis of malnutrition.

Conclusions

The activation and maintenance of the immune response requires adequate energy consumption. PEM is a critical – but underestimated – factor contributing to susceptibility to infection, causing impaired immune function, as well as alterations in the normal development of children. Once the infection is established, it, in turn, contributes to further malnutrition, therefore perpetuating a vicious cycle. An adequate micronutrient status is important for the prevention of certain infections; therefore, in impoverished countries where malnutrition represents a great burden, mainly in children, strategies must be implemented in order to reduce the morbidity and mortality caused by malnutrition and, more particularly, chronic undernourishment.

See also: Food and Agriculture Organization of the United Nations; Food Poisoning: Epidemiology; Foodborne Pathogens; HIV Disease and Nutrition; Malnutrition: Concept, Classification and Magnitude; Malnutrition: Prevention and Management; Nutritional Epidemiology; Parasites in Food: Occurrence and Detection.

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Nutritional Epidemiology

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Nutritional Epidemiology

Nutritional epidemiology is the application of epidemiological methods to the study of how diet is related to health and disease in humans at the population level. Nutritional exposures have been studied in relation to a wide variety of health outcomes, such as infectious diseases, chronic diseases, cancer, and congenital malformations. Epidemiologists investigate how nutrition affects the occurrence of disease by collecting data on and comparing large groups of people. Statistical methods are employed to estimate the extent to which a factor influences risk of disease in a population. This estimate is often expressed as a measure of association. Epidemiological methods are also used to estimate the nutritional status of populations.

In epidemiological research, diet can be studied at different levels comprising intake of nutrients, foods, food groups, and/or patterns. These exposures can be measured by directly ascertaining what people eat (e.g., through the administration of questionnaires), by measuring markers of intake in biological specimens, or by estimating body size and the relative size of body compartments.

The exposure measure of interest in nutritional epidemiology is usually long-term diet, since the effects of intake on most health outcomes, especially those related to noncommunicable diseases, are likely to occur over extended periods.

Findings from epidemiological studies can contribute to the development of policies on diet and health, ranging from governmental dietary guidelines to food fortification and banning of substances from food. Randomized controlled trials provide the strongest evidence of a causal relationship between an exposure and health outcome. However, randomized trials of dietary interventions have several limitations and are often unfeasible for ethical or practical reasons. Thus, nutritional policy decisions are often guided by findings from a combination of sources, including observational epidemiological studies in which participants have been followed prospectively.

Methods of Measuring Diet

A major challenge faced in nutritional epidemiology is the limited availability of methods to accurately measure diet at the population level. Studying diet as a cause of disease can be difficult for several reasons. First, diet is not a single exposure, but instead comprises a group of exposures (e.g., nutrients, foods, or food groups) that are often highly correlated. Second, in many cases, nearly everyone in a population is exposed to the same dietary factors; thus, dietary exposures are continuous variables often with a limited range of variation. Third, people generally do not make sudden changes to their diet at easily identifiable time points. Rather, eating patterns change gradually over several years. Finally, recalling what one eats on a given day can be difficult, and most individuals do not know the exact contents of the food they eat. Despite these challenges, several methods are currently available to assess diet at the population level.

Report of Food Intake

Diet Record

Diet records are used to assess short-term intake. Individuals prospectively record their food and beverage intake over a period of 3–7 days, providing detailed descriptions of the foods, preparation methods, and quantities consumed. Quantities are assessed by actual weighing of food and volumetric measurements or estimated with the use of photographs. Diet records provide quantitatively accurate information and often serve as the 'gold standard' to validate other dietary assessment methods. Individuals do not have to rely on memory to record their diet and, therefore, are less likely to omit foods. Diet records require significant training of participants, who need to be literate and highly motivated. The burdensome nature of diet recording can lead to participant fatigue, reduced cooperation over time, and alteration of typical eating habits to simplify recording. Diet records are costly and consequently are most commonly used in clinical or research settings to monitor compliance to dietary interventions or to validate other instruments.

Twenty-Four-Hour Recall

Twenty-four-hour recalls are also used to assess short-term intake. The method consists of asking study participants to recall and report their food and beverage intake in the preceding 24 h. Interviewers use standard questions to obtain detailed information from participants through an interview conducted in person or by telephone. This method is easily implemented through a quick, inexpensive interview and often has a high response rate. Participants are not required to have high literacy to complete 24 h recalls. Although long-term memory is not needed, accurate recall of the previous day's intake and quantities of food may still be difficult. Detailed, sensitive probing by well-trained interviewers in a relaxed, unhurried setting can reduce erroneous recall of foods. Interviewers may relate a participant's activities to food consumption and refer to a list of commonly forgotten foods to support recall. Food models and photographs can be used to aid recall of portion sizes. Subjects may also be trained prior to the interview in estimating portion sizes and providing adequate descriptive detail to improve accuracy in recall. Twenty-four-hour recalls can provide detailed information without altering eating behaviors; however, the dietary information supplied by this method does not represent an individual's usual diet and does not account for day-to-day variation. This method is often used to describe the average diet of a group or population.

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A 24 h recall allows researchers to assess mean absolute nutrient intake and compare mean intake over time.

Food Frequency Questionnaire

Food frequency questionnaires (FFQs) are used to measure long-term dietary intake. In general, one's usual average intake over an extended period is easier to recall than specific intake over a short time. Thus, dietary information supplied by an FFQ over a long period, although crude, is often more helpful than precise information obtained over a short period. FFQs do not quantify absolute intake, but instead rank individuals within a population with respect to intake. FFQs are composed of a food list accompanied by a question, typically close-ended, on the frequency of consumption for each item, usually over the previous year. Several factors are involved in selecting foods to include in an FFQ. They should be eaten reasonably often in a population, but their consumption must still vary between subjects. Included foods should have substantial nutrient content and not be highly correlated with another food on the list. Frequency categories must be mutually exclusive and have adequately narrow time intervals to capture variability of consumption between subjects. To allow estimation of absolute intake, portion sizes may be included by providing specific size options for each food, from which the participant can choose. However, portion sizes in FFQs are often poorly estimated. Moreover, portion size is correlated with frequency since the variability of intake in a population is largely driven by the frequency of intake rather than absolute amount consumed. Thus, including portion size does not appreciably contribute to ranking a population's intake, and it is often omitted from FFQs. In this case, an average or typical serving size, as provided in food composition tables, is used to compute nutrient intakes. FFQs can be self-administered or completed in an interview. They are inexpensive and relatively easy for participants to complete. FFQs may misclassify individuals with unusual diets, and they must be adapted for different populations. Used and validated in many settings, FFQs are region-specific and may require nutrient databases similarly specific to a region.

The reliability, or reproducibility, of an FFQ may be evaluated by computing the correlation of responses from repeated administrations of the questionnaire to the same subjects in a population. The validity, or accuracy, of an FFQ can be determined by comparing the instrument to another method considered superior to the FFQ, such as the diet record.

In all of these methods, food composition tables are used to compute nutrient intake from the reported consumed foods. Food composition tables and nutrient databases have been constructed for diets in several countries. A key assumption in using these tables is that the nutrient content of a food is constant. Databases can differ in the estimation of the nutrient content of the same food, which can be a source of systematic error in epidemiological studies.

Biomarkers

A biochemical indicator, or biomarker, is the concentration of a nutrient or a substance affected by intake of a nutrient or food in blood or tissue. Biomarkers are used in epidemiological studies as a measure of nutrient intake. Biomarkers may be used to complement the methods based on reported food intake described earlier. Studies that rely on participants to remember what they consumed may be subject to bias; for example, participants who have the disease under study may unintentionally recall food intake differently than participants who did not have the disease. Biomarkers, therefore, can be useful surrogates of intake in studies in which recall bias and participant compliance in reporting food consumption may be concerns. Biomarkers are also used as a measure nutrient intake when food composition tables are inaccurate or unavailable.

Epidemiologists also use biomarkers to measure nutrient status. Nutrient status is determined by nutrient intake in addition to many other factors. Nutrient concentrations are influenced by physiological processes including absorption, metabolism, transport, and excretion. Genetic and lifestyle factors can further modify these processes. Some nutrients, such as vitamin D, are both consumed and synthesized endogenously. Biomarkers for these nutrients, therefore, may better reflect the dose to which tissues are exposed than dietary intake alone.

To be an indicator of nutrient intake, a biomarker concentration should correlate well with nutrient intake. A biomarker of nutrient intake should not be regulated so tightly by homeostatic mechanisms that its concentration is constant over the entire range of normal consumption and cannot be used to discriminate between different levels of intake. Given long-term nutrient exposures are generally most relevant in epidemiological studies, an ideal biomarker concentration reflects dietary intake over time and is insensitive to short-term fluctuations in intake. Time integration is partially a function of the tissue specimen from which the biomarker is sampled. For example, fatty acids are stored in adipose tissue, which has a long half-life compared to plasma. The concentrations of many fatty acids measured in adipose tissue correlate higher with dietary intake than samples from other compartments.

Biomarkers are also employed in epidemiological studies as gold standards to validate against other methods of dietary assessment. The biomarker concentration must reflect intake for this use. To have an accurate estimation of validity, the measurement error of the gold standard must be uncorrelated with the measurement error of the test under evaluation (e.g., report of intake). Biomarkers are particularly useful in validation studies because errors in the measurement of a biomarker, such as technical measurement errors or random physiological variation, are uncorrelated with the typical errors that occur with other dietary assessment methods, including recall of diet.

Anthropometry

Anthropometric measurements, due to the ease of their assessment in the field, are frequently used as a measure of nutritional status in epidemiological studies. As measures of dietary intake, anthropometric measurements generally represent energy balance (e.g., body mass index and size of body compartments) or diet and environmental influences during childhood (e.g., height).

Self-reported height and weight are useful proxy measures in large epidemiological studies in which it is not possible to

obtain measurements on all study participants. A tendency for underestimation of weight and overestimation of height by participants can introduce systematic bias into studies using self-reported data. The prevalence of obesity may be underestimated as a result of this bias. Nonetheless, high correlations between measured and reported weight, including recall of weight several years earlier, have been observed in validity studies.

Sources of Variation in Diet

Long-term dietary exposures, as opposed to short-term intake, are usually of primary interest to epidemiologists studying a particular disease outcome. Although diet can be extremely variable from day-to-day, one important assumption in nutritional epidemiology is that this day-to-day variation is superimposed on an underlying, consistent pattern in intake over time. Due to the variability in intake from one day to the next, measuring an individual's diet for a single day provides a poor assessment of one's long-term intake. A person's true long-term nutrient intake is better estimated by the mean intake for multiple days. Therefore, the population distribution of intake is better represented when several days are measured.

The extent to which nutrient intake varies from day-to-day depends on the nutrient. Total energy intake is physiologically tightly regulated and, therefore, has a low day-to-day variation. Since macronutrients contribute substantially to total energy intake, macronutrient intake also has low day-to-day variation. By contrast, there is greater variability in micronutrient intake. Micronutrients are present in specific foods, of which consumption can vary widely on a day-to-day basis.

Variation in dietary intake can be large in developing countries where food intake is often directly related to income. Socioeconomic inequities among people in low- and middle-income countries result in large between-person variation in dietary intake. Day-to-day variation can be large if people are able to afford particular foods sporadically. Furthermore, the effects of seasonal variation on availability of foods can be greater in developing countries compared to industrialized countries.

Most studies indicate that the majority of day-to-day variability in intake occurs at random; that is, there are not clearly identifiable sources to explain it. This random 'within-person variation' accounts for most of the variability in intake at the population level. The second component consists of true differences in intake between persons in the population, and it is the variability component of interest in epidemiological research. When measures of intake rely on assessing only one day per person, within-person day-to-day variation results in an overestimation of extreme intakes. This affects estimations of the proportion of people in the population with intake above or below a given nutrient value and tends to attenuate measures of association between nutrient intake and health outcomes.

The effects of random within-person day-to-day variation on estimates of prevalence or association can be ameliorated in a number of ways. First, in nutrition surveys aimed at determining the proportion of the population with absolute intake above or below a given nutrient value, collecting more than one day of intake in a subgroup allows one to identify the contribution of within-person variation to the total variance and to adjust the prevalence estimates. Available methods accommodate for uneven number of measures per person and deviations of the nutrient distributions from normality. Second, in epidemiological studies in which short-term intake measures are used as the exposure or the outcome, replicate measures within-person can be averaged, and this average can be used to represent each participant's data point for analysis. Finally, the assessment of long-term diet with the use of the FFQ tends to average out within-person variation and should be the method of choice in studies of diet and disease.

Total Energy Intake

At a population level, the primary determinants of variability in energy, or caloric, intake between people include body size, physical activity, metabolic efficiency, and weight change. The relative contributions of these factors to energy intake depend on the population under study.

Total energy intake is important in nutritional epidemiology for several reasons. First, individual differences in energy intake may lead to extraneous between-person variation in intake of other nutrients because the consumption of all nutrients is correlated to some extent with total energy intake. This correlation occurs because larger, more active people tend to eat more than smaller, less active people. Second, energy intake may be an independent primary cause of disease, and it may be relevant to measure its effect. Third, when caloric intake is an independent predictor of disease, the effects of specific nutrients may be confounded, or distorted, by total energy intake.

Adjustment for total energy intake should be conducted in most epidemiological studies assessing diet composition. Adjusted analyses allow us to remove the extraneous variation described earlier from estimates of association, as well as account for potential confounding by total energy intake. Some nutrients, such as macronutrients, are metabolized or consumed in proportion to total caloric intake; therefore, interpreting associations with these nutrients relative to energy intake is appropriate.

There are several analytic methods to adjust for energy intake. Some of the most widely used include the nutrient-residual method and the multivariable density model. In the nutrient-residual method, residuals are computed from a regression model in which nutrient intake is regressed on total caloric intake. Since the residuals do not have a reasonable interpretation on their own, they are often scaled by adding a constant value, such as the expected nutrient intake at the mean caloric intake. The nutrient residual, a measure of nutrient intake uncorrelated with total caloric intake, is then used as an independent variable in a regression model predicting a disease outcome. Models including the nutrient residual are isocaloric or adjusted for total caloric intake. However, if energy intake is associated with the disease, the model should include total caloric intake as a predictor in addition to the nutrient residual. This inclusion decreases random error of the residual and allows estimation of the independent effect of total energy intake.

The multivariable density model also controls for total energy intake. In this model, a disease outcome is regressed on two predictor variables, a nutrient density and total caloric intake. A nutrient density is equal to the nutrient intake value divided by total caloric intake. An advantage of the multivariable density model is that it allows public health recommendations to be provided relative to total energy intake. Long-term total caloric intake on the average is relatively constant. Consequently, people are more effectively able to reduce intake of a given macronutrient, such as saturated fat, by substituting calories they consumed from foods containing that macronutrient with other foods rather than reducing their total food intake.

In very few instances, absolute intake of a nutrient, regardless of total energy intake, may be of primary interest. This is the case of determining the specific requirements for an organ or biological function, such as iron requirements to prevent or treat anemia.

Measurement Error

All epidemiological data are subject to errors. Thus, any measurement of a dietary exposure, whether it is from a questionnaire on dietary intake, biochemical assay, or assessment of body composition, may contain some form of error. The implications of an error in the measurement of an exposure on measures of association with disease depend on the type of error. Error can be random or systematic, and in epidemiology, both kinds of error can occur within individuals or between people.

The day-to-day variation in a person's dietary intake is an example of random within-person error. Additionally, random within-person error arises from errors in measuring dietary intake on a given day. Systematic within-person error occurs when a person consistently over- or underreports a food item on repeated questionnaires. Systematic within-person error may also happen if a food item frequently consumed by a study participant is not included in a questionnaire.

Systematic within-person error can be distributed randomly in a population. For example, random between-person error occurs when consistent underreporting of a food item by some people is canceled out by overreporting of that item by others. As a result, the observed mean for the study group is the true mean, but the standard deviation of the distribution is larger than the true value.

Systematic between-person error occurs when systematic within-person error is not distributed randomly among study participants. When an item frequently consumed in a population is not included in a questionnaire, the item is underreported for those who consume the food. This omission affects everyone systematically in the same direction but not necessarily equally since it is likely that some people consume more of the food than others.

Random error, whether within- or between-person, usually attenuates correlations and biases measures of association toward the null value. When systematic errors affect all subjects equally, measures of association are not affected; however, this is probably rare in practice.

Measures of association, including correlation coefficients, regression coefficients, and relative risks, can be corrected for measurement error when an exposure has been assessed with an imperfect method, such as an FFQ, and additional information is available from reliability or validation studies. Correction for random within-person error requires a single replicate measure. One can correct for systematic within-person error by conducting a calibration study using a gold standard method, such as a diet record.

Systematic errors can also occur between groups being compared in an epidemiological study, such as participants with and without disease. These errors are usually the result of biases, either in selecting study participants or in measuring exposures. Systematic between-group error should be avoided through careful study design since it is difficult to correct for it analytically.

See also: Dietary Exposure Assessment; Energy: Intake and Energy Requirements; Food Composition Databases.

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Nuts: Brazil Nuts

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Sources and Production

Brazil nut (*Bertholletia excelsa* HBK), a plant in the family of *Lecythidaceae*, is native to the Amazon rain forest, is exploited commercially, and is geographically distributed in adjacent areas in Brazil, Bolivia, and Peru. It is an extractive product, collected directly from the native forest, with high ecological, social, economic, and nutritional values. Brazil nut is considered a good nutritional source for functional food and an alternative source of protein and energy for some consumer groups. It is a significant product of the Amazon rain forest and in production chains of various foods, with worldwide production of 78 000 tons, with Brazil accounted for ~40% of this value in 2008. The Brazil nut production chain starts in the forest communities, sometimes indigenous, with the harvesting of its fruit, known as 'pods,' which falls freely from the trees to the ground during the rainy season and from which the seeds are removed and transported for industrial treatment in processing plants. In the processing plants, there are several different steps to remove the visually stained or moldy nuts and to reduce moisture content. In-shell or shelled Brazil nuts (Figure 1) are then packaged for sale. A brown skin rich in protein, lipids, and selenium is obtained as a waste product resulting from processing. It can be used in the dietary supplement industry due to its antioxidant potential and also in the area of biotechnology to obtain enzymes with industrial potential. The kernels that do not meet the size standards (small, medium, and large) or have undesirable sensory characteristics (color, shape, or stains) according to industry standards can be otherwise used in pieces and/or as ingredients for the production of other products, such as cereal bars and cookies. The Brazilian production has two different purposes in terms of trade: domestic consumption (70–75%) and export (25–30%). Other importing countries of Brazil nut are Bolivia, the United States, Australia, Hong Kong, and some European countries. Other products can be obtained from the industrial processing of whole nuts or pieces of nuts, for example, oil and cake (partially or fully defatted), which are obtained by mechanical press and/or by extracting the fatty material using solvents or CO_2. The Brazil nut flour is another product that showed high energy value (431.48 kcal), protein content of 45.92 g%, and fiber of 17.14%. Brazil nut oil can be used in cosmetics, foods, and pharmaceuticals. The brown residue of the oil extraction by pressing the nut, generally called cake, has aroused great interest among researchers given its high protein content. An aqueous extract, popularly known as 'milk,' is obtained by dehulling previously dehydrated fresh nuts, which are then shredded producing thick white 'milk' that is subsequently diluted in water. This product is similar to coconut milk; it is rich in protein and is used as an ingredient in foods. In addition to the aqueous extract, a dry extract, a high-protein product, can be obtained by freeze-drying; it is an alternative product to cow's milk and soy milk. Therefore, Brazil nut products are an important alternative for specific groups of consumers, such as those who have high energy and protein requirements or lactose intolerant individuals.

Patterns of Consumption

Adequate intake of selenium (Se) is essential for normal activity of numerous enzymes involved in the protection against oxidative stress, immune system, and regulation of the thyroid. It was demonstrated that 100 μg day^{-1} of selenium (equivalent to two units of Brazil nuts) for 3 months was effective in increasing plasma selenium concentrations and glutathione peroxidase in healthy individuals. According to previous work, the ingestion of a single serving of Brazil nut (5 g) a day for 3 months seems to be effective in increasing the concentration of selenium and glutathione peroxidase activity in patients with selenium deficiency improving the antioxidant capacity of this mineral. Therefore, according to many scientific studies, the daily consumption of Brazil nut has beneficial effects on human body metabolism. In addition to Se, other minerals such as barium (Ba) and radium (Ra) can also be found in Brazil nuts; it is noteworthy that the radioactive dose–effect relationship associated with their consumption does not pose risks to consumers.

Availability, Absorption, and Metabolism

Table 1 shows the nutritional composition of Brazil nut according to several authors. Among the bioavailable nutrients are the vegetable proteins with high digestibility and sulfur amino acids, methionine and cysteine (Table 2). The high content of lipids (over 60%) can also be observed, and the ratio of saturated, monounsaturated, and polyunsaturated fatty acids is 25:41:34. This indicates that the content of unsaturated fat in the Brazil nut is higher than that of any other nut, and due to its high lipid content, its intake should be moderate. Brazil nut is a great source of vitamin E, bioactive compounds, and other micronutrients, such as phytosterols, tocopherols, squalene, and phenolic compounds. Beta- and gamma-tocopherols are the most abundant tocopherol isomers present in Brazil nuts. Brazil nut also contains numerous minerals such as calcium, magnesium, phosphorus, and potassium. With regard to its influence on human metabolism, the consumption of Brazil nut daily improves both the selenium status and the lipid profile, especially high-density lipoprotein and cholesterol levels, thus reducing cardiovascular risks in obese people.

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Figure 1 The Brazil nut.

Table 1 Composition of Brazil nuts

Components	*Results (g%)*
Moisture content	3.10
Lipid	64.94
Protein	14.11
Carbohydrate	6.27
Total dietary fiber	8.02

Values refer to the literature data average.

Table 2 Amino acids in Brazil nut total protein content

Amino acids	*%*
Aspartic acid	8.4
Glutamic acid	17.7
Serine	5.7
Glycine	8.8
Histidine	1.8
Arginine	11.8
Threonine	4.3
Alanine	6.2
Proline	5.0
Tyrosine	2.4
Valine	6.3
Methionine	2.4
Cysteine	1.0
Isoleucine	4.0
Leucine	7.9
Phenylalanine	3.8
Lysine	2.5

Health Effects

Brazil nut has significant nutritional properties that can help in the prevention of certain chronic diseases such as heart diseases. The nutrient composition of Brazil nut can produce beneficial effects due to its antioxidant and antiproliferative activities that are associated with a lower risk of developing atherosclerosis and cancer. The consumption of three to five nuts a day for 16 weeks improved the lipid profile and microvascular function in obese adolescents, possibly due to the high content of unsaturated fatty acids and bioactive substances. A long-term decrease in inflammatory markers after a single intake of Brazil nuts in healthy individuals had been reported. Despite its recognized nutritional properties, its protein level can be associated with allergic reactions to the 2*S* albumin content in sensitive individuals. When considering the protein content of the Brazil nut as potentially high (13–19 g%), food allergy related to its protein fractions deserves special attention in order to keep customers informed about the protein content of either in-shell or shelled Brazil nuts, mainly on the product labeling. Another important factor when acquiring this product is information from the supplier to avoid the consumption of nuts contaminated by aflatoxin, a metabolite carcinogenic to humans produced by fungi present in the production chain. In conclusion, it is important to emphasize that the Brazil nut has commercial importance as an export product of some countries and cultural importance to the people of the rain forest in addition to consuming it, using the collection of Brazil nuts as income. Several studies have been conducted focusing on the prevention of contamination ensuring the safety of this product from forest to production and consumption. The research is necessary in order to improve the quality of this important Amazon nutritional source for functional food.

See also: Aflatoxin: A Global Public Health Problem; Allergies: Public health; Antioxidants: Role on Health and Prevention; Energy: Intake and Energy Requirements; Fatty Acids: Metabolism; Food Allergies: Occurrence and Analysis; Food Allergies; Functional Foods; Mycotoxins: Classification; Mycotoxins: Occurrence and Determination; Mycotoxins: Toxicology; Nuts: Health Effects; Phenolic Compounds: Bioavailability and Health Effects; Phenolic Compounds: Occurrence, Classes, and Analysis; Protein: Food Sources; Selenium: Properties and Determination; Toxins in Food: Naturally Occurring; Vegetable Oils: Types and Properties.

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Nuts: Health Effects

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Introduction

Extensive research on nuts and health outcomes has been conducted in the last two decades since the results of the prospective Adventist Health Study relating frequent nut consumption to a lower risk of coronary heart disease (CHD) were published in the early 1990s. Together with cereal grains and legumes, nuts are high energy, nutrient-dense seeds, rich in nutrients, minerals, vitamins, and bioactive phytochemicals destined to protect the plant's DNA and sustain the growth of the future seedling. Nut constituents are bioavailable after consumption by humans and synergize in the body to beneficially affect metabolic and vascular physiology pathways leading to protection from cardiometabolic disorders.

The scientific evidence supporting the definition of nuts as heart-healthy foods stems from observations from both epidemiological studies and randomized clinical trials. Large observational studies have shown that the frequency of nut consumption relates inversely to incident CHD and diabetes, while many feeding trials comparing diets enriched with various nuts to control diets have demonstrated a consistent cholesterol-lowering effect associated with a beneficial impact on other intermediate markers of cardiovascular risk. The accumulating evidence on the cardiovascular benefit of frequent nut consumption has prompted the inclusion of this food group in many guidelines on lifestyle and health. In the summer of 2003, the US Food and Drug Administration issued a health claim for nuts in general and walnuts in particular based on the link between nut consumption and a reduced risk of CHD. Nuts have also been included in the American Heart Association (AHA) report setting goals for health promotion and disease reduction for 2020 and the newest AHA/American College of Cardiology guideline on lifestyle management to reduce cardiovascular risk; furthermore, nuts are an important component and part of the definition of one of the healthiest dietary patterns, the Mediterranean diet. In this article, current knowledge on the expanding topic of nut consumption and health will be summarized.

Nutrient Content of Nuts

Nuts are one of the natural plant foods richest in fat after vegetable oils, with a total fat content that ranges from 46% in cashews and pistachios to 76% in macadamia nuts (Table 1). Importantly, the saturated fatty acid content of nuts is low (range, 4–16%), while most fat is made up of unsaturated fatty acids, predominantly monounsaturated fatty acids, although polyunsaturated fatty acids, mainly linoleic acid, abound in walnuts, pine nuts, and Brazil nuts. Walnuts also contain sizable proportions of α-linolenic acid, the vegetable omega-3 fatty acid. The beneficial lipid profile of nuts is believed to contribute to the salutary effects of its frequent consumption.

Nuts also contain other bioactive macronutrients with potential health benefits. Thus, approximately 25% of the energy provided by nuts is made up of protein with a high content of the amino acid L-arginine, the substrate for endothelium-derived nitric oxide synthesis, a main regulator of vascular tone and blood pressure. This might explain the beneficial effects of nut consumption on vascular reactivity, as discussed below. Nuts also are a good source of dietary fiber, which ranges from 4 to 11 g per 100 g (Table 1). Among nut constituents, there are relevant amounts of essential micronutrients that are associated with an improved health status when consumed at doses beyond those necessary to prevent deficiency states. They include the B-vitamin folate and antioxidant vitamins (e.g., tocopherols) and polyphenols. Most antioxidants in nuts are located in the pellicle or outer soft shell, which means that significant quantities are lost when nuts are peeled or roasted. These facts should be considered when giving advice on nut consumption for health promotion.

Like all plant-based foods, nuts are cholesterol-free, but they contain sizeable amounts of phytosterols, compounds structurally related to cholesterol that have bulkier and more hydrophobic molecules, which confer them a higher affinity for intestinal micelles than has cholesterol. Thus, when phytosterols are present in the intestinal lumen, cholesterol is displaced from micelles and the amount available for absorption is limited, the end result being a reduction of circulating cholesterol. The richness of nuts in these compounds probably contributes to their cholesterol-lowering effect, as discussed below. Also, like most vegetables, nuts have very low sodium content but contain a high proportion of beneficial minerals, such as calcium, magnesium, and potassium (Table 1). A low sodium intake together with a high intake of calcium, magnesium and potassium is associated with protection against hypertension, insulin resistance, and overall cardiovascular risk, besides bone demineralization.

In summary, the nutrient and phytochemical components of nuts have all been documented to contribute to a reduced risk of CHD and related metabolic alterations. This might explain why nut consumption is associated with beneficial health effects in both observational studies and clinical trials.

Nuts and Cardiovascular Health

There has been much research on nut consumption as the exposure of interest in observational studies of diet and heart health and on nut-enriched diets as intervention in randomized clinical trials with outcomes on intermediate biomarkers of cardiovascular risk. Clinical cardiovascular endpoints (CHD, stroke, type-2 diabetes, hypertension, and metabolic syndrome) have been investigated in several prospective studies, while clinical trials have mainly explored the effects of nut diets on the lipid profile, but also on adiposity, blood pressure, insulin

Table 1 Average composition of nuts in macro and micronutrients and selected phytochemicals (per 100 g)

Nuts	Energy (kJ)	Protein (g)	Fiber (g)	Fat (g)	SFA (g)	MUFA (g)	PUFA (g)	LA (g)	ALA (g)	PS (mg)	Sodium (mg)	Potassium (mg)	Calcium (mg)	Total polyphenols (mg)
Almonds	2418	21.3	8.8	50.6	3.9	32.2	12.2	12.2	0.00	120	1	728	248	287
Brazil nuts	2743	14.3	8.5	66.4	15.1	24.5	20.6	20.5	0.05	NR	3	659	160	244
Cashews	2314	18.2	5.9	46.4	9.2	27.3	7.8	7.7	0.15	158	12	660	37	233
Hazelnuts	2629	15.0	10.4	60.8	4.5	45.7	7.9	7.8	0.09	96	0	680	114	687
Macadamia nuts	3004	7.9	6.0	75.8	12.1	58.9	1.5	1.3	0.21	116	5	368	85	126
Peanuts	2220	25.8	8.5	49.2	6.8	24.4	15.6	15.6	0.00	220	18	705	92	396
Pecans	2889	9.2	8.4	72.0	6.2	40.8	21.6	20.6	1.00	102	0	410	70	1816
Pine nuts	2816	13.7	3.7	68.4	4.9	18.8	34.1	33.2	0.16	141	2	597	16	58
Pistachios	2332	20.6	9.0	44.4	5.4	23.3	13.5	13.2	0.25	214	1	1025	107	1420
Walnuts	2738	15.2	6.4	65.2	6.1	8.9	47.2	38.1	9.08	72	2	441	98	1558

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LA, linoleic acid; ALA, α-linolenic acid; PS, plant sterols; NR, not reported.
Sources: US Department of Agriculture Nutrient Data Base at: http://ndb.nal.usda.gov/ndb/foods/list (accessed on 24 March 2014); National Institute for Health and Welfare, N.U. Fineli. Finnish Food Composition Database at: http://www.fineli.fi (accessed on 24 March 2014); and Phenol-Explorer 3.0. Data base on polyphenol content in foods at: http://www.phenol-explorer.eu (accessed on 24 March 2014).

resistance, endothelial function, oxidation, and inflammation. The PREDIMED (PREvención con DIeta MEDiterránea) randomized clinical trial of primary cardiovascular prevention has been an important exception because it included nut supplementation in one study arm and had cardiovascular events as the main outcome. The scientific evidence collected in these studies is summarized here.

Epidemiological Studies

Coronary heart disease

Six large prospective studies, five of them conducted in the United States and one in Spain, investigated the associations of dietary factors with health outcomes and described a protective effect of nut consumption on fatal and nonfatal CHD after follow-up ranging from 6 to 30 years. Based on recent meta-analyses of these studies, the consumption of nuts was inversely associated with fatal CHD in six studies including 6749 events, with a risk ratio (RR) per four weekly servings of nuts (one serving equals 28.4 g) of 0.76 (95% CI, 0.69–0.84) and with nonfatal CHD in four studies comprising 2101 events, with an RR of 0.78 (95% CI, 0.67–0.92). When expressing the results for each serving per day of nut consumption, the pooled RR for CHD (fatal and nonfatal) was 0.72 (95% CI, 0.64–0.81). A dose–response relationship between nut consumption and reduced CHD mortality was observed in all studies (Figure 1). The consistency of findings in all prospective cohort studies strongly suggests a causal association between nut consumption and reduced CHD rates.

Stroke

The findings of prospective studies are less consistent regarding nut consumption and the risk of stroke. Meta-analytical evidence from four studies indicates that there was no association between the consumption of each one serving of nuts per day and risk of stroke (RR: 0.90; 95% CI, 0.71–1.14) or for the comparison of extreme quantiles of nut intake and incident stroke (RR: 0.91; 95% CI, 0.81–1.02). The findings of the PREDIMED trial, however, indicate that nut consumption within the context of the Mediterranean diet reduces the risk of stroke, as discussed below.

Type-2 diabetes

The association between nut consumption and diabetes risk has been investigated in six epidemiological studies. Findings from the large prospective Nurses' Health Study cohort suggested that nut and peanut butter consumption was associated with a reduced incidence of diabetes in women, but this relationship was not observed in post-menopausal women from the Iowa Women's Health Study, another large U.S. observational cohort. In a prospective Chinese study of nearly 64 000 women it was also shown that nut consumption protected from diabetes risk. These findings, however, were not confirmed in male participants in another large prospective U.S. cohort, the Physicians Health Study. A further report from the Nurses' Health Study with extended follow-up for 10 years showed that consumption of walnuts, but not other nuts, was associated with reduced diabetes rates. The more recent study was a cross-sectional assessment of the PREDIMED cohort of older persons at high-cardiovascular risk and it showed a reduced prevalence of diabetes with increasing nut consumption. A meta-analysis of these six studies totalling 13 308 events showed an RR of diabetes per four weekly servings of nuts of 0.87 (95% CI, 0.81–0.94). Another meta-analysis showed that the RR of type-2 diabetes for each incremental serving per day of nuts was 0.80 (95% CI, 0.69–0.94) without adjustment for body mass index (BMI); with adjustment, the association was attenuated, with an RR of 1.03 (95% CI, 0.91–1.16). Clearly, the epidemiological evidence of a protective effect of nut consumption on diabetes risk is weaker than for CHD.

Metabolic syndrome

The association of nut consumption with metabolic syndrome, a high-cardiovascular risk cluster of at least three risk factors

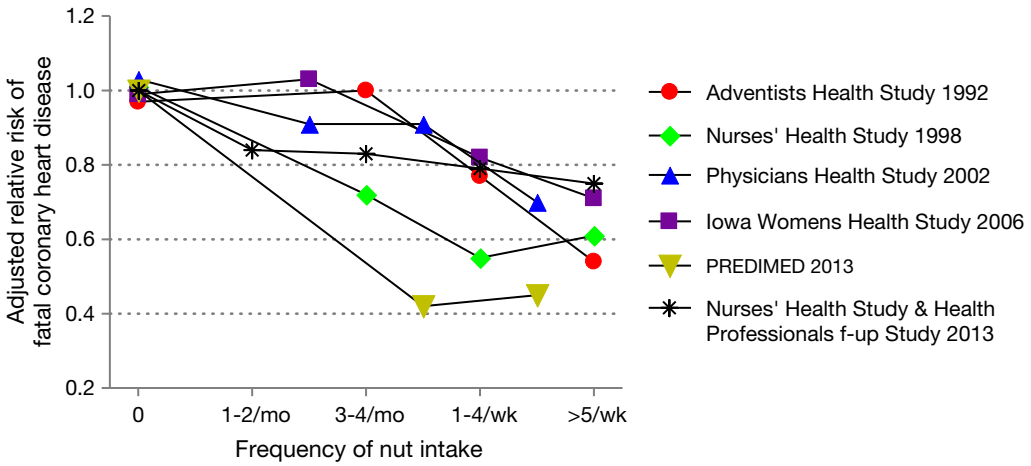

Figure 1 Incidence of fatal coronary heart disease (CHD) by frequency of nut intake. The results of six large prospective studies in which nut consumption was the exposure of interest and the outcome was death from CHD are shown.

related to insulin resistance (abdominal obesity, high blood pressure, impaired fasting glucose, high triglycerides, and low HDL-cholesterol), has been assessed in three epidemiologic studies. Frequent nut eaters had a lower prevalence of metabolic syndrome in a preliminary report of the PREDIMED study and higher reversion rates in a report of the full cohort, and the main driver was decreased visceral adiposity. A lower prevalence of the metabolic syndrome in frequent nut consumers was also described in a large cross-sectional report from the U.S. National Health and Nutrition Examination Survey (NHANES) for 2001–04. Finally, lower incident metabolic syndrome was reported for frequent nut eaters in the prospective Seguimiento Universidad de Navarra (SUN) cohort of Spanish university graduates. These findings further support nuts as heart-healthy foods.

Hypertension

Four prospective studies, three conducted in the United States and one in Spain, have examined the frequency of nut consumption in relation to incident hypertension. In the Coronary Artery Risk Development in Young Adults (CARDIA) study with 15 years of follow-up, participants in the upper category of nut consumption had a 15% decreased risk of hypertension compared those eating nuts infrequently. In the large men cohort of the Physicians Health Study, the risk of hypertension was reduced by 18% in those who consumed nuts daily compared with non consumers and were lean at baseline, but not when they were overweight or obese. A prospective study of university graduates in Spain reported a non significant 23% lower risk of incident hypertension for the highest versus lowest nut-consumption category after 4.3 years of follow-up. Another prospective U.S. study of middle-aged adults showed a 13% lower risk of incident hypertension in the top quintile of nut consumption compared with the bottom quintile after a 9-year follow-up. The cross-sectional NHANES 2001–04 study also reported a lower prevalence of hypertension in nut consumers compared with non consumers. These studies have been recently meta-analyzed with the conclusion that the consumption of each one serving of nuts per day was significantly associated with lower incident hypertension rates with an RR of 0.66 (95% CI, 0.44–1.00; $P=0.049$).

Inflammation

Chronic low-grade inflammation, which can be conventionally estimated from levels of circulating biomarkers, is believed to be a key process in atherogenesis and the occurrence of ischemic events. Three cross-sectional studies have investigated circulating inflammatory biomarkers in relation to nut consumption. In the Multi-Ethnic Study of Atherosclerosis, an analysis of data from nearly 6000 participants showed that the frequency of nut consumption related inversely associated to levels of inflammatory markers, C-reactive protein (CRP), interleukin-6 (IL-6), and fibrinogen. Another study of close to 1000 diabetic women from the NHS cohort showed an association between nut consumption and increased circulating adiponectin, an anti-inflammatory cytokine secreted by adipose tissue. In a cross-sectional assessment of the PREDIMED study cohort involving 772 older individuals at high-cardiovascular risk, increasing nut consumption related to decreasing serum levels of intercellular adhesion molecule-1 (ICAM-1), but not those of CRP or IL-6. Thus, lessening the inflammatory status might be one mechanism by which nut consumption decreases CHD risk.

In summary, the results of several prospective studies strongly suggest that regular consumption of nuts is beneficial for CHD risk. The risk of stroke in relation to nut consumption has been less investigated than CHD risk and epidemiologic data do not consistently support a protective effect; confirmation of a protective role in stroke must await further studies. Diabetes is another high-cardiovascular risk condition that has been favorably affected by nut consumption in prospective studies. Epidemiologic evidence also points to a protective effect of nut consumption on the metabolic syndrome, as well as in hypertension, one of the components of the syndrome. Finally, there is also suggestive epidemiologic evidence that frequent nut consumers display reduced circulating markers of systemic inflammation.

Randomized Clinical Trials

After publication of the results of the first observational studies suggested that nut consumption protected from CHD, clinical trials began to explore the underlying mechanisms by investigating the effects of diets supplemented with nuts on intermediate biomarkers of cardiovascular risk. Most randomized clinical trials have compared nut-enriched diets with control diets for outcomes on blood lipid changes in individuals with normal or moderately elevated blood cholesterol levels. Some studies have also evaluated nut effects on glycemic control in patients with type-2 diabetes. Other studies have examined whether unrestricted consumption of high-energy nuts has any effect on body weight and other adiposity measures. Additional clinical trial have evaluated the effects of nut-enriched diets on intermediate markers of cardiovascular risk, such as blood pressure, insulin sensitivity, endothelial function, and inflammatory status. Almonds and walnuts have been the nuts most studied. The long-term PREDIMED trial targeted the effects of nut consumption on both intermediate cardiometabolic markers and clinical outcomes, such as the metabolic syndrome, diabetes, and cardiovascular diseases. Given the relevance of PREDIMED for the topic of nuts and cardiovascular health, its results will be detailed in a separate section.

Blood lipids

The first controlled clinical trial with nuts, published in 1993, investigated the lipid effects of a walnut diet versus a control diet in healthy volunteers and showed that it was associated with a reduction in blood cholesterol levels. Since then the effects of the consumption of different nuts on blood lipids and lipoproteins have been examined in many clinical studies. A pooled analysis of 25 studies assessed the lipid effects of diets enriched with different nuts versus control diets during periods of 3–8 weeks. The results demonstrated that nuts had a consistent cholesterol-lowering effect that was related to both dose and baseline cholesterol level, similar by gender and across all age groups, and independent of the type of nut tested. Specifically, consumption of 67 g (2.4 oz) of nuts per day was associated with mean reductions of 0.28 mmol l^{-1} (5.1%) in total cholesterol, 0.26 mmol l^{-1} (7.4%) in LDL-cholesterol, and 0.22 (8.3%) in LDL:HDL ratios ($P<0.001$, all). Nuts had no effect on HDL-cholesterol or triglycerides, except in participants with serum triglycerides >1.68 mmol l^{-1}, in whom a significant mean 0.11 mmol l^{-1} reduction was observed. A separate meta-analysis of 13 studies examined the effect of walnuts on the lipid profile and showed that, compared to control diets, diets enriched with walnuts in amounts ranging from 30 to 108 g day^{-1} (10–24% of energy) were associated with weighted mean reductions of total cholesterol and LDL-cholesterol of 0.27 and 0.248 mmol l^{-1} ($P<0.001$, both), respectively. Thus, the lipid effects of walnuts were similar to those described in the pooled analysis for different nut types. Clinical trials examining nut diets for lipid effects have usually been short term, but a few studies have shown that efficacy is maintained in the long term. For instance, adding walnuts (12% of energy) to the usual diet of free-living individuals to whom no dietary advice was given during a 6-month period reduced total cholesterol by 0.13 mmol l^{-1} ($P=0.02$) and triglycerides by 0.09 mmol l^{-1} ($P=0.03$), with a nearly significant decrease of 0.09 mmol l^{-1} in LDL-cholesterol ($P=0.06$). Likewise, a walnut diet compared to a control diet for 1 year in obese patients with type-2 diabetes showed maintained, albeit small, LDL-cholesterol reductions and HDL-cholesterol increases.

Glycemic control

Acute feeding studies using test meals with a high glycemic index with or without added nuts have shown reduced postprandial glucose responses following nut meals, suggesting that nuts might be useful in diabetic control. Feeding nut-enriched diets to patients with type-2 diabetes fed diets for up to 12 weeks has also been associated with improved glycemic control. It may seem counterintuitive to recommend energy-dense nuts to diabetic patients for the fear of gaining weight. However, as discussed below, there is no evidence that frequent nut consumption promotes weight gain, thus they can safely be recommended to patients with type-2 diabetes, who are usually overweight.

Other markers of cardiometabolic risk

The unique composition of nuts in bioactive nutrients and phytochemicals also underlies the effects of their consumption on cardiometabolic markers other than the lipid profile and glycemic control. A few studies have shown beneficial effects of nuts on vascular reactivity, oxidative stress, and inflammation. Small clinical trials, mostly testing walnuts, have demonstrated beneficial effects on both fasting and postprandial endothelial function, as ascertained by flow-mediated dilatation studies in the brachial artery, reduced circulating levels of endothelial activation molecules or inhibition of proinflammatory cytokine production by blood mononuclear cells. An effect of nut diets on resting blood pressure has been difficult to uncover in clinical studies, but testing hemodynamic responses to stress has detected beneficial effects on blood pressure and total peripheral resistance. Improved vascular reactivity in studies with walnuts may be attributed to their richness in polyunsaturated fatty acids, particularly α-linolenic acid, L-arginine (the precursor of nitrous oxide) and potent polyphenols.

The consumption of antioxidant-rich nuts has also been associated with an improved oxidative status. This has been reported for monounsaturated fatty acid-rich nuts, such as almonds, pistachios and hazelnuts, but not for polyunsaturated fatty acid-rich walnuts, albeit no deleterious effects on oxidation have been described. Nut feeding studies have documented reduced circulating concentrations of inflammatory cytokines but no consistent changes of CRP. A novel mechanism by which walnut consumption may reduce cardiovascular risk has recently been reported: increased cholesterol efflux from cholesterol-laden macrophages exposed to human serum collected after a walnut meal, suggesting improved functionality of HDL, the main lipoprotein particles involved in reverse cholesterol transport. In summary, the emerging picture is that nut consumption has beneficial effects on cardiovascular risk factors beyond well known lipid lowering without incurring undue increases in adiposity.

Cardiovascular effects of nuts in the PREDIMED trial

The PREDIMED study is a large, parallel group, multi center, 5-year field trial of nutrition intervention conducted in Spain.

Eligible participants were men, 55–80 years of age, and women, 60–80 years of age at high risk of cardiovascular disease but who had not suffered any ischemic event at enrolment. Participants were randomized into three groups, two high-fat, high-unsaturated fat Mediterranean diets, which were supplemented with either extra-virgin olive oil or mixed nuts, or a control diet (advice to reduce all types of dietary diet). They received quarterly individual and group educational sessions and, depending on group assignment, free provision of extra-virgin olive oil (1 l/week), mixed Mediterranean nuts (30 g day^{-1}, 15 g walnuts, 7.5 g almonds, and 7.5 g hazelnuts), or small non-food gifts. The recommended diets were non energy restricted and increased physical activity was not promoted, thus the full effect of dietary intervention alone could be ascertained. The primary end point was an aggregate of major cardiovascular events (myocardial infarction, stroke, and cardiovascular death).

Since its inception in October 2003, the trial's results have provided significant evidence on the beneficial health effects of Mediterranean diets enriched with both supplemental foods. The efficacy of the PREDIMED nutritional intervention has generally been similar for both the extra-virgin olive oil and nut-supplemented Mediterranean diets in comparison with the control diet. The main results concerning the nut-supplemented Mediterranean diet are summarized below.

A study involving nearly 750 participants completing intervention for 3 months showed that, compared to the control diet, the two supplemented Mediterranean diets were associated with significant reductions of both systolic and diastolic blood pressure and total and LDL-cholesterol; increased HDL-cholesterol; decreased fasting glucose in diabetic participants and increased insulin sensitivity in those without diabetes; and reduction in circulating levels of inflammatory cytokines. The nut-enriched diet also reduced fasting triglycerides and both intervention diets resulted in reduced oxidized LDL levels. While results on lipids, glycemic control, oxidation, and inflammation were essentially confirmatory, the blood pressure-lowering effect of the nut-enriched diet was novel. Noticeably, reduced blood pressure by both PREDIMED Mediterranean diets after treatment for 1 year was confirmed in a sub study using 24-h ambulatory blood pressure monitoring, the gold standard to ascertain the effects of interventions on blood pressure.

Another PREDIMED sub study showed both reduction in circulating inflammatory biomarkers and reduced expression of ligands for inflammatory molecules in circulating monocytes after 3-month intervention with the two Mediterranean diets, thus beginning to unravel the molecular bases for their anti-inflammatory effects. The PREDIMED Mediterranean diet supplemented with nuts has also demonstrated an anti atherosclerotic effect, namely delayed progression of carotid intima-media thickness and plaque after intervention for 2 years.

The effects of the interventions on metabolic syndrome status and incident diabetes were secondary end points of the PREDIMED trial. In the first 1224 trial participants completing intervention for 1 year, the nut-rich Mediterranean diet was associated with a 14% reduction in the prevalence of metabolic syndrome and in the full cohort, a 28% increase in reversion rates was documented; in both studies, the beneficial effect of the nut-enriched diet on metabolic syndrome was mainly due to reduced visceral adiposity. Because there were no weight changes, this suggests fat redistribution away from the abdominal compartment. In one of the PREDIMED recruiting centers, yearly oral glucose tolerance tests were performed in non-diabetic participants to detect incident diabetes. Results in 418 subjects followed for a mean of 4 years showed that both Mediterranean diets were protective of diabetes, and the reduction of incidence in the nuts arm was 52% compared with the control diet. Again, this beneficial effect took place in the absence of weight loss or increased energy expenditure in physical activity. However, the results for the full PREDIMED cohort showed only a non significant 18% reduction of incident diabetes for the Mediterranean diet enriched with nuts.

The results of the PREDIMED randomized trial on the primary end point have shown for the first time a reduction of incident cardiovascular diseases after long-term consumption of the two Mediterranean diets. After a median follow-up of 4.8 years in 7447 participants, those assigned the two Mediterranean diets showed a 30% reduction in cardiovascular disease events (myocardial infarction, stroke or cardiovascular death) compared to the control diet. As seen in Figure 2, the curves of the two Mediterranean diet groups and the control group diverged soon after the beginning of the study. Such an early and sustained reduction in cardiovascular events fits with the improvement of intermediate cardiovascular risk markers observed after follow-up for only 3 months. The nut-supplemented diet also resulted in a significant 49% reduction in stroke risk, which differs from the generally null findings of epidemiologic studies on nut consumption and incident stroke. The two PREDIMED Mediterranean diets were associated with non significant reductions in myocardial infarction and no effect could be discerned on total mortality, although the trial was not powered for these outcomes. It must be underlined that the Mediterranean diet interventions were conceived to improve the overall dietary pattern, but the principal between-group differences in food consumption were for the supplemental items. Thus, nuts probably accounted for most of the observed benefit in the corresponding Mediterranean diet group. A protective effect of both Mediterranean diets on peripheral arterial disease, another cardiovascular outcome, was also reported. However, as opposed to the olive oil-enriched diet, no beneficial effect of the nut-supplemented diet on atrial fibrillation, an additional PREDIMED outcome, could be discerned.

The beneficial effects shown in the PREDIMED trial for the nut-enriched Mediterranean diet on intermediate cardiovascular risk biomarkers include blood pressure, the lipid profile, insulin resistance, lipoprotein oxidation, and systemic inflammation. This diet was also associated with reduction in visceral adiposity, metabolic syndrome and diabetes, as well as delayed atherosclerosis progression. Such multiple beneficial effects provide a mechanistic explanation for the observed protection of long-term consumption of a Mediterranean diet supplemented with nuts against cardiovascular diseases. Because PREDIMED was a controlled, randomized clinical trial, its results provide first class evidence on the potential of nuts and other healthy foods such as extra-virgin olive oil to improve cardiovascular health.

Mortality

Besides reporting on nut consumption and fatal CHD, several prospective studies have provided data on all-cause mortality.

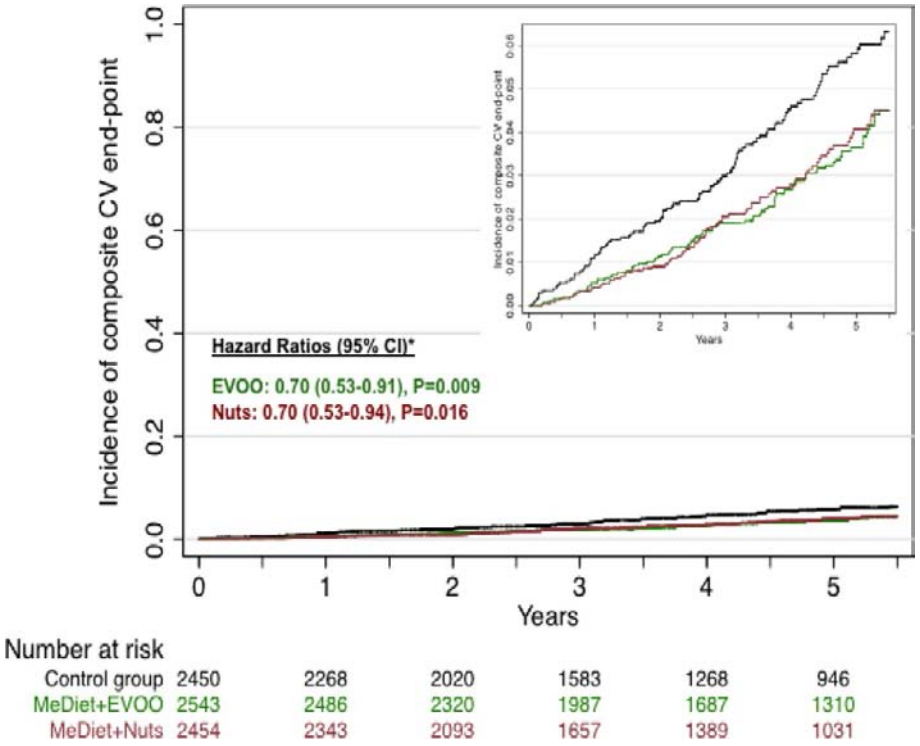

Figure 2 Incidence of cardiovascular disease by intervention group in the PREDIMED study. Med Diet, mediterranean diet; EVOO, extra-virgin olive oil. Reprinted from Estruch, R., Ros, E., Salas-Salvadó, J., et al. (2013). Primary prevention of cardiovascular disease with a Mediterranean diet. *The New England Journal of Medicine* 368, 1279–1290, with permission by Massachusetts Medical Society, Copyright © (2013).

Among them stand two large cohorts of women from the Nurses' Health Study ($n = 76\,464$) and men from the Health Professionals Follow-up Study ($n = 42\,498$) with 27 429 overall deaths after follow-up of 30 and 24 years, respectively. A meta-analysis of prospective cohort studies with high-quality scores, large sample sizes, and long-term follow-up that reported on nut consumption and total mortality showed a pooled RR (95% CIs) for each serving per day of 0.83 (0.76–0.91) for all-cause mortality and a pooled RR for the comparison of extreme quantiles of 0.85 (0.79–0.91). There are no clinical trials reporting on nut consumption and mortality because the size and duration of follow-up of a study with this outcome would make it unfeasible. Nevertheless, the epidemiological evidence strongly suggests that frequent nut consumption has a beneficial impact on chronic conditions that shorten life span well beyond cardiovascular diseases.

Cancer

The fact that, among edible plant foods, nuts are among those richest in phytochemicals with significant antioxidant and immunomodulatory properties suggests that their customary consumption might have a chemopreventive potential by either blocking tumor initiation or preventing the progression of cancer. Indeed some *in vitro* and animal studies have shown that nut phytochemicals promote apoptosis in cancerous cells and stop cancer cell growth. Other nut components, such as folic acid, are important in the process of repair of cancer-inducing DNA damage, while some polyphenols abundant in nuts have shown an antitumor cell invasion effect, thereby limiting metastasis. Studies in animal models of cancer fed whole nuts have also shown reduced cancer growth, particularly with walnuts and mammary tumors.

However, the epidemiological evidence of a protective effect of nuts on cancer incidence is weak and inconclusive; this is partly due to difficulties in the interpretation of epidemiological data, since most of the studies often associated nuts with legumes and seeds in a single food group. There is some evidence suggesting that nuts may be anticarcinogenic, especially on colorectal cancer in women and prostate cancer, but data are insufficient to draw any conclusion on the potential protective effects of nuts on cancer development or progression.

Remarkably, besides a reduction in fatal CHD rates and all-cause mortality, nut consumption was associated with decreased cancer deaths in two large prospective studies. In the pooled cohort of women from the Nurses' Health Study and men from the Health Professionals Follow-up Study, the multivariate-adjusted hazard ratio for cancer death ($n = 10\,293$) among study participants who consumed nuts two or more times per week versus those who never consumed nuts was 0.91 (95% CI, 0.85–0.97). In the PREDIMED study considered as an observational cohort, the adjusted hazard ratio for cancer death was 0.60 (0.37–0.98) for consumers of more than three servings of nuts per week compared to those who never consumed nuts, and walnuts were more protective than other nuts. Given that

incident cancer was a pre specified outcome of the PREDIMED trial, its extended follow-up may shed light on the potential anticarcinogenic effect of nuts.

Neurodegenerative Diseases

The nutrient density of nuts, particularly their high polyphenol content, and their beneficial effects on vascular risk factors and function, which are well known to affect cognition, also suggest that their increased consumption might promote brain health and thereby relate to improved cognition and/or decreased rates of dementia. *In vitro* studies have shown that polyphenolic compounds found in nuts reduce the oxidant and inflammatory load on brain cells, improve inter neuronal signaling, and increase neurogenesis. Data from experimental animal models fed whole nuts, especially walnuts, indicate improved performance in cognitive and motor tasks involving working memory compared to control diets. Animal studies have also shown that nut diets enhance clearance of toxic protein aggregates from the brain.

Few prospective studies have focussed on the relation of nut intake to cognition. A study from a Dutch cohort with 2613 participants reported that total nut intake was associated with better cognitive function at initial assessment, but not with decline after follow-up for 5 years. Similar results were reported from a sub cohort of 15 467 older women from the Nurses' Health Study followed for 6 years. Preliminary data from subsets of the PREDIMED study indicate that the Mediterranean diet with nuts is associated with lower rates of depression (a surrogate of impaired brain health), improved concentrations of plasma brain-derived neurotrophic factor, and better overall cognition. Again, incidence of neurodegenerative diseases, including Alzheimer's disease, vascular dementia, and Parkinson disease, was a pre specified outcome of the PREDIMED study and results are eagerly awaited.

Nuts and Body Weight

There is a widespread perception that all fatty foods provide excess energy and promote obesity, which has had a negative effect on the image of nuts. However, there is no epidemiologic or clinical trial evidence that frequent nut consumption promotes weight gain; rather, data from large prospective studies suggest inverse associations between the frequency of nut consumption and BMI or weight gain over time after adjustment for confounders. Similar findings regarding BMI together with reduced waist circumference have been shown for increasing nut consumption in cross-sectional studies and in the PREDIMED cohort considered as a longitudinal study. A meta-analysis of clinical trials that tested nut diets versus control diets showed small non significant associations of nut intake with reduced adiposity measures. The mechanism for the lack of weight gain after consuming nuts lies in their prominent satiating effect, which results in food compensation accounting for up to 75% of the energy they provide. An additional factor that curtails energy acquisition after eating nuts is fat malabsorption, documented as increased fecal fat excretion, which is attributable to incomplete digestion of their matrices because the fat of nuts is enclosed within cell membranes, which are not readily available to digestive enzymes. In conclusion, there is a substantial body of evidence to alleviate the fear of undue weight gain when eating high-fat nuts.

Conclusion

Nuts are seeds, energy-dense foods with an optimal composition in macronutrients, micronutrients and bioactive phytochemicals and this is likely to be of utmost importance for their wide range of health effects. As summarized in Table 2, there is consistent evidence from epidemiologic studies and clinical trials of the beneficial effects of nuts on risk for cardiovascular diseases, diabetes, and intermediate risk factors. Increasing evidence indicates that including nuts in a healthy

Table 2 Summary of scientific evidence on the effects of nut consumption on the risk of chronic diseases and cardiovascular risk factors

Disease/factor	*Effect*	*Level of evidence*
Epidemiological studies		
Coronary heart disease	Decrease	++
Stroke	No change	+
Type-2 diabetes	No change/decrease	+/−
Metabolic syndrome	Decrease	+
Hypertension	Decrease	+
Inflammatory markers	Decrease	+
All-cause mortality	Decrease[a]	+
Cancer	No change/decrease	+/−
Neurodegenerative diseases	Decrease	+
Body weight	No change/decrease	++
Randomized clinical trials		
Blood lipid profile		
Total cholesterol	Decrease[a]	++
LDL cholesterol	Decrease[a]	++
HDL cholesterol	No change/increase[a]	+
Triglycerides	Decrease[a]	+
Insulin sensitivity	Increase[a]	+
Blood pressure	Decrease[a]	+
Oxidation	Decrease[a]	+/−[b]
Inflammation	Decrease[a]	+
Endothelial function	Increase	+
Body weight	No change[a]	++
Visceral adiposity	Decrease[a]	+
Metabolic syndrome	Decrease[a]	+
Type-2 diabetes	Decrease[c]	+
Cardiovascular diseases	Decrease[c]	+
Stroke	Decrease[c]	+
Peripheral arterial disease	Decrease[c]	+

+/−, equivocal evidence; +, limited evidence from few studies; ++, evidence from several studies.

[a]Evidence collected in the PREDIMED trial, among others.

[b]Oxidation improved with nuts rich in monounsaturated fatty acids (i.e., almonds, pistachios, hazelnuts) and unchanged with nuts rich in polyunsaturated fatty acids (i.e., walnuts).

[c]Evidence collected only in the PREDIMED trial.

diet provides protection against cardiometabolic disorders beyond that attributable to the other components of a healthy diet. Data on critical outcomes such as cancer and neurodegenerative diseases is still emerging, but there are reasons to believe that nut consumption will also protect against these devastating conditions. Nut-rich diets have also been related to reduced all-cause, cardiovascular and cancer mortality. Contrary to popular belief due to the high energy content of nuts, their consumption does not induce undue weight gain, which is attributable in part to increased satiation and lesser intake of other high-energy foods in subsequent meals. Nut components such as unsaturated fatty acids, L-arginine, non-sodium minerals, and polyphenols target multiple cardiovascular risk factors and metabolic pathways, providing a mechanistic explanation for cardiovascular risk reduction. The results of the PREDIMED trial have provided first class evidence that long-term compliance with a healthy diet enriched with one daily serving of nuts reduces the incidence of type-2 diabetes and cardiovascular diseases, particularly stroke. Ongoing research in the context of this landmark trial will eventually show whether the salutary effects of nuts reach to the prevention of other epidemic chronic diseases, including cancer and neurodegenerative disorders.

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See also: Antioxidants: Role on Health and Prevention; Calcium: Physiology; Cashew Nuts; Dietary Exposure Assessment; Dietary Fiber: Bran; Dietary Practices; Fatty Acids: Fatty Acids; Fatty Acids: Metabolism; Hypertension and Diet; Magnesium; Mediterranean Diet; Nuts: Brazil Nuts; Obesity Management; Peanuts; Phenolic Compounds: Occurrence, Classes, and Analysis; Potassium: Physiology.

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O

Oats

SM Tosh and SS Miller, Agriculture and Agri-Food Canada, Guelph, ON, Canada

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Production, Trade, and Consumption

Oat is a cereal grain that is widely grown for use as animal feed and for human consumption. In 2013, 10.45 million hectares of land was planted with oats worldwide (Figure 1). The European Union (EU-27) produces approximately one-third of the world's oat supply, mainly in Poland, Finland, Spain, Sweden, and Germany. In 2011, production in the EU-27 increased by 1.7% over the previous year to 11.6 MT. Russia, the largest producer, grows about 20% of the world's oats. Canada is the largest exporter of oats (1.6 million tons in 2012), whereas the United States is the largest importer (1.6 million tons in 2012).

Although oats are grown in many countries, the total production is only about 1% of the world's grain production. It currently ranks sixth in the world's production behind corn (40%), wheat (29%), rice (20%), barley (6%), and sorghum (3%). Of the oats grown, the majority are used for animal feed. Only about 25% of the crop is milled for human consumption. Human consumption is highest in the European Union (1.8 million tons per year) followed by Russia (1.5 million tons per year), the United States (1.1 million tons per year), and Canada (0.75 million tons per year).

It is believed that oats, *Avena sativa*, have been cultivated in Europe and Asia for 4000–5000 years. Another species, *A. byzantina*, was domesticated in northwest Africa and performs better in tropical latitudes. Much of the breeding activity in recent years has targeted adapting oats for particular regions. To this end, varieties are being produced that are drought-tolerant, winter-hardy, resistant to lodging caused by wind or rain, and resistant to diseases and pests found in different areas. Breeding programs also aim to increase yield and improve milling quality. Varieties of naked oats have been developed for Canada and China. The advantage of naked oats is reduced shipping costs because the hull need not be shipped and dehulling is not required during processing. However, without the protective hull, the pericarp is more susceptible to damage, which can lead to a decrease in quality due to breakage and off-flavors. Breeding programs are also attempting to produce oats with higher content of mixed-linkage β-glucan (hereafter referred to as β-glucan), the cell wall component that is mostly responsible for the oat's reputation as a health food. The content of β-glucan in commercially available varieties ranges from 2 to 8 g per 100 g and breeding programs for high β-glucan varieties with good agronomic traits continue.

Nutrition

Oats are higher in oil and protein and lower in starch than wheat (Table 1). The oil is high in phospholipids and glycolipids that have high emulsifying capacity, and oat protein has a better amino acid balance and thus superior protein quality (protein efficiency ratio = 1.8) than wheat protein (protein efficiency ratio = 0.8). Because the amino acid sequence is sufficiently different from gluten, hordein, and secalin, the avidin protein in oats does not contain the epitopes that elicit reactions in persons with celiac disease. Therefore, it can be consumed by celiac patients provided that it is not contaminated with wheat, barley, or rye. Oats tend to be higher in calcium, iron, magnesium, phosphorus, zinc, and folate than wheat, barley, and rye, but lower in niacin.

Health Benefits of Oats

Beyond providing good basic nutrition, oats also provide additional health benefits. There is solid evidence that the soluble fiber in oats, β-glucan, reduces serum LDL cholesterol in people with mildly elevated cholesterol levels and attenuates postprandial blood glucose concentrations. In addition, a group of polyphenols found in oats, known as avenanthramides, are potent antioxidants that have been shown to reduce inflammatory responses. β-Glucans may also have anti-inflammatory capabilities. There is also growing evidence that oats extend the sense of satiety after a meal and thus reduce calorie intake at a subsequent meal.

Cholesterol Reduction and Heart Health

The soluble fiber β-glucan has been identified as the bioactive component of oats that facilitates the reduction of serum cholesterol levels in persons with mildly elevated cholesterol levels. The ability of oats to reduce total and LDL cholesterol has been associated with β-glucan's capacity to form very viscous solutions at low concentrations. Two mechanisms of action appear to be involved. In the upper intestine, the viscosity of the meal bolus hinders the emulsification of fat into small droplets and reduces absorption of cholesterol. Thus, an increased amount of ingested cholesterol is excreted. At the same time, bile acids are secreted by the gall bladder to aid

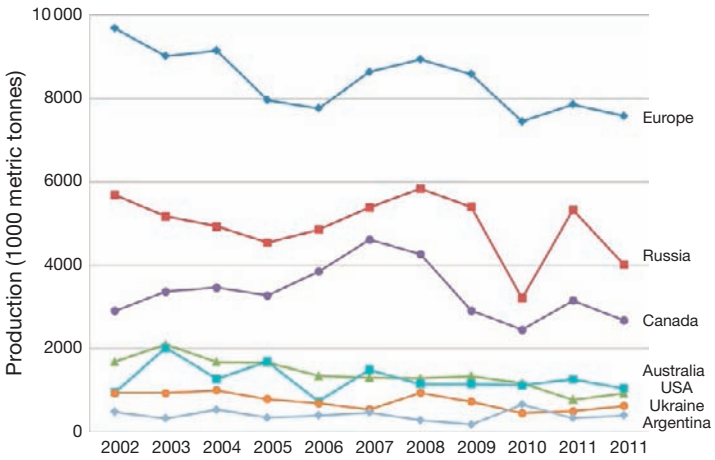

Figure 1 Historical production trends for the seven largest oat producers in the world. Data from USDA.

Table 1 Nutrient content of oats as compared to other cereal grains

	Oats	*Wheat*	*Rice*	*Barley*	*Rye*
Water (g)	8.2	12.8	11.6	9.4	10.6
Energy (kcal)	390	330	365	355	340
Protein (g)	16.9	15.4	7.13	12.5	10.3
Total lipid (fat) (g)	6.9	1.92	0.66	2.3	1.63
Carbohydrate (by difference) (g)	66	68	80	73	76
Fiber, total dietary (g)	10.6	12.2	1.3	17.3	15.1
β-Glucan (g)	4.4[a]	0.8[a]	0.1[a]	4.2[a]	2.1[a]
Sugars, total (g)	–	0.41	0.12	0.8	0.98
Minerals					
Calcium (Ca) (mg)	54	25	28	33	24
Iron (Fe) (mg)	4.7	3.6	0.8	3.6	2.6
Magnesium (Mg) (mg)	177	124	25	133	110
Phosphorus (P) (mg)	523	332	115	264	332
Potassium (K) (mg)	429	340	115	452	510
Sodium (Na) (mg)	2	2	5	12	2
Zinc (Zn) (mg)	4.0	2.8	1.09	2.8	2.7
Vitamins					
Thiamin (mg)	0.76	0.50	0.07	0.65	0.32
Riboflavin (mg)	0.14	0.11	0.05	0.29	0.25
Niacin (mg)	0.96	5.71	1.6	4.60	4.27
Vitamin B_6 (mg)	0.12	0.34	0.16	0.32	0.29
Folate, DFE (μg)	56	43	8	19	38
Vitamin A (μg)	0	9	0	22	11
Vitamin E (α-tocopherol) (mg)	1.2[a]	1.01	0.11	0.57	0.85
Vitamin K (phylloquinone) (μg)	–	1.9	0.1	2.2	5.9
Lipids					
Fatty acids, total saturated (g)	1.22	0.31	0.18	0.48	0.20
Fatty acids, total monounsaturated (g)	2.18	0.30	0.21	0.30	0.21
Fatty acids, total polyunsaturated (g)	2.54	0.77	0.18	1.11	0.77

Nutrients per 100 g of cereal.
[a]Data taken from 'Oats: Chemistry and Technology' Webster and Wood editors.
Source: USDA nutrient database.

emulsification and digestion of fat. The reabsorption of the bile acids is also inhibited by the increased viscosity and the rate of excretion increases. In preparation for digestion of the next meal, new bile acids are synthesized from cholesterol stores in the liver. The consumption of 3 g of oat or barley β-glucan daily is recommended to lower cholesterol levels.

Blood Sugar Control and Type 2 Diabetes

The increased viscosity associated with β-glucan also influences the digestion and absorption of carbohydrates. Oat foods have been shown to reduce the concentration of glucose in the bloodstream in the 2 h following a meal. The postprandial

blood glucose levels are significantly lower than a control meal in a dose-responsive manner. The glycemic index (GI), a ranking of carbohydrate foods on a scale from 0 to 100 according to the extent to which they raise blood sugar levels after eating, is frequently used to assess oat foods and many oat foods are considered to have low GI (<55).

It is believed that the mechanism of action involves a number of elements. The increased viscosity of the meal bolus slows gastric emptying rate and impedes the mixing of digestive enzymes with the food, slowing the release of glucose from starch. It also slows the rate at which glucose is transported to the gut wall and absorbed. Reduction in the rate of glucose absorption means that the pancreas is able to respond with insulin to remove glucose from the bloodstream more effectively, thus blunting postprandial spikes in blood glucose levels. The attenuation of blood glucose and insulin levels has been associated with a reduction in the risk of the development of type 2 diabetes.

Satiety and Obesity

The extension of satiety is desirable to prevent hunger between meals and reduce calorie intake at the following meal. In addition to extending the time required for blood glucose concentrations to return to baseline and attenuating insulin levels, oats appear to influence other gut hormones. Both protein and increased viscosity have been associated with extended satiety and may contribute to this effect.

Antioxidants and Anti-inflammatory Potential

Avenanthramides are a class of phenolic compounds found uniquely in oats. They are phenolic acids conjugated with amides that have been shown to be potent antioxidants. They are found as both free and bound forms in the groat. Anti-inflammatory and antiatherogenic activities have been demonstrated by avenanthramides at parts per million levels, suggesting that they may reduce the risk of cardiovascular disease. Avenanthramides decrease the expression of endothelial proinflammatory cytokines at least in part through the inhibition of nuclear factor-κB activation and proteasome activity suppression.

Oat Ingredients and Foods

Oats are consumed in a wide variety of food products. Whole groats can be boiled and eaten as a rice substitute. Frequently, oats are milled and flaked to produce oatmeal used to make porridge or baked goods. Oat flours are used for breads, breakfast cereals, and baked goods. Oat bran is made by removing part of the endosperm to enrich the nutrients found in the subaleurone and aleurone layers.

Oat Milling and Processing

When harvested oats arrive at the mill, they are subjected to a series of procedures before they can be used in food products. Although they may have received some preliminary cleaning prior to transportation, the first step at the mill is a cleaning process to remove unwanted materials (e.g., seeds from other crops, weed seeds, stones and dust, and metal fragments). Various devices are used to accomplish this, including screens, gravity tables, aspirators, and magnets. At the end of this process, the clean milling oats, or green oats, are graded into up to four size fractions based on density and weight, which optimizes the efficiency of the next step: dehulling. The most common method of hull removal uses impact dehullers, in which oats are fed onto a rotating disk that propels them onto the cylindrical housing of the dehuller, where the impact separates the hulls from the groats. The lighter hulls are removed by aspiration, and undehulled oats are separated from groats by a density table system and returned to the dehulling stream. A set of scouring brushes may be used to further clean the groats and remove trichomes before kilning. During the kilning process, steam is used to inactivate enzymes in the raw groats, which are then roasted to develop a desirable toasted flavor, and the moisture content is reduced sufficiently for product storage. Kilned groats can be flaked whole, to produce large flake oats, or cut to produce steel-cut groats, which can be used as is or flaked further to produce quick-cooking oats. The oats (whole or steel-cut) are first steamed or tempered to toughen the groat and minimize the production of fines, before flattening between two large rollers. Further processing to either oat flour (used mostly for baby foods and ready-to-eat cereals) or oat bran can start with either stabilized whole groats or flakes. Whole oat flour or a combination of low bran oat flour and oat bran can be produced. Hammer mills are most commonly used to grind the product, although rollers can also be used.

Oat Products

Oat porridge is probably the oldest and best known oat food. It is made by boiling oatmeal in water and/or milk until the starch is gelatinized. Oatmeal is available in a range of flake sizes from large flake, which results in a coarse, chewy texture, to instant oatmeal, which has a smooth, creamy texture. Steel-cut oats are cut rather than flaked. They require a longer cooking time than oatmeal and result in a coarser texture. Oat bran is also used to make porridge with higher fiber content.

Oatmeal, oat bran, and oat flour are used in a wide range of products, from breakfast cereals to bakery products. Oat flour and bran, alone or in combination with other flours, are extruded to make ready-to-eat breakfast cereals. The heat and pressure of the extrusion process gelatinize the starch and increase the solubility of β-glucan. Extruded whole oat and oat bran cereals have been shown to reduce cholesterol in people with mildly elevated cholesterol levels when they consume at least 3 g of β-glucan daily. Oat bran cereal containing at least 4 g β-glucan per serving has low GI, whereas whole oat cereal has high GI. Oat bran cereal has also been demonstrated to extend satiety and influence gut hormones associated with hunger.

A wide range of bakery products containing oats and oat fractions are now available. Breads can be found on supermarket shelves containing both whole oats and oat bran. The long proofing time required for the yeast fermentation has been shown to result in partial depolymerization of β-glucan in breads containing wheat flour in addition to oat ingredients.

The depolymerization appears to be caused by endogenous β-glucanase enzymes in wheat. Despite the partial degradation of β-glucan, several studies have found that oat bran bread significantly reduced LDL cholesterol levels in people with mildly elevated cholesterol, although some studies failed to demonstrate significant reductions. Other bakery products incorporating oatmeal, oat bran, and oat flour include muffins, granola bars, cookies, and crackers. Unlike yeast breads, chemically leavened products have a shorter preparation time and tend not to exhibit depolymerization of β-glucan. Oat bran muffins with at least 4 g of β-glucan per serving significantly reduced postprandial blood glucose response. Whole oat muffins and muffin mixes have low to medium GI. Oat bran, which is composed of the outer layers of the groat (the pericarp, aleurone, and subaleurone layers), has been shown to contain the highest levels of β-glucan, as well as avenanthramides, the bioavailability of which has been demonstrated in oat bran muffins.

Increasingly, oats are being used in less conventional applications. Oat pasta is still a novelty in North America and Europe, but it is common in some areas of China. A host of novel food products incorporating oats or oat fractions have been developed in Scandinavia, such as yogurt-like fermented products and high β-glucan drinks. Oat milk is also available. The soluble components are extracted from oats in a process similar to the manufacture of soy milk. There is preliminary evidence that oat milk reduces cholesterol when consumed on a regular basis.

Microstructure

Raw Groat

The oat groat is packaged much like most of the cereals (Figure 2), with a protective pericarp surrounding the kernel, inside of which is the aleurone layer, made up of a single layer of thick-walled, cuboidal cells. This layer contains the bulk of the minerals and vitamins of the oat, as well as significant amounts of protein and lipid. These outer layers, as well as a small part of the endosperm (typically just one or two layers of thick-walled subaleurone cells), make up the bran fraction in milling. At the proximal end of the kernel is the embryo, or germ, which contains the scutellum and the embryonic axis. The germ is also high in lipid and protein and, in the scutellum, some minerals as well. The remainder of the kernel is composed of the starchy endosperm, which is the main storage tissue of the kernel.

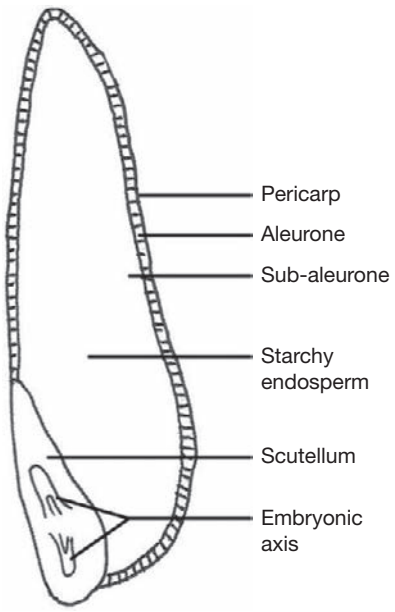

Figure 2 Diagram showing the different tissues of the oat groat.

Like most other cereals, the starchy endosperm contains starch granules embedded in a protein matrix. Figure 3(a) shows a cross section of oat groat stained to demonstrate starch (purple) and protein (green). Starch is present mainly in compound grains, which are composed of multiple smaller granules, although some larger single granules are also present. Individual protein bodies can be distinguished in the protein matrix, particularly in the subaleurone region, where there is less starch. Unlike most cereals, the endosperm also contains significant amounts of lipid distributed as small lipid bodies throughout the protein matrix, although in much lower concentrations than the protein and starch. The cell walls of the starchy endosperm are also rich in β-glucans (up to 85% of the wall), particularly in the subaleurone region, where the walls are typically thicker than in the central endosperm. Figure 3(c) shows a section of oat in the subaleurone region, stained with calcofluor white to demonstrate β-glucans. The walls are thickest immediately adjacent to the aleurone layer (at the top of the micrograph) and become progressively thinner into the endosperm.

Cooked Groat

Although the majority of oats for food use are cut and flaked before packaging or incorporation into other products, there is a growing trend for the consumption of whole cooked groats as a rice alternative. In the whole cooked groat, the nutritional components remain mostly in place, although the starch granules within the larger compound grains swell and often fuse in varying stages of gelatinization. In some areas, amylose can leach from some granules and aggregates on the outside of the granules, visible as a darker purple outline in the upper left corner of Figure 3(b). There is also some disruption of the cell walls and leaching of β-glucans (Figure 3(d)). The β-glucans released from the wall would be more available for physiological action in the gut.

Oat Products

Figure 4 presents cryosections of some common oat cereals. Although a proportion of whole, large groats are flaked after kilning to make large flake, or 'old fashioned' oatmeal, most groats enter the production line as steel-cut oats. Despite being kilned, the microstructure of the raw steel-cut oat differs very little from a raw groat (Figure 4(a)). The starch granules are very slightly swollen, but little gelatinization has occurred. However, after cooking in water to make porridge, the starch granules are swollen and fused, and the cell walls are swollen as well (Figure 4(b)). The level of gelatinization of starch

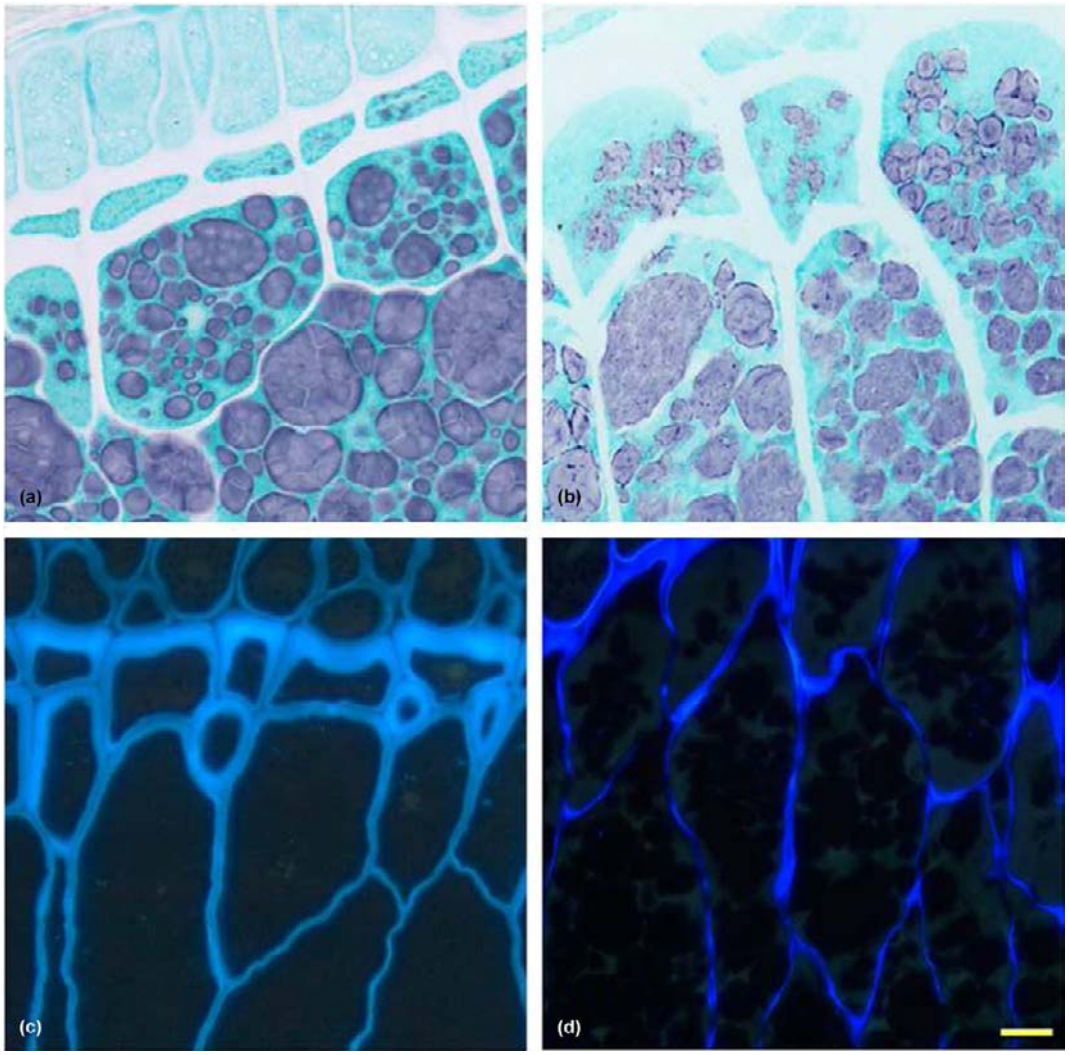

Figure 3 Raw (a, c) and cooked (b, d) oat groats. (a, b) Resin-embedded sections stained with light green to demonstrate protein and iodine/potassium iodide (IKI) to demonstrate starch. (c, d) Stained with calcofluor white to demonstrate mixed-linkage β-glucan. Bar = 20 μm.

granules varies depending on how close to the cut surface they are. Starch, protein, and cell wall material released from the cut surfaces of the oats form a matrix of sorts for the cut oat pieces in the steel-cut oat porridge (see bottom of Figure 4(c)).

Steaming and flaking of whole groats to make large flake oats cause many ruptures in the endosperm, and some gelatinization of starch granules is evident, even before cooking. Although there is some cell wall fragmentation due to tissue disruption, the β-glucans in the wall are essentially intact (not shown). Oat flakes in this form are often incorporated into muesli, granola mixes, and granola bars, with little change in microstructure or nutrient distribution. When the large flake oats are cooked in water to make porridge, the starch granules swell and fuse, ultimately becoming completely gelatinized (Figure 4(d)). In areas of the endosperm with intact cells, the cell walls are swollen but intact.

When the pieces of steel-cut oats are steamed and flaked, quick-cooking oatmeal is produced. Raw flakes of quick-cooking oatmeal show more extensive tissue disruption and swelling and gelatinization of starch granules (Figure 4(e)). Like the large flakes, quick oats can be used in muesli, granola mix, or granola bars as is. Cooking as porridge continues the gelatinization of starch, with granule swelling, fusion, and gelatinization, accompanied by leaching and aggregation of amylose from the starch granules (Figure 4(f)). Where there are intact cells, the walls are disrupted, and β-glucans are leached from the walls. This is particularly evident in the thick subaleurone walls (Figure 5(a)). Leaching of β-glucan from cell walls was observed in all cooked oat cereals.

Cooking of the various porridges renders the starch and proteins more easily digestible and also partially solubilizes the β-glucans. The viscosity increase caused by the increase in soluble β-glucan blunts glycemic response, while the heat and moisture increase the fraction of starch that is rapidly broken down to glucose.

Raw oat flakes are often further milled into flour and/or oat bran to make infant cereals or extruded ready-to-eat cereals. Even in a formulated extruded cereal, fragments of oat kernel

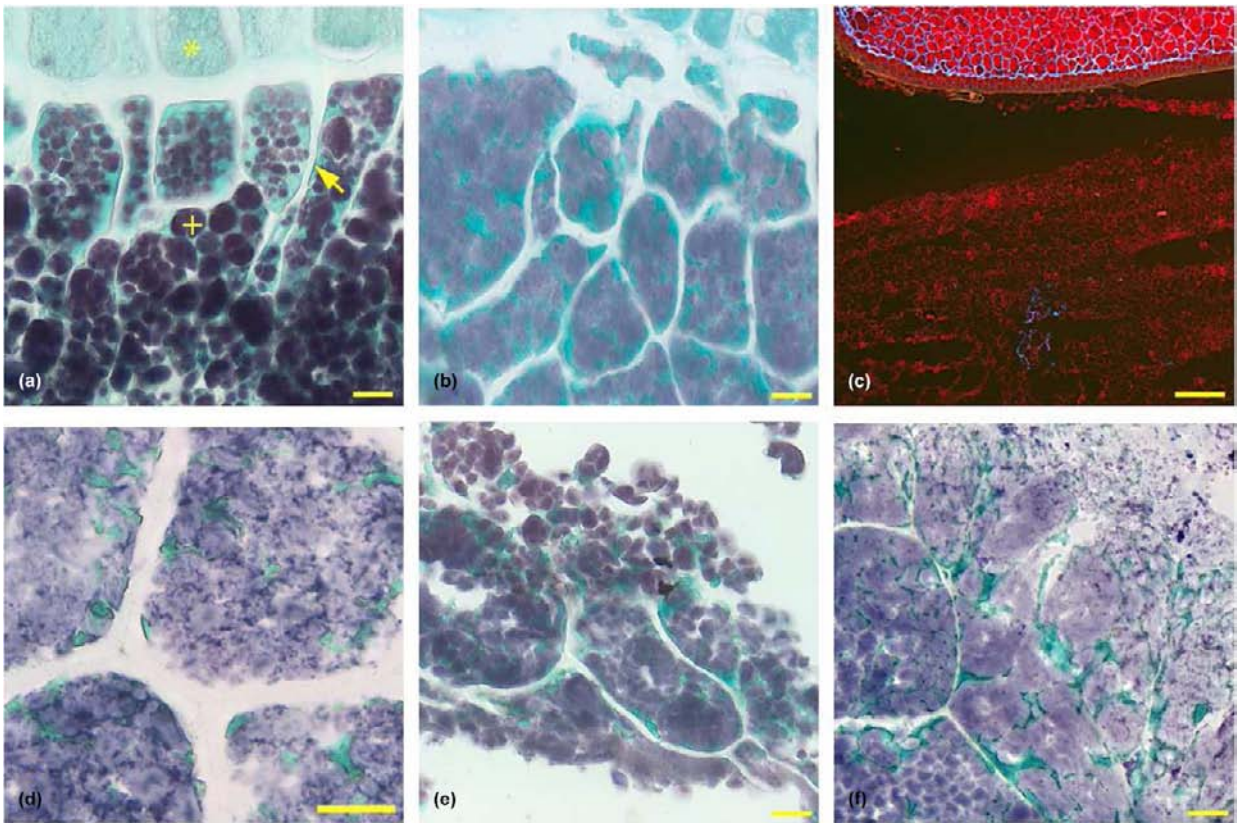

Figure 4 Raw and cooked oat cereals. (a–c) Steel-cut oats. (a) Raw, stained with IKI; *, aleurone cell; +, starch granule; arrow, subaleurone cell wall. Bar = 20 μm. (b) Cooked, IKI, bar = 20 μm. (c) Cooked, CF & FG, bar = 200 μm. (d) Large flake 'old fashioned' oats. Cooked, IKI, bar = 20 μm. (e, f) Quick oat flakes. (e) Raw, IKI, bar = 20 μm. (f) Cooked, IKI, bar = 20 μm. IKI, iodine/potassium iodide reagent; FG, fast green; CF, calcofluor white. Cryosections.

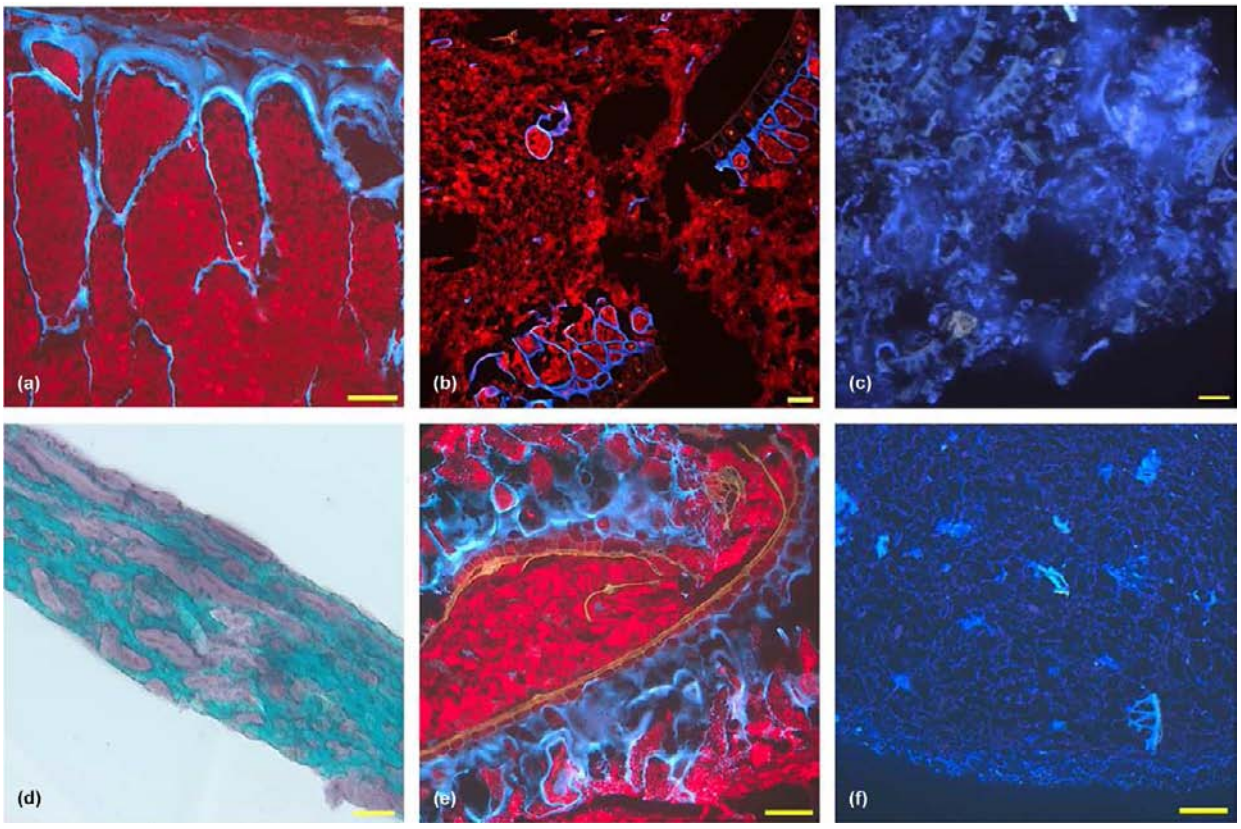

Figure 5 Oat products: (a) Quick oat flakes, cooked, CF & FG, bar = 50 μm. (b) Ready-to-eat cereal, CF & FG, bar = 50 μm. (c) Oat bran muffin, CF, bar = 100 μm. (d) Oat bran bread, IKI, bar = 20 μm. (e) Oat bran bread, CF & FG, bar = 50 μm. (f) Oat bran pasta – cooked, CF, bar = 100 μm. IKI, iodine/potassium iodide reagent; FG, fast green; CF, calcofluor white. Cryosections.

are recognizable (Figure 5(b)), although gelatinization of starch granules is extensive. Large pieces of oat bran can be seen, in which the β-glucans are clearly visible in the intact subaleurone walls. Smaller fragments of the cell wall (blue flecks) are also visible in the starch matrix. Disruption of the cell walls may affect physiological function.

Some products that are primarily wheat-based, such as quick breads, yeast breads, and pasta, can be enriched with oat bran, thus combining the processing functionality of wheat with the physiological benefits of oats. The physiological efficacy of β-glucan in quick breads such as muffins is affected by storage conditions. Figure 5(c) shows a section of a fresh oat bran muffin. Many fragments of aleurone can be distinguished; however, the subaleurone cell walls are not distinct. Instead, the β-glucan has been solubilized and is present as an amorphous matrix throughout the muffin. Yeast breads have a spongelike microstructure, with air cells surrounded by 'walls' with starch granules embedded in a fibrillar gluten (protein) matrix (Figure 5(d)). At the junctions where the air cells are connected, thicker areas of protein matrix can be found. Figure 5(e) shows one of these junction areas in bread enriched with oat bran. During the long fermentation process required for the dough to proof and rise before baking, the β-glucan in the dough is partially depolymerized by enzymes in the wheat (Figure 5(e)), which may compromise the physiological effectiveness of the product. Pasta is another wheat-based product that can be enriched with oat bran for putative health benefits. Figure 5(f) shows a cross section of a cooked noodle enriched with oat bran. When the cooked pasta is stained to demonstrate β-glucan, fragments of oat cell wall can be seen throughout the protein–starch matrix. In addition, there is a layer of solubilized β-glucan that has migrated to the surface of the noodle during cooking (bottom), which would tend to make the β-glucan more available.

Summary

Oats are a globally produced crop, although they account for only 1% of world's cereal production. They provide excellent nutrition from both macro (protein, starch, lipid, and fiber) and micro (vitamins, minerals, and antioxidants) components. Whole oats and a variety of oat products and fractions are available for use as is or the incorporation into oat-enriched foods. Consumption of oats and oat products has been shown to be beneficial for both lipid metabolism and carbohydrate metabolism in humans. Much of this benefit is attributed to the content of mixed-linkage β-glucan, as well as antioxidants such as avenanthramides. The relative availability (and therefore physiological functionality) of these and other nutritional components are impacted by both industrial processing and home preparation practices, which are reflected in microstructural changes.

See also: Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Barley; Carbohydrate: Digestion, Absorption and Metabolism; Cereals: Dietary Importance; Cereals: Types and Composition; Cholesterol: Absorption, Function and Metabolism; Dietary Fiber: Bran; Dietary Fiber: Determination; Functional Foods; Glucose: Metabolism and Regulation; Proteins: Chemistry, Characterization, and Quality; Starch.

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Obesity Management

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Introduction

The prevalence of obesity has nearly doubled in the past generation, with two-thirds of American adults now overweight or obese. If current trends continue, it has been projected that essentially all Americans will be overweight by 2030. The recent increase in prevalence is even more dramatic among children and the extremely obese.

What is causing this epidemic? Despite recent discoveries in the molecular genetics of obesity, genetic influences are not a likely explanation for the rapid recent changes in the prevalence of obesity. Obesity is a result of a complex interplay between genetic, behavioral, and environmental factors that include diet and exercise.

In the United States, although the percent of energy intake derived from fat is decreasing (from a high of 40% to about 32% today), total daily caloric intake is increasing, and refined carbohydrate intake is increasing. Meanwhile, growing numbers of adults and children engage in virtually no physical activity. The built environment is a large contributor to this inactivity and, thus, plays a significant role in the US obesity epidemic.

Obesity is close second to cigarette smoking as the most important modifiable cause of death. It is a risk factor for diseases of virtually every organ system, including certain cancers, and the most important risk factor in the development of diabetes and multiple other health complications. The risk of comorbid medical conditions increases with the degree of obesity, although for some complications – notably dyslipidemias, which are associated with cardiovascular risk factors, type 2 diabetes, and hypertension – the risk correlates best with the regional distribution of fat.

Obesity also increases overall mortality and shortens life expectancy by at least several years in individuals who are $>25\ \mathrm{kg\,m^{-2}}$; this reduction can be dramatic for those who have a BMI of $>35\ \mathrm{kg\,km^{-2}}$. In addition to the medical risks, often more motivating for many people seeking to lose weight are the untoward psychosocial consequences of obesity.

This article aims to provide an overview of the assessment and treatment of obesity, with the primary focus on dietary, exercise, and behavioral interventions. Pharmacological and surgical therapies are a second-line option in the treatment of obesity and are reviewed in less depth.

Evaluating Obesity

Obesity is defined as an excess of body fat (>25% of body weight for men and >30% for women) rather than an excess of body weight per se. However, the measurement of percent body fat is more difficult to obtain and not as intuitive as body weight. Measuring weight adjusted for height, or BMI, defined as weight in kilograms divided by the square of the height in meters, is usually the first step in assessing obesity. While BMI is the standard measure of relative weight, it may overstate the actual degree of adiposity in very muscular people (e.g., certain types of athletes and laborers) and may understate adiposity in very sedentary individuals with little muscle mass. A BMI of 25–30 $\mathrm{kg\,m^{-2}}$ is defined as overweight, 30–40 $\mathrm{kg\,m^{-2}}$ as obese, and 40 or more $\mathrm{kg\,m^{-2}}$ as severely/morbidly/grade III obese.

Waist circumference is the second step in the assessment of obesity. In women, a waist circumference of greater than 35 in. and, in men, greater than 40 in. constitutes abdominal/visceral obesity and is associated with an increased risk of health complications. Waist circumference can be easily measured with a tape measure around the widest point above the hips. Note that visceral obesity can exist even in the absence of overall obesity (i.e., at BMIs below the cutoff points for obesity or overweight). With visceral obesity, even with a normal BMI, it is probably best to encourage weight loss for medical reasons.

Dietary Assessment

A formal dietary assessment, best conducted by a registered dietitian with training and expertise in weight management, can identify problem areas in diet requiring modification for weight loss to occur. It may consist of a 24 h recall of food intake, a food frequency assessment to determine adequacy and composition of the diet, anthropometric assessment, and an interview assessing meal patterns, beverage consumption, food/taste preferences, portion sizes, grocery shopping/cooking pattern, restaurant dining, and any religious or cultural customs that may affect the patient's diet.

The results should be interpreted cautiously, because both underreporting and restrained eating while under observation (either intentional or unintentional) are common. Despite these shortcomings, the information gathered can be quite valuable. For example, if an individual reports drinking almost 1000 calories per day in juice and soda, simply switching to noncaloric beverages would promote a significant weight loss (assuming dietary compensation does not occur).

Metabolic Testing

Assessment of the patient's resting metabolic rate (RMR) via a metabolic cart (indirect calorimetry) is recommended if the necessary equipment and staffing are available. RMR (also called resting energy expenditure (REE)), obtained by either metabolic cart or estimation formulas, is then used to determine daily caloric needs by multiplying RMR by an activity factor ranging from 1.3 (for very sedentary) to 2.0 or more (for very physically active individuals). This estimate of total daily energy expenditure is used by the dietitian to determine the patient's initial caloric requirements and an estimated rate of weight loss, given a specific reduction in energy intake in the diet.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00744-3

Nutrition

Nutritional Management of Mild to Moderate Obesity

Nutritional management of obesity entails an individualized approach, based on the dietary assessment, within a multidisciplinary setting whenever possible. For patients with mild to moderate obesity, a moderate caloric deficit of at most 500–1000 calories per day is recommended to promote a 0.4–0.85 kg weight loss per week. A low-calorie, individualized food-based diet that is either balanced-deficit (reducing the total number of calories while keeping proportions from carbohydrate, fat, and protein basically the same as before) or fat-deficient diet, with most of the caloric reduction resulting from restriction of fat, can be prescribed. The latter approach is preferable for Americans, whose typical diet is too high in fat. Also, a greater volume of food can be eaten on a diet that emphasizes complex carbohydrates and reduces fat intake to 20–25% of calories consumed.

The diet must be realistic – that is, based on dietary modification and practical changes in eating habits. Nutritional recommendations should be determined by the patient's current eating habits, preferences, lifestyle, ethnicity and culture, other coexisting medical conditions, and potential nutrient–drug interactions. The patient should be advised to drink at least 1.5–2.0 l of water daily, unless contraindicated (e.g., by congestive heart failure, edema, or renal insufficiency). Patients should also be encouraged to self-monitor their food intake, which may include measuring portion sizes and recording and calculating calories, fat grams, and/or carbohydrate grams. If energy intake is prescribed below 1200 calories per day, taking a daily multivitamin and mineral supplement is helpful to ensure adequate intake.

Nutritional Management of Severe Obesity

A medically supervised very low calorie diet (VLCD) (fewer than 800 calories per day) weight management approach is appropriate for individuals who are severely overweight (generally BMI $\geq$ 35) and/or those with serious medical conditions related to their obesity. Obese patients who have failed multiple weight reduction attempts through traditional programs may also be considered for aggressive strategies. A VLCD may consist of food, commercially available liquid supplements, or a combination of the two. It is recommended that patients being considered for VLCDs undergo a comprehensive medical examination and an ECG. Absolute contraindications include the presence of untreated or severe cardiac, hepatic, renal, or thromboembolic disease; type 1 diabetes; cancer; and severe eating disorders. Weekly medical supervision is recommended while on the VLCD. With full adherence, weight loss averages 1.1–1.8 kg per week, depending on body mass and physical activity level. A high protein content consumed during a VLCD may minimize muscle wasting as the rapid weight loss occurs. The diet period is followed by progressive refeeding. Throughout the course of dieting, education on nutrition, exercise, and behavior modification will help the patient maintain his or her weight loss during and beyond the refeeding phase. For this reason, multidisciplinary treatment is strongly recommended in combination with the VLCD. Without this education and support, weight gain will likely occur almost as rapidly as weight loss.

Exercise

Exercise/Fitness Assessment

The exercise/fitness assessment should be performed by a trained exercise physiologist and should explore the patient's usual degree of physical activity, any limiting factors such as joint disease or injuries, preferred types of activity, and measurement of the patient's current level of fitness. A fitness test consisting of a Harvard step test, test of flexibility, and bioelectric impedance testing may be used to assess body composition. A formal stress test is not required unless active cardiovascular disease is suspected. The exercise physiologist may also explore lifestyle factors such as usual work hours, social support, and nearby facilities or other resources that could promote increased activity level.

Exercise Treatment

Although regular, moderate physical activity alone results in limited weight loss over the long term, it is an essential component of any weight management program. Research has shown that patients who diet and exercise regularly are much more likely to maintain weight loss than those treated with diet alone. When performed in conjunction with caloric restriction, regular, moderate physical activity achieves the following effects: increases 24 h energy expenditure, maintains (or minimizes loss of) lean body mass, reduces cardiovascular risk by producing beneficial changes in the lipid profile, has positive psychological effects, improves insulin sensitivity, and may provide other health benefits independent of weight loss.

In determining an appropriate, individualized exercise program, the exercise physiologist and the patient together should consider a plan that (1) fits into the patient's schedule and lifestyle, (2) is liked or disliked by the patient, (3) makes use of the patient's resources, and (4) is based on the patient's current level of fitness. The exercise physiologist should ensure that, before beginning a fitness program, all patients can recognize and deal with abnormal physical responses to physical activity.

When beginning an exercise program, it is worthwhile to start with small goals and increase the level and intensity of exercise gradually. Eventually, the exercise goal of any weight management program should be 30–60 min of continuous, moderate-intensity physical activity five to seven times per week. Until a patient can tolerate 30 min of continuous activity, several 10 min periods of physical activity throughout the day can help build the patient's endurance while still burning calories. Additionally, short exercise and rest intervals can be alternated. The intensity, duration, and frequency of activity should be progressively increased, according to the patient's increasing ability. Patients should be encouraged to self-monitor their daily physical activities, including type of activity, time performed, and duration, so that progress can be appreciated and the goal reset a bit higher from time to time.

Resistance Training

Resistance (strength) training can be added to an aerobic exercise regimen and has been shown to help preserve muscle mass. Studies have shown that resistance training along with

aerobic exercise in addition to calorie-restricted diet results in weight loss along with muscle strength preservation and improvements in physical functioning. While resistance training may not correlate with body weight changes during the weight loss phase and/or may result in weight gain since muscle mass (which is denser than fat) increases with resistance training, it is important for weight maintenance. One study reported that those who incorporated resistance training during the weight maintenance phase were likely to regain fat mass at a slower pace or were less likely to regain fat mass at all, compared to those who did not, in spite of overall body weight regain. Additionally, resistance training is associated with lower levels of visceral fat, which corresponds to a lower risk of cardiovascular disease. While a lot of emphasis has been placed on aerobic training to lose body weight/fat, combining resistance training with calorie-restricted regimen for weight loss can indirectly aid in both weight loss and maintenance by improving muscle mass, strength, and endurance.

Behavior Therapy

Psychological and Behavioral Assessment

A psychosocial and behavioral assessment is an important component of multidisciplinary treatment that can provide considerable information about the patient's readiness for lifestyle change, emotional health, and triggers for inappropriate eating. The assessment explores the patient's history of weight gain and loss, history of previous diet attempts, patterns of weight maintenance or relapse, and motivation for weight loss. Current eating behaviors are assessed, and antecedents or 'triggers' that cause the patient to eat when not hungry or to overeat are examined. Triggers might be environmental (e.g., seeing or smelling tempting food), mental (e.g., thoughts about food), emotional (e.g., eating for comfort and eating for reward), social (e.g., dining out with friends and alcohol consumption), habitual (e.g., eating at a certain time), or physiological (e.g., poor sleep and hormones). The stages of change model for behavior change intervention may be useful in assessing the readiness state of the patient to make behavior changes, while motivational interviewing techniques can be used to identify reasons for wanting to change behavior, as well as barriers that have a negative impact on motivation.

A licensed clinician should conduct a thorough psychological evaluation and screen for mental health conditions, past or present. Both mood disorders and eating disorders are prevalent in this population. Obese persons with significant mood-related symptoms should be provided appropriate treatment (cognitive behavior therapy and/or pharmacotherapy) prior to or concomitant with weight reduction efforts. Individual or group psychotherapy may also be beneficial to address other issues related to obesity, such as low self-esteem, body image, and relationship problems. Binge eating or other eating disorders often coexist with obesity and may require separate treatment by a specialist.

Behavioral Treatment

Behavioral treatment of obesity aims to identify triggers for excess caloric intake/inadequate physical activity and to develop approaches to address these behaviors. Studies show that behavior therapy as an adjunct to exercise and diet therapy for weight loss produces significantly better results than exercise and diet alone. Typically, a psychologist or mental health professional on the team suggests or implements behavioral techniques, although these tools may be useful in all components of treatment. The principles used in behavior modification typically include goal setting, self-monitoring, stimulus control, contingency management, self-care (including stress management and sleep hygiene), cognitive restructuring, social support, and relapse prevention.

Goal Setting

Whether the goal is a target weight loss, daily calorie, or exercise goal, goal setting is a common component in behavioral weight loss treatment. Research clearly demonstrates that goals can favorably enhance performance, including weight loss outcomes. Larger goals can often be broken down into smaller more manageable goals that are easier to implement and monitor.

Self-Monitoring

Self-monitoring for weight management generally involves recording dietary intake and physical activity to increase the patient's awareness of his or her own behavior and to promote positive behavior change. Research has consistently demonstrated that self-monitoring is associated with improved treatment outcomes, and patients often report that it is one of the most helpful obesity management tools. Patients may be encouraged to record the time of eating episodes, type and amount of food eaten, feelings of hunger and satiety, place, context, and/or comments in order to better understand behavioral patterns and triggers. Self-weighing allows participants to assess the effectiveness of their current weight loss strategies and to make corrective action if necessary. Regular self-weighing is associated with lower BMI and greater weight loss.

Stimulus Control

Evidence for the impact of the food and physical activity environment on eating and sedentary behaviors is well documented. Eating cues in the environment are numerous, ever-present, and often outside of an individual's conscious awareness. Stimulus control strategies might include recommendations such as only eating at the kitchen table and not in front of the television, keeping tempting, high-calorie foods out of the house (or, at least, out of sight), and laying out exercise clothes the night before as a reminder to exercise in the morning.

Problem Solving

Motivational interviewing techniques can be used to help patients identify and overcome barriers to behavior change (i.e., no safe place to exercise outside the home and dislike of vegetables).

Contingency Management

Contingency management involves rewarding desired changes in behavior, such as increasing frequency of exercise or reducing intake of fast foods. The rewards can be granted by the patient, the patient's family, and/or the weight management center; however, rewards must be of value and reinforcing to the patient in order to be effective. One contingency management technique used to enhance motivation is incentives (usually money).

Cognitive Techniques

Behavioral-based therapies have evolved over time to emphasize cognitive elements. For example, participants may be taught to stop dichotomous thinking (e.g., I blew my diet; I'm off the program), to ban perfectionist attitudes and imperatives (e.g., I will *never* eat more than 1200 calories a day), and to be aware of attitude traps (e.g., my life will be perfect when I lose weight; I can't wait for the program to end so I can get back to normal eating). Mindfulness-based (e.g., acceptance) and control-based (e.g., distraction and delay) techniques may be taught as a means of helping participants manage cravings or thoughts about food. In addition, problem-solving techniques are often taught to patients to help them to break down more difficult or larger barriers to successful behavioral change.

Self-Care

Behavioral intervention targeting self-care focuses on improving sleep hygiene, time management, and stress management. Sleep and stress are closely and reciprocally linked. In addition, both sleep deprivation and high levels of stress appear to have a detrimental effect on weight management. One recent study, involving a 6-month behavioral weight loss intervention, found that lower baseline stress levels and longer sleep duration predicted greater weight loss over the course of the intervention.

Social Support

Social support, usually from the patient's family or a support group, is used to maintain motivation and provide reinforcement for appropriate behavior changes. Facilitated support groups are often an integral part of comprehensive weight loss treatment for these reasons, and they further serve to 'normalize' the ups and downs of the treatment process. Studies show that group treatment can produce significantly greater weight loss (~13%) than individual sessions (>11%) and are more likely to help maintain weight loss.

Relapse Prevention

Given the high rates of dietary relapse following weight loss, researchers have attempted to provide relapse prevention training to improve long-term weight loss maintenance. Based on models such as Marlatt and Gordon's relapse prevention model, weight loss researchers have attempted to help participants identify situations with high risk for a dietary lapse and to teach coping skills to successfully cope with the relapse crisis.

In summary, comprehensive psychological and behavioral therapy is crucial in the treatment of obesity. The aforementioned strategies, employed throughout treatment, help patients to better understand and modify problematic thoughts, moods, and behaviors. The resulting lifestyle changes can effectively bring about modest, yet clinically significant weight loss.

Pharmacological Treatment for Weight Loss

Lifestyle modifications discussed earlier form the cornerstone and first stage of any weight loss plan. Adjunctive anorectic medications may be useful, if this approach (lifestyle modifications including diet, exercise, and behavior therapy) alone does not result in weight loss. Pharmacotherapy may be helpful at the point where compliance to lifestyle modification begins to waver or physical hunger becomes an issue during dieting. There is little doubt that such medications significantly increase weight loss during the period in which they are used and may help with maintaining weight loss (though there tends to be regain even with continued use). However, pharmacological treatments are best suited for those who are severely obese ($>40\ \mathrm{kg\ m^{-2}}$) or who have significant medical comorbidities. The agents available are growing in number in very recent years and include appetite suppressants and fat-absorption blockers.

Surgical Treatment of Obesity

While lifestyle modifications that include combinations of diet, exercise, behavior therapy, and pharmacological agents for the severely obese can produce changes in body weight, these changes may prove to be inadequate, leaving weight loss surgery as a second-stage approach. The NIH consensus conference on gastrointestinal surgery for severe obesity concluded that weight loss surgery may be an appropriate option for those who are severely obese ($\mathrm{BMI} \geq 40\ \mathrm{kg\ m^{-2}}$) or those who have a BMI of $>35\ \mathrm{kg\ m^{-2}}$ with two or more obesity-related comorbidities.

Before an obese individual decides upon surgery to lose weight or their physician decides to recommend surgery as an option, it is important to thoroughly evaluate the patient using a multidisciplinary approach. This should ideally involve not only the surgeon and referring physician but also dietitians, psychologists, nurses, and an anesthesiologist. The patient's medical and nutritional history must be evaluated, as well as psychological assessment to better understand any underlying issues before a decision should be reached regarding suitability for surgery. Contraindications include issues that can hinder adherence to the recommended postoperative care needed to achieve expected outcomes. These issues include psychological disturbances, medical problems that may worsen or contraindicate major surgery, and a nonsupportive environment.

In summary, surgical treatments are the most efficacious option for individuals who have severe obesity and show

weight loss superior to other approaches. However, the rate of success following bariatric surgery is highly variable, and risks of postoperative complications are present. Therefore, a multidisciplinary approach needs to be adopted so that proper evaluation and selection of the patients occurs preoperatively and long-term postoperative care and monitoring is ensured.

Conclusions

Maintenance

Maintaining weight loss seems to be inherently more difficult than losing weight. Unfortunately, the long-term success rate for all weight loss methods (excluding surgery), defined as losing weight and keeping the majority of it off for at least 5 years, is low – perhaps 5–15%. Even after the most state-of-the-art, medically monitored comprehensive treatment, it is likely that patients will gradually return to their baseline weight within 3–5 years after treatment termination. Only the continuous-care model of management, which views obesity as a chronic disease requiring continuous support or contact after the end of formal treatment, has been found to produce significant maintenance of weight loss.

There are many factors that contribute to this relapse, including low motivation and support from the provider diminishing or ending. While weight regain is very difficult to prevent entirely, continuation of some form of exercise may slow and diminish its degree. Those who exercised regained ~7 lbs less in body weight compared to those who did not exercise. Other studies show that physical activity alone may not help in maintaining weight loss, though. Dietary restraint along with physical activity may prove to be more successful in maintaining weight loss and slowing/preventing weight regain than physical activity alone. Restricting calories from fat along with high physical activity may prove to be the most effective strategy to maintain weight loss on a long-term basis.

One study showed that teaching individuals to take responsibility for weight maintenance by developing problem-solving skills to overcome setbacks improves weight loss maintenance. These techniques and skills include providing incentives for motivation, learning how to preplan weekly low-calorie meals, how to control portions, providing additional support from peers, increasing provider and patient contact, and encouraging self-monitoring. Social support, along with these techniques, improves weight maintenance after initial weight loss. Thus, several studies suggest that behavior therapy beyond the initial weight loss phase is important in maintaining the weight loss.

While several diets have been reported superior for achieving weight loss, the choice of a specific dietary approach needs to be individualized based on an individual's goals, medical needs, and preferences. No one dietary approach appears to be substantially better or worse than the others. Many people are motivated to lose weight faster during the initial phases of weight control, which can help guide the choice of dietary approach toward a more aggressive level of energy restriction, if medically appropriate. It is probable that some form of continuous or punctuated long-term care will be necessary for many (if not all) obese patients to maintain the dietary and lifestyle changes that produced their weight loss. With ongoing support from a physician or other health care providers, patients will be better able to maintain their changes in eating and activity behavior and the resultant weight loss.

Regrettably, optimal strategies for weight maintenance are in a state of evolution and represent the key limitation of our current treatment of this highly prevalent, medically hazardous, and chronic condition. Research has identified continued program contact (e.g., monthly), regular physical activity, nutritional sophistication, and self-monitoring as four predictors of weight maintenance. It not surprising that the strategies helpful in promoting weight loss, such as self-monitoring, physical activity, regular weigh-ins, and attendance at a multidisciplinary program, will be the same strategies necessary for long-term maintenance of weight loss.

Summary

Obesity management is perhaps the greatest challenge faced by health care professionals today. With the rise in obesity rates come a plethora of medical complications. Almost all adults who become obese will do so not because of a specific medical or metabolic condition, but because of lifestyle behaviors leading to increased food consumption and/or decreased energy expenditure. Further research is needed in understanding the basis of certain behaviors adopted by obese individuals and ways to modify them more effectively to achieve long-term weight management.

To prevent obesity or slow its progression, efforts should be devoted to improving environmental factors and personal behavior. While the obesity treatment modalities discussed in this article can be successfully applied to obese individuals, changing/improving our environment is the key to preventing and reversing this public health epidemic.

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Obesity: Causes and Prevalence

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Why We Care about Obesity

Obesity is one of the most challenging health issues facing society today. Almost every country around the world has seen increasing rates of this problem. Obesity affects people of all ages, ethnicities, and sociodemographic backgrounds. In the last 30 years, rates have doubled throughout the world. Contrary to popular perception, 65% of the world's population lives in countries where more people die from overweight than from underweight. Although obesity was once considered a disease of affluence, levels are increasing in low- and middle-income countries (LMICs), particularly in urban settings.

Overweight and obesity are defined as an abnormal or excessive accumulation of fat that may impair health. Body mass index (BMI) is a standardized, population-based measure used to categorize individuals as overweight or obese. An individual with a BMI of 25 or more is considered overweight, and someone with a BMI of 30 or more is considered obese. Obesity is classified further based on grades of severity: obese class I (BMI 30–34.9), obese class II (BMI 35.5–39.9), and obese class III (BMI 40 or greater). The higher a person's BMI, the greater the health risks. Among children, weight status is determined using age- and sex-specific percentiles for BMI. Overweight is defined as a BMI above the 85th percentile and obesity as a BMI above the 95th percentile for children of the same age and sex.

The obesity epidemic has immense consequences for both individuals and society. Obesity has negative effects on an individual's health, well-being, and quality of life because of resulting chronic conditions, which lead to increased disability and premature death. Obesity is the fifth leading mortality risk factor worldwide, and ~3.4 million adults die each year as a result of being overweight or obese. Obesity is associated with higher risks of developing hypertension, high cholesterol, osteoarthritis, respiratory disorders, and fertility problems. Furthermore, obesity increases the risk of type 2 diabetes, cardiovascular disease, and some cancers. Obesity also adversely affects an individual's mental and psychological well-being.

Obesity-related costs continue to increase globally, which compromises the economies and health-care systems of all nations. Annual US health-care costs attributed to obesity are estimated at approximately $190 billion. Health-care costs for obese patients are 40% higher than for those with normal body weight, and it is estimated that if obesity trends in the United States continue at the current rate, by 2030, obesity-related medical costs could increase $48–$66 billion per year. The increase in prevalence of type 2 diabetes, which costs the US economy an estimated $180 billion per year, has paralleled the increase in obesity. A large proportion of these costs are borne by overburdened public health-care systems, whose resources could be better utilized for nonpreventable conditions. In addition to direct health-care and disability costs, obesity is linked to increased costs in the workplace due to loss of productivity and excessive absenteeism and higher costs of adapting to an increasingly obese population. The environmental costs of obesity are also escalating as a result of greater food production, fuel use, and greenhouse gas emissions. A greater dependency on technologies that limit physical activity such as manufacture of cars and labor-saving machinery, production of processed foods, and adaptation of the infrastructure to support heavier individuals contributes to cumulative negative environmental effects. Annual greenhouse gas emissions from food production and car travel associated with increased adiposity in a population of 1 billion people are estimated at ~0.4–1 additional gigatons of carbon dioxide equivalents.

The global burden of obesity will continue to grow unless action is taken to reverse current trends. The future health, social, economic, and environmental costs could be overwhelming and will affect everyone adversely, from individuals, families, and schools to small businesses, governments, and large corporations. The difficulties of treating obesity and the slow progress made to date in reversing the trend emphasize the need to prioritize obesity prevention and management worldwide. Warranting particular attention is the fact that many LMICs now face the multiple burdens of infectious diseases and undernutrition coupled with growing rates of obesity. Developing countries, particularly in urban settings, have undergone a 'nutrition transition' whereby traditional diets have been replaced with diets high in refined, energy-dense, yet nutrient-poor foods. The public health capacity of LMICs is significantly strained to accommodate this additional burden.

Causes

Whereas the underlying mechanism of obesity at the individual level is an imbalance of calorie intake and expenditure, social, cultural, and environmental influences on individual calorie intake and expenditure greatly affect the incidence of the condition. These influencing factors include the importance of individual and household food preferences, availability of fast food in the neighborhood, and cultural expectations regarding food and physical activity. Increases in obesity prevalence follow an established pattern: in LMICs, as gross domestic product (GDP) increases, high-income groups in urban areas are the first to have high obesity prevalence, followed by low-income groups and those in rural areas. This

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00500-6

phenomenon, known as the 'nutrition transition,' occurs when individuals move from eating local staple foods to having access to calorie-dense, nutrient-poor foods and concurrently become less active. As GDP increases, mean BMI increases linearly up to \$5000 year^{-1} per person GDP; above that level, the relationship is flat.

Economic success facilitates obesity but is not always required; obesity prevalence is high in some low-income countries and areas such as the Pacific Islands nations. A unique challenge for developing countries in the nutrition transition is that mothers are often undernourished, and this is known to influence an infant's birth weight and genetic ability to metabolize glucose effectively. Evidence supports this *thrifty phenotype* hypothesis, in which poorly nourished infants become 'thrifty' and are unable to compensate later in life when nutrient intake increases in an environment in which high-calorie food is widely available.

The drivers of the obesity epidemic in the food system, including the increased supply of cheap, high-calorie foods, greater access to foods, and persuasive food marketing, are complex and interconnected. In the framework developed by Swinburn and colleagues, energy imbalance is affected by largely individual behavior patterns, including high energy intake and low physical activity; these behaviors are influenced by important environmental and system drivers, including the food supply. These drivers, in turn, are moderated by environmental characteristics such as culture, activity, and transportation. At the highest level, systemic drivers, including policy and economic systems, impact the environment (Figure 1).

Prevalence

According to the World Health Organization, obesity rates have nearly doubled since 1980. In 2008, an estimated 1.5 billion adults over the age of 20 years (35%) were overweight, and 500 million (11%) were obese. In the United States, which has one of the highest rates of overweight and obesity in the world, ~35% of adults and 17% of children (almost one in every five) are obese (Figure 2).

Halting the global increase in obesity is one of the targets of the Global Monitoring Framework for noncommunicable diseases (NCDs), an international framework that was formally adopted in 2013 following the adoption of the Political Declaration on NCDs by the United Nations General Assembly in 2011. The framework comprises a list of 25 key indicators, including prevalence rates of adult and child obesity, that member countries are encouraged to track systematically to assess progress in preventing and controlling major NCDs.

In the United States, data from the National Health and Nutrition Examination Survey between 2011 and 2012 showed the first evidence that obesity rates have started to level off during the 2000s. During the same period, more than two-thirds of adults and one-third of children were overweight. There was a significant increase in the prevalence of obesity among women 60 years and older (Figure 3). Non-Hispanic black and middle-aged women had significantly higher obesity levels than did other population subgroups (Figure 4).

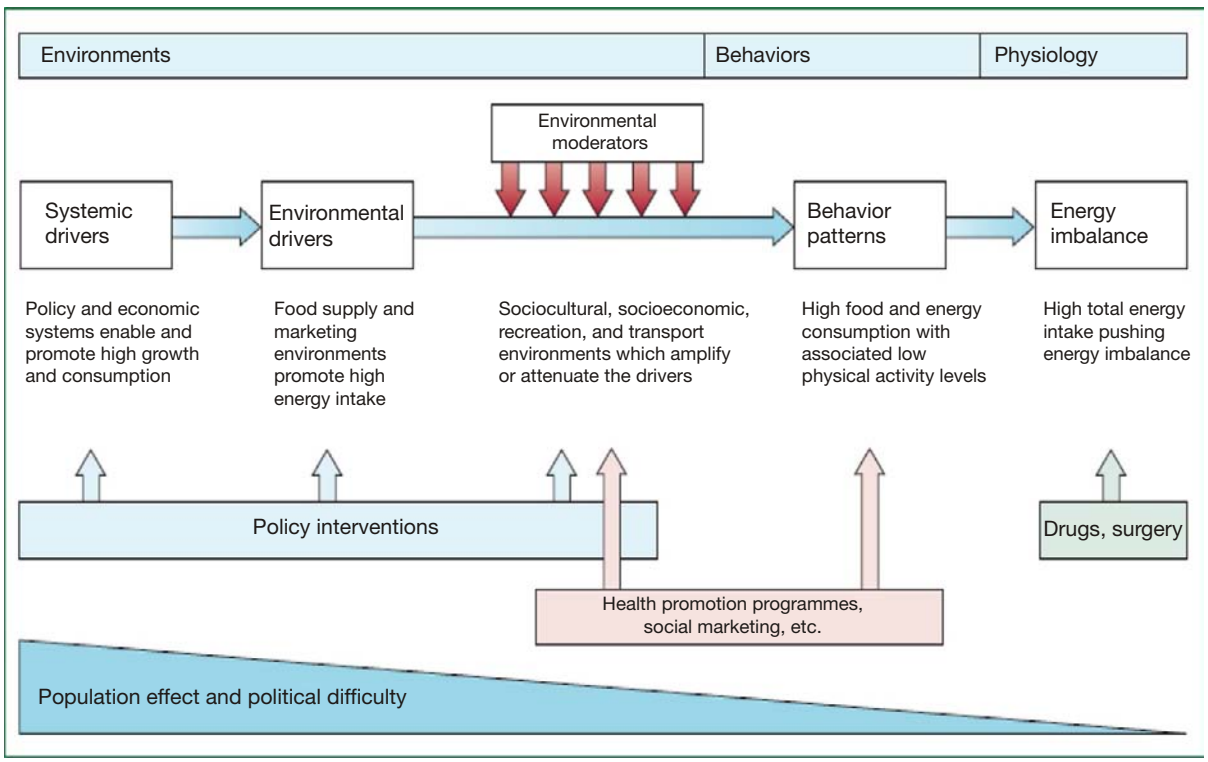


Figure 1 Framework for categorizing determinants of and solutions for obesity. Reproduced from Swinburn, B. A., Sacks, G. A., Hall, K. D., et al. (2011). The global obesity pandemic: shaped by global drivers and local environments. *Lancet* **378**, 804–814.

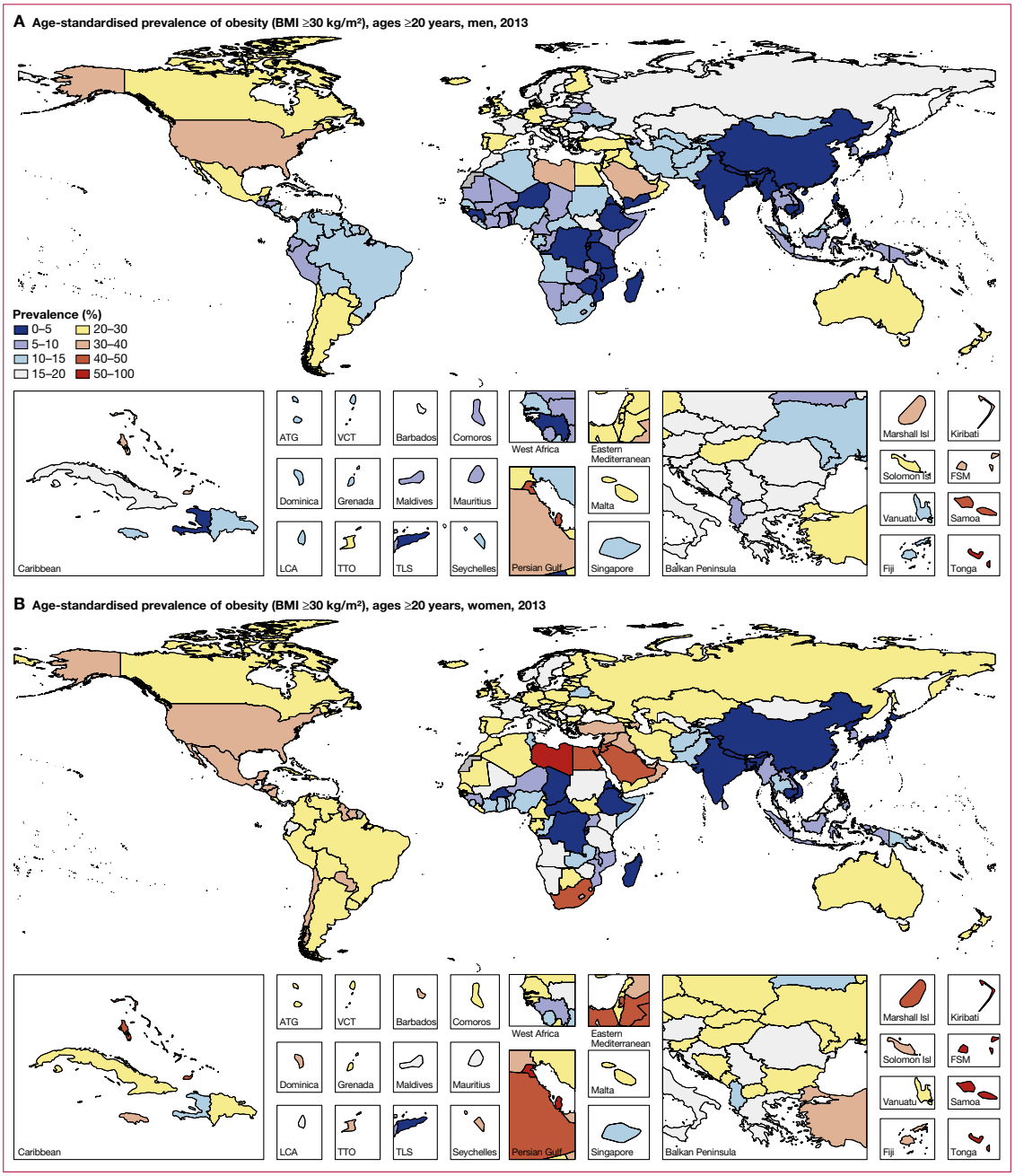
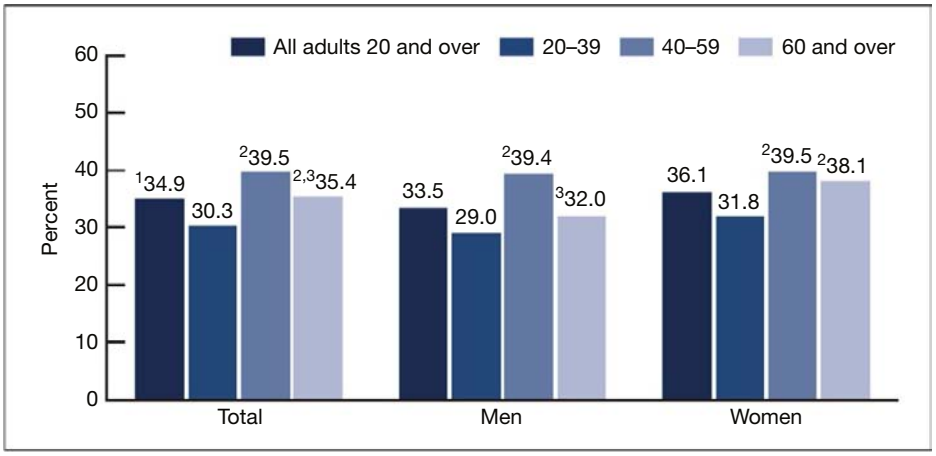


Figure 2 Age-standardized prevalence of obesity, ages ≥20 years. (a) Men, (b) women. Reproduced from Ng, M., Fleming, T., Robinson, M., et al. (2014). Global, regional and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **384**(9945), 766–781.

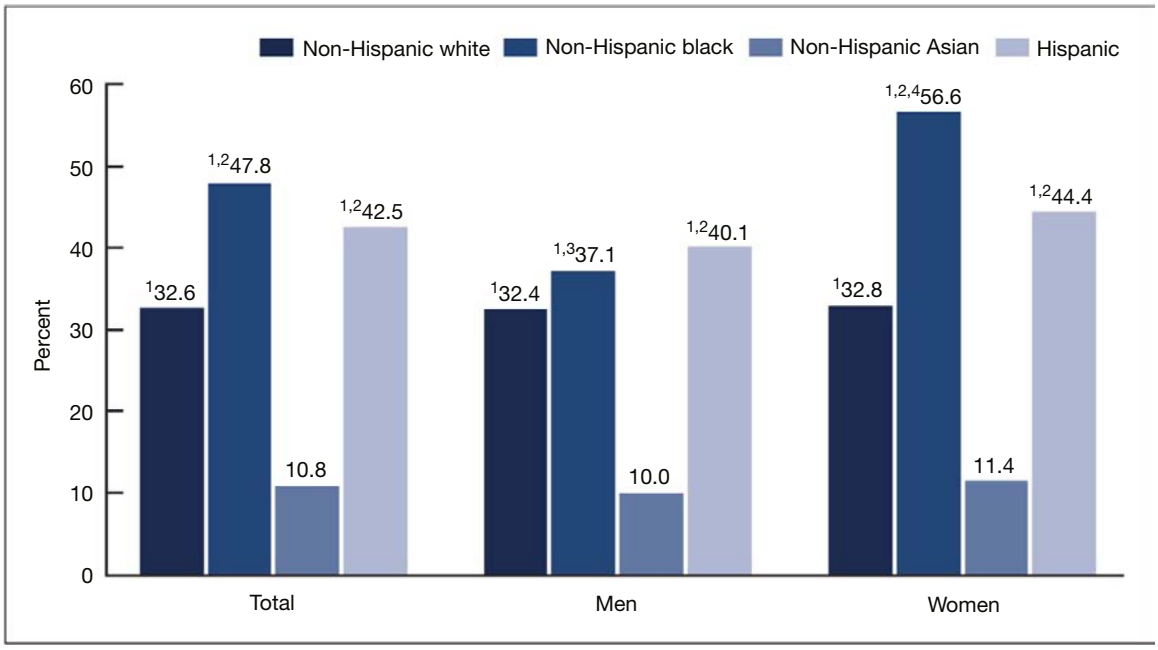
The overall prevalence of obesity in youth remained unchanged from rates between 2009 and 2010, and there has been no significant change since 2003–04; the same trend was observed among adults. Analyses show that the flat growth trend among children was primarily due to a decrease in obesity prevalence among those aged 2–5 years, from 14% to 8% between 2003–04 and 2011–12. No significant differences were observed between boys and girls; however, Hispanic youth showed significantly higher rates of obesity than did non-Hispanic Asians (22.4% vs. 8.6%). The overall declining prevalence of obesity among toddlers observed in the National Health and Nutrition Examination Survey is consistent with other reported data from the US Virgin Islands and Massachusetts and with recent declines in extreme obesity among US preschoolers in low-income families. Data for middle and high school students appear to be more mixed. Recent studies from Europe have also shown a leveling off of obesity prevalence, including a decline among German children and a slowing of the rate of increase in adult obesity in England.

Previous studies of the US population have shown an association between obesity rates and socioeconomic status as measured by education levels and income; however, a recent



[1]Crude estimate 35.1%.
[2]Significant difference from ages 20 to 39.
[3]Significant difference from ages 40 to 59.
Note: Estimates are age-adjusted for all adults aged 20 and over by the direct method to the 2000 U.S. census population using the age groups 20–39, 40–59, and 60 and over.

Figure 3 Age-adjusted prevalence of obesity, by sex and age group, among adults aged 20 and over: the United States, 2011–12. Reproduced from Ogden, C. L., Carroll, M. D., Kit, B. K., and Flegal, K. M. (2013). Prevalence of obesity among adults: United States, 2011–2012. NCHS Data Brief No. 131. http://www.cdc.gov/nchs/data/databriefs/db131.pdf.



[1]Significant difference from non-Hispanic Asian.
[2]Significant difference from non-Hispanic white.
[3]Significant difference from women.
[4]Significant difference from Hispanic.
Note: Estimates are age-adjusted by the direct method to the 2000 U.S. census population using the age groups 20–39, 40–59, and 60 and over.

Figure 4 Age-adjusted prevalence of obesity, by sex and race, among adults 20 and over: the United States, 2011–12. Reproduced from Ogden, C. L., Carroll, M. D., Kit, B. K., and Flegal, K. M. (2013). Prevalence of obesity among adults: United States, 2011–2012. NCHS Data Brief No. 131. http://www.cdc.gov/nchs/data/databriefs/db131.pdf.

study that investigated the association by poverty income ratio (PIR) found that only 20% of obese US adults have low incomes (below 130% of the federal poverty level). There were striking differences in prevalence rates by race/ethnicity and gender. Among men, obesity levels were generally similar across all income groups, although they were slightly higher at higher income levels. Among non-Hispanic black and Mexican-American men, obesity levels decreased as income level decreased. In contrast, among women, obesity prevalence increased as income decreased: for example, 29% of women who are obese live in households with PIRs of at least 350%, whereas 42% of obese women live in households with PIRs smaller than 130%. This trend was only significant among non-Hispanic white women. Similar gender differences were

observed regarding educational attainment. There was no significant association between education and obesity prevalence among men; however, obesity prevalence among women increased as education level decreased.

Although rates of obesity are leveling off in some developed nations, they continue to rise in developing countries. The prevalence of childhood overweight and obesity in the developing world increased from 8.1% of boys and 8.4% of girls in 1980 to 12.9% and 13.4%, respectively, in 2013. Distinct geographic patterns are evident, with higher rates in the Middle East, North Africa, and some Pacific Islands and Caribbean nations. In Latin America, the highest rates of childhood obesity among boys were observed in Chile (11.9%) and Mexico (10.5%), and the highest rates among girls were seen in Uruguay (18.1%) and Costa Rica (12.4%). It was estimated that in 2013, obesity rates among adults exceeded 50% of men in Tonga and of women in Kuwait, Samoa, Qatar, Libya, Tonga, Kiribati, and the Federated States of Micronesia. In China and India, although obesity prevalence has increased over time, rates remain relatively low, with 3.8% of men and 5% of women classified as obese in China and 3.7% and 4.2%, respectively, in India in 2013.

Projections

Despite evidence of stabilizing obesity levels in some populations, the existing high rates have far-reaching consequences for population health and the economies of nations. Because the aging populations of most countries are increasing, the projected health and economic burdens of obesity are of great concern and add urgency to the need to implement effective and sustainable individual- and population-based interventions. For example, it has been estimated that rising obesity levels accounted for 27% of US health-care expenditures between 1987 and 2001. A systematic review of the worldwide costs of obesity by Withrow and colleagues concluded that direct medical costs of treating obesity accounted for between 0.7% and 2.6% of a country's total national health-care expenditures. A study by Wang and colleagues suggested that there would be an additional 65 million obese adults in the United States by 2030, which would accrue 6–8 million excess cases of diabetes, 5–6.8 million cases of coronary heart disease and stroke, and ~500 000 additional cases of cancer. The projected health-care costs of treating obesity-related disorders in by 2030 are in the region of $66 billion, increasing the 2009 level of US health-care spending ($2.5 trillion) by an additional 2.6%. For the United Kingdom, Wang and colleagues projected an increase of 11 million more obese adults, which would result in an excess of 544 000–668 000 cases of diabetes, 331 000–461 000 cases of coronary heart disease and stroke, and 87 000–130 000 cases of cancer. This increased disease burden in 2030 would represent ~2% of total annual health-care spending in 2009 (approximately £110 billion).

Impact

Health Consequences

Overweight and obesity are the leading causes of death worldwide, with an estimated 3.4 million adult deaths each year attributed to being overweight or obese. Approximately 44% of diabetes, 23% of ischemic heart disease, and between 7% and 41% of cancers are due to overweight and obesity. Overweight and obesity are related to more deaths than is underweight. High BMI is an independent risk factor for chronic diseases, including cardiovascular disease, diabetes, musculoskeletal disorders, and some cancers. Obesity increases the risk of depression by 55%. Childhood obesity is associated with adult obesity and related risks. Obese children are also at risk of hypertension, early cardiovascular disease markers, insulin resistance, breathing difficulties, and bone fractures. Obese adolescents have more mental health problems, including anxiety, depression, and reported suicide attempts.

Stigma and Discrimination

Obese individuals are stigmatized and discriminated against. Over the past decade, weight discrimination has increased 66%. Negative stereotypes include those of overweight and obese individuals as lazy, sloppy, and less competent than are normal-weight individuals. Weight stigmatization has been observed in employment, health care, and education. Overweight and obese employees are discriminated against in the workplace in terms of hiring, wages, promotions, and termination. One study found that 25% of overweight and obese women report job discrimination because of weight; 54% report stigmatization by coworkers; and 43% report stigmatization by employers or supervisors. A nationally representative study found that overweight adults were 12 times more likely to face employment discrimination than were normal-weight adults; obese adults were 37 times more likely, and severely obese responders were 100 times more likely. Studies have found that overweight negatively affects wages. One study of more than 12 000 workers found a wage penalty for obese employees, ranging from 0.7% to 3.4% for men and from 2.3% to 6.1% for women. A study of more than 25 000 workers found that a weight increase of 64 lb was associated with a 9% wage decrease for white women. This is equivalent to 1.5 years of education or 3 years of work experience.

Physicians have negative attitudes towards obese individuals, with beliefs aligning with current stereotypes. A study found that more than half of physicians viewed obese patients as awkward, unattractive, ugly, and noncompliant. One-third viewed obese patients as weak willed, sloppy, and lazy. Studies in France, Australia, Britain, and Israel have found similar negative attitudes of physicians towards obese patients. Nurses, medical students, fitness professionals, and dieticians have also been observed to harbor negative bias towards obese patients. Overweight and obese students face stigma in educational settings from teachers, peers, and parents. Obesity has been associated with lower educational attainment in Sweden, England, and the United States.

Economic Impact

Obesity costs are increasing worldwide. In 2010, annual costs were calculated to be $4879 for obese women and $2646 for obese men. This includes direct medical costs, reduced wages, short-term disability, disability pension insurance, absenteeism, presenteeism, gasoline, and life insurance. Direct per-

person medical costs of overweight and obesity in 2008 were estimated to be $266 and $1.723, respectively. Direct costs of overweight and obesity to the National Health Service in England have increased from approximately £479 million in 1998 to roughly £4 billion in 2007. Estimates of indirect costs have increased from £2.6 billion in 1998 to £15.8 billion in 2007 and are projected to increase to £27 billion in 2015. Obesity costs, including medical care and absenteeism, for a company with 1000 employees are estimated to be $\$277\,000\ year^{-1}$. Even the military is affected by obesity: ~25% of young adults are too heavy to be eligible for military service. The military spends more than $1 billion per year treating weight-related diseases in the military forces.

Addressing Obesity

A great deal of research has been devoted to interventions to address obesity among both children and adults. The World Obesity Federation highlights the SPOTLIGHT project, which has undertaken a survey throughout Europe on obesity interventions to improve diet and physical activity. In the United States, The Community Guide has reviewed interventions in community settings to prevent and control obesity. These include behavioral interventions to reduce screen time among children, school-based programs, worksite programs, and technology-based interventions.

The contexts in which people live, work, learn, play, and pray impact eating and physical activity behaviors. Family exposure and behaviors affect calorie intake and obesity risk for children, as parents or adult caregivers prepare meals for children and set taste preferences, often for life. Adults spend nearly one-quarter of their lives at work, which offers the potential for long-term intervention through well-designed, comprehensive health promotion programs. Schools offer a great opportunity for obesity prevention by improving food options and including physical activity. In the United States, nutrition standards for school meals were changed through the Healthy, Hunger-Free Kids Act of 2010. Organizations such as the GENYOUth Foundation work to improve nutrition and increase activity in schools. GENYOUth, in partnership with the National Dairy Council and the National Football League, offers Fuel Up to Play 60, a program encouraging a nutrient-rich diet and 60 min of activity daily, to 70 000 US schools, impacting 36.6 million students.

The EPODE International Network, an approach for communities to implement effective and sustainable strategies to prevent childhood obesity, has 25 programs involving 150 million people in 15 countries. EPODE helps communities change social norms, behaviors, and environment to encourage healthy lifestyles. In France, obesity and overweight were reduced by 10% between 2005 and 2009; in Belgium, implementation of the community-based program Viasano resulted in a 20% reduction of overweight in the population between 2007 and 2010.

The costs and resources required to deliver effective in-person behavioral therapy have led increasingly to technology-supported programs for weight management. Mobile devices and the Internet show promise as effective platforms. Participant engagement in, and adherence to, many technology-based weight management programs is strengthened by the incorporation of self-monitoring tools, assistance with goal setting, real-time feedback, and support from fellow participants or coaches. Further benefits are evident from the low implementation costs and widespread availability of such programs, particularly in developing countries where the uptake of mobile phone technology has increased rapidly. The long-term effect sizes of these technology-assisted programs compared with those of intensive lifestyle modification, however, remain unclear.

Modeling has been used to assess cost-effectiveness of interventions in addition to effectiveness. The review by Swinburn and colleagues indicated that policy-level interventions (such as taxes and regulations) showed greater cost-effectiveness than did clinical interventions. This finding is consistent with a 2014 study that estimates that a national $0.01 per ounce sugar-sweetened excise tax in the United States is a better tactic to reduce childhood obesity than are after-school physical activity programs and advertising bans on unhealthy foods. A study of health effects and cost-effectiveness of efforts in LMICs concluded that population-based strategies (health information and communication), fiscal measures (price increases for unhealthy foods), and regulation (e.g., restricting food marketing directed towards children) would be most cost-effective in reducing unhealthy diets and increasing physical activity.

Clearly, interventions alone are not enough to address obesity; rather, a comprehensive strategy is needed that includes multisectoral action. Gortmaker and colleagues highlighted a systems approach to addressing obesity that comprises action by governments, international agencies, the private sector, civil society, health professionals, and individuals. Governments can introduce and implement policies to prevent obesity in the health and other sectors such as agriculture, transportation, and urban planning. Government leadership is needed to accelerate obesity prevention. Local policies have had more success in moving from proposal to action than have federal or even state-level policies. For example, the nation's first soda tax was passed in 2014 in Berkley, California. Other cities have proposed similar actions to tax soda or limit beverage sizes, although none have yet passed, and similar policies are not politically feasible at the federal level. Tax policies to address obesity have been introduced in other countries, such as Mexico, where soda is now taxed at 1 peso per liter and high-calorie foods at 8% of cost. The food industry has taken action to address obesity on several fronts, including reformulating products to reduce sugar, salt, and fat; developing more healthy products; and reducing marketing directed towards children. The International Food & Beverage Alliance comprises food and beverage companies committed to addressing health and wellness in support of the World Health Organization's Global Strategy on Diet, Physical Activity and Health.

Conclusion

Obesity is a continuing challenge because of its increasing prevalence, its health and economic impact, and the lack of sustainable solutions. Comprehensive approaches that address environmental, economic, social, and individual factors affecting obesity will need to be taken. Addressing the complex issue

of obesity will require a solution that includes interventions and policies from multiple sectors. Although there is evidence showing that policies and interventions can improve diet and increase physical activity, other factors, such as cost, feasibility, and sustainability, must be considered. Further research is needed to develop successful interventions to prevent obesity as well as to support sustained weight loss for currently obese individuals.

See also: Appetite Control in Humans: A Psychobiological Approach; Dietary Practices; Energy: Intake and Energy Requirements; Food Environment; Hunger; Obesity: Epidemiology of; Obesity Management; Obesity: The Role of Diet; Satiety.

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Obesity: Epidemiology of

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Introduction

Epidemiological studies conducted over the last decades have clearly documented the widespread occurrence and consequences of obesity in the United States and worldwide. These studies have allowed us to gain insights into the causes of obesity and the causes of the epidemic of obesity. The epidemic and universal nature of the problem of obesity is now clear. Moreover, the serious and numerous complications associated with obesity place an excessive burden at both the individual/clinical level and the population/health system level. Over the same period, definitions and measurement methods have evolved in an attempt to improve the characterization of the burden of the disease and the consequences. While body mass index (BMI) has been widely used because of its simplicity, the cutoffs have been somewhat controversial in particular in individuals of Asian or African origin. Major concerns of the epidemiology of obesity are the inequalities seen throughout the world showing a dual pattern, with obesity generally affecting more economically developed nations and, at the same time, economically disadvantaged populations within these countries. Major advances in the control of infectious diseases and the aging of the population have helped to increase the number of susceptible individuals. In addition, major changes in the food systems, availability, and affordability of healthy foods and changes in patterns of physical activity have played a major role in the development of the epidemic. Most of the work related to strategies for the prevention and treatment of obesity has had a focus at the individual level, with more recent push on macrosocial and upstream level of prevention and control.

Definition and Measurement of Obesity

The World Health Organization defines obesity as abnormal or excessive body fat accumulation (adiposity) that may impair health.

The exact definition of 'excessive' or the threshold for labeling abnormal accumulation of body fat is ill-defined. Adiposity, as other human traits such as blood pressure and blood glucose, is a continuous trait, and therefore, the sorting of individuals into disease categories is not straightforward. In spite of that, there has been an emphasis on defining cut points, and these cut points and their resulting diagnoses are fundamental in clinical care and practice as they result in treatment, prognosis, and administrative justification of health-care resource use.

Tools to measure body composition including body fat more directly (e.g., densitometry, bioelectrical impedance, dual-energy x-ray absorptiometry, and dilution techniques) are not widely available and are costly and laborious, and therefore, their use is limited to small clinical or laboratory settings. For practical reasons, most epidemiological studies rely on simpler anthropometric measurements, in particular the measurement of weight, skinfold thickness, and circumference of the trunk and limbs. Most of the studies define obesity using a measure of body weight adjusted by height. The most common one is the BMI (or Quetelet's index), calculated as follows: weight in kilograms divided by height in meters squared. Studies have shown that there is a high correlation between BMI and body fat percent. However, the main limitations of BMI are the lack of distinction between fat mass and fat-free mass and differences in muscle and adipose tissue distribution by age, sex, race/ethnicity, and other factors. As it will be discussed in the succeeding text, these differences have led to conclude that different cutoffs by race/ethnicity are needed to more accurately characterize obesity and to classify risk in certain subgroups.

Studies of individuals using very sophisticated methods such as magnetic resonance imaging have demonstrated that at any given BMI, the degree of fatness may be very different. Therefore, certain populations (like aging populations) may have a trend for increased adiposity accompanied by a stable (or even decreasing) BMI trends.

The other important threat to the accuracy of BMI is the use of self-report information weight and height information. Large epidemiological studies in many countries are often limited to the use of self-report data, and it is important to be aware of biases in the reporting, with more important misclassification at the extremes.

Notwithstanding its limitations, several studies have shown the prognostic value of elevated BMI for identifying individuals or populations at risk of health consequences, and BMI remains the most important and widely used indicator in surveillance systems for measuring the burden of the disease, for tracking trends over time, and for evaluating the effects of interventions.

Among adults, two other complementary and widely used measures of excess adiposity include waist circumference and waist-to-hip ratio. Both highlight the relevance of abdominal fat mass (also referred to as abdominal or visceral obesity). On the basis of the large body of evidence demonstrating an increased risk of health complications with increased waist circumference or waist-to-hip ratio, the WHO and other organizations have recommended their use. A very important issue for the use and interpretation of both waist circumference and waist-to-hip ratio is the protocol used in the assessment. Critical elements of the protocol include the anatomic placement of the measuring tape, the tightness, the type of tape, the subject's position, phase of respiration, abdominal tension, and clothing. It is of paramount importance to follow standardized protocols for their measurement and in the report of the findings, to explicitly provide information of the placement of the tape.

The WHO proposed and established the classification of body weight based on BMI in 1993 and 1997, respectively

(Table 1). While that classification has been considered universal, there have been controversies around the cutoffs as they have been considered inappropriate in certain populations, in particular for those of Asian descent. The 2 main motivations for suggesting to move the cutoffs are (1) the increased evidence of an association between BMI and increased risk of outcomes at a lower BMI and (2) the increased evidence of different association of BMI, percentage of body fat, and body fat distribution across the populations.

In light of these facts, in Japan, obesity is defined as BMI ≥ 25 kg m^{-2}, and in China, it is defined as BMI ≥ 28 kg m^{-2}. Medical organizations are also starting to modify guidelines, the American Diabetes Association 'Standards of Medical Care in Diabetes – 2015' made a specific change in 2015 and lowered the BMI cut point to 23 kg m^{-2} (vs. 23 kg m^{-2}) for screening for diabetes among Asian Americans.

Based on the waist circumference and waist-to-hip ratio, the WHO has also provided cut points to define abdominal obesity (Table 1).

Table 1 World Health Organization classification of body weight based on body mass index and cutoff points based on waist circumference, waist-to-hip ratio, and risk of metabolic complications

Indicator	*Cutoff points*	*Classification*
Body mass index (kg m^{-2})	<18.5	Underweight
	18.5–4.9	Normal weight
	25.0–29.9	Overweight
	30.0–34.9	Class I obesity
	35.0–39.9	Class II obesity
	≥40	Class III obesity
Waist circumference (cm)	>94 (M); >80 (W)	Risk of metabolic complications increased
	>102 (M); >88 (W)	Risk of metabolic complications substantially increased
Waist-to-hip ratio (cm)	≥0.90 (M); ≥0.85 (W)	

Magnitude of the Problem

Epidemiological studies are the foundation for disease control and prevention, and national surveys of weight, height, and other anthropometric indicators have been a constant resource of data at the national level. Although most of these studies were first undertaken to document problems related to the opposite problem of obesity, namely, undernutrition, these sources then were well positioned to measure the magnitude of the problem of obesity and for the identification of trends over time.

Using data from these national surveys, the epidemic of obesity was first appreciated in the United States and started between 1970s and 1980s when the prevalence of obesity started to increase at an alarming rate (Figure 1). Large increases in average BMI were observed between surveys conducted in the late 1970s and the mid-1980s and have continued to increase thereafter (Figure 2). Shortly after, other developed countries also started to see a steep increase in the prevalence of obesity (Figure 1). At the same time, developing countries faced two opposing problems: the problem of undernutrition and that of obesity (nutritional transition). By 2000, the number of adults with excessive weight surpassed the number of those underweight. Consequently, it has been estimated that the prevalence of obesity in the world nearly doubled between 1980 and 2008 (from 6.4% to 12%) with the largest increase between 2000 and 2008.

Although obesity is considered a global epidemic, there are substantial variations in the prevalence across countries, ranging from ~1% in Bangladesh, the Democratic Republic of Congo, and Ethiopia to ~75% in some countries in Oceania (Tonga, Nauru, and the Cook Islands) (Figure 3). Overall, economically developed nations exhibit higher prevalences of obesity compared to emerging or developing countries.

A second pattern emerges when looking at obesity prevalence within developed countries, where an inverse gradient between obesity and socioeconomic position is apparent.

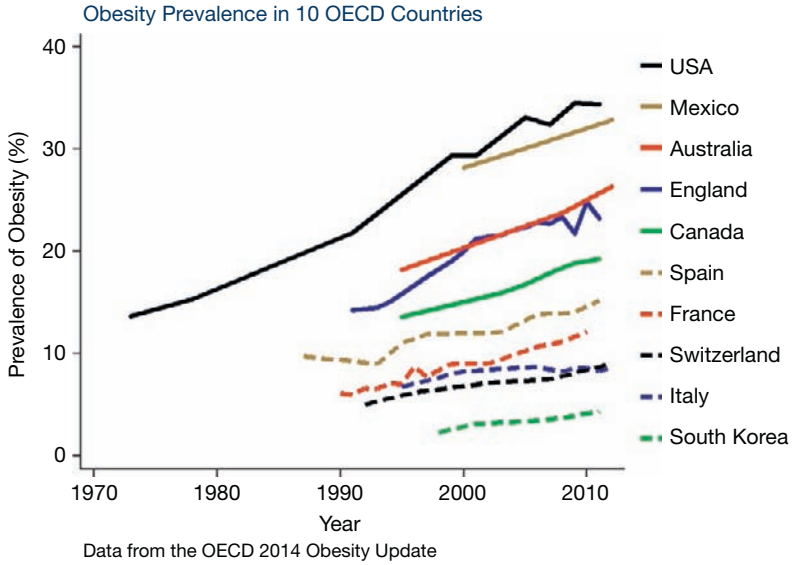


Figure 1 Obesity trends over time.

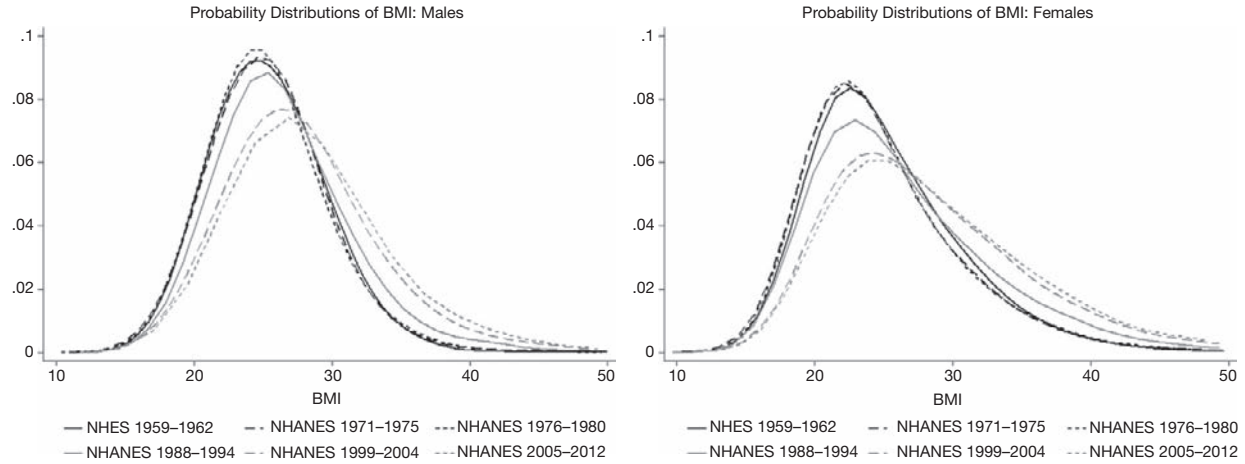
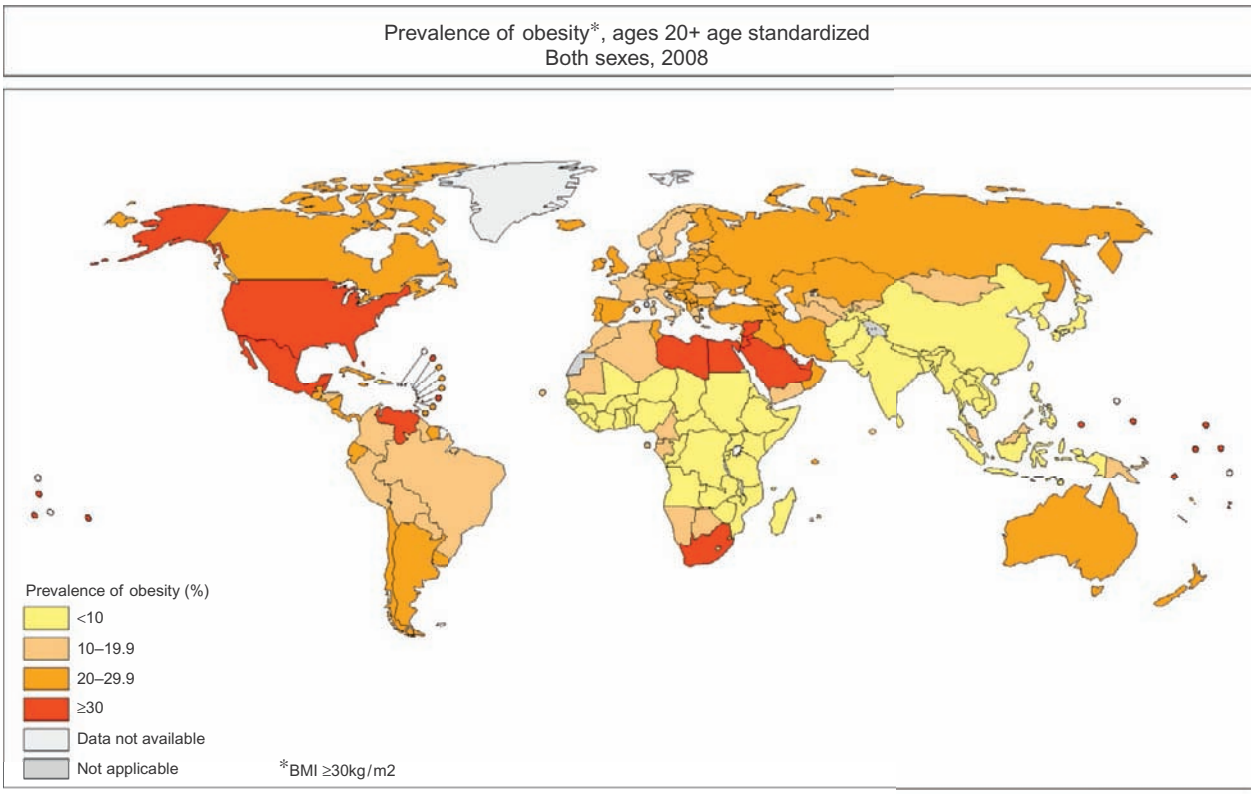


Figure 2 Temporal changes in body mass index distribution in the United States by sex.



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on the maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

Figure 3 Worldwide prevalence of obesity.

Population groups with low socioeconomic status (education, income, or occupational class) show increased rates of obesity, likely contributing to the mortality differentials that exist in most developed nations.

The causes of these international and national disparities are diverse and complex. In the next section, we elaborate on a general framework to study these causes and list potential determinants of obesity.

Causes of Obesity

As stated by Geoffrey Rose in his article 'Sick individuals and sick populations,' when studying the origins of a disease, it is important to differentiate two types of causes: the causes of cases and the determinants of incidence. In this case, the first relates to why some individuals become obese (as compared to individuals who do not), while the second looks at the causes

in the variation of obesity rates in different populations or the same population over time (e.g., the epidemic of obesity). These two types of causes need not be the same. Therefore, the causes that are relevant in the clinic may not be relevant in departments of health (and vice versa).

Causes of Obesity among Individuals

Among individuals, causes of obesity can be divided into two categories: medical and nonmedical causes. Medical causes are those derived from medical conditions that directly or indirectly increase adiposity. Table 2 lists potential medical causes for obesity. Most of these causes are rare in most populations and therefore are not major contributors to the epidemic of obesity.

Nonmedical causes are those not derived from medically recognized conditions. In simple terms, obesity is usually the result of an energy imbalance gap between calorie intake and calorie output, that is, an imbalance between diet (too many calories consumed) and physical activity (too few calories burned).

While it has been of great interest to determine whether specific dietary components (e.g., fat, refined carbohydrates, and high-fructose corn syrup) or dietary patterns have different effects on obesity, results of controlled clinical trials have resulted in conflicting results. Most clinical guidelines for the management of obesity currently recommend comprehensive lifestyle interventions with moderately reduced caloric intake and increased physical activity.

A second aspect of these nonmedical causes is genetic predispositions to obesity. Several candidate genes have been studied, including the FTO, MC4R, and LEPR genes. These genes have been shown to have significant (but weak) associations with an increased risk of obesity. In general, it is considered that genetic predisposition of obesity is the result of a polygenic expression not attributable to a single gene.

Causes of the Epidemic of Obesity

The study of the differences in obesity rates between populations or within the same population over time requires the identification of mass influences that affect entire populations and put people at risk for disease. In the case of obesity, if we want to understand why (a) most developed countries have seen an increase in obesity in the last four decades; (b) most developing countries have also seen an increase, delayed by a few decades; and (c) there are spatial within- and across-country differences, we need to look at the mass influences that have changed over time or that are different within/across countries.

Mass influences of obesity include (see Cutler's recommended reading) the following:

- On the energy intake (consumption) side
  - Increased availability of ultraprocessed foods that are able to have increased shelf life but lose nutritional value and are energy-dense
  - Shift from a food pattern based on the classic core (whole carbohydrate), pulse (legume), and fringe (spice, flavor, and meat) to one based on refined carbohydrates and animal protein and fat
  - Increased availability of cheap sweeteners (like high-fructose corn syrup)
  - Globalization of food consumption and production, which reduces reliance on seasons or agricultural capability of countries
- On the energy expenditure side
  - Increased reliance on motorized transport
  - Reduced demand for labor-intensive work
  - Increased demand for sedentary leisure activities (like TV watching)
- Other/miscellaneous
  - Increased work hours (which lead to decreased time availability for proper meal preparation and leisure time physical activity)

Although it is clear that there have been major changes in factors influencing the energy intake and energy expenditure at the population level, the contribution of each and their preponderance in the epidemic of obesity has been less clear and subject of heated debate. A number of studies have now documented the gradual increases of energy intake in the last four or five decades with relatively little change in the physical activity and therefore suggest that energy intake has been the major driver. Therefore, while not discouraging physical activity, as it has many health benefits (even in the absence of weight loss), the major efforts to address the obesity epidemic need to target energy/food supply. Additionally, while a number of clinical studies have been conducted to test the effects of different diet compositions or patterns among individuals, fewer studies have addressed this question at the population level. These studies will be key for formulating prevention policies.

More recently, with the realization of the complex nature of obesity and the epidemic of obesity, studies focused on examining the role of single factors on obesity have been subject to criticism. This epidemic involves a complex interplay between many of its biological, behavioral, social, economic, political, and cultural determinants. In an attempt to overcome that limitation, it has been proposed that complex systems methods may be more appropriate for addressing the problem of obesity. In complex system methods, determinants are

Table 2 Medical causes of obesity

Drug-induced
Antipsychotic
Antidepressants
Antiepileptic
Diabetes
Neuroendocrine
Hypothalamic obesity
Cushing's syndrome
Hypothyroidism
Polycystic ovary syndrome
Growth hormone deficiency
Genetic
Leptin deficiency

connected through feedback loops and exhibit nonlinear behaviors and emergence (patterns that cannot be predicted by looking at individual components of the system). For example, increased work hours that lead to decreased time availability may lead to increased demand of ultraprocessed ready-to-eat foods that shift food patterns to refined carbohydrates and meat. These increased demands for refined carbohydrates may lead to regulations and subsidies that emphasize their consumption even more, causing an amplification of these effects.

Complications of Obesity

Obesity has been shown to be associated with many health outcomes either directly (low back pain and arthritis) or indirectly (through a number of metabolic disorders) (Table 3). Particularly relevant are the strong and robust associations between obesity and type 2 diabetes, high blood pressure, and dyslipidemia, as they represent the major causes of cardiovascular disease, and cardiovascular disease is the leading cause of death in both developed and developing countries.

An area that has generated controversy is the effect of obesity among people with already established chronic diseases. Studies looking at the association between obesity and mortality in this subpopulation have been paradoxical. While in the general population, there is a benefit (or no harm) of being normal weight (BMI of 18.5–25) compared to overweight or obese, this association is not easily reproduced in observational studies of people with chronic conditions, where the most protective weight can be found in the overweight or mildly obese categories. This phenomenon has been termed the 'obesity paradox' and it has been found in populations with diabetes, chronic kidney disease, or heart disease. Some of the potential explanations for this phenomenon include the following:

- Mechanistic/physiological: postulates that the presence of fat in people with chronic diseases is due to its hormonal effects and it leads to protection against frailty.
- Residual confounding: highlights that the role of lack of adjustment or imperfect adjustment for important factors correlated with the level of adiposity and mortality (e.g., smoking).
- Reverse causality: as many chronic illnesses lead to unintended weight loss. Therefore, if not properly accounted for, a preexisting illness may cause weight loss and lead to increase risk of death.
- Selection bias/collider stratification bias: the most novel explanation. If an individual has diabetes and is nonobese, this individual may be more likely to have a third factor causing diabetes (and mortality). Therefore, a spurious (not causal) association between normal weight and higher mortality is created.

In summary, better designed observational and interventional studies are needed to understand the complex nature of this phenomenon and to better guide policy and clinical interventions in obese people with already established chronic diseases.

Table 3 Complications of obesity

Endocrinological
Type 2 diabetes
Infertility
PCOS
Rheumatologic/orthopedic
Osteoarthritis
Gout
Low back pain
Limited mobility
Ulcers
Gastroenterological
Gallbladder disease
Fatty liver disease
Gastroesophageal reflux
Respiratory
Obstructive sleep apnea
Urological and nephrological
Erectile dysfunction
Urinary incontinence
Chronic kidney disease
Hypogonadism
Cardiovascular system
High blood pressure
High cholesterol
Ischemic heart disease
Peripheral arterial disease
Heart failure
DVT/PE
Neurological/psychiatric
Depression
Others
Certain cancers
Quality of life
Stigma

Concluding Remarks

The medical paradigm has largely focused on sick or high-risk individuals. This thinking has shaped most of the research questions, planning of health expenditures, and provision of care. For example, we have continuously focused on answering the following question: Why did this individual develop obesity? As stated before, it is important, however, to distinguish that factors that explain the incidence of disease within a population and the risk factors for disease within an individual are not necessarily the same. We then need to ask ourselves, why does this population have this rate of obesity compared to another population? Or why has obesity increased in this population in the last decades? By answering that question, public health practitioners will then be able to effectively prevent or reduce the burden of obesity in our populations.

See also: Beverage: Health Effects; Energy: Intake and Energy Requirements; Food Environment; Hypertension and Diet; Obesity: Causes and Prevalence; Obesity Management; Obesity: The Role of Diet.

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Obesity: The Role of Diet

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Introduction

The incidence of overweight and obesity has grown to alarming rates worldwide. They are risk factors for serious chronic diseases and for several of the leading causes of preventable death, including cardiovascular disease, diabetes mellitus, and many types of cancer. The causes of obesity are multifactorial and not well understood. There have been numerous presumed contributors including the increased availability of inexpensive; highly refined, high-density foods; sedentary lifestyle; sleep apnea; endocrine function; smoking cessation; pharmaceutical iatrogenesis, adipose cellularity, increasing maternal age, and intrauterine and intergeneration effects. These factors are not only numerous but also interrelated and dynamic. Lifestyle changes including diet intervention, exercise, and behavior modification have been considered the cornerstone of therapy. These are achievable, modifiable behaviors that can reduce the incidence of preventable diseases. Although each of these cornerstone therapies is important in the care of these patients, this article will focus on diet modification for the treatment of obesity.

Metabolic Factors Affecting Weight Loss

To consider weight loss as merely a function of producing a caloric deficit regardless of dietary macronutrient composition or variances in biological systems is an oversimplification. The principles regarding regulation of heat, temperature, energy, and work are defined by the laws of thermodynamics. These laws are often applied to the induction and maintenance of weight loss such that when energy consumption is less than expenditure, there is mobilization of adipose tissue resulting in a reduction of body weight. However, when applied to living organisms, the equation becomes far more complex. The second law of thermodynamics dictates that there is an inevitable metabolic inefficiency in all biological and biochemical process. Therefore, it is impossible to convert a specific amount of energy into an equivalent amount of work. Energy is derived from chemical reactions in the body from the diet. But calories are not converted to energy on a one-to-one basis because of the inefficiency defined by the second law of thermodynamics. There is a larger loss of energy when one has to metabolize protein instead of merely oxidizing carbohydrate as an energy substrate.

Beyond the concept of thermodynamics, the biochemical, physiological, psychological, emotional, economic, and social factors surrounding mechanisms of weight loss are multifaceted, interrelated, and dynamic. Each of these factors has the potential to exert a significant biological and metabolic influence. For example, an increase in physical activity may influence metabolic rate that in turn affects insulin sensitivity and insulin resistance. There are other metabolic factors and changes that contribute to obesity. Alterations in endocrine function (e.g., decreases in leptin and increases in ghrelin) affect appetite and satiety level. There may be changes in neuroendocrine signaling (e.g., decreased satiety signaling) and changes in the responsiveness to food intake. With advancing age, there is a naturally occurring reduction in lean body mass, metabolic rate, energy expenditure, and physical activity, all of which will result in increased weight gain if the same level of calories is consumed throughout the life span. This along with many other factors affects the gut microbiota, which in turn influences the storage and release of energy. When oral intake is restricted to attempt to reduce body weight, there may be increased metabolic efficiency, making weight loss more difficult. There are also societal and economic factors that are intimately related to the biological and physiological factors. Our food supply has become more refined and highly processed often resulting in foods that are lower in nutrient concentration. This in turn affects satiety value due to the high concentration of refined sugars and the relatively low concentration of complex carbohydrates and dietary fiber. When there are financial limitations, people will purchase readily available low-cost foods, which may be low in nutrients but high in fat and refined carbohydrates. These are just some of the reasons why affecting a significant and sustained weight loss has been so difficult. Obesity is a very complex disorder with multiple factors, which are interrelated and dynamic such that a change in one factor will produce a change in another factor.

The Role of Diet

Although there are numerous factors affecting weight management, clearly, the role of diet is central in achieving and maintaining a healthy body weight. A plethora of weight loss diets have been proposed over the years. Many of these diets eliminate one or more of the essential food groups or recommend consumption of one type of food at the expense of other foods with little to no supporting evidence. These are commonly referred to as fad diets and are generally popularized in the lay literature. However, there are diets that are based on sound physiological and metabolic principles and studied under rigorous controlled conditions. Most of the modifications in diet have centered on altering the macronutrient composition (i.e., carbohydrate, protein, and fat) of the diet. These macronutrients cannot be considered in isolation. When the percentage of one component is changed, there will be a corresponding increase or decrease in the other components. Additionally, it is not simply a matter of increasing or decreasing the percentages of the three basic macronutrients, but it is also a matter of defining each of these macronutrients. A carbohydrate is not a uniform organic compound. For example, carbohydrates may be defined as simple or complex, by glycemic index (GI) and glycemic load, or by the fiber content (viscous vs. fermentable). Protein may be derived from animal, plant, or marine

sources, all with varying amino acid profiles. Fat encompasses monosaturated, polyunsaturated, saturated, and *trans* fats. They may include omega-3, omega-6, and omega-9 fatty acids or a combination. Along with altering the macronutrient content of the diet, attention must be given to the micronutrient portion to ensure adequate intake of vitamins, minerals, and trace elements.

Low-Fat and Very Low-Fat Diets

For many years, the traditional approach to weight loss had been with the use of the low-fat and very low-fat diet. Since fat is most calorically dense of the macronutrients (9 kcal g^{-1}), it was logical to assume that restricting its intake would result in weight loss. The exact composition of the low-fat diet varies but generally contains between 20% and 30% of calories from fat, and the very low-fat diet contains less than 19% of calories coming from fat. This means that the carbohydrate and in some instances the protein content of the diet will be higher. These diets tended to be lower in calories when properly initiated and resulted in weight loss when done correctly. Unfortunately, many individuals believed that a low-fat diet meant they could eat as many calories as desired as long as these foods were low in fat. The use of the low-fat diet without calorie restriction will not result in weight loss.

Since high-fat diets have been linked to inflammatory states, coronary artery disease, and various cancers, there was interest in determining if these diets could prevent or reverse disease in addition to reducing body weight. One of the most studied dietary interventions is the Ornish diet, which integrates a vegetarian diet with a fat intake of 10% of total calories. The program also integrates exercise, meditation, and smoking cessation. In an early pilot trial, Ornish was able to demonstrate positive effects in patients with ischemic heart disease including reductions in total cholesterol and a trend toward improved cardiac ejection fraction. However, high-density lipoprotein (HDL) levels decreased by 20%. To test the long-term effectiveness of this plan, they embarked on a 5-year randomized controlled trial of 48 patients with coronary heart disease. Subjects lost an average of 5.8 kg compared with no change in the control group, and the diet lowered low-density lipoprotein (LDL) levels by approximately 20%. Triglyceride and HDL levels were unchanged. Study participants following the Ornish diet had a 91% decrease in angina symptoms at 1 year and a 72% decrease at 5 years. The control group had a 186% increase in angina symptoms at 1 year and a 36% decrease at 5 years. The reduction at 5 years in the control group was attributed to greater rates of bypass surgery and angioplasty but not a function of the diet.

However, results from the low-fat arm of the Women's Health Initiative in which 20% of the calories came from fat with emphasis on fruits, vegetables, and grains failed to demonstrate any difference in cancer rates or incidence in cardiovascular disease, and overall, there was poor weight loss. Unfortunately, it was difficult for the participants to adhere to the diet and most of the subjects only achieved 24% fat consumption and, after 8 years, drifted up to 29% fat intake. The diet did not limit the calorie level nor did it define the type of fat to be consumed. Thus, individuals may have been following a lower level of fat, but the fat could have included harmful fats such as *trans* fats. The failure of the low-fat diet to induce weight loss and reverse or prevent disease in this long-term study reflects one of the major problems with low-fat diet. People often assumed that they could eat any level calories, as long as it was low fat. According to the CDC, energy intake increased by about 335 kcal per day from the years 1971 to 2000 with a significant increase in carbohydrate. This was partially fueled by an increase in the availability of commercially prepared low-fat convenience food items, which, although were low in fat, were often high calories and simple carbohydrates. The diet also tended to be high in highly processed white bread, white rice, and other rapidly digested carbohydrates, causing higher blood sugar levels and more hunger.

There have been numerous other studies that have looked at the use of low-fat diets to reduce body weight and comorbidities. A systematic review and meta-analysis of 33 randomized controlled trials demonstrated that diets lower in total fat were associated with lower relative body weight but the size of the effect varied. Greater reduction in total fat intake and lower baseline fat intake were associated with greater relative weight loss, lower body mass index, and waist circumference. The reductions in total fat intake were also associated with a small but statistically significant reductions in certain metabolic parameters including blood pressure, total cholesterol, LDL, and the ratio of total cholesterol to high-density lipoprotein cholesterol.

In spite of the inability to achieve and maintain a desirable body weight with the low-fat diet, it may become an important component of overall health and longevity. Onrnish and colleagues demonstrated that lifestyle intervention including a diet high in whole foods, plant-based protein, fruits, vegetables, unrefined grains, and legumes and low in fat (10%) and refined carbohydrate along with moderate aerobic exercise and stress management resulted in an increase in telomere length in men with biopsy-proved low-risk prostate cancer.

Low-Carbohydrate Ketogenic Diets

Carbohydrate restriction to induce weight loss has been extensively studied. The rationale for the restriction is that when the intake of carbohydrate is sufficiently reduced, there will be a corresponding reduction in insulin concentrations, which in turn will shift metabolism from lipogenesis to lipolysis, resulting in rapid weight loss. With a reduction in carbohydrate, there is a subsequent increase in the protein and fat content of the diet and production of ketones, which causes an increase in the satiety value and an overall voluntary caloric reduction. It is also possible that there is a metabolic advantage of inefficient protein and fat oxidation, which leads to extra energy loss as supported by the second law of thermodynamics. In many cases, lipolysis is maintained despite excess calories because glycerol from fat is needed as a gluconeogenic precursor. The exact carbohydrate level required to produce this metabolic shift is thought to be between 20 and 50 g per day in the initial (induction) phases of the diet in comparison with the carbohydrate content of the typical Western diet that often exceeds 300 g per day and may be in the form of simple, rapidly hydrolyzed carbohydrate.

There have been several randomized controlled trials (RCT) comparing weight loss and metabolic parameters of a low-carbohydrate ketogenic diet (LCKD) versus a low-fat diet. Sondike and colleagues examined the weight loss and the effects on cardiovascular risk factor of an LCKD in adolescents. Thirty-nine obese adolescents were randomized to receive either an ad libitum LCKD diet (<20 g day^{-1} of carbohydrate for 2 weeks and then <40 g day^{-1} of fat) or an ad libitum low-fat (LFD) diet (<40 g day^{-1} of fat) for 12 weeks. The LCKD subjects lost more weight than the LFD subjects (10 vs. 4 kg; $P<0.04$). There were no significant differences in the lipid profiles between the two groups and there were no serious adverse effects related to the intervention were reported.

Another RCT comparing an LCKD and a calorie-restricted low-fat diet was conducted to determine the effects on body weight, cardiovascular risk factors, and body composition in 53 healthy women. Twenty-two of 26 (85%) subjects from the LCKD and 20 of 27 (74%) subjects from the LFD completed the 6-month study, with both groups reducing their energy intake from baseline by approximately 450 kcal. Weight lost and reduction of body fat as measured by DXA were significantly greater in the LCKD group compared with the low-fat group at 3 and 6 months ($P<0.001$). There were no significant differences between groups in the effects on serum lipids, glucose, insulin, and leptin except triglycerides, which decreased significantly more in the LCKD group.

Samaha and colleagues randomized 132 severely obese (mean BMI, 43 kg m^{-2}) medical outpatients to either an ad libitum LCKD or a calorie-restricted LFD. Subjects received weekly group counseling sessions for 4 weeks and then monthly sessions, with 67% of LCKD subjects and 53% of LFD subjects completing the study. At 6 months, there was significantly greater weight loss (5.8 vs. 1.9 kg; $P=0.002$) and triglyceride reduction (20% vs. 4%; $P=0.001$) in the LCKD group compared with the LFD group. In addition, the diabetic subjects in the LCKD group demonstrated improved serum glucose (decrease of 25 vs. a decrease of 5 mg dl^{-1}; $P=0.01$) compared with their LFD group, whereas the nondiabetic subjects in the LCKD had improved insulin sensitivity (6% vs. -3%; $P=0.01$) compared with their LFD group. Seven LCKD subjects had a reduction of diabetic medication dosage compared with only one from the LFD group.

In a multicenter trial, 63 obese, healthy subjects were randomized to either the ad libitum LCKD or a reduced energy LFD (conventional diet) to examine the effectiveness of the LCKD. Each subject received a popular diet book and met with a dietitian at baseline and for three visits thereafter over the 1-year trial. Of the original 63 subjects, 37 completed the 12 months. The LCKD subjects lost more weight than the LFD subjects (7.0% reduction vs. 3.2% reduction; $P=0.02$) at 6 months, but the difference between groups was not statistically significant (-4.4% vs. -2.5%; $P=0.26$) at 12 months. The LCKD group experienced greater improvements in HDL cholesterol (increase of 11.0% vs. decrease of 1.6%; $P=0.04$) and triglycerides (decrease of 17.0% vs. increase of 0.7%; $P=0.04$) compared with the LFD group. Serum glucose and insulin measurements were not different between groups.

Yancy and colleagues conducted a number of studies to evaluate the effects of LCKD. In one study, the ad libitum LCKD combined with nutritional supplements was compared to a low-fat, low-cholesterol, reduced-calorie diet in hyperlipidemic individuals. LCKD participants were instructed to restrict carbohydrate intake to less than 20 g per day initially and gradually add carbohydrates back into their diet as goal body weight was approached. Energy intake was not restricted in the LCKD group and the emphasis was on the macronutrient composition of the diet. More subjects on the LCKD completed the study compared to the reduced-calorie subjects (75% vs. 53%; $P=0.03$). Weight loss was greater in the LCKD subjects who completed the study compared to the low-fat subjects (14% reduction vs. 9% reduction; $P<0.001$). The LCKD group also demonstrated beneficial changes in serum lipids: Triglycerides decreased by 42% and HDL cholesterol increased by 13%, whereas total cholesterol and LDL cholesterol did not change significantly. They conducted another trial in which community-dwelling overweight hyperlipidemic subjects randomly assigned to LCKD (<20 g) ($N=59$) or low-fat (<30%), low-cholesterol, reduced-calorie diet ($n=60$) for 24 weeks. Both groups received exercise and participated in group meetings. Patients in the LCKD group lost more weight, had a greater decrease in triglyceride levels, and had higher HDL-C levels. Levels of LDL-C remained stable in both groups.

Gardner and colleagues evaluated the 12-month effectiveness of four different weight loss diets among 311 overweight or obese, nondiabetic, premenopausal women. There was greater mean weight loss at 1 year in participants following the Atkins diet (4.7 kg) compared with those using the Zone low-carbohydrate (1.6 kg), Ornish very low-fat (2.2 kg), and LEARN (lifestyle, exercise, attitudes, relationships, and nutrition low-carbohydrate) (2.6 kg) diets. LDL levels were significantly reduced in the Atkins group, with no reduction in HDL levels or blood pressure. Insulin and fasting glucose levels did not differ among groups. Whether the improved risk profile was due to the absolute weight loss or diet composition is not clear as the study was not designed to answer this question.

A meta-analysis of very low-carbohydrate diets evaluated six trials with 447 overweight participants. In most cases, the control diet was a low-fat, calorie-restricted plan. Those following the low-carbohydrate plan had greater weight loss at six months, but no difference at 12 months. One of the concerns with the use of these diets was that there may be an increase in blood cholesterol, triglycerides, and LDL due to the high-fat composition of the diet. However, there was no significant difference in the levels of LDL between the diets. HDL and triglyceride levels improved at 6 and 12 months in subjects on the very low-carbohydrate diet.

In each of these well-controlled clinical trials, the LCKD demonstrated superior weight loss when compared to more traditional approaches. There are some adverse effects that could potentially occur even though they have not been observed in any of the clinical trials to date. These include the development of kidney stones, hypokalemia and hypomagnesemia, elevated fatty acids, and gout in susceptible individuals. When this diet technique is employed in individuals taking medications for diabetes mellitus or hypertension, meticulous monitoring is required in order to prevent hypoglycemia and hypotension as body weight is rapidly reduced.

Low-GI/Low-Glycemic-Load Diets

Related to the low-carbohydrate diet is the concept of the GI that was originally developed to aid those with diabetes manage their carbohydrate intake relative to insulin requirements. The index is a ranking of various carbohydrate foods, which measures the rate at which blood glucose levels rise when a particular food is ingested and how quickly the blood glucose levels drop. The GI should not be confused with the glycemic load, which is the mathematical product of the GI and the carbohydrate. Diets with a high glycemic load result in higher postprandial insulin concentration than those with a low glycemic load. Pure glucose has a rating of 100; thus, the closer a food is to 100, the higher the GI rating. Foods with a low GI rating will be absorbed more slowly, keeping blood glucose levels lower and more sustained. Certain low-carbohydrate diets such as South Beach, Zone, Sugar Busters, and Nutrisystem utilize the GI approach and are low in sugar and starch but not low in total carbohydrate. The diets are relatively high in fiber and are thought to be more satisfying than high-glycemic-index foods since they take longer to absorb and help increase the satiety value of the diet.

Several investigators have examined glycemic response, blood sugar, and insulin levels in subjects randomized to receive low- versus high-GI foods. The hormonal and metabolic responses to the low- and high-GI meals are different. Serum insulin concentrations are high following ingestion of the high-GI meal due to the rapid absorption of glucose. By contrast, plasma glucagon levels are suppressed. Additionally, the high-GI diet has been shown to result in a spike in counterregulatory (plasma epinephrine and growth hormone) hormones that trigger hunger. Weight loss with the low-glycemic-load diet has been shown to be greater than the low-fat diet, which has been associated with higher insulin concentrations. Low-glycemic-load diets have beneficial effects on HDL cholesterol and triglyceride concentrations but not on LDL cholesterol concentration. The use of the low-glycemic-load diet has been shown to produce larger reductions in HbA1c than those on the low-fat diet, which suggests that the quality and quantity of carbohydrate consumed can significantly enhance the effect of weight loss on HbA1c in patients with type 2 diabetes.

Use of the GI has been criticized due to some apparent irregularities. For example, white bread and whole wheat bread rank comparably on the index scale as do brown and white rice, yet clearly the whole grain choices are healthier. There are some scores that are confusing and inconsistent and will vary depending on how the food is prepared. Carrots, for example, are a nutrient-rich, high-fiber vegetable that can have a GI ranking of 47, plus or minus 16 depending on preparation. Pasta that is cooked al dente will rank lower than fully cooked pasta. Some candy and confections that include nuts have a better GI score than a potato because the composite score of the candy is made up of the lower carbohydrate content of the nuts plus the other ingredients in the candy. There are also variations within a food; ripe bananas have higher GI scores than underripe bananas presumably due to the changes in the sugar content during the ripening process. It should be noted that dietary foods are generally not consumed in isolation. When carbohydrates are paired with other foods, it impacts the glycemic load. Thus, ingestion of cheese and crackers together will produce a different GI than cheese or crackers alone. The fat content and food temperature will affect gastric emptying and GI. Thus, reliance on the GI/load can be helpful but can also be a source of confusion to many consumers.

Mediterranean Diet

Considering the relative lack of success of the low-fat diet and the questions with the long-term use of the low-carbohydrate diet, people became interested in the use of the Mediterranean diet when the Seven Countries Study showed low cardiovascular mortality rates in a cohort from Crete despite high levels of dietary fat intake (35–40% of total calories). The exact composition of the Mediterranean diet varies depending on the eating habits of the population in countries bordering on the Mediterranean Sea, but overall, it emphasizes fruits, vegetables, legumes, whole grains, nuts, olives, and olive oil along with some cheese, yogurt, fish, poultry, eggs, and red wine in moderation. Red meat is limited to no more than a few times a month. The diet is not simply a weight loss diet. Rather, it is a part of an overall healthy lifestyle that includes exercise, leisurely dining, and socialization during meal times. One of the major advantages of the Mediterranean diet is that most consider it to be palatable and compliance is generally good.

In a 2-year interventional trial, Shai et al. randomly assigned 322 moderately obese subjects to one of three diets: low-fat (<30%), restricted-calorie; Mediterranean (<35% fat), restricted-calorie; or low-carbohydrate ($<20\ g\ day^{-1}$), non-restricted-calorie. The rate of adherence to the assigned study diet was 95.4% at 1 year and 84.6% at 2 years. The 2-year adherence rates were 90.4% in the low-fat group, 85.3% in the Mediterranean diet group, and 78.0% in the low-carbohydrate group ($P=0.04$). The mean weight loss was 2.9 kg for the low-fat group, 4.4 kg for the Mediterranean diet group, and 4.7 kg for the low-carbohydrate group ($P<0.001$). Additionally, the relative reduction in the ratio of total cholesterol to HDL cholesterol was 20% in the low-carbohydrate group and 12% in the low-fat group ($P=0.01$). Among the 36 subjects with diabetes, changes in fasting plasma glucose and insulin levels were more favorable among those assigned to the Mediterranean diet than among those assigned to the low-fat diet ($P<0.001$). Although the rate of weight loss was greatest in the low-carbohydrate group, those following the Mediterranean diet were able to achieve substantial weight loss with favorable changes on other metabolic parameters.

The Lyon Diet Heart Study was a randomized, controlled trial of 605 free-living subjects with known coronary heart disease. Subjects were randomized to receive the Mediterranean diet or usual postinfarction low-fat diet. Participants in the Mediterranean diet group supplemented with alpha-linoleic acid, as a source of omega-3 fatty acids. Although the study was planned to be a long-term 5-year trial, it was terminated at 27 months' mean follow-up time because the benefits in the experimental group were so striking and included a 70% reduction in all-cause mortality including cancer, reduced cardiovascular disease events, and better weight loss than the control diet. The intake of dietary fiber was higher in the Mediterranean group, about 3 g, as well as a higher intake of antioxidants and B vitamins found in fruits and vegetables. The

Mediterranean group consumed significantly less lipids, saturated fat, cholesterol, and linoleic acid but more oleic and alpha-linolenic acids, which was confirmed by measurements in plasma. There were no significant differences between the groups in serum lipid levels, blood pressure, or BMI. High levels of serum alpha-linoleic acid were associated with improved outcomes. With the mean follow-up time of 46 months per patient, the initial beneficial effects of the Mediterranean diet continued compared with the group consuming the prudent low-fat diet.

Most of the studies on the Mediterranean diet were conducted in Europe. The first investigation of the Mediterranean diet to be conducted in the United States was published as part of the National Institutes of Health–AARP Diet and Health Study. There was an inverse association between adherence to a Mediterranean style diet and cardiovascular disease, cancer, and overall mortality rates.

Although the Mediterranean diet is palatable, some may find it more difficult to follow due to the inability to purchase fresh fruits and vegetables. The question becomes can these beneficial effects be replicated without the consumption of Mediterranean foods? The GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto) trial, which showed that supplementation with $n-3$ polyunsaturated fatty acids (fish oil) after myocardial infarction (MI) reduced the risk of MI recurrence, stroke, and death by 10%. In another study, rates of MI in high-risk patients following a Mediterranean diet supplemented with soybean and mustard seed oils were significantly reduced. These studies focussed on the anti-inflammatory properties of the poly- and monounsaturated fats. Thus, the exact mechanism for the beneficial effects of the Mediterranean diet is unclear and may comprise a variety of factors including a higher intake of fiber, naturally occurring antioxidants, and omega-3 fatty acids along with a reduction of highly processed foods.

Prescribing Diet Modification Interventions

Unfortunately, there is no one diet that has been shown to induce and maintain a healthy body weight in all circumstances. Those individuals who select a non-evidence-based fad diet plan will most likely lose weight in the short term but often at the expense of nutrient deprivation and unsustainable weight loss. Selection and implementation of a weight loss program should be based on the best available evidence for achievable and sustainable weight loss, positive effects on metabolic parameters, and overall reduction of morbidity and mortality. This is not a simple decision. There are numerous factors to consider such as the degree of obesity and associated comorbidities as well as medications affecting appetite (Figure 1). There are also some data to suggest that the degree of insulin sensitivity determines the effectiveness of dietary macronutrient composition in inducing weight loss. In a 4-month feeding trial, a high-carbohydrate low-fat diet was more effective in inducing weight loss for insulin-sensitive

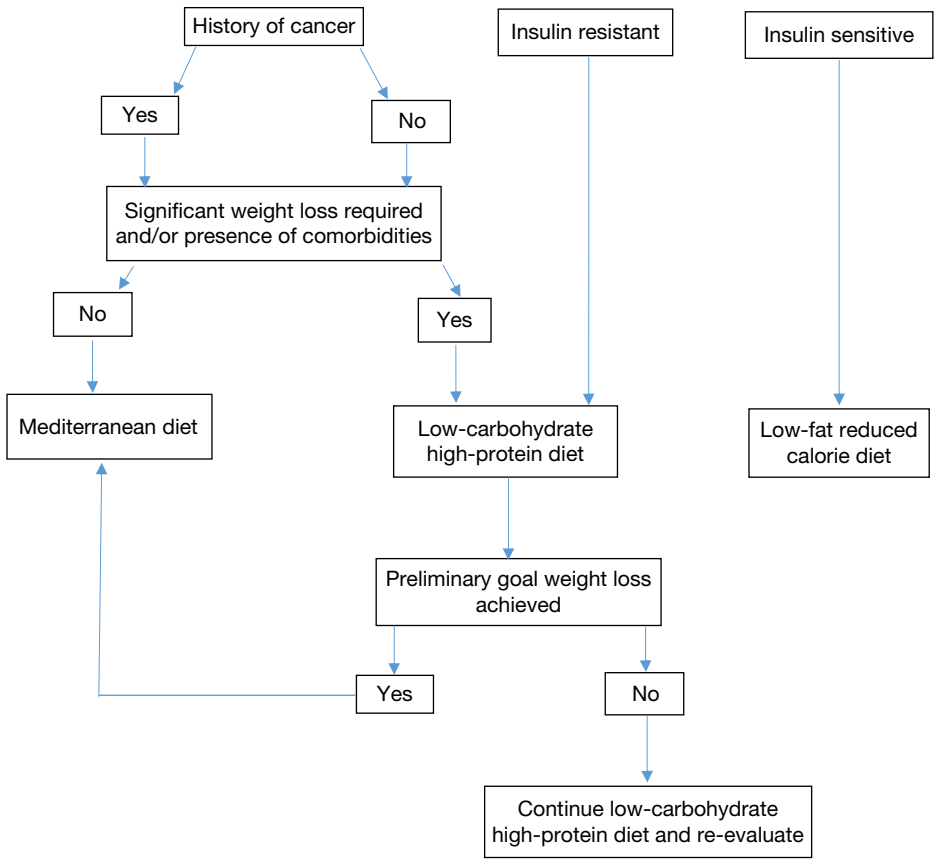

Figure 1 Proposed algorithm for prescribing weight loss diets based on metabolic and disease parameters.

women, but a low-carbohydrate high-fat diet was more effective for insulin-resistant women. Similar results were achieved by Ebbeling and colleagues in 6-month intensive intervention period with a 12-month follow-up period.

Obesity, as well as diets high in fat and red meat, has been linked to many cancers. Although a high-protein low-carbohydrate diet has been shown to produce the greatest weight loss in the shortest amount of time, this may not be the best choice for an overweight/obese individual with a history of cancer since these diets tend to be high in fat and often red meat. In this circumstance, the adoption of the Mediterranean diet may be more appropriate. This diet will result in a slower sustained weight loss and has the added benefit of being rich in foods that are high in antioxidants. Alternatively, a high-protein, low-carbohydrate diet can be used to induce rapid weight loss and then switched to a Mediterranean diet for continued and sustained benefits.

There are additional socioeconomic factors to consider, for example, the illiterate, totally noncompliant patient or those with limited financial resources who cannot or will not be able to follow a dietary modification program. There are several approaches that can be used. The patient can be instructed to eat half of their usual and customary diet and/or attempt to increase exercise simply by walking. The largest meal should be consumed early in the day. It may not be optimal, but it may be the best that can be achieved in this free-living situation. Some patients will do best with the use of a weight loss meal replacement formula such as Optifast (Nestle) provided once or twice daily plus one regular meal. If the diet is deficient in micronutrients, a daily multivitamin should be added. Additionally, the work or home environment should be considered. A construction worker or someone who works in a field may not have access to refrigeration making it difficult to bring lunch to work.

Summary

Obesity is a serious, complex, and highly prevalent global disease associated with increased morbidity and mortality. There are numerous factors that impact metabolism. These factors are interrelated and dynamic. Primary treatment should be directed at achieving and maintaining desirable body weight. Achievement and maintenance of a healthy body weight are difficult. Certainly, any diet that is hypoenergetic will result in short-term weight loss. Body weight can be reduced with a number of scientifically based weight loss diets if implemented correctly and with adequate compliance. The weight loss diet should be individualized for each patient based on BMI, metabolic risk factors, lifestyle, and cultural, religious, and food preferences. The diet should be nutritionally balanced and provide all necessary nutrients. A strong educational component is essential to include not only the mechanics of the diet but also portion control, eating in restaurants, and how to change behaviors for lifelong health. It is important to help the patient find a balance between food and physical activity for overall well-being and fitness. High-risk patients with multiple comorbidities should be medically supervised and carefully monitored. Ultimately, the best diet is the one the patient will follow and incorporate into their daily lives for lifelong maintenance of a healthy body weight.

See also: Appetite Control in Humans: A Psychobiological Approach; Cancer: Diet in Cancer Prevention; Mediterranean Diet; Obesity: Causes and Prevalence; Obesity: Epidemiology of; Satiety.

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http://win.niddk.nih.gov/index.htm – Weight Control Information Network.

Offal: Types and Composition

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Introduction

According to a traditional definition, offal can be described as the internal organs and entrails of a butchered animal excluding muscle and bone. Marti published that offal is a term used to categorize parts of a butchered animal that 'falls off' the carcass when being trimmed, such as the organs and entrails. Offal classification includes the distinction between red and white offal. Red offal comprised parts that are directly edible, can be consumed in a recognizable form, and are considered to be delicacies in certain parts of the world. They are widely used in many processed meats and as ingredients in sausages and spreadable products like pâtés. On the other hand, white offal refers to parts that are edible but require further processing, such as the stomach and intestines.

Based on the FAOSTAT latest results, the most abundant species of animals in 2013, which were used for human consumption, were, in number of livestock, chicken (21 billion); cattle (1.5 billion); lambs (1.2 billion); pigs, ducks, and goats (1 billion each one); rabbit (0.9 billion); and turkeys (0.4 billion). Weight of meat production shows the most important is porcine (109 million of tons or Mt), followed by chicken (92 Mt), cattle (63.2 Mt), turkey (5.6 Mt), and goat (5.3 Mt). The same organization published that the most captured fish species in 2011 were anchoveta (8, 3 Mt), Alaska pollack (3.2 Mt), skipjack tuna (2.6 Mt), herring (1.7 Mt), mackerel (1.7 Mt), Japanese anchovy (1.3 Mt), and largehead hairtail (1.2 Mt). Based on these figures, it can be assumed that large volumes of offal are produced every day in both industries: meat and fisheries. Table 1 summarizes the main offal parts from the most consumed animal around the globe. In general, average values are presented; however, yield may vary widely depending on species, sex, live weight, and method of recovery.

The main data used in this article have been obtained from the online database provided by the Agricultural Research Service of the US Department of Agriculture, National Nutrient Database for Standard Reference (web link in table). The data presented in this article are for raw offal. For details relating the cooked offal, the reader is directed to the USDA database.

Proximate Analysis of Offal

Proximate analysis provides a breakdown of a material into its major constituents: water, protein, fat, carbohydrates, and ash. In terms of caloric intake, undoubtedly, proteins and fats are the most important component in offal. In general terms, protein and fat contents tend to be lower than that of meat cuts. Those exhibiting higher protein contents than meat have a higher content of connective tissue (ears, blood vessels, tongue, or tail). Offal with higher fat content includes products such as tongue, skin, and feet that also tend to have the lowest water content. Because of this composition, most offal supply less energy than the equivalent weight of meat.

Protein Content and Amino Acid Profile

The average protein content in offal, regardless the source, is generally between 15% and 18%, and they contain both essential (EAA) and nonessential amino acids. Table 2 shows the most common offal and the associated protein, EAA profile, and EAA content. EAA content can vary from 38% (in porcine jowl) to 49% (beef liver, porcine heart, or sheep spleen). These values are very close to the EAA level in lean meat, which is ~50%. The main difference can be found in lysine and histidine concentration: In meat, their average value is 9.8 and 5.1 g/100 g of total protein, respectively; in offal, the concentration is between 7–8 and 2–3 g/100 g, respectively. Apart from these two amino acids, from a nutritional point of view, offal provides a source of good quality proteins. In the specific case of blood, from either cattle and porcine, it is notably the great amount of EAA (close to 60%) that is even higher than meat.

In products with a high connective tissue component, protein content generally is higher than 20%, for example, cattle lips (22%), ear (22–24%), feet (21–26%), and liver (21–22%) or porcine skin (22%). Collagen and elastin are the principal type of proteins that can be found in those products rich in connective tissue, with the proportion ranging from 93% of total protein (skin), to 88% in blood vessels, to 71% in ears. As a result, these by-products contain a high amount of proline, hydroxyproline, and glycine. Due to the amino acid profile of the collagen proteins in particular, these tissues also tend to be poor sources of certain EAAs such as histidine, tyrosine, methionine, and cysteine. Also, many of these products can contain relatively low overall EAA content, for example, cow lips (17.9%), feet (21.6%), skin (23.3%), or tail (27.6%).

While many offal with protein content higher than meat have high portion of connective tissue proteins and hence low ESS, a remarkable exception is seen in the liver. This organ has protein content of around 20% depending on the species, and 50% of total protein content is constituted by EAA.

Fat Content and Fatty Acid Profile

Total fat content in offal is highly dependent on the type of organ; it varies from lower values such as 0.1% in blood, 1.4% in spleen, or 1.9% in lung to very high levels as 10.6% in brain, 15.5% in lamb tongue, 33.5% in pig tail, or even 69.6% in porcine jowl.

The proportions of fatty acids (FAs) vary with species, diet, age, and gender. In general terms, oleic acid is the most abundant FA in the animal body of lamb, cattle, and pigs (20–47%); while in poultry, the most abundant is palmitic acid (26%).

Fish oil, extracted from either liver, viscera, or by-products, is the richest source of monounsaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs). In such products, <23% of total fats are saturated FAs (SFAs). Fish are the main source of eicosapentaenoic and docosahexaenoic acids (0.5–2% of total

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Table 1 Percentage of weight regarding total live animal weight of main offal in different meat, poultry, and fish species

Product	*Beef*	*Pig*	*Lamb*	*Duck*	*Turkey*	*Chicken*	*Product*	*Average % of fish offal*	*Mackerel*	*Tuna*	*Cod*
Blood	2.4–6	2–6	4.9	3.3–4.8	3.3–4.8	3.3–4.8	Backbone	14	7.7	10	9.7
Brain	0.08–0.12	0.08–0.1	0.26			0.2–0.3	Fins and lugs	–	0.3	–	–
Ears	0.02	0.04	0.03	–		–	Gut	7	7.5	9	5.6
Feet	1.9–2.1	1.5–2.2	2.0	1.75	2.85	3–4	Head	21	15.6	20	20.2
Genitals			2				Liver	5	–	6–10	–
Gizzard				1.75	1.21	1.9–2.3	Roe	4	–	6–10	0.7
Head	2.5–3	5.2	6.7	3.5	2.85	3–4	Skin	3	7.1	4	4.2
Heart	0.3–0.5	0.15–0.35	0.3–1.1	0.7	0.35	0.3–0.8	Tail	1	0.8	–	–
Intestines	2–3	1.8	1.3								
Kidney	0.07–0.24	0.2–0.4	0.3–0.6								
Liver	1.0–4.5	1.1–2.4	0.9–2.2	2.9–3.1	1.1	1.6–2.3					
Lung lobes	0.4–0.8	0.4–0.85	0.7–2.2			0.7					
Omasum	0.6		1–1.2								
Pancreas	0.06	0.4	0.2								
Spleen	0.1–0.27	0.1–0.16	0.1–0.4			0.15					
Tail	0.1–0.25	0.1	1								
Tongue	0.25–0.5	0.3–0.4	0.5								
Tripe	0.75	0.6–0.7	2.9–4.6								
Total (%)	18.9	17.6	26.3	13.6	13.2	15.6		65	39	55–63	40.3

Table 2 Total protein content (%), individual essential amino acids (g/100 g protein), and total essential amino acids (% EAA) in selected offal across a number of species and fish by-products

Product	*Species*	*Protein (%)*	*Leu*	*Ile*	*Lys*	*Met*	*Cys*	*Phe*	*Tyr*	*Trp*	*Thr*	*Val*	*His*	*% EAA*
			Essential amino acid content (g/100 g protein)											
Blood	A	17–18	13.2	0.9	9.7	2.4	n.d.	10.7	1.4	–	4.8	8.7	8.8	60.6
	B	18.5	13.0	1.3	9.0	2.3	n.d.	9.7	2.9	1.5	3.7	9.0	5.6	58
Brain	A	10.5	7.5	3.9	6.0	2.1	1.8	5.0	3.6	4.7	4.7	4.9	2.5	42.8
	B	10.3	8.7	4.6	7.8	2.0	1.3	5.1	4.2	4.2	4.7	5.7	5.7	46.8
	C	12.3	7.8	4.0	6.4	2.0	1.1	4.8	3.7	1.1	4.5	4.8	4.8	42.8
Ear	B	22.3	5.2	2.2	4.7	0.6	1.3	3.2	1.8	0.2	2.8	3.7	1.2	25.6
Feet	B	21.2	4.2	1.6	4.3	1.0	–	2.7	1.5	0.2	2.6	2.3	1.2	21.6
Heart	A	17	8.8	4.4	8.2	2.6	1.3	4.5	3.6	1.1	4.7	5.2	2.7	47.1
	B	17	9.0	4.8	8.3	2.6	1.8	4.4	3.4	1.2	4.4	5.3	2.5	47.7
	C	18	8.5	4.3	7.5	2.2	0.8	4.3	3.1	1.1	4.7	5.0	2.3	43.8
Kidney	A	15.3	8.0	4.1	6.6	2.1	0.8	4.8	3.8	1.4	4.8	6.2	2.6	45.2
	B	15.4	9.0	5.3	7.2	2.1	2.2	4.7	3.6	1.3	4.1	6.0	2.5	48.0
	C	18.0	7.5	4.0	6.5	2.0	1.1	4.7	3.5	1.4	4.7	5.9	2.6	43.9
Lips	A	21.8	3.1	1.8	4.1	–	–	3.1	–	0.5	2.0	3.3	–	17.9
Liver	A	21	9.4	4.6	6.9	2.5	1.5	5.3	4.0	1.4	4.6	6.2	2.7	49.1
	B	19	8..9	5.1	7.7	2.5	1.9	4.9	3.4	1.4	4.2	6.2	2.7	48.9
	C	20.3	8.2	4.3	5.4	2.1	1.0	4.5	3.6	1.2	4.5	5.5	2.4	42.7
Lung	A	17	7.3	4.8	7.1	2.0	1.5	4.1	2.2	0.9	3.7	4.9	3.0	41.5
	B	15	7.8	4.0	7.3	1.6	–	4.2	-	0.9	3.5	6.0	2.5	37.8
	C	12.5	8.0	3.2	6.5	1.8	1.6	4.1	2.8	0.9	3.7	5.5	2.5	40.6
Spleen	A	19	8.8	3.8	7.2	1.8	2.9	4.0	5.5	1.0	3.9	6.0	3.6	48.0
	B	17.9	8.2	4.5	7.5	1.8	-	4.3	2.8	1.0	4.0	5.4	2.4	41.9
	C	17.2	8.9	6.3	7.7	1.9	1.3	4.5	2.9	1.1	4.1	6.5	3.3	48.5
Tongue	A	17.1	7.5	4.3	7.7	2.1	1.3	4.1	3.2	0.8	4.4	4.8	2.6	45.8
	B	16.3	8.0	4.6	8.2	2.2	-	4.1	-	1.2	4.2	5.2	2.5	40.2
	C	15.3	7.1	3.9	7.1	2.1	1.1	3.7	2.9	1.0	4.5	4.8	2.2	40.4
Fish by-product	D	15-30	9.1	5.3	10.1	6.9	1.6	4.0	1.8	0.8	2.8	6.4	5.2	54.0
	E	15-30	9.6	5.3	7.5	3.2	1.9	5.0	2.4	1.0	3.2	5.9	2.6	47.6

A, cattle; B, porcine; C, sheep; D, commercial saltwater fish waste; E, commercial freshwater fish waste.

Table 3 Fat, cholesterol, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) per 100 g of raw material of several offal products

Product	*Species*	*Fat (%)*	*Cholesterol (mg)*	*SFA (g)*	*MUFA (g)*	*PUFA (g)*	*Linoleic (g)*	*Linolenic (g)*
Blood	A	0.4	90	0.1	0.1	0.1		
	B	0.4	40	0.1	0.1	0.1		
Brain	A	10.3	3010	2.3	1.9	1.6	0.01	0.0
	B	9.2	2195	2.1	1.7	1.4		
	C	8.6	1352	2.2	1.6	0.9		
Fat/tallow	A	100	109	49.8	41.8	4		
	B	99	95	39.2	45.1	11.2		
	C	80	78	32.3	21.7	2.3		
	D	99	85	29.8	44.7	20.9		
	E	99	100	33.2	49.3	12.9		
Feet	B	22	82	6.5	9	2		
	D	14.6	84	3.9	5.5	3.0		
Heart	A	3.9	124	1.4	1.1	0.5	0.25	0.06
	B	4.4	131	1.2	1.0	1.1	0.308	0.09
	C	5.7	135	2.2	1.6	0.6		
	D	9.3	136	2.7	2.4	2.7		
Kidney	A	3.1	411	0.9	0.6	0.5	0.19	0.04
	B	3.2	319	1.0	1.1	0.3	0.15	0.05
	C	3.0	337	1.0	0.6	0.6		
Liver	A	3.6	275	1.2	0.5	0.5	0.29	0.01
	B	3.7	301	1.2	0.5	0.9	0.15	0.14
	C	5.0	371	1.9	1.2	1.3	0.47	0.01
	D	4.8	345	1.6	1.1	0.8		
Lung	A	2.5	242	0.9	0.6	0.3		
	B	2.7	320	1	0.6	0.3		
	C	2.6	n.d.	0.9	0.7	0.4		
Spleen	A	3.0	263	1.0	1.2	0.5		
	B	2.6	363	0.9	0.7	0.2		
	C	3.1	250	1.0	0.8	0.2		
Tongue	A	16.1	87	7.0	7.2	0.9	0.27	0.13
	B	17.2	101	6.0	8.1	1.8	0.34	0.27
	C	17.2	156	6.6	8.5	1.1		
Fish oil	Herrin	100	766	21.3	56.6	15.6	0.6–2.9	0.2–1.1
	Cod liver	100	570	22.7	46.7	22.5	0.8–2.1	0.9–1.1
	Salmon	100	485	19.9	29.1	40.3	1.2	0.5
DV		65 g	300 mg	<10%[a]	15–20%[a]	8–20%[a]		

The content of linoleic and linolenic acid is shown in those products where data were available.
A, cattle; B, porcine; C, lamb; D, chicken; E, duck. DV, daily value according to the US Food and Drug Administration.
[a]Recommended amounts of fats based on the fact that it must supply 20–35% of total energy intake.

fat). Cholesterol content in oil extracted from fish by-products is the highest one, reaching values over 700 mg 100 g^{-1}; this value is only higher in brain tissue, where it could be as high as 2200 mg 100 g^{-1} as in porcine brain.

Table 3 summarizes the fat and cholesterol content, as well the proportion of SFA, MUFA, PUFA, and essential FAs in the most common offal. Detailed FA profile can be found in the USDA National Nutrient Database for Standard Reference.

Carbohydrate Content

With the exception of liver, where carbohydrates in the form of glycogen can account for between 8.5% (beef) and 15% (chicken) of weight, the content in carbohydrates in offal usually is lower than 1%: which are the same levels that can be found in meat cuts. Low sugar content can present some advantages as it does not cause secondary degradation reactions like the reaction of Maillard during cooking or heat treatments, racemization, or cross-linking reactions.

Mineral and Vitamin Content

Next to protein and fat, the nutritional interest in offal meat lies in the mineral and vitamin profile. Offal can often be considered as a low-nutrient-value product; however, it can contain higher vitamin or mineral content than meat. In this section, it is demonstrated that the best animal sources of vitamins and minerals are actually offal tissue. Average mineral content in 100 g of raw bovine muscle is 2.4 mg of iron, 3.7 mg of zinc, 0.1 mg of cupper, 3 mg of iodine, 7 μg of selenium, and 11 μg of manganese. In addition, the same beef muscle can provide certain amounts of vitamins in the diet; although lipid-soluble vitamins are found at very low levels in meat or offal (with the exception of vitamin A in the liver), the content of

water-soluble vitamins could be enough to cover the daily value (DV) as in the case of vitamin B_{12}. For comparative purposes the, average value contents of some water-soluble vitamins in meat are: niacin, 6.7 mg; thiamine, 0.05 mg; vitamin B_6, 0.6 mg; vitamin B_{12}, 2 μg; and vitamin C, <1 μg. When we compare values in meat with those in offal (Tables 4 and 5), it is clear that offal are a very rich reservoir for this nutrients.

Mineral Content

Minerals are inorganic micronutrients. We need them in small amounts to help our body function properly. There are 16 minerals that are essential nutrients and must be supplied by the diet. These minerals are divided into two groups: major and trace minerals. Major minerals, like calcium, chloride, magnesium, phosphorus, potassium, sodium, and sulfur, are found in our body in amounts larger than 5 g. Trace minerals, like chromium, copper, fluoride, iodine, iron, manganese, selenium, and zinc, are found in our body in amounts <5 g.

The content of minerals in offal is often higher than in muscle tissue; as an example, liver and kidney are a good source of essential minerals for human diet as Fe, Cu, Zn, and Se, especially iron and zinc, that are presented in higher concentration in offal than in meat. A remarkable case is pork liver where iron content is 233 mg kg^{-1}, which is up to 23 times higher than in meat; kidneys from beef, pork, and lamb are an excellent source of selenium, which is four of five times more concentrated than in meat. Liver, regardless of species, is a good source of manganese; its concentration can be six to ten times higher than in meat cuts. Both meat and offal are good sources of iron, but it may not always be recognized by consumers that they are even better sources of zinc.

There are two types of dietary iron that can be consumed: heme iron and nonheme iron. Heme iron is found in meat and fish in the form of myoglobin or hemoglobin. Iron from plant sources is nonheme iron, though this is also present in animal tissue. Nonheme iron has a very low rate of absorption, which can be increased by co-ingestion with vitamin C or heme iron. Taking into account that iron deficiency is the most common nutritional disorders in the world (in both developed and developing countries) affecting a 25% of total offal may play a role in the enhancement of iron intake and absorption in the world.

Regarding fish by-products, calcium and phosphorus are the most abundant minerals commanding ~5.8% and 2.04% of the total weight in dry basis, respectively. As calcium is deficient in the most of the regular diets, consumption of whole small fish or addition of fish by-products to improve calcium intake can be nutritionally valuable.

In the same way that beneficial minerals are accumulated in the offal, certain heavy metals such as cadmium, lead, mercury, and arsenic can be detected in these organs at higher levels than it can be found in muscles. However, the concentration of these metals is strongly dependent on the environment and diet, with the environment in particular (mining, heavy, or chemical industry) having a strong influence. Liver and kidney seem to accumulate most heavy metal, followed by lung, heart, intestines, and brain. The liver and kidneys are target tissues

Table 4 Mineral content in raw offal expressed as mg 100 g^{-1} – with the exception of selenium that is expressed as μg kg^{-1}

Product	*Species*	*Ca*	*Fe*	*Mg*	*P*	*K*	*Na*	*Zn*	*Cu*	*Mn*	*Se*
Brain	A	43	2.5	13	362	274	126	1.02	2.9	0.03	21.3
	B	10	1.6	14	282	258	120	1.27	2.4	0.10	15.9
	C	9	1.75	12	270	296	112	1.17	2.4	0.04	9.0
Heart	A	7	4.31	21	212	287	98	1.70	4.0	0.03	21.8
	B	5	4.68	19	169	294	56	2.80	4.0	0.06	10.4
	C	6	4.6	17	175	316	89	1.87	4.0	0.05	32.0
	D	12	5.96	15	177	176	74	6.59	3.4	0.01	4.3
Kidney	A	13	4.6	17	257	262	182	1.92	4.3	0.14	141.0
	B	9	4.89	17	204	229	121	2.75	6.2	0.12	190.0
	C	13	6.38	17	246	277	156	2.24	4.5	0.12	126.9
Liver	A	5	4.90	18	387	313	69	4.00	9.8	0.31	39.7
	B	9	23.3	18	288	273	87	5.76	6.8	0.34	52.7
	C	7	7.37	19	364	313	70	4.66	7.0	0.18	82.4
	D	8	8.99	19	297	230	71	2.67	4.9	0.25	54.6
Lung	A	10	7.95	14	224	340	198	1.61	2.6	0.02	44.3
	B	7	18.9	14	196	303	153	2.03	0.8	0.02	17.8
	C	7	5.23	12	288	274	108	1.16	2.4	0.02	17.2
Spleen	A	9	44.5	22	296	429	85	2.11	1.7	0.07	62.2
	B	10	23.2	13	260	396	98	2.54	1.3	0.07	32.8
	C	9	42.0	21	280	358	84	2.84	1.2	0.05	32.4
Tongue	A	6	2.95	16	133	315	69	2.87	1.7	0.02	9.4
	B	16	3.35	18	193	243	110	3.01	0.7	0.01	10.4
	C	9	2.65	21	184	257	78	2.32	2.1	0.05	15.0
Fish by-products[a]		5800	10.0	170	2040	680	610	62.0	10	60	–
DV		1000 mg	18 mg	375 mg	1000 mg	3500 mg	2400 mg	15 mg	2 mg	2 mg	70 μg

A, beef; B, porcine; C, lamb; D, chicken; DV, daily value according to the US Food and Drug Administration.
[a]In dry basis.

Table 5 Concentration of water-soluble vitamins in several types of offal

Product	*Species*	*Thiamin*	*Riboflavin*	*Niacin*	*Pantothenic acid*	*Vitamin B_6*	*Vitamin B_{12}*	*Folate*	*Vitamin C*
Brain	A	0.092	0.199	3.550	2.010	0.226	9.51	3	10.7
	B	0.155	0.275	4.275	2.800	0.190	2.19	6	13.5
	C	0.130	0.300	3.900	0.920	0.290	11.30	3	16.0
Heart	A	0.238	0.906	7.530	1.790	0.279	8.55	3	2.0
	B	0.613	1.185	6.765	2.515	0.390	3.79	4	5.3
	C	0.370	0.990	6.140	2.630	0.390	10.25	2	5.0
	D	0.152	0.728	4.883	2.559	0.360	7.29	72	3.2
Kidney	A	0.357	2.840	8.030	3.970	0.665	27.50	98	9.4
	B	0.340	1.697	8.207	3.130	0.440	8.79	42	13.3
	C	0.620	2.240	7.510	4.220	0.220	52.41	28	11.0
Liver	A	0.189	2.755	13.175	7.173	1.083	59.30	290	1.3
	B	0.283	3.005	15.301	6.650	0.690	26.0	212	25.3
	C	0.340	3.630	16.110	6.130	0.900	90.05	230	4.0
	D	0.305	1.778	9.728	6.233	0.853	16.58	588	17.9
Lung	A	0.047	0.230	4.000	1.000	0.040	3.81	1	38.5
	B	0.085	0.430	3.345	0.900	0.100	2.75	3	12.3
	C	0.048	0.237	4.214	-	0.110	3.93	3	31.0
Pancreas	A	0.140	0.445	4.450	3.900	0.200	14.00	3	13.7
	B	0.105	0.460	3.450	4.555	0.460	16.40	3	15.3
	C	0.030	0.250	3.700	1.000	0.070	6.00	13	18
Spleen	A	0.050	0.370	8.400	1.081	0.070	5.68	4	45.5
	B	0.130	0.300	5.867	1.055	0.060	3.26	4	28.5
	C	0.047	0.348	7.895	-	0.110	5.34	4	23.0
Tongue	A	0.125	0.340	4.240	0.653	0.310	3.79	7	3.1
	B	0.490	0.485	5.300	0.641	0.240	2.84	4	4.4
	C	0.150	0.380	4.650	0.970	0.180	7.20	4	6.0
DV		1.5 mg	1.7 mg	20 mg	10 mg	2 mg	6 μg	400 μg	60 mg

Units are given in mg 100 g^{-1}, with the exception of vitamin B_{12} and folate, which are presented as μg 100 g^{-1}.
A, beef; B, porcine; C, lamb; D, chicken. DV, daily value according to the US Food and Drug Administration.

for monitoring metal contamination in animals because both organs function in removing toxic metals from the body and therefore end up accumulating them.

Table 4 presents a summary of the mineral content in the most common offal according to species as well as the average mineral content in fish by-products.

Vitamins

Vitamins are a group of organic compounds that are essential to normal metabolic functioning in the body. They are not a source of energy and do not form a structural role in tissues. Instead they are essential as cofactors or coenzymes. Vitamins can be divided in two main groups depending on their solubility: those soluble in fat organic solvents and those soluble in water; they are known as fat-soluble and water-soluble vitamins, respectively. Vitamins A, D, E, and K form the fat-soluble group, with others being water-soluble. Sources of vitamins can be classified as high, rich, or excellent when their content in 100 g is higher than 20% of recommended DV according to the US Department of Agriculture; the term good is used when the content is 10–19% of DV (daily value).

Within the fat-soluble vitamins, the most abundant in the offal is vitamin A with liver and kidney having the higher amounts. One hundred grams of liver can provide 781% (veal), 492% (lamb), 433% (pork), or 338% (beef) of DV; meanwhile, the same amount of kidney provides 28% (beef), 6.3% (veal), 6% (lamb), and 4% (pork). The other three fat-soluble vitamins are absent or present at very low levels in meat and offal.

The most prevalent water-soluble vitamins in meat and offal are those from group B (thiamin or vitamin B_1, riboflavin or vitamin B_2, niacin or vitamin B_3, pantothenic acid or vitamin B_5, vitamin B_6, and cobalamin or vitamin B_{12}), together with folate and vitamin C. These have a low stability and loss after cooking can be detected depending on the time and temperature employed. The average content and DV of water-soluble vitamins in offal by species is presented in Table 5.

Thiamin is a cofactor for enzymes involved in carbohydrate and fat metabolism, and it can be found in high amounts in pork offal, especially in heart where a 100 g portion supplies 40% of the DV. Riboflavin acts as cofactor for enzymes of the Krebs cycle; liver and kidneys from pork and beef are an excellent source for this vitamin yielding more than 100% of DV in 100 g of product. Niacin is found in high concentrations in offal with highest levels in liver, kidneys, and spleen where values can range from 5.9 mg 100 g^{-1} (pork spleen) to 16.1 mg 100 g^{-1} (lamb liver). As comparison, niacin content in meat is ~5–7 mg 100 g^{-1}. Three pyridines (pyridoxine, pyridoxal, and pyridoxamine) and their 5′-phosphorylated derivatives that are interconvertible are known as B_6 vitamins; they play a role in protein and amino acid metabolism. Liver from beef and lamb are the best sources of vitamin B_6; however, the amount is quite similar to that found in meat cuts. Cobalamin or vitamin B_{12} is exclusively of animal origin and is created by bacterial fermentation in the gut; however, it can be found at

very high concentrations in pancrease, liver, kidney, brain, and heart. In beef liver, the content is such that 10 g can be sufficient to cover the DV.

With the exception that liver from pork and lamb and kidney from beef offal are not a good source of folate, the rest of offal rarely provides more than 3% of folate DV. Vitamin C can be detected as reduced ascorbic acid or oxidized dehydroascorbic acid; it is very susceptible to oxidation during the processing, storage, and cooking; maximum stability takes place at acidic pH between 4 and 6. Spleen and lungs are the most important source of vitamin C within offal products; 100 g of such products provides up to 75% of DV (beef spleen) or 64% (beef lungs).

Conclusion

An overview of the composition of various offal has been presented in this article with details presented on the protein, fat, mineral, and vitamin contents of the most relevant offal in terms of human consumption. While these products can be rejected by consumer for a variety of reasons, it is clear that they represent a valuable source of nutrition in particular for EAAs, heme iron, calcium, vitamins within group B, vitamin A, selenium, and even vitamin C. Some offal such as liver, kidney, heart, and spleen are richer in high-nutritive compounds than meat or meat products. Because of this, if offal has been correctly processed and cooked, and presented well there is much scope for increased consumer acceptance, which in turn can lead to better harnessing of products from livestock and increase the value for the meat sector. Data presented in this article and in 'Further Reading'/'Bibliography' can be supplemented by further in-depth analysis of the composition of macro- and micronutrients in offal from poultry or minority meat sources (as rabbits, camels, goats, etc.).

See also: Aluminum: The Toxicology of; Aluminum: Properties, Presence in Food and Beverages, Fate in Humans, and Determination; Amino Acids: Determination; Amino Acids: Metabolism; Beef; Bioavailability of Nutrients; Cadmium: Properties and Determination; Cadmium: Toxicology; Calcium: Physiology; Calcium: Properties and Determination; Cholesterol: Absorption, Function and Metabolism; Cholesterol: Factors Determining Blood Cholesterol Levels; Cholesterol: Properties, Processing Effects, and Determination; Cobalt: Properties and Determination; Cobalt: Toxicology; Copper: Physiology; Dietary References: US; Energy: Intake and Energy Requirements; Essential Oils: Isolation, Production and Uses; Essential Oils: Properties, Composition and Health Effects; Fats: Classification and Analysis; Fats: Production and Uses of Animal Fats; Fatty Acids: Determination and Requirements; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fish Oils: Composition and Health Effects; Food and Agriculture Organization of the United Nations; Heavy Metal Toxicology; Iron: Biosynthesis and Significance of Heme; Magnesium; Manganese; Meat: Role in the Diet; Meat: Structure; Pork Meat Quality, Production and Processing on; Potassium: Physiology; Potassium: Properties and Determination; Poultry: Processing; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Proteins: Chemistry, Characterization, and Quality; Sheep: Meat; Trace Minerals and Trace Elements; Triacylglycerols: Characterization and Determination; Triacylglycerols: Structures and Properties; Vitamins: Overview; World Health Organization; Zinc: Physiology and Health Effects; Zinc: Properties and Determination.

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Olive Oil: Its Role in the Diet

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A Historical Overview of Olive Oil

Cultivated olive probably developed about 6000 years ago from wild olive or acebuche (*Olea europaea* var. *oleaster*). Pliocene fossil remains of wild olives have been found in Italy, in higher Paleolithic strata in Northern Africa, and from Chalcolithic and Bronze Age in Spain. Archaeological remains locate the first cultivated olives in Asia Minor and from there spread towards Greece. However, other authors suggest that cultivated olive derives from *Olea chrysophylla*, a variety widely growing in the mountains in Ethiopia, Kenya, and Uganda. Its origin has also been suggested in Egypt and Ethiopia and then spread along Northern Africa. Nevertheless, genetic studies of existing trees indicate that its origin derived from cultivated olives from Palestine, Lebanon, Syria, Cyprus, and Crete.

Many ancient civilizations considered olive tree a sacred tree and carved wooden scepters of kings with olive wood, olive leaves were used to make fine crowns to distinguish the outstanding men, and olive nectar was a key element in food, beauty product, medical remedy, and substrate for lighting.

The Phoenicians started disseminating the olive throughout Mediterranean countries in the sixteenth century BC, beginning in Greece between fourteenth and twelfth centuries BC, and it was later introduced in Spain c.1050 BC. The Romans intensified olive cultivation throughout the Empire, especially in the Iberian Peninsula. Spain became the largest producer of olive oil that was mostly exported to Rome. In the Roman Empire, the olive tree cultivar was of strategic importance.

The Arabs arrived in the Iberian Peninsula in AD 711 and introduced new varieties to existing cultivars, encouraging oil production especially in the area of Andalusia. Etymology for the Spanish terms for oil 'aceite' and olives 'aceitunas,' among other terms, is from Arabic origin.

With the discovery of America, the olive culture passed the Atlantic. In 1560, olive cultivars began to grow in Mexico and later in Peru, California, Chile, and Argentina. In more recent times, olive cultivation has spread in widely dispersed geographic locations from its origin, such as Japan, China, Australia, and South Africa.

The progressive introduction of olive culture in territories inhabited by nomadic people led them to become sedentary tribes willing peace with the surrounding villages. Hence, olive became a universal symbol of peace.

The origin of the word 'olive' probably derives from the term elaiwa in a Cretan dialect, which derived from Ancient Greek term elaia (ελαια); the term for olive oil was elaialadah. Both terms come from the Semitic term ulu, which eventually led to the Latin oleum, precedent for the Romance languages word oli. This phonetic variant gave rise to the terms oil in English and öl in German.

Olive oil is an excellent culinary and gastronomic resource in the Mediterranean diet (MD). It has been used for medicinal purposes, alone or with addition of various substances and herbal remedies. Pliny the Elder and other medical texts of the Roman Empire document the uses of olive oil in different balsams and medicines. Later authors such as Averroes and Maimonides refer to the goodness of consuming olive oil in their medical works.

Olive Cultivation and Varieties

Wild olive tree is known as 'acebuche' (*Olea sylvestris*). It was one of the first varieties of olive trees in the Mediterranean area and after several crosses and upgrades originated the existing variety *Olea europaea*.

Olive trees are grown in warm, dry climates and olive harvest is collected in late fall, although in some varieties and in certain areas, the collection takes place in December. There are over 260 varieties of olive trees, among which 26 are the most representative varieties. These unique varieties are named according to the geographic origin. Usually, olive tree is a long-lived tree that can reach up to 500 years or more and bring extraordinary quality oil called 'ancient tree oil.' In the south of Catalonia, there are some 4400 trees aged 700–1000 years old and oil produced from these ancient trees is specially certified.

The most widely cultivated olive varieties are Picual, Cornicabra, Hojiblanca, Arbequina, Lenchin, Verdial, Empeltre, Moorish, Gordal or Queen, Manzanilla, and Farga.

Picual variety is the world's largest variety. Picual olive has a very high content of polyphenols, which gives great stability. Picual oil is characterized by fresh flavor and full-bodied, with a degree of bitterness and pungency.

Arbequina olive produces high-quality fruity oil. The Cornicabra variety, grown mainly in Toledo and Ciudad Real, produces golden oil with green tones, which contains 77% oleic acid. The Empeltre variety is grown in Aragon and produces high-quality oil. It is one of the oldest varieties of Spain. Farga olives are grown in Castellón, Valencia, Tarragona, and Teruel. Oil from ancient Farga olive trees is highly valued.

In Greece, Kalamata and Koroneiki varieties are among the best known. Additional varieties in Italy are the autochthonous Ascoli from Ascola and Leccino. Israel grows the biblical variety Barnea and two major varieties grown in France are Picholine and Nice.

Olive Oil Processing

Olive oil is extracted from the different varieties of olive (*Olea europaea*) yielding diverse quality and quantity oil depending on the ripeness, variety, type of collection, transfer, and storage. Olives generally contain between 25% and 32% oil; water content is 40–55% and olive pit with vegetal residues between

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23% and 35%. All varieties are capable of producing excellent virgin olive oil if the collection process and milling have occurred on the same day and it is stored following proper procedures.

The oil extraction process does nothing to improve quality or nutritional content of olive oil, but the neglect of this process favors considerable loss of quality. In the traditional press method for olive oil production, olives are crushed and malaxed into a paste, which is spread on mats. Pressure is applied to squeeze out the oil and wastewater, leaving a material on the mats called pomace. The oil and wastewater are then separated by gravity. Steps followed in olive oil production are described in the succeeding text:

(1) *Collection*. It is a key process influenced by the appropriate time to pick the fruit when optimal level of maturation is reached and by the procedure used. The best system is the one that least impairs the olive tree and less damages the fruit.
(2) *Grading and washing*. Olives are transported to the mill and incorporated immediately to a cleaning process, removing leaves and foreign bodies, washing, and grading. A higher proportion of leaves increase the content of chlorophyll pigments, give a greener color oil, and alter the organoleptic, olfactory, and gustatory sensations.
(3) *Crushing*. Olives are passed to grinding and pressing–squeezing process to extract olive juice through the crushing, mixing, and centrifugation. Premium oil only is obtained when olives are collected and grinded the same day.
(4) *Malaxing*. At this stage, oil droplets are grouped. Extending this process reduces the content of antioxidant compounds. Malaxing should occur at as low temperature as possible but not lower than 20 °C, ideally between 25 and 30 °C. According to EC regulations, cold extraction takes place <27 °C.
(5) *Separation and centrifuging*. After crashing and grinding step, two or three products are extracted, oil and alperujo (two phases) or oil, pomace, and alpechin (three phases). Pomace oil is extracted by chemical means from alperujo. The first extraction liquid obtained in the initial phase consists of oil and alpechin, which are then centrifuged. The oil thus obtained belongs to the categories 'virgin' and 'extra-virgin,' provided that it satisfies the physicochemical and organoleptic characteristics. It has been reported that the largest loss of phenolic compounds takes place at the crushing stage (60% of phenolic compounds and 70% of oleuropein) but only 21% of antioxidant activity. Malaxation does not cause significant losses of phenolic compounds but affects the antioxidant activity (43% loss). The resulting pomace retains 26% of the phenolic compounds, 21% of the oleuropein, and 33% of the antioxidant activity.
(6) *Storage*. The resulting oil is transferred to stainless steel tanks for preservation and storage, where it is stored at 15 °C protected from light and external stimuli.
(7) *Packaging*. Containers for packaging olive oil are made of transparent glass, opaque glass (amber or green), plastic (PET), or tin-coated paperboard (e.g., Tetra Brik®). The freshness and quality of the aromas of olive oil deteriorate over time, but the rate of deterioration depends on the packaging. The content of phenolic compounds is better kept when containers prevent the passage of light and oxygen and are stored in the dark at temperatures between 16 and 18 °C. The choice of a container cap that prevents the passage of oxygen is very important as well. Cork taps are permeable to oxygen and can cause premature aging of oil.

Olive oils with higher content of polyunsaturated fatty acids are prone to a shorter life, while those containing higher proportion of saturated fatty acids have a longer life. Higher content of free fatty acids, greater peroxide value, or higher humidity also favors oxidation and shortens oil life. Conversely, more phenolic antioxidant content lengthens shelf life. Olive oil quality, if kept in good condition, can last for months or even years without rancid processes.

Virgin olive oils with high acidity or deteriorated organoleptic qualities due to the quality of the fruit or extraction processes used must follow a refining process. This procedure consists of a physical decolorizing action by absorbing bleaching earth and further filtration, removing excessive pigmentation, deodorization, and neutralization by a physical distillation under heating vacuum to remove compounds that may infer bad taste and odor.

In the refining process, the product is heated so the solvent evaporates completely. If the heating does not exceed 90 °C, the final product is not likely to contain polycyclic aromatic hydrocarbons like benzopyrene. The risk of benzopyrene contamination occurs when the heating method used exceeds 300 °C. Specific standards have been set in various regions of the world limiting the allowable amount of benzopyrenes in pomace olive oil. Within the European Union and in member states of the International Olive Council (IOC), the limit is 2 ppb or 2 $\mu g\,kg^{-1}$ as decided in Resolution RES-1/93-IV/05 Madrid in November 2005.

Types of Olive Oil and Quality Control

If the olive oil obtained exclusively by physical processes, that is, the juice of the olive, meets the requirements in terms of characteristics of acidity and organoleptic properties, it may be classified as extra-virgin olive oil. In 1959, the United Nations supported the creation of the IOC to oversee standards, research, and development regarding olive oil production and consumption worldwide following the adoption of the International Agreement on Olive Oil in 1956. IOC controls 85% of olive oil production worldwide and ensures the quality control of olive oil production. The IOC sets the standards but cannot enforce governments to adopt any regulation.

In the European Union (EU), Article 118 of Regulation (EC) No 1234/2007 delineates the various categories of olive oil and considers the terms describing the organoleptic characteristics referring to taste and/or smell of extra-virgin and virgin olive oils defined by the IOC in its revised method for the organoleptic assessment of virgin olive oils. These are also

included in Regulation (EU) No 29/2012 of 13 January 2012 on marketing standards for olive oil – which states that "consumers should be informed about the types of olive oil offered." In 2010, the United States adopted new standards for grades of olive oil and olive-pomace oil. Australia and New Zealand, which are not IOC member countries, approved a new olive oil standard in 2011.

Since extra-virgin olive oil is simply fruit juice without any additives, its quality and taste are influenced by the varieties of olives, the terroir where they were grown, and the decisions and production practices of producers (Table 1).

In order for an oil to qualify as 'extra-virgin,' the olive oil obtained exclusively by physical processes must also pass both an official chemical test in a laboratory and a sensory evaluation by a trained tasting panel recognized by the IOC. The olive oil must be found to be free from defects while exhibiting some fruitiness. Sensory analysis informs about bitterness, pungency, and astringency. Oleocanthal is the polyphenol most associated with pungency in the throat. Tyrosol is among polyphenols causing astringency, derivatives of oleuropein produce bitterness, and volatile compounds from the lipoxygenase pathway are responsible for complexity of flavor.

Cooking Techniques and Uses of Olive Oil

Currently, the culinary use of olive oil is not restricted to areas growing olive tree cultivars, but it is much appreciated and its use is associated with quality cuisine. Most common uses of olive oil are raw and for frying. When added raw without any heating, olive oil retains all its nutritional and organoleptic qualities. Olive oil is considered the fat of choice for frying, a cooking technique very common in countries surrounding the Mediterranean basin. There are two frying techniques, shallow and deep frying. In the first one, oil does not cover all the food surface and food needs to be turned around. With this procedure, food is impregnated with larger amount of oil. Deep frying is usually performed in fryers. Food is completely covered with oil and absorbs less oil. Fatty acids in oils are

Table 1 Categories of olive oil as described on Article 118 of Regulation (EC) No 1234/2007 (1), the International Olive Council (2), and the US Standards for Grades of Olive Oil and Olive-Pomace Oil, 2010 (3)

1. Virgin olive oils	Oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alterations in the oil, which have not undergone any treatment other than washing, decantation, centrifugation, or filtration, and to the exclusion of oils obtained using solvents or using adjuvants having a chemical or biochemical action or by reesterification process and any mixture with oils of other kinds
(a) Extra-virgin olive oil[1,2,3]	Virgin olive oil having a maximum free acidity, in terms of oleic acid, of 0.8 g per 100 g, the other characteristics of which comply with those laid down for this category
(b) Virgin olive oil[1,2,3]	Virgin olive oil having a maximum free acidity, in terms of oleic acid, of 2 g per 100 g, the other characteristics of which comply with those laid down for this category
Ordinary virgin olive oil[2]	(2) Virgin olive oil having a maximum free acidity, in terms of oleic acid, of 3.3 g per 100 g, the other characteristics of which comply with those laid down for this category This designation may only be sold if permitted in the country of retail sale. If not permitted, the designation of this product has to comply with the legal provisions of the country concerned
(c) Lampante olive oil[1,2] or US virgin olive oil not fit for human consumption without further processing[3]	(1) Virgin olive oil having a free acidity, in terms of oleic acid, of more than 2 g per 100 g, the other characteristics of which comply with those laid down for this category (2) Virgin olive oil having a free acidity, expressed as oleic acid, of more than 3.3 g per 100 g, the organoleptic characteristics and other characteristics of which correspond to those fixed for this category in the IOC standard. It is intended for refining or for technical use (3) Virgin olive oil having poor flavor and odor, a free fatty acid content, expressed as oleic acid, of more than 2.0 g per 100 g, and meeting the additional requirements outlined
2. Refined olive oil[1,2,3]	Olive oil obtained by refining virgin olive oil, having a free acidity content, expressed as oleic acid, of not more than 0.3 g per 100 g, the other characteristics of which comply with those laid down for this category
3. Olive oil – composed of refined olive oils and virgin olive oils[1,2,3]	Olive oil obtained by blending refined olive oil and virgin olive oil other than lampante olive oil, having a free acidity content, expressed as oleic acid, of not more than 1 g per 100 g, the other characteristics of which comply with those laid down for this category
4. Crude olive-pomace oil[1,2,3]	Oil obtained from olive pomace by treatment with solvents or by physical means or oil corresponding to lampante olive oil, except for certain specified characteristics, excluding oil obtained by means of reesterification and mixtures with other types of oils, the other characteristics of which comply with those laid down for this category
5. Refined olive-pomace oil[1,2,3]	Oil obtained by refining crude olive-pomace oil, having free acidity content, expressed as oleic acid, of not more than 0.3 g per 100 g, the other characteristics of which comply with those laid down for this category
6. Olive-pomace oil[1,2,3]	Oil obtained by blending refined olive-pomace oil and virgin olive oil other than lampante olive oil, having a free acidity content, expressed as oleic acid, of not more than 1 g per 100 g, the other characteristics of which comply with those laid down for this category

modified by heat during frying, which can lead to the formation of toxic by-products. The alteration undergone by vegetable oils when heated for frying is quicker and more intense the higher their content of polyunsaturated fatty acids and the higher the initial acidity of the oil. Olive oil has a high proportion of monounsaturated fatty acids (MUFA) and antioxidants that have a protective effect in this regard.

The ideal frying temperature is 180 °C. The boiling point of olive oil stands at 210 °C. An added benefit of olive oil is that it forms a thin surface crust around the food that is fried, preventing the entry of oil inside. Different oils should never mix for frying, since the mixture will alter the boiling point. Oil used for frying must be renewed often and filtered before reuse; oil should not emit smoke during frying.

Nutritional Value of Olive Oil

In olive oil, two different fractions can be distinguished, oil fraction and nonoily fraction. The oil fraction is the major portion, 98–99% of the total oil. It consists of triglycerides containing MUFA (55–85%), mainly oleic acid but not only. It also contains palmitoleic acid; saturated fatty acids (12.7%), such as palmitic, stearic, and arachidic acids; and polyunsaturated fatty acids (10.1%), including linoleic and linolenic fatty acids. It contains smaller amounts of free fatty acids, monoglycerides, diglycerides (DG), phosphatides, waxes, and sterol esters. The levels of fatty acids vary during the different maturation stages of the olives, the variety, and the growing conditions. It is generally accepted that cooler areas will produce oil with higher MUFA content than warmer climates. The proportion of linoleic acid in oils from warmer regions tends to be higher (Table 2).

The non-oil fraction or unsaponifiable fraction is only 1–1.5% of the total olive oil. At least 230 substances have been reported in this fraction, some of them with important physiological action, which also determines the organoleptic characteristics of olive oil. Virgin and refined oils differ in the composition of the minor compounds.

This fraction includes terpenes as squalene (136–700 mg per 100 ml) and carotenes, especially β-carotene and carotenoids that also contribute to the typical green-yellowish color of olive oil. Squalene accumulates in the skin and helps eliminate singlet oxygen ($^1O_2$) or reactive oxygen species generated by the action of ultraviolet radiation.

Pigments (chlorophylls and pheophytins) contribute to the color of olive oil and have been attributed antioxidant actions.

Tocopherols are also present in olive oil. α-, β-, γ-, and δ-tocopherols can be found. α-Tocopherol (vitamin E) accounts for 90–95% (5.1 mg per 100 ml^{-1}) of tocopherols in olive oil. Temperatures above 35 °C decompose vitamin E.

Sterols include campesterol, stigmasterol, and β-sitosterol. The latter represents 93% of the total and has a cholesterol-lowering action with competitive antagonism with cholesterol for its intestinal transport mechanism (Table 3).

Table 2 Energy, fatty acids, and fat-soluble vitamin content in olive oil (BEDCA, Spanish Food Composition Data Base)

		Olive oil
Energy (kJ (kcal))		3696 (887)
Total lipid (fat) (g)		99.9
Fatty acids, total monounsaturated (g)		73.3
Fatty acids, total polyunsaturated (g)		10.52
Fatty acids, total saturated (g)		13.81
Cholesterol (mg)		0
Vitamin A, RAE (μg)		3
Vitamin D (μg)		0
Vitamin E (α-tocopherol) (mg)		12
Vitamin K (phylloquinone) (μg)		60.2
***Percentage of different fatty acids in olive oil*[a]**		
	Fatty acid	%
SFA	Myristic (14:0)	0.0–0.05
SFA	Palmitic (16:0)	7.5–20
SFA	Margaric (17:0)	0–0.3
SFA	Stearic (18:0)	0.5–5
SFA	Arachidic (20:0)	0–0.6
SFA	Behenic (22:0)	0–0.2
SFA	Lignoceric (24:0)	0–0.2
MUFA	Heptadecenoic (17:1)	0–0.3
MUFA	Palmitoleic (17:1*n*7)	0.3–3.5
MUFA	Oleic (18:1*n*9)	55–83
MUFA	Eicosenoic (20:1*n*9)	0–0.4
PUFA	Linoleic (18:2*n*6)	3.5–21
PUFA	Alpha-linolenic (18:3*n*3)	0–0.9

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[a]*Sources*: RedBEDCA. Base de Datos Española de Composición de Alimentos (BEDCA). http://www.bedca.net/ Guillén N, Acín S, Navarro MA, et al. (2009). Knowledge of the biological actions of extra virgin olive oil gained from mice lacking apolipoprotein E. Revista Española de Cardiología 62(3), 294–304. Other sources: Beltrán G, del Rio C, Sánchez S, Martínez L (2004); Inglese P, Famiani F, Galvano F, et al. (2011); Mataix J (2001); Montedoro GF, Taticchi A, Esposto S, et al. (2007); Oarada M, Igarashi M, Tsuzuki T, et al. (2009).

Table 3 Minority components in virgin olive oil

Compounds	*mg/100 ml olive oil*
Terpene compounds	100–350
Uvaol and oleanolic acid	
Erythrodiol and maslinic acid	
Sterols	100–250
β-Sitosterol	
δ-Avenasterol	
Campesterol	
Hydrocarbons	
Squalene	150–800
Carotenes	0.5–1
Phenol compounds	5–100
Simple phenols	
Hydroxytyrosol	
Tyrosol	
Vanillic acid	
Vanillin	
p-Coumaric acid	
Ferulic acid	
4-Ethylphenol	
Tyrosyl acetate	
Hydroxytyrosylacetate	
Polyphenols	
Flavonoids	
Apigenin	
Luteolin	
Lignans	
(+)-Pinoresinol	
(+)-1-Acetoxypinoresinol	
Secoiridoids	
Glycosylated oleuropein	
Dimethyl oleuropein	
Oleuropein	
Elenolic acid	
Ligstroside	
Deacetoxyligstroside	
Aliphatic alcohols	10–70
Tocopherols	0.5–30
α-Tocopherol	
β-Tocopherol	
γ-Tocopherol	
Esters	10–20
Aldehydes and ketones	4–8
Chlorophylls	0.1–2

Modified from Guillén, N., Acín, S., Navarro, M. A., et al. (2009). Knowledge of the biological actions of extra virgin olive oil gained from mice lacking apolipoprotein E. *Revista Española de Cardiología* **62**(3), 294–304.

Phenolic compounds can be found in varying proportions (5–80 mg per 100 ml^{-1}) in extra-virgin olive oil. Simple phenols (tyrosol, hydroxytyrosol, and phenolic acids such as caffeic acid and vanillic acid), complex phenols (oleuropein and verbacósido), lignans (acetoxypinoresinol and pinoresinol), and flavonoids can be found. Oleuropein is responsible for the bitter taste of olives and gives a brown color to their skin. Hydroxytyrosol and tyrosol are potent antioxidants that prevent oxidation of LDL. Hydroxytyrosol inhibits platelet aggregation *in vitro*, plasma accumulation of thromboxanes, leukotriene production by neutrophils, and arachidonic lipoxygenase action. Phenolics in olive oil help eliminate reactive oxygen species generated in the fecal mass and thus protect the colonic mucosa. The phenolic content in olive oil varies depending on the variety of olive fruit (Coratina variety is the richest in phenolics), region where it is grown, climate, agricultural techniques used, maturity of the olive fruit at harvest, and olive oil extraction, processing, storage methods, and time since harvest. Cooking methods have also been shown to alter phenolic concentrations in virgin olive oil.

Other compounds such as alcohols, ketones, esters, ethers, and furans are volatile compounds that contribute to the characteristic flavor of olive oil.

Digestion and Absorption

Fats rich in MUFA such as oleic acid have been found to cause pronounced postprandial lipemia, with a tendency for large TG-rich lipoprotein particles. In humans, hydrolysis of olive oil triglycerides begins in the stomach where it is catalyzed by an acid-stable gastric lipase. Triglyceride hydrolysis continues in the duodenum, by the synergetic actions of gastric and colipase-dependent pancreatic lipases and bile secretion. Gastric lipolysis leads to the hydrolysis of 10–30% of ingested triglycerides, generating mainly DG and free fatty acids. This facilitates subsequent triglyceride hydrolysis by pancreatic lipase by allowing fat emulsification. Different lipid metabolites are quickly absorbed along the epithelial cells of the small intestine. Compared to diets rich in polyunsaturated fatty acids, olive oil suppresses gastric acid secretion and is a potent releasing factor of cholecystokinin peptide.

Bioavailability assays in animals and humans show that 55–60% of phenolics from extra-virgin olive oil are absorbed. Absorption of most minor compounds of (extra-virgin) olive oil takes place in the small intestine, as native or derivatives. Research has shown that the phenolic compounds hydroxytyrosol and tyrosol are absorbed after ingestion in a dose-dependent manner. Different polarities of the various phenolics have been postulated to play a role in the absorption of these compounds. Phenolics tyrosol and hydroxytyrosol are polar compounds, and it has been postulated they are absorbed via passive diffusion. It has been suggested that oleuropein glycoside may diffuse through the lipid bilayer of the epithelial cell membrane and be absorbed via a glucose transporter. Phenolic compounds, oleuropein glycoside and oleuropein and ligstroside aglycones, are converted to hydroxytyrosol or tyrosol and excreted in urine. Hydroxytyrosol and tyrosol themselves are sometimes conjugated to glucuronic acid and excreted in urine as glucuronides. Excreted phenolics (mainly in the form of hydroxytyrosol and tyrosol) have been determined to be 5–16% of the total ingested.

Patterns of Consumption

Worldwide, around 11 million hectares of olive cultivar is grown in 47 producing countries. World production of olive oil is estimated at 3.1 million tons according to the IOC for the season 2013–14 (between 1 October 2013 and 30 September 2014). Spain is at the forefront of the global ranking of

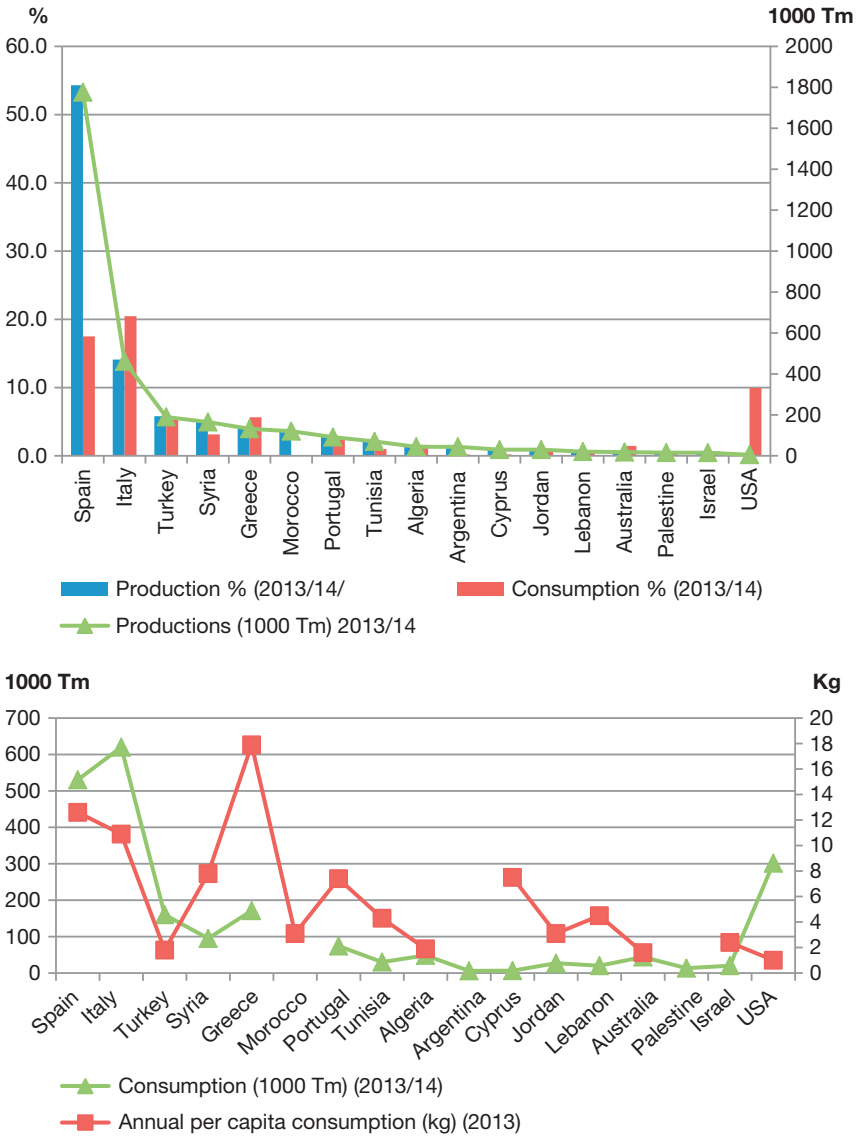

Figure 1 Olive oil production in highest producing countries in 2013/2014, percent production of world production by country, and percent consumption in the same period (a); olive oil consumption in highest consuming countries and annual per capita consumption in period 2013 (b). *Source*: International Olive Council.

countries producing olive oil since the mid-1990s, with 1.5 million tons in 2013–14, 50% of total world production. Two other Mediterranean countries, Italy and Greece, rank second and third, with 450 and 230 thousand tons of olive oil, respectively. Turkey, Syria, and Morocco also produce over 100 thousand tons and close to the top ten producing countries Tunisia, Portugal, Algeria, and Chile, with output ranging from 80 thousand tons of Tunisia to 32 thousand tons of Chile. Argentina and Jordan are between 25 000 and 30 000 tons, while Australia, Libya, Palestine, Lebanon, Israel, Albania, the United States, and Iran produce between 9000 tons of Iran and 19 000 tons of Australia. Italy and Spain are the main exporters of olive oil (Figure 1).

Currently, olive oil is consumed in 160 countries. In the last two decades, there has been increasing interest in olive oil in many countries, first as an echo of accumulated scientific evidence on possible beneficial health effects. On the other hand, its organoleptic properties have focused on culinary trends. In fact, new regulations regarding standards of quality control and marketing strategies have been debated among producing EU countries, the United States, and Australia.

Italy is the country with the highest consumption of olive oil, followed by Spain, the United States, and Greece, but considering per capita consumption, Greece leads the ranking with an average annual consumption of more than 24 l per person per year, followed by Spain, Italy, and Portugal. Countries in North Africa, southern coast of the Mediterranean Sea, show consumptions around 4.3 l per person per year for Tunisia and 3.1 l per person per year in Morocco. A consumption of 2 l per person per year is estimated in France, and the estimated

consumption in the United States is 1 l per person per year, slightly higher than in Canada (1.4 l per person per year) and the United Kingdom (1.1 l per person per year).

Differences in the consumption of olive oil are mostly explained by differences in food habits, cultural and gastronomic traditions, and income level, although to a lesser extent.

The US consumption of olive oil has tripled in the last 20 years, conditioned mainly by increased overall interest in the MD and by favorable health effects of olive oil and culinary properties, as evidenced by the abundance of information on these topics published in general media of general information, both on paper and online, on blogs, etc., published in recent years, which have been estimated at around 50 news per month.

Olive Oil, MD, and Health

Fats play an important role in the diet, providing calories; help in the absorption of fat-soluble vitamins A, D, E, and K; and contribute to add taste and texture to foods, as well as satiety and sense of fullness after eating a meal. There is sound evidence that it is not only the amount of fat content in the diet but also the quality of fat that has a relevant influence for health. Dietary fat affects the fat composition of cell membranes.

The authors of the Seven Countries Study, started in the 1950s by Ancel Keys and other researchers, reported that the people in Crete consumed some of the largest amounts of fat but had the lowest cardiovascular mortality rate and a long life expectancy. The finding led to the development of the concept of MD. The traditional MD is the dietary pattern prevailing among the people of the olive tree-growing areas of the Mediterranean basin before the mid-1960s. The traditional MD is characterized by high consumption of vegetables, fruits, and nuts, legumes, and unprocessed cereals; low consumption of meat and meat products; and low consumption of dairy products except for long-preservable cheeses and moderate alcohol consumption, generally wine during meals. Olive oil was then used liberally. Total intake of lipids could be high or moderate, but the ratio of monounsaturated to saturated lipids was high. Fish consumption depended on the distance from the sea but overall was moderate.

The MD should be understood as something else than a food pattern; it is a cultural model that involves the way foods are selected, produced, processed, and distributed. The MD has recently been acknowledged by UNESCO as an Intangible Cultural Heritage of Humanity. Unfortunately, current diets in Mediterranean countries are departing from the traditional MD.

The most widely researched health benefits of the MD are the reduction in cardiovascular disease, including peripheral artery disease, with strong evidence supporting a causal vascular protection. Other benefits extensively researched include the prevention of type 2 diabetes and metabolic syndrome, cognitive impairment, and unipolar depression. The EPIC study has also provided some benefits against cancer (Figure 2).

The *Pre*vencion con *Die*ta *Med*iterranea (PREDIMED) trial compares a low-fat diet to either the MD supplemented with either olive oil or that with nuts. Authors of this large trial have

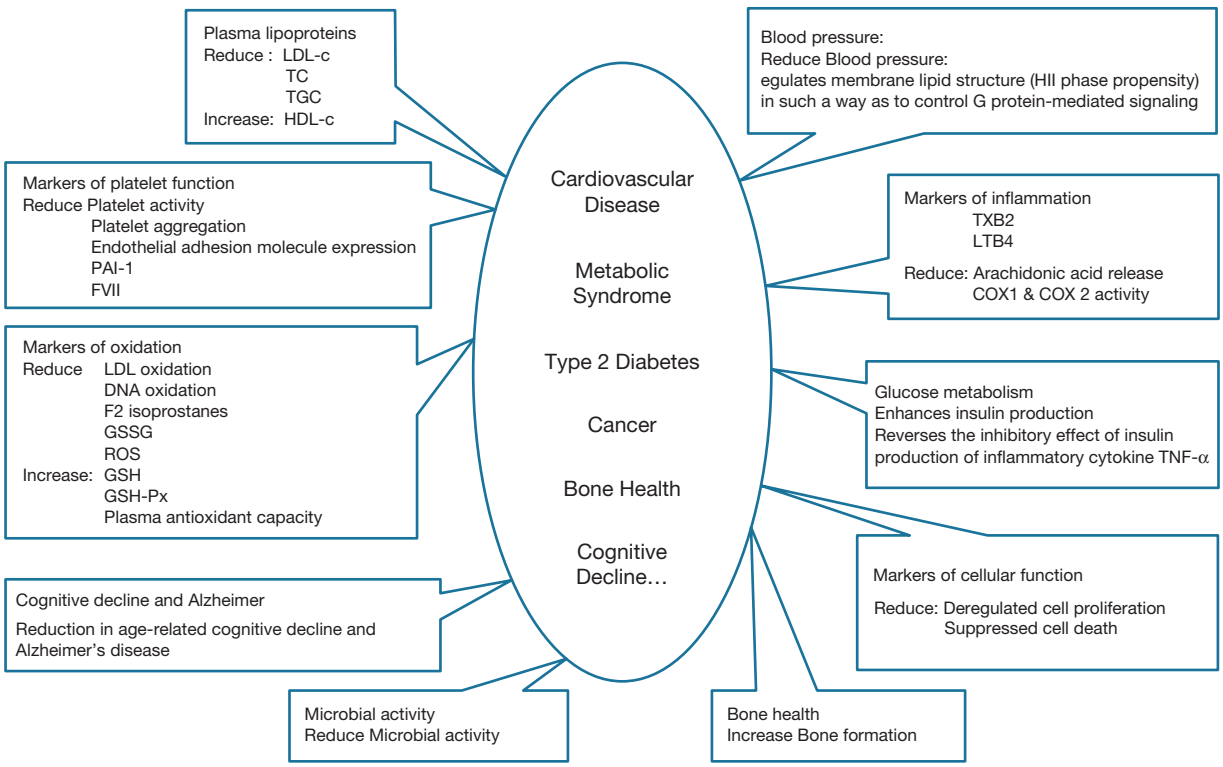

Figure 2 Health effects of olive oil and suggested mechanisms. *Sources:* Caramia G et al., 2012; Cicerale et al., 2010; Estruch R et al., 2013; López-Miranda J et al., 2010; Rees K et al., 2013; Salas-Salvado J et al., 2014; Widmer et al., 2014.

reported a significant reduction in cardiovascular disease events in the MD arms of the trial.

One of the most relevant aspects of the MD is the high content of unsaturated fats in its sources of fiber and protein, along a paucity of saturated fats. Olive oil, a good source of MUFA, contains phenolic compounds that may have antioxidant, anti-inflammatory, and antithrombotic properties. There is some evidence that oleic acid itself can reduce inflammatory markers like C-reactive protein. However, the main anti-inflammatory effects seem to be mediated by the antioxidants in olive oil, primarily oleocanthal.

The cardioprotective effects of olive oil are now thought to be attributed to the presence of its phenolic compounds obtained from the water-soluble fraction and include mostly the low-molecular-weight molecule hydroxytyrosol and also oleuropein, which are both potent antioxidants, free radical scavengers, and enzyme modulators. Two randomized, blinded crossover trials have assessed the antioxidant effect of dietary supplementation of extra-virgin olive oil in humans: EUROLIVE and the Virgin Olive Oil Study. Results show that not only does the MD improve endothelial function and reduce systemic inflammation, but also it improves endothelial progenitor cell numbers.

Early meta-analyses primarily evaluating the effects of fatty acids on surrogate markers for cardiovascular disease such as cholesterol indicated that MUFA intake increased lipid levels, including HDL. Phenolic compounds in olive oil protect blood lipids from oxidation and vitamin E protects cells from oxidative damage. A large meta-analysis suggests an inverse correlation between blood pressure and MUFA intake.

Beneficial effects of olive oil in the digestive system have been reported: minor reflux of gastric content, partial inhibition of gastric motility, increase of satiety, promotion of digestion of food, and prevention of the sudden increase in blood glucose after digestion. It reduces heartburn, which is a protective factor for the formation of gastric ulcers. Olive oil improves intestinal absorption of nutrients, particularly minerals, reduces intestinal absorption of cholesterol by competitive antagonism of sitosterol, and reduces intestinal reabsorption of bile salts, which stimulates the synthesis of cholesterol and stimulates hepatic synthesis of bile salts. It also stimulates the contraction of the gallbladder by stimulating hormone cholecystokinin (CCK-PZ), previously called pancreozymin, and moderately stimulates the exocrine pancreas.

Phenolic compounds in olive oil have shown antioxidant and anti-inflammatory properties, prevent lipoperoxidation, induce favorable changes of lipid profile, improve endothelial function, and show antithrombotic properties. Cohort studies in the Mediterranean suggest that dietary MUFA may be protective against age-related cognitive decline and Alzheimer's disease.

Notwithstanding the beneficial health effects of olive oil, it should be considered that olive oil is an energy-dense food and should be used in moderation in order to avoid excessive energy and fat intake. Olive oil should be used to replace other fat sources in the diet. Cooking techniques should be adjusted to get the flavor and the benefit of olive oil but avoiding excess fat. Beneficial health effects of olive oil are best gained when used in the context of a balanced and varied diet, rich in fruit and vegetables, such as the MD along with adequate levels of physical activity.

See also: Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Bioavailability of Nutrients; Cancer: Diet in Cancer Prevention; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Consumer Protection Legislation; Cooking: Domestic Techniques; Dietary Practices; Dietary Surveys: National Food Intake; Fats: Classification and Analysis; Fatty Acids: Determination and Requirements; Fatty Acids: Fatty Acids; Fatty Acids: Metabolism; Food Composition Databases; Food Fraud; Lipoproteins; Mediterranean Diet; Nutritional Epidemiology; Olives: Types and Consumption; Oxidation of Food Components; Phenolic Compounds: Bioavailability and Health Effects; Phenolic Compounds: Occurrence, Classes, and Analysis; Polycyclic Aromatic Hydrocarbons; Quality Control in Food Processing; Sensory Evaluation: Sensory Rating and Scoring Methods; Tocopherols: Physiology and Health Effects; Tocopherols: Properties and Determination; Traditional Foods; Vegetable Oils: Composition and Analysis; Vegetable Oils: Dietary Importance; Vegetable Oils: Oil Production and Processing; Vegetable Oils: Types and Properties; Vitamins: Overview.

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Olives: Types and Consumption

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Table Olives: Origin and Production

Table olives are a traditional fermented vegetable with many centuries of history, particularly in the Mediterranean Basin, where this food has had a great influence on the culture and diet of many countries. Most likely, the culture of wild olive tree (*Olea europaea*) originated from Asia Minor, but the Phoenicians started disseminating the olive throughout the Greek isles and mainland. Later, the olive spread throughout the Mediterranean area reaching Tripoli, Tunis, Sicily, and southern Italy. The Roman civilization continued the expansion of the olive culture to the countries bordering the Mediterranean Basin. In Spain, olive growing was introduced by the Phoenicians at ~1050 BC. During the fifteenth and sixteenth centuries, the Spanish extended olive planting to America where there is nowadays an increasing production. Recently, the olive tree has also expanded to Australia, Japan, and South Africa.

The oldest written reference on the preparation of table olives is due to Lucius Columella in his manuscript '*De Re Rustica*,' in the year AC 54. Therefore, the production of table olives originally started at small domestic scale millennia ago, and it was a craft process until the middle of the twentieth century, when the mass fermentation and storage systems were introduced together with the pitting and stuffing automation. At this point, the progressive mechanization in the industries has led to a rapid expansion of the sector.

Nowadays, table olives are one of the most important fermented vegetables of the food industry, with a production above 2 400 000 tons year^{-1}. According to the last consolidated report for 2011/2012 season, most of the table olives were produced in the Mediterranean region with Spain (521 500 tons), Turkey (400 000), Egypt (384 500), Syria (172 000), Algeria (145 500), Greece (130 000), and Morocco (100 000) as the main contributors, although there were also important productions in Argentina (150 000), Peru (81 000), and the United States (26 000). Thus, table olive processing is worldwide spread and represents an important economic source for olive-growing countries.

Types of Elaborations

The olive fruit is a fleshy drupe. The major components (in average concentration (g kg^{-1})) in the flesh olive fruits are, depending of the olive variety and maturity degree, moisture (650–800), fat (100–300), sugars (30–60), proteins (10–20), fiber (20–50), ash (10–15), and phenolic compounds (30–60). Because of its peculiar characteristics, the presence of a bitter compound, it cannot be consumed directly from the tree. For this reason, diverse processes were developed to make them eatable; although sharing the same objective, they can slightly differ between areas of production. The Trade Standard Applying to Table Olives defines table olives as "the product obtained from suitable olive cultivars, processed to remove their natural bitterness, and preserved (by natural fermentation, heat treatment or preservatives) with or without brine until consumption."

According to the International Olive Council, table olives can be classified as a function of the type of olive fruit used (turning color, green, and natural black olives) and the method of processing used. Processing alludes to the system used for fruit debittering, the way they are kept, and the preservation procedure. The trade standard includes (i) treated olives (fruits are debittered using lye treatments, followed by fermentation in brine), (ii) natural olives (fruits are brined directly and the debittering process occurs only by dilution or natural hydrolysis), (iii) dehydrated or shriveled olives (fruits, regardless of lye treatment or not, are subjected to dehydration by salt, heat, or any other technological process), and (iv) olives darkened by oxidation (fruits are darkened by oxidation and preserved by sterilization).

Trade preparations may be offered in several presentations forms such as (i) whole, which includes cracked and split olives; (ii) pitted olives, which includes halved, quartered, divided, sliced, chopped (or minced), and broken fruits; (iii) stuffed olives, with different products (pepper paste, anchovies, etc.); (iv) salad olives, a mixture of olives with pieces of stuffing material and/or cappers; (v) olives with capers; (vi) olive paste; and (vii) other styles.

The most important table olive industrial preparations are (i) alkali-treated green olives (the so-called Spanish-style), which represent about 50–60% of production; (ii) ripe olives by alkaline oxidation (the so-called Californian-style); and (iii) untreated or directly brined olives (green, turning color, or naturally black). There are also many other traditional/industrial ways of processing table olives distributed around the world. In the succeeding text, a brief description of the main processing styles according to the volume of production is presented.

Spanish-style green table olives: In this type of elaboration, green fruits are treated with alkali, washed, and put in brine where they undergo lactic acid fermentation. The lye treatment consists of a NaOH solution (18–35 g l^{-1}), which penetrates up to 2/3 of olive flesh. The main goal of the debittering process is the chemical hydrolysis of the glucoside oleuropein, which is transformed into elenolic acid and hydroxytyrosol. The lye treatment also favors the solubilization of olive components (sugars, minerals, organic acids, etc.) from olive flesh into the liquid. Then, olives are washed to remove excess alkali and brined in a 100–120 (g l^{-1}) NaCl solution, which is rapidly enriched with the nutrients leached from flesh. The fermentation of this style of olive processing is usually spontaneous, with a predominance of lactic acid bacteria (LAB) and yeasts. Throughout fermentation, the main process is the transformation of sugars into lactic acid by LAB, which drops the pH up to values that make the product safe (below 4.5) by preventing growth of undesirable microorganisms (*Clostridium* and Enterobacteriaceae). Most of the olives are then commercialized as pitted, pitted/stuffed with diverse products from vegetal or animal origin, or pitted/sliced olives.

Ripe olives: In this type of elaboration, green or turning color fruits are stored in brine or acidic solutions or directly darkened by oxidation in an alkaline medium. Storage of olives for long periods of time can be achieved in diverse holding solutions at room temperature. In the case of olives kept in NaCl solutions (50–70 g l^{-1}), brines must be acidified with acetic acid or supplemented with calcium chloride to prevent olive softening. The initial stages of this type of storage are dominated by Gram-negative bacteria, but after 4–5 days, LAB begin to appear and the other groups of microorganisms to decline. This period in brine can be combined with the application of an aerobic process, which favors the growth of oxidative yeasts and reduce possibility of spoilage. On the contrary, in acidic solutions, the storage is achieved by a combination of acids (lactic or acetic) and food preservatives (sorbate or benzoate) or also using an aerobic process without preservatives. With this system, the Gram-negative bacteria and yeast growth is markedly reduced, while the growth of acetic bacteria and LAB is clearly favored. The darkening process consists in a series of alkaline treatments, which progressively penetrate into the olive flesh up to the pit, with washing periods between them to remove the excess of alkali. Then, a solution of ferrous gluconate or lactate is added for 10–24 h to fix the black color. Hydroxytyrosol and caffeic acid are mainly responsible for the color formation during darkening. Olives are then packed in light brine (20–50 g l^{-1} NaCl) with ferrous gluconate or lactate added. The product has a final pH above 4.6 and its preservation is only achieved by sterilization. The most common commercial presentations in retail are whole and pitted olives.

Directly brined olives: In this type of elaboration, green, turning color, or mature black fruits are directly brined, where they undergo spontaneous fermentation. Initial salt concentration in brines ranges from 50 to 100 g l^{-1} NaCl, according to the sensitivity of the olive cultivar, but its content must be periodically increased as salt is absorbed by fruits. Initial pH correction with acids (lactic or acetic) to prevent excessive growth of undesirable microorganisms is also fairly common. The leaching of nutrients from the olive flesh into the solution is slow because of the strong barrier of the untreated skin, but microbial growth begins just after brining. The debittering process in this type of elaboration is due to the hydrolysis of the bitter glucosides present in the fruits and their diffusion and solubilization into the brines. In green–turning color olives, the main groups of microorganisms involved in the fermentation are, like in other table olive processing, Enterobacteriaceae, yeasts, and LAB, but with a higher importance of yeasts instead of LAB because these last microorganisms are partially inhibited due to the presence of phenolic compounds. Usually, directly brined olives are offered as whole or cracked and sold in just brine or seasoned with vinegar, garlic, pepper, aromatic herbs, etc. They are relatively unstable and need pasteurization or addition of preservatives for their complete stabilization. In the case of natural black olives, they can be subjected to two fermentation systems: (i) the traditional anaerobic (sugars are quickly consumed as they pass into the solution, with a presence of both yeasts and LAB) and (ii) the aerobic process to prevent gas pocket formation (basically consists in the injection of air into the brine through a column device, which prevents the growth of anaerobic *Clostridium* and *Propionibacterium* and favors the growth of yeasts). After fermentation, olives lose most of their initial bitter taste and are ready for commercialization.

Dry-salted olives: This is a product obtained by mixing coarse salt and freshly harvested olives without liquid. The microorganisms present at the beginning of the process can be LAB, yeasts, Enterobacteriaceae, and *Pseudomonas*, but at the end of fermentation, only osmophilic yeasts are usually isolated. Dry-salted olives can also be obtained by drying in oven.

Patterns of Consumption

Table olives are strongly embedded in the Mediterranean culture and diet. Because of their daily intake, this food is considered as snacks/appetizers, albeit this fermented vegetable is also normally used as ingredient of some dishes. Thus, they can be ingested directly or in salads, cocktails, pate, and pizzas (slices). The Spanish Association of Processors and Exporters (ASEMESA) considers seven olives (about 25 g of olive flesh) as a satisfactory daily intake.

Spain, Turkey, Egypt, and other major table olive-producing countries are strongly committed with their national markets. In this way, only a few producers require importations to cover their internal demand (e.g., the United States and Italy). According to the last consolidated report for 2011/2012 season, the main consuming countries were Turkey (350 000 tons), Egypt (300 000), Spain (217 900), the United States (210 000), Italy (139 800), and Syria (132 000). Among the nonproducing countries with high demand for consumption of table olives, Brazil (101 500 tons), Russia (68 000), Germany (65 100), and the United Kingdom (41 600) are the leaders. The total worldwide consumption for 2011/2012 season was 2 552 000 tons.

Nutritional Value

Table olives should have all the benefits attributed to olive oil, as well as other important components with proved biological activities. In this way, olives are a good source of natural antioxidants (hydroxytyrosol, oleuropein, methyloleuropein, etc.) and triterpenic acids, all of them with unquestionable biological value. From the nutritional labeling point of view, olives are mainly constituted of moisture, fat, ash (mineral), fiber, and proteins. On the contrary, olives after fermentation process are low in carbohydrates.

Several works have recently revised the nutritional composition of table olives. The fat content ranges from 100 to 300 g kg^{-1} of flesh (in cultivars also destined to olive oil extraction or double use). Most of this fat is monounsaturated (50–190 g kg^{-1}) due to its abundance in oleic acid. It is followed by saturated fat (15–60 g kg^{-1}), with the palmitic acid as the most outstanding component of this fraction, and polyunsaturated fat (5–39 g kg^{-1}), with linoleic acid being the major contributor. Table olives have also small proportions of *trans* fat (0.8–4.4 g kg^{-1}), mainly elaidic acid. Therefore, table olives are a good source of monounsaturated fatty acids with a reasonable level of polyunsaturated fat. Fiber is also an important constituent of the flesh (20–50 g kg^{-1}). Some stuffing material (hazelnut, almond, etc.) can even produce an

increment in the fiber content. The levels of lactic acid are ~5 g kg^{-1} in Spanish green table olive flesh, but the proportion of sugars is negligible after fermentation. Average protein levels are between 10 and 20 g kg^{-1}, its proportion being obviously higher in the presence of animal origin stuffing materials (anchovies, salmon, etc.). Protein proportion is relatively low but has all the essential amino acids with lysine as the limiting amino acid, followed by cysteine and methionine.

Table olives are also a good source of minerals like calcium (3–8 g kg^{-1}), potassium (0.5–1.2 g kg^{-1}), magnesium (0.5–2 g kg^{-1}), and phosphorous (0.5–1.4 g kg^{-1}), containing important proportions of microelements (copper 1.7–11.0 mg kg^{-1}, zinc 1.5–3.6 mg kg^{-1}, and manganese 0.2–1.5 mg kg^{-1}). Fermented olives are a good source of phytosterols (200–520 mg kg^{-1}), β-sitosterol being the most abundant, vitamin E (13–52.3 mg kg^{-1}), and vitamin A (1970–13 870 μg kg^{-1}), which is particularly abundant in those presentations including red pepper as stuffing material. Therefore, a recommended daily intake of 25 g of olives provides to the metabolism, as mean values, 45 kcal, 0 g carbohydrates, 0.25 g proteins, 5.5 g fats, 0.65 g fiber, and 0.3 g of sodium.

Table olives are also a good source of biologically active compounds (polyphenols and triterpenic acids). The proportion of polyphenols depends on the type of presentation. The highest proportions were found in directly brined turning color Manzanilla (~1.5 g kg^{-1}), while the content in plain green Spanish-style was slightly lower (around 1.1 g kg^{-1}). Green Hojiblanca had concentrations in the range 0.35 (pitted)–0.70 (plain) g kg^{-1}. Both cultivars had markedly lower proportions when prepared as ripe olives (~0.25 g kg^{-1}). In general, directly brined olives are richer in polyphenols but the content decreases as the number of aqueous treatments for processing increases. Naturally directly brined olives from Kalamata and Thassos cultivars were also good source of phenols (1.0 and 0.7 g kg^{-1}, respectively). Triterpenic acids are also abundant in some table olives. In general, maslinic acid, which is present in higher proportion in the wax of olive skin, has proved to have important anticancer properties. The content of triterpenic acids in diverse commercial presentations of table olives ranged from 0.46 to 1.47 g kg^{-1} flesh. The levels of these acids are mainly influenced by the lye treatments, which solubilize them. Thus, naturally directly brined natural black olives are among the richer products in triterpenic acids with concentrations around 2 g kg^{-1}. The presence of these nutritional and functional compounds in table olives may contribute to their revaluation because of the promising bioactivity properties attributed these substances.

Maybe, the main drawback of table olives is their relative high content in sodium (5–18 g kg^{-1}), which is partially absorbed by the flesh during the elaboration process (lye treatment and brining). This is not a problem for persons with a high daily physical activity, but it is not convenient for persons with cardiovascular diseases. NaCl substitution by other mineral salts may be achieved during fermentation or in packing. The use of salt mixtures in 'Gordal' has shown to affect the sugar solubilization and the general fermentation profile. The sodium content (which decreased up to ~50%) was inversely related to the concentration of KCl and $CaCl_2$ used in the initial brine. Salt mixtures may be also used in packing. Seasoned packed cracked olives had up to 31% lower Na content, while the proportions of K and Ca in the product were doubled (>0.45 and >0.30 g kg^{-1}, respectively). Finally, table olives may also be fortified with other mineral nutrients. The use of 0.75 mg l^{-1} of $ZnCl_2$ in packing brine produced seasoned cracked table olives, which could be considered as a good source of Zn for metabolism (79% daily requirements per 50 g olive flesh). Therefore, these researches may lead to new commercial presentations with improved and healthier nutritional value.

Table Olives as Carrier of Beneficial Microorganisms

In the last years, diverse researchers have tried to turn olives into a delivery vehicle of probiotic microorganisms to the human body. The first study in this direction was carried out by inoculating olives with exogenous lactobacilli strains, as it was the case of the human-origin strain *Lactobacillus paracasei* IMPC2.1. This microorganism showed high adaptability to the fermentation process adhering to the surface of the olives and being recovered from the feces of people who were fed for 10 days with olives fermented in the presence of this bacterium. Thus, the Italian health ministry allowed its application as a starter culture in table olives. Various strains of *L. paracasei* have also been used for the fermentation of green olives, but always with exogenous bacteria to the olive fermentation process.

However, the greatest developments on the matter have been obtained recently thanks to the European project Probiolives (FP7-SME-243471). In the framework of this project, diverse native LAB strains were isolated from the fermentation of different types of table olives elaborations and molecularly identified and characterized according to their potentially probiotic characteristics. Although the project was originally aimed to obtain LAB strains with probiotic characteristics that could potentially be used as starter cultures during the fermentation, the results obtained have opened new and interesting research lines. In this way, recent studies have proved, using scanning electron microscopy techniques, that the capacity of fruits to act as vehicle was due to the formation of biofilm by microorganisms on the olive surface. This biofilm was a polymicrobial ecosystem, consisting essentially of lactobacilli and yeasts, which were attached to the skin of the fruit forming a community characterized by the excretion of a protective adhesive extracellular matrix mainly composed of exopolysaccharides (Figure 1). These exopolysaccharides isolated from olives have also shown the ability to inhibit the adhesion of the enterohemorrhagic strain *Escherichia coli* K88 to the intestinal mucosa of pigs. Thus, it could have a large application in both animal feed and humans for the treatment of diarrhea.

The study of mixed biofilms between yeast and LAB on olive surfaces is a recent issue, with a direct application because an excellent vehicle of beneficial microorganisms to the final consumer can be made from this food. Recent studies conducted with table olives found that the major species that are part of these mixed biofilm during fermentation were *L. pentosus* among LAB and *Geotrichum candidum*, *Pichia galeiformis*, *Candida sorbosa*, *Saccharomyces cerevisiae*, and *Wickerhamomyces anomalus* among yeasts, reaching population levels on the olives up to 8 $\log_{10}$ CFU g^{-1}. However, our knowledge of the genes regulating the different steps during biofilm formation, the interaction

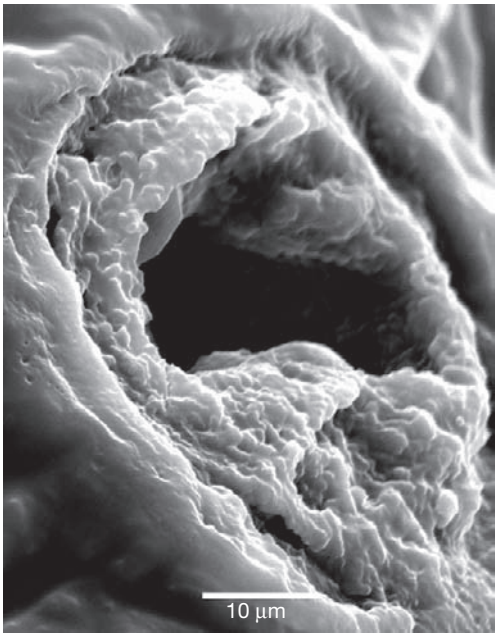

Figure 1 Photography of scanning electron microscopy showing bacteria cells adhered to Gordal olive stomata during fermentation.

between microorganisms, population dynamics, and composition of the matrix surrounding the microorganisms is still scant, despite the large application that both yeasts and LAB species play during table olive processing.

See also: Biofilms; Fermented Foods: Fermented Vegetables and Other Products; Olive Oil: Its Role in the Diet.

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Organic Food: Effect on Nutrient Composition

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Introduction

There are several definitions of organic agriculture, for example, used by FAO "Organic farming is a holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity" or IFOAM "Organic agriculture is a production system that sustains the health of soils ecosystems and people." The main idea is that organic agriculture is enhancing human and environment health because there is no use of synthetic fertilizers and pesticides. Legal regulations and principles of the organic food production are described in another chapter.

Organic food production is one of the fastest growing sectors with an increase of approximately 250% in 10 years. The global organic food market reached a value of € 45.8 billion in 2013; 47% of the global market was in Europe; there was increasing sale also in the United States and Canada. In Europe, the majority of the sales are occurring in the large economies (mainly Germany, France, the United Kingdom, and Italy). Although organic market growth rates are different across countries, it is clear that organic food is becoming a significant sector in the food production chain. Consumers are looking for organic food because they believe it has better nutritive value and taste; in addition, organic farming is more environment-friendly and provides better animal welfare. The main obstacles hampering the sale of organic products are high price premiums, insufficient availability, low confidence in certification bodies and organic labels, and satisfaction with current food source.

Factors Influencing the Quality of the Organic Food

Quality and safety of the organic food rely on many factors; they are presented in Figure 1.

Abiotic factors comprising the quality of the environment are basic for the food production in general and in organic food in particular. Air, groundwater, surface water, and soil have to meet high qualitative demands; first of all, the level of contamination with chemical unwanted substances has to be low and safe for plant production. Next, biotic factors as cultivars of plants and races of animals are important, as well as the occurrence of the diseases and pests. The methods of soil fertilizations together with the methods of animal raising are fundamental for the quality of the produced crops and animal materials. Here, the farmers' knowledge, skills, and scrupulousness are also of high importance.

The food processing methods are basic for the food products quality – in organic food processing, only c.50 natural substances are allowed, while in the conventional processing, several hundreds of the artificial synthetic substances are admissible. It makes the situation of the organic producers very difficult – the shelf life of the organic products is much shorter and the production is more expensive. On the other hand, the health merits of organic products are bigger.

Last but not least, the functioning of the control and certification systems is the basic factor guaranteeing the high quality of the organic products. The production process has to be in agreement with demands comprised by the relevant legal regulations. Strict control system is necessary in order to exclude the dishonest producers from the market; fraud is very seldom but sometimes happens, spoiling the whole sector.

If all described conditions are fulfilled, the high quality of unprocessed and processed organic food products can be expected.

Fertilization and Plant Protection as Factors Determining the Chemical Composition of the Obtained Organic Crops

In the organic crop production, there is a ban on the use of synthetic chemical crop protection products and certain mineral fertilizers (all N, KCl, and superphosphate) in order to diminish environmental impacts and the risk of pesticide residues being present in crop plants. The fertilization is based on organic fertilizers (mainly manure and composts) and legume crops in rotation (to enhance soil N levels). Crop protection is based on the preventative and nonchemical crop protection methods (e.g., the use of crop rotation, more diversified agricultural landscape, more resistant varieties, mechanical and flame weeding, and biological disease and pest control products).

The effect of such management on plant composition is strong, and nitrogen fertilization is considered as the main factor. Most of the obtained data indicate that the content of antioxidants is higher when plants are grown under limited N-fertilization regime (organic system and lower N-fertilizer dose). It is consistent with the GDBH (growth–differentiation balance hypothesis) – nitrogen fertilization is the key factor influencing growth and differentiation processes in plants. When nitrogen is less available, plants are growing slower and final biomass is lower, but differentiation understood as synthesis of many bioactive compounds important for plant metabolism is more intensive. As a result, the nutritive value of these plants is higher, which is important for human health.

Plant protection is also important, as the use of synthetic pesticides is diminishing the content of antioxidants in plants. The probable mechanism is that plants grown in organic system have to produce more polyphenols as the protective substances against pests, especially herbivorous insects, and diseases.

Methods of Animal Husbandry as Factors of the Animal Products' Quality

Organic raising of animals differs significantly from conventional breeding. First of all, it is necessary to apply grazing as far

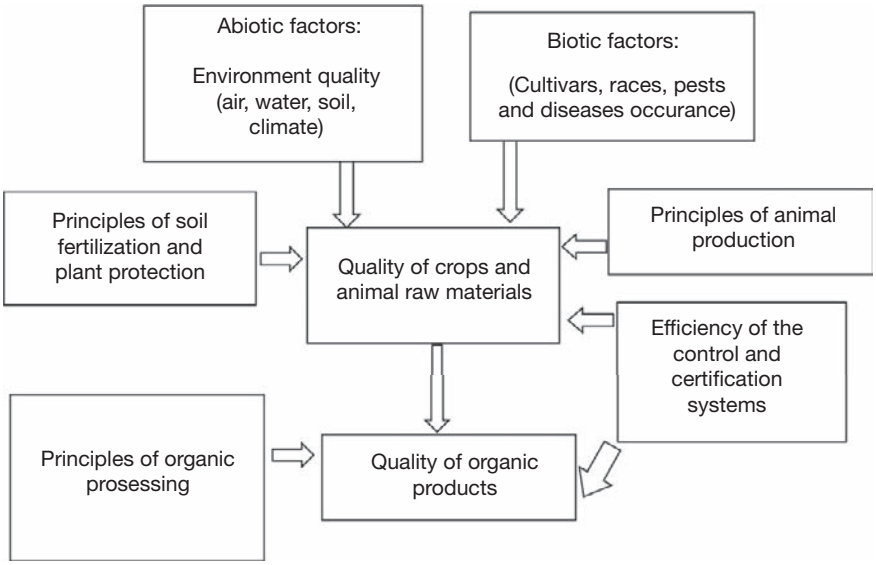

Figure 1 Factors influencing the quality of the organic raw materials and products, own elaboration.

as possible. This concerns mainly ruminant animals such as cattle, sheep, and goats; horses; to a lesser extent, swine; and poultry. However, all species of animals must be ensured a daily exercise in the air, outside the inventory building – this ensures a regular movement and the possibility of implementing the natural behavior for the species and social interactions. In addition, all species of animals have to be provided with a natural litter layer on the floor inside the inventory building – in most cases the cereal straw. Feed for animals must come only from organic production, without containing any synthetic ingredients. Described conditions ensure higher animal welfare in organic husbandry compared to the conventional one.

In the case of organic animal raw materials (milk, eggs, and meat), the main factors affecting their composition are animal nutrition and in particular a wide access to grazing in case of the ruminants (cattle, sheep, and goats). Animal feed made with fresh-stalk meadow grass affects the increased content of antioxidants and polyunsaturated fatty acids in milk and meat of animals from organic farming in comparison to the conventional one; animals in the last case have much lower access to open pasture and are fed to a large extent with silage and concentrates.

Comparison of the Content of Harmful Substances in the Organic and Conventional Crops

Nitrates and Nitrites

Source of nitrates V and nitrates III (nitrites) in raw materials and plant products are nitrogen fertilizers used in agricultural production. In the nitrates III, nitrogen is at +3 oxidation state, and in the nitrates V, nitrogen is at +5 oxidation state. Scientific reports confirm a higher content of nitrates in food from conventional production methods, where crop fertilization takes place with the use of synthetic fertilizers.

Mineral fertilizers have easily available forms, and as a result, the excessive intake and accumulation take place in the root system and next in the leaves and other plant organs.

In organic production, only organic fertilizers (compost, manure, and green manure) are applied; in such case, nitrogen is less available to plants. Organic fertilizer in the soil is first decomposed by microorganisms and edaphon; next, humus is created, which is composed of the organic–mineral compounds. This process allows the plant collection of nitrogenous compounds in adequate amounts and in appropriate plants for time.

Therefore, the concentration of nitrogen compounds proved to be significantly lower in plants from organic farming than from the conventional one – the concentration of total nitrogen by 10% lower, nitrates by 30%, and nitrites by 87%. Health danger of excessive accumulation of nitrates (V) in plants is due to the fact, among other things, that these compounds are easily turning into nitrites (nitrates III) and may cause serious disease called methemoglobinemia, dangerous especially for infants, young children, and older people.

In turn, nitrites may be subject to further chemical changes and can react with tertiary amines creating nitrosamines, which are carcinogenic and mutagenic, causing gastrointestinal disease and leukemia.

Pesticides

The use of pesticides that are synthetic compounds, not found in nature, increases yields through protection against pests and diseases. However, on the other hand, pesticides do not have impact only on the unwanted organisms. Residues of these substances accumulate in plants and move along the food chain, disturbing the balance of natural environment and damaging the animal and human organisms.

Health effects depend on the dose that has been taken by the body; however, it is known that pesticides, even at very low doses, can cause diseases such as cancer or birth defects. In order to cut down the intake of pesticides together with the polluted food, the maximum permissible residue level has been established (MRL). Unfortunately, the MRL is usually based on relatively short-term tests for checking the impact of the individual substances on the lab rats.

There is a lack of knowledge on the consequences of consuming a wide variety of pesticides, which a man is exposed during the whole life; the synergistic action of a mixture of these compounds is possible.

The way to reduce health risks from pesticides is to avoid their consumption, which is possible thanks to the consumption of products from organic production methods, where it is not allowed to use pesticides. This recommendation should be followed especially by pregnant women, breast-feeding mothers, and children under the age of 3. Organic products very clearly differ from conventional products in terms of both quantities and the incidence of pesticide residues.

Recent studies have shown that the probability of the detection of pesticide residues was four times higher in conventional crops than in organic ones. The comparison of the levels of pesticide residues in vegetables and fruits in different countries indicates that it depends on the general level of pesticide use in the country concerned; the highest values were reported in the United States, where it was used and high doses of pesticides were applied; slightly lower in Belgium; and the lowest in Sweden, where low dose of synthetic plant protection products was always applied.

The results of the presented study may lead to the conclusion that a diet containing mainly organic products can decrease the levels of pesticide residues in the tissues of the human body.

Studies have confirmed that, together with the increasing share of organic products in the diet of lactating women, the content of pesticide residues in breast milk decreased. It has also been found that in body fluids (blood and urine) of children eating conventional products, the organophosphate pesticides' residues were up to six times more than in the case of children fed organically. The data mentioned earlier indicate that consumption of organic products is an effective way to reduce exposure to pesticides.

Heavy Metals

Sources of heavy metals, including cadmium, lead, arsenic, mercury, and zinc, are industry, transport, municipal waste, and agriculture. For example, cadmium in soil and food can come not only from phosphorous mineral fertilizers used in conventional agriculture but also from industry and transport. Therefore, test results were previously ambiguous – generally, more cadmium has been found in the raw conventional materials than organic, but sometimes, it was the other way around.

Recently, however, in the latest meta-analysis based on a large amount of data, it has been shown that the raw organically grown materials contain significantly lower cadmium concentrations (on average by 48%) than the conventional plants. This is essential for health because cadmium is an extremely toxic element: it damages the kidneys, resulting in anemia, bone disease, and proteinuria, and also affects the cardiovascular system. Cadmium accumulates in the pregnant smoking mothers disturbing the development of the fetus; carcinogenic activity of cadmium was also noted in rats. In the case of other heavy metal (lead, arsenic, and mercury) concentrations, no significant differences between conventional and organic raw materials have been found. More research is needed to clarify what is the impact of such treatments as composting on the increase of the organic matter and pH in soil and on the possibility to cut down the intake of heavy metals by plants.

Mycotoxins

The source of mycotoxins in foods is mushrooms produced by genera *Aspergillus*, *Penicillium*, and *Fusarium*. Environmental conditions such as high temperature and humidity are beneficial for the development of fungi.

Mycotoxins in food can pose a serious risk to the health of the consumer, causing, among other, carcinogenicity and impairment of the functioning of the immune system. Results of studies comparing the contents of mycotoxins in organic and conventional products are divergent. Some research indicates lower contamination with mycotoxins in the organic products compared to the conventional products. In other studies, organic products were more contaminated with mycotoxins than conventional ones; however, these were minor differences, and the contents of mycotoxins never exceeded acceptable values. Much of the comparative studies were cereals, including wheat, the results of which indicated lower contamination of the organic grain than conventional ones. In organic farming, there are practices dwarfing the formation of mycotoxins in agricultural crops as, for example, proper crop rotation and cultivation of varieties resistant to the toxin-producing fungi.

Comparison of the Nutritive Compounds Content in the Plant Organic and Conventional Crops

Vitamin C

Vegetables are a very rich source of vitamin C, which belongs to the antioxidant compounds. Vitamin C participates in many metabolic chains in human body. It stimulates the immune system (most important function), enhances detoxification of the organism, impedes the initial stages of the carcinogenesis, and helps to fight against heart diseases, allergy, and diabetes. In one of the recently published meta-analyses, comprising 33 available scientific publications comparing organic and conventional systems, it was found that the organic crops contained on average 6% more vitamin C in relation to the conventional crops and it has significant difference. Similar results were obtained in two other new studies based on meta-analysis.

Secondary Metabolites

Secondary plant metabolites, which include phenolic compounds, are compounds with a high antioxidative activity and with a wide range of health-related properties, for example, anticarcinogenic.

Secondary metabolites are produced by plants in response to external stimuli, for example, the attack of pests and other stressful conditions. They fulfill the role of regulators for physiological changes occurring in the plant. On the other hand, humans need to consume plants containing secondary metabolites, because they help to prevent infectious and noninfectious diseases (the last also called civilization diseases). One of the big groups of plant metabolites are phenolic compounds not containing nitrogen in their molecule, such as

phenolic acids, flavonoids, and terpenoids (carotenoids and xanthophylls).

All these compounds play an important role in suppressing the development of cancer, preventing atherosclerosis, strengthening the walls of blood vessels, reducing the risk of stroke, supporting the immune system, and preventing bacterial viral and infections. The second group of secondary metabolites is compounds containing nitrogen in the molecule as alkaloids, amines, amino acids, glycosides, and nonprotein glucosinolates. Their impact on human health is less known than the previously described group of substances.

The most recent meta-analysis of different 343 investigations concerning the chemical composition of organic and conventional crops showed that the content of a number of key antioxidants in organic raw materials and based on these products is up to 60% higher than in conventionally grown plants.

In the case of carotenoids, this analysis showed significantly higher content in organic versus conventional fruits; for vegetables and cereals, the differences were not statistically significant. A lot of research on the peppers and tomatoes indicates that the level of lutein and beta-carotene is usually higher in organic crops than in conventional ones, while a reverse situation is found with the level of lycopene.

Mineral Compounds

The first review paper concerning the content of mineral compounds in organic and conventional vegetables has shown that organic crops contained a higher level of trace elements such as iron, magnesium, and phosphorus than conventional ones. This may be due to the fact that the soil cultivated according to the principles of organic agriculture contains more microorganisms, which may contribute to the increasing availability of necessary mineral compounds to the plants.

The latest elaboration on the basis of meta-analysis shows that organic fruits and vegetables contain on average 5.5% more minerals (primarily boron, copper, magnesium, molybdenum, potassium, phosphorus, selenium, sodium, and zinc) than conventional crops. This may be important for human health in the case of ingredients such as magnesium, potassium, selenium, and zinc, which are dietary deficiencies recognized for the countries of central Europe.

Carbohydrates, Dry Matter, Keeping Quality, and Proteins

Many studies show a higher content of total and reducing sugars in the organic versus conventional crops. The latest big meta-analysis also detected significantly higher concentrations of total carbohydrates and reducing sugars and moreover significantly lower concentrations of proteins, amino acids, and dietary fiber in organic crops/crop-based compound foods. It also detected significantly higher concentrations of DM (dry matter) in organic crops.

The content of sugars in fruits and vegetables is very important. For example, the more sucrose the sugar beets contain, the higher is the technological value of this crop. Sugar level in crops is also important for the nutritional value and sensory quality. In the case of dietary fiber, it is not so obvious. The diet of Europeans today is too little of this component, and organic crops – according to research – provide even less dietary fiber than conventional ones.

Organic crops usually contain more dry matter than their conventional counterparts, which follows from the effect of 'water swelling' – tissues of the conventional plants collect more water than those of organic ones, because the plants absorb a lot of water together with mineral fertilizers that are applied. The higher dry matter content in organic than conventional crops is positive because it means more energy and nutritive density of organic food, which contains less water, but more nutrients per unit of mass. The higher dry matter content translates to higher palatability of the organic crops due to the greater concentration of fragrance and taste, including essential oils. Less water in the tissues means also lower susceptibility of organic crops to decomposition processes during the keeping period compared to conventional materials. From a review of over 50 different studies, it appeared that conventional crops showed 38% loss of their initial biomass during the winter, while organic only 28%.

Several studies indicate that the amount of total proteins is lower in the organic crops than in the conventional ones. The latest meta-analysis showed a significant difference in this respect for cereals. However, the attention should be paid to the quality of the proteins in the organic crops, which is characterized by a higher content of essential amino acids (lysine, cysteine, and methionine), reflecting their higher biological value, compared to conventional crops. The differences in the composition of proteins and amino acids are derived from different nitrogen fertilizations used in the organic versus conventional systems.

Sensory Values of the Plant Products

Both regular consumers and specially trained panelists in many cases much better judge the sensory properties of organic crops than conventional ones. This mainly applies to vegetables such as carrots, potatoes, tomatoes, and peppers and fruits such as apples, black currants, grapes, and strawberries. Food preference tests carried out on rats have shown that animals given a possibility to select feed of organic or conventional origin clearly prefer and select more frequently organic feed, for example, wheat, carrots, and beetroots. These results are probably associated with a higher concentration of fragrance and taste in organic versus conventional crops, including the higher content of sugars and more favorable ratio of sugars/organic acids found in the organic crops.

Quality of the Animal Products from Organic versus Conventional Production

Milk

The composition of milk depends mainly on the diet and maintenance of animals, different in organic and conventional system. A number of studies have shown that proteins in organic cow milk are more beneficial than conventional milk; examples of these proteins are α-lactalbumin, β-lactoglobulin, and lactoferrin. Milk from the cows grazed or fed with fresh

feed, rich in different species of plants, is characterized by a significantly higher ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) and a more favorable ratio of PUFA Ω-6 to Ω-3.

Organic milk contains more PUFA, CLA, cis-9, trans-11, t-vaccenic acid (TVA), and α-linolenic acid (LNA) compared with conventional milk. These substances are recognized as inhibiting development of atherosclerosis, cancer, and inflammatory processes.

During the period of grazing, cow milk from organic farms has a higher content of fat-soluble vitamins (A, D, and E) than the milk of animals that do not utilize pasture. The concentration of minerals in milk depends on their contents in soil and green fodder. Some reports indicate too low content of minerals in organic milk because of the prohibition on the use of the mineral components in the feed supplementation of cows. However, the milk from organic production should be considered as very valuable. Other things to determine are how much impact has the management of husbandry and how much natural fresh feed is produced without the participation of the synthetic mineral fertilizers and pesticides. Distinguishing of these factors in the experimental layout is very difficult due to the legal regulations, prohibiting to keep animals only within the buildings in organic farming.

Meat

The factors mentioned earlier have an impact on the quality of not only the milk but also meat from animals of organic farms. The main factors are also the composition of the feed and the animals' access to an open pasture for many months of the year, which mainly affects ruminants. Impact on the quality of the meat is similar as in the case of milk. The nutritional quality of organically produced meat is affected by organic diet and breeding conditions. A number of studies confirm the lower total fat content of organic farm meat and this means a decreased calorific value. Carcasses from organically reared animals have usually higher content of intramuscular fat, positively affecting sensory properties, better in comparison with the conventional meat, which has been confirmed in several studies. Moreover, the fatty acids' profile in organic meat is considered to be more beneficial to human health. Organic meat contains more favorable proportions of unsaturated fatty acids, including *n*-3 acids, and decreased level of saturated ones. The ratio of n-6:n-3 PUFA in meat from organic and conventional husbandry differs significantly, for example, in conventional beef[29] 2:1 and in organic one[4] 6:1. The last ratio is much more recommended for human health by nutritionists – consumption of organic meat can cause anticancer effects, stimulate the immune system, and prevent coronary heart diseases. A negative side is that organic animals have lower total weight of carcasses (lower weight gain) at the slaughter time, making organic husbandry less profitable from the economic point of view. Moreover, organic meat and meat products have higher TBARS value and faster lipid oxidation processes, shortening their shelf life period. Some consumers are complaining because of the lower tenderness in case of organic pork compared to the conventional meat.

Eggs

So far, relatively little research comparing the composition and nutritional value of eggs from organic and conventional production has been published. A few studies show that organic eggs have a lower unit weight than conventional eggs, which have proportionally thicker and stronger shell, smaller egg white, and bigger egg yolk. It has been proved also in the Netherlands, Denmark, and Italy that egg yolks from organically raised hens contained significantly more carotenoids than conventional ones; this was primarily lutein, as well as beta-carotene and zeaxanthin. At the same time, it has been demonstrated that the level of these valuable substances increased as the growing access of hens to natural pasture, confirming the impact of the consumption of roughage (grass and other plants) on the level and composition of carotenoids in eggs. Organic yolks contained also more α-tocopherol and flavonoids compared to the conventional ones.

In the Netherlands, it was found that carotenoid profile was so characteristic for eggs from organic farming that could be used to identify these eggs for the needs of the market. This is needed because of a commercial fraud that sometimes happens; organic eggs are more expensive, and dishonest traders try sometimes to sell conventional eggs as organic. This practice also applies to many other products, for example, wine and various fruits and vegetables. As in the case of milk and meat, the composition of fatty acids in eggs depends on the access of hens to the open pasture. The fatty acid composition of the yolk is significantly affected by pasture. The eggs of hens having bigger access to the pasture showed lower concentrations of polyunsaturated fatty acid (PUFA) *n*-6 and a higher percentage of PUFA *n*-3 than eggs of hens with limited access to the pasture.

Summary

Improper diet, unhealthy lifestyle, and food and environment contamination are the main causes of the so-called civilization diseases, also known as chronic noninfectious diseases: diabetes, obesity, atherosclerosis, cancer, and allergies. The prevention of these diseases and their treatment require not only the observance of proper diet (diet and lifestyle) but also eating the best quality food. From most of the comparative studies, it appears that food from organic production can bring beneficial effects in the prevention of the mentioned diseases.

Food with a high biological value contains the right amount of nutrients with the lowest content of harmful substances. Organic food has these features (Figure 2) – low content of contaminants and high content of vitamin C, carotenoids, and phenolic compounds having strong antioxidative properties.

In addition, higher content of sugar and dry matter in organic crops means that they have a higher technological quality and, at the same time, better and more intense flavor than the conventional ones.

Current research also indicates that despite the fact that synthetic fungicides are not used in the organic farming to combat fungal diseases, the level of mycotoxins in organic and conventional cereals is similar. Also, studies on the microbiological contamination indicate parallel level of pathogenic bacteria in organic and conventional crops. Therefore,

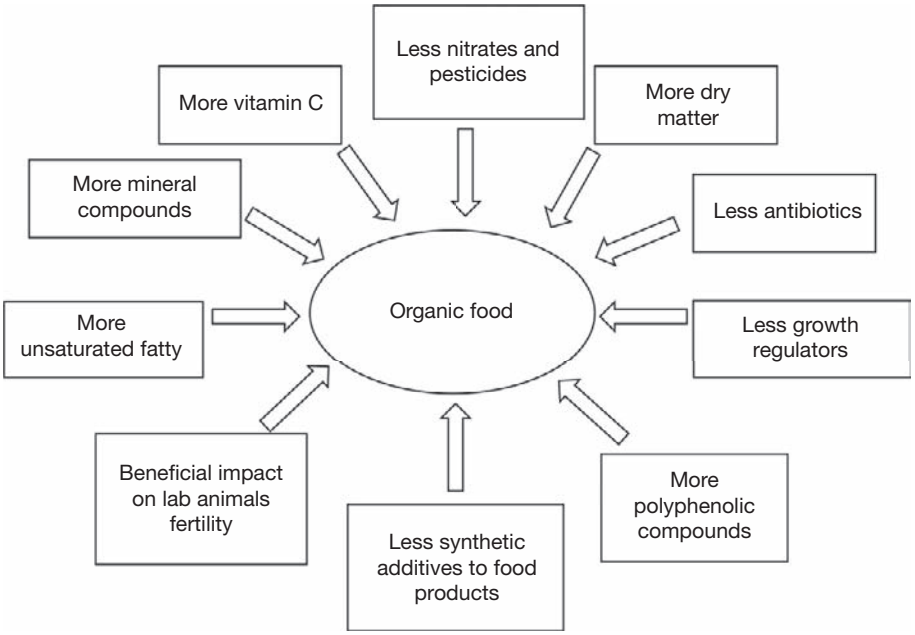

Figure 2 Quality features of the organic food in comparison to the conventional food, own elaboration.

consumption of organic products compared to conventional ones does not represent a higher risk to health, particularly if they are subjected to adequate heat treatment.

Described quality features of organic food (Figure 2) constitute a good basis to formulate a hypothesis that regular consumption of organic foods should positively affect the health of consumers. Preliminary results from the research on experimental animals suggest more favorable characteristics of fertility and survival rates of animals fed with organic feed compared to those fed with conventional feed. Also, studies in humans suggest that the regular consumption of milk and milk products from organic production by mothers of children up to the age of 2 was positively correlated with the lower incidence of atopic diseases among these children. Preliminary studies on large groups of consumers indicate, furthermore, that the persons eating predominantly organic products better evaluate their health status than the consumers basing on the conventional products.

The mentioned results seem to confirm the initial hypothesis of the positive impact of organic food on the consumer health, but further studies conducted on animals and on humans are necessary in order to confirm the preliminary results and to understand the mechanisms of the organic diet impact on animal/human health.

See also: Antioxidants: Role on Health and Prevention; Ascorbic Acid: Physiology and Health Effects; Cadmium: Toxicology; Cancer: Diet in Cancer Prevention; Carotenoids: Occurrence, Properties and Determination; Nitrites and Nitrates; Organic Food: Production and Control; Pesticides and Herbicides; Phenolic Compounds: Bioavailability and Health Effects; Pregnancy: Dietary Guidance for Pregnancy.

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Organic Food: Production and Control

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Organic Food: An Introduction

Agricultural products typically have a price dictated by the international commodities market and it does not meet often the income needs of farmers with small production volumes. To add value to these products, organic production may be an alternative way to increase the income, helping the rural development and environmental integrity.

It is widely recognized that patterns of food consumption have experienced rapid change over the past few years as the result of concerns on environmental sustainability, development, nutritional aspects, and also health issues. The consumption of organic food cannot guarantee itself to improve the consumer's health, but they can represent an important part of a sustainable and healthy diet.

Organic food can be defined as the product, both fresh and processed, obtained or made according to the standards of organic agriculture. According the International Federation of Organic Agriculture Movements (IFOAM) (2009), organic agriculture is a production system that sustains the health of soils, people, and ecosystems, using organic pesticides and avoiding, for instance, the use of antibiotics and growth hormones. It relies on tradition, innovation, and science, looking up to benefits for people and for the environment, promoting the quality of life of all stakeholders involved in this chain. The organic producer must respect established norms in all production stages, from the seeding process to packaging, always concerning about the process' impact on the natural environment.

The main difference between conventional and organic farming refers to the way of land use. The conventional one is based mainly on the inputs (insecticides, herbicides, fungicides, bactericides, and fertilizers are all based on soluble salts and organic raw materials). The organic farming technology is based on the process technology, based on food production without the use of pesticides and chemicals in a self-sustainable environmental fertilizer.

Among the various possible strategies, large and small producers have sought the development of organic market, the alignment with the global trends of organic production, and the appreciation of food safety, through the implementation of traceability programs, quality patterns, and certification. Apart from these systemic initiatives, it is still necessary to coordinate and support a large number of small organic producers, seeking strategies that allow better income opportunities and greater chances of inclusion of its production in distinguished distribution channels. Internationally, one of the successful strategies has been the adoption of certified organic system. Organic agriculture is one of the best practices in ensuring environmental sustainability; it sustains the fertility of soils, ecosystems, and sustains people's health. As an ultimate goal, this system helps to develop ways to protect small producers from the market fluctuations, guaranteeing better economical margins and greater sustainability in their activities.

Organics' Global Market

Despite the fact that the largest domestic markets of organic food are located in North America (the United States primarily) and Europe (mainly in Germany, with 7 billion euros in 2012), the largest number of organic producers, however, is located outside those markets.

In 2012, the distribution of the organic agricultural land was Oceania (32%), Europe (30%), Latin America (18%), Asia (9%), North America (8%), and Africa (3%), the principal countries being Australia, Argentina, the United States, and China. About one-third of the world's organic producers are located in developing countries and emerging markets. Data from 2012 show that organic producers (about 1.5 million) are located in Latin American, Asia, and Africa, with a higher production growth rate, especially in the food sector, such as fruits, vegetables, grains, herbs, and spices. India was the country with the largest number of organic producers in 2012, followed by Uganda and Mexico. Not only production but also demand growth in developing countries and emerging markets is also remarkable.

Organic Agriculture: The Origins

The history of organic agriculture began in the 1920s with the work of the British researcher Albert Howard on the agricultural practices in composting and use of organic fertilizers used by farmers in India. Around the same time, organic farming was widespread in France through the use of techniques such as crop rotation, green manures, manure, crop residues, straw, and other plant or animal wastes and natural control of pests and diseases. It also involved avoiding the use of fertilizers, synthetic fertilizers, and pesticides in the management of crops or artificial growth accelerators or fattening in the handling of animals, applying only the mandatory vaccines. The herbal medicine, homeopathy, and acupuncture treatments are used in cases of diseases. A similar concept is the biodynamic farming that began in Germany in 1924 with Rudolf Steiner and is based on harmonization and balance of the plant (land, plants, animals, and man) using the influences of the sun and moon. The farm is considered as a systemic organism and one should therefore only use organic elements produced there to establish the link between the forms of matter and energy in the natural environment. Mokiti Okada, in 1935, defined what would be a natural agriculture. According to his philosophy, there are spirit and feeling in all living things (plants and animals). Soil is the primary source of life and used the inputs available at the production site for composting it, thus strengthening its natural energy. Its goal is to get products for agricultural systems that are similar to the original ecosystem conditions. In parallel, the concept of permaculture was developed in Australia in 1971 by Bill Mollison; it consists of an integrated model of agriculture to the environment; however, it

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00506-7

also uses information on the direction of the sun and wind to determine the spatial arrangement of plants. These first movements are not similar to the current organic production because they had no standards, regulations, or interests in environmental issues and food safety. This set of production practices came to be known as alternative agriculture. This term originated in 1977, when the publication of a report containing an analysis of all unconventional streams of agriculture gathered under such generic name. This report was made by the Ministry of Food, Agriculture and Fisheries of the Netherlands and is known as the 'Dutch report.' In the 1980s, agroecology defines a set of alternative farming practices also incorporating a social discourse. Its authors Dr Miguel Altieri and Dr Stephen Gliessman, the University of California, the United States, emphasize the breadth of the concept dealing with complex interactions between people, cultures, soil, and animals. In the late 1980s and throughout the 1990s, the concept of sustainable agriculture became popular. This was very broad and full of contradictions and should be considered more as a goal to be achieved than simply a set of agricultural practices.

The various terms mentioned earlier in the text (organic, biodynamic, natural, permaculture, agroecology, sustainable agriculture, etc.) are considered forms of organic agriculture, provided that they suit the production methods and marketing standards. In short, these different streams are the basis of the current organic agriculture. A sustainable production system through the management and protection of natural resources, without the use of harsh chemicals to humans and the environment, keeping the increase of fertility, soil life, biological diversity, and respecting the cultural integrity of farmers are the core principles of organic agriculture.

Organic production, in the 1970s, is related to the philosophical movements linked to alternative forms of life in contrast to the consumerism of modern society. The products were sold directly from producer to consumer. With growing environmental awareness and the search for a healthier diet, there was an increase in the consumption of organic products. Similarly, production cooperatives of organic products were organized in the 1980s, as well as the emergence of restaurants dedicated to natural food. However, it was at the end of the 1990s that organic products are now sold in supermarkets. As in the rest of the world, the existence of a profitable and growing market has attracted new entrepreneurs, aimed mainly to the profits from the activity, and, while maintaining the technical principles of organic agriculture, the philosophy that originated from the movement is increasingly farther.

Organic Certification

Within the food market, information asymmetry is present; consumers do not have usually the same level of information that producers have about the product itself, and this may cause an opportunistic behavior or negotiations' difficulties. Every asymmetry implies incomplete information and increases transaction costs throughout the supply chain, resulting in losses for one or more agents. Thus, certification has emerged as an alternative to eliminate or reduce these asymmetries.

A certification can be understood as a clear definition of the attributes of a product, process, or service associated with instrumental guarantee that they fit into predefined rules before the transaction. The concern over food security has given rise to many requirements to ensure organic food patterns. Certification and traceability have become commonplace in this new context. This is linked to the specificities related to organic products: due to their intrinsic characteristics, the differentiation of organic products cannot be easily identified and evaluated. Organic food can be classified as credence good, as its key attributes cannot be evaluated by consumers even after purchase and consumption. This feature justifies the need for monitoring of the production by an independent agent as the certifying companies, which are responsible for the warranty offered with respect to established procedures for organic farming in an organic label. The organic label is not owned by the producer who receives it. The certifying organization is who owns it and both parties have to sign a contract. However, the label is an achievement of the producer and reflects that the productive system complies with national (and international standards in some cases) of organic production, but normally, even the internal standards are based on international ones. Therefore, their use must be nurtured so that their credibility is always preserved, and they can be recognized by consumers as a highly desirable notion and added value.

Assuring the Organic Standards

The IFOAM is a global organization that accredits certifiers, creating an acceptable international organic standard. It includes the International Organic Accreditation Services (IOAS), which is responsible for the accreditation of the certification bodies and ensures the equivalence of accreditation programs in an international standard. In 1972, the agency developed a guarantee scheme based on a democratic process of consultation with all the agents involved in organic production. This process resulted in a structure called the *Organic Guarantee System*, whose content is divided into (1) basic standards, (2) criteria for accreditation, (3) accreditation program, and (4) seal.

Certification audit process is the mechanism most widely used to protect organic farming from fraud. Public or private accredited certification bodies use internationally recognized criteria and procedures for conformity assessment such as the ISO 65, recognized by IFOAM, and the European standard CEC 2091/91, in addition to the technical requirements established by the specific country legislation for organic agriculture, if needed. The basis of the certification audit is that the conformity assessment has to be made by an independent body with no direct link with anyone who buys or produces. After approval, the certification body is responsible for including the producer in the official register list and allows him or her to use the label.

The certifying bodies draw up an institutional framework that can be called 'self-regulation,' since adherence to their respective certification programs is voluntary. This logic allows

some standardization of the requirements so that there are legitimacy and trustworthiness among agents in the market, particularly consumers, producers, traders, and certifiers themselves.

Concluding Remarks

The certification label of an organic food assures the consumers a product free of chemical contamination. It also ensures that this product is the outcome of an agriculture that works on behalf of the environment protection, besides biological and nutritional values. Furthermore, it also attends to social aspects related to the quality of life in the land (for producers) and in the community (for consumers). The label ensures that the whole process was checked.

However, the organic certification process has different standards depending on the country, representing the need of recertifying organic products according to the regulations of the importing country. In the European Union, for example, each country has a distinguished certifying body (usually private companies) and standards do overlap. In the United States, the Department of Agriculture is the main certifying body. On the other hand, each country in the European Union has different certification organizations. The lack of harmonization of production standards and control makes the organic certification process complex and involves high costs, especially for the exports. In this way, the organic production might represent a potential huge international market for producers from developing and emerging countries, but the decision of which certification to comply with involves several factors.

Large retailers perceived the importance of this growing market and offer a diversity of organic food products on their shelves. The supermarkets might help organic suppliers to get the certification. Besides the technical process, the knowledge of attributes more valued by consumers helps retailers to play the role of coordinators of the organic supply chain stimulating more small producers to adhere to organic certification, helping them to upgrade their production practices and improve their income. Street markets are an important distribution channel for organic product, but some of them use self-auditing. There is no third-party or certification body to control if the production processes really follow the organic production guidelines. In this way, consumers need to trust that no environmental damage was involved in the production of the food product.

The next step for organic production and control is a global standardization of the processes that will motivate more food producers to convert their conventional production to a more sustainable one, delivering a safe, healthy, and less expensive food to society.

See also: Genetically Modified Foods: Risk Assessment, Legislation, Consumer Behavior, and Ethics; Organic Food: Effect on Nutrient Composition; Pesticides and Herbicides: Residue Determination.

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Osteoporosis

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Introduction

Osteoporosis is a disease characterized by a loss of bone mass (BM) and strength below the threshold level required for mechanical support of normal activity, as well as an increased occurrence of non-traumatic fractures. It is a disease that gradually causes the bones to become fragile and break easily. It is a metabolic bone disorder characterized by a diffuse decrease in the amount of bone. It affects both men and women, mainly as they grow older. Loss of BM occurs as a normal part of the aging process; however, in the individual with osteoporosis, the loss is so extensive that it falls below the threshold of a fracture. By their 30s, most people begin to slowly lose more bone than can be replaced. As a result, bones become thinner and weaker in structure.

Osteoporosis is the most common disease in elderly women, affecting millions of white women in the US. More than 25 million Americans have osteoporosis, and 80% of them are women. Women of northern European descent who have an inadequate dietary calcium intake are at the greatest risk of developing osteoporosis. Fractures that result from osteoporosis are a major and growing concern for public health systems. As the population ages, the number of fractures worldwide will double or triple in the next 50 years. Osteoporosis causes 1.3 million fractures, with 500 000 vertebral, 250 000 hip, and 240 000 wrist fractures costing $10 billion per annum. In addition to this direct expenditure on treatment, enormous economic costs and incalculable losses occur in terms of disability, pain, and premature death. Only about 25% of women over 45 years of age who sustain a hip fracture regain prefracture mobility.

An increased risk of falling contributes to a high incidence of fragility fractures in osteoporotic patients. Osteoporosis is likely to be caused by complex interactions among local and systemic regulators of bone cell function. The heterogeneity of osteoporosis is due to differences in the production of systemic and local regulators, enzymes that produce or inactivate local regulators, changes in receptors, nuclear transcription factors, and signal transduction mechanisms. An increase in the elderly population is directly proportional to the number of cases of osteoporosis.

Historical Background

Osteoporosis has probably existed throughout human history, but only recently – as the human lifespan increases – has it become a major clinical problem. In the early nineteenth century, Sir Astley Cooper – an English surgeon – noted the lightness and softness of bones, which is observed in the more advanced stages of life, making them prone to fractures. The term osteoporosis was first coined by Johann Lobstein at about the same time. Dr. Fuller Albright, an American physician and endocrinologist in 1940, described the postmenopausal osteoporosis, and proposed that it was the consequence of impaired bone formation due to estrogen deficiency. Furthermore, the concept of two forms of osteoporosis (one related to estrogen deficiency at the menopause stage and the other to calcium deficiency and aging of the skeleton) was proposed. This was replaced by the more recent concept stating that osteoporosis represents a continuum in which multiple pathogenic mechanisms congregate to cause the loss of BM and microarchitecture deterioration of the skeletal structure.

Hip fracture rates are the highest in Caucasian women living in temperate climates. These rates are somewhat lower in women from Mediterranean and Asian countries, and are lowest in African women. Countries like Hong Kong have seen significant increases in age-adjusted fracture rates in recent decades, while those in western countries largely appear to have reached a plateau. Within the last decade, genetic studies have facilitated the identification of many of the regulatory mechanisms that have been linked to osteoporosis.

Human Skeleton

The human skeleton is a collection of bones – held together by ligaments, tendons, muscles, and cartilage – in which crystals of calcium phosphate are embedded, providing a framework for the body. It holds and protects the organs. The adult skeleton consists of 206 bones. A newborn has more than 300 bones, but many of these fuse together as a child grows into an adult. Bones are of two types. First is cortical bone, also called compact bone. With a hard outer layer making up to 80% of the total BM, it gives white color to the bones. Second is the trabecular bone, also called cancellous or spongy bone. This forms the inside and makes up the remaining 20% of the total BM. It is light and porous, and makes up most of the bone material. This tissue also has space for blood vessels and bone marrow. Both cortical and trabecular bone contribute to the overall strength of a bone. The trabeculae function as a storage site for calcium phosphate crystals. As osteoporosis progresses, the cortex also thins and weakens. These bones can be injured easily, even without a serious accident or fall. Fractures have been known to result from an affectionate hug.

Bone Strength

Bone mineral density (BMD) is determined by the amount of bone present in the skeletal structure, that is, the higher the

BMD, the stronger the bones, and vice-versa. It is greatly influenced by genetic factors which, in turn, are sometimes modified by environmental factors and medications. Normally, BMD accumulates during childhood and reaches a peak by around age 25, which is maintained for about 10 years. After age 35, both men and women normally lose 0.3–0.5% of their BMD per year as part of the aging process. Estrogen is important in maintaining BMD in women. When estrogen levels drop after menopause, loss of BMD accelerates. During the first 5–10 years after menopause, women can suffer up to 2–4% loss of BMD per annum. This can result in the loss of up to 25–30% of their BMD during that time period. The accelerated bone loss after menopause is a major cause of osteoporosis in women, referred to as postmenopausal osteoporosis.

Osteoporosis: Role of Diet and Nutrition

Many nutrients and food components can potentially have a positive or negative impact on bone health. They may influence bones by various mechanisms, including: alteration of bone structure, the rate of bone metabolism, the endocrine system, and homeostasis of calcium and possibly of other bone-active mineral elements. Among the essential nutrients, vitamins A, B, C, and K also play important roles in bone health. Reduced levels of calcium, vitamins, estrogen, and physical activity have been implicated in the increased incidences of osteoporosis (Table 1).

Calcium

Calcium is one of the main bone-forming minerals, and an appropriate supply to bones is essential at all stages of life. Calcium is required for normal growth and development of the skeleton; 99% of the calcium in the body is in the skeleton. Inadequate calcium intake during childhood and adolescence can impair bone development and may prevent the attainment of optimal peak BMD during early adulthood. In older adults, inadequate calcium intake accelerates bone loss and likely contributes to the development of osteoporosis. Sufficient calcium intake is critical to achieving optimal peak BMD, which modifies the rate of bone loss associated with aging. Adequate levels of calcium may slow the development of osteoporosis, as it suppresses bone resorption and it promotes bone mineralization/calcification – leading to the strengthening of the bones. A very small amount of calcium is required for muscle contraction, nerve impulse transmission, and other regulatory functions of the body. Although the importance of calcium to bone health is well-recognized, adequate calcium intake alone is not enough to prevent bone loss; loss that could lead to osteoporosis and osteoporotic fracture.

In addition to the amount of calcium in the diet, the absorption of dietary calcium in foods is also a critical factor in determining the availability of calcium for development and management of bones. The calcium that is lost daily through the urine, sweat, and feces must also be replaced by the calcium from the skeleton. To prevent a reduction in BM, calcium intake and absorption must balance calcium losses. With increasing age, calcium absorption decreases, and is lower in women who have osteoporosis than in women who do not have osteoporosis. There is a need to identify the foods and the food ingredients that may positively influence calcium absorption, and to ensure the calcium's bioavailability from these foods. This approach is important for individuals who fail to achieve the recommended dietary level of calcium, and for those with a low efficiency of intestinal absorption of calcium.

The current recommended daily allowance of calcium is 1 g a day. It is recommended that women after menopause increase their calcium intake to 1.5 g a day to maintain calcium balance. Calcium in food occurs as salts, or with other dietary constituents in the form of complexes of calcium ions. Calcium

Table 1 Potential nutritional determinants of bone health

Beneficial factors	*Potentially detrimental dietary factors*
Nutrients	Dietary factors/nutrients
Calcium	Excess alcohol
Copper	Excess caffeine
Zinc	Excess sodium
Fluoride	Excess fluoride
Magnesium	Excess/insufficient protein
Phosphorus	Excess phosphorus
Potassium	Excess/insufficient vitamin A
Vitamin C	Excess n-6 PUFA
Vitamin D	
Vitamin K	
B vitamins	
n-3 fatty acids	
Protein	
Novel bioactive food compounds	
Whey-derived peptides	
Phytoestrogens	
Nondigestible oligosaccharides (especially inulin-type fructans)	

Source: Cashman, K. D. (2006). A prebiotic substance persistently enhances intestinal calcium absorption and increases bone mineralization in young adolescents. *Nutrition Reviews* **64**:189–196.

must be released in a soluble, and probably ionized, form before it can be absorbed. The increased bone resorption that occurs with reduced levels of calcium affects both cortical and trabecular bone. The incidence of fractures occurring at sites composed of substantial amounts of both cortical and trabecular bone (the hip, pelvis, proximal humerus, and tibia) increases slowly with aging until late in the life cycle, when it rises exponentially.

Phosphorus

Phosphorus is an essential bone-forming element. Its adequate supply is necessary throughout life. Both calcium and phosphorus are required for the appropriate mineralization of the skeleton. A depletion of serum phosphate leads to impaired bone mineralization and compromised osteoblast function. In the case of very low birth-weight infants, the dietary intake of phosphorus influences the risk of osteoporosis.

Magnesium

Magnesium is required for bone and mineral homeostasis, and in bone crystal growth and stabilization. Magnesium intake has been reported, in some of the studies, to be positively associated with both BMD and bone resorption markers in middle-aged women. Magnesium is one of the nutrients in fruits and vegetables that contribute to an alkaline environment, which may promote bone health by a variety of mechanisms, making it difficult to examine the effects of magnesium alone.

Fluorine

Fluorosis causes joint stiffness, limb deformities, and staining of the teeth. Due to naturally high levels of fluoride in drinking water, fluorosis occurs in several parts of the world, such as South Africa, Tanzania, and India. Because of its effects on stimulating osteoblastic activity and inhibiting bone crystal dissolution, there has been considerable interest in the use of pharmacologic doses of sodium fluoride for the treatment of osteoporosis. At levels below those associated with fluorosis, and when combined with a low calcium intake, high fluorine intake has been associated with widened bones, reduced BMD, and osteoporosis of cortical regions of the skeleton—possibly due to excessive urinary calcium excretion.

Vitamins

Vitamin D is synthesized in the skin when exposed to ultraviolet radiation from sunlight, and can be obtained from the diet. Older people tend to have reduced endogenous production of the vitamin for a variety of reasons, and they become more dependent on dietary sources to maintain adequate vitamin D status. Younger people also have a reliance on dietary sources of vitamin D, especially if they have limited exposure to sunlight or if they are dark skinned and are living outside of the tropics. It is a well-established fact that marginal vitamin D deficiency is common, and it increases the risk of osteoporosis. The active form of this vitamin increases the intestinal absorption of calcium and prevents urinary calcium loss. In the absence of vitamin D, calcium absorption is not efficient enough to satisfy the body's needs, even when calcium intake is adequate. Vitamin D deficiency contributes to accelerated bone loss, increasing fragility, and also neuromuscular impairment leading to the increased the risk of falling. Calcium and vitamin D supplementation is now advocated as the basic minimum for treatment of osteoporosis and secondary fracture prevention in women several years after menopause.

Among the vitamins, thinning of the cortices and loss of trabecular architecture are common features of stark vitamin C deficiency. Ascorbic acid is a cofactor in the hydroxylation of lysine and proline, and is therefore important in the cross-linking of collagen fibers in bone.

Vitamin K is a cofactor in the gamma carboxylation of glutamic acid, which is important in the production of osteocalcin—one of the main noncollagenous proteins of bone. Inverse relationships have been reported between low vitamin K intake in older people, BMD, and the risk of fragility fractures—possibly through an increase in the amount of osteocalcin produced in its under-carboxylated and less fully functional form.

It is reported that a high intake of vitamin A as retinol has been associated with hip fracture in Sweden. There are studies linking low intakes of vitamin B6 and other B-vitamins with low BMC and hip fracture.

Estrogen

Estrogen has a protective effect on the bone by suppressing resorption. Estrogen deficiency is a major contributory factor to the development of osteoporosis in women, and hormone replacement therapy (HRT) remains the mainstay for prevention of bone loss in postmenopausal women. Deficiency of estrogen causes bone loss after menopause in women in their 70s and 80s, as evidenced by the fact that estrogen treatment rapidly reduces bone breakdown in these older women. This effect occurs for several reasons. Estrogen improves calcium absorption in the intestinal tract and decreases calcium loss in the urine.

Increased levels of estrogen lead to elevated levels of vitamin D in the circulatory system in healthy human subjects. The hypothesis that the estrogen deficiency is critical to the pathogenesis of osteoporosis was based initially on the fact that postmenopausal women, whose estrogen levels naturally decline, are at the highest risk for developing the disease. Estrogen stimulates the production of calcitonin, which prevents removal of calcium from the bone. The level of estrogen required to maintain relatively normal bone remodeling in older postmenopausal women is lower than that required to stimulate classic target tissues, such as the breast and uterus.

Trabecular bone tends to be more sensitive than cortical bone to changes in the level of estrogen. Decreased levels of estrogen have been implicated in the high incidence of Colles' and vertebral fractures noted in women. These sites are composed primarily of trabecular bone. Clinical trials involving older individuals at high risk for calcium and vitamin D

deficiency indicate that supplementation of both can reverse secondary hyperparathyroidism, decrease bone resorption, increase BD, decrease fracture rates, and even decrease the frequency of falling.

Proteins

Protein intake is a determinant of urinary calcium excretion. Animal protein (which is rich in sulfur-containing amino acids) contributes to an acidic environment, leading to higher excretion of calcium in urine. High protein intakes have been linked with hip fracture because the consumption of protein, particularly in the form of meat and dairy products, is greatest in countries where hip fractures are common. A low protein intake was associated with the greatest bone loss. At present, there is no firm evidence on which to base recommendations about optimal protein intake for bone growth, or for the prevention of osteoporosis.

Physical Activity

Physical activity enhances bone strength by optimizing BMD, and improving bone quality reduces the risk of falling. Resistance training increase BM and prevents age-related declines in BMD. Bone atrophy with accentuated calcium losses has been reported after periods of inactivity, either from prolonged bed rest or from immobilization. The emphasis of physical exercise programs in elderly osteoporotic patients should be on improving muscle strength and balance. They should be encouraged to participate safely in any activity in a frequent, regular, and sustained manner. The exercises should be weight bearing and easy to complete and should fit into their daily routine. A program of walking, sitting, and standing exercises (or water aerobics) can be recommended to start with, and gradually increased to more rigorous activity. For patients with an osteoporotic fracture, physical exercise programs can help reduce pain and increase functional capacity. The program should increase the patient's ability to perform routine daily activities while minimizing the risk of further fractures. For patients with vertebral fractures, back flexion exercises have been found to be harmful and to increase the risk of new vertebral fractures. These patients will benefit from resistance exercises that strengthen back extensor muscles.

Lifestyle

An inactive lifestyle or extended bed rest tends to weaken bones. Cigarette smoking is bad for bones as well as the heart and lungs. Similarly, excessive consumption of alcohol increases the risk of bone loss and fractures.

Risk Factors for Osteoporosis

Low BMD is only one of several risk factors underlying osteoporotic fracture. Other factors include both skeletal and non-skeletal factors. The BMD in the later portion of life of an individual depends on the peak BMD achieved during growth, and the rate of subsequent age-related bone loss. Development of maximal BMD during growth and reduction of loss of bone later in life are the two main strategies of preventing osteoporosis. Consequently, any factor that influences the development of peak BMD or the loss of bone in middle-age will affect later fracture risk. The factors like low BMD, physical frailty, propensity to fall, and a history of fracture also appear to be strong predictors of osteoporosis.

Those factors that influence the BMD are broadly grouped into non modifiable factors (gender, age, body [frame] size, genetics, and ethnicity) and modifiable factors (hormonal status [especially sex and calciotropic hormone status], lifestyle factors including physical activity levels, smoking and alcohol consumption patterns, and diet). The interactions of these genetic, hormonal, environmental, and nutritional factors influence both the development of bone to peak BD at maturity and its subsequent loss.

Symptoms of Osteoporosis

Osteoporosis is often called a silent disease because bone loss occurs without symptoms. The lack of any symptoms may continue for decades because osteoporosis does not cause symptoms until a bone fractures. Patients may not be aware of their osteoporosis until they suffer a painful fracture. The symptom associated with osteoporotic fractures usually is pain at the site of fracture. The symptoms in men are similar to those in women. The disease remains unnoticed until the bones become so weak that a sudden strain, bump, or fall causes a hip to fracture, or a vertebra to collapse. Collapsed vertebrae may initially be felt or seen in the form of severe back pain or spinal deformities.

Diagnosis of Osteoporosis

The BMD refers to the amount of minerals in a specific area of the bones. It gives an overall picture of bone health. A BMD test compares the person's results with the optimal peak bone density of a healthy young adult of that sex. Tests are safe and painless, with no needles or other invasive instruments. Typically, the test is performed with a central dual energy x-ray absorptiometry machine. It also may be done with computed tomography or an ultrasound.

Treatment of Osteoporosis

A comprehensive treatment program includes a focus on proper nutrition, exercise, and safety issues to prevent falls that may result in fractures. Treatments with medication can be broadly divided into two categories: antiresorptive (or anti-catabolic) and anabolic agents. Antiresorptive drugs help slow bone loss, and studies show they can decrease the risk of fractures.

Antiresorptive agents (which include oestrogen), selective estrogen receptor modulators, and bisphosphonates reduce bone resorption (and subsequently bone formation), preserving BMD. Anabolic agents, including full-length parathyroid

hormone (PTH1-84) and teriparatide (PTH1-34), stimulate bone formation (and subsequently bone resorption), thereby increasing BMD. Strontium ranelate is another agent that reduces fracture risk. It has weak effects on bone remodeling and probably improves bone strength mainly through effects on bone material properties.

HRT may consist of estrogens alone, or in combination with progestin. Even at low doses, HRT helps to slow bone loss, reducing the risk of osteoporosis and fractures in women who have gone through menopause. HRT is safe and effective for most women under the age of 60 who have osteoporosis and who also need hormonal treatment to relieve the symptoms of menopause.

It was demonstrated in recent studies that HRT decreases fragility fracture risk by 20–35%. However, its discontinuation results in acceleration of bone turnover, decrease in BMD, and eventual loss of anti-fracture efficacy. It was also reported that even low doses of HRT protects bone turnover markers levels and prevents bone loss. At present, HRT is regarded as the acceptable treatment for osteoporosis only after all other treatments have been considered, and when all the risks and benefits have been carefully explained to the patient. It has also been observed in some of the studies that the HRT can also induce vaginal bleeding and breast tenderness.

Conclusion

Osteoporosis is a treatable disease and in some cases, preventable. It is a complex, polygenic disorder. The contributions of specific gene polymorphisms are likely to be relatively small, but may still be clinically important. If treatment begins early, patients can be spared pain and suffering, and will likely lower the overall health cost incurred from treatment of osteoporosis complications. Preventative and therapeutic treatment should be given before signs occur, or as early in the disease as possible. To keep pace with the modern developments in research, large cohort studies using standardized genotyping methodology are needed to better define the role of specific genes in pathogenesis of osteoporosis.

See also: Alcohol: Metabolism and Health Effects; Calcium: Physiology; Dahi; Dairy Products: Dietary and Medical Importance; Fish: Dietary Importance and Health Effects; Milk: Role in the Diet; Mushrooms and Truffles: Role in the Diet; Vitamins: Overview; World Health Organization.

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Oxidation of Food Components

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Introduction

Originally, oxidation meant a reaction with oxygen to form an oxide, since oxygen was the first known oxidizing agent. However, later, the term oxidation has been expanded to include oxygen-like substances that accomplish parallel chemical reactions. Ultimately, the meaning has been generalized to include all processes involving loss of electrons.

Oxidation is defined as the loss of electrons or an increase in oxidation state by a molecule, atom, or ion. However, this definition is not completely correct, and oxidation should be referred to as an increase in oxidation state.

Many different components of food, lipids, proteins, nucleic acids, and different vitamins, are prone to oxidation. Since lipids and proteins are two of the major components in food, the focus of this article will therefore be on the oxidation of these components.

Lipid Oxidation

Lipid oxidation is a highly complex set of free radical reactions between fatty acids and oxygen, which results in oxidative degradation of lipids, also known as rancidity. Lipid oxidation intermediate products (free radicals) and end products (reactive aldehydes) may interact with other food constituents, such as proteins, sugars, pigments, and vitamins, and negatively modify their properties. The reaction mechanisms and the rate of lipid oxidation depend on many factors, such as fatty acid composition, the presence of prooxidants and antioxidants, type of lipid (triacylglycerols, phospholipids, and others), and storage conditions, for example, temperature, light, oxygen availability, and water activity.

Mechanisms of Lipid Oxidation

Lipid oxidation is initiated by the reaction of unsaturated fatty acid with oxygen (O_2) forming a primary oxidation product – lipid hydroperoxide (LOOH). Thermodynamically, atmospheric oxygen cannot react directly with double bonds on a fatty acid, because the spin states are different – the oxygen is in a triplet state ($^3O_2$), whereas the double bond is in a singlet state. Therefore, the reaction between an unsaturated fatty acid and oxygen (oxidation substrates) can only occur when one of the oxidation substrates is converted into an activated form – either a fatty acid radical ($L^\bullet$) or activated oxygen species – for instance, singlet oxygen ($^3O_2$), hydroxyl radical ($^\bullet OH$), or superoxide radical anion ($O_2^{\bullet -}$), the latter two belonging to a category of reactive oxygen species (ROS). Based on the reaction mechanism, lipid oxidation can be classified as

- enzymatic oxidation,
- singlet oxygen (light-induced) oxidation,
- autoxidation.

Enzymatic Oxidation

Several enzymes can catalyze lipid oxidation:

Lipoxygenases are iron-containing enzymes present, for example, in fish gill and skin tissues, beans, or grains. The enzymes catalyze the insertion of O_2 into an unsaturated fatty acid producing lipid hydroperoxides (LOOH).

Myeloperoxidases can initiate lipid oxidation in the presence of hydrogen peroxide and halides, such as bromides and iodides, and can be critical during the processing of meat/muscle tissues, when interaction between air (oxygen), blood, and lipids (oxidation substrates) is increased.

Singlet Oxygen (Light-Induced) Oxidation

In the presence of photosensitizers (porphyrins and riboflavins) and under exposure to light, the nonreactive triplet oxygen can be converted into reactive singlet oxygen ($^1O_2$). In addition, singlet oxygen can be formed chemically and enzymatically. Singlet oxygen can directly attack the —C═C— double bond on a fatty acid and thereby initiate photooxidation. Singlet oxygen-induced oxidation leads to the formation of both conjugated and nonconjugated hydroperoxides, which further decompose by the same mechanisms as peroxides formed from triplet oxygen in autoxidation (described in the succeeding text). The rate of lipid oxidation mediated by singlet oxygen is much higher than the rates in autoxidation, resulting in drastically increased rates of oxidation even at very low temperatures, which lowers the quality of foods during processing and storage.

Autoxidation

The most important mechanism in lipid oxidation is autoxidation, which occurs via free radical chain mechanism in an autocatalytic manner. It involves the reaction of unsaturated fatty acids with atmospheric oxygen and occurs in three phases: initiation, propagation, and termination.

In the initiation step, lipid radicals may be formed by thermal cleavage, by reactions with chemical oxidizers (activated oxygen species), or by interactions with transition metals or enzymes. Once an initial lipid radical is formed, it reacts with atmospheric triplet oxygen, generating peroxyl radicals. These act as chemical oxidizers and react with a new unsaturated fatty acid, thereby propagating the chain of autoxidation (Figure 1), which leads to branching reactions. The chain is terminated once the free radical is reacted into a stable molecule.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00508-0

Primary Oxidation Products

The primary product of the autoxidation cascade is LOOH; each peroxide group is accompanied by the formation of conjugated diene structure on the fatty acid. The chain also produces intermediate and short-lived lipid radicals, such as $L^{\bullet}$ and $LOO^{\bullet}$. As the number of double bonds in polyunsaturated fatty acid (PUFA) increases, more complex mixtures of LOOH are produced. Lipid hydroperoxides are tasteless and odorless (Figure 2).

Secondary Oxidation Products

In the presence of metals and nonheme iron and at high temperatures, lipid hydroperoxides break down to an array of nonvolatile and volatile secondary oxidation products. As a result of this breakdown, new radicals are formed ($^{\bullet}OH$, $LO^{\bullet}$, and $LOO^{\bullet}$) that can reinitiate and branch the autoxidation reactions. $LO^{\bullet}$ can also be cleaved in a β-scission reaction into various nonvolatile and volatile secondary oxidation products. Peroxyl radicals ($LOO^{\bullet}$) of fatty acids or esters that contain three or more methylene-interrupted double bonds can be oxidized by cyclization into hydroperoxy epidioxides and bicycloendoperoxides, which then may be broken down into malondialdehyde.

Due to the variety of lipid hydroperoxides that can be formed during autoxidation and the type of oxidative cleavage of double bonds, the mixture of volatile secondary oxidation products formed after the breakdown of hydroperoxides is very complex. Volatile decomposition products from long-chain $n-3$ PUFAs from marine organisms have extremely low flavor threshold values and have readily detectable odors at very low levels of oxidation. During homolytic β-cleavage of the peroxides, aldehydes are left on the triacylglycerol or phospholipid molecule, and these aldehydes are called core aldehydes.

Tertiary Oxidation Products

In the final termination stage, relatively unreactive tertiary oxidation products are formed, including hydrocarbons, aldehydes, and ketones. However, unsaturated aldehydes and ketones may also undergo further oxidation forming volatile oxidation products. Additionally, hydroperoxy radicals formed during the propagation stage may react with triacylglycerols forming dimeric and trimeric triacylglycerols linked via peroxidic-type bonds.

Factors Influencing the Oxidation Rate and Mechanism

As the degree of unsaturation of fatty acid increases, the susceptibility to oxidation of fatty increases (Table 1).

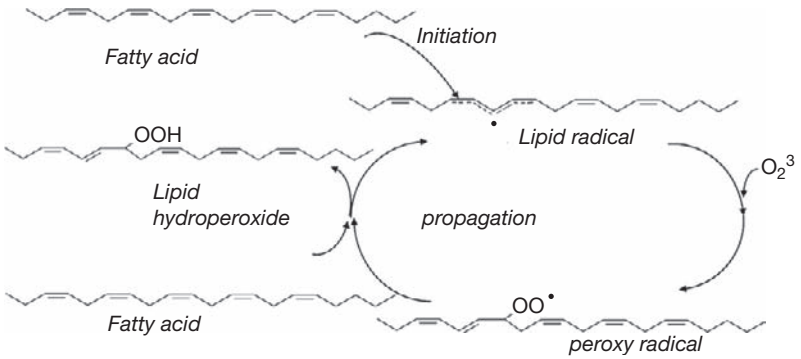

Figure 1 Autoxidation of fatty acids. Reproduced from Frankel, E. N. (2005). *Lipid oxidation*. Bridgewater: Oily Press.

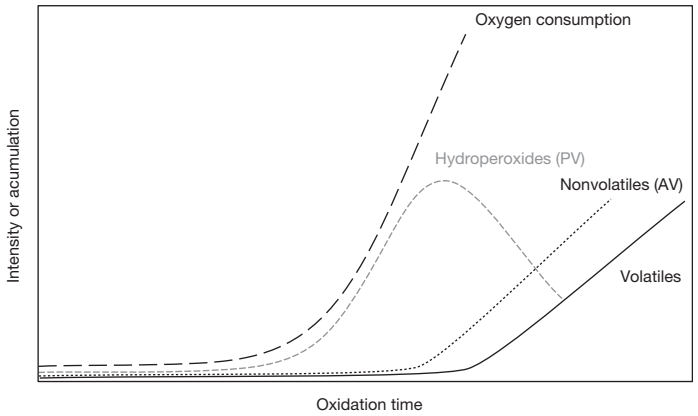

Figure 2 The kinetic curve of autoxidation of polyunsaturated fatty acids. PV, peroxide value; AV, anisidine value. Adapted from Kamal-Eldin, A. (2003). *Lipid oxidation pathways*. Champaign, IL: AOCS Press.

Table 1 Relative oxidation rates of unsaturated fatty acids subjected to photooxidation and autoxidation

	Degree of unsaturation		
Oxidation mechanism	*C18:1*	*C18:2*	*C18:3*
Autoxidation	1	27	77
Photooxidation	3×10^4	4×10^4	7×10^4

Oxygen

Oxidation of oils also increases with an increased amount of dissolved oxygen. The concentration of dissolved oxygen in the oil is dependent on the oxygen partial pressure in the headspace of the oil. Therefore, in order to stabilize unsaturated oils, oxygen should be omitted during processing and storage of unsaturated oils. Due to this, tall and narrow tanks are suggested for the storage of oils. Air-filled headspace in storage tanks should be held at a minimum, and all pipelines are recommended to be filled with inert gas.

Light

Photochemically induced oxidative changes are directly related to the light source, wavelength, light intensity, exposure time, and temperature. UV light was observed to be more harmful than visible light. Packaging of oils into colored (dark) glass containers or plastic vessels with UV absorbers efficiently reduces light-induced oxidation.

Temperature

Autoxidation of oils and decomposition of LOOH increase as the temperature increases. As a rule of thumb, autoxidation rates double for each increase in temperature by 10 °C. Therefore, variations in storage temperature will lead to significant reduction in shelf life.

Prooxidants and Antioxidants

Prooxidants

Prooxidants are compounds that initiate, facilitate, or accelerate lipid oxidation. Prooxidants are considered ubiquitous and are efficient in catalyzing lipid oxidation even at trace concentrations.

Transition Metals

Transition metals, such as low-molecular (free) iron and copper ions, are common catalysts of lipid oxidation. Iron is normally found in greater concentrations than copper, but copper is a stronger prooxidant than iron. Oxidizing metals ($M^{(n+1)+}$) decompose LOOH at rates several orders of magnitude slower than reducing metals (M^{n+}). Iron catalyzes lipid oxidation via the decomposition of LOOH into free radicals through redox cycling pathways. In the absence of oxygen, metal ions can still cause the breakdown of already formed hydroperoxides, facilitating the formation of secondary oxidation products (via β-scission), such as aldehydes.

Hemoproteins

Hemoproteins, that is, proteins containing a porphyrin structure with embedded iron atom, are known to catalyze lipid oxidation at much higher rates than low molecular weight iron. The most common heme compounds are derivatives of hemoglobin, myoglobin, catalase, and peroxidase. Hemoglobin and myoglobin are typically present in meat tissues and tissues rich in blood, such as organs.

In living tissues, hemoglobin exists in the reduced form (Fe^{2+}), either saturated with an oxygen molecule (oxyhemoglobin) or devoid of oxygen (deoxyhemoglobin). After death (postmortem), the concentration of oxy- and deoxyhemoglobin progressively decreases as it is converted (oxidized) to brown-colored methemoglobin (metHb) that does not have the ability to bind oxygen.

It is believed that intact porphyrin–Fe structure inside a pocket formed by surrounding proteins is an absolute requirement for heme-catalyzed lipid oxidation and that hypervalent iron complexes – mainly ferryl iron complexes (Fe^{4+}=O and Fe^{4+}(OH)) – are responsible for the rapid catalysis. The basic reaction mechanism involves binding of preformed LOOH to Fe^{3+}-heme, which generates the ferryl iron complex in a very fast reaction ($K \sim 10^9\ M^{-1}\ s^{-1}$). The LOOH is then decomposed either heterolytically or homolytically inside the heme pocket, producing lipid alcohols (R–OH) or $LO^{\bullet}$, respectively. The resulting ferryl iron complex is an extremely strong oxidant and rapidly abstracts H from either a new LOOH or the fatty acid directly, which generates lipid radicals for autoxidation reactions. The hypervalent iron complexes can be maintained by electron transfers, thus keeping their oxidizing power. Eventually, they are reduced back to Fe^{3+}-hemes.

The composition and arrangements of amino acids in the heme pocket, as well as heme pocket size and orientation, affect lipid binding and proton abstraction, while the protein structure and ligands influence electron transfer processes and stabilization of the ferryl iron complex. The reaction environment influences whether the LOOH cleavage is homolytic or heterolytic. Therefore, variable catalytic activity between different heme compounds and the same heme compounds from different animals has been observed.

Antioxidants

Antioxidants are compounds that prevent or delay lipid peroxidation. Antioxidants are usually classified into primary and secondary.

Primary antioxidants act as free radical scavengers via their ability to donate electron/hydrogen to peroxyl or alkoxy radicals, preventing them from reacting with a new fatty acid, thereby blocking the branching reactions in autoxidation. Tocopherols (vitamin E), ascorbic acid (vitamin C), and carotenoids (vitamin A) are common antioxidants in plants. Plant extracts are rich sources of natural antioxidants that contain compounds with multiple OH groups (phenolic acids, flavonoids, and anthocyanins), which act as hydrogen donors. Examples of these are rosemary extract where the active compounds are carnosol, carnosic acid, and rosmarinic acid or flavonoids found in green tea (catechins), red wine, seeds, and spices.

Secondary antioxidants act by a number of different mechanisms including metal chelation and scavenging of singlet oxygen and ROS. Examples are ethylene-diamine-tetraacetic

acid and citric acid, which complex iron and copper ions and thereby prevent metal-catalyzed lipid oxidation. Carotenoids absorb energy from singlet oxygen, transferring it to triplet oxygen, without changing their chemical structure (quenching) and therefore protect oil against light-induced oxidation.

Antioxidants assist to maintain low oxidative status of oils when added after an effective refining process, when most oxidation products and prooxidants have been removed from the oil. If the oxidation process in the oil is already started, addition of an antioxidant may only to some extent slow down oxidation or even have no effect.

Oxidation of Proteins

Oxidation of food proteins has received less attention than oxidation of lipids and the studies of oxidation of proteins have mainly been focused on the role of protein oxidation in age-related diseases.

The discovery that myofibrillar proteins in beef were oxidized led to many studies of protein oxidation in muscle foods. Early studies on protein oxidation in food showed that oxidation of food proteins could lead to significant losses in the functionality of proteins. Oxidation may lead to changes in both the primary and the secondary structures of proteins. Protein oxidation may lead to loss of solubility, changes in surface hydrophobicity, and changes in texture and affect water binding and also how susceptible proteins are toward proteolytic degradation. It has also been shown that some of the changes occurring during frozen storage of fish can be explained by protein oxidation.

The reactions between proteins and radicals in the presence of oxygen lead to changes both in the amino acid side chains and in the backbone of the proteins. The amino acids that are most often affected are cysteine, tyrosine, lysine, arginine, and histidine. The amino acids may be oxidized both as free amino acids and as part of the protein backbone.

The oxidation of proteins is believed to occur via a free radical chain reaction that is similar to the mechanism of lipid oxidation. However, there is an even higher complexity of pathways and therefore also a larger variation in reaction products. Reaction between a protein and a ROS leads to the formation of protein-centered radicals (called $P^{\bullet}$). In the presence of oxygen, this is converted to a peroxyl radical ($POO^{\bullet}$), and by abstracting a hydrogen atom from another molecule, it is converted to an alkyl peroxide (POOH). Further reactions can lead to the formation of alkoxy radicals ($PO^{\bullet}$) and hydroxy compounds. Protein radicals may react with other macromolecules in food like DNA, starch, lipids, and other proteins.

The thiol group in cysteine is highly susceptible to oxidation, and this may lead to the formation of sulfenic acid, sulfinic acid, and disulfide bonds. Oxidation of tyrosine may lead to the formation of dityrosine cross-linkages. The formation of disulfide and dityrosine cross-linkages may lead to both textural changes and reduced solubility. Oxidation of lysine, arginine, and histidine may lead to the formation of carbonyl groups. In addition to changes in the amino acid side chains, protein oxidation may also lead to breakage of the protein backbone.

Oxidation may change hydrophobicity, conformation, solubility, and susceptibility toward digestive enzymes – the latter may reduce nutritional value.

Some proteins are more susceptible toward oxidation than others and studies have shown that large proteins are more prone to oxidation than proteins with lower molecular weight.

There are several methods to determine protein oxidation, by detection of carbonyl groups, determination of changes in sulfhydryl groups, and formation of dityrosine. In addition, advanced methods like electron spin resonance, fluorescence spectroscopy, and high pressure liquid chromatography coupled with fluorescence detection as well as mass spectrometry have been used to increase the understanding of the mechanisms of protein oxidation.

As already mentioned, protein oxidation affects food properties, both texture and water-holding capacity. It has also been found that protein oxidation affected the gel strength of transglutaminase (TG)-mediated restructured meat. The combination of oxidation and high-salt content increased the TG-mediated cross-linking of myofibrillar proteins, probably because TG had increased accessibility to glutamine and lysine residues. A study on pork meat showed that mild oxidation of myofibrillar proteins improved gelling properties probably due to the formation of disulfide bonds; however, a negative effect was found on the water-holding capacity of the gels. Oxidizing agents are also used to improve baking properties of wheat (gluten) due to the importance of disulfide interchange for dough strength. Other studies have, however, shown negative effects of protein oxidation on the gel-forming properties of proteins showing that more studies are needed.

Several enzyme systems, the main ones are the calpains and the cathepsins, influence the tenderness of muscle foods. The only endogenous meat enzyme system that has been evaluated with regard to oxidation in meat is the calpain. The decrease in tenderness due to protein oxidation has been ascribed to either inactivation of μ-calpain or cross-linking of myosin resulting in strengthening of the myofibrillar structure.

Studies of lipid and protein oxidation in rainbow trout have shown that protein and lipid oxidation followed the same trend and that protein carbonyls developed together with the formation of LOOH indicating that these two processes occur simultaneously.

Protein oxidation has been studied during different food processing operations. For salted herring, cross-linking of myosin was found to be responsible for changes in texture, and an increase in protein carbonyls with increased ripening period was also found.

Storage of processed food such as pâtés and cooked sausages was also found to result in increased hardness; this has been ascribed to onset of protein oxidation, especially to the formation of cross-linkages between myofibrillar proteins. A relationship between protein carbonyls and instrumental hardness of sausages and packaging of beef in high-oxygen atmosphere has been found to have a negative effect on meat tenderness.

In rainbow trout stored at different storage temperatures, no protein oxidation was detected while storage at −20 °C resulted in the formation of protein carbonyls.

Controlling Protein Oxidation

The use of antioxidants – for instance, as part of the feed of the animals – has been found to increase oxidative stability of the food products. Reducing the PUFA/FA in animal tissues and supplementing the feed with antioxidants have also been

suggested as strategies to control protein oxidation. Different results have been found with regard to the effect of modification of the fatty acid composition in the muscle; some studies show only minor effect while the results of other studies indicate positive correlations between the ratio between PUFA and tocopherol as antioxidant. Therefore, controlling the protein oxidation by managing the feed composition seems to be a promising possibility. In a study on chilled mackerel, catechin was found to inhibit both lipid oxidation and protein oxidation; however, the antioxidative effect varied for the different myofibrillar proteins. It is also important to bear in mind that protein oxidation may lead to desired changes in food and might enable the production of foods with new and desirable textural properties.

Several recent studies on protein oxidation in food have led to increased understanding of both the mechanisms and the effect of protein oxidation on food quality. However, the existing methods for detection of protein oxidation are not sufficiently sensitive. There is therefore a need to develop new and sensitive methods to characterize the oxidation products generated during oxidation of proteins in food.

See also: Antioxidants: Characterization and Analysis; Fish Oils: Production and Properties; Fish: Processing; Phospholipids: Properties and Occurrence; Proteins: Chemistry, Characterization, and Quality; Vegetable Oils: Oil Production and Processing.

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Packaging: Aseptic Filling

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Introduction

Packaging plays an important role in the food processing industry to make the packed foods more convenient and to give the food greater safety assurance from microorganisms and biological and chemical changes such that the packed foods can have longer shelf lives. The demands of high-quality aseptic prolonged shelf-stable and safe foods have created huge development of aseptic processing and packaging (APP) systems.

APP of food products is one of the important processing operations in the food processing industry for producing and packaging the food products that can be rendered safe with an assured quality and extended shelf life. In general terms, APP is defined as the sterilization of the packaging materials and filling and hermetic sealing of commercially sterile products in an aseptic environment, which can prevent reinfection. For products expected with a long shelf life at room temperature, the package must also give complete protection against light and atmospheric oxygen. An APP system generally consists of sterilizer for the product, product filling assembly with ancillary piping and filler bowl, sterile gas lines and filters, aseptic packaging material sterilization zones to sterilize laminates of packaging materials/containers and lid materials, and a sterile zone where containers are filled and sealed. An overpressure of sterile air or inert gas is maintained in the aseptic zone to facilitate product removal and to deny any ingress from the environment. The term 'aseptic' is implemented when the unwanted organisms are either absent or excluded from the food product, packaging material, and product filling and sealing zone or other specific areas, while the term 'hermetic' signifies the suitability and compatibility of the packaging material to avoid the entry of microorganisms into the food package more specifically and/or prevent the passage of gas or vapor into or from the container, and these properties are totally dependent upon the mechanical properties of the packaging material. The most important example of an aseptic food is ultrahigh-temperature (UHT) processed milk, which undergoes a heat treatment at 135–150 °C for a varying holding period of 2–6 s, which changes per the type of food product. After sterilization, the food products are delivered through aseptic pipes to the product filling and sealing zone where the product is filled into presterilized containers under aseptic conditions, which assures a shelf life of about 3–6 months even if it is stored/distributed under ambient conditions. The types of food products that are processed by UHT treatment followed by aseptic filling include milk and milk products, drinks, concentrates, wines, teas, mineral water, nutritional beverages, sauces, fruit and vegetable juices, soups, tomato products, products with particulates, and ready-to-eat breakfast. The Tetra Pak system is the most widely used among the aseptic packaging systems, and the brick-shaped cartons fabricated from paperboard/polyethylene/aluminum foil lamination dominate the market.

The packaging materials used for filling perishable food products are normally produced under hygienic, but nonsterile, conditions. Contaminating microorganisms present in food packaging materials and equipment surfaces cause spoilage of aseptically packed food products. Therefore, sterilization of aseptic food packaging materials, food contact surfaces, and the environment directly surrounding these materials is of paramount importance. Sterilizing agents such as chemicals, heat, irradiation, or combinations of these will ensure the microbiological safety, stability, and quality of food over time. The agents to be used must destroy relevant microorganisms to the required level in the shortest possible time, be compatible with the food packaging materials and food contact surfaces, be easy to apply in the aseptic system, be inexpensive, be easy to remove from the treated surface, and, if residues are left, be within the accepted tolerance level.

Types of Aseptic Packaging Material

Commercial manufacturers of aseptic packaging include Tetra Pak, Scholle, and the Dole aseptic canning system (Table 1).

The types of aseptic packaging used for packing food products are discussed as follows:

- *Carton Boxes*: they are used to fill products under sterile conditions, which contain particulates. The carton boxes dominate among the packaging materials and are generally used to pack white milk and low- and high-acid food products such as fruit juices. Carton boxes are generally made of six layers that give an effective shelf life to the food products when packed aseptically.
- *Bags and Pouches*: pillow pouches are usually used for the packaging of milk, which is sealed from three sides, and it is also possible to pack products with particulates of 12 μ size.

Table 1 Aseptic packaging systems

System	*Package*	*Sterilizing agent*
Asepak	Bags	Heat
ASTEC	Bins and tanks	Pressurized steam
CKD	Cups	H_2O_2
Combibloc	Cartons	H_2O_2 + heat
Dole aseptic canning system	Steel/aluminum cans and lids	Superheated steam
DuPont Canada	Bags, pouches	H_2O_2
ERCA	Cups	H_2O_2 + heat
Evergreen	Cartons	H_2O_2 + heat
Gasti	Cups	High-pressure steam
Gaulin	Bags	Ethylene oxide
Hamba Manufacturing	Cups	Ultraviolet rays
Hassia	Cups	H_2O_2 + heat or pressurized steam
Ingko	Bags	Chlorine solution + heat
Inpaco	Pouches	H_2O_2 + heat
International Paper Co.	Rectangular packages	H_2O_2 + heat
Lieffeld & Lemke	Cups	H_2O_2 + heat
Liqui-Box Corp.	Bags	Gamma radiation
Mancini	Bags	Gamma radiation
Mead Packaging Co.	Cups	Citric acid + heat
Metal-Box Freshfill (Autoprod)	Cups	H_2O_2 + heat
Pure Pak Inc.	Cartons	H_2O_2 + heat, oxonia
Purity Packaging Co.	Cups	H_2O_2
Remy	Cups	H_2O_2 + heat
Remy	Bottles	H_2O_2 or oxonia
Scholle Corporation	Bags	Gamma radiation or ethylene oxide
Serac	Bottles	H_2O_2
Tetra Pak Inc.	Cartons	H_2O_2
Wright Sel	Bags	Gamma radiation or ethylene oxide

- *Cups and Trays*: these are used either premade or formed, filled, and sealed in thermoform/fill/seal machines. Usually, polypropylene-based multilayer materials with ethylene vinyl alcohol (EVOH) barrier are applied for this purpose.
- *Bottles and Jars*: glass bottles may be aseptically filled with food containing small particles, for instance, baby food. In recent developments, Tetra Top bottles, which combine the convenience of bottle and all the characteristics of carton packaging, can now be employed for packing thicker products for easy pourability. For the packaging of milk, a bottle made in the form of carton is made available in the form of Tetra Evero.
- *Metal Cans*: the Dole system is used for packing food products into cans made from steel/aluminum using aseptic filling. The existing slit filler, however, limits applications to liquids with very small particles, such as rice.
- *Plastic Cans*: an aseptic machine for filling and closing of two-piece plastic cans, 'gourmetans,' was recently developed. Cans and lids with easy opening features consist of polypropylene (PP)/EVOH/PP.
- *Composite Cans*: they are generally used to pack products and fruit juices containing fibers and not the particulates.
- *Retortable Carton*: an innovative packaging system is developed as Tetra Recart, which has utilized carton packaging for packing products like vegetables, tomatoes, soups, and sauces, which can stay fresh for about 24 months.
- *Bag-in-Box*: it is best suited for liquid and paste-like products for bulk packaging. In addition to the all conventional requirements, the bag-in-box aseptic system must have a flexible primary packaging, as rigid primary containers cannot guarantee sterility. It can be used for the packing of high- and low-acid products like fruit juices, concentrates, purees, tomato products, and milk and cream.

Factors that are considered vital in the case of the packaging material, which affect the shelf life of the products, are the strength of the seal, integrity of the packaging material, package shape, stiffness, and durability of the packaging material and barrier properties. Recent demands and the changing nature of consumers have driven the packaging material manufacturers to consider the special need of plastics, different types of closures and packs, environmental impact, raw material for packaging material, new product applications, and even alternatives for aseptic packaging.

Filler Sterilization

Aseptic fillers have sections containing sterile contact pipes and valves along with noncontact sections (sterile chambers) that need to be sterilized prior to production and must be able to maintain sterility. Aseptic fillers and associated pipes are usually sterilized with heat in the form of steam, and usage of saturated steam is the most dependable sterilant, as microorganisms are more resistant to dry heat, which necessitates higher temperatures.

Once sterilized, the product is transported under aseptic conditions to the aseptic filling equipment, and reinfection is avoided. The cleaning of aseptic transfer equipment is done by cleaning-in-place (CIP) systems, and its effectiveness depends upon the kind and concentration of detergent, time of circulation, and flow rate. Reinfection is possible only by leakage in the system, so attention is required to be paid on gaskets, membranes, cracks, and pinholes. Some aseptic filling plants use steam barriers in the aseptic transfer system, particularly so if a sterile tank is part of the transfer line and the steam barriers are controlled by thermosensors and/or inspection by the machine operator.

The packaging material is supplied in the form of reels that are then loaded in a magazine at the rear part of the Tetra Brik machine. The packaging material web (Figure 1) travels from the magazine, where a longitudinal seal strip is applied to one edge of the packaging material web by using hot air.

The longitudinal seal strip has the task to reinforce the longitudinal seal and to prevent the product from coming into contact with the paper layer of the packaging material. The packaging material web then passes through the hydrogen peroxide bath (30% concentration) at a temperature of 70 °C for about 7–8 s for effective sterilization. Excess H_2O_2 is removed by means of a pair of pressure rollers, which is followed by 'air knives.' Air knives are the narrow slits through

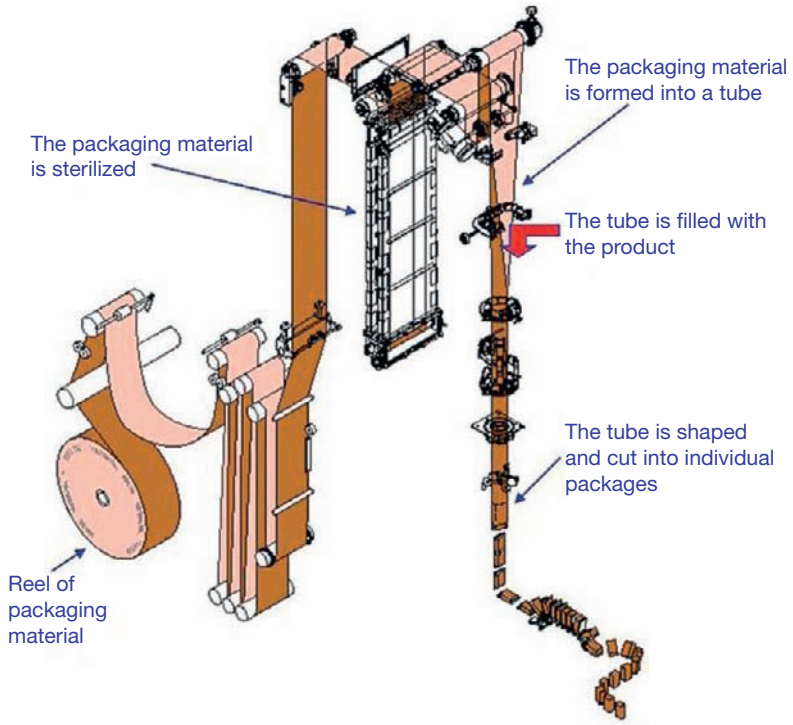

Figure 1 Packaging material web path in aseptic packaging machine.

which hot sterile air is blown, which dries the surface. The sterile packaging material first travels down in the aseptic chamber on the front side of the Tetra Brik machine. By means of forming rings, a tube is formed, which is longitudinally sealed, using hot sterile air or induction heating, and the product is supplied by means of a filling pipe inserted in the tube of the packaging material. In order to minimize foaming and splashing, the filling pipe extends below the product level, and a floating valve, or a regulating valve, helps to keep the product at a constant level. Two pairs of sealing and pressure jaws constantly pull the packaging material through the machine. The first step in the transversal sealing operation is the closing of the jaws around the cylindrical packaging material tube. The tube simultaneously acquires a rectangular shape by way of the volume flaps placed on the jaws. The transversal seals are performed below the product level to ensure a completely filled package, and the inner plastic coating (polyethylene) is heated up by induction heating. When the transversal seal has been executed, a knife releases each package from the tube. The package is transported to the final folder where the ears of the package are sealed with hot air at both the top and bottom.

Required Log Cycle Reduction

The sterilization process employed should be established in terms of the number of log cycle reductions for the most resistant organisms present on the food contact surfaces. Factors like the type of the food product, storage temperature, and expected shelf life decide the required log cycle reduction. For nonsterile acidic products of pH <4.5 and for sterile, neutral, and low-acid products of pH >4.5, a minimum of four- and six-log cycle reductions of bacterial spores is required, respectively. Commercial requirements for effective sterilization demand less than one faulty package out of 10 000 produced. Factors that affect commercial sterility are the efficiency of the sterilizer for food product and packaging material, initial microbial load of the raw material and packaging material, procedures of cleaning and sterilization of the plant and filling section, and service and maintenance of the plant and operational care. Another factor that is used in determining the appropriate value for container sterilization is container size. Smaller containers with a smaller food contact surface area will have correspondingly less initial contamination, and hence, a less severe sterilization process will be needed and vice versa. Calculations of minimum sterilization effects necessary are often based on the assumption that all microorganisms on food contact surfaces are bacterial spores.

However, even though the organism reduction rate of a system can be calculated with some confidence and precision, this cannot be used to give a precise measure of the proportion of nonsterile packs that will be produced by the systems. There are a number of reasons for this: (a) the proportion of nonsterile packs will vary according to pack size, that is, the larger the pack, the higher the proportion that will be nonsterile at a given rate of organism reduction, (b) the condition of the packaging material before sterilization will also affect the incidence of nonsterile packs, and (c) the failure of the sterilization process to kill the bacteria is not the only, or even the most important, cause of pack nonsterility. Other causes that may cause to produce nonsterile packs are physical defects in the packs, particularly in seals and seams, and technical failure in the aseptic packaging system.

Defective packages are generally classified as damaged caused by operation of the aseptic packaging machine, maintenance of the equipment, faulty packaging material, internal transportation, outer wrapping equipment, handling during storage, or no package defect detectable.

Packaging Materials and Food Contact Surface Sterilization Processes

The packaging material sterilization is dependent upon the type and concentration of the chemical used, contact time, and temperature during contact. The important sterilization processes for food packaging materials and food contact surfaces are as follows:

Chemical Sterilizing

Chemical sterilization is extensively practiced for sterilizing the food contact surfaces. In order to deliver the packaging material and contact surface commercially sterile, chemicals are used to kill bacterial forms (vegetative cells and spores), yeast, and molds. The chemical sterilizing agents that have been used for sterilizing packaging materials and food contact surfaces include the following:

- *Hydrogen peroxide*: Hydrogen peroxide (H_2O_2) is the most widely used chemical agent for the decontamination of food packaging materials and contact surfaces before aseptic filling and subsequently dried with hot sterile air (Figure 2).

The oxidative potential of hydrogen peroxide is the principle behind the antimicrobial effect. The lethal effect of H_2O_2 and heat for the sterilization of the surface of paperboard laminate cartons was used by Tetra Pak in 1961. The lethality of H_2O_2 is relatively low at room temperature but can be increased considerably by raising either the temperature to 85–90 °C or concentration. Most aseptic packaging systems use H_2O_2 at concentrations from 30% to 35%, followed by hot air (60–125 °C) to augment the sterilizing effect and to dissipate residual H_2O_2. The bactericidal effectiveness of H_2O_2 is influenced by the temperature, duration of treatment, pH, concentration, quantity applied to the packaging material per unit area, intensity of the radiant or irradiant heat or the temperature and quantity of the drying air, microbial load, and species of organisms present. Sterilizing effectiveness of H_2O_2 is usually assessed against spores of either *B. subtilis* or *B. subtilis* var. *globigii*. The action mechanism of hydrogen peroxide is credited to the formation of either oxygen or hydroxyl radicals. The lethal effect can be enhanced by heat, which also reduces the exposure time necessary for the lethal effect. Under commercial conditions, since the rapid rate of spore destruction is required to obtain the commercial sterility of the packaging material, several aseptic filling and packaging systems utilize hydrogen peroxide in conjunction with heat and/or other agents to sterilize the packaging material. Since regulation requires residuals of less than or equal to 0.1 ppm H_2O_2, hot sterile air is commonly used to dissipate hydrogen peroxide. Per US Food and Drug Administration, the concentration of H_2O_2 must not be >500 ppb at the time of filling and must be reduced to $\sim$1 ppb within 24 h for the packaging material used to pack the food products.

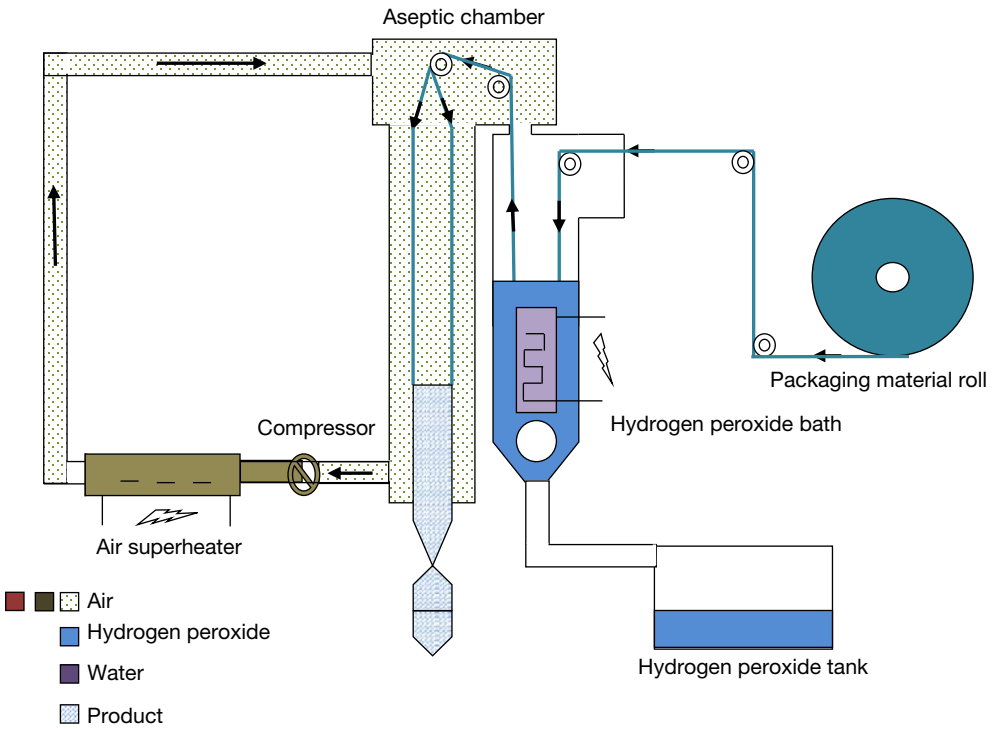

Figure 2 Sterile system of aseptic packaging machine using hydrogen peroxide.

There are three commercial methods for applying hydrogen peroxide to packaging materials: dipping, spraying, and rinsing.

- *Dipping*: It is universally used for the sterilization of food packaging materials when the packaging material is in the form of roll stock, and then the packaging material is immersed in a bath of 30–35% H_2O_2 solution containing a wetting agent to ensure uniform wetting of plastic surfaces, followed by heating to vaporize the peroxide before the filling operation. A large amount of the sterilizing liquid is removed mechanically, for example, by rollers or air blasts, and the remainder is generally removed by drying with hot sterile air or radiant heat. To increase the efficacy, especially in the case of dusty or slightly soiled materials, prior treatment of the material with rotating brushes or sterile compressed air jets may be used.
- *Spraying*: It is normally used for preformed containers in which sterilization is carried out by spraying or atomizing H_2O_2 into the containers. In this method, a measured amount of H_2O_2 solution (35%) is applied into each container through a nozzle to ensure that a uniform film coats the inside surface of the package. The peroxide is then dried using hot sterile air. The death rate is dependent on the volume of sprayed H_2O_2 (larger volumes require longer drying time) and the temperature of the hot sterile air. The new trend is to use a mixture of hot air (130 °C) and vaporized peroxide. Sterility of the aseptic zone during production is maintained by an overpressure of sterile air.
- *Rinsing*: It is used for prefabricated containers of an intricate shape, which are rinsed with an aqueous 30–33% H_2O_2 solution or a mixture of peroxide and peracetic acid (PAA). After spraying, the container is drained and then completely dried by hot sterile air and is used to sterilize glass containers, metal cans, and blow-molded plastic bottles. Live steam and disinfectant are used for presterilizing the filling station, piping, valves, and others into the filling chamber, followed by drying with hot sterile air, which is then maintained by an overpressure of a laminar sterile air flow.

- *Vapor-phase hydrogen peroxide (VPHP)*: The potential use of hydrogen peroxide vapor is to decontaminate surfaces that are hard to reach and complex surfaces giving good surface coverage of the material, and it is used as aqueous solution (30% w/w) that is evaporated into the heated carrier gas stream to produce hydrogen peroxide concentrations in excess of 4.5 mg l^{-1}. *Geobacillus stearothermophilus* and *Bacillus subtilis* are commonly used indicator organisms to verify the efficacy of VPHP sterilization. A 6-log cycle reduction can be induced within 1.2 min for the spores of *Bacillus subtilis* var. *niger* by using air saturated with H_2O_2 having a concentration of 7.6 mg l^{-1} at 70 °C. Sterilization of preformed containers by hydrogen peroxide vapor is cost-effective, as fewer amounts are adsorbed on the treated surface from the vapor phase. Flushing the vapor-treated surface with low-temperature sterile air free of H_2O_2 vapors can effectively eliminate residues, which can be used effectively for the in-line sterilization of aseptic packaging materials.
- *Corona discharge with hydrogen peroxide*: The sterilization effect can be achieved by using pulsed corona discharge in combination with H_2O_2 at atmospheric pressure. Positive ions and fine droplets (10–100 nm) of H_2O_2 are useful for sterilization, and bioindicator for estimating the sterilization effect is *E. coli*, and it is usually more than 100 times as compared with H_2O_2 alone. For high-speed sterilization of aseptic packaging materials, a combination of H_2O_2 droplets (30 wt%) and corona discharge is found to be very effective, with an added advantage of time reduction for soaking.
- *Food acids*: The shelf life of food products can be increased by inactivating the vegetative cells, spores, yeast, and molds that can grow at room temperature. PAA is a peroxide of acetic acid and, on decomposition, forms acetic acid and water, which is effective against spores of aerobic and anaerobic bacteria. PAA at a concentration of 0.1% is more effective than 23.6% (H_2O_2 solution) at room temperature. At 20–45 °C with a concentration of 0.5–1%, PAA can reduce the number of microbes on polyvinyl chloride (PVC) or polyethylene terephthalate (PET) bottles by 5–6 logs within 30 min. Synergistically, H_2O_2 (5.9%) and PAA (0.2%) can achieve a 4-log cycle reduction of the *B. subtilis* spore within 1.4 min at 20 °C. PAA can be used as a sporicidal agent in treating surfaces of both aseptic filling machine and packaging materials such as PET.
- *Ozone*: Ozone is a feasible alternative to H_2O_2 due to its wide range of antimicrobial properties against a wide spectrum of bacteria, fungi, viruses, protozoa, and bacterial and fungal spores. An added advantage of using ozone is that it does not leave any residues after its decomposition. Microbial cell inactivation is achieved by attacking the cell membrane and wall constituents (e.g., unsaturated fats), which ultimately leads to increased cell permeability of microbial cells and eventual death. Ozone can disinfect food contact surfaces, and its effectiveness can be evaluated by using spores of *B. stearothermophilus* as indicators. The antimicrobial property of ozone increases with a decrease in temperature; even at lower concentrations, a large amount of microbial populations can be killed within a short span of time. In recent years, the introduction of ozonation equipment for surface cleaning and disinfection in the food industry has made significant advances, and it has proved to be an effective sanitizer.

Physical Sterilizing Agents

Sterilization by chemical agents is a tedious method, and on several occasions, unacceptable amounts of residue on the packaging material may interact with polymers or the components of packaged food coming in contact with the surface of the packaging material. Hence, physical agents as alternative sterilization for the decontamination of packaging materials and food contact surfaces may be adopted, and some of them are discussed as follows:

- *Thermal sterilization*: Thermal sterilization processes generally use either moist heat (steam) or dry heat (hot air) as a sterilizing medium. Microorganisms, especially spores, show greater thermal resistance when exposed to dry heat than moist heat. The effectiveness of sterilization at 121 °C for 20 min using steam is equivalent to dry heat

sterilization at 170 °C for 60 min, which signifies that steam is much more efficient. Dry heat produces microbial death as a result of dehydration, followed by protein oxidation, while death by moist heat is caused by denaturation and coagulation of essential cell proteins.

- *Saturated steam*: Sterilization of metal cans and lids by saturated steam under pressure was used as early as 1920 in the United States, and saturated steam is also used for sterilizing thermostable plastic cups. A reduction by 5.5–7 log cycles can be achieved in bacterial spores by using a saturated steam at a temperature of 147 °C and 3.5 bar pressure with a contact time of 4 s. To attain adequate destruction in high-speed packaging units, the surface temperature of the material must reach 135 °C, and it is also necessary to select an appropriate steam sterilization cycle. Factors like microorganisms present (type and number), food package (type and size), type of packaging material, and also those that can influence the performance must be considered before selecting a steam sterilization cycle. Moist heat when used as a sterilant, on many occasions, can cause blistering or delamination of the laminates, which may directly affect the heat-sealing characteristics of plastics, causing leaky packets. Hence, saturated steam is a preferred method for sterilizing metal food contact surfaces downstream from the holding tube including sterile hold tank, homogenizers, fillers, and the aseptic packaging zone. Equipment sterilization can be attained by exposure of the surfaces to an appropriate time–temperature sequence (e.g., 30 min/121 °C) by superheated water, saturated steam, superheated steam, or other appropriate treatments prior to the start-up of the filling operation.
- *Superheated steam and hot air*: A major challenge of using superheated steam or hot air, instead of moist heat, as a sterilizing agent is the requirement of a higher time–temperature combination. To guarantee the sterilization when superheated steam or hot air is used, it is necessary to provide longer holding time and high temperatures, as the machines consist of relatively large masses of metal. Since the resistance of microorganisms to dry heat is much greater than to wet heat, much higher temperatures are needed for thermal inactivation by dry heat than for inactivation in a same time by wet heat. Spores of *Bacillus subtilis* are used as indicators in dry heat, and these spores are used commercially in sterility testing of aseptic fillers. The Martin–Dole process continuously sterilizes tinplate cans by passing through superheated steams (220–256 °C) at normal pressure for 45 s and a minimum time–temperature condition in superheated steam of 1 min at 177 °C or 10 min at 160 °C to give satisfactory container sterilization in an aseptic canner.

  Hot air is generally preferred as a sterilizing agent while sterilizing the paper-based packaging materials over superheated steam due to the drawbacks like delaminating, which are associated with superheated steam. Combi cans of cardboard laminates are sterilized using hot air at 145 °C for 3 min. Hot air at a temperature of 315 °C has also been used to sterilize paperboard laminate cartons and is suitable for products that have a pH < 4.6. *C. botulinum* is often used as an indicator for low-acid food products. Hot air sterilization units are commercially available and are used for the packaging of juices and beverages.

- *Heating by extrusion*: The coextrusion process is used to produce multilayer packaging materials for the aseptic packaging system by utilizing the heat required to form–fill–seal operation by thermoplastic resin. The high temperature attended by the resin during its forming assures the sterility of the packaging material. During the extrusion of plastic granules prior to the blow molding of plastic containers, temperatures of 80–230 °C are reached and maintained up to 3 min. Sterile air under a positive pressure maintains the sterility of the forming, filling, and sealing areas. Extrusion results in a three- to four-log cycle reduction in microbial spores, and therefore, extruded containers should only be aseptically filled with acidic products with a pH < 4.5, while for products with a pH > 4.5, the extruded containers must be further sterilized with H_2O_2/PAA. The heat of extrusion (230–240 °C) during the manufacturing/blow molding of plastic pouches/bottles is sufficient to guarantee its surface sterilization and can be affected by the initial microbial load of the granules and the environmental conditions surrounding the blow molding machine.

Irradiation

When neither heat nor chemicals can be used to sterilize a given material, irradiation is the best alternative and is used by manufacturers in Europe. Irradiation is used to sterilize bag-in-box (plastic or plastic–aluminum laminates), cups of cardboard or plastic, valves of metal or plastic, plastic or paper bags, cardboard, plastic tanks, rolls of plastic, aluminum, and coated cardboard. Irradiation involves the action of ionizing radiation or pulsed light (PL) and UV radiation and infrared rays to reduce the number of microorganisms. The types of ionizing radiation used are gamma rays, emitted from a radioisotope such as ^{60}Co or ^{137}Cs, or electron beams generated from an electron accelerator. The processing time for electron beam sterilization is generally shorter than that of gamma ray sterilization. As irradiation does not leave any residue on the treated surface nor affect the immediate environment, it appears to be a perfect solution for the sterilization of packaging materials.

Ionizing radiation: Ionizing radiation is becoming increasingly popular as the deep penetration properties of the gamma rays make it highly suitable for sterilizing the food packaging materials and the contact surfaces that cannot resist and get deformed or delaminated by the high temperatures of the sterilizing mediums. Gamma irradiation at a dosage of 25 kGy (2.5 Mrad) or more can assure the sterility of bag-in-box packaging materials in which acid food can be packed for bulk vending. The principle behind achieving the sterility is that when microorganisms are exposed, gamma rays disrupt the DNA structure of microorganisms present on the surface, which then eliminates the ability to reproduce life-sustaining cells. Besides gamma ray treatment, decontamination with accelerated electron beams has proved to be a safe and effective method for sterilization. Electron beams work by directing a

shower of accelerated electrons through a high-voltage emitter toward a target. The advantages of using the method are that it can be used at room temperature and also it reduces the usage of chemical agents. Implementing the ionizing radiations as a sterilant source imposes a challenge of high installation and uncertainty of the sterilization effect in the case of preformed containers. For commercial usage in decontaminating the food packaging materials by using ionizing radiations, factors like reduction in the cost of the equipment producing the radiations and the shielding of the radiations must be considered.

Pulsed Light

PL is used as a nonthermal technique for processing and increasing the shelf life of different food items, and it has also found its way in sterilizing food packaging materials and food contact surfaces. PL can be used to decontaminate surfaces by using very-short-time pulses of high-power broad-spectrum light and rich in UV-C light. The process is reported to be highly successful in killing bacteria, yeast, molds, viruses, and spores and in deactivating enzymes. The material to be sterilized can be exposed to at least 1 pulse of light (typically 1–20 flashes per second) having an energy density in the range of about 0.01–50 J cm^{-2} at the surface with a duration ranging from 1 μs to 0.1 s. Microbial inactivation by using PL occurs by several mechanisms such as photochemical and photothermal mechanisms, chemical modification and cleavage of DNA, protein denaturation, and alteration of cellular materials. With an increase in the light intensity and fluency, the lethal effect on spores is also increased. As compared with UV light (42 600 μW-s cm^{-2}), for achieving a sterilization effect of 99.999% for *B. subtilis*, PL only requires a dosage of 4500 μW-s cm^{-2}. The PL system can be deployed in the aseptic packaging machine that uses preformed containers like bottles, plastic jars, or paper packs for the packing of milk and juice products and where continuous sterilization of inner surfaces is required.

UV Irradiation

UV radiation is effective in inactivating vegetative bacteria but relatively ineffective in reducing bacterial spores, as a long exposure time is required to achieve desired sterility. Sterilization by UV irradiation is becoming increasingly prevalent, especially for packaging materials and machines that are less readily sterilized by H_2O_2. UV radiation promotes lipid oxidation in food; hence, safety issues need to be considered in designing sterilization processes. UV irradiation induces a mutation of DNA in microorganisms, which ultimately gives a germicidal effect. To accomplish four to six decimal reductions in microbial counts on a flat surface, a dosage of 250 mW cm^{-2} is required. The factors that assure the effectiveness of UV irradiation are smoothness of packaging materials and dust-free surfaces.

Combination of UV Irradiation with Hydrogen Peroxide and Heat

Sterilization of cartons can also be achieved by using H_2O_2 either alone or in combination with UV lamp. Spores are more resistant to UV, so to ensure an adequate sporicidal activity, a combination of H_2O_2 and UV radiation is used. The Liquid Pak system utilizes the synergistic effect for sterilizing the cartons to be used in the carton forming, filling, and closing system in a sterile chamber. The combination of H_2O_2 with UV can be used commercially for the sterilization of cartons during aseptic filling, as the UV irradiation promotes the breakdown of the peroxide into hydroxyl radicals, which results in the lethal DNA disruption of vegetative cells and resistant spores. The lethal effect of UV plus H_2O_2 treatment is affected by the concentration and temperature of H_2O_2, wavelength of UV radiation, and smoothness, reflectivity, and geometry of the surface being treated. The advantages of combined treatment include usage of a lower concentration (5%) of H_2O_2, minimum sterilization time, minimum H_2O_2 residue in products, chemical-free sterilization, and an alternative for high-temperature sterilization.

Control Points and Control Parameters for Effective Sterilization of Packaging Material

Control procedures and points are necessary to assure the repeatability and sterility of the packaging material. To ensure the reliability of the chemical to be used as sterilization medium, the concentration is measured either by the machine operator or by laboratory staff, usually only at the beginning of the packaging operation. The consumption of the sterilization agent must also be determined by the machine operator either at the end of the production run or, better, at regular intervals. The packaging area in which the packages are formed, filled, and sealed must be sterilized prior to the start of production and must be maintained during the entire production run. In this process stage, a number of control parameters are involved, which are essential for the safety and adequacy of the operation. Two positions can be selected when the packaging machine is prepared for cleaning, intermediate cleaning, or final cleaning. Intermediate cleaning can be used whenever the sterile product remains in the product line in which a steam barrier protects the sterile product from being contaminated by the cleaning solution and is to be done immediately after a stop, except when cleaning according to the final cleaning can be accomplished within 1 h. Final cleaning is used in order to clean all parts in contact with the product in the AP valve; cleaning of the steam barrier space must be done every day or as soon as production is finished.

See also: Bioavailability of Nutrients; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; Condensed Milk; Fermented Foods: Fermented Milks; Foodborne Pathogens; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Heat Treatment: Effect on Microbiological Changes and Shelf Life; Heat Treatment: Principles and Techniques; Milk: Processing of Milk; Pasteurization: Principles and Applications.

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Palm Oil

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Historical

There is fair agreement that the oil palm (*Elaeis guineensis* Jacq) originated in West Africa. It grows 10° north and south of the equator and its utilization as basic nourishment had always been of vital importance to the inhabitants of the equatorial regions in Africa; its existence is reported as long as 3000 BC, when palm oil was known to the Egyptians under Pharaoh's reign.

Africans probably brought the oil palm to Madagascar in the tenth century and slave traders introduced the oil palm to South America. The Dutch had a trading post in Ghana and the oil palms grown in the botanical gardens in Amsterdam may well have originated from Ghana. Two of the oil palm seedlings that were planted in 1848 in the botanical garden near the palace of the governor in Buitenzorg (now Bogor) on Java in the former Dutch East Indies came from Amsterdam. Two more came from Madagascar or the isle of Bourbon (now Réunion). These seedlings form the basis of the palm oil industry in Malaysia and Indonesia, their progeny having been widely distributed from 1853 onward.

The material bred from these palms is referred to as *Deli dura*. It is very stable and uniform in oil and kernel contents. The tree was freely distributed in Sumatra, and oil palm cultivation was introduced into Malaysia (then the British colony of Malaya) in 1910 by Scotsman William Sime and English banker Henry Darby.

The foundation of the palm oil industry is generally attributed to Adrien Hallet, a Belgian with some knowledge of the oil palm in Africa. In 1911, he planted palms of Deli origin in the first large commercial plantation in Sumatra. He also recognized that the avenue palms growing in Deli not only were more productive than palms in Africa but also had a fruit composition superior to the ordinary *dura* palms of the west coast. In the meantime, H. Fauconnier, who had been associated with Hallet, had established two palms of Deli origin at Rantau Panjang in Kuala Selangor in 1911 and 1912. These palms were in full bearing by 1917, and in that year, the first seedlings were planted on an area later to be known as Tennamaram estate. It was during this period that the *dura* palm and *pisifera* palm were crossed and that a hybrid progeny *tenera* was introduced to the oil palm industry.

A slow development of oil palm plantations occurred in Indonesia after World War I, followed by Malaya and Belgian Congo. By 1938, Indonesia had 90 000 ha and Malaya 30 000 ha under oil palm. After 1960, the area under oil palm increased rapidly in Malaysia, reaching 3.2 million ha by 2001. Developments in Indonesia were slower, with 2.4 million ha in 2001. These two countries are by far the largest producers of palm oil and the main suppliers to the world market. Plantation developments in Central and South America have reached about 500 000 ha in all, with Colombia, Ecuador, and Brazil having the largest areas. Total word area devoted to oil palm stands at 17 million ha at the end of 2013 with Indonesia on first place with 7 million ha, Malaysia second at 4.5, and Nigeria third at 2 million ha. As pointed out by the Malaysian Palm Oil Board, this accounts for just over 0.3% of the total world area used for agriculture and 6% of the area devoted to oil crops.

Botanical

E. guineensis is a monoecious, erect, one-stemmed palm tree, usually 20–30 m high, with an adventitious root system that forms a dense mat in the upper 35 cm of the soil with only a few roots penetrating deeper than 1 m. The stem is cylindrical and up to 75 cm in diameter and covered with petiole bases in young palms and smooth in older trees (>10–12 years old). Juvenile leaves are lanceolate and entire but gradually become pinnate; mature leaves spirally arranged, paripinnate, up to 7.5 m long; petioles 1–2 m long, spinescent, clasping the stem at base; and leaflets linear, 35–65 × 2–4 cm, up to 376 per leaf.

Inflorescences are unisexual, axillary, and pedunculate and until anthesis are enclosed in 2 fusiform or ovate spathes 10–30 cm long, with 3-merous flowers; the males have numerous cylindrical spikes forming an 15–25 cm long ovoid body and bearing flowers with 6 stamens, connate at base, with linear anthers, whereas the females are subglobose and 15–35 cm in diameter, with numerous lanceolate, spiny bracts, each subtending a cylindrical spikelet with 10–20 spirally arranged female flowers, each with two rudimentary male flowers. The stigma is sessile and 3-lobed. Fruits are ovoid–oblong drupes, 2–5 cm long, and tightly packed in large ovoid bunches with 1000–3000 fruits; drupes have a thin exocarp, an oleiferous mesocarp, and a lignified endocarp containing the kernel with embryo and solid endosperm.

The stem has a single growing point from which a leaf primordium develops about every second week. Succeeding primordia are separated by a divergence angle of 137.5° (Fibonacci angle), causing leaf bases to be arranged in various sets of spirals, of which a set of eight parastichies is normally obvious. Rate of leaf production is up to 40 per year in the first 2 years, dropping to a rate of 18–24 per year from year 8 onward. From leaf primordium to fully expanded leaf (2–10 m^2) takes about 2 years. The normal photosynthetically active life of a leaf is about 2 years, so under natural conditions, up to 50 leaves are present per palm. In plantations, this number is usually kept at about 40. In the first 2 years, lateral growth of the trunk dominates, giving a broad base up to 60 cm in diameter. After that, the trunk starts growing in height, 35–75 cm per year, reducing its diameter up to 40 cm.

The rate of height increment and rate of leaf production appear to be independent. All leaf bases contain inflorescence primordia, but the first fully developed inflorescence does not appear before leaf 20 and usually much later, some 3 years after germination. The length of male and female phases in

individual palms is very variable and irregular, but a population of palms shows clear seasonal trends.

The mean interval between sex differentiation and anthesis is around 20 months, and between anthesis of female flowers and fruit ripeness 4–5 months. Fruit ripening on the bunch proceeds simultaneously from top to bottom and from outer to inner fruits, and ripe fruits become detached. In well-managed plantations in Malaysia and Sumatra, on soils with a reasonable availability of nutrients and a good water-holding capacity under uniform and adequate rainfall, annual yields of bunches of 24–32 tons ha^{-1} are common.

Production of Palm Oil

The production of palm oil starts with a fresh fruit bunch (FFB). This is harvested in the plantation when it starts to drop loose palm fruits. So when these fruits are observed on the soil, a skilled worker uses a knife on a long stick to cut the FFB free from the palm tree so that it falls on the ground from a height that can be 25 m or more. The worker collects the loose fruits in a net, and a small lorry fitted with a crane that runs in between the palm trees collects the FFBs. At that time, the fruits have changed from almost black to a reddish orange.

The fruits are ripe some 14–17 weeks after pollination but pollination is a continuous affair. So bunches are ready to be harvested all the time. This means that each plantation parcel has to be visited every 6–8 days to detect the bunches that are ready for harvesting.

The small lorry may unload its FFBs onto a larger lorry that delivers the FFBs to the oil mill or rather 'palm oil separation plant' since the fruitlets are not milled like olive fruits are milled. Olives arriving at an olive oil mill are first washed and then sent to a hammer mill that crushes the stones in the fruits. When palm FFBs are processed, the kernels are left intact.

Large palm oil mills can have a capacity of 90 tons FFBs per hour. However, these FFBs can only be harvested and transported during daylight. This means that in practice, harvesting will commence at dawn and that the oil mill will be started up around noon, when the first FFBs arrive at the mill. The oil mill will continue processing until the last FFBs that have been delivered during that day have been processed and then shut down until fresh fruit bunches are delivered to the mill on the next day.

Accordingly, palm oil mills only operate about half of the time so that the 90 ton/hour plant will process some 300 000 tons of FFBs per year leading to a production of about 70 000 tons crude palm oil and 45 000 tons of palm kernel nuts. Assuming a yield of 30 tons FFBs per year this corresponds to a plantation of 10 000 ha or a piece of land of 10 km square requiring a fair number of lorries to transport the FFB to the oil mill.

Before and after the lorries have been unloaded, they pass over a weighbridge. This is especially important when the oil mill processes FFBs delivered by smallholders. At unloading, the FFBs are dumped into the intermediates storage that consists of an inclined hopper at the ramp that will hold 900 tons of FFBs (2 lines of 15 bays $\times$ 30 tons FFB).

This intermediate storage affects the crude palm oil quality because storing fruits causes the FFA of the oil to increase. The oil hydrolysis is enzyme catalyzed so it is unlikely that the FFA formed increases the rate of hydrolysis, but even so, care should be taken to minimize FFB storage and to maintain a first-in, first-out schedule.

The first process step in the palm oil mill is the sterilization of the FFBs. To this end, the bunches are loaded into cages that can be wheeled into an autoclave that can hold 3–10 cages of 1.5–7.0 tons per cage. After the autoclave has been closed, steam is introduced until a pressure of 2.5–3.0 bar has been reached. To expel air from the sterilizer, the pressure is released one or two times. Then, the pressure is held for 20–30 min or even longer and released so that the sterilizer cages can be forwarded to the thresher. This sudden release exerts a beneficial drying effect on the fruits. FFB sterilization is a fully automated batch process with a cycle time of 100–120 min including loading and unloading, thus permitting 12–14 batches per day. The patent literature and PIPOC conference proceedings describe several continuous sterilization processes but few of them have been realized industrially, if any.

The sterilization process leads to a condensate. The amount of steam is about 200 kg ton^{-1} FFB, some steam is flashed off, and some water is liberated from the FFB leading to a final liquid effluent of 150 kg ton^{-1} of FFB. This effluent contains some oil, equivalent to 0.5 kg ton^{-1} FFB.

During the sterilization process, the lipase enzyme is denatured, and at the same time, the fruits are loosened from the bunches and inside the fruits, proteins are coagulated so that the mesocarp structure is altered in a way that can be compared to the rendering of animal fats. After the sterilization process, the cages are hoisted and emptied in turn into the feed hopper of the thresher or bunch stripper. This is a slowly rotating horizontal drum fitted with bars that lift the fruit bunches and then drop them so that the loosened fruit separates from the bunches and is removed by a screw conveyor. The empty bunches leave the drum at the other side. However, they still contain some oil, which is estimated at 4.5 kg oil per ton FFB.

The screw conveyor feeds the fruits into a digester. This is a cylindrical vessel with a steam-heated jacket, where the oil and nuts are separated from the fruit pulp by gentle agitation by a paddle stirrer at a temperature of 90–100 °C for a period of 20 min. At the same time, cell walls in the fruit pulp are broken and oil droplets coalesce like during the malaxation step in olive oil production. The resulting mash is then fed to a continuous screw press in which the solid fraction consisting of fibers and nuts is separated from the liquid fraction consisting of fruit water and palm oil.

The separation is far from perfect in that the fiber/nuts fraction is wet and also contains an amount of oil that corresponds to 5.0 kg oil per ton FFB and that the liquid fraction contains some solids. This liquid fraction can be diluted with hot water such as the condensate of the sterilization process. Then, it can be screened to remove fibers and course contaminants before being pumped to a settling tank where the oil is collected as supernatant, clarified by centrifugal separators, and dried to a residual moisture content of $< 0.1\%$ in a vacuum dryer. At some stage, the oil is cooled to storage temperature of preferably 32–40 °C.

The solid fraction is dried and separated into nuts and fibers by a winnowing process in the depericarper. The fibers can be sent to the boiler house, or they can first be further dried so that the oil adhering to these fibers can be recovered by hexane

extraction and then sent to the boiler house. The nuts are cracked to recover the kernels from which the palm kernel oil is produced by screw pressing and solvent extraction.

Palm Oil Refining

Crude palm oil tends to have a low (5–20 ppm P) phosphatide content and a relatively high (>3%) FFA content. Accordingly, the most profitable way to refine this oil is by a combination of the dry degumming process that removes both phosphatides and color and physical refining. The latter process has the advantage that it almost completely avoids the entrainment of neutral oil, which is the case with soapstock.

In the dry degumming process, the oil is heated to 80–100 °C, mixed with 0.05–0.1% (w/w) phosphoric acid, and then mixed with up to 4% of bleaching earth. After a mixing period of 20–30 min, the spent earth is removed by filtration using pressure leaf filters. To minimize oil retention, the filters are blown with steam before being discharged.

When vegetable oils originating from oil seeds are bleached, the cost of bleaching can be reduced by mixing the spent earth with the oilseed meal and selling the earth at meal value. With palm oil, this is not possible so reducing bleaching earth usage is even more important for palm oil than for seed oils. Because the extent of color reduction in palm oil is quite large, the bleaching process can be made more efficient by using a countercurrent process such as the Öhmi-bleach process. In this process, oil is first of all bleached with spent earth, and afterward, the partially bleached oil is bleached with fresh earth to final bleached oil color. The spent earth resulting from this final bleaching step is then used in the prebleaching stage.

The physical refining of the bleached oil is invariably executed as a continuous process because then, the use of heat exchangers and thermosiphons allows a heat recovery of up to 90%. The vacuum stripping equipment can be fitted with a packed column to remove the bulk of the FFA with a minimal amount of steam by making it flow countercurrently through this column.

Because the distillate comprises the free fatty acids, its relative amount is much larger than in installations that deodorize neutral oil. The scrubber section of the equipment has to take this difference into account. Because the distillate can be sold at a higher price when its FFA content is high, a two-stage vapor scrubber/condensation system has been introduced. In a first, high-temperature scrubber, the least volatile compounds are condensed with a small fraction of the FFA. This fraction will contain the tocopherols and tocotrienols and can thus be sold as a tocol concentrate. In a second, low-temperature scrubber, the bulk of the FFA is condensed, thus yielding a valuable by-product that can be used in the oleochemical industry or as a raw material for biodiesel production. This second scrubber can also be one of two surface condensers operating alternately meaning that if one of these condensers is active as low-temperature condenser, the other is being heated to melt its surface condensate and to prepare it for taking over from the active one when that is 'full.'

A health aspect of palm oil refining that should be mentioned is the possibility of forming the potentially toxic fatty acid esters of 3-monochloropropanediol (3-MPCD) and glycidol (2-oxyranemethanol). Both 3-MPCD and glycidol are derivatives of glycerol, hence their occurrence in edible oils. Palm oil is more prone to their formation than other vegetable oils because the high FFA content of crude palm oil is accompanied by a relatively high diglyceride content. Using bleaching earth that has been activated with sulfuric acid rather than hydrochloric acid reduces/eliminates a source of chlorine and thus 3-MPCD formation. The use of naturally activated bleaching earth instead of acid-activated bleaching earth can also help. A slight reduction in deodorization temperature also helps and reduces the formation of *trans* isomers at the same time. Not that this is a serious matter for palm oil since it does not contain any linolenic acid, the fatty acid that is most prone to thermal isomerization to *trans* isomers, and only little linoleic acid.

The Composition of Palm Oil

Like other vegetable oils, palm oil consists mainly of triacylglycerols or triglycerides. However, before being isolated from the oil palm fruits, palm oil suffers some lipase-catalyzed hydrolysis that manifests itself in a relatively high free fatty acid content and a concomitantly high partial glyceride content. Because the relative molecular mass of a diglyceride is more than twice the relative molecular mass of a fatty acid, an FFA content of 1% corresponds to a diacylglycerol (DAG) or diglyceride content of >2%. This is only part of the story. A DAG molecule may be hydrolyzed further to produce another free fatty acid and a monoacylglycerol (MAG) or monoglyceride. This causes 1% FFA to correspond to 0.5% MAG. But then, kinetically, MAG formation will be slow since it requires DAG to be present, unless the hydrolysis rate constant of MAG is much larger than the DAG hydrolysis rate constant.

The stereochemical fatty acid composition of palm oil is given in Table 1.

As with most vegetable oils, there is a preference of the saturated fatty acids for the outer positions and of the unsaturated fatty acids for the middle, 2-position. However, the palmitic acid (16:0) content of the middle position at 24.5% is still substantial. This has repercussions on the triglyceride composition of palm oil. From this stereochemical fatty acid composition, it can be calculated that the tripalmitin content of the oil equals $(0.61)^2 \times 0.245 = 9\%$. This is higher than analytic data that show a tripalmitin content of 6%. In addition to the tripalmitin, palm oil also contains 1% of dipalmitoyl-stearoyl glycerol.

The high palmitic content of the 2-position also causes the P_2O triglycerides (dipalmitoyl-oleoyl glycerol) to contain a fraction of about 0.4 of the nonsymmetrical PPO triglyceride. This has implications for the crystallization behavior of confectionery fats in which palm oil fraction with a high P_2O content is used.

Table 1 Stereochemical fatty acid composition of palm oil

Position	*14:0*	*16:0*	*18:0*	*18:1*	*18:2*	*18:3*
sn-1,2,3 position	1.2	48.8	4.8	36.0	9.1	0.1
sn-1,3 position	1.3	61.0	6.0	25.7	5.8	0.2
sn-2 position	1.0	24.5	2.5	56.5	15.6	Tr

Source: Table 2.27 in Gunstone, F. D., Harwood, J. L. and Dijkstra, A. J. (eds.) (2007). *The lipid handbook* (3rd ed.). Boca Raton: CRC Press.

The phosphatide content of crude palm oil is low because the oil has been 'water degummed' during its production. It has been stated that only 4% of the phospholipids present in the palm fruit end up in the crude oil. The phosphorus content of crude palm oil is 5–20 ppm, but this includes inorganic phosphate that may have arisen from the enzymatic breakdown of phospholipids. The phosphatide composition of crude palm oil has been reported as listed in Table 2.

Crude palm oil is dark red because of the presence of carotenes. The quality specification of crude palm oil lists a maximum value of 600–700 ppm. This is very much lower than the values found in palm fiber extracts, which can be as high as 5500 ppm depending on the extraction solvent. The main component is β-carotene (56% of total) followed by α-carotene (35%). The carotene contents decrease during bleaching and, because they are thermally unstable, especially during the vacuum stripping process.

In comparison with other vegetable oils, palm oil has a rather low phytosterol content at about 700 ppm. Table 3 lists the sterols in crude palm oil and makes a distinction between free sterols and esterified sterols.

With respect to the tocols (tocopherols and tocotrienols), palm oil is similar to coconut oil in that it contains tocotrienols but the tocotrienol content in palm oil (530 ppm) is much larger than in coconut oil (30 ppm) (Table 4).

Given their volatility, some tocols will be vaporized during the vacuum stripping process. However, since this process is likely to be used for the physical refining of the oil, the resulting condensate will have a high FFA content and a very low tocol content. A two-stage scrubber should therefore be used to concentrate the tocols to a useful level.

Table 2 Phospholipid composition of crude palm oil

Phospholipid	*Range (%)*
Phosphatidylcholine (PC)	34–35
Phosphatidylethanolamine (PE)	22–25
Phosphatidylinositol (PI)	21–25
Cardiolipin (CL)	7–8
Phosphatidylglycerol (PG)	5–7

Source: Chapter 16, Zou, Y., Jiang, Y., Yang, T., Hu, P. and Xu, X. (2012). Minor constituents of palm oil: characterization, processing, and application. In: Lai, O.-M., Tan, C.-P. and Akoh, C.C. (eds.) *Palm oil*, *production*, *processing*, *characterization*, *and uses*. Urbana, IL: AOCS Press.

Table 3 Sterol content of crude palm oil

Phytosterol	*Esterified*	*Free*	*Total*	*% Free*
β-Sitosterol	97	325	422	77
Campesterol	37	98	135	72
Stigmasterol	18	69	87	79
Δ5-Avenasterol	11	–	11	0
Total	163	492	655	

Source: Verleyen, T. (2002). PhD thesis. *Stability of minor components during vegetable oil refining*, Ghent University.

Table 4 Tocol content of crude palm oil

	Tocopherols	*Tocotrienols*
α-	260	140
β-		30
γ-	320	290
δ-	70	70
Total	650	530

Source: Table 2.48 in Gunstone, F. D., Harwoord, J. L. and Dijkstra, A. J. (eds.) (2007). *The lipid handbook* (3rd ed.). Boca Raton: CRC Press.

Palm Oil Modification Processes

Four different processes have been developed to convert vegetable oils into fat blends with the required physicochemical properties. The most simple process involves blending two or more different components, like a hardstock and a liquid oil. In the partial hydrogenation process, a fraction of the double bonds present in the fatty acids are saturated or isomerized. This increases the solid fat content profile of the oil being hydrogenated to a level required for the intended application. Hydrogenation changes the fatty acid composition of an oil but does not alter its composition according to carbon number.

A third modification process is the interesterification process. In this process, the fatty acid moieties are reshuffled over the glycerol moieties so that the composition according to carbon number is likely to be affected but the fatty acid composition does not change. An oil with a high content of trisaturated triglycerides, such as a low IV palm stearin, has a high melting point. It gives products an often undesirable, waxy mouthfeel. This can be remedied by interesterifying this high-melting product with an oil that has a high content of medium chain fatty acids like palm kernel oil or with a liquid oil. These oils reduce the fraction of saturated long-chain fatty acids in the mixture and thus the fraction of high-melting triglycerides in the interesterification product. Whereas palm stearin on its own is not suitable as hardstock because of poor mouthfeel and palm kernel oil on its own is not suitable because of its low melting point, their interesterification product can be made to meet a whole range of hardstock requirements.

The interesterification reaction needs a catalyst. This can be a base like sodium methanolate or a lipase enzyme. The base-catalyzed process can be carried out as a batch process or as a continuous process; the enzymatic route is only continuous. The base-catalyzed process uses neutral oil as starting material, which in the case of soybean oil can be alkali-refined oil that has not yet been bleached but, in the case of palm oil, a neutral oil that is likely to have been degummed, bleached, and steam-refined. The oil to be used in the enzymatic interesterification process must be carefully purified to prevent the enzyme from being inactivated.

The importance of the interesterification process is growing because it provides an alternative to the partial hydrogenation process and offers the presumed advantage of avoiding the formation of *trans* isomers. That holds even stronger for the

fourth modification process, the fractionation process, which process can also claim to be purely physical.

The early fractionation processes of edible oils and fats used a solvent like acetone, hexane, or 2-nitropropane. Their flammability required X-proof fractionation plants, which are expensive. Solvent evaporation requires additional equipment and energy, which is also expensive. Consequently, the solvent fractionation process is only used for very special products that can carry the heavy fractionation cost. Besides, fractionation leads to two or more different products, only one of which may be the product that can carry the cost. The other fraction or fractions may have to be sold at much lower price.

The fractionation process consists of two steps: partial crystallization and separation. This separation is nearly always by filtration, thus leading to a filtrate, the olein, and a filter cake, the stearin. The filter cake will never consist of just fat crystals; there will always be some olein in between these crystals. The residual olein content of the filter cake determines the stearin properties and the yields of both fractions. The properties of the olein fraction, on the other hand, are mainly determined by the temperature down to which the crystallization was allowed to proceed.

In the solvent fractionation process, the liquid in between the crystals of the filter cake is a solution of olein in the solvent. Removal of that solvent by evaporation will leave relatively little olein so that the stearin properties are close to those of the pure crystals. This is called a selective fractionation. Working without a solvent in the so-called dry fractionation process formerly led to poor selectivity since the filter cake as obtained by a vacuum drum or band filter could contain as much as 70 wt% olein. Now, the use of membrane filter presses has greatly reduced the olein content of the filter cake to values close to 30%. This has improved the selectivity of the fractionation process so that it can now be used to produce products that formerly required solvent fractionation.

In the field of crystallizers, there has also been steady development. The early crystallizers used for the dry fractionation of oils and fats were cylindrical vessels, fitted with a central agitator and internal cooling coils near the outer walls. They had the disadvantage that on emptying, crystals remained between the

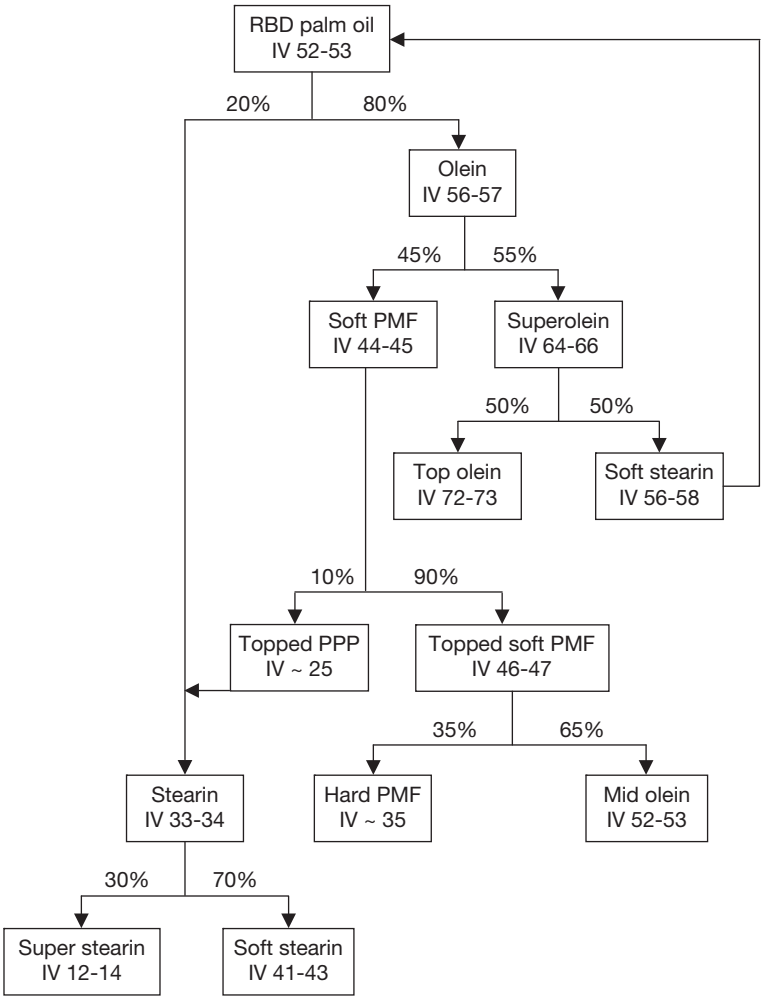

Figure 1 Multistage fractionation of palm oil. Adapted from Figures 12.11–12.13 in Chapter 12, Gibon, V. (2012). Palm oil and palm kernel oil refining and fractionation technology. In: Lai, O.-M., Tan, C.-P. and Akoh, C. C. (eds.) *Palm oil*, *production*, *processing*, *characterization*, *and uses*. Urbana, IL: AOCS Press.

coils and the wall and on top of the coils. So the coils were replaced by vertical, hollow fins that did not hold up any crystals.

However, the limited cooling surface did not permit fast cooling so that in a subsequent crystallizer design, the vessel was divided into coaxial annular compartments with annular cooling compartments in between. A centrally driven agitator extended into the compartments filled with oil. This design had the disadvantage that the linear speed of the agitator was much faster in the outer compartments than in the compartment closer to the central axis. It was also expensive to construct because the cooling compartments had to be fitted with a labyrinth for the cooling water.

The latest crystallizer design comprises a rectangular vessel, the contents of which are agitated by a bundle of cooling tubes that is moved slowly to and fro. This is the so-called Mobuliser®. This crystallizer has a large cooling surface per crystallizer volume and the agitation speed is uniform throughout the crystallizer. Its use has shown that there is no basic need to maintain the same temperature throughout the crystallizer. It therefore permits continuous crystallization with the warm, precooled oil being fed to the top of the crystallizer and the 'cold' slurry leaving at the bottom. This is the iConFrac® process developed by Desmet Ballestra in Belgium.

Its use has also shown that the replacement of the fast-moving agitator blades by the slow movement of the 'agitator *cum* heat exchanger' suppresses secondary nucleation. Consequently, the crystals that were formed initially go on growing at about the same rate so that they maintain a fairly uniform size. As a result, crystal slurries with a high solid fat content can still be agitated, and this allows a fractionation that formerly required two steps to be carried out in a single step. Whereas formerly, the first fractionation of palm oil would produce an olein with an IV of 58 and the second fractionation would produce a second olein with an IV of 62, this is now possible in a single step. This reduces the fractionation cost because it increases the throughput and lowers energy requirements. The more uniform crystals also improve the filter performance.

To make special, high-value products, palm oil is subjected to a multistage fractionation as illustrated in **Figure 1**. It shows that palm oil with an IV of 52–53 is first of all fractionated into a stearin and olein and that both fractions can be fractionated again. If the first olein fraction is refractionated, it yields a superolein, but as explained earlier, this can also be achieved in a single step. This superolein is already a commercial product that can be used as salad oil since it has a cloud point of 4 °C. It can also be refractionated to yield a top olein with an IV of 72–73 and a cloud point of −5 °C.

Figure 1 also shows how a palm mid fraction that can be used as cocoa butter equivalent (CBE) can be produced by multistage fractionation. The stages are the following:

- Palm oil: 80% palm olein (IV 56–57) plus palm stearin
- Palm olein: 45% soft PMF plus palm superolein
- Soft PMF: 90% topped soft PMF plus topped PPP
- Topped soft PMF: 35% hard PMF plus mid olein

The overall yield equals $0.80 \times 0.45 \times 0.90 \times 0.35 \times 100\% =$ 11%. This is not that much but fortunately, the CBE does not have to carry all the raw material and fractionation costs. One of the coproducts is the superolein that commands a premium over palm oil. The soft stearin (IV 41–43) that results from the fraction of the first stearin can be used in margarines and shortenings, and the first stearin and especially the superstearin are highly suitable fats for interesterification with lauric oils to provide hardstocks.

Fractionation is a versatile process that can be adapted to market requirements and opportunities. It also has the advantage that it is reversible. When the fractions do not live up to expectations, they can be mixed and reconstitute the starting material, which can then be subjected to a fresh fractionation.

See also: Carotenoids: Occurrence, Properties and Determination; Cocoa: Production, Chemistry, and Use; Colors: Properties and Determination of Natural Pigments; Enzymes: Analysis and Food Processing; Fatty Acids: *Trans* Fatty Acids; Fatty Acids: Fatty Acids; Fruits of Tropical Climates: Biodiversity and Dietary Importance; Heat Treatment: Principles and Techniques; Lauric Oils; Mayonnaise; Phospholipids: Properties and Occurrence; Tocopherols: Properties and Determination; Triacylglycerols: Characterization and Determination; Triacylglycerols: Structures and Properties.

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Pantothenic Acid

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Introduction

Pantothenic acid (vitamin B_5) in its pure state is water-soluble, viscous, and yellow. It is stable at neutral pH but is readily destroyed by acid, alkali, and heat. It is an essential growth factor for both prokaryotes and eukaryotes and widely found in living materials. Pantothenic acid is ubiquitous in nature (derived from the Greek term pántothen, which means 'from all quarters'). The calcium salt, calcium pantothenate (white, odorless, and crystalline) is the form of pantothenic acid usually found in commercial vitamin supplements.

By the 1920s, it was appreciated that water-soluble factors or 'vitamines' were not a single entity, but compounds with differing functions. The first to be identified was thiamin, initially designated as B_1; the remaining compounds, including pantothenic acid, were found to be a complex of several factors. The identification of pantothenic acid as an essential dietary factor largely evolved from the efforts of research groups associated with Roger J. Williams, Conrad A. Elvehjem, and Thomas H. Jukes. Williams' group established that pantothenic acid was required for the growth of certain bacteria and yeast and later for animals. These observations were confirmed and amplified by Elvehjem and Jukes and coworkers. Next, Williams' group eventually isolated and partially characterized pantothenic acid using 250 kg of liver as starting material. By the 1950s, coenzyme A (CoASH), which contains pantothenic acid as a component, was identified as a cofactor essential for the acetylation of sulfonamides and choline and later of essential importance in the synthesis and oxidation of fatty acids. Pantothenic acid was next identified as a component of acyl carrier protein (ACP), a part of the fatty acid synthesis complex. More recently, there has been an appreciation that additional enzymes utilize pantothenic acid (usually in the form of 4′-phosphopantetheine) as their cofactor component (cf. Figure 1). Examples include 10-formyltetrahydrofolate dehydrogenase and enzymes important to polyketide synthesis. Polyketides are a large class of diverse compounds that are characterized by more than two carbonyl groups connected by single intervening carbon atoms, which include many plant and bacterial substances with antibiotic, anticancer, cholesterol-lowering, or immunosuppressive activity. Steps in the biosynthesis of polyketides share similarities with steps in the synthesis of fatty acids. Also, in addition to the major endoplasmic reticular pathways for fatty acid synthesis, mitochondria have recently been shown to synthesize fatty acids in a malonyl CoA/ACP-dependent manner. In eukaryotic cells containing mitochondria, the pathway resembles that in bacteria, that is, the fatty acid synthesis type II pathway.

Dietary Sources, Production, and Requirements

About 6000 tons of calcium D-pantothenate are produced annually. It is mainly used as an animal feed additive. It is also used for pharmaceutical, health care, and food products. D-Pantothenate (the active form) is prepared by chemical synthesis from D-pantolactone and calcium β-alaninate. However, racemic separation of L form and D form of the final product is often necessary (e.g., separation by chromatography or recrystallization). The commercial product resulting from the chemical synthesis is usually the calcium salt of D-pantothenic acid, calcium D-pantothenate. In addition, many of the current approaches take advantage of biotechnological preparation using microorganisms in which there is synthesis of only the D form of pantothenic acid. As a consequence, the separation of racemates (as is required in chemical synthesis) is not necessary.

It is estimated that most human diets provide 4–6 mg of pantothenic acid per day, which is sufficient to maintain normal serum levels. About 1–2% of the total body pool of pantothenic acid is excreted each day. The recommendation for the human requirement is based on the concept of adequate intake. An adequate intake is based on observed or experimentally determined approximations or estimates of daily nutrient intake or amounts to balance total excretion that result in no obvious metabolic perturbations and when a recommended daily amount cannot be determined.

The adequate intake recommendations for humans are, for 1–3-year-olds, 2 mg day^{-1}; 4–8-year-olds, 3 mg day^{-1}; 9–13-year-olds, 4 mg day^{-1}; 14-year-olds and over, 5 mg day^{-1}; and for pregnant and lactating women, 6 mg day^{-1} and 7 mg day^{-1}, respectively. For reference, human milk contains about 5–6 mg of pantothenic acid per 1000 kcal. These values are in keeping with the reported requirements for monogastric animals, that is, about 2–3 mg pantothenic acid per 1000 kcal or 4.2 kJ of typical diets. There is currently insufficient scientific evidence on which to base a tolerable upper intake level for pantothenic acid.

Availability, Absorption, and Metabolism

Intestinal Absorption, Cellular Uptake and Efflux, Plasma Transport, and Excretion

Pantothenic acid in food is usually present as CoASH or peptide-bound 4′-phosphopantetheine. In order to be absorbed, intestinal enzymes must hydrolyze these substances. This occurs by the sequential activity of two intestinal hydrolases, a pyrophosphatase and a phosphatase. Pantetheine, the product, can be further metabolized to pantothenic acid by another intestinal hydrolase, pantetheinase. There is a near-quantitative release of pantothenic acid or pantetheine from proteins and peptides, such as those derived from ACP. Following enzymatic release, the pantothenic acid or pantetheine absorption involves a sodium-dependent multivitamin transporter with a K_m for pantothenic acid intestinal uptake of 10–20 μM.

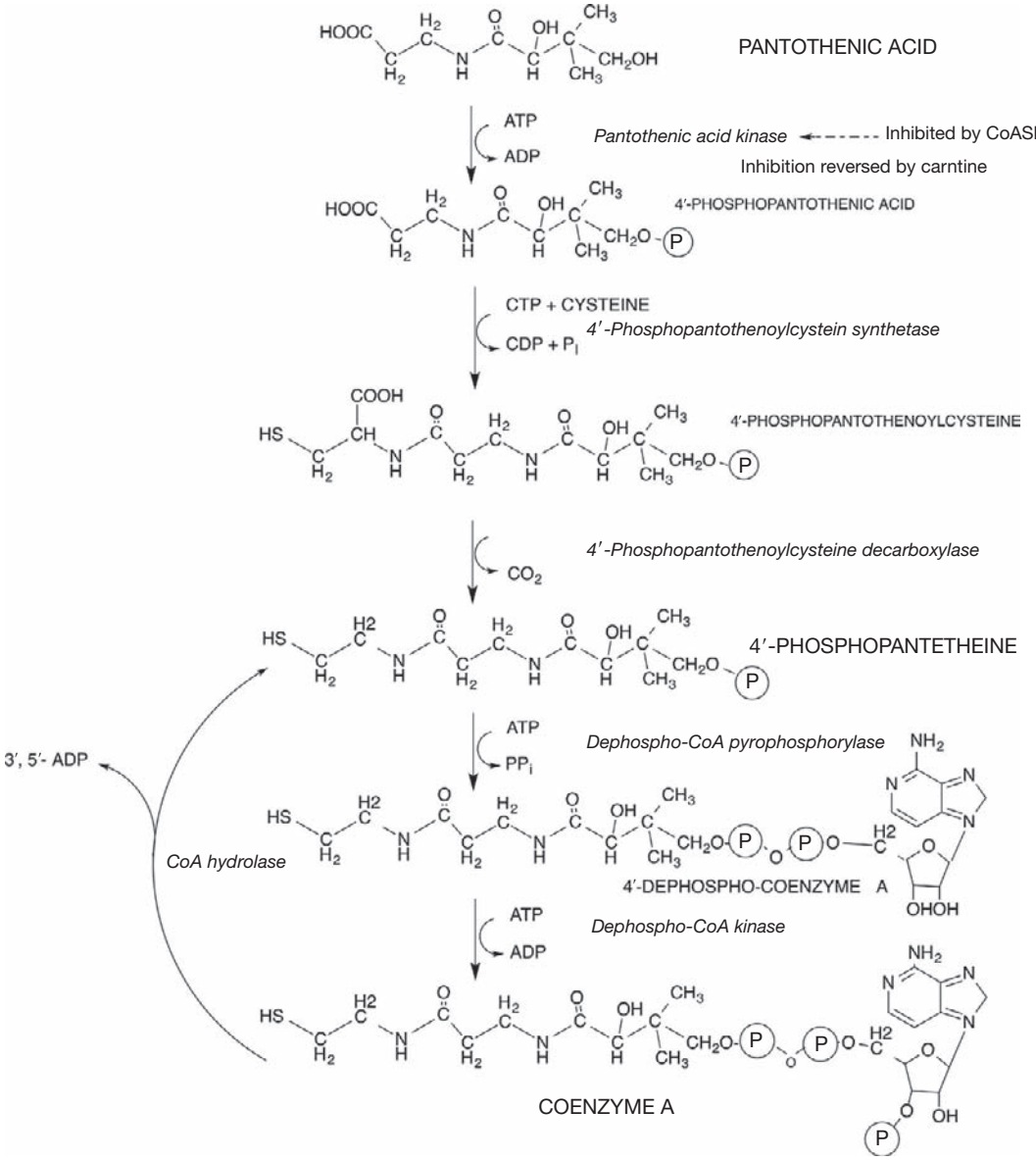

Figure 1 Pantothenic acid: metabolic conversion to coenzyme A and ACP. Forms of pantothenic acid that are important to the synthesis of coenzyme A (CoASH) and acyl carrier protein (ACP).

At a dietary intake of ~10–15 mg of CoA (the amount of CoASH in a typical meal), the pantothenic acid concentration in luminal fluid is ~1–2 μM. At this concentration, the transport system is not saturated, and as a consequence, pantothenic acid is efficiently and actively absorbed. In an analogous fashion, cells in tissues take up pantothenic acid from the plasma by a saturable process with an apparent K_ms in the low μM range. For example, kinetic analysis of human placental choriocarcinoma cells indicates the presence of a single saturable transport system with a Michaelis–Menten constant of ~2 μM. The dependence of uptake on the Na^+ concentration exhibits sigmoidal kinetics, indicating interaction of more than one Na^+ ion with the transporter. In the brain, pantothenic acid is transported through the blood–brain barrier by a low-capacity, saturable transport system with a half-saturation concentration approximately 10 times the plasma PA concentration. In the liver, the cellular concentration of free pantothenic acid is 10–15 μM and in the heart ~100 μM compared with the 1–5 μM concentrations observed in the plasma. For comparison, the concentrations of CoASH and ACP are 50–100 μM and 10 μM, respectively, in the cytosol of typical cells. In mitochondria, the CoASH concentration can be as much as 10- to 20-fold higher, that is, 70–90% of the total cellular CoASH content. Although much remains regarding the details of cellular influx, internal regulation, and egress from cells, pantothenic acid cellular uptake has been linked to protein kinase C activity and calmodulin-dependent regulatory and signaling pathways. As a final step in whole-body regulation, the vitamin is excreted in the urine primarily as pantothenic acid. This occurs after its release from CoASH by a series of hydrolysis reactions that cleave off the phosphate and β-mercaptoethylamine moieties.

Most Important Cellular Functions

As a component of CoASH and ACP, pantothenic acid is involved in over 100 known reactions central to intermediary metabolism. As a general rule, CoASH is involved in acetyl and acyl energy-related transfer reactions and processes related to oxidative metabolism and catabolism, whereas ACP is involved primarily in synthetic reactions, essential to either fatty acid or isoprenoidal structures. Moreover, CoASH-dependent acetylation of certain lipids, amino acids, and proteins is essential to many aspects of cell membrane formation and changes in protein structure related to compartmentalization and stability, for example, acetylation of histone.

Properties of CoASH and ACP Important to Function

The adenosyl moiety of CoASH provides a site for tight binding to CoA-requiring enzymes, while allowing the phosphopantetheine portion to serve as a flexible arm to move substrates from one catalytic center to another. Similarly, 4′-phosphopantetheine in ACP also functions as a flexible arm that allows for a systematic presentation of acyl derivatives to each of the active centers of the fatty acid synthase complex. It is also important to appreciate that intermediates arising from the transfer reactions catalyzed by CoASH and 4′-phosphopantetheine in ACP may be viewed as 'high-energy' compounds. Typical esters (O–CO–R) or amides (–N–CO–R) are thermodynamically more stable than thioesters (–S–CO–R). The double-bond character of the C–O bond in –S–CO–R does not extend significantly into the C–S bond. This causes thioesters to have relatively high-energy potential, and for most reactions involving CoASH or ACP, no additional energy, for example, from ATP hydrolysis, is required for transfer of the acetyl or acyl group. For example, consider that at pH 7.0, the $-\triangle G$ of hydrolysis is ~7.5 kcal for acetyl-CoA and 10.5 kcal for acetoacetyl-CoA compared with 7–8 kcal for the hydrolysis of ATP to AMP and pyrophosphate or ADP and phosphate. The terminal thiol group of CoASH and ACP is also ideally suited for nucleophilic substitution reactions involving activated carboxylic acids and α- and β-carbonyl functions.

Regulation of CoASH and ACP via Pantothenic Acid Kinase

Pantothenic acid kinase serves as a primary control point for pantothenic acid utilization. There are three distinct types of pantothenic acid kinase (PanK): PanK-I (bacterial), PanK-II (mainly found in eukaryotes and also in the *Staphylococci*), and PanK-III (also bacterial). The eukaryotic PanK-II enzymes occur as different isoforms (PanK1, PanK2, PanK3, and PanK4). Pank2, expressed in mitochondria and found on chromosome 20p13, is the best characterized of the various isoforms. Alternatively spliced transcript variants encoding multiple isoforms have also been observed for Pank2. Mutations (particularly in PanK2) can result in pantothenate kinase-associated neurodegeneration, such as Hallervorden–Spatz syndrome.

Feedback inhibition of the kinase by CoASH or CoA derivatives is a principal factor governing the flux through the subsequent steps in the CoASH synthesis pathway and defines the upper threshold for intracellular CoASH cofactor levels. Inhibition by acetyl-CoA is greater than that of free CoASH. The inhibition by free CoASH is uncompetitive with respect to pantothenate concentration.

In addition to regulation at the substrate level, PanK2 is regulated by tumor protein p53, also known as p53, a protein that is crucial in multicellular organisms, where it regulates the cell cycle and, thus, functions as a tumor suppressor. Peroxisome proliferator-activated receptors, such as PPARα, also influence pantothenic acid kinase activity. Using metabolomic approaches, for example, high-resolution NMR and mass spectrometry technology, it has been shown in humans that significant depletion of both pantothenic acid (as much as fivefold) and acetylcarnitine (as much as 20-fold) occurs in response to PPAR agonists, such as fenofibrate.

Substrates are added to pantothenic acid kinase sequentially with ATP as the leading substrate. The allosteric regulatory domain responsible for acetyl-CoA inhibition also binds the substrates, pantothenate and pantetheine, and the various small-molecule inhibitors and activators that can influence pantothenic acid kinase activity. As it relates to acetyl-CoA production and utilization, the sensitivity to carnitine is an important observation. Carnitine relieves the feedback product inhibition of pantothenate kinase through its role in transporting acyl groups from cytosol to mitochondria for β-oxidation. Carnitine has no direct effect by itself, but specifically reverses the inhibition by CoASH. In the heart muscle, the free carnitine content varies directly with the phosphorylation of pantothenic acid. Thus, the interactions involving carnitine are additional mechanisms for the control of CoASH synthesis and the regulation of cellular pantothenic acid content. Moreover, fasting and low insulin increase pantothenic acid kinase activity and the total content of CoASH.

Following 4′-phosphopantothenic acid formation, the remaining steps in CoASH synthesis are carried out on a protein complex (~400 000 Da) with multifunctional catalytic sites. Enzymatic features of the complex include dephospho-CoA pyrophosphorylase activity and coenzyme A hydrolase activity (Figure 1). Less is currently known about the regulation of ACP, often described as 'macro-cofactor.'

Health Effects

Symptomatic nutritional deficiencies of pantothenic acid are rare. Pantothenic acid deficiency in experimental animal models (both avian and mammal) results in impaired energy production, most likely due to low CoASH levels and impaired acetylcholine synthesis. Pantothenic acid deficiency can also cause hypoglycemia and an increased sensitivity to insulin. Given a requirement for pantothenic acid that appears to be ~5 mg day^{-1}, it may be predicted that 5–6 weeks might be required before signs of deficiency are observed in humans.

There is only a limited amount of clinical data for experimental pantothenic acid deficiency in humans. What is known about pantothenic acid deficiency in humans comes primarily from indirect lines of evidence. For example, malnourished prisoners of war (World War 2) experienced numbness and burning sensations in the extremities, which seemed reversed primarily following pantothenic acid supplementation, in contrast to other B vitamins. Moreover, the administration of

pantothenic acid kinase inhibitors, such as ω-methylpantothenate or calcium hopantenate, in combination with a diet low in pantothenic acid, exacerbates signs of pantothenic acid deficiency, such as numbness, burning sensations, and changes in lipid metabolism.

In animals, the classic signs of pantothenic acid deficiency include growth retardation and dermatitis, sometimes accompanied by the graying of hair and/or alopecia. A consistent finding, when a deficiency is severe, is progressive morphological and functional changes in adrenal glands. The adrenal cortex becomes enlarged, and there is a progressive depletion of ketosteroids. In later stages of pantothenic acid deficiency, adrenal atrophy occurs.

Pantothenic acid at abnormally high levels is well tolerated; the acute oral LD50 is 10 g kg^{-1} in mice and rats. A chronic intake of calcium pantothenate at 3% of the diet is the lowest observed adverse effect level in rats. At this level, enlargement of the testes, diarrhea, and hair damage may be observed. In humans, such a dose would be the equivalent of 2–3 g of pantothenic acid per day.

See also: Bioavailability of Nutrients; Coenzymes and Cofactors; Dietary References: US; Dietary Surveys: National Food Intake; Fatty Acids: Metabolism; Vitamins: Overview.

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Papayas

IGN Hewajulige, Industrial Technology Institute, Colombo, Sri Lanka
SA Dhekney, University of Wyoming, Sheridan, WY, USA

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Sources and Production

Papaya (*Carica papaya* L.) belongs to the family Caricaceae and is the most economically important species of the genus *Carica*. Papaya is native to tropical America, and seeds of papaya were taken from the Caribbean, to Malacca or Philippines, then to India. Subsequently, papaya was introduced as a plantation crop to Australia, Hawaii, Sri Lanka, and other tropical and subtropical countries in the world. Papaya is an important fruit crop in the tropics and is cultivated for consumption in the fresh or processed form and for use in pharmaceutical, leather, and textile industry. The fruit is a good source of vitamins and minerals while being low in sodium, fats, and calories.

Global production of tropical fruits (excluding banana) reached 73.02 million metric tons (Mt) in 2010, and papaya ranked third with 11.22 Mt or 15.36% of the total annual tropical fruit production. Global papaya production is highly concentrated, with the top ten countries averaging 82.32% of the total production during the period 2008–10. India is the leading papaya producer, with a 38.61% share of the world production (2008–10), followed by Brazil (17.5%), Indonesia (6.89%), Nigeria (6.79%), Mexico (6.18%), Ethiopia (2.34%), Congo (2.12%), Colombia (2.08%), Thailand (1.95%), and Guatemala (1.85%). Economic value of papaya has potential to be exploited as an income generator besides providing food to the people. However, papaya industry in some tropical countries is still relatively small, and trees continue to be grown in backyards and home gardens mainly for local consumption. Commercial planting started during the last few decades in Malaysia, Thailand, the Philippines, Indonesia, and India with the introduction of new and better-yielding varieties. Constraints that hinder the expansion of the papaya industry in these countries include unavailability of suitable land for growing crops, pathogen infections, insufficient handling techniques, short life of the fruit, and plant quarantine restrictions imposed by some importing countries. Major papaya-exporting countries in the world are Mexico, Brazil, Malaysia, India, and the United States (mainly via re-exports). The major papaya varieties dominating the world trade are Solo, Sunrise, Maridol (red and golden), Red Lady, Linda, and Tainung.

Papaya is a large herbaceous plant generally with a single erect stem, which can grow up to 9 m, terminating with a crown of large leaves. There is a wide diversity of biological types of cultivated papaya. The plants can be dioecious, with male and female flowers on separate plants; monoecious, with male and female flowers on the same plant; or hermaphroditic, with male and female parts on the same flower. Papaya is a melon-like fruit, which varies greatly in shape and size. The skin of unripe fruit is smooth, green, and thin and changes to deep orange or yellow when ripe. The flesh varies from 2.5 to 5.0 cm in thickness and yellow to orange in color. Numerous round, black seeds with wrinkled seed coat are enclosed in a gelatinous membrane (sarcotesta), which is attached to the inner wall of the fruit.

One of the major problems faced in papaya fruit marketing is the identification of the optimum harvest maturity to ensure adequate fruit ripening and good eating quality. The harvest maturity depends upon distance to markets. To supply for the local markets, fruit may be harvested at one-quarter to one-half ripe or yellow stage. Fruit to be transported for long distances or exported are harvested at color break to one-quarter ripe, depending on the cultivar's ripening characteristics and season. Fruit should be harvested when peel color changes from dark green to light green and when one or two yellow streaks begin developing from the posterior side of the fruit to the pedicel end. This stage is known as color break stage, and fruit in this condition will continue to ripen normally after harvest. Fruit harvested before this stage will fail to show complete ripening, and those harvested after are more susceptible to damage and bruising during handling. The Hawaii grade standards require fruit to have 11.5% total soluble solids (°Brix) with color break stage normally meeting this standard. Papaya is harvested by hand alone or with knives or specialized tools, depending on the size and age of the tree. As the trees become taller, some form of harvesting aids such as poles and ladders must be used. A pole with a small hoop and a small mesh bag at the end will be useful to minimize the mechanical damage that can happen to the fruit during harvest. Harvested fruit could be collected in field bins made of a soft material or plastic crates to prevent damage to the papaya skin.

Grading and packing should be carried out as soon as possible after harvest. This should be done within 3 h after which the fruit should be kept under ambient tropical conditions (25–28 °C) to continue ripening or cooled and stored at 10 to −12 °C. Depending on the country of origin, some importing countries require the fruit be treated for fruit flies. Treatments are determined by importing country requirements and may include long exposure to hot water treatment or vapor heat treatment. Postharvest treatment with fungicides is used to prevent the development of anthracnose and phytophthora neck rot.

Papaya is packed in single-layer cartons, each with a net weight of 4–5 kg, depending on importer and importing country requirements. Internal packing materials can be included with shredded paper placed at the base of the carton and individual tissue wrap or with an expanded polystyrene sleeve. If papaya is intended for export, precooling should be done before shipment. Room cooling and forced air cooling are commonly used. Hydro cooling is also possible. However, rapid cooling after insect disinfestation treatments can lead to skin scalding. Optimum storage temperature for papaya is in the range of 7–13 °C with 90–95% RH. However, at the lower range of 7–10 °C, chilling injury may occur, while at 10–13 °C,

ripening occurs slowly. At the color break stage, papaya can be stored at 7 °C for 14 days and will ripen normally when transferred to room temperature. Papaya is a climacteric fruit with typical respiratory and ethylene production patterns during ripening. During ripening, the color, firmness, carbohydrate composition, and production of secondary compounds, which are responsible for fruit color and flavor, in papaya fruits change.

Papaya fruits are very susceptible to invasion by certain pathogenic fungi and bacteria due to high moisture and nutrients. The development of fungal infection during the postharvest phase can depend upon the physiological age of the fruit, mechanical injuries, and storage conditions. Anthracnose is a major cause for the postharvest loss of papaya particularly when attempting to extend the storage life. The causal organism *Colletotrichum gloeosporioides* is of common occurrence in home garden environments. Symptoms of the disease are known to be particularly destructive once ripening has been initiated. Incidence of the disease limits the storage life of the commodity in both local and export markets during the ripening process, storage, and transportation.

Patterns of Papaya Consumption

Papaya is rapidly becoming an important fruit crop as a fresh fruit or in the processed form. In Asia, green fruits are served in salads and as a vegetable or made into preserves. Papaya is processed into various forms such as slices for fruit salads and cocktails, dehydrated slices, chunks, and fruit leathers or bars. Papaya can also be processed into puree for juices, nectar, mixed drinks, and jams. Papaya provides a cheap source of vitamins and minerals in the daily diet of the people. The fruits of certain cold-hardy genera in the Caricaceae family are used as a source of food in Central and South America on a limited basis where the fruit might be consumed directly as fresh, roasted, or processed products. Supplements made from fermented papaya are known to have good nutraceutical properties. Papaya seed, which accounts for up to 50% of the waste from the fruit, can be ground into flour. The seed kernel flour is known for its high protein content and can moderate carbohydrate levels while having good water and oil absorption capacities. Papaya also has several industrial uses. Leaves and fruit are valuable sources of several proteins and alkaloids that have applications in the pharmaceutical industry. Of particular importance is papain, an important proteolytic enzyme that occurs in the milky latex produced by immature fruit. The latex is obtained by scarifying immature fruit to induce flow of the milky latex, which is then collected and dried prior to processing. Papain has several applications including its use in chewing gum, chill proofing of beer, tenderizing meat, and preparation of drugs for digestive ailments and treatment of wounds. It is also used in the textile and cosmetic industry. Papaya seed has been explored as a source of biodiesel in countries with high papaya production. The seed oil composition is familiar to that of olive oil and appears to be a suitable candidate for biodiesel production due to its appropriate proportions of fatty acids for cold flow properties and oxidative stability.

Availability, Absorption, and Metabolism

Being a nutritious fruit, papaya ranks first among 13–17 fresh fruits for vitamin C content per 100 g edible portion. It is reported that most of the papaya cultivars contain higher levels of vitamin C compared to passion fruit (1.4 mg/100 g), banana (8–20 mg/100 g), annona fruit (11.5–37.3 mg/100 g), and pineapple (0.93 mg/100 g). Papaya is an excellent source of provitamin A (carotenoids), which is important for good health especially on eye sight, helping to prevent early blindness in children. Papaya has more carotene compared with other fruits such as apple, guava, and plantain, which help to prevent damage by free radicals. Papaya ranks first among the fruits for vitamin C, vitamin A, riboflavin, folate, calcium, thiamin, iron, niacin, potassium, and fiber. The fruit is also popular with obese people who are into weight-reducing regimes since it is low in fat, carbohydrate, and calories. The antioxidant activity of fermented papaya is highly appreciated due to its nutraceutical properties (Tables 1 and 2).

Nonvolatile organic acids formed the major portion (about 80–90%) of total acidity in fruit. Citric and malic acids, present in equal quantities, are the predominant acids. However, the presence of tartaric, malonic, fumaric, and succinic acids has also been reported. The concentration of total and nonvolatile acids decreased during fruit development, reaching a

Table 1 Nutritional composition of papaya (per 100 g edible portion)

Nutrient	*Composition*
Water	90.8 g
Energy	32.8 k cal
Protein	0.6 g
Fat	0.1 g
Carbohydrates	7.2 g
Calcium	17.0 mg
Phosphorus	13.0 mg
Iron	0.5 mg
Vitamin C	57.0 mg
Carotene	666.0 μg
Thiamine	40.0 μg
Riboflavin	250 μg

Table 2 Vitamin C content of different papaya cultivars

Papaya cultivar	*Vitamin C content (mg/100 g^{-1} fresh weight)*
Maradol	46–82
Criolla	42–55
Tainung 01 Formosa	59.9–68.2
Dampit	108.1
Jingga	94.7
Paris	35.4
Kapoho	45.3
Laie Gold	51.2
Rainbow	51.8
Sunrise	55.6–187
Golden Solo	103.1
Baixinho do Santa Amelia	147–151

minimum at the ripe stage. An increase in total acidity observed during ripening is believed to be associated with an increase in free galacturonic acids. Tissue pH of papaya increases from 5.2 to 6.0 in 50% yellow fruit and then decreases to 5.2 in 100% yellow fruit.

During ripening, the color of the pulp turns yellow to reddish. Carotenoids content estimated as β-carotene showed a five- to tenfold increase in yellow-fleshed cultivars, between the mature green stage and the full ripe stage. In red-fleshed cultivars, however, the change in color is associated with a marked increase in lycopene content, which has the highest radical scavenging ability among carotenoids. The major difference between the yellow- and red-fleshed papayas is the complete absence of lycopene in yellow-fleshed types. The antioxidant properties of lycopene in red-fleshed papaya varieties have a greater contribution to human health than vitamin A. A recent report of Hawaiian papaya analysis revealed that other than vitamins C and A, papaya is an important source of magnesium. A cup of papaya (140 g) contains 8–11% of the dietary reference intake (DRI) of magnesium, and it would provide 80% and 96% of the DRI for vitamin C for the average adult male and female, respectively.

The principal carbohydrates in papaya fruit are sucrose, glucose, and fructose. In the early stages of fruit development, glucose is the predominant sugar, while at the preripe and ripe stages, sucrose increases by two- to fivefold, reaching higher levels than those of fructose and glucose. Early reports indicated much lower amounts of sucrose and higher percentages of glucose and fructose. This may be due to the presence of an invertase enzyme that is active during extraction. A recent evaluation where samples were heated in a microwave oven to deactivate the enzyme has given the distribution of sugar as 48.3% sucrose, 29.8% glucose, and 21.9% fructose. Papaya fruit contain a small amount of starch, which shows very little change during fruit development.

About 106 volatile components in small concentrations are present in papaya fruit. Amounts vary according to the cultivar and locality. Linalool has been found as the major volatile component. The relative proportions of different volatile components vary with the ripeness. The volatile compound methyl butanoate is considered responsible for the sweaty odor quality of papaya, but only methyl benzoate is described as having papaya qualities on odor assessment.

Softening of papaya fruit is characterized by an increase in the solubility of cell wall pectin, and the extent of softening depends on pectin depolymerization. The ripening of papaya, as characterized by changes in tissue softness, is believed to be initiated on the inner mesocarp tissues close to the seed and to progress outward. While pectin solubilization in inner and outer mesocarp tissues was comparable, pectin depolymerization appeared and begins earlier in the inner than in the outer mesocarp tissues. The polygalacturonase (PG) enzyme activity increases during ripening as the tissue softens. PG activity increased with the increasing depth of the mesocarp tissue and was the highest in the inner mesocarp tissue, suggesting that formation and activation of the enzyme began in the inner tissues and progressed toward the outer regions. The activity of β-galactosidase also increased during ripening, but unlike PG activity, it was found to be consistently higher in the outer mesocarp than the inner mesocarp. It is thought that β-galactosidase significantly contributes to pectin and hemicellulose modification and thereby softening of fruits.

Fatty acid content of phospholipids increased with increasing fruit maturity. The increase in the relative amounts of lower-melting point unsaturated fatty acids such as palmitoleic acid and linoleic acid among papaya phospholipids is thought to increase the resistance to chilling injury. Several thiol proteases, namely, papain, chymopapains A and B, and papaya peptidase, were found in papaya latex. The milky latex, which is most abundant in immature fruit, contains mainly papain and chymopapain. Both are active principles in breaking down or digesting protein. This property is being used by cooking meat with raw papaya to make it tender and digestible.

Polyphenol oxidase activity in papaya decreased during the climacteric rise and then increased up to the ripe stage. A decrease in bitterness during ripening could result from the decline in phenol content, but the peel still contained bitter compounds at the ripe stage.

Health Effects

Papaya extracts from various plant parts are well known for their medicinal properties and have been used as traditional medicine for the treatment of various diseases. The milky latex, which is most abundant in immature fruit, contains several thiol proteases, namely, papain and chymopapain A and B, and papaya peptidase. Both papain and chymopapain are active principles in breaking down or digesting protein. A number of volatile and aroma compounds have been identified from papaya fruit extracts. Papaya juice and fruit extracts are known to exhibit antioxidant and anti-inflammatory activities that could potentially provide protection against a number of debilitating cardiovascular and neurological disorders. Papaya juice extracts contain compounds responsible for relaxation of vascular tissues and may have beneficial properties in alleviating heart disease and related ailments. Carparine, an alkaloid found in papaya leaves, has also been used for medicinal purposes. Carparine is known to cause a strong depression in the activity of heart. A number of chemical compounds with antitumor properties have been isolated from papaya leaf, fruit, and seed extracts. Such bioactive compounds exhibited chemoprotective properties under *in vitro* and *in vivo* conditions. Microarray analyses of tumor cell lines treated with papaya leaf extract revealed the upregulation of several genes with immunomodulatory properties. Papaya fruit, leaf, and seed tissues are known to exhibit wide range of antimicrobial properties. The seeds and unripe fruit are rich in benzyl isothiocyanate, a sulfur-containing chemical that has been reported as an effective germicide and insecticide. Antibacterial properties of leaf and stem tissues have been attributed to the presence of cyanogenetic glycosides, which could be a potential source of the toxic cyanide compounds. Seed extracts have been demonstrated to exhibit properties against gram-positive bacteria. Papaya leaf and seed extracts exhibit significantly high levels of antifungal activities. Antifungal properties of papaya latex can be attributed to the presence of glycosidase compounds, which may cause a degradation of the fungal cell wall. Studies have demonstrated the synergistic effects of papaya latex with antifungal drugs, which could

potentially reduce the dose of such drugs administered to treat fungal diseases. Alcohol extracts of papaya leaves and seeds exhibit antifungal activity against a number of plant pathogens and could have potential applications for reducing postharvest diseases that lower fruit quality in storage and during transit.

Papaya extracts have been actively studied for their hypoglycemic properties. Papaya fruit extracts administered to test animals significantly reduced blood sugar and lipid levels. Similar results were observed with aqueous extracts of papaya seed. The hypoglycemic index of papaya leaf extracts revealed that an aqueous leaf extract significantly decreased blood glucose levels in diabetic rats and was attributed to either a decrease in intestinal glucose absorption or an increase in peripheral glucose utilization.

Crude extracts of papaya tissues are effective in controlling parasitic infections and have been attributed to the cysteine proteinases that are present in the latex that is released upon injury to the plant. Administration of extracts containing papaya latex was effective in controlling intestinal parasites in sheep and pigs. Both studies indicated the potential use of papaya latex as an oral application for controlling human parasites that were transmitted via soil. Treatment of free-range chicken with papaya latex significantly reduced nematode infections and resulted in a subsequent increase in weight gains and could be used to substitute currently used chemicals for nematode control in poultry. The hydrolase enzyme tightly bound to the water-insoluble fraction of crude papain, papaya lipase, is considered as a 'naturally immobilized' biocatalyst. Crude extracts of papaya seed were found to be effective in controlling pathogenic parasites of fish and could have potential application to reduce diseases occurring in aquaculture. In other studies, papaya seed extracts were effective in treating intestinal parasites in children without any apparent side effects.

Herbal preparations of papaya extracts have been evaluated for their ability to treat burn-induced wounds. Topical application of fermented papaya extracts significantly reduced inflammation in rats with burn wounds and accelerated wound healing. Such effects were attributed to the antibacterial and antioxidant activities of the extract applied to the wound region. The proteolytic fraction of latex from immature fruit of mountain papaya exhibited potential for skin regrowth of wound tissues. Similar properties were exhibited by latex obtained from *C. papaya*.

Fermented papaya extracts are reported to possess antiviral and neuroprotective properties. Extracts have also been used to treat malaria and amoebiasis. Crude extracts of papaya tissues are also reported to have galactopoietic and contraceptive activities. Crude papaya latex could be used as a catalyst for the synthesis of triacylglycerols resembling human milk via transesterification of vegetable oils.

See also: Antioxidants: Role on Health and Prevention; Bioavailability of Nutrients; Carotenoids: Occurrence, Properties and Determination; Carotenoids: Physiology; Colors: Health Effects; Fermented Foods: Fermented Vegetables and Other Products.

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Parasites in Food: Illness and Treatment

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Introduction

Foodborne diseases are a major cause of morbidity and mortality worldwide, representing a major public health problem due to their multiple implications. Knowledge of infectious etiological agents transmitted due to contamination of food consumption represents a major challenge in terms of control policies on the handling and marketing of food. Unfortunately, most of them are due to pathogens predominantly present and prevalent in developing countries and are the cause in many cases of diseases considered as neglected. Neglected diseases typically occur in developing countries, imposing a significant estimated burden. However, because in most of those countries such diseases are not notifiable and under surveillance, the real impact in terms of burden of public health can be difficult to determine.

Foodborne diseases can be caused by a wide range of etiological agents, including bacteria, fungi, viruses, parasites, and even prions. The relevance of foodborne parasitic diseases, caused either by helminths or protozoans, has been highlighted during recent years. In 2014, the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations, initially focussing on the ten foodborne parasites with the greatest global impact, developed a joint report 'Multicriteria-Based Ranking for Risk Management of Foodborne Parasites,' based on the burden on human health and other factors and including information on where they can be found. The top ten are

1. *Taenia solium* (helminth: cestode; pork tapeworm): In pork
2. *Echinococcus granulosus* (helminth: cestode; hydatid worm or dog tapeworm): In fresh produce
3. *Echinococcus multilocularis* (helminth: cestode; a type of tapeworm): In fresh produce
4. *Toxoplasma gondii* (protozoa): In meat from small ruminants, pork, beef, game meat (red meat and organs)
5. *Cryptosporidium* spp. (protozoa): In fresh produce, fruit juice, milk
6. *Entamoeba histolytica* (protozoa): In fresh produce
7. *Trichinella spiralis* (helminth: nematode; pork worm): In pork
8. *Opisthorchiidae* (helminth: trematode; family of flatworms): In freshwater fish
9. *Ascaris* spp. (helminth: nematode; small intestinal roundworms): In fresh produce
10. *Trypanosoma cruzi* (protozoa): In fruit juices

However, this is a global approach. In some countries and regions, some of them can be of higher relevance (e.g., in rural areas of Brazil *Trypanosoma cruzi* can be more risky than *Opisthorchiidae* which is not even endemic in the country; or the opposite in countries of Southeast Asia, where *Opisthorchiidae* infections can be prevalent but *Trypanosoma Cruzi* is not present).

Even more, its incidence and presentation depend largely on the sociocultural conditions of affected populations, such as feeding habits, and the main determinants of living and social conditions. This article aims to review the main pathophysiology, clinical manifestations, and etiological management of some of the most important foodborne parasitic diseases, five of the mentioned top ten risky foodborne parasites (*Taenia*, *Toxoplasma*, *Cryptosporidium*, *Trichinella*, and *Trypanosoma cruzi*), and three additional that are highly important in terms of prevalence, not just in developing countries but also in developed regions (*Giardia*, *Cyclospora*, and *Sarcocystis*).

Taeniasis and Cysticercosis

Cysticercosis is considered as one of the most fatal but also preventable parasitic diseases, caused by the larval stages of *Taenia solium* (cysticerci). The global burden of the disease and the possible clinical outcomes make cysticercosis an important foodborne and zoonotic disease that requires more attention, especially from the public health sector.

Transmission Mechanism and Pathophysiology

The transmission of *Taenia solium* is usually reported in well-known rural endemic areas like Asia, Africa, and Latin America, but lately it has been reported as an emerging infectious disease in some developed countries. Infection by *Taenia solium* larvae (cysticerci) occurs through the consumption of pig meat containing the parasites, by person-to-person transmission, or by autoinfection. In the case of taeniasis (intestinal infection caused by *Taenia*), once a human is infected by ingesting raw or undercooked cysticerci-infected meat, the adult form of this parasite can reside in the human intestine, where they reproduce into eggs. In a second cycle, these embrionated eggs can be ingested, and the oncospheres hatch, penetrate the intestinal wall, and disseminate to several tissues, developing cysts or cysticerci. The cyst can occur anywhere, but the most common localizations include muscles, subcutaneous tissues, or the brain.

Clinical Signs

A symptomatic patient with cysticercosis usually develop neurocysticercosis, in which seizures are the most common

manifestation. There are also several manifestations including headaches, neurological signs, or hydrocephalus. The diagnosis includes the traditional radiological finding of cerebral calcifications.

Treatment and Prevention

Treatment, particularly of neurocystocercosis, is complex, but is mainly focussed on the control of the seizures. The whole therapy includes anticonvulsants and steroids. The administration of anthelmintic drugs, especially albendazole, is considered the first-line treatment, with praziquantel as a second-line treatment.

Possible fatal outcomes in neurocysticercosis give this zoonotic disease a high relevance. Its epidemiology includes occurrence in developing and developed countries, and it is important that health authorities focus on the prevention and ultimately the eradication of cysticercosis.

Toxoplasmosis

Toxoplasmosis is considered one of the most important and common parasitic diseases worldwide. It is caused by a protozoan, *Toxoplasma gondii*, which owes its high global prevalence to its capacity of infecting all animal species and birds. *Toxoplasma* has been commonly associated with congenital disease since the 1930s, but it has taken longer to understand the real epidemiological and clinical implications of its foodborne transmission and its importance to human beings. The life cycle is complex, using the cat as the definitive host and humans as intermediate hosts.

Transmission Mechanism and Pathophysiology

Felids are the key in the life cycle of *Toxoplasma gondii* due to their ability to excrete oocysts (the resistant stage) in their feces. Human infection occurs from the ingestion of contaminated food, principally of undercooked meat containing tissue cysts of *Toxoplasma gondii* or by the accidental ingestion of oocysts from the environment. Meat containing the parasite may be derived from lambs, goats, pigs, and chickens, and environmental transmission occurs via food, soil, or water contaminated by cat feces.

Clinical Signs

Once a human gets the infection it tends to be chronic, with a wide spectrum of clinical manifestations, including an initial subclinical stage, characterized by nonspecific symptoms such as lymphadenopathy, fever, and malaise. The chronic stage is associated with syndromes such as epilepsy, Parkinson's disease, psychomotor deficits, and schizophrenia.

Treatment and Prevention

Toxoplasmosis treatment varies and depends on the severity of the illness, but currently the prevention strategies are playing an important place in the approach to the disease. In adults with mild disease, who have normal immune system function, treatment is rarely required, but in cases of severe disease or in immunosuppressed persons, a combination of antiparasitic drugs is a necessary intervention.

As part of a prevention strategy, the US Department of Agriculture recommends adequate cooking of food. For example, beef meat should be cooked at 71.1 °C (160 °F) for at least 10 min, and others like pork or lamb should be cooked at 62.8 °C (145 °F) or higher for at least 10 min, all of them with a 3 min rest at environmental temperature after the cooking process. Other recommendations include that all fruits and vegetables should be washed and peeled before eating, pasteurized milk should always be consumed, and untreated water should not be consumed.

Cryptosporidiosis

Cryptosporidiosis results from infection by *Cryptosporidium parvum* (Figure 1), a coccidian parasite. Worldwide, the prevalence is 1-4.5% in developed countries and 3-20% in developing countries.

Transmission Mechanism and Pathophysiology

Transmission is usually by the fecal–oral route but can also occur by aerosols. Sporulated oocysts are excreted in the feces throughout the illness and for several weeks afterward and are immediately infectious; they may survive for 2-6 months in a moist environment. Contaminated food or water can result in outbreaks, and transmission can also occur via contaminated fomites.

The largest outbreaks of cryptosporidiosis have been attributed to contaminated drinking water, from surface water, groundwater sources, recreational water, and swimming pools. Also outbreaks have occurred through consumption of contaminated food (raw milk and meat, farm-made apple

Figure 1 *Cryptosporidium*, acid-fast staining.

cider, fermented milk, salads, raw vegetables). Oocysts have also been found in shellfish, such as oysters.

Clinical Signs

Cryptosporidiosis is clinically characterized by profuse, watery diarrhea with cramping, abdominal pains, nausea, anorexia, flatulence, and malaise. Some individuals may also experience vomiting, weight loss, fever, or myalgia. It is generally self-limiting, resolving after 2-4 days, but in those who are immunosuppressed it may be chronic, debilitating, and severe.

Treatment

Drinking plenty of fluids to prevent dehydration is the main treatment strategy. Nitazoxanide has been FDA-approved for treatment in people with healthy immune systems.

Trichinellosis

Trichinellosis is caused by the nematode *Trichinella* spp., whose transmission to humans occurs by the ingestion of raw or undercooked meat from infected mammals, mainly pigs. However, other potential sources include horses, dogs, foxes, walruses, wild boars, and bears in countries where the consumption of these animals is popular.

Transmission Mechanism and Pathophysiology

Once the female *Trichinella* nematode releases the larvae in the intestinal mucosa of the host, they are disseminated by lymphatic and blood streams to the striated skeletal muscle, where they develop into infective muscle-stage larvae and may remain there for several years until the tissue is consumed by a new host. After the ingestion of contaminated meat, the larvae are released in the gastrointestinal tract of the new host, where they reach their adult stage and start reproducing. After a couple of weeks an immune response occurs in the host across the intestinal mucosa, which ultimately triggers the symptoms that characterize the intestinal phase of trichinellosis which has the possibility to migrate through the blood vessels into the striated skeletal muscle or systemic parenteral triggering phase trichinellosis.

Clinical Signs

The clinical spectrum of trichinellosis can range from a completely asymptomatic course to death. The clinical manifestations are closely related with the amount of inoculum, the response of the host, and the time of initiation of an appropriate treatment. The inflammatory response produced after infection occurs via Th2 cells, eosinophils being frequently found to determine the intensity and the presence of the severe clinical manifestations. The release of histamine and prostaglandins plays a major role in the inflammatory response, as their presence is closely related with clinical manifestations such as edema (for increased capillary permeability), urticarial reaction, and vasculitis. During the acute phase associated symptoms are nonspecific, including fever for 1-3 days, headache, chills, malaise, and gastrointestinal symptoms such as diarrhea and abdominal pain. Other important manifestations include facial edema, conjunctivitis, vasculitis, subconjunctival bleeding, petechiae, eosinophilic myocarditis (associated with sinus tachycardia), elevated cardiac biomarkers, and nonspecific conduction disturbances. Ventricular repolarization and thromboembolic disease are some of the more severe complications, which are presented during the systemic phase. Pain associated with eye movements is caused by infiltration of the extraocular muscles. In the chronic phase symptoms may appear as encephalitis and neurological manifestations and to a lesser extent, multiple cortical infarcts, anisocoria, facial paralysis, and Babinski reflex.

Treatment

Anthelmintics drugs of choice for treatment of trichinellosis are albendazole, as first-line drug. Another alternative includes mebenzazole; however, these drugs are contraindicated during pregnancy. Although pyrantel has no effect on newborn larvae and those who live on the muscle, it can be used in pregnant women and children in two 10-20 mg kg^{-1} doses separated by 2-3 days of dosing.

Chagas' Disease

Chagas' disease is caused by the protozoan parasite *Trypanosoma cruzi*. Transmission occurs mainly through triatomine bug vectors typically found in homes built with mud, so the disease tends to affect poor countries. The disease can also be acquired congenitally, by blood transfusions, by organ transplants, through biological accidents, and orally due to the intake of food contaminated with the parasite (due to feces of triatomine and complete triatomines). This has been particularly reported in fruit juice in Brazil and Venezuela, but also in other South American countries.

Transmission Mechanism and Pathophysiology

According to WHO, the definition of a case of Chagas' disease by oral transmission involves having excluded other forms of transmission, having epidemiological evidence that the affected were exposed to food or drink contaminated with the parasite, and the absence of chagoma. Oral transmission occurs by direct consumption of triatomines, breastfeeding, or consumption of food or beverages that have been contaminated by the feces of infected triatomine or infected secretions from the anal glands of marsupials. Some studies have estimated that the parasite can survive in such food between approximately 12 and 24 h before being consumed. Once ingested, the infection would occur through contact of metacyclic trypomastigotes (infecting form of the parasite) in the gastric mucosa, where the severity of the infection will depend on the amount of the seed and the host defense mechanisms at this level.

Clinical Signs

The oral transmission of Chagas' disease is usually associated with high severity and mortality. In the reported series signs and symptoms have been frequently dyspnea, fever, periorbital edema, headache, myalgia, cough, hepatomegaly, chest pain, abdominal pain, vomiting, palpitations, and edema in the lower limbs. The ECG findings include right bundle branch block, sinus bradycardia, and atrial fibrillation, while usually evident on echocardiography pericardial effusion, mitral or tricuspid insufficiency, impaired regional contractility, and/or left ventricular systolic dysfunction.

Treatment

The etiologic treatment may be with nifurtimox or benznidazole, the latter being the drug of first choice. The same is given at a dose of 5 mg kg^{-1} day^{-1} with a maximum dose of 300 mg daily for 60 days and can be extended for longer in patients weighing more than 60 kg. Such treatment should be initiated as soon as possible because the time of onset influences the prognosis. Currently multiple candidate drugs, used in other infectious and noninfectious diseases, are being tested and with anti-*Trypanosoma* activity, such as new antifungal azoles and some anti-arrhythmic drugs.

Giardiasis

Giardiasis, a gastrointestinal disease characterized by acute or chronic diarrhea, is caused by protozoan parasites of the genus *Giardia*. *Giardia intestinalis* is the only species known to cause illness in humans. This organism is carried in the intestinal tracts of many animals and people, which are considered to be the most important reservoir hosts for human giardiasis. Recently environmental and particularly climate conditions appears to be significantly associated with the epidemiology of giardiasis.

This infection is more prevalent in developing countries; the infection rate was typically 8-30% higher in children. Among adults, the prevalence was reported as with lower incidence. Approximately 60-80% of *G. intestinalis* infections are asymptomatic. Chronicity has been reported to occur in less than 4% of the patients.

Transmission Mechanism and Pathophysiology

Giardia spp. has two stages, cysts and trophozoites. The infection is acquired by ingesting cysts, which are excreted in the feces. Trophozoites are released from the cyst in the small intestine, where they multiply. Many of them are carried toward the colon and encyst along the way, in response to bile salts and other stimuli. Cysts can appear in the feces from 3 days to 3 weeks after infection.

Giardia cysts can be transmitted directly between hosts or on various fomites including contaminated water or food, which are responsible for many outbreaks; over 160 waterborne outbreaks of giardiasis and cryptosporidiosis have been reported, with the greatest documentation in the United States and United Kingdom. Individuals at increased risk include those who drink contaminated water or swallow surface water during recreational activities, children (especially in day care facilities), travelers to regions where human infections are prevalent, men who have sex with other men, and contacts of infected individuals.

Food-related outbreaks have been reported less frequently and often involve contamination by a food handler. Person-to-person transmission can propagate outbreaks, especially among young children.

Clinical Signs

Most *G. intestinalis* infections are asymptomatic, with affected individuals either clearing the infection spontaneously or shedding cysts subclinically.

Symptomatic cases are characterized by mild to severe gastrointestinal signs; some patients develop acute or chronic diarrhea, with loose, foul-smelling stools. The feces may have a greasy appearance, but blood is rarely seen. Other symptoms may include abdominal cramps or pain, bloating, flatulence, nausea, anorexia, vomiting, weight loss, or dehydration. Most infections are self-limited and last for a few days to a few weeks; however, cases may occasionally persist for months or even years. Chronic infections may lead to malabsorption syndromes that can include disaccharide deficiency and vitamin deficiencies, as well as severe weight loss and debilitation. Fatal cases have been related with extreme dehydration, mainly in infants or malnourished children.

Treatment

Giardiasis can be treated with a number of drugs; however, the best clinical results are with the nitroimidazole drugs: metronidazole, tinidazole, and ornidazole, which are highly effective. The recommended dosing for metronidazole is 250 mg t.i.d for 5-7 days; pediatric dose is 5 mg kg^{-1} t.i.d for 5-7 days. For tinidazole the adult dose is 2 g in a single dose, and the pediatric dose is 50 mg kg^{-1} in a single dose. Most common adverse effects of these drugs are headache, vertigo, nausea, metallic taste, urticaria, and disulfiram-like reaction with alcohol ingestion. Supportive care, such as fluid and electrolyte management, may also be necessary. Asymptomatic cases may be treated to reduce transmission of the organism.

Prevention strategies include good hygiene, such as hand washing, avoidance of fecal contact with skin or mucous membranes during sexual activity, and washing vegetables and fruits before eating them. Also, people with giardiasis should not swim in recreational water for at least 2 weeks after the symptoms end. In water, *G. intestinalis* cysts can be killed by a rolling boil maintained for 1 min.

Cyclosporiasis

Cyclosporiasis is an intestinal illness caused by the microscopic parasite *Cyclospora cayetanensis*.

Transmission Mechanism and Pathophysiology

The mature oocyst is ingested (infective form), then occurs as asexual and sexual reproductions in the small intestine's epithelium, producing immature oocysts. These oocysts must spend several days in favorable conditions to mature; therefore human-to-human infection is not possible. Transmission occurs by intake of mature oocysts present in water, vegetables, or contaminated food.

Most outbreaks have been related to the consumption of vegetables, raw raspberries and other berries, basil, and raw salads.

Clinical Signs

It causes acute and prolonged diarrhea in immunocompetents, traveler's diarrhea, outbreaks, and chronic diarrhea in immunocompromised patients. Main symptoms are fever $<38\,^{\circ}C$, nausea and occasional vomiting, and diarrhea of varying intensity, approximately five to seven stools per day. It can last 5–14 days but also 1 month or more.

Treatment

Indicated treatment for adults is cotrimoxazole (160/800 mg) twice a day and for children trimethoprim 8 mg kg^{-1} sulfamethoxazole 40 mg kg^{-1} divided into two doses per day, both groups for 7–10 days. In patients with HIV/AIDS, treatment must last until disappearance of symptoms. As an alternative, ciprofloxacin 500 mg twice a day for 1 week can be used.

Sarcocystosis

Sarcocystosis is known as an important zoonotic disease caused by intracellular protozoa of the genus *Sarcocystis*. It is recognized as a common parasite in the musculature of herbivores and carnivores, being described at first in a pig muscle. They have a two life cycle hosts according to prey and predator, being their potential definitive host: dogs, cats, and humans.

Transmission Mechanism and Pathophysiology

The intermediate host development of *Sarcocystis* occurs in both cardiac and smooth muscle that came in contact with humans by eating the meat or by the contamination of soils by feces of the host. Once they get into the human, they can cause a large spectrum of clinical manifestations including muscular *Sarcocystis*, representing the majority of the cases in the tropical or subtropical areas, and intestinal *Sarcocystis*, occurring more frequently in Europe.

Clinical Signs

The symptoms can be gastrointestinal nonspecific manifestations such as vomiting, diarrhea, and abdominal pain, occurring between the first 3-6 h after eating the meat. More serious symptoms include cardiomyopathy and bronchospasm.

Treatment and Prevention

There is no specific treatment for *Sarcocystis*, so prevention is the principal key of control of this disease. An adequate cooking process of the meat and not drinking untreated water are important strategies for prevention. A few drugs like sulfadiazine and pyrimethamine have demonstrated some efficacy.

Conclusion

Even though, parasitic diseases represent an important public health problem mainly in developing countries, little attention is given by governments and communities. Multiple routes of transmission have been described for each microorganism; waterborne and foodborne transmission are not the main route. However, because they are responsible for the occurrence of outbreaks, these transmission routes are the most important for public health, and efforts should be addressed to control them. Although environmental monitoring of water and food, and regulations to minimize protozoan parasite contamination of potable water have been implemented in many countries, the potential for contaminated food and water to cause extensive outbreaks still remains, due to the breadth of populations served.

See also: Food and Agriculture Organization of the United Nations; Food Poisoning: Classification; Food Poisoning: Epidemiology; Food Poisoning: Tracing Origins and Testing; Foodborne Pathogens; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Parasites in Food: Occurrence and Detection.

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Parasites in Food: Occurrence and Detection

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Introduction

Despite causing a high burden of disease and resulting in considerable suffering, parasitic infections have generally not received the same level of attention as other foodborne hazards. Nevertheless, in September 2012, an expert meeting was convened by FAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization) that attempted to rank foodborne parasites in order of importance from a global perspective and identify most relevant transmission vehicles.

A total of 24 parasites (species, genera, or families) were ranked as listed in Table 1. Weighting was predominantly driven by public health importance; other factors included global distribution, potential for increases in disease rates, trade impacts, and socioeconomic effects of infection.

As demonstrated in Table 1, all classes of endoparasites are covered, with eight protozoa, seven nematodes (roundworms), five cestodes (tapeworms), and four trematodes (flukes) listed. Although protozoa and nematodes predominate, the three parasites ranked as being of greatest importance are all cestodes, due to the potentially severe pathology that may be caused when humans act as accidental, aberrant intermediate hosts in the parasites' life cycles.

Among the range of food matrices that are of relevance for these parasites, all the major food types are included, such as meat, fresh produce, fruit juice, milk, and fish. The latter includes both freshwater fish and marine fish, depending on parasite species. And it is also worth noting that for the Anisakidae, which includes the species *Anisakis simplex*, *Anisakis pegreffii*, and *Pseudoterranova decipiens*, the potential hazard to human health is limited not only to the possibility of infection occurring but also to the potential for gastroallergic reactions. Such reactions, which may be severe, are usually facilitated by a hypersensitivity reaction, most frequently to a major secretory/excretory antigen/allergen of the Anisakidae, and may even be activated if the parasite is not alive.

In the following sections, the eight most highly ranked foodborne parasites are described in greater detail. For each parasite, a brief overview of the parasite is provided (life cycle, prevalence, global distribution, and disease) and then some details on foodborne infection, including, where applicable, a description of methods of detection in the relevant food matrices.

The Highest-Ranked Foodborne Parasites

Taenia solium

Taenia solium is also known as the pork tapeworm because transmission to humans, the definitive host in the life cycle, is by ingestion of infective larval cysts in undercooked pork. Prevalence of *T. solium* infection varies greatly according to local practices regarding consumption of pork (occurs very rarely in Muslim or Jewish communities), as well as pig husbandry practices and general sanitation. *T. solium* is endemic in several developing countries, including Central and South America, sub-Saharan Africa, Southeast Asia, and Western Pacific. It is considered an emerging disease in United States and parts of Europe due to increased immigration and international travel; over 50 million people are estimated infected worldwide.

Although the sheer size of adult *T. solium* (between 2 and 7 m in length) inhabiting the human intestine sounds disagreeable, taeniasis produces only mild abdominal symptoms, the main clinical sign being passive passage of proglottids – the mature segments of tapeworm that pass out with the feces and contain around 50 000 eggs each. These eggs are infective to pigs and, when ingested, hatch in the intestine, invade the intestinal wall, and migrate to the tissues where they encyst into cysticerci. The cysticerci are infective to humans and, when ingested, develop into adult worms in the intestine, thereby completing the life cycle.

If *T. solium* eggs are ingested by humans (not pigs), they also hatch and invade the intestinal wall and migrate to the tissues and develop into cysticerci. They are more common in subcutaneous tissues but are also relatively common in the liver, eyes, and central nervous system (CNS). This clinical disease, cysticercosis, is far more serious than taeniasis, with symptoms resulting from the development of cysticerci. The most important clinical manifestation occurs when the cysticerci are in the CNS. This disease, neurocysticercosis, can result in various symptoms including epilepsy-type seizures, mental disturbances, and death. Ocular, cardiac, or spinal lesions and associated symptoms occur when cysticerci occur in those organs.

While taeniasis is exclusively a foodborne infection, associated with the consumption of undercooked pork containing the larval stages (cysticerci) of the parasite, cysticercosis requires consumption of eggs excreted from an infected human. This can be direct hand-to-mouth or via a vehicle such as food. It is particularly likely to occur when human excreta (night soil) is used for manure on vegetables that are not cooked before consumption.

Routine meat inspection of pigs for cysticerci is conducted at slaughter, and, if detected, the meat is condemned (or, in countries where infection is common, might be passed for cooking). The cysticerci are around 10×5 mm (pea size) or larger. Although relatively easy to see, it should be noted that in countries where they are not expected, inspection may be cursory and light infections may be overlooked.

No methods are in place for inspecting vegetables or other possible transmission vehicles for *T. solium* eggs; reduction of taeniasis by appropriate control of pork is a sensible approach to reducing cysticercosis and also encouraging thorough cooking to inactivate the tissue cysts (heating to a core temperature of at least 60 °C). Tissue cysts are also inactivated by freezing to

Table 1 Foodborne parasites ranked in order of global importance

Parasite	*Group*	*Order of ranking*
Taenia solium	Cestode	1
Echinococcus granulosus	Cestode	2
Echinococcus multilocularis	Cestode	3
Toxoplasma gondii	Protozoa	4
Cryptosporidium spp.	Protozoa	5
Entamoeba histolytica	Protozoa	6
Trichinella spiralis	Nematode	7
Opisthorchiidae	Trematode	8
Ascaris spp.	Nematode	9
Trypanosoma cruzi	Protozoa	10
Giardia duodenalis	Protozoa	11
Fasciola spp.	Trematode	12
Cyclospora cayetanensis	Protozoa	13
Paragonimus spp.	Trematode	14
Trichuris trichiura	Nematode	15
Trichinella spp.	Nematode	16
Anisakidae	Nematode	17
Balantidium coli	Protozoa	18
Taenia saginata	Cestode	19
Toxocara spp.	Nematode	20
Sarcocystis spp.	Protozoa	21
Heterophyidae	Trematode	22
Diphyllobothriidae	Cestode	23
Spirometra spp.	Nematode	24

Source: WHO/FAO (2014). Multicriteria-based ranking for risk management of foodborne parasites. Report of a Joint FAO/WHO Expert Meeting, 3–7 September 2012, FAO Headquarters, Rome, Italy. Microbiological Risk Assessment Series. ISBN 978 92 4 156470 0 (WHO); ISBN 978-92-5-108199-0 (print) (FAO); E-ISBN 978-92-5-108200-3 (PDF) (FAO); ISSN 1726-5274.

−10 °C or lower for at least 10 days. Additionally, use of human excreta for fertilizer on crops should be discouraged, particularly in places where taeniasis is endemic.

Echinococcus granulosus

Echinococcus granulosus is a small (3–5 mm) tapeworm, also called dog dwarf tapeworm, with canids as definitive hosts. A range of mammals are suitable as intermediate hosts, including sheep, goats, buffalos, cattle, camels, pigs, kangaroos, and cervids. Eggs passed in the feces of an infected dog may be ingested by an intermediate host, and the oncosphere that hatches in the intestine penetrates the intestinal wall and migrates into various organs, especially the liver and lungs. Here, it develops into a hydatid cyst that gradually enlarges, producing protoscolices and daughter cysts that fill the cyst interior. Canids are infected by consuming parts of an animal that contains these cysts. A common source of infection for dogs is offal from infected livestock.

Infection of humans occurs due to accidental ingestion of *E. granulosus* eggs from dog feces and results in the disease cystic echinococcosis (CE). The strain usually associated with human infection is the common sheep strain (G1). Transmission is influenced by extent of domestic and wildlife reservoirs and is closely affected by human activities, behavior, environmental factors, and local policies.

E. granulosus has a global distribution; endemic foci are found on every continent. However, local distribution and prevalence depend on the presence of large numbers of sheep, cattle, goat, or camel flocks (intermediate hosts) and close contact between them and canine definitive hosts. Prevalence of CE is highest in temperate zone countries, including several parts of Eurasia, Australia, some areas of America (especially South America), and North and East Africa.

Clinical manifestations of CE are variable and determined largely by site, number, and size (can be over 20 cm diameter) of cysts. Strain of parasite may also affect the symptoms. Nevertheless, unless cysts are located in the brain or eye, clinical manifestations of most cases of CE are generally mild for a considerable period. Most cysts (>65%) are located in the liver, followed by the lungs (25%), but cysts have also been reported from the spleen, kidneys, heart, bone, and CNS. For cysts in the liver, signs include hepatic enlargement, right epigastric pain, nausea, biliary duct obstruction, and vomiting. Pulmonary involvement can result in chest pain, cough, and hemoptysis. Although CE is rarely fatal, death may occur due to anaphylactic shock or cardiac tamponade. Furthermore, if a cyst ruptures, release of cyst contents can cause allergic reactions and produce fever and mild to fatal anaphylactic shock. It has been estimated that CE results in the loss of 1–3 million disability-adjusted life years (DALYS) per annum.

E. granulosus is not strictly a foodborne pathogen, and contact with infected dogs seems to be a common risk factor for infection. However, the eggs are highly resistant to environmental factors, remaining infective for many months in a moist environment. Thus, if eggs contaminate a food product that is not cooked before eating, then food may act as a transmission vehicle.

There are no data on the occurrence of *E. granulosus* eggs on food, and there is no standard method for analyzing food products for these eggs. Minimizing *E. granulosus* infection is directed toward breaking the life cycle. The main approaches are frequent deworming of dogs in endemic areas and not feeding raw offal to dogs or leaving raw offal accessible to scavenging canids.

Echinococcus multilocularis

The fox tapeworm, *Echinococcus multilocularis*, has a similar life cycle to that for *E. granulosus* but is mostly associated with a sylvatic life cycle, with foxes usually serving as definitive hosts. Other canids (e.g., dogs, raccoon dogs, wolves, and coyotes) may also act as definitive hosts. Various different genera of rodents (also some lagomorphs) may act as intermediate hosts, being infected by ingestion of eggs released from the tapeworms in the definitive hosts. A number of nonrodent mammals, including humans and pigs, may also be infected as aberrant or accidental intermediate hosts; in humans, this may result in the disease state known as alveolar echinococcosis (AE).

Data on the prevalence of AE in humans are scattered and patchy, probably partly due to diagnostic challenges, particularly in early stages of infection. *E. multilocularis* infection apparently does not occur in Australasia, Africa, and South or Central America, but countries in Asia and Europe, as well as North America, are important endemic areas. In particular, Russia and adjacent countries, various regions in China, and the Japanese island of Hokkaido are of importance. By far the

largest numbers of human cases are reported from foci in China, with prevalences ranging up to 4% in Gansu and northwestern Sichuan. Although the number of human cases of AE in Europe remains low, there appears to be widespread, expanding prevalence in wildlife and trends of AE in humans appear to follow parasite abundance in wildlife.

While the tiny (3–6 mm) adult *E. multilocularis* tapeworms normally cause little harm to canine definitive hosts, in intermediate hosts, including humans, ingested eggs develop to oncospheres, which penetrate the intestinal wall and are carried via blood to, in particular, not only the liver but also other organs where they form multilocular cysts. From ingestion of eggs to onset of clinical symptoms in people may be from months to years, or even decades, depending on cyst location and speed of growth. In most AE cases, metacestodes of *E. multilocularis* initially develop in the liver. These cysts, varying from a few millimeters to 15–20 cm or more in diameter, can also reproduce aggressively by asexual lateral budding. This gradual invasion of adjacent tissue in a tumorlike manner is the basis for the severity of AE. Metacestodes may also spread from the liver to other organs, such as the lungs, spleen, heart, and kidney. Symptoms of severe hepatic dysfunction appear in the advanced clinical stage, in addition to symptoms from other affected organs. While there is negligible acute morbidity, the chronic morbidity of AE is severe and potentially fatal.

As with CE, the proportion of AE cases that are actually foodborne is difficult to estimate, as diagnosis usually occurs long after infection; it is difficult to associate an infection with a foodborne event many years previously. However, the tapeworm eggs may contaminate various types of food, including fruits and vegetables. Although sensitive to desiccation and heat, the eggs are extremely tolerant of environmental conditions, including cold; freezing at −20 °C does not affect egg infectivity. Thus, there is a large potential for foodborne infection via raw produce. However, there are currently no tools for detecting *E. multilocularis* eggs on food. Current control regimes include deworming of dogs in endemic areas, monitoring prevalence in wild canids, and, in some areas, treating wild canids with impregnated bait.

Toxoplasma gondii

Toxoplasma is a protozoan parasite, infectious to practically all warm-blooded animals, including humans, livestock, birds, and marine mammals. Although the genus has only one species, *Toxoplasma gondii*, there are different genetic groups of differing virulence. *T. gondii* is perhaps the most widespread protozoan parasite affecting humans; between 1 and 2 billion of the world's population have been estimated to be infected at any one time. It should be emphasized that most of these infections do not cause clinical illness.

The life cycle of *Toxoplasma* is somewhat complicated, containing two distinct cycles, a sexual cycle and an asexual cycle. The definitive hosts are members of the cat family (Felidae); thus, the sexual cycle occurs only within the intestinal epithelial cells of felids. Oocysts are excreted unsporulated (uninfectious) in cat feces. Oocyst sporulation in the environment usually takes around three days, depending on factors such as temperature and humidity. Oocysts are environmentally robust and can retain infectivity in a cool damp environment for months.

The asexual cycle occurs when consumption of tissue cysts (see succeeding text) or oocysts results in infection of the intestine, and the tachyzoite form of the parasite multiplies asexually in the cells of lamina propria until the cells rupture, releasing tachyzoites into the surrounding tissues and resulting in systemic infection. Circulating tachyzoites infect new cells throughout the body, and after several more rounds of asexual division, tissue cysts are formed. Tissue cysts of *T. gondii* are microscopic (5–100 μm) and contain bradyzoites, which are infectious when ingested with the surrounding tissue. If ingested by a felid, then the sexual cycle occurs and the life cycle is complete; if ingested by any other host, then the asexual cycle occurs, as previously described. If a female host is pregnant when first infected, then circulating tachyzoites may move to the fetus (intrauterine or congenital transmission).

The clinical picture of *Toxoplasma* infection is greatly influenced by the immune status of the infected person and also by the virulence of the parasite strain. In immunocompetent people, *T. gondii* infection is usually asymptomatic but may cause mild to moderate flu-like illness. In some cases, ocular toxoplasmosis may occur, possibly with partial or total loss of vision. Ocular disease appears to be more severe in South America, presumably due to more virulent *Toxoplasma* genotypes. Although latent *Toxoplasma* infection is generally considered benign in the immunocompetent, some studies indicate that the parasite may affect behavior, being a contributory, or even causative, factor in various psychiatric disorders including depression, anxiety, and schizophrenia.

In the immunocompromised and immunodeficient patients, severe or life-threatening disease can result from acute *Toxoplasma* infection or reactivation of previously latent infection. Encephalitis is the most clinically significant manifestation. Congenital toxoplasmosis is another serious potential manifestation, usually occurring when *Toxoplasma* is passed to the fetus from a first infection acquired by the mother immediately before or during pregnancy. Symptoms commonly associated with transplacental infection include spontaneous termination, fetal death, and intracranial calcification. Congenitally infected children that are asymptomatic at birth may suffer from mental retardation or retinochoroidal lesions later in life.

With the exception of congenital transmission, most *T. gondii* infections are considered to be foodborne, although waterborne infection has been suggested to be the major source of *Toxoplasma* infection in developing countries.

There are three potentially infectious stages of *Toxoplasma*: tachyzoites, bradyzoites, and oocysts, two of which – bradyzoites and oocysts – are particularly relevant to foodborne transmission. Bradyzoites may be ingested with tissue of an infected intermediate host, while oocysts may be ingested with any foodstuff that has been contaminated with feces of an infected felid; this is particularly likely for fruit and vegetables that are eaten raw, and *Toxoplasma* oocysts are particularly robust, being able to survive in the environment for weeks or months.

Human infection via bradyzoites in meat depends on various factors, including prevalence of *Toxoplasma* infection in

meat animals (most livestock species are susceptible to infection), cultural factors regarding meat consumption and meat preparation, and factors of the person exposed (e.g., age and immunologic status). Parasite factors are probably relevant also. In some countries, sheep and goats are the most important hosts of *T. gondii* and the main source of infection to humans. In other countries, for example, the United States, lamb and mutton are relatively minor food commodities; of the major meat animal species investigated in the United States to date, pig (particularly organically raised) is the only species frequently found to harbor the parasite. Although poultry are also susceptible to *T. gondii* infection and theoretically pose a source of infection to humans, the relatively limited life span of poultry and the fact that they tend to be well-cooked before consumption limit their importance as sources of infection for humans. Game animals are also considered as potentially important sources of meatborne toxoplasmosis, particularly as such meat is often consumed undercooked; wild boar and venison are particularly implicated in Europe, while consumption of undercooked meat from marine mammals is an important risk factor for human infection in Arctic regions.

There is no systematic monitoring of meat for *Toxoplasma*, as the cysts are very small and can occur in almost all tissues. For high-risk groups, ensuring that meat is thoroughly cooked is important. Similarly, no standard method exists for monitoring for contamination with oocysts, although some research surveys have demonstrated quite widespread contamination.

Cryptosporidium spp.

Cryptosporidium spp. are protozoan parasites that have been reported from a large variety of different hosts, including humans, globally. Around 25 species of *Cryptosporidium* have been identified to date, of which approximately 50% have been reported as infectious to humans. However, most reported human infections involve *Cryptosporidium parvum* (an important zoonotic species, particularly associated with infections in calves) or *C. hominis*, which is found primarily in humans. Other species considered important for human infection in some areas are *C. meleagridis*, particularly in children in South America, but primarily considered as infecting turkeys, and *C. cuniculus*, also associated with infections in rabbits. Although prevalence data are patchy, *Cryptosporidium* infection has emerged as a global public health problem and has been reported from over 100 countries.

The life cycle of *Cryptosporidium* is direct (no intermediate host). When a viable oocyst is ingested by a susceptible host, it excysts in the small intestine where the resultant sporozoites invade epithelial cells and locate epicellularly (within the cell but not within the cytoplasm). Repeat cycles of asexual reproduction result in destruction of these cells and the production of vast numbers of parasites. A sexual cycle results in oocyst production. These are excreted in the feces and are immediately infectious but are also very robust and can survive for long periods under cool, moist conditions.

Cryptosporidiosis is an enteric disease, characterized by watery diarrhea, abdominal pain, and related symptoms. It is self-limiting in immunocompetent individuals, although a high relapse rate has been reported. However, in some immunocompromised patients, the symptoms may become chronic, debilitating and potentially life-threatening; drug development for this vulnerable group has been largely unsuccessful.

Waterborne transmission is important in cryptosporidiosis; numerous waterborne outbreaks of cryptosporidiosis have occurred worldwide due to oocyst contamination of drinking water sources. The largest waterborne illness outbreak of any kind in the United States occurred in 1993 when over 400 000 people acquired cryptosporidiosis in Milwaukee. More recently, around 27 000 people suffered from waterborne cryptosporidiosis in Östersund, Sweden, in 2010.

Although foodborne transmission of cryptosporidiosis is considered much less common than waterborne or person-to-person transmission, it is emerging as a public health issue. Several foodborne outbreaks of cryptosporidiosis have been reported, mainly in United States and northern Europe, and in some instances, tens or hundreds have been infected. Implicated foods have largely been fresh produce (e.g., ready-to-eat spinach, green onions, parsley, lettuce, and salad bar items), but there have also been outbreaks associated with apple cider and fresh milk. Some outbreaks have been attributed to contamination from infected food handlers, but others have implicated other contamination routes. Surveys performed worldwide have reported *Cryptosporidium* oocysts, albeit at low concentrations, on a wide variety of fresh produce items, in irrigation water, and also in shellfish. Different methods have been proposed and used in research products for the identification of *Cryptosporidium* oocysts on fresh produce, often in combination with the detection of *Giardia* cysts. A standard ISO method for the analysis of leafy green vegetables and berry fruits is under development and is based broadly on elution from the product, concentration of eluate, isolation from debris by immunomagnetic separation, and detection using immunofluorescent staining.

The likelihood of contamination of produce can be reduced by implementing appropriate measures both preharvest and postharvest and also at the consumer level. Although robust, oocysts do not survive prolonged freezing at below −15 °C and are killed by cooking.

Entamoeba histolytica

Many species of *Entamoeba* have been described, of which several may inhabit the human intestinal tract, but only *E. histolytica* is a proved human pathogen causing the disease amebiasis, although only around 10% of individuals infected with *E. histolytica* develop symptoms. *E. dispar* is considered apathogenic; *E. moshkovskii* may be pathogenic under some conditions. That these pathogenic and nonpathogenic species are morphologically indistinguishable means that the occurrence of amebiasis was previously overestimated, and therefore, older data or data not obtained with molecular tools are unreliable. Nevertheless, *E. histolytica* is considered to occur globally and is considered a particular health risk in countries where sanitation is poor. Disease burden has been estimated to be approximately 50 million infections, resulting in an estimated 40 000–110 000 deaths, annually.

The life cycle of *E. histolytica* includes the infectious cyst form and the trophozoite, the invasive, disease-causing stage. Following ingestion of an infectious cyst, trophozoites are released in the small intestine and multiply through divisions.

The trophozoites generally migrate to the large intestine, localizing to the cecum and adjacent ascending colon, where encystation occurs. Both trophozoites and cysts may be excreted in the feces, although the former rapidly disintegrate and are not considered infectious. Cysts, however, can survive for considerable periods outside the host, and thus, new infection can occur via contamination of food and water. *E. histolytica* is not considered as zoonotic.

For infected individuals who experience clinical amebiasis, symptoms range from mild colitis to severe invasive and/or disseminated infection. Amoebic colitis includes bloody diarrhea with multiple mucoid stools and abdominal pain. Chronic infection, with persistent nondysenteric diarrhea, has also been reported. Fulminating amoebic colitis occurs in about 0.5% of infections and is a serious form of invasive infection, usually presenting with profuse bloody diarrhea, abdominal pain, and fever. Colonic perforation and peritonitis may occur, and a mortality rate of around 40% has been estimated. Extraintestinal spread of *E. histolytica* trophozoites occurs through the portal venous system. Amoebic liver abscess (ALA) is the most common extraintestinal manifestation, and associated symptoms include fever, right upper quadrant pain, and hepatic tenderness, usually not concurrent with amoebic colitis. Spread to other sites is rare and usually occurs secondarily to ALA; pleuropulmonary amebiasis, amoebic brain abscess, and amoebic skin abscess have all been reported.

Although infection is generally considered to occur most frequently from ingestion of contaminated food or water, only a few outbreaks of waterborne amebiasis have been reported and no foodborne outbreaks. This could be because around 90% of people infected would not experience symptoms. Studies of parasitic infections in food handlers have often reported *E. histolytica* infection, and unhygienic food handling is commonly considered a contamination route. However, those very few studies that have investigated food for a range of parasites, including *Entamoeba*, tend not to report contamination with *E. histolytica* cysts, and no standard methods have been developed for examining food products for this parasite.

Trichinella spiralis

Trichinella spiralis is an intracellular parasitic nematode of mammalian striated muscles, found worldwide in many carnivorous and omnivorous animals. There is no stage in the life cycle that is external to the host (in the environment), and transmission is solely from ingestion of another infected host. Human infections are mainly associated with the consumption of undercooked pork, and the parasite is endemic in pig-breeding/pork-producing facilities in several countries in eastern Europe, Russia (some areas), China (various provinces), Southeast Asia (Laos and Thailand), and South America (except Brazil). Other species of *Trichinella* are also infectious to humans and are often particularly associated with specific geographic regions or species of game animal.

The life cycle commences with the ingestion of encysted larvae in the striated muscle tissue of another infected host. Following exposure to gastric acid and pepsin, the larvae are released from the cysts and invade the mucosa of the small intestine where they develop into adult worms, just a couple of millimeters in length. Hundreds of new larvae are produced and these are spread with the blood around the body, settling in the muscle cells where they become encapsulated into tissue cysts. These infective encysted larvae can survive for years in tissue and also remain infectious for several weeks in dead animals, thus enabling infection of carrion eaters.

Although animal infection with *T. spiralis* is considered asymptomatic, human infection causes a serious clinical disease, trichinellosis, which can cause much suffering and may be fatal. Symptoms of trichinellosis are related to life cycle stages, with enteric, migratory, and muscle phases. During invasion of the intestinal epithelium, abdominal pains, diarrhea, and vomiting may occur. During larval migration, symptoms may include fever, facial edema myalgias, rashes, and tachycardia. Establishment of larvae within the muscle cells and the encystment of muscle larvae are associated with symptoms such as myalgia and severe asthenia. The most frequently affected muscles are those of the cervix, trunk, and upper and lower extremities. The severity of muscle pain reflects the intensity of the infection, and mobility may be restricted. Severe myalgia generally lasts for 2–3 weeks. Although the infection is persistent, as dictated by the life cycle, only a few cases become 'clinically chronic' with recurrent muscle pain. Brain abnormalities have also been reported. Although case mortality is low, for severe infection without treatment, it may rise to over 10%.

Infection with *T. spiralis* can occur only via the foodborne route, and pigs are submitted to compulsory veterinary controls to ensure meat is *Trichinella*-free. EU regulations require that pigs are systematically sampled at the slaughterhouses, with samples taken from the predilection sites and examined by the recommended method, 'the magnetic stirrer method for pooled sample digestion,' which is considered to have the best sensitivity (EU Commission regulation EC 2075/2005; International Commission on Trichinellosis Recommendations). In this method, larvae are identified after artificial digestion of muscle samples from carcasses. Derogations from testing are possible for meat from domestic swine when the pig holdings have been officially recognized as *Trichinella*-free by the competent authorities. This requires compliance with a considerable number of rules. In industrial countries, most marketed pigs are reared in high-containment-level farms and consequently are *Trichinella*-free, but backyard pigs that have more environmental contact are more likely to be infected, and 'organic' pork is also more likely to be infected. Meat from domestic swine that has been frozen under the supervision of competent authorities can also be exempted of *Trichinella* examination.

Prevention of human infection is accomplished by meat inspection, meat processing, and prevention of exposure of food animals to infected meat. Although freezing can kill *T. spiralis* larvae, other species of *Trichinella* are resistant to freezing. Ensuring that pork is thoroughly cooked before eating ensures against infection with both *T. spiralis* and *Taenia solium*.

Opisthorchiidae

The Opisthorchiidae is a family of fishborne zoonotic trematodes that includes the Southeast Asian liver fluke (*Opisthorchis viverrini*), the cat liver fluke (*O. felineus*), and the oriental liver fluke (*Clonorchis sinensis*). For all these flukes, the definitive

hosts are fish-eating mammals (including not only humans but also cats and dogs). Millions of people have been estimated to be infected with these trematodes: approximately 15 million with *C. sinensis*, 10 million with *O. viverrini*, and about 1 million with *O. felineus*. Up to 700 million (10% of the global population) have been considered to be at risk of infection. Geographically, populations in Asia and Europe are most at risk, with *C. sinensis* endemic in southern China, Korea, Taiwan, northern Vietnam, and Russia; *O. viverrini* endemic in areas of Thailand, Laos, Cambodia, and central Vietnam; and *O. felineus* reported from the former USSR and central–eastern Europe, with endemicity in over 12 European countries.

The life cycles of these trematodes include two intermediate hosts; eggs excreted in the feces of the infected definitive hosts are ingested by the first intermediate host, a freshwater snail. In the snail, an asexual cycle occurs that enables considerable multiplication from the single miracidium released from the egg. The final stage is the cercariae, and these emerge from the snail host and seek out the second intermediate host that includes various species of freshwater cyprinid fish (e.g., carp) and penetrate their flesh. Here, they encyst as metacercariae. When these are ingested with undercooked or raw fish, then they excyst in the duodenum and ascend into the biliary ducts, where they develop into adults, later producing eggs and thereby completing the life cycle.

When humans are infected by consumption of fish containing viable metacercariae, the infection primarily results in hepatobiliary pathology, which may subsequently cause bile duct cancer (cholangiocarcinoma (CCA)). Benign hepatobiliary infection may be asymptomatic and is characterized by cholangitis, obstructive jaundice, hepatomegaly, cholecystitis, and cholelithiasis, most of which are mild. In heavy infections, acute symptoms might occur with epigastric pain, fever, jaundice, and diarrhea. However, the main characteristic of these infections is chronic morbidity, with chronic illness occurring in around 10% of infected individuals who may develop severe disease (weakness, abdominal pain, chronic cholecystitis, gall stone, pyogenic cholangitis, and abscess) and also CCA. Once CCA develops, it is usually fatal; curative treatment is not available, and even with surgical treatment, the prognosis is very poor. Most CCA patients survived less than 5 years, and it is a leading cause of death in Asia; both *O. viverrini* and *C. sinensis* are described as type 1 carcinogens.

Opisthorchiidae are transmitted to humans solely via the foodborne route, with the consumption of the flesh of freshwater fish that is undercooked or has been prepared by salting, pickling, or smoking, all of which may be insufficient to inactivate the metacercariae. In some countries, control programs have been instigated. Strategies combine public health aspects with socioeconomic and cultural dimensions. No standard method has been established for examining fish for metacercariae, but researchers have used artificial digestion methods followed by microscopy for identification.

See also: Diarrheal Diseases; Emerging Foodborne Enteric Bacterial Pathogens; Food and Agriculture Organization of the United Nations; Food Poisoning: Tracing Origins and Testing; Foodborne Pathogens; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Parasites in Food: Illness and Treatment; Risk Assessment of Foods and Chemicals in Foods; Water Supplies: Microbiological Analysis; World Health Organization; Zoonoses.

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Parenteral Nutrition

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Definition

Parenteral nutrition (PN) therapy can be defined as a set of therapeutic procedures for the maintenance or recovery of nutritional status in malnourished patients or in those suffering from malnutrition risk either in hospital, ambulatory, or home settings. PN is provided through the intravenous administration of sterile and pyrogen-free solutions or emulsions composed of water, electrolytes, carbohydrates, amino acids, lipids, vitamins, and minerals, and packaged in a glass or plastic container, designed to support the synthesis or maintenance of tissues, organs, or systems.

History

The routine use of PN in clinical practice was initiated in 1968, after Dudrick and collaborators demonstrated the safety and efficacy of the prolonged central vein administration of a parenteral solution containing 50% glucose and 10% amino acids, combined with electrolytes, minerals, and vitamins, in achieving growth and body weight gain in children. Later on, it was found that the PN solution IV-infused with highly concentrated solutions of glucose could cause hyperglycemia, particularly in severely ill patients. This intercurrence was associated with adverse events such as immunosuppression and an increase in complications related to infection. Accordingly, the energy provided by glucose was replaced, in part, by the addition of parenteral fat emulsions. The parenteral administration of fat as a lipid emulsion (LE) formula, in combination with glucose and amino acids in a single bag constitutes the so called all-in-one PN.

Indication

PN is of utmost importance for the nutritional recovery of patients who cannot eat, should not eat, or may not be sufficiently fed by the enteral route. Providing PN perioperatively can reduce the morbidity of seriously malnourished surgical patients, and a meta-analysis of nine randomized clinical trials showed that PN can significantly decrease mortality in critically ill patients. However, the intestinal disuse due to the exclusive use of the parenteral route for nutrients supply can lead to the atrophy of the mucosa, and it can promote increased gut permeability and sometimes bacterial translocation.

Exclusive PN regimens or PN solutions complementary to enteral nutrition (EN) therapy are usually prescribed when oral or EN feeding is impossible, contraindicated, or insufficient. These indications apply to malnourished patients and those at risk of malnutrition with no gastrointestinal tract function, disorders requiring complete bowel rest, or those unable to achieve their energy targets by oral/enteral feeding after 2–8 days, respectively (see Table 1 for absolute and relative indications, and contraindications of PN). While used as a unique food source, the PN solution must contain all necessary macro and micronutrients to ensure the patient's homeostasis.

Indications of PN should also consider its possible negative influence on the evolution of the patient's disease or treatment, and the wishes and needs of patients and their families in critical situations such as terminal cancer without a clear prospect of improved survival or quality of life by using PN. For patients requiring PN for long periods without the need for hospitalization, home PN (HPN) is recommended and should be used in conjunction with EN whenever possible, in order to maintain the intestinal tropism. Indications for HPN have increased worldwide as an alternative to improve the life quality of patients with intestinal failure or insufficiency, but it implies adequate patient, family, and caregiver training, to avoid HPN-related complications, such as metabolic abnormalities, organ dysfunction, and derived infection of the central venous catheter, which is largely responsible for morbidity and mortality in these patients.

Planning

The formulation of the PN must meet the patient's energy–protein requirements and provide essential nutrients in adequate amounts to maintain life, cell, and tissue growth. These nutritional requirements may vary according to nutritional status, the disease, and the metabolic condition of patient, as well as the length of nutritional therapy. Therefore, the planning of PN therapy must calculate the energy and nutrient requirements on an individual basis, according to the patient's clinical condition.

The American Society of Parenteral and Enteral Nutrition (ASPEN) states that adult patients should receive 20–35 kcal kg^{-1} day^{-1}, divided into carbohydrates, proteins, and fats (see Figure 1 for a step-by-step guide). Vitamins, trace elements, and electrolytes for parenteral infusion in adults are offered based on the recommendations of the Dietary Reference Intakes (DRIs), which are accepted as a reference point to find the individual estimation of these micronutrients. As DRIs were formulated based on the needs of healthy subjects, patients with specific diseases may require increased amounts of certain micronutrients for wound healing, tissue recovery, and anti-free-radical production, which may be provided by diluting in saline and infusing these micronutrients through a peripheral vein.

In addition, beside standard formulas of PN, which are composed mostly of amino acids, glucose, lipids, and electrolytes, there are special formulations developed to address the specific needs of morbid conditions that involve metabolic disorders, such as liver and kidney diseases. The special solution for liver failure is rich in branched chain

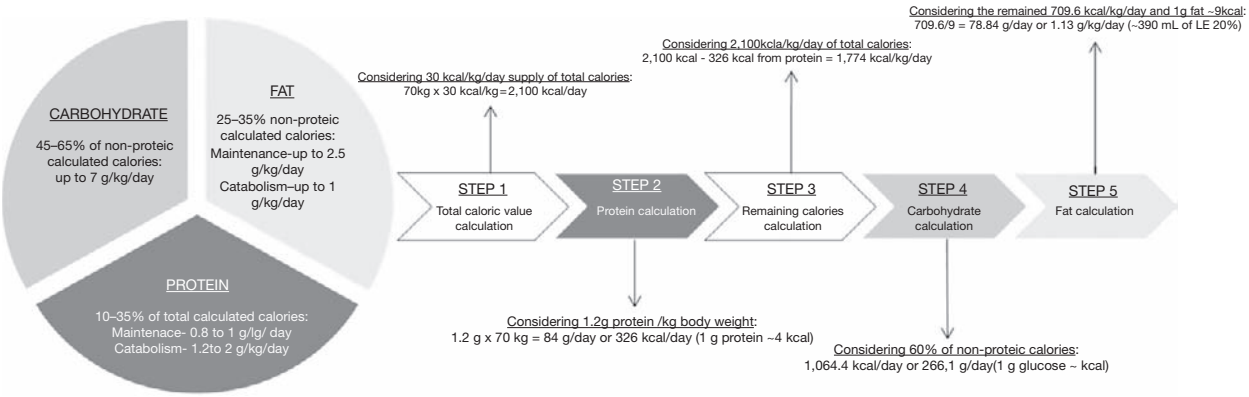

Figure 1 Steps for calorie distribution between carbohydrates, proteins, and lipids during parenteral-nutrition planning. Calculated based on a male patient with a 70-kg body weight and without specific restrictions regarding macronutrients and micronutrients.

Table 1 Indications and contraindications for prescribing parenteral nutrition

Absolute indications	*Relative indications*	*Contraindications*
Impossibility for enteral access due to gastrointestinal obstruction or prolonged ileus	Gastrointestinal bleeding requiring prolonged gastrointestinal rest	Functional gastrointestinal tract
Inability to absorb nutrients through the gastrointestinal tract due to massive intestinal resection (early stage)	Anorexia or severe mucositis due to chemotherapy, radiotherapy, or bone marrow transplantation	Hemodynamic instability
Inability to absorb nutrients through the gastrointestinal tract due to severe short bowel syndrome	Extensive surgery with a prediction of postoperative prolonged ileus for more than 5 days	Allergy to any of its components
Inability to absorb nutrients through the gastrointestinal tract due to active inflammatory bowel disease (requiring 5–7 days of bowel rest)	Severe diarrhea by malabsorption Severe pancreatitis requiring bowel rest for more than 5 days	Severe hyperlipidemia, blood clotting disorders, acute shock, and other unstable conditions (e.g., acute myocardial infarction, stroke, and severe sepsis), when their lipid component is used

amino acids (BCAAs), to increase the BCAA/aromatic amino acids (AAAs) ratio. In this condition, the decreased hepatic metabolism increases plasma levels of AAAs and peripheral metabolism, resulting in a decrease in BCAAs. This situation allows AAA and its metabolites to enter the brain, potentially favoring hepatic encephalopathy.

Special formulations for patients with renal failure are characterized by the highest amount of essential amino acids and histidine; one of them stands out by the addition of arginine, and another by containing small amounts of nonessential amino acids. The design of these formulations is based on the principle that the treatment of patients with chronic renal failure is benefited by offering low-protein diets and essential amino acid supplementation. However, due to metabolic differences between acute and chronic renal failure, offering solutions containing only essential amino acids cannot meet the protein needs of the patient.

Different formulations of LEs are also available as sources of fat in PN therapy and can be infused alone or associated to amino acids and glucose (3:1 system), as the source of non-glucose high-calorie energy and essential fatty acids. The different available LEs are distinguishable by the type and amount of fatty acids present in their composition, based on the availability of essential fatty acids and the property that certain fatty acids have to influence the immune response. Therefore, understanding the biological properties of the different fatty acids that comprise LEs is required to determine those that will contribute to the clinical evolution of the patient.

Critically ill patients are more susceptible to oxidative stress, alterations in cell-mediated immunity, inflammation, and thrombosis. Therefore, these patients may benefit from LEs based on fish oil containing adequate amount of the antioxidant alpha-tocopherol, as source of the potentially anti-inflammatory and antithrombotic omega-3 fatty acids, eicosapentaenoic and docosahexaenoic. On the other hand, LEs based on soybean oil should be avoided in this patient population, because they provide larger amounts of potentially proinflammatory and prothrombotic omega-6 fatty acids.

Another nutrient with pharmacologic functions available for PN is glutamine, which is considered to be an essential amino acid in some clinical conditions (i.e., a conditionally essential amino acid). Glutamine plays a key role in various stages of human metabolism, such as the transport of nitrogen and protein synthesis, and it serves as the energy source for rapidly proliferating cells such as enterocytes and immunologic cells. Due to its low water solubility, the parenteral use of glutamine is commercially available as dipeptides, in which glutamine may be associated with another amino acid, such as alanine (alanil-glutamine) and glycine (glycil-glutamine).

Recommendations suggest a dose of 0.3–0.5 g kg^{-1} of body weight for parenteral supplementation of glutamine; however, this dose may vary according to the individual condition and

patient needs. According to the Canadian Guidelines (2013), when the PN is prescribed to a critically ill patient, glutamine supplementation should be considered. However, the authors strongly recommend that glutamine should not be used for patients with shock or multiple organ failure.

Finally, when planning PN solutions, we must consider that, independent of the formulation, the nutrients are dissolved in water. Therefore, PN can also influence the patient's hydration. Considering that some clinical conditions can increase (i.e., fever, sunburn, and diarrhea) or decrease (i.e., renal and cardiovascular failure, respiratory disorders) the need for water, water content is part of the PN planning in order to provide adequate water amounts based on the patient's metabolic condition.

Administration

After planning the PN, in order to start, the patient must have an adequate central or peripheral venous access. The PN solution is usually administered into a large-diameter venous vessel, accessed via the jugular or subclavian vein. The peripheral route for the infusion of PN should be limited to the administration of PN solutions with low osmolarity (<850 mOsmol l^{-1}) to cover only part of the nutritional needs and mitigate negative energy balances.

PN should not be initiated before unstable conditions, such as hypokalemia, hyperhydration, hypotonic dehydration, metabolic acidosis, and hemodynamic instability, are corrected. There is no definitive available indication to define the ideal moment to start PN infusion, except for critically ill patients, but still without consensus between the available nutrition international society's guidelines. For these patients, the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines recommend starting PN after 24–48 h in subjects, with EN intolerance, who are not expected to achieve adequate nutrition within 3 days. On the other hand, ASPEN guidelines recommend PN before reaching 8 days of hospitalization, with the exception of patients who have malnutrition on hospital admission.

The infusion of PN is the individual responsibility of the nursing staff, who must be trained to properly attend the medical recommendations and follow the good hygiene protocols to ensure adequate and safe infusion. On both the first and last day of PN infusion, a half of the prescribed volume should be infused to allow metabolic, enzymatic, and hormonal adjustment and to avoid the occurrence of abnormalities, such as hyperglycemia and hypoglycemia, and electrolyte disturbances, among others.

Monitoring

During the period of PN infusion, the treatment efficacy should be monitored closely. PN is a complex procedure subject to complications that may jeopardize its benefits. The occurrence of adverse events (mechanical, metabolic, and infectious) and clinical changes that may require changes in the PN formulation should be controlled during PN infusion. In addition to specific laboratory tests to identify the risk of metabolic complications (see the main population risk for metabolic complications in Table 2, as well as the key laboratory tests to identify its risk in Table 3), it is necessary to periodically observe the degree of patient hydration; clinical signs of electrolyte disturbances; the occurrence of edema; changes in consciousness level; the thermal curve; the number

Table 2 Main population risk for developing metabolic complications related to parenteral nutrition

Population	*Metabolic complication*	*Main consequence*
Patients undergoing prolonged partial fast, whose body has adapted to the use of free fatty acids and ketone bodies as energy sources	Hypophosphatemia Hypomagnesemia Hypokalemia	Refeeding syndrome, with impairment of hematologic, neuromuscular, and respiratory functions
Patients with pre-existing diabetes or significant physiological stress	Hyperglycemia	Impairment of immune functions, with an increased risk of infectious complications
Patients receiving parenteral lipid emulsions, either alone or in 3-in-1 systems	Hypertriglyceridemia	Development of pancreatitis and altered pulmonary function

Table 3 Laboratory tests to measure the potential risk in developing metabolic complications related to parenteral nutrition (PN)

	Monitoring from the beginning of PN	
Laboratory test	*Stable patients*	*Critically ill patients*
Sodium, potassium, and chlorine	Three times per week	Daily or at physician criteria
Ionized calcium, phosphorus, and magnesium	Twice weekly	Daily or at physician criteria
Glucose	Daily	At physician criteria
Capillary blood glucose	Daily	Each 6 hours or at physician criteria
Plasma osmolarity	Weekly	Daily
Plasma urea and creatinine	Weekly	Twice weekly
Total protein and fractions	Weekly	Weekly
Total bilirubin and fractions	–	Weekly
Plasma ALT, AST, GGT, and alkaline phosphatase	Twice weekly	Weekly or as required
Hemoglobin, hematocrit	Weekly	Daily
pH and blood gas	–	Daily
Plasma triglycerides	Weekly when using lipid emulsion	Weekly
Urinary glucose	4–6 times per day	4–6 times per day
Density or urine osmolality	2–4 times per day	2–4 times per day
Hydrous balance	Each 12 hours	Each 6 hours

of bowel movements; the abdominal workup; the introduction of nutrients; concomitant pharmacological treatments; signs of PN intolerance; and anthropometric, hematological, and hemodynamic changes, as well as functional changes in organs and systems. Whenever possible, the urine-output monitoring and calculation of water balance should be accessed.

Table 4 summarizes the main PN-related complications, with an emphasis on etiology, symptoms, treatment, and prevention. Complications associated with PN include the risk of developing infections in the central venous catheter. The control of common infection symptoms, such local changes, leukocytosis, fever, and hyperglycemia, may support the early

Table 4 Complications related to parenteral nutrition (PN)

Complications	*Possible etiology*	*Symptoms*	*Treatment*	*Prevention*
Mechanical				
Pneumothorax	Inadequate catheter insertion	Tachycardia, dyspnea, persistent cough, and diaphoresis	Small pneumothorax can resolve spontaneously; larger pneumothorax may require a thorax drain	Catheter placement by experienced professional
Air embolism	Air entering in central venous system by inadequate opening of the infusion system	Cyanosis, tachypnea, hypotension, and heart murmur	Immediately turn the patient to his left side and lower the headboard, encouraging the air to remain in the right ventricular apex until reabsorbed	Placing and maintenance of CVC and the extreme care of the entire infusion system by an experienced professional
Venous thrombosis	Mechanical vein trauma, hypotension, high-osmolality solution, hypercoagulopathy, sepsis, trauma	Edema or pain in neck, in one or both arms, or in shoulders	Anticoagulation therapy with urokinase or streptokinase; remove CVC	Silicone catheter use, addition of heparin to PN when possible, low-dose warfarin therapy
Infectious				
Phlebitis	Peripheral administration of hypertonic solution (osmolality $\geq$900 mOsm kg^{-1}), infiltration of venous access	Redness, swelling, and pain in peripheral locations	Change the place of peripheral access	Minimize osmolarity of peripheral solution by using lipids as the primary source of calories and reducing the addition of electrolytes and other additives, if possible
Catheter-related sepsis	Improper CVC placement or maintenance care; contaminated solution	Systemic fever, chills, redness and swelling around the site of catheter insertion	Change the catheter placement	Development of strict protocols for CVC placement and care
Matabolic				
Hyperglycemia	Rapid infusion of concentrated glucose solution, sepsis, pancreatitis, postoperative stress, chromium deficiency, steroid use, advanced age	Blood glucose >200 mg dl^{-1}, metabolic acidosis, polyuria, polydipsia	Use insulin, reduce the concentration of glucose in NP	Start slowly and advance the infusion of NP
Hypertriglyceridemia	Supply of lipids exceeds the capacity of the bloodstream (>4 mg kg^{-1} min^{-1}); sepsis, multiple organ failure, severe hyperlipidemia	Serum triglycerides 300–350 mg dl^{-1} 6 h after beginning lipid infusion; high triglyceride levels in previously stable patients (e.g., sepsis)	Decrease the volume and time of lipid infusion; simultaneous infusion with glucose	Hyperlipidemia history before NP infusion; avoid lipid administration >2.5 g kg^{-1} day^{-1} or >60% of total calories
Refeeding syndrome	Excessive administration of carbohydrates and/or proteins in severely malnourished patients	Excess carbohydrates, CO_2 retention, cardiac tamponade, hepatic dysfunction Excess protein; blood urea nitrogen (BUN), excessive nitrogen excretion; high BUN/creatine ratio	Reduction of excessive supply of carbohydrate/protein	Avoid excessive administration of carbohydrate/protein

recognition of an infection episode related to the central venous catheter, and compliance with best practice during installation and the infusion of PN can reduce this risk.

Final Comments

Currently, PN therapy, for the specific treatment of malnourished patients who cannot use their oral and enteral routes for nutrient supply, provides more benefit than harm when performed according to the available guidelines. The new all-in-one concept, in which nutrients are premixed in a single bag, greatly promotes the safety, efficacy, tolerance, and convenience of PN. However, if done at the hospital pharmacy, this preparation must be carried out on demand, due to the limited stability of this mixture (24 h).

New commercial multichannel PN bags were designed to overcome this limitation and to ensure the PN solution stability by separating its reactive components in different chambers. The individual components of the PN are mixed in a closed aseptic system only at the time of their use, through the breaking of the seals separating these chambers. It is noteworthy, however, that multichannel PN formulations have the disadvantage of not being designed to meet every single patient's needs, mainly infants, in a personalized way, as traditional formulations can be. Therefore, multichannel PN bags must be used based on specific criteria.

PN therapy should not be seen as an emergency intervention and therefore must be designed to adequately meet the individual energy and nutrient needs of patients. PN planning and execution must be performed by qualified professionals from the very beginning, meaning parenteral route access, to a proper ending. Different clinical and biochemical markers should be closely monitored for these professionals to avoid potential mechanical, metabolic, and infectious complications related to PN throughout the infusion period. They also must consider the PN, for most patients, as a temporary treatment that should be discontinued as soon as conditions for oral or enteral diet reintroduction are achieved.

See also: Amino Acids: Determination; Amino Acids: Metabolism; Carbohydrate: Digestion, Absorption and Metabolism; Diarrheal Diseases; Dietary Fiber: Determination; Dietary Practices; Dietary References: US; Energy: Intake and Energy Requirements; Energy Metabolism; Fatty Acids: Determination and Requirements; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fatty Acids: Metabolism; Fish Oils: Composition and Health Effects; Malnutrition: Concept, Classification and Magnitude; Malnutrition: Prevention and Management; Nutrition and Infection; Protein: Digestion, Absorption and Metabolism; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Proteins: Chemistry, Characterization, and Quality; Storage Stability: Mechanisms of Degradation; Storage Stability: Shelf Life Testing; Vitamins: Overview.

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Passion Fruit

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Introduction

Passion fruit is a tropical fruit belonging to the dicotyledonous family Passifloraceae. The name was given by Spanish missionaries to South America who used the exotic flower to describe and explain their religious convictions while trying to convert the indigenous inhabitants to Christianity. The most well-known types of this fruit are the purple passion fruit (*Passiflora edulis* Sims) also known as the granadilla (see Figure 1(a) and 1(b)), the granadilla (*Passiflora ligularis* Sims), the gulupa (*Passiflora edulis* Sims. Fo edulis), and the yellow passion fruit (*Passiflora edulis* var. flavicarpa Degener) (see Figure 2(a) and 2(b)). When mature, the passion fruit is round to oval, either yellow or dark purple fruit with a soft to firm, juicy interior filled with numerous seeds. The purple passion fruit (*Passiflora edulis*) is adapted to the coolest subtropics or to high altitudes in the tropics, while the golden passion fruit (*P. edulis* f. *flavicarpa*) is more suited to tropical lowland conditions. The fruit has many health benefits and is both eaten as a fresh fruit and consumed as juice. Passion fruit juice is often added to other fruit juices to enhance flavor and aroma.

Sources and Production

The origin of the edible commercial species of passion fruit is thought to have been on the edges of South American rain forests in the Amazon region of Brazil and possibly in Paraguay and North Argentina. The Inca people are known to have cultivated passion fruit plants in the Peruvian region around AD 1000, and the mashed fruit combined with water has been consumed in Peru, as a nutritious drink for many centuries.

Passion fruit was introduced to Europe in the seventeenth century by early travelers to the Americas and was grown as an ornamental plant under greenhouse conditions for its attractive flowers. Passion fruit became a naturalized crop in most of the tropical and subtropical world covering South Africa; Hawaii, California, and Florida; Sri Lanka; and Fiji by about the middle of the twentieth century.

Passion fruit requires a mild climate free from extremes of heat and cold and plenty of rain. Extreme cold and frosty conditions can damage vines permanently, while fruiting is poor in regions with very warm climates. The plant is now cultivated commercially in many tropical and subtropical regions for its sweet, seedy fruit. The edible fruit is borne on a shallow-rooted woody perennial vine, which climbs by means of axillary tendrils. The fruit itself is round or slightly oval with a hard, thick shell 4–7 cm in diameter. The fruit is produced on 'new growth' parts of the vine and careful pruning is required for optimum yield. The average productive commercial life of a vine is considered to be three-and-a-half years. Growth of the vine from seed to the flowering stage takes approximately 6 months. Fruits develop after the stages of flowering and pollination, which usually takes a further 70–80 days. The fruit may be harvested manually when ripe, but due to high labor costs, the fruit is generally harvested after it falls to the ground. In such situations, the fruit must be collected daily in order to keep it from pests and spoilage, which is enhanced by soilborne organisms.

While propagation of the plant can be carried out using cuttings, the propagation by seeds is generally the preferred method, as cuttings are prone to carrying diseases. Passion fruit flowers are cross-pollinated by insects. They are most effectively pollinated by different species of carpenter bees depending on locality. In locations where carpenter bee species are present, decaying logs are placed in plantations among the vines as they provide nesting spots and encourage colonization by bees. Cross-pollination encourages a greater variety of offspring with better resistance to disease, higher tolerance for climatic variations, and enhanced seed production.

There are two commercially grown varieties of passion fruit – the purple and the yellow (see Figures 1(a) and 2(a)). The passion fruit vine has a very distinctive, fragrant flower and alternate, finely toothed leaves, which are deep-green and glossy above, but they are paler and dull on the under surface (see Figure 3). Leaves can be tinged with red or purple, especially in the yellow form, are deeply lobed when mature, and can be 8–20 cm long. Fruits are round to oval in shape and, at maturity, are either yellow or dark purple in color depending on variety. Fruits have a thick peel and a soft to firm, juicy interior, which is filled with many seeds. The purple form has black seeds, while the yellow form has brown seeds (see Figures 1(b) and 2(b)). The pulp of purple passion fruit is less acidic and richer in aroma and flavor and has a higher proportion of juice (35–38%). It is grown in the United States, Kenya, and Australia, but is less well known although the fresh market for this variety is said to be growing. Most commercial production centers on the yellow variety, which has a more vigorous vine and generally larger fruit than the purple. Some of the older cultivars as well as some of the more recent ones are listed in Table 1. Variations in seasonality occur according to cultivar and location. In some areas, vines bear throughout the year although peak periods are observed. In Ecuador the harvest is year-round but the volume peaks during the period from March to September, while in Brazil, peak harvest occurs between November and February. In Colombia and Peru, harvest peaks between March and June. In India, the first peak occurs between August and December, with a second peak from March to May. During the latter, the fruits are somewhat smaller, with less juice. In Hawaii, passion fruits mature from June to January, with heaviest crops harvested between July and November.

Passion fruit production is reported under the category of minor tropical fruits together with lychees, durian, rambutan, and guava, which are traded in smaller volumes compared with the major tropical fruits such as avocado, mango, papaya, and pineapple, as reported by the FAO Economic and Social Development Department. Minor fruit crops are reported as

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Figure 1 (a) Yellow passion fruit – *Passiflora edulis f. flavicarpa*. Whole fruit soon after harvest on left and after 4 days at ambient temperature (26–30 °C) shriveled appearance indicating moisture loss. (b) Yellow passion fruit – *Passiflora edulis f. flavicarpa*. Cross section soon after harvest (left), after 4 days at ambient temperature (26–30 °C).

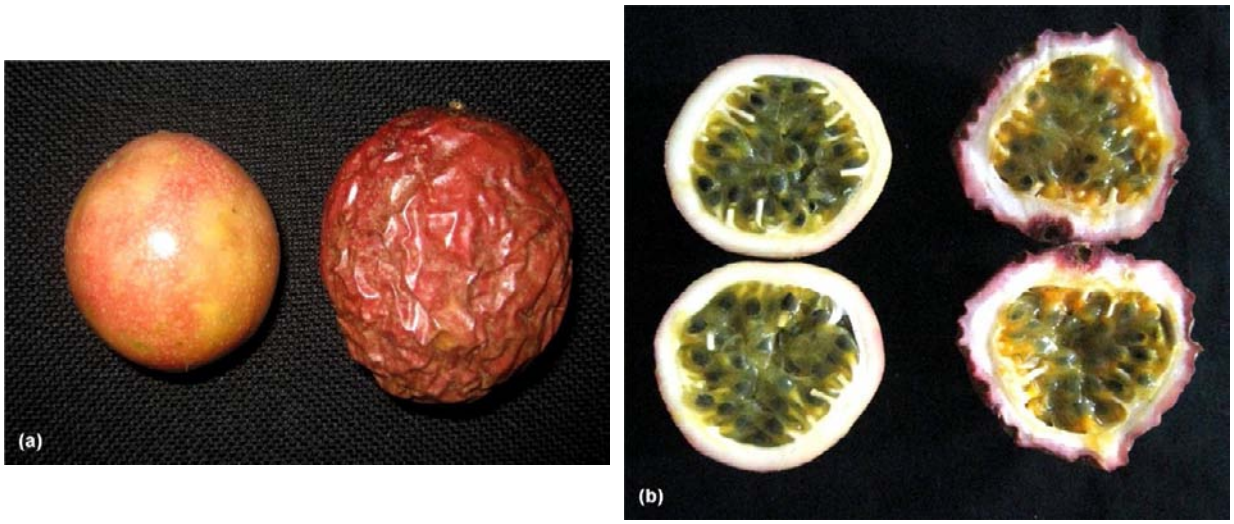

Figure 2 (a) Granadilla – *Passiflora edulis*. Whole fruit soon after harvest on left and showing moisture loss after 4 days at ambient temperature (26–30 °C). (b) Granadilla – *Passiflora edulis*. Cross section soon after harvest (left), after 4 days at ambient temperature (26–30 °C).

being traded mostly at the regional level. International trade classifications generally do not provide sufficient detail to desegregate global figures. FAO estimates that production of minor tropical fruits reached 15 Mt, or 24%, of total world tropical fruit production in 2000.

Passion fruit production statistics from other sources indicate an annual production of 0.852–1.2786 Mt year^{-1} of which only 0.5 Mt is produced in the developed world.

Major passion fruit-producing countries include Brazil, Ecuador, Colombia, Peru, Mexico, Australia, Zimbabwe, and Kenya (see Table 2). Over 95% of the production is the yellow form for juice extraction, while purple fruit contributes predominantly to the fresh market trade.

Patterns of Consumption

Passion fruit is grown in many tropical countries where it is a popular commodity. It is consumed in the fresh and processed form. Brazil accounts for 50–60% of world production and is the largest producer and consumer of fresh and processed passion fruit. Domestic consumption in Brazil is largely in the processed form as fruit drinks.

Ecuador is a major producer and is the largest global exporter of processed passion fruit juice. Colombia is also a major producer of passion fruit and is the largest exporter of fresh passion fruit to Europe, while Peru is becoming a leading producer and international exporter of passion fruit juice.

Indonesia is a large producer, but is not an established exporter, with fruit being consumed within the country. Although Kenya is considered a minor producer, significant exports of the fresh fruit are made to Europe as well as regional markets. Passion fruit production is being promoted in Queensland, Australia.

In many other tropical countries, passion fruit is grown largely for domestic consumption in both the fresh and processed form. In general, the sweeter purple passion fruit referred to as the granadilla is preferred for consumption as a fresh fruit, while the yellow passion fruit is grown mainly for the production of juice concentrate or single-strength juice, fruit preserves, and jams and as a flavoring agent. Apart from the unique flavor of the latter (Brix value range of 12–20), the comparatively high acid content of the juice (2.4%) is its most discouraging characteristic in the processing and development of value-added products. Passion fruit seeds are edible, and the oil extracted from seeds is consumed for medicinal and health purposes in some countries. Passion flowers are used to brew herbal tea, while the leaves are used as a vegetable in many Asian countries.

Figure 3 The passion fruit vine has deeply 3-lobed, finely toothed leaves, which are deep-green and glossy above, but paler and dull on the under surface with axillary tendrils originating adjacent to leaves.

Availability Absorption and Metabolism

Information on the availability of beneficial nutrients in individual fruit crops is important to researchers, dietitians, and nutritionists. Passion fruit is a rich source of a range of nutrients. The fruit contains a high amount of antioxidants and of fiber, which is effective in improving digestion and the nutrient-absorbing capacity of the body. Passion fruit has a high mineral content and is considered to be a useful source of provitamin A, ascorbic acid, riboflavin, and niacin. It has a high mineral content. In-depth analyses of nutrient composition of passion fruit are presented in Table 3.

Passion fruit pulp when consumed with seeds contains 24.5 g of fiber per cup, which is equivalent to more than 4 cups of bran cereal and more than 5 g of protein. Consuming the seeds also enables better absorption of vitamin A. On removal of seeds, the fiber content is reduced to 0.5 g with protein content reduced to less than 1 g. Passion fruit seeds are rich in polyphenolic compounds such as piceatannol and scirpusin B, and they deliver plenty of insoluble dietary fiber and

Table 1 Some common varieties of passion fruit[a]

Variety	*Description*
Australian Purple, or Nelly Kelly	A purple selection of mild, sweet flavor, grown in Australia and Hawaii
Common Purple	Naturalized in Hawaii; thick-skinned, with small seed cavity, but of fine flavor and low acidity
Sevcik Selection	A golden form of the yellow selected in Hawaii; a heavy bearer, but subject to brown rot and the juice, has a peculiar woody flavor
Kapoho Selection	A cross of 'Sevcik' and other yellow strains in Hawaii. A heavy bearer of large fruits but subject to brown rot; many fruits contain little or no pulp and the juice has the off-flavor of 'Sevcik' though not as pronounced
University Selection No. B-74	A Hawaiian hybrid between 'Pratt' and 'C-77,' usually yellow, occasionally with red tinges; resembles 'Waimanalo'; has good juice yield and very good flavor
Pratt Hybrid	Thought to be a natural cross between the 'Common Purple' and a yellow strain; subject to rot, but juice is of fine color and flavor, low in acid
University Round Selection	A Hawaiian crosses of 'Waimanalo' and 'Yee' – fruit smaller than 'Yee'; not as attractive but yields 10% more juice of very good flavor
University Selection No. B-74	A Hawaiian hybrid between 'Pratt' and 'C-77,' usually yellow, occasionally with red tinges; resembles 'Waimanalo'; has good juice yield and very good flavor
Waimanalo Selection	Consists of 4 strains: 'C-54,' 'C-77,' 'C-80,' of similar size, shape, color, and very good flavor, and 'C-39' as pollinator
Yee Selection	A yellow, round, very attractive, highly disease-resistant, but fruit has thick rind and low yield of juice, which is of very good flavor
Kahuna	Very large, medium purple fruit. Sweet, subacid flavor. Good for juicing. Vigorous, productive self-fertile vine. Produces over a long season. Large, attractive foliage
Purple Giant	Very large fruit, dark purple when mature
Brazilian Golden	Large, golden-yellow fruits, larger than standard forms. Flavor somewhat tart. Extremely vigorous vine, requiring cross-pollination. Extra large, fragrant flowers, white with a dark center, blooming during mid-summer. Produces one large crop beginning in late August or early September
Golden Giant	A large yellow-fruited cultivar that originated in Australia

[a]Compiled from Morton, J. F. (1987). *Fruits of warm climates*. Creative Resources Systems Inc., pp. 320–328; California Rare Fruit Growers Incorporated, 1996.

Table 2 World production of passion fruit by country (2010)

Country	*Tons × 1000*
Asia	165.8
Indonesia	127.4
Israel	1.1
Malaysia	5.0
Pakistan	0.2
The Philippines	0.6
Sri Lanka	0.5
Thailand	10.0
Vietnam	21.0
Africa	35.3
Kenya	32.8
South Africa	0.4
Zimbabwe	2.1
Central America and the Caribbean	4.8
Dominican Republic	4.8
South America	1072.5
Brazil	563.7
Colombia	93.7
Ecuador	394.1
Peru	15.7
Suriname	0.3
Venezuela	5.0
Oceania	0.1
New Zealand	0.1

Source: FAO Committee on Commodity Problems; Intergovernmental group on Banana and tropical fruits 5th Session Cameroon, May 2011.

Table 3 Approximate nutrient composition of passion fruit cultivars

Energy	97 kcal	5%
Carbohydrates	23.38 g	18%
Protein	2.20 g	4%
Total fat	0.70 g	3%
Cholesterol	0 mg	0%
Dietary fiber	10.40 g	27%
Folates	14 μg	3%
Niacin	1.500 mg	9%
Pyridoxine	0.100 mg	8%
Riboflavin	0.130 mg	10%
Thiamin	0.00 mg	0%
Vitamin A	1274 IU	43%
Vitamin C	30 mg	50%
Vitamin E	0.02 μg	<1%
Vitamin K	0.7 mg	0.5%
Sodium	0 mg	0%
Potassium	348 mg	7%
Calcium	12 mg	1.2%
Copper	0.086 mg	9.5%
Iron	1.60 mg	20%
Magnesium	29 mg	7%
Phosphorus	68 mg	10%
Selenium	0.6 μg	1%
Zinc	0.10 mg	1%
Carotene-β	743 μg	–
Cryptoxanthin-β	41 μg	–
Lycopene	0 μg	–

Passion fruit (*Passiflora edulis*), fresh, nutritive value per 100 g.
Source: USDA National Nutrient database.

magnesium. Piceatannol and scirpusin B in passion fruit seeds provide antioxidant protection.

Vitamin A present in the fruit helps maintain cell growth, good vision, and a healthy skin. A cup of passion fruit contains up to 25% of the recommended daily allowance (RDA) of vitamin A per person. Carotenoids present in passion fruit include zeta-, beta-, and alpha-carotene, β-cryptoxanthin, and lycopene. These compounds are fat-soluble and help the body produce vitamin A.

Passion fruit contains vitamin C, which helps the body scavenge harmful proinflammatory free radicals, strengthens the immune system, and prevents premature aging, as it is a powerful water-soluble antioxidant. Passion fruit contains up to 30 mg vitamin C, per 100 g, with one cup providing a days' recommended requirement of this vitamin. However, being water-soluble nutrients, vitamin C and folate are prone to gradual depletion after harvest. Thus, a freshly picked passion fruit is more nutritious than one that has been stored and transported.

Daily requirement of iron is in the range of 8–12 mg for both women and men. Passion fruit is a rich source of nonheme, plant-based iron, and a cup of raw passion fruit can provide up to 4 milligrams of this micronutrient and will make a useful addition to the diet, particularly with respect to vegetarians. The vitamin C present in passion fruit facilitates iron absorption, prevents loss of iron, and increases the production of blood.

Magnesium ensures proper gene function and plays an important role in carbohydrate metabolism, heartbeat, and nerve transmission. It is also necessary for the development of strong bones. The RDA for magnesium in adults ranges from 310 to 420 mg, and a regular consumption of passion fruit seeds can make a significant contribution to an individual's daily magnesium intake. Consumption of adequate quantities of magnesium-rich foods is particularly important to people with an increased risk of magnesium deficiency. Incorporation of passion fruit seeds in the diet in such cases will be of benefit and will help combat risk factors that increase the likelihood of magnesium deficiency such as stress, high alcohol consumption, long-term use of certain diuretics, and the incidence of gastrointestinal disorders such as Crohn's disease.

Health Effects

Written records of the medicinal use of passion fruit date back thousands of years. The anti-inflammatory properties of passion fruit were understood by Native Americans who used the flowers to heal bruises and wounds and the leaves and root as a poultice for injuries and boils. Leaves, roots, and flowers were also brewed as teas to calm and help settle the nerves and encourage sleep. Including passion fruit consumption in the diet is credited with boosting immune function, improving eyesight, increasing skin health, regulating fluid balance in the body, lowering blood pressure, boosting blood circulation, and improving bone mineral density. It is also accredited with reducing signs of premature aging, improves sleeping habits, and eliminates asthma.

Fiber is an essential component of a healthy diet, since it facilitates healthy digestion of food and the regulation of bowel movements. Passion fruit is a good source of soluble

and insoluble fiber, both in the pulp and in the rind. Fiber acts as a bulk laxative, which moves food through the digestive tract and regulates bowel movements, thereby reducing exposure time of the colon to any toxins.

Passion fruit seeds provide protection against damage caused by the accumulation of free radicals in the system, which cause many degenerative and chronic diseases such as immune system problems, atherosclerosis, Alzheimer's disease, heart disease, arthritis, dementia, and diabetes. The body produces *metalloproteinase enzymes in response to* exposure to UV rays, which help repair sun damage. However, some of these enzymes are not beneficial as they degrade collagen and elastin, which leads to wrinkling of the skin. Free radicals are known to stimulate the production of the latter category of *metalloproteinase* enzymes. Thus, the free radical-destroying properties of passion fruit seeds also provide beauty benefits by removing free radicals from the body, thus preventing premature wrinkling of the skin caused by UV radiation.

The antioxidants present in passion fruit help with improving eye sight and protect the body from aging and other damages caused by free radicals, which are produced due to the increase in environmental pollution. High oxidative stress increases production of free radicals in the body. These free radicals damage normal cell DNA and are able to turn it into cancerous forms.

The antioxidants protect arteries from free radicals. Passion fruit being rich in flavanoids and vitamins A and C, when consumed at recommended levels, is reported as providing protection from oral and lung cancer. Vitamin A is also linked to eye health and the prevention of macular degeneration, cataracts, and night blindness, while vitamin C is highly effective in increasing immunity against common cold, flu, and infections. High sodium levels in the body increase the risk of high blood pressure, heart attack, and stroke. High amount of antioxidants also protects arteries from free radicals and proves to be highly beneficial to the cardiovascular system.

Soluble fiber present in passion fruit. 100 g passion fruit provides 27% of daily fiber required by the body and is known to improve digestion. Insoluble fiber acts as a bulk laxative, which helps protect the colon mucous membrane by decreasing exposure time to toxic substances as well as binding to cancer-causing chemicals in the colon. It is thus considered to be an effective colon cleanser as this fiber is capable of removing the toxins stored in the colon, which are responsible for the development of colon cancer.

Dietary fiber is also beneficial to the cardiovascular system as it helps remove excess cholesterol and reduce cholesterol levels in the blood. It increases high-density lipoprotein (HDL) referred to as good cholesterol and reduces low-density lipoprotein (LDL) referred to as bad cholesterol. A higher level of HDL:LDL ratio tends to prevent plaque formation in arteries, thereby reducing blockage of blood flow to the heart and preventing heart attacks. Insoluble fiber in passion fruit seeds promotes intestinal health. It has been observed that adding insoluble fiber from passion fruit seed to a fiber-free diet enhances the amylase activity, which converts complex sugars to simple sugars during digestion and reduces cecal pH and cecal ammonia content. Passion fruit seeds are rich in insoluble dietary fiber (64.1 g/100 g). Chau et al. had suggested that insoluble fiber from passion fruit seeds might offer an effective functional ingredient to promote intestinal function and health. A decrease in cecal pH and cecal ammonia content helps balance bacteria in the intestines and reduces the potential risk of tumors in the colon.

Potassium is a vasodilator and an important component of cells and body fluids. It relaxes the tension of blood vessels and promotes increased blood flow and helps regulate heart rate and blood pressure. Passion fruit contains 7% of RDA of potassium and when incorporated into the diet can help maintain the balance of sodium levels in the body. Fresh granadilla is rich in potassium, with 100 g of fruit pulp containing up to 348 mg of potassium, and is a valuable good source of minerals such as iron, copper, magnesium, and phosphorus, which are present in adequate amounts in the fruit.

Minerals such as iron, copper, magnesium, and phosphorus contribute to bone density and bone strength. Passion fruit is a rich source of these minerals and when incorporated into the diet will contribute to bone health and the elimination, prevention, or alleviation of the symptoms of osteoporosis and the associated pain and inflammation that occur when bones deteriorate with age and activity.

Medicinal harmala alkaloids such as harmine (monoamine oxidase inhibitors) present in passion fruit function as a sedative. This compound has been connected to a reduction in restlessness, insomnia, sleeplessness, and nervous anxiety.

Bioflavanoids present in the peel of the purple passion fruit have been found to have an expectorant, sedative, and soothing effect on the respiratory system. Extracts from the peel have been positively connected to a reduction in asthma attacks, wheezing, and whooping cough.

See also: Antioxidants: Role on Health and Prevention; Dietary Fiber: Determination; Folic acid and Folates: Physiology and Health Effects; Food Composition Databases.

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Pasta: Manufacture and Composition

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Introduction

Pasta is one of the most diffused foods in the world, since it is one of the largest forms of wheat consumption. The use of durum wheat in pasta making, traditionally limited to local areas, mainly Italy, has increased throughout the last few decades. This is due to several specific characteristics, and among them, we recall nutritional value, low cost, easy storage, product consistency, and being synonymous with the Mediterranean diet. The amount of pasta consumed throughout the world is continuously increasing and reached about 13 million tons in 2011; thereof, nearly a quarter was produced in Italy. Italy can be undoubtedly considered the reference country for this product from all points of view. Pasta consumption was between 4 and 10 kg per capita per year for many countries in 2011, but this value was about 27 in Italy (UniPI data). A quite high number of both pasta and pasta machine producers are Italian.

Pasta may be considered a commodity product, because its storage does not require either any temperature-controlled device or a particular package. Even the preparation for consumption is very easy consisting essentially of rehydration performed at normal water boiling temperature and the addition of any suitable sauce. The consistency of pasta after cooking seems to be the most important claim of pasta quality for consumers. Pasta 'al dente,' for instance, is a classic requirement.

Pasta is made essentially with durum wheat, even though in some countries, it is allowed to use other flours or a combination of them. Legislation or production guidelines govern this matter: for example, in Italy, pasta is produced with a maximum of 3% of nondurum wheat; on the opposite, in the United Kingdom and Germany, there are no specific legislations governing pasta production, whereas in Austria, pasta can be made with durum wheat or other wheats. The choice of cultivars producing excellent semolina quality is linked to its capability to give a final product that maintains a good consistency after cooking. This is related to the protein and gluten content. In fact, a high-quality pasta should have a relatively high protein content, at least between 13.5 and 14.5 g/100 g, and a good balance between glutenins and gliadins, the two protein components of gluten.

Dough formation is obtained by mixing the right amount of water to semolina flour to create a homogeneous mass. Owing to the size of the granules, this stage can be very difficult to perform in the right way avoiding lumps and small spots of undissolved flour. Then, after mixing, water is removed by drying to achieve a final value of 12.5% w/w corresponding to equilibrium with the ambient air (relative humidity 60%). In these conditions, it is not necessary to use any particular package to prevent drying or wetting of the final product, as it is necessary, on the other hand, with bread or biscuit, respectively. Drying is one of the oldest methods for food preservation used by humans over the centuries. It uses damp warm air as a vector to extract water from the dough and is a crucial stage in the pasta production process.

Raw Materials

Pasta is made by mixing semolina and water under energy application at controlled temperature and moisture conditions. The main use of durum wheat (*Triticum durum*) is for pasta, and the end-product quality is related to the durum grain characteristics, which, in turn, are mainly determined not only by the genotype, but also by the environment (weather and nutrition) and crop management. Durum is generally considered to have the hardest kernel among wheats; it has an amber color and high protein content. Three different parts constitute the grain seed: the endosperm, the bran, and the germ. The kernel is constituted by almost 80–85% by weight by the endosperm. Inside, not only proteins and starch, but also, to a lesser extent, fibers, vitamins, and minerals can be found. The endosperm is rich in proteins and enzymes. Gliadins, glutenins, albumins, and globulins are the major proteins present. The glutenins and gliadins form the gluten complex, responsible for dough rheological properties. Moreover, the endosperm contains about 30% of glutamine and 10% of proline, followed by leucine (6.5%), another important amino acid, and minor quantity of other amino acids. Flour from the endosperm contains about 82% starch, which is present as granules of various sizes and shapes. Starch is the main carbohydrate present in the endosperm cells. The external layers and the internal kernel of the grain have their own specific chemical and morphological characteristics. In the bran, which is mainly composed by the outer layers of the caryopsis, the chemical characteristics are given not only by the concentration of fibers and in particular insoluble fibers (cellulose and hemicellulose), but also by minerals and proteins. Finally, the germ is rich in fats that can easily go rancid.

Starch

Starch, in the form of granules, is deposited in the cereals and comprises about 70% (w/w) of endosperm in wheat, and it is an energy source. The starch is a mixture of two polymers, amylose and amylopectin, classified as D-glucans. The molecular weight of amylose is around 250 000 and it is a mostly linear polymer of α-(1,4)-linked glucose with a degree of polymerization (DP) of 1000–5000 glucose units. The structure of this polymer was assumed to be mainly linear, but really appears to be partially branched. On the contrary, amylopectin is branched to a much greater extent than amylose, and the molecular weight is 10^7–10^8. The ratio of amylose to amylopectin is relatively constant, at about 23. Amylopectin is a much larger glucose polymer (DP 105–106) in which α-(1,4)-linked glucose polymers are connected by 5–6% α-(1,6)-linkages. Normal wheat starch contains 20–30% amylose and 70–80% amylopectin.

Starch granules in wheat are frequently classified into two size groups: large, elliptically shaped A granules (30–40 μm in dia) and small, round B granules (<10 μm in dia), which are formed later in the grain-filling process, which gives a durum

biphasic distribution. B starch granules make up about 25–30% of the total starch volume but about 90% of the number of starch granules. Intact starch granules are almost insoluble in water, but when heated in excess water, they gelatinize. Starch gelatinization causes the granules to swell and eventually to rupture. This change is detected by microscopy and differential scanning calorimetry in a ranging gelatinization temperature of ~50–70 °C. Upon heating, starch loses its rigid structure, becomes rubbery after its glass transition, and can readily absorb water. This causes an increase of viscosity as the granules swell and release soluble material from the granule. When the percentage of B granules is increased in durum starch, the dough absorbs more water, because the smaller B granules have a higher surface to volume ratio and are able to hydrate and swell more efficiently and bind more water than A granules. Therefore, increased B granule content should increase flour water absorption.

Proteins

Protein is considered the most important nutrient for humans and animals, as manifested by the origin of its name, from the Greek *proteios* for primary. The protein content of wheat grains may vary between 10% and 18% of the total dry matter. Wheat proteins are classified according to their extractability and solubility in various solvents. Proteins can be classified into albumin (water-soluble), globulin (soluble in salt solution), gliadin (extractable in aqueous ethanol solution), and glutenin (soluble in dilute acid), conforming to Osborne's protein classification. The largest fraction (80%) of durum is gliadins and polymeric glutenins, whereas the lesser fractions (20%) are albumins and globulins. The group of proteins in wheat that exerts the most influence on the strength and elastic properties of dough are the glutenins and gliadins (gluten complex). The polypeptide complex made up of glutenin, gliadin, and lipid is defined as the viscoelastic mass remaining after removal of the starch. The glutenin function is able to form an extensive 3-D network of molecules through disulfide bonding, hydrogen bonding, and hydrophobic interactions. All contribute to the formation of a cohesive elastic dough. The gliadin, being globularly shaped, acts as a lubricant between glutenin strands, allowing strength and extension of the network. Proteins, such as those present in gluten, are denatured by heat and extreme changes in pH. Hence, in order to retain its strong viscoelastic properties, wet gluten should be dried carefully. The distribution of total gliadins among the different types is strongly determined by wheat variety and growing conditions (soil, climate, and fertilization). The polymeric protein (glutenin) is mainly responsible for the elasticity of the dough, whereas the monomeric gliadins are the extensibility-related characters. Thus, the ratio of glutenin to gliadin can be directly related to the balance of dough strength and extensibility. The types of glutenins present can be undissociated (MW 15 000–150 000) and associated fraction (MW 150 000–3 000 000), many having extensive disulfide bonding. Reduction of the intermolecular disulfide bonds yields subunits, and their molecular weight can be high and/or low (HMW and LMW subunits). The degree of cross-linking by intermolecular disulfide bonds varies considerably leading to proteins with a wide range of molecular weights. Differences in the amino acid composition of the LMW and HMW subunits have been reported. The gliadin can be classified in α-, β-, γ-, and ω-gliadins, in order of their electrophoretic mobility or in α-, γ-, and ω-gliadins for the amino acid N-terminal sequence. The gliadin proteins have a higher content of proline residues than glutenin and yet do not display such strong viscoelastic behavior.

Finally, the nongluten proteins are albumins and globulins. The former are the smallest wheat proteins, followed in size by globulins, and it is covalently bound into the glutenin matrix.

Minor Elements

Lipids, nonstarch polysaccharides, enzymes, vitamins, pigments, and minerals are present in a small amount in wheat. Lipids are 1–3% (dry matter) of the grain and can be both starch-bound and non-starch-bound. They are able to associate themselves both with proteins, owing their amphipathic nature, and with starch, forming inclusion complexes. In durum, semolina free lipids are 64% of the total amount. They are important for the color of pasta due to pigments and lipoxygenase. In fact, a variable number and variety of pigments are present in wheat. The xanthophylls are present in a quantity of about 1.5 ppm. Lutein is a xanthophyll pigment, which is abundant in wheat and present also as its esters. Carotenoids are members of a class of hydrocarbons (carotenes), and their oxygenated derivatives (xanthophylls) consist of eight isoprenoid units. Flavones are almost insoluble in water, but soluble in 85% (v/v) ethanol and WSB (1-butanol saturated with water). They are present mainly in the bran and their color ranges from yellow to brown.

The yellow color of pasta is due to the pigments present in the endosperm with a slight reduction caused by the oxidation of yellow pigments catalyzed by the enzyme lipoxygenase. Protease is another enzyme capable of reducing the consistency of dough after mixing and resting. Cytase, lipase, phosphatase, and oxidase are other enzymes present in wheat germ.

Finally, grains are good sources of insoluble fiber. Similar values (3–8%) to those of soft wheat were found in durum wheat: cellulose (2.7% of dry weight), glucans (1%), arabinogalactan peptides, and arabinoxylans (7.6%). Then, pentosans, nonstarch polysaccharides, the main constituent of cell walls of the lignified bran layer, are mainly mixed arabinoxylans, and as a consequence of their interfacial activity, they play a crucial role in air entrapment. Vitamins, such as B_{12}, B_{16}, C, and E, and minerals are also present in durum wheat.

Pasta Dough: Forming and Development

The first stage of pasta production is mixing, and it appears to be very important. The durum wheat semolina is mixed with water in a paddle mixer, fed into an extrusor, then compressed, and extruded through a die. The rheological behavior of dough after extrusion is related to the gluten development matrix. In fact, the rheological properties of polymers reflect the degree and type of cross-linking of the polymers as shown in Figure 1. At the early stage of mixing, the water, in the right quantity to reach a final moisture content of about 33% on a wet basis, is distributed among

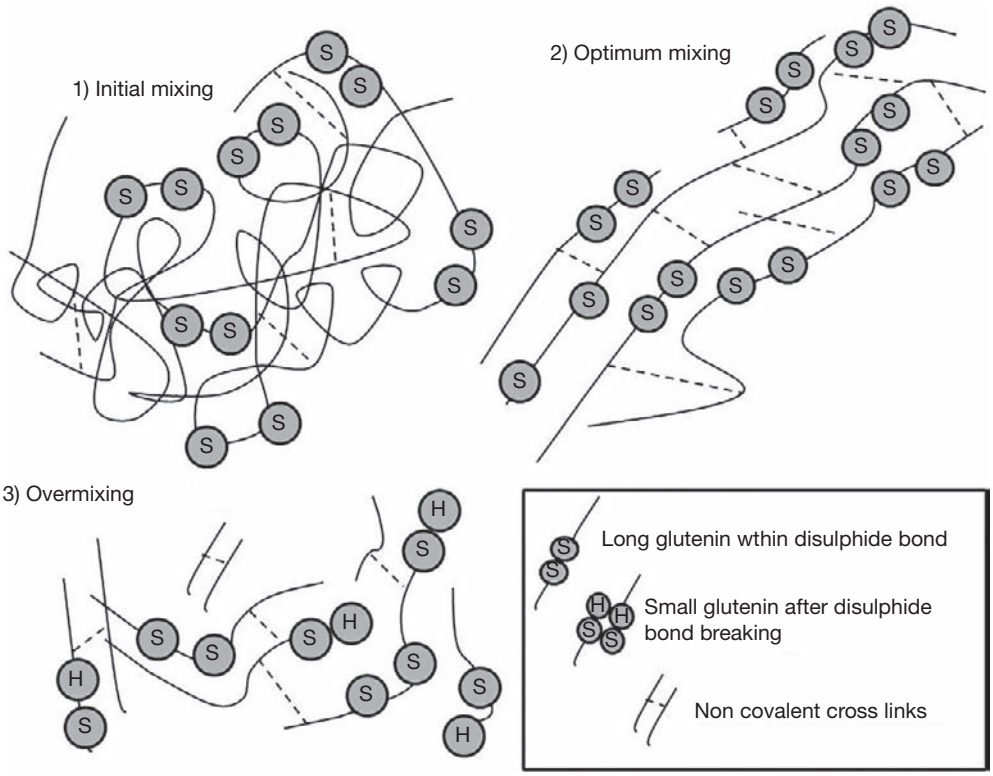

Figure 1 Model for structure of gluten.

the semolina particles. At the initial mixing time, glutenins, which are the long polymeric proteins, are folded, and the chains are in random orientation, but as mixing proceeds, more proteins become hydrated and the glutenins tend to align, because of the shear and stretching forces imposed by the paddles. For the network development, during mixing, the balance of gliadin and glutenin is very important so that chemical bonds may develop. In fact, the optimum dough structure involves bonds like disulfide and hydrogen bonds and cross-links involving dityrosine. Indeed, as reported in the literature, by adding hydrogen bond-breaking agents, it is possible to observe a drastic effect on dough rheology and the strengthening effect of heavy water compared to ordinary water. Another unique feature of hydrogen bonds is their ability to interchange under stress and thereby facilitate re-orientation of gluten proteins. Hydrophobic bonds result from the interactions of nonpolar groups in the presence of water. Their functionality is similar to that of hydrogen bonds, but the overall effect is much less. Disulfide bonds play a key role in dough formation and development. They form strong cross-links within and between polypeptide chains, thereby stabilizing hydrogen bonds and hydrophobic interactions. The interchange reaction requires a 'mobile' sulfhydryl-containing substance to initiate the series of disulfide interchanges. The total number of S—S bonds does not change; only their location in the glutenin molecule is altered. When the durum is hydrated and well mixed, the monomeric proteins, gliadins, form a matrix within the long polymer networks and contribute to resistance to extension and between protein polymers increase gluten viscous resistance and resistance to extension. Glutenins, and in particular omega-gliadins, seem to interact with the glutenins during dough processing through noncovalent interactions.

Then, when the dough is at the optimum mixing time, the gluten network has a maximum cross-linking of protein with disulfide bonds, the interaction between the polymer cross-links is becoming stronger, and, as a consequence, a maximum resistance to extension and restoring force after deformation can be observed. On the contrary, when mixing is too long, the dough can go beyond its optimum development; the cross-links begin to break due to disulfide bonds breakage. Consequently, glutenins depolymerize and become sticky.

Mechanical Properties: Rheological and Empirical Measurements

The mechanical properties of pasta dough and final pasta quality are determined by gluten network formation during mixing and then by durum wheat composition (low or high protein content), processing parameters (mixing time, energy, temperature, and speed rate), and ingredients (e.g., water and salt). Mixing time and energy input are used to adjust dough properties, but the optimum mixing time varies with durum semolina. The widespread belief is that a stronger dough requires a longer mixing time and greater energy input. During the mixing process, the dough goes from 'underdeveloped,' with a poor gluten structure; to 'properly developed,' with an appropriate gluten network; to, finally, 'overdeveloped,' with soft, extensible and sticky properties that is undesirable because of its reduced machinability. A level of work above

the optimum induces significant changes in the mechanical properties of the dough.

Dough properties are also determined by the amount of water added. An optimum water level is required to develop cohesive, viscoelastic dough with optimum gluten strength. Owing to a higher protein and dense particle content, a strong semolina requires a higher water level than a weak one. The heat generated by the frictional forces and the heat of hydration of the semolina during mixing cause a temperature increase, which influences the elastic properties of the dough. Temperature increase is mainly according not only to mixing speed, but also to the type of semolina and the type of mixer. It is well accepted that an optimal temperature should be 28–30 °C. In fact, when the temperature is relatively high, the dough becomes soft and viscous, showing less elastic properties, because elasticity and firmness change due to the fermentative activity increasing. If the temperature is relatively low, the dough becomes too hard to push and rather difficult to extend, with an evident loss of the final quality of the products.

The mechanical properties of pasta dough after mixing are measured in terms of gluten strength by several empirical instruments: farinograph, mixograph, and alveograph tests, eventually adapted or modified.

Even though these tests are purely descriptive according to the type of instruments, size and geometry of the sample, and the specific conditions under which the tests are performed, they are still useful, especially for the determination of the relative strength and extensibility of different cultivars. Fundamental tests are not widely used because they require complex expensive instrumentation and a high-level technician; often, they are time-consuming and difficult to maintain in an industrial environment; moreover, the deformation condition test may be inappropriate, and consequently, the interpretation of results may be rather difficult. Nevertheless, at present, the use of rheological measurements has increased because the apparatus has become less expensive and more automatized. Usually, with the same device, it is possible to perform small deformation dynamic oscillatory measurements at several frequencies, which are useful to characterize not only dough and wheat flour–water mixtures at equilibrium but also stress relaxation, creep, and creep recovery for investigating the fundamental mechanical properties.

Small deformation dynamic oscillatory measurements are performed under mild conditions to preserve the structure during the test, thus allowing one to obtain information on the bulk properties of the dough. From dynamic oscillation tests, it is possible to obtain the elastic modulus (G′) and the viscous modulus (G″), the former being in closer agreement to the cross-linked polymer behavior exhibited by such systems. This test method has been found very useful to study the effect of protein content, water level, mixing time, and the different amount of gluten in the dough. This experimental technique requires a linear link between stress and strain that usually is found at strain levels smaller than 1%. Beyond this limit, both the elastic and loss moduli G′ and G″ decrease. In the linear region, the frequency sweep tests have shown that the modulus follows a power law behavior. The water level and the protein/starch ratio influence the values of the moduli and, particularly, G′ that decreases when increasing the water. It should be noted that G′ is a good measure of the dough strength. By performing strain sweep tests, it appears that the storage modulus shows a significant increase as the proportion of starch in the dough increases. Moreover, the value of the phase angle tangent, defined as the ratio of G″ to G′, reveals that gluten–starch mixtures behave as elastic solids and consequently the linear strain region limit decreases exponentially as the starch content increases. G′ decreases with increasing protein and gluten concentration, whereas the opposite happens when constant water levels are used. Studies about the influence of mixing time still do not provide any general conclusions, and most of the works refer to empirical measurement of mixer motor torque, voltage, or power consumption during mixing as a qualitative indication of structural change. Many problems are involved, for instance, owing to a poor accounting for motor and drive losses, frictional and surface effects between the dough and the mixer, varying signal damping and data acquisition rates, effects of aeration on rheology, and rheological relaxation effects. Alternatively, measurement of rheological changes at some time after mixing can be performed.

To measure dough properties under uniaxial extension, often, a rather empirical but useful method is used, clamping the material at two ends and pulling or extending it by a hook at the center of the sample at a constant strain rate. During stretching, the material undergoes an extensional deformation up to its breakage known as the tensile failure. The stress–strain curves obtained show that stress increases with increasing strain and reaches a maximum at sample fracture point. It is often found that the stress increases are more than proportional to the strain thus showing a so-called strain hardening, which is very important for the final pasta quality.

Production Technology: From Mixing to Packaging

The transformation of durum into pasta involves dosing, premixing, mixing and extrusion stages, drying, and finally packaging. A sketch of the pasta production phases is shown in Figure 2. This sequence of operations was developed on an industrial automated scale in the early 1930s with the

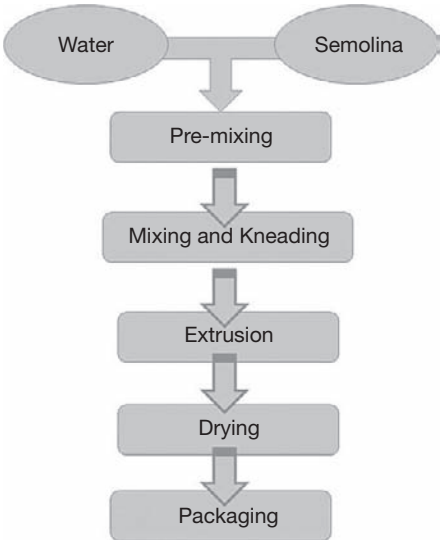

Figure 2 Dry pasta production line.

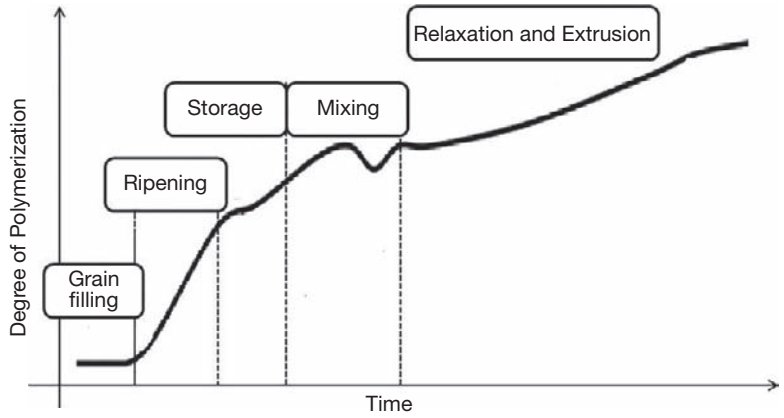

Figure 3 Degree of polymerization of glutenin protein.

introduction of the continuous press, then with the use of automatic dryers, and finally, in the mid-to-late 1940s, with the manufacture of continuous, automatic production lines. The resulting wet pasta is modeled as a network of proteins that encapsulates the starch granules, to produce a structure that has the minimum of cracks and voids. The pasta dough has completely developed disulfide bonds and then a gluten network, which continue the architecture development even during storage. Figure 3 shows a sketch of disulfide bond formation during the production process.

The initial raw material dosing is of primary importance for the subsequent dough formation and pasta manufacture steps. The water–semolina ratio determines the rheological behavior of the semolina dough and the mechanical energy input for each product. Semolina dosers can be volumetric or gravimetric, while water dosing can be achieved using either a mechanically or a volumetrically based system that is synchronized. Water temperature is controlled by a simple thermostatic system and usually ranges between 20 and 50 °C. All the components of solid and fluid dosers are built of stainless steel and food-grade materials. In some plants, water is supplied as small drops to facilitate semolina wetting. Then, a mixing–kneading process is used to produce homogeneously hydrated dough with a moisture content of ~33% (w/w). To obtain the most homogeneous hydrated mass possible, semolina and water first undergo high-speed premixing (using a single or double shaft, vertical or horizontal) to ensure intimate contact between semolina particles and water droplets. Further mixing takes place in the main mixing unit, generally under vacuum, before sending the mass to the extrusion section. A vacuum chamber is used to remove air bubbles from the pasta before extruding. If the air is not removed before the extrusion, the pasta dough shows small bubbles that reduce the mechanical strength.

In the mixer–kneaders, as well as in vacuum mixers, the residence times range from 10 to 15 min, according to the semolina particle size distribution. Once the first mixing phase starts, the gluten proteins are stretched, and as the temperature rises, an interlinked protein framework is established. After hydration and mixing, the wetted semolina particles are sent to the extrusion stage, where pasta dough development largely occurs. During extrusion, the pasta dough is transported by a suitably designed screw rotating inside a cylinder and a transition occurs from hydrated granular semolina to a homogeneous material, with an associated increase in specific weight along the screw from 750 to 1350 kg m^{-3}. The extrusion system consists of a screw-barrel subsystem, capable of transporting the dough and pushing it into a die that restricts the outflow of the dough. As a consequence, the die creates a back pressure along the screw, which results in dough development. Further energy input is transferred to the forming dough to push it through the die hole and to shape the product after the gluten network is completely developed. Single or double screws are normally used. The extrusion controls the rate of production and influences the overall quality of the finished product. Friction must remain at low levels to prevent dough temperature from exceeding 44 °C; thus, the cylinder and head of the extruder are cooled, avoiding starch damage. If the extruder temperature rises above 55 °C, the gluten becomes tough and stiff and forms an irreversible gel, with the consequence of making the pasta strands weaker. It is well accepted that to give the pasta some resistance to overcooking, the protein network must not be damaged. There are grooves along the sides of the barrel to increase the shear effect and make the dough adhere to the barrel, which generates a gentle kneading action.

Finally, the homogeneous dough is extruded through die holes designed to give the desired pasta shape and size. The material used to make dies is bronze with inserts made of Teflon or bronze, the design and construction of which are arts in themselves and influence pasta shape and smoothness. Fresh pasta is then dried in order to reduce its moisture content to about 12.5%, to reduce moisture to a water activity value where microbial growth is impossible and there is equilibrium with ambient conditions (a_w~0.60).

Nowadays, the majority of pasta-drying processes adopt air temperatures ranging from about 70 to 100 °C while keeping product temperatures between 60 and 90 °C. The difference between product temperature and air temperature is mitigated by air moisture content and ongoing evaporation through the water-removal process. Drying is the most difficult and critical step to control in the pasta production process. The objective of 12% is hard. Drying temperature and relative humidity increments are important factors in drying. Since the outside surface of the pasta dries more rapidly than the inside, moisture gradients develop across the surface to the interior of the

pasta. If pasta is dried too quickly, a crust is formed and the pasta may crack, giving the product a poor appearance and a very low mechanical strength. Cracking can occur not only during the drying process but also after several weeks in the package. If the pasta is dried too slowly, it tends to spoil or become moldy during the drying process. Therefore, it is essential that the drying cycle is successful: the pasta will be not only firm but also flexible enough so that it can bend to a considerable degree before breaking.

In the first phase of drying, the pasta is preheated under severe conditions to form a thin crust capable of preventing sticking between pasta pieces. Short pasta is then put on suitable trays, whereas long pasta hangs on sticks, and those devices come into the drier passing through zones at different temperatures and humidities in accordance with drying characteristic diagrams. Temperature and humidity are continuously measured, and signals are sent to the controlling unit to maintain drying conditions as programmed. Thermal energy is transferred to the pasta by airflow coming from ventilation units, and the product passes through successive drying and stabilization phases. Sometimes, it is necessary to perform humidification along the drier by adding moisture through steam injectors to eliminate residual tensions and enhancing product stabilization.

The last phase of the pasta-drying process is the cooling phase, where pasta is slowly brought to equilibrium with the ambient outer environment, making it ready for packaging. Outer panels, made of material with high insulation power, cover drying system modules. They may be covered with stainless steel sheeting with watertight portholes at the dryer end for inspecting the inside. The bottom sections of the dryers are equipped with a condensation collection and a removal system. The storage section has an air circulation system to maintain homogeneous temperature and humidity conditions during storage.

In the packaging phase, the product has to be free of contamination. Packages must protect the pasta from damage during shipment and storage. The principal packaging material is the cellophane bag, which provides moisture-proof protection for the product and is used easily on automatic packaging machines. It should be noted that the package itself is a necessary means of providing all the needed information to the consumer.

Pasta Drying: Process Parameter Setting and Control

During drying, it is required that the operating conditions of the process do not introduce any mechanical damage, so in the past, slow processes, characterized by high water content (70–80%) and not very high temperature (60–70 °C) of the extracting wet air, were used. The need to reduce process time and consequently plant productivity has driven pasta producers to use stronger conditions with temperature, even higher than 100 °C and low relative humidity. These severe process conditions can induce mechanical damage resulting in poor quality. Even though pasta drying is a very common technology, the operating conditions and the choice between slow and fast processes are very questionable. In fact, the final quality of dry pasta is largely affected by the temperature and humidity conditions during the drying process and by the drying velocity. During this stage, pasta is subject to rather severe conditions that are fully responsible for the end-product quality. According to the market request for high-quality production, severe process conditions can cause a so-called thermal damage that is evidenced by a poor texture, a decrease in the mechanical resistance, a lower nutrient content, a darker color, etc. This kind of product obviously does not fit with consumer expectations, even though safer from a microbiological point of view, owing to the poorness of most of the quality indices. Then, a correct design and conduction of the drying process are important, which requires an adequate model for describing the overall phenomena involved.

Some mathematical models have been proposed in the literature to describe and control the process, and an engineering approach seems to be helpful to simulate the process steps, making it possible to understand better the optimal process variables with the target of high product quality. The drying process involves a simultaneous heat and mass transport and, as a consequence, a humidity profile within the pasta dough with a volume contraction, which is proportional to the removed water. The volume shrinkage occurs by beginning from the external layers of dough, where free water concentration is smaller compared to the inside layers. The differential equations used for process modeling describe the fundamental two transport phenomena: heat is transmitted to the solid phase for liquid evaporation and water is transferred as liquid through the solid and evaporation phase. During this process, the external layers of the dough begin to lose water more quickly with respect to the inner ones, up to a determined value of humidity, and some tests showed that a humidity value of 15% is a threshold for dough behavior that is completely elastic. When humidity becomes $<15\%$, the external crust shows elastic behavior, while the inner layers still maintain the viscoelastic characteristics with the tendency to shrink. This causes the generation of stressed zones, which do not relax and can even cause pasta breakage. A careful control of the temperature and humidity is needed to prevent these conditions.

While there are a lot of both scientific and technological works dealing with pasta nutrient content, the literature is very poor about mechanical stability. In fact, on the one hand, this latter requires a deep knowledge of dough properties according to humidity and temperature; on the other hand, it is needed to know the kinematic field arising from the shrinkage following water removal. The so-called thermoviscoelastic effect can deeply influence shrinkage and final quality.

Conclusions

Pasta production has been summarized in terms of ingredients and production features. It appears that even though the food product is to some extent very old, nevertheless, its knowledge still requires hard work by both researchers and technologists. A good semolina is not directly transformed into an optimum dough, with the risk of frustrating the raw material choice by process damage. Actually, very automatized plants allow a very finely controlled process, but the best profile temperature/humidity is still found by trial and error. A thorough application of rheology and thermodynamics transport phenomena

with the aim of achieving high-quality products is the new frontier for pasta production.

See also: Biscuits, Cookies and Crackers: Nature of the Products; Bread: Dough Mixing and Testing Operations; Pasta: Role in Diet; Rheological Properties of Food Materials; Starch: Sources and Processing; Starch: Structure, Property, and Determination.

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Pasta: Role in Diet

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Introduction

Pasta is an ancient food widely consumed in the world and represents one of the staple foods of the Mediterranean diet. There are many theories concerning its origin. Some researchers place its origin in the thirteenth century by Marco Polo, who introduced pasta in Italy upon returning from one of his trips to China in 1271. However, pasta dates much further back, maybe to the ancient Etruscans, who made pasta by grinding several cereals and grains and then mixing them with water, a blend that was later on cooked producing a tasty and nutritious food. The term 'macaroni,' which is currently referred to as a long type of pasta, is found in the documents of Roman writers since the first centuries 'after Christ.' However, both the Etruscans and the Romans baked their noodles in an oven, so boiled pasta had yet to be born in Italy. As the centuries went by, pasta continued to appear on the tables of Italy with different names. For example, in 1400, pasta was called 'lasagna' and pasta manufacturers were referred to as 'lasagnare,' while in 1800, their name turned into 'vermicellai.' Between 1400 and 1800, a new kind of pasta, that is, 'fidelli,' started being manufactured besides 'lasagne' and 'vermicelli.' 'Fidelli' were pasta threads with a cylindrical shape, and pasta manufacturers became known as 'fidellai.'

According to the American historian Charles Perry, who has written several articles on the origins of pasta, the first clear western reference to boiled noodles is in the Jerusalem Talmud of the fifth century AD written in Aramaic for which the term 'itriyah' was used. By the tenth century, 'itriyah' in many Arabic sources referred to dried noodles bought from a vendor, as opposed to fresh ones made at home.

Pasta Ingredients and Production

Pasta is mainly produced with wheat flour and water: eggs and/or other optional ingredients can also be added. Pasta manufacturers typically use milled durum wheat (semolina) although soft wheat flour and other cereal flours are also used. Pasta traditionally consumed in Italy is made exclusively with durum wheat flour, while in other countries, pasta made from soft wheat and/or other cereal flours are also frequently consumed. Durum wheat pasta is made from durum wheat semolina (*Triticum turgidum* L. var. *durum*) to which water is added, the reactant, that allows modification of gluten via the input of mechanical and thermal energy to produce pasta. Wheat proteins help with strengthening pasta structure by means of entangled aggregation. Starch, the major component of semolina and pasta, occurs in the form of granules dispersed within the protein network.

Pasta processing consists of three main operations: (1) hydrating and mixing–kneading of semolina to make a dough (formation of gluten network), (2) shaping the dough through extrusion, and (3) stabilizing the shaped pasta pieces, usually by drying. This sequence of operations is developed in an industrial automated scale. The process of hydrating and mixing–kneading of semolina produces a homogeneous hydrated dough that is essential for producing high-quality pasta. To obtain the most homogeneous hydrated mass, semolina and water first undergo high-speed premixing (using a single or double shaft, vertical or horizontal) to ensure intimate contact between semolina particles and water droplets. Further mixing then takes place in the main mixing unit, generally under vacuum, that removes air bubbles before the mass passes in the extrusion section.

After the dough is mixed, it is transferred to the extruder in order to obtain a specific form (spaghetti, macaroni, etc.) with the development of a continuous network of gluten protein that surrounds the starch granules. This process facilitates maintenance of pasta structure during cooking. The extrusion auger forces the dough through the die under high pressure, followed by cutting. The die holes are designed to give pasta the desired shape and size. Compression and extrusion are obtained with different machines. Until recently, most dies were made of bronze, which was relatively soft and required repair or periodic replacement. Recently, dies have been improved by fitting the extruding surface of the die with Teflon® inserts to extend the life of the dies and improve pasta quality.

Drying is the most difficult and critical step to control in the pasta production process. Drying consists of passing a flow of hot or warm air over the fresh pasta product, so that the thermohygrometric properties of the air progressively decrease the pasta moisture content at a level acceptable for dried pasta. Nowadays, the majority of pasta-drying processes adopt air temperatures in the range of about 70–100 °C while keeping product temperatures between 60 and 90 °C. The objective of drying is to lower the moisture content of pasta from ~31% to 12–13%, so that the finished product is hard, retains its shape, and can be stored without spoiling. Dried pasta stored at room temperature is stable for years at room temperature.

The best pasta is produced from durum wheat semolina, due to the dough excellent rheological properties, pasta superior color, cooking quality, and consumer acceptance. Whole grain wheat and other grains such as rice, buckwheat, barley, spelt, millet, oats, quinoa, and corn are also sometimes used to make pasta, which can be made also from more than one type of grain, and, in such a case, it is called 'multigrain.'

Pasta can be enriched, supplemented, or fortified or remain conventional. In recent years, several ingredients have been developed and are being used to improve the nutritional quality of pasta, for example, fibers, proteins, and vitamins.

On the basis of scientific evidence showing beneficial effects on health of whole grain product consumption, production of whole wheat pasta is increasing in recent years and many efforts have been made to improve its rheological and organoleptic properties and to implement its consumption even if this product is not yet widely consumed. Whole wheat pasta is

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00523-7

significantly higher in fiber and nutrients than its refined counterpart: during the refining process, wheat loses roughly 60% of its grain, a portion that includes its most nutritious components, the bran and the germ. Consequently, refined wheat provides less than half the amount of B vitamins, minerals, and fibers than whole grains. At present, there are no food labeling regulations setting the minimum whole grain content required for a given food to be considered whole grain. The Whole Grains Council recommends manufacturers to label as 'whole grain' only products containing a higher percentage of whole grain than refined grain (i.e., at least 51% whole grain). The HEALTHGRAIN Forum proposes two labeling categories, one for bread and pasta indicating 100% whole grain if the content of whole grain flour is almost over 90% of dry material and the other category for foods defined as 'rich in whole grain' where the amount of whole grain must be specified.

Production and Consumption

The last report of the International Pasta Organization (IPO) (www.internationalpasta.org/index.aspx) states that in 2012, about 13.5 million tons of dry pasta has been produced in the world. Among the pasta-producing countries, Italy ranks first with an annual production of about 3.33 million tons, followed by the United States (2.00 million tons), Brazil (1.19 million tons), Russia (1.08 million tons), and Turkey (1.00 million tons). The European Union produces 36.4% of pasta, followed by Central and South America with 21.9% and by North America with 16.9%; Asia and Australia are the countries with the lowest pasta production, that is, 1.8% and 0.4%, respectively. Italy is the first pasta-exporting country with about 1.8 million tons, followed by Turkey (~436 thousand tons), Belgium (~137 thousand tons), the United States (~133 thousand tons), and China (~109 thousand tons). Italy is the largest per capita/year pasta consumer in the world (26 kg), followed by Venezuela (13.2 kg), Tunisia (11.1 kg), and Greece (10.6 kg); Switzerland (9.2 kg) and Sweden (9.0 kg) consume more pasta per capita than the United States (8.8 kg).

Composition and Nutritional Qualities

Pasta is an important source of carbohydrates, especially starch. A portion of 100 g of uncooked pasta contains about 68.1 g starch, 4.2 g soluble sugars, 2.7 g fiber, 10.9 g protein, and 1.4 g fat, providing about 353 kcal (1478 kJ). Pasta also contains vitamins B_1 and B_2, as well as some minerals. In the United States, however, pasta is vitamin- and mineral-enriched. During cooking, some of the nutrients get lost either in the water or by heat, especially some starch, proteins, phosphorous, and vitamin B_1.

The biological value of proteins in durum wheat pasta is not particularly elevated given its low content of essential amino acids: in particular, wheat proteins are deficient in lysine. Based on high or low temperature used in drying, 100 g of pasta contains from ~22% to 26% lysine recommended dietary intake. However, pasta is usually consumed with other foods with different protein contents that improve the nutritional value of the pasta dish. The addition of eggs to the basic ingredients increases its protein content (about 20% higher compared to wheat pasta) and its biological value since eggs contain about 139% of the recommended dietary intake for lysine. However, the addition of eggs increases also the fat content of pasta.

Whole grain pasta contains fewer calories for 100 g of dry product (about 324 kcal per 1356 kJ) and supplies more fiber (~6 g) and 25% more proteins than the traditional refined pasta. Whole grain pasta is an excellent source of B vitamins and iron and provides significantly higher levels of the essential trace mineral selenium, a micronutrient with antioxidant properties. Whole wheat pasta also contains appreciable levels of alpha-linolenic acid. The most common whole grain pastas are made from whole wheat, brown rice, or buckwheat, but quinoa, spelt, and kamut are also used to make whole grain pasta. Not all whole grain pasta is made from 100% whole grain flour, however. Some are made from a mixture of whole grain flour and ground legumes and flax, ingredients that improve the product texture while boosting its protein and unsaturated fatty acid content.

Compared to wheat, buckwheat contains proteins with a high biological value due to a well-balanced amino acid composition (rich in lysine and arginine) and antioxidant molecules, such as rutin (a flavonol glycoside), which is recommended for people with capillary fragility. Corn pasta has about half as much proteins as traditional wheat pasta, but it is otherwise nutritionally comparable. Pasta from rice flour does not contain any gluten. Brown rice flour compared to white rice flour is made from rice that has only the inedible hull removed; therefore, it is high in fiber and produces pasta dough similar to whole wheat pasta, with the exception that dough made from brown rice flour is stickier. This kind of pasta is consumed in Asian countries and available in Asian markets or health food stores. Compared to common wheat, the composition of the kamut grain is richer in several minerals, including magnesium and zinc and selenium, a powerful antioxidant. Compared to wheat flour, kamut flour is also characterized by a percentage of vitamin E higher than 30%, as well as a higher content of proteins, lipids, and fatty acids. Oat and barley flours are mixed with wheat flour because they are rich in beta-glucan, a type of viscous fiber that improves pasta texture. Soy flour can be used to supplement proteins in macaroni and to produce a quick-cooking pasta product. Spaghetti with high protein and high lysine content can be obtained by adding up to 35% soy flour without adverse effects on flavor and texture and could result in greater acceptability of a soy-based diet.

Among the different types of pasta, the inulin-enriched pasta is to be counted for its content of nondigestible fructo-oligosaccharide with prebiotic effects.

Starch is the most abundant nutrient in pasta and the source of rapidly released glucose during digestion, regardless of the type of cereal used. The starch of pasta contains two major glucose polymers, amylose and amylopectin, which differ in the degree of polymerization (DP) of glucose chains and in the frequency of branches. In particular, wheat endosperm contains ~75–80% of starch as amylopectin and 20–25% as amylose. Amylose is a predominantly linear molecule with glucose chains linked through alpha-1,4 linkages in the range of 1000–2000 DP, while amylopectin is a complex and highly branched molecule in the range of 50 000–500 000 DP. In amylopectin, glucose units are linked in a linear way with alpha-1,4

linkages. Branching takes place with alpha-1,6 bonds occurring every 24–30 glucose units, resulting in a soluble molecule that can be quickly degraded since it has many end points for enzymes to attach onto. In contrast, amylose contains very few alpha-1,6 bonds or even none at all. This causes it to be hydrolyzed more slowly but to have a higher density and to be insoluble. In raw products, native starch occurs naturally as insoluble semicrystalline granules assembled in some way to form an ordered granular architecture and is not digested by human bowel enzymes. When it is heated in excess water, the starch granules undergo an irreversible phase transition, referred to as gelatinization, in which the highly ordered structure is disrupted.

The extent of starch gelatinization is the key factor in determining the digestibility and nutritional properties of starchy foods. The starch, before being absorbed in the small intestine, must be hydrolyzed to its monosaccharide components. The digestion of starch begins with salivary amylase, but this activity is much less important than that of pancreatic amylase in the small intestine. Amylase hydrolyzes starch, with the primary end products being maltose, maltotriose, and alpha-dextrins; some glucose is also produced. The products of alpha-amylase digestion are hydrolyzed into their monosaccharide components by enzymes expressed on the brash border of the small intestinal cells, the most important of which are maltase, sucrase, isomaltase, and lactase. However, starch digestibility is influenced also by the physical structure of food. In the case of pasta, molecular rearrangements of proteins may lead to the formation of large protein aggregates, which reduce pasta starch accessibility to the action of alpha-amylase during digestion that results in a lower but prolonged increase of blood glucose concentrations in the postprandial period. Addition of viscous fiber or inulin in pasta further reduces the rate of starch digestion.

Health Effects

Pasta is a key component of the traditional Mediterranean diet, a dietary pattern that confers health benefits compared to current Western dietary patterns.

Several decades of observational and intervention studies have clarified the characteristics of lifestyles able to prevent chronic diseases and extend longevity. It is well known that both an excess of calorie intake and an inappropriate composition of the habitual diet are relevant for the development of obesity and other chronic diseases. There is a general consensus among nutrition scientists and related experts concerning the optimal distribution of macronutrients: carbohydrates at 45–60% of calories, fats at 25–30% of calories, and proteins at 15–20% of calories. However, appropriate food choices may be more important than the overall amount of each of these macronutrients. This implies that avoiding extreme and/or unbalanced diets and favoring macronutrient-balanced eating patterns are as important as emphasizing the preferential use of unsaturated fats, whole grain and low-glycemic index (GI) foods, fiber-rich fruit and vegetables, and a combination of plant and animal protein sources.

The GI is a measure of the blood glucose-raising ability of the available carbohydrates in foods. A large body of evidence shows that a diet with a low GI may be able to prevent weight gain and reduce the risk of type 2 diabetes and coronary heart disease. GI is calculated by dividing the incremental area under the curve (AUC) of blood glucose concentrations, measured after the ingestion of a portion of the test food by the AUC of blood glucose concentrations achieved with a portion of the reference food (glucose or white bread). The test and the reference food must contain the same amount (50 g) of carbohydrate and are evaluated in the same group. This index was proposed for the first time in 1981 by Jenkins as an alternative system for classifying carbohydrate-containing foods.

Among carbohydrate-rich foods, spaghetti has a lower GI than bread, pizza, and other cereal foods. The average GI of durum wheat spaghetti from different manufacturers, cooked 10–15 min in water, is 46%. Other types of durum wheat pasta (i.e., macaroni, penne, and fusilli) or fresh pasta, made from soft wheat flour and eggs, also have a low GI that ranges from 32% to 47%. These values are also superimposable to those of whole grain boiled spaghetti (42%) and soy spaghetti (47%). As mentioned earlier, the low GI of these products is consistent with a low rate of starch accessibility to digestion, due to the formation of large protein aggregates surrounding the starch. This is relevant since it is able to reduce insulin secretion and consequently the risk of reactive hypoglycemia during the postabsorption period; moreover, it contributes to increase glucagon-like peptide-1 (GLP-1) that regulates postprandial satiety and glucose metabolism.

The majority of the intervention studies, mainly performed in high-risk subjects – obese subjects or people with insulin resistance/metabolic syndrome – and in diabetic patients, have shown that a low-GI diet induces favorable changes on body weight regulation, blood glucose, and insulin metabolism and lipid control.

A meta-analysis of randomized controlled trials has demonstrated that a diet with low GI, at least in the medium term (<6 months), promotes a greater weight gain and total fat mass loss compared to diet with high GI. This result has also been confirmed by a systematic review in which the authors evaluated the independent role in body weight reduction of GI and other variables of diet in healthy subjects, in glucose-intolerant subjects, and in type 1 and type 2 diabetic patients.

Epidemiological evidence also suggests that a diet with a low GI and rich in fiber contributes to diabetes prevention and to an improvement of the plasma lipid profile. The benefits of low-GI foods on blood glucose control and other metabolic parameters are particularly evident in intervention studies performed in diabetic patients and in other high-risk individuals compared to healthy subjects. In relation to blood glucose control, data clearly show that in diabetic patients, low-GI/high-fiber diets improve blood glucose concentrations, especially in the postprandial state, reduce the number of hypoglycemic events, and also lower the level of glycated hemoglobin. The beneficial metabolic effects of low-GI foods are independent of the fiber content. However, a combination in the diet of both low-GI and fiber-rich foods may enhance the beneficial effects of each component. These findings have led some food industries to develop new types of pasta rich in fiber, like those made from whole grain wheat flour or barley and oat flour. In particular, pasta made from whole grain wheat flour, beyond its content of dietary fiber, is also rich in oligosaccharides, minerals, vitamins, and antioxidant components that may have an additional beneficial influence on several biological functions. As a matter of fact, the habitual consumption of

whole grain foods is consistently associated with reduced risk of developing obesity, type 2 diabetes, and cardiovascular diseases in several epidemiological studies. These benefits have been related to multiple mechanisms; worth considering are (1) the lower energy content of whole grain products, as well as the higher satiating effect, favoring a lower energy intake per eating occasion and, in the long term, playing an important role in body weight regulation, and (2) the higher fiber, resistant starch, and oligosaccharide content fermented by gut microbiota with production of short-chain fatty acids (SCFA), as acetic, propionic, and butyric acids, which improve the control of glucose and lipid metabolism, decrease gastric emptying rate, and increase satiety. In particular, an intervention study, performed in subjects with the metabolic syndrome, showed that whole grain wheat pasta consumed in the context of a mixed meal significantly reduces postprandial insulin and triglyceride plasma levels, compared to refined cereal products. For these reasons, the consumption of whole grain foods in the diet has been included in the nutritional recommendations of the United States and other countries as a dietary strategy targeting the whole population.

Beneficial effects on glucose and lipid metabolism have been also reported in subjects consuming pasta made from barley or oat flour, two cereals particularly rich in beta-glucan, or pasta added with guar. These types of pasta, compared to traditional refined wheat pasta, induced an even lower increase of plasma glucose, insulin, and triglyceride levels in the postprandial period and decreased even more both fasting and postprandial plasma cholesterol concentrations. The benefits on glucose metabolism of barley and oat pasta are related to the high viscosity of beta-glucans, which delays gastric emptying and limits starch accessibility to digestive enzymes, thus prolonging nutrient digestion and absorption in the small intestine.

Finally, consumption of pasta enriched in fructo-oligosaccharides and inulin has been proposed as a useful tool to prevent inflammatory gastrointestinal diseases and colon cancer, thanks to its prebiotic effects. The prebiotic activity might have a beneficial impact also on fat storage, insulin resistance, and subclinical inflammation.

However, despite the potential health benefits of new pasta formulations, it needs to be underlined that the types of pasta commonly available in food shops represent a healthy choice among carbohydrate-rich foods for their low GI and high satiating ability. Moreover, according to the Mediterranean tradition, pasta is the main ingredient of a complex dish in which other ingredients are also present, among these being olive oil, vegetables such as tomatoes, and products of animal origin. When animal products like fish, cheese, and meat are present in the dish, their amount is usually small and it can be considered optimal from the nutritional standpoint, being a good source of low-GI carbohydrates, rich in fiber, and with a balanced content of high-quality proteins and unsaturated fats, not to mention micronutrients and polyphenols.

In conclusion, scientific evidence supports the health benefits of regular pasta intake, in particular if it is consumed in the context of a diet balanced for energy density and macronutrient distribution, according to the Mediterranean dietary pattern. In fact, pasta, due to its low GI, represents an ideal source of carbohydrates in the diet of healthy individuals, obese subjects, and diabetic patients. In this context, the alarm about the possible dangers of a high consumption of dietary carbohydrates certainly does not apply, on the basis of the available scientific evidence, to healthy carbohydrate-rich foods like pasta. In contrast, it is strongly advised to favor pasta consumption, in those countries where its consumption is low, in substitution of other carbohydrate foods with higher GI. Furthermore, in order to enhance whole grain intake in the daily diet, whole grain pasta consumption should be considered in addition or in substitution of refined grain pasta.

See also: Carbohydrate: Digestion, Absorption and Metabolism; Cereals: Dietary Importance; Cereals: Types and Composition; Dietary Fiber: Bran; Dietary Fiber: Determination; Glucose: Metabolism and Regulation; Gut Hormones; Obesity: The Role of Diet; Pasta: Manufacture and Composition; Starch: Modified Starches; Starch: Sources and Processing; Starch: Structure, Property, and Determination; Starch; Wheat: Grain Structure of Wheat and Wheat-based Products.

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Pasteurization: Effect on Sensory Quality and Nutrient Composition

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The pasteurization concept emerged as a mild heat treatment that aims the removal of pathogenic and spoilage bacteria and inactivation of undesirable enzymes from food products, in order to assure food safety. This process derives from the discovery of Pasteur in 1857, when he observed that souring in milk could be delayed by heating it to 50.0–61.0 °C. Nowadays, thermal pasteurization is the method commonly used for food preservation and shelf life extension. The severity and duration of heat treatment depend on food type, equipment, and method used. However, thermal processing has several disadvantages, such as the destruction of nutrients, development of off-flavors, and other losses on product sensory quality. Despite this, thermal processing remains the prevailing method for food preservation due to its high availability, low cost, and effectiveness.

The consumer is increasingly demanding for nutritious, fresh-like food products, with high organoleptic quality and extended shelf life. Therefore, many efforts have been taken to optimize pasteurization conditions, in order to achieve bacterial inactivation and reduce the loss of nutrients. Additionally, novel technologies have emerged for food processing, in an attempt to complement or even replace conventional thermal pasteurization. These novel pasteurization methods include high pressure processing (HPP), high-voltage pulsed electric field (PEF), ohmic heating (OH), microwave (MW) and radio-frequency (RF) heating, ultraviolet (UV) exposure, irradiation, and membrane filtration.

Nowadays, the most well-established nonthermal processing technique is HPP, which uses pressures of 100–1000 MPa, at room temperature or below. As pressure has a limited effect on covalent bonds of low-molecular-mass compounds (e.g., color and flavor compounds), HPP allows a high retention of food quality attributes, namely, sensorial and nutritional properties. Therefore, food products treated with HPP usually have fresh-like characteristics, without added preservatives. However, HPP also has several disadvantages, such as the resistance of food enzymes, leading to possible enzymatic and oxidative degradation of food components during storage and distribution.

PEF is another well-established nonthermal processing technique that applies high-voltage electric fields for a short duration of time. This treatment results in the inactivation of enzymes and destruction of spoilage and pathogenic microorganisms, due to electroporation of cell membranes. Since PEF processing only causes a small increase in temperature, there is no appreciable loss of flavor, color, and bioactive compounds.

OH is defined as a process where electric currents are applied directly to the food. Heat is internally generated due to electrical resistance of the food to the current. The advantage is that much shorter heating times can be applied, and the food will maintain its nutritional and sensory characteristics.

Concerning MW and RF heating, electromagnetic waves of certain frequencies are used to generate heat within food products, resulting in a high and homogeneous heating rate and subsequently a shorter processing time. This advantage often yields an increased processing rate and/or an improved product quality. Comparing MW with RF heating, RF has a longer wavelength, and thus, RF power will penetrate deeper into products than MW.

UV exposure for food preservation is due to the absorption of short-wave UV light by microorganisms, inhibiting cell replication processes. When compared with thermal processing, UV presents several advantages, such as improved destruction of spores that are not susceptible to heat. But it also has limitations, including a very short penetration capacity and possible generation of free radicals by organic photochemical reactions. These reactions can adversely affect food, due to damage of vitamins and proteins, destruction of antioxidants, lipid oxidation, color change, and formation of off-flavors.

Irradiation is another method used for the preservation of food products, which allows microbial inactivation at an ambient temperature, avoiding the deteriorative effects of heat on flavor, color, and nutrient value of foods. However, it is often associated with the development of off-flavors, softening and increasing permeability of some fresh fruits and vegetables, since irradiation can affect all the major components of food, that is, carbohydrates, proteins, and lipids.

Membrane filtration is a preservation method mostly used for juice processing. The advantages associated with this treatment include the extension of shelf life of food with energy savings and reduced waste generation. However, it has high operational and maintenance costs, due to the need of membrane cleaning and replacement.

The aim of this article is to review the effect of conventional and nonconventional pasteurization techniques, specifically on nutritional and sensorial properties of several food products. For that purpose, information is organized into different food categories, which include fruits and vegetables, dairy products, eggs, meat and seafood products, and alcoholic beverages.

Fruits and Vegetables

Many fruits and vegetables are low-acid foods with very short shelf lives. However, their processing and subsequent storage may represent significant quality losses in terms of sensorial, nutritional, and functional properties. For instance, thermal pasteurization applied to carrots resulted in significant hardness decrease compared with raw carrots, leading to a final product that would certainly be rejected by the consumer. Therefore, application of nonthermal processing technologies on fruits and vegetables has been investigated, especially HPP and gamma irradiation. Table 1 shows the effects of thermal and nonthermal pasteurization treatments on sensorial and nutritional attributes of fruits and vegetables.

In most cases, gamma irradiation caused no changes in the organoleptic properties of vegetables and fruits. However, the effects of irradiation on these attributes depended on the applied dose. For instance, carrots irradiated at 1 kGy had no

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Table 1 Nutritional and sensory effects caused by different pasteurization treatments in fruits and vegetables

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
Carrot	Thermal (70, 90, or 117 °C)	β-carotene bioaccessibility increased after intense thermal treatment (sterilization), but not with pasteurization
		Hardness was negatively affected by temperature
	Gamma irradiation (1 or 2 kGy)	No significant differences in ascorbic acid content and total carotenoids between irradiated and nontreated samples
		Irradiated and nontreated carrots were not differentiated by sensorial analysis
	Gamma irradiation (<2.0 kGy)	No significant effect on color, lightness, flavor, sweetness, odor, and taste
		During storage, overall acceptability of irradiated carrots similar to nontreated samples
	Gamma irradiation (2.0 kGy)	No effect on textural properties
		Slight increase in the sweetness
	Gamma irradiation (3.0 kGy)	Appearance scores were lower for irradiated samples than for nontreated ones
Cabbage	Gamma irradiation (0.5–3.0 kGy)	Appearance of irradiated cabbage was not significantly affected
Onion bulbs	Gamma irradiation (0.05–0.5 kGy)	No appreciable effects on the total free sugar content (sucrose, glucose, and fructose)
	Gamma irradiation (0.1–0.5 kGy)	Drastic reduction in ascorbic acid content
Garlic bulbs	Gamma irradiation (0.1 kGy)	No influence on the total sulfur and thiosulfonate content
Potato	Gamma irradiation (0.1 kGy)	Increase in sucrose content
		Ascorbic acid levels decreased during the initial period of storage
		Decrease in carotenoid content
		Aspartic acid, asparagine, threonine, serine, alanine, isoleucine, leucine, lysine, and arginine showed increases 24 h after irradiation, while glutamic acid, proline, methionine, and phenylalanine decreased
		Lysine content showed a sixfold increase after 1 week of storage, and at 1 month, the concentration was still three times higher than the control values
Sliced tomato	Gamma irradiation (1 kGy)	No significant effect on total carotenoid and ascorbic acid content
		40% decrease in α-tocopherol
Broccoli and mung bean sprouts	Gamma irradiation (1 kGy)	Appearance, color, texture, taste and overall acceptability did not undergo significant changes after 7 days of storage
Okra, baby corn, chaom, bird pepper, goat pepper, eggplant (brinjal), bitter cucumber, asparagus, and basil	Gamma irradiation (0.3, 0.6, or 0.9 kGy)	Organoleptically acceptable after 7 days of storage
Cucumber	Gamma irradiation (2 kGy)	No change in the organoleptic properties
Picked asparagus	Microwave (88 °C, 9 or 15 min) and thermal processing (88 °C, 30 min)	The required temperature for pasteurization was achieved twice as fast as conventional heating
		Microwave pasteurization reduced thermal degradation of asparagus
Leek, cauliflower, and green beans	HPP (200 MPa, room temperature, 5 min)	Increased proportion of folates in the monoglutamate forms, that is, higher folate bioaccessibility
Green beans	HPP (500 MPa, ambient temperature, 1 min)	Large retention of firmness and ascorbic acid content after treatment
		More intense green color after HPP treatment
		Green color of the HPP-treated vegetables turned into pale yellow
Apple, pear, orange, pineapple, carrot, celery, green pepper, and red pepper	HPP (100–400 MPa, room temperature, 5–60 min)	Rapid firmness loss during compression
		During the pressure holding period, the firmness either decreased further or recovered gradually
		At 100 MPa, pear was the most pressure-sensitive fruit, while at 200 MPa, apple was the most pressure-sensitive
Strawberry	HPP (200–800 MPa)	There is not an HPP treatment for the simultaneous retention of all flavor volatiles
		Acid retention was more effective at 400 MPa
		Alcohol retention was more effective at 800 MPa
		The most effective range of pressure for ketone retention was 200–800 MPa

(*Continued*)

Table 1 (Continued)

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
Raspberry	Gamma irradiation (0.5, 1, or 1.5 kGy, 60 °C)	Phenolic content increased after irradiation and decreased during storage Decrease in firmness No effect on most of the physicochemical and sensorial properties
Papaya	Gamma irradiation (0.5 kGy)	Irradiation caused a 2-day delay of ripening time Irradiated fruits ripened normally in terms of sugar content, color change, firmness, and general appearance
Grapefruit	Gamma irradiation (0.07, 0.2, 0.4, or 0.7 kGy)	Soluble solid and ascorbic acid contents of early-season fruit were not affected by irradiation Irradiation $\geq$0.2 kGy caused a reduction in soluble solid and ascorbic acid contents of late-season fruit Sensory qualities (such as appearance and flavor) of early-season grapefruit exposed to irradiation ($\leq$0.4 kGy) were similar to the control
	Gamma irradiation (0.3 kGy)	No considerable changes of the content of total soluble solids Decline in acid content Enhanced or maintained flavonoid content
Pineapple	Gamma irradiation (0.05, 0.1 or 0.15 kGy)	Maintained their texture better than the controls through storage
Apple	Gamma irradiation (1.5, 4.5, 5 or 6 kGy)	Higher doses of irradiation caused a decrease in ascorbic acid content
	Gamma irradiation (0.5–3.0 kGy)	Hardness of apples decreased during increasing irradiation dose and during storage
	Gamma irradiation (0.15–0.90 kGy)	Irradiation doses 0.30–0.90 kGy reduced apple firmness Doses of $<$0.30 kGy had no effect on apple firmness External color of apples was not affected
Melon	Gamma irradiation (2.5 kGy)	The sensory qualities were maintained within acceptable limits
Mango	Gamma irradiation (0.4 or 0.6 kGy)	Effect of irradiation on hardness was dependent on mango variety Gamma irradiation had no effect on skin or flesh color
Pears	Gamma irradiation (0.30–0.90 kGy)	Firmness loss due to irradiation was dose-dependent
Citrus	Gamma irradiation (0.15, 0.3, or 0.45 kGy)	Irradiation caused softening of some cultivars, while others remained unaffected The appearance of all cultivars was negatively affected with the 0.45 kGy dose, due to loss of glossiness
Papayas, rambutans, and oranges	Gamma irradiation (0.75 kGy)	Aroma and flavor tended to be more intense in the irradiated fruit Firmness decreased as a result of irradiation and storage
Pineapple and watermelon in cubes	Gamma irradiation (1 or 2.5 kGy)	Pineapple sourness was not affected Watermelon sweetness was not affected

changes in sensorial properties, while at 2 kGy, a slight increase in sweetness was observed, and at 3 kGy, a clear negative impact on carrot appearance was detected. The effect on apple firmness was also dose-dependent, since at lower doses (0.15–0.30 kGy), it did not change, while at higher doses (0.30–0.90 kGy), it was significantly reduced. This decrease in firmness was also observed for raspberries, pears, papayas, rambutans, and some cultivars of citrus fruits. Changes in flavor were observed for papayas, rambutans, and oranges, in which more intense flavor and aroma were developed after irradiation treatment (0.75 kGy). Regarding irradiation effects on fruit appearance, citrus fruits had loss of glossiness after treatments at 0.15, 0.3, or 0.45 kGy. In what concerns to nutritional properties, some changes have been identified in irradiated fruits and vegetables: (1) reduction of ascorbic acid content in onions, potatoes, apples, and late-season grapefruits; (2) increase in sucrose content, changes in amino acid contents, and decrease in carotenoid content in potatoes; (3) decrease in α-tocopherol content in sliced tomatoes; (4) increase in phenolic content in raspberries; (5) increase in flavonoid content and decline in acid content in grapefruits. However, several other studies report no nutritional changes in irradiated fruits and vegetables.

During HPP of fruits and vegetables, different pressure and temperature combinations can be used to achieve desired effects on texture, color, and flavor. The quality of HPP fruits and vegetables can, however, change during storage due to coexisting chemical and biochemical reactions, when endogenous enzymes or microorganisms are incompletely inactivated.

The effect of HPP (100–400 MPa, room temperature, 5–60 min) on firmness was evaluated on a great variety of fruits and vegetables (Table 1). A firmness loss was observed during compression, and, in some cases, firmness was recovered gradually during pressure holding time. Pectin methylesterase activity was suggested to be the major reason for the

increase in firmness. Upon HPP treatment, pectin methylesterase was liberated and contacted with the substrate, leading to demethylation. The de-esterified pectin was capable of forming a gel network with divalent ions resulting in increased hardness. However, this effect was more prominent in samples treated at 100 or 200 MPa, since pressures above 200 MPa caused the softening of most fruits and vegetables tested. HPP was also applied on green beans, showing no effect on firmness and ascorbic acid content. However, a more intense green color was acquired, which represents a positive feature for consumer acceptability. This green color turned into pale yellow during storage, showing that storage conditions of HPP products must be optimized to avoid the undesirable modifications that can occur. Application of HPP (200–800 MPa) on strawberry was not suitable for simultaneous retention of all important flavor volatiles: (1) Acids (such as butanoic acid, 2-methylbutanoic acid, and hexanoic acid) were better retained at 400 MPa; (2) alcohol retention was better at 800 MPa; (3) ketone retention was more effective at pressure ranges from 200 to 800 MPa. Therefore, the choice of HPP conditions should have in consideration which type of flavor volatiles are important to retain.

In terms of nutritional properties, HPP can be used to increase folate bioaccessibility in vegetables, since the pressure increased the proportion of monoglutamate folate (form with higher bioaccessibility) in leeks, cauliflower, and green beans. However, the resulting monoglutamate forms after treatment were degraded during storage at low temperatures; thus, it would be important to identify proper storage conditions to minimize this problem.

Few studies focus on MW pasteurization of fruits or vegetables. This approach has already been tested on picked asparagus, and the required temperature (88 °C) for MW pasteurization was achieved twice as fast as (15 min for 1 kW and 9 min for 2 kW) conventional heating (30 min). In addition, the thermal degradation of asparagus was more intense in samples subjected to conventional treatment than in MW-treated samples, showing that this technology has some potential for application in vegetables.

While not much information is available on the pasteurization of vegetable and fruit products, the application of thermal and nonthermal technologies for processing of purees and juices is extensively studied, as discussed in the next sections.

Purees

Table 2 shows the impact of different pasteurization treatments (conventional thermal processing, OH, MW, and HPP) on nutritional and sensorial properties of fruit/vegetable purees. Thermal pasteurization usually causes loss of several important nutritional properties, such as β-carotene, chlorophyll, and phenolic content. Deteriorative effects on organoleptic characteristics were also observed, more specifically in terms of color, viscosity, and flavor.

Spinach puree pasteurization by OH (10–40 $V\,cm^{-1}$) and thermal processing (60, 70, 80, or 90 °C, ≈2 min) was tested, and a more intense browning was observed in samples treated with OH processing. However, these samples showed higher retention of color and β-carotene and chlorophyll content. This increase could be caused by the possible enhancing effect of OH on β-carotene biosynthesis and the formation of chlorophyll derivatives. Therefore, OH could be applied on vegetable purees, resulting in high retention of some important attributes.

Few studies are available focusing on the MW pasteurization of fruit and vegetable purees. When intense MW treatments (in the range of 200–1000 W, 60–340 s) were applied on kiwi fruits, changes in consistency and viscosity were observed, as well as an atypical flavor and color. Regarding nutritional composition, MW (652 W, 35 s) applied on apple puree did not cause modification of total polyphenols and ascorbic acid during the treatment, but these parameters decreased during storage. In contrast, titratable acidity and viscosity were modified by the treatment, but not during storage. Samples also suffered oxidation during storage, since the treatment was not able to inactivate browning-related enzymes.

HPP has also been tested on fruit purees, usually allowing a higher retention of total antioxidant capacity, carotenoids, and polyphenolic content, when compared with thermally treated purees. HPP-treated samples also showed minor changes in color, viscosity, and aroma. However, some studies report modification of aromatic volatile profiles (≈800 MPa), possibly because of the generation of undesirable flavors.

Several studies were performed on HPP for tomato puree pasteurization. At 400 MPa, lycopene, β-carotene, and total carotenoid content were increased, while total antioxidant capacity was decreased. However, longer holding times led to different results: HHP treatments of 300/600 MPa for 60 min showed no effect in lycopene and β-carotene content, while total antioxidant capacity decreased after the first week of storage but, during the next 2 weeks, it was better preserved than in nontreated samples. The effects of HPP on important nutritional properties are also highly dependent on the pressure intensity: (1) Lycopene content increased (58%) at 500 MPa, while it remained stable at 700 MPa; (2) carotenoid content increased with a treatment of 600 MPa for 15 min, but it significantly decreased at 400 and 500 MPa, compared with thermal pasteurization. In all cases, HPP caused a less significant loss of ascorbic acid than thermal treatment. In terms of sensory properties, HPP (300, 500, or 700 MPa, 2 min) improved color and viscosity compared with thermal processing (75 °C, 4 min). This viscosity improvement was attributed to heat or enzymatic degradation of pectins.

Thermal pasteurization of strawberry purees increased viscosity, particularly when intense treatments were applied, being the taste quality usually superior in fresh puree, due to a greater aromatic intensity. The application of HPP on strawberry puree showed several advantages compared with thermal processing, including minor differences in color, low losses of ascorbic acid, no changes in anthocyanins, and high retention of phenolic compounds through storage. However, HPP caused modifications on volatile profiles, affecting the flavor of strawberry purees. For example, HPP at 400 MPa more than doubled the hexanal content (characteristic smell of foliage and grass), while HPP at 800 MPa resulted in a slight decrease in hexanal content. Additionally, at 800 MPa, the volatile compounds contributing to fresh strawberry flavor (nerolidol, furaneol, linalool, and some ester compounds) were decreased, but the most important flavor compounds (e.g., 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one) were not affected.

Table 2 Nutritional and sensory effects caused by different pasteurization treatments in fruit and vegetable purees

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
Carrot puree	HPP (400–600 MPa, 10–30 °C, 15 min) and thermal (70 °C, 2 min)	Increase in total carotenoids and total antioxidant capacity compared with thermally treated and untreated samples
Spinach puree	Ohmic heating (10–40 V cm^{-1}) and thermal (60, 70, 80, or 90 °C, ≈2 min)	Ohmic heating caused more browning than conventional heating Ohmic heating allowed higher retention of color attributes, β-carotene, and chlorophyll content
Pumpkin puree	HPP (400 or 600 MPa, 5 min) and thermal (85 °C, 15 min)	No color changes at 400 MPa HPP caused lower color modifications compared with thermal treatment Higher carotenoid and polyphenol contents in HPP
Avocado puree	HPP (600 MPa, 23 °C, 3 min)	Decrease in pH No significant color modifications Increases in the activity of polyphenol oxidase and lipoxygenase may cause generation of undesirable flavors and nutritional losses
Tomato puree	HPP (300, 500, or 700 MPa, 20 °C, 2 min) and thermal (75 °C, 4 min)	Pressure-treated samples had improved color and viscosity compared with thermally treated ones Lycopene content increased at 500 MPa, but it remained unaffected at 700 MPa
	HPP (400 MPa, 25 °C, 15 min)	pH, °Brix, and viscosity were increased Ascorbic acid losses of ≈29% were found Lycopene, β-carotene, and total carotenoid contents were increased A significant decrease in total antioxidant capacity was obtained
	HPP (400–600 MPa, 10–30 °C, 15 min) and thermal (70 °C/2 min)	Lower decrease in ascorbic acid in HPP-treated tomato puree compared with thermally processed samples Significant increase in total carotenoids at 600 MPa, while a significant decrease was observed at 400 and 500 MPa
	HPP (300 or 600 MPa, 20 °C, 10–60 min)	No effect in the total concentrations of the main water-insoluble antioxidants, lycopene, and β-carotene Enhanced water binding Total antioxidant capacity decreased after the first week of storage, but it was better preserved during the next 2 weeks, compared with untreated samples
Guava puree	HPP (400–600 MPa, room temperature, 10–15 min)	Decrease in pectin content and turbidity at 400 MPa No significant changes in aroma Untreated and HPP-treated (400 MPa) samples had a decline in ascorbic acid content Ascorbic acid content remained stable in thermally treated samples and samples at 600 MPa
Nectarine puree	HPP (450 or 600 MPa, 10 °C, 5–10 min) and thermal (85 °C, 5 min)	pH, titratable acidity, and °Brix were not affected by HPP Thermal processing caused higher color changes than HPP Increase in zeaxanthin and lutein at 450 MPa, compared with other treatments HPP increased the total phenolic content and total antioxidant activity during storage
Strawberry puree	Thermal (75 or 90 °C, 15 s or 20 s)	Viscosity was increased as a consequence of more severe thermal pasteurizations Taste quality was superior in untreated puree, without significant differences among the thermally treated samples Thermally treated samples maintained their sensory quality during 2 months of storage at 3 °C
	HPP (400–600 MPa, 10–30 °C, 15 min) and thermal (70 °C, 2 min)	Color differences were minor for HPP-treated purees than in thermally treated ones HPP caused ascorbic acid losses of 5–9% No changes in anthocyanins (cyanidin-3-glycoside and pelargonidin-3-glucoside) Total antioxidant activity decreased after HPP
	HPP (400 MPa, ambient temperature, 20 min)	Hexanal content of strawberry puree more than doubled after HPP
	HPP (200, 500, or 800 MPa, ambient temperature, 20 min)	No significant changes in all the aromatic volatile compounds at 200 and 500 MPa Synthesis of new aromatic compounds responsible of the aroma at 800 MPa Slight decrease in the hexanal content at 800 MPa
	HPP (200, 400, 600, or 800 MPa, 18–22 °C, 15 min)	Ester compounds not detected in HPP samples
	HPP (600 MPa, 20 °C, 5 min) and thermal (88 °C, 2 min)	No significant difference between HPP and thermal pasteurization in terms of phenolic compounds after processing

Table 2 (Continued)

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
		Thermal pasteurization resulted in higher retention of phenolic compounds during storage
	HPP (800 MPa, 20 °C, 20 min)	HPP modified the flavor profile of strawberry puree Volatile compounds contributing to fresh strawberry flavor (nerolidol, furaneol, linalool, and some ester compounds) were decreased at 800 MPa HPP did not affect one of the most important flavor compounds in fresh strawberry (2,5-dimethyl-4-hydroxy-2*H*-furan-3-one)
Strawberry jam	HPP (400–600 MPa, room temperature, 10–30 min)	No changes in ascorbic acid content
Blackberry puree	HPP (400–600 MPa, 10–30 °C, 15 min) and thermal (70 °C, 2 min)	Color differences were minor for HPP-treated purees than in thermally treated ones No changes in anthocyanins (cyanidin-3-glycoside and pelargonidin-3-glucoside) Increase in total antioxidant content
Raspberry puree	HPP (200–800 MPa, 18–22 °C, 15 min)	No significant changes in anthocyanins (cyanidin-3-glucoside and cyanidin-3-sophoroside) Highest stability of the anthocyanins at samples treated at 200 and 800 MPa and stored at 4 °C
Raspberry, strawberry, and black currant purees	HPP (600 MPa, 5 min) and thermal (80 °C, 5 min)	Both physical treatments affected the volatiles of the berry fruits Thermal treatment had a more significant effect on volatile compounds than HPP
Persimmon fruit puree	HPP (50–400 MPa, 25 °C, 15 min)	Carotenoid content was increased, mainly due to an increase in β-carotene and β-cryptoxanthin Total antioxidant capacity was increased
Kiwi puree	HPP (400–600 MPa, room temperature, 10–30 min)	No changes in ascorbic acid
	Microwave (200–1000 W, 60–340 s)	Intense treatments (high power, long treatment periods, and a combination of these factors) affect the consistency, viscosity, color, and taste of the product
Mango puree	HPP (100–400 MPa, 20 °C, 15 or 30 min)	Viscosity of mango pulp increased at 100 and 200 MPa Reduction in viscosity was observed at 300 and 400 MPa
Apple puree	HPP (400 or 600 MPa, 20 °C, 5 min) and thermal (75 °C, 10 min)	Ascorbic acid was unaffected by thermal pasteurization and by HPP at 400 MPa Total phenolic content was stable at 400 MPa but was affected by HPP at 600 MPa and by pasteurization treatment HPP raised viscosity HPP caused discoloration during storage
	Microwave (652 W, 35 s)	Total polyphenols were not modified by the microwave treatment and suffered only a small decrease during storage Vitamin C remained unchanged by the treatment, but it was degraded during storage Titratable acidity and viscosity were modified by the treatment, but not during storage

The effects of HPP (600 MPa, 5 min) and heat pasteurization (80 °C, 5 min) on volatile compositions of raspberry, strawberry, and blackcurrant purees were measured by an 'electronic nose.' Both treatments affected the berry volatiles, but heat treatment had a more significant effect on volatile composition than HPP. Therefore, HPP treatment can pasteurize berry products while retaining their volatile profiles.

Juices

Most studies concerning the pasteurization of fruit/vegetable products were performed in juices. Thermal pasteurization is traditionally used for juice preservation, but it can cause detrimental effects on important attributes. The most frequent effects reported in literature were losses of bioactive compounds (vitamins, carotenoids, and phenolic compounds) and diminished sensory acceptance, due to changes in flavor profile (production of off-flavors and degradation of flavor compounds) and in color (nonenzymatic browning and loss of pigments). As other food products, the application of nonconventional pasteurization methods could bring significant improvement to processed fruit/vegetable juices (Table 3).

Several pasteurization methods were tested on apple juice. Ultrafiltration allowed the removal of microorganisms without compromising the physicochemical and sensorial properties, and thus, it can be suitable for apple juice processing. HPP in the range of 200–600 MPa showed no significant changes in total antioxidant activity, but a treatment of 400 MPa caused an increase in phenolic acids, particularly hydroxycinnamic

Table 3 Nutritional and sensory effects caused by different pasteurization treatments in fruit and vegetable juices

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
Apple juice	Ultrafiltration (2.5 nm pore size)	Juice retained total soluble solid, pH, and acid contents
	Ultrafiltration (4 nm pore size)	Color and flavor remained stable
	HPP (600 MPa, 60 °C, 30 min)	No changes in total antioxidant activity immediately after HPP and during storage
	HPP (200–600 MPa, 15–65 °C, 0.5–10.5 min)	Phenolic acids increased significantly after 10 min at 400 MPa
	PEF (50 kV cm^{-1})	No significant sensorial differences between PEF-treated and untreated juices
	PEF (35 kV cm^{-1}) and thermal (90 °C for 30 s)	PEF retained better most of the compounds responsible for the color and flavor of apple juice
		Phenolic compounds were better retained by PEF than by thermal treatment
	UV (30 W, 30 min) and thermal (72 or 94 °C, 26 s)	UV-treated samples had a 29% reduction in total phenolic content, which was much lower than that of thermal processing (48%)
		Juice color was less affected by UV than by thermal treatment
	UV (400 W, 120 min)	No variations were observed in pH, soluble solid content, formol index, total phenolics, and sugars after UV treatment
		Color of the juice from King David apples was unchanged by UV
		UV-treated juice from Golden, Starking, and Fuji apples showed changes in color
		The content of ascorbic acid in juices from Golden, Starking, and Fuji apples slightly changed, but juice from King David apples showed significant losses
Pineapple juice	HPP (600 MPa, 40–75 °C, 40 min)	At constant pressure, increasing temperature enhanced ascorbic acid degradation, for example, loss of 20–25% at 40 °C, 45–50% at 60 °C, and 60–70% at 75 °C
Blueberry juice	HPP (200–600 MPa, 20–42 °C, 5–15 min)	No significant changes in pH, °Brix, and color of HPP-treated juice
		Ascorbic acid losses lower than 8%
		Increase in total phenolic content after treatments at 200 and 400 MPa
		Total antioxidant activity decreased after treatments of 400 and 600 MPa
Watermelon juice	HPP (300–900 MPa, 60 °C, 5–60 min), UV (2421–9685 J l^{-1}, 3–12 min), and thermal (60 °C, 5–60 min)	HPP showed the lowest changes in color, viscosity, browning degree, and lycopene content of watermelon juice compared with the other treatments
Cantaloupe juice	Gamma irradiation (1–5 kGy)	Slight off-odor after treatment at 1 kGy
		Strong off-odor at 2 kGy and above
Pomegranate juice	HPP (400–600 MPa, 25–50 °C, 5–10 min)	Anthocyanin concentration was affected by pressure and temperature level
	PEF (35 or 38 kV cm^{-1}) and thermal (88 °C for 30 s)	No significant differences in pH, °Brix, anthocyanin content, and total phenolic content between the PEF-processed and nonprocessed juices
		PEF processing had significantly less impact in color than thermal processing
		PEF-treated juice had the same consumer satisfaction scores as the nonprocessed juice, being significantly better than the thermally processed samples
	Gamma irradiation (0–10 kGy)	At higher doses of 3.5–10 kGy, a decrease in the total content of anthocyanins was observed
Strawberry juice	PEF (35 kV cm^{-1}) and thermal (90 °C, 30 or 60)	Increase in ellagic acid content in PEF-treated juice during storage
		Less phenolic degradation by PEF (49%) than by thermal pasteurization (55%) after storage
Guava juice	HPP (600 MPa, 25 °C, 15 min) and thermal (95 °C, 5 min)	HHP maintained the original flavor distribution of the juice after treatment
		Heat processing caused a decrease in most of the flavor components
		HPP-treated samples showed differences in the concentrations of methanol, ethanol, ester, and aldehyde compounds during storage, probably due to residual enzyme activity
Mango juice	HPP (400 MPa, room temperature, 10–40 min) and thermal (80 °C, 30 s)	HPP-treated and nontreated samples had similar color values, which were brighter than heated samples
		Vitamin C loss of heated samples (29.1%) was greater than that of HPP samples (2.1–8.3%)
		Small increases in viscosity were observed in HPP samples

Table 3 (Continued)

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
		Both the HPP and the heated sample had less α-terpinolene and more myristic acid than the nontreated samples
Grape juice	Microfiltration (0.2 μm pore size)	Color and flavor were slightly changed
	HPP (300–900 MPa, 20 °C, 2–10 min)	No significant changes in browning index after HPP
	HPP (400–500 MPa, 2–40 °C, 10 min)	The sensory characteristics of HPP-treated juice remained more stable than untreated juice during storage
	HPP (300–900 MPa, 20 °C, 2–10 min)	HPP at 600 and 900 MPa slowed degradation of nonflavonoid phenolics during storage
Citrus juice	Ultrafiltration (1 nm pore size)	Aroma, color, and natural antioxidant content were preserved
	PEF (28 kV cm^{-1})	pH, °Brix, electric conductivity, viscosity, nonenzymic browning index, and HMF[a] practically did not change, compared with nontreated juices
		Color and volatile flavor remained unchanged for all samples
		No significant change in organic acid content
Mandarin juice	HPP (400 MPa, ambient temperature, 10 min)	HPP had little effect on the volatile flavor components
		Typical off-flavor of heat-treated mandarin juice was not detected in HPP-treated samples
Orange juice	HPP (600 MPa, 20 °C, 1 min) and thermal (85 °C, 25 s)	pH, °Brix, alcohol insoluble acids, ascorbic acid, and β-carotene concentrations were not affected after treatment and subsequent storage
		Color and viscosity were stable after treatment and storage
		The degree of clarification and browning index increased over time
	HPP (500–800 MPa, room temperature, 5 min)	HPP caused no significant differences in antioxidant capacity and ascorbic acid, carotene, and sugar contents
		Odor and flavor of HPP-treated orange juice were occasionally altered, depending on treatment and cultivar
	HPP (500–900 MPa, 60 °C, 10 min)	Turbidity was maintained after HPP for longer times
	HPP (600 MPa, 5 °C, 1 min)	No changes in sensory properties
	HPP (500 MPa, 35 °C, 5 min) and thermal (80 °C, 30 s)	Ascorbic acid degradation rates were lower for HPP juice, compared with thermal-pasteurized juice
		Color was not substantially affected by type of processing
		HPP juice had higher viscosity, and its sensory characteristics were judged superior
	HPP (600 MPa, 40 °C, 4 min) and thermal (80 °C, 60 s)	HPP juices had better retention of ascorbic acid and total antioxidant activity, compared with thermally treated samples
		HPP caused less intense flavor changes than thermal treatment
		Higher viscosity values were determined for HPP juice
	HPP (100–400 MPa, 30–60 °C, 1–5 min)	No changes in ascorbic acid content after HPP, but significant losses were observed during storage
		Total carotenoids increased after HPP and decreased during storage
		Increase in hesperetin concentration after HPP
		Increase in flavanones during storage
	HPP (400 MPa, 40 °C, 1 min)	Naringenin and hesperetin increased 20 and 40%, respectively
		No changes observed in total antioxidant capacity
		Flavanone concentration increased after HPP and decreased during storage
	HPP (400–600 MPa, 20 °C, 15 min)	Ascorbic acid losses lower than 6% after HPP
		First-order degradation kinetics for ascorbic acid and anthocyanin (cyanidin-3-glucoside) during storage
		The cyanidin-3-glucoside concentration was higher in HPP-treated than in untreated juice
	HPP (500–800 MPa, 25–50 °C, 1 min)	No significant changes in ascorbic acid after HPP
		Losses lower than 20% were observed during storage
		Color values remained stable during storage
	HPP (100–800 MPa, 30–100 °C, 0–90 min)	Pressure induced thermal degradation of folic acid
		Total antioxidant capacity decreased as a function of treatment time
	HPP (50–350 MPa, 30–60 °C, 2.5–15 min)	Increase in 20–43% in total carotenoids at 350 MPa
		HPP juice had better preservation in total carotenoids during storage than untreated samples
		Total antioxidant capacity decreased during storage

(*Continued*)

Table 3 (Continued)

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
		Vitamin A content of orange juice was increased by 38.74% after HPP treatment (400 MPa, 40 °C, 1 min)
	HPP (200–500 MPa, 30 °C, 1 min)	Vitamins B_1, B_2, B_3, and B_6 are stable during HPP
	HPP (600 MPa, 25 °C, 5 min)	Excess of ascorbic acid strongly protected folates against pressure and heat
	UV (298.9 mJ cm^{-2})	UV-treated juice retained folic acid and vitamin E
		Destruction of riboflavin and β-carotene (≈50%) was observed
	UV (0–100 mJ cm^{-2})	Color and pH were not significantly impacted by UV treatment
		Degradation of ascorbic acid was 17% under UV exposure of 100 mJ cm^{-2}
	PEF (40 kV cm^{-1}) and thermal (90 °C, 90 s)	PEF-processed juice retained more ascorbic acid, flavor, and color than thermally processed samples
	PEF (30 kV cm^{-1}) and thermal (90 °C for 3 min)	Some important volatile compounds suffered losses for the PEF treatments but always in less proportion than the heat-pasteurized juice
		PEF-treated juice retained a higher content of ascorbic acid than heat-pasteurized juice during storage
	PEF (30 kV cm^{-1}) and thermal (90 °C, 20 s)	Color variations during storage were greater in heat-pasteurized than in PEF-treated orange juice
		PEF-treated juice had less nonenzymatic browning than the thermal-pasteurized juice
		No significant variations in the HMF[a] content of the juices pasteurized or treated by PEF with respect to the untreated orange juice
	Gamma irradiation (0.89, 2.24, 4.23, and 8.71 gGy)	Irradiation reduced the concentration of acyclic monoterpenes during storage, following a linear decrease pattern with irradiation dose
	Gamma irradiation (0.5, 1.0, 2.0, and 3.0 kGy)	The production of several sulfur compounds was induced by irradiation
		Volatile sulfur compounds were involved in the development of irradiation-induced off-odor
	Ultrasonication (20 kHz, 2–10 min)	Ultrasonication had a minimal effect on color
		The treatment slightly decreased ascorbic acid content
Orange–lemon–carrot juice	HPP (500–800 MPa, room temperature, 5 min)	No significant differences in antioxidant capacity and ascorbic acid, sugar, and carotene contents
		A significantly more intense carrot aroma was reported after 800 MPa
Carrot juice	Ultrafiltration (1 nm pore size)	Aroma, color, and natural antioxidant content were preserved
	HPP (100–800 MPa, 30–100 °C, 0–90 min)	Folate is unstable at pressures higher than 500 MPa
	HPP (600 MPa, 75 °C, 40 min)	Small losses of carotenes after HPP
Carrot juice and kale juice	Gamma irradiation (1, 2, 3, and 5 kGy)	No differences in amino acid composition
		Decrease in ascorbic acid immediately after the treatments
		After 3 days of storage, ascorbic acid content was higher in irradiated samples than in nontreated ones
		Similar sensory scores between irradiated and nontreated samples
Carrot juice and tomato juice	HPP (250 MPa, 35 °C, 15 min) and thermal (80 °C, 1 min or 60 °C, 5–15 min)	HPP did not cause a significant change in ascorbic acid content
		Heat treatments resulted in a significant loss in antioxidant activity compared with HPP-treated and untreated samples
		Color changes were minor for HPP juices, but it was more intense for heat-pasteurized samples as a result of insufficient antioxidant activity
Tomato juice	HPP (500–900 MPa, room temperature, 3–9 min)	Total pectin content and viscosity were dependent on the pressure applied but independent of treatment time
		Generation of a strong rancid taste, possibly due to the production of *n*-hexanal
	HPP (100–500 MPa, 4–50 °C) and thermal (60 °C, 2 min)	Thermal pasteurization caused significant changes in color, viscosity, and antioxidant capacity, compared with untreated juice
		HPP treatments at and below 200 MPa maintained the color, total carotenoids, lycopene content, and antioxidant capacity
		HPP at 500 MPa improved the quality attributes
Vegetable beverage	HPP (100–400 MPa, 20–42 °C, 2–9 min) and thermal (90–98 °C, 15 or 21 s)	HPP retained significantly more ascorbic acid than thermal treatment
		No significant changes in total phenolics between HHP and thermally processed samples
		Less color changes in HPP-treated samples
		Total antioxidant activity decreased with increasing pressure

[a]HMF – hydroxymethylfurfural.

and procyanidin acids. Pasteurization with PEF showed several advantages relative to thermal treatment: better retention of phenolic compounds and most of the compounds responsible for color and flavor. Moreover, no significant sensory differences were detected between PEF-treated and nontreated juices. In what concerns to UV, two different treatments were applied to apple juice: (1) a less intense treatment (30 W, 30 min), which affected the total phenolic content and color, but in a much lower extension than thermally treated samples, and (2) a more intense treatment (400 W, 120 min), which caused no variation in total phenolic content, pH, soluble solid content, formol index, and sugars. The effect of the latter treatment on juice color was highly dependent on apple variety, as observed in Table 3.

Most studies on juice pasteurization were performed using orange juice, one of the most appreciated and consumed worldwide. HPP is extensively reported for orange juice preservation, showing minimal impact on the physicochemical, nutritional, and sensory properties, compared with thermal pasteurization or even with other nonthermal technologies. Despite no changes are usually observed in the sensory properties (color, turbidity, viscosity, odor, and flavor) of HPP juice, viscosity, odor, and flavor may be occasionally altered by HPP. In terms of nutritional properties, alcoholic insoluble acids, β-carotene, and sugar content were unaffected by HPP. On the other hand, carotenoid and flavanone contents were both increased after HPP treatment and decreased during storage. In particular, naringenin and hesperetin (two important flavanones in orange juice) significantly increased after HPP. Vitamin content, which is highly sensible to high temperatures, was unaffected by HPP. For example, vitamins B_1, B_2, B_3, and B_6 were stable after HPP treatments of 200–500 MPa. The effect of HPP on vitamin C (ascorbic acid) was not always in accordance: in most cases, no significant changes were observed, but some studies reported ascorbic acid degradation during HPP treatment and subsequent storage. For instance, after a treatment of 400–600 MPa, ascorbic acid followed a first-order degradation kinetics during storage. Even so, the loss of ascorbic acid was lower in HPP samples compared with thermally treated ones. In what concerns to vitamin A, its content was found to increase under HPP, showing an increment of 38.74% at 400 MPa. A particularly interesting case is the low stability of folic acid (vitamin B_9), since pressure induced its thermal degradation. However, an excess of ascorbic acid in orange juice protected folic acid from degradation. The impact of HPP was also evaluated in terms of other parameters of orange juice, such as degree of clarification, browning index, and antioxidant activity. The first two were found to increase after HPP treatment, while the latter remained stable or it was only slightly affected.

Several studies reported the use of PEF in orange juice, usually leading to good retention of important quality parameters. Comparing with thermally processed samples, PEF-processed juice was able to retain more ascorbic acid, flavor, and color after the treatment and also during storage. PEF pasteurization showed no impact on the juice flavor compounds, decanal and octanal, contrary to the observed for heat treatment. Other compounds, such as ethyl butyrate, suffered losses with PEF treatments but to a lower extent than with thermal pasteurization. Nonenzymatic browning was also lower in PEF-treated juice, compared with thermal-pasteurized ones. However, the hydroxymethylfurfural content (a typical product of non-enzymatic browning) showed no significant variations in thermally treated or PEF-treated juices with respect to untreated ones.

UV light can adversely affect food constituents and sensorial attributes. Few information is available on UV technology for orange juice pasteurization: (1) UV treatment of 73.8 mJ cm^{-2} may extend the shelf life, without significantly affecting the color and pH; (2) UV treatment of 298.9 mJ cm^{-2} was able to preserve folic acid and vitamin E contents while destroying riboflavin and β-carotene; and (3) UV treatment of 100 mJ cm^{-2} caused loss of ascorbic acid (17%), similarly to thermal treatment.

Irradiation of orange juice may induce undesirable chemical changes, leading to negative flavor changes, usually related to the production of volatile sulfur compounds. For instance, irradiation of 0.5–3.0 kGy induced an increase in methyl sulfide, methanethiol, dimethyl disulfide, and dimethyl trisulfide. On the other hand, carbon disulfide was reduced by irradiation, while hydrogen sulfide was not consistently affected. Different levels of irradiation (0.89, 2.24, 4.23, and 8.71 gGy) were shown to reduce acyclic monoterpene concentrations, following a linear decrease pattern with an irradiation dose and correlated with an increase in thiobarbituric acid-reactive substrates. It was suggested that many of the undesirable effects of irradiation could be reduced by using low temperatures, the addition of antioxidants, and a combination with other techniques.

The influence of sonication was already evaluated on the quality attributes of orange juice. Ultrasonic treatments of 20 kHz for 2, 4, 6, 8, and 10 min affected the color minimally and decreased the ascorbic acid content slightly. Therefore, ultrasonication may be used to extend the shelf life of orange juice, but more studies are needed to fully understand its effects on juice quality.

There are several other studies in literature analyzing the impact of pasteurization on different fruit/vegetable juices, being the results often similar to the ones previously analyzed. More detailed information about these studies is shown in Table 3.

Overall, nonconventional methods seem to be more suitable for the pasteurization of fruit and vegetable products relative to thermal processing. But it should be taken into account that different pasteurization treatments have different effects on each product, and therefore, the assessment of the most suitable approach should be performed on a case-by-case basis.

Dairy Products

Milk is remarkably rich in many essential food constituents, such as amino acids, lipids, sugar, vitamins, and minerals, and thus, it is particularly extend to microbial growth and consequent spoilage. Therefore, milk processing is essential to ensure safety and extend shelf life, being thermal treatment the most common pasteurization technique used. The main effects of processing techniques on nutritional and sensorial qualities of milk and dairy products are presented in Table 4.

Until now, bovine milk is the most studied milk type and, because of that, different pasteurization methods were tested, including HPP, PEF, UV, MW, and conventional thermal

Table 4 Effects of pasteurization techniques on the nutritional and sensory properties of milk and other dairy foods

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
Bovine milk	Thermal (73 °C, 30 s)	Denaturation of whey proteins Degradation of labile vitamins Changes in flavor (due to formation of sulfhydryl groups)
	Thermal (85 °C, 15 min)	Denaturation of whey proteins Alterations of casein micelle size Changes in milk viscosity
	Thermal (65 °C, 10 s or 72 °C, 15 s)	No significant differences in sensorial properties
	HPP (200 MPa, −4 °C, 10–30 min)	Decrease in vitamins C, B_2, and B_3 with increase in treatment time Increase in short-chain FFA[a] content with increase in treatment time No changes in viscosity
	HPP (100–600 MPa, 20–60 °C, 30 min)	Micelle size reduced Phosphate concentration increased Denaturation of whey proteins Gel formation rate and gel strength increased Organoleptic quality maintained or improved
	HPP (100–800 MPa, 20 °C, 0–60 min)	No changes in fat globule size Protein content and % total protein decreased Viscosity increased with pressure and treatment time increase
	HPP (400 MPa, 25 °C, 30 min)	No significant losses of vitamins B_1 and B_6
	HPP (100–400 MPa, 25–60 °C, 10–60 min)	No changes in lactose Increase in phosphate concentration Denaturation of whey proteins
	HPP (482, 586, or 620 MPa, 25 or 60 °C, 1–5 min) and thermal (25, 60, or 80 °C, 1–5 min)	Significant differences on volatile profile: ○ Thermal processing promotes the formation of methanethiol, hydrogen sulfide, methyl ketones, and aldehydes ○ HPP promotes the formation of hydrogen sulfide and aldehydes
	PEF (40 kV cm^{-1})	No detrimental degradation of taste and flavor
	PEF (34.7 kV cm^{-1})	No significant changes in color, viscosity, and density
	PEF (15–35 kV cm^{-1})	No significant changes in vitamins A, B_1, B_2, and E
	PEF (35.5 kV cm^{-1}) and thermal (75 °C, 15 s)	Similar denaturation of whey proteins in both treatments FFA[a] not affected by the treatments
	PEF (35 or 38 kV cm^{-1}) and thermal (63 °C, 30 min; 72 °C, 15 s or 97 °C, 10 min)	Casein micelle and fat globule sizes unaffected Rheological and coagulation properties change less in PEF treatment
	PEF (18.3–27.1 kV cm^{-1}) and thermal (63 °C, 30 min or 75 °C, 15 s)	Vitamins B_1, B_2, D, and E remain constant Vitamin C content decrease (in a greater extent on thermal treatment)
	HPP (400 or 500 MPa, 20 °C, 0–20 min), PEF (36 or 42 kV cm^{-1}) and thermal (63 °C, 30 min or 97 °C, 7 min)	Alterations in fat globule size and distribution: ○ Similar effects of PEF and thermal treatment ○ Fat globule flocculation with HPP
	UV (10.75 mJ cm^{-2}) and thermal (73.5 °C, 15 s)	UV decreased cholesterol content Vitamins B_2 and B_{12} reduction with thermal treatment, but no reduction with UV No significant changes in protein content Differences in sensorial properties, namely, increase in 'tallowy' flavor
	MW (80 or 92 °C, 15 s)	Similar volatile content between MW-treated milk and raw milk No adverse effects on milk flavor, taste, and odor
Ewe milk	HPP (100–500 MPa, 4–50 °C, 10 min)	Alterations in fat globule size and distribution FFA[a] content reduced Lightness (L*) decreased; (−a*) and (+b*) values increased
Goat milk	UV (15.8 mJ cm^{-2})	FFA[a] concentration increased, not enough to detect off-odors Significant differences in sensorial properties Production of volatile compounds (pentanal, hexanal, and heptanal) increased
Cheese made from bovine milk-treated	Thermal (63 °C, 30 min)	Effects on cheese: ○ Changes in texture (less firmer) for thermal milk cheese (due to higher moisture)

Table 4 (Continued)

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
		○ More intense flavor for raw milk cheese (than thermal milk cheese)
	HPP (345 or 586 MPa, 1–15 min) and thermal (72 °C, 15 s)	Effects on cheese: ○ Defects on HPP milk cheese texture (due to excess moisture) ○ No significant differences in flavor scores
	PEF (35 kV cm^{-1})	Effects on cheese: ○ Hardness and springiness increased in PEF milk cheese ○ No significant changes in cheese adhesiveness and cohesiveness ○ Significant differences in cheese flavor (compared with raw milk cheese)
	PEF (20 or 30 kV cm^{-1}) and thermal (63 °C, 30 min)	Effects on cheese: ○ PEF milk cheese has an intermediate concentration of free amino acids (between raw milk and thermal milk cheeses) ○ PEF milk cheese has a curd firmness value between raw milk and thermal milk cheeses
Cheese made from ewe milk-treated	Thermal (75 °C, 15 s)	Effects on cheese: ○ Lower FFA[a] content in thermal milk cheese ○ Volatile compounds and moisture similar in thermal milk cheese and raw milk cheese ○ Higher values of fracturability, elasticity, and hardness for thermal milk cheese ○ Flavor quality and intensity affected negatively by thermal treatment
Cheese made from goat milk-treated	HPP (500 MPa, 20 °C, 15 min) and thermal (72 °C, 15 s)	Effects on milk: ○ No changes in fat globule size Effects on cheese: ○ Free fatty acid content increased on raw and HPP milk cheeses ○ No significant differences in all sensorial analyses
Yogurt made from bovine milk-treated	HPP (100–600 MPa, 1 h)	Effects on yogurt: ○ Texture (rigidity and resistance to breaking) and syneresis resistance were improved

[a]FFA – free fatty acid.

treatment. Thermal processing influenced important nutritional properties, such as whey proteins and vitamin content, since these compounds are highly thermolabile. Additionally, the thermal treatment of milk caused changes on sensorial attributes (namely, viscosity and flavor), with the changes being more intense at more severe processing conditions. Due to the negative impact of thermal processing on milk quality, several nonthermal techniques have emerged.

Nonthermal techniques also influenced the nutritional properties of bovine milk: (1) denaturation of whey proteins with HPP and PEF processing caused a decrease in micelle size and release of the phosphate ion from the micelle matrix, increasing the free phosphate concentration; (2) FFA (free fatty acid) content increased with HPP and not with PEF processing, possibly as a result of lipolysis; (3) vitamins C, B_2, and B_3 content decreased, and vitamins B_1 and B_6 were not influenced with HPP; (4) vitamin C content decreased, and vitamins A, B_1, B_2, D, and E were not affected by PEF processing. On the other hand, UV processing caused no changes in the vitamin and protein contents of bovine milk. Regarding the sensorial properties of processed bovine milk, HPP, PEF, and MW maintained or even improved its organoleptic and rheological properties (compared with raw milk), while differences were detected with UV processing.

For other milk types, little information is available, being only HPP and UV applied on ewe and goat milk, respectively. In ewe milk, the FFA content decreased after HHP, while in goat milk, the FFA increased after UV processing. The sensorial properties of both milk types differed from raw milk after processing. Therefore, the milk type also influenced the effects of pasteurization on the nutritional and sensorial properties of food products.

The pasteurization of milk may affect not only its own nutritional and sensorial properties but also the characteristics of dairy products made from treated milk. The studies evaluating the effect of milk pasteurization for the production of dairy products (such as cheese and yogurt) are detailed in Table 4.

Egg Products

Egg products are an important source of amino acids, vitamins, and minerals. However, they are responsible for a large number of food illnesses, with *Salmonella* being responsible in most cases. In order to guarantee egg safety, conventional thermal

Table 5 Effects of pasteurization techniques on the nutritional and sensory properties of egg products

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
Liquid whole egg	Thermal (56.6–61.1 °C, 3.5 min)	Increase in viscosity Decrease in protein content
	HPP (100–400 MPa, 5 or 25 °C, 10 min)	No protein coagulation at 100–150 MPa Protein coagulation at pressures higher than 250 MPa
	HPP (100–400 MPa, 1–120 min, 20 °C) and thermal (60 °C, 2.5 or 3.5 min)	HPP affects physical properties Viscosity increases with increasing pressure and treatment time: ○ Noticeable increase at 350–400 MPa with formation of macroscopic coagulates ○ Viscosity increase with HPP is lower than with thermal treatment
	PEF (37 kV cm^{-1}) and thermal (66 °C, 4.5 min)	Protein content and conformation less affected with PEF treatment Decrease in foaming capacity in both treatments Increase in viscosity with thermal treatment Color was not affected by PEF but turns opaque with thermal appearance
	UV (4.2 J cm^{-2})	No significant changes in rheological properties No protein denaturation and aggregation
Shell eggs	Thermal (56 °C, 32 min)	Detrimental effects on quality Changes in protein structure and composition Increase in albumen opacity
Whole egg	HPP (50–600 MPa, 30 min, 25 °C)	Effect on egg yolk: ○ Gel formation ○ Retention of color ○ Increase in softness and adhesiveness Effect on egg white: ○ Irreversible denaturation of proteins Effect on whole egg: ○ Appearance of cooked eggs ○ No presence of cooked flavor ○ No formation of unusual amino acids ○ No destruction of vitamins
Egg white	HPP (100–700 MPa, 10–60 °C, 20 min)	Changes more pronounced at 400–700 MPa: ○ Increase in turbidity ○ Decrease in protein content
Egg yolk	HPP (100–500 MPa, 25 °C, 10 min)	Increase in viscosity Decrease in protein content

treatment may be used, but the range of temperatures applied is limited by the precipitation of soluble proteins (at 57 °C). In order to overcome this limitation, alternative nonthermal technologies are being explored for egg product pasteurization, and their effects on nutritional and sensorial properties are represented in Table 5.

The thermal pasteurization of liquid whole egg and shell egg caused a decrease in protein content, due to denaturation, aggregation, and coagulation, causing deterioration of functional properties, changes in color (which becomes more opaque), and increased viscosity. In fact, viscosity is normally used as an indicator of pasteurization impact: an increment of 20% resulted in undesirable quality changes.

HPP and PEF applied on liquid whole eggs also affected the protein content and physical properties. However, these changes were observed to a lesser extent than thermal treatment. Nevertheless, the most suitable treatment for liquid whole eggs seems to be UV, since the viscosity and protein content were not significantly affected.

HPP was also applied to whole egg pasteurization (without shell), resulting in protein denaturation and consequent viscosity increase in both egg white and yolk. While the yolk color was retained after HPP, the egg white had an increased turbidity. As a result, the HPP whole egg had the appearance of cooked eggs, indicating the possibility of cold cooking with pressure. But, when compared with heat-treated (boiled) eggs, this cooking process is advantageous, since there were no development of sulfur flavor, no formation of unusual amino acids, and no destruction of vitamins. Therefore, HPP can be applied to whole eggs, allowing the retention of the nutritional properties of raw eggs but with the appearance of cooked eggs.

Meat and Seafood Products

Meat and seafood products have a rich nutrient matrix, being a suitable environment for the proliferation of microorganisms. In consequence, adequate pasteurization methods are needed

to extend the shelf life without compromising quality. The specific purpose of these techniques is the elimination or reduction of *Listeria monocytogenes* on meat products and *Clostridium botulinum* and *Vibrio vulnificus* on seafood products.

As most food products, meat and seafood are usually processed by thermal treatment, but heat causes the degradation of vitamins, coagulation and denaturation of muscular proteins, and changes in textural properties. The color of meat depends on the myoglobin form present in the muscle: reduced or deoxymyoglobin (purple-red), oxymyoglobin (red), and metmyoglobin (brown). Thermal processing caused color changes since it favored the metmyoglobin formation due to myoglobin oxidation. For example, in case of meat, there were changes from red to brown/gray and from red to red/pink in noncured and cured products, respectively. In addition, temperatures above 70 °C promoted hydrolysis and oxidation of polyunsaturated fatty acids, increasing free fatty acid contents. Consequently, the formation of different volatile compounds occurred, and thus, there was flavor modification from fresh to cooked and, in some cases, development of rancidity.

Several novel processing technologies have been applied to the preservation of meat and seafood products (Table 6). HPP was widely applied and, in case of meat products, different

Table 6 Effects of pasteurization techniques on nutritional and sensory properties of meat and seafood products

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
Pork and poultry	HPP (0.1–800 MPa, 5–30 min, 10–50 °C)	Lipid oxidation induced by pressure increasing Color changes: ○ Increase in lightness and decrease in redness No significant changes in aroma profile
Beef and poultry	HPP (0.1–800 MPa, 1–20 min, 10–70 °C)	Lipid oxidation induced by pressure increasing Increase in hardness Color changes: ○ Increase in lightness No significant changes in aroma profile
Beef	HPP (100–500 MPa, 5 or 10 min, 25 °C)	Texture changes: ○ Meat tenderization with pressurization
	HPP (520 MPa, 260 s, 10 °C)	Increase in proteolytic activity Texture changes: ○ Meat tenderization ○ Increase in toughness
	HPP (50–600 MPa, 20–300 s, 10–30 °C)	Color changes: ○ Increase in lightness ○ With pressures up to 350 MPa, redness increases, but with pressures up to 600 MPa, it decreases
Minced beef	HPP (100–500 MPa, 2–30 min, 10 °C)	Color changes: ○ Increase in lightness and decrease in redness
Poultry	Gamma irradiation (0–10 kGy)	Significant decrease in thiamine content Color changes: ○ Increase in redness
Beef and pork	Gamma irradiation (0–10.5 kGy)	Increase in lipid oxidation Denaturation of proteins Production of sulfur-containing volatiles, causing off-odors Color changes: ○ Formation of brown, green, and bright-red colors in fresh meat
Dry-cured ham and loins	HPP (200–800 MPa, 3–15 min, 20 °C)	Decrease in lipid oxidation Decrease in proteolytic activity Flavor changes, turning saltier Color changes: ○ Increase in lightness and decrease in redness after pressurization
Dry-cured ham slices	HPP (600 MPa, 6 min, 15 °C)	No significant changes in oxidation stability and protein content Texture changes: ○ Increase in hardness and chewiness Color changes: ○ Increase in lightness Flavor changes: ○ Increase in odor intensity and saltiness

(*Continued*)

Table 6 (Continued)

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
Cooked ham	HPP (100–700 MPa, 10 min, 5–40 °C)	No significant changes in physicochemical composition: ○ Protein profile and content ○ Fatty acid composition and cholesterol content ○ B-group vitamin content ○ Mineral composition, only a decrease in calcium and iron content Increase in syneresis No significant changes in texture, color, and sensory evaluation
Cured ham	RF (60 MHz)	Quality improvement compared with hot water processing Decrease in moisture loss
Ham slices	RF (27.12 MHz)	Decrease in moisture loss No significant change in color parameters No changes in sensorial quality
Dry-cured loins and ham slices	Gamma irradiation (0, 5, or 10 kGy)	Increase in lipid oxidation Changes in color parameters: ○ Samples with a redder appearance
Raw sausages	HPP (100 or 300 MPa, 5 or 20 min, 6–8 °C)	Decrease in protein content Texture changes: ○ Decrease in hardness Color changes: ○ Increase in lightness
Cooked sausages	HPP (500 MPa, 5 or 10 min, 65 °C) and thermal (80–85 °C, 40 min)	Texture changes: ○ Increase in cohesiveness and decrease in firmness on HPP sausages No significant changes in color parameters in both cases HPP sausages with better acceptance in sensory analysis, due to better appearance, taste, and texture
Fermented sausages	HPP (400 MPa, 10 min, 17 °C)	Changes in textural properties: ○ Increase in cohesiveness, chewiness, and springiness
Turkey frankfurters	IR (70, 75, or 80 °C)	No significant changes in color Change of color: ○ Surface turns slightly brownish
Surimi	Thermal (90 °C, 30 or 60 min)	Texture softening – loss of firmness Changes in color, turning yellowish No changes in flavor
Anglerfish	HPP (150–200 MPa, 5 min, 0 °C)	Cooked appearance
Bluefish	HPP (100–300 MPa, 5–30 min, ambient)	Decrease in protease activity Decrease in firmness with pressure increase Changes in color (higher L-values and lower a- and b-values)
Carp	HPP (100–500 MPa, 10 min, 20–22 °C)	Decrease in hardening Visual differences: loss of transparency and higher L-value
Cod	HPP (150–200 MPa, 5 min, 0 °C) HPP (100–800 MPa, 5 or 20 min, 0 °C or ambient)	Cooked appearance Decrease in protease activity with pressure increase Decrease in oxidative stability with pressure increase Denaturation of proteins Increase in hardening with pressure increase Cooked appearance
Hake	HPP (200–400 MPa, 15 min, 7 °C)	Cooked appearance
Mackerel	HPP (150–200 MPa, 5 min 0 °C)	Cooked appearance
Octopus	HPP (200–400 MPa, 15 min, 7 or 40 °C)	Decrease in protein content Increase in shear strength
Oysters	HPP (400–800 MPa, 5 min, 0 °C) HPP (100–800 MPa, 10 min, 7 or 20 °C)	Cooked appearance No significant inhibition of lipases Increase in shear strength More voluminous, shucked Increase in L-value
Prawn	HPP (200 MPa, 10 min, 7 °C)	Increase in shear strength Earlier browning Opaque muscle

Table 6 (Continued)

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
Plaice and pollack	HPP (150–200 MPa, 5 min, 0 °C)	Cooked appearance
Salmon	HPP (150 or 200 MPa, 30 or 60 min, 1–5 °C)	Changes in rancidity Increase in cutting strength Changes of color (increase in L-value and decrease in a-value)
Salmon trout	HPP (150–200 MPa, 5 min, 0 °C)	Cooked appearance
Sardine	HPP (100–400 MPa, 30 min, ambient)	Changes of color (increase in L-value and decrease in b-values and variable b-values)
Sea bass	HPP (100 or 300 MPa, 5 min, 10 °C)	No inhibition of proteases No changes in protein profile
Sea bream	HPP (200–400 MPa, 10 min, 20 °C)	No significant changes in protein Changes in texture; decrease in elasticity
Sheephead	HPP (100–300 MPa, 0–30 min, ambient)	Changes in color (increase in L-value) Cooked appearance
Squid	HPP (150–400 MPa, 15 min, ambient)	Production of biogenic amines delayed Skin reddening and muscle whitening
Tuna	HPP (150–200 MPa, 5 min, 0 °C)	Cooked appearance
Turbot	HPP (100–200 MPa, 15–30 min, 4 °C)	Decrease in protein content No effect on fatty acid content Changes in oxidative stability of lipids Changes in color (increase in L-value) Cooked appearance

effects were observed for different products, such as fresh meat, cured meat, and sausages.

For fresh meat, HPP induced lipid oxidation, with higher effects when higher pressures were applied. Proteolytic activity was also increased, mainly due to the activation of two enzymatic systems: cathepsins and calpains. Since these enzymes are also responsible for meat tenderization, HPP induced changes in texture. HPP also increased hardness and toughness, possibly due to modifications in the structural properties of meat proteins. Regarding sensorial properties, HPP caused no changes in aroma profile, while color was significantly affected. Meat discoloration was a result of myoglobin denaturation and oxidation. Therefore, HPP for fresh meat has disadvantages to final product acceptability, since drastic sensorial modifications were observed relatively to fresh meat.

Different effects were observed after HPP application on cured meat and sausages. For example, the application of HPP on dry-cured ham and loins decreased lipid oxidation and proteolytic activity, changing color, texture, and flavor (turns saltier). On the other hand, both nutritional and sensorial properties of HPP cooked ham were not changed. Therefore, these products were less modified by pressure, which resulted in good sensory acceptance.

Other novel techniques have already been studied for the pasteurization of meat products, such as irradiation, RF heating, and IR. Irradiation had deteriorative effects on meat quality by decreasing the vitamin content, increasing lipid oxidation, denaturing proteins, and changing color and flavor. RF applied to cured meat products reduced the moisture loss and did not change sensorial properties, being therefore advantageous for its pasteurization.

HPP was tested for the preservation of different seafood products, as observed in Table 6. The main effect of HPP was the modification of visual characteristics, due to the development of a cooked appearance. Texture was also affected by pressure: firmness and hardness increased and elasticity decreased, which are usually caused by the denaturation of cytoskeletal proteins. In addition, HPP affected the oxidative stability of lipids, with increasing pressure inducing oxidative reactions, possibly because of the presence of metal ions that promote lipid autoxidation.

Since HPP effects are different according to the food matrix, different seafood products show different pressure sensibilities. For example, the application of HPP to sea bass and sea bream caused no change in protein profile and proteolytic activity. Another example is HPP application to raw oysters, which caused denaturation of the adductor muscle, inducing the shell to open spontaneously. Thus, oysters were shucked with HPP, reducing the need of manual shucking and increasing the amount of meat removed from the shell. In addition, this treatment extended the shelf life of oysters while retaining its sensory characteristics. For all these reasons, the application of HPP to oysters is increasingly utilized.

As a conclusion remark, for fresh meat and seafood, even the novel processing techniques may cause drastic changes in visual properties, which may affect the overall consumer acceptability. However, for cured meat, these novel techniques seem to be a good method for its processing since nutritional and sensorial properties are mostly retained.

Alcoholic Beverages

Thermal pasteurization is usually applied to ensure the microbiological safety of beer. However, such as observed for other food products, thermal treatments cause the development of off-flavors and degradation of important quality parameters. Despite the influence of thermal pasteurization (70 °C,

30 min) on beer volatile profile, differences between pasteurized and nonpasteurized samples were attenuated during storage. Naturally, the negative impact of thermal pasteurization was more pronounced at higher temperatures: beer treated at 60 °C had more intense flavor and higher aldehyde content, compared with higher temperatures (72 and 84 °C). However, beer treated at 60 °C showed lower flavor stability through storage compared with samples treated at higher temperatures, possibly because heat improved the oxidative stability of beer.

Several studies have tested the application of HPP on beer (Table 7), in order to minimize the negative effects of processing on aroma and flavor. HPP effects on beer are highly dependent on several factors (such as beer type, pressure level applied, and holding time), but the main attributes of beer (ethanol content, density, extract, fermentation degree, and pH) were not affected by pressure. In most cases, color, foam durability, bitterness, and flavor were also stable during HPP treatment, but some studies reported changes in these parameters. Comparing HPP with thermal processing, several differences were observed in terms of their impact on beer quality. Heat treatment significantly changed color and permanent haze relative to nontreated and HPP-treated samples.

A study performed with PEF (22 kV cm^{-1}) on beer pasteurization showed a significant increase in the amount of several metallic ions, which caused a significant degradation of flavor and mouthfeel. Therefore, PEF pasteurization, as well as conventional thermal processing, showed a negative impact on the nutritional composition and organoleptic properties of beer. In consequence, HPP seems to be a more suitable technology for beer pasteurization, since it allows the development of a microbiologically stable product with sensorial properties similar to nontreated beer.

The development of spoilage microorganisms in must and wine causes great economic losses in the wine industry. Unlike beer, wine is not treated with heat, due to its temperature sensitivity, except in the case of lower quality wines. Therefore,

Table 7 Nutritional and sensory effects caused by different pasteurization treatments in beer, red wine, and white wine

Food products	*Pasteurization treatments*	*Nutritional and sensory effects*
Beer	Thermal (70 °C for 30 min)	Pasteurization influenced beer volatile profile after processing During storage, the differences between the volatile profiles of pasteurized and nonpasteurized beers disappeared Pasteurization had a positive influence on beer oxidative stability
	Thermal (60, 72, or 84 °C)	Pasteurization at 60 °C led to a more pronounced flavor, higher aldehyde content, and lower flavor stability compared with higher temperatures
	HPP (300, 500, or 700 MPa, 5 min)	No change in color, foam durability, and the spectrum of flavor materials Potential for arising the turbidity decreased at 300 and 500 MPa and increased at 700 MPa Filterability became better (filtration time decreased to 50%)
	HPP (600 MPa, 5 min) and thermal (60 °C, 10 min)	No effect on main chemical constituents of the beer HPP had no significant effect on color contrary to the thermal treatment HPP retained permanent haze throughout storage
	HPP (300, 400, 500, or 600 MPa, 20 min)	Increase in the foaming and haze characteristics of the beer
	HPP (200, 250, 300, or 350 MPa, 20 °C, 3 or 5 min)	Main attributes of the beer were not affected The color, protein sensitivity, and chill haze changed as pressure and time increased No effect on bitterness up to 300 MPa
	HPP (350 MPa, 20 °C, 3 or 5 min) and thermal (60 °C for 15 min)	Similar results in terms of pH and color for HPP and heat during storage HPP-treated samples had lower bitterness and protein sensitivity and higher chill haze than the thermally pasteurized samples
Beer	PEF (22 kV cm^{-1})	Significant increase in the amount of Cr, Zn, Fe, and Mn ions in the beer samples Degradation of flavor and mouthfeel
White wine	HPP (200–400 MPa)	No change in viscosity Optical density of HPP-treated wine was lower than that of nontreated samples Oxidation took place in HPP-treated samples during storage
	HPP (300–600 MPa, 20 °C)	No variation of the color parameters
Red wine	HPP (100–350 MPa, 25 °C, 0–30 min)	No differences in the aroma, taste, mouthfeel, and overall sensory quality between HPP-treated and nontreated samples
	HPP (600 MPa, 70 °C, 10 min or 1 h)	Decrease in the concentration of malvidin *3-O*-glucoside No differences in anthocyanic composition or antioxidant activity
	PEF (5 kV cm^{-1})	Higher color intensity, total polyphenol index, and total anthocyanic composition after treatment No differences in sensory attributes After 12 months of storage, there were no significant differences in the content of monomeric anthocyanins between both wines; however, the content of flavan-3-ols, flavonols, and hydroxycinnamic acids and derivatives was higher in PEF-treated wine
White wine and red wine	HPP (400 or 500 MPa, 4 °C or 20 °C, 5 or 15 min)	No effect on polyphenol oxidase activity, alcohol level, total and volatile acidity, free and total SO_2, protein stability, malic acid, lactic acid, reducing sugars, and pH No organoleptic differences between nontreated and HPP-treated wines

the addition of sulfur dioxide (SO_2) is a usual practice to final product preservation, but it must be moderated because of the negative effects on human health. In order to solve this problem, HPP and PEF were tested on wine pasteurization (**Table 7**).

White wine treated with HPP (200–400 MPa) showed no differences in viscosity compared with nontreated wine, while the optical density decreased with HPP. During storage, HPP-treated samples had higher oxidation levels, which increased the optical density values, but these values were still lower than in nontreated wine. Sensory evaluation showed significant flavor differences between sulfide-added and HPP-treated wines, with the latter being easily recognized by the tasters because of the oxidation. The application of HPP treatments in the range of 300–600 MPa in white wine resulted in no relevant variation of the color parameters compared with nontreated wine. In a study performed in both white wine and red wine, HPP-treated (400 or 500 MPa) wine showed no changes in the physicochemical and sensory parameters.

In terms of nutritional composition, the application of HPP on red wine had no effect on anthocyanic composition and antioxidant activity. However, significant differences were observed in the concentration of malvidin 3-*O*-glucoside. PEF technology (5 kV cm^{-1}) was also tested for red wine pasteurization, causing no differences in sensory attributes. However, color intensity, total polyphenol index, and total anthocyanic composition were increased by this treatment.

In general, HPP and PEF seem to have a great potential interest for wine pasteurization, since they are able to reduce microbial growth while minimizing the impact of processing on wine quality.

See also: Ascorbic Acid: Physiology and Health Effects; Browning: Enzymatic Browning; Browning: Non-enzymatic browning; Carotenoids: Occurrence, Properties and Determination; Carotenoids: Physiology; Colors: Health Effects; Colors: Properties and Determination of Natural Pigments; Enzymes: Analysis and Food Processing; Fatty Acids: Determination and Requirements; Fatty Acids: Fatty Acids; Folic acid and Folates: Physiology and Health Effects; Heat Treatment: Effect on Microbiological Changes and Shelf Life; Heat Treatment: Principles and Techniques; Irradiation of Foods: Processing Technology and Effects on Nutrients: Effect of Ionizing Radiation on Food Components; Maillard Reaction; Minimally Processed Foods; Pasteurization: Principles and Applications; Phenolic Compounds: Bioavailability and Health Effects; Phenolic Compounds: Occurrence, Classes, and Analysis; Preservation of Foods; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Proteins: Chemistry, Characterization, and Quality; Storage Stability: Mechanisms of Degradation; Storage Stability: Shelf Life Testing.

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Pasteurization: Principles and Applications

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Introduction

Milk is the most widely consumed pasteurized food and the process was first introduced commercially during the 1930s. Treatments of the order of 63 °C for 30 min were used. Modern milk pasteurization uses an equivalent process of 72 °C for 15 s (in the United Kingdom) and is usually delivered through a heat exchanger. Pasteurization is nowadays used extensively in the production of many different types of food, which includes fruit products, pickled vegetables, jams, soups, sauces, and ready meals. Food may be pasteurized in a sealed container as with in-pack processing or in a continuous process analogous to an aseptic filling operation.

The aim of a pasteurization process is to reduce the number of pathogenic and spoilage organisms by a specific amount, usually 5 or 6 log reductions. This, together with one or more microorganism growth hurdles, is sufficient to ensure that a satisfactory shelf life is attained.

Pasteurization treatment for foods involves the delivery of a thermal process that is less severe than the full sterilization process of 3 min at 121.1 °C. This is the F_0 3 concept that has its origins in canning. F_0 3 is applied to ambient stable low-acid foods in which mesophilic strains of *Clostridium botulinum* could grow. Examples of pasteurization processes could be 70 °C for 2 min for a short shelf life chilled food or 93.3 °C for 5 min for an ambient food with pH control. Thus, a requirement is that there is at least one additional preservation hurdle present within the food or its environment that prevents microorganism numbers reaching levels at which they pose a threat to health or reduce the food quality below an acceptable level. Pasteurized foods represent one of the largest groups of preserved foods and include foods such as

- fruit juices and smoothies;
- canned fruit products;
- cooked chilled and frozen foods;
- baked products;
- beer and soft drinks;
- ice cream and dairy products;
- pies, pasties, and pastry products;
- cooked meat, fish, and poultry products.

As mentioned previously, the efficacy of heat in destroying organisms is influenced by various factors. It is for this reason that mild heat alone cannot be used to produce safe stable pasteurized foods.

The following statements are usually true:

- Most spore-forming bacteria are inhibited by a combination of pH less than 4.5 and a_w less than 0.90.
- Acid-tolerant spore-forming bacteria will survive and grow at pH > 3.8. This includes many of the butyric anaerobes, a group of bacteria that under anaerobic conditions can ferment sugars to butyric acid, and includes *Bacillus macerans* and *Bacillus polymyxa*.
- Some xerophilic and osmophilic spore-forming yeasts and molds can grow below a_w 0.85.
- Antimycotic agents (preservatives) have been successfully used in foods to prevent the outgrowth of yeasts and molds.
- *C. botulinum* does not usually germinate and grow in foods with a pH of less than 4.5 or a water activity (a_w) of less than 0.94, although some experimental conditions have shown growth and toxin production by *C. botulinum* at pH < 4.5.
- Chilling is only a short-term barrier to microbial growth.

Pasteurization Values

Mathematical analysis of pasteurization is similar to that for sterilization, except for the fact that the target microorganisms are less heat-resistant. Some pasteurization processes are described by pasteurization units (PU), similar to the F_0 value for a low-acid product. The PU concept was originally designed for the pasteurization of beer where yeasts are the target microorganisms. It has since expanded to include many types of food product, and so the term *P*-value is more general and used widely. Calculation of PUs is useful to give the process a reference thermal exposure, but there is no international minimum as there is for low-acid canned foods (i.e., F_0 3).

A target *P*-value for a process to achieve is calculated from microorganism heat resistance data. This is characterized by the *D* and *z* concept, where *D* is the decimal reduction time (min) and *z* is the kinetic factor (°C). *D* is the time it takes to reduce the microorganism population by one log, at a constant temperature (T_{ref}). It is correct to refer to *D* as D_T because the value is at the reference temperature only. *z* is the temperature change required to change the D_T by one log, in effect changing the killing power of the process by a factor of 10.

The first step is to decide on the target microorganism for the pasteurization process, which will be based on the relevant hurdle to growth for that food and its environment. Literature sources are invaluable in making this decision. **Tables 1–3** present a selection of industrially relevant data for pasteurization processes. These are the heat resistance data used to set up the target *P*-value.

Data on microorganism heat resistance are usually taken from work published by researchers, because it is time-consuming and expensive work. It is rare for food companies to commission heat resistance studies unless the food product is unusual or there is a reason to justify lowering the process severity. Using values from the literature, the time required to process a product to achieve a specific reduction in microbial load can be calculated using eqn [1]:

$$P = D_T \cdot n \qquad [1]$$

where *P* is the required pasteurization value (min); D_T is the *D*-value at the reference temperature, T_{ref} (min); and *n* is the number of log reductions required (usually 6).

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Table 1 Heat resistance data for *Bacilli* and *Clostridia*

Organism	*Heating substrate*	*Heating temperature (°C)*	D-*value (min)*	z-*value (°C)*
Bacillus cereus	Buffer (pH 7.0)	100	8.0	10.5
Bacillus coagulans	Red pepper (pH 4.5)	100	5.5	–
Bacillus licheniformis	Buffer (pH 4.0)	100	1.05	10.2
Bacillus polymyxa	Buffer (pH 7.0)	100	~18	–
Bacillus subtilis	Buffer (pH 6.8)	121	0.57	9.8
Clostridium botulinum nonproteolytic type E	Water	80	3.3	9.4
Clostridium butyricum	Buffer (pH 7.0)	85	23	–
Clostridium pasteurianum	Buffer (pH 4.5)	95	3.95	–
Clostridium tyrobutyricum	Buffer (pH 7.0)	90	18	–

Table 2 Heat resistance data for other bacteria

Organism	*Heating substrate*	*Heating temperature (°C)*	D-*value (min)*	z-*value (°C)*
Enterococcus (*Strep*) *faecalis*	Fish	60	15.7	6.7
Escherichia coli	Broth	56	4.5	4.9
Lactobacillus plantarum	Tomato juice	70	11	12.5
Listeria monocytogenes	Carrot	70	0.27	6.7
Pseudomonas fluorescens	Broth	60	3.2	7.5
Salmonella senftenberg	Pea soup	60	10.6	5.7
Staphylococcus aureus	Pea soup	60	10.4	4.6

Table 3 Heat resistance data for yeast and mold

Organism	*Heating substrate*	*Heating temperature (°C)*	D-*value (min)*	z-*value (°C)*
Byssochlamys fulva	Grape drink	93	5.0	7.8
Saccharomyces cerevisiae (ascospores)	Buffer (pH 4.5)	60	22.5	5.5
Zygosaccharomyces bailii (vegetative cells)	Buffer (4.5)	60	0.4	3.9
Zygosaccharomyces bailii (ascospores)	Buffer (4.5)	60	14.2	3.9

Preservation by Pasteurization

Manufacture of a pasteurized food can be broken down into two basic processes:

(1) The food is heated to reduce the numbers to an acceptably small statistical probability of pathogenic and spoilage organisms capable of growth under the intended storage conditions.
(2) The food is sealed within a hermetic package to prevent reinfection. Preservation methods, such as traditional canning, seal the food in its package before the application of heat to the packaged food product, whereas other operations such as aseptic, cook–chill, and cook–freeze heat the food prior to dispensing into its pack.

Retorting and canning are terms that are still widely used in the food industry to describe a range of thermal processes where the food is heated within its package to achieve a commercially sterile packaged food. The heating usually takes place in retorts that are batch or continuous hot water- and/or steam-heated pressure cookers. The principal concept of food retorting is to heat a food in a hermetically sealed container so that it is commercially sterile at ambient temperatures. In other words, so that no microbial growth can occur in the food under normal storage conditions at ambient temperature until the package is opened. Once the package is opened, the effects of retorting are lost, the food will need to be regarded as perishable, and its shelf life will depend on the nature of the food itself.

Hurdles to Microbial Growth

Food preservation is the process of treating food to stop or slow down spoilage (loss of quality or nutritional value) caused or accelerated by microorganisms and/or intrinsic enzymes. A number of hurdles to microbial growth are described, including water activity, pH, storage temperature, and the use of preservatives such as salt and sugar. These can be used alone or in combination.

Heat treatment is an extremely effective means of food preservation, but a full sterilization of the food is not always appropriate, as the nutritional value, texture, and/or color of the food could be unacceptably changed. In these instances, a mild heat treatment, together with at least one other method of food preservation, is used. When a combination of two or more food preservation factors is used, generally, each factor

can be used at a slightly lower level. Using more than one food preservation factor or hurdle is termed 'hurdle technology.' Hurdle technology uses the intelligent combination of different preservation factors or techniques to achieve multitarget, mild, but reliable preservation effects. It is usually more effective than single targeting and allows hurdles of lower intensity, with the benefits of improved product quality.

The number of already applied and potential hurdles for the preservation of foods amounts to over 100. As our understanding of the mode of action of food preservation improves, factors such as microbial metabolic exhaustion, stress reactions, and homeostasis are taken into account when considering food preservation. The traditional methods of heating, chilling, drying, curing, acidification, oxygen removal, fermenting, and adding chemical preservatives are frequently used (see Table 4), but newer methods like pulsed technologies and 'natural' preservatives are also being employed. To use all of these food preservation methods, a good understanding of the biochemistry of the mode of action is required.

The most commonly used hurdles are discussed in the succeeding text (those that have relevance to pasteurization are in bold and are discussed in the following sections):

1. Control of the load of microorganisms
2. Use of restrictive pH
3. Use of anaerobic conditions
4. Use of low temperatures
5. Drying/low water activity
6. Chemical preservation
7. Irradiation

Control of the Microorganism Loading

Shells, skins, membranes, and fat are the natural defenses that prevent microbial contamination of meat, fish, fruit, and vegetables prior to harvesting. Once these barriers are removed, there are a number of methods that can be used to physically remove microorganisms from foods, which include the following:

- Washing of raw foods, which is an important part of most ingredient preparation but may result in increasing the microbial load if the water used for washing is not clean. Washing can also increase the food's moisture content and thus increase the spoilage potential.
- Trimming includes the removal of spoiled portions. It is an important step in ensuring that the load of an ingredient batch is kept as low as possible.
- Filtration is limited to clear liquids but is used successfully with some fruit juices, beer, wine, soft drinks, and water.
- Centrifugation or sedimentation is used in some milk and juice production.

Reducing the initial microbial load is critical because it determines the extent of heat process time and temperature that must be applied to achieve the condition of commercial sterilization. Although it is important to ensure that a food process starts with a low load, it is also important to understand why each step of a process is being done, because this helps to understand the importance of that step. This is where the HACCP plan is used to ensure levels of microbial contamination are low. However, it is important to note that even if microorganisms are removed from food, various preformed enzymes and/or toxins might remain and present problems.

Use of Restrictive pH Levels

Most microorganisms grow best at neutral pH (6.5–7.5) and only a few are able to grow at $pH < 4.0$. Bacteria are more fastidious about their pH requirements than are yeasts and molds. The fact that pH can limit microbial growth is a basic principle of food preservation and has been used for thousands of years. Fermentation and pickling extend the shelf life of food products by lowering the pH. The fact that no known spore-forming pathogenic bacteria can grow at $pH < 4.6$ is the basis for the food sterilization principle for low-acid foods.

Under normal conditions of storage, bacterial spores that survive commercially adequate processing are incapable of development in acid foods, and this fact has led to the adoption of the term 'commercial sterility.' This indicates that the microbiological condition is not necessarily completely sterile, but is a safe, marketable food. Commercially sterile food may be defined as food, which has been so processed, that, under ambient storage conditions, will neither spoil nor endanger the health of the consumer.

Table 4 Selected food preservation techniques and their effect on spoilage microorganisms

Method	*Effect on microbial growth or survival*
Refrigeration	Low temperature to retard growth
Freezing	Low temperature and reduction of water activity to prevent microbial growth, slowing of oxidation reactions
Drying	Reduction in water activity sufficient to delay or prevent microbial growth
Vacuum and oxygen-free modified atmosphere packaging	Low oxygen tension inhibits strict aerobes and delays growth of facultative anaerobes
Modified atmosphere packaging (enriched CO_2)	Specific inhibition of some microorganisms
Addition of weak acids, for example, sodium lactate	Reduction of the intracellular pH of microorganisms
Sugar preservation	Too high osmotic pressure for most microbial survival
Ethanol preservation	Ethanol is toxic to microorganisms and causes inhibition of growth or death
Addition of preservatives (e.g., nitrite or sulfite)	Inhibition of specific groups of microorganisms by interfering with cell membrane functioning
Irradiation	Delivery of ionizing radiation to disrupt cellular RNA
Pulsed electric field processing	Short bursts of electricity for microbial inactivation

Table 5 Suggested *P*-values (T_{ref} 93.3 °C, z 8.9 °C) for fruit and vegetables

Product	*pH*	P-*value (minutes at 93.3 °C)*
Lemon juice	2.5	0.1
Plums	2.8	0.2
Pickled vegetables	3.0	0.5
Greengages	3.2	0.8
Mandarins	3.2–3.4	1.0–2.0
Grapefruit juice	3.2	0.2–0.4
Apricots	3.2–4.0	1.0–8.0
Apples	3.3	0.2–0.6
Orange juice	3.5–3.8	0.2–0.6
Pineapples	3.5	0.6–0.8
Strawberries	2.3–4.0	0.8
Jams	3.5	0.8
Sauerkraut	3.5–3.9	0.5
Pickled gherkins	3.5–3.8	0.5–1.0
Sweet cherries	3.8	0.6–2.5
Nectarines, peaches	4.0	1.5–8.0
Pears	4.0	1.3–10.0
Tomatoes	4.2–4.5	2.0–10.0
Tomato paste	4.2–4.5	1.0–5.0

Products of different pH can support the growth of different spoilage organisms, and so different *P*-values are often used. Table 5 provides processes for products over a wide range of pH ($2.5 < pH < 4.5$).

Another source of information was the National Food Processors Association (the United States) that gave the following recommendations for processing acid products that may contain butyric anaerobes (e.g., tomatoes):

For products between pH 4.0 and 4.3, the process should be equivalent to 5 min at 200 °F (93.3 °C):
P-value > 5 min (T_{ref} 200 °F, z 15 °F; T_{ref} 93.3 °C, z 8.3 °C).
For products between pH 4.3 and 4.6, the process should be equivalent to 10 min at 200 °F (93.3 °C):
P-value > 5 min (T_{ref} 200 °F, z 15 °F; T_{ref} 93.3 °C, z 8.3 °C).

Low Temperatures

There are categories of temperature sensitivity of microorganisms that help to define and position the thermal processes that are applied to foods:

- Psychrotrophic (cold-tolerant), which can reproduce in chilled storage conditions, sometimes as low as 4 °C. Having evolved to survive in extremes of cold, these are the easiest to destroy by heat.
- Psychrophilic (cold-'loving'), which have an optimum growth temperature of 20 °C.
- Mesophilic (medium range), which have an optimum growth temperature between 20 and 44 °C. These are of greatest concern with ready meals.
- Thermophilic (heat-'loving'), which have an optimum growth temperature between 45 and 60 °C. In general, these organisms are only of concern with ready meals produced or stored in temperate climates.
- Thermoduric (heat-enduring), which can survive above 70 °C, but cannot reproduce at these temperatures.

Table 6 Minimum growth temperatures for some pathogenic bacteria

Organism	*Minimum growth temperature (°C)*
Bacillus cereus	4.0
Clostridium botulinum (psychotropic)	3.3
Escherichia coli 0157	7.0
Listeria monocytogenes	−0.4
Salmonella species	4.0
Staphylococcus aureus	6.7
Vibrio parahaemolyticus	5.0
Yersinia enterocolitica	−1.0

Most of the pathogenic microorganisms fall within the mesophilic category, such as *Salmonella*, *Listeria*, and *Escherichia coli O157*, and so food production conditions will be designed to minimize their growth and survival during manufacture. The scheduled thermal processing conditions of low temperature and time are designed specifically with the intended storage conditions that dictate microorganism growth.

Low temperatures are used to retard chemical reactions such as the actions of food enzymes and to slow down or stop the growth and activity of microorganisms in food. The lower the temperature, the slower will be the chemical reaction, the enzyme actions, and the microbial growth. A temperature below 0 °C will prevent the growth of any microorganisms although in practice 3 °C is often considered to be a temperature sufficiently low to control (stop) microorganism growth. Low temperatures are inhibitory to growth, rather than lethal to the microorganisms. Table 6 presents minimum growth temperature for selected microorganisms.

Two of the main categories of heat-preserved chilled foods are those with shelf life less than 10 days and those in which the shelf life can go beyond 10 days, sometimes up to 30 days. Foods given these processes are varied and include ready meals, fruit products, ready-to-eat cooked meat, fish, and poultry products. Food products with up to 10-day chilled shelf life are, currently in the United Kingdom, required to receive a pasteurization treatment at least equivalent to 2 min at 70 °C. These products include cooked poultry, meat as an ingredient for chilled foods, ready meals, a wide range of pies and pastries, and soups and sauces for the high-quality end of the market. Microorganisms of concern with this group of foods are the aerobic pathogens; these include *Listeria monocytogenes*, *Salmonella* spp., and *E. coli*. These organisms exist only as vegetative cells and as such are relatively easy to kill by heat.

As shelf life is extended beyond 10 days, the possibility exists that those psychrotrophic strains of *C. botulinum* can grow. Sous vide was one of the first technologies to take shelf life beyond 10 days. It originated in France as a mild thermal process (e.g., 70 °C for 40 min) for the manufacture of high-quality foods sold to the catering sectors. More recent sous vide processes have targeted the psychrotrophic strains of *C. botulinum* that have required the process severity to be increased beyond 70 °C for 40 min. A process equivalent to 10 min at 90 °C is designed to achieve at least 6-log reductions in numbers of *C. botulinum* spores.

Frozen storage stops multiplication of microbial cells. The water activity (a_w) of the product will drop due to crystallization of the available water. Although there might be an initial decrease in cell numbers due to ice crystal formation, which may rupture the cells, many microorganisms may survive freezing and continue proliferation and growth once the product is thawed. The spoilage potential of the product will actually increase on thawing, as the texture changes and drip water forms. Either a frozen ready meal is likely to have been pasteurized as a complete meal, or the individual components will be pasteurized separately and then combined prior to being frozen.

Freezing does not destroy microbial toxins and viruses. Food that shows signs of microbial spoilage is not necessarily poisonous, and food that contains microbial toxins does not always appear spoilt. When low temperature is used for food preservation, either solely or in conjunction with mild heat processing, it is important to note the 'use by' date.

Dehydration or Low Water Activity

Microorganisms cannot grow in a water-free environment, because enzyme activity is absent, and most chemical reactions are greatly slowed down. This is the principle behind dehydration or low-water-activity foods.

Fresh vegetables, fruit, meat, and fish have naturally high moisture content, which averages about 80%. Drying reduces the amount of moisture, thereby limiting the numbers and types of microorganisms that can grow, and reduces the rate at which they can do so. There are different methods of drying that can reduce the moisture content slightly or virtually complexly, depending on the desired effect, for example,

- sun drying;
- drying by mechanical means, for example, hot air drying, spray drying, and roller drying;
- freeze drying, sublimation of water from a frozen food by means of a vacuum.

Some foods (e.g., jams and marmalades) have relatively high moisture content, but the moisture is chemically bound (to sugar in the case of jam) and not available to the microorganisms. A measure of the availability of water is called *water activity* and is defined by the ratio of the water vapor pressure in the food substrate to the vapor pressure of pure water at the same temperature and is denoted by a_w. Pure water has a water activity of 1.0, while most fresh foods have a water activity of about 0.99.

In general, bacteria require a higher a_w than yeasts and molds. Most spoilage bacteria cannot grow at $a_w < 0.91$, with *C. botulinum* having a minimum growth level of 0.94. *Staphylococcus aureus* has, however, been found to grow at a_w as low as 0.84. The lowest reported a_w value for a bacterial growth was 0.75. Most spoilage molds cannot grow at $a_w < 0.80$, with the lowest reported a_w for any mold growth at 0.65 and for yeasts at 0.61.

High-salt and high-sugar mixtures preserve food because they either kill the microorganisms directly due to the difference in osmotic pressure or bind the free water and lower the water activity, so that there is no water left for the microorganisms to use for growth. They effectively dry the product out. With salt or sugar, one can theoretically preserve food for decades without it being consumed by bacteria. The value of this method to reliably preserve food cannot be understated. Before refrigeration (about 100 years ago), sugar preserves were one of the only ways to keep fruit throughout the winters. Similarly, salt was, and still is, used to preserve fish, meat, and vegetables for many months.

In thermal processing, it is common to use low water activity as a hurdle technology that is applied in combination with a thermal process. Modern jams contain sufficient acidity and sugar to avoid the need for a full sterilization process; however, a mild pasteurization process is required to provide a shelf-stable product. Other examples of low water activity in combination with a mild process include pesto, sun-dried tomatoes, and fermented meats such as salami. Heat treatment targets yeast and mold, which requires processes equivalent to 5 min at 70 °C for the 6-log reduction.

Chemical Preservation

Some chemicals have specific antimicrobial activity and can be used to control the growth of microorganisms in food. Naturally occurring antimicrobial chemicals are present in herbs and spices and have been used traditionally for thousands of years. More recently, as our understanding of food science has improved, a range of specific active chemicals are used. The use of chemical preservatives is strictly controlled by legislation in the appropriate country, as is the quantity that may be used.

Chemical preservatives may inhibit microorganisms by interfering with the cell membranes (influencing cell permeability), enzyme activity, and/or genetic mechanisms. Most chemical preservatives have the highest activity at low pH.

Properties required of an ideal chemical preservative include the following:

- Wide range of antimicrobial activity
- Nontoxic
- Economical
- Must not affect the sensory qualities of a product
- Kill rather than inhibit

Examples of chemical preservatives are many and include the following:

- Benzoic acid, propionic acid, sorbic acid, and their salts
- Sulfur dioxide and methyl and ethyl parabens
- Nitrates and nitrites
- Antioxidants, for example, BHA, BHT, and TBHQ
- Antibiotics, for example, pimaricin (Delvocid) and nisin (Nisaplin) (however, no antibiotics of medical significance may be used in foods)

The mode of action of preservatives can be very specific and varies from compound to compound, and so the desired effect must be carefully considered when using preservatives. It is common to apply a mild heat treatment to the food product in addition to the hurdle of the preservative. Process times and temperatures do not tend to be as defined as with hurdles such as low pH or low a_w. An example of this is the baking process in which products exit the oven with microbiologically clean

surfaces. Recontamination from airborne yeast and mold spores can happen during cooling and packaging, and so growth of these organisms is controlled using propionic acid.

Organic acids

Acetic acid (vinegar) is known to cause disruption of the cell membrane. It also causes denaturation of proteins inside the cell. Acetic acid is highly effective and widely used as an acidulant and preservative. Only some bacteria (*Acetobacter* spp. and some lactic acid bacteria) and some yeast and mold are resistant to acetic acid. The growth of most food poisoning bacteria is inhibited by 0.1% acetic acid. Many pickles, mustards, and dressings use acetic acid in their formulation, with a pasteurization treatment of 2 min at 70 °C for products below pH 3.7.

Sorbic acid causes membrane damage of many microorganisms and disrupts enzyme activity inside the cells. It is widely used to inhibit the growth of yeast, mold, and some aerobic Gram-positive bacteria. It is, however, relatively infective against lactic acid bacteria. Sorbic acid is more effective at low pH and has virtually no effect above pH 6. It is widely used in pH-controlled drinks, such as fruit squashes, with a mild process of 2 min at 70 °C.

Benzoic acid has a similar mode of action to sorbic acid but is used mainly to control the growth of yeast and mold (at levels of 0.05–0.10%). Often, both benzoic and sorbic acids are used together so their effect is increased.

Propionic acid is similar in action to the other organic acids but is used primarily to inhibit mold growth (0.05–0.10%). It is also effective against many bacteria. It is most commonly used in baking to prevent ropiness caused by *Bacillus subtilis* survival through the baking process.

Sulfites and nitrites

Sulfites in the form of sulfur dioxide, bisulfites, or metabisulfites are used extensively to control the growth of undesirable microorganisms in fruit, fruit juice, wine, meat, fish, and pickles. At low pH (<4.0), it inhibits yeast and mold, and at higher pH values, it inhibits spore-forming bacteria. As the pH gets lower, more of the compound is in the sulfur dioxide form (from bisulfite form). The sulfur dioxide is believed to pass through the cell membrane by diffusion and then disrupt normal cell metabolism.

Nitrites enter the cell via the cell membrane and disrupt normal metabolic pathways and cellular respiration via formation of a variety of complex compounds. Nitrates are mainly used to inhibit bacterial growth. The effectiveness is related to pH, with lower pH being more effective. Nitrites are used widely with meat products so that a lower process can be applied than the full sterilization F_0 3, usually of the order of F_0 1.

Antibiotics

Nisin is a peptide antibiotic produced by some strains of *Lactococcus lactis*. Its inhibitory effect is restricted to Gram-positive bacteria where it causes death of the target organism by cell lysis. Nisin can be used to prevent the spoilage (by *Clostridium* and *Bacillus* spp.) of cheese, milk products, some canned products, cured meats, and alcoholic beverages.

See also: Acids: Natural Acids and Acidulants; Canning: Process of Canning; Chilled Foods: Packaging Under Vacuum; Chilled Foods: Principles; Cooking: Domestic Techniques; Drying: Principles and Types; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; Fermented Foods: Origins and Applications; Heat Treatment: Effect on Microbiological Changes and Shelf Life; Heat Treatment: Principles and Techniques; *Listeria*: Properties and Occurrences; Pasteurization: Effect on Sensory Quality and Nutrient Composition; Pickling; Preservation of Foods; Preservatives: Classifications and Analysis; *Salmonella*: Properties and Occurrence; Spoilage: Bacterial Spoilage; Spoilage: Yeast Spoilage of Food and Beverages; Sterilization of Foods.

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Peaches and Nectarines

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Introduction

Health benefits of fruit always seem to be in the news. Fruits are excellent functional foods as they are high in antioxidant and nutritional compounds. The regular consumption of fruit as part of a balanced diet has been associated with health benefits in different epidemiological studies. In fact, there is consensus among nutritionists and medical professionals that increased consumption of fruits will result in improved health. The health benefits associated with fruit are mainly associated with the bioactive components contained within them. There has been a growing interest in bioactive substances because of their antioxidant potential and association between their consumption and the prevention of some diseases. These health effects have been associated with reduced risk of diabetes, cancer, stroke, arthritis, Alzheimer's disease, vision problems, obesity, arthritis/joint pain, high cholesterol, and aging. A clear inverse relationship between the consumption of fruits and the incidence of cardio- and cerebrovascular, degenerative, and proliferative diseases and mortality has been demonstrated in many studies. Here, we report the nutritional value and health-promoting characteristics of peaches and nectarines.

Phytochemicals in fruit are thought to act as complex mixture rather than individually to provide protective health benefits through a combination of additive and/or synergistic effects. These phytochemicals can interact in many different ways, during food intake as part of a complete meal, changes in the gastrointestinal tract, interaction with gut microbiota, and interaction with other food constituents (lipids, proteins, carbohydrates, vitamins, etc.). The phytochemical content of fruit is influenced by numerous factors such as genotype, rootstock, climatic conditions, agronomic practices, harvesting time, and postharvest conditions.

Peaches and nectarines are a rich source of bioactives including ascorbic acid (vitamin C), carotenoids (provitamin A), and phenolic compounds. The bioactive compound profiles in peaches and nectarines show similar trends. Peaches can be categorized into clingstone and freestone varieties. Clingstone peaches are primarily used for processing, while freestone peaches are consumed fresh. Clingstones differ from freestone peaches in that they are considerably firmer in texture and, owing to a lack of the enzyme polygalacturonase, the stone clings to the flesh. Here, we report the nutritional value and health-promoting characteristics of peaches and nectarines.

Sources and Production

Peach originated in China and not Persia (now Iran) as its botanical name might reveal. Peach trees were introduced into Europe 2000 years ago from Persia and were brought to North America in the sixteenth century. Peaches are frost-sensitive, semihardy perennials of the Rosaceae. Peach trees require a chilling period of at least 25–42 days to induce flower production. Peach trees can tolerate cold winter temperatures, but flower buds are less cold hardy. Spring frosts can cause flower drop and trees may not be productive in growing seasons where spring frosts occur during flowering. Peaches are somewhat drought-tolerant but require irrigation during extensive periods of heat. Irrigation is particularly necessary during flower and fruit development, as flower buds and fruit will senesce if the tree begins to wilt. Peach is self-fertile and naturally self-pollinating; however, cross-pollination is also possible; nectarine and peach trees can cross-pollinate. From a genetic perspective, peaches and nectarines are almost identical; nectarines differ from peaches as they possess a recessive gene that prevents pubescence from forming on fruit. Peach trees may begin fruiting after 1 year if a rootstock is used; however, trees are usually short-lived and usually do not fruit for more than 15–20 years if conditions are favorable.

After apples, peaches and nectarines are the second most economically important fruit crop in the European Union (EU) and the most important within the genus *Prunus*. China is the leading producer of peaches and nectarines followed by Italy, the United States, and Spain. The top peach-exporting countries are Spain, Italy, EU, and the United States. The top four peach importers are Germany, Russia, France, and Poland. Canada is the 8th-largest importer of peaches producing about 25 000 tons of peaches annually and hence ranks well below the top 20 peach-producing countries. Peach and nectarine production in the EU lies in access of 4.2 million tons. Spain is the second-largest producer of nectarines and peaches in Europe producing over one million tons annually. Peach production and breeding is a growing industry and is the most dynamic among the temperate fruit crops with new cultivars being released yearly.

Fruit Anatomy and Development

Understanding peach fruit anatomy and development is a crucial component of studying the nutritional value of this crop. The nutrients in peach are not evenly distributed throughout the fruit. Therefore, identifying the key nutrients in each part of the fruit is essential in determining the parts of the fruit that harbor particular health benefits. Furthermore, peaches undergo a variety of physiological and biochemical changes throughout fruit maturity. These changes influence the relative type and amount of phytochemicals and bioactive compounds that accumulate in peach fruit over time. Peach fruit anatomy consists of two main organs, the seed and the pericarp. The seed consists of the endosperm, embryo, and seed coat, while the pericarp consists of the endocarp, mesocarp, and exocarp. The majority of health-promoting compounds in peach fruit are found within the pericarp.

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Peach fruit undergoes three distinct stages, stages I, II, and II, of development where growth can be explained by a double sigmoid pattern. Fruit in stage I undergoes rapid cell division followed by a rapid increase in the volume of the pericarp as a result of cell expansion toward the end of this period. In stage II, the pericarp remains predominantly quiescent in terms of growth and development, while the embryo develops rapidly. Stage III is marked by the completion of endocarp development and a rapid increase in pericarp volume, which is mainly due to cell expansion. Most bioactive components of the fruit develop and accumulate in the endocarp, mesocarp, and exocarp of the fruit during stage III. Early-maturing cultivars tend to have a reduced stage II period of development, and hence, maturity resumes at a faster pace. However, this phenomenon may have implications in the relative amounts of phytochemicals present in early-maturing varieties compared with mid- or late-maturing varieties.

Fruit maturity and ripening are marked by several biochemical and physiological changes. Fruit maturity precedes fruit ripening, and these two events are separately controlled. Fruit maturity is defined as fullness of development, can only occur while the fruit is attached to the tree, and is marked by termination of cell expansion and the cessation of dry matter accumulation. Fruit maturation occurs unevenly from the tip down and from the inside outside. Ripeness is defined as readiness for use, can occur while the fruit is attached or detached from the tree, and is marked by fruit softening, coloration, and development of aroma.

Consumer Consumption Trends of Peach and Nectarine

Identifying peach and nectarine consumption frequencies between nationality, age, and gender groups is important in understanding studies related to health benefits associated with these fruits. Several studies have evaluated peach consumption frequency within different European countries in relation to age and gender. In one study, 499 respondents from five countries answered questions relating to frequency of peach and nectarine consumption. France had the highest consumption of peach and nectarine where 48% of respondents declared a peach consumption of 3–5 per week with 40% eating more than 5 fruits per week. The lowest peach intake was declared in Germany followed by Poland. Data analysis of self-reported fruit intake shows significant differences between nationalities. Women declared a higher peach consumption than men. Peach and nectarine consumption decreased with age with the lowest consumption occurring in individuals between 16 and 35 years of age. In general, there has been reduced intake of fruit by the younger generation and a persistent tendency of lower frequency of fruit consumption among men than women. In some Europeans countries, an association between lower fruit intake as reported by men and a higher risk of chronic diseases in this group has been identified. This trend indicates an urgent need for a targeted informational campaign on the beneficial effects of fruit consumption on health.

Availability, Absorption, and Metabolism

Bioactives are compounds that produce physiological effects when present in living material. These compounds exert physiological benefits that relate to promoting health and preventing effects of a disease. Bioactive compounds must withstand digestion by the gut that would otherwise destroy the active structure of the compound, therefore, rendering it physiologically inactive when ingested. Sometimes, however, the inactive part of the compound becomes active once consumed. This phenomenon is a result of the action of digestive enzymes present in the gastrointestinal tract. In some cases, bioactives may not be absorbed by the digestive tract, but still exert their physiological effect within the digestive tract. Usually, however, the bioactive compound must be absorbed from the gastrointestinal tract into the blood circulatory system. The compound is then carried via the blood circulatory system to target organs where the bioactives can exert their effect. Functional foods are defined as foods that contain bioactives and consumed as part of a normal diet. Plants are a rich source of bioactive compounds. Fruit-derived bioactive products are becoming accepted because of their abundance and low cost. Furthermore, these products are becoming more and more popular due to the wider acceptability of plant products as a result of religious, social, or moral reasons that prevent many people from consuming animal-derived products. Fruit-derived bioactives show antimicrobial activity, anticancer activity, anti-inflammatory activity, immunostimulatory activity, and antioxidant activity. Peaches and nectarines are a rich source of bioactives including ascorbic acid (vitamin C), phenolic compounds, carotenoids (provitamin A), anthocyanins, and dietary fibers (DFs).

Vitamin C is a water-soluble antioxidant and is referred to as an antioxidant vitamin. Humans are unable to synthesize vitamin and, therefore, are entirely dependent upon dietary sources to meet their daily Vitamin C needs. More than 90% of the vitamin C in the human diet is supplied by fruits and vegetables. The vitamin C content in peach and nectarine flesh ranges between 1 and 14 mg of AsA per 100 g of FW depending on cultivar. These values are higher when peach peels are included in the test because skin tissues have more vitamin C to protect the fruit from outside stress caused by light and oxidation. The ranges of total ascorbic acid in mg per 100 g of FW were 5–14 (white-flesh nectarines), 6–8 (yellow-flesh nectarines), 6–9 (white-flesh peaches), and 4–13 (yellow-flesh peaches). The genetic background of the genotype assessed is a much more important factor compared to the climatic conditions and cultural practices used in producing fruit with high vitamin C content. There appears to be no correlation between vitamin C and any other phytochemical trait. There are no clear differences in the vitamin C content between peaches and nectarines, but the vitamin C content of white-flesh peaches is significantly higher than that of yellow-flesh fruits. Vitamin C does no show significant correlation with relative antioxidant capacity (RAC). Studies also indicate a positive correlation between vitamin C and TA due to the contribution of ascorbic acid to the fruit acidity.

The sugar content of peach fruit largely determines consumer acceptance and can be a good indicator of physiological maturity and ripeness. Peach fruits contain three main sugars; the dominant sugar is sucrose, and glucose, fructose, and sorbitol are also important components of the sugar content making up 54–75%, 4–11%, 9–21%, and 3–25% of the total soluble solid content (SSC) of fruit, respectively. 65–85% of

the soluble solids (SSs) of fruit are sugars, and therefore, SSC is a good indirect measure of fruit sugar content. SSC is typically measured on a refractometer and recorded as a unit of degree Brix. Hence, Brix is a measure of the sugar content of an aqueous solution; 1 °Brix is 1 g of sucrose in 100 g of solution. When measuring the sugar content of fruit, SSs other than sucrose are present in solution, and therefore, SSC measurements are only an indirect assessment of the sucrose content within fruit. Sucrose is important as an energy source and as a preservative of fruit flavors. Fructose is rated higher (1.75) than sucrose (1) and glucose (0.75) in terms of sweetness. Cultivars that have a high fructose percentage present the highest sweetening power. Sorbitol plays an important role in the texture and flavor of peach and nectarine. Sorbitol is an interesting polyalcohol in terms of nutrition for special dietary purposes like diet control or dental health.

Acids present in peach and nectarine fruit are mainly malic acid, citric acid, quinic acid, and shikimic acid. Malic acid is the most abundant acid at maturity (42.92–84.30% of the total acid content), followed by quinic acid (14.56–57.54%), citric acid (3.72–31.61%), and shikimic acid (0.14–1.89%). However, some studies have reported citric acid and quinic acid to be the second and third most abundant organic acid, respectively, in most peach and nectarine cultivars. Some studies suggest that quinic acid imparts a slightly sour and bitter taste and has antibacterial properties beneficial to health. Titratable acidity tends to be higher and pH lower in yellow-flesh peaches compared to white-flesh cultivars.

Carotenoids are lipid-soluble compounds that cause yellow, orange, red, and violet colors in various kinds of fruits. The bioactives present in carotenoid are classified into carotenoid hydrocarbons (carotenes) and carotenoid alcohols (xanthophylls). The prevention of chronic disease such as cardiovascular disease and skin cancer may be prevented via the provitamin A activity of some carotenoid. In peaches and nectarines, the main carotenoids present are carotene and cryptoxanthin. Carotenoids are more abundant in peel tissues than in flesh tissue. In peach and nectarine, carotenes are more abundant than cryptoxanthin. Yellow cultivars contain close to ten times more carotenoids (95–197 mg per 100 g serving) than white-flesh cultivars (8–18 mg per 100 g).

Phenolics are natural plant metabolites, which are found in many fruit, including peaches, and function as plant defense against insect and animal herbivory and oxidative damage. Fruit phenolic compounds play an important role in peach and nectarine quality because they have a role in the visual appearance of the fruit (pigmentation and browning), taste (astringency), and health-promoting properties (free-radical scavengers). Additionally, phenolics can be used to increase the shelf life of food and inhibit the growth of pathogenic microorganisms due to their natural antimicrobial properties. Phenolic compounds are not uniformly distributed within the tissue of fruits. Most phenolics are concentrated in the epidermal and subepidermal layers of fruit. Phenolic distribution is an important aspect of the overall phenolic composition and antioxidant capacity because peach skin is usually not eaten and, therefore, does not contribute to the human diet intake. The distribution of phenolics in the peach is 30% in the flesh and 70% in the peel, and on average, unpeeled fruit contained 1.5-fold higher levels of phenolics than peeled fruit. Phenolics in fruit are mainly in soluble free form (62–96%); however, 2–46% of phenolics existed in bound-E forms and 2–24% in bound-W forms. Bound phytochemicals cannot be digested by human enzymes and may survive stomach and small intestine digestion to reach the colon, providing site-specific health benefits.

Nectarines and peaches generally exhibit identical phenolic profiles, containing the same individual compounds. However, some studies found higher phenolic content in peaches than in nectarines and higher phenolic content in white-flesh fruit compared with yellow-flesh fruit. These findings support the idea that white-flesh varieties have significantly higher antioxidants content than yellow-flesh peaches and nectarines. However, it is important to note that these findings differ among studies, with some studies finding no significant difference in phenolics between white- and yellow-flesh fruits. Peaches and nectarines contain larger quantities of chlorogenic acid than neochlorogenic acid. Some peach and nectarine cultivars that contain high levels of phenolics have a corresponding bitter taste, which indicates that too high a level of phenolics may be detrimental.

Many reports suggest that phenolic compounds are mainly responsible for the antioxidant activity of peaches and nectarines, as described in many studies for stone fruits in general. Recently, much attention has been given to antioxidants as an eligible parameter for fruit quality. Cells in humans and other organisms are constantly exposed to several types of oxidizing agents. Some of these oxidizing agents are necessary for life. Overproduction of oxidants can result in oxidative stress, which is associated with chronic diseases. Therefore, increased consumption of fruits containing high levels of antioxidants (mainly phytochemicals) is recommended to prevent or slow the oxidative stress caused by free radicals. RAC of flesh samples among peach and nectarine cultivars ranges between 100 and 1000 μg of Trolox per g of FW. RACs of peach and nectarine samples that include the peel range between 700 and 6000 μg of Trolox per g of FW. Therefore, the antioxidant capacity of the peach fruits decreases when they are peeled. The antioxidant activity of peaches and nectarines ranges from 2074 to 13 505 μg of Trolox per g of FW in red-flesh peaches and between 437 and 1128 μg of Trolox per g of FW in lighter-flesh peaches. By increasing the phenolic content of nectarines and peaches by genetic manipulation, breeders can increase the antioxidant capacity of these fruit. However, there is a limit after which increased phenolic concentration may cause undesirable levels of astringency in these fruits. Due to the notable health benefits associated with nectarines and peaches, these fruit should be included in the range of fruits selected by consumers to meet the recommended two to four servings of fruits per day.

Anthocyanins are one of the primary components responsible for the flesh and skin coloration pattern of peach fruit. Anthocyanins are a class of flavonoids that are synthesized via the phenylpropanoid pathway. This important class of water-soluble vacuolar pigment is found in all tissues of higher plants including leaves, stems, roots, flowers, and fruits. They are odorless and nearly flavorless and give rise to red, purple, and blue tinges to tissues where they are expressed; color largely depends on pH. In peach, anthocyanins are localized in vacuoles of the epidermis or the flesh. Although anthocyanins contribute to

the mainly reddish tinge of mature peach fruit, they are independent of the skin ground color, which is influenced by other pigments that cause a yellow or white coloration pattern that lies below the coloration pattern expressed by anthocyanins. Anthocyanins play a major role in the appearance, taste, and flavor of fruits, including peach and nectarine. Anthocyanins are well known for their health-promoting properties. In addition to their colorful characteristics, they have potent antioxidant properties modulated by their different hydroxylations and glycosylations. The two most common anthocyanin pigments found in peach and nectarine are cyanidin-3-glucoside (major component) and cyanidin-3-rutinoside (minor component). However, other anthocyanins, namely, cyanidin 3-acetyl glucoside, cyanidin 3-galactoside, peonidin-3-glucoside (1.1–1.2 mg per 100 g), and peonidin derivatives (1.9–11.5 mg per 100 g), have also been identified in some peach and nectarine varieties in smaller amounts.

Total anthocyanins in peeled peach and nectarine varieties greatly vary among peach and nectarine genotypes (0.1–26.7 mg of cyanidin-3-glucoside equivalents per kg of FW). These values are much higher if peach peels are included in the analysis because peach peels contain higher levels of anthocyanins than flesh. The anthocyanin content greatly depends on the red pigmentation of the flesh. Genotypes with red flesh have higher anthocyanin content. There are no clear differences in anthocyanin content between peaches and nectarines, but the anthocyanin content in the flesh of white-flesh peaches is significantly higher than that of yellow-flesh fruits. These findings could be explained by the red pigmentation due to anthocyanins in white-flesh fruit, particularly in the flesh area surrounding the stone in some varieties. By contrast, anthocyanin content in the peel is reportedly higher in yellow-flesh fruits than in white-flesh fruits.

DFs refer to carbohydrate polymers containing ten or more monomeric units that are neither digested nor absorbed in the small intestine. DFs possess many health-benefiting properties. They can help decrease intestinal transit time, increase stool bulk fermentable by colonic microflora, reduce blood total and LDL cholesterol levels, prevent stroke, reduce postprandial blood glucose, reduce insulin levels, and prevent constipation. Approximately 25–38 g per day is encouraged to promote health benefits associated with fiber. DF consists of cellulose, lignin, hemicellulose, pectins, gums, and mucilages, and all of these components are present in the cell wall material of fruits. Total dietary fiber (TDF) is subdivided into insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) depending on their solubility in water. TDF, IDF, and SDF play an important role in human nutrition. Presently, the food composition tables give crude fiber (CF) content but not DF content. The TDF values are much higher than the CF values of corresponding fruits. According to various studies, the CF values for peach fruit using the CF and TDF methods were found to be 1.2 and between 1.9 and 3.9 g per 100 g of peach fruit tissue, respectively. This variation could be due to differences in the methodology applied for the determination of fiber content in foods.

Fresh Versus Processed Fruits

Most consumers believe that fresh fruits have more vitamins, minerals, and phytochemicals than processed fruits, and therefore, processed fruits are nutritionally inferior to fresh products. However, the commonly held belief that 'fresh is best' may not be the case. In fact, many some studies demonstrated that processed fruits are nutritionally similar and sometimes have more nutrients compared to their fresh and frozen counterparts. This misperception may limit consumer food choices considering that many products, including peaches, are seasonal, which limits their availability. In reality, processed peaches can deliver high-quality, nutrient-dense foods on a year-round basis, instead of the limited seasonal availability of fresh peaches.

Fruits are typically composed of over 90% water. Once harvested, fruits begin to undergo higher rates of respiration, which results in moisture loss, quality deterioration, and potential microbial spoilage. Harvesting separates the fruit from the tree, which is its source of nutrients, and therefore, the fruit uses itself as a source of calories. Many fresh fruits, including peach, have a shelf life of a few days before they are unsafe or undesirable for consumption. Storage and processing technologies are used to transform perishable fruits into safe, delicious, and stable products. To slow down respiration and increase shelf life, fruits are refrigerated. Freezing, canning, and drying allow fruit to be consumed year-round and transported safely to consumers worldwide. Processing arrests the progression of respiration, which then stops the consumption of nutritious components, the loss of moisture, and the growth of microorganisms.

Freezing and canning processes may preserve the nutrient value of fruit. Water-soluble and oxygen-labile nutrients such as vitamins C and the B may be lost during the initial thermal treatment of processed fruit. These nutrients are relatively stable during subsequent canned storage owing to the lack of oxygen. Initially, frozen products lose fewer nutrients due to the short heating time in blanching, but they may lose more nutrients during storage due to oxidation. Phenolic compounds are water-soluble and oxygen-labile. Changes in moisture content during storage, cooking, and processing may misrepresent changes in the nutrient components of fruit. Retention of nutrients is highly dependent on cultivar, production location, maturity stage, season, and processing conditions. Fresh fruits, including peaches, usually lose nutrients more rapidly than canned or frozen products.

According to some studies, the nutritional content of canned peaches is comparable to that of fresh peaches. In one study, the vitamins and phytochemicals were found to be present at statistically significantly higher levels in canned cling peaches versus fresh freestone (vitamin C, antioxidants, and folate), higher but not statistically different levels (vitamin A), or lower but not statistically different levels (vitamin E, total phenolics, and total carotenoids). There were no statistically significant changes in nutrient content during storage for 3 months (Table 1). Therefore, canned peaches can provide comparable nutrient levels to the consumer as fresh peaches.

Health-Promoting Benefits

Promoting longevity and health span has always been attributed to the consumption of fruits containing high antioxidant capacities and other bioactive components. One of the greatest environmental factors that affect life span is human diet. The

Table 1 Comparison of fresh freestone with fresh and canned cling peaches

Analyte and method	*Fresh freestone (FF)*	*FF vs. FC*	*Fresh cling (FC)*	*FF vs. T0*	*FC vs. T0*	*Canned cling (T0)*	*T0 vs. T3*	*Canned cling (T3)*
Carotenoids								
HPLC (RAE kg^{-1})	105 ± 44	NS	150 ± 75	NS	S	115 ± 54	NS	110 ± 67
Spectrophotometric (mg kg^{-1})[a]	16.3 ± 5.7	NS	16.7 ± 4.2	NS	S	13.3 ± 4.9	NS	13.3 ± 4.8
Vitamin E								
Alpha (mg kg^{-1})	1.42 ± 0.42	NS	1.68 ± 0.24	NS	S	1.12 ± 0.31	NS	0.93 ± 0.10
Gamma (mg kg^{-1})	0.11 ± 0.01	NS	0.13 ± 0.02	S	NS	0.13 ± 0.07	NS	0.14 ± 0.02
Total (mg kg^{-1})	1.53 ± 0.42	NS	1.82 ± 0.24	NS	S	1.24 ± 0.35	NS	1.06 ± 0.11
Total phenolics								
Folin–Ciocalteu (mg kg^{-1})[b]	281 ± 92	NS	306 ± 73	NS	NS	265 ± 73	NS	227 ± 39
Vitamin C								
HPLC (mg kg^{-1})	9.5 ± 4.5	NS	19.5 ± 18.3	S	NS	34.1 ± 24.1	NS	55.0 ± 17.1
Antioxidants								
FRAP (μmol l^{-1} Trolox equivalent kg^{-1})	9620 ± 5700	NS	11 990 ± 3840	S	NS	15 580 ± 5450	NS	12 180 ± 3400
Folate (μg kg^{-1})	2.0 ± 0.8	S	5.9 ± 4.3	S	S	22.4 ± 4.2	NS	

Results are given as the average ± standard deviation.
NS, not significant ($P \geq 0.05$); S, significant ($P < 0.05$). Comparisons were made using the two-sample *t*-test.
[a]As β-carotene.
[b]As gallic acid equivalents.
Source: Durst and Weaver (2012).

addition or increase of certain fruits to the human diet has been shown to have human health benefits including an increased life span. One study has evaluated the effects of nectarine fruit extract on *Drosophila melanogaster* life span. In this study, wild-type flies were fed with standard, dietary restriction, or high-fat diet supplemented with 0–4% nectarine extract. Life span, food intake, locomotor activity, fecundity, gene expression changes, and oxidative damage indicated by the level of 4-hydroxynonenal–protein adduct were evaluated in the flies. Supplementation with 4% nectarine extract extended life span, increased fecundity, and decreased expression of some metabolic genes. Nectarine extract reduced oxidative damage in wild-type females fed with high-fat diet. Together, these findings suggest that nectarine promotes longevity and health span partly by modulating glucose metabolism and reducing oxidative damage. These results suggest that nectarines and likely peaches might promote a healthier, longer life by modulating glucose metabolism and reducing oxidative damage.

The anticancer activity of many fruit extracts has been studied using *in vitro* cell line model systems and *in vivo* systems. Fruit consumption provides an array of bioactive phytochemicals that can help lower the risk of cancer. A cohort study regarding the effect of fruit and vegetable intake on head and neck cancer risk in the United States showed that those volunteers placed in the first tertile of peach and nectarine consumption, together with other Rosaceae fruits, had a decreased risk of head and neck cancer.

Different plant species have compounds that are active against cancer propagation by blocking cell cycle progression and/or inducing apoptosis, which is directly associated to DNA fragmentation. A desirable feature of these bioactive compounds is that they have selective cytotoxicity against cancer cells while not affecting normal cells. In stone fruits, many phenolics have been reported such as anthocyanins, hydroxycinnamic acids, flavan-3-ols, and flavonols. The main flavonols reported in stone fruit include chlorogenic acid, neochlorogenic acid, catechin, epicatechin, and quercetin 3-rutinoside. Many of these phenolics act as antioxidants, antimutagenics, and anticarcinogenics.

To demonstrate the effectiveness of peach fruit phenolics to treat cancer, one study evaluated the phenolic composition of the yellow-flesh peach 'Rich Lady' (RL). RL extracts were collected from mature firm fruit and used to study their effects at suppressing cancer cells in mice. The extract from RL contained a mixture of polyphenolics like chlorogenic acid, neochlorogenic acid, cyanidin 3β-glucoside, quercetin 3β-rutinoside, quercetin 3β-glucoside, and catechin derivatives. Peach RL extracts (0.2–1.6 mg CAE per day) did not exert adverse effects on body weight gain and appearance of major organs including in the nonxenografted controls, which confirms their lack of toxicity. Results showed that treatment with peach phenolics (0.8–1.6 mg CAE per day) inhibited tumor growth of the highly metastatic MDA-MB-435 cells of xenografted animals after 12 days compared to the xenografted control group. However, only tumor volume (v) but not tumor mass (m) was reduced. The inhibitory effects of peach phenolics on tumor growth were accompanied by decreased blood vessel formation compared to the xenografted control, which is characteristic of angiogenesis. This observation indicates that tumor volume decreased and not mass due to the effects in density of the tumor tissue (m/v).

The mixture of phenolics present in some peach fruit variety extracts may constitute a novel chemopreventive tool in the combination therapy when primary cancer is diagnosed because these polyphenols do not have toxic side effects. The generated phenolic extract dose information for mice in *in vivo* studies could be converted to equivalent human intake and considered as a reference for future clinical studies by using the body surface area normalization method. For example, the use of 6.17 mg kg^{-1} per day of peach fruit phenolic extract from

the genotype RL will potentially decrease breast cancer cell volume and mass in humans. Therefore, for an average human adult weighing 60 kg, the intake of RL peach phenolics would be ~370.6 mg day^{-1}. The phenolic content for an average RL peach fruit is in the range of 111.6–180 mg per fruit. Therefore, the human equivalent dose of phenolic extract required to suppress breast cancer volume and mass (~370.6 mg day^{-1}) could be supplied by consuming only two to three peach fruits a day. Alternatively, supplements free of sugars could be supplied by an aqueous or peach phenolic extract powder if sugar intake is a concern.

In a second study, polyphenolic extracts and fractions of selected peach genotypes were evaluated for cell viability and antiproliferation activity *in vitro* against an estrogen-independent MDA-MB-435, which is a very aggressive and invasive breast cancer cell line, and estrogen-dependent MCF-7 breast cancer cell lines, which are less aggressive and invasive compared to MDA-MB-435, and one noncancerous breast line MCF-10A. The extracts showed a phenolic dose-dependent cytotoxic effect against MDA-MB-435, weak activity against MCF-7, and small or no activity against MCF-10A. HPLC analysis of the peach fruit extract from the genotype BY00P6653, which had the strongest anticancerous effects from the genotypes used, revealed four fractions. The first fraction (F-I) contained hydroxycinnamic acid derivatives including chlorogenic acid and a caffeic acid derivative, which is likely neochlorogenic acid. Chlorogenic acid and caffeic acid derivatives are reported to have anticarcinogenic activity on hepatic, HL-60, U937, and Jurkat cells as well as apoptosis-inducing activity on leukemia cells. The second fraction (F-II) contained anthocyanins with cyanidin-3-glucoside, the most abundant combined with small amounts of flavonols. The third fraction (F-III) contained quercetin derivative compounds. The fourth fraction (F-IV) contained polymer compounds, mainly polymerized anthocyanins.

Metalloproteinases (MMPs) are important targets in breast cancer progression as they play a role in the degradation of the extracellular matrix facilitating tumor invasion and metastasis. The gene expression of MMPs in tumor tissues was investigated. Treatment with peach phenolics (1.6 mg CAE per day) inhibited gene expression of MMP-2, MMP-13, and MMP-3 down to 0.68-, 0.57-, and 0.54-fold of the xenografted controls. These findings indicate that peach phenolics downregulated the gene expression of MMPs.

Obesity is a serious health issue affecting both developed and underdeveloped nations. The factors associated with increased occurrence of obesity include diminished physical exercise and an increased consumption of saturated fats and refined carbohydrates. Obesity is associated with several clinical complications and diseases including insulin resistance, hypertension, inflammation, oxidative stress, and dyslipidemia. Several *in vitro* and *in vivo* studies have demonstrated the antilipidemic and antiobesity effects of polyphenols, including suppression of adipogenesis and adipocyte proliferation, inhibition of fat absorption, and modulation of energy metabolism and inflammation. Research suggests that dietary polyphenols can modulate the composition and metabolic activity of intestinal microorganisms. This modulation may be involved in the underlying mechanisms for the associated health benefits. This hypothesis is supported by the close association between energy harvest, obesity, and the complex assembly of microorganisms residing in the intestinal tract.

Peach and plum fruits contain high amounts of polyphenols, which possess antiobesity effects. Successful treatment of obesity using polyphenols is thought to involve an alteration of the intestinal microbiota. Zucker rats have been very well characterized as a model for studies of obesity and are an attractive animal model for studies of the gut microbiota. These rats possess a mutation in the leptin receptor and develop metabolic syndrome symptoms, including insulin resistance and dyslipidemia, at 4–5 weeks of age. Obese individuals have been shown to contain distinctive intestinal microbiota when compared to nonobese subjects. In one study, three groups of Zucker rats were used to study the effects of polyphenols on obesity by assessing the weight gain by each of the rats and conducting qPCR analyses of their fecal material. The rat groups were an obese control group, a lean group, and a peach group. Rats in the peach group were fed with carbohydrate-free fruit juices from peaches or plums, while the control group and lean group were given water. Fecal samples were taken after 11 weeks of control or fruit juice was administered. The qPCR analyses identified statistically significant differences in the abundance of several fecal bacterial groups between treatment (peach and plum) and control and lean groups. This study suggested that the consumption of carbohydrate-free peach and plum juices containing polyphenols as well as vitamins and minerals has the potential to modify fecal bacterial composition in an obese animal model. These changes, in turn, may contribute to the prevention of obesity in rats and potentially in other animals.

Antiallergic inflammatory property of peach extract has been demonstrated in some model systems. Mast cell-derived mediators play an essential role in the initiation and progression of the allergic inflammatory reactions connected with atopic asthma and other allergic disorders. The mast cell-based *in vivo* allergic inflammation model and its possible mechanism of action were investigated using ethanol extract of fruits of peach. Fruits of *Prunus persica* (FPP) were found to dose-dependently inhibit compound 48/80-induced systemic anaphylaxis (used as a model of induction for systemic fatal allergic reaction – the synthetic mast cell stimulator) and immunoglobulin E-mediated local allergic reactions. Anaphylaxis is a life-threatening syndrome induced by the sudden systemic release of inflammatory mediators like histamine, heparin, and various cytokines from mast cells.

In this study, mice were injected with compound 48/80 and monitored for 1 h, after which the mortality rate was determined. The injection of compound 48/80 into mice induced fatal shock in all animals. When FPP was intraperitoneally administered for 1 h at a concentration of 10–1000 mg kg^{-1} body weight, the mortality with compound 48/80 was dose-dependently reduced. The effect of FPP on the compound 48/80-induced serum histamine release was also investigated in this study. FPP was administered at concentrations between 10 and 1000 mg kg^{-1} 1 h before the compound 48/80 was injected. When compound 48/80 was injected, there was marked increase of serum histamine release, and this induction was inhibited by FPP treatment in a dose-dependent manner. The results of this study demonstrated that FPP has antiallergic inflammatory properties. FPP inhibited mast cell-mediated allergic

reactions and decreased PMACI-stimulated expression of pro-inflammatory cytokines, therefore suggesting that FPP might be useful in the treatment of allergic inflammatory diseases.

See also: Acids: Natural Acids and Acidulants; Acids: Properties and Determination; Allergies: Public health; Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Bioavailability of Nutrients; Calcium: Physiology; Calcium: Properties and Determination; Carbohydrate: Digestion, Absorption and Metabolism; Carotenoids: Occurrence, Properties and Determination; Carotenoids: Physiology; Chilled Foods: Effects on Shelf-life and Sensory Quality; Chilled Foods: Modified Atmosphere Packaging; Chilled Foods: Principles; Colon: Diseases and Disorders; Colon: Structure and Function; Controlled Atmosphere Storage: Effect on Fruit and Vegetables; Copper: Physiology; Dietary Fiber: Physiological Effects; Dietary Fiber: Properties and Sources; Dietary References: US; Fats: Classification and Analysis; Fatty Acids: *Trans* Fatty Acids; Fatty Acids: Determination and Requirements; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fatty Acids: Metabolism; Folic acid and Folates: Physiology and Health Effects; Food Allergies: Occurrence and Analysis; Freezing Theory; Fructose and High-Fructose Corn Syrup; Fructose: Sources, Metabolism, and Health; Fruit Juices; Functional Foods; Glucose: Properties and Analysis; Heat Treatment: Effect on Microbiological Changes and Shelf Life; Heat Treatment: Principles and Techniques; Iron: Biosynthesis and Significance of Heme; Iron: Properties and Determination; Magnesium; Manganese; Obesity: Causes and Prevalence; Obesity: Epidemiology of; Obesity: The Role of Diet; Pantothenic Acid; pH: Principles and Measurement; Phenolic Compounds: Bioavailability and Health Effects; Phenolic Compounds: Occurrence, Classes, and Analysis; Phospholipids: Physiology; Phospholipids: Properties and Occurrence; Phytic Acid: Properties, Uses, and Determination; Potassium: Physiology; Potassium: Properties and Determination; Preservation of Foods; Probiotics; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Proteins: Chemistry, Characterization, and Quality; Riboflavin: Physiology; Riboflavin: Properties and Determination; Sucrose: Dietary Importance; Sucrose: Properties and Determination; Thiamin: Physiology; Thiamin: Properties and Determination; Vitamin K: Physiology; Vitamin K: Properties and Determination; Vitamins: Overview; Zinc: Physiology and Health Effects; Zinc: Properties and Determination.

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Peanuts

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History of Peanut Cultivation

Several earlier works provide a detailed account of the history of peanut cultivation. Briefly, people native to Brazil, Bolivia, and Peru were cultivating wild peanut species well before the cultivated peanut, *Arachis hypogaea* L., existed. Since 1977, explorations to collect *Arachis* germplasm in South America revealed numerous sites where hunter-gatherer–farmers had transplanted the wild species, *A. stenosperma* Krapov. and W.C. Gregory, to new sites and maintained it as a semi-cultivated crop. Many of these sites are located in the littoral, south of Rio de Janeiro, extending to near Puerto Alegre. This same species was found growing near old Jesuit mission ruins in central Brazil and also northward to São Félix. All of these sites are distant from the native site of this species in central Brazil, near Barra do Garças. Another species that is still cultivated by native tribes in the western Mato Grosso of Brazil is *A. villosulicarpa*. Archaeological evidence in north Argentina and in Peru indicates that wild *Arachis* were being cultivated well before the cultigen existed.

Little doubt remains that the cultivated peanut that is so widely distributed around the tropics and warmer temperate parts of the world today originated in the gardens (fields) of hunter-gatherer–farmers in the time frame of 3500–3800 BP. The strongest evidence that *A. hypogaea* did not evolve in the wild is the fact that the cultigen cannot survive indefinitely without man's intervention. The location of the formation of *A. hypogaea* is not determined, and some discussion has mentioned that the same event of cultivar formation could have occurred twice in different locations, thus possibly accounting for the two subspecies recognized today: *A. hypogaea* ssp. *hypogaea* and *A. hypogaea* ssp. *fastigiata*. Molecular biology has aided in identifying the two wild species that were probably the parents of the cultigen: one, *A. duranensis* Krapov. and W.C. Gregory, contributing the A genome and the other, *A. ipaensis* Krapov. and W.C. Gregory, contributing the B genome.

Recorded history of peanut cultivation begins with the discovery of the Americas by Columbus because the natives on the islands were growing peanuts for food. A significant amount of selection and differentiation occurred in the plantings as farming techniques and farmers' knowledge advanced from 3800 BP to 1500 AD. As the conquests progressed, several accounts document natives growing peanuts. Hammons cited 24 recordings of peanut cultivation in the new world from 1502 to 1697. The first recording of peanut in Europe was in 1574 by N. Monardes, but most likely, it was there earlier. From Spain and Portugal, the peanut was spread across Africa, Asia, and China. Archaeological evidence from China indicates that the peanut may have been carried from the west coast of the Americas with ancient Chinese sailors and was grown in China during the Han dynasty, well before the time of Columbus. The evidence of these visits to the Americas is questionable, but many different authors refer to the possibility. The peanut reached Australia in 1870 with Chinese gold diggers.

There is no documented evidence that the native peoples of what is now the United States were growing peanuts when the colonists arrived. Thus, the crop probably came to the colonies with early settlers or more likely with the slaves as the peanut was sometimes used to feed slaves during the journey from Africa to the Americas because it required a small amount of space.

Consumption Trends

Peanuts are ranked fifth in the world for total oil production and are produced for edible oil and food in more than 100 countries worldwide. Because of its adaptability and high nutritional value, peanuts remain an important crop for small farmers in South America, Africa, India, and China, as well as many island nations. It is grown for home consumption as whole peanut, cooking oil, and the meal remaining after the oil is removed. It is also sold or bartered for other essential needs, including other foods. For modern, mechanized farming, peanut production has become an important cash-crop business in countries such as Argentina, Australia, Brazil, and the United States. However, China remains the leading producer of peanuts (~15 million metric tons per year) in the world simply due to the large number of farmers growing peanuts. India remains second in production (8+ million metric tons) for the same reason, with Nigeria ranking third (3.8 million metric tons) and the United States fourth at 1.9 million metric tons. The total world production of peanuts increased steadily between 1963 and 2012 from 15 879 053 to 41 269 156 tons, respectively, according to FAO statistics.

In the United States, the first people reported eating peanuts were slaves. Peanuts, however, became more mainstream during the pre-Civil War period, with consumption steadily escalating after the Civil War. By the twentieth century, the medical profession convinced Americans that peanuts not only tasted good but were a healthy food. Currently, four different market types of peanuts are cultivated, including Runner, Virginia, Spanish, and Valencia. The Runner types are generally grown in the Southeast (Georgia, Florida, Alabama, and Mississippi). The Virginia types are grown in the VC region (Virginia, North Carolina, and South Carolina). The Southwest (including Oklahoma, Texas, and New Mexico) is the source of Spanish and Valencia peanuts. Overall, in the United States, 83% of the production area was planted with Runner cultivars in 2012, whereas Virginia peanuts were produced on 15%, Spanish 1%,

and Valencia 1% of the production area. The primary use of Runner-type peanuts is to produce peanut butter. Based on the peanut stock and processing report compiled by the USDA National Agricultural Statistics Service (NASS), the primary products of shelled peanuts were 60.6% peanut butter, 19.8% peanut snacks, 18.7% peanut candy (includes peanut butter manufactured for candy), and 0.9% other products from 2010 to 2012.

Botanical Description of Peanut

Early descriptions of the peanut showed drawings/pictures of the peanuts growing on the roots of the plant and had nothing to do with the aboveground stems and flowers. Smith was one of the first to correct this error and described the peanut as an herbaceous legume, annual in growth habit but can be maintained for long periods if the fruits are removed regularly. Rao and Murty presented a technical description of the *Arachis* genus.

The fruits can be very small or quite large, and seed size is directly related to fruit size. The seed has two fleshy cotyledons covered by a testa, or skin, beneath which are several layers of parenchyma cells that include a one- or two-layer integument. At the base of the cotyledons is an embryonic plant with a straight axis, unlike other legumes, with a developed taproot primordia and two to four embryonic quadrifoliate leaves. As the seed germinates, the hypocotyl lengthens to push the cotyledons upward and the taproot down. As the cotyledons reach light at the soil surface, the hypocotyl stops growing and becomes fibrous, drying to a stemlike tissue as the plant ages. Meanwhile, buds on the central axis in the axel of the cotyledons initiate growth to form the cotyledonary lateral branches. The main axis (epicotyl) continues to grow, as do the taproot and secondary root. Two main differences develop at this point to distinguish the two subspecies – *A. hypogaea* ssp. *hypogaea* and *A. hypogaea* ssp. *fastigiata*. The *A. hypogaea* ssp. *hypogaea* will not produce inflorescences on the main axis and the first one to three, usually two, nodes of each cotyledonary lateral will not have inflorescences either. However, subsequently vegetative (V) and reproductive (R) nodes will occur 2×2, in alternating fashion. In *A. hypogaea* ssp. *fastigiata*, nodes of the central axis will/may exhibit inflorescences, and each node of lateral branches will/may also have inflorescences, referred to as sequential branching. Generally, the main axis of *A. hypogaea* ssp. *hypogaea* is relatively short, and those of *A. hypogaea* ssp. *fastigiata* are taller. These branching patterns hold true in primitive germplasm collected in South America, but in modern cultivars that have been intercrossed between the two subspecies, the branching pattern is less consistent.

Inflorescences are generally reduced to less than 2–3 mm in length and under some environmental conditions may grow to as long as 200 mm. Also, some *A. hypogaea* ssp. *fastigiata* types have inflorescences that are 40–70 mm. The number of flowers in an inflorescence varies from four to an observed thirteen.

Flowers are typically papilionaceous, with a calyx tube that varies in length due to genetics and environment (temperature); two calyx bracts, one axial and one abaxial (front and back); a large standard petal and two wing petals on either side of the keel petal that encloses the stigma; and the nine or ten anthers. The anthers are of four types: two sterile, three larger oblong biloculate, one oblong uniloculate, and three or four globose, like a ball on a stick.

The ovary is at the base of the calyx tube on the inflorescence in the leaf axil and has ovules that develop a typical *Polygonum*-type embryo sac containing two polar nuclei, three antipodal nuclei, two synergids, and the egg nucleus. The difference for the peanut ovary is that it can contain multiple ovules. In most modern cultivars, the most common is two or three, but in primitive germplasm, the range is from one to seven ovules per ovary, the larger numbers occurring in the *A. hypogaea* ssp. *fastigiata*. If fertilization is withheld or incompatible pollen is applied, the sexual apparatus degenerates, and apomictic embryo sacs have been observed and documented, but apomictic reproduction has not been confirmed.

The process of development from flower bud through fertilization, embryo development, fruit development, seed formation, and maturation for the peanut is a bit unusual. As a flower bud develops on an inflorescence the day before the flower becomes mature, the calyx tube initiates growth, raising the bud vertically into the air. This growth accelerates so that by sunrise of the day, when the flower opens and the anthers dehisce, the tube is 40–150 mm long. At anthesis, the filaments are elongating, dispersing the pollen the length of the stigma where the pollen grains will germinate and grow down through the style to the ovule(s). Fertilization occurs within 6–16 h after dehiscence, and the zygote will divide two or three times but then become dormant. At that time, a meristem at the base of the basal ovule becomes active and begins to divide and grow, forming what is called the peg. The growth of the peg is positively geotropic and, depending upon the distance to the soil, will grow into the soil a few days later, with the ovule(s) and embryo(s) at the tip of the peg. As the peg continues growth into the soil, it will reach a point where the peg tip is in total darkness, and then, the meristem will position the ovule(s) horizontally and cease growing. When the growth of the peg ceases, the embryo becomes active again and growth continues until it becomes a mature seed. Seed growth and maturation will usually take from 70 to 150 days past fertilization of the egg(s).

The harvested seeds have varying lengths of dormancy, from almost none in some *A. hypogaea* ssp. *fastigiata* types to several months in some *A. hypogaea* ssp. *hypogaea* types.

Nutrient and Chemical Properties

Regular peanut consumption has positive impacts on human health. The nutrient composition is presented in Table 1. Dietary intake of peanut seeds provides a significant source of protein, tocopherol (vitamin E), antioxidants, flavonoids, folate, fiber, and edible oil. Flavonoids such as quercetin, kaempferol, and genistein vary significantly among peanut lines. Resveratrol, a phytoalexin, is a powerful antioxidant reported to have anticarcinogenic properties. The resveratrol concentration varies considerably in different peanut products (e.g., roasted, ground, and boiled peanuts). Factors such as agronomic, postharvest conditions and the degree of roasting can influence chemical composition, as well as sensory properties. Therefore, specific

Table 1 Mean concentration of selected nutrients in raw peanuts

Nutrient	*Amount per 100 g*
Water (g)	6.50
Energy (kcal)	567
Protein (g)	25.80
Total lipid (g)	49.24
Carbohydrate (g)	16.13
Dietary fiber (g)	8.5
Total sugars (g)	3.97
Magnesium (mg)	168
Sodium (mg)	18
Potassium (mg)	705
Niacin (mg)	12.066
Folate (μg)	240
Vitamin E (mg)	8.33

Source: National Nutrient Database for Standard Reference, Release 26, USDA Agricultural Research Service 2014.

nutrients in cultivars produced in different environments and processed for an assortment of food products found in the market will vary.

Cultivated peanuts (*A. hypogaea* L.) are important oilseed crops worldwide, because they typically contain 50% oil in the seed. The majority (~80–90%) of the extracted oil is composed of saturated, monounsaturated, and polyunsaturated fats in the form of three primary fatty acids, palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acids, respectively. The fatty acid composition in the oil extracted from peanuts tends to range between 36% and 80% oleic acid and 2–43% linoleic fatty acid. In general, high-oleic lines are characterized by 74–80% oleic acid (C18:1) and less than 7% linoleic acid (C18:2), whereas normal-oleic peanuts can range from 30% to 60% oleic acid and greater than 10% linoleic acid. In normal-oleic peanuts, the fatty acid composition can vary, depending on genotype, seed maturity, and environmental conditions such as time of year planted, seasonal variation, insect damage, and soil conditions.

The flavor, stability, shelf life, and nutritional quality of peanuts and peanut products are dependent on the fatty acid composition (ratio of saturated, monounsaturated, and polyunsaturated lipids) of the extracted oil. Unsaturated fats, especially polyunsaturated fats, are prone to oxidation over time, leading to the production of aldehydes, ketones, and hydrocarbons with noxious odors and off-flavors in stored peanut products. Peanuts that have a high percentage of oleic acid (monounsaturated Ω-9 fatty acid) and a low percentage of linoleic acid (polyunsaturated Ω-6 fatty acid) in the oil are less susceptible to rancidification, which results in a longer shelf life for stored food products. Therefore, an emphasis from manufacturers and consumers has been placed on breeding peanuts with high oleic acid and low linoleic acid.

Due to the efforts of the peanut breeding program in Florida in the 1980s, the first high-oleic peanuts F435-2-1 and F435-2-2 were identified having oleic levels (~80%) comparable to that found in olive oil (*Olea europaea* L.), and since its discovery, the majority of high-oleic peanut cultivars have been derived from F435 or F435. Studies following the discovery of the F435 high-oleic peanut established that the trait segregated in either a digenic (15:1) or a monogenic (3:1) fashion-dependent on the normal-oleic parental genotype (ol1ol1Ol2Ol2 or Ol1Ol1Ol2Ol2). In most normal-oleic US Runner- and Virginia-type cultivars (predominantly *A. hypogaea* subsp. *hypogaea* var. *hypogaea*), one of the mutant alleles, ahFAD2A, is often fixed in the homozygous state, so that the high-oleic characteristic behaves as if controlled by a single gene pair. Although numerous high-oleic cultivars have been developed whose pedigrees include F435 as the high-oleic donor, elite high-oleic cultivars are still needed to thwart disease and abiotic challenges that exist in different environments. Due to shelf life stability and health benefits, some countries (i.e., Australia, Argentina, South Africa, and Israel) are mostly or solely producing high-oleic peanut products for consumption. Breeding multiple traits of interest, such as disease resistance and other quality traits, into high-oleic cultivars can be time-consuming due to the generation time, phenotyping for the traits of interest, and maintenance of large populations that are needed to obtain desired trait stacking. This can potentially be overcome by selecting lines with the desired genotypes at key loci by using molecular markers, a process known as marker-assisted selection (MAS). MAS has been effectively utilized to select high-oleic lines in large peanut populations.

Nutrient Availability and Absorption

Lipids within peanuts are aggregated in globules that are contained within the cellular matrix of the parenchyma. To fully access this lipid (i.e., emulsify and transport it from the intestinal lumen into the enterocyte), the cell walls must be disrupted. These structural elements are resistant to enzymatic and microbial degradation in the human gastrointestinal tract. Thus, mechanical destruction by chewing is an important process to access the cellular lipid. The availability of other components, especially fat-soluble nutrients, would be similarly affected.

In part because chewing is not thorough, absorption of lipids from whole peanuts is incomplete. The analysis of fecal fat and energy reveals both are elevated when whole peanuts are incorporated into the diet, relative to fecal losses with a comparable diet containing no peanuts. Additionally, the energy and fat loss is greater compared to when peanut oil, flour, or butter is consumed. Fecal fat increases by just over 5% with whole peanuts and is elevated by 3–4% with peanut butter, oil, and flour. This is associated with an increase in fat loss of just over 5.0 kcal g^{-1} for whole peanuts and 4.4 kcal g^{-1} for peanut butter and products (oil and flour). There is a daily energy loss of about 37 kcal day^{-1} with whole peanuts, 19 kcal day^{-1} with butter and oil, and 1–2 kcal day^{-1} loss with flour. The fat loss represents just over 50% of the energy loss, suggesting losses of carbohydrate and protein also occur. With very high intake of peanuts, fat losses may be greater. In one trial providing peanuts at a level that provided 95% of dietary fat energy, the percent daily dietary fat excretion approached 18%, with values for butter and oil at 7% and 4.5%, respectively.

The composition of the diet excluding peanuts also influences the energy absorbed from the peanuts. High fiber content reduces energy absorption, because the fiber binds with fatty acids in the intestinal lumen, leading to increased

excretion. In one trial, the increment in fat excretion amounted to 1% with whole peanuts, 2.8% with peanut butter, and 2.7% with peanut oil. The food matrix in which peanuts may be embedded also modifies chewing behavior, which alters the distribution of resulting peanut particle sizes and, as a result, lipid bioaccessibility.

Processing of peanuts may alter the energy yield as well. Roasting makes the peanut more brittle, so when chewed, it will be more likely to fracture into smaller particles. This would be expected to enhance the efficiency of energy and nutrient absorption. However, roasting also alters sensory properties, and in one trial, this led to reduced chewing and larger particle sizes in the bolus about to be swallowed. Flavors added to peanuts (e.g., salt, sugar, and spices) alter the palatability of peanuts and modify oral processing time and effort as well. The addition of salt and a sweetener (honey-roasted) may reduce chewing time, resulting in larger particle sizes before swallowing. Collectively, it is clear that processing practices differentially alter energy availability from peanuts, but data are too incomplete to predict the net effect of these multiple influences.

Metabolism

Incorporation of peanuts in the diet may increase energy expenditure. This does not occur via increased physical activity or an increase in the thermogenic effect of feeding, the energy required to digest and utilize food energy. Rather, they appear to increase resting energy expenditure. Daily consumption of peanuts (500 kcal day^{-1}) for 19 weeks raised resting energy expenditure 11% in one study. Another trial reported that daily consumption of peanut oil in a shake that provided 30% of daily energy requirements led to a 5% increase in resting energy expenditure among overweight individuals, but not lean individuals. The increment was 11% among males. While the increment in resting energy expenditure is small, because this component accounts for approximately 75% of daily expenditure, small changes are of potential importance. A mechanism for this effect has not been identified.

There are data, not uncontested, indicating that there is a hierarchy wherein unsaturated fat is preferentially oxidized relative to saturated fats. An early study noted greater satiation effects with polyunsaturated fats than with other types of fats. This prompted speculation that the rapid oxidation of unsaturated fats generates a satiation signal. Because peanuts are a rich source of both poly- and monounsaturated fatty acids, it was hypothesized that this would contribute to their high satiety value. However, this has not been substantiated. At present, the satiety value of peanuts is attributed to the synergy between its chemical and physical components.

Impact on Appetite and Obesity

Epidemiological data generally reveal an inverse association between nut consumption and BMI or body weight. Often, the peanut contribution to these findings cannot be determined. Studies measuring the effect of including peanuts as part of a moderate-fat diet for the purpose of weight loss report similar weight declines between individuals consuming peanuts and moderately reducing fat intake and those who reduce dietary fat intake to a greater degree, but did not include peanuts in the diet. The peanut consumers also had improved blood lipid profiles, while less robust declines were noted in the group not consuming peanuts.

Because snacking is a large portion of energy intake in the United States and elsewhere and peanuts are frequently consumed as snacks, it is important to determine their influence on energy intake and body weight when eaten in this manner. The limited available data indicate peanuts consumed as a snack do not have any differential impact on appetitive ratings and energy intake when compared to peanuts ingested as part of a meal. However, they tend to promote more precise dietary compensation when consumed as a snack. That is, individuals eat somewhat less energy over the day when peanuts are consumed as a snack compared to when they are included as part of a meal. Compensation scores of approximately 50% are reported when peanuts are consumed with a lunch meal, whereas this improves to 74% when peanuts are eaten as an afternoon snack.

Strong dietary compensation is also observed when peanuts are provided with no dietary recommendation about when they are to be consumed during the day. In one trial, participants given approximately 500 kcal day^{-1} peanuts compensated for two-thirds of the energy the nuts provided. That is, when peanuts were incorporated into the diet, roughly 330 kcal of the usual intake was omitted. Due to this compensation, weight gain was minimal. A weight gain of 1.0 kg was observed, while the predicted gain was 3.6 kg. Other studies have reported dietary compensation values ranging from roughly 50% to greater than 100%. Peanut oil alone elicits a weaker, yet still significant, effect on compensation. Approximately 46% of its energy was offset by reduced intake at later eating occasions. Thus, other components of the whole nut contribute to the compensatory dietary response.

The high satiety value of peanuts is likely attributable to multiple properties. They are high in fiber, which enhances satiety by multiple mechanisms including activation of stretch receptors by augmenting gastric distention and slowing gastrointestinal transit. Peanuts are also a good source of protein and fat, and these nutrients stimulate the release of gut peptides associated with satiation. Additionally, as a solid food, peanuts require greater masticatory effort, which may contribute to satiety.

Collectively, consumption of peanuts in moderation does not have a major impact on energy balance and thus weight gain due to three main factors: Approximately 55–75% of energy from nuts is offset in the diet by reduction of energy intake of other foods after peanut consumption, another 10–15% of the energy contained in peanuts is excreted in the feces due to its low bioaccessibility, and approximately 10% of their energy is hypothesized to be lost as a result of increases in resting energy exposure following frequent peanut consumption.

Impact on Glucose Regulation

Peanut consumption may help to moderate blood sugar concentrations and reduce the risk of developing type 2 diabetes.

Individuals consuming five or more combined servings of peanuts and tree nuts per week reduced their risk for developing diabetes by 27%, and those consuming the equivalent amount of peanut butter had a reduction of 21%. The moderating effect of whole peanut and especially peanut butter consumption on glycemia was recently documented in a trial demonstrating that the inclusion of peanut butter in a high-glycemic-index breakfast meal significantly reduced the excursion of blood sugar after the morning and midday meals relative to the breakfast without added peanut butter. The effects were of slightly lower magnitude with whole peanuts, but consistent with responses to the peanut butter. Over the approximately 8 h study period, the incremental area under the blood glucose curve was 18.7% and 14.4% lower for the peanut butter and whole peanut treatments compared to the breakfast without peanuts.

The components of peanuts responsible for the glycemia-moderating effects have not been established but are hypothesized to include their high total lipid, unsaturated lipid, protein, fiber, and phytochemical contents. All could slow gastric emptying of carbohydrate and its rise in the circulation. Additionally, peanut products in the intestinal lumen may stimulate the release of gut peptides such as GLP-1 with incretin effects; that is, they enhance insulin release and activity. Likely because the lipid and other nutrient contents of whole peanuts are not fully bioaccessible, ground peanut (butter) shows even greater effects on glycemic control than whole nuts. Studies with mixed nuts, though not including peanuts, eaten with meals indicate the reduction of glycemia is dose-dependent and occurs in normoglycemic and diabetic individuals.

Impact on Cardiovascular Health

Because cardiovascular disease is the leading cause of death in developed countries and will be in developing countries in the next few decades, the benefits of nut consumption on the cardiovascular system have been well studied in both epidemiological and interventional studies. Epidemiological evidence consistently reveals that individuals with the highest mixed nut intake have a roughly 35% reduced risk of coronary heart disease as a result of improved blood lipid profiles and vascular reactivity. The contribution of each nut type in such studies is difficult to ascertain; however, an independent effect of peanuts has been demonstrated. Increasing peanut consumption was associated with reduced risk of coronary heart disease in women enrolled in the Nurses' Health Study. Participants consuming peanuts at least twice a week were 43% less likely to develop heart disease. Trends linking intake of other nuts with a decreased incidence of heart disease were less robust when peanut consumption was removed from analyses.

In an 8-week free-feeding intervention trial, serum triacylglycerol concentrations decreased by up to 20% in healthy participants consuming 500 kcal of peanuts daily; total cholesterol in the same study decreased by 7.2%. Other studies report lower, but significant, decreases in serum triacylglycerol concentrations, for example, 13%. Another study, comparing the health effects of daily consumption of peanuts in various physical forms over 4 weeks, revealed a significant increase in high-density lipoprotein (HDL) cholesterol, from 530 mg l^{-1} at baseline to 560 mg l^{-1} across the peanut forms. This study also noted that individuals with initially elevated cholesterol concentrations had significantly greater decreases in serum low-density lipoprotein (LDL) cholesterol and total cholesterol than individuals with lower resting concentrations.

The component of peanuts responsible for lipid lowering is not well characterized. Reductions in LDL cholesterol following frequent peanut consumption are greater than expected given the fatty acid profile of peanuts alone. Trace minerals may contribute to lipid lowering and cardiovascular disease risk. Additionally, peanut consumption increases dietary intake of magnesium, folate, alpha-tocopherol, and copper, all of which have been associated with a decreased risk of cardiovascular disease. In particular, alpha-tocopherol is thought to act as an antioxidant that can inhibit the oxidation of LDL cholesterol and thereby mitigate its promotion of atherosclerosis. Folate reduces homocysteine levels, a risk factor for coronary heart disease. Magnesium is required for lipase activity, promoting lipid clearance from the circulation, and it helps reduce platelet aggregation. Copper can prevent lipoprotein oxidation and maintain enzymes crucial for preventing free radical formation in the circulation.

Recent work indicates that daily consumption of peanuts may also lead to a decrease in blood pressure. In a 12-week intervention where participants consumed 42 g of peanuts daily, diastolic blood pressure decreased by approximately 2.0 mmHg. The proposed mechanism of blood pressure reduction with peanut consumption draws on their high arginine content. Arginine promotes increased circulating levels of nitric oxide, and this relaxes smooth muscle and improves blood flow.

Peanut Flavor

Realization of the health benefits attributed to peanuts requires their regular consumption. Implementing such a dietary change is potentially problematic as regular consumption of a single food may lead to monotony and rejection of the item. To combat this problem, peanuts are marketed in a variety of flavors (e.g., salted, honey-roasted, and spicy). Recent work indicates that the palatability of peanuts offered as either a single flavor or varied flavors is well maintained over a 12-week period. Further, the health benefits were not compromised by the addition of the flavors. Concerns have been expressed that the addition of salt may exacerbate hypertension, sugar can contribute to wider swings of blood sugar and contribute to obesity or diabetes, and spices may enhance stress responses. However, no such adverse effects have been documented. Indeed, as noted earlier, blood pressure was significantly reduced after 12 weeks of peanut consumption, regardless of the addition of salt or other flavorings to the peanuts. The absence of deleterious health effects is likely attributable to the low concentration of flavor ingredients actually added to the peanuts. Because they are on the surface of the nut, they have a strong sensory impact, so small quantities can impart strong flavor sensations.

See also: Bioavailability of Nutrients; Cashew Nuts; Hypertension and Diet; Legumes in the Diet; Nuts: Health Effects; Obesity: The Role of Diet; Snack Foods: Role in Diet; Snack Foods: Types and Composition.

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Peas and Lentils

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Introduction

Legumes are a food group consisting of nearly 19 000 species consumed by both humans and animals due to their low cost, high protein contents, complex polysaccharides, and minerals. However, legumes also contain other substances that may lower its nutritional value, such as enzyme inhibitors, oxalates, phytates, and polyphenols. Mediterranean countries in particular are noted for their high consumption of legumes in the form of beans, chickpeas, peas, and lentils, usually available in two forms – dry and overcooked. Furthermore, lentils in particular are highly valued in the countries of Southern Europe. All varieties that are grown and produced in these countries are of the species *Lens esculenta*.

About a third of the lentils produced worldwide is in India and consumed in the Indian domestic market. The biggest consumers are in Asia, North Africa, Western Europe, and parts of Latin America. Canada is the largest exporter of lentils in the Americas, with the main production region being Saskatchewan. The Palouse region of Eastern Washington and the Idaho Panhandle region, with their collective commercial center in Pullman (WA), constitute the largest lentil-producing region of the United States. Algeria and Egypt are the largest importers of lentils, followed by Bangladesh, Sri Lanka, India, and Pakistan. In Europe, the largest importers are Spain, France, Italy, and Germany. Finally, in Latin America, the countries of the Andean Community and Brazil all imported about 140 000 tonnes of lentils.

The FAO reported the world production of lentils in 2008 at 3.874 million tonnes, with the largest producer being India (with 29.0% of global production), followed by Canada (24.5%), Turkey (14.5%), Australia (5.7%), the United States (4%), and China (3.9%), together accounting for 81% of the global production of lentils. The National Agricultural Statistics Service (NASS) reported that in 2007, US production was 154 500 tonnes in North Dakota, Montana, Washington, and Idaho combined. Canada estimated its 2009–10 production to be a record 1.5 million tonnes.

In the case of peas, they are produced in almost every country in the world. Canada is the largest producer (about 25% of total world production) and the main exporter (40% of total world exports) of dry peas in the world. More than half of all Canadian pea production is exported as seed without subject to any processing. About 10% of the peas produced are used as seed for crops. Whole and cracked seeds are also used for stews and soups and as canned food. The cover is added to breads rich in fiber; however, most peas become animal feed. The largest use of dried peas in Europe and North America is in the food industry, where whole seeds are ground and mixed with cereal flours to produce food products. The main use of peas in Asia and South America is for human consumption, where the whole or cracked seeds are first boiled and then eaten.

Carbohydrates

The concentrations of total carbohydrates found in lentil flour and pea flour are between 625 and 657 mg g^{-1} dry matter with a higher concentration in peas than in lentils. Among these carbohydrates, starch stands out as the major component, with percentages of between 59.1% and 53.6%, respectively, of the total carbohydrate content. However, the digestibility of this starch is affected by many factors and is generally low. Among these factors, we can include firstly granule size, which is mainly defined by the relationship between the surface area and volume. As the grain size increases, the contact between the substrate and amylases decreases and thereby the rate of enzymatic digestibility ($r = 0.791$, $p < 0.05$). This result is consistent with the fact that peas, with an average granule size of about 20 μm, present a lower digestibility than lentils with starch granule diameters of about 17.5 μm.

A further factor that also influences the digestibility of starch is the degree of crystallization of the granule. Starch consists of amylose chains, a linear polymer made of glucose units linked through α-1,4, and amylopectin chains and a branched polymer consisting of glucose units linked by 1,4 bonds α-bonded to the branching main chain by α-1,6 glycosidic bonds. These chains may be in the amorphous phase or the crystalline phase. The higher the content of amylopectin in the structure of the starch granule, the higher the crystallinity ($r = 0.914$, $p < 0.01$). Similarly, the presence of amylose reduces this crystallization degree ($r = 0.775$, $p < 0.05$). It is expected therefore that the existence of a higher crystallinity in starch granules will decrease the rate of enzymatic digestion by hindering access to glycolytic enzyme substrate. Accordingly, several studies have shown an increased presence of amylose in peas and lentils, which justifies its rapid hydrolysis.

This starch digestibility can be improved by cooking treatment. Thus, it has been observed that the digestibility can alternate between 37% and 42% in the case of raw legumes, 70–77% for the standard cooked variation (100 °C for 90 min), and even 87–91% with autoclaving at 121 °C for 10 min. However, it is not apparent that increasing the cooking time leads to enhanced starch digestibility. This improvement in the digestibility of starch could be attributed to hydrolysis as a result of heat treatment and the destruction and removal of antinutritional substances, such as phytic acid and tannins, thus creating more space in the matrix, increasing the susceptibility to enzymatic attack. Heat treatment can also decrease the percentage of resistant starch from 2.4% to 1.9% for peas and from 3.3% to 2.5% for lentils. The fraction of starch that is not hydrolyzed by amylases after approximately 2 h resists enzymatic digestion and becomes part of the dietary fiber. Germination treatment can also decrease the resistant starch content by reducing the concentration of α-amylase inhibitors.

Fiber

Dietary fiber is a concept widely used to refer to a complex mixture of indigestible polysaccharides including cellulose, hemicelluloses, oligosaccharides, pectins, gums, waxes, and lignin commonly found in the cell walls of plant cells. The properties of dietary fiber in relation to human nutrition are associated with distinct physiological responses including viscosity, water-holding capacity, fermentability, bulk, and the ability to bind bile acids.

Dietary fiber can be classified into two groups based on solubility in a pH-controlled enzyme solution, each one with distinct physiological properties and nutritional benefits. Insoluble fiber is mainly related to intestinal transit, thereby improving laxation. In addition to this, insoluble fiber is fermented in the large intestine by promoting the growth of flora in the intestine (prebiotic effect). The formation of short-chain fatty acids originating from the fermentation process can also prevent the development of colon tumor cells. Moreover, the soluble dietary fiber can help to lower blood cholesterol (hypocholesterolemic effect) and regulate blood glucose levels. Several human studies have shown that the inclusion of pea fibers can decrease blood glucose peak. Pea fibers have been also used in the treatment of hypercholesterolemic patients.

The total dietary fiber content ranges between 180 and 350 g kg^{-1} for peas and 90 and 300 g kg^{-1} for lentils. Within this content, the highest percentage is represented by the insoluble fraction (about 70% of total dietary fiber) comprising cellulosic glucose, poor lignin, and xyloglucans (predominant hemicelluloses in dicotyledonous plants). Arabinoses (also known as rich pectins) are abundant in the soluble group of dietary fibers.

Another important group of compounds of dietary fiber also found in significant concentrations in lentils, peas, and other pulses is α-galactooligosaccharides, also known as the raffinose family oligosaccharides.

Finally, with regard to simple carbohydrates, the following sugars have been identified in the flour of lentils and peas: monosaccharides (0.085–0.124 fructose, 0.042 glucose, and mainly 0.722 galactose and 0.305–0.521 ribose g/100 g) and disaccharides (0.647–0.697 sucrose, 0.039–0.191 maltose and 0.159 melibiose g/100 g). Saccharose concentrations were also analyzed with results between 20.8 and 31.9 mg g^{-1} in dry matter. Also found in oligosaccharides is alpha-galactoside and within this, the family of rafinosas. But in this last group, the links between monosaccharides are produced by links α -1,2, which cannot be digested by humans, becoming part of dietary fiber as discussed in the succeeding text.

These carbohydrates are characterized by intestinal gas production in humans, that is, cause flatulence consumption, because the gastrointestinal tract does not synthesize α-galactosidase, the enzyme acting on these oligosaccharides. High concentrations of raffinose, stachyose, and verbascose were found in raw lentils (RL).

Processed lentils and peas can reduce the concentration of these oligosaccharides responsible for flatulence. Soaking them for 3–12 h can show reductions of up to 75% of content. Thus, it appears as if soaking can reduce the raffinose family oligosaccharides and ciceritol by 75% in cooked lentils and peas soaked for 3–12 h. Furthermore, cooking also reduces levels of saccharose, raffinose, and stachyose. Germination can also cause a decrease in these substances that results in an increase in the activity of alpha-galactosidase. It has been shown that an addition of commercial α-galactosidase enzyme or endogenous action to pea flour and lentil flour reduces the same raffinose, stachyose, and verbascose substantially (from 41% to 100%), as well as ciceritol in lentils.

It has also been shown that both culture and the environment have an effect on the sugar content of lentil seeds. Thus, different concentrations of raffinose family oligosaccharides between cultivars may be due to differences between biosynthetic enzyme and raffinose family oligosaccharides. However, there is so far no comparative study. It has been observed that the total concentration of lentil-cultured raffinose family oligosaccharides may also vary according to different environmental conditions. According to the results shown, it can be ascertained that lentils, which receive less precipitation, show higher content of raffinose family oligosaccharides compared with those who received more precipitation in their environment. However, no conclusive correlation between the surface area in which they were grown and the concentration of raffinose family oligosaccharides was obtained.

Proteins

Legumes have a high protein content, with lentils recording between 22% and 31% and peas between 26% and 32% (expressed as dry matter). Yet, despite this high protein content, its quality is not as high as proteins of animal origin. It should be noted that there are factors that influence the lower digestibility of these proteins, such as the presence of protease inhibitors and very compact quaternary structures, which make difficult the action of enzymes. Similarly, some authors observe the existence of noncovalent interactions or disulfide bonds between the proteins as being associated with the fraction of insoluble dietary fiber.

Another factor that affects the quality of protein in peas and lentils is their deficiency in many essential amino acids. With regard to the content of amino acids, it has been found that RL and peas are rich in aspartic acid, glutamic acid, asparagine, and arginine. However, as with other legumes, they are deficient in sulfuric amino acids, such as methionine, cysteine, and tryptophan. Therefore, the combination of vegetables (which contain a high proportion of lysine compared with cereals) and foods rich in sulfuric amino acid, such as cereals, is a common practice that provides all essential amino acids. This combination increases the nutritional quality of protein available so that it is similar to the values found in animal proteins.

As is the case for starch, the nutritional value of these proteins can also be increased during culinary preparation, because protease inhibitors are inactivated by heat and other structural changes occurring that facilitate access to the enzymes, thus promoting digestive absorption of proteolysis and nitrogen compounds.

Germination treatment may also increase the protein content of free amino acids depending on the circumstances in which it is conducted. With respect to lentils, it has been observed that in the presence of light during germination, higher amounts of free amino acids and protein–protein

amino acids may occur (with some exceptions), which would not be found if germinated in darkness. However, in peas, the opposite effect has been observed. It has also been found that germination time affects the content of free amino acid proteins in legumes differently. Thus, a study shows that in lentils, germination time primarily increases the content of serine, asparagine, glycine, threonine, proline, and valine. In peas, the amino acid contents that are increased over time are proline, histidine, and tyrosine. In both peas and legumes, the content of other amino acids, such as lysine, over time decreases during germination, in both the presence and the absence of light. These changes in the amino acid content during germination could be related to the hydrolysis of proteins, synthesis, and reorganization.

Germination involves the mobilization of reserves of protein in the cotyledons, along with the synthesis of new proteins for shoot growth. The new proteins can be composed of various amino acids stored in protein, altering the amino acid content pattern with respect to the raw legume. Furthermore, amino acids produced by the hydrolysis of protein reserves are not only used exclusively for the synthesis of new components but also used as an energy source, especially in the early stages of germination.

Furthermore, the results indicate that bleaching, sterilization, and storage of peas show no effects on the protein content, compared with that of fresh peas. Nor are losses observed in the total protein content in bottled or canned peas stored under different conditions (10 °C and room temperature) for a period of 6 months. However, a slight variation is produced in the total amino acid in legumes. The results suggest that Maillard reactions or other reactions occurred, including hydroperoxides formed from occurring unsaturated fats.

Lipids

As with many plant foods, lentils and peas have a low fat content ranging from 0.7% to 6.1%. When it comes to the lipid profile of RL, mono- and polyunsaturated fatty acids – unsaturated oleic, linoleic, and linolenic – stand out at a rate of about 20%, 38%, and 7%, respectively, compared with the total of fatty acids. The contents of saturated fatty acids, more atherogenic potential, are about 0.5%, 18%, and 2% for myristic, palmitic, and stearic fatty acids, respectively. Similarly, a fatty acid profile present in raw peas has a content of 13% oleic acid, 35% linoleic acid, and 5.5% linolenic acid. The percentage of saturated fatty acids is approximately 22%, highlighting palmitic acid (14%). In the case of canned peas, with a heat treatment applied prior to being cooked, linoleic acid remains the principal fatty acid.

In some varieties of peas, it has been observed that this fatty acid profile may vary depending on the size of the seed. In the early stages of growth (peas with a size of between 4.7–7.5 mm and 7.6–8.2 mm), the ratio of unsaturated to saturated fatty acids increases slightly due to a series of changes in enzyme systems involved in the biosynthesis of fatty acids. Afterward (pea size of 8.3–8.8 mm), this ratio stabilizes and finishes by declining sharply in the final stage (pea size of 8.9–10.2 mm). Accordingly, some industries order these peas for the manufacture of flours, as this latter size would be the required size in order to decrease the susceptibility to autoxidative deterioration.

The content of fatty acids increases during storage because of the lipolysis of triglycerides, polar lipids, and sterol esters, although a higher temperature of storage favors all these processes. It has been observed in red lentils that the percentage of free fatty acids can reach up to six times its original levels within 16 weeks at a storage temperature of 40 °C, while storage temperatures at 10 or 20 °C show no increase in fatty acids. Meanwhile, the moisture content of lentils also influences this lipolysis of fat as a decrease of moisture content from 17.5% to 12.5% increases the content of free fatty acids by a factor of 3, rather than 6 at 40 °C. Furthermore, the material in which they are packaged also changes the conditions of all these aforementioned processes. When packaged in glass jars, the concentration of free fatty acids is significantly higher than in a container of lacquered tinplate. While this influence of packing material has not been sufficiently studied, it is possible that the transparent nature of the glass containers favors the action of light on lipolysis from fat.

Trace Elements

Many legumes, such as lentils and peas, have relatively high levels of phosphorus, magnesium, iron, zinc, and copper. However, the mineral bioavailability of pulses is generally poor, as a result of its low digestibility, high content in dietary fiber, or antinutritional components, such as phytic acid, oxalates, and polyphenols, all of which can interfere with mineral absorption. Thus, an *in vitro* study to determine mineral bioaccessibility, using a simulated gastrointestinal tract, found iron dialyzability percentages of 2.6% for chickpeas and lentils. In the case of zinc, zinc dialyzability percentages were better, being 32.2% and 48.5% for chickpeas and lentils, respectively.

Phytate is the major storage form of phosphorus in legumes. The inhibitory effect of phytate on the absorption of minerals in the small intestine is due to the formation of insoluble mineral–phytate complexes. It has been found that phytate concentrations ranged between 0.06 and 0.33 g per 100 g for peas and between 0.08 and 0.30 g per 100 g for lentils, which causes high content but is still however lower than those found in beans (between 0.34 and 0.58 g per 100 g). However, the negative effect of phytic acid on mineral bioavailability depends on the amount of phytic acid present in the legume as a molar ratio of phytate/mineral. Phytate/zinc molar ratios below 5 are designated as high zinc availability, ratios within the range of 5–15 as moderate availability, and ratios above 15 as low availability. In the case of iron, phytate/iron molar ratios are recommended as being lower than 1 for good bioavailability. A phytate/mineral molar ratio for zinc and iron of around 18 and 2.1 in lentils and 22 and 6.5 in peas, respectively, has been reported, which is in accordance with the low rates of mineral bioavailability found in these legumes.

Oxalate salts are very insoluble at intestinal pH, and it has been widely documented that oxalic acid decreases the absorption of minerals in monogastric animals. The oxalate content of cooked peas ranged between 6 and 4 g per 100 g and in

lentils, around 8 g per 100 g. Although the concentration of antinutritional substances is again lower than those determined in other legumes such as beans (85–26 g per 100 g), also, it is expected that this oxalate content affects the decrease in its bioaccessibility in the intestinal lumen.

Peas and lentils are also rich sources of polyphenols – natural antioxidants that can replace synthetic antioxidants used in food. It has been observed that the seed coat of lentils has a higher content of flavonoid compounds than cotyledon that is primarily rich in nonflavonoid compounds. The ability of the polyphenols to decrease mineral bioavailability has been studied mainly with regard to iron and calcium. It has been observed, however, that not all polyphenols have the same capacity to reduce mineral absorption. Thus, several studies suggest that flavonoids and their polymers do not interfere with iron absorption in humans, whereas polyphenols with a high galloyl ester content exhibit a potent inhibitory effect. In this sense, it can be seen that these areas of study need to be further researched.

Different studies have evaluated the effect of a series of treatments such as soaking, cooking, extrusion, and germination to reduce the inhibitory capacity of phytic acid, oxalate, and polyphenols on mineral bioavailability. These treatments can promote or inhibit the development of phytases, significantly affecting the hydrolysis of phytic acid.

One study shows that during the soaking, all phenolic components identified in peas and lentils decrease drastically. This decrease was due to leaching of water-soluble phenols into the soaking water. Likewise, traditional cooking of lentils (cooking by boiling (the ordinary method)) can reduce the concentration of tannins and phytic acid by up to 25%. Cooking lentils in the autoclave results in a reduction of 33–46% for tannins and 28–52% for phytic acids, with losses increasing in line with increased cooking time (10–90 min) and increases in temperature (121–128 °C). Meanwhile, the germination of lentils and peas increases the activity of endogenous enzymes (such as hydrolases, polyphenol oxidases, and phytases) resulting in a reduction of phenolic compounds and degradation of phytic acid forms with a lower content of phosphate groups, as inositol tri- and tetraphosphates with less chelating ability. Various *in vitro* and *in vivo* studies show that these treatments improve the mineral bioavailability of these legumes.

Phytic acid can also be reduced by endogenous phytase activity, which works by breaking inositol hexaphosphate into different forms that possess less mineral chelating ability, thus leading to an increase in iron dialyzability resulting from this enzymatic dephytinization in lentils. Similar results were found for pea flour, with enhanced iron absorption after treatment with exogenous microbial phytase, leading to inositol hexaphosphate levels below those reported by other authors.

A study using an exogenous microbial phytase in optimum conditions (pH 5.5 and 37 °C) shows dephytinization and subsequent removal in the soaking solution after transformation and the effect on iron bioavailability meal in pea (*Pisum sativum* L.). This experiment was conducted on rats by examining the chemical composition of pea flour and the digestive and metabolic utilization of the mineral mentioned.

The rats were fed with three types of diets: raw pea meal, raw pea meal that underwent soaking treatment without phytase, and raw pea meal that underwent soaking treatment with phytase.

Soaking pea flour leads to a considerable reduction in the iron content (33%), while a smaller reduction in the iron content (7%) is observed, associated with a higher total phosphorus concentration that is obtained with an additional phytase treatment. The digestive utilization by growing rats of iron from raw pea meal and soaked peas was negligible but increased significantly as a result of treatment with phytase. The low absorption of iron obtained by the first two dietary treatments during an experimental period of 10 days was not reflected in any hematologic indexes (red cell count, hemoglobin, and hematocrit) or tissues (the femur, heart, and kidney) studied, with the exception being the sternum.

Finally, an *in vivo* study using growing rats observed a significant increase in digestive calcium utilization as a result of lentils being germinated for 6 days.

A total of 40 rats were divided into four groups of ten, and each group was fed with one of the four types of diets: control, diet containing casein (20%) + methionine (0.5%), the tested diet with raw lentils (RL), and diet with dry-heated lentils (HL) and germinated lentils (GL).

It has been reported that diets containing lentils provide a lower quality of the protein casein, due to lentils being deficient in sulfuric amino acids. The macropeptides that may be formed during consumption reduce calcium absorption. Therefore, a low absorption of calcium in sprouted lentils was expected, because the protein quality is lower when compared with RL or HL. However, this increased absorption can be explained due to the possible effects of germination on other factors such as fiber, phytic acid, and oxalic acid.

The results show a drastic drop in hemicellulose. This indicates that it is possible to achieve maximum absorption of calcium when lower hemicellulose content in the diet was found. Also, the content of inositol hexaphosphoric acid or phytic acid (IHP) was decreased compared with HL. It is possible that this decrease plays an important role, since the absorption of calcium in sprouted lentils was 20% higher than in animals fed with RL. Furthermore, rats fed with GL also showed a major transformation of phytic acid. It has been shown that calcium bioavailability is related to the levels of phytic acid. In GL, the transformation of phytic acid increased significantly, which can be translated into a greater total phosphorus content, expressed as a percentage of total phosphorus present as IHP (% IHP-P/total P), following the reduction of 26% of IHP per g of dry matter. Thus, the results show increased calcium absorption in sprouted lentils (value of 82) that had a lower content of IHP, compared with diets of HL.

It has been shown that the Ca/P ratio affects the absorption of Ca. A low Ca/P ratio observed in RL and HL compared with lentils from the control diet can be a cause of the low absorption of calcium (see **Table 1**).

Vitamins

Different literature sources show lentils and peas as good sources of soluble B vitamins, especially niacin and pantothenic acid. However, they are deficient in vitamin C, carotene, and retinol. With respect to folic acid, although readily

Table 1 Mineral contents (mg/100 g)

	Lentils				*Peas*			
Minerals	*Raw*	*Soaked*	*Cooked*	*Germinated*	*Raw*	*Soaked*	*Cooked*	*Germinated*
Ca	42–97.3	19–69	15.4–50.21	86–94	55–91	85.9	14	NE
P	380–541	203–418	180–462	283–318	295–335	317–365	99	NE
Mg	129	45–115	36–118	NE	115–132	117–129	109–120.5	NE
Zn	3.6–5.11	NE	1.27–3.13	4.77–5.49	2.67–3.68	2.37–3.16	2.71–3.7	NE
Cu	0.852–1	NE	0.25–0.94	NE	0.87–6.33	0.94–5.87	0.181–0.78	NE
K	753–905	295–752	422–520	NE	981	861–909.5	488–599	NE
Fe	7.5–9.02	NE	1.29–2.4 3.3	9.03–11	7.36 1.5–5.2	3.56–4.96	1.29–4.8	NE

NE: Not examined.

Table 2 Vitamin contents (mg/100 g)

	Lentils				*Peas*			
Vitamins	*Raw*	*Soaked*	*Cooked*	*Germinated*	*Raw*	*Soaked*	*Cooked*	*Germinated*
Thiamine	0.427–0.439	0.38–0.412	0.162–0.212	0.48–0.53	0.70–0.78	NE	0.19	0.68–0.78
Riboflavin	0.03–0.41	0.102–0.121	0.056–0.072	0.24–0.49	0.15–0.22	NE	0.056	0.23–0.40
Niacin	0.88–2.06	0.427–0.924	0.405–0.929	NE	2.89–3.15	NE	0.89	NE
Folic acid	0.03–1.5 433	NE	181	NE	101.5	21	24.5–25.1	NE
Vitamin A	17–112 39 IU	NE	8 IU	NE	149 IU	NE	7 IU	NE
Vitamin E	0.33–0.62	NE	0.11	NE	0.3–1.02	NE	0.39	NE
Vitamin C	ND 7	NE	1.5	NE	1.8–40	NE	0.4	NE

occurring in natural sources, the folic acid is not actually available due to binding with other biomolecules to the form complexes.

It has been shown that concentrations of thiamine, riboflavin, and niacin can be more changed during processing than before consumption. Study shows a percentage reduction in the content of thiamine (54–62%) and niacin (25–61%) during soaking and cooking of lentils and peas, with the percentage of losses being greater the higher the rate of hydration.

However, one study shows that germination produces no significant change in the content of thiamine in lentils and peas, while an increase in the content of riboflavin of between 20% and 135% for lentils and from 60% to 166% in peas is observed – possibly due to an increase in microbial activity.

These changes in the vitamin content of these legumes can be explained by several factors. In relation to losses during soaking, these are easily accountable, based on their solubility in water soaking, with the loss being greater the weaker the strength of its link to cell structures. Accordingly, thiamine (consisting of a pyrimidine ring and a thiazole ring interconnected by a methylene bridge) and niacin (in the form of nicotinic acid and/or nicotinamide) are simpler chemical molecules with a higher water solubility than riboflavin – this is associated with complex molecular structures such as flavin mononucleotide and flavin adenine dinucleotide, which account for the lower solubility. Cooking also increases the loss of these water-soluble vitamins as a result of high rates of leaching due to the high cooking temperatures achieved and the increase in levels of sodium ions (see Table 2).

Antinutritional Substances

Despite the potential nutritional value of pulses as a relatively inexpensive source of significant amounts of protein, carbohydrates, vitamins, and minerals, the use of these as a food source has been limited due to the presence of certain antinutritional factors. These include the presence of phytic acid, condensed tannins, and polyphenols, as discussed in the preceding text, plus protease inhibitors (trypsin and chymotrypsin) and α-amylase inhibitors, which reduce the digestibility of proteins and carbohydrates.

Protease Inhibitors

Protease inhibitors have been extensively investigated in legumes, as they may have an important impact on the nutritional value of these pulses. Protease inhibitors may inhibit the function of digestive enzymes, mainly acting on trypsin and chymotrypsin (it may affect other enzymes although to a lesser extent). This results in affecting the digestive utilization of the protein, causing pancreatic hypertrophy, and instigating weight loss and nitrogen removal in feces.

Although the values of trypsin inhibitor activity were observed in both peas and lentils, these are lower than those in other legumes. Thanks to their character, these thermolabile protease inhibitors may be reduced by standard cooking procedures of seeds, such as soaking, or conventional low-pressure cooking. One study shows how the roasting and boiling of flour,

lentils, and peas affect the activity of trypsin inhibitor. The roasting and boiling processes reduced the activity of trypsin inhibitor; a maximum of 95.6% reduction was found in roasted shelled green lentils, while the lowest (37.8%) was found in the flour of boiled shelled yellow peas. The effects of roasting do not significantly differ from those of boiling, except for red lentils with husks, which had the lowest values of trypsin inhibitor activity after roasting compared with those after boiling.

Furthermore, it has been shown that during the soaking process, certain losses are produced in the trypsin inhibitor activity as a result of the leaching process. In soaked peas and lentils, this treatment can produce a reduction of trypsin inhibitor by up to 15.4%. Variations in the percentage of losses of the inhibitor can be attributed to changes in the permeability of the seed shell that allows antinutritional solubilization of this substance in the soaking liquid. Germination for 72 h can also potentially reduce the occurrence of trypsin inhibitor by between 58% and 76%. However, treatment by extrusion processing produces a greater reduction (about 95%).

Inhibitors (α-Amylase)

α-Amylase plays an important role in the metabolism of carbohydrates, by hydrolyzing the type of linkages between monosaccharides and providing glucose as an energy source for humans and animals. It has been shown that the inhibitor reduces α-amylase digestion of starches, thus reducing postprandial glucose, and increases the circulation of insulin. This can be beneficial in treatments for obesity or diabetes mellitus; however, in other cases, it decreases the nutritional and energy values of these foods.

Soaking was observed to have reduced the activity of alpha-amylase inhibitor. This may be due to leaching in the steepwater. Similarly, germination for 72 h was also found to have reduced the activity of α-amylase inhibitor by up to 48%; this reduction may become a complete extrusion treatment.

See also: Phytic Acid: Properties, Uses, and Determination; Pulsed Electric Fields.

Further Reading

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Pectin and Health

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Sources and Production

In the food industry, commercial pectin is broadly segregated into high methoxyl, a degree of esterification (DE) greater than 50%, or low methoxyl (LM), a DE of less than 50%, and is typically used as a gelling and stabilizing agent in jams, jellies, preserves, confections, and beverages. In the food science literature, pectin is typically described based on solubility. The high DE pectin is water-soluble; low DE pectin is soluble in chelator-containing extractants (CSP); insoluble pectin, also called protopectin, is soluble in dilute acid, dilute alkali, or more concentrated alkali. The latter is likely to include other cell wall fractions such as hemicellulose and xyloglucans. The commercial pectin extraction and isolation process are often tailored for specific functions in foods and beverages. The main commercial sources of pectin are apple pomace, citrus peel and pulp, and sugar beet pulp, which provide an abundant supply of raw materials as a functional or bioactive ingredient in food. However, pectin is ubiquitous in plant material, and alternative sources with unique functional properties are described in the literature. The heterogeneity of pectin structure *in situ* and the more holistic understanding of structure–function relationships of pectin have led to multifaceted research in the area of pectin and health.

Patterns of Consumption

Conventionally, pectin is consumed as an ingredient in food products, most notably jams, jellies, and preserves. Historically, the commercial value of pectin was based on the 'jelly grade' of pectin and categorized based on the gelling mechanism and on the rate of gel formation from ultrarapid set pectin to slow set pectin. More recently, pectin is fractionated by viscosity, charge, and charge distribution, according to use in specific applications, such as stabilizing acidified dairy drinks, stabilizing colloidal dispersions and emulsions, increasing water-holding capacity in gelled foods like yogurts and sour creams, and controlling ice crystallization in low-fat frozen dairy desserts. Pectin is rich in whole fruits and vegetables and is an excellent source of soluble dietary fiber with health benefits. A diet rich in whole fruit and vegetable foods is rich in health benefits associated with pectin and other bioactive ingredients such as phenolic compounds. As a stabilizer or texturizer in food products, there is not likely to be a sufficient amount of pectin to have a quantifiable health benefit in foods. However, commercially extracted pectin is added to further processed foods, such as baked goods, cereals, and beverages, and can favorably influence health outcomes. Pectin is also used as a dietary supplement and consumed in tablet form or topically added to foods. Despite the known health benefits of consuming fruits and vegetables, consumers do not typically consume the recommended daily servings. Further, food products enriched in added dietary fiber, such as pectin, are not as palatable and often not consumed as an integral part of the healthy diet and healthy lifestyle for many consumers. Developing food products that meet the consumers' criteria of taste, cost, and convenience, and still provide health benefits, remains the food product challenge for favorably influencing the consumption of health-promoting, pectin-rich foods.

Availability, Absorption, and Metabolism

Pectin is a water-soluble fiber and is virtually nondigestible during passage through the upper gastrointestinal (GI) tract. Upon entrance to the colon and the diverse microflora and under the anaerobic conditions, colonic bacteria metabolize pectin to generate microbial by-products, including short-chain fatty acids (SCFAs). Despite the limited metabolism of pectin in the stomach and small intestinal tract, pectin exerts an influential, systemic effect by the interaction with other constituents of food and body fluids. These include lowering of lipid and cholesterol levels, lowering serum glucose and moderating the glycemic index, exerting anticarcinogenic activity, and increasing the viscosity and gut transit time, thereby influencing nutrient absorption and, potentially, satiety. Viscous fibers like pectin may be involved in the cell signaling of gut hormones associated with hunger (ghrelin) or satiety (glucagon-like peptide and peptide YY) and potentially regulate food intake. Because pectin passes the upper GI system virtually intact, there are applications of pectin related to the ability to serve as a delivery vehicle to the colon of nutraceuticals or drugs via emulsions or as hydrogels. Finally, although it is less well documented, pectin binding and delivery of phenolic compounds may have a systemic antioxidant effect and protection until delivery to the colonic bacteria. Dietary fiber is defined in several ways, and the conventional definition is based on the solubility or insolubility in water or digestibility or solubility in detergent containing solvents of different pH. Pectin is a diverse polysaccharide, and health benefits are linked with different structural domains in yet undefined variables. Molecular weight, charge and charge domain, substituent sugars, sugar linkages, and the presence of phenolic compounds influence the digestive fate of pectin and subsequently influence the availability, absorption, and metabolism of other nutrients and body fluids. Linking known health benefits with known structural domains would greatly facilitate the fractionation of pectins tailored for specific health benefits.

Health Effects

Structure of Pectin and Health

Pectin is a complex polysaccharide composed of a backbone of α-1,4 linked D-galacturonic acid units, interrupted α-1-2-L-rhamnose inserts, and neutral sugar side chains. Pectins may also be esterified with the methyl ester at carbon six or

acetylated at positions two or three, and some pectins contain ferulic acid and protein. Pectin is typically classified in domains composed of homogalacturonan (HG), rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), xylogalacturonan (XG), arabinogalactan I (AGI), and arabinogalactan II (AGII). In some pectins, such as sugar beet pectin, the arabinose and galactose residues may be substituted with ferulic acid, which, in addition to a substantial amount of protein, contributes to the strong emulsifying activity of sugar beet pectin. RG-I is rich in rhamnogalacturonan, which is substituted with neutral sugar side chains of galactosyl and arabinosyl residues, as single units or as polymers of arabinans, AGI, or AGII. Other cell wall proteins are associated with AGII and are known as arabinogalactan proteins. The RG-II region consists of at least 12 different side chains and more than 20 different linkages and is highly conserved. XG is similar to HG except that it is has branches of single xylose or short β-1,4-linked xylose chain. HG is the predominant domain in commercially extracted pectins. Acidic hydrolysis at temperatures near 60–90 °C for times up to 6 h in the commercial extraction process results in pectin rich in polygalacturonic acid of high molecular weight with less substitution of side chains than possible with more gentle extraction techniques. HG is also called the 'smooth region,' due to the fact that it is composed of the simplest α-1,4-galacturonic acid backbone with partial methyl esterification and acetylation but with few neutral sugar side chains attached. With milder extraction conditions, the neutral sugar side chains and diversity of pectin structure are better preserved and are more reflective of pectin structure *in situ*. While the health benefits of pectin and other dietary fibers are well documented with *in vitro* to clinical trials, the exact nature of the health effects are not well defined. A part of the uncertainty is surely related to the heterogeneity of pectin structure within the cell wall architecture of plant material and the further heterogeneity of pectin structure associated with pilot or commercial extraction techniques. Another contribution to the uncertainty is the void of pectin structural information and incomplete methods on sample preparation, such as rehydration, as part of the scientific literature dealing with health and wellness. Future trials on the health effects of pectin that include physicochemical characterization would greatly facilitate the understanding of the mechanism of the beneficial effects of pectin. In addition, knowledge of pectin domains of structure that have beneficial health outcomes would facilitate the development of pectin fractions with specific, tailored functions. In the development of health-promoting, fiber-rich foods, the product developer is limited in the amount of fiber that can be added and still makes a product that does not adversely affect the palatability. If the bioactive domain of pectin can be identified and isolated from the ballast, then potentially more consumer-friendly, fiber-rich foods can be developed (Figure 1).

Pectin as Soluble Fiber

The primary health benefit of pectin is as a source of soluble fiber. Increased fiber consumption is inversely connected to cancer, cardiovascular disease (CVD), type II diabetes, obesity, and other chronic diseases of inflammation. The

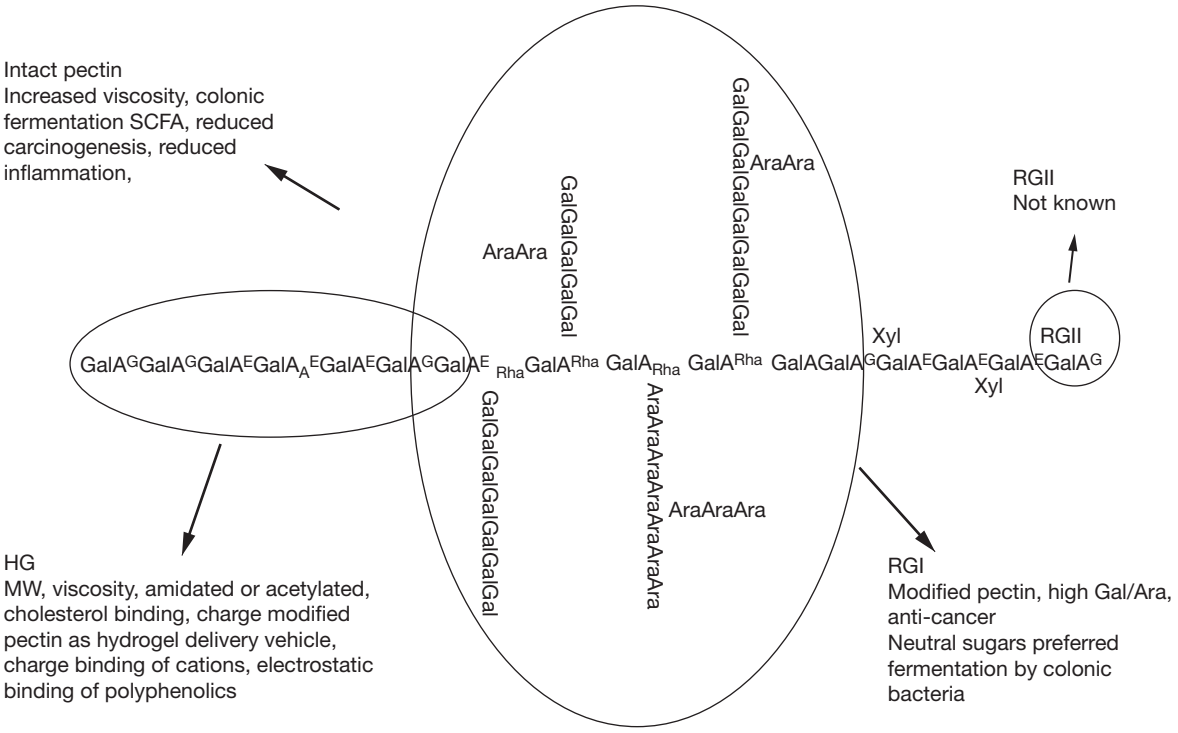

Figure 1 GalA^E and GalA^G and GalA$_A$ refer to esterified, deesterified, and amidated galacturonic acids, respectively. HG refers to the homogalacturonan region. RG-I consists of rhamnogalacturonan backbone with side chains of arabinose, galactose, and other neutral sugars. Only the arabinan and galactan side chains are shown. GalA-Xyl refers to the xylogalacturonan region. RG-II is a highly conserved structure; individual sugars are not shown.

FDA allows two health claims associated with increased fiber consumption. In addition, the health claims for fiber also are dependent on other dietary changes. The FDA recommendation for fiber intake is 25–35 g day^{-1} with 6 g soluble fiber. The health claim associated with anticancer properties also recommends a diet with increased consumption of fruits, vegetables, and whole grains; the health claim associated with decreased CVD has the additional recommendation for a diet low in saturated fat. While soluble fiber, including pectin, is virtually nondigestible in the upper gastrointestinal system, it increases viscosity, increases gut transit time, and influences nutrient absorption in the upper GI. There is some evidence that smaller oligomers of pectin can be absorbed in the small intestine, but the majority of pectin transits to the large intestine for metabolism by the colonic bacteria and production of SCFAs. Additionally, the production of gut hormones and increased satiety by delayed gastric emptying are linked with the increased viscosity of soluble fiber. While pectin and other fiber sources have an associated health claim, it is difficult to elucidate specific bioactivity with specific pectin structures. Pectin itself is a heterogeneous molecule; strong structural information is typically not provided in research studies; isolation processes and subsequent modifications yield unique structures. The next sections deal with pectin's role in preventing cancer and CVD, the impact on fermentation by colonic bacteria, and pectin as a colonic delivery vehicle (Table 1).

Pectin and Cancer

As a soluble fiber source, there are several modes by which pectin influences the development of cancer. The increase in viscosity in the GI system by soluble fiber reduces the contact between the mucosal cells and potential carcinogens. Dietary fiber increases the binding of potential carcinogens and increases the level of antioxidants. The fermentation of dietary fiber in the colon and production of SCFAs have anticarcinogenic properties. Of the studies on dietary fiber and cancer, few deal specifically with pectin to generate significant scientific agreement on efficacy. An exception in the area of pectin and anticancer activity is the binding of pectin to galectins, specifically a prometastatic protein, galectin 3 (GAL3). GAL3 is a small, 30 kD protein, with a carbohydrate recognition domain (CRD) at the C-terminus. It is present extra- and

Table 1 Pectin structural features and potential bioactivity

Structural features		*Bioactivity*	*References*
Intact pectin/dietary fiber		Many	
Homogalacturonan			
Linear galacturonic acid polymer, methyl-esterified, charge domains	Viscosity increase	Increased gut transit time, absorption of nutrients, barrier to antagonists, satiety	Logan et al. (2014), Wicker et al. (2014)
	Charge domain	Cholesterol and bile acid binding	Dongowski et al. (2007), Pau Robot et al. (2010)
	Amidation, acetylation	Cholesterol and bile acid binding via hydrophobic interaction	Dongowski et al. (2007), Pau Robot et al. (2010)
	Electrostatic binding	Charged bioactives, increased systemic anti-inflammatory effect	Fernandes et al. (2014)
	Charge domain	Binds toll-like receptor inflammatory cells, prevents the binding of lipopolysaccharide	LeClere et al. (2013)
Rhamnogalacturonan I			
Highly branched, ramified region, rich in neutral sugars	Preferential fermentation by colonic bacteria	SCFA production	Dongowski et al. (2007), Wicker et al. (2014)
Modified pectin			
Alkali and acid-treated pectin, modified RG-I	Reduced molecular weight, increased charge, RG-I enhanced in galactose, lower in arabinose	Binds GAL3 and inhibits carcinogenesis at multiple levels	Glinsky and Raz (2009), Maxwell et al. (2012), LeClere et al. (2013)
Heat-modified pectin	Reduced molecular weight	Antiproliferation, apoptosis increase	LeClere et al. (2013)
Charge-modified pectin	Increased total charge, increased charge domains, viscosifying, gelling	Delivery vehicle of exogenous bioactives for colonic delivery	Wong et al. (2011), Jung et al. (2013), LeClere et al. (2013)

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intracellularly, and its presence on the cell surface and in the cytoplasm or nucleus will vary according to the type and stage of cancer. GAL3 involvement in cancer is multifold and ranges from inhibition of cell death, regulation of cell growth, differentiation and apoptosis, to stabilization of beta-catenin, which promotes cell migration and invasion. Pectin, and specifically modified pectin, binds the CRD domain of GAL3 and inhibits the ability of GAL3 to promote cell aggregation and migration and to prevent apoptosis. Animal and clinical trials have established the efficacy of this modified pectin, and other modified pectins are available commercially. The pectin modification involves chemical treatment that results in the hydrolysis of the pectin backbone to form oligomers, saponification of the methyl ester, and removal of arabinose from the RG-I domain. The likely result is a small oligomer of pectin that can be absorbed in the upper GI tract, with a galactose-rich RG-I domain that recognizes the CRD on GAL3 and prevents the multiplicity of GAL3 involvement in the development and metastases of various cancers.

Pectin and CVD

Risk factors of CVD include high blood serum cholesterol, hypertension, obesity, and type II diabetes. A diet high in dietary fiber is associated with a reduced risk of CVD. Important effects of dietary fiber include increased bile acid excretion and lowering of total and low-density lipoprotein (LDL) cholesterol. The sources of serum cholesterol are *de novo* synthesis and the diet, with the former accounting for about twice the cholesterol contributed by the diet. Cholesterol is removed from the body primarily by excretion, conversion to bile salts, and synthesis of steroid hormones. Dietary fiber reduces blood serum cholesterol by preventing the reabsorption of bile salts into the enterohepatic circulation, by increased excretion, and by inhibition of cholesterol synthesis in the liver by SCFAs, produced from the fermentation of fiber in the colon. The high viscosity in the digestive tract contributed by dietary fiber may influence cholesterol levels in two ways. High viscosity may result in the formation of larger micelles that are not as effectively absorbed. Also, dietary fiber moderates the glycemic response and less insulin is produced. Less available insulin, as an activator of the rate-limiting enzyme in cholesterol synthesis, results in less cholesterol synthesis.

Pectins with different physicochemical characteristics, such as molecular weight and DE, have varying viscosities and charges. The degree of polymerization (DP) of the HG region and the absence of the methyl ester influence the size and charge, respectively. High-molecular-weight pectin is more viscous than low-molecular-weight pectin, and charge modified pectin forms more viscous to gelled systems. The influence on total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, and bile acid binding varies with the type of pectin, suggesting that pectin structure influences the propensity to reduce CVD. Highly charged, LM, viscous pectin had no effect on LDL cholesterol but increased fecal bile acid excretion. Total cholesterol was decreased more effectively by high methoxyl pectin than LM (higher charge) pectin, but neither had an effect on HDL cholesterol or triglycerides. While the beneficial effect of pectin on markers of CVD is well documented, the absence of physicochemical characterization hinders the elucidation of how dietary pectin minimizes CVD.

Most typically, studies that evaluate pectin and cholesterol-lowering effects report at most the manufacturer specifications of molecular weight and DE. However, it is likely that molecular weight, charge domains, and substitutions influence bile acid and cholesterol binding. When pectins with different structural domains were evaluated for the binding of bile acid or cholesterol, the nature of binding seemed to be hydrophobic. The binding of bile acid was more favorable for pectins with a high DP, high methoxyl, or randomly charged pectin and substituted by amidation or acetylation. The hydrophobic nature of the interaction was confirmed by NMR, showing that cholesterol binding to pectin was at ring A of cholesterol at a hydrophobic pocket formed at uronic residues between C-3 and C-5 on pectin chains.

Pectin and Colonic Bacteria

The human gut contains over 10^{14} microorganisms; the beneficial bacteria and those found in healthy individuals include bifidobacteria, lactobacilli, Firmicutes, *Eubacterium*, and Bacteroidetes. The gut microbiome is a dynamic ecosystem, and the disease state, inflammation state, age, weight status, level of physical activity, and diet influence the colonic flora. Diets high in dietary fiber favorably influence the balance of beneficial bacteria and minimize the etiology of pathogens. The pervading role of the gut microflora in chronic diseases of inflammation and the potential to manipulate the gut flora with lifestyle and dietary changes have catalyzed interest in the structure–function relations of dietary fiber, like pectin, and synergism with other food constituents. Notably, the low bioavailability of polyphenolic compounds, like anthocyanins, is incongruent with the favorable effects that are documented in diets that are rich in anthocyanins. Recent research suggests that many of these polyphenolics that bind dietary fibers, including pectin, are protected during passage through the upper gastrointestinal tract and likely enter the colon to provide local and systemic antioxidative and anti-inflammatory effects during transit.

Pectin as a dietary fiber source is metabolized by the gut microflora and the production of SCFAs, such as propionate, butyrate, and acetate. The heterogeneity of pectin that results from source and preparation methods greatly influences the gut microbes and potential health implications. Parent pectin can be subdivided into fractions that are smaller in molecular weight, are charged, are neutral, have varying ratios of galacturonic acid to rhamnose or varying ratios of galacturonic acid to neutral sugars, have varying amounts of ferulic acid or other phenolic compounds, or are modified to be more linear or branched. All are fermentable, but different fractions show different rates of fermentation and stimulate the growth of different bacteria, but not in a systematic way. In some cases, SBP arabinooligosaccharides with degrees of polymerization greater than eight stimulated *Bifidobacterium* spp. but enzyme-treated potato fiber, with MW values greater than 100 kDa, also stimulated *Bifidobacterium* spp. The presence of phenolics, like ferulic acid, and neutral sugar sides seemed to be more closely associated with rapid and complete fermentation than charge or molecular weight.

Pectin as Hydrogel or Emulsion-Based Delivery Vehicles

Pectin has unrealized potential as a delivery vehicle of hydrophilic and hydrophobic bioactives for protected transit through the upper gastrointestinal system and colonic delivery. The highly charged LM pectin forms gels with cations, such as calcium, via the egg-box structure. Pectin methylesterase (PME) deesterifies the pectin methyl ester to carboxylic acid group, and if sufficient total charge or zones of charge are created, the ionized carboxylic acid groups on adjacent chains of pectin form a gel via an egg-box structure. The pattern of deesterification will vary according to extrinsic conditions such as pH, ionic strength, temperature, and type of PME isozymes and result in variations of multichain and multiattack and single-chain and single attack. Modification conditions and source of enzymes influence the pattern of deesterification; fungal PMEs and NaOH saponification tend to yield random patterns, and plant PMEs tend to form blockwise deesterification patterns. The extent and pattern of deesterification influence the total charge and charge distribution, which in turn influence the propensity to gel with cations, such as calcium. Even within pectins that are LM and at a similar total charge, block deesterified pectins show higher encapsulation efficiency and lower release rates under upper GI conditions than commercially prepared, NaOH-saponified pectins. Whether commercial LM or PME-modified pectin is used to encapsulate addenda, results are consistent that addenda are stable against release during transit through the upper gastrointestinal system. Fermentation by colonic bacteria facilitates the release of addenda and influences the gut flora.

Sugar beet pectin, and to a much lesser extent, citrus and apple pectin, has been studied for the emulsifying activity and encapsulation of bioactives. The entrapment of hydrophobic bioactives in an o/w emulsion, stabilized and protected by pectin during gastric transit, offers excellent potential as a delivery vehicle for colonic fermentation that would be superior to any emulsifiers that are small-molecular-weight amphiphiles or any protein-based emulsifier. SBP is unique to other pectins in that it is acetylated, which minimizes the gelling ability but contributes to the hydrophobic nature and thus the amphiphilic nature of SBP. SBP also contains ferulic acid, within the RG-I domain, which may contribute to hydrophobic interactions at the oil surface. In addition, SBP contains up to 15% protein, compared with 1–2% protein in citrus or apple pectin. The emulsifying activity is thought to reside in the protein moiety of SBP, and it remains the most consistent variable associated with emulsifying activity. However, other intrinsic variables, such as molecular weight and neutral sugar side chains, and extrinsic variables, such as pH and type of oil, contribute to the emulsifying activity and subsequent encapsulation of bioactives, in a yet undefined manner.

Conclusions

As a dietary fiber source, pectin serves a critical role in health and wellness. Despite the heterogeneity of pectin structure depending on the source, extraction methods, and use, pectin contributes to health, not only in sustaining health primarily as a dietary fiber but also in treating disease conditions such as cancer or CVD. Much of the beneficial effect of pectin relies on the viscosity and gelling ability of pectin, which is preserved as much as possible by commercial extraction methods. The identification of specific domains of structure that are more favorable to treating cancers and CVDs, minimizing inflammation, and promoting the growth of specific, beneficial bacteria in the colon are emerging areas of high interest. The latter suggests that much of the dietary fiber relies within small structural domains, and the other portions are ballast. Continued research into the structure–function relationships of pectin would facilitate fuller use of the potential of pectin in health.

See also: Carbohydrate: Digestion, Absorption and Metabolism; Cholesterol: Factors Determining Blood Cholesterol Levels; Glucose: Metabolism and Regulation; Gums: Dietary Importance; Gut Hormones; Obesity: Causes and Prevalence; Obesity: Epidemiology of; Obesity: The Role of Diet; Pectin: Properties Determination and Uses; Phenolic Compounds: Bioavailability and Health Effects; Prebiotics; Probiotics.

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Pectin: Properties Determination and Uses

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Introduction

Pectin is generally considered as a safe food ingredient: according to European Union regulation (EC 1333/2008), it may be used under 'quantum satis' conditions, that is, it has to be used at a level not higher than the necessary amount, according to the good manufacturing practice. The US Food and Drug Administration (FDA) recognizes it as GRAS, that is generally regarded as safe; the Joint FAO/WHO Expert Committee on Food Additives (JECFA) attributed to pectin a 'not specified' acceptable daily intake.

Its name comes from the Greek word 'pektos,' which means firm and hard, suggesting pectin's ability to form hydrogels. Pectin properties are strictly related to its microstructure even though this is not yet completely understood. Two main descriptions of pectin structure are currently proposed: The first one is commonly accepted, while the other is quite new and has been discussed in the last few years. The traditional explanation of pectin structure assumes that the polysaccharide is mainly constituted of α-(1–4)-linked D-galacturonic acid chains (GalA, at least 65% for commercial aims) also called homogalacturonan, interrupted by 1,2-linked rhamnose units. From the rhamnose units, side chains of different neutral sugars branch off, forming the so-called hairy region. A different nomenclature can also be used to identify the traditional structure of a pectin. Thus, *rhamnogalacturonan II* is attached to the main chain of homogalacturonan. They consist of complex side chains attached to the GalA residues of the backbone of homogalacturonan, as shown in **Figure 1(a)**. In the same figure, the structure called *rhamnogalacturonan I* consists of the repeating disaccharide [→4)-a-D-GalA-(1→2)-a-L-Rha-(1→] to which a variety of different glycan chains (principally arabinan and galactan) are attached to the rhamnose unit residues.

According to the alternative description of the pectin structure, homogalacturonan could also be considered as a long side chain of *rhamnogalacturonan I* (**Figure 1(b)**).

It is commonly accepted that the carboxyl groups of the galacturonic acid units are, to a certain extent, esterified with methanol. The degree of methyl esterification (DM) is commonly used to classify pectin into high-methoxyl (HM) pectins, containing more than 50% esterified carboxyl groups (DM > 50), and low-methoxyl (LM) pectins with < 50% esterified carboxyl groups (DM < 50).

Since, in higher plants, pectin normally has the capability of being a hydrating agent and cementing material for the cellulosic network, pectin is widely used for its thickening and gelling properties. Generally, the main sources of pectin are apple pomace and citrus peel, but recent investigations have suggested many other sources for pectin extraction such as sugar beet pulp, mango peel, grapefruit, cocoa husks, palm fruit, gold kiwifruit, and others (see **Table 1**).

Industrial Pectin Extraction

In plant structure, in particular in the wall cell, pectin is present as an insoluble pectic substance or protopectin. Protopectin is a complex structure in which pectin is attached to other cell wall components such as cellulose, hemicellulose, and proteins by covalent bonds, hydrogen bonds, and/or ionic interactions. The residual carboxyl groups are partly neutralized with cations of calcium, potassium, and magnesium, naturally included in the plant tissues.

Pectin extraction is commercially carried out by treating the raw material with hot mineral acid at a pH value of about 2. The process time is determined according to both the raw materials adopted and the type of desired product. The pectin extract, not easily separated from the viscous liquid phase where it is contained, is further clarified through filtration and then concentrated under vacuum in order to provide efficient concentration at low temperatures without damaging the pectin. Powdered pectin is produced by mixing the concentrated liquid with an alcohol, such as isopropanol, methanol, or ethanol. The resulting mass is a gelled material, which is, then, pressed and washed to remove the mother liquid, dried, and ground. An alternative precipitation process uses colloidal aluminum hydroxide, with the main advantage that pectin does not have to be concentrated to be separated from the matrix. The extract is cooled and mixed with a solution of an aluminum salt and ammonia or sodium carbonate to reach a pH of about 4. Pectin flocculates and tends to float on the liquor, and, therefore, it is separated by a screen or by flotation and finally pressed to remove as much as liquor as possible. In order to remove aluminum traces, the pectin mass is suspended in alcohol and treated with acid in a series of stages and then partially neutralized before drying. The final pectin is generally characterized by a DM close to 70; lower values can be obtained either by modifying the extraction conditions or by using a subsequent hydrolyzation by the action of acid. If the hydrolyzation is carried out in the presence of ammonia, some of the esters groups are converted into amide groups, obtaining the so-called amidated LM pectin.

HM pectin is produced in the largest amount (∼62% of the total production in 2011), followed by LM pectins (32%), whereas amidated pectins are a small fraction (only 6%) of the total production.

It is worth noting that acid hydrolysis is a nonselective process; therefore, reducing DM leads to a reduction of chain length. A more selective process could be based on enzymatic deesterification; in fact, it was observed that pectin methylesterase (PME) can reduce the DM without affecting the molecular weight. According to the source of PME (e.g., from citrus fruits or from fungi), a blockwise or a random deesterification can be obtained.

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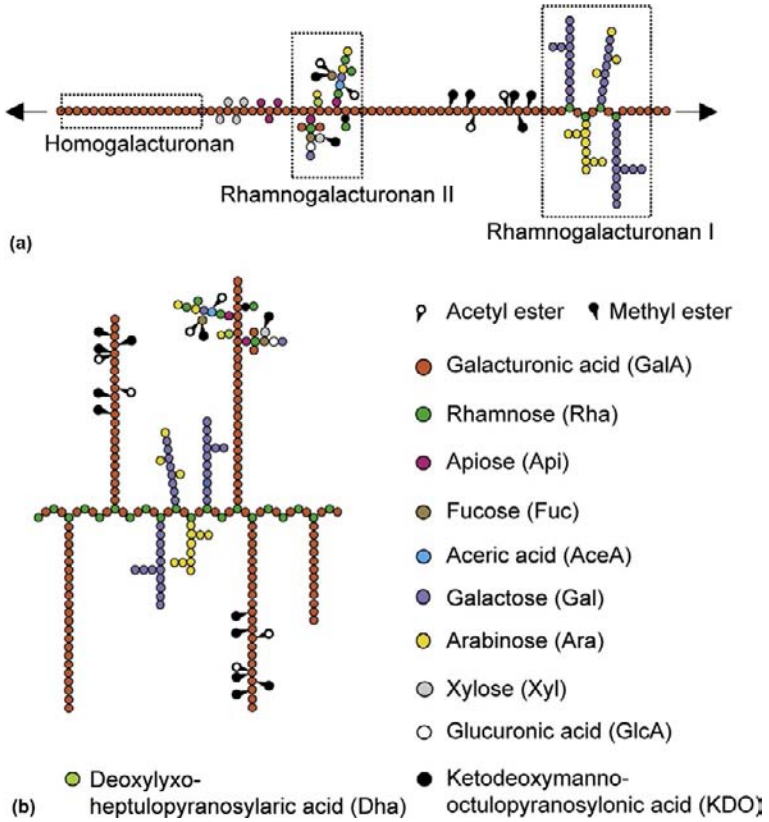

Figure 1 Schematic representations of the conventional (a) and recently proposed alternative (b) structures of pectin. It is important to note that the polymers shown here are intended only to illustrate some of the major domains found in most pectins rather than definitive structures. Reproduced from Willats, W. G. T., Knox, J. P. and Mikkelsen, J. D. (2006). Pectin: new insights into an old polymer are starting to gel. *Trends in Food Science and Technology* **17**, 97–104, with permission from Elsevier.

The improved and deep knowledge about chemical composition of pectin and process conditions for its extraction and also for modifying its structure in order to tune the final properties of gelled systems are enlarging the field of use where this additive can be employed.

Pectin Properties

The main industrial use of pectin is related to the gelling ability of this polysaccharide and to the gelation mechanism that is determined by the degree of methoxylation. LM pectin has a high number of free carboxylic acid groups; they can interact with divalent ions forming strong links between the pectin chains and, therefore, creating the gel network structure usually represented as an 'egg box' (see Figure 2).

Besides the primary interactions, which can be established by pectin chains through the methyl-esterified groups, also secondary interactions among free carboxyl groups should be considered to widely explore the final properties of gels. In fact, according to the literature, three possible kinds of links can be formed in LM pectin: a weak hydrophobic interaction between methyl-esterified groups, hydrophilic interactions between undissociated carboxyl groups and hydroxyl groups via hydrogen bonds, and stronger interactions like ionic linkages with divalent ions.

HM pectin is characterized by a high number of methyl-ester groups: it is generally accepted that the gelation occurs through the cross-linking of the polymer in junction zones, in which not only mainly hydrogen bonds are significant but also hydrophobic interactions between the methyl-ester groups form a high-ester pectin gel. Since the pectin chains are negatively charged, they normally repel each other. A low pH is, therefore, necessary for high-ester pectin gelling because of chain repulsion and the impossibility of forming hydrogen bonds between ionized pectin chains. Moreover, the water activity of the system also has to be lowered to attain sufficient hydrophobic interactions to stabilize the molecular network. Cosolutes like sucrose are usually added for this purpose. Thus, typically, high-ester pectins will only form gels when the pH is below 3.6 and soluble solids are above 55%.

Gelation phenomena depend on biopolymer characteristics (intrinsic parameters) such as polymer molecular weight, galacturonic acid content, degree and pattern of methoxylation, degree of acetylation, and degree of amidation.

Table 1 Pectin content of some fruits

Fruit	*% Pectic substances (wet weight)*
Apple (*Malus* spp.)	0.5–1.6
Apple pomace	1.5–2.5
Banana (*Musa acuminata* L.)	0.7–1.2
Beet pulp (*Beta vulgaris*)	1.0
Carambola (*Averrhoa carambola*)	0.66
Carrot (*Daucus carota*)	0.2–0.5
Giant granadilla (*Passiflora quadrangularis* L.)	0.4
Guava (*Psidium guajava* L.)	0.77–0.99
Lemon pulp (*Citrus limon*)	2.5–4.0
Lychee (*Litchi chinensis* S.)	0.42
Mango (*Mangifera indica* L.)	0.26–0.42
Orange peel (*Citrus sinensis*)	3.5–5.5
Papaya (*Carica papaya*)	0.66–1.0
Passion fruit (*Passiflora edulis* S.)	0.5
Passion fruit rind	2.1–3.0
Peaches (*Prunus persica*)	0.1–0.9
Pineapple (Ananas *comosus* L.)	0.04–0.13
Strawberries (*Fragaria ananassa*)	0.6–0.7
Tamarind (*Tamarindus indica* L.)	1.71
Thimbleberry (*Rubus rosifolius*)	0.72
Tomato fruit (*Lycopersicon esculentum*)	0.2–0.6

Source: Thakur, B. R., Singh, R. K. and Handa, A. K. (1997). Chemistry and uses of pectin – a review. *Critical Reviews in Food Science and Nutrition* **37**, 47–73, with permission from Taylor & Francis.

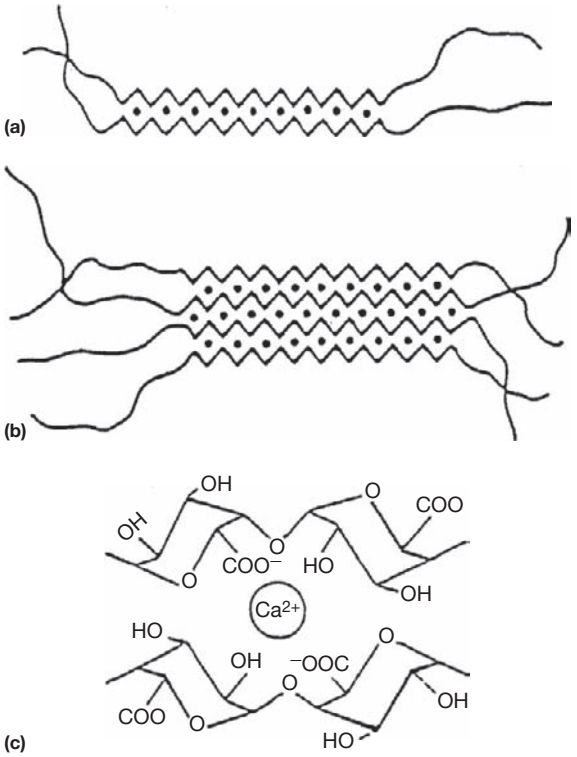

Figure 2 Schematic representation of calcium binding to polygalacturonate sequences: (a) 'egg-box' dimer, (b) aggregation of dimers, (c) an 'egg-box' cavity. Reproduced from Thakur, B. R., Singh, R. K. and Handa, A. K. (1997). Chemistry and uses of pectin – a review. *Critical Reviews in Food Science and Nutrition* **37**, 47–73, with permission from Taylor & Francis.

Pectin Molecular Weight Determination

Molecular weight strongly affects pectin behavior and it is, therefore, an important parameter for pectin characterization. It is worth noting that it should be better to discuss the molecular weight distribution, or an average molecular weight, to take into account that commercial pectin is made by different molecules with different molecular weights.

High-pressure size exclusion chromatography (HPSEC), also known as gel permeation chromatography, is probably the most common technique adopted for molecular weight determination: it is based on the different elution times, from a packed column, of molecules having different volumes. A detector (usually a refractometer), at the exit of the column, is used to determine the polymer amount. A preliminary calibration of the system has to be carried out using (monodisperse) polymers with a known molecular weight. Even though the system is fast and simple, it is a measure of volume rather than of mass. In order to avoid the calibration procedure, the same system can be equipped with a light scattering detector.

A different procedure is based on the determination of polymer intrinsic viscosity ($[\eta]$) that can be considered as a measure of the polymer hydrodynamic volume. This property can be obtained by measuring (usually through capillary viscometry) the viscosity of diluted polymer solutions and extrapolating the experimental data to zero polymer concentration, according to the well-known Huggins (eqn [1]) or Kraemer (eqn [2]) equations:

$$\frac{\eta_{sp}}{c} = [\eta] + k'[\eta]^2 c \qquad [1]$$

$$\frac{\ln(\eta_r)}{c} = [\eta] + k''[\eta]^2 c \qquad [2]$$

where c is the polymer concentration and η_{sp} is the specific viscosity that can be obtained from the relative viscosity η_r (i.e., the solution, η_{sol}, to solvent, η_s, viscosity). Intrinsic viscosity can be related to the so-called viscometric average molecular weight by using a two-parameter empirical correlation (the Mark–Houwink equation). The parameters, depending on the solvent–polymer pair, can be estimated by a calibration with polymers with a known molecular weight. It is worth noticing that this procedure, widely adopted for synthetic polymers characterization, is based on some assumptions, concerning polymer behavior, including the electrical neutrality of the chain. On the contrary, many polysaccharides, including pectin, exist in aqueous solutions in a dissociated form, and the presence of charges makes the relationship between viscosity and polymer concentration more complex. Consequently, salts are usually added to the solution to reduce these effects.

With the aim of improving the molecular weight estimate, an intrinsic viscosity detector can be coupled to HPSEC and multiangle laser light scattering.

Degree and Pattern of Methoxylation

The degree of methoxylation is an important parameter that strongly affects pectin behavior and, as a consequence, is one of the most used, even at commercial level, to describe different pectins. The most known determination is based on a titration procedure suggested by Joint FAO/WHO Expert Committee on Food Additives. Titration allows also the determination of galacturonic acid content.

Alternative methods were also suggested in the literature, for example, the alkaline hydrolysis to split the ester linkage and the following determination of methanol released using high performance liquid chromatography (HPLC) analysis, nuclear magnetic resonance (NMR) spectroscopy of pectin solutions, capillary electrophoresis (CE), and infrared spectroscopy. In particular, some authors used Fourier transform infrared spectroscopy (FTIR) and found that the absorbance of the ester carbonyl groups increased with increase in the DM and that the band area seemed linearly related to the DM.

The distribution of methoxylated units is also an important parameter when gelation of LM pectin, occurring through calcium ions bridges, is considered. It was observed that the bonds are stable when blocks of at least seven nonesterified galacturonic acid units are available; therefore, the greater the number of nonesterified units close each to the other, the greater the interaction among different polymer chains. One method, largely adopted in the literature to determine the so-called degree of blockiness (DB), is based on a selective depolymerization performed by using an endopolygalacturonase degrading GalA backbone only when more than four adjacent non-methyl-esterified GalA units are present. The length of the resulting pectin segments is evaluated through high-performance anion exchange chromatography. The larger the DB, the more blockwise-distributed are the esterified units. Alternative methods are based on high-frequency H-NMR spectroscopy by dissolving pectin in deuterated water.

Degree of Acetylation and Amidation

The degree of acetylation is important because a large number of acetylated groups reduce the gelling properties of pectin, thereby improving, apparently, its emulsifying characteristics. The degree of amidation affects calcium reactivity toward pectin and therefore the gel properties, and according to the current regulation, it cannot exceed 25% of total carboxyl groups of pectin.

Both parameters are determined, at industrial level, by using the titration procedure suggested by JECFA, even though it is quite time-consuming.

The degree of amidation was also measured, in the literature, using a CE or an FTIR method based on the different absorption wavelengths, at pH 6, of methyl esters, free carboxyl groups, and amide groups. Some authors investigated also the distribution pattern of amidated groups, by using a method similar to that described for the distribution pattern of methoxylated groups.

The degree of acetylation can be also determined by using HPLC with a resin-based column.

Industrial Uses of Pectin

Pectin is mainly used in the food area where in 2011, jams, fruit preparations, and confectionery covered ~60% of the total pectin market (more than 47 000 tons of pectin produced in 2011) evidencing the significant role played by this biopolymer as a gelling agent; pectin is also used as a thickening agent and stabilizer in beverages (25% of the market) and dairy products (4%).

Its best-known use is probably in jams, and often, pectin is the only permitted gelling agents in these products. According to type and quality of fruit, the required amount of pectin can change; for instance, cherry and strawberry usually require a large addition, whereas lower amounts are necessary for apple or blackcurrant. Typical values usually range between 0.2% w/w and 0.4% w/w. In addition to commercial jams, particular products, to be used as fillings or topping in bakery, are usually made. In this case, the products have to meet the specific requirements of the particular use; therefore, the amount and type of pectin are significantly determined by the specific case.

In the confectionery industry, pectin is used to make flavored candies. Usually, a neutral pectin (i.e., without specific flavor) is added to a mixture of water and sugar, and an external flavor is then added.

In beverages, pectin is often used to give texture to juices or soft drinks, mainly when a low sugar content could yield a low viscosity and therefore be unattractive to the consumer. In the dairy industry, pectin is used to stabilize dairy beverages, drinkable yogurts, and drinks containing both milk and fruit. In these systems, owing to heat treatments and acidification, casein particles are altered and unstable dispersion (with potential separation phenomena) can be obtained. Pectin seems to stabilize these products probably owing to electrostatic interactions with casein particles.

Recently, pectin was used also to prepare edible films or coatings to improve the shelf life of portioned fruits and vegetables by creating a barrier able to reduce water and oxygen transfer.

Even though use in food is still dominant, it is worth noting a growing use of this polysaccharide in novel areas, such as pharmaceutical (or biomedical) products (~5%), where pectin properties seem potentially interesting for many uses. For example, they were investigated as potential carriers for controlled drug delivery in nasal, oral, and ocular applications, for cancer-targeted drug delivery, in tissue engineering, and as a base for bone substitutes.

Nasal drug delivery is commonly used to deal with local diseases (such as allergy and congestion), and it is often based on liquid systems, which have very low residence time; therefore, different systems could be more useful. Pectin has shown itself to be very interesting owing to its mucoadhesive properties, which allow a controlled release of the active ingredients on the mucosal surface. In oral drug delivery, pectin gels have exhibited interesting characteristics because they are able to be resistant against digestive enzymes and to be degraded by intestinal microflora. In ocular uses, the pectin ability to gel at low pH is suitable to promote the gelation of pectin solution inside the eye (allowing a controlled release of the drug) owing to the low pH of the tears and their electrolytes content. The cancer-targeted delivery seems due to the interaction between the galactan component of the

polysaccharide and galectin-3 (Gal3), a component involved in many steps of cancer progression. Owing to these interactions, pectin itself seems able to inhibit cancer growth, and it could be useful to deliver, in a specific way, conventional anticancer drugs. In bone tissue engineering, pectin gels seem able to promote the nucleation of a mineral phase yielding a final system more similar to the bones than other systems are.

Finally, recent studies have investigated the use of pectin to remove heavy metals in remediation technologies and as gelling (and stabilizing) agents in cosmetic emulsion production.

Pectin Gel Properties

Since the main effect of pectin in food (and also nonfood) products is based on the structure and texture, it is important to be able to determine some characteristics, such as the use of pectin to form gels, the setting time, and the gel strength, as a function of the adopted operating conditions.

From the industrial point of view, some standard empirical procedures are commonly adopted to characterize the strength of commercial pectin gels; the most common are the SAG method, the Leatherhead Food Research Association (LFRA) texture analyzer, the Food Industry Research Association (FIRA) tester, the Bostwick consistometer, and different universal testing machines. In addition to the strength of the gel, other parameters such as the setting temperature and the setting time are determined by using the SAG method. Since the pectin gelation is affected by solute content and pH level, standard values for sugar amount, pH, and cation addition have to be adopted. Nevertheless, they can be different depending on the adopted empirical procedure and on the pectin type (LM or HM).

The IFT SAG method is, maybe, the most used for standardization of HM pectin, it was adopted by the Institute of Food Technologists (IFT) in 1959, and it estimates the gel strength through the definition of SAG grade. A pectin solution, prepared in standard conditions (i.e., 65% sucrose, pH value 2.3), is poured in specific vessels (the Ridgelimeter glasses) and allowed to cool down at room temperature. After 24 h, the gel obtained is placed on a plate and the change of the height, under its own weight, in 2 min is measured by using the Ridgelimeter. From this value, the SAG grade (°SAG) is calculated according to the following equation:

$$^{\circ}\mathrm{SAG} = \frac{A}{B} \cdot F \qquad [3]$$

where A and B are the amounts of sugar and pectin in the gel and F is a factor depending on the Ridgelimeter result (i.e., the 'sagging percentage'). F can be obtained by specific figures or tables as a function of the sagging percentage; for example, a gel having a sagging percentage of 23.5% is considered as standard and $F = 1$, a gel with higher percentage has $F < 1$, and a gel with lower percentage has $F > 1$. The pectin concentration, to be used in the test, is chosen assuming an expected (or estimated) grade and assuming $F = 1$.

A similar procedure is adopted to estimate the so-called setting time. A warm pectin solution, containing pepper grains, is poured in a glass immersed in a water bath. Periodically it is removed from the bath and the potential presence of a gel is checked by slightly twisting the gel by moving the tube. When the gel is formed, the pepper grains, owing to the viscoelastic properties of the material, swing back before going to rest. The time necessary to observe this behavior in the system considered is assumed as the gel setting time. The setting temperature can be estimated by using a similar procedure, measuring the temperature occurring inside the system. Based on the setting time, commercial pectins can be classified as rapid set, medium rapid set, slow, and extra slow set. Anyway, this classification is quite arbitrary and it can change from producer to producer.

These procedures are extremely empirical and, therefore, strongly affected by the practical experiences and by the adopted standard conditions. For example, the SAG degree is time-dependent, being determined 2 min after inversion of the gel from the recommended glasses dimensions, and it is based on an inhomogeneous and complex deformation of the sample. As a consequence, alternative methods, based on more fundamental measurements, have been investigated.

Pectin Gels: The Sol–Gel Transition

A significant contribution to the knowledge of biomolecular structure in several biopolymer is given by rheological techniques that are able to relate macroscopic parameters to material microstructure. Rheological techniques, such as small-amplitude oscillations, being nondestructive tests, seem very suitable to investigate the change of behavior during the gelation process and the final microstructure. During these tests, a sinusoidal deformation (or a stress) is applied to the sample and the resulting stress (or deformation) is measured and, as result of the test, two dynamic moduli are obtained: G' is defined elastic (or storage) modulus and is related to the energy stored in the sample, whereas G'' is defined viscous (or loss) modulus and is a measure of the energy dissipated during a cycle. Small-amplitude oscillations are used to investigate the linear viscoelastic behavior of the material and, therefore, the material microstructure.

Dynamic tests in linear conditions are widely adopted to investigate gelation phenomena and gel microstructure, and different methods were proposed to determine the sol–gel transition.

From a theoretical point of view, the 'critical gel' behavior (i.e., the material at gel point) was well described by Winter and coworkers that gave the experimental demonstration of a power law behavior of dynamic moduli. This criterion has been applied to many different gel systems, both chemically and physically linked polymers, finding a good agreement.

Nevertheless, other criteria, often simpler than Winter's, were proposed in the literature, and, probably, among them, the most used is the crossover of dynamic moduli at a constant frequency. This simple method suggests that the crossover of loss and storage modulus can mark the transition from a liquid-like to a solid-like behavior without invoking the onset of a gel. It is considered, sometimes, questionable because the G'–G'' crossover may be a function of frequency, whereas an intrinsic property of the material, such as the real gel point, should be independent of frequency. Nevertheless, being a simple method, it was applied to many systems.

However, the crossover value seems to be a function of biopolymer concentration. Therefore, in some situations, it cannot be easily detected because it does not occur in the investigated temperature window being G' greater than G'' since the beginning of the test even if the initial temperature is larger than 90 °C.

Independently of the pectin and according to the literature, it is generally assumed that in a time cure test of pectin gels (small-amplitude oscillatory test carried out decreasing temperature at a fixed cooling rate and maintaining linear viscoelastic conditions), storage modulus G' first increases rapidly and subsequently more slowly approaching pseudo-equilibrium, whereas the loss modulus G'' is always lower than G'. With increasing pectin concentration, the largest changes occur at an earlier stage, while, if pectin content is lowered, the sharp increase of G' is preceded by a period of time during which G' increases slowly.

The incipient gelation, at constant temperature, can be evaluated by time sweep tests (analysis of moduli during time, in linear viscoelastic conditions). At the beginning of the tests, pectin aqueous solutions show a typical liquid-like behavior, with G'' higher than G'. As time goes by, G' progressively increases, and a crossover of moduli is encountered. It seems that crossover clearly decreases with pectin increase following a pseudo-power law behavior.

Different rheological methods were, also, proposed in the literature to determine the pectin gel point. Some authors suggested the extrapolation, to the temperature axis, of storage modulus values in the rapid increase zone. Other authors assumed the transition as the temperature value where the phase angle was halved, while in some other literature references, a 'structure development rate (SDR)' was evaluated, based on the first derivative of storage modulus, to describe the junction formation and, therefore, the material gelification. In a recent work on LM pectin gelation, moreover, the determination of two new parameters could be complementary or alternative for the classical gel point identification: the initial structuring temperature and the critical structuring temperature that can be estimated on the basis of the SDR.

SDR is defined as

$$\mathrm{SDR} = \frac{\mathrm{d}G'}{\mathrm{d}t} \qquad [4]$$

and it is a simple and efficient way to describe gelation rate of these systems clearly evidencing, also, the way in which temperature affects the magnitude of G' and, most of all, its rate of change with time.

Pectin Gel Strength as a Function of Operating Conditions and Intrinsic Factors

Pectin gel characteristics are affected by both intrinsic properties, related to biopolymer structure, and operating conditions. Different mechanisms are involved in gelation of LM or HM pectin; therefore, it can be useful to discuss separately their effects on final gel.

LM pectin gels

Low-ester pectin gel formation is the result of ionic linkage through calcium bridges between carboxylic groups from two pectin chains. This linkage, generally, takes place upon cooling a pectin solution of calcium. Even though a number of positive ions can link pectin molecules, especially magnesium and potassium, calcium is particularly effective in gelling carbohydrates, mostly because its ionic radius (0.1 nm) is big enough to coordinate with oxygen atoms and because of flexibility with regard to the direction of its coordinate bonds. One of the most important intrinsic factor, in pectin gelation, is the DM value because a significant number of nonmethoxylated GalA units have to be present to promote the formation of calcium bridges. Nevertheless, the DM is not sufficient to describe pectin–calcium interactions because it gives only the total fraction of GalA units. On the other hand, according to the recent literature, a relevant role is even played by GalA distribution: when a blockwise pattern is present, stronger gels are formed, owing to the larger number of units involved in junction zones. On the contrary, a random distribution yields less strong gels, probably owing to shorter and weaker junction zones. It seems that at a constant DM, gel strength depends on DB, and a maximum strength can be obtained for a specific pattern that results in the maximal cross-link density. For these reasons, both DM and DB should be known even though the latter is more difficult to estimate. Gel strength is also affected by chain length, that is, the molecular weight: it was observed that, decreasing the polymer length, a weaker gel is obtained, owing to the lower number of junction zones. Under a critical molecular weight, it seems that gelation cannot occur. Finally, the degree of acetylation and degree of amidation modify gel properties: acetyl groups decrease the propensity to gelation of pectin (highly acetylated pectins cannot gel), whereas amide groups, introduced during demethoxylation, improve gel formation owing to the presence of hydrogen bonds among them.

Concerning operating conditions, the ratio between calcium and biopolymer plays a relevant role in pectin gelation; usually, it is described by defining the stoichiometric ratio R:

$$R = \frac{2[\mathrm{Ca}^{2+}]}{[\mathrm{COO}^{-}]} \qquad [5]$$

It was observed that gel strength increases with R value up to 0.5 because a larger number of calcium ions are available for junction formation. When $R = 0.5$, theoretically, all calcium ions are involved in egg box formation. For larger R values, the calcium can be available for less specific and weaker interactions that should not improve significantly gel strength, even though positive effects were observed by some authors. Finally, for further relevant increases in R, a decrease in gel strength is observed probably because of a too fast local gelation yielding phase separation phenomena. The increase in calcium content also increases the temperature of gel formation. When calcium level is kept constant and pectin concentration is modified, an increase in gel strength is observed with increasing fraction of polysaccharide. Nevertheless, when the pectin concentration becomes too high (i.e., a low R value is obtained), a decrease in gel consistency can be also observed.

LM pectin gels are in a wide range of pH values, at least from pH 3 to 6. At low pH, hydrogen bonds and hydrophobic interactions significantly contribute to gel formation, and for pH lower than 3.5, gelation can occur even without calcium

addition. At high pH (approximately larger than 4.5), gel properties are less dependent on pH level.

Cosolute addition is less relevant than in HM pectin anyway; also, in this case, some effects can be observed, owing to their interaction with water, and increase in gel strength, when adding sugar, was observed also for LM pectin. Nevertheless, the effects are according to the specific adopted solute.

High-ester pectin gel formation is due to the presence of hydrogen interactions and hydrophobic bonds between polymer chains, and therefore, it is affected by factors able to modify these interactions.

HM pectin gels

HM gel formation is favored by low pH solutions able to prevent the dissociation of carboxyl groups and, by the addition of sugar (from about 55 to about 80%) thanks to which pectin chains are available to cross-link, to form hydrogen bond and the other hydrophobic interaction. In fact, cosolutes promote pectin gelation by bonding water, which become less available to maintain carbon chains in solution and, as a consequence, induce them to build the network. So far, it is well understood that pH variation effect is very important for final gel properties: at first, if pH is above the value of pK for the acid groups, charges of the carbon chain are negative, because of dissociation, and allow gelation to be prevented. If pH is gradually lowered, very high sugar content (about 80%) is necessary to form weak gel structures, and gradually, sugar content can be reduced if pH is lowered as well. At a pH value below 3, for pectin with a DM above 72%, a gel can be formed starting from a sugar content of about 55%.

Considering this experimental evidence, it can be deduced that, fixing the sugar content, the effect of pH is evidenced by a loss of strength above a certain critical value and a decrease of setting temperature with the pH increase.

During gelation, the structure development is important for producing many food products. For example, in jams, rapid-setting pectin is necessary to ensure the entrapment of large pieces of fruit within structure network to ensure a proper distribution in the product. Moreover, if clear gels have to be produced, no air bubbles should be included in the network: this could be achieved through the use of slow-setting pectin.

Apart from pH value dependence, another important factor affecting strength and setting of HM pectin gels is the kinds of sugar used as cosolute for gel formation, as well as their concentration. In fact, it is known that glucose can be successfully substituted by sucrose, fructose or corn syrups, maltose, or liquid cosolutes as glycerol, xylitol, and sorbitol. According to the literature, polymer–cosolute interactions, in competition with polymer–polymer interaction, are lowered by cosolute–cosolute interactions, which are weaker for liquid cosolutes than for solids: Therefore, in general, if gels are formed with solid cosolutes, strong cosolute–cosolute interaction are expected, and, as a consequence, a stronger gel system is formed thanks to an improvement in polymer–polymer interaction.

Pectin Interfacial Activity

Even though pectins are mainly known and used for their bulk properties, that is, as thickening and gelling agents able to entrap water, recently, their interfacial characteristics were investigated with the aim of understanding if they are usable to modify the structure of interfacial layers (air/water or oil/water) as other biopolymers (such as protein) can do. Interesting results were found mainly for sugar beet pectin, even though these polysaccharides have poor gelling properties owing to their high content of acetylated groups. On the contrary, significant interfacial activity (at oil/water interface) was observed for pectin with different DM and molecular weight values. The origin of these properties is so far not completely understood: some authors attribute them to the number of acetylated groups that should enhance the hydrophobic behavior of the polymer; nevertheless, also, pectin from other sources (such as citrus pectin) with a low degree of acetylation seems to exhibit interesting emulsifying characteristics. It seems that a significant role should be played by the protein fraction of pectin; in fact, it is known that proteins exhibit good interfacial activity, and therefore, they could be the main responsible of pectin interfacial behavior. From an experimental point of view, the most promising results were observed for HM pectin, probably owing to the larger number of hydrophobic methoxyl groups, and for low-molecular-weight polymers, probably because smaller molecules can diffuse faster from the bulk to the interface. Even though pectin interfacial activity has still to be investigated, current results suggest potential interesting uses for pectin also in this area.

See also: Fruit Juices; Gelatin; Pectin and Health; Rheological Properties of Food Materials.

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Peppers and Chilies

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Introduction

The terms 'pepper' and 'chili' have been used to nominate different types of plants and plant products, all of them with pungent fruits or seeds. It was originally used to designate seeds of *Piper nigrum* from the *Piperaceae* family, commonly known as black, green, and white peppers. Nevertheless, this article primarily discusses chilies and peppers from the *Capsicum* genus from the *Solanaceae* family, which includes potatoes, tomatoes, tobacco, and petunias. They are native to the tropics and subtropics of America, and the first record of their consumption dates from approximately 9000 years ago in Mexico. European visits to the New World by shipping routes in the period between 1492 and 1600 disseminated this ancient crop around the world. Nowadays *Capsicum* is an important agricultural crop with numerous varieties widely cultivated.

Capsicum genus includes a large range of species from sweet green bell peppers to habanero peppers, the reason why their naming terminology is confusing, and names like pepper, chili, chile, aji, paprika, and capsicum are often used. These species are classified by characteristics of the fruit like pungency, shape, color, flavor, size, and use. The name chili or 'chile' is generally applied in the current literature to those fruits that are pungent, and they are used as fresh or dry fruits and also in powder form. Chili is mainly spread out in tropical regions of America, Asia, and Africa, where they are valued for their flavor and pungency, representing a culinary tradition and a cultural symbol. There is a gradation of pungency, and some varieties show a slight pungent taste or no pungency at all. The nonpungent species are commonly known as peppers, sweet peppers, or sweet bell peppers (used as fresh) and are preferred by consumers from Europe and North America, although they have been spreading to the tropics through a Western influence on its diet.

Capsicum Fruits

Botanic

Among the 31 species identified in the *Capsicum* spp. genus, five are domesticated, including *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens*. *C. annuum*, *C. chinense*, and *C. frutescens* are the most widespread in America and the world. *C. annuum* specie includes most of the Mexican chilies like *poblano* and *jalapeño*, most of the hot peppers of Africa, and the various cultivars of sweet peppers grown in temperate countries. These fruits are used to extract paprika and paprika oleoresin, products applied currently in cuisine, food industry, drugs, and cosmetics. Because *C. annuum* is not well adapted to humid countries, it was replaced by *C. frutescens* and *C. chinense*. *C. frutescens* is cultivated in Latin America, Asia, and Africa either for fresh consumption of intact fruits or oleoresin extraction. This specie produces peppers known by the names of *malagueta*, *tabasco*, and *arbol*. *C. chinense* is extensively cultivated in West India and South America and includes Scotch bonnet and habanero peppers. This specie is characterized by its flavor and pungency, although nonpungent forms of *C. chinense* are grown commercially in Amazonia. The other two domesticated species, *C. baccatum* and *C. pubescens*, are practically unknown outside America. They are prevalent in Andean South America and southern Brazil.

Morphology and Anatomy

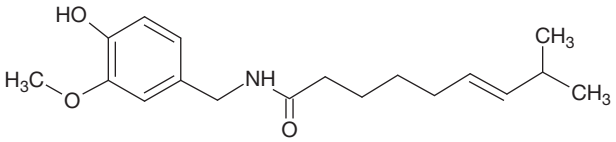
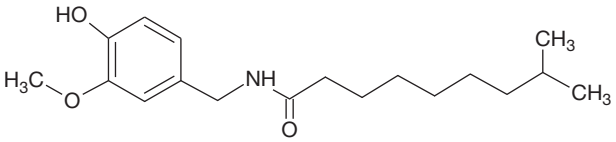
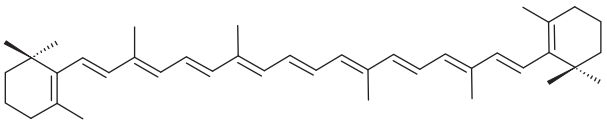
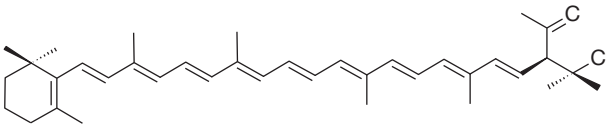
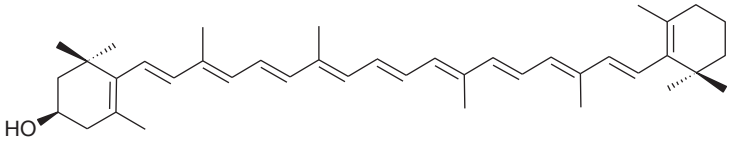
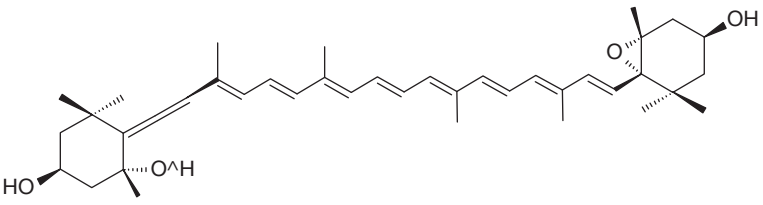
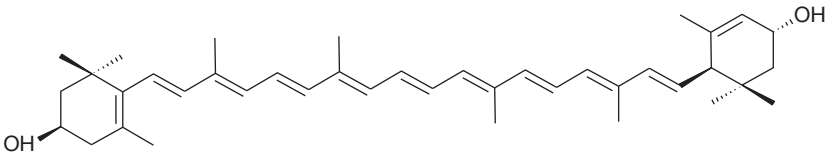
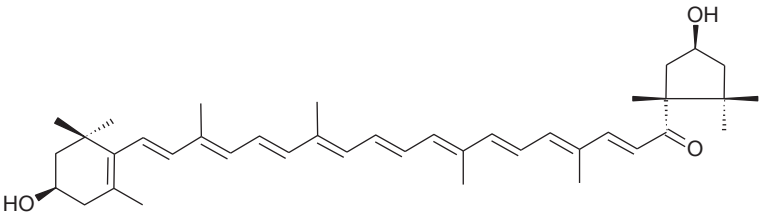
Fruits of *Capsicum* are conical or semispherical berries with different sizes (a few grams up to 250 g) depending on the varieties, and wild peppers have smaller fruits than domesticated species. Size and pungency are genetically independent characters. The space between mesocarp and endocarp is full of air, and the interior has two to four central ribs that support the seeds. Fruits that have a thick pericarp are consumed fresh, whereas those with a thinner pericarp are consumed dry or after grinding. The pericarp has an impermeable outer cuticle to protect the fruits and retard water losses, which contributes to extend their shelf life. This cuticle is indigestible and firm, so frequently it is removed before the canning process.

Sensory Characteristics

The color of *Capsicum* changes according its variety and ripening stage, starting from green, yellow, or white in unripe fruit turning to red, dark red, brown, and almost black in the ripe stage. Cells of mesocarp of immature fruit contain chlorophyll, carotenoids as lutein and neoxanthin, and occasionally anthocyanins, so the color of unripe fruits usually varies from creamy white to green. Overall, during fruit ripening, levels of chlorophyll are reduced, whereas carotenoids as capsorubin and capsanthin (red carotenoids), and β-carotene and β-cryptoxanthin (yellow carotenoids) are massively synthesized, and consequently most of ripe fruits show intense red color (Table 1). Concentration of carotenoids in ripe red pepper is estimated as being 35-fold higher than in unripe fruit. Capsanthin, capsorubin, and cryptocapsin are ketocarotenoids characteristic from *Capsicum* genus. During ripening, carotenoids undergo diverse degrees of esterification with different fatty acids, a process that confers to them more stability.

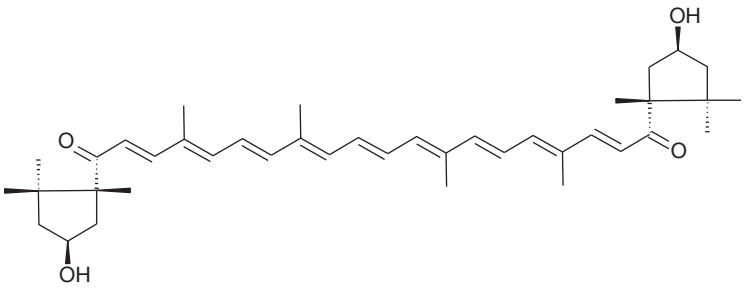
Pungency of chilies is conferred by capsaicinoids, an alkaloid group that includes capsaicin and dihydrocapsaicin, which represents 90% of the total capsaicinoids. Chemically they are amides of vanillyamine and carboxylic acids with 9–11 carbon atoms and are responsible for spiciness of pungent peppers. These compounds are synthesized in the epidermal cells of the placenta and accumulate in cuticle covering the placenta. Cuticle rupture spreads capsaicinoids over the seeds and internal wall of fruit, which leads to the belief that these are pungent parts of fruit. Three structural characteristic

Table 1 Structural formula of major phytochemicals present in fruits of *Capsicum*

Compound	*Group*	*Structural formula*
Capsaicin	Capsaicinoids	
Dihydrocapsaicin	Capsaicinoids	
β-Carotene	Carotenoids	
α-Carotene	Carotenoids	
β-Cryptoxanthin	Carotenoids	
Neoxanthin	Carotenoids	
Lutein	Carotenoids	
Capsanthin	Carotenoids	

(*Continued*)

Table 1 (Continued)

Compound	*Group*	*Structural formula*
Capsorubin	Carotenoids	

features make capsaicin one of the most pungent compounds known: the vanillyl group, the acid–amide linkage, and the alkyl side-chain. Any alteration in one of these groups supposes a decrease in pungency. The capsaicinoid burning sensation is produced by binding to vanilloid receptors VR-1, responsible for pain perception. A repeated exposure to capsaicinoids leads to desensitization of these receptors. Determination of pungency is very important for consumers and industrial purposes. The Scoville Organoleptic Test is a method that measures pungency by human perception, expressing pungency value in Scoville heat units. Measurement is correlated to capsaicin content, so a sweet pepper containing no capsaicin has a Scoville rating of zero, whereas hottest chilies like *habanero* have a rating about 200 000 or more. Because human perception of pungency is subjective, a modern universal scale was built by the American Spice Trade Association (ASTA) based on measurement of capsaicinoids amount by high-performance liquid chromatography. Pungency is expressed as ASTA units and can be easily converted to the most common scale of Scoville units by multiplying the content of each capsaicinoid by a conversion factor.

Although color and pungency are the most valuated aspects of chilies and peppers, aroma has also been focussed on as an important attribute for quality of fruits and vegetables and consequently their acceptance by consumers. The characteristic aroma of the *Capsicum* is due to the presence of droplets of volatile oils in the mesocarp, which are a combination of methoxypyrazines, aliphatic alcohols, and esters, with more than 125 volatile compounds currently identified both in fresh and processed fruits. Like pigment concentration, the volatile composition depends on the maturity stage of the fruit. Immature fruits have grassy and herbal aromas attributed to compounds as 2-isobutyl-3-metoxypyrazyne and hexanal. As fruit ripening, their concentrations decrease or disappear, whereas levels of compounds responsible for sweet aroma increase. Among volatile compounds present in ripe peppers are octanal (fruity), *trans*-2-hexenal (sweet), 2,3-butanedione (caramel), 3-carene (red bell pepper, lemon), and 1-penten-3-one (pungent/spicy).

Nutritional Composition and Absorption

Composition of nutrients of *Capsicum* genus varies extremely according species, variety, stage of maturity, and part of plant consumed (Table 2). Raw vegetables contain about 90% water, 6–9% carbohydrates, and very low or no content of fat and protein, so their consumption does not suppose a great caloric intake. On the other hand, after cooking and drying processes, they supply more calories than equal amounts of fresh peppers due to water losses. In general, these fruits are good sources of phytochemicals like vitamin C, E, and A, as well as phenolic compounds and carotenoids.

Vitamin C

Fresh peppers are among best sources of vitamin C in the human diet. In fact, 100 g of green sweet peppers can provide practically 100% of recommended daily intake (RDI) of vitamin C for both adult males and females (ages 19–70 years), which are 90 and 75 mg day^{-1}, respectively. Furthermore, the same quantity of red and yellow sweet peppers supposes an intake of two and three times the RDI of vitamin C, respectively. In general, an increase in the ascorbic acid content is observed for fresh fruits as fruit mature. Nevertheless, the drying process results in losses of almost 50% of vitamin C content, whereas canned *Capsicum* retain about 70% of initial content.

Vitamin A and Carotenoids

The content of vitamin A depends on the presence of carotenoids with provitamin A activity, so it is strictly related with the maturation stage and color of fruits. In general peppers are good sources of the provitamin A carotenoids as β-carotene, α-carotene, β-cryptoxanthin, and oxygenated carotenoids as lutein. As capsorubin and some carotenoids present in ripe stage do not exhibit provitamin A activity, not even red peppers provide higher amounts of vitamin A, if compared with its corresponding green variety. Studies concerning the nutritional composition of chilies and peppers report concentrations of carotenoids that can provide 60% of RDI of vitamin A for an adult, by consumption of 100 g of fruits. However, steps in the processing of red pepper fruits into paprika like trituration and heating, as well extraction with an organic solvent or oil, produce pigments that are much more labile. Moreover, heat increases the rate of thermo-oxidation reactions, which are highly effective in thermal degradation of carotenoids. As a result, manufactured products of *Capsicum* may not contribute

Table 2 Nutritional composition of some chilies and peppers

	Unit	*Sweet pepper (green, raw)*[a]	*Sweet pepper (yellow, raw)*[a]	*Sweet pepper (red, raw)*[a]	*Hot chili (green, raw)*[a]	*Hot chili (red, raw)*[a]	*Jalapeño (raw)*[a]	*Serrano (raw)*[a]
Proximates								
Water	g	93.89	92.02	92.21	87.74	88.02	91.69	90.25
Protein	g	0.86	1.00	0.99	2.00	1.87	0.91	1.74
Total lipid	g	0.17	0.21	0.30	0.20	0.44	0.37	0.44
Carbohydrate[b]	g	4.64	6.32	6.03	9.46	8.81	6.50	6.70
Dietary fiber	g	1.70	0.90	2.10	1.50	1.50	2.80	3.70
Sugars, total	g	2.40	–	4.20	5.10	5.30	4.12	3.83
Minerals								
Calcium (Ca)	mg	10	11	7	18	14	12	11
Iron (Fe)	mg	0.34	0.46	0.43	1.20	1.03	0.25	0.86
Magnesium (Mg)	mg	10	12	12	25	23	15	22
Phosphorus (P)	mg	20	24	26	46	43	26	40
Potassium (K)	mg	175	212	211	340	322	248	305
Sodium (Na)	mg	3	2	4	7	9	3	10
Zinc (Zn)	mg	0.13	0.17	0.25	0.30	0.26	0.14	0.26
Vitamins								
Vitamin C[c]	mg	80.4	183.5	127.7	242.5	143.7	118.6	44.9
Thiamin	mg	0.057	0.028	0.054	0.090	0.072	0.040	0.054
Riboflavin	mg	0.028	0.025	0.085	0.090	0.086	0.070	0.081
Niacin	mg	0.480	0.890	0.979	0.950	1.244	1.280	1.537
Vitamin B_6	mg	0.224	0.168	0.291	0.278	0.506	0.419	0.505
Folate, DFE	μg	10	26	46	23	23	27	23
Vitamin B_{12}	μg	0	0	0	0	0	0	0
Vitamin A[d]	μg	18	10	157	59	48	74	47
Vitamin A	IU	370	200	3131	1179	952	1078	937
Vitamin E (α-tocopherol)	mg	0.37	–	1.58	0.69	0.69	3.58	0.69
Vitamin D (D2 + D3)	μg	0	0	0	0	0	0	0
Vitamin D	IU	0	0	0	0	0	0	0
Vitamin K (phylloquinone)	μg	7.4	–	4.9	14.3	14.0	18.5	118

[a]Value per 100 g.
[b]By difference.
[c]Total acid ascorbic.
[d]Retinol activity equivalent.
Source: USDA National Nutrient Database for Standard Reference.

as significantly to vitamin A ingestion as fresh fruits because losses of carotenoids with provitamin A activity occurs.

Only a part of carotenoids ingested is really absorbed, and the magnitude of this fraction depends on several factors related to the chemical structure of carotenoid, food matrix, and host. Absorption of carotenoids is similar to other dietary lipid compounds and occurs in three phases: solubilization, micellization, and absorption. After ingestion, they are released from food matrix and grouped into fat droplets in gastric emulsion, where they undergo enzymatic hydrolysis. A reduction in the size of droplets occurs, and carotenoids are incorporated into mixed micelles formed from lipids and bile salts, and then they are transferred into intestinal mucosa cells. Data regarding bioavailability of carotenoids from peppers and chilies are still scarce and controversial. Generally, xanthophylls as lutein and zeaxanthin are most bioaccessible both from fresh and dried fruits of *C. annuum*. From fresh fruits, more than 80% of lutein and zeaxanthin are absorbed, whereas β-carotene and β-cryptoxanthin show rates of about 25%. On the other hand, amounts of carotenoids absorbed from paprika (dried fruits) from different cultivars are lower, ranging from 33% to 49% for β-carotene, from 20% to 41% for β-cryptoxanthin, and from 25% to 49% for zeaxanthin.

Phenolic Compounds

Fruits of *Capsicum* are rich in phenolic compounds, and many authors have worked to elucidate their phenolic composition, which still remains incomplete. Capsaicinoids are the most important group of phenolic compounds because they characterize *Capsicum* fruits. The major flavonoids are glycosides of quercetin and luteolin, and some studies also report the presence of derivatives of cinnamic, coumaric, and caffeic acids.

Phenolics from *Capsicum* present a great bioavailability (about 70%) as compared with other fruits and vegetables. This occurs probably because pepper fruits do not contain proanthocyanidins, which are phenolic compounds resistant to the action of digestive enzymes. The remaining phenolics (nonbioaccessible) could reach the colon and be fermented. Data regarding capsaicinoids show that capsaicin is

preferentially absorbed in the jejunum part of small intestine, and it is a fast and nonenergy consumed absorption. *In vivo* research showed that capsaicin could be detected in the circulation 10 min after ingestion.

Health Effects

The *Capsicum* plant has been historically employed in traditional medicine and currently has being used in modern herbology and conventional treatments, principally due to its antimicrobial, anticonvulsive, and sedative properties. First reports date back to Mayan and Azteca civilizations, which used fruits from the pungent and nonpungent peppers in treatment of asthma and coughs, as well as relieving sore throats and toothaches. Positive health benefits from *Capsicum* fruits are related to their phytochemical composition because they have high content of compounds as carotenoids, capsaicinoids, phenolics, and ascorbic acid. Many of these compounds are antioxidants that exert their biological effects through free-radical scavenging, protein binding, and interaction with human signal transduction pathways.

Several biological effects and pharmaceutical properties, including antioxidant, anticarcinogenic, and anti-inflammatory effects, have been attributed to capsaicinoids. It is known these alkaloids act in pain mechanism by depletion of substance P and other neuropeptides from sensory nerve terminals. This fact justifies *Capsicum* application as a neuropharmacological tool in preventing chronic pain associated with postherpetic neuralgia, diabetic neuropathy, and also in topical creams to treat chronic pain syndromes and osteoarthritis. Studies showed the effectiveness of capsaicinoids in the treatment of cystitis, human immunodeficiency virus, and disorders related to sensory nerve fibers. Intake of capsaicinoids at low levels has been related to a decrease in myocardic and aortic cholesterol levels. Moreover, ingestion of capsaicin can reduce body-fat mass in dose-dependent ways by decreasing the desire to eat, as well as protein, fat, and carbohydrate intakes. It enhances energy metabolism by stimulation of the sympathetic nervous system. Some authors associated ingestion of 5 g of fresh capsicum with lower plasma glucose levels and maintenance of insulin at basal levels and attributed this effect to capsaicin content (about 26.6 mg capsaicin/5 g *Capsicum*).

It is well known that carotenoids play important biological functions as potent quenchers of singlet oxygen and effective scavengers of reactive of oxygen and nitrogen species (ROS and RNS), compounds that are implicated in pathogenesis of chronic degenerative diseases such as cardiovascular diseases and some types of cancer. In fact, capsanthin and capsorubin, major carotenoids in the *Capsicum* genus, have been reported in literature to quench singlet oxygen, suppress free radical induced lipid peroxidation, and suppress generation of superoxide and nitric oxide. An *in vivo* study showed the antitumor-promoting activity of capsanthin and its mono and diesters. Moreover, this carotenoid showed strong quenching activity for singlet oxygen and effective ability to suppress the generation of ROS in mouse skin by irradiation of ultraviolet A. Lutein, a carotenoid predominant in green peppers and immature fruits, is involved in prevention and delay of progression of age-related macular degeneration and cataracts.

Ascorbic acid, first isolated from paprika, is probably the most effective and least toxic antioxidant identified at the biological system. Similar to other phytochemicals presents in *Capsicum* fruits, it seems to be important in protecting against such oxidative stress-related diseases as coronary heart disease, cancer, and cataracts, as well as in the natural aging process and modulating immune system function. High intakes of acid ascorbic have been related to lower blood pressure. In addition, it acts in iron metabolism by preventing formation of insoluble iron compounds or reducing ferric to ferrous ions, then enhancing iron absorption.

Production and Consumption Patterns

Capsicum is an important agricultural crop around the world, not only due to its economic relevance as a flavoring and coloring agent to the food industry and cuisine, but also for the nutritional value of the fruits, medicinal applications, and ornamental uses. The average annual world production from 2000 to 2011 was about 25.7 million tons for green products (fresh) and 2.9 million tons for dry products (Table 3), with an average annual growth rate of 3.2%. Data from 2011 point to China as the largest producer of fresh products with more that 50% of world production. In turn, India stands out as major producer of dry products (42%), and the top 10 producers accounted for about 80% of production. This country produced more *Capsicum* than it consumed, so 17% of the 1.2 million metric tons were destined for export.

Despite the fact that *Capsicum* is the most consumed spice in the world, its consumption tends to be concentrated within a few countries. The top 5 consumers of *Capsicum* absorbed 54% of the market, and India heads it with 941 000 metric tons consumed in 2009. Rounding out the list are Thailand, Pakistan, Bangladesh, and China. Market composition of the top 10 consumers and producers is very similar, with nine countries in common. The differences are the United States and Peru, which only appear on the top 10 consumers and producers lists, respectively. Considering per capita consumption, Bosnia and Herzegovina is at the first place with 5.5 kg year^{-1}, followed by Jamaica (3.7 kg year^{-1}), Peru (3.6 kg year^{-1}), Ghana (3.2 kg year^{-1}), and Bermuda (3.1 kg year^{-1}).Only

Table 3 World production and harvest area of chilies and peppers (2011)

	Chilies and peppers (dry)		*Chilies and peppers (green)*	
Continent	*Production quantity (tons)*	*Area harvested (ha)*	*Production quantity (tons)*	*Area harvested (ha)*
Africa	550 026	509 757	2 485 530	294 481
America	247 541	50 438	3 691 003	216 791
Asia	2 542 310	1 402 073	20 510 332	1 205 142
Europe	117 656	69 995	2 857 593	118 584
Oceania	–	–	56 717	2706
Total	3 457 533	2 032 263	29 601 175	1 837 704

Source: FAOSTAT (2013). Food and Agricultural Organization of the United Nations. Agricultural production.

Thailand, Myanmar, and Ghana appear in both lists of top 10 consumers and top 10 per capita consumers.

Global Uses in the Food Supply

Chilies and peppers are used worldwide as a spice, salad, complementary dish, medicine, industrial product, or decoration. Their primarily application is in the food industry, which in developed countries comprises about 55–60% of fruit production, whereas 35–40% are consumed by the retail sector and about 10–15% by the catering sector. In developing markets the main consumers of peppers are households.

Capsicum is employed in the food industry to improve taste, color, or aroma of sauces, soups, processed meats, snacks, frozen dairy desserts, candies, gelatins and puddings, soft drinks, and alcoholic, and nonalcoholic beverages. The most economically important species are *C. frutescens* and *C. annuum*, and intensity and quality of color, as well pungency, are the main characteristics determining commercial value of pepper fruits. They are generally processed and used as natural colorant in the form of paste, concentrated extracts (oleoresin), or powder (paprika), which can increase shelf life of fruits from days to months. Paprika is a fine, brilliant, and red-colored powder obtained by dehydration and milling of red-fruited cultivars. It is valuated for its coloring power by spectrophotometric measuring. The higher the coloring capacity, the higher the price. Oleoresins are red-colored oils produced from dried or ground fruits of red pepper. The traditional process is carried out by extraction using organic solvents followed by distillation to remove the solvent. This product is considered by some food manufacturer as more aseptic (less contamination by microorganisms, insects, etc.) and more standardized than paprika (regarding pungency).

Besides applications in food industry, the nonpungent species, named sweet peppers, also are used in fresh form. They are eaten raw in salads or can be processed, cooked, or fried like vegetables and consumed together with other foods. Most pungent peppers are considered spices or condiments and are used in small quantities for seasoning food, as well as commonly pickled in salt and vinegar.

There are applications of *Capsicum* not related to food supply. Pungent species are used to produce defensive spray because the irritant effects of capsaicinoids are dangerous for mucosa. Some herbal formulas contain *Capsicum* as a synergistic agent, like general tonics, laxatives, and sedatives. In addition, these plants are currently used in aquariums for koi fishes or in zoos for improving the feather color of flamingoes.

See also: Alkaloids: Properties and Determination; Antioxidants: Role on Health and Prevention; Ascorbic Acid: Properties, Determination and Uses; Carotenoids: Occurrence, Properties and Determination; Colors: Properties and Determination of Natural Pigments.

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Pesticides and Herbicides

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Pesticides and herbicides are intended to keep our food supply safe. Ideally, the pesticides and herbicides we use on our foods will eliminate the target pest without contaminating the food itself. Pesticides and herbicides are designed also to prevent pests from lowering the yield of the food we produce. Maximum yields are expected from the inputs required to raise a food crop.

The Food and Agriculture Organization (FAO) has defined a pesticide as "Any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during, or otherwise interfering with, the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal foodstuffs, or which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit, substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport."

Herbicides specifically refer to products used to control plants and not other pests.

Insects, animals, plants, fungi, bacteria, and viruses all can have a negative impact on our food supply. Pesticides used to control these include herbicides, insecticides, insect growth regulators, nematicides, termiticides, molluscicides, piscicides, avicides, rodenticides, bactericides, insect repellents, animal repellents, antimicrobials, fungicides, and antimicrobial disinfectants. There are always compromises when using pesticides and herbicides on our food supply because there are no pesticides or herbicides that do not involve a risk. Biologically based pesticides can be safer than traditional pesticides but even these are not without risk. The biological pesticides include pheromones and microbial pesticides. Natural products are not necessarily without risk, and synthetic products can be just as safe as natural products.

Pesticide Use

There is a long history of human's use of pesticides. Elemental sulfur was the first known pesticide that was used about 4500 years. A 4000-year-old Hindu text mentions the use of poisonous plants for pest control. The heavy metals, arsenic, mercury, and lead, were used on crops to kill pests in the fifteenth century. In the seventeenth century, nicotine sulfate from tobacco leaves was used as an insecticide.

From the 1900s to the 1950s, arsenic-based pesticides were the main pesticides used. One of the first uses of a chemical as a pesticide was 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT). In 1939, Paul Müller, a Swiss chemist, discovered the insecticidal action of the organochlorine, DDT. Organochlorines were extensively used during WWII and after, but they were replaced in the United States by organophosphates and carbamates by 1975.

Herbicides became common in the 1960s, led by "triazine and other nitrogen-based compounds, carboxylic acids such as 2, 4-dichlorophenoxyacetic acid, and glyphosate. A list of pesticides and the targets are listed in Table 1."

Pesticides Containing Minerals and Metals

The Chinese used sulfur around 1000 BC to control bacteria and mold. Sulfur is used today in fungicides to control diseases on agricultural and ornamental plants. The Chinese also used arsenic-containing compounds to control insects. Arsenic has a history of use as an insecticide and herbicide and also as a medicine. Arsenic trioxide has been used as a herbicide in the late 1800s, and lead arsenate, containing both lead and arsenic, was used as an insecticide prior to the development of synthetic pesticides following WWII. Arsenic in the form of chromated copper arsenate (CCA) was used as a wood preservative from the 1940s to 2003 when it was voluntarily removed from the residential market. Wood treated with CCA is still available primarily for industrial use. CCA is still registered with the EPA for the treatment of wood products that may be found in residential settings like shake shingles, wooden shingles, and structural members other than decks. In the agricultural market, CCA-treated wood is used in posts, sawn timbers for fence posts, or structural supports.

Plant-Based Pesticides

Nicotine from tobacco leaves was used in the late 1600s as an insecticide and is still in limited use as a pesticide. *Pyrethrum*, derived from the chrysanthemum plant, is used as an insecticide. Synthetic pyrethrins have been developed that are more potent. Strychnine from the strychnine tree, *Strychnos nux-vomica*, is used as a rodenticide. Rotenone, from the root of *Derris elliptica*, is used as an insecticide and fish poison. These natural pesticide products are potent chemicals and in some cases more potent than synthetic products.

Chemicals Used as Pesticides

The primary chemicals used as early pesticides include organochlorines, organophosphates, carbamates, and sulfonylurea herbicides.

The development of DDT as an insecticide during WWII started the era of chemicals used as pesticides. The synthetic chemistry advanced rapidly in the 1930s, and by the early 1940s, a range of new pesticides called the organochlorine

Table 1 Types of pesticides and the targets

Pesticide	*Target*
Algicide	Algae
Avicide	Birds
Bactericide	Bacteria
Fungicide	Fungi
Herbicide	Plants
Insecticide	Insects
Miticide	Mites
Molluscicide	Snails
Nematicide	Nematodes
Rodenticide	Rodents
Virucide	Viruses

insecticides were developed. These very potent organochlorines were originally developed as potential chemical warfare agents. After WWII, the organophosphate and carbamate compounds were repurposed as insecticides, and many organophosphate and carbamate insecticides continue to be used today.

Herbicides were developed after WWII to increase food production and as possible warfare agents. In 1946, the first commercially available chlorine-based herbicides were marketed to kill broad-leaved plants. This class of compounds includes 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). 2,4,5-T was used extensively during the Vietnam War as Agent Orange to defoliate the jungle. During the manufacturing process, 2,4,5-T was often contaminated with the persistent and very toxic chemical called dioxin, TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin). Dioxins, like other chlorinated compounds including DDT, bioaccumulate in body fat and persist in the environment for many years. Dioxins are classified as carcinogens and are also known to affect the reproductive and immune systems. The organochlorines biomagnify in the food chain. Larger predators accumulate more of these chemicals than the prey in the food chain.

The US Environmental Protection Agency (EPA) canceled the use of 2,4,5-T in 1979 in the United States, but 2, 4-D is still a widely used herbicide.

Neonicotinoids are the newest class of insecticides with widespread use in crop production. The neonicotinoid insecticides include imidacloprid, acetamiprid, dinotefuran, thiamethoxam, nithiazine, and clothianidin. From 2000 to 2010, neonicotinoid insecticides have been the fastest growing class of insecticides in modern crop protection because of their widespread use against a broad spectrum of sucking and certain chewing pests. Neonicotinoids have a relatively low risk for nontarget organisms and the environment, a high target specificity to insecticide, and versatility in application methods. The neonicotinoids have been implicated in the colony collapse disorder in bees, but this has not been proved. The neonicotinoids have a wide safety margin in mammals, and the insecticidal activity can be targeted so this class of compounds can have wide future use as an insecticide.

Fipronil, a phenylpyrazole, is also a new generation of insecticides. Fipronil, like the neonicotinoids, can be applied at very low dose rates.

Biologically Based Pesticides

Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal applications and are considered biopesticides. At the end of 2001, there were approximately 430 registered biopesticide active ingredients and 1320 products.

The most widely used microbial pesticides are subspecies and strains of *Bacillus thuringiensis*, or Bt. The genes for Bt have been inserted in the genome of plants, and these plants are called genetically modified organisms (GMOs). Each strain of this bacterium produces a different mix of proteins and specifically kills one or a few related species of insect larvae. While some Bts control moth larvae found on plants, other Bts are specific for larvae of flies and mosquitoes. The target insect species are determined by whether the particular Bt produces a protein that can bind to a larval gut receptor, thereby causing the insect larvae to starve. Biochemical pesticides include substances, such as insect sex pheromones that interfere with mating as well as various scented plant extracts that attract insect pests to traps. Because it is sometimes difficult to determine whether a substance meets the criteria for classification as a biochemical pesticide, the EPA has established a special committee to make such decisions.

Glyphosate

Glyphosate, N-(phosphonomethyl)glycine, is a widely used nonselective herbicide discovered by a Monsanto chemist, John Franz, in 1970. Monsanto has marketed glyphosate as a product called Roundup. In 2007, glyphosate was the most used herbicide in agriculture in the United States and the second most used herbicide in the lawn and garden market. Glyphosate inhibits an enzyme involved in the production of tyrosine, tryptophan, and phenylalanine in plants but not mammals. It is absorbed through foliage and translocates to growing portions of the plant. Crops have been produced that are glyphosate-resistant; this resistance allows the crop to be sprayed with glyphosate, but the crop will be unaffected and only the weeds in the field will be killed. There are concerns that glyphosate can cause adverse health effects in humans from acute and chronic exposure. More data however are needed to completely answer this question.

Health Effects

Human Health and Pesticides

The World Health Organization estimates that 3 million workers in agriculture in the developing world have severe poisoning from pesticide use. Of the 3 million exposed, 18 000 die. One of the worst pesticide-associated death losses occurred in Bhopal, India, on December 2, 1984. Methyl isocyanate leaked from a Union Carbide plant producing carbaryl and killed 2259 people immediately and over 8000 died from gas-related diseases. Long-term, low-dose exposure to pesticides has been linked to human health effects like immune

suppression, hormone disruption, reproductive abnormalities, cancer, and diminished intelligence. In studies conducted from 2001 to 2011 in Asia and Africa, dichlorodiphenyltrichloroethane (DDT) was found in concentrations exceeding the Codex Maximum Residue Limits/Extraneous Maximum Residue Limits for milk (20 ng g^{-1} lipid wt. whole milk) in human breast milk. Pesticides can cause risks for human fetuses.

There were 3017 calls about pesticide exposures in the 2010 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 28th Annual Report. Pesticides were included in 3.3% of calls about poison exposure.

Suicide using pesticides is a major public health problem in the developing world. The World Health Organization recognizes pesticide poisoning to be the single most important means of suicide worldwide. In areas of the world where pesticide poisoning is a public health concern, the use of pesticides has to be balanced with the agricultural uses. A classification of toxicity of oral and dermal dose of pesticides is given in Table 2.

Environmental Effects

Pesticide use results in widespread distribution of chemicals that regulatory agencies evaluate for risks to environmental and human health. The widespread concern for pesticide use and the impact on human and animal health initially occurred with Rachel Carson's publication of Silent Spring in 1964. Approximately 245 000 000 kg of active pesticides was applied in the United States in 1964. Worldwide, approximately 3 billion kg of pesticides is applied each year at a cost of over $40 billion.

From 1992 to 2011, pesticides were found at concentrations that exceed regulatory limits in waterways that drain agricultural, urban, and mixed land use watersheds. For agricultural and mixed land use waterways, the proportions of waterways contaminated with pesticides did not change from 1992 to 2001 compared to 2002–11. During this same time, pollution of urban streams increased 53% largely because of fipronil and dichlorvos. More than 400 different pesticides are used in the United States every year.

In Europe, the effect of pesticides on the environment is measured by microcosm and mesocosm studies. These studies are dependent on the no observed effect concentrations (NOECs). The studies using these systems are artificially constructed systems from portions of natural ecosystems. These systems assess the effect of chemicals on the ecosystem. The statistical reliability of the conclusions from these studies depends on the power of the test conducted. Laboratory studies are useful but must be accompanied by field studies to evaluate all the complex interactions that occur in nature.

Economics

Human health and environmental cost from pesticides in the United States is estimated at $12 billion. This estimate is from 2009. Losses associated with pesticide use in the United States include impacts on public health; livestock and livestock product losses; development of pesticide resistance in pests; crop pollination problems; honeybee problem; bird, fish, and other wildlife losses; and the governmental cost of reducing the impact on the environment and society.

In the United States, there are approximately 500 million kg of over 600 different pesticides applied at a cost of $10 billion. In 2009 alone, an investment of approximately $10 billion dollars in pesticide control saved $40 billion dollars in US crops annually.

Regulation

International

At a conference of the United Nations Food and Agriculture Organization in 1985, a code pertaining to pesticide use was adopted. The International Code of Conduct on the Distribution and Use of Pesticides created voluntary standards of pesticide regulation for different countries. The code was updated in June 2013.

Pesticide safety education and pesticide applicator regulation are methods of protecting the public from pesticide misuse. Education and regulation have the potential to reduce the risk of pesticides by using less toxic pesticides. Integrated pest management (IPM) is the use of multiple approaches to control pests. IPM recognizes the more widespread impacts of an action on an ecosystem.

The United States

The Environmental Protection Agency (EPA) is responsible for regulating pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Food Quality Protection Act (FQPA).

Table 2 WHO classification of toxicity of pesticides

Class	*Description*	*LD50 for the rat (mg kg^{-1} body weight)*			
		Oral		*Dermal*	
		Solids	*Liquids*	*Solids*	*Liquids*
Ia	Extremely hazardous	≤5	≤20	≤10	≤40
Ib	Highly hazardous	5–50	20–200	10–100	40–400
II	Moderately hazardous	50–500	200–2000	100–1000	400–4000
III	Slightly hazardous	>500	>2000	>1000	>4000

Reproduced from Dawson, A. H., Eddleston, M., Senarathna, L. et al. (2010). Acute human lethal toxicity of agricultural pesticides: a prospective cohort study. *PLoS Med*, 7, e1000357.

The EPA regulates pesticides under two main acts, both of which amended by the Food Quality Protection Act of 1996. The US Department of Agriculture (USDA) and the US Food and Drug Administration (USFDA) set standards for the level of pesticide residue that is allowed on or in crops.

Pesticides are applied at individual sites, but chemicals can be widely distributed by atmospheric drift, runoff, and movement through the food chain. The EPA evaluates the risks and benefits of pesticides to human and environmental health.

See also: Arsenic: Properties and Determination; Arsenic: Toxicology and Health Effects; Fungicides; Genetically Modified Microorganisms; Heavy Metal Toxicology; Insect Pests; Pesticides and Herbicides: Types of Pesticide; Pesticides and Herbicides: Types, Uses, and Determination of Herbicides.

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http://www2.epa.gov/pesticide-registration/pesticide-registration-manual – EPA, Pesticide Registration Manual.

http://extoxnet.orst.edu/ – Extoxnet, the Extension Toxicology Network.

http://www.who.int/ipcs/publications/pesticides_hazard/en/– The WHO Recommended Classification of Pesticides by Hazard.

Pesticides and Herbicides: Residue Determination

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Introduction

Pesticides are a general class of chemicals or biological agents used to control, repel, attract, or kill pests. They are often classified according to the organism they are used to control. Although the term pesticide is frequently used interchangeably with insecticide, it has a much broader meaning. In addition to insecticides, pesticides comprise herbicides, fungicides, fumigants, rodenticides, nematicides, algicides, and molluscicides. This group includes more than 1000 substances active against pests that present different chemical structures and properties. Herbicides are a broad category of pesticides that cover nearly 70% of all pesticides used by farmers, the reason why sometimes this group deserves a special attention.

In the present agricultural practice, pesticides provide an unquestionable benefit by increasing the agricultural production. However, each year, 140 000 tons are sprayed onto crops in the European Union (EU) alone, and even though pesticides are degraded or dissipated, certain amount remains in crops and vegetables. Then, food is the primary source of pesticide exposure for humans. Raw materials are directly contaminated through the application and transmission of the pesticide and its residues through the food chain.

Pesticide residue is defined by the World Health Organization as "any substance or mixture of substances in food for man or animals resulting from the use of a pesticide and includes any specified derivatives, such as degradation and conversion products, metabolites, reaction products, and impurities that are considered to be of toxicological significance." The sustainable use of pesticides is achieved through fixation of the maximum residue limits (MRLs). These limits restrict the concentration of a residue permitted on a commodity and constrain the type of commodity on which it is allowed. It is the responsibility of the government authorities to register and set MRLs and to implement pesticide residue monitoring programs in order to control the pesticide quantity in food and to enforce tolerances.

In this context, analytic methods are needed to screen, quantify, and confirm pesticide residues in food for both research and regulatory purposes. In the recent years, significant evolution was noted in the extraction and determination of pesticide residue analysis in food. Since years ago, some major premises and basic rules have made up these analyses, in such a way that they were reliable, rapid, and feasible. Multiresidue methods (MRMs), which determine a wider range of multiclass pesticides as possible, are preferred over single-residue methods because the products applied and the regularity of the treatments are generally unknown and residues of more than one pesticide can be present in samples.

This article provides a review of the current status of the routine methods in the monitoring and control of pesticide residues and discusses the analytic problems arising at each stage of the procedure. Reported information is focused on the methodology applied in the screening of pesticide residues within the monitoring programs and its innovations. The role of mass spectrometry (MS), a universal and selective approach, within this field is highlighted. This article examines the capabilities of MS to determine pesticide residues in food and its possibilities to compensate the lacks still presented by the MRMs. Consideration is given to new developments and future trends.

Multiresidue Methods

A number of analytic methods designed to determine multiple pesticide residues have been developed since the 1950s and 1960s. Chromatographic methods represent the most suitable approach for determining pesticide residues in food samples, and MRMs represent an efficient and widely used approach for pesticide analysis. In general, MRLs in most of the current legislation are in the range 0.01–10 mg kg^{-1}, depending on the commodity–pesticide combination, the lowest level being characteristic of banned or very toxic compounds – because it is considered that this would be the minimum limit of detection. However, these MRLs can be even lower for some particular cases, as for baby food.

The low parts per billion level at which a pesticide residue should be analyzed in a complex food matrix, such as fruits, vegetables, milk, meat, or eggs, represents a major analytic challenge. Another problem that should be considered in residue analysis concerns the pesticide metabolites and/or degradation products included in the residue definition that need to be detected by the analytic methods. Most pesticides are oxidized, reduced, or hydrolyzed by different mechanisms to more polar forms. In addition to these known metabolites, the presence of unknown metabolites may interfere with the analysis, and further identification of these unknown and confirmation for each compound are required. Pesticide residue definitions are periodically revised to incorporate all metabolites of concern. The analysis of the metabolites is so complicated that there are two residue definitions established: one used for monitoring and enforcement and the other for risk assessment to ensure better evaluation of the risk for human beings.

An ideal method for residue analysis should be sensitive, selective, accurate, precise, automated, cheap, applicable to a wide range of pesticides and matrices, and capable of providing unambiguous structural information. However, such ideal methods are not encountered in practice. Analytes must be isolated from matrices and then enriched before the final determination to eliminate matrix components and to ensure the appropriate sensitivity. The complete procedure for determining pesticide residues in food is complex and consists of several stages, which, together with the different possible analytic techniques, are summarized in Figure 1. Of these possibilities, this article will only describe those used for routine monitoring.

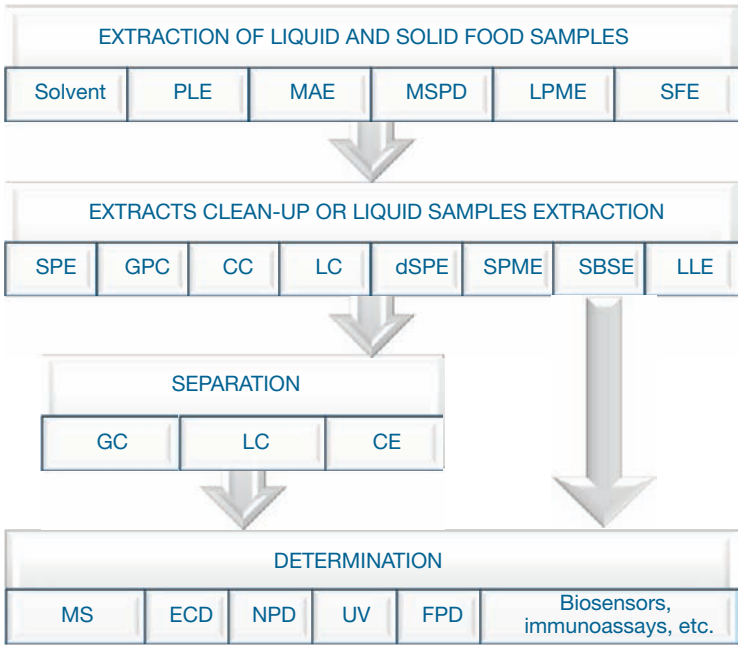

Figure 1 The main stages in analytic procedures for pesticide residue determination in food.

Extraction

Factors relating to the choice of the extraction procedure are (a) the determination techniques, (b) the nature and number of pesticides, and (c) the complexity of the matrix. The first prominent MRM, the Mill's method, was developed at the Food and Drug Administration (FDA) in the United States during the 1960s to measure nonpolar organochlorine pesticides in nonfatty food. The Mill's method utilized acetonitrile extraction and acetonitrile/water partitioning. Follow-up research in the 1970s used NaCl for more efficient extraction and partitioning.

The disadvantage of the Mill's and other similar methods was their inefficiency as screening procedures. These methods are too complex, labor-intensive, and time-consuming, and, many times, they do not generate relevant data in time to prevent contaminated foods from entering the marketplace (it should be taken into account that most food samples are highly perishable). These schemes required an amount of chemicals and toxic solvents that is usually greater by a factor of 10^8–10^{10} than that of the pesticide to be determined. Furthermore, increasing polar and/or thermodegradable pesticides should be incorporated into the existing MRMs.

In this sense, the increased sensitivity, selectivity, and specificity of analytic instrumentation for the final determination of pesticide residues have reduced the sample treatment. Nonselective detectors in gas chromatography (GC) or liquid chromatography (LC) (such as nitrogen–phosphorus detector (NPD), electron capture detector (ECD), or UV) need more extensive sample cleanup. In contrast, current MS techniques in combination with GC or LC simplify, or even eliminate, the cleanup of the sample extract.

Solid-phase extraction (SPE) methods have been used since the 1980s to replace liquid–liquid extraction (LLE) methods, which have been limited due to mounting environmental and health concerns.

The amount of research on the ways to reduce solvent usage and laboratory time has increased since the 1990s, and several alternative extraction methods including matrix solid-phase dispersion, supercritical fluid extraction, liquid-phase microextraction (LPME), stir bar sorptive extraction, and solid-phase microextraction have been introduced. However, at the moment, these methods are not robust and reliable enough to replace more conventional solvent extraction.

The efficiency of the extraction methods could be improved by the application of alternative sources of energy such as microwaves or high temperatures, which are applied in techniques such as microwave-assisted extraction and pressurized liquid extraction. However, the additional amount of laboratory material, as well as the extra energy required, is not proportional to the provided efficiency increment. Manual or mechanical shaking and ultrasounds are preferred to guarantee an appropriate efficiency.

During the 2000s, new advanced technologies incorporating a rapid and simple pretreatment step were developed to detect pesticides in foods. These methods are characterized for being as generic as possible, achieving the extraction of the majority of pesticides and even the extraction of other toxic organic compounds from food such as antibacterials, mycotoxins, and toxins. The required specificity will be provided by the determination technique. Among these types of procedures, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method has become the most popular. This two-step system based on partitioning with acetonitrile, followed by a dispersive SPE (dSPE) cleanup with primary–secondary amine (PSA) and/or other sorbents, provides a clean extract appropriate for both GC and LC. This approach has been verified and

modified at several US FDA (AOAC Official 2007.01 Method) and EU (European EN 15662 Method) laboratories, and the method now is widely accepted for many types of pesticide residue samples. Reviews of QuEChERS are starting to appear in the literature, and the original method has evolved into a flexible template for modification in several applications. Validation and proficiency data are available for a wide variety of pesticides in several common food matrices. Commercially available QuEChERS extraction and dSPE products in a variety of standard sizes and formats make this approach even simpler. The straightforwardness of this method is schematized in Figure 2.

Currently, QuEChERS methodology makes possible the simultaneous determination of more than 800 pesticides and even other contaminants in crops. This extraction procedure conforms to the principles of green analytic chemistry because it

- enables a wide range of analytes to be determined in a single analysis using MRMs;
- ensures maximum removal of interferences from extracts;
- yields high recoveries of compounds, is very sensitive, and guarantees good precision; and
- consumes the smallest possible amounts of chemical reagents, especially organic solvents.

Despite the widespread use of the QuEChERS method in the control of pesticide residues, the extraction step is not yet a closed chapter. The current trend toward the use of smaller amounts of sample and organic solvents will continue to develop in the future to save the bottleneck that still represents the extraction of samples.

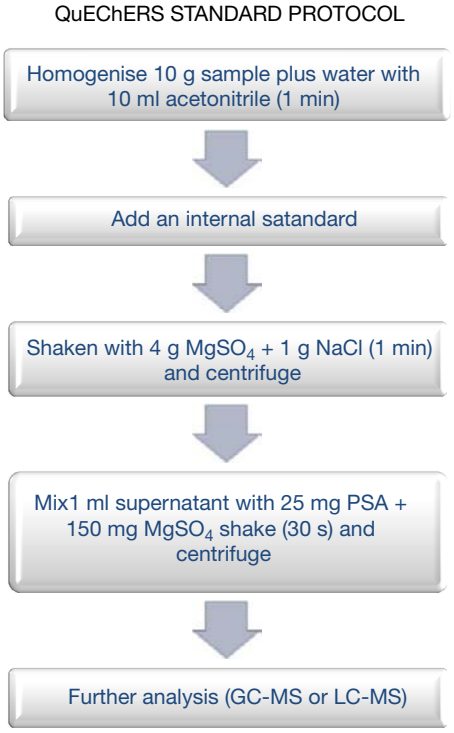

Figure 2 Scheme of the Mill's and QuEChERS multiresidue methods.

Cleanup methodology

Food extracts contain, in addition to pesticides, many other compounds, mostly coming from the matrix, which produce several analytic problems. Their loading onto chromatographic columns can deteriorate the separation efficiency and cause irreparable column damage. Furthermore, with nonspecific detectors, these compounds can interfere with the detection systems and give rise to false-positives. Therefore, direct analysis is not always possible without further purification for cleaning up the extract and enriching the target analytes. LLE, gel permeation chromatography, column chromatography (CC), and even LC (either normal- or reversed-phase) were extensively developed in the past to further purify trace pesticides from crude extracts.

If the determination is carried out by MS, these coextracted materials are responsible for the matrix effect characterized by enhanced or more frequently suppressed analyte signals that can heavily affect detection and quantification. Despite these effects, nowadays, there is a trend to keep the cleanup part as simple as possible or even eliminate it. LPME has been used as an alternative to LLE for cleanup and as a replacement for CC; SPE with small commercial cartridges is often applied after the different extraction techniques to purify the extracts of fruits, herbs, and other vegetable samples. An effective cleanup step is still required in order to eliminate interfering compounds (mainly lipids and pigments) present in the extracts. However, currently, laborious and time-consuming cleanup procedures are being replaced with LPME, SPE, and dSPE.

dSPE was included as a novel cleanup technique for the QuEChERS. It is based on the addition of the sorbent material into an extract aliquot to remove the matrix interferences, which is then separated from the extract bulk by centrifugation. In this way, dSPE avoids passing the extract through a SPE column, using a much smaller quantity of sorbent and solvent, saving time and labor. Mostly, PSA was used as dSPE sorbent that could remove various polar organic acids, polar pigments, some sugars, and fatty acids. Further research modified the original method with changes of sorbents used in the dSPE step. Modified QuEChERS cleanup steps with GCB or C_{18} as sorbents were reported; in these cases, sterols and pigments, such as chlorophyll, may be absorbed by GCB and nonpolar interfering substances like lipids could be removed by C_{18}. Although the dSPE cleanup is faster and easier, it is not as effective as the use of SPE columns. dSPE procedure could not be sufficient in removing food colorings such as chlorophyll, carotene, and water-soluble materials. In some cases, the QuEChERS was modified, changing dSPE to more conventional SPE.

Although the current trend is to reduce to the maximum cleanup process, routine practice highlights its extreme importance to obtain a correct identification and quantification of pesticides in food. However, this is a long and tedious stage of the process that is difficult to optimize. Currently, it is still needed to reach a balance between the spent time and the quality of the results.

Determination

Since the early 1970s, most routine pesticide residue determination has been conducted by GC with different detectors, ECD, NPD, flame photometric detector, etc. During the

1980s, these detectors were quickly replaced by MS detector, given that this detector is very specific and provides enough structural information to achieve confirmation of compound identity.

However, since the 1990s, there has been a clear trend to increase the number of LC applications in pesticide residue analysis, both in specific/individual methods and in MRMs, especially after the introduction into the market of robust, easily operated LC–MS instruments that provide a new way of analyzing pesticides that is more sensitive and efficient and with greater scope (~80% of currently used pesticides can be determined by without long and tedious derivatization). Furthermore, LC–MS showed better performance than GC for all pesticides except organochlorine. However, GC is still an unquestionable tool within pesticide residue determination since LC has some disadvantages compared with GC such as lesser resolving power.

Capillary electrophoresis emerged some years as an interesting alternative but it is not applied in routine monitoring, mostly because it is always less sensitive than LC or GC.

There are also nonchromatographic or miniaturized methods for residue detection. These methods consist of a wide variety of techniques. For illustrative purposes, these may be divided into 'biological'- and 'physical'-based methods, depending on whether or not biological reagents are involved. Biological techniques include immunoassays, biosensors, bioassays, and enzyme assays. However, these techniques, despite their interesting prospects within the field, are not well suited for multiresidue analysis because they can only be used to simultaneously determine a limited number of compounds.

MRMs enabling the determination of pesticide residues at low concentration levels need highly sophisticated MS instrumentation. The first mass spectrometer available to determine pesticide residues at trace level was the quadrupole. However, the development of new mass spectrometers has created the appropriate background to improve the scope of the MRMs. Within this situation, two emerging trends in the mass analyzer design are valuable to get better quality and quantity of structural information:

- High-resolution (HR) or high-accuracy mass spectrometers, which calculate elemental composition because they can determine the exact mass of analytes, are a tool to identify unknown substances in the samples. These instruments easily provide the full-range mass spectrum with high sensitivity. Most used in pesticide residue determination are the time of flight (ToF) and the orbitrap.
- Tandem mass spectrometry (MS/MS) allows the mass spectrometer to isolate an ion and fragment it to obtain its mass spectrum again. The mass spectrometers capable of carrying out MS/MS only with nominal mass are the triple quadrupole (QqQ), ion trap (IT), and quadrupole–linear ion trap (QTrAP). The quadrupoles have the inconvenience of low sensitivity using scan, but ITs and QTrAPs can provide full-range mass spectra with appropriate sensitivity. Furthermore, the hyphenation of different mass analyzers has also achieved the possibility of combining HR and MS/MS as it is the case of the quadrupole–ToF (QqToF) and the QTrAP-orbitrap.

These mass analyzers can be combined indistinctly with either GC or LC. However, orbitrap is still considered as a research tool within LC–MS. Thanks to these combinations and their wide range of possibilities, two types of analysis can be established, depending either on the way the analysis is approached or on its final purpose; the determination of pesticide residues may be targeted and nontargeted as schematized in Figure 3. Each of these analyses can be performed by either LC or GC and by either nominal or HRMS with some limitations that will be discussed later in the text.

Target analysis

This is a conventional analysis based on establishing a method with standards prior to analysis and monitoring real samples. Target analysis can be carried out with all types of mass spectrometers, and commonly, it achieves better sensitivity because the chemical background is reduced using selected ion monitoring in low-resolution quadrupole or selected reaction monitoring (SRM) in MS/MS. The main problem to confirm the identity of the compounds in these cases is the presence of isobaric interferences that are compounds with the same nominal mass and different chemical structure. The normal practice to confirm the identity of the compounds is to select several characteristic fragments (commonly three). Better specificity is obtained for MS/MS because even if several coeluting compounds have identical molecular mass, usually (nominal) isobaric ions can be distinguished by the SRM experiments, because the fragmentation in the collision cell frequently results in different product ions. The main advantages of MS/MS are their very good quantitative capabilities and their great sensitivity in the SRM mode and the possibility to select simultaneously multiple transitions. For this reason, QqQ mass analyzers have been the most often applied MS detectors. It should be kept in mind that target analysis can also be carried out using HRMS. In this case, the background signals produced from sample matrix and LC solvent clusters are reduced by the use of very narrow mass ranges for the extraction of the chromatogram.

Nontarget analysis

All compounds eluted from the analytic column are detected and identified without any previous selection. This nontarget approach has the advantages of identifying unexpected pesticides and metabolites as components present in the sample and allowing the proposal of their elemental compositions. In general, this approach is limited by sample treatment step and also by ionization mode. Only compounds working on selected ionization conditions and compatible with the sample-extraction procedure can be detected.

Any instrument able to provide full spectrum in an m/z range can be used for nontarget analysis, including quadrupole. However, HRMS has opened new possibilities because the accurate mass measured allows us to obtain the empirical formula of the pesticide facilitating its identification. Extensive screening and searching of a wide range of pesticide residues or even other compounds can be afforded using databases. Depending on the detector degree of sophistication, these databases include library search, isotopic signature information, element-specific information from isotopic signatures, relative intensities, and areas from isotopes (ratios and

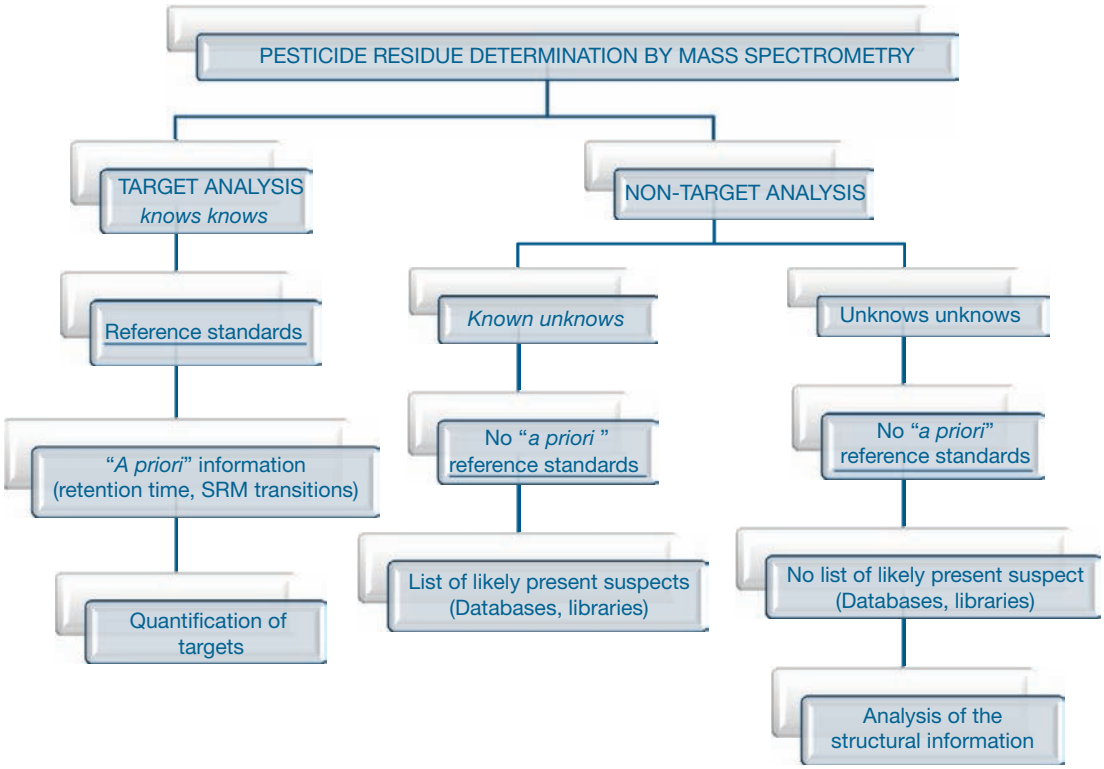

Figure 3 Target and nontarget pesticide residue determination.

abundances), making an accurate identification of the compounds without the 'a priori' need of analytic standards.

The acquired full-scan MS data can be used to retrospectively examine data for nontarget pesticides and/or metabolites. The information of the accurate molecular ion, isotopic pattern, and detected fragment ions can be used to characterize the structure of nontarget ions. Figure 4 shows the residue of imazalil in an apple sample, detected and identified in a nontarget way by GC-ToF-MS. Accurate mass confirmation automatically performed for four representative ions and the library forward match (>700 used as criterion) led to the confirmation of the identity of imazalil with mass errors below 1.8 mDa for all ions. In addition, the structures proposed for at least four fragment ions observed in the EI spectrum were compatible with the chemical structure of imazalil. The injection of reference standards allowed the presence of imazalil in the samples to be confirmed.

Gas chromatography–mass spectrometry

Nowadays, more than 60% of the pesticide residues are still detected by GC. With more benefits such as simple manipulation, fast speed, and high separating efficacy, GC is widely used to analyze volatile and thermostable pesticide residues with relatively small molecular weight, such as organochlorine, organophosphorus, and pyrethroids.

Typical GC–MS methods use a relatively long, narrow-bore analytic column (30 m, 0.25 mm i.d.), which generally requires >30 min run time per sample, especially when analyzing >100 GC-amenable pesticides. However, faster chromatographic separation reduces the analysis time, which can be translated in a significantly increased sample throughput. Among the alternatives, low-pressure GC–MS has advantages of increased ruggedness, sensitivity, and speed (150 pesticides were separated in <6.5 min) with the only sacrifice of reduced chromatographic separation efficiency.

Another possible approach to faster GC is to optimize selectivity (ability to distinguish between compounds) with respect to the utilization of time. Two-dimensional (2D) GC coupled to ToF-MS (GC × GC-ToF-MS) provides an additional separation because coeluting peaks, resulting from a one-dimensional separation, can be separated by a second column possessing a different stationary phase. The resulting GC × GC chromatograms form narrow peaks and require a mass spectrometer such as high-speed ToF to be fast enough to scan enough data points for each peak. This high-speed ToF only provides nominal mass differently to the high-resolution ToF. This approach is not extensively used within the pesticide residue field because the difficulties in processing the high amounts of obtained information.

For analytes amenable to GC–MS, identification of nontarget compounds is sometimes feasible with low-resolution quadrupoles, as forward-search methods enable library searching (e.g., the large library of the National Institute of Standards and Technology). However, to obtain an unbiased dataset, full-spectrum acquisition techniques are required. Single-quadrupole and QqQ instruments generally do not operate in full-scan mode when applied to residue analysis because of the lack of sensitivity. IT instruments normally have higher sensitivity in scan mode, but they have low selectivity because they acquire mass spectra in nominal mass, a drawback that also applies to single-quadrupole and QqQ analyzers.

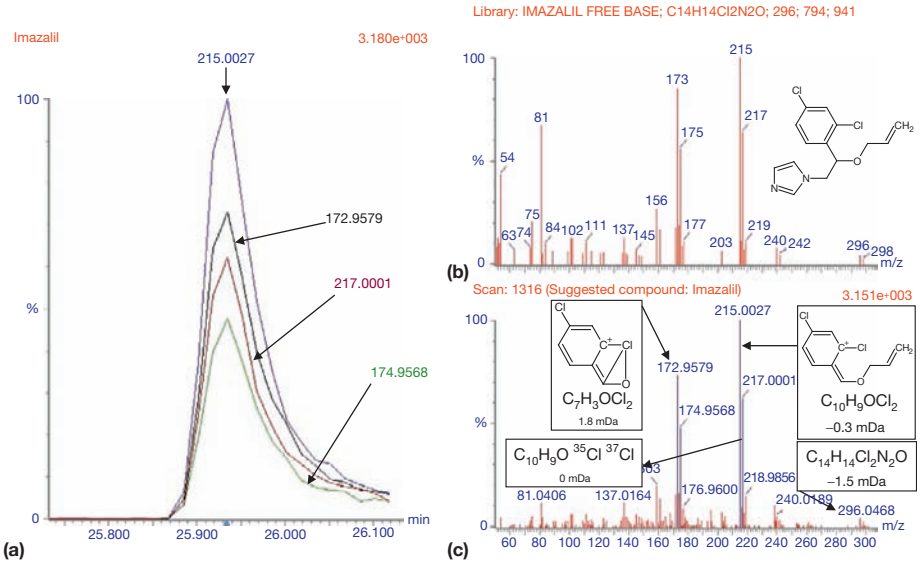

Figure 4 Identification of nontarget imazalil in apple. (a) Extracted ion chromatograms for four imazalil ions used for deconvolution. (b) Library mass spectrum of imazalil at nominal masses (match 839). (c) Deconvoluted accurate mass spectrum of imazalil from the sample and chemical structures proposed for four representative EI fragment ions together with mass errors. Reproduced from Cervera, M. I., Portolés, T., Pitarch, E., Beltrán, J. and Hernández, F. (2012). Application of gas chromatography time-of-flight mass spectrometry for target and non-target analysis of pesticide residues in fruits and vegetables. *Journal of Chromatography A* **1244**, 168–177, with permission from Elsevier.

GC has been combined with HR-ToF-MS (GC-HR-ToF-MS) for nontarget screening of GC-amenable pesticides. This MS analyzer provides the selectivity and the sensitivity required for efficient, wide-range screening, as it combines high, full-spectral sensitivity with high mass resolution to measure accurately the mass of any ionizable component in the sample. Elemental compositions can be proposed with low mass errors (typically below 5 ppm). ToF-MS can provide a notable amount of chemical information in a single experiment, so this technique is very attractive for performing nontarget analysis, as illustrated in Figure 4.

Liquid chromatography–mass spectrometry

LC–MS has become one of the reference techniques in pesticide residue analysis, matching to the GC or even surpassing it. The column is the soul of an LC system. Traditional LC columns are 50–300 mm long and have internal diameters between 3 and 4.6 mm. However, the combination of LC and MS has been a motor in the miniaturization of the LC columns as the MS interfaces require low flow rates (to form smaller droplets in the electrospray). Miniaturization in LC is also an important strategy and is mostly based on reducing the inner diameter of the LC columns or the particle size. Medium, narrow-bore LC columns (50×2.1 mm) and intermediate flow rates (~ 0.2 ml min^{-1}) are often ideal.

The reduction of the particle size, the emerging issue in the field of pesticide separation, is the introduction of new ultra-high-performance liquid chromatography (UHPLC) platforms. This instrument has been shown to give superior chromatographic resolution, reduced analysis time, and increased response. The low degree of band broadening in UHPLC also benefits mass spectrometric detection, concentrating the analyte at the peak center and thereby increasing response. Thus, UHPLC coupled to MS offers improvements in performance for quantitative analysis over existing high LC–MS/MS methodologies. Pesticide residue analysis in food can also benefit from the high sample throughput obtained in UHPLC methods, keeping the high multiresidue capability required for such applications. Although UHPLC technology was launched only recently, many applications to determine pesticide residues can be found, which illustrates the interest in this technique. It has almost replaced most conventional LC. 2D LC approaches have also been developed to improve separation between pesticides and also can favor the separation between pesticides and matrix compounds. These 2D systems are simpler than their GC homologous and shown better perspectives within the field.

LC–MS/MS is most widely employed for the simultaneous qualitative and quantitative profiling of multiple classes of pesticides because of its high sensitivity and selectivity. Compound identification and quantification largely depend upon separation and the uniqueness of the precursor–product ion transitions selected for SRM. This approach has been the most widely applied for the simultaneous determination of individual or multiple classes of pesticides and their metabolites. It has allowed the determination of a great number of pesticides and metabolites, especially polar ones that were previously difficult or even impossible to analyze.

This approach, however, does not enable to identify nontargeted compounds. In other words, the detection of analytes for which a reference substance is not available is a rather complicated (or even impossible) task. Another problem encountered when using a number of MS/MS is moving an analyte out of an originally fixed time window, which requires its readjustment.

ToF-MS, QqToF-MS, and orbitrap in combination with LC are used in HR mode (typical mass error <2 mDa), which provides better discrimination of background and more accurate identification of unknown peaks in a sample, even if analytic standards are not available. The ToF capability of interrogating full-spectrum data postacquisition seems more interesting, as it also allows screening for unexpected metabolites at the time of injection without reanalyzing the sample. Unlike GC–MS, LC–MS still does not have commercially available or standardized spectral libraries due to the absence of a normalized interface. The differences in ionization between existing interfaces, together with variability in results, depending on the composition of the mobile phase or the cone voltage applied, make it difficult to use standardized libraries. This means that it is necessary to build homemade libraries to facilitate searching, focusing on particularly relevant compounds.

The application of a hybrid QqToF-MS technique to pesticide residue analysis in food allows an unequivocal confirmation of the contaminants detected. Eliminating false-positives and avoiding interpretation ambiguities are possible because of its unique characteristic of generating full-scan product ion spectra with exact masses. The main fields of application are the identification of unknowns and elucidation of structures proposed for transformation products, where the amount of information obtained allows secure identification of the identity of compounds.

Conclusions and Future Trends

In the recent years, the detection technology of pesticide residues in food has developed rapidly. At present, some new analytic methods and techniques are making great progress, especially in the multiresidue analysis for many types of pesticides. Particularly, extraction procedures that are simple, generic, and ecofriendly have been fully implemented thanks to the advances in the determination techniques. Nowadays, extraction is not the narrow bottleneck that was before.

MS has clearly modified the strategy applied for the quantification of pesticide residues in food, biological, and environmental samples. MS is now playing a pivotal role in solving analytic problems concerning pesticide residues. Because both GC–MS and LC–MS allow the achievement of high-throughput analysis with high sensitivity and selectivity, the sample preparation can be simplified and sped. The enhanced selectivity of MS/MS techniques is almost mandatory for complex sample matrices like fruits and vegetables.

With the introduction of QqQ-MS, IT-MSn, LIT, ToF, QqToF, and orbitrap instruments, all major classes of pesticides can be detected, identified, and quantified satisfactorily. Currently, the first option is typically QqQ-MS or LIT-MS in multiple SRM for target pesticide analysis. However, the gradual introduction of the newly developed accurate mass spectrometers, such as the QqToF or the orbitrap, with the distinctly enhanced selectivity and the possibility of calculating elemental composition will improve the performance of the analysis and open the door to the identification of nontarget and unknown pesticides and metabolites.

No doubt, in the next decade, LC–MS and GC–MS will develop new analytic tools for the characterization of a pesticide in parts per trillion levels or lower, and the detection of a pesticide with a very low threshold will be among the leading options.

See also: Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Codex Alimentarius Commission: Role in International Food Standards Setting; Codex Alimentarius; Consumer Protection Legislation; Food and Agriculture Organization of the United Nations; Fumigants; Fungicides; Immunoassays: Principles; Infrared Spectroscopy: Applications; Insect Pests; Legal Requirements for Food Hygiene; Mass Spectrometry: Applications; Mass Spectrometry: Principles and Instrumentation; Pesticides and Herbicides: Types of Pesticide; Pesticides and Herbicides: Types, Uses, and Determination of Herbicides; Pesticides and Herbicides; Quality Control in Food Processing; Spectroscopy: Types; Supercritical Fluid Extraction; World Health Organization.

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Pesticides and Herbicides: Types of Pesticide

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Introduction

The use of synthetic pesticides started in 1948–49 with the use of 1,1,1-trichloro-2,2-bis (chlorophenyl)ethane (DDT; Figure 1) for malaria and hexachlorobenzene (BHC) for locust control. Since then, pesticides play a key role in agriculture and animal husbandry. Following the end of the Second World War and the introduction of synthetic organic insecticides for the control of insect pests, a great number of companies took up the research for the synthesis of new pesticides, mainly insecticides. In 1950, more than 30 companies in the United States and Europe were involved in pesticide discovery, and by the end of 1960, this number had risen to 50 companies. The next decade, this number declined slightly. Later on, there has been a substantial degree of reduction in agrochemical companies. There were many reasons for this steady reduction, among them the most important was the increasing regulatory requirements as regards the human and environmental safety leading to escalating costs in the development and registration of new products.

Pesticides are defined by the FAO (Food and Agricultural Organization) as substances or mixtures of substances intended for controlling, preventing, destroying, repelling, or attracting any biological organism deemed to be a pest. The term pest includes insects, mice, and other animals, weeds, and fungi, among others. Pesticides are usually chemical substances, although they can be sometimes biological agents such as viruses or bacteria. The term 'pesticides' is often used as a synonym for plant protection products, which are mainly used in agriculture to keep crops healthy and prevent them from being destroyed as a consequence of disease and infestation. However, pesticides include also the class of biocides that are used for similar purposes but in fields other than agriculture. For example, pesticides have been used in the human health sector for the control of insect-borne diseases such as malaria, dengue, and encephalitis, especially in tropical countries.

Plant protection products were initially applied to cereal crops, and now, they are used in the agriculture sector including horticultural, ornamental, and vegetable crops. The active portion of a plant protection product, known as active ingredient, is usually the only component of the formulation listed on the label and is generally formulated by the manufacturer as liquid or solid formulation. The most widely available formulation types are listed in Table 1. The active ingredients used in plant protection products are the chemicals or microorganisms including viruses that are the essential component enabling the product to be effective. For example, they may destroy unwanted plants in the case of herbicides or protect the plant against insects or fungi that destroy plants or reduce crop yields.

Agricultural yields strongly depend on crop protection measures. Great productivity gains can be achieved in agriculture with the use of pesticides. Indeed, they are needed to meet the world's demand on foodstuffs, and there are no other alternatives that can be used in such a large scale. Pesticides help farmers and growers to produce good-quality food at reasonable prices. The main purpose of pesticide use is to

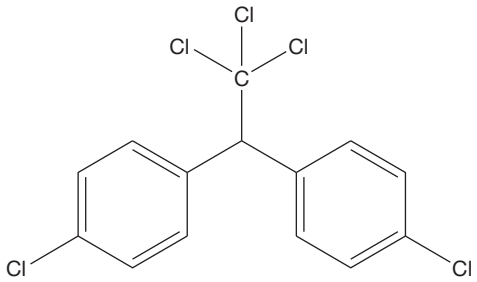

Figure 1 Molecular structure of DDT.

Table 1 Catalog of the most widely available pesticide formulation types and their international coding system as reported in the 'Manual on development and use of FAO and WHO specifications for pesticides'

Formulation types	*Definition*
Capsule suspension (CS)	A stable suspension of capsules in a fluid, normally intended for dilution with water before use
Dustable powder (DP)	A free-flowing powder suitable for dusting
Emulsifiable concentrate (EC)	A liquid, homogeneous formulation to be applied as an emulsion after dilution in water
Emulsion, oil in water (EW)	A fluid, heterogeneous formulation consisting of a solution of pesticide in an organic liquid dispersed as fine globules in continuous water phase
Granule (GR)	A free-flowing solid formulation of a defined granule size range ready for use
Oil dispersion (OD)	A stable suspension of active ingredient(s) in a water-immiscible fluid, which may contain other, dissolved active ingredient(s), intended for dilution with water before use
Water-soluble granule (SG)	A formulation consisting of granules to be applied as a true solution of the active ingredient after dissolution in water, which may contain insoluble inert ingredients
Soluble concentrate (SL)	A clear to opalescent liquid to be applied as a solution of the active ingredient after dilution in water
Technical material (TC)	A material resulting from a manufacturing process comprising the active ingredient, together with associated impurities. This may contain small amounts of necessary additives
Water-dispersible granules (WG)	A formulation consisting of granules to be applied after disintegration and dispersion in water
Wettable powder (WP)	A powder formulation to be applied as a suspension after dispersion in water

increase food security, with a secondary goal being an increased standard of living. Other benefits from their use include reductions in agricultural labor and energy expenses.

However, pesticides have also negative impacts on human health and the environment. Humans are exposed to pesticides by different routes, such as inhalation, ingestion, and dermal contact, which may result in acute or chronic health problems. Additionally, the environmental contamination from pesticides provokes nontarget species poisoning and disturbance of the ecosystems. For these reasons, the pesticide regulatory authorities have established regulations for their authorization, proper use, and disposal.

In this article, several types of pesticide classification are presented, and certain related definitions are given for clarification purposes. Their main adverse health effects and the environmental contamination and its impact are described. Finally, current regulations are reported with emphasis to those of the European Union.

Definitions

A pesticide formulation is a mixture of different components prepared properly for safe use. Definitions regarding basic terms, useful to understand what components constitute a pesticide product, are presented and are based on those outlined in the 'Manual on development and use of FAO and WHO (World Health Organization) specifications for pesticides' published in November 2010 (second revision of the first edition), prepared by the FAO/WHO Joint Meeting on Pesticide Specifications.

The term 'pesticide' is considered to embrace active ingredients in any form, irrespective of whether, or to what extent, they have been formulated for application. The term is usually associated with materials intended to kill or control pests (insecticides, fungicides, herbicides, etc.), but it also embraces certain materials to control the behavior or physiology of pests (e.g., insect repellents and insect growth regulators) or of crops during production or storage.

The term 'active ingredient' refers to the component(s) of a formulation responsible for the direct or indirect biological activity against pests and diseases or in regulating metabolism/growth. A single active ingredient may be composed of one or more chemical or biological entities that may differ in relative activity. A formulation may contain one or more active ingredients.

'Formulation' is a pesticide preparation containing technical-grade active ingredient(s) and formulant(s) in a form suitable for use.

'Formulant' is any substance, other than the technical-grade active ingredient, intentionally incorporated in a formulation.

In most of the cases, pesticide products contain impurities; among them, only the case of what is a relevant impurity should be clarified. As 'relevant impurity' is considered a by-product of the manufacture or storage of a pesticide, which, compared with the active ingredient, is toxicologically significant to human health and the environment, it is phytotoxic to treated plants, affects the stability of the pesticide, or causes any other adverse effect. In order to be clarified, an impurity may be nonrelevant in one pesticide product and relevant in another, even though it occurs in both, because relevance is determined by hazards relative to those of the active.

Classification of Pesticides

Pesticides can be classified in a number of ways. Basically, they can be divided into plant protection products and biocidal products. The former are used in agriculture to protect crops and in forestry to protect trees from pests and diseases. In contrast, the field of application of biocidal products involves human and veterinary hygiene, food preservatives, and antifouling products, among others, but not agriculture and related areas. Both of these main categories can be further classified according to the target organism, the chemical structure, and the source of the active substance. Another classification concerning the formulation type of pesticides has already been illustrated in Table 1. Other classifications involve the developmental stage affected, acute and chronic toxicity, general action on pests, biochemical or physiological activity, and application method. The most representative classifications of the general term pesticides are described in the succeeding text. At the end of this section, a classification concerning only biocidal products is presented in Table 2.

Target Pest Species and Pesticide Function

One of the most typical classifications of pesticides is based on the type of pest against which they are directed. The main categories are herbicides, insecticides, and fungicides, which are applied in order to kill weeds, insects, and fungi, respectively. Other types of pesticides are rodenticides, bactericides, nematicides and acaricides. Plant growth regulators are also included in the category of pesticides.

Among the different types of pesticides, herbicides are the most widely used, accounting for more than 60% of all pesticides applied in the agricultural sector. The first systemic herbicides 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-T, and MCPA had been discovered before the Second World War around 1940. These compounds killed selectively many weeds in small quantities and in a relatively inexpensive way. For that reason, herbicides were considered among the greatest scientific discoveries of the twentieth century. In 1951, Willard called 2,4-D "the catalyst which generated the recent and continuing explosive reaction concerning plant control by chemical means." Half a century later, it remains one of the most widely used herbicides in the world. The finding of the first hormone herbicides was a multiple independent discovery. A group of scientists in the United Kingdom and separate groups in the United States made the discovery, without knowledge of the others' efforts. In 1945 started the market of 2,4-D in the United States, under the trade name Weedone, and ICI in the United Kingdom began selling MCPA as Methoxone. Since then, the annual commercial production of herbicides grew from less than one million pounds in 1945 to more than 30 million in 1952 and to more than 60 million in later years.

The increasing demand of food for the world's ever-growing population coupled with allocation of arable lands for biofuel production necessitates increasing global agricultural yields. Because weeds are responsible for the major part of agricultural

losses worldwide, maintenance of world agricultural production is directly dependent on the chemical control of weeds. Thus, research for new herbicides with different modes of action from herbicides already available in the market is increasingly important.

Chemical Structure

Based on their chemical structure, pesticides can be classified as organic and inorganic compounds. Organic pesticides are complex chemical compounds with a carbon backbone in their molecular structure, often bonded to heteroatoms such as oxygen, phosphorus, sulfur, chloride, and fluorine, whereas inorganic pesticides consist of the inorganic compounds of sulfur, copper, and other elements. Although the earliest chemical pesticides were inorganic, the vast majority of modern pesticides belong to the class of organic compounds. Organic pesticides can be further divided into many other subcategories based on their functional groups, with the most characteristic ones presented in the succeeding text.

Organochlorines

The organochlorine pesticides (OCPs) are insecticides and characterized by three types of compounds: DDT analogs (Figure 1), benzene hexachloride (BHC), and cyclodiene compounds. OCPs are persistent and toxic chemicals, belonging to the group of persistent organic pollutants (POPs). Although these compounds were widely used in the 1940s in large quantities, they were banned in developed countries in the 1970s because of their high persistence in the environment and their harmful effects in human health. OCPs are semivolatile compounds, and they have the ability for wide range transportation in the environment and bioaccumulation in food crops and animal tissues. They tend to move from warmer to colder climate regions, even as far as the poles.

Organophosphates

The organophosphate pesticides are the general name for esters of phosphoric acid. They are broad-spectrum compounds, with a very specific action, characterized by their high water solubility in comparison with OCPs. Because of that, they are readily hydrolyzed and dissipate from soil within a few weeks after their application. This group of pesticides is highly applied worldwide due to their low persistence and high effectiveness. Organophosphate pesticides are generally regarded as safe for use on crops and animals due to their relatively fast degradation rates. Their ability to degrade made them an attractive alternative to the persistent OCPs previously described. Although organophosphates degrade faster than the OCPs, they have greater acute toxicity, posing risks to people who may be exposed to large amounts. Organophosphate pesticides irreversibly inactivate acetylcholinesterase, which is essential to nerve function in insects, humans, and many other animals. They have been characterized as compounds with very high acute toxicity to bees, wildlife, and humans. Recent studies suggest a possible link to adverse effects in the neurobehavioral development of fetuses and children, even at very low levels of exposure. In this class, well-known compounds such as parathion, malathion, chlorpyrifos (Figure 2), diazinon, dichlorvos, phosmet, and azinphos-methyl are included.

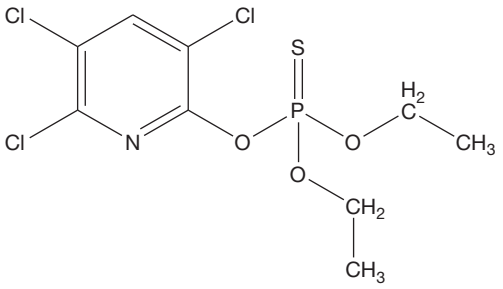

Figure 2 Molecular structure of chlorpyrifos.

Triazines

Triazines belong to one of the most important group of herbicides that is widely used throughout the world. These compounds are applied in agriculture as selective pre- and postemergence weed control for corn, wheat, barley, sorghum, and sugar cane, but they are also widely employed for nonagricultural usage (railways and roadside verges). In some cases, they are used in combination with other herbicides. Triazine herbicides and some of their transformation products are considered as one of the most important classes of chemical pollutants owing to their widespread use and toxicity. Moreover, some compounds of this chemical family are highly persistent in all environmental media and in organisms. Additionally, atrazine, one of the most representative members of triazines, has been classified as a possible human carcinogen. Other representatives of this group are the herbicide simazine and the insecticide cyromazine.

Ureas and substituted ureas

Urea and substituted urea herbicides belong to one of the most important agricultural herbicide groups. Diuron, linuron, and isoproturon are representative compounds of this class that have been widely used in agriculture. There are several subcategories of substituted ureas with phenylureas being the most important one. The general structure of a phenylurea herbicide is phenyl–NH–C(O)–NR_2. The phenyl ring is often substituted with chlorine or bromine atoms, but methoxy, methyl, trifluoromethyl, or 2-propyl substitution is also possible. Phenylurea herbicides are used as selective and nonselective herbicides in substantial amounts, including the use as systemic herbicides to control broadleaf and grassy weeds in cereals and other crops.

Sulfonylurea constitutes another group of substituted ureas, which belongs to the group of selective herbicides with the following general structure R_1–NH–C(O)–NH–SO_2–R_2. R_1 and R_2 are generally substituted by heterocyclic rings. The mode of action of these herbicides consists of inhibiting acetolactate synthase – a key enzyme in the biosynthesis of branched amino acids (valine, leucine, and isoleucine). They are low-dose herbicides used to control broadleaf weeds in cereals exhibiting very low acute and chronic mammalian toxicities.

Benzoylureas are also a group of substituted ureas introduced in the early 1970s. This group belongs to the class of insect growth regulators that act on the larval stages of most insects by inhibiting or blocking the synthesis of chitin. Diflubenzuron is the prototype of benzoylurea chitin synthesis inhibitor insecticides. Benzoylureas are characterized by low acute toxicity to mammals along with their high biological

activity, and for that reason, they are included in integrated pest management programs.

Representatives of the group of ureas and substituted ureas, widely used in agriculture, are diuron, linuron, and isoproturon.

Carbamates

Carbamate herbicides were developed during the early nineteenth century, but their effects were discovered in 1932. Carbamates are organic compounds derived from carbamic acid (NH_2COOH). Specifically, they are esters of carbamic acid. Carbamates and thiocarbamates represent a broad group of herbicides that are applied to soil as preemergence. They are used to control annual grasses and broadleaf weeds in dome horticultural crops. They are inhibitors of the acetylcholinesterase enzyme, which terminates the action of the neurotransmitter acetylcholine. They are normally decomposed quickly in soil by microorganisms. This group includes pesticides such as carbofuran, aldicarb, oxamyl, and methomyl (Figure 3). Carbamates contain two oxygen atoms ROC(=O)NR_2. There are analogs of carbamates with both oxygen atoms replaced by sulfur, which are called dithiocarbamates (RSC(=S)NR_2). There are two different structurally isomeric types of thiocarbamate: O-thiocarbamates (ROC(=S)NR_2), where the carbonyl group (C=O) is replaced with a thiocarbonyl group (C=S), and S-thiocarbamates (RSC(=O)NR_2), where the R–O– group is replaced with an R–S– group. O-thiocarbamates can be isomerized to S-thiocarbamates.

Chloroacetanilides

Chloroacetanilides are an important class of preemergence herbicides used to control annual grasses and broadleaf weeds in various crops; some are soil-incorporated and others surface-applied. Chloroacetanilides represent a large group of herbicides with the general chemical formula $C_xH_yClNO_2$. These compounds are aniline derivatives where the nitrogen is a tertiary amine forming an acetanilide-based compound. On the alpha carbon of the amide functional group is a chlorine substituent. Because of this halogen, chloroacetanilides are considered halogenated alkyl compounds. Each herbicide differs in that their amide side-chain substituents to the benzene ring are of different lengths and types. Because of their structural similarities, these herbicides are expected to possess comparable physical/chemical properties, transformation mechanisms, modes of action, and selectivities. Among them, alachlor was one of the first herbicides widely used, while other important chloroacetanilide pesticides are acetochlor, metolachlor, and dimethenamid.

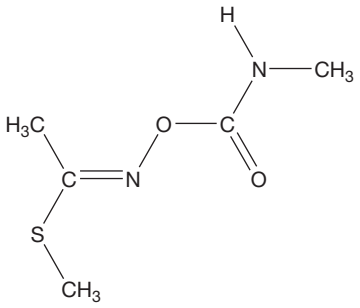

Figure 3 Molecular structure of methomyl.

Pyrethrins and pyrethroids

Pyrethrin and pyrethroid pesticides are insecticides that are mainly used in and around households, in mosquito control, and in agriculture. Their use has increased worldwide with the declining use of organophosphate pesticides, which are more acutely toxic to birds and mammals. Pyrethroid pesticides were developed as a synthetic version of the naturally occurring pesticide pyrethrin, which is found in chrysanthemums. They have been modified to increase their stability in the environment. Some synthetic pyrethroids are toxic to the nervous system.

Pyrethrins are divided into two groups: the natural and the synthetic pesticides. Natural pyrethrins – a single pesticide active ingredient, containing six components with insecticidal activity (pyrethrins I and II, cinerins I and II, and jasmolins I and II) – are botanical insecticides derived from chrysanthemum flowers most commonly found in Australia and Africa. They work by altering nerve function, which causes paralysis in target insect pests, eventually resulting in death. On the other hand, pyrethroids are synthetic chemical insecticides whose chemical structures are adapted from the chemical structures of pyrethrins and act in a similar way to pyrethrins. Pyrethroids are modified to increase their stability in sunlight. They include insecticides such as bifenthrin, cyfluthrin, lambda-cyhalothrin, and cypermethrin.

Source

Pesticides can be classified into natural and synthetic according to their source. In synthetic pesticides, the active compound is produced artificially by chemical synthesis, whereas natural pesticides are derived from naturally occurring sources such as plants, animals, and bacteria. A representative of naturally occurring pesticides is the pyrethrum, a complex of chemicals extracted from *Chrysanthemum cinerariaefolium*. Some other examples are nicotine and nicotinoid alkaloids that come from tobacco.

Biopesticides is a term widely used for natural pesticides that encompass also certain minerals like baking soda. Biopesticides are also known as biologicals, biological pesticides, biocontrol agents, or biochemical pesticides. This class of pesticides plays an important role in providing pest management tools in areas where pesticide resistance, niche markets, and environmental concerns limit the use of conventional chemical pesticide products. They can be used as insecticides, fungicides, herbicides, nematicides, plant or animal growth regulators, plant strengtheners, biostimulants, biofertilizers, and more.

Biopesticides can be distinguished into the following major categories: 'microbials' based on living organisms such as bacteria, fungi, viruses, and viroids; 'macrobials' based on macroorganisms; 'botanicals' based on plant extracts; and 'biochemicals,' covering naturally occurring substances that control pests by nontoxic mechanisms and may contain pheromones and other semiochemicals, as well as other natural products such as hormones, minerals, and enzymes. As an example, the most widely used microbial pesticides are subspecies and strains of *Bacillus thuringiensis* (Bt). Each strain of

this bacterium produces a different mix of proteins that specifically kills one or a few related species of insect larvae. While some Bt's control moth larvae found on plants, other Bt's are specific for the larvae of flies and mosquitoes. The target insect species are determined by the protein produced by each particular Bt that can bind to a larval gut receptor, thereby causing the insect larvae to starve.

Although biopesticides are used globally, the regulations involved in their registration processes vary at a regional or national level worldwide. In Europe, they can be regulated as agrochemicals under Regulation EC 1107/2009 or as biocides under Regulation 528/2012.

Biocidal Pesticides

According to the Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998, biocidal products are defined as active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means. Biocidal products are authorized according to EU Regulation 528/2012, which is different from the regulation concerning the authorization of plant protection products, EU 1107/2009. According to the Biocidal Products Regulation (BPR) 528/2012, biocidal products are classified into 22 product types grouped in 4 main categories: disinfectants, preservatives, pest control, and other biocidal products. Table 2 presents a detailed classification of the biocidal products.

Human Exposure and Health Effect

People are exposed to the described types of pesticides through various ways. The population that receives the greatest exposure is the pesticide handlers, the workers that mix, load, and apply the formulated pesticides. The primary route of their exposure is through the skin and subsequently through inhalation of contaminated air. Families of pesticide handlers are also a vulnerable group with increased potential of exposure. Their exposure may involve the storage and mixing of concentrated formulations near home and several 'take-home' pathways, like the introduction of contaminated clothing into home and poor hygienic practices. On the other hand, the general population may be directly exposed, through inhalation or dermal contact, to pesticides during the application for agricultural or residential pest control and during public or domestic use for sanitation purposes. Exposure to pesticides may also occur via consumption of contaminated water and food.

The exposure to pesticides has been associated with a wide range of adverse effects to human health, from skin irritation to several types of cancer, with the last one being the dominant and the most severe reported effect. Additionally, an elevated rate of other diseases, such as diabetes, neurodegenerative disorders like Parkinson's and Alzheimer's diseases, and reproductive disorders and birth defects, has also been associated with pesticide exposure.

As already stated, one of the most severe potential risks for human health that has been associated with pesticide exposure is several types of cancer like prostate cancer, non-Hodgkin's lymphoma, leukemia, multiple myeloma, breast cancer, and brain cancer. Moreover, parents' exposure to pesticides at work has been related to kidney cancer in children, particularly for high and prolonged exposures, and to brain tumors in children and young adults.

Pesticide exposure has also been linked to neurotoxic effects, particularly with Parkinson's disease. Additionally, prenatal exposure and to a lesser extent postnatal exposure to organophosphate pesticides have been associated with neurodevelopmental and behavioral deficits in preschool and school children.

The reproductive and endocrine systems are also deemed to be adversely affected by organochlorine and organophosphate pesticide exposure among other groups of pesticides. Concerning diabetes, although it has been reported that organophosphates increase the risk of type 2, evidence relating nonpersistent pesticides to diabetes in Asian populations has been found to be equivocal.

Regarding the reported adverse effects of pesticide exposure, it should be pointed out that there are many limitations in the epidemiological studies, and the necessity for further research in order to draw more reliable conclusions regarding the human health risk assessment is frequently highlighted in the literature.

Table 2 Types of biocidal products

Disinfectants	*Preservatives*	*Pest control*	*Others*
Human hygiene	Preservatives for products during storage	Rodenticides	Antifouling products
Private area and public health area disinfectants	Film preservatives	Avicides	Embalming and taxidermist fluids
Veterinary hygiene	Wood preservatives	Molluscicides, vermicides, and products to control other invertebrates	
Food and feed area	Fiber, leather, rubber, and polymerized materials preservatives	Piscicides	
Drinking water	Construction material preservatives	Insecticides, acaricides, and products to control other arthropods	
	Preservatives for liquid-cooling and processing systems	Repellents and attractants	
	Slimicides	Control of other vertebrates	
	Working or cutting fluid preservatives		

Environmental Effect

Pesticide and herbicide application leads inevitably to environmental contamination through direct application of the products and also through spray drift and runoff. The adverse environmental effects of this contamination include nontarget organism poisoning and disturbance of the sensitive ecosystems. The severe adverse effects of the indiscriminate use of pesticides, particularly of DDT, were already described in *Silent Spring* of Rachel Carson (1962). Pesticide environmental contamination has been associated with reproductive and developmental effects in wildlife like birds, reptiles, and amphibians, classifying some pesticides as potential endocrine disrupters. The presence of pesticides may also disturb sensitive marine ecosystems. A representative example of such an environmental impact is that of the Great Barrier Reef in Australia, the largest reef system in the world, which has been seriously contaminated by pesticides. It should be noted that the environmental pesticide contamination has even more severe adverse effects when the active substance or its harmful metabolites are persistent, since this creates a permanent and cumulative hazard to the environment and eventually to human health.

Regulations

Authorization of Plant Protection Products

Authorities worldwide establish regulations and guidelines regarding the authorization and use of pesticides in order to protect the environment and human safety. In the European Union, pesticides are authorized according to Regulation EC No 1107/2009, concerning the placing of plant protection products on the market. According to this regulation, substances should only be included in plant protection products if they present a clear benefit for plant production and harmful effect on human or animal health or any unacceptable effects on the environment are not expected.

An active substance is permitted to be used in plant protection products only if the approval criteria related to mutagenicity, carcinogenicity, toxicity for reproduction, and endocrine-disrupting properties are satisfied according to EC 1107/2009. Additionally, the active substance must not be considered a POP, a persistent bioaccumulative and toxic substance (PBT), or a very persistent and very bioaccumulative substance (vPvB), according to specific criteria. Provided that these requirements are fulfilled, an evaluation of the environmental fate of the active compound of the pesticide and its metabolites and a corresponding environmental risk assessment, along with a human health risk assessment, are performed in order to authorize a pesticide product for specified agricultural uses.

In addition to these evaluations and in order to ensure the lowest possible consumer exposure to pesticide residues in treated products such as fruits, vegetables, cereals, milk, eggs, and animal tissue, maximum residue levels (MRLs) are established by Regulation (EC) No 396/2005. According to EFSA, the MRLs are the upper legal levels of a concentration for pesticide residues in or on food or feed. MRLs are derived after a comprehensive assessment of the properties of the active substance and the residue levels resulting from the good agricultural practices defined for the treated crops.

Apart from the aforementioned prerequisites, the product is evaluated as regards its intended use and efficacy, its identity, composition, physicochemical and technical properties, and finally the existing methods for identification and quantification of the active substance and its degradation products in the commercial formulations, food, and environmental and biological samples.

In the United States, pesticides must be registered with the Environmental Protection Agency (EPA), which regulates the use of pesticides under the authority of two federal statutes, the Federal Insecticide, Fungicide, and Rodenticide Act and the Federal Food, Drug, and Cosmetic Act. Registration assures that pesticides will be properly labeled and that, if used in accordance with specifications, they will not cause unreasonable harm to the environment.

In order to harmonize the data requirements for pesticide registration among different countries, FAO and WHO have adopted an International Code of Conduct on the Distribution and Use of Pesticides, which was last updated in 2013.

Monitoring and Market Control

According to Regulation EC 1107/2009, member states should carry out official controls in order to enforce compliance with the regulation. There are specific provisions for the controls regarding the production, packaging, labeling, storage, transport, marketing, formulation, parallel trade, and use of plant protection products. For the control of the pesticide products regarding the determination of the active ingredient, relevant impurities, and their physicochemical properties, competent authorities apply the official CIPAC methods (Collaborative International Pesticides Analytical Council) in case they are available. These methods are collaboratively tested by laboratories all over the world, and after their evaluation and adoption, they are published in the CIPAC handbooks. There are detailed specifications proposed by the FAO and WHO that pesticides must comply with, like the tolerance of the active ingredient content and limits for their physical properties.

Enforcement methods for the determination of pesticide residues in food of plant and animal origin and environmental (water, soil, and air) and biological samples are also required by the regulation. The required methods need to be reliable, sensitive, specific, and confirmatory, taking into consideration that the MRLs of pesticides in these complex matrices involve low concentrations ($\mu g\, kg^{-1}$). The most widely applied analytical techniques for enforcement purposes that fulfill these requirements are gas and liquid chromatography coupled to mass spectrometry (GC–MS/(MS) and LC–MS/(MS)). Other analytical techniques for the determination of pesticide residues involve immunoassay techniques and capillary electrophoresis. Prior to the determination of the pesticide compounds by the aforementioned techniques, a sample preparation step including isolation of the compounds of interest and purification of the sample is required. The isolation of the target compounds can be accomplished using an extraction solvent performing solid–liquid or liquid–liquid extraction, depending on the physical state of the sample. Other extraction techniques utilizing supercritical fluid and accelerated solvent extractions are also used. Subsequently, for the purification of the liquid extract, solid-phase extraction is the predominant technique applied.

In conclusion, high-tech instrumentation, sensitive and robust analytical methods, qualified personnel, good laboratory practice, and interlaboratory tests are very significant for the successful implementation of the market control of pesticide products and the monitoring of pesticide residues.

Closing Remarks

Taken all together, it becomes apparent that the proper use of pesticides can improve significantly the quality of human life in various ways. However, special attention should be drawn on the awareness of pesticide users regarding the precautionary statements and the label directions, which must be followed with consistency, in order to eliminate the adverse effects of pesticide misuse.

See also: Fungicides; Pesticides and Herbicides; Pesticides and Herbicides: Residue Determination; Pesticides and Herbicides: Types, Uses, and Determination of Herbicides.

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Pesticides and Herbicides: Types, Uses, and Determination of Herbicides

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Introduction

Herbicides or weed killers are chemicals used to kill or control the growth of undesired plants by acting as plant-hormone mimics, which can control the cell enlargement or division, bud or shoot formation, root initiation, etc. An ideal herbicide should kill the unwanted weed while maintaining the target crop relatively unharmed, favoring the normal development of the species of interest.

The benefits of the use of herbicides in agricultural production are unquestionable. However, their presence in the surface of food products constitutes a potential risk for consumers. In fact, this has become a constant cause of concern for the different regulatory agencies around the world, which have made efforts to establish good agricultural practices (GAPs) and GAP-based maximum residue limits (MRLs) that ensure suitable nourishment from a toxicological point of view.

Although current herbicides are not highly persistent, the principal problem is that several farmers do not follow GAPs and employ them in a concentration higher than that advised, or even collect the crops before the security harvest interval has been reached. To achieve the compliance of the current legislations and to monitor the presence of these harmful substances in edible materials, a good number of analytic researchers have focused their efforts on the development of robust methodologies that permit the determination of low concentration of herbicide residues in food matrices.

This article pretends to provide a general vision of the types, uses, and mode of action of the most utilized herbicides and to present an overview of the different extraction techniques that have been used for their isolation and preconcentration from foods and of the separation or detection strategies employed for their later quantitation.

Types and Uses of Herbicides

Herbicides are a subtype of pesticides that can be itself classified into other groups depending on its moment of application, activity, selectivity, mode of action, and chemical family.

Classification by the Time of Application

Regarding the moment of application, herbicides can be classified into early preplant or preplant-incorporated, preemergence, and postemergence herbicides. Early preplant and preplant herbicides (e.g., sulfentrazone, atrazine, and alachlor) are added to the soil months, weeks, or days before the crop of interest is planted. In this way, the dissipation of the pesticide by photodecomposition or volatilization is avoided. Preemergence herbicides (e.g., acetochlor, prodiamine, and glyphosate) are applied to soil after planting the crop and before its germination in order to prevent the growth of foreign weeds by the inhibition of a key enzyme, while postemergence herbicides (e.g., fluazifop-p-butyl, paraquat dichloride, and glyphosate) can be used to kill weeds that have already germinated and small seedlings. Despite this classification, a herbicide can be used as both pre- and postemergence depending on the crop, the soil, and the climatological conditions, among others.

Classification by Activity

Depending on the way they act, herbicides can be categorized as contact or systemic. Contact herbicides (e.g., diquat, bentazon, and glufosinate) affect only the part of the weed on which they are physically deposited, not moving throughout the plant tissues. They are in general very rapid and effective to remove annual weeds. On the contrary, systemic herbicides (e.g., glyphosate, imazaquin, 2,4-D, and dicamba) are completely absorbed through the foliage or roots of the foreign plant and transported through the phloem to other parts of it. They are effective against all types of weeds but are especially useful to control perennials' growths.

Classification by Selectivity

Herbicides can also be distinguished by their selectivity against the crop of interest. In this way, selective herbicides respect a specific plant while destroying other extraneous weeds (e.g., butachlor for rice and metribuzin for potato). On the other hand, nonselective herbicides cause death to all sprayed plants (e.g., glyphosate), so they are normally applied to noncultivated soils or with extreme precaution to avoid the damage of the crop of concern.

Classification by Mode of Action

Another interesting way in which herbicides can be classified is attending to their mode of action, which particularly depends on the biological processes that they inhibit in the target undesired plant.

In this way, herbicides can act as inhibitors of the photosynthesis of the weed (e.g., paraquat and atrazine), producing free radicals, membrane destruction, necrosis, and desiccation. They can also inhibit the synthesis of pigments (e.g., acifluorfen and diflufenican), interfering with the enzymes and causing the loss of protection against radicals. Some herbicides can avoid the formation of amino acids (e.g., glyphosate and glufosinate) or the synthesis of lipids (e.g., dalapon and sethoxydim) that negatively affects the cell membrane and increases the susceptibility to the attack of pests. Still, more aggressive are probably those that hinder cell division interfering in the mitosis (e.g., asulam and

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alachlor) or the inhibitors of growth (e.g., 2,4-D and dicamba), which mimic plant hormones, forming tumors and eliminating absorption and translocation mechanisms.

Classification by Chemical Family

As can be assumed, herbicides comprise a very large group of pesticides with different functional groups and structures. For this reason, chemical classification is perhaps the most complex and extensive, but it is not absolute, since certain herbicides could be classified into different groups and other compounds within these groups may not be herbicides. However, they are usually divided in the following families:

- Aliphatic carboxylic herbicides (acids, esters, and salts)
- Amides (including anilides, sulfonamides, and thioamides), amino acids, and quaternary ammonium salts
- Benzoic and phthalic herbicides (acids, esters, and salts)
- Benzonitriles
- Carbamates and thiocarbamates
- Cyclohexanediones
- Dinitroanilines
- Diphenyl ethers
- Halogenated herbicides
- Imidazolinones
- Inorganic herbicides (including arsenical herbicides)
- Phenols (including dinitrophenols)
- Phenoxy herbicides (acids, esters, and salts)
- Pyridines and pyridazines
- Ureas and sulfonylureas
- Triazines
- Other herbicides

Figure 1 shows the chemical structure of some representative herbicides belonging to the different groups previously mentioned.

Herbicide Determination

Extraction Methods

Sample preparation is probably the most important step of any analytic method especially when dealing with complex matrices as foods. In general, when analyzing liquid food samples like wine, fruit juices, and milk, less sample treatment is normally required: filtration, pH adjustment, dilution, centrifugation, etc., followed by a simple extraction procedure if the analysis cannot be directly performed. However, when solid or semisolid samples like fruits, vegetables, or cereals are considered, the first step in sample preparation is the extraction of the target analytes from the matrix. The amount of coextracted substances is usually high, especially for nonvolatile lipids, which in some cases can damage the analytic instrumentation. For this reason, a postextraction cleanup of the extract is nearly always necessary.

Classical methodologies for the extraction of herbicides from solid or semisolid food matrices often consist in a solid–liquid extraction (SLE) carried out by homogenization of the sample with an organic solvent or water (depending on the herbicide's properties) and a cleanup of the extract, normally by liquid–liquid extraction (LLE), column chromatography (Florisil, alumina, silica, etc.), gel permeation chromatography (GPC), or even tandem combination of them. In this way, different families of herbicides have been determined in fruits, vegetables, cereals, and other food matrices.

Though such classical approaches are still used up to some extent, they have been replaced over the years by an initial solvent extraction assisted by pressure (pressurized liquid extraction), microwaves (microwave-assisted extraction (MAE)), or ultrasounds (ultrasound-assisted extraction (UAE)), followed by a cleanup procedure that is usually done by solid-phase extraction (SPE). In this sense, SPE's benefits over LLE or GPC cover the lesser consumption of solvents, the higher analyte enrichment or preconcentration, and the specificity of the stationary phases. For this purpose, typical commercially available stationary phases have been used and selected depending on the mechanism of interaction between the sorbent and the analytes of interest: bonded silica sorbents for acidic herbicides; graphitized carbon black for neutral, basic, or acidic herbicides; polymeric resins for polar ones; etc. However, the trends in SPE have been lately focused on the use of more selective sorbents that avoid the retention of other interfering sample components. In this respect, immunosorbents, carbon nanotubes, and especially molecularly imprinted polymers (MIPs) have reached importance in the last decade. MIPs constitute one of the most selective ways to extract and clean food extracts, since the retention of foreign molecules is avoided.

Miniaturized sorbent-based extractions, for example, solid-phase microextraction (SPME) and stir-bar sorptive extraction (SBSE), have been much less used in this field for several reasons. On the one hand, commercially available SPME fibers are mechanically fragile, are less stable in highly acidic or alkaline samples, and lack selectivity, a fact that has been partially solved by the preparation of tailor-made fibers covering metal wires with MIPs. However, in SPME, like in SBSE, extraction is normally carried out under nonequilibrium conditions and absolute recoveries are expected low, also in pesticide analysis and especially for complex matrices like foods.

Regarding other techniques based on the use of sorbents, matrix solid-phase dispersion (MSPD) emerged as an alternative and direct extraction approach for the determination of the main herbicide families in different solid, semisolid, or liquid–viscous food matrices, using diverse solid support materials (C_{18}- or C_8-bonded silica, Florisil, NH_2-silica, or even MIPs). This technique includes sample homogenization, sample architecture disruption, fractionation, and purification in a single step, so it overcomes the disadvantages of SPE and SPME in which liquid extracts are mainly needed. However, in some cases, after elution, an additional cleanup step (washing of the column prior to elution, an SPE in the same column or in a different one – which is called co-column clean-up, etc.) has been found necessary, depending on the nature of the solid support, the sample matrix, and the elution solvent.

Although such recent approaches provide clear advantages over classical methods, the truth is that current trends in sample preparation tend to minimize extraction time and to develop simpler and cheaper extraction methods. In this respect, since its introduction in 2003 for the determination of pesticides in vegetables, the 'Quick, Easy, Cheap, Effective, Rugged, and Safe' (QuEChERS) method has been extensively

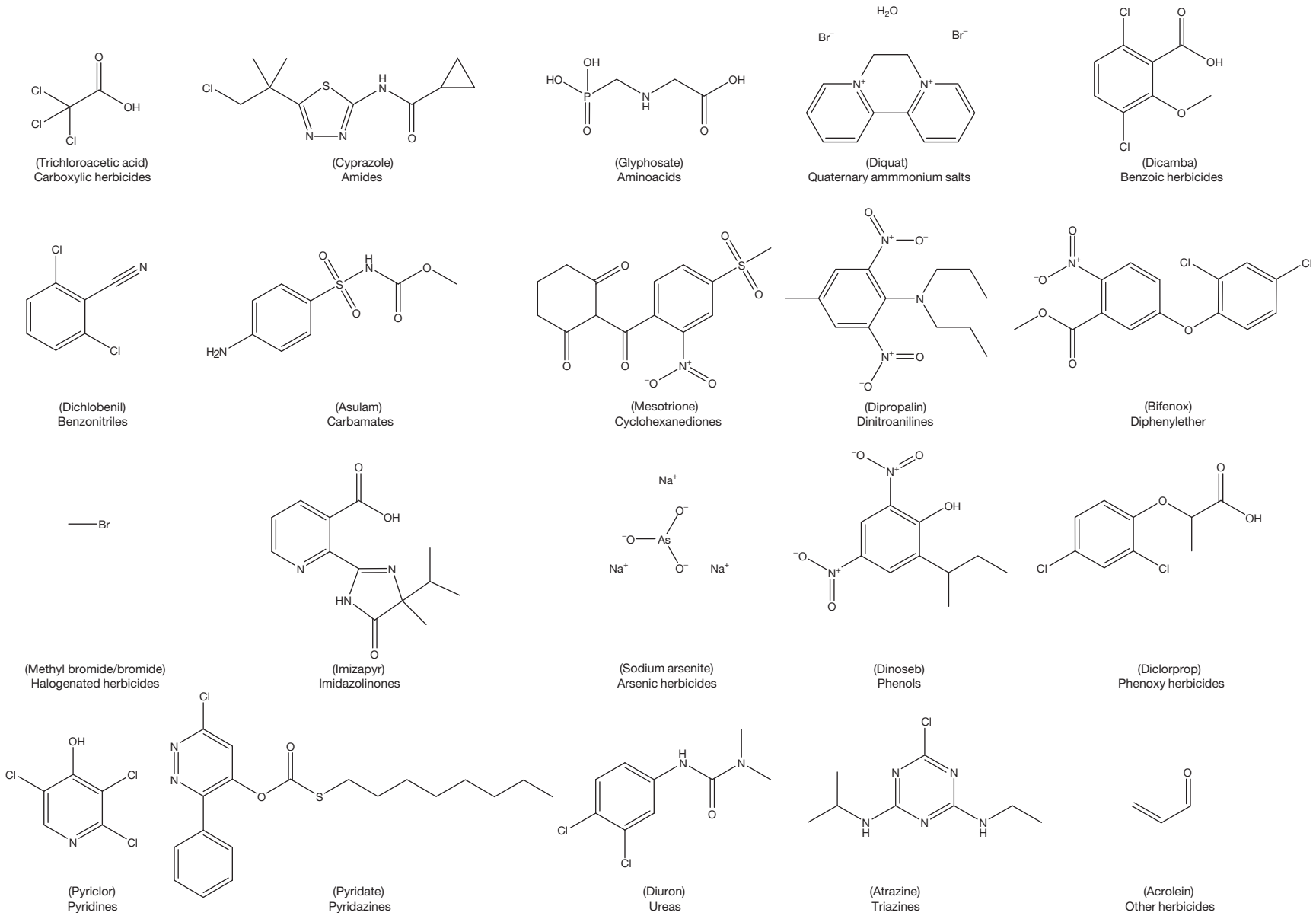

Figure 1 Representative examples of herbicides (between parentheses) belonging to different chemical families.

used also for other agroalimentary matrices. It is very effective and has permitted to cover a wide range of analytes, from highly polar to nonpolar pesticides, and highly acidic and basic substances, just studying slight modifications of the two principal steps of the method: ACN extraction/partitioning and dispersive SPE. In general, this method provides good results in terms of time, solvent expense, and laboriousness with respect to other extraction/cleanup techniques, being also very successful as a multiclass pesticide analysis option, herbicides obviously included.

Another important tendency in this field has been the miniaturization of the extraction devices and the diminution of the consumption of harmful solvents. In this sense, liquid-phase microextraction (LPME) techniques in its different application modes (single-drop microextraction, dispersive liquid–liquid microextraction (DLLME), and hollow fiber-LPME (HF-LPME)) have lately experienced a huge success. However, the application of LPME to food matrices requires an aqueous media, which means that a previous extraction of the analytes (MAE, UAE, etc.) should be developed followed by a suitable reconstitution in water. Regarding the analysis of herbicides in food, DLLME and HF-LPME have been the LPME modes that have found higher application.

Nowadays, the real aim is to achieve very low LODs, avoiding matrix interferences using rapid and easy-performance extraction procedures with low consumption of solvents, reagents, and sample. However, the selection of an extraction/cleanup combination depends also on the separation technique considered and on the characteristics of the detection system, which will be discussed later in the text.

Separation Techniques

In the literature, a great number of methods to separate and determine herbicides in food can be found. Such methodologies include chromatographic techniques (gas chromatography (GC) and liquid chromatography (LC)), electromigration methods (capillary electrophoresis (CE), capillary electrochromatography (CEC)), electrochemical and spectrophotometric techniques, biochemical assays, and immunoassays. Among them, both chromatographic and electrophoretic techniques have been the most popular and they have been frequently combined with mass spectrometry (MS) to identify and quantify a great variety of herbicides.

It is worth mentioning that these techniques have been used to determine herbicides in different food matrices of vegetable origin like cereals, tubers, vegetables, legumes, fruits, nuts, flours, oils, pasta, bread, fruit juices, and coffee; food matrices of animal origin as milk, fish, shrimp, honey, and infant formulas; or even in alcoholic beverages as beer and wine. It is also important to highlight that, although in some cases the developed methods have focused only on the analysis of herbicides, in most manuscripts, they have been determined together with other pesticides, metabolites, degradation products, or even with other organic contaminants as phenolic compounds or antibiotics.

Analysis by LC

LC has been one of the most widely used techniques to analyze herbicides in foods for both individual and multiresidue analyses. In the majority of the methods, LC has been performed in reversed-phase columns based on chemically modified silica stationary phases with C_{18}, C_8, or even NH_2 and pentafluorophenyl. However, other separation modes, for example, mixed mode, ion exchange, or hydrophilic interaction chromatography, as well as MIP stationary phases, have also been occasionally used for the separation of selected amino herbicides (diquat, paraquat, chlormequat, mepiquat, etc.). It is noteworthy to mention that in some cases, particles with diameters lower than 2 μm have also been used, exploiting the important advantages of ultrahigh-performance LC, such as higher separation efficiency and lower retention time.

Regarding the detection systems, UV/DADs have been widely employed due to their inherent characteristics. In this sense, herbicides tend to present maximum absorption in the 190–310 nm region. On the contrary, very few herbicides (e.g., bentazone, some phenoxy acid herbicides – 2,4-D, 2,4-DB, MCPA, MCPB, mecoprop, and dichlorprop – naphthylacetic acid, and flurecol) show native fluorescence, and as a result, the use of fluorescence detection is subjected to the application of a previous derivatization step employing, for example, *o*-phthalaldehyde, 2-mercaptoethanol, 9-fluorenylmethyl chloroformate, or dansyl chloride, which can be done either pre- or postcolumn. Besides, electrochemical detection has also been selected though in a very low number of occasions, for example, for the determination of 2,6-diethylaniline and 2-(1-hydroxyethyl)-6-ethylanyline (metabolic products of alachlor) or ethylene thiourea, which are electrochemically active.

However, and as it also happens for the analysis of other organic compounds, MS is the preferred detection system for the analysis of herbicides in food. The selection of a particular LC-MS interface is often determined by the matrix properties, instrumentation availability, and personal preferences derived from experience. The preferred interface selected to couple LC to MS is, without any doubt, the electrospray ionization (ESI) interface, although several examples using atmospheric-pressure chemical ionization (CI) can be found. Both positive and negative modes have been used depending on the analyte and ionization conditions. In this regard, certain compounds of the families of the amides (e.g., alachlor, propyzamide, butachlor, and flufenacet), triazines (e.g., ametryn, cyanazine, simazine, and terbutryn), ureas and sulfonylureas (e.g., amidosulfuron, diuron, and chlorimuron ethyl), carbamates and thiocarbamates (e.g., butylate, carbetamide, and molinate), imidazolinones (e.g., imazapyr or imazaquin), quaternary ammonium salts (e.g., mepiquat, diquat, or paraquat), nitriles (e.g., bromoxynil or ionoxynil), diphenyl ethers (e.g., bifenox), phenoxy herbicides (e.g., quizalofop-p-ethyl), and other herbicides (such as lenacil, metosulam, and oxadiazon) have been determined in the positive mode, while herbicides belonging to the phenoxy acid (e.g., 2,4-dichlorobutyric acid, dichlorprop-p, and MCPA), sulfonylurea (e.g., flazasulfuron), amide (e.g., oryzalin), and other families (e.g., picloram, dicamba, and bentazon) have been determined in the negative ionization mode. Moreover, several herbicides have also been determined in both ionization modes (such as fluroxypyr, pentachlorophenol, glyphosate, propanil, clopyralid, and fluazifop). Regarding the mass analyzer used for tandem MS (MS/MS), the triple quadrupole (QqQ) is the most used for pesticide analysis (and also herbicides) because it was early

commercialized, is easy to operate, and has a small size. Also, quadrupole ion trap, ion trap (IT), and time-of-flight (TOF) analyzers are among the most used for herbicides determination.

Analysis by GC

GC has been widely used for the analysis of herbicides due to the adequate volatility and high-temperature stability of many of these compounds. In this way, various herbicides have been separated by GC, basically pesticides belonging to the following families: amides (e.g., alachlor, isoxaben, or propyzamide), carbamates and thiocarbamates (e.g., chloropropham, molinate, and prosulfocarb), dinitroanilines (e.g., trifluralin or fluchloralin), triazines (e.g., simazine, ametryn, or cyanazine), ureas (e.g., diuron, amidosulfuron, and tribenuron-methyl), and phenoxy herbicides (e.g., MCPA, dichlorprop-p, and haloxifop). The examples of the separation of diphenyl ethers (e.g., oxyfluorfen), quaternary ammonium salts (e.g., difenzoquat methyl sulfate), pyridines (e.g., clopyralid or fluroxypyr), imidazolinones (e.g., imazamethabenz-methyl), and other herbicides (e.g., metribuzin and oxadiazon) by GC can also be found. The columns more frequently used for this purpose are based on (5%-phenyl)-methylpolysiloxane and 100% dimethylpolysiloxane or phenyl arylene polymer (virtually equivalent to (5%-phenyl)-methylpolysiloxane), although columns based on trifluoropropyl polymer, (50%-phenyl)-dimethylpolysiloxane, (5%-phenyl-polysilphenylene)-siloxane, (1%-phenyl)-methylpolysiloxane, or (14%-cyanopropyl-phenyl)-methylpolysiloxane have been used in certain applications.

Among the different detectors that can be used in GC, flame ionization detection (FID) has been selected when a more specific detector was not available, since an intensive cleanup step is usually needed. Besides, flame photometric detection (FPD), which is more appropriate for the analysis of triazines or carbamates containing sulfur or phosphorus in their structure, has been used several times in combination with other detection techniques. On the other hand, electron capture detection (ECD), which is very sensitive for halogenated compounds, has been used to determine polyhalogenated herbicides like oxyfluorfen, fluchloralin, diuron, or oxadiazon and herbicides derivatized with methyl iodide–triethylamine (1/1, v/v), diazomethane, or pentafluorobenzyl bromide (among others). Some examples are flumetsulam, dicamba, and picloram. Finally, nitrogen–phosphorus detection (NPD) has also been used in some applications for the determination of herbicides containing nitrogen atoms. This is the case of pesticides like anilofos, ametryn, vernolate, or terbacil.

Nonetheless, an important number of works have also used MS and MS/MS detection alone or in combination with other detection systems like FID, FPD, ECD, or NPD. In most of these applications, ionization has been carried out via electronic impact although it is also possible to find examples that employ CI. These ionization modes have been used in combination with either IT, TOF, or QqQ analyzers.

Regarding derivatization for GC analysis, herbicides that have hydroxyl, carboxylic, or amino groups (e.g., benzonitriles, phenoxy acids, or phenylureas) are those that often require such step. A particular case is the determination of glyphosate. Due to its high polarity and water solubility, it requires exhaustive derivatization pretreatments as the use of trifluoroacetic anhydride and trifluoroethanol or diazomethane, isopropyl chloroformate, and diazomethane or heptafluorobutyric anhydride and 2-chloroethanol, because standard derivatizations in GC are ineffective. Despite this, it is also noteworthy that, in general, a large number of pesticides (much higher than in most applications with other techniques) have been determined in a single run by GC, due to the high-separation power of this technique for multiresidue routine analysis.

It is important to mention that several herbicides, for example, some quaternary ammonium salts, ureas, and their metabolites or degradation products, are not amenable by GC-MS, not even by subjecting them to a derivatization process.

Analysis by CE

Despite the fact that in the literature, there are a large number of manuscripts devoted to the analysis of herbicides in environmental matrices by CE, the number of articles that deal with the determination of herbicides in food matrices is still scarce compared to those using LC or GC.

CE represents an interesting alternative because it offers not only high-separation efficiency but also short analysis time, low consumption of solvents, simplicity, and almost all pesticides may be determined without derivatization (unless it is necessary to facilitate the detection). By contrast, the major drawback is the low sensibility associated with the short optical path length of the capillaries when UV–vis detection is used and the small sample volumes injected. The use of sensitive detectors and/or online preconcentration techniques (*stacking* and *sweeping* modes) allows solving this problem, and in fact, both stacking and sweeping procedures have also been used in the determination of herbicides. Regarding the stacking phenomena, electrokinetic injection has been mainly used, although normal stacking mode, large volume sample stacking, or stacking with reverse migrating micelles has also been applied in several occasions.

Among the different operational modes of CE, capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), nonaqueous CE (NACE), and CEC have been used to separate herbicides. Although CZE prevails over the rest, especially for pesticides that have ionizable groups, the separation of closely structurally related polar herbicides in aqueous solution is difficult. NACE has been assayed to overcome this problem, since the acid–basic properties of the analytes change in organic media and the existence of other phenomena that do not occur in the aqueous background electrolytes is useful to discriminate separation. MEKC, which is based on the interactions between the analytes and the micelles present in the separation medium, can be used to separate both charged and neutral solutes with either hydrophobic or hydrophilic properties, which is perhaps the reason why it has been widely used in this field. Among the available micellar agents, sodium dodecyl sulfate has been the most employed in these methodologies (even used in NACE applications), although others like tetradecyltrimethylammonium bromide have also been used. With respect to CEC, despite the potential advantages of the inclusion of a stationary phase, its use to determine herbicides in foods has not been very common.

Due to the ease of handling, simplicity, and low cost, UV-Vis/DAD is the most extended detection mode in these CE applications. Nevertheless, and as it was previously mentioned,

the low sensibility of CE has favored the use of more sensitive detectors, such as MS or MS/MS. The interface that has been normally used in these cases is also the ESI but modified to add a sheath liquid that helps to maintain the electric circuit and the analyzers varied from quadrupoles to ITs. There are also some applications that used alternative detectors such as those based on electrochemiluminescence for the determination of amine herbicides like isoproturon, linuron, glyphosate, or aminomethylphosphonic acid (major metabolite of glyphosate).

Analysis by other techniques

The need of rapid and accurate analytic methodologies to determine herbicides in foods has rapidly led to the development of techniques alternative to LC, GC, or CE. Among them, immunoassays and biosensors have been those that attracted more interest and further development.

Immunoassays, which are based on the recognition reaction between the molecule of interest (antibody) and the antigen adsorbed onto a specific support, offer rapid, sensitive, and relatively low-cost measurements while reducing the need of sophisticated equipment. Perhaps, this is the reason for its extensive development in this type of applications. Among them, methodologies based on enzyme-linked immunosorbent assays (ELISAs) are those that have achieved higher success. In some cases, ELISA experiments could take several hours and its implementation could require a set of stationary devices. For this reason, novel formats of immunoassays, such as immunobiosensors (including optical, piezoelectric, and electrochemical immunosensors), fluoroimmunoassays, and immunomembranes, have also been tested. However, the vast majority of these methods are limited to a single analyte or to a small group of analytes structurally closely related, due to the specificity of antigen–antibody recognition. In fact, without any doubt, a great number of manuscripts are devoted to the analysis of triazine herbicides (mainly atrazine). However, if cross reactivities or matrix interferences are not observed, the immunoassays could be an interesting alternative.

Regarding the use of electrochemical methods and apart from electrochemical immunosensors, several authors have developed different methodologies based on voltammetry, polarography, or amperometry. For this purpose, several different electrodes (based on gold, highly boron-doped diamond, graphite-poly(tetrafluoroethylene), MIPs, or mercury drop) have been used. As also happened with immunoassay techniques, almost all published manuscripts are devoted to the analysis of a single compound or a low number of structurally related ones (mainly nitrogenous herbicides like paraquat, cyanazine, propazine, or pendimethalin). Besides, and due to the nature of the electrodes, many of the methods have been developed to analyze liquid matrices have also been tested after a suitable extraction process. The principal disadvantage of these methods is the possible adsorption of the reagents or products of the electrochemical reaction onto the surface of the electrode, decreasing the sensitivity and reproducibility of the procedure.

Finally, spectroscopic and spectrometric techniques have also been used to determine herbicides in several occasions. Thus, the advantages of different methodologies, such as

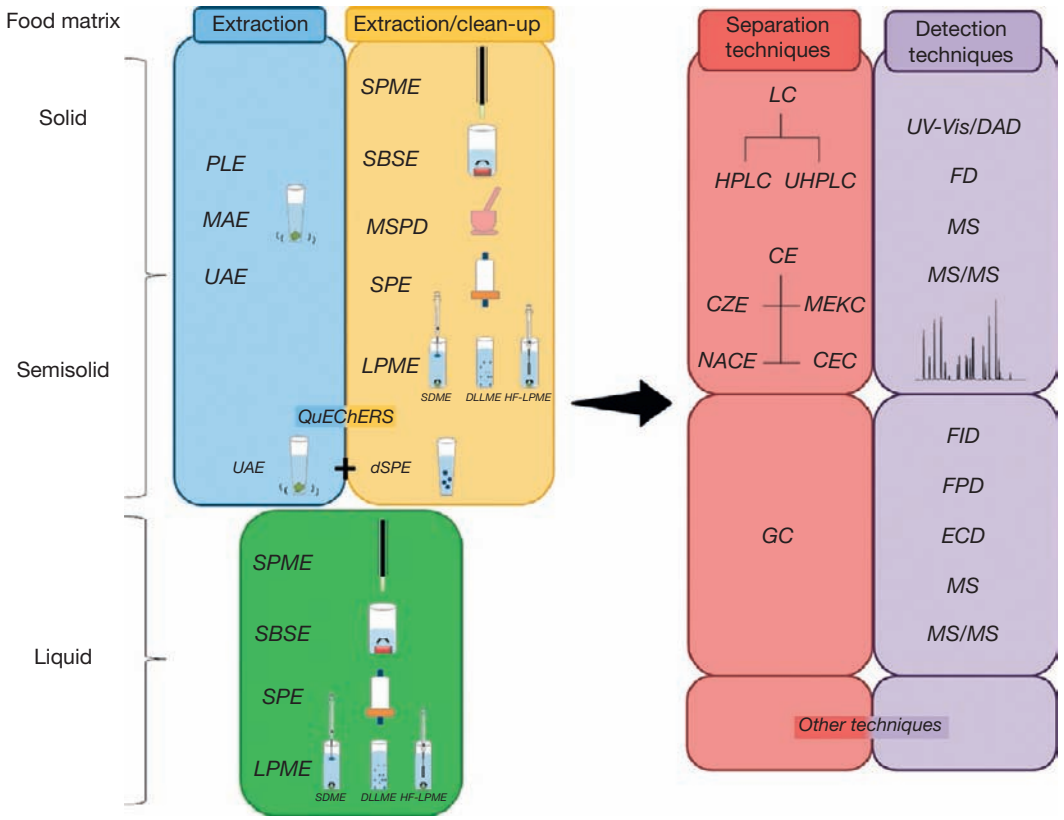

Figure 2 Schematic representation of the different methodologies applied to herbicide determination in foods.

Raman spectroscopy, MS, MS/MS, fluorescence, UV–vis spectrophotometry, and plasma ionization MS, have been exploited. In many papers, the sample introduction was made by means of automated flow injection. The mentioned methods have the advantage of being less time-consuming, but on the contrary, only one or two herbicides are determined (except for MS and MS/MS detectors).

Figure 2 shows a schematic summary of the different extraction and separation methodologies applicable to the herbicide determination in liquid, solid, and semisolid food matrices.

Conclusions

The analysis of herbicides and, in general, pesticides is a relevant issue because of their controversial use in agriculture being focused on the development of new and effective analytic methods able to determine such compounds at the required MRLs.

Herbicides, which can be classified depending on different criteria, comprise a big number of compounds of diverse structures and properties. As a result, there is not a universal analytic method for their determination and different strategies are applied depending on the food sample and also on the determination technique.

In general, herbicides are initially extracted from food by different procedures: SLE (assisted or not by ultrasounds, microwaves, etc.), LLE, or sorbent-based extraction techniques (SPE, SPME, MSPD, etc.). A suitable cleanup or a second extraction may also be necessary, depending on the application. The procedure is simpler when samples are liquid. Afterward, it is very frequent to analyze the final extract using a separation technique, being GC and LC the ones of choice, although CE and other procedures/techniques like immunoassays or biosensors have also been used with success.

In general, it is clear that new analytic methods are constantly being developed/suggested on this challenging subject. Like in any other field, suitable and continuous revision of the literature is extremely necessary to achieve a general overview of its past, present, and future.

See also: Alkaloids: Properties and Determination; Antibiotics and Drugs: Residue Determination; Antioxidants: Characterization and Analysis; Biosensors; Cancer: Diet in Cancer Prevention; Carcinogenic: Carcinogenic Substances in Food; Carcinogens: Identification of Carcinogens; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Codex Alimentarius Commission: Role in International Food Standards Setting; Codex Alimentarius; Consumer Protection Legislation; Food Additives: Classification, Uses and Regulation; Food and Agriculture Organization of the United Nations; Fumigants; Fungicides; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Herbs: Composition and Dietary Importance; Immunoassays: Principles; Infrared Spectroscopy: Applications; Insect Pests; Legal Requirements for Food Hygiene; Mass Spectrometry: Applications; Mass Spectrometry: Principles and Instrumentation; Pesticides and Herbicides; Pesticides and Herbicides: Residue Determination; Pesticides and Herbicides: Types of Pesticide; Preservation of Foods; Preservatives: Classifications and Analysis; Preservatives: Food Use; Quality Control in Food Processing; Spectroscopy: Types; Supercritical Fluid Extraction; World Health Organization.

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pH: Principles and Measurement

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Definition and Basics

pH Basics and Notional Definition

The acidity and alkalinity of aqueous media, where most reactions in nature occur, depend (according to the Arrhenius definition of acids and bases) on the concentration of the hydronium (H_3O^+) and hydroxyl (OH^-) ions. The former is quantified by the more general concept of pH, initially proposed by the Danish chemist Søren Peder Lauritz Sørensen in 1909 and revised in 1924 to adjust definitions in relation to the electrochemical cells used for its measurement. The pH is notionally defined for any medium as the decimal logarithm of the reciprocal of the hydrogen ion activity, α_{H^+}, in that medium:

$$\mathrm{pH} = -\log_{10}\alpha_{H^+} = \log_{10}\left(\frac{1}{\alpha_{H^+}}\right) \quad [1]$$

It is only for very dilute solutions that the pH can be directly related to proton concentration:

$$\mathrm{pH} = -\log_{10} c_{H^+} \quad [2]$$

This approximation holds for pure water where, due to its self-dissociation constant being $K_w = 10^{-14}$ at 25 °C, proton and hydroxyl concentrations are as low as 10^{-7}. Thus, pure water at 25 °C has a pH value very close to 7; therefore, a value of pH = 7 defines a 'neutral' medium where the proton and its hydroxyl counterion are present in equal concentrations. Media with pH < 7 are characterized as acidic, whereas those with pH > 7 as alkaline. The common pH range is between 0 and 14 since cases with H^+ or OH^- concentrations higher than 1 M are not often encountered. It should be stressed that a standard pH reference value has been set by IUPAC with respect to which primary and operational standard solutions are further defined; this is the value of pH = 4.21 for a 0.05 mol kg^{-1} potassium hydrogen phthalate aqueous solution.

Operational pH Definition

The notional definition of pH according to hydrogen ion activity (eqn [1]) is based on the fact that pH is accurately determined by pH-sensitive ion-selective electrodes, which, strictly speaking, respond to activity rather to concentration changes. Ideally, the electrode potential, E, of a pH-sensitive electrode follows the Nernst equation, which, for the hydrogen ion, can be written as

$$E = E' + \frac{RT}{F}\ln(\alpha_{H^+}) = E' - \frac{2.303RT}{F}\mathrm{pH} \quad [3]$$

where E is the measured potential, E' is a formal electrode potential (i.e., the constant value of the electrode potential for $\alpha_{H^+}=1$ and the specific electrode/solution conditions for each case), R is the gas constant, T is the temperature in Kelvin, and F is the Faraday constant. It follows that the potential of such a pH-sensitive electrode is a linear function of the pH when the latter is defined in terms of activity.

According to the international standard ISO 31-8, the standard methodology for pH measurements involves the construction of a liquid junction-free galvanic cell where the pH-sensitive electrode is the hydrogen electrode ($Pt/H_2/H^+$: H_2 gas bubbled at 1 atm through a H^+-containing solution in contact with a high surface area Pt electrode) and the reference electrode is a Ag/AgCl in a KCl solution. The following notation refers to such a cell:

$$\mathrm{Ag|AgCl|concentrated\ solution\ of\ KCl,}$$
$$\mathrm{test\ solution|H_2(g,\,1\,atm)|Pt}$$

where vertical lines denote the interface between two different phases. The cell is filled with a standard solution of known pH/hydrogen ion activity, pH_s, and its electromotive force (emf), E_S, is measured. Then, the test solution is replaced by the sample solution of unknown pH_x and the new emf of the cell, E_X, is measured. It follows (applying eqn [3] twice and taking into account that the same reference electrode is used) that the pH of the unknown solution is a linear function of the difference between the two measured emf values:

$$\mathrm{pH_X} = \mathrm{pH_s} + (E_S - E_X)/(2.303RT/F) \quad [4]$$

Equation [4] describes the operational definition of pH.

The pH values of the standard solutions (of both primary and secondary/operational standards), used in the operational definition of the pH of unknown solutions, are estimated by direct measurements of the emf of cells similar to the one described earlier, following the methodology adopted by IUPAC Recommendations 2002.

Buffer Solutions

A (pH) buffer solution refers to an aqueous solution containing a mixture of a weak acid and its conjugate base or vice versa. The most outstanding characteristic of a buffer solution is its ability to keep its pH almost constant with the addition of a small amount of strong acid or base. This is the reason that buffer solutions are applicable in a variety of chemical procedures where the prevention of changes in the pH of a solution is needed. Many living organisms grow in a relatively small pH range, and in order to keep the pH constant, they utilize a buffer solution (e.g., blood). This remarkable property of buffer solutions is attributed to the presence of equilibrium between the acid HA and its conjugate base A^-:

$$HA \rightleftarrows H^+ + A^- \quad [5]$$

According to Le Chatelier's principle, the addition of a small amount of strong acid to this equilibrium mixture will

Table 1 Standard buffer solutions at 25 °C

Buffer solution composition	*pH value*
Potassium tetraoxalate (0.05 m)	1.68
Potassium hydrogen tartrate (saturated)	3.56
Potassium hydrogen phthalate (0.05 m)	4.01
Potassium dihydrogen phosphate (0.025 m) + disodium hydrogen phosphate (0.025 m)	6.87
Potassium dihydrogen phosphate (0.08695 m) + disodium hydrogen phosphate (0.03043 m)	7.41
Borax (0.01 m)	9.18
Calcium hydroxide (saturated)	12.45

Source: Bates, R. G. (1964). *Determination of pH: theory and practice* (1st ed.), p. 123. London: Wiley; Westcott, C. C. (1978). *pH measurements*, p. 81. Michigan: Academic Press.

force the equilibrium to shift to the left side of reaction [5]. In this way, the concentration of the hydrogen ion increases by less than the amount expected for the quantity of strong acid added. On the other hand, if a portion of strong alkali is added to the mixture, the hydrogen ion concentration decreases by less than the amount expected for the quantity of alkali added, since more protons are produced as reaction [5] is shifted to the right.

Table 1 gives the characteristics of seven standard buffer solutions that cover the usually encountered pH range (between ~2 and 12).

Measurement

pH Meters

pH is a measurable parameter and the electronic device that is used for measuring the pH of a liquid (or in special cases of semisolid compounds) is called a pH meter. The most important component of a typical pH meter is its special measuring probe (a glass electrode or, for special applications, an ion-selective field-effect transistor (ISFET)), which is connected to an electronic meter that measures and displays the pH reading. All pH meters are calibrated against buffer solutions of known hydrogen ion activity. The use of a set of buffer solutions (operational pH standards) has been proposed by IUPAC.

A crude estimate of pH in laboratories can also be performed by pH indicators and sometimes in connection with holographic pH sensors, which allow the measurement of pH colorimetrically.

Glass Electrode

Construction and principle

.The most widely used pH meter is based on the glass electrode, which belongs to the large family of ion-selective electrodes, that is, electrodes that contain a material sensitive to changes with respect to the concentration of ions in the test solution, in such a way that the potential drop between the electrode and the outer surface of the material depends on this concentration. Glass electrodes are the main components of potentiometric sensors and are used in the determination of univalent cations. The glass electrode used in pH measurements has a glass bulb membrane with a typical three-component composition of 72% SiO_2–22% Na_2O–6% CaO (or 80% SiO_2–10% Li_2O–10% CaO for highly alkaline media).

The interaction of the protons in solution with the surface of the glass membrane is based on the ion exchange property of the latter since, when its main silica component comes in contact with water, it is hydrated and dissociates to Si–O– and this in turn can be partially protonated:

$$\mathrm{Si-O^- + H_3O^+ \leftrightarrows Si-O-H + H_2O} \qquad [6]$$

The name of the glass electrode may be misleading to the nonspecialist operator in that two misconceptions may sustain: that a pH meter contains a single electrode and that the electrode material is made of glass. Both of these are obviously not true: glass is an ion conductor and not an electronic one (hence, it is not an electrode material) and a potential difference can only be measured between two points (hence, there are two electrodes in a pH meter). In reality, the voltage between the two sides of the glass membrane is measured with the help of two electrodes immersed in the solutions on either side of the membrane. In Figure 1, where (a) a simple glass electrode coupled with a reference electrode and (b) a combined system of them are shown.

The glass electrode is made of a plain glass or plastic tube with the pH-sensitive specialty glass membrane (~0.1 mm thick) blown from its melt and sealed at the end of the tube. The latter contains an internal standard acidity solution (typically 0.1 N HCl, sometimes with the addition of a AgCl saturated solution too) and an internal reference electrode, RE_{in} (typically a Ag/AgCl salt-covered wire electrode). The glass membrane is in contact with the test/sample solution, and in the plain configuration of Figure 1(a), the second external reference electrode, RE_{ext} (again a Ag/AgCl electrode), is also immersed in the test solution. Alternatively, the external electrode can be placed in an external tube, concentric to the glass electrode tube and containing a KCl solution, which is in contact with the test/sample solution via a porous ceramic frit (Figure 1(b)); in that case, the system is termed as a combination pH electrode. Hence, a pH meter consists of an electrochemical cell that, in the case of a combinatorial system, can be denoted as

$$\mathrm{Ag/AgCl\ (RE_{in})|0.1\ N\ HCl|glass\ membrane|sample\ solution\ (H^+_s)||KCl\ solution|Ag/AgCl\ (RE_{ext})}$$

with vertical lines signifying the interface between two different phases and the double vertical line an electrolytic junction. The measured potential, E, between the two cell terminals can then be written as

$$E = E_{in} - \Delta E_m - \Delta E_{lj} - E_{ext} \qquad [7]$$

where E_{in} and E_{ext} are the potentials of the reference electrodes, ΔE_m the potential drop between the two sides of the glass membrane, and ΔE_{lj} the liquid junction potential drop at the electrolytic junction. Since E_{in}, E_{ext}, and ΔE_{lj} are expected to be constant, eqn [7] becomes

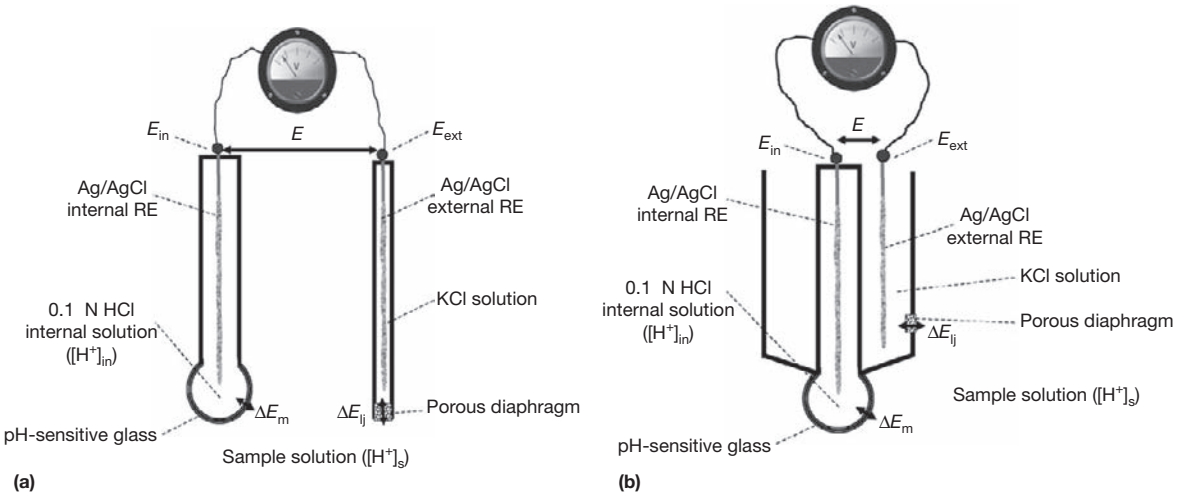

Figure 1 Schematic representation of a pH meter comprised of (a) a glass electrode coupled with a separate reference electrode and (b) a combined system.

$$E = E_{const} - \Delta E_m \quad [8]$$

The ΔE_m potential drop is due to the development of different surface concentrations of chemisorbed H^+ at opposite glass surfaces, as a result of the establishment of different equilibriums for reaction [6], due to different bulk solution concentrations in the internal and sample solutions.

This potential drop may be given by the Nernst equation for the potential of a concentration cell:

$$\Delta E_m = 2.303\frac{RT}{F}\log\frac{[H^+]_{in}}{[H^+]_s} \quad [9]$$

Taking into account that $[H^+]_{in}$ is a constant and known quantity and that for the sample solution it holds $pH_s = -\log [H^+]_s$, eqn [9] becomes

$$E = E'_{const} - 2.303\frac{RT}{F}pH_s \quad [10]$$

indicating that the pH is a linear function of the measured potential.

The specific form of the equation ($y = a + bx$) is behind the need for a two-point calibration of glass pH meters, using two buffer solutions of known pH values (typically around 4 and 7). It should be noted that, since the pH of standard buffers is reported with two decimal places accuracy, pH readings should also be rounded to two decimals.

As the resistance of such a cell (primarily due to the glass membrane) is very high (in the 10^7–10^9 Ω range), the corresponding pH meter cannot be equipped with a common voltmeter, but instead, a high impedance device is needed. The commercialization and widespread use of pH meters was only possible when cheap, miniaturized electronics, based on field-effect transistors, became available and were employed in the demanding electronic part of the device.

The most serious practical limitation of the common glass electrode is its inability to work at pH values above ~11 as a result of the 'alkaline error,' that is, the interference from alkali metals such as Na^+ and K^+ usually present in alkaline media and competing with H^+ for silica sites due to their similar size. Specialty glasses, rich in Li, partially alleviate this problem. Other problems encountered include attack by high F^- levels, noble metal deposition, protein deposition, and debris fouling of the ceramic frit. Cleaning the surface of a fouled glass electrode can be done by brief immersion in HF, but nowadays, replaceable affordable glass membrane tips have appeared in the market too.

ISFET pH Sensors

Construction and principle

There are certain applications for pH measurements where the use of glass cannot be tolerated and miniaturization is also essential. Among these, food testing and biomedical testing are the most usual cases where breaking of the thin glass membrane of a glass electrode would be catastrophic and inclusion of the meter in a catheter is often needed. The first of these requirements is fully met by nonglass pH electrodes, whereas the second one can be partially met if, together with a miniature pH-sensing device, a miniaturized reference electrode is also used. The most typical nonglass electrodes (apart from the Sb-based ones) are those based on ISFETs, which originated from the metal oxide semiconductor field-effect transistors (MOSFETs) that are widely used in modern electronics.

A MOSFET consists of a semiconductor (e.g., a p-type Si) covered by a thin insulating layer of an oxide (e.g., SiO_2) and four metal terminals: the substrate (located at the free face of the semiconductor), the source and drain (located between the other face of the semiconductor and the oxide), and the gate (located on top of the oxide layer (Figure 2(a))). The application of an appropriate gate voltage V_G changes the charge/conductivity of the semiconductor channel between the drain and the source. For example, for an enhancement-mode MOSFET (where the semiconductor is not heavily doped) and for a p-type semiconductor, a positive V_G bias above a certain threshold value V_T results in the mobile hole h^+ intrinsic carriers being pushed away from the oxide/semiconductor interface into the bulk of the latter; this leaves behind a thin

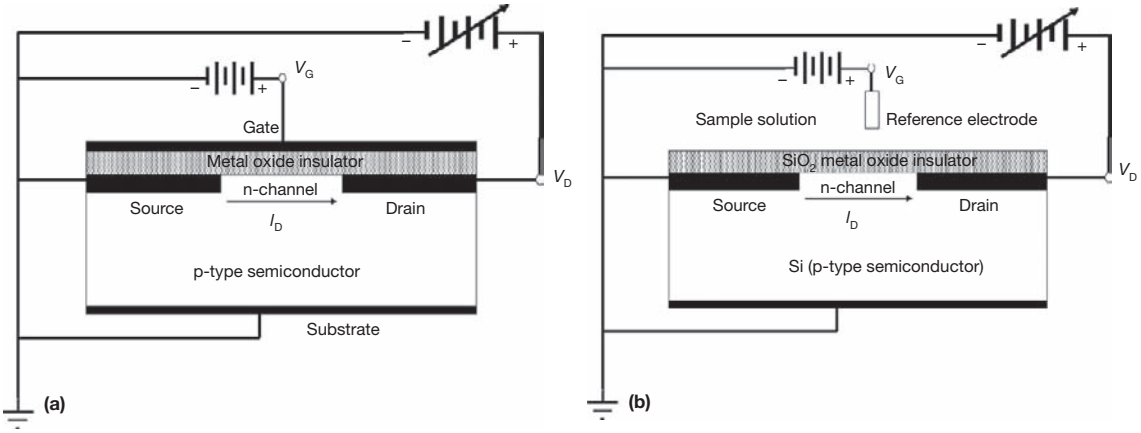

Figure 2 Schematic representation of (a) a MOSFET device and (b) of a SiO_2-based ISFET used for pH measurements.

electron-rich (n-type) channel between the gate and drain terminals. If a positive bias, V_D, between the drain and the source is applied, then the electrons flow through the n-channel giving rise to a drain current, I_D. For a constant value of V_D, this drain current depends on the gate bias V_G (more accurately on expressions containing its difference from the threshold potential, $V_G - V_T$).

In an ISFET used as a pH meter, the gate electrode terminal is replaced by a reference electrode, and the sample solution is between this electrode and the insulating oxide, which is pH-sensitive (e.g., SiO_2, $Si_3N_4O_x$, Al_2O_3, or Ta_2O_5), as depicted in Figure 2(b). (For other ion-sensitive ISFETs, there is an additional ion-selective membrane on top of the oxide layer.)

In an ISFET pH meter, changes in the pH of the sample solution alter the surface potential of the oxide layer (via ion exchange reactions similar to [6]), which in turn modifies V_T and, as a result, the drain current I_D. There are two modes of operation: a constant V_G voltage operation whereby I_D is monitored via a current follower circuit and a constant I_D current mode, whereby the changes in V_G needed to maintain a constant I_D via a feedback circuit are recorded. In either case, the measuring circuit need not be characterized by a very high impedance, as is the case of the potentiometric glass electrode. A practical implication of this is that the meters of a glass and an ISFET electrode are not interchangeable in a straightforward manner.

The voltage output of a pH ISFET (either after conversion to V_T values or as directly related to changes of V_G, depending on mode of operation) should theoretically follow a Nernstian dependence on sample proton concentration. However, most of the metal oxide materials used do not exhibit the Nernstian/maximum sensitivity of $\sim 59\ \text{mV dec}^{-1}$ (e.g., SiO_2 has a $30\ \text{mV dec}^{-1}$ value, $Si_3N_4O_x$ has $\sim 55\ \text{mV dec}^{-1}$, and only Ta_2O_5 approaches the theoretical value).

The reduced sensitivity is perhaps the only drawback of ISFET pH meters (the other is their higher cost) with respect to the glass electrode. However, their nonfragile property (especially if a miniaturized reference electrode is also embedded in an all-plastic body), their fast response time, and their ability to operate at extreme pH values make them the only viable solution for many applications.

pH Indicators

pH indicators are halochromic compounds, weak acids or bases, that occur as natural dyes and indicate the concentration of H^+ (H_3O^+) ions in a solution through color changes. They exist in the form of dissolved dyes, which are added directly to the solution or dye-infused paper strips, which are dipped into the solutions and then removed for comparison against a color–pH key. The general idea of pH indicator functionality is that the nondissociated form of the indicator has a different color than the ionic form of the indicator. It must be stressed that the color of the indicator changes over a range of hydrogen ion concentrations, which is called the *color change interval*. The corresponding pH interval (for an acid indicator) is between $pK_a + 1$ and $pK_a - 1$, where K_a is the indicator's acid dissociation constant. Equation [11] describes the dissociation of an acidic pH indicator HA_{ind}:

$$HA_{ind} + H_2O \leftrightarrows H_3O^+ + A_{ind}^- \qquad [11]$$

In eqn [11], HA_{ind} represents the acid form and A_{ind}^- the conjugate base of the indicator. It must be stressed that the ratio of these determines the color of the solution and connects the color to the pH value. The weak protolyte pH indicators have a pH that can be calculated from the Henderson–Hasselbalch equation:

$$pH = pK_a + \log \frac{A_{ind}^-}{HA_{ind}} \qquad [12]$$

From eqn [12], it can be deduced that in the case where $pH = pK_a$, both acid and conjugate forms are present in a 1:1 ratio. When pH is above the pK_a value, the concentration of the conjugate base is greater than the concentration of the acid, and the color associated with the conjugate base dominates. In the opposite case, where pH is below the pK_a value, the converse is true. More precise measurements are possible if the color is measured by spectrophotometry. pH indicators work efficiently at their designated pH range, and they are usually destroyed at the extreme ends of the pH scale due to undesired side reactions. In Table 2, some common indicators are summarized.

Table 2 Common pH indicators

Indicator	*pH range*	*Acid*	*Base*
Thymol blue	1.2–2.8	Red	Yellow
Pentamethoxy red	1.2–2.3	Red-violet	Colorless
Methyl yellow	2.9–4.0	Red	Yellow
Methyl orange	3.1–4.4	Red	Orange
Bromophenol blue	3.0–4.6	Yellow	Blue-violet
Tetrabromophenol blue	3.0–4.6	Yellow	Blue
Alizarin sodium sulfonate	3.7–5.2	Yellow	Violet
Bromocresol green	4.0–5.6	Yellow	Blue
Methyl red	4.4–6.2	Red	Yellow
Bromocresol purple	5.2–6.8	Yellow	Purple
Chlorophenol red	5.4–6.8	Yellow	Red
Bromophenol blue	6.2–7.6	Yellow	Blue
p-Nitrophenol	5.0–7.0	Colorless	Yellow
Azolitmin	5.0–8.0	Red	Blue
Neutral red	6.8–8.0	Red	Yellow
Cresol red	7.2–8.8	Yellow	Red
α-Naphtholphthalein	7.3–8.7	Rose	Green
Thymol blue	8.0–9.6	Yellow	Blue
Phenolphthalein	8.0–10.0	Colorless	Red
Thymolphthalein	9.4–10.6	Colorless	Blue
Nile blue	10.1–11.1	Blue	Red
Alizarin yellow	10.0–12.0	Yellow	Lilac

Source: Bates, R. G. (1964). *Determination of pH: theory and practice* (1st ed.), pp. 138–139. London: John Wiley & Sons.

Applications

The measurement of pH is important for many applications in medicine, biology, chemistry, agriculture, forestry, environmental science, oceanography, civil engineering, chemical engineering, water treatment and water purification, food science, and nutrition.

pH in Nature

The role of pH in nature is closely related to that of water, and as such, it is extremely important for living organisms and the environment. The pH of natural water and soils controls the form of life sustained in these environments. The pH of the various parts of plants and living organisms defines their function, whereas that of foodstuffs their taste and function once in the food chain.

Examples of the environmental importance of pH are acid rain (a result of industrial pollution, with detrimental effects on life and buildings) and ocean acidification as a result of increased carbon dioxide emissions (detrimental to living organisms in aquatic environments). Typical examples of biological processes involving pH changes include the production of carboxylic acids, such as lactic acid by muscle activity, the protonation of phosphate derivatives such as ATP, and the function of the oxygen-transport enzyme hemoglobin. Finally, an example of pH-related properties of foodstuff is the acidity of some juice fruits due to the presence of citric acid.

Living Systems

pH is extremely important for living systems through its role in biochemical reactions. The pH of various parts and fluids of an organism is regulated by the acid–base homeostasis. For example, human blood should have a pH value in the 7.36–7.42 range, mainly controlled by the bicarbonate/carbonic acid buffer. A pH change as low as 0.2 pH units can result in death (via acute acidosis or alkalosis).

Certain definite pH values are needed for the activation of many enzymes in the body and the trigger of associated reactions. The parts and fluids of the human body have pH values that span the entire pH range, starting from gastric acid (pH = 1.0), to human skin (pH = 5.5), urine (pH = 6.0), and blood (pH = 7.4), to pancreatic fluid (pH = 8.1).

Food and pH

pH is also very important for foodstuff in many perspectives. It is a factor of major importance in water absorption, emulsification, and gelation of different protein sources. It affects significantly the physical and chemical properties of food ingredients such as proteins, sugars, and amino acids, to mention a few.

The vast majority of foodstuffs, apart from egg whites and soda crackers, have a pH value < 7. Alkaline foods (pH > 7) are limited, though the pH of some staple foods lies in the range of 4.5–7.0. Generally, foods that are products of plant origin have a pH that is lower than those of animal origin. On the basis of their pH, foods can be classified as high-acid (pH = 3.7), acidic (pH = 3.7–4.6), medium-acid (pH = 4.6–5.3), or low-acid (pH = over 5.3). The pH of some food is listed in Table 3.

pH gives also information about food stability and preservation. It can be used to retard microbial spoilage that could happen in the presence of some pathogens such as bacteria, molds, and yeasts. Microorganisms usually show their best growth rate in the pH range of 6.5–7.5. Furthermore, the growing capability of molds and yeasts lies in a much broader pH range than that of microorganisms such as bacteria. In consideration of the fact that almost all of the pathogenic agents and most of deterioration bacteria cannot grow at pH < 4.5, foods are divided into two categories, which are low-acid or acidic. The *low-acid foods* with pH > 4.5 are less stable. Their stabilization by heat treatment demands a heat sterilization to remove all pathogens and corruption, including bacterial spores. *Food acids*, at pH < 4.5, are relatively stable. Their stabilization by heat treatment needs less severe procedures, such as pasteurization, in order to eliminate mold, yeast, and some acidophilic bacteria.

The reduction of pH of food products is usually necessary and can be carried out using two methods. One of them is acidification, which is a direct method, and its aim is to lower the pH by adding organic acids, such as acetic acid or vinegar and citric acid or lemon. The second method, which is an indirect method, utilizes microorganisms for fermentation. *Fermentation* is a food preservation procedure that uses selected nonpathogenic microorganisms producing acid or alcohol to alter food organoleptic and/or antibacterial characteristics.

Table 3 pH values of some foodstuffs

Foodstuff	*pH*
Apple, eating	3.30–4.00
Bananas	4.50–5.20
Beans, vegetarian, tomato sauce, canned	5.32
Bread, white	5.00–6.20
Bread, Boston, brown	6.53
Bread, whole wheat	5.47–5.85
Cabbage	5.20–6.80
Carrots	5.88–6.40
Cauliflower	5.60
Cheese, Camembert	7.44
Cheese, Cheddar	5.90
Cheese, Cottage	4.75–5.02
Cheese, Parmesan	5.20–5.30
Cherries, California	4.01–4.54
Chicken	5.8–6.4
Cornflakes	4.90–5.38
Eggs, new-laid, whole	6.58
White	7.96
Yolk	6.10
Fish	6.5–6.8
Garlic	5.80
Grapes	2.80–3.80
Grapefruit	3.00–3.75
Honey	3.70–4.70
Ketchup	3.89–3.92
Lemon juice	2.00–2.60
Milk, cow	6.40–6.80
Milk, goat's	6.48
Milk, peptonized	7.10
Mushrooms	6.00–6.70
Mustard	3.55–6.00
Olives, black	6.00–7.00
Onions	5.30–5.80
Oranges, Florida	3.69–4.34
Peaches	3.30–4.05
Peanut butter	6.28
Pears	3.50–4.60
Peas, chick	6.48–6.80
Peppers	4.65–5.93
Pineapple	3.20–4.00
Potatoes	5.40–5.90
Rice (cooked)	6.00–6.80
Salmon, fresh, boiled	5.85–6.50
Sardines	5.70–6.60
Soy Sauce	4.40–5.40
Soybean milk	7.00
Spaghetti, cooked	5.97–6.40
Spinach	5.50–6.80
Strawberries	3.00–3.90
Tea	7.20
Tomatoes	4.30–4.90

Source: US Food and Drug Administration; http://www.fda.gov/Food/default.htm.

See also: Acids: Natural Acids and Acidulants; Acids: Properties and Determination; Biosensors; Fatty Acids: Determination and Requirements; Fatty Acids: Fatty Acids; Nucleic Acids.

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Phenolic Compounds: Bioavailability and Health Effects

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Introduction

Phytochemicals are plant secondary metabolites, that is, substances that have little or no role in photosynthesis, respiration, or growth and development. In humans, they are nonnutrient xenobiotics that are quickly eliminated. The majority of phytochemicals are antioxidant compounds.

Phenolic compounds ('phenols' and 'polyphenols') comprise a major class of phytochemicals. They are essential to plants for pigmentation, growth, reproduction, resistance to pathogens, and several other functions. Hence, phenolic compounds are ubiquitously present in the plant kingdom, representing the main source of exogenous-dietary antioxidants in our diet. In general, dietary – exogenous – antioxidants are effective at very low concentrations, while at higher concentrations, most of them act as pro-oxidants possibly due to their involvement in the initiation reactions. Recent studies indicate that plant-derived phytochemicals, ingested by humans at subtoxic doses, induce mild cellular stress responses. This dose–response phenomenon, characterized by a low-dose stimulation and high-dose inhibition, is described in biology and medicine as 'preconditioning' or 'hormesis' and results in inducing the expression of genes that encode several cytoprotective proteins, including antioxidant enzymes, protein chaperones, phase-2 enzymes, and neurotrophic factors.

The terms 'phenol' and 'polyphenol' are defined chemically as substances that possess at least an aromatic ring bearing one (phenol) or more (polyphenol) hydroxyl substituents. However, in the strict context of plant phenolics, such a definition is not satisfactory, since it would include terpenoid compounds like the phenolic carotenoid 3-hydroxyisorenieratene and the phenolic female sex hormone estrone.

Phenolic compounds are synthesized mainly through shikimate and acetate pathways and are generally found conjugated to sugars and organic acids, with conjugates linked to hydroxyl or aromatic carbon moieties. Besides glucose, which is the most common conjugate, galactose, rhamnose, xylose, arabinose, and glucuronic or galacturonic acids are also found. The structure of plant phenolics and polyphenols varies from simple molecules, such as phenolic acids, to highly polymerized compounds, such as proanthocyanidins. Several thousand different compounds have been so far identified (among them over 8000 flavonoids), bearing a large range of structures, and many of them occur in food.

According to number and arrangement of carbon atoms, naturally occurring plant phenolics can be classified into flavonoids and nonflavonoids. In addition, traditionally processed foods and beverages, such as coffee, red wine, and black tea, may contain products of their transformation that are described as 'derived polyphenols.' Different types of phenolics predominate in certain foods; for example, flavan-3-ols are the main phenolics found in tea, flavanones in citrus fruits, and isoflavones in soy, whereas other phenolic compounds, such as gallic acid and quercetin, are ubiquitously found in plant foods.

Several classes of phenolics have been categorized on the basis of their basic skeleton: C6 (simple phenols and benzoquinones), C6-C1 (phenolic acids), C6-C2 (acetophenones and phenylacetic acids), C6-C3 (hydroxycinnamic acids, coumarins, phenylpropenes, and chromones), C6-C4 (naphthoquinones), C6-C1-C6 (xanthones), C6-C2-C6 (stilbenes and anthraquinones), C6-C3-C6 (flavonoids and isoflavonoids), $(C6\text{-}C1)_2$ (hydrolyzable tannins), $(C6\text{-}C3)_2$ (lignans and neolignans), $(C6\text{-}C3\text{-}C6)_2$ (biflavonoids), $(C6\text{-}C3)_n$ (lignins), $(C6)_n$ (catechol melanins), and $(C6\text{-}C3\text{-}C6)_n$ (condensed tannins). Flavonoids (Figure 1) are the most widespread among the phenolic compounds. The most abundant subclasses of dietary flavonoids are flavones, flavonols, flavanones, flavan-3-ols, anthocyanidins, and isoflavones (Figure 2). Dietary flavonoids found in relatively lower amounts are flavan-3,4-diols, dihydroflavonols, aurones, coumarins, chalcones, and dihydrochalcones.

Bioavailability of Phenolic Compounds

Food components are categorized to nutrients and nonnutrients with distinguished roles in human body. Nutrients are mainly utilized as substrates for energy production, while nonnutrients exhibit a variety of other biological functions essential for human homeostasis. Plant-derived foods, for example, fruits, vegetables, and whole grain fruits, contain large quantities of nonnutrient phytochemicals that have been extensively studied for their beneficial health effects on the prevention of chronic inflammation, cardiovascular diseases, and some types of cancer.

Phenolic compounds are the most studied type of phytochemicals. The majority of *in vitro* studies consent that phenolic compounds are potent antioxidant and anti-inflammatory agents, but they must be biologically available in order to exhibit their health functions *in vivo*. Transport through the gastrointestinal tract, release from the solid matrix, absorption across the gastrointestinal epithelium, and transport into the circulation are the normal paths of dietary phenolics.

In line with other nutrients and nonnutrients, ingestion of phenolic compounds is followed by both bioaccessibility and bioavailability. Bioavailability is a concept that comes from a fusion of the words 'biological' and 'availability' and was initially defined as "the fraction of administered drug that can reach plasma and body tissues in an unchanged form." When it comes to the bioactivity of food components intended for oral ingestion, either as food supplements or as parent foods, the impact of bioavailability is crucial since the digestive tract constitutes the primary barrier between an active ingredient and the systemic circulation. The internal environment of the digestive tract (e.g., pH and microbial flora) can also affect absorption. Thus, bioavailability should always be considered when the effects of ingested food components are studied in animal

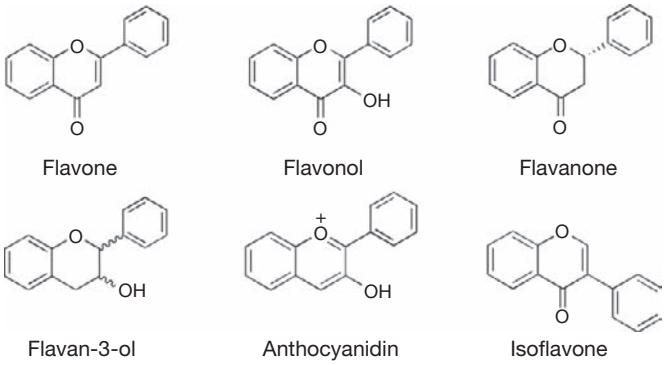

Figure 1 Flavonoid skeleton indicating the numbering of carbon atoms and the naming of rings.

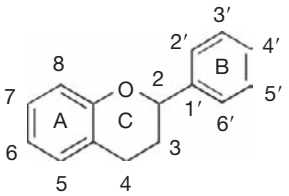

Figure 2 Main subclasses of dietary flavonoids.

models or human clinical trials. In order to be available for absorption in the gastrointestinal tract, the food component must also be bioaccessible. The term bioaccessibility refers to the amount of any food constituent that is readably present in the gut, as a consequence of its release from the solid matrix, and that may be able to pass through the intestinal barrier.

The 'rule of five' introduced by Lipinski et al. (2001) describes the ideal molecular properties of a drug molecule for gastrointestinal absorption. According to this rule, a molecule displays optimal absorption if it has (a) a molecular weight of less than 500 Da, (b) no more than five hydrogen bond donors, (c) no more than ten hydrogen bond acceptors, and (d) an octanol–water partition coefficient (log P) less than five. Molecules absorbed by specific transporters are an exception to this rule. Additionally, the presence of adjuvants, for example, food, can also affect bioavailability. Catechin is an example of phenolic compound that meets the aforementioned rule for oral bioavailability.

The bioavailability of phenolic compounds has been extensively reviewed in the bibliography. A great number of reviews are focusing on phenolic compounds from individual sources, such as green tea, cocoa, beer, wine, and fruits, while others discuss the bioavailability of phenolic compounds in total. Nevertheless, the overall bioavailability of phenolic compounds is relatively low, mainly due to their low absorption in the gastrointestinal tract, instability, biotransformation within the gut, excessive metabolism, and rapid clearance from the body. As described earlier herein, phenolic compounds occur mostly as soluble conjugates (glucosides) and insoluble forms covalently bound to cell wall structural components. Absorption for bound phenolic compounds in the gastrointestinal tract greatly depends on the liberation of sugar moieties. Food processes such as fermentation, malting, thermoplastic extrusion, and enzymatic, alkaline, and acid hydrolyses occasionally assisted with microwave or ultrasound treatment have potential to release phenolics associated to cell walls. Additionally, food processing may modify the naturally arranged microstructure of most plant foods. Phenolic compounds can be present in the matrix as individual molecules, either bound to cell organelles or entrapped in complex macromolecular matrices with other macronutrients such as carbohydrates or proteins. The food matrix affects bioaccessibility and bioavailability.

Overall, the main factors affecting the bioavailability of phenolic compounds in humans are (a) environmental factors (e.g., sun exposure, and degree of ripeness) and food availability, (b) food processing (e.g., thermal treatments, homogenization, lyophilization, cooking and culinary methods, and storage), (c) food-related factors (e.g., food matrix), (d) interactions between food components (i.e., bonds with proteins or with polyphenols with similar mechanism of absorption), (e) chemical properties (chemical structure, concentration in food, and amount introduced), (f) host-related factors such as intestinal factors (e.g., enzyme activity, intestinal transit time, and colonic microflora), and systemic factors (e.g., gender and age, disorders and/or pathologies, genetics, and physiological condition).

In vitro evaluation of the activity of phenolic compounds does not have to be in accordance with the activity demonstrated in humans. Bioactivity is influenced by metabolic transformation and bioavailability and the main reason is that phytochemicals are processed as xenobiotics.

There are several *in vitro* approaches for evaluating bioavailability of phenolic compounds, which use a barrier membrane separating the donor compartment, that is, the gastrointestinal lumen from the receiver compartment, that is, the blood circulation, through which the molecule has to pass. Thereafter, an analytic method of appropriate selectivity and sensitivity is used to quantify the amount of components transported across the membrane. The *in vitro* assimilation approaches have been developed as simple, cheap, and repeatable tools for the study of food component bioavailability. These models aim to reproduce the absorption processes in humans, and the stability of the *in vitro* conditions achieved is their major advantage. Nevertheless, there is a significant limitation; the intestinal mucosa is not only a barrier on a physical sense but also a biochemical barrier, which carries out a series of complex metabolic reactions that are part of the presystemic metabolism and affect bioavailability.

There are various types of models including those based on physical reproduction of the membrane (artificial membrane),

those based on cell cultures and isolated organs, and those using isolated or reconstituted cell membranes. Data obtained from the last two types of studies demonstrate higher correlation with those obtained from *in vivo* trials.

In the case of models based on cell cultures, the human colon cancer cells type 2 ($CaCo_2$) are more commonly used. Although this cell line originates from human adenocarcinoma colonocytes, when cultured under appropriate conditions, these cells differentiate into polarized monolayers that present morphological and functional characteristics of intestinal absorptive epithelium, including intercellular tight junctions and apical microvilli, and carriers for nutrient as well as efflux proteins and a number, but not all, of phase-2 enzymes required to xenobiotic disposition. Other cell lines, such as Madin–Darby canine kidney cells, rat fetus intestinal cells (2/4/A1), and pig kidney epithelial cells (LLC-PK1), are also used.

Furthermore, suspension brush border membrane vesicles prepared from the intestinal tissue of mammalians (e.g., pigs, rabbits, chickens, rats, mice, and humans) are used to study the transport of compounds through the cell membrane. A weakness of this model is that it predicts only the step of the absorption process that occurs on the interior of the membrane.

In order to detect and quantify the compounds transported across the gastrointestinal barrier, the most common analytic methods used are gas and liquid chromatography with UV or MS detection. For *in vivo* studies, MS detection is more appropriate than UV detection because of the high sensitivity of the method. Finally, both sensitivity and selectivity are significantly enhanced when tandem mass detectors are used (MS–MS).

In vivo evaluation of bioavailability provides safer conclusions about the fate of phytochemicals in humans. These studies are usually conducted in mammalian models (e.g., mice, rats, dogs, and monkeys), nonmammalian models, and human clinical trials. Blood, urine, and/or feces samples are collected at regular time intervals usually until 24 h after ingestion. The phenolic compounds, as well as their metabolites, are detected in the biological samples, while total plasma antioxidant capacity is usually evaluated. One limitation of these studies is that the methodology includes a high-dose consumption of the particular food or compound(s) after implementing a washout diet restricted in phytochemicals. Such high doses may cause different pharmacokinetics from the usual followed by ingestion of normal food quantities. Additionally, the restriction diets may be difficult to implement for several days leading volunteers to withdraw from the study.

Despite disadvantages, bioavailability studies serve to understand the different absorption mechanisms, as well as which phenolic compounds are absorbed intact or have to be first transformed. For example, it was initially assumed that the glycosylated phenolic compounds are not able to cross the enterocyte wall due to their hydrophilic nature. Now, the presence of specific transporters that assist their transmembrane transport is well documented.

Digestion and Metabolism of Phenolic Compounds

The first step of digestion is mastication, a process that turns food to smaller pieces facilitating partial release of food components and passage through the esophagus toward the stomach. The salivary secretion contains digestive enzymes that help the release of phenolic compounds. Hydrolysis of glycoside flavonoids already starts in mouth by means of β-glucosidase activity; however, its effectiveness depends on the sugar type present in the molecule. Most flavonoids in food occur as *O*-glycosides. Glucose is the most common glycosidic unit, and also glucorhamnose, galactose, arabinose, and rhamnose may occur. Glucose conjugates are rapidly hydrolyzed as opposed to others such as those of rhamnose. Absorption may as well occur in the oral mucosa. Tea catechins are detected in the salivary phase at levels ranging from 74.8% to 99.5% of undigested samples, indicating good stability under oral biochemical conditions *in vitro*. Degalloylation, that is, the conversion of epigallocatechin gallate to epigallocatechin, occurs in the oral cavity, which leads to subsequent absorption through the oral mucosa. A catechin esterase activity that converts epigallocatechin gallate to epigallocatechin has been identified in the saliva. A plethora of polar and hydrophobic compounds can rapidly reach the blood circulation, bypassing the gastrointestinal tract, while possible underlying mechanisms have already been studied. Interaction with proteins (glycoprotein mucin, albumins, immunoglobulins, and lactoferrin), also present in the salivary secretion, can lower bioaccessibility. Chemical structure seems to affect the degree of this interaction. *In vitro* interaction of catechin with saliva proteins is stronger than that of epicatechin, an observation mostly attributed to the stereochemistry of flavan-3-ols. Similarly, procyanidin dimers linked through a C(4)–C(8) interflavanoid bond present greater affinity for saliva proteins than those linked through C(4)–C(6). Esterification of a galloyl group to the C(3) hydroxyl function of (−)-epicatechin or to the epicatechin moiety of procyanidin dimer B2 increases the binding affinity to proteins.

In the stomach, peristalsis and chyme production under low pH environment generally enhance the release of phenolic compounds from the food matrix. Hydrolysis and/or transformation, such as hydrolysis of proanthocyanidin oligomers or cleavage of sugar moieties in the stomach, may occur and facilitate bioaccessibility. Gastric pH plays significant role in phenolic compound stability. Total phenolic content in fruit juices is decreased at least 47% after *in vitro* digestion compared with predigestion. Significant reductions in gallic, caffeic, and ferulic acid levels are observed after digestion of pineapple and red fruit juices, probably due to poor stability to pH changes. Anthocyanins are stable in the acidic conditions of the stomach but are less stable at the elevated pH of the small intestine. Generally, flavonols and proanthocyanidins remain intact, but they may also be broken down when pH is substantially low in the stomach. Among all the flavonoids, the flavan-3-ols exist as aglycones and pass intact into the duodenum without requiring the action of β-glucosidase prior to absorption. Phenolic compounds may show different stability to pH changes when tested solely, meaning that the interaction with other food components can affect the stability during digestion. Codigestion of raspberry extract with other foodstuffs such as bread, breakfast cereal, ice cream, and cooked minced beef alters bioavailability. In particular, the total phenol content is slightly reduced by codigestion with ice cream or breakfast cereal but is not affected by codigestion with bread or minced beef. In the case of small phenolic compounds lightly bound to food matrix, absorption is also observed in the stomach.

Absorption across the enterocyte wall depends strongly on the food matrix, the physiology, and the anatomy of the consumer, as well as the characteristics of phenolic compounds, such as molecular dimension, polymerization, stereochemistry of the glycosidic moiety, and hydrophobicity. In particular, procyanidin dimer and trimer may cross the gut barrier, but procyanidin polymers do not. While phenolic compounds are not subjected to major transformation in the stomach, as the chyme passes to the alkaline environment of the duodenum, partial degradation and loss of phenolic compounds occur. Anthocyanins, flavonols, and flavan-3-ols are unstable in the alkaline environment of the small intestine, which results in 43%, 26%, and 19% reduction, respectively. Deglycosylation, glucuronidation, methylation, sulfonation, and hydroxylation of flavonoids take place. Epigallocatechin gallate may also be oxidized to dimerized products possessing higher superoxide radical scavenging activity than epigallocatechin gallate itself. The food matrix can buffer the pH and oxidation of the flavanols may occur only to a limited extent during intestinal transit. It has been documented that vitamin C significantly increases the stability of flavanols incubated with intestinal juice *in vitro*. Galloylated catechins are rapidly eliminated, not by degalloylation, a process not less occurring in humans, but rather excretion of these compounds in bile (feces). Similarly, phenolic acid esters such chlorogenic acid cannot be degraded in the small intestine due to the absence of esterases; therefore, they are only metabolized by the colonic microflora.

Regarding phenolic compound glycosides, two main absorption paths have been proposed: (a) along the gastrointestinal tract and (b) inside the enterocytes. Metabolisms along the gastrointestinal tract are mainly aiming at deglycosylation. Phenolic compound glycosides are more hydrophilic than their corresponding aglycones and cannot readily cross the enterocyte cellular wall via passive diffusion. For this purpose, hydrolase enzymes available in intestinal lumen, brush border, and enterocyte cleave the glycosidic moieties. This deglycosylation also takes place by microbiota in the colon. Lactase–phlorizin hydrolase (LPH), a brush border enzyme on the surface of enterocytes, is very potent toward flavonoid-*O*-β-D-glycosides. Consequently, the aglycone products with their lipophilic nature can cross the cell membrane via passive diffusion.

Inside the enterocyte, phenolic compound glycosides are transported across the membrane cell wall via an active sodium-dependent glucose transporter. There, glycosidic moieties are cleaved by cytosolic β-glucosidase, and the aglycone forms are either passed to the bloodstream via passive diffusion or transformed to phase II metabolites. Conjugation of phenolic compounds into glucuronides, sulfates, or methyl conjugates is the most studied pathway. These phase II metabolites are either effluxed into the gut lumen or transported to the bloodstream via apical and basolateral multidrug/lipid transporters, respectively. As xenobiotics, phenolic compounds undergo phase II transformation in the small intestine, liver, and bile contributing to detoxification. This transformation with conjugation in hydroxyl groups of glucuronidic acid, sulfate, and/or methyl is mainly catalyzed by UDP-glucuronosyltransferases, sulfotransferase, and catechol-*O*-methyltransferase mainly found in the liver. There is evidence that conjugation retains bioactivity of phenolic compounds but is generally weaker than that of the aglycone form. For example, quercetin-3-glucuronide, one of the three major plasma quercetin conjugates, is able to delay the Cu(II)-induced oxidation of human low-density lipoprotein, but two other major conjugates (quercetin-3-sulfate and 3-methylquercetin-3-glucuronide) are ineffective.

There are significant differences between the administration of drugs (usually at a high dose) and the consumption of dietary phenolic compounds (usually at a low dose). Drugs saturate the metabolic pathways that rely on phase II metabolism and circulate in the blood in the unconjugated form. In contrast, dietary phenolic compounds are not able to saturate these metabolic pathways and undergo conjugation. However, when phenolic compounds are administered at high doses, they can be found in free-form in the blood. For example, after the intake of a large dose of (1)-catechin, free (1)-catechin is detected in the plasma after 30 min. On the other hand, after the consumption of a few milligrams of (1)-catechin, normally present in red wine, catechin is found only in the conjugated form.

Absorption of flavonoid glycosides also depends on the type of sugar moiety. Quercetin-3-*O*-rhamnoglucoside is slower absorbed than quercetin-4-*O*-glucoside, since the glycoside is absorbed in the small intestine, while the rhamnoglucoside in the colon after hydrolysis by the gut microbiota.

Undigested polyphenols pass into the large intestine, where they are degraded by the colonic microflora. The colon contains 1012 microorganisms per cubic centimeter, which have catalytic and hydrolytic potential. Biotransformation in the gut depends on phenolic compound chemical structure, microbiota population, and food consumed. Generally, phenolic compound polymers degrade to phenolic acids; flavonols produce hydroxyphenylacetic acids; flavones and flavanones degrade to hydroxyphenylpropionic acids; and flavanols produce both phenylvalerolactone and hydroxyphenylpropionic acids. Procyanidins are also degraded to phenolic acids of lower molecular weight. Additionally, colonic bacterial enzymes catalyze reactions of hydrolysis, dehydroxylation, demethylation, and decarboxylation. For example, quercetin-3-*O*-rhamnoglucoside and quercetin-3-*O*-rhamnoside are hydrolyzed by a-rhamnosidase and β-glucosidase. Bacterial enzymes (β,D-glucuronidase, β,D-glucosidase, and a,L-rhamnosidase) also deconjugate phase II metabolites produced in enterocytes and hepatocytes. Then, the bacterial derivatives can be absorbed into the circulation and transported to the liver, where they are subjected to first-pass and phase II metabolism.

Human clinical trials and animal studies confirm that phenolic compounds and their metabolites are found in various tissues, for example, brain, endothelium, heart, kidney, liver, pancreas, prostate, uterus, ovary, mammary gland, testes, bladder, bone, and skin tissues. Nevertheless, only a small amount of ingested phenolic compounds is detected in tissues, while excretion is rapidly occurring via urine and bile within 24 h after consumption. The biological activities of phenolic compound metabolites may also be different from those of the parent compounds depending on their transformation (methylated, sulfated, or glycosylated). However, these metabolites account for the increase in total plasma antioxidant capacity. Further bioavailability studies are needed to elucidate the pharmacokinetics of dietary phenolic compounds and how they are distributed to peripheral tissues and exert their biological effects.

Phenolic Compounds Health Effects

Research on the health effects of polyphenols has developed significantly in the past decades, supporting their antioxidant, anti-inflammatory, and antimicrobial properties in humans. However, their role in human health is centered chiefly in the prevention of atherosclerotic disease and the resulting cardiovascular disease or some forms of cancer. The antioxidant properties of polyphenols in chemical and biological assays have been most widely studied, and it has become clear that the mechanisms of action of polyphenols go beyond their radical scavenging capacity. Many clinical trials have shown that to some extent, their properties evidenced in observational studies or shown *in vitro* may apply to entire organisms, yet their effect in human disease remains a controversial topic.

Effects on Atherosclerosis

Based on the 'oxidation theory' for atherosclerosis, polyphenols have attracted considerable attention as preventive and therapeutic agents as there is sufficient evidence that consumption of polyphenol-rich foods results in a reduction in oxidative stress and myocardial infarction biomarkers. Observational studies serve to correlate dietary polyphenol intake with disease prevention or disease risk reduction. An example of such is the Zutphen Elderly study, in which intake of flavonoids (30 mg day^{-1}) was correlated with 50% reduction in cardiovascular disease mortality compared with 19 mg day^{-1} flavonoid intake. However, observational studies are based upon measurements of micronutrient intakes through the use of dietary recall questionnaires that may be imprecise. Thus, the strong hypotheses driven by such observations are verified by *in vitro* or *in vivo* intervention studies.

Oxidized LDLs are associated with the pathogenesis of atherosclerosis. Oxidation takes place when naturally occurring antioxidant agents such as alpha-tocopherol and carotenes that normally inhibit LDL oxidation do not occur. The role of oxidized LDLs in atherosclerosis, and consequently in cardiovascular disease, is manifold as they induce cell adhesion molecule expression in aortic endothelial cells and subsequently enhance endothelial cell expression of chemotactic and adhesion molecules and stimulate T cells through the major histocompatibility complex and CD4+ helper T-cell receptor. Further, they trigger proinflammatory cytokine secretion that increases smooth muscle cell proliferation, activates monocytes, and increases T-cell proliferation. Under the stimulation of oxidized LDLs, activated macrophages influence the expression of mediators of endothelial activation, such as nitric oxide. Besides, the aldehydic products of LDL oxidation exert a direct toxic effect on the endothelium and platelets. Several *in vitro* studies strongly suggest that polyphenols protect LDL from oxidation. Additionally, LDL oxidizability is reduced after consumption of several natural products that are rich in polyphenols, such as not only olive oil and its oleuropein and hydroxytyrosol or red wine with its quercetin and resveratrol but also grape juice or tea or cocoa. Flavonoids have been found to act against LDL oxidation, and their antioxidant capacity is related to their chemical structures. The most hydrophilic do not bind LDL molecule; instead, they either scavenge free radicals; or act as chelating agents; or protect vitamin E, carotene, and lycopene in the LDL particle; or preserve serum paraoxonase activity, which itself promotes hydrolysis of lipid peroxides. Concerning isoflavones, present mainly in soy products, the clinical results are controversial, as some studies do show that they are effective inducers of *ex vivo* LDL oxidation after supplementation, while others do not.

Apart from oxidation of LDLs, the vascular endothelium plays a key role in atherosclerosis, particularly in the regulation of vascular tone by the release of vasodilator substances, mainly nitric oxide and prostacyclin, and vasoconstrictor substances, mainly free radicals and endothelin. In normal endothelium, nitric oxide mediates vasodilation, platelet desegregation, and antiproliferation and inhibits inflammation. In endothelial dysfunction, vasoconstriction, inflammation, proliferation mediated by several growth factors, platelet aggregation mediated by adhesion molecules, such as selectins, and thrombosis result in detrimental functional consequences and adverse long-term effects, including vascular remodeling. Endothelium dysfunction of the coronary arteries may cause or contribute to myocardial ischemia. The clinical trials carried out as to the potential of dietary flavonoids to counter heart disease risk by inhibiting platelet aggregation result in contrasting evidence. Hence, no clear effectiveness has been shown after dietary supplementation with genistein- and daidzein-rich soy in males or quercetin and apigenin in platelet aggregation, despite the increase in respective plasma concentrations. In coronary artery disease patients, no alterations in platelet aggregation have been observed by acute or chronic tea consumption, regardless of the fact that plasma flavonoids increased with both acute tea consumption and chronic tea consumption. Contrarily, cocoa or chocolate supplementation in healthy subjects not only increased plasma flavonoid levels but also decreased platelet aggregation, increased plasma prostacyclin, and decreased plasma leukotrienes in humans, evident of an antithromboembolic activity. In human trials, isoflavones were found to significantly increase forearm vascular resistance, lower postocclusion peak flow velocity of the brachial artery, and reduce arterial stiffness with improved systemic arterial compliance attributable to a reduction in total peripheral resistance and a corresponding reduction in central pulse wave velocity.

Moreover, the capacity of polyphenols to modulate gene expression has been investigated. Nutrients can influence gene expression directly or via gene promoters, via control of regulatory signals in nontranslated regions, and via posttranscriptional pathways. All the genes encoding proteins and the genes associated with transcriptional activation and signals modulating the transcription of the respective genes are potential candidates for gene–diet interaction. Phenolic compounds have been found to modulate the expression of cyclooxygenase-2 through suppression of activator protein-1, to increase the expression of serum high-density lipoprotein-associated paraoxonase 1 and of antioxidant enzymes, to downregulate vascular endothelial growth factor in human aortic endothelial cells, and to downregulate inflammatory cytokine gene expression in mononuclear cells and in macrophages.

Chemopreventive properties of phenolic compounds

Carcinogenesis is generally recognized as a complex and multistep process in which oxidative stress and inflammation play

crucial roles, and distinct molecular and cellular alterations occur. The factors affecting cancer may be not only genetic but also environmental, including a large body of physiological and biochemical factors, such as ultraviolet and ionizing radiation, smoking, viruses, bacteria, parasites, and mycotoxins contained in foods. Carcinogenesis may result from the action of any one or a combination of chemical, physical, biological, and/or genetic insults to cells. Independently of the etiologic factor, cancer is characterized by uncontrolled cell proliferation. Unlimited cell proliferation results in the development of a cell mass known as tumor, characterized as benign when lacking the ability to invade neighboring tissue or metastasizing or malignant when being anaplastic, invasive, and metastatic. The process of carcinogenesis may be divided into at least three stages: initiation, promotion, and progression. Initiation results from an irreversible genetic alteration, most likely one or more simple mutations, transversions, transitions, or deletions in DNA. Promotion is reversible and does not involve changes in the structure of DNA but rather in the expression of the genome mediated through promoter–receptor interactions. The final irreversible stage of progression is characterized by karyotypic instability and malignant growth.

Chemoprevention is a new approach to cancer prevention. Cancer chemoprevention attempts to inhibit the progress of the disease by using natural or synthetic substances. In terms of chemoprevention, many food components of the Mediterranean diet gained the 'food-borne anticarcinogens' title due to the phenolic phytochemicals with chemopreventive properties they contain. Phenolic compounds have been shown to interrupt or reverse carcinogenesis by acting in intracellular signaling network molecules involved in the initiation and/or promotion but also may arrest or reverse the progression stage of cancer. Those 'food-borne anticarcinogens' can be defined as either 'blocking agents,' which act immediately before or during the initiation of carcinogenesis by chemical carcinogens, or 'suppressing agents,' which act after initiation during the prolonged stages of promotion and progression. Blocking agents scavenge reactive oxygen species (ROS) by potentiating the antioxidant enzyme system, inhibiting carcinogen activation or enhancing their detoxification and excretion. Suppressing agents may prevent DNA damage, induce cell-cycle arrest or apoptosis, and inhibit inflammatory invasion or angiogenesis.

Many forms of cancer are attributed to oxidative stress meaning the presence of free radicals, which affect genes involved in cell apoptosis and proliferation and cause oxidative damage to proteins, lipids, and DNA. Prolonged oxidative stress and increased ROS production is one of the characteristics of cancer cells, shown in both *in vitro* and *in vivo* experiments and attributed to hypoxia where tumor grows or increased glucose metabolism. Hence, the chemopreventive activities of polyphenols are believed to be closely related to their ability to scavenge ROS. Further, phenolic compounds have been found to reduce the proliferation of cancer cells. For example, resveratrol has been found to regulate the concentrations of major molecules of the cell cycle, thereby interrupting cell growth phase in G1/S via upregulation of cyclins D1/D2/E and Cdk2/4/6, or to inhibit cell growth in phases S and G2/M through the inhibition of Cdk7 and p34Cdc2 or cyclin B and Cdk1 in the case of prostate cancer.

Very recent research suggests that dietary polyphenols act as chemopreventive agents in part due to their ability to modulate epigenetic alterations in cancer cells. The epigenome is nowadays considered just as important as the genome to normal development. Epigenetics is defined as the study of heritable changes in gene function that are not dependent on DNA sequence. The molecular basis of epigenetic processes is complex involving modifications of histones, DNA methylation, positioning of histone variants, and gene regulation by noncoding RNAs. Cancer development is owed to the epigenetic modifications triggered by carcinogens and metabolites. Epigenetic modifications are potentially reversible, and therefore, controlling cancer development at this stage is of immense importance. Flavonoids may act at this stage; however, the mechanism of how they do regulate epigenetic modifications in cancer cells is a topic still in its infancy. Catechol-containing coffee phenolics caffeic acid and chlorogenic acid have been shown to be demethylating agents. Acting as demethylating factors, phenolic compounds may inhibit chronic inflammation that has its molecular basis in epigenetic modifications on the one hand and underlies several forms of cancer on the other. Further, they act as chemopreventive factors by inhibiting cancer-linked inflammatory enzymes such as cyclooxygenase or lipoxygenases and other factors such as nuclear factor kappa B, the synthetase of nitric oxide or the activator protein-1. Specifically, the nuclear factor kappa B is one of the most important target molecules for phenolic compounds functioning as a transcription factor for at least 200 genes encoding proteins committed to inflammation, cytokines, chemokines, adhesion molecules and enzymes, cell proliferation, differentiation, and apoptosis. This molecule is activated in many tumors, suggesting that it is an important molecule in carcinogenesis and in tumor promotion and progression stages and augmenting the chemopreventive role of the phenolics that inhibit its activation.

Conclusive Remarks

Considerable progress has been made concerning our knowledge of phenolic compounds derived from foods, specifically the digestion, absorption, and bioavailability of these phytochemical compounds. Their contribution to disease risk reduction or disease prevention is supported by the evidence of absorption and bioavailability. Although research on their health effects is often contrasting and in the case of epigenetic modifications even in early infancy, their use in the future as agents to induce cardiovascular risk reduction or chemoprevention of various human cancers may be a strong possibility from a clinical standpoint. Since there is a significant unmet need in the existing disease prevention and treatment strategies, the evolution of treatments with phenolic compounds that are absorbed and bioavailable in human tissues and with relevant effectiveness offers an exciting opportunity to reduce the global burden imposed by cardiovascular events and cancer and remains a scientific challenge. If these agents are well absorbed, have no toxic effects, but have high efficacy against targeted disease, with known mechanism of activity, they may realistically be complementary treatment options for subjects in high risk.

See also: Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Bioavailability of Nutrients; Cancer: Diet in Cancer Prevention; Phenolic Compounds: Occurrence, Classes, and Analysis.

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Phenolic Compounds: Occurrence, Classes, and Analysis

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Introduction

Dietary phenolic compounds comprise a huge number of substances having several common structural features. They not only do have specific functions in the plant cell or plant organ but also can interact with the human metabolism being either health-promoting or toxic. In the food, these substances can have specific functions. In some cases, they can be used as food colorants (e.g., quercetin being yellow and anthocyanins being red or blue). However, due to the phenolic group, they can act as antioxidants being very effective oxygen radical quenchers.

The biosynthesis of polyphenols in plants is based on the shikimate pathway. The aromatic ring systems in flavonoids – ring B and chromane – are originating from phenylalanine. Ring A is formed from three units of malonyl CoA. The phenylpropanoid biosynthesis leads to p-coumaroyl-CoA, which is the first key intermediate in the further synthesis of higher polyphenols (naringin, flavonols, anthocyanins, and flavanols). A detailed representation of the biosynthetic pathways of flavonoids can be found at the KEGG pathway database (Reference pathway, map 00941).

Phenolic compounds are needed in plants for a variety of tasks that comprise pigmentation, growth, reproduction, and resistance to pathogens. They are also responsible for UV light protection, serve as signal compounds and internal regulators or chemical messengers, and act as defense against fungal pathogens and phytophagous insects.

Polyphenols contribute not only to the chemical and textural properties of foods but also to the sensory properties. These are the constituents that contribute mostly to astringency and bitterness. Astringency and bitterness are elicited from a variety of foods including nuts, fruits, chocolate, tea, wine, and beer. Alpha acids are the principle bitter compounds in beer, whereas in the other foods, catechin, epicatechin, and the oligomers (proanthocyanidins) are the primarily responsible constituents for astringency and bitterness. The bitter taste is a result of the interaction of the respective compounds with specific taste receptors, whereas astringency is perceived through touch, via mechanoreceptors. In contrast to bitterness, astringent compounds are chemically defined as compounds precipitating proteins (e.g., tannins).

Phenols

Due to their specific chemical structure, the phenols can act as antioxidants. In contrast to their action in physiological systems, they can interact directly with the radicals of oxidized foods and thereby inhibit or reduce further oxidation. Depending on the position and number of hydroxyl groups, the number of conjugated double bonds, and glycosylation, they have different reduction potentials resulting in stronger or weaker antioxidants. In the case of flavonoids, the critical structural features for an optimal antioxidant potential were identified as (i) the presence of a catechol group in the B-ring (Figure 1), (ii) a 2,3-double bond, and (iii) 3- and 5-hydroxy groups adjacent to the 4-keto structure.

Phenolic Acids

The phenolic acids can be divided into two groups, that is, the hydroxybenzoic acids (Figure 2) and the hydroxycinnamic acids (Figure 3). Either they can be found as free substances in, for example, fruits and vegetables, or they are conjugated, mainly in grains and seeds. The conjugates can release the phenolic acids upon acid, alkaline, or enzymatic hydrolysis.

Hydroxybenzoic acids are typically found in berries at low concentrations. Blackberries contain up to 250 mg kg^{-1} comprising protocatechuic acid, *p*-hydroxybenzoic acid, and gallic acid, red raspberries and sweet cherries up to 40 mg kg^{-1}, and gooseberries up to 20 mg kg^{-1}. Other fruits contain significantly lower amounts, if any. In general, *p*-hydroxybenzoic acid and protocatechuic acid are the dominating hydroxybenzoic acids in ripe fruits. In the literature, a huge variation in the content of phenolic compounds can be found. This might be due to a number of parameters that are not described in detail and that comprise, for example, the variety, state of ripeness, geographic location, and climate during ripening. This is true for the other phenolic compounds as well.

The free hydroxycinnamic acids are found in low concentrations in the plant foods. Normally, they are conjugated with quinic acid forming chlorogenic acid (5′-caffeoylquinic acid), cryptochlorogenic acid (4′-CQA), or neochlorogenic acid (3′-CQA). They can also be linked to glucose as esters (e.g., p-coumaroyl glucose) or as glycosides (p-coumaric acid glucoside).

Quinic acid esters are mainly found in blueberries (<2000 mg kg^{-1}); kiwis, cherries, and plums (<1000 mg kg^{-1}); eggplants and pears (<600 mg kg^{-1}); peaches (<400 mg kg^{-1}); and apples (<500 mg kg^{-1}). Glucose conjugates are occurring at lower concentrations, for example, in tomatoes (<100 mg kg^{-1}) and in strawberries, raspberries, gooseberries, and black currant (30–60 mg kg^{-1}). Exceptionally high amounts of chlorogenic acids are found in green coffee (*c*.65 g kg^{-1} in arabica and 100 g kg^{-1} in robusta coffee). During roasting, the content of chlorogenic acids is reduced by 50–70%.

Isoflavones

Food products containing isoflavones are limited to soybeans and products thereof. The first specific reaction in the biosynthesis of isoflavones in plants is the 2-hydroxylation, coupled to aryl migration, of a flavanone. The worldwide application of soybeans as food and feed resulted in an uncountable number of varieties optimized for practically all culinary, nutritional, and technological purposes. It is not only the protein and oil

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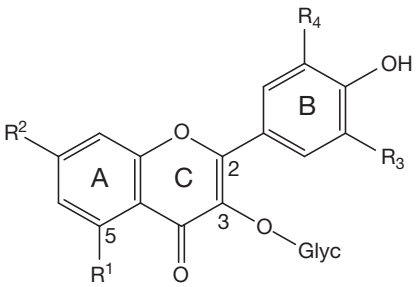
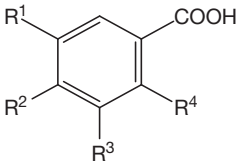


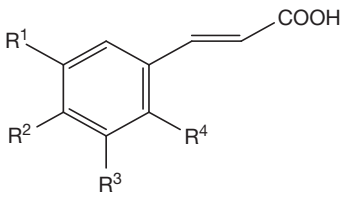
R^1	R^2	R^3	R^4	Common name
OH	OH	OH	H	Quercetin
OH	OH	H	H	Kaempferol
OH	OH	OH	OH	Myricetin

Figure 1 Flavonols occurring in plant foods.



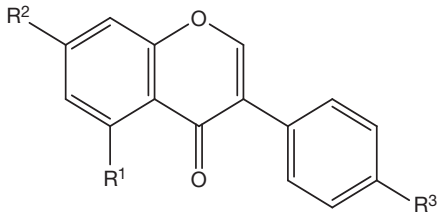
R^1	R^2	R^3	R^4	Common name
H	H	H	OH	Salicylic acid
OH	OH	OH	H	Gallic acid
OCH_3	OH	H	H	Vanillic acid
OCH_3	OH	OCH_3	H	Syringic acid
OH	OH	H	H	Protocatechuic acid
H	H	OH	OH	Gentisic acid

Figure 2 Commonly occurring hydroxybenzoic acids in foods.



R^1	R^2	R^3	R^4	Common name
H	H	H	OH	o-Coumaric acid
H	H	OH	H	m-Coumaric acid
H	OH	H	H	p-Coumaric acid
OCH_3	OH	H	H	Ferulic acid
OCH_3	OH	OCH_3	H	Sinapic acid
OH	OH	H	H	Caffeic acid

Figure 3 Commonly occurring hydroxycinnamic acids in foods.



R^1	R^2	R^3	Common name
OH	OH	OH	Genistein
H	OH	OH	Daidzein

Figure 4 Main isoflavones occurring in soybeans.

content but also the isoflavones concentration in the soybeans and products and many other traits relevant for agricultural production and further processing. Isoflavones can act as antioxidants and have an estrogenic activity.

The two dominating isoflavones in foods are daidzein and genistein. These compounds are only found in foods containing soy products. Up to 1.4 g kg^{-1} occurs in soybeans and products thereof. This includes also glycitein, which is a similar compound methoxylated at C6. Daidzein and genistein can be used as indicators for the presence of soy in foods. Due to the high variation of the isoflavone content in soybeans, it is difficult to determine the exact percentage of soy as ingredient, but a rough estimate is possible. In addition, it can be used as an indicator for adulteration (Figure 4).

Flavonols

Flavonols are flavonoids that have a double bond between C2 and C3 and a carbonyl at C4. Having this large conjugated double bond system, these compounds absorb in the visible range, which means that they appear yellow. They have a strong absorption in the range of 340–380 nm, which is

especially useful for attracting insects that are sensitive to UV light. The flavonols are occurring widespread in the plant kingdom and therefore also in plant foods. The flavonols are strong antioxidants as they have all the molecular features mentioned earlier. Normally, the flavonols are glycosylated, preferentially at C3 as mono-, di-, or triglycosides. Glucuronidation is also possible. The most widespread flavonol diglycosides are rutinosides (e.g., rutin). Rutinose is a disaccharide in which rhamnose is glycosidically bound to glucose by a $1 \rightarrow 6$ linkage.

The total alimentary daily uptake of flavonols (calculated as aglycones) is *c.*13 mg. The concentrations in the foods vary strongly. In grapes (*Vitis vinifera*), the content of the glycosylated flavonols is in the range of 10–100 mg kg^{-1} depending mainly on the grape variety. Good sources of flavonols in the diet are onion (350–1200 mg kg^{-1}), kale (<600 mg kg^{-1}), broccoli (40–100 mg kg^{-1}), tomatoes (<200 mg kg^{-1}), apples (20–40 mg kg^{-1}), berries (15–160 mg kg^{-1}), tea infusion (20–45 mg kg^{-1}), and red wine. In vegetables, the intensive green color is a good indicator for higher flavonol content. There are large differences between different varieties in their flavonoid content. Among cruciferous crops, curly kale is known for the highest flavonoid content.

Flavanols

Flavanols are often called catechins. There is no double bond between C2 and C3 and no carbonyl in the ring C (C4). As a result, the flavanols have two chiral carbons (C2 and C3) and therefore four possible diastereomers: (+)-catechin (2*R*,3*S*), (−)-catechin (2*S*,3*R*), (+)-epicatechin (2*R*,3*R*), and (−)-epicatechin (2*S*,3*S*).

(+)-Catechin and (−)-epicatechin are commonly found in plant foods. The two monomeric flavanols and their analogs (gallocatechins) are occurring abundantly in tea leaves (green tea, 25–35% in dry leaves), cocoa beans (*c.*1.4 g kg^{-1}), and grape seeds (*c.*1.7 g kg^{-1}). In addition, the flavanols are found in many fruits like grapes (*c.*10 mg kg^{-1}), apples (*Malus domestica*, 30–100 mg kg^{-1}), apricots (*Prunus armeniaca*, *c.*10 mg kg^{-1}), and different berries (10–100 mg kg^{-1}) (Figure 5).

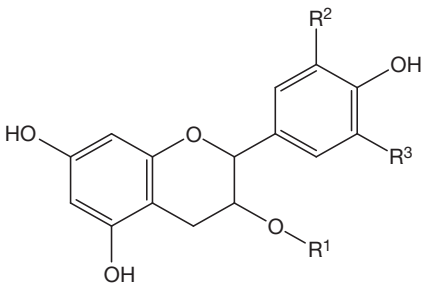
Anthocyanidins

The anthocyanidins comprise a group of flavonoids that have a specific absorption in the visible range. At low pH, the chroman ring is charged positively resulting in a strong absorption at *c.*520 nm. At pH values above 4.5, the charge is lost concomitantly with a significantly reduced absorption. Because of the intensive red color of the anthocyanins, they are used as colorant for acidic food mainly. The anthocyanins are strong antioxidants and therefore of limited stability. Depending on the glycosylation at the positions 3, 5, and 7, the stability increases with increased glycosylation and bulkiness of the carbohydrate moieties. A further stabilization can be obtained by copigmentation with hydroxycinnamic acids (caffeic acid) or with intramolecular copigmentation, which can be observed in the anthocyanins of the black carrot.

Due to the intensive absorption of the anthocyanins at around 520 nm, it is very easy to identify anthocyanin-rich foods. Especially, some berries are intensively colored. Cyanidin is the most widely spread anthocyanidin, which occurs in nearly every anthocyanin-containing fruit. Some fruits contain many different anthocyanidins, and they show also a great variety in glycosylation (grapes, blueberry, crowberry, and bilberry). The anthocyanins can be located in the skin of the fruits but could also be distributed throughout the whole fruit. High concentrations of anthocyanins can be found in eggplant (*Solanum melongena*), blackberry (*Rubus fruticosus*), black currant (*Ribes nigrum*), blueberry (*Vaccinium corymbosum*), bilberry (mainly *Vaccinium myrtillus*), elderberry (*Sambucus nigra*), chokeberry (*Aronia melanocarpa*), black crowberry (*Empetrum nigrum*), grape (*Vitis vinifera*), and cherry (*P. avium*). The concentration ranges from 1000 to 5000 mg kg^{-1}. Medium concentrations are found in rhubarb (*Rheum rhabarbarum*) with 1000–2000 mg kg^{-1}. Lower concentrations are occurring in strawberry (*Fragaria* × *ananassa*), plum (*P. domestica*), red cabbage (*Brassica oleracea*), and onions (*Allium cepa*) with concentrations of 10–1000 mg kg^{-1} (Figure 6).

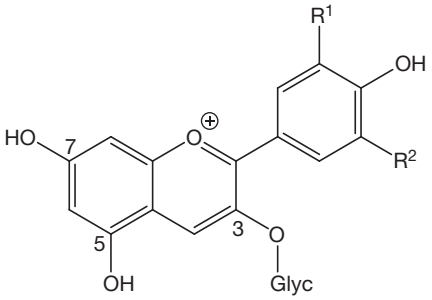
Proanthocyanidins

The proanthocyanidins are a class of compounds that are condensed flavanols. The name originates from the acid hydrolysis during which anthocyanidins are formed and a red color appears. There are different groups of proanthocyanidins that refer to the linkage of polymerization. Type A is an interflavonoid linkage with the bridges $4\beta \rightarrow 8$ and $2\beta \rightarrow O \rightarrow 7$ (e.g., proanthocyanidin A2, epicatechin-$(2\beta \rightarrow 7,\ 4\beta \rightarrow 8)$-epicatechin). This A-type linkage is not as common as the B type, which has a single linkage of the type $4\beta \rightarrow 8$ (e.g., proanthocyanidin B1, epicatechin-$(4\beta \rightarrow 8)$-epicatechin). In B-type proanthocyanidins, α-configurations are also possible (B3, catechin-$(4\alpha \rightarrow 8)$-



R^1	R^2	R^3	C-2	C-3	Common name
H	H	OH	R	S	(+)-Catechin
H	H	OH	R	R	(–)-Epicatechin
H	OH	OH	R	R	(–)-Epigallocatechin
gallate	OH	OH	R	R	(–)-Epigallocatechin gallate
gallate	H	OH	S	S	(–)-Epicatechin gallate

Figure 5 Common flavanols in plant foods.



R^1	R^2	Common name
H	H	Pelargonidin
OH	H	Cyandin
OCH_3	H	Paeonidin
OH	OH	Delphinidin
OCH_3	OH	Petunidin
OCH_3	OCH_3	Malvidin

Figure 6 Commonly occurring anthocyanidins in plant foods with additional glycosidation on C-5 and C-7.

catechin; B4, catechin-(4α→8)-epicatechin; B6, catechin-(4α→6)-catechin; or B8, catechin-(4α→6)-epicatechin). Procyanidin C1 is a B-type proanthocyanidin. It is an epicatechin trimer found in, for example, grapes (*Vitis vinifera*).

High concentrations of oligomeric proanthocyanidins (dimers and higher) can be found in cocoa-containing products (cocoa beans contain *c*.70 g kg^{-1}), apples (*M. domestica*; 500–1000 mg kg^{-1}), and chokeberries (*Aronia melanocarpa*; 6 g kg^{-1}). Grape seeds contain mostly monomers and dimers (*c*.4 g kg^{-1}) whereas in the juice, the higher oligomers and polymers are dominating. Polymeric proanthocyanidins are mainly found in cranberries (*Vaccinium macrocarpon*; with a total amount of *c*.4 g kg^{-1}) or chokeberries (*c*.6 g kg^{-1}). In different kinds of nuts (hazelnuts, almonds, and pistachios), the polymers are also dominating with a total amount of *c*.5 g kg^{-1}; walnuts contain significantly less proanthocyanidins (*Juglans regia*; < 1 g kg^{-1}). Data on foods containing proanthocyanidins can be found in a specialized database of the USDA (United States Department of Agriculture, http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/PA/PA.pdf) (**Figures 7** and **8**).

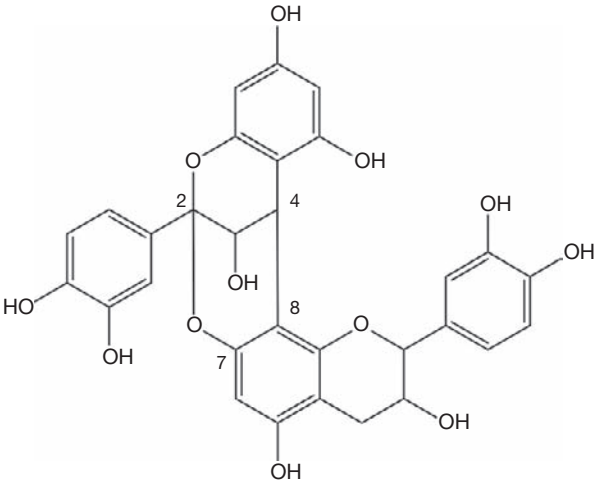


Figure 7 A-type proanthocyanidin (2β→7, 4β→8), for example, epicatechin-(2β→7, 4β→8)-epicatechin.

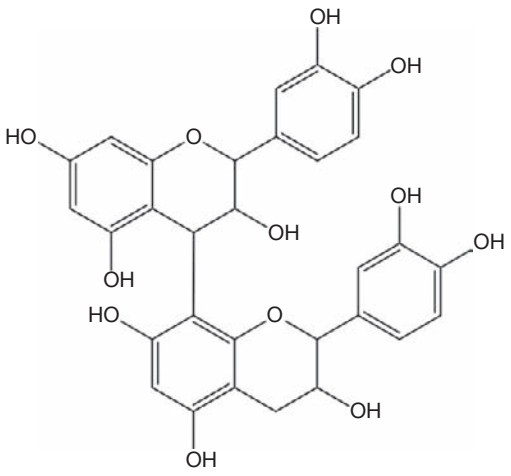


Figure 8 B-type proanthocyanidin (4β→8), for example, epicatechin-(4β→8)-epicatechin.

Analysis

Identification and Analysis of Phenols in Foods

The identification of the substance of interest is essential before the analysis. As liquid chromatography is the method of choice for the analysis of phenols, there are a range of detectors that can give some structural information. As long as reference substances are available, the UV/vis absorption spectra in addition to the retention time can be very helpful for identification. This has been widely used in flavonoid analysis (**Table 1**). Besides the UV absorption, the detection of the polyphenols can also be done electrochemically due to their low potential of oxidation. Especially, the coulometric detection not only is sensitive but can also be very selective resulting in high-quality chromatograms. Furthermore, the hyphenation of chromatography with mass spectrometry gives the possibility of deeper analysis of the molecular structure. In addition, when analyzing a selective fragmentation pattern – as can be done by using a triple quadrupole mass spectrometer – detailed information on glycosylation and the aglycone can be obtained. The use of very high-resolution liquid chromatography in combination with high-resolution MS (mass accuracy 1–5 ppm, resolution > 60 000) is the state of the art of unambiguous identification of these compounds.

If no or limited structural information is available, it will be necessary to use nuclear magnetic resonance (NMR) with the limitation that high amounts or concentrations of the

Table 1 UV absorption of flavonoids

	Band II (nm)	*Band I (nm)*
Flavones	250–280	310–350
Flavonols (3-OH-substituted)	250–280	330–360
Flavonols (3-OH-free)	250–280	350–385
Isoflavones	245–275	310–330
Flavanones	230–270	300–330
Anthocyanidins	270–280	465–560
Flavanols	207–280	–

substances of interest are needed. A comprehensive collection of NMR data on food phenols can be found in the database MetIDB (http://www.metidb.org). However, this approach will allow a definite elucidation of the phenolic compound. The identification of the plant metabolites can be done by liquid chromatography hyphenated with mass spectrometry. The recently commercially available high-resolution high performance liquid chromatography (HPLC) in combination with MS/MS is an excellent tool for identification and quantification. For the more polar (water soluble) compounds, the application of hydrophilic interaction liquid chromatography (HILIC) can be advantageous. This type of separation can also be used as orthogonal method for reversed-phase (RP) chromatography. Especially, the recent advancements of 2-dimensional (2-D) liquid chromatography can be applied for complex separations like procyanidins in apples or cocoa.

Extraction of Phenols for Analysis

In plant foods, the phenols are located in different organs and within the cells in vacuoles. The biosynthesis is normally taking place in the cytosol.

For efficient extraction, it is necessary to break up the structures and cells as completely as possible. In addition, the phenoloxidases present within the cells in the chloroplasts or in plastids – as it was shown for apples – start oxidizing the phenols (mainly o-diphenols) as soon as they come in contact with the substrates. Normally, the phenoloxidases are quite thermostable (up to 90 °C) and have a pH optimum at *c.*6.5. Therefore, it is difficult to inactivate the enzymes without compromising the polyphenol content. Using low-pH solvents, these enzymes can be inactivated and the concomitant stabilization of, for example, anthocyanins in acidic conditions can result in high recoveries of the analytes during extraction. Ascorbic acid can also be used to limit the activity of phenoloxidases, and especially, the combination of ascorbic acid with divalent cations (Mg^{2+}) can be used to stabilize the analytes during the extraction.

Freeze-drying of the food samples prior to grinding can also be advantageous for two reasons. On one hand, the phenoloxidases cannot catalyze the oxidation without water, and on the other hand, the polarity of the extraction solvent is not changed during the extraction. Care has to be taken when the pH of the solvent is optimized since it will be changed due to the cellular internal buffer systems that will be extracted as well. The selection of the solvent depends on the type of phenol and also on the glycosylation. For extraction of more polar compounds like the glycosides, acidified mixtures of water with methanol or ethanol are commonly used. This combination also limits the coextraction of proteins and other biopolymers. The clarified (Carrez solutions) and centrifuged extract can be purified further using solid-phase extraction (SPE) or can be analyzed directly.

Besides the direct (or Soxhlet) extraction, it is possible to increase the efficiency by using ultrasound or higher temperatures. Automated procedures like accelerated solvent extraction using higher temperatures and pressures can improve the efficacy. The use of supercritical solvents (e.g., CO_2) can also be advantageous since this can easily be combined with a subsequent SPE (e.g., C18) for the purification of the extracted analytes. The complete extraction of the phenols can be a challenging task due to the limited solubility of some of the phenols or the strong interaction with food structures or other food components (e.g., interaction of catechins with proteins).

HPLC Analysis

The columns used for HPLC analysis in literature are almost exclusively RP. Elution systems are usually binary, with an aqueous acidified polar solvent such as aqueous acetic acid, phosphoric acid, or formic acid and a less polar organic solvent such as methanol or acetonitrile. Isocratic elution is mainly used for routine analysis if only a few polyphenols want to be determined. Recent developments in HILIC broadened the applicability to the analysis of highly polar substances and also for polyphenols. HILIC separation is characterized by a highly polar stationary phase and a moderate polar eluent. The eluents are normally composed of high concentrations of acetonitrile and low concentrations of water. In this mode, water is a strong eluent, which means that during a gradient run, the water content of the eluent increases in contrast to RP separations.

For detection and quantification after separation, the absorption of UV or visible light is normally used. For flavonoids, two absorption bands are characteristic: the first one in the range of 240–285 nm, which is believed to arise from the A-ring, and the second one with a maximum in the range of 300–550 nm, which arises from the absorption of the B-ring. Since the absorption spectrum depends on the chemical substitution and the conjugated system, the different groups of flavonoids (flavanones/isoflavones, catechins, flavones/flavonols, etc.) have distinct absorption bands. The anthocyanins show absorption above 500 nm, whereas the catechins have only a small maximum at 280 nm and no absorption at higher wavelengths.

In general, the analysis can be limited to the determination of the aglycone after acidic hydrolysis or the substances are analyzed as they are present in the foods. This means that the glycosylation and conjugation to phenolic acids have to be considered in the analytic methods. In some cases, the aglycones (e.g., anthocyanidins) are more sensitive to oxidation and care has to be taken to protect these compounds during the analysis using even more powerful antioxidants or reducing the exposure to light and oxygen.

The analysis of the phenols is not only necessary in foods but also in metabolically relevant tissues in humans or experimental animals. In general, the concentrations are

significantly lower in the biological samples, and in many cases, the phenols are metabolized by the organism or the intestinal microorganisms. The result is a conjugation different from the naturally occurring glycosides. The breakdown of the carbon backbone is also common by the intestinal bacteria leading to smaller molecules that can also interact with the metabolism.

Other separation techniques can also be used (e.g., capillary electrophoresis and thin layer chromatography). Both of these techniques can also be hyphenated to mass spectrometry so that structural information can be obtained. Especially, thin layer chromatography is powerful since a simple setup for 2-D chromatography is available, and in addition, post-separation derivatization can give additional information or can add selectivity to the analysis. The limitation of thin layer chromatography is definitely the limited sensitivity compared to HPLC.

Occurrence

The concentrations of phenols in plant foods can be found in several databases that are available online like the eBASIS database, USDA database for the flavonoid content of selected foods, Japanese database, and the phenol database initiated by INRA (French National Institute for Agricultural Research). These databases do collect data not only on the occurrence of phenols but also on chemical properties or biological activities. NMR data of phenols can be found in the database MetIDB.

The concentrations of the phenols in foods vary enormously. Depending on the species, variety, harvesting time, state of ripeness, climate, and many other factors, the variation can be two orders of magnitude or even higher. A comprehensive overview of the data is given in the aforementioned databases.

Conclusion

Phenolic compounds from plant foods are a group of substances that are of interest because of their interaction with the human metabolism and their interaction with other food constituents. The main activity is related to the scavenging of different radicals and thereby inhibiting the oxidation of unsaturated fatty acids. Different chemical groups comprising phenolic acids, flavonoids, and many other phenols (e.g., tocopherols, phenylethanoids, stilbenoids, lignans, coumarins, and chalcones) show this common reactivity. Due to the high antioxidant activity, these compounds are not stable in foods during storage, preparation, and processing. They have specific sensory as well as textural properties that contribute to the appearance and taste of these foods. During breeding of new varieties, the content of the phenols was reduced significantly. This is due to the taste and the enzymatic browning that is related to the phenols. Today, we have more stable varieties and better-tasting varieties but with a lower content of potentially health-promoting substances.

See also: Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Berries and Related Fruits; Grapes; Olive Oil: Its Role in the Diet; Olives: Types and Consumption; Phenolic Compounds: Bioavailability and Health Effects; Tocopherols: Physiology and Health Effects; Tocopherols: Properties and Determination; Wines: Wine and Health.

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Phospholipids: Physiology

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Introduction

Phospholipids are ubiquitous molecules that participate in innumerous cellular events. The major chemical feature of phospholipids is their polarity, and for that, they are part of the lipid class of polar lipids. The biochemical and physiological properties of phospholipids are largely related to this chemical feature, which is named amphipathy, meaning phospholipids interact both with lipids and with water and other polar compounds. Mammalian cell membranes contain more than 1000 different phospholipids, presenting both structural and regulatory functions. Phospholipids exert structural functions in cellular membranes, which vary in phospholipid composition, according to cell and organelle functions. Phospholipid regulatory functions are both direct and indirect, through their metabolites. Phosphatidylcholine (PtdCho) was the first phospholipid to be discovered, and it was originally named lecithin after the Greek word *lekithos* (egg yolk). Because phospholipids were considered for a long period mostly serving structural functions in membranes, along with methodological difficulties, research interest in these compounds was delayed. It was then in the mid-1940s when the biosynthetic pathway of phospholipids was described that phospholipid research started to develop. The role of phospholipids in cell signaling events was initially described in the early 1970s with the association of membrane phosphoinositide turnover with intracellular calcium stores. Nowadays, it is well established that phospholipids participate in a broad range of cellular events such as apoptosis and regulation of mitochondrial physiology and that phospholipid-derived messenger molecules are crucial for the transduction of extracellular signals to intracellular events.

Distribution of Phospholipids among Organisms, Organelles, and Membranes

Phospholipids are polar lipid compounds in which both fatty acids and phosphoric acid are esterified to an alcohol (Figure 1). Phospholipids are divided into two classes: phosphoglycerides or glycerophospholipids, in which the alcohol is glycerol, and sphingolipids, in which sphingosine is the alcohol. Base groups such as choline, ethanolamine, inositol, and serine are esterified with the second acid group of phosphoric acid of the glycerol or sphingosine. The charges provided by the base (positive) and the acidic phosphate (negative) form the hydrophilic head, and hydrocarbon chains form the hydrophobic tail of the phospholipids (Figure 1). These physical–chemical properties allow phospholipids to spontaneously form special structures in aqueous media. Among these structures, extended bilayers with a hydrophobic core are major constituents of all cellular membranes, and micelle-like structures are seen in blood lipoproteins and cellular lipid bodies and are necessary for lipid and liposoluble vitamin absorption.

PtdCho and phosphatidylethanolamine (PtdEtn) are the most abundant phospholipids in blood plasma and organelle membranes from yeast to mammalian cells (Figure 2). In bacteria, the predominant phospholipid is PtdEtn. On the other hand, phosphatidylserine (PtdSer) is found mainly in the plasma membrane and in the Golgi apparatus and cardiolipin in mitochondrial membranes. Besides the differences in phospholipid distribution among organisms and organelles, there is a distinct distribution among organs. For example, in the brain, 45% of total phospholipids are PtdEtn, whereas this phospholipid accounts for 20% of total phospholipids in the liver. The maintenance of this distinct distribution of phospholipids among organelles, cells, and organisms requires a fine regulation of phospholipid synthesis, remodeling, transport, and degradation.

All cellular membranes show very different lipid composition between the inner and outer leaflets (e.g., Figure 2). Phospholipid asymmetry in membranes has obvious implications for membrane function, because it impacts on several of its physical–chemical properties. For instance, membrane asymmetry affects surface charges, and membrane phase transition, which is related to membrane fluidity and mobility of membrane components. A common feature of mammalian cell membranes is that the aminophospholipids PtdEtn and PtdSer and phosphatidylinositol (PtdIns) are concentrated in the cytoplasmic leaflet, whereas the outer leaflet is greatly enriched with PtdCho and sphingolipids (Figure 2). Conversely, exposure of PtdSer to the outer leaflet induces apoptosis and serves as a signal to phagocytes to target and destroy cells. The distinct asymmetrical distribution among the leaflets of cellular membranes is kept by the activity of three phospholipid exchange proteins (or translocators): flippase, floppase, and scramblase. Flippases and floppases translocate membrane phospholipids against concentration gradients, using energy from ATP. The former move aminophospholipids (PtdSer and PtdEtn) from the outer to the inner leaflet, whereas the latter translocate PtdCho, sphingolipids, and cholesterol in the opposite direction. In contrast, scramblases are less substrate-specific and facilitate lipid translocation along concentration gradients and without ATP use.

Metabolism of Phosphoglycerides

The bulk of phosphoglyceride synthesis occurs in the cytoplasmic leaflet of the endoplasmic reticulum (ER) and to a lesser extent in the Golgi complex. In the case of phosphatidylethanolamine (PtdEtn), part is synthesized in the mitochondria (Figure 3).

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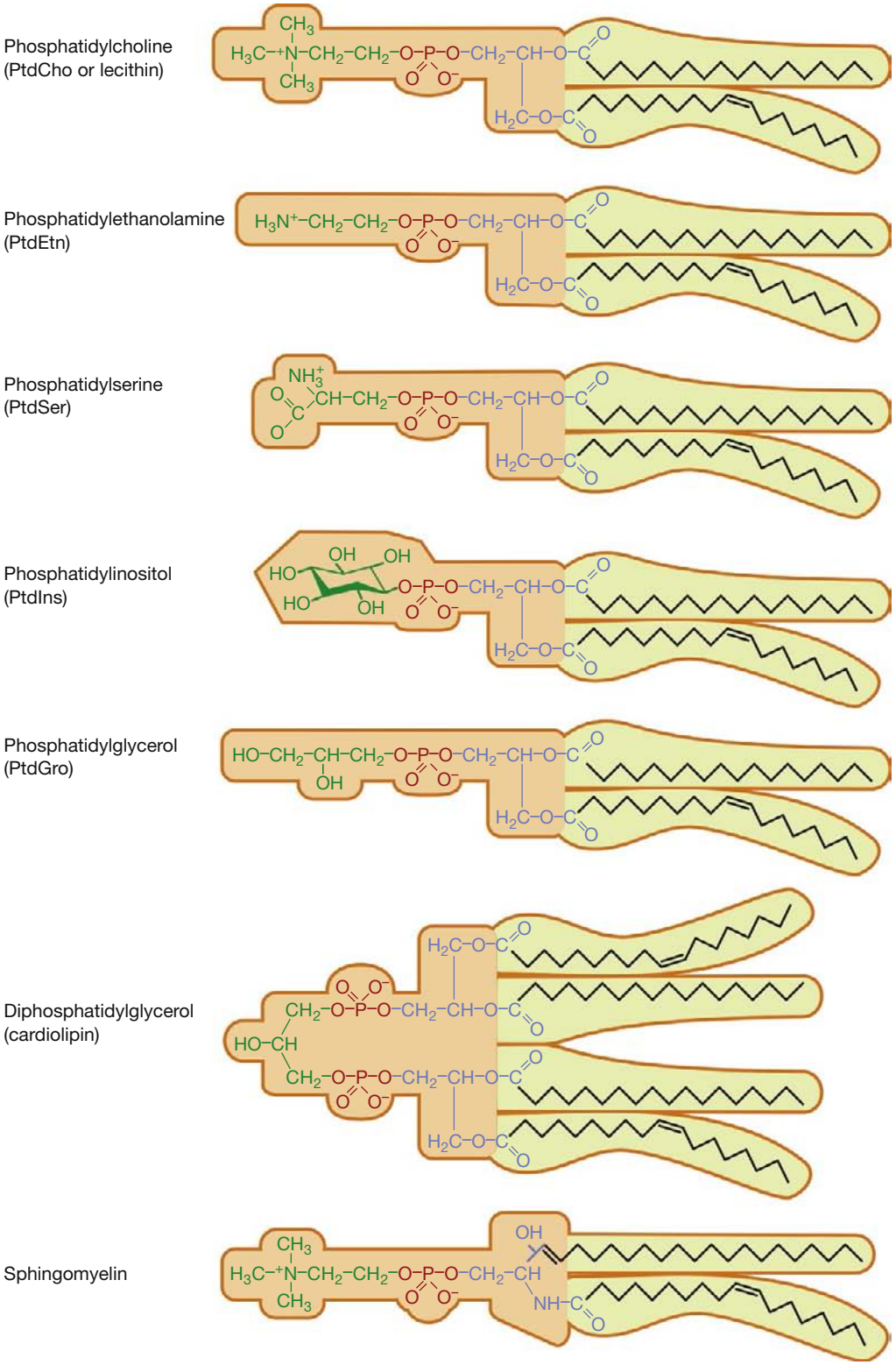

Figure 1 Structure of phospholipids. The hydrophilic heads of the phospholipids are formed by the base, phosphoric acid, and alcohol groups and are highlighted in light orange. The hydrophobic tails of the phospholipids are the fatty acyl chains and are highlighted in light yellow. The presence of a double bond produces a kink in the fatty acyl chain. Adapted from Da Costa, T. H. M. and Ito, M. K. (2003). Phospholipids: Physiology. In: Caballero, B., Trugo, L. C., and Finglas, P. M. (eds.) *Encyclopedia of food sciences and nutrition* (2nd ed.). London, UK: Elsevier Science Ltd.

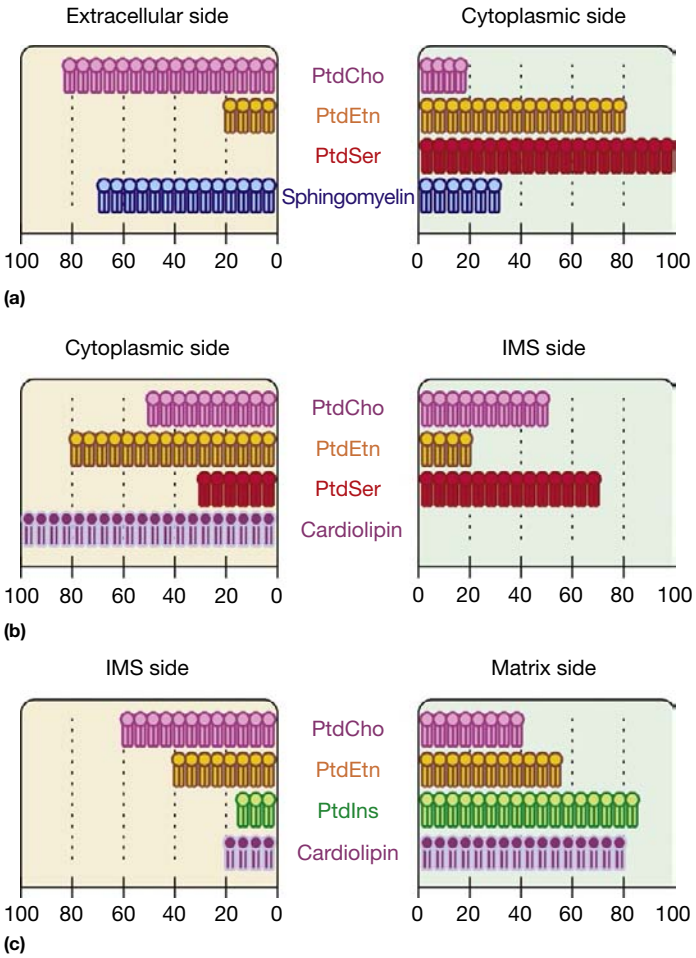

Figure 2 Asymmetry of membrane phospholipids. Transbilayer orientation of phospholipids in the (a) plasma membrane of human erythrocytes and (b) outer and (c) inner mitochondrial membranes of rat liver. (a) Relative distribution of phospholipids facing the extracellular side and cytoplasmic side of human erythrocytes. (b) Relative distribution of phospholipids facing the cytoplasmic side and intermembrane space (IMS) of the outer mitochondrial membrane (OM). (c) Relative distribution of phospholipids between IMS and the matrix side of the inner mitochondrial membrane (IM) from mammalian cells. PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine; CL, cardiolipin. Adapted from Horvath, S. E., Daum, G. (2013). Lipids of mitochondria. *Progress in Lipid Research* **52**(4), 590–614; Harwood, J. L. and Gurr, M. I. (1991). *Lipid biochemistry: an introduction* (4th ed.). London: Springer (Chapman & Hall).

Usually, phosphoglycerides contain a saturated fatty acid esterified at the *sn*-1 position of glycerol, whereas an unsaturated fatty acid is esterified at the *sn*-2 position, forming a diacylglycerol (DAG) moiety (Figure 1). The fatty acid chains usually have an even number of carbon atoms with up to 80% being 16–20 carbon atoms long. In most phosphoglycerides of cell membranes, the fatty acid at the *sn*-2 position is polyunsaturated (e.g., arachidonic acid, eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA)). This structural feature of phospholipids is very important in membrane function and in cell signaling events. The degree and the amount of unsaturated fatty acids have a direct influence on membrane fluidity. Moreover, 20-C unsaturated fatty acids in the *sn*-2 position in glycerol are precursors of cell signaling molecules.

Phosphoglycerides are named after the moiety attached to the phosphate group esterified at the *sn*-3 position of glycerol (Figure 1). The simplest phosphoglyceride, phosphatidic acid (mostly phosphatidate in physiological pH), contains only phosphoric acid attached to the DAG. Phosphatidate is the precursor of all phosphoglycerides and also triacylglycerol. It is synthesized *de novo* from the dual acylation of glycerol 3-phosphate using acyl-CoA as fatty acid donor (Figure 3). Glycerol-3-phosphate acyltransferase (GPAT) and 1-acylglycerol-3-phosphate acyltransferase catalyze, respectively, the first and the second acylation of glycerol 3-phosphate. GPAT is specific for saturated fatty acids and 1-acylglycerol-3-phosphate acyltransferase is specific for fatty acids with one or two double bonds. Glycerol 3-phosphate arises either from glycerol or from dihydroxyacetone phosphate, which is produced in glycolysis. This fact highlights the intimate association between energy metabolism and phospholipid synthesis. Besides *de novo* synthesis, phosphatidate is produced via DAG phosphorylation by DAG kinases or via degradation of PtdCho by phospholipases.

Upon its synthesis, phosphatidate is used directly to synthesize PtdIns or cardiolipin, or it is dephosphorylated to DAG,

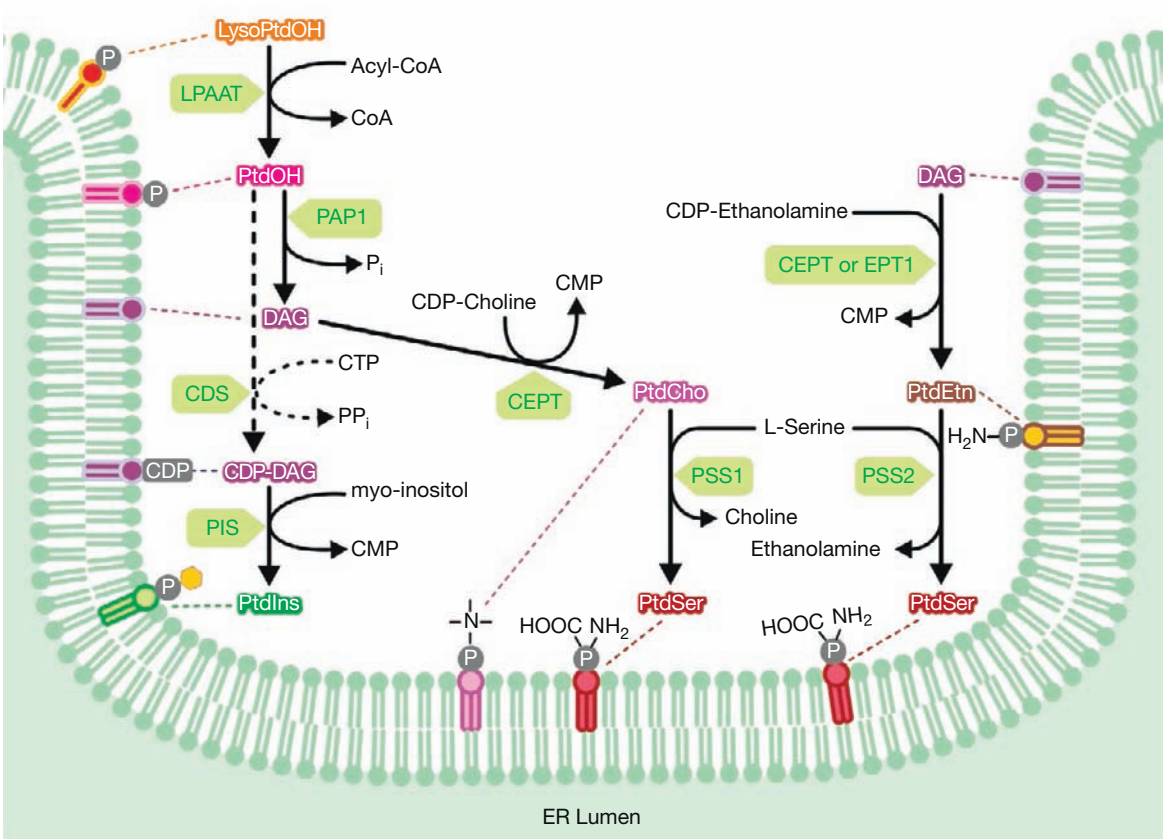

Figure 3 Phosphoglyceride synthesis. Phosphoglycerides are predominantly synthesized in the cytoplasmic leaflet of the ER membrane. PtdOH is the precursor of phosphoglycerides. LysoPtdOH produced from glycerol 3-phosphate is acylated to PtdOH. PtdOH is used directly to synthesize PtdIns. PtdOH dephosphorylation produces DAG, the precursor of PtdCho, PtdSer, and PtdEtn. PtdCho is also made in the Golgi complex, and PtdEtn is also produced in the mitochondria from PtdSer. See text for details. LysoPtdOH, lysophosphatidic acid; PtdOH, phosphatidic acid; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine; DAG, diacylglycerol; CDP-DAG, cytidine diphosphate-diacylglycerol; LPAAT, lysophosphatidic acid acyltransferase; CoA, coenzyme A; NAD^+, nicotinamide adenine dinucleotide; PAP1, phosphatidic acid phosphatase 1; CDS, CDP-DAG synthase; CTP, cytidine triphosphate; CDP, cytidine diphosphate; CMP, cytidine monophosphate; PIS, PtdIns synthase; CEPT, choline/ethanolaminephosphotransferase; PSS, PtdSer synthase; EPT1, ethanolaminephosphotransferase 1; CPT1, cholinephosphotransferase. Adapted from Bohdanowicz, M., Grinstein, S. (2013). Role of phospholipids in endocytosis, phagocytosis, and macropinocytosis. *Physiological Reviews* **93**, 69–106.

by phosphatidate phosphohydrolase, for the synthesis of PtdCho or PtdEtn (Figure 3).

Phosphatidylcholine

PtdCho is the precursor of various signaling molecules and it is the major phospholipid in bile. The first step in PtdCho synthesis is phosphocholine production from choline and ATP. Phosphocholine then reacts with cytidine triphosphate (CTP), producing cytidine diphosphocholine (CDP-choline). This reaction is catalyzed by choline-phosphate cytidyltransferase. The phosphocholine group is then transferred from CDP-choline to DAG by choline phosphotransferase, producing PtdCho (Figure 3). PtdCho can also be synthesized by methylation of PtdEtn, with S-adenosyl methionine (SAM) as the methyl donor. Phosphatidylethanolamine *N*-methyltransferase catalyzes this reaction. The importance of this pathway for PtdCho synthesis is evident in vitamin B12 and/or folic acid deficiency, which affects the production of SAM, decreasing the formation of PtdCho.

Phosphatidylethanolamine

PtdEtn is synthesized in a similar manner. Phosphoethanolamine reacts with CTP producing cytidine diphosphoethanolamine (CDP-ethanolamine), and the phosphoethanolamine group is transferred from CDP-ethanolamine to DAG (Figure 3). PtdEtn can also be synthesized by decarboxylation of PtdSer in the mitochondrial intermembrane space (IMS). This reaction is catalyzed by PtdSer decarboxylase, an enzyme located at the inner mitochondrial membrane. Since PtdSer is synthesized in the ER, its translocation to the mitochondria is a crucial step for PtdEtn synthesis by this pathway. Indeed, this pathway seems to be important for cellular functions since mitochondrial membranes are enriched with PtdEtn, despite that this phospholipid is found in most subcellular compartments.

Phosphatidylserine

PtdSer is synthesized in the ER. PtdSer synthases 1 and 2 catalyze the synthesis of PtdSer from PtdCho and PtdEtn,

respectively, in a calcium-dependent exchange reaction with L-serine (Figure 3). PtdSer is confined to the inner leaflet of cellular membranes. Under certain pathophysiological conditions, PtdSer is exposed to the outer leaflet. One such condition is apoptosis, which is one form of programmed cell death. Exposure of PtdSer functions as an 'eat me' signal, which is recognized by phagocytic cells. Moreover, increased PtdSer exposure in platelets regulates the blood coagulation cascade, and it is associated with tumor progression.

Phosphatidylinositol

PtdIns is synthesized from phosphatidate, rather than DAG. Phosphatidate is converted to the high-energy phosphoanhydride intermediate CDP-diacylglycerol in the ER by the enzyme CDP-DAG synthase. PtdIns synthase (CDP-DAG–inositol 3-phosphatidyltransferase) catalyzes the direct reaction of CDP-diacylglycerol to *myo*-inositol, thus forming PtdIns. PtdIns is unique among phosphoglycerides since it undergoes reversible changes to its inositol head group. Inositol can be phosphorylated on positions 3, 4, and 5, producing polyphosphoinositides. PtdIns and polyphosphoinositides comprise the phosphoinositide family, and their role as mediators of signal transduction is crucial (see succeeding text). PtdIns is present in most organelle membranes, but its concentration changes considerably. This fluctuation occurs due to the role of PtdIns in signal transduction and is regulated by phosphoinositide kinase, phosphoinositide phosphatase, and phosphoinositide synthase activities.

Sphingolipids

In place of glycerol, sphingosine is the long-chain amino alcohol of sphingolipids (Figure 1). Additionally, unlike glycerol, regarding phosphoglyceride synthesis, sphingosine is not a substrate for sphingolipid synthesis. Sphingomyelin is the major sphingolipid in mammalian cells, and it is synthesized from ceramide and PtdCho by sphingomyelin synthetase. Myelin is a complex structure composed of sphingolipids and proteins and surrounds some neurons and their axons. Myelin provides for the high rates of conduction of electrical activity along nerves.

Remodeling of Phosphoglycerides: Phospholipases and Acyltransferases

PtdCho (and most phosphoglycerides) in cell membranes presents a very long polyunsaturated fatty acid at the *sn*-2 position of glycerol. This is achieved by the concerted activity of two classes of enzymes: phospholipases and acyltransferases (Figure 4). Phospholipases hydrolyze phospholipids on water–lipid interfaces. Phospholipase A2 specifically removes the fatty acyl group at the *sn*-2 position, producing a lysophosphoglyceride and a nonesterified fatty acid. Then, acyltransferase reesterifies the available hydroxyl group from the glycerol residue with another fatty acid. This fatty acid is in the acyl-CoA form, and it is usually arachidonic acid.

Incorporation of phospholipids in the plasma membrane occurs mainly through membrane fusion with secretory vesicles. Additionally, membrane phospholipids are turned over through the action of membrane enzymes with phospholipase, lysophospholipase, and acyltransferase activities. Phospholipid remodeling, achieved by sequential removal and re-esterification of the fatty acyl group at the *sn*-2 position, is called the Lands cycle (Figure 4). The concerted activity of phospholipase A2 and acyltransferases (acyl-CoA:lysophospholipid acyltransferase) is crucial for cellular function for several reasons. As previously described, phosphoglycerides are synthesized from phosphatidate and DAG. Upon synthesis, these molecules present at the *sn*-2 position a fatty acid with one or two double bonds. Therefore, the formation of more

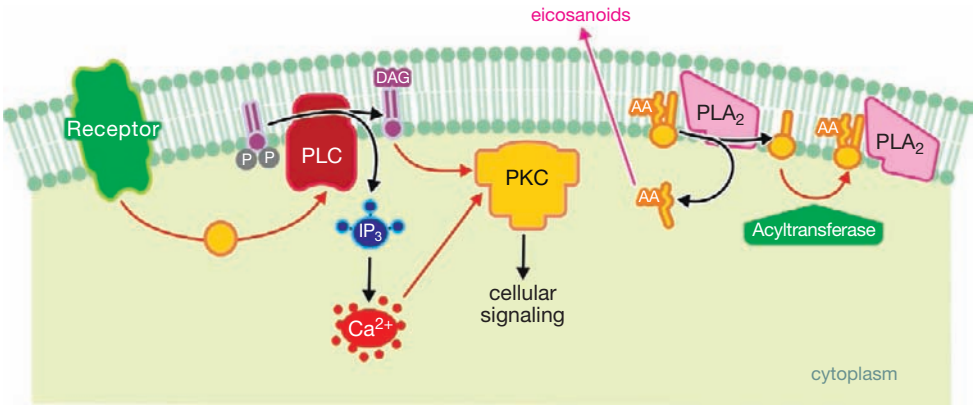

Figure 4 Phospholipids in cell signaling and membrane remodeling. Activated membrane receptor (e.g., G protein-coupled receptor or tyrosine kinase receptor) activates PLC, which in turn cleaves phosphoinositides in cell membranes generating DAG and IP_3. IP_3, a soluble mediator, induces the increase in intracellular calcium. Calcium and DAG activate PKC, a membrane kinase involved in many regulatory functions. Another phospholipase, PLA_2, can also be activated by membrane receptors, leading to hydrolysis of fatty acids at the *sn*-2 position of glycerol, producing free fatty acids and lysophospholipids. Lysophospholipids, by the action of acyltransferases, can be reacylated, maintaining cell membrane composition. AA is shown as the fatty acid at the *sn*-2 position that is both hydrolyzed from and acylated to the glycerol moiety of the phospholipid. AA (or EPA) serves as substrate for lipoxygenases and cyclooxygenases to produce eicosanoids, which are important signaling molecules. Lysophospholipids are also signaling molecules involved in a wide variety of cellular events (see text for details). PLC, phospholipase C; purple PP, phosphoinositide biphosphate; DAG, diacylglycerol; PKC, protein kinase C; IP_3, inositol triphosphate; AA, arachidonic acid; PLA_2, phospholipase A_2.

unsaturated phosphoglycerides occurring via the Lands cycle is crucial for the building of typical cellular membranes. Long-chain polyunsaturated fatty acids in cellular membranes play very important roles for increasing membrane fluidity and as signaling molecules.

Cellular membrane fluidity increases the rate of lateral movement and activity of proteins. The amount and the degree of unsaturation of fatty acids in the component phospholipids affect membrane fluidity. The higher the number of double bonds, the higher membrane fluidity is. By evaluating phospholipids' fatty acid composition in a particular tissue, one can suppose the importance of membrane fluidity and channel and transporter activities to cell function. In this regard, the brain and retina have high contents of the very long-chain polyunsaturated fatty acids, AA, EPA, and DHA. These fatty acids are essential for cognitive and visual functions.

Another important reason for membrane phospholipid remodeling is removal of oxidized PUFA. Unsaturated and especially highly unsaturated fatty acids, such as AA, EPA, and DHA, are readily oxidized or peroxidized by superoxide anion and other reactive oxygen species (ROS). Hydroperoxides are extremely reactive and can promote oxidative damage to membrane proteins, which may disturb membrane structure and function and possibly integrity. Phospholipase A2 targets the removal of oxidized fatty acids and terminates the propagation of membrane oxidation, thus protecting the membranes from peroxidative damage. Acyltransferase regenerates the phospholipid by esterifying a new, undamaged fatty acid to the lysophosphoglyceride. Interestingly, glutathione peroxidase acts concertedly in this pathway. The oxidized free fatty acid coming from PLA2 activity reacts with reduced glutathione, by the action of glutathione peroxidase. Therefore, the concerted action of PLA2 and glutathione peroxidase is important for the integrity and function of cellular membranes.

Phospholipids in Cell Signaling

DAG, phosphoinositides, lysophospholipids, eicosanoids, and ceramides are some of the fatty messenger molecules derived from phospholipids. Phosphatidylinositol in its biphosphorylated form is abundantly present in the inner leaflet of the plasma membrane. Upon external stimulation of some receptor types, phospholipase C is activated, which promotes the hydrolysis of PtdIns biphosphate into inositol triphosphate (IP_3) and DAG. IP_3, which is soluble, binds to a receptor on the ER and leads to the release of calcium. Increased cytosolic Ca^{++} signals to several cellular processes. DAG, which remains in the membrane, activates protein kinase C (PKC), a kinase attached to the cell membrane. PKC stands for protein kinase Ca-dependent. Therefore, activation of PKC is achieved by both DAG and IP3 signaling (Figure 4). PKC participates in different important cellular events. Receptors that activate PLC are G protein-coupled receptors, of which catecholamines are classic agonists, and also receptors from the tyrosine kinase family, which have growth factors as natural agonists. Therefore, PtdIns and its phosphorylated forms participate in the signaling cascade of hormones such as epinephrine and insulin. Consequently, phospholipid-derived mediators are involved in the regulation of several cellular functions.

Another important class of fatty messenger molecules derived from phospholipids is the so-called eicosanoids (Figure 4). The prefix *eicosa-* stands for their, and precursor fatty acids', chain length. Eicosanoids are potent inflammatory mediators derived from 20-C highly unsaturated fatty acids, AA and EPA. PLA2 activity on the *sn*-2 position of membrane phospholipids generates two products: arachidonic acid (or EPA) and lysophospholipid (Figure 4). Arachidonic acid and less commonly EPA serve as substrates for lipoxygenases and cyclooxygenases, producing the eicosanoids. Eicosanoids of the even series, with an even number of double bonds, come from AA (20:4$n-6$), and those coming from EPA (20:5$n-3$) are of the odd series. Generally, the former eicosanoids are proinflammatory and strongly stimulate platelet aggregation. In contrast, odd series eicosanoids are anti-inflammatory and much less aggregatory. This underlies part of the strong effects of habitual dietary intake of *n*-3 PUFA for the prevention of inflammation-related chronic diseases, such as atherosclerosis. These effects are especially strong in the Western world, where $n-6$ PUFA in the diet is in great excess over $n-3$ PUFA, in several cases at over tenfold.

Lysophospholipids, produced by hydrolysis of membrane phospholipids, also participate in cell signaling. Differently from DAG, lysophospholipids are partially water-soluble and therefore might bind to cytosolic ligands or be secreted and act in autocrine and paracrine signaling. Cellular action of lysophospholipids depends on several structural features, such as the group in glycerol *sn*-3 position, the fatty acid, and if it is esterified in the *sn*-1 or *sn*-2 position, among other factors. For instance, lysophospholipids are signaling molecules involved in many diverse processes from vascular and neuronal development to tumor progression. Additionally, blood lysophospholipids are carriers of DHA to the brain.

Ceramide can be produced by the activity of specialized phospholipases, named sphingomyelinases, on sphingomyelin phospholipid. Ceramide is a signaling molecule presenting a potent tumor suppressor activity, since it induces apoptosis and cell cycle arrest.

Cardiolipin and Mitochondrial Physiology

Mitochondria are organelles that synthesize ATP via oxidative phosphorylation and also regulate cell survival and death. Perturbations in mitochondrial structure directly affect mitochondrial functions. The mitochondrion has a complex structure containing two membranes: the inner membrane (IM) and outer membrane (OM). The OM forms a highly permeable smooth lipid surface of mitochondria. The IM, in contrast, is extremely folded and presents higher protein and lower lipid contents compared with the OM. The mitochondrial IM is virtually impermeable and, therefore, highly selective to only few compounds. As for all membranes, phospholipid distribution in mitochondrial membranes is asymmetrical (Figure 5). Regarding OM distribution, in rat liver mitochondria, PtdCho is equally distributed between both leaflets. Additionally, PtdIns and PtdSer are preferentially oriented toward the IMS, whereas cardiolipin is exclusively exposed to the cytoplasmic side. As for the IM distribution, PtdCho and PtdEtn are equally distributed between the two leaflets, and cardiolipin and

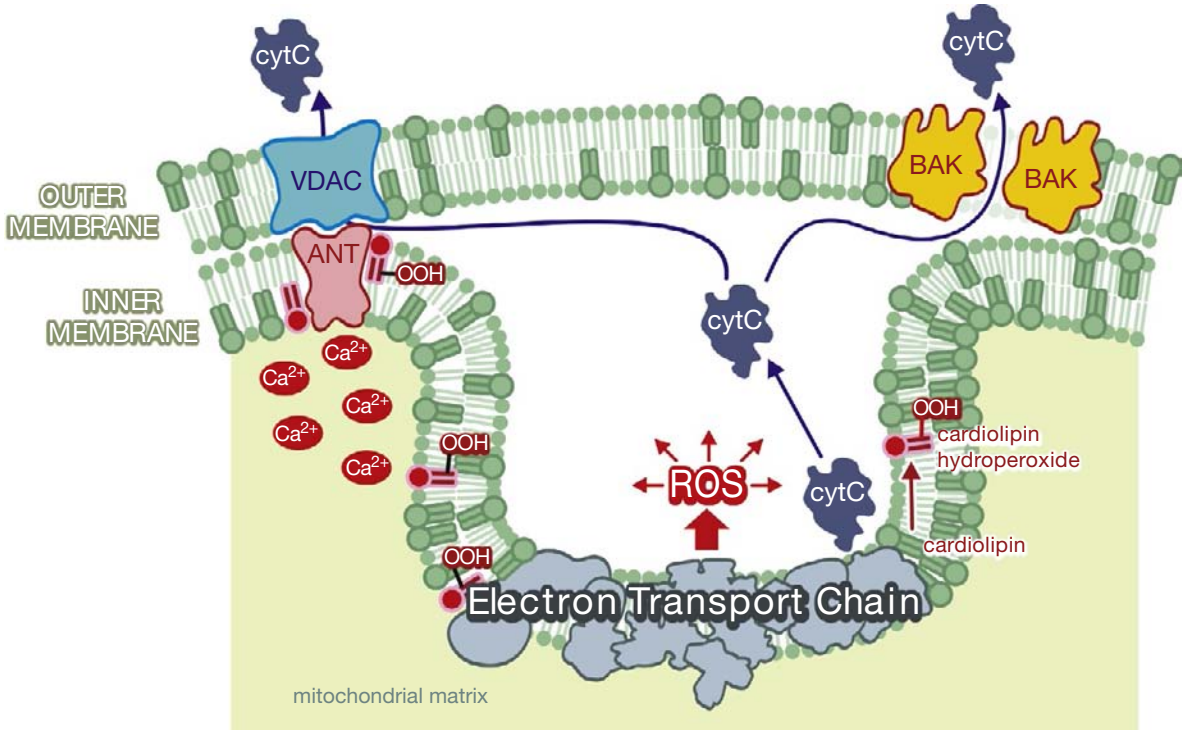

Figure 5 Oxidized cardiolipin and cytochrome *c* release from the mitochondria. Reactive oxygen species produced by the electron transfer chain induces cardiolipin oxidation. Oxidized cardiolipin causes alterations in the mitochondrial inner membrane structure, which promotes detachment of cytochrome *c*. Cytochrome *c* is then released into the cytosol through the mitochondrial outer membrane, via permeability transition pore, represented by ANT and VDAC or Bax–Bak oligomerization. Calcium ion influx into the mitochondrial matrix also induces the formation of permeability transition pore and cytochrome *c* release from the mitochondria. These events signal to apoptosis. ROS, reactive oxygen species; ANT, ADP/ATP translocator; cardiolipin-OOH, cardiolipin hydroperoxide; Cytc, cytochrome *c*.

PtdIns are predominantly exposed to the matrix side. The enrichment of cardiolipin in the IM, specifically in the matrix side, is of great significance for the organization and operation of the electron transport chain and oxidative phosphorylation (Figure 5).

Cardiolipin present in prokaryotes and in eukaryotes is enriched in mitochondria. This fact may support the endosymbiotic hypothesis, according to which mitochondria were 'inherited' from prokaryotes living inside eukaryotic cells. On the other hand, cardiolipin function differs from prokaryotes to eukaryotes. In prokaryotes, cardiolipin content increases only in certain conditions, such as environmental stress, whereas in eukaryotes, high amounts of cardiolipin are required for proper mitochondrial function. Consistently with these physiological differences, cardiolipin synthesis in prokaryotes is a near-equilibrium reaction, and in eukaryotes, this reaction is at nonequilibrium with large and negative ΔG.

Cardiolipin (diphosphatidylglycerol) is commonly referred to as the signature phospholipid of mitochondria. In this phospholipid, a glycerol molecule acts as a bridge between the phosphate groups of two phosphatidic acid molecules (Figure 1). As it occurs for PtdIns, phosphatidate is the precursor for cardiolipin synthesis. Biosynthesis of cardiolipin in mammalian cells takes place entirely in the mitochondria. In the OM, phosphatidate is activated to CDP-diacylglycerol by CDP-diacylglycerol synthase. The subsequent steps occur in the matrix side of the IM. The activated phosphatidyl group is transferred to the *sn*-1 hydroxyl group of another glycerol 3-phosphate to yield the formation of the intermediate phosphatidylglycerolphosphate, which in turn is hydrolyzed to phosphatidylglycerol forming premature cardiolipin. Phosphatidylglycerol undergoes a reacylation cycle to obtain its specific acyl composition, receiving a new phosphatidyl group from CDP-diacylglycerol. This reaction is catalyzed by cardiolipin synthase, an integral IM protein exposed to the matrix side.

Phosphatidylglycerol synthesis depends on the fatty acid composition of CDP-diacylglycerol. In general, CDP-diacylglycerols with unsaturated fatty acids as dioleoyl (C18:1) and dilinoleoyl (C18:2) or medium-chain fatty acids as dilauroyl (C12:0) and dimyristoyl (C14:0) are preferred substrates.

Cardiolipin interacts with innumerous molecules of the IM such as the electron transport complex proteins and ADP/ATP translocator. An interesting observation is that cardiolipin is required for the optimal activity of the aforementioned proteins suggesting that it plays an important role in mitochondrial bioenergetics. Indeed, an even increasing number of evidences show that cardiolipin is very susceptible to oxidative damage, with several consequences to mitochondrial and cellular functions. Cardiolipin is enriched in polyunsaturated fatty acids; for instance, in rat heart mitochondria, linoleic acid contents in cardiolipin are approximately 70% of total fatty acids. This high content of polyunsaturates and the fact that cardiolipin is in close contact with electron transport chain complexes, which are sources of reactive oxygen species in either physiological or pathological situations, render this phospholipid very sensitive to oxidation

(Figure 5). Oxidation of cardiolipin promotes destabilization of the IM, which may lead to derangements of mitochondrial electron transfer complexes and to an abrupt increase in the permeability of the IM. These effects have a direct impact in mitochondrial bioenergetics since they promote a decrease in mitochondrial membrane potential. Increased ROS production and oxidation of cardiolipin are also related to an increase in calcium influx to the mitochondria, which aggravates this scenario (Figure 5). Altogether, these events may lead to cytochrome *c* release from the mitochondria to the cytosol, which is a signal for the intrinsic apoptotic cascade pathway and, therefore, cell death. Cytochrome *c* release may occur via permeability transition pore (PTP) or Bax–Bak oligomerization and insertion into mitochondria (Figure 5). It appears that degenerative diseases and heart ischemia/reperfusion are physiopathologic conditions associated with oxidized cardiolipin. Thus, antioxidant compounds targeted to the mitochondria, and able to prevent cardiolipin oxidation, may open new perspectives for the treatment of these disorders.

See also: Choline: Physiology; Emulsifiers: Types and Uses; Fats: Classification and Analysis; Fatty Acids: Essential Fatty Acids; Fish Oils: Composition and Health Effects; Phospholipids: Properties and Occurrence; Vegetable Oils: Dietary Importance.

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Phospholipids: Properties and Occurrence

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Introduction

Phospholipids have been scientifically studied since the 1700s and became commercially available as lecithin in the 1930s. Their primary commercial source today is the soya bean, but phospholipids can be found in all living cells as part of the cellular membranes. This article covers the properties and occurrence of phospholipids as commercial lecithins, their chemistry, manufacture, composition, specifications, and their potential use in food systems.

Occurrence

The International Lecithin and Phospholipid Society defines lecithin as 'a complex mixture of glycerophospholipids obtained from animal, vegetable or microbial sources, containing varying amounts of substances such as triglycerides, fatty acids, glycolipids, sterols, and sphingophospholipids.' Lecithins are natural surfactants primarily derived from soya beans and eggs. They are found most abundantly in seeds and nuts, eggs, brains, and cell walls, in a concentration range of 0.5–2%.

Properties of Phospholipids (Lecithins)

There are three types of properties necessary to define phospholipids and lecithins: (1) chemical, (2) physical, and (3) functional.

Chemical Properties

The chemical composition of deoiled and liquid soya bean lecithin is shown in Table 1. There are approximately 17 different compounds in commercial lecithin, including carbohydrates, phytosterols, and minor phytoglycolipids. The three major phospholipids are phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol.

Structure of the Major Phospholipids

The chemical backbone of the major phospholipids is a diacylglycerol molecule with the third carbon attached to a phosphate molecule. Choline, ethanolamine, serine, and inositol can be attached to the phosphate group to change the physical and functional properties, leading to the formation of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, respectively. The groups attached to positions 1 and 2 (α or β) are C_{14}–C_{18} fatty acids with double bonds associated with the lecithin source. The second carbon of the glycerol molecule, the β-position, usually contains linoleic acid.

Physical Properties

There are two major physical classes of lecithins: (1) fluid, and (2) waxy solids.

The fluid lecithins can have viscosities from 5000 to 100 000 cP, depending on processing conditions and diluents. The low-viscosity products are made through the addition of fatty acids and vegetable oil, depending on the function and stability required. Divalent metal ions like calcium can be added during drying to decrease viscosity. The moisture content can also make a difference. Water levels above 1% will increase the viscosity, eventually to a plastic state.

Deoiled lecithins are waxy solids that can be ground to various particle sizes. They are stable, free-flowing granules or powders.

Functional Properties

Lecithins are multifunctional agents. They can be used for many purposes in a food system, as shown in Table 2. The most popular functionalities are discussed below.

Antidusting agents

Lecithins reduce static electricity by wetting dusty particles. They can be used alone or in conjunction with vegetable oils. Oils can be selected for the degree of shelf-life required.

Crystal formation modifier

Lecithins retard nucleation in fats and even monoglycerides, reducing graininess in texture.

Emulsifiers

Lecithins are most often used as amphoteric emulsifiers. They promote stable formation of oil-in-water and water-in-oil emulsions by reducing the interfacial surface tension between immiscible liquids.

Mixing and blending aids

Lecithins decrease time and increase efficiency of mixing of unlike ingredients such as sugar and shortening by providing lubricity as well as viscosity reduction at the contact surfaces of the incompatible solids.

Release agents

Lecithins provide easy release from metallic surfaces by attaching to the metal surface during hot or cold cooking. They assist in the cleaning of hot surfaces where proteins or batters are

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Table 1 Chemical composition of soya bean lecithin

	Granular lecithin	*Typical liquid lecithin*
Phosphatides (acetone-insolubles) (%)	95 (minimum)	60 (minimum)
Soya bean oil (%)	2–3	39
Moisture (%)	1	0.7
Fat (g per 100 g product)	90	93
Monounsaturated (oleic acid) (%)	9.2	17.9
Polyunsaturated (linoleic, linolenic acids) (%)	65.9	60.7
Saturated (palmitic, stearic acids) (%)	24.9	20.3
Carbohydrates (g per 100 g of product)	8	5
Approximate composition (100-g sample)		
Fatty acid content (g)	50	66
Fatty acid content (relative composition) (%)		
Linoleic	58.9	54.0
Linolenic	7.0	6.7
Oleic	9.2	17.9
Palmitic	20.3	15.6
Stearic	4.6	4.7
Other fatty acids	0.0	1.1
Total	100.0	100.0
Primary acetone insolubles (g)		
Phosphatidylcholine	23	15
Phosphatidylethanolamine	20	12
Phosphatidylinositol	14	9
Elemental analysis (mg)		
Calcium	65	40
Iron	2	1
Magnesium	90	60
Phosphorus	3000	2000
Potassium	800	440
Sodium	30	10

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applied. They also reduce sticking between frozen food products.

Separating agents

Lecithins prevent the adhesion of products that normally stick when in contact, like cheese slices and caramel confectionery.

Viscosity modifiers

Lecithins reduce viscosity by coating particles to reduce particle–matrix friction such as in chocolates.

Wetting agents

Lecithins provide complete wetting of fatty or hydrophilic powders in aqueous systems. The fatty acids are attracted to the fatty portion and the hydrophilic portions of the molecules actively imbibe water and control the hydration of the powder.

Manufacture

The majority of phospholipids are solvent-extracted from their source. Usually, nonpolar hydrocarbons, like hexane, are used. Soya beans, for example, are cleaned, cracked, and dehulled before flaking and extraction. The hexane is removed from the solvent micelle and the crude soya bean oil is cooled for further refining. Figure 1 shows a flow diagram for the degumming of crude soya bean oil and the production of lecithin. Approximately 2–3% water is added to the crude oil and agitated for at least 30 min. The phosphatides hydrate, swell, and are separated by centrifugation. The wet gums with 50% moisture are dried through a thin-film drier. The important points here are careful drying temperatures and cooling of the product below 20 °C. Lightening the color of the product can be achieved with hydrogen peroxide in the gum stage. The hydrogen peroxide is removed through drying.

Modification

The chemistry and functionality can be altered by simple chemical additions with acids and bases as well as hydrogen peroxide and acetic anhydride. These modifications increase the dispersion and hydration properties of the lecithin. Enzyme modification is also possible with lipases and phospholipases. These changes markedly affect the functionality in emulsification.

Composition of Lecithins

The composition of lecithins and their phospholipids will vary depending on their source: vegetable, animal, or bacterial. There are minor differences within a class but major differences

Table 2 Functionality of lecithins

Adhesion aid
Antibleed agent (as in fat bloom)
Anticorrosive
Antidusting agent
Antioxidant
Antispatter agent
Biodegradable additive
Biologically active agent
Catalyst
Color intensifier
Conditioning agent
Coupling agent
Dispersing agent, mixing aid
Emollient, softening agent
Emulsifier or surfactant
Flocculant
Grinding aid
Lubricant
Liposomal encapsulating agent
Machining aid
Modifier
Moisturizer
Nutritional supplement, vitamin source
Penetrating agent
Plasticizer
Promoter
Release agent, antisticking agent
Spreading agent
Stabilizer
Strengthening agent
Suspending agent
Synergist
Viscosity modifier
Water repellent
Wetting agent

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between the sources. Vegetable lecithins are high in phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid, but very low in phosphatidylserine and contain no sphingomyelin. Animal lecithins are high in phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and sphingomyelin but contain no phosphatidylinositol. Microbes have phospholipids similar to the plant kingdom with high levels of phosphatidylethanolamine and phosphatidylcholine and phosphatidylserine or sphingomyelin.

Specifications of Lecithins

Lecithins may be qualified in several ways, chemically, physically, and functionally, but there are also specifications used to assess quality and purity (Table 3). These include acetone-insolubles (AI), acid value (AV), hexane-insoluble matter (HI), peroxide value (PV), moisture, color, free fatty acids (FFA), divalent metals (DVM), iodine value (IV), and phosphorus. Most of these analytical methods are found in the American Oil Chemists' Society (AOCS) *Official Methods and Recommended Practices*, Section J.

Acetone-Insolubles

Phospholipids are nearly insoluble in cold acetone. This quantitative method should measure the active ingredients in lecithin. Depending on the type of lecithin, the range is 35–98%.

Acid Value

The phosphorus group in lecithins have a titratable acidity that is measured with this volumetric method. FFAs are also measured in this test and should not be confused with phospholipid acidity. The AV range is 20–36 mg of potassium hydroxide per gram.

Hexane-Insolubles

In the processing of soya bean lecithin, particulate matter finds its way through some processes and gives the product a hazy appearance. The HI can be determined by dissolving the product in hexane, centrifuging and gravimetrically measuring the insolubles. The HI range for soya bean lecithins is 0.05–0.3%.

Peroxide Value

The PV is a measure of oxidation in fats and oils. In lecithin, however, the PV usually measures the residual hydrogen peroxide from processing. The range in unbleached products is 0–10 mmol kg^{-1} and in bleached products is 10–75 mmol kg^{-1}.

Moisture

The water content of lecithins is quite low, at 0.1–1.0%. It can be measured by oven drying but is more accurately determined by the Karl–Fischer method. This water content is so low that there is no measurable water activity for microbial growth. Moisture contents above 1% will change the viscosity from a fluid to a plastic state.

Viscosity

The Brookfield viscosity at 25 °C will have a range of 150–20 000 cP. Diluents and divalent metals can alter the viscosity to usable levels.

Free Fatty Acids

This method measures the true fatty acid levels in lecithins which range from 1 to 5 mg of potassium hydroxide per gram. Fatty acids are added as additives to adjust the viscosity.

Iodine Value

The IV is a traditional method for qualifying lecithin sources. The more unsaturated fatty acids are found in soya bean lecithins, which have a range of 95–110 in natural fluid lecithins, to 80–90 in deoiled lecithin.

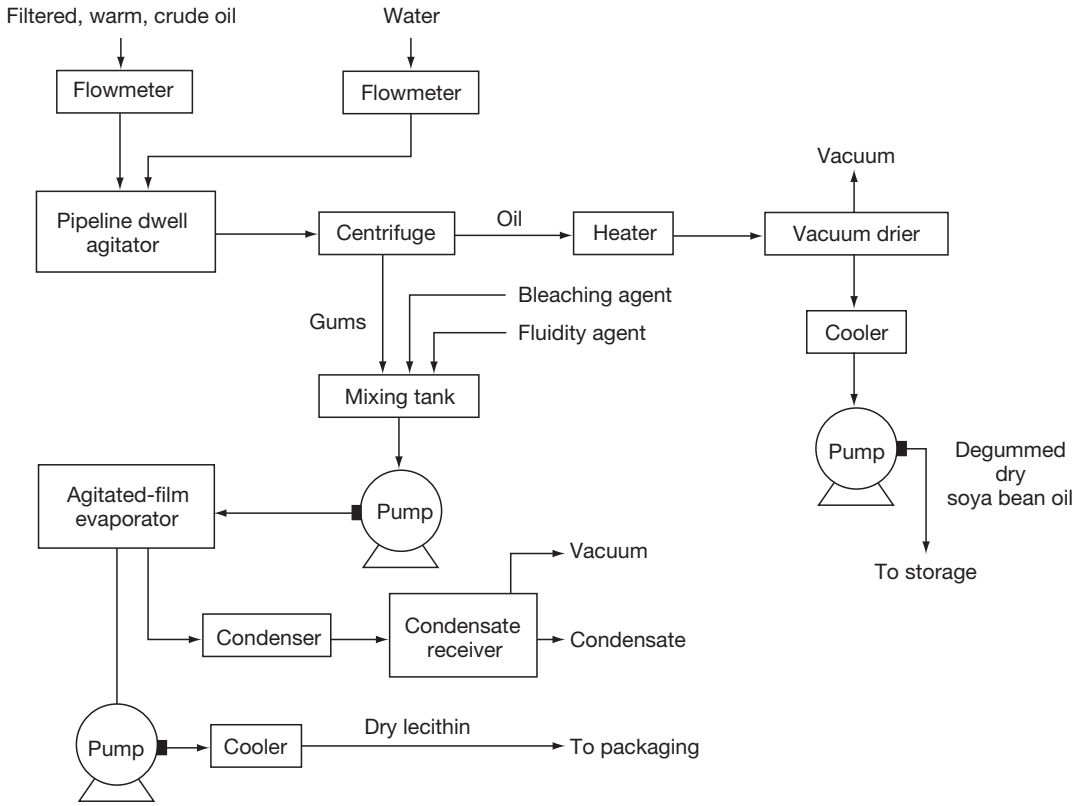

Figure 1 Flowsheet for degumming and crude lecithin production. Reproduced from List GR (1989) In Szuhaj BF (ed.) *Lecithins: Sources, Manufacture and Uses*, p. 149. Champaign: American Oil Chemists' Society, with permission.

Table 3 Specifications range for commercial lecithins

Analysis	*Typical range*
Acetone-insolubles (%)	35–98
Acid value (mg KOH g^{-1})	20–36
Hexane-insolubles (%)	0.05–0.3
Peroxide value (mmol kg^{-1})	
Unbleached	0–10
Bleached	10–75
Moisture (%)	0.1–1.0
Viscosity (Brookfield, 25 °C) (cP)	150–20 000
Free fatty acids (mg KOH (g^{-1})	1–5
Iodine value (cg I g^{-1})	
Natural	95–110
Oil-free	80–90

	Fatty acid composition (%)		
	Soya bean oil	*Natural*	*Oil-free*
$C_{16:0}$	10.3	15.6	20.3
$C_{18:0}$	4.4	4.7	4.6
Total saturates	14.7	20.3	24.9
$C_{18:1}$	24.5	17.9	9.2
$C_{18:2}$	53.8	54.0	58.9
$C_{18:3}$	7.0	6.7	7.0
Total unsaturates	85.3	78.6	75.1
Unsaturated: saturated ratio	5.8:1	3.9:1	3.0:1

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Phosphorus

This wet chemical method is an indirect way of measuring the phospholipid content. The typical level of phosphorus is 2.0% in fluid lecithin and 3.0% in deoiled products. The AOCS method Ca 12–55 has an approximation for converting percent phosphorus to the phosphatides in soya bean oil. The equivalent phosphatides content is equal to percent phosphorus $\times$ 30.

Uses of Lecithin

There are many uses of phospholipids in the food industry. As seen from the functional properties, there are multiple functions for lecithins. The following is a listing of the major areas of use:

- margarines – emulsifier, stabilizer, and antispatter;
- confectionery and snack foods – crystallization control, viscosity control, antisticking;
- instant foods – wetting and dispersing agent, emulsifier;
- commercial bakery products – crystallization control, emulsifier, wetting agent, release agent;
- cheese products – emulsifier, release agent;
- meat and poultry processing – browning agent, phosphate dispersant;

- dairy and imitation products – emulsifier, wetting and dispersing agent, antispattering and release agent;
- packaging aid – release agent, sealant;
- processing equipment – internal or external release agent, lubricant.

Applications of lecithins in foods are clearly supported by the Food Chemicals Codex, the European E322 regulations, and they are considered as generally regarded as safe substances by the US Food and Drugs Administration.

Storage and Handling

Lecithins are very stable products. They are shipped in drums or in bulk containers. They can be stored at ambient temperatures for up to 2 years without loss of activity or becoming rancid or spoiling. The water activity is so low that no microbial growth can occur. The products may be heated to 25 °C for easier application.

See also: Eggs: Use in the Food Industry; Fatty Acids: Determination and Requirements; Fatty Acids: Fatty Acids; Soy Beans: Properties and Analysis; Spices and Flavoring Crops: Uses and Health Effects; Triacylglycerols: Structures and Properties.

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Phytic Acid: Properties, Uses, and Determination

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Sources and Properties

Phytic acid (*myo*-inositol-1,2,3,4,5,6-hexa-dihydrogen phosphate; $InsP_6$; Figure 1) is the major storage form of phosphorous, inositol, and cations in plant foods including cereals, nuts, legumes, and vegetables. Cereals are high in phytate, whole grain products in particular since phytate is abundant in the bran. Due to its acidic properties, phytic acid is only fully protonated at high pH conditions (>12), and therefore, its anionic form, phytate, is the chemical form encountered in plant-based foods. The charged phytate molecule has high affinity for divalent cations such as Fe^{2+}, Zn^{2+}, Ca^{2+}, and Mg^{2+}. This property is important for the ability of plants to store metals and results in that the mineral concentration of plants is directly related to phytate content. This has implications for human nutrition research, in which plants are genetically modified or bred to decrease its phytate content, which also decreases its metal content, and may thus be regarded as a contraproductive strategy to improve mineral absorption from plants. In plants, phytate is almost always found as metal complexes, which have different compositions depending on origin, for example, phytate salts in oats are mostly composed of magnesium and manganese, while in wheat, phytate–calcium complexes are dominating. However, the composition of phytate salts changes during food processing and in the harsh environment of the gastrointestinal tract (pH gradient and enzymatic degradation). At small intestinal pH (6–7), where metal and mineral absorption occurs, phytate carries 8 (out of 12) negative charges and is therefore prone to form insoluble complexes with metals making the cations unavailable for absorption by humans. In addition to the high affinity of phytate to metals, phytate salts easily form insoluble aggregates, which further aggravate their absorption.

During food processing, some of the phytate is degraded into lower inositol phosphates ($InsP_{5-3}$), which also form metal chelates, but to a smaller extent. Since phytate degradation and the formation of inositol phosphate chelates are pH-dependent, the pH during food processing and the digestion in the gastrointestinal tract will determine the extent of metal chelation at the site of nutrient absorption, in the human small intestine.

Determination of Inositol Phosphates

Traditionally nonspecific methods have been used for phytate determination involving extraction of the food/feed by hydrochloric acid (HCL) and subsequent precipitation of ferric iron phytate in dilute acid solution. The content of iron or phosphorus was then determined in the precipitate. In the current AOAC (Association of Analytical Chemists), the extraction by HCl and anion exchange chromatography on AG1 resin for purification and stepwise elution with NaCl was introduced. The phosphate content of this fraction is then used to calculate the phytate content; also, with this AOAC method, there is a lack of specificity. Disadvantage is that this method does not discriminate between inositol phosphates and various organic phosphates. The nutritional and biological significance of phytate ($InsP_6$) and its degradation products have driven the need for the development of specific and reliable analytic methods, which differentiate between the different inositol phosphates. Adequate procedure for sample preparation from different matrices and high sensitivity methods for determination of the low concentrations occurring particularly in cells and tissues are required.

With the development of ion-pair HPLC procedures, quantitative determination of phytate and some of its degradation products ($InsP_6$–$InsP_3$) formed during food/feed processing or digestion in the gut became feasible. Later, isomeric-specific high-performance ion-exchange chromatography (HPIC) methods have been developed using gradient elution and post-column reaction with Fe^{3+} ions and UV detection. This system allows the analysis of isomers of $InsP_2$–$InsP_6$ in the same run (Figures 2–4). These HPLC methods are most commonly used in food and nutrition research. Using HPLC with metal-dye detection, the high sensitivity makes it applicable to biological samples and also the use of HPLC-mass spectrometry (MS), inductively coupled plasma-MS (ICP-MS), or ICP-atomic emission spectrometry (ICP-AES). Reliable determination of inositol phosphates in food can also be performed by 31P-NMR spectroscopy, but the low sensitivity makes it less useful for biological samples.

Availability, Absorption, and Metabolism

Most human cell types contain inositol phosphates ($InsP_{(1-6)}$). Whether phytate ($InsP_6$) is absorbed in the gastrointestinal tract or produced by cells has been debated for years. However, there are now several studies in humans, animals, and cell lines that suggest that inositol phosphates are indeed absorbed. $InsP_6$ has been detected in human urine and plasma in which the levels were correlated with dietary ingestion or deprivation of phytate. In human cells, $InsP_{5-6}$ are present at relatively high levels (10–100 μM), in which they are degraded to lower inositol phosphates, which play roles as second messengers in cellular processes. $InsP_3$ and $InsP_4$ are important for intracellular Ca^{2+} mobilization and sequestration during events such as mitosis. Decreased rates of mitosis lead to a slower rate of cell proliferation. In several cellular processes, Ca^{2+} may be seen as an on switch and $InsP_{3-4}$ as off switches. The mechanism of absorption of inositol phosphates has not yet been established, but there is some evidence suggesting that $InsP_6$ may be internalized into cells by means of pinocytosis. Bioavailability data for inositol phosphates are scarce, but the formation of salts seems to have an impact, for example,

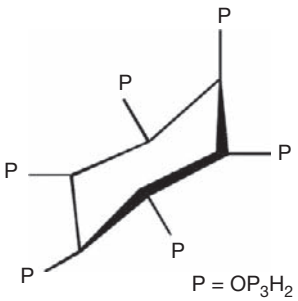

Figure 1 Structure of phytic acid.

Ca^{2+}phytate decreases the availability of phytate in rats and hamsters. This may also be a result of the favorable aggregation of phytate salts, especially calcium phytate.

Health Effects

The most significant effect of phytate in human nutrition is its potential to reduce mineral absorption. This is particularly a problem in low-income countries where the diet is based on staple foods (cereals and legumes). Phytate constitutes typically 1–3% of cereal grains, legumes, oil seeds, and nuts

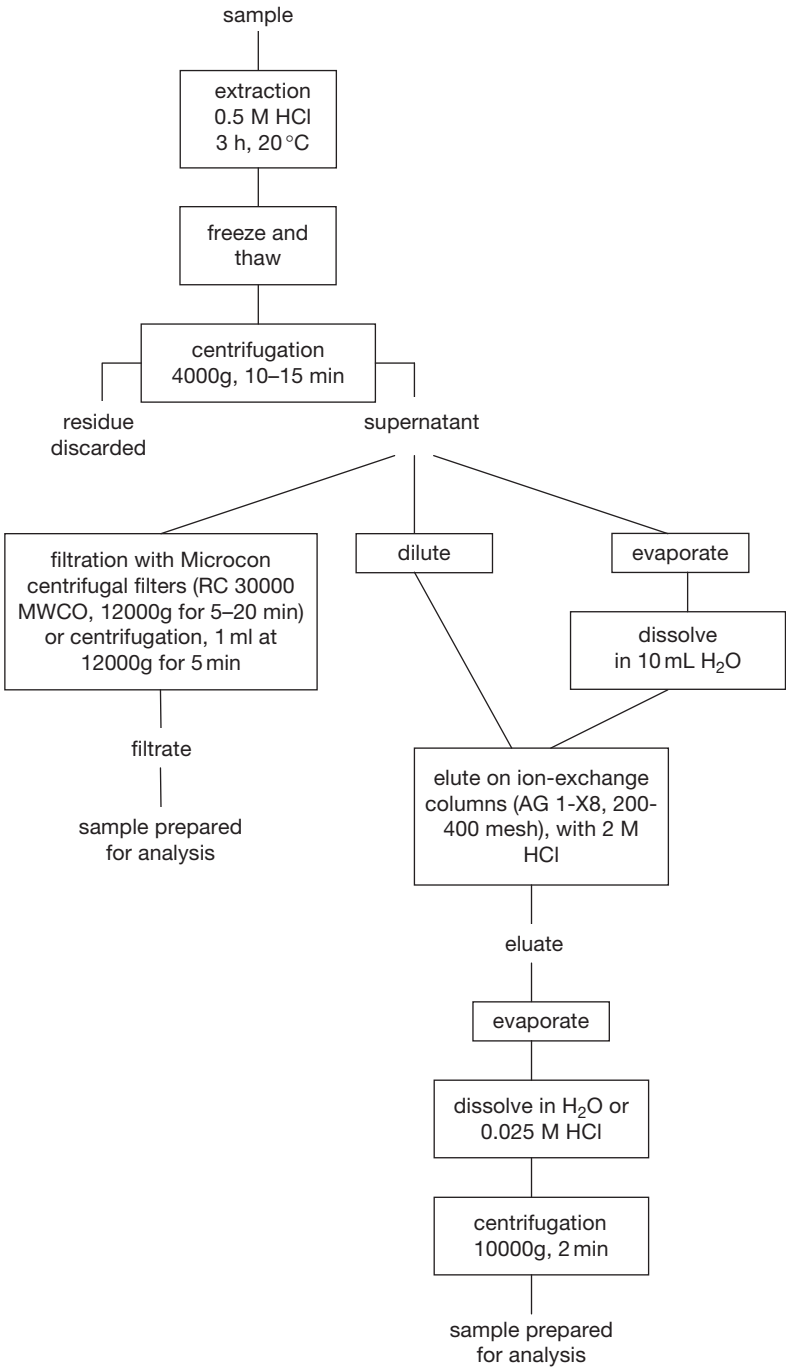

Figure 2 Schematic illustration of the preparation of samples for HPIC analysis. Reproduced from Skoglund E, Carlsson N-G, and Sandberg A-S (2008) Phytate. In: Shewry, P. R. and Ward, J. L. (eds.) *Analysis of bioactive components in small grain cereals*, chapter 11, p. 129–139. St. Paul, MN: AACC International.

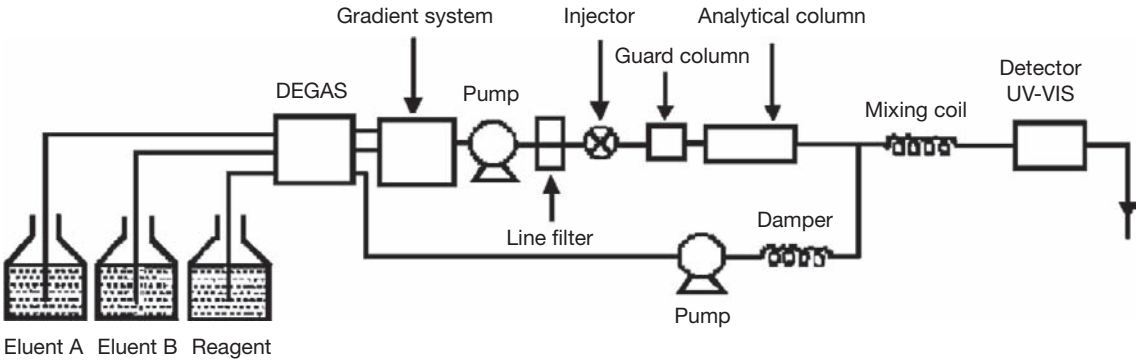

Figure 3 Schematic illustration of the analytic system: A = H_2O, B = 1 M HCl, and Reagent = 5 mM $Fe(NO_3)_3$*$9H_2O$ in a 0.35 M solution of $HClO_4$. Reproduced from Skoglund E, Carlsson N-G, and Sandberg A-S (2008) Phytate. In: Shewry, P. R. and Ward, J. L. (eds.) *Analysis of bioactive components in small grain cereals*, chapter 11, p.129–139. St. Paul, MN: AACC International.

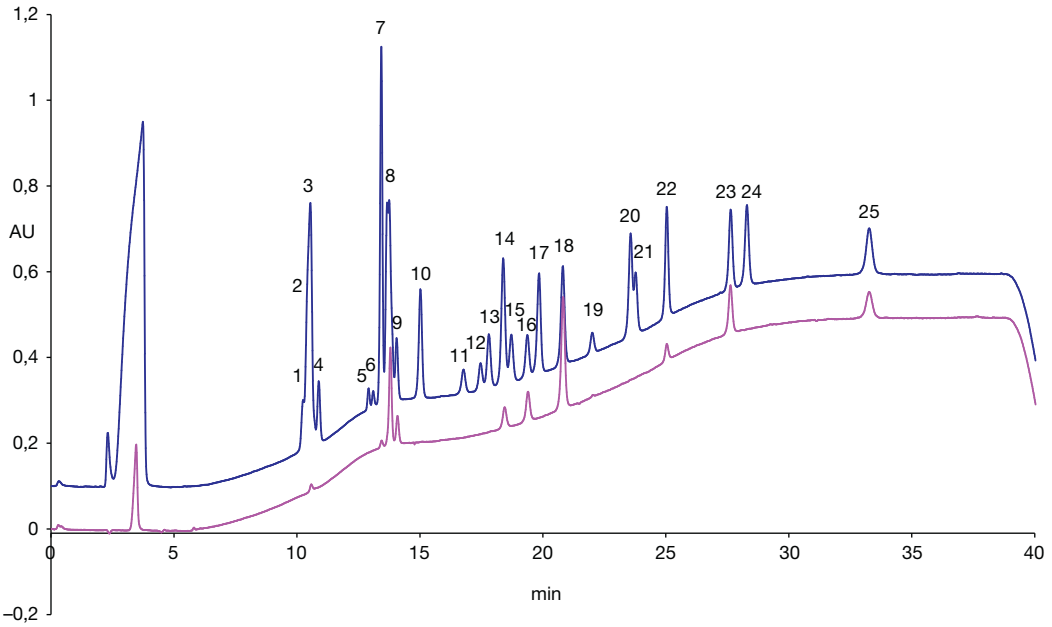

Figure 4 Chromatographic profiles of (a) $InsP_6$ reflux boiled with diluted HCl; (b) $InsP_6$ degraded by *Aspergillus niger* phytase (incubated 240 min). Peaks: (1) DL-Ins(1,6)P_2, Ins(1,3)P_2; (2) DL-Ins(1,2)P_2; (3) DL-Ins(1,4)P_2, DL-Ins(2,4)P_2; (4) DL-Ins(4,5)P_2; (5) DL-Ins(1,3,5)P_3; (6) DL-Ins(2,4,6)P_3; (7) DL-Ins(1,2,4)P_3, Ins(1,3,4)P_3; (8) DL-Ins(1,2,6)P_3, Ins(1,2,3)P_3, DL-Ins(1,4,6)P_3; (9) DL-Ins(1,4,5)P_3, DL-Ins(2,4,5)P_3; (10) DL-Ins(1,5,6)P_3; (11) DL-Ins(4,5,6)P_3; (12) DL-Ins(1,2,3,5)P_4; (13) DL-Ins(1,2,4,6)P_4; (14) DL-Ins(1,2,3,4)P_4; (15) DL-Ins(1,3,4,6)P_4; (16) DL-Ins(1,2,4,5)P_4; (17) DL-Ins(1,3,4,5)P_4; (18) DL-Ins(1,2,5,6)P_4; (19) Ins(2,4,5,6)P_4; (20) DL-Ins(1,4,5,6)P_4; (21) DL-Ins(1,2,3,4,6)P_5; (22) DL-Ins(1,2,3,4,5)P_5; (23) DL-Ins(1,2,4,5,6)P_5; (24) Ins(1,3,4,5,6)P_5; (25) $InsP_6$. Reproduced from Skoglund E, Carlsson N-G, and Sandberg A-S (2008) Phytate. In: Shewry, P. R. and Ward, J. L. (eds.) *Analysis of bioactive components in small grain cereals*, chapter 11, p. 129–139. St. Paul, MN: AACC International.

(Table 1) and occurs also in low concentrations in roots, tubers, and vegetables. The high prevalence of iron and zinc deficiency is a main nutritional issue in these countries especially in women of fertile ages, infants, and adolescents resulting in premature deliveries, morbidity of mother and child, retarded brain development in infants, prevention of normal child growth, and reduced immune and cognitive function. Phytate is the major inhibitor of iron and zinc absorption from food and feed. It has been demonstrated in humans that the inhibition of zinc and iron absorption by phytate is dose-dependent. Also, $InsP_5$ was shown to have an inhibiting effect. Even $InsP_4$ and $InsP_3$ contribute to the negative effect in processed food and feed probably due to binding of minerals between molecules. For this reason, efforts have been made to remove phytate, especially in infant foods. Controlled degradation of $InsP_6$–$InsP_3$ during processing was shown to markedly increase zinc and iron absorption from single meals. This is technically feasible by enzymatic degradation (soaking, germination, fermentation, or addition of phytase), but processing has to be optimized and removal of phytate extensive to improve zinc absorption and virtually complete to improve iron absorption. Combining dephytinization with enrichment strategies (adding meat and ascorbic acid) and breeding/genetic engineering for iron and zinc biofortification can be a means to improve mineral nutrition. Dephytinized cereal–legume-based complementary foods for infants are options to improve iron and zinc nutrition in low-income countries. However, in high-tannin cereals, it is not enough to remove phytate; the phenolic compounds still have a strong inhibiting effect on iron absorption. Also, if milk is added to the infant formulas, as often is the practice in industrialized countries, the iron absorption is markedly reduced.

In animal nutrition, two aspects are of importance: First, phosphates need to be liberated from phytate to meet the

Table 1 Content of phytic acid in selected plant foods

Common name	*Phytic acid (g per 100 g dry weight)*
Cereals	
Wheat	0.39–1.35
Rice	0.06–1.08
Barley	0.38–1.16
Sorghum	0.57–3.35
Oat	0.42–1.16
Rye	0.54–1.46
Legumes	
Kidney beans	0.61–2.38
Peas	0.22–1.22
Chickpeas	0.28–1.60
Lentils	0.27–1.51
Oilseeds	
Soybeans	1.0–2.22
Linseed	2.15–3.69
Sesame seed	1.44–5.36
Nuts	
Peanuts	0.17–4.47
Almonds	0.35–9.42

Adapted from Schlemmer, U., Frölich, W., Prieto, R. M., Grases, F. (2009). Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. *Molecular Nutrition Food Research* 53, S330–375.

phosphorus requirements of the growing animal, and secondly, the animal needs iron and zinc. Dietary phytate is resistant to degradation in the stomach and small intestine if active dietary phytate-degrading enzymes (phytases) are not present. Monogastric animals and humans have very limited phytase activity in the gastrointestinal tract. Therefore, microbial phytases are often used as additives to animal feed to degrade phytate in the feed or in the gut, thereby increasing mineral absorption. Microbial phytases are more stable to gastric juice than plant phytases and are active in a broader pH area. What is also priority for use in animal nutrition is high specificity for $InsP_6$ and $InsP_5$. The commercial phytases used in animal nutrition are often extracted from molds or produced by genetically engineered organisms. So far, microbial phytases are not used in human nutrition but may have a potential use in the future.

In contrast to its antinutritional properties, dietary phytate has also been suggested to have beneficial properties such as antioxidative and anticancerogenic effects. Although demonstrated antioxidative effects *in vitro*, such as the inhibition of the hydroxyl radical formation by iron binding, it is difficult to show this *in vivo*. Anticancer activity of dietary phytate has so far been demonstrated in carcinogen-induced cancer models and cell line experiments. Thus, there is a need to evaluate the significance of the anticancer activity by phytate in humans.

See also: Anemia: Prevention and Dietary Strategies; Antinutritional Factors in Legume Seeds: Characteristics and Determination; Bioavailability of Nutrients; Trace Minerals and Trace Elements.

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Pickling

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Introduction

Pickling is the process of preserving edible products in an acid solution, usually vinegar, or in salt solution (brine). In the latter case, the acid that does the preservative action (lactic acid mainly) is produced by fermentation. The process of pickling is also known as brining and the resulting foods as pickles. However, in the United States and Canada, the word 'pickles,' unless otherwise specified, generally means cucumber pickles. Usually, pickles refer to vegetable products, but sometimes, fish, eggs, or meat is also subjected to pickling. The process applied to meats is usually called curing. The focus in this article will be on pickled vegetables.

Most major vegetables can be preserved by pickling, either commercially or in households. Cucumbers, cabbage, and green olives account for the largest volume of vegetables and fruits commercially pickled in Western countries. Capers, garlic, onions, carrots, cauliflower, beans, and other vegetables are also pickled, albeit in lesser quantities. In Asian countries, fermented vegetables are very popular too, scientific research being mainly concentrated on kimchi, which is the general name given to a group of acid-fermented vegetable foods that have a long tradition in Korea. Therefore, the emphasis in this article will be on the following groups of fermented vegetables: fermented cucumbers, cabbage, and green olives, as well as kimchi. Information about two interesting nonfermented pickled vegetables, that is, capers and garlic, is also included.

The History of Pickling

Pickled vegetables were, probably, originally designed to preserve food and especially as a means to preserve surplus agricultural products for winter when supplies were scarce. Vegetable fermentation may have started in China, which can be deduced from references to the mixing of vegetables, which were given as rations to coolies during the construction of the Great China Wall in the third century BC. The origin of pickled cucumbers is unclear, but it is believed that cucumbers were first fermented around 2000 BC in the Middle East. Early written records of pickled cucumbers come from paper fragment remains of a play (*The Taxiarchs*) by the Greek writer Eupolis (429–412 BC), and this product is also mentioned in the Christian Bible (Old Testament books Numbers and Isaiah). Pliny the Elder, in the first century AD, is said to have been the first who described the production of sauerkraut by preservation of the so-called salt cabbage in earthen vessels. The method used today for sauerkraut could have been developed between AD 1550 and AD 1750 in eastern Europe. Korean-style fermented cabbage, kimchi, is thought to have originated in the primitive pottery age from the natural fermentation of withered vegetables stored in seawater. Kimchi is mentioned in the book *Samkuksaki*, published in AD 1145. The oldest written reference to the preparation of pickled green olives dates back to the first century before the Christian era, and different ways of pickling olives were described by Columela in his agriculture treatise *De Re Rustica* (AD 42). The history of the international trade of this product began when olives were sent to America in 1540.

Types of Pickled Vegetables

Pickled vegetables are divided into two types: fermented and nonfermented.

Fermented Vegetables

The general procedure for the preparation of fermented vegetables is shown in Figure 1.

Most, if not all vegetables, may be fermented in brine. The fermentation process for vegetables can result in foods that may be stored for extended periods, that is, 1 year or more, without refrigeration. Vegetables may be treated in various ways before fermentation, depending on the nature of the fresh vegetable and the product desired. Examples of such prebrining treatments include washing, cutting, peeling, blanching, and exposure to NaOH solution. Vegetables may be brined at various salt concentrations by either the dry-salt method (e.g., cabbages) or the brine method (e.g., cucumber, green olives, carrots, and caper berries). When vegetables are brined at salt concentrations of up to about 8% (w/v) and allowed to undergo natural fermentation, the brine solution supports fermentation by a sequence of various types of microorganisms: several gram-negative and gram-positive bacteria, mostly lactic acid bacteria (LAB), and yeasts. After fermentation, vegetables are packed with fresh packing brine in proper containers in order to be consumed or commercialized. Depending on the final conditions of the fermented product, a pasteurization treatment can be applied to further stabilize the packed product. Factors that affect fermentation are acid content, pH value, salt concentration, temperature, natural inhibitory compounds that are present in the vegetable, chemical additives, sugar content, redox potential, and availability of nutrients in the brine. Four species of LAB have historically been associated with the natural fermentation of vegetables: *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*, *Lactobacillus brevis*, and *Lactobacillus plantarum*. Nevertheless, many other LAB (e.g., *Lactobacillus fermentum*, *Lactobacillus pentosus*, and *Weissella* spp.) and yeast species play also important roles. Information about the preparation of the major fermented vegetables is summarized later in the text.

Cucumbers

Cucumbers for pickling are harvested while still immature. Sorting to remove blossoms and broken, defective, and

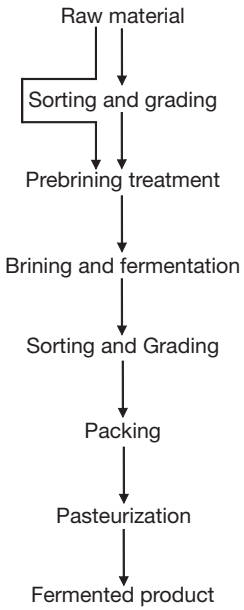

Figure 1 General procedure for the preparation of fermented vegetables. Pasteurization depends on the final conditions prevailing in the fermented vegetable.

distorted cucumbers should be done before brining to minimize spoilage during the fermentation. Sorting is followed by size grading. After that, cucumbers are covered with salt brine. Fermentations are typically carried out in brine equilibrated at about 6% NaCl. Most commercial cucumber fermentations rely upon growth of the LAB that are naturally present on the surface of cucumbers. Cucumber fermentations typically undergo a homolactic fermentation by *Lactobacillus plantarum* and related LAB. Carbon dioxide may be generated from the respiration of cucumbers and by the decarboxylation of malate during the initiation of fermentation. In an effort to prevent bloater formation, cucumber fermentations are purged with air to remove excess carbon dioxide from the tank. In addition to lactic acid, the LAB produce a variety of metabolites (e.g., bacteriocins) that can be inhibitory to other bacteria. At the end of the fermentation, there may be 1.5% lactic acid, a pH of 3.1–3.5, and little or no residual sugar. Prior to sale, cucumbers are washed to remove excess salt and then packed in a variety of containers (plastic pails, pouches, or jars) with appropriate cover liquor. The cover liquor typically contains acetic acid and spices in addition to residual lactic acid. Fermented pickles may be pasteurized to ensure long-term stability.

Sauerkraut

Sauerkraut is the product resulting from the natural lactic acid fermentation of salted, shredded cabbage. The German word literally translated means acid (sauer) cabbage (kraut). The heads of cabbage are trimmed to remove the outer green, broken, or dirty leaves, and the core is bored or partly removed. Subsequently, the cabbage is shredded into 0.7–2 mm wide strips that, in most cases, are salted with 0.7–2.5% salt. The shredded cabbage is placed into fermentation containers. Filling and pressing the cabbage into the fermenters together with added salt lead to an osmotic withdrawal of water out of tissue cells and, as a consequence, brine is formed. Finally, the surface of the filled containers must be covered carefully in order to exclude oxygen and microbial contamination. Usually, a sheet of plastic large enough to cover more than the area of the top of the fermenter is used. Depending on the temperature, a 'spontaneous' fermentation will start within a few hours to 1–2 days and will continue between 7 days and several weeks. The microbial growth sequence of spontaneously fermenting cabbage is invariably initiated by *Leuconostoc mesenteroides*, followed by heterofermentative LAB, and finally homofermentative LAB. A completely fermented sauerkraut contains 1.8–2.3% acid (calculated as lactic acid) giving a pH of 3.5 or less. Lactic and acetic acids are the predominating acids, but other organic acids such as succinic, malic, and propionic acids may also be formed in smaller quantities. Ethanol, mannitol, CO_2, and other compounds are produced in variable amounts as a result of the metabolism of heterofermentative LAB. Sauerkraut may be packaged in cans, glass jars, or plastic bags. Refrigerated sauerkraut, packaged in glass or plastic, is an unpasteurized product to which preservatives (e.g., sodium benzoate or potassium metabisulfite) are added. Pasteurized sauerkraut, packaged in cans or glass jars, can be made by pasteurization at 74–82 °C for 3 min.

Kimchi

The ingredients for kimchi preparation include four major groups: the main vegetable (mostly Chinese cabbage or white radish), spices, seasonings, and additional materials. The spices may include garlic, hot chili, ginger, onion, and others, whereas seasonings form a group of optional ingredients that include salt, pickled seafoods, sesame seed, soy sauce, vinegar, and sugar. Optional ingredients include small amounts of other vegetables, fruits, cereals, nuts, meats, seafoods, and mushroom, which may influence the rate of fermentation, flavor, and nutritional value of kimchi, as well as the fermentation process.

For cabbage kimchi (known as *baechu* kimchi or referred to simply as 'kimchi'), the trimmed Chinese cabbage is cut 3–5 cm in length, washed, mixed with salt (8–15%) for 2–7 h, rinsed with freshwater, and then drained. Sliced radish, onion and bunching onion, chopped garlic, ginger, hot chili, and salt-pickled seafood and dry salt are combined together to make a premixture in an appropriate ratio according to the formula. These minor ingredients are mixed well with the treated cabbage. The final salt concentration of the mixed vegetable fermentation is usually adjusted to ~2–3%. The mixed vegetables are placed in a fermentation vessel with a closed lid and left to ferment for 1–3 weeks at a low temperature (2–10 °C) or for 2–3 days at room temperature (20–25 °C). The microorganisms mainly responsible for the fermentation process are LAB, although fermentation by aerobic bacteria, yeasts, and molds occurs simultaneously. The pH of optimally fermented kimchi is 4.2–4.5 corresponding to organic acid contents of 0.4–0.8% (as lactic acid), respectively. Pasteurization is a successful method in preventing overacidification and can be used to extend the storage period of kimchi. However, possible flavor defects caused by heating may occur.

Green olives

The most common preparation is known as 'treated green olives in brine,' also known as Spanish-style or Sevillian-style green olives. Green olives must be harvested when the fruits have reached their maximum development (size) and prior to color change. The fruits are sorted, partially graded by size, and finally treated with a dilute 2.0–3.5% (w/v) NaOH solution (lye) to chemically remove bitterness by the chemical hydrolysis of the oleuropein. The penetration of the lye into the flesh is considered sufficient when the lye reaches about two-thirds of the distance from the exterior to the pit. After this treatment, olives are washed with tap water several times for variable periods of time, to eliminate the excess of alkali. Later, olives are immersed in a 9–10% (w/v) brine in which olives are maintained during the fermentation and preservation periods. Brine stabilization is fairly rapid, and in a few days, the NaCl concentration stabilizes at a level of 5–6%. *Lactobacillus pentosus* is considered the species mainly responsible for the fermentative process. Former microbiological research on olive fermentation was carried out before this species was finally recognized as a different species from *Lactobacillus plantarum*. Nowadays, most strains isolated from Spanish-style green olive fermentations are allocated to the species *Lactobacillus pentosus*. When the active fermentation has finished, fruits are sorted and graded by size again and, finally, conditioned to be packed as plain (whole), pitted, or stuffed with diverse products. The levels of acidity and salt determine the product stability. Traditionally, this has been obtained by setting high values of acid and salt (0.5–0.7% and 5–7%, respectively) and low pH (<3.5 units). However, the progressive preference of consumers for milder conditions (low salt and acid percentages) has modified such conditions. In these cases, the stability of the product is guaranteed by pasteurization. It has been established that a value of accumulated lethality of 15 pasteurization units (15 min at 62.4 °C) is enough to assure correct pasteurization. Specific heating times depend on the container characteristics and heat penetration curves.

Nonfermented Vegetables

Nonfermented vegetables are prepared according to the procedure outlined in Figure 2.

After preliminary operations (sorting, grading, etc.), vegetables are directly packed or can be preserved in concentrated brine with as high as 20% of salt before packing. At this high salt concentration, virtually no lactic acid fermentation takes place but the occurrence of a film of yeasts is not impossible. In this case, a desalting operation must be carried out prior to packing. Vegetables are packed using acidified brine as packing solution. The microbial inhibitor effect of acid pH is usually complemented, when necessary, by heat treatment (pasteurization) or by refrigeration plus the addition of antimicrobials (sorbates, benzoates, or sulfites). Acetic acid is commonly used as an acidulant at a concentration of 0.5–2%. More details about the preparation of selected pickled vegetables by this procedure are given in the succeeding text.

Cucumbers

Fresh-pack cucumber pickle products typically contain between 0.5% and 1% acetic acid. The salt concentration varies from 0% (in dietetic pickles) up to about 3%. A recommended pasteurization procedure consists of heating to an internal temperature of 74 °C for 15 min. Refrigerated, nonfermented cucumbers are preserved by the addition of low concentrations of vinegar and a chemical preservative (e.g., sodium benzoate), in addition to refrigeration at 1–5 °C.

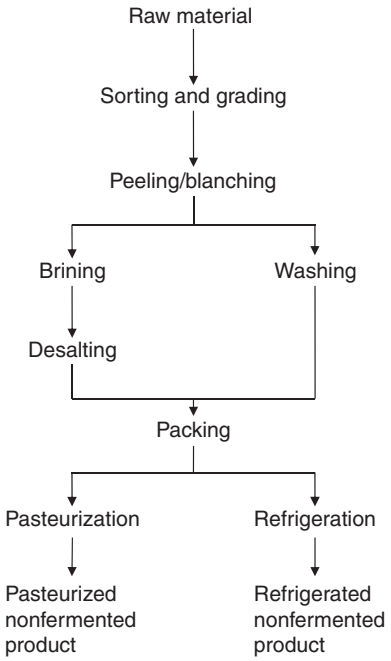

Figure 2 General procedure for the preparation of nonfermented vegetables. Peeling/blanching depends on the type of vegetable.

Capers

Capers are flower buds of the Mediterranean shrub *Capparis spinosa* L. The young buds are harvested when they are still tightly closed. The usual commercial procedure for the manufacture of pickled capers includes a first step of pretreatment of high brine (ca. 16% NaCl w/v, at the equilibrium, increasing 20% after changing the first brine). At the end of the pretreatment stage, capers are desalted and packed into a finished product containing about 6% salt and 1% acid w/v, as acetic acid.

Garlic

After cracking the bulbs to separate the garlic cloves and peeling the outer covering, the defective and smallest cloves are discarded. The peeled cloves are washed, blanched in hot water to eliminate the enzymatic activity responsible for pungent flavor, and then packed. Blanching can be carried out in water at 90 °C for 15 min, but less heating time (4 min) is also effective. Brine acidified with a lactic acid–acetic acid mixture (to give equilibrium values of 1.5% acidity, expressed as lactic acid, and 3% salt) can be used as packing solution. The corresponding packed product can be successfully stored at room temperature, at least for 4 months, without the need for pasteurization or refrigeration.

The Nature of the Preservative Action

Common salt and organic acids are primary preservatives for most types of pickled vegetables.

Common Salt

Common salt (NaCl; solubility in water at 25 °C, 35.7%) lowers the water activity (a_w value) of a system and thus renders conditions less favorable to microbial life. However, since the a_w value of a saturated common salt solution is only in the region of 0.75 and some microorganisms continue to grow below this limit, it is impossible to protect a foodstuff reliably from all microbial deterioration by using common salt alone, apart from the virtually unacceptable restrictions imposed on taste. Common salt also reduces the solubility of oxygen in water, which is of relevance for aerobic microorganisms.

Organic Acids

Organic acids are weak acids naturally found in vegetables, fruits, and fermented foods and have been used for decades as food additives for preservation. Lactic and acetic acids are the most used in pickled vegetables. Acidic pH environments can alter intracellular metabolic activities in bacteria. The diffusion of a protonated organic acid through cell membranes followed by intracellular dissociation of the acid can result in the acidification of the cytoplasm and intracellular acid anion accumulation, with eventual disruption of pH homeostasis and cellular metabolism.

Lactic acid ($pK_a = 3.79$) is a hygroscopic, syrupy liquid having a moderately strong acid taste. In the pickled vegetable industry, lactic acid is widely used to adjust acidity in brines. Lactic acid is produced naturally in fermented foods by LAB. As an end product in natural fermentations, lactic acid is probably one of the oldest known antimicrobial agents. The inhibitory capacity of this acid lies in its reduction of pH to levels below which bacteria cannot initiate growth. In fermented foods, lactic acid coupled with other antimicrobial factors excreted by lactic acid-producing microorganisms inhibits competing microorganisms.

Acetic acid ($pK_a = 4.75$) is used in food preservation in two forms, namely, as 5–10% vinegar and as 25–80% aqueous solutions of synthetic acetic acid. Vinegar is a dilute solution of acetic acid produced by a two-stage fermentation process. Vinegar contains a large number of secondary constituents that contribute to its flavor. As well as the sharpness conferred by acetic acid, individual vinegars each have their own particular flavor. This reflects the process by which it was made and the raw material used. Normally, a vinegar takes its name from the raw material used (malt vinegar, wine vinegar, etc.). The preservation action of vinegar is due to its acetic acid content, but is not simply a pH effect. Activity is associated with the unionized, lipophilic molecule that can penetrate the cell membrane, disrupting membrane transport processes and dissociating within the cell to increase acidity and produce toxic levels of the anion. Besides its preservative action, acetic acid is very important as a flavoring and acidulant.

Effect of Pickling on Sensory Quality and Nutritional Properties

Historically, pickled vegetables have played an important role in providing safe, preserved food commodities. With the introduction of new preservation techniques (freezing, canning, etc.), pickling has become less commonplace. However, pickling remains an important technology because it provides unique, desirable organoleptic traits, requires low energy, and allows the processing of fruits and vegetables to be extended over a longer period of time. Aside from their appreciated organoleptic characteristics, fermented vegetables are nutritious, owing to the presence of the vegetable nutrients and viable LAB, which may in some cases beneficially affect the human intestinal system. The nutritional value of nonfermented vegetables is generally lower than that of the fermented type, since these vegetable products are frequently preserved in high salt brine with exudation of cell contents and then desalted with excessive water leaching and nutrient losses.

The chief nutritive value of vegetables is in their content of vitamins, which may include important amounts of β-carotene, ascorbic acid, and folic acid and less important amounts of riboflavin and some of the other B vitamins. In general, vegetables used for fermentation are an important source of fiber but contain low levels of protein and calories. More specific information regarding the effect of pickling on sensory quality and nutritional properties for specific pickled vegetables is given later in the text.

Cucumbers

In the pickling process, the color of cucumbers changes from bright green to an olive or yellow-green color due to pigment degradation. Chlorophylls are converted to pheophorbides and pheophytins, whereas the carotenoids, consisting principally of β-carotene and the xanthophylls lutein and neoxanthin, undergo some destruction. The tissue changes to translucent from the normal chalky white and opaque due to the expulsion of gas during brine storage. This translucent appearance is not desired in pasteurized or refrigerated pickles since this is associated with a perception of loss in freshness. Retention of firmness is a key quality issue in the fermentation and storage of cucumbers. Calcium chloride (0.1–0.4%, equilibrated) is added to the cover brine to maintain the firm, crisp texture of the fermented cucumbers during fermentation and storage. Apart from acid formation due to sugar metabolism, numerous secondary fermentation reactions also occur in fermenting cucumbers that generate by-products such as fusel oil components, esters, and carbonyl compounds that impart unique flavor attributes to the pickled cucumber. A notable effect of fermentation on cucumber volatiles is the inhibition of the production of (*E*, *Z*)-2,6-nonadienal and 2-nonenal, the two most important odor impact compounds in fresh cucumbers, because the low pH of the brine inactivates lipoxygenase activity. Inactivation of lipoxygenase suppresses the pathway for the biogenesis of cucumber flavor compounds. The most potent odorants that define the typical characteristic brine aroma of pickled cucumbers are *trans*-4-hexenoic acid and *cis*-4-hexenoic acid.

With regard to nutritional aspects, pickling normally results in losses of vitamins. Losses of 86%, 82%, and 28% for ascorbic acid (vitamin C), thiamin, and carotene, respectively, have been reported in the case of desalted salt-stock cucumbers. When cucumbers are not desalted (e.g., fermented product at low salt concentration), 40–67% losses for ascorbic acid have been reported.

Sauerkraut

Acidity and salt levels influence greatly the sensory quality of this product. Ideal ranges for titratable acidity (1.1–1.5%), salt (1.7–2.4%), salt/acid ratio (1.0–1.7), and lactic/acetic acid ratio (3.0–5.0) have been proposed for canned sauerkraut. Textural characteristics of the final product are influenced by the salt concentration. Less than 1.8% (w/w) NaCl produces sauerkraut of soft texture, whereas applications in excess of 2.5% impart fibrous and tough textural properties to the product. It suggests that salt may serve to partially inactivate pectinolytic enzymes inherent in cabbage and/or reduce the pectinase activities of salt-intolerant microorganisms. Volatile sulfur compounds, such as hydrogen sulfide, methanethiol, dimethyl sulfide, carbon disulfide, dimethyl disulfide, allyl isothiocyanate, and dimethyl trisulfide, can be detected in the headspace of sauerkraut, having a great impact on the product flavor.

Sauerkraut is usually considered to be a healthy product. A health-promoting effect of sauerkraut may be linked to the high content of glucosinolates (up to 1% of dry weight) of the white cabbage. These compounds are hydrolyzed by the enzyme myrosinase during fermentation, resulting in the formation of indoles and isothiocyanates. The latter compounds were shown to be powerful inhibitors of carcinogenesis in animals. Ascorbigen, a compound formed from the reaction of a degradation product of indole glucosinolate (glucobrassicin) and ascorbic acid, is the dominant glucosinolate degradation product in sauerkraut, and is considered to be one of the most potent anticarcinogens of the glucosinolates family. Because sauerkraut is not desalted before use, good retention of water-soluble vitamins (especially vitamin C) would be expected. In fact, during the last centuries, sauerkraut was one of the major sources for vitamin C in the diet. The losses of this vitamin from the fresh cabbage to canned and pasteurized sauerkraut have been reported to range from 13% to 53%.

Kimchi

Numerous biochemical changes occur during kimchi fermentation, forming flavor compounds and changing the texture of the vegetables. A firm, crisp texture is highly desired in the vegetable components of kimchi. As can be predicted from the numerous ingredients of kimchi, this product is an important source of vitamins and their precursors, such as certain vitamins from the B group, β-carotene, and ascorbic acid. Kimchi is also a good source of dietary fiber. Kimchi consumption also seems to have hypolipidemic effects in humans. Some of the raw vegetables used for kimchi, such as cabbage, parsley, perilla leaf, green pepper leaf, garlic, and red pepper, have been tested and shown to be antimutagenic with an *in vitro* assay system. Kimchi extracts have shown anticarcinogenic activities in animal studies. This may be due to an enhancement of the immune system of the host or to kimchi's ability to affect the metabolism of mutagens. Further, it has been found that LAB isolated from kimchi block the adherence of *Helicobacter pylori* to a human gastric epithelial cell line and inhibit the growth of *H. pylori in vitro*.

Green Olives

Spanish-style processing of green olives includes several steps (lye treatment, washing, and fermentation in brine) that influence the sensory and nutritional characteristics of the final product. Lye treatment produces a marked loss of texture that persists during washing but recovers partially during fermentation due to the effect of salt and the absorption of divalent cations from the brine. As found in pickled cucumbers, fermentation causes total transformation of the chlorophylls initially present in the fruits and degradation of some carotenoids. As a result, the olives lose their original green color and become pale yellow. The unique and pleasant flavor of this product is probably the most important characteristic that makes Spanish-style green table olives so enjoyable and directly influences consumer acceptability. Flavor compound formation is a dynamic process that develops mainly during fermentation by LAB and yeasts, together with a variety of contaminating microorganisms, which produce volatile compounds from major fruit constituents through various biochemical pathways.

Soluble sugars disappear during the fermentation and are absent from the final product. The same happens to the bitter principle, oleuropein, which is mostly hydrolyzed during the alkaline treatment. The fiber content tends to decrease slightly, while the oil and protein contents do not significantly change during processing. Protein has been found to have a high content of essential amino acids, except for lysine. Contents of water-soluble vitamins such as ascorbic acid are low or negligible due to losses during processing, but green olives can be considered a reasonable source of provitamin A carotenoids (due to their content in β-carotene) and vitamin E (α-tocopherol mainly), which remain practically unaltered during processing.

Although the loss in total polyphenols during Spanish-style processing can reach some 90%, an important content still remains in the final product. This content is similar to the total polyphenol content (about 400 mg kg^{-1}) of commercial virgin olive oils. Phenolic compounds of olives have been shown to possess antioxidative, antimutagenic, anticarcinogenic, and antiglycemic beneficial properties. Because of their natural polyphenolic content, green olives are an important part of the health-promoting Mediterranean diet.

Capers

The color of capers shifts from bright green to a more yellowish color as a consequence of the brining treatment. In addition, the surface of capers is covered by tiny grayish spots, which are typical of pickled capers after a few days in brine. Pickling results in lower contents of fiber, protein, and water-soluble vitamins in pickled capers compared to fresh capers. Pickled

capers are a very rich source of the flavonols quercetin and kaempferol. During brining, some hydrolysis of flavonoids occur releasing quercetin and kaempferol from the original glycosides. Quercetin derivatives have received special attention as dietary constituents, as epidemiological studies point out that they might have a role in preventing cardiovascular diseases and cancer. Similarly to sauerkraut, pickled capers are rich in isothiocyanates, which derive from glucosinolates by enzymatic hydrolysis.

Garlic

Blanching step modifies the color and firmness of garlic. Immediately after this treatment, garlic color is less white than raw garlic. Firmness decreases by 70% compared to raw garlic. The nutrient most affected is ascorbic acid, which is totally lost during processing. It is known that the health properties of garlic depend on its organosulfur compounds, namely, γ-glutamyl peptides, *S*-alk(en)yl-L-cysteine sulfoxides, and *S*-allyl-L-cysteine. In general, the concentration of these compounds decreases significantly during processing, mainly as a result of their solubilization into the packing brine, but a relatively high concentration of each compound still remains in the final product. In the case of the packed product, 65% total identified sulfur compound retention has been found. Therefore, it is reasonable to assume that a large part of the health benefits of garlic is maintained following pickling.

Conclusion

The preservation of vegetables by pickling is one of the earliest and most widespread technologies developed by humans. Both fermented and nonfermented pickled vegetables are produced and consumed in every culture and society around the world, usually based on traditional processing methods. This is because the products produced are safe, due to the presence of inhibitory compounds, primarily NaCl and organic acids, and have appealing sensory characteristics. Aside from these features, there are other reasons nowadays to recommend the consumption of this type of foods, taking into account the growing interest by consumers in healthy foods. Thus, some pickled vegetables such as sauerkraut, kimchi, green olives, capers, and garlic appear to be a rich source of compounds (e.g., polyphenols, glucosinolates, and organosulfur compounds) with beneficial properties. Another health-promoting property may result from the LAB involved in fermentation in case of fermented vegetables, although the probiotic efficacy of these microorganisms still have to be demonstrated in most cases.

See also: Fermented Foods: Composition and Health Effects; Fermented Foods: Fermented Vegetables and Other Products; Fermented Foods: Origins and Applications; Fermented Foods: Use of Starter Cultures; Functional Foods; Garlic; Olives: Types and Consumption; Preservation of Foods; Vinegar.

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Pine Kernels

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Pine Kernels

Pine kernels are the edible seeds of pine trees. Pine kernels are among the most expensive and priced nuts on the market because of its high harvest costs and limited supply. Recently, pine kernels have gained much popularity and attention as health food. Pines are evergreen trees that bear cones. Pine trees belong to the Pinaceae family in the genus *Pinus*. Pine kernels are released by cracking open the seeds of cones from pine trees. Nuts from three types of pines, the stone pine (*P. pinea*), Korean pine (*P. koraiensis*), and chilgoza pine (*P. gerardiana*), have been widely traded internationally for many years. Siberian pine nut (*Pinus sibirica*) is a recent addition to international trade as well. Stone pines traded globally are primarily collected from domesticated trees, while seeds from other species are mostly gathered from the wild. China is the world's largest exporter of pine nuts (mainly Korean pine). However, a major share of China's exports originates from other countries that ship unshelled pine nuts to china where they are processed and exported. In 2011, about 36 080 tonnes of pine kernels were produced globally. China and the Russian Federation contribute 55% and 14%, respectively, to world pine nut production. Spain, Portugal, and Italy also export nuts from stone pine. Prices of pine nuts fluctuate greatly in international markets.

Sources and Production

Most of the marketed pine kernels are collected from the wild forests. Different pine species have seeds of different sizes and with different flavors. Cones are woody and contain numerous seeds. Cones are made up of cone scales arranged in spirals around a central axis. The upper surface of the cone scales bears seeds. Seeds of most of the pine species have membranous wings. Piñon and some other pines lack the wings. Based on the wood and leaf anatomy, pines are classified as soft and hard pines. The wood of soft pine is soft, even-textured, and creamy white, and their summerwood is light in color. Most of the edible nut-bearing pines are soft or haploxylon pines, and some of these are also of the hard or diploxylon group. Being a gymnosperm, pine nuts are not a true nut and they lack a carpel. Approximately 29 species of *Pinus* produce edible seeds. Only a few species produce edible nuts of sufficiently high quality with desirable flavor. The most important sources of pine nuts are *P. sibirica* (Siberian pine), *P. koraiensis* (Korean pine), *P. pinea* (Italian stone pine), *P. gerardiana* (chilgoza pine), *P. monophylla* (single-leaf piñon), *P. edulis* (Colorado piñon), and other piñon species. *P. gerardiana*, *P. koraiensis*, *P. pumila*, and *P. sibirica* are important nut-bearing pine species in Asia. *P. koraiensis* is the most internationally traded species, and they are produced and harvested in China. The seeds of *P. gerardiana* (chilgoza pine) are long and boat-shaped with one sharp end. The Asian pine nuts are shorter and stubbier than the European pine nuts. *P. koraiensis* (Korean pine) is a native of eastern Asia. It is an evergreen monoecious tree growing 40–50 m tall. Needles are dark bluish green and occur in bundles of five. Cones are borne on a short peduncle and are ovoid or cylindrical. Triangular ovoid seeds are 12–16 mm long with a hard seed coat covering a shiny yellowish kernel. A list of edible nut-bearing pine species are presented in Table 1.

The Mediterranean Stone Pine

The most commonly consumed pine nuts are derived from *P. pinea*. *P. pinea* L. is commonly known as the stone pine. The section *Pinea* under the subgenus *Pinus* includes the other true Mediterranean pines (*P. pinaster*, *P. halepensis*, *P. brutia*, and *P. canariensis*). The stone pine (*P. pinea*) has a long history of cultivation and is the most widely used species in Europe and in the Middle East. Italy is the largest producer of stone pine followed by Spain and Portugal. It is often called umbrella pine because of its widespread umbrella-shaped foliage. This evergreen tree can grow to a height of about 80 ft, and its reddish-brown bark is scaly and deeply furrowed. Needles are in twos and round ovoid cones are borne singly or in groups of 2 or 3. These trees are slow-growing and start production in 7–8 years, and it may sometimes take longer (10–12 years) to start bearing under poor conditions. Cones are about 80–150 mm long, up to 110 mm wide and 153 mm in height. The cones of stone pines generally take 3 years from pollination to ripening. Each cone contains 100 s of narrow cylindrical seeds with hard brown covering. Thick-shelled seeds are dull brown with wings. The kernel is whitish yellow and possesses a sweet flavor. Stone pines are propagated from seeds.

Piñon Pines

The piñon pines of northern Mexico and southwestern United States are the largest group of edible nut-bearing pine species. Among the 13 species of known piñon pines, three species (*P. cembroides*, *P. edulis*, and *P. monophylla*) have a widespread distribution, being the largest group. Other piñons are used as food sources locally and have limited distribution. Other edible nut-bearing North American pines include *P. albicaulis*, *P. flexilis*, and *P. strobiformis* of the Rocky Mountains and Great Basin regions and *P. coulteri*, *P. lambertiana*, *P. sabiniana*, and *P. torreyana* of California, the United States, and Baja California del Norte. The single-leaf piñon (*P. monophylla*) has needles arranged singly, and the Colorado piñon (*P. edulis*) has two needles per bundle and produces a hard-shelled pine nut. Some other species of pinion pines are border piñon (*P. discolor*), Texas piñon (*P. remota*), Nelson piñon (*P. nelsonii*

Table 1 Some common edible nut-bearing pine species

Species	*Natural range*	*Uses*
P. pinea (stone pine)	Mediterranean Europe	Global trade
P. coulteri (Coulter pine)	The United States (California)	Traditional food
P. ponderosa (ponderosa pine)	The United States and western Canada	Traditional food
P. sabiniana (gray pine)	The United States (California)	Traditional food
P. roxburghii (chir pine)	India	Traditional food
P. torreyana (Torrey pine)	The United States (California)	Traditional food
P. ayacahuite	Central America, Mexico	Traditional food
P. albicaulis	Western Canada and the United States	Traditional food
P. cembra	Europe	Locally used
P. flexilis	Western Canada and the United States	Traditional food
P. gerardiana	Afghanistan, Pakistan, India	Global trade
P. lambertiana	West United States (California and Oregon)	Traditional food
P. monticola	NW United States and Canada, SW United States	Traditional food
P. koraiensis	Russia, China, Korea, Japan	Global trade
P. sibirica	Russia, Mongolia	Locally and globally used
P. pumila	East Siberia, China, Korea, Japan	Locally used
P. strobiformis	N Mexico, southwestern United States	Locally used
Piñon pines		
P. cembroides (Mexican piñon)	Mexico	Locally used
P. culminicola (Potosí piñon)	Mexico	Locally used
P. edulis (Colorado piñon)	Mexico and the United States	Imp nut producer
P. johannis (Johann's piñon)	Mexico	Locally used
P. monophylla (single-leaf piñon)	Mexico and the United States	Important nut producer
P. orizabensis (Orizaba piñon)	Mexico	Locally used
P. quadrifolia (Parry piñon)	Mexico and the United States	Locally used
P. remota (Texas/papershell piñon)	Mexico and the United States	Locally used
P. catarinae	Mexico	Locally used
P. juarezensis	Mexico and the United States	Locally used
P. maximartinezii	Mexico	Locally used
P. nelsonii (Nelson's piñon)	Mexico	Locally used
P. pinceana (weeping piñon)	Mexico	Locally used
P. remota	Mexico and the United States	Locally used

Sources: http://en.wikipedia.org/wiki/List_of_Pinus_species; http://www.fao.org/docrep/x0453e/x0453e12.htm

Shaw), Pince piñon (*P. pinceana* Gord.), Potosí piñon (*P. culminicola* Andresen & Beaman), and Martinez piñon (*P. maximartinezii* Rzedowski).

Collection, Storage, and Processing

Pine nuts are collected in several ways. The majority of pine nuts are picked by hand. Cones are also gathered while they are still green; pickers climb the trees and collect the cones. Pickers also use a long pole with hooks to pull the cones off the tree. Harvesting the green cones is difficult and time-consuming. Dry picking of the brown cones takes place later in the season once the cones have dried. When the cones turn brown, their scales open up and the seeds in the opened cones fall down. The seeds can also be removed from the brown cones by hitting the cones over a blanket to collect the seed. Collections of brown cones are easier but there is a chance of birds and mammals consuming a large portion of the seeds. Collectors also spread sheets, blankets, and tarps under individual trees, and trees are shaken to dislodge seeds or cones. Some commercial pickers also gather seeds directly from the ground beneath the trees particularly in bumper crop years when the seeds are abundant. Pickers rake or sweep up the seeds and then sift the seeds from the litter. Some pickers also collect seeds from the packrat middens, and these seeds are more likely to have fecal matter contamination. There is a great fluctuation in the quality and quantity of pine nut harvests from year to year. Demand for pine nuts often exceeds production, which often leads to high prices and price fluctuation.

Processing of Pine Kernels

Pine kernels need processing before they are ready to be eaten. Processing method depends on the stages of the harvested cones. Green cones are sun-dried or machine-dried prior to processing. Dried green cones and brown cones are shaken to extract the nuts using a tumbling machine. Separated nuts are then sorted to remove empty shells and poor quality seeds. Sorted nuts are again dried to reduce their moisture content to prolong their shelf life. Pine kernels are covered with a hard shell or seed coat when first isolated from the pine cone. This shell has to be removed prior to consumption. Dried nuts are milled to remove the kernel from the outer shell. Seeds collected from ground or rodent caches need to be washed before they are processed further. All nuts are best stored in a cool dry

environment. Fresh unshelled nuts can be stored at temperatures between −5 and +2 °C for a year or more preserving their flavor and texture. Unshelled nuts have a long shelf life under dry and refrigerated conditions. Shelled nuts can turn rancid within a few weeks especially under warm conditions. Nuts of different species vary in size, taste, and nutritional composition. Pine nuts of different species are generally mixed together during marketing and sold generally as pine nuts.

Methods of Consumption

Pine kernels are nutritious healthy snacks and are consumed raw or roasted. The bulk of the piñon nuts is sold as whole nuts for human consumption. Nuts are sold shelled, unshelled, fresh and dried, and raw and roasted. Some vendors soak unshelled nuts in brine giving them a salty taste. Whole nuts and pieces are also included in breads, candies, cookies, cakes, and sauces such as pesto. They are also added to gourmet chocolates. Pine nut flakes are by-products of pine nut oil, and they are used for making granolas, chocolates, and crunch bars. Pine nut flakes are further pressed and crushed to extract oil, and the by-product is pine nut meal or flour that has a variety of uses. It can be used as a substitute for wheat or rye flour and can be used for making pastries and pancakes. A dairy-free milklike drink with a rich nutty flavor is obtained by mixing pine nut meal with water. It can serve as a thickening agent for sauces and also a nutritious soup. Pine nut oil is extracted from pine nuts, which is valued for its many health benefits such as antioxidant and anti-inflammatory properties. Pine nut oil is used as gourmet cooking oil or as a medicine. Oil extracted from *P. sibirica* and low-grade seeds of *P. yunnanensis* and *P. koraiensis* is used as cooking oil, which contains polyunsaturated fatty acids (PUFAs) such as pinolenic, linolenic, and linoleic acids. Pine nut oil is also used in beauty, cosmetic, and spa products.

Nutritional Composition

There is great variation in the nutritional composition of pine nuts with species and subspecies depending on geographic location and climatic conditions. The nutritional profile of some edible pine nuts is presented in Table 2. Pine nuts have a high nutritional value being particularly rich in protein, lipids, and vitamins such as B_1, B_2, K, and E. It also serves as a good source of minerals such as potassium, phosphorus, and magnesium. Zinc, iron, and manganese have also been detected. In *P. pinea* seeds, potassium was identified as the most prominent mineral followed by phosphorus and magnesium. The content of thiamine and riboflavin was high in pine kernels and has only traces of vitamin C. The protein content of some pine nuts (e.g., *P. edulis* and *P. pinea*) exceeds that of all other commercial nuts, except the cashews. Proteins account to about 25% of *P. pinea* seeds on dry weight basis. Among the various edible pine nut species, *P. pinea* seeds have the highest protein content that is comparable to that of beefsteak. Seeds from *P. monophylla*, *P. quadrifolia*, *P. edulis*, and *P. koraiensis* show a protein content of about 9.5, 11.0, 14.3, and 17.0–18.0 g per 100 g dry mass, respectively. Of the piñon pines, *P. cembroides* has the highest protein content and lowest starch content. Protein quality of pine nuts is also high. All 20 of the amino acids are found in the protein of the nuts of both *P. edulis* and *P. monophylla*. Essential amino acids such as lysine, threonine, and tryptophan are also present in the pine nut oil. Carbohydrate content ranges from 2.4 to 5.4% of *P. pinea* seeds. Sucrose (4.3%) is identified as the main sugar component of *P. pinea* seeds.

Table 2 Nutritional composition of some edible pine kernels. Value per 100 g

Nutrient	*Unit*	Pinus pinea *(per 100 g)*	Dried Pinus pinea *and* P. koraiensis[a]
Proximates			
Moisture	g	1.8	2.28
Energy	kcal	2925 (kJ)	673
Protein	g	13	13.69
Total lipid	g	70	68.37
Carbohydrate	g	4.5	13.08
Total fiber	g	5.1	3.7
Total sugars	g	3.4	3.59
Minerals			
Calcium, Ca	mg	11	16
Iron, Fe	mg	4.1	5.53
Magnesium, Mg	mg	230	251
Manganese	mg	6.9	
Phosphorus, P	mg	560	575
Potassium, K	mg	600	597
Sodium, Na	mg	3	2
Sulfur	mg	110	–
Zinc, Zn	mg	5.3	6.45
Vitamins			
Thiamin	mg	0.57	0.364
Riboflavin	mg	0.19	0.227
Niacin	mg	4.3	4.387
Pantothenic acid, B_5	mg	0.48	–
Vitamin B_6	mg	0.02	0.094
Biotin, B_7	μg	1.7	–
Folate	μg	34	34
Vitamin B_{12}	μg		0
Alpha-tocopherol	mg	12	9.33
Gamma-tocopherol	mg	9.4	0
Vitamin A, RAE	μg	–	1
Beta-carotene	μg	10	0
Vitamin C	mg	0	0.8
Vitamin K (phylloquinone)	μg	–	53.9
Lipids			
Total saturated fatty acids	g	4.2	4.899
Total monounsaturated fatty acids	g	22.95	18.764
Total polyunsaturated fatty acids	g	39.8	34.071
Cholesterol	g	0	0

[a]The USDA National Nutrient Database for Standard Reference Release 27. NUTTAB 2010 Online searchable database http://www.foodstandards.gov.au/science/monitoringnutrients/nutrientables/nuttab/Pages/default.aspx.

Total lipid content of various pine species ranges from 23% to as high as 68% (by weight). Oil comprises about 60% of pine nuts. Korean pine nut oil is composed of more than 92% of PUFAs like pinolenic acid (C18:3) and linoleic acid (C18:2) and monounsaturated fatty acids (MUFAs) like oleic acid (C18:1). Approximately 15% Korean pine nut oil is pinolenic acid (C18:3). According to various studies, oil from *P. pinea* is rich in unsaturated fatty acids. Oleic and linoleic acids account for more than 85% of the total fatty acids of *P. pinea*. Saturated oil constitutes about 13% of total fatty acids of *P. pinea*, and the predominant saturated acids were palmitic, stearic, and lignoceric acids with trace amounts of arachidic, myristic, and behenic acids. Triacylglycerols with chain lengths of 44 and 48 were predominant in the oil. The nuts of *P. sibirica* contained about 65% of oil on a dry weight basis. The unsaturated fatty acids accounted for about 90% of the total lipids of *P. sibirica* nut oil. About 66% of *P. sibirica* oil is PUFAs, and palmitic and stearic acids were the predominant saturated fatty acids. *P. koraiensis* contains oleic and linoleic acids as predominant fatty acids. Seed oil of conifers contains considerable amounts of an unusual group of PUFAs, and these PUFAs contain Δ18 or Δ20 carbon atoms with their first double bond located at Δ5 and the second one at either Δ9 or Δ11. In most pine nut species, pinolenic acid is the most predominant Δ5 polymethylene-interrupted PUFA. Its content varies greatly with pine species. The unique nature of Korean pine nut oil is that it contains approximately 15% of pinolenic acid.

Substantial amounts of tocopherols and triacylglycerols were present in the *P. pinea* seeds. The total tocopherol content of the pressed *P. sibirica* oils was 49.6 mg per 100 g oil. Tocopherols were mostly α- and γ-tocopherols. Tocopherols are powerful antioxidants, which can reduce the risks of cardiovascular diseases (CVDs). Pine nuts are good sources of phytosterols. Among various edible nuts, pine nut has very high total phytosterol content. Beta-sitosterol is identified as the predominant phytosterol in most pine nuts, and the European *P. pinea* contain about 429.8 mg per 100 g oil. Research reports suggest that phytosterols can reduce blood cholesterol and risks of certain types of cancers such as colon, breast, and prostate and enhance immune function. Phytosterols can inhibit intestinal absorption of low density lipoproteins (LDL), thus reducing plasma cholesterols and LDL levels. Studies show that pine nut also contains low amount of squalene of about 400 mg/100 g oil. Squalene acts as a biosynthetic precursor for all steroids in plants and is implicated to have important health benefits such as decreasing serum cholesterol levels and the risks of cancer. The total phenolic acid in defatted pine nut kernel was determined as 0.137 mg gallic acid equivalents (GAEs) per 100 g and reported to contain caffeic acid as the predominant phenolic acid. Among commonly consumed tree nuts and peanut, pine nuts contained the lowest total phenolic content of 0.68 mg of GAE per gram fresh sample, and an antioxidant activity of 7.19 μmol of TE per gram fresh sample. Pine nuts do not contain anthocyanins. Studies showed that pine nuts contain 32 μg isoflavones. Also, pine nuts have no detectable proanthocyanidins.

Health Effects

In general, a large body of research is available on the health benefits of nuts, but there has been little information available on the pine nut specifically. A number of investigations have been carried out to study the effect of intake of pine nut oil on plasma lipoprotein levels, and the biological mechanisms involved were studied using the French maritime pine (*Pinus pinaster*). Some of these studies done using animal systems demonstrated the potential benefits of pine nut and its oil in improving the plasma lipid profiles. In a study conducted in 2008, Pasman and coworkers evaluated the appetite suppressant effects of Korean pine nut free fatty acids (FFAs) and triglycerides (TGs) in STC-1 cell culture for their ability to increase cholecystokinin (CCK-8) secretion against several fatty acids from Italian stone pine nut FA. FFAs from Korean pine nuts had the greatest ability to increase CCK-8 release from the STC-1 cells compared to the other fatty acids tested and the negative control. Korean pine nut FFA showed approximately eightfold potency in releasing CCK-8 than the other fatty acids tested. Evidence from this study suggests that pine nut FFA and TG may act as an appetite suppressant in overweight women. A human intervention study performed in 18 overweight postmenopausal women who consumed Korean pine nut FFA, pine nut TG, or placebo (olive oil) in combination with a light breakfast showed enhanced secretion of CCK-8 after pine nut FFA (after 30 min) and pine nut TG (after 60 min) when compared to placebo.

In animal models, Korean pine nut oil improved the activities of superoxide dismutase (SOD) and glutathione peroxidase activities and reduced the content of malondialdehyde in serum indicating its antioxidant activities. Additionally, studies conducted in animal models showed that supplementation of *P. pinaster* oil reduced the levels of TG and very low density lipoprotein in rats, suggesting the potential of *P. pinaster* in lowering the high blood triglyceride levels. Procyanidins from *P. koraiensis* exhibited antitumor activity in animal models of cancer. PinnoThin is an oil preparation from *Pinus koraiensis* that consists of 66% PUFAs like linoleic and pinolenic acids (C18:3) and 26% MUFAs. A study conducted with PinnoThin™ (pine nut oil with high content of pinolenic acid) showed that it increased the release of satiety hormones CCK and glucagon-like peptide 1 and suppressed appetite. No effect was noticed on hunger-inducing factor ghrelin. Further, toxicity study also showed that pine nut oil was safe to use for obesity treatment and not toxic. Another double-blind placebo-controlled randomized study conducted in 42 overweight female volunteers suggested that Korean pine nut oil, PinnoThin™, may exert satiating effects and no effects of PinnoThin TAG on appetite ratings were found. More human intervention studies are required to investigate into the effects of major pine nut components on CVD risk and health.

Traditional Medicinal Uses

Pine seeds have analgesic, antibacterial, and anti-inflammatory properties. Resin and turpentine are obtained from the trunk of stone pine. Turpentine has a variety of uses such as use in medicinal products and for making varnish. The turpentine is antiseptic, diuretic, rubefacient, and vermifuge. It is used externally as a rub in rheumatic conditions and also for the treatment of a variety of skin complaints such as wounds, sores, burns, and boils.

Pine Mouth/Pine Nut Syndrome

Some individuals experience a bitter, metallic taste disturbance usually lasting a few days after consuming pine nuts, which is exacerbated by the consumption of food and drink. This taste disturbance has become referred to as 'pine mouth' or 'pine nut syndrome'; however, medically, it is known as dysgeusia, metallogeusia, or cacogeusia. The symptoms can last from a few days to 2 weeks and the symptoms decreased over time with no adverse clinical effects. Reports from consumers suggest that the taste disturbance is quite pronounced that it significantly decreases appetite and enjoyment of food causing considerable discomfort to the sufferer. Patients also occasionally complained of abdominal cramp and nausea after eating raw, cooked, or processed pine nuts. While pine nuts are potentially allergenic, as such, pine mouth is not considered a food safety issue. There is an increase in the reports of occurrence of pine mouth worldwide. Although the cause of pine mouth is not exactly known yet, mixing of pine nuts that are regarded as inedible with edible nuts may be a contributing factor. Evidences also show that consumption of *Pinus armandii* nut that is not listed among the edible pine nuts by the FAO may lead to dysgeusia.

See also: Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Nuts: Brazil Nuts; Nuts: Health Effects.

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Pineapple

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Introduction

Pineapple, *Ananas comosus* L., is a monocotyledon, belonging to the large genus *Ananas* of the family Bromeliaceae, which includes many ornamental plants. It is an exotic fruit with origins from South America. It is mentioned in the chronicles of Pedro Martyr d'Anghiera in 1530 as being found in a tiny Indian village on the island of Guadeloupe during the second voyage of Christopher Columbus in 1493. The name pineapple is thought to have originated because of the fruit's resemblance to a pine cone.

The pineapple plant is a drought-tolerant herbaceous perennial plant, with a rosette of waxy leaves and a short stem. The pineapple fruit is a compound fruit that develops through the fusion of many individual fruitlets. These small fleshy fruitlets are fused together around the fibrous central stem referred to as the core. Each fruitlet develops from a hermaphrodite flower, which is self-sterile although it has both male and female parts. Self-sterility is advantageous in terms of fruit quality and palatability. Each fruit is borne on a peduncle, which is an extension of the stem of the pineapple plant. Fruits can vary in size. The flesh of the fruit is juicy, has an exotic sweet flavor, and can be pale cream to yellow in color. The top of the fruit is known as the crown. It is an extension of the apical meristem and can be used as planting material.

Sources and Production

Pineapple is known to have originated in the tropical Americas, where the fruit was a stable component of the Native American diet long before it was discovered by European explorers. It was also used for the production of alcoholic beverages, for medicinal purposes, and for the production of fiber. It was apparent to the explorers that considerable selection had taken place with regard to domesticated pineapples. The American Indians readily distinguished land types from wild types and developed agronomic practices based on the production cycle of the fruit. By the end of the sixteenth century, increasing popularity and interest in this exotic commodity in the West resulted in the introduction of pineapples to many parts of the world including Asia, Africa, and the South Pacific colonies by Spanish and Portuguese explorers.

Commercial production of pineapple began in the nineteenth century in the West Indies, Brazil, and Mexico. Prior to the First World War, pineapples were supplied to North America by Cuba, the Bahamas, Florida, and Puerto Rico, while Europe was supplied by the Azores. However, production shifted to other regions in Africa and Asia after the Second World War. With the development of processing technology, production expanded in Hawaii, Malaysia, Taiwan, the Philippines, Australia, South Africa, the Caribbean, and Kenya. The emergence of improvements in refrigeration in the mid-twentieth century and the launching of refrigerated reefer containers facilitated sea shipment, opened new markets, and created an increased demand for the fresh fruit.

Pineapple contributes to over 20% of the tropical fruit produced in the world. Large-scale production is managed by multinational companies operating in several pineapple-producing countries. As such, it is considered to be the third most important tropical fruit crop after banana and citrus. The total world production of pineapple was estimated as amounting to 23.33 million MT in 2012. While 85 countries are listed in FAO records as pineapple-producing countries, Thailand, Costa Rica, Brazil, the Philippines, and Indonesia together produce over 66% of the total. Thailand, the Philippines, Indonesia, and Malaysia also account for over 75% of canned pineapple exports and 55% of the juice concentrate exports. FAO records indicate that 70% of the global production is consumed in pineapple-producing countries. Costa Rica is the largest exporter of fresh pineapples, while the United States and the European Union (EU) together account for 88% of imports. Supermarkets are the main outlets for pineapple sales, in most importing countries although they are also sold through greengroceries, street markets, and catering outlets. Large supermarkets are supplied by large agro-multinationals. However, many other importers in Europe, North America, Japan, and Singapore are also active in the pineapple trade.

Pineapples are cultivated on a range of different aerated and well-drained soils with soil pH ranging from 4.5 to 5.5. The plant is a xerophyte and is adapted to withstand periods of drought. The leaves of the plant are covered with trichomes and equipped with small stomata situated in furrows under the surface. Leaves also have a highly cutinized upper dermis. However, despite these characteristics, the plant is adversely affected by prolonged periods of drought. On average, pineapple plants need 1–1.5 m of rain per year. The optimum temperature for vegetative growth of the plant is between 28 and 32 °C although varieties are known to differ in response to climatic conditions. Prolonged exposure to low temperatures slows down vegetative growth in most instances.

Mature fruits ready for harvest and fruits reaching maturation develop postharvest physiological disorders when exposed to continuous nighttime temperatures below 18 °C. While exposure to low temperatures induces a high degree of flowering in some varieties, flowering is artificially induced in commercial plantations at an appropriate stage of development to meet quality requirements of respective markets and facilitate harvest and distribution operations (Figure 2). Cultivars such as the Queen-type Mauritius variety respond better to shade and are successfully grown as an intercrop in coconut plantations (Figure 1). Other varieties such as the Cayenne-type Kew pineapples show low Brix values when grown in the shade.

Dry leaves, coir dust, or straw can be used as a mulching material. Mulching with black polythene or saw dust has also

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Figure 1 Queen-type Mauritius variety pineapple plantation intercropped with coconut.

Figure 2 Queen-type Mauritius variety pineapple fruit at early stage of development following flower induction.

been found to be very effective. Mulching is practiced in many plantations to prevent the growth of weeds around the plant. Mulching also reduces soil erosion and water loss and helps regulate soil temperature. The maturing fruits may be covered with rice straw or pineapple leaves in order to reduce both sunburn and damage caused by birds.

Pineapples are cultivated as a ratoon crop, where replanting occurs usually after the second ratoon crop is harvested. Crowns, stem shoots or suckers (aerial and terrestrial), and slips from the peduncle are used as planting material. Crowns are used in most commercial plantations where the fruit is grown for processing purposes and harvested 18–24 months after planting. Slips are harvested at 20–22 months and tissue-cultured plants at 15–18 months after planting. Many smallholders prefer to plant ground and stem suckers, which can be harvested between 12 and 14 months and 14–16 months, respectively. Planting material must be selected according to size to ensure uniform development of plants.

Cultivars selected for commercial production may be classified into five distinct groups based on plant characteristics, fruitlets, fruit shape, fruit flesh, number and size of bracts, and leaf morphology. These groups include Cayenne, Spanish, Queen, Abacaxi (Pernambuco), and Maipure (Perolera). The Smooth Cayenne is thought to have originated in India and has smooth leaves with spines limited to the tip of the leaf compared with the Queen types, which have numerous spines distributed along the entire edge of the leaf. Cayenne group cultivars continue to be the most widely grown commercial variety. They are grown for processing purposes as they are cylindrical in shape, juicy, and low in fiber with a sweet to mildly acidic flavor and are less prone to chilling injury. However, the less acidic and sweeter flavored hybrid variety MD2 is also resistant to chilling injury and has become the dominant variety in European markets. Other Queen-type pineapples with sweet flavor are also becoming increasingly popular in the fresh fruit market, despite the latter group being particularly susceptible to a complex physiological disorder referred to as internal brown spot (IBS) or endogenous brown spot.

There are a number of climatic, production, and postharvest conditions that contribute to the development of this disorder. While crops grown under low sunlight conditions and exposed to cool temperatures either in the field or during storage develop the characteristic browning symptoms, pineapple cultivars with high Brix and low titratable acidity are more prone to develop the disorder. Stress conditions also predispose fruit to IBS, for example, where extremes in water availability occur with the commencement of heavy rains following the end of the dry season. Thus, more incidence of the disorder occurs in fruit harvested during the rainy season due to the low sunlight, cool temperatures, and high water availability. Increased incidence of the disorder also occurs when flower induction is practiced during wet periods.

Patterns of Consumption

Fresh and processed pineapples are appreciated and consumed by people of all ages in producing and importing countries alike. Fresh pineapples are a common product in many ethnic and urban supermarkets the world over. Although consumption levels of the fresh fruit continue to be higher in high-income Western markets, consumption trends are expected to grow in low-income European countries as they follow the consumption trends of their more affluent neighbors. Meanwhile, processed pineapples are exported for convenient consumption as canned or frozen concentrates and IQF frozen chunks, rings, or quarters. While pineapple juice is incorporated into tropical juices and concentrate blends, chunks are included in fruit salads. Pineapples are also consumed as dehydrated products and often incorporated into trail mix snacks.

Nearly 80% of the world pineapple production output continues to be utilized by the processing industry, with the bulk of the world production being used by the canning industry. Among canned fruits, pineapples are important next only to peaches. In contrast to the foremost pineapple-producing countries, where over 90% of the production is absorbed by the processing industry, the major portion of the pineapple produced in India and many other developing countries is for consumption in fresh form with less than 10% of the production being used by processing industries.

Major constraints faced by the pineapple-processing industries in these countries include

(a) high cost of canning due to the high cost of raw materials, containers, and overheads;
(b) nonavailability of a continuous supply of fruits throughout the year.

Marketing of fresh pineapple also poses problems due to its highly perishable nature. Mature pineapple fruits cannot be stored for more than 4–5 days after harvest under warm humid ambient environmental conditions.

The international trade in fresh fruits is limited although an increasing demand for fresh fruit has been observed in the major export markets in recent years. The United States is the largest single consumer nation of fresh pineapple, importing over 29% of the world's production with per capita consumption of pineapple estimated at 2.5 kg. In the EU, the market for pineapples is observed to be growing. Germany, Italy, and the United Kingdom are the largest EU markets, with potential for further market growth in the new EU countries. Belgium and the Netherlands are the leading importers for distribution of pineapples in the EU.

There is also an increasing demand for organic pineapples and pineapple products. However, trends in the supply and demand of fresh and processed organic pineapple are difficult to assess due to the lack of official statistics and the resulting reliance on anecdotal information. Organic producers and processors catering to international markets need to obtain certification for their respective products from internationally recognized organic certification bodies and need to meet prescribed standards set by them.

Globalization and the growing consumer quest for a healthier lifestyle are important drivers for exotic fresh fruit consumption and have contributed to the increased demand for pineapple in international markets. While the Cayenne-type pineapples respond better to low-temperature storage and transportation, the current overall preference is for a less acidic, sweet, and juicy fresh pineapple. It is also noted that pineapple is not the most convenient of fruits to handle or to convert into consumable portions. This obstacle to consumer preference is overcome by some retailers who offer precut and ready-to-eat fresh pineapple products. The introduction of the popular baby pineapple into many international markets also meets the demand for smaller portions and single servings. Pineapple juices are subjected to fermentation procedures and used to produce wine or alcohol and are also used to extract citric acid.

Availability Absorption and Metabolism

Fruit crops available for human consumption are estimated in trade and socioeconomic reports as a measure of supplies of the crop moving from production to marketing channels for domestic human consumption. These estimates are often used in food availability databases as a popular proxy for actual fruit consumption. However, information on the availability of beneficial nutrients in individual fruit crops is important to dietitians and nutritionists.

On average, pineapples are estimated to have close to 50 calories per 100 g of weight. The fruit is rich in the essential vitamins A, B, C, E, and K, beta-carotene, folate, and pantothenic acid. One cup (US) of raw pineapple can contain 31% of the daily requirement of vitamin C. It is also a useful source of minerals such as iron, calcium, copper, potassium, and magnesium and an excellent source of manganese as one cup serving (US) of raw pineapple can contain up to 76% of the daily value requirement of this mineral. Pineapples are also rich in dietary fiber and low in calories and contain no saturated fat.

The sugar content changes from 4% to 15% during the final stages of fruit ripening and in some cultivars rise to 18%. The sugar–acid ratio and the ascorbic acid content could also vary with cultivar.

Bromelain present in pineapples is associated with many health benefits and contains a collection of protein-digesting enzymes including proteinases, peroxidase, and acid phosphatase, as well as several protease inhibitors. Bromelain is absorbed in the gastrointestinal tract. Significant amounts of bromelain can be absorbed by the human body without ill effects. Bromelain contains organically bound calcium, which remains stable over a pH range of 2–9 and is considered to be readily absorbed orally. It is used as a phytomedical compound. Though it is present in the fruit, pineapple stems contain higher concentrations of bromelain, and as stems are a waste by product, they prove to be a cheaper source of the substance for commercial extraction. Fruit bromelain and stem bromelain are prepared differently and are of different enzymatic properties.

Pineapples are known to contain bioactive compounds such as alkaloids, flavanoids, and tannins, which are present in both pineapple flesh and skin. Nutrients available in this fruit are listed in Table 1.

Health Effects

Fruits, nuts, and vegetables play an important role in providing the nutrients essential to humans and must be incorporated into the daily diets of men, women, and children. It is currently estimated that over three billion people lack adequate micronutrients in their diets. Lack of these nutrients increases morbidity and mortality rates, results in lower worker productivity, and causes learning disabilities in children. This also results in increased healthcare costs, diminishes human potential, and affects overall national economic development. Among tropical fruits, many health benefits are associated with pineapples. Per the US Food and Drug Administration, the nutrient content descriptors approved for pineapples are fat-free, saturated fat-free, very low sodium, cholesterol-free, and high in vitamin C. The fruit contains calcium, potassium, and magnesium. It is a good source of vitamins B1 and B6, copper, and dietary fibers. Pineapple has an abundance of anti-inflammatory properties. The juice is taken as a diuretic, to expedite labor, and as an antidote for sea sickness.

Pineapples as a Source of Vitamins

Pineapples are a useful source of vitamins. A cup of pineapple chunks can contain up to 28 milligrams of vitamin C, which is

Table 1 Nutritional value and calories in pineapple[a]

Nutrients – basic components	*Amount*
Proteins	891 mg
Water	142 g
Ash	0.4 g
Phytosterols	9.9 mg
Calories	
Total calories	83
Calories from carbohydrates	78
Calories from fats	1.7
Calories from proteins	3
Carbohydrates	
Total carbohydrates	22 g
Dietary fiber	2.3 g
Sugar	16 g
Fats and fatty acids	
Total fat	198 mg
Saturated fat	15 mg
Monounsaturated fat	21 mg
Polyunsaturated fat	66 mg
Omega-3 fatty acids	28 mg
Omega-6 fatty acids	38 mg
Vitamins	
Vitamin A	96 IU
Vitamin C	79 mg
Vitamin E	33 mcg
Vitamin K	1.2 mcg
Thiamine	130 mcg
Riboflavin	53 mcg
Niacin	825 mcg
Vitamin B6	185 mcg
Folate	30 mcg
Pantothenic acid	351 mcg
Choline	9.1 mg
Betaine	0.2 mg
Minerals	
Calcium	21 mg
Iron	479 mcg
Magnesium	20 mg
Phosphorus	13 mg
Potassium	180 mg
Sodium	1.7 mg
Zinc	198 mcg
Copper	182 mcg
Manganese	1.5 mg
Selenium	0.17 mcg

[a]http://nutrition.indobase.com/articles/pineapple-nutrition.php – Pineapple nutrition facts.
Amount: 1 cup; weight: 165 g.

31% of the recommended daily vitamin C intake for men and 38% for women. The water-soluble antioxidant properties of vitamin C help prevent scurvy, increase immunity, and build resistance against infectious disease. Vitamin C is a useful free radical scavenger and helps remove harmful free radicals from the body. Vitamin C is also needed for the production of collagen, which is the major structural protein required in the human body for maintaining the integrity of blood vessels, skin, organs, and bones. Vitamin C present in pineapples also contributes to oral health and helps with the prevention of gum disease. Vitamin C reduces the risk of colon, esophagus, and stomach cancer. Vitamin A and beta-carotene, though present in smaller quantities, also have antioxidant properties and are required for good vision and retarding age-related macular degeneration, healthy skin, and maintaining mucus membranes. Pineapple is also a good source of thiamine. This component of the vitamin B complex acts as a cofactor in enzymatic reactions central to energy production and red blood cell formulation. Antioxidant-rich foods in the diet can improve fertility. The bioavailability of nutrients is the proportion of a nutrient that is absorbed from the diet and used for normal body functions. Both external and internal factors govern the bioavailability of the nutrient. External factors include the food matrix and the chemical form of the nutrient in question, whereas gender, age, nutrient status, and life stage are among the internal factors. Ascorbic acid and S-containing amino acids present in pineapple are agents that promote micronutrient availability in the human body, while polyphenolics and phytates are known to inhibit micronutrient bioavailability.

Bromelain

Bromelain belongs to a group of protein-digesting enzymes that attack the internal peptide bonds of the protein chain. While the fruit bromelain, which is an acidic protein, is present in modest amounts in the fruit, the bromelain widely used as a food supplement is extracted from the pineapple stem. It is a mixture of different thiol endopeptidases and other components such as phosphatase, glucosidase, peroxidase, cellulase, escharase, and several protease inhibitors. Bromelain is readily absorbed by the body in the gastrointestinal tract and remains functionally intact.

Bromelain enhances the absorption of drugs, particularly antibiotics. *In vitro* and *in vivo* studies demonstrate that bromelain exhibits various fibrinolytic, antiedematous, and antithrombotic activities. Bromelain has anti-inflammatory properties and is used to prevent postsurgical inflammation and trauma.

It is used in the treatment of arthritic patients, for pain relief for those suffering from osteoarthritis and joint injury. It is able to dissolve mucus and is used in the treatment of bronchitis and sinusitis. Bromelain supplement is used to reduce the incidence of various cardiovascular disorders as it is an inhibitor of blood platelet aggregation. It prevents the coagulation of blood and is used to combat thrombophlebitis and to minimize the severity of angina pectoris and transient ischemic attack.

It is effective in curing burn wounds where the formation of layers of burned and traumatized tissue if not removed will serve as an infection site and a medium for growth of bacteria. In such instances, bromelain is administered to remove the dead and infected tissue and bring about effective healing of the wounds.

Bromelain contains proteolytic enzymes called cysteine proteinases, which break down proteins and help digest food. It is also used to combat diarrhea and is thought to counteract some of the effects of intestinal pathogens such as *Vibrio cholera* and *Escherichia coli*.

Bromelain also possesses some anticancerous activities and is known to prevent the growth of certain types of tumors as it promotes apoptotic cell death.

Manganese and Copper

One cup of pineapples contains 2.6 mg of manganese, which covers the entire recommended daily intake of this mineral. It is instrumental in the creation and activation of certain enzymes. It activates clotting factors and helps prevent excessive bleeding. This trace mineral is an important cofactor in many enzymes that are involved in energy production. It thus helps the body convert nutrients such as fat and carbohydrates into usable energy. It is also a necessary cofactor in antioxidant defense enzymes as with the oxidative enzyme superoxide dismutase, which disarms harmful free radicals produced within mitochondria. Manganese is also essential for the development of strong bones and connective tissue. Copper present in pineapples helps with absorption of iron and regulates blood pressure and heart rate.

Fiber

A medium-sized pineapple can provide up to 13 g of fiber. Individuals with type 1 diabetes and who consume high-fiber diets have lower blood glucose levels, while type 2 diabetics show improved levels of blood sugar, lipids, and insulin. The fiber present in pineapple is effective in curing constipation and irregular bowel movements. Consumption of the fruit is known to make bowel movements easy and regular. Pineapples can contribute to high-fiber intake, which is associated with a lowered risk of colon cancer. Pineapple fiber has many other uses. It is extracted from leaves and used in the manufacture of thread, fabrics, and fishing nets and for stringing jewels.

In Africa, the dried, powdered root is used to treat edema and the crushed rind is applied on fractures. Corns and calluses on the feet or rough skin on elbows and heels may be treated with the fruit pulp as follows – repeated application of the pulp to the affected area for an hour, followed by rinsing off the pulp and gently scrubbing the affected area.

The flesh of immature fruits is toxic and can cause severe diarrhea and vomiting. Excessive consumption of pineapple could cause swelling and tenderness of lips. Consumption of unripe fruit can cause throat irritation and drastic bowel discharge. In some cultures, the toxic flesh of very young fruits is ingested to expel intestinal worms and achieve abortion. Excessive consumption of pineapple cores can cause fiber balls to develop in the digestive tract.

See also: Antioxidants: Role on Health and Prevention; Ascorbic Acid: Physiology and Health Effects; Ascorbic Acid: Properties, Determination and Uses; Bioavailability of Nutrients; Fruits of Tropical Climates: Biodiversity and Dietary Importance.

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Pitaya (*Stenocereus* spp.)

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Definition

Pitaya (*Stenocereus* spp.) (Figure 1) is a fruit that belongs to the Cactaceae family (Table 1). It is a crop of paramount importance for the inhabitants of semiarid zones of Mexico, mainly because of its nutrimental, therapeutic, and decorative value. The columnar cactus producing this fruit, named pitayo, is from the tribe Pachycereae and subfamily Cactoideae, with Stenocereae being the most important subtribe and *Stenocereus* the most relevant genus.

Cactaceae are prosperous crops in arid and semiarid regions due to their low water requirements and the good use of the natural resources. Moreover, the ancient populations of these areas, mainly in Mexico and Southern parts of the United States, have traditionally selected cacti according to their usefulness; that is, it has been used as biomass, as a source of water, as building material, as a source of carbohydrate and as decorative plants. The use of water by pitayos is five to ten times more efficient compared with regular crops. This characteristic is due to its CAM metabolism (*Crassulaceae*-type metabolism), based on the optimal opening of stoma (overnight) for CO_2 fixation when respiration is low. The thickness of the plant skin (14 μm) is another adaptation for pitayo's survival in arid and semiarid regions, allowing the plant to cope with long periods of drought.

In Mexico, the predominant Cactaceae with fruits are garambullo (*Myrtillocactus geometrizans* – Martius Console), nopal (cactus pear, *Opuntia* spp.), and pitayo (*Stenocereus* spp.). The genus *Stenocereus* (from the Greek words 'stenos,' meaning narrow, and 'cereus,' meaning candle) includes a heterogeneous group of cacti found from Southern Arizona, the Baja California peninsula and other parts of Mexico, Costa Rica, Guatemala, to northern Colombia and Venezuela. *Stenocereus* are creeping, group-forming, and treelike cacti. Stems of the *Stenocereus* cacti are cylindrical and often show numerous ribs. This plant yields funnel-shaped flowers, typical from various Cactaceae. *Stenocereus* includes at least 24 species distributed from the southern part of the United States to Peru and Venezuela. In Mexico, at least 19 species are found throughout the country (Figure 2). *S. thurberi* grows in the Baja California peninsula, in Sonora, and in some areas of Sinaloa; *S. montanus* flourishes from Sonora to Nayarit; *S. queretaroensis* is found in Jalisco, Michoacán, Guanajuato, and Queréraro; *S. quevedonis* flourishes from Sinaloa to Guerrero (Pacific coast); *S. griseus* is found not only within the Mixteca region (Oaxaca, Guerrero, and Puebla) but also in the states of Tamaulipas and San Luis Potosí; *S. dumortieri* is found in Hidalgo, Morelos, Puebla, Oaxaca, and Chiapas; *S. weberi* can be found in Puebla, Oaxaca, and Guerrero; *S. stellatus* can be found in Morelos, Puebla, and Oaxaca. In Mexico, the most important cultivated *Stenocereus* species are *S. queretaroensis*, *S. griseus*, *S. stellatus*, and *S. fricii*.

Despite the wide geographic area of pitaya's production, the most important zones for pitaya production are the Sayula Basin (Jalisco, Zacatecas, Guanajuato, Michoacán, and Querétaro) and the Mixteca (between the states of Oaxaca and Puebla) (Figure 2).

The common name for *S. griseus* is pitaya de mayo, while *S. queretaroensis* and *S. stellatus* are known as pitaya de Queretaro and pitaya agria, respectively. Pitaya is known locally by various colorful common names such as amarilla (yellow), espina negra (black spine), cántaro (pitcher), Acateca, borrega (sheep), hormiga (ant), pitaya de flor o chica (flower pitaya or small), agria (sour) or espina amarilla (yellow spine), Copal, Pitaya Señora (Mistress Pitaya), Pitaya Xoconostle, and cantarito (little pitcher).

Sources, Production, and Agronomical Aspects

Pitayo requires few agronomical inputs. In fact, most of the production in America is based on harvesting wild plants (as opposed to an intensive and controlled farming process). *Stenocereus* grows well in slightly acidic and sandy soils, but it can also grow well in stony ground and soils where no other crops can prosper. The optimum temperature for its growth is in a range from 10 to 34 °C, with an average of 22 °C in the Sayula Basin.

The average rainfall in the pitaya production area in Mexico is 700 mm, mostly in summer. The flowers and fruit of pitaya reach their development during late winter and spring, when rains are scarce, and mature from May to August. The production of flowers and fruits starts between the first and third year after plantation, but in order to obtain a large production for commercial viability, the plant requires 10 years of development. Pitayo's lifetime production is from 25 to 30 years, and for *S. stellatus*, there are some documented cases with 100 years of production.

Fertilizers are almost never applied, and although irrigation can increase fruit production, this practice is not used regularly in Mexico. Trimming to control plant height is not practiced either, even when this is recommended to reduce harvesting time.

Most of the pitaya produced in Mexico is wild type, although some varieties are selected and grown in family backyards for decorative purposes, and the practice helps to enhance the genetic diversity.

Compared with other fruits, pitaya is not very productive. The average yield per hectare is 3.44 metric tons (mt), while primary production of fruit worldwide is 27 mt ha^{-1} and 7.23 for prickly pear, the most similar cactus fruit. There is no information available about worldwide pitaya production, but in Mexico, according to SINAREFI, pitaya crop has increased steadily from 448 mt in 2000 to 3428 mt in 2010 (Table 2).

Yield, in terms of number of pitaya fruits per pitayo plant and also average weight, varies widely according to the *Stenocereus* species or variety (Table 3).

The annual harvest for a middle-age pitayo plant ranges from 45 to 98 fruits per plant and their weight from 89 to 165 g. Fruits

Figure 1 Pitaya fruit and pitayo cacti.

Table 1 Classification of *pitaya* (*Stenocereus* spp.)

Kingdom	*Plantae*
Division	Magnoliophyta
Class	Magnoliopsida
Order	Caryophyllales
Family	Cactaceae
Genus	*Stenocereus* (pitaya)
Species	Griseus, queretaroensis, stellatus, and fricii (as the most important species)

Figure 2 Main areas for pitaya production in Mexico. Areas cross hatched are regions with traditional wild fruit harvest and dappled regions are areas with pitaya commercial production. Adapted from Pimienta-Barrios and Nobel (1994).

of *S. griseus* are larger than those of *S. queretaroensis*, generally from 200 to 350 g; nevertheless, this depends on the genetic selection made with traditional propagation.

The yield of pitaya increases with the plant's age, because of the increase in number and length of stems along which fruits are borne. When a pitayo is about 20 years old, the production can reach up to 17 mt ha^{-1}.

Physicochemical Characteristics

The pitaya fruit consists of three main anatomical parts: pulp (in its interior chamber); seeds, located within the pulp; and high-fiber skin or peel (Figure 3). The pulp is very aromatic, juicy, and sugary. Pulp color can be white, pinkish, yellow, red, or purplish. As discussed before, the fruit weight varies widely; in a mature pitaya, it could be anything between 69.3 and 148.3 g (*S. stellatus*), of which 53.9–111.8 g would be pulp, 15.3–36.5 g would be skin, and 1.63–3.42 g would be seeds. Compared with cactus/prickly pears (tuna), a larger fraction of the pitaya fruit is edible. The peel of pitaya represents 18% to 24% of the fruit weight, whereas in the tuna, it is up to 45%. Furthermore, in the case of tuna or cactus pear, the seeds (4%) are usually not eaten, while pitaya seeds (1.6–3.4% of fruit weight) are chewed and digested.

Regarding the geometry of the fruit, pitaya is almost spherical, with transverse and longitudinal diameters of 4.4–6.2 and 4.4–6.8 cm, respectively. Based on our experimental data over *S. griseus* specimens, the ratio for transverse and longitudinal diameters was 0.79–0.81, the volume 97–119 cm^3, and density 1015–1019 kg m^{-3} (Table 4).

Skin or peel texture or resistance (Table 4; Figure 4) and color are also a distinctive trait. Color can range from green to yellow as the fruit develops, and it can be described as reddish when the pitaya matures (Figure 5).

If a color index is calculated with the three parameters of a lab color scale ($color\ index = (a \times 1000) \times (L \times b)^{-1}$), differential values can be obtained, and if a value ranges between +2 and +20, it describes a pitaya from pale yellow to orange, whereas values >+20 are related to intense orange to red.

The chemical characteristics of pitaya are shown in Table 5 for fifteen varieties or species as reported by different authors. Acidity and pH are related traits, ranging from 0.09 to 0.64 for the former and from 3.95 to 5.20 for the latter. This is a very wide range of pH, higher compared with apples (3.3–3.9) and blueberries (3.1–3.4); similar to pear (3.5–4.6), cherries (3.2–4.5), and bananas (4.5–5.2); and lower than watermelon (5.2–5.6). For prickly pear, also a cactus fruit, pH ranges from 5.3 to 7.1, higher than pitaya.

According to this characteristic, some pitaya varieties can be classified as low acidic because their pH is higher than 4.5,

Table 2 Pitaya production in Mexico (metric tons)

Year	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Pitaya production (mt)	448.0	748.5	1803.9	1947.5	1680.3	2595.5	2536.0	2941.9	3275.1	3270.9	3428.3

SINAREFI, available at: http://www.sinarefi.org.mx/redes/red_pitaya.html.

Table 3 Number of fruits per plant for five principal commercial varieties of *S. queretaroensis*

Variety	*Pulp color*	*Number of pitaya per plant*	*Mean fresh fruit weight (g)*
Amarilla	Yellowish	68	97
Blanca	Whitish	45	95
Mamey	Dark red	93	165
Morada	Purple	90	89
Roja	Red	98	162

Source: Pimienta and Tomas (1993).

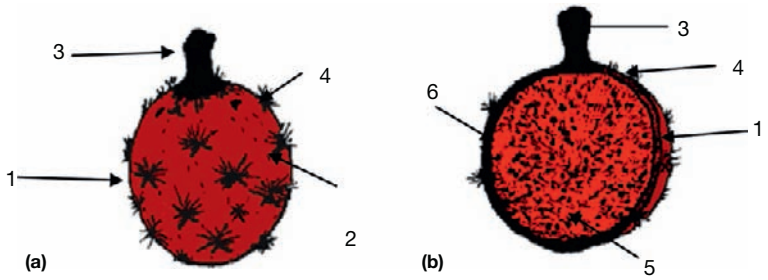

Figure 3 The anatomical parts of pitaya, fruit of *Stenocereus stellatus*. (a) Whole fruit. (b) Longitudinal cut: (1) pericarpel (peel), (2) scales, (3) parts of flower (androecium, gynoecium, and perianth), dry and adhered on fruit, (4) spines, (5) seeds, and (6) pulp. Reproduced from Cruz-Hernández (1985).

Table 4 Some physical parameters for two different batches of *S. griseus* collected in the municipalities of Ahuatlán and Santa Clara Huitziltepec, Puebla, Mexico[a]

Batch no.	*Weight (g)*	*Long. diameter (mm)*	*Transverse diameter (mm)*	*Ratio of diameters (sphericity index, %)*	*Volume (cm^3)*	*Density ($kg\ m^{-3}$)*	*Skin resistance (N)*	*Pulp resistance (N)*
1	98.6 ± 22.5	50 ± 5	62 ± 5	81.1 ± 5.6	97 ± 22	1015 ± 17	2.2 ± 1.6	0.6 ± 0.6
2	120.9 ± 31.9	54 ± 6	68 ± 6	79.9 ± 5.5	119 ± 32	1019 ± 27	–	–

[a]Values are the average of at least three samples ± standard deviation.
Source: Mayorga-Martínez, not published yet.

Figure 4 Texture determination for pitaya fruits (*S. griseus*) from Santa Clara Huitziltepec Puebla, Mexico.

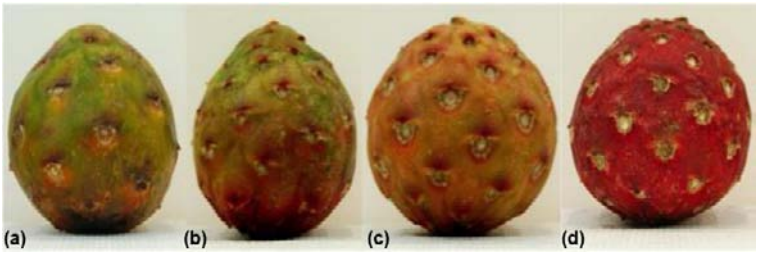

Figure 5 Color index for different pitaya specimens (*S. griseus*): (a) −2.91, (b) −0.65, (c) +5.48, and (d) +48.25. Reproduced from Mayorga-Martínez, not published yet.

Table 5 Chemical characteristics of the pulp of different varieties of *S. queretaroensis*[a] (1–5), *S. griseus*[b,c] (6–8), *S. stellatus*[c,d] (9–13), and *S. pruinosus*[e] (14 and 15)

No.	*Variety*	*pH*	*Acidity ([f]malic acid and [g]citric acid %)*	*Total sugars (%)*	*Reducing sugars (%)*
1	Amarilla	3.90	0.50[f]	11.0	10.0
2	Blanca	4.70	0.18[f]	11.0	11.0
3	Mamey	5.00	0.15[f]	10.0	9.0
4	Morada	4.60	0.29[f]	11.0	10.0
5	Roja	4.90	0.17[f]	10.0	10.0
6	Ahuatlán municipality	4.96	0.11[g]	8.8[h]	–
7	Santa Clara Huitziltepec municipality	4.97	0.09[g]	8.9[h]	–
8	*S. griseus*	5.20	0.12[g]	12.2[h]	8.6
9	Roja	4.20	0.53[g]	9.2[h]	10.1
10	Blanco	4.40	0.39[g]	10.0[h]	11.2
11	Amarillo	4.46	0.50[g]	9.1[h]	10.2
12	Solferina	3.70	0.47[g]	9.0[h]	9.6
13	*S. stellatus*	3.95	0.64[g]	9.1[h]	8.1
14	Red	–	0.17[f]	9.3[h]	
15	Orange		0.13[f]	10.3[h]	

[a]Pimienta and Tomas (1993).
[b]Mayorga-Martínez, not published yet.
[c]Mercado and Granados (2002).
[d]Cruz-Hernández (1985).
[e]García-Cruz et al. (2013).
[f]Malic acid, %.
[g]Citric acid, %
[h]Expressed as total soluble solids (°Brix).

yielding important considerations to make during processing, mainly in thermal-related operations as pasteurization.

Another important characteristic is its sugar concentration. Similar to other fruits, the main sugars in pitaya are fructose and glucose, with a total content of 9.9% on average. Other fruits with similar sugar content are prickly pear fruit, 10%; pear, 10%; apricot, 9.3%; kiwi fruit, 10.5%; and orange, 9.2%. This percentage (almost 10%) of sugars is higher than strawberry (6%), but lower than other fruits as apples (13.3%) and banana (15.6%).

In the case of *S. stellatus*, a total sugar content of up to 15.92% has been reported (Table 6). This species is also one of the most acidic, so as in other fruits, the acidity/sugar ratio is closely related.

Table 6 shows that the sugar content in pitaya *S. stellatus* ranges from 12% to almost 16%. Cultivars of *S. griseus* typically

Table 6 Total sugar content and composition for four pitaya varieties from *S. stellatus* species (common name: xoconostle)

Sugars (%) (moisture basis)	*Variety (*S. stellatus*)*			
	Roja	*Blanca*	*Amarilla*	*Solferina*
Glucose	7.02	6.89	6.93	7.04
Fructose	3.10	4.26	3.29	2.52
Sucrose	2.44	4.76	3.84	3.12
Total reducing sugars	10.13	11.16	10.22	9.56
Total sugars (reducing + nonreducing)	12.58	15.92	14.07	12.68

exhibit contents around 10–11 expressed as percentage of fresh weight. As Table 5 shows, most of the sugars are reducing (glucose and fructose), more readily digested than nonreducing ones. Sugar content of 10–14% is generally considered acceptable for fruits that are eaten raw.

Table 7 shows the approximate composition of different pitaya varieties. Protein ranges from 1.0 to 1.7 and crude fat and fiber from 0.1 to 0.3 and 0.25 to 6.1, respectively. Protein is somehow higher in pitaya compared with fruits as apples, cherries, and prickly pear (0.2, 1.0, and 0.7, respectively), and this can be related to the high content of protein of the pitaya seeds. Regarding fat and fiber, Table 7 shows similar content to other fruits, particularly to prickly pear (0.51 and 3.6 for fat and fiber, respectively).

The moisture content of pitaya is between 85% and 89%, similar to apples and cherries (86%) and prickly pear (88%).

The vitamin C content in pitaya ranges from 8.49 to 17.04 mg/100 g of fresh pulp in *S. stellatus* and up to 21.7 mg for *S. griseus*. Oranges, however, contain approximately 59 mg/100 g fruit. According to the National Nutrient Database for Standard Reference (USDA), the vitamin C concentration in cherries is only 10 mg/100 g, in apples 4.6 mg/100 g, and in prickly pear 14.0 mg/100 g of fruit.

No data regarding concentrations of other vitamins were found in the literature, except for niacin: 0.47 mg/100 g, fairly similar to oranges (0.425 mg/100 g), cherries (0.400 mg), and prickly pears (0.460 mg).

Health-Related Properties: Antioxidant Activity and Nutraceuticals Within Pitaya (*Stenocereus* spp.)

Data available for the phenolic content and antioxidant activity of pitaya's cultivars show the importance of this crop as a

Table 7 Approximate analysis of different pitaya varieties

Content[a]	*Olla*[b] (S. griseus)	*Jarra*[b] (S. griseus)	*Ceniza*[b] (S. griseus)	*Nonidentified variety* (S. griseus)[c]	*Nonidentified variety* (S. stellatus)[c]	*Red variety* (S. pruinosus)[e]	*Orange variety* (S. pruinosus)[e]
Protein	1.6	1.6	1.7	1.3	1.0	1.3	1.2
Carbohydrates	5.0	6.7	4.6	9.0	11.7	10.2	8.5
Crude fat	0.2	0.1	0.2	0.1	0.3	0.1	0.1
Crude fiber	5.64	4.37	6.10	3.29	0.25	0.67	0.53
Moisture	86.5	86.5	85.8	85.8	86.3	87.1	89.0
Ash [d]	1.08	0.74	1.66	0.46	0.48	0.63	0.61

[a]Expressed as grams for 100 g of fresh pitaya pulp (moisture basis).
[b]Armella et al. (2003). Samples obtained in San Juan Joluxtla Oaxaca, México.
[c]Mercado and Granados (2002).
[d]Calculated for the first three samples as: 100 − (protein + carbohydrates + crude fat + crude fiber + moisture), because it was not reported in the reference.
[e]García-Cruz et al. (2013).

Table 8 Total phenolic content in *Stenocereus* fruit

Stenocereus *species*	*Variety*	*mg gallic acid eq./100 g dry sample*
S. stellatus[a]	Red	1384.3 ± 10.4
	Cherry	1552.2 ± 37.0
	Yellow	2129.1 ± 5.5
	White	2395.3 ± 42.6
S. griseus[b]	Red	1482.37 ± 14.59
	Yellow	1704.85 ± 3.82
	Orange	2006.17 ± 11.32

[a]Concentration based upon gallic acid as standard (Beltrán-Orozco et al., 2009).
[b]Ayala-Camarillo et al. (2008).

Table 9 Total soluble phenolic content in two different *Stenocereus* species: *S. pruinosus* and *S. griseus*

Species	*Variety*	*Total soluble phenolic content*
S. pruinosus	Red	1.60 ± 0.20[a]
	Orange	1.20 ± 0.20[a]
S. griseus	Red	1.66 ± 0.14[b]
	Orange	0.52 ± 0.03[b]

[a]Gallic acid eq. mg/g fresh sample (García-Cruz et al., 2013).
[b]Gallic acid eq. mg/g dry sample (García-Cruz et al., 2012).

Table 10 Antioxidant capacity in red, cherry, yellow, and white varieties of pitaya (*Stenocereus stellatus*)

Species	*Variety*	*Antioxidant capacity (mg trolox/g)*[a]
Stenocereus stellatus	Red	2763.2 ± 50.7
	Cherry	3047.1 ± 38.2
	Yellow	4202.1 ± 52.1
	White	4336.8 ± 36.3

[a]Milligram per gram of concentration based upon trolox as standard (Beltrán-Orozco et al., 2009).

potential source of beneficial compounds, in particular for inhabitants of arid regions. Information about the total phenolic content for two different *Stenocereus* species is shown in Table 8. The highest total phenolic content has been reported for yellow and white varieties belonging to the species *S. stellatus:* 2129–2395 gallic acid equivalents (GAE). Red and cherry varieties of the same species of pitaya, on the other hand, have lower amounts of total phenolic compounds: 1384–1552 GAE (Table 8).

The phenolic content in red and cherry varieties of *S. stellatus* and *S. griseus* pitaya is similar to that of apple and strawberry, whereas the yellow, white, and orange varieties show levels similar to cranberries. It is worth mentioning that cranberry is one of the fruits with the highest antioxidant capacity reported worldwide.

Regarding total soluble phenolics, the highest content was found in the red varieties of both *S. pruinosus* and *S. griseus* as compared with orange varieties of the same species (Table 9).

There is conclusive evidence that the antioxidant capacity in pitaya is highly correlated with the level of total phenolic compounds. A higher antioxidant activity for yellow and white *S. stellatus* has been reported (Table 10): expressed in μmol g^{-1}, such activity would be 16.8–17.3 μmol g^{-1} for yellow and white varieties (4202.1 and 4336.8 mg trolox g^{-1}) and 11–12.2 μmol g^{-1} (2763.2–3047.1 mg trolox g^{-1}) for the cherry and red varieties. Reported activity for the genus *Vaccinium* (cranberries) is 17.0 μmol g^{-1} at the lowest end of the range, overlapping somewhat the yellow and white *Stenocereus stellatus* varieties.

The level of polyphenolic compounds in pitaya is so high that 100 g of fresh pulp (14% solids in average) can provide up to 9.3% of the antioxidant levels consumed in a Mediterranean diet (average of 2590–3016 mg/person/day), which is traditionally high in antioxidants. The high antioxidant capacity of pitaya has the potential to suppress the effects of free radicals, providing protection against diseases like colon cancer and cardiovascular problems.

Other compounds of interest present in pitaya are the pigments responsible for the bright color of the pulp. Betalains are water-soluble nitrogenated compounds and are divided in two groups: betacyanins (red tones) and betaxanthins (yellow tones). Five types of betalains have been found in pitaya: two betaxanthins and three betacyanins.

Betalains play an important role in the induction of quinone reductase, an enzyme involved in cancer prevention. The antiproliferative effect of betalains in cells of malign melanoma has been reported, and the highest inhibitory effect is obtained with extracts from pitaya skin. Pitaya red pulp has 347.3 mg of total betalains per 100 g of dry sample, while the orange pulp has 215.0 mg (Table 11). Betacyanin contents for red and orange pulps are 286 mg and 47 mg/100 g of dry sample, respectively; the betaxanthin contents are 321 and 267 mg/100 g for the red and orange pulp, respectively. Total betalain content is higher in the red pulp.

Table 11 Betalains content within different pitaya cultivars (*S. griseus*)

Species	*Variety*	*Total betalains (mg/100 g dry sample)*	*Betacyanin (mg/100 g dry sample)*	*Betaxanthin (mg/100 g dry sample)*
S. griseus[a]	Red	347.3 ± 21	199.6 ± 24.3	147.6 ± 18.2
	Orange	215.0 ± 36.2	37.6 ± 7.5	177.4 ± 30.2
S. pruinosus[b]	Red	610.0 ± 100.0	286.0 ± 38.0	321.0 ± 56.0
	Orange	310.0 ± 40.0	47.0 ± 22.2	267.0 ± 27.0

[a]García-Cruz et al. (2012).
[b]García-Cruz et al. (2013).

The content of betalains in pitaya is significantly lower than in beetroot (*Beta vulgaris*). Total betalains in beetroot are 1227 mg/100 g of dry sample, where 788 mg corresponds to betacyanins and 438 mg to betaxanthins. This represents two to four times higher concentrations of betalains than red and orange pitaya, respectively. Nevertheless, from all cacti, pitaya has the highest betalain concentration. Combined with its agronomic characteristics (efficient land and water use), pitaya is a very advantageous crop for regions with high hydric stress and where arid and semiarid climates are predominant.

In summary, the level of phenolic compounds is highly related to their antioxidant capacity ($R^2 = 0.97$). In the case of pitayas, their content of phenolic compounds puts them on a level similar to some berries from the genus *Vaccinium* and, equally, can be similarly considered as functional foods. In addition to the advantages of pitaya in terms of its nutritional qualities, from the sustainable agriculture perspective, this native crop represents an alternative use of idle land with minimal maintenance and water requirements.

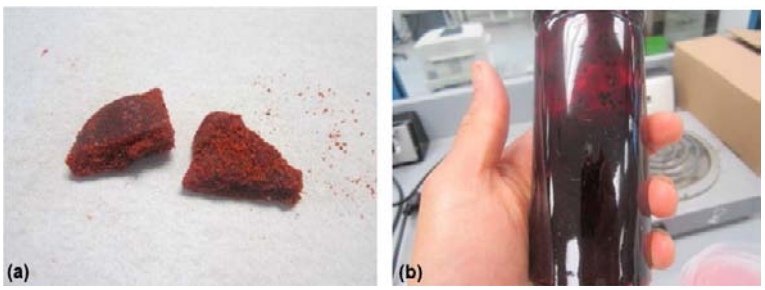

Figure 6 Products obtained from pitaya fruit (*Stenocereus* spp.). (a) A traditional Mexican candy and (b) pitaya jam.

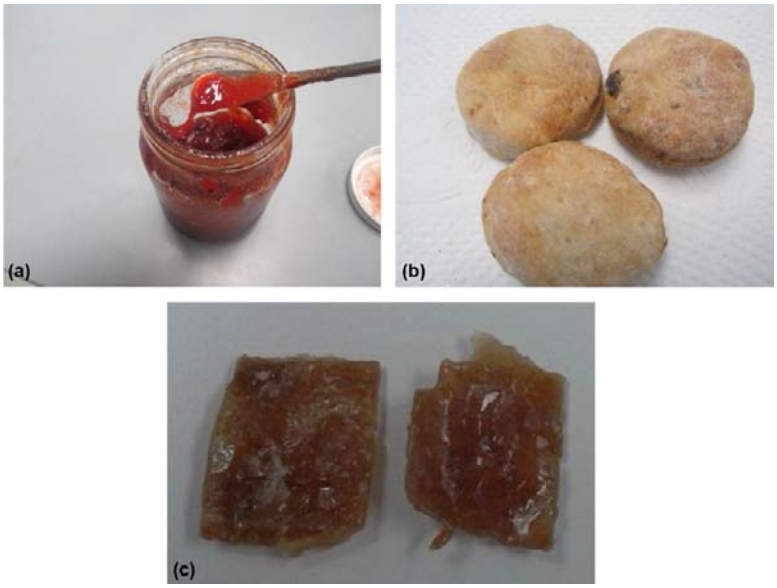

Figure 7 Products obtained with skin or pitaya peels: (a) jam, (b) cookies, and (c) candies, crystallized pitaya skins.

Uses and Commercialization

In some countries like Mexico, pitaya is consumed just as a fresh fruit; however, it can be processed for foods such as jams, frozen pulp, drinks, and ice cream (**Figure 6**). In some areas of Mexico, an alcoholic beverage, called 'ponche,' is traditionally made using underripe fruits mixed with sugar and mezcal.

Fresh pitaya fruit is highly appreciated in exotic fruit markets; however, its short shelf life presents a challenge for postharvest treatments. There are not only proposals for the consumption and industrial utilization of the pulp but also innovative designs to include the pitaya skin or residues in new products, thus supporting local producers (**Figure 7**). Despite the efforts, the pitaya is still mainly commercialized in traditional and local markets only as a fresh fruit, but the antioxidant compounds are an excellent opportunity for its development as a functional food in the international market.

See also: Antioxidants: Role on Health and Prevention; Apples; Bananas and Plantains; Berries and Related Fruits; Cherries (*Prunus* spp.): The Fruit and Its Importance; Fructose: Sources, Metabolism, and Health; Functional Foods; Glucose: Metabolism and Regulation; pH: Principles and Measurement; Phenolic Compounds: Occurrence, Classes, and Analysis; Strawberries; Vegetables of Temperate Climates: Carrot, Parsnip, and Beetroot.

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Pituitary Gland: Pituitary Hormones

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Introduction

Along with the nervous (central nervous system (CNS)) and circulatory systems, the endocrine system coordinates and integrates a huge variety of vital body functions. The blood circulation is a main carrier for the hormones' distribution to target tissues. The *neurosecretory* cells of the hypothalamus produce and release the *neurohormones* that through the anterior and posterior lobes of the pituitary gland (PG) stimulate the synthesis and secretion of different hormones into the bloodstream, which regulate and maintain the control of the adrenal and thyroid glands and gonads through the cascade of complex neuroendocrine feedback and a number of neuronal peripheral mechanisms. The focus of this article is the organization of the PG, the major elements of the neuroendocrine pathways, and the role of the PG in the control and modulation of a variety of vital body functions.

The Brief History of the PG

The first historical description of the PG (hypothesis) was provided by Galen (AD 150) who proposed the potential role for the found anatomical structure as a draining passage for the phlegm from the brain to the nasopharynx. In 1742, Joseph Lieutaud discovered the pituitary–portal blood system (hypothalamic–hypophyseal axis), and Saucerotte was the first to describe acromegaly in 1772. The gland was for the first time fully described in 1778 by Soemmering and called 'hypophysis cerebri.' The anatomical formation of the PG was described by Rathke in 1838. The posterior part of the PG was investigated by Oliver and Schafer in 1895 and by Dale in 1906, who discovered the vasopressor and oxytocic activities of the posterior pituitary, showing antidiuretic and galactokinetic properties. The first solid description of the clinical symptoms and pathology of the PG was provided by Minkowski in 1887 and Vassale and Sacchi in 1892, who demonstrated that surgically removing the PG (hypophysectomy) causes irreversible change on the water and mineral metabolisms of the body, and it also has been stated that overdevelopment and hyperfunction of the PG cause acromegaly. The first experimental links between the reproductive organs and PG were demonstrated via hypophysectomy leading to dwarfism in growing animals in 1909, by Aschner, and by Cushing in 1910.

Development and Basic Anatomy of the PG

Development

Complex development of the PG is occurring at the early stage of embryogenesis and linked to that of the forebrain. The PG contains three lobes that have dual embryonic origins: *anterior lobe* (*adenohypophysis*), *intermediate lobe* derived from Rathke's pouch (the primitive pharynx), and *posterior lobe*, which develops from the infundibulum (a stalk derived from the region of the ventral diencephalon). The anterior pituitary lobe (adenohypophysis) and posterior pituitary lobe (neurohypophysis) both develop from Rathke's pouch. During the development anterior pituitary loses contact with the oral cavity and the posterior pituitary stays connected with the base of the hypothalamus. Adenohypophys and neurohypophysis basically are joined together in the sella turcica (turkish saddle) at the base of the middle cranial fossa. The forming of the anterior pituitary (*adenohypophysis*) primordium is controlled by a complex signal emanating from the ventral diencephalon/infundibulum and from Rathke's pouch, and after that, Rathke's pouch forms a sac that differentiates and forms the *adenohypophysis*, connecting to the hypothalamus via stalk. The molecular nature of extrinsic signals and especially *transcription factors/morphogenes* that are involved in the patterning of many other organs, lineage specification, and cell type-specific gene expression in pituitary organogenesis has been recently investigated. The *ectodermal primordium* of Rathke's pouch is found to be induced by two major signals emanating from the diencephalon: *BMP4* (bone morphogenetic proteins), inducing the formation of the pouch rudiment, and *FGF8* (fibroblast growth factor), activating the key regulatory gene and development of the rudiment into a Rathke's pouch. Highly regulated gene expression of the controlling processes of cell proliferation and appearance of specific transcription factors allow the formation of gland cell types. A human PG embryogenesis and forming of the neuronal posterior pituitary and nonneuronal anterior pituitary tissues are usually complete at 12 weeks of gestation.

Basic Anatomy of the PG

The PG, which is a relatively small anatomical structure that is situated deep within the head, at the base of the skull, deep in the pit of the sphenoid bone, is surrounded by the optic chiasm, blood vessels, and other important brain structures. It consists of the larger part (80% of the PG size) called *anterior pituitary lobe* (*adenohypophysis*), *intermediate lobe*, and *posterior pituitary lobe* (or neurohypophysis), connected to the hypothalamus by the pituitary stalk. The adenohypophysis is composed of (a) *pars distalis*, containing epithelial cells surrounded by capillaries and fibers, with the cells being divided into *acidophils* (predominantly located in the lateral part of the lobe), *basophils* (concentrated in the central portion of the pars distalis), *chromophils*, and *chromophobes*, (b) *pars intermedia*, and (c) *pars tuberalis*. Acidophils consist of somatotrophs which produce growth hormone (GH), mammotrophs which produce prolactin (PRL), basophils consist of gonadotrophs which produce follicle-stimulating hormone (FSH), thyrotrophs which produce thyrotropin releasing hormone (TSH), and corticotrophs which produce adrenocorticotropic hormone (ACTH). Cromophils consist of cytoplasm

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with a large number of secretory granules in difference from chromophobes which consist of cytoplasm with no secretory granules. *Pars intermedia* is located between the *pars distalis* and *pars tuberalis* and contains small basophilic cells. *Pars tuberalis* is a part of the adenohypophysis around the *infundibulum*, composed of basophilic cells that secrete gonadotrophs (luteinizing hormone (LH) and FSH). The adenohypophysis is responsible for the majority of the signaling hormones released into the bloodstream through the well-developed hypothalamic–-hypophyseal portal capillary system that drains into hypothalamic–hypophyseal portal veins (Figure 1(a) and 1(b)). The posterior pituitary lobe (or neurohypophysis, consisting of three distinct regions: *pars nervosa* (posterior lobe, which includes Herring bodies and *pituicytes*), *infundibulum* (pituitary *stalk*, which connects the hypothalamic and hypophyseal systems), and median eminence (ME) (which is included as part of the posterior pituitary). The pituitary stalk contains the fibers of the neurohypophysis connecting the posterior lobe to the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus and the portal venous system, transmitting hypothalamic peptides that control anterior lobe secretion.

The pathology of the PG leads to the development of symptoms dependent on its change of secretion or symptoms dependent on the new anatomical intracranial relations that arise (lesions, tumors, etc.). It has been found that lack of balanced PG hormonal secretion could lead to *acromegalic gigantism* (first time described by Pierre Marie in 1886), *dwarfism*, Frohlich's disease (dystrophia adiposa genitalis), acute pituitary insufficiency (causes fevers and toxemias), and polyglandular syndrome (autoimmune disease leading to inflammation, lymphocytic infiltration, and partial or complete

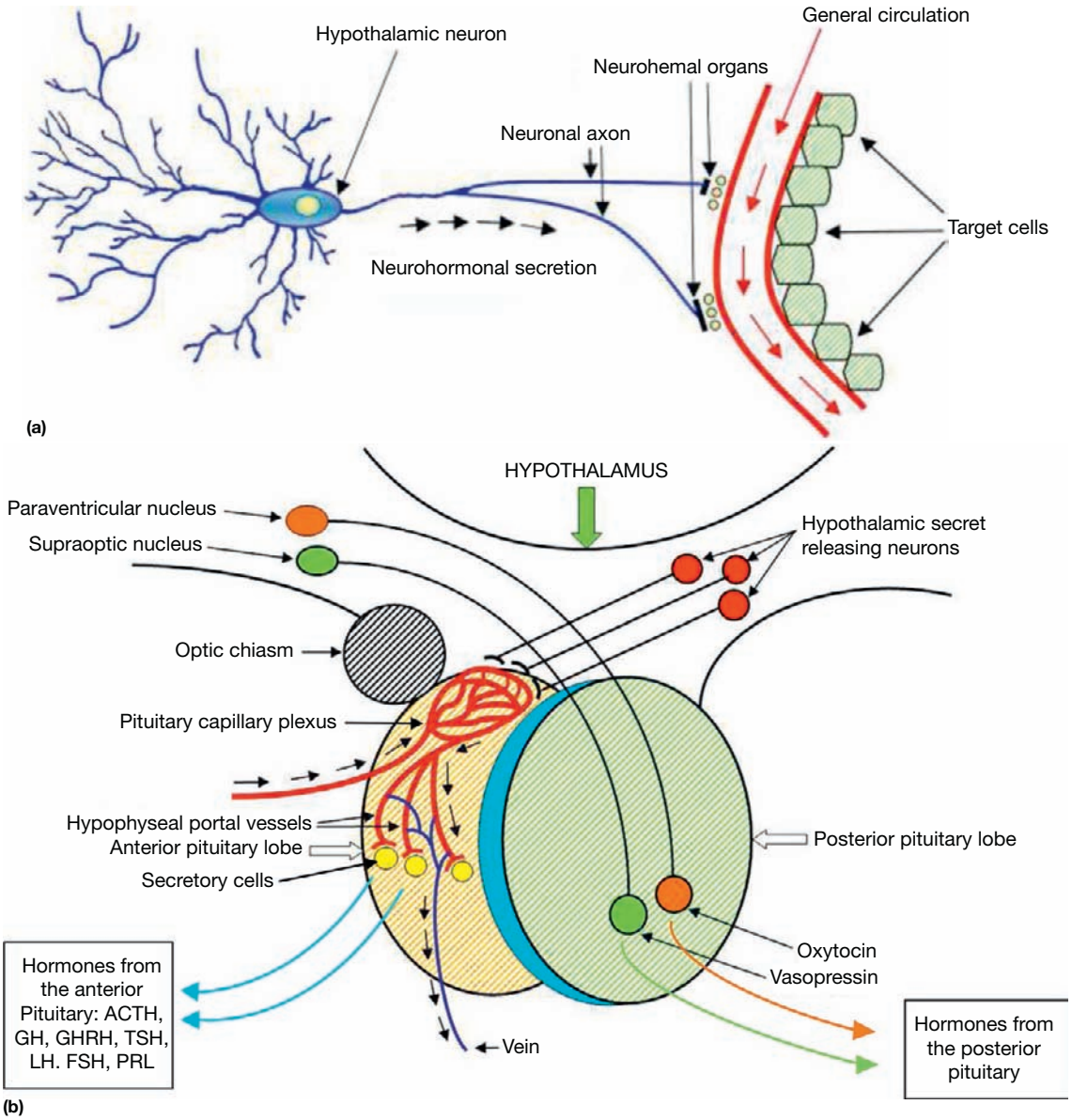

Figure 1 (a) The release of neurohormones from neurosecretory neurons. (b) Hypothalamic–hypophyseal (HPH) axis: hormones of the mammalian pituitary gland. Copyright @ by Dr. M. Emerald, 2015.

gland destruction). The typical anatomical pathology of the PG is a tumor (Cushing's disease) indicative of increased intracranial pressure causing headache, vomiting, vision impairment, nausea, and partial blindness, with main symptoms such as weak immune system, osteoporosis, extreme hair growth on face, fatigue, and cognitive dysfunctions.

Neuroendocrine Pathways

The proper function of the PG is regulated by the hypothalamus releasing *trophic factors* that modulate cell proliferation, hormone synthesis, and secretion. The hypothalamic–pituitary axis represents a complex organization of interactions between the hypothalamus and pituitary and major endocrine glands that regulate a wide variety of the vital body processes via hormones and neurotransmitters. The neuroendocrine pathways are a basic core of the human neuroendocrine system that controls and modulates the adaptation of organisms to changes in the internal or external environment, homeostasis, hormonal and nervous system activities, energy release, mood, the immune and digestive systems, sexual functions, our reaction to stress, etc. The pathology of the neuroendocrine pathways contributes to the manifestation of many chronic diseases. The neuroendocrine pathways are the core part involved in the regulation and control of the (a) hypothalamic–pituitary–adrenal axis (HPA axis, or limbic–hypothalamic–pituitary–adrenal axis), (b) hypothalamic–pituitary–thyroid axis (HPT axis), and (c) hypothalamic–pituitary–gonadal axis (HPG axis, or hypothalamus–pituitary–gonadal–liver axis, typical for females when chorionic proteins such as *vitellogenin* and *choriogenin* synthesized in the liver are vital for the growth and development of *oocytes*).

The HPA axis plays an important role in the regulation of digestion, immune response, response to 'social and physical stress,' mood, energy storage, and the development of anxiety, depression and insomnia, borderline personality disorder, bipolar disorder, posttraumatic stress disorder, Attention-deficit hyperactivity disorder (ADHD), chronic fatigue syndrome (CFS), irritable bowel syndrome (IBS), and other pathological conditions. Produced by the PVN of the hypothalamus, corticotropin-releasing hormone (CRH) and vasopressin (VP) stimulate the release of stored ACTH (from *corticotroph* cells of the *adenohypophysis*) transported by the blood to the adrenal cortex of the adrenal gland, where it stimulates *cortisol* production, which is a main stress hormone.

The HPT axis is responsible for growth, reproduction, intelligence, and regulation of metabolism. The hypothalamus produces and releases thyrotropin-releasing hormone (TRH) in response to low circulating levels of triiodothyronine (T3) and thyroxine (T4) hormones in the blood, which is a part of the negative feedback loop and hypothalamus and anterior lobe of the PG controlling the release of both TRH from the hypothalamus and thyroid-stimulating hormone (TSH) from the adenohypophysis. The HPG axis plays a critical role in the development, regulation, and proper functioning of the reproductive and immune systems and regulation of life cycle, menstrual cycle, behavior, and sexual dimorphism and aging. The importance of the neuroendocrine pathways and hypothalamic control of the endocrine functions is impossible to underestimate, and it has a long history of experimental research. 'Hypothalamic releasing factors' or 'releasing hormones' were introduced to the world in 1905 and in 1936. Marshall proposed an involvement of the brain in controlling pituitary functions, which was proved later using a variety of morphological and functional methods. It has been demonstrated that growth hormone (GH), ACTH, PRL, and TSH were stored in separate special types of cells that actually showed immunoreactivity for both LH and FSH. The classical description of the hormonal function based on their ability to be basically 'chemical messengers' discharged from different glands via endocrine secretion, following release into the bloodstream and targeting specific tissues reacting on their small concentrations, was significantly extended with time. The process of hormonal regulation was suggested to include two main important processes: neuroendocrine regulation, with a control of the hypothalamus over the PG (characterized by quick response, localized effect, and short duration), and endocrine regulation (characterized by slower development but prolonged effect widely distributed through the body). The mechanism of central neuroendocrine regulation is based on neuronal connection between the neurosecretory cells of the hypothalamus, which translate neural signals into chemical stimuli by producing neurohormones that are passed through the neuronal axons and released into the bloodstream at *neurohemal* organs (specific region where axon endings contact with blood capillaries) (**Figure 1(a)**). The *neurohormones* belong to a group of peptide hormones which consist of short or polypeptide chains, such as antidiuretic hormone (ADH) and oxytocin (OT), small proteins such as Growth Hormone (GH), or large glycoproteins, such as follicle stimulation hormone (FSH). The releasing or release-inhibiting hormones synthesized by neurons of the supraoptic, paraventricular, periventricular, and arcuate hypothalamic nuclei projecting their axons into the tuberoinfundibular tract are a major force driving the secretion of anterior pituitary hormones (**Figure 1(b)**). The neurohormones are produced and released by the different hypothalamic nuclei: CRH, prolactin-releasing hormone, and prolactin-inhibiting hormone are synthesized and secreted by PVN neurons of the hypothalamus, somatostatin (GHIH) is synthesized and secreted by cells of the periventricular nucleus (which is a layer of nerve cells adjacent to the third ventricle), growth hormone-releasing hormone (GHRH) is synthesized and secreted by the arcuate nucleus, gonadotropin-releasing hormone (GnRH) is synthesized and secreted by the medial preoptic area and in the arcuate nucleus, and TRH is synthesized and secreted by the neurons of preoptic nucleus and PVN of the hypothalamus (**Figure 2**). Hormones released from the different hypothalamic nuclei cause specific effects on the hormones of the PG and represent the important 'driving part' of the complex neurohormonal 'positive' and 'negative' feedback loops, which regulate multiple endocrine and physiological functions (**Table 1**). The regulatory pathways of the HPA axis are a great example of self-regulating neuroendocrine feedback loops (**Figure 2**). The PVN of the hypothalamus initiates the glucocorticoid secretion of CRH and arginine vasopressin (AVP), which activate the stimulatory circuit. CRH and AVP are released into the anterior pituitary lobe portal circulation at the ME and stimulate the secretion of ACTH (corticotropin), which stimulates the synthesis and secretion of cortisol at the adrenal cortex, which has transsynaptic negative feedback inhibition of the HPA axis at the

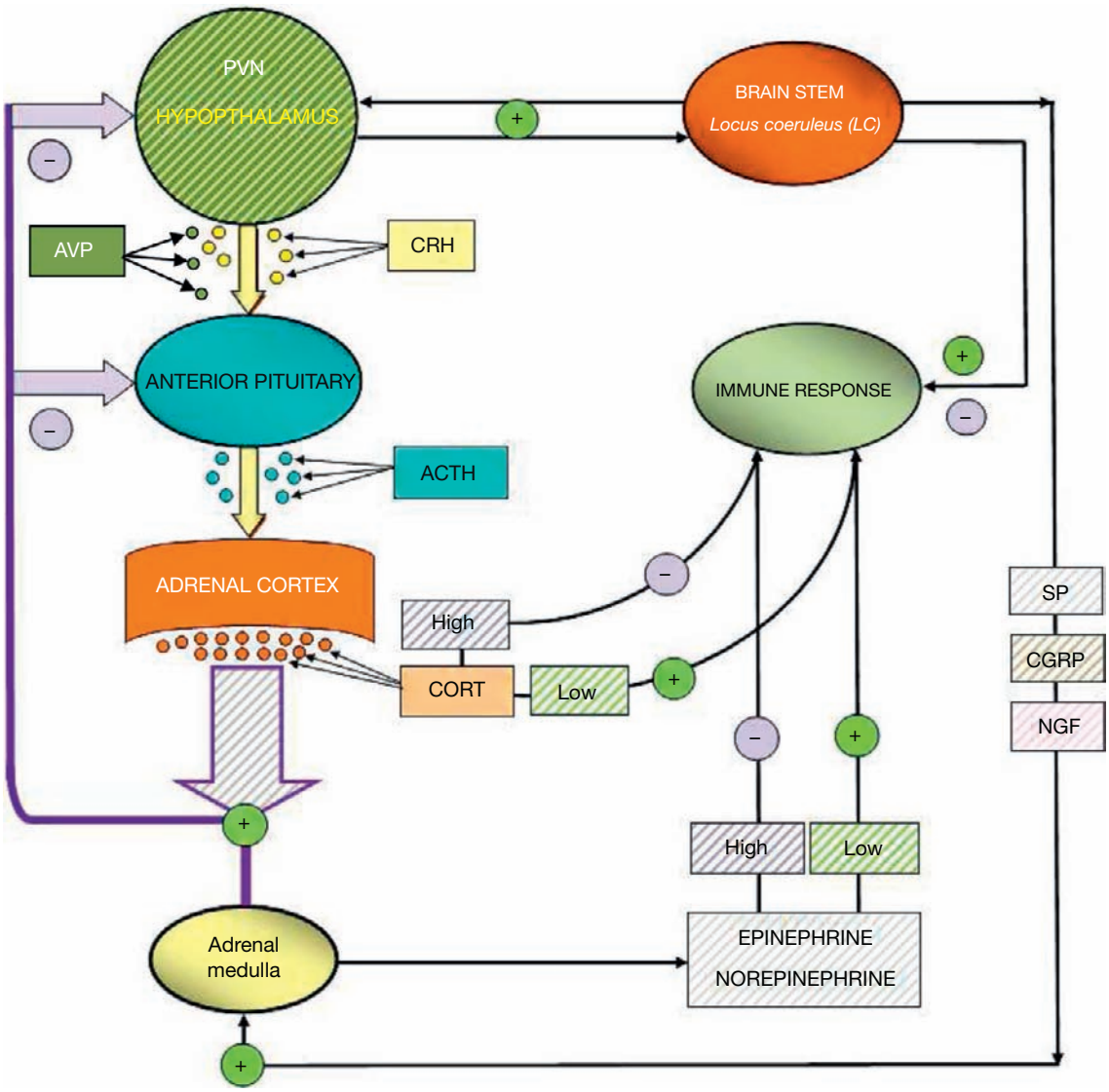

Figure 2 Hypothalamic–pituitary–adrenal (HPA) axis neurohormonal feedback loop and its interconnection with the immune system. CRH, corticotropin-releasing hormone; AVP, arginine vasopressin; PVN, paraventricular nucleus of the hypothalamus; ACTH, adrenocorticotropic hormone; SP, substance P; CGRP, calcitonin gene-related peptide; NGF, cutaneous nerve growth factor. Copyright @ by Dr. M. Emerald, 2015.

Table 1 Hypothalamic releasing factors and their effect on the anterior pituitary hormones

Hypothalamic releasing hormone	*Effect on pituitary gland*
Gonadotropin-releasing hormone (GnRH): Stimulated by estrogen and inhibited by high levels of testosterone and progesterone	Stimulates LH and FSH secretion
Thyrotropin-releasing hormone (TRH): Stimulates to produce and secrete thyroid-stimulating hormone (TSH)	Stimulates TSH, GH, and prolactin secretion
Growth hormone-releasing hormone (GHRH): GHRH inhibited by high levels of somatomedins	Stimulates GH secretion
Corticotropin-releasing hormone (CRH): Stimulated by low levels and inhibited by high levels of glucocorticoids	Stimulates ACTH secretion
Prolactin-releasing hormone (PRH): Inhibited by high levels of prolactin	Stimulates PRL secretion
Prolactin-inhibiting hormone (PIH): Stimulated by high levels of prolactin	Inhibits PRL secretion
Somatostatin (GHIH): GHIH stimulated by high levels of somatomedins	Inhibits GH and TSH secretion

LH, luteinizing hormone; FSH, follicle-stimulating hormone; TSH, thyroid-stimulating hormone (thyrotropin); GH, growth hormone; ACTH, adrenocorticotropic hormone (corticotropin); PRL, prolactin.

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different brain levels, and induces epinephrine and norepinephrine from the adrenal medulla. Cortisol, epinephrine, and norepinephrine stimulate immune responses at low concentration and suppress at high concentration. They also found a positive feedback loop between the HPA axis and locus coeruleus norepinephrine cells of the sympathetic nervous system (the brain stem), which is stimulated by stress (Figure 2). One of the examples for other feedback loops is PRL secretion, which is depressed by the adrenal steroids as a result of inhibition of CRH release and a reduction in ACTH secretion by the anterior lobe of the PG and inhibited by dopamine release, which is stimulated by TRH. The direct endocrine regulation is based on the hormone synthesis and release from the adenohypophysis through the portal vascular system. Via 'lock and key' mechanism, hypothalamic hormones can bind to a specific glycoprotein containing receptors on the anterior pituitary cells, which have hundreds of thousands of receptors for one type of hormone. When there is too high level of circulating hormones, cellular receptors reduce their sensitivity ('downregulation'), and if there is too low level of circulating hormones, cellular receptors increase their sensitivity when there is an excess of the specific hormone in the blood ('upregulation'). The hypothalamic secretion is a complex mechanism controlled by multiple stimuli including neural (olfaction, pain, stress, etc.), hormonal feedback, and chemical stimuli (electrolytes, water concentrations in the blood, osmotic pressure, temperature, plasma glucose, etc.). Some of the neurohormones are found not only in the hypothalamus but also in other parts of the body, where they are acting as neurotransmitters and play an important regulatory role. For example, somatostatin (GHIH) is found in the pancreas, where it acts as an inhibitor of the release of insulin and glucagon, and in the gut as well.

Hormones of the Adenohypophysis

The different cells of the adenohypophysis synthesize and release into the bloodstream vital endocrine hormones such as ACTH (secreted by corticotrophs), TSH (secreted by thyrotrophs), FSH (secreted by gonadotrophs), LH (secreted by gonadotrophs) in females also known in males as interstitial cell-stimulating hormone, growth hormone (GH, secreted by somatotrophs), GHRH (secreted by somatotrophs), PRL (secreted by lactotrophs), and melanocyte-stimulating hormone (α-MSH, secreted by melanotrophs). All those hormones can be classified into three major groups: *glycoproteins* (TSH, LH, and FSH composed of a common α-subunit and a hormone-specific β-subunit), growth hormones (GH and GHRH), and *proopiomelanocortins* (α-MSH, regulating the production and distribution of melanin by melanocytes) (Figure 3).

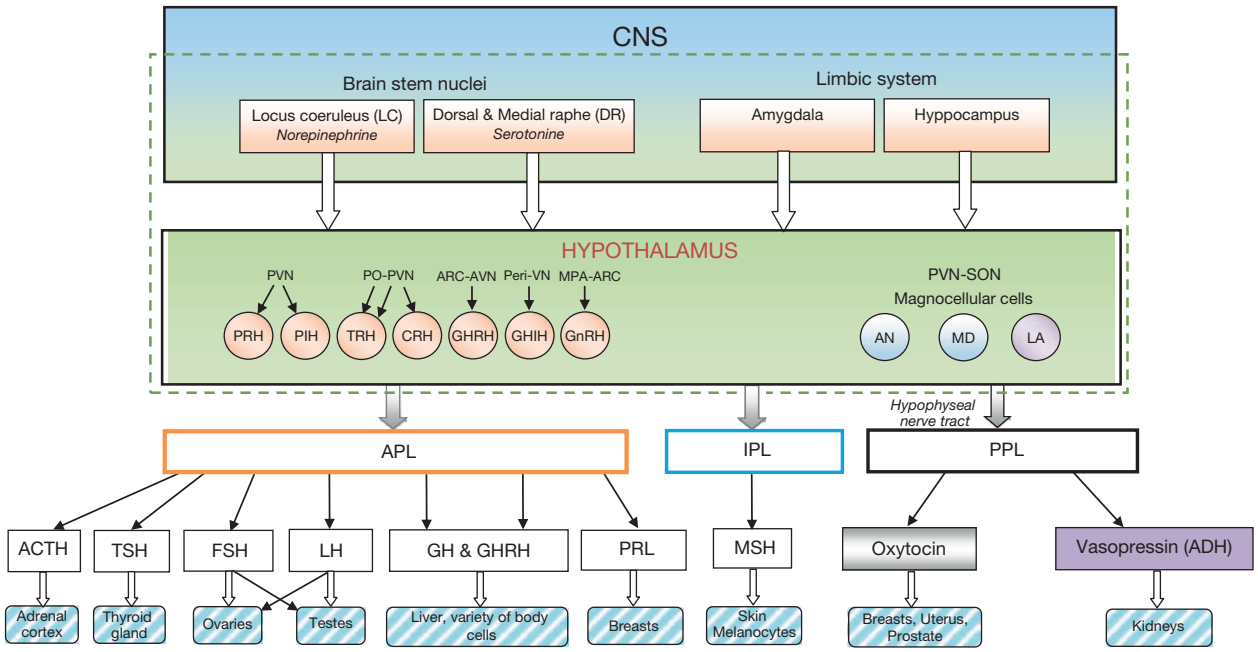

Figure 3 CNS–hypothalamus–pituitary gland diagram. AN, anterior part of the magnocellular division; MD, medial part of the magnocellular division; LA, lateral part of the magnocellular division; CNS, central nervous system; APL, anterior pituitary lobe; IPL, intermediate pituitary lobe; PPL, posterior pituitary lobe; PVN, paraventricular nucleus of hypothalamus; ARC, arcuate nucleus; AVN, adjacent ventromedial nucleus; SON, supra optic nucleus of the hypothalamus; Peri-VN, periventricular nucleus of the hypothalamus; MPA-ARC, medial preoptic nucleus and arcuate nucleus of the hypothalamus; PO, preoptic nucleus of the hypothalamus. Hormones released by the parvocellular neurosecretory cells: PRH, prolactin-releasing hormone; PIH, prolactin-inhibiting hormone (dopamine); TRH, thyrotropin-releasing hormone; CRH, corticotropin-releasing hormone; GHRH, growth hormone-releasing hormone; GHIH, somatostatin (growth hormone-inhibiting hormone); GnRH, gonadotropin-releasing hormone. Hormones released by the anterior part of the pituitary gland: ACTH, adrenocorticotropic hormone (corticotropin); TSH, thyroid-stimulating hormone (thyrotropin); FSH, follicle-stimulating hormone; LH, luteinizing hormone; GH, growth hormone; GHRH, growth hormone-releasing hormone (somatoliberin); PRL, prolactin; MSH, melanocyte-stimulating hormone; ADH, antidiuretic hormone (vasopressin). Copyright @ by Dr. M. Emerald, 2015.

Adrenocorticotropic Hormone

The properties of ACTH were discovered in 1933 by J. Collip, H. Evans, and B. Houssay. ACTH is based on 241 amino acids and was isolated first in 1943 and synthesized in 1963. The mass production of ACTH for medicinal use and reduction of inflammation and swelling started during World War II. In 1950, Hench and Kendall received the 1950 Nobel Prize in physiology or medicine for their discovery and successful use of ACTH in treating arthritic patients. Presently, ACTH is intensively used for diagnostics of the adrenal gland disorder, and it is sometimes used to treat inflammatory disorders, such as arthritis, ulcerative colitis, and hepatitis. ACTH stimulates the adrenal gland cortex cells to produce (a) *glucocorticoid cortisol* as a response to low blood glucose and stress, which increase blood sugar by stimulating gluconeogenesis; (b) *mineralocorticoids*, which control vascular volume and serum electrolyte (Na^+ and K^+) concentrations; and (c) *aldosterone* and *androgen* (male sex hormones and testosterone). On the adrenocortical cells, ACTH binds to the melanocortin 2 receptor (MC2R) in the middle zone of the adrenal cortex, causing three phases of effects: *immediate effect*, which leads to increase in pregnenolone levels; *chronic effect*, occurring during several hours; and *trophic effect*, occurring during a period of weeks and months. It is well known that cortisol plays an important role in maintaining immune homeostasis; the balance of fat, protein, and carbohydrate metabolisms; increasing bone resorption; decreasing the function of the reproductive axis at the hypothalamic, pituitary, and gonadal levels; inhibiting fibroblast proliferation and collagen formation; increasing the glomerular filtration rate by increasing cardiac output and acting on the kidney; causing muscle weakness and pain due to hypokalemia, which has a trophic effect on the GI mucosa; and stimulating appetite and often causing weight gain. Cortisol also stimulates whole-body lipolysis, an effect that is caused by proportionate stimulation of femoral and abdominal subcutaneous adipose tissues. Through glucagon, cortisol affects liver and muscle glycogenolyses and is usually inactivated in the liver and excreted in the urine. Overproduction of ACTH could be a result of Cushing's disease, which is one of the causes of Cushing's syndrome (characterized by abnormally high levels of the hormone cortisol), which is a result of the excessive production of ACTH by a noncancerous adenoma (tumor) of the anterior lobe of the PG or in the lungs and adrenal gland as well. The overproduction of ACTH stimulates the adrenal glands to produce excessive amounts of cortisol. The genetic mechanism plays an important role in a significant fraction of cases of Cushing's syndrome, which provides a great and reliable diagnostic tool and may also lead to new types of treatment. Underproduction of ACTH leads to a poorly functioning adrenal gland or Addison's disease due to insufficient production of cortisol (*hypocortisolism*). The pseudo-Cushing's disease is usually caused by prolonged taking of steroid medications (such as prednisone) used to treat asthma, lupus, rheumatoid arthritis, alcoholism, and depression.

Melanocyte-Stimulating Hormone

The α-MSH, in which the main functions are mediation of immunity and inflammation, is produced and secreted by the anterior pituitary corticotrophs involved in the synthesis and secretion of ACTH as well, and their chemical structure is similar. In spite of the fact that ACTH demonstrates some MSH-like activity, α-MSH does not share any of the actions of ACTH at all. α-MSH consists of 13 amino acids and can bind with various affinities to five MCRs that are believed to be involved in regulating pigmentation and inflammation, sexual behavior, appetite regulation (appetite suppression), exocrine function and energy homeostasis, and pathogenesis of restless legs syndrome via stimulation of motor activity and increasing body movements in sleep. The main function of the α-MSH (regulating pigment (melanin) production in the skin) is based on binding α-MSH with the MC1Rs on melanocytes and the involvement into the complex signaling cellular pathways through activating adenyl cyclase via G protein signaling, leading to an increase in intracellular cAMP. It stimulates a cascade reaction inducing stimulation tyrosinase synthesis, and increase in the catalytic efficiency of the enzyme. a-MSH triggers the synthesis of cAMP, which initiates the pigmentation process and stimulates the synthesis of melanin granules and their disposal in melanosomes transferred from the dendrites of the melanocytes to nearby keratinocytes. The granules are released and spread to form a protective pigmented barrier over the nucleus to protect the DNA against damage by ultraviolet radiation (UV). α-MSH also plays an important role in enhancing the repair of DNA damage and reducing the generation of free radicals, which both are important in reducing the risk factors for some types of skin cancer. After UV-R exposure, melanocytes increase the proopiomelanocortin (POMC, the precursor of MSH), melanocortin 1 receptor (MC1-R), TYR and TYRP1, protein kinase C (PKC), and other signaling factors' production, as well as endothelin-1 (ET-1) production by keratinocytes. Many factors, including fibroblast growth factor (bFGF), nerve growth factor (NGF), endothelins, hepatocyte growth factor (HGF) and others are involved in iregulating melanogenesis and melanocyte dendrite formation. Keratinocytes produce and release NGF involved in regulating the melanogenesis and dendritogenesis of melanocytes. α-MSH demonstrates a great spectrum of antimicrobial, antipyretic, immunomodulatory, and anti-inflammatory properties. α-MSH was found to be elevated in the plasma of HIV-infected patients, and during sepsis syndrome, the concentrations of α-MSH were reduced. A significant number of independently conducted studies have demonstrated the antimicrobial activity of α-MSH and its C-terminals (containing Lys–Pro–Val) against a wide range of microorganisms including *Candida albicans*, *Candida vaginitis*, *Cryptococcus neoformans*, *Staphylococcus aureus*, and *Escherichia coli* by inhibition of the formation of the germ tube via increasing the level of cAMP (damaging the membrane of the microorganisms) and through anti-inflammatory action of carboxy-terminal tripeptide (KPV). The increase in α-MSH production was reported not only following UV light exposure but also after injury, trauma, and infection and in patients with psoriasis and inflammatory skin conditions. A higher concentration of α-MSH occurred in the synovial fluid of patients with *juvenile arthritis*, acute myocardial infarction, and CFS. α-MSH deficiency is noticed in the early stages of Alzheimer's disease, early-onset obesity, adrenalin sufficiency, and pigmentation alteration, and α-MSH could be used as a potential agent for the treatment of

inflammatory, autoimmune, and allergic diseases, and its analogs could have therapeutic value due to their anti-inflammatory actions in the vasculature in cardiovascular diseases. α-MSH has effects on the MC1R-expressing monocytes, dendritic cells, and macrophages via downregulation of the proinflammatory and immunomodulating cytokine production and demonstrates potent capacity to modulate the function of antigen-presenting cells; α-MSH should be considered and investigated further.

Thyroid-Stimulating Hormone

Thyrotropin, or TSH, is a glycoprotein secreted by the basophilic thyrotrophs of the anterior lobe of the PG, and its secretion is regulated by the negative feedback of thyroid hormones. TSH consists of an *alpha* molecular subunit that is chemically identical to human chorionic gonadotropin hormone (GH), LH, and FSH; the *beta* subunit determines the function of the hormone. TSH is a member of the glycoprotein hormone family, which is structurally classified as a part of the CKGF superfamily of structurally related proteins with very important biological activities. TSH controls the production and secretion of the thyroid hormones, T4 and T3, by the thyroid gland, which are released into the bloodstream and control the body's metabolism, brain development and state (decreased and increased concentrations of thyroid hormones lead to alterations in mental state), bone development, heart (increases heart rate, cardiac contractility, and cardiac output), digestive functions, reproductive system (normal reproductive behavior and physiology are dependent on having essentially normal levels of thyroid hormone), etc. TSH enters the cells through membrane transporter proteins using ATP hydrolysis or other carriers and binds to specific receptors (there are the multiple forms of thyroid hormone receptors). The TSH, which activates the secretion of the hormone T4 via binding to transmembrane G protein-coupled receptors on the surface of the cells of the thyroid gland, is stimulated by TRH but inhibited by somatostatin released from the hypothalamus. The hormone–receptor complex interacts with specific sequences of DNA, which gives an opportunity to modulate gene expression by stimulating or inhibiting the transcription of specific genes. Inappropriate levels of TSH hormone can be caused by problems in the pituitary or thyroid gland itself and can result in different pathological conditions and autoimmune diseases, such as Hashimoto's *thyroiditis* (when the body itself produces antibodies destroying the thyroid gland), which could lead to *vitiligo* (chronic skin disease characterized by part of the skin losing their pigment). More than 2 billion people worldwide suffer from hypothyroidism, which is a result of pituitary and hypothalamus problems and iodine deficiency. Severe hypothyroidism sometimes can develop in people exposed to physical stresses, infections, cold temperatures, and trauma and lead to myxedema coma with loss of consciousness, inhibited breathing, and seizures. Hypothyroidism may be associated with incident coronary heart disease (CHD), especially in men, independently of conventional cardiovascular risk factors. Hyperthyroidism is a main cause of Graves' disease (when the immune system produces an antibody that stimulates the entire thyroid gland) and toxic goiter or toxic thyroid adenoma. It has been shown that higher TSH levels are associated with risks of thyroid tumors (papillary, follicular,anaplastic, and medullary), especially in advanced tumor stage. The ectopic TSH-secreting pituitary adenoma (TSH-oma) has been shown to raise levels of TSH as well as free thyroxine.

Follicle-Stimulating Hormone and Luteinizing Hormone

In females, the FSH assisted by LH stimulates the release of estrogen and is activated by GnRH released by the hypothalamus. In males, the FSH activates *spermatogonia*, which with testosterone input stimulate the production of sperm. In females, LH helps complete meiosis I of the egg and ovulation and stimulates follicles to develop into the *corpus luteum* secreting *progesterone* during the second half of the menstrual cycle. In males, LH stimulates the Leydig cells of the testes to secrete the male sex hormone, testosterone. The release of the regulatory hormones from the hypothalamus to the anterior pituitary lobe and secretion of the anterior pituitary hormones are controlled by negative feedback or classical neuroendocrine loop. When the parvocellular CRH and VP-containing neurons of the PVN of the hypothalamus initiate the glucocorticoid secretion, the anterior pituitary lobe releases ACTH, which promotes the synthesis and secretion of glucocorticoids at the level of the adrenal cortex, which are exerting transsynaptic negative feedback inhibition of the HPA axis at multiple levels in the brain. Low FSH and low LH levels in women are a result of the pituitary or hypothalamic problem leading to ovarian failure. The symptoms of *gonadotropin* deficiency are hot flashes and lack of libido, which cause infertility, amenorrhea (absence of menstruation), or oligomenorrhea (infrequent menstruation) in women and impotence in men, and osteopenia (weak bones), which need hormone replacement therapy (gonadotropin deficiency could be diagnosed in the presence of low or normal LH and FSH). The hypersecretion of LH can lead to polycystic ovary syndrome with typical menstrual irregularities, infertility, acne, and hirsutism.

Prolactin

PRL (luteotropin) is a protein, in which its chemical structure is similar to growth hormone, that is known in three different forms varying in size, and the level of larger ones is higher during early development. PRL is detected in epithelial cells of the mammary gland and in the milk and is produced at the PG, breast, lymphocytes, leukocytes, prostate, and other tissues. In spite of the fact that the main function of PRL is lactation, it also can be an indicator of relaxation and sexual satisfaction. Increased levels of PRL decrease estrogen and testosterone and are responsible for impotence and loss of libido. PRL influences the hypothalamus, decreasing the release of growth hormone-releasing hormone (GnRH) and subsequently LH. PRL stimulates the process of myelin coating formation on axons in the CNS. The role for pituitary and lymphocytic PRL in immune response and in transplant rejection needs further detailed investigation.

Growth Hormone

The main and vital function of the growth hormone (GH) produced by somatotropic cells in the anterior lobe of the PG

is development and growth. The GH synthesis is initiated by the GHRH, secreted by the cellular structure of the hypothalamus called a *porosome*, which induces the production and pulsatile secretion of GH. The GH is traveling via the bloodstream, demonstrates affinity to specific GH receptors on the surface of a large variety of cells, and causes the cellular division of the cartilage, bone, muscles, and other tissues as a result of insulin-like growth factor 1 (IGF-1, somatomedin C) production by the liver. However, the GH production is also inhibited by somatostatin (GHIH) released by the anterior PG, pancreas, and GI tract. The glucocorticoids and IGF-1 are also able to suppress the release of GHRH and diminish the sensitivity of the GHRH receptors. Most of GH's effect occurs through the stimulation of IGF-1 (somatomedin C), primarily by the liver. IGF-1 causes the growth of muscle, bone, and cartilage, protein synthesis, amino acid transportation, DNA and RNA syntheses, and cell proliferation. IGF-1 also suppresses the production of GH as a part of a negative feedback loop. The decreased muscle strength, diminished libido, metabolic disturbances, social isolation, decrease of the bone mineral density (increased total cholesterol, low-density lipoprotein, apolipoprotein B, and triglyceride levels), dwarfism, acromegaly, gigantism, multiple endocrine neoplasia, McCune–Albright syndrome, and neurofibromatosis are the conditions of GH deficiency in adults, often caused by pituitary tumors. The typical symptoms of GH deficiency are carpal tunnel syndrome, weakness, joint pain, fatigue, sleep apnea, enlarged hands and feet, weight loss, etc. The GH deficiency can be evaluated by insulin tolerance and GHRH/arginine test. The growth disorders, which are the result of GH deficiencies, are presently treated by synthetic GH produced by recombinant DNA technology.

Hormones of the Neurohypophysis

The large (magnocellular) neurons located in the SON and PVN of the hypothalamus synthesize and transport along the axons to terminals situated within the posterior lobe of the PG (neurohypophysis) two vital hormones: *oxytocin* and VP (or antidiuretic hormone (ADH)), which are secreted by calcium-dependent exocytosis. The posterior pituitary hormones are transported in association with specific proteins, the *neurophysins*, via the 'unmyelinated' nerve fibers that extend through the infundibulum along with small cells called *pituicytes* to end in nerve terminals that lie within the posterior lobe; prior to secretion, they are stored in secretory granules in the Herring bodies. The oxytocin stimulates contraction of the uterus during labor, prevents posttraumatic hemorrhage, and is involved in lactation and milk ejection from the nipples, sexual arousal, suppressing appetite, etc. Oxytocin is also called the 'love and bonding hormone' that plays an important role in social recognition (especially in trust, empathy, rejection, and suspicions in the group), increasing positive attitudes, the formation and realization of maternal instinct, orgasm, depression and anxiety, wound healing, and soothing inflammation. Oxytocin receptors are expressed by neurons not only in the neurohypophysis but also in the septum, nucleus accumbens, brain stem, amygdala, and other parts of the brain and body. The chemical structure of VP (ADH) is very similar to oxytocin, and this is why oxytocin has mild diuretic properties, and high levels of ADH can cause uterine contractions. The main functions of the ADH are water retention and maintaining normal body fluid volume via specific renal (V2) and vascular (V1) receptors, regulation of body temperature, initiation of aggressive behavior, and male pair-bonding behavior. ADH produces vasodilation in the renal, pulmonary, cerebral, and mesenteric vascular beds by stimulating endothelial nitric oxide (NO) release and increases the systemic blood pressure. ADH is also involved in the modulation of the corticosteroid release from the adrenal gland in response to stress during pregnancy and lactation and also may cause an analgetic (nociceptive) effect during sex and stress. ADH demonstrates anti-inflammatory properties via inhibition of the inflammatory cytokine interleukin-1 released in response to trauma or infection. ADH deficiency, as a result of the hypothalamic–neurohypophyseal lesions or insensitivity of the kidney to ADH in nephrogenic diabetes insipidus, leads to dehydration, diarrhea, hyperosmolality, and eventual death. ADH overproduction promotes excessive water retention and hyponatremia, which may cause convulsions and coma. ADH could be effective in reversing hypotension produced by septic shock and could be a useful therapy for hypovolemic cardiac arrest.

Pineal Gland and Biological Clocks

The human primary circadian 'clock' is located in the hypothalamic pacemaker suprachiasmatic nucleus (SCN), where the information on the lengths of the day and night is passed from the retina to the pineal gland, where the hormone melatonin is secreted with peaks at night and ebbs during the day. The pineal melatonin is rhythmically released onto the SCN, which helps modulate circadian patterns of activity and other vital processes throughout the body. Disruption to rhythms usually has a negative effect and significant adverse health consequences on peripheral organs especially in the development or exacerbation of cardiovascular diseases and greater risks of metabolic syndrome, hypertension, and gastrointestinal disturbances.

Conclusion

Recent advances using a wide range of methodologies provide more detailed knowledge and generate new valuable insights about the cellular, molecular, and genetic mechanisms involved in the anatomy, organization, development, and function of the human PG. The recognition of the fascinating role of the PG and its sophisticated hormonal system, which is a retrograde regulator of hypothalamic and peripheral endocrine functions, raises important questions regarding the extraordinary complexity and vital role of the PG in neuroendocrine regulation of a huge variety of human physiological functions on molecular, organic, and systemic levels that remain virtually not fully explored.

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Plums and Related Fruits

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Introduction

Plums are one of the ancient and long cultivated fruits and belong to the 'stone fruit' family, which includes peach, nectarine, cherry, and apricots as well. They come in a variety of colors, although red and black are the most common ones seen in the market. Most plums ripen in the summer/fall months due to their production cycle; however, we are currently seeing plums in the market almost year round due to modern storage and shipping worldwide. In addition to being a delicious and juicy dessert fruit, a number of products, both homemade and industrial, are made out of plums, and thus, they find a way in our food system. Information from recent research on their health benefits helps in increasing the consumer interests in plums.

Origin and History of *Prunus* Species

The family Rosaceae includes a number of fruits, including plum and prune, in addition to cherry, apple, nectarine, peach, pear, and several berry crops. In Rosaceae, the genus *Prunus* is an important member to which all the true plums belong. It is estimated that around 200 species of *Prunus* are found predominantly in the northern temperate regions of the world. There are a number of plums, among which European plums (*Prunus domestica* L.) and Japanese plums (*Prunus salicina* Lindl) are the most important. In addition, there are also American plums, damsons, mirabelle plums, myrobalan plums, etc. More recently, there are also a few 'interspecific hybrids,' by crossing plums with apricots or sweet cherries often marketed as Pluots™, Apriums™, or plumcots. The basic chromosome number of *Prunus* species is $x = 8$. While a number of them are true diploids, European plums are hexaploid. The polyploidy species emerged from two more ancient wild plums, which were the diploid cherry plum, *Prunus cerasifera* Ehrh, and the tetraploid blackthorn *Prunus spinosa* L. The product of this crossbreeding was cultivated and propagated and carried westward to Europe and later to other continents by the immigration of Europeans to other countries, including the Americas. Although several opinions are aired regarding the classification and the relationship between various plums, recent molecular evidences suggest that *Prunus* is monophyletic and descended from some Eurasian ancestor. The fruits of this family are rich in polyphenolic components, including anthocyanins, vitamin C, and fiber, which has led to increasing research into the functional properties of these widely consumed stone fruits and berries.

Plums and the related *Prunus* species are important international commodities due to their broad consumer acceptance and a number of value added preparations including canned, dried, and processed for cooking use or occasionally distilled into brandy. Similar to the other stone fruits, the history of prunes dates back thousands of years since prunes were used as a ready snack for centuries. Some varieties of the numerous cultivars were dried and preserved by desiccation in the sun or warm oven for continuous consumption after the harvest season. The virtues of prunes were extolled for its use in aiding digestion and curing mouth ulcers. European plums were introduced only relatively into North America when French horticulturist, Louis Pellier, brought European plums to California in the mid-to-late nineteenth century, resulting in the first-known prune production in North America.

Botany and Horticulture of Plums

Plums are deciduous temperate species and can tolerate zone 2 temperatures. In cultivation, they are grown in subtropic climates and also in higher elevations in the tropics where the temperature goes to near-zero temperatures, required for dormancy of these species. Most of the plums in nature are about 8–10 m tall with occasional spiny stems, although no spines or thorns are usually left in cultivation. The leaves are simple, alternate, elliptic to lanceolate, unlobed with a serrated margin, and often with nectaries on the leaf stalk. The color, size, and texture of the leaves vary slightly between cultivars. Leaves range from light green to dark green when mature and are quite tough and leathery. Young leaves are relatively soft and are often bronze or pale green in color. There are a few genotypes that produce dark, wine red leaves, which are often used as ornamentals. The flowers are mostly white and rarely light pink with five petals and five sepals. There are numerous stamens. Flowers are usually in umbels of two to six or sometimes more on racemes. While the flowers are generally perfect with functional stamen and pistil, incompatibility is very common among plums. The fruit is a fleshy drupe with a single hard-coated seed, often referred to as the stone. The fruit skin colors among Japanese plums are yellow, red, and black with various shades in between. Mostly, they exhibit solid color with some varieties showing mottled or freckled skin. The flesh is usually golden yellow to amber. There are some varieties with dark red flesh as well. Although they are known as 'Japanese plums,' they originated in China, but due to its popularization through Japan, they got the name.

The European plums are native to Eastern Europe around the Caspian Sea area. They are often blue black in color with a strong ashy coating and hence they are referred as 'blue plums.' Some varieties also exhibit green, yellow, and occasionally pink to purple colorations in skin. The flesh of European plums may be green, yellow, to amber brown. Plum fruit is about 5–6 cm in diameter and weighs 70–80 g at maturity. Externally, a central depression is often found at the stem end of the fruit. The fruit pulp is juicy with a sweet, tarty taste and a pleasant aroma. European plums are prone to lower incidences

of frost damage as they bloom later than in the spring than the Japanese plums.

Plums generally propagated by grafting as seedlings are not true to type. Most of the plums are grafted to another plum or a closely related species that provides the root system, called the rootstocks. Traditionally plums have been grafted on another species called myrobalan plums. However, modern plum growing involves a number of other rootstocks developed by breeders to address the industry demands. Japanese plums require a well-drained soil, while European plums can be grown even in fairly heavy soils. Typically, grafted trees are planted in the beginning of spring. Care must be taken to have the graft union at least an inch above the soil. It is important to take the wind direction into consideration as it will greatly aid in pollination as well as tree architecture. As the young trees grow into their second or third year, they are carefully thinned by selectively removing some branches to encourage proper passage of sunlight as the trees get older. This process is called training. The most common method of training for plums is 'open center' where three to four scaffolds are allowed to grow around leaving the central area for smaller branches to fill in. Another method that is popular is the 'central leader,' where a main scaffold grows in the center allowing branches to spread around it. Modern plum cultivation is gearing towards mechanization to facilitate ease of harvest and maintenance, although it tends to be expensive and is not practiced by small growers. Since plums produce copious amounts of branches every year, unwanted branches that would otherwise hinder cultivation practices are often removed in a systematic manner called pruning. Pruning is an annual event that helps to keep the growth to crop load ratio balanced. Pruning is mostly done just at the beginning of spring or in some cases at flowering. Proper training and pruning are the key to maintain the health, productivity, and longevity of the plum trees. It is very important to plant more than one variety of plum as plums are often cross-pollinated and requires a pollenizer. When choosing the pollenizer, only compatible cultivars should be chosen. Compatibility of any plum cultivar should be readily available with the nursery or any regional extension agents.

Production of Plums and Prunes

Although plums are primarily cultivated in the subtropic and temperate regions, there are producer countries across the world. The major prune producers in the world include the United States and France following the predominant production in China, as seen in Table 1. Asia is the largest producer of plums worldwide; North American producers contribute close to 5% of the worldwide average and the United States alone is the second highest exporter of plums and the top exporter of prunes internationally with 28% and 32% of the worldwide average, respectively. In particular, California has become the top producer over the past decade, producing about 67% of the world's dried prune supply, 178 million kilograms of prunes per year (Table 1).

Food and Nutritional Attributes of Plums

Both Japanese and European plums are largely consumed as a fresh fruit, either as a healthy snack or as a dessert. Since they come in a range of vibrant colors, both inside and out, they are preferred in fruit salads and bowls. The sweet and tangy taste of the plums coupled with their unique flavor adds to their food value. Both types of plums are processed into jams, spread, and chutney that are often classified as low-sugar products. Sliced Japanese plums go well in a number of entrées as they add their unique flavor and color to the food. Dried European plums constitute the prunes and hence people refer them as 'prune plums.' Not all varieties of European plums are good for drying and only a few varieties do well for prune making. The term prune is being phased out in favor of 'dried plums,' especially in California, where a study revealed that more than 80% of the respondents in the 25–54 age group preferred the term 'dried plum.' This is largely due to the perception that 'prunes' or prune products such as prune juice are associated with bowel movement and other health issues.

Current consumer appeal of plums is largely attributed to taste, texture, and soluble sugar content. However, plums also have low caloric content and relatively high nutritive value. These fruits contain carbohydrates, including sucrose, glucose, fructose, sorbitol, and isatin, as well as organic acids such as citric and malic acids, in addition to soluble fibers (pectins), tannins, aromatic substances, and enzymes. These substances contribute to nutritive value and taste of plums. The fruits are also high in health-promoting antioxidants such as flavonoids, polyphenols such as anthocyanins, and carotenoids such as cryptoxanthin, lutein, and zeaxanthin. These compounds are known scavengers of oxygen-derived free radicals and reactive oxygen species that are associated with aging and chronic diseases. Zeaxanthin is an important dietary carotenoid that is selectively absorbed into the retina and is considered to provide antioxidant and UV light-filtering properties. Their dried counterparts, prunes, contain significant quantities of soluble fiber in the form of pectin with at least 3 g per 100 g of fruit in a serving, which has been shown to decrease plasma concentrations of cholesterol, possibly through increased bile acid excretion in the feces. Plums are also rich in a variety of mineral and vitamins, including vitamins C, B1, B2, A, and PP, that are essential for certain functions in the human body. The minerals with known recommendations that are present in physiologically significant amounts include iron, magnesium, copper, zinc, manganese, and potassium. Specifically, the amount of iron that is bioavailable is also among the top in fruits and vegetables, which is attributed to the ascorbic acid content in prunes. Although some vitamins are degraded during the dehydration process at high temperatures, such as vitamin C, others may be concentrated by dehydration, resulting in more nutrient density in prunes than plums and other fresh fruits. In addition to vitamin C, a significant contribution may be made by riboflavin, niacin, and pyridoxine, which are present at levels of 10% of the recommended daily intake per serving of prunes. Prunes are one of the richest sources of antioxidants and soluble fibers and hence could qualify very well as a 'super food.'

Table 1 Production of plum crops worldwide in 2011

Country	*Production (tonnes)*	*Area harvested (Ha)*
Africa	324 342	49 751
Asia	7 090 653	1 820 729
Australia and New Zealand	17 686	3361
Europe	2 817 981	526 843
Northern America	562 288	35 491
Oceania	17 686	3361
South America	475 369	44 004
Canada	2464	401
World	11 359 707	2 495 351

Breeding and Improvement of Plums

Plum breeding or improvement is done largely by publicly funded academic or research institutes in several parts of the world. However, there are a few private breeders who have been successful in developing new and improved varieties as well. Being a perennial crop, breeding and improvement take a long time with much less success rate than other horticultural crops. Unlike several other fruits, a successful pollination can at best result in only one seed in *Prunus*. Hybridization is also dependent on a number of intrinsic factors such as compatibility and extrinsic factors among which weather is a major player. Published records show that plum breeding has been done for at least 150 years now. Among the major countries that are involved in plum improvement are Japan and China in Asia, Eastern European countries, England and Germany in Europe, South Africa and Canada, and the United States in North America. In Australia and South America, plum breeding is more recent and is very limited. It is safe to say that Japanese plum breeding is performed in more places than the European plums, which are currently performed only in a few places.

The general objective of any plum breeding program is to develop a variety that can be successfully grown for that specific locality with good marketability. As for Japanese plums, the focus largely is on fruit quality and their capacity for shipping since most of the Japanese plums are consumed as fresh fruit or in desserts. Since most of the Japanese plums are grown in relatively warmer regions, breeding programs aim for lower chilling requirements for a good production, while varieties developed for the northern latitudes should exhibit sufficient cold hardiness. In regions where several varieties are grown and there is a need to maximize the market potential, breeding cultivars to fit the gaps in the marketing window is often a priority. In addition to these, there are niche markets for certain types of plums that are also addressed in the breeding program. For instance, yellow-skinned Japanese plums such as Shiro and Early Golden or the red-fleshed varieties such as Red Heart and Vampire are preferred in certain local markets, and hence, breeding complementary varieties to these is addressed.

European plum breeding is often oriented towards the end use. Fresh market European plums are often bred with size, shape, and color of both skin and flesh in mind. Uniform-looking plums are preferred by retail markets so that they can be sold as 'club packs.' Often, three to four varieties that look extremely similar but ripen at different times are promoted in a particular region so that the consumers get similar fruits for an extended period. These varieties should be 'free stone' where the seeds (or pit) separate from the flesh easily. Further, such free stone varieties are also preferred for baking purposes where plum halves are often used and also for jam making. A large amount of European plums however goes for prune making and 'prune' cultivars should have a fairly high degree of sugars, have less water at ripening, and be easy to pit. Self-fertility is another factor that is increasingly important that precludes the planting of multiple varieties in a region or that of pollenizer.

Just like any other crop, both types of plums are affected by a number of diseases. Thus, resistance to these diseases is a common thread in any breeding program. Brown rot, a fungal disease caused by *Monilinia fructicola* that can cause serious economic losses, and bacterial spot caused by *Xanthomonas campestris* pv *pruni* are two important diseases for which field tolerance is needed in a good variety. With mechanization on the rise, breeding for tree architecture with smaller, compact trees with less pruning requirements is currently explored. Value addition is an important area that is being addressed in breeding as well. As mentioned earlier, plums are a rich source of beneficial phytochemicals. Tapping into these for marketing purposes is a good strategy in plum breeding, and thus, antioxidant- or nutrient-rich plum varieties can be expected in the market soon.

Related Fruits

A number of other fruits that are closely related to plums are grown and available in the market for various reasons. Some of them are just interesting variants of either Japanese or European plums or some are hybrids – mostly man-made. Among these, the most noteworthy are damsons, plum hybrids, and 'yellow European plums' (or YEPs) (Figure 1). Further, there are several other species of *Prunus* that are closely related to cultivated plums (Table 2).

Damsons

Damsons are often thought as a subspecies of *P. domestica* var. *insititia* or a species itself *P. insititia*. It is thought to have been in cultivation for much longer and is believed to have been brought from Syria to Britain where it gained most popularity

Figure 1 Plums related fruits. Top left – red Japanese plum, top right – yellow Japanese plum, middle left – European plum or blue plum, middle right – yellow European plum (YEP), bottom left – damson, bottom right – interspecific hybrid plumcot.

Table 2 Classification of cultivated plum species based on geographic distributions

Class	Prunus *species*	*Pomological*	*Cultivar*
European/Asian	*P. domestica* L.	Lombards Prunes	Victoria, Pond, Bradshaw D'Agen, Hungarian
	P. domestica var. *italica*	Greengages	Reine claude, Jefferson
	P. domestica var. *intermedia*	Yellow egg	Monroe, Golden Drop
	P. domestica var. insititia	Damsons	Shropshire, Crittenden
	P. domestica var. *syriaca*	Mirabelles St. Julians	Late Mirabelle, Drap d'Or
	P. cerasifera	Myrobalans (cherry plums)	Marianna, Lindsayae
American	*P. americana*	Common wild	
	P. nigra	Wild goose (Chickasaw)	Golden Queen, Cherokee
	P. angustifolia		Crimson, Oxford, Aitkin
	P. munsoniana		Caddo chief, Ogeechee
	P. hortulana		Jewel, Osage
	P. maritime	Beach plums	Golden beauty, Cumberland, Bassett's American, Sisson
	P. subcordata	Pacific Coast plums	
Oriental/Japanese	*P. salicina*	Japanese plums	Early Golden, Shiro
	P. simonii	Apricot plums	Wickson, Climax

in middle age. Stones of damsons have been found in excavation sites in and around Britain confirming its presence in the eleventh to thirteenth centuries. Other than Britain, it is grown sparingly throughout Europe and more so in Eastern European countries and Germany. It is not as popular in other continents including North America, although one can see small stands in these places, often sold in local markets as a niche product. Damsons were grown for their heavy cropping and small fruits with a rich color that were used in a number of ways. Other than the usual jam, jelly, and spreads that can be made out of these sugar-rich fruits, they were extremely coveted for dyeing clothes in those days. Further, the somewhat short and sharp branches made them ideal candidates for hedges and they were also very good as wind breaks. A number of varieties were grown, but Shropshire and Westmorland damsons were among the popular varieties. The damson fruits are quite small −10–15 g and 2.5–3.5 cm long. They are mostly oval with occasional round types in cultivation. There are also other 'fruits' often mixed up with damsons. 'Bullace' and sloe are commonly mixed with damsons. It is accepted that both are *P. insititia*. However, 'bullace' is considered as the wild form and the 'damson' refers to the cultivated form. If the fruits are small and roundish, it is likely to be the wild form as most of the cultivated forms are oval or elliptic. Sloe is another species, *P. spinosa*, and their fruits are probably the smallest of the group. It is very likely that 'sloe' and 'bullace' were hybridized to get some of the modern damsons.

Hybrid Plums

It is not uncommon to see a variety of Japanese plums in the market, especially in the slightly warmer growing regions such as California or eastern United States. Although they are mostly listed as 'plums,' in reality, many tend to be interspecific hybrids. Quite often, such varieties are the result of a cross between *P. salicina* and *P. americana* or *P. simonii*. More recently, there are a lot of hybrids between plum and apricot, originally called as plumcots by Luther Burbank, a renowned breeder of horticultural crops. In the current market, we see a number of hard plums in very unique colors both inside and out, often referred as Pluots® or Apriums® – hybrids between plum and apricots (*P. armeniaca*). Pluots® are more plum than apricot, while Apriums® are more apricots than plums. These were developed in the 1970s and still being continued by a private breeding firm, Zaiger Genetics in California, who have trademarked both the names. More recently, they have also developed NectaPlums, which are hybrids between plums and nectarines (*P. persica*). Most of these have a firm flesh and are fairly large in size and there are several varieties of these in the market.

Yellow European Plums

As mentioned earlier, European plums are generally blue black in color with rare variants of yellow or green color. Some of the earlier off-color varieties include 'greengage' and 'yellow gage,' which are not cultivated extensively. Recently, the plum breeding program in Canada has identified a number of such off types that range widely in color from yellow to green with intermittent pink blush and started calling them YEPs, an acronym for yellow European plums. These types are preferred by consumers as fresh fruit since they are quite sweet and do not have the slightly bitter aftertaste that typical blue plums exhibit due to the presence of phenolics. It was hypothesized that the phenolics may be less in these YEPs, thus making them less bitter. While the blue-black plums are rich in anthocyanins, YEPs may lack the anthocyanins and associated phenolic compounds. However, these YEPs may have more of other flavonoids and thus compensate for the lack of phenolics, and thus, their antioxidant potential is quite comparable to that of regular European plums. It is anticipated that using suitable preservation methods, prunes that are different in color and taste will be developed from these YEPs in addition to being a good fresh fruit.

Future of Plum Cultivation

As consumer awareness toward healthy eating is on the rise, it can be safely said that plum cultivation and availability will be steady in the market. However, changes are bound to happen as the improvement programs are always looking to meet the consumer and marketing demands. Thus, there will be an emphasis on breeding plums that have higher nutritional and health-promoting benefits as well as improved postharvest shelf life, which will help in shipping. With the world looking for continuous changes, newer products such as the interspecific hybrids and YEPs will find a place in the market shelf, while the traditional varieties will still be cultivated and eventually getting 'premium' status. The global push for 'local' foods will help the expansion of plums in wherever possible to attract the local food market. The postharvest treatment and processing of plums is also a critical factor that can help to optimize the quality of the fresh and dried plums. The storage conditions of the fresh fruit are imperative to preserve quality, including optimal temperature and light conditions. The typically cooler temperatures and reduced light conditions favor extended shelf life for both the fresh and dried fruit but may adversely affect the retention of natural phenolic compounds present. Modified atmospheric packaging, which has produced very good effects on retaining heath compounds in a number of fruits and vegetables, may be another area that will boost plums and prunes.

Further Reading

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Polycyclic Aromatic Hydrocarbons

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General Overview

Polycyclic aromatic hydrocarbons (PAHs) are a large group of carcinogenic organic compounds, which originated from incomplete combustion or pyrolysis of organic matter. They are formed by two to six fused aromatic rings (linear, cluster, or angular arrangement), and they solely consist of carbon and hydrogen. They can present alkyl substituents, depending on the generating process.

Although the International Union of Pure and Applied Chemistry defined a systematic nomenclature for these compounds, many of them have maintained their common name, reflecting the initial isolation from coal tar (e.g., naphthalene and pyrene), their color (e.g., fluoranthene and chrysene; the latter erroneously, because contaminated with naphthacene, which is orange, when first isolated), and their shape (e.g., coronene and ovalene).

The numbering and naming of PAHs are based on a few basic rules: Rings are drawn with two vertical sides (when possible), with as many rings as possible in a line and the rest of the structure sitting in the top right quadrant; the numbering starts from the first carbon atom not engaged in ring fusion in the right-hand ring and proceeds clockwise; the name of a fused-ring system is made up of a prefix of the fixed part (e.g., benzo) followed by an italic letter denoting the bond of the base (e.g., *a* refer to the 1,2-bond) and the name of the parent compound follows (e.g., benzo[*a*]anthracene reported in Figure 1). A list of the most analyzed ones is reported in Table 1, along with their chemical structure, molecular formula, molecular weight (mw), solubility in water at 25 °C, partition coefficient octanol–water (log K_{ow}), and abbreviation (that will be used all over the chapter).

PAHs are solid at ambient temperature and are the least volatile of the hydrocarbons (the boiling points of the PAH are markedly higher than those of the *n*-alkanes of the same carbon number). Nonetheless, light PAHs (2–3 rings) are present in the environment mainly in the vapor form, while heavier PAHs (more than five rings) are predominantly adsorbed on organic particulate matter, usually on small particles (<2.5 μm); four-ring compounds have an intermediate behavior. The high mw and the absence of polar substituent groups make these compounds very insoluble in water, but the presence of detergent or organic matter causing emulsions in water, or PAHs adsorbed on suspended particles, can increase the content of PAHs in wastewater or in natural waters.

Although PAHs are chemically stable and poorly degraded by hydrolysis, they are susceptible to oxidation and photodegradation, and this behavior is particularly relevant to possible losses during analysis.

Legislation

In the European Union, the necessity of a harmonized European legislation appeared evident after 2001, when a batch of pomace oil entering the Czech Republic was found highly contaminated. Following this event, some states fixed their own limits for PAH contamination in edible oil, mainly considering the 16 PAHs highlighted by the US Environmental Protection Agency (EPA) (called 16 EPA PAHs). These different limits among the European Member states caused several commercial problems.

In 2002, the Scientific Committee on Food (SCF) published an opinion where 15 PAHs, over 33 assessed for toxicity information, were identified as both genotoxic and carcinogenic. Furthermore, BaP was pinpointed as a suitable marker for PAH contamination in food. Subsequent to the opinion, the European Commission (EC) introduced a new regulation (Reg. 208/2005, shortly followed by Reg. 1881/2006) harmonizing the PAH legislation among all the member states and fixing a limit for the presence of BaP only, used as a marker of PAH contamination. The EC also published a recommendation (Rec. 2005/108/EC), requiring all the member states to investigate the presence of the 15 PAHs highlighted by the SCF plus the one (BcF) considered by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (called the 16 EU PAHs) to review the limits already set and to add new food classes by 1 April 2007. No new limits were set in 2007, but the European Food Safety Authority (EFSA) raised doubts on the suitability of BaP as a marker. In 2008, it was definitely concluded that BaP was not appropriate, while the sum of eight heavy PAHs (PAH8) (BaA, Ch, BbF, BkF, BaP, DBahA, BghiP, and IP) or of a subgroup of four (PAH4) (BaA, Ch, BbF, and BaP) was suggested. Following the EFSA opinion, the EC enacted a new regulation in 2011 (Reg 835/2011) fixing new limits considering both the BaP only and the PAH4. Additional food classes compared to the previous legislation were added, but the limits for these newly introduced classes came into force later than the previously regulated classes, giving more time to adapt the production technologies. The complete overview of the considered food classes and respective limits are shown in Table 2.

Meanwhile, the EC, not dictating any official method, fixed the performance criteria required by any analytic method applied for PAH analysis (Reg. 836/2011), extending the parameters set only for BaP in previous regulations (Reg. 333/2007 and Dir. 2005/10/EC), to all the analytes included in the PAH4 sum (Table 3). Finally, if any of the four PAHs is at the limit of quantification (LOQ), it would be considered as zero in the summing of the four PAHs.

Outside Europe, regulations are much less restrictive. In the United States, even though EPA has always paid a great attention to PAHs as environmental pollutants, no standards governing the PAH content of foodstuffs have been established by the US Food and Drug Administration (FDA). The only exception regards the level of PAHs in seafoods after the Deepwater Horizon oil spill, which occurred in the Gulf of Mexico in 2010. In response to this event, the FDA developed risk criteria and established threshold for allowable levels (levels of concern) for

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00550-X

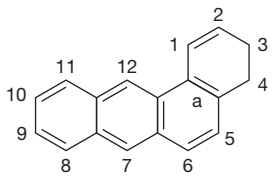


Figure 1 Numbering of benzo[a]anthracene.

Table 1 Compound name, abbreviation, molecular formula and weight (mw), solubility at 25 °C, log K_{ow}, and structure of some of the most studied PAHs

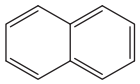
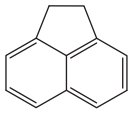
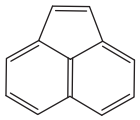
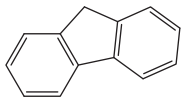
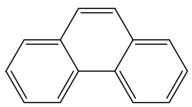
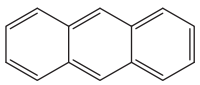
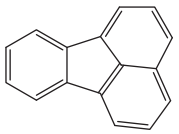
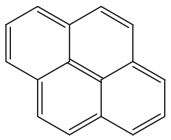
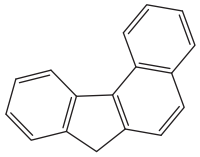
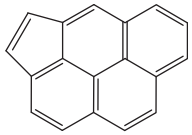
Compounds (abbreviation)	*Molecular formula (mw)*	*Solubility at 25 °C ($\mu g\ l^{-1}$)*	*Log K_{ow}*	*Structure*
Naphthalene (Na)	$C_{10}H_8$ (128)	12500–34000	3.37	
Acenaphthene (Ac)	$C_{12}H_{10}$ (154)		3.98	
Acenaphthylene (Ap)	$C_{12}H_8$ (152)	3420	4.07	
Fluorene (F)	$C_{13}H_{10}$ (166)	800	4.18	
Phenanthrene (Pa)	$C_{14}H_{10}$ (178)	435	4.46	
Anthracene (A)	$C_{14}H_{10}$ (178)	59	4.5	
Fluoranthene (Fl)	$C_{16}H_{10}$ (202)	260	4.90	
Pyrene (P)	$C_{16}H_{10}$ (202)	133	4.88	
Benzo[*c*]fluorene (BcF)	$C_{17}H_{12}$ (216)	103	5.19	
Cyclopenta[*c,d*]pyrene (CPP)	$C_{18}H_{10}$ (226)	33.52	5.70	

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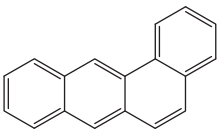
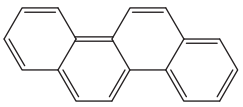
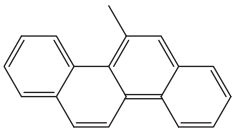
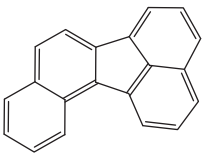
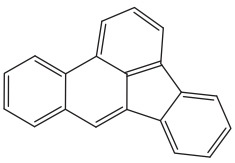
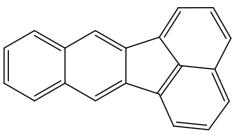
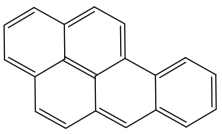
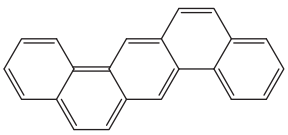
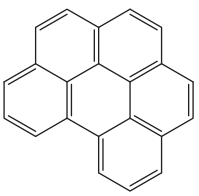
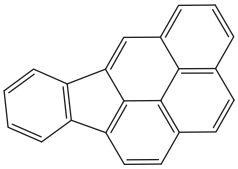
Table 1 (Continued)

Compounds (abbreviation)	*Molecular formula (mw)*	*Solubility at 25 °C ($\mu g\, l^{-1}$)*	*Log K_{ow}*	*Structure*
Benzo[*a*]anthracene (BaA)	$C_{18}H_{12}$ (228)	11.0	5.63	
Chrysene (Ch)	$C_{18}H_{12}$ (228)	1.9	5.63	
5-Methylchrysene (5MeCh)	$C_{19}H_{14}$ (242)	65	6.07	
Benzo[*j*]fluoranthene (BjF)	$C_{20}H_{12}$ (252)	2.4	6.21	
Benzo[*b*]fluoranthene (BbF)	$C_{20}H_{12}$ (252)	2.4	6.04	
Benzo[*k*]fluoranthene (BkF)	$C_{20}H_{12}$ (252)	0.8	6.11	
Benzo[*a*]pyrene (BaP)	$C_{20}H_{12}$ (252)	3.8	6.06	
Dibenzo[*a,h*]anthracene (DBahA)	$C_{22}H_{14}$ (278)	0.4	6.86	
Benzo[*g,h,i*]perylene (BghiP)	$C_{22}H_{12}$ (276)	0.3	6.78	
Indeno[*1,2,3-cd*]pyrene (IP)	$C_{22}H_{12}$ (276)	-	6.58	

(*Continued*)

Table 1 (Continued)

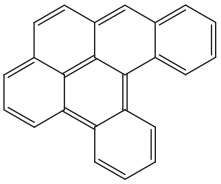
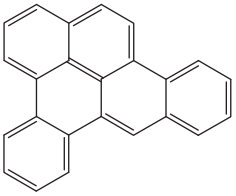
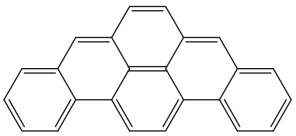
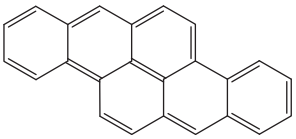
Compounds (abbreviation)	*Molecular formula (mw)*	*Solubility at 25 °C (μg l^{-1})*	*Log K_{ow}*	*Structure*
Dibenzo[*a,l*]pyrene (DBalP)	$C_{24}H_{14}$ (302)	0.24	7.71	
Dibenzo[*a,e*]pyrene (DBaeP)	$C_{24}H_{14}$ (302)	0.24	7.71	
Dibenzo[*a,i*]pyrene (DBaiP)	$C_{24}H_{14}$ (302)	0.5	7.28	
Dibenzo[*a,h*]pyrene (DBahP)	$C_{24}H_{14}$ (302)	-	6.26	

Table 2 Limits fixed by EC Regulation 835/2011 for BaP and the sum of PAH4 (BaA, Ch, BbF, and BaP)

	Maximum levels (μg kg^{-1})	
Foodstuffs	*BaP*	*Sum of PAH4*
Oils and fats intended for direct human consumption or use as ingredient in food (excluded cocoa butter and coconut oil)	2	10
Coconut oil for direct human consumption or use as ingredient in food	2	20
Cocoa beans and derived products	5	35[a] 30[b]
Processed cereal-based foods and baby foods for infants and young children Infant and follow-on formulae, including infant and follow-on milk Dietary foods for special medical purposes, intended specifically for infants	1	1
Smoked sprats and canned smoked sprats Bivalve mollusks (fresh, chilled, or frozen) Heat treated meat and meat products sold to the final consumer	5	30
Smoked bivalve mollusks	6	35
Muscle meat of smoked fish and smoked meat and meat products	5[c] 2[d]	30[c] 12[d]

[a]Until 31/3/2015.
[b]From 1/4/2015.
[c]Until 31/8/2014.
[d]From 1/9/2014.
Adapted from EC Regulation n 835/2011.

Table 3 Performance criteria required for analytic methods by the EC Regulation 836/2011

Parameter	*Value*
Applicability	Foodstuffs listed in Reg 1881/2006
Specificity	Free from matrix or spectral interferences, verification of positive detection
LOD	$\leq 0.3\ \mu g\ kg^{-1}$ for each PAH
LOQ	$\leq 0.9\ \mu g\ kg^{-1}$ for each PAH
Precision (repeatability and reproducibility)	$HORRAT_r$ or $HORRAT_R < 2$ in ring test
Recovery	50–120%

Adapted from EC Regulation n 836/2011.

shrimp, crabs, oyster, and finfish. Levels of concerns were calculated for 13 PAHs and their alkylated homologues selected among the most studied PAHs in petroleum mixture, namely, Na (including C1, C2, C3, and C4 homologues), F (including C1, C2, and C3), A and Pa (including C1, C2, C3, and C4), P, Fl, Ch, BkF, BbF, BaA, BaP, DBahA, and IP. A daily consumption of $13\ g\ day^{-1}$ for shrimp and crab, $12\ g\ day^{-1}$ for oyster, and $49\ g\ day^{-1}$ for finfish was considered for a reference person of 80 kg (http://www.fda.gov/food/ucm217601.htm).

Since 2001, the Canadian legislation has settled a limit of $3\ \mu g\ kg^{-1}$ for the sum of heavy PAHs (BaP, DBahA, BaA, BbF, BkF, IP, Ch, and BghiP) calculated on the basis of the toxic equivalence to BaP only in pomace oil. The toxic equivalence factor approach considers the risk of any single PAH compared to that of BaP. This approach assumes the additivity of the risks, but some studies showed that PAH mixtures can present not only additive but also synergistic and/or antagonistic effects. On the other hand, the toxicity of most environmentally relevant PAHs has not yet been quantified; therefore, the risk may be underestimated.

The Chinese government set a maximum level for the only BaP content in different classes of product, namely, $5\ \mu g\ kg^{-1}$ for cereal and cereal products, meat and meat products, and fish and fishery products both smoked and roasted and $10\ \mu g\ kg^{-1}$ for fats and oils (GB2762-2012).

In Australia and New Zealand, no regulatory limits have been set for PAHs, but the general approach is to minimize the exposure following the ALARA (as low as reasonably achievable) principle and applying good operational practices.

Source and Occurrence in Food

PAHs are formed from both natural and anthropogenic sources. The former includes natural combustion, such as forest fires and volcanoes, and long-term degradation followed by synthesis (fossil fuels contain extremely complex mixtures of these compounds, with a prevalence of highly alkylated ones). The anthropogenic sources are mainly related to incomplete combustion of organic material (forming mainly parent PAHs). The formation mechanism occurs primarily by partial cracking of organic compounds to smaller unstable fragments at high temperatures (pyrolysis), mostly radicals, which recombine to give relatively stable PAHs (pyrosynthesis). The main rate of formation occurs linearly between 500 and 750 °C, whereas above 3000 °C, they are not formed. The ratio between alkylated PAHs and parent PAHs can be an indication of a source of contamination.

Foods can be contaminated by PAHs through two main routes, namely, environmental pollution and lack of good manufacturing practices during food production. The two sources are discussed separately in the following sections.

Environmental Sources

Natural sources of PAH emissions in the environment are forest fires, volcanoes, hydrothermal process, oil seepage, and carbonization. Nevertheless, they are present in the environment mainly due to anthropogenic sources, such as exhaust from motor vehicles; domestic combustion of wood, oil, gas, and charcoal; industrial biomass combustion; open burning of agricultural debris; tobacco smoke, laying tar, coke oven, and roofing tar emission; asphalt roads; and coal, coal tar, and hazardous waste sites.

Once released to the atmosphere, PAHs may be transported quite far from the emission source and can be removed from the atmosphere by wet and dry deposition onto soil, water, and vegetation. In the air, PAHs are subjected to reactions with oxidant gases, such as NO_2, O_3, and SO_3, and are photooxidized. Many PAHs, such as BaP, are rapidly destroyed by UV light.

After deposition, PAHs may undergo different fates: In surface water, they can be volatilized, photolyzed, biodegraded, and bound to suspended particles or sediments or accumulate in aquatic organisms; in soil, they can be volatilized or undergo biotic or abiotic degradation, mainly photolysis and oxidation. PAHs in soil can also enter groundwater and be transported within an aquifer.

The relative solubility in water and organic solvents (characterized by the log K_{ow} value) affects transport and distribution of PAHs between different environmental compartments and their uptake and accumulation by living organisms. The chemical reactivity influences their adsorption to organic matter or their degradation. The PAH mixture profile, which may be found in foods and feeds contaminated by environmental sources, can be very different from the original source, due to the different properties within the large group of PAHs.

Due to the lipophilic characteristics of PAHs, high-water-content plant tissues have a limited amount of these contaminants, but the waxy surfaces of vegetables and fruits can concentrate PAHs through surface adsorption. The superficial contamination of vegetables in urban areas can be up to 100 times higher than in rural areas. Some authors found that the amount of BaP in fruits from rural areas ranged between 0.19 and $0.34\ \mu g\ kg^{-1}$, while in urban areas, it ranged between 30 and $60\ \mu g\ kg^{-1}$. Up to 50% of these kinds of contamination can be removed by careful washing of the surface of vegetables and fruits.

Technological Processing

Heat-processing procedures during food preparation, in particular grilling, roasting, and smoking, involving direct contact with combustion gases, are among the major sources of PAH

contamination in food. The level of contamination in smoked products is related to the time and temperature of processing, humidity, types of control and smoke used, and the design and types of kilns. Modern kilns have strict electronic control of the entire process. Different types of wood or woodchips used to generate smoke produce different amounts of PAHs, with the highest concentration found when spruce, hazel tree, plum tree, and aspen are used, whereas they are lowest when apple tree, alder, and maple are burned. Over the last decades, commercial smoked food production has generally replaced the traditional smoking process with the use of liquid smoke flavoring. The latter derives from smoke subjected to fractionation and purification; thus, the content of PAHs on the final product is greatly reduced and strictly controlled.

It has been demonstrated that in canned smoked fish, over 70% of the entire amount of BaP migrates from the fish to the oil fraction.

Roasting may also lead to direct or indirect contamination, but to a small extent, considering that the temperature reached is relatively low for PAHs formation (up to 260 °C). It has been proven that strong coffee bean roasting conditions lead to significant levels of P, Ch, and BaA but low BaP (approximately 0.2 μg kg^{-1}). However, the transfer from the coffee bean to the coffee brew was reported low (in general lower than 140 ng l^{-1}) and obviously related to the water solubility of the specific PAH.

Differently from industrial process, considerable variability was found in the PAH level in domestic grilled products, depending on the heat source, the type and geometry of the grill, the composition of the food grilled, and the cooking time. However, since PAHs arose mainly from the pyrolysis of fat dropped into the flame, as long as the food is protected from direct contact with flames, contamination can be controlled.

Another important source of contamination of PAHs is related to the mineral oil contamination, which is composed of two main groups of compounds, mineral oil saturated hydrocarbons (MOSH) and (mainly alkylated), mineral oil aromatic hydrocarbons (MOAH). Mineral oils, previously purified of the aromatic fraction (food-grade quality), are widely employed in the food industries as releasing agents, antidust agents, etc. However, accidental or intentional contamination with nonfood-grade mineral oil may occur. The main sources of the latter are jute bags, recycled cardboard, plastics, lubricating oils, printing inks, and environmental contamination.

Occurrence and Dietary Intake

Several reports have been published concerning surveys of PAH contamination in foodstuffs. The most recent one is the report published by EFSA in 2008 on the data collected from the European member states on request of the EC (Rec. 2005/108/EC). Figure 2 outlines a summary graphic on the BaP content (average and the maximum amount found) in different Codex food categories, extrapolated from the most relevant data in the report (considering only the food categories in which more than ten samples were analyzed).

Among the main categories, the most contaminated ones, on average, were coffee and tea, food supplements, herbs and spices, fresh and treated mollusks, and fats and oils. There is no specification of the type of lipids included in the two distinct categories reported, namely, fats and oils and vegetable oil. However, in a previous report published in 2004 by the EC, olive pomace oil, grape seed oil, and seed oils resulted much more contaminated compared to virgin olive oil, due mainly to the drying process carried out before oil extraction and the raw material handling. The refining process reduces the amount of PAHs; specifically deodorization removes light PAHs, while bleaching in the presence of activated charcoal reduces heavy PAHs.

A high level of contamination can be found in smoked meat and fish, which is related to the method of smoking. The liquid smoked products are practically noncontaminated, while the traditionally smoked products can present over 5 μg kg^{-1} of BaP.

Seawater may be highly contaminated with PAHs due to oil spills, industrial and urban effluents, and leaking from creosote wharfs and pilings. However, bluefish species, such as tuna, mackerel, and salmon, generally present lower contamination than mollusks. Indeed, fish, in contrast with bivalves, oxidize and metabolize PAHs to water-soluble compounds, which are gradually excreted. Since bivalves filter large volumes of water, their levels of BaP can be significantly high (several hundreds of ng g^{-1}).

There are not many studies on the dietary intake of PAHs, and it is difficult to generalize since it is strictly related to the availability of food consumption surveys and their criteria and the PAH occurrence data in the national diet. In 2005, the EFSA claimed the necessity of a common database on food consumption to improve the consistency and reliability of exposure assessment carried out by the various EFSA Panels. As a result, in 2007, the development of the Concise European Food Consumption Database started, which was published in 2008.

Upon processing of such data, cereals and seafoods were the main contributors to the dietary intake, mainly due to high consumption, rather than a high contamination level of these products. In particular, cocoa butter and pomace oil were excluded from the food categories examined, since they were not consumed as such; therefore, the fats and oils category, which was among the main sources in a previous survey by the JECFA, was surpassed by seafood.

Notwithstanding the general low consumption, some foodstuffs present very high levels of contamination and may affect the overall exposure in particular cases. Some food supplements (such as fish oil, ginseng, bee products, and plant extract), whose consumption is very variable depending on their intended use, were found highly contaminated (up to 270 μg kg^{-1}). Equally, some spices were found with very high level of PAHs (up to 75 μg kg^{-1}, with an average of 4.75 μg kg^{-1}).

Considering all the European Countries, an average daily intake can be estimated in the 185–255 ng day^{-1} range for BaP and in the 936–1449 ng day^{-1} and in the 1415–2136 ng day^{-1} range for PAH4 and PAH8, respectively.

Toxicity and Risk Assessment

PAHs, once absorbed in the organism, become toxic during the attempt to eliminate them through feces or urine. In fact, some intermediates of the metabolic pathway are very reactive,

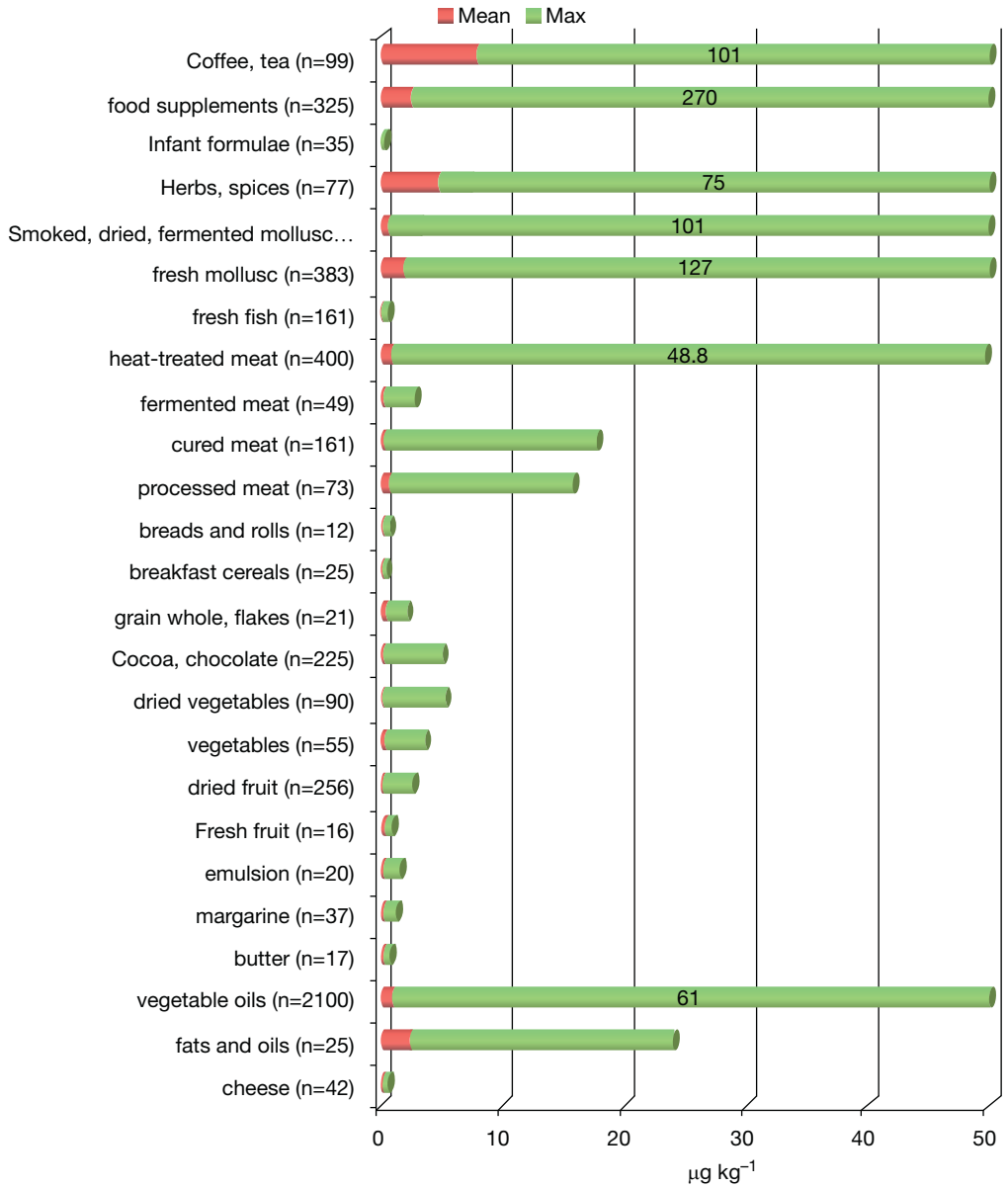


Figure 2 A summary graphic on the BaP content (average and the maximum amount found, μg kg^{-1}) in different Codex Food Categories, extrapolated from the most relevant data reported in the report published by EFSA in 2008. Reproduced from European Food Safety Authority (EFSA) (2008). Polycyclic aromatic hydrocarbons in food, scientific opinion of the panel on contaminants in the food chain (Question N° EFSA-Q-2007-136), The EFSA Journal 724, 1–114.

forming adducts with DNA, while the final product can be used as an exposure biomarker.

Absorption

There are three main routes of absorption of PAHs in humans: inhalation, skin contact, and ingestion. The rate of absorption by the lungs depends on the structure of the PAH, the size, and the chemical nature of the particles where PAHs are adsorbed. The absorption through skin contact is characteristic of occupational exposure, like coal cooking, petroleum refining, and road paving, and it involves both diffusional and metabolic processes. The main route that will be discussed hereafter is ingestion of PAHs occurring through food and water. Some authors have suggested two phases involved in the absorption process: uptake by the mucosa, followed by diffusion through the intestinal wall. The composition of the diet affects PAH absorption. In fact, it has been shown that lipophilic foods increase absorption of ^{14}C-BaP, while cellulose, lignin, bread, rice flakes, and potato flakes suppress it in Wistar rats.

Due to the lack of functional groups, these compounds can easily pass through lipoprotein membranes and reach all internal organs. Several authors have shown that detectable levels of PAHs can be identified in almost all internal organs. Organs rich in adipose tissue can store hydrocarbons and gradually release them, while the gastrointestinal tract contains high

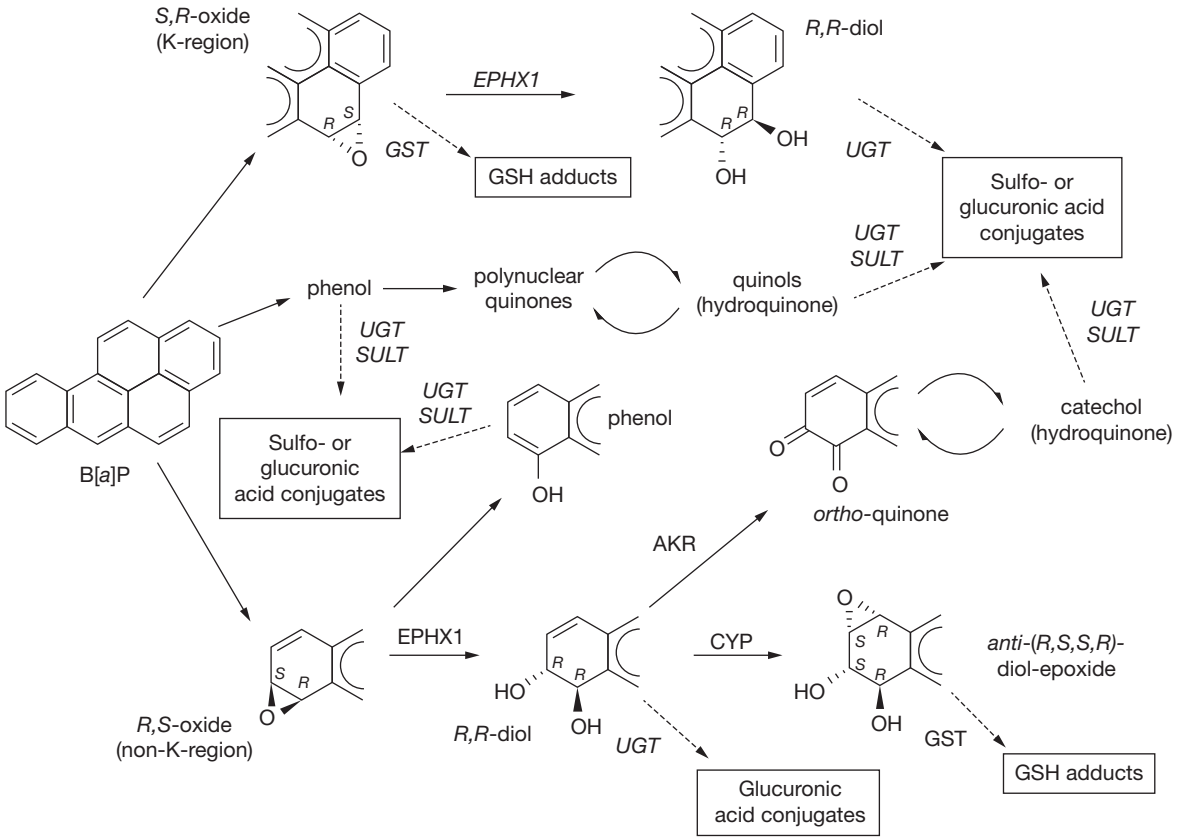


Figure 3 Scheme of benzo[a]pyrene metabolism. Reproduced from Luch A., Baird W.M. (2010). Carcinogenic polycyclic aromatic hydrocarbons. In McQueen C. A. (ed.) Comprehensive Toxicology (2nd ed.), pp. 85–123. Elsevier.

levels of PAHs and their metabolites, independently of the route of absorption.

Metabolism and Excretion

The metabolism of PAHs, using BaP as an example, is schematized in Figure 3.

The general scheme of the PAH metabolism involves oxidation to a range of primary (epoxides, phenols, and dihydrodiols) and secondary (diol epoxides, tetrahydrotetrols, and phenol epoxides) metabolites, followed by conjugation with glutathione, glucuronic acid, or sulfate. A large number of inducible enzymes are involved in these stages (microsomal cytochrome P450-dependent monooxygenase, glutathione transferases, dehydrogenases, hydrolases, peroxidases, and oxidoreductases). The most important metabolite of BaP is benzo[a]pyrene-7,8-diol-9,10-epoxide, which presents the highest tumor-inducing activity, because it forms adducts with proteins or DNA. The main site for bonding is the amino group of guanine or adenine (Figure 4). If the adduct PAH–DNA is not repaired, it can start a carcinogenic process.

The major metabolites of BaP excreted through different routes are 3-hydroxy- and 9-hydroxy-BaP, part of them conjugated to sulfate or glucuronic acid.

Most of the metabolites are excreted through feces, while a smaller proportion by urine. Excretion of metabolites through milk may occur as well, but to a lower extent. The proportion among the excretion routes varies with the PAH congener.

Although the use of several biomarkers in urine, such as 1-hydroxypyrene, hydroxynaphthalene, and hydroxyphenanthrene, is widely accepted to estimate the PAH exposure in workplace and in smokers, there are still few studies on dietary exposure evaluation. Several methods have been developed to assess human exposure through urinary biomarkers, such as 1-hydroxypyrene, a metabolite of pyrene. However, the relative amount of P and BaP may vary considerably between different sources of contamination, and proofs of correlation are still weak. Quantification of adducts of PAHs with DNA in peripheral lymphocytes, and other tissues and with proteins, such as albumin, has also been used as an indicator of the dose of the reactive metabolite.

Toxicity Evaluation and Risk Assessment

Most of the toxicity studies on PAHs have been carried out on dermal, subcutaneous, and inhalation exposure, while only limited studies have dealt with oral administration.

Table 4 reports the evaluation of toxicity (genotoxicity and carcinogenicity) according to the International Programmme on Chemical Safety (IPCS) of the WHO, the International Agency for Research on Cancer (IARC), and the SCF.

Fourteen of the 33 PAHs considered by the study of the SCF published in 2002 showed clear evidence of both genotoxicity

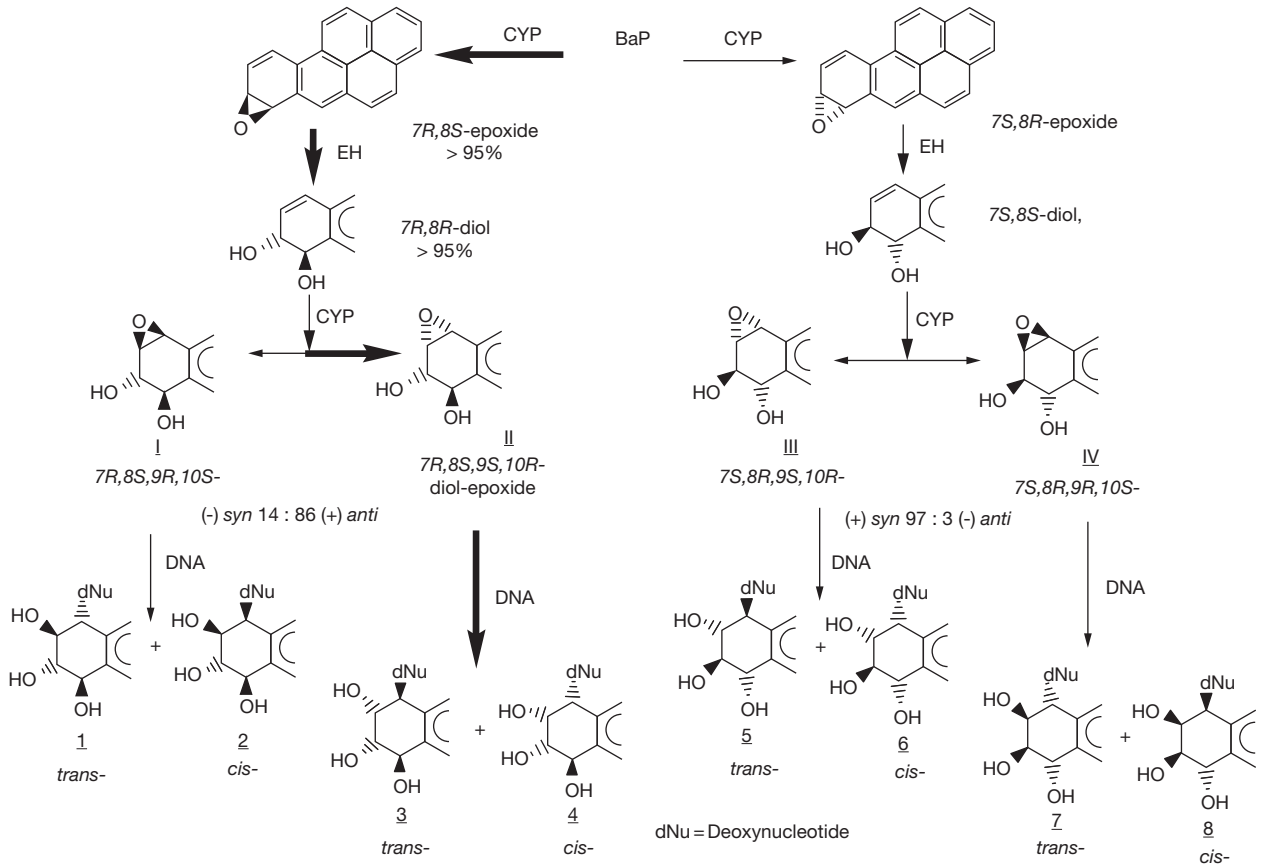


Figure 4 Formation of BaP-DNA adduct: configurational isomers of BaP-7,8-diol-9,10-epoxide and DNA adducts. Reproduced from Xue W., Warshawsky D. (2005). Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. *Toxicology and Applied Pharmacology* 206(1), 73–93.

and carcinogenicity, according to both SCF and IPCS. The PAHs are BaA, BbF, BjF, BkF, BaP, Ch, CPP, DBahA, DBaeP, DBahP, DBaiP, DBalP, IP, and 5-MeCh. BghiP is considered genotoxic, but not carcinogenic due to the limited data. According to IPCS, the carcinogenicity of Ac, Ap, benzo[g,h,i] fluoranthene, benzo[a]fluorene, benzo[b]fluorene, benzo[e] pyrene, coronene, Na, Pa, and P was considered questionable. Among these, Na was considered to be noncarcinogenic due to its negative genotoxicity. Finally, A, benzo[g,h,i]fluoranthene, F, 1-methylphenanthrene, perylene, and triphenylene were evaluated as noncarcinogenic.

According to the experiments carried out using the same route of exposure, the most potent carcinogenic PAHs seem to be DBahA, DBahP, DBalP, BaP, BbF, and 5-MeCh. BjF and BkF are of moderate potency, while BaA and Ch are relatively weak.

Tumor formation is related to the route of administration but not restricted to the site of application, that is, oral administration can induce tumor of the gastrointestinal tract, dermal application can induce mainly skin tumors, and inhalation can induce mainly lung tumors; however, liver and mammary gland tumors may be developed as well.

The risk assessment process consists of several steps including hazard identification, hazard characterization, exposure assessment, and risk characterization.

For most toxic process, there is a critical level of interaction, below which the homeostatic mechanisms would be able to avoid any structural or functional changes. Nevertheless, when genotoxic substances interacting with DNA, directly or after metabolic transformations, are considered, the absence of a threshold in their mechanism of action is generally assumed. This means that there is no dose without a potential effect. The general approach to minimize the exposure to these xenobiotics is expressed in the ALARA (as low as reasonably achievable) principle. However, this principle is not helpful to the risk management, which should establish a reasonable level of risk.

To evaluate the cancer risk of any xenobiotic, epidemiological data are the more informative and reliable way. However, when the latter are not available, animal assays are used to determine the risk. These experiments are carried out by exposing animals to high levels of toxic compounds in order to compensate for the low number of experimental groups and to detect significant tumor incidence in a reasonable time frame. Therefore, the extrapolation of low levels of exposition to which a human population may be exposed is necessary and very delicate. A wide range of models can be used to extrapolate these data, but unfortunately, each leads to a very different conclusion. In 2005, the SCF suggested the possibility to use the margin of exposure (MoE) approach. It is similar to the

Table 4 Evaluation of genotoxicity and carcinogenicity of several PAHs according to different references

Compounds (abbreviation)	*Genotoxicity (SCF, 2002)*[a]	*Genotoxicity (IPCS, 1998)*[b]	*Carcinogenicity (IPCS, 1998)*[b]	*Carcinogenicity (IARC, 1987)*[c]
Naphthalene (Na)	Probably not genotoxic	Negative	Questionable	
Acenaphthene (Ac)	Inadequate data	Inconsistent, limited database	Questionable	
Acenaphthylene (Ap)	Inadequate data	Inconsistent, limited database	No studies	
Fluorene (F)	Inadequate data	Negative, with few exception	Negative	
Phenanthrene (Pa)	Equivocal	Inconsistent	Questionable	3
Anthracene (A)	Not genotoxic	Negative, with a few exceptions	Negative	
Fluoranthene (Fl)	Equivocal	Positive	Positive, limited database	
Pyrene (P)	Not genotoxic	Inconsistent	Questionable	3
Cyclopenta[*c,d*]pyrene (CPP)	Genotoxic	Positive	Positive	3
Benzo[*a*]anthracene (BaA)	Genotoxic	Positive	Positive	2A
Chrysene (Ch)	Genotoxic	Positive	Positive	3
5-methylchrysene (5MeCh)	Genotoxic	Positive	Positive	2B
Benzo[*j*]fluoranthene (BjF)	Genotoxic	Positive	Positive	2B
Benzo[*b*]fluoranthene (BbF)	Genotoxic	Positive	Positive	2B
Benzo[*k*]fluoranthene (BkF)	Genotoxic	Positive	Positive	2B
Benzo[*a*]pyrene (BaP)	Genotoxic	Positive	Positive	2A
Dibenzo[*a,h*]anthracene (DBahA)	Genotoxic	Positive	Positive	2A
Benzo[*g,h,i*]perylene (BghiP)	Genotoxic	Positive	Negative, limited database	3
Indeno[*1,2,3-cd*]pyrene (IP)	Genotoxic	Positive	Positive	2B
Dibenzo[*a,l*]pyrene (DBalP)	Genotoxic	Positive, limited database	Positive	2B
Dibenzo[*a,e*]pyrene (DBaeP)	Genotoxic	Positive	Positive	2B
Dibenzo[*a,i*]pyrene (DBaiP)	Genotoxic	Positive	Positive	2B
Dibenzo[*a,h*]pyrene (DBahP)	Genotoxic	Positive, limited database	Positive	2B

[a]SCF, Scientific Committee on Food (2002). Opinion of the Scientific Committee on Food on the risks to human health of polycyclic aromatic hydrocarbons in food. 4 December 2002. European Commission, Brussels.

[b]IPCS, International Programme on Chemical Safety (1998). Selected non-heterocyclic polycyclic aromatic hydrocarbons. Environmental health criteria 202. Geneva: International Programme on Chemical Safety, World Health Organization.

[c]IARC, International Agency for Research on Cancer (1987). Overall evaluation of carcinogenicity: an updating of IARC Monographs Volumes 1 to 42. IARC monographs on the evaluation of carcinogenic risks to humans. Supplement 7. Lyon: International Agency for Research on Cancer, World Health Organization.

margin of safety (MoS), where the no observed adverse effect level is compared to human exposition to the xenobiotic. However, the MoS approach is not appropriate for carcinogenic and genotoxic substances, where there is not a level without effect, while the MoE is. The MoE is the ratio between a defined point on the dose – response curve for the adverse effects and the human intake. To establish the appropriate point on the dose – response curve the SCF considered the benchmark dose (BMD), which is calculated on the best-fitting curve to the experimental data. In particular, it has been recommended to use the BMDL10 (BMD lower confidence limit 10%), which is the lower limit of the interval of confidence at 95% of the BMD, because this is the lowest effect measurable as statistically significant. Furthermore, considering that at low doses the carcinogenic process seems to be nonlinear, and the assays are carried out on animals, other two factors of 100-fold have been added to include the inter- and intraspecies variability and the uncertainty of the carcinogenic process. Therefore, an MoE of 10 000 or higher would generally be of low concern for the public health.

Comparable approaches have been used by Health Canada for Priority Substances under the Canadian Environmental Protection Act, the National Health and Medical Research Council in Australia and New Zealand for the Toxicity Assessment for Carcinogenic Soil Contaminants, and the JECFA.

Analytic Determination

Sample Preparation

Analytic methods have to be carefully optimized and be very sensitive and selective, minimizing handling to avoid spurious contributions from solvents and glassware and manipulation. Plastic materials have to be avoided since PAHs can be adsorbed (especially in polyethylene); thus, inert materials, such as aluminum, glass, and stainless steel, previously rinsed with high purity acetone or hexane, should be used. Extracts have to be protected from light by using amber or foil-wrapped vials to avoid photooxidation of analytes, and drastic evaporation should be avoided to minimize loss of more volatile compounds. The use of a keeper, such as toluene, may reduce such a loss.

For PAH determination in complex samples, the first step is to extract the lipid fraction to which PAHs are associated. Traditionally, Soxhlet extraction or saponification using alcoholic KOH, followed by a purification step performed by gel

permeation or, recently, by solid phase extraction (SPE), was employed.

Over the last decades, more efficient and/or rapid techniques, which minimize sample manipulation and solvent consumption, have been largely employed for PAHs analysis.

Many papers report the use of ultrasound-assisted extraction, a cheap and easy technique, which, if properly optimized, can be a satisfactory alternative to Soxhlet and saponification extraction, depending on the nature of the contamination and the complexity of the matrix. Generally, the sample has to be lyophilized to optimize the extraction yield.

More innovative techniques have been used exploiting the effect of pressure and temperature to improve the extraction efficiency and rapidity, such as pressurized liquid extraction (PLE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) using CO_2. To obtain a satisfactory extraction yield over an extended range of PAHs by SFE, the addition of a modifier (e.g., methanol) was reported, leading to a necessity of a further purification step. More effective results were obtained by mixing a sorbent (e.g., C18 beads) with the ground sample directly on the extraction thimble (Figure 5).

Applying classical PLE extraction (with the extraction vessel packed with the ground sample and inert material), a further cleanup step is required to purify the analytes from the coextracted lipids. Many works proposed the use of a sorbent directly mixed in the vessel to perform a single extraction/purification step. In general, activated silica (over Florisil®, sulfuric acid-impregnated silica, and neutral, basic, and acidic alumina) gave better results.

A different approach to reduce coextracted lipid matter can be applied in the MAE technique. A simultaneous saponification and extraction method can be performed in the same extraction vessel. The approach succeeded for simple samples, such as vegetables and propolis, but still a mild purification was needed when complex samples, such as fish and meat, were analyzed.

SPE is the most applied approach for purification, not only for lipid extracts but also for fatty and nonfatty liquid foods, such as edible oil, milk, water, and beverages (wine, beer, and spirits). Many different sorbent phases have been employed to exploit different retention mechanisms according to the nature of the sample. Among the traditional stationary phases, silica, C_{18}, C_8, Florisil®, styrene–divinylbenzene, or mixture of these phases has been the most employed, giving satisfactory purification and recovery results. Recently, the applications of novel sorbent phases for PAHs analysis have been published, such as molecular imprinted polymers, sulfonated graphene sheets, and carbon nanotubes.

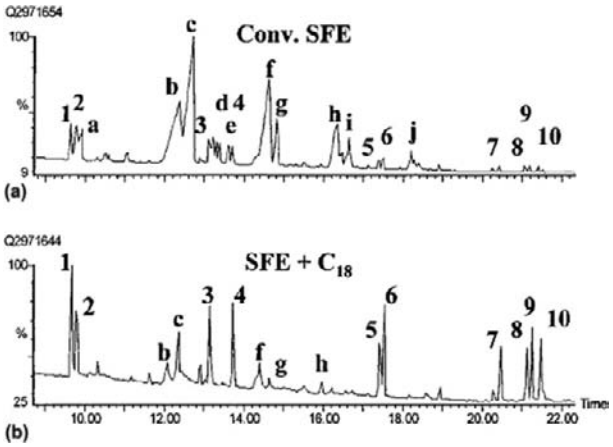


Figure 5 GC-MS chromatograms of a spiked crab tissue extracted by SFE. (a) Conventional SFE; (b) SFE plus C_{18} as sorbent. Peaks: (1) Pa. (2) A, (3) Fl, (4) P, (5) BaA, (6) Ch, (7) BbF, (8) BeP, (9) BaP, (10) perylene; letters are natural lipids. Reprinted with permission from Ali M.Y., Cole R.B.(1998). *Analytical Chemistry* 70, 3242–3248.

Solid-phase microextraction (SPME) has been largely employed thanks to its peculiar characteristics. In fact, it is easy to use, rapid, and practically solvent-free. It can be used both in the head-space (HS) and in direct immersion (DI) mode by carefully selecting the fiber coating. HS-SPME is obviously more selective for more volatile PAHs (2–4 rings compounds) rather than a heavy one. However, even in the DI mode, the selectivity does not improve much when a polydimethylsiloxane (PDMS) fiber is employed for analyzing PAHs in water (Figure 6). Sensitivity can be much improved using a stir bar sorptive extraction approach, which exploits the same principle as SPME, employing a larger amount of coating on a magnetic stir bar, but the discrimination between light and heavy PAHs persists.

Interesting results were obtained using a noncommon fiber coating, namely, Carbopack Z/PDMS, which has a high affinity for heavy PAHs, while very low, almost none, for light PAHs. The primary mechanism, when dipped in a nonpolar solvent, is the π–π interaction between the carbon surface and the planar aromatic compounds. Such a fiber was used directly immersed in a diluted oil solution, obtaining very good limits of detection and repeatability. Sensitivity can be further improved by performing a rapid liquid–liquid extraction of the edible oil to reduce the bulk of triglycerides, thus improving the signal-to-noise ratio in the gas chromatographic (GC) analysis.

Chromatographic Techniques

Both liquid chromatographic (LC) and GC techniques can be used to analyze PAHs after sample purification. The LC systems are usually equipped with fluorimetric detectors (FLD), which give very high selectivity and sensitivity, although in recent years, the use of the mass spectrometer (MS) has been increased, obtaining sensitivity comparable to FLD. Despite the significant lower sensitivity, UV detector is still used in several works.

LC equipped with dedicated C_{18} columns were widely employed to easily resolve the 16 EPA PAHs, but since the focus has moved to the 16 EU PAHs, a proper separation of all the isomers has become more cumbersome. Some critical pairs, such as P and BcF, BjF and BeP, and DBahP and BghiP, are not resolved even using a different step elution gradient; thus, more FLD detectors in series are employed by setting different wavelengths for the eluted PAHs in each FLD, thus exploiting the selectivity of the detector. Furthermore, the use of a UV detector is required to detect CPP, which does not give rise to fluorescence. GC-MS is a valid alternative to circumvent the absence or the low intensity of the fluorescence signal, as is the case of CPP and alkylated PAHs. Sensitivity of the pg order can be reached using the single ion monitoring (SIM) mode in

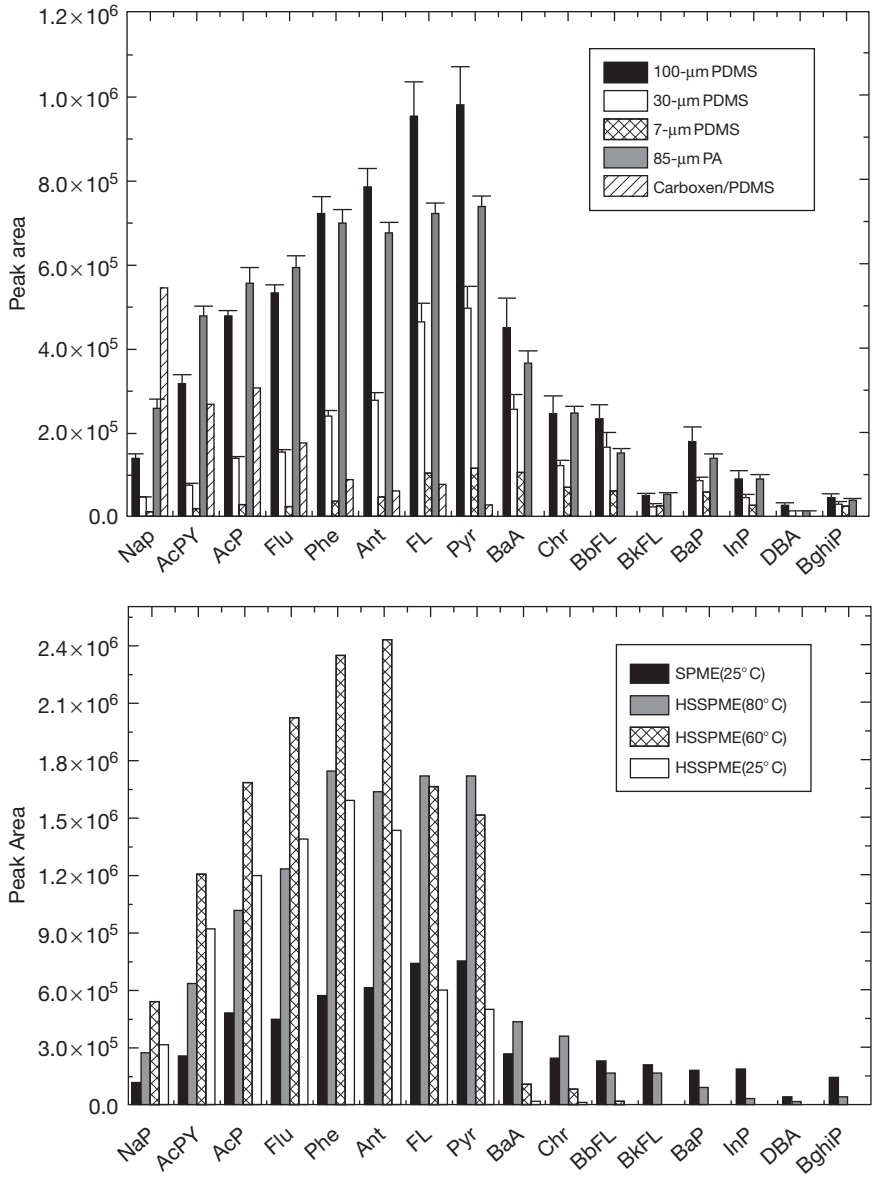


Figure 6 Profile of PAH uptake obtained using different fiber coating in SPME in (a) head-space mode and (b) comparison between direct immersion and head-space at different temperature. Reprinted with permission from Doong R., Chang S., Sun Y. (2000). *Journal of Chromatography A* 879, 177–188.

the MS detector. The US EPA did not consider the GC reliable enough due to several coelution problems, thus suggesting the use of LC, but it has been largely proved that a careful selection of the stationary phase and column dimensions gives an optimal resolution of critical pairs. Good results can be obtained using a 50% phenyl column. The selection of the injection mode (splitless, PTV, or on-column), along with the proper injection liner (multibaffle, packed, etc.), is fundamental in avoiding discrimination, especially of the lighter PAHs.

Hyphenated Chromatographic Techniques

An interesting approach to reduce sample manipulation and thus the risk of cross contamination is the application of hyphenated techniques. The most exploited technique is the coupling of an LC step (equipped with one or two LC columns) for purification purposes, followed by GC analysis for the final determination. This technique has been mainly applied to analyze alkylated PAHs.

The analysis of parent PAHs can be performed using a silica column in the LC purification, retaining triglycerides, which are then removed by backflushing the column, while PAHs are eluted and analyzed in GC-MS, obtaining a limit of detection of about 0.5 $\mu g\ kg^{-1}$ in SIM mode. Coupling two LC columns of different selectivity prior to GC analysis made it possible to analyze alkylated PAHs in edible oil. A first silica column removed triglycerides, while a second amino one allowed to fractionate PAHs according to the ring number.

Several applications coupled two LC systems or similar chromatographic systems, such as the donor-acceptor complex chromatography (DACC). The working principle of DACC is based on the π–π interaction between PAHs and the sorbent, which was exploited to retain PAHs while removing the matrix interferences, and then PAHs were backflushed directly onto the LC analytic column to be analyzed. A silica column was also applied as preparative chromatography prior to a C_{18} column for PAH separation.

Although comprehensive multidimensional GC ($GC \times GC$) cannot disregard a preparation step, the increased separation power, obtained by coupling two columns with different selectivity, can enable the isolation of matrix interferences; thus, in several cases, a less intensive cleanup step can be performed. $GC \times GC$ has been often applied to characterize alkylated PAH contamination, rather than to analyze parent PAHs, even if some few papers have been published on this.

Conclusion and Future Perspective

PAHs are a class of compounds that have been under investigation for a long time. However, further investigation is still needed, in particular considering the high toxicity of such compounds, along with the possible occurrence in rather complex mixtures. In particular, the EFSA is still requiring data on oral carcinogenicity of mixtures relevant for dietary exposure and occurrence data for less considered and often less abundant compounds, such as BcF. Therefore, to satisfy such requirements, improved analytic methods, in terms of both selectivity and sensitivity, are required to study the occurrence of PAHs in different food matrices and of metabolites in biological fluids. Miniaturized and hyphenated techniques will lead the development of further methods in the next future following the general trend of reducing solvent and time consumption and reducing sample manipulation avoiding artifacts formation.

An interesting future challenge will be to extend the study to the reliable identification and quantification of not only parent PAHs but also the alkylated ones, for which a detailed toxicological evaluation is still lacking.

See also: Carcinogenic: Carcinogenic Substances in Food; Carcinogens: Identification of Carcinogens; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Codex Alimentarius; Risk Assessment of Foods and Chemicals in Foods.

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Sources and Production

A Brief History

Although the remains of domestic species of pigs were not discovered until Neolithic times, fossilized pig skeletons have shown their emergence as early as 2.6–5.3 Ma (Pliocene period) in Europe and Asia. The domesticated pigs of Europe and North America are descendants of two species of wild pigs: *Sus scrofa* and *Sus scrofa vittatus*, the wild boar of Europe found north of the Alps and wild pig found only in the Malay Peninsula, respectively. Pigs are a nonnative species to the Americas and were transported across by early explorers and settlers of the new world. Other wild pigs, such as *S. scrofa cristatus*, are found in India and Ceylon, and there is also a number of subspecies of wild swine currently recognized, for example, Sus scrofa scrofa (Europe), S. scrofa meridionalis (Mediterranean), S. scrofa barbarous (North Africa), and S. scrofa attila (East Asia). Consumption of pigs (wild, large and small) is evidenced in the archaeological investigations surrounding prehistoric civilization eating places, but evidence of their domestication is not apparent until man's transition from hunter to settled farmer around 6900–6400 BC. The reason for this may be due their intractable nature and that they do not herd easily like ruminants. Another factor could have been the parasites they carry, such as pork trichinella, which could also explain the underlying reason for religious movements preventing their consumption. However, a number of factors may have endeared them to primitive farmers, such as their rooting habits (reducing woodland undergrowth and allowing grass to grow), used to hunt for underground mushrooms (truffles), or their passing over seeded ground to embed the seeds before plowing equipment was discovered.

Pork Quality

The eating and technological quality of pork will ultimately determine the success of the commodity in global markets. Pork quality can be considered as a series of interacting chemical, biochemical, physical, and biological components that determine the suitability of pig meat for further processing and storage. The technological quality would be affected by attributes such as breed, genotype, preslaughter handling, stunning, and slaughter method, while quality attributes (fat content, oxidative stability, and uniformity) would be affected by genotype and feeding strategy. Other key characteristics, such as water holding capacity (WHC) and color, would be affected by most or all of these factors.

Primary Production

Breeding and Breeds

Breeding is the act of selecting specific animal types for reproduction in order to promote certain desirable traits considered to be advantageous. Producers have had to adapt to the demands of consumers as early as the eighteenth century. For example, pigs that were able to store large quantities of fat were then prized due to differing consumption patterns of the era, that is, its high calorific content was ingested for energy due to the considerably more common labor-intensive working day (preindustrial revolution). Other reasons for their popularity were the widespread use of animal fats in cooking, soap making, and candle making, as grease in burning lamps, and as lubrication in simple machinery such as pulleys and gears. This demand was replaced in the 1900s when people began to utilize more plant-based oils (e.g., vegetable oil). Other more desirable traits in pigs were sought, and they were soon recognized for their efficiency in converting feed into protein. Therefore, contemporary demand dictates an intensive selection against fatness, where lean muscle and characteristics that promote flavor development are favored. Table 1 shows a mixture of traditional and commercial breeds, and while the list is not exhaustive, it tries to cater for the most commercially relevant and some historically significant pig breeds still in use and previously used by producers. While some of these breeds are now of little numerical importance, supporters carry on with dedication the maintenance of these heritage breeds. As economic factors and consumer preferences fluctuate, so to the fortunes of particular breeds rise and fall. Furthermore, it is prudent to maintain small distinct populations of the rarer breeds in case they are required to provide some particular characteristic(s), which may be lacking in the current commercial population.

The structure of most national herds is pyramidal in nature. A small number of prominent breeders occupy the apex of the pyramid who supply stock to a large number of other breeders further down the hierarchy. These breeders multiply the breeding stock, which finds its way to the commercial herds forming the majority of the herds at the base of the pyramid. Most EU countries have tried to manipulate this structure by ensuring that the breeds that occupy the apex make the greatest possible progress in pig improvement, for example, body responsible for this in the United Kingdom is the Agriculture and Horticulture Development Board (previously the Meat and Livestock Commission (MLC)). Pedigree breeds are ones where the ancestry of the stock has been recorded accurately. It is usually administered by a central agency, such as a breeding society. These groups, for example, the Tamworth Breeders' Club, are responsible for all aspects pertaining to administration/record keeping, such as birth records and herd book numbers to adult breeding stock, specifying correct breed (color/conformation), and also for promoting the breed both nationally and internationally. In the mid-1960s, there was a movement towards developing pig quality by geneticists who worked on a successful development strategy in poultry. It was felt that this success could be replicated in the pig industry, and this pioneering

Table 1 Pig breeds considered commercially or of historical importance

Breed	*Origin*	*Characteristics*	*Type*
Berkshire	The United Kingdom	Black, six white points (legs, nose, and tail), erect ears, upturned noses, long carcasses	Traditional
British Saddleback	The United Kingdom	Black head and neck, white belt extending over the shoulders to the forelegs, body returns to black, white feet and tip of the tail, good grazing and mothering, very hardy breed	Traditional
Chester White	The United States	Solid white, droopy ears, most durable of white breeds, good mothering, large litter size and percentage ham	Commercial
Duroc	The United States	Red, large-framed, medium length and muscular, with partially drooping ears, one of the least aggressive of all the swine breeds, superior growth rate, and feed efficiently	Commercial
Gloucestershire Old Spot	The United Kingdom	Predominantly white with black spots, long head length with a slightly dished nose, ears well set apart and dropping forward to the nose, known for docility, profligacy, and intelligence	Traditional
Hampshire	The United States	Black with white belt around the shoulders, erect ears, muscular, rapid growers, and exhibiting good carcass quality, good mothering, and good-tempered	Commercial
Iberian	Portugal/ Spain	Red or dark in color, if black ranging from dark to gray, with little or no hair and a lean body, high capacity to accumulate intramuscular and epidermal fat – prized for their flavor due to IMF and its traditional feeding based on acorns	Traditional
Lacombe	Canada	White, medium-sized, docile temperament, large drooping ears, long bodied, comparatively short legs, meaty in conformation, noted for rapid gain (especially sows)	Traditional
Landrace	Denmark	White, grow well producing long acceptable carcasses, large droopy ears, and surpasses most breeds for litter size and mothering ability	Commercial
Large Black	The United Kingdom	Black, hardy, docile, known for large litters	Traditional
Large White (Yorkshire)	The United Kingdom	Big, solid white, erect ears, dished face, rugged and hardy, large litters, good length and mothering, excellent for cross breeding. Originally developed for outdoors but is one of the most favored by commercial pig breeders today, lending uniformity to pigs produced for meat on a large scale	Commercial
Mangalica	Hungary	Characterized by its hairy 'fleece'/long coat, direct descendant of the wild boar, originally bred as a lard pig that would explain its decline in popularity	Traditional
Middle White	The United Kingdom	White, pricked ears, short snout, sharply upturned snub nose, docile, outdoor grazer, known as a pork producer (rather than bacon or lard type pigs)	Traditional
Piétrain	Belgium	Spotted, black and white in color, with droopy ears and long, wide body. Widely recognized as the world's leanest, heaviest muscle breeds	Commercial
Poland China	The United States	Typically black, occasionally with 6 white patches, known for their large size. America's oldest breed	Traditional
Taihu/Meishan	China	Large, black, heavily wrinkled face, large head with broad forehead, large folded ears, high reproduction rate (litters size up to 20), and highly disease-resistant making it a breed of considerable interest to research	Traditional
Tamworth	The United Kingdom	Pale ginger to dark mahogany red, elongated head shape, long narrow body, erect, pointed ears, face has rectilinear lines as well as the snout, considered medium-sized porcine, small litter sizes compared to other commercial breeds	Traditional
Welsh	The United Kingdom	Large, white, hardy outdoor, long, pear-shaped body, wide head with lopped ears and straight nose, regarded for quality bacon it produces – despite this, it is not extensively found outside of the United Kingdom	Traditional

work inspired both meat processing companies and traditional breeders to compete with pedigree breeders for a larger stake in the breeding business.

Pale, Soft, Exudative (PSE)

Pale, soft, exudative (PSE) meat is a condition that has classically been associated with pork (although it is known to occur in other species), which has been studied for over 50 years. The development of PSE in pork is described in the succeeding text: all muscle tissue undergoes a reduction in pH as a result of ATP hydrolysis during the conversion of muscle to meat. The typical pH profile in pork would be between 6.5 and 6.7 with a temperature of 37 °C at 45 min postmortem. However, in aberrant carcasses, the pH may drop to 6.0 in the same time period. It is this latter condition of rapidly decreasing pH in combination with high carcass temperature that results in denaturation of some of the contractile proteins, with consequent loss of water holding capacity (WHC). It has significant implications on fresh and processed pork, that is, the former is more likely to be rejected by consumers due to its physical appearance, and its compromised technological performance also makes it a less attractive raw material for the processing industry in the latter. The main causes for the condition are known to be antemortem stressors for the animal, for example, handling, welfare, heat, physical exercise, transportation, and mixing of unfamiliar animals. There is also evidence that genetic factors within a specific breed may further its

predisposition to the condition. These are discussed in more detail in later sections of the text.

Dark, Firm, Dry (DFD)

Dry, firm, dark (DFD) meat is a meat quality defect that is associated with beef, but it is known to also occur in pork. It is characterized by high pH_{24} values resulting from low muscle glycogen and creatine phosphate levels present at the time of slaughter (restricting lactate formation). The result is meat that has a considerably higher WHC than normal as the pH (6.0) is further away from the isoelectric point of myosin (5.0). This advantage is largely offset by its susceptibility to increased microbial growth and its color that are more likely to be rejected by consumers. The dark color is a result of the high postmortem pH that keeps the muscle protein highly charged, thereby maximizing the separation of the muscle myofibrils and reducing the light scattering. Furthermore, mitochondrial respiration at higher pH also reduces the fraction of oxymyoglobin in the tissue. DFD can be caused by a number of factors such as hunger (starvation), exercise (exhaustion), and long-term stress in the live animal. Its incidence rates tend to be seasonal, with higher rates reported when animals are subjected to long periods of cold and damp weather.

Genotype

Genetics play an important role in determining pork quality, which can comprise differences between breeds and differences between animals of the same breed. Genetic effects can be categorized as follows:

1. Polygenic: large numbers of genes having relatively small effects (this characterizes most of the effects associated with meat quality traits).
2. Monogenic: these 'major genes' are genes that are necessary and sufficient by themselves to cause a condition, for example, halothane, RN^-, and androstenone (boar taint) genes.

The Halothane Gene

Otherwise known as the porcine stress syndrome gene, the halothane gene causes malignant hypothermia, which can be triggered by stress or exposure to the anesthetic gas halothane. It has been studied for half a century due to its close association with PSE. In the 1960s, some breeds were noted for containing a large proportion of PSE-prone animals (Piétrain and Poland China), which gave rise to the study of and resultant discovery of the halothane gene. Subsequently, the presence of the halothane gene has been eliminated from many selection lines.

The RN^- Gene

The RN^- gene was identified in the Hampshire breed and is closely related to a reduction in technological yield. The term 'RN' originates from the French 'Rendement Napole,' which translates as Napole yield (Napole being a method for estimating technological yield in cooked ham). The condition is characterized by high-muscle-glycogen stores and an extended pH decline postmortem. While there is limited effect in the early postmortem period, the low pH_{24} results in low WHC and high reflectance (lightness) in the meat. The term 'acid meat' was coined to describe the phenomenon. When compared to the halothane gene that dramatically hinders WHC, the RN^- gene only results in small increases in drip loss ($\approx$1%). However, technological yield is decreased by 5–6% compared to noncarriers and halothane gene carriers (2–3%). A similar strategy to the approach applied to the halothane gene that is selectively eliminating the RN^- gene from selection lines has been discussed.

Boar Taint/Odor

Boar taint is an objectionable odor or taste that can be evident during the cooking or eating of pork or pork products derived from noncastrated male pigs (boars) once they reach puberty. The flavor is produced by a testosterone derivative and by skatole, produced by the intestinal microbial breakdown of the amino acid tryptophan. The testosterone derivative, 5-alpha-androstenone, starts to accumulate in the fat of male pigs when they are sexually mature or exceeding 90 kg. Skatole on the other hand can be detected in pork from males and females. Most boars (80%) do not have taint and are slaughtered before puberty so it is not an issue. However, research suggests that 75% of consumers are sensitive to boar taint, with women and certain ethnic groups showing a higher degree of sensitivity than men. The high sensitivity shows that it is necessary for producers to control it despite its low prevalence in the pig population. Although castration was the preferred method of control in the past, research is under way to find alternatives (e.g., selection) to reducing boar taint without resorting to castration.

Polygenic Effects

Excluding those mentioned earlier, the heritability of most attributes relating to pork quality is low to moderate. The notable exception is intramuscular fat (IMF). Increased IMF content is usually associated with improved eating quality. While the heritability of IMF percentage and fat tissue is high (0.5 and 0.69, respectively), the genetic correlation between them is low (0.11). This suggests that selection for high IMF in lean carcasses should be possible.

Production Systems

The profitability of a pork production system depends on three main factors:

1. High sow productivity (a number of piglets reared per sow per annum)
2. Conversion of feed to pig meat
3. Lean carcass production (currently most favored by consumers)

Many of the improvements are simple to address and pertain to animal welfare issues, such as correct management of feed, housing, and health. As the margin between processors has narrowed, genetic-based improvements of performance traits have been the favored method of increasing the profitability of

the production system. The generally available tools that breeders have to bring about this genetic improvement are selection of superior individuals as parents within a breed and choice of breeds or composite lines to be used in crossbreeding systems. Performance traits that determine system efficiency can be grouped into two broad categories:

1. Reproductive traits, for example, age of puberty, fertility, and litter size, are expressed only in breeding animals.
2. Production traits, for example, growth rate, feed efficiency, and carcass composition, are expressed in market animals and animals destined for the breeding herd.

Most pork quality traits are only expressed in market animals. Genetic improvement of reproductive and production traits through the selection of superior parents is possible and dependent on a number of factors, such as the heritability of the trait, the amount of variation between potential replacements, and the superiority of chosen replacements.

Another very important option to breeders is crossbreeding. This offers the potential to exploit the differences between breed lines and enhance the inherited traits by mixing the genetic contribution of the parents (heterosis). A terminal static cross in which all the offspring are market animals takes the greatest advantage of the differences in the strength of the breeds. Lines with increased genetic merit for the reproductive traits provide the females for the crossbreeding system, while lines with superior genetic merit for the production traits provide the males. For example, the first cross gilt of a large white boar and a Landrace sow is one of the most popular crossbred lines and generally gives excellent prolificacy, good feed efficiency, and progeny with a highly lean carcass when mated back to one of the two parent breeds.

Production Phases

Pigs breed at all times of the year so pork production can be considered a continuous cycle. Producers also fall into a number of categories depending on their farming operation. Some operations keep and mate sows, farrow litters, and raise piglets to market weight, that is, farrow-to-finish operations. However, some specialized operators choose to concentrate on specific stages of the production sequence. For example, weaner producers maintain breeding herds but sell their pigs' offspring at 10 weeks. The recipient of these piglets is often a finishing/feeder operator who feed the pigs until they reach market weight. Pork production can be broadly characterized into four main phases and their satisfactory completion will result in animals ready for market:

1) Breeding: Historically, sows have been bred by placing a number of them in a pen with one or more boars. The boars are often rotated between sow pens in order to ensure all sows are fertilized while they are in heat. Sows that are kept in enclosures enter estrous (the period when a sow can be bred – also termed standing heat) 3–5 days after their young are weaned. In general, sows will display signs outwardly that she is willing to accept the boar that is holding her ears erect and standing still if a producer applies downward pressure her back. If the sow is not bred during this time, she is considered unproductive (as the animal is required to be fed and housed). However, she will return to estrous approximately 3 weeks later. These two periods are termed 'first' and 'second' -heat breeding, respectively. Modern operations only have sows bred on first heat with unproductive sows sent to market and replaced with gilts (young female) removed from the grow–finish phase. Thereafter, the sow will gestate her litter for approximately 115 days until the pigs farrow.
2) Farrowing: Before giving birth, sows are generally moved to a farrowing room where she will birth 8–12 piglets, collectively referred to as a litter. Most confinement operations place the sow in a temperature-controlled environment and usually a farrowing pen/crate that restricts her movement to protect the baby pigs (from crushing when she lies down). Average reproductive rates for sows are approximately three to five litters over their lifetimes. However, they may be culled due to health problems, age, failure to conceive, or low litter ratio. Pigs are born with eight sharp teeth that are clipped to prevent injury to the sow's udders and other piglets and curly tails that are shortened to prevent biting. Piglets weigh between 1.1 and 1.3 kg when they are born and are weaned from the sow at two to three weeks.
3) Nursery pigs: After weaning, pigs can be placed in a nursery (although joint nursery/grow–finish operations are now common) – a temperature-controlled environment (to keep the animals warm and dry throughout the year) with a plastic or plastic-covered steel slotted floor, which provides the young pigs a more comfortable environment than a concrete floor space. About 0.25 m^2 is afforded for each pig, and they have easy access to feed and water. Slotted floors can sometimes be raised to prevent chilling to the animals, while temperatures range from 29 °C (after weaning) to 21 °C as the pigs start to grow. After six to ten weeks, pigs are moved from the nursery to the grow–finish building.
4) Grow–finish: Pigs are fed until they reach their market weight c.150 kg and their space requirements increase to approximately 0.75 $m^{2.}$ On average, duration to market is five to six months, which is dependent on intrinsic factors such as breed and susceptibility to disease. Some gilts are removed at this point to replenish the sow herd (breeding phase), and the older sows are culled. Pigs that are in the grow–finish phase produce a considerable amount of heat. Therefore, adequate building ventilation is required to keep the animals from overheating. This can be managed naturally (wind-driven) or mechanically (wall fan) depending on the climate the producer lives in.

Feeding

Pigs, like humans, are monogastric animals and many of the components of their diets are easily transferred from the feed they ingest to the muscle and fat tissues. This has a subsequent effect on pork eating quality, and a number of feeding strategies have been researched to exploit this trait. In some cases, it is used to counteract a resultant technological problem that stems from the processing of the animal into meat or is inherent in the breed (e.g., dietary intervention to offset the effects of

stress on meat quality) or to enhance or change the underlying composition of the muscle tissue (supplementation with ingredients known to have a beneficial effect for health in consumers):

1. Technological: In order to offset deleterious effects of conditions such as DFD meat, feeding strategies involving the manipulation of the muscle glycogen levels have been investigated. For example, feeding a carbohydrate-rich feed (sucrose or other digestible carbohydrates) has been shown in a number of studies to have resulted in increased muscle glycogen and subsequently decreased pH_{24}. It should be noted that other researchers also concluded that care is needed when applying this approach (sugar feeding), particularly with breeds known to be carriers of the halothane gene, as it may increase the incidence of PSE meat. Creatine monohydrate has also been investigated as an alternative to sugar-based feeds to increase muscle energy stores (and offset early glycogen metabolism and resultant slow pH decline).
2. Physiological: Stress responses can have a direct influence on the quality of meat. When an animal is subjected to stress, it releases brain neurotransmitters that in turn release stress hormones into the blood. These hormones can stimulate muscle metabolism that can have detrimental effects on meat quality. This is particularly relevant in pork as certain breeds can be very susceptible to stress factors. Research into magnesium supplementation prior to slaughter has shown it has beneficial effects for WHC and pork quality. Magnesium appears to reduce neuromuscular stimulation by interfering with calcium. Tryptophan supplementation has also shown positive effects on the number of reduced aggressive acts prior to slaughter and PSE incidence in pork. This may be due to serotonin (popularly thought to be associated with feelings of well-being and happiness), which is biochemically derived from tryptophan. While these stress-reducing feeding strategies do show promise as potential tools for improving pork quality, very little is still known and it requires further research.
3. Health: This will be detailed in the 'Health Effects' section.

Secondary Production

Preslaughter Handling

Preslaughter handling involves the handling, mixing, transit, and storage of animals. All handling practices have the potential to induce psychological and physiological stress, and it represents both an animal welfare and a subsequent quality issue. Preslaughter-induced stress can be broadly categorized into the following:

1. Long-term stress factors: on-farm handling, mixing of unfamiliar animals, loading, and transport. These factors mainly lead to the development of DFD meat.
2. Short-term stress factors: lairage conditions and driving towards the stunner. These factors mainly lead to the development of PSE meat.

Reduction or effective management of these factors has been shown to have a positive effect on meat quality. For example, optimum lairage time has been reported to be between two and three hours, to allow time for the animals to calm down but not afford them too much time to begin to fight.

Slaughter

Healthy animals that have reached their finishing weight are sent for slaughter. In the EU, there are a number of regulations governing the hygiene of preparing foodstuffs, which included the slaughter of pigs, such as 852/853/854-2004. Pigs are generally fasted (16–24 h) prior to slaughter in order to reduce complications (contamination) and to ensure completeness of bleeding and easy evisceration (removal of internal body parts and organs). The animal's sex, breed, ID number, and live weight are recorded. Dressing percentage is calculated from the carcass weight (typically taken warm/hot as chilling will decrease the weight by up to 2% through moisture evaporation) divided by the live weight multiplied by 100. Typically, pigs have a dressing percentage of 70%, of which only 57% of the original live weight goes on to become retail cuts. Animals must be stunned that is rendered unconscious, prior to slaughter. Therefore, the animals must be immobilized in some fashion. In small operations, a small confinement pen and a squeeze gate are all that are required, while larger operations may require a series of chutes, restrainers, and conveyers. Three primary methods of stunning are available:

1. Electrical stunning: the application of electrical tongs to deliver between 70 and 90 V (0.3 A) for 2 to 10 s is sufficient to render a pig unconscious.
2. Mechanical stunning: the application of a compressed air/cartridge propelled compression bolt (fitted with mushroom-shaped or penetrating heads) to the forehead of the animal.
3. Chemical stunning: fast becoming the standard practice throughout the industry, it involves the exposure of the animals to carbon dioxide (CO_2)-rich environment that starves the animal's blood of oxygen, rendering them unconscious. Danish research has shown that common injuries sustained by animals during electrical stimulation, such as shoulder and backbone fracturing, are greatly reduced, and it also appears to minimize the incidence of pale, soft, exudative (PSE) meat. Most setups involve animals entering a conveyer cage, which is lowered into CO_2 confined chamber (CO_2 is heavier than air).

The stunned animal is shackled and suspended at the Achilles tendon and exsanguinated (bled) by severing the carotid artery and jugular vein using a 'sticking' knife. A precise cut is required to avoid cutting into the muscle tissue and exposing it to blood flow (contamination) and also to ensure a quick, humane death. Pig carcasses must undergo scalding, which is the process of hair removal by drenching the carcass in a hot water device. The most effective and uniform results are achieved when the carcass is fully submerged and continuously rotated. Water temperature of approximately 61 °C is reported to give adequate results; however, care should be taken to avoid overscalding, which can result in contaminated scalding water (with blood) and damage to the animal tissues. Additional hair removal can be carried out manually using clippers/

blades and a blow torch if necessary. In order to adequately chill and facilitate ease of cutting and cut fabrication, carcasses are split in half using a circular saw. The internal and external surfaces should be washed top to bottom with water or a mild sanitizing solution (low salt, e.g., 0.1 M KCl, or weak organic acid, e.g., 2% lactic acid) to remove any residual blood, bone fragments, and in particular bacteria. At this point, the carcasses are inspected for the final time, checking for abscesses/growths and contaminants, weighed, and stamped for identification of wholesale cuts. They can be boned directly at this point (hot boning) or transferred to commercial chilling facilities (0–1 °C; air speed 2.2-6.7 ms^{-1}) for 24 h.

Postslaughter Handling and Interventions

The manner in which animal carcasses are handled postslaughter can also have a significant influence on the meat quality. As muscle pH and temperature are intrinsically linked, the thermal conditions that they are subjected to postslaughter have a direct effect on their physicochemical and eating quality performance thereafter. Hence, pH and temperature conditions should always be considered in full cognizance of each other to avoid inducing unwanted conditions, such as PSE and DFD meat. Postslaughter interventions like electrical stimulation and accelerated (hot) boning can be applied in order to improve the technological or financial performance of meat. However, chilling regimes should be specifically designed to accommodate any of the following postmortem interventions as they may have a fundamental effect on the underlying biochemistry, that is, pH.

Electrical stimulation

Electrical stimulation is when an electrical current is applied through a carcass. It effectively hastens the process of rigor mortis by accelerating postmortem metabolism, changing the initial pH and the rate of pH decline. In this regard, it is an invaluable tool for offsetting deleterious conditions such as cold and heat shortening and subsequently improve tenderness. Significant improvements in other meat quality characteristics, such as improved WHC and color, have also been reported. High- or low-voltage applications are available and effective, although the latter are often favored due to operator and plant staff safety. Electrical parameters such as waveform and pulse frequency, duration, delay prestimulation, subsequent chilling regime, and species can be considered for optimizing final meat quality. In pork, electrical stimulation has been shown to encourage a greater incidence and extent of PSE and is therefore considered much more successful for beef and lamb applications. Electrical stimulation should be considered as part of a total process (from slaughter to sale) as it has particular advantages for ultrafast cooling and hot boning applications.

Hot boning

Hot boning involves the removal of muscles or cuts up to about 90 min postmortem, before the onset of rigor mortis. The main meat quality advantage of hot boning is the reduction of weight loss during chilling and drip loss during storage, due to increased WHC of the muscle proteins at the higher pH. Furthermore, significant financial savings could be made as space requirements could be reduced as much as 50% (individual muscles rather than whole carcasses), while refrigeration costs could be up to 80% lower (faster chilling times and more uniform cooling) than conventional boning practices. Much of the early hot boning research was carried out using pork. Unlike other meats, hot boning of pork frequently goes beyond the fresh meat application (initial carcass fabrication) to include further processing, such as injected whole muscle and comminuted products. Combinations of hot boning and electrical stimulation are often used with beef and lamb. However (as mentioned earlier), due to the inherent susceptibility of PSE condition in pork muscles, this can be exacerbated by combining the two techniques in pork processing. Alternative techniques to electrical stimulation that have been investigated in beef and lamb, such as high-temperature conditioning (less dramatic pH decline than electrical stimulation), may be useful to alleviate the potential undesirable effect of PSE in pork.

Altered hanging (aitch-bone/pelvic suspension)

The principle of suspending carcasses from the aitch-bone (pelvic), rather than the more conventional Achilles tendon, for improving tenderness in beef and lamb has been known for many years. By suspending the carcasses in this manner, some of the commercially more important muscles are stretched, thereby preventing them from shortening when *rigor mortis* sets in. It has also been suggested as a method for improving pork tenderness. By stretching the muscles, the resulting increase in sarcomere lengths is associated with increased tenderness. Pelvic suspension also appeared advantageous over electrical stimulation, as it not only prevented toughening but also consistently reduced drip. However, research has shown considerable variation between muscles in the relative length change induced by pelvic suspension. Sarcomere length changes were much larger in topside (m. *semimembranosus*) and silverside (m. *biceps femoris*) than in the loin (m. *longissimus dorsi*) without producing proportional tenderness improvement. M. *rectus femoris*, a major muscle in the leg, actually shortens after pelvic suspension. The practice of pelvic suspension has been commercially adopted by both the beef industry and the pork industry to improve tenderness.

Carcass Grading

The aim of carcass grading is to ensure fair payment to producers, obtain transparency, and compare/standardize across different markets. The set conditions are specific to market requirements for the commodity. In pork, lean content is the most highly sought attribute, and the basis for its assessment is uniformity in the following criteria: (a) the definition of grading standards, (b) systems of assessment, and (c) estimation formulas. Up until the mid-1980s, carcass grading was carried out subjectively by visual determination. However, a more objective approach has been carried out since then by the use of instrumental measurement. Despite this, the systems of pig carcass grading are far more developed than beef and lamb. A number of technologies are used to determine lean content of carcasses, and these vary greatly from country to country. Some examples include the Fat-o-Meater, the Hennessy probe, ultrasound system, x-ray absorptiometry, AutoFOM (automatic Fat-o-Meater), and a simple ruler to measure fat thickness. The

adoption of the technologies will depend on initial cost, training required (operators), and the overall resultant benefits of its adoption. For example, some technologies such as x-ray absorptiometry would be impractical in a large-scale fast-moving production facility. Regardless of the technology adopted, fat thickness plays an important role in the determination of lean carcass percentage as it is the most variable tissue in the carcass, and as such, it is included in every calculation. In Europe, lean meat content is determined using the EUROP classification, which is based on muscle and fat thickness. In this system, lean meat occupies the following percentages: E = >55%, U = 50–55%, R = 45–50%, O = 40–45%, and P = < 40%.

Patterns of Consumption

Despite its consumption being prohibited by Judaism, Islam, and certain Christian denominations, pork is an important commodity in most parts of the world. It ranks first among all meat sources and accounts for approximately 40% of global meat consumption. Its high palatability, nutritional value, and versatility for processing and cooking for many different cultures contribute to its nearly worldwide appeal. The main markets for pork are in East Asia and the Pacific region. In particular, it is held in high esteem in Chinese cuisine (mostly for aesthetic reasons). China is both the largest producer of pork and also its largest consumer (Tables 2–3). Domestic consumption in China exceeded 56 million tons in 2014 and it accounts for more than half of global pork consumption. The large growth in Chinese consumption has been attributed to increasing disposable income and urbanization over the past decade. China is one of the largest net importers of pork and their imports are forecast to exceed a record 1 million tons in 2015.

The combined nations of the European Union (EU) collectively account for about a fifth of the world production of pork. Of these, Denmark, Belgium/Luxembourg, Holland, Spain, and Austria have the highest per capita production of pork in the EU. Denmark exports up to 80% of its pork (although its per capita consumption is high) to major markets in the United Kingdom (a net importer of pork) and Asia (particularly Japan). The United States accounts for about 10% of global pork production. However, it consumes substantially less and is mostly exported. It should be noted that up until the mid-1990s, the United States was a net importer of pork.

Table 2 Pork production by country (2010–15)

Country	*2010*	*2011*	*2012*	*2013*	*2014 (prov.)*	*2015 (est.)*
World	103 032	103 629	107 016	108 863	110 606	111 845
China	50 712	50 604	53 427	54 930	56 500	57 350
EU	22 627	22 953	22 526	22 342	22 400	22 365
The United States	10 186	10 331	10 554	10 524	10 329	10 858
Brazil	3195	3227	3330	3280	3344	3494
Russia	1981	2064	2175	2400	2650	2820
Vietnam	2217	2262	2307	2349	2425	2450
Canada	1779	1812	1840	1819	1830	1860
The Philippines	1260	1288	1310	1340	1365	1390
Mexico	1175	1202	1239	1281	1280	1290
Japan	1292	1267	1297	1309	1273	1279
South Korea	1110	837	1086	1252	1182	1148
Others	5498	5782	5925	6037	5918	5531

Figures are on a carcass weight equivalent basis and expressed in thousands of metric tons (e.g., 50 712 represents 50 712 000, Source USDA).

Table 3 Domestic pork consumption by country (2010–15)

Country	*2010*	*2011*	*2012*	*2013*	*2014 (prov.)*	*2015 (est.)*
World	102 898	103 274	106 437	108 464	109 882	111 174
China	50 799	51 108	53 802	55 406	57 010	58 250
EU	20 951	20 822	20 382	20 125	20 262	20 175
The United States	8654	8338	8441	8662	8455	8874
Russia	2896	3035	3208	3267	3109	3194
Brazil	2577	2644	2670	2696	2760	2795
Japan	2488	2522	2557	2550	2558	2552
Vietnam	2199	2238	2275	2315	2389	2414
Mexico	1784	1710	1850	1953	1975	2005
South Korea	1539	1487	1546	1628	1631	1640
The Philippines	1418	1432	1446	1511	1554	1589
Canada	810	800	834	803	863	895
Others	6783	7138	7426	7548	7205	6680

Figures are on a carcass weight basis and expressed in thousands of metric tons (e.g., 50 799 represents 50 799 000). Source USDA.

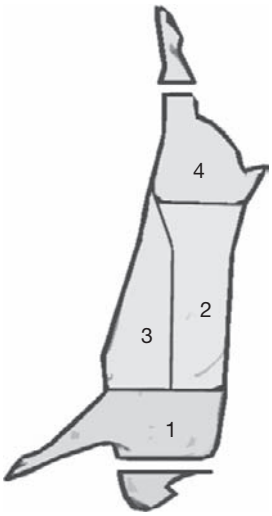

Figure 1 Carcass position of popular pork cuts.

Common Fresh Cuts of Pork

The methods of cutting pigs for wholesale markets may differ slightly from region to region, but in general, four large sections are recognized (Figure 1):

1. Shoulder: It accounts for approximately 25% of the carcass weight and is typically subdivided into two sections, that is, upper ('Boston butt/blade Boston') and lower ('picnic'). The cuts are considered flavorful but connective tissue is significant due to the considerable work carried out by the animal in the shoulders. Therefore, roasting, braising, and marinating are popular cooking techniques with these cuts.
2. Loin: It contains the rib, loin, and sirloin sections and accounts for 22% of the carcass weight. Typically, these are the most highly prized cuts as they are characterized by being very lean and tender (e.g., tenderloin). The loin can be whole or cut, that is, pork chops, while the ribs are noted to have higher meat content than their spare rib counterparts from the side.
3. Side ('belly'): It accounts for 23% of the carcass weight and contains both the spare rib and the belly. The meat is streaked with fat and is often cured and/or smoked to produce bacon, for example, pancetta. Spare ribs are usually trimmed of excess belly fat and slow baked or barbecued.
4. Leg ('ham'): It contains large muscles with low connective tissue content that account for 30% of the carcass weight. Hams can be purchased either bone-in or boneless, and in some cases, the skins can be left on. Typically, hams are consumed cured or smoked but fresh hams are also available.

Some of the other popular meat types include minced/ground pork, which is a mixture of trimmed meat and fat predominantly from the shoulder and leg. The lard of the pigs is also still heavily used for artisan baking, particularly in pastry. Furthermore, specialty meats such as pork liver, tongue, heart, feet/knuckles, neck bones, jowls, and tails are often utilized in fine dining restaurants, where they are considered delicacies.

Cured Meats

Pork is used as the primary constituent of many traditional cured meat products. Curing involves the addition of salt, sugar, nitrate/nitrite, and other curing adjuncts for the purposes of preservation, flavor, and color development. Historically, curing literally is translated as 'saving of meat,' and the first written evidence was documented by the ancient Greeks who described the preservation of meat by packing in salt. Saltpeter (sodium/potassium nitrate), a commonly occurring impurity in salt, was reduced by the natural microorganisms present to nitrite allowing for the formation of its characteristic color. However, the emergence of cured meats in Europe occurred in the sixteenth century when the nitrite compound became readily available (primarily used for medicinal applications). This is thought to be the origin of the now established food curing processes of drying, smoking, and salting. The basic principle is the application of salt in order to reduce the available water and make the environment less ideal for microbial growth and subsequent spoilage. Salt can be applied to the meat as a dry rub or submerged in a salted liquid (brine). The meat cell fluids are less concentrated than the salt water. Water flows out of meat and salt passes into the meat (osmosis). The salt action helps dissolve some of the muscle proteins. The cell's fluids become more concentrated, which draws water back inside. The advantages include preservative effects, reduction in rancidity, and imparting of characteristic flavor and color. Cured meats can be divided into four many categories:

1. Dry cured: Very much rooted in the traditional curing methodology. Ingredients are applied dry in the form of a rub to the outside of the meat over a number of time intervals. The rate of curing is dependent on the diffusion of salt from the exterior to the interior of the meat. The products are often time-intensive, with some products taking years to be ready, which is often reflected in the price they command at retail and their status as premium specialty meats. Common examples in pork include prosciutto and serrano hams.
2. Wet cured: Has largely superseded dry curing and now represents the standard industry practice for most cured products due to its flexibility and time-saving. Brines are injected directly into the meat using a multineedle injector and often tumbled for a short time to ensure good distribution of the cure ingredients. Common examples in pork are bacon (belly or shoulder regions of the pig) and hams (pork leg).
3. Smoked meats: Having a strong characteristic flavor, smoking also has a preservative effect on meats. Nonresinous woods are deemed suitable for the smoke generation, although hard woods such as oak, beech, and hickory have been traditionally favored. Modern smokers are usually carried out in special units that are separate from the cooking chamber so they can be controlled independently of oven temperature and humidity. Another time-consuming process, modern advances include the use of smoke concentrates/essences that can be sprayed onto the product instead of producing the natural smoke. Common examples in pork are smoked bacon and hams.
4. Fermented meats: In curing, selective microbiological effects are commonly termed as 'active' or 'live' brines. The addition of starter cultures is a common practice in the meat

fermentation industry to increase uniformity of the batches. The most commonly used bacterial species are lactic acid bacteria, which are designed to give a unique flavor profile through acidification and related changes in the meat. Common examples in pork are Wiltshire bacon that uses a 'live' brine in the immersion stage of the Wiltshire process.

Sausages

Sausages represent one of the oldest forms of processed food that predate Roman times. The primary economic purpose of these products is to present relatively large proportions of fat in palatable ways. Comminution of the fat is therefore a common feature. Sausages are defined in the United Kingdom as cylindrical products with hemispherical ends. The word is derived from the Latin word salus, meaning salted, and sausages consisted of meat, blood, and other ingredients, which were filled into animal gut and consequently preserved. The products' composition has remained relatively similar throughout the millennia, and the basic principle behind sausage production is outlined in the succeeding text (although some additional/reduction of steps may be required for specialty sausages). Meat is primarily responsible for binding the product together. It is modified by its treatment with salt and water and mechanical action to bind water, hold fatty tissue, and free fat in the sausage matrix. Water (usually added as ice for moderating temperature during processing) and fat are added. Sausages are emulsion-like products (not true oil in water emulsions like mayonnaise). The meat is chopped (comminuted) to varying degrees to help extract the proteins. Salt is added to help further extract salt-soluble proteins (like actin and myosin) and for flavor. The extracted proteins coat the fat and water forming a structure similar to that of an emulsion. At this point, other ingredients and additives can be added to help improve technological performance or cost effectiveness. A wide range of cereals (flours, starches, bread, and bread crumbs) have been traditionally used in sausages. More common today is the use of rusk, a product derived from biscuit dough that can be ground to various sizes, which is employed for texture and water-binding properties (up to three times its own weight under the right conditions). Other additives, such as polyphosphates, can be added to improve water binding and lessen cooking losses, while others can be added to enhance flavor (monosodium glutamate and spices) and extend shelf life (sulfur dioxide). Protein substitutes (blood plasma, skimmed milk powder, casein, and whey) can also be added subject to regulation. The resultant batter is filled in to sausage casings and tied in sausage links. Modern sausage casings (skin) are usually made from one of the following:

1. Natural casings: cleaned intestines
2. Artificial casings: (a) regenerated collagen (derived from animal hide or other collagenous materials) or (b) cellulose (plant wall material), which is usually peeled off after cooking (skinless sausage type).

Heating (if applied) stabilizes the system by denaturing the proteins and gelatinizing heat-labile proteins (collagen).

There are broadly three categories (although among all the sausages made in various countries there are many variations in detail and much overlapping among the types):

1. Fresh: Typically uncured and sold uncooked. As a result, they have a short shelf life and examples come with varying degrees of comminution (although they are most commonly coarsely comminuted). Common examples include British and Irish types of fresh sausage that contain varying levels of meat content.
2. Emulsion: These are usually made from finely comminuted meat/fat and are more highly flavored than fresh sausages (spices). Emulsion sausages usually undergo some additional processing, such as cooking, curing, and smoking/drying. Therefore, they have an intermediate shelf life. Popular examples include frankfurter, Vienna sausage (wiener), bratwurst, and saucisson.
3. Fermented/dried: Lactic acid is produced by fermentation of microorganisms introduced in the form of a starter culture. These organisms were also historically important in the curing process – by reducing unrefined nitrate salts (saltpeter) to nitrite. Finally, the products usually undergo a drying step in the latter stages of manufacture. This gives the products a long shelf life (up to 1–2 years), but the process is complex and difficult to manage (often reflected in the price and high-quality status of such products). Common examples include salami, coppa, cervelat, and rosette.

Availability, Absorption, and Metabolism

Meat that has been prepared using similar principles but originating from the different species has comparable amounts of nutrients. For example, pork from modern, muscular pig breeds has comparable cholesterol to its bovine, ovine, and poultry counterparts. However, this was not always the case as previously discussed. Significant changes in pork over relatively few generations are evidenced in research published by the National Pork Producers Council in 1999, which found that fresh pork is 31% lower in fat, 14% lower in calories, and 10% lower in cholesterol than pork produced in 1983. Many of these reductions can be attributed to improved production practices such as better genetic selection, nutrition, welfare, parenting, and changes in consumer demands. Table 4 shows the general composition of pork, fresh, composite of retail cuts (leg, loin, and shoulder), separable lean only, and cooked.

Protein and Amino Acid Metabolism

Pork, like all meats, is a good source of dietary protein. When ingested, protein macromolecules are broken down into amino acids and simpler derivative compounds by a digestion process termed protein catabolism. Dietary proteins are first acted upon hydrochloric acid and nonspecific endo- and exoproteases such as pepsin, trypsin, chymotrypsin, and elastase to form smaller peptide chains and amino acids by various enzymes such as carboxypeptidase, aminopeptidase, and dipeptidase in the gastrointestinal tract. This breakdown into smaller peptides (tri- and dipeptides) is to facilitate their absorption into the intestines. Different enzymes are selective for specific substrates, for example, trypsin cleaves peptide chains predominantly at the carboxyl side of the amino acids arginine and lysine (except when either is followed by proline). In order to prevent

Table 4 Composition of pork, fresh, composite of retail cuts (leg, loin, and shoulder), separable lean only, and cooked (source USDA nutrient database, 2014)

Proximates	*Units*	*Value per 100 g*
Water	g	62.53
Energy	kcal kJ^{-1}	201/840
Protein	g	27.51
Fat (total lipid)	g	9.21
Carbohydrate (by difference)	g	0.00
Total dietary fiber (TDF)	g	0.00
Sugars	g	0.00
Ash	g	1.11
Minerals		
Calcium (Ca)	mg	18.00
Iron (Fe)	mg	1.00
Magnesium (Mg)	mg	25.00
Phosphorus (P)	mg	231.00
Potassium (K)	mg	357.00
Sodium (Na)	mg	55.00
Zinc (Zn)	mg	2.90
Copper (Cu)	mg	0.08
Manganese (Mn)	mg	0.01
Selenium (Se)	μg	44.20
Vitamins		
Total ascorbic acid (vitamin C)	mg	0.20
Thiamine	mg	0.64
Riboflavin	mg	0.25
Niacin	mg	5.57
Pantothenic acid	mg	0.72
Vitamin B_6	mg	0.64
Folate (DFE)	μg	2.00
Vitamin B_{12}	μg	0.64
Vitamin A (RAE/IU)	μg IU^{-1}	1.00/2
Vitamin D ($D_2 + D3$)	μg IU^{-1}	0.05/19
Vitamin E (α-tocopherol)	μg	0.17
Vitamin K (phylloquinone)	μg	0.00
Lipids	*Units*	*Value per 100 g*
C4:0	g	0.00
C6:0	g	–
C8:0	g	–
C10:0	g	0.01
C12:0	g	0.00
C14:0	g	0.11
C16:0	g	1.92
C18:0	g	1.00
Total SFA	g	3.09
C16:1	g	0.25
C18:1	g	3.56
C20:1	g	0.07
C22:1	g	0.00
Total MUFA	g	3.91
C18:2	g	1.28
C18:3	g	0.03
C18:4	g	0.00
C20:4	g	0.06
C20:5 (EPA)	g	–
C22:5 (DPA)	g	–
C22:6 (DHA)	g	0.00
Total PUFA	g	0.86
Cholesterol	mg	84.00
Amino acids		
Tryptophan	g	0.32
Threonine	g	1.25
Isoleucine	g	1.32
Leucine	g	2.28
Lysine	g	2.51
Methionine	g	0.74
Cystine	g	0.33
Phenylalanine	g	1.13
Tyrosine	g	1.00
Valine	g	1.46
Arginine	g	1.77
Histidine	g	1.14
Alanine	g	1.61
Aspartic acid	g	2.62
Glutamic acid	g	4.35
Glycine	g	1.27
Proline	g	1.11
Serine	g	1.16

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; red text denotes essential amino acids.

unwanted or premature digestion, the enzymes are secreted in an inactive form, which is activated only in very specific circumstances. For example, pepsinogen (inactive precursor of pepsin) is secreted by the stomach and only becomes active in the high-acid environment of the stomach. The amino acid compounds have to constantly be degraded as there is no store for excess quantities in the body due protein turnover. They are degraded by the removal of the α-amino group (transamination) and subsequently converting the resultant carbon skeleton into one or more metabolic intermediates. If the carbon skeletons give rise to the net gain in glucose, they are termed glucogenic and ketogenic if they give rise to ketone bodies. The acceptor for the transamination reaction is typically α-keto acid, which results in the formation of glutamate and α-keto acid. The coenzyme of all transaminases is pyridoxal phosphate, which is derived from vitamin B_6 and is converted to a transient intermediate during transamination. The glutamate produced from transamination is oxidatively deaminated by glutamate dehydrogenase to produce ammonia. This enzyme can utilize either NAD+ or NADP+ and the coenzyme is used in the biosynthesis (NADP+) or degradation (NAD+) of glutamate. It is subject to allosteric regulation, that is, GTP and ATP are allosteric inhibitors, while GDP and ADP are allosteric activators. Therefore, when the energy of the cell is low, that is, more ADP and GDP than the triphosphate form, glutamate dehydrogenase is activated and the oxidation of amino acids increases. The resultant carbon skeletons are utilized as a carbon fuel feeding into the citric acid cycle and ultimately to energy production through oxidative phosphorylation. The amino acids can also be broken down and synthesized into other amino acids and macromolecules, such as fat (which will not be discussed here).

Fat and Fatty Acid Metabolism

For fat to be absorbed, it must first be broken down (emulsified) into a more digestible form. Emulsification takes place

in the duodenum, when bile is secreted (from the liver) into the small intestine on ingestion of food. The bile acts as a surfactant, aggregating around lipids and preventing the lipids from reaggregating together to form larger fat particles. The increased surface area of the micelles makes it easier for pancreatic lipases (enzymes specific for breaking down fat) to act upon the fatty core of the triglycerides, resulting in the formation of two fatty acids and a monoglyceride, which is absorbed by the villi. A process known as β-oxidation breaks down fatty acids in the mitochondria. The rate of degradation is dependent on the availability of free fatty acids in the blood, which arise from the breakdown of triglycerides. The fatty acids are converted into their acyl coenzyme A (CoA) derivatives before entering the mitochondria. The fatty acid breakdown involves a repeating sequence of four reactions:

1. Oxidation of the acyl CoA by FAD to form trans-Δ^2-enoyl CoA
2. Hydration to form 3-hydroxyacyl CoA
3. Oxidation of NAD^+ to form 3-ketoacyl CoA
4. Thiolysis by a second CoA molecule to form acetyl CoA and acyl CoA shortened by two carbons

The pathway produces $FADH_2$ and NADH directly, which are then oxidized by the respiratory electron transport chain to yield energy in the form of ATP. The energy yield from the degradation processes, for example, for palmitate (C16:0), is 129 ATP molecules (taking into account the two ATP equivalents required to activate the C16:0 to its acyl CoA derivative). Pork has a higher degree of unsaturated fatty acids than lamb or beef. Unsaturated fatty acids require additional enzymatic breakdown (isomerase (unsaturated fatty acids) and 2,4-dienoyl CoA reductase (polyunsaturated fatty acids)) before they can be completely broken down by β-oxidation. Excess acetyl CoA produced from β-oxidation is converted to other compounds collectively known as ketone bodies that provide an alternative energy source for the brain under starvation conditions or in diabetes.

Vitamin Metabolism

Pork is an excellent source of B vitamins that along with vitamin C are classified as water-soluble vitamins. These water-soluble vitamins are partially enzyme-digested and typically absorbed in the duodenum and the jejunum, respectively (parts of the small intestine). They then pass through the intestinal wall into the blood stream. Many of the B vitamins are water-soluble but are bound to proteins that must be removed before absorption. This is carried out by digestion with proteinases and stomach acids as outlined earlier. When separated from their protein complex, B vitamins are absorbed in the ileum. However, due to their water-soluble nature, they are much more easily excreted; thus, a regular source in the diet and/or supplement is recommended, particularly in the vulnerable groups like the elderly. Vitamins A, D, E, and K are categorized as fat-soluble vitamins. Like protein-bound water-soluble vitamins, they must be separated from the fats before they can be absorbed (as described earlier). Unlike their water-soluble counterparts, fat-soluble vitamins can be readily stored (in the liver or fatty tissues around the body), and therefore, there is less of a requirement for them to be ingested regularly as part of the diet.

Health Effects

Meat is a very good source of essential amino acids and, to a lesser extent, certain minerals from a nutritional standpoint. While it is also a good source of essential fatty acids and vitamins, the body often relies on other dietary sources for their absorption. Despite this, its long-term consumption from a health standpoint is still widely debated due to its apparent association with the development of many chronic diseases, for example, carcinoma, cardiovascular disease, and hypertension. However, implicating meat consumption as the direct cause of such conditions is not borne out by the available medical literature, which has found contradictory results in many of the studies.

In general, pork is more unsaturated (softer) than beef and lamb. Its fatty acid composition varies directly in response to diet, that is, it can become gradually more unsaturated when pigs are supplemented a diet high in polyunsaturated fatty acids (PUFA), for example, fish meal or plant oils. Pork is high in protein content and contains a better balance of amino acids than most plant proteins, while its amino acids are more efficiently utilized by the human digestive system. It is an excellent source of water-soluble vitamins but less so of fat-soluble vitamins. Pork is high in thiamine, providing nearly 75% of an adult human's guideline daily allowance (GDA) in a 100 g serving. As with all animal proteins, it is an excellent source of vitamin B_{12}, riboflavin, and niacin compared to plant-based alternatives. As a source of minerals, pork is an excellent source of potassium, phosphorus, iron, zinc, manganese, and magnesium. However, it is a relatively poor source of calcium and sodium. Despite this, bioavailability of minerals from pork is considered high.

Improving Healthy Characteristics of Pork through Dietary Intervention

In pigs, the fats ingested in the diet are absorbed in the intestine and incorporated into tissue lipids unaltered. Certain polyunsaturated fatty acids (PUFA), linoleic and α-linolenic acids, cannot be synthesized *in situ*, and therefore, the tissue concentration can vary greatly with changes in dietary habits. On the other hand, saturated and monounsaturated fatty acids (SFA and MUFA, respectively) are adequately produced, leading to a less direct effect of diet. Modification of the native animal fat composition through feeding has been known for many years. More recently, emphasis has focused upon changing these characteristics for human health, that is, optimizing the ratio of saturated and unsaturated fatty acid profile. However, excessive supplementation can lead to the onset of undesirable quality traits:

1. Texture modification (softer meat), which is perceived as inferior by consumers. A standard value of 16 g of linoleic acid per kg feed has been agreed to avoid this quality defect.

2. Increased oxidation due to increased unsaturation. This can reduce the shelf life, particularly in products that have gone for further processing.

In recent years, much research has focused on specific or essential fatty acids in feed supplementation work. The potential of conjugated linoleic acid (CLA – a group of isomers (>20) of linoleic acid (C18:2) mostly derived from the meat and dairy products of ruminants) as a food supplement in pork has been investigated in a considerable amount of studies. This is due to its positive health effects in animals, which may have potential for human health through ingestion. In pigs, CLA supplementation has shown to improve performance, reduce fat deposition, and increase lean meat composition. In pork meat, CLA has been shown to increase the saturated–unsaturated ratio, pH_{24}, and WHC and improve belly firmness. However, it has been also shown to decrease the perceived eating quality of the pork (flavor, tenderness, juiciness, and overall acceptability).

Vitamin E as a supplement in feed has also gained much attention. Traditionally, it was considered important for animal nutrition and was added in order to maintain normal animal reproduction. More recently, however, it has been noted more for its potential radical chain-breaking antioxidant properties. In this regard, it is seen as important in protecting the integrity of tissues and it has a fundamental role in life processes. It also has functionality independent of its antioxidant/radical scavenging properties, that is, protein kinase C inhibitor, promotes the growth of certain cells, and is involved in the transcription of some genes. Furthermore, it protects against lipid oxidation in fresh and processed pork when supplemented to pigs at a level of 200 mg kg^{-1} feed. Lipid oxidation and microbial spoilage are the primary cause of pork quality deterioration. The rate and extent of the lipid oxidation are dependent on the vitamin E concentration and the native fatty acid composition of the meat. Discoloration in meat is the result of both oxidation and enzymatic metmyoglobin reduction systems. While vitamin E supplementation in beef was shown to be effective in promoting color stability in beef, the results in pork have been inconsistent. WHC in pork is generally considered to be improved with vitamin E supplementation. The suggested mechanism is that the vitamin E preserves the muscle cell membranes by preventing oxidation of the membrane phospholipids during storage.

Reformulation of Pork Products and Functional Foods

There are a number of approaches that can be taken to improve the health profile of pork, such as changes during the animal production phases, that is, genetic strategies (selection, breeding, and modification) and nutrition and feeding considerations as previously discussed. However, pork is often the major component of many processed meats, such as sausages, and cured and fermented products. Altering the components of one of these products during one of their preparation stages offers the most flexible opportunity to reformulate. It should be noted that this is in no way limited to pork and is being investigated across all meat types. The most investigated strategies are reductions of the added-fat content; modification of the fatty acid profile; reductions of cholesterol, calories, sodium chloride, and nitrites; and fortification with vitamins and minerals. In some of these cases, ingredients that are termed 'clean label' (ingredients considered naturally occurring or additive free) are used as substitutes for some of the removed additives in order to maintain technological quality of the products, for example, walnut, seaweed, carrot, and herb extracts. The use of pork and its products to act as a carrier of functional ingredients (an ingredient that offers an additional benefit other than that of basic nutrition) such as pro- and prebiotics, bioactive peptides, plant sterols, dietary fibers, and antioxidants has been investigated. Finally, the conditions in which these products are processed, stored, and consumed are also important. Maximizing the retention of bioactive compounds (vitamins, minerals, and added-functional ingredients) and retaining their bioavailability while simultaneously minimizing the formation of compounds known or suspected to be unhealthy (polycyclic aromatic hydrocarbons, heterocyclic amines, biogenic amines, nitrosamines, and lipid oxidation products) could be achieved through the use of smarter storage conditions (active packaging) and novel nonthermal and thermal processing technologies.

Negative Health Effects

While pork is inherently a safe food to ingest as part of a balanced diet, it does have safety issues specific to its species. There are three critical safety issues regarding pork:

Trichinosis

Trichinosis is a condition caused by a genus of parasitic roundworms (phylum: Nematoda) called *Trichinella spiralis*. These parasites encyst in the muscle of host. If present in pork, humans can be infected by consuming the pork. Human digestive juices dissolve the cyst, releasing the parasite into the body. The larvae penetrate the body where they mature into adult worms and mate. It is at this point that some initial symptoms (diarrhea and abdominal cramping) may be experienced. After approximately 1 week of ingestion, the adult female worms produce larvae that penetrate the intestinal wall, enter the bloodstream, and eventually burrow into muscle/tissue. The tissue invasion may cause the onset of other symptoms, such as fever, muscle pain/tenderness, facial swelling, weakness, headaches, photosensitivity, and conjunctivitis. Only in rare severe cases do symptoms lead to more serious complications (myocarditis, encephalitis, meningitis, bronchopneumonia, nephritis, sinusitis, and pneumonia). Treatment is usually in the form of corticosteroids and inflammation reduction strategies. The problem persists worldwide because pigs and other hosts (rodents/other mammals) are frequently infected, making it difficult to eradicate. The incidence and infection rate can vary from region to region. Asian countries can report 100s or 1000s of cases per year. However, the number of cases in the West has decreased markedly over the past decades, for example, the United States records only a few cases annually. The decreased incidence of trichinellosis in the United States is attributed to changes in pork industry management standards and government regulations. Proper culinary preparation of pork ensures protection from *Trichinella* infection. Cooking pork to internal temperature of 77 °C or freezing for 20 days at −15 °C destroys

the parasite. Irradiation treatments have also proved successful in eradicating the parasite in the United States.

Foodborne Pathogens

Like all foodstuffs, pork is susceptible to contamination from serious pathogens, for example, food poisoning incidences in pork and pork products have been previously attributed by pathogenic strains of *Salmonella*, *E. coli*, and *Campylobacter*. This is due to the fact they are ubiquitous in the environment, and as a result, contamination can occur at any point in the processing and retail cycle (farm to fork). Accepted regulatory standards of hygiene are in place at local, regional, national, and international level to ensure the curtailment of microbial contamination. As with *Trichinella*, adequate handling and proper cooking are key to their destruction prior to consumption.

Antibiotic Residues and Antibiotic-Resistant Bacteria

Subtherapeutic antibiotics are used in some territories throughout the world to treat, combat, and/or control disease and help promote growth in pigs. For example, the United States has maintained such a practice for over 50 years. The Food and Drug Administration (FDA) regulates the sale of 'antibiotic-free' pork, meaning that preslaughter withdrawal times for each approved antibiotic must be adhered to, in order to guarantee that maximum recovery limits are not exceeded in tested market animals. Unlike in the United States, the administration of subtherapeutic antibiotics to pigs in the EU is prohibited. The ban, which came in to force from 1999, was in response to the perceived risk of an increasing number of antibiotic-resistant strains of microorganisms. Opponents of such a ban being applied in the United States assert that eliminating the use of antibiotics would lead to more diseased animals, an increased bacterial load in meat, and an increase in the overall production price of pork.

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Potassium: Physiology

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Introduction

Potassium is the most abundant intracellular cation in the human body and plays an important role in a variety of cell functions. This article summarizes the main aspects of potassium physiology and nutrition, including absorption, transport, distribution, storage, and excretion, as well as homeostatic mechanisms of K balance.

Role in the Body

Potassium is essential for the muscular, cardiovascular, nervous, endocrine, respiratory, digestive, and renal systems. Cell metabolism depends on the maintenance of a high intracellular K^+ concentration. Many of the body functions of potassium are due to its ionic character: it generates gradients of concentration, potential, and pressure. Potassium is the predominant osmotically active species inside the cell. Together with other ions such as sodium and chloride, which are characteristic of the extracellular fluid (ECF), potassium determines osmolarity and plays a major role in the distribution of fluids inside and outside the cell and hence in the maintenance of cellular volume. In addition, potassium participates in the regulation of the acid–base balance and is involved in cellular growth and division, energy transduction, glycogenesis, protein synthesis, hormone secretion, etc. Consequently, its deficiency causes growth retardation, with a pronounced decrease in circulating levels of growth hormone and somatomedin C and inhibition of protein synthesis.

Cell excitability and muscle contraction depend on potassium. The transmembrane electrical potential is determined by the ratio of the intracellular to extracellular potassium and sodium concentrations, in particular that of potassium. Differences in K^+ and Na^+ concentrations across cell membranes are maintained by the specific permeability to each of these ions and by K^+/Na^+-ATPase activity (K^+/Na^+ pump). Thus, K^+ is critical for the excitability of nerve and muscle cells.

During vigorous exercise, potassium is released from muscle cells, leading to an increase in extracellular potassium concentrations, which facilitates ongoing muscle contraction and induces vasodilatation, increasing local blood flow. However, liberation of potassium also leads to muscular fatigue. Training reduces the exercise-induced rise in plasma K^+ concentration and also increases the total activity of Na/K pumps in muscle. The potassium internal balance helps to delay the onset of fatigue during exercise and to restore homeostasis during recovery.

Potassium can be defined as a cardioprotector nutrient. It acts directly on the heart, regulating its mechanical and electrical properties. Epidemiological data suggest that potassium intake and blood pressure are inversely correlated. The greatest hypotensive effect of potassium occurs in hypertensive patients and in subjects with a high sodium intake. The mechanisms involved include enhancing natriuresis, baroreflex sensitivity, direct vasodilatation, catecholaminergic functions, improvement of glucose tolerance, and effects on the central nervous and the renin–angiotensin–aldosterone systems.

Potassium also protects against stroke, which is the third leading cause of death worldwide (after coronary artery disease and cancer). This electrolyte may decrease ischemic as well as hemorrhagic stroke risk through its effect on blood pressure on the one hand and by inhibiting the formation of free radicals at the endothelial cell level, thus affecting vasomotion, on the other hand. Moreover, potassium inhibits the proliferation of smooth muscle cells, platelet aggregation, and arterial thrombosis. Potassium may also prevent the death of cerebellar neurons.

Interrelationships exist between potassium and other nutrients. Potassium and sodium are strongly metabolically interrelated, principally due to K^+/Na^+-ATPase. This enzyme also provides the driving force for the transport of other solutes, such as amino acids, phosphate, vitamins, and glucose. Potassium depletion, which is more intense if there is a simultaneous excess of sodium, enhances urinary loss of calcium. This interaction may have adverse effects on the bone and blood pressure.

Requirements and Daily Intakes

To date, no recommended potassium intake has been unanimously established. The UK report on dietary reference values established a reference nutrient intake of 3500 mg day^{-1} for adults. Lower amounts were recommended for infants (mg day^{-1}): 800 and 850 for 0–3 months and 4–6 months, respectively; 700 for 7–12 months; 800 for 1–3 years; 1100 for 4–6 years; 2000 for 7–10 years; and 3100 for 11–14 years. No specific levels were recommended during pregnancy and lactation or for the elderly. The Nutrition Working Group of the European International Life Sciences Institute suggested a higher recommended daily intake (3900 mg). This recommendation was aimed at promoting a potassium intake similar to that of sodium, in molar terms, that is, an intake of 100 mmol day^{-1} of each cation (2300 mg and 3900 mg for Na and K, respectively). However, more recently, a Na:K ratio below 1 (1600 mg and 3500 mg for Na and K, respectively) has been recommended to prevent hypertension, stroke, and cardiovascular disease.

During exercise, potassium needs may be increased owing to higher losses in sweat, especially in hot climates and in

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unaccustomed individuals. These needs should be satisfied by eating food rich in potassium and exceptionally by potassium supplements.

Published values of nutrient consumption demonstrate that in some populations, sodium intake is higher than recommended, while potassium intake should increase. In the United Kingdom, the 1991 report of the National Food Survey Committee showed that the average sodium intake was 170% of the reference intake, while that of potassium was 80% of the reference value. In Spain, data from the same year revealed that the estimated potassium intake was satisfactory but that of sodium was 140% above the reference value.

Potassium is ubiquitous in all kinds of food, but the best sources are vegetables and fruits because they combine a high potassium content with low sodium concentration (Table 1). Potassium is an essential element of all forms of life, whereas sodium and chlorine are essential for animals but not for many plants. Meat and fish contain important amounts of potassium because they are highly cellular tissues, but they also contain large amounts of sodium chloride, due to the ECF present in animal food. Raw foods are preferable to processed ones because during food preparation, salt is usually added to enhance flavor and retard bacterial growth (e.g., bread, bacon, tomato ketchup, and cookies). Potassium salts, however, are not palatable and are not added to food during processing.

Table 1 Potassium and sodium content of various foods (mg per 100 g of edible portion)

	Potassium	*Sodium*
Vegetables		
Potatoes	320	11
Cabbage	275–310	7–12
Carrots	235	60–90
Spinach	500–633	65–140
Legumes		
Red kidney beans	1370	18
Soya beans	1730	5
Lentils	940	12
Fruits		
Banana	400	1
Melon	100–310	5–32
Avocado pear	400–600	2–4
Orange	195	2–3
Apple	120	2–3
Dried fruits		
Raisins	1020	60
Figs	970	62
Almonds	780	14
Walnuts	450	7
Cereals		
White bread	100	460–540
Brown bread	175–240	540–636
Rice	110	6
Meat and fish		
Beef, veal, lamb	230–360	52–110
Chicken	320	81
Bacon	350	1860
Herring	320	120
Halibut	410	60
Tuna	400	47
Mussels	320	290
Milk		
Whole cows' milk	140	55
Various		
Chocolate	300	11
Tomato ketchup	590	1120

Legumes represent a good source of potassium because they provide at least 1 g potassium per 100 g portion and very little sodium. Dried fruits, for example, apricots, figs, and prunes, should also be mentioned, since they often exceed 0.5 and even 1 g potassium per 100 g portion. Nuts such as pistachios and almonds contain a considerable quantity of potassium, but the addition of salt during roasting upsets the ideal sodium-to-potassium ratio normally present in these foods. Bananas are known to be one of the fruits richest in potassium, together with coconuts, melons, avocados, and kiwi fruit. Milk is the largest dietary source of potassium for infants.

All these data should be considered, taking into account the amount of each food item consumed. For example, a ration of legumes is ~80 g, while those of meat and milk can easily reach 200 g. Although cereals are low in potassium, they account for almost 75% of potassium ingestion in Western diets. There is a wide range of potassium intake by Western populations (1560–5850 mg). It is higher in vegetarians and people who consume more food from plant than animal sources. The Mediterranean diet provides a mean daily intake of potassium equivalent to the recommended value (3500 mg).

Although thermal treatments have no effect on dietary potassium, during processes such as boiling, soaking, and canning, it can be reduced as a result of leaching, particularly if food is to be cut into pieces and the liquid is not consumed.

In order to protect against hypertension and stroke, except in clinical hypokalemia, potassium from good dietary sources should be encouraged rather than potassium from supplements, because such supplements may have severe adverse effects. Fruits and vegetables also contain magnesium and fiber, which seem to have protective effects.

Absorption

Normal subjects absorb about 90% of ingested potassium in the intestinal tract. The net absorption is the difference between fluxes from lumen to blood and from blood to lumen. In the human small intestine, K^+ permeability is high and potassium absorption is carried out across the epithelium of duodenum, jejunum, and ileum by passive mechanisms in response to electrochemical gradients and solvent drag. In the proximal small bowel, K^+ is concentrated through the absorption of water, providing a driving force for the movement of this cation across the intestinal mucosa, preferably through the tight junctions between enterocytes. Duodenum and jejunum absorb this ion even more rapidly than water. Indeed, shortly after a meal, the K^+ concentration $[K^+]$ in jejunum rapidly reaches plasma levels. In the ileum, the transepithelial electrical potential difference strongly influences its movement. There is no evidence of active potassium absorption in the small intestine, but the existence of an apical membrane H^+/K^+-ATPase could suggest active K^+ transport.

In the colon, K^+ may be secreted or absorbed in response to variations in potassium status: net secretion occurs when the luminal concentration is $<20–25$ mmol l^{-1}, while net K^+ absorption takes place when levels are above 25 mmol l^{-1}. There are two mechanisms for K^+ secretion; the major mechanism involves potential-dependent, passive flux, mostly via the tight union or by facilitated transcellular diffusion. The second is an active serosa-to-mucosa secretory mechanism in the proximal and distal colon, which is the result of uptake across the basolateral membrane mediated by Na^+/K^+-ATPase and $Na^+/K^+/Cl^-$ cotransport and movement across the apical membrane through potassium channels. Active K^+ absorption also occurs in the distal colon, in which the K^+ uptake appears to be an exchange for H^+ across the apical membrane. This process is energized by adenosine triphosphatases (ATPases) located in that membrane. Passive potassium absorption may also take place in the proximal colon.

Bioavailability

The availability of dietary potassium is high because potassium salts are entirely soluble and few dietary factors modify its digestive utilization. Olive oil favors potassium uptake, while some fibers and certain ion exchange resins may decrease K^+ absorption.

Physiological status also affects the nutritive utilization of potassium. Infants absorb a greater proportion of salts than adults. This may be due to the increased permeability of the immature small intestinal epithelium of newborns, and especially to a higher activity of the K^+-absorptive pumps in the colonic apical membrane, while K^+-secreting channels are less relevant. During the second half of pregnancy, fecal potassium decreases. Urinary K^+ output increases in parallel with Na^+ retention; nevertheless, enough potassium is retained to cover gestational needs. Glomerular filtration rates fall with age, accompanied by limitations of K^+ secretion and Na^+ conservation. Many drugs used in the elderly alter potassium homeostasis.

Transport

Potassium, being an electrolyte, is transported mainly under ionic form in the body fluids. Intracellular potassium concentration is 110–160 mmol l^{-1} of cells, while plasma concentration averages 4–5 mmol l^{-1}, of which only 10–20% is bound to proteins.

Figure 1 schematically illustrates the intracellular and paracellular mechanisms of potassium transport. Different potassium transport mechanisms can operate simultaneously. They are usually interrelated and are also linked to other ion transport systems. Each name, except the last one, corresponds to an entire family of transporters.

Na^+/K^+-ATPase activity is responsible for the maintenance of the extra- and intracellular Na^+ and K^+ concentrations against electrochemical gradients. This ATPase is found in the plasma membrane of virtually all animal cells and represents an enormous metabolic energy cost, which increases the entropy of the system. It is a carrier protein that pumps $2K^+$ in and $3Na^+$ out of the cell during each cycle of conformational changes driven by adenosine triphosphate (ATP) hydrolysis. This process is electrogenic, meaning that one net positive charge is removed from the cell every pump cycle. Many

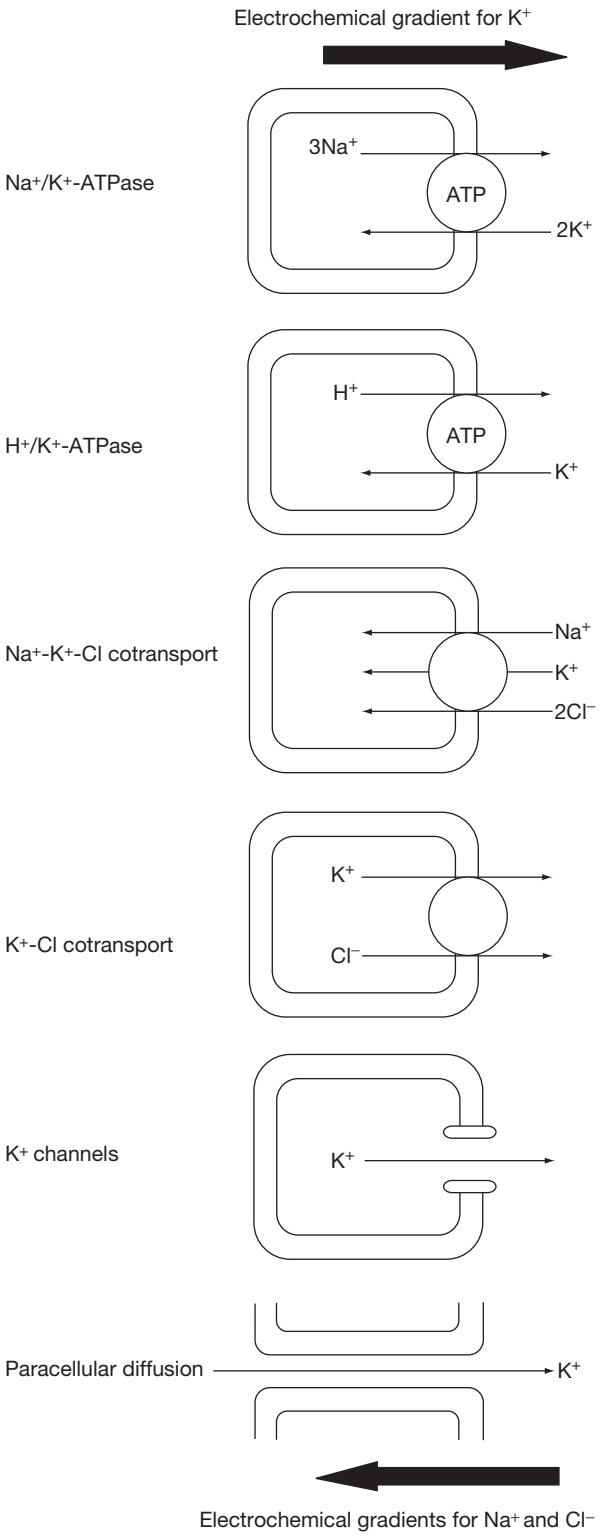

Figure 1 Potassium transport via intracellular and paracellular pathways. ATP, adenosine triphosphate.

hormones and various neurotransmitters can modulate K^+/Na^+-ATPase activity.

Another active K^+ transport is controlled by the H^+/K^+-ATPase, which ejects H^+ in exchange for K^+. This pump has an important role in some gastrointestinal cells and in the renal tubules.

Other potassium transport systems are driven by the force of ion gradients. The free energy gained during the movement of an inorganic ion down an electrochemical gradient (sum of the concentration gradient and the electrical potential difference) is used as the driving force to pump other solutes against their electrochemical gradient. Thus, the carrier protein acts as a coupled transporter and electroneutrality is maintained. Cotransport depends on ATP only indirectly. Various $Na^+/K^+/Cl^-$ cotransporters, which carry $1Na^+$, $1K^+$, and $2Cl^-$ inside the cell, have been identified in salivary glands, gastrointestinal tract, and renal tubules. The K^+/Cl^- cotransporter, related to the former ones, plays an important role in erythrocytic volume. The first cotransport mediates K^+ influx, while the second mediates efflux.

Passive transport of K^+ occurs via intracellular and paracellular pathways. The intracellular mechanism involves K^+ channels, which are pores in the plasma membrane made by a specific protein. Channels have 'gates' that open and close in response to specific stimuli: voltage, stretch, ATP, Ca^{2+}, hormones, neurotransmitters, etc. Various stimuli sometimes act together on a channel. K^+ channels exhibit great diversity and participate in many cell functions.

Distribution and Storage

Body potassium concentration is 45–55 mmol kg^{-1} of body weight; thus, a 70 kg adult man contains ~135 g potassium. Women have 35–40 mmol kg^{-1} of body weight, and in the elderly and children, body potassium concentration is also lower than that for young men. These differences are due to the lower muscle mass in these groups. Almost 98% of the potassium in the body is inside the cells, making it the major intracellular cation. More than 95% of total potassium is exchangeable.

Total potassium is an index of lean body mass because potassium is only present in the fat-free compartments of the body. It can be measured with K^{40}, a natural isotope present as a small fraction of the total potassium, which emits γ-rays and can be detected by a sensitive whole-body counter, although other isotopes and other techniques that are not based on total potassium are also available.

Studies of body composition in healthy humans have demonstrated that the total body potassium/total body nitrogen ratio is constant. However, in chronic protein-energy malnutrition, this ratio can decrease owing to the reduction of muscle proteins and loss of intracellular potassium. The cellular exchange of sodium and potassium is altered, leading to potassium loss and increased intracellular sodium and water. In contrast, exercise and training increase total lean body mass, thereby causing a rise in potassium body content. Increase in muscular mass is only possible if proteins, as well as potassium, phosphorus, and magnesium, which are the main intracellular elements, are present in the diet in adequate amounts.

In the ECF, an increase of potassium equivalent to 1% of total body potassium may double the concentration of potassium in plasma, resulting in muscle hypopolarization. However, if such an increase is stored in the cells, only minor intracellular changes would result, with little change to the potassium concentration difference across the cell membranes. To accomplish this buffer function, the major reservoir of potassium is the muscle, followed by the liver and erythrocytes, although each cell possesses the capacity for accumulating potassium.

Excretion

About 90% of daily potassium intake is excreted in the urine and 10% in the stool, although this last percentage can be somewhat higher in cases of diarrhea. When dietary potassium is severely restricted, its fecal loss decreases to ~3.5 mmol day^{-1}. This presumably represents obligatory potassium losses related to K^+ digestive secretions (salivary, gastric, biliary, and pancreatic), cell desquamation, and mucus secretion. Potassium concentration in sweat is about 10 mmol l^{-1}, so loss through perspiration is small, provided that the climate and exercise conditions are not extreme.

Renal Potassium Handling

The kidney plays the main role in potassium excretion. Renal potassium excretion shows a circadian rhythm, characterized by peak output during the subject's activity.

K^+ is freely filtered by the glomerulus. Usually, urinary potassium excretion is 5–15% of the amount filtered, which indicates the existence of tubular potassium reabsorption. The renal tubules are capable of reabsorbing and secreting potassium in response to various stimuli.

Proximal tubule

The proximal tubule is responsible for the reabsorption of ~60% of previously filtered potassium. Solvent drag and diffusion have been proposed as the major driving forces of this process, and K^+ is reabsorbed largely via the paracellular pathway. However, some observations suggest participation of a transcellular route of potassium reabsorption by an active mechanism. The fractional rates of K^+ reabsorption are similar to those of sodium and water along the proximal tubule, and changes in fluid and potassium transport are closely coupled.

In this region, the basolateral K^+ channels have higher conductance than the apical ones, and this is essential to maintain a negative intracellular potential and hence promote reabsorption of positively charged carriers (Na^+–glucose cotransport).

Loop of Henle

The concentration of K^+ increases as the filtrate passes through the loop of Henle, where permeability for potassium is very high. At the end of the descending limb, the amount of K^+ present usually exceeds that of the glomerular filtrate. There is a net passive secretory entry of potassium into the proximal

straight tubule and the descending thin limb of Henle, which arises from reabsorption in the collecting tubule and partly in the ascending limb. These pathways of K^+ transport constitute potassium recycling in the renal medulla.

In the thick ascending limb, potassium is mainly reabsorbed. The mechanism of active transport includes apical uptake by $Na^+/K^+/Cl^-$ cotransport and passive paracellular exit across the basolateral membrane by diffusion. Driven by transepithelial potential difference, reabsorption also occurs through the paracellular pathway.

The role of this limb in potassium secretion has also been recognized. The K^+ secretion through apical K^+ channels is important for net Na^+ and Cl^- reabsorption through $Na^+/K^+/Cl^-$ cotransport, since luminal Na^+ and Cl^- concentrations are higher than luminal K^+ levels.

Distal tubule and collecting duct

These tubules are the major determinants of urinary K^+, since they are able to either reabsorb or secrete potassium at rates that depend on K^+ intake and other factors.

Potassium secretion occurs in 'principal cells' by active uptake across the basolateral membrane by Na^+/K^+-ATPase and passive diffusion into the lumen across the apical membrane by K^+ channels or using a K^+/Cl^- cotransport (Figure 2). K^+ transport follows this route toward the lumen thanks to a favorable electrochemical gradient and the greater K^+ permeability of the apical membrane in comparison with the basolateral one. High Na^+ concentration in the lumen favors K^+ secretion, as the entrance of Na^+ into the cell generates a potential difference that tends to make the interior positive, so K^+ diffuses in the opposite direction. Simultaneously, the enhanced delivery of Na^+ into principal cells facilitates the action of Na^+/K^+-ATPase, and the accelerated potassium uptake favors its secretion toward the lumen.

Potassium reabsorption mechanisms have been detected in the intercalated cells of the distal tubule and collecting duct. It appears that there is a component of potassium reabsorption even during net secretion. Several isoforms of H^+/K^+-ATPases are able to reabsorb K^+ in exchange for H^+. The $Na^+/K^+/Cl^-$ cotransport may also introduce K^+ into the cell, while the basolateral K^+ channels enable potassium to pass from the cell into the interstitial space. Proton secretion by H^+/K^+-ATPase, together with that by H^+-ATPase, contributes to urine acidification. These enzymes participate in the acid–base balance and potassium status.

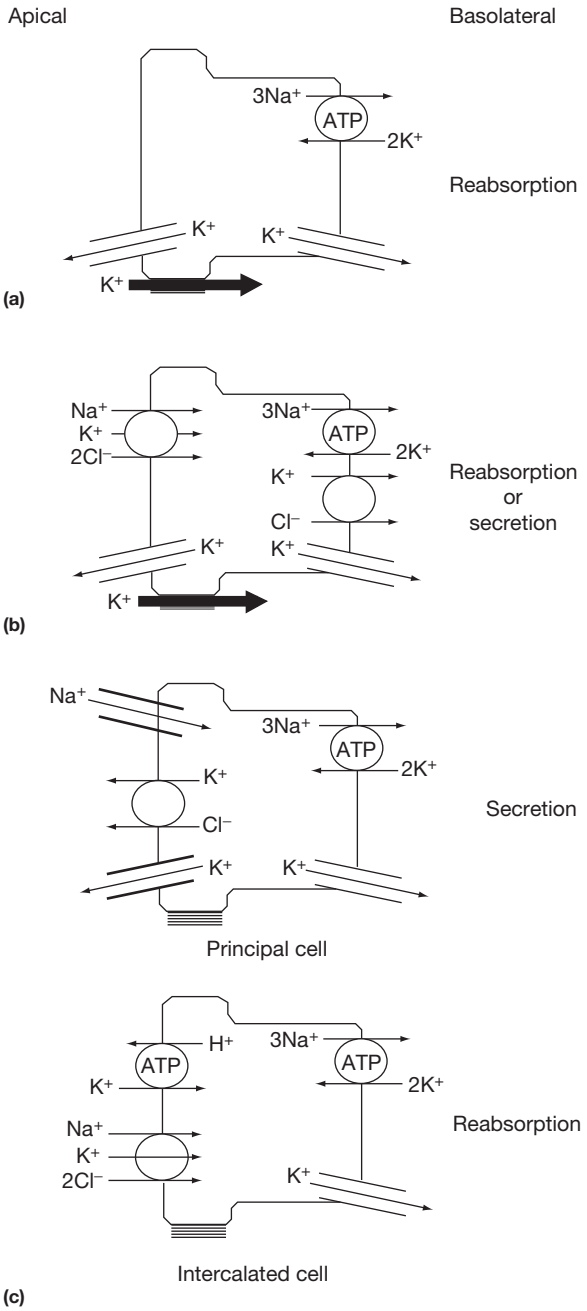

Figure 2 Principal mechanisms of potassium transport in the major tubular segments of the nephron: (a) the proximal tubule; (b) thick ascending limb; (c) distal tubule and collecting duct. ATP, adenosine triphosphate.

Potassium Balance and Homeostasis

Intra- and extracellular potassium concentrations must be maintained within narrow limits, despite wide fluctuations in dietary intake. This is achieved, on the one hand, by 'internal balance' (distribution of K^+ between the intracellular fluid and ECF carried out mainly by Na^+/K^+-ATPase) and, on the other hand, by 'external balance' (maintenance of the amount of K^+ in the body through equilibrium between K^+ intake and excretion, which is achieved principally not only in the kidney but also in the intestine). After a meal, the absorbed K^+ rapidly enters the ECF. The subsequent rise in plasma $[K^+]$ is attenuated by a rapid cellular K^+ uptake (which occurs within minutes). To maintain external K^+ balance, all of the K^+ absorbed in excess must be excreted slowly by the kidneys.

Potassium Homeostasis

To maintain the equilibrium previously described, several factors that act at different levels contribute to potassium homeostasis (Figure 3).

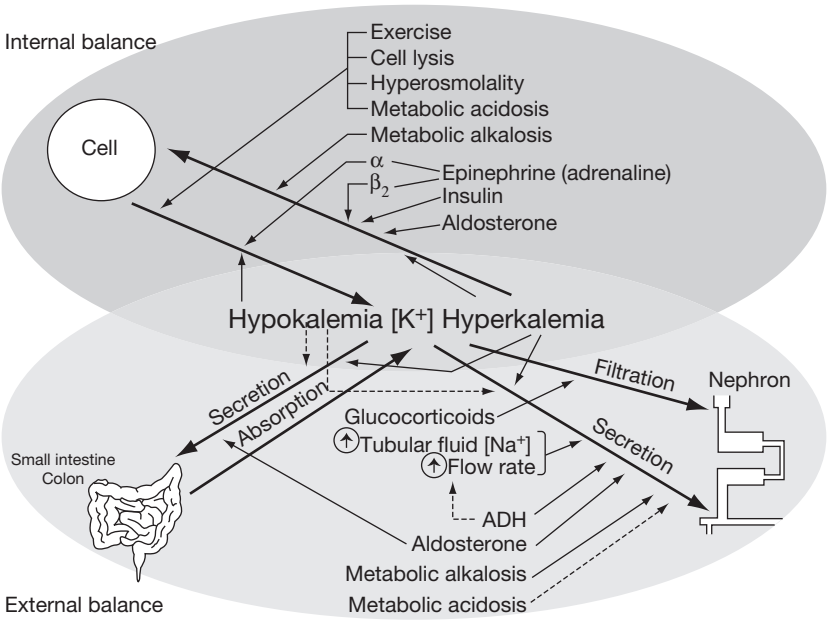

Figure 3 Potassium homeostasis. ADH, antidiuretic hormone. Continuous arrow, increase; dashed arrow, decrease.

Internal balance

A rise in plasma [K^+], especially with hyperkalemia, and the hormones insulin, epinephrine or adrenaline (by activating β_2-receptors), and aldosterone promote K^+ uptake by the muscle, liver, bones, and red blood cells. In contrast, a decrease in plasma [K^+], such as with hypokalemia, and stimulation of α-adrenergic receptors induce K^+ transport from cells to the ECF. Moreover, hyperkalemia stimulates insulin, aldosterone, and epinephrine secretions, while hypokalemia has the opposite effect. These are the major physiological factors involved in internal K^+ balance homeostasis.

Furthermore, other factors that are not normal homeostatic mechanisms influence K^+ movements across the plasma membrane: metabolic acidosis promotes exit of K^+ from cells, whereas metabolic alkalosis favors its uptake. Increased osmolality of the ECF enhances K^+ release by cells. Cell lysis (severe trauma, burns, etc.) induces K^+ release and may produce hyperkalemia. During intense exercise, K^+ is also released from skeletal muscle cells.

External balance

There are two hypotheses concerning the homeostatic mechanisms, which regulate external K^+ balance:

1. The first involves a peripheral mechanism without central nervous system (CNS) intervention. In this case, [K^+] would stimulate urinary K^+ excretion and aldosterone secretion directly.
2. The second attributes K^+ regulation to a reflex mechanism initiated by K^+ receptors in the portal vein and liver stimulated by high [K^+]. Afferent fibers would transmit signals to CNS – 'K control center' – and efferent limbs, mediated by several hormones and other factors, would modulate urinary K^+ excretion in response.

In any case, plasma [K^+] and aldosterone are the major physiological regulators of K^+ excretion. High [K^+] increases aldosterone secretion and both act synergistically, stimulating K^+ elimination by the intestine (particularly increasing colonic secretion) and, above all, by the kidney (promoting secretion in the distal tubule and collecting duct). Renal secretion is due to stimulation of the Na^+/K^+-ATPase pump, increased permeability of the apical membrane to K^+, and greater K^+ uptake across the basolateral membrane. Hypokalemia decreases K^+ secretion by antagonist action. Under repletion conditions, potassium transported by Na^+/K^+-ATPase and H^+/K^+-ATPase recycles back into the lumen through K channels; however, when potassium levels are low, little potassium is wasted through luminal channels and passage of K through basolateral channels predominates.

Aldosterone secretion is favored by angiotensin II. On the other hand, aldosterone stimulates Na^+ and water reabsorption and consequently decreases tubular flow at first. However, flux is restored in time, permitting aldosterone to increase K^+ renal excretion.

Antidiuretic hormone (ADH) increases K^+ secretion in the apical membrane of the principal cells in exchange for Na^+ absorption. However, because ADH also decreases tubular flow, changes in ADH levels do not substantially alter urinary K. K^+ secretion rises along the distal tubule in a flow-dependent manner and it is influenced by dietary potassium intake. This means that diuretics may enhance urinary K^+ excretion. However, K^+ excretion and distal flow rate are not completely coupled, and the K^+ balance is maintained through compensatory mechanisms when necessary.

Physiological [Na^+] does not alter K^+ secretion but high [Na^+] in tubular fluid increases K^+ secretion, whereas a fall in concentration has the opposite effect. Acid–base balance is another factor that modulates K^+ secretion: alkalosis increases secretion, whereas acidosis decreases it. Glucocorticoids are

kaliuretic, but their effects appear to be a consequence of an increase in the glomerular filtration rate.

K^+ itself exerts a feedback regulation over most of its own control factors. Together, all these mechanisms allow the human body to maintain normal plasma [K^+]. However, the equilibrium can sometimes be altered, causing either hyperkalemia (extracellular [K^+] over 5.5 mmol l^{-1}) or hypokalemia (extracellular [K^+] below 3.5 mmol l^{-1}).

Hyperkalemia may be the consequence of a K^+ shift from cells to ECF or the effect of excessive K^+ retention. The first signs are flaccid paralysis, natriuresis, and other minor effects. The most important consequences are ventricular fibrillation and cardiac arrest. Hyperkalemia may be either of the following:

- Without potassium excess: caused by K^+ shift from cells (acute acidosis, hyperosmolality, insulin deficiency, β-adrenergic blockers, vigorous exercise, etc.)
- With potassium excess: impaired renal excretion (renal failure, aldosterone deficiency, and primary tubule dysfunction) (inappropriately high oral or parenteral K^+ administration)

Hypokalemia may be the result of either intracellular shift or K^+ depletion or both. Among its clinical sequelae is hyperpolarization of membrane, which affects nerves and muscle activity and produces renal, cardiac, and metabolic alterations. The hypokalemia may be caused by the following:

- Intracellular shift: alkalosis, insulin excess, familial periodic paralysis, catecholamine increase, intoxications, etc.
- Extrarenal causes: insufficient K^+ intake, diarrhea, vomiting, skin loss, etc.
- Excessive renal losses: renal tubular acidosis, hyperaldosteronism, syndrome of chloride depletion, and diuretic condition

See also: Colon: Structure and Function; Diarrheal Diseases; Hypertension and Diet; Potassium: Properties and Determination; Vegetarian Diets.

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Potassium: Properties and Determination

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Properties and Determination

Potassium is a light, soft metal and a strong reducing agent, discovered by Davy in 1807 in caustic potassium (KOH). It is widely distributed in minerals such as sylvin (KCl), carnallite ($KMgCl_3$ or $MgCl_2{\cdot}KCl$), langbeinite [$K_2Mg_2(SO_4)_3$], or polyhalite [$K_2Ca_2Mg_2(SO_4)_4{\cdot}2H_2O$]. Potassium is an abundant metal and is the seventh most abundant element in the earth's crust. Seawater contains 380 p.p.m. of potassium, making the mineral the sixth most abundant in solution.

Potassium is used extensively, mainly in the form of potassium salts, in various fields such as medicine (iodide as a disinfectant), photographic processing (carbonate), explosives (chlorate and nitrate as powder), defreezing agents, poisons (potassium cyanide), metallurgy, basic chemistry (hydroxide in, e.g., strong bases, oil sweetening, CO_2 absorbent, chromate and dichromates as oxidants, and carbonate in the glass industry), fertilizers (nitrate, carbonate, chloride, bromide, and sulfate), detergents and soaps (hydroxide and carbonate), and coolants in nuclear reactors (NaK alloy), etc.

Potassium (atomic number = 19) forms part of the group of alkali metals classified between sodium (atomic number = 11) and rubidium (atomic number = 37). It has an atomic weight of 39.098, an oxidation state 1, and an electronic orbital structure of [Ar] $4s^1$. This electronic configuration (one electron in the 4s orbital) enables potassium to ionize easily to the cation K^+ owing to the loss of its most external electron. It does not appear as a native metal in nature. Potassium exists in nature in three isotopes: ^{39}K (93.26%), ^{40}K (0.0117%), and ^{41}K (6.73%). ^{40}K is radioactive and responsible for most of the naturally occurring internal radioactivity in the body. This property enables researchers to monitor total body potassium values as a function of age and disease.

The behavior of potassium in its metallic form is similar to that of sodium, with which it is closely related. Both are alkali metals essential for life owing to their involvement in cellular physiological development and the regulation of body fluids, together with chlorine, thus explaining why it is common for both alkali metals to appear in the form of their corresponding chloride salts. Given this relationship with cellular liquids, potassium, sodium, and chlorine are also known as electrolytes (ions with greater proportions in the composition of organic fluids).

The K^+ ion is the main intracellular cation, with an approximate [K^+] of 5.6 g l^{-1} in cellular fluids, ~30 times more concentrated than in plasma or interstitial liquid (0.15–0.20 g l^{-1}). Its high intracellular concentration is regulated by the cell membrane through the sodium-potassium pump. The intracellular K^+ regulates the catalytic action of numerous enzymes, through the attachment of the cation to active locations with negative charges of the enzymatic proteins, modifying the conformation of the molecule and its activity, as well as participating in cellular division processes.

The small percentage of extracellular potassium (2% of body potassium) is of great physiological importance since it is a critical determinant of neuromuscular excitability (nervous impulse and contraction of bone muscles). At a cellular membrane level, the transport and permeability of energy-dependent potassium, with simultaneous excretion of sodium linked to the Na/K enzyme ATPase, is essential for generating the potentials of membranes required for the proper functioning of nervous and muscular cells. It also helps to maintain the acid–base equilibrium and blood pressure.

Homeostasis of potassium is still the subject of research, although it is known that 90% of dietetic potassium is absorbed in the small intestine and that body potassium (1.6–2 g kg^{-1} of body weight) is regulated by renal glomerular filtration and tubular secretion, with potassium being lost on a daily basis through urine, gastrointestinal secretions (ileum and colon), and, to a lesser degree, sweat. Provided that renal function is normal, it is practically impossible to reach an excessive level of potassium with normal dietary intake, since the kidney is capable of excreting more potassium than it can filter.

The effect of potassium on blood pressure has been discussed in recent reports on metaanalysis; these have confirmed that increases in doses of potassium from 60 to 80 mmol day^{-1} (2.3–3.1 g day^{-1}) may prompt a decrease of 4 mmHg in systolic blood pressure and possibly reduce the number of deaths related to high blood pressure by 25%.

The use of potassium chloride salts as substitutes for sodium chloride, in individuals with whom the intake of sodium is restricted because of problems relating to hypertension, is a questionable alternative, since cardiac arrhythmias have been reported in association with the excessive intake of salt substitutes containing potassium chloride. Although hyperkalemia is highly unlike in normal diets, it can be produced with healthy individuals for intakes exceeding 17.5 g day^{-1}. In the case of individuals with renal dysfunctions this disorder may appear with intakes of potassium exceeding 5.9 g day^{-1}. Cardiovascular or neuromuscular complications arising from situations of hypo- and hyperkalemias are resolved favorably by correcting the plasmatic potassium levels.

The mean potassium intake in Western populations ranges between 1.6 and 5.9 g day^{-1}, and the required dietary intakes of this are met without any problem, thanks to the ubiquitous presence of potassium in both vegetable- and animal-based foods. The Scientific Committee for Food of the European Community recognizes that the minimum recommended intake of potassium is 1600 mg day^{-1}, and the reference intake for the population is 3100 g day^{-1}.

Analysis of Potassium

Potassium can be analyzed using a number of different methods, although many of these are not commonly used for

routine analysis of potassium in food and biological samples. In the case of potassium, there has been a shift from the old gravimetric methods, based on the precipitation of potassium using chloroplatinate or tetraphenylboron, to the spectroscopic methods, mainly atomic emission and atomic absorbance spectroscopy, and which, according to the scientific community, are the most commonly used.

However, there are other techniques for analyzing potassium that are not as 'popular' as those mentioned above, and are equally applicable to other mineral elements. These include selective electrodes, nuclear magnetic resonance (NMR) spectroscopy, x-ray analysis, helium glow photometry, inductively coupled plasma atomic fluorescence, ion-scattering spectrometry and other methods. Another procedure can be used – namely, radioactive dilution of potassium isotopes – when determining both potassium content and its distribution in extra- and intracellular compartments.

Emission and absorbance spectrometry have been the most widely used techniques for analyzing trace elements in biological and food samples. Their widespread use is justified by their analytical specificity, good detection limits, excellent accuracy, and relatively low cost. Both techniques are based on energy modifications of the electronic orbital structure of the atoms of mineral elements in response to certain stimuli (flame, plasma, and incidence of a beam of light of specific wavelength for absorption).

The determination of potassium by spectroscopy requires a stage of preparation of samples for analysis that entails the drying, grinding, and destruction of the organic matter of the sample. There are two alternatives for destroying the organic matter of biological and food samples, both with different variants: wet oxidation and dry ashing. Both techniques yield reasonably comparable results and it is often the analyst who decides which technique to employ.

Atomic Absorption Spectroscopy

In atomic absorption spectroscopy (AAS), electromagnetic radiation at a specific wavelength is passed through a cell containing gaseous free atoms. Atoms are produced in two different ways using AAS: flame atomization (FAAS) and electrothermal atomization (ETAAS) (graphite furnace and a L'vov platform). Thus, the determination of potassium using an atomic absorption spectrophotometer requires a light source (wavelength for $K = 766.5$ nm) and an atomization source. For the light source, arc discharge lamps may be used for *K*, although hollow cathodes are preferred. Lineal calibration is required to obtain analyte concentrations in the sample. Standard working conditions for analyzing potassium by AAS are listed in Table 1.

Atomic Emission Spectroscopy

The determination of potassium in biological and food samples, as in the case of sodium, may be performed using flame photometers and atomic absorption spectrophotometers (with the option of working in emission conditions). In recent years, some manufacturers have named atomic emission spectroscopy (AES) instruments as 'optical emission spectrometers' (OES) because they measure the emitted light when excited atoms return to the ground state. Since this latter term is more widely used, we will use OES rather than AES onwards in the article, but the terms are practically interchangeable. There are two ways to produce the excitation of the atoms in food analysis: flame emission spectroscopy or the use of electrically generated plasmas (highly ionized gases) resulting in inductively coupled plasma–optical emission spectroscopy (ICP–OES). Flame emission can be used for the thermal excitation of elements with relatively low excitation energies such as potassium. A specific wavelength of 766.5 nm is used in order to determine potassium, and the equipment reading resolution is adjusted to the response given to the pattern of greatest concentration used. An effort must be made to avoid possible interference problems due to the matrix characteristics of the sample in flame emission photometry; this can be achieved by separating the element from the object of study, eliminating the ions responsible for the interferences, or using a compensatory technique. For this purpose, recommended practice for correcting interferences includes the addition of lithium (as an internal standard) either to the samples or to the standards. It is important to obtain a suitable calibration curve, either linear by diluting the samples, or by logarithmic adjustment between emission values and potassium concentration.

When comparing flame emission spectroscopy and AAS, the detection limits for potassium ($\mu g\,ml^{-1}$) are 0.0005 and 0.005, respectively. Using an atomic absorption spectrophotometer, the accuracy and precision of potassium analysis by flame emission can be obtained (Table 2).

Table 3 shows a series of official methods for determining potassium, based on flame emission and absorption spectrophotometry.

Table 1 Standard conditions for potassium analysis by AAS

Spectrometers setting	*Potassium*
Wavelength/slit (nm)	766.5/0.2
Nebulizer	Spoiler
Oxidant	Air
Fuel	C_2H_2
Flame condition	oxidizing
Optimum concentration range in solution ($\mu g\,ml^{-1}$)	1–10
Detection limit ($\mu g\,ml^{-1}$)	0.005
Sensitivity 1% absorption ($\mu g\,ml^{-1}$)	0.05
Interferences	Ionization

Inductively Coupled Plasma–Optical Emission Spectrometry

The above analytical techniques are shown to be sensitive enough to detect potassium and other trace elements, however, as mono-elemental techniques; they become slow and tedious when a large number of elements are analyzed. ICP–OES is a multi-elemental technique that is able to determine concentrations of multiple elements in a sample with a single aspiration obtaining a high degree of variability and minimum interferences. As already mentioned, argon plasma is needed to produce atomization. The nearly complete atomization of the sample minimizes chemical interferences. Samples can be prepared by either wet oxidation or dry ashing, and a wide range of concentrations can be used without the need to dilute or

Table 2 Accuracy and precision of the potassium analysis by flame emission

Accuracy	*Certified (g kg^{-1})*	*Found (g kg^{-1})*	*IC (95%)*[a]	*Rec (RSD)*[b]
Citrus Leaves SRM-1572	18.2 ± 0.6	17.7 ± 0.5	16.8–18.7	97 (3)
Non-Fat Milk Powder NIST-1549	16.9 ± 0.30	16.3 ± 0.41	15.5–17.1	96 (5)
Precision	Relative standard deviation			
Plant food	1.35			
Dairy food	2.55			

[a]Intervals of confidence (95%).
[b]Recovery percentages and relative standard deviation (RSD).

Table 3 Official methods recognized for potassium determination

Official methods	
Sodium and potassium in seafood	Flame photometric method, AOAC Official Method 969.23
Potassium in fruits and fruit products	Rapid flame photometric method, AOAC Official Method 965.30
Potassium and sodium in wines	Flame spectrophotometric method, AOAC Official Method 963.13
Potassium in distilled liquors	Flame photometric method, AOAC Official Method 963.08
Potassium in beer	Atomic absorption spectrophotometric method, AOAC Official Method 987.02
Potassium in water	Atomic absorption spectrophotometric method, AOAC Official Method 973.53
Potassium in infant formula, enteral products and pet foods	Atomic absorption spectrophotometric method, AOAC Official Method 985.35

concentrate the sample. For example, it is possible to measure samples varying in concentration from 1 μg l^{-1} to 1 g l^{-1} without having to recalibrate the instrument. Also, many of the other mineral elements can be quantified, thus reducing the time of analysis. These advantages, together with the high speed and excellent instrument stability, make ICP–OES highly attractive.

For potassium determined by ICP–OES in plant tissues a solution detection limit of 0.06 (μg ml^{-1}) and a sample detection limit of 2.0 (μg g^{-1}, based on a 2-g sample diluted to 50 ml) are recommended. There is also an AOAC Official Method (984.27) for the determination of potassium in infant formula by means of ICP–OES, which recommends the following ICP emission spectrometer operating parameters for potassium: wavelength (nm) = 766.5, no background correction, and low standard = 0 and high standard = 200 (μg ml^{-1}). The AOAC also recommends an Official Method (985.01) for the analysis of potassium in plants and pet foods by inductively coupled plasma spectroscopy.

ICP can also be used coupled with a mass spectrometer leading to inductively coupled plasma–mass spectrometer (ICP–MS). MSs are instruments that separate ions according to their mass to charge ratio (m/z) and then accurately count these ions as they strike a detector. ICP–MS is also a multielement technique with very low detection limits and being highly appropriate for the determination of elements present in low levels. Thus, although ICP–OES is a widely used method for the analysis of potassium, ICP–MS can also be used when other elements found in foods in trace amounts are determined simultaneously.

Near Infrared Spectroscopy

The near infrared (NIR) radiation is the electromagnetic spectrum radiation encompassing the interval 780–2500 nm being situated between the middle infrared (MIR) and visible radiations. In spite of the early discovery of NIR radiation in 1800, NIR spectroscopy has not gained interest for chemical analysis up until recently. The main reason has been the high complexity of NIR spectra, which is the result of overtones and combination bands generated by different functional groups such as C–H, N–H, and O–H. Classical statistical methods, such as univariate analysis, are not considered valid to obtain this information. Instead, this disadvantage can currently be overcome using suitable chemometrics techniques, such as multivariate analysis (partial least squares (PLSs) or modified-partial least squares (MPLSs)).

As already mentioned, NIR spectrum of an organic material gives a global signature of composition based on the assessment of the organic chemical structures containing C–H, N–H, and O–H bonds. However, the determination of inorganic compounds such as potassium is more challenging compared to organic compounds owing to the lack of a direct relation between the element involved and the spectra obtained. In this case, NIR spectroscopy could be used for determining mineral concentration, due to association between minerals and organic functional groups in the food matrix and the effect on O–H bonding. The determination of potassium and other mineral elements by NIR is, therefore, generally dependent on the presence of those elements in mixtures of organic or hydrated compounds and salts (cations and anions).

NIR spectroscopy is being widely adopted for potassium analysis of many food products, such as wine, cheeses, and human diets, among others. The main advantage of this analytical technique lies on its speed, accuracy, and low cost (it does not require samples processing). Besides, it is non-destructive and more environmentally respectful when compared with other analytical techniques. Three different measurement setups can be performed to obtain a NIR spectra: reflectance, transmittance, and interactance modes. In reflectance mode, the light source and detector are mounted under a specific angle (e.g., 45°) to avoid specular reflection. In transmittance mode, the light source is positioned opposite to the detector. Finally, in interactance mode, the light source and detector are positioned parallel to each other in such a way that, due to specular reflection, light cannot directly enter through the detector. For potassium analysis, reflectance mode has been widely reported in literature as having an acceptable prediction of the potassium content in foods.

Potassium-selective electrodes

Potassium-selective electrodes have been developing from the first ion-selective glass electrodes, with little ion selectivity, to potassium-sensitive electrodes based on valinomycin, a highly-specific neutral carrier compound for analyzing potassium, to potassium-sensitive electrodes with ion-exchange compounds dissolved in organic solvents that are not specific due to significant interferences induced by the presence of sodium and other cations in organic solutions. Ion-exchange electrodes have faster response times than those with membranes of valinomycin, although the latter show fewer interferences from other ions. However, neither type of electrode is effective in organic solvents. Furthermore, electrodes measure ion activity and therefore, do not detect ions linked to other molecules or potassium that is not in solution.

Ion-selective electrodes are also incorporated into gasometric equipment for measuring electrolytes (Na, K, Cl, and ionized Ca), oxygenation, ventilation, basic – acidic state, and electrolytic metabolism of patients with respiratory problems. In this context, it is useful to quantify the exchange of gases and electrolytes through cellular membranes. For this purpose, the so-called *anion gap* is calculated, which interrelates with sodium (*anion gap Na*), potassium (*anion gap K*), bicarbonate, and chlorine. This parameter provides information on electrolytic alterations and the presence of toxins in blood, and also helps to control quality in laboratories by interrelating standards.

Some developments in the field of potassium-selective electrodes have focused on the appearance of novel potassium-selective valinomycins in the development of ion-selective sensors based on new technologies and on the combination of these ion-selective electrodes with other analytical methodologies, as shown in Table 4.

Carbon materials, such as graphene sheets, have been used as the intermediate layer between an ionophore-doped solvent polymeric membrane and a glassy carbon electrode. The results showed that the graphene layer is capable of both ionic and electronic charge transfer, providing a good Nernstian response to potassium, with a detection limit of 10^{-5} M. Similarly, carbon nanotubes, with a high surface area, have been used as ion-selective electrodes for other elements, such as calcium. Finally, a potentiometric microelectrode method for intracellular K^+ determination based on ZnO nano-wires has recently been reported. These new materials offer promising results to developed ion-selective electrodes with excellent potential stability, low cost, ability to miniaturize, and mass fabrication.

Nuclear Magnetic Resonance

NMR is used to studying the relationship between metabolism and function in living systems as simple as cells in a culture and as complex as human subjects (e.g., the effect of temperature on the sodium/potassium pump in red blood cells). As a non-invasive, non-destructive spectroscopic technique, NMR offers

Table 4 Analytical methodologies with ion-selective electrodes for potassium analysis

Investigation	*Reference*
A potassium-ion selective electrode with valinomycin-based poly(vinyl chloride) membrane and a poly(vinyl ferrocene) solid contact	Hauser, P. C., Chiang, D. W. L. and Wright, G. A. (1995). *Analytica Chimica Acta* **302**(2–3), 241–248
Miniaturized ion-selective sensor chip for potassium measurement in a biomedical application	Uhlig, A., Dietrich, F., Schnakenberg, U., Hintsche, R. and Lindner, E. (1996). *Sensors and Actuators B: Chemical Abstract Export* **34**, 1–3, 252–257
Synthesis of novel potassium selective valinomycins	Dawson, J. R., Dory, Y. L., Mellor, J. M., McAleer, J. F., et al. (1996). *Tetrahedron* **52**(4), 1361–1378
Optical sensors for sodium, potassium and ammonium ions based on lipophilic fluorescein anionic dye and neutral carriers	Wang, E., Zhu, L., Ma, L. and Patel, H. (1997). *Analytica Chimica Acta* **357**, 85–90
Recording of neuronal network properties with ner-infrared dark-field microscopy and microelectrodes	Holthoff, K. and Witte, O. W. (1997). *Electrochimica Acta* **42**(20–22), 3241–3246
Flow injection analysis of potassium using an all-solid-state potassium-selective electrode as a detector	Komaba, S., Arakawa, J., Seyama, M., Osaka, T., Satoh, I. and Nakamura, S. (1998). *Talanta* **46**, 1293–1297
Transferability of results obtained for sodium, potassium and chloride ions with different analyzers	Rodriguez-Garcia, J., Sogo, T., Otero, S., and Paz, J. M. (1998). *Clinica Chimica Acta* 275, 151–162
Plasticizer-free all-solid-state potassium-selective electrode based on poly (3-octylthiophene) and valinomycin	Bobacka, J., Ivaska, A. and Lewnstam, A. (1999). *Analytica Chimica Acta* **385**, 195–202
Simultaneous detection of monovalent anions and cations using all solid-state contact PVC membrane anion and cation-selective electrodes as detectors in single column ion Chromatography	Isildak, I. and Asan, A. (1999). *Talanta* **48**, 967–978
Novel sensors for potassium, calcium and magnesium ions based on a silicon transducer as a light-addressable potentiometric sensor	Seki, A., Motoya, K., Watanabe, S. and Kubo, I. (1999). *Analytica Chimica Acta* **382**, 131–136
Reference ranges of electrolyte and anion gap on the Beckman E4A, Beckman Synchron CX5, Nova CRT, and Nova Stat Profile Ultra	Lolekha, P. H., Vanavanan, S., Teerakarnjana, N. and Chaichanajarernkul. (2001). *Clinica Chimica Acta* **307**, 87–93
Intracellular K(+) determination with a potentiometric microelectrode based on ZnO nanowires	Ali, S. M. U., Asif, M. H., Fulati, A., Nur, O., Willander, M., et al. (2011). *IEEE Transactions on Nanotechnology* **10**, 913–919
All-solid-state potassium-selective electrode using graphene as the solid contact	Li, F., Ye, J., Zhou, M., Gan, S., Zhang, Q., et al. (2012). *Analyst* **137**, 618–623
A dual K(+)–Na(+) selective Prussian blue nanotubes sensor	Ang, J. Q., Binh, T. T. N. and Toh, C. S. (2011) *Sensors and Actuators B: Chemical* **157**, 417–423

a powerful approach to the study of ion balance in intact biological systems. The aims are to validate new NMR methods, to elucidate tissue-specific mechanisms, and to draw conclusions with respect to basic concepts and design in tissue metabolism and function.

The possibility of determining potassium by NMR is due, on the one hand, to the relatively high concentrations of the cation in cellular tissue and, on the other hand, to the natural abundance of ^{39}K nuclide (NMR receptivity is about 2000 times less than that of one hydrogen atom) and the short longitudinal relaxation time. The concentrations of intracellular and extracellular potassium in tissues may be quantified by applying impermeable cell-membrane chemical shift reagents.

However, this quantification by NMR measurement depends on the NMR invisibility of the potassium actually present, in accordance with other techniques. Several previous studies have shown that only ~20% of cardiac intra-cellular potassium is visible with current NMR techniques. This NMR sensitivity of potassium (about 60%) represents a serious obstacle to its proper quantification in biological tissues.

A study of factors affecting ^{39}K NMR detectability in rat thigh muscle showed that the signal may be substantially higher (up to 100% of total tissue potassium) than values previously reported of around 40%. These signals present two superimposed components – one broad and one narrow – and involve improvements in spectral parameters (signal-to-noise ratio and baseline roll), together with computer simulations of spectra, that enable a spectrum quality with a major effect on the amount of signal detected, largely due to the loss of detectability of the broad signal component.

Biological and biochemical cellular research in connection with the quantification of ions in intracellular and extracellular compartments, transport, and ionic metabolism at the level of different cells and biological tissues, as well as on the union capacity of cations Na^+ and K^+ to other cellular components, are based on the use of ^{39}K NMR spectroscopy, together with ^{23}Na and ^{31}P NMR studies.

Proper ion equilibrium between intra- and extracellular compartments is required for normal physiological function. Conversely, alterations in membrane ion transport occur in numerous pathological states. By introducing an anionic paramagnetic shift reagent into the medium, NMR signals of intra- and extracellular Na^+ and K^+ could be resolved, enabling ion transport processes to be studied by NMR. Unfortunately, rare NMR active nuclides that are isotopes of the 100% naturally-abundant $^{23}Na^+$ and $^{39}K^+$ are unavailable for tracer kinetic studies of Na^+ and K^+ transport. However, Cs is a biologically active analog of K^+ and the 100% naturally-abundant NMR active $^{133}Cs^{++}$ nuclide can be employed to examine K^+ transport. Other studies have shown the potential of ^{39}K NMR as a useful tool in the study of protein-cation interactions and the binding of K^+ to double-helical DNA.

x-Ray Fluorescence Analysis

x-Ray fluorescence (XRF) can be successfully used for the qualitative and quantitative elemental analysis of various agricultural products. It belongs to a family of atomic spectrometry techniques but has some distinctive analytical advantages compared with other techniques, such as AAS and ICP–OES. Its simplicity, high throughput and automation possibilities make it useful for screening large numbers of samples. Another major advantage is that it is able to determine almost all the elements in the periodic table – including potassium – simultaneously at very low concentrations. XRF is based on bombarding a small area of the sample with x-ray photons, which are sufficiently energetic to cause ionization of inner electrons of the atoms. Because of this unstable atomic configuration, this vacancy of an inner electron is filled by an electron from an outer orbital, which emitted the difference of energy between the two orbitals as a 'secondary' (or fluorescent) x-ray photon. The main difference with other spectrometry techniques (i.e., AAS or ICP–OES) is that, in the latter, the absorbed or emitted radiation covers the ultraviolet–visible region of the spectrum.

The methods for preparing the specimens of liquid media of the organism and XRF analysis are simple and fast, entail no disintegration of the sample, and allow measurements of elements at sigma $1 = 0.02$ at concentrations of 12 $\mu g\ ml^{-1}$ to be obtained. The bibliography describes different XRF analysis techniques for measuring potassium in food and biological samples, as indicated in Table 5.

Wavelength dispersive x-ray fluorescence (WDXRF) spectrometry has universal application in a wide range of environmental and geo-chemical samples, such as soils, plants, and water. WDXRF instrumentation uses an x-ray tube as the excitation source and is available with one or two scanning goniometers or, alternatively, with a number of multiple fixed goniometer channels for rapid simultaneous elemental detection. Currently, WDXRF has been used for determination of potassium in human placenta and food samples such as beans.

Energy dispersive x-ray fluorescence (EDXRF) incorporates a solid-state semiconductor detector, which offers different detection characteristics with parallel detection of the full x-ray spectrum. Another difference with WDXRF is that adequate excitation is provided by a lower–power x-ray tube arranged for direct or secondary target excitation. Potassium content in human blood, spinach, and human diets has been studied using EDXRF. Furthermore, ED spectrometers have no moving parts and have benefited extensively from the miniaturization that has taken place in integrated circuit technology.

Table 5 x-Ray techniques for potassium analysis in food and biological samples

Samples	*x-Ray analysis*
Human placenta Beans	Wavelength dispersive x-ray fluorescence (WDXRF)
Walls of the mosaic virus infected wheat leaf cells Perisperm tissues of seeds Human blood and human diets Vegetables (spinach)	Energy dispersive x-ray fluorescence (EDXRF)
Cancerous and normal tissues In vegetable foodstuffs and their respective cell fractions Cancerous breast tissue	Total reflection x-ray fluorescence (TXRF)
Human brains Human atherosclerotic artery wall	Proton induced x-ray emission (PIXE)
Breast tissue	Synchrotron x-ray fluorescence (SRXRF)

The use of total reflection x-ray fluorescence analysis (TXRF) in life sciences is considered a powerful analytical tool for simultaneous multielement determination. TXRF is basically an energy-dispersive technique, with the sample being excitated in total reflection geometry. This technique only requires a few nanograms of sample, with simple preparation, and involves a large dynamic measuring scale. Simultaneous detection of almost all chemical elements and lower limits of detection are achievable in optimized excitation conditions. The preferred sample types are aqueous and acidic solutions, particularly samples digested with HNO_3. Special preparation techniques are required for solids and other samples.

The application of the proton-induced x-ray-emission (PIXE) method has enabled us to determine not only the concentrations of elemental composition, including K, but also their localization in different artery-wall regions. Furthermore, the usefulness of the micro-PIXE method for studying biomedical materials has been also considered.

Finally, synchrotron x-ray fluorescence has also been used in studies on trace elements and potassium in breast tissue. Synchrotron radiation is emitted when charged particles are accelerated by means of a magnetic field perpendicular to their direction of motion. The brightness and intensity of synchrotron x-rays are many orders of magnitude more than x-rays produced in conventional x-ray tubes. A promising tool is the combination of high flux and low divergence provided by synchrotron source with microscopic analysis for elemental mapping of biological samples.

Radioactive Isotopes

The dilution of radioactive isotopes of potassium is applied to establish the concentration and distribution of the amounts of potassium in the different body compartments. For example, the differences in potassium concentrations between the extra- and intracellular spaces may be studied by such techniques.

For this purpose, it is important to take into account that animal and plant tissues present different potassium compartments in which the ions accumulate, and this conditions the radioactive isotope dilution process. In animal tissue, three cellular compartments (plasma + extracellular fluid, cells, and spaces where ions are closely fixed or form complexes) are described, whereas in plant tissue only two spaces (cytoplasm and vacuoles) must be considered since the extracellular space is small in size. This compartmental distribution conditions the process of radioactive isotope dilution.

In animal tissue, the method involves incorporating a known amount of the tracer isotope of potassium into the extracellular compartment and monitoring the changes in plasma isotope concentration until equilibrium is reached with all the compartments. This entails estimating the distribution of potassium (1) in extracellular fluid, using tracer polymers (inulin or polyethylene glycols) that cannot penetrate cell membranes, (2) in cellular volume, by subtracting extracellular volume from total distribution space (determined by using labeled water, tritiated, or deuterated), and (3) in the space where the ion is strongly fixed or forms complexes (this is calculated by subtracting the amount of extracellular potassium + cellular potassium from the amount of potassium isotope injected).

In plant tissue, the dilution of radioactive isotopes of potassium presents limitations due to the difficulty involved in terms of separating vacuolar volume and cytoplasmic volume. For this reason, more appropriate methods than radioactive dilution methods have been developed for determining cytoplasmic and vacuolar potassium concentrations, namely ion-selective microelectrodes or x-ray microprobe analysis.

A related technique with the use of radioactive isotopes is neutron activation analysis (NAA). It has become an important analytical tool in the food science and nutrition field, providing sensitive trace element analysis. The technical basis is that samples are first irradiated with the flood of neutrons generated by a nuclear reactor, creating radioactive isotopes of present elements. By monitoring the subsequent decay of these radioisotopes, it is possible to identify and accurately quantify the elements in the sample. It is also a non-destructive technique that only requires sample preparation with a high sensitivity and accuracy.

NAA has been selected for analyzing potassium of cereals and legumes samples with a limit of detection 14.4 $\mu g\, g^{-1}$. It has also been used for determining potassium content in other biological samples, such as human hair and kidney stones, among others. Finally, a promising prospect about this technique in quality control of the food industry is that NAA could be used in multielement analysis – including potassium – combined with chemometric methods to characterize the geographical origin of foodstuffs and reject possible imitations.

See also: Authenticity of Food; Hypertension and Diet; Infrared Spectroscopy: Applications; Mass Spectrometry: Applications; Mass Spectrometry: Principles and Instrumentation; Potassium: Physiology; Sodium: Properties and Determination; Spectroscopy: Types.

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Potatoes and Related Crops

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Introduction

The potato is the world's number one nongrain food commodity and is the world's fourth most important food crop after wheat, rice, and corn. Potato occupies third position in terms of consumption after rice and wheat. It is a staple food for the human diet and as such is an integral part of the global food security system. In order to emphasize the importance of potato in providing world food security and alleviating poverty, the United Nations General Assembly officially declared 2008 as the 'International Year of the Potato.' Potatoes originated in the Andes of South America near Lake Titicaca, located between Peru and Bolivia, and were brought to Europe by the Spanish in the sixteenth century. It was domesticated between 10 000 and 7000 years ago. The Incan people (thirteenth to sixteenth century AD) regarded the potato as crucial to their food security since the crop could easily be stored as dehydrated, freeze-dried, and fresh forms for consumption during times when other crops failed. The Spanish introduced potato to Spain after conquering Peru, and then, it spread to European countries by the end of the sixteenth century. Despite the initial aversion to potato consumption in many parts of the continent, by the 1700s, potato was gradually accepted as a high-yielding stable crop that was a high-calorie food source for feeding both livestock and humans. Ireland was particularly dependent on this crop by the mid-1800s. 'Late blight' caused by the fungi *Phytophthora infestans* first appeared in 1845, and it devastated potato crops in Ireland resulting in three consecutive crop failures between 1845 and 1848 disrupting its highly vulnerable agricultural-based economy. During this period, more than 1.5 million people died of starvation or were forced to emigrate from Ireland. Late blight disease virtually wiped out the European descendants of the original *S. tuberosum* ssp. andigena by the mid-nineteenth century, and they were replaced by *S. tuberosum* ssp. tuberosum. Potato also reached Asia, New Zealand, and Africa through various colonial activities. In this article, we are providing an overview of the nutritional composition and bioactives of potato and their health benefits.

Current Trends in Production (FAOSTAT)

Potato cultivation has expanded globally and is currently grown in more than 160 countries around the world. China is ranked as the top producer (became the top producer) with 85 860 000 million metric tons contributing 23.3% of world production. China is followed by India, the Russian Federation, and Ukraine. The United States occupies fifth position. Demand for potatoes and potato products continues to increase in developing countries. In the United States, the consumption of the fresh potato has declined, while processed potato consumption has increased over the last 10 years. Almost half of the potato produced globally is consumed in Asia and is increasing on a per capita basis. Over the past 20 years, potato-growing areas have declined in North America, Europe, and the former Soviet Union. In developing countries including those in Africa, Asia, and Latin America, production areas have doubled. In Asia, potato production is increasing at a fast pace due to increasing interest in processed potatoes and use as animal feed for the livestock industry. There has been an increase in potato production at an annual rate of 4.5% over the last 10 years. Currently, the potato is the most predominant vegetable in the United States in terms of production, sales, and consumption. In the United States, fresh potato consumption has decreased by 50%, while processed potato consumption has increased by approximately two-thirds. Potato became the first vegetable to be grown in space in October 1995, with a goal of feeding astronauts during long space journeys.

Solanaceae Family

The potato (*Solanum tuberosum* L.) is a member of the Solanaceae or 'nightshade' family, genus *Solanum*, and section Petota. Section Petota are divided into two subsections, Potatoe and Estolonifera. Potatoe are characterized by the presence of underground tubers and include the potato and its wild relatives. Estolonifera are non-tuber-bearing and nonstoloniferous species that resemble the true potatoes. Solanaceae also includes other economically important plant species such as the tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), and eggplant (*Solanum melongena* L.). *Solanum tuberosum* is divided into two cultivar groups: Andigenum (Andean) and Chilotanum (Chilean). Andigenum is adapted to short-day conditions and is mainly grown in the Andes, while Chilotanum is the European potato adapted to the long-day conditions. It is believed to have developed from Andean cultivars introduced first into Chile and then into Europe.

Potato Plant and Tubers

Solanum tuberosum is an herbaceous perennial that can grow to about 60 cm in height. In modern-day farming, potato is cultivated as an annual species grown and harvested every year. Plants are characterized by long pinnate leaves, and they bear white, pink, blue, or purple flowers with yellow stamens in clusters. Potatoes are mostly pollinated by insects such as bumblebees, though self-fertilization also occurs. Fruit of *S. tuberosum* resembles green cherry tomatoes with or without seeds. Fruits are unsuitable for human consumption as they contain substantial amounts of the toxic alkaloid solanine. As the potato plant grows, it produces enlarged underground stems known as the tubers, which are the edible portion of the plant. Tuber is the only commercially important part of this

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plant. Tubers store and reserve food that enables the plant to survive the cold and are also involved in vegetative plant propagation. The skin of the tuber consists of two layers, an outer epidermal layer of single cells and an inner layer called periderm, consisting of several layers of corky cells. Beneath the periderm is the cortex followed by the vascular ring. Internal to the vascular ring is the medulla that represents the primary storage area for the potato tuber. Each tuber has two to ten 'eyes' or buds arranged spirally around its surface. The eyes are capable of producing new shoots and plants under favorable conditions. Potato plants are usually vegetatively propagated by planting tubers rather than from true seeds. Plants grown from seeds show wide variations, while tuber-propagated plants are clones of the parent plant. Seed propagation is a time-consuming and labor-intensive process, and the resultant progenies have poor vigor and lower yields. For propagation, tubers are cut into pieces with at least one or two eyes (seed tubers) before planting. Each section sprouts and develops into a new plant. However, tubers can carry pests and diseases that affect the tuber quality and yield of the harvest. High costs of transportation and storage and low multiplication rate are some other constraints of using seed tubers. True potato seed (TPS) is an alternative to seed tubers for growing potato. Ease of handling and transportation and low incidence of diseases are some of the advantages of TPS over tubers. However, TPS are produced as a result of a sexual generation and are genetically different from the parents.

Cultivation

Potato is generally a 'cool-weather crop,' but they are cultivated in more than 160 countries under temperate, subtropical, and tropical conditions. The potato can thrive and adapt well even without the ideal soil and growing conditions. Temperature is the main limiting factor for its production. Tuber growth is inhibited below 10 °C and above 30 °C, while optimum mean daily temperature is in the range of 18–20 °C. Potato is a very adaptable plant; however, it is also prone to a number of pest and diseases. For that reason, farmers avoid growing potatoes in the same land from year to year. Growth of potato plant occurs in five stages. Cultivation of potato begins with planting of cut and healed seed tubers with a strong sprout. The initial phase of growth starts with sprouting of seed potatoes and the beginning of root growth. Next, the vegetative growth phase starts and proceeds with the development of vegetative aboveground parts of the plant. During this phase, leaves, branches, roots, and stolons are formed. Soil is mounted to the base of the plant (hilling) to promote tuber production and to keep the plants in an upright position. Stolons will grow into the soil and tubers develop as swellings on these stolons. Hills ensure that the tubers will remain underground and away from sunlight to avoid greening and production of toxic glycoalkaloids. Plants will enter into flowering phase and then initiate tuber growth. In the fourth stage, tubers undergo bulking or size increase. Maturity depends on the cultivar and environmental factors. At the end of the maturity period, the aboveground vegetation (or vines) dies and the tubers are left in the ground for 2 weeks for hardening. Tubers are harvested after hardening and used as fresh or stored for use in the future. Vine killing can also be induced by applying chemical vine killer 2 weeks prior to harvest. Vine killing allows tuber skin maturation, thus reducing bruising of tubers during transportation and storage.

Harvesting and Postharvest Handling

During harvesting and handling, bruising of tubers should be avoided. In the early stages of postharvest handling, potatoes can get nicked and become susceptible to pathogens and microorganisms causing severe losses. Before storage, any damaged, diseased, or frozen tubers should be culled. Potatoes bound for storage should also be cured by exposing to temperatures between 10 and 15.5 °C and 95% relative humidity for 10–14 days. Curing heals the potato skin, making it less susceptible to infections and injury. During the curing process, tubers form new cell layers that protect the internal living tissues from injuries and pathogens. Potatoes are then stored in refrigerated warehouses or in bulk bins.

Tuber Specific Gravity

Specific gravity is an important potato quality attribute used as a basis for nondestructive quality evaluation especially in processing. This attribute of a tuber is an indication of maturation that the industry uses as a reference to judge fry quality, baking characteristics, and storability. Specific gravity is the weight of the tuber compared with the weight of the same volume of water. There is a high correlation between the tuber specific gravity, the starch content, and also the percentage of dry matter or total solids. The potato processing industry values high tuber specific gravity. Specific gravity and solid content have also been shown to have an effect on fat uptake by French fries. The higher the specific gravity, the higher will be the rate of recovery and the better will be the quality of the processed product. It has been shown that potatoes with high specific gravity produce a high yield of French fries with a lower fat content than potatoes with lower specific gravity.

Potato Cultivars

Today, vast numbers of potato cultivars exist. Potatoes are genetically very diverse with many favorable genes introgressed from wild to the cultivated species. Potato varieties can be classified based on a number of criteria. They can be classified based on days of maturity as very early (65–70), early (70–90), mid-season (90–100), late (110–130), or very late (110–130). Varieties can also be classified based on tuber qualities. Cultivars used for frying can be separated into chipping (round) or French fry (elongated) types.

Uses of Potato

Potatoes are the world's most popular and versatile vegetable that is used in the cuisines of countries around the globe. Indigenous people developed processing methods to extend

the storability and portability of potato products. *Chuño* is a freeze-dried potato product that is without any bitter glycoalkaloids and can be stored for several years. During *chuño* preparation, tubers undergo several rounds of freezing and drying followed by washing and soaking in running water and finally sun-dried for 10–15 days. Another native potato product is *papa seca* (dehydrated potato). To prepare *papa seca*, tubers are boiled, peeled, cut into chunks, sun-dried, and ground into starchy powder and consumed with pork, tomatoes, and onions. Potatoes are used for a variety of purposes other than a vegetable for cooking at home. Less than 50% of potatoes cultivated worldwide are consumed fresh. The rest are processed into various food products and ingredients, used to feed livestock and for industrial purposes and used as seed tubers to grow next season's crop. Potatoes should be cooked because of the indigestibility of raw starch, and cooking can involve baking, boiling, steaming, roasting, deep-frying, or microwaving. They are used to make mashed potatoes, potato pancakes, dumplings, soups, and salad. Potato processing has grown into an expanding global industry. The majority of processed potato products are potato chips and French fries. Industrial production of crisps (chips) started in 1920 and French fries in the 1950s. Potatoes can also be made into frozen products including most of the French fries. To make frozen French fries, potatoes are peeled, cut, parboiled, air-dried, par-fried, frozen, and then packaged. Potato crisp, another processed potato product, is made from thin slices of deep-fried or baked potato. Dehydrated potato flakes and granules are used as ingredients in snacks and are made by drying, mashing, and cooking potatoes to a moisture level of 5–8%. Potato flour, also made from ground, whole-cooked potatoes, is used as a thickener in gravies and soups. Potato starch is also used to make fermented alcoholic beverages such as vodka. Besides the food use, potato starch is widely used by industries to make adhesive, animal feed, and fuel-grade alcohol.

Nutritional Profile of Potato

Carbohydrates

Potatoes are regarded as good dietary sources of carbohydrates. Nutritional composition of various potato products is presented in Table 1. Potatoes are touted as an energy-dense food due to their high carbohydrate content, which makes up to 75% of their dry weight contributing 90% of their total energy. Without the addition of extra fats during preparation, lipid content of potatoes is very low (0.1% of FW). Saturated fatty acid forms one-third of the total fat in white potatoes, and the remaining is primarily polyunsaturated fatty acids. Additionally, the fat content of potatoes is lower than that of pasta (0.9%) or rice (0.2%). However, they are a cheap source of energy due to their carbohydrate-rich composition. Potatoes are not typically considered as a good dietary protein source due to their low overall protein content. The low protein content of potatoes constitutes about 1–2% of tuber FW. Although low in content of sulfur amino acids, potatoes are good sources of lysine. Patatin is the principal storage protein and is an allergen to some people. Heating and cooking reduce its allergenicity.

Starch is the principle carbohydrate in potato, and it is composed of a mixture of amylose and amylopectin in a 3:1 ratio. Amylopectin, the predominant branched form, allows greater digestibility. Amylose, the linear form, is the 'resistant' starch and is resistant to digestive enzymes. Raw potato starch is indigestible and has a crystalline structure. Cooking gelatinizes the starch, increasing the solubility and digestibility of starch. Cooking followed by cooling will result in a change in starch structure called retrogradation, making the potato starch more resistant to digestive enzymes. These changes are related to the amount of glycosyl residues and to the relative ratio of amylose to amylopectin in potato. This explains the lower glycemic index of cooked and cold versus cooked and hot potatoes. Only a small portion of potato starch is resistant starch, which is resistant to digestion by enzymes in the stomach and small intestine. Resistant starch is fermented in the large intestine to produce short-chain fatty acids that act as a prebiotic by promoting the growth of colonic bacteria. Resistant starch occurs naturally in other foods such as legumes, underripe bananas, and some whole grains. Resistant starch is considered to have health benefits as it is considered a form of dietary fiber offering protection against colon cancer and improvement of glucose tolerance and insulin sensitivity. It also lowers plasma cholesterol and triglycerides and increases satiety. In potato, resistant starch amounts are dependent on methods of preparation. Boiled potatoes were reported to have a resistant starch content of 2.0% of total starch, while commercially processed potato products had a higher resistant starch content of 4.8–9.0% of total starch. Dietary fiber can be obtained from the cell walls in the peel of potatoes. Cooked potato with and without the skin can supply 2.1 and 1.8 g fiber per 100 g, respectively. Potato contains more fiber than white rice.

Vitamins and Minerals

Potatoes are crucial sources of vitamins including B_6, B_1, and C. The most abundant vitamin in potato is vitamin C, which ranges between 84 and 145 mg per 100 g dry weight. Potatoes supply about 13 mg of vitamin C per 100 g (14% RDA for adult men). In many parts of the world, potatoes serve as an important dietary source of vitamin C. Potatoes can be considered as a good source of vitamin B_6. The amount of ascorbic acid in potato differs widely among the various cultivars and in planting site and postharvest storage conditions and is also influenced by the cooking methods. About 25–50% of the daily recommendation of vitamin C can be obtained from 100 g of cooked potatoes. Additionally, they also provide minerals, such as potassium, phosphorus, calcium, and magnesium, and micronutrients, such as iron and zinc. Among the minerals, potassium is the most abundant, while the concentration of iron and zinc is low compared with that in cereals and legumes. Interestingly, the bioavailability of iron in potato is greater than that in cereals and legumes due to the presence of vitamin C and low levels of phytic acid. One serving per day of potato cultivars such as 'Russet Burbank,' 'Freedom,' and 'Yukon Gold' can significantly contribute to the intake of microminerals including magnesium, phosphorus, and potassium and trace minerals including copper, iron, selenium, and zinc.

Table 1 Nutritional profiles of potatoes cooked by various methods (value per 100 g; nutrient values and weights are for edible portion)

Nutrients	*Unit*	*Russet potato flesh and skin baked*	*Potato white flesh and skin baked*	*Potato red flesh and skin baked*	*Potato, boiled cooked with skin, flesh, without salt*	*Potato, boiled, cooked without skin, flesh, without salt*	*Potato, French fried, all types, salt added, frozen, as purchased*	*Potato, microwaved, with skin and flesh, without salt*	*Potato, roasted, salt added in processing, frozen, unprepared*
Water	g	74.45	75.43	76.67	76.98	77.46	66.61	73.55	68.08
Energy	kcal	97	94	89	87	86	147	100	130
Protein	g	2.63	2.10	2.3	1.87	1.71	2.26	2.10	2.22
Total lipid	g	0.13	0.15	0.15	0.10	0.10	4.66	0.10	1.81
Carbohydrate	g	21.44	21.08	19.59	20.13	20.01	24.81	23.28	26.15
Fiber	g	2.3	2.1	1.8	1.8	1.8	1.9	1.6	2.6
Total sugars	g	1.08	1.53	1.43	0.87	0.85	0.20	5	0.69
Calcium	mg	18	10	9	5	8	9	0.41	15
Iron	mg	1.07	0.64	0.70	0.31	0.31	0.62	25	0.50
Magnesium	mg	30	27	28	22	20	21	109	
Phosphorus	mg	71	75	72	44	40	83	411	
Potassium	mg	550	544	545	379	328	408	7	450
Sodium	mg	14	7	12	4	5	332	0.33	298
Zinc	mg	0.35	0.35	0.40	0.30	0.27	0.35	15.1	
Vitamin C	mg	8.3	12.6	12.6	13.0	7.4	17.3	0.129	1.7
Thiamin	mg	0.067	0.048	0.072	0.106	0.098	0.098	0.025	0
Riboflavin	mg	0.048	0.043	0.05	0.020	0.019	0.048	1.625	
Niacin	mg	1.348	1.528	1.595	1.439	1.312	2.038	0.319	
Vitamin B_6	mg	0.354	0.211	0.212	0.299	0.269	0.178	12	
Folate	μg	26	38	27	10	9	35	0	
Vitamin B_{12}	μg	0.0	0	0	0	0	0	0	
Vitamin A	μg	1	1	1	0	0	0	0	
Vitamin A	IU	10	10	10	3	3	4	0	
Vitamin E	mg	0.07	0.04	0.08	0.01	0.01	0.10	0	
Vitamin D	μg	0	0	0	0	0	0	0	
Vitamin K	μg	2.0	2.7	2.8	2.1	1.6	2.2	0	
Total saturated fatty acids (FA)	g	0.034	0.022	0.026	0.026	0.026	1.005	0.026	0.167
Total monounsaturated FA	g	0.003	0.002	0.002	0.002	0.002	3.164	0.002	
Total polyunsaturated FA	g	0.056	0.037	0.043	0.43	0.43	0.303	0.043	
Cholesterol	mg	0	0	0	0	0	0	0	0

Source: National nutrient database for standard reference Release 27.

Potato Phytochemicals

In addition to the vitamins and minerals, potatoes also contain a number of health-promoting phytonutrients such as phenolics and carotenoids. Potatoes can supply 25% of the vegetable phenolics recommended in the American diet. Both the skin and flesh of potatoes contain phenolic constituents, the concentration being higher in skin. Phenolic compounds in potatoes were reported to be in the range of 530–1770 μg g^{-1}. The presence of lignin, coumarins, anthocyanins and flavones, tannins, monohydric phenols, and polyhydric phenols was identified in potato. Red and purple varieties of potato are particularly rich in phenolic compounds. Phenolic acids constitute the most abundant polyphenolic compound in potato. Chlorogenic acid is the predominant phenolic acid accounting for about 90% of the total potato phenolic compounds. Caffeic, protocatechuic, vanillic, sinapic, p-coumaric, salicylic, and ferulic acids also occur in potato, and they are concentrated in the skin. Generally, purple-fleshed cultivars tend to have the highest phenolic acid content compared with white-, yellow-, or red-fleshed types. Colored potatoes such as red- and purple-skinned potatoes contained approximately twice the concentration of phenolic acid than white-skinned tubers.

Flavonoids such as catechin, epicatechin, kaempferol, and naringenin have been detected in potatoes. Higher flavonoid contents were reported for purple- (~900 mg per 100 g FW) and red-fleshed (500 mg per 100 g FW) varieties. Studies have shown that flavanone and flavonoid extracts had high free radical scavenging actions. Potatoes also have aglycones, which are antioxidant compounds. The most abundant flavonols are rutin and kaemferol-3-rutinose. Quercetin, a potent antioxidant flavonol, was identified in the potato peel. Anthocyanins are present in negligible amounts in white potatoes. Generally, pigmented potatoes have higher anthocyanin contents than white- or cream-colored varieties. Anthocyanin content of 5.5–35 mg per 100 g FW has been reported. Red-fleshed potato varieties contain acylated glycoside derivatives of pelargonidin and peonidin as the predominant anthocyanins. Glycosylated delphinidin and malvidin also occur at low concentrations. Purple cultivars have acylated petunidin and malvidin glycosides and lesser amounts of glycosylated delphinidin.

Potato also contains carotenoids and their derivatives, xanthophylls. Lutein, zeaxanthin, violaxanthin and neoxanthin are the principal carotenoids in potatoes. But only trace amounts of β-carotene is present in potato. Zeaxanthin and lutein imparts orange or yellow color to the potato tubers. Yellow- or orange-fleshed potato varieties have more carotenoids than the white-fleshed varieties. The carotenoid contents of potato tubers are related to flesh color. White- or cream-colored potatoes typically have low concentrations of carotenoids, with lutein, violaxanthin, and beta-carotene being the predominant forms. Zeaxanthin is the predominant carotenoid in yellow-fleshed potatoes and the total carotenoid concentration is about 1840 μg per 100 g FW. Yellow-fleshed potato has about 1290 μg per 100 g zeaxanthin content. This is of particular importance as dietary sources of zeaxanthin are quite scarce. Cooking seems to have no negative effect on the lutein and zeaxanthin concentrations. The nutrient profiles of potatoes are greatly influenced by the genotype. Navarre and coworkers reported that a wide variation exists among potato genotypes in their phenolic content. Purple-fleshed wild varieties contain higher amount of phenolics (>5–6 mg g^{-1} DW) than white-fleshed varieties (<4 mg g^{-1} DW). In another study conducted in 26 pigmented potato cultivars, anthocyanin content of about 7 mg g^{-1} FW was detected in the skin and 2 mg g^{-1} FW in the flesh.

Glycoalkaloids

Glycoakaloids are steroidal nitrogen-containing metabolites found in potatoes and in many solanaceous plants. They are antinutritional substances highly concentrated in tissues with high metabolic activity including flowers, young leaves, unripe berries, and sprouts. Small immature tubers are also high in glycoalkaloids being metabolically active. Two major glycoalkaloids in potatoes are α-solanine and α-chaconine, which comprise about 95% of the total glycoalkaloids content in the plant. Solanine is a combination of a sugar molecule (solatriose) with an alkaloid (solanidine), and chaconine is chacotriose combined with solanidine. Mechanical injury or pest-induced injuries, fungal attack, poor growing environment, and poor storage conditions can influence the concentration of glycoalkaloid content in tubers. They are often produced in tubers on exposure to sunlight and characterized by greening of the skin of tubers. Glycoalkaloids at lower levels enhance the flavor of tubers, while at higher concentration, they confer bitterness. When present in large amounts glycoalkaloids are toxic and undesirable. Most potato tubers for human consumption have about 7.5 mg per 100 g FW of α-solanine and α-chaconine. The allowed level of glycoalkaloids in tubers is 20 mg per 100 g FW. At high concentrations, these concentrations may be lethal to humans causing gastrointestinal and neurological disorders. Their mechanism of toxicity includes inhibition of acetylcholine esterase activity and disruption of membranes. Glycoalkaloids impart protection to the plants against various pathogens. The levels of glycoalkaloids are dependent on a number of factors including genotype, climate, storage conditions, maturity, temperature, light, and tuber damage. Glycoalkaloids are not uniformly distributed in tubers. Concentration is relatively lower towards the pith and higher in the periderm and cortex.

Acrylamide

Formation of acrylamide was noticed in starchy foods that are baked, fried, or roasted at high temperature. Acrylamide is formed when asparagine, a free amino acid, reacts with reducing sugars at high temperature via a complex process involving Maillard and related reactions. Potato cultivars have considerable amounts of reducing sugars and free asparagine at varying levels. Presence of acrylamide was reported in French fries and potato chips. Concerns about the health issues associated with the intake of acrylamide have been expressed because acrylamide is known to be a neurotoxin and genotoxin, and it is also classified as a probable carcinogen in humans. A number of factors such as the amount of asparagine and reducing sugars, cooking process, temperature and time, the pH, and the surface to volume ratio of food materials influence acrylamide formation. Various effective measures have been suggested to reduce levels of acrylamide in foods. Reducing the pH by pretreatment with citric and other organic acids has been suggested as an effective strategy.

Effects of Cooking/Processing on Nutrient Composition

Digestibility of starch in potato is greatly improved by cooking and processing. Unpeeled potato shows better nutrient retention than peeled potato. Generally, boiling of cut and peeled potatoes result in loss of water-soluble vitamins and minerals including potassium and copper due to leaching. This loss was further increased with prolonged cooking time and very high temperature. Baking, frying, and roasting leads to lower nutrient losses than boiling. Peeling reduces the fiber content of potatoes. Frying or chipping of potatoes in oil and addition of extra fat raise the fat content and caloric intake of potatoes, making it unhealthy. Steaming and microwaving are the best methods for cooking potato to minimize the loss of nutrients. The method of cooking reduces the vitamin C content in potato. Microwaved or baked potatoes have approximately twice the amount of ascorbic acid compared with fried or boiled potatoes (Table 1). Studies have found that tubers boiled with skin on had higher vitamin C content than those baked or cooked in a microwave oven.

Health Benefits

Despite contributing significant amounts of vital vitamins and minerals to human diet, potato has been mistaken as a contributor to the high prevalence of obesity, diabetes, and cardiovascular diseases in the United States.

Antioxidant Activity

Consumption of antioxidant compounds through fruits and vegetables in our diet could potentially prove to be an effective strategy towards defending our bodies from illness such as cardiovascular diseases, cancer, and diabetes by limiting oxidative stress. Although potatoes contain a variety of antioxidant compounds exhibiting a multitude of anti oxidant mechanisms, the light-skinned potatoes are not known as a food item with high antioxidant activity. Total antioxidant potential of potato is highly correlated with total phenolic

contents contributing greatly to the total antioxidant activity. Its daily consumption can contribute to high total phenolic content to our diet. Carotenoids and ascorbic acid also serve as major antioxidants in potato. Antioxidant activity varies among potato cultivars. The antioxidant capacity of the potato cultivars is dependent on the color of the skin and flesh. Colored-fleshed potato varieties are particularly high in anthocyanins. Anthocyanins are mostly concentrated on the peel of potatoes, and anthocyanins are 3–4 times higher in potato peel than in tuber. Anthocyanins are known to have antioxidant, antimicrobial, and antiobesity potentials.

Cardiovascular Health and Diseases

Consumption of potato products with high lipids such as French fries and chips is often discouraged by nutritionists. For cardiovascular benefits, cooking of potatoes with less fat and sodium is also suggested. A human intervention study examined the effects of pigmented potato consumption on antioxidant status, oxidative stress, and inflammation in healthy adult males. Consumption of both yellow- and purple-fleshed potato decreased oxidative damage and inflammatory cytokine concentration compared with those participants who consumed white-fleshed potato. Additionally, purple-fleshed potato consumption also decreased C-reactive protein concentration. Some animal studies provided evidence that chlorogenic acid reduces plasma total cholesterol and LDL cholesterol levels, and potato peptides have the ability to alter serum lipids.

Lowering of Blood Pressure

Potatoes contain several nutrients that help lower blood pressure. Potatoes are good sources of potassium compared to other fruits and vegetables and are very low in sodium. Evidence from animal studies, observational studies, and clinical trials has associated intake of adequate amounts of potassium with reduced blood pressure. Human studies have shown that two small helpings of purple-fleshed potatoes a day decrease blood pressure without affecting weight gain while increasing plasma antioxidant capacity. In another human interventional study, intake of potatoes (versus refined starch) caused an increase in blood and urine antioxidant capacity, whereas refined starch caused a decrease in both. Additionally, a drop in blood pressure was noticed in a study carried out in overweight individuals with high pressure after potato consumption. More studies are needed to confirm these beneficial effects of potatoes. Proteins isolated from potatoes and potato products have shown angiotensin-converting enzyme inhibitory activity *in vitro*.

Anticancer

Evidence from some animal and *in vitro* studies points towards the anticancer properties of potatoes, but this is an area where more research is required. One study reported the antiproliferative properties of colored potato extracts and an anthocyanin fraction on prostate cancer cells. Similarly, potato extracts also suppressed the proliferation of colon and liver cancer cells. Research carried out with individual phenolic compounds has suggested that chlorogenic acid may be the primary compound responsible for the antiproliferative activity. Also, purple-fleshed potatoes were more effective in conferring antiproliferative activity on colon carcinoma cells than either white- or yellow-fleshed potatoes. This is likely related to the higher antioxidant potential of purple-fleshed potatoes. Research showed that chlorogenic acid could selectively inhibit matrix metalloprotease, an angiogenic enzyme responsible for tumor progression and metastasis. Chlorogenic acid also showed anticarcinogenic property in a number of studies using cell models.

Antidiabetic

The association between potato consumption and the risk of type 2 diabetes has been evaluated. Data from a Nurses' Health Study showed that substituting one serving of potato per day for 1 serving of whole grains per day increased the risk of type 2 diabetes in obese women by 30%, but this association risk was not found for nonobese women. Another study found that high intake of energy-dense foods including fried potatoes was associated with higher levels of biomarkers of inflammation and increased risk of type 2 diabetes. On the other hand, the Shanghai Women's Health Study found that the intake of potato tubers was associated with lowering the risk of type 2 diabetes. A few other studies also found no association between potato intake and type 2 diabetes. Chlorogenic acid has been studied for its health benefits, and it might be helpful in lowering the glycemic index of potatoes as it slows down the release of glucose into the bloodstream as suggested by some animal studies. It appears that chlorogenic acid increases insulin sensitivity rather than affecting the release of insulin.

See also: Solanaceous Fruits Including Tomato, Eggplant, and Peppers.

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Relevant Website

www.potatogoodness.com.

Potatoes and Related Crops: Role in the Diet

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Origins and Historical Context

South America (Center of Origin)

Potato (*Solanum tuberosum* L.) is a versatile staple crop with a unique and historically significant story of domestication. Originating in the Andean highlands almost 10 000 years ago, potato has since spread to all continents with a vast range of climate conditions, ranging from temperate to tropical regions. Even Charles Darwin commented on the wide adaptation of potato in *The Voyage of the Beagle* writing, "It is remarkable that the same plant should be found on the sterile mountains of Central Chile, where a drop of rain does not fall for more than six months, and within the damp forests of the southern islands."

Potato domestication began in South America, where potatoes were planted on high-altitude lands in raised beds to protect from frost damage. Initially, cultivated potatoes were adapted to long-day conditions, but converted to short-day conditions following hybridization with a wild species. This adaptation was crucial for the spread of potato production to other continents, particularly regions of North America, Europe, and Asia, where most production occurs today.

Europe and North America

It is postulated that potato was first introduced to Europe by Spanish explorers *c.*1565. As part of the notoriously toxic nightshade family, it was regarded with skepticism and thought to cause leprosy, gas, and other ailments. Despite these superstitions, potato was quickly adopted as a staple food crop in England when wheat became too expensive. Potato offered a satiating, cheap, and sustainable food supply during famine and had a key role as a food source for workers during the industrial revolution in the nineteenth century.

The utilization of potato as a staple food in Europe was not entirely a success story, as evidenced by the Irish potato famine from 1845 to 1852. Spanish explorers introduced the potato to Ireland in the late sixteenth century, where it was rapidly implemented as a staple food to feed the starving population. The cool, damp climate in Ireland was ideal for potato production, providing high yields and a reliable food supply for nearly a century, during which time the population of Ireland increased by almost five million, approximately 60%. Ireland relied almost exclusively on potato for sustenance, with consumption estimated at up to 12–14 pounds (~5.4–6.4 kg) per day for an adult male. Unfortunately, the climate was also ideal for the late blight pathogen, *Phytophthora infestans*, which swiftly descended across Ireland and devastated crop production. The resulting crop failure, coupled with other socioeconomic issues, resulted in the Great Irish Famine, during which an estimated one million lives were lost from starvation or disease. Much of the crop failure is attributed to a lack of genetic diversity, as farmers relied almost entirely on the cultivar Lumper, which had poor resistance to late blight.

Details about the introduction of potato to North America are sparse. Potato was planted in North America as early as the 1600s, but was not widely adopted until the eighteenth century, when Scotch-Irish immigrants introduced it to New England. As in Europe, initial acceptance of potato as a food source was slow but, by the time potato was included on the US Census in 1840, production had progressed to 728 100 tonnes.

Asia and Africa

Mariners from Europe are credited with the dissemination of potatoes throughout the remainder of the world. Introduction to China occurred in the seventeenth century, most likely via Dutch trading routes, where the higher yielding potato quickly usurped traditional crops such as barley and oat.

Although reported on the Canary Islands as early as 1567, European colonists and missionaries did not introduce potato to Africa on a large scale until the late nineteenth century. Again, as with other parts of the world, the uptake was slow but gradually increased as potato was adopted as a staple crop.

Production Trends

Current Production Statistics

Potato is the most important vegetable crop and the fourth most important food crop worldwide, outranked only by maize, rice, and wheat. Worldwide, more than 150 countries produce potatoes, with over half of global potato production in developing countries. According to the Food and Agriculture Organization of the United Nations (FAO), the highest production of potatoes is observed in Asia (49%), followed by Europe (30.7%), the Americas (11.6%), Africa (8.2%), and Oceania (0.5%).

Prior to the 1990s, most potato production and utilization occurred in Europe, North America, and countries formerly part of the Soviet Union. Since then, potato production and popularity has markedly increased in Asia, Africa, and Latin America. As of 2005, production in the developing world began to exceed that of the developed world, with China and India contributing almost a third of world production. This is not surprising as potatoes are an essential part of food security for millions of people in the developing world.

Management Practices

Potatoes are most commonly grown from seed potatoes, which consist of small tubers or sections of a larger tuber with buds, and undergo a period of dormancy to induce sprouting. As a highland tropical crop that thrives in cooler temperatures, potato cannot tolerate frost and is grown during the spring in temperate regions and as a winter crop in hot, tropical

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climates. Potatoes have adapted to a wide range of growing conditions, but perform best when grown with irrigation in a sandy loam soil. During cultivation, potatoes are often treated with a variety of pesticides to control insects, most notably the infamous Colorado potato beetle, and pathogens. Additional chemical applications include fertilizers, which can also function to improve the mineral content of tubers. Following harvest, tubers are frequently treated with sprout inhibitors and can be stored for several months, with care to avoid disease development and physical damage, such as bruising. Optimal storage conditions consist of a dark, well-ventilated, and humid area around 4 °C. Colder temperatures should be avoided to prevent the conversion of starch to sugar.

As an underground tuber, potatoes are highly susceptible to many fungal, bacterial, and viral pathogens. Disease mitigation strategies include crop rotation, chemical control, and use of disease-free seed tubers. Because seed potatoes are derived from the previous crop year, virus load increases with each successive generation. Thus, it is important to obtain certified, virus-free seed tubers every few generations. Tubers afflicted with common ailments such as early blight, late blight, and soft rot most often result in crop loss, while tubers with silver scurf, scab, and *Rhizoctonia*, colloquially known as 'the dirt that won't wash off,' are still marketable provided the damage is minor. Zebra chip, a relatively new disease, causes losses due to plant death and darkening in fried products.

Breeding Resources

Maintenance of genetic diversity is paramount to continued improvement of existing potato cultivars and to prevent disasters such as the Irish potato famine. Breeding efforts for potato focus on disease resistance, quality traits, yield, and adaptation to diverse growing environments. Wild species and indigenous cultivars are valuable sources of genetic variation for breeding efforts. Central and South America, particularly Bolivia and Peru, are still home to the greatest range of potato diversity, boasting over 4000 varieties of native potatoes and more than 180 wild potato species adapted to various altitudes and climatic conditions. The International Potato Center (CIP), based in Lima (Peru), is part of the Consultative Group for International Agricultural Research (CGIAR) and houses the largest collection of potato germplasm *in vitro*, with 80% of known wild potato species and over 1500 accessions of native Andean root and tuber crops. This collection is available for distribution to breeding programs as a valuable resource to improve adaptation to changing climates and to aid farmers. Additionally, the NRSP-6 Potato Genebank in Sturgeon Bay (WI, the United States), a part of the USDA National Genetic Resources Program (NGRP), maintains over 5000 entries available for breeding efforts.

Patterns of Consumption

Potato is widely consumed as a dietary staple around the world. Less than half of potatoes grown are consumed fresh, while the remainder are used for processed products, animal feed, starch, and seed tubers. Based on data from FAO, Asia accounts for almost half of world utilization, with an average annual per capita consumption of 23.9 kg, while the highest annual per capita consumption is observed in Europe and North America, at 87.8 kg and 60 kg, respectively. Consumption in Europe and North America is relatively stable, while consumption in Asia and Africa is steadily increasing. As the wealth status of a country improves, consumers tend to prefer 'convenience' foods, such as fried and processed products. More detail on global utilization and per capita consumption by region can be found in Figure 1.

Culinary Applications

Potatoes have a wide range of culinary applications and are most often consumed baked, boiled, or fried. Potatoes are categorized based on harvest date, optimal cooking method, storage properties, and appearance. Common market classes and traditional culinary uses include russet (baking and French fries), white (boiling/mashed and chipping), red (boiling/mashed and roasting), and novelty classes (e.g., purple/blue) with various shape, pigment, and texture combinations (e.g., waxy or mealy) (Figure 2). Additional consumption is in the form of processed commodities, such as frozen potatoes, French fries, chips, and dehydrated products. According to the US National Potato Council, potato consumption in the United States is highest for frozen products (42.8%), followed by fresh (30.5%), chips (14.5%), dehydrated (11.4%), and

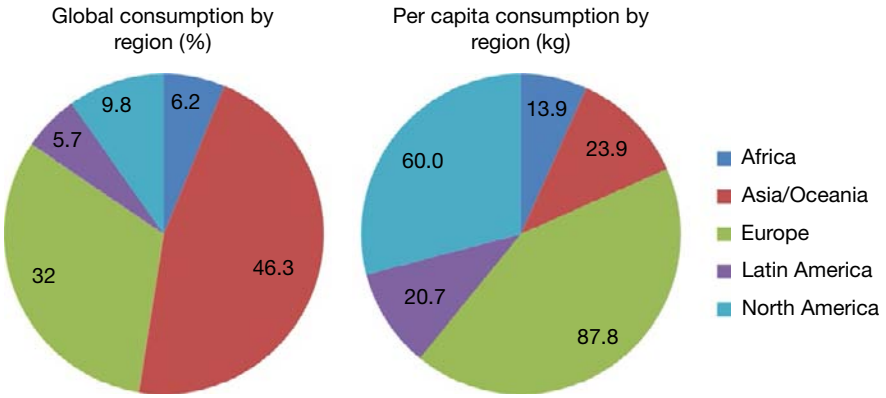

Figure 1 Global potato consumption (%) by region contrasted with per capita consumption (kg) by region. Data were adapted from FAOSTAT.

Figure 2 An image showing tuber diversity for skin color, flesh color, shape, and texture. Image courtesy of the Texas A&M Potato Breeding and Variety Development Program.

canned (0.01%) products. Patterns in the United Kingdom are similar according to the Agriculture and Horticulture Development Board (AHDB), but consumption of fresh potatoes is highest (52.3%), followed by frozen (27.1%), chips (17.4%), dehydrated (2.7%), and canned (0.5%) products. Consumption of processed or convenience products is highest in the developed world and expected to rise in countries with increasing wealth.

Nutritional Content

As part of a balanced diet, potatoes offer a nutrient-dense, satiating, and phytochemical-rich source of calories. Considerable variation exists for nutrient composition among cultivars, which can also be influenced by growing environment, storage conditions, and cooking method. Nutritional values for a standard, medium sized (138 g) potato are provided in Figure 3. Breeding efforts and management practices can be used to increase nutritional value and decrease undesirable qualities, such as glycoalkaloid content.

Carbohydrates, Proteins, and Lipids

Historically, potato obtained status as a staple crop by providing a high yielding source of carbohydrates. Additionally, potato supplies more protein per unit area than cereal crops. Carbohydrates, mainly starch, contribute around three-fourths of the dry matter content in tubers. Raw potatoes contain mostly resistant starch, which has low digestibility and essentially functions as dietary fiber. During cooking, starches solubilize and become highly digestible, rapidly increasing blood sugar following ingestion. For this reason, potato is described as having a moderate to high glycemic index (GI), depending on the preparation method. Other major carbohydrates include sucrose and the reducing sugars glucose and fructose. Cold temperatures during storage can increase the ratio of reducing sugars to starch and contribute to darkening after cooking, which is discussed in more detail later.

Nutrient	Unit	Flesh (100 g)	Skin (38.0 g)
Water	g	83.29	31.65
Energy	kcal	58	22
Energy	kj	243	92
Protein	g	2.57	0.98
Total lipid (fat)	g	0.1	0.04
Ash	g	1.61	0.61
Carbohydrate, by difference	g	12.44	4.73
Fiber, total dietary	g	2.5	1
Minerals			
Calcium, Ca	mg	30	11
Iron, Fe	mg	3.24	1.23
Magnesium, Mg	mg	23	9
Phosphorus, P	mg	38	14
Potassium, K	mg	413	157
Sodium, Na	mg	10	4
Zinc, Zn	mg	0.35	0.13
Copper, Cu	mg	0.423	0.161
Manganese, Mn	mg	0.602	0.229
Selenium, Se	µg	0.3	0.1
Vitamins			
Vitamin C, total ascorbic acid	mg	11.4	4.3
Thiamin	mg	0.021	0.008
Riboflavin	mg	0.038	0.014
Niacin	mg	1.033	0.393
Pantothenic acid	mg	0.302	0.115
Vitamin B-6	mg	0.239	0.091
Folate, total	µg	17	6
Folic acid	µg	0	0
Folate, food	µg	17	6
Folate, DFE	µg	17	6
Vitamin B-12	µg	0	0
Vitamin A, RAU	µg	0	0
Retinol	µg	0	0
Vitamin A, IU	IU	0	0
Vitamin D (D2 + D3)	µg	0	0
Vitamin D	IU	0	0
Lipids			
Fatty acids, total saturated	g	0.026	0.01
10:00	g	0.001	0
12:00	g	0.003	0.001
14:00	g	0.001	0
16:00	g	0.016	0.006
18:00	g	0.004	0.002
Fatty acids, total monounsaturated	g	0.002	0.001
16:1 undifferentiated	g	0.001	0
18:1 undifferentiated	g	0.001	0
Fatty acids, total polyunsaturated	g	0.043	0.016
18:2 undifferentiated	g	0.032	0.012
18:3 undifferentiated	g	0.01	0.004
Cholesterol	mg	0	0

Figure 3 Nutritional values for raw potato (100 g) with skin (38 g). Data were sourced from the US Department of Agriculture (USDA) National Nutrient Database for Standard Reference.

Potatoes contain relatively little dietary protein, which constitutes around 1–2% of the dry matter in the tuber. While only present in small amounts, protein in the tuber has a high biological value (BV), meaning most of the nitrogen absorbed during digestion is retained by the body. Most of the protein content is stored as patatin, which is allergenic for some people, but usually innocuous after cooking. Potatoes also contain minimal levels of fat, most of which is present as unsaturated and saturated fatty acids, and no dietary cholesterol. The fat content of potatoes is often increased by preparation methods, such as frying with oils, and high-fat additives, such as butter.

Vitamins and Minerals

The primary vitamin in potatoes is vitamin C, or ascorbic acid, which functions as an antioxidant and can facilitate uptake of iron, an essential nutrient limited in many diets. Potatoes may have helped prevent scurvy in early European sailors and continue to supply an important source of vitamin C in many parts of the world, with one medium potato (138 g) providing 26% of the daily value (DV) based on the US Food and Drug Administration (FDA) standards. Although the content is less than other sources (e.g., citrus and peppers), potatoes constitute the primary source of vitamin C in many countries. Potatoes are also a good source of pyridoxine (vitamin B6) and contain many other B vitamins, including folate (B9), niacin (B3), riboflavin (B2), and thiamin (B1).

Important minerals in potato include potassium, phosphorus, calcium, magnesium, iron, and zinc. Comparatively by weight, potatoes contain more potassium than bananas, spinach, or broccoli, with a medium potato providing around 16% of the DV. Mineral content can be improved by mineral fertilization, exploiting existing variation for mineral accumulation, or genetic engineering.

Phytochemicals

Phytochemicals have been studied widely because of their role as bioactive dietary compounds in the prevention/risk reduction of chronic diseases, such as cancer and cardiovascular disease. The most common phytochemicals in potatoes include phenolics, carotenoids, and alkaloids. Both phenolics and, to a lesser degree, carotenoids contribute to tuber coloration, while alkaloids are monitored strictly to prevent toxicity.

Phenolic compounds, primarily phenolic acids and flavonoids, are the most abundant phytochemicals in potato. Most of the phenolic compounds are concentrated in the skin and decrease towards the center of the tuber. Phenolic acids, particularly chlorogenic acid, constitute the majority of the total phenolic content. Within the skin, primary phenolic acids include chlorogenic, protocatechuic, and caffeic acids, along with lower levels of vanillic, *p*-coumaric, ferulic, sinapic, and salicylic acids. Tuber flesh has a similar profile, containing predominantly chlorogenic acid and low levels of caffeic, protocatechuic, *p*-coumaric, ferulic, gallic, and sinapic acids. Flavonoids are present to a much lesser degree, with moderate amounts of eriodictyol and naringenin in tuber skin, along with trace amounts of catechin, epicatechin, and eriodictyol in tuber flesh.

Flavonoids, specifically anthocyanins, also contribute red, blue, and purple pigments to potato skin and flesh, which is particularly important for the specialty potato market. Anthocyanins are putative antioxidants, and red- and purple-flesh potatoes typically have higher antioxidant activity than their white-flesh counterparts. Common anthocyanins in potato include pelargonidin, peonidin, malvidin, delphinidin, peonidin, and petunidin.

Carotenoids are lipid-soluble compounds that determine the amount of yellow or orange pigmentation in tuber flesh. Potatoes contain primarily oxygenated carotenes (xanthophyll), specifically lutein, neoxanthin, zeaxanthin, antheraxanthin, and violaxanthin, along with trace amounts of β-carotene. Of these compounds, zeaxanthin and lutein are found at the highest concentrations in yellow- and orange-flesh tubers.

Effects of Postharvest Storage and Cooking Method

Potatoes are commonly stored and cooked prior to consumption, both of which impact phytochemical levels. Existing literature is contradictory on the effects of long-term cold storage to increase anthocyanin, phenolic acid, and carotenoid content, but there is a consensus that no degradation occurs during storage. Removal of the tuber skin not only reduces glycoalkaloid content but also reduces the amount of phytochemicals and fiber. Common preparation and cooking methods, such as baking, boiling, microwaving, and frying, influence phytochemical levels and antioxidant capacity to varying degrees. Boiling tends to result in the loss of water-soluble nutrients but may increase antioxidant capacity by increasing bioavailability of compounds. Although the application of heat degrades vitamin C, methods such as baking, frying, and microwaving typically preserve the majority of phytonutrients. Diverse results in cooking studies may be due to different cooking temperatures and duration as well as differences in the cultivars used or original content.

Relation to Human Health

As a staple in the human diet, potato has a large influence on health. While potato is a valuable addition to a balanced diet, many people are concerned about its associations with weight gain, acrylamide, and toxicity. However, potato also provides notable levels of phytochemicals, which are linked to numerous health benefits.

Glycemic Index (GI) and Weight Gain

Potatoes often receive negative press for association with weight gain and diabetes. Concerns related to weight gain and diabetes both stem from the relatively high starch content in potatoes, which is approximately 15% by weight. As a result, potatoes have an intermediate to moderately high rank on the glycemic index (GI) and consumption may cause rapid increases in blood sugar. Several studies have also identified an association with weight gain, most often when consumed as French fries and potato chips.

Although the starch content in potato is high, the digestibility is contingent on cooking method and genotype. Raw potatoes mostly consist of resistant starch, which is not easily digested, has a low glycemic load, and essentially serves as a source of dietary fiber. After cooking, this resistant starch is converted into rapidly digestible starch and, thus, results in a higher GI. Cooling potatoes after cooking can cause a reversion back to slowly digestible starch, which could be used to develop products with improved nutrition. Concerns about GI and weight gain can be addressed by selecting varieties with reduced starch content, limiting intake of fried products, reducing the use of high-fat toppings, such as sour cream, butter, or cheese, and consuming cooled or reheated potatoes.

Acrylamide

Currently, fried potato products are under scrutiny for high levels of acrylamide, which is a potential carcinogen. Acrylamide is produced during the frying process from the combination of asparagine and reducing sugars at high temperatures, known as the Maillard reaction. The amount of acrylamide in fried potato products is influenced largely by storage conditions and genetic background. During cold storage, the starch in potato is converted to glucose and fructose, increasing the potential for acrylamide production. Additionally, some market classes of potato (e.g., red potatoes) contain more reducing sugars and are better suited to baking or boiling. Although the evidence is inconclusive on the effects of dietary acrylamide in humans, efforts are being made to reduce acrylamide content in potato products through changes in breeding, molecular genetics, production practices, and postharvest storage.

Glycoalkaloid Toxicity

Concern about toxicity stems from the presence of glycoalkaloids, which are naturally occurring secondary metabolites produced by the nightshade family. Glycoalkaloids function as plant defense compounds to protect against a variety of pathogens and herbivores but can induce toxicity in humans. As a result, glycoalkaloid content is monitored strictly in commercial potatoes. Total glycoalkaloid content in potatoes is estimated as the combined amounts of the most common glycoalkaloids in potato (i.e., α-solanine and α-chaconine), which cannot exceed 200 mg kg^{-1} of fresh potato, the point at which potatoes develop a bitter taste and consumption causes adverse symptoms. Green or sprouting potatoes, which accumulate glycoalkaloids, can also cause toxicity and should not be eaten. The toxicity of glycoalkaloids results primarily from disruption of cell membranes and inhibition of acetylcholinesterase, which is a neurotransmitter. Mild exposure to glycoalkaloids results in gastrointestinal discomfort (nausea, vomiting, and diarrhea) and may aggravate irritable bowel syndrome, while significant exposure may cause a neurological response. However, it is possible that the different types of glycoalkaloids have variable toxicity in mammals. Potential benefits of glycoalkaloids include the capacity to lower cholesterol, anti-inflammatory activity, antibiosis against food-borne pathogens, and anticancer activity.

Potential Health Benefits

With high levels of consumption, and notable levels of beneficial phytochemicals, potatoes are an ideal delivery system for beneficial compounds. Small increases in nutrient and antioxidant content in potato can have a significant impact, especially in regions with micronutrient deficiencies. Genetic variation exists for phenolic, carotenoid, and mineral content, including essential minerals such as zinc and iron, which are common nutrient deficiencies. Increasing the antioxidant content in potatoes may also improve consumer perception of potatoes as a functional food.

Phenolic compounds are well known for their association with the prevention of chronic diseases, such as cancer and cardiovascular disease. Currently, there are no long-term studies confirming the effects of potato consumption on cancer in humans, but several studies have demonstrated the anticancer activity of potato extracts on human prostate, stomach, and breast cells *in vitro*. Although potentially toxic, there is evidence that glycoalkaloids also promote apoptosis and reduce proliferation in human liver, colon, stomach, and prostate cancer cell lines. Glycoalkaloids are a diverse group of compounds, and further characterization may allow the selection of potato cultivars containing less toxic, more beneficial glycoalkaloids.

Despite the association of potatoes with weight gain, potatoes can contribute to satiety, or a feeling of fullness and satisfaction, following a meal. Reducing the consumption of fried potato products and the addition of fattening toppings may improve the role of potato as part of a balanced, healthy diet.

Summary

From humble origins in the South American highlands, potato has become a dietary staple around the world. Initially regarded as a famine food, potato has developed into a nutritious and satiating source of calories with a wide range of culinary uses. Production and consumption remain relatively stable in the developed world but are steadily increasing in developing countries, where potatoes offer a practical solution for food security. Major dietary concerns include weight gain, high GI, glycoalkaloid toxicity, and acrylamide content, all of which can be mitigated by altering consumption habits, cooking methods, and plant breeding. Potatoes also contain notable levels of health-promoting phytochemicals and there are opportunities for breeding to improve the nutritional content.

See also: Salad Crops: Dietary Importance; Salad Crops: Root, Bulb, and Tuber Crops.

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Poultry: Processing

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Introduction

The poultry processing industry has seen a lot of changes over the past half a century. New equipment has helped to reduce labor cost and increase efficiency and line speed. The steps involved in a typical primary processing plant are shown in Figure 1.

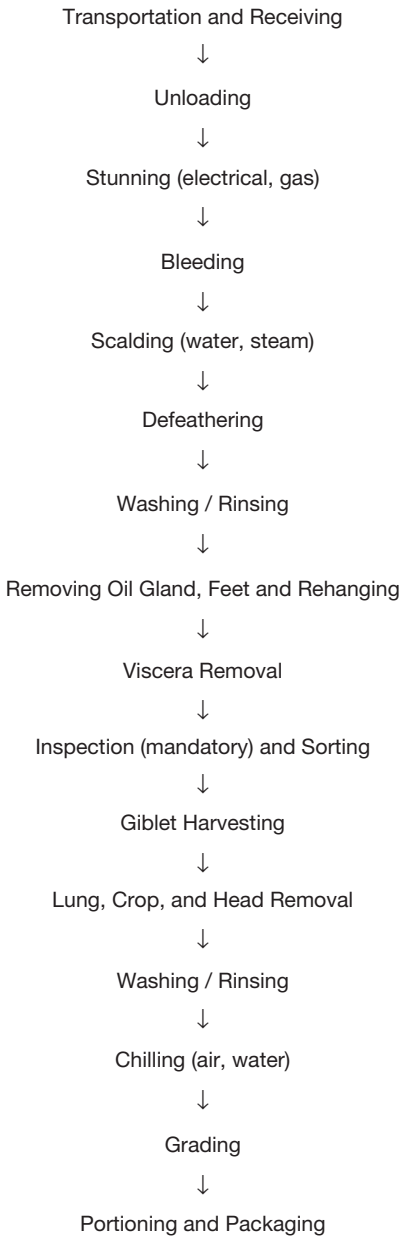

Figure 1 Typical sequence of operations in a poultry processing plant. Based on Barbut, S. (2010). Past and future of the poultry meat harvesting technologies. *World's Poultry Science Journal* **66**, 399.

Table 1 Increase in broiler processing line speed (bird per hour) from 1970 to 2015

Year	*Line speed*	*Equipment development*
1970	3000	Mostly manual operation
1975	4500	Semiautomatic evisceration
1980	8000	Fully automatic evisceration
1990	9000	Giblet harvesting
2000	10500	Cut-up machines together with in-line chilling
2010	12000	Automated unloading and gas stunning
2015	14000	More computer control

Mechanization and automation of different steps has helped improve output. In the 1970s, line speed was about 3000 birds per hour, and today, it can reach 14000 on a single line (i.e., can be split at certain areas such as at the point of inspection).

Some of the major developments in machinery and processes are outlined in Table 1. One of the first processes to be mechanized was the evisceration process where previously many people were needed to carefully perform the opening cut (e.g., in order to eliminate gut spills that would contaminate the carcass) and withdraw the viscera. A few generations of machines have gone through extensive developments, and today, this process is fully automated with a line speed four times higher than 50 years ago. Giblet harvesting systems were also developed at the same time with the challenge of handling soft tissues. Later, more attention was given to semiautomated/fully automated cutting and portioning equipment as the demand for deboned meat increased (both for the fast food industry and for home consumption of raw and convenience-type poultry products). Other areas of great importance are the unloading and stunning of animals. These areas have received more attention over the past few years as there has been a major focus on animal welfare and meat quality issues. To address these points, semiautomated and fully automated unloading systems have been developed to minimize stress to the birds, help increase efficiency, and reduce labor cost. In some of the new gas stunning systems, birds are either kept in the crates during stunning or automatically removed from the crates by gentle tilting.

The following sections will describe the different processing stages in more detail and focus on new developments.

Transportation and Receipt of Poultry at the Plant

Poultry is commonly transported from the growing farm to the processing plant by a truck where the birds are loaded into small crates (4–10 birds) or large modules (40–60 birds). Prior to loading, feed and water are removed from the growing barn, so collection is easier and more efficient. The birds are usually kept off feed for 4–8 h before they arrive to the plant. The time off feed and conditions on the truck should be carefully monitored to avoid dehydration, extreme temperature conditions,

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00557-2

and problems with air-flow fluctuation, which could stress and hurt the birds. On arrival, it is recommended to allow a rest period (1–3 h) for the birds in order to reduce transportation stress (i.e., rest period also depends on distance traveled, outside temperature, and feed withdrawal time). Optimally, this should be done in a temperature-controlled environment (20–25 °C) and under dim light (i.e., especially important if controlled atmosphere stunning is used; see discussion in the succeeding text) as high stress levels can ultimately and negatively affect meat quality. Prior to unloading, the weight of the birds is determined and used as the basis for calculating the payment to the farmer.

Unloading and Stunning

Unloading the birds from the crates and placing them on a moving shackle line are done manually by people standing by the truck or by the unloaded crates onto a conveyor belt (most common method) or semiautomatically by carefully tilting the modules. In some plants, a fully automated system is used to place the crates on a conveyer belt and move them through a special controlled atmosphere tunnel. In this situation, the birds inside the crates are stunned by CO_2, argon gas, or a mixture of both and later removed while they are unconscious (this can help reduce bruising of the more excited birds taken out of a crate).

Stunning is common in most plants, except when religious considerations are involved (e.g., kosher or halal regulations usually do not permit stunning). Stunning is usually carried out by utilizing electrical current or gas; less often is this process carried out using mechanical means. Stunning should produce a rapid onset of stress-free insensibility of sufficient duration, to allow the animal to remain unconscious, until it is dead. Currently, electrical stunning is the most common method used for poultry. The equipment is relatively inexpensive and simple to use (a fiberglass brine/water bath fitted under the overhead shackle line from which the suspended birds are moved forward), does not require much space, and is compatible with current line speeds. The birds pass through the stunner in a continuous procession (e.g., 140–180 birds/min in a modern processing plant). The voltage is applied between the submerged electrode and the ground. A renewed interest in gas stunning has emerged over the past few years, mainly to overcome problems (e.g., hemorrhages) encountered when high electrical currents are mandated. Carbon dioxide, argon, and nitrogen are used in different ratios to render the birds unconscious. Currently, carbon dioxide is the most commonly employed gas because at concentrations of 20–30%, this gas has been shown to have an anesthetic effect. Later, its concentration can be raised to achieve a longer stunning period. In this instance, unloading should be done fairly quickly after stunning so that sufficient time is not allowed for the birds to regain consciousness.

Bleeding

Bleeding is done by cutting the blood vessels in the neck. The so-called modified kosher is one of the most common methods, which results in cutting the jugular vein just below the jowls, so that the windpipe and esophagus remain intact (this can be important when automated equipment is later used to pull out the windpipe). The 'modified kosher' method is easy to perform (with both manual and automated equipment) and results in a good bleed-out. Automated high-speed equipment employs a railing system that positions the neck, of the suspended bird, in such a way that precise cutting can be performed. For kosher and halal slaughter, only manual cutting of the blood vessels is permitted.

The bleed-out phase takes anywhere between 2 and 5 min, depending on bird type and size. During the process, 35–50% of the total blood is lost; however, considerable variation can exist between individual birds and flocks. Other factors affecting blood loss include stunning method and time interval between stunning and bleeding. It is important to note that a poor bleed-out can increase the prevalence of carcass downgrading owing to conditions of muscle blood spots and, in particular, engorged or hemorrhagic wing veins.

Scalding

Scalding is done to loosen the feathers prior to plucking, and this process is performed by immersing the birds in warm water or through the use of a newly developed process that involves exposure to steam. In a small plant, manual scalding is done by placing the carcasses in a hot water tank. In large plants, this is done in a continuous manner where the birds are dipped in a single or multistage scalding bath while suspended from a moving shackle line (Figure 2). There are three commonly employed scalding schemes: The first is the so-called soft scalding/semiscalding, which is performed at water temperature of 50–53 °C for 1–3 min and used for young broilers and turkeys. The second is medium scalding/subscalding, which is performed at 54–58 °C for 1–2 min and is used for mature birds (where feathers are more tightly attached). The third is hard scalding where a temperature of 60–63 °C is used for 0.75–1.5 min and is used for waterfowl (ducks, geese, etc.). Selecting one process over another depends on the degree of difficulty in removing the feathers, chilling method to follow (e.g., water or air), and age of the bird. Higher scalding temperatures are better for loosening feathers from their follicles, but this is also more harsh on the skin (i.e., the outer layer of the skin, epidermis, becomes loose and is later removed by the automated plucking equipment; see next section of this article). The removal of the epidermis can also result in skin discoloration if the skin is allowed to dry during a subsequent air chilling operation. However, hard scalding is the only satisfactory way to release the feathers of waterfowl. Generally speaking, hard scalding does not cause as much discoloration in the thicker skin of waterfowl as it does in young broilers or turkeys. As indicated earlier, soft scalding/semiscalding is commonly used for young broilers and turkeys since it does not damage as much of the epidermis while still allowing for relatively easy feather removal. Adequate agitation of the scald water and uniform application temperature are essential to ensure good feather removal. To improve meat hygiene, careful equipment design is required as contamination (e.g., 1 g of fecal material can contain 10^8–10^9 bacteria) from one bird can affect the other birds in the flock. In the new steam scalders, there is less of a problem since the condensed water

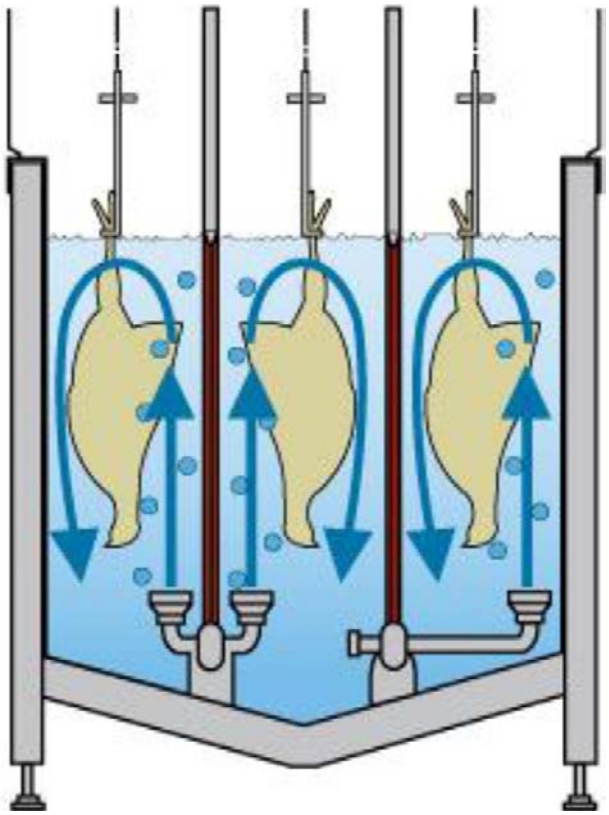

Figure 2 Scalding operation showing how birds move through the scalding tank while streams of air bubbles help to agitate the birds and assure good water penetration in between the feathers. Courtesy of Marel.

vapor drips off the bird. Controlling the temperature in the conventional water bath scalder is also a key feature in keeping bacteria load under control. Another way to reduce bacterial load on bird carcasses is through the use of a counterflow design (i.e., clean water introduced at the exit end of the tank, and water flows toward the entrance where the birds are introduced). Installing a multistage scalding tank system can further reduce contamination problems (i.e., two to four water tanks, where the birds are moved from the initial, more contaminated tank to successively cleaner tanks while water drips off the carcasses in between).

Defeathering

Feather removal is commonly performed by mechanical pickers equipped with rubber fingers that rub the feathers off the carcass. In a continuous operation, this is carried out while the birds are hanging upside down and moved in between two/three sets of rotating disks equipped with plucking fingers (Figure 3). The fingers are made of rubber and also contain a certain degree of lubricating agent that controls their hardness and elasticity. The hardness varies depending on the task required, machine speed, type of birds, etc. The fingers can also be mounted on drums that rotate toward the center. The space between the two sides is adjusted to accommodate bird size variations of different flocks.

Figure 3 Different types and arrangements of feather-picking fingers used by the industry. The disks are positioned along the line, at different heights, and the birds are passed in between the rotating fingers. See text for further explanation.

As indicated previously, scalding significantly reduces feather attachment, and it has been reported that scalding broilers at 50 °C (122 °F) reduced feather pulling force by about 30% compared to pulling similar feathers from a stunned bird. When scalding temperature is raised to 53 °C (128 °F), force is reduced by about 50%, and when 60 °C (140 °F) is used, about a 95% reduction can be seen. It is also known that the force required to pull the feathers in the femoral area is higher than in the pectoral area, with sternal feathers requiring the least force.

When pinfeathers are a problem, as with waterfowl, wax dipping is common after mechanical picking (this is performed by suspending the carcasses in hot wax, followed by cold water immersion and peeling off the wax); the wax can be reused after reheating and filtering. Singeing is used when only a small amount of pinfeathers exists and the process consists of burning small feathers with a clean gas-operated burner (no off-flavor or odors generated from the burning gas). The carcasses are then washed (Figure 1) to remove any soiling left after defeathering and singeing. Note that manual picking of the feathers is often carried out in small-scale operations.

Removing Oil Gland, Feet, and Rehanging

Once birds have been defeathered, feet are removed and birds are transferred to another line to minimize contamination in the following steps. Feet removal can be done manually or by automated equipment. In an automated process, the knee joint is positioned, by guiding bars along the shackle line, on an angle and severed when passing by a circular rotating blade. It is important that the cut be done between the bones and not through a bone, since the latter will appear dark/red in the chilled bird and almost black in the cooked product. Some of the new automated leg cutters first bend the leg and then perform a small incision, which allows further bending prior to cutting through the joint with a rotating circular blade. The oil gland is usually removed at this point by first positioning

the bird at a certain angle (by a set of metal bars/rails, along the shackle line) allowing a rotating blade to cut off the oil gland from the tail area. After removing the oil gland and feet, the carcasses are moved to another line. This can be done manually as the carcasses fall onto a sorting table or automatically by transferring the birds to another line where they are suspended from the knee joint. It should be pointed out that when birds are initially unloaded from the crates, they are placed on the line with their feet suspended from a shackle. As indicated earlier, this also assists in reducing contamination since the dirty shackles, used for the live birds, are replaced with clean ones.

An automated rehanging system consists of a large wheel with slots for grabbing the birds from underneath the knee joints and a device to push them into the evisceration line. Using automated rehanging equipment allows for labor savings and better hygiene (i.e., birds are not piled on a sorting table). In addition, fast rehanging can prevent variation in meat quality since rigor starts to set in at this stage, and if rehanging is done without delay, all birds are processed the same way.

Viscera Removal

During this stage, the body cavity is opened and the viscera (intestines, gizzard, gallbladder, and crop) are withdrawn. It can be done manually by using a knife and a pair of scissors, semiautomatically (with a circular rotating blade), or fully automatically by using a circular cutting blade and a scoop-like arm to withdraw the viscera. Fully automated processes can be done on high-speed lines (e.g., up to 14 000 birds per hour). In all procedures, special care should be taken not to damage the viscera and contaminate the carcass. In some countries, such a contamination results in condemnation of the whole bird or parts, whereas in other countries, washing and reprocessing of moderately contaminated carcasses are permitted.

During a manual operation, the abdominal skin is cut open along the midline (anterior part of the breast bone toward the cloaca), while the skin around the cloaca is trimmed in a circular pattern to minimize gut content spilling. Automating the process requires precise control of the same operation. Adjusting the equipment to accommodate size variations is of great importance, since unadjusted equipment can result in tearing of the intestines, causing gut spills and damaging the carcass itself. In semiautomated or fully automated evisceration processes, the first step is to cut around the cloaca, using a circular rotating blade. Some of the new devices are equipped with a vacuum device so potential fecal contamination is reduced, and the knife is rinsed after each insertion. The viscera are then withdrawn from the body cavity and remain attached to the carcass for inspection purposes. Alternatively, automated equipment can also be used for viscera separation right after its withdrawal and placement on a parallel line (i.e., can further improve the hygiene of the process). Once the viscera pack is exposed, the birds are inspected.

Meat Inspection

Inspection requirements can somewhat differ among countries and are usually carried out by a government official. This process is essential in ensuring that only wholesome birds, free of disease, will get to the marketplace. In some countries, it is required to inspect each individual bird by a qualified veterinarian, whereas in other countries, inspection is performed on a whole flock basis, and only certain carcasses are inspected by a designated veterinarian/inspector. Inspection is then performed when all the internal organs are visible to the inspector to reveal diseases/problems with the carcass itself and/or the internal organs. The inspection area should be provided with adequate bright light, a handwashing station, a rake for placing suspected birds, and a bin for condemned birds. The suspected birds receive a more thorough inspection, and all or some parts can be salvaged. Requirements about line speed or splitting the line so each inspector can check an adequate number of birds can usually be found in the regulations of each country. A mirror is often installed on the far side of the carcass, so the inspector can view both sides of the bird without touching it. Some processors and government agencies are currently evaluating computer vision systems (producing a digital image that can be compared with a reference) to assist inspection. It is important to note that many new lines already use a camera system to help with grading as well as assisting decisions about how to market each bird (e.g., cut-up and whole bird).

Giblet Harvesting

After inspection, the viscera are removed and the giblets (liver, heart, and gizzard) are salvaged and washed using a separate processing line. This is an optional step, where all or only some organs are harvested, depending on market demand and price. In the past, the process could only be done manually, but today, semiautomated or fully automated equipment is used. The gizzard (stomach used to grind the food, since birds have no teeth) is first cut open, the contents are removed, and the lining is peeled off. This can be done today by equipment that peels off the lining. The machine consists of two rollers that 'catch' the lining and pull it away. The equipment is showered all the time to keep it clean and cold. The hearts and livers can also be collected (manually or automatically), inspected, washed, and chilled. The chilled giblets are then packed in a paper bag (sometimes the neck is included) and inserted back into the eviscerated whole bird, collected and sold in bulk, or used for further processing in the plant.

Lung, Crop, and Head Removal

Removing the lungs, head, and crop is commonly done after inspection; however, in some operations, one or all are removed prior to inspection. The lungs can be removed manually by using a rake-like device that is inserted into the abdominal cavity. A semiautomated process employs a vacuum gun, which is inserted, by a plant employee, into each bird. A fully automated process employs the same type of gun, guided by a machine. Head removal can be performed by using a knife or a pair of shears. Automated systems usually consist of a puller that results in detaching the neck (of the forward moving carcass) at the weakest point between the atlas and the axis vertebrae. The

advantage of this device is that the esophagus and trachea (windpipe) can also be pulled out at the same time.

Bird Washing/Rinsing

Rinsing off all processing debris after removing the internal organs helps to keep the process clean. There are different devices that can be used at certain stages along the processing line. One of the most common control washing/rinsing stages is prior to chilling. The washing device usually consists of multiple spray nozzles positioned to cover the whole bird or critical carcass areas for washing off debris or blood residues. The carcass cavity area is also commonly rinsed by spray nozzles or by a retracting shaft equipped with high-pressure nozzles. The water can then be drained through the abdominal opening (created during evisceration) or by tilting the carcass. In the case where a retracting shaft is used, it can be pushed toward the neck opening (formed after pulling out the windpipe and the crop), and the carcass does not have to be tilted to drain the water. For rinsing the surface, some designs consist of spray heads positioned along the moving shackle line, washing first the upper part of the carcass and gradually the lower parts. Where permitted, antimicrobial agents such as chlorine and organic acids are also used. Chlorine is one of the most commonly used agents and is typically used at 20–50 ppm. The washing operation is critical in keeping the carcasses visibly clean and reducing the number of microorganisms on the carcass. The importance of obtaining low microbial counts (including pathogens) for food safety and shelf-life reasons are discussed in subsequent sections.

Chilling

Meat is a perishable item and therefore must be quickly chilled to minimize microbial growth. End-point temperatures and times to reach them are mandatory in many countries. The most common methods used include water immersion chilling, air chilling, and spray chilling. In a large-scale operation, immerse chilling is performed in long chillers with a counterflow of cold water (Figure 4), sometimes supplemented with ice, to help bring the carcass temperature to about 4–5 °C within 50–75 min. The carcasses are placed into a trough-like structure, which usually contains a large diameter auger that moves the birds forward; other designs employ large paddles. Most chillers today employ counterflow of products and water since it is more efficient (coldest water at the exit end) and more hygienic compared with parallel-flow chillers (i.e., product and water flow in the same direction). The microbial quality of birds coming out of a water chiller is often better than before chilling, since the water allows for washing off of bacteria.

Employing a prechiller and/or a postchiller is another improvement in obtaining a cleaner product. In the prechiller, water is used for the initial chilling and washing of the carcasses. The product is then lifted, drained, and passed into a long chiller where clean cold water is used to continue cooling the product. The amount of water overflow in the chilling tank is regulated in some countries (e.g., in Europe, 2.5 l for birds $\leq$2.5 kg). A short postchiller tank is now also becoming popular in North America (i.e., where the use of antimicrobial agents such as peracetic acid is allowed), to further help reduce microbial counts, including pathogens such as *Salmonella*. The short configuration allows for the use of high enough concentration of acid in an economical way. The product coming out of the chiller is allowed to drip for a few minutes so excess water is drained. The amount of water picked up, during the chilling process, is regulated in various countries, and is usually based on a percentage of the carcass weight (e.g., in the United States, 6% for birds up to 4.5 kg).

Using cold air chilling is more commonly used in Europe, but such operations are now starting to appear in North America and elsewhere. Since cold air, at medium flow rate, is used as the chilling medium, care must be taken not to overdry the product surface and increase weight loss. That can be achieved by wetting the product at some point(s) along the line (also helps to increase the environment's relative humidity). A normal chilling operation should usually result in a 0 to −1% carcass weight loss. A typical air chilling tunnel includes an overhead railing that goes back and forth along a chilling room, the overall length of the line can be a few km. Depending on the tunnel capacity, size of the birds, and volume of the product going through, chilling can be achieved within 75–150 min. The air-chilled birds do not, and should not, show much exudation (drip loss) in tray packs. Some processors claim that the microbial quality of the air-chilled product is better than that of a water-chilled product, but this is not always the case.

Spray chilling is a hybrid between water and air chilling. During the process, cold water is constantly sprayed over the carcasses while they are moved along the line. Moisture pickup is less than during water chilling but higher than during air chilling with only intermittent moisturizing of the carcasses.

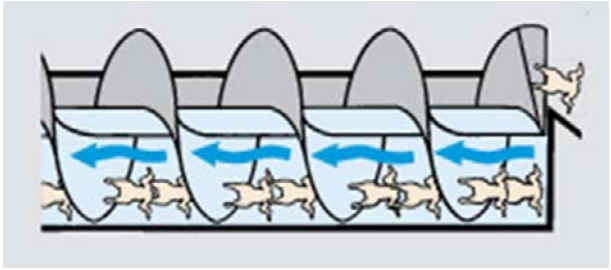

Figure 4 Water-chilling operation showing a spin chiller with a counterflow of clean cold water from the exit side, Courtesy of Stork.

Grading, Portioning, and Packaging

Poultry carcass grading is not mandatory in all countries, but is done in most large markets to facilitate sales. The grade is based on carcass conformation, relative muscling, the presence of tears/bruises/pinfeathers, and missing parts. Different countries usually have their own specifications, but generally speaking, a bird (chicken, turkey, or duck; see also meat compositional differences in Table 2) with adequate muscle deposition and no missing parts/esthetic defects will be classified as grade A. Minor defects will result in a grade B, and more

Table 2 Composition and nutritional value of different raw poultry meats

Source of meat							
Species	*Meat*	*Skin*	*Water (%)*	*Protein (%)*	*Fat (%)*	*Calcium (mg)*	*Calories (kcal)*
Chicken	White	+	68.6	20.3	11.1	0.86	186
		−	74.9	23.2	1.6	0.98	114
	Dark	+	65.4	16.7	18.3	0.76	237
		−	75.9	20.1	4.3	0.94	125
Turkey	White	+	69.8	21.6	7.4	0.90	159
		−	73.8	23.5	1.6	1.00	115
	Dark	+	71.1	18.9	8.8	0.86	160
		−	74.5	20.1	4.4	0.93	125
	All	+	70.4	20.4	8.0	0.88	160
Duck	All	+	48.5	11.5	39.3	0.68	400
Goose	All	+	50.0	15.9	33.5	0.87	370
		−	68.3	22.7	7.1	1.10	160
Quail	All	+	69.7	19.6	12.1	0.9	192
Pheasant	All	+	67.7	22.7	9.3	1.3	180
Pigeon	All	+	48.1	15.7	20.2	1.0	250

Expressed on a 100 g portion of meat with/without skin.
Source: USDA (2011). *National Nutrition Database for Standard Reference*. Washington, DC: United States Department of Agriculture. www.nal.usda.gov.

serious defects will result in a grade C. The last two categories are usually not sold as whole birds, but rather as parts (no grade labeling required) or used for further processed products. Grading can be done by a qualified person or with the assistance of a computerized machine vision system, as indicated previously. The appearance of the carcass and final grade can also be affected by processing conditions used (e.g., high electrical stunning can result in some bone dislocation and blood spots), as well as feeding, growing, and transporting parameters (e.g., bruising during loading of birds) described in more details in other sections.

Weighing of the ready-to-sell birds (whole bird or portions) is done manually or by automated weighing equipment connected to a computer network. A computerized system can also be employed to automatically print labels on individual/bulk packages. Automated portioning equipment is used in large plants where simple portioning is done (e.g., nine cuts commonly used for fast food chains), or more sophisticated cutting and deboning (e.g., breast meat fillets, drumstick meat) are performed by high-speed equipment guided by cameras/laser sensors that first produce accurate 3-D images of the portions to be cut. Selling portioned poultry to consumers interested in only buying certain parts (skinless breast fillets, drumstick meat) has allowed the industry to significantly increase sales and also move some items such as whole turkey (i.e., traditionally consumed during Thanksgiving and Christmas) to an all year-round product.

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Prebiotics

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Introduction

There has been a remarkable increase in people having gastrointestinal and digestive problems without showing the characteristic clinical symptoms (i.e., associated with changing life style and age). These are caused due to imbalances in the intestinal microflora, referred to as dysbiosis. To overcome this problem, probiotics and prebiotics play a major role in prevention of dysbiosis. Probiotics are beneficial bacteria that colonize in human intestines (mainly the colon) and provide health related benefits to the host. These probiotic microorganisms may not be able to survive the harsh, acidic conditions of the stomach; thus, making it difficult to colonize in the intestine. Most of these bacteria are strict anaerobes and obtain their energy by fermentation of food. Therefore, food and energy in the form of dietary supplementation work to restore the intestinal ecology of the probiotic microorganisms. The food and dietary supplements that stimulate the growth and multiplication of the microorganisms that are beneficial to our health (mainly lactobacilli and bifidobacteria) are termed as prebiotics. Thus, prebiotics are nondigestible food ingredients which enhance the growth and colonization of probiotic bacteria, and contribute to the improved health of the host. Prebiotics were recognized in the 1950s, but the term 'prebiotics,' was coined by Gibson and Roberfroid in 1995. The contribution of colonic microbiota to the health associated benefits to the host has been a breakthrough in the field of medical and nutrition research.

The main types of fermentative substrates of dietary origin are as follow:

- Nondigestible carbohydrates such as resistant starch, nondigestible oligosaccharides, non-starch polysaccharides.
- Undigested proteins that escape digestion in the small intestine and reach the colon.

Out of these, the dietary carbohydrates (10–60 g day^{-1} reaches the colon) serve as the major substrate for the bacterial growth. They include resistant starch (8–40 g day^{-1}, fermented by most of the colonic bacterial species including *Bacteriodes* sp., *Eubacterium* sp., *Bifidobacterium* sp., etc.), non-starch polysaccharides (8–18 g day^{-1}), unabsorbed sugars (2–10 g day^{-1}), and oligosaccharides (2–8 g day^{-1}). Out of these, prebiotics such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), xylo-oligosaccharides, lactulose, etc. are selectively fermented by the probiotic microorganisms, thereby resulting in their growth and multiplication and hence are termed as prebiotics.

Sources of Prebiotics

The short chain prebiotic oligosaccharides occur widely in nature and can be obtained in small amounts by direct extraction from natural sources such as plants – including chicory, onion, garlic, asparagus, artichoke, bananas, tomatoes, and leeks. These are reported to be present in animal sources such as human milk and colostrum (in relatively smaller amounts). They can also be produced in comparatively larger amounts by using microorganisms and their enzymes at large scale. Enzymes, mainly hydrolases (E.C. 3.2.) and transfereases (E.C. 2.4.), of plant origin can also be used for enzymatic synthesis of prebiotic oligosaccharides. Prebiotic oligosaccharides can also be produced by controlled hydrolysis of natural polysaccharides. To make the production process economical, prebiotics can also be synthesized by using agro-industrial waste/byproducts like whey, wheat and rice straw, and sugarcane bagasse.

Characteristics of Prebiotics

Prebiotics are short, have low molecular weight, and are heat resistant bio-molecules with three to ten sugar moieties. They possess β-glycosidic bonds which enable them to resist hydrolysis by human salivary and pancreatic enzymes. They have low caloric value – 4.l2–8.4 kJ g^{-1}. Figure 1 represents the structure of some common prebiotics. The characteristics of ideal prebiotics are:

(i) They should not be absorbed or hydrolyzed by mammalian tissues or enzymes.
(ii) They should selectively enhance the growth and multiplication of beneficial/probiotic microorganisms.
(iii) They should beneficially alter the activity of the intestinal micro-flora.
(iv) They should have beneficial effects on the host defense system.
(v) They should be stable across a wide range of pH in the stomach and colon.
(vi) They should be advantageous to the host's defense system.

Types of Prebiotics

Common prebiotics in use include inulin, FOS, GOS, soya-oligosaccharides, xylo-oligosaccharides, pyrodextrins, isomalto-oligosaccharides, lactulose, pectic oligosaccharides, lactosucrose, sugar alcohols, gluco-oligosaccharides, levans, and resistant starch. These can be produced using various substrates such as lactose, sucrose, starch, and xylan by both chemical and enzymatic methods through transgalactosylation activity on respective carbohydrates (Figure 2). Enzymes from different microbial sources, such as bacteria, yeast, and fungus, are responsible for the production of prebiotics.

Prebiotics have been classified into two categories: established prebiotics and emerging/tentative prebiotics. Established prebiotics include inulin, GOS, FOS, and lactulose. Emerging/tentative

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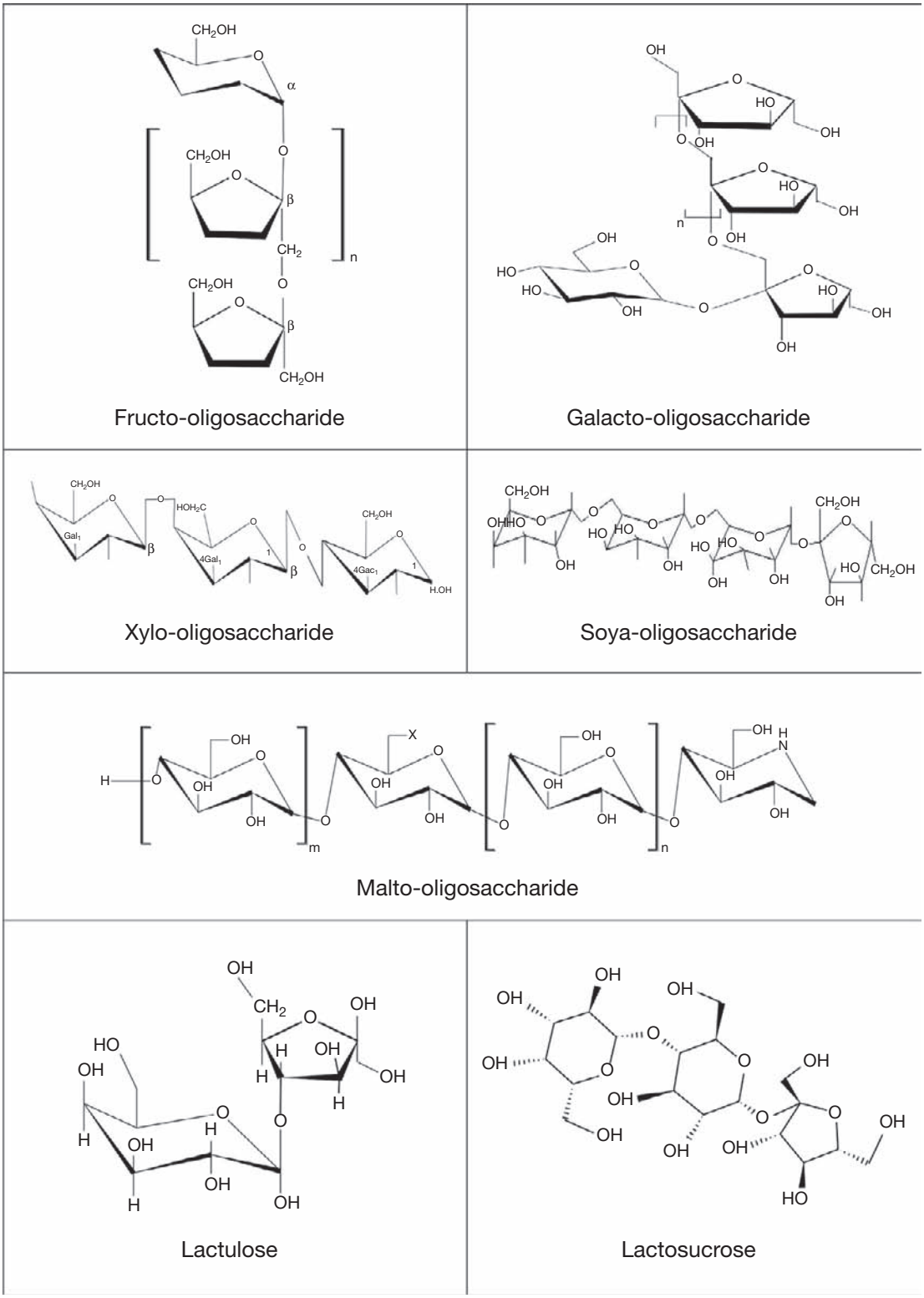

Figure 1 Some common prebiotics and their structure.

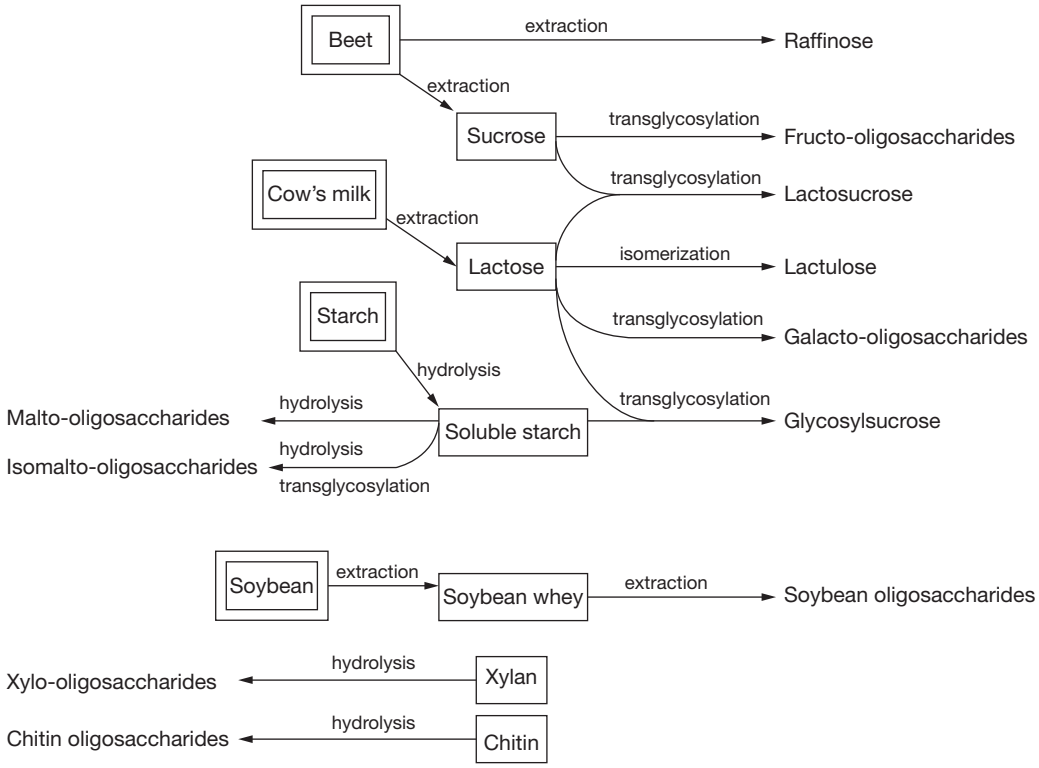

Figure 2 Production process of nondigestible oligosaccharides. Reproduced from Sako, T., Matsumoto, K. and Tanaka, R. (1999). Recent progress on research and applications of non-digestible galacto-oligosaccharides. *International Dairy Journal* **9**, 69–80, with permission.

prebiotics include isomalto-oligosaccharides, xylo-oligosaccharides, and soya-oligosaccharides. Prebiotics can also be distinguished on the basis of the substrate used for their production, such as:

(i) lactose derived prebiotics, including GOS, lactosucrose and lactulose;
(ii) sucrose derived prebiotics, including FOS, isomaltulose;
(iii) starch derived prebiotics, including isomaltose-oligosaccharide, isomaltotriose; and
(iv) xylan derived prebiotics including xylo-oligosaccharide.

Galacto-oligosaccharides

GOS made up of β-linked (mostly β-[1→4], but β-[1→2]-, β-[1→3]-, or β-[1→6] linkages are also observed) galactose units with mainly glucose residue at its reducing end. These are synthetically produced by the trans-galactosylation activity of β-galactosidase (E.C. no. 3.2.1.23), or β-glycosidase (E.C. no. 3.2.1.21), enzymes using lactose, glucose, or galactose as a substrate having lactose as a galactosyl donor. These enzymes can be obtained by using different microorganisms such as *Lactobacillus reuteri*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, or *Kluyveromyces lactis*. Oligomate 55, Cup-oligo H-70, Vivinal-GOS, and TOS-syrup, are some of the commercial available GOS. They are mainly used as cosmetic additives, bulking agents, low-calorie sweeteners, and food ingredients in breads, jams, marmalades, desserts, etc.

Fructo-oligosaccharides

FOS, generally known as fructans, are of the inulin and levan type, including 1-kestose (GF2), nystose (GF3), and 1-β-fructofuranosyl nystose (GF4) composed of fructosyl (F) units linked by β-(2→1) glycosidic bonds. These are mainly produced by the transfructosylation activity of β-fructofuranosidase (E.C. no. 3.2.1.26) or fructosyltransferase (E.C. no. 2.4.1.9) enzymes using sucrose as a substrate. These can also be synthesized by the hydrolysis of inulin using endoinulase as enzymes. Enzymes from microbial sources, such as *Aspergillus japonicus*, *A. niger*, *Penicillium citrinum*, *Arthrobacter* sp., *Zymomonas mobilis*, *Lactobacillus reuteri*, and *Candida*, are used for the production of prebiotics. FOS occur naturally in wheat, onions, bananas, garlic, artichoke, dahlia tubers, etc. Commercially available FOS includes Actilight, NutraFlora, Raftilose, Inulin FOS, Fibrulose, etc. These have low calories, are safe for diabetics, are not cariogenic, have bifidus-stimulating factors, and aid immune-regulatory functions.

Xylo-oligosaccharides

These constitute oligosaccharides made up of xylose units (2–10) that are linked through β-(1→4) linkages. They also possess side groups such as α-D-glucopyranosyluronic acid and 4-*O*-methyl derivative. They are produced through the hydrolysis of xylan by xylanase enzymes, and mainly consist of xylobiose, xylotriose, and xylo-tetraose. Enzymes involved in their production are endoxylanase (E.C. no. 3.2.1.8), β-D-

xylosidases (E.C. no. 3.2.1.37), and debranching enzymes like esterases (E.C. no. 3.1.1.72). These enzymes are produced by microorganisms such as *Aspergillus* sp., *Trichoderma* sp., *Penicillium* sp., *Thermomyces lanuginosius*, *Bacillus halodurans*, and *Pichia stipiti*. Xylo-oligosaccharides are present in fruits, vegetables, bamboo, honey, and milk and can be synthesized on a large scale from xylan-rich substrates or agricultural waste/by-products such as cornstalks, bagasse, and corncobs. Xylo-oligosaccharide 95P, 70L, 35P, 20P, etc. are commercially available xylo-oligosaccharide products. These have an acceptable odor, as well as non-cariogenic and low-caloric properties.

Soya-oligosaccharides

Soya-oligosaccharides are composed of trisaccharide raffinose (consists of one unit of D-galactose, D-glucose, and D-fructose) and tetrasaccharide stachyose (consists of two molecules of D-galactose and one of D-glucose and D-fructose), which are linked by α-(1→6) linkages. These oligosaccharides have degrees of polymerization ranging from 3 to 4 (trisaccharide raffinose and tetrasaccharide stachyose). These can be extracted from soya beans, peas, and some other beans. They may have a role in the prevention of allergies and may be used in the treatment of galactosemia and lactose intolerance.

Lactulose

Lactulose is a ketose disaccharide, chemically known as 4-*O*-β-D-galactopyranosyl-D-fructose. The enzymes used for the biocatalytic production of lactulose are β-galactosidase (EC 3.2.1.23) and β-glycosidase (EC 3.2.1.21) – using lactose and fructose as a substrate. It can also be produced by the alkaline isomerization of lactose. Microorganisms used for the production of lactulose are *Kluyveromyces lactis*, *Sulfolobus solfataricus*, *Arthrobacter* sp., *Pyrococcus furiosus*, etc. Some of the commercial preparations of lactulose include Duphalac, Bifiteral, Chronulac, Cephulac, etc. It is mainly used as a laxative and may be used for prevention of hepatic encephalopathy.

Lactosucrose

Lactosucrose (lactosylfructoside) is a trisaccharide that consists of galactose, glucose, and fructose units, chemically known as *O*-β-D-Galactopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside. Being an artificial oligosugar, it is low in caloric content and stimulates the growth of bifidobacteria present in the colon. It can be synthesized by transfructosylation of lactose with sucrose using levansucrase (E.C. no. 2.4.1.10) and fructofuranosidase (E.C. no. 3.2.1.26) enzymes; or, by transgalactosylation of sucrose with lactose using β-D-galactosidase enzymes (E.C. no. 3.2.1.23). Microbial sources used for their production are *Zymomonas mobilis*, *Paenibacillus polymyxa*, *Bacillus subtilis*, *Bacillus circulans*, etc. The commercially available lactosucrose products are Nyuko-Oligo LS-40L, Nyuko-Oligo LS-55P, Pet-Oligo L55, Newka-Oligo LS-35L, etc. These may be used as pet foods, table sugar, drinks, or confectionery ingredients.

Isomalto-oligosaccharides

These are oligosaccharides having α-D-(1→6) linked glucose moieties (and rarely α-D-(1→4) glucosidic linkages) with degrees of polymerization ranging from 3 to 6. These occur naturally in honey and fermented food products such as sake and soybean sauce. These oligosaccharides can either be produced by using dextranase (E.C. no. 3.2.1.11) and dextransucrose (2.4.1.5) as enzymes with starch as a substrate; or, by transglucosidase (E.C. no. 2.4.1.24) enzymes using sucrose as a substrate. Transformation of maltose into isomalto-oligosaccharide can also be achieved using α-glucosidase enzymes (E.C. 3.2.1.20). *Penicillium lilacinum*, *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, etc. are the microbial sources for the enzymes. The commercially available isomalto-oligosaccharide products are IMO-900, IMO-800, etc. It has been reported that they may be used in the treatment of constipation and hyperlipidemia in elderly and hemodialysis patients.

Isomaltotriose

Isomaltotriose, chemically known as α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-D-glucose, consists of three molecules of isomaltose. Isomaltotriose can be synthesized by the hydrolysis of dextran using dextranase enzymes (E.C. no. 3.2.1.11). *Flavobacterium* sp., *Streptomyces anulatus*, etc. are the microbial sources for the production of isomaltotriose using dextrans as a substrate. The commercially available isomaltotriose products are available under the brand name Isomaltotriose, manufactured by Santa Cruz Biotechnology Inc. (California), Advanced Technology & Industrial Co. Ltd (Hong Kong), etc.

Isomaltulose (Palatinose)

Isomaltulose, chemically known as 6-*O*-α-D-glucopyranosyl-D-fructose, is a structural isomer of sucrose constituting of glucose and fructose units linked by α-(1→6) glycosidic bonds. It is commonly produced by the enzymatic rearrangement of sucrose using α-glucosyltransferase (i.e., isomaltulose synthase [E.C. no. 5.4.99.11]). Isomaltulose can also be synthesized by palatinose synthetase followed by the interamolecular dehydration of palatinose. The microorganisms responsible for the conversion of sucrose into isomaltulose are *Erwinia* sp., *Klebsiella planticola*, *Serratia*, etc. Isomaltulose occurs naturally in products such as sugarcane and honey. The commercially available isomaltulose products of are available under the brand name Isomaltulose, manufactured by Shaanxi Sciphar Hi-Tech Industry Co. Ltd (China), Xian Yuensun Biological Technology Co. Ltd (China), and Palatinose manufactured by Shanghai Honghao Chemicals Co. Ltd (China) etc.

Resistant Starch

Resistant starch is a small fraction of the starch that escapes digestion in the small intestine and reaches the colon. This portion of starch that is resistant to digestion is then fermented in the large intestine, mainly the colon, by the endogenous microflora. Starch is basically glucose molecules linked by α-D-(1→4) or α-D-(1→6) glycosidic bonds, and is digested in the small intestine. It comprises mainly amylose (straight chains

that form 15–20% of starch possessing α-D-[1→4] linkages) and amylo-pectin (highly branched and a major component possessing both α-D-[1→4] and α-D-[1→6) linkages]. Amylopectin is a highly branched structure that provides more surface area available for digestion in the small intestine. Resistant starch possesses a higher amount of amylose, which limits the exposed surface area. Therefore, starch with a high amylose content is digested slowly, and some parts of it escapes digestion in the small intestine and reaches colon. On the basis of its structure and complexity, resistant starch is further classified into four classes. Type I includes physically inaccessible starch granules. Type II includes resistant starch granules. Type III includes retrograded starch. Type IV includes chemically modified starches. Resistant starch naturally occurs in under-ripe bananas, oats, lentils, white beans, etc. Commercially available resistant starch products include Hi-maize, CrystaLean, Novelose, Amylomaize VII, etc. These are reported to contribute in promoting apoptosis and reducing the risk of colon cancer.

The Prebiotic Effect

Movement of food is rapid through the stomach and small intestine (~4–6 h) compared to colonic transit time, which is around 48–70 h. This results in the formation of a stable microbial community in the large intestine. Prebiotics (along with other dietary fibers and undigested proteins) escape the upper part of the gastro-intestinal tract undigested, thus serving as a potential substrate for probiotic endogenous bacteria (bifidobacteria and lactobacilli) in the colon. Most of the microflora in the colon are strict anaerobes and derive their energy through fermentation. Prebiotics increase the population growth rate of bifidobacteria and lactobacilli from tenfold to a hundredfold. The presence of 'osidic' bonds makes prebiotics nondigestible by human salivary and intestinal enzymes; therefore, they reach the colon in the undigested form. The hydrolysis and fermentation of these prebiotic oligosaccharides in the intestine by colonic microflora results in their breakdown – mainly into short chain fatty acids. Prebiotics provide energy, metabolic substrate, and essential micronutrient selectively to the endogenous probiotic bacteria. These probiotic bacteria have health related effects on the host by reducing the risk of intestinal infections. The end product of these prebiotics include short chain fatty acids (such as acetate, butyrate, and propionate), lactate, pyruvate, ethanol, succinate, polyamines, bacitracin, and gasses such as H_2, CO_2, CH_4, H_2S. Short chain fatty acids are mainly responsible for causing the prebiotic effect. Short chain fatty acids lower luminal pH, which restricts the growth of disease-causing and putrefactive microorganisms like *Escherichia coli*, *Salmonella*, *Listeria*, and *Shigella*. Short chain fatty acids have also been reported to contribute in the regulation of absorption of sodium and water, maintenance of cholesterol, and increased absorption of calcium and other minerals by the host.

Health Benefits for Human

Changes in dietary habits influence the balance of colonic bacteria in the human gut. Probiotics and prebiotics, together known as synbiotics, have associated health benefits. Prebiotics have been studied for their role in reducing the risks of cancer, acute gastroenteritis, osteoporosis, cholesterol, and hyperlipidaemia; as well as for their bifidus-stimulating ability and immuno-modulatory effects.

Production of Short Chain Fatty Acids

The fermentative end products (short chain fatty acids) of prebiotic oligosaccharides produced by probiotic microorganisms are in the order of acetate > propionate > butyrate in the ratio of 3:1:1 (Figure 3). Probiotic microorganisms use fermentation as their energy source. Production of short chain fatty acids results in a decreased pH, which leads to the reduced levels of pathogenic bacteria, decreases bile acid solubility, and reduces ammonia absorption. Short chain fatty acids are water soluble and are readily absorbed into the blood stream by diffusion or through anion exchange. They also influence intestinal motility. Only 5–10% of short chain fatty acids are eliminated in the feces. Short chain fatty acids contribute to the growth and physiology of intestinal tissues and systemic metabolism. Table 1 represents the function of various short chain fatty acids.

Acute Gastroenteritis

Acute gastroenteritis is a disease state that occurs when food or water that is contaminated with pathogenic microorganisms (such as *Clostridium perfringens*, *Vibrio cholera*, *E. coli*,) or their toxins is consumed. Some of its symptoms are nausea, vomiting, diarrhea, and abdominal pain. Prebiotics help in overcoming this problem by stimulating the growth of bifidobacteria and lactobacilli, which act as a barrier against the invasion of food pathogenic microorganisms. The probiotic bacteria compete for nutrition and colonization sites, produce short chain fatty acids, lower the pH, and secrete antimicrobial agents against the pathogenic bacteria, thereby reducing their invasion in the gut.

Cancer Risk

Colorectal cancer has been reported mostly in people having a family history of colon cancer, high smoking rate, high body weight, greater consumption of alcohol and meat, lower physical workload, and lower intake of fruits and vegetables. Prebiotics have been reported to enhance detoxification, reduce toxic metabolite production, and provide protection against tumor development in the colon. Prebiotics, along with probiotics (bifidobacteria), have been studied to reduce a significant number of colonic aberrant crypt foci and inhibit the propagation phase of the carcinogen. The activity of colon cancer causing bacterial enzymes such as β-glucuronidase, β-glucosidase, and nitroreductase (which convert pre-carcinogens to proximal carcinogens) was observed to be reduced by prebiotics by way of reducing the pH of the colon. Prebiotics also reduce indole and isovaleric acids levels, which are markers of putrefaction.

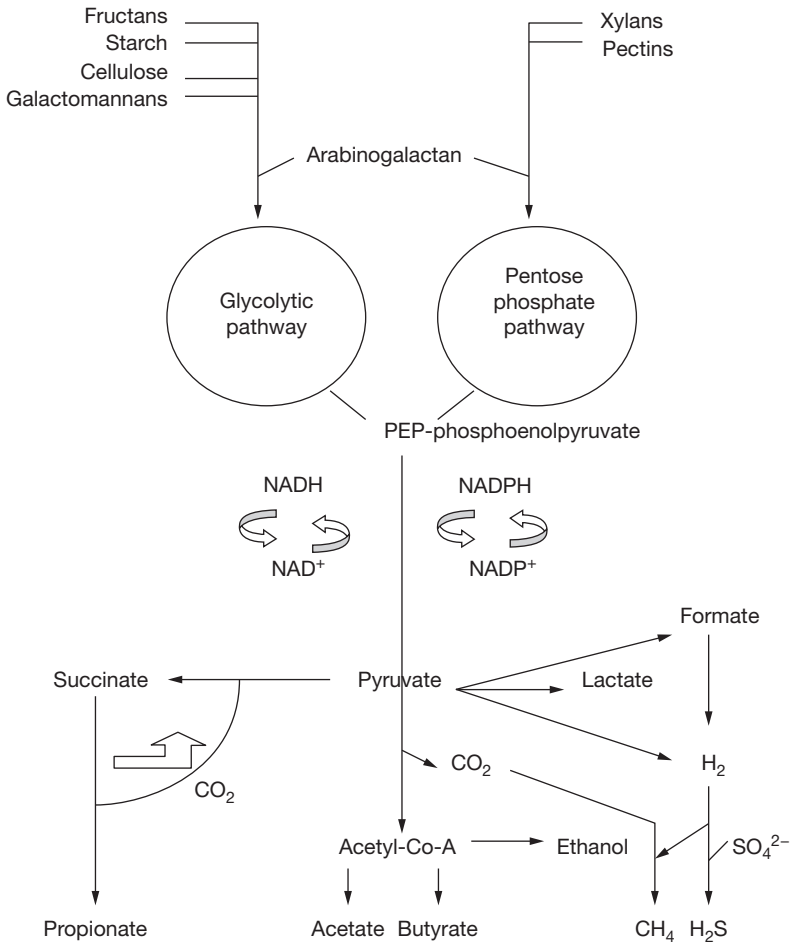

Figure 3 The basic pathway for production of short chain fatty acid metabolites by bacterial fermentation in the colonic lumen. Reproduced from Macfarlane, G. T. and Gibson, G. R. (1997). Carbohydrate fermentation, energy transduction and gas metabolism in the human large intestine. In: Mackie, R. I. and White, B. A. (eds.) *Ecology and physiology of gastrointestinal microbes: gastrointestinal fermentations and ecosystems*, pp. 269–318. New York: Chapman and Hall, with permission.

Table 1 Functions of short chain fatty acids

Short chain fatty acid	*Function*
Acetate	Energy source for body; metabolized by skeletal muscle, heart, kidney, and brain
Propionate	Reduces hepatic glucose output and cholesterol biosynthesis, regulates adipose tissue deposition; metabolized by liver
Butyrate	Energy source for colonocytes; modules colonocyte differentiation and proliferation, regulates gene expression and transcriptional protein

Mineral Absorption

Prebiotics stimulate the absorption of minerals, such as calcium, magnesium, zinc, and iron, which improves bone mineralization. Prebiotic oligosaccharides (3–6 sugar moieties) can bind minerals in the small intestine and release them in the large intestine, where they are absorbed in a much better way. Short chain fatty acids, which are formed in the intestine due to fermentation, can also assist the absorption of these minerals by modifying the electrolyte exchange, thus lowering the risk of osteoporosis. Their effect on bones is further dependent on the time, dose administered, and age of the host.

Reduction in Constipation

Elderly people, pregnant and nursing women, travelers, and people on weight loss diets commonly face the problem of constipation (or irritable bowel disease). Prebiotics play a significant role in overcoming the problem of constipation by increasing the fecal biomass which, in turn, increases the fecal microbial mass, thereby further reducing the transit time of stool. This results in the reduction of colonic water resorption which, in turn, results in a softer and heavier stool with increased frequency. Prebiotics, like oligofructose, inulin, xylo-oligosaccharide, and lactulose, have significant roles in reducing constipation.

Lipid Regulation

Prebiotics lower total cholesterol, LDL-cholesterol, and triglyceride levels, thus showing hypolipidaemic and

hypotriacylglycerolaemic effects. The increased production of short chain fatty acids through fermentation of prebiotics decreases, as well as inhibits, liver lipogenesis in hepatocytes. This may be due to modified gene regulation, which further leads to down regulation of all hepatic lipogenic enzymes. Prebiotics, like GOS, xylo-oligosaccharide, and isomalto-oligosaccharide, have been reported to have a significant role in reducing serum cholesterol and triglycerides.

Immune Modulation

Fermentative end products of prebiotics (i.e., short chain fatty acids) have been reported to contribute to boosting the immune response by selective stimulation of specific membranes of probiotic microflora. The G protein coupled receptors of immune cells contain receptors that interact with short chain fatty acids. These short chain fatty acids also play a significant role in cytokine production, up regulation of gut-associated lymphoid tissue expression of secretory IgA, anti-inflammatory profile, and increased chemotactic response of neutrophiles, thereby modulating the immune system of the host.

Effect on Dental Caries

The nondigestible oligosaccharide cannot be fermented by *Streptococcus mutans*, which is a causative agent of dental caries and plaque formation in humans and animals. Thus, prebiotics such as nystose have been reported to show low cariogenic properties and may serve as alternative sweeteners with reduced dental problems.

Global Status

Due to increasing awareness in consumers, and the wide applications of prebiotics in various fields (including health drinks, dairy products, beverages, bakery and pet food), demand for these products is increasing globally. According to Multi-Sponsor Surveys' 2012 Gallup Study of Probiotic & Prebiotic Consumers, 31% of the population is aware of prebiotics (http://www.nutraceuticalsworld.com/issues/2013-03/view_trendsense/getting-ahead-of-the-curve-prebiotics/#sthash.PoGUV4Zw.dpuf). The number of new prebiotic products has increased almost threefold from 2009 to 2013. The prebiotics market value (Nutraceuticals) in Europe and North America has been estimated at $355.69 million USD and $321.18 million USD, respectively, for the year 2018 – as per the reports of Micromarket Monitor (http://www.micromarketmonitor.com/). In Asia, the market of prebiotics was estimated to be $2.3 billion in 2012, and is likely to grow at CAGR of 11.4% and reach $4.5 billion by 2018 (http://www.nutraceuticalsworld.com/issues/2014-04/view_features/flourishing-flora-probiotics-prebiotics-market-update/#sthash.2zJ6hHlH.dpuf).

Conclusions

Prebiotics are potent agents that contribute to the nutritional, physiological, and digestive functions of the host. Prebiotics improve and maintain the balance of beneficial intestinal microflora in the gut of animals and humans. Because of their properties, prebiotics (along with probiotics) can be helpful in the treatment of various diseases such as acute gastroenteritis and osteoporosis. They can also reduce the risk of cancer, and help to lower cholesterol and hyperlipidaemia. Prebiotics also possess the characteristics of having bifidus-stimulating ability, immuno-modulatory effects, and low cariogenicity. These properties of prebiotics, along with their safety aspects, have created countless new opportunities in both the food industry, and in academia. With their low cost of production, the future is open for designing new prebiotic, functional food products, as well as studying their structure, function, relationship, and their mode of action. Also, the increasing awareness of consumers is making prebiotics a promising market in the coming times.

See also: Bifidobacteria in Foods: Health Effects; Colon: Structure and Function; Dahi; Dairy Products: Dietary and Medical Importance; Fatty Acids: Metabolism; Functional Foods; Lactic Acid Bacteria; Lactose; Probiotics; Soy Beans: Dietary Importance.

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Pregnancy: Dietary Guidance for Pregnancy

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Introduction

Nutrition is a major environmental factor that influences the health of both mother and infant during pregnancy. Foods and drink consumed during pregnancy play an important role in maternal health and fetal growth and development. A mother's food choice affects the supply of nutrients to the fetus and has a primary influence on fetal nourishment throughout each stage of gestation. The pattern of nutrient demand varies in relation to maternal and fetal metabolism during each stage of pregnancy and the growth of the fetus. Macronutrients provide energy and protein for fetal growth, while micronutrients are involved in the metabolism of macronutrients and the structural and cellular metabolism of the fetus. Changes in diet at different stages of gestation may lead to developmental adaptations that permanently alter fetal physiology and metabolism. This can influence the child's risk of metabolic, cardiovascular, and/or endocrine diseases in later life. Therefore, supporting women to adopt healthy eating patterns needs to be prioritized. Making smart food choices is a huge responsibility, but fortunately, it is fairly simple.

Nutrient Partitioning during Pregnancy

Historically, it was accepted that a fetus acts as a parasite that could successfully compete with its maternal host for nutrients in the event of reduced availability. However, in the last 30 years, evidence from animal and human models has contradicted this theory by demonstrating that the division of nutrients favors the maintenance of maternal body weight, rather than normal fetal growth. Biological competition may exist when nutrient restriction leads to the loss of maternal body stores, although proportionally, the mother will continue to receive a larger share of the nutrients. When a mother exhibits excess maternal body/nutrient stores, fetal growth may still be maintained when her nutrient supply is inadequate. This suggests maternal nutritional status at conception will influence how nutrients are partitioned between mother and offspring during pregnancy. If maternal diet at conception and during pregnancy is inadequate, fetal growth will only be sustained when dietary intake is able to support some maternal weight gain. Thus, when maternal nutrient stores are restricted, then fetal growth will be restricted. The quantity and quality of maternal diet are fundamental for the maintenance of optimal fetal growth because fetal parasitism does not sustain fetal growth at the expense of maternal tissue.

Dietary Recommendations during Pregnancy

Dietary recommendations for pregnancy differ by geographic region. In 1991, the United Kingdom became the first country to develop a set of estimated dietary requirements for pregnancy using the dietary reference values. More recently, the Institute of Medicine's Food and Nutrition Board adopted a similar approach on behalf of the US and Canadian governments, with similar guidelines also now available in other developed countries including Australia, New Zealand, Japan, and Europe.

Energy and Macronutrients

Table 1 summarizes the daily dietary recommendations for energy and macronutrient intakes during pregnancy and the food sources providing those nutrients. Dietary guidelines for macronutrient intakes contain population nutrient intake goals that are expressed as a percentage of total energy intake and referred to as either an adequate macronutrient distribution range (AMDR) or a dietary goal. AMDRs and dietary goals are associated with reduced risk of chronic disease while ensuring adequate intakes (AIs) of essential nutrients. If dietary intake exceeds the AMDR or the dietary goal, there may be an increased risk of chronic diseases and/or inadequate intake of essential nutrients.

The systematic review by Blumfield and colleagues showed that in developed countries (the United States, Canada, the United Kingdom, Europe, Australia, New Zealand, and Japan), energy and macronutrient recommendations do not match with reported intakes of pregnant women. Energy and fiber intakes were consistently lower than recommendations, total fat and saturated fat intakes were generally above recommendations, and carbohydrate and PUFA intakes were either lower or marginal compared to recommendations. Due to limited experimental data, many nutrient targets for pregnancy have been set on the basis of theoretical calculations to establish what is believed to optimize the health and well-being of pregnant women and their offspring. Therefore, until epidemiological studies demonstrate consistent associations between exposure and health or disease outcomes, the implications of such macronutrient intakes for mother and offspring will be unknown.

Interestingly, a recent Australian study has shown that a moderate intake of protein (18–20% of total energy intake) allows pregnant women to eat the best range of foods across all the healthy core food groups in the Australian Guide to Healthy Eating (Australia's national food selection guide) while optimizing vitamin and mineral intakes. The ratio of protein to carbohydrate intake in the maternal diet during pregnancy has also been related to the amount of muscle and fat tissue in the developing fetus. While more research is needed, macronutrient intakes during pregnancy may contribute to offspring risk of developing diabetes in later life.

Micronutrients

Table 2 summarizes the daily micronutrient intakes recommended during pregnancy and the food sources providing

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Table 1 Daily dietary recommendations for macronutrient intakes during pregnancy by geographic region[a–c] and the food sources providing those nutrients

	Recommendations					
Nutrients	*The United States/ Canada*	*The United Kingdom*	*Europe/WHO*	*Japan*	*Australia/NZ*	*Food sources*
Energy[c]						
1st trimester 2nd trimester 3rd trimester	– +340 kcal/ day +450 kcal/ day	– +360 kcal/ day +475 kcal/ day	– +360 kcal/ day +475 kcal/ day	+50 kcal/ day +250 kcal/ day +500 kcal/ day	– +335 kcal/ day +455 kcal/ day	Additional energy increment recommended in the 3rd trimester only. All food sources provide energy
Protein	10–35% E	10–35% E	10–35% E	<20% E	15–25% E	Meat, poultry, fish, cereal and cereal-based products, eggs, dairy products, soy products, seeds, nuts, beans, lentils
Total fat	20–35% E	15–30% E	15–30% E	20–30% E	20–35% E	See food sources for fat types below
Saturated fat	Minimize	≤10% E	<10% E	4.5–7.0% E	≤10% E	Avoid foods high in saturated fat such as fatty cuts of meat, full-fat milk, cheese, butter, cream; most commercially baked products such as biscuits and pastries; most deep-fried fast foods; coconut and palm oil
Polyunsaturated fat						
N−6 fatty acids *N*−3 fatty acids	5–10% E (13 g/ day*) 0.6–1.2% E (1.4 g/ day*)	2% E 0.5% E	2% E 0.5% E	≤10% E (9 g/day*) 2.1 g/day (AI)	10 g/day* 115 mg/ day*	Fish; seafood; polyunsaturated margarines; vegetable oils such as safflower, sunflower, corn, or soy oils; nuts such as walnuts and Brazil nuts; and seeds
Monounsaturated fat	–	–	–	–	–	Margarine spreads such as canola- or olive oil-based choices; oils such as olive, canola, and peanut oils; avocado; nuts such as peanuts, hazelnuts, cashews, and almonds
Carbohydrate	45–65% E	55–75% E	55–75% E	50–70% E	45–65% E	Whole grain breads and cereals, fruits, vegetables, milk and milk products, legumes
Fiber	28 g/day*	>25 g/day*	>25 g/day*	21 g/day*	28 g/day*	Whole grain foods, fruits, and vegetables

WHO, World Health Organization; E, energy; g/day, grams per day; *, adequate intake; –, no dietary reference values derived

[a]Macronutrient values expressed as a percentage of total energy intake, unless otherwise specified.

[b]Macronutrient recommendations derived from the Institute of Medicine (2005). Dietary reference intakes for energy, carbohydrate, fibre, fat, fatty acids, cholesterol, protein and amino acids. Washington, DC: National Academies Press; World Health Organization (2003). Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases. WHO Technical Report Series, 916. Geneva; Sasaki, S. (2008). Dietary reference intakes (DRIs) in Japan. *Asia Pacific Journal of Clinical Nutrition* **17**: 420–444; National Health and Medical Research Council (2006). Nutrient reference values for Australia and New Zealand. Canberra: Commonwealth of Australia.

[c]Energy values expressed as the additional estimated energy requirement. 1 cal = ~4.2 kJ.

those nutrients. Each dietary guideline contains nutrient intake values suitable for comparison with group population intakes, which are referred to as an estimated average requirement (EAR), an AI, or an acceptable range of intake (ARI). The EAR is the daily nutrient level estimated to meet the requirements of half of the healthy individuals in a particular life stage and is used to estimate the prevalence of inadequate intakes in groups. The proportion below the EAR provides an approximation of the prevalence of inadequacy. When an EAR could not be set, either an AI or an ARI, which is the average daily nutrient intake level or the range of intakes that are assumed to be adequate, respectively, is used instead. The distribution of nutritional requirements within the population is illustrated in Figure 1.

Specific micronutrients are required during different periods of pregnancy for optimal fetal development, which influences fetal metabolism, organ growth, development, and function. The systematic review by Blumfield and colleagues showed that in high-income countries (the United States, Canada, the United Kingdom, Europe, Australia, New Zealand, and Japan), pregnant women consistently report intakes of folate, iron, and vitamin D that are below national recommendations. Thus, these nutrients require particular attention prior to conception to build up maternal nutrient

Table 2 Daily dietary recommendations for micronutrient intakes during pregnancy by geographic region[a,b] and the food sources providing those nutrients

	Recommendations					
Nutrients	*The United States/ Canada*	*The United Kingdom*	*Europe/ WHO*	*Japan*	*Australia/ NZ*	*Food sources*
Vitamin A (μg RE)	550	500	500	450	550	Liver, dairy products, fish, palm oil, dark green vegetables/ fruits, orange vegetables/fruits, yellow vegetables/fruits, and red vegetables/fruits
Thiamin (mg)	1.2	0.3/1000 kcal	1.0	1.1	1.2	Yeast extract, pork, fortified breakfast cereals, enriched grain products, and meatless soy products
Riboflavin (mg)	1.2	1.2	1.4	1.3	1.2	Yeast extract, milk and dairy products, cornflakes, fermented soy products
Niacin (mg NE)	14.0	5.5/1000 kcal	14.0	12.0	14.0	Yeast extract, meat, fish, poultry, pumpkin seeds, peanuts, fermented soy products
Pantothenic acid (mg)	6[c]	–	3–12	6[c]	5.0[c]	Chicken, beef, potatoes, oat cereals, tomato products, yeast extract, egg yolk, broccoli, and whole grains
Vitamin B_6 (mg)	1.6	13 μg g^{-1} protein	13 μg g^{-1} protein	1.7	1.6	Yeast extract, meat, fish, poultry, organ meats, enriched cereals, meatless soy products, nuts, lentils, bananas, potatoes
Vitamin B_{12} (μg)	2.2	1.25	1.2	2.3	2.2	Eggs, milk, cheese, milk products, meat, fish, shellfish, poultry
Folate (μg)	520	250	400	370	520	Green leafy vegetables; dried legumes such as chickpeas, beans, and lentils; baby soybeans; oranges; yeast extract; and enriched breads and cereals
Vitamin C (mg)	70	35	40	95	40	Fruits and vegetables. Especially red and yellow peppers, broccoli, cabbage, Brussels sprouts, guava, papaya, kiwifruit, oranges, lemon, strawberries
Vitamin D (μg)	10.0	10.0	0–10.0[c]	7.5	5.0[c]	Oily fish (such as salmon, mackerel, and sardines), eggs, and vitamin D-fortified foods (e.g., some breakfast cereals, margarine, and milk)
Vitamin E (mg)	15	3[c]	–	8[c]	7[c]	Wheat germ oil, polyunsaturated vegetable oils, peanut oil, olive oil, nuts, seeds, whole grains
Vitamin K (μg)	90[c]	–	–	60[c]	60[c]	Spinach, soybeans, cabbage, wheat bran, green beans
Biotin (μg)	30[c]	–	15–100[c]	47[c]	30[c]	Yeast extract, egg yolk, soybeans, bread, fish, milk
Calcium (mg)	800	525	550	700	840	Milk and milk alternatives, collards, spinach, turnip greens, kale, canned fish with bones (e.g., salmon, sardines, and anchovies), tahina, baked beans, almonds, enriched foods (e.g., orange juice and tofu)
Chromium (μg)	30[c]	–	–	25[c]	30[c]	Cereals, bran products, fruits, and vegetables
Copper (mg)	0.8	1.2[c]	0.8	0.6	1.3[c]	Organ meats, seafood, nuts, seeds, whole grains, and cocoa
Iodine (μg)	160	140[c]	100	170	160	Fish, shellfish, iodized table salt, processed foods containing iodized salt (e.g., bread)
Iron (mg)	22.0	11.4	19.7[d]	16.5	22.0	Meat; fish; poultry; eggs; dried beans, peas, lentils; green vegetables; tomato puree; asparagus; and enriched breads and cereals
Magnesium (mg)	290	200	150–500[c]	260	290	Legumes, spinach, Swiss chard, soy cheese, nuts, seeds, fish, whole grains
Manganese (mg)	2.0[c]	–	1–10[c]	3.5[c]	5.0[c]	Grain products, legumes, nuts, vegetables, fruits, and brewed tea
Phosphorus (mg)	580	525	400	900[c]	580	Milk, milk products, meat, poultry, seafood, meat alternatives, beans, lentils, nuts, whole grains, mushrooms, baby soybeans, potatoes
Potassium (mg)	4700[c]	3500[c]	1600–3100[c]	1600[c]	2800[c]	Bananas; papaya; sweet potato; dark green leafy vegetables; avocado; milk; yogurt; dried beans, chickpeas, lentils; beef; pork; fish; nuts; seeds
Selenium (μg)	49	60[c]	40	24	55	Brazil nuts, mixed nuts, eggs, canned tuna, oysters, salmon, pork, poultry

(*Continued*)

Table 2 (Continued)

Nutrients	Recommendations: The United States/ Canada	The United Kingdom	Europe/ WHO	Japan	Australia/ NZ	Food sources
Sodium (mg)	1500[c]	1600[c]	575–3500[c]	600	460–920[c]	Avoid foods high in sodium. Table salt, processed meats, canned foods, cheese, bread, cereals, sauces, pickled foods, commercial rice or pasta mixes, condiments
Zinc (mg)	9.5	5.5	5.5	6.0	9.0	Yogurt, milk, cheese, dried beans. Soybeans, lentils, pumpkin seeds, sunflower seeds, meat, poultry, fish, and seafood

[a]Micronutrient values expressed as reference nutrient intakes, unless stated otherwise.
[b]Micronutrient recommendations derived from the Institute of Medicine (1997). Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D and fluoride. Washington, DC: National Academy Press; Institute of Medicine (1998). Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B12, pantothenic acid, biotin and choline. Washington, DC: National Academy Press; Institute of Medicine (2000). Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids. Washington, DC: National Academy Press; Institute of Medicine (2001). Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, magnesium, molybdenum, nickel, silicon, vanadium and zinc. Washington, DC: National Academy Press; Institute of Medicine (2005). Dietary reference intakes for water, potassium, sodium, chloride and sulphate. Washington, DC: National Academy Press; Institute of Medicine (2011). Dietary reference intakes for calcium and vitamin D. Washington, DC: National Academy Press. Department of Health (1991). Dietary reference values for food energy and nutrients in the United Kingdom. HMSO: London. Scientific Committee for Food (1993). Reports of the scientific committee for food, thirty-first series. Luxembourg: Commission of the European Communities. National Health and Medical Research Council (2006). Nutrient reference values for Australia and New Zealand. Canberra: Commonwealth of Australia; Sasaki, S. (2008). Dietary reference intakes (DRIs) in Japan. *Asia Pacific Journal of Clinical Nutrition* **17**: 420–444.
[c]Adequate intake or acceptable range of intake. $\mu g\ g^{-1}$, micrograms per gram; –, no dietary reference values derived.
[d]Based on 9.7 mg/day for adult women plus 10 mg/day increase in the 3rd trimester.

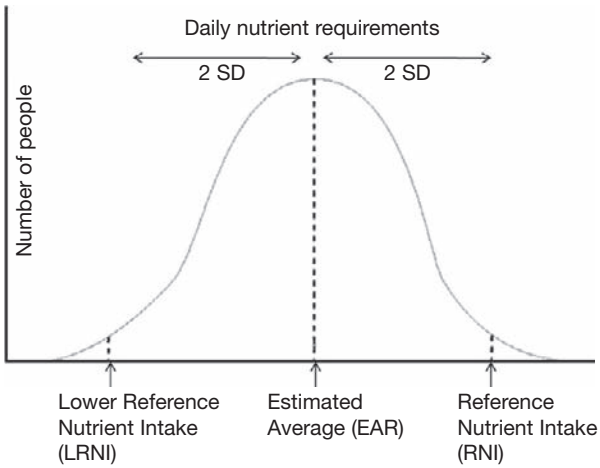


Figure 1 The distribution of nutritional requirements within the population.

stores and during pregnancy to ensure women consume sufficient amounts.

Alcohol

Alcohol consumption during pregnancy can lead to miscarriage, stillbirth, premature birth, and/or fetal alcohol syndrome. Currently, there is no safe level of alcohol consumption during pregnancy identified. The National Institute for Health and Clinical Excellence (NICE) advises that pregnant women and women trying to conceive a baby should avoid alcohol altogether, especially in the first 3 months of pregnancy.

Table 3 Caffeine intake during pregnancy

The amount of caffeine found in some foods and drinks is as follows:
- One mug of instant coffee: 100 mg
- One mug of filter coffee: 140 mg
- One mug of tea: 75 mg
- One can of cola: 40 mg
- One can of energy drink: up to 80 mg
- One 50 g bar of plain chocolate: up to 50 mg
- One 50 g bar of milk chocolate: up to 25 mg

So caution is needed. For example, in one day, a woman will reach the 200 mg limit of caffeine if she has one of the following:
- One bar of plain chocolate and one mug of filter coffee
- Two mugs of tea and one can of cola
- One mug of instant coffee and one can of energy drink

Caffeine

Caffeine is present in tea, coffee, chocolate, cola drinks, energy drinks, and many over-the-counter remedies for colds and flu. Research by the CARE Study Group in 2008 suggests that small amounts of caffeine (up to 100 mg/day) are safe during pregnancy, but high levels (greater than 200 mg/day) increase the risk of miscarriage, premature birth, and low-birth-weight babies. The Food Standards Agency (FSA) recommends that pregnant and breastfeeding women limit their caffeine intake to 200 mg/day (Table 3).

Dietary Supplements

Women trying to conceive are advised by the NICE guidelines to supplement their dietary intake with 400 μg of folic acid daily 1 month before pregnancy and throughout the first trimester to prevent neural tube defects (NTD), which include anencephaly, spina bifida, and myelomeningocele. If there is a

family history of NTD, or the woman has previously had a baby with an NTD or has diabetes, then 5 mg of folic acid daily is recommended. Vitamin D supplements (10 μg/day) are recommended to support optimal fetal bone development and prevention of rickets during childhood. Women with dark skin or who always cover their skin are at particular risk of vitamin D deficiency.

The World Health Organization and other international government authorities (the United States, Australia, and New Zealand) recommend pregnant and lactating women consume a daily iodine supplement of 150 μg. Evidence from the Avon Longitudinal Study of Parents and Children (ALSPAC) suggests that pregnant women in the United Kingdom are at risk of mild–moderate iodine deficiency, which increases the risk of poor neurodevelopmental outcomes in children. Iodine deficiency should be treated as an important public health issue and iodine supplementation recommended for pregnant women. However, for pregnant women with preexisting thyroid disease, it should be managed individually to ensure normal thyroid function.

There is currently not enough evidence to support the recommendation of other supplements routinely during pregnancy, including multivitamin supplements. Multivitamin supplements may be recommended when there is a fairly high chance of not meeting nutrient needs from food. This is more likely for pregnant adolescents; vegetarians; those on preexisting special diets; individuals with drug, tobacco, and alcohol addiction; or obese pregnant women on medically restricted diets to limit weight gain. Pregnant women are at particularly high risk of low iron levels and iron-deficiency anemia. Thus, iron status needs to be regularly monitored and supplements commenced in cases of deficiency. Importantly, supplements containing vitamin A should be avoided during pregnancy, as they are teratogenic in the immediate postconception period. If inadequate nutrient intake is suspected or nutrient deficiency present, referral to a dietician is recommended for assessment and advice.

Food Recommendations during Pregnancy

Since individuals consume whole foods not just individual nutrients, food recommendations are often provided in a food selection guide to accompany dietary recommendations. A food selection guide is an educational and promotional tool, which converts scientific knowledge of food composition and nutritional requirements for health into a practical guide for food selection.

In the United Kingdom, the eatwell plate can assist individuals to choose a healthy, balanced diet by encouraging daily consumption of a wide variety of foods from each of the four core food groups (Figure 2). The core food groups include (1) bread, rice, potatoes, and other starchy foods (33% dietary intake); (2) fruit and vegetables (33% dietary intake); (3) milk and dairy foods (15% dietary intake); and (4) meat, fish, eggs, beans, and other nondairy sources of protein (12% dietary intake). A noncore, fifth food group contains foods and drinks that are high in fat and/or sugar (8% dietary intake).

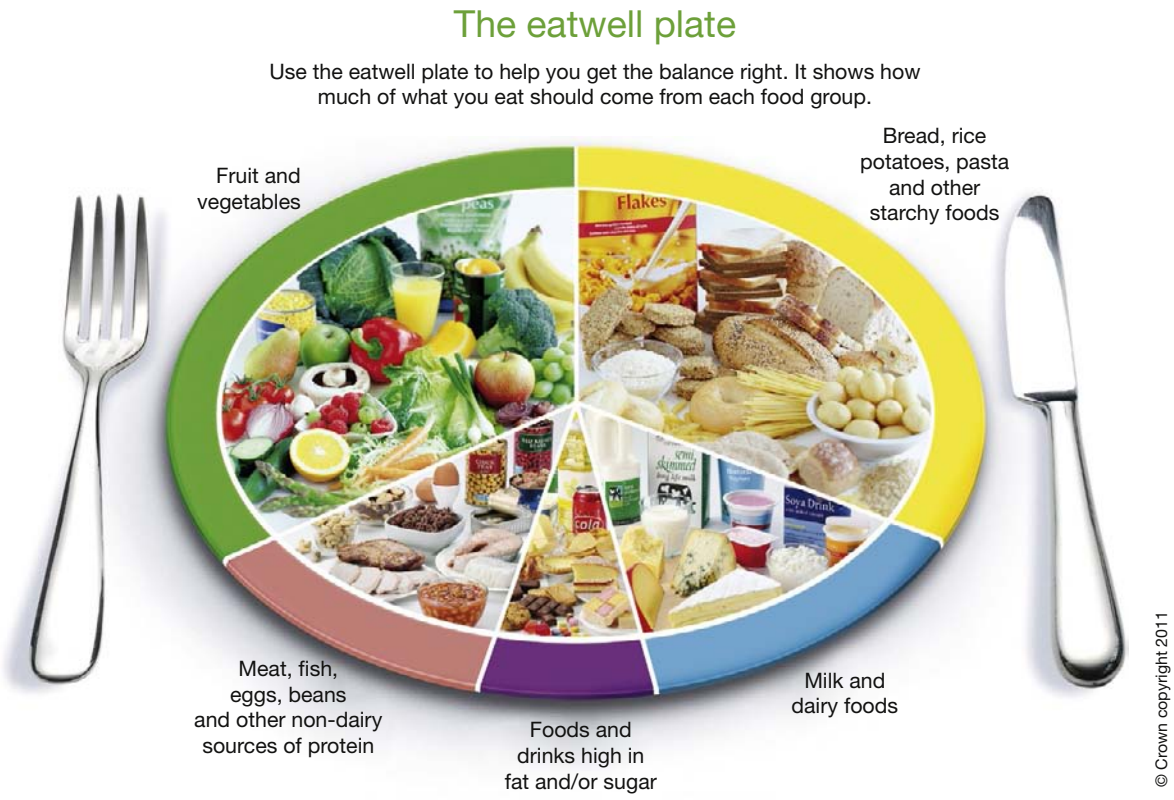


Figure 2 The eatwell plate.

Foods/drinks within this food group are not essential to a healthy diet and are only recommended in limited amounts due to being high in calories (energy) but low in nutrients. The eatwell plate is a visual tool that illustrates the proportion of total dietary intake that should come from each food group, including snacks. However, it does not include recommendations for number of daily servings or portion sizes, except for fruit, vegetables, and fish (see Figure 2).

In 2011, the US Department of Agriculture (USDA) and the US Department of Health and Human Services created MyPlate to provide food guidance to Americans, including individualized recommendations for pregnant women (see Figure 3). MyPlate is based on the 2010 Dietary Guidelines for Americans and illustrates five food groups that include grains, fruits, vegetables, protein, and dairy. The MyPlate resources are available in multiple languages that include English, Spanish, Chinese (simplified and traditional), Filipino, Hindi, Indonesian, Japanese, Korean, Malay, Pashto, Thai, Urdu, and Vietnamese. Promotion materials such as cookbooks are also available to translate recommendations to differing cultural eating patterns (www.choosemyplate.gov/).

During pregnancy, women are advised to follow a healthy diet that includes a large variety of foods from each food group. A similar illustrated food selection guide, adapted to the food habits and choices of other countries in the north and south of the planet, would benefit gestational nutrition worldwide. If individualized nutritional advice is required or women have specific issues that affect their eating habits or nutritional status, then consultation with a registered dietician is recommended.

Eating for Two during Pregnancy

Women do not need to 'eat for two' during pregnancy. While a woman's nutrient needs are higher during pregnancy, total energy requirements are only slightly higher (Table 1). Therefore, to meet the high nutrient demands of pregnancy, nutrient-dense foods need to be chosen from the five food groups (see Figure 2) to ensure excess calories are not consumed. Total energy requirements during pregnancy are dependent on many factors including physical activity. While energy is required for fetal growth and maternal fat deposition throughout pregnancy, women compensate for these additional demands by slowing down, becoming less active, and becoming more metabolically efficient. The best way to determine whether a pregnant woman is consuming an adequate energy intake is to monitor her weight gain and/or have her dietary intake assessed by a registered dietician.

Whole Foods versus Supplements

Nutritional requirements can be met through consumption of whole foods or whole foods plus supplementation. A recent review of nutrition research and practice has found that health benefits may be stronger when nutrients are consumed in a synergistic dietary pattern rather than in their purified form. The theoretical framework surrounding this concept has been termed 'food synergy.' Food synergy is based on the notion that the interrelations between constituents in foods are significant. This significance is dependent on the balance of constituents in food, the ability of the constituents to survive digestion, and their bioavailability at a cellular level. Different effects are likely to be evident between constituents in foods taken directly from their biological environment and those formulated through technological processing.

While supplementation may be beneficial in states of insufficiency, each nutrient has an asymmetrical U-shaped curve characterizing deficiency, safe range, and toxicity, which vary by individual and life stage. The risk of harm depends on the safe intake range of the nutrient, the susceptibility of the individual, and the absorption from alternative forms of dietary intake such as other supplements and whole foods. The UK dietary guidelines advocate that nutritional needs during pregnancy are to be met primarily through consumption of nutrient-rich foods within a varied diet. Supplements are not a food substitute, but may be useful when adverse health outcomes can be prevented or treated by a therapeutic nutrient dose when it is unable to be obtained through dietary intake alone. Therefore, to promote optimal maternal and fetal health during pregnancy, it is recommended that foods be considered first rather than supplements to address nutritional requirements. If nutrient needs are unable to be met through food intake alone, as is often the case with iron, supplements should be considered to ensure AIs are achieved.

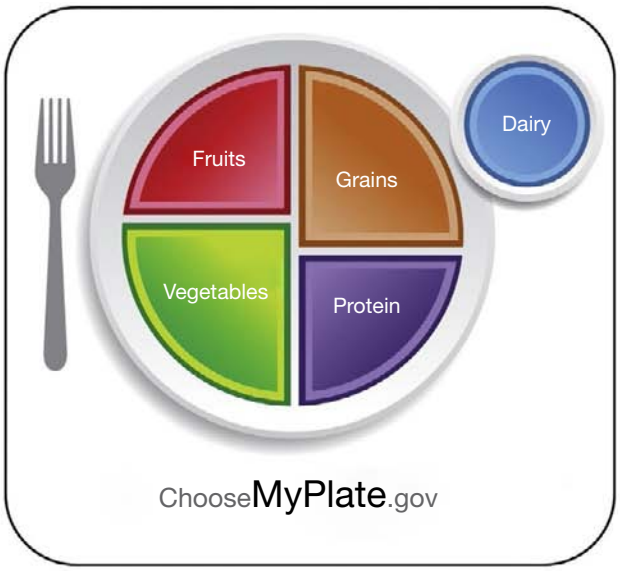
Food Safety during Pregnancy

There are some foodborne illnesses that cause adverse outcomes or harm the fetus during pregnancy. Pregnant women need to be especially careful to protect themselves and their unborn fetuses from listeriosis, salmonellosis, and toxoplasmosis. During pregnancy, women are more susceptible to these infections due to some suppression in their immune function. Additional awareness surrounding mercury is also required to ensure safe fish consumption. However, there is much confusion among the public and health professionals about which foods are safe to eat during pregnancy.

Listeriosis

Listeria monocytogenes is a microorganism found widely in the environment and can cause foodborne illness in pregnant women if ingested in a large dose. While the actual clinical burden of listeriosis is low, the consequences of infection are severe. Listeriosis during pregnancy can cause miscarriage, premature birth, neonatal infection, and/or fetal death following transmission of the organism across the placenta. *Listeria* is managed by hygienic preparation, storage, and handling of food. To reduce the risk of listeriosis during pregnancy, it is recommended that pregnant women wash their hands carefully and frequently and avoid specific 'high-risk' foods such as soft cheeses, cold meats, seafood, unpasteurized dairy products, and preprepared fruit and vegetables while consuming safer alternatives. Recommendations are summarized in Table 4.

However, the perceived risk of *Listeria* infection in pregnancy for many women appears to be much greater than the actual risk. This leads some women to severely restrict all



Food Group	1st Trimester	2nd and 3rd Trimester	What counts as 1 cup or 1 ounce?	Remenber to...
	Eat this amount from each group daily.*			
Fruits	2 cups	2 cups	1 cup fruit or juice ½ cup dried fruit	*Focus on fruits* — Eat a variety of fruits.
Vegetables	2 ½ cups	3 cups	1 cup raw or cooked vegetables or juice 2 cups raw leafy vegetables	*Vary your veggies* — Eat more dark-green and orange vegetables and cooked dry beans.
Grains	6 ounces	8 ounces	1 slice bread 1 ounce ready-to-eat cereal ½ cup cooked pasta, rice, or cereal	*Make half your grains whole* — Choose whole instead of refined grains.
Meat &	5 ½ ounces	6 ½ ounces	1 ounce lean meat, poultry, or fish ¼ cup cooked dry beans ½ ounce nuts or 1 egg 1 tablespoon peanut	*Go lean with protein* — Choose low-fat or lean meats and poultry.
Milk	3 cups	3 cups	1 cup milk 8 ounces yogurt 1½ ounces cheese 2 ounces processed cheese	*Get your calcium-rich foods* — Go low-fat or fat-free when you choose milk, yogurt, and cheese.

*These amounts are for an average pregnant women. You may need more or less than the average. Check with your doctor to make sure you are gaining weight as you should.

Figure 3 MyPlate food recommendations for pregnancy, published by the United States Department of Agriculture.

potential food sources of *L. monocytogenes*, which has implications for diet quality and nutrient intakes. The avoidance of foods potentially containing *L. monocytogenes* has been associated with lower nutrient intakes during pregnancy. This presents a challenging issue for public health authorities and pregnant women. Therefore, pregnant women must be encouraged to consume safer alternatives of high-risk foods to ensure nutritional adequacy is not compromised.

Table 4 Listeriosis during pregnancy. Examples of higher-risk foods and safer alternatives

Food type	*Higher-risk examples*	*Safer alternatives*
Cold meats	Unpackaged ready-to-eat from delicatessen counters, sandwich bars, etc. Packaged, sliced ready-to-eat	Home cooked meats. Store in fridge and use within a day of cooking
Chicken	Purchased (whole, portions, or diced) ready-to-eat	Home cooked chicken. Ensure chicken is cooked thoroughly, use immediately. Store any leftovers in the fridge and use within a day of cooking Hot takeaway chicken (whole or portions). Use immediately or store any leftovers in fridge and use within a day of purchase
Pate	Refrigerated pate or meat spreads	Avoid. No safe alternative
Salads (fruit and vegetables)	Preprepared or prepackaged salads from salad bars, smorgasbords, etc.	Homemade, freshly prepared salads. Wash all vegetables and fruits thoroughly. Store any leftover prepared salads in fridge, and use within a day of preparation
Chilled seafood	Raw (e.g., oysters, sashimi, or sushi), smoked ready-to-eat. Ready-to-eat peeled prawns (cooked) in prawn cocktails, sandwich fillings, prawn salads, etc.	All freshly cooked seafood. Use immediately. Store any leftovers in fridge and use within a day of cooking
Cheese	Soft, semisoft, and surface ripened cheeses – prepackaged and from delicatessen (e.g., brie, camembert, ricotta, feta, and blue)	Hard cheese (e.g., cheddar and tasty), processed cheese, cheese spreads, plain cream cheese, plain cottage cheese. Store in the fridge. Purchase cheeses packaged by the manufacturer
Ice cream	Soft serve	Packaged frozen ice cream. Maintain the ice cream frozen
Other dairy products	Unpasteurized dairy products (e.g., raw goats milk)	Pasteurized dairy products (e.g., yogurt, pasteurized milk, custard, and dairy dessert). Store in the fridge
Canned and similarly packaged foods	None	All. Store unused portion in the fridge in clean, sealed containers and use within a day

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Table 5 Tips for safe food handling during pregnancy to prevent foodborne illness

- Always wash and dry hands thoroughly before and after handling food to prevent the spread of bacteria
- Keep cold food below 5 °C in the fridge and hot food above 60 °C (steaming) before serving. This will stop the growth of food poisoning bacteria
- Keep raw and cooked food separate. Do not use the same utensils, especially cutting boards and knives, for both
- Defrost frozen food in the fridge or microwave – never on the bench or in the sink
- Cook foods thoroughly. Poultry and meats should be well done with no pink visible and juices should be clear
- Avoid foods past their 'best before date' or 'use by' date

Salmonellosis

Salmonella is a common cause of food poisoning. Pregnant women are not at an increased risk of contracting salmonellosis, but severe cases can trigger intrauterine sepsis, miscarriage, or premature labor. To reduce the risk of salmonellosis, pregnant women should avoid foods containing raw egg, ensure animal products (meat, chicken, and eggs) are cooked thoroughly, and adopt good hygiene practices. Table 5 provides some tips for safe food handling to prevent foodborne illnesses.

Toxoplasmosis

Toxoplasma gondii is an organism that can be found in unpasteurized milk, undercooked meat, and unwashed fruit and vegetables and most commonly in cat and dog feces. Pregnant women have an increased susceptibility to *Toxoplasma gondii* infection due to alterations in cell-mediated immunity that occur during gestation. Toxoplasmosis during pregnancy is often asymptomatic and can lead to fetal brain damage and/or blindness. Therefore, pregnant women should wear gloves when gardening, wash hands after touching animals, not handle cat litter, and keep pets out of food preparation areas. Further tips to avoid toxoplasmosis include consumption of pasteurized dairy products, well-cooked meat, and freshly washed fruit and vegetables.

Mercury and Other Contaminants in Fish

Fish and seafood can be an important part of a healthy pregnancy diet. Fish is rich in protein and minerals, low in saturated fat, and a major source of healthy omega-3 fatty acids. During pregnancy, omega-3 fatty acids play an important role in the developing fetal central nervous system and are building blocks for the brain and retina. Research shows that maternal omega-3 fatty acid consumption during pregnancy (diet and/or supplements) is associated with improved neurodevelopmental outcomes in the child, while animal studies suggest deprivation of omega-3 fatty acids during pregnancy is associated with irreversible visual and behavioral deficits in offspring. Population surveys indicate that pregnant women do not consume adequate amounts of omega-3 fatty acids and eat minimal fish, in part due to fears surrounding adverse effects of mercury and other harmful toxins (e.g., polychlorinated biphenyls).

Table 6 Guidelines for how pregnant women can safely include fish in their diet

1 Serve equals 140 g
2 Serves per week of any fish and seafood not listed below
OR
4 Medium cans per week of tuna (140 g each) and 2 serves per week of oily fish
OR
1 Serve per week of orange roughy (deep-sea perch) or catfish and no other fish that week
AND
Pregnant women should not eat shark (flake) or billfish (swordfish, broadbill, and marlin)

Mercury occurs naturally in the environment and accumulates in fish as methylmercury. Fish absorb the methylmercury as they feed on other fish, with levels dependent on the amount of fish eaten and the levels of mercury in those fish. Processing techniques such as canning, freezing, and cooking do not reduce the mercury content in fish. Table 6 presents guidelines for how pregnant women can safely include fish in their diet to ensure they consume the necessary important nutrients to optimize fetal development.

Barriers to Nutritional Adequacy

Three reviews have evaluated the effectiveness of nutrition education interventions to promote healthy eating in pregnancy. The reviews report that pregnant women appear motivated to improve dietary intakes and make lifestyle changes in line with recommendations when provided with education. However, pregnant women commonly described barriers to making changes to achieve nutritional adequacy that included understanding the importance of good nutrient intakes, limited nutrition knowledge, anxiety with avoiding the risk of *Listeria* infection, quality of experiences with health professionals, and physical health. In focus group sessions conducted in Australia, pregnant women were aware that a healthy diet was important but were unsure what this meant in practical terms during this life stage. There was uncertainty surrounding food serving sizes. Many women believed the importance of maternal diet was secondary, as fetal nutritional needs would be met irrespective of maternal dietary intake. Pregnant women reported knowledge of the consequences of listeriosis but found it difficult to get consistent information, had difficulty making suitable food choices, and ultimately experienced a large amount of guilt and responsibility attached to making possible 'wrong' food choices. General practitioners were viewed as having an important role in distributing quality nutrition information. However, the quantity and quality of the nutrition information received were highly variable, and printed material often lacked the practical information required to select and achieve a healthy diet. Lastly, food cravings and conditions such as hyperemesis gravidarum, constipation, and heartburn also influenced dietary intake during pregnancy.

Common Nutrition-Related Concerns of Pregnancy

Nausea and vomiting, constipation, heartburn, and food sensitivities are common nutrition-related symptoms in pregnancy. Dietary strategies to minimize these maternal discomforts are listed in Table 7.

Nausea and Vomiting

Nausea and vomiting (often called morning sickness) is experienced by most pregnant women and can range in intensity from slight to severe. While symptoms are more common in the morning and early stages of pregnancy (4–16 weeks gestation), they can occur at any time of the day or night and at any stage of pregnancy. Possible causes include high levels of hormones (including estrogen), fluctuations in blood pressure (particularly lowered blood pressure), and altered carbohydrate metabolism. Severe vomiting during pregnancy or hyperemesis gravidarum can cause weight loss and dehydration, and women may require hospitalization, intravenous fluids, and parenteral nutrition. Health professionals are urged to reassure women that nausea and vomiting during pregnancy will have no adverse effect on their developing fetus. Strategies to minimize nausea and vomiting during pregnancy are listed in Table 7. Although some women may benefit most from simply consuming the foods they enjoy when they feel like eating. Comfort may also be found in more temperate environments.

Heartburn

Gastroesophageal reflux disease, or heartburn, occurs in 40–85% of pregnant women. Similar to nonpregnant women, reflux during pregnancy is due to a decrease in lower esophageal sphincter pressure (caused by estrogen and progesterone changes) and/or an increase in intra-abdominal pressure (caused by the growing fetus). This combination enables the stomach acid to back up into the lower esophagus and creates a burning sensation near the heart. Although heartburn is occasionally experienced in the first trimester, it is more common in the third trimester. Ways to help relieve heartburn are included in Table 7.

Constipation and Hemorrhoids

Constipation can develop or increase in severity during pregnancy, with prevalence reported as 11–38%. Constipation during pregnancy has been linked to rising hormone levels (both progesterone and estrogen) relaxing the muscles of the bowel, low fluid and fiber intakes, parity, and previous cesarean delivery. Women usually prone to constipation are more likely to become constipated during pregnancy and may further develop hemorrhoids (swollen veins of the rectum). Hemorrhoids can be painful and straining during bowel movements may cause bleeding. Pregnancy-induced constipation is usually mild and can be self-treated with a mix of high-fiber foods, including soluble (oats, lentils, dried peas and beans, and psyllium) and insoluble (whole wheat and whole grain breads and cereals, wheat bran, vegetables, and fruit) fibers or nonprescription methods (Table 7).

Table 7 Institute of Medicine weight gain recommendations for pregnancy[a]

Prepregnancy weight category	*Body mass index*[b]	*Total weight gain range (kg)*	*Rates of weight gain*[c] *in the 2nd and 3rd Trimester (mean range in kg/week)*
Underweight	<18.5	12.5–18.0	0.5 (0.5–0.6)
Normal weight	18.5–24.9	11.5–16.0	0.5 (0.2–0.5)
Overweight	25.0–29.9	7.0–11.5	0.3 (0.2–0.3)
Obese (includes all classes)	≥30.0	5.0–9.0	0.2 (0.2–0.3)

[a]Rasmussen, K. M., and Yaktine, A. L. (eds.). (2009). Weight gain during pregnancy: re-examining the guidelines. Washington DC: Institute of Medicine, National Academy of Sciences.
[b]Body mass index is calculated as weight in kilograms divided by height in meters squared or as weight in pounds multiplied by 703 divided by height in inches.
[c]Calculations assume a 0.5–2 kg (1.1–4.4 lbs) weight gain in the first trimester (based on Siega-Riz et al., 1994; Abrams et al., 1995; Carmichael et al., 1997).

Food Cravings and Aversions

Pregnant women often experience food cravings and aversions, with worldwide prevalence reported between 50% and 90%. The most common cravings include dairy and sweet foods such as fruit and fruit juices, sweet desserts, and chocolate, whereas the most common aversions are to alcohol, caffeinated drinks, meats, fish, poultry, and eggs. These behaviors are particularly prominent during the first and third trimesters of pregnancy.

Despite significant research in the field, the etiology of these behaviors remains inconclusive. It is likely that the cause for food cravings and aversions is multifactorial and involves a combination of biological, psychological, and environmental factors, as opposed to purely reflecting physiological needs. Therefore, common cravings of ice cream during pregnancy are unlikely to signify a calcium deficiency. Importantly, not all food cravings and aversions contribute to a nutritious diet. Some women develop cravings for nonfood items such as detergents, clay, or soil, which is known as pica. Pica is particularly common in African American women and is often associated with iron-deficiency anemia. However, whether iron deficiency leads to pica or pica leads to iron deficiency remains unclear. Additionally, women often report aversions for meats and eggs, which could lead to a reduced protein intake, a key macronutrient for fetal growth and development. Knowledge of a pregnant woman's total dietary intake is required before the nutritional effect of food cravings or aversions can be assessed. In general, most food cravings increase calcium and energy intakes, while food aversions decrease intakes of alcohol, caffeine, and animal protein. Generally, these behaviors do not have a deleterious effect on diet quality. However, repeated intakes of energy-dense foods may promote excessive weight gain during pregnancy and increase the risk of pregnancy complications.

Weight Gain during Pregnancy

Weight gain during pregnancy plays an important role in the growth and development of the fetus, placenta, amniotic fluid, maternal tissues (uterus and breasts), blood supply, extracellular fluid, and maternal fat stores. Sufficient maternal weight gain is essential to prevent low-birth-weight babies, a significant determinant of neonatal mortality, infant/childhood morbidity, and child's risk of chronic disease in later life. The World Health Organization states that maternal weight gain between 10 and 14 kg during pregnancy is associated with optimal birth weight outcome (between 3.1 and 3.6 kg) and will reduce the risk of fetal and maternal complications.

However, weight gain recommendations for women during pregnancy are largely dependent on prepregnancy weight. Underweight women are encouraged to gain more weight and overweight women may need to put on less weight. Importantly, excessive weight gain should be avoided. Excessive weight gain during pregnancy increases the risk of complications such as gestational diabetes, pregnancy hypertension, preeclampsia, and congenital defects. It also increases the risk of birth complications including induced labor, instrumental delivery, shoulder dystocia leading to obstructed labor, cesarean delivery, and postpartum hemorrhage. Weight loss during pregnancy is not recommended as the relationship between weight change during pregnancy and fetal outcomes is not fully elucidated and sufficient dietary consumption is required for optimal fetal growth and development. Therefore, women should ideally commence pregnancy at a healthy body weight (body mass index (BMI), 18.5–24.9 kg m^{-2}). The Institute of Medicine weight gain recommendations for pregnancy are presented in Table 8.

Ideally, women with a BMI over 30 would be encouraged to lose weight before pregnancy and need to be informed about the increased risks to themselves and their babies. If already pregnant, then encourage moderate weight gain only, with weight loss delayed until after pregnancy. Refer pregnant obese women (BMI ≥ 30) to a dietician for assessment and individualized dietary advice. It is important to monitor weight gain during pregnancy by weighing women regularly.

Fetal Origins of Noncommunicable Diseases

The rapid worldwide shift towards a population with a greater obesogenic phenotype suggests it may be driven by environmental factors as opposed to genetic contributions. Although current lifestyle factors play a vital role in the progression of

Table 8 Dietary strategies to minimize common maternal discomforts during pregnancy

To minimize nausea:	*To prevent/minimize constipation*:	*To prevent/minimize heartburn*:
Eat small, frequent meals (i.e., every 2 h). An empty stomach can cause nausea	Eat a variety of high-fiber foods (fruits, vegetables, whole grain cereals, legumes, nuts, and seeds)	Eat small, frequent nutritious meals (avoid large meals)
Avoid foods with offensive odors	Try a mix of brans including wheat bran, oat bran, and psyllium. Aim for 1–2 tablespoons/day	Drink between meals rather than with meals
Avoid large drinks. Have frequent small drinks, preferably between meals to avoid dehydration	Aim for a fiber intake between 25 and 28 g/day	Avoid high-fat foods, spicy foods, and caffeine
Limit fatty, spicy, and fried foods	Drink plenty of fluids (preferably water)	Eat slowly and chew food thoroughly
Have someone else prepare meals or prepare food at times of the day when you feel better	Aim for fluid intake between 1.5 and 2.0 l/day	Always sit down to eat.
Eat dry toast or plain crackers on waking and arise slowly	Exercise regularly	Avoid eating on the run
Eat a healthy snack before bed at night	Respond promptly to the urge to defecate	Wait 1–2 h after finishing a meal before lying down or exercising
Drink ginger root tea, but limit intake to no more than 3 g of raw ginger per day (approx. three teaspoons)	If laxatives are required, only use those prescribed by your doctor. Not all laxatives are safe during pregnancy. Only occasional/short-term use is advised to avoid dehydration, electrolyte imbalances, and dependency	Elevate the head with a couple of pillows while sleeping
	Oral iron supplements can sometimes cause constipation. If you suspect this may be causing constipation, consult your doctor	Avoid wearing tight clothing

adverse health outcomes, mounting evidence indicates that early life environmental factors such as maternal nutrition during pregnancy can program the health outcomes of an individual in later life. Presently, the maternal nutritional insults that have been found to program a range of adverse health outcomes in later life are overnutrition, hyperglycemia, and undernutrition of energy, protein, vitamins, or iron. Specific evidence indicates that the intrauterine environment can program a child's body composition and metabolic-endocrine axes. This has implications for the susceptibility and adaptability of the child to future environmental conditions. Therefore, maternal insults that influence the intrauterine environment have the potential to play an extremely important role in the prevention of noncommunicable diseases.

Maternal Undernutrition

Maternal undernutrition was first investigated in pregnant women exposed to the Dutch famine during World War II in 1944–45. Retrospective human data showed that famine during pregnancy had adverse effects on adult health, and outcomes were dependent on the gestational period in which the dietary restriction occurred. Exposure to undernutrition in the first trimester produced children with an increased susceptibility to coronary heart disease compared with those exposed in other trimesters. Undernutrition in the second trimester was associated with an increased incidence of renal disease, while exposure in the third trimester was associated with reduced birth weight and decreased glucose tolerance. These differential effects align with the chronological development of the fetus. Cardiovascular growth takes place during early gestation, kidney development during midgestation, and adipose and muscle tissue growth during late gestation.

David Barker and colleagues reported an association between lower birth weight and increased risk of cardiovascular disease, stroke, hypertension, glucose intolerance, and T2DM. It was concluded that the fetal environment played a critical role in susceptibility of cardiometabolic disease. Rodent studies have used overall calorie restriction to model maternal undernutrition during pregnancy. Results reported were dependent on the timing, length, and degree of dietary restriction. A 50% calorie restriction in the first and second trimester led to hyperphagia and delayed-onset obesity in male (not female) offspring independent of postnatal diet. A strict 30% of ad libitum diet throughout pregnancy resulted in offspring with adult-onset obesity, hyperinsulinemia, and hyperleptinemia associated with hyperphagia and hypoactivity, whereas a mild 30% calorie restriction throughout gestation reduced birth weight and increased catch-up growth and sensitivity to high-fat diet (HFD)-induced obesity.

Maternal Overnutrition

Maternal overnutrition is characterized as excess dietary intake and may lead to diet-induced obesity. Epidemiological evidence indicates a positive relationship between maternal obesity during pregnancy and the onset of obesity and metabolic syndrome in offspring. This relationship has been found to be independent of gestational diabetes. In human studies, offspring adiposity has been positively related to a woman's BMI during gestation, while weight gain and BMI between pregnancies can influence a child's susceptibility to obesity in both directions, compared to their siblings. This disproportionate risk in children from the same mother under different intrauterine conditions supports the effect of environmental stimuli on the physiological programming of offspring phenotype.

In animal studies, the most common model of maternal overnutrition has involved feeding animals an HFD or obesogenic diet (high fat and sugar). Rhesus macaques fed with an HFD during pregnancy produced offspring that developed nonalcoholic fatty liver disease that included triglyceride accumulation and a twofold increase in percent body fat. Interestingly, in subsequent pregnancies, when these mothers consumed a control diet, the adverse effects in offspring were prevented. In rats, an HFD during pregnancy produced offspring with increased adiposity, insulin resistance, and

hypertension. Consumption of a junk food diet (high energy, fat, sugar, and salt) during gestation led to offspring with hyperphagia, reduced skeletal muscle mass, a postnatal preference for junk food, and increased weight gain when weaned onto a junk food diet. In sheep, maternal diet-induced obesity led to fetuses with increased triglyceride levels and offspring with upregulated inflammatory signaling and lipogenic genes, increased fat deposition, and reduced skeletal muscle development. Metabolic syndrome constituents were intensified in offspring exposed to maternal overnutrition *in utero* and an obesogenic postnatal environment. However, interpretation of the evidence remains complicated by the different durations of exposure and dietary compositions reported in each model. An increase in one dietary component will result in the reduction of another. It is unknown whether the causal factor is the increased fat content or the reduced carbohydrate or protein contents or simply the excess energy content in maternal diet.

It remains inconclusive as to whether the effect on fetal phenotype is due to the maternal diet itself or the maternal metabolic variables associated with obesity. The effects of maternal weight gain and diet during gestation have proved difficult to segregate in animal models due to models generally using an obesogenic diet to induce maternal weight gain and obesity. Thus, outcomes may be produced by diet, maternal obesity, or a combination of the two. While evidence is building to suggest the gestational diet may impart independent effects on offspring phenotype, the critical timing of exposure remains unknown.

See also: Dietary References: US; Pregnancy: Metabolic Adaptations and Nutritional Requirements.

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Pregnancy: Metabolic Adaptations and Nutritional Requirements

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Introduction

Pregnancy is a dynamic anabolic state with increased nutritional requirements to support growth and metabolism of maternal and fetal tissues, nutrient storage in the fetus, and preparation of maternal tissues for lactation. Although these additional nutrient needs may be met by increased maternal dietary intake, part of the increased demands is met by complex physiological adjustments and changes in nutrient metabolism that evolve continuously throughout pregnancy according to the changing requirements at different stages of gestation.

Metabolic adjustments depend on each nutrient and may include uptake and accretion in new tissues, deposition in maternal stores, redistribution among tissues, and increased turnover or rate of metabolism. For certain nutrients, physiological adjustments favor a more efficient utilization of the nutrient from the diet by increased intestinal absorption and/or reduced excretion through urine or the gastrointestinal tract. For each nutrient, more than one adjustment may be acting (Table 1).

Metabolic and physiological adjustments during pregnancy respond to the hormonal changes during gestation, the fetal nutrient demands, and the maternal nutrient supply through diet or mobilization of nutrient reserves. Adjustments vary widely between women depending on several factors such as lifestyle behavior, genetic background, habitual diet, use of supplements, and prepregnancy nutritional status.

Within the physiological capacity of adaptation, the developing fetus is protected from nutrient deficiency or excess in the maternal diet. However, a limit in the capacity for adjustment of the maternal organism during pregnancy may be reached depending on complex factors and interactions not yet fully studied. Limited access to a variety of foods, intense physical activity, adolescent motherhood, poor nutritional status prior to pregnancy, and environmental exposure to toxic substances (i.e., mercury, lead, pesticides, and dioxins) or infectious agents (i.e., foodborne pathogens such as listeria as well as salmonella, tuberculosis, malaria, intestinal parasites, and HIV) impose additional stress on the physiological capacity of adaptation during pregnancy. When this capacity is exceeded, fetal growth and development and pregnancy outcome may be impaired. If undernutrition or excess of certain nutrients affects the fetus, there may be short-term and lifelong adverse health effects.

Table 1 Metabolic and physiological adaptations affecting nutrition utilization during pregnancy

Adaptation	*Example of nutrients affected*
Increased tissue uptake	Amino acids, iodine, folate, iron
Redistribution among tissues	Zinc, iron, calcium, vitamin A, vitamin D, vitamin B_{12}
Increased turnover/ metabolism	Protein (amino acids), zinc, folate, calcium, iodine, vitamin D
Increased maternal stores	Fat
Increased intestinal absorption	Iron, calcium, zinc
Reduced endogenous losses	Protein (nitrogen), zinc

Changes in Maternal Physiology and Metabolism during Pregnancy

Hormonal Changes

Changes in maternal hormone secretion during pregnancy contribute to maintain the pregnancy state and to modify the utilization of nutrients (especially carbohydrates, fat, and protein) in order to stimulate maternal tissues to make greater use of lipids as energy-yielding substrates and to increase the availability of glucose, amino acids, and micronutrients for fetal growth. Hormonal changes also contribute to ensure that maternal lean tissues are conserved and spared for providing energy or amino acids for the fetus.

Soon after implantation, the corpus luteum and the placenta secrete hormones to ensure the maintenance of pregnancy and to influence metabolism in preparation for the developing fetus. Serum human chorionic gonadotropin increases rapidly during the first 2 weeks of gestation, reaches a peak at about 8 weeks, and then gradually declines but remains elevated until delivery. Serum human placental lactogen increases continuously throughout pregnancy parallel to the increase in placental mass, reaching a concentration at term about 20 times higher than before conception. This hormone affects the metabolism of carbohydrates and lipids, antagonizing the action of maternal insulin and stimulating maternal lipolysis, thus contributing to direct energy-yielding substrates to the fetus.

The corticotropin-releasing hormone (CRH) is produced by the placenta by 8 weeks after conception but remains usually undetectable in maternal serum until the last trimester of pregnancy when there is a gradual increase to about 50-fold. Placental CRH stimulates the production of fetal cortisol that in turn blocks the inhibitory effect of progesterone on placental synthesis of CRH and fetal cortisol. Maternal serum CRH is increased in preeclampsia and by maternal stress and infections and is associated with higher risk of preterm delivery.

Progesterone and estrogens serum concentrations rise progressively throughout pregnancy increasing over fivefold and tenfold, respectively, from 10 to 40 weeks of pregnancy. After 8–10 weeks of gestation, the main source of these hormones is the placenta although estrogens are also originated in the fetus and other maternal tissues. Progesterone stimulates maternal respiration and relaxes smooth muscle contributing to the atony of the uterus and the urinary and gastrointestinal tracts.

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Estrogens (estradiol, estrone, and estriol) influence carbohydrate and lipid metabolism, increase the rate of maternal bone turnover, stimulate the conversion of maternal pituitary somatotroph cells into prolactin-secreting cells (in preparation for lactation), and help prepare the uterine muscle cells for delivery.

Blood Volume and Composition and Other Physiological Changes

As gestation progresses, several maternal organ systems adapt to the changing needs. The size of the heart increases by about 12% and cardiac output increases by about 40% in response to the increased oxygen demands. Although lung capacity is reduced by about 5% (due to elevation of the diaphragm by the enlarged uterus), alveolar ventilation increases as pregnancy progresses resulting in a more efficient gas exchange. Gastrointestinal motility is reduced, and consequently, transit time is longer allowing for increased nutrient absorption. The kidneys increase slightly in size, and renal plasma flow and glomerular filtration rate increase by about 75% and 50%, respectively, resulting in an increased urinary excretion not only of metabolic end products but also of certain nutrients. Maternal blood volume gradually expands with a modified cell and plasma composition that serves to increase the tissue supply of oxygen and nutrients and also to prepare for blood loss at delivery.

Compared to the nonpregnant state, total blood volume increases 35–40% during pregnancy, mainly by expansion of plasma volume (45–50%) and proportionally less expansion of red cell mass (15–20%). As a result of this 'hemodilution of pregnancy,' hemoglobin concentration and hematocrit values decrease especially at midpregnancy (20–30 weeks), when there is the largest increase in plasma volume, but increase slightly again in the third trimester. For these reasons, the recommended hemoglobin and hematocrit cutoff values for screening for anemia in pregnant women are trimester-specific and lower than for nonpregnant women.

Plasma volume starts to increase at 6–8 weeks of gestation, expands rapidly up to 30–34 weeks, and tends to plateau thereafter. By the end of pregnancy, the total increase in plasma volume is about 1500 ml. Red cell mass increases by about 200–250 ml during pregnancy driven by the increase in maternal erythropoietin production. The increase in red blood cell mass is greater with the use of iron supplements. Total white blood cell count increases during pregnancy although within the normal range. Neutrophils are the major type of leucocytes increased, possibly due to reduced apoptosis, and have increased oxidative metabolism. Monocyte-to-lymphocyte ratio markedly increases during pregnancy. Platelet count decreases particularly during the third trimester, a condition named 'gestational thrombocytopenia.'

Plasma concentration of total protein, particularly albumin and γ-globulin, most amino acids, some minerals, and water-soluble vitamins, decreases during pregnancy due in part to hemodilution, increased glomerular filtration rate, and/or changes in turnover or metabolism. In contrast, plasma concentration of α1-, α2-, and β-globulins, lipid fractions (particularly triacylglycerol and also VLDL, LDL, and HDL), and fat-soluble vitamins usually increases during pregnancy. Most of these changes occur during the first 10 weeks of gestation. For those nutrients that have a decreased concentration during pregnancy, reduction is much less than the 40-fold increase in plasma volume. Therefore, the total amount of energy-yielding substrates, vitamins, and minerals in the maternal circulation increases during pregnancy.

Fetal Demands

The demand for nutrients gradually evolves during gestation both in total amount and in particular nutrients required. Three main physiological stages of fetal development are usually described during pregnancy: implantation, organogenesis, and growth. Animal studies indicate that the presence or absence of certain nutrients particularly at the early stages of gestation is critical for normal development. The total amount of nutrients needed becomes critical during the second half of gestation when most of the fetal growth occurs.

During implantation and up to 2 weeks of gestation, the fertilized ovum embeds in the uterine wall and receives nutrients directly provided by the uterine secretions. At this stage, there are specific nutritional needs, but quantitative requirements are negligible.

During tissue differentiation and organogenesis, mostly occurring from 2 to 6 weeks of gestation, nutrients are provided from the maternal circulation. At this stage, lack or excess of certain nutrients can cause specific congenital abnormalities. For example, evidence from animal studies indicates that folate, vitamin B_{12}, vitamin A, and niacin deficiencies cause defects in the central nervous system; vitamin B_6 and manganese deficiencies impair neuromotor development; riboflavin deficiency affects the formation of the skeleton; and zinc deficiency or excess of vitamin A causes multiple organ malformations and embryonic death. In contrast, much less is known about the effects of specific nutrient deficiencies on organogenesis in humans. Only folate has been clearly recognized as a critical micronutrient for the prevention of neural tube defects.

During the remaining 30–32 weeks of pregnancy, most fetal growth occurs, initially by hyperplasia (rapid increase in the number of cells), later by simultaneous increase in cell size, and finally by hypertrophy (cells growing mostly in size). Critical nutrients for cell division are folate, vitamin B_{12}, and zinc, while sufficient supply of amino acids, vitamin B_6, and glucose is required for cell growth. During this rapid growth period, nutrients are delivered from mother to fetus through the placenta. Some nutrients are transferred across the placenta by simple diffusion (e.g., free fatty acids, cholesterol, and fat-soluble vitamins) whereas others by facilitated (carrier-mediated) diffusion (e.g., glucose) or active transport (e.g., amino acids, most water-soluble vitamins, calcium, iron, and zinc). In the latter case, nutrient concentrations are usually higher in fetal than in maternal circulation. Similarly to the phase of organogenesis, little is known on the effect of specific nutrient deficiencies during fetal growth in humans, although there is strong evidence that nutritional deficiency during this phase causes intrauterine growth retardation and low birth weight and increased risk of impaired immune function. However, inadequate nutrition at this stage will not cause developmental abnormalities.

Changes in Maternal Body Composition and Weight Gain

Gestational weight gain usually follows a sigmoid curve. Approximately 5% of the total weight gain occurs during the first trimester of pregnancy and the remainder 95% is gradually gained at an average rate of about 0.45 kg per week during the second trimester and 0.40 kg per week during the third trimester. The average total weight gain in full-term healthy primigravidas is about 12 kg although the amount of weight gain varies widely among women. Total weight gain is inversely related to maternal prepregnancy body mass index. Mean total weight gain during a full-term pregnancy ranges between 10 and 16.7 kg in normal weight adult women and is lower than 11 kg in overweight and obese women. Higher total weight gains normally occur in adolescent women, in thin women, and in twin or multiple pregnancies. Current recommendations of weight gain during pregnancy are set according to prepregnancy maternal weight for height categories. A normal weight gain during pregnancy is more likely to be associated with optimal reproductive outcome, fetal and infant growth, and development.

Two major components contribute to weight gain during pregnancy: the products of conception (fetus, amniotic fluid, and placenta) and the accretion of maternal tissues (expansion of blood volume and extracellular fluid, enlargement of uterus and mammary glands, and increased adipose tissue). Of the total weight gain, the fetus accounts for ~27%, amniotic fluid 6%, and the placenta 5%. Maternal tissue accretion contributes mainly with fat deposition (average 27%, although highly variable between women). The majority of the maternal fat deposited during pregnancy is subcutaneous. In healthy pregnant women, fat appears to be deposited mainly in the hips, back, and upper thighs. This pattern of fat deposition appears to be unique of pregnancy. Fat deposition occurs mainly during the first 30 weeks of gestation under progesterone stimulation. This early fat deposit acts as an energy store for late pregnancy and during lactation. The increase in fat-free mass during pregnancy represents mainly an increase in body water. The amount and pattern of gestational weight gain are strongly influenced by changes in maternal physiology and metabolism and by placental metabolism.

Nutritional Requirements during Pregnancy

Approaches for Estimating Nutritional Needs during Pregnancy

Assessment of nutritional requirements during pregnancy is challenging due to the continuous alteration in nutrient metabolism and turnover that affect concentrations in tissues and fluids and the gradual shift in the efficiency of nutrient utilization that contributes to attain homeostasis.

For most nutrients, including energy, protein, and minerals, requirements during pregnancy have been assessed using the factorial approach. This involves estimates of the total amount of the extra energy and nutrient accumulated in the mother (blood, breast, uterus, and adipose tissue) and the products of conception (fetus, placenta, and amniotic fluid) during the whole pregnancy, above the nonpregnant nonlactating state, and correction by a factor to cover for the inefficient nutrient use for tissue growth. This approach relies on indirect data and does not allow for estimates of requirements by gestational stage.

For protein and some minerals, balance studies have been used to assess nutrient retention at different stages during pregnancy. In these studies, measurement of the nutrient intake and losses from the body is done as precisely as possible during a period of time (in general during 3 weeks), and the difference is taken to indicate net retention (or loss) during the assessed period. Because not all possible sources of body losses are measured, balance studies tend to overestimate intake and underestimate losses. Also, because balance studies are carried out over short periods of time at specific stages of pregnancy, results cannot be extrapolated to the entire pregnancy.

For a few nutrients, such as folate, evidence has been obtained from experimental human studies on the amount of intake needed to attain adequate tissue concentrations (e.g., erythrocytes) and nutrient-dependent functions (e.g., enzyme activity). For many vitamins and minerals, observational studies and intervention trials have related maternal intakes to pregnancy outcomes.

Recommended nutrient intakes during pregnancy are set by expert scientific committees of different countries or organizations, based on the interpretation of the available evidence on nutritional requirements during pregnancy. The most widely used set of nutrient recommendations are those developed by the Food and Agriculture Organization/World Health Organization (FAO/WHO) and by the Institute of Medicine (IOM) for the United States and Canada (Dietary Reference Intakes, DRIs). The percent increase of recommended daily nutrient intake during pregnancy over the nonpregnant nonlactating state differs between nutrients and may differ between scientific organizations (Figure 1).

Specific Nutrient Metabolism and Requirements

Energy

Theoretical estimations of the overall energy cost of pregnancy in well-nourished women have been done based on the contribution of three main components: (1) the energy deposited as new tissue in the products of conception (placenta, uterus, amniotic fluid, fetus, mammary glands, and expansion of blood volume), approximate cost 5000 kcal; (2) the energy deposited in fat, approximate cost 36 000 kcal; and (3) the energy required to maintain the new tissues, approximate cost 36 000 kcal.

Several longitudinal studies have measured changes in basal metabolic rate (BMR), energy expenditure, and energy deposition during pregnancy in different populations, thus providing experimental evidence for estimating requirements. These studies have shown that energy requirements during pregnancy have a large variability within individuals and between populations and are influenced by several factors, including prepregnancy body weight, body mass index and body fat, maternal age, BMR, and level of physical activity.

Estimated energy requirements (EERs) during pregnancy by the FAO/WHO have been based on multiples of BMR and the level of physical activity. For each trimester of pregnancy, studies indicate that the average increase in BMR is 5%, 10%, and 25%, and the average increase in total energy expenditure (TEE) is 1%, 6%, and 17%, respectively, over prepregnancy values. EERs during pregnancy have been also derived from studies using the double-labeled water technique. EER is

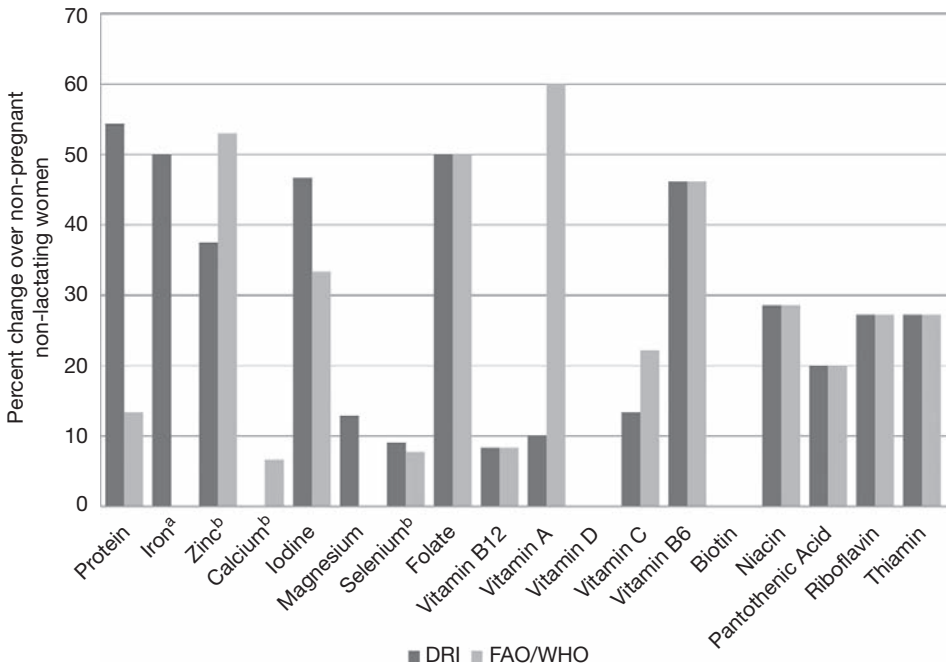

Figure 1 Percent changes in recommended nutrient intakes during pregnancy over the nonpregnant state. The [a]FAO/WHO recommends taking iron supplements up to 100 mg to all pregnant women. [b]For the FAO/WHO, percent change over nonpregnant nonlactating women was calculated considering the average recommended intake throughout pregnancy.

estimated as the sum of TEE of the nonpregnant woman plus the estimated median change in TEE of 8 kcal per gestational week, plus 180 kcal per day for energy deposition in maternal and fetal tissues during pregnancy. Taken together, these approaches translate into recommended increases of energy intake of 340 kcal per day in the second trimester and 452 kcal per day in the third trimester.

The capacity for physiological adaptation in energy metabolism during pregnancy is complex and highly variable. Adequate maternal weight gain and fetal growth are good indicators that maternal energy intake is adequately meeting energy requirements during pregnancy.

Protein

Adaptation in maternal nitrogen metabolism starts early during pregnancy and favors nitrogen conservation and protein deposition in the fetus and maternal tissues. There is a shift in the partitioning of amino acids for oxidation and protein synthesis favoring the latter. Maternal urea synthesis is reduced, therefore, with less utilization of amino acids as energy substrates. The rate of transamination of branched-chain amino acid is also reduced. During the second half of pregnancy, insulin secretion increases associated with greater insulin resistance. Thus, there is an increase in circulating glucose available for transfer to the fetus that indirectly spares amino acids for protein synthesis. Isotopic studies indicate that protein turnover increases during pregnancy and that the increase in protein synthesis is about 25% by late pregnancy compared to the nonpregnant state. There is evidence that metabolic changes in amino acid utilization during pregnancy may be limited by micronutrient status.

Using the factorial approach, the estimated total protein accretion during a full-term normal pregnancy is 925 g. Most of protein is accrued in the fetus (42%) and also in the uterus (17%), blood (14%), placenta (10%), and mammary tissue (8%). Balance studies in women before and during pregnancy indicate that nitrogen retention increases gradually during gestation particularly from the second to third trimesters. The increase in nitrogen retention during pregnancy is due to a reduction in urinary nitrogen excretion, with no change in fecal and integumental losses.

The recommended increase in protein allowance during pregnancy over the nonpregnant requirement varies (+25 g per day, the United States and Canada; +6 g per day, the United Kingdom and FAO/WHO/UNU). Most pregnant women worldwide consume at least the recommended intake. Moreover, there is little evidence that healthy women already consuming an adequate amount of protein need to consume more during pregnancy.

Iron

Pregnancy has a marked impact on iron metabolism due to the expansion of maternal erythrocyte mass that is proportional to the increased need for oxygen transport. Besides the iron need for the additional synthesis of hemoglobin (450 mg), tissue formation (placenta, 50 mg), and restitution of blood loss during delivery (250 mg), there is a need of 200–400 mg to be transferred from mother to fetus in order to ensure infant iron stores during the first months of life. Therefore, it is estimated that iron requirements over the course of pregnancy may reach 1000 mg, not equally distributed over its duration. The average requirement for iron absorption increases from

about 0.8 mg per day at early pregnancy to about 3–8 mg per day during the third trimester.

Maternal–fetal transfer of iron is especially increased during the last two-thirds of pregnancy and involves serum iron binding to transferrin receptors in placenta. Maternal serum transferrin increases almost twofold over nonpregnant values during pregnancy, possibly to facilitate iron uptake by the placenta. Hepcidin is a systemic regulator of iron metabolism that controls the efflux of iron into plasma through ferroportin regulation and therefore may have an important role on iron homeostasis during pregnancy. Liver production of hepcidin is simultaneously regulated by circulating and stored iron, erythropoietic activity, and inflammation. The limited evidence from studies during pregnancy indicates lower concentrations of hepcidin compared to the nonpregnant state and also a decrease in circulating hepcidin as pregnancy progresses with the lowest levels observed in the third trimester.

During the initial phase of pregnancy, cessation of menstrual iron losses contributes to meeting iron requirements and preserves maternal iron. Thereafter, mobilization of maternal iron stores is stimulated and theoretically could make a significant contribution. During the second and third trimesters, there is also a marked increment in the efficiency of intestinal iron absorption with an average increase of two- to threefold, or more, over nonpregnant values. It is estimated that women would need an iron store of ~500 mg at conception in order to ensure meeting the iron demands of pregnancy together with the iron supplied in the diet. However, almost half of women worldwide enter pregnancy with no iron reserves, therefore requiring increased iron intakes. The IOM recommends an increase of iron intake from 18 mg per day for nonpregnant women to 27 mg per day during pregnancy, a level difficult to achieve from food intake, therefore requiring the use of iron supplements. The WHO recommends iron supplementation 30–60 mg per day of elemental iron during pregnancy. However, recent studies indicate that the benefits of iron supplementation on maternal and infant health are inconsistent across populations and may have side effects such as reduced absorption of nonheme iron and other minerals and increased oxidative stress. Therefore, iron supplements during pregnancy may not be universally recommended.

Zinc

The additional zinc requirement during pregnancy estimated by the factorial approach is about 100 mg, more than half accrued in the fetus (57%) and the remainder in the uterus (24%), placenta (6.5%), expanded blood volume (6.5%), mammary tissue (5%), and amniotic fluid (<1%). This requirement corresponds to about 5–7% of the total body zinc in a healthy nonpregnant adult woman.

There is a physiological decline of 15–35% in plasma zinc concentration as a result of hemodilution, increased urinary zinc excretion, increased zinc uptake by maternal tissues, and transfer to fetus. However, the total amount of circulating zinc rises due to the increased blood volume and the increased (10–15%) erythrocyte zinc concentration. Transfer of sufficient zinc to the fetus is dependent on the maternal serum zinc concentrations. It appears that placenta zinc uptake adapts to fetal needs, being higher in periods of rapid growth and also when maternal zinc status is low. The efficiency of intestinal zinc absorption increases from early to late pregnancy, especially when habitual zinc intake is low. Renal zinc conservation is an additional adaptation in pregnant women accustomed to low-zinc diets.

It is estimated that an additional zinc intake of 2–10 mg per day is needed during pregnancy, depending on zinc bioavailability in the diet (FAO/WHO). The IOM recommends an additional 3 mg daily for both adult (from 8 to 11 mg per day) and adolescent (from 9 to 12 mg per day) pregnant women.

Calcium

During pregnancy, there is a marked increase in maternal calcium demand for fetal skeletal mineralization. Fetal calcium accumulation rate varies from about 2–3 mg per day in the first trimester, 50 mg per day at midgestation, to 250–300 mg per day in the third trimester. This implies in a total fetal calcium accrual of 20–30 g.

The additional calcium is obtained primarily from increased efficiency of intestinal absorption that usually doubles during pregnancy and may be more efficient when maternal calcium intake is low. Concomitantly, there is an increased expression of enterocyte Ca-binding protein. The increase in calcium absorption starts early in pregnancy and therefore precedes fetal calcium needs for bone growth. This is consistent with some calcium storage on maternal skeleton for future mobilization. Bone turnover is increased during pregnancy, as indicated by several markers of bone resorption and bone formation, especially during the last trimester. Although bone turnover may contribute to maintaining calcium dynamics, it results in very little or no net calcium loss from bones during this period. Urinary calcium excretion is increased as a consequence of the higher intestinal uptake and glomerular filtration rate. When corrected by creatinine clearance, fasting calcium excretion is normal or decreased.

Evidence from different studies indicates that the physiological adaptations for providing calcium to the fetus are largely independent of maternal calcium intake. Therefore, recommended calcium intakes during pregnancy are not increased over those for nonpregnant nonlactating women (IOM and FAO/WHO). However, special consideration should be given to adolescent mothers and women habitually consuming very low-calcium diets.

Iodine

Physiological alterations in thyroid function include the requirement of an increased production of thyroid hormone that, in turn, depends on thyroid integrity and adequate availability of dietary iodine. Regulation of thyroid function during pregnancy includes a marked increase in serum thyroxine-binding globulin, increased renal clearance of iodine, and stimulation of the thyroid gland by human chorionic gonadotropin.

Iodide (the reduced form of iodine) pool of the body is constituted by iodide from diet and from catabolism of thyroid hormones. In the nonpregnant state, and conditions of adequate intake (150 μg per day), this pool is in dynamic equilibrium with the thyroid gland and kidneys. During pregnancy, renal iodide clearance increases 30–50% and iodide requirement for thyroid hormone production increases by 50%, from the first weeks of gestation. Later in gestation, iodide is transferred from the maternal circulation to the fetus by placenta.

Increased iodide uptake and glandular hypertrophy are the main mechanisms by which the thyroid gland adapts to increased requirement and/or decreased supply.

In situations of iodine sufficiency, the thyroid gland is able to adjust hormone synthesis to the increased needs reaching a steady state, as a physiological adaptation of pregnancy. In contrast, with restricted iodine supply, pregnancy may lead to an excessive glandular stimulation. Depending on severity of iodine deficiency, pathological alterations may occur resulting in different degrees of impairment in fetal neural system development.

Iodine intake recommendation is based on estimated daily thyroid iodine uptake of ~75 μg per day by the fetus and the thyroid iodine content of the newborn, together with data on iodine balance during pregnancy and studies of iodine supplementation that prevent increased thyroid size. Compared to the nonpregnant state, both the IOM and the FAO/WHO recommend an additional intake of 70 μg per day during pregnancy.

Folate

Pregnant women have a high folate requirement as a result of increased folate utilization, catabolism, and urinary excretion. 5-Methyltetrahydrofolate (5-MTHF) is the biologically active folate metabolite that affects DNA synthesis, amino acid metabolism, and methylation of genes, proteins, and lipids via S-adenosylmethionine-mediated one-carbon transfer reactions.

Circulating 5-MTHF can be transported into cells either by the reduced folate carrier, a facilitative anion-exchange carrier, or by an endocytic process mediated by folate receptors. Folate receptors are expressed in the neural folds during the neurulation phase of embryonic development, demonstrating the importance of folate uptake for proper neural tube development. Adequate folate nutrition, especially in the periconceptional period, is thus necessary to prevent neural tube defects besides other congenital defects.

Serum 5-MTHF is the main determinant of placental transfer to the fetus. A critical concentration >7 nmol l^{-1} appears to ensure adequate folate fetal transfer. Serum folate concentrations decline in pregnant women as a physiological response to pregnancy, although mechanisms have not been fully elucidated. Erythrocyte folate concentration is an indicator of long-term folate status and tissue stores during pregnancy. A physiological decline in erythrocyte folate concentration is observed at early pregnancy followed by a slight increase at midpregnancy.

The IOM and FAO/WHO recommend an intake of 400μg dietary folate equivalents (DFEs) per day for all women of reproductive age. This is based on strong evidence that ensuring an adequate folate intake for women who are capable of becoming pregnant contributes to reduce the risk of neural tube defect occurrence. During pregnancy, both organizations recommend an additional 200 μg of DFEs daily. In the United Kingdom, recommendations are substantially lower, with an additional 100 μg per day of folate during pregnancy over the 200 μg per day recommended for nonpregnant, nonlactating women.

Vitamin B_{12}

Vitamin B_{12}, or cobalamin, is a crucial nutrient for fetal development due to its role in DNA synthesis. Maternal serum cobalamin concentrations begin to decline in the first trimester of pregnancy, more than would be expected from hemodilution alone, and continue to decrease to about half the nonpregnant concentrations until the end of second trimester. Despite this decrease, serum homocysteine is not elevated as would be expected in vitamin B_{12} deficiency. A concomitant increase in erythrocyte cobalamin and decrease in saturation of cobalamin-binding serum proteins suggest a redistribution of cobalamin during pregnancy. Transcobalamin I and transcobalamin III increase during the second and third trimesters, and transcobalamin II, considered the best biomarker of active or available B_{12}, increases sharply in the third trimester to about one-third of nonpregnant values.

During pregnancy, absorption of vitamin B_{12} remains unchanged or increased due to an increase in the number of intrinsic factor–B_{12} receptors in response to placental lactogen. Newly absorbed vitamin B_{12} is preferably concentrated in the placenta and then transferred to the fetus down a concentration gradient so that serum vitamin B_{12} concentrations in the newborn are about twice the level found in mother, although correlated with each other. Maternal liver stores contribute less to the placental–fetal transference of vitamin B_{12}.

Total fetal vitamin B_{12} requirement during pregnancy is estimated to be 50 μg, while maternal stores may reach >1000 μg in healthy women with mixed diets. The estimated deposition rate of vitamin B_{12} in fetal liver during intrauterine life is 0.2 μg per day and is the basis for current recommendations. An intake of 2.6 μg per day of vitamin B_{12} is recommended by both the IOM and the FAO/WHO during pregnancy, corresponding to an 8% increase compared to the nonpregnant state. Meeting fetal demands may be difficult for pregnant women consuming diets restricted in animal food sources as in many developing regions of the world and in the case of vegetarians.

Vitamin A

During pregnancy, an extra amount of vitamin A is required for tissue growth and also to ensure fetal stores to be used after birth. Requirements are highest during the third trimester, when fetal growth is most rapid. The absence or excess of active vitamin A metabolites (isomers of retinoic acid) at critical periods of gestation can lead to abnormal development of the embryo and the placenta.

The major maternal circulating forms of vitamin A are retinol bound to retinol-binding protein and retinyl esters packaged in chylomicrons and their remnants. Changes in maternal status and/or dietary vitamin A intake affect these circulating retinoid levels and thus the amount of vitamin A available to cross the placenta. Maternal serum homeostatic mechanisms involving lecithin–retinol acyltransferase activity and binding proteins contribute to maintain fetal acquisition of vitamin A relatively stable over a wide range of maternal dietary vitamin A intake. Maternal serum retinol concentrations <0.7 μmol l^{-1} and >3 μmol l^{-1} indicate deficiency or excess, respectively.

In less developed countries, providing pregnant women with adequate amounts of vitamin A contributes to reduce maternal and infant mortality due to infection. In contrast, in developed countries, the potential adverse effects of excessive consumption of vitamin A associated with fetal malformation are the main concern. The risk of fetal abnormalities due to vitamin A toxicity is higher during the first trimester of

gestation and associated with excessive retinol (and not β-carotene) intake from supplements.

Vitamin A requirements during pregnancy are based on adequate fetal liver vitamin A content at birth, assuming that the liver contains approximately half the body's vitamin A when liver stores are low, as in the case of newborns. Considering an efficiency of maternal vitamin A absorption of 70% and an estimated increased requirement of 50 μg per day during the last trimester, vitamin A DRI for pregnancy is increased by 10% over the nonpregnant state, totalizing 770 μg RAE (retinol activity equivalents) per day. The recommended safe intake by the FAO/WHO is increased from 500 to 800 μg RE (retinol equivalents) per day.

Vitamin D

The main circulating form of vitamin D, 25-hydroxyvitamin D, is transferred from mother to fetus through the placenta in relatively small amounts that do not appear to cause maternal depletion. Serum concentration of 25-hydroxyvitamin D does not generally change during pregnancy, although there are physiological changes affecting both transport and metabolism of vitamin D by mechanisms not fully understood. Changes include increased circulating levels of vitamin D-binding protein and 1,25-dihydroxyvitamin D, the major active vitamin D metabolite.

Compared to prepregnancy state, 1,25-dihydroxyvitamin D serum concentration increases two- to threefold starting in the first trimester and reaching a maximum in the third trimester. Although this increase is mainly due to maternal synthesis by the renal 1α-hydroxylase, there is also contribution from increased placental and decidual 1α-hydroxylase activity. During pregnancy, parathyroid hormone (PTH)–related peptide (rather than PTH), which is produced in fetal parathyroid glands and placental tissues, could be a potential signal for placental synthesis of active vitamin D and consequently for placental calcium transfer. Although closely related, the increase in intestinal calcium absorption during pregnancy cannot be explained solely by the increased 1,25-dihydroxyvitamin D because intestinal calcium absorption doubles early in pregnancy, well before the increase in free 1,25-dihydroxyvitamin D concentrations late in pregnancy.

Given that the available evidence does not suggest that pregnant women are at increased risk of vitamin D deficiency compared with nonpregnant women, recommended intakes are not increased during pregnancy compared to the nonpregnant state (IOM and FAO/WHO). However, recommended intakes of vitamin D (cholecalciferol) for nonpregnant women are set higher by the IOM (15 μg per day) than by the FAO/WHO (5 μg per day).

Conclusion

The complex physiological adjustments and changes in nutrient metabolism during pregnancy appear to be highly integrated for maintaining homeostasis and ensuring that fetal and maternal needs are met. Studies investigating these adjustments in different populations and under different conditions in relation to dietary intake have provided the basis for establishing nutritional requirements during pregnancy. However, much remains to be known on the interacting effects of factors such as nutritional status prior to pregnancy, maternal immaturity, habitual diet, physical activity, genetic background, and environmental exposure to toxic substances and infectious agents, on the capacity for physiological/metabolic adaptation during pregnancy. Understanding the limits of this capacity is crucial for warranting fetal and maternal health in the short and long term.

See also: Calcium: Physiology; Dietary References: US; Energy Metabolism; Folic acid and Folates: Physiology and Health Effects; Food and Agriculture Organization of the United Nations; Hypovitaminosis A; Iodine: Physiology; Iron: Physiology of Iron; Pregnancy: Dietary Guidance for Pregnancy; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Retinol: Physiology; World Health Organization; Zinc: Physiology and Health Effects.

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Preservation of Foods

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Introduction

Foods are perishable commodities containing enough nutrients and water to be altered by microorganisms or support deteriorative enzymatic reactions. This explains the need for and development of preservation processes. Agents that can spoil or alter foods can be divided into physical, chemical, and biological agents.

- Physical agents: Mechanical processes, temperature, dryness, and light
- Chemical agents: The Maillard reaction, lipid rancidity, and oxidation of vitamins, among others
- Biological agents: Enzymes, parasites, and microorganisms in general

Among the agents mentioned earlier, microorganisms (spoilers and pathogens) have been the prime targets of preservation processes because of the serious consequences that their presence has on human health.

Various preservation methods have been used in human history, many of them taking advantage of natural circumstances, and they have been consolidated and improved. The most common food preservation methods include salting, curing, smoking, marinating, freezing, refrigeration, drying, and heat treatment. There are now new emerging technologies that were developed toward the end of the twentieth century, being the most important high hydrostatic pressure (HHP), irradiation, and pulsed electric fields (PEF).

In this contribution, a review about concepts related to food preservation is addressed, and a brief description of some of the most important traditional and new emerging technologies is given.

Food Preservation

What does food preservation mean? Although the concept of preservation is very broad, it can be said that it consists in performing actions to maintain the desired natural properties of foods as long as possible, protecting them from pathogens and other agents responsible for spoilage, thus allowing later consumption. These actions lie squarely in the heart of food science and technology, and they are the primary purpose of food processing.

The processing and storage of food are vital for a continuous supply of nourishment during growing or production seasons and afterward. The main reasons for food processing and preservation are the following:

- To absorb agricultural surplus and reduce losses generated as a result of the highly perishable nature of foodstuffs
- To provide products with added value (vegetables and fruits, meats, eggs, and milk, among others)
- To provide variations in the diet and, in the case of fruit and vegetables, to make them available outside the growing season

Food preservation goes back to very remote times. In ancient times, salt, ice, and cold air were used to preserve food. In ancient Egypt, liquids such as oil, vinegar derivatives, and even honey were used to increase the life of foodstuffs; and salting and smoking were also part of their preservation techniques. In Persia, sugar was added to certain foods, and in Greece, the fruit and vegetable were also coated with sugar. In Gaul, the druids put fruit in baskets covered with leaves to kept them fresh longer, probably as a result of the action of CO_2 evolving from the respiration of the fruit, while in Rome, wine was preserved by adding sulfur dioxide. Between the fifteenth and the eighteenth centuries, sauces and borax were used, but it was in the nineteenth century that all the preservation technology began to develop, with the application of sulfite to meat; the use of organic acids such as boric, salicylic, and benzoic acid; and a great development the appertization invented by Nicolas Appert in 1810. Appertization was the first direct attempt by man to intentionally create a new method for preserving food. Progress was also made in the effective recognition of microorganisms as the main source of alteration of food with the discovery of bacterial spores. Appertization was the beginning of a huge industry based on the use of heat to preserve food. Canning soon became established throughout the world, although it was not until 80 years later that science started to apply it to the development of preservation procedures and their control. Until the mid-twentieth century, the main form of preservation was heat treatment, although salting, drying, fermentation, cold smoking, and other preservation methods were also used. Subsequently, other methods were developed, such as freezing, the use of modified atmospheres, aseptic food packaging, and high-temperature short-time (HTST) or ultrahigh-temperature short-time (UHT) treatments, as well as a variety of innovative technologies not based on heat, such as high hydrostatic pressure, irradiation, electrical pulses, and light pulses. Chemical preservatives and, more recently, natural antimicrobials (essential oils, polyphenols, etc.) appeared and have been used as preservatives for a long time by the food industry.

Biological Food Spoilage

Many of the microorganisms that alter food are pathogenic to humans and animals, and it is necessary to remove them or control their development throughout the food's shelf life. Microorganisms can reach food in various ways, basically before, during, and after processing.

Before processing:

- Soil, sea water, or freshwater
- Raw materials (in the field or orchard)
- During harvesting and storage

During and after processing:

- Processing equipment (blanching and peeling)
- Added ingredients (spices, sugar, and salt)
- Water used during processing
- Packaging, storage, and distribution of processed foods (cross contamination)

The organisms involved in food spoilage or that can cause or contribute to foodborne illness can be divided into the following groups:

Microorganisms:

- Yeast and mold
- Bacteria
- Viruses
- Protozoa

Insects and mites:

- Directly, by consuming foods they produce infestation
- Indirectly, through the spread of disease

Rodents:

- Directly, by consuming food
- Indirectly, by disseminating disease

From the point of view of preservation processes, bacteria are the microbial group that produces most outbreaks or sporadic cases of foodborne illness. These microorganisms can be classified by their need for oxygen as (i) aerobic, (ii) anaerobic, (iii) facultative, and (iv) microaerophilic.

In general terms, according to their growth temperature, microorganisms can be classified as follows:

(a) Psychrophilic, growth at low temperature −15 °C
(b) Psychrotrophic and mesophilic, growth at low temperatures 0–7 °C
(c) Mesophilic, growth at average temperature 20–45 °C
(d) Thermophilic, growth at optimum temperature between 45 and 80 °C
(e) Hyperthermophilic, growth at high temperature 80 °C or more

This classification is important because the growth temperature directly affects the preservation procedures, from the point of view not only of their growth capabilities but also of the heat treatment applied; because thermophilic microorganisms are more resistant to heat than mesophilic microorganisms; and so on.

Among all the microorganisms, the genus *Clostridium* and the genus *Bacillus* produce spores and have species that produce toxins harmful to humans. These are mainly the proteolytic and nonproteolytic strains of *Clostridium botulinum*, *C. perfringens*, *Bacillus cereus*, and others.

Spores of these microorganisms resist high temperatures, so they are a problem in the case of foods that receive pasteurization processes of around 90–96 °C for several minutes. After the pasteurization process, the psychrophilic and psychrotrophic strains of these microorganisms can grow at very low temperatures, even below 4 °C, and produce their toxins.

In addition to microorganisms that produce spores, there are vegetative cells, less heat-resistant than spores, which fundamentally alter food during refrigerated storage. Many of these vegetative cells are pathogenic for humans and animals and can grow at refrigeration temperatures, so their control is essential in the prevention of foodborne diseases. Among this group of organisms are *Salmonella*, *Yersinia*, *Campylobacter* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Aeromonas hydrophila*.

Certain viruses that cause foodborne illness, such as norovirus, rotavirus, and the hepatitis A virus, are becoming quite important.

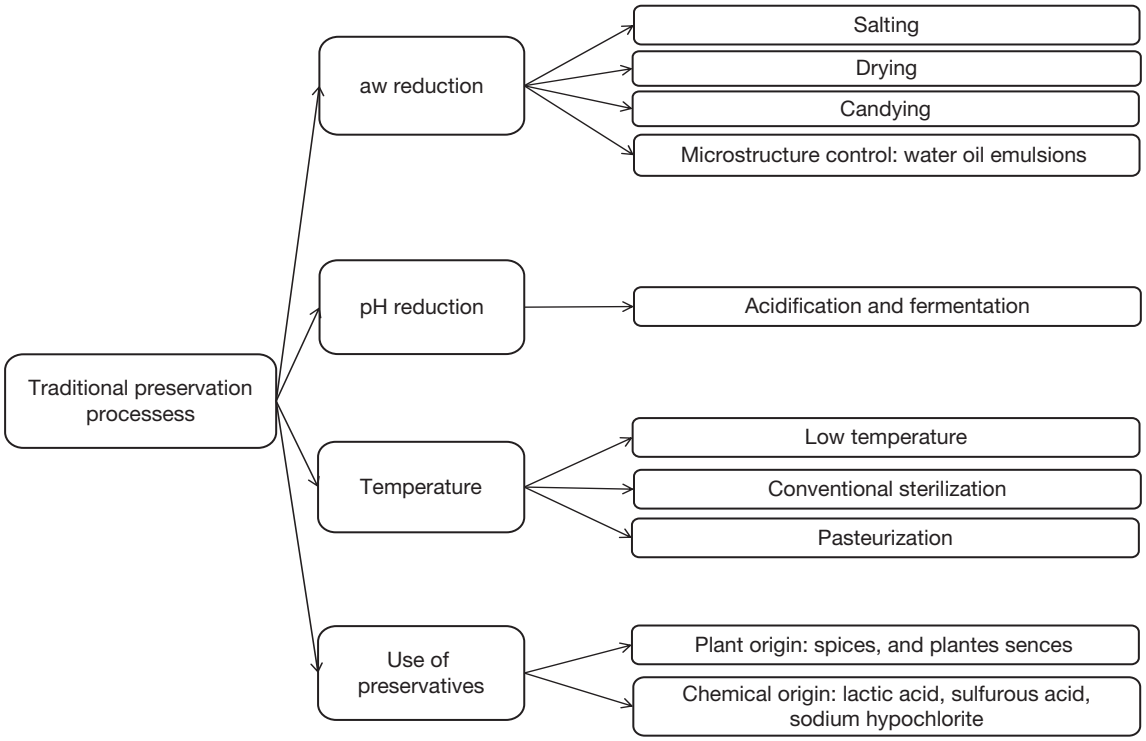
Although in the most of cases preservation processes are aimed to destroy or control pathogenic or spoilage microorganisms, preservation processes have also an important role in the inactivation of enzymes that can affect the sensorial properties of the preserved foods (color, texture, or flavor). Blanching and the conventional heat treatment applied to the foods serve also to reduce or inactivate the enzymes present in them (pectin methylesterase, peroxidase, and polyphenol oxidase, among others). In many cases, enzymes exhibit greater resistance than sporulated microorganisms; for this reason, the enzymatic inactivation is in some cases used as an indicator of the effectiveness and intensity of the heat treatment. Regarding the treatment of enzymes, it has been observed that inactivation may be affected by several factors including the composition, enzyme structure, pressure, temperature, pH time of treatment, and a_w.

Preservation Processes

The shelf life of food is increased by preservation processes. Preservation processes begin in the field through what is known as preventive measures (good agricultural practices and good hygiene practices) applicable to raw materials and the good manufacturing practices applied in the industry.

Preservation processes based on the destruction of microorganisms, including heat, biocides, and new conservation technologies, are applied in the industry. There are also procedures whose preservative power relies on slowing the growth of microorganisms, among which are low temperatures, reducing water activity, reducing pH, preservatives (natural or synthetic), and modified atmospheres. Sometimes, a combination of various preservation procedures may be used, improving the effectiveness of the preservation procedure.

Broadly speaking, these processes can be classified into traditional processes (Scheme 1), including those based on the effect of temperature, reduction of water activity or pH, and the addition of preservatives, and the so-called emerging preservation procedures. The most important forms of the latter are based on irradiation; emission of pulsed energy; the use of high pressure; new ways of applying heat and/or packaging, in addition to various antimicrobials; and applications of nanotechnology. Finally, there are combined processes or 'hurdle technologies' that combine different preservation technologies, increasing inactivation, reducing the treatment intensity of each



Scheme 1 Traditional preservation processes.

process, and thus reducing the impact of the process on the quality of the food.

The main advantages of the traditional processes are that they are economical, safe, and well established and in the case of heat treatments have a very wide application field. However, in some cases, there are some problems, such as lack of uniformity in treatment and changes that occur in chemical, physical, and nutritional properties. According to the latest market research, consumers demand 'fresh,' healthy, safe, and quickly prepared food. Given the limitations of traditional processes, especially thermal processes, and consumer demands, the future of food preservation lies in moving toward 'processing on demand,' consisting in designing a specific treatment for each food. In fact, there are foods for which traditional technologies are, and will remain, the best preservation alternative, but niche markets have appeared for emerging technologies because, when applied to certain products, they result in food that is safe from a microbiological point of view, that is healthy, and that maintains the properties of the fresh product better. These are the foods that can best meet consumer demands (Scheme 2).

A description of all preservation procedures is beyond the scope of this article. Consequently, the most commonly used methods are summarized, although technological advances are constantly being developed.

Reducing Water Activity

This mechanism for preserving food is aimed at the control of water (use of salt; drying; candying; concentration; and microstructure control, water–oil emulsion). Bacteria and other microorganisms need water to grow; thus, if there is a moisture control, the possibility of growth is reduced. The same thing happens with enzyme activity: enzyme reactions decrease when there is less free water in the medium. Consequently, dehydration is a mechanism that helps in the preservation of foods.

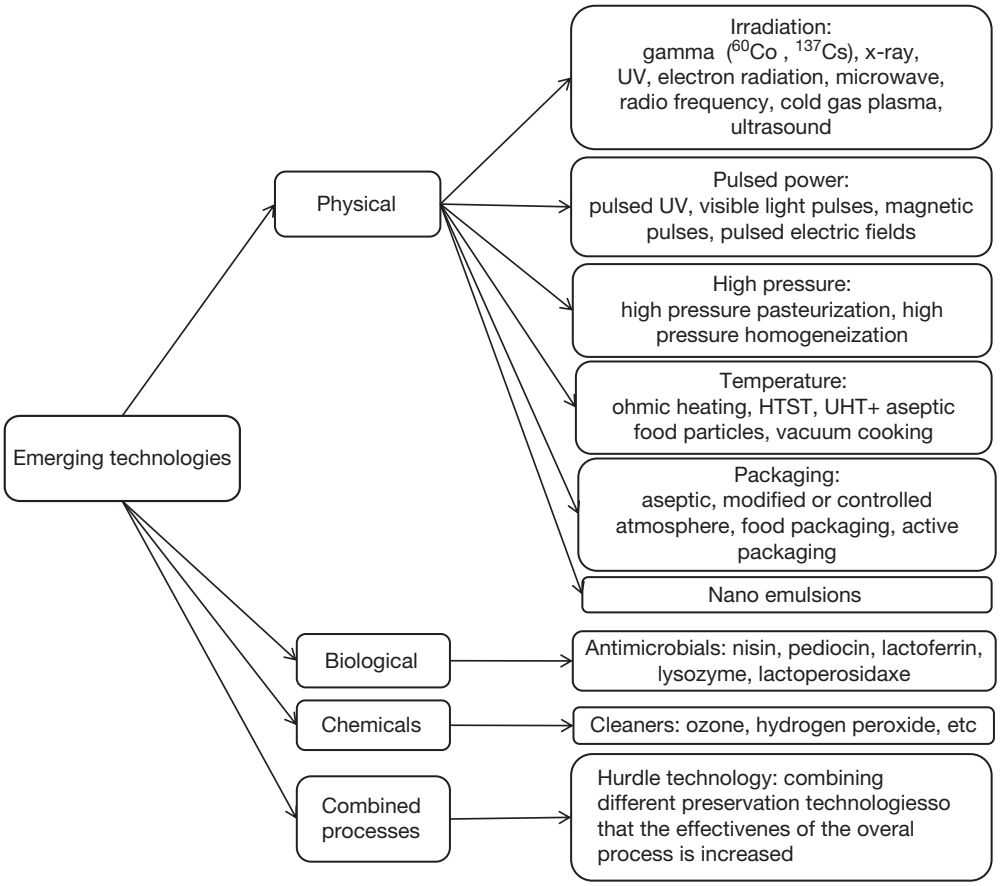
Use of Temperature to Control Microorganisms

Within the classical preservation technologies, the use of temperature to prevent the growth or activity of microorganisms and to inactivate and destroy enzymes is widespread and is one of the most common forms of food preservation. The main preservation processes based on the use of temperature are sterilization, pasteurization, cooking, freezing, and cooling.

For many years, heat treatment at high temperatures played a leading role as a preservation process. Over the years, the process has not changed, but the way in which it is used has undergone important modifications. What was initially merely a pressure cooker became a retort in its various forms, batch, continuous, and rotating and, in recent years, with heat treatment, a heat exchanger, with processes at HTST and UHT in conjunction with aseptic packaging.

There have also been changes in the way in which heat is generated, using steam, microwaves, or ohmic heating, in which the food acts as a resistance and becomes hot. It is important to point out that these treatments modify product characteristics such as taste, texture, and color to a greater or lesser extent and also increase the digestibility of components and the coagulation of protein.

Low temperatures have also been used in food preservation. This procedure has been used since time immemorial,



Scheme 2 Emerging preservation processes.

although the real development of it took place during the twentieth century. The effect of this technology is primarily based on slowing the chemical reactions and enzymatic activity inside cells. Those changes produce a limitation in the pathogenic or spoilage growth of microorganisms, slowly increasing by this way the shelf life of the products. In many cases, the growth is inhibited and the microbial concentration decreases with the storage time. Low temperatures also affect enzymes that can deteriorate foods. Low temperatures can be applied as refrigeration, which is short-term storage. In this case, the Food and Drug Administration (FDA) requires that refrigerated foods should be kept at a temperature of 4 °C or lower. Under these conditions, organoleptic and nutritive characteristics are slightly modified, the changes depending directly on the type of food, protective packaging, or treatment applied prior to storage. Freezing is the other system of preservation at low temperature. This long-term preservation method uses temperatures between −18 and −22 °C, so this technique prevents the development of spoilage and pathogenic microorganisms during the shelf life of the product.

Chemical Methods

The use of the preservative properties of many chemicals has resulted in numerous preservation methods. The main purpose in the use of chemicals is the preservation of foods by acting against microorganisms inhibiting their growth or even killing them. In some cases, chemical additives produce organoleptic changes that are pleasant for the consumer by modifying their original organoleptic properties.

Chemical preservatives (sorbic acid or benzoate) and substances with antiseptic activity can be included as methods that do not modify the sensory properties of products. Salting, curing, smoking, glazing, coating, the addition of organic acids, and fermentation processes that produce organic acids can be considered preservation methods that change the sensory properties of foods.

Owing to growing consumer concern about chemical preservatives, the industry is trying to bring to market natural antimicrobial substances from plants, animals, or bacteria that can be added to foods. Flavonoids and essential oils are compounds of plant origin that have potential as antimicrobials in the food industry. Milk proteins such as lactoferrin, lactoperoxidase, and casein have antimicrobial activity. Recently, chitosan has been gaining popularity as an antimicrobial of animal origin.

Various bacteria are being investigated in order to identify and increase the production of antimicrobials of microbial origin. Examples of these compounds are nisin, natamycin, subtilin, and plantaricin, generated by lactic acid bacteria.

Some of these natural antimicrobials are used in combination with other preservation procedures to originate hurdle technologies. The philosophy of hurdle technologies relies on

the control of microorganisms by using the addition or synergism of low doses of stressing factors.

Other chemical methods, such as ozone or chlorine, are used in the sanitation of vegetables for prepared food products.

Packaging Technologies

Packaging technologies developed rapidly during the last century. The main purpose of the package, to protect food from recontamination after being processed by other technologies, made it a preservation technology in itself.

Modified atmosphere packaging (MAP) was a great contribution to preservation processes made during the twentieth century. It is based on the preservative capability of CO_2 and a reduction in the O_2 concentration inside a package. Today, it is possible to find a great variety of vegetable, meat, fish, milk, and pasta products packed and preserved under MAP.

Various packaging systems are now being developed, such as active packaging that exhibits characteristics of absorption, retention (humidity, ethylene, oxygen, and carbon dioxide), release of components (antioxidants, antimicrobials, flavors, and preservatives), and temperature control (self-cooling and self-heating). Edible packages and intelligent packages are another step forward; the latter incorporate intelligent systems that indicate the quality and safety of the packed product, detect secondary metabolites of microbial growth, and can also have systems that monitor the temperature during production and storage, using chips, labels, or radio frequency systems.

Emerging Technologies

Among the emerging technologies, HHP is increasingly gaining more popularity as a preservation method. This technology belongs to the group of nonthermal processes, characterized by the long shelf life they provide, maintaining quality and sensory characteristics of the food. HHP now appears to be an alternative to heat treatment. This technology is being used throughout the world for the processing of fruit juices, fruit beverages, fruit purees, meat products, guacamole, and ready-to-eat foods, including dairy products. The method is based on applying pressures ranging from 100 to 800 MPa with relatively short times (from a few seconds to several minutes), combined with moderate temperature (20–50 °C). In the industry, HHP is now applied in batch processes. The resistance of microorganisms to pressurizing treatment depends directly on the type of organism and the characteristics of the matrix. In general, molds and yeasts are more sensitive to pressure, Gram-negative bacteria have a relatively medium strength, Gram-positive bacteria are among the most resistant vegetative cells, while bacterial spores require high pressures with high initial temperature to obtain significant reductions in initial concentrations. However, various germination methods prior to pressurization are now being studied in order to remove food spores. Advantages of this technology include the high nutritional quality and sensory characteristics of the products obtained, achieving sufficient microbial and enzyme inactivation at moderate temperatures. Furthermore, combinations of time, temperature, and pressure level allow the development of new products that can reach new markets.

Another nonthermal preservation technology is high-intensity PEF. This technology is based on the application of a potential difference in the food, so that an electric current is created in the product. The electric current causes a change in microbial cell membrane potential and consequently in the formation of pores in the membrane. When these pores are permanent, massive microorganism inactivation is caused by the difficulty in maintaining osmotic balance.

This technology applies electric pulses between 10 and 80 kV cm^{-1} and short treatment times between 50 and 2000 ms. There are certain limiting factors that directly affect PEF treatment: the food to be treated must comply with certain values for parameters such as pH, water activity, fat, and sugars; it is also necessary to standardize values of viscosity and electrical conductivity, because high values can cause an increased electric field that leads to a cessation of treatment.

Ionizing irradiation was considered as a promising food preservation technology. Irradiation up to 10 kGy can destroy microorganisms and bacterial spores. Irradiation is highly effective, but it has two major limitations: one is its high cost, and the other has to do with safety in the performance of the procedures. There is also some consumer concern about the consumption of foods preserved by means of this technology.

Future of Food Preservation

As new technologies are developed or the traditional ones are refined, new opportunities will be open to new products. HHP is a reality today and it is present in many preservation industries (meat, vegetables and fruits, pasta, and seafoods, among other products). Other technologies as PEF are still under development although some industrial equipment have been manufactured and are in production plants. Despite those and other advances in food preservation, heat preservation still remains as an important form of enlarging the shelf life of foods with an incredible record on food safety. Improvements on this traditional technology have permitted to produce preserved foods with better organoleptic properties than those made by using old still retorts without reducing the level of safety.

Probably, the future of food is the 'tailor-made foods' meeting the needs of some special groups of population (children, young people, and elderly). New products with new textures could be produced by using nonthermal technologies as the HHP, and new outcome technologies are being sighted and are still in its conceptualization phase.

See also: Biosensors; Chilled Foods: Principles; *Clostridium botulinum*; Controlled Atmosphere Storage: Applications for Bulk Storage of Foodstuffs; Drying: Principles and Types; Foodborne Pathogens; Freeze-drying: The Basic Process; Freezing Theory; Heat Treatment: Principles and Techniques; Packaging: Aseptic Filling; Pasteurization: Principles and Applications; Preservatives: Classifications and Analysis; Pulsed Electric Fields; Spoilage: Bacterial Spoilage; Spoilage: Yeast Spoilage of Food and Beverages; Sterilization of Foods; Storage Stability: Shelf Life Testing.

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Preservatives: Classifications and Analysis

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Chemical Preservatives

Chemical preservatives are defined in the Code of Federal Regulations (CFR) by the US Food and Drug Administration (FDA; 21 CFR 101.22 (a) (5)) as "any chemical that, when added to food, tends to prevent or retard deterioration thereof, but does not include common salt, sugars, vinegars, spices, or oils extracted from spices, substances added to food by direct exposure thereof to wood smoke, or chemicals applied for their insecticidal or herbicidal properties." Preservatives are used to prevent or retard both chemical and biological deteriorations of foods. The FDA includes in their list of food preservatives (21 CFR 172, subpart B) compounds with different preventive functions, such as antioxidant, color, flavor, and texture retainers, as well as antimicrobial agents. Only two functional classes of preservatives are clearly defined by the agency, antioxidant that is defined as "substances used to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation" (21 CFR 170.3 (o) (3)) and antimicrobial agents as "substances used to preserve food by preventing growth of microorganisms and subsequent spoilage, including fungistats, mold and rope inhibitors" (21 CFR 170.3 (o) (2)).

On the other hand, the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) of the United Nations through the *Codex Alimentarius* international food standards define preservative as "a food additive, which prolongs the shelf life of a food by protecting against deterioration caused by microorganisms" and include the following categorization: antimicrobial preservative, antimicrobial synergist, antimold and antirope agent, antimycotic agent, bacteriophage control agent, fungistatic agent, and preservative. A more specific definition is defined and established by the FAO/WHO for preservative, which only considers the biological deterioration, neglecting chemical deterioration. The approved preservatives for use in foodstuffs by two main regulatory agencies FAO/WHO and FDA are presented in Tables 1 and 2, respectively. Therefore, according to these two major regulatory agencies and their consistency in preservatives definition, preservatives in this article will refer only to chemical compounds that prevent biological food spoilage.

Classification of Preservatives

Antimicrobial agents can be classified with regard to different aspects, for example, based on their spectrum of action as bacteriostatic, bactericides, fungistatic, and fungicides; based on their origin as synthetic or natural; and based on chemical structure. Most preservatives are utilized to delay or inhibit the growth of a group of microorganisms, but there are some that are assigned to a particular microorganism, as in the case of nitrite against *Clostridium botulinum* in cured meat products, nisin against *C. botulinum* in pasteurized milk cheeses, and lactate and diacetate to inactivate *Listeria monocytogenes* in processed meats. There are not established criteria for classifying preservatives; in some cases, more than one classification is utilized according to their function, intended used, chemical structure, and origin. For example, the FAO/WHO established a classification based on their action spectrum and function. Furthermore, the FDA classifies preservatives based on their function. Some scientific reports utilize a classification based on the origin (natural or synthetic), if they want to highlight the nature of the compound. However, the classification most commonly reported in the literature is based on their chemical structure. Preservatives are classified as benzoic acid and benzoates, sorbic acid and sorbates, organic acids, sulfur dioxide and sulfites, nitrates and nitrites, nisin, natamycin, parabens, dimethyl dicarbonate, lysozyme, and miscellaneous antimicrobials.

Antimicrobial properties of preservatives depend on several factors such as antimicrobial spectrum, chemical and physical properties, concentration, and mechanism of action. Further, their activity depends on composition, pH, a_w, and storage temperature of food. Besides these factors, some other issues for choosing a preservative for use in foods must also be considered, such as genus and microorganism load, cost of preservative, and its effect on quality of the specific food. A proper assessment of these factors should be performed before choosing a preservative for a particular food. It is also necessary to revise if the preservative is permitted for use in the particular food because regulatory agencies establish restrictions. In addition, it should be reviewed the maximum permitted concentration of preservative intended to be used. Therefore, preservative maximum permitted concentrations in different kinds of food have been regulated in many countries. Regulations about maximum permitted concentrations of food preservatives are established by FAO/WHO and FDA among other agencies. Hereby, in order to assess their concentrations in foodstuffs and ensure food safety, it is important and necessary to develop effective, convenient, inexpensive, reliable, and rapid analytic methods to monitor the preservative levels in foods. A wide variety of analytic methods for determining preservatives have been developed to quantify and assess their presence in a foodstuff. These methods use the physicochemical properties of each preservative. However, before the compound or analyte of interest can be quantified, it is necessary to separate or remove it from the food matrix. The process to remove or separate the analyte depends on the composition of each food, the technique used to quantify the preservative, and the kind of preservative. Some preparatory processes for separating preservatives from a foodstuff include ultrasonication, solvent extraction, distillation, ion exchange electrophoresis, digestion, centrifugation, dilution, and filtration. Moreover, there are many methods to quantitatively determine the preservative; the most common are titration, spectrophotometry, high-performance liquid chromatography (HPLC), gas chromatography, and capillary electrophoresis (CE),

Table 1 Additives approved as the functional class preservative by FAO/WHO in the *Codex Alimentarius*

INS No.	*Food additive or group*	*INS No.*	*Food additive or group*
260	Acetic acid, glacial	212	Potassium benzoate
210	Benzoic acid	228	Potassium bisulfite
928	Benzoyl peroxide	224	Potassium metabisulfite
263	Calcium acetate	252	Potassium nitrate
213	Calcium benzoate	249	Potassium nitrite
385	Calcium disodium ethylenediaminetetraacetate	283	Potassium propionate
227	Calcium hydrogen sulfite	202	Potassium sorbate
282	Calcium propionate	225	Potassium sulfite
203	Calcium sorbate	280	Propionic acid
290	Carbon dioxide	262 (i)	Sodium acetate
242	Dimethyl dicarbonate	211	Sodium benzoate
230	Diphenyl	262 (ii)	Sodium diacetate
386	Disodium ethylenediaminetetraacetate	222	Sodium hydrogen sulfite
214	Ethyl parahydroxybenzoate	223	Sodium metabisulfite
236	Formic acid	251	Sodium nitrate
239	Hexamethylenetetramine	250	Sodium nitrite
384	Isopropyl citrates	232	Sodium ortho-phenylphenol
243	Lauric arginate ethyl ester	281	Sodium propionate
1105	Lysozyme	201	Sodium sorbate
218	Methyl parahydroxybenzoate	221	Sodium sulfite
235	Natamycin	200	Sorbic acid
234	Nisin	220	Sulfur dioxide
231	Ortho-phenylphenol	339 (iii)	Trisodium phosphate
261	Potassium acetates		

among others. Procedures for both qualitative and quantitative determinations of various preservatives can be found in the Association of Official Analytical Chemists' (AOAC) Official Methods of Analysis handbooks.

Another approach to the analysis of preservatives is based on its identity and purity for which regulatory agencies established particular test methods. The FAO/WHO provides a technical sheet for each preservative, which includes its chemical name, chemical structure, physical description, level of purity, solubility, melting point, levels of metal waste, and the assay method to determine its identity and purity. Furthermore, FDA also provides several features of preservatives and test methods for identification. This information is found in the sections that describe the use of food additives and generally recognized as safe (GRAS) substances in title 21 of the CFR.

Benzoic Acid and Benzoates

The FAO/WHO defines benzoic acid as a white crystalline solid, usually in the form of scales or needles, having not more than a faint characteristic odor, with a molar mass of 122.12 g mol^{-1} and a purity of 99.5% on the dried basis, a pH of 4.0 in aqueous solution, a slight solubility in water, and free solubility in ethanol. The assay method is based on acid–base titration using phenolphthalein as an indicator. Calcium and sodium benzoate are also described.

The amount of the benzoic acid and benzoates used as preservatives during food processing is important for consumer health. Benzoic acid and benzoates are generally used to inhibit mold and yeast growth, and they are also effective against bacteria. Benzoic acid and sodium benzoate are most suitable for foods and beverages that naturally are in a pH range below 4.5 or that can be brought into this range by acidification; therefore, they have been widely used to preserve beverages, fruit products, bakery products, fish products, liquid eggs, margarine, mayonnaise, mustard, salad dressings, and sauces. The maximum concentration permissible of benzoic acid and benzoates in most foods is 0.1%. The AOAC methods for determining benzoic acid and benzoates are based on different principles, and the quantification can be performed with various equipment, which facilitates the analysis of these preservatives at different laboratories, food processors, and regulators. Table 3 presents the official AOAC methods for determining sets of benzoic acid and benzoates. These methods are based on the ability of benzoic acid and benzoates to react with certain compounds under specific condition and absorb light at a specific wavelength. To separate the benzoic acid or benzoates of food, distillation must be performed (967.15), and extraction operations should be done (983.16, 960.38, and 963.19), centrifuged and filtered (994.11), or solubilized and diluted (980.17 and 960.38). The qualitative test (ferric chloride test, 910.02) uses the ability of the benzoic acid to react with $FeCl_3$ in an alkali medium in order to produce ferric benzoate (a salmon color precipitate). The spectrophotometric techniques take advantage of benzoic acid absorbing light in a range of 265–280 nm. This same feature is used by the chromatographic methods. To determine benzoic acid by gas chromatography, it must be converted to trimethylsilyl ester.

As an alternative to the official methods of analysis, several researchers proposed improvements to the traditional methods to reduce time, reduce reagent consumption, reduce analyte separation steps, improve the detection limit, or detect more than one analyte simultaneously. For instance, HPLC to determine benzoic and sorbic acids in various food like jam, candied chestnut, carbonated drinks, black table olive, green table olive, pickles, whole meals, brown bread, and white bread using a C18 column and to detect benzoic and sorbic acid was carried out at the wavelengths of maximum absorption of the compounds, 235 and 254 nm, respectively. The detection limit was 0.19 and 0.64 mg kg^{-1} for benzoic acid and sorbic acid, respectively. In addition, they proposed a simple sample preparation, which involves homogenization with methanol and filtering. Another method consisted of a continuous system for the simultaneous determination of benzoic and sorbic acids using CE with a dynamic pH junction-sweeping strategy in liquid food with a detection limit of 0.001 mg and a very simple sample preparation, which consists in food dilution with water (100–1000 times) and filtering. They successfully analyzed fruit juices, soft drinks, soy sauces, and wines.

Table 2 Additives approved as functional class preservative and antimicrobial agents by US Food and Drug Administration (Title 21 of the Code of Federal Regulations, CFR)

Compound(s)	*Principal food applications*	*CFR designation*
Calcium disodium EDTA, disodium EDTA	Dressings nonstandardized, egg product, oleomargarine, potato salad, and salad dressing, canned kidney beans, and mayonnaise	172.120, 172.135
Dehydroacetic acid	Cut or peeled squash	172.130
Sodium nitrite, sodium nitrate	Smoked, cured sablefish; smoked, cured salmon; smoked, cured shad; and meat and meat products	172.170, 172.175
Quaternary ammonium chloride combination	Raw sugar cane juice	172.165
Benzoic acid, sodium benzoate	Beverages, fruit products, and margarine	184.1021, 184.1733
Lactic acid, lactates	Meats and fermented foods	184.1061, 184.1207, 184.1639, 184.1768
Propionic acid, calcium propionate, sodium propionate	Bakery products and dairy products	184.1081, 184.1221, 184.1784
Sorbic acid, sorbates	Most foods, beverages, wines	182.3089, 182.3225,182.3640, 182.3795
Acetic acid, acetates, diacetates	Baked goods, condiments, confections, dairy products, fats/oils, meats, and sauces	184.1005, 182.6197, 184.1754, 184.1185, 184.1721
Hydrogen peroxide	Milk, cheese and related products, and wine	184.1366
Dimethyl dicarbonate	Beverages	172.133
Methylparaben, propylparaben, heptylparaben	Beverages, baked goods, syrups, and dry sausage	184.1490, 184.1670, 172.145
Nisin preparation	Pasteurized cheese spread, pasteurized process cheese spreads, pasteurized cheese spread with fruits, vegetables, or meats	184.1538
Natamycin	Cheese	172.155
Lysozyme	Cheese, cooked meat, and poultry products	184.1550
Sulfites	Fruits, fruit products, potato products, and wines	Various

Table 3 Official methods for qualitative and quantitative determination of benzoic acid and benzoates by AOAC

Number of method	*Analytic method*	*Food*
994.11	Liquid chromatographic method	Orange juice
960.27	Titrimetric method	Milk
910.02	Qualitative tests (ferric chloride test or the modified Mohler test)	Beverages
963.19	Titrimetric method	Ketchup, jellies, jams, preserves, marmalades, cider containing alcohol, salted or dried fish
960.38	Spectrophotometric method	Ketchup, other tomato products, jams, jellies, beverages containing small amounts of alcohol, soft drinks, and fruit juices. Not applicable to solids
967.15	Thin-layer chromatographic method	Liquids, solids, semisolids, and solid–liquid mixtures
983.16	Gas chromatographic method	Apple juice, almond paste, and fish homogenate (at 0.5–2 g kg^{-1} levels) representing carbohydrate-rich, fat-rich, and protein-rich foods
980.17	Spectrophotometric method	Ground beef

Sorbic Acid and Sorbates

2,4-Hexadienoic acid and 2-propenylacrylic acid are the chemical names of sorbic acid. FAO/WHO describes it as colorless needles or white free-flowing powder, having a slight characteristic odor with a purity not less than 99.0% and molar mass of 112.12 g mol^{-1}, with a melting point between 132 and 135 °C, and being slightly soluble in water and soluble in ethanol. The assay method is based on acid–base titration using phenolphthalein as an indicator. Calcium and potassium sorbates are also described.

Sorbic acid is active against yeasts, molds, and many bacteria. Microbial inhibition by sorbate is variable and depends on species, strains, composition of food, pH, a_w, food-processing treatments, temperature of storage, and concentration of sorbate. The antimicrobial action of sorbate depends on pH and is most effective approaching its dissociation constant ($pK_a = 4.76$); however, it is possible that it has a good inhibitory effect at pH as high as 6.5–7.0, which is an advantage over other preservatives such as benzoic and propionic acids that loss their effectiveness at pH 4.5–5.5. The most commonly used forms include sorbic acid and its potassium salt. Sorbates have found wide application in various foods, including dairy products, bakery products, fruit and vegetables products, meat and fish products, beverages, food emulsions, and sugar and confectionery products. The maximum permitted concentration of sorbates for most foods is between 0.1% and 0.3%.

As already mentioned, AOAC established the official methods for the determination of sorbates in most foods; only quantitative methods are described for sorbates. In this case, the methods of analysis are based on the ability of sorbate

Table 4 Official methods for quantitative determination of sorbates by AOAC

Number of method	*Analytic method*	*Food*
971.15	Oxidation method	Cheese
974.08	Spectrophotometric method	Wines
974.10	Spectrophotometric method	Dairy products
975.10	Colorimetric method	Wines
980.17	Spectrophotometric method	Ground beef
983.16	Gas chromatographic method	Apple juice, almond paste, and fish homogenate (at 0.5–2 g kg^{-1} levels), representing carbohydrate-rich, fat-rich, and protein-rich foods

to absorb light under certain reaction conditions in a range of wavelengths ranging from 250 to 550 nm. Only one method used gas chromatography for the detection of sorbic acid. AOAC methods are summarized in Table 4. Detection methods require quantitative extraction and separation of sorbic acid from the food material without food ingredient interference. Extraction methods include acid-steam distillation, selective gas diffusion, and solvent extraction; in some foods, only filtration and dilution have been used. Extraction by steam distillation has been used extensively, but it is time-consuming; in addition, the compounds generated by the degradation of lipids in this process can cause interference in the colorimetric and spectrophotometric methods. Method 983.16 determines sorbic and benzoic acids simultaneously in fat-, carbohydrate-, and protein-rich foods and has as principle that acids are isolated from food by extraction with ether and successive partitioning into aqueous NaOH and CH_2Cl_2. Acids are converted to trimethylsilyl esters and determined by gas chromatography.

Recent proposals of new analytic methods include chromatographic methods (HPLC, thin-layer chromatography, paper chromatography, capillary chromatography, and ion chromatography). More recent approaches used chemiluminescence (CL) coupled with an automated method of analysis such as flow injection analysis using a flow-through column packed with a molecularly imprinted polymer for sorbic acid. CL detection was based on the enhancement of CL intensity of the analyte with acidic potassium permanganate with 3-o-methoxyphenyl-5-(20-sulfonophenylazo) rhodanine acting as sensitizer. This method has been probed for determined sorbic acid in milk and fruit juices. An analytic method using solvent extraction and quantitative proton nuclear magnetic resonance spectroscopy was used for the absolute quantification of sorbic acid in processed foods. The limit of quantification was 0.063 g kg^{-1} for foods and 0.0063 g kg^{-1} for beverages containing *Lactobacillus* species. More ambitious methods include the determination of nine additives in red wine with chromatography (ion-suppression reversed-phase HPLC) or determined nine preservatives in various foods by liquid chromatography–mass spectrometry, including benzoic and sorbic acids. Alternatively, new extraction methods are being developed in order to reduce cost and time of analysis like the monolith-based stir cake sorptive extraction and liquid chromatography analysis.

Organic Acids

Organic acids are referred to a specific group of short-chain acids, where acetic, citric, lactic, malic, tartaric, succinic, fumaric, and propionic acids are the most important ones. The salts of sodium, potassium, and calcium derived from these acids are also considered within the group. Only propionic acid is widely recognized as an antimicrobial agent. According to regulatory agencies (FDA and FAO/WHO), the other acids may or may not be considered as antimicrobials. These acids are considered acidulant or pH control agents, whose antimicrobial effect is due to their nondissociated form. The dissociation constants of these acids are in the range of pH 3–5; therefore, its main application is in acidic foods. Choosing an acidulant as antimicrobial agent depends on its secondary effects, because they contribute to the taste and acidity in the product. The inhibitory effect of acids depends on pH, concentration, chain length, type, microorganism, strain, and food. Organic acids are most effective in inhibiting bacteria. They have also shown inhibitory effect on yeasts and molds. Selected organic acids (acetic acid, citric acid, and lactic acid) have been used exclusively as spray sanitizers against pathogens on beef carcasses. Its application is limited by its effect on taste; however, they can be used in a wide variety of foods such as fruit products; fresh, frozen, and fermented vegetables; egg products; coffee, tea, and herbal infusions; dairy products; fish products; grain beverages; pastas; and complementary foods for infants. Most organic acids have no maximum permitted concentration, and its use is based on good manufacturing practices (GPM). Nevertheless, some acids have maximum permitted concentration for particular foods; for example, tartaric acid and tartrates must not exceed 0.4% in fruit products, propionic acid must not exceed 0.3% in whey protein cheese, citric acid must not exceed 0.3% or 0.5% in some fruit products, and fumaric acid must not exceed 700 ppm in pasta.

The various organic acids can be assayed by simple procedures. Volatile acids can be separated from foods by steam distillation and titrated for the particular acid (when one acid is present); for a mixture of acids, the distillate is separated on a silicic acid column, and the component acids can be identified by other methods. Several techniques used for the analysis of acids include spectrophotometry; potentiometry; spectrofluorimetry; HPLC; gas, paper, and thin-layer chromatography; and CE. Official methods from the AOAC to determine organic acids are presented in Table 5. Qualitative or quantitative methods can be used depending on the required degree of sensitivity.

Nowadays, HPLC is the most widely used technique in organic acids analysis due to the advantage of simplicity, short analysis time, and sensitivity. In most cases, HPLC method uses a C18 column with an acid solution as mobile phase. Organic acids are detected at wavelengths ranging from 210 to 220 nm. The sample preparation starts with homogenization followed by solvent extraction or direct dissolution in

Table 5 AOAC official methods to determine organic acids in foods

Number of method	*Analytic method*	*Food*
942.09	Qualitative tests Monochloroacetic acid	Carbonated beverages, orange juice, wine, and commercial preservatives
942.10	Titrimetric method Monochloroacetic acid	Carbonated beverages, fruit juices, and wine
950.35	Chromatographic method Acetic and propionic acids	Air-dried bread and fresh bread
953.12	Qualitative test Dehydroacetic acid	Cheese
953.13	Spectrophotometric method Dehydroacetic acid	Cheese
970.36	Chromatographic method Propionates	Food

Table 6 AOAC official methods to determine sulfites in food

Number of method	*Analytic method*	*Food*
892.02	Titrimetric method (free sulfurous acid)	Meats
961.09	Qualitative test (sulfites)	Meats
962.16	The modified Monier–Williams method (total sulfurous acid)	Food, not applicable to dried onions, leeks, and cabbage
963.20	Colorimetric method (total sulfurous acid)	Dried fruit
975.32	Qualitative test (sulfurous acid)	Food
980.17	Spectrophotometric method (sulfites)	Ground beef
987.04	Differential pulse polarographic method (total sulfites)	$\geq$10 μg total SO_2 per gram in shrimp, orange juice, peas, dried apricots, and dehydrated potatoes
990.28	The optimized Monier–Williams method (sulfites)	$\geq$10 ppm (μg g^{-1}) sulfites in foods; not applicable to dried onions, leeks, and cabbage
990.29	Flow injection analysis method (total sulfites)	$\geq$5 μg g^{-1} total SO_2 in shrimp, potatoes, pineapple, and white wine
990.30	Flow injection analysis method (free sulfite)	Wines
990.31	Ion exclusion chromatographic method (sulfites)	Food and beverages with SO_2 at $\geq$10 μg g^{-1}

the mobile phase or centrifugation and filtration. The type of separation depends on the complexity of the food matrix. This technique has been used in fruit juices, cheese, soy sauce, cider, soft drinks, salad dressings, and fresh and dried vegetables. CE is another method that is being widely utilized for organic acid analyzes. The method is relatively fast with typical analysis time of 5 min. Kits are available from a number of suppliers and contain pre-prepared reagents and standards, which provide reproducible separations. The separations are performed on standard CE equipment and capillaries, which may prevent the need to purchase specific equipment and consumables such as ion exchange chromatography systems and columns. This method has been proposed to determine organic acids in fruits, juices, nectars, wines, beer, and honey.

Sulfur Dioxide and Sulfites

Sulfur dioxide, sulfite, and metabisulfite are included in this group of preservatives. SO_2 is a broad spectrum antimicrobial to inhibit bacteria, yeasts, and molds. It is especially used in the fermentation of wine to prevent malolactic fermentation. Sulfites are used as preservatives in foods and beverages to prevent oxidation and bacterial growth. Sulfites are also used to prevent enzymatic and nonenzymatic browning in raw, dried, frozen, and canned vegetables and fruits. The Codex General Standard for Food Additives allows the use of SO_2, sulfite, and metabisulfite in the following products: fresh, frozen, fermented, and dried fruit; fruit products; fresh, frozen, fermented, canned, and dried vegetables; starches; precook pastas; fish and certain shellfish products; sugar products; herbs and spices; seasonings and condiments; vinegars; mustards; sauces; beer and malt beverages; cider; wines; water-based flavored drinks (including 'sport,' 'energy,' or 'electrolyte'); distilled spirituous beverages containing more than 15% alcohol; aromatized alcoholic beverages; and snacks. The maximum concentration permitted is in a range from 15 to 1000 ppm and depends on the product.

There are many methods for analyzing sulfites in beverages and foods, involving titration, electrochemistry, fluorimetry, CL spectrometry, colorimetric, gas–liquid chromatography, and HPLC, among others. The most commonly used techniques are based on the classic Monier–Williams method. Although this method has sufficient sensitivity, it also presents certain limitations due to the long analysis time (with important repercussions on the analytic capacity of the laboratory) and the extreme control needed in some of the analytic phases (e.g., N_2 stream adjustment). AOAC official methods of analysis for sulfites are presented in Table 6.

Faster methods are being investigated; ion exchange chromatography with column and conductivity detector has been proposed to determine free sulfites in dried fruits processed with sulfur dioxide. This method uses aqueous 0.2 N NaOH ($pH > 13$) as the solvent for standard preparation and sample extraction. Five different fruit matrices (apricot, golden grape, white peach, fig, and mango) were successfully tested and analyzed with this method. Nowadays, the most common technique proposed for determining sulfites is HPLC (reversed-phase

ion-pairing liquid chromatography with postcolumn detection or HPLC–fluorescence spectrometry).

Nitrates and Nitrites

Nitrates and nitrites are used as curing and antimicrobial agents and in cured products. Their main purpose as an antimicrobial agent is to inhibit the growth of *Clostridium botulinum* and the production of botulinum toxin in these cured products. Its use is restricted to these products. There are four permissible salts of these compounds for use in foods: sodium nitrate, potassium nitrate, sodium nitrite, and potassium nitrite. The use of nitrite and nitrate in the processing of fish products is specified in title 21 of the CFR: potassium nitrate can be used as a curing agent in the processing of cod roe, without exceeding 200 ppm in finished roe (21CFR172.160). Furthermore, sodium nitrate can be used as a preservative and color fixative, with or without sodium nitrite, in smoked, cured sablefish; smoked, cured salmon; and smoked, cured shad and in meat-curing preparations for the home curing of meat and meat products (including poultry and wild game) so that the level of sodium nitrate does not exceed 150 ppm and the level of sodium nitrite does not exceed 150 ppm in the finished product. Sodium nitrite has the same specifications as sodium nitrate. In addition, sodium nitrite could be use as color fixative in smoked, cured tuna fish products without exceeding 10 ppm in the finished product. Sodium nitrite can also be used in processing smoked chub; the maximum content in the edible portion of the finished smoked product is 150 ppm, as measured in the loin muscle. Procedures for calculating the amount of nitrite and nitrate that can be added to curing solutions and meat products are specified by the USDA.

Since control and regulation of nitrates and nitrite use in meat products is crucial to ensure consumer safety, various methodologies have been utilized to determine nitrates and nitrites, including ion chromatography, CE, differential pulse voltammetry, enzymatic reduction, and electrochemistry. The extraction of nitrate and nitrite from meat products is critical in terms of obtaining adequate recoveries and reducing possible interferences. When nitrite is added to a biological material, such as meat products, it reacts with various components present naturally in the matrix, which means that the levels of nitrite analytically detectable (known as residual) necessarily vary. Some compounds in meat products (ascorbic acid, fat, protein, etc.) that can interfere in the determination of nitrates and nitrites may be present in the extract, which is why purification or clarification steps are necessary. Several clearing techniques have been applied for the analysis of nitrite and nitrate in food, especially in meat products; these are based on deproteinization and clarifying agents, using reagents, such as potassium aluminum sulfate, borax (anhydrous sodium tetraborate), mercuric chloride, zinc sulfate, or potassium hexacyanoferrate plus zinc acetate. Other clearing techniques include active carbon or gel filtration on Sephadex G-25, the latter used for beverages only.

AOAC official methods established one method to determine nitrates and nitrites in meat (935.48 xylenol method), one method to analyze nitrites in curing preparations that includes dry cure mix and curing pickle (964.13 titrimetric method), and one method for nitrites in cured meat (973.31 colorimetric method). These methods are simple.

Nisin

FAO/WHO describes nisin A as an antimicrobial preservative with chemical formula $C_{143}H_{230}O_{37}N_{42}S7$ and molar mass of 3354.12 g mol^{-1}; it physically appears white to light brown micronized powder, is soluble in water and insoluble in nonpolar solvents, and is not less than 900 IU of nisin per milligram. The assay method consists in a bioassay that compares the activity of a standard solution against unknown sample by fluorescent light. FDA permits its use to inhibit the outgrowth of *C. botulinum* spores and toxin formation in pasteurized cheeses; its maximum concentration is determined by GMP (<250 ppm in the finished product). The Codex General Standard for Food Additives permits its use in cheeses (12.5 ppm max. level), clotted cream (10 ppm max. level), and cereal and starch desserts (3 ppm max. level). Many more applications have been suggested in foods like fresh pizza, salad dressings, soy and vegetarian products, pasteurized liquid egg, fruit juices, fish and shellfish, and meat products. Nisin antimicrobial spectrum is against Gram-positive bacteria. FDA established the quantitative method for nisin, the one designated by British Standards Institution 'Methods for the estimation and differentiation of nisin in processed cheese.' Alternatively, there are several suggested methods for quantification in foods (milk, cheese, liquid egg, canned tomatoes, and salad dressings), which include horizontal agar diffusion, immunochemistry, nisin-induced reporter gene expression, and turbidimetric bioassay. The widely used agar diffusion assay is not able to distinguish nisin from other interfering substances present in the food, causing false-positive results. Although immunochemical methods are more sensitive than the agar diffusion assay, they are not totally reliable, due to cross-reactions with compounds structurally related to nisin that can be present in the testing material.

Natamycin

Natamycin, also known as pimaricin is defined as a fungicidal antimycotic of the polyene macrolide group. It is produced by several species of *Streptomyces*. The commercial product may contain up to three moles of water. Its chemical formula is $C_{33}H_{47}NO_{13}$; its molar mass is 665.74 g mol^{-1}; it is a white to creamy-white, almost odorless, crystalline powder; is practically insoluble in water, liquid, and mineral oils; and is slightly soluble in methanol and soluble in glacial acetic acid and dimethylformamide. The assay method is by HPLC with a column packed with octadecylsilanized silica and ultraviolet detector measuring at 303 nm. FDA permitted its use in cheese in amounts not exceeding 20 ppm in the finished product. FAO/WHO permits its use in unripened, ripened, processed, analogue, and whey protein cheeses at maximum level of 40 ppm and in cured and dried non-heat-treated processed meat, poultry, and game products in whole pieces or cuts at a maximum level of 20 ppm. Other applications are suggested for natamycin in fruit juices, bakery products, olives, and wine.

FDA established as quantitative the one method designed by International Dairy Federation (IDF) Standard 140A:1992, 'Cheese and cheese rind – determination of natamycin content: method by molecular absorption spectrometry and by HPLC.' Other suggested methods are ultraviolet spectrometry, liquid chromatography, and HPLC for natamycin in cheeses.

Parabens

Methyl, ethyl, propyl, butyl, and heptyl esters of *p*-hydroxybenzoate are denominated parabens. They are odorless, almost colorless crystals or white crystalline powders. Water solubility is inversely related to alkyl chain length. The pH range of antimicrobial activity is 3–8; their antimicrobial activity is mainly against bacteria and fungi. Combinations of two or more parabens are often used since they have synergistic effects. FDA sets the maximum level of application of methyl (21CFR184.1490) and propyl (21CFR184.1670) esters of *p*-hydroxybenzoic acid at 0.1%. Both methylparaben and propylparaben are permitted as antimycotic agents in food packaging material (21CFR181.23). Heptylparaben (21CFR172.145) is also approved for use in fermented malt beverages at a maximum of 12 ppm and in noncarbonated soft drinks and fruit-based beverages at a maximum level of 20 ppm. FAO/WHO permits the use of methylparaben, ethylparaben, and propylparaben in foods. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established the assay method of methylparaben and ethylparaben as a titrimetric method. Regulatory agencies have not established an official method for its determination in foods. Nevertheless, a great number of methods are proposed to quantify parabens in foods, such as spectrophotometry, injection gas chromatography, HPLC–mass spectrometry, and CE with amperometric detection.

Dimethyl Dicarbonate

Dimethyl dicarbonate ($C_4H_6O_5$) is described by the FAO/WHO as a colorless liquid with a molar mass of 139.09 g mol^{-1} and soluble in water and miscible with toluene. The method of assay is titrimetric; dimethyl dicarbonate (DMDC) is mixed with an excess of diisobutylamine, which it reacts quantitatively. The excess of amine is backtitrated with acid. DMDC has antimicrobial activity against yeast and bacteria. FDA approves the use of DMDC in certain beverages in which the microbial population has been reduced to 500 microorganisms per ml or less by current GMP; in wine, dealcoholized wine, and low-alcohol wine at maximum of 200 ppm; and in ready-to-drink teas at a maximum of 250 ppm. For carbonated or noncarbonated, non-juice-containing ($\leq$1% juice), flavored and unflavored beverages containing added electrolytes (5–20 mequiv.l^{-1} Na+ and 3–7 mequiv.l^{-1} K+), a maximum of 250 ppm is permitted, while for carbonated, diluted beverages containing juice, fruit flavor, or both, in which the juice content does not exceed 50%, a maximum of 250 ppm. FAO/WHO permits its use in water-based flavored drinks including 'sport,' 'energy,' or electrolyte drinks and particulate drinks, coffee, coffee substitutes, tea, herbal infusions, grain beverages, cider, perry, and wines at a maximum of 250 ppm and in grape wines and mead at a maximum of 200 ppm. AOAC official methods are not available for the determination of DMDC in food. The FDA designates gas chromatography to determine DMDC.

Lysozyme

Lysozyme is defined by FAO/WHO as a polypeptide obtained from hen's egg whites consisting of 129 amino acids, having a molar mass of about 14 000 g mol^{-1} and an isoelectric point of 10.7 and possessing enzymatic activity to hydrolyze the β(1–4) linkages between *N*-acetylmuramic acid and *N*-acetylglucosamine in the outer membranes of bacterial species, in particular Gram-positive organisms, usually obtained in the hydrochloride form for food use. It is described as a white, odorless powder, as a preservative to prevent the late blowing of cheese caused by *Clostridium tyrobutyricum*, and as an assay $\leq$950 μg mg^{-1} and is soluble in water and insoluble in organic solvents and in concentrated saline solutions. The assay method is based on the changes of turbidity of a suspension of *Micrococcus luteus* ATCC 4698. Codex General Standard for Food Additives permits lysozyme use in ripened cheese, and maximum level is according to GMP, while for cider, perry, and grape wines, a maximum of 500 ppm is permitted. Several food applications have been suggested for lysozyme in meat products, dairy products, seafood, tofu, vegetables, kimchi, sake, and wine. Official methods are not available for the determination of lysozyme in foods. Recent studies show that online capillary zone electrophoresis and mass spectrometry can be used as a method for quantifying lysozyme in cheese.

See also: Acids: Properties and Determination; Bacteriocins; Bioactive Peptides in Foods; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Consumer Protection Legislation; Food and Agriculture Organization of the United Nations; Infrared Spectroscopy: Applications; Natamycin; Nitrites and Nitrates; Preservation of Foods; Preservatives: Food Use; World Health Organization.

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Preservatives: Food Use

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Introduction

Quality loss and spoilage of food products can be caused by a wide range of physical, chemical, enzymatic, and microbial reactions. Food preservatives are used to avoid spoilage during storage, distribution, retailing, and consumption. They are compounds that have been intentionally added to avoid deteriorative reactions and thus protect quality and extend the shelf life of foods and beverages. Preservatives are used in controlled amounts and usually at low levels, ranging from parts per million (ppm) to 1–3% by weight. Many food preservatives are multifunctional, serving to help extend shelf life by inhibiting oxidation, browning, or enzymatic reactions and to ensure microbial safety by inhibiting or killing pathogens as well as spoilage microorganisms. Food preservatives may be extracted from natural sources or synthetically produced.

Several factors have to be considered when choosing an appropriate preservative for a specific food product, including the type of microorganism present (pathogen or deteriorative), pH, composition, physical state and, product shelf life required, use, and application. It is important to also consider their possible effects altering organoleptic properties of the product like flavor or color.

Antimicrobial Agents

Microbiological damage to food products is by far the most economically and important problem related with food losses; a loss of about one-quarter of the world's food supply due to microbiological activity is estimated annually. The Food and Drug Administration (FDA) categorizes an antimicrobial agent as a substance used to preserve food by preventing growth of pathogenic and deteriorative microorganisms and subsequent spoilage. Most available antimicrobial agents do not fully inactivate microbial populations, but rather work as growth inhibitors providing inhospitable environments for microorganisms avoiding the microbial development. Antimicrobial activity of specific agents may vary widely within different food systems, thus requiring experimental validation in the food product intended for its use.

Acids are the most effective and widely used preservatives. Among the most used are the weak lipophilic organic acids such as sorbate, benzoate, and propionate or the inorganic ones such as sulfite or nitrite, all of which are most effective at pH values lower than about 5.5. There are no antimicrobial preservatives with sufficient activity at varied pH values, with the exception of parabens. Emerging concerns on the extensive use of these types of preservatives and their effects on human health as well as their inefficacy in pH levels closer to neutrality have generated a market demand for natural alternatives.

Acidulants and Organic Acids

The pH and acidity of foods play an important role in the type of microorganisms that may survive as well as their ability to proliferate and cause spoilage reactions. Microorganisms have defined pH ranges for growth and survival. Acidity in food products may be increased artificially to prevent or suppress microbial growth. Generally, spoilage bacteria and pathogens will not proliferate in pH values lower than 3 or 4, with yeasts and molds able to survive at pH values up to 2.

Acidulants and organic acids constitute the two major types of acidifying agents used to prevent food spoilage, each with different modes of action. Acidulants are acids employed to increase acidity in food products by decreasing the pH of the food matrix, whereas weaker organic acids can have an important effect in decreasing internal cytoplasmic pH. Fully dissociated acids increase proton concentrations and result in decreased pH values, negatively affecting cell walls, bacterial outer membranes, and surface membrane proteins. Protons, however, are insoluble in lipids and diffuse slowly through the phospholipid bilayers of the cell membrane, not affecting microorganisms from changes in cytoplasmic pH levels. Extracellular pH values sufficiently low to kill yeast and bacteria with acidulants would render food unpalatable, so other agents are used to effectively inhibit microbial proliferation without compromising organoleptic attributes.

Lipophilic weak acids are able to diffuse, with some actively transported through the microbial cellular membrane in their undissociated form. They have bacteriostatic and bactericidal effects below their pK values at a pH under 4.5. In the cytoplasm, at pH values near neutrality, weak acids dissociate and effectively lower cytoplasm internal pH. Changes in internal pH will cause protein misfolding, enzymatic inhibition, and metabolic alterations. Lowering internal pH also neutralizes the electrochemical gradient across the plasma membrane needed for the active transport of many essential nutrients. Alternative inhibitory mechanisms may include sequestration of metal ions and damage to membrane fluidity. Inhibition of *Clostridium botulinum* by citric acid is reversible by the addition of calcium and magnesium to the media, suggesting that inhibition was mainly as a result of the chelating properties of citric acid. For more hydrophobic acids with larger chains such as sorbic and benzoic acids, inhibitory action may be due to the effect dissolved free fatty acids have on the microbial membrane fluidity. All of these combined effects effectively control microorganism growth in foods and beverages.

The amounts required for effective antimicrobial activity vary greatly among acids. Organic acids and their salts such as benzoic acid inhibit yeast at pH 4 at concentrations of 2–6 mM, whereas acidulants, like citric acid, are required at levels of above 400 mM. The most common organic acids used as preservatives are (a) benzoates (sodium benzoate) used at concentrations from 0.01% to 0.10%, (b) sorbates (sorbic and potassium sorbate), 0.03–0.10%, and (c) propionates (sodium and calcium propionate), 0.20–0.50%. Commonly used acidulants with antimicrobial activity are acetic, citric, fumaric, lactic, and phosphoric acids. It is important to consider not only the antimicrobial properties of acidulants but also their

flavor profiles given that they are used at far greater amounts and may have a considerable effect on the palatability of the product. A combination of acidulants along with weak organic acids or their salts may be used to potentiate antimicrobial activity.

Lactic, citric, and acetic acids are permitted for their use in the European Union with the E numbers E270, E330, and E260, respectively. Lactic acid is most commonly used as a food additive in fermented meat and dairy products, carbonated drinks, dressings and sauces, and pickled vegetables. Citric acid can be found in nonalcoholic drinks, jams and jellies, sauces, cheese, and canned vegetables. Citric acid may also be used in bakery products, dry soup, or cake mixes but requires special packaging care due to its high hygroscopic properties. Acetic acid is mainly used in pickles, chutneys, sauces, mayonnaise, and dressings.

Parabens

Parabens are a group of esterified molecules of hydroxybenzoic acid at the C-4 position used as food, pharmaceutical, and cosmetic preservatives due to their antimicrobial effect. Methyl paraben occurs naturally in cloudberry, yellow passion fruit, white wine, and bourbon vanilla. Commercially used parabens are artificially synthesized through the esterification of *p*-hydroxybenzoic acid with methyl, ethyl, propyl, butyl, heptyl, and benzyl groups. While longer-chained parabens possess greater antimicrobial properties, they also exhibit decreased water solubility, thus making the shorter-chained methyl and propylparabens better suited for their application in foods.

Parabens are effective against the proliferation of yeasts and molds at much lower concentrations than weak acids, although they are less active against bacteria, especially Gram-negative. Unlike benzoic acid, from which they are derived, parabens retain their efficacy at pH levels of up to 8.0, making them a wide-spectrum antimicrobial agent and a popular choice to use in foods closer to a neutral pH. Parabens are used in cakes, pastries, pie crusts, icings, toppings, and fillings (0.03–0.06%); soft drinks (0.03–0.05%); creams and pastes (0.1%); jams and jellies (0.07%); olives and pickles (0.1%); and syrups (0.07%). Combinations of methyl and propylparabens are commonly employed to achieve better antimicrobial results.

Parabens antimicrobial activity is generally considered to be pH-independent under values of 8.0 where they are fully undissociated. No evidence has been shown linking parabens to a specific target, but experimental results suggest they act upon the cell membrane and affect the functionality of enzymes. Possible mechanisms include interactions with membrane proteins and generation of disorder on membrane lipids.

The FDA, the European Community, and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have been approved the use of parabens in food, and they are generally recognized as safe (GRAS). Parabens are rapidly metabolized without accumulating in the body, are relatively nonirritating and nonsensitizing, and present low toxicity. Recent studies have raised concerns about the use of parabens, with special focus on propylparaben as possibly having estrogenic potential; however, no conclusive evidence suggests any risk to people under current used levels.

Sulfur Dioxide and Sulfites

Sulfur dioxide (SO_2) has long been used as a food additive due to its multifunctional properties. SO_2 and soluble sulfite salts are employed as antimicrobial and antioxidant agents, enzymes, and Maillard reaction inhibitors. Sulfites are most commonly applied in drinks (alcoholic and nonalcoholic), fruits, and vegetables. In wine production, sulfites play a major role by inhibiting growth of yeast and other spoilage microorganisms, while wine yeast are relatively unaffected. Another major application is in dehydrated fruits and vegetables preventing oxidation and microbial growth.

Various similarities between sulfite salts and organic salts exist regarding their mode of action. As with organic acids, in low pH foods, sulfites are used to prevent the proliferation of yeasts and molds, and their activity is pH-dependent. Unlike organic salts and acids, sulfites are effective antimicrobial agents against Gram-negative bacteria. This advantage is employed in meat products to avoid pathogens' growth. It is important to note that the efficacy provided by sulfites' high reactivity also makes them susceptible to easy degradation. Sulfites may be rapidly oxidized into sulfates with no antimicrobial or antioxidant properties, which should be considered for shelf life evaluation. No unique cellular target for sulfites is responsible for their antimicrobial activity, but rather a combination effect over several metabolic systems.

Approved forms of sulfite additives are SO_2, sodium sulfite, sodium bisulfate, sodium metabisulfite, potassium metabisulfite, calcium sulfite, calcium bisulfate, and potassium bisulfate. Concentration of sulfite additives is evaluated in terms of SO_2 equivalents, since sulfite salts release molecular SO_2, and the concentration in foods is highly dependent on the maximum approved levels for specific products. SO_2 concentrations range from 10 ppm in wines to a maximum of 2000 ppm in dried fruits. GRAS status in the United States has been revoked for certain applications of sulfites, mostly when added to fresh products. These regulations are also prevalent in the European Union, and in the past decades, legislations controlling the approved amounts employed in different foods have been reformed internationally to lower daily intake and human exposure.

Natural Antimicrobials

The use of natural antimicrobial compounds in food has acquired great attention from the consumers and food industry. The potential negative impact of synthetic preservatives on health, the benefits of natural preservatives, and the consumers' demand for natural and minimally processed food have generated interest among researchers in the development of natural products used as preservatives. Some natural compounds with antimicrobial activity can be obtained from plants, animals, bacteria, fungi, and algae. Nisin, natamycin, and essential oils are some natural preservatives used in food processing. Nisin a bacteriocin and natamycin a polyene antibiotic are both obtained from bacteria, while essential oils are plant-derived.

Plant-derived antimicrobials

Throughout history, herbs and spices have been attributed with functional characteristics such as medicinal and

antimicrobial effects. Research in this area has proven the microbiological efficacy of many plant-derived compounds. Essential oils are considered as one of the most promising natural alternatives to synthetic preservatives. They consist of aromatic and volatile oily extracts obtained from different plant tissues, including flowers, buds, roots, bark, and leaves. Essential oils from thyme, clove, balm, ginger, oregano, rosemary, basil, coriander, citric fruits, marjoram, and lemon grass have shown a greater potential to be used as antibacterial and antifungal agents. Given that essential oils have been extensively used for centuries in food products, they are classified as GRAS and present very low risk of microorganism resistance development. Despite their approval for use in the United States as well as in Europe, currently few commercially available essential oils are marketed as food preservatives; nonetheless, their usage is expected to rise with the trending demand for natural and minimally processed foods.

Phenylpropenes and terpenoids constitute the majority of antimicrobial compounds found in essential oils, the most active being thymol, carvacrol, and eugenol. Molecular targets and mechanism of action are not yet fully understood, although many essential oil constituents have been proposed to act on the cell membrane. Aldehydes, such as cinnamaldehyde, and other nonphenolic substances may be responsible for important synergic activity as suggested by greater antimicrobial activity of crude essential oils compared to blends of their major individual components.

Concentrations needed to achieve significant antibacterial effect range between 0.5 and 20 $\mu l\ g^{-1}$ in foods and about 0.1–10 $\mu l\ ml^{-1}$ in solutions for fruit and vegetable washing. Microbial inhibition by essential oils has greatly focused on dairy and meat products where they may also impart flavor to the final product. Special considerations have to be taken when using essential oils as preservative agents. Given the hydrophobicity of many components in essential oils, application in foods with a high fat content may impair antimicrobial activity. As a result, much higher inhibitory concentrations are typically required to reproduce in food the effects observed *in vitro*. This has been reported from twofold in semiskimmed milk to 100-fold in soft cheese. In certain food products, the required inhibitory concentrations can strongly affect the product's flavor profile and even render unpalatable.

Animal-derived antimicrobials

Natural antimicrobials in animals are used as the first chemical barrier against bacterial and fungal infections. They are synthesized by all animals mostly in the form of peptides, polysaccharides, sterols, terpenes, prostaglandins, and fatty acid derivatives. Some of these compounds, such as lysozyme, chitosan, and lactoferrin, have applications in the food industry as food preservatives. Lysozyme and lactoferrin are bioactive peptides mainly extracted from hen eggs and bovine milk, respectively, whereas chitosan is a polysaccharide derived from crustacean shells and fungal cell walls.

Lysozyme is an enzyme naturally present in eggs and mammalian milk used for its bacteriolytic properties. Commercially available lysozyme is extracted from hen eggs and used as a preservative for meat and fish products, milk and dairy products, fruits, and vegetables. Lysozyme is predominantly used to prevent late-blowing defects in cheeses and has been approved by the FDA and in the European Union.

Antimicrobial activity results from lysozyme's ability to compromise Gram-positive bacterial cell walls by hydrolyzing the β-1, 4 linkages between *N*-acetylglucosamine and *N*-acetylmuramic acid from peptidoglycan. Synergic activity against Gram-positive bacteria has been reported when lysozyme is applied in combination with chelating agents (EDTA). In cheese production, lysozyme is used in concentrations of 40–500 $mg\ kg^{-1}$ of product.

Another animal-produced peptide is lactoferrin, an 80 kDa iron-binding glycoprotein, which possesses strong chelating properties. Its application as preservative has been reported in a variety of meat and dairy products. Lactoferrin is naturally occurring in mammalian milk, with concentrations in human milk of nearly ten fold the amounts found in bovine milk. Lactoferrin has strong antimicrobial activity against a wide spectrum of microorganisms such as bacteria, fungi, yeasts, and viruses. As a chelating agent, it restricts the amount of iron available for microbes in food products, resulting in decreased bacterial counts and limited growth.

The European Food Safety Authority approved the use of bovine-extracted lactoferrin in 2012, but so far, it has not received safe recognition by the FDA. Maximum levels of bovine lactoferrin for foods vary from 0.66% for baby foods, 4.0% in energy bars, 0.1% for infant formula, 0.12% for nonalcoholic beverages, 0.2% for dairy products, to 0.3% for sports nutrition beverages.

In addition to bioactive peptides, polysaccharides have gained considerable attention for their functional properties. Chitosan, a deacetylated derivative of chitin, is a natural antimicrobial agent with promising applications in the food industry as an additive or processing aid and in active packaging. Chitosan applications, however, are limited by its insolubility at neutral or higher pH. As a preservative agent, most research has focused on bioactive chitosan films alone or in combination with antimicrobials such as nisin, thymol, carvacrol, and eugenol. In winemaking, chitosan is employed to remove suspended solids, heavy metal content, and contamination microorganisms, especially *Brettanomyces*.

Chitosan is obtained from crustacean shells or extracted from the cell wall of *Aspergillus niger*. Both forms have been approved as processing aids and food additives in the European Union, the United States, Japan, Australia, and New Zealand. Antimicrobial activity of chitosan has been reported effective against spoilage yeasts and bacteria, showing stronger bactericidal effects against Gram-positive bacteria. At 0.025%, water-soluble chitosan derivatives are able to cause extensive cell surface damage *in vitro*. Applied in beverages, 0.3 $mg\ ml^{-1}$ effectively eliminated yeasts for 13 days in pasteurized apple juice.

Bacterial-derived antimicrobials

Evolutionarily, several microorganisms have developed antimicrobial defense mechanisms. Antibiotics, which have been extensively obtained from molds such as penicillin, are one of many examples. Bacteriocins are antibacterial peptides or proteins produced by lactic acid bacteria (LAB). They differ from antibiotics in their biological synthesis, application, spectrum, and mode of action. Unlike antibiotics, which are produced as

secondary metabolites, bacteriocins are peptides of ribosomal synthesis with a narrow spectrum of activity and no known toxicity. Bacteriocins possess multiple desirable properties as food preservatives. They exhibit a broad antimicrobial spectrum against spoilage and pathogenic bacteria and bactericidal activity, are inactivated by digestive proteases, and present no toxicity to eukaryotic cells. Despite a large quantity of recognized bacteriocins, so far, nisin is the only one permitted as a food preservative. Since nisin is naturally produced by starter cultures in many fermented meat products such as sausages, it has been unknowingly consumed for centuries, giving it a distinction of GRAS. Few countries have imposed maximum levels for the use of nisin at values around 10 mg kg^{-1}.

Application of bacteriocins in preservation techniques can be in the form of coculturing bacteriocinogenic LABs in food products or nisin as a food additive. LABs are utilized in table olives, sourdough, miso, sauerkrauts, refrigerated pickles, mung bean sprouts, fermented sausages, and cheese. As a food additive, nisin is used in cheeses, meat products, and kimchi. Nisin is commonly incorporated into foods as a concentrate from dairy cultures rather than a purified additive.

Natamycin, also known as pimaricin, is a polyene antibiotic produced by aerobic fermentation of *Streptomyces natalensis* used to control fungal growth with no efficacy against bacteria or viruses. Due to its extremely low solubility in water, natamycin is primarily applied as a surface preservative for cheese and meat products. Natamycin has been used for decades and is approved as a preservative agent in the European Union, Latin America, and the United States. Despite its approval in meat and cheese products in Europe, its application in the United States is limited to cheese in amounts not exceeding 20 ppm.

Polyene antibiotics damage eukaryotic cells through interactions with membrane sterols such as ergosterol. Natamycin binds irreversibly to ergosterol in yeasts and fungi, which increases permeability and results in rapid cell leakage. While fungal resistance to natamycin has been reported in research, no resistant strains have been observed from its commercial use. Natamycin may be used in hard, semihard, and soft cheeses. Its actions are to surface level and it presents very limited diffusion, allowing desired fungal species in cheese maturation such as *Penicillium* to remain unaffected. Cheese is typically immersed in a 0.2–0.4% aqueous solution before maturation. When used in meat products such as salami and other dry sausages, natamycin is commonly applied by immersion or spray with aqueous solutions of 1–4 g l^{-1}. Maximum final surface concentration for any product should not exceed 1 mg dm^{-2}.

Incorporation of antimicrobials to packaging material is considered as a promising area with major interest in the use of natamycin-based coatings. Several studies have been carried out on the potential use of natamycin, either by itself or in conjunction with organic acids or nisin, as a preservative in alginate, pectin, and other polymer films.

Antioxidants

Oxidative reactions produce substances that affect flavor, odor, and color, decreasing shelf life and consumer acceptance of food products. Oxidation may be minimized by the removal of compounds such as metals and free fatty acids, along with the protection from air and light. A more practical approach consists in the addition of antioxidants, substances employed to preserve food by retarding deterioration, rancidity, or discoloration. Enzymatic browning and lipid rancidity are the two most significant forms of oxidative problems in foods. Browning is caused by the enzymatic oxidation of phenolic compounds by polyphenol oxidase (PPO) in fruits and vegetables, whereas oxidative rancidity results from the decomposition of unsaturated fatty acids in lipid-containing foods.

Enzymatic Browning Inhibitors

Enzymatic browning in foods and beverages is a key factor contributing to quality loss. Browning is a major challenge in minimally processed vegetable products such as ready-to-use salads, fresh-cut fruit, and fresh juices. Antibrowning agents may be added to foods and beverages or used as pretreatments to prolong shelf life and avoid changes in coloration.

Browning in fruits and vegetables is caused mainly by the enzyme PPO, which in the presence of oxygen catalyzes the oxidation of *o*-diphenols to *o*-quinones. Brown pigments are produced by the polymerization of *o*-quinones. Ascorbic acid, citric acid, sodium chlorite, and chelating agents are commonly used PPO inhibitors added to foods to prevent browning. Ascorbic acid, acting as a reducing agent and a competitive enzymatic inhibitor, reduces *o*-quinones back to *o*-diphenols preventing the formation of brown pigments. Apple slices developed only moderate browning after 14 days of storing at 4 °C following a 2 min bath in a 0.5 M ascorbic acid solution, and its use in fruit-based beverages, at concentration of 0.1%, inhibits browning. Other inhibitors such as cysteine and sulfites have been reported, but their use is legally restricted.

Lipid Antioxidants

Oxidative rancidity produces low-molecular-weight compounds that negatively affect a product's odor and flavor potentially producing toxic by-products. Rancidity in foods can vary greatly, ranging from noticeable changes in flavor, loss of color and change in texture, to subtler decreased 'freshness.' Nitrites, sulfites, and synthetic phenolic compounds (SFCs) butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) are the most commonly used antioxidants in lipid-containing foods. Increased demand in natural products in addition with allergenic response to some of these agents has led to an increased use of natural antioxidants such as α-tocopherol (vitamin E), phenolic acids, phenolic diterpenes, flavonoids, and volatile oils.

Oxidation of lipids is provoked mainly from autoxidation, photosensitized oxidation, and thermal oxidation, all of which affect double bonds in unsaturated fatty acids. Once lipid peroxy radicals are formed due to heat, metals, light exposure, or reactive oxygen species, they may catalyze a free radical chain reaction. Phenolic compounds function as free radical scavengers. Each molecule of BHT, for example, is able to react with two alkylperoxy radicals, which normally act as chain propagators, thus preventing further oxidation. Phenolic

compounds are able to donate hydrogen to free radicals and remain as relatively stable radicals due to resonance delocalization by the phenolic ring. Some natural antioxidants, due to the multiple rings and arrangement of –OH in their structure, also serve as chelating agents.

Synthetic phenolic compounds

BHA, BHT, and TBHQ are the most widely used food antioxidants due to their low cost, high performance, and wide availability. They can be found in a great variety of products containing lipids. TBHQ, BHA, and BHT are approved food antioxidants in the European Union, the United States, Australia and New Zealand, and many other regions. TBHQ, BHA, and BHT can be found in oils, margarines, and fat-containing products. A major advantage in SFCs is their stability at high temperatures, retaining their antioxidant capabilities during and after baking and frying. While effective in both fats and oils, BHA and BHT are more recommended for use in fats, whereas TBHQ has a broader application in vegetable oils. Their use is highly regulated, and usually, up to 100–200 $\mu g\ g^{-1}$ of SFCs is allowed either singly or in combination.

Natural antioxidants

Compounds from natural sources used as antioxidant agents in foods include phenolic acids, flavonoids, tocopherols, and carotenoids. They inhibit oxidation by acting as reductants, chelating agents, and free radical scavengers with strong H-donating capacity. The most commonly used natural antioxidant is α-tocopherol, followed by herbs and spices with high concentrations of phenolic compounds. Vitamin E is mainly composed of α-tocopherol, a fat-soluble carotenoid with antioxidant capacity. Vitamin E adoption commercially as an antioxidant agent is extremely popular given the consumer impression of added health and wellness to food products.

Herbs with antioxidant properties are normally within the *Lamiaceae* family, including rosemary, basil, oregano, marjoram, sage, and thyme, all containing rosmarinic acid. Rosmarinic acid, a phenolic acid with 2 aromatic rings and 2 –OH groups in each ring, is capable of chelating metals and donating H+. As a free radical scavenger, rosmarinic acid is more potent than BHA, BHT, TBHQ, and α-tocopherol. Herbs' potential as antioxidants is not limited to their purified components. Polar extracts of different salvia species, including sage, have demonstrated antioxidant activities similar to BHT. For spices, oleoresins and extracted compounds show promising activity, with special focus on cinnamon, nutmeg, clove, cumin, and turmeric. These spices are rich in the phenolic acids of gallic acid and caffeic acid, as well as a wide range of volatiles like pinene, linalool, and cymene.

Conclusions

Food preservatives are an essential constituent of processed foods serving the purpose of extending the shelf life of products and preventing deterioration. While this has led to a ubiquitous use of highly characterized synthetic preservatives, governments and research centers have become increasingly concerned with their possible effects on human health. A public demand for 'more natural' preservative technologies and minimally processed foods has seen an unprecedented increase. As alternative antimicrobial and antioxidant agents are increasingly adopted and approved for their use by international regulators, some of the major challenges to arise will be focused in the discovery of broad-spectrum agents, which are able to maintain the highest standards in food safety.

See also: Acids: Natural Acids and Acidulants; Acids: Properties and Determination; Antioxidants: Characterization and Analysis; Ascorbic Acid: Physiology and Health Effects; Ascorbic Acid: Properties, Determination and Uses; Bioactive Peptides in Foods; Browning: Enzymatic Browning; Essential Oils: Isolation, Production and Uses; Food Additives: Classification, Uses and Regulation; Food Fraud; Fungicides; Lactic Acid Bacteria; Natamycin; Niacin; Phenolic Compounds: Occurrence, Classes, and Analysis; Preservatives: Classifications and Analysis; Spices and Flavoring Crops: Uses and Health Effects.

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Probiotics

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Introduction: Definition of Probiotics

Microbes have been part of daily diet for centuries, but the concept of 'probiotics' has evolved relatively recently. In 1907, Élie Metchnikoff, the Russian-born Nobel Prize winner, suggested the use of beneficial microbes to replace harmful microbes and pathogens in the human gut. The term 'probiotic' was extensively acknowledged in 1960 by Lilly and Stillwell, who suggested probiotics as substances produced by microorganism that promote the growth of other microbes. Fuller defined a probiotic in 1989 as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal balance." Another definition was suggested by Havenaar and Huis in't Veld in 1992, which defines a probiotic as "a viable mono or mixed culture of bacteria which when applied to animal or man, beneficially affects the host by improving the properties of indigenous flora." Keeping all the recent developments and trends in consideration, FAO/WHO 2002 guidelines defined probiotics as "live microorganisms which when administered in adequate amount confer a health benefit on the host." Today, the question of successful fecal transplants is likely to further redefine the concept and future of probiotics as mixed cultures of microorganisms.

Common Probiotics

All probiotic strains marketed for human consumption should possess minimum properties laid down by WHO/FAO 2002, which focus on strain identification, functional characterization, safety assessment, and effective human trials.

It has been deemed desirable that probiotic isolates should be of human origin, that is, isolated from the mouth, gastrointestinal (GI) content, or feces, as these microbes could reach highest density in humans. As such, probiotics should ideally be members of normal healthy gut microbiota. For example, *Lactobacillus fermentum*, *Lactobacillus gasseri*, *Lactobacillus vaginalis*, *Lactobacillus reuteri*, and *Lactobacillus salivarius* have been isolated from the human stomach and showed promising probiotic properties. However, traditionally fermented milk and related products have also been explored for the isolation of lactic acid bacteria, bifidobacteria, and other microorganisms, including yogurt, cheese, kefir grains, Maasai milk, koumiss, and naturally fermented yak milk. Interestingly, breast milk has also been explored as a source of probiotic *Lactobacillus* spp. with immunomodulatory activity.

Conventionally, dairy products have been considered as both sources and excellent vehicles for probiotic microorganisms. Maasai milk, a traditional fermented milk from Kenya, was previously used for the isolation of probiotic *Lactobacillus* spp. that were subsequently applied as starter cultures. *Kule naoto*, or *kwerionik*, and *amasi* (*mukaka wakakora* or *zifa*), traditional fermented milks with probiotic properties, are produced and consumed in Kenya and Zimbabwe, respectively. Kefir grains are extensively used for the isolation of potential probiotic strain and in the preparation of fermented milk products. Cheeses from different countries, like Italy, Argentina, and Bulgaria, have also been used for the isolation of *Lactobacillus plantarum* strains that exhibited tolerance to relevant physiological factors, including survival in milk-based media. Yogurt is another example of traditionally prepared fermented milk product with high consumer acceptance. During the processing of yogurt, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* exhibit symbiotic relationship and are considered as probiotic microbes. Industrially, the incorporation of probiotic microbes in milk or related products should not affect the major characteristics of the final products. The latter include safety, fermentability, flavor, texture, and shelf life of the formulation. Other characteristics such as the oxygen content, redox potential, and water activity are also very important to ensure the survival of probiotic microorganisms during storage. Moreover, interactions between conventional starter cultures and probiotic microorganisms, that is, synergistic or antagonistic effects, could also affect the overall sensory properties, as well as the survival of probiotic microbes, both in the product and in the human GI tract.

Apart from milk-based probiotic formulations, there are many varieties of traditional beverages used worldwide for the isolation and characterization of probiotic bacteria with specific attributes. Several of these traditional beverages are cereal based. An example is *Yosa*, an oat-based fermented cereal product that has gained widespread acceptance in the Nordic countries and provides an alternative for lactose-intolerant subjects and those who avoid animal products, soy, or milk protein. The fermented *Yosa* product has been reported to normalize bowel movements in elderly nursing home subjects. *Bosa*, a Bulgarian beverage mainly made from cereals, has been used for the isolation of *Lactobacillus* spp. *Mageu* (*amaHewu*), a traditional South African nonalcoholic drink, is made from maize porridge and is predominated by *Lactococcus lactis* subsp. *lactis*, which could be further characterized for probiotic attributes. Worldwide, many other cases where cereal-based fermented beverages have been used for the isolation of probiotic microorganisms can be found, including *Pozol* (Mexico) and *Togwa* (Tanzania). Moreover, herb- or fruit-based probiotic beverages have gained popularity. *Hardaliye* is a grape-based fermented beverage and many lactobacilli were isolated and incorporated for the standardization of its production. Other than beverage-based probiotics, there are some evidences of using meat and vegetable as potential source for probiotic microorganisms. Furthermore, *Lactobacillus plantarum* strains were isolated from Indian traditional herbal beverages, such as *Kutajarista*, for probiotic characterization.

Due to recent advancements in high-throughput technologies for microbiota analysis, a great number of studies have highlighted microbial dynamics during fermentation and the possible role of probiotic microorganisms during fermentation,

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storage, and consumption. One illustrative example is the use of probiotic strain *Lactobacillus paracasei* IMPC2.1 during fermentation of green olives. In this fermentative process, the probiotic strains predominated in the total microbial flora and exhibited a dual role as starter and probiotic culture.

Types of Microorganisms

Lactobacillus and *Bifidobacterium* genera constitute the majority of the probiotics products available in the market at present. Moreover, most of them have a long history of safe use in food and are listed in the European QPS list and/or the US GRAS notification list as safe for specific food uses. Therefore, lactic acid bacteria and *Bifidobacterium* are probably the best models for probiotic use or health effect characterization. However, members of the genera *Streptococcus*, *Enterococcus*, *Saccharomyces*, *Bacillus*, *Escherichia*, *Propionibacterium*, and *Lactococcus* have also been explored for probiotic attributes. In each case, the assessment should start from the potential safety issues and then continue to the demonstration of efficacy and health benefits.

Lactobacillus and *Bifidobacterium*

The major *Lactobacillus* species that are considered or studied for probiotic attributes include *Lactobacillus acidophilus*, *Lactobacillus amylovorus*, *Lactobacillus casei*, *Lactobacillus crispatus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus gallinarum*, *Lactobacillus gasseri*, *Lactobacillus johnsonii*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, and *Lactobacillus rhamnosus*. Lactic acid bacteria are acid-tolerant and fermentative in nature and have been used as starter cultures in the majority of fermented foods. Lactic acid bacteria in general have the intrinsic capacity to tolerate low pH values and high bile concentrations and to adhere to intestinal cell lines. Molecular studies have revealed that a large proportion of their genomes are attributed to sugar utilization and transportation. Additionally, intestinal adaptation is also apparent, for example, with respect to the presence of mucous-binding protein and bile salt hydrolase genes in *Lactobacillus plantarum* and adhesion protein in *Lactobacillus acidophilus*. Moreover, *Lactobacillus casei* differentially expresses genes in the mouse intestine for a better adaptability to the intestinal conditions.

Bifidobacterium spp. have also been extensively used in product formulations and probiotic applications as they share many phenotypic characteristics with lactobacilli. In comparison with lactobacilli, bifidobacteria inhabit very limited niches, such as human and animal GI tracts and, in some cases, insect intestines. The major species that were isolated and studied include *B. adolescentis*, *B. angulatum*, *B. animalis* subsp. *lactis*, *B. bifidium*, *B. breve*, *B. catenulatum*, *B. dentium*, *B. longum*, and *B. pseudocatenolatum*. Genomic studies of *Bifidobacterium* strains revealed that they can utilize a broad range of complex carbohydrates such as inulin-type fructans, arabinogalactans, arabinoxylans, and starch. Exclusive operons have been evolved for specialized purposes, as the degradation of amylopectin by *B. breve* strains and the utilization of a broad range of simple sugars by *B. dentium*. *Bifidobacterium* strains also show changes at transcriptome and proteome level in intestinal conditions or gut environment.

Strain Selection and Health Benefits

As per recent WHO/FAO guidelines 2002, probiotic properties are possibly not only species-specific but also strain-specific. Similar, but binding regulatory guidelines have been given by the European Food Safety Authority (EFSA). Therefore, health benefits conferred by one strain cannot be extrapolated to another, not even within a same species.

Probiotics are typically selected for their ability to pass through the upper GI tract (characterized by low pH and high bile salt concentrations), to colonize the intestinal epithelium, and to express antimicrobial activity against pathogens. Additionally, all initial screening and detailed characterization should follow WHO/FAO guidelines. The major steps include strain identification using molecular tools, functional characterization using *in vitro* and *in vivo* models, double-blind controlled human trials, and formulation assessment and quality control.

Highlights of the WHO Guidelines (2002)

Strain Identity

It is now widely accepted that probiotic properties are strain-specific. Therefore, in addition to identifying probiotic microorganism till genus and species level, strain identity is considered to be one of the fundamental aspects of probiotic characterization. Biochemical characterization should be supplemented with genotypic characterization based on molecular tools for strain identification or differentiation. Whole genome sequencing of probiotic strains may act as reference for validation and commercialization. Similar guidelines are also given by EFSA.

History of Safe Usage and Recommendations

Strains used for probiotic characterization should be safe in each particular application, which could in principle be ascertained by identifying the respective source of isolation and history of use. However, there were some independent reports of infections that might have been caused by administration of probiotic strains. It has been recommended that probiotic strains should also be screened for antibiotic resistance, metabolic potential, and production of mammalian toxins. In general, probiotic strains isolated from traditional food or beverages are considered to be safe because of their long history of human consumption. Nevertheless, these probiotic strains are not exempted from the WHO guidelines including *in vitro* and *in vivo* tests.

Tolerance to GI Conditions

Survivability of any probiotic strain is characterized by its ability to withstand GI conditions like acidity and high bile concentrations. Probiotic strain may also be screened for antimicrobial potential against pathogens. Many studies have highlighted secretion of bioactive factors with potential to

inhibit a broad range of pathogens. Adherence to intestinal cell lining can be determined by *in vitro* adherence tests based on various intestinal cell line models and *in vivo* by different animal models.

Human Intervention Studies

Based on animal studies, human trials have to be designed to validate claimed health benefits and their biological and statistical significances. Apart from claimed health benefits, general well-being or improvement in health condition should also be taken in account. The different phases of a clinical study include phase 1 (safety), phase 2 (efficacy), phase 3 (effectiveness), and phase 4 (surveillance of potential side effects). However, in case of foods and food supplements, slightly less regulatory framework is required (i.e., in comparison with pharmaceutical product) for the demonstration of health benefits.

Health Effects

Apart from the positive effect of probiotics on general well-being, there are several particular clinical symptoms or conditions that are reported to be beneficially affected by the use of specific probiotics. Such conditions include diarrhea, gastroenteritis, inflammatory bowel disease, irritable bowel syndrome, Crohn's disease, and alleviation of symptoms of lactose intolerance (Figure 1).

Effects on Intestinal Disorders

Probiotics find applicability in diarrheal cases that could be caused by viral or bacterial infection or could be antibiotic-associated diarrhea. It should also be noted that efficacy of probiotic strains depends on the type of diarrhea. For instance, probiotics are found to be effective against rotavirus-associated diarrhea. The major probiotic strains used for clinical applications in children belong to *Saccharomyces boulardii*, *Lactobacillus rhamnosus*, and *Lactobacillus reuteri*. For instance, some studies highlight the use of *Lactobacillus rhamnosus* GG for *Clostridium difficile*-associated diarrhea and the prevention of the onset of pouchitis.

The possible mechanism could be due to an immunomodulatory role, probably by increase in secretory IgA. Some probiotic bacteria also secrete antimicrobial peptides, that is, bacteriocins, which could inhibit the growth of certain enteric pathogens. It is also believed that probiotic microorganisms compete with pathogenic viruses or bacteria for intestinal binding sites.

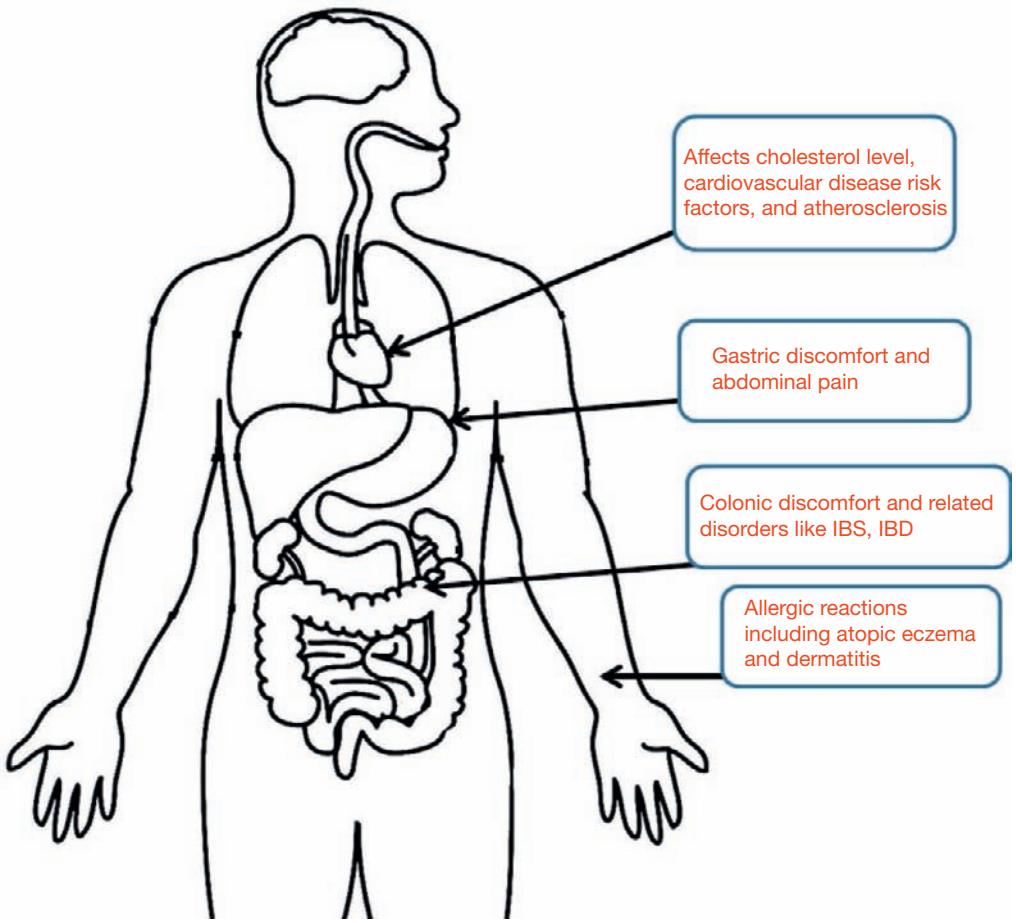

Figure 1 Major areas of studies for human health effects for probiotics: reduction of cardiovascular disease risk factors and allergies; alleviation of gastric discomfort, colonic discomfort, and related syndromes including IBS, IBD, and Crohn's disease.

Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are marked by high immune responses to gut microbes. Probiotics have found application in alleviating inflammatory conditions in the gut, which could be due to the stimulation of host immune response that may include immune cell proliferation, enhanced phagocytic activity, increase in secretory IgA, and shifts in overall microbial composition.

Some lactic acid bacteria, including *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, also may improve lactose intolerance through the production of lactase. Therefore, fermented dairy products like yogurt are widely recommended for regulating lactose intolerance.

In case of constipation, it is believed that lactic acid bacteria secrete organic acids that modify the intestinal microbiota and increase intestinal motility due to lowering of pH or shortening of transit time.

Prevention of Allergies

Probiotics may exhibit antiallergic effects by degradation or structural modification of enteral antigens, stabilization of aberrant microbiota, improvement of the gut-barrier function, regulation of the secretion of proinflammatory mediators, and development of the immune system. Probiotic intervention studies in children have shown that there is significant reduction in cumulative incidence of eczema and IgE-associated eczema. Evidence also suggests that probiotic administration during pregnancy may reduce the risk of eczema during the first year of life. In the case of food allergies, infants that were fed with *Lactobacillus rhamnosus* GG showed an increase in tolerance to cow's milk protein. Interactions between gut microbiota and probiotics have significant roles in immune regulation and predisposition to allergy. Moreover, present knowledge suggests that singular clinical reports cannot be extrapolated for general usage or as a therapeutic alternative for allergy treatment yet. Indeed, properties are strain-specific and the exact mechanisms of action remain to be elucidated.

Anticarcinogenic Activities

Several probiotic strains belonging to *Bifidobacterium* spp. and *Lactobacillus* spp. were claimed to have anticarcinogenic, antimutagenic, and antitumorigenic activities in different animal models. Short-chain fatty acids produced by the intestinal microbiota play very important roles in the maintenance of colonic mucosal health. Yet, probiotic strains in general do not produce anticarcinogenic butyrate, in contrast to gut bacteria from the clostridial clusters IV and XIVa. The latter butyrogenic bacteria may nevertheless be stimulated by the administration of certain probiotics through cross-feeding mechanisms. Mechanism for anticarcinogenic activity of butyrate may be due to the inhibition of proliferation, induction of apoptosis, or differentiation of tumor cells. Probiotics also influence carbohydrate and protein fermentation, enriching microbiota with high saccharolytic activity and low proteolytic activity. Proteolytic fermentation end products or metabolites are potentially toxic and microbial manipulation or dietary modification toward saccharolytic fermentation is highly desirable. However, there are very few studies that claim efficacy of probiotics, prebiotics, or synbiotics with potential anticarcinogenic activity in humans.

Use of Probiotic in Controlling Blood Cholesterol

Elevated blood cholesterol levels signify increased risks for cardiovascular and related diseases. It has been suggested that incorporation of probiotics in dietary supplements may help control serum cholesterol levels. *Lactobacillus acidophilus* and *Bifidobacterium lactis* that were incorporated in yogurt may thus lower total cholesterol levels in serum. *In vitro* assays revealed that lactobacilli can precipitate cholesterol from the media and deconjugate bile salts. However, the cholesterol-lowering capacity is possibly strain-specific and, more importantly, human intervention studies are not conclusive (Table 1).

Future Developments

The market currently offers a range of probiotic-based products and formulations with varied health benefits and claims, but several challenges still remain, not in the least on the legislative level. For example, there are a number of recommendations for probiotic application by scientific societies such as the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the corresponding society in North America (NASPGHAN) for probiotic use in treatment and prevention of specific diseases. However, in the European Union, no probiotic health claims for the general public have been approved yet. The discrepancy does not mean that probiotic products are not useful for the general population, but rather suggests that studies until now have been conducted in at risk populations or subjects with specific diseases. Further studies are thus required for the documentation of health claims. Furthermore, viability of probiotic strains to ensure adequate dosage is very important for industrial standardization. Still, food matrix composition and general processing condition are not mentioned on product labels, although they may affect efficacy, viability, or stability of probiotic strains. Encapsulation technology is very promising in maintaining the stability of probiotic strains in different food matrices. Also, the incorporation of probiotic strains should not affect overall flavor and texture of the marketed product. Therefore, it is important to meet technological and sensory demands for manufacturing probiotic-based formulations.

The efficacy of probiotics based on intervention studies should be evaluated by their clinical outcome and reduction of risk factor. In case of obesity, specific changes in gut microbiota can be promoted by probiotics, which may help in controlling fat mass and decreasing insulin resistance. Immunostimulatory activity of lactobacilli was reported to enhance immune function in case of elderly subjects. However, specific health claims of probiotics in control of obesity or immunogenicity have yet to be established. Meta-analysis of clinical trials reveals that it is extremely difficult to achieve consensus on general health claims as most trials are based on different strains or combinations of strains, used at different concentrations and in different target groups. Specific claims against major diseases, such as HIV infection or cancer, should be

Table 1 Some of the commercially available probiotic microorganisms

Probiotic strain	*Source of isolation and commercial application*	*Claimed benefits or applications*
Lactobacillus acidophilus LA-5 and *Bifidobacterium animalis* ssp. *lactis*, BB-12	LA-5 was selected by Chr. Hansen for the production of probiotic dairy products, which majorly includes infant formula, dietary supplements	LA-5 and *Bifidobacterium animalis* ssp. *lactis* BB-12 reduce diarrhea and constipation in elderly patients
Lactobacillus acidophilus NCDO 1748	Isolated from human pharynx and used in food products during 1970s. *Lactobacillus acidophilus* NCDO 1748 in fermented milk products were patented by Arla Foods	Prevents negative impact of antibiotics and immobilizes food mutagen
Lactobacillus acidophilus NCFM	Isolated at North Carolina Food Microbiology, that is, NCFM. Commercialized in supplements and fermented foods	Maintains intestinal health
Lactobacillus casei Shirota	Dr. Minoru Shirota isolated at Kyoto Imperial University's School of Medicine and later on developed fermented mild drink called Yakult	Beneficial effect on chronic constipation and short bowel syndrome (SBS)
Lactobacillus gasseri OLL2716 (LG21)	Warren and Marshall isolated in 1982 to find an alternative to antibiotic against *H. pylori* infection	Beneficial in *Helicobacter pylori* infection and gastric inflammation
Lactobacillus paracasei ssp. *paracasei*, F19	Isolated from healthy colonic mucosa. Used in fermented milk products like Cultura in Sweden and Denmark	Helps in stabilizing gut flora during antibiotic treatment
Lactobacillus paracasei ssp. *paracasei*, *Lactobacillus casei* 431	Isolated from feces of healthy child	Found effective in infant and young children diarrheal cases
Lactobacillus rhamnosus GG, LGG	Isolated from healthy human intestine and commercially available through Valio in Finland	Prevention and treatment of antibiotic-associated and traveler's diarrhea
Lactobacillus rhamnosus, GR-1, and *Lactobacillus reuteri* RC-14	GR-1 was isolated from the distal urethra of a healthy woman and RC-14 was isolated from vagina of a healthy woman	Shown significant improvement in women's urogenital health
Lactobacillus rhamnosus HN001 and *Bifidobacterium lactis* HN019	Selected from culture collection of the New Zealand Dairy Research Institute. Marketed by Danisco and by Fonterra	Beneficial in gastrointestinal diseases and have positive effect on immune health
Bifidobacterium longum BB536	Isolated from healthy infant and commercially available as Morinaga Bifidus milk	Immune stimulatory activity and stabilize gut microbiota

based on clinical trials based on large cohorts. Analysis based on biomarkers should be correlated with clinical endpoints. For example, variation in inflammatory cytokines should be backed by reduction or prevention of disease.

Probiotics have complex modes of action and can affect a number of different pathways; therefore, it is difficult to apply conventional pharmacological regulations. It becomes increasingly apparent that probiotic claims are more toward 'soft' health benefits rather than treatment of specific disease symptoms. Therefore, claims range from shortening the duration of acute diarrhea, improvement of gut well-being, immune stimulation, and control of cholesterol levels to the prevention and treatment of atopic dermatitis and eczema.

The role of gut microbiota is increasingly been recognized as one of the major factors affecting overall health status. Due to advent of 'omics' technologies, the impact of gut microbiota in different health conditions can be studied in greater detail, which will eventually lead to enhancement of our basic understanding of the microbial role in host physiology. For example, it has recently been shown that *Faecalibacterium prausnitzii* exhibits strong anti-inflammatory activity in patients with Crohn's disease, that spore-forming members of the clostridial clusters IV and XIV induce regulatory T cells in the colon and could be of therapeutic potential, and that *Akkermansia muciniphila* seems effective in controlling high-fat, diet-induced metabolic disorders. Another intriguing example is the application of fecal microbiota transplants to treat *Clostridium difficile* infections. Such studies may lead to the formulation of novel probiotics or innovative combinations of microbes that could be exploited for therapeutic utilities.

Conclusions

Recently, the field of probiotics has witnessed constant advancement with respect to product development and targeted health benefits. As such, probiotics are emerging as an alternative medicine for metabolic diseases. It seems that the whole concept of probiotics will be revisited to incorporate unconventional microbes or groups of microbes with specific health benefits. However, common probiotics are currently still predominated by members of the genera *Lactobacillus* and *Bifidobacterium*. Traditional health foods still act as major resource for the isolation and characterization of probiotic microorganisms. In the future, gut microbiota studies will lead to the design of new probiotics with proven health benefits. Importantly, probiotic strains have to be well characterized and follow guidelines set up by international organization as WHO and EFSA.

See also: Acidophilus Milk; Authenticity of Food; Bacteriocins; Bifidobacteria in Foods: Health Effects; Colon: Diseases and Disorders; Dahi; Emerging Foodborne Enteric Bacterial Pathogens; Fermented Foods: Composition and Health Effects; Fermented Foods: Origins and Applications; Fermented Foods: Use of Starter Cultures; Functional Foods; Lactic Acid Bacteria; Novel Foods; Prebiotics; Traditional Foods; World Health Organization; Yogurt: The Product and its Manufacture.

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Protein Quality and Amino Acids in Maternal and Child Nutrition and Health

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Introduction

The role of nitrogen and related compounds in life processes was first unraveled in the 1700s when French chemists identified nitrogen in air (naming it *azote*), with protein as a chemical class being identified about 50 years later. Protein is defined as a complex high-molecular-weight organic compound, consisting essentially of combinations of amino acids (AAs) in peptide linkages that contain carbon, hydrogen, oxygen, and nitrogen. Jakob Berzelius identified individual AAs as early as 1830, but it was not around the early 1900 that all the essential AAs were fully identified. Since then, considerable research on protein and AAs at the biochemical, molecular, and pharmacological levels has been undertaken, and more recently, with a better understanding of the molecular basis of the genetic coding of AA sequences, the role of protein and AAs in human health and disease is being established. While the skeletal muscle constitutes the major compartment of body proteins accounting for over 40% of the whole-body protein pool, the skin and blood proteins are also relevant.

Populations in the developing world, subsisting primarily on cereals and cereal products, are more vulnerable to protein deficits compared with those eating flesh foods. Recent food prices and financial crises have increased risk of food insecurity and hunger. Whereas energy needs may be fully met by cereals, such diets are poor not only in micronutrients but also in high-quality protein and thus have limited value in satisfying protein needs. This article presents the current understanding of protein and AA needs, their sources and production, worldwide patterns of consumption, requirements, and assessment methods and examines evidence relating protein and AA needs to health and nutrition with the aim of applying this knowledge to optimize health and well-being of human populations.

Sources and Production

From the perspective of nutritive value, dietary proteins are the source of indispensable AA and supply nitrogen for the synthesis of dispensable AAs. Indispensable AAs are those that are not produced at all or not made in sufficient amounts to support health by humans. Of the 20 AAs, 9 AAs are indispensable, one is considered as conditionally indispensable, and 10 are dispensable (Table 1).

The quality of a protein depends on its digestibility and on its indispensable AA composition, both the absolute amounts present and the proportions of the AAs. Protein-containing foods are designated as complete (high-quality) or incomplete (low-quality) on the basis of indispensable AA content. Dietary sources of animal protein such as meat, poultry, fish, and dairy products are complete proteins, while plant foods such as grains, legumes, and vegetables are incomplete proteins since they lack one or more essential AAs. The latter when consumed in specific combinations, generally cereal–legume mixes (e.g., corn and beans, rice, and lentils), provide an AA profile similar to that of a complete protein, thus the term AA complementarity. More information on food sources can be obtained from the article on protein food sources.

The capacity of food protein sources, mixed diets, or both to satisfy metabolic demand is estimated through evaluating their quality. Overall protein utilization (net protein utilization) reflects both digestibility (a function of digestion and absorption) and biological value (the degree with which absorbed nitrogen is utilized). Rat growth assays have been used to estimate protein quality in foods through the determination of the protein efficiency ratio (PER), defined as the weight gain of the test rat group divided by the protein consumed by the test group compared with a reference protein (commonly casein or egg protein). PER is based on achieved growth rate and thus has been criticized for its inability to account for protein used for maintenance purposes. Furthermore, values of PER of proteins of differing quality are not proportional to each other, that is, a PER of 2.0 cannot be assumed to be as twice as good as a PER of 1.0. Through several consultations from 1985 to 1991, a method based on measures of digestibility and AA composition, called the protein digestibility-corrected AA score (PDCAAS) method, has been adopted. The PDCAAS method is a scoring system that assesses the quality of protein in mixed diets, mixed formulations, or both based on its digestibility and essential AA composition, thus correcting for total protein and giving an estimate of 'utilizable protein.' Diets that are high in quality and digestibility such as animal-source foods are likely to have total protein and utilizable protein values closer to each other compared with those diets that are poor in quality and digestibility such as cereals and starchy food crops. It is now known that in diets in which over 50% of protein comes from cereal sources, the protein quality is relatively poor, adversely affecting biological value and protein utilization. Recent consultations have led to the recommendation of a new protein

Table 1 Indispensable and dispensable amino acids for adult mammals

Essential (indispensable)	*Nonessential (dispensable)*
Valine	Cysteine[a]
Leucine	Glycine
Isoleucine	Alanine
Threonine	Serine
Phenylalanine + tyrosine	Proline
Methionine + cysteine	Glutamic acid
Tryptophan	Aspartic acid
Lysine	Glutamine
Histidine[a]	Asparagine
Arginine[a]	Tyrosine[a]

[a]Cysteine, arginine, and tyrosine may be conditionally dispensable and histidine indispensable in infants.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00574-2

quality measure called the digestible indispensable amino acid score (DIAAS). This will replace the PDCAAS and is defined as DIAAS % = 100 × ((mg of digestible dietary indispensable AA in 1 g of the dietary protein)/(mg of the same dietary indispensable AA in 1 g of the reference protein)). Further information on measures of protein quality can be obtained from the 2014 protein quality evaluation report by the Food and Agriculture Organization.

Protein and AA Requirements

Protein requirements are a function of metabolic demand reflected by maintenance (metabolic processes that utilize AA and generate N compounds excreted via urine, feces, sweat, and other miscellaneous losses), special needs (growth, lactation, and pregnancy), and the efficiency of utilization (factors related to digestion of proteins and absorption of AAs). Intakes at which balance is achieved vary within and across individuals due to variability in metabolic demand, genotype, and factors that affect phenotype and physiologic conditions (growth, pregnancy, and lactation). Efficiency of utilization (net protein utilization), dietary intakes of other nutrients, lifestyle, and environmental stress (including infection) also alter the protein requirements.

The composition and pattern of AAs in a diet are important to generate a suitable mix that will match metabolic demand for protein synthesis with other needs. AA requirements in the past were established using nitrogen balance techniques (N equilibrium defined by an intake that matched N excretion (urine + fecal + integument losses) expressed in grams of protein per kg of body weight). Presently stable isotope techniques that allow assessing protein synthesis and breakdown are used to define optimal AA and protein intakes. Carbon balance and AA oxidation methods are now recommended methods for the estimation of essential AA requirements. Compared with previous estimates, current protein requirements are lower in both adults and children; however, the requirements for AA remain the same in children and are significantly higher in adults. Effectively smaller quantities of higher-quality protein (to achieve the AA pattern) are now recommended. Protein and AA requirements as defined by the current FAO/WHO 2007 report for all age groups are provided in Table 2. For further reading on protein requirements, please refer to the article on protein requirements.

Interactions between energy deficit and protein needs also affect nitrogen equilibrium. Energy imbalance (both excess and deficit) affects body nitrogen balance. At a protein intake of 0.57 g/kg^{-1} body weight, N equilibrium is achieved if energy intake is ~10–15% above the energy requirement. Conversely, individuals with energy deficit need additional protein, and even a modest energy deficit increases protein needs by about 10%. Such fluctuations in needs are not accounted for in the estimation of normative requirements; thus, adjustments need to be made if the target population is under stress or other conditions that may increase requirements. Other possible factors that may have an impact on protein and AA needs include infections or environments where individuals have persistent immune activation. In subjects with chronic enteric infections or inflammation leading to a decline in intestinal absorptive capacity; even in the absence of overt clinical symptoms, there is still an increased demand of protein. Thus, for vulnerable populations such as women and children commonly affected by acute and chronic infections, protein and AA needs are likely to be higher than what is recommended. Growth rate (especially in length) is likely to be affected by repeated infections (bacterial and parasitic) with the long-term implications associated to stunting. These include impaired learning capacity, poorer school performance, decreased work productivity, and other functional deficits. It is known that requirements of AAs such as lysine are significantly higher in chronically undernourished adults suffering from intestinal infections.

Protein and AA Metabolism

Protein and AA metabolism is a complex system. Movement of AAs through the system includes the flow to protein synthesis and other metabolic pathways; excess AAs undergo oxidation

Table 2 Protein and amino acid requirements in children 6 months to 18 years of age

	Protein requirements (g/kg^{-1} per day) for		*His*	*Ile*	*Leu*	*Lys*	*SAA*	*AAA*	*Thr*	*Trp*	*Val*
Age (years)	*Maintenance*	*Growth*	*Amino acid requirements (mg/kg^{-1} per day)*								
0.5	0.66	0.46	22	36	73	64	31	59	34	9.5	49
1–2	0.66	0.2	15	27	54	45	22	40	23	6.4	36
3–10	0.66	0.07	12	23	44	35	18	30	18	4.8	29
11–14	0.66	0.07	12	22	44	35	17	30	18	4.8	29
15–18	0.66	0.04	11	21	42	33	16	28	17	4.5	28
Adults	0.66		10	20	39	30	15	25	15	4	26
Pregnant women											
First	0.66	+1 g per day	10	20	39	30	15	25	15	4	26
Second	0.66	+9 g per day	10	20	39	30	15	25	15	4	26
Third	0.66	+31 g per day	10	20	39	30	15	25	15	4	26
Lactating women											
First 6 months	0.66	+19 g per day	10	20	39	30	15	25	15	4	26
Next 6 months	0.66	+12.5 g per day	10	20	39	30	15	25	15	4	26

Source: Adapted from the WHO (2007).

and are excreted as urea. AAs to satisfy demand are derived from dietary protein ingestion, body protein degradation, and de novo synthesis of nonessential AAs. As most of the AAs derived from protein degradation match the amount and pattern going to protein synthesis, demand is affected primarily by flow from dietary intake and de novo synthesis. Finally, the pathways of AA metabolism and interchange are critically dependent upon an adequate micronutrient status and hence upon the amount and quality of food consumed (Figure 1). The digestion, absorption, and metabolism of proteins are covered in a separate article in this volume as is the metabolism of AAs.

Global Patterns of Consumption and Access

A commonly used method to estimate nutrient availability at the global, regional, and country level is the estimation of nutrient availability using country level food balance sheets from the Food and Agriculture Organization (FAO) of the United Nations. Analyses conducted on 40 years of data from FAO (1961–2005) correcting for protein quality using the PDCAAS method show differences in the availability of energy and utilizable (good-quality) protein across regions and countries. For example, in 2005, total energy availability ranged from 2090 to 4497 kcal (2789 kcal per capita available globally) with least developed countries exhibiting the lowest levels. Regionally, Africa had the lowest availability at 2522 kcal, while countries in Northern America had the highest at 4497 kcal available per capita per day. Total protein followed total energy with a range of 53–137 g per capita available (83 g available globally), while utilizable protein (total protein corrected for AA score and digestibility) ranged from 46 to 125 g with an average of 74 g available globally. Correcting for quality of protein led to a reduction in total protein by 11% globally with as much as a 17% reduction in total protein availability in Africa (Table 3).

Examining the data for trends (1961–2005), we found that the global availability of utilizable protein (good-quality protein) has steadily increased from 57 to 74 g per capita per day (Figure 2). This increase was mirrored in Northern America, Latin America and the Caribbean, and Asia. In Europe and Oceania, utilizable protein availability has remained consistently high since 1961. In contrast, utilizable protein availability in Africa was low (47 g per capita per day) in 1961, with small gains in the following years with a per capita availability of 56 g per day in 2005. While Asian countries were below African countries with respect to utilizable protein availability from the 1960s to 1970s, the steady increase in availability in Asia is opposite to that in Africa, where there is stagnation in utilizable protein availability.

Health Effects

Clearly, there are limitations in the quality of protein available in developing country diet, which are predominantly plant- and cereal-based, and these have health ramifications. This is especially the case for infants (low-nutrient-density complementary foods) as well as foods available to pregnant and

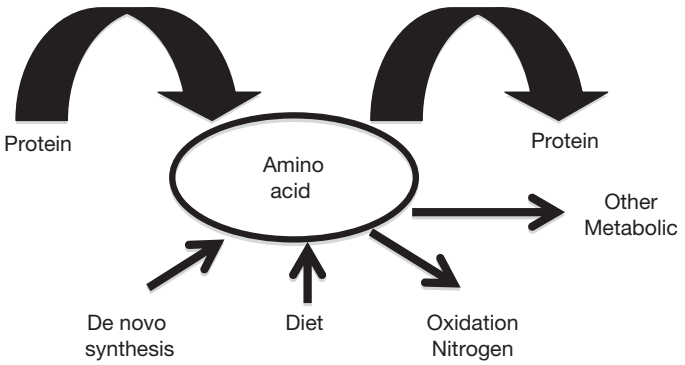

Figure 1 Protein and amino acid metabolism and interchange. Adapted from the WHO (2007).

Table 3 Comparison of protein availability across geographical regions, 2005

Country	*Total energy (kcal)*	*Total protein (g)*	*Utilizable protein (g)*[a]
World	2789	83	74
Northern America	4497	137	125
Europe	3674	115	104
Oceania	3542	116	107
Latin America and the Caribbean	3144	88	79
Asia	2550	75	67
Africa	2522	67	56
Least developed countries	2090	53	46

[a]Adjusted for limiting amino acid score and digestibility (PDCAAS).

Source: Adapted from Gunaratna et al. (2010).

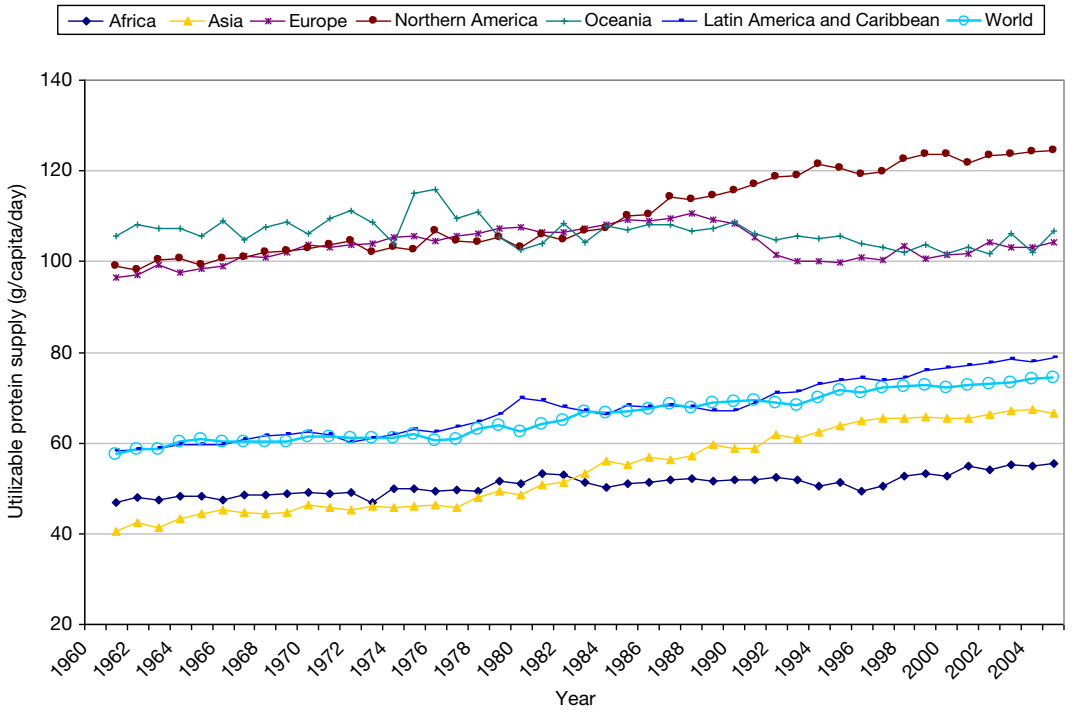

Figure 2 Utilizable protein availability across regions (1961–2005).

lactating women, preschool, and school-aged children, all of which are also low in micronutrients. The role of protein in the context of nutrition in infants and young children and maternal nutritional status and birth and pregnancy outcomes that also affect long-term growth and cognitive development in children are explored in the following sections.

Protein Effects in Pregnancy

Approximately 30 million newborns are affected by intrauterine growth retardation (IUGR, defined as being born with a birth weight under the 10th percentile of the birth-weight-for-gestational-age reference curve) in the least privileged of developing countries, with the highest prevalence in south-central Asia. Low birth weight/IUGR newborns are four times more at risk of neonatal death compared with those weighing 2500–3000 g at birth and 10 times more likely than infants weighing 3000–3499 g.

The consequences of maternal energy and protein restriction are widely documented. Maternal nutritional status has an impact on infant weight and body proportions, with low weight, height, or BMI on the mother's side being associated with lighter, shorter, and thinner babies; this has a further strong link between composition of the mother's diet and birth outcomes. Maternal protein deficiency during early or midgestation results in decreases in placental and fetal growth and may permanently retard postnatal growth and lead to permanent alterations in their structure, metabolism, and function. In vivo examination (porcine model) of the effect of maternal protein deficiency on AA availability to the fetus and potential retarded growth indicates the following: while maternal protein deficiency maintains maternal plasma concentration of AAs, it may impair placental transport of AAs from the maternal to the fetal blood with subsequent decreases in the concentrations of essential and nonessential AAs in the fetus. This could be the mechanism by which maternal protein restriction results in fetal growth retardation. More recent in vitro work using murine embryonic cells shows that AA deficiency (specifically that of lysine) increases the steady-state levels of several mRNAs (by 5- to 40-fold) that are elevated in growth-arrested cells within 24 h of exposure to AA deficiency. The provision of a high-protein diet or a diet supplemented with branched AAs to pregnant mice, in conjunction with calorie restriction, partly ameliorated the fetal growth restriction induced by the maternal calorie restriction through an increased IGF gene expression (IGF-1 and IGF-2 upregulation) in the fetal liver and was found to be independent of fetal glucose levels.

Consumption of a high percentage of energy from protein in early pregnancy is positively and significantly associated with birth weight and placental weight, an association independent of the effects of energy intake, weight gain, and confounders such as maternal age, parity, and smoking, and was specific to women who had met prespecified criteria of reliable data. For every one percent (isoenergetic) increase in protein in the diet, a 16 g increase was observed in birth weight (95% CI, 2.8–29.2 g) and a 4.2 g increase was found in placental weight (95% CI, 0.4–8.5 g). Furthermore, percent energy from carbohydrates in early and late pregnancy is negatively associated with the ponderal index. Percent of energy from dairy protein is also positively associated with birth weight and with ponderal index more strongly associated than other sources. For every 1% increase in dairy protein consumption, a 24 g increase in birth weight ($P=0.02$) and a 0.12 kg m^{-3} increase in ponderal index ($p=0.05$) have been observed. Similarly, women with high intakes of carbohydrate in early

pregnancy and low intakes of dairy protein in late pregnancy were likely to have infants that were thin at birth; women who had been low birth weight babies themselves were likely to have thin infants with the infant's ponderal index falling from 28.3 to 26.2 kg m^{-3} as the mother's birth weight decreased from 4000 to 2500 g or lower.

Several intervention studies have indicated that balanced protein and energy supplementation can affect birth outcomes and could reduce the risk of small-for-gestational age infants (as an indicator of IUGR by 32%). According to a Cochrane review of balanced protein–energy supplementation trials, providing nutritional advice to increase energy and protein intakes did not have a consistent benefit on pregnancy outcomes (5 trials with 1134 women); however, in 13 trials (4465 women) with balanced protein–energy supplementation, small increases were found in maternal weight gain and mean birth weight, with a substantial reduction in risk of Small for gestational age (SGA) births, still births, and neonatal deaths; preterm birth risk, however, was not affected. High-protein supplementation (2 trials, 1076 women) and isocaloric protein supplementation (3 trials, 966 women) led to increased risk of SGA births. Furthermore, high-protein supplementation alone or isocaloric protein supplementation may have a negative impact on fetal and neonatal outcomes. A meta-analysis has shown positive effects of balanced protein–energy supplementation on SGA births, mean birth weight, and neonatal mortality. Trials included providing nutrient supplements to pregnant women, with protein content ranging from 30 to 44 g of protein per day and contributing 25% or less to energy. The meta-analysis reports a significant reduction of 31% in the risk of giving birth to SGA infants (RR = 0.69, 95% CI 0.56–0.85) using SGA as a proxy for IUGR. Infants in the balanced protein–-energy supplementation group gained more weight compared with the control group infants, an effect that is more pronounced in malnourished women compared with those adequately nourished women. Another study providing prenatal food supplementation to undernourished or anemic pregnant women (fortified spread with peanut butter, soy flour, vegetable oil, and sugar) fortified with multiple micronutrients compared with only a multi-micronutrient supplement found improved birth length – but not weights – in the fortified supplement group. The total energy provided was 372 kcal, with 15% from protein (14.7 g of protein) and 67% from fat.

Low-protein diets, protein-restricted environments during gestation, or both could induce a nongenomic growth and adaptive response. A moderately low-protein diet (7.6% PE ratio) during gestation in rats activates a placental AA response pathway, which could be a cue for the fetus to develop an adaptive response suited to a predicted postnatal environment (a more favorable phenotype for their survival) as observed in the case of the growth of pups. Pups born to dams with a moderately low-protein diet through gestation are lighter than control pups starting from week 2 of lactation, whereas pups born to dams with a moderately low-protein diet during both gestation and lactation are significantly lighter than control pups from the 1st week of lactation. Analysis of the placental mRNA showed increases in the expression of specific genes that could be connected to the developmental capacity of the growing neonate, which may have implications in the programming of adult-onset diseases. Alterations have also been found in the expression of the liver transcriptome over three generations of offspring following a protein-restricted diet in dams (generation 0) during pregnancy; this was associated with differences in the number of genes being altered per generation, magnitude of the difference, and the distribution of up- and down-regulated genes. While not all the same genes were altered in each generation and some genes changed in the third – but not in the first – generation, all three generations had altered fasting glucose homeostasis and changes in the methylation of phosphoenolpyruvate carboxykinase promoter. The findings are indicative of the complex nature of the effects of altered nutrition (with the case in point being altered protein intakes) on the phenotype of subsequent generations of offspring. There is an association of high dietary protein intake in pregnancy (late) on infant ponderal index, later body composition, and the risk of chronic disease. In an analysis of Tasmanian Infant Health survey data, for every 10 g increase in daily maternal intake of protein during late pregnancy, a reduction in birth weight of 17.8 g was observed (95% CI: −32.7, −3.0; $p = 0.02$). Protein intake was negatively associated with ponderal index (beta = −0.01; 95% CI: −0.02, −0.00; $p = 0.01$) in large birth weight infants, but not in low birth weight infants.

Protein Effects in Infancy and Childhood

Protein and AAs in infancy and childhood play a role in growth, including muscle protein synthesis and linear growth. Studies have shown a positive impact of incorporating high-quality foods into the diets of young schoolchildren. An ecological analysis examining the relationship of stunting at the national level and protein quality and availability (in 2005) has shown that total energy, total protein, and total utilizable protein estimates are all independently and significantly associated negatively with the prevalence of stunting ($p < 0.001$). Total energy explains 41% of the variation in stunting, total protein explains 34% of the variation, and utilizable protein explains 40% of the variation in prevalence of stunting (separately). When total energy and total protein were incorporated into the model together to control for energy availability, only total energy emerged significant; however, when total energy and utilizable protein are incorporated in the model together, both are significant ($p = 0.006$ and $p = 0.017$, respectively), thus demonstrating that good-quality (utilizable) protein is a predictor of stunting controlling for energy availability.

High protein intakes (three to five times the current European requirements) are reported in children in European countries during the complementary feeding period and are indicated of having a growth stimulating role (especially that of linear growth) through effects on insulin-like growth factor-1 (IGF-1), with protein restriction leading to low levels of IGF-1 in healthy children. When older children are energy-restricted (50% reduction in intake) or protein-restricted (reducing protein from 1.0 to 0.66 g/kg^{-1} body weight per day), both forms of restriction lead to a significant decrease in nitrogen balance and decline in IGF-1 and specific IGF binding proteins. There is also a significant impact on the type and quality of protein on gene expression especially genes associated with IGF-1 and insulin-like growth factor-binding protein-1 (IGFBP-1), both of which play an important role in whole-body protein synthesis with implications for growth promotion and body

composition. High protein intake in infancy, however, could also increase BMI, early adiposity rebound, and the risk of adiposity later in life through the stimulation and subsequent release of higher levels of IGF-1. The type of dietary protein seems to have a specific stimulating effect on weight and length gain. Milk intake is positively associated with serum IGF-1 concentrations and height, suggesting a stimulating effect of milk on the hormonal pathway and subsequently on growth. Eight-year-old boys receiving a high milk intake have higher IGF-1 levels compared with boys receiving protein from meat. It is postulated that AAs, peptides specific to milk and/or other milk components (e.g.,β-lactoglobulin, α-lactalbumin, immunoglobulins, and lactoferrin) are likely to be the active stimulating components. There is a strong association between IGF-1 and body size during infancy and childhood with IGF-1 mediating a positive association between protein intake and growth. IGF-1 has been shown to regulate the partitioning of overall weight gain into linear growth compared with adiposity in infants and promote skeletal muscle formation and appears to protect against gains in BMI and adiposity (whether infants are breast-fed or formula-fed). Furthermore, there is a strong positive association between protein intake at 9 months of age and body size, height, and weight, but not percentage of body fat at 10 years of age.

There is a relationship between the quality of the protein being provided and growth patterns of infants and young children. Schoolchildren in New Guinea, receiving a regular diet (three meals of taro and sweet potato) with skim milk powder supplement (25 g protein) for 5 months, had significantly greater gains in height and weight compared with those with no milk powder supplement. Similarly, schoolchildren (in a separate study in New Guinea) provided with skim milk powder versus margarine (both isocaloric amounts) or two additional meals of taro and sweet potato daily exhibited the highest height and weight increases compared with a control group. While small weight increases did happen in the margarine group, this was accompanied by substantially higher skinfolds and the children receiving taro and sweet potato exhibited small increase in height but not to the degree of the skim milk powder group. The authors hypothesized (given the high levels of tubers in the diet) that in the case of this particular population, protein was limiting. The effect of milk-based formula has also been observed in younger children in Jamaica, where 9–24-month-olds who were initially stunted and matched (age and sex) to stunted controls were provided a milk formula for 1 year. Supplemented children gained an additional 0.9 cm and 0.31 kg during the first 6 months of the study (only), and the effects were more pronounced in younger infants than older ones. Linear growth improved in Peruvian toddlers consuming marginal diets when given complementary foods, breast milk, or animal product foods at 12–15 months of age. In toddlers with low intakes of animal product foods, breast milk was positively associated with linear growth, whereas infants with low intakes of complementary foods (of all types) benefited in length gain from having animal product foods in the diet.

Besides animal-source foods, well-formulated (e.g., cereal–legume mixture with good protein quality and a complete micronutrient profile) complementary foods also have a beneficial effect on growth patterns.

A study in Ghana found that infants receiving a cereal–legume porridge alone or with fish powder added or with a micronutrient premix added compared with those who received a cereal porridge with fish powder (no legume or micronutrients added) showed similar rates of weight and length gains and similar improvements in major indicators of iron and zinc but not iron and vitamin A stores. In contrast, nonintervention comparison infants had significantly lower Z-scores at 9–12 months of age. Another study in Ghana comparing two forms of micronutrient delivery (powders vs. tablets) to an energy-dense spread (peanut butter- and milk-based) found improved weights and lengths in infants receiving the energy-dense spread. In a study in Zambia, infants receiving a cereal–legume blend with or without amylases were found to have similar weights and heights compared with each other, and only the cereal–legume blend (with no amylase) had significantly higher length gain than the infants in the control group. Work conducted through the *Oriente* studies in Guatemala compared the skim milk-based high-protein, high-energy supplement *atole* to a nonprotein, low-energy supplement fresco with both supplements receiving additional micronutrients. The authors reported that *atole* group had greater length (2.45 ± 0.10 cm) at 3 years of age than those receiving *fresco*. The effects of protein quality on growth (height and weights) have been examined through interventions utilizing biofortified maize (quality protein maize (QPM)). A meta-analysis of randomized, controlled studies in target communities found that intake of QPM instead of conventional maize in communities where maize was the major staple food led to a 12% (95% CI: 7–18%) increase in the rate of growth in weight and a 9% (95% CI: 6–15%) increase in the rate of growth in height in infants and young children with mild to moderate malnutrition. Lysine-fortified wheat flour provided to households in China and Pakistan led to improvement in several nutrition indexes including immune parameters, iron status, and weights and heights in children under 10 years of age.

AA Effects in Infancy and Childhood

Specific AAs both indispensable and those considered 'dispensable' have also been shown to have an impact on linear growth possibly through effects on GH release. Dietary restriction of single essential AAs including leucine, lysine, methionine, and threonine decreases plasma IGF-1 production, does not affect plasma IGFBP-1 production, but does decrease its production in hepatocyte cultures. Arginine and lysine could have individual or combined effects within this mechanism via the somatotropic axis. Oral ingestion of dietary protein, AA mixtures to resemble soy protein, and arginine–lysine test drink have shown to increase growth hormone (GH) release in normal women. Soy protein ingestion has also been found to increase GH secretion (an effect similar to direct arginine supplementation); however, this effect is reduced when soy protein is ingested as part of a meal. Ingestion of soy proteins with a carbohydrate or fat alone increased secretion at the same level as soy and/or arginine alone; however, when soy was combined with both carbohydrate and fat, the effect is reduced. Peak plasma arginine concentrations were higher, indicating a role for arginine in the somatotropic activity of protein.

An association between intake of dietary protein and linear growth and high arginine intake and linear growth has been

observed in prepubertal girls over a 3-year and a 6-year follow-up period. High arginine and lysine intakes (ranging from 3.8 to 4.6 g per day) were inversely associated with fat mass index in prepubertal lean girls in both studies. This is supported by animal model studies that have found that a 1.25% L-arginine supplementation of diets in Sprague–Dawley rats leads to a reduction in retroperitoneal fat mass and an increase in lean body mass separate from the effects observed by supplementing diets with conjugated linoleic acid, another regulator of adipose tissue metabolism. Similarly, L-arginine supplementation (1.5% supplementation level) was found to shift nutrient partitioning such as promoting muscle over fat gain.

AAs and Intestinal Health

Lysine supplementation has been shown to reduce diarrheal morbidity in women and in children, as observed in studies in Syria and Ghana. Lysine supplementation (16 weeks, 1 g per day) in Ghanaian children reduced the number of days ill per child with diarrhea in the lysine group compared with the placebo group ($p = 0.034$). In addition, a significantly lower weight gain was observed in the placebo group (0.70 ± 2.18 kg) compared with the lysine group (0.87 ± 0.92 kg). The mean days ill per child with diarrhea per week was also significantly and negatively associated with weight change over the study period, when controlling for baseline weight and study group ($p = 0.04$). These findings are especially relevant from the perspective of children given that there are an estimated 5 billion cases of diarrhea in children per year, equating to at least 2.5 billion preventable deaths under 5 years of age. Every additional diarrheal episode before age 2 years contributes to stunted growth, and diarrhea is a risk factor for poorer school performance and diminished long-term cognitive function later in life. Diarrhea and protein–energy malnutrition are synergistic, especially in developing countries where cereal-based diets are marginal or deficient in protein. The effects observed on diarrheal morbidity could be due to improvement of protein status or an individual AA effect (such as lysine, tryptophan, glutamine, and arginine). The direct AA effect is a more rapid and potent mechanism. In the case of lysine, studies in rats have shown that lysine deficiency per se can strongly enhance diarrheal incidence and that lysine administration alone blocked gastric emptying and stress-induced diarrhea through a direct intestinal action on serotonin receptor 4. It is known that dietary components such as fiber can influence gut motor functions and that oral AA solutions inhibit gastrocolonic and small intestinal transit. In 'in vitro' models of rotavirus diarrhea, arginine has been suggested as a possible stimulator of protein synthesis during diarrheal episodes, and a new class of human G-protein-coupled receptor (GPRC6A) with high affinity for arginine and lysine has been described in the intestine. Protein–calorie malnutrition delays small intestinal recovery in neonatal pigs infected with rotavirus; moreover, the provision of high-quality animal plasma proteins reduces intestinal damage and reduces the effect of the rotavirus infection. Another mechanism of AA effects on diarrhea is through effects on the central and the gastroenteric systems. Lysine and tryptophan are both known to have an effect on chronic anxiety and stress in animals and humans. Lysine fortification of wheat reduces anxiety and stress response in family members of rural households in Syria with diarrheal morbidity and plasma cortisol levels reduced in women and sympathetic arousal in males. Chronic anxiety was significantly lower in men in the lysine-fortified households.

A major fuel in the gut, glutamine also becomes conditionally essential and has an impact on the immune system when the body is stressed. Glutamine supplementation improves intestinal mucosal repair and function. It has been shown to improve intestinal mucosal function and improve weight gain in HIV-positive patients with wasting and also help in rebuilding or maintaining the GI mucosal function in infectious diarrhea, cryptosporidiosis, and cholera. While glutamine itself is unstable in solution, its dipeptide form, alanyl glutamine, is stable and has been found to be well tolerated, safe, and effective in different clinical and experimental settings. Recent work in the area of HIV/AIDS indicates that indole-2,3 dioxygenase (a cytokine-inducible enzyme) contributes to enhanced tryptophan catabolism, thereby increasing immune dysregulation, accelerating disease progression, and increasing risk of other complications of HIV infection. Free glutamate is also an important signaling taste substance that facilitates efficient protein digestion, playing an important role in numerous physiological and metabolic functions such as protection and maintenance of tissue within the gastrointestinal tract. A double-blind clinical study conducted in Japan revealed that glutamate facilitates the gastric emptying of a protein-rich meal compared with a carbohydrate meal or plain water. Thus, it is suggested that free glutamate may play an important role in both protecting the GI tract and facilitating general ingestion.

Conclusion

This article provides an overview of protein and AAs, with a brief analysis of the sources of protein, their pattern of consumption worldwide, and their health effects. We also provide background on the potential role of protein and AAs within the context of food-based interventions that target maternal and child nutrition and health. Protein needs in infancy and early childhood include maintenance and growth and are the highest across all age groups in terms of g per kg of body weight. This is also the case for the AA requirements as expressed in mg per kg of body weight and the AA pattern (mg of AA/g of protein). Furthermore, additional protein needs are considerably high in gestation (especially the last trimester). A key concern identified in the setting of the current requirements is the lack of accounting for energy deficit and repeated infections, both very likely scenarios in infants transitioning from exclusive breastfeeding to breastfeeding in combination with a diet of complementary foods that are low in energy density and nutrient quality. Evidence on the effects of balanced protein and energy supplementation is moderately strong, and the impact of protein quality and quantity needs to be considered within the context of interventions that target pregnant women for prenatal services. Evidences on the specific effects of protein and AAs on infant growth and development were examined, and associations have been found between the type of dietary protein (milk vs. meat) and linear growth, and the effect of milk is said to be mediated via the GH and IGF-1. Individual AAs (arginine and lysine) and proteins (e.g., soy) are also postulated to affect GH release.

Further areas of consideration that were not covered in this article include the role of AAs in the control of stress, depression, and anxiety and effects on sarcopenia, aging, and obesity. Considerable research also exists on the role of specific AAs (such as the branched AAs) within the context of muscle protein synthesis and the role of dispensable AAs in HIV/AIDS therapy. Some nonessential AAs classified as dispensable are now being considered conditionally essential, especially when the body is under catabolic stress. Requiring further examination are the role of indispensable AAs such as lysine and the conditionality of the dispensable AAs such as arginine and glutamine within this context. The effects of individual AAs and protein have been observed on intestinal health, although more work needs to be done within the applied realm.

See also: Amino Acids: Metabolism; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein: Requirements.

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Protein: Digestion, Absorption and Metabolism

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The Central Role of Protein in Body Metabolism

Chemically, the human body comprises protein, lipid, ash (minerals), and water, and the body's metabolism is primarily associated with the protein component, often determined as the lean body mass (LBM). Dietary protein, which provides the building blocks (amino acids) for body protein synthesis, is thus metabolically transcendental. Dietary protein is needed for body growth and for the maintenance of LBM, and because protein is central to body metabolism and because many of the noncommunicable or so-called lifestyle diseases (e.g., diabetes, hypertension, and cardiovascular disease) are ultimately related to disturbances in body metabolism, dietary protein has an important role in the maintenance of optimal body composition, muscle mass and strength, visceral-organ function, and general health.

Amino acids, released from protein during digestion in the gastrointestinal tract (GIT), are absorbed and fuel body protein metabolism. In infants and children, the amino acids are used to maintain existing protein and to synthesize new protein; this is deposited during growth, whereas in adults, the absorbed amino acids are generally used to replace amino acids that are lost from the body during metabolism. Apart from their role in body protein synthesis, amino acids also have specific metabolic roles (e.g., leucine and muscle protein synthesis; glutamine and gut energy supply; tryptophan and serotonin synthesis; and arginine and nitric oxide production). Although the demand for amino acids for these specific metabolic roles may be low quantitatively, their supply is critical to normal body function.

Twenty amino acids are needed for protein synthesis in humans. Some, referred to as the dietary essential or dietary indispensable amino acids, must normally be supplied by the diet preformed, whereas others (the dietary nonessential or dietary dispensable amino acids) can usually be synthesized by the body from other amino acids or from simpler precursors. Nine amino acids are dietary indispensable in humans (lysine, methionine, threonine, valine, isoleucine, leucine, phenylalanine, tryptophan, and histidine). It is important to note that all of the twenty amino acids participate in body protein synthesis, but in adults, 11 do not need to be supplied in the diet. These remaining dietary dispensable amino acids are arginine, proline, serine, glycine, glutamine, glutamic acid, tyrosine, cysteine, alanine, asparagine, and aspartic acid. Some amino acids, those that are normally dietary nonessential, may become dietary essential under certain metabolic conditions when the cell may not be able to synthesize them at a sufficient rate. These amino acids are referred to as being 'conditionally essential.'

Amino acids not only are used by the body to make proteins and for specific physiological and regulatory purposes but also can be catabolized to provide the body with energy. Moreover, the synthesis of body protein is energetically costly. That body protein synthesis is energy-demanding and that amino acids can be used as a substrate both for protein synthesis and for energy supply are the bases for a protein/energy interaction during metabolism. It is also important to understand that protein in the body is not a static entity, but rather body proteins are continuously being broken down and resynthesized at different rates. This is referred to as body protein 'turnover,' with synthesis, degradation, and resynthesis. The process is very significant quantitatively. In a 70 kg adult human, some 200–400 g of body protein is broken down and resynthesized each and every day. As discussed, this process is energetically costly and constitutes an important component of basal energy needs. Also, body protein turnover is not completely efficient with attendant losses of amino acids; this accompanies other losses of amino acids from the body due to other metabolic processes. A dynamic equilibrium is achieved by matching these cumulative losses of amino acids from the body with the uptake of absorbed amino acids from the diet.

An understanding of the role of amino acids and protein in protein synthesis, protein turnover, and whole body energetics gives further insight to the central metabolic role of dietary protein. Dietary protein supports LBM and a normal body weight, with higher dietary protein intakes promoting and maintaining LBM. As basal energy expenditure is related to LBM, a greater LBM at the same body weight supports a higher basal energy expenditure, which assists maintenance of body weight. Higher dietary protein intakes are also related to higher rates of body protein turnover, which in turn translate to higher energy demand, which assists with the maintenance of body weight. Dietary protein is thought to be more satiating than starch, sugars, or lipids (fats and oils), and higher protein intakes may assist in maintaining lower food calorie intakes. Also, protein and amino acids yield less energy after catabolism compared to glucose and fatty acids on a per-gross energy basis and thus provide relatively lower amounts of dietary calories per unit gross energy. All of these effects of higher protein diets underline an important role for dietary protein in promoting normal body weight and composition and in the avoidance of obesity.

Dietary Protein Digestion

Traditionally, protein digestion and amino acid absorption were considered as distinct and separate processes; it is now recognized that the breakdown of protein into smaller units and their subsequent uptake into the enterocyte with the release of amino acids into the portal blood is a highly organized and tightly integrated process. Digestion involves multiple interactions between the food matrix, itself, physical processes, and chemical processes involving endogenous mammalian and bacterial enzymes, all occurring within the complex milieu of the GIT.

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Throughout the GIT, protein of body origin (endogenous) is either secreted or sloughed into the lumen, roughly doubling the total amount of protein available to be digested. The origins of gut endogenous proteins include enzymes, mucoproteins, sloughed intestinal cells, and proteins (e.g., albumin and immunoglobulins) that either leak or are secreted into the gut lumen from the blood. Bacterial bodies, nondietary though not strictly endogenous, are also an important component of the proteinaceous material present in the gut lumen.

Protein digestion is a remarkably efficient process. For the average Western male, around 200 g day^{-1} of protein (100 g dietary and 100 g endogenous) is effectively digested and absorbed by the enterocytes as free amino acids and dipeptides and tripeptides. Typically, around 85% of the total protein is absorbed prior to the end of the small intestine (terminal ileum), with around 30 g protein entering the large bowel. There is also extensive microbial metabolism of protein in the colon. Nitrogen derived from the breakdown of protein is absorbed from the colon in the form of ammonia, and the equivalent of around 10–20 g day^{-1} of protein is excreted as fecal nitrogen giving overall digestibility values of 90–95%. The fecal protein represents mainly the fraction of gut endogenous and dietary protein not absorbed in the small intestine, but because this unabsorbed material is used by the large intestinal microbiota for microbial growth, the fecal protein is present mainly as bacterial protein.

Protein digestion occurs throughout the GIT. Ingested food is subjected to physical breakdown by mastication in the mouth, and food is mixed with saliva and liquids, generating a food bolus that can be swallowed to enter the stomach. The lumen of this organ, moreover, also provides a medium suitable for the action of digestive enzymes. Gastric digestion involves further physical breakdown of the food and denaturation and hydrolysis of protein due to the action of gastric hydrochloric acid and the pepsins secreted by the parietal chief cells. The outcome of the gastric digestion of protein is a mixture of protein, mainly large polypeptides and some free amino acids, with virtually no amino acid absorption. The digested mixture is emptied from the stomach over time and enters the small intestine (duodenum, jejunum, and ileum), wherein further hydrolysis occurs catalyzed by a family of protease enzymes secreted by the pancreas and by enzymes localized to the intestinal cells. The latter are either secreted into the gut lumen or attached to the brush border of the intestine or are active within the mucosal cells. Small intestinal digestion is facilitated by the secretion of pancreatic bicarbonate, which neutralizes the acidic gastric chyme and establishes a pH in the intestinal digesta of 6–7. The pancreatic proteases comprise trypsin, chymotrypsin, elastase, and the carboxypeptidases A and B. The epithelial cells' brush border membrane-bound enzymes comprise a number of aminopeptidases and a carboxypeptidase and act on any oligopeptides left after pancreatic digestion. Amino acids are absorbed mainly as free amino acids, dipeptides, or tripeptides, with the peptides being acted upon by cytosolic aminopeptidases, such that amino acids are mainly absorbed into the portal blood in the free-form. It is now considered, however, that very small amounts of some peptides and protein fragments may also enter the portal blood intact. These peptides may have specific bioactivities. Protein and oligopeptides remaining undigested at the end of the ileum pass into the colon where they are subjected to extensive microbial metabolism, but amino acids *per se* are not absorbed from the human colon, at least not in nutritionally significant amounts.

Digestive enzymes are also secreted by bacteria inhabiting the GIT, and the gut microbiota undoubtedly contributes to overall gut proteolytic activity. They also make proteins more accessible to mammalian enzymes by breaking down plant fiber. Bacteria can both catabolize and synthesize amino acids and some GIT bacteria have ureolytic activity and are involved in body urea recycling. The role of bacteria in protein digestion has not been studied extensively, but it seems likely that bacterial enzymes complement, to some extent, the action of the mammalian enzymes. Bacteria, although predominantly found in the large intestine, are actually present throughout the GIT and can be intensely metabolic. The role of bacteria in supplying dietary essential amino acids to the host net of dietary supply, through microbial amino acid synthesis, is widely debated, but it is generally considered that such supply is quantitatively low.

Amino Acid Absorption

The products of protein digestion, mainly free amino acids, dipeptides, and tripeptides, are absorbed from the small-intestinal lumen by several transport mechanisms. Free amino acids are taken up by transporters located in the luminal-facing, apical membrane of enterocytes on the villi and exit the cell via basolateral membrane transporters. There are a number of brush border (apical membrane) and basolateral membrane transporter systems, specific to particular amino acids or groups of amino acids. Transepithelial movement of amino acids involves interactive participation among multiple transport systems, multiple subunits (a transporter protein encoded by a specific gene), and ion electrochemical gradients. Amino acid movement via the transport systems occurs by both secondary active transport (dependent upon ionic gradients and electrical potentials across the enterocyte membranes) and facilitated (ion-independent) transport, depending upon the system.

It is critical that the body receives a net positive flow of amino acids from the dietary proteins, and so the small intestine has the ability to adaptively upregulate its capacity for amino acid absorption. Both dietary protein intake and physiological state (influencing the amino acid requirement) may change over time in an individual, and amino acid absorptive mechanisms can adapt accordingly at both tissue and cellular levels. The combined effects of the upregulation of transporters and mucosal hyperplasia can lead to a severalfold increase in amino acid absorptive capacity in comparison with the fasting state. In addition to free amino acids, dipeptide and tripeptide products of protein hydrolysis are also transported across the apical membrane of absorptive enterocytes. In fact di- and tripeptides are absorbed to a significant extent and more rapidly than the free amino acid counterparts. Once absorbed, however, they are in the main hydrolyzed to free amino acids by the aminopeptidases in the cytoplasm of the enterocyte. The uptake of peptides is primarily due to the action of the high-capacity apical membrane Pept 1 transporter (secondary active transport), expressed throughout the small bowel, but only to a

low degree in the colon. Overall, the ability of the enterocyte to take up amino acids in the free-form and as dipeptides and tripeptides, via multiple transporters and systems, ensures an efficient absorption of the amino acids released from proteins during digestion.

Amino acids either entering the enterocyte directly or arising from intracellular peptide digestion largely pass through the enterocyte unchanged but may be used for protein synthesis within the enterocyte or be metabolized (oxidized or converted to another amino acid, metabolite, or compound). In particular, the enterocyte metabolizes amino acids such as glutamate, glutamine, aspartate, and arginine quite extensively. Following basolateral membrane transport to the interstitial fluid, amino acids move via the villus capillaries to the portal vein. The portal vein supplies the liver directly, where the amino acids may be further metabolized or transported to other organs and tissues.

Determining dietary amino acid digestibility in humans

Although dietary proteins are generally digested efficiently within the GIT, they are not completely digested and absorbed. Also, different amino acids are both released from proteins and absorbed across the intestine to different extents. It thus becomes necessary to be able to determine the digestibility (disappearance from the GIT) of dietary proteins and amino acids. Both the extent and rate of protein breakdown are a function of the amino acid sequence of the protein and various posttranslational modifications and are also influenced by the structure of the food matrix within which the protein is found. The processing, storage, and cooking of food, moreover, can lead to chemical (e.g., Maillard products or D isomers) and structural (e.g., cross-linking) changes, which can result in an impaired digestibility.

Although traditionally, the digestibility of amino acids has been determined by comparing fecal amino acid output with dietary amino acid input, this approach is now known to be inaccurate. The role of the colonic microbiota in colonic protein metabolism has been discussed earlier. The microbes may both degrade and synthesize amino acids in the large intestine, and two-thirds to three-quarters of fecal protein is actually present as microbial bodies. This means that only a small proportion of fecal amino acid excretion has any direct relationship to the flow of undigested dietary amino acids. The measurement of amino acids remaining unabsorbed at the end of the terminal ileum (posterior portion of the small intestine) is now widely recognized as generating more accurate observations of amino acid digestibility. Amino acid digestibility values determined by sampling digesta from the end of the ileum are referred to as ileal digestibility coefficients. In most simple-stomached animal species, multiple studies have demonstrated considerable differences between fecal and ileal amino acid digestibility for the same protein. For most foods and amino acids, ileal amino acid digestibility is lower than the fecal counterpart, indicating a net degradation of the amino acid in the large intestine. For some amino acids in some foods, however, a net microbial synthesis may be observed. The latter is often seen for methionine. Regardless as to whether there is a net disappearance or net appearance of an amino acid in the hindgut, fecal digestibility values are affected by the microbial metabolism of amino acids entering the colon and do not properly reflect amino acid absorption.

Although ileal amino acid digestibility provides accurate estimates of amino acid uptake from the digestive tract, its determination in humans is not straightforward. Ileal digesta can be sampled using a nasointestinal tube or directly in adult ileostomates whereby the colon has been surgically removed due to disease. Neither method can be regarded as routine or entirely satisfactory, and because of this, animal models of ileal protein digestibility have been developed. The growing pig is an established model for protein digestion in the adult human and the milk-fed piglet for human infants. Ileal digesta can be sampled directly from the ileum of an anesthetized pig or be collected from animals via surgically implanted gut cannulas. When dietary amino acid input is corrected for the total ileal amino acid output, digestibility coefficients are determined that are termed 'apparent' digestibility coefficients. This term indicates that the value is only an approximation rather than the 'true' digestibility coefficient. It is recognized that ileal digesta is a mixture of both undigested dietary protein and 'endogenous' protein that has entered the digestive tract from the animal, and this endogenous protein influences the measurement of digestibility. There are a number of ways of determining the endogenous protein, and when this is corrected for in determining digestibility, 'true' or 'standardized' coefficients of digestibility can be derived. Recommended best practice is to determine protein and amino acid digestibility using true ileal digestibility. True ileal amino acid digestibility values are available for numerous foods consumed by humans, with values ranging from around 50% to up to 95–98%. Within a protein source, the true ileal digestibility of individual amino acids can vary quite markedly.

During food processing and storage, or during the cooking of foods, some amino acids (lysine is the classic example) can undergo structural alterations. The structurally altered compounds are often not digested and absorbed and, when absorbed, are generally not utilized but are broken down and excreted in the urine. The traditional methods used for determining amino acids in food and ileal digesta can give rise to erroneous digestibility coefficients in these circumstances. During chemical analysis, the structurally altered compounds can revert back to the parent amino acid, but this occurs to different extents in food and digesta and with different foods. Because of this, specific analytic methods have been developed that determine only the structurally unaltered or available amino acid, which allows the determination of the true ileal digestibility of the structurally unaltered (available) amino acid. This can be particularly important for lysine and methionine in foods whereby protein may have been 'damaged.'

Bioactive Peptides

During dietary protein digestion, large quantities of small peptides (di- and tripeptides) and larger oligopeptides are produced within the GIT lumen. Many of these peptides possess amino acid sequences that confer bioactive functions, and some are quite resistant to digestion. The peptides may interact with mucosal cells or indeed with bacterial cells within the gut lumen and may elicit a wide variety of bioactivities (e.g., mineral-binding, cholesterol-lowering, immunomodulation,

antioxidant, antithrombotic, antimicrobial, and antifungal). It also appears that small quantities of especially the smaller peptides may be absorbed intact from the GIT and may affect body processes systemically. Larger opioid peptides are known to influence the digestive process itself through modulation of gastrointestinal motility and the stimulation of gut secretory processes. Many studies have recorded the generation of bioactive peptides during dietary protein digestion, their presence in digesta, and multiple biological effects. These bioactive peptides may have a role as complementary physiological modulators and regulators. They have been shown to be produced from the digestion of a wide variety of foods. What has not been so widely considered, however, is that the endogenous proteins entering the GIT from the body are also an important source of bioactive peptides and may provide the GIT lumen and body with a more constant and consistent supply of peptides.

Bioactive peptides are inactive within the parent protein molecule until released by enzymatic hydrolysis, generally during digestion but potentially also during food processing. Once released, the peptides are potentially capable of influencing a range of behavioral, gastrointestinal, hormonal, immunologic, neurological, physiological, vasoregulatory, and nutritional responses, and such peptides have been put forward as candidates for a variety of food and pharmaceutical applications.

There is no doubt that the generation of bioactive peptides during the normal digestion of both foods and the endogenous proteins in the GIT is an important function of proteins, and these peptides have numerous regulatory, stimulatory, and inhibitory effects. Moreover, dietary protein itself stimulates the production of gut endogenous proteins, leading to further peptide production, suggesting a highly interlinked process. Food proteins are thus far more than merely a supply of amino acids for body protein synthesis. However, the importance of the overall role of these peptides produced in the GIT lumen external to the body cells remains to be fully elucidated.

Protein Metabolism

Once absorbed, amino acids may be used for the synthesis of body protein or, in small amounts, as precursors for a multitude of nitrogen-containing compounds with unique biological activities or be catabolized with their nitrogen ultimately incorporated into urea, which is finally excreted in the urine. After deamination, the carbon skeleton from the amino acid can be oxidized to provide energy or, depending upon body energy status, can be used to synthesize fatty acids, which are stored as body lipid. The balance achieved between catabolic and anabolic pathways involving the first-limiting amino acid determines overall dietary nitrogen retention. In the growing child, there is a need for amino acids for body protein maintenance and for new tissue deposition, whereas in the adult, amino acids are needed to replace amino acids lost from the body. For pregnant and lactating women, there are additional amino acid needs for growth of the conceptus and milk production.

The absorption and metabolism of amino acids is complex and highly integrated, with continuous flux within and among body cells and within and among tissues and organs. However, it is useful to view amino acid metabolism in terms of several discrete processes that explain the utilization of amino acid postabsorption (Table 1).

Table 1 Biological processes giving rise to a requirement for absorbed amino acids in the adult human

- Integumental amino acid losses
- Gut endogenous amino acid losses
- Urinary amino acid losses
- Other miscellaneous amino acid losses
- Synthesis of nonprotein nitrogen-containing compounds
- Irreversible modification of amino acids
- Inevitable amino acid catabolism (oxidation)
- Preferential amino acid catabolism

In the adult human, relatively small amounts of amino acids are lost from the body due to the shedding of skin cells and when hair is cut or removed from the body. There is also a small loss of intact amino acids via the urine and very small losses through other miscellaneous routes. Of greater significance, quantitatively, are the losses of amino acids via the GIT. Relatively large amounts of body protein enter the GIT daily; whereas some of this material is digested and reabsorbed, considerable amounts of amino acids are catabolized in the GIT or are incorporated into microbial protein, which along with some undigested endogenous protein is lost from the body in the feces. These losses of amino acids need to be made good using absorbed amino acids from the diet, so as to maintain the body's protein mass. Amino acids are also used by the body to synthesize a wide range of nonprotein nitrogen-containing compounds essential for normal metabolic functioning (e.g., lysine for carnitine, arginine for creatinine, and methionine as a methyl donor). The use of amino acids for these purposes is usually low, though for some amino acids in some circumstances, the rate of utilization may be quantitatively significant. Also, amino acids may become irreversibly modified and thus unavailable for protein synthesis. Examples of this are the methylation of histidine and the hydroxylation and methylation of lysine. Of much greater quantitative significance than the latter two processes is the loss of amino acids due to oxidation, arising from a number of processes collectively referred to as inevitable catabolism. Inevitable catabolism refers to the oxidation of an amino acid, not for the express purpose of energy supply, but arising rather as an inevitable consequence of the action of catabolic enzymes. Even the first-limiting dietary amino acid given with a surfeit of nonprotein energy sources is subjected to a considerable degree of inevitable catabolism. Amino acids enter the body's free amino acid pool from body protein turnover and from the supply of absorbed dietary amino acids, and a proportion of the amino acid flux will be subjected to inevitable catabolism. The extent of inevitable catabolism may be affected by habitual protein intake and thus the background activity of the catabolic enzymes. First-pass intestinal amino acid catabolism may be an important contributor to inevitable catabolism, whereby some absorbed amino acids are specifically targeted for oxidation within the enterocyte. Also, it is thought that some absorbed amino acids function in having a transitory regulatory influence on body protein synthesis (anabolic drive), before being catabolized. Finally, and in situations whereby nonprotein energy sources may be limiting in terms of meeting the body's energetic needs, amino acids may be catabolized expressly to meet the energy need (preferential catabolism).

Collectively, these various biological processes translate to a metabolic requirement for amino acids, which must ultimately be met by amino acids absorbed from the diet. Many of the processes discussed earlier are affected by dietary factors. By means of example, the GIT amino acid losses are affected by the protein and fiber contents of the diet and by the presence of dietary antinutritional factors. Higher dietary protein intakes lead to higher rates of body protein turnover and likely greater fluxes of amino acids. Amino acid oxidation in the postabsorptive phase occurs at a rate determined by habitual protein intake. The kinetics of uptake of amino acids from a protein ('fast' and 'slow' proteins) can have important effects on postprandial metabolism. It is thus axiomatic that dietary amino acid requirements for the adult human at maintenance are not fixed values, but rather are diet-dependent values. This is not usually recognized in traditional tables of requirement estimates, whereby static values are prescribed.

Protein and amino acid requirements

Over the years, much work has been undertaken to determine the dietary amino acid requirements of humans, and this work has often relied on conducting nitrogen balance studies. An accurate determination of dietary amino acids in humans has proved to be elusive, and there has been much debate about the accuracy of the nitrogen balance approach. Over the last three decades, requirement estimates have been made using direct or indirect measures of amino acid oxidation. These techniques give rise to much higher estimates of amino acid requirements than the N-balance approach, but are also not without controversy related to their accuracy. The FAO, WHO, and UNU recommend 0.83 g high-quality protein per kg bodyweight as a 'safe' daily intake of dietary protein, which translates to 58 g protein per day for the reference 70 kg bodyweight man and 47 g protein per day for the reference 57 kg woman. In the United States, the approximate median intake of protein for adults (31–50 years of age) is 100 g day^{-1} for men and 65 g day^{-1} for women, suggesting a surfeit of dietary protein. In parts of the developing world, however, habitual dietary protein intakes are much lower.

Lately, research has focused on health, bodyweight management, and body function attributes as parameters to assess protein and amino acid requirements, and based on this approach, protein requirement estimates often much higher than current official recommendations are promulgated. Dietary requirements for individual dietary essential amino acids as published by the FAO, WHO, and UNU and based largely on amino acid oxidation studies are given in Table 2. As discussed, for purposes of practical necessity, single average values of requirements are given; in reality, however, there is considerable variation in requirement values due to differences among people and due to the metabolic effects of different foods and diets.

Table 2 Estimates of the daily amino acid requirements (mg kg^{-1} bodyweight) of the adult human

	FAO/WHO/UNU (2007)
Histidine	10
Isoleucine	20
Leucine	39
Lysine	30
Methionine and cysteine	15
Phenylalanine and tyrosine	25
Threonine	15
Tryptophan	4
Valine	26

Dietary Protein Quality

The extent to which amino acids present in a food can be used to meet the body's metabolic need for amino acids is referred to loosely as the food's 'protein quality.' Measures of protein quality are used for predicting the amounts of protein (first-limiting amino acid) that can potentially be utilized by a person for a particular food or protein source. They are also used to value or score proteins for trade and regulatory purposes. Essentially, protein quality evaluation aims to describe the amount of each dietary amino acid present in a food in an available (structurally unaltered) form and the amount of that amino acid, which can be absorbed from the food and based on this predict the value of the food under different conditions.

Recognizing that, in addition to serving as building blocks for body protein synthesis, amino acids also have specific physiological functions, the most recent FAO recommendations on dietary protein quality evaluation state that first and foremost, "dietary amino acids should be treated as individual nutrients and wherever possible data for digestible or bioavailable amino acids should be given in food tables on an individual amino acid basis." It is further recommended that amino acid digestibility be based on true ileal digestibility determined either in humans directly or using the growing pig as an animal model for the adult human. For foods susceptible to damage from processing, determination of the true ileal digestibility of structurally unaltered or available rather than total (conventional amino acid analysis) lysine is recommended. Also recognizing that it is important to be able to assess the contribution a food protein or mixture of food proteins makes toward meeting nitrogen and amino acid requirements, the FAO recommend a scoring procedure for proteins. Here, the predicted balance of dietary essential amino acids reaching the sites of protein synthesis is compared with the individual's ideal amino acid balance deemed to be required for body protein synthesis. The system for scoring dietary protein quality used over the last 20 years called 'protein digestibility corrected amino acid score' has been recently replaced by the FAO with a new system called 'digestible indispensable amino acid score' (DIAAS). DIAAS is defined as follows: DIAAS% = 100 × ((mg of digestible dietary indispensable amino acid in 1 g of the dietary protein)/(mg of the same dietary indispensable amino acid in 1 g of the reference protein)). Amino acid digestibility should be based on true ileal amino acid digestibility, and for processed foods, lysine availability should be taken into account. Three recommended amino acid scoring patterns (amino acid pattern of the reference protein) are given for the calculation of DIAAS, one for infants, another for young children, and another for older children, adolescents, and adults. In determining DIAAS, the ratio (described earlier) is calculated for each dietary

indispensable amino acid and the lowest value designated as the DIAAS. DIAAS can have values below or above 100%. Values above 100% are not truncated except when calculating DIAAS for mixed diets or sole source foods. A DIAAS value of 120% would indicate that the protein source supplies 120% of the individual's requirement for the first-limiting amino acid, while a value of 50% would indicate that the protein source supplies only half of the required amount of the first-limiting amino acid.

The amino acid requirements for humans of different ages and physiological states are not known with certainty. Also, there is always some degree of uncertainty in the digestible or available amino acid contents of specific foods. Because of this, DIAAS values can only be regarded as approximations or best current estimates of dietary protein quality. Other aspects of proteins, such as their ability to generate specific bioactive peptides during digestion, may also need to be considered in evaluating a protein. The 'quality' of a protein from a nutritional or physiological perspective is multifactorial, and all aspects of a protein's quality cannot be captured in a single score. Nevertheless, such scores are important for understanding a food protein's potency as a source of amino acids to meet daily metabolic amino acid requirements.

See also: Adolescent Nutrition; Amino Acids: Determination; Amino Acids: Metabolism; Antinutritional Factors in Legume Seeds: Characteristics and Determination; Bioactive Peptides in Foods; Bioavailability of Nutrients; Browning: Enzymatic Browning; Browning: Non-enzymatic browning; Colon: Structure and Function; Energy: Intake and Energy Requirements; Maillard Reaction; Obesity: The Role of Diet; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Satiety.

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Protein: Food Sources

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Introduction

Food proteins have two important functions, namely, those that serve to meet essential nutritional requirements and those relating to the physicochemical properties essential for maintaining product quality. In relation to food products, proteins possess unique functionalities such as rheological properties (i.e., viscosity, hardness, and gel strength) and interfacial properties that allow stability of emulsion formation, and baking and film formation.

Production and Consumption

Protein is one of the essential macro nutrients for the human body and constitutes about half of the human body's dry weight. The different amino acids that are the building blocks of proteins are essential for human growth, tissue repair, and replacement. Protein can also serve as an energy source.

The major food sources of dietary protein are from animals and plants. Specifically, these include dairy products like milk, yogurt, and cheese; meat, poultry, and seafood; eggs; soy foods such as tofu; seeds and nuts; legumes and pulses; and grain- and cereal-based products (Table 1).

The world's supply of animal proteins as meat has shown an increase of 77% over the last 40 years, from 8 to 14.2 g/capita/day (Figure 1). However, cereals are still the major protein sources, particularly in the developing world and in Asia and Africa where cereals form the major part of staple diets. On average, cereal proteins account for 32 g/capita/day in the human diet (Figure 1).

Proteins derived from animal sources are considered to be nutritionally superior to those from plants because they contain a better balance of the dietary essential amino acids. Of these, egg and milk proteins (i.e., caseins) are frequently used as reference proteins for evaluating protein quality.

The most common sources of animal proteins for humans are in the form of meat from domesticated animal species such as cattle (beef), pigs (pork), poultry (chicken, duck, or turkey), and sheep (lamb). The production and consumption of pork and poultry meat have increased considerably over the last four decades and overtook the consumption of beef in late 1990s (Figure 2). Nowadays, poultry meat is the highest contributor to the animal protein supply for human consumption. This is probably due to the improvement in production efficiencies in intensive farming and the relatively lower cost of poultry meat compared with beef and lamb. However, the utilization and subsequent consumption of different animal species vary throughout the world due to different cultural preferences and religious beliefs.

The global per capita food supply of fish and seafood was estimated at 18.4 kg, in which fish accounted for 6.4% of the global population's intake of food proteins consumed and almost 20% of the average per capita intake of animal proteins (Figure 1).

The contribution of dairy to overall dietary protein consumption was relatively steady from the 1960s to the early 1990s but has since shown a steady increase from 7 g/capita/day in 1995 to 8.1 g/capita/day in 2011 (Figure 1). In the past, increases in demand for milk and dairy products were driven mainly by population growth, whereas they are now increasingly fueled by rising per capita milk consumption in developing countries. It may be expected that increasing income levels in Asia, particularly in China, will stimulate an increase in dairy product consumption and overall dairy protein intake.

The contribution of eggs to global protein consumption has also shown a dramatic increase, particularly over the last 20 years, from 1.7 g/capita/day in 1980 to 2.7 g/capita/day in 2011. This trend of increasing egg consumption is expected to continue with increasing world population, especially in countries with improving economies, such as China. However, total egg consumption in developed countries has actually been declining due to health concerns related to high cholesterol and fat content.

Among the cereal crops, wheat is the single most important food crop in the world. Wheat is eaten in various processed forms by more than one billion people currently and makes the largest contribution to the protein available to human beings compared with any other single food source (Figure 3). However, the consumption of wheat-based foods has decreased during the last two decades. This trend may be partly due to the increased consumption of animal proteins with increasing income levels, particularly in the Asian region. In addition, the consumption of rice is continuing to rise (Figure 3), possibly driven by the increasing popularity of Asian cuisine in Western countries and rice becoming more regularly consumed as part of the diet.

Although soybeans are one of the most important agricultural commodities, their use as a food protein source is still relatively low (Figure 3). A large amount of defatted soybean meal is produced after extraction of oil and most defatted soybean meal is used for animal feed. Only a small portion is further processed into various types of soy protein ingredients for human consumption. Thus, soybeans account for a relatively low human protein supply.

Dairy Proteins

Bovine milk serves as an important source of proteins in the human diet, in particular for infant nutrition, for much of the past century. Milk contains ~3–4.4% protein. Milk proteins are classically divided into two major groups, that is, caseins and whey proteins. Caseins are the most abundant group, which account for 80% of total milk proteins and are insoluble at pH 4.6, whereas whey proteins remain soluble at pH 4.6.

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Table 1 Sources of food proteins

Sources	*Subcategory*	*Common food source*	*Protein ingredients*
Animal	Dairy	Milk, cheese, yogurt, and other dairy products	Casein and whey
	Meat, poultry, and seafood	Muscle proteins Proteins in the connective tissue, restructured products	Blood proteins, collagen, and gelatin
	Eggs		Whole egg powder, egg white, and egg yolk
Plant	Cereals	Wheat, corn, barley, oats, and rice	Wheat gluten and texturized proteins
	Legumes and pulses	Soybeans, peas, lupins, lentils, chickpeas pinto beans, kidney beans, and navy beans	Soy protein isolate, pea protein isolate, and texturized proteins
	Oilseeds	Canola, cottonseed, and flaxseeds	
	Nuts	Various ground nuts and tree nuts; raw, roasted, and salted	

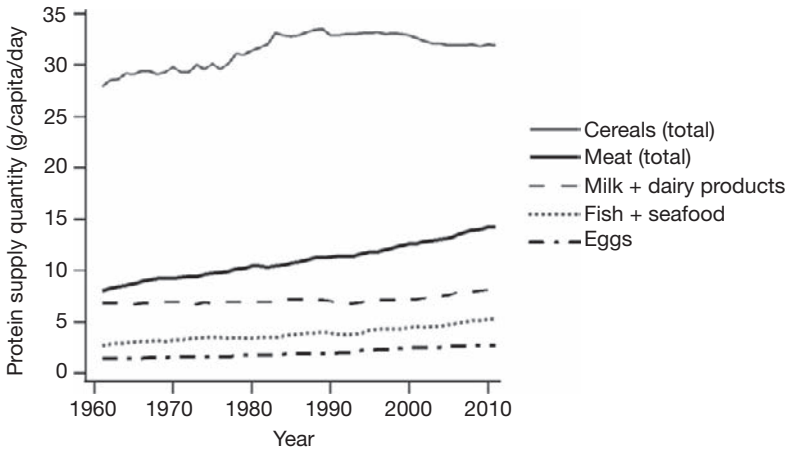

Figure 1 World protein supply quantity as food (g/capita/day) in the last four decades (1961–2011). Food and Agriculture Organisation of United Nation FAOSTAT (2014) at http://faostat3.fao.org/.

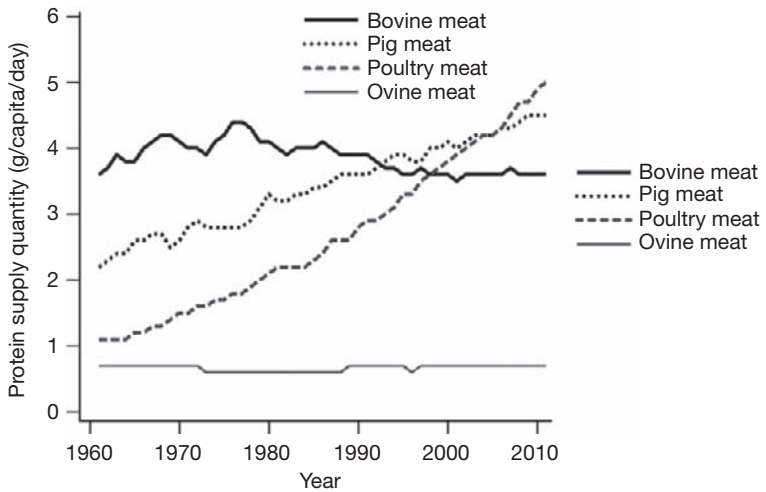

Figure 2 Changes in protein supply quantity as food (g/capita/day) from animal meat sources between 1961 and 2011. Food and Agriculture Organisation of United Nation FAOSTAT (2014) at http://faostat3.fao.org/.

Dairy proteins are also widely used as protein ingredients by the food industry in a variety of manufactured food products because they provide desirable sensory properties, physical functionalities, and nutritional values for foods.

Due to the liquid nature of bovine milk, the dairy industry has developed a range of processing technologies to fractionate, concentrate, and dry dairy proteins into an array of functional ingredients for food applications (Table 2). The most

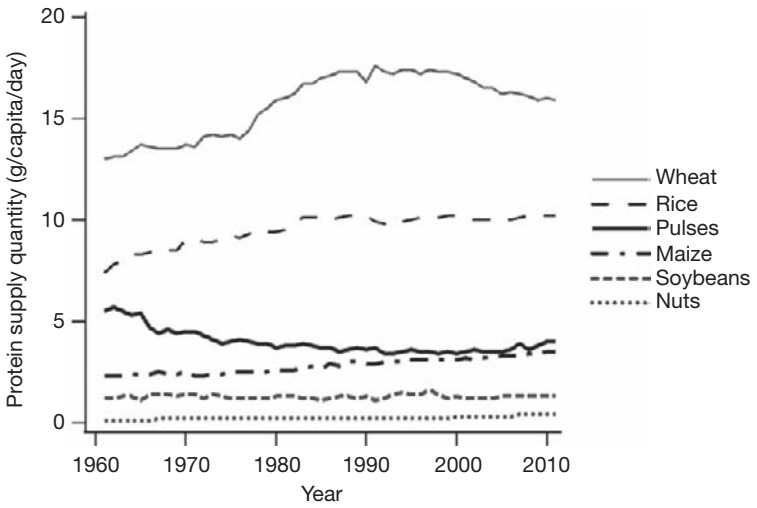

Figure 3 Protein supply quantity as food (g/capita/day) from different plant sources between 1961 and 2011. Food and Agriculture Organisation of United Nation FAOSTAT (2014) at http://faostat3.fao.org/.

Table 2 Dairy protein based ingredients and its manufacturing process and applications in food products

Product	*Protein content (%)*	*Manufacturing process*	*Food applications*
Whole milk powder			Chocolate
Skimmed milk powder	35		Chocolate
Milk protein concentrates	42–90	Membrane filtration from fresh skim milk. They contain both casein and whey protein in the same ratio as skim milk	Cheese, cultured products, dairy-based beverages, nutrition foods, weight management products, powdered dietary supplements, and sports nutrition products
Acid casein		Acidification of skim milk to pH 4.6 by adding a mineral acid or through lactic fermentation	
Rennet casein		Precipitation of skim milk through the action of microbial rennet enzymes	Processed cheese and nutritional foods
Caseinate			Beverages, food bars, soups, sauces, whipped toppings, bakery products, cultured foods, and nutritional foods
Whey protein concentrates	>80	From fresh cheese or acid whey using an ultrafiltration process	Yogurts, beverages, and dairy desserts Nutritional products and infant food
Whey protein isolates	>90		Low pH nutritional beverages, dry mix beverages, nutritional bars, protein fortified food, and sports nutrition products

frequently used technique for the dehydration of dairy products is spray drying. The purpose of dehydrating milk and dairy protein fractions is to extend the shelf life of these products for later use and reduce the cost of transportation.

Caseins

Caseins are composed of α_{S1}-, α_{S2}-, β-, and κ-casein in a percentage ratio of 40:10:40:10. Casein proteins are present in milk as self-assembled colloidal particles, commonly known as casein micelles. These micelles are in the range of 100–600 nm in diameter and provide milk with its white appearance. The casein micelle is a natural nanoparticle – self-assembled within vacuoles of the mammary epithelial cells, containing 92% proteins and 8% inorganic salts, primarily calcium phosphate, in dry weight. The major functions of the micelles are to transport proteins, calcium, and phosphate in high concentrations that would otherwise be insoluble in water and to provide adequate nutrients to young calves. The association between the mineral and protein components gives the native casein proteins their nutritional values as well as their unique functional properties that have been extensively exploited to produce dairy foods such as yogurts and cheeses.

The stability of casein micelles is due to their inherent zeta potential (~−20 mV) and from their structural steric hindrance caused by the protruding (the 'hairy' layer) C-terminal segments of glycosylated κ-casein, which prevent the close approach of nearby micelles. This creates a unique characteristic of milk in its 'white' color due to the light reflection of micelle particles. Removal of these protruding segments by

chymosin, the main enzyme present in the neonate calf stomach, results in coagulation of the modified casein micelles.

Casein is the major functional contributor in a range of dairy products. The destabilization of the casein micelles through manipulation of the pH by fermentation using bacterial cultures or enzymatically by rennet addition is an integral part in the manufacturing of the products such as yogurts and cheeses.

The casein proteins are also commercially produced as high-protein ingredients through separation from other components in bovine milk. There are three principal ways to produce casein ingredients on an industrial scale. They are isoelectric precipitation, rennet coagulation, and membrane filtration (Table 2). The isoelectric precipitation by the use of hydrochloric or sulfuric acid or by lactic acid produced by bacteria to reduce the pH of the milk to 4.6 produces the acid casein. Rennet casein is made by adding sufficient rennet to skim milk to cause it to clot. Acid or rennet casein may then be dried in a cabinet, tunnel, or spray dryer to a moisture content of 4%. The dried casein is then milled and screened to an appropriate particle size. The casein ingredients are widely used in coffee/tea whiteners, whipping powders, and imitation milks (Table 2). Rennet casein is a major ingredient in cheese analogue production.

Whole milk can also be dried to produce milk powder ingredients. Skim milk powder usually contains 35% milk protein. Milk powder ingredients are widely used for recombined milk products such as retorted or ultra high temperature (UHT)-treated long shelf life products where stability to secondary heating is paramount.

Whey Proteins

The whey from bovine milk also contains several distinct proteins. The main proteins are β-lactoglobulin (β-Lg), α-lactalbumin (α-La), bovine serum albumin, and immunoglobulin, representing approximately 0.27, 0.12, 0.025, and 0.065% in milk, respectively.

Whey proteins are highly functional and nutritional ingredients and have become increasingly valuable in formulated food products for human nutrition. There are two major types of whey protein ingredients, that is, whey protein concentrates (up to 80% protein) and whey protein isolates (90% protein). Whey proteins are manufactured as a by-product of the manufacturing of other dairy products such as cheese and casein. Low-temperature processing is needed to ensure the retention of both nutritional and functional properties because all the major whey proteins are globular proteins with defined molecular conformations that are susceptible to heat denaturation.

Whey proteins have excellent functional properties that are widely utilized in food applications such as yogurts, beverages, and dairy desserts (Table 2). They are highly soluble and able to stabilize oil/water and air/water interfaces, thus providing excellent foaming and emulsifying properties. Whey proteins can also form gels and have good water-binding properties. The excellent gelling properties of whey proteins make it an ideal gelling agent for meat products.

Whey proteins have also been increasingly recognized for their nutritional value as a superior source of protein fortification for specialized nutritional products and infant food. The nutritional value of whey proteins lies in their favorable amino acid compositions and their optimal digestibility. Whey proteins are used as a balancer for other proteins in specific nutritional applications, such as infant formula, and as a rich source of highly digestible branched-chain amino acids in sports beverages or nutritional bars (Table 2), to enhance protein synthesis in muscles, particularly after exercise.

Meat Proteins

Meat can be part of a balanced diet that contributes valuable nutrients that are beneficial to human health. Meat and meat products contain important levels of protein, vitamins, minerals, and micronutrients that are essential for growth and development of human bodies. The meat proteins, ~20% of a muscle's weight, are the major components that constitute the structure of the meat product. From a nutritional point of view, meat's importance is derived from its high-quality protein, containing all the essential amino acids and its highly bioavailable minerals and vitamins. Meat is rich in vitamin B12 and iron, which are not readily available in vegetarian diets.

Muscle proteins can be divided into three groups: myofibrillar, sarcoplasmic, and connective tissue proteins. The myofibrillar proteins account for 50–55% of the total protein. The two most abundant myofibrillar proteins are myosin and actin. They are responsible for the muscle's overall structure. The sarcoplasmic proteins account for 30–34% of the total meat protein and are soluble in water. Most of the sarcoplasmic proteins are the glycolytic enzymes, creatine kinase, and myoglobin. Myoglobin is a type of globular protein and gives meat its characteristic red color. The remaining 10–20% protein mass consists of connective tissue proteins such as collagen, reticulin, and elastin, which are all fibrous proteins.

Whole muscle meat is the food source of protein. Comminuted and reformed meat products such as burgers, sausages, and nuggets are also a common source of meat protein, although the protein content in these products is generally lower and the fat content is higher than the muscle meat cuts.

Meat protein ingredients that are derived from either meat processing by-products or lean tissue components are also used as high-protein ingredients in processed meat and other food products.

Collagen

Collagen is a family of insoluble fibrous proteins found in all multicellular organisms. It is one of the most abundant proteins in mammals, accounting for 25–30% of total body protein. It is also a major component of the skin, bone, cartilage, tendon, and blood vessels.

Collagen is a rod-shaped molecule, ~1.5 nm in diameter and 300 nm in length, consisting of three helical polypeptide chains coiled around one another into a triple-stranded superhelix stabilized by hydrogen bonds. Collagen is completely insoluble in water.

Collagen has a very unique amino acid composition and sequence. In terms of its amino acid composition, it has approximately 33% glycine, 12% proline, 11% hydroxyproline, and 11% alanine. A collagen chain has three amino acid residues per helical turn and is made up of an almost continuous repeating

of the Gly-X-Y-sequence, where X is mostly proline and Y is mostly hydroxyproline. Every third amino acid is a glycine residue, which, being small, occupies the helix's interior positions. The proline and hydroxyproline content of the collagen dictates its thermal stability.

Collagen for use in foods is extracted from bones, beef hides, pork skins, and skeletal muscle connective tissue. It is primarily used for its water-binding and gel-forming properties in reformulated meat products such as frankfurters and ham so as to increase cooking yields. Another application of collagen is in the manufacture of skinless or coextruded sausages.

Gelatin

One of the most commonly used meat protein ingredients is gelatin, a soluble protein obtained by partial hydrolysis of collagen. Gelatins are produced mainly from bovine and porcine sources; however, they can also be extracted from fish and poultry. Depending on the sources and manufacturing processes, the amino acid composition and the functional properties of gelatin can vary considerably. The content and distribution of proline and hydroxyproline are the major factors determining the physical properties of the gelatin, which in turn influence its quality and potential food applications.

Gelatin is one of the most versatile biopolymers and has numerous applications in food, confectionery, pharmaceutical, medical, and cosmetic products. The important functional properties related to the application of gelatin in food are its gel-forming, texturizing, thickening, and water-binding properties. A critical parameter for both collagen and gelatin is the helix-to-coil temperature or the denaturation temperature, which is around 36 °C. Above this temperature, all triple helical structure is lost due to the destabilization of the intra- and intermolecular hydrogen bonds leading to the unfolding of the triple helices. Below this critical helix-to-coil temperature and above the critical gelatin concentration (c*), the gelatin molecules reassemble into a network of triple helices, forming a thermoreversible viscoelastic gel upon cooling. The critical concentration and gel strength for a gelatin will also depend on factors such as the molecular weight of the gelatin, cosolutes, and pH of the solution.

It is this unique thermoreversible gelling property that makes gelatin such a useful food ingredient. Gelatin desserts and all types of jellies are examples of food products that take advantage of the thermoreversible gel formation and the 'melt in the mouth' texture of gelatin. Gelatin is also widely used in the confectionery industry. Examples of products containing gelatin are fruit gums, marshmallows, meringues, caramels, bar products, and sugar-coated candies. Gelatin can also be added to dairy products to improve the texture and reduce the secretion of aqueous whey from yogurt gels, cheese curds, and cream cheeses. Gelatin is also used in ice creams to control the size and distribution of ice crystals and thereby influence the texture, mouthfeel, and consistency of the product. In addition, gelatin is widely used in reformed meat products to bind water and meat juices and to enhance good texture and taste, in particular, as a fat replacer and/or binding agent in low-fat, low-carb, and low-calorie meat products.

From a nutritional point of view, gelatin is a good protein source that is easily digested and absorbed by the human body. However, gelatin is unusually high in the nonessential amino acids glycine and proline (i.e., those produced by the human body) while low in certain essential amino acids. The addition of gelatin in food products can increase the protein content and reduce the amount of carbohydrates, the salt content, and the amount of fat in low-fat food products.

Proteins from Seafoods

Seafood includes fish, crustaceans, and mollusks, which are considered as a low-fat and protein-rich source, as part of a healthy and balanced diet. Protein from seafood sources is of high quality, containing all of the essential amino acids in good quantity and in balanced amounts.

The amount of protein present in most fish products used for human consumption ranges from 15% to 20% on a wet weight basis (Table 3). There are no major differences between the amino acid compositions of freshwater fish and those of marine fish.

The muscle of different aquatic animals is very similar, containing similar protein and amino acid profiles. There are slight differences between fish muscle and the muscle of land animals. These are mainly associated with the differences in muscle structure required for swimming and buoyancy. Fish muscle tends to have less connective tissue than muscles from terrestrial animals, resulting in a more tender texture. About 70–80% of fish muscles are structural proteins. These structural proteins are soluble in cold neutral salt solutions of fairly high ionic strength. The remaining 20–30% contain sarcoplasmic proteins that are soluble in water and dilute buffers and a final part of the structural protein, 2–3%, being insoluble connective tissue proteins. Myofibrillar proteins are the primary food proteins of fish, comprising 66–77% of the total protein in fish meat, of which myosin comprises 50–60% of the myofibrillar contractile proteins and actin only 15–30%.

The major restructured seafood product is surimi, which is made from fish flesh by the removal of soluble protein, lipids, pigments, and other odorous compounds and with the addition of cryoprotectants, so that it can be stored in a frozen state. Currently, about 2–3 million metric tons of fish is used for surimi production. The gelling of actin and myosin on heating gives the surimi product a good resilient texture. During the

Table 3 Typical nutritional composition of fish and meat products (100 g)

Source	*Energy (Kcal)*	*Protein (g)*	*Fat (g)*
Fish	100–120	18–23	1–4
Chicken	100–160	20–25	6–15
Duck	130–210	17–24	4–15
Turkey	110–190	21–28	2–7
Beef tenderloin	200–220	30–31	7–10
Beef steak	150–260	21–28	6–16
Veal	134	21.7	4.6
Pork loin tenderloin	110–140	21–26	2–4
Pork chop	130–200	21–30	4–9
Lamb	200–300	16–25	12–20

Source: FAO.

last 10–20 years, the range of food products prepared from surimi has increased in number and quality.

Fish mince is another source of fish protein. It is a less-refined product compared with surimi. The mince retains all the flesh components, with resulting flavor and texture differences to surimi products. Fish mince is commonly used in the manufacturing of restructured products such as fish cakes and fish fingers.

Egg Protein

Hen egg is one of the most versatile foods. The hen egg, in the form of table eggs and egg products, forms an important part of the human total protein consumption. Egg products as a rich source of protein ingredients are also widely used in the food industry for their nutritional value, for their organoleptic characteristics, and mainly for their functional properties, hence their wide incorporation into manufactured food products.

Whole egg is composed of 75% water, 12% protein, 12% lipids, and about 1% carbohydrates and minerals. Proteins present in egg are distributed among the egg white and yolk. The egg white makes up ~60% of the total egg weight, of which water (88%) and protein (11%) are the major constituents. Proteins represent more than 90% of the egg white dry matter. The major proteins in egg white are ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), ovomucin (3.5%), and lysozyme (3.5%). Proteins present in egg yolk consist of lipovitellins (36%), livetins (38%), phosvitin (8%), and low-density lipoproteins (17%).

Egg white has no specific macrostructure. It is more or less a colloidal suspension of different protein constituents. They are highly functional physically and biologically and have high potential for industrial applications, if separated. Lysozyme is amongst the first egg white proteins that have been isolated and used by industry. The separation of lysozyme is achieved mainly through ion exchange chromatography due to the high isoelectric point value of lysozyme. Due to its antimicrobial activity, lysozyme is used as a natural food preservative against bacteria involved in food spoilage and food-borne disease.

Pasteurized whole egg, separated egg white, and egg yolk are further processed in liquid, frozen, or powder forms. Its foaming, emulsification, gelation, and thickening properties make egg product a universal ingredient for the food industry. Egg white is still considered as the reference for foaming properties, compared with other animal and plant protein ingredients. Both egg white and egg yolk have very good coagulation properties, when exposed to heat. Egg white, egg yolk, or whole egg is a traditional ingredient for desserts such as cakes, pastries, batter, and fresh pasta and sauces such as mayonnaise. The egg white proteins represent more than 90% of the total dry matter in egg white; thus, it is a high-quality nutritional source of protein.

Proteins from Plant Sources

Soy Protein

Although the overall contribution of soy protein in the average human diet is not as great as wheat protein (Figure 3), soy protein is, however, the most popular plant protein source to serve as an ingredient in formulated foods for their functional properties, such as water and fat binding, emulsification, foaming, and gelation. Soybeans contain ~35–40% of which 90% are storage proteins mostly consisting of β-conglycinin and glycinin. β-Conglycinin has the sedimentation coefficients of 7S, whereas glycinin has 11S. β-Conglycinin is a glycoprotein and is a trimer that consists of three subunits with molecular masses of 150–200 kDa, whereas glycinin is a hexamer with molecular masses of 300–380 kDa. Each glycinin subunit is composed of acidic (~35 kDa) and basic (~20 kDa) polypeptides, which are linked together by a disulfide bond.

The main soy protein products are soy flour, soy protein concentrate, soy protein isolate, and texturized and hydrolyzed soy proteins. Soy protein concentrate, which is produced from defatted soy flakes, after removing most of the soluble cell wall materials, has >65% protein (Table 2). Soy protein isolate, which is produced by alkali extraction and isoelectric precipitation, is the most refined form of soy protein with a protein content higher than 90%. Textured soy protein is produced by extrusion to resemble the texture of meat chunks (Figure 4). The principal function of texturized soy protein is to partially or totally replace animal proteins in various food products and is used as a protein source in vegetarian meat alternatives. Soy flour, soy protein concentrate, soy protein isolate, and their texturized products are mainly used as ingredients in formulated foods for their functional properties, such as water and fat binding, emulsification, foaming, and gelation. Common soy protein foods are soy milk that is used as a replacement for cow's milk by vegans and persons with intolerance to milk protein and tofu that is a protein curd made by precipitating the protein from soy milk.

Although traditional foods made from soybeans such as soy milk and tofu have been consumed throughout East Asia for more than 2000 years, in Western countries, soybean protein has only became an economical and high-quality vegetable

Figure 4 Texturized soy protein.

protein source for human diets during the last few decades. Soy protein ingredients have high protein levels and a well-balanced amino acid composition, which makes soy protein an important source of plant protein, with a great potential to replace meat and dairy proteins in human diets.

Wheat Gluten

The main storage protein in wheat is the gluten protein. Gluten protein is not a single protein, but a complex mixture of proteins, comprising several hundreds of polypeptides. About half of the proteins are monomeric gliadins and the other half are disulfide cross-linked polypeptides that form the polymeric glutenin fraction. The combination of these two protein fractions gives the gluten its unique physical functional property that none of other plant proteins have, that is, it forms a cohesive, viscoelastic proteinaceous network that provides the unique ability of wheat to produce leavened products. It is this precise ability of forming an insoluble protein mass when wheat flour is mixed with water that allows gluten to be separated from starch through a simple water wash process. Commercial gluten (known as vital wheat gluten) with a protein content as high as 75–80% is widely used in a variety of manufactured food products.

The major usage of gluten in Western countries has traditionally been in baked products, pasta, noodles, and breakfast cereals. In these product categories, the gluten is used to fortify flours of lower-than-desirable protein content by flour millers and bakers, in order to supplement lower-protein local wheats and to improve the quality of the flour so as to equal those with higher protein content. Gluten is attractive as a fat- and water-binding ingredient in restructured meat, fish, and poultry products. A major use of gluten in nonbakery foods is as a meat replacement in vegetarian foods and in the production of analogues of expensive foods such as seafood and crab meat, particularly in Japan. Texturized wheat protein, which is produced via extrusion, is used increasingly as plant protein to replicate the look and texture of meat products.

One major issue associated with gluten is its link with celiac disease, which is characterized by inflammation of the small intestine resulting from an inappropriate immune response to wheat gluten (and similar proteins from barley and rye). This disease can cause affected persons to lose weight and suffer from deficiencies of vitamins and minerals, which can lead to anemia and skin problems. At present, the only treatment is a strict diet avoiding all products containing gluten, and to cater for this market, other nongluten cereals or legumes are used commercially to produce gluten-free breads, pasta, flour, and other foods.

Proteins from Other Cereal Grains and Pulses

Although a variety of cereal grains are consumed as the basis of staple human diets either in the form of manufactured foods such as breads and breakfast cereals or by direct cooking of the grains such as rice, the protein content of cereal grains or the food prepared from the grains is generally low, in a range of 7–15% (Table 4). Conversely, pulses including pea, lupin, chickpea, lentil, bean, and other dry edible seeds from the pods of legume plants contain higher amounts of protein (20–36%; Table 4) compared with cereal grains and are important sources of protein in many diets around the world.

Table 4 Protein, carbohydrate, and fat content of major cereals, legumes, and oil seeds (g/100 g)

	Protein	*Carbohydrate*	*Fat*
Wheat flour	8–15	70–80	1–2
Rice	7–9	~80	~1
Maize (corn)	7–9	~75	4–5
Barley flour	10.5	74.5	1.6
Sorghum	11.3	74.6	3.3
Oats	16.9	66.3	6.9
Rye	10.3	75.9	1.6
Soybeans	36.5	30.2	19.9
Canola			
Pea	24.6	60.4	1.2
Chickpea	19.3	60.7	6.0
Lupin	36.2	40.4	9.7
Cowpeas	23.9	59.6	2.1
Lentils	25.8	60.1	1.1
Kidney beans	23.6	60.0	0.8
Broad beans	26.1	58.3	1.5
Mung beans	22.9	61.5	1.6

Flours, protein concentrates, or protein isolates have been produced from peas, chickpeas, and lupins. Pea flour is produced by dry milling of dehulled peas. Pea protein concentrate is produced by dry separation methods, whereas pea protein isolate is produced by wet processing using either alkali or acid solubilization, followed by isoelectric precipitation or an ultrafiltration process, which produces a protein fraction with a much higher protein content of 85–95%. Pea proteins have found applications in a range of food products such as cereal and bakery products, nutritional snack bars, pasta, meal replacement beverages, baby food formulations, vegetarian applications, meat, and seafood products. The increasing popularity of using pea proteins is largely due to their positive fat- and water-binding capabilities, emulsification properties, and gelation, texture, and nutritional values. Like other flours derived from pulses, chickpea flour is a potential high-protein source for use as an extender in emulsified meat products due to its superior technological functionality and minimal effects on flavor.

Lupins are nonstarch leguminous seeds with a similar protein content to soybean, at about 40%. Yellow lupins grown in Europe and South America are mostly used for animal feed. The presence of quinolizidinic alkaloids, which may be removed by soaking, prevents the direct consumption of yellow lupins as food. However, the white lupins, commonly consumed in the Mediterranean countries, are primarily grown for direct food uses. Australian sweet lupin (*L. angustifolius*) has a low alkaloid content and hence is more suitable for direct human consumption. When lupin protein is extracted, the resulting protein isolate is free of alkaloids and therefore can be used as a functional ingredient in human food. Lupin flour (i.e., free of alkaloids) and protein isolates have been successfully tested as ingredients in various food products such as in muffins in which egg and milk proteins are totally substituted, in biscuits to achieve high protein contents, and in dairy, bakery, and meat products.

Table 5 Protein, carbohydrate, and fat content of nuts (g/100 g)

	Raw			*Roasted*		
	Protein	*Carbohydrate*	*Fat*	*Protein*	*Carbohydrate*	*Fat*
Peanuts	25.8	16.1	49.2	26.4	18.9	49.3
Walnuts	24.1	9.9	65.2			
Almonds	21.2	21.6	49.9	21.2	21.1	52.5
Pistachios	20.3	27.5	45.4	21.0	29.4	44.8
Cashews	18.2	30.2	46.4	15.3	32.7	46.4
Brazil nuts	14.3	12.3	66.4			
Hazelnuts	13.7	17.0	60.8	15.0	17.6	62.4
Pine nuts	11.6	19.3	61.0			
Pecans	9.2	13.9	72.0	9.2	13.0	75.2
Macadamia nuts	7.9	13.8	75.8	7.8	13.4	76.1

Source: US Department of Agriculture Nutrient Database (2013) at http://www.ars.usda.gov/ba/bhnrc/ndl.

Proteins from Nuts

The total protein content in nuts is relatively high, which makes them a good source of plant protein (Table 5). Peanuts, walnuts, almonds, pistachios, and cashews have the highest protein content, about 20% or higher (g/100 g raw and roasted products), followed by Brazil nuts, hazelnuts, and pine nuts in the range of 11–15%. Pecan and macadamia nuts have the lowest protein content at below 10%. However, due to their high fat content, nuts are energy-dense.

Nuts are part of the Mediterranean diet, although their average consumption is relatively low. Nuts are most commonly eaten as snacks (raw or roasted and salted). They are also used in foods such as soups, cakes, pastries, cookies, and pasta sauces such as 'pesto.'

The nutritional value of nut proteins is not very high since they are limiting in some essential amino acids, in particular lysine. Thus, for the human body to make good use of nut proteins, they need to complement other food proteins, such as pulses or other vegetable proteins in vegetarian diets, and dairy proteins in order to provide a high protein value.

See also: Beef; Casein and Caseinate: Methods of Manufacture; Cereals: Types and Composition; Cheese: Composition and Health Effects; Eggs: Composition and Health Effects; Fish: Fish in the Human Diet; Food Composition Databases; Gelatin; Legumes in the Diet; Lupine; Maize; Meat: Role in the Diet; Meat: Structure; Milk Powder; Milk: Processing of Milk; Milk: Role in the Diet; Milk: Sources and Composition; Nuts: Health Effects; Pork Meat Quality, Production and Processing on; Poultry: Processing; Protein: Digestion, Absorption and Metabolism; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Proteins: Chemistry, Characterization, and Quality; Pulsed Electric Fields; Sheep: Meat; Soy Beans: Dietary Importance; Sports Nutrition; Wheat: Grain Structure of Wheat and Wheat-based Products; Whey and Whey Powders: Production and Uses; Whey and Whey Powders: Protein Concentrates and Fractions; Yogurt: Yogurt Based Products.

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Protein: Requirements

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Introduction

Discussions in the area of nutritional recommendations are seldom without some degree of controversy, and protein is an exemplar of the difficulties and debates that can occur. The amount and type of dietary protein needed to allow optimal expression of the genome during growth and development remain poorly understood. Even the question of the nature and consequences of protein deficiency has proved controversial. For example, clinical manifestations long thought to reflect a protein deficient diet, such as edematous infantile malnutrition, have now been redefined as a reductive adaptation to a combination of infection and micronutrient deficiency. Also, there are virtually no effective markers of protein deficiency in adults. At the other end of the spectrum of public health issues, within health clubs worldwide, more money is spent on protein and amino acid supplements than on any other ergogenic aid, even though the effectiveness of such expense is poorly documented to say the least.

Values for protein and amino acid requirements derive from human and animal nitrogen (N) balance and growth studies that began early in the twentieth century and identified both estimates of dietary requirements and the pattern of amino acid essentiality. From the 1970s, research expanded to include stable isotope tracer studies of human protein and amino acid metabolism and turnover. This research has provided insight into the regulation of protein synthesis, proteolysis, and amino acid and N metabolism, in terms of quite detailed accounts of signal transduction pathways and their effectors. However, the most recent expert report from WHO on human protein and amino acid requirements concluded that, of the methodologies available, N balance (NB) remains the only available method for estimating the requirement for total protein (N); as a consequence, no current method is entirely reliable for determining the dietary requirement for indispensable amino acids (IAAs). Because of these limitations, the latest recommendations for protein and amino acid requirements are not entirely satisfactory and the nature and limitations of commonly accepted protein requirement values are by no means widely understood. In terms of the most recent appropriate research, the focus has been much more on identifying optimal protein requirements, especially for older adults. The difficulty is that virtually all new information derives from acute experimental studies of the amino acid requirements for maximizing protein synthesis in the skeletal muscle. However, the relationship with such measurements and the long-term maintenance of lean body mass in muscle and other tissues has not been established. It is the case, as indicated in Figure 1, that dietary protein intakes can vary markedly even within diets that would be judged 'healthy' and between population groups who choose plant-based rather than animal source foods. In fact, given $\geq$3-fold variation in dietary protein content indicated in Figure 1 and the fact that food energy intake can vary by up to 1.7 times due to lifestyle, overall protein intakes can vary over a fivefold range in ostensibly healthy individuals. The challenge is to identify the intake at the lower end of this range, which satisfies the criteria of a requirement value.

Terminology

Protein requirements are best discussed in terms of metabolic demand, dietary requirement, and dietary allowances. Metabolic demand is for amino acids, and the magnitude and amino acid pattern of this demand are determined by the extent of those metabolic pathways (e.g., net protein synthesis) that irreversibly consume amino acids. Thus, the demand varies with the phenotype and the developmental and physiological state of the individual. The dietary requirement is the amount of protein and/or its constituent amino acids that must be consumed in order to satisfy the metabolic demand. Because digestion and absorption may be incomplete (with dietary nitrogen (N) lost in the feces) and the absorbed amino acid pattern may not exactly match cellular needs (with some absorbed amino acid N lost as urinary N excretion), the dietary protein requirement will usually be greater than the metabolic demand. Dietary allowances are intakes derived from estimates of the average amount and between-individual variability within a population group that meets the dietary requirements of most individuals within the population. The most recent dietary allowances are those published by WHO in 2007, the values of which have been reproduced in the 2012 report of the European Food Safety Authority (EFSA).

Metabolic Demands for Amino Acids: General Aspects

Current evidence supports the representation of the metabolic demands as in Figure 2. The metabolic demand for amino acids is to maintain tissue protein at appropriate levels and to provide for all amino acid-derived metabolites and nonprotein products and any special protein needs during growth, rehabilitation, pregnancy, and lactation. Tissue proteins are diverse, including structural or fibrous insoluble types and soluble globular species, with characteristic properties and functions that are determined by their amino acid sequence. All proteins are in a dynamic state of constant turnover (i.e., breakdown to constituent amino acids and resynthesis), although for the structural proteins, this is slow or minimal. The tissue protein pool is not static even when body weight is constant, contracting and expanding in the postabsorptive and postprandial state. Nonprotein products/derivatives include nucleic acids and a diverse range of smaller molecules, such as creatine, taurine, glutathione, hormones (e.g., catecholamines and thyroxine), neurotransmitters (serotonin and dopamine), and

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00573-0

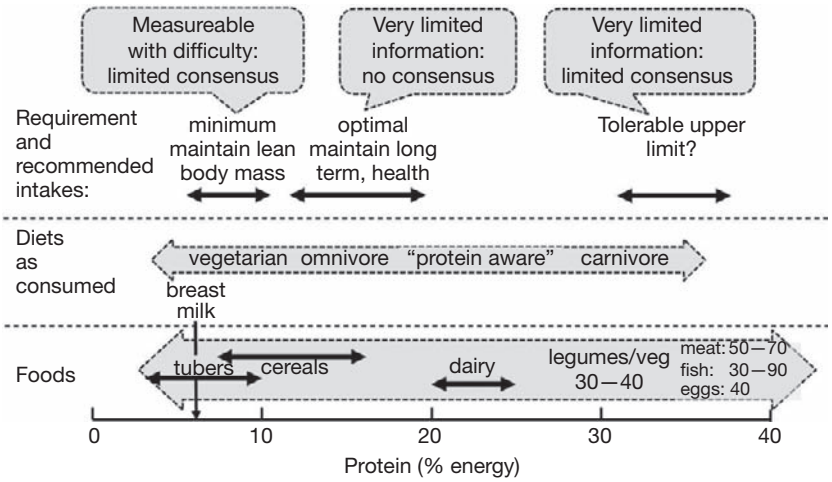


Figure 1 Protein requirements in relation to protein intakes from foods and diets.

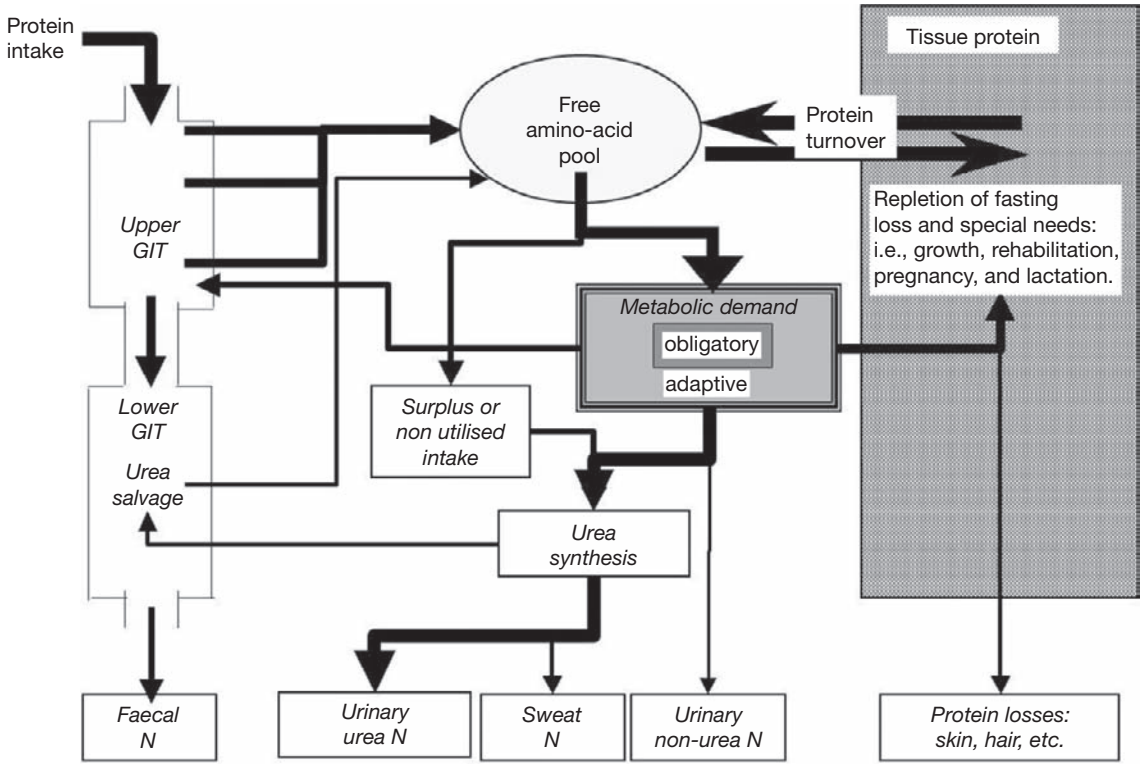


Figure 2 Schematic representation of the metabolic demands for amino acids.

nitric oxide, a key regulator of blood flow and other physiological processes.

The metabolic demand is supplied from the free amino acid pool, the size of which, for most of the IAAs, is regulated within narrow limits. This regulation involves supply from three sources: dietary proteins after digestion and absorption from the upper gastrointestinal tract (GIT), tissue protein after proteolysis during protein turnover, and urea salvage in the large bowel. Within the free amino acid pool, there are also interconversions of several of the dispensable amino acids (DAAs), especially during transfer between organs and in the course of amino acid absorption from the GIT. Removal of free amino acids occurs by reactions in which they act as substrates, and these reactions are shown as three pathways, one of which is the metabolic demand. This pathway involves a number of irreversible pathways, including net protein synthesis, and other irreversible metabolic transformations of individual amino acids. The second and quantitatively largest pathway is the removal for protein synthesis during protein turnover. At N equilibrium, because turnover involves the reversible removal of amino acids, with replacement through proteolysis, it does not exert a net metabolic demand (other than for those amino

acids irreversibly modified during or subsequent to protein synthesis). The third pathway is the irreversible removal of amino acids by oxidation and N excretion provoked, for example, by the transient increases in some or all free amino acids after a protein meal. This would represent an inefficient utilization. Although amino acid oxidation and urea synthesis are assumed to be irreversible, this is not entirely true because of urea salvage in the lower GIT. Thus, the rate of urea synthesis is usually in excess of the rate of urea excretion because some urea enters the large bowel and is hydrolyzed by bacteria. Most of this N is utilized by bacteria, and since little is lost as fecal N, it is eventually returned to the systemic pool as ammonia and amino acids after bacterial death and proteolysis, including some IAAs. Although the extent and nature of this salvaged urea N are poorly understood, ammonia predominates and this contributes to the *de novo* synthesis of DAAs.

The metabolic demand for amino acids appears to involve obligatory and adaptive components. The obligatory component for subjects at equilibrium (i.e., maintenance) comprises conversion of some individual amino acids into important metabolites that are further transformed into nitrogenous end products, mainly urea and other compounds in urine, feces, or sweat, as well as net synthesis of proteins lost from the body as skin, hair, and any other secretions including those within the upper GIT such as mucin proteins lost into the large bowel. These diverse biological demands for amino acids for maintenance represent an essential but probably quite small intrinsic metabolic demand for an equivalent amount of protein. The magnitude of this maintenance component is assumed empirically to be equal to the obligatory nitrogen loss (ONL) – that is, the sum of all N losses from the body observed in subjects fed with a protein-free but otherwise nutritionally adequate diet after 7–14 days. By this time, N losses have declined to a stable and reproducible low level with the subjects losing body protein at a constant daily rate. In normal adults, the daily obligatory urinary, fecal, and subcutaneous and other N losses are approximately 33, 12, and 7 mgN kg^{-1}, respectively (i.e., 52 mg kg^{-1} day^{-1}), in total equivalent to 0.33 g protein/kg/day tissue protein mobilized to meet such demands. The ONL is a function of body weight and, as far as is known, varies little with age. After adaptation to a protein-free diet, net tissue proteolysis is assumed to provide for the nonprotein components of the obligatory demand at a rate determined by the metabolic consumption of the rate-limiting amino acid (the amino acid with the highest ratio of molar proportion in the metabolic demand to molar proportion in protein). Because the obligatory metabolic demand is for a mixture of amino acids with a profile that is unlikely to match that of tissue protein, the actual N content of the metabolic demand is likely to be less than that indicated by the ONL (i.e., less than an equivalent of 0.325 g protein/kg/day). This is because all amino acids mobilized to provide for the metabolic demand must be oxidized and will contribute to the N excretion, whereas only some of them will serve useful functions. The evidence for this is the lowering of the ONL in response to feeding selective amino acids, such as threonine, tryptophan, and methionine. In addition to these metabolic demands for maintenance, any net protein synthesis associated with growth, pregnancy, and lactation also constitutes an obligatory metabolic demand.

The adaptive component of the metabolic demand represents amino acid oxidation at a rate varying with the habitual protein intake. Although this aspect of amino acid metabolism is not well understood, it is likely to occur because humans grow slowly or maintain constant weight on diets that may contain protein considerably in excess of minimum needs. Thus, in order to be able to rapidly dispose of excess protein and maintain the very low tissue concentrations of those amino acids that may be toxic at higher concentrations, that is, the branched chain (e.g., isoleucine), aromatic (e.g., phenylalanine), and sulfur amino acids (e.g., methionine), the capacity of the pathways of oxidative amino acid catabolism adapts (increasing their maximum initial velocity of enzymatic reaction $\{V_{max}\}$), enabling them to operate at the appropriate rate set by habitual protein intakes. Crucially, the adapted rate of amino acid oxidation changes minimally with feeding and fasting and changes only slowly in response to a change in dietary protein intake level. This has two main consequences.

First, because the adaptive rate of amino acid oxidation continues to some extent into the postabsorptive state, there are postabsorptive losses of tissue protein and N excretion – that is, a diurnal cycle of postabsorptive losses and postprandial gains with an amplitude that increases with the increasing habitual level of protein intake as shown in Figure 3. As such, the adaptive metabolic demand includes a component of net protein synthesis that repletes postabsorptive losses. The magnitude of this varies in a complex way with eating pattern and with the amount and quality (amino acid score) of the habitual protein intake.

Second when intake falls below habitual intake, mobilization of tissue protein occurs with a negative NB for as long as it takes to adapt amino acid oxidation rates to the lower level of intake. This was previously identified as the labile protein reserves. It can be assumed that for intakes greater than the minimum protein requirement (MPR), full adaptation to the new level will include not only a change in the adaptive metabolic demand to match intake but also repletion of most tissue N lost during the adaptive transition, that is, a period of positive NB.

The main practical implication of this adaptive metabolic demands model is that the true MPR will be that associated with the lowest possible adaptive metabolic demand. It is not known with any certainty how long such adaptation would take, but the limited existing evidence suggests it is considerably longer than the 2-week periods employed in short-term balance studies. This implies that short-term balances, from which our estimates of the MPR derive, may overestimate the value. It also means that some of the variability in protein requirements between studies may reflect variable completeness of adaptation to the test diets.

The Amino Acid Pattern of the Metabolic Demand: Plant Versus Animal Sources

With continuous and extensive amino acid interconversion, the pattern of amino acids in dietary protein need not exactly match the amino acid pattern of the demand. This is because some amino acids are dispensable and can be replaced by sufficient total amino acid N supplied from other amino acids or sources of nonessential N such as ammonium

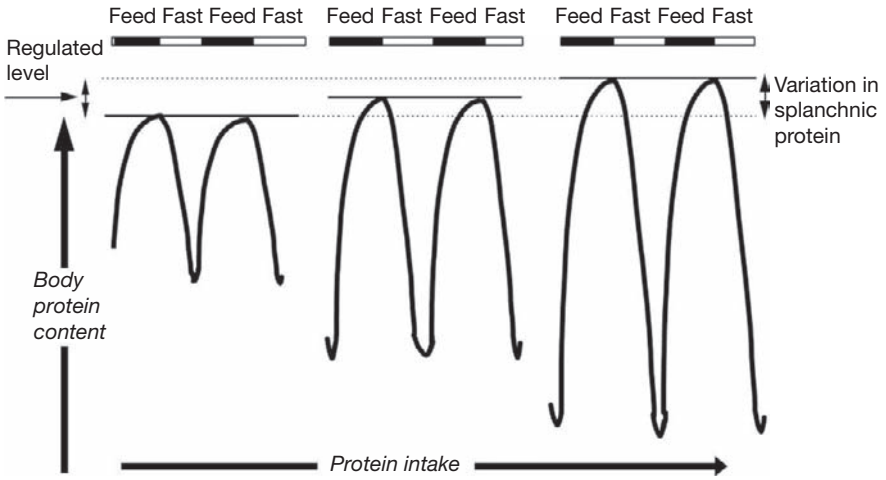


Figure 3 Balance regulation throughout the diurnal cycle with increasing habitual protein intakes.

compounds. These amino acids include aspartic acid, asparagine, glutamic acid, alanine, and serine. However, there will be a minimum dietary requirement for other amino acids, that is, those amino acids that cannot be formed from other amino acids and are therefore classified as indispensable. These are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. In addition, some amino acids are formed only slowly from other amino acids and become indispensable under specific physiological or pathological conditions. These are the conditionally IAAs, for example, cysteine, tyrosine, glycine, arginine, glutamine, and proline.

However, the definitions 'dispensable' and 'indispensable' for amino acids need to be interpreted with care. It might be assumed that excess IAAs can provide nonessential N after their carbon skeletons are oxidized. In fact, it has long been known that dietary amino acid mixtures rich in IAAs like egg protein are less effective in meeting the demand than mixtures with added nonessential N. Such diluted mixtures will maintain overall balance with lower levels of IAAs. This indicates that the demand includes an absolute need for nonessential N, which is less efficiently provided for by the oxidation of excess IAAs than by the DAAs. The metabolic basis of this demand for nonessential N is poorly understood.

There is a widespread view that there are significant differences in the relative nutritional value (quality) of plant compared with animal protein sources in human nutrition, mainly because of relative deficiencies of some IAAs. This view, which almost certainly overestimates the extent of any such differences, derives from studies that assessed the quality of dietary proteins in animal growth assays. In such assays, marked differences are observed between animal and many individual plant protein sources. For example, compared with animal source proteins, cereal proteins generally have low levels of lysine and in some cases tryptophan because of the structure of the main storage proteins – the prolamins, for example, gluten in wheat and zein in maize. In legumes, while the main storage proteins, the globulins, contain much higher levels of lysine than cereals, they often have relatively lower levels of the sulfur amino acids, methionine, and cysteine. These differences influence their individual performance in animal growth trials identifying them as lower-quality proteins. However, when different plant protein sources are combined in these assays, such as cereals and legumes, near maximal growth can be observed. This gave rise to the concept of 'complementation,' in which the appropriate balance of essential amino acids is provided from combinations of plant proteins.

It is now recognized that in human nutrition, with the rapid growth of the newborn infant slowing markedly after weaning, the nutritional demand for IAAs for tissue growth becomes a minor component of the metabolic demand after the first year of life. Little of the metabolic demand for amino acids is generated by protein turnover because of amino acid recycling. Some net protein synthesis is associated with skin and hair growth and with gastric secretions (e.g., threonine-rich mucus glycoproteins) that pass into the colon to be utilized for bacterial metabolism and with the postprandial replacement of postabsorptive tissue protein losses as shown in Figure 3. The metabolic demand for maintenance of normal function and composition is a poorly understood pattern of amino acids utilized in the various metabolic pathways other than protein synthesis. It has long been known, however, that this pattern differs from that required for growth (the tissue protein pattern) and contains a much lower overall amount of IAAs. The currently recommended amino acid pattern for human adults, the maintenance pattern, contains 28% IAAs compared with 42% in tissue proteins. Furthermore, nitrogen balance studies of different plant protein sources have consistently failed to show marked differences between plant-based and animal protein-based diets. Indeed long-term studies of healthy active adults on diets where wheat provided most of the protein have shown that body composition and physical fitness is maintained over periods of several months. This means that protein quality in terms of the amino acid profile may be less relevant in human nutrition, at least after the first few years of life when the rapid growth of infancy has subsided.

In fact, it is likely that the main difference between animal and plant dietary protein sources reflects the lower digestibility of some plant protein sources due to a combination of tough

plant cell walls that reduce the accessibility of plant proteins to digestive enzymes and antinutritional factors that can inhibit digestion.

In the opinion of this author, the currently accepted amino acid pattern of the maintenance requirement for IAAs defined in the 2007 WHO report is not entirely satisfactory. This is because each value is the average of several values that derive from different methodologies. These vary considerably, over a twofold range in the case of lysine, for example. This means that considerable uncertainty remains about the amino acid requirement pattern, which is used currently to judge the quality of dietary protein sources.

Nitrogen Balance

NB studies have been central to the definition of protein requirements within a model of nutrient balance: that is, a protein intake that balances all losses observed in a healthy, weight-stable subject. N is the proxy measure of protein intake and losses, and health is identified only in terms of an appropriate lean body mass at a healthy body weight. Thus, the MPR is determined as the intake for N equilibrium (zero balance), as indicated in NB studies. There are no other measureable physiological or biochemical indicators of health that are exclusively and unequivocally indicators of protein intake and no disease states that have been identified as having a quantifiable relationship with protein intakes.

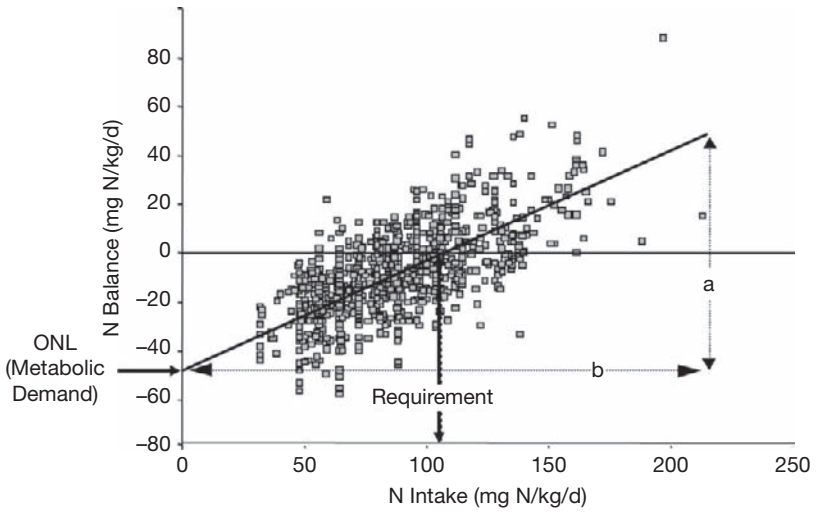
The aim of NB studies is simple – to define the relationship between intake and all losses (urinary, fecal, and surface – mainly sweat, skin, hair, breath ammonia, nail clippings, etc.) in subjects fed with three or preferably more intake levels, usually at or below the expected intake for equilibrium. This allows the intake (I), which matches all losses (L), and maintains N equilibrium to be identified as the requirement (R). Thus,

$$\text{when } I = R, \quad \text{balance} = I - L = 0$$

As indicated previously, the lowest level of losses observed, the ONL, equates to a daily loss of about 0.33 g protein/kg/day. When such subjects are refed with protein, losses of body protein decrease as the dietary protein provides for some of the metabolic demand. However, the apparent efficiency of utilization of dietary protein to meet the metabolic demand is much $<100\%$ so that N losses increase with intake and the required intake for balance is more than the ONL. The literature on human NB studies has been assessed in a meta-analysis, with all the reported individual balance points and the analytic principle shown in Figure 4. The protein intake that will balance all losses and produce N equilibrium, the maintenance requirement, is identified by linear regression of balance against intake, as intercept/slope. The currently accepted maintenance requirement for adults ($0.66\,\text{g}\,\text{kg}^{-1}\,\text{day}^{-1}$) is the median value of all individual subject linear regressions of the three or more levels of protein intake. Although the majority of the studies were young men studied with animal proteins, some studies with mixed plant proteins, with women, and with older adults were included. However, no significant influences of sex, age, or dietary protein source were observed. Subsequent NB studies examining age and sex specifically have confirmed that these factors do not influence the MPR.

Inherent Difficulties with N Balance Studies

This apparently simple – but laborious – approach is in fact beset with a large number of quite serious problems, as listed in Table 1. Because balance is a small value compared with the much larger values of N intake and N excretion, there is a lack



Balance = e x intake-ONL
(e = efficiency of utilisation = Δbalance(a)/Δintake(b))

Balance = 0.47.intake – 48.1mgN/kg/d
Requirement = intake for zero balance = ONL/e
= 105mgN/kg/d = 0.66g protein/kg/d

Figure 4 Meta-analysis of N balance studies: individual balance points and principle of analysis.

of precision in its measurement with considerable overall error. The various systematic errors mean that balance may be overestimated, often with unrealistic positive balances (protein gains) at high intakes. In practice, prediction of a zero balance–intake intercept is made from a few balance points by linear regression. Of the various difficulties relating to the design and interpretation of NB studies, the most important are (a) ensuring subjects are at energy balance, (b) allowing sufficient time for adaptation of amino acid oxidation rates to the change in protein intake, and (c) interpreting the slope of the regression as a true measure of the efficiency of protein utilization.

Energy Balance and N Balance

Body protein equilibrium is influenced by energy intake, with excess leading to weight and lean-tissue gain and with inadequate energy intake leading to weight loss, with the protein intake diverted to oxidation as an energy source. Thus, the protein requirement for N equilibrium is a function of energy balance and the actual influence is quite marked. According to one analysis of NB on intakes of energy (EI; kcal kg^{-1}) and N (NI; mg N/kg), NB = 0:171NI + 1.006EI – 69:13, implying that the intake for N equilibrium (the requirement) will vary from 1.4 to 0.32 g kg^{-1} day^{-1} according to whether energy intakes are equal to the resting metabolic rate (RMR) or equal to twice the RMR. In fact, most (85%) of the estimated between-individual variability used to calculate the safe protein intake shown in Figure 5 could be accounted for by an error of only ±10% of basal metabolic rate (BMR) in estimating the true energy needs of a subject. Such errors might not be observed in terms of weight change during the few days in which NB is measured. It is highly likely that many of the positive NBs shown in Figure 4 reflect excess energy intake. This dependence of NB on energy intake raises difficult and currently unanswered questions about whether the protein requirement should be defined in terms of a specified energy intake level, to maximize dietary protein utilization. For example, for populations with low-protein staples, the consumption of more energy would allow body protein equilibrium to be achieved: this could, however, predispose to obesity if energy expenditure is insufficient to match the increased intakes. In any case, the extent to which variation in energy intakes at energy balance (i.e., with increasing levels of physical activity) influences NB is unknown.

Table 1 Potential problems relating to N balance methodology

Imprecise: Balance is small difference between two much larger amounts
Systematic errors: Intake overestimated, loss underestimated due to difficulty of accounting for all losses:
Skin surface and secretions
Loss of N_2 gas
Expired ammonia
Endogenous NO production gives urinary nitrate, fecal ammonia, and nitrite (not always measured)
Changing size of the body urea pool
Design: Balance influenced by
dietary energy intake and physical activity
extent of adaptation of amino acid oxidation
Analysis: Linear regression slope markedly underestimates efficiency of utilization

Adaptation to Changes in Protein Intake

With the metabolic demand for amino acids including both fixed and variable demands, the relationship between intakes and balance will be a function of time and the rate of adaptation. This is undoubtedly why the determination of human protein requirement by NB has proved to be so difficult. Thus, when protein intake changes, the metabolic adjustments involved with matching amino acid oxidation and urea excretion rates to the new intakes take considerable time to adapt to the new level of intake. In practice, most balance studies are short-term, with dietary periods of 2 weeks at each intake studied and with diet periods randomized to minimize metabolic carryover of prior diets. The actual time taken for complete adaptation is poorly understood, but it is likely to be much longer than the 2 weeks usually allowed for in balance studies. While 2 weeks allows N excretion to stabilize in

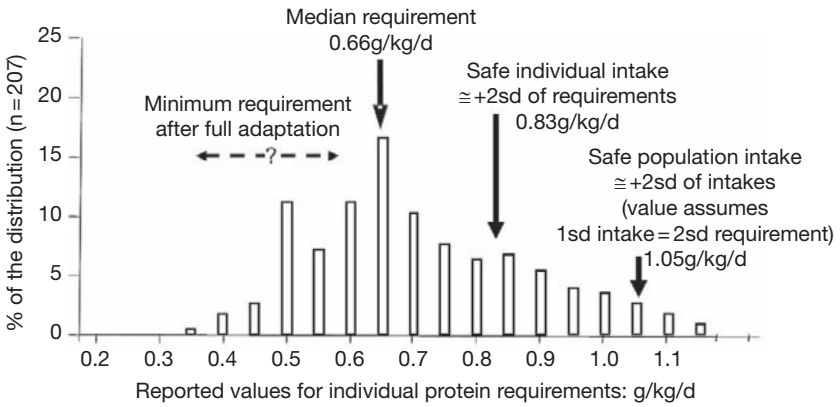


Figure 5 Distribution of reported values for the protein requirements showing values for safe individual and population intakes. The bars are the distribution of individual values of intakes for N equilibrium expressed as protein equivalents ($n = 224$ individual subjects from $n = 32$ studies, after a 5% trim of outliers), from a meta-analysis of N balance data. The minimum requirement after full adaptation is not known but likely to be less than the median value within the range shown.

subjects fed with a protein-free diet while establishing the magnitude of the obligatory N losses, it appears that this adjustment to a protein-free diet, an extreme metabolic change, occurs more rapidly than the adjustment from one intake to another. The evidence suggests changes in N excretion continue over several months after reduced protein intakes. Incomplete adaptation to a reduced intake at the end of a 2-week dietary change will result in a more negative NB, increasing the apparent protein requirement.

Adaptation and the Efficiency of Protein Utilization

While considerable NB studies have been performed in healthy adults, there are only a limited number of studies in any other population group, for example, children and pregnant and lactating women. In these cases, protein requirements have been derived with a factorial model in which the special needs for growth, pregnancy, and lactation have been calculated and added to the maintenance requirement derived for adults, which is assumed to be the same in these other populations. Calculation of the additional special needs involves adjusting measured rates of protein deposition, in children or during pregnancy, or rates of breast milk production, by an efficiency factor to determine the extra dietary intake necessary to meet the special needs. Historically, these efficiency factors were decided somewhat arbitrarily at 0.7. However, in the recent WHO report, much lower specific values were chosen from the slopes of the linear regressions of the limited NB studies reported for the different population groups. As discussed in the succeeding text, the calculated special needs added to the maintenance requirement are now much larger than previously identified.

Clearly, most balance studies are performed with high-quality protein sources, and this raises the question as to why protein utilization should be so inefficient in healthy adults. In fact, direct studies of the efficiency of utilization of proteins with ^{13}C-1 leucine oxidation (leucine labeled on its 1-carbon with the stable isotope, carbon-13) balance studies have shown that the true efficiency of utilization of dietary protein is as would be expected, that is, 95–100% for milk and high-quality proteins and lower (60–70%) for wheat. The shallow slope (low efficiency) of the NB regression has been explained in terms of the adaptive metabolic demand, which results in an increasing demand above the ONL (the intercept) with increasing intakes. This means that the slope underestimates the actual efficiency of utilization and it is consequently incorrect to evaluate the balance data in terms of an efficiency of utilization. The slope of the regression is only of value in terms of defining the intake for equilibrium (i.e., the requirement = intercept/slope = intake for zero balance). The consequence of this conceptual error is that the special needs for children and pregnant and lactating women are all overestimated in the current WHO report and these values have been adopted by other international and national bodies that invariably tend to adopt the WHO values.

Dietary Protein Allowances for Individuals and Populations

NB studies aim to determine both an individual MPR and a recommended allowance for protein, which, if consumed by any individual, would be associated with a very low risk of deficiency (deficiency = an intake < requirement). As shown in Figure 5, the average or median requirement is the midpoint of the range of individual requirements (0.66 g kg^{-1} day^{-1}). The safe individual intake (recommended nutrient intake in the United Kingdom) is calculated from the interindividual variability of the distribution of requirement values, as the 97.5th percentile, nominally the average + 2 SD, that is, 0.83 g kg^{-1} day^{-1}. Thus, any individual receiving such an intake will have a very low (<2.5%) risk of deficiency (defined as an intake < requirement). However, it is not generally appreciated that the safe intake for a population will differ from the safe individual intake. This is because the safe population intake needs to take into account the distribution of individual intakes as well as requirements. Because the variability of intake is usually greater than that of the requirement, the safe population intake is greater than the safe individual intake, usually approximating to the average requirement + 2 SD of intakes or plus 3–5 SD of requirements (i.e., 1.05 g kg^{-1}/day^{-1}). This does not mean that every individual in the population should consume the safe population intake; it means merely that the mean intake should be at least this level. In fact, the average or median requirement can be used to judge the adequacy of the population intakes. This is because, on the assumption that intakes and requirements are not correlated, the prevalence of deficiency approximates to the proportion of the population with intakes below the average requirement. For a population within which the median intake equates to the safe population intake, this proportion would be about 2.5% (this is called the 'cut-point' method of calculating the approximate deficiency prevalence).

A tolerable upper limit (TUL) has not been identified for protein, but the evidence suggests that intakes of three to four times the safe individual intake are consumed without obvious harm. This would suggest as shown in Figure 1 that the TUL may be quite high (e.g., ≈35% protein energy) and certainly higher than the value of 2 × safe intake often assumed in the past.

The serious implication of lack of complete adaptation in short-term multilevel balance studies is that because of the very wide range of protein intakes in the human diet, mainly through variable meat intake, the apparent requirement indicated in a study may still reflect the prior habitual diet: that is, the actual metabolic demands are higher than minimum levels because the adaptive component of amino acid oxidation remains influenced by previous intakes. This may explain the very wide range of reported apparent requirements shown in Figure 5 from below 0.4 to >1.1 g protein/kg/day. If adaptation, rather than variation in actual protein requirements, does account for the variability, then a quite different analytic model would be implied in which the MPR and safe intake are much lower. In this case, risk of deficiency for fully adapted individuals would remain low until intakes fall to quite low levels. Such adaptive models pose difficult questions for public health nutrition.

Protein Requirements for Growth and Special Needs: The Factorial Model

As indicated earlier, for infants, children, adolescents, and pregnant and lactating women, protein requirements are derived by a semifactorial model in which the requirement

comprises the components of the metabolic demands shown in Figure 2, identified as maintenance (the adult MPR identified as the intake for N equilibrium as discussed earlier), plus special needs. Special needs, that is, growth in children, the products of conception in pregnancy, milk production during lactation, and catch-up growth during nutritional rehabilitation, are calculated from measured rates of protein deposition (or secretion) adjusted with an assumed efficiency of dietary protein utilization to meet these amounts. In practice, the amounts of protein deposition, or milk protein secretion, are known reasonably accurately.

Infants, Children, and Adolescents

The main components of the safe individual intake during growth and development as reported in the recent WHO report are shown in Figure 6. The requirements include maintenance, assumed to be the adult MPR for all age groups, and growth. This is derived from measured rates of protein accretion in infants and children adjusted for an efficiency of utilization of 58%, derived from the slope of NB studies in children. To account for interindividual variability, the safe intakes include the addition of 2 SDs for maintenance and dietary growth needs, calculated from a coefficient of variation (CV) that is the weighted mean of the CVs for maintenance and growth. Overall, the safe intake falls from 1.31 g kg^{-1} day^{-1} at 6 months to 0.83 g kg^{-1} day^{-1} in adults.

Pregnancy and Lactation

The values reported by WHO allow for protein retention in the products of conception and in the maternal tissues associated with an average gestational weight gain in healthy women of 13.8 kg; this has been estimated from body composition analyses and for the increased maintenance costs of the increased body weight. The efficiency of dietary protein utilization to meet these growth costs is assumed to be 42%, a value observed in NB studies in primiparous teenagers, and the safe intake is calculated assuming a CV of 12%. This equates to additions of 0.7, 9.6, and 31.2 g protein per day. Lactation requirements are 19 g day^{-1} for the first 6 months and 12.5 g day^{-1} after this time; these values derive from the demand, observed as average milk protein output of well-nourished women breast-feeding adjusted for an efficiency of utilization of 47% as observed in NB studies in adults.

Uncertainty About and Safety of Requirements for Growth and Special Needs

Because the values selected for the efficiency of dietary protein utilization are very much lower than would be expected, the requirements for growth, pregnancy, and lactation are much higher than would be if more physiologically realistic values were used. This means that there are uncertainty and controversy about these requirement values.

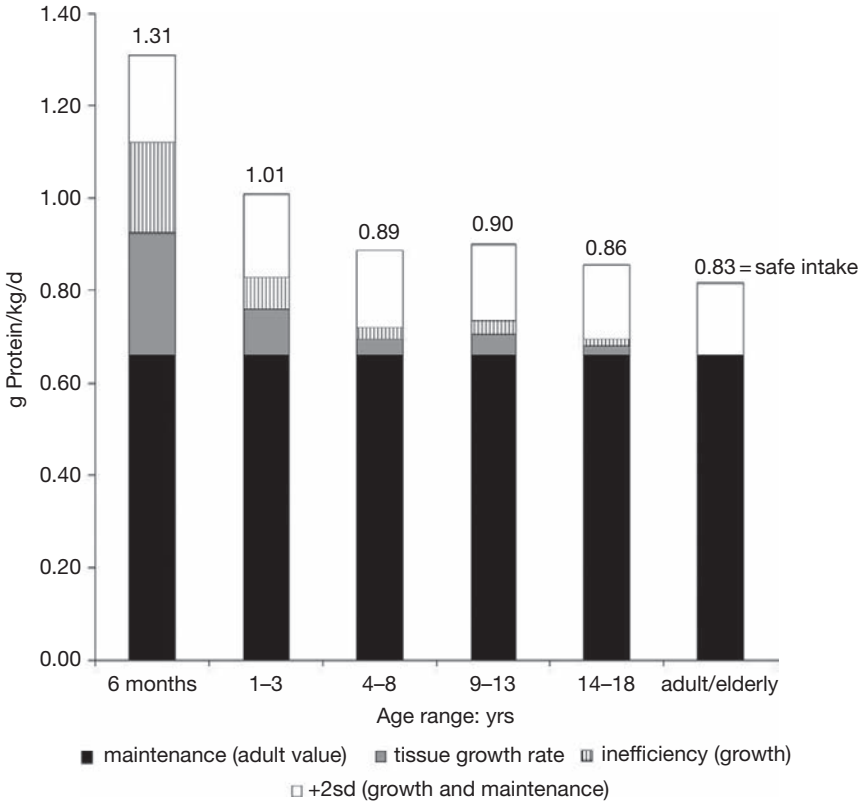


Figure 6 Factorial estimates of protein requirements throughout the lifecycle. Overall values are averages of the separate values for boys and girls.

Clearly, any argument that the true adult MPR may be less than the median value of 0.66 g kg^{-1} day^{-1} with a value toward the lower end of the distribution shown in Figure 4 will also apply where the adult maintenance value is used in the factorial calculation (i.e., child growth and pregnancy). This means that for children and adolescents, the true MPR values are likely to be lower than values shown in Figure 6. However, it is the case that the factorial model used for children was evaluated in the WHO report by modeling the values from birth to 6 months of age against protein intakes of healthy breast-fed infants. This indicated that the factorial model predicted a safe protein requirement value that is similar to, but lower than, the 'crude' breast milk protein intake at all ages, which is what would be expected given that breast milk intakes from healthy mothers can be assumed to be adequate. However, in this modeling, the values for maintenance were 12% lower (0.58 rather than 0.66 g kg^{-1} day^{-1}) and the efficiency of protein deposition assumed to be 14% higher (66% rather than 58%), so that at 6 months, the safe intake was identified as 1.14 g kg^{-1} day^{-1} compared with 1.31 g kg^{-1} day^{-1} as shown in Figure 6. This 15% overestimation is probably acceptable, however, given that utilization of protein from a mixed diet after weaning is likely to be less efficient than during breast-feeding. It is also the case that any need for dietary protein to provide an anabolic drive on linear growth is not included in the factorial model for children. Taken together, this means that there is little reason to be concerned about any overestimation of the protein requirements for children.

In the case of pregnancy, however, there is every reason to be concerned that the requirement has been seriously overestimated. This is because of evidence that excessive protein intakes in pregnancy, including high-protein supplements, can have adverse effects on pregnancy outcomes. Thus, if any additional dietary protein intakes are recommended for pregnant women, these should comprise extra intakes of a normal healthy diet. In the WHO report, advice is given that the additional protein during pregnancy should consist of additional normal food, rather than high-protein supplements. During the third trimester, an extra 31 g day^{-1} of protein would represent an extra 3.6 MJ of a mixed diet assuming it contains 15% protein energy. This is considerably more additional energy than is recommended at this stage of pregnancy by any agency. Indeed, given the concern for adverse influences of overweight and obesity on pregnancy outcomes and given that pregnant women often have successful pregnancy outcomes without any increase in food intake, it is likely that this amount of additional food would result in excessive weight gain. On this basis, it is clear that these new WHO requirement values for pregnancy need a serious review.

Optimal Protein Intakes and Implications of Adaptation for Nutrition Policy

In general, protein requirements serve two purposes. One is as a basis for prescription (i.e., advice on safe diets through recommending appropriate dietary intakes). Adaptation implies a low – but difficult to define – MPR. Indeed, since natural diets, providing sufficient energy and other nutrients, usually provide considerably more than the minimal amount of protein, the magnitude of the MPR becomes to some extent an issue of scientific curiosity only. Formulation of policy in relation to prescriptive matters will inevitably and correctly be most concerned with satisfying the upper range of demands for protein and, where there is uncertainty, include positive margins of error. In this case, it is arguably unwise to adopt an adaptive model and reduce the MPR, even if agreement could be reached on the likely lower limit of adaptation. Indeed, an adaptive model does not imply that protein is an unimportant nutrient for the maintenance of human health and well-being, but rather that indicators other than balance (N, protein, or amino acid) need to be identified. Thus, the most relevant measure is an optimal requirement allowing balance and supporting both optimal body function and minimum risk of chronic disease as shown in Figure 1. There is increasing experimental evidence for the potential benefit of protein intakes considerably higher than the current MPR for bone health in the elderly and epidemiological evidence for benefit with respect to hypertension and ischemic heart disease. However, such influences are unproven, with no plausible mechanism identified in the latter cases. In any case, there are no quantifiable indicators. This results in a dilemma for those attempting to frame prescriptive dietary guidelines. From this perspective, it is probably wise to retain current values as an operational expedient (at least those that are safe) until it becomes possible to quantify the benefits (and any risks) of protein intakes within the adaptive range.

The other purpose of requirement recommendations is as a diagnostic indicator of deficit risk, often within an epidemiological context in which population groups rather than individuals are considered. In this case, indicators used to estimate prevalence of disease states or deficit risk are carefully chosen so as to strike an acceptable balance between false positives and false negatives. The main implication of adaptation for estimating risk of deficiency as intakes fall to less than requirement levels is a dramatic reduction in the prevalence of risk for most populations compared with that assessed according to the traditional model, which does not account for adaptation. This occurs because the requirement and intake can be assumed to be correlated and because the actual MPR and safe intake calculated from it will be lower. As in the prescriptive context, this low risk of deficiency applies only to that of being unable to maintain NB after full adaptation with otherwise nutritionally adequate diets satisfying the energy demands. Nutritionally adequate diets containing protein intakes at such low levels are likely to be very rare, and whether such populations enjoy optimal protein-related health in terms of immune function, bone health, or any other function is a separate issue and needs to be addressed as such. From this perspective, it follows that maintenance of NB can no longer be used as a surrogate of adequate protein-related health and that current lack of quantifiable alternative indicators is no excuse for ignoring the issue of adaptation.

See also: Amino Acids: Determination; Amino Acids: Metabolism; Dietary References: US; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Proteins: Chemistry, Characterization, and Quality.

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Proteins: Chemistry, Characterization, and Quality

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Proteins in Foods

Proteins are one of the major compound classes in raw agricultural materials (e.g., milk, eggs, soy, and cereals) as well as in meat and fish products. The proteins present in raw products serve different purposes, related to the structure and properties of the proteins. The first distinguishing feature of proteins is their solubility. This was used in the classical separation of wheat proteins by Osborne. The milled wheat can be sequentially extracted by water, 5% NaCl, and 70% ethanol to yield the albumin, globulin, and prolamin fractions. The remaining alcohol-insoluble fraction contains the glutelins, which combined with the prolamin fraction are referred to as gluten. For milk whey, obtained after the removal of the caseins (either by chymosin or by precipitation at pH 4.6), a similar separation of proteins is obtained by increasing the concentration of ammonium sulfate. The globulins precipitate in 50%, and the albumins in 100% $(NH_4)_2SO_4$. Proteins that are insoluble in aqueous solutions often have the function to provide structure (e.g., myosin in muscle tissue). Soluble proteins such as found in milk are often used for metabolic purposes (e.g., enzymes, transport proteins, and nutrition). In addition to proteins, which are polymeric chains of amino acids or polypeptides, there are also free amino acids and oligopeptides present in food raw materials. It is important to note that while up to 300 different natural amino acids have been identified, only 20 amino acids are commonly included in the protein amino acid sequence.

The most common sources of proteins that are used as ingredients in food products are milk, egg(white), and soy. In addition to these traditional protein sources, research is done to develop the potential of other protein sources, such as alternative leguminous seeds (e.g., pea), potato, or algae. The composition of these food materials is reported in Table 1.

Chemistry of Proteins

Food proteins are an essential component of the diet needed for the survival of animals and humans. Their basic value in nutrition is to supply adequate amounts of needed amino acids.

Proteins are a major source of energy, nitrogen, and essential amino acids, which are essential to human health, but which the body cannot synthesize.

The proteins are present in natural products in what is called their native structure. For globular proteins, this means that the proteins have a more or less spherical shape, where the more hydrophobic amino acids are buried in the interior, while the charged and polar amino acids are mostly present on the solvent exposed surface of the proteins. During processing, different reactions can occur that change the properties of the protein. These are (1) denaturation, which means a transition from the native (globular) state to a different folding state, (2) hydrolysis of the peptide bonds in the proteins, and (3) chemical reactions to the side chains of amino acids. Denaturation occurs, for instance, during the boiling of an egg and can be used to make aggregates or gels. The hydrolysis of proteins is often used to reduce allergenicity or to improve digestibility. Chemical side reactions, such as the Maillard reaction, can be positive with respect to technofunctional properties (e.g., solubility) but may negatively affect digestibility.

In addition to the natural presence in food raw materials, proteins are often extracted from such materials to be added in processed food products (e.g., bread, ice cream, and mousse). In these applications, the proteins do serve not only as source of essential (and other) amino acids but also as ingredients to give products a desired structure (e.g., gels, emulsions, or foams).

Alteration of the chemical structure of food proteins can be caused by deteriorative reactions arising during processing and storage and by modifications with chemical or enzymatic reagents intentionally done to alter the properties of the proteins. The application of chemical and enzymatic methods intentionally to modify chemical and functional properties of food proteins has a long history of usage, as illustrated by the enzymatic modification of milk proteins to produce yogurt and cheese.

The Maillard reaction, which was first observed by the French chemist, Louis-Camille Maillard (1912), is probably one of the best-known reactions of proteins in the processing, drying, and storage of foods containing both proteins and carbohydrates. It is known as nonenzymatic browning, resulting from the reaction between the carbonyl group of a carbonyl compound (usually glucose, fructose, or pentose) and the amino groups of proteins. Glycation results in the formation of stable compounds through the Amadori rearrangement, which further evolve in other degradation products. Several reaction factors, such as temperature, time, pH, water activity, intrinsic properties of protein, and sugar composition, influence the yields and types of the Maillard reaction products and consequently physicochemical, structure, and functional properties of modified food proteins. Moreover, the Maillard reaction alters the nutritional content of the proteins, mostly depleting lysines, which, in later stage of Maillard reaction, are modified to generate compounds such as lysinoalanine, furosine, and carboxymethyllysine.

Protein cross-links form an interesting subset of the Maillard reaction products since, in addition to the loss of essential amino acids such as lysine, proteins can be cross-linked to form aggregates, which are thought to be less accessible to digestive enzymes reducing the digestibility of the proteins.

Chemical methods of derivatization that have been reported in the literature include (a) acylation (acetylation and succinylation) or alkylation of amino groups; (b) esterification or amidation of carboxyl groups; (c) acylation or electrophilic substitution of phenolic groups; (d) oxidation or alkylation of sulfhydryl, thioether, imidazole, or indole groups; (e) oxidation or reduction of disulfide groups; and

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Table 1 Proximate composition of food materials used as traditional and novel sources of proteins

% (w/w DW)	Egg white	Meat	Fish	Milk	Soy	Algae	Pea	Nuts	Potato
Moisture	90	70–75	68–80	88	~10	~10	~10	~5	75
Protein	85	74–94	78–90	27	41	36	28	13–27	10
% Nonprotein nitrogen/total N	0.6	3–6	9–18	3	3	25	8	n.d.	24
Lipids	1.5	2–22	4–17	28	20	22	2.3	44–70	0.1
Mono-, di-, and oligosaccharides	3	n.d.	n.d.	38	15	5	13	10	1
Nonstarch polysaccharides	0	0	0	0	17	13	7	6–14	7
Starch	0	0.4–4[a]	1[a]	0	0	5	42	0; 23[b]	76
Phenolic compounds[c]	0	0	0	0	0.04	n.d.	n.d.	0.1–2.6	2.8
Ash	5	4	5	6	6	15	4	3	4

[a]Glycogen.
[b]Cashew.
[c]Data in g kg^{-1} dry weight.
Data are given, except for moisture, on dry weight.

(f) glycosylation or phosphorylation through hydroxyl (O-linked) or amino (N-linked) groups.

However, most of these chemical modifications are not approved or acceptable for the modification of proteins that are intended for human consumption. Notable exceptions are oxidizing and reducing agents, such as sulfite, hydrogen peroxide, ascorbic acid, and cysteine, which are commonly used for food protein modification, for example, in controlling dough properties in the baking industry. In addition, acid or alkali treatments are currently being used for the purpose of deamidation or peptide bond cleavage to produce hydrolysates.

In contrast to chemical modification, enzymatic modification is generally considered to be milder, more specific, and less prone to yield undesired side reactions. Furthermore, enzymes can possibly be inactivated after the desired functional property has been achieved, leading to better control of the final product quality.

Proteolysis, or hydrolysis of the peptide bond, is probably the most common application of enzymatic modification of proteins. A variety of enzymes from microbial, animal, and plant sources are commercially available for the modification of food proteins, with different specificities and sites of action. The optimum degree of hydrolysis depends on the desired product, with limited proteolysis being used to enhance functional properties such as emulsification, foaming, or curd formation, while extensive proteolysis yielding amino acids and peptides used to produce flavor hydrolysates (in combination with the Maillard reaction) or predigested nutritional products for feeding or for individuals who have protein hypersensitivity, gastrointestinal disorders, or other special medical concerns. Proteolysis, besides decreasing the molecular weight, also increases the number of ionizable groups and can expose hydrophobic groups, which can change physical or chemical environmental interactions. However, the hydrolysis conditions need to be controlled to avoid excessive protein hydrolysis that can impair functionality and cause unfavorable effects, such as the production of bitter-flavored peptides.

Nutritional Quality of Proteins

The basic function of proteins in human nutrition is to provide the adequate amount of the amino acids needed for the synthesis of our own proteins. The nutritional value of the proteins is related to several different parameters: amino acid composition, ratio of essential amino acids, the susceptibility to digestion by the enzymes during gastrointestinal passage, and modifications introduced during food processing in the amino acids. The different sources of proteins highly differ in the nutritional quality of the provided proteins. Although amount can vary according to metabolic state, age, sex, diet, and health conditions, roughly speaking, the amount of proteins required by young adult males is 0.6 g kg^{-1} body mass. This number anyway is highly theoretical, since it refers to 'ideal' proteins having a perfect amino acid composition and being perfectly digestible. If the intake is based on proteins having not a perfect amino acid composition (which is almost always the case), this number must be raised to 0.8 g kg^{-1}, and if the average digestibility (which of course most of the time is far from perfect) is also taken in consideration, a more realistic value of 1 g kg^{-1} body mass should be considered.

Among the 20 amino acids found in proteins, some of them are released and used as such, since the human metabolism cannot synthesize them. Others can be further metabolized obtaining nitrogen compounds useful as building blocks of the amino acids that human metabolism can synthesize. The amino acids that the human metabolism cannot synthesize and thus must be introduced with the diet are histidine, isoleucine, leucine, valine, phenylalanine, tryptophan, methionine, threonine, and lysine. They are named essential amino acids. Other amino acids that are not considered essential, in the sense that the human body can synthesize them, can nevertheless be obtained only by metabolic conversion of essential amino acids and not really synthesized from scratch, like cysteine (obtained by methionine) and tyrosine (obtained by phenylalanine). These amino acids, together with some other amino acids, are considered to be 'conditionally' essential, since they are usually not essential, but they can become essential in certain conditions, like growing children or in the presence of metabolic disease. The conditionally essential amino acids are tyrosine, arginine, cysteine, glycine, glutamine, proline, serine, and tyrosine. This only leaves alanine, aspartic acid, and glutamic acid as truly dispensable amino acids.

In proteins, the greater the prevalence of essential amino acids is, the greater their nutritional value, but also, the distribution of the single essential amino acids is of paramount importance. Proteins that are deficient in one or more essential amino acids, even if rich in all the others, are considered to be

of poor quality. The essential amino acid that has the lowest abundance in specific proteins as referred to an ideal abundance is often named the 'limiting' amino acid. Tryptophan and lysine are limiting in corn, lysine in wheat and other cereals, and methionine in soybean and legumes.

There are many available methods to estimate the nutritional quality of proteins, aimed at determining the net biological use of a protein as related to the amount consumed and its quality. Some are based on the composition, like the chemical score for single essential amino acids (the mg of that amino acid in 1 g of a test protein as related to the mg of the same amino acid in a reference protein), and some on *in vivo* trials, like the protein efficiency ratio (the ratio between the weight gain and the protein consumed).

Before a protein can be used as nutritional source of amino acids, it must be digested, that is, it must be susceptible to hydrolysis to single amino acids by the enzymes present in the gastrointestinal tract. Resistance to digestion of course lowers the nutritional value of a protein, by lowering the amount of essential amino acids available for the human metabolism. The food matrix is further complicating this picture, since the matrix can both affect the susceptibility of proteins to be digested by the gastrointestinal enzyme, as well as the availability of released amino acids to be absorbed. The presence of protease inhibitors, amylase inhibitors, lectins, phytates, and tannins can thus impair the true nutritional value of proteins ingested, as compared to the theoretical one in the absence of these compounds.

Food processing can both improve and reduce the nutritional value of proteins, depending on the type of food and processing. Highly proteolyzed foods, such as cheese, usually have an increased nutritional value due to the increased digestibility due to the presence of 'predigested' proteins. On the other side, processes that destroy essential amino acids can have an impairing effect on the nutritional value of a proteinaceous material. In particular, extreme pH values or heat treatments highly affect the nutritional value of proteins. These conditions can trigger the Maillard-type reactions involving amino groups or oxidative degradations of sensitive amino acids. Racemization can also occur, as well as the formation of cross-linked amino acids. Lysine, methionine, and tryptophan are particularly sensitive to degradation. Lysine easily reacts through the primary amine side chain, particularly in the presence of carbohydrates; methionine easily undergoes oxidative degradation; and tryptophan is very sensitive to acidic pH in combination with extreme heat.

In order to be considered a good source of proteins, a food must contain large amounts of high-quality, easily digestible proteins. According to the FAO/WHO standards, white egg and milk whey proteins are considered to be the most optimal protein source when looking at nutritional characteristics also including digestibility. For this reason, egg proteins are often used as reference in all methods for evaluating the nutritional properties of proteins. Also, meat is usually considered to be an optimal source of good amounts of high-quality proteins, but in this case, the true availability of amino acids is often hampered by digestibility issues. Some plant proteins (soybean, peas, and beans) have interesting nutritional characteristics when considering protein amount, quality, and digestibility, even if certain essential amino acids are usually limiting (cysteine, methionine, tryptophan, threonine, and lysine).

Besides basic nutrition properties, proteins can have a role in satiety (they are reported to induce satiety more efficiently than other food compounds, although the issue is still controversial). Proteins can also be a source of peptides having biological properties. A huge amount of literature exists on positive biological properties of peptides, such as antihypertensive, anti-inflammatory, opioid, calcium-binding, antibacterial, and antioxidants. Some peptide sequences can actually escape digestion and be absorbed as such, suggesting that these functions might also be exerted *in vivo*, even if often the amount absorbed is very low. Despite the relevance of the subject and the extensive researches done, the issues about resistance, absorption, distribution, and biological effects of food-derived peptides are still a matter of much debate.

Allergenic properties of food proteins are specifically treated in another chapter.

Technological Quality of Proteins

Proteins are major structural components of many natural foods, often determining their overall texture, for example, tenderness of meat or fish products. Isolated proteins are often used in foods as ingredients because of their unique technofunctional properties, that is, their ability to provide desirable appearance, texture, or stability. Typically, proteins are used as gelling agents, emulsifiers, foaming agents. Some food proteins are enzymes that are capable of enhancing the rate of certain biochemical reactions. These reactions can have either a favorable or an unfavorable effect on the overall properties of foods. Protein functional properties are attributed to both intrinsic (molecular structure and composition) and extrinsic (temperature, pH, and environmental chemicals) factors. When these factors are changed, food proteins' structure is modified and functional properties vary accordingly.

Proteins isolated from different sources (e.g., milk, eggs, and soy) are often added in food products to form and stabilize certain textures of the food, such as foams, emulsions, or gels. It is important to note that such protein preparations are never pure. Typically, they contain many different types of proteins. For instance, milk contains whey proteins and caseins. In each class, again, a subdivision of proteins is made (e.g., β-lactoglobulin and α-lactalbumin). Each individual protein can be present in different states. This may be due to genetic variation (β-lactoglobulin A and β-lactoglobulin B), posttranslational modification (i.e., phosphorylation and glycosylation), or processing (i.e., heat-induced unfolding and aggregation). In addition, due to processing, chemical reactions may have taken place such as the Maillard glycation (nonenzymatic) and decarboxylation.

Protein preparations are sold as concentrates or isolates. The distinction is often based on the isolation procedure and the resulting differences in protein purity. Concentrates contain 35–80% protein. Isolates contain 80–95% protein. The remaining part may contain fat but mostly contains carbohydrates. Due to the variation in protein content, composition, and the state of the protein, even seemingly similar protein isolates (such as whey protein isolates) may show a large variation in their technofunctional properties, that is, their ability to form and stabilize foams, emulsions, or gels. The first characterization of protein ingredients is often done by determining the solubility of the material, perhaps as function of the pH. Since proteins contain

both acidic and basic amino acid side chains, the charge of the protein changes from positive at low pH values to zero (at the isoelectric point) to negative at high pH values. Proteins usually have lowest solubility around the isoelectric point, since the absence of electrostatic repulsion will not counteract the hydrophobic attraction. However, whey proteins can be obtained from milk after acid precipitation of the caseins at pH 4.6. In this case, the caseins do precipitate, while the whey proteins stay in solution, even though for all the proteins the pH is close to their isoelectric point. After heat treatment, the proteins may unfold (and partially refold), after which hydrophobic groups that were previously buried in the protein interior became exposed. This increased hydrophobicity results in a higher attraction between proteins and consequently a lower solubility. Unfolded whey proteins, for instance, can be separated from the native whey proteins by precipitation at the isoelectric point.

For foams and emulsions, again, the charge of the proteins and the exposed hydrophobicity seem to be dominant factors that determine the ability of the proteins to adsorb to the air–-water or oil–water interface. This adsorption results in an increase in surface pressure and surface dilatational modulus. These properties have been typically related to increased stability of foams and emulsions. However, recently, it has become apparent that for certain systems (e.g., heated lysozyme), the interfacial properties of several samples may be similar, while the foam properties are very different. Consequently, the determination of the technofunctional properties of proteins is still best tested under the conditions and the system that most closely reflects the final application. In addition, it is very important to realize that there may be changes in the protein structure that can influence the observed behavior. Many proteins do not occur as single polypeptide chains, but have a so-called quaternary structure. β-Lactoglobulin, for instance, occurs mostly as a dimer, and soy glycinin occurs as a hexamer of a disulfide bridge-linked subunit consisting of an acidic and a basic polypeptide chain. In both cases, changes in pH, temperature, or ionic strength can result in the dissociation of the quaternary structure. In addition to these changes in the protein state, it is of extreme importance to realize that the presence of nonprotein compounds also has a significant effect on the properties of the protein isolate or concentrate. Partly due to the name, people are lead to believe that the properties may be attributed to the protein part, but carbohydrates and phenolic compounds can also have significant effects. For instance, the presence of salt in the protein isolate can result in changes in the ionic strength of the protein solution when protein solutions are prepared at different concentrations. Such effects are often overlooked. Based on the wide variety of properties observed even in seemingly similar protein ingredients, any comparison of functionality between proteins from different sources should be considered with appropriate care. It may be that while testing a whey protein isolate, a soy protein isolate, and a caseinate in one case, the whey protein isolate seems best, while with another set of samples, the soy protein isolate is best.

Analysis of Proteins

w?>Food analysts are interested in knowing the total concentration, type, molecular structure, and functional properties of the proteins in foods. The first and most relevant information on protein content in a given food is of course the crude amount of proteins. This is usually done by methods determining the nitrogen content, since, among the three major classes of macronutrients (proteins, fats, and carbohydrates), the nitrogen atom is contained almost exclusively in proteins. Also, the most relevant methods are those that analyze the whole product, without extractions, and that can handle solid materials, in order to minimize or even skip the sample preparation. Among them, Dumas and Kjeldahl methods are the most widespread ones.

The Kjeldahl method, developed in 1883 by a brewer named Johan Kjeldahl, is usually considered to be the standard method of determining the total protein content. A food is completely hydrolyzed by boiling in concentrated acid so that it releases all protein nitrogen in the form of ammonia, which can then be determined by a suitable titration technique. The amount of protein present is then calculated from the nitrogen concentration of the food. The same basic approach is still used today, although a number of improvements have been made to speed up the process and to obtain more accurate measurements. Because the Kjeldahl method does not measure the protein content directly, a conversion factor (F) is needed to convert the measured nitrogen concentration to a protein concentration. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) is used for many applications; however, this is typically not correct. Each protein has a different conversion factor depending on its amino acid composition.

The Kjeldahl method is widely used internationally and is still the standard method for comparison against all other methods. Its universality, high precision, and good reproducibility have made it the major method for the estimation of protein in foods. However, it does not give a measure of the true protein, since all nitrogen in foods is not in the form of protein, so a slight overestimation is generally the rule.

Recently, an automated instrumental technique has been developed that is capable of rapidly measuring the protein concentration of food samples based on a method first described by a scientist named Dumas over a century and a half ago. A sample of known mass is combusted in a high-temperature chamber, leading to the release of gasses (including N_2 deriving from proteins) whose thermal conductivity is measured, allowing to determine the amount of nitrogen. As with the Kjeldahl method, it is necessary to convert the concentration of nitrogen in a sample to the protein content, using suitable conversion factors, which again depend on the precise amino acid sequence of the protein.

The Dumas method is nowadays competing with the Kjeldahl method as the standard method of analysis for proteins for some foodstuffs, since it has the advantage of being much faster than the Kjeldahl method, it does not need toxic chemicals or catalysts, it can handle many samples at time, and it is easy to use. On the other hand, the Kjeldahl method is much better for inhomogeneous samples and can analyze larger samples sizes.

Besides these two, a number of methods based on UV–vis spectroscopy have been devised in order to measure protein concentration after extraction from a food matrix. These methods either use the natural ability of proteins to absorb light in the UV–visible region of the electromagnetic spectrum or chemically or physically modify proteins in order to make

them absorb light in this region. One example is the direct measurement at 280 nm, which is used for proteins that contain tryptophan and tyrosine, which absorb ultraviolet light strongly at this wavelength. Another possibility is the use of compounds that bind proteins making them absorbing in the UV–visible region: the biuret method (based on Cu^{2+} complexes), Lowry method (based on the biuret and the Folin–Ciocalteu phenol reagent), and methods based on anionic dyes. In all these methods, the quantitation is obtained by calibration with a reference protein, typically bovine serum albumin (BSA). However, the response factors for different proteins may be different from that of BSA, giving inaccurate results. In addition, hydrolyzed proteins do not bind the compounds in the same way as full proteins, again altering the results. Furthermore, the reagents used to bind proteins might bind to other matrix components, giving rise to unreliable results.

UV–visible techniques are fairly rapid and simple to carry out and are sensitive to low concentrations of proteins. However, it is necessary to use dilute and transparent solutions, which contain no contaminating substances, which absorb or scatter light at the same wavelength as the protein being analyzed. The need for transparent solutions means that most foods must undergo significant amounts of sample preparation before they can be analyzed, for example, homogenization, solvent extraction, centrifugation, and filtration, which can be time-consuming and laborious. In addition, it is sometimes difficult to quantitatively extract proteins from certain types of foods, especially after they have been processed, so that the proteins become aggregated or covalently bound with other substances. As a consequence, while the Kjeldahl method and increasingly the Dumas method have been officially approved for a wide range of food applications, only a small number of applications of UV–visible spectroscopy have been officially recognized.

Besides bulk protein content, food analysts are also often interested in the type of proteins present in a food, because each protein has unique nutritional and physicochemical properties. Gel electrophoresis techniques, such as typically one-dimensional (1-D) SDS-PAGE or 2-D electrophoresis, provide a nice overview of the complexity of the protein mixtures. Individual proteins are usually visualized by staining with Coomassie blue or silver and possibly identified by immunoblotting procedures or by mass spectrometry. The analysis of single proteins can be also performed by chromatographic methods by exploiting the different affinities of different proteins for the solid matrix. Ion-exchange chromatography and reversed-phase chromatography are the most used general methods, although hydrophobic interaction chromatography and Hydrophilic Interaction Liquid Chromatography (HILIC) are also used. Size exclusion chromatography is also largely used, although more for preparative purposes.

Amino acid analysis is used to determine the amino acid composition of single proteins and protein mixtures and also to determine the true protein amount in a mixture in a way that is independent from the Kjeldahl and Dumas determination, albeit more cumbersome. A protein sample is first hydrolyzed (e.g., using a strong acid) to release the amino acids, which are then separated by using chromatography, typically ion exchange (as such), or reversed-phase chromatography (after derivatization).

The differences among proteins in their physicochemical properties, such as size, charge, adsorption characteristics, solubility, and heat stability, can also be exploited to isolate individual proteins from a complex mixture. Many separations are done by exploiting differences in their solubility in aqueous solutions. Proteins can be selectively precipitated or solubilized by altering the pH, ionic strength, dielectric constant, or temperature of a solution. Some examples are salting out, isoelectric precipitation, solvent fractionation, and denaturation.

In the salting out, proteins are precipitated from aqueous solutions when the salt concentration exceeds a critical level. Ammonium sulfate $(NH_4)_2SO_4$ is commonly used because it has a high water solubility. The protein of interest can be then separated by centrifugation.

The isoelectric point (pI) of a protein is the pH where the net charge on the protein is zero. Proteins tend to aggregate and precipitate at their pI because there is no electrostatic repulsion keeping them apart. Proteins have different pI's because of their different amino acid sequences (i.e., relative numbers of anionic and cationic groups), and thus, they can be separated by adjusting the pH of a solution.

The solubility of a protein depends on the dielectric constant of the solution that surrounds it because this alters the magnitude of the electrostatic interactions between charged groups. As the dielectric constant of a solution decreases, the magnitude of the electrostatic interactions between charged species increases.

In the solvent fractionation, by adding water-soluble organic solvents, such as ethanol or acetone, it is possible to decrease the dielectric constant of the aqueous solution and indeed favor the protein isolation by precipitation.

Finally, many proteins are denatured and precipitate from solution when heated above a certain temperature or by adjusting a solution to highly acid or basic pH. Proteins that are stable at high temperature or at extremes of pH are most easily separated by this technique because contaminating proteins can be precipitated while the protein of interest remains in solution.

See also: Amino Acids: Determination; Food Allergies: Occurrence and Analysis; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements.

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Proteomics: Contribution of Proteomics Techniques to Understanding the Interrelationship between Food and Health

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Introduction to Proteomics

The term and concept of proteomics were introduced by Marc Wilkins et al., in the 1990s, the term proteome referring to the entire protein complement expressed by a genome or by a cell or tissue type.

One main difference between the concepts of genome and proteome is that the proteome varies in different cell types, from tissue to tissue, and it changes during development and under different conditions, but the genome does not. Moreover, the number of proteins in a proteome can exceed the number of active genes due to alternative gene splicing and posttranslational modifications (PTMs). Proteomic analyses were initially based on protein separation by two-dimensional electrophoresis (2-DE) on polyacrylamide gels (isoelectric point (pI) and molecular mass) followed by image analysis of the gels in order to catalog all the resolved spots to form reference maps. Initially, the analyses were mostly descriptive: for example, one could analyze the 2DE protein maps of the control and the case samples (e.g., a biopsy from the liver of animals fed with diet A and another from animals fed diet B) and examine the differences, which often required multivariate data analyses. This approach, the most frequently used in the early 1980s, was mainly descriptive and the results were lists of differentially present spots, whose pI and molecular mass had been estimated and cataloged as present, absent, increased, or decreased intensity in relation to the issue under study (type of diet, disease, etc).

The proteome, the total protein content of one particular biological system, is highly dynamic and constantly changes according to different stimuli. Proteomics includes not only the structural and functional knowledge of proteins but also the study of their modifications, the quantification of their abundance, the interactions between them, and the study of their localization.

A general challenge for proteomic analyses is the large complexity of the protein mixtures, which usually is accompanied by a wide range in their relative abundances. Therefore, the comprehensive characterization of complex proteomes often necessitates their fractionation into less complex subproteomes (selecting proteins with similar characteristics or with similar abundances). The integration of the subproteomes will then give a picture of the total proteome of the complex sample. The complexity presented by most proteomes makes the need for further technical advances in the near future clear.

What Do You Need in Your Lab to Work in Proteomics?

The provision of a proteomics lab includes expensive instruments for protein separation and fractionation, imaging, robotics sample preparation, mass spectrometers, computers, advanced software, and expert personnel in such processes and results interpretation or management. Furthermore, electrophoretic, liquid chromatographic, and mass spectrometry (MS) apparatuses require accessory material and facilities such as gas supply, fume cupboards, thermostatic baths, centrifuges, air-conditioned rooms, vacuum pumps, and sonication baths, without which it would be impossible to prepare the sample in good conditions to be analyzed using that instrumentation. Table 1 compiles the most important material needed for each proteomic step in the proteomic workflow.

However, it must be kept in mind that a good sample is the most valuable item for the analysis, and it is easy to forget that the protein extraction from their source and the subsequent fractionation or purification steps are the most important processes in the procedure. Indeed, that is the starting point, to ensure that the relevant proteins or peptides have been successfully extracted and will be characterized.

What Proteomics Can Do

Depending on the general objectives of the study, proteomics can be used to identify and characterize a given protein, to study variations in the proteome caused by stimuli (differential proteomics), or to study the functional interactions between different proteins and the networks to which the proteins belong to (functional proteomics).

Protein Identification and Characterization

MS is the method of choice for the identification and characterization of proteins. The analysis of any kind of proteome usually relies on one or several separation steps followed by MS analysis. MS experimental data are compared with the data included in protein sequence databases using appropriate software, known as search engines, of which Mascot, SEQUEST, and ProFound are the most frequently used. Matches are scored and the peptide or protein with the best score may be assigned to the experimental data after a judicious probabilistic calculation is made by the selected program.

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Table 1 Major lab requirements for proteomic workflow

Proteomic step	*Instrumentation*	*Fungible material*	*Reagents*
Protein extraction and solubilization	Homogenizers, tissue disrupters, vortex, mortars, liquid nitrogen, centrifuges	Glasses, tubes, balances, freezers, automatic pipettes, test tubes	Protease inhibitors, detergents, chaotropes, reducing agents, TCA, ethanol, commercial extraction kits, commercial cell lysis kits, commercial fractionation kits, commercial cleanup kits, reduction–alkylation kits, devices to avoid high-abundance proteins and to concentrate low-abundance proteins (i.e., phosphoproteins)
Protein quantitation	UV–Vis, NanoDrop, spectrophotometer, fluorometer, centrifuge	Quartz or glass cuvettes, disposable pipettes, tubes	Protein commercial assay reagents. Protein standards
Electrophoresis	Power supplies, thermostatic baths, vertical or horizontal electrophoretic systems, IEF cells	Bottles, test tubes, glass plaques, combs for sample deposition, separators	Precast gels; acrylamide/bisacrylamide; electrophoresis buffers; ampholytes; urea; molecular weight and isoelectric point protein standards; protein stains: Coomassie, silver nitrate, commercial dyes. Others: methanol, acetic acid, ethanol, detergents, DTT (dithiothreitol), ammonium persulfate, sodium thiosulphate
Protein digestion	Incubator, ultrasonic sound, pressure-cycling technology, vacuum concentrators	Low-peptide-bound plastic or glass material, pipettes, filters, tubes	Trypsin, V8 enzyme, lysine-C, cyanogen bromide, other proteases, TCEP (tris 2-carboxyethyl-phosphine hydrochloride), IAA (iodoacetamide), C18 cleanup devices
MALDI-TOF–MS	MALDI mass spectrometer	Low-peptide-bound plastic or glass material, pipettes, filters, MALDI target plates	C18 reversed-phase resin beds; alpha-cyano-4-hydroxycinnamic acid (a-cyano or CHCA) and 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid or SA), 2,5-dihydroxybenzoic acid (DHB) and 2-(4-hydroxyphenylazo) benzoic acid (HABA), 2,6-dihydroxy acetophenone, and ferulic and caffeic acids. Peptide standards
LC–ESI-MS/MS	Micro-HPLC, UPLC, autosampler, and mass spectrometers with electrospray ionization	Columns, syringes, bottles, peak tubing, micropipettes	C18 reversed-phase resin beds, CAN acetonitrile, FA (formic acid), TFA (trifluoroacetic acid), peptide standards, AQUA™ peptides, helium supply, nitrogen supply
Software	Image analysis; protein database; protein identification, quantification, monitoring, and *de novo* sequencing analysis	Good resolution monitors, robust computers and servers	Examples of patented and public software: PdQuest™, UniProt, ExPASy, SEQUEST™, Mascot, X! Tandem™, Trans-Proteomics Pipeline, MaxQuant, OpenMS, PeptideAtlas, MRM Atlas™, Progenesis™, DeNovoX™, PEAKS™, Spectral Libraries, Skyline, R-statistical software, Spotfire®

In the peptide mass fingerprinting (PMF) approach, the protein previously isolated (usually, but not only by 2-DE) is enzymatically digested. The masses of the peptides obtained from the MS spectrum are the data used for the identification. Another approach, known as peptide fragmentation fingerprint, uses tandem mass spectrometry (MS/MS) fragment ion data from one or more peptides, allowing the sequencing of the peptide(s) and thus yielding the unambiguous identification of the protein. Alternatively, MS/MS fragment ion data from the whole protein can be used in a similar way, in the approach known as top-down proteomics. For all these approaches, the sequence(s) of protein(s) under study must be contained in the database. However, if the protein is absent from the database, identifications can still be made if homologous proteins, usually from related species, are present in the database. When the experimental sequence remains unassigned, the peptides must be sequenced *de novo*, meaning that the MS/MS spectrum has to be interpreted manually or by computer-assisted identification of the fragmented ions whose mass differences correspond to the residue masses of the amino acids.

Proteins can suffer PTMs in response to a wide range of extra- and intracellular signals, or as a result of processing treatments, as may be a frequent case in food products. PTMs play crucial roles, since they alter the protein activity, localization, or stability. More than 300 different types of PTMs are known, although only few of them are being extensively investigated, such as phosphorylation, acetylation, glycosylation, and oxidation. The mass shift in the modified amino acid with respect to the unmodified one is the basis for the detection and characterization of PTMs by MS. However, since PTM peptides are usually present in very low amounts when compared to non-PTM peptides, modification-specific enrichment techniques, such as antibody-based affinity enrichment, special chromatographic procedures, or chemical tagging, are needed.

Differential Proteomics

Proteomes are highly dynamic. Protein patterns change from cell to cell or tissue to tissue, through cell and organism cycles, and in response to different environmental stimuli. Quantitative information at the protein level, such as the relative abundance in a specific protein in different samples, or the absolute amount of the protein, turns out to be very helpful when looking for differences between different conditions (e.g., control vs case). Relative quantification can be achieved with different methodologies, which can be classified as gel-based, label-based, and label-free approaches. In gel-based methods, the protein spots from different samples are compared after 2-DE. The samples to be compared may be run either in different gels or, alternatively, in the same gel after being differentially labeled (technique known as difference gel electrophoresis, DIGE). DIGE allows the comparison and quantification of the proteins contained in up to three different samples, and it increases the confidence in terms of detection and quantification of differences in protein abundance, in addition to reducing the number of gels needed per experiment.

Other label-based methods relay in the relative quantification of peptides labeled with the different isotopes of the same chemical element (i.e., isobaric tags for absolute and relative quantification (iTRAQ), ^{18}O). Peptides or proteins previously labeled using a mass tag that has been introduced metabolically, enzymatically, or by a chemical reaction produce differences in the intensity of the signals obtained from the MS or MS/MS readout, based on the intensity ratio of heavy to light isotope in the peptide pairs.

Finally, label-free approaches avoid using labeling and stable isotopes. The protein amount is calculated based on the MS-derived ion current signal of the peptides/proteins or on the number of identified MS/MS spectra (spectral count) for the protein. However, the absolute quantification of proteins needs isotopically labeled synthetic peptides as internal standards for each targeted protein. Quantitative proteomics methodologies have been greatly improved with the introduction of selected reaction monitoring (SRM), a high-sensitivity LC-MS/MS acquisition mode that is commonly used to verify and validate candidate biomarker proteins.

Functional Proteomics

Most proteins work in close collaboration with other proteins and molecules and are assembled in dynamic complexes. Functional proteomics deals with the integrated analysis of the functional interactions between different proteins and the networks made up by them. Thus, the term interactomics refers to the discipline that studies the interactions between a specific protein and the others, or between a protein and other molecules, and the consequences of these interactions. In this transition from structural to functional proteomics, different platforms are being developed and not all of them are MS-based: protein microarrays, yeast two-hybrid assay, phage display, affinity pull-down, and capture compound MS are some of them.

Activity-based proteomics is another area related to functional proteomics, studying the specific activities of the proteins in a sample, such as their function and inhibition. Activity-based protein profiling uses small-molecule probes to label and analyze active enzymes in lysates, cells, and whole organisms. MALDI imaging MS, a new imaging modality that allows mapping proteins within a tissue section, has turned out to be a relevant tool for functional proteomics, since the location of the different protein isoforms can help to understand their roles.

Proteomic Workflow

As stated earlier, the analysis of the proteome is commonly done after one or several sample preparation steps. As already mentioned, a great challenge for MS technology is the complex nature and highly dynamic range of proteomes. This makes it convenient to select extraction protocols that allow the enrichment in the proteins of interest. Partial purification, depletion of the high-abundance proteins, and selective enrichment of those in low abundance are some of the common protocols when working with complex samples. At this point, two different proteomic workflows can be followed: *bottom-up* or *top-down* as shown in Figure 1.

Using the classical workflow, referred as the bottom-up approach, the protein/s of interest is converted into peptides using enzymes such as trypsin, and the peptides produced are analyzed by MS. For that reason, this approach is sometimes

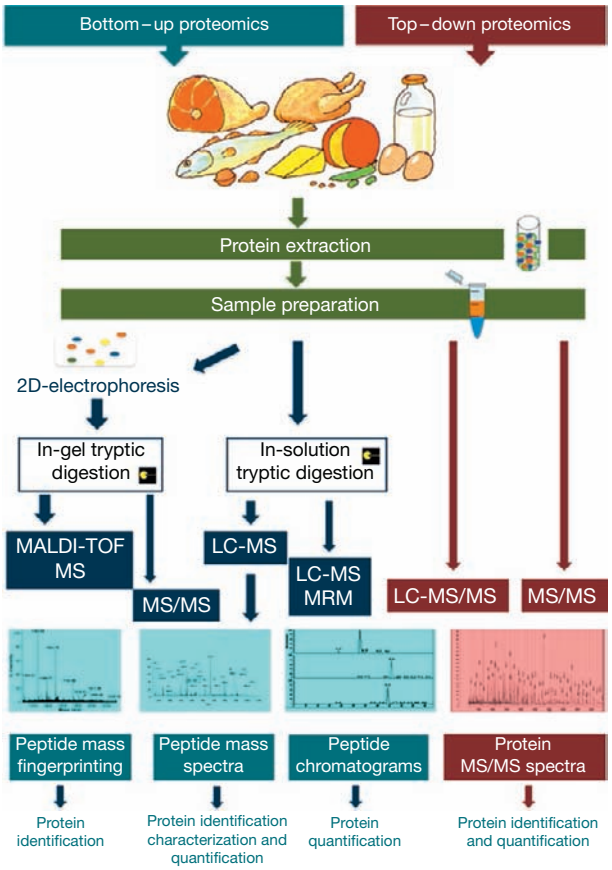

Figure 1 Proteomic workflows commonly used in food technology research.

known as peptide-based proteomics. Bottom-up approaches can be further divided depending on whether the fractionation steps are performed before (at the protein level) or after (at the peptide level) the enzymatic digestion. The former strategy has its more representative example in the use of the gel-based 2-DE technique, where proteins are separated according to their pI and molecular mass, so that protein spots can be individually excised from the gel and digested into peptides that are analyzed by MS. It must be noted that proteins may comigrate, and it is not infrequent to find that one single spot in a gel contains more than one protein or peptide.

In an alternative bottom-up approach, also referred as *shotgun proteomics*, the protein mixture is enzymatically digested without previous fractionation, and the resulting mixture of peptides separated by reverse-phase (RP) HPLC or UPLC coupled to an ESI tandem mass spectrometer. However, the chromatographic separation of peptide mixtures becomes very complex, and usually, the peak capacity of one-dimensional chromatography is not enough for highly complex protein samples. Therefore, multidimensional liquid chromatography coupled to mass detection is widely used. Separations combining strong cation exchange chromatography, or based in the isoelectric points of peptides (OFFGEL), are usually combined with RP separations.

Top-down proteomics uses intact, isolated, and undigested proteins for direct fragmentation by MS/MS. The fragment ions produced are used to reconstitute amino acid sequences by any of the methods previously described. Top-down proteomics is possible thanks to the high mass accuracy and resolution attainable with Orbitrap or FTICR mass spectrometers.

MS Analysis

MS analysis relies on the ionization of analytes at an ion source (i.e., ESI and MALDI) and the separation of the analyte ions according to their mass to charge (m/z) ratio at one or several mass analyzers (i.e., quadrupole (Q), ion trap, time of flight (TOF), and FTICR). Subsequently, a detector registers the ion current coming from the analyzers and generates the corresponding mass spectrum. Applying a variety of bioinformatics tools to the analysis of the mass spectra obtained, it is possible to obtain information about the peptide sequence and its abundance.

MS techniques provide valuable mass data from peptides and proteins and can be used for PMF identification of an isolated protein, to measure the exact mass of a whole protein, or for the selected ion monitoring (SIM) quantification method. Nevertheless, most of the protein identifications are produced nowadays using hybrid mass spectrometers. These instruments combine different types of analyzers, such as Q, TOF, linear ion traps (LIT), or any of the high-resolution instruments (Orbitrap or FTICR). Some examples are triple quadrupole (QQQ), quadrupole-time of flight (Q-TOF), tandem time of flight (TOF–TOF) and, for high resolution, Q or LIT coupled with either Orbitrap or FTICR. MS/MS or even more MS stages (MSn) are possible with these instruments. This means that a specific peptide (in bottom-up approaches) or protein (in top-down approaches) can be isolated and fragmented (MS/MS) using different activation approaches (e.g., collision-induced dissociation, CID; higher-energy collisional dissociation, HCD; and electron transfer dissociation, ETD) to obtain the fragment ions needed for their identification. Using ion traps, the fragmentation cycle (precursor isolation, activation, and analysis of the produced fragments) can be repeated several times selecting specific fragments from the previous cycle (MSn).

Several data acquisition approaches are possible depending on the nature and the stage of our research. The approach known as data-dependent analysis (DDA) is the most frequently used when the objective is to study the highest possible number of proteins present in the sample. This method consists on the MS/MS fragmentation of the most abundant ions detected during a survey MS scan, and it is often followed by discovery analyses, where a massive identification of proteins is pursued, and quantitative data can also be obtained from the MS/MS spectra using a proper methodology such as chemical isotopic labeling or spectral counting. However, the detected peptides are biased towards those coming from high-abundance proteins. To avoid this, one can use the data-independent analysis acquisition mode, where, instead of selecting one specific ion for fragmentation, all peptide ions present at a time in the mass analyzer are fragmented. This latter approach is currently growing due to recent advances introduced in MS instruments, such as elevated-energy MS (MSe) and sequential window acquisition of all theoretic mass spectra acquisition modes (where multiplexed fragmentation spectra are obtained), and in spectral deconvolution software.

When previous information about the m/z of the proteins/peptides under study is available, data can be obtained using several acquisition modes included under the term-directed analysis. Directed analyses focus on specific ions selected by the operator, thus increasing reproducibility, sensitivity, and selectivity. They can be used to identify, monitor, and quantify low-abundance peptides not detected in DDA analyses, in acquisition modes such as SIM or selected MS/MS ion monitoring, where only the selected m/z values are detected or fragmented, respectively.

Finally, when the m/z of both the precursor ion and one or several product ions are known, a targeted proteomic analysis can be made using the SRM scanning mode. The high-sensitivity LC-MS/MS acquisition mode, usually performed on QQQ instruments, is the gold standard method for the quantification of proteins and is commonly used to verify and validate candidate biomarker proteins in a hypothesis-driven approach following a discovery study. Absolute quantification can be achieved in SRM using isotopically labeled synthetic peptides as internal standards for each targeted protein.

Proteomic Studies on the Main Food Groups

Cereals, Fruits, Wine, and Beers

Proteomic studies on cereals focus on relevant biological aspects that go from assessing biodiversity, to (1) crop improvement, authenticity, and nutrition assessment; (2) the characterization of seed development, germination, and growth; (3) responses to stress (biotic and abiotic); (4) mapping organelle proteomes and secretomes (secreted proteins); (5) the screening of transgenic plants (e.g., nutritional and safety assessment of transgenic plants); (6) food safety proteomics; and (7) the identification

of proteome characteristics that keep a relationship with grain quality. Technically, there is a need for improvements in sample preparation methods (for tissue and organelle isolation) and protocols for protein extraction, separation, and detection of proteins, PTMs, and the redox proteome (related to protein oxidoreduction and the characterization of the redox control systems). The methods used include MALDI-TOF–TOF, ESI-MS/MS and multidimensional protein identification technology, isotope-coded affinity tags, and iTRAQ protein separation.

Proteomic studies on fruits (tomato, grape, citrus, prunes, and apples) have mostly focused on maturing and ripening processes, and most species seemed to share profiles involving sugar and oxidative metabolism and stress responses, but there is still a clear need for further studies to optimize the quality and nutritional value of the fruits.

In wines and beers, the proteinaceous material is made up of native, denatured, chemically modified, and partially hydrolyzed proteins and peptides. Many of the proteomic studies aim at characterizing their quality (formation of haze, protein denaturation, aggregation, and precipitation) and safety (detection of allergens that may originate in the fruit or be formed during processing and of hordeins and gliadin-like proteins in beer, which make the product unsuitable for celiacs). Combinatorial peptide ligand library (CPLL) technology (to detect very low-abundance proteome), nano-LC–ESI-MS/MS, and competitive ELISA assays using specific antibodies have been used to detect traces of harmful proteins in these beverages.

Other relevant properties examined include how changes in the proteome may be affected by adaptation to different stresses and during berry ripening (i.e., the induction of antioxidant proteins; changes in amino acid, proteins, and energy metabolism; and production of by-products and changes in structural proteins) and the influence of these changes on the product's nutritional value. Both the abundance and the PTM of some proteins (disulfide bond reduction, partial proteolysis, glycosylation, and phosphorylation) seem to be of high relevance for the quality of the beverages.

The yeasts used to produce the beverages have a large impact on their quality and have been subject to proteomic studies: most of the changes in yeast proteomes affect proteins involved in stress responses; glycolysis and ethanol production; glycerol, carbohydrate, and amino acid metabolism and protein degradation (most of which were upregulated); and nucleotide metabolism and protein synthesis (most of which were downregulated).

Dairy Products and Eggs

Milk is one of the important biological fluids and contains considerable amounts of nutrients. All types of milk and derivatives contain proteins in colloidal dispersion as micelles, fats in emulsified globules coated with a membrane, and easily digested minerals, vitamins, and other components. The application of proteomics tools to milk research took place in the 1990s, and while the major protein components of human, and animal and particularly bovine milk were biochemically characterized two decades ago, the analyses of the less abundant milk proteins have only just recently been reported for bovine and porcine milk and included with the human data in the PeptideAtlas repository. Significant advances have been made in (1) the detection and identification of new proteins, in particular minor proteins or proteins located in subcellular compartments such as the milk fat globule membrane; (2) variations in the protein profiles depending on the mammalian species or on the stage of lactation; and (3) PTMs (e.g., glycosylation and phosphorylation), which occur at extremely high frequency, either naturally or induced by processes such as lactosylation.

The proteomic milk workflow includes essential prefractionation steps and strategies for every milk fraction (Figure 2) (caseinomics, milk fat globule proteins, and whey proteins), although other recently strategies such as ion exchange and SDS-PAGE-based protein fractionation have been also described. From the food composition, nutrition, and technology perspective, proteomics has been applied to the dairy industry in every phase and product obtained from raw milk in order to enhance their quality and safety and the knowledge about milk protein functionality. Regarding product safety, mayor and minor allergenic milk proteins have been detected with different proteomics tools and classical strategies. Proteomics has been used to study milk adulteration, bacteriologic characterization, protein modifications due to processing (including yogurt and cheese production), and aspects related to milk quality and functional properties.

Most of the proteomic studies on eggs have targeted hen eggs and their allergenicity, changes during storage, and characterization of proteins in the white, yolk, and eggshell using CPLL.

Meat Products

Meat being a major source of proteins in the human diet, proteomics has been applied in meat research from many different perspectives. The investigations have been related to factors influencing sensory quality properties, mainly juiciness, flavor, and tenderness. These properties are closely related to the proteolytic state and degree that in turn are dependent on pre- and postmortem processes taking place in the carcass. Many works monitor skeletal muscle biochemistry and physiology of different animal farm species using proteomics methodologies including glycolytic and proteolytic pathways by 2-DE and

Figure 2 Milk essential prefractionation steps for proteomic analysis.

MALDI-TOF–MS. Alternatively, as manufacture and storage of meat products involve different and sophisticated processes affecting the integrity of their proteins, proteomics contributes to understand and resolve many of the protein modifications and is becoming a valuable tool to identify a large amount of proteinaceous biomarkers related to safety, such as protein oxidation, microbiological contamination, adulteration by growth promoters, and authenticity. However, the main proteomic work about meat products has been carried out in meat from farmed species such as beef, veal, poultry, rabbit, and, the most studied, pork.

Seafood

Seafood comprises a large number of very heterogeneous species and products, and that heterogeneity has been an impediment to the application of proteomics technologies to seafood research because the databases have been and are still scarce. Fortunately, basic studies on model animal species such as zebra fish and on relevant commercial species (carp, salmon, and eel) are contributing to increase the number of sequenced proteins from aquatic organisms. The main issues addressed by proteomics of seafood products include quality (influence of the feed composition, fish welfare, capture method, and handling and storage on the quality of the fillet), safety (identification of allergens and spoilage microorganisms and of exposure to contaminants and biotoxins), authentication, and fish processing monitoring (e.g., changes happening during surimi manufacture, ice and frozen storage, and high-pressure treatment).

Bioactive Compounds

In opposition to toxic compounds, bioactive compounds are substances, or compounds, that have a biological activity with positive direct effects on living organisms (e.g., cholesterol-lowering or anti-inflammatory activities). Bioactive compounds are present in foodstuffs of both plant origin and animal origin and may have different nature: lipids, vitamins, carbohydrates, phenolic compounds, and evidently proteins, peptides, and amino acids. Protein bioactive sequences are normally quiescent within the parent proteins. Active peptide fragments are released from their matrix via proteolytic digestion by means of gastric, fermentative, enzymatic, and other chemical processes. The most representative bioactive peptides from food proteins are angiotensin-converting enzyme inhibitory peptides with antihypertensive action and opioid, antioxidative, and immunomodulating peptides.

Proteomic studies on bioactive peptides are still scarce, but they are increasing rapidly through the application of both traditional and high-throughput electrophoretic and MS methodologies, such as microchip electrophoresis or nano-LC–Q-TOF, ICP-MS, and 'precursor acquisition independent from ion count and imaging' coupled with bioinformatics and genomics database in order to characterize those bioactive substances.

From Proteomics to Systems Biology

The significant technical improvements in analytic methods for proteomics have gone hand in hand with significant advances in genomics, transcriptomics, metabolomics, and other -omic approaches, and this has generated an enormous amount of very valuable data that can no longer be processed by traditional statistical methods or classical multivariate data analyses. Fortunately, these analytic improvements have found a counterpart in comparable developments in computer sciences, and the vast amount of information provided has led to redefinition of proteomics. For example, Patterson and Aebersold defined it in 2003 as "the systematic study of the many and diverse properties of proteins in a parallel manner with the aim of providing detailed descriptions of the structure, function and control of biological systems in health and disease." What is interesting in this last definition is the inclusion of the term 'biological systems,' which indicates the shift that has taken place in the last decade from the analyses of the function and interactions of single components towards a holistic approach focusing on how biological systems function and interact as whole entities.

To take into use this new way of understanding proteomics and its role in nutrition and health requires first the delimitation of which is the biological system under study (it may be a cell or a metabolic route, but also a person) and then, considering the system in a holistic manner, the application of tools that come from a variety of disciplines (such as mathematics, physics, computer science, and control systems engineering). These new tools are essential to map the interactions between the individual constituents of the system. The concept of systems biology was introduced as a distinct discipline in the 1960s, and it has been increasingly used ever since.

Systems biology requires the knowledge and the data produced within experimental biology (including proteomics techniques) and mathematical modeling to identify the relationships between the data. Computational technology and numerical techniques are used to simulate complex biological networks. The information gained from simulations can then be used to design targeted and more efficient *in vivo* and *in vitro* experiments (lowering the cost of the study and use of experimental animals), to further improve the model. The final goal is to understand the processes, mechanisms, and principles of the system, and this in turn may help to predict the outcome and to control the system.

Proteomics is one of the main set of tools (together with genomics, metabolomics, lipidomics, and other high-throughput analytic techniques) that have become essential in systems biology studies.

Application of Systems Biology to Nutritional Science

Nutritional science deals with the organism's responses to the diet. It includes studies not only on the nutrients themselves but also on molecular interactions, metabolic and signaling pathways, physiology, pathology, and toxicology. As in other disciplines, it became clear that the application of the systems concept and modeling were necessary to expose and understand the intricate interrelationships between the diet and its result on the organism and also to perform quantitative or semiquantitative studies. One major challenge is that a model on nutrition must contribute to understanding the relationship between nutrition and health, but this relationship occurs at

different organizational (e.g., biochemical and cellular interactions and metabolic routes) and temporal (e.g., from immediate responses to the intake of a given nutrient to the response after a chronic ingestion of deficiency in the diet) levels. Even though the level of complexity is enormous and currently no single mathematical model can integrate all the information available and generate all the possible knowledge, it is possible to develop suitable computational models based on the use of the virtual patient concept, together with accurate phenotyping and the development of biomarkers generated from the -omics techniques. Then, all these data must be integrated at multiple organizational levels and timescales.

The application of proteomics in a systems approach to nutritional sciences and human health has been used to study the relationships between muscle proteomics and obesity and type 2 diabetes (T2D) (by 1DGE and LC-MS/MS), urinary proteomics in type 1 diabetes versus T2D (by CE, LC-MS/MS), plasma proteomics after folic acid supplementation (2DGE and LC-MS/MS), plasma proteomics in T2D, serum proteomics after fish oil supplementation, adipocyte proteomics after energy restriction, and peripheral blood mononuclear cell proteomic response after flaxseed and after soy-isoflavone supplementations (by 2DGE and MALDI-MS).

Any systems-based nutrition study must first define the system under study (cell, tissue, organ, and individual) and then determine which information should be obtained from the system (proteomics, behavior, genetic makeup, and cholesterol levels) and the interactions among component (e.g., how the fat content in the diet influences the cholesterol level in an individual with genotype X and a proteome Y in the tissue Z). Then, a model needs to be constructed for the system to explain and help understand the interactions between the different data. Finally, the model needs to be improved with further data and finally validated.

Needless to say, we are still far from having a complete model integrating all the components and interactions relevant to human nutrition and health that must also take into account individual differences, that is, taking into account gene expression–nutrient interactions. But, eventually, we will get there.

See also: Allergies: Public health; Amino Acids: Metabolism; Antinutritional Factors in Legume Seeds: Characteristics and Determination; Antioxidants: Characterization and Analysis; Authenticity of Food; Beer: Fermentation; Berries and Related Fruits; Bioactive Peptides in Foods; Cereals: Types and Composition; Cherries (*Prunus* spp.): The Fruit and Its Importance; Dairy Products: Dietary and Medical Importance; Eggs: Composition and Health Effects; Eggs: Use in the Food Industry; Enzymes: Analysis and Food Processing; Enzymes: Functions and Characteristics; Fish: Dietary Importance and Health Effects; Fish: Fish in the Human Diet; Fish: Processing; Food Allergies; Food Allergies: Occurrence and Analysis; Food Environment; Food Fraud; Mass Spectrometry: Applications; Mass Spectrometry: Principles and Instrumentation; Meat: Conversion of Muscle into Meat; Meat: Eating Quality and Preservation; Meat: Structure; Milk: Role in the Diet; Milk: Sources and Composition; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Protein: Food Sources; Proteins: Chemistry, Characterization, and Quality; Wines: Wine and Health; Wines: Wine Production.

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Pulsed Electric Fields

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What Are Pulsed Electric Fields?

High-energy pulsed electric fields (PEFs) deliver high-energy discharges at very short periods. Low-voltage energy from the DC power supply is transformed to high-intensity pulsed fields. The high-intensity energy is stored in capacitors to be discharged to the target at different electric strengths (20–80 kV cm^{-1}) and times (<1 s), which depend on the purposes to be attained in the food. The food is passed between two electrodes, hosted in a chamber, which could be of different designs; the high-intensity pulses are discharged to the food mainly for inactivating microorganisms and enzymes. Supposedly, the food's physicochemical, nutritional, and sensory characteristics should be kept as those of fresh products during delivery. However, attention should be paid since the PEF processing may increase temperature suddenly and fast; therefore, a combination of the type of food, electric fields, processing times, flow rate, and refrigeration systems should be taken into account for delivering foods maintaining their nutritious and sensory characteristics similar to fresh foods.

PEF Production

Circuits for generating high-energy electric fields are simple. Different types of pulses can be produced by the PEF system, namely, square wave, exponential decay, oscillatory, or bipolar pulses (Figure 1). However, the square waves and exponential decay pulses are the more commonly used and they are produced differently. The exponential decay pulses are produced in the circuit formed by a DC power supply, a bank of capacitors connected in series with a charging resistor, and a discharge switch to deliver the high-energy pulses to the treatment chamber hosting the food. On the other hand, the square wave pulses, which are more energetic, are produced in an array of capacitors, inductors, and switching devices.

Pulse Generation and Treatment Chambers

The PEF treatment chambers are the heart of the PEF system. There, pulses are delivered to the food passing between two electrodes. There are different designs. Figure 2 illustrates a batch treatment chamber (a) and two continues chambers (b, c). Caution should be paid since a breakdown may occur if the electric field strength exceeds the food electric strength; as a result, sparks produced may damage the electrode's surface.

In a simplest pulse-forming system, an electric strength (E) between two points is created when an electric potential difference (V) crosses two points separated by a nonconductive material, which is directly proportional to V and inversely proportional to the distance (d):

$$E = \frac{V}{d} \quad [1]$$

In a coaxial treatment chamber, the electric field varies according to the radial distance from the inner electrode:

$$E = \frac{V}{r \ln\left(\frac{R_2}{R_1}\right)} \quad [2]$$

where r is the distance to measure the electric field and R_1 and R_2 are the radii of the inner and outer electrodes, respectively. The charged capacitors (C_o), where the high energy γ is loaded before being delivered to the treatment chamber, are discharged (Figure 1) through the resistor (R); therefore, the voltage through the food decreases exponentially with a pulse width (τ):

$$R = RC_o \quad [3]$$

The treatment time (t) is obtained taking into account the pulse width and number of pulses (n):

$$t = n\tau \quad [4]$$

The discharge of pulses to the food and its control are controlled by a computer coupled to the pulse generator.

Energy and Temperature Release

The energy requirements and temperature rise during a PEF treatment are complicated. For a single-pass process with no recirculation, the energy (Q) released during the capacitor discharge (C) at the charged voltage (V) would be

$$Q = \frac{1}{2}CV^2 \quad [5]$$

Ideally, the total capacitor energy is discharged to the treatment chamber. However, only part of the total energy is set to the food and this defines the maximum voltage in the treatment chamber:

$$V_{Chamber} = V_{Charging}\frac{R_{Chamber}}{R_{Total}} \quad [6]$$

As a result, the pulse energy dumped to the chamber is

$$Q_{Pulse} = \frac{1}{2}\frac{R_{Ch}}{R_T}CV^2 \quad [7]$$

During the delivery of energy to the food, throughout the process, the food temperature increases due to ohmic heating. The heating of food product is

$$Q = mC_p\Delta T \quad [8]$$

where m is the mass, C_p is the specific heat capacity of the food product, and ΔT is the rise of temperature. In a continuous PEF system, the pulse energy is

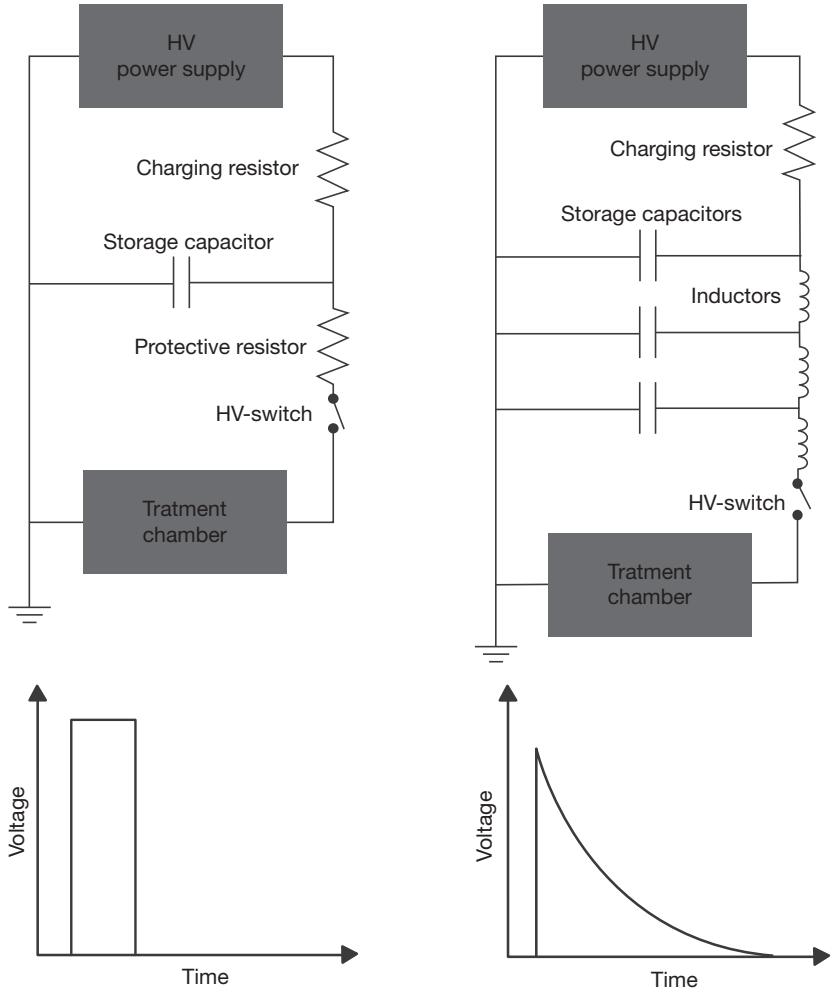

Figure 1 Exponential decay and square wave pulse generation.

$$Q_{\text{Pulse}} = \frac{\rho v C_p \Delta T}{f} \quad [9]$$

where ρ is the density, v is the flowing volume per second, and f is the frequency of pulses.

What PEF Are Used for?

The PEF technology is used for treating foods with different purposes. The microbial and enzymatic inactivation is of paramount importance to deliver microbiological and physicochemical safe food products to consumers. PEF technology is used for inactivating pathogenic and spoilage microorganisms, and the effect on all of them will depend on the treatment (electric field intensity (E), pulse width, treatment time (the number of pulsed (n) per the time of each pulse), t, temperature, pulse shape and pulse polarity, microorganism (type, concentration, growth phase), and medium characteristics (pH, preservatives, conductivity, and ionic compounds)). PEF is also valuable for inactivation of enzymes such as pectin methyl esterase, polygalacturonase, polyphenol oxidase, peroxidase, and lipoxygenase found in fruit and vegetable juices and alkaline phosphatase, protease, and lipase in milk.

PEF Effect on Cells

Electroporation (Figure 3) is the phenomenon in which electric field is applied to biological cells. The number of pores formed around the cell membranes, due to the electric field, depends on the medium and on the PEF variables – electric field strength, pulse intensity, pulse width, pulse number, and processing time – and the type and strain of microorganism and size and geometry of cells (Figure 4). Electroporation may compromise cells at different levels; therefore, electric field strength higher than 2 kV cm^{-1} generates irreversible recovery in microbial cells and more than 14 kV cm^{-1} is required for plant cells.

Microbial Inactivation Modeling

Microbial inactivation can be described using different mathematical models. The type of model used will depend on the

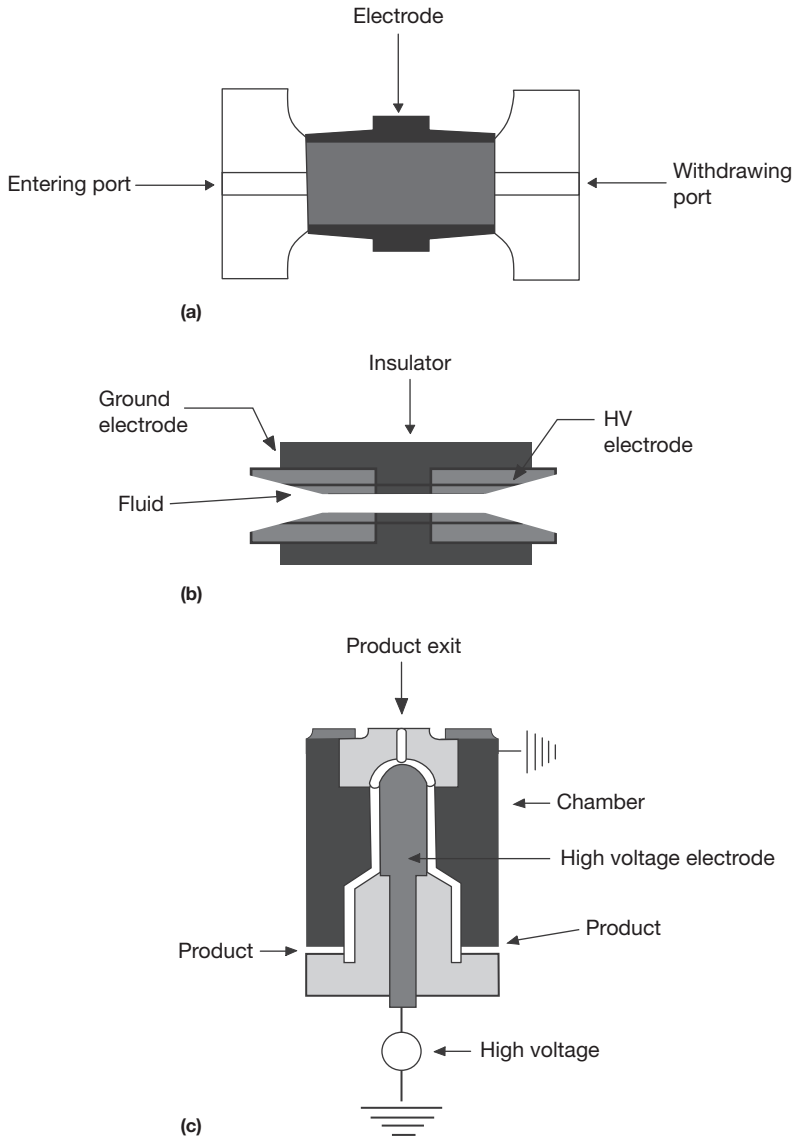

Figure 2 Batch parallel plate (a), cofield continuous (b), and concentric cylinder continuous treatment chambers (c).

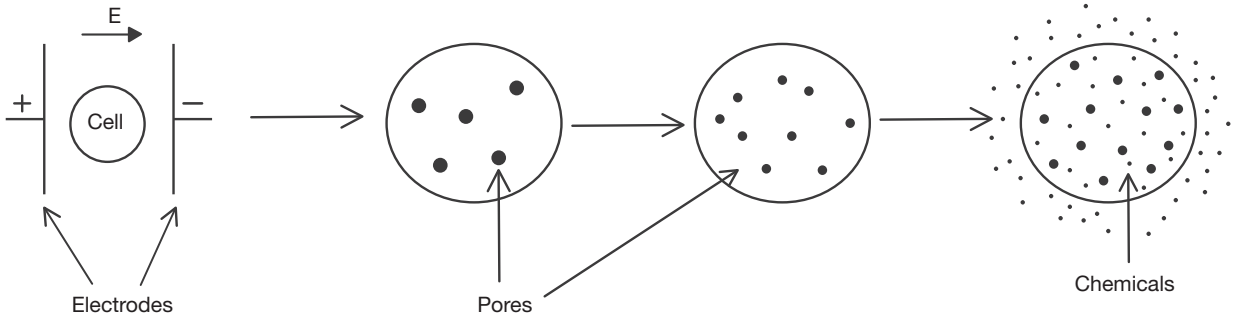

Figure 3 Electroporation of cells.

manner the microorganism is being injured along the time of treatment or any other variable used in the PEF methodology. Some models used for describing the microbial load survivals are as follows:

- First-order kinetics model

$$\log\left(\frac{N}{N_0}\right) = -kt \qquad [10]$$

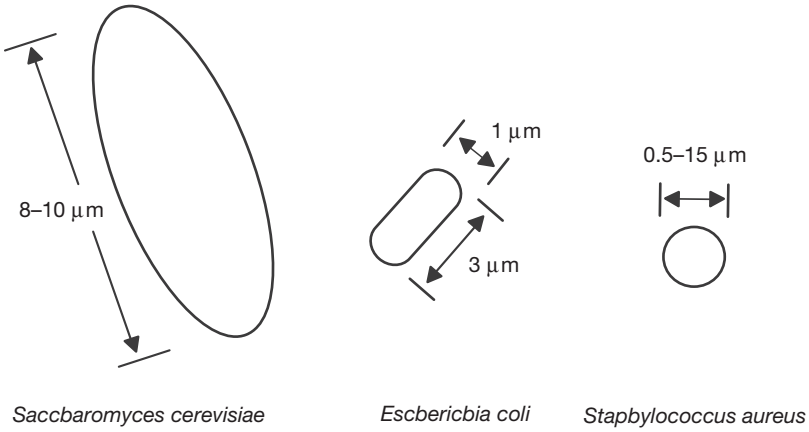

Figure 4 Microorganism size and geometry.

- Weibull distribution

$$\frac{N}{N_0} = e^{-(bt)^a} \quad [11]$$

- Log-logistic distribution

$$\log\left(\frac{N}{N_0}\right) = \alpha + \frac{\omega - \alpha}{1 + e^{[4\sigma(\tau - \log t)]/\omega - \alpha}} \quad [12]$$

- Modified Gompertz equation

$$\log\left(\frac{N}{N_0}\right) = Ce^{-e^{BM}} - Ce^{-e^{B(t-M)}} \quad [13]$$

- General model

$$s = \left(\frac{t}{t_c}\right)^{-\left(\frac{E-E_c}{K}\right)} \quad [14]$$

where N is the microbial load after some treatment (CFU ml^{-1}); N_0 is the initial microbial load; t is the time (product of the number of pulses and pulse width); k is the inactivation rate constant; a and b are the shape and scale constants, respectively; α and ω are the upper and lower asymptotes, respectively; σ is the maximum slope of the survivals curve; τ is the logarithm of time at the maximum slope; C, B, and M are constants; s is the survival fraction; E is the electric field strength; and E_c, t_c, and K are constants evaluated experimentally or empirically.

PEF Effect on Enzymes

Enzymes found in foods can be inactivated by applying PEF. Some enzymes could damage the food systems and some of them are only part of the product metabolism and non-dangerous; however, unwanted enzymes should be inactivated or depleted to ensure an adequate food product shelf life. The PEF effect on enzymes may depend on the electric conditions. In most of the cases, a high amount of enzyme was inactivated; however, in some cases, no inactivation or an increased activation was observed. PEF electric conditions for treating foods with the aim of affecting enzymes are higher than for inactivating microorganism. The levels of field strength that have been applied range from 10 to 87 kV cm^{-1} for several times or pulse number for attaining some purpose in a food system, model food systems, or in solutions. Since enzymes are proteins, denaturation may occur due to PEF effects on the structure. In a general way, the mathematical models reported to describe microbial inactivation are similar for describing the enzymatic inactivation.

Effect of PEF Treatment on Functional, Nutritional, and Sensory Properties

Functional compounds such as antioxidants found in foods could be altered after PEF treatment. Many fruit and vegetable products have phenolic compounds, vitamins, pigments, and other chemical compounds found in essential oils of parts of plants. These types of compounds may remain unaltered, depleted, or increased in the food product after PEF treatment, which will depend on the treatment conditions. In some cases, phenolic compounds are depleted after PEF treatment (apple juice) for accomplishing the microbial or enzymatic inactivation or increased when the objective is disintegration of cells for increase the liberation of them (anthocyanin of grapes and their products) or increase such as in the case of soy milk. Carotenoids and vitamin C could decrease. Therefore, antioxidant capacity related with those compounds may decrease or remain in the food product after PEF treatment as the thermal treatment does.

In general, the sensory characteristics of foods remain unchanged when there is no increase in temperature during PEF processing at different treatment conditions. Milk, orange juice, and cranberry juice, just to mention some, have been evaluated for their sensory characteristics after PEF treatments. In all cases, no significant sensory changes were noticed.

PEF Food Safety Features

Foodborne diseases and rotting of foods due to microorganisms can be controlled by applying PEF to foods. Food spoilage microorganisms and pathogens can be decreased or eliminated due to impairment of their normal metabolic functions. PEF methodology has been used for processing foods or model

solutions with pathogens such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella dublin*, *Salmonella enteritidis*, *Escherichia coli* O157:H7, *Bacillus cereus*, *Yersinia enterocolitica*, *Clostridium tyrobutyricum* (spores), and *Pseudomonas aeruginosa* and spoilage microorganisms (or fermenters) such as *Corynebacterium* spp., *Xanthomonas maltophilia*, *Lactobacillus delbrueckii*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Listeria innocua*, *Leuconostoc mesenteroides*, *Zygosaccharomyces bailii*, *Lactobacillus brevis*, *Lactobacillus plantarum*, and *Lactobacillus plantarum*.

What Types of Foods Are Treated with PEF?

The PEF technology is used for treating liquid and solid or semisolid foods. For instance, for liquid foods, orange juice and apple cider and juice are two of the more studied products by researchers in PEF as well as milk and other fluid products such as liquid egg; tomato, grape, mango, and cranberry juices; and beer. The disintegration of vegetable cells is another field of study for improving the liberation of the cell content, such as fruit or vegetable juices or other high-value products from cells.

PEF-Treated Liquid Foods

Due to the chamber's design and PEF system, most of the treated foods are liquids. The electric system (high-voltage pulse generator) consisting of the high-energy reservoirs which are delivered throughout the treatment chamber (electrode system) to target the liquid food which is flowing throughout the chamber as well. Hence, a number of liquid foods have been treated by the PEF technology, bringing microbiological and physicochemical safe and nutritious with good sensory quality products. Milk, egg, fruits, and vegetables and their products have been the most PEF-studied products alone or in combination with other preservation factors.

PEF-Treated Solid and Semisolid Foods

As mentioned earlier in the text, cells from solid foods such as plants can be treated to ease the liberation of valuable chemicals. However, food products other than those from plants could be also PEF-treated to accomplish other purposes. However, foods such as meat and fish do not resist electric field strengths beyond 10 kV cm^{-1} because their texture may change. Such low-electric field intensities are not enough for inactivation of microorganisms or enzymes for delivering satisfactory PEF-treated foods. PEF treatment has been used to electroplate cell from herbs for improving the extraction of essential oils.

Final Remarks

Even though the PEF technology has been studied for more than one decade, more research is required for processing and delivering physicochemical and microbiological safe food products with good sensory characteristics to consumers.

See also: Enzymes: Functions and Characteristics; Spoilage: Bacterial Spoilage; Sterilization of Foods.

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Quality Control in Food Processing

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Introduction

Food processing refers to a series of actions involved in the preparation and preservation of a food supply by some continuous operation or treatment to achieve as a goal a safe, high-quality product with extended shelf life. Changes occurring in foods could be of two types: deterioration and spoilage. The former involves changes in quality induced by physicochemical and/or biochemical reactions taking place with or without the intervention of a physical environment (CO_2, O_2, light, temperature, etc.), while the latter refers to changes in quality due to action of biological agents such as bacteria, molds, and yeasts. These changes induce modifications in the quality characteristic of foods at the macroscopic (sensory and rheology), molecular (protein–protein, protein–lipid, and protein–water interactions), and microscopic levels.

Food control is defined as a mandatory regulatory activity of enforcement by national or local authorities to provide consumer protection and ensure that all foods during production, handling, storage, processing, and distribution are safe, wholesome, and fit for human consumption; conform to safety and quality requirements; and are honestly and accurately labeled as prescribed by law.

Quality control is not an optional extra in food processing, neither is it something that is only done by large manufacturers. It is an essential component of any food processing business. The purposes of quality control are to:

- protect the customers from dangers (e.g., contaminated foods) and ensure that they get the weight and quality of food that they pay for;
- protect the business from cheating by suppliers, damage to equipment (e.g., stones in raw materials), and false accusations by middlemen, customers, or suppliers;
- be sure that food laws operating in a country are complied with.

The determination of the quality of food products during processing is a critical attempt due to the presence of and interaction between different components (e.g., proteins, lipids, and carbohydrates). These interactions influence the quality of food products. The quality of foods could be determined by several analytical methods including microscopic, physical (sizes, weight, density, etc.), physicochemical (pH, vitamin content, fat content, etc.), microbiological, rheological (firmness, elasticity, fragility, etc.), sensory (appearance, taste, flavor, texture, etc.), etc. Among these methods, the physicochemical methods are most commonly used. Although heavy, these techniques are considered among the most interesting ones for the determination of the quality of food products during processing. With regard to rheological methods, they are being used to determine the texture of food products (firmness, adhesivity, gumminess, etc.).

However, most of the previously mentioned techniques are tedious and destructive and time-consuming and require highly skilled operators. As technology advanced, food processing technology also geared up to meet productivity, quality, safety, and economic requirements. Objective testing of quality attributes like odor and taste is difficult to carry out by subjective methods. Present-day technology continues to develop and use various modern tools for both monitoring and control of the various food quality parameters. In addition, the variation in texture properties of food products depends strongly on their structure. Recently, more attention has been paid to the development of noninvasive and nondestructive instrumental techniques such as infrared, fluorescence, nuclear magnetic resonance, and Raman spectroscopy. These techniques are fast, of relatively low cost, and environmentally friendly and provide a great deal of information with only one test, making them suitable for on-line and/or at-line process control. In addition, spectroscopic techniques often require little or no sample preparation and are relatively easy to operate.

This article will provide a comprehensive overview of the applications of different analytical techniques (i.e., physicochemical, rheological, fluorescence, and infrared), in combination with multivariate data analysis, to determine the quality of food products (Figure 1). Actual examples illustrating the utilization of these techniques in both laboratory and industrial environments will be discussed as well as their advantages and disadvantages.

Physicochemical Methods

The physicochemical properties are considered as an important tool to determine the quality of food products (Table 1). Indeed, food products may be altered by several factors related to the area of production and characteristics of the production systems, such as feeding, microbial contamination, and the technological process.

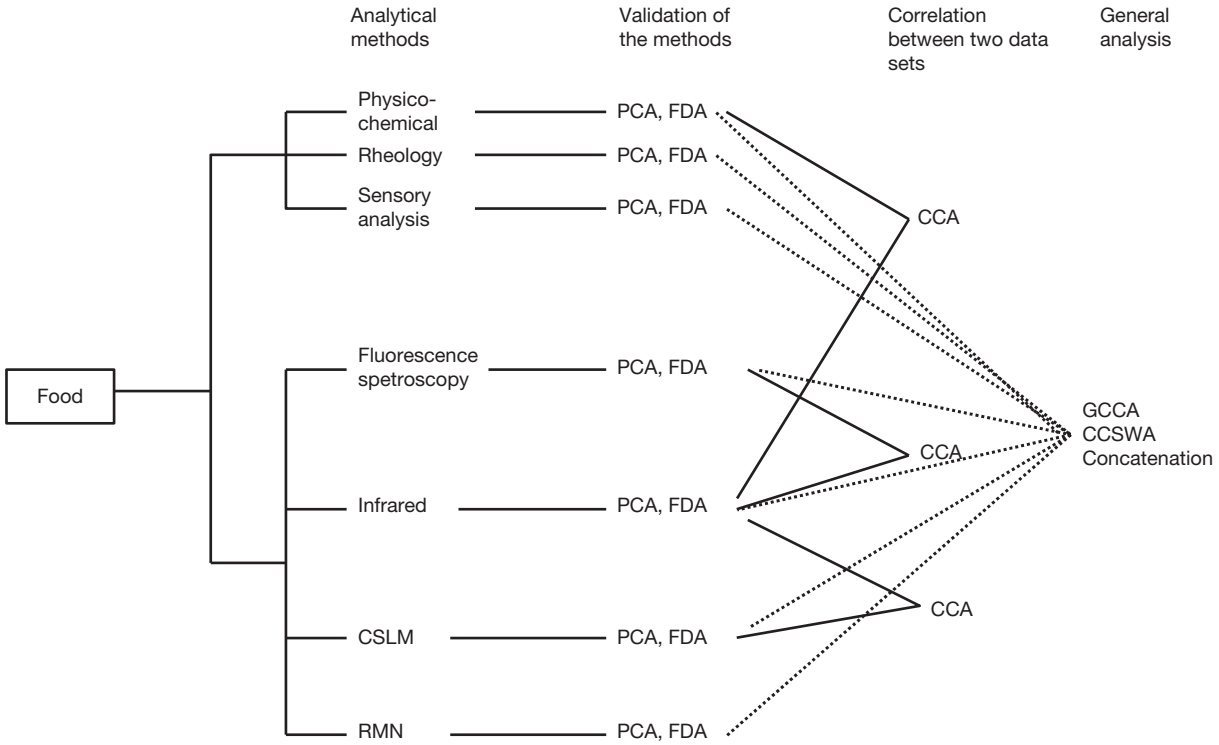

Figure 1 Different analytical techniques coupled with chemometric tools used for the evaluation of food quality. CCA, canonical correlation analysis; CCSWA, common component and specific weight analysis; CSLM, confocal scanning laser microscopy; FDA, factorial discriminant analysis; GCCA, general canonical correlation analysis; NMR, nuclear magnetic resonance; PCA, principal component analysis.

The physicochemical characteristics of semiskimmed UHT milk kept at different storage temperatures (4, 20, and 40 °C) for up to 6 months were investigated. No change for milk samples stored at 4 and 20 °C even after 6 months was found in contrast to those kept at 40 °C where, on the one hand, a gradual drop to pH 6.21 was observed compared with freshly processed UHT milk presenting a pH of 6.7 and, on the other hand, an increase in noncasein nitrogen content with the increase of storage temperature (i.e., 3.54, 3.87, and 5.47 g kg^{-1} for milks kept at 4, 20, and 40 °C).

The impact of milk maturation on the physicochemical characteristics in three different conditions was studied. Full cream milk samples collected from the evening milking were stored in the farm and delivered to the cheese factory in churns or in thermos-regulated tanks at a temperature not lower than 18 °C. The natural creaming (10–12 h overnight) was performed in a traditional large flat vat or in thermos-regulated large flat vats at ∼15 °C. Compared with full cream milk, skimmed evening milks showed lower values of casein and casein number and higher contents of whey proteins. Recently, the physicochemical characteristics of raw bulk milk samples kept at different conditions have been investigated: 12, 24, 36, and 48 h at 4–6 °C and 8–10 °C and 12 and 24 h at 13–15 °C. A significant increase in pH value was observed in the first 12 h of storage, regardless of the considered temperature. No significant change of milk samples kept at 13–15 °C during 24 h was observed, whereas a gradual decrease of this parameter was noted during the first 36 h of milk samples stored at lower temperatures (4–6 °C and 8–10 °C) due to the dissociation of calcium from the casein micelle, which increased milk pH.

The impact of technological process on the formation of biogenic amines in fish products has been investigated by several authors. The effect of different freezing treatments on the formation of biogenic amines of cultured bighead carp heads was investigated. Fish heads were packaged in polyvinyl chloride bags and kept randomly at two temperatures −40 and −18 °C during 3 months. After being stored for 3 months, fish heads were moved to boxes and stored in refrigerated chambers at 4 °C. Among the eight biogenic amines investigated in this study (i.e., tryptamine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, and spermine), spermine and spermidine were found to be the two major amines present in fish head samples attaining levels of, respectively, 56.9 and 11.1 mg kg^{-1} after 18 days storage. One of the main conclusions of this study was that freezing treatments had a significant lower value of spermine and spermidine than the fresh ones.

Although the physicochemical analyses are considered as reference methods providing accurate and reliable results on quality and authenticity of fish samples, they are time-consuming and need a lot of pollutant reagents, are relatively expensive, cannot be utilized at-/on-line, and involve the use of chemicals and trained labor.

Rheological Methods

Rheology is the study of deformation and flow of matter. Foods can be classified in different ways, such as solids, gels, homogeneous liquids, and suspensions of solids in liquids and

Table 1 Some examples of destructive and nondestructive methods used for quality assessment of food products

	Analysis	*Examples of most used methods*	*Approximate percentage (wet weight basis)*
Destructive methods	Moisture and total solids	Over drying methods Distillation procedure Chemical method (Karl fisher titration) Refractometer	Wheat flour: 10.3 Milk: 88 Egg: 75.3 Honey: 17.3
Nondestructive methods		Infrared (NIR and MIR) Fluorescence Nuclear magnetic resonance	
Destructive methods	Ash	Dry ashing Microwave ashing	Bread and pasta: 1.5 White rice: 0.6
Nondestructive methods		Infrared (NIR and MIR) Fluorescence Nuclear magnetic resonance	Milk: 0.7 Yogurt: 1 Apples: 0.3 Fish filet: 2.5
Destructive methods	Fat	Soxhlet method Gerber method for milk fat Refraction index method for processed meat Babcock method for milk fat Goldfish method	Skimmed milk: 2.5 Milk: 3.3. Cheddar cheese: 33.1 Butter: 81.1 Beef: 10.6
Nondestructive methods		Infrared (NIR and MIR) Fluorescence Nuclear magnetic resonance	Egg: 10
Destructive methods	Protein	Kjeldahl method Dumas method Biuret method	Rice: 7.9 Corn starch: 0.3 Yogurt: 6.3
Nondestructive methods		Infrared (NIR and MIR) Fluorescence Nuclear magnetic resonance	Chicken: 23.1 Dried beef: 29.1 Egg: 12.5 Mature seeds: 36.5
Destructive methods	Carbohydrate	Phenol sulfuric acid method High performance liquid chromatography Gas chromatography Enzymatic methods Mass spectrometry	Bread: 86 Ice cream: 28.2 Grape: 17.2 Fish filet: 17 Honey: 82.5
Nondestructive methods		Infrared (NIR and MIR) Nuclear magnetic resonance	

emulsions. Many of the food products exhibit both viscous and elastic properties and can be described as viscoelastic materials. In contrast, if fluids contain dissolved low-molecular-weight compounds (e.g., sugars) and no polymer or insoluble solids, they may show Newtonian behavior.

Processed foods may be viewed as edible structures that are created as a result of the responses of proteins, polysaccharides, and lipids in aqueous media to different processing methods, such as thermal processing, homogenization, and other physical treatments. Most, if not all, of those responses are physical in nature. The measured rheological responses are those at the macroscopic level. However, they are directly affected by the changes and properties at the microscopic level. Thus, it would be helpful to understand the role of structure of foods on their rheological behavior. Rheological properties are based on flow and deformation responses of foods when subjected to normal and tangential stresses.

Texture is a very important quality parameter of food products and it is of interest to producers, processors, and consumers. Since rheology represents the properties and characteristics of both liquid and solid foods, in strict terms, texture is the rheology related to solid foods, while viscosity is the rheology related to fluid foods. Changes occurring in heated UHT milk with low levels of calcium chloride addition ($\leq$20 mM) have been monitored. One of the main conclusions of this study was that the addition of 10–20 mM $CaCl_2$ caused thickening and/or gelation of milk heated at 70 °C. Some authors have monitored during ripening at 4 °C (5 days, 8, 16, and 24 weeks) the rheological properties of cheddar-like cheese made from caprine milk collected at different lactation periods and found that a minimum of 8 weeks of aging was needed to stabilize the cheese texture.

Regarding fish and fish products, the impact of smoking and freezing–thawing treatments on the rheological properties of fish samples has been determined and the obtained results indicated that hardness, gumminess, and chewiness decreased within 5 days of storage.

In a different approach, the incorporation of specific probiotic bacteria, together with selected additives, has been conducted. The whey cheeses have been produced with probiotic bacteria from combinations of bovine whey and milk, following protein denaturation at 90 °C; they were subsequently

inoculated at 10% with the previously mentioned strains and homogenized and additives such as salt and sugar were then incorporated. The resulting solid matrices were stored at 7 °C for up to 21 days. Their results showed that whey cheeses inoculated with *L. casei* were always more elastic and viscous than those inoculated with *B. animalis*. Other research studies determined the impact of cream heat treatment prior to butter manufacturing as well as the fluctuation of temperatures during storage. The obtained results showed that low cooled cream maturation significantly increased the storage modulus (G′) from 0.30 to 0.38 MPa compared to butter produced from nonmatured cream. One of the main conclusions of this study was that cooled cream maturation did not cause any changes in the rheological profile, while fluctuating temperatures significantly affected the G′.

Rheology measurements avoid the high costs of highly trained personnel and do not suffer from fatigue or adaptation as sensory evaluation could do. However, some artifacts such as slippage and sampling procedure could occur during the measurements since solid food products samples could present different dimensions during the application of rheological methods inducing difference in rheological values.

Front-Face Fluorescence Spectroscopy

Fluorescence spectroscopy is a rapid, sensitive analytical method for characterizing molecular environments of food products. Fluorescence is the light emission subsequent to absorption of ultraviolet or visible light of a fluorescent molecule or substructure, called a fluorophore. The fluorophore present naturally in food products absorbs energy in the form of light at a specific wavelength and liberates energy in the form of light emission at a higher wavelength.

With classical right-angle fluorescence spectroscopy, the measurements are carried out in dilute solutions where the absorbance is below 0.1. At a higher absorbance rate, a decrease of fluorescence intensity and a distortion of emission spectra are observed due to the inner filter effect. To overcome such problems, front-face fluorescence spectroscopy (FFFS) was developed where only the surface of the material is illuminated and examined. To minimize artifacts generated by the photons of excitation reflected from the sample, the emitted photons are collected at an angle between 30° and 60° to the surface of the sample; this technique allows a quantitative investigation of fluorophores in powders as well as in concentrated or even opaque samples.

The potentiality of fluorescence spectroscopy coupled with multivariate statistical methods to characterize milk samples collected from different manufacturers and stored at four different temperatures (i.e., 4, 22, 35, and 50 °C) for 8 weeks has been determined. To extract information from the fluorescence spectra, the authors have applied multivariate statistical methods, that is, principal components analysis (PCA), to the different acquired spectra (i.e., Maillard products, tryptophan, and riboflavin). One of the main conclusions of this study was that FFFS could be used as a tool for monitoring changes occurring in milk samples during storage. In another study, FFFS has been utilized to monitor structure during milk acidification kinetics of skimmed milk samples at two coagulation temperatures (30 and 40 °C). For each coagulation temperature, synchronous fluorescence spectra were recorded in the 250–500 nm excitation wavelength range using offset of 80 nm between excitation and emission monochromators during 300 min coagulation kinetics. By applying PCA to the spectral data sets, the authors pointed out the impact of the coagulation temperature on the molecular structure and the interaction between the different macromolecules present in the coagulum, which was confirmed after by others who assessed to determine the effects of heat treatment (60 and 80 °C during 20 min) and rennet-induced coagulation temperatures (30 and 40 °C). One of the main conclusions reached was the clear discrimination observed between (i) raw milks and heated milks and (ii) milks renneted at 30 °C and those renneted at 40 °C. These results were recently confirmed by others who also succeeded to discriminate milk samples according to heating (72–120 °C).

Recently, some authors have determined using the emission spectra of vitamin A the impact of heat treatment on the evolution of fluorescence spectra. As shown in Figure 2, heat

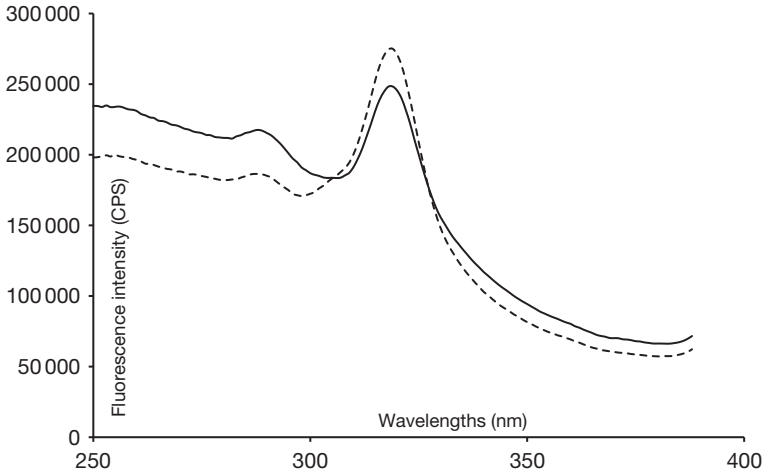

Figure 2 Evolution of vitamin A spectra acquired on milk samples after heating at 55 °C (---) and 75 °C (___) during 10 min.

treatment induced a decrease in the fluorescence intensity at both 320 and 290 nm since milk samples heated at 75 °C for 10 min presented the lowest intensity compared with milk samples heated at 55 °C during the same time.

Regarding cheeses, the impact of technological process on the molecular level of both the external and central zones of 15 different soft cheeses (i.e., two traditional soft cheese varieties and one stabilized soft cheese variety) has been investigated. By applying a series of chemometric tools, the authors succeeded to discriminate cheeses according to their manufacturing process and sampling zone. The obtained results were confirmed after by others who monitored changes occurring in Cantal cheeses throughout ripening period (30, 120, and 200 days) using synchronous fluorescence spectra. A clear differentiation between cheeses according to their ripening stage was observed since 100% of correct classification was obtained; the authors have attributed this trend to the modifications occurring in the physicochemical properties of cheese matrix.

Reported studies on the use of FFFS as a method to monitoring fish and egg freshness are scarce. The impact of storage conditions on the evolution of fish and egg freshness has been monitored. The shape spectra of egg and fish samples illustrated some differences according to storage time. By applying factorial discriminant analysis (FDA) to the NADH data sets acquired on fish samples, correct classification amounting to 92% has been obtained. The authors concluded that NADH spectra could be considered as fingerprints that may allow discrimination between fresh and aged fish fillets. The same research group has later succeeded using FFFS to differentiate between fresh and frozen–thawed fish samples. By applying PCA to the normalized NADH spectra, a clear discrimination between fresh and frozen fish samples was observed, which was confirmed by FDA model since complete (100% and 100%) correct classification was obtained for the calibration and validation data sets, respectively.

As illustrated earlier, the environment of intrinsic fluorophores recorded on food products contains valuable information regarding the composition and nutritional values of food products. The huge potential for the application of fluorescence spectroscopy combined with multivariate statistical analyses could be an alternative to the destructive techniques for the evaluation of food products quality. The principal advantages of fluorescence spectroscopy are its rapidity and specificity since this technique is considered to be 100–1000 times more sensitive than other spectrophotometric techniques. However, the major disadvantage of fluorescence is its strong dependence on light scatter and environmental factors such as temperature, pH, and viscosity.

Infrared Spectroscopy

The infrared region comprises that part of the electromagnetic spectrum in the wavelength range varying between 780 and 100 000 nm and is divided into near-infrared (NIR) region (780–2500 nm) and mid-infrared (MIR) region (4000–400 cm^{-1}). Absorptions in the fingerprint region are mainly caused by bending and skeletal vibrations, which are particularly sensitive to large wave number shifts, thereby militating against unambiguous identification of specific functional groups.

Sixteen semihard cheeses varying in their protein (20.2–24.1%), fat (23.7–31.1%), and dry matter (50.2–57.9%) contents were sampled at different ripening times (1, 21, 51, and 81 days) and analyzed by MIR. To extract information from the MIR spectral data sets, PCA was applied to the 1700–1500 cm^{-1} spectral region, and the similarity map showed incomplete differentiation between the investigated samples. By combining mid-infrared and fluorescence spectra using canonical correlation analysis, a clear discrimination of cheese samples has been obtained. One of the main conclusions of this study was that the protein network characteristics of the ripened cheeses were found to be related to the initial composition of young (1-day-old) cheeses. The authors suggested that FT-MIR and fluorescence spectroscopies provided a common description of cheese samples throughout ripening, allowing their potential use as tools for providing useful information related to protein structure in cheese.

In a similar approach, some authors have used FT-MIR to monitor the ripening process of camembert soft cheeses at different ripening stages (i.e., after 10, 13, 15, 17, 20, and 27 days). No changes were observed in the case of core samples, although those collected from under the rind did show some spectral modification throughout the ripening period studied.

Regarding meat and meat products, the potential of FT-MIR has been explored for the analysis of comminuted beef meat contaminated at low spoilage levels. Using the 1500–700 cm^{-1} spectral region, the developed models were found to be not accurate. Another application related to authentication issues of red seabream was performed by others who conducted a study to determine whether VIS/NIR spectroscopy in a reflection mode could differentiate fresh fish samples from frozen–thawed ones. By applying PCA to the spectral collection, a clear differentiation between frozen–thawed samples and fresh ones was observed. The obtained results confirmed previous studies that succeeded by using FT-MIR to differentiate between fresh and frozen–thawed fish samples in the 3000–2800 cm^{-1}. By applying FDA to this spectral region, 100% and 87.5% of correct classification was obtained, respectively, for the calibration and validation data sets.

Regarding cereal products, some authors have applied NIR and MIR spectroscopy to study dough proofing, after mixing. On the basis of these studies, the authors concluded that FT-IR spectroscopy revealed information about the relative importance of protein and starch moieties during proofing.

In another study, infrared has been used to determine the effect of heat treatment on the quality of apple juice samples. The authors have applied a series of chemometric tools, and correct classification in the 77.2–100 range has been found depending on the technique used (NIR or MIR).

The infrared technique provides more information compared to traditional ones, as it allows simultaneous identification of quantitative and qualitative changes of various chemical components (proteins, fat, water, etc.) involved in the determination of food quality. The use of MIR is characterized by a high repeatability and very easy sampling. However, the disadvantage of infrared technique is its inability to measure molecules with very low concentrations. Moreover,

the strong absorptions of water due to the O–H bending band could obscure potentially useful absorptions from protein for foods containing high level of water content.

Conclusion

During the past decade, a considerable interest has been paid to determine food product quality. Food quality could be determined by different analytical techniques. Nowadays, the need for rapid analytical techniques to determine quality and authenticity is greater than ever. Recently, a considerable effort has been made by researchers to explore the possibilities of using spectroscopic techniques for the determination of the quality of food products. These techniques as depicted in this article are mostly of relatively low cost and can be applied in both fundamental research and the factory as on-line sensors for dairy process monitoring and product quality assessment.

See also: Authenticity of Food; Cheese: Types of Cheese – Medium; Cheese: Types of Cheeses – Hard; Cheese: Types of Cheeses – Soft; Chemometrics; Infrared Spectroscopy: Applications; Proteins: Chemistry, Characterization, and Quality; Rheological Properties of Food Materials; Sheep: Milk; Storage Stability: Shelf Life Testing.

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Quinoa

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Introduction

Quinoa, *Chenopodium quinoa* Willd., is an Amaranthacean, stress-tolerant plant cultivated along the Andes for the last 7000 years, facing highly different environmental conditions ranging from Bolivia, up to 4.500 m of altitude, to sea level, in Chile. Quinoa, called 'the mother grain,' constitutes a strategic crop with potential to contribute to food security and sovereignty due to nutritional quality, genetic variability, adaptability to adverse climate and soil conditions, diversity of methods of use, and low production cost.

The nutritional benefits and agricultural versatility of quinoa have been stressed by the United Nations Food and Agriculture Organization (FAO). The expansion of the crop from South America to other continents supports that it is a crop with high potential to contribute to food security in various regions worldwide, especially in those countries where the population does not have access to protein sources or where production conditions are limited by low humidity, reduced availability of inputs, and aridity. After the submission of the report 'Quinoa: An Ancient Crop to Contribute to World Food Security' in July 2011 by the Regional Office for Latin America and the Caribbean to the 37th FAO Conference in support of the declaration of the 'International Year of Quinoa,' 2013 was declared the International Year of Quinoa.

Its grains have higher nutritive value than traditional cereals, and it is a promising worldwide cultivar for human consumption and nutrition. Quinoa has been called a pseudocereal for botanical reasons but also because of its unusual composition and exceptional balance between oil, protein, and fat. Quinoa is the only plant food that contains all the essential amino acids, trace elements, and vitamins and contains no gluten. Essential amino acids are found in the core of the grain, unlike other cereals, like rice or wheat, in which they are located in the hull. Quinoa is an example of a 'functional food' that aims at lowering the risk of various diseases. Functional properties are given also by minerals, vitamins, fatty acids, and antioxidants that can make a strong contribution to human nutrition and health, particularly to protect cell membranes, with proven good results in brain neuronal functions. Its minerals work as cofactors in antioxidant enzymes, adding higher value to its rich proteins. Quinoa also contains phytohormones, which offer an advantage over other plant foods for human nutrition.

Furthermore, the crop has a remarkable adaptability to different agro-ecological regions. It can grow at relative humidity from 40% to 88% and withstands temperatures from −4 to 38 °C. It is a highly water-efficient plant, is tolerant and resistant to lack of soil moisture, and produces acceptable yields with rainfall of 100–200 mm. There are more than 3000 varieties or ecotypes of quinoa both cultivated and wild, which can be grouped in five basic categories according to the altitudinal gradient: ecotypes from sea level, the valleys, the Yungas, the salt flats and the Altiplano (high plain). Although the main producers are Bolivia, Peru, and the United States, quinoa production is expanding to other continents, and it is currently being cultivated in several countries in Europe and Asia with good yields.

Quinoa, like other Andean grains, has traditional and nontraditional uses as well as value-added industrial innovations generated through research that are now commercially available.

Sources and Production

The quinoa (a dicot plant) is not a true grain, like cereal (monocot) grains; it is rather a fruit, so that it has been called a pseudocereal and even a pseudo-oilseed. This is also because of its unusual composition and exceptional balance between oil, protein, and fat. Quinoa is a drought-tolerant crop that has low water requirements, although yield is significantly affected by irrigation. Quinoa can be seen as an oligocentric species with a broad center of origin and multiple diversification.

As a plant, quinoa grows three to seven feet high and produces a small, flat, circular-shaped seed. It has been considered 'one of the world's most perfect foods.' Compared to other cereal grains, it is higher in protein content (14–18%) and has a nutritionally attractive amino acid balance. The seed is high in lysine, methionine, and cysteine, making it complementary to both other grains and to legumes, which are deficient in these nutrients. It also has higher levels of energy, calcium, phosphorus, iron, fiber, and B vitamins than barley, oats, rice, corn, and wheat.

The seeds have a bitter saponin in the outer coat that must be removed before consumption. They can be washed or milled to remove the seed coat, adding to the total cost of the final product. On average, quinoa yields 5.8% oil by weight.

Quinoa production is in a process of expansion into different geographic areas of the planet due to its extraordinary adaptability (Figure 1). Quinoa is grown mainly in cool mountainous regions because air temperatures above 90–95° cause sterility of the pollen. Although most quinoa is still grown in South America, it is also cultivated in the United States, China, Europe, Canada, and India. It is also cultivated experimentally in Finland and the United Kingdom. Increasing amounts are being exported to the developed world like Europe and the United States. Quinoa has been cultivated since the early 1980s and commercially produced since the mid-1980s in the Colorado Rockies, especially in the San Luis Valley. Production has also been attempted in California, New Mexico, Oregon, and Washington. Until 2008 the production of Bolivia and Peru accounted for 90% of world quinoa production. Following them are the United States, Ecuador, and Canada with about 10% of global production volumes. Quinoa is also cultivated in England, Sweden, Denmark, the

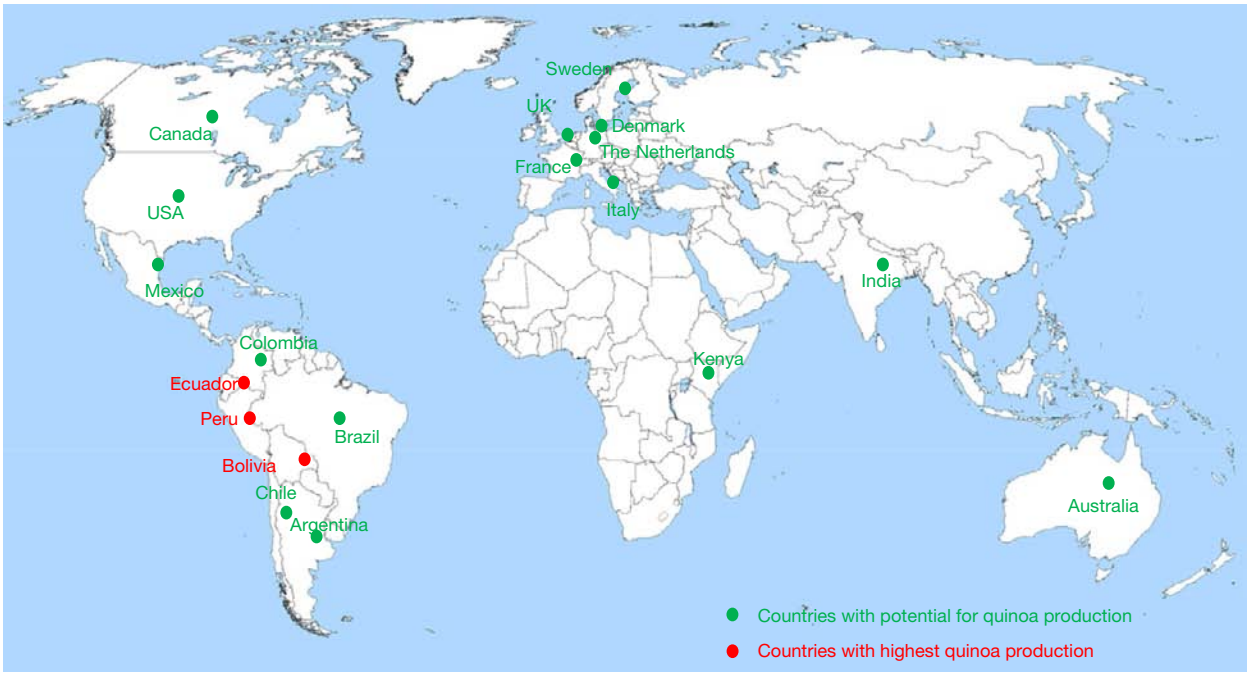

Figure 1 Geographic distribution of quinoa production worldwide. Adapted from FAO (2011). Quinoa: an ancient crop to contribute to world food security. Regional Office for Latin America and the Caribbean. http://www.rlc.fao.org/en/publications/quinoa-ancient-crop-food-security/.

Netherlands, Italy, and France. Recently France has reported an area of 200 ha with yields of 1080 kg ha^{-1}, and Kenya has shown high seed yields (4 ton ha^{-1}). In the Himalayas and the plains of Northern India, the crops can develop successful and with high yields. In tropical areas such as the savannas of Brazil there has been experimental quinoa cultivation since 1987 reportedly obtaining higher yields than in the Andean area. In Europe quinoa was introduced in England in the 1970s, and later research projects focussed on its production for humans and/or as a fodder crop under temperate conditions. Quinoa production has increased in the last 20 years, especially in Bolivia. The main producing countries are Bolivia, Peru, and Ecuador, which in 2007 produced 61 490 tons, up from 19 000 tons in 1973. During 2007 quinoa production was 34 000 tons in Peru, 26 800 tons in Bolivia, and 690 tons in Ecuador. Most quinoa consumed in the United States is imported from South America. Current estimates of US production are minimal, at less than 10 000 pounds. According to the FAO, world production of quinoa in 2012 was 82 510 metric tons (MT) on 102 745 ha. Peru produced 44 210 MT in 2012 on 38 495 ha followed by Bolivia with 37 500 MT on 63 000 ha. The price of quinoa has increased dramatically over the past 10 years due to high demand by the United States and European consumers. Higher prices that help farmers can also adversely affect local Andean consumers. Poor people in Bolivia and Peru have less access to quinoa, a traditional and healthy component of their diet. The higher price might force them to shift consumption to other types of food, as has been reported in Bolivia. Bolivia is the largest exporter of quinoa in the world followed by Peru and Ecuador. In 2009 Bolivia exported a value of over US $43 million. The major importers of Bolivian quinoa grain are the United States (45%), France (16%), the Netherlands (13%), Germany, Canada, Israel, Brazil, and the United Kingdom. In 2007, Peru exported volumes somewhat greater than 400 MT of quinoa grain with values equivalent to US $552 000. In 2008 Ecuador showed similar export levels: 304 MT worth US $557 000.

Value-Added Uses

Quinoa, like other Andean grains, is used in many different ways. It has traditional and nontraditional uses as well as value-added industrial innovations generated through research that are now commercially available (Table 1).

Quinoa can be eaten as a rice replacement, as a hot breakfast cereal, or boiled in water to make infant cereal food. The seeds can even be popped like popcorn. Seeds can be ground and used as flour or sprouted. The sprouts need to get green before they can be added to salads. Quinoa is made into flour for baked goods, breakfast cereals, beer, soups, pastas, desserts, and even livestock feed (Figure 2). Quinoa flour can be mixed with maize or wheat flour. Several levels of quinoa flour substitution have been reported, for instance, in bread (10–13% quinoa flour), noodles and pasta (30–40% quinoa flour), and sweet biscuits (60% quinoa flour), to obtain products of acceptable quality. Quinoa flour can be also drum-dried and extruded, providing products with good physical, sensorial, and nutritional qualities. Solid-state fermentation of quinoa with *Rhizopus oligosporus Saito* provides a good-quality tempeh. The seeds are used boiled like rice or used to thicken soup or as porridge. Quinoa flour was made into noodles. Such a use is, however, complicated due to the bitter taste of the seeds because of their saponin content, which forms a soapy solution in water. The seeds can be boiled, added to soups, or even

Table 1 Traditional, nontraditional, and industrial uses of quinoa

Traditional uses
Quinoa soup: Cooked quinoa with meat or dried meat, tubers, and vegetables
Lawa: A semithick porridge-like preparation with raw flour, water with lime, and animal fat
P'esque: Quinoa grain cooked with water, without salt, served with either milk or grated cheese
Kispiña: Steamed buns of different shapes and sizes
Tacti o tactacho: Fried buns, a kind of doughnut made with flour and llama fat
Mucuna: Steam-cooked balls made from quinoa flour with seasoning in the center
Phiri: Roasted and slightly dampened rough quinoa flour
Phisara: Lightly roasted and cooked quinoa grain
Q'usa: Quinoa chicha, a macerated cold drink
El Ullphu, *Ullphi*: Cold drink prepared with roasted quinoa flour diluted in water with sugar added
Kaswira de quinua: Flattened bread fried in oil, made with katahui (lime) and white quinoa
Kaswira de ajara: Flattened bread fried in oil, made with lime and black quinoa or ajara
K'api kispiña: Steamed bun, made with quinoa ground in a K'ona and cooked in a clay pot
Turucha quispiña o Polonca: Large steamed breads, made with katahui and quinoa
Mululsito quispiña: Steamed bread, made with katahui and quinoa flour, cooked in a clay pot
Quichi quispiña: Steamed and fried bread, made with katahui and quinoa flour, fried in a pan
Juchacha: Andean soup based on ground quinoa and katahui, with roasted barley flour
Chiwa: Young leaves of quinoa used as a vegetable in the preparation of soups and salads
Nontraditional uses
Gluten-free goods, pasta, cakes, tawas, pancakes, doughnuts, juice, api, bread, biscuits, confectionary, chili, and nectar
Industrial and other potential uses
Cosmetics, pharmaceuticals, and other uses

Source: FAO (2011). http://www.rlc.fao.org/en/publications/quinoa-ancient-crop-food-security/.

fermented. The cooked seeds have a nutlike flavor and remain separate, fluffy, and chewy. Quinoa breads and flours are popular products for their gluten-free, high-protein qualities. The plant is sometimes grown as a green vegetable, and leaves are eaten fresh or cooked. The leafy plant materials can be used for human consumption or for livestock feed. Leaves, like the seed, can also be cooked, made into a spinach-like dish, or served raw in a salad. Tonics, puddings, and syrups can also be prepared from the leaves. The foaming qualities of saponin are sometimes used to produce a frothier chicha. In industry, saponins from quinoa have multiple purposes. They are used as soap for washing hair or clothes, in a compound for a fire extinguisher, or in photo processing. Dried stalks of the plant are used as fuel or may be used in preparing bleach or dyes.

Future uses of quinoa can be wide ranging, like textured and fermented products. There are many ways in which it can be consumed: cooked, flour, extruded. Quinoa meat substitute has been introduced in Europe. There are several developments with quinoa flour at a smaller scale, like bread, cookies, muffins, pasta, snacks, drinks, flakes, breakfast cereals, baby foods, beer, diet supplements, and extrudates. Extruded corn grits–quinoa blends that had high-protein quality and solubility and an acceptable sensory evaluation have been obtained. Gluten-free quinoa spaghetti was developed and a product obtained without loss of solids and acceptable weight and volume increase on cooking, whereas the adhesiveness of the cooked product was not very high.

Quinoa flour does not have good baking properties like wheat gluten proteins. The wheat proteins are able to form a viscoelastic network when flour is mixed with water to form dough, and these viscoelastic properties allow the use of wheat to produce bread and other processed foods. The enzyme transglutaminase is promising for developing a protein structure. Use of the enzyme reduced the polypeptides of the low-molecular-weight fractions and the soluble protein fractions when producing pasta. There are some gluten-free products without good baking properties for celiac groups, and quinoa provides an opportunity to develop gluten-free cereal-based products. It has been proven that quinoa can be used to make novel, healthy, extruded, snack-type food products. It produces a good product with maximum expansion, minimum density, a high degree of gelatinization, and a low water solubility index.

Chemical, Nutritional, and Health-Related Aspects of Quinoa

Quinoa seeds are a complete food with high nutritional value due mainly to their high content of good-quality protein. There is an extensive literature on quinoa seeds and flours covering different aspects, including the composition of reserves, chemical characterization of proteins, fatty acid composition of the oils, mineral content, and functional and nutritional values (Table 2).

Biopolymers are found in specific parts of the grain. Starch granules occupy the cells of the perisperm, whereas lipid bodies, protein bodies with globoid crystals of phytin, and proplastids with deposits of phytoferritin are the storage components of the endosperm and embryo tissues. The embryo that surrounds the perisperm is dicotyledonous and is part of the bran fraction of the seed; it is high in proteins and lipids and contains most of the ash, fiber, and saponins. The shape of quinoa seed is similar to a flattened sphere; their mean equivalent diameter varies from 1.4 to 1.6 mm. Carbohydrates, proteins, and lipids are the main components of the seeds, and they are mostly responsible for the functional properties that have made them new ingredients in the development of new products.

Proteins

The mean protein content reported in the literature for quinoa seeds is 12–23%. Compared to cereal grains, the total protein content of quinoa seed (16.3% dry basis, db) is higher than that of barley (11% db), rice (7.5% db), or corn (13.4% db) and is comparable to that of wheat (15.4% db). Quinoa contains relatively minor proteins compared to legume seeds. Relative to cereal grains, quinoa proteins are particularly high

Figure 2 Assorted quinoa-based products (a) and loaves, slices, and magnified crumb grain of breads made of wheat:rye (50:50, w:w) (left) and wheat:rye:quinoa (50:50:5, w:w:w) (right) flours (b).

Table 2 Chemical, functional, and nutritional composition of flours from wheat (*Triticum aestivum*) and quinoa (*Chenopodium quinoa* Willd.) (mean of three replicates ± SD)

Parameter	*Quinua*	*Wheat*
Moisture (g/100 g flour, as is)	12.05 ± 0.36^a	14.32 ± 0.32^b
Fat (g/100 g flour, as is)	3.44 ± 0.19^b	1.34 ± 0.09^a
Ash (g/100 g flour, as is)	2.10 ± 0.10^b	0.54 ± 0.06^a
Protein (g/100 g flour, as is)	11.32 ± 0.16^a	12.11 ± 0.21^b
Insoluble fiber (g/100 g flour, as is)	9.13 ± 0.96^b	1.28 ± 0.09^a
Soluble fiber (g/100 g flour, as is)	5.37 ± 1.03^b	0.91 ± 0.09^a
Total dietary fiber (g/100 g flour, as is)	14.5 ± 0.98^b	2.19 ± 0.09^a
*Digestible carbohydrates (g/100 g flour, as is)	57	70
Water holding capacity 3000 rpm (g water/g flour)	1.57 ± 0.09^b	0.84 ± 0.06^a
Water holding capacity 5000 rpm (g water/g flour)	1.16 ± 0.06^b	0.61 ± 0.09^a
Fat adsorption capacity (g/g)	1.2 ± 0.10^a	1.38 ± 0.12^a
Foam capacity (ml)	3 ± 1^a	14 ± 2^b
Foam stability 30' (%)	100 ± 7	36 ± 2^a
Foam stability 60' (%)	100 ± 4^b	36 ± 3^a

Within each row of single flour samples, values with different letters differ significantly from each other ($p < 0.05$).

*Calculated by difference.

Source: Collar, C. and Angioloni, A. (2014). Pseudocereals and teff in complex breadmaking matrices: impact of lipid dynamics on the bread functional and nutritional profiles. *Journal of Cereal Science* **59**, 145–154, with permission from Elsevier.

in lysine, the limiting amino acid in most cereal grains. Their essential amino acid balance is excellent because of a wider amino acid range than in cereals and legumes, with higher lysine (5.1–6.4%) and methionine (0.4–1%) contents. Quinoa proteins have higher histidine content than barley, soy, or wheat proteins, whereas the methionine and cysteine content of quinoa is adequate for children (2–12 years old) and adults, it is similar to that of barley and soy, and lower than the amounts in wheat. According to the FAO/World Health Organization (WHO), suggested requirements for 10-year-old children, quinoa proteins have adequate levels of aromatic amino acids (phenylalanine and tyrosine) and similarly in histidine, isoleucine, threonine, phenylalanine, tyrosine, and valine contents. By comparison, lysine and leucine in quinoa seeds are limiting amino acids for 2- to 5-year-old infants or children, whereas all the essential amino acids of this protein are sufficient according to FAO/WHO suggested requirements for 10- to 12-year-old children. In comparison,100 g of quinoa contains nearly fivefold lysine; more than double isoleucine, methionine, phenylalanine, threonine, and valine; and much larger amounts of leucine (all essential amino acids along with tryptophan) than 100 g of wheat. In addition, it also exceeds wheat, in some cases by triple, in the amounts of histidine, arginine, alanine, and glycine as well as containing amino acids not present in wheat such as proline, aspartic acid, glutamic acid, cysteine, serine, and tyrosine (all nonessential amino acids).

Bioavailability of lysine in quinoa is very high, whereas in wheat, rice, oats, millet, and sesame it is significantly lower. Quinoa also contains interesting amounts of phenylalanine, threonine, and tryptophan. Other amino acids present in quinoa but uncommon in other cereals are aspartic acid, glutamic acid, cysteine, serine, and tyrosine.

The protein digestibility or bioavailability (true digestibility) of quinoa's amino acids varies depending on the variety and the treatment they are subjected to. Comparative studies carried out by FAO/WHO using the balance method in rats ranked the values of true digestibility of protein in three ranges: high digestibility from 93% to 100% for animal foods and soy protein isolate; intermediate digestibility values of 86–92% for polished rice, whole wheat, oatmeal, and soy flour; and low digestibility of 70–85% for corn and different types of pulses including beans and lentils. According to this classification, quinoa grain is in third position, that is, with low digestibility.

Active biopeptides have been obtained from quinoa by enzymatic hydrolysis, and it has been suggested that short-chain peptides are more active than long-chain peptides. Low-molecular-weight peptides possess higher potential than high-molecular-weight peptides as antihypertensive agents or as compounds that reduce the amount of free radicals.

Fats

Quinoa contains from 2% to 10% fat. The study of 555 accessions of the Bolivian quinoa collection found that the fat content ranged from 2.05% to 10.88% with an average of 6.39%. Quinoa's fat content is very valuable due to its high proportion of unsaturated fatty acids. These values from quinoa germplasm are expected in the extraction of fine vegetable oils for culinary and cosmetic uses. The relatively high amount of oil in quinoa, a very understudied aspect of the crop, makes it a potential source for oil extraction. Studies carried out in Peru to determine the fatty acid content of quinoa found that the highest percentage of fatty acid in this oil is omega-6 (linoleic acid), 50.24%, very similar to the values found in corn germ oil, which has a range of 45–65%. Omega-9 (oleic acid) is second in quantity, accounting for 26.04% of quinoa oil. The values found for omega-3 (linolenic acid) are 4.77%, and palmitic acid accounted for 9.59%. In addition a small proportion of fatty acids such as stearic acid and eicosapentaenoic acid were also found. The composition of these fatty acids is very similar to corn germ oil. Quinoa helps reduce low-density lipoprotein cholesterol in the body and raise high-density lipoprotein cholesterol due to its omega-3 and omega-6 content. In some cases, 82.71% of fatty acids in quinoa oil are unsaturated.

Carbohydrates

The carbohydrate (by difference, db) content of quinoa sees is comparable to that of barley and rice. Starch is the major component of quinoa carbohydrates, and it is present between 32% and 69.2%. Besides, total dietary fiber of quinoa is near that of cereals (7–9.7% db), and the soluble fiber content is reported between 1.3% and 6.1% db. There is about 3% of simple sugars, mostly maltose, followed by D-galactose and D-ribose, and also fructose and glucose.

Carbohydrates play a basic nutritional function, and they may have different physiological health effects, such as provision of energy, effects on satiety/gastric emptying, control of blood glucose and insulin metabolism, protein glycosylation, and cholesterol and triglyceride. Carbohydrates from quinoa can be considered a nutraceutical food because they have beneficial hypoglycemic effects and induce lowering of free fatty acids. Studies made in individuals with celiac disease showed that the glycemic index of quinoa was slightly lower than that of gluten-free pasta and bread. Besides, quinoa induced lower free fatty acid levels than gluten-free pasta and significantly lower triglyceride concentrations compared to gluten-free bread. *In vitro* digestibility of raw quinoa starch was reported at 22%, whereas that of autoclaved, cooked, and drum-dried samples was 32%, 45%, and 73%, respectively. Saponins did not affect the digestibility of the starch. The total dietary fiber content in quinoa flour is affected by thermal treatment, whereas the insoluble dietary fiber fraction does not change with heat treatment.

Fiber accounts for 6% of the grains' total weight, and quinoa intake therefore promotes intestinal transit, regulates cholesterol, stimulates the development of beneficial bacterial flora, and helps prevent colon cancer. It has a high percentage of total dietary fiber, which makes it an ideal food for helping to eliminate toxins and waste products that can damage the body. It therefore acts as a purifier of the body. It produces a feeling of fullness. Grains in general, and quinoa in particular, have the ability to absorb water and stay longer in the stomach, and in this way fullness is achieved with a low volume of food.

Minerals

If a comparison is made between wheat, corn, rice, barley, oats, rye, triticale, and quinoa, quinoa stands out for its high content

of calcium, magnesium, and zinc. Quinoa is a food rich in calcium, which is easily absorbable by the body (containing more than four times that of corn, almost triple that of rice, and much more than wheat), and quinoa can therefore help to prevent decalcification and osteoporosis. Calcium is responsible for many structural functions of hard and soft tissues of the body as well as the regulation of neuromuscular transmission of chemical and electrical stimuli, cell secretion, and blood clotting. Thus calcium is an essential component of the diet. The recommended daily intake for calcium is 400 mg day^{-1} for children 6–12 months to 1300 mg day^{-1} for adults and is covered with an average consumption of food from 800 to 1000 mg day^{-1}. Quinoa provides 114–228 mg day^{-1}, with a calculated average of 104 mg/100 g of edible portion. The calcium content of quinoa is between 46 and 340 mg/100 g of dry matter. Quinoa contains three times more than wheat and five times more than rice, whereas corn lacks this mineral completely. It contains twice the potassium amount of wheat, four times more than corn, and eight times more than rice. Magnesium is also in much higher amounts than in the other three grains. An adult male of 70 kg contains about 20–28 g of magnesium and the recommended daily intake is about 300–350 mg day^{-1} for adults. Quinoa contains 270 mg/100 g of dry matter. Figures ranging from 170 to 230 mg/100 g of dry matter are presented. Magnesium is a component and activator of many enzymes, especially those that convert energy-rich phosphates. It is also a stabilizer of nucleic acids and membranes. Quinoa almost doubles the amount of zinc found in wheat and is four times that of corn, whereas rice does not contain this mineral. The zinc content of an adult male weighing 70 kg is 2–4 g. Zinc acts on the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids. If the body can make use of 20% of the zinc available in food, recommended intakes are 8.3 mg day^{-1} (children under 1 year), 8.4 and 11.3 mg day^{-1} (preschool and schoolchildren), 15.5 and 19.5 mg day^{-1} (adolescents), and 14 mg day^{-1} (adults). Therefore, an intake of between 6 and 20 mg day^{-1} is sufficient, and in this sense, quinoa provides 4.8 mg/100 g of dry matter.

Vitamins

The vitamin content (mg/100 g) of quinoa seeds is also valuable because quinoa seeds have high levels of vitamin B_6 (0.487) and total folate (0.184), whose amounts in 100 g can cover the requirements of children and adults. The riboflavin content in 100 g (0.30–0.32) contributes 80% of the daily needs of children and 40% of those of adults. The niacin content (1.24–1.52) does not cover the daily needs, but is beneficial in the diet. Thiamin values in quinoa (0.29–0.36) are lower than those in oats (0.763), but those of niacin, riboflavin, vitamin B_6, and total folate are higher than in oats and barley.

Other Bioactive Components in Quinoa

Recently, much attention has been given to naturally occurring antioxidants, which may play an important role in inhibiting both free radicals and oxidative chain reactions within tissues and membranes. Pseudocereal seeds and sprouts show relatively high antioxidant activity. The antioxidant activity of various extracts from quinoa (Japan) and from its relative Amaranthus were foundto be different.. Quinoa presents higher antioxidant activity than amaranth using different methodologies: ferric reducing/antioxidant potential, 2,2-azinobis(3-ethylbenzothiazoline 6-sulfonate) and 2,2-diphenyl-2-picryl-hydrazyl. Antioxidant activity of quinoa might be of particular interest to medical researchers and needs further attention regarding its utilization as a natural potent antioxidant. Quinoa seeds from different origins show different isoflavone concentrations, particularly daidzein and genistein. These hormones are implicated in plant physiology (protection from pathogens, from UV light and nitrogen-limited soils) and could be recognized by alpha- and beta-receptors of estrogens in humans. These endoplasmic reticulum-linked receptors are implicated as inhibitors of tyrosine kinase enzymes and as antagonists of vessel contraction. They also reduce arterial resistance, benefit bone density, and stimulate osteoprogerin secretion by osteoblasts, in addition to their antioxidant properties. Saponins are strongly bitter-tasting, surface active compounds with a structure consisting of a steroid or triterpenoid aglycone and one or more sugar chains, which are present in quinoa seeds at significant levels. Saponin levels in different quinoa varieties vary within the range 0.01–4.65%. Due to the bitter taste imparted by saponins, quinoa seeds are normally processed by means of abrasive dehulling or water extraction to remove the outer layers of the seeds where saponins are concentrated, which in turn causes a reduction in the vitamin and mineral content. Although traditionally considered as antinutrients due to their mineral binding activity, recent research has shown that saponins may have anticarcinogenic and cholesterol-lowering properties.

Functional Properties of Quinoa

Some functional properties of quinoa flour have been described, mainly solubility, water-holding capacity, gelation, foaming, and emulsifying capacity (Table 2). Solubility is related to the hydrophilic–hydrophobic balance of the proteins and the thermodynamics of its interaction with the solvent. It has been found to have solubility values of about 15–52%, corresponding to minimum solubility at pH 6 and maximum at pH 10. The solubility values of quinoa flour in the acid pH region imply that the protein may be useful in the formulation of beverages, dehydrated soups and sauces, and low-acid foods. Quinoa flour may not be a good gel-forming agent. However, it was observed that the addition of different salts at low concentration (0.5%) improved the gel-forming property of quinoa, and this effect was better with KCl. The other functional properties measured in quinoa flour are those related to surface tension, like foaming and emulsifying capacity. Quinoa has a low-foaming capacity and stability, and salt addition may improve this property, but high concentrations around 10% are not useful for human consumption. Water and oil absorption are good, enhancing the potential of quinoa seeds in human food and formulations like beverages, sauces, desserts, and sausages.

Conclusions

The outstanding physicochemical, nutritional, and functional properties of quinoa have been emphasized and currently rediscovered by modern scientific approaches. Its uses account for traditional and nontraditional food and industrial applications. The need for intensive cultivation of quinoa should be stressed and encouraged, as it could meet the quality and quantity requirements by the food industry. Quinoa could play a key role in the nutritional behavior of either developing countries or the developed world. In the Andean countries, quinoa crops could play an important role in their economies in the future, giving a new export market, as well as in national subsistence. In addition, quinoa could be a strategic crop used to complement the diet in rural/marginal regions where energy–protein malnutrition affects most of the population of the developing countries. Quinoa, as the 'mother grain,' represents an exotic and healthy rediscovery (gluten free) in the developed world. Bitter seed coat saponins, although probably giving slower speed to quinoa recovery, might now be helpful for its 'take-off' among farmers for a broader range of consumers, as such saponins also have important agropharmacological and cosmetic industrial uses. From the point of view of vegetarian consumers, quinoa in combination with other cereals might easily replace meat, with a great future in modern, conscient, and more ecological food habits. Functional properties given by strongly active compounds like minerals, vitamins, fatty acids, and antioxidants make of this small and noble grain a strong contribution to human nutrition, particularly for all cell processes requiring antioxidant protection of membranes, like neuronal activity, with minerals and amino acid contents with potential implications for aiding memory and lowering anxiety under stressful conditions.

See also: Amaranth; Cereals: Dietary Importance; Ethnic Foods.

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Rapeseed Oil/Canola

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Introduction

Canola and rapeseed belong to one of the most widespread and diverse cultivated plants known as the *Brassica* genus. The development of canola from rapeseed originated in Canada and is often referred to as *Canada's Cinderella* crop. Prior to World War II, rapeseed was totally unknown to Canada as well as North America. The first recorded planting of rapeseed in Canada occurred in Saskatchewan during the 1930s by an immigrant farmer from Poland, Fred Solvoniuk, with seeds he received from a friend back in Poland. The seeds were from *Brassica campestris* (now known as *Brassica rapa*) and adapted well to the soil condition in western Canada. The oil from rapeseed proved invaluable during World War II as it was an excellent lubricating oil for marine engines. No other oil adhered to the metal surfaces of the marine engines under the extreme heat and steam conditions better than rapeseed oil. Because of the blockade of Europe and Asian sources of rapeseed oil, a high priority was given by the Canadian government for the commercial production of rapeseed in Canada. In order to meet the desperate shortage of rapeseed oil, rapeseed was also imported from Argentina of the type *Brassica napus*. After the conclusion of the war and with the conversion of ships to diesel engines, the demand for rapeseed oil decreased precipitously. Possible alternative food uses were sought for rapeseed oil. However, these proved unsuitable because of the oils' green color, sharp taste, and very high erucic acid content. Nothing was done until the late 1950s when several plant breeders in Manitoba and Saskatchewan conducted groundbreaking work on rapeseed that would change the landscape of Canadian agriculture in the prairies. This article will discuss the development of canola oil, its composition, processing, and functional and health properties that make it the second most important oilseed crop in the world.

Conversion of Rapeseed to Canola

Rapeseed suffered from two basic problems that limited its use for human or animal consumption. Firstly, the oil was very high in erucic acid, a C22:1 fatty acid that was found to cause heart lesions in several animal models. Secondly, the meal was high in glucosinolates, which limited its utilization for animal feed. Consequently, there was a need to drastically reduce the level of erucic acid level in rapeseed oil (which ranged from 40% to 70% of the total fatty acids) and glucosinolates in the meal. This challenge was undertaken by the outstanding work of two Canadian plant breeders, Dr. Baldur S. Stefansson and Dr. R. Keith Downey, who both developed rapeseed varieties that were low in erucic acid and glucosinolates. This resulted in the Canadian government placing a maximum of 5% erucic acid in rapeseed oils for use in foods. The permitted level for erucic acid was further reduced to 2% in both Canada and the United States in 1985. The stellar work by both these breeders earned them the title of 'fathers of canola,' which led to the development of a multibillion dollar canola oilseed industry with hundreds of thousands of jobs created in Canada and around the world. Not to be forgotten is the important contribution of Dr. Frithjof Hougen, a professor at the University of Manitoba, whose analysis of the canola varieties and crosses for erucic acid using gas chromatography (GC), a Nobel-winning development in science that occurred at this time, made the achievements by Dr. Stefansson possible. A key discovery in the development of canola was an important discovery by Dr. Downey and his graduate student that genotype of the developing embryo controlled erucic acid rather than the maternal plant. This meant that seeds from the same plant could actually differ in erucic acid content. The development of low-erucic-acid varieties was accelerated using the 'half-seed method' to measure the erucic acid content. A very low-erucic-acid sample was finally identified in *B. campestris* by Dr. Downey and grown into a new plant using the half-seed technique. This resulted in the release of the first low-erucic-acid variety of *B. campestris* in 1971. Attention was then focused on reducing the glucosinolate content in the seed. Using a new GC method for measuring glucosinolates, Dr. Stefansson developed the first zero-erucic acid, low-glucosinolate *B. napus*, Tower. This was followed by the release of a corresponding zero-erucic acid, low-glucosinolate *B. campestris* variety, Candle, which was far better suited to northern growing conditions than Tower.

The successful development of double-low rapeseed varieties from *B. napus* and *B. campestris* necessitated their differentiation from the high-erucic-acid and high-glucosinolate rapeseed varieties. In 1978, the Western Canadian Oil Crushers' Association trademarked the new double-low rapeseed varieties as canola, a contraction of Canada and ola, meaning oil, which was later transferred to the Canola Council of Canada (previously known as the Rapeseed Association of Canada). The official definition for canola is the seed from *B. napus* (L.) or *B. rapa* (L) that

produces an oil that contains less than 2% erucic acid, and solid component of the seed must contain less than 30 μmol of any of a mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate, and 2-hydroxy-4-pentenyl glucosinolate per gram of air-dried, oil-free solid.

Since rapeseed was not a traditional food of the United States, canola had to convince the US Food and Drug Administration (FDA) that it was safe for humans. Luckily, the large number of nutritional studies conducted in Canada and other countries combined with the endorsement by the American Heart Association of canola oil, due to it being very low in saturated fatty acids (SFAs), led to the successful granting of generally regarded as safe status by the FDA in 1985. In Europe, it took longer to convert the high-erucic-acid rapeseed varieties to the low-erucic-acid varieties, which are now referred to as low-erucic-acid rapeseed or LEAR.

After palm and soybean oil, canola oil is now the third-largest oilseed crop worldwide with Europe accounting for 63% of world production and Canada 31%.

Composition of Canola Oil

Fatty Acids and Triacylglycerols

Canola oil comprises around 44% of the total seed and is composed mainly of triacylglycerols (TAGs), which account for 94–99% of the total oil. Table 1 illustrates the fatty acid changes occurring when the high level of erucic acid (C22:1) in rapeseed was reduced in canola oil and replaced by substantial increases in levels of C18 fatty acids. The most notable difference was a substantial increase in oleic acid (C18:1) from 14.8% in rapeseed oil to 61.6% in canola oil followed by linoleic acid (C18:2), which almost doubled to 27.7% in canola oil. Of particular note was the very low level of SFAs (7%) in canola oil, which made it quite unique among the other edible oils. Canola oil was also very high in linolenic acid (Ln,C18:3, 9.6%) making it similar to soybean oil, although the latter has much higher SFAs (14.9%) and linoleic acid (L,53.3%) and much lower oleic acid (O, 23.8%).

Table 1 Fatty acid composition of canola oil (w/w%)

Fatty acid	*Canola oil*	*Rapeseed*
C14:0	0.1	–
C16:0	3.6	4.0
C18:0	1.5	1.0
C20:0	0.6	0.6
C22:0	0.3	1.0
C24:0	0.2	0.3
Saturated	*6.3*	*7.1*
C16:1	0.2	0.3
C18:1	61.6	14.8
C20:1	1.4	10.1
C22:1	*0.2*	*45.1*
Monounsaturated	*62.4*	*69.7*
C18:2n−6	27.7	14.1
C18:3n−3	9.6	9.1
C18:3n−6		*1.0*
Polyunsaturated	*31.3*	*23.2*

Source: Adapted from Przybylski and Eskin (2011).

The marked reduction in erucic acid (C22:1) in canola oil resulted in substantial changes in the types and location of fatty acids in the TAG molecules. TAGs are esters of glycerol with three molecules of fatty acids, with each fatty acid located at one of the three positions in the molecule referred to as *sn*−1, *sn*−2, and *sn*−3. The higher amounts of linoleic acid in canola oil replaced erucic acid in the *sn*−1,3 positions in the TAGs. Regular canola oil was also reported to be composed of five main TAG molecules ranging in decreasing order from triolein (OOO), linoleo-diolein (LOO), linoleno-diolein (LnOO), dilinoleo-olein (LnLnO), and linoleno-linoleo-olein (LnLO).

As will be discussed in the processing section, the commercial processing of canola oil is designed to remove all those minor components that have a detrimental effect on its stability. These include free fatty acids, phospholipids, chlorophyll, oxidized products, and trace metals. Thus, top-quality commercially refined canola oil is composed mostly of triacylglycerols (96–99%) with less than 0.1% free fatty acids. The main minor components naturally in canola oil are tocopherols, as these serve as very efficient antioxidants and are usually related to the content of unsaturated fatty acids, particularly linoleic and linolenic acids. There are four isomers of tocopherols, with the greatest degree of antioxidant activity following the order $\delta > \gamma - > \beta > \alpha$. The main ones in canola oil are γ- and α-tocopherols. Other minor components include phytosterols as well as the pigments, chlorophyll, and carotenoids.

Canola oil processing

The processing of canola oil is similar to most edible oil processing operations and is designed to remove deleterious minor components. The ultimate goal is to produce as pure a solution of TAGs as possible, which is essentially what is in the bottle of vegetable oil purchased in the supermarket. Each source of edible oil, including canola oil, is unique and requires some process modifications as one process does not fit all oilseeds. These differences are often subtle but extremely important for the particular type of oil processed.

Flaking, Cooking, and Pressing

The mature and cleaned canola seeds are very small ranging from 2 to 3 mm in diameter. In order to release oil, the cell wall must first be broken down by passing through a set of rollers. Further treatment with rollers continues rupturing of the cells, which allows the oil to be released and coalesce into larger particles. At this stage, the changes on surface of the flaked seed are minor as they still appear fairly dry. The canola flakes are then cooked and held at 85–95 °C for a short period of time to inactivate the enzyme myrosinase and prevent it from hydrolyzing glucosinolates. Some of the hydrolyzed products may contain sulfur, which could have a deleterious effect on the flavor and odor of finished canola oil. Following cooking, the canola flakes are transferred to a screw press. This applies pressure to the canola flakes and removes 60–70% of the total oil leaving behind a press cake.

Solvent Extraction

The residual press cake, containing the remaining oil (39–40%), must be subjected to solvent extraction with *n*-hexane. Concerns to minimize the use of n-hexane have led to using alternative

solvents such a mixture of 2-methylpentane, 3-methylpentane, hexane, and a small amount of other solvents sometimes referred to as 'isohexane.' Countercurrent solvent extraction of the press cake will remove most of the residual oil leaving the cake with as much as 25–35% solvent. Total removal of this solvent is achieved by then subjecting the press cake to countercurrent steam flows in a desolventizer toaster vessel to make the cake meal solvent-free for use for feed.

Degumming

The solvent extracted oil is then mixed with the screw-pressed oil. The combined oil, known as the crude oil fraction, contains phospholipids. Because phospholipids are gums that are hydrophilic, that is, water-soluble, so they can be treated with steam by degumming. This process effectively removes the phospholipids, which can be then added back to the meal or press cake. If mineral salts, particularly calcium and magnesium ions, are extracted with the oil during the extraction and pressing processes, they can make the phospholipids nonhydratable. A super-degumming process using a small amount of citric acid (1500–2500 ppm) at 50–65 °C is available in canola plants to chelate the nonhydratable phospholipids so that they can now be removed from the crude oil along with the hydratable phospholipids. Another reason for removing most of the phospholipids is that they are very high in polyunsaturated fatty acids (PUFAs) and would oxidize readily causing the oil to become rancid.

Canola Oil Refining

The crude-degummed oil now undergoes refining to remove free fatty acids, any phospholipids still remaining, color bodies, iron and copper, and in the case of canola oil also any sulfur compounds. In the traditional alkali-refining process, there are at least five major steps.

Acid Pretreatment

The first step involves heating crude-degummed canola oil to 50–60 °C while at the same time injecting 300–2000 ppm phosphoric acid into the oil followed by mixing vigorously. Once the nonhydratable phospholipids are acidified, they can then be separated. In addition to phospholipids, chlorophyll as well as prooxidants iron and copper are also removed.

Neutralization

The free fatty acid content of the oil as well as the amount of phosphoric acid added will determine the amount of sodium hydroxide added to neutralize the oil. The level of sodium hydroxide added will vary between 8% and 12%. To ensure complete saponification of these fatty acids, however, further alkali may be required. The overall result is that some triacylglycerols are hydrolyzed by sodium hydroxide to produce glycerol and fatty acid salts or soaps.

Soapstock Removal

Further reduction of the soapstock from the oil is achieved by washing with water at 85–95 °C. Citric or phosphoric acids may also be added to the remaining oil to remove any traces of soapstock.

Drying Oil

This is achieved by agitating the oil at 105 °C until the oil is dried.

Physical Refining

An alternative to alkali refining is to use steam distillation directly to remove free fatty acids from canola oil. This process, known as physical refining, is carried out on canola oil after it is acid-degummed and bleached in a specially designed deodorizer. It is more environmental-friendly than alkali refining by avoiding the production of soap and the disposal problems associated with the latter.

Bleaching

Once the canola oil is refined and dried, it then undergoes bleaching. The bleaching process is primarily geared to reducing and removing chlorophyll from canola oil. Acid-activated bleaching absorbent or bleaching clay is used to absorb chlorophyll as well as any phosphatides still remaining after degumming, color pigments, metal ions, magnesium, calcium, iron, and any oxidized fatty acids such as peroxides. Of these minor components, chlorophyll reduction is the primary goal as unless removed it can act as a prooxidant making the oil rancid. Consequently, the level of chlorophyll must be reduced to less than 30 ppb (parts per billion).

Deodorization

Until fairly recently, hydrogenation was regularly carried out after bleaching to reduce the PUFAs and enhance the oxidative stability of canola. This practice has since been discontinued due to the formation of *trans*-fatty acids, which have been shown to be deleterious to human health. Consequently, following bleaching, the oil is now subjected to steam distillation at very high temperatures (240–270 °C) under vacuum (3–8 mmHg). Under these deodorization conditions, any free fatty acids or off-flavor products are completely removed from the oil. The final product is a bland oil to which citric acid (0.005–0.01%) is added to chelate any residual trace metals to prevent oxidation.

Cold-Pressed Canola Oil

In Europe, there is an increasing niche market in virgin cold-pressed canola oil. It simply involves extracting the oil by screw pressing. There is no further treatment of the oil, so it is important to select top-quality canola seeds. Cold pressing is normally

carried out in small- and medium-sized plants, and the oil yields obtained are much lower at around 75–85% of the oil produced from the traditional oil-refining plants. Once the oil is collected, it is cleaned, by removing any particles of seed material, and then carefully stored to minimize loss of quality. Virgin cold-pressed canola oil represents a small but expanding niche market, particularly in Germany, and has slowly been developing in North America for the health food market.

Functional Properties

Canola oil accounts for 50% of all edible oils consumed in Canada. Around 65% of canola oil produced in Canada is exported for use in salad oils and dressings, soft and hard margarines, and baking shortenings. It is a natural salad that generally remains clear at refrigerated temperatures.

The high level of linolenic acid (C18:3) in canola makes it very susceptible to oxidation. Generally, the more double bonds a fatty acid has, the greater is the rate of oxidation. For example, linoleic acid (C18:2) with 2 double bonds oxidizes 25 times faster than oleic acid (C18:1) with one double bond. In the case of the more PUFA, linolenic acid (C18:3) with three double bonds, it oxidizes twice as fast as linoleic acid. Consequently, linolenic acid oils, such as canola and soybean oils, were generally subjected to light hydrogenation that added hydrogen to saturate the double bonds and reduce the level of linolenic acid. The overall effect was to enhance the shelf life and frying life of the oil by preventing oxidative rancidity. Unfortunately, this process, in addition to saturating some of the natural *cis*-double bonds, also resulted in the formation of *trans*-fatty acids. The *trans*-double bond behaved like a saturated bond so that the oil became solid by a combination of newly saturated and newly *trans*-double bonds. The recognition of the deleterious effects of *trans*-fatty acids has essentially removed hydrogenation as a safe process for edible products. An alternative to hydrogenation is to develop tools that will block the formation of linolenic acid and produce canola oil with much lower levels of this PUFA. This resulted in the release of a more stable canola oil, high-oleic-acid canola oil, in 2012. This oil is used extensively in commercial deep frying by food service establishments in North America and has excellent stability. While oleic acid accounts 80% of the total fatty acids, linoleic and linolenic acids account for around 23–27% and 3% for linoleic and linolenic fatty acids in high-oleic-acid canola oil, respectively. In addition to its enhanced frying stability, it is also used extensively as an ingredient in foods.

Health Aspects

The fatty acid composition of canola oil makes it quite unique nutritionally (Table 1). It has very low levels of SFAs, very high amount of monounsaturated fatty acids (MUFAs), and significant levels of the PUFAs, linoleic and linolenic acids, as well as substantial amounts of tocopherols (700–1200 ppm) and phytosterols (0.53–0.97%). This combination has made canola oil extremely healthy because of these cardioprotective substances. Early understanding of the cardioprotective effects of fatty acids was based on the P/S ratio, the ratio of PUFAs (P)/SFAs (S), which suggested you need twice as much P to S to prevent your cholesterol levels from increasing. This ratio proved too limited and oversimplified as it ignored the fact that not all SFAs raised cholesterol and did not take into account the role of the MUFA, oleic acid. This view changed drastically when it was shown that MUFAs were as effective as PUFAs in lowering blood total and low-density (LDL) cholesterol and that they contributed to cardiovascular health in contrast to some of the SFAs. Thus, MUFAs were subsequently shown to exert cardiovascular benefits by regulating plasma lipids and lipoproteins, the susceptibility of LDL lipoproteins to oxidation, and insulin sensitivity. The combined benefits of these canola oil components on the biomarkers of cardiovascular health (total cholesterol and LDL cholesterol) resulted in the following qualified claim for canola oil authorized by the US Food and Drug Administration in 2006: "Limited and not conclusive scientific evidence suggests that eating about 1½ tablespoons (19 gram) of canola oil daily may reduce the risk of coronary heart disease due to the unsaturated fat content in canola oil. To achieve this possible benefit, canola oil is to replace a similar amount of saturated fat and not increase the total number of calories you eat in a day."

A recent study by Jenkins and coworkers in Canada examined the combination of linolenic acid, MUFA in canola oil as part of a low-glycemic-index diet on participants with type 2 diabetes. The test diet, 4.5 slices of canola oil-enriched whole wheat bread, was provided as a supplement and contributed 31 g canola oil or 14% of the total dietary calories of a 2000 cal diet. The control diet supplement was 7.5 slices without canola oil. The canola oil-enriched low-glycemic-index diet improved glycemic control, particularly for those with high systolic blood pressure. Overall, a greater reduction in the Framingham CVD risk score (FRS) was reported for the test diet. FRS is based on a long-term and ongoing cardiovascular study of 5209 adult residents in Framingham, Massachusetts, started in 1948 and now in its third generation of participants. It is a gender-specific algorithm for estimating the 10-year cardiovascular risk for an individual based on total cholesterol, HDL cholesterol, smoking, and systolic blood pressure.

DHA-Enriched High-Oleic-Acid Canola Oil

Plant breeders have been developing new varieties of canola, such as low linolenic acid and high oleic acid, that improve oxidative stability. Because of the increase in metabolic syndrome (a cluster of conditions that increase the risk of heart disease, stroke, and diabetes) and the prevalence of coronary heart disease, breeders have focused their attention on developing canola oil varieties with unique fatty acids such docosahexaenoic acid (DHA), an invaluable $n-3$ fatty acid with important cardiovascular benefits. The deficiency of DHA in North American diets led to a recent multicenter randomized controlled human clinical trial conducted in Canada and the United States using a novel DHA-enriched high-oleic-acid canola oil. Consumption of the DHA-enriched high-oleic-acid canola oil was found to improve HDL cholesterol (the good cholesterol), triglycerides, and systolic blood pressure and reduce FRS compared to other oils with varied unsaturated fatty acid composition.

Conclusion

The conversion of high-erucic-acid rapeseed oil to canola oil had a dramatic effect on the economics of Canadian agriculture. It not only made Canada a major producer of oilseed for the world market but also provided employment for hundreds of thousands of people both in Canada and around the world. The oil also had a unique fatty acid profile that made it one of the healthiest oils by lowering cholesterol and improving heart health. New breeding developments introducing new varieties of canola with even lower levels of SFAs as well as the presence of important fatty acids such as gamma linolenic acid and DHA that may further improve cardiovascular health.

See also: Bread: Types of Bread; Functional Foods; Soybean Oil; Triacylglycerols: Structures and Properties; Vegetable Oils: Composition and Analysis; Vegetable Oils: Oil Production and Processing; Vegetable Oils: Types and Properties.

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Relevant Website

www.canolacouncil.org – Canola Council of Canada.

Raspberries and Related Fruits

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Introduction

Raspberries are an aggregate fruit commonly grown and consumed throughout the Northern Hemisphere and, as members of the rose family, are closely related to blackberries and other brambles or caneberries. Although many species and types of raspberries exist, red and black are the most common. Here, we discuss the origins and traditional uses of raspberries, raspberry biology, specific raspberry species and varieties, raspberry production, raspberry nutrient composition, and the potential effects of raspberries on health.

Raspberry Origins and Traditional Uses

Raspberries belong to the rose family (Rosaceae) and the genus *Rubus*, which also includes other brambles or caneberries, such as blackberries, dewberries, and boysenberries. It is thought that the scientific name for red raspberries (*Rubus idaeus*) came from Mount Ida in Turkey because of the myth that Greek gods went there in search of berries and came back with raspberries. There is a belief that Romans cultivated raspberries in the fourth century and that raspberries appeared in gardens across Europe by the 1500s. Throughout their long history of consumption, various parts of the plant have been used not only as food but also as medicine. The raspberry fruit and raspberry leaves and blossoms have been used for medicinal purposes for many centuries. Raspberry leaves have been used traditionally to treat gastrointestinal disorders, respiratory disorders, heart problems, the flu, fever, and diabetes and to treat morning sickness and to aid with labor. Raspberry leaves were also traditionally used to promote sweating, urination, and bile production. Red raspberry leaves have been applied directly to treat sore throat symptoms and skin rashes. Raspberry blossoms were used to make eye ointments or to treat stomach ailments. Some of these traditional uses are now being investigated using modern scientific methods (see the section '**Raspberry Health Effects**').

Raspberry Biology

Raspberries are woody shrubs that have a perennial root system and long, thin stems or canes, which are biennial. This means that while roots grow year by year, the canes have a 2-year life span. Raspberry plants produce a number of new canes, known as primocanes, each year. The second-year canes are called floricanes. Some raspberry varieties are known as primocane-fruiting, while others are known as floricane-fruiting. Primocane-fruiting raspberry plants can produce a limited number of flowers and fruits the first year, from late summer to fall, and then produce a second, larger crop of berries from floricanes in the second year. In contrast, floricane-fruiting raspberry canes produce no fruit in the first year. Instead, floricane-fruiting plants produce only nonfruiting primocanes in the first year. These nonfruiting primocanes emerge from crown buds or adventitious roots (roots that emerge from aboveground stems). Nonfruiting primocanes grow rapidly during spring and early summer and then form flower buds in the fall of the first year. Buds on the nonfruiting primocanes break and plants produce leaves, flowers, and new, fruiting canes (floricanes) in the spring of the second year. After fruiting, the floricanes on floricane-fruiting plants senesce and die.

In addition to primocane- and floricane-fruiting types, raspberry plants can also be characterized by the amount of support their canes need to remain off the ground. Keeping raspberry canes off of the ground helps to prevent disease by keeping the berries dry. It also makes machine harvesting or handpicking raspberries easier. Raspberries are hand-harvested for fresh market and can be machine-harvested for processing. Machine harvesting is accomplished by tall tractors that straddle raspberry rows and shake the berries off of the canes using long, flexible plastic rods. The berries that are shaken off of the canes land on conveyor belts that transport them into boxes or baskets for storage. Based on how much support is needed, raspberry plants are classified as erect, semi-erect, or trailing type. Just as it sounds, the erect and semi-erect canes do not require support. The trailing type of canes, however, requires a stake or trellis system for support.

Like blackberries, boysenberries, and the other so-called caneberries, raspberries are not considered true berries. Instead, they are classified as 'aggregate fruits,' because they are composed of 75–125 small fruits, called drupelets. **Figure 1** shows the characteristic aggregate form of the 'berries.' When the fruit ripens, an abscission (separation) layer develops between the fruit and the 'receptacle' (a fingerlike projection that attaches them to the plant during ripening). When the ripe raspberry fruit is removed from the plant, the receptacle is left behind, resulting in a hollow fruit. This is one of the physical characteristics that differentiates raspberries from blackberries. Blackberries do not develop an abscission layer in the same way as raspberries do and are, therefore, not hollow. Raspberry fruits undergo color change as they ripen due to the degradation of chlorophyll and accumulation of the anthocyanins that are primarily responsible for the red to dark purple colors of the ripe fruits. Raspberry fruits are highly perishable, with a shelf life of 3–6 days at 0 °C and 90–95% relative humidity. Therefore, raspberries need to be handled properly so the respiration rate is reduced and shelf life can be extended as much as possible. The fact that fresh market berries must be harvested by hand, along with their limited shelf life, may explain their high price, relative to other commonly sold fresh fruits.

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Figure 1 Black raspberries at various stages of ripeness.

Raspberry Species and Varieties

More than 200 raspberry species have been identified. Raspberry fruits can have several colors including red and yellow (both *R. idaeus*), purple (*R. neglectus*), and black (*R. occidentalis*), but red raspberries are the most widely grown type. Black raspberries are a distant second. Red raspberries are more widely grown than black raspberries because they are generally more disease-resistant and more productive than black raspberry plants. Raspberry breeding experienced major advances in Britain in the nineteenth and early twentieth centuries. Red raspberries cultivated in North America before 1800 originated in Europe. In 1866, the US-developed cultivar Brinckle's Orange was released. Cultivars such as Latham, Newburgh, and Chief were also developed during the twentieth century. Primocane-fruiting raspberries were bred, and cultivars such as Heritage, Amity, and Autumn Bliss were released. Currently grown floricane-fruiting red raspberry cultivars include Claudia, Emily, Encore, Esta, Lauren, Prelude, and Qualicum, and primocane-fruiting cultivars include Autumn Bliss, Autumn Britten, Caroline, Dinkum, Heritage, Josephine, and Polana. Fewer black raspberry cultivars have been developed compared with red raspberries, primarily due to the greater popularity of the red varieties. Currently, popular black raspberry cultivars include Allen, Black Hawk, Bristol, Jewel, Mac Black, and Munger. There are currently established breeding programs in 21 countries, so that raspberries with enhanced disease resistance (fungal diseases and viral diseases transmitted by aphids are major concerns) or desirable traits (e.g., thornless canes or larger, sweeter berries) can be produced.

Raspberry Production

Raspberries are grown worldwide, primarily in the Northern Hemisphere, although there is some commercial production in Chile. Fresh market raspberries are grown to be bright and glossy, whereas raspberries destined for processing are selected for their color intensity. Black raspberries are grown to be dark purple so that they can be used for their natural colorant properties. Frozen seedless raspberry puree can be used as a base for products such as ice cream and sorbet. Raspberry juice can be mixed into many beverages to enhance flavor and color and to balance sweetness and tartness in mixed fruit beverages. Machine-harvested raspberries can be processed for fruit juice, puree, jam, jelly, concentrated syrups, dried forms, individually quick frozen units, and frozen bulk fruits. According to the FAO (the Food and Agricultural Organization of the United Nations), Russia is the number one raspberry producer in the world, followed by Serbia, Poland, and the United States. Russian production in 2010 was reported to be 125 000 metric tons, one and a half times greater than that of either Serbia or Poland and four times greater than that of the United States. Serbia is the world's top raspberry exporter, followed by Russia and Poland. In recent years, raspberry production has increased markedly. Worldwide raspberry production and total production area increased from 320 861 metric tons and 63 762 ha in 1995 to 543 421 metric tons and 100 556 ha in 2011, respectively.

According to USDA National Agricultural Statistics Service data, 31 400 metric tons of red raspberries and 1000 metric tons of black raspberries were produced in the United States in 2012, with an estimated total worth in excess of $220 million. Of these, 590 metric tons of red raspberries and 23 metric tons of black raspberries were sold for fresh market purchase. Freezing (whether into individually quick frozen berries or into solid blocks) represents 50% of the processed raspberry market in the United States, followed by juice and puree. In the United States, raspberry production is dominated by three Western states, Washington, California, and Oregon. In 2010, Washington state produced 61 million pounds ($50 million) of red raspberries, while Oregon produced 5 and 1.8 million pounds of red and black raspberries, respectively. California produced 81 million pounds of raspberries ($200 million). About 95% of raspberries produced in the United States in 2001 were processed and only about 5% were sold for fresh market purchase. Despite the large quantity of raspberries produced, the United States imports raspberries from Canada, Mexico, and Chile. This may be due partly to the proximity of these countries (raspberries have a very short shelf life) and in the case of Mexico and Chile, may also be due to the availability of fresh berries in winter months. With regard to processed berries, about 40% of raspberries and blackberries processed in the United States are ultimately used for preserves, jam, and jelly, followed by bakery products (25%), individual quick frozen products (18%), juices and extracts (7%), ice cream and yogurt (5%), and canned berries (5%). In summary, raspberry production and their monetary value are increasing worldwide and most raspberries are processed, rather than eaten fresh. This raises the question of how the nutrients found in the fresh berries are affected by the various types of processing.

Raspberry Nutrient/Phytonutrient Composition

A variety of nutrients and other potentially beneficial compounds are found in raspberries, including vitamins,

essential minerals, fatty acids, and dietary fiber. These nutrients not only determine the fruit's health properties but also can affect their flavor, texture, and appearance. As with other fruits, water is the major constituent of raspberries. Sugars, in particular glucose and fructose, and citric acid are the major soluble solids. The sugar-to-acid ratio is important to the taste of raspberries and thus for consumer acceptance. Fruits grown in mild, dry summers (daytime temperature around 77 °F or 25 °C) have the best sensory characteristics in that they are sweeter, less acidic, and have more aroma and intense color than raspberries grown in other conditions. The *Rubus* species are the sources of the water-soluble vitamins C and folic acid and the fat-soluble vitamins A and E. Depending on the type of soil in which they are grown, they contain minerals, including calcium, magnesium, manganese, and selenium. They are also rich in soluble fibers, such as pectin. Although nutrient content is influenced by many factors, one cup of raspberries, on average, provides 54% of the daily recommended value (DV) of vitamin C, 12% DV of vitamin K, 6% DV of folate, 41% DV of manganese, 7% DV of magnesium, 5% DV of potassium, and 32% of the DV of dietary fiber. Raspberries are low in total calories, containing 52 cal, on average, in a 100 g serving or ~120 cal per cup.

In addition to their nutrient composition, raspberries are an excellent source of nonnutrient phytochemicals, also known as 'nonessential' nutrients, because they are not technically necessary to sustain life but may communicate health benefits if consumed in the right amounts. Berries contain a variety of monomeric and polymeric phenolic compounds. Monomeric compounds found in raspberries include flavonoids, phenolic acids, and carotenoids. Polymeric raspberry compounds include ellagitannins, gallotannins, and proanthocyanidins. Flavonoids are a diverse group of over 6000 compounds that includes anthocyanins and flavanols. Anthocyanins are of particular interest in raspberries, since they provide a red to purple color and may be some of the most 'bioactive' in terms of potential anticancer and other health effects. Depending on the cultivar, cyanidin 3-glucoside, cyanidin 3-sambubioside, and cyanidin 3-sophoroside have all been reported as major anthocyanins in red raspberries. Black raspberries contain the anthocyanins cyanidin 3-sophoroside-5-rhamnoside, cyanidin 3-sambubioside-5-rhamnoside, and cyanidin 3-rutinoside. Carotenoids such as lutein, zeaxanthin, b-cryptoxanthin, and a- and b-carotene are also present in raspberries. The importance of carotenoid pigments is most evident in yellow raspberries (which are an albino form of the red raspberry), where the red to purple anthocyanin pigments are absent. Although carotenoid colors are typically masked by anthocyanins, they do contribute to the aroma of raspberries via the formation of compounds known as ionones and damascenones. Interestingly, ethyl formate, one of the principal flavor components in raspberries, was detected at the center of the Milky Way galaxy, proving once and for all that raspberry flavor is out of this world. Table 1 gives a general summary of the nutrient/phytonutrient contents reported in raspberries. It is important to keep in mind that, as indicated previously, the nutrient content varies based on the type of raspberry in question and its degree of ripeness, length of storage, degree of processing, etc.

Table 1 Approximate raspberry nutrient/phytonutrient content by percent

Nutrient/phytonutrient	*Reported content*
Water	86%
Fiber	6.5%
Sugars	4.4%
Organic acids (primarily citric acid)	1.5%
Protein	1.2%
Lipids	0.7%
Total minerals (ash)	0.5%
Ellagitannins	0.1%
Anthocyanins	0.1%
Salicylates	<0.1%

Raspberry Health Effects

As mentioned previously, raspberry leaves and fruits have been used as part of traditional medicines to treat a wide range of illnesses for millennia. In contrast, the study of the health effects of raspberries by modern science is relatively new, going back only to the early 1980s. These newer studies have identified potentially beneficial compounds and chemical properties in raspberry plants and have examined raspberry's health effects in cell culture, in animal models, and, to a limited degree, in humans. Based on these newer studies, raspberries and the compounds contained in them have been reported to possess a wide variety of potential benefits, including anticancer, antidiabetic, antiobesity, antihypertensive, anticoagulant, anti-inflammatory, antiviral, antidepressant, and neuroprotective effects. The weight of evidence for most of these effects is weak, relying primarily on cell culture or animal models. The strongest evidence presented so far relates to the cancer preventive (chemopreventive) effects of black raspberries in humans. These effects are influenced by raspberry variety, growth, storage, and processing conditions and by individual differences in the genetics and intestinal flora of the consumer. While raspberries may provide health benefits, a number of outbreaks of foodborne illnesses have also been linked to fresh and frozen raspberry consumption. This section examines the potential health benefits of raspberries and weighs these benefits against the risk of foodborne illnesses that may be associated with their consumption.

The recognized leaders in the area of cancer chemoprevention with raspberries are Dr. Gary Stoner and his colleagues. The groundbreaking work done by Dr. Stoner and his collaborators at the Cleveland Clinic at Ohio State University and his current work at the Medical College of Wisconsin have shown that raspberries are highly effective chemopreventive agents in cell culture and in animal models. More importantly, the information gathered from cell culture and animal models is now being translated into human clinical trials. His primary focus in human studies has been on cancers of the digestive tract. The first step in these types of food-based human studies is to assure that no harm results from the amount of a food or food components consumed. Some of his early work in human trials indicated that the black raspberry constituents ellagic acid and anthocyanins were found in blood and urine after large amounts (equivalent to about 1 pound of fresh berry consumption per day) of

freeze-dried black raspberry slurry were consumed by human volunteers over several days. It also indicated that even this large amount of berries was well tolerated when the freeze-dried berries were reconstituted in a small amount of water and consumed as a slurry. Studies by other researchers have indicated that the majority of anthocyanins consumed are rapidly absorbed and rapidly cleared from the body but that some are absorbed and remain in the tissues for extended periods.

Dr. Stoner's work examining the preventive effects of whole raspberries against digestive tract cancers in humans has included studies of oral, esophageal, and colon cancer. An oral cancer prevention trial conducted by his lab showed that the anthocyanins in black raspberry-based mouth rinses are taken up by human oral tissue and metabolized by it. They are also metabolized by bacteria present in the mouth. Individuals differ in the way that this metabolism occurs, which may, in turn, alter their risk for oral cancers. Raspberries have been reported to affect antioxidant measures in patients with Barrett's esophagus, a precancerous condition that can lead to esophageal cancer in humans. Some of his most recent work, which involved feeding freeze-dried black raspberry slurries to colon cancer patients, indicated that raspberries have the ability to beneficially affect tissue- and blood-based markers in colon cancer. Blood samples taken from colon cancer patients indicated that there were positive changes in the plasma levels of granulocyte–macrophage colony-stimulating factor and interleukin 8, two blood markers associated with cancer growth. Consumption of black raspberry slurries was also associated with the methylation of tumor suppressor genes in cancerous colon tumors of human subjects. Gene methylation is important because it can determine whether a gene is 'turned on' or 'turned off.' Turning 'on' tumor suppressor genes in tumor cells can cause them to die off. In addition to work with whole, freeze-dried berries, Dr. Stoner's group also examined the effects of black raspberry extracts. A black raspberry extract was found to reduce polyp formation in the colon by 59% in human patients with familial adenomatous polyposis, a genetic condition that dramatically increases the risk of developing colon cancer. Although some effort has been made to identify an 'active ingredient' in black raspberries, one of the unique aspects of this work is the apparent success achieved with whole berries.

Despite the success of the 'whole food approach,' a number of compounds present in raspberries have been identified as important to their health effects, including anthocyanins, ellagitannins, and salicylates. There is some evidence that the dark purple pigment compounds, anthocyanins, may be the active anticancer ingredient in the black raspberries. It is also possible that any health benefits provided by raspberries may result from a combination of compounds (anthocyanins, ellagic acid, etc.) or metabolites of multiple compounds (e.g., the ellagic acid metabolites, urolithins, that are discussed later in this section) contained in the raspberry fruit. Salicylates are similar to aspirin in chemical structure and have similar anti-inflammatory and pain-relieving effects. While it would be premature at this point to say that raspberries have cancer preventive effects in humans, the results of preliminary clinical trials examining the chemopreventive effects are encouraging and warrant further study.

One area of recent research interest has been the realization that because freshly harvested, unprocessed, or minimally processed berries are alive and biochemically active, they can respond to environmental stimuli. It has long been recognized that phenolic compounds, like flavonoids, are produced by plants as a stress response to factors such as sunlight, heat, drought, and pests. Man-made stresses, such as radiation, ultrasound, or high pressure, can increase the level of potentially beneficial compounds. One study reported that gamma-irradiated raspberries showed significant decreases in bacterial and fungal content and increased the phenolic and antioxidant properties. A loss in firmness appeared to be the only negative effect observed. Irradiation of raspberries may, therefore, have two benefits: one with regard to increased content of phenolic compounds and another with regard to decreased bacterial load and enhanced food safety. Ultrasound, at both low and high frequencies (20 and 986 kHz, respectively), was shown to increase the content of potentially beneficial compounds in raspberry puree. At the lower frequency, total phenol content, antioxidant content, and anthocyanin content each increased by 10% or more. At the higher frequency, only total phenol content increased. With an improved understanding of the ways in which raspberries respond to specific types of stresses, it may be possible to maximize the production of specific compounds, thus standardizing and maximizing the content of potentially beneficial compounds or biological effects.

Antioxidant activity has been used extensively as a marketing tool for raspberries and may also influence their health effects. Antioxidants are compounds that can react with and inactivate highly unstable molecules known as free radicals or that prevent the formation of free radicals. Total phenolic content, which is a measure of a whole food's chemical reducing capacity, is closely related to antioxidant activity and, in raspberries, to anthocyanin content. Some essential nutrients contained in raspberries, such as vitamin C and other 'nonessential' nutrients, including flavonoids and carotenoids, possess antioxidant properties. Antioxidants may play a role in reducing the risk of chronic diseases, such as cardiovascular disease, cancer, and diabetes via the prevention of oxidative stress and effects on cell signaling. Raspberries have been reported to possess greater antioxidant properties than strawberries, kiwi, broccoli, leeks, apples, and tomatoes. Recently, questions have been raised about whether antioxidant properties alone can communicate any health benefits. For this reason, the USDA has decided to remove their oxygen radical absorbance capacity database from their website. Despite the changing views over the importance of antioxidant properties in relation to direct health effects, it is clear that many foods, like raspberries, with proved health benefits also possess significant antioxidant activity. In addition, antioxidants may indirectly benefit health by acting as natural 'preservatives' for other food components, protecting them from destruction by processing and storage.

Microbial flora and their interaction with the foods we eat have now been recognized as one of the most significant contributors to health, for both positive and negative reasons. Raspberries are no exception. One of the most intense areas of investigation has focussed on nutrient absorption and how nutrient and nonnutrient compounds are metabolized to other compounds not only by human digestion but also by the trillions of microbes present in the human gut. It is well known that only a very small percentage of flavonoids,

carotenoids, and other 'nonessential' nutrients are absorbed by the body, such that they are detectable in blood or urine after a berry-containing meal. Research interests are now beginning to examine whether the microbial metabolites of 'nonessential' nutrients, rather than the compounds found in food, may be the active compounds that may influence disease prevention. One example that illustrates this idea is the conversion of raspberry ellagitannins to ellagic acid and ellagic acid to urolithins by intestinal bacteria. Depending on the type of raspberry and its degree of ripeness, raspberries contain varying levels of ellagitannins and ellagic acid (pale, unripe raspberries contain more ellagitannins and less anthocyanins than ripe raspberries). Ellagitannins are not absorbed by the body and ellagic acid is only poorly absorbed. Urolithins are relatively well absorbed compared with ellagic acid. Urolithins are formed from the breakdown of complex ellagitannins to ellagic acid. In this way, raspberries are analogous to pomegranates, walnuts, and muscadine grapes, in that they are all sources of ellagitannins that may be converted to urolithins by intestinal microbes. Urolithins may be responsible for some of the antioxidant, anti-inflammatory, and anticancer effects attributed to ellagitannins and ellagic acid.

Despite all of the reported health benefits of raspberries, there are also risks associated with their consumption. These risks are related primarily to foodborne illnesses but may also be associated with pesticide application and heavy metal contamination. Raspberries can serve as vectors for foodborne illnesses, particularly in fresh or fresh frozen berries. Raspberries have been associated with norovirus, hepatitis, and *Cryptosporidium* outbreaks. The most thoroughly studied and frequently reported foodborne illness associated with raspberry consumption is norovirus. Typical norovirus symptoms include short-term bouts of vomiting and diarrhea. While rarely fatal, the symptoms of this viral illness can be quite severe. Raspberries may be contaminated with norovirus in the field, during picking and handling, or during packaging and freezing processes. For this reason, every effort must be made to assure that not only the berries but also the workers, the processing machinery, and the packaging be norovirus-free. Interestingly, some black raspberry compounds may have both antibacterial and antiviral effects. Although it is clear that raspberries can be sources of foodborne illnesses, it is unclear whether individuals who eat contaminated raspberries are more or less likely to become ill than if they consumed other foods (i.e., do the potential antimicrobial effects of berries have any real benefits in terms of decreasing food poisoning incidents?).

In addition to microbial safety, chemical contamination and allergies to raspberry proteins can also affect raspberry safety. Depending on the types of minerals found in the soil in which they are grown, raspberries may contain metals, including chromium, cobalt, and lead, each of which may be toxic at high levels. The issue of metal contamination is not unique to raspberries but certainly deserves consideration. Strawberries, blueberries, and raspberries contain similar proteins that may act as allergens in sensitive individuals. This means that those who are allergic to one of these berries may be allergic to all of them. Some individuals are also sensitive to salicylates. Whether the issue is microbial food safety, environmental contamination, or food allergies and sensitivities, the most important question regarding the safety of raspberries is whether the potential health benefits of eating raspberries outweigh the potential risks. Given that raspberry-related foodborne illnesses, metal contamination, and allergies appear to be relatively rare events, the answer would seem to be that the benefits do outweigh the risks.

As with other fruits, one of the primary issues with raspberry's health effects is their availability and expense relative to other foods. From 2003 to 2004, 87% of fruits in the United States were consumed in the homes of the buyers. The total US consumption of fruits is 9.65 g day^{-1}. American male adults from 40 to 59 years old have the highest consumption of fruits. Higher educated families have a higher consumption of fruits than families with lower education levels. Caucasians eat more fruits than all other races. Underweight children eat more fruits than children in other weight classes. Since many of the human studies conducted with raspberries have subjects consuming the equivalent of a pound of berries per day in freeze-dried form, this presents two problems. The first is the cost of consuming one pound of berries per day. The second is the need to consume either a pound of fresh berries or the equivalent (about 45 g or 2 oz) in order to reap the potential benefits of the berries. For this reason, research to determine the active ingredients in raspberries is important, because it may allow for the consumption of a much smaller amount a material, if daily dosing is needed.

Summary

Raspberries have a long history of human consumption and cultivation in what is now Europe. Today, world raspberry production is focussed in Eastern Europe and Russia, with Russia being the leading producer. Various species of raspberries may produce red, yellow, purple, or black fruit. Of these, red raspberries are the hardiest and most commonly grown. Only a small percentage of raspberries are consumed fresh. Most raspberries are frozen or processed into jams and other products. Raspberries are sources of essential vitamins and minerals and 'nonessential' compounds that may benefit health. In addition to the use of raspberry fruit as food, many parts of the raspberry plant have also been used for medicinal purposes. Although not conclusive, recent human studies suggest that raspberries may show promise in cancer prevention. Although raspberries are generally considered to be 'healthy' foods, they can also be sources of foodborne illnesses if contaminated in the field or during processing and storage. Future research will continue to produce new varieties of raspberries and to examine their potential health effects.

See also: Antioxidants: Role on Health and Prevention; Berries and Related Fruits; Strawberries.

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Renal Function and Disorders

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Background

The kidneys are bean-shaped retroperitoneal organs located in the paravertebral gutters. The kidneys are highly vascular organ systems and they receive 20% of the cardiac output. They are supplied by the right and left renal arteries, which are direct branches from the abdominal aorta. The main functions of the kidney include the following:

- *Excretion of metabolic end products*: They play a key role in excreting products of protein breakdown most notably urea, toxin excretion, and excretion of drugs and metabolites.
- *Maintenance of body homeostasis*: They regulate body fluid volume, osmolarity, acid–base balance, and electrolyte balance. Electrolytes regulated by urinary excretion include sodium, potassium, chloride, calcium, magnesium, and phosphate.
- *Production of hormones and enzymes*: Renin and erythropoietin are the hormones produced by the kidney. Renin is produced by granular cells of the juxtaglomerular apparatus. Renin acts on a plasma globulin called angiotensinogen and converts it to angiotensin. Angiotensin is a potent vasoconstrictor and plays a key role in blood pressure and sodium regulation. Erythropoietin is produced by the renal cortical interstitial cells and stimulates maturation of erythrocytes in the bone marrow.

Body Fluid Composition

The human body is largely made of water. But the quantity of water is variable depending on the fat mass. Adipose tissue is low in water content. An obese individual may have a lower water content compared to a nonobese individual. Similarly, women have lower water content (55%) compared to men (60%). Simple bedside estimates for body fluid compartment volumes are as follows:

Total body water (liters) = 60% × body weight
Intracellular water (liters) = 2/3 of total body water
Extracellular water (liters) = 1/3 of total body water
Plasma water (liters) = ¼ of extracellular water
Blood volume (liters) = plasma water/(1 − hematocrit)

Basic Structure of a Nephron

Please refer to Figure 1 for a basic structure of a nephron. Structurally, each kidney is composed of 1 million filtering units called nephrons (Figure 1). Each nephron comprises a glomerulus, proximal convoluted tubule, loop of Henle, distal convoluted tubule, and collecting duct. The renal artery is a direct branch of the abdominal aorta. Then, the renal artery branches into the lobar, the interlobar, and then the arcuate arteries, which further branch into the afferent arterioles that take blood to the glomerulus and form the glomerular tuft of capillaries. From the glomerular tuft of capillaries arise the efferent arterioles, which join the arcuate vein, then the interlobar vein, and finally the renal vein, which joins the inferior vena cava. The uniqueness of this system is that the glomerular tuft of capillaries gives rise to the efferent arteriole rather than a venule. The resistance offered by this efferent arteriole increases pressure within the glomerular capillaries aiding in ultrafiltration where fluid and electrolytes are filtered out of the glomerular capillaries into the Bowmen's capsule.

Glomerular Ultrafiltration

Substances encountered by the glomerulus are broadly divided into large molecules (>60 000 Da molecular weight), middle molecules (between 500 and 60 000 Da), and small molecules (<500 Da). The large molecules are usually immunoglobulins and are not filtered by the kidney. This is considered an evolutionary protective mechanism to retain immunoglobulins in the body. Small molecules usually are nitrogenous waste products that are highly water-soluble and are easily filtered. They can be easily dialyzed when the kidney is diseased. The middle molecules are uremic toxins and drug haptens that resist filtration via the glomerulus. Accumulation of these middle molecules is considered the predominant reason for uremic symptoms such as nausea, vomiting, and lack of appetite. Dialyzing these middle molecules, though possible, is difficult.

The rate at which blood is filtered in the glomerulus is called the glomerular filtration rate (GFR). GFR is the most commonly used marker to denote kidney function in clinical practice. GFR can be accurately assessed by measuring the clearance rate of a solute that has a constant concentration in the blood, which is filtered but neither reabsorbed nor secreted. Inulin clearance measured by infusing inulin at a rate so as to maintain a constant blood concentration is an accurate method to assess GFR. But this technique is not commonly used in routine clinical practice considering the hassle that goes with infusing inulin. The commonest and easiest method to estimate GFR is to use creatinine clearance. Creatinine is endogenously produced, filtered in the glomerulus, and not reabsorbed and we have assays to easily measure creatinine. Hence, it fits most criteria for the solute that will make an ideal ultrafiltrate. However, creatinine is secreted by the peritubular capillaries, and hence, creatinine clearance potentially overestimates actual GFR by 10–15%. There are two general methods of estimating creatinine clearance: (1) Direct measurement: from a 24 h urine collection assessing the quantity of creatinine excreted over a 24 h period. Though considered accurate, the technique is cumbersome and not used in regular

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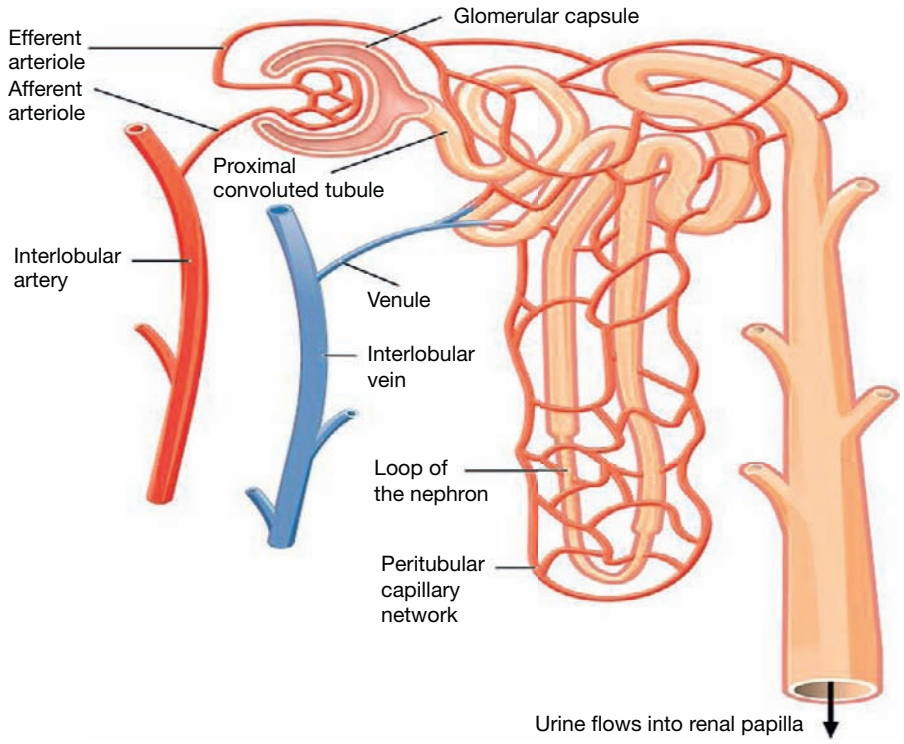

Figure 1 Microscopic structure of a nephron.

clinical practice. (2) Estimated glomerular filtration rate (eGFR). There are multiple formulas to assess eGFR. Broadly, they are classified into creatinine-based equations and cystatin-based equations. Common creatinine-based equations are (1) the Cockcroft and Gault equation, (2) Modification of Diet in Renal Disease (MDRD) equation, and (3) Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) equation. Creatinine-based equations, though clinically applicable and inexpensive, may overestimate GFR as discussed earlier. Also, these equations have not been validated for use for ages >75 years. The most valid among these is the CKD-Epi equation:

$$\text{eGFR} = 141 \times \min(SCr/k, 1)^{a} \times \max(SCr/k, 1)^{-1.209} \times 0.993^{\text{Age}} [1.018 \text{ if Female}] \times [1.159 \text{ if Black}]$$

Cystatin alone or cystatin combined with creatinine-based estimated GFR prediction has been found to perform optimally for GFR estimation.

Irrespective of the method used, eGFR has been shown to predict outcomes that include cardiovascular events, stroke, incident diabetes, and mortality. Further, rate of eGFR decline has been identified to predict future events better than a single measure of GFR.

Tubular Reabsorption and Tubular Secretion

Tubular reabsorption is the process by which there is movement of solute or water from tubular lumen to blood. This is the predominant process in the renal handling of Na^+, Cl^-, bicarbonate, water, glucose, amino acids, protein, phosphate, calcium, magnesium, urea, uric acid, and other molecules. Tubular secretion is the process of movement of solutes from the blood or the renal tubular cell interior to the tubular lumen. Secretion is important in the renal handling of hydrogen ion, potassium, ammonium ion, and a number of organic acids and bases. Briefly, the glomerular filtrate passes through the proximal convoluted tubule. Here, water, sodium, chloride, amino acids, and sugars are reabsorbed by active transport mechanism involving Na/K-ATPase pump. Nearly 90% of NaCl and water reabsorption happens in the proximal convoluted tubule. Also, in the proximal convoluted tubule, secretion of organic ions and drugs happens. Then, the filtrate passes through the loop of Henle where passive movement of NaCl and water happens from the tubular lumen to the tubular cells and then to the tubular interstitium. Then, at the distal convoluted tubule, further reabsorption of NaCl and water happens. The remaining filtrate comes out as urine. Understanding these absorption sites is important to understand diuretic action. Osmotic diuretics inhibit Na and water reabsorption at the proximal tubule. Loop diuretics like furosemide act at the ascending loop and inhibit the sodium potassium cotransporter. Thiazide diuretics inhibit Na/Cl^- cotransporter at the distal convoluted tubule. Amiloride blocks epithelial sodium channel at the collecting duct.

Acid–base balance

An arterial pH lower than 7.36 is considered as acidemia and a pH more than 7.44 is considered alkalemia. The blood pH is determined by the ratio of serum bicarbonate concentration and the PCO_2. When a metabolic disorder decreases the serum bicarbonate (metabolic acidosis) or increases serum bicarbonate (metabolic alkalosis), compensatory PCO_2 response follows the same direction as the bicarbonate. In a patient with metabolic

acidosis, the PCO_2 falls approximately 1.2 mmHg for every 1 meq l^{-1} reduction in serum HCO_3 concentration. In a patient with metabolic alkalosis, the PCO_2 raises by 0.7 mmHg for every 1 meq l^{-1} increase in serum HCO_3 concentration. In acute respiratory acidosis, for every 10 mmHg increase in PCO_2, the serum HCO_3 increases by 1 meq l^{-1}, whereas in chronic respiratory acidosis, for every 10 mmHg increase in PCO_2, the serum bicarbonate increases by 3.5–4 meq l^{-1}. In acute respiratory alkalosis, for every 10 mmHg increase in PCO_2, the HCO_3 increases by 2 meq l^{-1}, whereas in chronic respiratory alkalosis, for every 10 mmHg drop in PCO_2, the HCO_3 decreases by 4–5 meq l^{-1}. Metabolic acidosis is broadly divided into two types: (1) raised anion gap metabolic acidosis and (2) normal anion gap metabolic acidosis. The anion gap is the difference in the measured cations (positively charged ions) and the measured anions (negatively charged ions) in serum, plasma, or urine. Raised anion gap acidosis happens when acid accumulates in the body. Examples of raised anion gap metabolic acidosis include lactic acidosis, diabetic ketoacidosis, kidney disease, toxic alcohol ingestion including methanol and ethylene glycol, and salicylate poisoning. Normal anion gap metabolic acidosis happens due to loss of body reserves of bicarbonate. Normal anion gap acidosis happens in conditions like diarrhea and renal tubular acidosis, conditions associated with bicarbonate losses. Metabolic alkalosis happens secondary to hydrogen ion loss in the gastrointestinal track or the urine. Common causes include removal of gastric contents via vomiting or nasogastric suctioning and use of loop and thiazide diuretics. Respiratory acidosis happens in chronic obstructive pulmonary disease and secondary to respiratory muscle fatigue, conditions associated with CO_2 retention. Respiratory alkalosis happens in conditions associated with CO_2 washout secondary to hyperventilation. Examples include anxiety and panic attack, acute asthma, and hyperventilation.

Nutritional Management in Kidney Disease

Acute kidney injury

Acute kidney injury (AKI) represents a syndrome of acute derangement in kidney functions and usually reversible decline in GFR. The causes for AKI include (a) prerenal causes (due to hypovolemic states such as acute hemorrhage, diarrhea, and gross insensible losses such as burns), (b) intrinsic renal vascular disease (includes acute insults such as small vessel vasculitis, microangiopathies such as thrombotic thrombocytopenic purpura, and chronic vascular insults such as hypertensive nephrosclerosis), (c) intrinsic glomerular diseases (includes primary glomerulonephritides or secondary to paraneoplastic and drug-induced or systematic rheumatologic etiologies), (d) intrinsic tubular/ interstitial (the commonest being acute tubular necrosis), and (e) obstructive nephropathy (includes urolithiasis, prostate disease, and retroperitoneal fibrosis).

Nutrition in AKI

Nutrition in AKI is an important component in the management:

(a) *Energy requirement*: Though requirements may vary, the general consensus is a calorie requirement of 25–30 kcal kg^{-1} day^{-1}.

(b) *Protein requirement*: Increases with severity of illness and initiation of dialysis. Nondialysis patients require 0.8–1.2 g kg^{-1} of protein a day and the critically ill require 1.2–1.5 g kg^{-1} per day or more. Also, enteral nutrition is preferred compared to parenteral nutrition as evidence supports the survival benefit of enteral nutrition over parenteral nutrition.

(c) *Vitamins and mineral requirement*: Patients with AKI receiving renal replacement therapy (RRT) have low plasma levels of vitamin C, thiamine, and folic acid compared to patients with AKI who do not need RRT. The significance of these deficiencies and outcomes upon replacement of these nutrients are unknown. Hypocalcemia is common among patients with AKI, the causes being increase in serum phosphorus levels caused by reduced GFR, skeletal resistance to parathormone, and decreased synthesis of 1,25(OH)2D3. Treatment of hypocalcemia depends on the severity and symptoms. In asymptomatic cases, correction of hyperphosphatemia with use of phosphate binders will correct serum calcium spontaneously. Patients with symptomatic hypocalcemia and a serum phosphorus between 8 and 10 should be dialyzed to correct hypocalcemia and hyperphosphatemia. Intravenous therapy with calcium chloride or calcium gluconate is only indicated in the presence of life-threatening hypocalcemia characterized by tetany, confusion, seizures, and QT interval prolongation in electrocardiogram.

(d) *Sodium and potassium requirement*: Sodium and potassium are restricted depending on urinary excretion, edema, serum sodium levels, and dialysis needs. During the oliguric phase of acute kidney injury, sodium and potassium may be restricted to 500–1000 mg per day and 1000 mg per day, respectively.

Chronic kidney disease

Chronic kidney disease (CKD) is defined as the presence of kidney damage or decreased kidney function for 3 or more months irrespective of the cause. The time duration of 3 months is necessary to differentiate AKI from CKD.

Nutrition Assessment in Patients with Chronic Kidney Disease

Nutrition assessment is a major component of kidney disease management. There is no single measure to assess malnutrition in a patient with kidney disease. The following criteria have been proposed to identify kidney disease-related protein energy wasting:

Serum chemistry

- Serum albumin < 3.8 g dl^{-1}
- Serum prealbumin < 30 mg dl^{-1} (transthyretin) (applicable only for patients on maintenance hemodialysis since the serum levels may vary depending on GFR in patients with CKD stage 2–4)
- Serum cholesterol < 100 mg dl^{-1}

Body mass index (BMI)

- BMI < 23 kg m^{-2}

- Unintentional weight loss over time: 5% over 3 months and 10% over 6 months
- Total body fat percentage < 10%

Muscle mass

- Muscle wasting: reduction in muscle mass by 5% over 3 months or by 10 % over 6 months
- Reduced midarm muscle circumference area (>10% reduction in comparison to 50 percentile of reference population)
- Creatinine appearance

Dietary intake

- Unintentional low dietary protein intake $<0.80\ g\ kg^{-1}\ day^{-1}$ for at least two months for dialysis patients or $<0.6\ g\ kg^{-1}\ day^{-1}$ for patients on CKD stages 2 to 5
- Unintentional low dietary energy intake $<25\ kcal\ kg^{-1}\ day^{-1}$ for at least 2 months

At least three out of the four listed categories (and at least one test in each of the selected categories) must be satisfied for the diagnosis of kidney disease-related PEW. Optimally, each criterion should be documented on at least three occasions, preferably two to three weeks apart. In short, PEW may be suspected if the serum albumin is $<3.8\ g\ dl^{-1}$, serum prealbumin $<30\ mg\ dl^{-1}$, and serum cholesterol $<100\ mg\ dl^{-1}$.

Nutrition Management of Patients with CKD

(a) *Energy requirement*: Though energy requirement largely depends on metabolic demand depending on the stage of CKD, 25–30 kcal $kg^{-1}\ day^{-1}$ is acceptable energy requirement in CKD.

(b) *Protein requirement*: Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend protein restriction of 0.6–0.8 $g\ kg^{-1}$ in predialysis patients. However, the benefit of protein restriction in patients receiving maintenance dialysis is unclear. Protein intake <0.6 or >0.8 $g\ kg^{-1}$ did not show any significant benefit in terms of reduced CKD progression or mortality benefit. Protein restriction in CKD patients with nephrotic range proteinuria is unclear. Evidence-based recommendations in this subgroup are scanty, though it is generally believed that protein restriction of 0.8 $g\ kg^{-1}\ day^{-1}$ may be safe in this subgroup.

(c) *Sodium requirement and potassium requirement*: The 2012 KDIGO guidelines recommend sodium intake of $<2\ g\ day^{-1}$ in all adult patients with CKD and a potassium intake of 40–70 mEq day^{-1} (1500–2700 mg day^{-1}).

(d) *Calcium, phosphorus, and vitamin D*: Hyperphosphatemia, hypocalcemia, and hypovitaminosis D are the abnormalities commonly noted in patients with CKD. KDIGO guidelines recommend a moderate phosphate dietary restriction of 900 mg day^{-1} in patients with CKD. The target serum phosphorus goal in CKD patients is between 3.5 and 5.5 mg dl^{-1}. If dietary restriction fails to correct phosphorus to normal levels, then phosphate binders are indicated. Calcium generally corrects with use of phosphate binders. Calcitriol or activated vitamin D deficiency is common in CKD. CKD patients with parathormone levels (PTH) > 300 pg ml^{-1} should receive calcitriol according to KDIGO guidelines. Oral doses of calcitriol (0.5–1.0 μg) or doxercalciferol (2.5–5.0 μg) can be given two or three times weekly. Alternatively, a lower dose of calcitriol (0.25 μg) can be administered daily. The goal of therapy is to keep PTH to a target range of 150–300 pg ml^{-1}.

Dietary Management of Renal Stones

In general, increased fluid intake is associated with reduced recurrence of kidney stones. The exact quantity of fluid intake needed to achieve this remains unclear. One study noted that the maintenance of urine output $>2\ l\ day^{-1}$ decreases recurrence of kidney stones. Another study showed that an increase in urine output of 320 ml day^{-1} from baseline was associated with a decreased incidence of kidney stones, thereby emphasizing the concept that any increase in fluid intake decreases risk for recurrent kidney stones. Grapefruit juice is associated in observational studies to be associated with increased risk for kidney stones, whereas coffee, tea, and alcohol lower risk for kidney stones. Lowering animal protein intake, increasing consumption of fruits and vegetables, lowering sodium intake (80–100 meq day^{-1}), a normal calcium intake (1200 mg day^{-1}), and decreasing sucrose and fructose intake decreased risk for stone formation.

Nutrition and Its Effects on Kidney

Obesity and kidney disease

Obesity has become a pandemic. Obesity adversely affects multiple morbidities that in turn result in deranged kidney functions. Obesity increases risk for hypertension, diabetes, and metabolic syndrome, which are all risk factors for CKD. Data from the Framingham Offspring Study showed the independent association of obesity and kidney disease in spite of adjustment for the confounders such as hypertension, diabetes, and metabolic syndrome. Focal segmental sclerosis and obesity-related glomerulopathy are two specific types of kidney diseases that are known to be independently associated with morbid obesity. An interesting observation in the association between obesity and kidney disease is the obesity paradox observed in patients with ESRD and receiving hemodialysis. Obese ESRD patients (BMI > 30) have better survival when compared to ESRD patients with BMI < 30. Though the exact mechanism is not known, the malnutrition–inflammation complex syndrome (MICS) has been indicted in this association. Nonobese ESRD patients are at increased risk for malnutrition. Malnutrition is known to be associated with systemic inflammation in this group, which increases risk for cardiovascular disease and all-cause mortality.

Common Food Stuffs and Effect on Kidney (Coffee, Tea, Alcohol, Tobacco, and Chocolate)

The association between coffee, tea, and kidney disease is unclear. Small studies have reported an increased GFR with

increasing coffee and tea intake, but the effect of this increased GFR associated with coffee intake on cardiovascular and mortality outcomes is unknown and yet to be studied. Alcohol consumption, especially moderate–excess consumption, has been shown to decrease risk for kidney disease. Several observational studies and a large meta-analysis confirmed these results. The exact mechanism underlying this association is unknown and patient characteristics that promote heavy alcohol consumption are suspected to confound this association. Randomized studies assessing this association are lacking and may not be viable considering the ethical issues surrounding the conduct of such a randomized trial. Tobacco in any form increases risk for incident CKD and is associated with CKD progression. Molecular evidence and small observational studies on healthy individuals show a protective effect of chocolate on kidney functions. However, the effect of chocolate on incidence of CKD, progression of CKD, cardiovascular, and mortality outcomes is unknown and needs to be studied.

Weight Loss Diets and Effect on Kidney

As discussed earlier, patients with MICS have increased risk for kidney disease, but those attempting intentional weight loss have decreased incidence of kidney disease. The Look AHEAD (Action for Health in Diabetes), a randomized controlled trial that assessed weight loss in patients with diabetes, showed that in a median follow-up of 8 years, intensive weight loss efforts decreased both CKD incidence and progression, hence alluding to a need to consider intentional weight loss as a adjuvant to other efforts to prevent CKD. There are many diets and medications claiming to achieve effective weight loss in obese patients. Weight loss diets and medications that aid with weight loss are unlikely to adversely affect kidney functions in healthy individuals with no prior kidney disease. However, the use of these modalities is not recommended in patients with existing kidney disease because most weight loss diets recommend high protein intake (some as high as 1.9 g kg^{-1} day^{-1}), which can have deleterious effects on kidney functions in this group of obese people with existing kidney disease.

See also: Adipose Tissue: Structure and Function of Brown Adipose Tissue; Caffeine: Consumption and Health Effects; Coffee: Health Effects; Obesity: Epidemiology of.

Further Reading

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Retinol: Physiology

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Introduction

'Vitamin A' is the collective term for compounds that show the biological properties of retinol, including visual function and maintenance of epithelial tissues. This classification includes retinol (vitamin A alcohol), retinyl esters (vitamin A esters), and retinal (vitamin A aldehyde and retinaldehyde). Retinoic acid (vitamin A acid) is included, even though it does not sustain visual function, because it has been found to be the active metabolite that carries out many of the functions of vitamin A. These are isoprenoid compounds, having in common a polyene chain attached to a trimethyl-substituted cyclohexenyl ring (Figure 1). The term 'retinoids' refers to all compounds, natural or synthetic, that show some biological activity typical of vitamin A, such as promoting differentiation of cells; but not all retinoids can support all the functions of vitamin A, such as vision. Vitamin A compounds are not found as such in plant tissues, but rather are characteristic of the animal kingdom; the notable exception is 13-*cis* retinal, which serves as a chromophore in the purple membrane of certain halobacteria.

Vitamin A deficiency is still a major public health problem especially in developing countries, although much progress has been achieved during the past 50 years. It has been estimated that some 127 million preschool children have vitamin A deficiency and more than 6 million women develop night blindness during pregnancy.

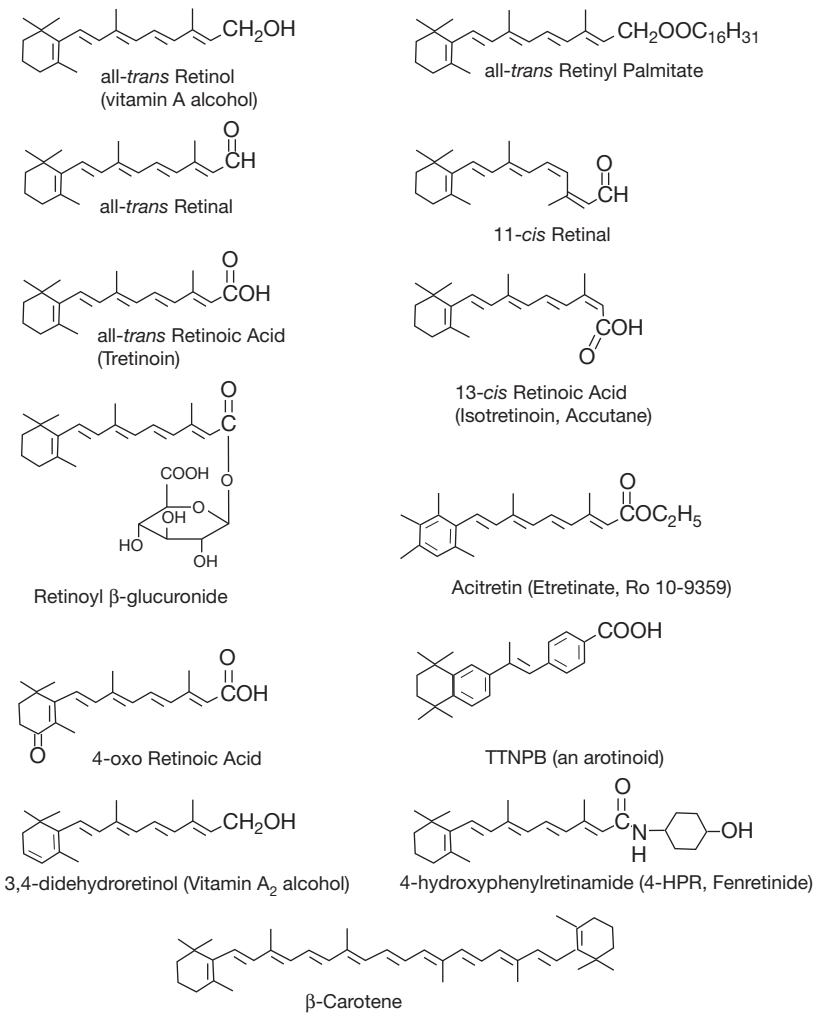

Figure 1 Chemical structures of some representative retinoids.

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Major Dietary Sources

Provitamin A carotenoids from plant sources are the major source of vitamin A for much of the world's population. These are provided by green leafy vegetables (GLV) and by orange-yellow fruits. Provitamin A carotenoids arise mostly from plant products: β-Carotene is the most active precursor of vitamin A and is widely distributed in plants, but other carotenoids (such as α-carotene and β-cryptoxanthin and β-apo-carotenals) can be important sources of vitamin A from particular foods. Other carotenoids, such as lycopene and xanthophylls (including lutein and zeaxanthin), are major carotenoids in some foods and can have other important physiological functions, but they have no provitamin A activity in higher animals. Biofortified foods with enhanced β-carotene content have been produced by conventional plant breeding (e.g., maize, carrots, and cassava) or by genetic engineering (golden rice).

Good food sources of preformed vitamin A (as retinyl esters) include liver, fish liver oils, egg yolks, and fortified foods (such as milk and breakfast cereals in the United States). Human milk is a very important source of vitamin A for the breast-fed infant. Other foods, such as sugar, monosodium glutamate, and cooking oils, are used as vehicles for fortification in other settings. Retinyl esters, for example, retinyl acetate and retinyl palmitate, are more stable chemically than is free retinol and hence are used in food fortification and food supplementation. The relative importance of these sources of vitamin A is very dependent on diet, that is, on agricultural, cultural, and behavioral patterns.

Typical estimates of absorption efficiency of preformed dietary vitamin A are 70–85%. Estimates of carotenoid absorption are usually much lower but are confounded by slow intestinal absorption and rapid metabolism; there is considerable species variability in absorption efficiency and in metabolism of carotenoids. Animal feeding studies show that the biological matrix of food carotenoids has profound effects on their bioavailability.

Metabolism, Storage, and Excretion

The importance of vitamin A in multiple physiological pathways is reflected in the redundancy of mechanisms for interconverting its forms and transporting it. This brief article cannot give full justice to these complementary pathways; the reader is referred to the Supplemental Reading for details.

Since both vitamin A and carotenoids are lipids, intestinal micelle formation with bile acids is essential for their absorption. Human subjects with impaired bile acid formation or flow (e.g., with biliary atresia) may require intramuscular supplementation with vitamin A and the other fat-soluble vitamins. Within the intestinal lumen, vitamin A esters are hydrolyzed to free retinol, which is absorbed as such; free retinol and carotenoids apparently are taken into intestinal cells by membrane-bound lipid transporters. Provitamin A carotenoids, such as β-carotene, are often cleaved within intestinal cells; this metabolism is predominantly by central cleavage (mediated by the enzyme carotene 15,15′-monooxygenase, CMO1 – formerly called carotene 15,15′-dioxygenase), although asymmetrical cleavage followed by chain shortening may play a role. Retinal, the final product of carotenoid cleavage, is enzymatically reduced to retinol. Free retinol is mostly reesterified within the intestinal cells, although some may be released as such into the blood circulation (Figure 2). The physiological ligand for this esterification process seems to be retinol bound to an intracellular retinol-binding protein (CRBP II, molecular weight approximately 14 600 Da, one of several small intracellular retinoid-binding proteins); the primary retinyl ester-synthesizing activity transfers fatty acid from phosphatidylcholine (lecithin retinol acyltransferase (LRAT)), although an acyl-coenzyme A-dependent esterifying activity (acyl-CoA:retinol acyltransferase (ARAT)) is also present.

Regardless of their dietary source, the retinyl esters are transported in the core of chylomicra in the lymph from intestinal cells. After removal of triacylglycerols by lipoprotein lipase as the lipoprotein particles circulate through peripheral tissues, the chylomicron remnants are rapidly taken up by

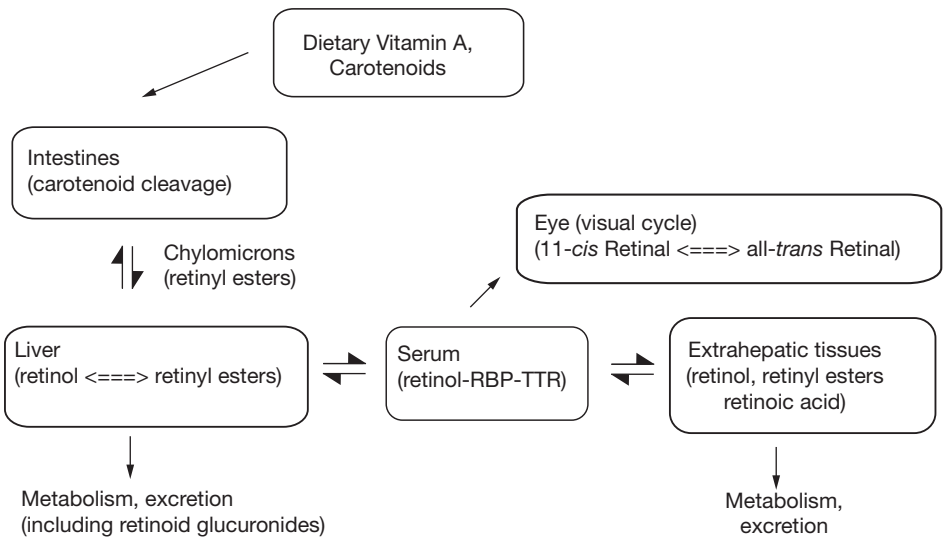

Figure 2 Metabolic interconversions of vitamin A forms.

hepatocytes in the liver, and the vitamin A esters are converted to retinol by retinyl ester hydrolase there. The resulting retinol is then either reesterified (primarily by LRAT, although ARAT activity may be important at high retinol concentrations) and stored in the liver or released into the plasma as a complex with plasma retinol-binding protein (RBP; also called RBP4). The two cell types involved in liver vitamin A metabolism are the hepatocytes (parenchymal cells) and the lipocytes (also called stellate cells, Ito cells, or fat-storing cells). The hepatocytes are the major site of RBP synthesis and retinol-RBP release; some retinyl esters are also found here. The lipocytes store retinyl esters in cytoplasmic lipid droplets, which also contain triacylglycerols and some cholesteryl esters within a phospholipid coat. After requirements of other tissues have been satisfied, vitamin A is stored in the liver, typically 50–80% of total body vitamin A in the well-nourished individual. Vitamin A in excess of immediate requirements is stored in the liver as esters of long-chain fatty acids (Figure 2); the ratio of liver retinol to retinyl esters decreases as total liver vitamin A increases, but typically, 95% of total liver vitamin A is present as the esters. Retinyl palmitate is the primary ester in the livers of most species, although significant amounts of other esters are found (especially oleate and stearate), and the liver retinyl ester composition can be affected by dietary fatty acid composition. Other organs, such as kidney, testes, and adrenal glands, also contain detectable retinyl esters. The concentration of retinyl esters in adipose tissue is low, but the significant mass of adipose tissue in the human body can account for a substantial amount of total vitamin A storage. The efficiency of liver storage of vitamin A is particularly noteworthy in the polar bear, where concentrations as much as 36 μmol g^{-1} (10 380 μg g^{-1}) of liver have been reported. Typical values for human liver vitamin A (autopsy specimens) are 0.44–0.74 μmol g^{-1} (126–211 μg g^{-1}) in the United States of America.

Because most forms of vitamin A are hydrophobic, the transport, metabolism, and function of vitamin A are dependent on a series of binding proteins, each specific for its ligand and tissue. Intracellular binding proteins serve as transporters and chaperones (CRBP forms for retinol, CRABLP and interstitial retinoid-binding protein (IRBP) forms for retinal, and CRABP forms for retinoic acid). Retinol-RBP is the major form of transport from liver to peripheral tissues; gene knockout studies have shown that delivery of retinyl esters via lipoproteins may be adequate for maintaining vitamin A in some tissues but that retinol delivered via RBP is essential under conditions of stress. Although the transport of vitamin A from other tissues back to the liver may be via retinyl esters transported by lipoproteins (normally present at about 5–10% the serum concentration of retinol-RBP), transport via retinol on RBP synthesized in the outlying tissues may be an important pathway (mRNA for plasma RBP has been detected in a number of other tissues in addition to liver). Plasma RBP typically has molecular weight of approximately 21 000 Da in mammalian species; the complete amino acid composition has been determined for several species, and the gene from some species has been cloned. RBP binds retinol with 1:1 stoichiometry. The hydrophobic retinol molecule fits into a 'barrel' within the protein, shielded from interactions with the aqueous environment. Holo-RBP, in turn, binds transthyretin in plasma (TTR; formerly called prealbumin); a TTR tetramer can bind up to four molecules of RBP. Usual concentrations of human plasma RBP are 1.9–2.4 μM (40–50 μg ml^{-1}); typically, total circulating RBP is 80–90% saturated with retinol ligand. Although retinol, retinal, and retinoic acid bind to RBP with similar affinity, retinol is present in highest concentrations in plasma and is the predominant ligand. Retinyl esters have much less affinity for RBP and are transported by serum lipoproteins instead.

The release of holo-RBP from liver hepatocytes is carefully controlled to maintain levels of circulating retinol within narrow limits, but the mechanism of this control is not yet clear. In the absence of adequate vitamin A, apo-RBP accumulates in the liver hepatocytes, ready to be released as soon as vitamin A is available (i.e., the basis of the relative dose response (RDR) assay for vitamin A deficiency, as discussed in the succeeding text). Clearly, this mechanism has developed because of the dichotomy of vitamin A action: vitamin A is essential, in small amounts, for proper differentiation and maintenance of epithelial cells, but excesses of vitamin A are toxic and must be avoided by the organism. A cell surface receptor, STRA6, facilitates the uptake of retinol from plasma RBP in tissues that are heavily dependent on retinoids; STRA6 may be coupled with intracellular LRAT to trap vitamin A as retinyl esters within cells.

Some studies have suggested linkage between RBP (known as RBP4 in this context) and obesity, diabetes, and insulin signaling. It has been proposed that activation of the JAK/STAT cascade by RBP-retinol, via a peroxisome proliferation-activated receptor (PPAR β/γ), upregulates target genes including those that control lipid homeostasis and inhibition of insulin signaling.

Retinol can be reversibly oxidized to retinal (vitamin A aldehyde) by aldehyde dehydrogenases (either cytosolic or microsomal) and retinal can be reduced back to retinol; retinal can be oxidized to retinoic acid by aldehyde dehydrogenases, but retinoic acid cannot be converted back to the other forms. Thus, retinol, retinyl esters, and retinal have equal biological activity because they are freely interconverted; retinoic acid fills some (but not all) of the functions of retinol, but it and its derivatives are not stored. Concentrations of retinoic acid are carefully regulated, by regulation of both production and catabolism. Not surprisingly, genetic expression of many of the enzymes involved in retinoid metabolism is regulated by retinoic acid (see succeeding text). Typical human serum concentrations of retinoic acid are 5–10 nM (1.5–3 ng ml^{-1}), compared with typical retinol concentrations of 1–2 μM. Retinoic acid presumably can be transported in plasma by albumin as well as by RBP. Retinoyl β-glucuronide and retinyl β-glucuronide (formed in the liver from retinoic acid and retinol, respectively) are secreted into the bile but can be hydrolyzed and reabsorbed in the intestine (enterohepatic circulation); both these compounds have vitamin A activity in a variety of tests, are found in human blood, and may have regulatory roles in vitamin A function.

In rats and mice, catabolism of vitamin A occurs by oxidation of the cyclohexenyl ring (particularly at the 4-position), epoxide formation at the 5,6-positions, hydroxylation of the ring methyl groups, and chain shortening. A set of cytochrome P450 enzymes (CYP26A1, CYP26B1, and CYP26C1) are primarily responsible for catabolism of retinoic acid, although

other cytochrome enzymes can also be involved. All-*trans* retinoic acid induces its own catabolism by upregulating CYP enzymes. The resulting metabolites are generally inactive, although some may have a little biological activity. These more polar retinoids are excreted in the urine and in the bile.

The rat has served as a valuable model for vitamin A metabolism in the human, particularly for aspects that cannot be studied readily in humans. Mathematical modeling of kinetic studies carried out in rats has shown that there is extensive cycling of liver vitamin A stores and extensive recycling of vitamin A between liver and other tissues; this assures an ample supply of retinoid to tissues in response to immediate needs. Kinetic models of human vitamin A metabolism are consistent with these findings.

Functions in the Body

Vision

The major roles of vitamin A are (a) in vision and (b) in control of gene expression (including differentiation of epithelial tissues, support of reproductive functions and the immune system, and a host of other systems). Metabolism of vitamin A in the retina of the eye is unique, in keeping with the unusual role of vitamin A in that tissue. Vitamin A is stored in the retinal pigment epithelium as retinyl esters. All-*trans* retinyl esters are simultaneously hydrolyzed and isomerized to 11-*cis* retinol by an isomerohydrolase enzyme (RPE65) that uses the free energy of hydrolysis of the retinyl esters to drive the isomerization reaction. 11-*cis* Retinol, a compound unique to the eye, is then oxidized to 11-*cis* retinal by an alcohol dehydrogenase. 11-*cis* Retinal is transferred from the pigment epithelium cells to the rod cells by IRBP, a distinct binding protein (MW 140 000). In the rod cells, 11-*cis* retinal binds (as a Schiff base with the ε-amino group of a specific lysine residue) to the protein opsin to form the visual pigment, rhodopsin. When a photon of light is absorbed by a rhodopsin complex, the 11-*cis* retinal is isomerized to all-*trans* retinal and released from the protein complex; the resulting conformation change of the protein initiates a cascade of reactions, resulting in a neural signal to the brain. The protein opsin is then available to bind another molecule of 11-*cis* retinal for another round of the visual cycle. The all-*trans* retinal that was released from the protein complex is transferred back to the pigment epithelium via IRBP, enzymatically reduced to all-*trans* retinol, and esterified again. In contrast to the high turnover rates of vitamin A in other tissues, vitamin A in the eye is highly conserved, with little leakage back to the liver. Prolonged vitamin A deficiency, however, leads to reduced sensitivity to light, usually first noted as impaired vision at night (night blindness). These effects of vitamin A deficiency are generally reversible by subsequent vitamin A supplementation.

In a very different role of vitamin A in the eye, the cornea depends on vitamin A for proper cell differentiation and for secretion of protective glycoproteins. In vitamin A deficiency, these tissues lose integrity and are susceptible to attack by opportunistic bacteria; such attack may result in permanent scarring and permanent vision loss. These effects of vitamin A deficiency, unlike those in the retina, may not be reversible by subsequent vitamin A supplementation. Such vitamin A-dependent corneal degeneration (given the general name 'xerophthalmia') accounts for an estimated 500 000 new cases of blindness in preschool children in the world each year.

Control of Gene Expression

Many functions of vitamin A are the result of its regulation of gene expression, mediated via retinoic acid. To date, more than 500 genes, with a great variety of functions, are known to be under control of retinoic acid; proteomic analyses have revealed unanticipated targets. The dominant nuclear receptor proteins responsive to retinoids are RAR (retinoic acid receptors), RXR (retinoid x receptors), and PPAR. The three RAR forms (RARα, RARβ, and RARγ) are activated by all-*trans* retinoic acid. The three RXR forms (RXRα, RXRβ, and RXRγ) initially were believed to be activated by 9-*cis* retinoic acid, but intracellular concentrations of this retinoid are generally undetectably low; however, some synthetic analogs ('rexinoids') and various other natural lipids are effective ligands. RARs form heterodimers with RXR to bind to a specific retinoic acid response element (RARE) in the control element of a gene; RXR can form homodimers or heterodimers with a variety of nuclear receptor proteins (vitamin D receptors, thyroid hormone receptors, and PPARs, among others) (**Figure 3**), providing an exquisite mechanism for control of multiple cellular pathways.

In addition to these genomic, transcriptional roles of retinoic acid, function of some proteins can be controlled via retinoylation, covalent addition of retinoic acid to a protein via a thioester bond. These modifications are cell-specific and posttranslational. Effects apparently include cell differentiation, cell growth, and steroidogenesis.

Human Health

Infection and Disease

From the early days of vitamin A research, it was observed that vitamin A-deficient animals are more susceptible to disease. Supplementation of children with vitamin A reduces the severity of measles and diarrhea (although vitamin A supplementation may not reduce the incidence and duration of diarrhea). Supplementation of measles patients with vitamin A (200 000 international units (IU), i.e., 60 000 μg of RE, 209 μmol) during infection reduces hospital stay and mortality rate and is now recommended by WHO in communities where vitamin A deficiency is prevalent. On the other hand, infectious diseases such as measles, chickenpox, and respiratory diseases can lead to vitamin A deficiency. Currently, it is not believed that vitamin A supplementation can provide any benefit for other respiratory diseases (non-measles pneumonia). The mechanistic role of vitamin A status in tuberculosis, malarial infection, and HIV infection is not clear. However, it has been shown that vitamin A public health programs can have a tremendous impact on child morbidity and mortality in developing countries.

The mechanism(s) by which retinoids affect the immune system have not yet been delineated, but it seems safe to assume that control of gene expression is involved. In at least

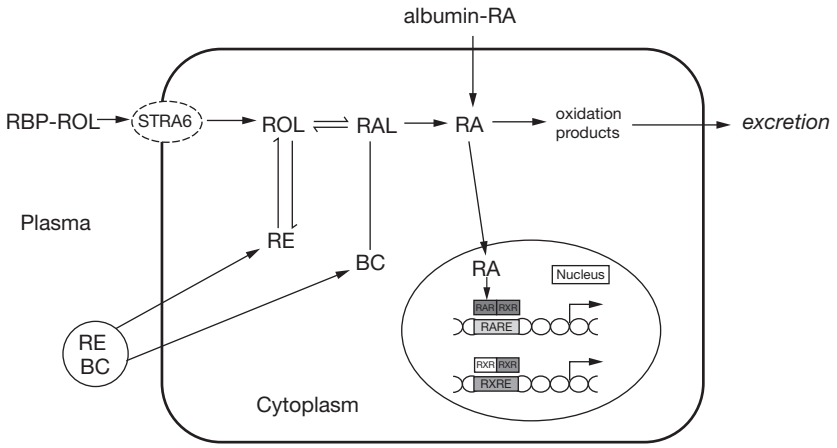

Figure 3 Intracellular metabolism and nuclear mode of action of retinoids. ROL, retinol; RAL, retinal; RA, retinoic acid; RE, retinyl esters; BC, β-carotene; RBP, plasma retinol-binding protein; STRA6, cell surface receptor for retinol-RBP; RAR, retinoic acid receptor; RXR, retinoid X receptor; RARE, retinoic acid receptor element; RXRE, retinoid X receptor element.

some experiments, the function of vitamin A can be filled by all-*trans* or 13-*cis* retinoic acid, consistent with current knowledge of retinoids and gene expression. Both antibody-mediated and cell-mediated immune responses are reduced in vitamin A deficiency. Interpretation of human studies of the relationship between vitamin A status and immune response is complicated by the fact that RBP is an acute-phase protein; the release of retinol-RBP from liver and thus plasma retinol concentrations (often used as a convenient measure of vitamin A status) fall during infection even in vitamin A-replete subjects.

Dermatology

One of the first pharmaceutical applications of retinoids was the use of 13-*cis* retinoic acid (isotretinoin, Accutane) to combat cystic acne, ichthyosis, and follicular keratosis; 13-*cis* retinoic acid promotes CRABP II mRNA expression and inhibits sebaceous gland size and activity. Routine use of 13-*cis* retinoic acid, however, is restricted because of dangers of teratogenicity. The process of keratinization (differentiation and maturation) of skin cells is affected both by deficiency and by excess of retinoids. Retinoids are now used clinically to treat psoriasis, ichthyosis, Darier's disease, and other disorders of keratinization, acne, and photoaged skin. 3,4-Didehydroretinol (dehydroretinol or vitamin A_2) and its esters, as well as trace amounts of retro-retinoids, are found in human skin (more so in epidermis than in dermis) as well and may have specific roles there. Synthetic retinoids, such as etretinate and the ethyl ester of the arotinoid TTNPB (Figure 1), as well as the naturally occurring retinoyl β-glucuronide, have been developed in attempts to reduce the toxicity of the naturally occurring retinoic acids without losing efficacy. Retinol (or retinyl esters) is frequently used as an ingredient in skin lotions.

Cancer Prevention and Treatment

At times, there has been great interest in retinoids for the prevention and treatment of cancer. They can inhibit cell transformation and tumor development in a variety of cell and animal models, but the effects are not uniform with all malignancies. In general, it seems that retinoids may not prevent initiation of tumors, but more likely interfere with the promotion process. They exert these effects by modulating growth, differentiation, and apoptosis of cells; most, if not all, of these effects may be explained by the role of retinoids in control of gene expression.

Retinoids have been tested against a variety of cancers in human and animal studies. *N*-(4-Hydroxyphenyl)-retinamide (4-HPR; fenretinide) has been tested clinically for the prevention of recurrence of human breast cancer; although results are promising, some toxicity (owing to interference with normal retinol metabolism) has been observed. Incidence of skin tumors associated with xeroderma pigmentosum is reduced by treatment with 13-*cis* retinoic acid. Perhaps the most dramatic application is the use of retinoic acid (tretinoin) against promyelocytic leukemia; this disease results from a chromosomal translocation producing the fusion of the RARα gene with the PML gene to produce an oncogene and responds well to high doses of all-*trans* retinoic acid.

Requirements and Recommended Intakes

Vitamin A deficiency is still a significant public health problem in many parts of the world, especially in young children because they are born with low body stores of vitamin A (placental transfer is limited). A variety of public health interventions are used to combat vitamin A deficiency, including dietary diversification (increased consumption of GLV and orange-yellow fruits and vegetables), food fortification, biofortification with provitamin A carotenoids (as mentioned earlier), and supplementation (periodic dosing with vitamin A in oil).

Liver concentrations provide the best estimate of vitamin A status. Human liver specimens can be difficult to obtain, but analysis of autopsy specimens can be useful in evaluating the vitamin A status of a population. As indicated earlier, serum retinol levels are maintained nearly constant (via an as-yet unclarified mechanism) and are not generally useful in

assessing vitamin A status, except when liver vitamin A reserves fall well below 0.07 μmol g^{-1} (20 μg g^{-1} of liver). Serum (or plasma) retinol levels are normally 1–2 μM (290–570 ng ml^{-1}) across a wide range of mammalian species.

Because liver concentrations of vitamin A are not readily measured, and serum retinol values are not an adequate indicator of an individual's vitamin A status, several indirect methods have been developed. Conjunctival impression cytology (CIC) evaluates the development of squamous metaplasia (enlarged epithelial cells) and loss of goblet cells from the conjunctiva of the eye by histological examination of cells transferred from the cornea to filter paper. The relative dose response (RDR) depends on the release of retinol-RBP from the liver into the serum after a large oral dose of vitamin A (typically, 450 μg of retinyl acetate in human studies); as described earlier, apo-RBP accumulates in the liver in vitamin A depletion. The RDR is calculated as

$$\text{RDR} = \{(A_5 - A_0)/A_5\} \times 100\%$$

where A_5 represents the serum retinol concentration at 5 h after the oral dose and A_0 represents the fasting serum retinol concentration at the time of the dose. Studies in both humans and rats indicate that an RDR value greater than 20% indicates liver vitamin A reserves less than 0.07 μmol g^{-1} (<20 μg g^{-1} of liver), that is, inadequate vitamin A status. The RDR assay requires, of course, that the oral dose be absorbed normally (an intramuscular injection has been used in human subjects with impaired absorption) and that protein metabolism is normal: protein deficiency or zinc deficiency or liver cirrhosis impairs the dose response. An alternate approach, the modified relative dose response (MRDR), uses the vitamin A analog 3,4-didehydroretinol (vitamin A_2, a form found in some freshwater fish and found in very low levels in human skin). The ratio of vitamin A_2 to vitamin A_1 in serum retinol at 5 h after an oral dose is used as a measure of vitamin A status, with high values indicating poor vitamin A status. Chlorinated vitamin A analogs have been used in a similar fashion. The RDR and MRDR, as well as CIC, have been used successfully in human population studies. Isotope dilution of tracer-labeled vitamin A (radioactive or stable-isotope labeling) has been used to estimate human vitamin A status, and recent developments in mass spectrometer technology are making this technique more useful.

Because of confusion among the various units for presenting vitamin A values, the concept retinol equivalent (RE) has been proposed. One RE equals 1 μg of all-*trans* retinol, either free or as the retinyl component of a retinyl ester; 1 RE = 3.33 IU of vitamin A, or 3.5 nmol of retinol or retinyl ester. Although the exact vitamin A value of carotenoids depends on several factors, the RE was defined as 6 μg of all-*trans* β-carotene or 12 μg of other provitamin A carotenoids (10 IU of provitamin A carotenoid). To account for updated findings on provitamin A value of carotenoids, the retinol activity equivalent (RAE) has been defined more recently as 1 μg of all-*trans* retinol, 12 μg of all-*trans* β-carotene, or 24 μg of other provitamin A carotenoids.

Nutritional requirements for vitamin A have not been well defined because of the diversity of vitamin A functions. In animal studies, intakes of 3–8 RE per day per kilogram of body weight (10–28 nmol $day^{-1}kg^{-1}$ of body weight) cure deficiency symptoms, and intakes of 30–60 RE per day per kilogram of body weight (100–210 nmol $day^{-1}kg$ of body weight) produce optimal growth. Kinetic studies of vitamin A metabolism in rats show that irreversible loss of vitamin A is decreased on low vitamin A intakes. Studies on vitamin A requirements have not yet addressed functional criteria such as immune function and control of genetic function.

Recommended intakes of vitamin A (preformed plus provitamin A carotenoids) are given in Table 1. Because of differences in assumptions, recommendations by the WHO/FAO are slightly different from the Canadian/US recommendations.

Toxicity

Numerous cases of human vitamin A toxicity have been reported (and doubtless many go unreported), owing to either acute (single or a few large doses ingested over a brief period of time) or chronic intake (moderately high doses taken frequently for periods of months or years). Acute toxicity in human adults is reported from doses of 300 000–10 000 000 RE (1000–35 000 μmol); chronic doses of 15 000–300 000 RE (50–1000 μmol) have produced hypervitaminosis A. Symptoms include headache, vomiting, diplopia, alopecia, dryness of mucous membranes, desquamation, bone abnormalities,

Table 1 Dietary intake recommendations

Population	*WHO/FAO recommended nutrient intake (RE/day)*[a]	*US and Canadian dietary reference intakes (RAE/d)*[b]
0–6 months	375	400 AI
7–12 months	400	500 AI
0–1 years	400	
1–3 years	450	300
4–6 years	450	
4–8 years		400
7–9 years	500	
9–13 years		600
10–18 years	600	
14–18 years, male		900
14–18 years, female		700
Adult males	600	900
Adult females	500	750
Pregnant women	800	770
Lactating women	850	1300

AI, adequate intake; RE, retinol equivalents; RAE, retinol activity equivalents.
[a]FAO/WHO. (2004). Vitamin and mineral requirements in human nutrition.
[b]Institute of Medicine, Food and Nutrition Board. (2001). Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc.

and liver damage. However, single oral doses of 60 000 RE in oil have been successfully used in vitamin A intervention programs for preschool children, with transient toxic symptoms observed in no more than 3% of subjects. Massive doses of β-carotene are not toxic, but may be less efficiently absorbed and used than vitamin A itself. (Conversion of provitamin A carotenoids is reduced when vitamin A status is high.) Rodent animal models have been extremely valuable in elucidating vitamin A requirements and metabolism, but the rat seems to be much less susceptible to hypervitaminosis A than is the human.

The toxic effects of high vitamin A intakes are mediated by serum retinyl esters; retinol-RBP concentrations are maintained at normal levels in hypervitaminosis A, but serum retinyl esters are markedly elevated, bypassing the normal homeostatic controls on vitamin A transport. Some carnivores, including the dog, are unusual in having high fasting levels of serum retinyl esters, presumably reflecting differences in lipoprotein metabolism in these species; the implications of this for vitamin A metabolism and for resistance to hypervitaminosis A are not clear.

The most tragic consequences of excessive vitamin A intake are teratogenicity (specifically, malformations of the cranium, face, heart, thymus, and central nervous system) and embryotoxicity. Acidic retinoids such as those that have been used in dermatology are particularly potent, as they can attain high serum levels and can readily pass the placental barrier. Although large intakes of vitamin A itself (>7500 RE day^{-1}, 26 μmol day^{-1}) early in human pregnancy can cause birth defects (probably because of metabolism of retinol to retinoic acid), serum concentrations of retinol and retinyl esters are normally maintained at moderate levels during pregnancy. It is assumed that these teratogenic effects are related to the important role of retinoids in the differentiation of cells and that these effects are mediated via the nuclear receptor proteins. (Interestingly, the retinoid β-glucuronides are much less teratogenic than retinoic acid.) In view of these teratogenic effects occurring at less than 10 times the recommended daily intakes, a consensus of several professional organizations is that women should avoid vitamin A supplements during the first trimester of pregnancy and that subsequent supplements, if taken at all, should be prudently limited to 8000 IU day^{-1} (2400 μg RE, 8.5 μmol), although 10 000 IU (3000 μg RE, 10 μmol) appears to be safe.

Future Directions

The pathways by which vitamin A is needed for vision and for control of gene expression have been elucidated. It is possible that new roles for vitamin A and retinoids will be discovered. The molecular role of vitamin A in immune function remains to be explained. Many aspects of vitamin A metabolism (including the mechanisms maintaining plasma concentrations of retinol-RBP) need further clarification. A current pressing need is improved methods for assessing human vitamin A status so that nutrition intervention programs can be more efficiently targeted; it seems likely that functional assays of vitamin A status (based on gene expression) will be developed. Such functional assays of vitamin A status will also lead to better estimates of vitamin A requirements and dietary allowances.

See also: Carotenoids: Occurrence, Properties and Determination; Carotenoids: Physiology; Hypovitaminosis A; Retinol: Properties and Determination.

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Retinol: Properties and Determination

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Introduction

Vitamin A is a fat-soluble micronutrient required in small amounts for embryonic development and for maintenance of nearly all tissues throughout the life span. The chemical and physical properties of vitamin A are major determinants of its metabolism, and they also dictate the procedures used for its determination. The International Union of Pure and Applied Chemistry specifies that "the term vitamin A should be used as the generic descriptor for retinoids exhibiting qualitatively the biological activity of retinol. This term should be used in derived terms such as vitamin A activity, vitamin A deficiency, vitamin A antagonist." The term retinoid, which is now commonly used to describe vitamin A and its metabolites and analogs, was coined as a generic term to cover a large number of synthetic analogs that have structural similarity to retinol, but not necessarily vitamin A activity.

Vitamin A in foods is found two forms. Certain carotenoids, also known as provitamin A, are present in foods of plant origin and, when ingested, as a precursor for the conversion to vitamin A in vertebrate tissues. Retinol and its esterified form, retinyl ester, which are also known as preformed vitamin A, are present in certain foods of animal origin. Much of the retinol or retinyl ester that is used as a component of feedstuffs, special diets, and formulas and for the fortification of foods is of synthetic origin, identical chemically to the vitamin A in foods. This article will focus on retinol and retinyl esters because these are the two major forms of vitamin A in foods and tissues. While the vitamin A metabolites retinal and retinoic acid are both extremely important physiologically, neither are quantitatively significant forms in the diet. *In vivo*, the function of retinyl esters and retinol appears to be exclusively to serve in the storage and transportation of vitamin A, which can be converted to these active metabolites in appropriate tissues.

Specific Forms of Vitamin A

Retinol

Retinol, Figure 1(a), is a lipid alcohol of empirical formula $C_{20}H_{30}O$ and molecular mass 286.44 g mol^{-1}. Although the first report of the biological requirement for vitamin A was published in 1913, it was not until the 1930s that the structure of retinol was known and its complete chemical synthesis was accomplished. A Nobel Prize in Chemistry was awarded to Paul Karrer in 1937 for his research that elucidated the structures of retinol, β-carotene, and other vitamins. Purified retinol is crystallized as a pale-yellow solid. The retinol molecule is composed of three parts, an ionone ring; a conjugated, methyl-substituted side chain; and a terminal hydroxyl group (Figure 1(a)). If retinol is produced by the metabolism of β-carotene, then its ring will be a β-ionone ring. However, some foods such as carrots contain a portion of carotene, as the α-carotene isomer (structurally α, β-carotene) and the ring of the retinol produced by its metabolism may be in the α-configuration, with the double bond between carbons 4 and 5.

The double bonds of retinol are generally in the all-*trans* configuration. While *cis* double bonds at position 9 or 11 are known to exist, such isomers are mainly found in the metabolites of retinol, such as 11-*cis*-retinal. The system of conjugated double bonds in the retinoid structure allows for very efficient absorption of light in the UV range (see the section 'Spectrophotometry of Vitamin A'). This intrinsic absorptive property is critical to the visual function of retinal *in vivo*. Light absorption is also the basis of many of the methods used for the detection and determination of retinol and related metabolites in various analytic procedures.

In vivo, the properties of retinol are modified by their interactions with specific proteins and lipid-containing structures. Retinol present in plasma is bound to a specific transport protein, retinol-binding protein (RBP), while retinol present within the cytoplasm of cells is mostly bound to one of several specific intracellular RBPs. The retinol molecule, being lipophilic, is 'membrane-seeking,' and thus, in the absence of sufficient binding capacity by these proteins, the retinol molecule can intercalate into the membrane phospholipid bilayer. Various adverse effects including membranolytic properties on the cell's plasma membrane and intracellular organelle membranes have been described.

Other forms of vitamin A found in nature include 3,4-dehydroretinol, a form present in tissues of freshwater fish and also produced *in vivo* in skin. The isomer of retinol, α-retinol, is present in some foods and has been described as having partial vitamin A activity.

Retinyl Esters

Retinyl esters are the most abundant form of vitamin A in most tissues and the form often used in preparing diets, supplements, and fortified foods. In a retinyl ester, the C-15 alcohol group of retinol is esterified with a fatty acid, usually a long-chain aliphatic acid such as palmitic acid. Esterification increases the lipophilicity of the retinyl ester molecule significantly, with retinyl palmitate being more lipophilic than, for example, retinyl acetate. Purified retinyl palmitate is a very viscous oily compound and dark red-orange in color. The empirical formula and molecular mass of retinyl palmitate are $C_{36}H_{60}O_2$ and 525 g mol^{-1}, respectively. The esterification of retinol improves its stability toward oxidation.

In vivo, retinyl esters are usually found dissolved in other neutral lipids. In plasma, retinyl esters constitute a minor component of the core lipid of plasma lipoproteins. In cells, retinyl esters are found within lipid droplets admixed with triglycerides and cholesteryl esters. Most of the data available on retinyl esters are from studies in rats and some from human tissues that, in general, show a similar pattern of esters among tissues. In the

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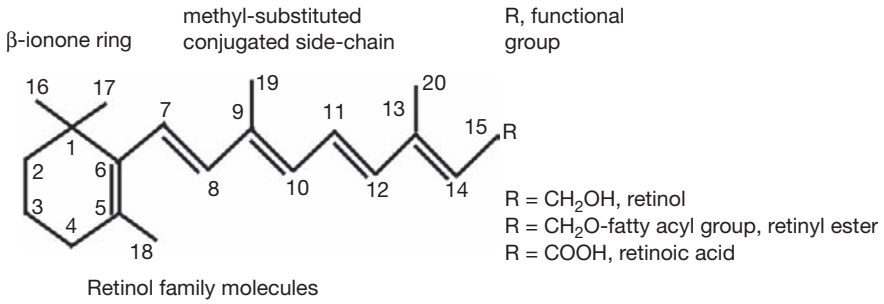

Figure 1 All-*trans*-retinol illustrating the IUPAC numbering system.

liver, where the majority of vitamin A is stored, retinyl palmitate and some retinyl stearate are the predominant retinyl esters, mostly contained in vitamin A-containing lipid droplets within the cytoplasm of hepatic stellate cells. In chylomicrons, which are formed during vitamin A absorption, retinyl palmitate, retinyl stearate, and retinyl oleate are predominant, while in milk, the variety of retinyl ester species was broader and included medium-chain fatty acid esters of retinol. Other organs that have significant stores of retinyl esters include the kidney and lung, although their concentrations are generally lower than those in the liver, while numerous other tissues may contain even lower concentrations. The retinal pigment epithelial cells of the retina comprise a concentrated, but small, retinyl ester pool. Although these patterns are common in the rodent and appear similar in humans, several interesting species-related differences have been reported. For example, some carnivores store more vitamin A per g weight in the kidney than in the liver. Dietary vitamin A intake is an important determinant of vitamin A concentrations in most tissues.

Retinal

Retinal is a metabolite of retinol, formed by the oxidation of the C-15 alcohol group. It is also the first metabolite of β-carotene cleavage that is formed by oxidative cleavage of the C-15,15′ double bond. Except for the aldehyde group, the structure of retinal is identical to that of retinol. Its empirical formula is $C_{20}H_{28}O$ and molecular mass 284.4 g mol^{-1}. Through redox reactions that are catalyzed by different enzymes, retinal can be either reduced to form retinol or oxidized to form retinoic acid. The reductive reaction appears to be predominant, as feeding retinal results in an increase in the concentration of retinyl esters in vitamin A-storing tissues. Normally, however, the concentration of retinal is very low, even undetectable, in most tissues because all-*trans*-retinal is present only transiently as a metabolic intermediate. In the retina, however, the concentration of retinal, in the form of the 11-*cis* isomer, is relatively high. Most of this 11-*cis*-retinal is bound as a Schiff base to one of the visual pigment proteins, either rhodopsin, which is a major constituent of the outer membrane disks of rod cells, or one of the iodopsins in the outer membrane disks of cone cells. The binding of 11-*cis*-retinal serves to sequester retinal within the protein structure in a manner that shifts the wavelength of maximal light absorption from 365 nm, as found for 11-*cis*-retinal in organic solvent, to higher wavelengths for rhodopsin, 500 nm, and for each of the three major cone pigments, resulting in selective 'tuning' of the red, green, and blue cone cells for their specific color vision. During the process of phototransduction, also known as photobleaching, 11-*cis*-retinal becomes released from the visual pigment proteins and then is regenerated through a series of reactions referred to as the visual cycle. This cycle involves the metabolism and isomerization of the retinoid and then the binding, again, of newly generated 11-*cis*-retinal with a visual pigment protein.

Retinoic Acid

All-*trans*-retinoic acid (vitamin A acid) was first reported in 1946. It is a crystalline yellow solid of empirical formula $C_{20}H_{38}O_2$ and molecular mass 300.0 g mol^{-1}. The UV absorption properties of retinoic acid are similar to those of retinol, but the absorption maximum is slightly redshifted to ~345 nm, and, due to the carboxylic acid nature of the molecule, the absorption spectrum is sensitive to changes in pH.

In plasma and cells, retinoic acid is typically present at much lower concentrations than either retinol or retinyl ester. Similar to fatty acids, retinoic acid binds to albumin in plasma. Essentially, all of the biological functions of retinol, except for its role in vision, can be replaced by retinoic acid. This was first shown in studies in which retinoic acid was fed to vitamin A-deficient rats, which thereafter resumed growth and were generally healthy. However, visual function was still impaired after retinoic acid treatment, indicating that *in vivo* there is no mechanism for the reduction of retinoic acid to retinal.

The functions of retinoic acid *in vivo* are mediated to a significant extent through its binding to nuclear receptor proteins known as retinoic acid receptors (RARs), RAR-α, RAR-β, and RAR-γ, which are transcription factors. A larger number of genes, estimated at over 300, are responsive to retinoic acid under physiological conditions, with most showing increased but some decreased transcriptional activity in the presence of retinoic acid. Well-defined DNA sequences, known as retinoic acid response elements, have, however, been characterized for only a small proportion of these genes. Receptors may also be involved in indirect mechanisms, such as through protein–protein interactions and nongenomic mechanisms.

Oxidized Metabolites

The retinoid structure is subject to oxidation by several types of enzymes, including cytochromes P450 that introduce epoxide,

hydroxyl, and keto groups onto the ring structure or the side chain of the molecule, creating molecules that are referred to collectively as polar metabolites. The polar metabolites of retinol and retinoic acid have been found. While their function is still debatable, it is generally thought that they are formed as elimination products during the normal metabolism of vitamin A, especially when higher amounts of retinol are consumed or after pharmacological administration of retinoic acid. Very polar metabolites, which are soluble in aqueous media, can be formed by the addition of groups such as glucuronic acid, forming retinyl or retinoyl β-glucuronide. Polar and aqueous-soluble metabolites are found physiologically in bile and are excreted mainly in the feces and, in lesser quantity, in urine.

Vitamin A for Use in Foods and Supplements

Stability. For use in the food industry, vitamin A is added as carotene or as retinol or retinyl ester. Antioxidants may be added to vitamin A premixes. Such compounds are butylated hydroxyanisole, butylated hydroxytoluene (BHT), and tocopherols (vitamin E). Chelators may be added to bind trace metals (especially iron and copper) that accelerate the degradation of vitamin A. Gelatin and food starches may be added to commercial preparations of vitamin A as carriers and/or to improve stability. The stability of vitamin A is also adversely affected by acidity, and below a pH of 5.0, vitamin A is very unstable. Thus, compounds that maintain a neutral pH are likely to improve the stability of vitamin A. Carotenoids and vitamin A compounds are easily degraded by light, oxidants, and heat. Therefore, they should always be stored in the cold under conditions that exclude light and air (see succeeding text). This is especially true for any reference standards, which should be checked periodically for quality.

Preparations Used Specifically for Vitamin A Supplementation

Certain specialized preparations of vitamin A have been used for various purposes, especially for supplementation in populations with a low intake of vitamin A.

Vitamin A capsules

Vitamin A in the form of retinyl palmitate is the major form that has been used for reducing vitamin A deficiency in at-risk populations. Because retinol is stored in the body as retinyl ester, which can be hydrolyzed to release retinol as needed, it is possible to deliver a relatively large dose of vitamin A, but below an amount that would result in acute toxicity, on a periodic basis. Doses equal to 100 000–200 000 international units, 30–60 mg of retinol, in the form of retinyl ester, given at 4–6-month intervals, have been shown to reduce morbidity and mortality in children and pregnant women. Capsules have been designed to provide this quantity of vitamin A for use in public health programs. The supplement is packaged into small single-dose gel caps that protect the vitamin A from light.

Vitamin A admixed with other ingredients

The topic of micronutrient supplementation of foods, including fortification with vitamin A, was reviewed by a United Nations Advisory Group on Nutrition in 1998. A general approach to micronutrient supplementation for vitamin A has been its addition to products that are widely used in a population, consumed in rather standard quantities, of low cost, and amenable to appropriate packaging and distribution. Examples include vitamin A fortification of monosodium glutamate, an approach used in Indonesia, vitamin A-fortified sugar as produced and tested in Guatemala for reduction of vitamin A deficiency, and vitamin A-fortified margarine in the Philippines.

Spectrophotometry of Vitamin A

UV Light Absorption Properties

Retinol possesses a very characteristic UV absorption spectrum. The spectrum is slightly influenced by the solvent in which the molecule is dissolved but altered rather markedly when retinol is bound to proteins such as RBP. Most published data are for all-*trans*-retinol dissolved in either absolute ethanol or hexane (petroleum ether). In these solvents, the UV absorption maximum is at 324–326 nm. There is a slight shoulder at ~318 nm, and the absorption drops off significantly at wavelengths above the maximum. Thus, determining the UV absorption spectrum using a spectrophotometer that can scan from ~260 to 400 nm, through a UV-transparent cuvette or flow cell, such as quartz, will give a good first indication of the purity of a preparation. If the oxidation product, retinal, is present, then the absorption will be increased at wavelengths above the UV maximum for retinol, as the spectrum for retinal is much stronger at these higher wavelengths. There should be no absorption of retinol above 395 nm.

Determination of Mass Using Extinction Coefficients

Because the researcher is often dealing with small amounts of retinol or retinyl esters, the most accurate means of determining the quantity or concentration is by using spectrophotometry, rather than by attempting to weigh the sample. When using larger quantities, determination by weighing may be suitable. However, spectrophotometry has the additional advantage that the retinol solution can be kept out of the air, in the dark, and in the presence of a suitable antioxidant, if desired. The extinction coefficients for retinol and its major metabolites are shown in Table 1. For retinol, the molar extinction coefficient (value for the absorption of a 1 M solution under standard conditions in a 1 cm light path) is 52 770 in ethanol and 51 770 in hexane. Absorption maxima and extinction coefficients for a variety of other retinoids, including both naturally occurring forms and numerous synthetic analogs, can be found in references such as by Furr et al.

Because the molar extinction coefficients for retinoids are relatively high, it is necessary to dilute the stock sample(s) before reading on a spectrophotometer. Solutions must be diluted into the low micromolar range to give a suitable reading. For example, using a 1 cm cuvette, a 1.0 μM solution of pure retinol in ethanol is expected to have an absorbance reading at 325 nm of 0.527, which is within the range of good sensitivity for spectrophotometry. After reading to

Table 1 Maxima of absorption spectra and molar extinction coefficients of retinol, retinyl esters, and selected biological metabolites of vitamin A

Analyte	*Solvent*	*Maximum absorbance (nm)*	*Molar extinction coefficient ($mole^{-1}\,cm^{-1}\,l$)*
All-*trans*-retinol	Ethanol	325	52 770
All-*trans*-retinol	Hexane	325	51 770
α-Retinol	Ethanol	311	47 190
All-*trans*-retinyl palmitate	Ethanol	325	49 260
All-*trans*-retinal	Ethanol	383	42 880
11-*cis*-retinal	Ethanol	380	24 935
All-*trans*-retinoic acid	Ethanol	350	45 300
All-*trans*-retinoyl β-glucuronide	Methanol	360	50 700
All-*trans*-retinyl β-glucuronide	Methanol	325	44 950

Data from Furr et al. (1994), where data for many nonbiological retinoids can also be found.

determine the experimental absorbance, the Lambert–Beer law (absorbance = molar extinction coefficient × path length (cm) × concentration, M) can be applied to calculate the exact concentration of the diluted material. The dilution factor is then applied to calculate the exact concentration of the stock solution. Before using retinol, the solvent can be evaporated under inert gas, nitrogen or argon, and a suitable vehicle added to achieve the desired concentration. The vehicle selected might be ethanol or dimethyl sulfoxide if the purpose is to add retinol to aqueous media, for example, for a cell culture experiment, or it might be an oil or purified triglyceride for the addition of retinol to a diet or for oral administration.

Handling and Determination of Vitamin A

Storage

Crystalline retinol should be stored in a tightly capped vial, either low-actinic red or brown glass or clear glass wrapped in foil, after introducing a stream of nitrogen just long enough to evacuate the air, at −20 °C or lower, inside a desiccator or within a tightly capped container containing a desiccant such as calcium chloride. Purging the sample or the secondary container with nitrogen may also help to stabilize the compound during storage.

Retinyl esters can be stored neat (dry), provided they are kept cold and protected from air and light. Retinyl ester is also a suitable material for the preparation of retinol, by hydrolysis, when small quantities are needed, as for research purposes.

Liquid stock solutions can also be stored for a relatively long time, provided they are protected from light, heat, and oxygen. For research purposes, it is often convenient to store retinol as a solution in organic solvent, with ethanol being preferred because it is less volatile than hexane. In the author's laboratory, we typically prepare retinol beginning with a high-quality commercial retinyl ester, which is very stable on storage, and then generate free retinol by saponification. The nonsaponifiable lipid fraction containing the retinol is isolated by partition extraction into hexane. The hexane is evaporated under nitrogen, and ethanol is then added to prepare a stock solution at a concentration of about 3–10 mM, stored in the dark at −20 °C, purged of air. When retinol is needed for an experiment, we then dilute the sample and determine its UV spectrum and concentration, as described in the preceding text, and then dilute the retinol to the desired concentration in a solvent of choice for the experiment to be conducted.

Retinol has been reported to be stable for years in plasma or serum in samples stored at −70 °C. Due to the UV sensitivity of retinoids, care should also be taken to prevent light exposure when collecting tissues, transferring and storing samples, and weighing them for analysis. The problem is most severe for samples that contain small amounts of vitamin A compounds or samples that are of small size so that light may more readily penetrate them.

Room lighting can be controlled by using a yellow or red light source that does not emit UV light or, alternatively, placing a UV filtering material over the ambient lights. The author's laboratory has used plastic sheets of UV filtering material, cut to fit over the overhead lighting fixtures, and has found that this is sufficient for blocking UV light for most work with vitamin A, such as for the analysis of plasma and most tissues.

Determining Vitamin A in Samples by Extraction, High-Performance Liquid Chromatography, and UV Detection

Numerous published methods provide details, and thus, only some of the principles of analysis and examples are discussed here. Analysis generally consists of five sequential processes: (1) denaturation of the matrix (foodstuff, plasma, or tissue) to liberate the retinoid and solvent extraction to obtain the broad class of lipids containing the vitamin A for analysis; (2) partitioning or transfer of the retinoid into a solvent that is ideal for high (or ultra)-performance liquid chromatography (HPLC or UPLC); (3) an HPLC separation step capable of resolving the molecular species of the retinoids to be detected; (4) detection, usually done on-line, using UV, fluorescence, or mass spectrometry for analysis; and (5) finally, the processing of the resulting data for quantification, usually accomplished by integration of the peaks using various software. Because few biological molecules possess both the lipophilicity and absorption characteristics of retinol, it often is possible to determine retinol levels in foods, plasma/serum, or tissues even in the presence of other molecules, like vitamin E or β-carotene, which are generally coextracted into organic solvents in the initial separation but later can be resolved by HPLC and further defined by differences in their light absorption characteristics. Several methods have been described for the simultaneous analysis of retinol, carotenoids, and vitamin E in various matrices.

Solubility

Retinyl esters are maximally soluble in hexane, petroleum ether, or another highly nonpolar solvent, while retinol and retinoic acid are most soluble in organic alcohols such as ethanol or methanol. When considering solubility, it is important to consider the entire lipid in the sample. For example, the solubility of vitamin A in extracts of lipid-rich adipose tissue or lipoproteins may be limited by the other lipid components of these materials.

The initial denaturation step typically uses an organic alcohol capable of denaturing proteins and of solubilizing the total lipids in the sample. Methods for the initial denaturation/extraction of tissues, such as liver tissue, include maceration in ethanol, lyophilization followed by extraction, and grinding the wet tissue with anhydrous sodium sulfate to remove water, followed by several extractions with a mixture of organic solvents such as hexane, ethyl acetate, dichloromethane, and methanol. Some methods use ethanol for the denaturation step followed by the addition of water and partitioning from 50% ethanol to hexane to extract (partition) the neutral lipids, which include retinyl esters, retinol, and other lipids such as triglycerides, cholesteryl esters, cholesterol, and tocopherol. Other methods, usually used for plasma, are based on the use of a more polar solvent initially, such as acetonitrile or dichloromethane/methanol, followed by centrifugation to eliminate protein residues from the organic phase that can be used directly for HPLC. The choice of method is largely determined by the type of sample, and, for each new type of sample, a recovery test should be conducted using one or more 'spiked' samples to which a known amount of the analyte of interest, or a very similar compound as surrogate, has been added, in comparison with the same sample without this addition.

Retinol and retinyl ester separation and detection

For separation, most methods are based on reversed-phase HPLC in which the column matrix (stationary phase) is nonpolar and the solvent (mobile phase) is relatively more polar. Common combinations are an octadecylsilyl, octyl, or phenyl stationary phase and either ethanol, methanol, or acetonitrile mixed with a small percentage of water, for the elution of retinol and similar molecules. Solubility is also an issue regarding the choice of mobile phase used for HPLC. The polarity of the mobile phase may need to be reduced (e.g., to 100% methanol) for the elution of the less polar molecules including retinyl esters. Even within the class of retinyl esters, the molecular polarity of various esters differs in a systematic way by fatty acyl chain length, and the methods used for retinyl ester separation, for example, for a complete analysis of milk, may need to be modified to account for this.

Detection may be either at a single wavelength or at dual or multiple wavelengths simultaneously, such as by using a photodiode array detector. For serum retinol, generally monitoring at 325 nm is sufficient. Mass spectrometry is not necessary for routine retinol or retinyl ester determination but it may become necessary if the study has used a heavy isotopic compound and isotopic enrichment must be determined. Integration is generally based on peak area after subtraction of suitable background and is generally automated within the analytic software of the HLPC or UPLC instrument.

Application to determination of plasma or serum retinol

For retinol in plasma, either plasma (anticoagulated) or serum is acceptable, and results are similar. Several approaches for retinol analysis are possible, and the first choice to be made is whether to measure the retinol fraction, without use of saponification, for example, alkaline hydrolysis. The second choice is to saponify and then measure the total retinol in the sample, which will include both the unesterified retinol and whatever fraction is present as retinyl ester. As plasma total retinol is generally >90% unesterified, free retinol and total retinol are generally similar, but in a sample collected postprandially, the retinyl ester may increase especially if a meal or diet high in vitamin A has been consumed. The third option is to measure both the unesterified retinol and retinyl ester in the unsaponified sample; this is feasible but it substantially increases the run time and thus the amount of solvent that is required. Reversed-phase HPLC is the separation method of choice. Either an internal standard method or external standard method can be used. We prefer the internal standard method because it eliminates the need for transferring precise volumes of solvent after the internal standard is added. The internal standard should be a material that is not present endogenously in the sample, but that has essentially identical extraction characteristics as retinol, and that will separate from retinol on the HPLC system that is used. Either a synthetic derivative of retinol, tocol, or potentially another UV-absorbing compound can be used as the internal standard. Reference materials suitable for use in the analysis of human plasma are available, such as SRM 968d from the National Institute of Standards and Technology, and can be used for external validation for retinol and several other plasma lipid analytes. Alternative methods are under development, especially for use in field studies. A portable fluorometer was compared with HPLC, with adequate correlation, for serum retinol analysis in a field survey situation.

Retinol in other tissues

For tissues other than plasma, or for samples of diet, feed, etc., it is important to consider the matrix (tissue or sample type – whether fibrous, easily homogenized, etc.) and the approximate concentration of retinol that is expected. For tissues that can easily be macerated, it may be preferable to pulverize/grind a weighed portion of tissue in absolute ethanol, which may contain 1 mg ml^{-1} of BHT as antioxidant, in a glass tube, using either new tubes or acid- or ethanol-washed tubes. For tougher tissues such as the lung or muscle, the same procedure may be used but the tissue may be minced beforehand, ground for a longer time, or mechanically disrupted in the solvent with a tissue homogenizer. For adipose tissue, due to the high level of total fat, it may be necessary to add another low-polarity miscible solvent such as benzene to the ethanol for complete extraction of the tissue lipids; this should be determined on trial samples beforehand. In the experience of the author's laboratory with tissue retinoid analysis, recoveries were better using direct extraction into ethanol, either by tissue maceration or mechanical homogenization, than after use of a traditional chloroform/methanol extraction method with washing. For analyzing vitamin A in diets, a sufficiently large sample should be taken, sampling from different batches or lots of the feed for the most accurate sample, and then, if the diet is pelleted, it

should be finely ground beforehand. A small weighed amount is then transferred to a glass tube and extracted with ethanol. Extraction from ethanol into hexane is as previously described. Samples of hexane are then taken for the evaporation of solvent and preparation of the sample for analysis by HPLC.

Retinyl esters

In tissues that contain retinyl esters, it is likely that several different esters may be present. For some of these esters, there is no commercially available retinyl ester to use in coelution studies in order to identify the peak. The synthesis of specific standards is an option, and acyl chloride- or acyl anhydride-based methods have both been used; these standards can be purified and kept frozen as reference materials. However, our experience has shown that the order of retinyl ester peak elution is quite reproducible, since it is based on polarity, and, therefore, if a standard method is used, the order of elution will be the same each time, and thus, comparison with published chromatograms in which multiple retinyl ester standards have been adequately identified may be sufficient to make a reasonable identification of each retinyl ester peak. In most tissue samples, retinyl palmitate and retinyl stearate will constitute the major peaks, and since retinyl palmitate is available commercially, it can be used in a comigration study to identify this major peak. In reversed-phase chromatography, the order of elution is retinol (and internal standard, if added), followed by a long elution time before retinyl esters are eluted. Generally, a gradient to a less polar mobile phase is used to speed up the elution of the retinyl esters and sharpen their peaks. Several minor esters of retinol may appear before a larger peak of retinyl palmitate and, following that, retinyl stearate. If retinyl oleate is present in the sample, it may migrate with or as a shoulder on the retinyl palmitate peak. Retinyl esters can be identified provisionally by comparison of the mass of retinol in a saponified sample with the sum of the masses of eluted retinyl esters. The UV absorption spectrum of retinyl esters and their molar (but not mass) extinction coefficients are essentially identical to the spectrum and extinction coefficient of retinol. The use of a photodiode array detector to determine the absorption spectrum of each peak can be very helpful in identifying whether or not a peak is a retinyl ester.

Analysis of retinal and retinoic acid

These compounds are not dietary components per se, and thus, their analysis is almost always performed on tissues, cultured cells, or reaction mixtures after *in vitro* assays. Retinoids such as retinal and retinoic acid require a similar but yet somewhat different approach from retinol for their determination, both because they are sufficiently different in polarity and because they are relatively reactive.

Retinal, as an aldehyde, is intrinsically reactive with amines, and methods have been developed to extract retinal and form a derivative by reaction with hydroxylamine or an acylhydroxylamine such as ethylhydroxylamine. The hydroxylamine is added to cleave the Schiff base of retinal with proteins, such as rhodopsin, or to derivatize free retinal in organic solution. The retinal oxime that is formed is much more stable than retinal and can be extracted and analyzed by HPLC. The oxime formed will represent the isomers present in the sample. *Syn*- and *anti*-conformers are formed for each geometric isomer of retinal, but they can be added up to represent the total amount for each isomer.

Although retinoic acid is a qualitatively important metabolite, it is generally present in low concentrations, for example, in the nanomolar range in plasma, as compared with retinol, which is in the micromolar range. An issue for analysis is the existence of several isomers, including all-*trans*, 9-*cis*, and, potentially, di-*cis* isomers of retinoic acid. Since all of the isomers share the same molecular weight, their separation on HPLC is very challenging and is necessarily based on small differences in polarity due to the conformational change caused by isomerization. Methods have been developed to separate these forms by liquid chromatography with quantification by mass spectrometry, in which the different fragmentation patterns of the *cis* and *trans* isomers aid in separation, identification, and quantification. Extra care is needed in preparing samples to prevent the isomerization of the native forms during processing.

See also: Retinol: Physiology.

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Rheological Properties of Food Materials

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Introduction

Rheology is a science dealing with the mechanical properties of materials, with a particular emphasis on those showing a complex behavior that cannot be classified as a Newtonian liquid or an elastic solid. Constitutive equations and mechanical models are the main objective of this science, the cradle of which is polymer science. Rheology has become more and more important for foods because they usually show a complex mechanical behavior ranging from simple liquid (fruit juices, tapered water, beverages) to hard solids (candies, dry fruits). This specific behavior of foods, is due to the existence of an internal structure, operating at supramolecular dimension, and the mechanical properties exhibited by the material mainly depend on it; consistency, hardness, and viscosity, for instance, are fully determined by the extension and strength of this network. This structure is what is perceived by consumers and is better called 'texture.' It is something different from what is usually assumed as chemical structure because it is linked directly to consumer perception, more than to the presence of micro- or macrocomponents. Even flavor is determined by this structure because its release is according to its diffusivity in the food matrix. In turn, the process conditions are capable of controlling the material parameters, which allow the food to be produced with the desired 'texture.' Food rheology has the objective to make a bridge between texture and the chemical structure because rheological parameters are defined on a macroscopic scale, and from this point of view they are strictly linked to texture, but they are determined by the strength and extension of the internal network.

Because food properties are a combination of different contributions that can hardly be reduced to simple rheological parameters such as viscosity or elastic modulus, food rheology has been widely approached in a rather empirical way. Consequently, an incredible amount of experimental or imitative devices and fitting models have been proposed even though poor theoretically. In the last few decades the requirement of quality products has become more exacting to remain competitive; therefore researchers have been asked to tackle food production using a higher degree of technology and science. Consequently, the use of rheological techniques has also increased. Fitting equations and empirical or imitative devices are increasingly substituted by fundamental measurements and rheometers in industrial use. This has been possible because, despite apparent big differences, common aspects and features can be easily recognized, and consequently, foods can now be classified as a unique material class to which similar theoretical models and experimental techniques apply: material properties will differ only for their intensity. It should be admitted, however, that there is still a trend to approach this task in an empirical way both experimentally and theoretically; therefore, in the following, the two approaches called 'diversifying' and 'unifying' are both detailed.

Finally, it should be observed that the unifying approach is very helpful in process design, particularly when producing new products of a desired texture, whereas the diversifying approach allows only the final product properties to be determined.

Diversifying Approach

This approach has been used for many years and was necessary to tackle rheological characterization when the availability of instruments was very poor and very expensive. Any food is considered as a single system, and properties are found by iterating a simple test several times to find an acceptable result. The experimental solutions are based on experience and linked to technological or consumer evidence. This way to operate can be very helpful for well-consolidated productions, but it is useless for new products and process design because, as already stated, it needs a lot of measurement to be reliable.

Experimental Aspects

There are an incredible number of experimental devices measuring the mechanical properties of foods in an empirical fashion. The difficulty to have a complete assessment of the rheological parameters has driven researchers in the past to find rather empirical solutions to characterize foods, instead of using well-defined physical parameters. One of the most used classes of these devices is the 'penetrometer' based on the physical idea that the material, according to its structure, can more or less resist the penetration of a solid rod or needle. Thus, several geometries such as cylinders, cones, flat disks, or spheres are used. The penetration depth under a fixed applied force is an empirical easy way to measure food consistency. Very similar to those apparatus are 'compressimeters,' the difference being that the material is compressed, avoiding penetration. If a cylindrical tool is forced on a material, the resulting compression is proportional to the dimension of its base area (Figure 1(a)); on the contrary, if it penetrates there is the additional contribution of the lateral surfaces, and if this so-called shear deformation, defined as the deformation occurring when two parallel surfaces move one in respect to the other, is dominant, obviously, its contribution is proportional to the contact perimeter (Figure 1(b)). Because during penetration the active surface is changing, the experimental data are often reported in terms of 'yield value,' computed as the depth penetration obtained under a given force for a given time. Yield value, often defined as force minimum value needed to start flowing, is then associated with a perceived assessment: low values are associated with soft, just pourable materials, middle values to spreadable materials, and high values to hard ones.

To measure flow properties of consistent materials sometimes a squeezing flow apparatus is used. In this device

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the testing material is put between two plates that are pushed one against the other at constant velocity causing the squeezing out of the material (see Figure 2). The data are reported as the measured load to maintain a constant velocity versus the height of the gap. Of course, a sudden increase of the load is found as far as the height becomes smaller. In Figure 2 the squeeze of a biscuit dough is shown, from which it is possible to obtain a sort of measurement of its resistance to flow.

Another very popular apparatus, is the so-called back extrusion device that seems to be very helpful to determine the viscosity of soft materials. In fact, the material is put into a cup and then is forced by a plunger to flow out at constant velocity through the narrow annular gap between cup and plunger (Figure 3(a)). The resistance to flow can be assumed as a sort of empirical viscosity. This test is very helpful when treating with rather liquid dough; as an example the results obtained for a batter dough are shown in Figure 3(b), where the values of the measured force are plotted versus the cell height.

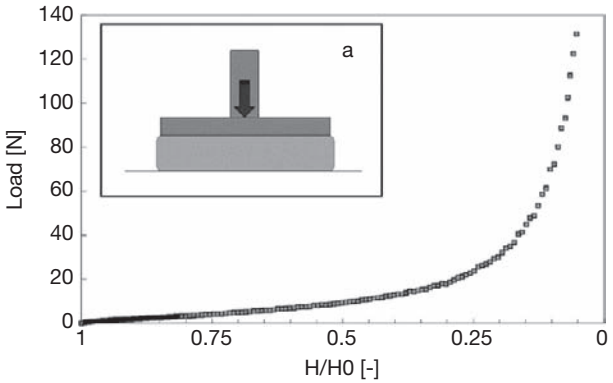

Figure 1 Sketch of compressimeters (a) and penetrometers.

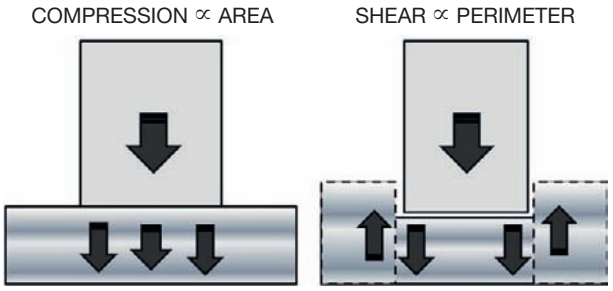

Figure 2 Squeezing flow: sketch of the device (a) and experimental data for a biscuit dough (b).

Special attention has been dedicated to dough characterization due to its importance in several foods. There are many devices used in the different applications. Farinograph, amilograph, mixograph, Brabender mixer, extensimeter, and Chopin alveograph are well-known devices widely used in the industry. However, despite their specific importance in several uses and the availability of a rather great amount of industrial data, they are very empirical, and consequently they need a lot of measurement to be well standardized. The Brabender mixer is very useful to establish the so-called rheological optimum of a dough. With this device the mechanical power adsorbed during water mixing is measured and a power versus time plot is obtained (Figure 4). During the experiment the power grows due to the increase of the consistency, attaining a maximum within a couple of minutes and falling down more or less significantly. The so-called Brabender units (BUs) are defined as a measuring scale of mechanical power, and a value of 500 is taken as reference for optimum rheological behavior. Three parameters are considered to qualify flours: development time (time needed to reach 500 BU), stability (time interval between the initial exceeding of 500 BU and before the falling down), and relaxing degree (difference between BU peak and BU value after 10 min). Strong flours are characterized by a small degree of relaxation and are stronger when the 500 BU are achieved in shorter times. Weak flours show significant values of the degree of relaxation. A strong or weak flour is preferred according to the use considered. In conclusion, one should admit that this apparatus is useful to determine the amount of water needed to form an optimal rheological dough.

Because doughs are subjected to processes implying extension, another apparatus is often used to measure the processing capability of different flours. 'Chopin alveograph' is a very popular device that consists of blowing a dough sheet to

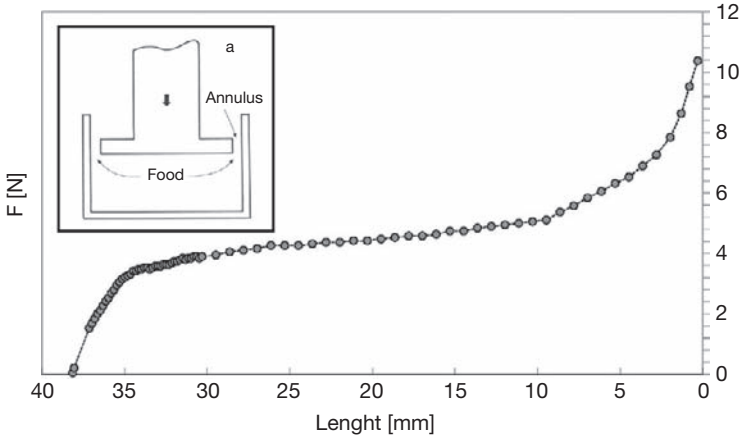

Figure 3 Back extrusion: sketch of the device (a) and experimental data for a batter dough.

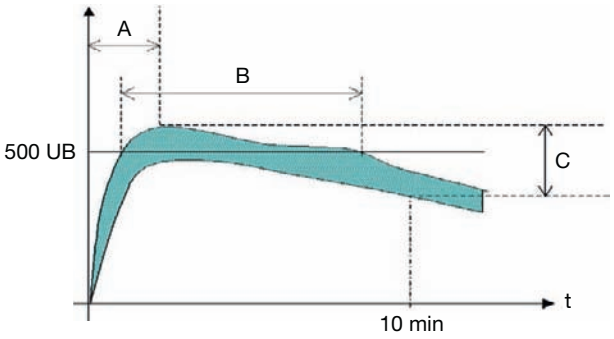

Figure 4 Measurement by a Brabender mixer: force versus time plot.

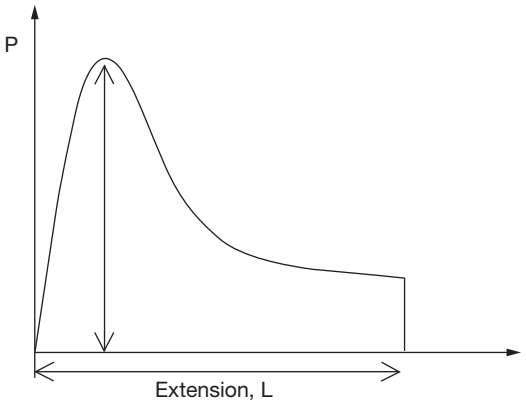

Figure 5 Measurement by a Alveograph apparatus: pressure versus extension plot.

make a bubble and measuring both pressure and extension continuously up to rupture. The shape of the resulting force versus extension plot (Figure 5) has been standardized to define some empirical parameters whose result is very helpful to address flours to different uses. Terms such as short or long flour are associated with the value of the rupture extension L, whereas strong or weak are associated with the maximum pressure P exhibited during the experiment. Even though the experimental procedure is scientifically rather questionable, an optimized ratio between P and L, based on experience, is found useful for industrial uses. For instance, based on experience, a biscuit dough is expected to have a lower P and a greater L to facilitate leavening and bubble growth, whereas a French bread should require shorter L and higher P.

It must finally be recalled that there are also a great number of devices built on an imitative base. For instance, the so-called texturometers are built to make a compression cycle that imitates human mastication. In this way the mechanical response is associated with the first and the second bite, and a qualitative measurement for the hardness, fracturability, and adhesiveness of foods can be obtained.

Theoretical Aspects

Diversifying approach theories mainly stand on data fitting being absent from any sort of generality. Rheological behavior is considered to come from the combination of three basic mechanical elements: an elastic spring, a Newtonian dashpot,

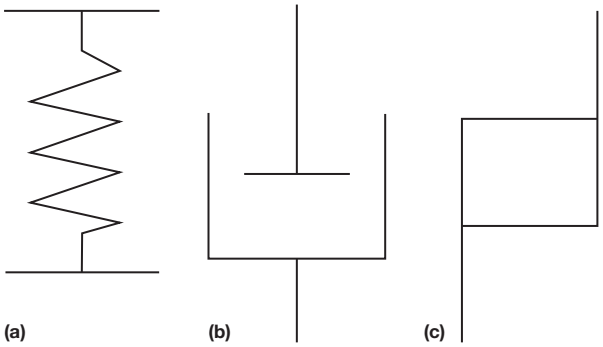

Figure 6 Basic mechanical elements: elastic spring (a), viscous dash pot (b), and plastic body (c).

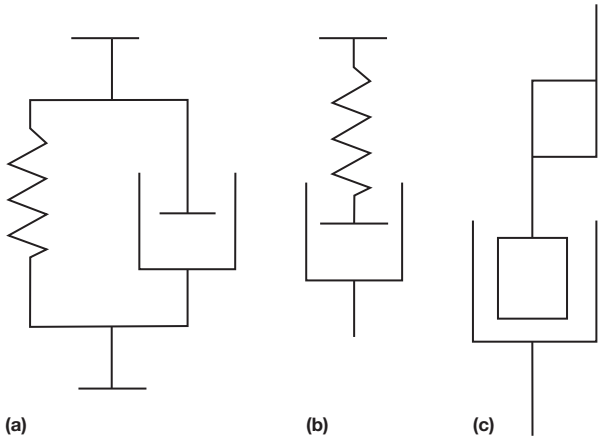

Figure 7 Combined mechanical elements: Maxwell element (a), Voigt element (b), and viscoplastic element (c).

and a plastic body (Figure 6). For instance, if the elastic element is combined with the dashpot in a serial fashion, the so-called Maxwell element is obtained, which when subjected to a fixed deformation over time relaxes the acting force. Viscoelastic behavior is well represented by this combination (Figure 7(a)). On the contrary, when the assemblage is parallel, the so-called Voigt element is obtained that, again, represents viscoelasticity but when subjected to a constant stress, defined as the force divided the area of the surface on which it acts (Figure 7(b)). The yield stress, that is, the minimum stress necessary to flow, is modeled by a friction element and takes the viscoplasticity into account (Figure 7(c)). It is possible to propose more complex combinations to fit data with the aim to separate the contributions of single components. For instance, in the case of ice cream, the contributions to the mechanical behavior of ice and fat crystals, of stabilizers, of air bubbles, of proteins can be split into elastic and viscous elements. The quantities of different ingredients in any given recipe is assumed to be a function of their contribution to the proposed mechanical model. This approach, although to some extent fascinating, seems rather questionable because of the excessive number of parameters and because the same result can be obtained by a simple numerical fitting technique.

Because a lot of rheological measurements are done with simple rheometers based on the application of selected shear

deformation rates and geometries, numerous rheological fitting equations are found that define some rheological parameters. The most-used monodimensional fitting equations are Newtonian fluid and Power law fluid. For instance the Power law equation assumes that the measured shear stress, that is, the tangential force divided by its application surface, is proportional to a power of the deformation rate: If the value of the power is 1 the behavior is Newtonian and a constant viscosity is found. Some examples of data usually found are reported in Table 1. It clearly appears that the greater is *K*, the greater is the perceived consistency, but also that *n* gives an idea of the resistance to flow at different shear rates.

Unifying Approach

The deepening knowledge of food behavior and the availability of more sophisticated but less expensive devices to measure mechanical properties has allowed researchers to collect foods in a unique frame to find both experimentally and theoretically unique solutions. This in turn has had a positive effect on food product design responding to the actual requirement of more complex systems, especially those addressed to new uses, such as healthy foods, baby food or foods for the elderly, nutraceutics, controlled release foods, and so on. In these cases it is necessary to predict the rheological properties for a proper design of the production process.

Table 1 Power law fitting parameters for foods

Food	n	K *(dynes* s^n *cm^2)*
Tomato purée	0.55	10.80
Apricot purée	0.31	200
Applesauce	0.64	5.0
French mustard	0.40	334
Tomato ketchup	0.28	187
Guar gum (1.5%)	0.16	464
Xanthan gum (1.2%)	0.26	39.8
Sweetened condensed milk	0.83	36

Experimental: Rotational and Oscillatory Shear Flow

Two rheological functions characterize complex materials: non-Newtonian viscosity and viscoelastic moduli. Viscosity is measured by rotational devices as the ratio of the measured stress to the applied shear velocity of deformation. The difference with the diversifying approach is essentially the use of rotational rheometers with specific standard geometries like cone-and-plate, plate and plate, and coaxial cylinders. Applied kinematics control allows the measurement of physically well-defined properties. The experimental test is done at different shear rates, and a so-called flow curve is obtained giving the shear viscosity as a function of the shear rate. In principle also the normal stresses difference should be measured, but up to now there have not been any suitable instruments capable of reliable measurement. Often, a pseudoplastic behavior is exhibited, which means that the shear viscosity decreases when the shear rate is increased; more rarely the opposite dilatant behavior is observed in foods. The flow curve is usually reported as shear viscosity versus deformation rate, and often fitting is done using a power law equation or a cross equation. Often food viscosity shows a constant value at low shear rates called zero shear viscosity, then it decreases, sometimes attaining another constant value at high shear rates. This latter is interpreted as the material resistance to flow when the internal structure is completely destroyed. In Figure 8 the shear viscosity of a chocolate sample is shown.

When fitting is done with the power law, the value of the constant *K* is interpreted as the measure of the consistency of the considered food, whereas the value of the power *n* is linked to the rate of destruction of the internal structure. In Table 2 the most-used models are reported.

Another rotational test is the creep test, consisting of applying a constant force and measuring the resulting deformation: the rheological parameter is the compliance defined as the deformation divided by the applied stress, the force per unit of surface. When compliance is not determined by the applied stress, a linear behavior is evidenced, and compliance assumes the meaning of a material property: Very often the linearity range is limited to very low stresses. In this test the material always starts to show a solid behavior and at long times can

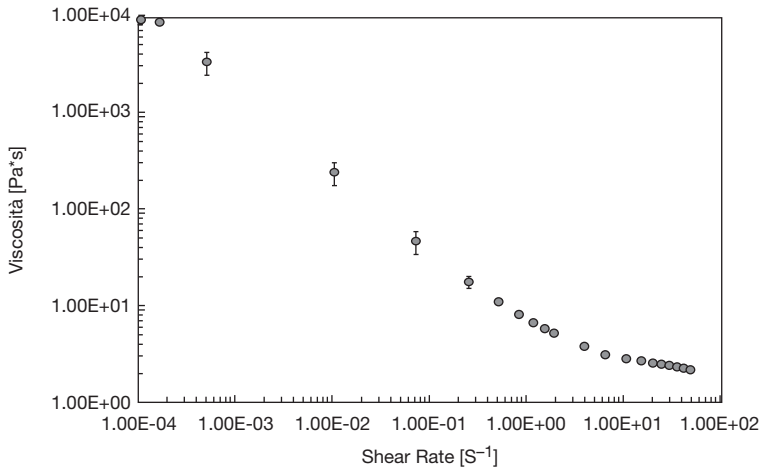

Figure 8 Flow curve of a chocolate sample.

exhibit a liquid-like behavior if the slope becomes constant, the reciprocal of this being viscosity, or a solid-like effect when the slope is zero. The test is very useful to measure yield stress because the transition between no flow and flowing is evidenced by the slope change.

To get more information about food behavior, creep is often followed by a creep recovery following the sudden removal of the applied force. If the deformation, called in this case shear strain, is reported versus time, the mechanical response of the material may be interpreted as an instantaneous strain just at beginning when the stress is applied, followed by a primary creep and a secondary one when the relationship becomes linear. At this time the applied stress is removed with the consequence of an instantaneous recovery, followed by a time-dependent recovery, finally ending, after some time, with a permanent strain. The test depends on the applied stress, and any different shown behavior may be linked to specific material parameters. Semolina dough creep and creep recovery test is shown as an example in Figure 9.

It should be noticed that from a theoretical point of view, the aforementioned parameters are rheological properties according to the deformation history; therefore they can be used strictly only when the material is subjected to an equivalent flow or deformation. This can be overcome by measuring equilibrium properties. The solid–liquid characteristics at equilibrium are measured by applying small oscillation shear deformation in the linear region and measuring the phase angle δ of the mechanical response respect to the applied deformation. Because an ideal solid is characterized by a phase angle 0° whereas a liquid will show phase angle 90°, the measure of δ will give the relative weight of the two behaviors. Usually the mechanical response is reported as the specific force divided by the deformation called modulus and is subdivided into two contributions called G', storage modulus, and G'', loss modulus. G' is associated with elastic (solid) behavior, whereas G'' is associated with the liquid one. A sketch of a typical viscoelastic response is shown in Figure 10, reporting, in a log–log plot, the values of the two dynamic moduli against the oscillation frequency. At low frequencies liquid behavior prevails with respect to the solid behavior, whereas at high frequencies the contrary occurs. The cross frequency is assumed as a rough measure of the material inversion behavior.

It is worth noting that seldom do foods show that sharp viscoelastic behavior, and often a linear behavior of both dynamic moduli G' and G'' in a log–log plot is observed within a large range of frequencies. This specific behavior is associated with the mechanical properties of a macrostructure spread into the material that contributes to solid–liquid characteristics according to its strength and extension. Typically foods show values of G' at most one decade higher than G''.

Table 2 Shear stress models

Model	$\tau(\dot{\gamma})$
Power law	$\tau = k\dot{\gamma}^n$
Cross	$\tau = \eta_\infty\dot{\gamma} + \frac{(\eta_0 - \eta_\infty)\dot{\gamma}}{1 + (\lambda\dot{\gamma})^{1-n}}$
Casson	$\tau^{1/2} = \tau_y^{1/2} + \eta_\infty^{1/2}\dot{\gamma}^{1/2}$
Carreau	$\tau = \eta_\infty\dot{\gamma} + \frac{(\eta_0 - \eta_\infty)\dot{\gamma}}{\left(1 + (\lambda\dot{\gamma})^2\right)^{\frac{1-n}{2}}}$
Herschel–Bulkley	$\tau = \tau_y + k\dot{\gamma}^n$

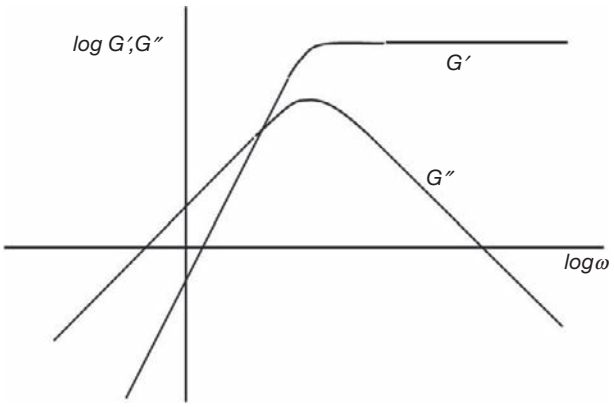

Figure 10 Small amplitude oscillation dynamic moduli G' and G'' versus oscillation frequency for a linear viscoelastic material.

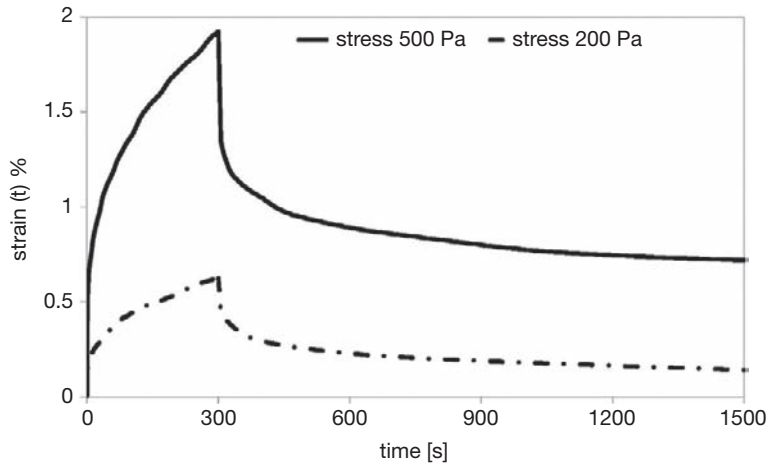

Figure 9 Nonlinear creep and creep recovery test of a semolina dough for pasta making.

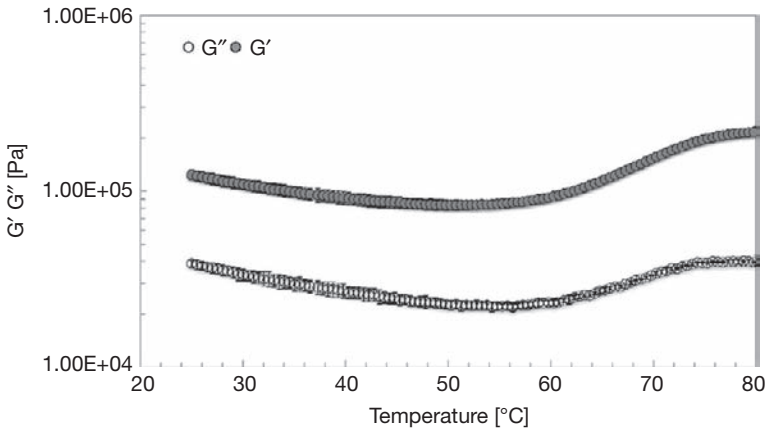

Figure 11 Dynamic moduli G' and G'' at 1 Hz versus temperature for a semolina dough sample.

In addition, a test can be carried out at a fixed frequency, usually 1 Hz, changing temperature by a suitable ramp, usually 1 °C min^{-1}, with the aim to show the structural changes that can occur. In Figure 11, for instance, the values of G', G'' are shown in the case of a semolina dough. It clearly appears that both G' and G'' moduli decrease by increasing temperature up to 60°, where protein and wheat coagulation occurs, with the consequence of a sudden and relevant increase of both moduli. The material becomes harder, and both moduli G' and G'' may become an order of magnitude higher than those shown by the dough.

Coming back to flow curves, they are very important in process design because in many operations shear flow is dominant, and shear viscosity must be determined in a large range of shear rates. To do this, different tests must be used. In fact, shear creep test is capable of covering a low rate of deformation because it is possible to apply a stress as low as possible depending on the used rheometers. For a large shear rate of deformation, a shifting technique may be adapted from polymer science using the complex dynamic modulus defined as the square root of the sum of the squares of G' and G'' and assuming that oscillation frequency is equal to shear rate.

Viscoelastic and Weak Gel Theoretical Models

A network operating at supramolecular scale and changing according to applied deformation is a well-accepted rheological model. The continuum medium is modeled as a series of topological points (entanglements) linked each to the other with strands. When applying a deformation rate, if the system remains at equilibrium (small amplitude oscillation), the number of strands and entanglements remain dynamically constant. The applied deformation is transferred from the continuum to the network in an affine way, so that what is applied to the continuum contour applies identically to the discrete network. To write the constitutive equation, some additional hypothesis is necessary regarding the dynamic and kinetic behavior of nodes and strands, the relationship between stress and deformation, the link between material parameters, and the node concentration. The interactions present in foods are rather weak when compared to chemical bonds because they are essentially van der Waals and London forces, hydrogen bonds, and so on; consequently, the network is temporary and very sensitive to deformation. A linear viscoelastic model seems to be a good approximation to represent the rheological behavior of these systems by assuming that at equilibrium the entanglements form or disappear with the same velocity, their number (network connectivity) remaining constant. By assuming a Gaussian distribution of the rheological units (nodes and strands), the effect superposition principle can be used at any time to evaluate their contribution to the total stress. It should be noted that when the behavior is solid-like, the stress will not change with time, whereas solid–liquid behavior is characterized by a relaxation following the imposition of the deformation. Materials can exhibit several different relaxing mechanism that contribute separately to their mechanical response and that can be summed in accordance with an assumed suitable distribution. Usually a so-called relaxation time spectrum $H(\lambda)$, a sort of distribution of the different relaxing mechanism, can be used to represent the modulus decreasing mechanism with time at any assumed relaxation time λ. The time-dependent modulus $G(t)$ is then obtained by means of the effect superposition principle summing all the mechanism, that is, integrating over λ if an infinite number of relaxation times is considered. This model implies that at low frequency the material should show a liquid behavior (G' proportional to the square of the frequency and G'' to the frequency) and at high frequency behaves as a solid (G' constant and G'' proportional to the reciprocal of frequency) as sketched in Figure 10; at a given frequency a cross between G'' and G' occurs, this latter thereafter becoming definitively greater. Sometimes it is possible to find also a solid behavior at low frequency then an extraelastic modulus G_E term can be added to take into account this effect. This is ascribed to the possible existence of a permanent cross-linked network with fixed node. In conclusion, in accordance with the viscoelastic network model, the rheological properties of foods are the relaxation spectrum $H(\lambda)$ and the elastic modulus G_E that can be obtained by G' and G'' measurement.

To find the relaxation spectrum this model requires the inversion of the integral defining the spectrum, and this is easily done if the liquid behavior is attained. This means that at the lowest frequencies experimentally tested it is necessary to find that the modulus G'' is proportional to frequency and G'

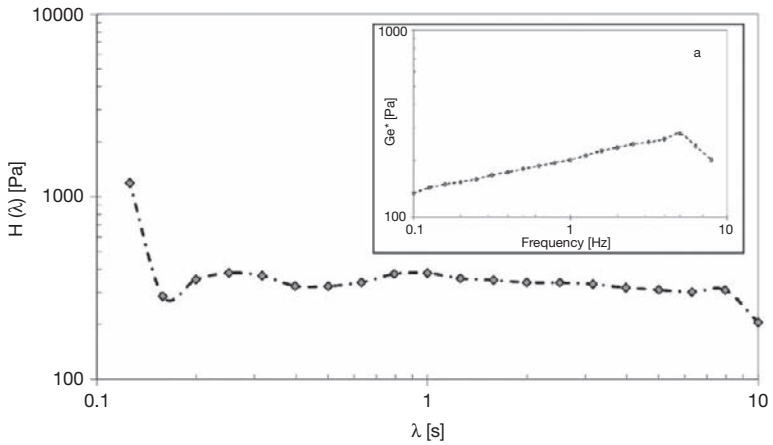

Figure 12 Rheological characterization of a yogurt sample by relaxation spectrum (a) and apparent elastic modulus.

to the square of frequency. It is a common experience that when considering foods, this occurs only for very liquid systems; on the contrary, a constant slope of both moduli G' and G'' is evidenced. However, if the relaxation spectrum is computed in these conditions, it is necessary to define an apparent elastic modulus G_E^* that, depending on the applied frequency range, allows to take into account the approximation. The two parameters $H(\lambda)$ and G_E^* are shown in the case of a yogurt (Figure 12). It must be noticed that if the frequency range is increased, the value of G_E^* decreases. From a physical point of view, G_E^* represents the mechanical response of a part of the network assumed to be permanent in the time window considered.

More recently another approach has been proposed that seems to be more realistic for foods. The viscoelastic network model is taken from polymer theories that consider bonds so strong that the strand will never break, therefore the rheological properties stand only on the variation of the entanglements that can break and be reformed as a function of the applied deformation. In equilibrium the number of entanglements remains constant. When considering foods, according to the weakness of the forces involved, it appears that the strands are more likely to break than the nodes. This means that the behavior is always solid-like, but the value of G' decreases linearly with frequency in a log–log plot with a slope ranging from 2 to 0. The limiting value 2 refers to a viscoelastic liquid, whereas the value 0 refers to a viscoelastic solid. Therefore this slope is a measure of the weakness of the network. From the cooperative flow and the gel theories, it has been possible to propose a new model called 'weak gel' that has become very popular in the last decade to interpret food rheological properties. Weak gel is a two-parameters model stating that the value of the complex modulus G^*, defined as the square root of the sum of the squares of the two dynamic moduli G' and G'', is proportional to a power of the applied frequency. The proportionality constant A can be related to the strength of the network and the reciprocal of the power $1/z$ to the extent of the network. Often, because the value of G'' is smaller than G', the complex modulus is approximate to the latter. This model is very simple because it requires only two parameters avoiding to invert the integral. From a physical point of view this means that foods are considered weak solids at least within the time range considered.

Table 3 Weak gel parameters for jams at 25 °C

Jam	z	A *(Pa)*
Apricot	4.17	686.4
Cherry	6.72	479.6
Strawberry	6.33	522.8

Table 4 Temperature dependence of cherry jam weak gel parameters

Temperature (°C)	z	A *(Pa)*
25	6.72	479.6
40	7.41	323.1
60	3.92	85.0
80	4.72	84.9

The weak gel model, for instance, may be very useful in the product design of jams. The choice depends on the rheological matching between the product and the jam, taking into account the temperature behavior due to the cooking in an oven. In fact, krapfen are filled and therefore they need a jam capable of flowing and diffusing into the product during cooking, whereas cakes are covered with a top layer that should remain sticky over its surface. The rheological behavior of jams is consistent with a weak gel model. The values of the two parameters are reported in Table 3. Apricot jam shows a smaller network extension but a rather strong strength, whereas cherry and strawberry jams show larger extension and less strength. The temperature dependence of A and z is quite different because apricot jam is very stable, whereas strawberry jam becomes very liquid. Cherry jam has a particular behavior showing a minimum of the network extension (Table 4). This information suggests to use apricot jam as topping and strawberry jam for filling. Some active rheological ingredients may be added to the original recipe to vary these conditions.

Conclusions

The unifying approach is justified by several cases of experimental evidence out of the results obtained that clearly demonstrate the possibility of defining a unique class of materials. The majority of foods, when subjected to small oscillatory deformation, show similar features: G' values increase with the oscillation frequency and decrease when increasing the applied deformation. There is always a nonzero value of G'', and it is approximatively within one decade less than G'. When high deformations are applied, the following characteristics are often found: stress overshoot in starting shear, plasticity, thixotropy, elongational viscosity, syneresis, and low mechanical stresses. In this respect, from a rheological point of view, foods are very similar but differ each from the other only in the intensity of their properties. The main result is therefore that so far the structure present in the food can be fully measured by a unifying rheological approach, but it should be admitted that the link between texture and structure is still too weak and has to be developed to produce predictive models. The weak gel model is the closest to achieving this result and is very interesting not only due to the theoretical aspects, but also because of the few parameters involved. Actually the equilibrium behavior seems to be understood enough to allow a link to be made between chemical structure, process, and ingredients. This, in turn, allows the making of good rheological process models very useful for designing and controlling industrial product, but on the contrary, the link between texture and rheology is still suffering from a lack of suitable parameters that define attributes and descriptors closer to reality, even though in this matter some progress has been made.

See also: Aerated Foods; Biscuits, Cookies and Crackers: Nature of the Products; Bread: Dough Mixing and Testing Operations; Butter: Properties and Analysis; Food Colloids and Emulsions; Pectin: Properties Determination and Uses; Quality Control in Food Processing; Starch: Structure, Property, and Determination.

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Rhum–Ron–Rum: Technology and Tradition

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Introduction

Rum is an alcoholic beverage resulting from the distillation of fermented raw materials with high sugar contents. Its alcohol content varies from 35% to 54%V V^{-1}, measured at 20 °C. The various legislations of the countries where the beverage is produced determine the minimum alcohol content rum must have. For example, in Brazil, the legal contents range from 38% to 54%V V^{-1}; in Venezuela, the amount is 40%V V^{-1}; in Colombia, it is 50%V V^{-1}; in Cuba, it is not <35%V V^{-1}; and in Spain, the minimum is 37.5%V V^{-1}. For some special types of rum, such as those produced in the Canary Islands (Spain), the minimum content permitted is 30%V V^{-1}.

Rum-producing areas are mainly found in both the Spanish- and Portuguese-speaking regions of the Caribbean, as well as in areas of English and French influence, although there is a rum-producing tradition in the Spanish provinces of Granada, Malaga, and the Canary Islands. Generally speaking, areas where sugarcane has traditionally been grown are also rum-producing.

Although rum is one of the so-called congeneric beverages, that is, it has a distinctive aroma achieved during fermentation and distillation, it also has the requirement to be aged in oak barrels for a period ranging from a few months to several years, or even decades. Depending on the country of origin, other types of wood such as cherry can also be used. After aging, the rum finally acquires its final organoleptic characteristics.

The techniques involved in the production of rum can be summarized in the following stages: (1) harvesting and preparation of the raw material, (2) fermentation of the raw material, (3) distillation, (4) aging, and (5) standardizing and preparation for bottling.

Characteristics of the Raw Material

Rum must be made using sugary raw materials, usually supplemented with nutrients necessary for the initial fermentation.

The juice of sugarcane is used as the sugary raw material, as well as molasses and/or other by-products of the production of sugar from sugarcane, although molasses is the most commonly used. As a by-product of sugar production, molasses found its principal use in the making of rum. Its use in beverage production began over 300 years ago. The British Crown even passed the Molasses Act of 1733, which it imposed in all its possessions in the New World, in an attempt to exclude competition from other Caribbean regions.

In the process of sugar production, molasses consists of inorganic and organic by-products, left when most of the water (in several stages) and finally the sucrose has been extracted from the juice (*guarapo* in Cuba). Molasses is a high-density, viscose liquid, characterized by its sugars, total solids, and ash contents. The physical, chemical, and physicochemical characteristics of sugarcane molasses have an important effect on the resulting rum.

Generally speaking, sugarcane syrup mainly consists of water and sugars, such as sucrose and glucose, with smaller amounts of fructose. In an alkaline medium, fructose can produce furfural and hydroxymethylfurfural, which disrupts fermentation, and are therefore undesirable.

In addition to sugars, other elements and compounds are also present, such as metallic cations (potassium, calcium, sodium, magnesium, iron, zinc, and manganese as macrominerals and copper, iodine, cadmium, chrome, molybdenum, nickel, and vanadium as microminerals), anions (chloride, sulfate, nitrate, nitrite, and phosphate), nitrogen-containing organic compounds (glutamic acid, aspartic acid, valine, arginine, leucine, peptides, colorants, pyrazines, and pyrroles), organic acids (citric acid, malic acid, lactic acid, aconitic acid, propionic acid, and butyric acid), phenolic compounds (such as p-coumaric acid, ferulic acid, and syringic acid), fatty acids, and vitamins (biotin, pantothenic acid, inositol, thiamine, nicotinic acid, and pyridoxine). It should be pointed out that organic nitrogen is responsible for the formation of higher alcohols (such as isoamyl and isobutyl) during fermentation, which is not entirely desirable.

Many of the foregoing compounds, such as vitamins and minerals, are required for the metabolism of sugars during alcoholic fermentation. Although these nutrients are found both qualitatively and quantitatively in the molasses, other nutrients not found in molasses must be added. This is the case for inorganic nitrogen, which is rapidly assimilated and must be supplied to complete fermentation. Likewise, the addition of sulfur as a sulfate is recommended for the synthesis of the amino acids required by the yeasts, as well as phosphate.

Water and microorganisms, such as yeast and bacteria, are also essential for fermentation, but are present in molasses. Water is the medium in which other components are dissolved and it is indispensable for fermentative reactions. Yeast and bacteria are naturally present in molasses and play a fundamental role, inducing fermentation, and thereby a factor in the production of light or heavy rum, depending on the type and species of microorganism involved. The yeasts responsible for the fermentation of rum molasses must be able to generate a high degree of ethanol and the desired, balanced content of aromatic compounds. Table 1 summarizes some of the most common genera and species of yeast and bacteria found in molasses.

Apart from the bacteria naturally present in molasses, selected cultures of other bacteria may be added at the start of fermentation, mainly types of *Clostridium*, which can improve the development of alcoholic fermentation by acting in synergy with the yeast (*Saccharomyces cerevisiae*), as well as producing volatile acids (e.g., butyric acid) characteristic of heavy rums.

Alcoholic Fermentation in Rum Production

Fermentation is a metabolic process in which chemical changes take place in an organic substrate under the action of

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00598-5

cellular enzymes, typically in the absence of oxygen. It is characterized by being highly exothermic and the incomplete oxidation of large quantities of the original substrate.

In the case of alcoholic fermentation, the initial substrates are fermentable sugars such as glucose and fructose, and the end products consist mainly of ethyl alcohol, smaller amounts of other congeneric products, and the large-scale release of CO_2 (Figure 1).

In order for alcoholic fermentation to commence, a number of requisites must be met for both the raw materials and the conditions under which fermentation is to take place. One of these factors is the sugar content and both intrinsic and extrinsic nutrients found in the molasses. Other factors within the control of the rum producer are temperature, fermentation duration, the pH, the predominant type of yeast during fermentation, and the density of the medium (Table 2).

With the exceptions of temperature and fermentation duration, all these factors must be adjusted in the molasses prior to fermentation. Correspondingly, the molasses is diluted to the appropriate density to avoid malfunction of the fermentation apparatus. Particles in suspension are precipitated with aluminum sulfate; nutrients added, such as nitrogen (urea or ammonium salts) and phosphorus; and the pH adjusted with phosphoric and/or sulfuric acid. If selected yeasts or bacteria are to be added, pasteurization may be used to ensure the elimination of autochthonous microbial flora that might compete with the added flora and reduce their output. The characteristics of commercial yeast strains should promote the appropriate development of fermentation, such as high fermentation velocity, ethanol tolerance, high-temperature acidity, and osmotic pressure, as well as alcoholic output and the production of appropriate aromatic compounds. It is now also thought very desirable for these yeasts to have a 'killer' capacity, by controlling the development of other undesirable yeasts during fermentation.

In order to accelerate the start of fermentation, a mixture known as 'bottom of the vat' may be prepared at 4–10%V V^{-1} with yeasts selected from the molasses. This preferment is added to the vat and, when fermentation begins, diluted

Table 1 Most frequently bacteria and yeasts in molasses

Bacteria	*Yeasts*
Bacillus subtilis	*Schizosaccharomyces pombe* (heavy rums)
Bacillus licheniformis	*Schizosaccharomyces octosporus* (heavy rums)
Bacillus brevis	*Saccharomyces cerevisiae*
Bacillus cereus	*Saccharomyces rouxii* (light rums)
Bacillus megaterium	*Saccharomyces mellis* (light rums)
Bacillus pumilus	*Rhodotorula mucilaginosa*
Brevibacterium imperiale	*Kluyveromyces fragilis*
	Candida
	Kloeckera
	Pichia (*Hansenula*)
	Saccharomycodes
	Torulopsis

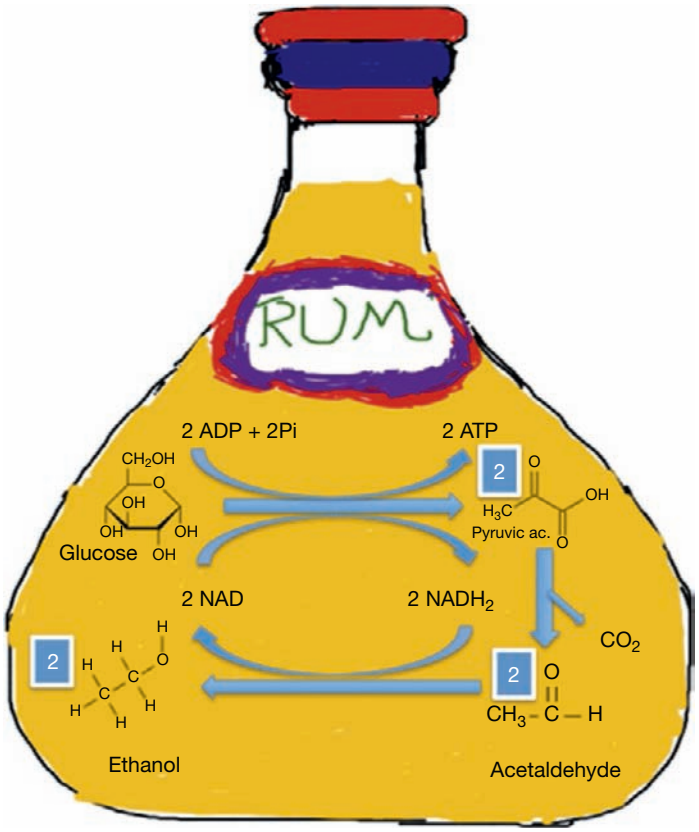

Figure 1 Alcoholic fermentation in rum molasses.

Table 2 Composition and factors molasses should gather for the start and good development of alcoholic fermentation

Nutrients			
	Contributing factors		
Sugars	*Intrinsic*	*Extrinsic*	*Conditions molasses*
Sucrose	Water	Inorganic nitrogen (ammonium salts in g 100 ml^{-1}): 2.0–2.5	Ideal fermentation temperature: 21 °C (33–42 °C max, for yeast)
Glucose	Vitamins	Sulfur (sulfate)	Fermentation time: Light rum 1.5–2 days Heavy rum: up to 12 days
Fructose	Minerals: Macros: 0.1–1 mM Micros: 0.1–100 μM	Phosphorus (phosphoric acid in mg 100 ml^{-1}): 600–750	Density (°Brix): 16–20
	Yeasts	Selected yeasts	pH: 2.5–5.5 (generally pH = 4)
Nonfermentable sugars Total sugars (g 100 ml^{-1}): 10–12	Bacteria	Selected bacteria (e.g., *Clostridium*)	

Table 3 Congener production under different yeast strains

Strain	*Acetaldehyde (g 100 l^{-1}a.a)*	*Ethyl acetate (g 100 l^{-1}a.a)*	*Propyl alcohol (g 100 l^{-1}a.a)*	*Isoamyl alcohol (g 100 l^{-1}a.a)*	*Isobutyl alcohol (g 100 l^{-1}a.a)*	*Total acids (g 100 l^{-1}a.a)*
S. pombe L/28-4-1	4.01	3.07	3.33	ND	2.31	577
K. fragilis KR 16	2.84	2.41	3.75	10.00	3.26	475
K. fragilis KR 1	1.20	2.11	2.01	5.36	2.79	517
S. cerevisiae 373	1.85	2.22	2.73	7.08	3.12	440

a.a, absolute alcohol.

molasses is gradually added to avoid excessive temperature buildup. Although the preferred temperature for fermentation is 21 °C (Table 2), the temperature range is quite wide, depending on the type of rum produced. For example, for light rums, temperature can vary from 28 to 33 °C, while for heavy, Jamaican-type rums, the range is from 30 to 33 °C, and cases have been recorded of fermentation at 37 °C. The production of dark or heavy rums always involves the use of higher temperatures and fermentation durations (Table 2), with even the possible addition of the 'dunders,' which are the nitrogen-rich residues or lees from previous distillations.

Just as there are nutrients in molasses that encourage alcoholic fermentation, other constituents may have the opposite effect. Compounds, such as hydroxymethylfurfural (≥0.05% V V^{-1}), butyric acid (≥0.1%V V^{-1}), valeric acid (≥0.1% V V^{-1}), and minerals, such as silver, arsenic, barium, mercury, lithium, nickel, osmium, lead, selenium, and tellurium (≥100 mM), can delay or even inhibit alcoholic fermentation. Acetic acid can affect yeast activity, modifying their tolerance to both alcohol and temperature. The higher the concentration of this compound, the lower the alcohol tolerance of the yeast, up to a maximum of 1%V V^{-1}, where tolerance is zero. Similarly, at a concentration of 1%V V^{-1}, the critical temperature of yeast falls to 26 °C, in comparison to a 42 °C maximum for some yeast in the absence of acetic acid.

Once alcoholic fermentation has begun, and the alcohol concentration has reached 3.5–4.5%V V^{-1}, the sugar has fallen below 6 g 100 ml^{-1}, and the fermentation temperature is below 30 °C, the *Clostridium* culture is added. The most efficient culture has a bacterial (*C. saccharobutyricum*) to yeast ratio of 1:5. The bacteria accelerate the formation of alcohol during fermentation and encourage the formation of a balanced aromatic profile. The function of other bacteria is not clear.

Table 4 Influence of pH on alcoholic fermentation

Product (g 100 l^{-1}a.a)	*pH 3*	*pH 4*	*pH 5*	*pH 6*	*pH 7*
Ethanol	171.0	177.0	173.0	161.0	150.0
CO_2	181.0	190.0	188.0	177.0	161.0
Glycerol	6.2	6.6	7.7	16.2	22.2
Acetic acid	0.5	0.7	0.8	4.0	8.7
Lactic acid	0.8	0.4	0.5	1.6	1.9

a.a, absolute alcohol.

The major end products of alcoholic fermentation are ethyl alcohol and CO_2. A number of other compounds, known as congeneric compounds, are also formed. These have an important effect on the bouquet of the rum, although others are present in the raw material. The congeneric compounds are mainly glycerol, *n*-propyl alcohol, organic acids (acetic and lactic), higher alcohols (isoamyl and isobutyl), aldehydes (acetaldehyde), esters (ethyl acetate), and fatty acids. The production of congeners is closely related to the type of yeast and the predominant pH during alcoholic fermentation. Tables 3 and 4 provide some examples of the foregoing.

A rest period of 24–40 h after the conclusion of fermentation is recommended to increase development of the rum's bouquet. Low fermentation temperatures and low pH values toward the end of the process likewise encourage the concentration of esters. It is also thought that significant concentrations of a higher alcohol, such as *n*-propanol, are the result of high concentrations of glutamic acid in the molasses.

Different fermenting systems are used in the production of rum. Discontinuous systems are commonly used in the closed batch and Melle–Boinot variants (Figure 2). These are closed processes in which the preferment and nutrients are added at the start of fermentation, allowing incubation to take place under optimal conditions. When the desired state has been reached, the fermentation vessel is emptied and cleaned and the process repeated. Multiple-stage continuous fermentation systems are also used, where the nutrients and preferments are continuously added and ferment is withdrawn from the vat or bioreactor. Conditions are thus kept uniform throughout the fermentation vessel, with a balance between nutrients, microorganisms, and desired end products obtained (Figure 3).

After the completion of fermentation, and before distillation commences, the fermented mass or wash (*batición* in Cuba) is left to stand in order for the yeast to settle at the bottom. Suspended insoluble matter is separated by centrifuge.

Distillation in Rum Production

After fermentation, the next stage is distillation. Its modern expression evolved variously in different geographic production areas. However, the industrial revolution brought about the most important change in distilling, allowing highly homogeneous beverages to be produced regardless of year, raw material, etc. A better understanding of chemistry, and especially the principles

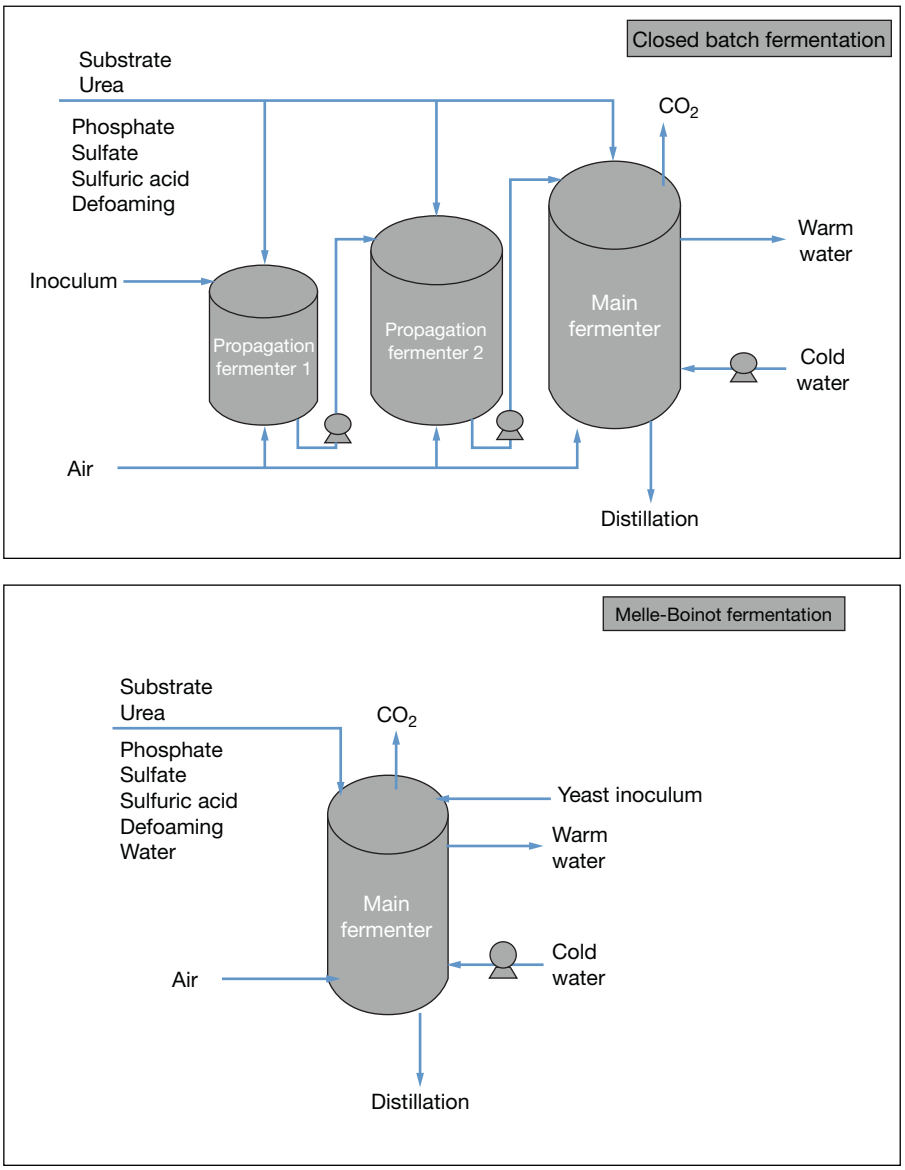

Figure 2 Discontinuous fermentation systems in rum.

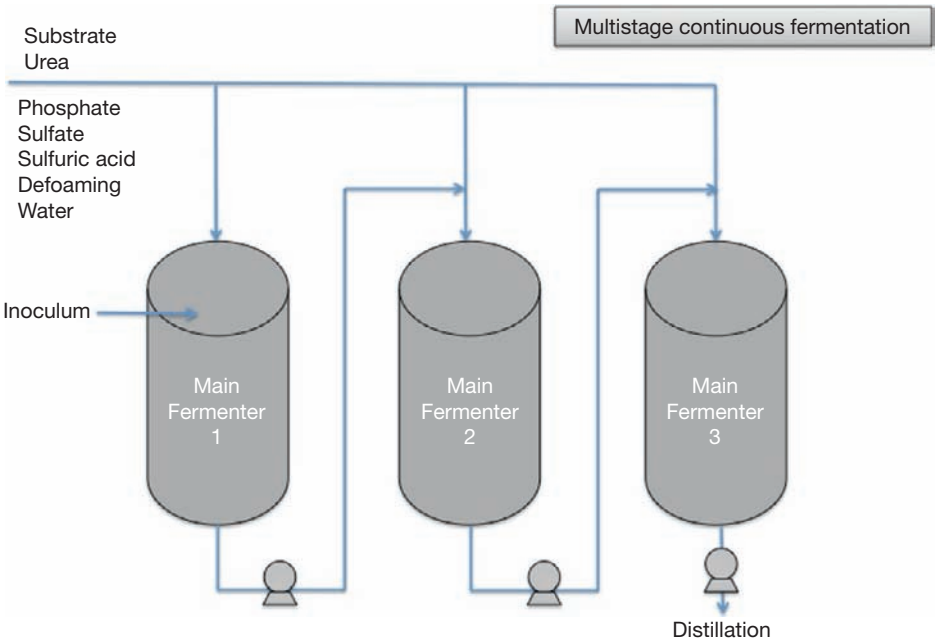

Figure 3 Multiple-stage continuous fermentation system in rum.

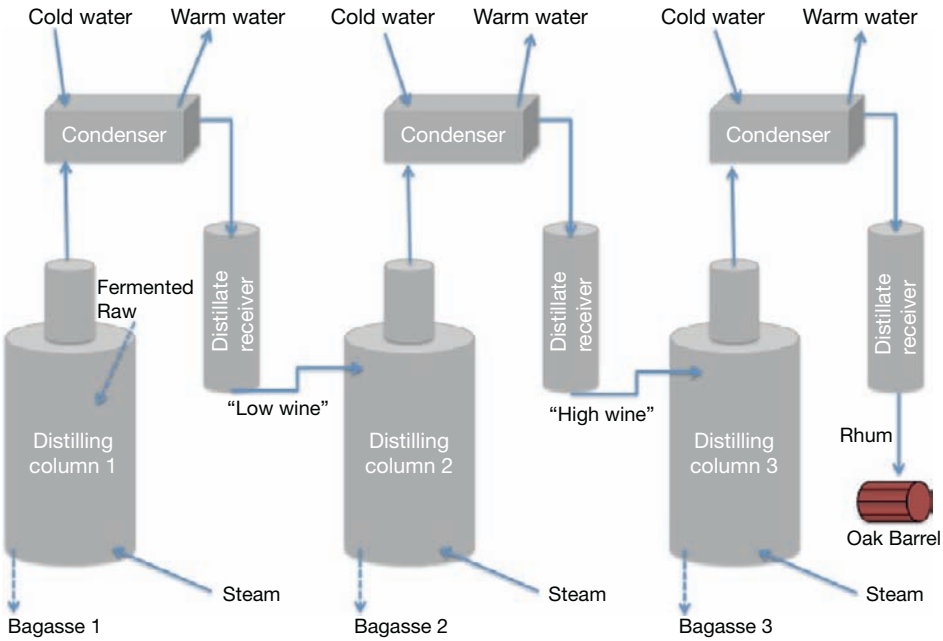

Figure 4 Batch distillation system in rum.

of evaporation and condensation, altered rum production forever.

Generally speaking, the term 'distillation' is applied to processes of vaporization, by which compounds removed with steam are recovered during condensation. More specifically, the constituents of a liquid mixture are separated by partial vaporization, and there is separate recovery of the steam and waste. Most of the volatile components of the initial mixture pass into the steam, while the less volatile constituents concentrate in the remaining liquid. In this way, the alcoholic and aromatic products of yeast fermentation (secondary aroma), as well as those from molasses (primary aroma), are separated, selected, and concentrated. Rum is made using either simple, discontinuous, batch distillation or continuous distillation in a single or multiple (3–5) column system.

Simple, discontinuous, or batch distillation (Figure 4) is used in French and English areas of the West Indies and in Brazil for heavy rums. It is typically a relatively simple,

low-cost, flexible system, producing high-quality rum, although production costs are high. A typical installation is similar to a simple pot still with three stages. The mixture to be distilled is pumped into a copper distillation vessel, from which the distillate obtained, known as 'low wine,' is collected in a second vessel where another distillation takes place, producing 'high wine.' The heads and tails of the second distillation are discarded and only the heart (middle cut) of the high wine is distilled to produce rum. The head fraction of the high wine is obtained at temperatures below 78 °C and is rich in very volatile substances such a methanol, acetone, acetaldehyde, and ethyl acetate. The tail fraction is the last to be obtained, at temperatures above 82 °C. It consists of the components with the highest boiling point: higher alcohols, furfurals, and aromatic compounds. These could impair the aromatic characteristics of the distillate if their recovery is prolonged, leading to high contents in the final product. The central fraction is collected between 78 and 82 °C and consists mainly of ethyl alcohol and desirable congeneric compounds, reaching an alcohol content of 90–95%V V^{-1}. The master distiller's skill consists in knowing how to collect only the central of the three fractions, determining when they begin and end. This ensures the absence of undesirable alcohols and the presence of a high percentage of aromatics, although correction can be carried out to obtain pure ethanol. Correction processes enrich alcoholic vapors by passing them countercurrent through liquids descending from the upper parts of a column. Inside the column, the vapors exchange heat and mass with the liquid and eventually condense upon leaving the column with partial reflux. This 'washes' components with highest boiling point out of the rising vapor flow. At the same time, some the compounds with low boiling point are lost.

Variations in continuous distillation are suitable for large-scale production (~10 000 l in 24 h), with consequent savings in fuel and time. However, a significant disadvantage is the inability to obtain a suitable degree of splitting. An installation of this type, which avoids the disadvantage, consists of a distilling column, a rectifying column, one or more purifying columns, and three or more condensers (Figure 5). The end product is complex, with alcoholic strength of 40–70%V V^{-1} and important amounts of aldehydes, acids, esters, higher alcohols, etc., and is known as *liquor*.

Distillation contributes decisively to the volatile composition of the rum distillate. For example, heating promotes oxidation, esterification, and partial dehydration, giving rise to new compounds, with an increase in the concentration of aldehydes, ethyl acetate, and other esters, as well as furfural. Sugarcane ferments (worts) contain very little furfural, but it can appear due to the dehydration of pentoses and pentosans during distillation. A wort with higher sugar contents and more acid gives rise to a higher furfural content in the distillate. Similarly, an increase in acetaldehyde content is due to the incomplete oxidation of ethyl alcohol. Acetaldehyde can also react with ethanol to produce acetal.

Another important consideration in the production of rum, and of any distilled beverage, is the presence of methanol. Because of its nerve toxicity, distillation is adjusted to keep its concentration very low in the final distillate. Methanol is metabolized by the liver to formaldehyde and subsequently to formic acid. The latter can be excreted (via urine) or oxidized to CO_2. Approximately 3–5% is eliminated in unmetabolized form via the kidneys and lungs. The minimum lethal dosage is 100 mg Kg^{-1} body weight (ranges from 20 to 100 ml methanol).

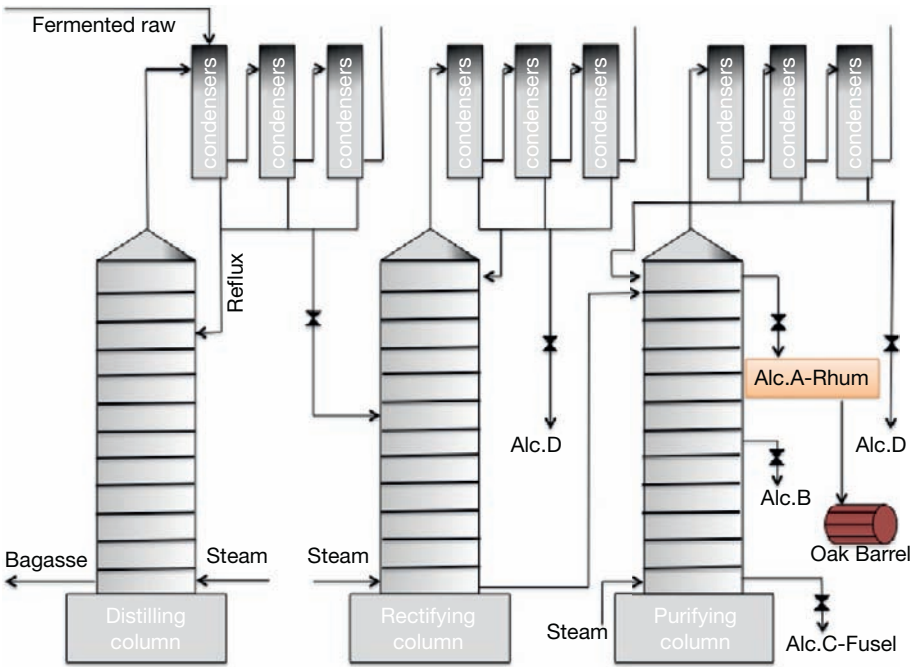

Figure 5 Continuous distillation system in rum. *Alc.A-Rhum*: The finest alcohol and used for rhum elaboration; *Alc.B*: Fine alcohol but not used for rhum elaboration; *Alc.C*: Fusel alcohol rich in superior alcohols and not used for rhum elaboration; *Alc.D*: The worst alcohol in distillation and not used for rhum elaboration.

In order to ensure the minimal concentration of methanol in the distillate, systems can be used consisting of columns that leach methanol by trichloroethylene in the liquid–vapor phase. Different regulations in rum-producing countries set maximum limits for the presence of methanol. In Cuba, for example, the maximum is set at 10 mg 100 ml^{-1}, and in the European Union and other Caribbean nations, it is 30 mg 100 ml^{-1}.

Another manner of purifying rum is by using activated carbon. This is a porous adsorbent, consisting mainly of carbon, with a high adsorption capacity for gaseous substances as vapor or in solution. This is the result of a particular thermal treatment known as activation, which increases the specific surface of the carbon and donates high porosity. The use of this product with rum decreases its acidity, as well as its phenol, ester, and fatty acid contents. It also creates a slight decrease in higher alcohol contents and eliminates lactones. If necessary, it can also have a bleaching effect.

Aging of Rum

Aging of rum involves storage in white oak barrels (cherry being used in some regions), the duration depending on the quality and type of product desired, with the aim of improving its sensory attributes. This improvement is the result of a number of transformations that take place between components of the distillate and those of the wood. These involve reactions between different compounds of the distillate, reactions between substances extracted from the wood and their oxidation, and reactions between the original compounds, extracted compounds, and those formed previously. The most influential reactions during aging are those of esterification, oxidation, and the decomposition of lignin.

Esterification depends on alcoholic strength, acid content, temperature, and duration, while oxidation is more intense at the start of the aging process. This results because of the large amount of oxygen dissolved during preceding stages. Oxidation begins by affecting the components of rum, with an increase in aldehydes and acetic acid, and over time, it also affects wood components.

The most common wood used for aging rum is oak (*Quercus*). The most widely used species are white oaks (*Q. alba*, *Q. minor*, *Q. platanoides*, *Q. iberica*, *Q. longipes*, *Q. robur*, and *Q. canariensis*) because of their excellent porosity and the contribution they make to the aromatic composition of rum, providing both volatile and nonvolatile compounds. The main role of most of the nonvolatile compounds is to act as precursors in the formation of flavorants during contact of the oak with the alcoholic beverage, whereas the volatile compounds can make direct contributions to the taste and bouquet of the rum.

Oak wood contains cellulose (40–45%), hemicellulose (20–25%), lignin (20–33%), and a heterogeneous group of soluble compounds (2–10%). The latter are important, as they can donate the characteristic color and smell of wood. This large group includes resins, volatile oils, terpenes, fatty acids, carbohydrates, polyhydric alcohols, nitrogenous compounds (proteins and alkaloids), phenolics, and inorganic components.

The decomposition of lignin (Figure 6) causes the appearance in the distillate of compounds such as coniferaldehyde and sinapaldehyde and their derived acids and aldehydes. These have an important role on the organoleptic characteristics of the rum. The longer the aging, the higher the presence of these compounds in the distillate, although extraction also depends to a large extent on the age and usage of the barrel. The same is true of other wood extractives, such as tannins and oak lactones. Similarly, the intensity of the scorching of the barrel's inner surfaces, part of its construction, also has an effect on the compounds that originate from the wood.

Although compounds that originate during aging have some active participation in its sensorial characteristics, oak lactones are possibly the most important. The two oak lactones recognized in rum are oak *trans*-lactone and oak *cis*-lactone. The

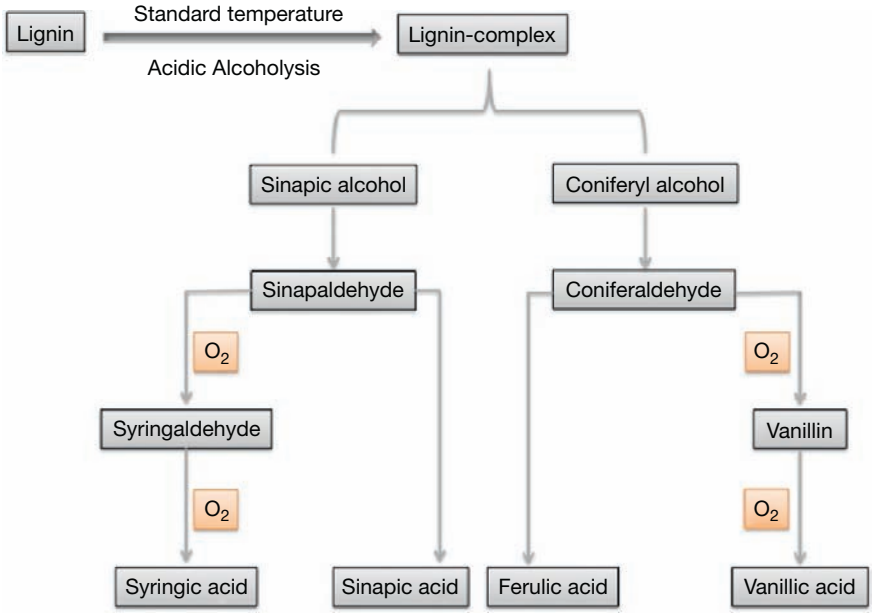

Figure 6 Decomposition of lignin.

former gives off aromatic notes of grass, straw, spices, a slight smell of firewood, and a moderate smell of coconut, while the second smells of cinnamon and strongly of coconut. The lactone contents of rum depend on the species of oak and the intensity of charring during construction. The species with highest lactone contents are Romanian and French oaks, while American oaks, which are the species used primarily for rum aging, have medium contents. The lowest concentration of lactones is found in Japanese oak, which is hardly ever used. The heavier the barrel charring, the higher the oak lactone contents. The maximum charring duration is 15 min (heavy charring). For light rums, barrels may be only lightly toasted, or not at all. Charring has several effects on wood composition: thermal decomposition of polyoxides, creating furanic aldehydes; thermal decomposition of lignin, creating volatile phenols, aldehydes, and phenolic acids; thermal decomposition of tannins, creating gallic and ellagic acids; and thermal decomposition of lipid components, creating lactones.

An important question regarding rum aging is evaporative loss. This depends on several factors, such as the density of the barrel wood, the evaporation area per unit volume, the humidity of the air in the storage area (70% is considered optimum for balance), ambient temperature, airtightness of the barrels, and the intensity of inner surface charring. Loss can be up to 12% per year, with a decrease in compounds such as ethanol, acetaldehyde, n-propanol, ethyl acetate, isobutanol, isoamyl acetate, isoamyl acid, ethyl caproate, and acetic acid.

The oak wood used in aging can have different morphological characteristics that can have an effect on the end product. The capacity of the barrels is usually 200 l or 450 l, which determines the contact surface of the distillate with the wood. In a 200 l barrel, the area of wood per liter of distillate is 90 cm^2, while for a 450 l barrel, the area is only 60 cm^2. This means that one or other type of barrel will be chosen, depending on the desired type of rum and aging period. The higher the contact surface, the more intense the organoleptic characteristics of the rum, and therefore, aging time in the barrel will tend toward the legal minima, whereas in a barrel with lower contact surface, aging times will increase for similar organoleptic results.

The barrels are stacked in different ways depending on the characteristics of the storage area. The most usual arrangements are known as *solera* and racks (Figure 7), each of which has its advantages and disadvantages. For example, the solera arrangement, also known as *solera gallega* in Cuba, has the advantage of economy of setup but is more awkward to work with. The rack arrangement has the advantage of improved barrel support, but it is expensive and complex to set up, sometimes requiring the use of forklift trucks to move the barrels.

The traditional arrangements for barrel storage (*solera* and racks) are the most common, but they have their disadvantages: large warehouses required to keep the barrels, the limited availability and cost of barrels, evaporative loss during aging, deterioration of warehouses and barrels, periodic reactivation of the barrels, and treatment of new barrels before use for aging.

In an attempt to reduce the effects of the foregoing, nontraditional aging systems have come into being. These aim to reduce evaporative loss and accelerate the aging process. To this end, these new aging techniques must achieve rapid extraction of oak compounds by the hydroalcoholic mixture, improve the conservation and increase the original volatile substances, and provide adequate oxidation of the components extracted from the wood and those present in the rum. Some of these nontraditional techniques include the use of oak extracts, oak wood chips, treatment with oxygen or ozone, the application of electrical currents to the distillate, and the application of nonionizing radiation. The most effective of these techniques are those using extracts and chips of oak wood. The others are of dubious utility.

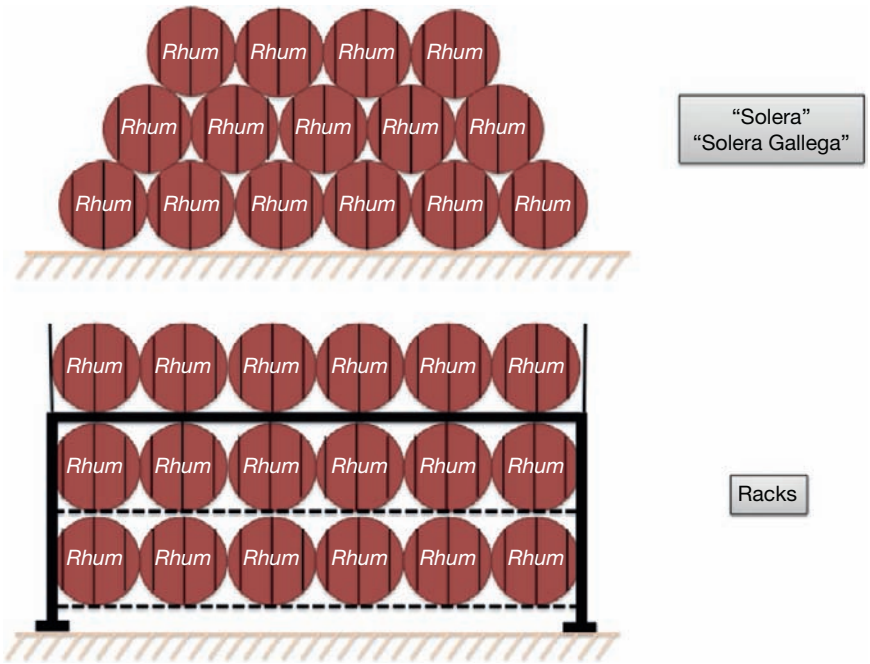

Figure 7 Most usual arrangements for aging of rum.

Final Treatments of Rum

At the conclusion of the desired aging process, and taking into account the change in alcoholic strength that may have occurred, the rum has to be diluted to the final strength permitted by the regional legislation. Adjustment of the alcoholic strength must be carried out with suitable water that does not contaminate the taste or smell of the rum. The same goal can also be achieved using rums of different alcoholic strengths to make a blend of the desired strength.

Other factors, such as the variation in the raw material, growth conditions of the sugarcane, the strain of yeast, and the techniques of distillation and aging, can lead to the same rum not always presenting homogenous characteristics. Standardizing the final rum is an important part of the producer's job. It can be achieved by blending different distillates with different characteristics from different barrels, ages, amounts of distillate, and aging processes or by using small amounts of additives that do not interfere with the product's character but provide it with an easily identifiable personality. The use of coloring syrup and caramel (E-150) is very common to standardize rum color, which can range naturally depending on aging from amber to mahogany color. The use of polysaccharides as coloring is not without risk, considering that if they have an unsuitable isoelectric point or they are not alcohol-resistant, they may precipitate. This can be avoided by the addition of alcohol prior to use, in order to ensure their suitability and stability. White or almost colorless rums can be made by decoloration with activated carbon.

The use of *solera* aging methods, similar to those used in the production of Sherry wines or Sherry brandy, can also help to standardize rum. This is the method used for rum made in Motril (Spain).

Having adjusted the alcoholic strength and blending, a number of operations are necessary before bottling to assure that the rum remains a clear, bright liquid, attractive to the end consumer. One of these operations is to allow it to rest for a variable period (from 48 h to 15 days), in order for sedimentation to eliminate components adversely affected by a change in solubility, the formation and sedimentation of some less soluble compounds, and the homogenization and balance of its blended aromatic components. Cold or coagulating agents are commonly used at this stage to achieve faster sedimentation.

Finally, the beverage is clarified using a filter system using diatomaceous earth or cellulose plates.

At present, the bottling process is no different to any other beverage. Modern machines fill and close the glass bottles, some of which can inject an inert gas (nitrogen) into the neck of the bottle to improve conservation of the beverage.

General Types of Rum

Heavy or Strong Rum

Maturation: charred oak or cherry. Aging time: 10 or 12 years up to 15 years. Final alcoholic strength: 40–60%V V^{-1}. Color: variable (colorless/amber/mahogany color). Additional coloring: caramel. Bleaching where necessary: active carbon.

Light or Smooth Rum

Maturation: oak or cherry, even uncharred. Aging time: short periods. Final alcoholic strength: 40–60%V V^{-1}. Color: variable (colorless/amber/mahogany color). Additional coloring: caramel. Bleaching where necessary: active carbon.

Commercial Types of Rum

White

These are clear, dry, light rums, although their alcoholic content is the same as in others. The liquor produced by the distilleries is colorless, so it could be said that white rums are the basis of the industry. Contact with the oak wood during aging gives the rum a slight amber color, which is eliminated by active carbon filtering before marketing, although some producers bottle without filtering, retaining the name of white rum. Due to its short aging (sometimes only 1 year), this is the cheapest rum with most neutral flavor and is therefore preferred for mixing and cocktails.

Dorado, Gold, or Ambré

It is similar to white rum, but with a more or less intense amber color. Because of its longer aging than white rum and lack of filtering, it has a more intense flavor due to the higher congeneric contents. Caramel is sometimes added to accentuate the color.

Black or Dark

They are heavy rums with strong body. Most are produced in pot stills and therefore retain a strong taste of molasses. The dark, almost black color is obtained by the addition of coloring to intensify the amber color acquired in the barrel. The main producing countries are Jamaica and Barbados. The Demerara variety is a dark rum produced in the Demerara River basin in Guyana.

Spiced or Flavored

In this category, the rum is blended with various extracts to give extra flavor. Both fruit flavors (orange, lemon, banana, pineapple, coconut, etc.) and spices (vanilla, nutmeg, cinnamon, etc.) are used. White rum is normally used for the fruit flavors, while gold or aged rums are used for spiced flavors.

Overproof

These are generally white rums bottled at an extremely high alcoholic strength (100 or more degrees British proof). There are no differences between British and Canadian overproof rums.

Premium

Premium rums are those in which aging and blending have been taken to the maximum degree of quality without economic loss. The term 'premium' is frequently used only for publicity purposes. Rums produced in small quantities for one

Table 5 Rum-producing countries

Country	*Rum kinds*	*Commercial brands*
Brazil	Sugarcane rum	Cachaça
Colombia	Old rum (light)	Caldas, Antioquía
Cuba	Carte blanche (light) Golden carte (light, colored)	Arecha, Bucanero, Caribbean, Havana Club, Isla del Tesoro
Dominican Republic	Light rum	Barceló, Brugal, Siglo
Guatemala	Light rum	Zacapa
Guyana	Demerara Courante	El Dorado, Dos Maderas, XM, Wood's, Lam's
Haiti	Simple distillation rum Double distillation rum	Clairin, Barbancourt
Jamaica	Light rum Blended rum Heavy rum (wedderburn, plummer)	Appleton, Capitán Morgan, Myer's, Lemon Hart
La Martinica	Heavy rums	Bardinet, Grappe Blanche, Clement, Gran Arome, Saint James, Negrita, Bacardí, Bailly, Duquesne
La Barbada	Light rum	Mount Gay, West Indian Rum, Barbados Dt.
The Philippines	Light rum	Pandy, Tanduay
Puerto Rico	White label Golden label	Bacardí, Boca chicacarioca, Don Q, Mérito, Ronrico, Matusalem, Bellows
Spain	Pale rum (light) Honey rum (sweet)	Montero, Bacardí, Bermúdez, Larios, Guanche, Aldea, Tropicana, Negus, Negrita, Bunti, Pujol
Trinidad	Light rum	Bacardí
Venezuela	Light rum	Aniversario, Cacique, Pampero, Santa Teresa, Estelar, Real Caruano

reason or another are special cases and may be meant for private consumption or special occasions.

Production Areas

Rum-producing areas coincide with the regions where sugarcane is grown. This mainly includes practically all of America, where Christopher Columbus introduced sugarcane from Andalusia. Apart from America, sugarcane is still grown in some parts of Europe, specifically southern Spain and the Canary Islands. There is also a small production of rum in the Philippines, doubtless the result of its colonization by Spain. Table 5 summarizes the different areas and countries where rum is produced.

See also: Alcohol: Metabolism and Health Effects; Alcohol: Properties and Determination; Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Beverage: Health Effects; Beverage: Patterns of Consumption; Brandy and Cognac: Consumption, Sensory and Health Effects; Brandy and Cognac: Manufacture and Chemical Composition; Caramel: Methods of Manufacture; Caramel: Properties and Analysis; Cellulose; Fermented Foods: Origins and Applications; Fermented Foods: Use of Starter Cultures; Flavor Enhancers: Characteristics and Uses; Food Additives: Classification, Uses and Regulation; Food Fraud; Fructose and High-Fructose Corn Syrup; Fructose: Sources, Metabolism, and Health; Gin; Glucose: Properties and Analysis; Liqueurs: Cream Liqueurs; pH: Principles and Measurement; Phenolic Compounds: Occurrence, Classes, and Analysis; Sucrose: Dietary Importance; Sucrose: Properties and Determination; Sugar Alcohols; Vodka; Whisky, Whiskey and Bourbon: Composition and Analysis of Whisky; Whisky, Whiskey, and Bourbon: Products and Manufacture; Yeasts.

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Riboflavin: Physiology

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Introduction

Riboflavin (7,8-dimethyl-10-ribityl-isoalloxazine) is the generic term used for the water-soluble vitamin B_2 but chemically describes the free-form of riboflavin. Derivatives of riboflavin, which are functionally important to humans, are riboflavin phosphate, also called flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD). Intracellularly, riboflavin is converted to FMN by ATP: riboflavin 5-phosphotransferase (flavokinase; EC 12.7.1.26); and FMN is converted to FAD by ATP: FMN adenylyltransferase (FAD pyrophosphorylase; 2.7.7.2). FAD and FMN are tightly bound, either covalently or noncovalently, at the catalytic site of numerous enzymes.

Dietary Sources and Patterns of Consumption with a Global Perspective

Riboflavin is distributed widely in foods, as free riboflavin or the derivatives FMN and FAD. In Western diets, milk and milk products generally make the largest contribution to dietary intakes; fortified cereals, meat, and fish make a useful contribution too. The fortification of some grain products with riboflavin means that certain breads and breakfast cereals are very good sources of this vitamin. Yeast extract such as Marmite can also be a very good source, even though generally consumed in small amounts.

Studies worldwide indicate that riboflavin intakes are likely to be low in populations consuming diets low in dairy and meat products. Although there are several reasons why individuals and communities consume dairy- and meat-poor diets, low income is a central determinant. However, even in resource-rich countries, riboflavin deficiency is prevalent in some groups, notably adolescents, which might be causally linked to a decline in the consumption of milk and dairy products. Although vegetarian diets can be nutrient-dense, riboflavin intakes have been reported as being lower in vegetarians than nonvegetarians in some settings.

Bioavailability, Transport, Metabolism, and Excretion

Most riboflavin is present in foods bound as FAD and FMN with small amounts as free riboflavin. Milk and eggs differ from most foods in that they contain riboflavin bound to specific binding proteins, but there is no evidence that this influences bioavailability. To be available for absorption, FAD and FMN in food are hydrolyzed in the intestinal lumen by phosphatases, releasing free riboflavin, which is transported into the enterocytes of the small intestine by an active, carrier-mediated process. The transport process is saturable at about 30 mg in a single meal. Flavokinase in the intestinal mucosa phosphorylates intracellular riboflavin, so that much of the riboflavin entering the plasma does so as FMN. Kinetic modeling following ingestion of stable isotope-labeled foods suggests that a large fraction of newly absorbed riboflavin is removed from the circulation on first-pass metabolism by the liver. Although the main site of absorption is the proximal small intestine, there is some capacity for absorption from the colon, so that riboflavin synthesized by colon microbiota may be an important source of this vitamin. Bioavailability is not known to differ between food sources, although this has been the subject of limited research activity.

Riboflavin is carried in the plasma bound to albumin and to certain immunoglobulins, which can also bind FAD and FMN. Recent data show that about 70–75% of total riboflavin in the plasma is present as FAD, 5–10% as FMN, and 15–20% as free riboflavin.

Uptake into tissues occurs by carrier-mediated diffusion of riboflavin, which is phosphorylated intracellularly to FMN and then to FAD, which constitutes the great majority of intracellular flavins. Very little intracellular riboflavin occurs in the free-form. FAD and FMN that are not bound to protein are hydrolyzed to riboflavin, which diffuses rapidly out of cells and is excreted. Evidence suggests that flavin-dependent enzymes show a differential response to progressive reduction in dietary riboflavin that is also tissue-specific. Generally, it appears that tissue concentration of FAD is conserved at the expense of riboflavin and FMN.

As riboflavin intake increases, there is a point at which urinary excretion increases markedly; this occurs at a daily intake of around 1.0 mg and is generally understood to reflect tissue saturation. About 25% of urinary riboflavin is as free-form. Riboflavin-8-α-histidine and riboflavin-8-α-cysteine may appear in the urine as a result of the metabolism of enzymes containing covalently bound FAD or FMN. The activity of hepatic cytochrome P450-linked mixed-function oxidases generates 7-hydroxymethylriboflavin and 8-hydroxymethylriboflavin, which may also appear in the urine.

Biological Function

Redox Reactions in Intermediary Metabolism

Attention has been drawn to the remarkable versatility of the flavoproteins, attributed to the wide variation in redox and electronic states in which the isoalloxazine ring of flavins can exist. Flavins act as electron carriers in a diversity of redox reactions important to intermediary metabolism. Notably, steps in the cyclical β oxidation of fatty acids and oxidation steps of the tricarboxylic acid cycle utilize FAD as an electron acceptor in two-electron transfer reactions. In the process, the substrate is oxidized, often to form a carbon–carbon double bond, and the flavin is reduced. FAD functions as an electron carrier in the iron–sulfur flavoprotein of the mitochondrial electron transport chain. Riboflavin is thus essential for energy

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generation in the aerobic cell through oxidative phosphorylation, and a lack of riboflavin compromises cellular energetics. Riboflavin-deficient mice exhibit impaired hepatic oxidative metabolism, and rats fed with a diet deficient in riboflavin show a reduction in the activity of flavin-dependent mitochondrial enzymes. Riboflavin-depletion of cultured cells leads to a decrease in ATP generation.

Disulfide oxidoreductases: FAD is also integral to a group of flavoproteins that contain an active disulfide site; these enzymes catalyze reduction of a disulfide group with the concomitant oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH). They include glutathione reductase, which is used as the basis of a functional assay for riboflavin status, and the associated generation of reduced glutathione has important antioxidant function. Lipoamide dehydrogenase and thioredoxin reductase also fall into this group.

Flavin oxidases: Flavin oxidases catalyze reactions in which the flavin is reduced by dehydrogenation of the substrate, reactions that involve an intermediate substrate–flavin adduct. D- and L-amino acid oxidases fall into this category. Reoxidation of the reduced flavins generates a flavin semiquinone radical and superoxide, which react further to generate perhydroxy radicals.

Other flavoproteins: Other flavoproteins include NADPH oxidase (the respiratory burst oxidase), important in the cytotoxic action of macrophages, and flavin-dependent mixed-function oxidases, which are active in the metabolism of xenobiotics.

Methylenetetrahydrofolate Reductase

FAD is a coenzyme for methylenetetrahydrofolate reductase (MTHFR), which is central to the function of the methyl cycle, which drives the very many and diverse methylation reactions in humans. Methionine is the direct precursor of *s*-adenosylmethionine, which serves as the methyl donor for numerous substrates including DNA, phosphatidylethanolamine (to form phosphatidyl choline), and guanidinoacetate (to form creatine). All methyltransferase reactions generate homocysteine, a sulfur-containing amino acid that is not incorporated into protein. Homocysteine remethylation to methionine depends on 5-methyltetrahydrofolate as the methyl donor, generated in a reaction catalyzed by MTHFR. Factors associated with a diminished MTHFR activity can lead to elevated plasma homocysteine concentrations. Riboflavin was identified as a factor influencing plasma homocysteine concentration in the Framingham Offspring Cohort. Plasma homocysteine concentration is responsive to riboflavin intake, but only in individuals who are homozygous for the relatively common MTHFR C677>T polymorphism.

Dietary Requirements for Riboflavin

Dietary requirements are variously expressed as dietary reference values (DRVs) in the United Kingdom, dietary reference intakes in the United States, and population reference intakes in Europe. In all cases, the intention is to convey need in terms of a daily intake that will satisfy the needs of the majority of the population, excluding those with particular requirements because of disease or metabolic abnormality. For riboflavin, values have been set between 1.0 and 1.6 mg day^{-1} for adults, varying according to the scientific body making the recommendations. There is no clear functional outcome associated with intakes less than recommendations; in the United Kingdom, requirements were based in part on the relation between riboflavin intake and urinary excretion. An inflection, at which urinary excretion increases sharply, thought to reflect tissue saturation with flavins, occurs at an intake of about 1.0 mg.

Because of the central role of riboflavin in energy-generating metabolism, riboflavin requirements are sometimes expressed on the basis of energy intake. However, in view of the wide range of riboflavin reactions unrelated to energy generation, it is difficult to justify this approach.

Consequences of Low Intakes

Prevalence of Deficiency Globally

At very low intakes of riboflavin, clinical signs of deficiency may occur, including lesions at the corners of the mouth (angular stomatitis) and at the margins of the lips (cheilosis), desquamation of the tongue, and a general seborrhoeic dermatitis. However, these clinical signs may also occur in association with other B vitamin deficiencies and cannot be considered specific for riboflavin. Clinical signs of deficiency are rare in Western countries but relatively common in some low-income countries, with some evidence of seasonal variation in incidence. The relation between dietary intake of riboflavin and appearance of clinical symptoms of riboflavin deficiency may be confounded by other dietary factors and by local infection, and the absence of clinical symptoms does not necessarily reflect adequate riboflavin status.

Biochemical riboflavin deficiency is usually assessed by measurement of the activity of FAD-dependent glutathione reductase, measured in red blood cell hemolysates. The activity of erythrocyte glutathione reductase is measured before and after the addition of the coenzyme FAD, and the ratio of the two activities (FAD-stimulated/FAD-unstimulated) is expressed as an activation coefficient, EGRAC. This test is very sensitive to changes in riboflavin intakes at the lower end of the intake range. An EGRAC value of >1.30 is the most commonly used cutoff for biochemical riboflavin deficiency. The detail in the conditions used in the EGRAC assay can have an important influence on the assay results, and this must be borne in mind when making comparisons between data describing prevalence of riboflavin deficiency in different populations.

Biochemical deficiency based on the EGRAC test has been reported in many countries and is not confined to low-income regions or populations. A high prevalence of deficiency has been documented in, for example, Gambia, India, Taiwan, Ivory Coast, and the United Kingdom. Deficiency is often associated with a low dietary intake of milk and dairy products and meat. Deficiency occurs across life stages from infancy to old age.

Many studies of the prevalence of riboflavin deficiency, particularly those conducted in low-income countries, are reliant on estimates of dietary intake. Compared with national or WHO DRVs, there is a high prevalence of inadequate intake of

riboflavin in many regions of the world, including China, Iran, several countries in Africa, and India.

However, despite evidence for low intakes and biochemical riboflavin deficiency across many regions of the world, there is still an incomplete understanding of the functional consequences of low intakes of riboflavin and poor status.

Growth

Impaired growth is a well-established hallmark of riboflavin deficiency in animals, presumed to be due to effects on energy-generating steps in metabolism and an associated reduction in ATP generation. Frank riboflavin deficiency in humans generally occurs in association with other micronutrient deficiencies, making it difficult to ascribe symptoms to riboflavin specifically. Certainly, while poor growth in association with low riboflavin intakes is common in many low-income countries, this is likely to be the result of inadequate intakes of several essential nutrients.

Lipid Metabolism (Organic Acidurias)

Much of the research into the effects of low riboflavin intakes on lipid metabolism and associated disorders has been conducted in animal models of deficiency. Rats fed a riboflavin-deficient diet exhibited altered fatty acid profiles in hepatic lipids, notably an increase in 18:2$n-6$ and a lower 20:4$n-6$. Feeding a riboflavin-deficient diet to weanling rats impaired β oxidation and led to the abnormal urinary excretion of dicarboxylic acids. Gene defects in humans, leading to flavin-dependent acyl-CoA dehydrogenase deficiencies, are associated with defects in fatty acid metabolism and organic aciduria, accompanied by a range of clinical abnormalities. People carrying these defects are responsive to high doses of riboflavin. Whether there is broader relevance to humans is unclear; β oxidation of fatty acids seems not to be impaired in riboflavin-deficient infants.

MTHFR Activity, Homocysteine Concentration, and Disease Risk

Riboflavin is one of several B vitamin determinants of plasma homocysteine concentration, which may be important because elevated plasma homocysteine concentration has been linked with a greater risk of coronary heart disease, stroke, hypertension, and some other conditions. There does however remain considerable uncertainty about the cause–effect relationship of some of these associations, which seem particularly weak when publication bias is taken into account.

The homocysteine-lowering effect of riboflavin seems largely confined to people with a reduced activity of MTHFR, and this seems to have particular functional relevance to hypertension and hypertensive disorders. A graded association between blood pressure and the MTHFRC677<T polymorphism has been reported across different populations, and evidence is accumulating to suggest that riboflavin supplementation, even at rather modest doses, can lower blood pressure in individuals with hypertension who also have the MTHFR C677T genotype.

In addition to its coenzyme role in MTHFR, riboflavin status may influence flux through the methylation cycle, through effects on the metabolism of vitamin B_6, which is essential for the metabolism of homocysteine to cystathionine, via transsulfuration. Interconversions of vitamin B_6 metabolites are influenced by riboflavin through the activity of flavin-dependent enzymes.

Cardiovascular Disease

Paradoxically, although riboflavin has the potential to generate free radicals on exposure to UV light, it is also known to have antioxidant properties. The action of flavin reductase on riboflavin generates dihydroriboflavin, which can reduce heme proteins containing ferric iron. This is potentially important because of the pro-oxidant activity of heme iron. This is thought to explain protective effects of riboflavin administered either *in vivo* or to tissues *ex vivo*, in three models of tissue damage – ischemia-reperfusion injury, activated complement-induced lung injury, and brain edema following hypoxia reoxygenation. However, although effects in animal models of tissue injury appeared promising, there has been little to suggest an application in humans. A small study showed that supplementation of patients with B-group vitamins immediately following an acute ischemic stroke mitigates oxidative damage; the supplement included riboflavin, folate, vitamin B_6, and vitamin B_{12} and so the effect cannot be ascribed solely to riboflavin.

Vision

There is a long history of reports of corneal vascularization, corneal opacity, and cataracts in animals fed with diets low in riboflavin. Whether there is a human corollary is not clear. Several studies have examined a possible relationship between cataracts in elderly people and riboflavin status; results are suggestive that higher riboflavin intake is associated with a lower risk of development of nuclear lens opacities and cataract. A long-term supplementation of riboflavin and niacin to elderly people in Linxian, China, was associated with a lower prevalence of cataract, but the study design does not make it possible to ascribe effects to riboflavin alone. Recent attention has been focused on the therapeutic role of flavins in corneal disorders. The nonenzymatic oxidation of reduced flavins leads to the production of reactive oxygen species, including superoxide. This potentially damaging reaction has been shown to have protective benefits when applied to the cornea, as the free radicals generated are thought to increase cross-linking of collagen molecules the cornea, which increases the biomechanical strength of the cornea. The treatment consists of the ultraviolet-A irradiation of riboflavin-soaked cornea; the riboflavin undergoes autoxidation, with the generation of superoxide and other radicals, which initiate the cross-linking. Results in patients have been so successful that treatment with riboflavin in its role as a photosensitizer has become standard treatment for progressive conditions of the cornea, such as keratoconus.

Iron Metabolism/Anemia

Suggestions of a mechanistic link between riboflavin metabolism, iron handling, and anemia date back to the 1950s, when riboflavin-responsive anemia was reported in humans. Subsequently, several groups conducted investigations in various animal models of riboflavin deficiency, which collectively confirmed that riboflavin depletion can lead to disturbances in iron handling. The mobilization of iron from the iron storage protein, ferritin, requires a reductive step, and this can occur through nonenzymatic reduction (e.g., by ascorbic acid) and by the activity of FMN:NADH-linked oxidoreductase (EC.1.5.1.42), which is riboflavin-dependent. Riboflavin deficiency in animals impairs iron mobilization from ferritin in the gastrointestinal mucosa, placenta, and liver, indicating a possible role for riboflavin in iron absorption, maternofetal transfer, and mobilization of iron from hepatic stores.

The relevance of these studies to humans has been the focus of some research endeavor in different populations. Findings from a large cohort in China show that low riboflavin intake is associated with an increased risk of anemia at the 5-year follow-up. An interaction between iron intake and riboflavin intake was apparent, such that when riboflavin intake was adequate, there was no relation between iron intake and anemia. Correcting a deficiency of riboflavin has been shown to improve hematological status in school-age children, pregnant women, and men, the effect being most convincing when marked riboflavin deficiency and poor hematological status exist at baseline. Understanding of the mechanisms of such associations, or response to riboflavin supplements, is incomplete; while it can be shown that riboflavin deficiency in animals impairs iron absorption, results of human studies of riboflavin and iron absorption have been less consistent. Findings from human studies can be more readily explained by an effect of correcting riboflavin deficiency on mobilization of iron from stores than by effects on iron absorption.

Gastrointestinal Structure and Function

There is a small but persuasive body of literature suggesting that riboflavin status influences aspects of gastrointestinal development. Riboflavin-deficient rats exhibit a number of disturbances to the normal structure and kinetics of the cells of the duodenum. Effects include crypt hypertrophy, a decreased villus number, and an enhanced rate of transit of cells along the duodenal villi, all of which would be expected to have a downstream effect on nutrient absorption and nutrient loss from the gastrointestinal tract. This may be a contributing factor to the well-established effects of riboflavin deficiency on growth. The human corollary is less clear, but there is some evidence that poor riboflavin status is associated with morphological disturbances to duodenal crypts and altered proliferative activity.

Cancer

Evidence for causal links between low riboflavin status and increased risks of cancer is rather limited. There has been long-standing interest in a possible link between low riboflavin intake and increased risk of esophageal cancer, with plausible mechanisms relating to effects of riboflavin deficiency on epithelial integrity and function. Early interest was based on epidemiological studies of micronutrient deficiencies and prevalence of esophageal cancer in Iran and South Africa. Subsequently, particular attention has focused on China and Iran, countries with a high prevalence of esophageal cancer and evidence for low dietary intakes of riboflavin. Most studies were case–control studies or cross-sectional studies and generally supported an inverse association between riboflavin intake and risk of this cancer. A large multinutrient supplementation trial in Linxian, China, showed a modest benefit on precancerous cell changes, but evidence from randomized controlled trials for a beneficial effect of riboflavin on incidence of esophageal cancer or progression of precancerous lesions is lacking. A case-control study nested in the large EPIC cohort (European Prospective Investigation into Cancer and Nutrition) revealed an inverse association between plasma concentrations of flavins and risk of colorectal cancer. There is modest evidence to associate low riboflavin concentrations in the circulation and tissue with increased risk of cervical cancer and also with an increased likelihood of cervical cell infection with the causal agent, human papillomavirus; this warrants further investigation. Other smaller studies of riboflavin and colorectal cancer risk have generally generated null findings.

Neurological Function

Severe riboflavin deficiency in rats and chickens can lead to neurodegeneration, in some cases linked with peripheral nerve demyelination. This may arise from impaired energy generation and deranged lipid metabolism, but this remains conjectural. Evidence of neurodegeneration in humans, arising from riboflavin deficiency, is lacking. There are a few isolated case histories describing neurological impairment in association with riboflavin deficiency, in some cases responsive to high doses of riboflavin. A group of rare and complex childhood-onset motor neurone diseases, called Brown–Vialetto–Van Laere syndrome, has been causally linked with a mutation in the gene SLC52A2, which encodes a riboflavin transporter RFVT2. Recent evidence suggests that high-dose riboflavin supplements can ameliorate some of the clinical and biochemical features and slow progression of the condition. A tenuous link has been proposed between poor riboflavin status and Parkinson's disease, with reported partial recovery of some motor functions in response to high-dose riboflavin, but this needs to be substantiated.

See also: Energy Metabolism; Folic acid and Folates: Physiology and Health Effects; Vitamins: Overview.

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Riboflavin: Properties and Determination

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Physicochemical Characteristics

Riboflavin, the prescribed name for vitamin B_2 by the IUPAC–IUB, is the biologically active component of the prosthetic group of flavoproteins. Older designations are lactoflavin, ovoflavin, and uroflavin, which originated from the first isolation. It comprises a tricyclic, nitrogen-containing ring system, chemically defined as a substituted isoalloxazine with a ribitol side chain at N_{10}. With the systematic nomenclature, the molecule is named as 7.8-dimethyl-10-(1-D-ribityl)-benzo[g]pteridin-2,4-dion. The empirical formula is given as $C_{17}H_{20}N_4O_6$ with an MW of 376.36 (Figure 1). The substance is a yellow to orange-yellow powder and crystallizes in sharp, optically active needles, melting under decomposition at ~275–282 °C. The optical rotation is $[\alpha]_D^{25} = -112°C$ to $-122°C$ (c = 50 mg in 10 ml 0.02 N alcoholic NaOH). Riboflavin is sparingly soluble in water with a yellow-green fluorescence, very sparingly soluble in ethanol and insoluble in other organic solvents. It is readily soluble in dilute alkali, but rapidly deteriorated, being accelerated by light. In neutral or acid solutions, it is more stable even against heat and oxygen. Nevertheless, all operations with riboflavin solutions should be handled in the dark or at red light.

Light sensitivity of the flavins has also consequences for the stability of vitamin B_2-containing foods. In milk stored under day light, the riboflavin content is dropping off by more than 80% within hours. Processing losses in foods are given with ~20%.

Light exposure of alkaline solutions decomposes riboflavin to lumiflavin (7,8,10-trimethylisoalloxazine); in neutral or acidic solutions, the blue fluorescent lumichrome (7,8-dimelthylalloxazine) is formed (Figure 2). Riboflavin is found in free-form only in the retina of the eye, in whey, and in urine. In other biological systems and tissues, the predominant forms are flavin mononucleotide (FMN) or riboflavin phosphate and flavin adenine dinucleotide (FAD). Free riboflavin and FMN-monosodium salt are the most important commercial products. FMN and FAD are effective as coenzymes or prosthetic groups in more than 100 enzymes in the plant and animal kingdom. The accepted designation for both compounds is incorrect, because FMN is not truly a nucleotide and FAD not a dinucleotide. The designations arose from the discovery of riboflavin and have been adopted ever since. Pure riboflavin was first isolated from yeast, egg white, and whey in 1933 (Kuhn and Wagner-Jauregg). Simultaneously, the so-called yellow coenzymes were discovered in yeast (Warburg and Christian), which could be identified as FMN. FMN is considerably more soluble in water than riboflavin. Its solubility in organic solvents as well as its stability against heat and light exposure is comparable to riboflavin.

The flavoproteins FMN and FAD belong to the enzymatic class of oxidoreductases. The conjugated structure of the pteridine moiety of the basic molecule determines its coloring and light absorbance in the UV and visible spectrum. Absorbance maxima of the flavins are listed in Table 1. The catalytic hydrogen transfer in the flavin enzymes takes place at both conjugated bound N_1 and N_5 atoms of the isoalloxazine molecule. The oxidized form of the flavoproteins, the flavoquinone, is thereby reversibly reduced to the colorless flavohydroquinone or leucobase (see Figure 1). The absorbance is lost, because double bonds are dissolved. As an intermediate product during this reaction, a sequinone radical is formed. During the redox process, the flavin molecule changes its conformation, which is planar in the oxidized and folded in a butterfly configuration in the reduced state.

The characteristic yellow-green fluorescence of riboflavin and FMN in aqueous solutions exhibits maximal intensity at pH 6–7. The fluorescence of FAD is about 10–20% of that of riboflavin with the same spectral distribution but exhibiting a maximal intensity at pH 3. The fluorescence of flavins is quenched when bound to proteins. The irradiation products of the flavins are also fluorescing, and their spectrum is shifted to the blue (lumichrome) or green (lumiflavin) spectral range. For quantification of riboflavin, particularly the conversion to lumiflavin, measurement of its fluorescence intensity in chloroform solutions is used (ex 270 nm, em 418 nm).

Content and Reactions in Food

Content

Riboflavin belongs to the group of water-soluble vitamins and is generally referred to as vitamin B_2. Riboflavin is an important compound of a healthy diet and is widely distributed in animal and vegetable foods. Protein-rich foods of animal origin are as rule considerable sources of this vitamin with good bioavailability. Particularly rich in riboflavin are offal, such as the heart, liver, and kidney, and eggs and dairy products, mostly as FMN and FAD. Of special importance for the dietary habits in Western countries are milk, dairy products, and meat, despite of a medium content of riboflavin. If those are omitted from the diet, problems may arise to cover an adequate vitamin B_2 intake for human beings (Table 2). Furthermore, riboflavin is used as a safe coloring agent for some processed foods and pharmaceuticals and is included in nutrient supplements. Meat, fish, and dark green vegetables contain also significant concentrations of riboflavin. Milk and cheese are in agreement with their content of riboflavin products for which light-induced quality deterioration is now widely recognized to be the result of riboflavin photosensitizing.

Stability of Riboflavin

Riboflavin and its physiological compounds are relatively stable during thermal and nonthermal food processing and

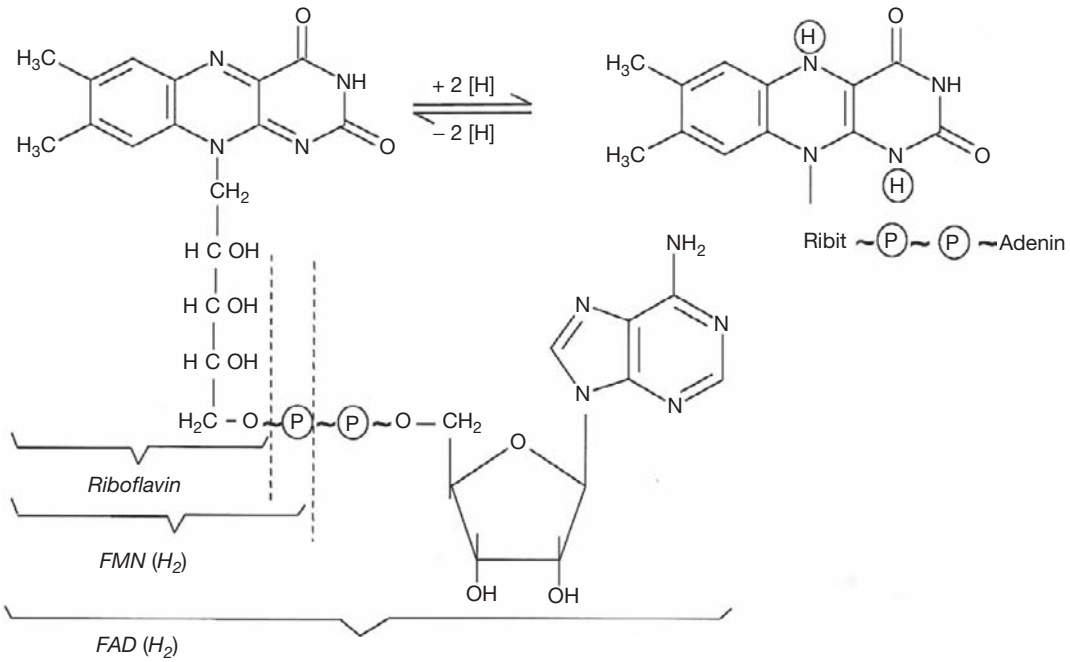

Figure 1 Riboflavin and its coenzyme forms.

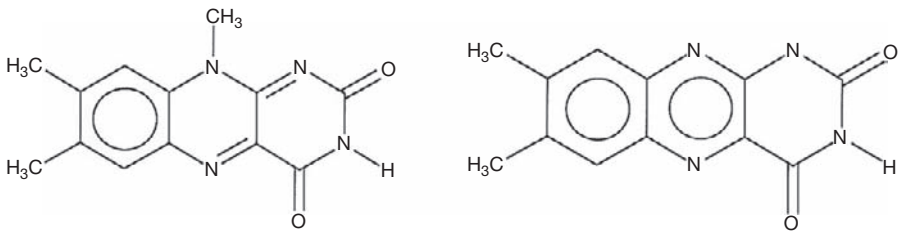

Figure 2 Photolysis products of riboflavin.

storage, in the absence of light even at high temperature. But they are very unstable to light even at room temperature. Particularly, aqueous solutions of these substances are sensitive to UV and visible light, and they are degraded through a variety of reactions resulting in a number of photoproducts under aerobic and anaerobic conditions. The major photoproducts obtained through normal photolysis of riboflavin in aqueous solvents include formylmethylflavin, lumichrome, lumiflavin, and 2,3-butanedione. With multicomponent spectrometric methods, several further photoproducts are to be identified. Lumichrome is the major product under neutral or acidic pH; lumiflavin is formed in basic pH; 2,3-butanedione is the major volatile compound with a typical buttery odor. Lumichrome and lumiflavin are formed from unstable diradical excited triplet riboflavin by dealkylation; 2,3-butanedione is produced from the reaction between electrophilic singlet oxygen and electron-rich riboflavin. The kinetics of riboflavin photolysis under light exposure and in the pH range of 1–12 indicate that riboflavin has maximum photostability around pH 5–6. Mostly destructive to riboflavin is exposure to light with wavelength of its absorption maximum at 450 nm.

Reactions of Riboflavin as a Photosensitizer

Riboflavin and other flavins are efficient photosensitizers, inducing oxidative damage to light-exposed food and several food compounds. When exposed to light of wavelength 420–560 nm, the absorbed energy does excite riboflavin to triplet state. Riboflavin can accept or donate two hydrogen atoms and acts together with lumiflavin as efficient photosensitizer. These so-called sensitized photoreactions are typical for foods and essentially involve two pathways. Direct interaction of the excited sensitizer with food compounds initiates radical processes through electron transfer or hydrogen abstraction and is known as a type 1 photo-oxidation. This mechanism is favored under low oxygen pressure and is, for example, responsible for light-struck flavor formation in beer. Under aerobic conditions, excitation energy is preferably

Table 1 Physicochemical data of flavines

Property	*Riboflavin*	*Riboflavin-5-phosphate-Na (FMN-Na)*	*Flavinadenindinukleotide-Na$_2$ (FAD-Na$_2$)*
Molecular weight	376.36	478.34	829.6
Melting point	278–282 °C (decomposition)	280–290 °C (decomposition)	
Solubility	Readily soluble in dilute alkalis (decomposition) Sparingly soluble in water (70–100 mg l^{-1}, 27 °C)	Soluble in water at pH 7 (30 g l^{-1}, 25 °C)	Soluble in water
	Very sparingly soluble in ethanol (45 mg l^{-1}, 27 °C)	Very sparingly soluble in ethanol	
	Insoluble in acetone, chloroform, ether, benzene		
Absorbance (UV/vis)	223, 267, 374, 444 nm (0.1 N HCl)		
Fluorescence	Yellow-green fluorescence of aqueous solutions at pH 6–7 (ex. 444 nm, em. 530–565 nm)		ex. 400–500 nm, em. 530 nm
Stability	In neutral and acidic medium relatively stable against heat and oxygen. Rapid photolysis by UV- and visible light to blue fluorescent lumichrome (acidic and neutral solutions) or yellow-green fluorescent lumiflavin (alkaline solutions). After reduction by dithionite, Zn-HCl, sodium hydrosulfite, etc., reversible conversion to leucoflavin (dihydroflavin)		

Source: *The Merck index: an encyclopaedia of chemicals, drugs and biologicals* (2014) (15th ed.). New Jersey: The Royal Society of Chemistry; Eitenmiller, R. R., Landen Jr, W. O. and Ye, L. (2007). *Vitamin analysis for the health and food sciences* (2nd ed.). Boca Raton, FL: CRC Press.

Table 2 Riboflavin content of foods

Content (mg/100) ingested food	*Milk and milk products*	*Meat and meat products*	*Cereals*	*Vegetables and legumes*	*Fruits and nuts*	*Fish*
>3.00		Pig's liver				
2.50–3.00		Beef liver, calf liver				
2.00–2.50		Beef kidney, calf kidney				
1.50–2.00		Pig's kidney				
1.00–1.50		Pig's heart				
0.70–1.00			Wheat germs			
0.50–0.70	Camembert type, cheddar type, Danish blue type, parmesan		Wheat bran			
0.30–0.50	Cream brie (50%), edam type, fresh cheese (skim milk)	Eggs, lamb				Eel, mackerel
0.20–0.30	Fresh cheese (50%), gouda cheese (45%), yogurt (low fat)	Goose, pork (lean) veal		Green cabbage, soybean meal	Cashew nuts, hazelnuts, sallow thorn	Flounder, herring, pilchard, plaice
0.15–0.20	Cottage cheese, cow's milk (fresh)	Beef (lean), chicken, duck, turkey	Oat flakes, whole meal flower (wheat, rye)	Broccoli (boiled), mangold, soybean sprouts, spinach (boiled)	Avocado	Haddock, salmon
0.10–0.15				Asparagus (boiled), Brussels sprouts	Peanuts (roasted)	

Source: Souci, S. W., Fachmann, W. and Kraut, H. (2008). *Food composition and nutrition tables* (7th ed.), compiled by Dr. Eva Kirchhoff. Stuttgart: Medpharm.

transferred to oxygen to generate singlet oxygen, the pivotal intermediate in a type II photo-oxidation. This reaction is responsible for oxygenation of unsaturated fatty acids in vegetal oils causing high peroxide values and eventually rancidity, while attack of amino acid residues in dairy proteins may give rise to sulfury and potato-like off-flavors. Both photo-oxidation pathways induced by triplet-excited riboflavin lead to premature aging or spoiling of foods and beverages. Photosensitizing of riboflavin causes production of reactive oxygen species such as super oxide anion, singlet oxygen, hydroxyl radical, and hydrogen peroxide. Radicals and reactive oxygen species accelerate the decomposition of food compounds

as amino acids, proteins, lipids, carbohydrates, and vitamins and cause significant alterations of nutritional value of foods. Carbohydrates are less sensitive to riboflavin-photosensitized oxidation than proteins, lipids, or vitamins. Exposed to visible light, riboflavin acts as a potent photosensitizer in food and beverages presenting a risk for their stability. Decomposition of the nutrients depends on the wavelength and energy of light, the concentration of oxygen and riboflavin, and the presence of activating or inactivating compounds. Riboflavin is an excellent photosensitizer for singlet oxygen formation and a superb reactant for singlet oxygen, with the reaction rate of 1.01 ± 10^{10}/M/s. The triplet-excited state of riboflavin is relatively long-living and as a biradical a very powerful oxidant compared to ground state riboflavin, leading to direct oxidation of most types of biomolecules.

Riboflavin Losses in Dairy Products

Due to the relative heat stability, only minor vitamin B_2 losses occur during processing. Likewise, the pasteurization of milk in the absence of light causes losses of $< 10\%$. On the other hand, exposure of dairy products to day light may result in a remarkable decrease of the riboflavin content being dependent on the surface exposed. In milk stored under daylight, the riboflavin content is dropping off by more than 80% within hours with both lipids and proteins being oxidized under photosensitized conditions. The photodegradation leads to the formation of carbonyl compounds such as pentanal, hexanal, heptanal, 2-nonenal, 2,4-nonadienal, and 2,4-decadienal all attributed to lipid photo-oxidation. The chemical reaction between methionine and singlet oxygen, which has been formed by riboflavin photosensitization, produces dimethyl-disulfide, which is responsible for the sunlight flavor of milk. In an actual study, it could be demonstrated that photodegradation of riboflavin in milk may be considerably reduced by the addition of small amounts of gum arabic microcapsules, avoiding large losses in the nutritional value of milk.

Riboflavin Losses in Beer and Cereals

Light exposure of beer generates an obnoxious off-flavor after a complex interplay of light, excited riboflavin, isohumulones and sulfur-containing amino acids, peptides, and proteins. The radical chemical degradation path of these compounds results in 3-methylbut-2-ene-1-thiol, the main off-flavor compound that characterizes light-exposed beers with the trivial name of skunky thiol. Vitamin losses occur also during the milling process of cereals. White flour with a low extraction rate contains about one-third of riboflavin of the whole grain flour.

Photodegradation of Amino Acids and Vitamins

The deactivation of triplet-excited state riboflavin by several food components leading to photodegradation of nutrients in food, beverages, and model solutions is described in actual studies. For instance, the amino acids cysteine, histidine, methionine, tyrosine, and tryptophan are rapidly photodegraded in aqueous solutions in the presence of triplet exited riboflavin. These amino acids are the primary targets of photochemical oxidation in proteins.

Both fat-soluble and water-soluble vitamins are sensitive to photo-oxidation as sensitized by riboflavin. The vitamins ascorbate, folate, thiamine, and pyridoxal phosphate deactivate triplet-excited riboflavin and are photodegraded as well as vitamins A and D3 and tocopherols in aqueous solutions. A good photostability in the presence of riboflavin was demonstrated for the vitamins biotin and niacin. Carotenoids seem to reduce the formation of triplet-excited riboflavin through an inner filter, which protects these substances against the riboflavin-induced photosensitizing damage.

How to Prevent Reactions

Polyphenolic compounds are efficient antioxidants with strong free radical scavenging ability. It was demonstrated that the plant polyphenols rutin and catechin, several flavonoids, and phenolic compounds present in beverages quench the triplet-excited state of riboflavin and protect the beverages that contain these compounds from light-induced off-flavor formation.

Methods of Determination

In earliest reports, the most frequently applied assays of flavins as vitamin B_2 active components made use of the direct fluorimetric assay of the intact flavins or after hydrolysis to FMN or free riboflavin. Alternatively, the lumiflavin method based upon the conversion of the chloroform-insoluble riboflavin into chloroform-soluble lumiflavin after photodegradation in alkaline solution was used, despite a transformation rate of only 60–70%. Currently, microbiological assays and high-performance liquid chromatography (HPLC) procedures with enhanced sensitivity are widespread utilized (see also **Table 3**).

In principle, riboflavin is extracted from dry materials by autoclaving in 0.1 N HCl for 30 min or on water bath for 1–2 h. By using acid extraction, flavins are released from their protein binding and FAD and FMN are converted to free riboflavin. In protein-rich materials, the acidic extraction may be followed by enzymatic hydrolysis for complete liberation of the flavins. It is of importance to carry out all operations relating to the extraction and quantitation of flavins and riboflavin under subdued light and in brown or dark vessels.

An optimal extraction procedure applicable for microbiological or HPLC determination of thiamine and riboflavin has been worked out by the European Measurement and Testing Program. The steps of the extraction are detailed in autoclaving, incubation with takadiastase, and filtration.

For biological samples, such as plasma/serum, the preferred extraction medium is 5–10% trichloroacetic acid, which is suitable for denaturing the relatively weak protein binding while keeping the phosphorylated forms intact.

Direct Fluorimetry

Direct measurement of the native fluorescence of riboflavin may be applicable for pharmaceutical preparations or food samples with a relatively high vitamin content. After acid extraction, the remaining proteins are removed by precipitation at pH 4.5. Interfering substances are subsequently oxidized with potassium permanganate and the excess oxidant

Table 3 Methods of riboflavin determination

Method	*Principles*	*Specials*	*Detection limits*
Direct fluorimetry	Fluorescence measurement of riboflavin at 440 (ex)/565 (em) nm	Conversion of FAD and FMN to riboflavin by acidic hydrolysis	0.1 μg B_2/g sample
Lumiflavin method	Fluorescence measurement after irradiation at pH 10–12 (ex 450, em 513 nm)	Lumiflavin is separated by chloroform extraction	0.02 ng B_2
Microbiological assay	Turbidimetric measurement of bacterial growth response dependent on the B_2 content	Interference of growth response with nutrients/food components	0.1–0.5 ng B_2
HPLC analysis	Separation of flavins on RP-C_{18} materials with aqueous/organic solvents and fluorimetric/UV detection	Differentiated analysis of flavins, combined analysis with other B vitamins	0.21 ng riboflavin 0.89 ng FMN 11.15 ng FAD 0.02 ng B_2 (precolumn irradiation to lumiflavin)

destroyed with hydrogen peroxide or sodium bisulfite. The fluorescence of the clear sample solution, commonly spiked with riboflavin as an internal standard, is registered by difference measurement before and after reduction of riboflavin with sodium bisulfite to the leuco form for correction by unspecific fluorescences (ex = 440 nm; em = 565 nm).

The detection limit of this method is about 0.1 μg riboflavin per gram sample. The direct fluorimetry of flavins had been adopted by the US Pharmacopoeia for the riboflavin analysis in tablets and injections and by the AOAC for the riboflavin determination in foods, as in milk-based formulas.

Lumiflavin Method

By this method, riboflavin is photodegraded in alkaline solution at pH 10–12 to the stronger fluorescent lumiflavin (ex = 450 nm; em = 513 nm). This reaction is specific for riboflavin since other flavins interfering with riboflavin fluorescence are not converted into lumiflavin. Lumiflavin is separated from riboflavin and other fluorescent substances by extraction with chloroform. The transformation into lumiflavin is not quantitative and ranges between 60% and 70%. Reliable results can actually only be obtained by comparison with a riboflavin standard under identical irradiation conditions for sample and standard solution.

While the original lumiflavin method is sparsely used, the principal methodology was applied to HPLC analyses of the riboflavin content in foods with detection limits of 0.02 ng riboflavin per injection.

Microbiological Assay

Besides bioassays, as chicken and curative rat growth tests, microbial assays belong to the first widely used test methods measuring the biological activity of preparations containing total vitamin B_2. The most frequently used test organism is *Lactobacillus casei* subsp. *rhamnosus* (ATCC No. 7469). The sensitivity limit is indicated to be 0.5 ng riboflavin absolutely. Occasionally, other test organisms are proposed such as *Enterococcus faecalis* (ATCC No. 10100) and *Leuconostoc mesenteroides* (ATCC No. 9135) with enhanced sensitivity of 0.1 ng ml^{-1} riboflavin. The microbiological assay with *L. casei* is approved by the AOAC International Methods for the riboflavin determination in vitamin preparations. Growth response of bacteria is proportional to the riboflavin content of the medium and measured turbidimetrically. Earlier techniques determined metabolically formed lactic acid by titration. However, comparisons of turbidimetry after 16 h incubation obtained results that agreed with those obtained by titration of lactic acid after 72 h. Thus, the turbidimetric measurement is at present preferred in view of its shorter incubation time and easier handling.

The growth response of lactobacilli differs significantly between free riboflavin and FMN and FAD. Acidic or enzymatic treatment is therefore needed for releasing and converting the flavins into riboflavin as described under methods of determination, followed by adjustment of the extract to pH 4.5 before incubation. In case of enzymatic release using takadiastase or clarase, it has to be considered that some enzyme preparations contain variable traces of riboflavin.

Starch, glycogen, free fatty acids, and other lipids and protein degradation products can interfere with the test by either stimulating or inhibiting the bacterial growth. Lipids in a relevant content can be removed either by filtration or by ether or petroleum ether extraction before hydrolysis. Proteins are precipitated at pH 4.5 and starch is split by the acidic or enzymatic hydrolysis step. As with the other analytic procedures, the microbiological test has also to be done under subdued light. Of importance is that the used glass vessels do not release any traces of alkali and should thus be cleaned with acid before use, because the flavins are alkali-sensitive. Hence, it is advisable to dissolve riboflavin standards in 0.02 M acetic acid.

An optimal extraction procedure applicable for microbiological or HPLC determination of thiamin and riboflavin has been worked out by the European Measurement and Testing Program. The steps are detailed in autoclaving in 0.1 N HCl at 121 °C for 30 min and adjusting thereafter to pH 4.0 with 4 M sodium acetate buffer (pH 6.1), addition of takadiastase (0.1 g g^{-1} sample), incubation at 37–45 °C (18 h), filtration, or centrifugation after cooling.

HPLC Procedures

HPLC methods enable separate quantification of individual flavins or simultaneous analysis of riboflavin including FMN

and FAD. A broad variety of HPLC techniques are described, varying the column material, mobile phase, separation mode, and detection system as well. Of the numerous analytic and technical variations communicated during the last 20 years, the separation techniques can be categorized as follows:

Reversed phase C_{18} materials are utilized as stationary phases in most cases, combined with aqueous/organic mobile phases on the basis of water, methanol, acetonitrile with or without phosphate, or acetate buffer in an isocratic mode. Rarely, ion pair chromatography with sodium salts of hexane or heptanesulfonic acid in the mobile phase (ion interaction chromatography) was utilized; in some cases, the separation of riboflavin and flavins was accomplished with gradient elution technique.

Ion-pair HPLC seems more favorable only when riboflavin is determined simultaneously with other B vitamins (e.g., B_1, B_6, niacin, folic acid, and B_{12}), because this technique results in better peak shapes.

Retention times of vitamin B_2 active compounds are between 5 (FAD) and 20 (riboflavin) minutes. Fluorescence monitoring is preferred for detection; UV or visible absorbance detection at 270 or 446 nm is restricted to early HPLC analyses or measurements of pharmaceuticals and enriched foods. Limits of fluorescence detection at 450/520 nm (ex./em.) for the native flavins were given to 0.55 pmol (0.21 ng) riboflavin, 1.96 pmol (0.89 ng) FMN, and 14.19 pmol (11.15 ng) FAD. In an earlier HPLC method, the detection limit could be increased to 0.02 ng riboflavin/injection by irradiation of the sample extract and conversion of the extracted riboflavin to lumiflavin prior to HPLC separation.

A specific and sensitive UHPLC/LC-MS method was developed for the simultaneous determination of riboflavin and pyridoxine in infant food.

In principle, extraction techniques and sample cleanup suitable for HPLC analysis resemble those used for quantitation by the other methods discussed. Mostly, vitamin B_2 active substances are released from the food matrix by autoclaving with 0.1 N mineral acid (HCl or H_2SO_4) followed by enzymatic digestion with papain, takadiastase, or claradiastase. Solid-phase cleanup procedures on C_{18} materials or Florisil are often used for support prior to injection. The extraction procedure is guided by the analytic aim, whether the total riboflavin content, that is, the sum of FAD, FMN, and free riboflavin, shall be determined or the differentiated quantitation of FAD and FMN in addition to free riboflavin is strived for. Treatment with potassium permanganate can be omitted, because possibly remaining extraneous fluorescent substances are chromatographically separated.

The combined acid and enzymatic hydrolysis is advantageous particularly for foods with high starch or protein content to liberate the bound flavins. Occasionally, autoclaving with dilute mineral acid is incomplete, particularly for the conversion of FMN to riboflavin, as indicated by the appearance of an FMN peak in the following chromatogram. Additionally, FMN may be partially converted during acid hydrolysis to biologically active isomeric riboflavin phosphates, which are separated from FMN by HPLC analysis and remain neglected for the calculation of total riboflavin. The combination of acidic and enzymatic digestion is thus advisable prior to HPLC analysis for the determination of total riboflavin. When using this extraction procedure and following HPLC analysis, the riboflavin content of various foods such as breakfast cereals, porridges, milk, and milk products correlated well with the microbiological assay using *Lactobacillus casei*. The simultaneous determination of FAD and FMN besides free riboflavin needs nondegradative techniques, such as extraction of flavins by methanol/dichloromethane followed by partitioning with citrate buffer (pH 5.5) or extraction with 6% formic acid containing 2 M urea. The HPLC separation and quantitation of the flavins was accomplished by using internal standards (7-ethyl-8-methyl-riboflavin, sorboflavin isoriboflavin, nicotinamide, and others). This analytic device enabled the flavin analysis (FAD, FMN, and riboflavin) of milk and dairy products, fruits and vegetables, meats, and cereal products. In many cases, HPLC methods were developed for the simultaneous or sequential determination of riboflavin and thiamin in foods. Both vitamins are extracted by a common procedure using acidic and enzymatic digestion.

For biological samples, as blood or plasma/serum, the preferred extraction medium is 5–10% trichloroacetic acid, which is suitable for denaturing the relatively weak protein binding while keeping the phosphorylated forms intact. In this way, flavins in whole blood can be analyzed by isocratic RP-HPLC against an external standard. A compilation of usual HPLC procedures is listed in **Table 4**. An overview on methods of riboflavin determination can be taken from **Table 3**.

European Standard Method

The European Technical Committee for Standardization (CEN/TC 275) specifies a method with the title "Foodstuffs: Determination of vitamin B_2 by high performance liquid chromatography and fluorescence detection." The document number is DIN EN 14152:2014, the publication date is 2014-08, the original language is German, and the responsible standards committee for food and agricultural products is NA 057-01-13 AA: Vitamins and Carotenoids.

The 'Deutsches Institut für Normung e.V. (DIN)' is the responsible committee for the standardization in the field of food as well as in the agricultural sector.

This method has been validated in two interlaboratory studies. The first study was for the analysis of samples of milk powder and pig's liver ranging from 1.45 to 10.68 mg/100 g. The second study was for the analysis of samples of tube feeding solution, baby food, powdered milk, meal with fruits, yeast, cereal, and chocolate powder ranging from 0.2 to 87.1 mg/100 g. Vitamin B_2 is the mass fraction of total riboflavin including its phosphorylated derivates.

In this European norm, riboflavin is extracted from foods after acidification, followed by an enzymatic dephosphorylation. The quantification takes place with subsequent HPLC separation, fluorimetric detection, and extern standards.

See also: Chromatography: High-Performance Liquid Chromatography; Enzymes: Functions and Characteristics.

Table 4 HPLC analysis of flavins

Food sample/matrix (compounds determined)	*Extraction procedure*	*Column*	*Mobile phase*	*Detection (nm)*	*Detection limit*
Dairy products (total riboflavin)	Disperse in water, clean up on C_{18} cartridge	Biosil ODS-5S C_{18}	H_2O/CH_3OH/acetic acid = 65/35/1	UV 270	10 ng
Blood (FAD, FMN, riboflavin)	10% TCA at 4 °C (30 min), adding NaOAc-buffer, centrifugation	Hypersil ODS 5 μm	0.3 M KH_2PO_4/CH_3OH = 83.3/16.7 (pH 2.9)	Fluorescence 470/525 ex. em.	FMN 15 nM FAD 20 nM Riboflavin 10 nM
Cereals, various foods (thiamin and total riboflavin)	0.1 N HCl, autoclave (15 min) at 125 °C, adjust to pH 4.0–4.5 (2 N NaOAc), incubation with claradiastase at 50 °C (3 h), 50% TCA and heating at 90 °C (15 min), adjust to pH 3.5 (2 N NaOAc), filtration	μ Bondapak C_{18}	CH_3OH/0.005 M phosphate buffer pH 7.0 = 35/65	Fluorescence 440/520 ex. em.	80–96% recovery
Dairy + meat products, fruits, vegetables, flour, baked products, beer, coffee (total riboflavin)	0.05 M (0.1 N) H_2SO_4, autoclave (20 min) at 121 °C, adjust to pH 4.5 (2.5 M acetate buffer), incubation with claradiastase at 45 °C (overnight); filtrated sample passed through C_{18} Sep-Pak, elution with 40–70% CH_3 OH	Spherisorb ODS 2.5 μm	H_2O/CH_3OH 0.65/35	Fluorescence 445/525 ex. em.	20 pg
Dairy products (FAD, FMN, riboflavin) sorboflavin as internal standard	Homogenize with 6% formic acid, containing 2 M urea pass through C_{18} solid phase, elute with 10% formic acid/CH_3OH = 4/1	LC-18, 3 μm (Supelco)	14% acetonitrile in 0.1 M KH_2PO_4, pH 2.9	Fluorescence 450/530 ex. em.	FMN 2.5 nM FAD 3 nM Riboflavin 2.5 nM
Dairy products, raw and cooked meats, cereals (FAD, FMN, riboflavin) 7-ethyl-8-methyl-riboflavin as internal standard	Homogenize with CH_3OH/CH_2Cl_2 = 9/10 mix with citrate-phosphate buffer, pH 5.5 (containing 0.1% sodium azide), centrifugation	2PLRP-S, 5 μm column	temperature 40 °C	Acetonitrile/ 0.1% sodium azide in 0.01 M citrate-phosphate buffer, pH 5.5 gradient elution	
Fluorescence 450/522 ex. em.	96–113% recovery				
Fruits, vegetables (total riboflavin)	0.1 N HCl (30 min) at 100 °C, incubation with amylase (38 °C)	Altex	Ultrasphere ODS 5 μm	H_2O/	
CH_3OH = 60/40 + 5 mM heptanesulfonic acid, pH 4.5	Fluorescence 450/530 ex. em.	0.2 ng/ inject.			
Potatoes, vegetables (total riboflavin)	0.1 N HCl (30 min) at 121 °C, incubation with takadiastase (45–50 °C)	μ-Bondapak C_{18}	CH_3OH/H_2O = 30/70	Fluorescence 450/510 ex. em.	0.1 ng/ inject.
Serum, urine (riboflavin) isoriboflavin as internal standard	Homogenize/mix with TCA (100 $g\,l^{-1}$), centrifugation, pass through Sep-Pak C_{18} (serum)	ROSIL C_{18} HL 5 μm	$CH_3OH/H_2O/CH_3COOH$ = 36.7/63.7/0.1	Fluorescence 450/530 ex. em.	10 $\mu g\,l^{-1}$

Source: De Leenheer, A. P., Lambert, W. E. and van Bocxlaer, J. F. (2000). *Modern chromatographic analysis of vitamins* (3rd ed.). New York: Marcel Dekker, Inc.; Ball, G. F. M. (2013). *Water-soluble vitamin assays in human nutrition*. New York: Springer; Eitenmiller, R. R., Landen, Jr. W. O. and Ye, L. (2007). *Vitamin analysis for the health and food sciences* (2nd ed.). Boca Raton, FL: CRC Press.

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Rice: Role in Diet

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Commercial Importance, Global Distribution, and Varieties

Rice is the most important crop in terms of total developing world production (480×10^6 tonnes of rough rice in 2012) and numbers of consumers (3.5 billion) dependent on it as a staple food. It is grown in over 100 countries in every continent (except Antarctica) from 53° N to 40° S and from sea level to an altitude of 3 km. In 2012, total production was 697×10^6 tonnes of rough rice with Asia accounting for $149/158 \times 10^6$ ha world area for cultivation; mean rough rice yield was 4.40 tonnes ha^{-1}. About 90% of the world's rice is grown and consumed in Asia across 200 million rice farms, most of which are smaller than 1 ha. The major rice producers in 2012 were China, India, Indonesia, Bangladesh, Vietnam, and Thailand.

Rice can grow in upland (aerobic) and flooded (anaerobic soil) conditions with air channels connecting to its roots. In 2010–12, about 57% of total rice cultivation was irrigated, 31% rain-fed lowland, 9% upland, and 3% flood-prone wetland corresponding to total rough rice production of about 75% (irrigated), 19% (rain-fed lowland), 5% (upland), and 1% (flood-prone wetland). Since rice-growing area is shrinking, rice production must keep up with the annual increase in consumption through increased yield.

Rice is mainly *Oryza sativa* L. with some *Oryza glaberrima* L. grown in West Africa. It is estimated that there are about 100 000 rice varieties, but only a small proportion are cultivated. These vary in grain weight, size and shape, translucency, degree of dormancy, longevity and seedling vigor, and pigmentation (none, to red, to purple–black). More than 75% of Asia's rice area is planted with semidwarf plant type with erect leaves. These newer, improved varieties have a similar yield potential to the first commercial variety (IR8) but have a better resistance/tolerance to biotic and abiotic stresses. Although essentially self-pollinated, three-line cytoplasmic male sterile hybrids and two-line thermogenic male sterile hybrids have been grown successfully in China with increased yields. Complete sequencing of the rice genome in 2002 has accelerated the use of molecular markers in breeding for yield, for abiotic and biotic stress tolerance, and, lately, for grain quality.

Structure of the Rice Grain

The rice grain (rough rice or paddy) consists of an outer protective covering (hull or husk) and the edible rice caryopsis or fruit (brown, cargo, dehulled, or dehusked rice) (Figure 1). Brown rice consists of the outer layers of pericarp, seed coat, and nucellus; germ or embryo, which are the maternal tissues; and the endosperm. The endosperm consists of the aleurone layer and the starchy or inner endosperm. Pigment is confined to the pericarp, but there are varietal differences in the retention of pigment with the degree of milling. The aleurone layer encloses the embryo.

The inedible hull constitutes 16–28% (mean 20%) of rough rice weight. Brown rice consists of 1–2% pericarp, 4–6% aleurone plus nucellus and seed coat, 1% embryo, 2% scutellum, and 90–91% endosperm. The aleurone and embryo cells are rich in lipid bodies (spherosomes, 0.2–1.5 μm) and in protein bodies (aleurone grains containing inclusions of phytic acid bodies or globoids (1–3 μm)).

The endosperm cells are thin-walled and packed with amyloplasts containing polyhedral compound starch granules (3–9 μm). Protein occurs mainly in the form of large (1–2 μm) and small (0.5–0.8 μm) spherical protein bodies and crystalline protein bodies (2–4 μm). Spherical protein bodies (PB I) are rich in prolamin (alcohol-soluble protein), while crystalline protein bodies (PB II) are rich in glutelin (alkali-soluble protein). High night ripening temperatures increase the PB II/PB I ratio. Spherosomes are present in the subaleurone or the two outermost cell layers of rice endosperm. High night ripening temperatures also reduce linoleic acid content of rice lipid.

Grading, Handling, and Storage

There is no international standard for milled rice size and shape. The International Rice Research Institute (IRRI) uses the following scale for size: extra long >7.50 mm, long 6.61–7.50 mm, medium 5.51–6.60 mm, and short <5.50 mm. For grain shape, based on the length–width ratio, the following scale is used: slender >3.0, medium 2.1–3.0, bold 1.1–2.0, and round ≤1.0. Grades are based on grain size and shape, degree of milling, percentage of head or whole-grain milled rice, immature grains, damages (discolored) and heat-damaged grains (chalky grains, red grains, and red-streaked grains), aroma, and organic and inorganic extraneous matter.

An important property of harvested grain is its moisture content: 14% on a wet-weight basis is considered a safe storage value. Grains become susceptible to fissuring from moisture adsorption stress below the critical moisture content (12–16%), depending on the variety. Aging of sun-/oven-dried rough rice for up to 2 months after harvest at ambient temperature (>15 °C) improves milling yields and makes milled rice expand more during cooking and become more flaky, with fewer soluble solids. Heating during grain drying accelerates aging.

About 20% of rice is consumed parboiled. Parboiling consists of boiling or steaming steeped aged rough rice until the hull starts to open and then cooling and drying the gelatinized grain. Diffusion of bran B vitamins into the endosperm occurs during parboiling, but with some heat decomposition, and many antioxidants are destroyed. Mycotoxins are a potential problem in parboiled rice.

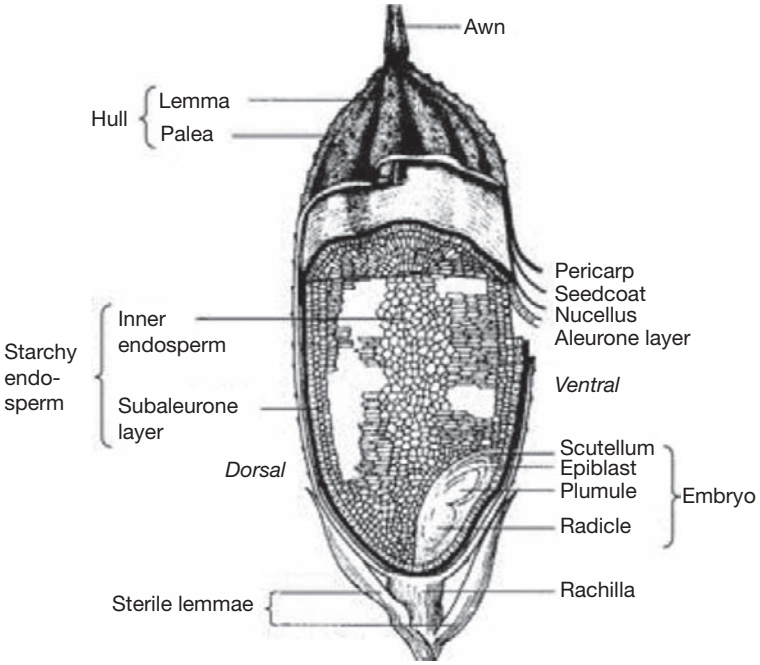

Figure 1 Longitudinal section of the rice grain. Reproduced from Juliano, B. O. (1984). Rice starch: production, properties and uses. In: Whistler, R. L., BeMiller, J. N. and Paschall, E. F. (eds.) *Starch chemistry and technology*, 2nd ed., p. 509. Orlando, FL: Academic Press, with permission.

Milling involves dehulling followed by removal of the outer 7–10% of the brown rice, by either friction or abrasion, removing most of the pericarp, seed coat, nucellus, aleurone layer, and the germ. In Engelberg mills, hull and bran are removed simultaneously with high grain breakage. In modern cone mills, milling is done in several steps with tempering between steps to minimize breakage. Many modern mills have also shifted to milling at >14% moisture to minimize breakage and use moisture to treat the rice (through hollow shafts) during milling to soften the bran and improve surface gloss.

Only about 4–5% of the world's rice production enters the international market. The major rice exporters in 2012 were Thailand, India, Vietnam, the United States, and Pakistan. In 2012, the major rice importers were Nigeria, China, Iran, the Philippines, Iraq, Saudi Arabia, Ivory Coast, and Malaysia.

Chemical and Nutritional Composition

Protein: Rice has one of the lowest protein contents (7%) among the cereals. The bran layers and embryo are richer in nonstarch constituents than the milled (white) rice (Table 1). The major nutritional advantage of brown rice, over milled rice, is its higher content of B vitamins and dietary fiber. Although higher in minerals, bran phytic acid forms complexes with minerals and proteins, reducing their bioavailability. Thus, the available iron (milligram per meal) is similar in brown and milled rice meals, but brown rice is probably higher in zinc (milligram per meal) than milled rice. The energy content of brown rice and bran is higher than milled rice, owing to the higher fat content. Rice contains no vitamin A, C, or D.

Although cereal proteins are deficient in lysine, rice protein has one of the staple foods having highest lysine contents, corresponding to an amino acid score of 65% in milled rice, based on the World Health Organization (2007) amino acid requirement pattern for 1–2-year-old children (5.2% lysine as 100%, Table 2).

The solubility fractions of milled rice proteins are about 15% albumin–globulin (water- and salt-soluble), 20% prolamin (PB I, alcohol-soluble), and 65% glutelin (PB II, alkali-soluble). Bran proteins are 66–98% albumins. Prolamin is poor in lysine, but rich in sulfur-containing amino acids. The high lysine content of rice protein is due to the low prolamin content. From studies funded by the United Nations University/FAO/WHO, the mean safe-level protein requirements of rice-based diets are 1.02 g protein per kilogram body weight per day in adults and 1.38 g protein per kilogram body weight per day in preschool children, while safe-level protein requirements are 0.85 g protein per kilogram body weight per day for adults and about 1.00 g protein per kilogram body weight per day for preschool children. Organic rice contains less protein than conventionally grown rice because of reduced nutrients from organic fertilizer.

Energy digestibility is higher in milled rice than in brown rice because of the reduced dietary fiber and phytic acid, as verified by the poor energy digestibility of rice bran (Table 2). The true digestibility (TD) of milled rice protein is also higher than brown rice, but the biological value (BV) is lower, resulting in similar net protein utilization (NPU). Bran protein has a lower TD, but a higher BV than brown and milled rice proteins. The amino acid score corrected for TD in rats, proposed by the Food and Agriculture Organization as a protein quality index, shows similar values to NPU for rice proteins. Black or purple rice has a lower NPU (72%) than brown rice and higher phenolics level (0.6%, anthocyanin) than red rice (NPU 83% and 0.2% phenolics, proanthocyanidins) and nonpigmented rice (NPU 97% and ≤0.02% phenolics), but these milled rice have identical NPUs. Japanese cooked milled rice protein has

Table 1 Comparison of nutrient composition of brown rice, milled rice, and rice bran

	Amounts (per 100 g)		
Property	*Brown rice*	*Milled rice*	*Rice bran*
Moisture (g)	14.0	14.0	14.0
Energy content (kJ)	1480–1610	1460–1560	1670–1990
Energy content (kcal)	355–385	349–373	399–476
Crude protein (g)	7.1–8.3	6.3–7.1	11.3–14.9
Crude fat (g)	1.6–2.8	0.3–0.6	15.0–19.7
Crude fiber (g)	0.6–1.0	0.2–0.5	7.0–11.4
Crude ash (g)	1.0–1.5	0.3–0.8	6.6–9.9
Available carbohydrates (g)	73–87	77–89	34–62
Total dietary fiber (g)	2.9–4.4	0.7–2.7	24–29
Water-insoluble fiber (g)	2.0	0.5	15–27
Sugars (g)	0.8–1.9	0.1–0.5	5.5–6.9
Thiamin (mg)	0.4–0.6	0.07–0.17	1.2–2.5
Riboflavin (mg)	0.04–0.14	0.02–0.06	0.18–0.43
Niacin (mg)	3.5–6.2	1.3–2.5	27–50
Pantothenic acid (mg)	1.4–1.6	0.8–1.3	20–60
Vitamin B_6 (mg)	0.5–0.7	0.1–0.4	3.7
Folate (μg)	16–20	4–9	40–140
Vitamin E, α-tocopherol (mg)	0.8–2.5	0.1–0.3	3–15
Calcium (mg)	10–50	10–30	30–120
Phosphorus (mg)	0.17–0.43	0.08–0.15	1.1–2.5
Phytic acid P (mg)	0.13–0.27	0.02–0.07	0.9–2.2
Iron (mg)	1.4–5.2	0.3–0.8	8.6–43
Zinc (mg)	1.9–2.8	0.8–2.3	4.3–26

Source: Juliano, B. O. (2007). *Rice chemistry and quality*. Muñoz, Nueva Ecija: Philippine Rice Research Institute; Champagne, E. T., Wood, D. F., Juliano, B. O. and Bechtel, D. B. (2004). The rice grain and its gross composition. In: Champagne, E. T. (ed). *Rice chemistry and technology* (3rd ed.), pp. 77–108. St. Paul, MN: American Association of Cereal Chemists; United States Department of Agriculture (2014). *National nutrient database for standard reference, Release 27*. http://www.ars.usda.gov/ba/bhnrc/ndl.

Table 2 Essential amino acid profile and energy and nitrogen balance in five growing rats of raw brown rice, milled rice, and rice bran

Property	*Brown rice*	*Milled rice*	*Rice bran*
Arginine (g per 16 g N)	7.2	7.9	7.5
Histidine (g per 16 g N)	2.4	2.2	2.5
Isoleucine (g per 16 g N)	4.0	4.1	4.0
Leucine (g per 16 g N)	7.9	7.8	7.3
Lysine (g per 16 g N)	3.6	3.4	4.6
Methionine (g per 16 g N)	2.1	2.2	2.2
Methionine + cystine (g per 16 g N)	3.3	4.2	4.4
Phenylalanine (g per 16 g N)	4.9	5.1	4.5
Phenylalanine + tyrosine (g per 16 g N)	8.5	8.3	7.5
Threonine (g per 16 g N)	3.5	3.4	4.0
Tryptophan (g per 16 g N)	1.2	1.1	0.8
Valine (g per 16 g N)	5.6	5.8	6.3
Digestible energy[a] (% of total)	94.3b	96.6a	67.4c
True digestibility[a] (TD, % of diet N)	96.9b	98.4a	78.8c
Biological value[a] (% of digested N)	68.9b	67.5b	86.6a
Net protein utilization[a] (% of diet N)	66.7b	66.4b	68.3a
Amino acid score[b] (%)	69lys	65lys	88lys
Amino acid score[b] × TD (%)	67lys	64lys	69lys

[a]Means in the same line followed by a common letter are not significantly different at the 5% by Duncan's multiple range test.
[b]Lysine is the first limiting amino acid. Amino acid score is based on 5.2 g per 16 g N as 100%.
Source: United States Department of Agriculture. (2014). *National nutrient database for standard reference, Release 27*. http://www.ars.usda.gov/ba/bhnrc/ndl; Eggum, B. O., Juliano B. O. and Maniñgat, C. C. (1982). Protein and energy utilization of rice milling fractions by rats. *Qualitas Plantarum Plant Foods for Human Nutrition* **31**, 371–376.

lower cysteine and slightly higher TD in rats than indica rice protein (tropical as compared with japonica, temperate, or Japanese rice). Rice complements legumes (deficient in sulfur-containing amino acids) in amino acid composition for human diets.

Cooking and parboiling reduce the TD in growing rats by 5–15%, with a corresponding increase in BV, but little change in NPU. However, lysine digestibility remains close to 100%, while cystine digestibility drops to about 82%. The fraction that remains in the feces, as fecal protein particles, represents

the lipid-rich core of large PB I, with less than 1% lysine in the protein, but is rich in cystine. PB II is readily digested.

Rice is hypoallergenic, but some individuals are allergic to the 14–16 kD alpha-amylase/trypsin inhibitor and 33 kD Oryza glyoxalase 1. Both allergens are still active in cooked milled rice.

Starch: Starch varies in apparent amylose content (true amylose plus long-chain amylopectin as determined by iodine colorimetry in acetate buffer pH 4.5): waxy 0–2%, very low 2–10%, low 10–20%, intermediate 20–25%, and high >25%, all on a milled rice dry-weight basis since the 1950s. Actual amylose content values, measured using differential scanning calorimetry (by melting or crystallization enthalpy of amylose–lysolecithin complex), are slightly lower and closer to values determined using iodine colorimetry (ammonium buffer pH 9) (low 10–17%, intermediate 17–22%, and high >22%).

Rice starches also differ in gelatinization temperature, the point when granules start to swell irreversibly in hot water: low 55–70 °C, intermediate 70–74 °C, and high >74 °C. Gelatinization temperature correlates positively with cooking time.

The glycemic index of cooked brown rice tends to be lower than cooked milled rice owing to the greater amounts of phytic acid and fiber in brown rice, but only in waxy and low-amylose content rice. Brown and milled rice have similar glycemic index in intermediate- and high-amylose content rice. Among cooked milled and brown rice, glycemic index decreases with increasing amylose content regardless of the cooking method. With high amylose content, glycemic indexes are lower in those cooked in excess water; when cooked in a rice cooker with the same water–rice ratio and cooking time, low-gelatinization temperature rice has a higher glycemic index than intermediate-gelatinization temperature rice. Processing, including parboiling, tends to reduce the glycemic index. Resistant starch *in vivo*, as determined in ileostomy patients, is ≤5% and may not be as discriminating as glycemic index for humans. Short-term satiety has been found to be similar in milled and brown rice cooked to the same hardness by adjusting water content, regardless of amylose content.

Rice bran: The use of rice bran and brown rice in cereal products has increased in recent years owing to its recognition as a whole grain and hypocholesterolemic effect of the unsaponifiable fraction (up to 4.4%) of bran oil, which contains gamma-oryzanol (cycloartenol, 24-methylene-cycloartanol, and campesterol esters of ferulic acid), tocopherols, and tocotrienols, an analog of tocopherol (vitamin E). Defatted bran has no hypocholesterolemic activity, unlike in oats where the active component is soluble β-glucan. Inactivation of antinutrition factors – trypsin inhibitor, oryzacystatin, and hemagglutinin–lectin – and lipase and lipoxygenase, which are concentrated in rice bran by heat treatment (including cooking) and extrusion, improves the shelf life of bran and its nutritional value to poultry. In contrast, phytic acid is heat-stable and, in rice bran and brown rice, has been reported to have some activity in preventing/reducing the risk of colon cancer. Phytic acid content of rice bran is the highest among cereal brans (3–8% phytic acid).

The major fatty acids of rice oil are palmitic, oleic, and linoleic. Essential fatty acid content of rice oil is 33.4% linoleic acid and 1.6% linolenic acid. Levels of oryzanol, tocopherols, tocotrienols, and unsaponifiable matter differ among crude rice bran oils, and oryzanol, tocopherols, and tocotrienols may be reduced by up to 90% by conventional refining and deodorizing. These antioxidants seem to be lower in Philippine rice than rice grown in the United States.

Breeding for improved nutritive value: Breeding efforts to improve the nutritional value of rice grain include a higher micronutrient density (higher iron, zinc, and vitamin A), low phytic acid (high inorganic phosphate) content, low prolamin content, elimination of lipoxygenase-3 activity and allergenic globulin, and transgenic rice endosperm with provitamin A (β-carotene) (golden rice 2), soybean glycinin, or ferritin genes. Efforts to increase protein content from 7% to 9% at IRRI in 1966–79 were not successful.

Processing and Food Uses

The mean worldwide per capita consumption of milled rice was 53 kg year^{-1} in 2009. It was 77 kg in Asia, 31 kg in South America, 21 kg in Africa, 14 kg in Oceania, 9 kg in North and Central America, and 5 kg in Europe. Per capita rice consumption was highest in Southeast Asia at 129 kg year^{-1} followed by South Asia at 71 kg year^{-1}. In 2009, more than 3.5 billion people depended on rice for more than 20% of daily energy. Rice provides more than half the daily energy for about 520 million people in Asia, most of them being poor to very poor.

There are various ways of cooking milled rice. In tropical Asia, rice is prewashed to remove dirt, which reduces B vitamins and fat content. Presoaking for 30 min reduces cooking time (particularly of parboiled and brown rice). Rice can be cooked in the amount of water it will absorb or boiled in excess water and the cooking water discarded. Steaming is used in Indonesia and elsewhere for waxy rice. Oil or ghee may be added in the Middle East, which reduces surface stickiness. Enriched rice premixes, containing iron and B vitamins resistant to washing, have been developed, but are not popular because of the additional expense; the enriched rice can be readily distinguished from ordinary rice.

Rice amylose content correlates positively with water absorption and volume expansion during cooking in the amount of water it will absorb and with hardness of boiled rice. South Asian consumers prefer high-amylose content, intermediate–high-gelatinization temperature (>70 °C) rice, while Southeast Asians prefer intermediate-amylose content, intermediate-gelatinization temperature rice. *Japonica* (temperate) short-grain rice, preferred in Japan, Korea, Taiwan, and northern China, are mainly low amylose content and low gelatinization temperature (≤70 °C), with some having an intermediate amylose content in Europe. Only in Laos and north and northeast Thailand is steamed waxy (glutinous) rice consumed as a staple.

The low- and intermediate–high-gelatinization temperatures result from differences in proportion of debranched amylopectin with chain lengths less than 24 glucose units: low-gelatinization temperature rice contain more of the shorter chains. Increased numbers of longer chains raise not only gelatinization temperature but also the staling of cooked starch. Among waxy and low-amylose content rice, low-gelatinization temperature rice is softer when cooked than those with high gelatinization temperatures (intermediate gelatinization temperature is rare at low amylose content), but intermediate-gelatinization

temperature rice is softer when freshly cooked rice than low-gelatinization temperature rice. High-amylose content rice, with a high content of long-chain amylopectin (blue staining with iodine), has a hard gel consistency because long-chain amylopectin behaves like amylose. Native long-chain amylopectin crystallizes from water-saturated 1-butanol only after debranching with isoamylase.

For some rice products, specific amylose content is preferred. Freshly, well-milled rice is preferred for rice products with a prolong shelf life, minimizing rancidity in the stored products. Parboiled rice should be aged with high or intermediate amylose content, whereas extruded and flat noodles use mainly aged, high-amylose content, low-gelatinization temperature rice. Rice with a low gelatinization temperature is better for rice puddings, breads and cakes, and beer adjuncts. Waxy and low-amylose content rice is preferred for rice wines (for a higher ethanol yield) and in frozen sauces, desserts, snacks, and sweets, because of the slower staling rate. Rice crackers are prepared from waxy and nonwaxy rice. Parboiled rice is preferred over raw rice for *idli* (pudding) and *dosai* (cake) with rice-black gram usually fermented at a 3:1 weight ratio. The thermophysical properties of starch, such as glass transition temperature, gelatinization temperatures, enthalpy of raw starch, staled amylopectin melting (45–60 °C), amylose-lipid complex I (<100 °C) and II (>100 °C) melting, and staled amylose melting (>130 °C) of gelatinized starch, affect the properties of rice products in addition to the amylose–-amylopectin ratio and protein content.

Specialty rice includes Italian Arborio rice for risotto, waxy rice (sweet rice with an opaque grain), Thai Jasmine rice (aromatic low-amylose content long grain), and Punjab Basmati (aromatic intermediate-amylose content long grain, which elongates when presoaked). 2-Acetyl-1-pyrroline is the first major aroma components to be identified in raw and cooked aromatic rice; others are being characterized. Endosperm 2-acetyl-1-pyrroline, which is bound to starch and more persistent, requires higher extraction temperatures than the free form, which is volatile at room temperature and lost during storage.

See also: Cereals: Dietary Importance; Cereals: Types and Composition; Food Allergies; Infrared Spectroscopy: Applications; Phytic Acid: Properties, Uses, and Determination; Prebiotics; Protein: Requirements; Proteins: Chemistry, Characterization, and Quality; Rice: Types and Composition; Satiety; Sensory Evaluation: Sensory Rating and Scoring Methods; Starch: Structure, Property, and Determination; Starch; Vegetable Oils: Dietary Importance.

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Rice: Types and Composition

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Introduction

Rice (*Oryza sativa* L.), a member of family Poaceae and subfamily Oryzoidea, is one of the major staple foods consumed by 70% of the world's population and occupies one-fifth of the total land covered under cereal crops. The importance of rice has been recognized for many centuries and rice is also known as 'dhanya,' which means the sustainer of the human race. It is not only used as a foodstuff but also is an ingredient in medicines and cosmetics and has a role in crafts and religious ceremonies. The adaptation of rice cultivars to wide climatic conditions has led to the development of thousands of varieties having diverse quality features in terms of physical, cooking, eating, and product development characteristics. Rice is also known as model crop due to fully sequenced genome, relatively small genome size, significant level of polymorphism, and large amount of well-conserved genetically diverse material. It gives unique opportunities to researcher for studying its varietal difference and history of crop improvement using different approaches. In natural form, rice is obtained as a paddy, which is further processed to get different forms (brown, milled, parboiled, enriched, etc.) depending on health issues and consumer choice. Many research efforts including genetic engineering have been put forward to improve the productivity and nutritional composition of rice.

Morphology and Taxonomy of Rice

Rice is an annual plant that can grow to 3.3–5.9 ft tall having long, slender leaves, and flowers are produced in branches bearing pendulous inflorescence. The edible seed is a grain that is a fertilized and ripened ovule containing a live embryo capable of germinating to produce a new plant. Grain is mainly composed of the ripened ovary, the lemma and palea, the rachilla, the sterile lemmas, and the awn, where lemma and palea along with their associated structures constitute the husk. The embryo present on ventral side of the spikelet next to the lemma contains the embryonic root, and the rest of the grain consists of edible portion, that is, the endosperm. The taxonomy of rice is given in Table 1.

Rice Forms and Nutritional Composition

Multilevel processing categorized rice into different forms, which include paddy rice (kernels are present inside the hull), brown rice (husk being removed), milled or white rice (bran layers being removed), parboiled rice (rice gone through steam-pressure process before milling to increase head rice recovery and vitamin content of milled rice), precooked rice (cooked and dehydrated rice after milling to reduce time required for cooking), and enriched rice (a blend containing ordinary milled rice and a small percentage of fortified rice with vitamins and iron content slightly above the level present in brown rice).

Rice is mainly composed of carbohydrates as energy source followed by fat. The carbohydrate in rice is mainly in the form of starch, which exists as either amylose or amylopectin. Amylopectin contains branches and is less resistant to digestion, whereas amylose is a straight chain molecule and harder for the digestive system to break up. It is also a good source of the B vitamins, thiamine, riboflavin, and niacin but contains little to no vitamin C or D or β-carotene (precursor of vitamin A). The amino acid profile of rice is high in glutamic and aspartic acids but low in lysine.

Rice Husk

Rice husk, a by-product of the rice milling industry, offers the valuable nutritional advantage, as it contains an antioxidant defense system to protect the rice seed from oxidative stress. The major phenolic compounds present in husk are ferulic acid and p-coumaric acid along with isovitexin, a potent antioxidant.

Brown Rice and Rice Bran

Brown rice is unmilled and a whole grain form of paddy with mild and nutty flavor. Brown rice and milled rice have similar amount of calories as well as carbohydrates. However, during the milling process, dietary fibers, minerals, and B vitamins present in the bran are stripped off. The rice bran fraction contains 12–13% oil and highly unsaponifiable components (4.3%). The oil fraction contains tocotrienols, gamma-oryzanol, and β-sitosterol, which play an important role in lowering of the plasma levels of the various parameters of the lipid profile. The main antinutritional factors concentrated in the bran are phytate, trypsin inhibitor, oryzacystatin, and hemagglutinin–lectin. The interesting feature related to mineral content of rice bran is its low sodium and high potassium content, which may be a useful supplement in diets for hypertensives.

Milled Rice

The milling and polishing process to get white milled rice results in the loss of some important nutrients. The highly milled white rice have reduced protein, mineral, and lysine content, and the zinc concentration remains half as compared with brown rice. However, the consumption of milled rice is generally more acceptable than brown rice due to difficult digestion, high cooking time, and the need for more water for cooking, and more importantly, the color and texture of cooked brown grains are not attractive to the consumers.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00596-1

The major use of milled rice is in daily meals, breakfast cereals, snacks, desserts, pet foods, and brewing.

Parboiled Rice

Parboiled rice (converted rice) has been reported to be nutrient-dense. During the processing (soak and steam the rice kernels under intense pressure) method, many of the nutrients from the rigid outer hull go to the inner part of the kernel, and the hull falls off. The end product (parboiled rice) is enriched with plenty of vitamins and minerals and is very low in the glycemic index. The parboiling has been mainly done to drive nutrients, especially thiamin, from the bran to endosperm, and the parboiled rice is 80% nutritionally similar to brown rice. Thus, parboiled rice is found to be a better source of fiber, calcium, potassium, and vitamin B_6 than milled white rice. They are used as refrigerated and frozen products, salads, health food products, snacks, and breakfast cereals.

Enriched Rice

During milling process, many nutritional components have been stripped off in bran, and the consumption of highly milled white rice results in nutritional deficiencies. To overcome the problems, different types of enriched white rice have been developed with fortification of lost components including thiamine, riboflavin, iron, niacin, pantothenic acid, vitamin B_6, calcium, and folate. The two commercially adopted techniques for rice enrichment are powder enrichment and whole grain enrichment. The stability of added nutrients may be varied by the method adopted, utilization and storage washing, and cooking conditions.

Table 1 Taxonomy of rice

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Poales
Family	Gramineae or Poaceae
Tribe	Oryzeae
Genus	*Oryza*
Species	*Sativa*

Source: Biology of Rice (http://dbtbiosafety.nic.in).

Colored Rice

The nutrient content of rice suggests that the nutritional value of rice varies based on a number of factors as shown in Figure 1. The nutritional content also depends on the color of the grain. Scientists have found that the color of rice is basically due to the compounds it contains as in purple rice, hydrophilic antioxidants represent a greater portion than the lipophilic antioxidants. Furthermore, studies showed that most of the useful substances are found in the inner part of the purple rice bran. Similarly, black rice and red rice contain pigments that reduce atherosclerosis development and cholesterol levels, which are however absent in milled white rice.

Cultivation of Rice

The genus *Oryza* has 21 wild and 2 cultivated species, that is, *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice). The varieties belonging to *O. sativa* spp. are grown across the world, while the cultivation of the *O. glaberrima* has been confined to Africa. *O. glaberrima* varieties are fast being replaced by the *O. sativa* due to weed tolerance, pest resistance, and fast maturing growth attributes of later varieties. Based on the number of morphological, physiological, biochemical, and molecular traits, Asian rice cultivars are broadly classified into two major subspecies, that is, *Oryza sativa japonica* and *Oryza sativa indica* as shown in Figure 2.

In addition to these major varieties, the minor groups include aus cultivars of India and Bangladesh, ashinas varieties of Bangladesh, and aromatic basmati varieties of India. These groups may be further subdivided into more types like aromatic and nonaromatic, waxy, and nonwaxy based on their distinct features. Each type of rice cultivars have different markets and within each type are special quality demands for specific uses.

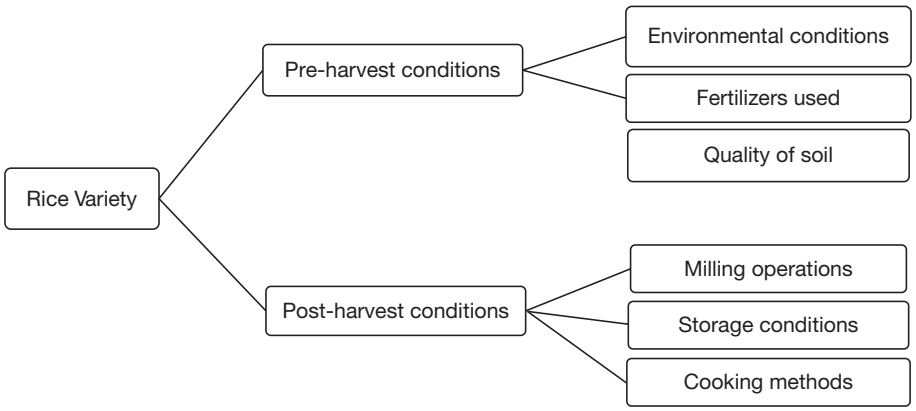

Figure 1 Different factors influencing nutritional composition of rice.

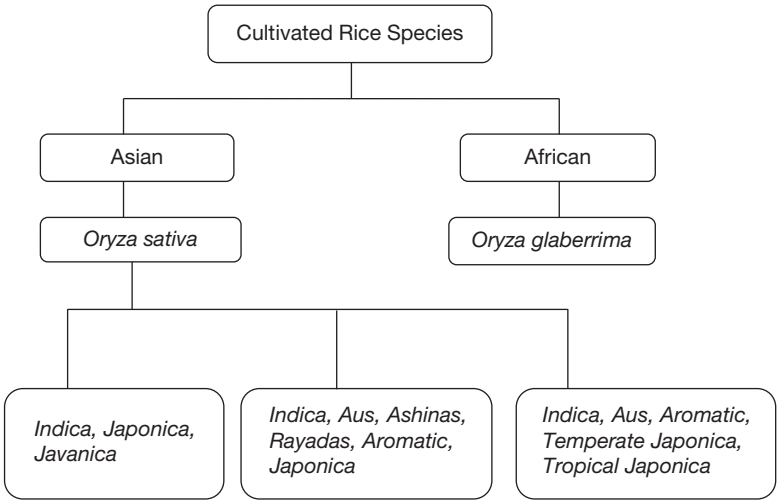

Figure 2 Classification of cultivated rice species.

Rice Varieties

With its long history of cultivation and selection under diverse environments, rice acquired wide adaptability, enabling it to grow in a wide range of environmental conditions. About 40 000 rice varieties and 500 000 accessions of rice genetic resources are conserved all over the world. The largest six gene banks are in Asia and together conserve around 70% of the total world rice germplasm. They are the International Rice Research Institute (IRRI) in the Philippines, National Bureau of Plant Genetic Resources (NBPGR) in India, Institute of Crop Germplasm Resources (CAAS) in China, China National Rice Research Institute (CNRRI), National Institute of Agrobiological Sciences (NIAS) in Japan, and Rural Development Administration (RDA) in the Republic of Korea.

Rice varieties are often classified into different groups on the basis of morphological, geographic, and quality variations, which are discussed in the succeeding text.

Japonica Rice

Japonica is one of the two major ecogeographic races of *O. sativa* (*O. sativa* japonica) from northern and eastern China and grown extensively in some areas of the world. It is found in the cooler zones of the subtropics and in the temperate zones. Japonica grains are short and roundish, do not shatter easily, and have 0–20% amylose content (AC), with awnless to long-awned spikelets. The two main categories of Japanese rice are ordinary rice (uruchimai) and glutinous rice (mochigome). Uruchimai is widely grown and is the staple foodstuff of the Japanese diet with short translucent grains and has sticky texture. Glutinous rice are short, round, and opaque grains with greater stickiness upon cooking but firmer and chewier texture when consumed.

Indica Rice

Indica rice is the other of the two major ecogeographic races of *O. sativa*. It is the major type of rice grown in the tropics and subtropics. They grow mostly in the Philippines, India, Pakistan, Java, Sri Lanka, Indonesia, central and southern China, and African countries. Indica grains are long to short, slender, and somewhat flat, and the spikelets are awnless. Indica grains shatter more easily and have 23–31% AC. The diverse ecological situations in areas of rice cultivation have given rise to the different ecospecific rice varieties of indica group with specificity for season, situation, and system.

Aromatic Rice

Aromatic rice constitute a small but special group of rice, which are considered best in quality. These varieties have long been popular in the Orient and are now becoming more popular in Middle East, Europe, and the United States. The fragrant rice other than basmati are found in different parts of the world. Basmati is one of the most well-known varieties of aromatic rice. It is cultivated in India, along with *Chinoor*, *Shakarchini*, and *Kalanamak*, among numerous other varieties. Basmati rice is different from other aromatic rice varieties because of the aroma and postcooking elongation attributes. No other rice has these combined characteristics. The other distinct characteristics of basmati rice include extra long, slender grain, lengthwise excessive elongation on cooking, and soft and fluffy texture of cooked rice grain. Studies on the unique quality traits of basmati rice found their gene expression only when they are grown in the northwestern foothills of the Himalayas in the Indian subcontinent. Due to the geographic specific manifestation of quality features, basmati is now a geographic indication belonging to a specific geographic area in the Indian subcontinent grown by farmers of Punjab region of India and Pakistan and Haryana and Uttar Pradesh regions of India for more than 250 years.

Similarly, jasmine rice, known as Thai fragrant rice (originally from Thailand), possesses long grain and has a nutty aroma and a subtle pandan (*Pandanus amaryllifolius*) like the flavor caused by 2-acetyl-1-pyrroline (2-AP). The grains will cling when cooked, though they are less sticky than other rice as they have less amylopectin. It is also known as Thai Hom Mali and has a very high glycemic index of 109, which is more than pure glucose.

In the United States, several companies have bred specific cultivars of aromatic rice, including wild pecan and Wehani rice. Wild pecan has a rich, nutty flavor and aroma, whereas Wehani smells like popcorn and is gray in color and the grain splits on cooking. These cultivars were developed in response to increasing consumer demand for this rice.

Glutinous Rice

This group of rice varieties is also famous as sticky rice or waxy rice. This rice is mainly grown in the regions of Southeast and East Asia. The grains have opaque grains and very low AC and are sticky when cooked. Amylopectin is responsible for the sticky quality of glutinous rice. Like all types of rice, glutinous rice does not contain dietary gluten (i.e., glutenin and gliadin) and should be safe for gluten-free diets.

Rice Grain Quality

The quality of rice grain assumes greater importance because most of the rice produced is cooked and consumed as a whole kernel, whereas the percentage of rice converted into flour or flakes is very small. The genetic diversity among rice cultivars from quality point of view can be assessed in three broad categories, that is, physical qualities, processing qualities, and nutritional qualities. The shape and size of grain, chalkiness, and alkali spreading values are considered under physical qualities. Alkali spreading value involves the treatment of rice grains with dilute alkali, which results in disintegration of starch molecules. The waxy and low-amylose-containing grains disintegrate fast as compared with high-amylose-containing rice grains. The change in shape of grain and its disintegration level are recorded on seven-point scale on the basis of which gelatinization temperature is calculated. Milling, cooking, and textural and thermal attributes are considered as processing qualities, which are in turn greatly influenced by AC and gelatinization temperature. The nutritional qualities of different rice grains depend upon protein, carbohydrate, starch, ash, fat, mineral, vitamin, and amino acid contents.

Milling yield is one of the most important criteria of rice quality especially from a marketing standpoint. A variety should possess a high turnout of whole grain (head) rice and total milled rice. Milling yield of rough rice is the estimate of the quantity of head rice and total milled rice that can be produced from a unit of rough rice. Thus, the milling quality of rice may be defined as the ability of rice grain to stand milling and polishing without undue breakage to yield the greatest amount of total recovery and the highest proportion of head rice to broken rice. The three main dimensions (length, width, and thickness) of paddy grains are useful in selecting sieve separators to calculate surface area and volume of kernels, which are important during modeling of grain drying, aeration, heating, and cooling and further in designing particular equipment for handling and storage of grains. Densities of different types of cereal grain kernels are of paramount interest in breakage susceptibility and hardness studies. The parameters related to the frictional properties (angle of repose and coefficient of friction) of cereal grains on different surfaces are very helpful in design of hoppers based on the flow ability of agricultural grains and also in safe designing of equipments related with processing, moving, and storage of grains.

Physical Parameters of Milled Rice

The rice grain length, width, and weight mainly determine the physical quality of rice grain. The classification of rice quality is based on the length of grain, that is, short, medium, and long grains. Grain shape is an important agronomic trait in cereal crops because it is directly or indirectly related to the quality and quantity of grain products. Moreover, grain size and shape are the first quality characteristics considered in developing new varieties. The length to breadth ratio ranging from 2.5 to 3.0 is widely acceptable, and the grain length >6 mm is preferred. The size and shape of the rice grain may vary from one group to another group of different regions. Some groups prefer short grains, some prefer medium grains, and mostly, the peoples like long, slender grains in Indian subcontinent regions and in international market. Medium and short grains are preferred by the peoples of South Asian regions. Thus, components of appearance traits are one of the first criteria for rice quality that breeders consider in developing new varieties for open release and commercial production.

The length to width ratio is also an important aspect to determine the shape of the variety, whereas grain weight gives the information about the size and density of the grain. The density of different rice grains affects the cooking quality.

Chemical Parameters of Milled Rice Grains

The most important quality criterion based on chemical characteristics of rice grain and flour of different cultivars is moisture, carbohydrate, protein, fat, crude fiber, and ash contents. The moisture content during storage showed changes with the length of storage and the temperature of storage. Proteins and starches are the two major components of rice, with approximately 8% and 80% composition, respectively. Rice protein is more nutritious because of its higher lysine content than any other cereal proteins. Micro-Kjeldahl analysis and different methods are used to determine the protein content in rice. Fat is the main source of food energy, which, rather than giving energy, helps in the absorption of fat-soluble vitamins. Rice contains very low amount of fat, but cooked brown rice contains higher fat contents than white rice.

Cooking and Textural Properties

The rice produced in different parts of world varies significantly in composition and cooking quality. Genetic and environmental factors are mainly responsible for the variation in composition and cooking quality of rice. Consumer preference regarding cooking attributes of rice grains may vary from region to region. Japanese like sticky rice, while Italians consume short-grain variety, which releases starch during cooking, making a creamy and smooth risotto, Americans prefer a semi-milled long-grain rice or even brown rice, Asians predominantly prefer spicy and scented basmati/jasmine rice, and the people of Indian subcontinent prefer a well-milled white rice. Cooking and eating characteristics are largely determined by the properties of the starch that makes up 90% of milled rice. Gelatinization temperature, AC,

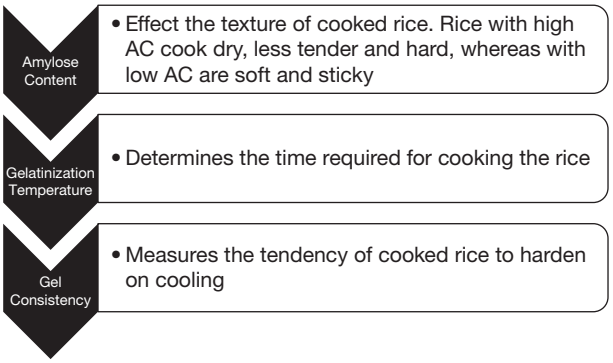

Figure 3 The effects of amylose content, gel consistency, and gelatinization temperature on cooked rice grains.

and gel consistency are the important starch properties, which influence cooking and eating characteristics as shown in Figure 3.

Amylose content

AC is considered as the most important character for predicting rice cooking and processing behavior. Many of the cooking and eating characteristics of milled rice are influenced by the ratio of two kinds of starches, that is, amylose and amylopectin. Amylose is the linear fraction of starch in the nonglutinous varieties, whereas amylopectin, the branched fraction, makes up the remainder of the starch. Amylose is almost absent from the waxy (glutinous) rice cultivars that do not expand in volume, are glossy and sticky, and remain firm when cooked. High-amylose-containing rice grains show high volume expansion and a high degree of flakiness. They cook dry, are less tender, and become hard upon cooling. Rice grains with intermediate AC cook moist and tender and do not become hard upon cooling. Rice varieties are grouped on the basis of their AC into waxy (0–2% AC), very low (3–9% AC), low (10–19% AC), intermediate (20–25% AC), and high (>25% AC).

Gelatinization temperature

Different diffusive processes like heating, water uptake, and swelling of the rice particles take place during cooking of rice grains. Starch granules are heated in water beyond a critical temperature; the granules absorb a large amount of water and swell to many times their original size. Over a critical temperature range, the starch granules undergo an irreversible process known as gelatinization, which is marked by crystalline melting, loss of birefringence, and starch solubilization. As soon as the temperature rises above the gelatinization temperature, the starch granules begin to swell and viscosity increases. The gelatinization temperature provides an indication of the minimum temperature required to cook for a given rice sample. The gelatinization temperature of rice varieties may be classified as low (55–69 °C), intermediate (70–74 °C), and high (>74 °C). The gelatinization temperature can be estimated by an alkali digestibility test in which the degree of spreading value of individual milled rice kernels in a weak alkali solution (1.7% KOH) is very closely correlated with gelatinization temperature. Rice with low gelatinization temperature disintegrates completely, whereas rice with intermediate gelatinization temperature shows only partial disintegration. Rice with high gelatinization temperature remains largely unaffected in the alkali solution.

Linear elongation of rice on cooking is one of the major characteristics of good rice. Some varieties expand more in size than others upon cooking. Lengthwise expansion without increase in girth is considered a highly desirable trait of rice. Soaked milled rice of high gelatinization temperature elongates less during cooking than rice of low and intermediate gelatinization temperature. Thus, gelatinization temperature correlates positively with grain elongation. However, gelatinization temperature does not correlate with the texture of cooked rice.

Gel consistency

The texture of cooked rice grains is mainly dependent upon the AC; however, the cohesiveness, tenderness, color, and gloss of cooked grains are greatly based on gel consistency, when the AC is high. Varietal differences in gel consistency exist among varieties of similar AC. The gel consistency test is based on the consistency of the rice paste, which is different among varieties with high AC. It measures the tendency of cooked rice to harden when it cools down. In gel consistency test, test tubes containing rice grain powder (100 mg), ethyl alcohol (95%), thymol blue (0.025%), and potassium hydroxide (0.2 N) are heated in a vigorously boiling water bath for 8 min. The test tubes are removed from the water bath and left to stand at room temperature for 5 min. The tubes are cooled in an ice water bath for 20 min and laid horizontally on a laboratory table lined with millimeter graphing paper. The total length of the gel has been measured in mm from the bottom of the tube to the gel front. This test separates rice grains into three categories:

1. Very flaky rice grains with hard gel consistency (length of gel, 40 mm or less)
2. Flaky rice grains with medium GC (length of gel, 41–60)
3. Soft rice grains with soft gel consistency (length of gel, more than 61 mm)

Cooked kernel elongation

The elongation ratio and elongation index are the important cooking quality traits of rice and are much preferred by the consumers. The quality of cooked rice grain is mainly associated with its length wise expansion as compared with its widthwise expansion. Aromatic long-grain basmati rice are known for their pleasant aroma and extra elongation on cooking with least breadthwise swelling. It has been also observed that if rice elongates more lengthwise, it gives a finer appearance, whereas it gives a coarse look if it expands girthwise.

Aroma

The aroma of rice plays a role in its consumer acceptability, and it draws a premium price in certain specialty markets. Most of the high-quality preferred varieties in major rice growing countries are aromatic in nature. Jasmine rice from Thailand, basmati rice from Pakistan and India, and Della rice from the United States are the main sources of aromatic imports. The scent of aromatic rice is a highly heritable trait and reportedly is under the control of one to four genes, depending on the population studied. More than 100 compounds that

contribute to the aroma of rice have been identified. Some of these volatile compounds contribute to consumer acceptance of certain type of rice, whereas other compounds contribute to consumer rejection. The popcorn-like smell of aromatic rice stemming primarily from its 2-AP content is considered desirable by many consumers.

Global Distribution of Rice

Rice is the world's leading food crop, cultivated over an area of about 155 million hectares with a production of about 596 million tons (paddy). In terms of area and production, it is second to wheat. Maximum area for rice cultivation is found in Asia, with India having the largest area (44.8 million hectares) followed by China and Indonesia. In respect to production, India ranks second with 131 million tons of paddy next to China (200 million tons of paddy). In regard to average yield per hectare, Egypt ranks first followed by the United States. The leading countries producing rice crop are Japan, Brazil, China, India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, and the Philippines. The details about milled rice production and consumption in tons at international level have been given in Table 2.

There are major world markets for rice in terms of quality, definite varieties, and processing types. The long-grain, higher-quality rice is sold mostly in Europe and the Middle East, the medium-quality long-grain in the deficit countries of Asia, the short-grain product in various special demand areas, the high-quality parboiled rice in the Middle East and Africa, and the lower-quality parboiled rice in special markets in Asia and Africa. Aromatic rice is demanded mostly in the Middle East, and glutinous rice meets the needs in Laos with smaller volumes to other countries.

Table 2 Statistical data on milled rice production and consumption in different countries

Countries	*Production (2013–14)*	*Consumption (2013–14)*
Bangladesh	34 200	34 700
Brazil	8500	7800
Burma	11 000	10 250
Cambodia	4900	3800
China	144 000	147 000
Egypt	4850	4000
India	108 000	98 500
Indonesia	37 700	40 300
Japan	7720	8150
South Korea	4220	4497
Nigeria	3100	3350
Pakistan	6200	5900
The Philippines	11 700	12 925
Thailand	21 100	10 600
Vietnam	27 850	20 600
Others	38 183	58 212
Subtotal	473 223	473 187
The United States	6038	3657
World total	479 261	476 844

Source: All India Rice Exporters Association (www.airea.net).

Rice Biotechnology

The rapid increase in global population and the ever-increasing demand for food force the researchers, industrialists, and government authorities to fulfill the demanding gap. This situation is true also to rice as it is a significant source of calories for half of the world. As a result, rice is receiving considerable attention by plant breeders who are using both conventional and transgenic techniques to increase rice yield and quality.

International Rice Genome Sequencing Project has developed and analyzed a complete, highly precise sequence of the rice, and the project came out with many prominent attributes of rice genome: the genome size is 389 Mb, sequencing covered 95% of the total genome including all of the euchromatin, two complete centromeres, and 37 544 nontransposable element-related protein-coding sequences, and 2859 genes were found to be unique to rice. It has recognized 80 127 polymorphic sites that can distinguish between two cultivated rice subspecies, *O. sativa japonica* and *O. sativa indica*. Along with the identification of genes, single-nucleotide polymorphisms and simple sequence repeats were additionally identified, which in the future will pick up the pace in improvements of rice production.

Some of the important interventions of biotechnology in rice (in terms of health) are as follows.

Nutritional Rice Varieties

Golden rice is a genetically modified variety of *O. sativa*, which has been developed to biosynthesize β-carotene, a precursor of vitamin A, in the edible parts of rice. The research was conducted with the goal of producing a fortified food to be grown and consumed in areas with a shortage of dietary vitamin A, a deficiency that is estimated to kill 670 000 children under the age of 5 each year. Golden rice was created by transforming rice with only two β-carotene biosynthesis genes:

1. *psy* (phytoene synthase) from daffodil (*Narcissus pseudonarcissus*)
2. *crtI* (carotene desaturase) from the soil bacterium *Erwinia uredovora*

The original golden rice was called SGR1, and under greenhouse conditions, it produced 1.6 $\mu g\ g^{-1}$ of carotenoids.

To improve the nutritional quality, the major focus is on developing rice varieties with provitamin A and high iron and zinc content in the polished grain. Biotechnology techniques such as plant transformation offer great opportunities for increasing the amounts of trace metals in the endosperm. Different types of genes (OsNAS2, SoyFer, TOM1, HvNAS1, etc.) have been used for rice biofortification, and these genes are encoding metal chelators and metal transporters, Fe storage protein (ferritin), and phytase.

Improvement of Quality Traits in Rice

The quality of grains is a major concern for consumers, which generally preferred long, nonsticky, and aromatic rice. Many traits related to the quality of cooked rice grains have been mapped, and genes/QTLs have been reported for AC, gel consistency (chromosome 6), kernel length after cooking, and

aroma (chromosome 8). Similarly, many QTLs have been reported for head rice recovery, protein content, grain length, and grain length to breadth ratio.

Expression of Human Proteins in Rice

Genetic modifications have been done in rice varieties to express lactoferrin and lysozyme proteins, which are usually found in breast milk and human serum albumin. These proteins have many medicinal benefits including antiviral, antibacterial, and antifungal effects. Grains of rice containing these proteins can be used as a component in oral rehydration solutions to treat diarrheal diseases and anemia.

Allergen-Free Rice

Rice seed proteins contain antigens that provoke allergic responses in some individuals with food allergy, particularly in those with cereal allergy. These antigens are responsible for clinical symptoms such as eczema and dermatitis. An attempt has been made to develop allergen-free rice variety by repressing the formation of high-molecular-weight allergen compounds.

Conclusions

The production and consumption of rice are variable at a global scale based on its forms, varieties, geographic origin, climatic conditions, and choice of consumer. The demand of rice is expected to be higher in the future to keep in pace with increasing population. Thus, technologies like biofortification and genetic modifications in rice are advancing at a rapid rate to make scientific progress, which would lead to both nutritional and economical benefits in rice-consuming countries.

See also: Cereals: Dietary Importance; Cereals: Types and Composition; Food Fortification: Rationale and Methods; Starch: Structure, Property, and Determination.

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Risk Assessment of Foods and Chemicals in Foods

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Introduction

Risk assessment has been defined by the International Programme on Chemical Safety in 2004 as

> A process intended to calculate or estimate the risk to a given target organism, system, or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system.

Risk assessment of food and food ingredients can range from assessment of the effects on health of whole foods and food ingredients, such as macro- and micronutrients, to individual chemical substances or physical agents such as radionuclides or foreign bodies present in food. The focus of this article is on individual chemical substances, such as food additives, processing aids, migrants from food contact materials, residues of plant protection products (pesticides) and of veterinary products, and undesirable contaminants originating from the natural or man-made environment. Risk assessment of novel foods and genetically modified (GM) foods has also become an important activity.

There is also increasing attention by public health bodies on the effects of the diet as a whole and of macroingredients in food on human health, particularly on serious, chronic, and prevalent conditions such as cardiovascular disease, diabetes, and cancer. Microbiological contamination of food is also an important and frequent cause of ill health. Both of these topics are beyond the scope of this article.

Risk assessment of individual chemical substances in food that are derived from products deliberately added to food or applied to food, crops, or food-producing animals (e.g., food additives, food packaging, processing aids, pesticides, and veterinary products) usually relies on information obtained from *in silico*/bioinformatic approaches, *in vitro* laboratory tests, and *in vivo* testing in laboratory animals. Similar approaches are used for the assessment of GM foods but with lesser emphasis on laboratory animal tests and greater emphasis on the characterization of the GM element(s). For common food contaminants (e.g., mycotoxins, heavy metals, and persistent organic pollutants), there are often human data available in addition to laboratory-derived data. Risk assessment of the diet or macroingredients is usually based on human epidemiological studies and observations.

General Principles of Risk Assessment as Applied to Food

The scientifically based process of risk assessment utilizes the following steps: hazard identification, hazard characterization, intake/exposure assessment, and risk characterization. This four-step paradigm for a systematic approach to risk assessment is widely adopted throughout the world by risk assessment bodies, such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Food Safety Authority (EFSA), and the US Food and Drug Administration.

Hazard Identification

Hazard identification is the first step in the risk assessment process. It identifies the types of adverse health effects that a chemical or physical agent may exert. The aim is to identify potential hazards to humans that the presence of a substance in food may pose. Potential hazards can be identified from data including *in silico* analysis of structure–activity relationships, read-across from toxicity data on closely structurally related substances, laboratory studies on the substance itself and *in vitro* or *in vivo* studies in laboratory or farm animals, human and domestic animal case reports, and other human observations and epidemiological studies.

Risk assessors consider all of the available data on chemical structure, physicochemical properties, kinetics and metabolism of the ingested substance in the body, and toxicity of the substance. In this manner, as much information as possible is assembled on the nature and severity of any toxic or adverse health effects observed, the affected target organs and/or tissues, and, if toxicity is evident, any information that may be available on the mode of action of the substance.

Hazard Characterization

Hazard characterization is the second step in the risk assessment process. It involves the qualitative and, where possible, the quantitative evaluation of the adverse health effects that have been identified in the first step. For chemicals, this is sometimes termed dose–response assessment. It describes the relationship between the magnitude of the administered dose of (or exposure to) a chemical and the incidence and/or severity of an adverse health effect. Where possible, risk assessors will try to identify from the range of studies available a dose or exposure below which no adverse effects have been observed.

During hazard characterization, each effect identified will be assessed for human relevance. Some effects observed in animal studies are not considered relevant for humans (e.g., the accumulation of alpha-2-microglobulin resulting in nephropathy in the kidney with exposure of rodents to hydrocarbons, adrenal medullary hyperplasia and neoplasia in rodents treated with large amounts of nondigestible substances, and the particular sensitivity of cats to hematologic effects from propylene glycol). Usually, a critical effect of potential relevance to humans will be identified. The critical effect is the first significant adverse effect that appears as dose or exposure is increased. This generally forms the focus of the risk assessment, but other identified adverse effects occurring at

higher doses may also need to be considered (see section 'Risk Characterization').

For most types of toxic effect, it is generally considered that there is a dose/exposure below which adverse effects will not occur (i.e., a threshold) in animals or humans. For toxic effects with a threshold, a no-observed-adverse-effect level (NOAEL) or a no-observed-effect level can usually be identified from animal studies. NOAEL is the preferred term used nowadays because it indicates that the effect observed at doses higher than the NOAEL is considered adverse and relevant to humans. Whether or not the NOAEL closely approximates the true threshold for the onset of a toxic action of a particular substance in the system or species under investigation depends very much on study design aspects, such as the duration of the study, the number of animals or human subjects per dose group, the dose intervals selected, the range of the toxicity parameters investigated, and the sensitivity of the methods used to detect changes in the body.

For chemical substances, the aim of hazard characterization is to identify, if possible, a numerical reference point or point of departure (POD) from which a health-based guidance value (HBGV) can be derived at the final risk characterization stage. The reference point or POD is often the NOAEL. If an NOAEL cannot be identified, it may be possible to identify the lowest-observed-adverse-effect level (LOAEL), which can then be used as the reference point or POD. More recently, risk assessors have introduced an approach in which the dose–response data for each potentially critical effect are mathematically modeled to derive a 'benchmark dose' (BMD) for a particular incidence of effect. The incidence selected is usually near the lower end of the observed range of data (e.g., a 5% or 10% incidence) and is termed the benchmark response. To allow for uncertainty, the computed lower 95% confidence limit on the modeled BMD (BMDL), rather than the BMD itself, is normally used as the reference point or POD for derivation of an HBGV. The critical effect to be taken forward for the risk characterization is usually the effect with the lowest BMDL. The use of the BMD approach is considered preferable nowadays to the NOAEL approach because it makes best use of the available dose–response data and is not unduly influenced by the dose interval selection.

For some types of toxic effect, such as genotoxicity or cancer caused by a genotoxic mode of action, it cannot be assumed that a biological threshold exists. For these types of effect, it is assumed that there is some probability of harm at any level of exposure. In the case of substances that are avoidable in foods (e.g., a proposed food additive, pesticide, and veterinary drug), which are shown to be genotoxic *in vivo*, a decision may be taken not to allow their use. In other cases, in which an unavoidable substance in food (e.g., a contaminant) is shown to be both genotoxic and carcinogenic, the BMDL derived from animal cancer bioassays may be used as the reference point or POD for risk characterization.

Intake/Exposure Assessment

Intake/exposure assessment is the third step in risk assessment, in which the known or anticipated extent of human intake of, or exposure to, the substance is determined. Quantitative determinations are essential for full risk assessment, but if data are sparse, as may be the case with contaminants, a qualitative assessment may be the best that can be achieved. Intake is the term usually applied when considering macroingredients or whole foods. Exposure is the preferred term when considering chemical or physical agents. In the case of chemical substances present in food, through either deliberate addition or application to food or their unintentional presence in food, exposure assessment takes into consideration the following information:

- Occurrence and concentrations of the chemical in the various foods in the diet
- The consumption patterns of the foods containing the chemical
- The likelihood of consumers eating large amounts of the food(s) in question (high consumers)
- The likelihood of the chemical being present in these foods at high levels

Single-point estimates of intake/exposure are of limited value because they do not reflect the likely range across the population. Normally, exposure assessors will provide a range of intake/exposure estimates (e.g., for average consumers and for high consumers), which will usually also be broken down by the subgroup of the population (e.g., infants, children, and adults).

Exposure estimates should, ideally, take into account not only exposure from food sources but also exposure to the same substance from all sources, by all routes and pathways. This is termed an aggregate exposure assessment. However, detailed information on other sources may often be lacking. For a comprehensive risk characterization, an estimate of total systemic exposure may be needed, particularly where nonfood sources make a significant contribution to overall exposure.

Risk Characterization

Risk characterization is the final step in the risk assessment process. All the data from the preceding three steps of hazard identification, hazard characterization, and intake/exposure assessment are assembled to provide a qualitative and, where possible, quantitative description of the probability of occurrence of adverse health effects in the population as a whole and/or in subgroups of the population, under different exposure scenarios. Risk characterization should include a narrative description of the nature and severity of any adverse effects. In considering risks to human health, a distinction is usually made between toxic effects and intolerance reactions such as pain and diarrhea from osmotic effects of polyol sweeteners or from consumption of bulky fibrous ingredients. Toxic effects are always taken into account when setting an HBGV, whereas intolerance reactions may not be, but may require a narrative warning.

The risk characterization advice should be in a form that is suitable to enable risk managers to make decisions about any necessary actions that may need to be taken to protect the population from ill health. Most importantly, risk characterization should include a clear description of the assumptions and uncertainties in the available data on hazards, dose–response, and exposure, including data gaps and any uncertainties in the derivation of an HBGV. Consideration of

any assumptions and uncertainties is a key aspect for risk management decision-making, particularly in cases where risk management actions may be indicated. Where risk management actions may need to be taken to avoid or reduce exposures, the risk characterization may usefully include the assessment of the effect on exposure of different scenarios for risk reduction.

Risk assessment is usually carried out by toxicologists, nutritionists, and exposure experts and requires a scientific background. Risk assessors advise and interact with risk managers who have the responsibility for ensuring that the food supply is safe and that consumers are not put at risk. Ideally, risk characterization should be based on quantitative information, but where this is not possible, advice can be given in a qualitative form.

Qualitative advice

Qualitative advice may include, for example, that the available data indicate that there are no human health concerns, or there are no toxicological concerns (or no safety concerns), even at high exposure levels. Alternatively, qualitative advice may need to state limitations, such as, for example, that there are no concerns from exposure to a particular substance used in a specified way up to a specified concentration in food. If the available data indicate that there are or would be human health concerns arising from certain exposures or particular uses in some or all sectors of the population, then qualitative advice should make clear the circumstances under which adverse effects could occur. When it is not possible to give a precise estimate of probability of occurrence of adverse effects on health, narrative guidance, based on professional judgment, is helpful for risk managers.

Quantitative advice

Quantitative advice may include the derivation of an HBGV and information on whether current population or subpopulation exposures are below or above the HBGV. Alternatively, quantitative advice may provide estimates of the level of risk at different exposures.

Health-based guidance values

HBGVs are levels of human exposure, which may be experienced over a lifetime, that are considered to be without appreciable risk to health. An HBGV for a chemical that is deliberately added to food or is applied to food crops or food-producing animals is termed acceptable daily intake (ADI). For chemicals not deliberately added to food, such as contaminants, the terms tolerable daily intake (TDI) and reference dose are preferred. For some chemicals, for which cumulative exposure causing a chronic toxic effect is the critical effect, the HBGV is set as a weekly or monthly intake, rather than a daily intake. For pesticides and veterinary products, in addition to an ADI, it may also be necessary to set an acute reference dose to cover possible acute effects from exposures to high amounts over a short period (24 h). This particularly applies to those chemicals whose mode of action as a pesticide or veterinary medicine is to inhibit acetylcholinesterase, as this effect is often seen at low exposure levels.

HBGVs, such as ADIs and TDIs for substances with toxic effects that are expected to have a threshold, are set by application of an uncertainty factor (UF), also known as a safety factor, to a reference point or POD; suitable PODs are the NOAEL, and, if the BMD approach has been used, the BMDL. An HBGV is calculated as follows:

$$\text{HBGV} = \frac{\text{POD (i.e., NOAEL or BMDL)}}{\text{UF}}$$

A default overall UF of 100 (10×10) has been routinely used for many years when extrapolating from animal data to humans, comprising a factor of 10 to cover possible interspecies variation and a factor of 10 to cover possible human interindividual variation. It was originally proposed in the 1950s, on an empirical basis, and has largely stood the test of time. Each of the subfactors of 10 can be further subdivided into toxicokinetic (TK) and toxicodynamic (TD) components. TK data describe the behavior of a chemical in the body; TD data describe the sensitivity of the target organs/tissues to toxicity. The default factors for TKs and TDs are as follows: interspecies variation, 4.0 for TKs and 2.5 for TDs, and human interindividual variation, 3.16 for TKs and 3.16 for TDs. If the data on a particular chemical are sufficient, they may allow replacement of some or all of the default UFs for TKs and TDs by specific, observationally derived values, known as chemical-specific adjustment factors. If the data used to derive an HBGV are from human studies, then the default UF of 10 for interspecies variation is not utilized.

If an LOAEL rather than an NOAEL is used as the reference point or POD, then it is usual to incorporate an additional UF (e.g., a factor of 2), in addition to the default UF of 100, giving an overall UF of 200. Similarly, some risk assessors may use additional UFs, ranging up to 10, to take account of gaps in the underlying toxicology database (e.g., absence of a reproductive study) or to extrapolate from subchronic to chronic exposure duration.

While the utilization of UFs in derivation of HBGVs is intended to take account of interspecies differences and human interindividual differences in TKs and TDs, it does not necessarily take account of specific medical conditions or all genetic differences in metabolic capacity. When it is known that a subgroup of the population with a specific metabolic condition may be at risk from exposure to a particular substance, then that substance may still be permitted in food, accompanied by an appropriate warning, as is the case for the intense sweetener, aspartame, for which those with phenylketonuria are advised to avoid.

HBGVs are normally set based on a single critical effect (see section '**Hazard Characterization**'), which will normally be the effect observed at the lowest dose across a range of studies. However, since HBGVs are derived by dividing the NOAEL or BMDL by an appropriately selected UF, there may be occasions when another toxic effect is first observed at a dose higher than that for the originally identified critical effect but requires the application of a higher UF, resulting in a lower HBGV. Thus, risk assessors may sometimes need to consider more than one toxic end point for a substance.

Once an HBGV has been set for a particular substance, the final task in the risk characterization is to compare the population exposure estimates with the HBGV. If all exposure estimates are below the HBGV, then the substance may be permitted for use in food, or if it is already in use, or it is a

contaminant, this indicates that risk management actions are not needed. If any of the exposure estimates exceed the HBGV, then that is an indication that risk management actions may be needed.

Margin of exposure approach

In the case of a substance for which the toxicity is considered to have a threshold, but for which some data are missing and so it may be inappropriate to set a numerical HBGV, the margin of exposure (MOE), also sometimes called the margin of safety (MOS), between an NOAEL or an LOAEL and the known or estimated human exposure can be calculated to provide guidance for risk managers. The MOE/MOS would be accompanied by a narrative describing the potential impact of the missing data on the risk assessment.

The MOE approach is also used for substances with toxic effects that are considered not to have a threshold, such as genotoxicity or carcinogenicity with a genotoxic mode of action. In such cases, the setting of HBGVs is not appropriate. Substances that are genotoxic and/or genotoxic carcinogens are not normally permitted in food unless they are unavoidable. In the case of genotoxic substances that have been tested in animal carcinogenicity bioassays, the data from such assays can be used to estimate the MOE between the BMDL value for the critical tumor site(s) and the known or estimated human exposure levels. The MOE is calculated as follows:

$$\text{MOE} = \frac{\text{BMDL}}{\text{Human exposure}}$$

MOEs of 10 000 or more generally indicate low health concern as the probability of the substance causing genotoxic damage or cancer is considered to be very low. MOEs of less than 10 000 will usually trigger risk reduction measures. MOEs of under 100, as, for example, in the case of acrylamide in food, would be considered to require urgent risk reduction measures.

Cumulative Risk Assessment

It has long been recognized that simultaneous exposure to more than one substance, as opposed to exposure to a single substance, may have an influence on the resulting toxicity of any of the substances involved. Substances that are very closely structurally related may have similar toxicities and, for food additives, this has been recognized by the allocation of group ADIs for such substances. In the case of dioxins and dioxin-like polychlorinated biphenyls, which are ubiquitous contaminants in the environment and thus present in food, a similar approach has been adopted of assessing the potential combined health effects from multiple exposures to closely related substances with similar toxic effects. For this, the concept of toxic equivalency factors has been developed, which allows comparison of the relative potency of different chemicals within the group on the same target and allows a combined HBGV to be set for the group.

Other substances that are not particularly closely related in structural terms may nevertheless exert their toxicity by a common mode of action. For example, various pesticides have a common mode of action to inhibit acetylcholinesterase, which forms the basis not only of their insecticidal activity but also of their toxicological activity. For such groups of substances, it is now recognized that a cumulative risk assessment is required. Cumulative risk assessment is the analysis, characterization, and possible quantification of the combined risks to health from aggregate exposure to multiple agents or stressors. During the last decade or so, regulatory authorities, such as the US Environmental Protection Agency and EFSA, have provided guidelines for the conduct of cumulative risk assessments.

Risk Assessment Approach for Substances with No Toxicological Data

An approach for the assessment of ingested substances of unknown toxicity was developed in the 1980s and 1990s, known as the Threshold of Toxicological Concern (TTC) approach. It has been further developed in recent years and is now widely used for substances for which toxicity data are few or completely lacking and for which human exposures are known to be low. It is particularly useful for low-level contaminants in food, on which risk assessment advice may be needed urgently but for which few toxicological data exist. It has also been used to assess substances migrating into food from food contact materials and to evaluate the acceptability of flavoring substances in food.

Application of the TTC approach requires only knowledge of the chemical structure of the substance concerned and information on human exposure. The exposure estimate(s) should include any likely high exposure. The approach uses generic human exposure threshold values (also called TTC values) for substances grouped according to their chemical structure and likelihood of toxicity. A range of TTC values have been developed covering both cancer and noncancer end points. A very low TTC value is used for substances that are genotoxic or have a structural alert for genotoxicity, and four higher TTC values are used for substances not likely to be genotoxic, which cover possible toxic effects that have a threshold. The TTC values have been derived using existing, published, toxicological information on substances that have been tested in animals in the past.

If the estimated human exposure to an untested substance is below the TTC value that is relevant for the structural class of the substance, it can be concluded with reasonable confidence that there is a low probability of adverse health effects. The TTC approach can be used to obtain a first approximation of risk or may be used (e.g., in the case of flavoring substances) to conclude that toxicity testing is not necessary. If estimated human exposure is above the relevant TTC value, then that is an indication that toxicity data would be needed for a reliable risk assessment.

Risk Assessment Approaches for Specific Food Categories

Micronutrients

In the case of essential vitamin and mineral micronutrients, HBGVs for the amounts needed to maintain good health

are set. These are variously termed guideline daily amounts, recommended daily amounts, recommended daily allowances, dietary reference intakes, or dietary reference values. The physiological requirements for a micronutrient may differ depending on the age group or physiological condition (e.g., pregnancy). Accordingly, guidance values are set for different age groups. In addition, some nutritional and risk assessment bodies set upper limits or tolerable upper intake levels (ULs), above which toxicity may occur. The UL is defined as the maximum level of total chronic daily intake of a nutrient (from all sources) judged to be unlikely to pose a risk of adverse health effects to humans. Unlike HBGVs for other chemicals that are deliberately added to food, such as ADIs, which may be 100-fold lower than the overall NOAEL derived from animal toxicity studies, for micronutrients, there may only be a narrow margin (<10) between the amount required to maintain good health and the UL, with a similar narrow margin between the UL and the onset of toxicity in humans. This situation is due to the essential nature and the inherent biological activity of micronutrients, but the ULs are set, where possible, on the basis of human data. High safety margins are not attainable.

For micronutrients, risk assessment advice may also include the discussion of risks to health from exposures that are below the minimum daily requirement and might result in adverse health effects because of deficiency, as well as the possible toxicity risks from exposures that are above the ULs.

Novel Foods

Novel foods are foods or food ingredients that are not yet consumed on a wide basis by any population or by a proposed target population. Examples include foods, such as fruits, with a history of use in some countries but not hitherto consumed widely by the target population or novel sources of oils, proteins or enzymes, and other substances that can be isolated from plants, microorganisms, fungi, or algae.

Risk assessments of novel foods usually use traditional foods and ingredients as reference points and focus on the differences between the novel food and the traditional food. This approach uses the 'substantial equivalence' concept, which aims to show that there is substantial similarity between the novel food and its relevant traditional counterpart, whose safety is accepted. Under this approach, a systematic comparison of the information available on the compositional analysis and nutritional and toxicological characteristics of the novel food and its traditional counterpart is undertaken. From this, any additional information that may be required to reach a view on whether the novel food would be safe for consumption is identified. That information may or may not involve biological or toxicological testing. Since these are whole foods, risk assessment advice is normally of a qualitative nature, offering advice on whether consumption can be expected to be as safe as the traditional counterpart or can reasonably be expected to result in no harm to health. In some instances, if there are particular molecular structures within the food that are novel, these may have been tested for toxicity and may provide data suitable for quantitative advice within the overall risk assessment.

GM Foods

Risk assessment of GM foods follows a similar approach to that for novel foods, using comparisons of the GM food with relevant traditional counterparts that have a history of safe use. It aims to identify intended and unintended differences and/or lack of equivalences between GM foods and their comparators, taking into account the range of natural variation. For GM plants, assessment of composition and phenotypic and agronomic characteristics and assessment of the genetic modification process and of the resulting introduced genetic trait, including its expression and stability, are key aspects. The majority of GM foods involve the addition of one or more novel proteins; thus, the focus of the risk assessments is on the novel proteins and their potential for toxicity, including allergenicity. Toxicological evaluation of GM foods normally includes laboratory animal testing, the extent of testing required differing under different legislative jurisdictions. Allergenicity evaluations may involve comparison of amino acid sequence homology of the novel protein with that of known allergens (followed up by specific serum screening if there is homology with known allergens), assessment of pepsin resistance, and *in vitro* digestibility tests. A nutritional assessment is also carried out. Risk assessment advice is usually of a qualitative nature.

Enzymes

Enzymes (GM or non-GM) can be used as food additives and as food processing aids. Risk assessment of enzymes requires information on the source material for the enzyme, which may be animal, plant, or microbial, usually some toxicity data, such as genotoxicity and subchronic toxicity, allergenicity assessment (as for a novel protein of a GM food), and exposure information. Risk assessment advice will usually be of a qualitative nature.

Engineered Nanomaterials

The use of engineered nanomaterials (ENMs) in food is in its infancy, but it is anticipated that formulations of many types of substances that are deliberately added to food, used on food or feed crops, or used in food packaging may in the future contain ENMs. Soluble ENMs that have conventional-form equivalents, which have been toxicologically tested, do not require testing because they will revert to the conventional substance on solubilization, and risk assessment can be done by read-across. Risk assessment of substances that are in the form of insoluble ENMs involves additional considerations to those for substances in the conventional form. These include technically difficult characterizations of the nature of the ENM before it is added to food and when it is in a food matrix or in body tissues and fluids and in the material used for toxicological testing. Additional complications of the risk assessment include the interpretation of toxicity studies, given current uncertainties about the validity of testing ENMs in *in vitro* and *in vivo* systems that were originally developed for the testing of substances in conventional chemical forms.

Foods with Inherent Toxicity

Natural toxins are found inherently in some foods of plant and animal origins. Examples include naturally occurring toxins in certain species of mushrooms and berries and in plants that may be used in herbal remedies or plant-based food supplements. Some plant-based foods may also be toxic if they are not processed or cooked appropriately before consumption (e.g., pulses or sprouts from pulses containing lectins that cause gastrointestinal symptoms and cassava and sorghum that release cyanide from cyanogenic glycosides). In some plant-based foods, the level of inherent toxins may build up if they are stored inappropriately for long periods (e.g., glycoalkaloids in sprouting and green potatoes). Foods of animal origin that may be inherently toxic include shellfish that may contain toxins that cause amnesia, paralysis, and other forms of neurotoxicity, various species of reef fish that may contain ciguatoxin that causes gastrointestinal and neurological symptoms, and puffer fish containing the neurotoxin tetrodotoxin.

The risks from consumption of such foods are well documented. Local knowledge of the problems associated with inherently toxic foods and ways of avoiding toxicity generally means that risk assessment is not necessary. In the case of fish and shellfish toxins, regular monitoring of the frequency of occurrence and of occurrence levels may be necessary for ongoing risk assessment.

See also: Campylobacter: Properties and Occurrence; *Clostridium botulinum*; *Clostridium*: Food Poisoning by *Clostridium perfringens*; Codex Alimentarius; Food Additives: Classification, Uses and Regulation; Food Allergies; Food Poisoning: Epidemiology; Foodborne Pathogens; Genetically Modified Foods: Risk Assessment, Legislation, Consumer Behavior, and Ethics; Heavy Metal Toxicology; *Listeria*: Listeriosis; Mutagens; Mycotoxins: Toxicology; Nanotechnology in Food Processing; Nutritional Epidemiology; Pesticides and Herbicides: Residue Determination; Pesticides and Herbicides; *Salmonella*: Salmonellosis; Shigella; Spices and Flavoring Crops: Uses and Health Effects; Trace Minerals and Trace Elements; Vitamins: Overview; World Health Organization.

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Saccharin – How Sweet It Is

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Introduction

For as long as man has inhabited this Earth, he has been enchanted by foods that taste sweet. In nature, a sweet taste in foods typically indicates higher energy concentration than those foods that do not taste sweet. Saccharin ($C_7H_5NO_3S$) is a chemical compound that is 300 times sweeter than table sugar (sucrose), yet provides no energy. It is in a class of compounds known as nonnutritive sweeteners (NNS) or intense sweeteners for their ability to impart a sweet taste with little to no energy. These compounds have been extensively studied for their effects on human health, yet experts continue to debate their safety.

History

Saccharin, the oldest NNS, was discovered by accident in 1879. Constantin Fahlberg was working on coal tar derivatives in the laboratory of Ira Remsen at Johns Hopkins University. One evening, after a long day of work in the lab, he ate dinner without first washing his hands. He noticed an unusually sweet taste on a roll that he was eating and then on his napkin and a cup. He ran back to the lab and tasted all the vials, beakers, and dishes that he had been using until he identified the source in a beaker in which o-sulfobenzoic acid had reacted with phosphorus, chloride, and ammonia, producing benzoic sulfimide that he called saccharin. Fahlberg and Remsen published an article describing methods of synthesizing saccharin with a taste that was sweeter than cane sugar. A few years later, Fahlberg left Remsen's lab without notice and applied for German and American patents for a new method to produce greater quantities of saccharin at lower cost. He began marketing saccharin in New York City where it was used for many purposes including as a treatment for headaches, nausea, and corpulence, a preservative in canning, and a sweetener for coffee or tea for people with diabetes. Fahlberg touted it for its ability to provide sweetness without spoilage or adverse health effects.

By 1906, Americans were wary of the food industry and the safety of their food so Congress passed the Pure Food and Drug Act in an attempt to regulate the nation's food supply. Responsibility for enforcing the law fell to the US Department of Agriculture, Bureau of Chemistry, led by Harvey Wiley. Wiley sought to ban saccharin, not because he deemed it unsafe, but because it was being used in commercial food production to replace the more expensive agricultural product, cane sugar. His efforts were not successful, largely, perhaps, because President Theodore Roosevelt opposed him. The president used saccharin to manage his weight, at the recommendation of his physician. By 1911, saccharin was considered to be an adulterant and was prohibited from use in processed foods. Its use was to be limited to those with a medical need, but it was still available for purchase by the public at drug stores and pharmacies. A sugar shortage during World War I and again during World War II led to a marked increase in the use of saccharin as a sugar substitute. As the United States recovered from WWII, dietary patterns began to change and there was a greater interest in modifying caloric intake. Saccharin, the only NNS on the market, became more popular as a method of decreasing calories without giving up the sweet taste.

In 1958, Congress passed the Food Additives Amendment to the Federal Food, Drug, and Cosmetic Act, which gave the US Food and Drug Administration (FDA) responsibility for evaluating the safety of food additives. This amendment required that all new food additives undergo a strict premarket approval process unless the substance is generally recognized as safe (GRAS) among experts qualified by training and experience to evaluate its safety under the conditions of its intended use. Food additives were defined as any substance that results, directly or indirectly, in its becoming a component of food. At the same time, Congress passed the Delaney Clause, which stated that no food additive would be deemed safe if it is found to induce cancer when ingested by man or animal and prohibited the FDA from approving such food additives. Food ingredients that were in common use prior to 1958 were listed as GRAS and were not considered food additives; saccharin met that criterion.

In 1970, a study from the University of Wisconsin reported a higher incidence of bladder cancer among rats that consumed saccharin daily, and the FDA removed it from the GRAS list. In 1977, a study out of Canada indicated that rats exposed to very high doses of saccharin over two generations had significantly higher rates of bladder cancer than rats that were not exposed. The FDA moved to ban saccharin under the provisions of the Delaney Clause. This resulted in a massive public campaign to persuade Congress to intervene. Although the evidence to support a ban was limited, the interpretation of the Delaney Clause was that no risk of cancer, no matter how remote, was

acceptable in a food additive. Marketers of saccharine framed the public debate as overreach by big government to deprive the public of their right to self-determination. Congress received more than 1 million letters opposing a ban on saccharin. They placed a 2-year moratorium on the ban and passed the Saccharin Study and Labeling Act of 1977 that required all products containing saccharin to include a warning on the label stating "Use of this product may be hazardous to your health. This product contains saccharin which has been determined to cause cancer in laboratory animals." Saccharin was delisted as a food additive in Canada, although limited access was maintained for saccharin as a tabletop sweetener and the European Commission Scientific Committee on Food restricted the acceptable daily intake (ADI) of saccharin. The unintended result of the proposed ban was a large increase in the purchase of saccharin by American consumers.

Safety studies on saccharin continued over the next several years and the moratorium on the proposed ban in the United States was renewed several times. The American Medical Association, Council on Scientific Affairs, and Division of Drugs and Technology supported the moratorium and its extensions until (1) stronger evidence of saccharin's carcinogenicity was forthcoming or (2) an alternative nutritive or NNS becomes approved. Studies showed a clear dose–response relationship between saccharin exposure during weaning and bladder cancer in male rats with 1% (equivalent to 500 mg kg^{-1} body weight per day) identified as the dose at which there were no observed adverse effects. However, these results could not be replicated in other animal models. Epidemiological studies were inconclusive, but those that controlled for other variables related to bladder cancer showed no consistent dose–response relationship with saccharin. Case–control studies showed no increased risk of bladder cancer related to the use of saccharin. The Danish population that was born during WWII, when the use of saccharin was high, showed no evidence that saccharin exposure *in utero* was associated with an increased risk of bladder cancer during the first 35 years of life.

The National Institute for Environmental Health Sciences determined that the mechanism by which saccharin caused bladder cancer in rats was unique to male rats that had been exposed to saccharin over two generations and was related to changes in rat bladder tissue that were seen with sodium saccharin and with high doses of other sodium salts. An increased risk was not seen in other animal models, and human studies had not shown an increased risk for cancer related to saccharin consumption in amounts that were relevant to people. In 1991, the FDA formally withdrew its recommendation to ban saccharin, although by this time, there was an NNS alternative, aspartame, on the market. In 1990, the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment reviewed the studies on saccharin and recommended a full ADI of 0–5 mg kg^{-1} body weight. In 1997, the European Commission Scientific Committee on Food did the same. On 15 May 2000, saccharin was removed from the list of suspected carcinogens, and on 21 December 2000, President Clinton signed legislation removing the warning label. In 2006, Health Canada notified stakeholders that they intended to propose regulatory changes that would allow saccharin to be used as a food additive.

Sources and Production

Saccharin is used in more than 100 countries around the world. Its use has been approved by numerous regulatory bodies including Health Canada, the Joint Expert Committee on Food Additives of the World Health Organization and the Food and Agriculture Organization of the United Nations, the European Union Scientific Committee on Food, and the USFDA. To put the assessment of health risk in perspective, it is important to understand the approval process for food additives in the United States. A manufacturer that wants to market a new food additive must assemble and present to the FDA all required safety data relevant to the proposed use of the additive in accordance with safety guidelines published by the FDA. The FDA considers probable intake, cumulative effect from all uses, and toxicological data required to establish safety. Data should include pharmacokinetics and metabolism to evaluate absorption, tissue distribution, metabolic pathways, and rate of elimination of the substance and any metabolites. Toxicity studies include short-term and subchronic toxicity tests with rodents, subchronic and long-term toxicity tests with nonrodents, reproductive and developmental toxicity tests, and tests of carcinogenicity.

The approval process is based on three safety concepts. The first concept is the highest no-effect level – that is, the highest level or threshold of intake at which no adverse effect occurs in the most sensitive of animal studies. The second safety concept is the acceptable daily intake (ADI). ADI is calculated from the highest no-effect level, given a 100-fold safety factor: tenfold because the studies were done in animal models and tenfold to account for genetic variation. ADI is set as the highest no-effect level divided by 100 and expressed as mg per kg body weight. The ADI represents an amount that is considered safe to consume every day over the course of a lifetime. The final safety concept is the estimated daily intake (EDI), which is derived from the amount of the additive to be added to foods assuming 100% replacement of sugars and other sweeteners and the typical consumption of those foods by people of different ages and health status. The EDI generally overestimates consumption because it assumes that the new additive will replace all sweeteners on the market (100% market penetration). The ADI is compared with the EDI to confirm that the ADI is well in excess of the EDI. If the EDI is determined to exceed the ADI, there may be limitations placed on the use of the additive. Once a food additive receives final approval, that approval is published in the Federal Register and the FDA may request additional data on consumption or other safety data during the post-market period.

In the United States, the FDA has approved saccharin, ammonium saccharin, calcium saccharin, and sodium saccharin as food additives on an interim basis pending additional studies (21CFR180.37, revised April 2014) and with some restrictions. The ADI is 5 mg kg^{-1} body weight and is based on the highest no-effect level in rat studies. It is approved for use in beverages not to exceed 12 mg per fluid ounce, in processed foods not to exceed 30 mg per stated serving size, and as a tabletop sweetener not to exceed 20 mg saccharin per teaspoon of sugar sweetening equivalency. Diet soft drinks often contain a blend of saccharin and aspartame and provide

8 mg saccharin in one 12 ounce serving. A 70 kg person would have to drink 15 12 ounce cans of diet soda or use 30 individual packets per day to reach the ADI.

Saccharin is produced for commercial use in Asia, Europe, and the United States, but China is the largest manufacturer and exporter of saccharin. In 2005, China produced 22 850 tons; 19 000 tons was exported. Two methods are used to produce saccharin. Most manufacturers use the Remsen–Fahlberg process in which toluene is treated with chlorosulfonic acid to produce ortho- and para-toluenesulfonyl chloride. The resulting product is treated with ammonia to separate the two isomers. Ortho-toluenesulfonamide is oxidized and heated to produce saccharin. The only producer of saccharin in the United States uses the Maumee process that begins with methyl anthranilate, a substance occurring naturally in grapes. Methyl anthranilate or anthranilic acid successively reacts with sodium nitrate, sulfur dioxide, and chlorine to produce 2-chlorosulfonylbenzoic acid methyl ester. In the presence of ammonia, this is converted to its amide that is de-esterified to yield saccharin. Saccharin alone does not dissolve well in water, so it is often converted to its sodium or calcium salt.

Patterns of Consumption

Saccharin and other NNS are used in human consumption to provide sweetening without sugar. Sugar has several important functions in food preparation including binding water to inhibit microbial growth, adding texture or volume, supporting the growth of yeast for leavening, and enhancing crystallization. Saccharin cannot replace sugar for any of those functions; it can only replace the sweetening power of sugar. In fact, because it is so much sweeter than sugar, it is used in very small quantities, and when packaged as a tabletop sweetener, it is usually mixed with a bulking agent to approximate the same volume that sugar would provide for the comparable sweetness.

Perception of sweet taste begins on the tongue and soft palate where taste receptors interact with food or beverages. Taste receptor cells are organized into taste buds that are distributed along the tongue. The sweet taste receptor is a heterodimeric G protein-coupled receptor composed of subunits T1R2 and T1R3, which have multiple active sites. These receptors respond to sugars (sucrose, fructose, galactose, glucose, lactose, and maltose), amino acids (glycine and D-tryptophan), sweet proteins (monellin and thaumatin), and NNS (acesulfame potassium, aspartame, cyclamate, dulcin, neotame, saccharin, and sucralose). Saccharin may also bind with bitter taste receptors on the same taste buds. After binding to taste receptors, a cascade of chemical changes translates the stimulus through the nervous system to the perception of sweet taste in the brain. The transduction of sweet taste from sugars uses cyclic AMP as a second messenger, and the pathway for nonsaccharide sweeteners such as NNS uses inositol 1,4,5-trisphosphate (IP3). Both mechanisms work through calcium ion channels and exist in the same taste receptor cells. Synergy exists among sweeteners because different sweeteners bind different subunits of the taste receptor. Binding a single subunit activates the sweet response, whereas a second sweetener binding a different subunit enhances the response. Saccharin is often used in combination with other sweeteners to enhance the sweet response making it possible to achieve a more palatable product with the use of less sweeter volume.

Saccharin sweetens with little bulk, is stable during storage, and maintains its sweetness on heating. Saccharin and its salts are used in a variety of foods, beverages, pharmaceuticals, and cosmetics, and saccharin is available as a tabletop sweetener in powder, tablet, or liquid form. It is used in fruit juices, soft drinks, and beverage bases or mixes. In foods, it is used in processed fruits, gelatin desserts, confections, jams, toppings, sauces, and dressings. It is used in chewing gum, toothpaste, and mouthwash. Manufacturers who used NNS in their products are required to include them in the list of ingredients, but they are not required to list the amount with the exception of saccharin. Saccharin is restricted so the amount must be listed on the label. Some diet soft drinks contain saccharin, often in combination with acesulfame potassium or aspartame.

Estimating the usual intake of an individual is difficult because of inherent weaknesses in dietary intake methodology. People who answer questionnaires or surveys about their usual food intake tend to overestimate intake of foods perceived as high value and under report intake of low-value foods. People who record their intake in real time often change their usual intake during the reporting period (which is one reason that recording food intake is often used as a weight management tool). When reading studies that describe usual intake, it is important to consider the limitations of the data collection methods. A 2006 review of intake studies from around the world indicated that the average intake of saccharin was well below the ADI in almost all groups. The only exception in this review was a study of children with diabetes in Sweden that showed a very small number of the highest consumers took in about 125% of ADI. The data were collected using a food frequency questionnaire and assuming the maximum permitted saccharin concentration for each product category – considered by the authors to be a worst-case scenario. A survey in a region of India, also published in 2006, showed heavy use of saccharin in products that were popular with children including ice candy and crushed ice. Overall, children aged 6–10 years who had the highest consumption of ice candy and crushed ice exceeded the ADI for saccharin by 50% and adults with the highest intake of other food products containing saccharin exceeded ADI by 37%. Reports of saccharin intake that exceeds the ADI are very rare and limited to the highest consumers of saccharin-containing products.

In the United States, it is estimated that more than 6000 products contain NNS, with sucralose, acesulfame potassium, and aspartame being the most frequently used. The most common source of NNS in the United States is carbonated beverages, and NNS intake is typically estimated based on reported intake of beverages and foods that contain NNS. However, exact formulations are proprietary and subject to change based on market forces, making it difficult to estimate exposure of any specific NNS. Data from US nutrient monitoring systems were used to locate items that contained NNS on food composition tables to estimate *per capita* use of NNS. In 2004, about 15% of people in the United States used any NNS.

By 2010, that increased to 20%, most as sugar-free beverages. Low-calorie beverages accounted for about 19% of beverage intake by children aged 2–18 years and about 32% of beverage intake among adults aged 19 years and older.

Availability, Absorption, and Metabolism

Saccharin is almost completely absorbed from the gastrointestinal (GI) tract and not metabolized; it is rapidly excreted, unchanged, by the kidneys. When radiolabeled sodium saccharin was given to rats and monkeys at a dose of 40 mg kg^{-1} orally, ~99% was recovered in urine after 96 h of collection, most within the first 24 h, and < 1% was recovered in bile. Daily oral doses of up to 500 mg sodium saccharin given to monkeys over 2 years had no effect on metabolism of a single dose of radiolabeled saccharin. There was no evidence of metabolites, leading to the conclusion that saccharin is not metabolized in rats or monkeys. Experiments in human volunteers given saccharin intravenously or orally confirmed that it was not metabolized and was rapidly excreted in urine.

Health Effects

The safety of saccharin has been studied extensively, in part because of its controversial history. It is used around the world as a low-cost, readily available sugar substitute. There is wide agreement among experts that it does not increase the risk for cancer. Nevertheless, questions of safety persist – related not to the risk of cancer, but to the risk for obesity and diabetes. How could a substance that is not metabolized contribute to obesity and diabetes? Hypotheses include binding sweet taste receptors on the GI tract, discrepancies in learned relationships between sweet taste and energy delivery, and alterations in gut microbiota.

Duodenal cells express the G protein-coupled receptor, gustducin, T1R2, T1R3, and other taste transduction elements. Sugars and sweeteners in the GI tract bind to taste receptors and induce secretion of glucagon-like peptide-1 (GLP-1) and glucose-dependent, insulinotropic peptide (GIP), which regulate the expression of sodium–glucose cotransporter 1 (SGLT1). SGLT1 actively transports glucose and sodium into the enterocytes. GLP-1 and GIP are rapidly destroyed by hydrolysis, but some may be absorbed into the bloodstream to act as systemic hormones. GLP-1 stimulates insulin release, inhibits glucagon secretion, reduces gastric emptying, and augments feelings of satiety. Animal studies have shown that NNS stimulate the release of GLP-1 and GIP, although the magnitude of the increase varies among the different NNS. Some have hypothesized that this increase in glucose uptake and concomitant release of GLP-1 stimulate insulin release that lowers blood glucose, increasing appetite, leading to increased weight gain. However, studies in human subjects have not consistently shown that NNS increase GLP-1 or serum concentrations of glucose or insulin.

Another hypothesis states that consuming sweet-tasting foods that do not deliver the expected caloric load interferes with learned responses that normally contribute to glucose and energy homeostasis and lead to increased rates of weight gain. To test the hypothesis, male rats were supplemented with yogurt containing saccharin or glucose. Those in which the sweet supplement was not associated with increased calories (saccharin) consumed more total calories and gained more weight and adipose tissue. The changes were attributed to reductions in energy expenditure and a decreased ability to compensate for energy intake from other sweetened foods in the diet. The researchers concluded that saccharin stimulated food intake by reducing the ability of rats to regulate energy balance in the presence of caloric-sweetened foods. Other researchers have reported increased weight gain, but no increase in energy intake in rats supplemented with saccharin compared to rats supplemented with sucrose.

A newer hypothesis states that saccharin induces glucose intolerance by altering gut microbiota based on studies in wild-type and germ-free mice from one laboratory. The researchers fed mice with a high-fat diet supplemented with saccharin equal to 5 mg kg^{-1} body weight. After 5 weeks, the saccharin-supplemented mice had higher serum glucose concentrations at 90 and 120 min and higher area under the curve (AUC) after an oral glucose tolerance test compared to control mice. Fecal microbiota differed in saccharin-fed mice compared to control. After treatment with antibiotics to destroy gut bacteria, the differences in glucose tolerance disappeared. Seven healthy human volunteers were fed with saccharin equal to 5 mg kg^{-1} body weight in three divided doses daily for 5 days. Four of the seven had poorer glycemic response (defined as higher AUC on glucose tolerance test) after the saccharin supplement compared to before, while the other three volunteers showed no change. Fecal microbiota differed in those that responded to saccharin compared to the nonresponders at the beginning of the study and changed over the course of the study. No change was seen in the microbiota of nonresponders. The researchers concluded that NNS consumption in mice and humans enhances the risk of glucose intolerance, possibly due to increased glycan degradation pathways that have been linked to increased energy harvest.

Studies in Humans

Clearly, humans eat differently from other animals and humans who use NNS do so for different reasons including to limit carbohydrate intake, limit intake of added sugars, or manage weight. If the use of NNS has the opposite effect of making weight management more difficult, many people would stop using them. Understanding the relationship between NNS and factors related to weight management and other health effects is critical, but research is complicated. There are a number of different NNS in use, preferences for one NNS over another change over time, and many low-calorie products use one or more NNS. There is no indication that saccharin has different effects than other NNS, so this section includes information on health effects of NNS in general and on saccharin specifically when information is available.

The use of NNS should lead to an energy deficit by replacing sugar in foods or beverages, but animal studies seemed to indicate that animals compensated for the energy deficit by increasing intake of other sweetened foods. One question is whether NNS affect appetite, hunger, and satiety differently than sugars do in humans. Studies to answer that question

typically involve providing preloads with different energy contents and measuring energy intake in the following meal or meals. If NNS affects satiety differently than sugar does, subjects should report greater feelings of hunger or appetite and should compensate for the lower-energy preload by eating more at subsequent meals. Studies that used food as the vehicle for NNS showed compensation for about one-third of the energy deficit over the next 24 h. When the vehicle was a beverage, only about 15% of the deficit was made up over the next 24 h, meaning that NNS were effective in reducing energy intake when substituted for sugar. Studies that specified saccharin as the NNS showed no increased food intake or appetite in human subjects. An analysis of evidence by the Academy of Nutrition and Dietetics concluded that saccharin did not increase food intake or appetite and substituting saccharin for sugar-sweetened products in a form that is lower in energy can result in modest energy savings.

Randomized controlled trials (RCT) are considered the gold standard of experimental design because they compare an intervention with a control and minimize bias by randomly assigning subjects to treatment arm and blinding researchers and subjects to assignment. A recent meta-analysis of 15 RCT that examined the effect of NNS on body weight and composition in a total of 1951 subjects noted a small decrease in body weight (−0.8 kg, 95% CI −1.17, −0.43) among all ages with significant decreases in body mass index, waist circumference, and fat mass. Most RCT replaced sugar-sweetened beverages (SSB) with beverages sweetened with NNS as the intervention. RCT have not shown any significant differences between the effects of NNS and control on glycemic response in people with or without diabetes.

Although considered the gold standard for medical research, RCT have limitations in public health research. They cover a limited time frame and use a research team to provide support to subjects. Consequently, the results may not be generalizable to other groups and they are too short to determine changes in long-term outcomes such as development of chronic disease. Population studies and long-term outcomes require prospective cohort studies. These are studies that follow a group of individuals with many similarities and few differences over longer periods of time to measure differences in the outcomes of interest and to determine if those differences in outcomes are related to ways in which the individuals differ. Prospective cohort studies that examined differences in the use of NNS and body weight, weight change, and obesity-related diseases have had mixed results. In some studies, higher use of NNS has been associated with a higher risk for obesity and diabetes. However, studies that controlled for other differences that would affect risk have not found an association between NNS consumption and obesity or obesity-related diseases, leading many to conclude that the measured association was due to reverse causality – as people begin to gain weight, they may use NNS in an attempt to manage their weight. A meta-analysis of nine prospective cohort studies showed a small increase in BMI (0.03 kg, 95% CI 0.01, 0.06) but no significant increase in body weight or fat mass. Well-controlled prospective cohort studies that examined relationships among NNS and risk factors for diabetes and cardiovascular disease have not shown an increased risk of adverse effects from NNS consumption, and a long-term study of previously obese people who have maintained significant weight loss shows that the majority consume NNS-sweetened beverages.

There is consensus among experts that added sugar in the diet and consumption of SSB are associated with increased weight gain and greater risks for obesity, diabetes, and metabolic syndrome. Although NNS can replace the sweetness of sugar without energy and can provide a calorie-free beverage to replace SSB, there is no consensus as to their overall health effects. Despite a lack of consensus, many health experts support their use as a replacement for sugar in the diet. In a combined scientific statement, the American Heart Association and the American Diabetes Association state that NNS could facilitate reductions in added sugar intake, thereby resulting in decreased total energy and weight loss/weight control and promoting beneficial effects on related metabolic parameters.

Conclusion

Saccharin has been used to replace the sweet taste of sugar for many years in many countries. Overall, the evidence supports its safety when consumed within guidelines.

See also: Acesulfame-K; Beverage: Health Effects; Beverage: Patterns of Consumption; Obesity: The Role of Diet; Sweeteners: Classification, Sensory and Health Effects.

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Salad Crops: Dietary Importance

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Introduction

A large number of vegetables that are consumed raw (without cooking) are considered to be salad crops. They are attractive and seasonal and are valued for their succulence, bulk, and nutritional importance in the human diet. Eaten regularly, these foods help keep the body healthy because they contain fiber along with minerals, vitamins, and phytochemicals – regular consumption of which helps reduce the risk of cardiovascular disease, diabetes, and some cancers.

Types of Plant in Salad Category

Because a vast number of salad vegetables are being cultivated and consumed globally, these vegetables need to be categorized in such a way that information about them can be readily gathered. By virtue of their raw consumption, one option is to classify salad vegetables according to the part consumed (Tables 1–3).

Nutritional and Dietary Significance

Salad vegetables are considered to be protective because they are rich in minerals, vitamins, fiber, and natural phytochemicals, which have a key role in maintaining good health. Salad crops are inexpensive and easy to grow, and they have relatively a long period of harvest and storage. Because of their high fiber content, these vegetables promote digestion and help prevent constipation. Most leafy vegetables, such as celery, cabbage, Chinese cabbage, lettuce, parsley, rhubarb, and spinach, are characterized by having a high water and cellulose content (a result of their succulence and bulk).

In salad vegetables, carbohydrates consist of starches and sugars that are easily available to the body. Vegetables also provide small quantities of unsaturated lipids that contain essential fatty acids, such as linoleic (omega 6), linolenic (omega 3), and arachidonic acids, which are required for normal growth and maintenance of the body. Salad crops provide sundry proteins, which are analogous to animal protein in terms of their amino acid make-up. Most proteins consist of enzymes, thus, plant leaves are the actively metabolizing organs. This protein content is more absolute (containing nine essential amino acids) than proteins derived from seeds; thus, consumption of leafy salads could make a major contribution toward the optimum health of an individual.

Salad crops ranking highest in vitamin A content are: carrot, turnip greens, spinach, mustard, and dandelion (Tables 4–6). Artichokes rank seventh, based on mineral and vitamin content. Spinach, kale, and collard contain more minerals and vitamins (especially vitamin A, calcium, phosphorus, and iron) than many other salad crops. A single serving of these vegetables meets the adult daily requirement for vitamins A and C, as well as 13% of the total calcium requirement (Table 1).

Beetroot (*Beta vulgaris* var. crassa) is rich in carbohydrates, protein, calcium, phosphorus, iron, and vitamins A and C. The tops of beetroot and Swiss chard are excellent sources of vitamins A and B_2, and are a good source of vitamin B_1. Beet leaves are particularly valued for calcium, iron, B group vitamins (especially riboflavin), ascorbic acid, and vitamin K content – because they are all excellent source of vitamin K_1 (5–16 $\mu g\, g^{-1}$). They also supply iron, manganese, zinc, and copper as well as appreciable quantities of protein, which is rich in essential amino acids (especially in lysine).

Broccoli (*Brassica oleracea* var. italic) is the most nutritious of the mustard family (Cole crops) because it contains relatively more minerals (especially calcium and iron), and vitamins. Its secondary inflorescence is characterized by higher dry matter, vitamin C, chlorophyll, β-carotene, carotenoids, and by low levels of nitrates. In comparison to other Cole crops, Brussels sprouts (*Brassica oleracea* var. gemmifera) have more protein and carbohydrates. Except for vitamin A and calcium, Brussels sprouts are comparable with broccoli; however, for most of the parameters measured, the leaves are better than the sprouts. Cabbage (*Brassica oleracea* var. capitata) is rich in carbohydrates, protein, minerals (such as calcium, phosphorus, potassium, sulfur, and iron), vitamins A and C, thiamine, riboflavin, and – above all – fiber. The young green heads are higher in vitamin A than older white cabbage. Like other Cole crops, cabbage contains glucosinolates (i.e.,sinigrin, progoitrin, and glucobrassicin). The peculiar flavor of cabbage is due to sinigrin, which contains sulfur. Chinese cabbage (*Brassica pekinensis* and *Brassica chinensis*) is rich in thiamine, riboflavin, and carotene. It contains more vitamin C than lettuce. Among the organic acids, citric acid is highest in Chinese cabbage. Kohlrabi (*Brassica oleracea* var. gongylodes) contains more sugars than broccoli. Besides carbohydrates, kohlrabi is rich in vitamins A, B_3, B_5, B_6, B_9, and C, as well as calcium, phosphorus, magnesium, potassium, sodium, iron, and chlorine. It is also superior to turnip and rutabaga, but is similar to red cabbage.

Carrot (*Daucus carota*) roots contribute a significant proportion of the daily requirement for vitamin A. In addition to vitamins and minerals, carrot roots are an important source of dietary fiber. They are rich in sucrose, having ten times more sucrose than glucose and fructose. The highest score for sweet taste is obtained in carrots grown at low temperatures; bitterness (terpenes) and sugar increases with the temperature. Terpenes suppress the perception of sweetness, but terpinolene decreases with increasing temperature, and probably has only a minor role in masking the sweet taste.

The leaves of celery (*Apium graveolens* var. duke) are a rich source of flavonoids, such as zeaxanthin, lutein, and beta-carotene, and are a good source of vitamin A. The herb is also

Table 1 Botanical names, common names, and edible plant parts of vegetables/salad crops

Common name	*Botanical name*	*Family*	*Edible plant part*
Horsetail family			
Water fern	*Ceratopteris thalietroides* (L.) Brongn	Parkeriaceae	Young leaf
Vegetable fern	*Diplazium esculentum* (Retz.) Swartz	Polypodiaceae	Young leaf
Onion family			
Chive	*Allium schoenoprasum* L.	Alliaceae	Leaf
Chinese chive	*Allium tuberosum* Rottler ex. Sprengel	Alliaceae	Leaf and immature flower
Lily family			
Asparagus	*Asparagus officinalis* L.	Liliaceae	Shoot
Carpetweed family			
New Zealand spinach	*Tetragonia tetragoniodes* (Pall.) O Kuntze	Aizoaceae	Tender shoot and leaf
Carrot family			
Chervil	*Anthriscus cerefolium* (L.) Hoffm	Apiaceae	Leaf
Celery	*Apium graveolens* L. var. dulce (Mill.) Pers		Petiole leaf
Celeriac	*Apium graveolens* L. var. rapaceum (Mill.) Gaud.		Root, leaf
Fennel	*Foeniculum vulgare* var. azoricum (Miller) Thell.		Leaf
Florence fennel	*Foeniculum vulgare* var. dulce Fiori		Leaf base
Parsnip	*Pastinaca sativa* L.		Root, leaf
Parsley	*Petroselinum crispum* (Mill.) nym. var. crispum		Leaf
Turnip-rooted parsley	*Petroselinum crispum* (Mill.) nym. *Tuberosum*		Root, leaf
Italian parsley	*Petroselinum crispum* (Mill.) Nym. Var. neapolitanum		Leaf
Sunflower family			
Endive	*Cichorium endiva* L.	Asteraceae	Leaf
Chicory	*Cichorium intybus* L.		Leaf
Indian lettuce	*Lactuca indica* L.		Leaf
Asparagus lettuce	*Lactuca sativa* L. var. asparagina Bailey		Stem
Head lettuce	*Lactuca sativa* L. var. Capitata L.		Leaf
Romaine lettuce	*Lactuca sativa* L. var. longifolia Lam.		Leaf
Dandelion	*Taraxacum officinale* Wiggers		Leaf, root
Mustard family			
Curled mustard	*Brassica juncea* (L.) Czernj. and Coss. var. crispifolia	Brassicaceae	Leaf
Small leaf mustard	*Brassica juncea* (L.) Czernj. and Coss var. foliosa		Leaf
Gemmiferous mustard	*Brassica juncea* (L.) Czernj. and Coss. var. gemmifera Lee & Lin		Stem and axillary bud
Involutes mustard	*Brassica juncea* (L.) Czernj and Coss. var. insoluta Yang & Chen		Leaf
Wide petiole mustard	*Brassica juncea* (L.) Czernj. and Coss. var. Patipa Li.		Leaf
Rutabaga	*Brassica napus* L. var. napobrassica (L.) Reichb.		Root and leaf
Chinese kale	*Brassica deracea* L. var. alboglabra Bailey		Young flower stalk and leaf
White cabbage	*Brassica oleracea* L. var. capitata L.		Leaf
Portuguese cabbage	*Brassica oleracea* L. var. Costala DC		Leaf and inflorescence
Brussels sprouts	*Brassica oleracea* L. var. gemmifera Zenk.		Axillary bud
Konlrabi	*Brassica oleracea* L. var. gongylodes L.		Enlarged stem
Broccoli	*Brassica oleracea* L. var. italica plenck		Immature flower stalk
Spinach	*Brassica perviridis* Bailey		Leaf
Chinese cabbage	*Brassica rapa* L. var. Pekinensis (Lour.) Olsson		Leaf
Garden cress	*Lepidium sativum* L.		Leaf
Water cress	*Nasturtium officinale* R. Br.		Leaf
Buck wheat family			
Rhubarb	*Rheum rhabarbarum* L.	Polygonaceae	Petiole
Valerian family			
Italian corn salad	*Valerianella eriocarpa* Desv.	Valerianaceae	Leaf
European corn salad	*Valerianella locusta* (L.) Laterrade em. Betcke		Leaf

Source: Kays, S. J. and Silva Dias, J. C. (1995). *Economic Botany* **49**(2), 121–152. The New York Botanical Garden.

rich in many vital vitamins, including folate, riboflavin, niacin, and vitamin C. Fresh celery is an excellent source of vitamin K. The herb is a very good source of potassium, sodium, calcium, manganese, and magnesium. Its leaves and seeds contain many essential volatile oils, which include terpenes, limonene (75–80%), and the sesquiterpenes such as β-selinene (10%) and humulene.

Chicory (*Chihorium intybus*) is low in saturated fat and very low in cholesterol. It is also a good source of thiamine, niacin, and zinc, and a very good source of dietary fiber vitamins A, C,

Table 2 Names of salad crops in nine languages

English	*Danish*	*Dutch*	*French*	*German*	*Italian*	*Portuguese*	*Spanish*	*Swedish*
Artichoke	artiskok	artisjok	artischauf	artischocke	carciofo	alcachofra	alcochof	kronartskocks
Asparagus	asparges	asperge	asperge	spargel	asparago	espargo	esparrago	sparris
Broccoli	broccoli	broccoli	chou-brocoli	brokkoli	cauolo broccolo	broculo	brocoli	broccoli
Brussel sprouts	rosenkal	spruitbool bruxelles	chou de	Rosen kohl Bruxelles	Cavolo di Bruxelos	Couve de Bruselas	Col de	brysselkal
Cabbage	Kal	Kool	Chou	Kohl	Cavolo	Couve	Col repollo	Kal
Celery	Selleri	Selderij	Celeri	Sellerie	Sedano da erbucci	aipo	apio	Selleri
Celeriac	Knoldselleri	Knolselderiji	Celeri-rave	Knollensellerie	Sedano rapa	aipo de cabega	apio nabo	rotselleri
Chinese cabbage	Kinesisk kal	Chinese kool	Chou de chine	China kohl	Cavolo cinese	Caouve da China	Col de China	Sallad kal
Endive	endivie	andijuie	Chicoree frisee	Endivie	Indivia	Chicoria	escarola	endiviesallet
Kale	gronkal	groene kool	Chou vort	Grunkohl	Cavolo a foglia riccia	Couve galega frizada	Sin cabeza	gronbal
Kohlrabi	Knude kal	Koolrabi	Chou-rave	Kohlrabi	Cavolo-rapa	Couve-rabano	Colirabano	Kalrabbi
Lettuce	Salat	Sla	Laitue	Salat	Pattuga	Alface	Lechuga	Sallad
Parsley	Persille	Peterselie	Persil	Petersilie	Prezzemola comune	Salsa frisada	Perejil	Persitja
Parsnip	Pastinak	Pastinaak	Panais	pastsinake	pastinaca	pastinaca	Pastinaca	Pasternacka
Rhubarb	rabarber	rabarber	rhubarbe	Rhabarber	rabarbaro	ruibarbo	ruibarbo	rabarber
Rutabega	kalrabi	kool raap	Chou-navet	Kohlrube	navone	rutabaga	colinabo	kal rot
Spinach	Spinat	Spinazie	spinard	Spinat	spinaci	espinafre	espinaca	spenat

Source: Stadhouders, P. J. (ed.) (1990). *Elsevier's dictionary of horticultural and agricultural plant production*. New York: Elsevier Science Publishing Co., Inc.

Table 3 Classification of salad crops on the basis of edible part and botanical family

Edible part	*Botanical name*	*Family*
Underground portion		
Enlarged tap root		
Beet	*Beta vulgaris*	Chenopodiaceae
Carrot	*Daucus carota* var. sativus	Umhelliferae (Apaiaceae)
Celeriac	*Apium grveolens* var. rapeceum	Umbelliferae
Radish	*Raphanus sativus*	Cruciferae (Brassicaceae)
Turnip	*Brassica compestris* var. rapifera	Cruciferae
Parsnip	*Pastinaca sativa*	Umbelliferae
Tubers		
Jerusalem Artichoke	*Helianthus trberosus*	Compositae (Asteraceae)
Bulbs		
Florence	*Foeniculum vulgare var. azoricum*	Umbelliferae
Onion	*Allium cepa*	Alliaceae
Leek	*Allium ampeloprasum var. porum.*	Alliaceae
Above-ground portion		
Stems		
Asparagus	*Asparagus officinalis*	Lilliaceae
Kohlrabi	*Brassica oleracea* var. gongylodes	Cruciferae
Petioles		
Rhubarb	*Rheum rhabarbarum*	Polygonaceae
Celery	*Apium graveolens var. dulce*	Umbelliferae
Leaves		
Cabbage	*Brassica oleracea* var. copitata	Cruciferae
Chicory	*Cichorium intybus*	Compositae
Cress	*Lepidium sativum* L.	Cruciferae
Dandelion	*Taraxicum officinale*	Compositae
Garlic	*Allium Sativum*	Alliaceae
Lettuce	*Latuca sativa*	Compositae
Mustard	*Brassica juncea*	Cruciferae
Onion	*Allium cepa group*	Alliaceae
Endive	*Cichorium endiva*	Compositae
Parsley	*Petroselinum crispum*[a] *var. neopolitanim*	Umbelliferae
Watercress	*Irorippa nasturtium var. aquaticum.*	Cruciferae
Flowers		
Cauliflower	*Brassica oleracea* var. botrytis	Cruciferae
Artichoke (globe)	*Cynara scolymus*	Compositae
Fruits		
Immature		
Cucumber	*Cucumis sativus* L.	Cucurbitaceae
Mature		
Tomato	*Lycopersicum esulentum*	Solanaceae

[a]Recently assigned to the genus, *Carum;* hence variety now recognized as *Carum petroselenum* var. *neopolitanum*.

"All the salad vegetables belong to the Angiospermae and Dicotyledonae except onion, which belongs to subclass Monocotyledonae".

E, B_2, B_3, B_5, and B_6, as well as calcium, iron, magnesium, phosphorus, potassium, copper, and manganese. One cup (29 g) of chicory greens contains 108% of the RDA of vitamin K and a third of the RDA for vitamin A. Other active compounds in chicory are inulin, sesquiterpene lactones, fat, mannitol, and latex. Constituents include fructan, sesquiterpene lactones, alpha-amyrin, taraxerone, baurenyl acetate, and β-sitosterol.

Chives (*Allium schoenoprasum*) are very low in energy (30 kcal/100 g leaves), but surprisingly high in vitamin A (4353 IU/100 g leaves), which is significantly more than any other Alliaceae plants. Its green leaves contain other flavonoid-phenolic antioxidants, such as carotenes, zeaxanthin, and lutein, as well as flavonoids and dietary fiber (2.5 g/100 g leaves). Like other alliums, they also possess thiosulfinites (diallyl disulfide, diallyl trisulfide, and allylpropyl disulfide), which are converted to allicin by an enzymatic reaction when the leaves are crushed. Fresh chives are a rich source of vitamin K (212.7 μg/100 g greens) and folate (105 μg/100 g leaves). Leafy greens contain essential minerals, such as calcium, iron, magnesium, phosphorus, potassium, zinc, copper, manganese, and choline, as well as vitamins, including thiamine, niacin, pantothenic acid, riboflavin, pyridoxine, and vitamin C.

Dandelion (*Taraxacum officinale*) greens are very low in energy (providing just 45 cal/100 g), saturated fat, and cholesterol. They are a good source of dietary fiber. Fresh dandelion provides vitamin A (10 161 IU/100 g), which is one of the highest sources among culinary herbs. Its leaves are rich in flavonoids such as β-carotene, α-carotene, lutein, cryptoxanthin, and zeaxanthin. The herb is a good source of potassium, calcium, manganese, iron, and magnesium. It is also rich in many vitamins, including folate, riboflavin, pyridoxine, niacin, vitamin E, and vitamin C. Dandelion is probably the richest herbal source of vitamin K.

Endive (*Cichorium endivia*), commonly known as escarole, is one of the richest sources of vitamin A, C, *etc*. and it is one of the very low calorie (17 kcal/100 g leaves) leafy vegetables. It contains B-group vitamins such as folate, pantothenic acid, pyridoxine, thiamine, and niacin, and is also a good source of manganese, copper, iron, potassium, calcium, chlorine, magnesium, phosphorus, zinc, copper, and sulfur. It is an excellent source of fiber and carotene. Endive provides several other important nutrients such as omega-3 and omega-6 fatty acids.

Fennel (*Foeniculum vulgare* var. dulce Alef) is very low in energy (31 kcal/100 g bulb). It contains a generous amount of fiber (3.1 g/100 g), very little fat, and no cholesterol. Its flavor is due to a high content of essential oils (anethole, estragole) and fenchone (fenchyl acetate). The bulb contains several vitamins, including pantothenic acid, pyridoxine, folate, niacin, riboflavin, and thiamine (in small amounts), as well as vitamin C (12 mg/100 g bulbs) and a small amount of vitamin A. The bulbs have good levels of potassium (414 mg/100 g) and a small amount of copper, iron, calcium, magnesium, manganese, zinc, and selenium.

Kale (*Brassica oleracea* var. acephala) contains oxalates, which are naturally occurring substances that interfere with the absorption of calcium. Thus, kale should not be consumed with calcium-rich foods. Like other mustard family plants, it also contains health-promoting phytochemicals (quercetin sulforaphane and indole-3-carbinol), which reduce the risk of some cancers and cardiovascular disease. It is a rich source of β-carotene, lutein, and zeaxanthin, and is rich in vitamin A and vitamin K. The leaves contain vitamin C (120 mg/100 g) and are a notable source of niacin, pyridoxine, thiamine, pantothenic acid, etc. as well as minerals such as copper, calcium, sodium, potassium, manganese, phosphorus, and iron.

Table 4 Nutritional constituents of some of salad crops (per 100 g edible portion)

	Macronutrients					*Vitamins*				*Minerals*			
	Water (g)	*Energy (kJ, kcal)*	*Protein (g)*	*Fat (g)*	*Carbohydrate (g)*	*A (IU)*	*C (mg)*	*Thiamin (mg)*	*Niacin (mg)*	*Ca (mg)*	*P (mg)*	*Fe (mg)*	*K (mg)*
Asaragis	91.7	100 (26)	2.5	0.2	5.0	900	33	0.18	1.5	22	62	10.0	278
Aritichoke	85.0	84 (20)	2.7	0.2	2.3	160	11	0.06	0.8	53	78	15.0	430
Rhubarb	95.0	67 (16)	0.6	0.1	3.7	100	9	0.03	0.3	96	18	0.8	251
Spinach	51.0	100 (26)	3.2	0.3	4.3	8100	51	0.10	0.6	93	51	3.1	470
Kale	83.0	220 (53)	6.0	0.8	9.0	10000	186	0.16	2.1	249	93	2.7	378
Mustard	90.0	130 (31)	3.0	0.5	5.6	7000	97	0.11	0.8	183	50	0.3	377
Turnip greens	90.0	117 (28)	3.0	0.3	5.0	7600	139	0.21	0.8	246	58	1.8	–
Beet greens	91.0	100 (24)	2.2	0.3	4.6	6100	30	0.10	0.4	119	40	3.3	570
Beet roots	88.0	180 (43)	1.6	0.1	9.9	20	10	0.03	0.4	16	33	0.7	335

In some cases, the analytical data relating to a given crop will appear to vary between Articles. These differences may arise because of contrasts in experimental techniques and/or raw materials employed by the original authors and hence no attempt has been made to standardize the available information.
Source: Howard, F. D., et al. (1962). *USDA National Food Review*, 1978.

Table 5 Nutritional constituents of some salad crop (per 100 g edible portion)

	Macronutrient					*Vitamins*					*Minerals*			
	Energy (kJ, kcal)	*Water (g)*	*Protein (g)*	*Fat (g)*	*Carbohydrate (g)*	*A (IU)*	*B_1 (mg)*	*B_2 (mg)*	*Niacin (mg)*	*C (mg)*	*Ca (mg)*	*Fe (mg)*	*Mg (mg)*	*P (mg)*
Mustard greens	63 (15)	91	2.7	0.2	0.1	5300	0.08	0.11	0.8	70	140	2.0	48	45
Turnip (tops and roots)	75 (18)	92	1.8	0.2	0.2	2700	0.05	0.07	0.5	70	125	1.5	45	45
Greens	58 (14)	91	1.5	0.3	0.1	3400	0.07	0.10	0.6	60	90	1.1	31	42
Radish	54–58 (13–14)	94	0.6–0.1	0.1	0.1–0.2	0	0.03	0.02	0.3	21	27	0.8	22	27
Carrot (roots)	130 (31)	89	0.8	0.2	6.6	1300	0.05	0.04	0.3	6	30	1.4	17	36
Celeriac roots	84 (20)	88	1.5	0.3	3.5	0	0.05	0.06	0.7	8	43	0.7	20	115
Celery self-blanching (petiole)	29 (7)	96	0.7	0.1	1.2	90	0.03	0.02	0.3	7	25	0.3	10	27
Green (petiole)	34 (8)	95	0.9	0.1	0.2	120	0.03	0.04	0.3	10	70	0.5	14	34
Florence fennel	63 (15)	93	1.1	0.1	2.6	100	0.04	0.02	0.4	9	44	0.8	23	38
Parsley leaves	67 (16)	90	2.2	0.3	1.3	5200	0.08	0.11	0.7	90	125	2.0	79	40
Tomato fruit	80 (19)	94	0.9	0.1	3.7	1700	0.10	0.02	0.6	21	6	0.3	10	16
Cucumber fruit (immature)	50 (12)	96	0.6	0.1	2.2	45	0.03	0.02	0.3	12	12	0.3	15	24

In some cases, the analytical data relating to a given crop will appear to vary between Articles. These differences may arise because of contrasts in experimental techniques and/or raw materials employed by the original authors, and hence no attempt has been made to standardize the available information.
Source: Howard, F. D., et al. (1962). *USDA National Food Review*, 1978.

Mustard greens (*Brassica juncea*) contain an array of nutrients, including carbohydrates, sugars, soluble and insoluble fibers, minerals (calcium, potassium, phosphorous, sodium, and magnesium), vitamins (vitamin A and C), fatty acids, and amino acids. However, the nutritional values differ from species to species.

Parsley (*Petroselinum crispum*) is an excellent source of vitamins K (1640 µg/100 g) and C, as well as a good source of vitamin A, folate, and iron. The herb is also a good source of potassium (100 g providing 554 mg (12%) of daily requirements), calcium, manganese, and magnesium. Parsley is also rich in vitamins (pantothenic acid, riboflavin, niacin, pyridoxine and thiamin, and vitamin E) as well as zeaxanthin, lutein, and cryptoxanthin. It contains no cholesterol or fat, but is rich in essential volatile oils (myristicin, limonene, eugenol, and alpha-thujene) and flavonoids (apiin, apigenin, crisoeriol, and luteolin).

Spring or Welsh onions (*Allium fistulosum*) are very low in energy (31 kcal/100 g leaves) and contain many noteworthy flavonoids, minerals, vitamins, and dietary fibers. Their leafy greens also contain ascorbic acid (vitamin C), pyridoxine, folate (64 µg/100 g fresh leaves), niacin, riboflavin, and thiamine. In fact, spring onions are one of the richest sources of vitamin K (207 µg/100 g greens). In addition, this salad crop is

Table 6 Nutritional constituents of some salad crop (data per 100 g basis)

	Macronutrient					*Vitamins*					*Minerals*		
Crop	*Water (g)*	*Energy (kJ, kcal)*	*Protein (g)*	*Fat (g)*	*Carbohydrate (g)*	*A (IU)*	*C (mg)*	*B_1 (mg)*	*B_2 (mg)*	*Niacin (mg)*	*Ca (mg)*	*P (mg)*	*Fe (mg)*
Lettuce	96	54 (13)	0.9	0.1	2.9	330	66	0.60	0.06	0.3	20	22	0.5
Chicory	95	63 (15)	1.0	0.1	3.2	Trace	–	–	–	–	18	21	0.5
Endive (curly)	93	84 (20)	1.7	0.1	4.1	3300	10	0.07	0.14	0.5	81	54	1.7
Parsley	85	184 (44)	3.6	0.6	8.5	8500	172	0.12	0.26	1.2	203	63	6.2
Spinach	91	109 (26)	3.2	0.3	4.3	8100	51	0.10	0.20	0.6	93	51	3.1
Mustard	90	130 (31)	3.0	0.5	5.6	7000	97	0.11	0.22	0.8	183	50	3.0
Chinese cabbage	95	58 (14)	1.2	0.1	3.0	150	25	0.05	0.04	0.6	43	40	0.6
Garden cress	82	280 (67)	5.8	1.0	8.7	–	–	0.15	–	–	360	110	26.6
Watercress	89	138 (33)	2.9	0.2	4.9	2803	13	0.12	0.38	0.8	290	140	4.6

In some cases, the analytical data relating to a given crop will appear to vary between Articles. These differences may arise because of contrasts in experimental techniques and/or raw materials employed in experimental techniques and/or raw materials employed by the original authors, and hence no attempt has been made to standardize the available information.

Source: USDA (1978). *National Food Reviews*; Goplalan, C., Ramasastri, B. V. and Balasubramanian, S. C. (1971). *Nutritive values of Indian foods*. Hyderabad: National Institute of Nutrition.

a good source of copper, phosphorous, magnesium, potassium, chromium, iron, manganese, and calcium. They contain vitamin A (997 IU/100 g) and other flavonoids such as carotenes, zeaxanthin, and lutein. Being leafy-greens, spring onions contain more plant-derived antioxidants than other Allium members, like the onion, shallot, etc. Spring onions contain thiosulfinates antioxidants – albeit less than garlic – and are a source of flavonoids (such as quercetin), and contain allyl propyl disulphide.

Turnip (*Brassica rapa* var. rapa) greens are a good source of protein, thiamine, niacin, pantothenic acid and phosphorus, and are a very good source of dietary fiber, vitamins A, C, E, and K, riboflavin, pyridoxine, folate, calcium, iron, magnesium, potassium, copper, and manganese; but they are very high in sodium. One cup of cooked greens provides carbohydrates (4.4 g), protein (1.2 g), dietary fiber (3.5 g), vitamin C (27 mg), folate (118 μg), potassium (203 mg), about 20 kcal, and calcium (137 mg), which is about four times more calcium than cabbage and about twice that of mustard greens.

Watercress (*Nasturtium officinale*) contains many natural phytonutrients, including *isothiocyanates*. It is thought to be one of the most nutrient dense foods. The leaves and stem contain gluconasturtiin, a glucosinolate, as well as vitamin C (47 mg/100 g leaves). It is an excellent source of vitamin K (100 g/100 g), vitamin A, and flavonoids including beta-carotene, lutein, and zeaxanthin. It is also rich in riboflavin, niacin, pyridoxine, thiamine, and pantothenic acid. Further, it is also a rich source of minerals such as copper, calcium, potassium, magnesium, manganese, and phosphorus.

Industrial Use

Vegetables are less acidic than fruits. Hence, vegetables are classified as low acid foods. A wide range of microorganisms are able to grow on moist, low-acid products which may lead to spoilage and/or food poisoning. To prevent spoilage, vegetables can be processed with heat to destroy bacteria, or preserved using pickling, salting, fermenting, or drying to inhibit bacterial growth. Care is needed while processing low acid commodities to minimize the risk of pathogenic bacteria consumption.

The continuous supply of fresh and processed vegetables in markets globally indicates the economic importance of the vegetable industry, and the contribution of vegetables to dietary requirements. With the expected increase in population, vegetable crops will continue to comprise a major part of the agricultural economy. For example, dehydration of onion bulbs is an important industry in the United States – especially in California. Asparagus, tomatoes, lettuce, cucumber, and carrot are produced and processed worldwide. Sauerkraut is the primary processed product prepared from cabbage. Some cauliflowers, cabbages, rat-tail radishes, turnips, and carrots are canned. Large quantities of chives are sold to processors and, sometimes, their chopped leaves are mixed with cottage cheese for the retail market. A fermented Korean dish called kimchi includes either Chinese cabbage or radish along with hot pepper, garlic, and other ingredients. The juice of a tropical carrot is used to prepare kanji (a fermented product) which is an appetizer consumed before meals in India.

Because of the different growing and harvesting seasons of vegetables globally, the availability of fresh vegetables differs greatly in different parts of the world. Processing can transform vegetables from perishable produces into durable foods with longer shelf lives, thereby aiding in the global distribution of many vegetables. The goal of processing is to deter microbial spoilage and natural deterioration of the plant cells. Techniques include blanching, dehydrating, canning, freezing, fermenting and pickling, and irradiation.

After proper washing, vegetables are blanched in hot water at 88 °C for 2–5 min, or with steam at 100 °C for 30 s to 1 min. Blanching inactivates the enzymes responsible for discoloration.

It also reduces the number of organisms and renders the vegetables flaccid for easier packing. For some vegetables, blanching also serves as a way to remove/reduce strong flavor(s). After blanching, vegetables are cooled rapidly in cold water to retain quality. The vegetables are then ready for:

- *Dehydration*: Drying is the oldest method of preserving vegetables. Water is removed by applying heat from the radiant energy of the sun or electric heaters. Some vegetables are blanched before they are dried and some are dried directly in sunlight. Removing water reduces the volume, making storage and transportation convenient, as well as reducing the risk of microbial growth. New drying techniques, including tunnel, vacuum-, drum-, spray-, and freeze-drying, are very sophisticated. Although freeze-drying produces outstanding quality, it is costly and not widely used.
- *Canning*: Keeping vegetables in metal cans or glass jars is a key processing method, which is useful particularly in developing countries where refrigeration is restricted. Vegetables are cut into pieces, packed in cans, and subjected to heat treatment to make them sterile. Processing targets the inactivation of heat-resistant spore-forming mesophilic bacteria. The temperature and time used for canning varies with the size and other characteristics of the vegetables as well as heat transferring principles. After heat treatment, the containers are hermetically sealed while hot to create a vacuum when they are cooled to room temperature. Canned vegetables can be stored at ambient room temperature for years; however, the slightest defect in the process will result in swelling of the cans during storage. In most cases, this is caused by the formation of gas from chemical reactions between the metal cans and their contents. Alternatively, insufficient heat may not destroy bacterial spores. Canned vegetables may be inferior in quality (less nutritious) to fresh and frozen products because of the conditions during processing.
- *Freezing*: Frozen vegetables have outstanding quality. Indeed, some frozen vegetables may be superior in flavor and nutritional content than fresh produce. Vegetables can be stored longer when frozen because temperatures below 0 °C remove not only limit bacterial growth but also reduce the water available. Most vegetables are blanched at 90–100 °C for 1–5 min before freezing to inactivate the enzymes and reduce bacterial load. Various freezing techniques (blast, plate, belt-tunnel, fluidized-bed, cryogenic, and dehydration) are used for the preservation of vegetables. Advances in packaging materials and techniques have led to bulk frozen products being stored in large retortable pouches.
- *Fermented and pickled vegetables*: Low pH has been used to preserve vegetables for centuries. In both fermented and pickled vegetables, acid is used to preserve the products. Varieties used for fermentation or pickling may not be the same as fresh market vegetables. Due to the acidic environment, fermented and pickled vegetables need less heat treatment before being placed in containers. Cabbage, cucumbers, cauliflower, carrots, beans, sweet peppers, onions, and radishes, are salted (dry, e.g., cauliflower) or soaked in brine (wet, e.g., cucumber) prior to preservation. The temperature of fermentation is generally 20–25 °C.
- *Irradiation*: Ionizing radiation, mostly gamma rays, has been used in several countries to preserve vegetables. The practice is common for preventing deterioration in potato, onion, garlic, ginger, and arum during long-term storage. Vegetables treated with a low dose of ionizing radiation are safe, but consumers are still concerned about this processing technology and, generally, it is not accepted.

Use in the Home as Raw or Industrially Processed Food

Freshness is the key to crisp and tasty salads. Salad crops are largely consumed raw. They are attractive and have value in their appetizing succulence, bulk, and nutrient content. Celery, lettuce, onions, and tomatoes are the most important salad crops available throughout the year; but carrots, cauliflower, cucumbers, radishes, and turnips are also used for preparing pickles.

Artichokes are the edible, immature flowers of a cultivated thistle, but they may also be consumed after boiling or steaming. Some people appreciate eating it raw, when it is young enough. Artichoke hearts are canned, frozen, marinated, or kept in oil. Pulp, paste, and purees are also available. The carbohydrate inulin, in Jerusalem artichoke tubers, yields fructose after hydrolysis and has been examined as a dietary treatment for diabetes mellitus.

Cabbage (*Brassica oleracea* var. capitata) is used in the frozen food industry for preparing salad. Chinese cabbage is used raw in salads, cooked as greens, or fermented (e.g., kimchi). Sauerkraut is the product of the lacto-fermentation of cabbage. Many traditionally fermented food items (kraut, ketchup, pickles, etc.) do not undergo lactic-acid-producing fermentation, but are instead preserved with vinegar, enhanced with sugar, and pasteurized. The health benefits of lacto-fermentation have, therefore, been lost in the industrialized use of vinegars and pasteurization. An increasing number of retailers now carry lacto-fermented products, but these need to be refrigerated during storage.

Celery, chervil, leeks, and tomato are used in soups and stews. Celery is a staple in professional kitchens around the world, adding a crunch when raw and a distinct, yet mellow flavor when cooked. Celery and radishes are used to make banh mi bun (a sandwich and a popular snack food in traditional Vietnamese cuisine). Rhubarb and tomato are also used for preparing sauces. Fringed-leafed endive, parsley, chervil, chicory, leeks, and fennel leaves are used for flavoring and garnishing. Cucumber, cabbage, lettuce, radish, and turnip are fermented to dill and sour pickles, salt stock, sauerkraut, sauerriipen, lettuce kraut, and pawtsay. These foodstuffs are more palatable than their raw forms. Chervil is an herb that is processed lightly to retain its fresh green appearance, as well as its distinctive aroma. It is used with other food items such as dairy products, herb mixtures, instant meals, and marinades. Purées and passatas have become major tomato products as ketchup, juice, puree, paste, sauce, and pickles.

Chicory roots are dried, roasted, and ground for use as a coffee substitute or blended in with coffee. Rhubarb is prized

for its use in pies, tarts, sauces, wines, and even tanning hides. A relative of the globe artichoke, a leafy plant known as cardoon is used for its edible roots (or more often its petioles), which may be cooked or eaten raw in salads. Appealing to the eye and nose, parsley may be used both on and in most foods, except sweets. It can be utilized as both a seasoning and a garnish in soups, salads, fish, meats, and vegetables.

Toxic Substances

Vegetables are the most abundant foodstuffs on earth, and are rich in minerals and vitamins. Raw vegetables are enzyme-rich and widely believed to aid digestion. However, the digestive system of many people is too weak to digest raw vegetables. For many, excess gas, bloating, and abdominal pain are common reactions after eating raw vegetables. This is most likely because of cellulose, a fiber that is poorly digested by humans. Though fiber has little nutritional value, it is important for our intestinal health, in particular for forming a healthy stool. But, unlike cows and other ruminants, humans do not have the enzymes necessary to properly digest the fiber in vegetables. Cellulase, produced by some intestinal bacteria, digests cellulose into sugars that can be absorbed. Fermenting raw vegetable is also a problem, because this breaks down (or predigests) the fiber. When consumed in excess, even beneficial substances can produce harmful effects, whilst some toxic compounds can be tolerated at low concentrations.

In adapting to their environment, plant foods have developed various repellent structures (e.g., spines), odors, bitter flavors, and toxins. Characteristics like flavor and nitrate content may also need to be assessed. Several reports have indicated that ingested nitrates may be converted to carcinogenic nitrosamines. Asparagus contains saponins and cholinesterase inhibitors. The rhubarb and sorrel leaves have a high oxalic acid content, and should not be consumed. White oca tubers also contain oxalic acid, which combines with metals (i.e., calcium) in the body to form oxalate crystals. These crystals can irritate the gut and kidneys. Many greens contain oxalic acid, which binds iron and calcium from within the body or from other food sources. Cooking can destroy the oxalic acid. Vegetables that contain oxalic acid include spinach, chard, parsley, chives, purslane, rhubarb, and beet greens. Raw potatoes contain hemagglutinins, which disrupts red blood cell function. Some raw mushrooms (e.g., edible white mushrooms) contain toxic substances such as agaritine – a suspected carcinogen. These substances are heat sensitive and neutralized by cooking.

On the other hand, while eating cruciferous vegetables is important, these vegetables contain natural goitrogens that can block the production of thyroid hormones. These chemicals are released when the raw vegetable is chewed or the intestines break down vegetables that are only lightly steamed. Thus, some vegetables should only be consumed in large quantities after cooking. Perhaps more importantly, cruciferous vegetables are a rich source of iodine, which is essential for thyroid function. By steaming, goitrogens can be reduced by 30–90%, while the iodine is largely retained.

Glucosinolates present in mustards can cause digestive disorders. Parsley and celery contain psoralens and terpenoids which have been linked with dermatitis. Lettuce is known to be a nitrate accumulator, and spinach contains oxalic acid, saponins, and tends to accumulate high levels of nitrates. These nitrates can interfere with blood oxygen transport, or can form nitrosamines, which are carcinogenic. Significant toxins are listed below:

Name of crop	*Toxins*	*Name of crop*	*Toxins*
Artichoke	Grosshemin	Garden beet	Malonic acid
Asparagus	L-Arginine	Lettuce	Lactucin and lactucopicrin
Brown mustard	Progoitrin	Mustard	Gluconasturtiin
Cabbage	Glucocapparin (progoitrin)	Parsley	Malonic acid
Cabbage leaves	Spirobrassinin	Parsnip	Xanthotoxol
Celery	Xanthotoxin	Rhubarb	Oxalic acid
Chicory	Cichoralexin	Spinach	Coumestrol, histamine, and oxalic acid
Fennel	Anethole		

See also: Salad Crops: Leaf-Type Crops; Salad Crops: Root, Bulb, and Tuber Crops; Salad Crops: Stem-Type Crops.

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Salad Crops: Leaf-Type Crops

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Leafy salad vegetables, also known as potherbs, greens, vegetable greens, leafy greens, or salad greens, are among the most important and universally used vegetable crops grown globally and available throughout the year. They are valued for their appetizing succulence, bulk, and nutrients. These vegetables, as a natural group, have mostly crisp, tasty, and tender edible leaves and have become a staple food. As a result, the acreage under leafy salad crops has expanded to a remarkable degree over the past few decades, making these crops an important segment of agricultural and marketing industries.

Leafy salad vegetables are plants the leaves of which are eaten as vegetable. Nearly a thousand species of plants with edible leaves are known. These vegetables most often come from short-lived herbaceous plants, such as lettuce, which contains many nutrients, especially vitamin K because phylloquinone is involved directly in photosynthesis, which can lead leafy salad vegetables to interact significantly with the anticoagulant warfarin. Leafy salad vegetables are low in energy and fat and high in protein, dietary fiber, iron, and calcium and very high in phytochemicals, such as vitamin C, carotenoids, lutein, folate, magnesium, and vitamin K.

Nearly all vegetables can be consumed raw, but only few are commonly referred to as salad crops, and, of these, only a few are considered as leafy salad based on the criterion that the leaves alone are consumed. Leafy salad crops have been defined as those crops from which the leaves are harvested for use as raw vegetable. However, most of these crops are considered high-risk vegetables since their leaves and petioles are susceptible to attack by pests, poor production and handling systems, and environmental extremes over which growers may have little or no control.

The major leafy salad crops are endive (*Cichorium endivia* L.); lettuce (*Lactuca sativa* L.) and a number of lesser vegetables used primarily for salads, flavoring, or garnishing foodstuffs; Brussels sprouts (*Brassica oleracea* var. *gemmifera*); cabbage (*Brassica oleracea* var. *capitata*); celery (*Apium graveolens*); celtuce (*Lactuca sativa* var. *asparagina*); chervil (*Anthriscus cerefolium*); chicory (*Cichorium intybus*); Chinese cabbage (*Brassica rapa* subsp. *pekinensis* and *B. rapa* subsp. *chinensis*); collard greens (*Brassica oleracea*); dill (*Anethum graveolens*); garden cress (*Lepidium sativum*); kale (*Brassica oleracea* var. *acephala*); mustard (*Brassica juncea*); parsley (*Petroselinum crispum*); sorrel (*Rumex acetosa*); summer purslane (*Portulaca oleracea*); Swiss chard (*Beta vulgaris* var. *cicla*); turnip greens (*Brassica rapa* var. *rapifera*); and watercress (*Nasturtium officinale*). These salad crops thrive best during cooler and moist seasons and do not tolerate hot weather.

Global Distribution, Commercial Importance, and Varieties

Brussels sprouts (*Brassica oleracea* var. *gemmifera*) are believed to have been cultivated in Italy during the Roman times and possibly as early as the 1200s in Belgium. They were cultivated first in Belgium (hence Brussels sprouts) as early as 1587 with their introduction into the United States in the 1800s. Brussels sprouts were grown in California during the early 1900s. With the development of frozen food industry in 1940s, Brussels sprout production in California increased to its highest levels over the next 20 years. This crop has been grown near Brussels (BE) for hundreds of years and is an important vegetable in most European countries, particularly the United Kingdom. Outside of Europe, the importance of the crop is reduced, although Australia and California grow the crop widely. Brussels sprouts are a very good source of many vitamins, especially vitamin C and folate. They, along with other cruciferous vegetables, have been shown to have protective effects against certain types of cancer, as they contain many different ingredients that are believed to help prevent diseases.

The word cabbage (*Brassica oleracea* var. *capitata* L.) is thought to derive from the French caboche, meaning head. One of the oldest recorded vegetables, cabbage was mentioned in early literature and was probably in use as early as 2000 or 2500 BC. The Romans introduced it to Europe where it was bred into the form recognized today. Explorers in the seventeenth and eighteenth centuries included cabbage in ships' stores because of the high vitamin C content, which helped stave off scurvy. By this time, a pickled form was popular in Europe and the French from the Alsace area gave it the name Choucroute (sauerkraut). Cato advised eating cabbage soaked in vinegar before embarking upon an evening of heavy drinking and the accepted remedy for a Roman hangover was simply more cabbage consumption. Caesar's armies carried cabbage with them and not only used it for food but also bound wounds with the leaves to reduce infection. Cabbage has antibacterial properties and reduces inflammation. Cabbage is easy to grow and stores well, and a single serving of cabbage contains nearly half of the daily vitamin C requirement as well as significant levels of manganese, iron, and vitamin B_6. It is high in dietary fiber and low in energy, which makes it an ideal food for those seeking to lose weight.

Beet greens (*Beta vulgaris* var. *crassa*) are the leafy tops of beetroot, which evolved from wild sea beet, a native of coastlines from India to the United Kingdom and the ancestor of all cultivated forms of beet. Sea beet was first domesticated in the eastern Mediterranean and Middle East, although only the leafy salad was eaten when the Romans began to cultivate it. Apicius, the renowned Roman gourmet, recommended it in salad with a dressing with mustard oil and vinegar in his book *The Art of Cooking*. In the early times, it was used to treat a range of ailments including fevers, constipation, wounds, and various skin problems. At that time, the roots were long and thin like a carrot. The rounded root shape that we are more familiar today was not developed until the sixteenth century and became widely popular in Central and eastern Europe 200 years later. Many classic beetroot dishes originated in this region, including the famous beetroot soup, known as borscht.

Chinese cabbage (*Brassica rapa* subsp. *pekinensis* and *B. rapa* subsp. *chinensis*, $2n = 20$) is a native to China and has been cultivated since the fifth century. It was introduced to the United States in the late nineteenth century and is grown throughout the year in California, Florida, and Hawaii. It is increasing in importance in European countries, notably the United Kingdom, France, and Germany. Two more or less distinct species are grown, namely, pe-tsai (*B. rapa* subsp. *pekinensis*) and pak choi (*B. rapa* subsp. *chinensis*). Varieties include Shantung cabbage, chefoo cabbage, pekin cabbage, chou de Chine, and celery cabbage. Of the two types of Chinese cabbage, *B. rapa* subsp. *pekinensis* (napa cabbage) and *B. rapa* subsp. *chinensis* (bok choi), the Pekinensis group is more common, especially outside Asia, such as napa cabbage (large white vegetable) and Baguio petsay or petsay wombok (Chinese white cabbage). Pekinensis cabbage has broad green leaves with white petioles, tightly wrapped in a cylindrical formation and usually forming a compact head. As the group name indicates, this is particularly popular in northern China, around Beijing (Peking). The Chinensis varieties do not form heads, but have smooth dark green leaf blades forming a cluster reminiscent of mustard or celery. Chinensis varieties are popular in southern China and Southeast Asia. Increasingly, they are grown in northern Europe because they are winter-hardy. Linnaeus originally classified this group as its own species under the name *Brassica chinensis*. However, Chinese cabbage used for salad is a term covering a variety of greens used in Chinese cuisine. In the West, this is commonly known as napa cabbage, which has a mild, almost sweet flavor that complements strong-flavored ingredients.

Celery (*Apium graveolens*) is an herb widely cultivated for its edible swollen leaf stalks. It is available in green and white forms. Celeriac (*Apium graveolens* var. *rapaceum*) is a variety of celery with an edible tuberous base to the stems. Celery was first cultivated as a medicinal crop as early as 400 BC in Egypt and the Roman Empire. The Greeks used it to make a celery wine called selinites. Crowns made of celery stems and leaves were awarded to winners of athletic games at the Isthmus of Corinth. Celery is believed to be originally from the Mediterranean Basin. Ancient literature documents that celery was cultivated for medicinal purposes in this region before 850 BC. The claimed medicinal values were probably attributable to its volatile oils, contained throughout but mostly in the seeds. During ancient times, Ayurvedic physicians used celery seed to treat cold, flu, indigestion, arthritis, and liver and spleen ailments. Woven garlands of wild celery have been found in early Egyptian tombs. The Romans valued celery more for cooking than for religious reasons, although much superstition was also connected with it: celery was thought to bring bad fortune under certain circumstances. Although celery is thought to be from the Mediterranean, indigenous wild relatives of celery are found in southern Sweden, the British Isles, Egypt, Algeria, India, China, New Zealand, California, and the southernmost portions of South America. The Italians domesticated celery as a vegetable in the seventeenth century. Early celery had a tendency to produce hollow stalks. Growers found that the naturally strong flavor could be diminished if grown in cooler conditions and blanched, not cooking in boiled water, but the practice of pushing earth up around the stalk base to prevent sunlight from turning the stalks green. There are two types of stalk celery varieties, namely, (i) self-blanching or yellow and (ii) green or Pascal celery. In North America, green stalk celery is preferred and mainly eaten raw although it is also eaten cooked. In Europe and the rest of the world, self-blanching varieties are preferred. Celeriac is very popular in Europe where it is eaten cooked or raw.

Chervil (*Anthriscus cerefolium*) has a long history, as do many herbs. It was once called myrrhis, because the volatile oils extracted from the plant resembles myrrh in both fragrance and taste. Chervil symbolizes new life and is linked to Easter ceremonies in Europe. During the Middle Ages, it was used to cure hiccups, a remedy that still survives today. For thousands of years, the young green leaves of chervil were used as a tonic. Chervil, dandelion, and watercress combined were used to combat vitamin and mineral deficiencies. Native to Europe, it was spread abroad by the Romans. Chervil is native to Europe and it was cultivated first in England in 1597 and in America by 1806. The origin of salad chervil is the region of southeastern Europe and western Asia.

Cress is also known as peppergrass or garden cress and is grown as a salad crop all over the world. It is native to Iran and Europe. From Persia, it spread to India, Egypt, and Greece. It was mentioned by Xenophobe in 400 BC and has been cultivated in England since 1548. It grows wild and is cultivated in the United States and elsewhere. There are four cultivars: (i) common, (ii) curled, (iii) broad-leaved, and (iv) golden.

Chicory (*C. intybus* L., $2n = 2x = 18$), also known as French endive, witloof, witloof chicory, and succory, is an important salad crop. The first mention of chicory culture was in 1616 in Germany. It is one of the oldest recorded plants and a native to northern Africa, western Asia, and Europe. Its cultivation is thought to have originated in Egypt. Medieval monks grew chicory in Europe and it was brought to North America in the 1700s. Chicory has been a popular coffee substitute or an ingredient in coffee in France since around the 1800s. It tends to be the preferred coffee substitute, and in some circles, it is used even when coffee is available and cheap. One historical and cultural example of its use as a coffee substitute was found in New Orleans, which was a major consumer of coffee prior to the Civil War. Today, chicory remains a popular coffee replacement or flavoring in New Orleans, and New Orleans coffee typically refers to chicory coffee. 'Radichetta' and 'Rosso di Verona' are the principal chicory cultivars in the United States and Italy, respectively. 'Penninck,' 'Christaens,' 'Mueninck,' and 'Van Espen' are Belgian cultivars, while 'Normato,' 'Malina,' 'Bubbal Blank,' and 'Slusia Meilof' are Dutch cultivars. Flambor, Bergere, and Zoom are chicory hybrids. In a study to assess the best cultivar for forcing, Tardivo and Mitado gave the highest yield and best quality produce. Best forced under a hydroponic system in terms of yield and quality were Tardivo, Mitado, F_1 hybrids Nunhems 825-76 and Nunhems 820-76, Zoom, Alvaro, and Barola. With very early forcing, Daliva was outstanding; Arnova, Zoom, Mitiva, and Wivro gave the next highest yield.

Collard (*Brassica oleracea* var. *acephala*) is a headless form of cabbage. It bears the same botanical name as kale, from which it differs only in leaf characters. Its leaves are much broader without frill and resemble the rosette leaves of head cabbage. Lower leaves are harvested progressively. While collard has a medium green color, smooth texture, and an oval shape, kale

has dark grayish green broad leaves with a crinkled texture. On flavor scale, collard is several times milder than kale, a strong bitter vegetable. Historians surmise that it was growing wild in Asia Minor, now Turkey, as well as in Greece along the Mediterranean long before recorded history. Collard, a type of kale, has remained almost the same for about 2000 years. The plant is a source of nutritionally important minerals and vitamins A and C.

Kale (*Brassica oleracea* var. *acephala*, $2n = 18$), also a nonheading type of cabbage, is a hardy plant and grown as food and fodder. It is closely related to wild cabbage, and most forms bear a rosette of leaves at top of the stem. There are various types of kale, but curly leaf kale is grown for human consumption. Its cultivation probably began seven millennia ago; the Greeks grew it as early as 600 BC. Eighty varieties from 17 countries were studied and classified into marrow-stem kale, leafy kale, thousand-headed kale, and curly kale. It is an excellent source of vitamins A, C, and K; a good source of calcium; and a source of some folate and potassium.

Endive (*C. endivia* L., $2n = 2x = 3x = 18, 36$) is an annual or biennial salad crop. It is believed to be a native of Egypt or India and indigenous to eastern Mediterranean areas and may have originated from the cross *C. intybus* × *C. pumitum*. The Egyptians used it during the early periods and it was brought to Europe in the thirteenth century. Its cultivation began in England in 1686 and in France in 1826 and was recorded first in the United States in 1806. Endive is an especially popular salad vegetable in Europe, and its popularity has increased in the United States. Endive is classified into two general groups, namely, (i) the curled- or fringe-leaved cultivars and (ii) the broad-leaved cultivars. The green curled 'Ruffec,' 'Deep Heart Fringed,' 'Green Curled Pancalier,' and 'White Curled' are curled- or fringe-leaved types. The varieties representing broad-leaved class are 'Broad-Leaved Batavian,' 'Full Heart,' 'Batavian or Escarole,' and 'Florida Deep Heart.'

Lettuce (*Lactuca sativa* L.) is a popular leafy salad crop, grown throughout temperate, tropical, and subtropical climates and is of great commercial importance. Its cultivated and wild species are native to Europe and Asia and have been in cultivation for the last 2500 years. Evidence suggests it was cultivated before 4500 BC. The Portuguese and British introduced lettuce to India in the sixteenth century. The crop spread throughout the Mediterranean region during the Greek and Roman eras and from there to the rest of Europe and the New World by 1494. Lettuce is available in different forms, namely, romaine, iceberg, oakleaf, and mâche. Romaine lettuce is high in vitamin A and provides some folate. Leaf lettuce has a softer texture than romaine, a crunchy variety used in Caesar salads. The darker the leaf, the more nutrients it contains, meaning red-leafed varieties are more nutritious than green. In the United States, four types of lettuce are grown, and each is recognized as a subspecies or botanical variety, namely, (i) head type, *L. sativa* var. *capitata*; (ii) curl leaf type, *L. sativa* var. *crispa*; (iii) cos or romaine, *L. sativa* var. *longifolia*; and (iv) asparagus type, *L. sativa* var. *asparagina*. Six morphological types of commercial cultivars are available, namely, crisphead, butterhead, cos, leaf, stem, and Latin. The crisphead type is the most common in the United States. About 70% of US crisphead lettuce is produced commercially in California, 16% in Arizona, and rest in other states. Butterhead and cos (Romaine) are popular in northern and southern Europe. The US crisphead cultivars are usually large, heavy, brittle-textured, and tightly folded and have a green outer and white or yellowish inner leaves. This type is becoming increasingly popular in the United Kingdom and Spain and, in the latter case, as a winter crop for export to the United Kingdom. Crisphead lettuce is often called iceberg lettuce, but this is somewhat misleading since there is also a crisphead cultivar called 'Iceberg.' Most of the important commercial varieties are the crisphead class; the best-known are New York 515; Imperial 44, 152, 456, 615, and 847; Great Lakes; and Pennlake. Big Boston, White Boston, May King, Salamander, and Wayahead are the best-known varieties of butterhead type but have relatively little commercial importance. Black-Seeded Simpson, Early-Curled Simpson, Grand Rapids, and Slow Bolt are the main varieties of leaf or bunching lettuce. Paris white (white cos) and Dark Green are the cos-type varieties. 'Great Lakes' (head type) and 'Chinese Yellow' (loose-leaf type) cultivars are commonly grown in India. 'Imperial 859' and 'Slow Bolt' are also improved cultivars of lettuce.

Mustard (*Brassica juncea*) is native to Central Asia and the Himalayas with secondary centers in India and eastern China. White mustard (*Sinapis alba*), black mustard (*Brassica nigra*), and Ethiopian mustard (*Brassica carinata*) are commercially important in many parts of the world and grown all over the world for a variety of uses. They are common in the United States, Canada, and the United Kingdom and most other European countries, India, China, and Southeast Asian countries. The inner leaves are milder and best suited for salad use. There are two principal salad or greens cultivars, namely, 'Florida' (broad leaf) and 'Southern' (giant curled leaf), which are grown in the United States. Mustard greens have scalloped edges and come in red and green varieties. They have a peppery taste and give off a mustardy smell during cooking.

Parsley (*Petroselinum crispum* (Mill.), $2n = 2x = 22$) is native to the central Mediterranean region (southern Italy, Algeria, and Tunisia). The name is derived from the Greek meaning rock celery and it is a relative of celery. Parsley was popular in early times among the Greeks and Romans. Its value was reputed to be in aiding digestion and suppressing odors of onion and wine. It has been in cultivation for over 2000 years. It was introduced to the United Kingdom from Sardinia in 1548 and to the United States in 1806. It is produced commercially in Texas, California, New Jersey, Florida, and New York. The ancient Greeks associated parsley with death, as it was supposed to have sprung from the blood of Archemorus, whose name means 'forerunner of death.' Homer tells of chariot horses being fed parsley by warriors prior to battle in the hope of making the animals more fleet of foot. Generally, the Romans did not eat parsley but they did wear its garlands on their heads during feasts to ward off intoxication. It was kept away from nursing mothers because it was thought to cause epilepsy in babies. Five types of parsley cultivars are available, namely, (i) plain-leaved, (ii) celery-leaved or Neapolitan, (iii) curled, (iv) fern-leaved, and (v) turnip-rooted type. The curled type is common in the United States where plain and turnip-rooted parsley is also grown. The best-known varieties are Moss, Extra Double Curled, Fern-Leaved, Curled Dwarf, Evergreen, Extra Triple Curled, and Plain and Dark Green Italian. Morgo was developed in Sweden, and

'Hamburg' is the only turnip-rooted cultivar. Curly-leaf parsley is often used as a garnish. In Central and eastern Europe and in western Asia, many dishes are served with fresh green chopped parsley sprinkled on top. Root parsley is very common in Central and eastern European cuisines, where it is used as a snack or a vegetable in many soups, stews, and casseroles.

Swiss chard (*Beta vulgaris* var. *cicla*), also called silver beet, is a green leafy biennial vegetable originally from Sicily. It established a place in kitchen gardens in England and America in the 1830s. Indeed, its first notice in American agriculture is Baltimorean Gideon Smith's letter of 30 October 1829 announcing receipt of seeds from Europe and his later reports about plantings, published in *The American Farmer*. Horticulturist Robert Buist was its primary antebellum promoter and it first found popularity in the Philadelphia area. As late as the 1850s, it remained a special plant. Broader cultivation came after the American Civil War. In the nineteenth century, chefs often separated the midrib from the leaves and prepared it like asparagus. Similar to beets with a flavor that is bitter, pungent, and slightly salty, it is truly one of the vegetable valedictorians with its exceptionally impressive list of health-promoting nutrients. Swiss chard along with kale, mustard greens, and collard greens is one of several leafy green vegetables often referred to as greens. It is a tall leafy green vegetable with a thick crunchy stalk that comes in white, red, or yellow with wide fanlike green leaves. Both the leaves and stalk of chard are edible, although the stems vary in texture with the white ones being the most tender. It is a good source of vitamins A and C. Swiss chard contains oxalates, which are slightly reduced by cooking but bind to calcium, a concern for people prone to kidney stones.

Watercress (*Nasturtium officinale*), which is unique among the vegetables, as it commonly grows in flowing water as a wild plant, is believed to be native to the eastern Mediterranean and Europe. In cool regions of the world, it grows wild in streams but it is also found growing wild above 1500 m in the Himalayas of Nepal and hilly areas of the United States, especially in Virginia, Maryland, and Pennsylvania. It is also grown in California and Europe. It was cultivated in England for the first time in 1808 and in France in 1811. According to Howard and Lyon, watercress formed one of the tributes to the high Kings of Erin (Ireland) during the Dark Ages. The first large-scale cultivation of watercress was suggested to have occurred in southern Germany, near Erfurt, in around 1750. Large-scale cultivation in England and France, to supply the markets of London and Paris, started in the early nineteenth century. It is a peppery nutritious vegetable, which is often used in sandwiches and salads. An elongating shoot induces vigorous basal branching to produce a sward crop. Repeated cutting leads to increased branching and as many as ten crops may be harvested in a year. Watercress enjoys worldwide distribution and, when grown commercially outdoors, it is planted in beds covered with slowly flowing, clean water. There are two main cultivars, namely, (i) 'Green Cress' (diploid and fertile) and (ii) 'Brown Cress' or 'Winter Cress' (triploid and sterile).

Morphology and Taxonomy of the Edible Parts

Dwarf varieties of Brussels sprouts have short stems, <50 cm long. 'Improved Long Island,' 'Catskill,' 'Early Morn Dwarf Improved,' and 'Friither Zwerg Kvik' are the most important varieties. In dwarf cultivars, sprouts are of medium size and the distance between sprouts is short. 'Bedfordshire'; 'Cambridge No. 1, 3, and 5'; 'Irish Elegance'; and 'Sherradian' are some of the important cultivars grown in the United Kingdom and Ireland. Early cultivars such as 'Breda' (the Netherlands), 'Weibulls Rapid' (Sweden), and 'Wilhelmsburg' (Germany) have light green sprouts. Late hardy cultivars include 'Red Vein,' 'Hild's Idea,' 'DeRoshy Amager,' and 'Polarstjernen.'

An improved Chihili (Michihili), the most common variety of Chinese cabbage, is uniform in size with heads around 46 cm long, 10 cm thick, and cylindrical in shape with a tapered tip. The outer foliage is dark green, inner leaves blanched, and the midrib white and broad. 'Won bok' with light green leaves is a very old Chinese cultivar. Pe-tsai resembles cos lettuce but produces a much larger, elongated, and compact head, and the leaves are slightly wrinkled, green, thin, and much veined. The midrib is broad and light in color. Pak choi resembles Swiss chard in its habit of growth. The leaves are long, dark green, and oblong or oval and do not form a solid head.

Endive grows as a loose head of leaves, which are strongly ruffled and serrated. The outer leaves are green and bitter, but the inner leaves are light green to whitish. In the case of Radichetta chicory, the leaves are dark green, narrow, and notched, and the Italian cultivar is red and nonforcing type. Endive is a plant with numerous radical leaves, which are smooth, lobed, and more or less deeply cut, spreading into a rosette, and the stem is hollow (50 cm to >1 m high), ribbed, and branching.

Kale produces edible inflorescence like cauliflower and broccoli. The most popular varieties of kale in the United States are Dwarf Blue Scotch and Dwarf Green Scotch.

The cultivated lettuce (*Lactuca sativa* L.) is related to wild lettuce (*Lactuca serriola*), a common weed in the United States. Heading lettuce was first described as cabbage lettuce in 1543. It forms a rosette of large, longish leaves, which are somewhat spoon-shaped and more or less toothed at the edges. From the center of rosette springs a nearly cylindrical stem, which narrows very rapidly and branches at about one-third of its height. It is furnished with clasping leaves, which encircle the stem and become narrower as they approach the top.

Mustard (*Brassica juncea*), a member of the cabbage family, is used both as a salad and as a green vegetable. In addition to being grown for its seeds, white mustard may be grown as a salad plant, cover crop, and green manure. Although principally an oilseed crop, it is commonly grown as a leafy vegetable in northern parts of India, China, and tropical and subtropical regions. 'Ford Hook Fancy,' 'Ostrich Plume,' and 'Southern Giant Curled' are the popular varieties of *Brassica juncea* grown in the United States. The basal rosette leaves vary in form, while the stem leaves are mostly intact. Leaves are large, broadening toward the apex, and have smooth or curled margins. 'Florida BroadLeaf' is a vigorous, large, and erect plant, with large, thick, broad, oval leaves with toothed margins. 'Southern Giant Curled' (curled leaf) is large and upright, with large leaves and curly margins.

Parsley forms a rosette of divided leaves on a short stem. The most common and attractive is the curled leaf-type containing three subtypes, namely, (i) doubled-curled, (ii)

evergreen, and (iii) triple-curled. The degree of leaf curling, coarseness of the leaf, and plant growth habit distinguishes these subtypes. The plain leaf parsley (Dark Green Italian) has deeply cut leaves but no curling or fringing. Double-curled lettuce has a stem 30.5 cm tall with compact and very dark green leaves, which are finely cut, deeply curled, and frost-resistant. Evergreen leaves are coarsely cut, while extra triple-curled leaves are finely cut and very closely curled. The triple-curled variety has closely curled shorter leaves. Plain leaf types include plain (singles) and have flat leaves. The leaves of the Dark Green Italian cultivar are heavy and glossy green.

Watercress (*Lepidium sativum* L.) has three sections, namely, (i) an aerial stem portion with an apex leaves but no roots that is projecting above the water, (ii) a stem portion beneath the flowing water that has foliage and a system of adventitious roots that arise from the leaf axils and remain free in water, and (iii) normal roots. Watercress leaves are pinnately compound with 3–11 leaflets, rounded or oblong, slightly fleshy, and with smooth margins. Cress is a much-branched annual with linear leaves. The moss curled (curled) variety is branching, dark green, and with a mild but distinct flavor.

Postharvest Handling and Storage

Baskets are used to pick and carry Brussels sprouts to the packinghouse. Individual boxes are wrapped with a transparent film, which is useful in preventing moisture loss. The optimum storage conditions for Brussels sprouts are 0 °C temperature and 95–98% relative humidity, which keep them in marketable condition for about 3–5 weeks. At −2 °C, sprouts can be stored for a longer period. A controlled atmosphere (CA) of 2.5–5.0% O_2 and 5–7.5% CO_2 is helpful in maintaining Brussels sprouts at 5 or 10 °C, but not at 0 °C. Oxygen levels $< 1\%$ can cause internal discoloration.

Cabbage heads are harvested with a sharp-edged sickle, leaving a couple of leaves below the head intact, which provide a degree of protection for the base of the head. For prolonged storage, due care is exercised to prevent injuries arising from bruising. Healthy heads are packed in perforated polyethylene bags and stored at low temperature soon after harvest. Refrigeration at 1.6 °C and low relative humidity are needed to control the growth of pathogens. The lower the temperature, the longer their shelf life, but freezing reduces quality. Solid heads are more susceptible to freezing injury than firm heads. A storage temperature of 0 °C with 98% relative humidity is recommended for storage up to 6 months. The prevention of moisture loss is essential during storage. Slight reductions in weight loss and retardation of senescence, such as yellowing, toughening, and loss of flavor, are major advantages for CA storage of cabbage. White cabbage stores well in 5% O_2 and 2.5–5% CO_2. If oxygen levels are near zero or CO_2 is maintained at 15% or higher for 1 month, the internal leaves of cabbage discolor even though the external leaves are normal. Some cultivars are grown for off-season marketing. These cultivars can be maintained in acceptable condition for 2–3 months and up to 6 months, with the use of modified atmosphere storage (1% O_2 and 5% CO_2 at 0–1 °C temperature and 98–100% relative humidity). All cole crops should be stored in an atmosphere free from ethylene to avoid premature senescence and tissue injury.

Chinese cabbage is harvested by hand at the base, and the outer, diseased, or injured leaves trimmed off. The head should be packed loosely and preferably upright. The vegetable can be stored at 0 °C coupled with 95% relative humidity for several weeks. A temperature of 0 °C coupled with 98% relative humidity is optimal for maintaining a marketable state for about 4–6 months. Spacing among containers in storage should be allowed for air circulation. A low concentration of oxygen is reported to be beneficial in extending the storage life.

Chicory is harvested when the heads are compact, bright, and well sized. The plants are cut at the base, and damaged or diseased leaves are trimmed. The trimmed heads are washed and cooled by vacuum or hydrocooling to 0 °C temperature before being packed in layers in wire-bound crates, cartons, or baskets for transit. They are stored at 0 °C and 90–95% relative humidity for 2–3 weeks. Chicory can be stored for a longer period with crushed ice in and around the packaging.

Endive and escarole are leafy salad greens not adapted to long storage. Even at 0 °C, they cannot be expected to be kept satisfactorily for more than 2–3 weeks. Vacuum or hydrocooling can help in maintaining a fresh appearance. They can be kept for longer if stored with crushed ice in or around the packaging. The relative humidity should be above 95% to prevent wilting.

Lettuce harvesting begins as soon as the plants reach acceptable size and it should be completed before the leaves become tough and bitter or the seed stalk starts to bolt. Lettuce grown for the market must be allowed to grow to full size and develop a solid head. For home use, it is often harvested before the head is fully developed. The heads are hand-cut just below the lowest leaf. Loose and damaged leaves are removed and the product is washed prior to packaging. Most commonly, lettuce is packed in cartons and vacuum-cooled soon after harvest. Lettuce intended for longer storage should be packed in perforated plastic films in cartons or crates with liners to maintain a high humidity. It is advisable to trim unfolded leaves before packing, as these are not needed to maintain quality and retailers discard wrapper leaves. Removing wrapper leaves at harvest and wrapping the heads with plastic film reduces injury during packaging. Shed packaging of two to six dozen heads of lettuce in wooden crates with ice has yielded to dry packing of 24 heads in fiberboard containers. Polyethylene-lined packaging reduces decay by 30–40%, compared with open polyethylene bags. Mechanical injury is one of the major causes of loss of quality and waste in unwrapped lettuce. Packaging of romaine lettuce in unsealed polyethylene-lined (0.04 mm thick) packaging causes a marked decrease in decay during storage in spite of high relative humidity. The beneficial effect of packaging has been attributed to high CO_2 and low O_2 levels in the package because of respiration. Lettuce should be precooled to −1 °C and stored at 0 °C and 98–100% relative humidity early after harvest. The rate of general deterioration at 15 °C is about 2.5 times greater than that at 0 °C. Precooling is commonly accomplished by vacuum cooling. To aid vacuum cooling, clean water is sprinkled on lettuce heads prior to carton closure if they are dry and warmer than 25 °C. Lettuce is highly perishable and deteriorates rapidly with increasing temperature. The respiration rate increases rapidly and storage decreases concomitantly as the storage temperature

increases over the range from zero to 25 °C. Leaf lettuce respires at about twice the rate of a head lettuce. Lettuce is not stored for a long period although it can be stored for 3–4 weeks at 0 °C if it arrives in good condition. It can be kept for about twice as long at 0 °C as at 3–4 °C. Lettuce is easily damaged by freezing, and thus, all storage must be above the highest freezing point (−0.2 °C). CA is of limited use in storing lettuce. Oxygen about 3–5% and CO_2 1.5% are recommended to prevent russet spotting and butt discoloration. Lettuce is sensitive to high CO_2 and low O_2; oxygen below 1% and CO_2 above 2.5% are injurious to lettuce. Problems, such as russet spotting, bacterial soft rot, and CO_2 injury, can cause serious losses. Kinetin application delays chlorophyll losses and slows down the increase in abscisic acid content. Application of gibberellic acid (10 ppm) and cytokinin isopentenyladenine (0.1 ppm) 2 days before harvest can delay senescence effectively.

In the case of mustard, small bundles made after discarding old and diseased leaves are handled like other greens at 0 °C. It is commonly shipped in package under ice to maintain freshness. Quality is retained so long as wilting is prevented.

Because of the high rate of respiration, broccoli and kale are prone to rapid desiccation. Therefore, it is important to cool rapidly to low temperatures under high humidity until the commodity is consumed.

Parsley can be harvested over a long period by cutting outer, larger leaves only and tying it in bunches for market. The leaves can be packed loose or bunched after being washed thoroughly and before packing into crates. Crushed ice is usually placed in the packing and shipment made under refrigeration to distant markets. Much of the crop is supplied to restaurants and hotels or dehydrated. At 0 °C and high humidity, parsley can be held for 2 months in storage.

The top 15 cm watercress shoots are harvested and precooled with ice water and shipped using refrigerated vehicle. Its leaves are highly perishable and need prompt handling and refrigeration after harvesting. When it is handled inappropriately, the leaves become slimy. Watercress should be precooled using either hydro- or vacuum cooling and stored at 0 °C with high relative humidity (95–100%). It is bunched and packed in alternate layers with flake ice and kept cool during shipment by spraying with cold water and/or ice. Watercress at 0 °C and high relative humidity in waxed cartons with top ice can be held for up to 2–3 weeks. A similar storage life is possible using permeable polyethylene crate liners and package icing to minimize wilting. Naked bunches are highly perishable even at 0 °C and may be kept only for 3–4 days. The use of polyethylene film liners may be justified to prevent moisture loss under some circumstances.

See also: Salad Crops: Dietary Importance; Salad Crops: Root, Bulb, and Tuber Crops.

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Salad Crops: Root, Bulb, and Tuber Crops

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Feeding the Future

The common root and tuber crops (potato, cassava, sweet potato, and yams) play a critical role in providing needed calories to the food-insecure of the developing world. The global population, as estimated by the United Nations, exceeded seven billion in October of 2011 with projected current growth to nine billion by the year 2050. The Intergovernmental Panel on Climate Change, a global consortium targeting a scientific and socio-economic understanding, predicts future stresses on human communities, agricultural production, and natural ecosystems by threat of increased climatic temperatures, drought, and flooding. As a result, agricultural systems will be under pressure to produce more under suboptimal conditions and climatic stress. Root and tuber crops have tremendous satiating power through their capacity to thrive in marginal growing environments. However, ensuring global food security goes beyond providing needed calories. Root and tuber crops, like cassava, can provide a medley of phytonutrients and phytochemicals. According to the Food and Agriculture Organization (FAO), globally, 870 million people, or one in eight, suffer from malnutrition and undernutrition. In addition, the FAO has stated that root and tuber crops, grown in over 140 countries, are capable of delivering the needed calories and many essential nutrients to the undernourished.

Root and tuber crops are produced, processed, and consumed in ways as unique as each urban and rural culture where they are cultivated. Rural consumers are those who grow, on a substance level, much of the food they rely on to meet nutritional needs. Urban consumers, those who purchase the majority of their food from producers, have developed a preference for root and tubers based on convenience and purchasing power. Rural consumers typically produce their own food and trade with surplus for additional income as compared to urban consumers who purchase food. The nutritional importance of a food for a given population can be evaluated, according to the FAO, by considering its contribution to the total energy supply, taking into account the availability of the food and total available energy for a given population. Measured nutritional value of starchy foods depends on nutrient content, starch properties, antinutritional factors, and toxic substances. Nutritional availability, digestion, and absorption from root and tuber crops can be impacted by cultural practices, storage conditions, and processing. Technological advances have been shown by researchers to allow for the rapid characterization of nutritional properties, allowing for improved health benefits through these crops.

The Consultative Group on International Agricultural Research (CGIAR) is a global partnership uniting organizations engaged in research for a food-secure future. The 15 CGIAR research centers, each with a specific crop mandate, focus on reducing rural poverty, increasing food security, improving human health and nutrition, and ensuring more sustainable management of natural resources. Root and tuber crops are the source of most daily carbohydrate intake for large populations globally; therefore, three CGIAR centers carry mandates focused toward their development: the International Center for Tropical Agriculture (CIAT) in Colombia, the International Institute of Tropical Agriculture (IITA) in Nigeria, and the International Potato Center (CIP) in Peru. CIP was established as a root and tuber research and development center originally focused solely on the potato due its global nature of production and the ability to produce more dietary calories and protein than any other staple crop. Research efforts at CIP have since been broadened to include root crops due to their critical role in the developing world and global agricultural systems. Research has shown that root and tuber crops are capable of growing with limited inputs and in marginal conditions providing one of the cheapest sources of calories and vital nutrients. Preempting a global food crisis will require adaptation of current agricultural systems beyond simply producing dietary calories. Collaborative research efforts, such as those modeled by the CGIAR system, between agronomist, biologist, entomologist, nutritionists, and social scientist must be conveyed all the way to the farmer to carry the needed impact.

Production and Processing

Root and tuber crops function as a dietary foundation supporting those most affected by hunger within the developing world. Foods eaten regularly in quantities that constitute a dominant part of the diet (major source of energy and nutrient needs) are considered food staples. Globally, according to the FAO, four of the top ten food staples are root and tuber crops with potato just behind the major cereal grains of maize, wheat, and rice. In many resource-poor and developing areas, food cultivation requires crops capable of performance in marginal soils, those supplemented with limited inputs. Excluding the potato, root and tuber crops are grown almost exclusively in the highly variable environments of the tropics.

Industrial production and processing of root and tuber crops is challenging due to the unique and often ununiformed physiological shape of the roots and tubers produced. Differences in root and tuber shape can largely be associated to the variable environments in which they are grown. The same phenoplasticity allowing these crops to thrive across contrasting environments may limit their use in large-scale commercial production; however, this adaptive trait is critical in feeding those struggling in developing nations.

Crop breeding is based on selection of hybrids evaluated for desired traits. Crosses are made between parental lines with

different and hopefully complementary genes in hopes of deriving progeny capable of outperforming either parent. The breeding process requires extensive time and efforts, making the development of new cultivars challenging. The danger of vegetative propagation, such as that in root and tuber crops is a challenge, as breeders must keep ahead of rapidly changing plant pathogens capable of devastating the needed harvest. Farmers are at the mercy of changing plant diseases given its ability to impact an entire crop lacking in genetic diversity.

The potato is the world's most productive crop when considering the needed inputs in comparison to the total caloric output. Potato consumption has more than doubled since 1993 with a global production of over 335 million metric tonnes a year. China leads the global potato harvest followed by India and Russia. Potato cultivars that have been adapted for large-scale production typically have shallow root systems to facilitating harvest yet limit their ability to access lower soil profiles containing water and nutrients. The potato tuber is an enlarged portion of the stolon that forms in response to hormones triggered by temperature and day length. Tubers grow rapidly once initiated although high temperatures can negatively impact formation.

In commercial production, potatoes are planted in hills or raised beds to optimize water drainage and uniformity of irrigation. Potatoes can be grown on a variety of soils contrasting in water holding capacity. Ideal soils would be those of good structure and drainage allowing root aeration limiting the impact of root disease. Soil pH can range from 4.5 to 8.5; however, 5.5–7.0 is ideal for production and nutrient uptake.

In North America, the potato season begins in March or April with the preparation of seed for planting in April or May. The growing season spans from around April to October with harvest beginning in late July. Once planted, initiation of sprouting and emergence will occur 15–30 days following planting. Stolon initiation begins at the same time of emergence; tubers will initiate 15–30 days following emergence. Filling of tubers begins around 45–90 days following emergence. Tuber development, plant maturation, and harvest will range from 90 to 120 days following emergence.

Early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*) are potato fungal pathogens that can negatively impact crop production if not managed correctly. Additional efforts to control pests such as the aphids and psyllids are critical to reduce crop damage. Nematodes can have an equally devastating effect on overall production if not suppressed through simple crop rotation.

Patterns of Distribution and Consumption

Tuber crops constitute a considerable part of the world's food supply and are a valuable source of animal feed. On a global basis, ~45% of tuber crop production is consumed as food, with the remainder used as animal feed or for industrial processing for products such as starch and distilled spirits.

The pattern of tuber crop utilization varies considerably among countries. In the developing countries (with the exception of China and Brazil), relatively small amounts (<20%) are fed to livestock. Most of the remainder is used locally as food. The relatively high cost of transportation and processing, as well as the time needed in food preparation, makes unprocessed tuber crops less attractive to urban consumers.

The consumption of tuber crops in developed countries is considerably smaller, but their use as animal feeds is higher. A small proportion of tuber crop production (<5%) is traded internationally. More than two-thirds of those exports come from developing countries. Apart from cassava, only potatoes are traded internationally mainly among developed countries.

There are considerable differences in the agroclimatic conditions suitable for the production of tuber crops. Cassava is cultivated across a range of agroclimatic conditions from sea level to 1800 m and from areas with as little as 500 mm of rainfall to tropical rain forest areas. Potatoes, on the other hand, are considered to be a high-latitude/high-altitude crop, originating in the Andes, but now grown in a range of environmental conditions, including irrigated production in Latin America, Asia, and portions of Africa.

Sweet potato originated in the Americas (as did cassava and potato), and it too is grown over a considerable range of latitude and elevation (up to 2500 m). Conversely, yams have a relatively narrower range of production, being mainly confined to the tropical regions throughout the world from sea level to 1400 m.

The dozen or so lesser known Andean root and tuber crops vary considerably in their ranges of adaptation and tolerance to environmental conditions. They are primarily considered to be medium- to higher-elevation crops of moderate temperature regimes and water requirements.

Health Benefits

As with all crops, nutritional content in tubers can vary greatly depending on the varieties cultivated and management practices used during production. Furthermore, the preparation and cooking of tubers may alter their nutritional content. Vitamin, mineral, and fiber content can change by peeling, or cooking. With these caveats in mind, the values discussed should be considered averages.

Tuber crops are some of the richest sources of starch and dietary fiber. Research has shown that many varieties of potato also contain a melody of vitamins and minerals. Potatoes, yams, and sweet potatoes provide around 70–100 calories per 100 g serving, while cassava has nearly twice the calories of potatoes, perhaps highest for any tropical starch-rich tuber (100 g providing 160 calories). Tubers are good sources of vitamin B complex such as pyridoxine (vitamin B_6), thiamin, niacin, pantothenic acid, and folates. Furthermore, fresh potato along with its skin is a good source of antioxidants. Most tubers contain adequate amounts of many essential minerals like iron, manganese, magnesium, phosphorous, copper, and potassium (Figure 1). Colored potatoes like red and russet potatoes contain adequate amounts of vitamin A and antioxidant flavonoids.

Sweet potatoes are an excellent source of flavonoid phenolic compounds such as beta-carotene and vitamin A. In fact, their vitamin A content is among the highest in the root-vegetable categories. Sweet potato leaves are more nutritious than the tuber itself. Weight per weight, 100 g of fresh leaves contains more minerals than the tuber.

ROOT CROPS

TUBER CROPS

Cassava

Nutrition Facts
Serving Size 100 grams

Amount Per Serving	
Calories 160	Calories from Fat 2
	% Daily Value*
Total Fat 0g	0%
Saturated Fat 0g	0%
Trans Fat	
Cholesterol 0mg	0%
Sodium 14mg	1%
Total Carbohydrate 38g	13%
Dietary Fiber 2g	7%
Sugars 2g	
Protein 1g	
Vitamin A 0% • Vitamin C	34%
Calcium 2% • Iron	1%

Sweet Potato

Nutrition Facts
Serving Size 100 grams

Amount Per Serving	
Calories 86	Calories from Fat 0
	% Daily Value*
Total Fat 0g	0%
Saturated Fat 0g	0%
Trans Fat 0g	
Cholesterol 0mg	0%
Sodium 55mg	2%
Total Carbohydrate 20g	7%
Dietary Fiber 3g	12%
Sugars 4g	
Protein 2g	
Vitamin A 284% • Vitamin C	4%
Calcium 3% • Iron	3%

Yam

Nutrition Facts
Serving Size 100 grams

Amount Per Serving	
Calories 118	Calories from Fat 1
	% Daily Value*
Total Fat 0g	0%
Saturated Fat 0g	0%
Trans Fat	
Cholesterol 0mg	0%
Sodium 9mg	0%
Total Carbohydrate 28g	9%
Dietary Fiber 4g	16%
Sugars 0g	
Protein 2g	
Vitamin A 3% • Vitamin C	28%
Calcium 2% • Iron	3%

Potato

Nutrition Facts
Serving Size 100 grams

Amount Per Serving	
Calories 58	Calories from Fat 1
	% Daily Value*
Total Fat 0g	0%
Saturated Fat 0g	0%
Trans Fat	
Cholesterol 0mg	0%
Sodium 10mg	0%
Total Carbohydrate 12g	4%
Dietary Fiber 3g	10%
Sugars	
Protein 3g	
Vitamin A 0% • Vitamin C	19%
Calcium 3% • Iron	18%

Figure 1 Nutrition facts of selected root and tuber crops. Nutritional values represent an uncooked serving size of 100 g. Nutrient data were sourced from the USDA National Nutrient Database. *Percent daily values (%DV) are for adults or children aged 4 or older and are based on a 2000-calorie reference diet.

Like all tubers, cassava is low in fats and protein. Nonetheless, it has more protein than that of other tropical food sources like potatoes and yams. As in other roots and tubers, cassava is free from gluten. Young tender cassava (yuca) leaves are a better source of dietary proteins and vitamin K.

Toxic Substances and Antinutritional Factors

Tuber crops contain small amounts of potential toxins and antinutritional factors such as trypsin inhibitors. Apart from cassava, which contains cyanogenic glucosides, cultivated varieties of most edible tubers do not contain any serious toxins. Wild species may contain toxins that must be correctly processed. These species are useful in times of food scarcity, and local people have developed techniques for detoxifying the roots before consumption.

Cassava Toxicity

The main toxic principle, which occurs in varying amounts in all parts of the cassava plant, is a rather stable cyanogenic glycoside. Researchers have shown that if absorbed from the gut to the blood as the intact glycoside, it can be excreted unchanged in the urine without causing any harm. However, it can liberate cyanide in the gut during digestion. The range of cyanogen content of cassava varies greatly between varieties and also with environmental and cultural conditions. The concentration of the cyanogenic glycosides of cassava increases from the center of the tuber outward; in general, the cyanide content is substantially higher in the cassava peel.

Traditional processing techniques practiced in cassava production are known to reduce cyanide in tubers and leaves. Drying is the most ubiquitous processing operation in many tropical countries. Sun drying has been shown to eliminate more cyanide than oven drying and soaking followed by boiling is better than soaking or boiling alone.

Nutritional Bioavailability

Biofortification is the process by which necessary daily micronutrients are delivered to the diet through food crops. Plants are versatile biochemical factories, capable of synthesizing a nearly full complement of essential dietary micronutrients. Regrettably, the plant-based foods most abundantly consumed by at-risk populations contain levels of several micronutrients that are insufficient to meet minimum daily requirements. Furthermore, often these nutrients are unevenly distributed among plant parts exacerbating the problem. Biofortification efforts are directed toward increasing the levels of specific, limiting micronutrients in edible tissues of crops by combining crop management, breeding, and transgenic approaches.

Global economists from the Copenhagen Consensus Center have deemed plant biofortification as one of the preeminent global challenges faced today. Furthermore, the economists predicted tremendous benefits compared to costs associated with developing this technology. Perhaps, the greatest challenge may be to get producers and consumers to accept biofortified crops and increase their intake of the target nutrients. With the advent of good seed systems, the development of markets and products, and demand creation, this can be achieved.

There are numerous examples of biofortification strategies that have been successfully implemented with tuber crops. Zn fertilizers have been used with potato varieties to increase Zn accumulate in their edible portions. Traditionally, white or yellow sweet potato varieties are grown and eaten in Africa, but these provide little, or no, vitamin A. Orange sweet potato has now been conventionally bred, not only to provide more vitamin A but also to be high-yielding and drought-tolerant. Utilizing transgenic approaches, researchers have developed 'golden potatoes' where carotenoid levels of 114 $\mu g\,g^{-1}$ dry weight and beta-carotene of 47 $\mu g\,g^{-1}$ dry weight were obtained. Similarly, it has been suggested biofortifying cassava with provitamin A (converted in the body to vitamin A) could significantly improve nutrition and overall health, especially among poorer communities.

Food scientists have shown that most crops are deficient in one or more essential amino acids that cannot be synthesized *de novo* by humans; for example, legumes tend to lack methionine and cysteine, whereas grains tend to be deficient in lysine and threonine. Plant biologists have used various methods to increase essential amino acids in these plants. For example, research has shown that expression of storage proteins, which contain high levels of desirable amino acids, has raised protein content in potatoes.

Plant calcium transporters within the cell have also been manipulated to increase calcium levels in potatoes by transgenic approaches. Researchers at Texas A&M University have modified potatoes expressing high levels of a deregulated transporter to accumulate almost threefold more calcium in the edible part compared to control plants, without perturbing growth, development, or fertility, under controlled lab conditions (Figure 2).

As mentioned at the onset of this section, traditional breeding approaches will find widespread acceptance and can be used to enhance the nutritional qualities of foods. However, breeding alone will not be an adequate approach because of the limitations of particular plant species.

Summary

Root and tuber crops are calorie- and nutrient-dense foods with a potential to mediate future food insecurities. Additionally, technology is focusing on improving the nutrient content and yield of these crops. Flexible growing requirements in conjunction with the ability to produce in marginal environments afford these crops an important role in feeding the world's poor.

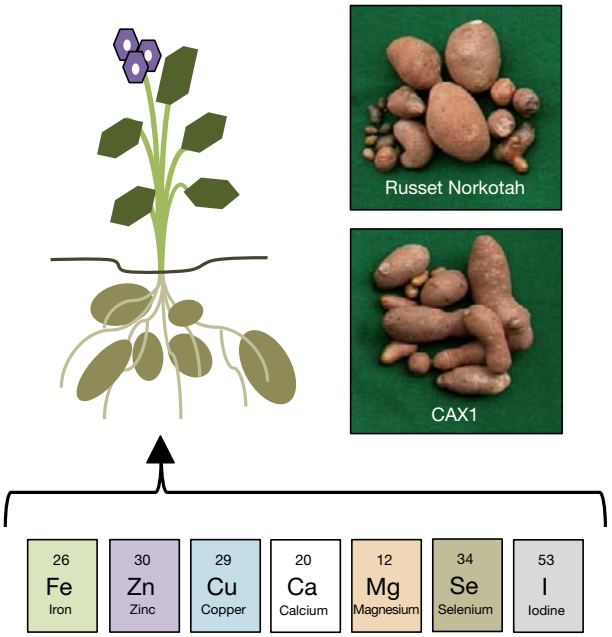

Figure 2 Target nutrients for crop biofortification. Researchers have demonstrated the capacity of Russet Norkotah potato tubers expressing the *Arabidopsis* H^+/Ca^{2+} transporter sCAX1 contain up to threefold more calcium without impacting growth or yield.

See also: Bioavailability of Nutrients; Cassava: The Nature and Uses; Cooking: Domestic Techniques; Functional Foods; Genetically Modified Microorganisms; Organic Food: Effect on Nutrient Composition; Potatoes and Related Crops: Role in the Diet; Potatoes and Related Crops; Salad Crops: Dietary Importance; Salad Crops: Leaf-Type Crops; Salad Crops: Stem-Type Crops; Vegetables of Temperate Climates: Carrot, Parsnip, and Beetroot.

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Salad Crops: Stem-Type Crops

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Among the leaf-stem type of salad crops, celery (*Apium graveolens*) is the most important. Other crops in this group are American wintergreen (*Gaultheria procumbens* L.), asparagus (*Asparagus officinalis*), baby corn (*Zea maize* var. crassa), broccoli (*Brassica oleracea* var. italica), dandelion (*Taraxacium officinale* Wig.), Florence fennel (*Foeniculum officinale* D.C.), globe artichoke (*Cynara scolymus* L.), kohlrabi (*Brassica oleracea* var. gongylodes), oca (*Oxalis tuberosa* Molina), and parsnip (*Pastinaca sativa* L.). These crops are consumed in different forms, especially in temperate countries.

Global Distribution, Commercial Importance, and Varieties

American wintergreen (*Gaultheria procumbens* L.) belongs to Ericaceae family, and Gaultheria was named after Dr Gaultheria, a physician in Quebec (CA) in about 1750. Its tender leaves and stems are consumed widely in America and other Western countries as well as in Asia and Australia.

Asparagus (*Asparagus officinalis*) is native to Europe and Asia. Its varieties produce green and purple spears. Forcing the plants with the exclusion of light produces white spears, which are very popular in Europe. Green asparagus is commonly canned and is most popular in the UK.

Baby corn (*Zea mays* var. saccharata or rugosa), a form of maize, is an important and popular vegetable, especially in the United States. Maize is an ancient crop, as revealed by cob remnants dating to about 5000 BC found at the Tehuacan cave excavations in Mexico; domestication is estimated to have begun about that time. Baby corn is most popular in Western countries, where it is imported from developing countries—mainly Thailand.

Broccoli (*Brassica oleracea* var. italica) is usually harvested when the curd is compact and immature. The name broccoli refers to the young shoots that develop in some species of the genus *Brassica* (*brocco* is an Italian word for shoot). Broccoli is believed to be the ancestor of cauliflower. Broccoli is gradually becoming a popular vegetable in other parts of the world. A favorite of Americans, broccoli is of commercial importance to the United States. A large portion of the United States' yield goes to the freezing industry. The top broccoli-producing states are California, Arizona, and Texas. In the US, it is also known as Italian broccoli, pointing to its origin. Italian green sprouting and calabrese are its most popular varieties. Strains of Italian green sprouting (midway, green mountain and grand central) are the early and medium-maturing type, which are dark bluish green and produce large compact heads and lateral shoots after the central head is cut. The cultivar De Cicco is light green in color and produces abundant lateral shoots; it is the main winter broccoli grown in Texas.

Celery (*Apium graveolens* L.) comprises three distinct cultivated varieties. They are grown in different parts of the world for different economic purposes. Based on the botanical part of the plant consumed, these varieties are classified as:

- *Apium graveolens var. dulce, common name celery*: develops succulent, solid petioles that are used mostly raw as salad
- *Apium graveolons var. rapaceum, common name celeriac or root celery*: characterized by the development of hypocotyls, or root tissue, and mostly consumed cooked in stews and soups or grated on salad
- *Apium graveolens var. secalinum, the common name smallage or leaf celery*: leafy, slender and often hollow petioles, its leaves are used as a condiment, garnish and for medicinal purposes.

The genus *Apium* is spread worldwide, particularly in the Mediterranean basin, Australia, New Zealand, South Africa, and South America. Among 14 species of *Apium*, graveolens is the only one to be cultivated. Argentina and Chile are the countries that are richest in *Apium* species. Celery is important in Europe and America. Its habitat extends southwards from Sweden to Algeria, Egypt, and Abyssinia. In Asia, the habitat is from the Caucasus (Bluchistan) to the mountains of India. Wild celery can be found in damp or marshy areas of various European countries, Egypt, and parts of Asia. It was first used as a medicinal plant and as food in AD 1500. Homer's *Odyssey* mentions celery grown in 850 BC, but this early form (later called smallage) was a leafy, pungent, and bitter plant used exclusively in medicines. Celery was demonstrated as a vegetable in Italy in the sixteenth century. At this time, the first report of the three horticultural varieties appeared. Selection for solid and succulent petioles resulted in celery types, whereas selection for enlarged hypocotyls gave rise to celeriac type. Celery is a popular crop widely cultivated throughout the world. The use of celery began in the eighteenth century and its use as a vegetable in the United States was recorded in 1806. It is used in mixed salads or as an appetizer. It is often stuffed with cream cheese or peanut butter. The petioles are sometimes cut up and served as a cooked vegetable, although its use is declining. In the US, a large part of the celery crop is consumed in its raw state; however, a considerable quantity is used in vegetable juices, soups and stews, and as a cooked vegetable. In the 18th century, two varieties of solid stem Italian celery were available in France, that is, solid celery and striped rose. In 1875, Chemin selected a line known as solid golden white celery, which became the variety Paris golden yellow—a self-blanching type. In 1884, Pascal selected a green celery line from this variety, which is known in the seed trade as Pascal celery. In 1887, both varieties were introduced to North America under the names white plume (the self-blanching type) and giant Pascal (the green type).

The word dandelion comes from the French phrase *Dent de Lion*, meaning lion's tooth—reflecting the jagged shape of the leaf. It is a great favorite in Europe and to a lesser extent in the United States. Previously, it was considered a noxious weed,

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but cultivated species are now available. The cultivated dandelion has been developed from the wild species (*Taraxacum officinale*), a member of the Compositae family. Its leaves are used raw in salads or cooked as greens. The flowers can be fermented to produce wine and the roots may be used as a coffee adulterant similar to chicory roots.

Fennel is produced commercially in the United States. Most fennel seeds are imported from India, Argentina, and Bulgaria. The volatile oil (anethole constituent) in fennel seed is an adaptable flavoring agent used in the preparation of pickles, perfumes, soaps, liquors, cough drops, and chicory candy. Florence fennel (*Foeniculum officinale*), a leaf-stem type salad crop, is native to southern Europe and resembles wild fennel. The Carosella, extensively used in Naples and scarcely known in any other place, is referred to by authors as *Foeniculum piperitum* D.C. It is closely related to *Foeniculum officinale*. The plant is used while running to bloom. The fresh and tender stems are served raw and are still enclosed in the expanded leaf stalks. It is only available from the end of March until June. Italian fennel (*Foeniculum dulce*), also called finnochio, is a short-lived perennial that is cultivated as an annual. The leaf base becomes enlarged and bulb-like and, when blanched, has the texture of celery. It is used primarily for flavoring.

The globe artichoke (*Cynara scolymus* L., $2n = 2x = 34$), a member of *Asteraceae* (compositae) family and *Cynarae* tribe, differs from the Jerusalem artichoke (*Helianthus tuberosus* L., $2n = 102$), which is an important vegetable in Mediterranean countries, where 90% of the world's artichokes are produced. Italy, Spain, France, and Argentina are the main producers of the globe artichoke. The genus *Cyanara* is very small, with only three recognized species. They are:

- *Cyanara cardunculus* L. var. sylvestris Lam (the wild cardoon), widely spread in the western Mediterranean basin (Spain, Morocco, Algeria, Tunisia, and Sicily),
- *C. sibthorpiana* Boiss and Heldr, located in the central part (the Peloponnese, Aegean Islands, and Cyrenaica), and
- *C. syiaca* Boiss, found in the eastern part (Palestine).

Jerusalem artichokes belong to the same family, but they bear tubers and are native to North America. They are a herbaceous-perennial but, again, are usually grown as an annual.

Kohlrabi (*Brassica oleracca* var. gongylodes), a member of Brassicaceae (cruciferae) family, is a biennial herbaceous vegetable crop grown as an annual. The kohlrabi produces a swollen bulb-like stem. It was described by writers in ancient Rome and was developed in northern Europe during the fifteenth century. It is a minor crop grown in temperate countries and in some subtropical areas. The tubers (swollen stems), which develop above the ground, may be due to secondary growth. The tuber is harvested before it becomes tough or woody.

Oca (*Oxalis tuberosa* Molina) belongs to the family Oxaliolaceae. It is an ancient crop cultivated by pre-Columbian people in the Andean highlands. Commercial production occurs in Mexico, Central America, and the South Island of New Zealand.

Parsnip (*Pastinaca sativa* L., $2n = 2x = 22$), a biennial crop, is native to Europe and western Asia. It is cultivated for its large and fleshy taproot. It was introduced to the West Indies in 1564, but it only grows in the tropics at high altitudes. The Romans and Greeks used it for medicinal purposes and for food in ancient times. It belongs to the family Umbelliferae (*Apiaceae*).

Rhubarb (*Rheum rhaponticum*) is an early and high vegetable crop well suited for cultivation in cool regions. UK, the Netherlands, Germany, the Nordic-countries, Canada, and the US cultivate the most rhubarb. It is a minor commercial crop grown in small areas, but it is very common in kitchen gardens. It is a long-lived perennial plant with edible stalks, and is native to Siberia.

Morphology and Taxonomy of Edible Part

American winter green plants are prostrate, with their creeping stems giving rise to erect branches (15 cm high and leafy at the top). The lower leaves are alternate and serrated with short petioles. Their upper leaves are oval, 2.5–5 cm long, apiculate, glaucous, and shiny. The flowers are solitary, or in racemes or panicles, and are scarlet in color.

The edible portion of asparagus is the spear, which develops from the crown. Green asparagus, which is the greater portion of crop, is cut 2.5 or 5.0 cm below the soil surface. Its spears are harvested before the excessive elongation occurs. Internode lengthening and loss of tip compactness and tenderness reduce quality. Blanched spear harvesting is more difficult. Spears are harvested when the tip is about to emerge or just protrudes through the soil surface. Even a slight amount of tip greening diminishes its perceived market quality.

Baby corn is an herbaceous monocot and a warm season annual. Plants are monoecious with male flowers borne as the terminal inflorescence (tassels) on the main stem. Female flowers are borne separately as a lateral inflorescence (ear) developing at a leaf axil. Plants produce one to several ears. Baby corn is the young ear of a maize plant harvested before fertilization, when silks have just emerged. Husked, light-yellow colored, and tender ears of baby corn are nutritious. It is an ingredient used in soup, vegetable biryani, mixed vegetables, Chinese food, and an array of others dishes. Crispy vegetable salad and pickles made from baby corn are also common. It provides diversity because of its texture and flavor, in addition to its significant nutritional value. Tender ears canned in 2% citric acid are exported to the US and European countries. Its recognized cultivars are Prakash and Composite Kesari.

Broccoli (*Brassica oleracea* var. italic) forms a head consisting of green buds and thick fleshy flower stalks. The terminal head is rather loose and green in color, and the flower stalks are longer than those of cauliflower. The internodes are long and the plant produces axillary flowering shoots in addition to the terminal inflorescence. The color of the flower buds, forming the edible portion, varies from white to green—depending on the cultivar. The inflorescence is made of fully differentiated flower buds. It is considered a good quality curd if these reach the open stage. The sprouts in the leaf axils develop sturdily, especially after removal of the terminal head. Both the terminal head and the sprouts with bud clusters are consumed as food.

Celery, a member of Apiaceae (Umbelliferae) family, is characterized by enlarged, tender, edible petioles, or leaf stalks.

The petioles are broad, with sheathing bases. It is biennial, but a few strains and related wild species are annual. The plants will bolt when subjected to chilling temperatures, producing a seed stalk and making them unmarketable. The most common form bears 5–12 thick petioles in a tight bunch. A less popular form is turnip-rooted celery, known as celeriac (var. rapaceum). It is a biennial cool season crop, but it is sensitive to prolonged chilling temperatures. Growth through the first year normally produces a tight cluster of petioles and leaves attached to a very compressed stem. Its leaves are divided, pinnatified, and smooth with almost triangular, toothed leaflets that are a dark green color. The leaf stalk is broad, furrowed, and concave on the inside. The stem, which appears in second year, is furrowed and branching. Early vegetative growth spreads, but the leaves arising from the apex of the short stem produce compact and elongated heads. The root system of celery is spread out and fibrous with many feeder roots close to the soil surface. The plant produces compound clusters (umbels) of small, white, perfect flowers with five petals and five stamens. The seeds (fruits formed from two compressed carpels enclosing the actual seed) are very small.

Dandelions produce leaves, which are deeply toothed, in a basal tuft and a single, composite, yellow flower atop a hollow stalk. Its cultivated forms are larger and leafier than the weedy forms. Improved cultivars (mostly of French origin) are larger, tenderer, less bitter, and slightly greener than the wild types.

Florence fennel is an annual, very distinct, low growing, and thickset plant with a very stout stem that has joints close together toward the base. The leaves are very finely cut, and green. The leaf stalks are very broad and have a whitish green hue. They overlap one another at the base of the stem, the whole forming a head, or enlarged areas that vary in size (the inside is white and sweet). The height of the plant, even when grown for seed, does not exceed 60–75 cm. The flowers are greenish and are in a broad umbel of stout, stiff rays. The seed, twice as long as it is wide, is flat on one side and convex on the other. It is traversed by five thick, yellowish ribs, which almost occupy the entire surface of the skin.

In Globe artichokes, the edible organ is the young capitulum, which is harvested when it is still in rapid growth. The enlarged receptacle (bottom) and the tender thickened base of the bracts are eaten. If the capitulum is picked at an early stage, the heart (after removal of tough outer bracts), the receptacle (lower part of inner bracts), and the undeveloped choke (immature florets, bristles, and pappus) are eaten. The stem just below the young head is also edible. In Jerusalem artichokes, the underground tubers can be oblong or elongated and weigh 80–120 g.

Kohlrabi (*Brassica oleracea* var. gongylodes) is often called turnip-rooted cabbage and is closely related to cabbage. The stem, which is edible, is enlarged immediately above ground and is an excellent vegetable if used before it becomes woody, tough, and stringy. The most popular varieties of kohlrabi in India are white Vienna, green Vienna, purple Vienna, and earliest ear furt.

The compact and bushy growth of oca (perennial) is between 20 and 30 cm in height. Tubers can be eaten immediately after harvest, but the flavor is better after they have been exposed to the sun (reduced bitterness). Oca is consumed after boiling, baking, frying, pickling, or uncooked. Leaves and young shoots are eaten in salads or as cooked vegetable.

Chemical and Nutritional Composition

The artichoke has interesting nutritional characteristics related to its high content of phenolic compounds, that is, flavonoids, inulin, fiber and mineral salts, which are derivatives of caffeic acid. Extracts containing cynarin (1,5-dicatteoyl quinic acid) affect hepatobiliary disease, hyperlipidaemia, and cholesterol metabolism. The pharmaceutical industry mainly uses the leaves. Baby corn is a good source of proteins and lipids, and is a source of pro-vitamin A (beta-carotene).

Celery is an important source of minerals, vitamins, and amino acids. Celery leaves are also characterized by higher levels of vitamin C, carotene, and essential oils. Many traits, such as stalk and petiole shape and color, crispness, pithiness, stringiness, how ribby it is, petiole cracking, and flavor contribute to quality. Having a low content of fat, celery is popular in the Western world. Of 100 g of celery, around 71 g is edible, made up of moisture (88 g), protein (6.3 g), minerals (2.1 g), carbohydrates (1.6 g) and fiber (1.4 g). It also contains energy (37 kcal), calcium (230 mg), phosphorus (140 mg), sodium (126 mg), iron (6.3 mg), carotene (3990 μg), vitamin A (240 IU), thiamine (0.9 mg), vitamin C (0.62 mg), niacin (1.2 mg), and riboflavin (0.11 mg). The composition varies at different stages of maturity, and total sugars show a marked decline in the leaf blades from harvest time to the end of storage. This decrease is accompanied by increased concentrations in petioles. Soluble nitrogen increases in leaf blades and petioles towards the end of storage.

Hardly any information is available, with respect to nutritional composition, for Florence fennel and American winter green vegetables. However, it is reported that dandelion leaves are an excellent source of vitamin A (14 000 IU per 100 g), vitamin C (35 mg per 100 g) and calcium (187 mg per 100 g edible part). Oca nutrition qualities are comparable to the potato; on a dry weight basis, about 85% of the tuber is carbohydrates and the starch is of good quality.

Post-Harvest Handling and Storage

Harvest maturity of asparagus is usually reached when the spears are about 12.5–20 cm above the ground. They should be cut before they are too long and the tips begin to spread. The spears should be harvested when they are rounded, and before they become ribby. Spears with open tips decay more rapidly, toughen, and are unattractive. Selective harvesting of asparagus is advocated since new shoots appear daily over a period of 6–10 weeks. Straight spears, green-tinged with purple on the bracts and with tightly closed tips, are preferred. Asparagus with white butts are less perishable than all green asparagus. Asparagus loses freshness and quality rapidly after harvest, meaning it is desirable to market or process it as soon as possible. The instant cooling of spears helps to maintain quality and considerably improves marketability. Hydro cooling can be used to reduce spear temperature rapidly. Generally,

chlorine is added to the water for cooling to reduce bacterial populations. The spears should be stored at low temperatures. At high temperatures, rapid elongation and senescence will occur; ethylene exposure also causes senescence. The loss of total sugars is pronounced at higher temperatures. In addition to general deterioration growth, loss of tenderness, flavor, and vitamin C, and decay take place at moderately high temperatures. An increased rate of fiber development is directly correlated with spear length and age, and with high post-harvest temperatures. Asparagus is packed in plastic film bags or crates without bunching. Butts are cut off and the spears are put in boxes lined with oily paper with a layer of wet moss at the bottom. Packing only spears with tightly closed tips can extend the shelf life. Packing in film bags maintains market quality by reducing moisture loss, keeps the edible quality by preventing fiber formation, and sustains the nutritional value by reducing ascorbic acid losses. However, impermeable film bags can produce off-flavors due to anaerobic decomposition. Such packaging leads to uncontrolled accumulation of CO_2 and depletion of O_2 with possible injury to the produce. The film must be sufficiently permeable to permit exchange of CO_2 and O_2 to dissipate the respiratory heat. Asparagus, if placed horizontally, will exhibit negative geotropism with the tips turning upward. Specially shaped containers, which are wide at the base and narrow at top, are often used to accommodate long tapered spears. High humidity is essential to preventing desiccation, particularly at the ends. Humidity is obtained by placing the butts on wet pads. Pre-packaging spears in perforated films can also obtain a high relative humidity. Spears continue to elongate after harvest, and headspace is provided to avoid tip damage. Asparagus is highly perishable and deteriorates rapidly at temperatures above 5 °C. Storage conditions at 0 °C with 95% relative humidity are recommended to extend the marketable life of asparagus to about 3–4 weeks, but it is subjected to chilling injury when held for longer at this temperature. The recommended handling temperature is 2 °C with the relative humidity greater than 95%. Chilling damage occurs at temperatures lower than 2 °C for prolonged periods. Controlled atmosphere storage of asparagus is known to reduce post-harvest deterioration because it prevents the development of off-flavor, water and chlorophyll losses, and toughening. Exposure of spears to CO_2 $7 \pm 2\%$ and O_2 2% for 24 h retards bacterial rot. No chilling injury is observed at temperatures of 1.7 °C and 15% CO_2 level. Controlled atmosphere storage of asparagus has three major advantages:

(i) the reduction of decay,
(ii) the retardation or even reversal of toughening, and
(iii) a more desirable color.

It provides a useful technique not only to retain a high quality in fresh asparagus, but also to improve the quality of the processed product. Controlled atmosphere stored spears retain more sugar, organic acids, and soluble proteins than spears stored in air. Shelf life of several leafy vegetables including asparagus has been reported doubled by immersing the cut ends, or dipping the whole spear, in solutions of 2-chloroethyl trimethylammonium chloride (CCC or cycocel).

Broccoli is harvested shortly before the buds begin to open while their heads remain compact and cut to a length of about 20–25 cm. The axillary shoots, as they develop, are cut at the same stage as the central head. The broccoli heads are further trimmed to a length of about 15 cm, and bunches weighing about 700 g are tied together for packing. Bunches are packed in crates that can hold up to 28–30 bunches each. The broccoli buds continue to develop and the flowers begin to open or turn yellow. Bunches are hydro cooled to 4.4 °C, packed with ice, and stored in refrigerated conditions. The respiration rate of freshly harvested broccoli is high compared with that of asparagus or sweet corn. Therefore, broccoli must be cooled soon after harvest to reduce the respiration rate and it must be stored at low temperatures in an oxygen-free environment to prolonged shelf life. Broccoli should be hydro-cooled or packed in ice immediately after harvest and kept at 0 °C to maintain good marketable conditions. Film wraps can be beneficial in maintaining high relative humidity and extending storage life.

There is no definitive stage of maturity at which celery should be harvested for satisfactory quality. Celery plants are cut off below the soil surface. The plants should be moved from field quickly because they wilt easily. Stripping to remove suckers and diseased and damaged leaves, washing, grading, packing, and pre-cooling are done for marketing. It is advisable to store celery near 0 °C to avoid decay. To avoid wilting, packaging in ventilated wraps is advantageous. Box or crate liners should have about one hole per square foot. Celery can be stored at temperatures between 0 and 0.6 °C with relative humidity at 90–95% for about 8 weeks. In controlled atmosphere storage, the oxygen level of $<5\%$ retards yellowing of celery only slightly and $CO_2 > 5\%$ may be damaging. Therefore, all wraps should be perforated to avoid potentially dangerous accumulation of CO_2 or the reduction in CO_2 levels to $<1\%$. Celery in storage absorbs foreign flavor. Hence, it should be kept away from the odor of other produce.

Dandelion plants are trimmed, washed, and stored in a container at a low temperature (0 °C) and high humidity (90–95%) to preserve their quality. Similar conditions may also be used for other crops.

Florence fennel plants are harvested just above the taproot. Upper petiole and leaf blades are trimmed relatively close to the bulb. Fennel has good post-harvest storage quality if maintained at a temperature of 0 °C with high relative humidity.

Globe artichoke flower buds require careful handling to avoid bruising and desiccation. The harvested buds are put in suitable containers to be emptied into bulk bins for transport to the packing shed, where they are graded according to their size and quality. Following harvest, the artichoke buds must be pre-cooled as quickly as possible to retain their quality. Hydro cooling is effective for both cooling and cleaning, but hydro- and room-cooling to 4.4 °C (within 24 h) are used widely. When storage is required, conditions providing temperatures of 0–2 °C and a relative humidity of 95% can maintain good quality for as long as 10 days. Globe artichokes are packed in different layers in paper-lined boxes. For prolonged storage, temperatures close to 0 °C are recommended, although 1.7–2.8 °C is satisfactory for normal marketing periods. Decay can be prevented effectively by holding artichokes below a temperature of 4.4 °C. Gentle handling aids in maintaining high quality because physical injury can accelerate decay as much as a 10 °C rise in temperature. Injured and decayed buds at harvest deteriorate 1.5 times more rapidly than sound buds, especially when they are held at 4.4 °C or

above. Wilting can be prevented by hydro cooling or washing the bracts in cool water.

Tubers of kohlrabi can be kept in good condition for 2–3 months at 0 °C and 98–100% relative humidity. High humidity is advocated to prevent shriveling and toughening. Packaging in perforated film can be used to reduce moisture loss. Kohlrabi with attached leaves has a storage life of only 2 weeks at 0 °C temperature.

Ploughing or hand-digging are used to harvest parsnip. Foliage is removed before lifting the roots. Harvesting is often delayed until lower temperatures have occurred, because root quality is improved under such conditions. With advancing maturity, the starch in roots is converted to sugars, and this is accelerated by exposure to cold temperatures. Parsnip can be stored below 0 °C with greater than 90% relative humidity. Since the roots easily desiccate, high humidity is important for extended storage. As with carrots, parsnips exposed to ethylene during storage become bitter. Oca storage characteristics are relatively good if they are properly cured, and moisture loss is minimized.

See also: Salad Crops: Dietary Importance; Salad Crops: Leaf-Type Crops; Salad Crops: Root, Bulb, and Tuber Crops.

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Salmonella: Detection

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Introduction

The genus *Salmonella* is a member of the Enterobacteriaceae family of enteric bacteria. They are a large group of closely related rod-shaped, Gram-negative, facultatively anaerobic bacteria, many of which are known to be potent human pathogens. The nomenclature of the *Salmonella* genus is complex and has undergone many revisions. In its current state, there are two species, namely, *Salmonella enterica* and *S. bongori*. Most pathogenic *Salmonella* belong to the species *S. enterica*, which is further divided into subspecies *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI). The subspecies themselves can be divided into serovars of which there are more than 2500. Typically, the names of *Salmonella* serotypes are abbreviated and so, for example, *S. enterica* subspecies *enterica* serovar typhimurium becomes *S.* typhimurium. *Salmonella* can grow in a temperature range of 6–46 °C with an optimum of 32–37 °C and in a pH range of 4–9.5 with an optimum of 6.5–7.5.

Salmonella is of significant worldwide concern as a human pathogen and results in numerous deaths and cases of severe disease annually. Illness in humans caused by pathogenic *Salmonella* is generally referred to as salmonellosis. Symptoms of the disease include watery diarrhea, nausea, abdominal pain, fever, and headaches. Complications can occur, and in some cases, *Salmonella* may invade the body and become systemic. The elderly, very young, and immunocompromised are at higher risk of complications and mortality from the disease. *Salmonella* generally infect individuals by the fecal–oral route with animals or animal products being the most common source of infection. *Salmonella* is routinely encountered in the gastrointestinal tracts of a wide range of animals and does not generally cause disease in them. Sporadic cases and outbreaks of salmonellosis are commonly associated with variety of foods including those of animal origin such as poultry, beef, eggs, pork, and milk. In addition, foods such as fresh produce, which are indirectly contaminated with *Salmonella*, also cause a substantial number of cases of disease.

The ability to detect the presence of *Salmonella* in food and in the food chain is important to verify process controls put in place by the industry. The presence or absence of *Salmonella* (as determined by growth and isolation on culture media) in a given amount of food (often 25 g) has traditionally been used as a criterion for this. The effectiveness of this approach is limited by a number of factors most notably (1) the great diversity of *Salmonella* described earlier, which makes it difficult to provide media suitable for recovery of them all; (2) the presence of sublethally injured *Salmonella* on foods, which require the inclusion of recovery steps; (3) the presence of *Salmonella* at levels too low for detection by these methods resulting in the requirement for selective enrichment steps; and (4) the presence of competing bacteria with similar characteristics, which makes it difficult to easily separate and visualize the *Salmonella*. Developments in detection technologies based on immunology, molecular biology, and other approaches have resulted in the introduction of techniques that overcome some of these problems. Implementation and uptake of these techniques is, however, relatively slow at both a regulatory level and an industry level. Factors that have slowed this uptake include the need to verify the efficacy of the new methods for regulatory approval, the cost of implementing the new technologies, and concern from industry over detection of significantly larger numbers of samples. The advantages of the new technologies are, however, becoming more apparent and their use is likely to become more widespread as time progresses. The range of different approaches used for the detection of *Salmonella* in foods, including their relative advantages and disadvantages, are described in the succeeding text.

Culture-Based Methods

Detection of *Salmonella* in food and food environments is still most widely achieved using culture-based methods. These methods may vary significantly with respect to the media and protocols used depending on the jurisdiction in which they take place. All methods require lengthy incubation times and the entire process generally takes from 5 to 7 days. The exact media and protocols employed in these methods vary, but the major steps in the detection process tend to be very similar in most cases (Figure 1).

Typically all culture-based methods initially entail taking a portion of food and subjecting it to a nonselective preenrichment step. This step allows any *Salmonella* present to recover from injury sustained during processing. The preenrichment step usually involves incubation of the food at suitable growth temperatures (usually ~37 °C) and time (usually 18–24 h) in a relatively rich medium (such as buffered peptone water). The number of *Salmonella* (and other bacteria) present in the medium increases during this process, making a higher number available for subsequent selective isolation. The importance of this step for culture-based and other detection methods that involve preenrichment is critical due to an increasing association of *Salmonella* outbreaks with foods of low water activity, such as spices and peanut butter. *Salmonella* survive well in these environments for a non-spore-forming Gram-negative bacterial genus but are likely to experience a higher degree of stress and sublethal injury than in some other foods. In addition and for the same reason, they may be present in lower numbers in these foods than in foods with a higher water activity. To mitigate these issues, methods used to detect *Salmonella* in low-water-activity foods may include a soaking step (to reduce the shock of sudden hydration), longer preenrichment times (to allow better recovery from stress), or the use of larger sample sizes (to increase overall numbers of *Salmonella*). The choice of method needs to be specific to the

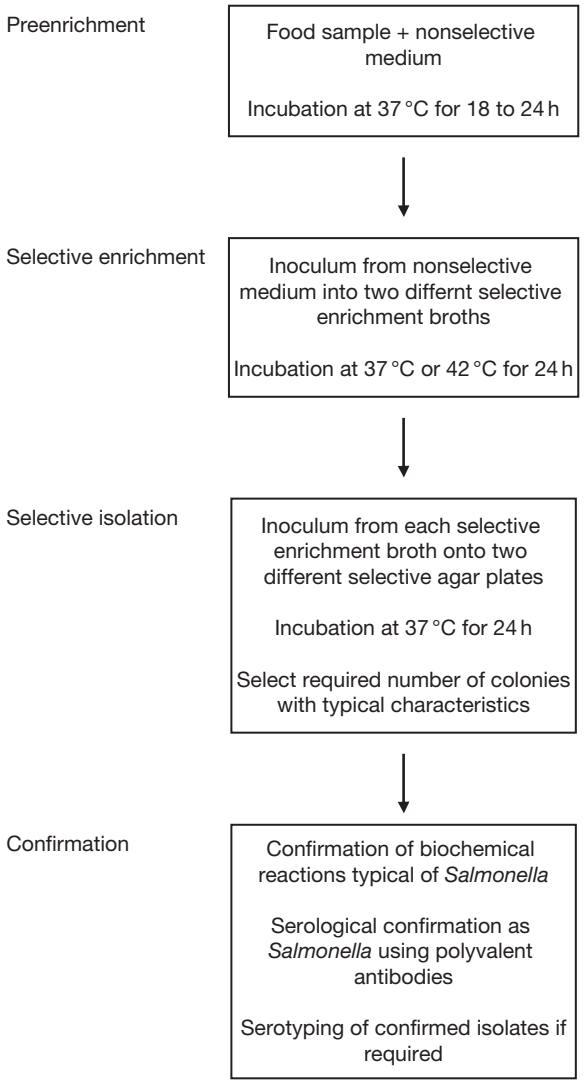

Figure 1 Generic scheme for detection of *Salmonella* in foods using culture-based methods.

product being examined as individual products have different properties and have been prepared using different stress-inducing methods. This is an active area of research, and better protocols for low-water-activity foods are likely to be developed in the near future.

Subsequent to the preenrichment step-selective primary enrichment for *Salmonella* is undertaken. This step is designed to specifically allow the growth of *Salmonella* while suppressing the growth of other bacteria that may otherwise be present in much higher numbers. Without selective enrichment, these other bacteria may either outcompete or simply mask the presence of *Salmonella* present in the sample. There are numerous enrichment broths available and these include Rappaport Vassiliadis soya broth and tetrathionate broth, to name but a few. Most protocols require the inoculation of more than one broth type from the preenrichment. This is done to assure that specific *Salmonella* strains that may be sensitive to the selective components in one type of broth have the opportunity to grow in the other. The enrichment broths are incubated (usually at 37 or 42 °C and for 18–24 h), and secondary enrichment and isolation of presumptive *Salmonella* on selective agar follow.

In the secondary enrichment step, the primary enrichment cultures are inoculated onto *Salmonella*-selective agars such as xyline lysine deoxycholate (XLD) agar, bismuth sulfite agar, or brilliant green agar, to name but a few. Incubation conditions for the secondary enrichments are generally similar to those of the primary enrichments. Most protocols require that more than one agar type is inoculated from each of the primary enrichment broths, once gain to ensure that all *Salmonella* strains present are able to grow and be differentiated from other bacteria. Based on the number of primary and secondary enrichment media available, a wide variety of combinations are possible. Some protocols are completely prescriptive but most specify media of one of each enrichment type and allow some choice with the second. Colonies displaying typical morphology and characteristics for *Salmonella* on the particular media (e.g., red yellow with black centers on XLD) are selected and isolated, generally on a nonselective medium. These colonies are subsequently taken through a battery test to confirm that they are in fact *Salmonella*. This step is necessary as other bacterial species may display the same characteristics as *Salmonella* on most media and if misidentified can result in false-positive tests being reported.

Confirmation is traditionally achieved by taking presumptive bacterial isolates from the secondary selective enrichment through a series of key biochemical and serological tests. The most common biochemical tests are for the production of H_2S (+) and indole (−), glucose and dulcitol fermentation (+), urease reaction (−), and the presence of lysine decarboxylase (+). The presumptive strains are also tested for agglutination with polyvalent H and O antisera. The correct biochemical and serological outcomes are sufficient to confirm the isolate as a *Salmonella*. Further characterization of the isolate to subspecies, strain, and serovar levels may be carried out if required and is generally done by specialist reference laboratories that have the necessary sera and equipment.

Ways to increase the speed and accuracy of the culture-based protocols described earlier have been developed. These are generally methods designed to amend or replace one of the steps described earlier. One of these is the use of immunomagnetic separation after the preenrichment stage. This technique uses magnetic beads coated in *Salmonella*-specific antibodies to trap, separate, and concentrate *Salmonella* in the medium. The beads can then be plated on the secondary enrichment-selective agars eliminating the need for the primary selective enrichment and reducing the time to complete the protocol. Other techniques that can be incorporated into the protocols include the use of miniaturized biochemical test kits to eliminate the preparation involved in the individual test required for confirmation. In addition, chromogenic agars based on unique characteristics of *Salmonella* can be used for more definitive and accurate isolation of these bacteria. While useful new methods add to the diversity of options available for the isolation and culture of *Salmonella*, establishing a clear and consistent approach to achieving this becomes more difficult.

The entire process described earlier is time-consuming and labor-intensive. For the food industry, this may increase costs and reduce productivity. In addition, food companies may face

requirements for different culture-based *Salmonella* testing protocols from different markets, particularly if they export. While many jurisdictions require (through standards) testing approaches to be followed in a very prescriptive way, others allow for leeway, which may speed up the process slightly. Attempts at harmonizing culture-based testing methods for *Salmonella* have been made, for example, through national and international standard setting bodies, and this has improved the situation for companies to some degree. There are a range of new and more rapid and accurate methods to detect *Salmonella* in food and food-related environments being developed, and the speedy dissemination of these into regulation should further reduce the burden on industry.

Nucleic Acid-Based Methods

Advances in the understanding and application of molecular techniques based on the specificity of nucleic acid (and most commonly DNA) sequences have resulted in the development of a variety of methods for the detection of *Salmonella* in food and food-related environments. These methods are generally much more rapid than the culture-based techniques described earlier as they are more sensitive and specific than them and therefore able to detect fewer cells of *Salmonella*. This reduces or eliminates the need for extensive enrichment procedures. These techniques were initially labor-intensive and very expensive, but numerous commercial platforms are now available, which means they are accessible for everyday use by the food industry. Despite their strong potential, nucleic acid-based techniques do suffer from a number of drawbacks depending on the method use. These include a higher rate of false-positive results, the inability of most techniques to differentiate between live and dead *Salmonella* in the sample being tested, and the inability of some methods to quantify *Salmonella* in the sample in cases where this is required. Solutions to these problems are being worked on, and future developments in this are likely to overcome these making the use nucleic acid-based techniques more powerful.

Most of the nucleic acid-based methods used are based on the polymerase chain reaction (PCR), which is a technique for amplifying nucleic acid. The exact protocols employed in these methods vary, but the major steps in the detection process tend to be very similar in most cases (Figure 2). Put simply, this method uses short pieces of added nucleic acid (primers) to identify and bind to a specific nucleic acid sequence in particular bacteria (e.g., *Salmonella*) present in the sample being analyzed. These primers, in the presence of an enzyme and under the influence of cycling temperatures, allow amplification of the target sequence to very high numbers. The amplified target is visualized; this is regarded as evidence of the presence of target sequence and by inference the bacteria of interest in the sample. The target sequence must be specific to the bacteria of interest and not appear in closely related bacteria. A good knowledge of the genetics of the bacteria of interest is therefore required. In the case of *Salmonella*, a number of genes have been used for this purpose, and these have, for example, included the gene encoding fimbrial subunits, a gene involved in host invasion, and a gene involved in virulence. It is a likely that other targets will be developed as our understanding of the molecular biology of *Salmonella* increases. In most cases, PCR-based techniques still require some enrichment (usually nonspecific) to boost numbers of *Salmonella* to levels at which detection of the target nucleic acid is easier. In addition, the target nucleic acid must be purified from the sample. This is generally easily achieved using commercial kits designed for this purpose. Depending on the food or other matrices that make up the sample to be examined, some of which may interfere with the PCR process, further purification steps may be required. Once enriched, however, PCR-based techniques allow confirmation of the presence of *Salmonella* in a sample in a matter of hours to a day, reducing the time required by 2–3 days as compared to conventional culture-based techniques. Using preenrichment, some PCR-based techniques are able to detect as few as 4 *Salmonella* cells in 25 g of sample, which is a very high level of sensitivity. At the end of this period, no bacterial isolate is available for further examination, however, and if this is required (e.g., if the serovar needs to be established), isolation from the sample by culture-based methods may still take place.

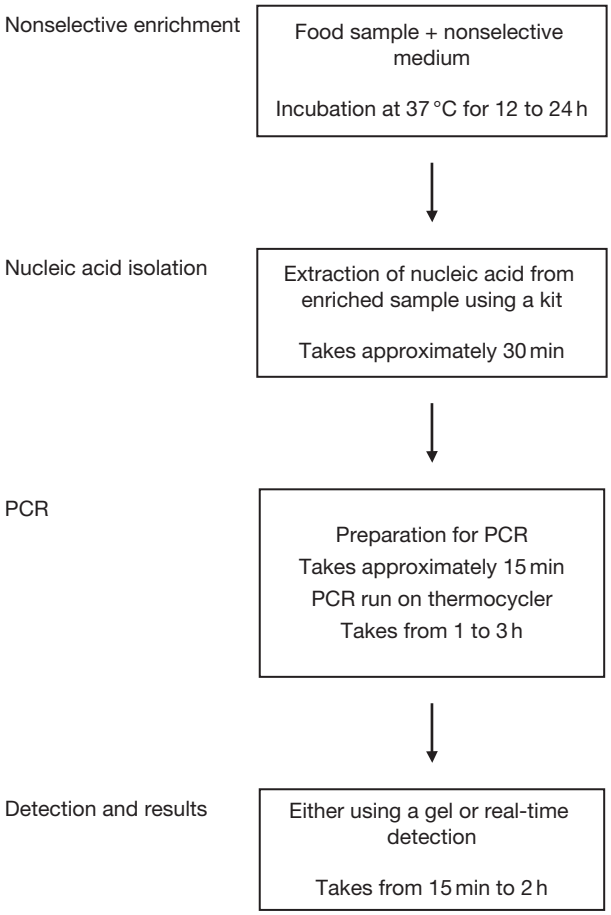

Figure 2 Generic scheme for detection of *Salmonella* in foods using nucleic acid-based (PCR) methods.

Traditionally, PCR products have been detected by separating them on polyacrylamide gels. This method is time-consuming and most commercial platforms for detection of *Salmonella* now use a modified PCR technique, called real-time

PCR, which eliminates the need for this step. This method entails the use of various fluorescent dyes that are incorporated in the PCR product as the PCR progresses. This incorporation is monitored in real time and allows the identification of positive samples in a shortened time and without the need for a second step. The inclusion of appropriate controls is very critical for this technique, but it has, together with the introduction of a kit format, made nucleic acid-based detection of *Salmonella* accessible and practical for most food testing laboratories, even those in small to medium enterprises. As indicated earlier, in some cases, for example, where the risks associated with particular foods are under investigation, quantification of *Salmonella* present in a food sample is required. A version of real-time PCR called quantitative PCR has been used to overcome this issue. For this technique, the real-time PCR platform is used on samples, generally without enrichment, but is also calibrated against series of controls with known quantities of bacterial template present in them. A standard curve established allows an accurate estimation of the numbers of *Salmonella* (or *Salmonella* templates) in a sample. This technique tends to be accurate only within a certain range of values and is less accurate when the number of *Salmonella* in the samples is lower.

One of the issues associated with many of the techniques using nucleic acid is the inability to differentiate whether the DNA present is from live bacteria or bacteria that may have died, for example, during processing, and no longer represent a risk to human health. This could result in the reporting of false-positive results, and possibly recalls, and imposes a cost burden on the industry. In order to overcome this issue, a specific PCR technique called reverse transcriptase PCR has potential. This technique entails converting messenger RNA (mRNA) into complimentary DNA (cDNA) and amplifying the resulting cDNA. The principle of this technique is based on the fact that mRNA only remains viable for a very short period of time and therefore will only be present in a sample if live cells are present. This technique has been applied to detect *Salmonella* in various matrices, but correlation between bacterial counts and this method remain poor. While not currently a commercial option, this method has substantial future potential.

Detection of the presence of *Salmonella* using specific DNA sequences is also possible without using PCR. Techniques of this type often entail the use of specific sequences of DNA labeled with fluorescent of other markers. The probes are allowed to bind to immobilized *Salmonella* cells or DNA (often after enrichment) and detected using colorimetric or other means. These techniques are not widely used in the industry and are more often used as research tools, although some commercial kits are available. Another area of interest is the use of the loop-mediated isothermal amplification (LAMP) technique. As the name suggests, like PCR, this method uses an enzyme to amplify nucleic acid, but unlike PCR, this technique does not require temperature cycling and can be measured by the simple production of turbidity. This has the advantage of allowing the development of methods that can be performed in the field and with minimal equipment. Currently, a number of techniques for the detection of *Salmonella* using LAMP are being evaluated, and despite some initial difficulties with sensitivity, this technique has substantial potential for the future.

Immunology-Based Methods

The specific interaction between antigens and antibodies is another approach used to detect *Salmonella* in food matrices and environments. Variations of this technique have already been mentioned as adjuncts to the culture-based techniques described earlier. Specifically, these interactions are the basis of immunomagnetic separation and the agglutination assays used for confirmation and serotyping. These techniques can, however, be used in their own right as integrated methods for the detection of *Salmonella*. Antibodies that target specific unique antigens and that are displayed on the cell surface can be used to 'capture' *Salmonella* cells. In the majority of methods using this technique, the specific antibodies are immobilized on a surface. The captured cells are detected by using additional antibodies (to the same or different antigens) conjugated to an enzyme. The enzyme is then used to convert its substrate to a color compound, the intensity of which is measured (by eye or a spectrophotometry) as a positive reaction. This technique is known as an enzyme-linked immunosorbent assay (ELISA). The exact protocols employed in these methods vary, but the major steps in the detection process tend to be very similar in most cases (Figure 3). A number of commercial systems using

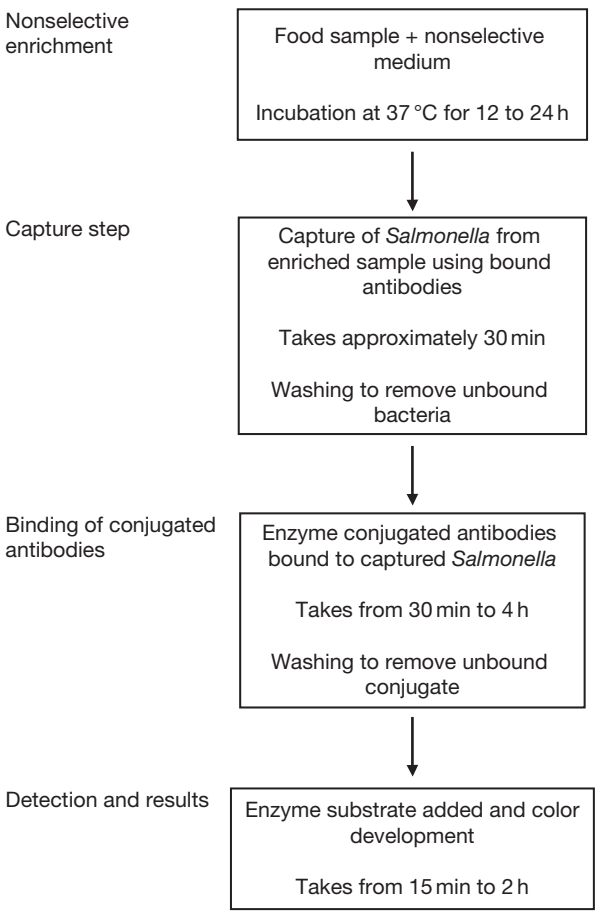

Figure 3 Generic scheme for detection of *Salmonella* in foods using immunology-based (ELISA) methods.

this method are available, and many of these have a relatively high degree of automation. The format of these tests may include microtiter plate-based assays, individual tube-based assays, or the use of once off membrane flow devices (similar to pregnancy testing kits) that produce a colored line if the sample is positive. Variations of the ELISA technique in which the antigens (*Salmonella* cells) are attached to a substrate and the antibodies added subsequently are available although less commonly used.

The steps in the ELISA technique are relatively rapid as compared to culture-based methods. The test itself can be performed in a matter of hours, but it has a relatively low sensitivity and still requires a nonselective enrichment step. As is the case for the nucleic acid-based techniques, the end result does not provide live cells for analysis, and if this is required by regulation, then isolation of *Salmonella* by culture-based methods will still need to be performed in the case of positive samples. The ELISA-based techniques can also suffer from other flaws such as cross-reaction with antigens on other bacteria resulting in false-positive signals and issue with detecting variable antigen production by some *Salmonella* strains resulting in false-negative results. Advances in technology and our understanding of *Salmonella* are reducing the occurrence of these issues, and a number of methods are accepted by regulatory authorities. Quantification of *Salmonella* using these techniques is currently not common although numerous hybrid methods to achieve this are under development.

Other Methods

Methods for the detection of *Salmonella* using techniques other than those fitting into the main groups described earlier are under development. The main one of these is bacteriophage-based detection. Bacteriophages are viruses that infect bacteria, many in a very specific way targeting individual genera, strains, or species. The specificity of these bacteriophage–bacteria interactions can be used in the same way as antibody–antigen interactions to detect bacteria of interest. These detection systems may operate in a variety of different ways. For example, they may use engineered bacteriophage designed to express luminescence when infecting their *Salmonella* host or bacteriophage proteins as a trap for *Salmonella* in a similar way to that used in antibody-based techniques. The potential of some of these methods lies in its ability to establish the viability of cells as bacteriophage signal will only be produced from live bacteria. While still in its infancy, this method has potential as it is more rapid than culture-based methods with the advantage described earlier.

Conclusion

The methods described earlier are diverse, and anyone wishing to detect the presence of *Salmonella* in food or food-related environments is faced with a dilemma of what to use. Often, the method to be used will be dictated by a regulatory authority, but even in this case, some choice in methodology is often allowed. The choice of method is necessarily a trade-off between a number of factors including speed, time, accuracy, cost, and relevance. Economies of scale are also important to consider and the approach used to examine only a few (or occasional) samples is likely to be different to that for routine processing of a large number of samples. The current trend is towards using the more rapid nucleic acid-based or immunology-based techniques to screen samples and then applying culture-based techniques only to samples found to be positive. Unless the rate of positive samples is expected to be high, this approach generally provides a happy medium between the factors listed earlier.

Advances in technology and our understanding of *Salmonella* biology continue to allow developments and improvements in the detection of this pathogen. Separation of *Salmonella* from other bacteria and from complex food matrices has improved but is an ongoing area of work. Increasing the rapidity and sensitivity of techniques and their ability to detect live cells is an area also receiving much attention. Improvements in, for example, sensitivity will necessarily result in an increased ability of regulatory authorities to impose stricter limits on the presence of this pathogen. The rapidly evolving technological and regulatory environment means that selection of techniques will become more specific to particular products and choosing the right method critical at all levels. Increasing globalization of the food supply will provide further challenges with respect to *Salmonella* detection and harmonization of the methods used, increasingly important.

See also: Emerging Foodborne Enteric Bacterial Pathogens; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; *Escherichia coli* and Other *Enterobacteriaceae*: Occurrence and Detection; Food Poisoning: Classification; Food Poisoning: Epidemiology; Food Poisoning: Tracing Origins and Testing; Foodborne Pathogens; Poultry: Processing; *Salmonella*: Properties and Occurrence; *Salmonella*: Salmonellosis; *Staphylococcus*: Detection; Zoonoses.

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Salmonella: Properties and Occurrence

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Introduction

Salmonella enterica infections are a cause of extensive morbidity and mortality throughout the world. In the United States, the Centers for Disease Control and Prevention (CDC) estimates that there are approximately 1 million cases of nontyphoid *Salmonella* infections leading to over 19 000 hospitalizations and nearly 400 deaths each year. *Salmonella* is the leading cause of bacterial foodborne illness, with approximately 95% of nontyphoid salmonellosis cases due to the consumption of contaminated food products. Estimates for the cost of salmonellosis range from approximately 2–11 billion US dollars each year due to loss of work, medical care, altered quality of life, and loss of life. The potential sources of *Salmonella* infection are numerous, as the bacteria have been found to persist in a wide range of environments and hosts including food animals, fruits and vegetables, legumes, nuts, pets, and reptiles.

Salmonella Basics

When humans are infected, salmonellosis can occur in the form of gastroenteritis, bacteremia, typhoid fever, and organ system infections. Typhoid fever, which is typically not considered a foodborne illness, is associated with *S. enterica* serotype Typhi and related serotypes and will not be extensively discussed in this article. Most cases of nontyphoid salmonellosis are limited to gastroenteritis that develops between 6 and 72 h following exposure. Gastroenteritis symptoms, including abdominal cramps, diarrhea, vomiting, and fever, typically resolve within a week without antimicrobial therapy. In some instances, a more invasive disease can occur, with patients developing bloodstream infections and infection of organs and tissues. These more severe manifestations most often occur in young children, the elderly, or those with a weakened immune system and often require antimicrobial treatment to resolve the infection. The typical antimicrobials used include the fluoroquinolones (which are not approved for use in children) or third-generation cephalosporins.

Members of *S. enterica* are generally identified based on their serotypes. Serotyping relies on the differential recognition of antigens present on the surface of the bacterium using antisera to distinguish serotypes. The antigen–antibody agglutination patterns separate *Salmonella* into at least 2600 distinct serotypes based on the Kauffmann–White scheme. Many of the serotypes are capable of causing human illness. In the United States, the most common serotypes associated with salmonellosis are Enteritidis, Typhimurium, Newport, Javiana, and Heidelberg. In Europe, *S.* Enteritidis is the predominant serotype, and in many parts of Asia, *S.* Choleraesuis is among the top serotypes, which causes relatively few cases in the United States. Most cases of salmonellosis are due to the consumption of contaminated foods such as meat and poultry products, eggs, unpasteurized dairy products, seafood, legume and nut products, and fresh produce. In addition, direct contact with infected animals, including reptiles and amphibians, can also be a source of infection.

Many of the serotypes commonly associated with human infections are also among the most commonly associated with food animal species. For example, of the ten most implicated serotypes for human infections in the United States, eight are among the top ten most isolated from one or more of the major meat or poultry species. Indeed, foods of animal origin are among the most commonly identified sources of human *Salmonella* infections; thus, there is a need for improved methods to limit contamination along the farm-to-fork continuum. This is especially important in light of the statistics that consumers are eating greater amounts of meat and poultry products throughout the world. In the United States, for example, the US Department of Agriculture (USDA) reported that there have been a greater than sixfold increase in per capita consumption of poultry products and a 20% increase in beef and pork consumption between 1910 and 2005. To combat the potential for increased foodborne illnesses, public health agencies, producers and processors, and consumers are working to limit *Salmonella* contamination and rapidly respond when a potential disease outbreak occur to limit the impact of foodborne illnesses.

Since *Salmonella* is primarily a foodborne disease, prevention of infections involves prevention of contamination of food products, either before/after harvest or through proper handling/preparation for both the food industry and the consumer. The optimum growth temperature for *Salmonella* is between 35 and 37 °C, but can grow in the range of 15–45 °C. For many food products, pasteurization is an effective way to kill *Salmonella.* For the consumer, improperly prepared and cooked foods are common sources of infections. Therefore, it is recommended that all meat products should be cooked to an internal temperature between 71 and 82 °C (depending on the type of meat). Also, care should be taken to avoid cross contamination with raw fruits and vegetables (e.g., use separate cutting boards to prepare meats and vegetables).

Salmonella Pathogenesis

Early Infection

Salmonella infections are typically due to ingestion of fecal material that may be present on contaminated foods or other vehicles. The estimated infectious dose, or number of organisms that need to be ingested to cause illness, is variable,

ranging from as few as 30 to greater than a billion organisms. The wide range is likely due to several factors, including the immune status of the patient, the genetics of the bacteria, and the type of food contaminated and consumed. For example, high-fat foods and those that can limit the impact of stomach acidity appear to allow for a lower infectious dose. *Salmonella* also have physiological properties that allow them to tolerate the low pH and presence of organic acids in the stomach to reach their sites of colonization in the intestinal tract. The production of acid shock proteins allow for *Salmonella* to elicit an acid tolerance response (ATR) mediated by regulatory factors including RpoS σ-factor, PhoPQ, and Fur proteins. These regulatory proteins allow the organisms to respond to the different inorganic and organic acids that are present in the stomach, by altering the expression of multiple ATR proteins. The exact roles that each of the regulated proteins, which impact protein expression and folding, metabolic function, and cell surface structures, play in the *Salmonella* ATR are not fully understood; however, it is evident that multiple factors contribute to the overall process.

Salmonella that survive passage through the stomach compete with members of the resident intestinal microbial populations (microbiota) for colonization sites within the small intestine or colon. When *Salmonella* attach to the intestinal epithelium, the adhesion is initiated by fimbriae or pili, which are located on the surface of the bacterium. Different *Salmonella* serotypes and strains can produce different types of fimbriae, which may contribute to some of the differences in the abilities of serotypes to infect various host species. Some common types of fimbriae associated with colonization include type 1 fimbriae, long polar fimbriae, curli fimbriae, and plasmid-encoded fimbriae. The different fimbriae bind to specific receptors on the host cells, which leads to differences in the cell types that *Salmonella* expressing different fimbriae are able to attach to and ultimately infect.

Following epithelial attachment, *Salmonella* can express a multiprotein structure termed as type III secretion system (T3SS), which initiates the uptake and invasion of intestinal cells. The T3SS is regulated by the HilA protein, whose expression is modulated in response to environmental factors that are important to cell survival. The structure of the secretion system is formed by the interaction of more than 20 structural and regulatory proteins to form the functional T3SS. The genes for the T3SS associated with intestinal cell invasion are carried on *Salmonella* pathogenicity island 1 (SPI-1). Pathogenicity islands are genetic regions that contain multiple genes that encode factors, such as host cell adhesion, invasion, and toxin production, which are important for increased virulence within a bacterium. The SPI-1 T3SS functions as a sort of molecular syringe that allows for the transport of protein effector molecules from the bacterial cytoplasm into the host cell. In the functional T3SS, a needlelike structure extends from a base anchored in the *Salmonella* cell membrane and the cell wall. An inner channel allows for effector molecules to be transported through the base structure into the needle, which interacts with the host cell. During the transport of effector molecules, chaperone proteins in bacterial cytoplasm enable interaction between the effector and T3SS-associated ATPases that drives the translocation of the effector into the needlelike structure. The tip of the needle interacts with a bacteria-derived translocation complex, which is inserted into the host cell membrane to facilitate delivery of effector proteins into the host's cytosol.

The translocated effector proteins have several effects on the host cells; SipA, SipC, and SopB proteins interact with actin to induce membrane ruffling associated with the engulfment and internalization of the bacterium. In membrane ruffling, the actin cytoskeleton undergoes rearrangement, and the cell membrane shape is altered to surround the *Salmonella* organisms. The internalized *Salmonella* subsequently reside in a membrane-bound compartment termed the *Salmonella*-containing vacuole (SCV). During the infection, the SCV migrates from the intestinal lumen to the basal membrane of the epithelial cell, where the *Salmonella* can interact with macrophages and/or dendritic cells in the submucosal tissue. The process of transfer from the epithelial cells to phagocytes remains poorly understood.

The SopB protein also appears to play an important role in the development of *Salmonella*-associated diarrhea, which is a key feature of gastroenteritis. SopB is likely involved with the attraction of neutrophils at the site of infection leading to inflammation and an alteration of the ion balance within the cells due to the disruption of chloride channels in the host cells. The altered ion balance leads to fluid secretion and subsequent diarrhea. In addition to SopB, several other effector molecules, including SipA, SopA, SopD, and SopE2, have been identified as potentially contributing to gastroenteritis.

Intracellular Survival and Systemic Infection

In addition to their function in epithelial cells, SCV are important for the survival of *Salmonella* within phagocytic cells, such as macrophages and dendritic cells, during invasive infections. Within the phagocytes, the SCV formation likely occurs distinct from the normal phagolysosomal processing pathways that immune system cells use to clear many bacteria from the host. The survival and persistence of *Salmonella* within the SCV are associated with a second T3SS that is encoded on SPI-2. The SPI-2 T3SS is only expressed inside the SCV and allows for the assembly of the secretion system apparatus and expression of affiliated effector proteins, chaperones, and regulatory proteins needed to function. A number of conditions within the SCV have been associated with SPI-2 T3SS expression, including changes in osmolarity and nutrients and acidification of the SCV.

SPI-2 T3SS effectors, such as SifA, SseF, and SseG, interact with the microtubule bundles and motor proteins within the host leading to morphological changes and the formation of vacuolar appendages that extend from the SCV, termed *Salmonella*-induced filaments (SIF). SIF are likely important in *Salmonella* pathogenesis, and their formation often coincides with the intracellular replication of *Salmonella*. In addition, the effector protein SpiC likely aids in the ability of *Salmonella* to persist intracellularly by interfering with phagolysosomal processing to avoid killing by reactive oxygen and nitrogen compounds that kill many other bacterial species.

Intracellular survival of *Salmonella* within macrophages and dendritic cells is an important attribute in the development of severe systemic infections. *Salmonella* present in the immune cells can be transported from the intestinal submucosa to other sites within the body. In contrast to macrophages, *Salmonella*

likely do not replicate within dendritic cells, but remain viable with reduced metabolic activity. SPI-2 T3SS effectors likely limit antigen presentation by dendritic cells, thereby further suppressing the immune response to *Salmonella*. When the infected phagocytes enter organs or certain tissues, they can spread to uninfected host cells leading to increased pathology associated with severe infections.

Antimicrobial Resistance

Resistance Trends of Salmonella

Salmonella are a public health concern not only due to the number of cases per year and the pathogenesis of the bacterium but also due to the number of strains resistant to antimicrobial agents. In the United States, the Food and Drug Administration, the USDA, and the CDC collaborate with the National Antimicrobial Resistance Monitoring System (NARMS) that tracks resistance trends of *Salmonella* and other enteric bacteria. The antimicrobials tested by NARMS include amikacin, amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. At the time of this writing, the most recent NARMS Executive Report summarized data collected in 2010 and indicated that in 2010, 15.3% of non-Typhi *Salmonella* isolates from humans were resistant to at least one antimicrobial agent. The most common multidrug resistance phenotype reported was resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines (ACSSuT), which was detected in 4.3% of isolates tested. The resistance trends for individual antibiotics are summarized in Figure 1. The antibiotics with the highest percentage of resistant isolates include ampicillin, streptomycin, sulfonamides, and tetracyclines; however, the percentage of isolates resistant to these drugs has either decreased or remained stable since 1999.

On the veterinary side, 51.9% of the *Salmonella* samples isolated from veterinary diagnostic sources were resistant to at least one antimicrobial agent with the ACSSuT phenotype also the most common resistance profile detected among veterinary isolates. Overall, when antimicrobial resistance data from both veterinary and human NARMS reports were compared, the trend appeared to indicate that the percentage of resistant isolates was greater in strains of veterinary origin. The trends for individual antibiotics are summarized in Figure 2. Veterinary-associated *Salmonella* isolates demonstrated the highest percentage of resistance to ampicillin, streptomycin, sulfonamides, and tetracyclines. Resistance to each of the drugs was on a general upward trend from 1999 to 2010, with the exception of sulfonamides. There have been sharp increases in resistance to amoxicillin/clavulanic acid, ceftriaxone, and ceftiofur among veterinary-associated *Salmonella* isolates. The increase in resistance among *Salmonella* to extended spectrum cephalosporins (ceftiofur and ceftriaxone) has become a significant public health concern due to the importance of ceftriaxone in the treatment of severe salmonellosis in children. Because ceftiofur-resistant organisms are cross resistant to ceftriaxone, the use of this antimicrobial agent in food animals has come under increasing scrutiny as a selective agent potentially responsible for the emergence and dissemination of ceftriaxone resistance in *Salmonella* and other enteric pathogens.

Mechanisms of Antimicrobial Resistance

The development of resistance in *Salmonella* to antimicrobial agents can be attributable to one of multiple mechanisms, which include production of enzymes that inactivate the drug, reduction of cell permeability, activation of efflux pumps, and modification of cellular targets. Resistance to penicillins and cephalosporins (i.e., ampicillin, cefoxitin, ceftiofur, and ceftriaxone) is commonly due to the production of β-lactamase enzymes that degrade the β-lactam ring of the antimicrobial agents. β-lactamases are a diverse group of enzymes, some with affinities for a limited number of antimicrobial agents, while others are able to degrade a wide array of antibiotics. Other enzymes inactivate the antimicrobial agents by modifying their structure, rather than degrading them. Resistance to aminoglycosides (i.e., amikacin, gentamicin, kanamycin, and streptomycin) in *Salmonella* is often the result of the production of modifying enzymes, which function by phosphorylating, acetylating, and adenylating certain aminoglycosides.

Other mechanisms of resistance involve the modification of the drug binding target of the cell. Resistance to quinolone and fluoroquinolone drugs (i.e., nalidixic acid and ciprofloxacin) is often associated with this mechanism. Mutations in the genes encoding the topoisomerase enzymes that are required for bacterial DNA replication alter the drugs binding site and prevent the antimicrobial activity of the drug. Resistance to tetracycline and chloramphenicol is often associated with the expression efflux pumps that reduce the level of antimicrobial drugs inside the bacterial cell. In addition to efflux-mediated resistance, some chloramphenicol resistance in *Salmonella* is associated with drug target modification by chloramphenicol acetyltransferases. The drugs trimethoprim and the sulfonamides function by competitively inhibiting different enzymes in the folic acid biosynthetic pathway in bacterial cells. Resistance to sulfonamides is often associated with genes that encode altered dihydropteroate synthetase enzymes that have a reduced affinity to the sulfonamides but function in folic acid biosynthesis. Likewise with trimethoprim resistance, resistance genes encode altered dihydrofolate reductases that have reduced affinity for the antimicrobial agent, allowing folic acid biosynthesis to occur in the presence of trimethoprim.

Mechanisms of Antimicrobial Resistance Dissemination

There are several mechanisms that are important for the dissemination of antimicrobial resistance; these include transformation, transduction, and conjugation. Natural transformation is characterized by the uptake, integration, and expression of free DNA from the environment. DNA is released into the environment from decomposing cells, damaged cells, or viruses and can be taken up by competent bacteria. Once DNA has entered the cell, it can be integrated into the chromosome or plasmids. If a resistance gene is incorporated into the recipient genome, the cell may develop resistance to the corresponding antimicrobial agent. In transduction, DNA is transferred from one bacterial cell to another by bacterial viruses (phages). Phages that have

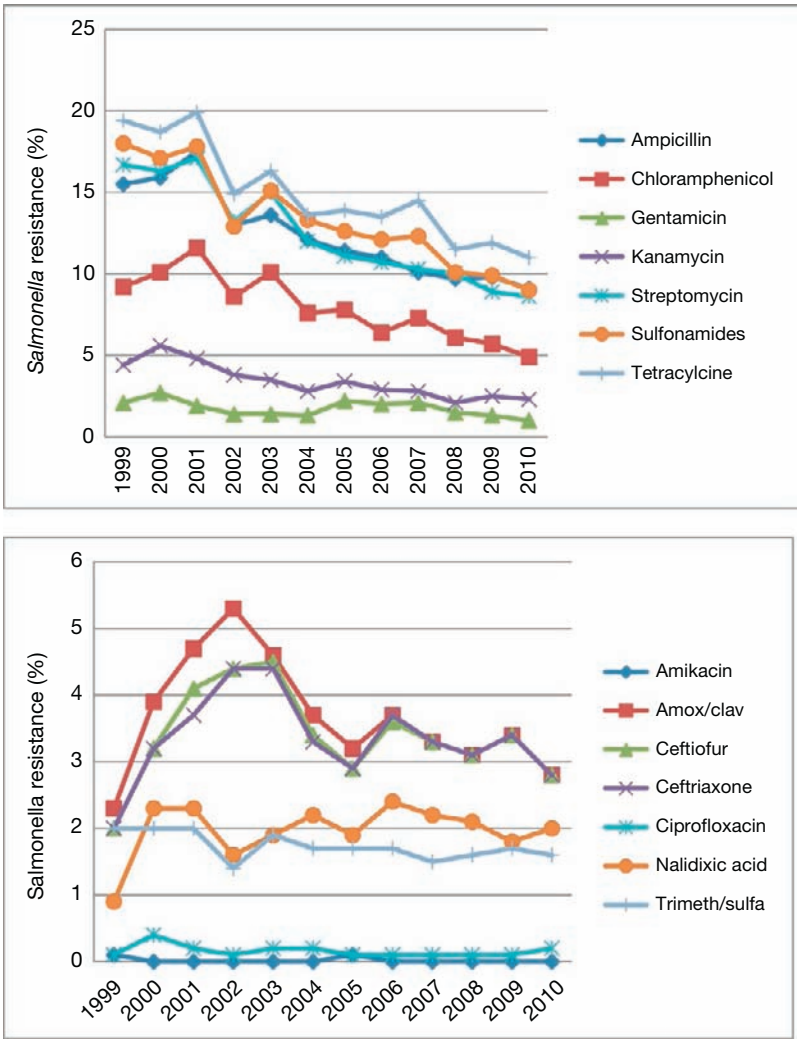

Figure 1 Trends in antimicrobial resistance in *Salmonella* from human sources from 1999 to 2010. Data are presented for Salmonella resistance to 15 different antimicrobial agents tested by the National Antimicrobial Resistance Monitoring System for Enteric Bacteria for all non-Typhi *Salmonella*. The abbreviations Trimeth/Sulfa and Amox/Clav are used in the symbol legend for the trimethoprim/sulfamethoxazole and amoxicillin/clavulanic acid drug combinations, respectively.

infected a bacterium can package small fragments of bacterial host DNA during viral replication. After the phage, with the host DNA fragment, is released into the environment, it can inject the new fragment of DNA into another bacterium it comes in contact with. As with transformation, the new piece of DNA can integrate into the host cell's chromosome and be expressed. With conjugation, transfer of plasmids is mediated by a cell-to-cell bridge (pilus), which is encoded by the plasmid. During conjugation, the donor cell produces a pilus that contacts a recipient cell and forms a mating pair. The plasmid begins replication and one strand of DNA is transferred to the recipient. Both strands finish replication in their respective cells resulting with the donor cell and a transconjugant (recipient cell with newly acquired plasmid).

Another important means of antimicrobial resistance dissemination is through integrons, which are mobile genetic elements that can be located on plasmids or the chromosome. Integrons contain the genes needed for insertion and excision of genetic material from plasmids or chromosomes and a complement of resistance genes. Class 1 integrons are the most common antimicrobial resistance integrons found in *Salmonella*. Class 1 integrons include a gene encoding an integrase (*intI*), a recombination site (*attI*), and a gene cassette. The gene cassette usually contains antimicrobial resistance genes and a short 59-base element (*attC*) that functions as a recognition sequence for the specific recombination site. The integrases can recombine the gene cassette into a specific recombination site. Immediately downstream of the gene cassette are the genes *qacE*Δ and *sul1*, which encode resistance to quaternary ammonium compounds and sulfonamides, respectively. Integron transfer of resistance genes has been shown to spread across serotype and even species barriers raising concern for potential resistance gene transfers from multiple sources to and among *Salmonella* strains.

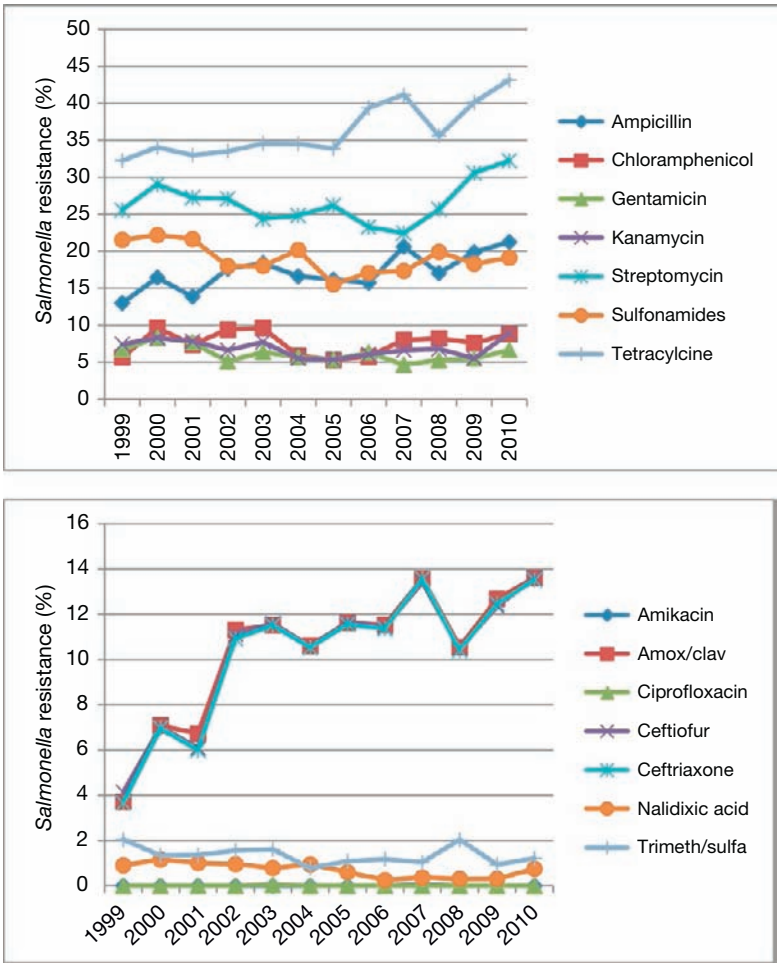

Figure 2 Trends in antimicrobial resistance in *Salmonella* from veterinary sources from 1999 to 2010. Data are presented *Salmonella* resistance to 15 different antimicrobial agents tested by the National Antimicrobial Resistance Monitoring System for Enteric Bacteria for all non-Typhi *Salmonella* tested. The abbreviations Trimeth/Sulfa and Amox/Clav are used in the symbol legend for the trimethoprim/sulfamethoxazole and amoxicillin/clavulanic acid drug combinations, respectively.

Conclusions

Salmonella are important human pathogens that cause millions of infections each year throughout the world. The pathogens have evolved to survive the hostile environment of the upper gastrointestinal tract and enter the intestines where they are often able to compete with intestinal microbiota and attach to the intestinal mucosa. This attachment facilitates the SPI-1 T3SS-mediated invasion of the intestinal epithelium. Effector proteins associated with the SPI-1 T3SS are likely responsible for the diarrhea that is typically associated with *Salmonella* gastroenteritis. Other effector proteins are involved in the engulfment and internalization of *Salmonella* that is the hallmark of infection. *Salmonella* can migrate through the intestinal epithelial cells and ultimately be taken up in macrophages or dendritic cells. *Salmonella* that are able to persist in these phagocytes can potentially spread to different parts of the body leading to increased pathology associated with the invasive infections.

Severe infections caused by *Salmonella* often require antimicrobial therapy. Resistance to antimicrobial agents is a public health concern in *Salmonella* especially with the increased resistance trends seen in veterinary isolates. Of great concern is the emergence of resistance to extended spectrum cephalosporins, such as ceftiofur and ceftriaxone, because ceftriaxone is the primary drug of choice for treating severe salmonellosis in children. *Salmonella* remains an important pathogen of both humans and animals, and research on understanding the mechanisms of pathogenicity and the development and dissemination of antimicrobial resistance is needed to help alleviate the burden of these pathogens.

See also: Antibiotics and Drugs: Drug–Nutrient Interactions; Antioxidants: Role on Health and Prevention; Dietary Exposure Assessment; Emerging Foodborne Enteric Bacterial Pathogens; Foodborne Pathogens; *Salmonella*: Detection; *Salmonella*: Salmonellosis.

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Salmonella: Salmonellosis

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Introduction

Salmonella remains one of the most important zoonotic pathogenic bacteria and is the causative agents of salmonellosis. The aim of this article is to give an overview of *Salmonella* and salmonellosis, starting by describing the characteristics of the microorganism *Salmonella*, including biochemical properties, physiology, classification, and nomenclature. Thereafter, the epidemiology of the organism is introduced, including the routes of transmission. Finally, the disease salmonellosis, the virulence mechanisms, and the prevalence in different types of food are described.

Characteristics of the Organism

Biochemical Properties

Salmonella are rod-shaped motile Gram-negative bacteria with flagella distributed uniformly over the cell surface (Figure 1). Nonflagellated variants and nonmotile strains containing dysfunctional flagella have been reported. *Salmonella*e are facultative anaerobic and nonspore-forming, able to utilize a wide variety of nutrients (chemoorganotrophic). They use both respiratory and fermentative metabolic pathways. Generally, *Salmonella*e are oxidase-negative and catalase-negative and grow on citrate as sole carbon source. Most strains produce hydrogen sulfide, decarboxylate lysine, and ornithine and do not utilize urea. Many of these traits are used when designing methods for identification, for example, selective xylose lysine deoxycholate agar plates used in the ISO 6579 standard method.

Classification and Nomenclature

The *Salmonella* genus is a member of the family Enterobacteriaceae and contains two species, *Salmonella enterica* and *Salmonella bongori*, each consisting of multiple serotypes. *S. enterica* is subdivided into six subspecies: *S. enterica* ssp. *enterica* (I), *S. enterica* ssp. *salamae* (II), *S. enterica* ssp. *arizonae* (IIIa), *S. enterica* ssp. *diarizonae* (IIIb), *S. enterica* ssp. *houtenae* (IV), and *S. enterica* ssp. *indica* (VI). *S. bongori* was formerly classified as subspecies V. *S. enterica* ssp. *enterica* is mainly associated with humans and warm-blooded animals, whereas the other subspecies are found in cold-blooded animals and the environment. This classification scheme has historically undergone a number of rearrangements over time, as a result of the methodological improvements to reveal the true relationship between bacteria in this group.

The subspecies of *Salmonella* are further separated into serovars according to the White–Kauffmann–Le Minor scheme. The separation of serovars is based on antigenic polymorphisms of lipopolysaccharide (O antigens), flagellar proteins (H antigens), and capsular polysaccharides (Vi antigens). Currently, about 2600 serovars of *Salmonella* have been identified, where most of the serotypes belong to *S. enterica* (Figure 2).

Physiology

Salmonella can be found in natural environments, such as water, soil, and plants, but it does not seem to grow significantly there. However, since *Salmonella* are known to be quite resistant toward different environmental factors, it can survive for weeks in water and several years in soil, as long as the conditions are favorable. It has also been reported to survive for extended time periods in foods stored frozen or at room temperatures. The optimal growth temperature for *Salmonella* is 37 °C, but the organism can grow in a quite broad range (7–45 °C). In extreme cases, *Salmonella* growth in food has been observed at 2–4 °C. *Salmonella* can grow at pH 4.5–9.5, with an optimum pH for growth at 6.5–7.5. The water activity (a_w) needs to be ≥ 0.93 to support the growth of *Salmonella* in food and it is generally inhibited by the presence of 3–4% NaCl.

Salmonella has a great ability to adapt to various more or less unfavorable environmental conditions, such as low pH, high temperature, and low a_w. This makes these bacteria able to outcompete other microorganisms in, for example, different food production chains possibly leading to problems with cross contamination and biofilm formation in food production environments.

Epidemiology

Salmonella is found worldwide and is an important zoonotic pathogen. It is estimated that *Salmonella* is responsible for 93.8 million cases of human gastroenteritis globally each year, and of these, 80.3 million cases are estimated to be foodborne. In the EU, 95 548 confirmed cases of human *Salmonella* infections were reported to the European Food Safety Authority in 2011. However, cases of salmonellosis are considered to be underreported as many of the sick individuals do not seek medical attention.

Approximately 50 serovars (all in subspecies *enterica*) account for 99% of all clinical isolates from humans and warm-blooded animals. The two serotypes, *Salmonella* ser. Enteritidis and *Salmonella* ser. Typhimurium, are the most frequently reported serovars, together accounting for almost 70% of all human *Salmonella* infections in Europe. Human *Salmonella* ser. Enteritidis cases are mostly associated with the consumption of contaminated eggs and poultry meat, whereas *Salmonella* ser. Typhimurium cases most commonly are linked with the consumption of contaminated pork, beef, and poultry meat.

The routes of *Salmonella* transmission to humans include the environment, contact with animals, and human-to-human contact (Figure 3). In industrialized countries, the main source of *Salmonella* infections is contaminated animal-derived food products, notably fresh meat and eggs as identified, for example, using source attribution models based on outbreak data. In developing countries, a large proportion of the human cases are from contaminated vegetables, water, and human-to-human

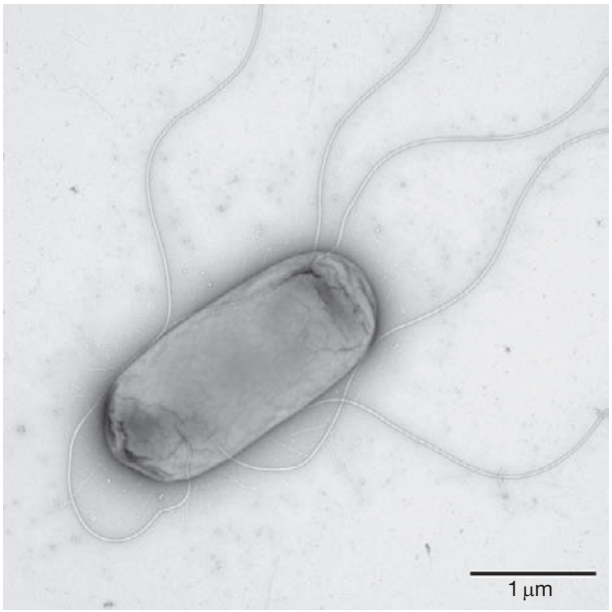

Figure 1 A light microscope picture of *Salmonella*. ©Dr. Jochen Reetz, Federal Institute for Risk Assessment (BfR), Berlin, Germany.

transmission. Recently, there has been an increase in the reported outbreaks of salmonellosis caused by contamination of fruits and vegetables possibly related to the greater worldwide trade with fresh produce.

Salmonellosis

Symptoms and Infectious Dose

S. enterica is a common agent of foodborne infections and the human infections can range from enteric fever (typhoid fever) to nontyphoidal *Salmonella*, which normally cause gastroenteritis. A few *Salmonella* can cause infection, especially for the more susceptible part of the population (e.g., young, elderly, and immunocompromised), but the infective dose is generally considered to be $>10^4$ CFU g^{-1}. Low infectious doses have been reported especially for food with a high fat content, including cocoa butter in chocolate and animal fat in meat or with food or ingredients with low a_w values.

Enteric fever is associated with serotypes Typhi and Paratyphi A, B, and C, and symptoms include diarrhea, prolonged and spiking fever, abdominal pain, headache, and prostration appearing after an incubation period of 7–28 days. After the initial acute phase of the disease, an asymptomatic chronic carrier state is common. Under certain conditions, it is possible for *Salmonella* to cause invasive (systemic) disease, entering the

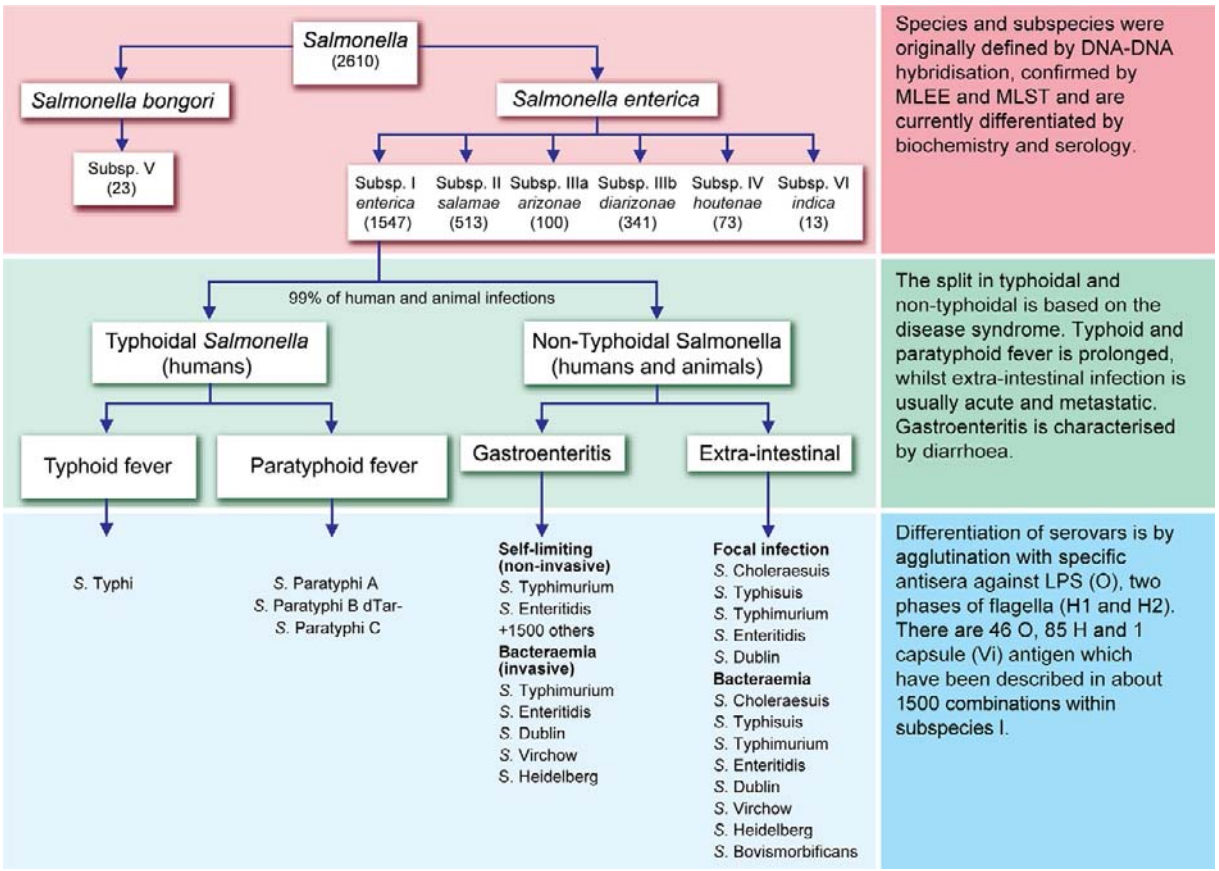

Figure 2 General overview of the current classification of *Salmonella enterica*. Reprinted from Achtman et al. (2012). Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. *PLoS Pathog* 8(6), p. e1002776.

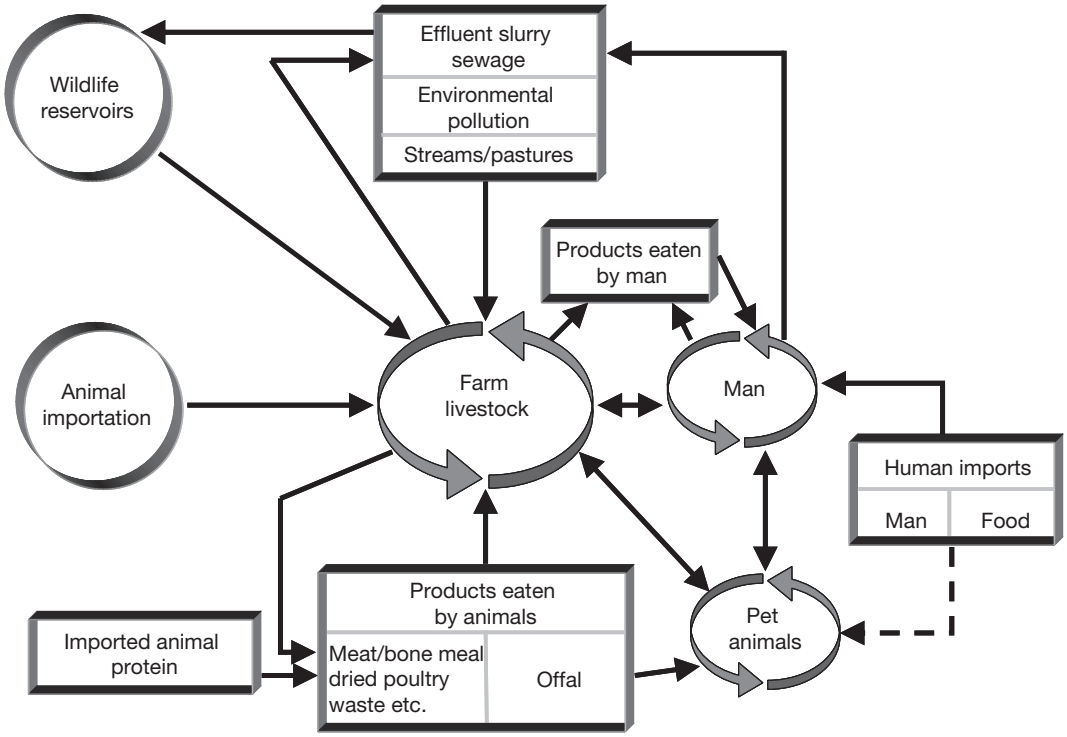

Figure 3 Routes of transmission for *Salmonella*. Reprinted with permission from the World Health Organization (1983). Guidelines on prevention and control of salmonellosis, A. H. Hinton (ed.), Geneva, p. 22. Available online: http://apps.who.int/iris/bitstream/10665/66402/1/VPH_83.42_%28p1-p66%29.pdf (Date: July 2014).

blood stream and spreading throughout the body. The nature and severity of *Salmonella* infections depend on several factors such as the infecting *Salmonella* serotype, virulence of the strain, infection dose, and the host. Treatments of enteric fever include the use of supportive therapy and/or antibiotics to avoid systemic infections. However, strains resistant toward several antibiotics have been increasingly found, limiting the usefulness of antibiotic treatment.

Salmonellosis with nontyphoidal *Salmonella* strains has a shorter incubation period of 8–72 h after ingestion of the pathogen. Gastrointestinal infections are primarily caused by serovars such as Enteritidis and Typhimurium. Usually, *Salmonella* colonize the intestine by attaching to the epithelial cells using the fimbria, followed by invasion of the intestinal mucosa and multiplication in the gut-associated lymphoid tissue. Symptoms include nonbloody diarrhea and abdominal pain and the illness is usually self-limiting. Usually, only supportive therapy is used to treat nontyphoidal salmonellosis since the use of antibiotics has been found to prolong the carrier state. Nontyphoidal salmonellosis can also sometimes result in systemic infections and chronic conditions such as aseptic reactive arthritis.

Pathogenicity and Virulence Factors

Salmonella enterica possesses a large set of virulence factors enabling it to withstand the host defense mechanisms, invade the gut epithelial layer, and persist in a localized area during gastrointestinal infections or even spread systemically in the case of typhoid fever. Because bacterial growth and the expression of those factors often lead to immune activation, it has to be well regulated depending on the invasion level by regulatory proteins or two-component-systems (TCSs).

One of the first lines of defense is the antimicrobial condition of the stomach. To survive especially at low pH, *Salmonella* uses a complex acid tolerance response (ATR) that consists of more than 50 acid shock proteins. The ATR is regulated depending on the growth phase by either *rpoS*-encoded σ^S (organic acids) or PhoPQ TCS (inorganic acids) during exponential growth and the OmpR/EnvZ TCS in the stationary phase, which is therefore also essential for persistence in the host cell phagosome.

Nevertheless, only 1% of the bacteria reach the gut and adhere to the epithelium by the fimbriae, nonfimbrial adhesins like SiiE or BapA, and autotransporter proteins like ShdA, MisL, or SadA. The repertoire of fimbriae is strain-specific and consists of more than 20 different loci, which might be important for tissue and cell tropism. Type 1 fimbriae (Fim) bind to enterocytes, long polar fimbriae (Lpf) bind to microfold (M) cells, whereas the plasmid-encoded fimbriae (Pef) and thin aggregative or curli fimbriae (Tafi) adhere to epithelial cells *in vitro*.

For the subsequent invasion of epithelial cells, macrophages, and dendritic cells, as well as for the intracellular persistence, *Salmonella* possesses two type III secretion systems (T3SSs). They are encoded on two *Salmonella* pathogenicity islands (SPI-1 and SPI-2), which are the most studied SPIs out of 21 known to date. Most of them are found in the majority of subspecies I *Salmonella* strains but a few are

serovar-specific, like SPI-14 for *Salmonella* ser. Typhimurium or SPI-7, SPI-15, SPI-17, and SPI-18 for *Salmonella* ser. Typhi. *Salmonella* uses its SPI-1 T3SS to secrete at least six effector proteins into the host cell cytosol that are important for the invasion process: *Salmonella* outer proteins SopB, SopE, and SopE2 induce by activating Rho GTPases Cdc42, Rac1, and RhoG an actin rearrangement, while *Salmonella* invasion proteins SipA and SipC stabilize the newly formed actin filaments. This leads to a membrane ruffling followed by an uptake of the bacteria into macropinosomes. Fission of the membranes is supported by SopD. Especially SopB, SopE, and SopE2 activity has, besides its role in the invasion process, other important consequences. SopE/E2 contribute to inflammation by inducing MAPK pathways leading to IL8 expression in epithelial cells and inducing IL1-β secretion and pyroptotic effects in macrophages by enhancing caspase-1 activation. SopB is directly involved in promoting diarrhea by elevating intracellular Ca^{2+} level and inducing a chloride and water efflux. Another SPI-1 effector, SipA, has also been shown to contribute to fluid efflux.

The SPI-2 T3SS and its effectors have been shown to promote intracellular survival although some effectors of both T3SSs are acting at the same time, which presumes a complex coordination of both SPI-1 and SPI-2 effector genes. HilA encoded by SPI-1 is known as master regulator of the SPI-1 effector expression, whereas three different TCSs SpiR/SsrB, EnvZ/OmpR, and PhoPQ are regulators of the SPI-2 virulence genes. The cross talk of the SPI-1/SPI-2 regulatory network is mediated by HilD, which controls both HilA expression and SsrAB expression. The main function of the 20 different SPI-2 effectors known to date is to facilitate intracellular replication by redirecting intracellular vesicular trafficking and maturation of the *Salmonella*-containing vacuole (SCV). The main effectors in that process are SifA, SseG, and SseF, which provoke the motion of the SCV in a juxtanuclear position, and SopD2, SifA, and PipB2, which are required for *Salmonella*-induced filament (Sif) formation by interacting with kinesin and the microtubule network. The function of Sifs for pathogenesis is still not completely understood but a Sif-forming ability has been shown to be necessary for full virulence in mice.

In addition to the SPIs, a lot of virulence factors are encoded on mobile elements like prophages and plasmids. Certain *Salmonella* serovars harbor a serovar-specific, 50–100 kb large, low-copy-number plasmid. Part of that plasmid is the 8 kb *Salmonella* plasmid virulence (*spv*) operon. Signals resulting in *spv* transcription are typically conditions of the SCV interior like iron or nutrient depletion, elevated temperatures, a low pH or the stationary growth phase. The operon is regulated by SpvR and the chromosomal *rpoS*-encoded σ^S. This plasmid seems to be an important component for intracellular persistence and growth, cytotoxicity, and the systemic spread although such a plasmid is missing in prominent serovars causing typhoid or paratyphoid like *Salmonella* Typhi and Paratyphi A. In addition to *spv*, some serovars harbor specific virulence genes on their plasmids like *rck* that influences the serum resistance of *Salmonella* ser. Typhimurium and Enteritidis. The fimbrial gene *pef* is encoded on *Salmonella* ser. Typhimurium, Enteritidis, and Choleraesuis but not on *Salmonella* ser. Dublin or Gallinarum plasmids.

Recently, a putative virulence plasmid called pR(ST98) has been described in *Salmonella* ser. Typhi, which supports drug resistance and cytotoxicity to macrophages. Even antimicrobial resistance is often transmitted by the so-called R plasmids. For example, the multidrug resistance (MDR) phenotype of *Salmonella* ser. Typhi is associated with an (Inc)HI1 plasmid. In addition to those factors, *Salmonella* ser. Typhi harbors some exclusive virulence factors, which might explain its strong virulence in humans. A so-called typhoid toxin (CdtB/PltA/PltB) similar to the pertussis toxin has been identified, which is expressed intracellularly and causes para- and autocrinely cytotoxic effects. Target specificity is accomplished by specific carbohydrate moieties on surface glycoproteins.

Another important factor is the expression of a virulence (Vi) capsular polysaccharide encoded by the *viaB* locus in a few *Salmonella* serovars. Depending on osmolarity, the expression of the Vi antigen is regulated by TviA and the EnvZ/OmpR TCS. Due to a regulatory network with RcsB, the induction of *tviA* and suppression of flagellin as well as T3SS genes are coregulated and support evasion from immune recognition.

Altogether, *Salmonella* evolved a complex network of virulence genes and regulators enabling it to successfully adapt to different environmental conditions and evade or rather manipulate the host immune defense mechanisms in a serovar-specific and host species-specific manner to form its own niche.

Occurrence in Food

Persistence in the Food Chain Environment

The adaptation and persistence of *Salmonella* in food production chains have been suggested to be partly a result of bacterial attachment and surface colonization. Colonization of *Salmonella* on surfaces in contact with food provides a reservoir of bacteria that can increase the risk of contamination in the production line. *Salmonella* present in biofilm are generally more resistant toward environmental stresses, for example, disinfections, desiccation, organics acids, and heat. Besides attaching to the materials used in the production line, *Salmonella* can also attach to surfaces of the food product or be localized inside the food item for products such as vegetables and fruits.

Salmonella in Meat and Eggs

Salmonella can enter at any stage of the food chain, from the livestock feed, at the slaughterhouse or packing plant; in manufacturing, processing, and retailing of food; and through catering and cooking at home. Colonized pigs constitute the main reservoir for carcass contamination in slaughterhouses and many of the carcasses harbor *Salmonella* on the skin or in the rectum. However, the slaughter line is an open process with many possibilities for cross contamination. Carcasses can throughout the slaughter line be contaminated from tools and equipment used for the slaughtering, and since the equipment can be very difficult to clean and sterilize, the *Salmonella* has the possibility to colonize and form biofilms. Eggs and egg products are another important source of *Salmonella* and salmonellosis. *Salmonella* are usually located on the shell surface but can also be found inside the egg making sanitation

difficult. Outbreaks associated with the consumption of eggs or egg products are common.

Recent data on monitoring of the *Salmonella* prevalence in the United States found the following percent positive rate of *Salmonella* per product class for the calendar year 2012: young chicken (4.3%), market hog (1.3%), cow/bull (0.0%), steer/heifer (1.1%), ground beef (1.9%), ground chicken (28%), ground turkey (11%), and turkey (2.2%). Data from the European Union report the following % positive rates: broiler meat and products thereof (5.9%), pig meat and products thereof (0.7%), and eggs and egg products (0.1%). Note that these data are difficult to compare as the sampling systems and analysis methods differ between different countries.

Salmonella in Fresh Produce

Recently, there have been an increasing number of reported cases and outbreaks due to *Salmonella* in fruits and vegetables, for example, herbs, melons, and tomatoes, and products derived from fruits and vegetables, for example, peanut butter. Moreover, *Salmonella* has the ability to attach and survive in or on fresh produce and might contaminate through cultivation practices, handling, or processing. A large number of outbreaks have been caused by the use of contaminated water sources used to irrigate and wash produce crops. Fresh produce is mostly consumed without a heating step that would kill *Salmonella*, and as *Salmonella* also can be found inside the crops, rinsing is sometimes ineffective.

Other Sources

Salmonella can also be found in a variety of other food items, such as milk, chocolate, and fish, and outbreaks due to these vehicles have been reported. Other routes of transmission include contact with animals and person-to-person transmission (**Figure 3**).

Conclusions

Salmonella and salmonellosis (both enteric fever and nontyphoidal salmonellosis) remain significant problems worldwide, leading to increased public health concerns and economic burden on society. *Salmonella* adapts easily to various environmental conditions, giving it a competitive advantage toward other microorganisms, and enables survival in many different food products and food production chains. The molecular mechanisms for salmonellosis are complex, combining several different systems. This also contributes to the success of *Salmonella* to survive and proliferate in its hosts. Important vehicles contributing to the spread of *Salmonella* are different types of food, in particular meat, eggs, and fresh produce.

Acknowledgments

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See also: Biofilms; Codex Alimentarius Commission: Role in International Food Standards Setting; Diarrheal Diseases; Eggs: Composition and Health Effects; Emerging Foodborne Enteric Bacterial Pathogens; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; *Escherichia coli* and Other *Enterobacteriaceae*: Occurrence and Detection; Foodborne Pathogens; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Laboratory Management: Microbiological Safety; *Listeria*: Listeriosis; *Listeria*: Properties and Occurrences; Meat: Eating Quality and Preservation; Milk Powder; Milk: Processing of Milk; Pasteurization: Principles and Applications; Pork Meat Quality, Production and Processing on; Poultry: Processing; Preservation of Foods; Risk Assessment of Foods and Chemicals in Foods; *Salmonella*: Detection; *Salmonella*: Properties and Occurrence; Shigella; Soy Beans: Processing; *Staphylococcus*: Food Poisoning; *Staphylococcus*: Occurrence and Properties; *Vibrio*: Types, Properties, and Determination; *Yersinia enterocolitica*: Properties and Occurrence; *Yersinia enterocolitica*: Detection and Treatment; Zoonoses.

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Sanitization

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The General Framework of Sanitation

Sanitation is the combination of activities and procedures conducted in a food-processing plant with the aim of obtaining a manufacturing environment free from hazards that may contaminate the foodstuffs processed therein and that could therefore threaten food safety for the consumer and shorten the shelf life of the foodstuff manufactured.

It is a broad-ranging concept that covers the correct choice of location for the construction of the food-processing plant, its hygienic design, careful selection of equipment designed to facilitate hygienic cleaning thereof, a pest-control plan, a waste-treatment plan, and, obviously, an effective cleaning and disinfection plan.

The final aim is to minimize the presence of contaminants in the manufacturing environment as far as possible, thereby preventing them from entering the foods processed. Sanitation is a basic and essential requirement for fully effective application of the Hazard and Critical Control Points (HACCP) system and to guarantee the safety and quality of the products manufactured in the food-processing plant.

In addition to allowing food companies to meet regulatory standards, the advantage of correct sanitation includes guaranteed food safety by eliminating the vast majority of physical, chemical, and microbiological contaminants present on tools, equipment, and surfaces, thereby preventing them from affecting consumer health. Sanitation also guarantees food quality, preserving its sensory characteristics and increasing its shelf life, and aids correct maintenance of the machinery, equipment, and facilities used by preventing corrosion caused by microorganisms or chemical reactions. In addition, correct sanitation transmits an impression of hygiene and order to both clients and consumers and improves the working environment. In short, it increases the efficiency of food production without compromising the brand name during possible food crises.

The cost of incorrect sanitation is a poor-quality product, increased maintenance costs, delays in completing customer orders, interruptions to production, the need for product reprocessing, client/consumer complaints, the possibility of product recalls, with their associated costs, including civil liability, and damage to the company's image.

Among the various actions and plans that allow correct sanitation of the food-processing plant, the cleaning and disinfection plan is perhaps the most important. Clearly, this plan will be more effective if the other actions have been correctly implemented. In other words, the plant should have been located at a site far from sources of contamination, should have an appropriate hygienic layout, and should use equipment and machinery purchased with a hygienic design that facilitates the cleaning thereof, and the pest-control and waste-treatment programs should be correctly implemented, all of which will favor and greatly improve the effectiveness of the cleaning and disinfection plan.

Cleaning and Sanitization: Situation of the Concepts

As its name implies, the process of 'cleaning and sanitization' comprises two parts. In the first step, cleaning comprises the removal of all soil from the equipment and manufacturing environment, enabling subsequent application of a sanitizer to reduce the number of microorganisms that have not been removed in the previous stage more effectively. The sanitization process then eliminates microorganisms that may contaminate foodstuffs from contact surfaces or the manufacturing environment. Sanitization is therefore a specific part of the broader process of sanitation referred to in the preceding text.

Cleaning covers the set of practices and processes used to eliminate all soil from surfaces and the manufacturing environment. Its main objectives are (1) to remove large foodstuff remnants such as shells, leaves, and pieces of food; (2) to remove food component residues, such as fat, blood, proteins, and sugars, which may serve as nutrients for microorganisms; (3) to remove other types of soil inherent to the foodstuff manufacturing process, such as containers, labels, dirt from the operators, machinery and equipment, dust, soil, and chemicals (lubricants, additives, etc.); (4) to treat the surfaces to ensure effective disinfection (free from organic matter and/or mineral deposits); and (5) to remove some of the microbial contamination by sweeping.

Disinfection, in turn, concerns elimination of the majority of microorganisms from work surfaces and facilities (manufacturing environment). Its objectives therefore include the total destruction of all pathogenic microorganisms and a reduction in nonpathogenic microorganisms to a level at which they are no longer detrimental to the quality or shelf life of the products.

Cleaning must always be performed prior to sanitization. Sanitizers must never be used on dirty surfaces as organic matter inhibits their action. As such, it is important to remove all soil particles that form on surfaces, such as biofilms and fat or protein films, deposits of mineral origin (e.g., limescale), or milkstone. Incorrect removal of these dirt films and incrustations contributes to the proliferation of microbiological contamination and promotes biofilm formation. Such films can form by way of various mechanisms, such as soil redeposition phenomena, chemical adsorption as a result of chemical reactions between surface molecules and soil molecules, physical adsorption as a result of van der Waal's-type electrostatic interactions, and adsorption to the porous structure formed by the surface.

A biofilm consists of microorganisms adhered to a surface and an extracellular polymeric substance (EPS), mainly comprising polysaccharides and glycoproteins, secreted by them. Biofilms are used by microorganisms to defend themselves against their surroundings by providing protection against the action of biocides and helping them to proliferate. They

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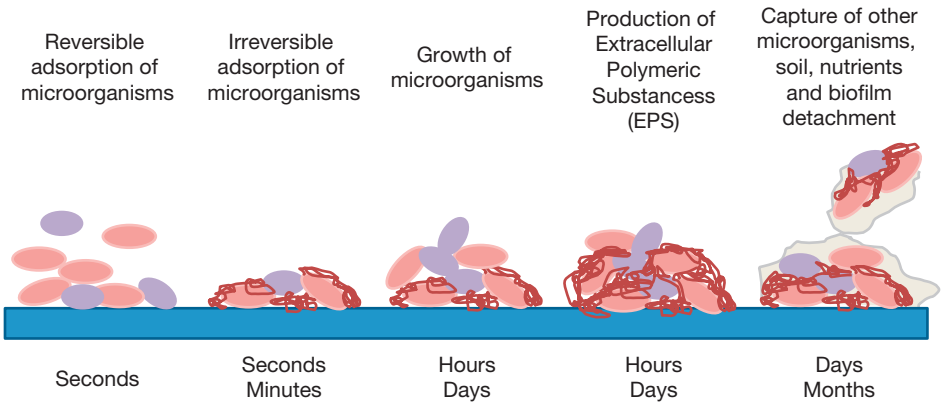

Figure 1 Biofilm formation.

usually contain various microbial species at the same time, including bacteria, molds, algae, and even protozoa. The presence of water or moisture on the surfaces is essential for biofilm formation. In the food industry, soil films and incrustations represent a highly suitable substrate for biofilm formation.

Biofilm formation is shown graphically in Figure 1. In the first stage, the microorganisms reversibly attach themselves to the surface. In some cases, if they encounter soil stuck to the surface or cracks in it, they may attach irreversibly. These microorganisms subsequently multiply and begin to excrete EPS, which envelops them in a matrix and protects them against external attacks. This matrix captures more soil and nutrients, thus allowing the biofilm to continue to grow. Once it reaches a certain size, pieces, which go on to colonize other surfaces or the foodstuffs themselves, thereby acting almost as microorganism reservoirs, begin to detach.

Biofilms provide four main benefits for the microorganisms, namely, (1) protection against the action of adverse agents, such as sanitizers, (2) the provision of nutrients for growth, (3) a reduced likelihood of dehydration (water represents ~95% of its composition), and (4) easier transfer of genetic material between microorganisms.

As these formations may contain pathogenic microorganisms and present greater resistance to disinfection, the probability of contaminating the foodstuff and provoking an outbreak of food poisoning is higher; therefore, the presence of biofilms on contact surfaces in the food industry is considered to be a clear health risk.

Cleaning: Dealing with Sinner's Cycle

As mentioned earlier, cleaning is the first step to follow for correct sanitization. However, as can be seen from Table 1, the main types of soil that can be found in a food-processing plant vary according to their source, composition, and removal characteristics.

In order to ensure correct cleaning, the five factors that constitute Sinner's cycle must be taken into account (Figure 2). These factors are water, temperature, chemical compounds, time, and mechanical action.

Table 1 Main types of soil found in the food industry

Criteria	*Type of soil*	*Examples*
Source	Animal	Blood, fats, proteins,
	Plant	Sugars, fats
	Mineral	Oxide, dust
Composition	Organic	Fats, proteins, sugars
	Inorganic	Mineral substances, carbonates
Removal methods	Soluble in water	Sugars, sodium chloride, calcium chloride, etc.
	Soluble in acid solutions	Iron oxides, Ca and Mg carbonates, milkstone
	Soluble in alkali solutions	Fats, proteins, blood
	Insoluble	Paints, inks, tars, wheel prints from pallet trucks, lubricants

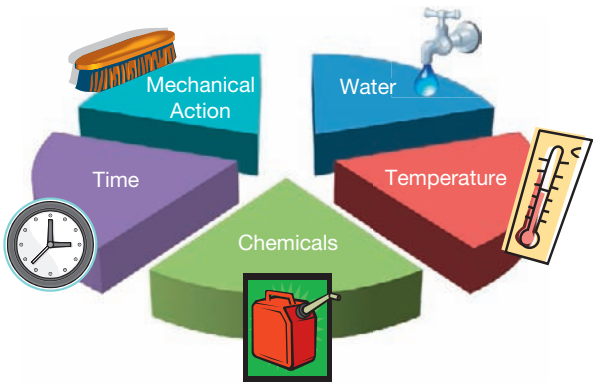

Figure 2 Sinner's cycle.

Water

Water is the most important and essential factor in the cleaning process as it acts as a vehicle via which cleaning is performed. It is the solvent for cleaning products and usually represents 95–99% of the cleaning solution. Moreover, it dissolves some of the components of soil. In addition, it often provides the mechanical energy (pressurized water or turbulence) or heat (temperature) for the process and is the vehicle via which remnants of soil dissolved, dispersed, or in suspension and remnants of cleaning products are removed.

In addition to its cleanliness, one of the most important characteristics of cleaning water to be taken into account is its hardness, in other words the concentration of Ca^{2+} and Mg^{2+} ions, which form insoluble salts that bind strongly to the substrate and result in the formation of limescale. As such, it is important to use soft water in the cleaning process and avoid the use of hard water ($CaCO_3$ concentrations higher than 250 ppm). Consequently, water hardness has an impact on the efficacy of cleaning and disinfection as well as on the performance of equipment at the plant. Some of the problems caused by hard water include a reduced efficacy of cleaning and disinfection products and a reduction in the efficacy of the equipment used to undertake thermal treatments (e.g., boilers or cookers) as a result of the deposition of limescale on the transfer surfaces and the contribution to biofilm formation on the equipment and surfaces.

Temperature

The use of heat is often necessary or advisable as it improves the efficacy of the mechanisms in the cleaning process. An increase in the temperature of the cleaning water increases the speed of the reaction between the detergent and the soil, reduces the viscosity of fats and oils and favors their dissolution, increases the solubility of the soluble components of soil, and decreases the surface tension of the water, thereby improving its wetting power. The recommended usage temperature for cleaning water is usually 15 °C above the melting point of fats, meaning that a water temperature of 55–72 °C should be used. However, it should be noted that higher temperatures may have the opposite effect as proteins are denatured at temperatures higher than 75–80 °C, thus allowing them to stick more strongly to the substrate to be cleaned and making their removal more difficult. In addition, very high temperatures may also degrade or deactivate some detergents and disinfectants, thereby reducing the efficacy of the cleaning and disinfection process.

Chemical Products

A cleaning product or detergent is a substance or mixture of substances that, when applied under specific conditions, is able to remove soil from the surface to be cleaned. The cleaning products used in practice are substances that adapt to the needs of each type of soil to be removed in the best manner possible. As such, the product to be used may vary markedly depending on the type of soil.

In order for a cleaning product to be as effective as possible, in addition to reacting chemically with the soil particles to be removed, it must also possess certain properties that enhance the cleaning action. For example, they must present a high wetting power, meaning that such products must contain surfactants that can reduce the surface tension of the cleaning solution in order to cover a greater cleaning surface and increase the penetrability of the product. They must also be able to dissolve, disperse, and/or emulsify the components of the soil and to reduce the hardness of water to prevent limescale formation (sequestering effect). In addition, they must be able to prevent redeposition of dispersed soil particles by adsorption and increase the rinsability in order to improve and promote fast drying of the surfaces to be cleaned. In general, no single substance possesses all these characteristics simultaneously. Therefore, cleaning solutions are usually a combination of different chemicals that present one or more of these actions to a greater or lesser degree. A list of these chemicals, with their most relevant actions, can be found in Table 2.

In light of the preceding text, an ideal detergent must be readily soluble in water at the temperature used, noncorrosive for equipment and facilities, nonirritant for the skin and eyes, nontoxic, free from added aromas, easy to rinse, biodegradable, stable when stored, inexpensive, and suitable for the type of soil and cleaning procedure. Detergents do not normally need to be bactericidal, although some compounds possess this property (Table 3).

Time

The cleaning process is a chemical reaction in which the cleaning products react with the soil to be eliminated. As with all chemical reactions, this is not instantaneous and a period of time is required for it to occur and to obtain an effective cleaning process.

The time that must be used for the cleaning process mainly depends on the temperature at which the cleaning process is performed and the capacity of the cleaning product. Thus, more powerful (or more concentrated) products require shorter contact times.

Time is normally a limiting factor related to the production capacity of the food-processing plant. However, it must be remembered that a lack of time must be compensated with more energy, higher temperatures, and/or higher concentration of cleaning agents (Sinner's cycle).

Mechanical Action

Mechanical energy is important during the cleaning process as it enhances separation of the soil from surfaces, including biofilms, divides and breaks up soil particles, and maintains them in suspension, thus preventing it from being redeposited. This mechanical action can be applied using various methods depending on the type of surface to be cleaned. For example, water at high pressure, manual scrubbing, circulation of the detergent solution through closed circuits, and automatic washing can be used.

Spraying water onto the surface to be cleaned under pressure means that soil is more readily swept away, separated, and broken down into small particles, thereby favoring its dispersion. Pressurized water can be sprayed manually when used to clean surfaces of the food-processing plant or equipment surfaces or automatically when washing machines are used to clean molds or boxes, for example. If a manual system is used, the plant must be equipped with a sufficient number of hookup stations with their corresponding hoses and spray guns, which themselves should be equipped with different types of nozzles in order to perform different functions during the cleaning protocol, such as washing, foam generation, or rinsing. These hookup stations are normally used to apply water and chemicals, either detergents or sanitizers. The working pressure used may be low (3–6 kg cm^{-2}) when water from

Table 2 Main cleaning substances used in the food industry

Cleaner/ detergent	*Action against soil*	*Most commonly used*	*Effects*
Alkali	Displacing, emulsifying, saponifying, and peptizing agents Prevents limescale	Sodium hydroxide and caustic soda (lye)	Corrosive to aluminum and galvanized steel (especially lye)
Phosphates	Emulsifying, peptizing agent, softening water, and preventing soil deposition and mineral precipitation		Harmful to the environment
Wetting/ surfactants	Promote wetting and rinsing Emulsify, disperse, and suspend soil such as oil as small droplets in water		Are noncorrosive, soluble in cold water, and not affected by hardness
Anionic	pH-neutral, compatible with acids or bases		
Nonionic	Better for oils Some will suppress foaming		
Cationic	Wetting agents	Typically quaternary ammonia compounds (QACs)	
Acids	Good for cleaning alkali soil and removing minerals, especially calcium and magnesium		
Inorganic		Phosphoric acid, hydrochloric acid, and sulfuric and nitric acids	Strong and corrosive, not recommended for food plant use
Organic		Strong and corrosive, not recommended for food plant use	More commonly used in food plants
Chlorinated cleaners	Increase peptization, minimize limescale Make alkaline cleaners more effective		
Chelating agents	Control mineral deposits through water softening Displace soil through peptization	Ethylenediaminetetraacetic acid (EDTA)	

Table 3 Main characteristics of an ideal cleaner or detergent used for food sanitation

Readily soluble in water at the use temperature
Noncorrosive for equipment and facilities
Nonirritant for the skin and eyes
Nontoxic
Free from added aromas
Easy to rinse
Biodegradable
Stable when stored
Inexpensive and easy to handle
Suitable for the type of soil and cleaning procedure
Sanitizing or disinfectant capability not necessary

the distribution network is used; medium (15–30 kg cm^{-2}), usually applied to generate foam; or high (>30 kg cm^{-2}) when high-pressure lances are used or in compact washing machines. High-pressure lances should not be used when food-processing plant surfaces are cleaned as they generate aerosols containing microorganisms in suspension, which may recontaminate zones that have already been cleaned.

During manual scrubbing, the mechanical action is generated by the operator performing the cleaning, using brushes, scourers, brooms, etc. Manual scrubbing is usually used for machinery in which water cannot be freely used, such as those that need to be dismantled in order for the internal parts to be correctly cleaned and also as support for other methods when the soil is deeply encrusted.

A cleaning in place system comprises circulating a cleaning solution through closed circuits that cannot be reached using standard methods, such as the cleaning of ducts, tubes, and pasteurizers. Circulation of a liquid (water or cleaning solution) through a tube at the appropriate flow rate generates turbulence by collision of its molecules against the walls of the tube. The solution is circulated in the opposite direction to that used normally in order to remove the soil from difficult regions such as bends and dead spaces. This type of cleaning is commonly used in liquid foodstuff processing plants, such as those used to manufacture fruit juices or dairy products.

Automatic washing is performed using rotating brushes that scrub the surface to be cleaned, thereby removing the dirt. This method is normally used to clean large surfaces, such as floors or conveyor belts. Floor cleaners are usually equipped with a brush to collect the water and dry the surface that has been cleaned. The use of squeegees and shovels is preferred for floor cleaning rather than brooms or brushes as squeegees are easier to clean than the latter, which can become encrusted with food material.

General Procedure

The cleaning process usually comprises three different phases: separation of the soil from the surface, dissolution of this soil in the cleaning solution, and prevention of soil redeposition. The factors described earlier play a role in these three phases to a greater or lesser extent. In the first phase, it is important to apply heat and mechanical energy together with the detergents in order to separate the soil stuck to the surface. Some of the compounds present in the formulation of the cleaning product play a role in the second phase by dissolving the soluble

components of the soil, emulsifying any fats and dispersing insoluble soil particles. Finally, the aim of the third phase is to retain the soil in the cleaning solution and prevent it from being redeposited on the surface to be cleaned. To this end, it is essential to maintain the cleaning solution in motion to prevent the precipitation of mineral substances and, finally, to remove the cleaning solution together with the soil from the surface to be cleaned. Once the surface has been cleaned, it is ready for the following disinfection phase.

To prevent cross contamination with the materials used during the cleaning phase, they must be regularly deep-cleaned and disinfected. Similarly, the cleaning solution must be changed regularly in order to maintain its active components in their optimal state.

Sanitizing and Disinfection

Once the cleaning step, including rinsing, has been correctly carried out, the process can proceed with the sanitizing step. It has been observed that some pathogens are able to survive for long periods of time on hard, nonporous surfaces. This is the case for hepatitis A virus and rotavirus, both of which can survive for up to 1 month on this type of surface. Moreover, noroviruses can survive for around 40 days on the same surfaces, and some foodborne pathogens, such as *Listeria monocytogenes*, can persist for periods ranging from several months to up to 10 years.

As was mentioned in the preceding text, the main aim of cleaning is not to kill or eliminate the microorganisms present in the food-processing plant. However, if cleaning is performed regularly and effectively, a significant reduction in the number of microorganisms of around 2–3 logs can be achieved. Moreover, additional agents, known as sanitizers and disinfectants, are required to sanitize or disinfect the surfaces of such plants. Sanitization follows cleaning and comprises the application of heat or chemicals to a correctly cleaned and thoroughly rinsed surface.

In a processing plant, it is necessary to distinguish between food-contact and non-food-contact surfaces (NFCS). Food-contact surfaces (FCS) are those belonging to any equipment or utensil that normally comes into direct contact with foodstuffs or surfaces normally in contact with them, such as food-processing equipment, conveyor belts, knives, cutting boards, and operators' hands. In contrast, non-NFCS are those that do not come into direct contact with food in the processing plant, such as equipment casings, floors, drains, walls, and walkways, among others.

Definitions

Although they are both used in the sanitization/disinfection step and normally with the same meaning, it is important to distinguish between sanitizers and disinfectants.

Sanitizers reduce, but do not necessarily eliminate, the number of microorganisms on surfaces that come into contact with food to levels considered safe according to public health codes or regulations. Sanitizers are not effective against viruses and fungi. They are ordinarily used in food-processing, food-handling, preparation, and service industries and include FCS and NFCS. Sanitizers tend to be used at lower concentrations for a shorter period of time and no perfumes are allowed in food-contact sanitizers.

Sanitizers with disinfectant properties used to sanitize food-contact surfaces must reduce the bacterial counts for specific bacteria by 5 logs or 99.999% in 30 s at 20 °C, whereas food-contact sanitizers with no disinfectant properties, or NFCS sanitizers, must only reduce bacteria by 3 logs or 99.9% within 5 min at the same temperature.

Disinfectants are used to destroy or irreversibly inactivate microorganisms, including bacteria, fungi, and viruses, but not necessarily spores. Disinfectants are used on all hard surfaces of a food-processing plant (NFCS), normally at much higher concentrations and with a longer contact time than sanitizers. In addition, perfumes can be added to their composition. The efficacy of disinfectants is based on the statistical significance of killing test bacteria on carriers, measuring the percentage of nonviable microorganisms in a set of test tubes under specific conditions.

Finally, sterilization is the process of destroying all living microorganisms, including bacterial spores. These compounds must demonstrate an absence of growth in test samples or a reduction of 6 logs or 99.9999% and include specialized chemicals such as glutaraldehyde and formaldehyde. Dry heat (use of dry-heat ovens) and moist heat (use of steam under pressure) are also used for sterilization.

These compounds show a different effect according to the different target microorganisms and resistant forms. Thus, their effect could be bacteriostatic (preventing the growth of a specific group of microorganisms but not necessarily killing them), bactericidal (killing a specific group of microorganisms), fungicidal (effective against molds), virucidal (effective for eliminating viruses), or sporicidal (destroying spores).

Methods

Sanitization may be achieved by physical or chemical methods, with heat and irradiation being the most widely used physical methods. Both dry heat and wet heat are used in the food industry, with the last being most commonly used to sanitize the pipelines of fillers or pasteurizers, for instance. Hot water at 80 °C or steam must be used, and it is important to ensure that all points of the pipeline reach this temperature for a minimum time. This method is normally combined with chemical sanitization. One disadvantage of the wet heat method is that it favors the formation of limescale when used with hard water.

UV irradiation is a disinfection method that uses short-wavelength ultraviolet radiation (UV-C) at 254 nm that is harmful to microorganisms, killing them by disrupting their DNA and leaving them unable to perform vital cellular functions. This method is used to disinfect water, air, and some food-processing surfaces, such as conveyor belts or some packaging materials.

However, chemical sanitization is more frequently used in food-production facilities than thermal or irradiation techniques. This method includes the use of active substances (chemicals) that are applied via aerosol or smoke to sanitize or disinfect ceilings and the air in the food-processing plant or in solution for other foods and NFCS.

Chemical Sanitizers Used in the Food Industry

A sanitizer/disinfectant contains one or more active compounds (chemicals with a bactericidal effect) together with other chemicals that add some interesting properties that facilitate their application and can also produce a synergistic effect. This is the case of certain compounds that increase wettability, thereby improving the penetrating power of the solution and favoring contact between the sanitizer and microorganism, substances with binding properties that decrease the negative effect of hard waters on sanitizer activity, pH correctors, and foam-forming compounds. The main characteristics for an optimal sanitizer/disinfectant are listed in Table 4.

Table 4 Main characteristics for a sanitizer/disinfectant used in the food industry

Easy application
Low toxicity
Noncorrosive
Good penetrating power
Fast acting
Not adversely affected by organic matter
Active before and after dilution with hard water
Compatibility with other chemicals and construction materials
Broad spectrum of activity toward Gram-positive and Gram-negative bacteria, fungi, and viruses
No harmful
No offensive odor
Stability (long shelf life)
Not persistent in the environment
Demonstrated residual activity
Cost-effective

Several chemical compounds can act as sanitizers or disinfectants depending on the concentration used. The most commonly used sanitizers in food-production environments are chlorine, quaternary ammonium compounds (QAC), and iodine. The dose, use conditions, and main advantages and disadvantages of these sanitizers are summarized in Table 5.

In order to eliminate the use of harsh chemicals at food-processing facilities, which often have negative environmental and safety impacts, various new methods have been proposed. These include the use of ozone (O_3), which has a demonstrated bactericidal effect against some foodborne pathogens and other microorganisms, the use of which was approved by the FDA in June 2001. Ozone has the advantage of not producing toxic residues on treated surfaces, although it can be corrosive at high concentrations on some types of surfaces and overexposure during its generation can result in bodily injury.

Electrolyzed water (EW) can be easily produced using common salt and an apparatus connected to a power source. The current, along with ion-selective membranes in electrolytic cells, produces two types of solution: sodium hydroxide, a high-pH, noncorrosive dirt and grease-cutting cleaner, and hypochlorous acid, a low-pH, high-dissolved-oxygen, chlorine-containing sanitizer and disinfectant. EW is effective against a wide range of microorganisms, including viruses, fungi, and spores, within 1–15 s of application and has been proved to be 80% more powerful at 50 ppm than chlorine bleach at 200 ppm. As the size of the machine is quite small, EW can be produced at the site where it is to be applied.

Peracetic acid (PAA), which is produced by the reaction of acetic acid with hydrogen peroxide, has been promoted as a potential chlorine replacement. The recommended usage dose concentration for food-contact surfaces is 150–200 ppm. At

Table 5 Main sanitizers used in the food industry

Sanitizing compound	*Conditions*	*Advantages*	*Disadvantages*
Chlorine			
Sodium hypochlorite, chloramines	50 ppm (maximum 200 ppm) in water 24–38 °C; 7 s >200 ppm rinsing is necessary	Effective against broad spectrum of vegetative bacteria and viruses (norovirus) Not affected by water hardness Effective in pH range of 6–8 Inexpensive (generally)	Corrosive, irritating to the skin Effectiveness decreases with increasing pH Activity decreases in the presence of organic matter
Iodine			
Iodine together with a stabilizing agent or carrier (iodophors)	12.5–25 ppm in water at least 24 °C, 30 s >25 ppm rinsing is necessary	Not affected by water hardness Active against a wide variety of non-spore-forming bacteria (vegetative bacteria and viruses) Relatively stable in the presence of organic matter Optimum pH range is 5.0 or less	Bactericidal effectiveness decreases greatly with an increase in pH (pH 7) Gram-negative bacteria may be able to survive Not very effective against spore-forming bacteria and bacteriophages Cannot be used at temperatures higher than 40 °C Discoloration of surfaces
Quaternary ammonium compounds			
Benzalkonium chloride	Maximum 200 ppm in water at least 24 °C; 30 s Rinsing is necessary	Nontoxic, odorless, colorless, noncorrosive, nonirritating Stable to heat and relatively stable in the presence of organic matter Active over a wide pH range Quite active against thermoduric organisms	Retarded effect on coliforms and psychrophilic bacteria Inactivated by proteins and soaps Not compatible with anionic detergents and hard water salts Not effective against bacteriophages

this concentration, it is capable of destroying microorganisms and removing milk stone deposits (dairy industries) and limescale, suppressing odors and removing biofilms from food-contact surfaces. However, this compound has no sporicidal effect and is more expensive than other sanitizers or disinfectants.

Factors Affecting the Effectiveness of Sanitization

When designed, a good sanitization procedure must take into account several factors in order to be more effective when applied at the food-processing plant. These factors include (1) previous cleaning status; (2) surfaces, facilities, and equipment to be sanitized; (3) type, counts, and possible niches of expected microorganisms; (4) concentration and stability of sanitizers used; and (5) physical parameters, such as temperature, pH, and water quality.

Previous cleaning status: It is important to properly clean and rinse the surface prior to sanitization in order to avoid the presence of organic and/or inorganic inactivators that may be present in dirt or detergent residues from an improperly cleaned surface. The presence of organic matter (food residues) on the surface can interfere with the antimicrobial activity of sanitizers and disinfectants by reducing the level of activity or by protecting the microorganisms from attack by acting as a physical barrier. Moreover, organic or inorganic debris can favor the appearance of biofilms on incorrectly cleaned surfaces. In this sense, the microbial load can affect sanitizer activity, with more time being needed when the number of microorganisms present is higher.

Surfaces: Before using a sanitizer or disinfectant, it is very important to determine its compatibility with the surfaces on which it will be used as these chemicals tend to react differently with plastic, glass, metal, and wood. Iodine, for instance, can be corrosive when applied to a metal surface and may stain porous surfaces and some plastics. Frequent or extended exposure to alcohols can also damage plastic materials. The surface area, topography, and geometry of the surface also have a major influence on the effectiveness of sanitizers and disinfectants. Thus, surfaces with cracks, pits, or crevices are more difficult to sanitize and disinfect than smooth surfaces because penetration to all areas may not occur. Food-contact equipment with multiple parts must be dismantled to ensure that all parts are correctly cleaned and sanitized.

Microorganisms: The different types of microorganism must be taken into account before applying a sanitizer or disinfectant. Gram-negative bacteria such as *E. coli*, *Campylobacter* spp., and *Salmonella* spp., for example, are more difficult to kill than Gram-positive bacteria due to the presence of the outer membrane, which act as a 'barrier' to sanitizers and disinfectants. These compounds also vary in their effectiveness against yeasts, molds, fungi, and viruses. Among the latter, nonenveloped viruses such as noroviruses or hepatitis A virus are less susceptible to disinfection than enveloped viruses because of their hydrophilic nature and lack of lipids. Finally, spores are more resistant to disinfection than vegetative cells due to the presence of the spore coat and cortex, which protects them.

Sanitizers and disinfectants (*chemicals*): When applying a sanitizer or disinfectant, it is very important to take into account the manufacturer's instructions for use, especially as regards concentration and contact time. Too low a concentration will result in an inadequate reduction of microorganisms and too high a concentration can be toxic, can be corrosive to equipment, and can lead to less effective cleaning over time. The more concentrated the chemical used, the greater its efficacy and the shorter the time required to kill the microorganism, with the exception of iodophors. As disinfectants are used at a higher concentration than sanitizers, they can achieve complete destruction of microorganisms, whereas sanitizers only achieve a 3–5-log reduction. To kill microorganisms, cleaned items must be in contact with the disinfectant for the time recommended by the manufacturer. Sanitizers and disinfectants have a minimum contact time for exerting their activity, with longer contact times generally being more effective than shorter ones.

Physical factors: The optimal temperature range over which chemicals are more effective is 20–50 °C. Nevertheless, the activity of most sanitizers and disinfectants increases with temperature, although there are some exceptions. For instance, chlorine-based solutions have higher killing rates at higher temperatures, although their corrosiveness also increases and the vaporization of chlorine is favored, thereby decreasing their effectiveness. As such, care must be taken not to exceed the optimal temperature range. In contrast, QACs are stable at high temperature. An increase in pH improves the antimicrobial activity of some sanitizers and disinfectants, such as glutaraldehyde and QACs, but decreases the activity of others, such as phenols, hypochlorites, and iodine. As mentioned previously, water hardness also affects the optimal performance of sanitizers and disinfectants, reducing the destructive activity of some of them.

Rinsing

It is important to determine whether rinsing is needed for each sanitizer and disinfectant after application and to follow the product label and manufacturer's instructions. This step is concentration-dependent, and rinsing is usually compulsory when a sanitizer is applied to FCS but not when applied to NFCS. In some food-processing plants, it may be of interest to allow the sanitizer to act overnight on FCS, for instance, to maintain a residual effect and avoid bacterial growth during night. However, it is always necessary to rinse the sanitizer off to avoid product contamination before starting production.

Resistance and Persistence

Phenotypic resistance to disinfectants at bactericidal concentrations occurs among all attached cells irrespective of whether they are involved in a biofilm or not. When an attached community of bacterial cells on surfaces from high-risk chilled food-processing plants is subjected to a deep cleaning and disinfection, only a few are able to resist. This fraction of surviving cells decreases as the disinfectant concentration increases but is greater for old established communities, which are referred to as 'persistent microorganisms.' These cells become persistent when their growth rate exceeds destruction by cleaning and disinfection, and the best way to avoid their presence is to prevent bacterial growth.

The most important recommendation is that premises and equipment must be hygienically designed so that bacteria cannot find harborage sites where they are protected from the action of chemicals. These sites are hard to eliminate and only a low initial bacterial load is required for subsequent persistence. In such cases, an early cleaning and disinfection intervention will be the only way to avoid persistence. According to this hypothesis, some authors believe that there are no bacterial strains with unique properties that lead to persistence, but simply harborage sites in food-processing plants and equipment where these bacteria can persist.

As most sanitizers and disinfectants are nonspecific, the development of resistance is mostly caused by innate factors, chromosomally controlled properties naturally associated with the organism, that include the existence of impermeable cell barriers that prevent the penetration of the sanitizer, cellular efflux mechanisms, lack of a biochemical target for antimicrobial attachment or microbial inactivation, and inactivation of antimicrobials by microbial enzymes. If resistance is discovered by specific testing, a disinfectant with a different active ingredient must be used. Higher concentrations of the same disinfectant are not recommended as this could promote the emergence of more resistant cells.

Finally, two key points must be taken into account when assessing the efficacy of a C&D procedure in order to avoid misinterpretation of the results. The first of these is that swabs used to sample surfaces do not detach all the microbial cells, and the second is that although some cells are not culturable, they may still be alive. As such, it is important to make use of a combination of not only culture-dependent but also culture-independent methods when sampling environmental food-processing facilities and equipment.

Conclusion

In all food manufacturing plants, cleaning and disinfection (sanitization) plays a key role in order to reduce or eliminate most of the microbiological hazards associated with food-production environment. This fact is extremely important to design a proper and effective HACCP system that guarantees the shelf life and safety of food products. Moreover, in order to obtain an optimum sanitization status of the food-processing plant, other prerequisite programs must be properly implemented such as proper layout of the food plant, waste treatment, pest control, and hygienic design of food-processing equipment and especially its implication to the food plant personnel.

See also: Biofilms; Food Environment; Foodborne Pathogens; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto.

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Relevant Websites

www.foodsafetymagazine.com – Food safety magazine

Saponins

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Background

Saponins are a heterogeneous group of glycosides that are widely distributed in plants of agricultural importance, particularly legumes. Many of these legumes are staple items of the human diet. Foods particularly rich in saponins are soya beans (*Glycine max*), chickpeas (*Cicer arietinum*), and beans derived from *Phaseolus vulgaris*.

When saponins are agitated in water, they form a soapy lather. Other properties generally ascribed to this wide group of compounds are hemolytic effects on red blood cells, cholesterol-binding properties, and a bitter taste. These properties characterize particular types of saponins and are not necessarily shared by all members of the group. From a biological point of view, some of these properties are beneficial, whereas others are considered to be adverse.

Of particular interest is the observation that dietary saponins reduce plasma cholesterol levels in primates, thus having the potential to lower the risk of coronary heart disease in humans.

Chemical and Physical Properties of Saponins

Saponins consist of an aglycone unit linked to one or more carbohydrate chains (Figure 1). The aglycone or sapogenin unit consists of either a sterol or the more common triterpene unit. In both the steroid and triterpenoid saponins, the carbohydrate side chain is usually attached to the 3-carbon of the sapogenin.

Saponins possess surface-active or detergent properties because the carbohydrate portion of the molecule is water-soluble, whereas the sapogenin is fat-soluble. The stability and strength of forage saponin foams are affected by pH, and this may have an effect on the development of bloat in ruminants. Saponins are remarkably stable to heat processing, and their biological activity is not reduced by normal cooking.

Isolation of saponins from plant material involves extraction with a polar solvent after removal of lipids, with petroleum ether or chloroform, followed by various purification techniques. A number of chromatographic procedures have been used to separate individual saponins.

The analysis of saponins is complex and potentially subject to considerable errors during their isolation, separation, and quantification stages. Thus, many early reports of the saponin content of food plants and processed food should be treated with caution. The data presented in Table 1 were obtained using rigorous methodology and are the most reliable data available at present. More recent studies have shown that many legume seeds contain several saponins, for example, five different saponins have been separated from soya beans.

Biological Effects of Saponins

Ingested saponins can influence animal performance and metabolism in a number of ways.

Sensory Properties of Saponins

Recent studies have shown that a bitter or astringent taste is related to amounts of soya saponin I isolated from pea and soya flour. It is possible that saponins are a contributing factor to the undesirable organoleptic properties that humans associate with some legumes and legume products. The bitter taste of saponins may be responsible for the low palatability of lucerne to ruminants and may explain the reduced feed intake observed in many experiments when using this material as forage. Many stocks will discriminate against feeds containing high levels of lucerne meal.

Erythrocyte Hemolysis

Saponins have pronounced hemolytic properties when given intravenously, the degree of effect on the red blood cells varying among different mammalian species. The release of hemoglobin from red blood cells is the direct result of the interaction of saponins with membrane-bound sterols, which causes an increase in the permeability of the plasma membrane, bringing about the destruction of the cell.

The hemolytic activity of saponins has been widely used as a means of detecting and assaying saponins in plant materials. The potentially toxic effects of intravenous injection of saponin extracts have resulted in this class of compounds being regarded as antinutritive factors in foods. Their low oral toxicity and the potentially useful nutritive value in foods have only recently been appreciated.

Effects on Blood and Tissue Cholesterol Levels

Since many legume saponins form insoluble addition complexes with cholesterol, the effects of dietary saponin on cholesterol might be expected. Saponin in the diet of chicks has been reported to reduce plasma cholesterol in cholesterol-fed animals. Saponin extracted from *Quillaia saponaria* has been shown to reduce liver (but not plasma) cholesterol levels in chicks. An exhaustive series of experiments, with a variety of saponins, have consistently demonstrated cholesterol-lowering effects. These include the feeding of lucerne saponins to rats, rabbits, and monkeys. Some workers have suggested that dietary saponins do not have any effect on plasma cholesterol concentrations. This is perhaps to be expected in experiments with rats that received no dietary cholesterol.

Experiments on humans have produced variable results. Different saponin levels in a dietary supplement based on soy flour had no effect on plasma cholesterol levels in

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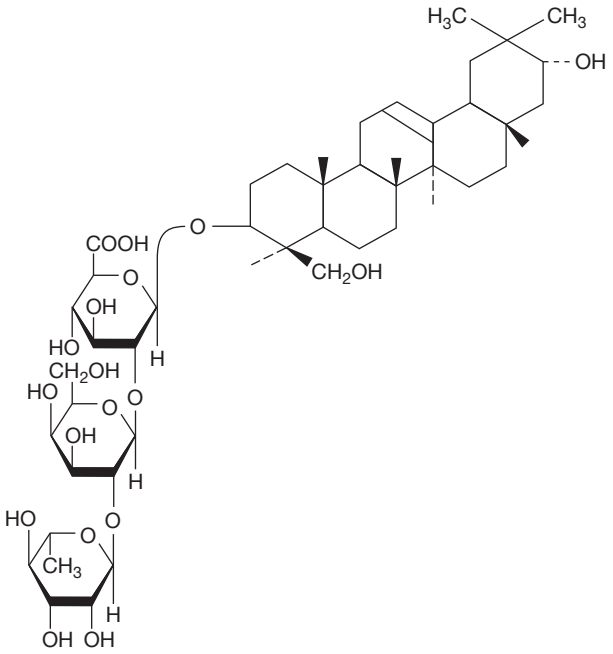

Figure 1 Structure of a typical saponin (from soya beans). Reproduced from Saponins. In: Macrae, R., Robinson, R. K., and Sadler, M. J. (eds.) *Encyclopaedia of Food Science, Food Technology and Nutrition*, Academic Press, 1993.

Table 1 Saponin content of some legume seeds

Legume	*Saponin content (g kg^{-1} of dry matter)*
Chickpeas (*Cicer arietinum* L.)	2.3
Green pea (*Pisum sativum*)	1.8
Haricot bean (*Phaseolus vulgaris*)	4.1
Kidney beans (*Phaseolus vulgaris*)	3.5
Lentils (*Lens culinaris* Medik.)	1.1
Mung bean (*Vigna radiata* L.)	0.5
Runner bean (*Phaseolus coccineus* L.)	3.4
Soya beans (*Glycine max* L. Merrill)	6.5
Yellow split pea (*Pisum sativum*)	1.1

hypercholesterolemic men. As the experiment was conducted on free-living subjects, it cannot be guaranteed that the subjects actually consumed the experimental diets.

In a more closely supervised trial, with subjects having normal blood cholesterol levels, it was found that a dietary supplement containing saponins did not have a significant effect on plasma cholesterol level. Increased fecal excretion of bile acids and neutral sterols was observed. Foods containing saponins, or diets supplemented with saponins, have been shown to reduce blood cholesterol levels in humans under conditions that would be expected to induce high levels of blood cholesterol.

Mode of Action

Saponins remain within the gastrointestinal tract. Some interact directly with dietary cholesterol, producing an insoluble complex that prevents the cholesterol being absorbed. Others appear to affect cholesterol metabolism indirectly by interacting with bile acids. An increased fecal excretion of bile acids is observed in response to feeding additional saponins in the diet. Bile acids thus diverted from the enterohepatic cycle would be replaced by hepatic synthesis from cholesterol.

In the digestive tract, saponins form mixed micelles with cholesterol and bile salts; their hydrophobic triterpene or steroid groups stack together like small piles of coins. These micelles are then too large to pass through the intestinal wall.

Bile salts normally pass through the wall of the small intestine by both passive diffusion and active transport. Passive diffusion takes place along the entire length of the ileum and jejunum; active transport is confined to the terminal ileum. Saponins can interact with cell membranes, as shown by their hemolytic activity. It is possible that they may also have an effect on the cell membranes of the intestinal mucosa. However, the effects of saponins on both passive absorption and active transport can be explained simply as being due to the reduction in the concentration of free bile acid, because bile acids assist lipid absorption. Low concentrations of free bile acids would also impair the efficiency of lipid absorption and presumably affect the absorption of fat-soluble vitamins. Thus, there may also be a significant metabolic effect within the animal as well as in the digestive tract.

Effects on Growth

When lucerne saponin was added at high levels to the diets of monogastric animals, reduced feed efficiency and growth rates were observed. It is clear that species differ considerably in their responses to dietary saponin, for example, poultry are more sensitive than rats. In contrast, soya bean saponins had little effect on the growth rate of experimental animals. Some saponins increase the permeability of the small intestinal mucosal cells, thereby inhibiting the active transport of some nutrients, but at the same time, they facilitate the uptake of materials to which the gut would normally be impermeable.

The biochemical mechanism that accounts for the growth-depressing effects has not been fully identified. In at least one experiment, the addition of 1% cholesterol to the diets of chicks completely overcame growth depression produced by 0.3% saponin.

It is possible that, in addition to their effects on lipid absorption, saponins also affect chymotrypsin and trypsin activity, which would affect the absorption of protein.

In addition to observing reduced intestinal uptake of cholesterol in rat intestinal perfusates on the addition of soy saponin extracts, some workers have also observed a significant reduction in cholate and glucose uptakes. It was observed that the saponins could be washed out of the intestinal lumen, suggesting that inhibition, at least in the short term, was not caused by modification of, or damage to, the intestinal mucosa.

Metabolism of Saponins

Toxicity studies indicate that only very low levels of saponin absorption occur. Saponins are between 10 and 1000 times more toxic when administered intravenously than when given orally. Destruction of saponins in the digestive tract of both ruminants and monogastric animals has been observed.

Saponins are degraded by rumen bacteria and by microflora found in the cecum of rats, mice, and chicks. Since saponin in the cecum is past the major sites of absorption, the release of sapogenins and sugars is considered to be insignificant.

Conclusion

The substantial evidence that saponins from a number of plant species can reduce plasma cholesterol levels in man is likely to encourage further interest in these plant foods. It is generally recognized that the overall nutritional value of many Western-type diets would be considered improved if more legumes or legume-based products were consumed regularly. The acceptance of more legumes in Western-type diets is limited by undesirable taste characteristics, some of which may be due to the higher levels of saponin found in many legume seeds.

There is little doubt that saponins can be incorporated into human diets at levels that can give a beneficial effect and would not entail a risk of acute toxicity. The fact that saponins can increase the permeability of intestinal mucosa raises the possibility of interesting nutritional and pharmacological uses.

See also: Cereals: Dietary Importance; Cholesterol: Factors Determining Blood Cholesterol Levels; Fats: Classification and Analysis; Legumes in the Diet; Protein: Digestion, Absorption and Metabolism; Pulsed Electric Fields; Soy Beans: Properties and Analysis.

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Satiety

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Defining Satiety

Satiety can only be understood with respect to the entire appetite control system. Thus, the state of satiety is reached by consuming an adequate amount of food in a specific period of time. Food intake is driven by a powerful biological need to obtain sufficient energy to sustain life. However, meals are initiated before significant food deprivation is experienced and intake terminated before nutrients are delivered to the system. Recent theories of what controls food intake have shifted from purely homeostatic to the proposal that there are both direct and indirect controls of food intake. An idea that was first suggested by Smith in his seminal article from 1996. In order to provide a context for research on satiety, a short historical background will be provided and then evidence from alternative theories discussed; then, sensory-specific satiety (SSS) is illustrated as one example of satiety linked to specific foods eaten. The implications for this research for the consumer will be discussed in relation to both weight management and other benefits of enhanced satiety.

Homeostasis

The early theories of food intake were predicated on the control of eating as a primary biological drive, a problem of nutritional homeostasis. Theories of the 'wisdom of the body' promoted by Walter Cannon and developed from Claude Bernard's concept of the 'milieu intérieur' in which internal systems function in harmony to achieve a stable, optimal state suggest that food intake is 'regulated.' Thus, the motivation to eat was initially conceptualized as an emergent property of a system with falling energy or nutrient stores. Put simply, food is sought when the animal is in a state of hunger caused by periods of deprivation. This conceptualization of the body defending against external threats such as famine, nutrient depletion, and seeking to maintain a stable energy supply to prevent deterioration provides the essence of a homeostatic system.

However, these early theories were then superseded by a greater focus on behavioral neuroscience in which the neural circuits and gut pathways to the brain were thought of as responding to the presence of nutrients both in the environment (behavioral responsiveness and indirect controls) and in the gut (direct controls). In this theoretical formulation, both direct and indirect controls of meal size are considered alongside the neural mechanisms underpinning a complex appetite control system.

It is clear that while body fat stores appear to be well defended and maintained over time, food intake is variable and can occur in response to environmental (indirect) cues. Thus, in laboratory settings, animals will eat in anticipation of deficit and in response to a conditioned stimulus even when tested in a satiated state. The idea then that food intake occurs *only* in response to low energy supplies, in order to maintain homeostasis, has been replaced with a more complex model of food intake acknowledging the primary importance of maintaining energy stores and at the same time the strong influence of environmental cues to initiate eating when food is available or when cues are associated with feeding.

Satiety Cascade

A central component of this more complex model is embodied within the notion of 'negative feedback,' a concept borrowed from engineering. Any behavior that is goal-directed and purposeful such as eating requires negative feedback in order to modulate the drive. Thus, in the example of a thermostat, energy is released in the form of heat to achieve the set temperature, and once, this is achieved, the heating is turned off. Similarly, satiation and satiety form part of the negative feedback loop from the gut to the brain to slow and then to stop eating. The problem for this analogy is that unlike the thermostat, there is an interval between seeking food, securing the food, eating the food, and then digesting the components of the meal to achieve the goal of satisfying the need for nutrients. Therefore, in the case of food intake, satiation and satiety are initiated in advance of metabolizable energy being made available, an observation elegantly explained by Blundell in his text on Physiological Psychology in 1975. The negative feedback loop relies upon sensory (smell and taste), mechanical (distension), and chemical (peptide) signals from the gut to the brain to prompt both satiation and satiety. In humans, satiety is also influenced by cognitive processes broadly incorporated into learning (expectancy) and memory (monitoring current and previous meal intake). It is this cognitive component of the satiety cascade that allows humans to control appetite, to resist the temptation to eat when food is so freely available in the environment, and to use what is known about satiety to select foods with a higher satiating capacity to enhance satiety for weight management (Figure 1).

The Negative Feedback Loop

If it is accepted that satiation and satiety provide negative feedback to slow and to stop eating behavior, how then are these processes defined and how are they distinguished?

Satiation is the process by which the drive to eat begins to wane. It is characterized by eating rate slowing down and by sensory, gastric, and physiological signals combining to inhibit further eating. Satiation determines how much is eaten within the bout or episode (meal size). Satiation is influenced by the characteristics of the food eaten, for example, it is thought that

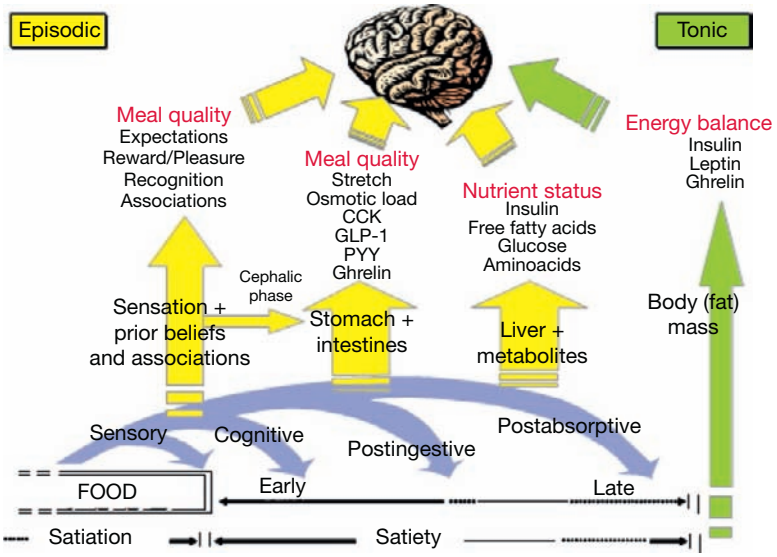

Figure 1 Satiety cascade as adapted by Blundell and Mela.

masticatory effort will enhance satiation. Liquids are less satiating than semisolids, which are less satiating than solid foods. Using this example, when foods are given to participants with the same underlying constituents – such as apple, given as juice, puree, or whole – the resulting effect on subsequent intake is that whole apple reduces energy intake due in part to the oral metering involved in chewing the solid form compared with the puree or liquid. Thus, a consistent finding across studies that systematically manipulate food form is that greater chewing or oral processing enhances satiation and satiety. This is confirmed by the elegant studies performed by Cees de Graaf and his colleagues at Wageningen University.

Satiety is the later state of inhibited eating that follows satiation and determines the interval until the next meal episode and how much is eaten at the next eating occasion. During this process, signals arising from the presence of nutrients in the gut feedback to the brain to inhibit further intake. Like the related process of satiation, satiety is influenced by the characteristics of the food eaten. For example, protein is considered the most satiating macronutrient and high protein foods are known to reduce subsequent food intake compared with high fat foods. Similarly, foods high in fiber are known to promote satiety, in part due to an increase in the release of satiety peptides – an observation made by Johannson and colleagues in 2013.

As the stomach is distended and the meal progresses, gut hormones are released, which then form part of the satiety circuit. One of the most well characterized of these signals is cholecystokinin (CCK). This is a peptide hormone secreted by I-cells of the epithelium of the small intestine. When the stomach is distended, CCK is released and the presence of nutrients delivered directly into the duodenum also stimulates the release of CCK. The pioneering work of James Gibbs and Gerard Smith in the Bourne Laboratory at Cornell University Graduate School of Medical Sciences from the early 1970s demonstrated the impact of CCK injected intraperitoneally into fasted rats, namely, that despite the fasted state known to drive intake, CCK inhibited food intake by up to 50% in the first 30 min of free access to food. This team showed that CCK induces the same behavioral satiety sequence as a meal, it slows gastric emptying, and it reduces food intake. There is now a substantial body of evidence linking CCK to satiety across many different species. Thus, acute administration of CCK to humans and other animals inhibits food intake. However, in the longer term, chronic administration fails to sustain this effect on satiety, and in experimental models, animals become resistant to the satiety-inducing effects of CCK. Thus, it has been suggested by Scott and his colleagues that there is little therapeutic benefit of CCK for the treatment of obesity. Nevertheless, CCK is a potent, short-term satiety signal and is a key component of the negative feedback loop determining how much is eaten within a meal.

A more promising therapeutic target is glucagon-like peptide-1 (GLP-1) – another satiety hormone, but one that is released in the ileum and colon and whose receptors are highly evident in the hypothalamus. The functional properties of GLP-1 as a satiety signal have been found reliably in humans. Thus, a meta-analysis conducted by Verdich and colleagues in 2001 examining seven studies of GLP-1 administration in lean and overweight participants demonstrated a dose-dependent effect of GLP-1 on satiety in part due to delayed gastric emptying. There appears to be less resistance to the effects of GLP-1 in the longer term. For example, diabetic patients given GLP-1 by subcutaneous infusion over 6 weeks reported reduced hunger and appetite, increased fullness, and weight loss relative to a saline control group. This outcome gives credence to the proposal that GLP-1 can be exploited as a drug target in the treatment of both diabetes and obesity.

It is also thought that high-fiber foods enhance satiety through the release of gut peptides. For instance, in the case of indigestible carbohydrates such as whole kernels of barley, the satiety effect (increased fullness, reduced hunger, and reduced intake) is linked to an increase in GLP-1 secretion and to a reduction in free fatty acids due to gut microbial fermentation of the indigestible carbohydrates, an observation made by Johansson and colleagues in 2013. This has direct

implications for clinical and consumer benefit. High-fiber foods offer enhanced satiety and the potential net outcome of reducing overall energy intake to assist in weight loss or weight management.

Other gut hormones such as oxyntomodulin have been found to enhance satiety. Oxyntomodulin is secreted by L-cells of the small intestine in response to food intake. It is known to suppress food intake by around 19% when administered intravenously. In a longer-term study, where participants were trained to deliver subcutaneous oxyntomodulin three times per day before meals, energy intake was reduced in the initial stages by 25%, and by the fourth week, intake had declined by 35%. This resulted in a significant weight loss compared to a saline control group as reported by Wynne et al. in 2005. Clearly, there is substantial potential for developing drug targets exploiting the action of satiety peptides in promoting weight loss through reduced food intake and in the case of oxyntomodulin by increasing energy expenditure.

Given the efficacy of gut hormones and their ligands to reduce food intake in experimental settings and clinical trials, other less invasive methods have been considered for weight management. For example, the use of ingredients to promote satiety in food products is an issue explored by Hetherington and colleagues.

Sensory-Specific Satiety

Returning to the satiety cascade, it is clear that the sensory characteristics of foods play an important role in influencing satiation and satiety. At the beginning of the meal, foods are experienced as pleasant and desirable, but as the food is eaten, the appeal of this food declines. Thus, the hedonic appeal of a food just eaten decreases with time and is less relative to other foods that have been tasted but not eaten. Changes in liking or pleasantness may signal the onset of satiation. This phenomenon has been described in detail by Barbara Rolls in her review paper published in 1986. It is known as 'sensory-specific satiety,' and it has been observed in a number of different species and across the life span in humans. It is considered important in determining how much of a food is eaten within a meal and it may influence food choice beyond the meal. Thus, typically within human eating patterns, foods eaten at one meal are avoided at the next. It is thought that this desire for variety is important to the omnivore ensuring that a diverse diet is consumed rather than just the most favored or available food.

Long-term studies of repeat consumption of food items demonstrate that variety is considered important. A study of Ethiopian refugees who had been maintained on a diet of three staple foods indicated that those who had just arrived at the camp rated the foods as acceptable relative to three other foods but those who had been in the camp for 6 months rated the staple foods as significantly less pleasant than the comparison foods. Similarly, in the twenty years of studying army personnel and their response to army rations, Meiselman and his colleagues found that army rations were discarded or traded to avoid monotony. Greater variety in the contents of the military ration was a consistent request from personnel. Variety is sought out to maintain health and well being, while SSS may be important in achieving the goal of a varied, nutritious diet.

Satiety can be specific to the particular foods eaten repeatedly or to a defined set of sensory features (appearance, smell, texture, and taste) associated with the eaten food(s). The classic, early studies by Barbara and Edmund Rolls reliably demonstrated that subjective pleasantness decreased for the food just eaten (and foods sharing similar sensory properties) but remained high for other foods in humans, that these same foods were rejected in favor of uneaten foods in both humans and monkeys, and that recording in single cells of the lateral hypothalamus reflected a reduction in firing rate in response to the eaten food relative to uneaten foods. Specific satiation to olfactory stimuli in humans was demonstrated by Blundell and Freeman in 1981. They used stimulus-induced salivation in response to food-related odors (honey, lemon, and meat) following ingestion of a glucose load (50 g in 200 ml water). Salivation in response to the sweet odor (honey) declined significantly after the sweet (glucose) load but not for the other odors. If salivation is a good indicator of willingness to eat a food, then this experiment revealed selective satiation related to the sensory component of the load (sweet taste). Thus, satiety can be specific to the sensory properties of the food eaten.

There are two central and reliable features of SSS: The first is that there is a significant response decrement (whether firing rate of single neurons, subjective pleasantness ratings, or stimulus-induced salivation) to the eaten food and the second that there is a consequence for consumption (rejection of the same or similar foods and acceptance and intake of different foods). The implications for dietary choice are obvious – if satiety is specific to a set of eaten foods, then the more variety offered, the greater the food intake, and in the longer term, variety might promote overeating and even weight gain. This is certainly true of the so-called cafeteria diet, first described by Anthony Sclafani at Brooklyn College in 1976. In this paradigm, the varied selection of high energy density, highly palatable foods produced hyperphagia and obesity over time. In earlier studies by Jacques Le Magnen in the 1950s, it was demonstrated that chow paired with specific odors had the 'specific capacity to satiate.' However, when rats were offered four different odors in succession, intake increased by 70% within the bout. This effect of variety illustrates the power of environmental cues to stimulate excessive food intake and the ways in which satiety signals arising from the gut may be overridden by the presence of food cues.

SSS is generally measured using subjective ratings of pleasantness or liking under laboratory conditions, but advances in technology in neuroimaging demonstrate that specific reward pathways can be investigated in relation to the affective valence of gustatory stimuli. SSS can be conceptualized as a process in which experienced pleasure changes during eating, thereby reducing the motivation to continue eating this food during the current eating episode and to influence when this food will be selected again. There have been attempts to harness these observations for weight management; thus, the 'Flavor Point Diet' seeks to reduce variety as a means to limit food intake for weight loss. While this is an attractive idea in theory, the potential effects of monotony as described by Meiselman and colleagues in addition to the lack of convincing data on long-term weight loss maintenance temper the practical application in practice.

Potential Benefits of Satiety

Rather than seeking to limit variety as a means to enhance satiety, there are other ways to promote satisfaction with different foods included in the diet, as a means to assist consumers in limiting intake for appetite control. For example, diets that contain whole grains, fruits, and vegetables and that are relatively high in fiber and low in energy density tend to promote satiation and satiety. The potential benefits associated with enhanced satiety include enhanced resistance to overeating tempting foods, strengthening weak internal satiety cues in consumers who have experienced binge eating, enjoyment of lower-energy foods without feeling 'deprived,' reduced hunger dysphoria, improved hunger management, and improved compliance with goal-directed behaviors such as eating a healthy, balanced diet. It can also serve as a potential adjunct to achieving weight loss or avoiding weight gain (see Figure 2).

Studies of consumer understanding of satiety reveal that most consumers do not use the term 'satiety.' Instead, feelings of fullness or satisfaction are considered as the postmeal subjective sensations, which are more commonly used. Using focus groups involving American students and staff, Murray and Vickers in their 2009 paper revealed that fullness is described as having both physical and psychological components, which are experienced as either typical or extreme. Thus, consumers describe fullness as 'a feeling of food in the stomach,' 'stomach stretch,' 'satisfaction,' or 'lack of desire to eat.' Not only are these descriptions mainly focussed on gastric signals at the end of a meal, but also they reflect the experienced absence of the desire to consume foods that extends for some duration following meals.

More recently, consumers across four different European countries were asked to interpret example satiety claims that could be found on product labels. The results indicated that consumers had a reasonable knowledge of what is meant by a satiety claim with most accepting that such products can only play a part in weight management and do not provide a simple, single solution to weight control. Most respondents understood that personal efforts are required to translate product attributes into potential weight control benefits. However, dieters were more likely than nondieters to misinterpret a satiety claim, which is of interest since this is precisely the consumer often targeted for satiety claims by food manufacturers.

As one example of satiety enhancement, adding fiber and protein to foods can promote satiety. Thus, a novel, low-fat yogurt enriched with protein and fiber was consumed by produced a significant reduction in appetite and reduced energy intake compared to a standard product. This is an observation reported by Lluch and her colleagues in 2010. However, whether enriched foods can be used in the longer term to control appetite, to resist overeating, to manage hunger dysphoria, or to assist in weight management is subject to some controversy.

It is possible that satiety claims may be misunderstood by some consumers. They might believe that 'enhanced satiety' claims equate to a slimming claim. Thus, Blundell has cautioned against claims that cannot deliver on weight loss even if food ingredients or products have been shown in the laboratory to enhance satiety in the short term. This is simply due to the components of energy balance. If satiety is enhanced within a meal under laboratory conditions, any short-term benefits observed do not guarantee that this will be sustained in a free-living environment or that consumers will lose weight overall since weight is regulated over the long term.

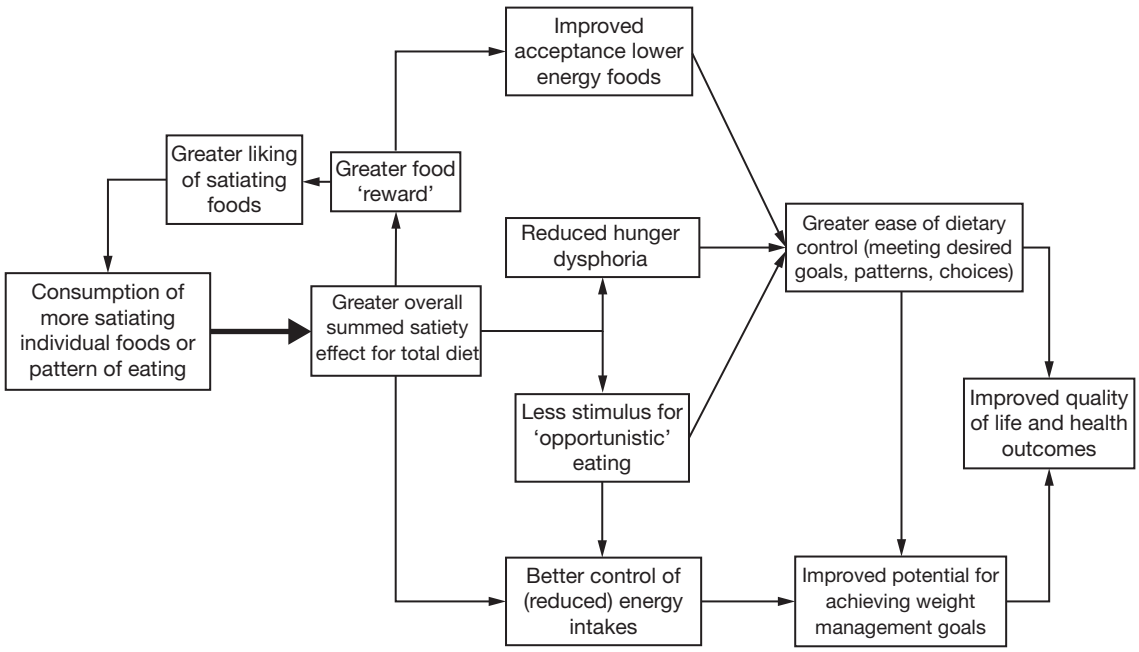

Figure 2 Benefits of enhanced satiety for consumers. Reproduced from Hetherington, M. M., Cunningham, K., Dye, L., et al. (2013). Potential benefits of satiety to the consumer: scientific considerations. *Nutrition Research Reviews* **26**: 22–38.

Conclusions

Overall, satiety is understood as part of the appetite control system. The related processes of satiation and satiety operate in tandem to reduce food intake within an eating episode. These processes determine how much is eaten within a meal and can influence the timing and content of the next meal. Over the past 40 years, research on the gut hormones that signal satiety has been targeted as potential therapeutic agents for obesity, and more recently, food manufacturers have sought to make claims about satiety enhancement to benefit consumers. It is likely that characterizing the mechanisms underpinning all aspects of the satiety cascade will provide potential targets for obesity treatment. It is also clear that consumers are aware of satiety claims and that there are potential benefits of satiety enhancement beyond the direct effects on food intake. Improved satisfaction with specific products and across the whole diet may indirectly benefit dietary control, weight management, or achieving a healthy, balanced diet through enhanced satiety.

See also: Appetite Control in Humans: A Psychobiological Approach; Gut Hormones; Obesity: Causes and Prevalence; Obesity: The Role of Diet.

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Sausages and Comminuted Products: Cooked Sausages

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Introduction

Sausage making has a rich history in most cultures. Sausage was a way to store meat for extended periods when mechanical refrigeration was unavailable. Today, the combination of meat and seasonings produces a broad type of sausages and generates different categories from fresh, smoked, cured, and cooked sausages. In addition, cooked sausages can generally be divided into two groups: those consisting of a homogeneous meat batter and another group consisting of a mixture of visible particles, principally comprised of meat and fat. The wide variety of sausages is due to differences in composition, shape, size, and cultural preferences and traditions.

Sausage Production

The manufacturing process of sausages has not changed over the years. But from the formulation point of view, manufacturers are always trying to improve the nutritional profile of these meat products.

Raw Materials

The selection of raw materials, lean meat and fat materials, for the manufacture of sausages is based on economic reasons. The main ingredient is meat, although other materials are used such as edible carcass by-products, liver, tongue, pork skin emulsion, and blood. Different types of carcass trimmings are employed in the manufacture, thereby creating a variety of sausages. The ingredients used in sausage formulations are different from substantial water content (in the form of ice) to a wide range of ingredients: meat from different species (beef, pork, poultry), salt, caseinates, vegetables, cereals, starch, and spices. The differences in ingredient composition frequently are dependent on cultural traditions and preferences.

Lean meat is the most expensive component in a cooked sausage, and its quality contributes positively to a good texture and development of red curing color. The first point in the selection of raw materials for sausage manufacture is the hygienic status of all the ingredients used and which should be as high as possible to avoid high microbial counts in the final sausage product. This factor will affect shelf life, and color stability, as well as flavor in the final sausage product. The handling of all raw ingredients will affect cooked sausage quality. The temperature of chilled meat and fat materials should be between 0 and 4 °C to delay bacterial growth and optimize the solubility of myofibrillar proteins from lean meat. In some cases, the raw materials, lean meat and fat, are stored frozen, but never for prolonged periods of time due to the fact that although freezing prevents microbial growth, it does not inhibit chemically based deteriorative processes like rancidity. The use of pale, exudative, and soft meat should be avoided as it not only affects the water-holding capacity of myofibrillar proteins, but also affects color. The use of meat from different species (pork, beef, mutton, etc.) may contribute differently to sensory characteristics. Sausage variety ranges from high-quality all-meat sausages to those economy-style forms that are manufactured using lower meat quality cuts augmented with higher fat levels, hydrocolloids, and other functional ingredients. The use of mechanical deboned meat (MDM) is widely used for cooked sausages, although its texture has to be built up with gelling materials (soy proteins, starch, etc.) to give firmness to the cooked sausages.

The fat used in cooked sausages stabilizes the solubilized protein network because it acts as a filler, thereby preventing protein shrinkage during the cooking process. Pork fat is widely used in cooked sausages from loin, belly, and neck due to its low unsaturated fatty acid content, although it is generally sourced from all parts of the carcass. The fat content of cooked sausages varies from 5% to 30%, with a popularly consumed sausage product like frankfurters having an average fat content of 28%. The type of fat used in cooked sausages depends on the type of sausage manufactured. In finely ground sausages the type of fat tissue is of less importance, whereas the requirements of fat with higher melting points is necessary when manufacturing cooked sausages with coarse cutting or with fat inclusions like mortadella.

Salt levels in sausages ranges from 1.5% to 2.5%; however, higher or lower percentages can be found in cooked sausages. Salt and phosphates play a role in the solubilization of myofibrillar proteins, which retain much of the water added during sausage formulation. During cooking, the solubilized proteins coagulate and aggregate producing the matrix, which is a three-dimensional gel network. The amount of salt in cooked sausages varies considerably, and current nutritional trends now target reductions in salt content to produce sausage products with low sodium content.

The use of phosphates in cooked sausages is common as, after common salt, it is the most efficient additive in solubilizing myofibrillar proteins. Phosphates are used during chopping or mixing at the beginning of the process to open up myofibrillar protein structure, thereby allowing salt to work more efficiently at solubilizing myofibrillar proteins.

Water (in the form of ice) is not an additive, but it is required to solubilize myofibrillar proteins. The use of ice counteracts heat generation during processing in the bowl cutter.

Nitrite is added to cooked sausages at levels of 120–150 mg kg^{-1} to develop curing color rapidly. The use of ascorbates is recommended together with nitrite, as it accelerates the curing process and reduces the risk of nitrosamine formation. Generally, ascorbic acid also reduces the levels of residual nitrites in cooked sausages. Originally nitrate was used in meat products; however, only nitrite is allowed for use in cooked sausages, and its usage is responsible for the curing

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00613-9

properties and distinctive flavor and color associated with the final products. Nitrite plays an essential role in meat quality and safety. Nitrite has been shown to be effective against spore-forming, anaerobically respiring bacteria like *Clostridium botulinum*. The antibotulinal properties of nitrite in meat products are further strengthened through interaction with other factors, such as; salt, pH, heat treatment, initial spore level, and original and residual nitrite level in the meat. Nitrite fixes color by the reaction of the nitric oxide generated by nitrite reduction with myoglobin and generating the cured color nitrosomyoglobin. It contributes to the cured meat flavor and effectively controls rancidity by inhibiting lipid oxidation. It has been indicated that the cured flavor differences among products with and without nitrite are due to the nitrite suppression of oxidation products, thereby controlling the generation of flavor compounds during processing. In addition, the antioxidant effect of nitrite is produced by its reduction to nitric oxide, which reacts with heme proteins, chelates trace metals and free radicals, and generates antioxidant nitrosyl compounds.

Since the twentieth century the use of nitrite has been progressively reduced due to controversies about its safety. One of the options proposed in the United States was the use of natural curing, which has grown in popularity in recent years. These natural-cured meat products are characterized by the use of vegetable sources of nitrate and nitrite such as celery instead of adding the synthetic chemical compounds. The vegetable addition is done together with a nitrate-reducing bacteria that produces the generation of nitrite from nitrate during processing.

Several color enhancers are used to intensify color such as ascorbic acid and glucono delta lactone. The use of acid additives is recommended toward the end of the cutting process because the pH of the mixture should be kept at optimum levels for protein solubilization. However, as acid additives lower the pH, they would consequently have a negative effect during processing.

Due to economic reasons, other proteins are added to cooked sausage formulations such as soy proteins. Soy protein is frequently used in cooked sausage formulations, and the levels used can range from 1% to 14% to compensate for the use of less expensive or fattier ingredients. However, the use of soy protein will affect texture, firmness, and flavor.

Other ingredients added to increase water retention in cooked sausages are carrageenan and various hydrocolloids due to their high water-binding capacity and their ability to reduce purge in the finished product. In addition, other thickeners such as starch and cereal flours are also used for enhancement of water-holding capacity in final sausage products.

Finally, the desired cooked sausage flavor is produced by the addition of spices and herbs. The combination of spices is very important, and in several cases, flavoring ingredients like liquid smoke or smoke flavor is added. A smoking process is typically applied to cooked sausages and relies on the penetration of smoke volatiles into meat products during the thermal combustion of wood. The wood used in the smoking process consists of a mixture of cellulose, hemicellulose, and lignin, the latter component of wood being responsible for the production of phenolic compounds, which are responsible for the characteristic organoleptic properties associated with smoked meat products.

Processing

During manufacture of cooked sausages (Figure 1), the objective is to solubilize as much myofibrillar protein as possible because this solubilized protein immobilizes the added water and emulsifies fat. The solubilized protein forms a thin layer around particles of fat preventing their separation during cooking. The thermal treatment causes the denaturalization of the protein thin layer, and the fat is maintained inside the layer forming a network. This network prevents the fat particles from joining with other fat particles. Emulsifying capacity is due to the solubilization of the myofibrillar proteins, principally actin and myosin.

As indicated earlier, protein solubilization depends on the amount of protein present and the level and type of additives used. Salt and phosphates increase the ionic strength producing an increase in myofibrillar protein solubilization.

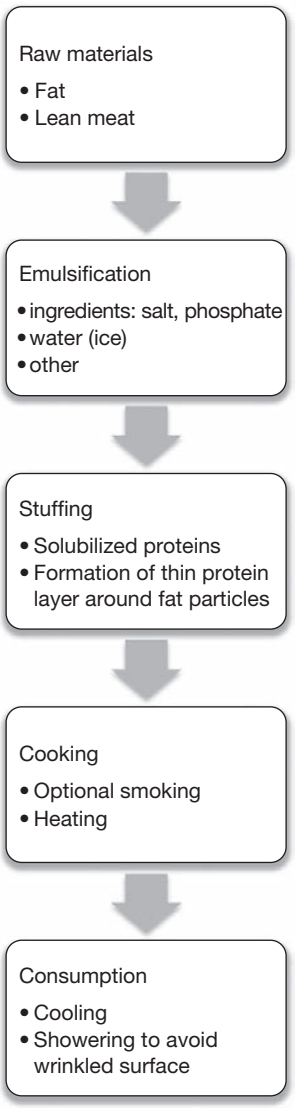

Figure 1 Process flow for the manufacture of cooked sausages.

The processing conditions used in the bowl cutter fractionate myofibrils and affect the partial solubilization of myofibrillar proteins. During chopping, the sausage mix should be cut for long enough that no particles are seen and, at the same time, ensure that as much protein as possible has been solubilized. During this process a stabilized emulsion should be obtained and the layer of proteins around fat particles fulfilled so that the risk of fat separation during the cooking process is reduced. Different interactions occur between protein–water, protein–fat, and protein–protein fractions that ensure the stability of the meat emulsion.

After the emulsion is formed, it should be filled firmly into casings and heated as soon as possible thereafter. The filling speed can be affected by sausage diameter, using lower speeds for high-diameter sausages. The filling process can affect the firmness of the product, and filling should avoid leaving air or free water in or around the final product, which would affect the final cooked sausage.

The cooking process used for cooked sausages varies according to the variety of sausages made. Large quantities of sausages are smoked, as this process produces high impacts on sensory characteristics (color, taste, flavor, and texture). The length of the smoking process depends on the desired color and smoked flavor required. This smoking process is usually carried out in smoking chambers or through the use of liquid smoke.

Afterward, the cooking of sausages can be done by steaming or water immersion. However, the products that are smoked are generally steamed, as they remain hung during all processing steps. During cooking, the solubilized proteins are denatured, thereby forming the three-dimensional matrix and stabilizing the fat particles. Generally, cooking is carried out between 74 and 80 °C, with a core temperature of 70–72 °C being targeted. The temperature range between 65 and 68 °C is not sufficient to stabilize the curing color, and undercooked products will fade over time. In general, the cooking temperature used is typically 6–10 °C higher than the core temperature being targeted.

Finally, the cooling process is applied by showering the product, especially products filled in natural casings and which can become wrinkled. Showering will depend on the casing-type used. All sausages should be cooled rapidly to an internal temperature of 10 °C to prevent bacterial growth. Finally, the completed products will be packed and storage for consumption.

To extend the sausage shelf life the final product is packaged and stored, and this packaging also delays lipid oxidation and microbial contamination. The preservation of sausages is based on hurdle technology as the preservation is achieved by the combination of preservation technologies such as low temperatures, high pressure, irradiation, and modified atmosphere packaging. A variety of packaging systems are available for cooked sausages, for long-term storage vacuum packaging, modified atmosphere packaging, or controlled packaging. Vacuum packaging is extensively used, as the preservative effect is achieved by the development of an anaerobic environment within the pack, and due to the barrier film used, the entrance of oxygen from the outside is restricted. The use of modified atmosphere packaging, where the air is replaced by a gas mixture, is to promote the overall quality of the packaged product, especially color stability.

Patterns of Consumption

The level of meat consumption within the diet is generally estimated for meat and meat products, and reports have described variable consumer consumption values ranging from 85 to 184 g per day, respectively. In general, the caloric contribution of meat and meat products is about 10–20% of the total calories in industrialized countries. Cooked sausages are not only consumed in Europe, but their consumption is increasing in the United States and not only for pork-based products, but also for beef sausages (http://www.hot-dog.org/ht/d/sp/i/38583/pid/38583). There are more than 800 types of sausages, and their consumption occurs globally. The U.S. patterns of meat intake and overall consumption differs among the population and is dependent on factors such as cultural, social, regional, and financial status. Overall, meat consumption has increased in the United States with red meat being the largest proportion of meat consumed. However, there are also differences in meat consumption patterns across Europe. Central and northern European countries have rather similar meat consumption patterns, whereas lowest meat intakes have been reported for Britain and France. In addition, processed meat consumption is higher than that for fresh meat among European consumers. In Europe, the sausage consumption rate is highest for German consumers, followed by Norwegian, Swedish, Danish, and Dutch consumers.

Impact on Nutrient and Bioactive Compounds Availability of Key Food Constituents

Fat Content

The fat content of lean meat is low (typically around 2.0–2.5%), whereas the fat content in cooked sausages is variable (Table 1); however, it is essentially high, and many efforts have been made to reduce it. From the nutritional point of view, fat is an energy source and provides essential fatty acids and liposoluble vitamins. However, the high content of saturated fatty acids in meat, together with a low polyunsaturated fatty acid content, has singled out meat as a food product for criticism.

In addition, cholesterol is an essential constituent of animal cells and therefore is present in meat products. Studies that linked dietary fat and cholesterol intake with chronic diseases have assisted in promoting a negative image for meat products due to their high fat and cholesterol content. The cholesterol content in meat products (Table 1) varies considerably due to numerous differences in their production, such as the use of different meat species, muscles, feeding regimens, levels of fat content, and formulations. In general, the higher the fat content in a processed meat, the higher the cholesterol contribution of the product to the diet. In addition, the use of carcass by-products such as liver in cooked sausages like 'pate' increases the cholesterol content. It has been shown that meat and meat products contribute from one-third to one-half of the daily recommended cholesterol intake (300 mg per day).

The profile of saturated, mono- and polyunsaturated fatty acids determines the nutritional quality of a cooked sausage. Although the content of monounsaturated fatty acids plays a

Table 1 Cooked sausage composition

	Frankfurter	*Liver Pate*	*Mortadella*
Composition (%)			
Fat	18.4	31.3	25.0
Protein	11.4	10.9	14.0
Carbohydrates	5.3	2.7	1.5
Fatty acid profile (g/100 g)			
Saturated FA	7.7	9.2	9.0
Monounsaturated FA	11.5	12.6	9.5
Polyunsaturated FA	3.1	4.1	4.0
Content			
Salt (%)	2.5	2.5	2.5
Phosphate (ppm)	3884.6	3232.9	–
Nitrate (ppm)	185.0	123.4	24.0
Nitrite (ppm)	12.9	19.5	13.0
Cholesterol (mg/100 g)	55.0	220.0	70.0

Source: Chizzolini, R., Zanardi, E., Dorigoni V. and Ghidini S. (1999) Calorific value and cholesterol content of normal and low-fat meat and meat products. *Trends in Food Science & Technology* **10**, 119–128; Honikel, K. O. (2010) Curing. In: Toldrá, F. (ed.) *Functional Meat Products. Handbook of meat processing*. Ames, IA: Blackwell Publishing.

neutral to favorable role in cardiovascular diseases, this is in contrast to saturated fatty acid content. In addition, the increase of polyunsaturated fatty acids at the expense of a reduction in the saturated fatty acid content in pork fat satisfies ideal dietary guidelines. Although the pork industry has developed leaner genotype pigs, meat processors (sausage industry) have improved the nutritional profile of meat products by reducing the fat content and also the level of salt.

However, one of the problems that can be caused through the reduction of fat content in meat products is an impact on flavor. The reason is that fat acts as a precursor of flavor compounds, and it can modify the flavor perception due to the changes in the interaction and release of volatile compounds, and finally, it produces changes in certain ingredients such as salt, spices, flavorings, and so on, due to the changes observed in the matrix.

The fat reduction in cooked sausages can be an effective way to reduce dietary fat intake. Fat reduction can be done by two ways: using raw materials with higher lean content, which makes the end product more expensive, or by adding other ingredients that act as fat substitutes. These ingredients should not adversely affect the sensory characteristics of the product as nonmeat proteins (blood plasma, egg protein, caseinate, soybean derivatives, etc.) and carbohydrates (fiber, maltodextrin, starches, gums, etc.). Many studies pertaining to fat reduction in meat products have been performed in cooked products, where the main effects observed were aroma/flavor losses and problems relating to texture, although acceptable sensory quality products have also been obtained. In some cases vegetable oil has been used as a substitute to solid fat; however, this approach produces lighter-colored cooked sausages.

Muscle Proteins and Peptides

Because connective tissue proteins have a lower content of essential amino acids than those of contractile proteins (myofibrillar proteins), products with a high percentage of collagen will also have a relatively lower nutritive value. However, connective tissue may not be nutritionally disadvantageous until the ratio of connective tissue nitrogen to muscular tissue nitrogen is >1. This can happen in cooked sausage where connective tissue is used to replace meat.

The high quality of meat proteins and their source of essential amino acids can be of interest in illnesses relating to protein turnover like sarcopenia. Sarcopenia produces a reduction in muscle mass and strength producing a physical disability and poor quality of life. Dietary interventions may have the potential to counteract sarcopenia, and meat may play an essential role.

Meat proteins are hydrolyzed extensively during gastrointestinal transit and are converted to amino acids and small peptides, which are transported through the gastrointestinal tract to be delivered to the systemic circulation. The functionality of these peptides generated during the gastrointestinal digestion has been studied recently with a focus on observing different biological functions. One of these biological functions is attributed to the presence of peptides with antihypertensive effect due to their inhibitory ACE activity (ACE, Angiotensin I-Converting Enzyme) originated from the hydrolysis of myofibrillar and sarcoplasmic proteins. In addition, the production of these ACE inhibitory peptides has been reported in other sources such as chicken collagen hydrolysates, but not as yet from other species. Apart from this inhibitory ACE activity, several antioxidative peptides have been obtained from myofibrillar hydrolyzates reporting different effects, like antifatigue activity in mice. These hydrolyzed peptides with biological activities can originate during the gastrointestinal transit of the ingested meat product or also during the processing of the cooked sausages. Further research is required in this field to show the presence of biological activities in cooked sausages.

Due to the contribution of meat ingredients to human health, the values for a recommended daily intake of protein has been indicated to be from 1.1 to 1.5 g per day to contribute to better muscle and bone maintenance and to improve quality of life. In this context, cooked sausages contribute to the daily protein intake.

Meat products, like cooked sausages, are also characterized by the content of endogenous antioxidants: glutation, uric acid, carnosine, and anserine. Carnosine and anserine are the most abundant dipeptides in meat and are not found in vegetal foods. The bioavailability of these compounds has been measured in human plasma after the ingestion of beef meat, and the highest dipeptide concentration was reached after 2.5 hr postingestion.

Health Effects

In terms of nutrition, meat provides the best quality of protein, and it is an excellent source of essential amino acids in adequate proportion. However, the preparation (high temperatures) and processing of meat products through the addition of certain ingredients, including salt, nitrites/nitrates, phosphates, and saturated fats, has contributed to indicate a reduction in meat product consumption due to health concerns in developed countries. An overview of the sausage composition and effect of ingredients on health is shown.

Minerals

The content of mineral components in meat products is in low proportion, although the most important is potassium followed by phosphorous and sodium. In addition the high content of iron is important in cooked beef sausages, but is less so for other sausage species. In addition, the presence of organs such as liver in the formulation of cooked sausages increases the content of several minerals such as iron, copper, and zinc. It is estimated that heme iron contributes to 10–15% total iron intake because of its higher bioavailability in relation to nonheme iron from plants. It is believed that diets containing red meat increase overall iron absorption. In addition, after meat-based diets, it has also been observed that a significantly higher calcium absorption occurs, which could have implications for bone health; however, this latest fact has not been clinically tested yet.

The use of MDM in sausages can modify their nutritional value. The reason for this is the variable levels of connective tissue and calcium present in MDM, and this results in an increase in the content of calcium, general ash components, iron, and bone-derived collagen.

Vitamins

Vitamins are also present in meat, but cooked sausages are not an important source of such, although the specific content of several vitamins like Vitamin B_1 in pork is higher than in other meat species. Even in cooked sausages, the percentages of vitamin B_1 recovered after processing are high.

Fatty Acids

Meat is also a source of essential fatty acids like linoleic (C18:2), linolenic (C18:3), and arachidonic (C20:4) acids. In cooked sausages, the content of this essential amino acid is affected by many factors like species used during manufacture, feeding regimens, muscle location, and so on. Therefore, the content of these essential fatty acids in cooked sausages will depend on manufacturing conditions. In addition, in meat derived from ruminants, a group of isomers from octadecadienoic acid (CLA) was identified as an anticarcinogenic compound in extracts of grilled beef and also was attributed with anti-atherosclerotic, antioxidative, and inmunomodulatory properties. However, the CLA content in beef meat varies according to breed, feed, and stage of lactation. This fact also means that its content in a cooked sausage will be affected.

Salt

The use of NaCl is very important for meat product flavor for a number of reasons. First, it is responsible for the salty taste that consumers perceive as sodium salt binds to protein receptors. Second, sodium has a synergetic effect with food matrix, and it enhances the sensory characteristics of meat as the typical desirable meat flavor. Third, salt produces the release of volatile aroma compounds from the food matrix (salting-out effect) because salt changes the ionic strength. In addition, chloride ions interact with meat proteins and favor the retention of water by the increase in the water-holding capacity and, finally, produce an impact on cooking yields. As indicated earlier, sodium chloride is a key ingredient in cooked sausages as it contributes to water-holding capacity, color, texture, and flavor. The main concern about its reduction is the loss of protein functionality and flavor. The increase in salt consumption in modern countries and its relationship with cardiovascular diseases has become the target of government authorities that have settled on different strategies to reduce salt content in processed foods. The reduction can be done over a period of years to produce an adaptation of the population to lower salty taste. These strategies have been adopted within and outside of Europe (http://ec.europa.eu/health/nutrition_physical_activity/docs/salt_report1_en.pdf) and also in the United States (http://www.nyc.gov/html/doh/html/diseases/salt.shtml).

Phosphates

The use of phosphates in cooked sausages is widespread as it contributes to protein functionally. Its use is associated with higher water retention and higher processing yields. More attention has been paid to salt reduction in cooked sausages, but the recent appearance of a scientific article suggesting an association between high intake of phosphates as food additives and increased cardiovascular risk in the general population has led to more attention being paid to phosphates. Therefore, a recent report from the European Food Safety Agency (EFSA) indicated that in the absence of actual information, it is not possible to make causal inferences for serum phosphate levels and adverse effects. The EFSA indicated the necessity to reevaluate phosphate usage as food additives.

Nitrites

Nitrite usage has been reduced in cooked sausages to reduce the risk of nitrosamine formation. The function of using nitrite in meat products has been indicated earlier, and it is considered an essential ingredient in cooked sausages. The concern over nitrite in cured meats came about when nitrosopyrrolidine was found in bacon. Later it was also found in other cured meats, but usually at lower levels than in bacon. Generally the residual nitrite levels in cooked meat products are 10 ppm or less at which nitrosamines are not likely to form.

In recent years concern about nitrite, nitrate, and nitrosamine exposure has led to several epidemiological investigations. The published results have been used in deliberations by governmental bodies. Several reports indicated that red meat and/or processed meat consumption was associated with higher rates of certain types of cancer. Therefore, several recommendations about limiting red meat and processed meat consumption were given. However, food researchers have challenged these conclusions and recommendations based on epidemiological associations of weak magnitude. Nevertheless, the meat industry has changed meat curing practices to minimize potential nitrosamine formation, such as adoption of additional regulatory limits and elimination of nitrate usage in cooked meat products. Disagreement and debate over the real health impacts of dietary nitrite and nitrate in cured meats still exist.

Polycyclic Aromatic Hydrocarbons

The impact of cooked sausages in health has been evaluated together with other meat products. But in numerous cases the results obtained may be confused by other dietary factors and cooking methods. Regarding cooking methods, the grilling of meat products can induce the development of polycyclic aromatic hydrocarbons (PAH) in the product, as it is affected by the cooking procedure employed. The contamination of PAH in grilled foods is mainly due to the pyrolysis of melted fat, and it can be reduced by avoiding excessive heating or keeping the melted fat away from the heat source. PAH has been demonstrated to be carcinogenic in experimental animal trials, and therefore, it is essential to minimize the contents of PAH in cooked sausages. However, high variation of PAH contents in smoked meat products has been detected. The relationship between PAH content and smoking conditions has been studied, and it has been reported that the PAH contents increases with smoke density and ventilator velocity. It has been recommended to achieve low smoke generation temperatures (below 600 °C) to lower PAH contents in hot smoked sausages; however, this approach requires longer smoking times with higher weight losses to obtain a comparable color. Benzo(a)pirene is one of the most important carcinogenic PAH compounds, and it has been used as marker, although a maximum level of 5 μg kg^{-1} has been established for smoked meat products. The intake of PAHs has been estimated for some European countries, and it has been shown that the major food contributors are cereals, oils, and vegetables. Grilled and barbecued meat products produce a significant proportion of the daily PAH intake (21%) in the United States. In this context, well-done red meat, and the intake of bacon and sausages has been associated with increased cancer risk and colorectal adenoma in the United States. However, the total intake of processed meats has not been associated with this risk. Once again, the high temperature used during cooking and the intake of benzo(a)pirenes were associated with this risk. The recommendation to reduce the exposure to these compounds are to use slow cooking for longer periods of time, turn meats more frequently to reduce surface temperatures, and add complementary consumption of foods rich in fiber and flavonoids.

See also: Amino Acids: Determination; Beef; Bioactive Peptides in Foods; Cholesterol: Properties, Processing Effects, and Determination; Cooking: Domestic Techniques; Cured Foods: Health Effects; Emulsifiers: Types and Uses; Fat Replacer; Fats: Production and Uses of Animal Fats; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Heat Treatment: Principles and Techniques; Iron: Biosynthesis and Significance of Heme; Low-fat Foods: Types and Manufacture; Low-salt Foods: Types and Manufacture; Meat: Conversion of Muscle into Meat; Meat: Eating Quality and Preservation; Meat: Role in the Diet; Meat: Structure; Nitrites and Nitrates; Polycyclic Aromatic Hydrocarbons; Pork Meat Quality, Production and Processing on; Skeletal Muscle; Smoked Foods: Principles and Production; Soy Beans: Processing; Spices and Flavoring Crops: Uses and Health Effects; Thiamin: Properties and Determination; Triacylglycerols: Structures and Properties.

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Sausages and Comminuted Products: Dry Fermented Products

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Dry Fermented Sausages: Introduction

The FAO defines 'dry fermented sausages' as uncooked meat products that consist of more or less coarse mixtures of lean meats and fatty tissues combined with salts, nitrite (curing agent), sugars and spices, and other nonmeat ingredients filled into casings. Their typical properties (flavor, firm texture, and red curing color) are obtained through fermentation processes. Shorter or longer ripening phases combined with moisture reduction ('drying') are necessary to safely build up the typical flavor and texture of the final product. These products do not receive any heat treatment during processing and they are mostly consumed raw.

As with other groups of foods, there is a great variety of dry fermented sausages and their variety is dependent upon the ingredients used in their formulation and also on the technological conditions applied during their processing. In most cases, these differences are related to the regions or countries from where the products are typically derived. Different meat species (pork, beef, deer, lamb, calf, and even goose), lean/fat ratios, curing salts, carbohydrates, spices, scents, starter cultures, and processing conditions (meat particle size, diameter and type of casings, and use of molds) give rise to a broad variety of sensory properties, which in turn creates a variety of very different products. Additionally, fermentation and drying conditions (temperature, humidity, time of drying, and smoking) can define the final characteristics of the products. For instance, high fermentation temperatures (around 26 °C), typical of that employed in Northern European countries, give rise to faster lactic acid production and lower ultimate pH products (pH from 4.8 to 5.2) as compared to using lower processing temperatures (<12 °C), as used in Mediterranean countries, that produce less acidic products (pH from 5.3 to 5.8). In the United States, very fast fermentation processes caused by high temperatures (35 °C) are typical. Additionally, in the United States, it is common for rapidly fermented dried sausages to be fully cooked in order to meet government regulations, thereby generating slightly different product forms to those found anywhere else in the world.

Types of Fermented Sausages: General Composition

Meat and meat products are recognized in the Western diet as the main source of essential amino acids, high-biological-value proteins, bioavailable iron and other minerals, and B vitamins. Dry fermented sausages are a group of processed meat products that supply these nutrients, providing these with the accompaniment of some rather unique sensorial properties derived from the lactic fermentation and the drying that takes place during their processing.

Table 1 reports the composition of some of the most common types of dry fermented sausages from different countries in the world. Differences in composition are derived from the raw materials used, the processing procedures (including the lactic acid bacteria strains responsible for the fermentation process), and the degree of drying.

As the main ingredients of dry fermented sausages are meat and back fat, protein and fat are the main macronutrients responsible for the high energetic value associated with these products.

Proteins are an essential component of the diet, meat being widely used as a nutritional source of this macronutrient. The amount and type of meat used in the sausage formulation give rise to different percentages of protein. In general, protein content is around 20% in the majority of product forms, with the highest percentages accounted to chorizo (a Mediterranean high-quality product from Spain that showed 27%) and the lowest to cervelat (from Norway, with 10.7%) and salami (from Denmark, with 13.9%). Moreover, the quality of these proteins is variable depending on the amount of connective tissue present in the meat. Protein from skeletal muscle has a high biological value, supplying significant amounts of essential amino acids. However, connective tissue has a much lower biological value, being rich in collagen, protein with hydroxyproline as characteristic amino acid contributors. It is generally stated that the higher the hydroxyproline amount in the protein fraction of the product, the lower the biological value of the protein. Additionally, the amino acid composition of meat can vary according to several factors related to anatomical location, breeds, sex, species, or feeding regime of the animals.

The importance of dietary protein cannot be underestimated in the diets of older adults; inadequate protein intake contributes to a decrease in reserve capacity, increased skin fragility, decreased immune function, poorer healing, and longer recuperation from illness. Additionally, it has been postulated that increased dietary protein has been shown to promote healthy body weight and composition, in part by increasing satiety and by improving vitality and stamina. The supply of protein from dry fermented sausages to the total diet could be considered significant.

Furthermore, it is important to take into account that dry fermented sausages could have a significant fraction of nitrogen compounds that are released from the protein fraction during the fermentation process. Among these compounds, bioactive peptides possessing interesting antioxidant, antimicrobial, and antihypertensive properties can be found.

Fat is the most significant fraction in every product, with percentages ranging from 17% to 49%. This high fat content is the main factor responsible for the negative health aspects relating to the high intake of these products and, in consequence, to the current recommendation that suggests an occasional intake of this type of product. In fact, fat is responsible for the greatest fraction of their energetic value. Furthermore, the amount and type of fat have a significant influence on the sensorial properties of food, being positively related to the

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Table 1 Composition data (per 100 g of product) of common types of dry fermented sausages

	Spain[a]				*France*[b]		*Portugal*[c]	*Turkey*[d]	*The Netherlands*[e]		*The United Kingdom*[f]	*Denmark*[g]	*Norway*[h]	*The United States*[i]			*China*[j]
	Fuet	*Chorizo*	*Salami*	*Salchichón*	*Cured meat/ sausages*	*Chorizo*	*Salpicao*	*Sucuk*	*Salami*	*Chorizo*	*Salami*	*Salami*	*Cervelat*	*Chorizo*	*Pepperoni*	*Salami*	*Cantonese sausage*
Energy (kcal)	475	323	435	422	230	477	412	431	392	358	438	503	218	455	494	407	
Protein (g)	19.5	27	17.8	25.8	17.6	21.5	20.5	20.4	18.7	20.7	20.9	13.9	10.7	24.10	22.9	22.58	28.6
CH (g)	5.5	1.9	1.3	2	1.87	3.5	0	9.5	1.5	3.4	0.5	3.4	3.3	1.86	0	1.6	7.7
Fat (g)	42	23.1	40.2	34.7	16.9	42.1	36.7	34.4	34.8	29.1	39.2	49	17.3	38.27	43.5	33.72	49.6
MUFA (g)	19.9	11	19.09	15.93	8.64	18.4	–	–	15.6	12.9	17.70	22.5	7.6	18.40	17.17	16	25.3
PUFA (g)	6.5	2.5	4	5.83	1.93	3.46	–	–	3.1	3.2	4.40	3	1.5	3.46	3.43	3.74	12.3
SAT (g)	15.5	9.6	14.86	12.3	5.66	14.6	–	–	13.8	11.3	14.6	10	7.2	14.38	14.86	11.89	18.2
Chol (mg)	87.5	72.6	80	72	79.5	108	84	–	66	nd	83	85	43	88	105	79	–
Ca (mg)	11	18.4	17	15	11.3	35.8	27	26	35	29	11	10	25	8	22	13	–
Fe (mg)	1	2.1	2.2	2.4	1.66	2.8	1.7	4.5	1.5	1.5	1.3	1.1	1.3	1.59	1.62	1.3	–
K (mg)	280	180	240	207	330	479	575	285	302	249	320	204	166	398	279	278	–
Na (mg)	1180	1060	1962	1060	1070	1730	4336	1069	1278	1537	1800	1900	904	1235	1761	2260	2044
Zn (mg)	2.3	1.2	1.7	1.7	1.93	2.3	3	4.7	3	2.34	3	1.47	–	3.41	2.5	4.20	–
Vit B_{12} (μg)	2.5	0.9	1.5	1	1.64	0.93	1	2	1.4	0.92	2	1.3	3.7	2	1.73	2.80	–

CH, carbohydrates; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SAT, saturated fatty acids; Chol, cholesterol.

[a]http://www.bedca.net/bdpub/index_en.php – BEDCA: Spanish Food Composition Database.

[b]https://pro.anses.fr/TableCIQUAL/index.htm – Ciqual French Food Composition Table (Anses-france).

[c]http://www.insa.pt/sites/INSA/Portugues/AreasCientificas/AlimentNutricao/AplicacoesOnline/TabelaAlimentos/Paginas/TabelaAlimentos.aspx – Portuguese Tabela da Composição dos Alimentos.

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Source: Data have been gathered from national databases.

sensory features of the final product. Products such as fuet, Cantonese, pepperoni, and some of the salamis and chorizo consist of more than 40% fat.

The fatty acid profile of fat depends on the animal species and the diet received by the specific animal species prior to slaughter. In most cases, the major part of all fat in fermented sausages corresponds to pork back fat, whose polyunsaturated fatty acid (PUFA) level can be modified by means of animal nutrition practices. As it can be observed in Table 1, the monounsaturated fatty acid (MUFA) fraction is, in general, the highest fat fraction in dry fermented sausages, accounting for values around 44–50% of the total fat content. This is in agreement with the fatty acid profile of pork back fat, which is rich in this lipid fraction due to the presence of oleic acid. The saturated fatty acid (SFA) fraction is also quite high, reaching values of 35–40% in most of the product forms. In contrast, PUFAs account for the lowest lipid proportion, ranging from 8% to 15% in most of the products. Regarding cholesterol contribution, amounts derived from dry fermented sausages can be considered similar to other products from animal origin and are far lower in content than foods such as shellfish and eggs.

Carbohydrate content is generally low in dry fermented sausages, and they are derived from the starches or other poly- and oligosaccharides added as ingredients to enhance the fermentation process. In some products, the amounts are more significant reaching 9.5% for sucuk or 7.7% for Cantonese sausage.

The mineral fraction of dry fermented sausages, and of processed meat in general, is very rich in some nutrients, such as iron, zinc, calcium, potassium, and especially sodium. The high amount of sodium is due to the use of common salt as ingredient, which plays an important role as preservative and also contributes to sensorial aspects. In recent years, a great effort to reduce the amount of salt used in dry fermented sausages has been undertaken. As it can be observed in Table 1, there are still some products on the market possessing sodium concentrations of around 1650 mg/100 g.

Iron is one of the micronutrients that still show significant deficiencies in some population groupings, even in developed countries. Meat and processed meats, including dry fermented sausages, constitute an interesting group of food supplying this nutrient. Sometimes, an intake of iron supplements is required to minimize the risk of deficiencies, but in some cases, as in the case of pregnant women, these intakes have been correlated with hyperglycemia.

Diets of high iron bioavailability contain generous amounts of meat and foods that enhance iron absorption (e.g., containing ascorbic acid (AA)), and it has been suggested that only meat intake has consistently (positively) been associated with higher iron stores (in the form of serum ferritin). There are two major forms of nutritional iron, heme and nonheme. Iron acquired from meat is 40% heme iron and 60% nonheme iron. Heme iron has a good bioavailability being very efficient in decreasing anemia, although in the last few years, some studies have indicated a relationship between this nutrient and some forms of diabetes and cancer. The amount of iron in dry fermented sausages is around 1.8 mg/100 g. The dietary reference intake for iron is 8 mg day^{-1} for men and 18 mg day^{-1} for menstruating women.

Zinc is another micronutrient that is present in significant amounts in dry fermented sausages. The DRI for zinc is 11 mg day^{-1} for males and 8 mg day^{-1} for females. The mean value for the sausages presented in Table 1 was 2.5 mg/100 g, a value that can be considered as significant when compared with levels found in other food groups, especially when compared with sources derived from vegetal origin rich in inhibitor factors that decrease its absorption, as well as that of iron and calcium. Other trace elements, such as selenium, could be supplied in some significant amounts by fermented sausages. The contribution of meat and meat products to daily selenium intake is around 21%.

In relation to calcium, the DRI is 1000 mg day^{-1}, so the intake of this nutrient from meat products (including dry fermented sausages) is in general low. However, the amount of vitamin B_{12} in dry fermented products (0.9–3.7 μg day^{-1}) significantly satisfies the DRI (2.4 μg day^{-1}). It is noteworthy that this vitamin is present only in foods from animal origin, meat and meat products being the main source in the diet.

In a recent paper, McNeill published a review about the beneficial aspects of red meat intake (which partially could be extrapolated to processed meats including fermented sausages) and its supply of proteins and micronutrients.

Controversial Aspects

Although the amounts and quality of different nutrients present in dry fermented sausages are of major interest, several factors operate against these products being among the most recommended foodstuffs.

Diseases as type 2 diabetes, high blood pressure, and coronary heart disease are correlated with overweight and in consequence with the intake of an excess of calories. So the high energetic value showed by most of the dry fermented products is a key factor limiting their intake.

Some studies have associated red and processed meat consumption with the development of two of the major chronic diseases in the Western world: CVD and colon cancer. The World Cancer Research Fund and the American Institute for Cancer Research have stated that there is epidemiological evidence of the relationship between red meat and processed meat consumption and colorectal cancer. In this sense, there are papers in which a deep research about the mechanisms explaining these effects is demanded.

Additionally, as previously stated, conventional formulations of this type of products include high amounts of fat. According to WHO, dietary fat intake should range between 15% and 35% of total energy value, with saturated fat making up to a 10% of this total and the polyunsaturated fraction making up between 6% and 11%. Regarding SFA, it has been long known that they primarily raise LDL-cholesterol in comparison to MUFA and PUFA, and suggestion to replace SFA by these other types of fat has been proposed. However, incipient epidemiological data challenge the concept that SFA increases the risk of CVD, suggesting the revision of current dietary recommendations for these fatty acids. In any case, the high fat content of traditional dry fermented sausages currently hinders their regular intake.

Another health concern related to the composition of dry fermented sausages deals with the use of nitrite (KNO_2/E249 and $NaNO_2$/E250) and, indirectly, nitrate ($NaNO_3$/E251 and

KNO_3/E252) in their formulations, not only basically to avoid the growth of *Clostridium botulinum* and other pathogens but also for technological reasons (nitrite contributing to adequate color and cured meat flavor). Despite these positive actions, the traditional use of nitrate/nitrite in the meat industry is being questioned due to their involvement in nitrosamine formation and inducement of methemoglobinemia with excessive intakes of nitrite. However, it has been also postulated that dietary amounts of nitrate may have robust NO-like effects in humans, including blood pressure reduction, inhibition of platelet aggregation, and vasoprotective activity, which could have beneficial health effects, including protection against cardiovascular disease and type 2 diabetes.

Healthier Fermented Sausages: Reformulation Strategies

As a consequence of these previously mentioned nutritional drawbacks, and in light of the strategies that propose food reformulation as one of the ways to improve the nutritional quality of the diet, extensive research has been conducted in order to achieve this objective. Consequently, it has been postulated that meat products could be excellent matrices for functional foods: They appeal to a wide range of consumers; they are versatile (e.g., minimally processed to comminuted products), and as has already been mentioned, they intrinsically contain high-quality nutrients (proteins, vitamins, and minerals).

Thus, dry fermented meat products are currently available in which addition, reduction, replacement, or increment of compounds with physiological or biological activity has been carried out to enhance final formulation. Additionally, other approaches dealing with animal nutrition practices for improving the nutritional quality of the resulting meat and meat products have also been carried out.

Lipid Fraction

Reducing the total fat content or substituting fat by nonlipid ingredients or healthier lipid sources is among the most usually studied strategies to improve the nutritional value of dry fermented sausages.

Replacement of pork back fat by healthier fat sources began with the use of olive oil (to increase the monounsaturated fat content), firstly as a direct ingredient in formulations and later by means of their incorporation into soy protein emulsions or soaking them in bread crumb, which resulted in better products. Products such as Greek slow-fermented sausages, chorizo, Italian salami, and sucuk have been elaborated with significant amounts of olive oil by different authors.

Regarding the incorporation of PUFAs, most of the scientific publications deal with the use of different oil sources rich in omega-3 fatty acids. Linseed oil was used to substitute 25% of pork back fat by an O/W emulsion in Spanish dry fermented sausages, achieving a relevant supply of a-linolenic acid (2.53 g/100 g product). Additionally, the use of different very long-chain omega-3 fatty acid sources has allowed the development of acceptable products if the amounts added were carefully adjusted. Thus, oil from microalgae *Schizochytrium* sp. was used to substitute 15% of pork back fat by a soy emulsion, thereby attaining a final formulation level of 1.30 g/100 g product of DHA, with an interesting $n-6/n-3$ ratio and good product stability with acceptable sensory properties storage under vacuum.

In sucuk sausage, hazelnut oil was directly incorporated to substitute an 11.11% of beef fat, contributing to a final PUFA concentration of about 7 g/100 g product, increasing both linoleic and linolenic acids as compared to the control product and reporting good sensory results. Additionally, sunflower oil and lard-based diacylglycerols have been tested as pork fat substitutes, at levels of 5%, giving rise to good sensory ratings, especially in the case of sausage formulations containing sunflower oil.

Not only have single oils been investigated, but also mixtures of oils have also been employed as a means of attempting to obtain a balanced lipid profile, closer to current lipid recommendations, without compromising the stability and sensory properties of products. A combination of 0.44 olive oil, 0.38 linseed oil, and 0.18 fish oil fractions was emulsified into konjac gel to be incorporated into low-fat fermented sausages. A significant fat reduction (up to 68%) and increments of linoleic, eicosapentaenoic, and docosahexaenoic acids as compared to the control product were reported. However, and due basically to the fat reduction, the sensory analysis of these products resulted in some sensory limitations (lower firmness and lower acceptability), so further modifications would need to be considered in order to improve the formulation.

Different fibers have also been used as fat replacers in different types of dry fermented products. Vegetable sources, such as inulin, cereal fibers, sugar beet, and fibers of red wine by-products, have been used with successful results at the doses tested. When amounts added were high, some textural defects were noticed. Moreover, a nitrite-reducing effect has been appreciated and enhancement of microbial quality was observed using orange fiber from different products.

Salt Reduction and Mineral Modification

The important role played by salt in dry fermented sausages challenges its reduction or replacement. In fact, there is no single ingredient that can fully substitute the effect of sodium chloride in foods, and several strategies have been generally tried to do so in processed foods.

The use of chloride and nonchloride salts has been the most common practice employed to date in an attempt to reduce salt, either by being used separately, or in combination, or with the incorporation of enhancers in the mixtures. KCl (alone or combined with potassium lactate) can substitute up to a 50% (molar basis) of NaCl in different small caliber products, with higher percentages causing bitterness and metallic aftertaste. KCl, in combination with $CaCl_2$ and Mg Cl_2, was also used in chorizo, reporting good technological properties, but lower saltiness perception. Consequently, in order to counteract the lower aroma perception of reduced salt products caused by lower sulfur compounds and higher aldehydes, inoculation with *Debaryomyces hansenii* has been explored in reduced fat and salt fermented sausages. These authors found better results when fat or salt was reduced than when the modification was carried out simultaneously.

Another yeast, *Saccharomyces cerevisiae*, was used in combination with KCl to replace up to 50% NaCl, achieving good sensory results due to relevant production of volatile compounds from amino acids and carbohydrate metabolism. A similar salt reduction was also reached by using lysine, disodium guanylate, and disodium inosinate as successful flavor enhancers in combination with KCl.

Interesting approaches that combined salt reduction with enrichment with calcium and other deficient minerals have also been tested. Thus, calcium ascorbate partially replaced NaCl in a 7:3 w/w mixture or in different mixtures up to 8:2 in chorizo. In this last example, which also modified the lipid fraction by increasing omega-3 fatty acids, sodium reduction reached 50% and calcium supply reached 5.2 g kg^{-1}, thereby resulting in a healthier product from a broader nutritional perspective.

Other works in which a combined modification of the lipid fraction and salt content have been performed simultaneously reduced fat (reduction to 10% of the initial fat content) used 3% sunflower oil instead of pork back fat and reduced salt (form 205% to 1.5%) in fuet (a small caliber non-acid fermented sausage).

Besides salt replacement, enrichment in other minerals has been successfully carried out by addition of salts (calcium lactate, citrate, and gluconate for calcium enrichment) or by addition of selenium yeast (for Se enrichment).

Nitrite Reduction

Alternatives to the use of additives have been explored to decrease direct nitrite addition without compromising the quality and safety of dry fermented products. Among them, the use of plant extracts and modification of the processing conditions have been examined.

Celery concentrate was used in combination with tocopherol (200 mg kg^{-1}) in an attempt to produce low-nitrite dry-cured sausages (salchichón), without creating oxidation or sensory-related problems. Another approach was attempting to use the nitrite-reducing effects of glucono-delta-lactone (GDL) and AA to lower residual nitrite levels in sucuk. Data from this study showed that nitrite reduction was achieved with AA but yield and sensory scores for sucuk sausages were compromised.

Curing with reduced amounts of nitrites/nitrates has been evaluated in formulations of salchichón (a typical Spanish sausage product), where the reduction accounted for 25% or 50% of the maximum amount allowable under European regulations (150 ppm each). Inoculation with *Listeria innocua* or *Salmonella* Typhimurium allowed these authors to conclude that the absence of nitrate/nitrite favored pathogens growth, with 2–2.5 log cfu g^{-1} higher counts at the end of ripening, compared to control sausages using normal commercial levels of nitrate/nitrite. Additives such as these represent essential tools in the growth control of pathogens, even when pH and water activity are below the values considered as minimum for their growth. Additionally, omission of curing agents without sufficient technological knowledge and modification of processes results in a product of poor quality and of questionable safety status.

Antioxidants

Some of the strategies that modify the lipid fraction lead to products with a higher unsaturated fatty acids content, which in turn may leave products open and susceptible to oxidation compared to traditional formulations, resulting in lower-quality products, with deleterious modifications in sensory and nutritional properties. The use of synthetic antioxidants, such as BHT and BHA, has been shown to control oxidation during the storage of these products. However, the demand for natural antioxidants in the meat industry has increased primarily because of concerns relating to potential adverse health effects associated with synthetic antioxidants.

In this sense, both vegetable by-products and edible medicinal plants have been explored as sources of antioxidant compounds. The use of green tea extract, *Urtica dioica* and *Hibiscus sabdariffa*, was investigated to control lipid oxidation and amine formation in sucuk. Water extracts from *Borago officinalis* leafs (340 mg kg^{-1}) controlled lipid oxidation in linseed oil-enriched chorizo, whereas *Melissa officinalis* (686 mg kg^{-1}) was effective in a similar product elaborated with an emulsion containing a mixture of linseed and algal oil as partial replacers of pork back fat. Both extracts showed high concentrations of rosmarinic acid, a substance known for its antioxidant capacity. In chorizo, the use of phytochemicals obtained from by-products of grape seed and chestnut processing has been reported. Studies concluded that, at the doses used (1 g kg^{-1}), extracts were more effective than synthetic antioxidants, with grape seed extract better accepted from the sensory point of view. Additionally, cocoa extracts (0.5% w/w) were found to be suitable for enrichment of dry fermented sausages (fuet and salchichón) with health-beneficial polyphenols (mainly flavan-3-ols), without modification of the sensory properties of the products.

Sausages as Probiotics

The commercialization, in 1998, of a German salami product containing three intestinal lactic acid bacteria strains (*Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium* spp.) and a Japanese equivalent containing *Lactobacillus rhamnosus* FERM P-15120 has been reported, claiming to have beneficial health effects. However, the commercial application of probiotic meat products is yet a challenge. Nevertheless, some papers discuss the possibility of using different strains of *Bifidobacterium*, *Lactobacillus*, or *Pediococcus* in several types of fermented meat products: dry sausages, salami, and sucuk.

One of the challenges when dealing with probiotic foods is to demonstrate their ability to survive passage through the alimentary tract and remain in the gut. A number of studies in this area using fermented meat products are noteworthy. A human intervention study demonstrated that the strain *Lactobacillus rhamnosus* CTC1679 was a suitable starter culture for producing safe potentially probiotic reduced fat and *sodium low-acid fermented sausages* (fuets), and it also showed its capacity to survive through the gastrointestinal tract. These products achieved a satisfactory overall sensory quality rating, without negative flavors and with the sensory properties typical of low-acid fermented sausages.

Additionally, *Bifidobacterium animalis* 241a, and especially *Lactobacillus acidophilus* CCDM 476, allowed for the successful manufacture of fermented mutton sausages with good sensory properties and produced viable microorganisms after a 14-day consumption period in healthy volunteers.

However, a debate continues about the actual amount of fermented meat products needed to be ingested to realize the

potential beneficial effects associated with probiotic consumption in this manner.

Conclusions

Dry fermented sausages are meat products produced globally with different procedures giving rise to a broad variety of products. They contribute significant amounts of nitrogenous compounds, minerals, and vitamin B_{12}. At the same, time they have a relatively high energetic value and supply high amounts of fat, a high SFA content, and sodium.

Reformulation of conventional dry fermented sausages allows counteracting some of their nutritional drawbacks, thereby becoming healthier products, without producing negative impacts on their sensory and technological performance. Products with low fat, enriched in MUFA and PUFA, and low sodium have been successfully developed to date, but there remains much scope for further compositional and nutritional improvement of dry fermented meat products.

See also: Cured Foods: Health Effects; Fermented Foods: Composition and Health Effects; Fermented Foods: Fermented Meat Products; Fermented Foods: Origins and Applications; Fermented Foods: Use of Starter Cultures; Low-fat Foods: Types and Manufacture; Low-salt Foods: Types and Manufacture; Meat: Eating Quality and Preservation; Meat: Structure.

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Selenium: Properties and Determination

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Chemical Properties and Biogeochemistry of Selenium

Selenium (Se) is an element of the group 16 and period 4 of the periodic table. It has four natural oxidation states: −II (selenide, SeH^-), 0 (elemental Se, Se^0), +IV (selenite, SeO_3^{2-}), and +VI (selenate, SeO_4^{2-}). The chemical and physical properties of Se are close to those of sulfur (S) as they belong to the same group in the periodic table. These two elements have similar outer valence-shell electronic configuration, atomic size, bond energies, ionization potential, and electron affinity. As Se shares numerous chemical behaviors with S, it is easily incorporated in biomolecules instead of the latter. Nevertheless, the two elements are not fully interchangeable because some key properties are significantly different. In biological systems, Se can replace S in the amino acid cysteine by means of a selenolic (−SeH) group, forming selenocysteine (SeCys). Selenols have stronger acidity compared to thiols (−SH) by up to three orders of magnitude and hence are negatively charged at physiological pH, while thiols are mainly neutral. Moreover, the bond stability of organic Se compounds is lower than in the S analogs, resulting in decreased stability. It follows that Se compounds are more reactive, easily oxidized, and liable to decomposition following exposure to air and light. SeCys is also much more susceptible to degradation under acid hydrolysis conditions than its sulfur analog.

Se is a low-abundance ($0.05\ mg\ kg^{-1}$) constituent of crustal rocks, mainly occurring as metallic selenides and as a minor component of metal-S minerals. The main sink and source of Se is rock material, from which the biogeochemical cycle starts with erosion and follows a number of pathways, as represented in Figure 1. Plants and microorganisms take up Se from soil, incorporating it into tissues, and release it into the atmosphere in the form of volatile methylated metabolites. Animals (and humans) who consume plants and cattle assume Se mainly through their diet. Se is relatively rapidly eliminated when consumed by higher organisms, and biomagnification in the higher levels of the food chain is therefore limited. Se compounds present in soil or released from the decomposition of biological tissues may be partly immobilized under reducing conditions in water or sediments, which makes them no longer bioavailable for plants. Like S, Se vaporizes at high temperatures and thus is also released in the atmosphere by volcanic activity as SeO_2. Upon cooling, volatile Se compounds condense onto PM's surface and undergo wet deposition, closing the cycle. Many anthropogenic sources were introduced into the biogeochemical cycle of Se over the last centuries, including fossil fuel combustion, soil fertilization, and many industrial processes. The addition of selenate to commercial fertilizers, aimed to improve the dietary intake in regions where soil Se is scarce, has an important impact on the whole food chain up to humans.

Se in Soil and Water

The level of Se in soil depends strictly on the parent rock material. The concentration varies geographically over a wide range, from a minimum of $0.01\ mg\ kg^{-1}$ to a maximum of $1250\ mg\ kg^{-1}$, but most soils contain between 0.01 and $2\ mg\ kg^{-1}$ of Se. A level below $0.1\ mg\ kg^{-1}$ identifies Se-poor regions, mainly located in northern Europe (Finland), Siberia, subequatorial Africa, Australia, and scattered areas of Nepal, China, and North Korea. Regions with Se-rich soils, up to the order of $g\ kg^{-1}$, are located in the North American Great Plains, Venezuela, Ireland, India, Nepal, China, and Australia.

Se in soil can exist in all oxidation states, mainly depending on the pH and redox state. Acidic conditions favor the formation of selenite, which has very low solubility and adsorbs to clays and Fe/Al/Mn hydroxides, remaining unavailable for plants. Elemental Se is assumed to form under further reducing poorly aerated conditions, whereas in well-drained and alkaline environment, the equilibrium shifts toward the soluble and bioavailable selenate. In most soils, selenate is the prevalent form and is easily taken up by plants. Organic compounds of Se, mainly amino acids, exist in soil as part of residues of vegetal tissues, but are less important than the inorganic forms.

Se concentrations in natural and domestic waters are generally very low, usually below $1\ \mu g\ l^{-1}$. The WHO has set the maximum limit of Se in drinking water to $0.01\ mg\ l^{-1}$, a standard later adopted also by the EU. With the exception of seleniferous or contaminated areas, such limit is very far from the average concentration of Se in drinking and even natural waters. Thus, water quality criteria for Se are primarily set on the basis of ecotoxicological criteria for organisms at the top of the aquatic food chain, rather than for human consumption. In oxic surface waters, Se speciation is dominated by the oxyanions selenite and selenate, still depending on the redox potential; chlorination in drinking water further favors the predominance of the selenate species. Speciation in freshwater impacts the acute toxicity of Se, a factor taken into account by the US EPA guidelines, but the total level of Se is so generally low that speciation is not determined under normal monitoring.

Se in Food

The main route for Se intake in humans is the diet, while the contribution from the water and air is negligible. The total amount of Se in the diet varies widely depending on the food type and composition, as shown in Figure 2. Bread and cereals, meat, fish, eggs, and milk/dairy products are the primary food sources of Se. The level of Se in crops correlates to that in the soil, whose conditions (pH, redox state, salinity, and organic matter) regulate its bioavailability. The crops, being consumed on a global scale, are an important source of Se. Nevertheless, Se in crops is generally in low abundance because such plants

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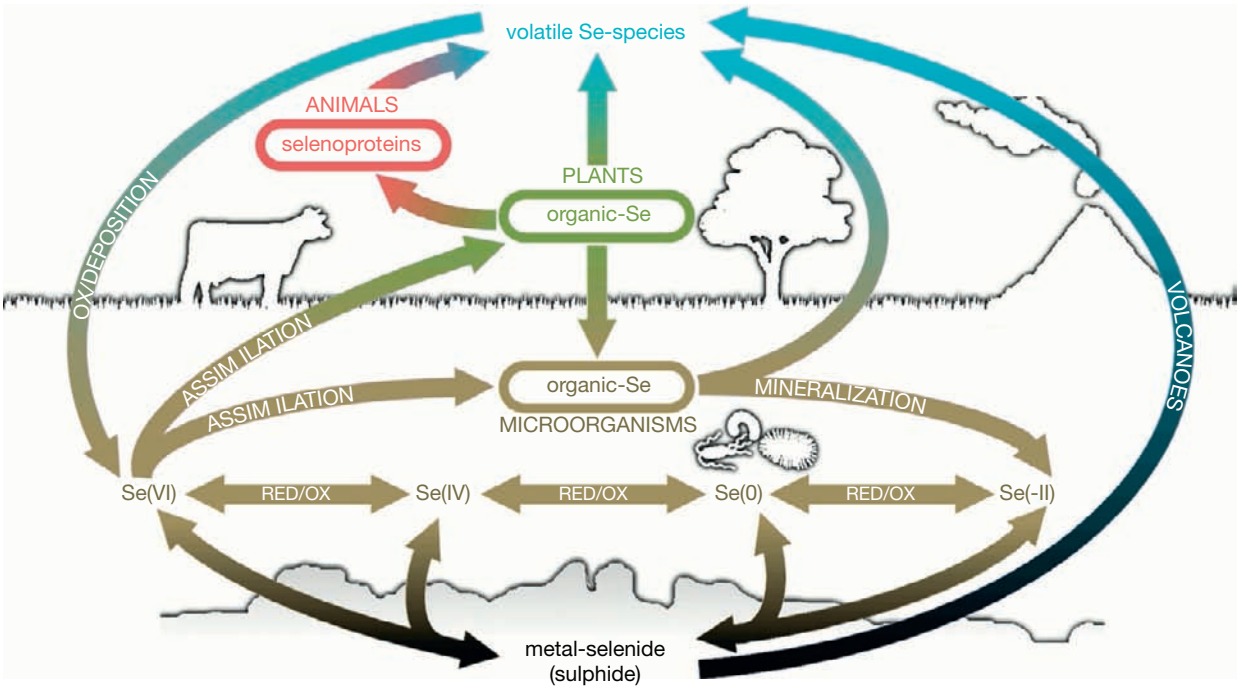

Figure 1 Main pathways of Se biogeochemical cycle.

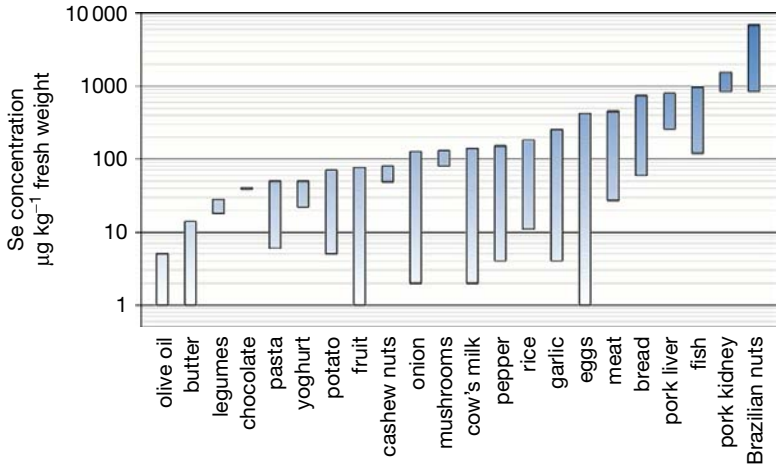

Figure 2 Range (min–max) of Se concentration observed in a variety of food types (the *y*-axis is in log scale).

do not require the element for growth and normally do not accumulate it. A level ranging between 10 and 550 μg kg^{-1} of Se was reported in cereals, whereas Se in bread is generally found at a level of ~60–160 μg kg^{-1}. Unlike crops, some vegetables such as those of the *Allium* family, including garlic and onion, can accumulate significant amounts of Se, reaching concentrations up to 68 and 96 μg g^{-1}. High amounts of Se can be accumulated also by mushrooms and broccoli. The richest natural source of Se is the Brazilian nut, in which this element can reach a concentration of 83 μg g^{-1}. In animal products, the level of Se reflects the one present in the cattle feed used. In meat, eggs, and particularly fish, which are rich in proteins, the Se content is relatively high, in the range of ~50–750 μg kg^{-1}. Additionally, specific organs, such as the liver and kidney, may contain a particularly high level of Se, up to 1500 μg kg^{-1}.

A crucial aspect is that different food types provide Se in distinct combinations of chemical forms (species), each one associated to a specific bioavailability of the element. The main Se species in vegetables are selenomethionine (SeMet) and selenate/selenite; minor species are SeCys, Se-methylselenocysteine (SeMCys), and γ-glutamyl-Se-methylselenocysteine (GGSeMCys). Selenate/selenite, SeMet, and SeCys are the prevalent species in animal products, with widely variable proportions depending on the animals' diet. Each species is characterized by specific absorption/assimilation routes and efficiency. Speciation analysis of Se in food is therefore a key requisite to estimate the actual bioavailability of the uptake.

Se in the Human Body

The concentration of Se in human tissues is below 1 $\mu g\,g^{-1}$, even in organs that play a key role in Se physiology. The adult whole-body amount of Se estimated for different countries reflects its uneven geographic distribution in soils and ranges between 2 and 20 mg. In good health conditions and sufficient dietary intake, the highest Se concentration was observed in the kidney, followed by the liver, spleen, pancreas, heart, brain, lung, bone, and skeletal muscle. Figure 3 summarizes the estimated level of Se in various parts of the human body. Notably, the distribution of the element may vary widely among distinct tissues in the same organ. For instance, the average level of Se in the brain can be estimated around 0.1 $\mu g\,g^{-1}$, but such a value is better representative of the cortex and white matter, whereas the cerebellum and pituitary gland exhibit much higher local levels of the element (0.28 and 0.55 $\mu g\,g^{-1}$, respectively). Relative to the body mass, 25–50% of Se was found in skeletal muscle, 16% in bone, 7–10% in blood, and 4% in kidney. Under conditions of Se deficiency, the distribution of the element within the body may change significantly, favoring those organs that play a key role in its utilization.

Se-Specific Detectors

A variety of detectors are available for the element-specific determination of Se. The most commonly used include inductively coupled plasma-mass spectrometry (ICP-MS) and atomic absorption spectrometry or atomic fluorescence spectrometry (AAS or AFS). Other analytic techniques that are occasionally used, but are not widespread in their application, include instrumental neutron activation analysis (INAA), inductively coupled plasma-atomic emission spectrometry (ICP-AES), and to an even less extent electrochemical and spectrofluorimetric methods. Most instrumental setups are conceived to provide a quantitative measure of the concentration of Se in a liquid medium. Hence, liquid extraction or wet acid mineralization is required for solid samples, whereas filtration, acidification, and dilution may be sufficient for liquids. Attention must be paid throughout the sample preparation, particularly mineralization, in order to prevent the generation and subsequent loss of volatile Se species; long times/low temperatures or closed environments are preferable for such operations. Solid samples can be directly analyzed in particular cases, for instance, when using graphite furnace (GF)-AAS, INAA, and ICP-MS with laser ablation (LA) interface.

ICP-MS is the most powerful technique for Se determination, as it provides high resolution and sensitivity and instrumental limits of detection (LODs) below 0.1 $\mu g\,l^{-1}$ and also enables multielemental analysis. The US EPA published standard methods for the analysis of trace elements, including Se, by ICP-MS in a variety of matrices. The most widespread mass analyzer for ICP-MS is the low-resolution quadrupole, although high-resolution systems such as the double-focussing magnetic sector field are also employed. Compared to other elements, the sensitivity provided by ICP sources for Se is

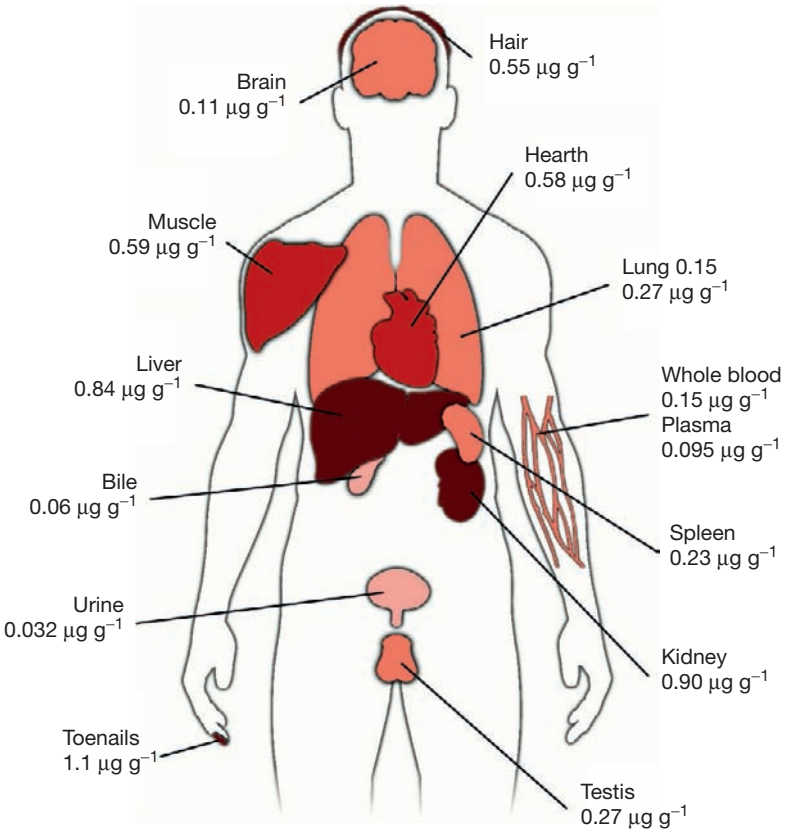

Figure 3 Average Se concentration estimated in various human organs.

relatively lower, due to the high ionization potential of this element. Se has six stable isotopes, all suffering from spectral interferences that must be carefully evaluated before analysis. High-resolution MS allows one to monitor the isotopes ^{77}Se and ^{82}Se free of their main interferences from $^{40}Ar^{37}Cl$ and $^{81}Br^{1}H$, whereas quadrupole MS does not. However, the major isotopes ^{78}Se and ^{80}Se can be resolved from their interferences ($^{38}Ar^{40}Ar$ and $^{40}Ar^{40}Ar$, respectively) only using quadrupoles equipped with dynamic reaction cell (using CH_4 or CO as reaction gas) or collision/reaction cell (using H_2) technologies. Matrix effects must also be taken into account when analyzing complex matrices such as soil or biological materials. For instance, the sensitivity to Se is strongly influenced by the level of organic carbon in the media; hence, the complete mineralization of organic material must be ensured when analyzing solid samples and can be useful also for liquid samples with elevated carbon content (e.g., plasma). The use of elemental internal standards (such as tellurium, Te), isotope dilution, matrix matching, or standard addition strategies for calibration are recommended to overcome possible matrix effects. ICP-MS enables the direct analysis of solid samples by coupling to LA but yields only qualitative or at best semiquantitative data.

AAS is a traditional reference method for the determination of Se, at a relatively low cost compared to ICP-MS. Boosted hollow cathode lamps and electrodeless discharge lamps are available to provide a specific and intense radiation for Se. GF and electrothermal ionization are often used in AAS, as they are more efficient than flame for the atomization of Se. Even if GF requires sample-specific operational procedures, it has the advantage of being applicable to the direct analysis of solid samples. Detection limits down to the range of 0.1–1 $\mu g\,l^{-1}$ can be achieved by GF-AAS or by combining AAS with hydride vapor generation (HG) interface, the latter being the most used setup for the determination of Se by AAS. In HG, all Se species are firstly converted into Se(IV) and then reduced to volatile H_2Se by reacting with $K_2S_2O_8$–NaOH, $KBrO_3$–HBr, or KBr–HCl. The resulting hydrogen stream can be used to create an H_2 microflame, where atomization takes place. In recent years, AFS has also become popular for Se determination. Theoretically, this technique yields even lower LODs compared to AAS, because it measures a small fluorescence signal relative to no background rather than a difference between two signals, and requires simpler and cheaper instrumentation. AFS for determination of Se is typically used only in combination with HG.

ICP-AES can also be used to measure Se, but its application remains limited because of the higher LODs (between 10 and 100 $\mu g\,l^{-1}$) compared to other techniques, caused by the relatively low emission intensity of the element. Such LODs complicate the measurement of Se in digested samples and make the analysis of waters almost impossible. The detection wavelengths for Se are in the deep UV region of the spectrum, potentially hampering the quantification, and the primary detection wavelength (196 nm) suffers from a minor interference from Fe.

INAA is an excellent method for the determination of total Se, as untreated and small weight samples are directly analyzable. INAA allows one to perform multielemental analyses with an LOD of ~0.05 ng but provides only a measurement of the absolute amount of the analyte rather than a concentration, which limits the comparability between samples. This technique requires the use of research reactors, because the samples must be irradiated for the subsequent detection of the 162 keV γ-rays from ^{77m}Se. Its application is therefore limited to very specific studies.

Determination of Se in Soil and Water

The techniques most commonly used for Se determination use liquid samples, and their application to soils therefore requires the previous extraction or mineralization of the sample. Unlike in rocks, Se in soil is generally not incorporated into the mineral lattice, but adsorbed on the surface. The absorption is also weak because selenate is typically the dominant form, having no high affinity for silicates, so HF is generally not necessary to digest soil for Se analysis. The main tasks for digestion are to reach a quantitative dissolution of the organic materials and to oxidize all forms of Se to Se(VI). The method with better efficiency, safety, and compatibility with detectors uses HNO_3/H_2O_2 mixtures for microwave digestion at high temperature and pressure in closed vessels. Dilution of the digests with water is generally sufficient to decrease the acidity, which is preferable in ICP-MS, and to not interfere with the subsequent chemical reduction when HG is used in combination to AAS or AAS. Calibration strategies based on matrix matching and/or internal standardization are recommended to overcome the variability of matrix effect associated to the heterogeneous composition of the samples. Contamination due to sampling/storage/preparation equipment and manipulation during sample preparation is usually not a problem for Se determination in soil. The only concern is the possible loss of volatile species, which is why drying/lyophilization and dry ashing should be avoided. Sample freezing is preferable, and digestion should be carried out on the wet sample, with the water content determined apart. Although 0.1 g of material may be sufficient for the determination of Se in soil, with LODs in the range of 0.01 mg kg^{-1} when using either ICP-MS or HG-AFS/AAS, it is preferable to use 0.5 g or more to guarantee the representativity of the sample.

From an analytic point of view, assigning exact chemical structures to the binding forms of Se in soils may be very difficult. However, their individual determination can be achieved on the basis of operational criteria by means of the so-called fractionation. A soil fractionation analysis typically consists in sequential extractions with media of increasing aggressiveness. The weekly adsorbed Se species can be firstly extracted in water media (phosphate or $MgCl_2$ solutions). Treatment with alkaline solutions allows one to decompose selectively the organic matter without oxidizing the reduced forms of Se (0 and –II). Fe–Mg minerals can be dissolved reductively to release the strongly bound Se species, whereas Se^0 can be extracted with CS_2 without attacking the sulfide mineral fraction. Finally, the sulfide mineral fraction can be dissolved by acid oxidation. For a fractionation study, preserving the redox conditions of the sample until analysis is crucial. Freezing, working in a glove box, and reducing as much as possible the time for storage and manipulation are valuable strategies.

Analyzing water for Se content is much easier. Since Se is not highly particle-reactive under typical oxic conditions, the

dissolved fraction approximates closely the total level. Acidification of freshwaters with 1–0.1% HNO_3 is a sufficient treatment for the determination of Se, aimed to prevent coprecipitation with major elements during storage. Even if contamination from sampling and storage containers and equipment does not remain a major concern, the level of Se in water may be very low, so that decontamination of plastics and working in clean environment may help to guarantee a good signal-to-background ratio.

Determination of Total Se in Biological Samples

The preparation of solid biological samples for the determination of total Se is analogous to that mentioned for soils, as it typically comprises microwave digestion with mixtures of HNO_3/H_2O_2. Liquid samples containing clots, cells, or proteins (e.g., whole blood and plasma) are also analyzed more reliably *previa* digestion; systems to perform on-line microwave digestion can be coupled to elemental detectors. The concentration of Se in many biological materials, particularly human tissues, is often very low (below 1 $\mu g\,g^{-1}$ level) and may be further reduced by dilution after digestion. Decontamination of laboratory equipment and working in a clean environment is therefore recommended. Loss of Se is also a major risk due to the presence of volatile metabolites (in urine, plant, and microbial materials) or to their generation by enzymatic reactions after sampling. Immediate freezing for storage is advisable, whereas drying should be avoided, and the percent moisture should be determined separately. If homogenization is necessary, grinding in liquid N_2 is a good option. Detection limits of the order of 0.1–1.0 $ng\,g^{-1}$ can be routinely achieved by AFS/AAS and even lower ones by ICP-MS using the reaction cell modes. The US National Institute of Standards and Technology (NIST), the National Research Council of Canada (NRC/CNRC), and the EU Community Bureau of Reference (BCR) provide a variety of biological matrices certified for Se concentration, which are very useful for validation and quality control.

Speciation of Se in Biological Samples

Unlike soils, biological matrices offer the opportunity to assign exact chemical structures to individual species of Se-binding compounds, each of which plays a specific role within the physiological processes, and corresponding analytic tools for their specific determination, the so-called speciation analysis. The Se species in biological matrices include assimilated inorganic and organic compounds, intermediate and final Se metabolites, and selenoproteins. The latter are the mediators of the biological action of Se as an essential nutrient for animals.

Sample Preparation

The sample preparation procedure for speciation of Se must be milder than the acid digestion employed for the total determination, in order to prevent species degradation and interconversion. Figure 4 summarizes the main sample preparation

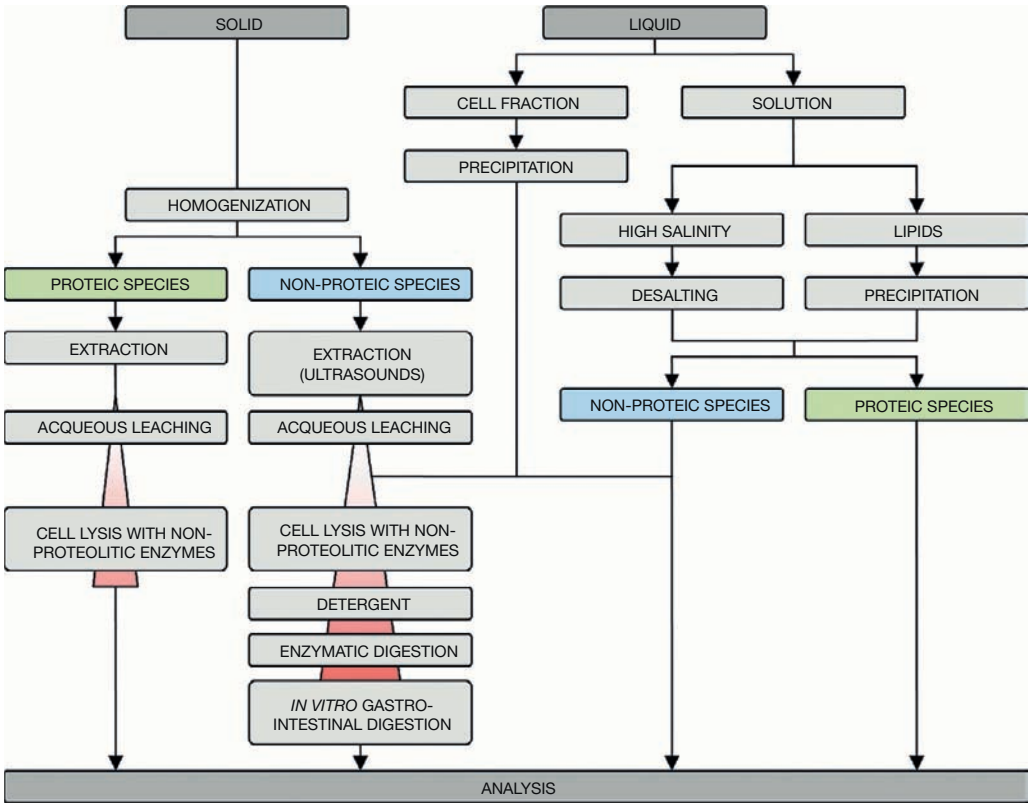

Figure 4 Flowchart of the sample preparation strategies for the speciation analysis of Se in biological matrices.

strategies used to perform speciation analysis of Se in biological matrices. Blood and its fractions (plasma, serum, and erythrocytes), breast milk, and urine are the bodily fluids most commonly analyzed for Se speciation. Plasma and urine have the advantage of containing soluble compounds, while erythrocytes must be lysed to release the Se species. Desalting or water dilution can be necessary for the analysis of urine, because the high salt concentration can be incompatible with chromatography. The normal constituents of urine are water-soluble and this enables direct chromatographic separation. Plasma/serum samples usually do not require a special treatment, a part of filtration to remove cell residues and cloths. For erythrocytes lysis, the most common method involves freeze–thaw cycles, followed by centrifugation and filtering. The analysis of milk requires the elimination of lipids by centrifugation and precipitation of casein with acetate.

The preparation of solid biological samples is more complex because the analytes must be extracted into a liquid phase. Some free Se amino acids or Se peptides are water-soluble and can be extracted with deionized water or various buffer solutions (Tris–HCl, phosphate, and HEPES). This procedure has been applied with variable recoveries to selenized yeast (10–15%), animal tissues (10–76%), plants and seeds (2–73%), and mushrooms (36%). Acid or alkaline hydrolysis can been applied to Se extraction with much higher recoveries, but degradation or transformation of the species can easily occur. Enzymatic hydrolysis using proteolytic enzymes or cocktails is the most frequently applied method for the pooled extraction of proteic and free Se amino acids, allowing one to operate at room temperature and neutral pH and thereby preventing species degradation. Conversely, when proteins must be preserved in their intact form, spiking the extraction buffer with inhibitors of proteases is important. EDTA inhibits metalloproteases by chelating Mg and other metal ions that are part of their active site, without affecting Se speciation because the element is covalently bound to its ligands. Detergents such as SDS, Triton-X, and Igepal break down the cell membrane and form ion pairs with proteins. This process not only increases the extraction of selenoproteins but also can denaturate them. Protein precipitation by organic solvent and size-exclusion chromatography can also be used as additional steps for size-based fractionation and concentration. A particular application of sequential extraction is the establishment of Se bioaccessibility by using an *in vitro* gastrointestinal digestion method, which consists in a first treatment with synthetic gastric juice containing pepsin, followed by a second treatment with synthetic intestinal juice containing pancreatin, amylase, and bile salts. Se amino acids, including SeCys and SeMet, and selenoproteins are susceptible to oxidative degradation with consequent loss of Se from the residues. To prevent the oxidation of amino acids, a feasible approach is the addition of iodoacetic acid or reducing agents such as dithiothreitol and β-mercaptoethanol, whereas selenoproteins are better protected by working under N_2 atmosphere.

Separation of Se Species and Coupled Techniques

The major techniques employed for Se-species separation in liquid samples or extracts are high-performance liquid chromatography (HPLC), gas chromatography (GC), and electrophoretic techniques, particularly capillary zone electrophoresis (CZE). These techniques are applicable to different classes of expected species and differ in the separation power (i.e., the number of compounds that can be separated in one single run). Theoretically, all these techniques can be coupled on-line to elemental detectors including ICP-MS, HG-AFS/AAS, and ICP-AES, leading to the so-called hyphenated techniques. The on-line coupling allows one to perform the separation and individual quantification of the various Se species in one single analytic run, saving time and minimizing manipulations. If the coupling is unavailable, Se speciation analysis can be performed using the same separation techniques, followed by fraction collection and subsequent off-line elemental analysis.

Generally, HPLC is more effective to separate relatively simple mixtures of small molecules such as inorganic species, small metabolites, and Se amino acids, providing a versatility derived from a wide variety of separation mechanisms. For studies in which only a few known Se species are monitored and eventually quantified, multidimensional HPLC methods offer higher specificity and technical flexibility that makes them adapted to a wide variety of compounds. Size-exclusion chromatography does not guarantee sufficient resolution for quantitative purposes, but is often applied to achieve a preliminary fractionation of Se biomolecules with Mw in the range 10–1000 kDa or to screen complex samples with unknown composition. Reverse phase is the most widespread mode used in HPLC and is particularly effective to separate Se amino acids and metabolites. Its coupling to ICP may be problematic because it requires eluents with high percentage of organic solvents. The diffusion of micro- and nanoflow HPLC systems and nebulizers allowed researchers to overcome this problem by maintaining the absolute amount of organic solvent carried to the ICP source at a low level. Another strategy consists in adding ion-pairing agents (TFA, HBFA, or room-temperature ionic liquids) to the mobile phases to increase the retention of the analytes with lower organic media. Ion exchange is another mechanism widely used in HPLC to separate inorganic species of Se and other easily ionizable Se compounds. In recent years, affinity and multiaffinity chromatography emerged as new powerful separation mechanisms, designed to retain individual selenoproteins with high specificity. Methods for the speciation of plasma/serum selenoproteins based on affinity HPLC-ICP-MS have been recently developed. GC coupled to elemental detectors can be used to separate and determine the volatile or readily derivatizable Se compounds. Solid-phase microextraction can be used to extract/preconcentrate Se metabolites from urine or particular volatile metabolites from plant extracts and can be directly combined to GC-ICP-MS for their determination.

Complex mixtures of Se compounds and intact selenoproteins are more efficiently separated by electrophoretic techniques. CZE is a separation technique based on differences in the electrophoretic mobility of target analytes and on electroosmotic properties. Its coupling to ICP-MS offers attractive features for Se speciation, including high resolution, a sample amount below 1 ng, absolute detection limits at pg levels, and the possibility of analyzing relatively labile species. So far, a few applications of hyphenated techniques based on CZE have been proposed for Se peptides (from enzymatically digested

selenoproteins), amino acids, and metabolites, but this technique is one of the most promising trends in the field.

Identification Techniques and Integrated Approaches

It is worth noting that Se speciation using elemental detectors is the best method for quantification but does not provide direct identification of the species because molecular information cannot be achieved. Identification can be only relative to the analysis of individual standards, but this approach may be unfeasible when appropriate standards are unavailable, particularly selenoproteins or unknown molecules. Molecular MS techniques such as electrospray ionization (ESI)-MS and ESI-MS/MS and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF)-MS are useful tools by which structural information can be achieved to reach the univocal identification of Se species. Theoretically, ESI-MS/MS can allow the direct detection of Se heteroatom based on its characteristic isotopic pattern, whereas this is practically impossible in MALDI-TOF-MS due to inadequate resolution. For low Mw Se-species identification, ESI-MS and ESI-MS/MS are more frequently used, since they allow high specificity and relative sensitivity for the unambiguous identification of the compounds by their exact mass and fragmentation pattern, as well as easy on-line coupling with HPLC and CZE. This technique can also be an alternative to ICP-MS for quantification purposes down to the ng g^{-1} order of concentration. Selenoproteins can be studied *previa* enzymatic digestion carried out either before or after separation, followed by the analysis of the resulting peptides. Carboxymethylation is essential in this case to protect the selenolic groups in SeCys, even if the derivatization agent may react with SeMet producing artifact peaks. The efficiency of species separation is also crucial, because a minor Se peptide must arrive virtually pure at the ESI source in order to be detected. Through fragmentations patterns, *de novo* sequencing of proteins can be achieved by ESI-MS/MS. MALDI-TOF-MS is a competitive method for the identification of selenoproteins: it not only provides the exact mass of their digests, which can be used to trace the peptide mass fingerprint, but also allows one to characterize the mass of their intact forms.

Approaches based on tandem total and speciation ICP-MS analyses and molecular MS for the study of complex matrices are extremely powerful strategies to provide information about the number of compounds that should be identified, their concentration, the efficiency of sample preparation, and analytic recovery. Such integrated ICP-MS-assisted approaches, also defined as 'synergic' or 'multimode' and schematically resumed in Figure 5, are the most actual trend in Se proteomics and metallomics.

Evaluation of the Se Nutritional Status

The action of Se as essential trace element is mediated by selenoproteins. Although their biochemistry is still partially unknown, it has become evident in recent years that each of them participates in distinct biological processes, according to a precise ranking in the incorporation of the element. According to the current interpretation, good health should not be defined as the absence of pathologies, but rather as optimum biological functionality. In this context, the diversity and specificity of selenoprotein action complicate the assessment of the status of Se due to the difficulty to establish (i) the actual correlation between intake (and speciation in food) of Se and the overall biological functionality; (ii) which biological function is the limiting process for the definition of the optimum health state; and (iii) which marker(s) better represents such biological functionality. Despite its widespread use until recent years, the total concentration of Se in body fluids cannot be considered a representative marker of Se status. Nowadays, the most frequently adopted parameter is the activity of the individual plasma selenoproteins glutathione peroxidase type 3 (GPx3) and selenoprotein P (SelP) compared to their

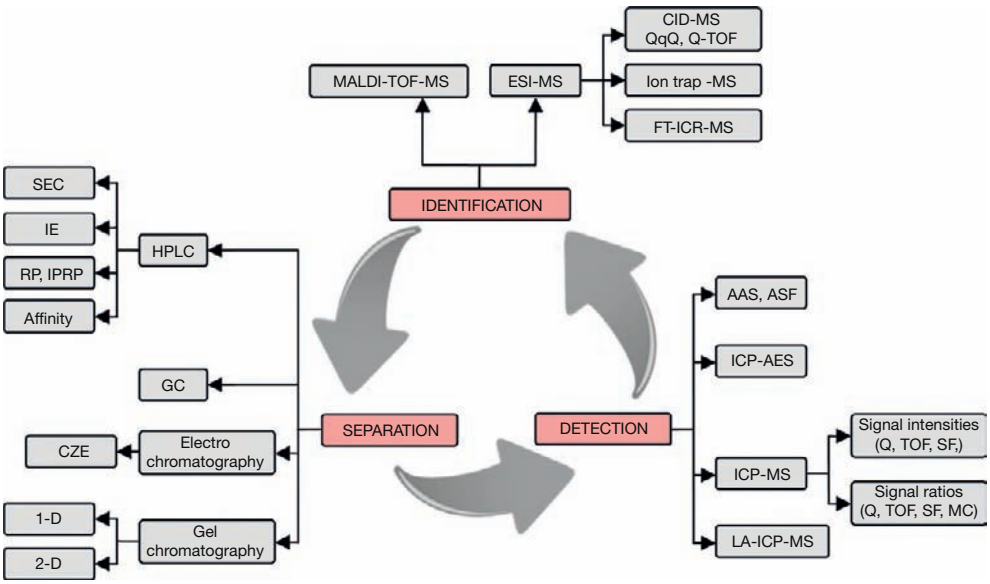

Figure 5 Flowchart of the integrated methodologies for the speciation analysis of Se in biological samples.

maximum (~100 and 124 ng ml^{-1} of Se, respectively), which is representative of the systemic distribution and correlates to the short-term intake (a few hours). However, plasma selenoproteins lose their validity as markers when Se overcomes their basal requirement, because they do not respond to additional increases of the element. New multiparametric markers, such as the expression of Se-protein mRNA in circulating leukocytes, integrated with the comprehensive analysis of health parameters, endocrine and immunological status, selenoprotein polymorphism, and other variables, are under evaluation, but introduction in a regulatory context is still to come.

Recommended Se Intake, Current Values, and Supplementation

Several institutions worldwide proposed reference values for the daily estimated average requirement (EAR, the intake level meeting the needs of 50% of the population) and recommended dietary allowance (RDA, the intake level meeting the needs of 97.5% of the population) for Se, taking into account the most reliable epidemiological studies. Figure 6 represents the range of theoretical dose–response curves used by various institutions to calculate such reference intakes, their hypothetical correspondence to plasma Se level, and possible endpoints for the evaluation of Se status. The basal requirement of Se is defined as the intake allowing the prevention of pathologically and clinically relevant signs of dietary inadequacy. An intake of ~20 μg day^{-1} for adults is generally accepted as the minimum needed to prevent Keshan disease onset and corresponds to the lower limit of the proposed EARs. Based on GPx3 activity maximization, the RDA adopted by various institutions ranges between 25 and 75 μg day^{-1} (most commonly between 50 and 60 μg day^{-1}), with small differences between genders and for particular categories (i.e., pregnant women) or age groups. The activity of other important selenoproteins such as SelP and iodothyronine deiodinases (DIOs) can provide an additional indication on the functionality of the Se system. Providing upper limits (ULs) for tolerable Se intake is more difficult due to limited data on Se toxicity for humans. So far, acute toxic symptoms were associated with extremely high Se intakes ranging between 3200 and 6700 μg day^{-1}. However, symptoms such as fingernail changes have also been reported for lower Se intakes, so that a threshold of 1260 μg day^{-1} is considered as the reference at which clinical selenosis appears, whereas the ULs

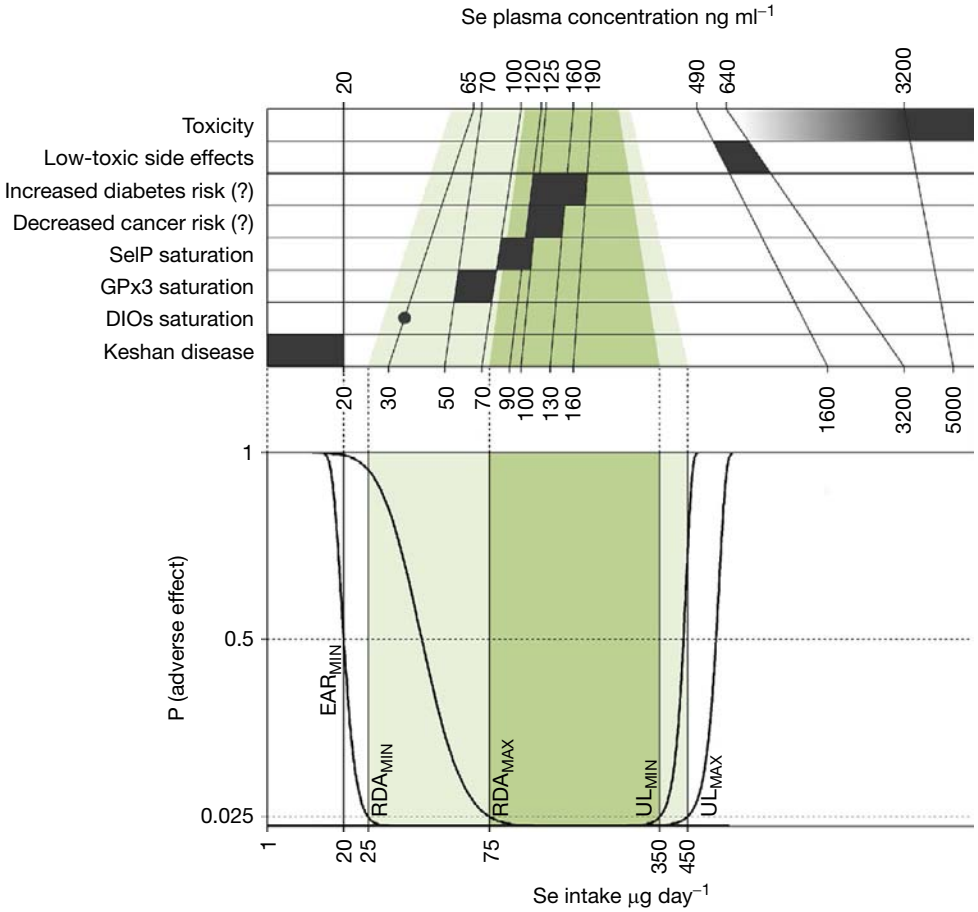

Figure 6 Indicative association among Se daily intake, plasma Se concentration, and possible endpoints for the evaluation of Se status. Two theoretical dose–response curves are represented to extrapolate the reference intake values (EAR, RDA, and UL), respectively, corresponding to the minimum and maximum of the range of values provided by the main institutions worldwide. The range of the endpoints is defined by the minimum and maximum values reported in the literature. The *x*-axis is in log scale.

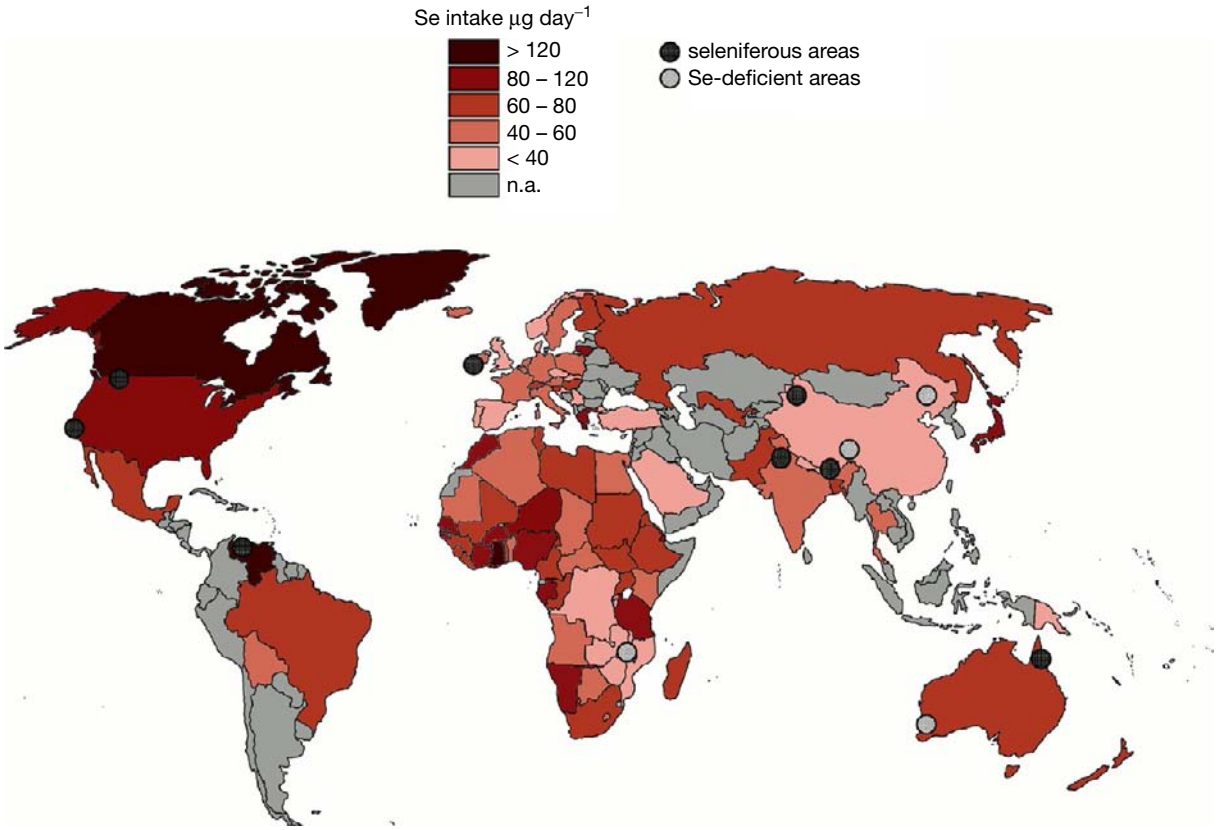

Figure 7 Estimated daily intake of Se for adults in various countries worldwide.

(corresponding to no adverse effect) usually range between 350 and 450 µg day^{-1}.

A number of approaches can be used to estimate the actual dietary intake of total Se, ranging from direct analysis of composite food types to indirect calculation using dietary or market basket surveys and food composition tables. Each approach has specific limitations, and in most large-scale evaluations, speciation, although important, is still overlooked. Since the total concentration of Se in food reflects primarily the soil conditions and secondly the nutritional habits, the dietary intake varies widely with the geographic localization, as shown in Figure 7. The individual dietary intake of Se was estimated between 3 and 7000 mg day^{-1} worldwide, with the highest levels in localized seleniferous regions of China, India, Venezuela, the United States, Australia, and Ireland. The average intake in Europe is ~50 mg day^{-1}, close to the typical RDAs. Some regions of northern Europe, the United States, and other countries exhibit relatively low levels of soil Se, but only a few extremely deficient regions were documented, mainly located in China, Australia, and Malawi. Because of the low abundance of soil Se in some areas around the globe, a wide variety of Se-enriched materials have been produced in order to supply populations with RDA adequate levels of Se. Among them, the use of fertilizers enriched in sodium selenite is a very effective, readily controlled, and widely used strategy to obtain vegetables with high Se concentrations. The total Se intake in Japan, Australia, Finland, and the United States, as well as in the Keshan areas in China, was significantly increased in the last decades thanks to this strategy.

See also: Bioavailability of Nutrients; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Dairy Products: Dietary and Medical Importance; Dietary References: US; Dietary Surveys: National Food Intake; Food Composition Databases; Food Fortification: Rationale and Methods; Infrared Spectroscopy: Applications; Mass Spectrometry: Applications.

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Sensory Evaluation: Sensory Rating and Scoring Methods

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Introduction

Sensations and hedonic experiences cannot be shared directly with other people, and therefore, it was thought that these experiences were inaccessible to direct measurements. However, after some years of studies, psychologists and researchers in the food area have accepted the use of methods with scales to measure sensory and hedonic responses. Since there are theoretical and practical differences among the various scales, many studies, discussions, and controversies existed and still exist.

The focus of most methods using scales consisted of measuring the intensity of sensations. The development of hedonic scales fell behind since the assessment of product acceptance was considered minor by psychophysicists. However, in recent years, the interest in measuring hedonic responses has grown dramatically, resulting in the development of several new methods.

Rating and scoring methods are the basis for the quantification of sensory information. Although these terms are often used as synonyms, actually, they have different meanings. Rating refers to the quantification of information through the use of ordinal categories, while scoring is a more definite way of rating, using a numerical interval or a ratio scale, whose properties are known. Thus, with rating information about the order of acceptance of the samples is obtained, while with scoring, it is possible to determine differences in acceptance among the products or consumers and the size of this difference, depending on the scale used.

Since the methods with scale to measure the intensity of sensations were always discussed, this article will focus on methods with scales that have been developed to measure hedonic responses. We intend to compare the different methods with scales for product acceptance assessment, showing their advantages and disadvantages, in order to help in the selection of the best method for each research.

Types of Scales

Four types of scale can be used to obtain sensory data of product acceptance: nominal, ordinal, interval, and ratio. Table 1 presents information about the different scales.

Nominal Scale

Nominal scale is that in which the collected data are categorized by name or number. The categories have no logical order, so the arrangement order in the evaluation sheet does not change the logic of the question or data processing. In addition, there is no quantitative relationship among them.

Nominal scales are hardly used in sensory analysis. An example of its use would be a test where assessors should indicate the number 1 for the products they consider good and 2 for those they consider bad. They can also be used to classify demographic data of the participants such as gender, age, education, and frequency of consumption of the product in question. The data analysis and interpretation are made through histograms, indicating the frequency of occurrence of responses in each category. Then, it is determined if there are more observations in one category than another through the chi-square test.

Ordinal Scale

Ordinal scale allows one to determine the order in which the products are accepted by consumers (e.g., product A, the most accepted; product B, the second most accepted; and product C, the least accepted). This type of scale cannot quantify the degree of difference among products. Ranking is considered an ordinal scale.

Interval Scale

Interval scale is used to measure magnitudes (different degrees of acceptance) assuming equal distances among the points of the scale and the existence of an arbitrary zero. Nine-point hedonic scale is often considered an interval scale, although this classification is debatable since the intervals are numerically but not psychologically equal.

Ratio Scale

Ratio scale is also used to measure magnitudes, and it is a special case of interval scale, wherein the acceptance of a

Table 1 Types of scales

Scale	*Basic operation*	*Number usage*	*Permissible statistics*	*Example hedonic scales*
Nominal	Categorization	Used as labels	Nonparametric	1: Good 2: Bad
Ordinal	Greater or less	Rank order	Nonparametric	Ranking
Interval	Differences	Degrees of difference	Parametric	Category scale
Ratio	Ratios	Relative proportions	Parametric	LAM, LHS

The numerical values for the semantic labels can be found in the original papers for each scaling method (Lim et al., 2009 [LHS], Schultz and Cardello, 2001 [LAM], Guest et al. (2007) [OPUS], and Green et al. (1993) [gLMS]).

Source: Adapted from Lim, J. (2011). Hedonic scaling: a review of methods and theory. *Food Quality and Preference* **22**, 733–747.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00617-6

product can be expressed as a percentage or ratio of acceptance of the other products. Ratio scale has an absolute zero. For example, a product obtaining score 8 is twice more accepted than that obtaining score 4. Labeled affective magnitude (LAM) and labeled hedonic scale (LHS) are examples of ratio scales.

Rating Methods

The rating methods involve quantifying the acceptance of products using ordinal scales. In this type of method, it is not possible to quantify the degree and ratio of the difference in acceptance, that is, you cannot know how much a sample is more accepted than the other is.

Ranking

This method is used when the objective is to compare several samples according to the preference, although it may be used when assessing any attribute, such as sweetness and crispness. It is a simple way to compare the samples, but the data are ordinal and no measure of the degree of difference is obtained from the participants. Products are presented all at once in balanced order, which can limit the number of samples due to sensory fatigue. The assessor is required to rank according to the attribute of interest or, for example, the preference.

The ordering of up to five or six products is possible; however, it depends greatly upon the nature of the sample. Data are analyzed using nonparametric statistics (e.g., the Friedman test) in order to determine whether there is difference in acceptance and among which samples. Ordering test does not provide data on the absolute values of acceptance or rejection. In addition, the products are ranked according to the degree of acceptance, but it is not possible to say whether all samples were accepted or rejected.

The judges can taste the products as often as necessary and review or change their scores, preventing inappropriate ratings caused by forgetting of previous samples. This is because, when the assessment is performed monadically, the scores given to each product, as well as the exact sensation it promotes, may be forgotten. With permission to try the products as often as they wish, the judges may change the order of products in the scale, until they are convinced that the degrees of acceptance of samples are properly represented.

When this protocol is compared to the monadic assessment, where tasting products more than once and monitoring assigned scores are not allowed, it presents fewer errors due to memory losses. In addition, it has the advantage that many judges find it easy to do the ordering of products as they are used to order 'things' in everyday life. Finally, human behavior as a data source would be more convenient than numerical estimates obtained with verbal scales.

However, this protocol may not be suitable in certain situations, such as when products subject to sensory adaptation are evaluated, precisely because there are multiple tastings of the same sample. In addition, it is considered a time-consuming method, and elderly or those with difficulty in reading or performing mathematical tasks would have difficulty using it.

Best–Worst Scale

A best–worst scale is an alternative preference elicitation method and is more readily understood through an example. Consider four products (A, B, C, and D) that will be evaluated for acceptance by consumers. An experimental design is used to generate sets of three samples from the four products (A, B, C, and D), which will be evaluated in four sessions with three products per session (Figure 1).

The participants of the test are required to indicate the most accepted (the most liked) and the least accepted (the least liked) product out of the three products available. Responses are converted into individual scales for each sample: (1) The number of times a certain sample has been indicated as the most accepted and the number of times it was considered as the least accepted is counted; (2) these numbers are subtracted. For example, a final value of 1 means that the sample was indicated twice as the most liked and once as the least liked ($2-1=1$), for example. A value of 0 means that the sample was indicated the same number of times as the most liked and the least liked. It is possible to assess the final results among participants or, overall, for the samples, indicating the most and the least accepted ones (Table 2).

The data from the first six assessors are shown in Table 2. For subject 1, the most accepted sample was sample A (value 3) and the least accepted sample was sample C (value -3). Overall (aggregated value), the preferred sample was C and the least preferred sample was A. The results of this scale can be transformed into a scale of probability when analyzed by multinomial logit.

Least liked sample	Set 1	Most liked sample
□	Sample B	□
■	Sample C	□
□	Sample A	■

Least liked sample	Set 2	Most liked sample
□	Sample D	□
□	Sample A	■
■	Sample B	□

Least liked sample	Set 3	Most liked sample
□	Sample A	■
□	Sample D	□
■	Sample C	□

Least liked sample	Set 4	Most liked sample
■	Sample C	□
□	Sample B	□
□	Sample D	■

Figure 1 Example of best–worst scale using four products. Reproduced from Jaeger, S. R., Jorgensen, A. S., Aaslyng, M. D. and Bredie, W. L. P. (2008). Best–worst scale: an introduction and initial comparison with monadic rating for preference elicitation with food products. *Food Quality and Preference* **19**, 579–588.

Scoring Methods

Scoring methods are those in which one can observe the acceptance differences and in some cases the size of such differences. Furthermore, in many cases, it is possible to evaluate differences in acceptance among groups of consumers.

Category Scales

Category scale is a measurement method in which the assessor is asked to 'measure' the intensity of a stimulus assigning a value (category) on a limited scale, which is usually numerical. Category scales are widely used in sensory analysis for affective responses (related to the preference or acceptance of products). These are scales where the degree of acceptance is evaluated through verbal (nine-point hedonic scale) or numerical labels (numerical nine-point hedonic scale).

Nine-point hedonic scale

Nine-point hedonic scale is widely used for acceptance testing when the goal is to determine how much a specific product is liked by the consumer. It consists of a balanced bipolar scale around the neutral category (5 = neither liked nor disliked), with four positive categories (6, 7, 8, and 9 = liked slightly, liked moderately, liked very much, and liked extremely, respectively) and four negative categories (1, 2, 3, and 4 = disliked extremely, disliked very much, disliked moderately, and disliked slightly, respectively) (Figure 2).

The categories are composed of sentences representing several degrees of affection and are arranged successively, suggesting continuity of liking and disliking of products. The sentences help consumers to respond adequately and researchers to interpret the responses regarding the acceptance of products.

Table 2 Example of data of six judges evaluating four samples using best–worst scale

Participant	*B–W (sample A)*	*B–W (sample B)*	*B–W (sample C)*	*B–W (sample D)*
1	3	−1	−3	1
2	0	0	3	−3
3	−3	−1	2	2
4	−1	−3	3	1
5	−3	3	1	−1
6	−3	2	−1	2
Aggregate	−7	0	5	2

Source: Jaeger, S. R., Jorgensen, A. S., Aaslyng, M. D. and Bredie, W. L. P. (2008). Best–worst scale: an introduction and initial comparison with monadic rating for preference elicitation with food products. *Food Quality and Preference* **19**, 579–588.

This scale can also be displayed vertically and be adapted to a seven-point scale or present 'faces' representing the degree of like/dislike, to facilitate its use with children or people who cannot read. This type of scale is often used for comparison among samples in *Food Science*, considering that if consumers 'liked extremely' sample 'A' and they 'liked slightly' sample 'B,' then sample 'A' is more accepted and preferred than sample 'B.' Thus, this scale infers the preference.

When using this scale, products must be presented in a monadic way, that is, one at a time, with no new assessment of the same sample or reference to the scores given to previous samples. The idea is that in purchasing situations, consumers do not test several products and then select the preferred one; they evaluate the products, one at a time, and select one based on their comparative memory of previous evaluations of products, that is, comparing the products to their internal and individual reference. The aim when evaluating samples monadically is to promote absolute evaluations that are not affected by comparisons with other products, thus removing contextual effects. Ideally, the samples should be evaluated on different days or weeks, but for practical conditions, products are evaluated one after the other and the evaluation sheet with the scores and samples are removed after each assessment. The order of samples presentation must be balanced to avoid positioning errors or carryover effects, and for this, designs are already available to facilitate the implementation of tests.

The main reason the nine-point hedonic scale is the most used in acceptance testing is that, compared to other scales (e.g., magnitude estimation), it has a limited number of categories, making it easy to use by different segments of the population and requiring no prior training. For researchers, the evaluation of the generated data is easier when compared to other techniques, which require measurement of lines or estimation of ratios that may include fractions. In addition, it was demonstrated that scales with simple categories are as sensitive as the other scales (magnitude estimation) in relation to the power of sample discrimination. So, if the main goal of a study is to measure differences in acceptance among samples or predict the acceptability, the nine-point hedonic scale has proved to be simple and effective. Many companies have stored data based on this kind of scale, which facilitates the comparison of products under development to those already available on the market.

However, although it is widely used in *Sensory Science*, several limitations are attributed to the nine-point hedonic scale. First, by having unequally spaced categories, the result indicates which samples are more accepted but does not quantify how much a sample is more accepted than another sample (because if a product obtained score 8, it does not mean that it is twice more accepted than a product obtaining score 4), nor compares the acceptance among individuals or groups.

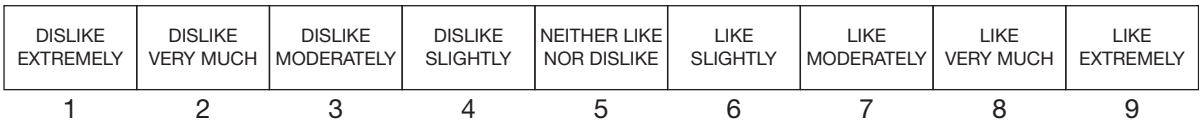

Figure 2 Nine-point hedonic scale with the numbers used in the statistical assessments. Reproduced from Wichchukit, S. and O'Mahony, M. (2014). The 9-point hedonic scale ranking in food science: some reappraisals and alternatives. *Journal of the Science of Food and Agriculture*. http://dx.doi.org/10.1002/jsfa.6993.

Figure 3 Numerical nine-point hedonic scale.

Secondly, due to the limited number of categories, it offers the assessors little freedom to express in full their hedonic experience when tasting the product. In addition, there is a general tendency among assessors of not using the extremes of the scale, reducing it from 9 to 7 points and limiting the ability to discriminate samples that consumers liked or disliked a lot (located at the scale extremes). This is because the assessors think that, once they assign a sample the maximum/minimum score, the following products that may be perceived as more or less accepted may not be adequately evaluated.

Frequently, there are errors by adaptation, that is, there is a tendency for the judges to repeat the same evaluation in conditions in which a series of products are displayed. The presence of a neutral category ('neither liked nor disliked') reduces the scale efficiency, since it encourages a certain degree of complacency by individuals, that is, they consider this category as being safe, which they can use without effect on the assessment.

Finally, considering the viewpoint of statistics, as the generated data are categorical and discrete, without a zero point, the type of statistics is restricted to nonparametric statistics. However, it is a common practice among researchers to use parametric statistics such as analysis of variance to analyze the collected data, although it is mathematically inappropriate because the homogeneity of variances and normality of experimental errors must be assumed.

Large numbers of consumers are required to evaluate the acceptability of a product with sufficient degree of reliability, since there is large subjectivity in the assessment.

When the nine-point hedonic scale shows only numbers, it is called numerical scale (Figure 3), and labels may be added at the extremes and in the middle of the scale. The judges are asked to taste the samples one by one and indicate the degree of acceptance, using the numerical values.

The numerical hedonic scale is useful when comparing a product under development to the market leader, as well as to products with intermediate and worst sales. If the new product reaches a score similar to that of the leader, it means that it is as accepted as the leader, no matter if the score obtained was 9, 8, or 7.

When using the traditional nine-point hedonic scale, the accepted products are positioned at the top of the scale (liked slightly to liked extremely). In the case of numerical hedonic scale (without using labels), products are expected to be positioned along the scale, which does not imply that a product at positions 1–4 is rejected. This is because the judge may have liked all products, positioning them on the scale in order of preference. When the labels are added, the numerical scale behaves similarly to the traditional scale, although the numbers do not represent the same hedonic sensations.

Continuous line scales

Unstructured or linear hedonic scales are represented by a line, anchored at their extremes with the minimum and maximum degrees of acceptance (disliked extremely and liked extremely) (Figure 4).

Figure 4 Unstructured or linear hedonic scale.

The line size is generally 100 mm; however, it may vary according to the test requirements. Very short lines can reduce the power of discrimination of products by judges, while very long lines prevent the judges from being accurate in their assessments.

The collected data are measured within an interval; however, they must be considered as ordinal, as explained for the nine-point hedonic scale.

The use of unstructured or linear hedonic scale has the advantage of giving judges more freedom to express their sensory perceptions; reduce contextual effects; accurately assess the acceptance, because it is possible to mark any point on the scale; and present data with smaller deviations from normality than the nine-point hedonic scale. However, its use is limited because it is more difficult to understand, thus slowing sensory evaluation.

Magnitude Estimation

In this method, the judges are required to provide numbers for the samples, without restriction, so the magnitude of values can reflect the magnitude of acceptance of the products. As with other hedonic scales, the scale used is bipolar, with positive numbers representing the acceptance of products, negative numbers representing rejection, and an intermediate value of 0 (neutral point). The magnitude of acceptance of a sample can be easily estimated and interpreted by comparison. For example, if a sample is positioned at +50 and another sample is positioned at +25, it means that the first one is twice more accepted than the second one.

Such method has some advantages when compared to the category or linear scales. First, as the judges can use any number to express the hedonic response, scale size is not restricted and therefore there is no problem of not using the extremes. Secondly, inferences can be made about the magnitude of the differences among samples. Therefore, in the case of product reformulation, it is possible to quantify how much an ingredient of higher cost would increase the acceptance of a product and thus assess whether its replacement would be convenient. In other methods (such as nine-point hedonic scale), this quantification of the difference in acceptance is not possible. Furthermore, the magnitude estimation method has higher power to reveal differences among samples when the number of products to be evaluated is large or the number of samples is few but very acceptable ones.

Obviously, the method has important limitations: (1) As all assessments are made comparing the products with each other and each judge can choose his/her own values on the scale, there is no general rule in the assessment, so there is no certainty that a magnitude of 9 means the same thing to all

judges, and therefore, comparisons among the values assigned by individuals have no meaning; (2) the absence of a label (e.g., 'disliked very much') makes difficult the translation of numbers into perceptions of acceptance; (3) it does not provide ordinal data on the acceptance or rejection of products; (4) the numerical nature of the method, using numbers to estimate the magnitude of the differences in acceptance, can be a difficult task for the general population, so the quality of the data obtained often depends on the judges' experience or training level; (5) there is a tendency among judges to use rounded values, such as 5, 10, 20, and 100, casting doubt on the validity of the method; and (6) data analysis is complex, requiring normalization and standardization even before the statistical analysis begins. Therefore, the magnitude estimation scale requires very detailed instructions and practice with the method, which cannot be achieved in experiments involving consumers. For this reason, it is not widely adopted in applied sensory research.

Hybrid Hedonic Scale

It is a linear scale (0–10) resulting from the combination of structured and unstructured scales (Figure 5). The hybrid hedonic scale is anchored with verbal labels on middle regions (5 = neither liked nor disliked) and extremes of scale (0 = disliked extremely and 10 = liked extremely), which makes it easier to be used by the consumer than the unstructured scale. The other portions are marked with equidistant points in order to better define the degree and direction of hedonic continuity.

Advantages attributed to this scale are the following: (1) Since it is not restricted to a limited number of categories, it provides better discrimination power; (2) it reduces errors by adaptation (repeating the answers when there are a number of products), encouraging the judge to conduct the evaluation in a more conscious and discerning manner; (3) it reduces or eliminates the numerical and contextual effects, allowing its use in cross-cultural studies; and (4) it generates continuous data, allowing a safer use of parametric or nonparametric analyses.

Category-Ratio Scales

Discussions focusing on the validity and superiority of category or magnitude scales fostered the proposition of a new type of scale containing the strengths of both. The category-ratio scale is linear and has verbal descriptors positioned according to the semantic magnitude, with the positions determined empirically by magnitude estimation.

The first category-ratio scale was developed for the evaluation of the intensity of oral sensations, which was called oral labeled magnitude scale (LMS). This scale had the term 'without sensation' at the lower end and 'strongest imaginable oral sensation of any kind' at the upper end, with five additional descriptors spaced in a quasi-logarithmic manner. After a few years, there were discussions about the top of the scale, as it was considered that oral sensations were different among individuals due to differences in the tongue anatomy and that the correct action should be anchoring the top end more generally. Thus, the upper anchor was replaced by 'strongest imaginable sensation of any kind,' and this scale was called general version (gLMS) (Figure 6).

One of the first scales developed for purposes of product acceptance was LAM, in which the labels were derived from the nine-point hedonic scale, with two additional anchors:

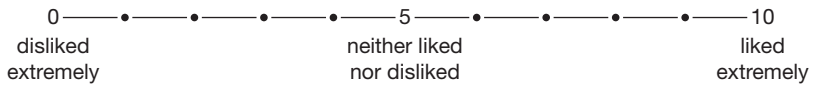

Figure 5 Hybrid hedonic scale. Reproduced from Villanueva, N. D. M., Petenate, A. J. and Silva, M. A. A. P. (2005). Performance of the hybrid hedonic scale as compared to the traditional hedonic, self-adjusting and ranking scales. *Food Quality and Preference* **16**, 691–703.

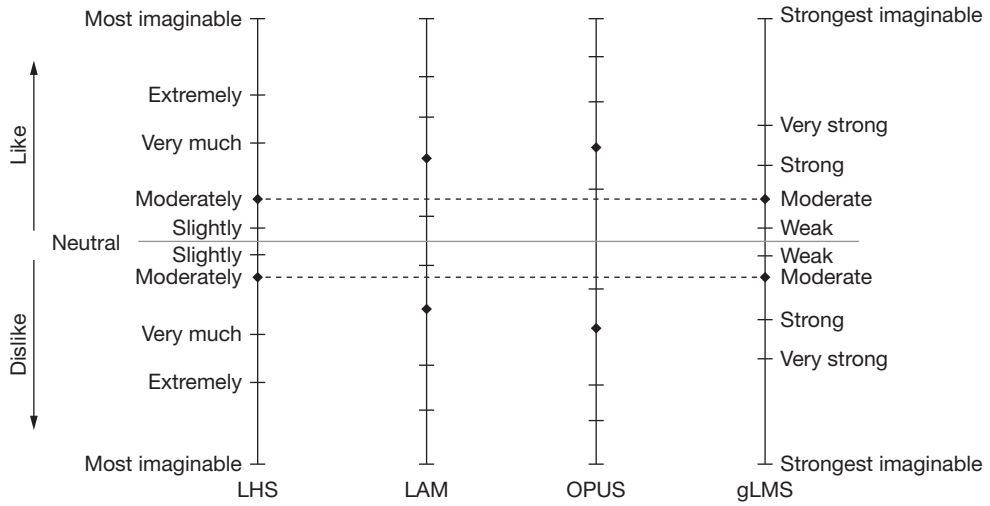

Figure 6 Category-ratio scales. Reproduced from Lim, J., Wood, A. and Green, B.G. (2009). Derivation and Evaluation of a Labeled Hedonic Scale. *Chemical Sciences* 34: 739–751.

'greatest imaginable like' and 'greatest imaginable dislike.' The development of the scale followed the procedures used in the creation of LMS.

After that, other scales have been developed ((hedonic) gLMS, OPUS, etc.), with advantages and disadvantages, such as incorrect quantification of the magnitude of acceptance, inappropriate descriptors, and inappropriate spacing among descriptors.

A few years ago, a group of researchers developed the LHS, using a procedure similar to that used in the development of LMS and comparing the magnitude estimation and nine-point hedonic scales. The LHS generated data identical to those obtained by the magnitude estimation, validating the positioning of descriptors and assuming that the magnitude of acceptance can be quantified. In addition, it had the power of sample discrimination slightly higher than that of hedonic scale and greater resistance to the effects of nonuse of the scale extremes, resulting in data with a more normal distribution.

The advantages of category-ratio scales, especially LHS, are as follows: (1) They generate magnitude data, making them possible, for example, to evaluate the relationship between the acceptance and the amount of a given ingredient and how much a sample is more accepted than another one (product A is three times more accepted than product B); (2) it is possible to translate the numerical data into perceptions of acceptance, because the positions of the category labels were determined; (3) since they are continuous scales, the judges are not confined to a limited number of categories; (4) by presenting more spaced anchors (most liked/disliked sensations imaginable), scale sensitivity is increased when discriminating samples, especially those that are very or little accepted; (5) data on the acceptance are normally distributed, so parametric analyses can be applied requiring no preliminary processing of data; and (6) the scales as well as the nine-point hedonic scale are easy to use, although they require instructions and practice so that the data obtained are reliable.

The disadvantages of this type of scale would be as follows: (1) As the anchors are far away from the other categories, there could be a compression of ratings in the center of the scale, reducing its power of discrimination, and (2) it can be misused by some groups of judges such as those with lower education or who are already used to other scales. For example, it was observed that many judges performed evaluations of samples only at the positions where the labels were located, making it a category scale. Therefore, instructions on the use of the scale are of vital importance for obtaining reliable data.

Conclusions

In this article, the four basic types of scales used in sensory evaluation of food were presented: nominal, ordinal, interval, and ratio. The main properties of the methods with scale were listed, emphasizing the use in acceptance testing. By describing the properties, advantages, and limitations of the methods with scales used in acceptance testing, we do not intend to show the superiority of a single method compared to others, but direct the researcher's choice of the appropriate and valid method, according to the study objective.

From demands of users of sensory analysis, it is expected that researches are conducted for the development of new methodologies with scales to evaluate the acceptance of products by consumers, in order to quantify the magnitude of differences among samples and segment consumers according to the preference. One has to keep in mind, however, that not always what is new is the best. On the other hand, it is not because a methodology has been used for years that it has no disadvantages.

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Sheep: Meat

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Introduction

Since antiquity, sheep (*Ovis aries*) have been domesticated for the production of meat, albeit often as a secondary product from wool production. This has resulted in a range of sheep breeds that vary in terms of production and climatic adaptation specializations. Therefore, there are inherent differences between sheep breeds regarding meat productivity and quality. For example, a purebred Australian Merino is a specialized wool breed that will produce lighter carcass weight with lean meat over a longer growth period when compared with a meat specialist breed such as Suffolk or Texel. Using this information, producers have tailored their production systems to optimize breed effects, in terms of feed usage and production localities, to achieve optimum meat yields and quality. Furthermore, tactical management of feeding systems prior to slaughter that offers optimum dietary nutrients as protein, energy, and micronutrients can provide more shifts in yield and quality and allow producers greater responsiveness to changes in market requirements or specification.

Sheep meat production is highly versatile and immediately employs many livelihoods around the globe, in both small holdings and large-scale extensive or intensive production systems. This is facilitated by its inclusion within various culinary diets, based upon sheep meat having a highly nutritious composition and distinctive flavor – although ideal flavor is debatable, depending on cultural backgrounds. Consequently, sheep meat is a key tradable commodity and the focus of much effort to enhance its nutritional and eating qualities and ensure these are preserved from production to consumption through processing and retail. To assist in the clarification of sheep meat, animals are generally divided into age-dependent categories: lamb, being meat from animal slaughter prior to 12 months of age; hogget or yearling, defining the age bracket between lamb and mutton; and mutton, being meat from animals once the age of 24 months old is surpassed. These definitions highlight the importance of animal age to carcass value, meat composition, and eating quality characteristics. Herein, the word sheep meat covers aspects of meat from both lambs (younger age animals – lamb) and sheep (older age animal – mutton).

This article aims to detail sheep meat, with reference to production and processing aspects, in terms of production types, nutritional composition and health implications, sensory and eating quality traits, and spoilage pathways that require preservative attention.

Production

In 2010, sheep meat production represented only 3% of the total global meat production, and this market share emphasizes the huge potential for growth available. This scope for advancement in sheep meat production is encouraging to traditional sheep meat-producing nations, such as New Zealand and Australia, which have a strong export focus and access to developing markets in the European Union, the Middle East, North America, and Asia. However, the global sheep meat industry faces limitations, which may undermine future growth, including animal and human welfare considerations, increased competition for land and feed resources, climatic impacts from intensified production, and economic restrictions on international trade. Overcoming these hurdles is the responsibility of all sheep meat stakeholders. Regardless, domestic consumption of sheep meats is increasing over recent years, and this has resulted in better prices being offered for sheep meat productions, especially lamb, in comparison with other meats.

Stakeholders across the supply chain from producer, processor, to retail are vital to the industry, but producers are particularly important. The production of sheep meat, broadly speaking, can be categorized into two groups: (1) small holdings, which are more common in developing countries or hobby farms and generally maintain a small flock (often <20 animals), or (2) large holdings, which can have flock numbers into the thousands and routinely manage animals on an entire flock basis. Animals are reared under two main feeding systems: (1) extensive grazing on traditional pasture feeding and (2) intensive production systems maintained on concentrate–grain-based diets. However, feeding systems can be combined depending on availability of resources, quality of feeds, and the purpose of production. Sheep maintained under extensive grazing receive diets with high protein (e.g., green pasture) and grow slower, while animals maintained under intensive systems receive high energy and grow faster. These in turn can impact on the muscle composition and eating quality traits of meat.

Historically, operations with a wool-only production focus would consider sheep meat as a by-product, but this is no longer universally true. The advent of synthetic fibers and narrow potential for expansion in traditional or new market share have both contributed to low wool prices. As a result, many traditional wool producers have adopted dual-purpose production focus. This entails flocks dominated by dual-purpose sheep breeds, such as Corriedale and Finnsheep, that equally specialize in both wool and meat production. Another approach to dual-purpose production uses a base wool specialist flock joined with a meat specialist breed to achieve progeny with hybrid vigor and both wool and meat quality characteristics. Under traditional Australian commercial practices, lambs under grazing conditions are slaughtered between 4 and 10 months of age, where they reach a live weight to produce carcasses of 18 kg or more. Sheep production in Australia uses different genotypes under various production systems.

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Table 1 Comparative percentage muscle (lean (%)) and fat (fat (%)) contents of different sheep genotypes that were slaughtered when having a hot carcass weight of 20, 30, or 40 kg

Weight (kg)	*Genotype*	*Age (months)*	*Lean (%)*	*Fat (%)*
20	M	8	76.4	20.4
	BL × M	8	74.9	22.2
	PD × M	4	77.5	19.5
	PD × M	8	77.1	20.1
	PD × (BL × M)	4	76.8	20.6
30	M	14	71.5	24.3
	M	22	67.2	28.5
	BL × M	14	68.3	28.1
	PD × M	14	71.3	25.2
	PD × (BL × M)	8	70.8	26.0
40	M	22	65.0	30.2
	BL × M	14	64.0	32.1
	BL × M	22	60.0	36.0
	PD × M	14	67.8	28.4
	PD × M	22	63.9	32.3
	PD × (BL × M)	14	65.9	30.7
	PD × (BL × M)	22	61.9	34.6

The genotypes investigated were Australian Merino (M), Border Leicester (BL), and Poll Dorset (PD).

Adapted from: Ponnampalam, E. N., Butller, K. L., Hopkins, D. L., Kerr, M. G., Dunshea, F. R. and Warner, R. D. (2008). Genotype and age effects on sheep production. 5. Lean meat and fat content in the carcasses of Australian sheep genotypes at 20-, 30- and 40-kg carcass weights. *Australian Journal of Experimental Agriculture* **48**, 893–897.

The major types of animals produced for sheep meat are about 40% from second cross (Poll Dorset × (Border Leicester × Merino)), 25% from first cross (Poll Dorset × Merino and/or Border Leicester × Merino), and 20% from pure Merino genotypes. This latter approach is popular among traditional wool producers as it allows their original wool flock genetics to be preserved, it avoids a full production system overhaul, and it allows better flexibility to match market flux (Table 1).

Sheep meat-only production focus routinely uses meat specialist breeds. This may include wooled breeds such as Dorset or Cheviot that produce coarse wool that can be harvested as a by-product of production. Otherwise, haired breeds that shed their coats may be used, for example, Dorpers and Barbados – these are gaining popularity as labor prices increase and wool prices remain low. As a sidenote, dairy sheep (e.g., East Friesian) are another source of sheep meat as they are typically slaughtered at the end of their primary usefulness and may be considered a by-product of the production focus just as described with a wool-only production focus. Selection of a production focus ultimately is regulated by market demands and preferences.

Composition

Sheep meat has a complex structure and consists of water (73%) and protein (21%) fractions, with lipid (5%), carbohydrate, and many vitamins and minerals also present but in smaller amounts. The major water, protein, and fat of meat (or muscle) composition can be affected by sheep genetics, feeding background, and age of sheep. The fat content of meat ranges from 2% to 10% and can substantially influence the meat quality aspects such as flavor/aroma, juiciness, and texture. It was observed that when sheep are reared under the same paddocks, within a given age and genotype category, sheep with smaller carcasses deliver less fat and more lean than sheep with larger carcass counterparts. This implies that within a genotype at a particular age, there is variation in fat and muscle deposition, with faster growing animals diverting more energy into fat deposition than muscle growth compared with smaller growing animals.

The protein-rich muscle fibers (myofibrils) of sheep meat mostly contribute to its distinctive texture (tenderness of meat). Myofibrils are arranged into bundles or sheets and are individually surrounded by an endomysium and sarcolemma (a plasma membrane); in turn, these are encapsulated in groups by a perimysium and as a whole muscle by an epimysium. A collective term for endomysium, perimysium, and epimysium is connective tissues. There are three myofibril types, and the prevalence of these has been found to vary depending on muscle function and location; these are (1) type 1/slow twitch, using aerobic metabolism and found in postural muscles; (2) type 2A/fast twitch, which are similar to type 1 but found mostly in limb muscles; and (3) type 2B/fast twitch, using anaerobic metabolism and found in locomotive muscles. The density of myofibril types can affect the sensory characteristics and nutritional quality of meat. This protein fraction is highly digestible, more so than plant protein, and provides consumers with the essential amino acids, lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, and valine.

As a red meat, sheep meat is a rich source of dietary iron (Fe) within a heme (Fe) complex – containing comparatively higher levels of Fe than beef. Heme (Fe) is readily absorbed when compared with nonheme (Fe), partly due to sheep meat being rich in glutathione in which its activity promotes iron absorption. Humans require iron to avoid anemia and the inhibition of several cellular processes – including oxygen transportation throughout the cardiovascular system. The consumption of heme (Fe) has, however, been associated with an increased incidence of colorectal cancer and congenital heart diseases. But evidence suggests that provided sheep meat such as trim lamb cuts or lean meat is consumed as part of a well-balanced diet, the risk from these diseases can be ameliorated. Sheep meat is also a valuable source of selenium and can provide approximately 20% of the daily recommended intake for an adult per 100 g serving. It also provides zinc that can be more readily absorbed than plant-sourced zinc because of the absence of many phytates and fiber that hinder its uptake. Sheep meat is also an important source of calcium, magnesium, copper, phosphorus, and potassium, all being essential to human health. Interestingly, the presence of these minerals does not only solely contribute to nutritional health benefits but also play a role in many sensory characteristics and keeping quality of meat.

Sheep meat, as with other red meats, has an unmerited notoriety for its high content of 'unhealthy' saturated fatty acids (SFA); these are unfavorable because of their association with cardiovascular disease and antagonism of several cancers. However, recent efforts are making progress to reverse this negative reputation by developing sheep meats with an improved 'healthy' fat content through genetics and nutritional improvement. It is also worthy to state that lean meat cuts from sheep are

low in fat and can contribute to the intake of key nutrients in a diet. Omega-3 polyunsaturated fatty acids ($n-3$) are one such 'healthy' fat, as these have been linked with improved resilience against coronary disease and diabetes, better mental status and infant development, and the maintenance of neural and optical tissues. Many types of $n-3$ exist, but those associated with these benefits are long-chain (>C13) $n-3$, which include eicosapentaenoic acid (EPA: 20:5$n-3$) and docosahexaenoic acid (DHA: 22:6$n-3$). In general, people fail to consume the recommended EPA + DHA levels by the World Health Organization (0.5–2% of total caloric intake), the United States (0.25–2%), or the United Kingdom and Australia (12%). Lamb has been identified as a 'good source' of these $n-3$'s – where a 'good source' refers to $n-3$ content above 60 mg/100g of food according to Food Standards Australia New Zealand. Furthermore, $n-3$ content can be improved by supplementing lambs with feeds rich in $n-3$ (e.g., some algae and flax) as part of a flock dietary management strategy. It should be noted that meat cut selection will influence the available $n-3$ as EPA and DHA are primarily located in membrane phospholipids and therefore are found in greater quantity in muscles rich in slow-twitching fibers (type 1 myofibrils). Another 'healthy' fat found in sheep meat is conjugated linoleic acid (CLA), which has been found to reduce the risk of some cancers, diabetes, and arthritis. The amount of CLA in sheep meat is highly correlated with fat content and is influenced by animal diet, but generally, 0.2–2% of total tissue fat is CLA. Animal genetics and rumen flora also play major roles in determining the fat content and composition of sheep meat (Table 2).

Table 2 This information was sourced from Food Standards Australia New Zealand (2010) and describes the nutrient content of raw meat (per 100 g fresh weight) sampled from 11 different cuts that were from various regions of the sheep carcass (lamb and mutton)

	Lamb	*Mutton*
Nutrient		
Energy (kJ)	2315	2522
Moisture (g)	31	28.8
Protein (g)	10.8	8.2
Total fat (g)	57.6	64.4
Saturated fat (g)	25.7	28.6
Polyunsaturated fat (g)	2.7	3.7
Monounsaturated fat (g)	20.8	24.1
Cholesterol (mg)	62	72
Total long-chain omega-3 (mg)	140.7	163.0
Minerals		
Sodium (mg)	22	33
Iron (mg)	0.41	0.77
Selenium (μg)	–	10
Zinc (mg)	22	0.99
Phosphorus (mg)	56	88
Calcium (mg)	5	10
Vitamins		
Retinol (vitamin A) (μg)	34	64
Thiamine (B1) (mg)	–	–
Riboflavin (B2) (mg)	0.07	0.07
Niacin (B3) (mg)	2	5
Pantothenic acid (B5) (mg)	0.43	0.46
Pyridoxine (B6) (mg)	0.1	0.1
Cobalamin (B12) (μg)	2.9	2.9
Vitamin E (mg)	1.1	0.7

Sheep meat is a valuable source of several key vitamins. A 100 g serving can provide nearly two-thirds of an adult's daily recommended intake of vitamin B12 and one-quarter of their daily vitamin B2, B3, B5, and B6 requirements. Sheep meat is also a useful source of many lipid-soluble vitamins, including vitamin A, but efforts to produce leaner sheep meat have had a negative influence on its content. Leaner sheep meat also has implications on some sensory characteristics and is described in the following section.

Quality

The quality of sheep meat is fundamentally determined by its appeal to consumers and their tangible experience following mastication. To better understand the appeal of sheep meat, consumer responses are routinely defined as major sensory characteristics: (1) tenderness, also known as mouthfeel or texture; (2) flavor, which is the intrinsic combination of both smell and taste senses; (3) appearance or color when sheep meat is retailed or on display; and (4) juiciness or moistness. These four sensory characteristics provide insight into the overall consumer acceptability of sheep meat.

Tenderness refers to the ease by which sheep meat can be severed and chewed during mastication. This is widely considered to have the most authority over perceived quality when compared with other sensory characteristics and hence acts as a key discriminating factor for consumers selecting sheep meat. In Australia, for example, lamb maintains a significantly larger portion of the sheep meat market than mutton, largely related to improved tenderness. This highlights the influence animal age has on tenderness, but other factors such as breed, genetics, muscle type, and even location within a muscle can affect tenderness. The pathway by which these shape meat tenderness revolves around their influence on myofibril type, distribution, and amount and intramuscular fat (IMF) content. Evidence suggests that tender sheep meat results from a greater density of thinner myofibrils with improved collagen solubility and when IMF levels exceed 3% – which is the threshold for consumer acceptability. Therefore, much investigation toward understanding genes controlling tenderness and their inheritability has been undertaken for inclusion into breeding programs. However, genetic contribution to tenderness must be balanced with management practices to achieve optimum tenderness.

The management of animal stress prior and during slaughter, particularly during lairage and on the kill floor, is important to improve meat tenderness. It is also common following slaughter and during the processing stage to apply other methods to further enhance tenderness. These methods include (1) mechanical stretching, wherein a carcass is positioned (e.g., pelvis hanging, or tenderstretch) or physically manipulated (e.g., carcass pull); (2) temperature manipulation, which avoids toughening in response to cold shortening or heat shortening in combination with various hygienic and spoilage considerations; (3) pH modification, to prompt a better and hygienic pH and temperature decline rate and prevent cold shortening; and (4) aging; increasing the period between slaughter and consumption of meat kept refrigerated allows myofibrils and muscle structures to tenderize due to changes

in enzymatic activity and provide less resistance to mastication – although this approach may allow contamination and faster spoilage. With the exception of aging, these methods aim to prevent myofibril shortening during which their density and therefore toughness increase. Research has found that a combination of these approaches results in most significant increases in meat tenderness, especially when electrical stimulation of a carcass is used to manage the pH of carcass post-slaughter. This proves an effective and cheap way to rapidly change pH via the stimulation of glycogen metabolism.

Flavor refers to the combination of consumer smell/aroma and taste sensations immediately prior to and during consumption. Smell is stimulated by the numerous volatile compounds released from sheep meat, mostly released following cooking, perceived by the olfactory receptors within consumer nasal cavities. These volatile compounds often result from protein and lipid lyses during cooking, but not much information exists linking specific compounds with sheep meat aroma – with exception to 2-ethyl-3,6-dimethylpyrazine and 2-pentylpyridine (heterocyclic compounds) and 3-methylindole acid and 4-methylnonanoic acid (MNA) and 4-methylphenol (skatole compounds), which have been associated with a mutton aroma. Nevertheless, it can be construed that their prevalence relies upon the base compounds of sheep meat, which may be influenced by animal diet. Taste refers to the stimulation of taste bud receptors within the mouth and throat to sheep meat compounds, especially branched-chain FA, which are identified as a precursor to the unique 'sheep' taste. Therefore, taste relies on many of the same compounds as smell and is also manageable with dietary controls. Hence, smell and taste are often referred to as flavor and are influenced by animal diet. For example, 4-methylphenol, methyloctanoic acid, and MNA content are intensified when animals are fed with pasture-based diets and result in a 'pastoral' sheep flavor. Also, it is general perception that when sheep are fed with canola and other oil-rich feeds, their meat can develop a foreign or atypical lamb/mutton flavor.

Nonvolatile compounds from sheep meat can also influence flavor, including antioxidants, carotenoids, and lipids. The contribution from these compounds can result from their opposing influence on lipid peroxidation from which many 'off-odor' and sour or off-tasting compounds are a by-product. As a rule, however, it is difficult defining an ideal sheep meat flavor because consumer culinary preferences, cooking methods, and cultural heritage can all influence its acceptability. This can affect the objective measurement of flavor, and therefore, it is typically measured using a trained sensory panel and reported within the context of the sample represented. This is necessary as, for example, British and Australian sheep meat consumers prefer stronger sheep flavors than Asian consumers, and if both these sampled the same sheep meat, they could provide different results on consumer perception and acceptability.

Many consumers' first encounter with sheep meat is in a retail environment, wherein an impression of freshness, quality, and value is often formulated based on appearance and color. Research has demonstrated that sheep meat must have a bright cherry red color and bright white fat content to match consumer ideal or preference. Achieving this appearance is essentially a function of myoglobin (Mb) oxidation – with Mb being a complex protein with a central iron atom and a role in oxygen transportation that is plentiful in sheep meat. This Mb will adopt a deoxymyoglobin (DMb) structure when sheep meat is stored in an anaerobic environment but will change to oxymyoglobin (OMb) when exposed to oxygen in a reversible reaction. This causes a color shift from purplish to cherry red. Prolonged exposure to oxygen, however, will result in OMb assuming a metmyoglobin (MMb) structure and will consequently instill a brownish color to sheep meat. The concentration of MMb is directly associated with this discoloration; hence, work has been undertaken to find a threshold value indicative of perceived quality and methods to prevent this threshold from being breached. For example, improving sheep meat antioxidant (e.g., vitamin E) and other micronutrient (e.g., beta-carotenoids) contents with dietary management (Figure 1) and using innovative meat packaging technologies have provided means to manage its appearance. This proves true for both meat and fat appearances.

When juiciness is used within the context of a sensory quality, it describes the amount of fluid released from sheep meat upon maceration in combination with that contributed from the consumer via salivation. Salivation is prompted by the IMF content, and a threshold level of 3% of IMF has been identified as necessary for juicy meat. The fluid discharged from sheep meat when it is chewed is a function of its water-holding capacity (WHC), which is the potential of meat to withhold liquid during (1) storage periods, otherwise lost as 'drip' and purge; (2) heating, referred to as 'cooking' loss although the cooking method can have significant implications on this factor; and (3) chewing and mastication, with fluid released promoting perceived juiciness. Ideally, sheep meat should be juicy and this requires a high WHC. The WHC can be influenced by animal carcass energy reserves, which are measureable as pH with high pH equating to high energy reserves and therefore heightened WHC. This may be improved using electrical stimulation technology. However, the WHC does contribute to carcass yield, and when the WHC is low, meat yield as well as subsequent profits are less. Not only the amount of water but also the distribution of water in muscle tissue has an effect on meat color, firmness, and drip loss. Under postmortem conditions with the onset of rigor, water moves from the myofibrillar compartment into the intermyofibrillar space and then in turn into the extracellular space. The degree of myofibril shrinkage will impact on the reflection of light from the muscle surface, and the ultimate pH will impact on this by affecting the amount of water loss.

Ultimately, an accurate indication of overall liking or achievement of a consumer-acceptable product can be provided by combining these four major sensory characteristics. However, this relies upon accurate assessment, which is often difficult due to the sometimes subjective nature of sensory panel protocols, experience of panel members conducting the evaluation, and expense needed to facilitate a statistically sound insight. As a result, efforts have been distinctly focused toward developing and validating objective measures as a proxy for consumer responses.

Spoilage and Preservation

All meat is susceptible to spoilage, and this can have detrimental implications upon perceived value, consumer safety, and wastage. Spoilage refers to the degradation or downgrade of

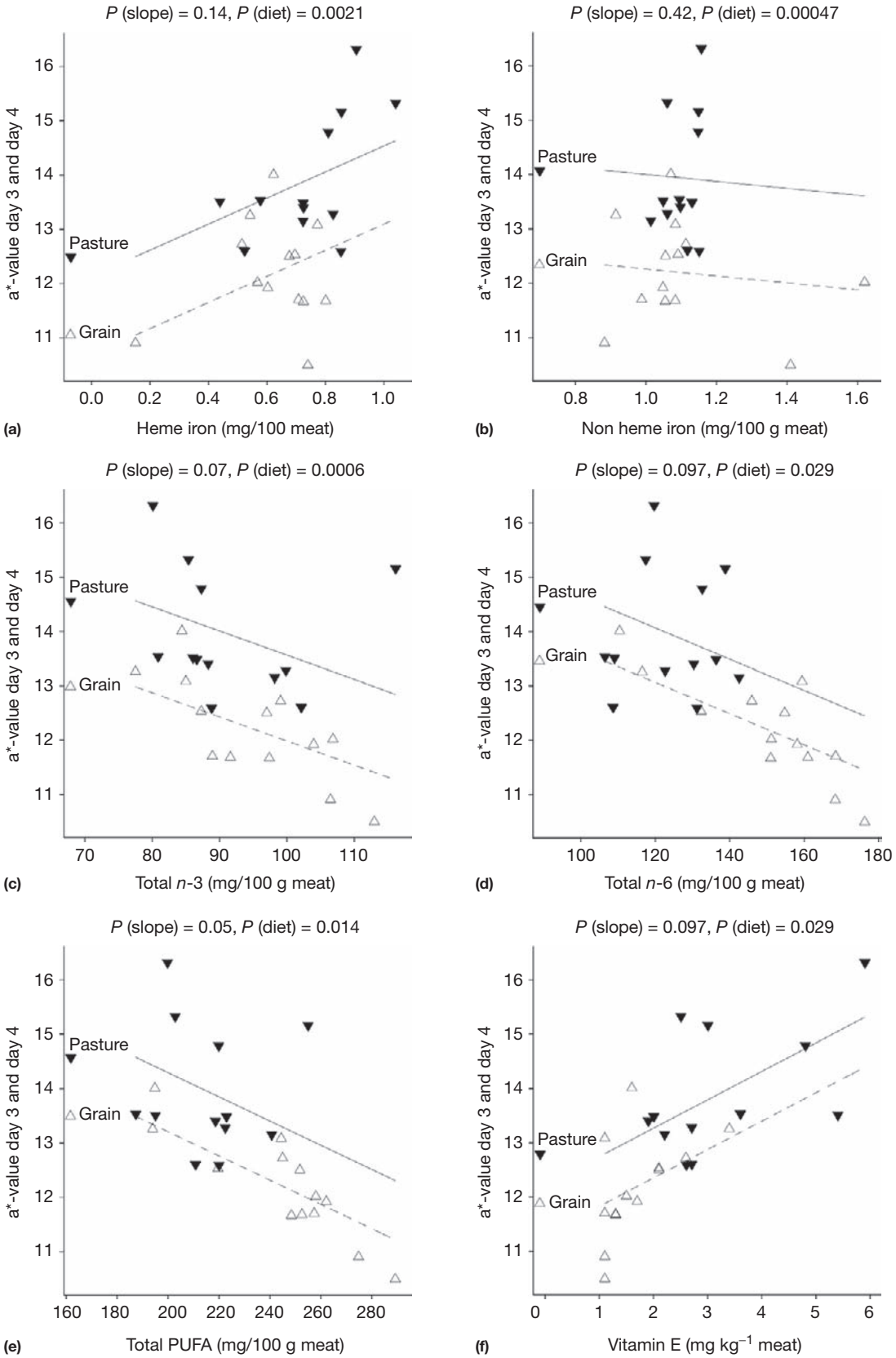

Figure 1 Scatter plots of redness (a*) of sheep meat after 3 and 4 days of retail display with (a) heme iron, (b) nonheme iron, (c) total omega-3 (*n*−3), (d) total omega-6 (*n*−6), (e) total polyunsaturated fatty acid (PUFA), and (f) vitamin E, measured in the *M. longissimus lumborum*. Parallel regression lines are fitted for lambs grazing pasture only (dashed) or offered with hay supplemented with grain (solid). Adapted from Ponnampalam, E. N., Butler, K. L., McDonagh, M. B., Jacobs, J. L. and Hopkins, D. L. (2012). Relationship between muscle antioxidant status, forms of iron, polyunsaturated fatty acids and functionality (retail color) of meat in lambs. *Meat Science* **90**, 297–303.

sheep meat quality, both sensory and nutritional, to an extent after which a meat product fails to meet safety and quality standards dictating consumption. The spoilage of sheep meat can be assessed as discoloration, a loss of integrity and texture, the development of sour and off-flavors and odors, loss of moisture, and/or visible bacterial and fungal infection. Bacterial and fungal infection could indicate the contamination of meat with pathogenic bacteria or toxic by-products from fungal and bacterial growth. As a general observation, meat spoilage results in a compromise of core compound structures, such as the lipid and protein fractions, from oxidation and/or microbial infection.

Sheep meat is a rich source of SFA and UFA, the latter being especially prone to lipid peroxidation. Lipid peroxidation is the oxidation of a fatty acid molecule and occurs as three sequential phases: (1) initiation, when a UFA reacts with a free radical species and donates a hydrogen ion to become a fatty acid radical; (2) propagation, which occurs during a fatty acid radical reaction and acceptance of an oxygen atom (or oxidizes) to become a peroxyl-fatty acid radical – this can then be used in the former phase and create a cyclic reaction; and (3) termination, when a fatty acid radical reacts with a nonradical (e.g., antioxidant), which interrupts the initiation and propagation reciprocal interactions. In instances where the termination phase fails to occur, the previous phases will continue until the original UFA is completely degraded into its many potential secondary oxidative products, which include reactive aldehydes, ketones, alcohols, hydrocarbons, epoxy compounds, and volatile organic acids. Lipid peroxidation of meat does compromise not only nutritional and health-promoting advantages from UFA but also meat structural integrity during prolonged storage and cooking and can affect perceived juiciness and tenderness qualities. Furthermore, the secondary products from lipid peroxidation can have a negative influence over sheep meat flavor and cause atypical and undesirable tastes and aromas. These can also be employed to initiate protein oxidation, and in this respect, lipid peroxidation acts as a precursor reaction.

Protein oxidation, or proteolysis, is catalyzed when protein peptides are exposed to oxygen and reactive radical species. Hence, this commonly follows lipid peroxidation because of the high reactivity of peroxyl-fatty acid radicals and other secondary oxidative products with protein peptides. This reaction causes the protein structure to change as peptide bonds are cleaved and amino acid side chains are modified – this latter change being the most common and can result in the synthesis of protein carbonyl groups. As an upshot from protein oxidation, many covalent intermolecular cross-linked protein secondary species are created. These include disulfide, dityrosine, and protein hydroperoxide species groups. An outcome from protein oxidation is a change in protein structure and a resultant inactivation of several enzymatic functions, although often the proteins become more prone to the effects of proteolytic enzymes – potentially sourced from microbial infections. It is interesting to note that some amino acids are more susceptible to oxidation than others, for example, cysteine, tyrosine, arginine, lysine, and methionine, and therefore, proteins rich in these amino acids will be preferentially oxidized. The presence of transition metals can also influence protein oxidation, and this has implications on heme proteins such as Mb. Mb is oxidized when, as OMb, it autoxidizes before MMb enzymatic reduction, when it is catalyzed by the degradation of lipid peroxyl-fatty acid radicals, or when Mb acts as a pseudoperoxidase. However, it is the central Fe molecule within Mb structures that significantly contributes to Mb oxidation, as this is readily oxidized from MbFe(II) to MBFe(III) as OMb becomes MMb. Nonheme Fe and other transition metal ions can contribute to protein oxidation, provided hydrogen peroxide is present – resulting in a 'Fenton' reaction.

The end result from protein oxidation on sheep meat quality is most apparent on its sensory qualities. For instance, when meat is aged, its structural proteins within myofibrils and connective tissues will degrade resulting in increased tenderness. It should be noted that this response is mediated by the calpain system wherein calpain enzymes, specifically μ-calpain and m-calpain, and several cathepsins sever myofibrils. Protein oxidation can interfere with the calpain system, with research showing that oxidation can inhibit μ-calpain activity and therefore causes the tenderness response to aging to be lessened. Protein oxidation also affects juiciness by prompting changes to myofibril diameters and protein cross-linkage connections, which can alter sheep meat WHC. Consequently, drip loss is found to increase during protein oxidation, and less fluid is retained within sheep meat, which can be released upon chewing; hence, consumer-perceived juiciness is reduced. The effect of protein oxidation on appearance is centered upon its effects on Mb as discussed.

Practicality dictates that sheep meat cannot be processed without experiencing some degree of bacterial or fungal (microbial) contamination. However, not all microbial contaminations contribute to spoilage, and therefore, efforts have been made to identify causal agents. Examples of spoilage bacteria that have been identified as hazardous to sheep meat preservation against spoilage include lactic acid bacteria (LAB), Enterobacteriaceae, *Brochothrix thermosphacta* sp., and *Clostridium* spp., as these often continue to proliferate during cold storage. As a rule, these contribute to spoilage as a result of their metabolism and subsequent breakdown of sheep meat protein and lipid fractions and glycogen reserves, which can influence integrity and texture. Furthermore, a by-product of metabolism by some microbes is the synthesis and deposition of often rancid-smelling or rancid-tasting compounds, which makes sheep meat unacceptable – these are commonly sulfur-rich metabolism products. In other instances, the by-products or the microbes themselves may be toxic to consumers and pose health risks. For instance, *E. coli*, *Salmonella*, and *Campylobacter jejuni* are three examples of pathogenic bacteria that if found above threshold levels on sheep meat will cause it to be inedible. Fortunately, microbial contamination can be controlled by direct application of health organization-approved antimicrobial agents to a sheep meat carcass or, more commonly, the manipulation of storage conditions to match those that inhibit microbial proliferation. This latter control focuses on temperature and oxygen availability factors primarily during the storage stage in the journey of sheep meat from processor to consumer through retailing sector.

With these effects of oxidation and microbial infection on sheep meat understood, it becomes apparent that spoilage is unavoidable. Instead, delaying spoilage onset and restricting its extent provide the best measure of combating spoilage until

consumption. This is routinely achieved by using specialized storage containments with inherent preservation and quality enhancement properties. For example, keeping sheep meat in chilled or frozen storage is a time-tested approach to restrict spoilage, as these low temperatures limit oxidation and microbial growth and therefore slow their contribution to spoilage. Recent efforts have used vacuum packaging or modified atmospheric packaging to shift the atmospheric gas surrounds of sheep meat to lower its exposure to oxygen – a necessity for propagation and growth of many spoilage bacteria and oxidation. Increasingly, intelligent sheep meat packaging is emerging as a means to restrict spoilage by better informing stakeholders along the supply chain to the extent of spoilage using embedded sensory equipment. These packages may also have antioxidants or antimicrobial agents incorporated into packaging films to directly reduce spoilage. However, preserving against spoilage is not solely a role for processors as producers make significant contributions using feeding management. Research has demonstrated that sheep fed with diets rich in antioxidants and vitamin E (α-tocopherol) will have increased antioxidant levels in their meat and hence better resistance to oxidation when compared with control animals (Figures 2 and 3). This has shown that when vitamin E levels exceed 2.95 mg kg^{-1} of muscle, sheep meat will be better preserved against lipid peroxidation. All these preservative methods share the common priority to interrupt or delay oxidation and microbial growth, and, in doing so, prolong sheep meat longevity.

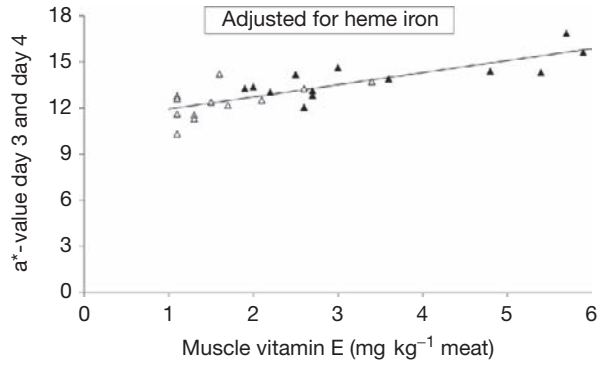

Figure 2 Relationship ($P = 8.9 \times 10^{-7}$) between redness (a*) of sheep meat after days 3 and 4 of retail display and *M. longissimus lumborum* vitamin E, after a* was adjusted for muscle heme iron for lambs offered with pasture only (▲) or offered with hay supplemented with grain (Δ). Adapted from Ponnampalam, E. N., Butler, K. L., McDonagh, M. B., Jacobs, J. L. and Hopkins, D. L. (2012). Relationship between muscle antioxidant status, forms of iron, polyunsaturated fatty acids and functionality (retail color) of meat in lambs. *Meat Science* **90**, 297–303.

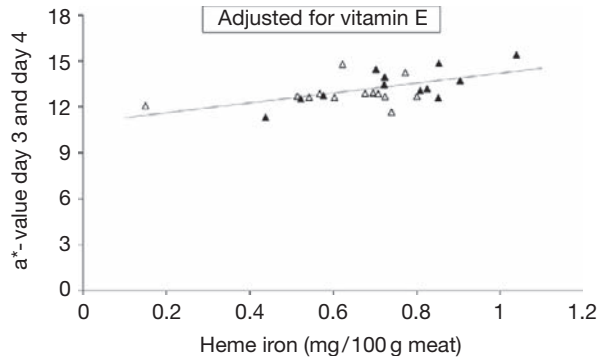

Figure 3 Relationship ($P = 0.0031$) between redness (a*) of sheep meat after days 3 and 4 of retail display and *M. longissimus lumborum* heme iron, after a* was adjusted for muscle vitamin E for lambs offered with pasture only (▲) or offered with hay supplemented with grain (Δ). Adapted from Ponnampalam, E. N., Butler, K. L., McDonagh, M. B., Jacobs, J. L. and Hopkins, D. L. (2012). Relationship between muscle antioxidant status, forms of iron, polyunsaturated fatty acids and functionality (retail color) of meat in lambs. *Meat Science* **90**, 297–303.

Conclusion

The production and consumption of sheep meat support many persons around the globe. This support may be economic, with a section of most communities basing their livelihoods on sheep meat retailing, processing, and/or producing, or support may be nutritional, as sheep meat proves to be a highly nutritious source of many vitamins, minerals, protein, and 'healthy' fats such as omega-3s. Consequently, it is meritorious for all stakeholders to explore production strategies, animal management, welfare of animals from farm to slaughter, and technological methods at processing and retail to improve sheep meat quality and achieve a shared priority to deliver a product that a consumer finds attractive and acceptable. This has resulted in much research into what describes consumer preferences for sheep meat and the underlying principles of preservation, spoilage, or the degradation of these quality traits. Understanding spoilage has allowed steps toward preserving or maintaining sheep meat quality until consumption to be successfully achieved, as fundamentally, it is consumer preference and satisfaction with a sheep meat product upon which the entire industry is based.

See also: Beef; Fats: Classification and Analysis; Fats: Production and Uses of Animal Fats; Fatty Acids: Determination and Requirements; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fatty Acids: *Trans* Fatty Acids; Iron: Biosynthesis and Significance of Heme; Iron: Physiology of Iron; Iron: Properties and Determination; Meat: Conversion of Muscle into Meat; Meat: Eating Quality and Preservation; Meat: Role in the Diet; Meat: Structure; Protein: Digestion, Absorption and Metabolism; Protein: Food Sources; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Protein: Requirements; Proteins: Chemistry, Characterization, and Quality; Sensory Evaluation: Sensory Rating and Scoring Methods.

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www.usda.gov – U.S. Department of Agriculture.

Sheep: Milk

SD Kalyankar, Government College of Dairy Technology, Udgir, India
AR Sarode, CD Khedkar, and SS Deosarkar, College of Dairy Technology, Pusad, India
RD Pawshe, Satpuda Shikshan Sansthan, Jalgaon Jamod, Buldhana, India

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Introduction

The sheep (*Ovis aries*) milk, which is the secretion of the mammary glands, is the only food of the lambs during the initial period of their life. The milk constituents provide both energy and the building materials necessary for its growth. Milk also contains antibodies that protect the lambs against infection. The milking of sheep dates back to the domestication of animals. In many countries, the cow has replaced the sheep as the major milk producer, but the sheep and goats remain important sources of milk in countries where land is too poor to support cows. With the search for alternative farm incomes, there has been a rekindling of interest in sheep dairying in northern Europe, Australia, India, New Zealand, and the United States. Attractions are low capital investment, limited land requirement, high profit margins per liter, and lack of quota limits on production. Milk plays a tremendous role in building a healthy society and can be used as vehicle for rural development, employment, and slowing down the migration of the rural population. Much less information is available on sheep milk composition than on cow, buffalo, and goat milk. Although some reviews cover both goat and sheep milk, most discuss goat milk in depth and sheep milk only superficially. Most studies are related to effects of animal feeding on fatty acid (FA) composition.

Production of Sheep Milk

Although China was the top producer of sheep milk in 2010 (17%), about 61% of the world's sheep milk is produced in the Mediterranean region and Middle East and mainly used as a raw material for producing cheese and other dairy products. In 1989, 8 778 000 tons of sheep milk was produced worldwide. Asia and Europe are the continents with the highest levels of production, the main producers being concentrated in the North Mediterranean Basin, the Black Sea area, Iran, and Central Asia. In many of these countries, the normal practice is to milk the ewes after the lambs have been weaned, the milk being an essential food in the diet of the local population. However, sheep can only be considered as dairy animals when they are milked for the greater part of their lactation, the lamb only suckling for 1–2 months. The concentration of sheep dairy production is at present mainly restricted to Europe and Israel. The trends in sheep population and milk production are shown in Table 1.

Breeds of Dairy Sheep

All breeds of sheep can be used for milk production, and in many localities, strains of sheep have been selected for their ability to produce significant quantities of milk. In the Mediterranean, at least 48 breeds can be classified primarily as milk producers; worldwide, the number would be much higher. The breeds most used for milk production in large commercial flocks are the Friesland and the Texel, although the Lacaune is favored in the Roquefort region of France and the Awassi in Israel. Many flockmasters cross a milk breed with a local breed to introduce hardiness or use breeds such as the Finn Dorset cross to encourage prolificacy.

Yields of Milk

Sheep milk yield varies depending on factors such as length of lactation, time and year of lambing, level of nutrition, and housing. However, yields of 120–1000 l per lactation are quoted, the normal yield being 200–300 l per lactation. To maintain a constant supply of milk, out-of-season lambing can be practiced. In this type of lambing management, each ewe produces three lamb crops in 2 years; thus, it will increase profitability. An alternative method is to keep two flocks, one lambing at the traditional season and the other out of season. Both methods require a high level of husbandry practices.

Milking

Milking is carried out mechanically in commercial flocks. Milking machines are similar to those used for dairy cows except that the vacuum level is set at 10 lb (4.5–5 kg) and the clusters are specialized units each with two lightweight teat cups. Milking is normally carried out in a parlor, which may be of static or rotary design. The milk is then transferred either to milk churns or to a bulk milk tank depending on the size of the enterprise. The milk must be cooled to below 5 °C as soon as possible after milking. In the case of churns, this can be achieved by passing the milk over a surface cooler before it enters the churn or by using an in-churn cooler. Churns should then be stored in a refrigerator. With a bulk tank, cooling is automatically carried out.

Composition of Sheep Milk

As with other milk, variations in composition with breed, stage of lactation, diet, season, milking times, and procedure have been recorded. The fat and total solids content tends to increase with the length of lactation leading to about 10% (w/v) fat content at the end of lactation. The average contents of protein (5.6%) and fat (6.4%) in sheep milk are high; only buffalo milk contains more fat on an average when milk from major dairy species is considered (Table 2). Sheep milk also contains more lactose than human, cow, buffalo, and goat

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00621-8

Table 1 Trends in world sheep population and milk production (2005–08)

Traits	*2005*	*2006*	*2007*	*2008*
Sheep population	1 091 375 497	1 104 192 341	1 105 610 121	1 086 307 458
Sheep milk production (tons)	194 101 483	207 399 826	210 343 406	205 304 768

Table 2 Proximate composition of human, cow, buffalo, goat, and sheep milk (per 100 g of milk)

		Cow		*Buffalo*		*Goat*		*Sheep*	
Proximate	*Human average*	*Average*	*Range*	*Average*	*Range*	*Average*	*Range*	*Average*	*Range*
Energy (kJ)	291	262	247–274	412	296–495	270	243–289	420	388–451
Water (g)	87.5	87.8	87.3–88.1	83.2	82.3–84.0	87.7	86.4–89.0	82.1	80.7–83.0
Total protein (g)	1.0	3.3	3.2–3.4	4.0	2.7–4.6	3.4	2.9–3.8	5.6	5.4–6.0
Total fat (g)	4.4	3.3	3.1–3.3	7.5	5.3–9.0	3.9	3.3–4.5	6.4	5.8–7.0
Lactose (g)	6.9	4.7	4.5–5.1	4.4	3.2–4.9	4.4	4.2–4.5	5.1	4.5–5.4
Ash	0.2	0.7	0.7–0.7	0.8	0.7–0.8	0.8	0.8–0.8	0.9	0.9–1.0

Source: http://www.fao.org/docrep/018/i3396e/i3396e.pdf (2013).

milk. The higher lactose content is compensated for by lower sodium and potassium levels, although most of the other minerals are present in higher amounts in sheep milk, in line with the higher ash content. The average fat globule size is reported to be even smaller in sheep milk than in goat milk, which makes the milk naturally homogenized. Milk composition is affected by various factors including stage of lactation, breed differences, number of calvings, seasonal variations, age and health of animal, and feed and management effects, which makes it difficult to compare compositional data between various studies. In order to permit such meta-analyses, published studies should include information on the factors mentioned earlier and analytic methods used and, where possible, include a control group for comparison. A detailed account of composition of sheep milk is summarized below.

Lipids in Sheep Milk

Lipids are one of the most important components of milk in terms of physical and sensory characteristics and in the nutritional properties that they confer to dairy products. Lipids are present in the form of globules, which in ovine milk are characteristically abundant in sizes of less than 3.5 μm. The average fat globule size in sheep milk is the smallest among all the ruminant milk. This is advantageous for digestibility and more efficient lipid metabolism compared with cow milk. The structure and composition of the globule membrane are similar to cow and goat milk fat, and it represents approximately 1% of total milk fat volume. Fat globules contain one hydrophobic lipid core, consisting mainly of triglycerides (TAGs), surrounded by a membrane made mainly of phospholipids and glycoproteins.

The FA profile of sheep milk is fairly similar to that of goat milk: five FAs make up more than 75% of the fat (C10:0, C14:0, C16:0, C18:0, and C18:1). SFA content (65–75 g/100 g total FA) is comparable to that in cow, buffalo, and goat milk. Sheep milk contains more retinol than cow and goat milk. As in goat milk, the nonprotein amino acid taurine is reported to be present in sheep milk. Sheep milk products can provide a profitable alternative to cow milk products owing to its specific taste, texture, typicity, and natural and healthy image. Nevertheless, consumers are requesting more and more information concerning the hygienic quality and nutritional composition of these products. All these characteristics can be influenced by several factors, such as breed, genetic, physiology, feed, environment, and technology.

Different fatty acids composed of fat globule core and membrane address a potentially healthy profile and have been detected in sheep milk. The C14:0 and C16:0 are present in greater amounts in the core, while polyunsaturated fatty acid (PUFA) omega-3, conjugated linoleic acid (CLA), and the precursors of the latter are more represented within the globule membrane. The unsaturated/saturated fatty acid ratio is lower in the fat globule core than in the membrane.

TAGs in Sheep Milk

The TAGs are the biggest group of lipids including a large number of esterified fatty acids. TAGs in sheep milk present a wide range of molecular weights when distributed according to the number of carbon atoms from C26 to C54. The TAG profile of sheep milk shows similarities to that for cow milk. However, sheep have a higher percentage of medium-chain TAG (C26–C36) than cow milk and a lower proportion of long-chain TAG (C46–C54). The unique characteristic of sheep milk fat is its content of about 25% medium-chain TAG.

Saturated Fatty Acids

The five most important fatty acids in quantitative terms (C18:1, C16:0, C10:0, C14:0, and C18:0) account for >70% of total fatty acids in sheep milk. It does not substantially differ in butyric acid (C4:0) content, but it contains more caproic (C6:0), caprylic (C8:0), and capric (C10:0) acids than cow milk. These fatty acids are associated with the characteristic flavor of cheeses and possess different biological properties.

Low concentrations of butyric acid can inhibit growth in a wide range of human cancer cell lines, including prostate.

Unsaturated Fatty Acids

Oleic acid (*cis*-9 C18:1), the second predominant fatty acid in sheep milk fat, is regarded as an antiatherogenic agent. Human diets high in oleic acid are mostly reported to decrease the level of low-density lipoprotein (LDL) cholesterol, whereas high-density lipoprotein (HDL) cholesterol levels are not affected notably. The PUFAs in sheep milk fat mainly comprise linoleic (*cis*-9 *cis*-12 C18:2) and α-linolenic (*cis*-9 *cis*-12, *cis*-15 C18:3) and smaller amounts of their positional and geometric isomers. Both are the essential fatty acids and they have many diverse functions in human metabolism and they promote an antiatherogenic effect. Sheep milk is not a rich source of linoleic and α-linolenic acids. However, other omega-3 PUFAs are hardly found in sheep milk fat, except when animal diets are supplemented with marine oil source.

Trans-Fatty Acids

Trans-fatty acid (TFA) content in sheep milk fat ranges from 2.5% to 5% of total fatty acids, mainly depending on diet and season. Monoene TFAs are the most abundant in all species. In ruminants, sheep milk presents the highest quantities, after cow and, finally, goat milk fat. The main source of TFA consumed daily by humans is partially hydrogenated vegetable fats and oils, although these compounds also occur naturally in ruminant milk as a result of partial biohydrogenation of PUFA caused by rumen microorganisms.

Conjugated Linoleic Acid

The generic name CLA is a collective term embracing all positional and geometric isomers of linoleic acid, which contain a conjugated double-bond system. Among ruminants, sheep milk fat could contain not only one of the highest levels of CLA but also the major content of vaccenic acid, its physiological precursor. The biological activity of other CLA minority isomers detected in sheep milk fat is marginal. Conversion of sheep milk to cheese has no effect on the final concentration of CLA. The isomeric profile and its content are primarily dependent on the CLA level of the unprocessed milk.

Other Minor Lipid Compounds

Along with TAG, sheep milk presents complex lipids (phospholipids) and different liposoluble compounds (sterols, β-carotene, and vitamins) with biological activity. The proportions of corresponding phospholipid classes in sheep milk are remarkably similar to other ruminants. Sterols are a minor fraction of sheep milk total lipids, the main sterol being cholesterol (about 300 mg/100 g fat, equivalent to approximately 10 mg/100 ml milk). The sterol fraction of milk is of nutritional interest because high levels of cholesterol in plasma are associated with an increasing risk of cardiovascular disease. Values for cholesterol content of sheep milk vary considerably and are associated with breed and the use of different analytic techniques. Small amounts of other sterols implicated in cholesterol biosynthesis have also been found in ovine milk.

Proteins in Sheep Milk

The sheep milk is a rich source of biologically active proteins. The amount of protein content in sheep milk is significantly higher (4.52–5.50%) than buffalo, cow, and goat milk. The protein components of milk have multiple functions. They provide amino acids, which are necessary for growth and development. The essential amino acids are present at concentrations similar to those in cows' milk, although isoleucine, leucine, serine, glutamic acid, and tyrosine may be slightly lower. In addition, milk proteins and peptides have more specific physiological functions. The biological properties of milk proteins include antibacterial activity, immunoglobulins, lactoferrin, lactoperoxidase, lysozyme, hormones, and growth factors. Most studies are performed with proteins of bovine and human origin, but these activities can be extrapolated to milk from other origins. Immunoglobulins are among the first host protein defense systems. The iron-binding protein, lactoferrin, is widely considered to be one of the most important defense proteins present in milk. Lactoferrin exhibits activity as an antimicrobial agent for host defense and as a physiological regulator with respect to both inflammatory and immune responses. Lysozyme and lactoperoxidase are also important antimicrobial proteins found in milk and colostrum. Lysozyme is mainly active against Gram-positive microorganisms, whereas Gram-negative microorganisms containing catalase, such as pseudomonads, coliforms, salmonellae, and shigellae, are killed by activated lactoperoxidase, provided that the hydrogen peroxide substrate is present in excess. Hormones, growth factors, and analogs are also present in milk, and they could act as development and metabolic regulators.

Carbohydrates in Sheep Milk

Lactose is quantitatively the main milk sugar in sheep milk. Its concentration is similar in horse, donkey, and human milk but lower in bovine or other ruminant milk. Lactose content in milk of buffalo, cow, goat, and sheep varies from each other. It is reported that the lactose content of buffalo milk ranges from 4.53% to 6.23%, cow milk 3.91–4.92%, goat milk 3.77–4.90%, and sheep milk 4.39–5.20%. Other milk carbohydrates, which are free or bound to lipids, proteins, or phosphate, include a small fraction of oligosaccharides. They are composed of galactose, fructose, *N*-acetylglucosamine, and/or *N*-acetylneuraminic acid (sialic acid) and contain mostly a lactose unit at their reducing end. They have the potential to modulate the growth of intestinal flora, to influence different gastrointestinal and inflammatory processes, and to provide protection against bacterial and viral infections. Their structural complexity however renders a functional comparison between species difficult.

Minerals in Sheep Milk

Interest in the nutritional significance of milk minerals and trace elements has markedly increased in recent years. There are about 20 minerals that are considered nutritionally essential

for humans (Na, K, Cl, Ca, Mg, P, Fe, Cu, Zn, Mn, Se, I, Cr, Co, Mb, F, As, Ni, Si, and B). Milk and dairy products can make an important contribution to the daily intake of some of them, especially Ca and P. The minerals in sheep milk have not been as extensively studied as in bovine milk, even though they may be of nutritional and health interest. Sheep milk has around 0.9% total minerals or ash, compared to 0.7% in cow milk. Sheep milk, goat milk, buffalo milk, and deer milk contain on average more Ca and P than bovine milk (Table 3). The most abundant elements in sheep milk are Ca, P, K, Na, and Mg; Zn, Fe, Cu, and Mn are the trace elements. In general, mineral contents of sheep milk seem to vary much more than those of cow milk. The mineral content of sheep milk is not constant but is influenced by a number of factors such as stage of lactation, nutritional status of the animal, and environmental and genetic factors due to feeding differences and seasonal variations. The salt balance in sheep milk is interesting as a contribution to the knowledge of nutritional characteristics of these types of milk and to the retention of these elements in the curd during cheese making. Because sheep milk is mainly used in cheese making and most of the soluble elements are lost in the whey during manufacture, the knowledge of element distribution would allow the evaluation of the influence of milk composition on the mineral content in cheese.

Vitamins in Sheep Milk

The total vitamin content of milk is highly variable and depends on the vitamin status and the feeding regime of the mother (with the level of water-soluble vitamins being more influenced by the feed than the level of the fat-soluble vitamins). The vitamin content of horse and donkey milk is on average lower than that of ruminant milk. The data on the vitamin content of sheep milk show that most of the known vitamins are contained in ovine milk, and for some of them, this foodstuff is a rich source. A comparative account of vitamin B content of sheep milk and cow milk is presented in Table 4. Daily riboflavin (B2) requirements, for instance, are completely covered by drinking just two cups of sheep milk without eating anything else. As consumption of sheep milk is not widespread, it is likely that two cups of sheep milk yogurt would meet those daily requirements or the milk equivalent in 90 g of sheep cheese. From the data available in published literature, it is evident that the sheep milk is richer than cow milk in most of the vitamins.

Table 3 Minerals in ewe milk and cow milk

Minerals	*Ewe milk*	*Cow milk*
Sodium (g l^{-1})	1.41–1.32	0.3–0.9
Potassium (g l^{-1})	1.01–1.52	1.1–1.7
Calcium (g l^{-1})	1.62–2.59	1.1–1.3
Magnesium (g l^{-1})	0.14–0.19	0.09–1.1.0
Phosphorus (g l^{-1})	0.82–1.83	0.9–1.0
Iron (mg l^{-1})	0.29–1.39	0.3–0.6
Copper (mg l^{-1})	0.19–1.30	0.1–0.3
Zinc (mg l^{-1})	4.66–11.8	2.0–6.0
Manganese (mg l^{-1})	0.032–0.13	NA

Source: Stubbs, A. J. (1992). Milk: sheep milk. In *Encyclopaedia of food science, food technology, and nutrition*, pp. 4080–4084. London: Academic Press.

Physicochemical Properties of Sheep Milk

A comparative appraisal of various physicochemical properties of livestock animals are depicted in Table 5.

Hygiene in Milk Production

High levels of hygiene must be maintained at every stage of production and handling of the milk, paying particular attention to the following:

1. Sheep should be kept as clean as is practicable and the hair and wool clipped away from the area around the udder.
2. Udders should be cleaned prior to milking; particularly, the attention should be given to teats and teat orifices.
3. Cleaning and sterilization of the milking equipment must be carried out thoroughly after each milking.
4. Milk must be cooled promptly and kept at less than 5 °C until used.

Nutritional Significance

From the compositional data, it can be seen that sheep's milk has a higher nutritive and energy value than cow and goat milk. Advantages to the processors are that yogurt can be made without increasing the levels of solids not fat, and cheese yields are high. The small size of the fat globules may enhance digestibility of the milk and products. Allergy sufferers who are intolerant to cow milk readily tolerate sheep milk.

Table 4 Vitamin B content of ewe milk and cow milk

Vitamins	*Ewe milk*	*Cow milk*
Riboflavin (mg l^{-1})	4.3–5.0	1.5–2.3
Thiamin (mg l^{-1})	0.6–1.2	0.3–0.6
Nicotinic acid (mg l^{-1})	3.9–5.4	0.6–1.3
Pantothenic acid (mg l^{-1})	3.5–5.3	3.5
Vitamin B6 (mg l^{-1})	0.7	0.4
Folic acid (μg l^{-1})	54.0	50.0
Vitamin B12 (μg l^{-1})	1.4–9.8	3.0
Biotin (μg l^{-1})	50.0–90.0	20.0

Source: Stubbs, A. J. (1992). Milk: sheep milk. In *Encyclopaedia of food science, food technology, and nutrition*, pp. 4080–4084. London: Academic Press.

Table 5 Physicochemical properties of milk of livestock animals

Type of milk	*pH*	*Specific gravity*	*Titratable acidity*
Sheep milk	6.63	1.034	0.23
Goat milk	6.55	1.030	0.16
Cow milk	6.64	1.029	0.17
Buffalo milk	6.75	1.033	0.21

Production of Processed Foods

Cheese and yogurt are the main products of sheep milk worldwide. Smaller markets exist for liquid milk and other products. Liquid milk may be pasteurized, frozen, or spray-dried. The main difference between the milk of sheep, cow, and goat is that the former contains almost double the solids because sheep milk is rich in fat, protein, minerals, and vitamins. However, the methods of production and processing and the potential microbiological problems are similar. The high level of the total solids contents in sheep milk makes it particularly suitable for manufacture of yogurt, cheese, butter, and ice cream.

Yogurt

The uniform small fat globules in sheep milk ensure that there is no separation of cream in the yogurt; consequently, there is no need of homogenization. The method of manufacture is the same as for cow milk; the set type is normally manufactured, although a small amount of the strained variety is made, particularly in Greece.

Cheese

Sheep milk can be successfully used to make cheese by any of the recipes used for cow milk, although the resultant cheese is unlikely to resemble the corresponding cow milk cheese. A wide range of cheeses (fresh, soft, brined, hard, and blue-veined varieties) have been made traditionally, many of which are produced in one locality. Roquefort, feta, pecorino, and ricotta are probably the best known. Until the introduction of modern technology, feta cheese, which should be white, could only be made successfully from sheep milk as there is too much natural color in cow milk. Pecorino is the hardest of the sheep milk cheeses and is associated with the production of ricotta. Ricotta, which is not technically a cheese as it contains no casein, is produced by heating the whey of any cheese that has not been salted to at least 85 °C. The resultant white foam is skimmed off and allowed to drain overnight in a 'ricotta' basket or strainer. In recent years, a wide range of 'new' cheeses have been developed from the sheep milk to suit modern tastes.

Butter

The production of butter from sheep milk is not yet commercially explored. But in some localities, sheep milk is utilized for butter making for domestic use, while in a few sheep belts in India, it is used to produce ghee.

Ice Cream

Sheep milk ice cream is not available commercially. However, some manufacturers make small quantities of ice cream from sheep milk, which they sell from their own premises as a cottage-level income-augmenting activity.

Public Health Significance of Sheep Milk

Relatively little information exists on the microbiology of sheep milk and its public health significance. However, such milk and its products are subject to similar public health and quality problems as is milk from other animals. Milk drawn aseptically from sheep udder is practically sterile, provided that the animal is free from any disease. However, after leaving the udder, it can be easily contaminated during unhygienic handling. The studies on freshly produced bulk milk showed poor picture of its microbiological quality with average viable counts of 10^5–10^6 CFU ml^{-1}. This is partly due to the preponderance of production in developing countries and rural areas where conditions are primitive and understanding of hygiene lacking. Milk produced under hygienic conditions can be of excellent microbiological quality. However, contamination from milking equipment can be a problem as the high fat content of the milk makes surfaces difficult to clean. The common cause of clinical mastitis and therefore the most likely contaminant of milk from this source is *Staphylococcus aureus*. However, *Pasteurella haemolytica* may be as or more important in some flocks. Mastitic streptococci and a variety of other bacteria may also be present in the milk owing to clinical or subclinical udder infections. The only report of disease of animal origin transmitted via sheep's milk or products in the United Kingdom has been of staphylococcal intoxication from raw milk cheese. Many strains of mastitic *S. aureus* have been shown to be enterotoxigenic, so this seems to be a public health risk. *Listeria monocytogenes* causes circling disease in sheep and can be excreted in the milk. This poses a potential health risk since infection is not usually associated with severe mastitis or organoleptic changes in the milk. Sheep can also become infected with salmonellas and campylobacters, and a carriage pattern of campylobacter in healthy sheep similar to that in cows has been found in the United Kingdom, creating a potential risk. Other pathogens involved in zoonosis through sheep milk and products are *Mycobacterium tuberculosis* (rare in sheep), *Brucella melitensis/abortus*, *Coxiella burnetii*, and *Toxoplasma gondii*.

Postpasteurization contamination of milk is thought to have been the cause of an outbreak of *Salmonella enteritidis* from cheese in Spain. Contamination of human origin has caused outbreaks of *S. aureus* intoxication and shigella infection.

See also: Bioactive Peptides in Foods; Buffalo Milk; Cheese: Processing and Sensory Properties; Cheese: Types of Cheeses – Soft; Fatty Acids: Essential Fatty Acids; Fatty Acids: *Trans* Fatty Acids; Goat: Milk; Human Milk: Composition and Nutritional Value; Milk: Role in the Diet; Milk: Sources and Composition; Yogurt: The Product and its Manufacture.

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Shellfish: Characteristics of Crustaceans and Mollusks

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Abbreviations

BM	Brown meat
DHA	Docosahexaenoic acid
EAA	Essential amino acids
EPA	Eicosapentaenoic acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acid
WM	White meat

Introduction

Shellfish are recognized as all exoskeleton-bearing aquatic invertebrate organisms harvested for human consumption, including crustaceans, bivalve mollusks, and gastropod mollusks. These organisms have high economic and nutritional relevance, being considered as delicacies and healthy food items, since they are important sources of proteins and have low fat levels with important levels of $n-3$ fatty acids. This article intends to provide an overview of the morphological, ecological, and nutritional characteristics of the most relevant shellfish consumed worldwide, namely, crustaceans and bivalves.

Crustaceans

Crustaceans (phylum Arthropoda, order Decapoda) can be mainly divided into four categories: crabs, clawed lobsters, spiny lobsters, and shrimps/prawns (see differences in Figure 1). All crustaceans are covered with a hard and rigid outer shell composed of calcium and chitin. The shell is periodically replaced by a large one enabling animal growth (molting). Generally, eggs are incubated in females' pleopods, and newly hatched planktonic larvae are released into the water.

Crabs

Worldwide, the most relevant crab species harvested are the horse crab (*Portunus trituberculatus*) (one-quarter of the total), followed by flower crab (*Portunus pelagicus*), snow crab (*Chionoecetes* spp.), blue crab (*Callinectes sapidus*), brown crab (*Cancer pagurus*), Dungeness crab (*Metacarcinus magister*), and mud crabs (*Scylla serrata*), each with more than 20 000 tons annually. The most relevant crabs consumed in Europe are the brown crab (*Cancer pagurus*), velvet crab (*Necora puber*), spider crab (*Maja* squinado or *Maja brachydactyla*), and green crab (*Carcinus maenas*). The main ecological (geographic distribution, habitat, and behavior) and morphological (biology) characteristics are shown in Table 1. They show marked sexual dimorphism, where the females' abdomen is broad with four pairs of hairy appendages on which the eggs are attached during spawning, whereas the males' abdomen is narrow with abdominal appendages modified in copulatory organs and larger claws (in adults).

Clawed and Spiny Lobsters

Several species are harvested globally, despite only four species account more than 20 000 tons annually, such as the American lobster (*Homarus americanus*), Norway lobster (*Nephrops norvegicus*), Caribbean spiny lobster (*Panulirus argus*), and tropical spiny lobsters (*Panulirus* spp.). The most relevant clawed lobsters consumed in Europe are the European lobster (*H. gammarus*), American lobster (*H. americanus*; imported from the United States and Canada), Norway lobster (*Nephrops norvegicus*), and spiny lobsters (represented by several *Palinurus* species; mostly imported). The main ecological (geographic distribution, habitat, and behavior) and morphological (biology) characteristics are shown in Table 1. Lobsters have long bodies with muscular tails and move by slowly walking on the seafloor or swim backward by quickly curling and uncurling their abdomen.

Shrimps and Prawns

Globally, the most relevant shrimps and prawns harvested or produced are the whiteleg shrimp (*Litopenaeus vannamei*), giant tiger prawn (*Penaeus monodon*), akiami paste shrimp (*Acetes japonicus*), northern prawn (*Pandalus borealis*), southern rough shrimp (*Trachysalambria curvirostris*), fleshy prawn (*Fenneropenaeus chinensis*), and banana prawn (*Fenneropenaeus merguiensis*). The most relevant shrimps and prawns consumed in Europe are the tiger prawn (*Penaeus monodon*; imported) and freshwater shrimp (*L. vannamei*; imported from Asian countries), among others. The main ecological (geographic distribution, habitat, and behavior) and morphological (biology) characteristics are shown in Table 1. Shrimps and prawns possess stalked eyes with long narrow abdomens, long antennae, and fragile legs adapted to swimming.

Bivalves

Bivalves (phylum Mollusca, class Bivalvia) include marine and freshwater organisms with laterally compressed bodies enclosed by a hard and rigid outer shell that grows with the animal, composed of calcium carbonate (secreted by the mantle tissue epidermis) and divided into two parts (valves) joined in one edge by a flexible ligament in conjunction with teeth.

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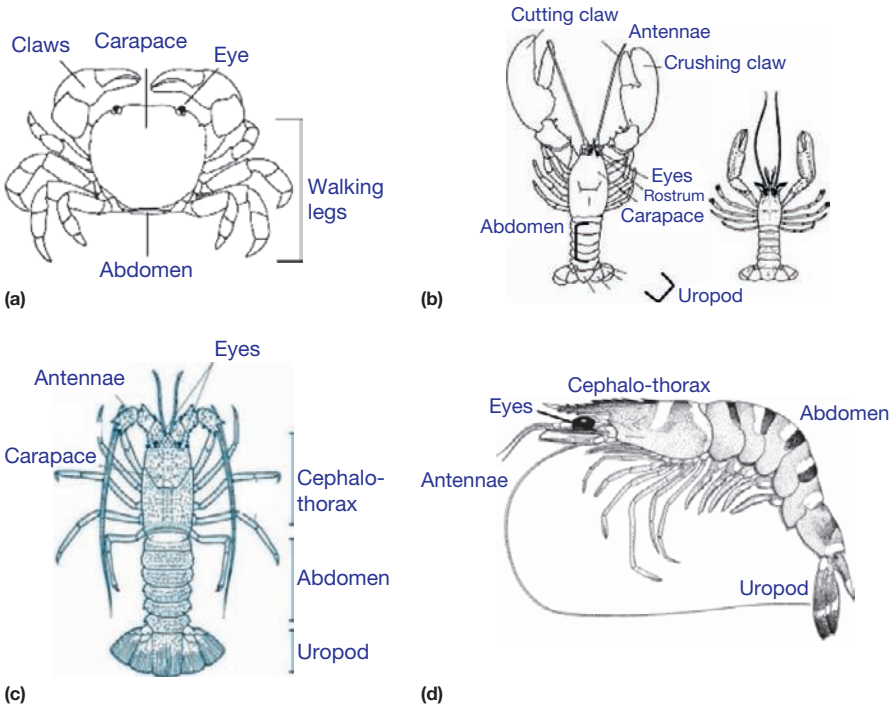

Figure 1 Schematic representation of different categories of crustaceans: (a) crabs, (b) clawed lobsters, (c) spiny lobsters, and (d) shrimps/prawns. Adapted from the Illustration of Jo Taylor; source: Museum Victoria, Australia.

Most species do not have heads, and the edible tissue is mainly composed of the mantle and viscera. Bivalves mostly consumed in Europe can be mainly divided into four categories: clams, mussels, oysters, and scallops (see differences in Figure 2). The bivalve categories have a notable variation in shape, size, thickness, and color.

Clams

Worldwide, the major species of clams for production are the Manila clam (*Ruditapes philippinarum*), razor clam (*Ensis*, *Siliqua*, and *Solen* genera), blood cockle (*Anadara granosa*), and northern quahog (*Mercenaria mercenaria*). The most important clam species consumed in Europe are the Manila clam (*R. philippinarum*), grooved carpet shell (*R. decussatus*), cockle (*Cerastoderma edule*), and Vietnamese clam (*Meretrix lyrata*; imported). The main ecological (geographic distribution, habitat, and behavior) and morphological (biology) characteristics are shown in Table 2.

Mussels

The New Zealand green-lipped mussel (*Perna canaliculus*), Chilean mussel (*Mytilus chilensis*), blue mussel (*M. edulis*), and the Mediterranean mussel (*M. galloprovincialis*) are the most relevant mussels produced worldwide. The most consumed mussels in Europe are the blue mussel (*M. edulis*) and the Mediterranean mussel (*M. galloprovincialis*). The main ecological (geographic distribution, habitat, and behavior) and morphological (biology) characteristics are shown in Table 2.

Oysters

Worldwide, the most important edible oysters are representatives of the genus *Crassostrea*, notably *C. gigas* in the western Pacific, *C. virginica* in North America, and *C. angulata* in Portugal. The two most important oyster species consumed in Europe are the Pacific cupped oyster (*C. gigas*) and the Portuguese oyster (*C. angulata*). The main ecological (geographic distribution, habitat, and behavior) and morphological (biology) characteristics are shown in Table 2.

Scallops

The Atlantic sea scallop (*Placopecten magellanicus*), Yesso scallop (*Patinopecten yessoensis*), Atlantic bay scallop (*Argopecten irradians*), Chinese scallop (*Chlamys* spp.), and Peruvian calico scallop (*Argopecten purpuratus*) are the most relevant scallops produced in the world. The king scallop (*Pecten maximus*) is the most important scallop species consumed in Europe. The main ecological (geographic distribution, habitat, and behavior) and morphological (biology) characteristics are shown in Table 2.

Nutritional Characteristics

The nutritional characteristics of shellfish vary among species. Also, the specimens' age, sex, maturation stage, origin, season, seawater physical–chemical properties, and feed availability may influence the levels of nutrients (example in Figure 3). Shellfish edible tissues are mainly composed of proteins (10–21%), lipids (0.3–20%), and carbohydrates (0.4–7%) (Tables 3 and 4). The energetic value in crustaceans' muscle

Table 1 Characteristics of the most consumed crustacean species in Europe in terms of distribution, habitat, behavior, and biology

Crustaceans	*Common name*	*Distribution*	*Habitat and behavior*	*Biology*
Crabs				
Cancer pagurus	Brown crab	European west coast (Portugal to Norway) and North Africa	Bottoms of sand, gravel, and rock up to 200 m depth, shallow in warm season and deeper waters in cold season, predators (bivalve mollusks, polychaetes, and crustaceans)	Heavy, oval-shaped body, with a 'piecrust' edge and massive black-tipped pincers, red-brown color, sexual dimorphism, grows up to 30 cm; females store sperm until eggs are developed
Carcinus maenas	Green crab	European west coast (Portugal to Norway) and North Africa	Marine and estuarine habitats in mud, sand, or rock substrates up to 60 m depth, predators (bivalve mollusks, polychaetes, and crustaceans)	Carapace with five short teeth along the rim behind each eye, sexual dimorphism, grows up to 10 cm; color varies from green to brown to gray or red; females store sperm until eggs are developed
Maja spp.	Spider crab	Mediterranean/European west coast (Portugal to Scotland) and North Africa	Rocky or coarse sandy areas up to 50 m depth, predators (bivalve mollusks, polychaetes, and crustaceans)	Red, brown-red, or yellow color, grows up to 20 cm, circular to oval carapace, broad at the back and narrow at the front, legs covered by hairs, shell covered with algae, sexual dimorphism; females store sperm until eggs are developed
Necora puber	Velvet crab	Mediterranean/European west coast (Portugal to Scotland) and North Africa	Stony and rocky substrata in shallow and sheltered water, predators (bivalve mollusks, polychaetes, and crustaceans)	Fast-moving swimming crab, blue-brown color, dorsal surface with velvety texture, red eyes, sexual dimorphism, grows up to 8 cm; females store sperm until eggs are developed
Lobsters				
Homarus gammarus	European lobster	European west coast (Portugal to Norway) and North Africa	Rocky substrata, living in holes and excavating tunnels up to 60 m depth, territorial and noctivagous, omnivores and eat fish, mollusks, crustaceans, worms, and algae	Blue-colored above with spots and yellowish below, body without strong spines or ridges, eggs fertilized during mating, grows up to 1 m; first pair of walking legs are massive but slightly unequal claws
Homarus americanus	American lobster	Atlantic coast of North America (North Carolina to Labrador)	Cold, shallow waters in rocky substrata; omnivores and eat fish, mollusks, crustaceans, worms, and algae; depths from 2 to 480 m	Dark bluish-green to greenish-brown color, red on the body and claws, and green on the legs, rostrum with spines, eggs fertilized during mating, grows up to 61 cm
Nephrops norvegicus	Norway lobster	Mediterranean/European west coast (Portugal to Norway) and North Africa	Muddy bottoms; digging hole to burrow; nocturnal; omnivores and eat fish, mollusks, crustaceans, worms, and algae; depths from 15 to 800 m	Color pale orange, grows up to 25 cm, head and thorax with a nonsegmented carapace, abdomen segmented, fanlike tail, first three pairs of legs with claws, two pairs of antennae (one longer and thinner), large black eyes, eggs fertilized during mating
Palinurus sp./ *Jasus* sp./ *Panulirus* sp.	Spiny lobster	South European, American, African, and Australasian coasts	Warm seas; live in crevices of rocks and coral reefs; only occasionally leaving at night to eat; omnivores and eat fish, mollusks, crustaceans, worms, and algae; depths from 5 to 200 m	Several colors, long, thick, and spiny antennae, lack of claws in the first four pairs of legs, slightly compressed carapace, lacking lateral ridges, eggs fertilized during mating, grows up to 60 cm
Shrimps				
Penaeus monodon	Prawn	Coasts of Australia, Southeast Asia, South Asia, and East Africa	Tropical marine coastal estuaries, lagoons, or mangrove areas, nocturnal activity, burrowing during the day, eggs fertilized during mating, muddy or sandy bottoms up to 20–50 m depth	Green, brown, red, gray, blue, and transverse blue or black and yellow band colors on abdomen and carapace, rostrum well developed and toothed dorsally and ventrally, grows up to 33 cm
Penaeus vannamei	Shrimp	Native to the eastern Pacific coast and farmed in America and Asia	Tropical marine habitats ($>$20 °C), ocean, coastal estuaries, lagoons, or mangrove, eggs fertilized during mating, muddy or sandy bottoms up to 72 m	Translucent, blue or olive with dusky bands, reddish-brown on the antennules, and white legs, grows up to 23 cm

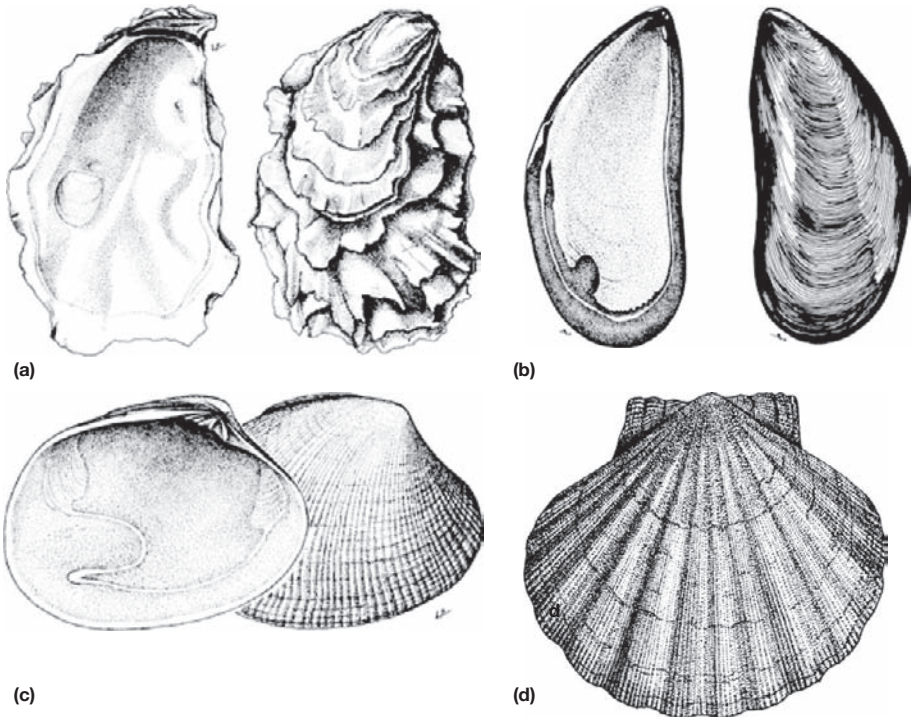

Figure 2 Schematic representation of different categories of bivalves: (a) oysters, (b) mussels, (c) clams, and (d) scallops. Adapted from FAO source.

Table 2 Characteristics of the most consumed bivalve species in Europe in terms of distribution, habitat, behavior, and biology

Bivalves	*Common name*	*Distribution*	*Habitat and behavior*	*Biology*
Cerastoderma edule	Cockle	European west coast (Portugal to Scotland) and North Africa	Able to burrow up to 5 cm depth in clean sand, muddy sand, mud, or muddy gravel, separate sexes, intertidal areas, filter feeder (phytoplankton)	Shell with radiating ribs, crossed by concentric ridges, outer surface white, yellowish, or brownish, two siphons and two adductor muscles, large muscular foot, external fertilization, grows up to 5 cm; shell is solid, thick, equivalve, globular, and broadly oval shape; gonads occupy a major portion of the visceral mass
Crassostrea spp.	Oyster	Mediterranean/European west coast (Portugal to Scotland), North Africa, Asia, America, and Australia	Intertidal and subtidal firm (attach to rocks, debris, or other oyster shells), mud or mud–sand bottoms, between 5 and 40 m depth, protandrous hermaphrodites (start as males), filter feeder (phytoplankton)	Shell solid, inequivalve, extremely rough, extensively fluted, laminated, white color with purple streaks and spots, white shell interior, irregular shell shapes, with a single adductor muscle, external fertilization, grows up to 30 cm
Mytilus spp.	Mussel	Mediterranean/European west coast (Portugal to Norway) and North Africa	Hard exposed intertidal substrates, attached by byssus threads to rocks and piers in sheltered harbors, estuaries, and coastal rocky shores, sometimes living in dense masses, separate sexes, up to 40 m depth, filter feeder (phytoplankton)	Extremely variable in shape and color, shell solid, equivalve, inequilateral, beaks at the anterior end, triangular shape, shells blue, purple, or dark brown, external fertilization, grows up to 15 cm
Ruditapes spp.	Clam	Mediterranean/European west coast (Portugal to Scotland), North Africa, America, and Asia	Buried in sand, muddy gravel, or stiff clay, below mid-tide level and littoral lagoons, separate sexes, up to 4 m depth, filter feeder (phytoplankton)	Shell solid, equivalve, inequilateral, beaks in the anterior half, broadly oval to quadratic shape, extremely variable in color and pattern, shell white, yellow, or light brown, sometimes with rays, steaks, blotches, or zigzags, two siphons, two adductor muscles, large muscular foot, external fertilization, grows up to 8 cm; gonads occupy a major portion of the visceral mass

Table 2 (Continued)

Bivalves	*Common name*	*Distribution*	*Habitat and behavior*	*Biology*
Pecten maximus	King scallop	European west coast (Portugal to Norway)	Clean firm sand, fine, sandy gravel, and occasionally muddy sand, active swimmer, functional hermaphrodite, between 4 and 100 m depth, filter feeder (phytoplankton)	Shell valves fan-shaped with an ear on either side of the apex of the valve, right valve strongly convex, with a white, yellow, or light brown color and often with bands or spots of darker pigment, left valve flat and light pink to red-brown, with radiating ribs, no siphons, one large and well-developed adductor muscle, conspicuous and well-defined gonads, external fertilization, grows up to 15 cm
Meretrix lyrata	Vietnamese clam	Indo-West Pacific coasts	Brackish tropical waters, buried in mud flats with sandy or muddy bottoms, separate sexes, up to 20 m depth, filter feeder (phytoplankton)	Trigonal ovate shape, equivalve, exterior color light tan to light brown, posterior dorsal margin dark brown, interior white with a purple or dark brown ray of pigment along the posterior dorsal margin, two siphons, two adductor muscles, large muscular foot, external fertilization, grows up to 5 cm; gonads occupy a major portion of the visceral mass

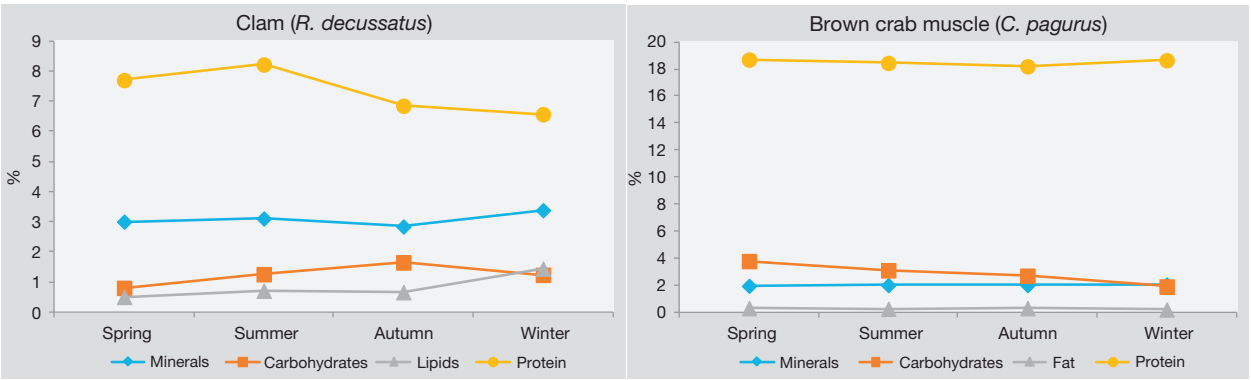

Figure 3 Seasonal variation of protein, lipid, carbohydrate, and mineral contents in a bivalve and a crustacean species.

and bivalves is generally reduced, containing less than 100 kcal (418 kJ) per 100 g portion but can be high in brown meat (243 kcal per 100 g portion). The lipid fraction does not exceed 5%, except crustaceans' brown meat (i.e., mixture of hepatopancreas and gonads). Despite considerable variations between species, shellfish fatty acid profile is particularly interesting, as the long-chain polyunsaturated fatty acids (PUFA) are predominant, such as eicosapentaenoic acid (EPA; 20:5$n-3$) and docosahexaenoic acid (DHA; 22:6$n-3$). Palmitic acid (16:0) is the prevailing saturated fatty acid (SFA) in shellfish species, whereas oleic acid (18:1$n-9$) is the main monounsaturated fatty acid (MUFA). Despite shellfish has long been assumed to possess high cholesterol levels, recent findings reported that only 25–40% of the total sterols in shellfish correspond to cholesterol. In fact, shellfish contains less cholesterol than meat or poultry, and the highest values are observed in crustaceans' brown meat. Protein contents in shellfish are, in general, more stable than lipids, revealing high levels of digestible proteins with essential amino acids similar to those of reference protein. Shellfish also have higher contents of carbohydrates compared with finfish, mainly stored as glycogen, exhibiting a clear seasonal variability related to reproduction. Other components responsible for the sapidity of shellfish are also found in shellfish, such as nonprotein nitrogen compounds (e.g., free amino acids, nucleotides, and volatile compounds). Shellfish contains most of the naturally occurring

Table 3 Biochemical composition of the most consumed crustaceans

	Brown crab (Cancer pagurus)		*Green crab* (Carcinus maenas)		*Spider crab* (Maja *spp.*)		*Velvet crab* (Necora puber)		*European lobster* (H. gammarus)		*American lobster* (H. americanus)		*Norway lobster* (Nephrops norvegicus)	*Spiny lobster* (Palinurus *spp.*)	*Prawn* (Penaeus *spp.*)
	WM	*BM*	*WM*	*BM*	*WM*	*BM*	*WM*	*BM*	*WM*	*BM*	*WM*	*BM*	*WM*	*WM*	*WM*
Nutritional data (per 100 g)															
Energetic value (kcal kJ^{-1})	89/372	175/735	78/328	84/351	83/347	144/604	71/298	85/357	85/356	193/807	80/336	243/1015	94/392	112/469	97/406
Lipids (g)	0.3	10.8	0.4	2.7	0.3	7.1	0.4	4.2	0.4	14.2	0.7	20.2	0.5	1.5	1.3
Proteins (g)	18.4	12.3	16.9	11.8	15.7	13.7	15.5	10.1	18.0	12.9	16.4	12.4	20.9	20.6	18.8
Carbohydrates (g)	2.4	7.4	0.7	3.8	2.2	3.2	0.5	1.1	1.1	2.1	1.3	2.1	0.4	2.4	1.2
Cholesterol (mg)	38.9	117.8	38.9	100.1	37.1	73.1	44.8	125.0	33.9	59.2	40.2	91.8	67.8	131.2	130.0

WM, white meat; BM, brown meat.

Table 4 Biochemical composition of the most consumed bivalve mollusks

	Oyster (Crassostrea *spp.)*	*Mussel* (Mytilus *spp.)*	*Clam* (Ruditapes *spp.)*	*Scallop* (Pecten *spp.)*
Nutritional data (per 100 g)				
Energetic value (kcal kJ^{-1})	81/339	86/360	58/243	105/440
Lipids (g)	2.3	2.2	0.9	0.8
Protein (g)	9.4	11.9	11.7	17.1
Carbohydrates (g)	4.9	7.0	2.6	6.0
Cholesterol (mg)	50	28	44	85

minerals (e.g., C, H, S, K, P, and Ca) and Se and Cu in high concentrations. Toxic elements (e.g., Hg, Cd, Pb, and As) are also detected in edible tissues, though the concentrations are usually low, with the exception of Cd in crustaceans' brown meat. Vitamins vary according to species, origin, and season, but vitamins A, B12, B3 (niacin), B2, and E are, generally, referred as the most abundant in shellfish.

Crustaceans

Most studies assessing the biochemical composition of crustaceans only consider the muscle (WM found in claws and legs) as edible tissue. However, crustaceans' BM (viscera) is also consumed. Major differences are encountered between both tissues (see Table 3), particularly in the lipid and mineral fraction. Fat and cholesterol contents can be extremely high in crustaceans' BM, whereas WM is a lean (fat $<0.2\%$) and low-cholesterol product. BM has also higher shares of carbohydrates, SFA (e.g., 14:0 and 16:0), MUFA (e.g., 16:1$n-7$, 18:1$n-9$, and 20:1$n-9$), and minerals (1.4–7%). In opposition, lower contributions of EPA and DHA are detected in BM, as well as lower protein (10–14%) and EAA levels. The most relevant minerals in BM are K, P (data not shown), Na, Ca, Zn, and Mg. High concentrations of Cu can also be found in crustaceans' BM. Regarding toxic elements, the levels of Hg, Pb, and As are usually similar in WM and BM, while Cd concentrations are much higher in BM. Despite Cd is only regulated in crustaceans' WM, Cd concentrations in BM can be 100 times above the maximum permissible concentration set by the EU. The information concerning vitamins is scarce for crustaceans' BM, whereas in WM, relevant contents of vitamins A, E, and B12, folates, and niacin have been found in brown crabs and European, Norwegian, and spiny lobsters. In this context, the assessment of crustaceans' nutritional value should also take into account the BM.

Bivalves

Bivalves are lean products due to the low lipid content in most species' edible tissue ($<3\%$; Table 4). Overall, high shares of PUFA are detected, namely, from the $n-3$ group. Other fatty acids are also typically found at high concentrations in bivalves, such as 16:0, 18:1$n-9$, and 20:4$n-6$. Low cholesterol content is usually detected in most bivalve species; however, higher values can be found in scallops (28–85 mg 100 g^{-1}). The protein content is stable (13–18%), though higher in scallops. The presence of some free amino acids, such as threonine, glycine, and alanine, confers the characteristically sweet taste of bivalves, whereas glutamic and aspartic acids are responsible for the acidic taste, and aspartate and glutamate are associated with the *umami* taste, also described as meat flavor. Carbohydrate content in bivalves also varies considerably mostly due to seasonal variations related to these species' reproductive cycle, reaching its lower peak immediately after spawning. The mineral content varies mostly according to species (0.8–3%) and origin, and the most abundant elements are K, P (data not shown), Na, Ca, and Mg. Within toxic elements, As is the most important, although the levels in bivalves are, generally, lower than those observed in crustaceans. Despite some variations within species, relevant contents of vitamins A (high in mussels: 360 μg 100 g^{-1}), B12 (high in oysters), B6, and B1 and niacin can be found in bivalves.

See also: Amino Acids: Determination; Arsenic: Properties and Determination; Cadmium: Properties and Determination; Calcium: Properties and Determination; Cholesterol: Properties, Processing Effects, and Determination; Cooking: Domestic Techniques; Copper: Physiology; Fats: Classification and Analysis; Fatty Acids: Determination and Requirements; Food Composition Databases; Iron: Properties and Determination; Magnesium; Manganese; Potassium: Properties and Determination; Protein: Requirements; Selenium: Properties and Determination; Trace Minerals and Trace Elements; Zinc: Properties and Determination.

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http://www.marlin.ac.uk/phylumdetails.php?phylum=2326 — MARLIN – Marine Life Information.
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http://nutritiondata.self.com/ — Food nutritional database.

Shellfish: Role in the diet

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Abbreviations

AI	Atherogenic index
BM	Brown meat
BW	Body weight
DHA	Docosahexaenoic acids (22:6$n-3$)
EPA	Eicosapentaenoic acid (20:5$n-3$)
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
TI	Thrombogenic index
TWI	Tolerable weekly intake
WM	White meat

Introduction

Seafood is one of the most important food commodities consumed worldwide. The health benefits of a diet rich in seafood have been extensively recognized. Namely, $n-3$ polyunsaturated fatty acids (PUFAs) are associated with decreased morbidity and mortality from various illnesses like reduced risk of coronary heart disease, hypertension, and stroke. The World Health Organization and Food and Agriculture Organization recommend a regular fish consumption of 1–2 servings per week in order to provide an equivalent of 200–500 mg of eicosapentaenoic acids (20:5$n-3$) (EPA) and docosahexaenoic acids (22:6$n-3$) (DHA). Within seafood, shellfish (including crustaceans and bivalves) are, together with fish and cephalopods, the most relevant items consumed worldwide. This article aims to describe the role of crustaceans and bivalves in the European diet, including the description of sources and production, consumption preferences, and hazards and benefits to consumers.

Sources and Production

Mollusks and crustaceans have gained increasing importance to European consumers, raising the annual *per capita* consumption from 300 to 400 g in 1961 to almost 2 kg in 2009 (Figure 1(a)).

In the last decade, mussels were the most relevant bivalve group produced in Europe (though showing wide variability; reaching 78% of the whole bivalves production in 2012; main producers: Spain, Italy, and France), followed by clams (12%; main producers: Italy, Spain, and Portugal), scallops (7%; main producers: the United Kingdom, France, and Ireland), and oysters (3%; main producers: Ireland, the Netherlands, and Spain) (Figure 1(b)). The overall production of bivalves in Europe accounted 423 000 tons in 2012. Mussels were also the most relevant bivalve species exported from the EU in 2012 (67%; main destination: Switzerland), followed by scallops (14%; main destination: Vietnam), clams (10%; main destinations: China and Switzerland), and oysters (9%; main destinations: China, Russian Federation, and Switzerland), with an overall exportation volume accounting 219 000 tons (Figure 1(c)). Similarly, mussels were the most relevant bivalve group imported by the EU in 2012 (60%; main suppliers: New Zealand and Chile), followed by scallops (19%; main suppliers: the United States, Peru, and Argentina), clams (15%; main suppliers: Vietnam, Chile, and Republic of Korea), and oysters (6%; main supplier: Republic of Korea), representing a global importation volume of 271 000 tons (Figure 1(d)). Therefore, the trade balance of bivalves in Europe is slightly negative due to mussels, scallops, and clams (−52 000 tons).

Concerning crustaceans, the most relevant group produced in the last decade in Europe was shrimps and prawns (60% of the whole crustacean production in 2012; main producers: Italy, the Netherlands, and Germany), followed by lobsters (22%; main producers: the United Kingdom, Ireland, and France) and crabs (18%; main producers: the United Kingdom, Ireland, and France) (Figure 1(e)). The European crustacean production accounted 121 000 tons in 2012. Shrimps and prawns were also the most relevant crustaceans exported by the EU in 2012 (91%; main destinations: Morocco, China, and Russian Federation), followed by lobsters (6%; main destinations: China, Vietnam, and Japan) and crabs (3%; main destinations: China, the United States, and Indonesia), with an overall exportation volume reaching 81 000 tons (Figure 1(f)). Similarly, shrimps and prawns were the most relevant crustaceans imported by the EU in 2012 (93%; main suppliers: Ecuador, India, and Argentina), followed by lobsters (5%; main suppliers: the United States, Canada, and Cuba) and crabs (2%; main suppliers: Greenland, Madagascar, and Norway), representing a total importation volume of 471 000 tons (Figure 1(g)). The crustaceans trade balance in Europe is very negative due to shrimps and prawns (−390 000 tons).

Consumer Preferences of Bivalve Mollusks and Crustaceans: Portugal as Case Study

European seafood annual *per capita* consumption has increased consistently over the past four decades, from 14 kg in 1961 to 22 kg in 2009. European seafood consumption varies considerably according to country and region, being generally higher and more traditional in southern countries than in northern and western Europe. Portugal is the eighth biggest seafood consumer worldwide and has the highest annual seafood *per*

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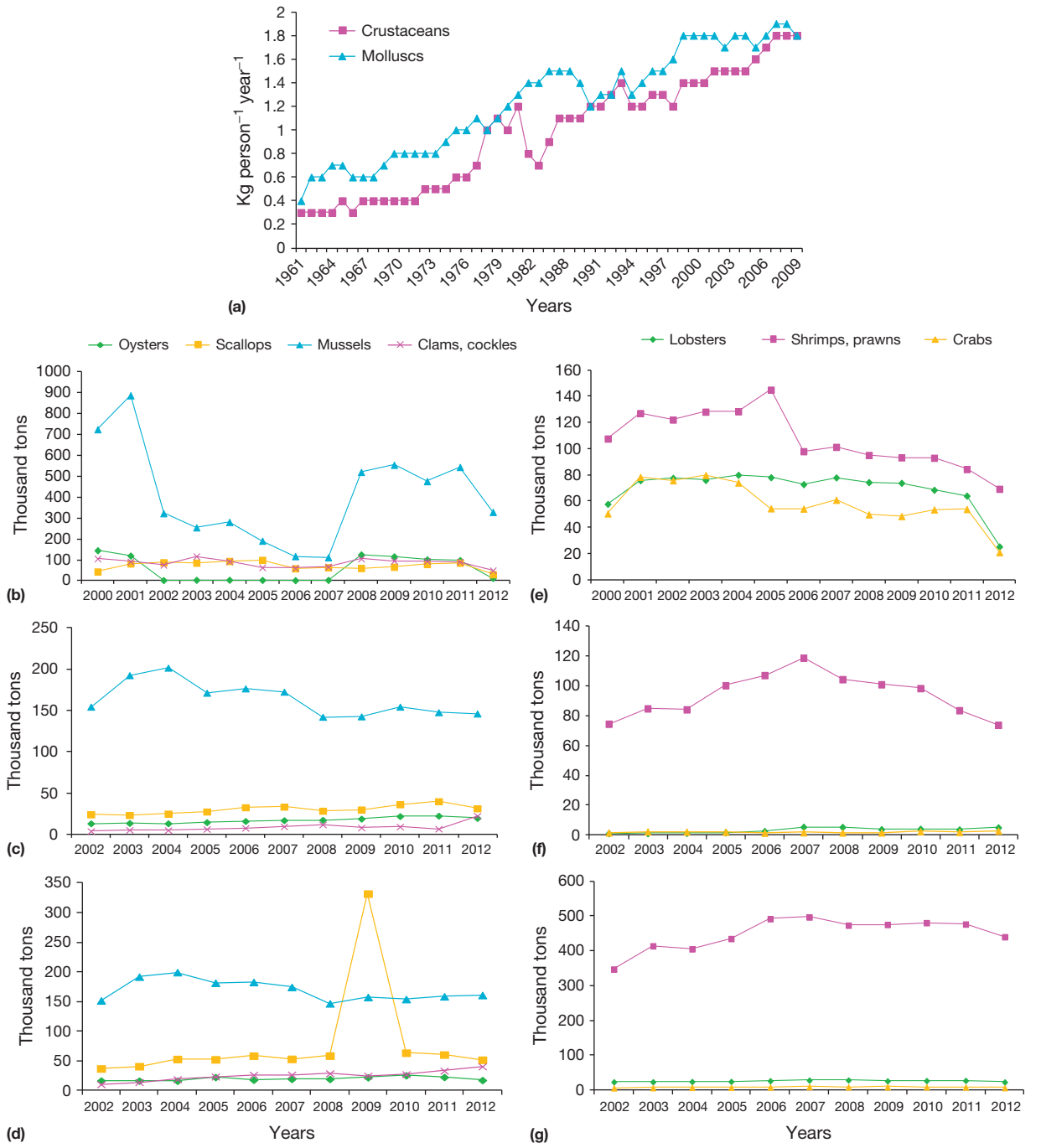

Figure 1 Trade balance, production, and consumption of crustaceans and bivalves in Europe: per capita consumption (a); bivalves production (b), exportation (c), and importation (d); and crustaceans production (e), exportation (f), and importation (g). Source: Eurostat.

capita consumption in the EU, with an annual average of 61 kg, well above the average world *per capita* consumption (18.4 kg, 2009). Among seafood, crustaceans and bivalve mollusks are greatly appreciated by European consumers from a social and gastronomic point of view, particularly in Southern Europe. Both items are usually maintained alive until the culinary preparation as a guarantee of freshness, but bivalves are preferably consumed either live/raw (e.g., oysters) or lightly cooked (e.g., clams and mussels).

Several studies have focussed the seafood consumer preferences in several European countries, such as seafood and bivalves in Portugal, shellfish in Greece, and oysters, mussels, and seafood in France. Most studies showed that consumers' behavior and attitudes vary considerably according to their demographic and socioeconomic characteristics. Portugal is an excellent case study to assess consumer preferences concerning seafood, since it is the largest *per capita* seafood consumption country in Europe. Therefore, information regarding

bivalve and crustacean consumers' preferences was collected through online surveys in Portugal (see Figures 2 and 3). In general, the Portuguese population widely consumes crustaceans (94%) and bivalves (80%) (Figures 2(a) and 3(a)). The most consumed crustacean groups are shrimps, prawns, and brown crab, representing more than 50% of the overall crustacean consumption in Portugal (Figure 2(b)). Concerning bivalves, the most important species consumed in Portugal are the pullet carpet shell clam (*Venerupis pullastra*), grooved carpet shell clam (*Ruditapes decussatus*), and Japanese carpet shell clam (*Ruditapes philippinarum*), representing 66% of the overall bivalve consumption (Figure 3(b)).

The less important species are lobsters, spider crabs, Norway lobster, velvet crabs, European lobsters, and green crabs, contributing with 36% of crustaceans' consumption, and mussels, oysters, hard clams, scallops, surf clams, and peppery furrow shell clams, accounting 32% of bivalves' consumption. The unpleasant flavor and high price are the main reasons for not consuming crustaceans (55%; Figure 2(c)) and bivalves (39%; Figure 3(c)). Additionally, Portuguese consumers also highlighted that crustacean consumption is a concern due to allergies (13%), whereas food poisoning can hamper bivalve consumption (18%). A high proportion of the population reported low consumption frequencies particularly of bivalves; that is, 39% of the population consumed bivalves one to five times per year (bimonthly to annually; Figure 3(d)), while crustaceans are mainly consumed (51%) 6–11 times per year (monthly to bimonthly; Figure 2(d)). Overall, the consumption of shellfish varies seasonally, increasing in summer particularly for bivalves (45%, Figure 3(e)), whereas crustaceans are also highly consumed in New Year festivities. The local markets seem to be the most convenient place to purchase crustaceans in Portugal (50%; Figure 2(e)), while bivalves are mostly purchased at supermarkets (51%; Figure 3(f)). In contrast, most European countries reveal other preferences to buy bivalves, principally mussels from traditional fish shops (viz., Greece, Switzerland, Poland, Norway, and France). Most consumers buy live (51%) or frozen (42%) bivalves, and the majority of consumers slightly cook them (52%) or use long cooking practices (47%) (Figure 3(g) and 3(h)). In contrast, concerning crustaceans, consumers buy mostly frozen shrimps and prawns (31%) and cooked-chilled products (28%) (Figure 2(f)), and most consumers prefer to boil (56%), grill (25%), and fry (19%) crustaceans (Figure 2(g)).

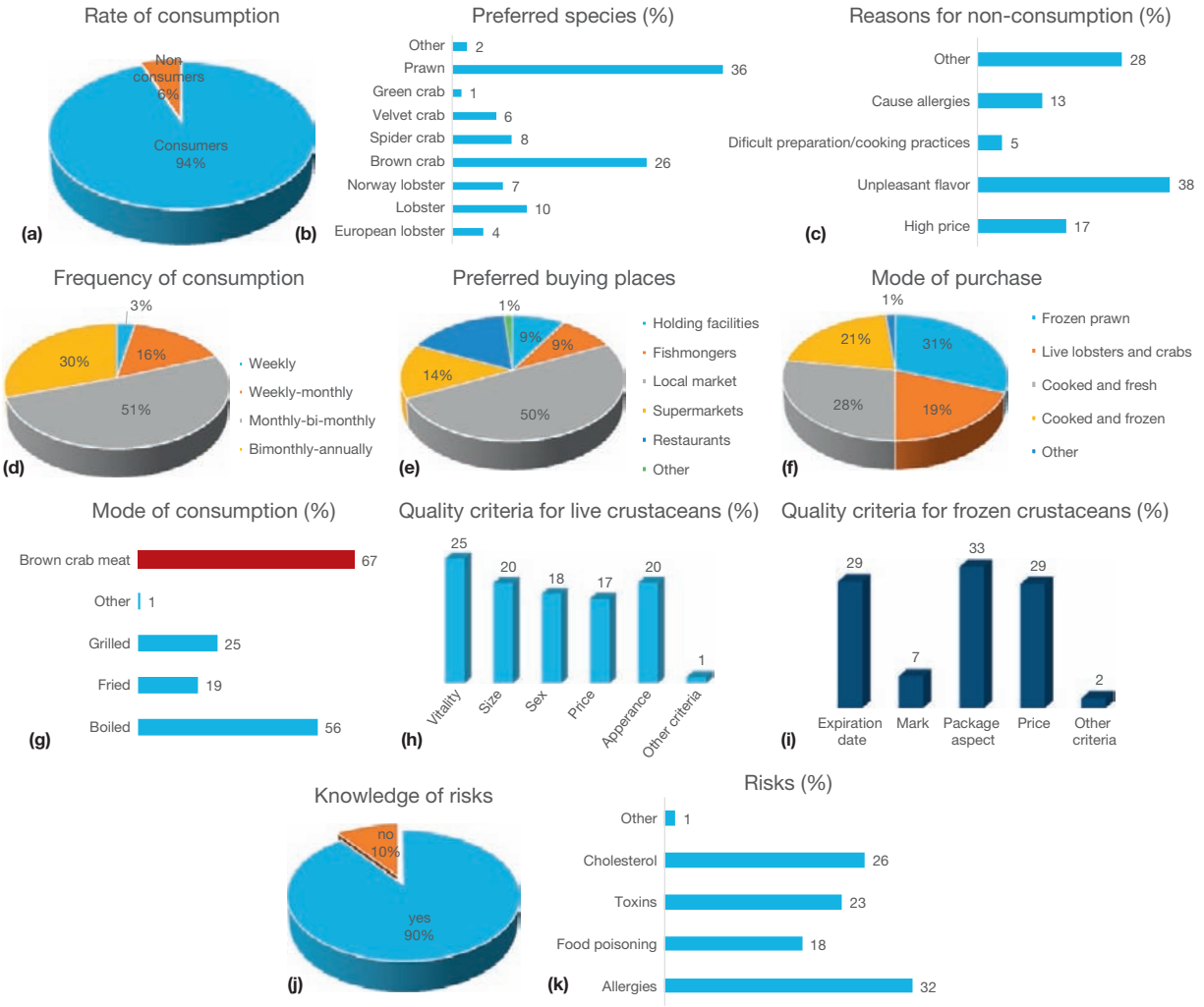

Figure 2 Consumers' patterns of crustaceans: Portugal as case study. Results from an online survey in 2009.

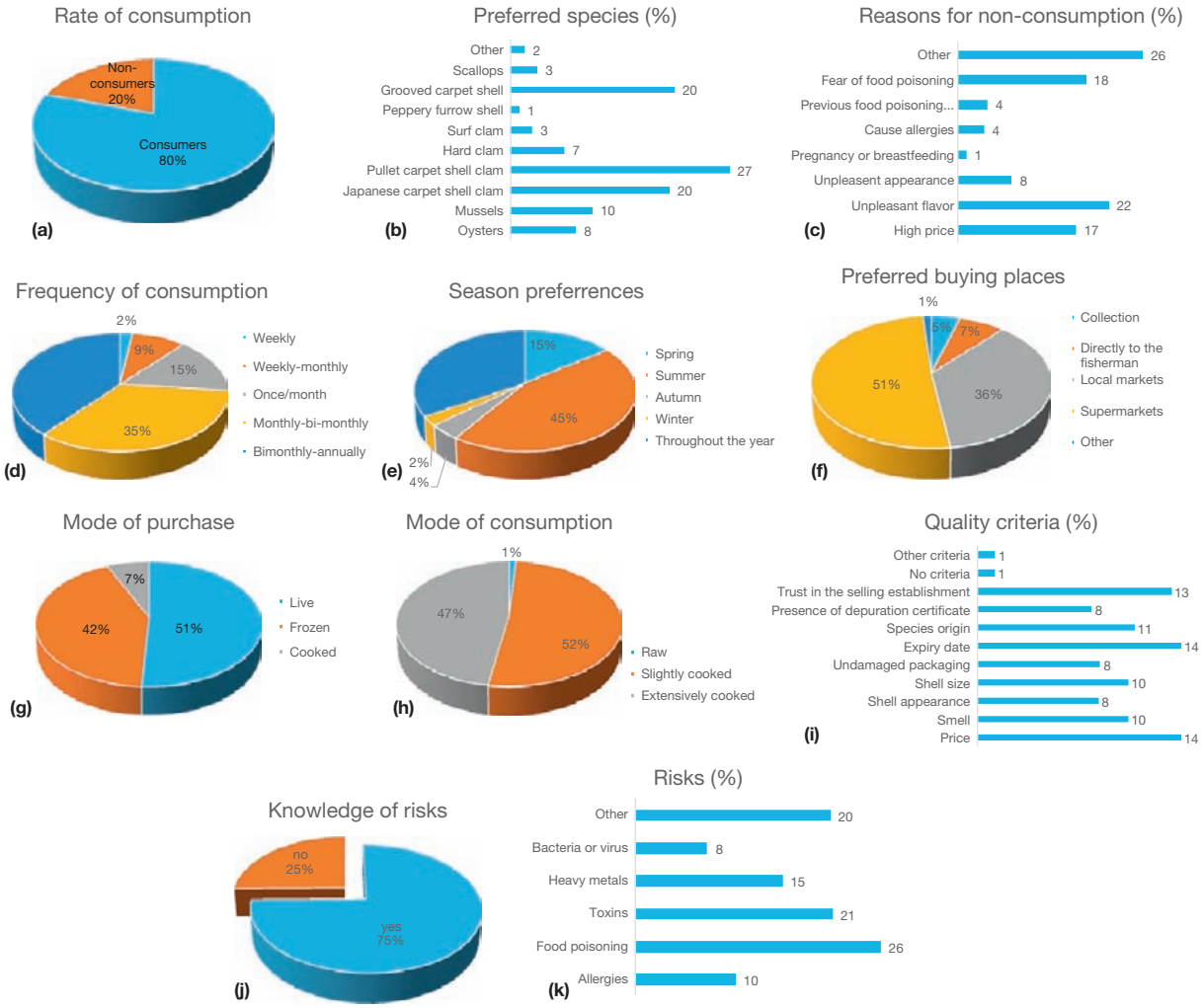

Figure 3 Consumers' patterns of bivalve mollusks: Portugal as case study. Results from an online survey in 2012.

Portuguese consumers are more exigent regarding the quality of shellfish. In this sense, the most important quality criteria for choosing live crustaceans are vitality, appearance, and size, accounting 64% of preferences, whereas frozen crustaceans are mainly chosen by consumers through package aspect, expiration date, and price, contributing with 91% of the preferences (Figure 2(h) and 2(i)). Concerning bivalves, price, expiry date, trust in the selling establishment, origin, size, and smell were the most crucial quality parameters for consumers, representing 73% of all items (Figure 3(i)).

In general, most consumers are aware of the hazards associated with the consumption of crustaceans (90%; Figure 2(j)) and bivalves (75%; Figure 3(j)), pointing out allergies and cholesterol as the highest hazards for crustaceans consumption (58%; Figure 2(k)) and food poisoning and toxins for bivalves (46%; Figure 3(k)). Even though consumers reveal, overall, a high level of hazards' awareness, inadequate practices often occur, such as harvesting/consuming shellfish from polluted areas, purchasing products directly to fisherman, performing short cooking procedures that do not effectively inactivate most pathogenic agents, and consuming bivalves with broken or closed shells. These practices may constitute a higher risk to human health than those highlighted by consumers, since there is a potential source of serious infections and diseases. Within crustaceans, crab species are particularly appreciated by Portuguese consumers, and their brown meat (BM) (i.e., hepatopancreas and gonads) is considered by most as a delicacy (67%). However, BM can pose serious risks to human health due to the high cadmium content.

Assessing consumer preferences is an excellent tool for stakeholders involved in the shellfish trade chain to adapt their products, processes, and practices according to consumers' requirements and preferences. Such understanding is also highly relevant to enable the evaluation of shellfish benefits and hazards for human health by health authorities and policy makers.

Hazards, Benefits, and Health Effects

Worldwide, consumers have been encouraged to increase seafood consumption due to several human health benefits of

Fat composition		Brown crab		Green crab		Spider crab		Velvet crab		European lobster		American lobster		Norway lobster	Spiny lobster	Prawn	Oyster	Mussel	Clam	Scallop
		C. pagurus		*C. maenas*		*Maja* spp.		*N. puber*		*H. gammarus*		*H. americanus*		*N. norvegicus*	*Palinurus* spp.	*Penaeus* spp.	*Crassostrea* spp.	*Mytilus* spp.	*Ruditapes* spp.	*Pecten* spp.
	Established values	WM	BM	WM	BM	WM	BM	WM	BM	WM	BM	WM	BM	WM	WM	WM	ET	ET	ET	ET
Lipids																				
Cholesterol(%)	RDI = 300 mg	13	39	13	33	12	24	15	42	11	20	13	31	23	44	43	17	9	15	28
EPA+DHA(%)	RDI = 500 mg	9	308	18	89	11	13	14	138	14	176	31	180	24	56	65	75	103	32	27
∑PUFA/∑SFA	RR>0.45	2.93	1.38	2.47	1.23	2.80	0.14	1.93	1.27	1.96	0.46	2.06	0.46	2.43	2.10	1.47	1.18	1.59	1.60	0.88
n–3PUFA/*n*–6PUFA	RR>0.25	3.76	3.11	6.30	3.50	5.24	2.50	4.80	3.60	4.12	2.57	5.84	3.08	9.93	0.83	2.34	2.83	8.62	3.97	5.27
AI	–	0.17	0.39	0.18	0.41	0.16	1.09	0.26	0.40	0.22	0.43	0.23	0.47	0.21	0.21	0.23	0.91	0.52	0.29	0.94
TI	–	0.12	0.23	0.12	0.24	0.12	1.24	0.17	0.24	0.16	0.46	0.14	0.38	0.11	0.20	0.18	0.28	0.17	0.21	0.42

WM: white meat; BM: brown meat; DHA: docoxahexaenoic acid; ET: edible tissue; EPA: eicosapentaenoic acid; SFA: saturated fatty acids; PUFA: polyunsaturated fatty acids; RDI: recommended daily intake; RR: Recommended ratio; AI: atherogenic index; TI: thrombogenic index

Figure 4 Fat and cholesterol composition (%) and indexes of bivalves and crustaceans taking into account a meal of 100 g.

Essential amino acids (%)		Brown crab		Green crab		Spider crab		Velvet crab		European lobster		American lobster		Norway lobster	Spiny lobster	Prawn	Oyster	Mussel	Clam	Scallop
		C. pagurus		*C. maenas*		*Maja* spp.		*N. puber*		*H. gammarus*		*H. americanus*		*N. norvegicus*	*Palinurus* spp.	*Penaeus* spp.	*Crassostrea* spp.	*Mytilus* spp.	*Ruditapes* spp.	*Pecten* spp.
	Established values	WM	BM	WM	BM	WM	BM	WM	BM	WM	BM	WM	BM	WM	WM	WM	ET	ET	ET	ET
Histidine	RDR= 15 mg g^{-1} p	174	163	112	110	85	97	140	990	149	155	163	162	121	129	248	142	112	171	117
Isoleucine	RDR= 31 mg g^{-1} p	127	136	118	113	144	100	172	99	130	129	163	135	112	162	426	142	140	171	136
Leucine	RDR= 59 mg g^{-1} p	107	109	100	86	111	75	131	84	113	92	135	96	114	132	288	126	114	130	119
Lysine	RDR= 45 mg g^{-1} p	109	108	124	72	136	49	172	88	136	103	150	90	159	194	71	165	168	190	169
Methionine+cysteine	RDR= 22 mg g^{-1} p	54	33	81	39	87	56	88	18	91	32	83	44	109	177	435	145	191	39	159
Phenylalanine+tyrosine	RDR= 38 mg g^{-1} p	186	220	156	134	184	131	204	130	191	184	209	170	176	115	560	168	177	202	169
Threonine	RDR= 23 mg g^{-1} p	234	262	180	147	205	143	196	215	170	236	186	176	146	169	254	185	183	223	178
Valine	RDR= 39 mg g^{-1} p	99	121	91	109	116	90	116	102	114	119	125	125	98	124	150	109	108	131	105

WM: white meat; BM: brown meat; p: protein; ET: edible tissue; RDR: reference daily requirement

Figure 5 Amino acid composition (%) of bivalves and crustaceans taking into account a meal of 100 g.

eating these products. Bivalves and crustaceans have a strong presence in the European gastronomy due to their sensory attributes greatly appreciated by consumers. Yet, safety and quality concerns regarding these products are often raised by consumers, partially due to the presence of contaminants and presumably high cholesterol levels. As filter-feeding species, bivalves can accumulate biological contaminants such as bacteria, viruses, and toxic microalgae, thus potentially representing a risk to consumers. Regular monitoring programs have been established in EU countries, banning the harvesting of bivalves whenever toxic microalgae blooms occur and, therefore, safeguarding consumer's safety. Sanitary control programs are also carried out regularly to monitor and classify bivalves' harvesting areas according to the levels of bacterial contamination, that is, category A (may be consumed immediately after harvesting), category B (may be consumed after depuration), category C (may be consumed after processing), and interdicted harvest. However, regardless of the area category, the consumption of raw bivalves is discouraged, and the duration of cooking procedures should be extended to ensure an effective inactivation of pathogenic agents, therefore avoiding health hazards to consumers. As discussed elsewhere in this Encyclopedia, bivalves' and crustaceans' white meat (WM) is considered lean products (<3%), and the consumption of 100 g of these products, generally, does not exceed 50% of the cholesterol recommended daily intake (see Figure 4).

On the other hand, these values can be much higher in crustaceans' BM. High shares of long-chain $n-3$ PUFAs, such as EPA and DHA, are one of the nutritional attributes of seafood, in general, as these fatty acids have proved to play a major role in the prevention and recovery from cardiovascular diseases, asthma, diabetes, arthritis, cancer, and fetal malformations. However, the consumption of 100 g of shellfish usually does not meet the recommended intake of 500 mg per day, except in mussels and some crustaceans' BM, which contain higher lipid contents. Considering the beneficial aspects of unsaturated lipids (monounsaturated fatty acids and PUFAs) in the prevention of cardiovascular diseases, opposed to the adverse effects of saturated ones (SFA), different quality indexes are used, such as $n-3$PUFA/$n-6$PUFA ratio, PUFA/SFA ratio, atherogenic index (AI), and thrombogenic index (TI). Despite the lower values in BM, shellfish products evidence balanced ratios of PUFA/SFA and $n-3$PUFA/$n-6$PUFA, as the minimum recommend values are exceeded for most species. Recommendations regarding AI and TI have not yet been proposed, though the values obtained with shellfish are similar to those found in other seafood products.

Bivalves and crustaceans contain adequate levels of high-quality protein, as 100 g meal composed of these species satisfies the adult daily requirements for most essential amino acids, with the exception of methionine and cysteine that are, usually, limiting amino acids in these species (Figure 5). Moreover, it should be highlighted that the high digestibility of shellfish proteins facilitates the absorption of essential amino acids during the human digestion process.

Shellfish are also important sources of macro and trace minerals, as they contain almost all essential minerals in their edible parts (Figure 6). Selenium, Cu, and Ca (in crustaceans' BM) are the most relevant minerals, since their concentrations often exceed the recommended daily allowances set for adults. Some shellfish species, such as oysters, crabs, and clawed and spiny lobsters, can also provide good intakes of Zn and Fe (Figure 6). As mentioned elsewhere in this Encyclopedia, Se levels are much higher in shellfish than in terrestrial animals, which is particularly beneficial for consumers since this essential mineral participates in the regulation of hormones, vitamin C, and other molecules and also acts as an antioxidant, protecting cells against oxidative stress. Despite that most minerals found in shellfish are required in several human metabolic processes, they must be uptake from the diet in

Essential and toxic elements (%)		Brown crab		Green crab		Spider crab		Velvet crab		European lobster		American lobster		Norway lobster	Spiny lobster	Prawn	Oyster	Mussel	Clam	Scallop
		C. pagurus		*C. maenas*		*Maja* spp.		*N. puber*		*H. gammarus*		*H. americanus*		*N. norvegicus*	*Palinurus* spp.	*Penaeus* spp.	*Crassostrea* spp.	*Mytilus* spp.	*Ruditapes* spp.	*Pecten* spp.
	Established values	WM	BM	WM	BM	WM	BM	WM	BM	WM	BM	WM	BM	WM	WM	WM	ET	ET	ET	ET
Ca	RDA = 800 mg (UL = 2500 mg)	3	149 (48)	15	70	10	49	6	72	6	4	6	4	9	6	3	34	5	6	4
Mg	RDA = 375 mg	5	12	11	14	16	16	11	7	8	–	–	–	11	11	10	6	9	27	13
Fe	RDA = 14 mg (UL = 45 mg)	2	30	4	21	8	122 (38)	2	7	1	11	1	21	3	9	9	123 (38)	13	61	23
Na	AI = 1500 mg (UL = 2300 mg)	16	18	9	22	26	24	27	13	36	–	–	–	30	12	–	7	19	16	20
K	RDA = 2000 mg	9	19	9	8	11	10	8	6	13	13	13	13	21	9	–	12	9	4	13
Mn	RDA = 2 mg (UL = 11 mg)	2	10	2	7	3	8	2	35	2	–	–	–	5	1	3	30	17	33	0
Cu	RDA = 1 mg (UL = 10 mg)	80	730 (73)	40	78	119 (12)	452 (45)	50	300 (30)	100 (10)	2700 (270)	100 (10)	1270 (127)	250 (25)	40	40	210 (21)	14	18	20
Zn	RDA = 10 mg (UL = 40 mg)	56	44	43	17	63	58	29	22	26	41	30	30	45	57	15	168 (42)	13	21	8
Se	RDA = 0.055 mg (UL = 0.4 mg)	167 (23)	545 (75)	73	91	167 (23)	309 (43)	36	73	109 (15)	182 (25)	164 (23)	364 (50)	–	91	–	127 (18)	76	36	73
Methyl-Hg	TWI=1.3 μg kg^{-1} bw	17.3	11.9	19.5	13.0	10.8	10.8	27.1	36.8	15.2	9.8	10.8	14.1	43.3	3.3	28.2	4.3	2.2	2.2	–
Cd	TWI=2.5 μg kg^{-1} bw	5.6	429.3	2.8	56.3	0.0	118.3	2.8	78.9	1.1	619.7	1.1	619.7	5.6	–	7.3	16.9	7.3	1.1	–

–Lack of data; WM: white meat; BM: brown meat; bw: body weight; p: protein; ET: edible tissue; RDI: RDA: recommended daily allowance; AI: adequate intake; UL: upper intake limit; TWI: tolerable weekly intake

Figure 6 Essential and toxic elements composition (%) of bivalves and crustaceans taking into account a meal of 100 g.

balanced doses, as high intakes can become toxic. For example, Cu upper intake level (the highest level of daily intake that is likely to pose no risk of adverse health effects to almost all individuals in a population) is exceeded when considering the consumption of 100 g of clawed lobsters' BM. Some adverse effects of high Cu intakes are gastrointestinal distress and liver damage. Toxic metals can also be found in shellfish as discussed previously. In order to regulate consumers' exposure to toxic elements, the European Food Safety Authority has established a tolerable weekly intake (TWI) of 2.5 μg kg^{-1} body weight (BW) for Cd and 1.3 μg kg^{-1} BW for the most toxic form of Hg (methyl Hg). According to the low Hg levels in shellfish, even if considered that 100% of the total Hg content in these species is in the methyl Hg form, the consumption of a shellfish meal (100 g) would place adult consumers (with 71 kg of average BW) well below the methyl Hg TWI. In contrast, the extremely high levels of Cd found in crabs and clawed lobsters' BM can represent a significant risk for consumers, since more than 100% of TWI for this contaminant is reached in a single meal (100 g). Nephrotoxicity, osteoporosis, carcinogenicity and genotoxicity, teratogenicity, and endocrine and reproductive disruptions are some of the adverse effects associated with high Cd intakes.

Overall, the potential hazards related to the consumption of shellfish are, largely, outweighed by the benefits, namely, considering the importance that these products have in the gastronomic traditions and consumers' social habits, holy days, and festivities. Nonetheless, bivalves' and crustaceans' WM should be preferred by consumers, whereas the consumption of BM should be moderated to avoid excessive intakes of cholesterol, Cu, and Cd.

See also: Cadmium: Toxicology; Calcium: Physiology; Cholesterol: Absorption, Function and Metabolism; Dietary Exposure Assessment; Heavy Metal Toxicology; Iron: Physiology of Iron; Potassium: Physiology; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Risk Assessment of Foods and Chemicals in Foods; Shellfish: Characteristics of Crustaceans and Mollusks; Toxins in Food: Naturally Occurring; Zinc: Physiology and Health Effects.

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Sherry Wines: Manufacture, Composition and Analysis

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Background

Sherry wines are among the most distinctive Spanish wines, mainly produced in southern Spain (particularly in Jerez and Montilla-Moriles regions). France (Jura), Italy (Sardinia and Sicily), the United States (California), and various South African and Australian regions are some of the other foremost producers of sherry-like wines in the world, and the quality of these wines is highly regarded. By international legal agreement, the name 'sherry' and some of the technical/style names associated are only accepted for producers operating within DO (Origin Denomination) Jerez.

Palomino Fino and Pedro Ximenez grape varieties are used to obtain high-quality base wines for sherry wine production. The main types of sherry wines are the Fino, Oloroso, and Amontillado, which are obtained from the same base wine but subjected to different aging procedures. Fino wines are obtained by biological aging under the action of *flor* yeast growing on the wine surface, while Oloroso wines are exclusively obtained by oxidative aging after fortification with ethanol to avoid the growth of *flor* yeast. Amontillado wines are produced by two consecutive biological and oxidative aging steps; therefore, they have chemical and sensory characteristics between Fino and Oloroso sherries. Other types of sherry wines are the darkest and sweetest Pedro Ximenez styles, where ripe grapes are further concentrated by raising on mats prior to fortification and barrel maturation.

The biological aging of sherry wines (Jerez-Xérèz-Sherry, Spain) is a unique and well established winemaking practice to obtain high-quality wines. It is mainly based on a microbiological process that occurs under a biofilm of a *Saccharomyces* yeast strain called the *flor* velum yeast. According to the industrial process, after the must fermentation, the young wine is fortified with wine alcohol to 15% (v/v) and is then clarified and transferred into oak casks for storage. Wine aging occurs in the *solera* and *criaderas* system under the *flor* film of yeast. During this period, a thick layer of *flor* yeast (1–3 cm) develops naturally on the surface of the wine in contact with air. The velum, which isolates and protects the wine from excess oxidation, is the origin of complex biochemical reactions that result from the oxidative metabolism of the *flor* yeasts and the reducing environment created in the wine.

Manufacturing Process for Sherry Wines

Fino wines are among the most characteristic sherry types. They are biologically aged following a unique process consisting of two consecutive steps. The first consists of grape must fermentation, which produces a 'young' wine using fermentative yeasts. Once a high-quality wine is obtained, a postfermentative treatment, in which this young wine is fortified with wine alcohol to approximately 15.0–15.5% (v/v ethanol), is carried out. This operation is known as *encabezado*. In Montilla-Moriles, no fortification is required due to the natural sugar content of musts, making it possible to reach naturally an adequate alcohol content (15% v/v).

Flor yeasts that grow in wine with a high ethanol content adapt to these conditions by forming a *flor* film (velum) on their surface, and their metabolism becomes oxidative. In the production of sherry wines, a few months after alcoholic fermentation has finished, the wine is transferred and yeast lees are removed. In the area of Jerez (Southern Spain), the wine is stored before starting the biological aging process. During the intervening time, it undergoes malolactic fermentation and spontaneously develops a yeast film. Wine stored in this way is referred to as *sobretablas* wine.

Biological aging takes place in American oak casks of variable capacity, depending on their position in the aging system, by a dynamic process called *criaderas and solera* (Figure 1). This involves stacking the casks in rows called *criaderas* (scales) in such a way that all casks in a row contain wine of the same type and are aged for an identical length of time. The casks are filled to four-fifths of their capacity in order to allow a biofilm of *flor* yeasts to develop on the wine surface. A series of casks hold wine in the process of maturing; the casks are arranged to facilitate a progressive, fractional blending. The row standing on the floor, which is called the *solera*, contains the oldest wine in the system. It is from this row that the commercial wine is withdrawn for bottling extraction, and never exceeds 40% of the cask contents per year. This process is carried out three or four times each year. The amount of wine extracted from the *solera* is replaced with an identical volume of wine from the upper row, which is called the first *criadera*. Likewise, the amount extracted from the first *criadera* is replaced with wine from the next row (the second *criadera*), and so on (Figure 1). Finally, the topmost *criadera*, which contains the youngest wine, is replaced with *sobretablas* wine. The number of scales typically ranges from 4 to 6; usually, the greater the number, the higher the quality of the final wine.

The *rocío* operation consists in the transference of wine from one scale to the next. The wine extracted from the casks in a scale is homogenized in a tank and then transferred to the casks in the next (older) row. This operation must be carefully performed in order to not disrupt the *flor* film on the wine surface. In addition, the *rocío* favors the adequate supplementation of nutrients from the young to the old wine, which in turn is required to form and maintain the yeast film. In addition, this process provides aeration, which is highly beneficial for the wine and *flor* yeasts. This dynamic process allows wine of similar sensory characteristics to be obtained year after year, irrespective of the particular vintage.

The Microbiological Process in the Biological Aging of Sherry Wines

The restrictive conditions of the biological aging process (low pH, presence of sulfite, high ethanol and acetaldehyde

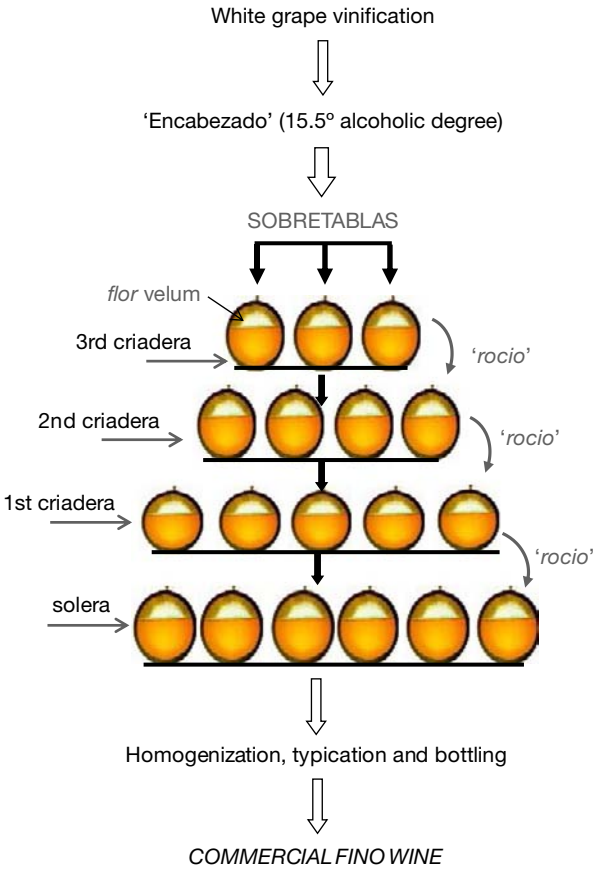

Figure 1 Main steps in the biological aging of sherry wines.

concentrations, lack of sugars, and low oxygen concentration) are compatible with only a few *Saccharomyces cerevisiae* races; therefore, more than 95% of the film microbiota usually consists of film-forming *S. cerevisiae*. Physiological and molecular characterization of the yeasts present in the velum of sherry wines has shown that most of them belong to different races of *S. cerevisiae*, mainly *beticus*, *cheresiensis*, *montuliensis*, and *rouxii*. These *flor* yeasts differ physiologically from the typical fermentative yeasts (which are unable to grow aerobically in wine), and they have different metabolic and genetic characteristic.

By using restriction mitochondrial DNA (mtDNA) analysis, it has been possible to study the dynamics of these yeast strains during specific steps or during the whole process of sherry winemaking. In these studies, it has been shown that other microbial populations, such as lactic acid bacteria (e.g., *Lactobacillus plantarum*, *L. casei*, *L. brevis*, and *L. Zeae*) and other *non-Saccharomyces* yeasts (e.g., *Debaryomyces*, *Zygosaccharomyces*, *Pichia*, *Hansenula*, and *Candida*), can coexist with the velum yeast. Moreover, species of the genera *Dekkera* and *Brettanomyces* have been related with an abnormally higher acidity in the casks containing biologically aged wines.

In recent years, genetic improvement in *flor* yeasts, such as studies on the genes responsible for flocculation, such as *FLO11*, have lead to advances in cell-immobilization technology. In 2006, a *S. cerevisiae* strain var. *capensis* was successfully coimmobilized with *Penicillium chrysogenum* in order to obtain biocapsules for potential use in a number of fermentation processes. On the other hand, the relatively large aging time necessary to obtain high-quality wines (4–5 years for Fino and more than ten for Amontillado wines) has promoted new research. Consequently, it has been shown that the expression of genes such as *SOD1* (superoxide dismutase 1) may facilitate the establishment of a more stable velum and a reduction in aging times. Likewise, the characterization of cell wall proteins and the encoding genes of *S. cerevisiae* and its relevance to *flor* formation have also been recently explored.

Analysis and Chemical Composition of Sherry Wines: Evolution During Biological and Oxidative Aging and Consequences for Wine Quality

The different production conditions involved in the manufacturing of the main types of sherry wines (Fino, Oloroso, and Amontillado) determine their differences in chemical composition and, therefore, in sensory characteristics. Some of the most important changes in chemical composition during the biological and/or the oxidative aging of sherry wines are revised as follows.

Polyphenols

Color is one of the most important characteristics of sherry wines, and the content and type of phenolic compounds are close behind. The type and amount of these compounds largely depend on the grape variety and winemaking technology employed in their production. For instance, the characteristic and appropriate pale yellow color of Fino wines is determined by the exclusively biological aging process. It has been suggested that *flor* yeast growing on the surface of the wines protects wines against oxidation because it minimizes oxygen diffusion into the wine, preventing the formation of browning pigments. In addition, *flor* yeasts have the ability to retain brown pigments produced during oxidation. After bottling, Fino wine tends to get browner, lowering its quality. This is one of the main sources of economical loss for producers of pale sherry wines. This is a natural process that inevitably occurs to some extent during bottled storage. In order to extend the commercial life of these wines and keep their pale color, different strategies have been evaluated. Some viticultural practices such as harvesting grapes during the night, or hyperoxidating musts in order to reduce the initial concentration of phenolic compounds, have been suggested. To avoid or minimize changes in color after bottling, the use of PVPP or activated charcoal is often employed in sherry production. In addition, the use of yeasts to retain previously formed browning compounds or specific yeast strains to decrease browning rate by inhibiting certain enzymatic reactions involved in the formation of colored compounds have been suggested. Another essayed strategy is the use of immobilized yeast in k-carragenate. It has been shown to be an effective alternative to the use of synthetic products to reduce oxidation in sherry wines, and is adequate for the production of organic wines.

In the absence of biological aging, Oloroso-type sherries are exclusively subjected to oxidative aging. Their high content in ethanol (between 18 and 20 °C) does not allow yeasts to grow.

They are submitted to extended dynamic oxidation, which gives these wines very particular organoleptic characteristics. Wood allows the slow diffusion of atmospheric oxygen into casks, which in the absence of *flor* yeast causes a slow oxidation of wine phenolic compounds. As a consequence, a progressive darker color develops, with mahogany cues. Particularly, the flavan-3-ol monomers and oligomers have been described as good substrates for browning by different chemical pathways. Basically, the oxidation of phenolic compounds produces quinones, which by polymerization lead to compounds showing a reddish-brown color. The rate of this process in the cellar depends on various factors, including the acidity of the wine, its SO_2 content, the presence of metals (Fe), the amount of available O_2, and the temperature. As aging progresses, the polyphenol oxidation reactions progress, thus increasing the dark color of the wine. On the other hand, during oxidative aging, phenols undergo oxidation and polymerization reactions, thereby decreasing the content of those compounds of low molecular weight. Brown color can also increase by condensation reactions of some polyphenols, such as flavan-3-ol monomers and oligomers with acetaldehyde. Moreover, flavanols are implied in the progression of browning via a condensation–oxidation process involving glyoxylic acid, which is formed by oxidation of tartaric acid in the presence of some metals. In addition, the concentration of some other phenolic compounds increases during prolonged aging because of the extraction of some compounds from the wood. Their nature depends on the type of wood, aging temperature, and so on. The length of time that wines spend in wood has been recently shown to be directly related to the antioxidant activity of these wines. However, it has also been suggested that the browning of the wines is more strongly dependent on the aging temperature than on the phenolic compounds supplied by the cask wood. The hydrolysis of oligomers, in particular the flavan-3-ol derivatives fraction, and the loss of water and ethanol through the wood and/or by evaporation might also explain the increase in the concentration of phenolic compounds during oxidative aging.

Because the Amontillado sherry type is produced by a two-stage aging process, combining a biological and an oxidative process, these wines exhibit a phenolic composition between Fino and Oloroso sherry wines.

Common to any wine, the analysis of sherry wine polyphenols requires a previous extraction step, usually performed by using C18 resins, and a further analysis with high-performance liquid chromatography. Normally, detection is performed using diode-array detectors (DADs), although currently mass spectrometers (MS) are widely employed. By using these techniques, it has been shown that the different sherry winemaking conditions generate a very different phenolic profile. For instance, in Fino sherry wines, the phenolic aldehydes typically associated with wines aged in wood are very low or almost absent. In addition, they do not experience great changes during the aging process. The only phenolic compounds that increase in these wines during aging are the benzoic acids, due to the contribution made by lignin and by the deamination of nitrogen compounds produced during the autolysis of the *flor* yeast. Benzoic and cinnamic acids are present throughout oxidative aging in Oloroso sherries. However, some other phenolic acids, such as gallic, syringic, and caffeic, experience greater changes during aging. In addition, the content of esterified derivatives is lower than in Fino sherries. Oloroso wines are also characterized by a high content of 5-(hydroxymethyl)-2-furaldehyde (HMF), mainly occurring at the end of the aging process. Amontillado sherries are characterized by a phenolic composition between Fino and Oloroso wines. Wines from the youngest scale of the *criadera* system (scale $n°7$) present a very similar profile to the samples of Fino wine taken from its final scale. However, in subsequent scales, their behavior becomes more similar to that of Oloroso wines than to Fino wines. In these wines, some aldehydes such as vanillin and p-hydroxybenzaldehyde show considerable increases during aging, whereas syringaldehyde remains constant. In addition, HMF shows a considerably increase during aging.

Volatile Compounds

Volatile compounds are responsible for the aroma of wines, and are therefore directly linked to wine quality and consumer preference. Similar to polyphenols, the different sherry wine production technologies produce wines with very different volatile compositions and sensory characteristics. In general, because of their low concentration, different types of extraction and concentration techniques are required prior to their analysis. Most of these techniques involve the use of organic solvents and/or the use of absorbent polymers, and further steps of concentration. The use of headspace solid-phase microextraction has been shown to provide good selectivity, and sensitivity avoiding the concentration steps. In any case, gas chromatography (GC) is the technique used for the analysis of volatile compounds. The use of GC coupled to flame ionization detectors or MS has provided a valuable tool for the characterization of the volatility profile of sherry.

Ethanol is the main volatile compound produced during yeast fermentation from grape sugars. After water, ethanol is the major component of wines. Its content is highly variable across wines, depending on the sugar content of the must and the winemaking technology involved in production. In the case of sherry wines and other fortified wines, its content ranges between 15% (in Fino wines) and 18–21% (in the case of Oloroso wines). Ethanol might have an impact on the sensory characteristics of wines because it occurs in sherry wines above its perception threshold. During the biological aging of sherry wines, the concentration of ethanol decreases because of its consumption by *flor* yeast. It is used as the main source of carbon and energy, and other secondary metabolites such as acetaldehyde (the main subproduct), acetic acid, butanediol, diacetyl, and acetoin. Ethanol can be also metabolized via the tricarboxylic acid pathway, and can be lost by evaporation through the oak cask.

Acetaldehyde constitutes one of the most important compounds produced during biological aging. Besides its contribution to the ethereal and overripe apple notes, it is responsible for the pungent aroma of Fino sherry wines. The acetaldehyde content allows the differentiation of Fino wines from other types of sherry wines. Its concentration can reach values between 350 and 450 mg l^{-1} and occasionally of 1000 mg l^{-1}. This compound is produced by *flor* yeast, mainly

from the oxidation of ethanol by alcohol deshydrogenase II (ADH II), which produces NADH. This enzyme is repressed by glucose. The acetaldehyde content increases with aging, although the most important changes occur in the scales containing the youngest wine, due to the intense metabolism of *flor* yeast. It also takes part in different reactions during biological aging, such as its combination with ethanol to produce 1,1-diethoxyethane. It can occur in wines at concentrations above 100 mg l^{-1}. This acetal has been shown to contribute to wine aroma, giving fresh, fruity, and green aromatic notes. Acetaldehyde is also involved in the formation of other aroma compounds, such as acetoin, 2,3-butanodiol.

It has been shown that *flor* yeasts increase the content of other aroma compounds, such as higher alcohols, ethyl esters, lactones, and terpenes. For instance, higher alcohols are very important contributors to the aroma of Fino wines, although the concentration of most of them (e.g., isobutanol, 2-phenylethanol, isoamylic alcohols) is quite stable through aging. One exception is propanol, which can dramatically increase during aging. The biosynthesis of higher alcohols is mainly produced in the fourth, third, and second *criaderas* from their corresponding amino acids. This is coincident with the maximum yeast activity. In addition, it has been suggested that their production can increase due to yeast autolysis.

Regarding esters, their concentration depends on the synthesis and hydrolysis reactions accounted for during aging, but also on the enzymatic activity of yeasts. These depend on the type of yeast, physiological state, and other factors. Many of these compounds contribute to fruity aroma notes. In general, the concentration of higher alcohol acetates decreases through hydrolysis over the first few months of aging, while ethyl esters of organic acids (lactic, succinic) increase.

Sotolon is an important lactone produced during the biological aging of Fino wines, but also is important in wines produced under oxidative aging conditions. This compound is produced from the aldolization reaction between α-ketobutyric acid (from the deamination of L-threonine by *flor* yeast) and acetaldehyde. Because of its low perception threshold (10 μg l^{-1}), this compound has been described as an important odor impact compound, with nut, curry, and candy cotton notes, in both biologically aged sherry wines and those produced under oxidative aging conditions. Its concentration in sherry wines depends on the duration of aging, but it normally occurs at concentrations above 200 μg l^{-1}. Recently, by using GC-MS and GC-olfactometry (GC-O), an ethyl analog of sotolon, identified as abhexonm (5-ethyl-3-hydroxy-4-methyl-5(*H*)-furan-2-one), has been associated with curry/spicy/honey nuances. It is also associated with two theaspirane oxidation products: dihydrodehydro-β-ionone with dried/fruit/Sauternes-like odors, and 4-hydroxy-7,8-dihydro-β-ionone. The latter exhibits a strong grenadine-like odor. They have also been identified in Jura Flor sherry wines ('yellow wines').

Other lactones detected in these wines include α-butyrolactone and pantonolactona (2,4-hydroxy-3,3-dimethyl-butyrolactone). They are typical of sherry wines. Besides the duration of aging, their concentration is closely linked with the yeast strain. Solerone (4-acetyl-γ-butyrolactone) was once believed to be an important contributor to biological aged sherry wines; however, its impact on wine aroma has been shown to be very low. Biologically aged wines are aged in oak casks that are rarely emptied or cleansed; therefore, the wines extract a few lactones from wood. It is only possible to find them in the scales containing the oldest wine.

Compounds released by the casks used during sherry wine aging can also be important contributors to the aroma of biological-aged sherry. They are absolutely essential when explaining the aroma of oxidative sherry wines (Oloroso). For example, some lactones such as *Z*-whisky lactone, also known as wood lactone, contributes to vanilla notes. Other compounds, such as the phenols eugenol and 4-ethylguaiacol, contribute to the spice aroma of clove. They are derived from precursors extracted by ethanol from the casks, and their concentrations increase with increasing wine wood contact time. The type of wood, the ethanol content of the wine, and the temperature of the cellar are the main factors that influence the efficiency of extraction in all type of wines.

The odor activity value (OAV) can be used to determine the odor impact of a volatile compound. It is calculated as the ratio of odorant concentration to odor threshold. From a sensorial point of view, by using the OAVs and odor descriptors, the sensory profile between the three main types of sherry wines was assessed (Table 1). Fino wines have a markedly floral and fruity character (because of the presence of compounds such as farnesol, β-citronellol, and β-ionone), as well as cheesy, rancid (butanoic acid), and pungent (acetaldehyde) aromatic notes. Oloroso wines exhibit smoky and ethereal notes associated to compounds such as ethylguayacol and ethyl acetate, respectively, whereas Amontillado wines are characterized by the presence of flavor notes from both aging processes, having the most complex aroma of the three.

Other Compounds

Organic Acids

In general, the content of organic acids decreases during biological aging. Prior to the incorporation into the biological aging system, wine is stored after its fortification with ethanol, and it is during this period that it undergoes malolactic fermentation. Therefore, most of the malic acid is converted into lactic acid. In addition, lactic and acetic acids can be metabolized by *flor* yeasts and converted into acetaldehyde and other by-products. In general, the climatic conditions in the southern regions of Spain where these wines are produced (Jerez and Montilla-Moriles) result in musts with low acidity (4–4.5 g l^{-1}); therefore, addition of tartaric acid is common during winemaking. Afterward, the concentration of tartaric acid decreases during biological and chemical aging due to its precipitation as potassium bitartrate.

Other acids, such as gluconic acid in sherries, can be used as a measure of the degree of grape rot; a concentration below 1 g l^{-1} shows wines suitable for biological aging. *Flor* yeast can use gluconic acid without provoking changes in the sensory quality of the wines. Acetic acid might be produced by yeast during fermentation, although its concentration is usually below 0.7 g l^{-1}. In addition, this acid is metabolized by *flor* yeast during biological aging, by incorporation via Acetyl-CoA into the Krebs cycle, or used in the synthesis of fatty acids.

Table 1 Perception threshold (mg l^{-1}), odor descriptors of the aroma compounds, odorant series in which they were grouped, and odor activity values (OAVs) calculated for the active odorant compounds identified in the three types of sherry wines

				Wine				
				Amontillado	*Biologically aged (Fino)*		*Chemically aged (Oloroso)*	
Compound	*Threshold*	*Odor descriptors*	*Odorant series*	*OAV*	*OAV*	*SL*	*OAV*	*SL*
Sotolon	0.005	Walnut, curry	Nutty, spicy	92 ± 8	40 ± 4	***	55 ± 4	***
Eugenol	0.005	Cinnamon, clove	Spicy	86 ± 13	130 ± 11	***	19 ± 6	***
Ethyl isobutanoate	0.015	Strawberry, melon	Fruity	36 ± 8	79 ± 12	***	30 ± 6	ns
Ethyl 3-hydroxybutanoate	67	Grape, marshmallow	Fruity, floral	27 ± 4	18 ± 3	***	31 ± 8	ns
Ethyl octanoate	0.002	Pineapple, pear, soapy	Fruity, fatty	26 ± 6	32 ± 10	ns	nd	***
1,1-Diethoxyethane	1	Green fruit, licorice	Fruity, balsamic	21 ± 10	60 ± 13	***	51 ± 15	***
Acetaldehyde	10	Overripe apple	Fruity	19 ± 6	56 ± 5	***	12 ± 2	ns
Ethyl hexanoate	0.005	Banana, green apple	Fruity	13 ± 4	20 ± 4	ns	17 ± 2	ns
β-Citronellol	0.1	Rose	Floral	9 ± 2	13 ± 4	ns	nd	***
Z-Oak lactone	0.035	Coconut, vanilla	Fruity, spicy	7 ± 3	4 ± 2	ns	5.7 ± 0.8	ns
Ethyl acetate	7.5	Pineapple, varnish, balsamic	Fruity, chemical, balsamic	6 ± 1	1.8 ± 0.7	***	25 ± 7	***
Isoamyl alcohols	30	Alcohol, nail polish	Chemical	6.0 ± 0.8	9 ± 2	***	10 ± 2	***
4-Ethylguaiacol	0.046	Clove	Spicy	3.4 ± 0.4	3.0 ± 0.8	ns	5 ± 1	***
2-Phenylethanol	10	Rose, honey	Floral	2.6 ± 0.6	4 ± 1	ns	3.6 ± 0.5	ns
Ethyl lactate	100	Strawberry, raspberry, buttery	Fruity, fatty	1.9 ± 0.6	1.17 ± 0.06	***	3.1 ± 0.8	ns
3-Methylbutanoic acid	3	Parmesan cheese, rancid	Fatty	1.0 ± 0.3	2.5 ± 0.8	***	nd	***

The OAV data are the average of nine values.

***, >99%; SL, significance level versus Amontillado wine; ns, <99%; nd, not detected.

Data are from Zea, L., Moyano, L. and Medina, M. (2008). Odorant active compounds in Amontillado wines obtained by combination of two consecutive ageing processes. *European Food Research and Technology* **227**, 1687–1692.

Glycerol

Glycerol is also one of the most abundant components of sherry wines. It can contribute directly to flavor perception because of its sweet taste. It also imparts viscosity to the wine. Therefore, it is involved in mass transfer phenomena and aroma release. It is mainly produced during a glycerol-pyruvic fermentation at the beginning of the alcoholic fermentation. *Flor* yeast can use it as a carbon source; therefore, its concentration decreases during wine aging. This could be useful as an indicator of wine age.

Nitrogen-containing Compounds

Nitrogen-containing compounds are important not only to yeast growth and metabolism, but are also known to be involved in the production of sluggish or stuck fermentations. *S. cerevisiae* can grow in a wide variety of nitrogen-containing substrates. The rate of consumption and the metabolism of these compounds largely depend on the yeast strain, its physiological state, and the physicochemical properties of the wine. *S. cerevisiae* can use amino acids, either in the biosynthesis of proteins, or as a nitrogen source. Amino acids are degraded by the cells, and the nitrogen that they contain is released (generally as ammonia). It is used it for the synthesis of other nitrogenous constituents. The carbon of the amino acids might also be used by the yeast for synthetic purposes. In this case, the compound acts as a carbon source that can be excreted into the medium. The biological aging of sherry wines reduces the content of amino acids and other nitrogenous wine components (ammonium and urea). The main source of nitrogen for yeasts during biological aging is L-proline, although yeasts differ in the amount of assimilable nitrogen they can use and have preferences for amino acid consumption. It has been shown that short aeration, used in accelerated aging conditions, did not increase the overall consumption of assimilable nitrogen. However, it accelerated the consumption of particular nitrogen compounds, such as L-proline, L-tryptophan, L-glutamic acid, ammonium ion, L-lysine, and L-arginine. Besides the use of amino acids as a nitrogen source, *flor* yeasts may use these compounds to balance the oxidation–reduction potential under conditions of restricted oxygen. This involves releasing some amino acids into the medium to restore the intracellular redox balance, by means of oxidizing the excess NADH.

See also: Alcohol: Properties and Determination; Grapes; Wines: Champagne and Sparkling Wines – Production and Effervescence; Wines: Madeira, Port and Sherry Fortified Wines – The *Sui Generis* and Notable Peculiarities. Major Differences and Chemical Patterns; Wines: Types of Table Wines; Wines: Wine Production; Wines: Wine Tasting.

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Shigella

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Historical Background

The first full description of shigellae is attributed to Kiyoshi Shiga (1898) following a large dysentery epidemic in Japan in 1897 that affected over 91 000 people, caused by *Bacillus dysenteriae* (now called *Shigella dysenteriae* serotype 1). Following work carried out over the next two decades, Murray (1918) proposed a classification for *Bacillus dysenteriae* that included four groups: Shiga, Kruse, Schmitz, and Flexner that later became *S. dysenteriae* serotype 1, *S. dysenteriae* other than serotype 1, *S. sonnei*, and *S. flexneri*, respectively, in the modern classification system. *S. boydii* was first described in 1938. *S. dysenteriae*, *S. sonnei*, and *S. boydii* had simple antigenic structures and were easily differentiated from each other, whereas the Flexner group had complex antigenic structures and differentiation was difficult.

The 1914–18 war was a key driver of research into bacillary dysentery and gastrointestinal symptoms caused by *Shigella* species, which continued to be a major concern for military personnel operating in high-risk regions, such as Africa, the Middle East, and the Indian subcontinent. Away from the military setting, shigellosis was endemic in both developed and developing countries throughout the twentieth century. During this time, *S. sonnei* and *S. flexneri* were responsible for most cases of endemic shigellosis in northwest Europe and North America, with outbreaks of *S. sonnei* traditionally associated with nursery schools and day-care centers. More recently, the majority of *Shigella* infections (and almost all *S. dysenteriae* and *S. boydii* infections) in developed countries are associated with cases reporting recent travel abroad. Nevertheless, outbreaks still occur in community settings mainly linked to person-to-person spread and foodborne transmission. Diarrhea caused by *Shigella* continues to be a major cause of morbidity and mortality in developing countries, particularly in children under 5 years old.

The Pathogen

Bacteria belonging to the genus *Shigella* in the Enterobacteriaceae family are small, unencapsulated, nonmotile, nonsporulating, facultative anaerobic Gram-negative bacilli and are part of the genomic species *Escherichia coli* (with the exception of *S. boydii* serotype 13).

In the contemporary classification scheme (Enterobacteriaceae Subcommittee Reports, 1954), the genus *Shigella* is divided into four subgroups based on the following biochemical and serological criteria:

- Group A, *S. dysenteriae*: all types that do not ferment lactose or mannitol (at least 15 serotypes)
- Group B, *S. flexneri*: all types that ferment mannitol but not lactose and possess type-specific antigens and group antigens (at least 15 serotypes)
- Group C, *S. boydii*: all types that ferment mannitol but not lactose and possess type-specific antigens but none of the antigens of *S. flexneri* (at least 18 serotypes)
- Group D, *S. sonnei*: all types that ferment mannitol and lactose (one serotype)

Incidence

A large proportion of all diarrheal episodes, an estimated 167 million worldwide including over a million deaths annually, are attributed to *Shigella*, with the greatest burden occurring in resource-poor countries in children under 5 years old. The Global Enteric Multicenter Study (GEMS) to identify the etiology and population-based burden of pediatric diarrheal disease in sub-Saharan Africa and South Asia found *Shigella* to be one of the four most common causes of moderate-to-severe diarrhea in this setting.

In the United States, between 14 000 and 22 000 laboratory-confirmed cases of shigellosis are reported each year with a population-based incidence of 3.99 per 100 000, although the true burden is likely to be nine times that number. Similarly, in the United Kingdom, the annual incidence of *Shigella* infection is estimated to be around 30 per 100 000, although recent studies show that only 2.8 per 100 000 are reported based on cultures submitted to Public Health England (PHE). Recent studies in Africa and Asia estimate 30.4 million diarrheal episodes (5308 deaths) in Africa and 58.1 million diarrheal episodes (10 158 deaths) in Southeast Asia due to shigellae in 2010. In Argentina, the incidence is 187 cases/year/100 000 children under 15 years old, and in Israel, the average annual incidence of culture-proved shigellosis is 97/100 000.

Globally, *S. dysenteriae* and *S. boydii* are less common than *S. sonnei* and *S. flexneri* in industrialized countries and are isolated mainly from travelers returning from abroad. *S. sonnei* is the dominant species of *Shigella* in industrialized countries. In England, *S. sonnei* is the most commonly isolated *Shigella* species (50%) with *S. flexneri*, *S. boydii*, and *S. dysenteriae* representing 38%, 7.5%, and 4.5% of the total number of strains, respectively. There is similar dominance of *S. sonnei* in the United States where *S. sonnei* accounts for 71% of all reports of laboratory-confirmed *Shigella* species (*S. sonnei*, 71.7%; *S. flexneri*, 18.4%; *S. boydii*, 1.6%; *S. dysenteriae*, 0.7%; and no serogroup reported, 7.6%).

Historically, *S. sonnei* has been predominantly responsible for dysentery in developed countries but is now emerging as a problem in the developing world, replacing *S. flexneri*, particularly in areas undergoing economic development. Data from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) in Dhaka showed that 63% of children with shigellosis were infected with *S. flexneri*, 20% with *S. dysenteriae* type 1, 10% with *S. boydii*, 4% with *S. sonnei*, and 3% with *S. dysenteriae* types 2–10. In China, the prevalence

of *S. flexneri* (76.2%) is decreasing, whereas *S. sonnei* (21.3%) is increasing in line with the rapid economic growth seen in recent years.

Clinical Symptoms

Infection with *Shigella* species affects the distal small intestine and results in loose stools of small volume accompanied by fever, nausea, vomiting, and abdominal cramps. Stools typically contain blood and mucus (dysentery) resulting from mucosal ulceration and colonic crypt microabscesses. Dysentery caused by *S. sonnei* usually causes a milder illness than the other three *Shigella* species. Previous studies have shown that *S. boydii*, *S. flexneri*, and *S. dysenteriae* cause more severe gastrointestinal symptoms. *S. dysenteriae* serotype 1 is associated with hemolytic uremic syndrome (HUS) and toxic megacolon. Post-infectious complications may include reactive arthritis and irritable bowel syndrome.

Transmission

The human gastrointestinal tract is the only significant reservoir of *Shigella* infection. Infection may follow ingestion of as few as ten cells. The incubation period is between 12 h and 4 days (usually 1–3 days, median 2 days) but may be up to 1 week for *S. dysenteriae* serotype 1. Cases of *Shigella* are potentially infectious for as long as the organism is excreted in the stool. Shigellae may survive for up to 20 days in favorable environmental conditions resulting in transmission through contact with contaminated fomites.

Transmission is predominantly via the fecal–oral route from cases with diarrhea in households or institutions, especially those with young children, and person-to-person spread is the most likely route of transmission. Outbreaks have been reported in specific settings, such as day-care centers and religious communities. The reported attack rate in school and day-care centers can be associated with a high risk of transmission. Outbreaks have also been associated with recreational water facilities.

Since the 1970s, shigellosis has also been reported as a sexually transmitted infection among men who have sex with men (MSM), and an increasing number of outbreaks of MSM-associated shigellosis have been reported in Northern America, Asia, and Europe. Oroanal contact and coinfection with HIV are key risk factors for shigellosis, with a significant number of MSM shigellosis cases being HIV-positive.

Foodborne Outbreaks

Gastrointestinal infection caused by *Shigella* species can be spread through the ingestion of contaminated food and water. Foodborne outbreaks caused by *Shigella* documented in the literature since 2002 have been mostly associated with *S. sonnei* (Table 1). Research from the United States also indicates that 72–79% of *Shigella* outbreaks relate to *S. sonnei*. Implicated food included imported lettuce, fresh basil, sugar snap peas, and baby corn, with contamination most likely occurring in the country of origin (Table 1).

Table 1 Documented foodborne outbreaks caused by Shigella since 2002

Year	*Implicated food product*	*Species*	*Country*
2001–09	Leafy greens, herbs, seed sprouts	*Shigella* sp.	Canada
1973–2008	Guacamole and salsa	*Shigella* sp.	The United States
2011	Imported basil	*S. sonnei*	Norway
2009	Sugar peas	*S. sonnei*	Norway, Denmark
2009	Baby corn	*S. sonnei*	Denmark, Australia
2004	Unpasteurized milk curds	*S. sonnei*	Lithuania
2006	Tomatoes	*S. flexneri 2a*	The United States
2004	Raw carrots	*S. sonnei*	International
2004	Oyster	*S. sonnei*	Japan
2003	Parsley	*S. sonnei*	The United States

Foodborne outbreaks of *Shigella* species commonly relate to an infected food handler and salad items are often implicated. Between 2007 and 2010, Greig and colleagues published a series of informative articles reviewing the role of food workers in foodborne outbreaks. They found that the most frequently reported factors associated with the involvement of the infected worker were bare hand contact with the food, failure to properly wash hands, inadequate cleaning of processing or preparation equipment or utensils, cross contamination of ready-to-eat foods by contaminated raw ingredients, and temperature abuse. Many of the workers were asymptomatic shedders or had infected family members and/or used improper hygienic practices. Multi-ingredient foods were most frequently implicated, most likely because of more frequent hand contact during preparation and serving, and usually were served in restaurants or hotels, at schools, and at catered events.

Shigella species are among the pathogens most likely to be transmitted by food workers as the infectious dose is low (<10 cells). Shedding of the pathogen during the prodromal phase of illness, lengthy postsymptomatic shedding periods, and excretion by asymptomatic individuals should be considered during the implementation of hygiene protocols and the overall management of food workers.

Management

Strategies for *Shigella* control in the public health setting include the sanitary disposal of feces, hand washing, hygiene measures, and the isolation/exclusion of infected cases. Where information indicates a point source, efforts aim to minimize ongoing exposure. Guidelines for the management of *Shigella* infections in the United Kingdom (2004) recommend the exclusion of cases of *S. sonnei* from school or work until 48 h after the first normal stool and clinical surveillance for both

cases and contacts. However, cases and contacts in risk groups (Group A: Any person of doubtful personal hygiene or with unsatisfactory toilet, hand washing, or hand drying facilities at home, work, or school. Group B: Children who attend preschool groups or nursery. Group C: People whose work involves preparing or serving unwrapped foods not subjected to further heating. Group D: Clinical and social care staff who have direct contact with highly susceptible patients or persons in whom a gastrointestinal infection would have particularly serious consequences.) of *S. dysenteriae*, *S. flexneri*, and *S. boydii* should be screened microbiologically for clearance, which is defined as having two negative stool samples not less than 48 h apart. Guidelines from other countries expected to have similar nonendemic rates of *S. dysenteriae*, *S. flexneri*, and *S. boydii* (e.g., the United States and Australia) vary in the degree to which cases and contacts are screened microbiologically.

In food service operations, direct contact with food by hands should be prevented and gloves can substantially reduce opportunities for food contamination. Other control measures include protecting the food and food preparation areas from insects and other pests, food shields to prevent aerosol contamination, work clothing designated strictly for work, and the use of utensils to prevent direct contact between hands and the food being prepared or served. Disinfectants should be used to remove microorganisms (including pathogens) from objects or materials used during food production and preparation.

The most convenient and efficient way of removing pathogens from hands is through hand washing. Hand washing should occur regularly and after any activity that soils hands and always before preparing, serving, or eating food. Antimicrobial soaps are marginally more effective than plain soaps, as constant use results in a build-up of the antimicrobial compound on the skin. The time taken to wash hands and the degree of friction generated during lathering are more important than water temperature or antimicrobial soaps for removing soil and microorganisms. Disposable paper or high-speed air blades should be used to dry hands, rather than cloth towels (risk of contamination is associated with the fact that towels are not changed after use) or conventional warm air dryers (conventional dryers take too long for efficient use). Be aware that excessive washing and scrubbing can cause skin damage leading to infections and an increased risk to contamination of the food.

Survival of *Shigella* in the Environment

Previous studies indicate that *Shigella* species are heat-sensitive, acid-resistant, and salt-tolerant and may survive in foods such as fruits, vegetables, cured ham, and certain cheeses. More recently, studies measuring the effects and interactions of temperature (4–37 °C), pH (2–6), and NaCl (0.5–9%) on survival kinetics of *S. flexneri* demonstrated that foods with low levels of organic acids stored at low temperatures support the survival of the bacterium for extended periods of time. Other recent studies have confirmed that the temperature range permitting growth for *Shigella* was 6.1–47.1 °C but that the pathogen could survive for extended periods of time when held at −20 to 4 °C. Traditional pasteurization and cooking temperatures are sufficient for inactivation.

Studies investigating the survival of shigellae in apple juice and tomato juice, strawberries, and a fresh fruit salad demonstrated that the ability of *S. flexneri* to survive and grow at a given pH is influenced by previous exposure to acidic environments and by incubation temperature. Acid foods, especially if kept at refrigeration temperatures, support the survival of *Shigella* spp. and may cause *Shigella* food poisoning. Early studies showed that a variety of fruit and vegetables can support the growth or survival of *Shigella*, including papaya, watermelon, raw cabbage, onions, peppers, and coleslaw. High survival rates have also been observed in *Shigella* in water, supporting the historical association of shigellosis outbreaks and water sources.

Experimental results have shown that the combination of antimicrobial bacteriophage cocktails with a commercial levulinic acid produce wash is a very effective method for treating produce contaminated with enteric pathogens, including *Shigella* spp., even in the presence of high loads of organic matter. Lactoperoxidase–thiocyanate was shown to be an effective bactericidal candidate at low pH for decontaminating fruit. Other studies concluded that detergent (0.1% Tween 80 and 0.1% sodium lauryl sulfate) was no more effective in removing organisms from produce than water.

Detection

Shigella grows as non-lactose-fermenting colonies on MacConkey agar, although a more selective agar is used for the isolation of *Shigella* from feces, such as deoxycholate citrate agar and xylose lysine deoxycholate agar. Biochemically, *Shigella* spp. are unable to ferment lactose or utilize citrate and urea or to produce lysine decarboxylase. They are nonmotile and produce acid from glucose but no gas. Biochemical identification is confirmed by serological reactions to specific *Shigella* antisera. Isolation of *Shigella* from food is difficult, as colonies can be obscured by more robust commensal microbiota, such as *Escherichia*, *Enterobacter*, and *Proteus* species, and the level of contamination can be low.

More recently, a polymerase chain reaction (PCR) targeting the invasion plasmid antigen H (*ipaH*) gene has been used to detect the pathogen directly in fecal and food samples. This PCR approach is much more sensitive than conventional culturing. The *ipaH* gene is the target of choice, as it is encoded multiple times on both the chromosome and the large virulence plasmid. Thus, the test has increased sensitivity over PCRs targeting single copy genes, and detection of the pathogen is possible in the event the large virulence plasmid is lost.

There are a variety of *Shigella* PCR assays described in the literature. Most incorporate primers targeting the *ipaH* genes located on both the virulence plasmid and chromosome and an internal amplification control and have been well validated and tested on several high-risk foods. The specificity and sensitivity of the assays are approximately 1–3 colony-forming units and 5–50 fg of target (total) DNA. Different post-enrichment extraction protocols have been compared, and the InstaGene matrix was found to be the most consistent and sensitive.

Multiplex real-time PCR assays for the simultaneous detection of *Campylobacter jejuni*, *Salmonella* spp., *Shigella* spp./

enteroinvasive *Escherichia coli (EIEC)*, and *Yersinia enterocolitica* in fecal samples have been described in the literature. The sensitivity of the PCR is approximately 61 colony-forming units per ml for *Shigella* spp. The use of PCR in clinical settings increases the overall clinical sensitivity from 78% to 100% when compared with conventional culturing. The specificity was 99.4% for the PCR, compared with 99.9% for conventional culturing. The real-time method for the amplification of the *ipaH* gene in food as recommended by the European Union Reference Laboratory can be accessed by following http://www.iss.it/binary/vtec/cont/EU_RL_VTEC_Method_07_Rev_0.pdf.

Typing

Strains are serotyped (*S. dysenteriae*, *S. boydii*, and *S. flexneri*) or phage-typed (*S. sonnei*) to facilitate outbreak investigations. The *S. sonnei* phage-typing scheme is a rapid and cost-effective approach to typing during outbreak investigations. However, it is a labor-intensive, low-level discriminatory method requiring a specific skill set, and few laboratories now report phage-typing data.

The *S. flexneri* serotyping scheme differentiates isolates serologically, based on the expression of the major type-specific somatic antigen (I–VI) and common group factor antigens (3,4 and 7,8). Currently, there are 15 established serotypes. Recently, a multiplex PCR approach for molecular serotyping of *S. flexneri* has been published. This method differentiates the 15 accepted serotypes based on known differences in their *gtr* genes (encoding the type-specific antigens, group factor antigen 7,8, and 1c), the *oac* gene that mediates O-acetylation modification, and the wzx_6 for detection of serotype 6.

The most common *S. flexneri* serotypes in England are 2a, 3a, and 6. The ICDDR,B reports that serotypes 2a and 2b are most common in their region. In China, *S. flexneri* serotypes 2a and 4c account for 21.5% and 12.9% of *S. flexneri* infections, respectively, in the past decade. In Israel, *S. flexneri* 2a and *S. flexneri* 6 are the most common serotypes accounting for 71% of the isolates. *S. flexneri* 2a (10.9%) and *S. flexneri* 3a (6.3%) are the most common types in Thailand.

S. boydii and *S. dysenteriae* are composed of 20 and 15 recognized serotypes, respectively. Globally, detailed serotyping data for these two species are scarce. In England, strains of *S. boydii* and *S. dysenteriae* are mostly isolated from travelers, and the most common serotypes are 4, 2, and 1, whereas serotype 2 and provisional serotype E112707 were the most frequently submitted *S. dysenteriae* serotypes. In Egypt, serotypes 7 (30%), 2 (28%), and 3 (23%) accounted for most of the *S. dysenteriae* isolates; 50% of *S. boydii* isolates were serotype 2. Many strains previously identified as *Shigella boydii* serotype 13 that were found not to harbor the *ipaH* gene and to be more active biochemically than *Shigella* species have been reclassified as *Escherichia albertii*.

If a higher level of discrimination is required, there are a variety of molecular typing methods available, including pulsed-field gel electrophoresis (PFGE), multilocus variable-number tandem repeat analysis (MLVA), and, more recently, whole genome sequencing (WGS).

WGS has been used to investigate outbreaks at the local level and to inform evolution and global transmission. Phylogenetic analysis shows that the current *S. sonnei* population descended from a common ancestor that existed less than 500 years ago and diversified into several distinct lineages. This diversification occurred in Europe and was followed by more recent establishment of local pathogen populations on other continents, predominantly due to the pandemic spread of a single, rapidly evolving, multidrug-resistant lineage.

Pathogenicity

Shigellae are facultative intracellular pathogens derived from *E. coli* by a combination of gene loss and acquisition. Deletions are associated with the loss of metabolic pathways (e.g., *cadA*) that are no longer required for an intracellular lifestyle, and antivirulence factors (e.g., *nadA* and *nadB*) that suppress pathogenicity. Acquisition genes are associated with bacterial invasion and intracellular survival (e.g., *virB* and *mxiE*), iron acquisition (e.g., *iuc* and *fuc*), and toxin production (e.g., *set1A* and *set1B*) encoded on the invasion plasmid pINV and the chromosomally located *Shigella* pathogenicity islands (SHIs) SHI-1 and SHI-2, SHI-O, and SRL.

Pathogenesis of *Shigella* involves four steps:

(i) Bacteria invade the epithelial cell in the large intestine via M cells.
(ii) On reaching the submucosa, they are engulfed by macrophages where they survive and multiply.
(iii) In the macrophage, they induce apoptosis and the release of cytokines that destroy the integrity of the epithelial layer.
(iv) On escaping from the macrophage, they invade the epithelial cells from the basolateral side, and traverse the intracellular cytoplasm of the invaded cell and neighboring cells, by formation of an actin tail on the bacterial surface that provides the propulsive force required for directed motility.

Diarrhea results from the breakdown of the integrity of the colonic epithelium and the subsequent reduction in its capacity to reabsorb fluid and the action of enterotoxins (ShET1 and ShET2) in the jejunum.

Three serine protease autotransporters of Enterobacteriaceae (SPATEs) potentially contribute to enterotoxic activity of *Shigella*:

- SigA is chromosomally encoded and exerts a cytopathic effect on HEp-2 cells.
- SepA may be associated with causing inflammation and tissue damage.
- Pic is implicated in mucinase activity, serum resistance, hemagglutination, enterotoxicity, and immune modulation.

Treatment

Oral rehydration is an essential therapeutic response to *Shigella* infection and with appropriate rehydration shigellosis is generally a self-limiting disease. Antibiotics are recommended in some cases when symptoms are severe or to reduce the likelihood of transmission. Antibiotics have been shown to reduce

the duration of symptoms from 5–7 to 3 days. It has been suggested that antibiotics may also reduce the period of asymptomatic carriage.

Resistance to ampicillin, tetracycline, and the sulfonamides is widespread. Despite some reservations concerning its use in children, ciprofloxacin has been the recommended drug of choice for many years although there is evidence of increasing resistance in strains isolated from cases returning from the Indian subcontinent. Ceftriaxone and azithromycin are alternatives, but strains resistant to both these antibiotics have been described.

Vaccines

A safe effective vaccine for *Shigella* is greatly sought after as oral rehydration therapy is less effective against this pathogen than against other toxin-producing gastrointestinal pathogens such as *Vibrio cholerae* and enterotoxigenic *E. coli*. More importantly, there are limited therapeutic options due to increasing resistance to antimicrobials. The genus *Shigella* comprises a wide range of serotypes, but studies have shown that a vaccine covering *S. sonnei* and *S. flexneri* serotypes 2a, 3a, and 6 would provide protection against more than 65% of *Shigella* infections. Vaccines targeting the variable lipopolysaccharide (O) antigens include polysaccharide conjugates and synthetic conjugates. An alternative strategy is to target the conserved antigens, such as the major outer membrane proteins and the invasion plasmid antigens.

Two factors are impeding progress:

(i) The lack of a small animal model – non-human primate (NHP) models mimic human disease but cost and availability prohibit the use of NHP in most studies.
(ii) The complexity of the immune response observed following *Shigella* infection that involves both humoral and cell-mediated antibodies and systemic and mucosal arms of the response.

Conclusion

Every year, *Shigella* species cause an estimated 167 million episodes of diarrhea worldwide and over a million deaths. *S. sonnei* is predominantly responsible for dysentery in developed countries but is now emerging as a problem in the developing world, replacing *S. flexneri*. MSM-related shigellosis is responsible for a significant proportion of domestically acquired cases in developed countries and is changing the epidemiology of *Shigella* in 'low-risk' regions. Foodborne outbreaks of *Shigella* species are often linked to salad items contaminated at the country of origin or by the food handler. Use of PCR targeting the invasion plasmid antigen H (ipaH) gene has improved the detection of *Shigella* in fecal and food samples. Highly discriminating and robust molecular typing methods are available including PFGE, MLVA, and, more recently, WGS. Increasing antibiotic resistance is driving the search for a safe, effective vaccine.

See also: Diarrheal Diseases; *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; *Escherichia coli* and Other *Enterobacteriaceae*: Occurrence and Detection; Food Poisoning: Epidemiology; Foodborne Pathogens.

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Single Cell Proteins

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Introduction

The term *single-cell protein* (SCP) refers to a dead, dry cell mass of microorganisms such as yeast, bacteria, fungi, and algae, which grow on different carbon sources. The term *SCP* was first coined by Carrol Wilson in 1966. These are the potentially important foodstuff-containing proteins, lipids, carbohydrates, and a variety of nucleic acids and other nonprotein nitrogenous compounds, vitamins, and inorganic substances. Microorganisms have been employed for many years in the production of high-quality proteinaceous food products like cheeses, fermented milk products, mushrooms, and soybean products. Proteins are the main nutritional components in all these types of foodstuffs. Owing to a large proportion of cell dry weight is accounted for protein, the nutritional value of SCPs is determined by the levels of protein produced. Use of microbes as a food source may appear to be unacceptable to some people but the idea of consumption of microbes as food for humans and animals is certainly innovative to solve the global food problem. Due to their ability to upgrade low-protein organic material to high-protein food, microorganisms are exploited for production of SCPs on an industrial scale. This phenomenon was employed in Germany during the First World War when the growth of *Saccharomyces cerevisiae* was exploited for human consumption. Moreover, *Candida arborea* and *C. utilis* were used during the Second World War, and about 60% of the country's prewar food input was replaced. The rapidly increasing world population generates the challenge of providing additional food sources. In particular, protein supply poses a problem because essential amino acids cannot be replaced. One possible solution to this problem is SCP production. Bacteria and yeast are candidates for the purpose. Protein contains the element N in addition to C, H, and O. The N comes from soil, but in limited quantum; however, the other elements are in the products of photosynthesis, which relies on CO_2 and water, which are reasonably easily available to plants. Production of protein from animal sources (meat/milk) depends on protein from plants: grass for grazing or animal feeds, which requires large areas of land. To grow the feed and fodders requires nitrogenous fertilizers, which can cause a variety of problems if not used in the prescribed doses. Similarly, stored and prepared animal feeds also pose economic and environmental problems. SCPs are gaining steady popularity as they require limited land area for growth. Similarly, the growth of microorganisms is faster than that of animals. A range of materials can be considered as suitable substrates depending on the microorganism chosen, and the microorganisms have the ability to upgrade low-protein organic material to high-protein food. Research on SCPs has been stimulated by a concern over the eventual food crisis or food shortage that will occur if the world's population is not controlled. Many fungal species are used as SCPs. They provide the B-complex group of vitamins and also show a low level of nucleic acid content.

SCP to Fulfill Protein Requirements of a Growing Population

Earth has always been subject to rapid and drastic changes under the name of development. Based on current trends, population experts predict that the world's population will increase by 38% from 6.9 billion in 2010 to 9.6 billion in 2050. The world population will continue to grow exponentially over the next 50 years. India is the second-most-populous country after China. During the last four decades Indian population has been doubled. At least 1.3 billion people will be added to the world population over the next 25 years (Figure 1). A jet of the population is considered both developing and developed countries, but with a difference in the rate of explosion. Countries in the developing world have about 98% of the total increase in the population, whereas developed countries add the remaining 2% difference. The World Health Organization estimates that 12 million people die due to hunger and hunger-related diseases each year, 50% of which are of 5 year age. The population explosion has adverse effects on the economy of the country and the social and political aspects. Population growth, rapid urbanization, and the rapid reduction of the area under agricultural production all pose problems to meet all our needs and rations, but the changes are so rapid that the resources provided are falling short due to population dynamics. The rapidly increasing world population generated the challenge of providing necessary food sources to fulfill the dietary needs of growing population, which suggested the need to explore the possibility of developing SCPs. In particular, protein supply poses a problem because essential amino acids cannot be replaced.

Substrates for SCP Production

Many industrial and agriculture waste substrates have been tried for production of SCPs. Though these wastes appear to be cheap, various factors like cost of transportation, pretreatment, seasonality of production, scattered production, and fluctuation in rates may act as limiting factors. Following are some of the substrates generally utilized for production of SCPs:

- Whey: Whey is a by-product of the cheese and paneer industry, which contains proteins and lactose. As per the stringent Laws under Environment Protection Acts, disposal of the whey is a big challenge for the dairy industry. The

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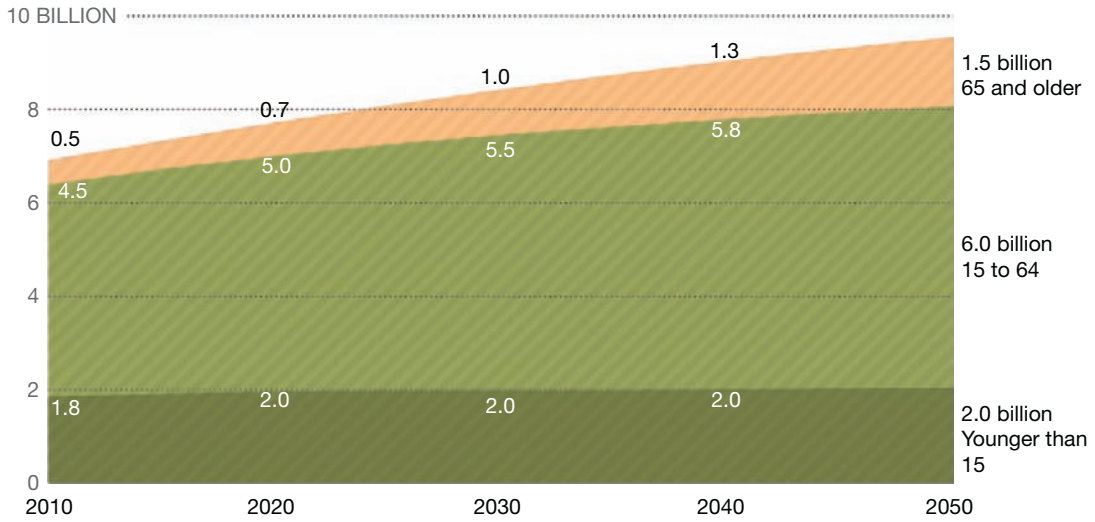

Figure 1 Estimated global population by age (2010–50). Source: UN, Department of Economics & Social Affairs, World Population Prospectus (June 2013).

dairy industry waste is liquid in nature and its transportation results into high costs. However, utilizing the whey for production of SCPs has proved to be a boon.

- Molasses: Generally molasses is available from four sources viz. cane, beet, corn, and citrus. The sugar industry utilizing cane produces large quantities of molasses, which are utilized for production of SCPs using *C. utilis* or *S. cerevisiae* as the starter cultures. Molasses is an excellent substrate for SCPs. Molasses may require pretreatment to reduce mineral content and remove suspended solids before use. Cellular yields from molasses are about 25%.
- Carbohydrates: Pure carbohydrates are not utilized for production of SCPs due to their high value. Cassava and other starchy products can be hydrolyzed to give a glucose syrup, which is used for production of SCPs. Similarly, in countries like India, clarified cane juice provides a cheap and readily available substrate for fermentation to produce SCP. In addition it provides a strategic source of proteins to replace imported products such as soya bean meal.
- Ethanol: It has many advantages over other substrates used for production of SCPs, but it is more expensive. It is probably the worthwhile substrate only where local overproduction of ethanol demands its disposal.
- Methanol: It presents the dual advantages of a clean and reliable substrate giving a high yield of SCPs together with availability and a reasonable source. The use of methanol as a substrate has been developed for full-scale commercial production of SCPs. This technology has a great potential for areas of the world with methane supplies and a need for an indigenous source of high-quality protein.
- Sulfite waste liquor: This is the waste product of wood-pulping industry. The effluent as the liquor can be fermented by *C. utilis* to produce SCPs.
- Methane: The natural gas methane at first sight appears to be an attractive substrate for SCPs because it is easily available and relatively inexpensive, it is pure, and it requires less quantities as compared to other substrates. But some of the bottlenecks of methane in SCP production includes that it is not readily water soluble, and its mass transfer is slow and energy intensive. Pure cultures of methane-fermenting organisms are difficult to cultivate, and there is no consistency in production. Similarly, the explosive nature of air–methane mixtures presents serious difficulty in design and operation of a large-scale plant, leading to excessive capital-related costs of production.
- Renewable plant sources: Glucose, cellulose, and starch are used as substrates/carbon sources for SCP production. They play an important role in the production of SCPs by microorganisms. The use of glucose as a carbon source is very attractive, considering its lower costs. Studies were conducted by using different concentrations of glucose as a source of carbon and revealed that the highest levels of SCP were produced by using 8% (w/v) glucose. It was observed that the protein content in the microbial cells was increased when the concentration of glucose was increased from 1% to 8% (w/v). By using glucose as a substrate, SCPs can be produced from *Fusarium graminearum*. The strategy is to use the fiber content of the mycelium to produce a range of products with value addition including analogues of meat for human consumption.

Cellulose has also great potential as a source of carbon for microorganisms. A mixed culture of Cellulomonas and Alcaligenes can be used for production of SCPs. Starches from potato wastes, tapioca, and cassava can be fermented symbiotically in a single-step continuation process by *Endomycopsis filburger*. It hydrolyzes starch and converts it into simple sugars, which are then used for the production of biomass *C. utilis*. Starch can be fermented directly by some amylolytic fungi and subsequently used for production of SCPs.

Criteria in Selection of Microbial Strains for Production of SCPs

Following are the parameters that are invariably considered while selecting the microbial strains for production of SCPs:

- Low generation time
- Ability to grow on different substrates without requirement of growth factors
- High nutritional content
- Low content of nucleic acid
- Nonpathogenic and nontoxin producing
- Suitability in downstream processing
- High digestibility
- Tolerance to high cell density
- Ease in handling
- pH tolerance
- Stability in growth rate during continuous cultivation
- Good organoleptic properties
- Ability to utilize complex substrate
- High fidelity with carbon source

Microbes Used for Production of SCPs

The following microbial genera are widely used for production of SCPs.

(a) Bacteria: Different types of bacteria are used for production of SCPs. Many strains, such as *Bacillus*, *Hydrogenomonas*, *Methanomonas*, *Methylomonas*, and *Pseudomonas* are commonly used for commercial scale production of SCPs. Bacteria have a slight advantage over other microbes in respect of requirement of energy source. This is due to their growth rate, relatively high protein content, and sulfur amino acids. About 80% of crude protein is present in total dry weight. Content of nucleic acids, particularly RNA, is based on the dry weight and is reported as 15–16%. In bacteria, more methionine is present than other groups, which is around 2.2–3.0%, which is relatively higher than in algae (1.4–2.6%) and fungi (1.8–2.5%).

(b) Yeasts: *Saccharomyces*, *Candida*, and *Rhodotorula* are commonly used for production of SCPs. Yeasts are widely used in production of biomass, which is used in both food and feed industries. For millions of years, we have used yeast as a food source in brewing, wine making, baking, and so on. An added benefit of yeast is that it will grow in acidic condition (pH 4.5–5.5), which is severe and inhibits growth of bacterial contaminants. This has an added benefit of no need to maintain strict aseptic conditions. Yeasts have many practical features such as the ability to use a wide variety of substrates, susceptibility for induced genetic variation, the ability to aggregate, and high nutritional value. One drawback of yeast is that the SCPs obtained are insufficient in sulfur-containing amino acids.

(c) Filamentous fungi: *Fusarium*, *Aspergillus*, and *Penicillium* are commonly used for SCP production. Filamentous fungi grow slowly and are highly prone to contamination by the yeast, so it should be grown under sterile conditions. They have some advantages like readily filtered from the liquid culture thereby reducing the cost of harvesting. As agricultural waste contains more carbohydrate, it is preferred for production of fungal SCPs.

(d) Algae: *Spirulina*, *Chiarella*, and *Scenedesmus* are generally used for production of SCPs. Algae (Cyanobacteria and unicellular eukaryotes) is autotrophic and synthesizes food by taking the energy of sunlight or artificial light and CO_2 as the source of carbon. In some countries, the culture is performed in large sections in the oxidation of sewage basins using sunlight or artificial light for use in life supportive systems for extended space exploration. The massive culturing of algae began in many countries, such as India, Japan, West Germany, Czechoslovakia, and Mexico. It can be harvested by simple and less-expensive methods. Algae is highly digestive (85–95%) due to thin wall and low nucleic acid contents. It contains a high percentage of digestible proteins (60–75%), vitamins, amino acids, and other nutrients.

Nutritive Value of SCPs

Changes in cultural conditions and medium play a crucial role in microbial composition like limitation of nitrogen content in medium, microbes accumulate more lipid content. Synthesis of fats and proteins in cells is totally dependent on C:N ratio in the culture medium. As the growth rate of microbes increases, RNA content also increases. In optimum conditions, the average contents of crude protein, nitrogen, fat, nucleic acid, and ash in microbes, plants, and animals are given in Table 1.

Proteins in SCP

Proteins are made up of different amino acids, and thus the nutritional quality of the protein is totally dependent on amino acids and their contents. In terms of amino acids, the bacterial protein is similar to the fish protein. In addition to this, yeast protein is almost identical to the soybean protein, and the fungal protein is smaller than a yeast protein. The essential amino acid composition of microbial sources is depicted in Table 2. A comparison with standard egg amino acid composition shows that these microbes are largely an amino acid motif, and it is well balanced but low-sulfur-containing amino acids.

Table 1 Composition of some microbes, plants, and animals

	Constituents (% on dry weight basis)				
Source	*Crude protein*	*Nitrogen*	*Fat*	*Nucleic acids*	*Ash*
Microbial sources					
Bacteria	72–78	11.5–12.5	1. 5–3	8–16	3–7
Yeast	47–53	7.5–8.5	2–6	6–12	5–9.5
Filamentous fungi	31–50	5–8	2–8		9–14
Algae	47–63	7.5–10	7–20	3–8	8–10
Plant sources					
Corn meal	7.0–9.4	1.1–1.5	2.0–2.8	2.1	0.5–1.6
Wheat flour	9.8–13.5	1.6–2.2	1.8–2.0	2.1	0.5–1.4
Rice	7.5–9.0	1.2–1.4	0.2–0.6	2.5	0.1–0.4
Animal sources					
Milk	22–25	3.5–4.0	1–16	0.273	5.8
Beef	81–90	13–14.4	12–15	0.5	4.0
Egg	35	5.6	11.2	0.1	3.9

Table 2 Average essential amino acid (EAA) composition of SCP

Sources	Average essential amino acid (% by weight on as-fed basis)											
	Arg	*Cyt*	*Hist*	*Iso*	*Leu*	*Lys*	*Met*	*Phen*	*Thr*	*Tryp*	*Tyr*	*Val*
Microbial sources of SCP												
Bacterial SCP												
Pseudomonas/Methylophilus spp. (methanol)	3.67	0.41	1.50	3.34	5.43	4.30	1.75	3.07	3.29	0.80	2.70	4.16
Filamentous fungi												
Aspergillus oryzae (waste starch)	1.91	0.34	0.82	1.54	2.55	1.95	0.49	1.20	1.57	0.49	2.14	1.87
Rhodotorula pilimanae	3.53	0.11	1.25	2.01	3.32	4.11	1.19	1.60	2.52	0.15	1.27	2.53
Yeasts SCP												
Saccharomyces cerevisiae	2.14	0.60	1.14	2.22	3.25	3.31	0.83	1.95	2.32	0.62	1.51	2.48
Torula utilis	2.62	0.60	1.36	2.88	3.51	3.77	0.79	2.93	2.62	0.51	2.00	2.93
Candida sp. (alkane substrate)	2.10	0.57	1.02	2.50	3.92	3.30	1.02	2.10	2.67	0.68	1.88	2.79
Mixed fungal SCP culture												
Geotrichum candidum, *Hansenula anomala*, and *Candida krusei*	2.24	0.56	1.04	2.33	3.63	3.27	0.53	3.15	2.39	–	1.91	2.81
Algal SCP												
Spirulina maxima	3.93	0.24	1.09	3.63	4.84	2.79	0.85	3.02	2.79	0.85	2.42	3.93
Mixed SCP												
Activated sludge: domestic sewage	1.60	0.25	0.69	1.41	2.46	1.67	0.25	2.07	1.85	–	0.72	2.21
Activated sludge: brewers waste	1.95	0.00	0.40	1.64	2.62	1.55	0.98	1.60	1.82	–	1.11	2.31
Activated sludge: paper proc. Waste	3.07	0.14	0.90	2.05	3.50	2.07	1.20	2.02	2.23	–	1.51	2.69

Source: http://www.fao.org/docrep/field/003/AB468E/AB468E05.htm.

Generally, in bacteria, there is higher methionine content than yeast, whereas in case of lysine it is the opposite. Lysine and threonine are present in higher concentration in microbes than in wheat or cereals adding value of SCPs as a supplement to cereal diet.

Microbial species possess high protein content, which is the main reason to consider them as an unconventional source of protein. The percentage of crude proteins is higher in bacteria than other microbial groups. It is 72–78% in bacteria and lowest in fungi (31–50%). However, this estimate is not entirely true because 20% of the total nitrogen is nonprotein in origin. This includes purine bases 8–13%, pyrimidine bases 4%, 0.5% choline, 0.5% glucosamine, and a few percent of other nitrogenous compounds.

Vitamins in SCP

Microorganisms mainly contain vitamin B. It plays key role in animal feed supplements (0.5–1.5%). Yeast is one of the rich sources of vitamin B. The vitamin B is generally lower in mycelial fungi than yeast. Algae are also good sources of vitamin B. They also contain tocopherol and β-carotene. Bacterial cell contains high amounts of vitamin B_{12}, whereas algae are generally rich in vitamin A. The most common vitamins in the SCP are riboflavin, niacin, thiamine, pyridoxine, choline, pantothenic acid, folic acid, inositol, biotin, p-aminobenzoic acid, and vitamin B_{12}.

Fatty Acids in SCPs

Fatty acids are primary metabolites of the acetyl CoA pathway. The fat content is higher in algae than other microbial group members. It is 7–20% in algae and lowest (1.5–3%) in bacteria. One difference between higher plants and algae is that many algae are capable of producing long-chain polyunsaturated fatty acids, which are beneficial for human health and nutrition. Algal fats and oils have very wide and potential applications. The algal oils resemble fish and vegetable oils. Some microbial species are rich in essential fatty acids such as C20 derivatives, eicosapentaenoic acid (20:5ω3), and arachidonic acid (20:4ω6) and C18 linoleic (18:2ω6) and γ-linoleic (18:3ω3). These fatty acids are important components in human diets and animal feeds. In aquaculture also, these fatty acids contribute an important role in fish growth.

Nucleic Acids in SCP

High nucleic acid content is a property of rapidly growing cells. Compared with traditional foods, microbes contain a high quantity of nucleic acids, which is between 8 and 25 g nucleic acid per 100 g proteins. Animal cells like intestinal mucosa, pancreas, liver, and kidney have 4 g nucleic acid per 100 g protein, whereas fishes like sardines and roe have 2.2 and 5.7 g nucleic acid per 100 g protein, respectively. As compared to animal sources, plant sources like wheat and rye flour have less nucleic acid (1.1 and 4.0 g per 100 g, respectively).

See also: Amino Acids: Metabolism; Bioactive Peptides in Foods; Bioavailability of Nutrients; Buffalo Milk; Cellulose; Dahi; Dairy Products: Dietary and Medical Importance; Eggs: Composition and Health Effects; Fatty Acids: Essential Fatty Acids; Fungi; Lactic Acid Bacteria; Mushrooms and Truffles: Role in the Diet; Nucleic Acids; Protein: Food Sources; Yeasts.

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Skeletal Muscle

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Introduction

The muscular system is responsible for the movement of the human body. Attached to the bones of the skeletal system are about 700 named muscles that make up roughly half of a person's body weight. Each of these muscles is a discrete organ constructed of skeletal muscle (SM) tissue, blood vessels, tendons, and nerves. Muscle tissue is also found inside the heart, digestive organs, and blood vessels. In these organs, muscles serve to move substances throughout the body. There are three main types of muscles: (1) SM, which is also called voluntary muscle because it can be consciously controlled; (2) smooth muscle, which is also termed as involuntary muscle as it is not under voluntary control; and (3) cardiac muscle, which is a specialized muscle. SMs differ in size and shape according to the jobs they have to perform. They are attached either directly or indirectly to bones. SM is the only voluntary muscle tissue in the human body (controlled consciously). It derives its name from the fact that these muscles always attach to the skeleton in at least one place. Every physical action that a person consciously performs (e.g., speaking, walking, writing) requires SM. The function of SM is to contract to move parts of the body closer to the bone that the muscle is attached to.

Most SMs are attached to two bones across a joint, so the muscle serves to move parts of those bones closer to each other. They work in opposite pairs, that is, one muscle in the pair contracts, whereas the other relaxes to produce body movements as diverse as walking, threading a needle, and an array of facial expressions. SM cells form when many smaller progenitor cells aggregate to form long, straight, multinucleated fibers. Striated just like cardiac muscle, these SM fibers are very strong.

Smooth muscles occur in the walls of internal body organs and perform actions such as forcing food through the intestines, contracting the uterus in childbirth, and pumping blood through the blood vessels. For voluntary muscles, all contraction (excluding reflexes) occurs as a result of conscious effort originating in the brain. The brain sends signals, in the form of action potentials, through the nervous system to the motor neuron that innervates several muscle fibers. In the case of some reflexes, the signal to contract can originate in the spinal cord through a feedback loop with the gray matter.

Anatomy of SM

Most SMs are attached to two bones through tendons (Figure 1). Tendons are tough bands of dense regular connective tissue whose strong collagen fiber strongly attaches muscles to bones. Tendons are under extreme stress when muscles pull on them, so they are very sturdy and are woven into the coverings of both muscles and bones. Muscles move by shortening their length, pulling on tendons, and moving bones closer to each other. One of the bones is pulled toward the other bone, which remains stationary. The place on the stationary bone that is connected via tendons to the muscle is called the origin. The place on the moving bone that is connected to the muscle via tendons is called the insertion. The belly of the muscle is the fleshy part of the muscle in between the tendons that does the actual contraction (Figure 2).

Types of SM Fibers

Three principal types of muscle fibers are found in varying proportions in human SM. These fiber types may be distinguished from one another on a histochemical, metabolic, morphological, and mechanical basis. Most muscles contain a mixture of fiber types, although one usually predominates.

Type 1 or Slow Oxidative Fibers

These fibers have a slow contraction speed and a low myosin ATPase activity. These cells are specialized for steady, continuous activity and are highly resistant to fatigue. Their motor neurons are often active, with a low firing frequency. These cells are thin (high surface to volume ratio) with a good capillary supply for efficient gas exchange. They are rich in mitochondria and myoglobin. Myoglobin is responsible for the red color of fibers. They are built for aerobic metabolism and prefer to use fat as a source of energy. These are the fibers of importance to the marathon runner.

Type 2A or Fast Oxidative-Glycolytic Fibers

These fibers have a fast contraction speed and a high myosin ATPase activity. They are progressively recruited when additional effort is required, but are still very resistant to fatigue. Their motor neurons show bursts of intermittent activity. These cells are thin (high surface to volume ratio) with a good capillary supply for efficient gas exchange. They are rich in mitochondria and myoglobin, which gives them a red color. They are built for aerobic metabolism and can use either glucose or fats as a source of energy. These are general-purpose muscle fibers, and they provide the edge in athletic performance. However, these fibers are metabolically more expensive to operate than type 1.

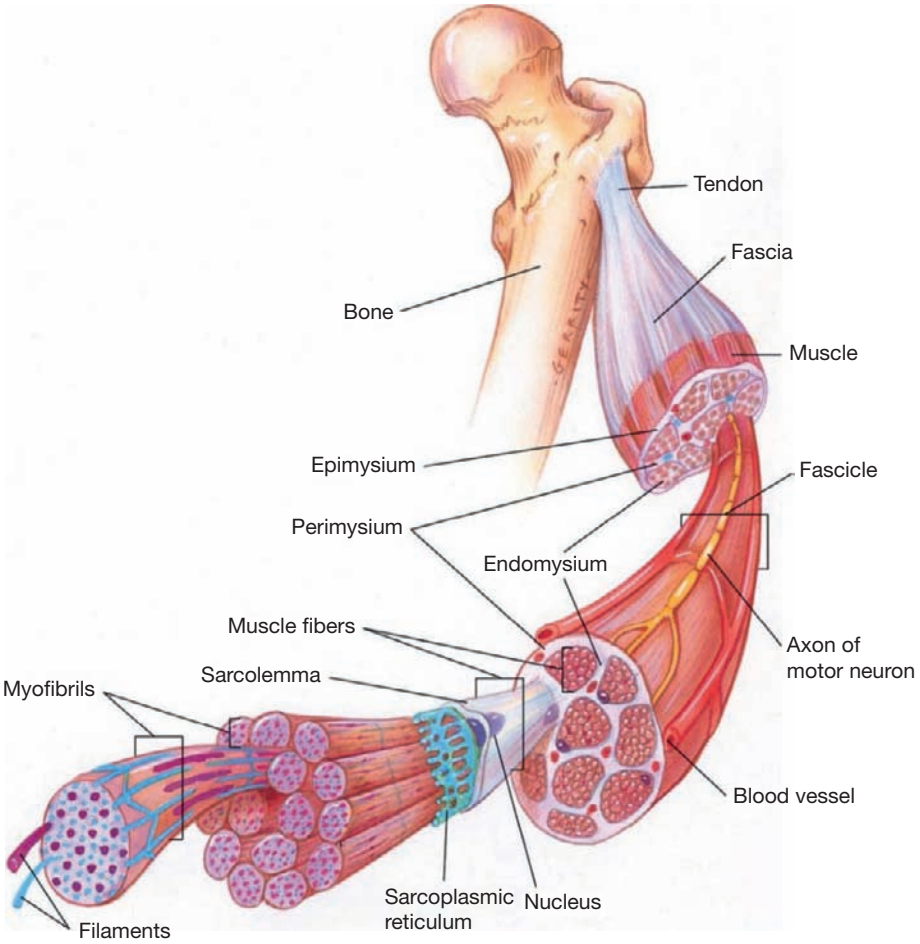

Figure 1 Connective tissue surrounding skeletal muscle.

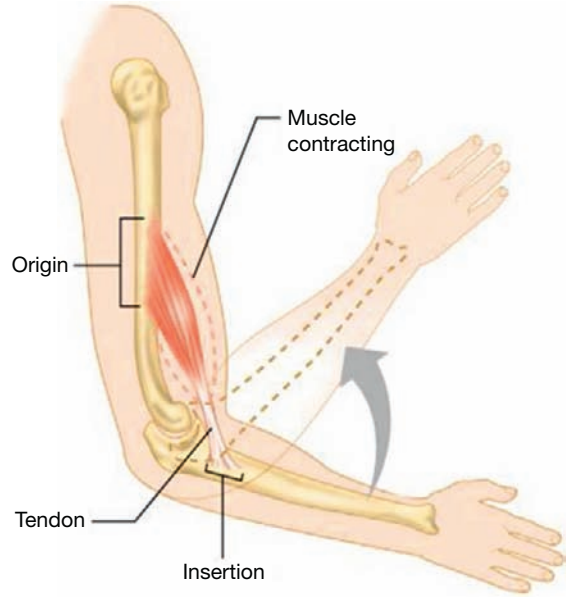

Figure 2 Isotonic muscle action.

Type 2B or Fast Glycolytic Fibers

These fibers have a fast contraction speed and a high myosin ATPase activity. They are only recruited for brief maximal efforts and are easily fatigued. Their motor neurons transmit occasional bursts of very high frequency impulses. These are large cells with a low surface/volume ratio, and their limited capillary supply slows the delivery of oxygen and removal of waste products. They have few mitochondria and little myoglobin, resulting in a white color. They generate adenosine triphosphate (ATP) by the anaerobic fermentation of glucose to lactic acid.

Nomenclature of SMs

The nomenclature of the SMs is based on various factors including the location, number of origins, shape, size, direction, and function.

Based on Region

Many muscles are named from their anatomical region. The rectus abdominis and transverse abdominis, for example, are

found in the abdominal region. Some muscles, like the tibialis anterior, are named after the part of the bone (the anterior portion of the tibia) that they are attached to. Other muscles use a hybrid of these two, like the brachioradialis, which is named after a region (brachial) and a bone (radius).

Number of Origins

Some muscles connect to more than one bone or to more than one place on a bone and therefore have more than one origin. A muscle with two origins is called a biceps. A muscle with three origins is a triceps muscle. Finally, a muscle with four origins is a quadriceps muscle (Figure 3).

Shape, Size, and Orientation of Fibers

Muscles are also classified by their shapes, for example, the deltoids have a delta or triangular shape. The serratus muscles feature a serrated or saw-like shape. The rhomboid major is a rhombus or diamond shape. The size of the muscle can be used to distinguish between two muscles found in the same region. The gluteal region contains three muscles differentiated by size – the gluteus maximus (large), gluteus medius (medium), and gluteus minimus (small). Finally, the direction in which the muscle fibers run can be used to identify a muscle. In the abdominal region, there are several sets of wide, flat muscles. The muscles whose fibers run straight up and down are the rectus abdominis, the ones running transversely (left to right) are the transverse abdominis, and the ones running at an angle are the obliques.

Origin and Insertion

Some muscles are named based on their connection to a stationary bone (origin) and a moving bone (insertion). These muscles become very easy to identify once you know the names of the bones that they are attached to. Examples of this type of muscle include the sternocleidomastoid (connecting the sternum and clavicle to the mastoid process of the skull) and the occipitofrontalis (connecting the occipital bone to the frontal bone).

Function

Muscles are sometimes classified by the function that they perform. Most of the muscles of the forearms are named based on their function because they are located in the same region and have similar shapes and sizes. For example, the flexor group of the forearm flexes the wrist and the fingers. The supinator is a muscle that supinates the wrist by rolling it over to face palm up. In the leg, there are muscles called adductors whose role is to adduct (pull together) the legs.

Architecture of the Muscle

The description of SM architecture includes the arrangement of the fibers in relation to the axis of force (amount of pennation), muscle fiber length, muscle length, muscle mass, and the physiologic cross-sectional area. These structural variations affect not only the overall shape and size of the muscles but also the function of the SM. Arrangement of fascicles (muscle fiber groups) varies among muscles. The fasciculi may be parallel to the long axis of the muscle, may spiral around the long axis, or may be at an angle to the long axis. Muscles that have a parallel fiber arrangement (parallel to the long axis and to each other) are designated as strap or fusiform muscles. Muscles that have a fiber arrangement oblique to its long axis are called pinnate muscles. In unipennate muscles, the obliquely set fascicles fan out on only one side of a central muscle tendon. In a bipennate muscle, the fascicles are obliquely set on both sides of a central tendon. In a multipennate muscle, the oblique fascicles converge on several tendons.

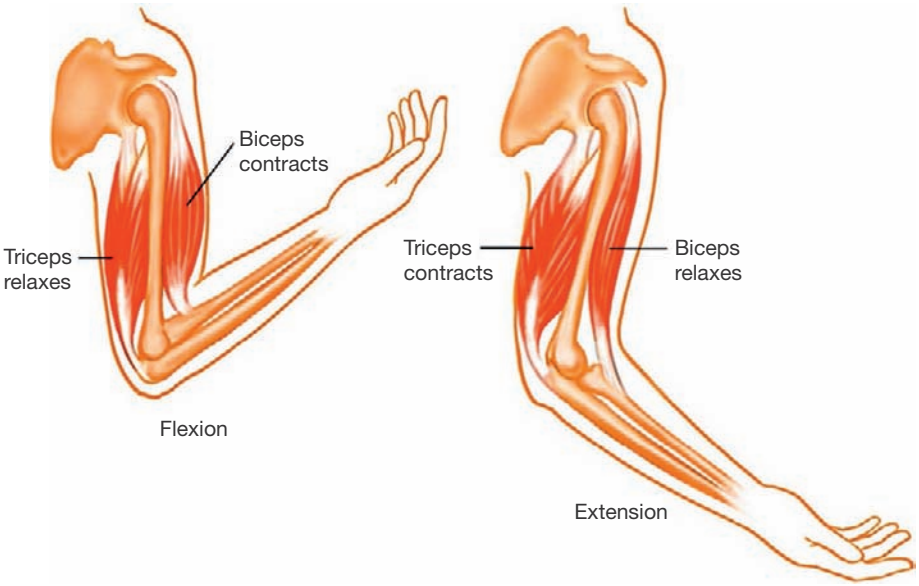

Figure 3 Biceps and triceps muscles.

Group Action in SM

SMs rarely work individually to achieve movements in the body. They work in groups to produce precise movements. The muscle that produces any particular movement of the body is known as an agonist or prime mover. It always pairs with an antagonist muscle that produces the opposite effect on the same bones. For example, the biceps brachii muscle flexes the arm at the elbow. As the antagonist for this motion, the triceps brachii muscle extends the arm at the elbow. When the triceps is extending the arm, the biceps would be considered the antagonist. In addition to the agonist/antagonist pairing, other muscles work to support the movements of the agonist (Figure 3). Synergists are muscles that help to stabilize a movement and reduce extraneous movements. They are usually found in regions near the agonist and often connect to the same bones. Because SMs move the insertion closer to the immobile origin, fixator muscles assist in movement by holding the origin stable.

Histology of the SM

SM fibers differ from other tissues of the body due to their highly specialized functions. Many of the organelles that make up muscle fibers are unique to this type of cell. A single muscle contains many fascicles, a group of muscle fibers (cells) surrounded by connective tissue. SM tissue is made up of individual muscle cells called myocytes. Myocytes are cylindrical and multinucleated cells that run parallel to each other within a muscle. An SM cell is surrounded by a plasma membrane called the sarcolemma with a cytoplasm called the sarcoplasm. It acts as a conductor for electrochemical signals that stimulate muscle cells. The transverse tubules (T tubules) are connected to the sarcolemma. They carry electrochemical signals into the middle of the muscle fiber. The sarcoplasmic reticulum acts as a storage facility for calcium ions (Ca^{++}), which are essential for muscle contraction (Figure 4).

Mitochondria are abundant in muscle cells to break down sugars and provide energy to activate muscles. Structure of muscle fiber is made up of myofibrils. These are the contractile structures of the cell made up of many proteins fibers arranged into repeating subunits called sarcomeres. It is the functional unit of muscle fibers made of two types of protein fibers: thick filaments and thin filaments. Thick filaments are made of many bonded units of the protein myosin. Myosin is the protein that causes muscles to contract.

Thin filaments are made of three proteins, namely, actin, tropomyosin, and troponin. Actin forms a helical structure that makes up the bulk of the thin filament mass. It contains myosin-binding sites that allow myosin to connect to and move actin during muscle contraction. The myosin filaments are slightly thicker and make up the dark band (or A-band). The actin filaments make up the light bands (I-bands), which are situated on either side of the dark band. The actin filaments are attached to the Z-line. This alternate arrangement of dark and light bands gives a striated appearance to muscle, which is why SM is also known as 'striated muscle.'

Tropomyosin is a long protein fiber that wraps around actin and covers the myosin-binding sites on actin. Troponin is bound very tightly to tropomyosin and moves tropomyosin away from myosin-binding sites during muscle contraction.

Working of Muscles: Sliding Filament Theory

A concentric contraction is a type of muscle contraction in which the muscles shorten while generating force. The muscle

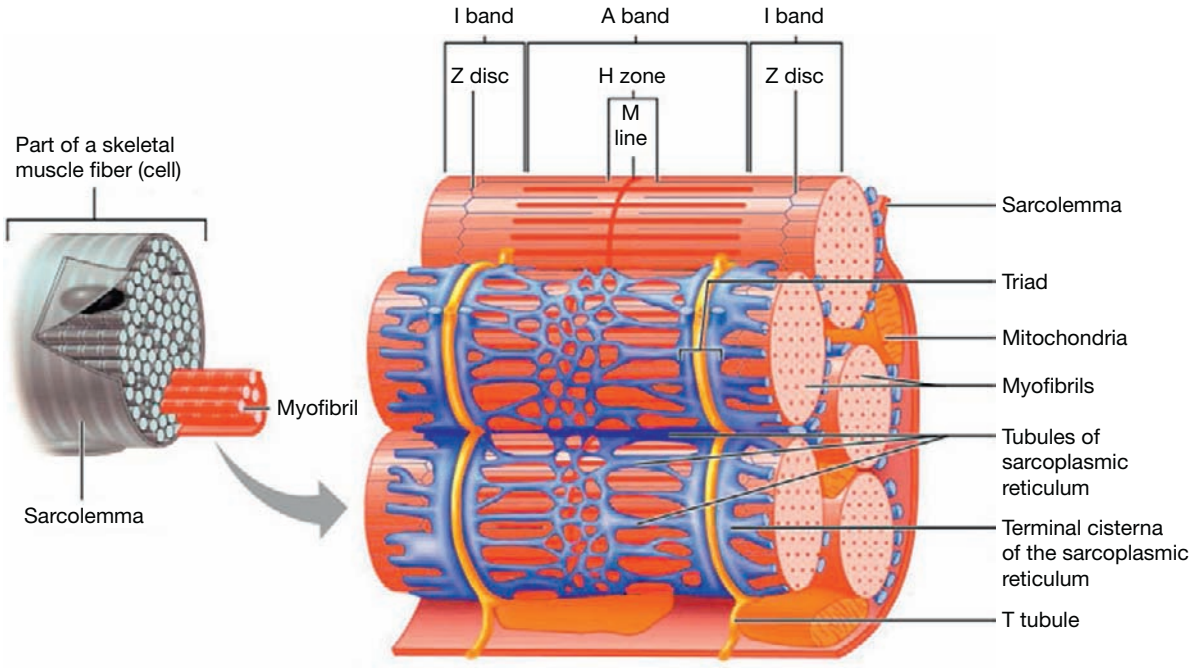

Figure 4 Sacroplasmic reticulum.

fiber will shorten (contract) if a sufficient number of sarcomeres actively shorten and if either one or both ends of the muscle fiber are free. In contrast to a shortening contraction, in which thin filaments are being pulled toward the thick filaments, the muscle may undergo an eccentric contraction, or lengthening contraction. In a lengthening contraction, the thin filaments are pulled away from the thick filaments, and cross-bridges are broken and reformed as the muscle lengthens. Tension is generated by the muscle as cross-bridges are reformed.

For the muscle as a whole to contract, the actin and myosin filaments must overlap, causing the sarcomere to shorten. This process is triggered by the release of calcium ions in the presence of troponin and tropomyosin. The release of calcium, which is provided by ATP, facilitates the interaction of myosin and actin at the molecular level. At high levels of magnification, it is detected that small bridges, known as myosin cross-bridges, on the thick filaments extend over to the thin filaments. These cross-bridges continually attach, rotate, detach, and reattach, which results in the sliding of the filaments, shortening of the sarcomere, and finally what we see to be muscle contraction (Figure 5).

Energy Required for Muscle Contraction

The energy for the muscular contraction is provided by the conversion of ATP into adenosine diphosphate (ADP) and inorganic phosphate, releasing energy. Enzyme myosins ATPase catalyze the reaction in the presence of Ca^{2+} and Mg^{2+} ions:

$$\text{ATP} \xrightarrow[\text{Myosin ATPase}]{Ca^{2+},\ Mg^{2+}} \text{ADP} + \text{Pi} + \text{energy}$$

The used-up ATP is restored for additional contractions, so phosphocreatine now comes into the picture. It donates its high energy–phosphate bond to ADP, producing ATP. This reaction is catalyzed by an enzyme creatine kinase

$$\underset{\text{(Phospho creatine)}}{\text{Creatine phosphate}} + \text{ADP} \xrightarrow[\text{Creatine Kinase}]{} \text{ATP} + \text{Creatine}$$

When creatine phosphate is used up, new ATP is generated by aerobic respiration in the muscle cells. If the ATP is used up faster than the muscle fibers can produce aerobically, then the muscle fibers start anaerobic respiration to provide ATP. This produces lactic acid. This lactic acid is diffused into the blood, leaving a small part to accumulate in the muscle fiber. The major part of lactic acid is passed into the liver, where it is oxidized to CO_2 and H_2O. The energy released from this oxidation is used for changing the remaining lactic acid to glycogen.

$$\text{Lactic acid} + \text{oxygen} \rightarrow CO_2 + H_2O + \text{ATP}$$

$$\text{Remaining lactic acid} + \text{water} \rightarrow \text{glygogen in liver cells}$$

Biochemical Changes During Contraction of Muscles

During muscle contraction a number of biochemical changes occurs. In a resting muscle fiber, sarcolemma is electropositive outside and electronegative inside. This potential difference

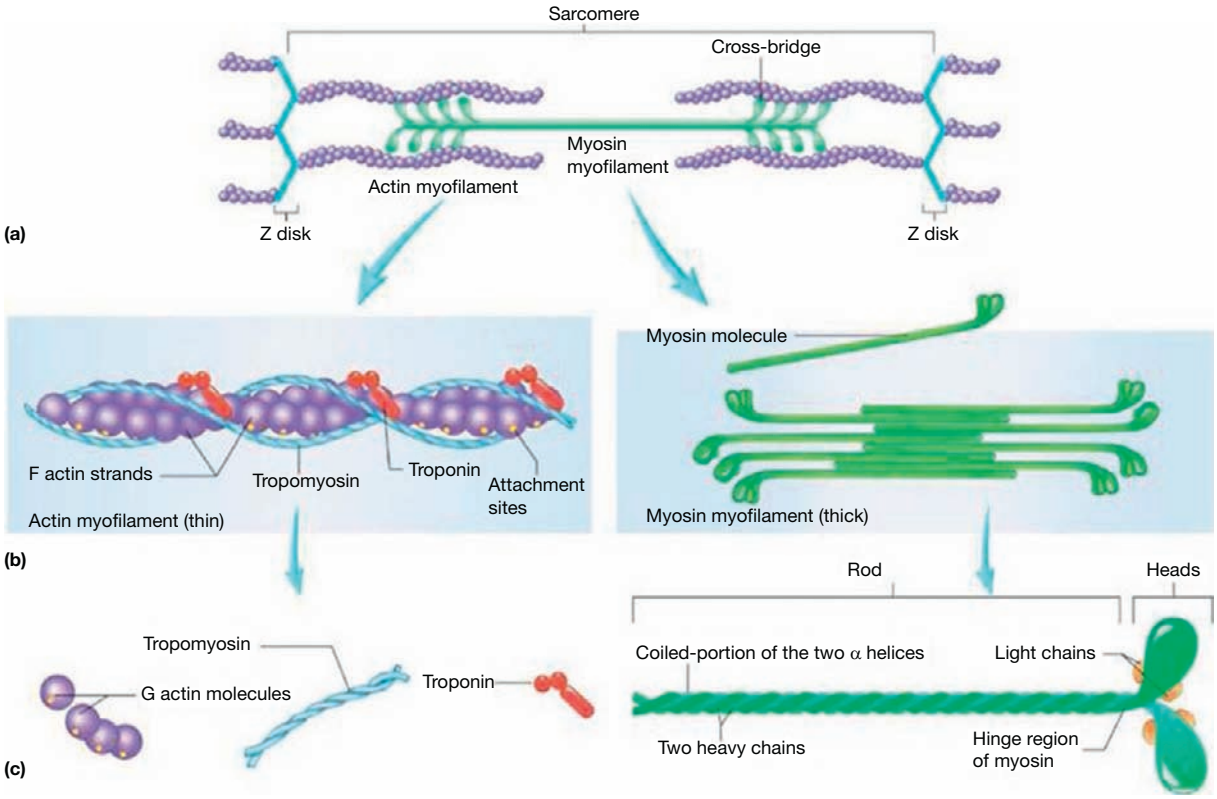

Figure 5 Sliding filament theory.

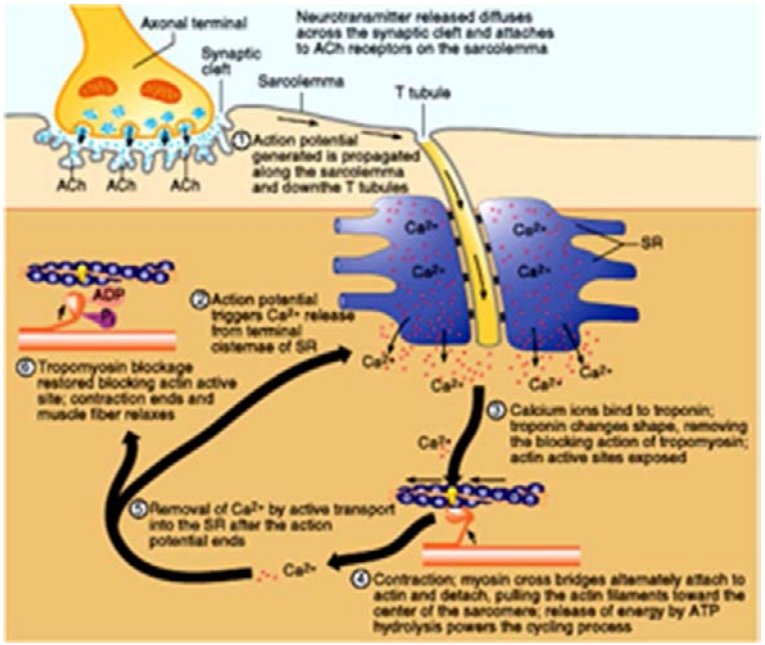

Figure 6 Schematic representation of contraction of muscle.

across a membrane is called resting potential. A membrane with such a resting potential is said to be polarized. Na^+ ions predominate the outside of the sarcolemma, and potassium ions predominate the inside. Due to the difference in concentration on the two sides of the sarcolemma, potassium ions leave and sodium ions enter the muscle fiber. Sarcolemma is more permeable to K^+ ions than to sodium ions. Hence the potassium ions leave the muscle fiber faster than sodium ions enter, and this builds a positive charge outside.

When the motor nerve impulse reaches the neuromuscular junction, the vesicles present in the motor end plate secrete a neurotransmitter chemical called acetylcholine (Ach). It binds to the receptors on the sarcolemma and makes it more permeable to Na^+ than K^+ so that sodium rapidly diffuses along the concentration gradient and the electrical gradient. Now sarcolemma becomes electropositive inside and electronegative outside. This new potential difference is called action potential. Such sarcolemma is called depolarized. Action potential stimulates the sarcoplasmic reticulum to release calcium ions, which then initiate the biochemical changes in muscle contraction. Calcium and magnesium ions act as cofactors for myosin ATPase enzyme, which hydrolyzes ATP into ADP and inorganic phosphate releasing energy (Figure 6).

Control of Muscle Contraction

The SMs are organized into hundreds of motor units, each of which is a motor neuron and a group of muscle fibers. A graded response to a circumstance will involve controlling the number of motor units. Whereas individual muscle units contract as a unit, the entire muscle can contract on a graded basis due to their organization into motor units. Neuromuscular junction is the point where a motor neuron attaches to a muscle. Ach is released from the axon end of the nerve cell when a nerve impulse reaches the junction. A wave of electrical charges is produced in the muscle cell when the Ach binds to receptors on its surface. Calcium is released from its storage area in reticulum. An impulse from a nerve cell causes calcium release and brings about a single, short muscle contraction called a twitch (Figure 7).

Disorders Affecting SMs

Some of the most common diseases affecting the SMs include myasthenia gravis, muscular dystrophy and fibromyalgia. Other primary muscle disorders include inflammatory myopathies, including polymyositis, which is characterized by inflammation and progressive weakening of the SMs. The most common symptom a muscle disorder is weakness. In addition the symptoms include abnormal fatigue with activity, as well as muscle spasms and pain. Electromyography is often used to diagnose muscular disorders. It helps to characterize the causes of nerve and muscle disorders by stimulating nerves and recording responses.

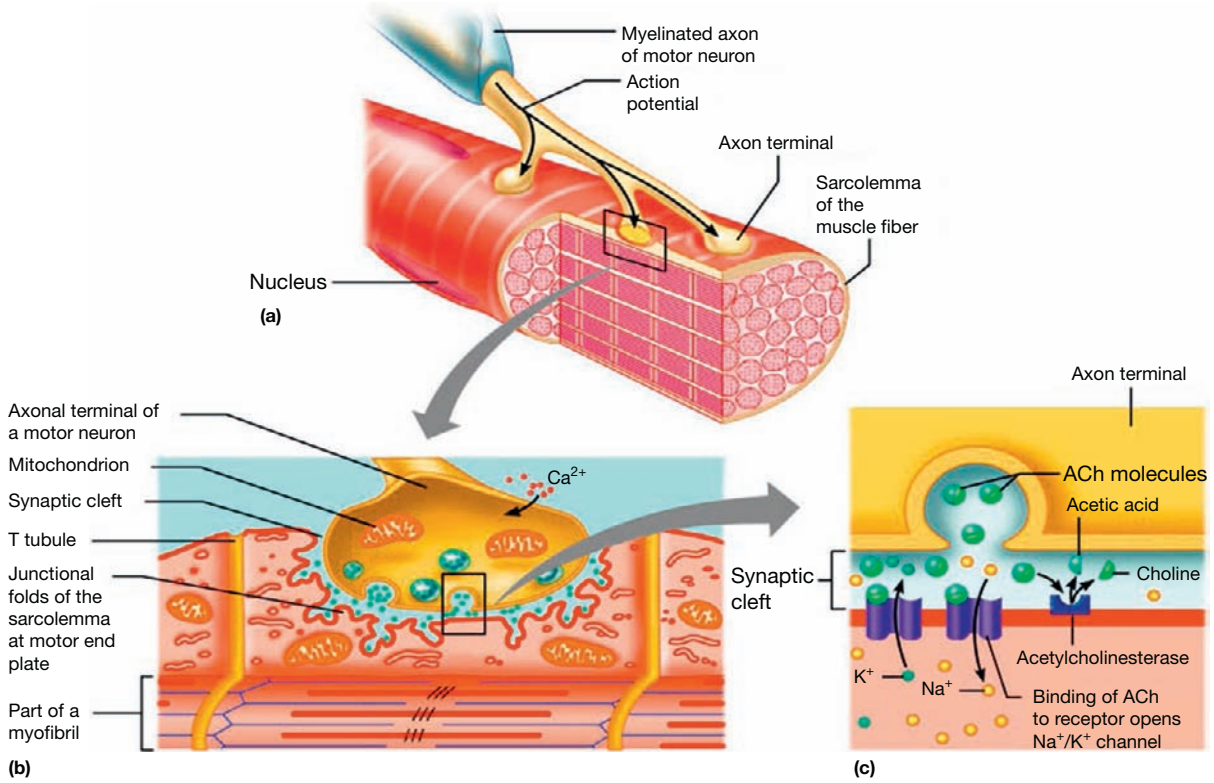

Figure 7 Schematic representation of mechanism of control of muscle contraction.

See also: Calcium: Physiology; Energy Metabolism.

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ENCYCLOPEDIA OF FOOD AND HEALTH

EDITED BY Benjamin Caballero, Paul M. Finglas and Fidel Toldrá

Volume 5: Smo–Z, Index

ENCYCLOPEDIA OF
FOOD AND HEALTH

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ENCYCLOPEDIA OF
FOOD AND HEALTH

EDITORS-IN-CHIEF

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PAUL M. FINGLAS

FIDEL TOLDRÁ

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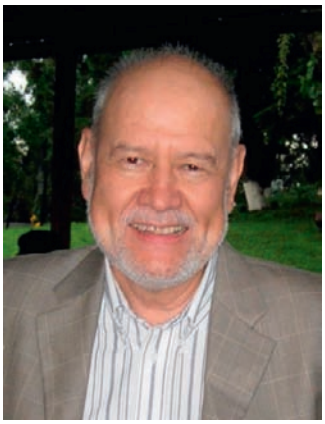


Benjamin Caballero is professor of International Health and of Maternal and Child Health (Bloomberg School of Public Health), and professor of pediatrics (School of Medicine) at Johns Hopkins University.

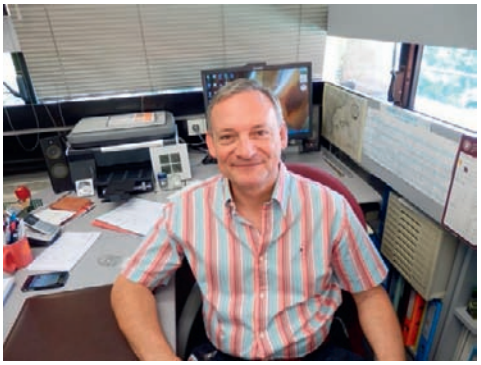
He obtained his MD from the University of Buenos Aires, his MSc in biochemistry from the University of San Carlos, and his PhD in neuroendocrine regulation from MIT, in Cambridge, MA. He started his academic career as assistant professor of pediatrics at Harvard Medical School and director of the Nutrition Unit of Boston Children's Hospital, and subsequently became the founding director of the Center for Human Nutrition at Johns Hopkins University, in Baltimore.

Prof. Caballero has focused his research on child nutrition and health in developing countries. In particular, he has explored the combination of undernutrition and overweight that has become increasingly prevalent in low- and middle-income countries. He was a member of the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences, USA, and of a number of expert panels created by the Institute, including the Dietary Reference Intakes (DRI) Committee, the Expert Panel on Macronutrient Requirements, and the Childhood Obesity Task Force. He was also a member of the Dietary Guidelines for Americans Advisory Committee, of the Scientific Advisory Board of the Food and Drug Administration (FDA), and of a number of advisory committees of the National Institutes of Health (USA).

He is the editor-in-chief of the *Encyclopedia of Food Sciences and Nutrition*, a 10-volume work on food production, consumption and biological effects. He is also editor-in-chief of the *Encyclopedia of Human Nutrition*, which received the Book of the Year Award from the British Medical Association. His *Guide to Dietary Supplements* summarizes the current scientific basis for the use of mineral and vitamin supplements. His book *The Nutrition Transition: Diet and Disease in the Developing World* explored the impact of demographic and economic development on diet- and lifestyle-related diseases in developing countries. His book *Obesity in China* summarizes research conducted in rural and urban China to track the impact of socioeconomic development on health outcomes. He is also coeditor of a widely used textbook on human nutrition, *Modern Nutrition in Health and Disease*.

He is a member of the Spanish Academy of Nutritional Sciences, and a Fellow of the American Society for Nutrition and of the Royal Society of Medicine (UK). Recent awards include the Donald Medearis Lectureship from the Massachusetts General Hospital/Harvard Medical School, the Mataix Prize for lifetime achievements in nutrition science from the Spanish Academy of Nutritional Sciences, the Ancel Keys Prize for achievements in international public health, and the Thompson–Beaudette Lectureship from Rutgers University.

Paul Finglas joined the Institute of Food Research in 1981 and is currently head of the Food Databanks National Platform and Research Leader in Food and Health at the Institute (http://www.ifr.ac.uk/science/platform/FD/default.html). He has, for most of his science career, been involved in a wide range of research in food composition and analysis, and the nutritional effects of micronutrients in food and health research. Paul has considerable experience of co-coordinating both national and international projects (e.g., EuroFIR, TDS-EXPOSURE, Bacchus and QualiFY (all EU FP7), and is currently of the spin-out EuroFIR AISBL, a non-profit international association based in Belgium, from one of these projects. Paul has a broad range of experience in science publishing and is currently editor of the journals *Food Chemistry* and *Trends in Food Science and Technology*, and was one of the coeditors for the *Encyclopedia of Food Science and Nutrition* (2nd Ed.). Paul has a degree in chemistry from Aston University in Birmingham and has published over 150 publications on a wide range of topics in food science and nutrition.



Fidel Toldrá holds a BSc in chemistry (1980), high degree on food technology (1981) and PhD in chemistry from the University of Valencia (1984). Professor Toldrá was a Fulbright postdoctoral scholar at Purdue University in West Lafayette (US, 1985–86) and visiting scientist at the University of Wisconsin-Madison (1991 and 1995), and the Institute of Food Research-Bristol (UK, 1987). Currently, he is research professor at the Instituto de Agroquímica y Tecnología de Alimentos (CSIC), in Paterna, Valencia (Spain). He is also associate professor of food technology at the Polytechnical University of Valencia.

Prof. Toldrá has focused his research on food biochemistry and its relationship with nutrition, quality and safety. He has filed 12 patents, directed 22 PhD thesis and published over 245 manuscripts in recognized scientific journals and more than 115 chapters of books. His h-index is 41. Prof. Toldrá has authored two books and edited/co-edited more than 30 books for major publishers like CRC Press, Wiley-Blackwell, Elsevier and Springer.

Prof. Toldrá is the European editor of *Trends in Food Science and Technology* (2005–) and associate editor of *Meat Science* (2014–); he was the editor-in-chief of *Current Nutrition & Food Science* (2005–2012), section editor of the *Journal of Muscle Foods* (2009–2010) and guest editor of 12 special journals issues. He is a member of the editorial boards of *Food Chemistry*, *Food Analytical Methods*, *Journal of Food Engineering*, *Journal of Food and Nutrition Research*, *The Open Nutrition Journal*, *The Open Enzyme Inhibition Journal*, *Recent Patents in Agriculture*, *Food and Nutrition*, *Food Science & Nutrition* and *Current Opinion in Food Science*.

He has been a member of the Scientific Panel on food additives, flavorings, processing aids and materials in contact with foods (periods 2003–2008) and the Scientific Panel on flavorings, enzymes, processing aids and materials in contact with foods (2008–2015) of the European Food Safety Authority (EFSA) acting as Chairman of the Working groups on Irradiation (2009–2010), Processing Aids (2011–2014) and Enzymes (2010–2015). He was a member of FAO/WHO group of experts to evaluate chlorine-based disinfectants in the processing of foods (2008–2009). He was a member of the Executive Committee of the European Federation of Food Science and Technology (EFFOST, 2002–2009). He is a Fellow of the International Academy of Food Science and Technology (IAFOST, 2008) and of the Institute of Food Technologists (IFT, 2009–). He received the Iber Award on Food and Cardiovascular Diseases (1992), the Institute Danone award in Food, Nutrition and Health (2001), the International Prize for Meat Science and Technology from the International Meat Secretariat (2002), GEA award on R+D activity from the Valencian Community (2002), and the Distinguished Research Award (2010) and Meat Processing Award (2014), both from the American Meat Science Association.

EDITORIAL ADVISORY BOARD

Siân Astley has worked extensively with individuals and organizations throughout Europe from a variety of disciplines including research, food and biotech industries, and the media. She is the author of more than 300 popular science articles for magazines and trade publications as well as 25 peer-reviewed papers, and she was awarded her Diploma in Science Communication in 2009 (Birkbeck University of London). After 14 years as a bench-scientist, Siân became communications manager for NuGO, one of the first FP6 networks of excellence, and was the European communications manager for the Institute of Food Research in Norwich (UK) until April 2012. Currently, she is the training and communications manager for the European Food Information Resource (EuroFIR AISBL) supporting training within EU-funded research projects and networks, and communication of research activities.

David J. Baer is a supervisory research physiologist with the US Department of Agriculture's Beltsville Human Nutrition Research Center located in Beltsville, Maryland. He serves as the research leader for the Center's Food Components and Health Laboratory and also serves as the director of the Center's Human Studies Facility.

Dr. Baer conducts controlled dietary intervention studies to investigate the relationship between diet and the risk for chronic degenerative diseases, especially cardiovascular disease, cancer, and diabetes in people. His research also includes studies on the health impacts of weight gain and determining the calorie content of foods. Some of the dietary interventions he has investigated include the effects of different types of protein, fats and fatty acids, fiber, margarine, butter, plant sterols, salad dressings, nuts, whole grains, berries, alcohol, and tea on overall nutrition and health. In addition to dietary intervention studies, Dr. Baer is involved in research studies to validate food survey methodologies and in developing new methods for dietary assessment.

Dr. Baer earned his bachelor's degree from the University of Illinois and his master's and doctorate in nutrition from Michigan State University.

Marina Carcea was awarded a master degree in agricultural sciences at the University of Pisa, Italy "cum laude" in 1980, and a PhD in food science also "cum laude."

She is currently a senior researcher in the Research Center on Food and Nutrition of the Council for research in agriculture and analysis of agricultural economy (CRA-NUT formerly INRAN National Research Institute on Food and Nutrition) and she was the director of the Cereals Research Programme in INRAN. CRA-NUT is a primary research institute in Italy under the egis of the Ministry of Agriculture. Dr. Carcea joined INRAN in 1989 after having worked in Italian and English universities (Queen Elizabeth College, King's College, and University of London) and after a two-year contract with the Food and Agriculture Organization (FAO) of the United Nations (UN), Rome.

She has a vast experience in the field of research on foods, cereals in particular. In recent years, her main research interests have been: chemical characterization and study of the functional properties of cereal components; study of the interactions between components and of the interrelationships between the biochemical properties of components and the technological properties of the raw material and derived products; development of new, cereal-based products; development of methods to assess technological parameters of the raw material; nutritional value of cereals; and developments of protocols for quality assurance of cereals, food authenticity.

She has taken part and/or co-ordinated several research projects within national or international programs (European Commission, FAO) involving several institutions. She is the author of more than 160 scientific publications, mostly in international journals, eight book chapters, and two scientific books. She delivered lectures on her research activity at about 150 national and international congresses and she seats in several national and international committees (Italian Ministry of Agriculture, Codex Alimentarius, and European Commission) regarding food and nutrition topics. She is also a member of the editorial board of scientific journals.

From 1994 to 2006, she has also been a lecturer of food science and technology at the University of Tor Vergata, Rome, Italy.

She is a founding member of AISTEC, the Italian Association of Cereal Science and Technology. Since 1996, she is an elected member in the Executive Committee of the same association and since 2009, president of the association.

Since 2000, she is the Italian National Delegate of the International Association for Cereal Science and Technology (ICC) and she was also the president of the same association for 2011–2012.

In 2004, she was the first woman to be awarded the International Harald Perten Prize for her excellent research achievements in the field of cereal science and technology.

She is also a member of the Georgofili Academy in Florence, Italy.

Lawrence J. Cheskin graduated from Dartmouth Medical School and completed a fellowship in gastroenterology at Yale–New Haven Hospital. He is an associate professor of health, behavior, and society at the Johns Hopkins Bloomberg School of Public Health, with a joint appointment in International Health–Human Nutrition, and in medicine (GI) at the Johns Hopkins University School of Medicine. Dr. Cheskin is also a founder and director of the Johns Hopkins Weight Management Center, a comprehensive treatment program for obesity.

In his research, Dr. Cheskin has studied the effects of medications on body weight, the gastrointestinal effects of olestra, how cigarette smoking relates to dieting and body weight, and the effectiveness of lifestyle and dietary changes in weight loss and weight maintenance.

He is also the author of four books: *Losing Weight for Good*, *New Hope for People with Weight Problems*, *Better Homes and Gardens' 3 Steps to Weight Loss*, and *Healing Heartburn*. Dr. Cheskin has appeared on television news programs and lectured to both professional and lay audiences on the topics of obesity and weight control.

Nigel Cook is a graduate of the University of Dundee. After postdoctoral research in the Universities of Aberdeen and Leicester, he moved to the Central Science Laboratory (now the Food and Environment Research Agency (FERA)) at the Food Science Laboratory, Torry, Aberdeen in September 1994, before relocating to new facilities in York. At FERA, he studies the transmission of pathogens, particularly enteric viruses, through foods and the environment. He has a visiting professorship at the Katholieke Universiteit Leuven in Belgium. He is a councilor of the International Association for Food and Environmental Virology. He is a project leader within the standardization working group ISO TC34 SC9 WG6, currently developing a standard for detection of *Cryptosporidium* and *Giardia* on berry fruits and leafy green vegetables. He was a coordinator of the European Framework 7 project "Integrated monitoring and control of foodborne viruses in European food supply chains (VITAL)," and a chair of COST Action 929 "A European Network for Environmental and Food Virology" from 2006 to 2010. Between 2009 and 2014, he was a member of various European Food Safety Authority's Working Groups preparing opinions on the risk of foodborne viruses, and represented the European Communities on the Codex Committee on Food Hygiene Working Group developing *Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food*. He was a member of the UK Advisory Committee on the Microbiological Safety of Food's Viral Infections Subgroup. He was the founding editor of the journal *Food and Environmental Virology*, published by Springer.

Luca Simone Cocolin graduated in 1994 in food science with a grade of 110/110 and remark followed by food biotechnology PhD studies from 1995 to 1998. In February 1999, he defended his thesis acquiring the title of PhD in food biotechnology. From 1998 to 2001, he received a scholarship from the Friuli Venezia Giulia region (Italy). From November 1, 2001, he was an assistant professor at the University of Udine, Faculty of Agriculture, Food Science Department, Italy, and in October 1, 2006, he became an associate professor at the University of Torino, Italy. In January 2014, he had the habilitation for full professor and from June 2015, he is the full professor in food microbiology at the University of Torino.

From September 2008, he is an executive board member of the International Committee on Food Microbiology and Hygiene (ICFM) part of the International Union of Microbiological Societies (IUMS) (http://www.icfmh.org/). From January 2008, he is the editor-in-chief of the *International Journal of Food Microbiology* and he is a member of the editorial board of *Applied and Environmental Microbiology*, *Food Analytical Methods*, *Frontiers in Food Microbiology*, and *Frontiers in Nutrition and Food Science Technology*. He regularly reviews paper for *Food Microbiology*, *Meat Science*, *Journal of Applied Microbiology*, and *Letters in Applied Microbiology*. He is a co-author of more than 180 papers on national and international journals and he attended national and international congresses with oral and poster presentations. On Scopus (www.scopus.com, consulted on March 2015) he has 172 documents reviewed, which were cited 3520 times, resulting in an h index of 33.

His scientific interests comprise:

development, optimization, and application of molecular methods for the detection, quantification, and characterization of foodborne pathogens;
study of the microbial ecology of fermented foods (mainly sausage, cheese, and wine) by using culture independent and dependent methods;
bioprotection: molecular characterization of bacteriocin production and its study *in vitro* and *in situ*;
selection of new putative probiotics from artisanal fermented foods; and
study of the human microbiome and its influence on human health.

Christopher Duggan, for the past 25 years, has been performing clinical trials in the fields of pediatric nutrition, gastroenterology, and global health. His early work centered on the management of diarrheal diseases in children, including trials that demonstrated the feasibility and efficacy of oral rehydration solutions (ORS) for diarrhea management in the United States and globally. In collaboration with colleagues at Harvard TS Chan School of Public Health and Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania, Dr. Duggan and colleagues are evaluating the efficacy of micronutrient supplementation in infants and young children born to women with or at risk of HIV infection. Recent studies include the development of new biomarkers of environmental enteric dysfunction as well as the evaluation of nutritional status on neurodevelopment. With colleagues at St John's Research Institute in Bangalore, India, he is evaluating the efficacy of maternal vitamin B12 supplementation on biochemical and clinical parameters during pregnancy and infancy. He is a course co-director of the Bangalore, Boston Nutrition Collaborative (http://bbnc.globalhealth.harvard.edu). Past and present research support has come from the National Institutes of Health, the Gates Foundation, and the World Health Organization.

In addition to his global health research interests, he is a pediatric gastroenterologist and a nutrition physician at Boston Children's Hospital where he directs the Center for Nutrition (http://www.childrenshospital.org/nutrition). He is a medical director of the Center for Advanced Intestinal Rehabilitation, one of the largest centers in the United States for the care of children with intestinal failure/chronic diarrhea syndromes (http://www.childrenshospital.org/cair). He is also the course co-director of an inaugural Harvard College course "Nutrition and Global Health" and mentors undergraduate, graduate, and postdoctoral students at HMS and HSPH (http://www.hsph.harvard.edu/nutrition-and-global-health/).

He is a professor of pediatrics at Harvard Medical School and a professor in the Department of Nutrition at the Harvard TS Chan School of Public Health (http://www.hsph.harvard.edu/faculty/christopher-duggan/).

Jed William Fahey's current research concerns elucidating the mechanisms of how plants protect themselves against unfavorable and stressful conditions, and how this understanding can be translated to chemoprotection of eukaryotic mammalian systems. This work draws on elements of natural product chemistry, enzymology, nutritional epidemiology, and clinical research in order with isothiocyanates (e.g., sulforaphane) and glucosinolates. His work led to the discovery that broccoli sprouts are an exceptionally rich and consistent source of phytochemicals that induce the detoxification of carcinogens, and to the development of methods for their detection and for assessing their metabolism in humans. He discovered that one of the inducers, sulforaphane, has potent antibiotic activity against *Helicobacter pylori*, a causative agent of peptic ulcer and stomach cancer, and followed up with trials in animals and in *H. pylori*-infected humans. Ongoing collaborations examine the effects of broccoli, Moringa, and the other plants and their phytochemicals against a range of chronic diseases. Dr. Fahey has for years taught courses in chronic disease prevention and nutrition at both medical and public health schools.

Manuel Franco is an associate professor at University of Alcalá in Madrid (Spain) where he leads the social and cardiovascular epidemiology research group (http://www3.uah.es/cardiosocialepi/). He is also an adjunct professor at Johns Hopkins University (Baltimore).

Prof. Franco's work focuses on the social determinants of cardiovascular diseases and its major risk factors as diet. His methodological interests include the measurement of the urban environment and large social and economic changes in relation to cardiovascular health. He is the lead investigator of the Heart Healthy Hoods, study funded by the European Research Council, that will study the urban environment in relation to cardiovascular health in Madrid (http://hhhproject.eu/). This longitudinal study will be collecting neighborhood level data (via audits, Google Street view, photovoice, and qualitative methods) and linking them to clinical outcomes collected from patients enrolled at the City of Madrid primary healthcare clinics. Prof. Franco trained in Spain and Germany to obtain his MD and obtained his PhD from Johns Hopkins Bloomberg School of Public Health working with Dr. Ana Diez-Roux in the MESA study on food environment and dietary patterns. He has published over 30 international high impact articles and collaborates with universities in the United States, Europe, and Latin America.

Maria Glibetic is a research director of Centre of Research Excellence in nutrition research, Institute for Medical Research in Belgrade, University of Belgrade, Serbia, and member of executive board of directors for food data association EuroFIR AISBL. She is an experienced basic and nutritional scientist with over 250 scientific publications and presentations. Maria has considerable experience in leading national and international projects and since 2006, she participated in nine EU funded projects including EuroFIR, EURRECA, BaseFOOD, CHANCE, BACCHUS, and ODIN. Maria and her team are responsible for the creation of the first online national food database, for designing food data management system, and for the development of different nutritional tools for intake analysis. She was a principal leader of many nutrition intervention studies evaluating the plant bioactive component effects on human cardiovascular health. She leads postgraduate department for integrated nutritional sciences at University of Belgrade, where she teaches two courses.

Linda Harvey obtained her PhD from the University of East Anglia, UK. She is currently the head of the Human Nutrition Unit at the Institute of Food Research, Norwich, UK. Her research interests include micronutrient requirements, bioavailability, and metabolism.

Ronald Jackson received his bachelor's and master's degrees from Queen's University and doctorate from the University of Toronto. His time in Vineland, Ontario, and subsequent sabbatical at Cornell University, redirected his interest in *Botrytis* toward viticulture and enology. As part of his teaching duties at Brandon University, he developed the first wine technology course in Canada. For many years he was a technical advisor to the Manitoba Liquor Control Commission, developing sensory tests to assess candidates of its sensory panel, and was a member of its external tasting panel. He is the author of *Wine Science: Principles and Applications, 4th edition* (2014), *Wine Tasting: A Professional Handbook, 2nd edition* (2009), *Conserve Water, Drink Wine*, and chapters and technical reviews in other multiple books and encyclopedia. He is retired in Bronte, Ontario, but remains active writing, cycling, doing yoga, and traveling, as well as being a fellow in the Cool Climate Viticulture and Oenology Institute, Brock University, St. Catharines, Ontario, Canada.

Joe P. Kerry is a senior college lecturer and the head of the food packaging research group in the School of Food and Nutritional Sciences at University College Cork (UCC). He received his doctorate in microbiology at University College Galway in 1995. Prof. Kerry is also a qualified member of the Institute of Packaging. He is very involved in national and international research projects both at fundamental and applied levels. Primary research interests address various aspects of food packaging, shelf-life stability, food composition, and numerous aspects of food quality, particularly in relation to muscle foods. He also has very strong links with industry and his research team assists companies in relation to many aspects of new food product development. He has over 220 publications in peer-reviewed international journals, over 300 presentations at major international conferences, along with several other significant publications. His expertise includes use and manipulation of modified atmosphere packaging systems for use with foods, use of extrusion technology for the manufacture of food products/packaging materials, and applications and sensor/new technology developments within the area of food packaging, especially in the area of smart packaging.

Frédéric Leroy, after studying bio-engineering at Ghent University, obtained a PhD in applied biological sciences at the Vrije Universiteit Brussel in 2002, where he continued his academic career at the research group of Industrial Microbiology and Food Biotechnology (faculty of sciences and bio-engineering sciences). As associate professor, his lecturing activities include courses in food science and technology (i.e., "Nutrition," "Technology of animal products," "Food microbiology and ecology," and "Quantitative and predictive microbiology"). Dr. Leroy's research primarily deals with the ecology and functional roles of bacterial communities in (fermented) foods, in particular with respect to the generation of quality, safety, and/or nutritional and health advantages. Focus is mostly on meat products, but other food systems are also being studied, including fermented milks and sourdough breads. In addition, his research interests relate to elements of tradition and innovation in foods, both from a technological and societal point of view.

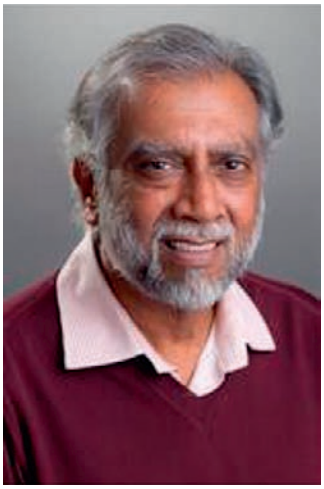
Catherine M. Logue completed her undergraduate and postgraduate degrees in Ireland and earned a PhD in meat microbiology from the University of Ulster, UK in 1996. Dr. Logue was a faculty member at North Dakota State University from 1999 to 2011 rising through the ranks of assistant to associate and full professor. In 2011, she re-located to Iowa State University's College of Veterinary Medicine and is a professor of veterinary microbiology and preventive medicine. Dr. Logue is also the director of faculty and staff advancement and equity for the college. Her research interests focus on foodborne pathogens of food animals and the contamination of meat and meat products destined for human consumption. Her research studies the detection, isolation, and characterization of a range of foodborne pathogens such as *Salmonella*, *Campylobacter*, *Listeria*, *Escherichia coli*, and methicillin-resistant *Staphylococcus aureus* (MRSA) in poultry, bovine, and swine. She also focuses her research on antimicrobial resistance in commensals and pathogens of production animals. She has been an author and a co-author on more than 90 peer-reviewed papers and book chapters as well as more than 150 abstracts and presentations at national and international meetings.

F. Xavier Malcata graduated in chemical engineering in 1986 from the University of Porto (Portugal), received a PhD in chemical engineering/food science from University of Wisconsin, Madison (USA) in 1991, and his habilitation in food science and engineering by Portuguese Catholic University in 2002. He was the dean of College of Biotechnology of Portuguese Catholic University, the chairman of Portuguese Society of Biotechnology, Portuguese representative at VI and VII European Union Framework Programs of research and development, expert for European Food Safety Agency, and a co-ordinator of Portuguese Engineering Accreditation Board in chemical engineering for Northern Region. He is currently a full professor at University of Porto.

His major research interests have focused on technological improvement of traditional Portuguese foods and upgrade of byproducts thereof, development of nutraceutical ingredients and functional foods, design and optimization of enzymatic reactors for edible oil processing, characterization of plant proteases toward cheese and whey cheese manufacture, production of starter and nonstarter cultures from adventitious microflora, and optimized application of unit operations to food processing.

With an academic career of independent research and teaching for more than two decades, Prof. Malcata published more than 450 papers in refereed journals worldwide, wrote 11 books, and prepared more than 45 chapters for edited books. Among many international distinctions, he was

recipient of Ralph H. Potts Memorial Award in 1991 by American Oil Chemists' Society (AOCS, USA), Foundation Scholar Award – Dairy Foods in 1998 by American Dairy Science Association (ADSA, USA), Young Scientist Research Award in 2001 by AOCS, Canadian/International Constituency Investigator Award in physical sciences and engineering in 2002 and 2004 by Sigma Xi (USA), Danisco International Dairy Science Award in 2007 by ADSA, Scientist of the Year Award in 2007 by European Federation of Food Science and Technology (the Netherlands), Samuel C. Prescott Award in 2008 by Institute of Food Technologists (IFT, USA), International Leadership Award in 2008 by International Association for Food Protection (IAFP, USA), Elmer Marth Educator Award in 2011 by IAFP, Distinguished Service Award in 2012 by ADSA, and William V. Cruess Award in 2014 by IFT. He has been elected for the honor societies of food science (Phi Tau Sigma, USA), scientific research (Sigma Xi, USA), and engineering (Tau Beta Pi, USA). He was also elected for fellow of IFT, ADSA, AOCS, and International Academy of Food Science and Technology.



Gopinadhan Paliyath is a professor at the Department of Plant Agriculture, University of Guelph, and the research program director for "Food for Health," under the UG/OMAFRA partnership. Dr. Paliyath is a biochemist and has an interest in various aspects of fruits and vegetables, specifically the nutraceutical components and their mechanism of action. He obtained his BSc Ed degree (botany and chemistry) from the University of Mysore, MSc degree (botany) from the University of Calicut, and PhD degree (biochemistry) from the Indian Institute of Science, Bangalore. Subsequently, he did postdoctoral work at Washington State University, University of Waterloo, and University of Guelph. Dr. Paliyath's research is focused on the biochemistry of plant senescence, specifically pertaining to postharvest biology and technology of fruits and vegetables. Investigations on the role of phospholipase D (PLD) in membrane homeostasis and signal transduction have led to advances in the understanding of the mechanism of membrane deterioration that occur during stress and senescence. Another aspect of his research is focused on understanding the mechanism of action of food components in disease prevention. The efficacy, bio-accessibility, bioavailability, and molecular mechanisms of action of nutraceuticals in fruits and processed products in relation to their cancer-preventive and anti-inflammatory actions are being investigated using mammalian cell lines, and animal model systems.

Dr. Paliyath has developed technologies and products for enhancing the shelf life and quality of fruits and vegetables based on PLD inhibition. R&D activities relevant to the industry sector include: (1) optimization of an enhanced freshness formulation for application to various fruits, vegetables, and flowers; (2) developing methods for nutraceutical carriers that would enhance the functional food quality and delivery (e.g., stabilizing lycopene in tomato juice, sauce, etc., for health beneficial effects); and (3) developing novel technologies to enhance the cancer-preventive ingredients in fruit products, etc. Patents awarded include: (1) # 6,514,914 (US) and 2,298,249 (Canada); (2) #7,198,811 (USA), 4141387-1 (Japan), 260738 (Mexico), 1469736 (Turkey), 028 284763 (China), and 223077 (India). The patents describe the use of nanoformulations based on hexanal and other generally regarded as safe (GRAS) ingredients for enhancing the shelf life and quality of fruits, vegetables, and flowers by pre or postharvest treatments. These technologies are currently being evaluated for extending shelf life and quality of mango in India and Sri Lanka with the assistance of the Canadian International Food Security Research fund. The collaboration involves researchers from Canada, India (Tamil Nadu Agricultural University), and Sri Lanka (Industrial Technology Institute).

Dr. Paliyath is also the research program director for the food and health theme-related activities under the OMAF/MRA/University of Guelph research partnership. He serves on the editorial board of several journals. He is a member of American Chemical Society and Canadian Society of Plant Biologists.

(Total-refereed publications in journals – 92; patents and intellectual properties – 2; disclosures – 4; chapters in books – 27; nonrefereed contributions – 10; research reports – 28; conference proceedings – 88; edited books – 9; book reviews – 6 (Google Scholar: h index – 31, i10 index – 68, citations – 4332; RG score – 35.63)).

Yolanda Picó is a full professor of nutrition and food science at the University of Valencia since 1998. She is currently the head of the research group on food and environmental safety of the University of Valencia. Her research interests are the development of new analytical methods to determine organic contaminants in food and the environment, identification of unknown compounds by liquid chromatography–mass spectrometry, micro-extraction separations, and environmental and food safety. To the date, she is the author of nearly 200 peer-reviewed papers, 170 scientific papers in journals of SCI, 25 book chapters, and editor of four books on food and environmental safety.

Vieno Piironen is a professor of food chemistry at the Department of Food and Environmental Sciences, University of Helsinki, Finland. She received her PhD in food chemistry at the University of Helsinki in 1987 and has approximately 35 years of experience in food research and education on bachelor, master, and PhD levels. She has participated actively in international research and education projects and networks. Her research has focused especially on chemical and nutritional properties, reactions and analysis of lipids, vitamins, and other bioactive compounds. Research on vitamins has been active from the beginning of 1980s. She has studied both lipid- and water-soluble vitamins; their chemical and nutritional properties and importance in foods and diets as well as factors influencing vitamin levels. In addition, development of analytical methods for different vitamers as well as validation and harmonization of the methods through international collaboration have been among the priorities. Recent collaboration projects have focused on enhancing vitamin contents in cereal-based foods by plant breeding, utilization of vitamin-rich grain fractions, and bioprocessing. Currently, the research focus lies on investigating microbial *in situ* synthesis of folate, vitamin B12, and other B vitamins in cereal and legume matrices as a means to improve nutritional quality of foods and to develop new food applications. In lipid research, different lipid classes and their chemical and enzymatic reactions in food matrices are studied. Diverse methods are used to study proceeding of oxidation from primary products to monomeric oxides, volatiles, and polymerization products and to study possibilities to control oxidation. Controlling enzymatic reactions leading to off-flavors in cereal and legume matrices is also among the interests. Phytosterols and their conjugates have been studied as natural food components belonging to the dietary fiber complex. On the other hand, questions related to sterol enrichment such as oxidation susceptibility and mechanisms as well as factors affecting oxidation reactions have been of interest. She has also studied nutrients and anti-nutrients in legumes and more recently started research on utilization of high value components in microalgae. She has approximately 160 papers in international journals and a number of other publications.

David Rodríguez-Lázaro is a doctor in veterinary medicine (DVM), specialized in food science (BSc and MSc) and molecular microbiology (PhD). He is a senior scientist at ITACyL and an assistant professor of microbiology at the University of Burgos. He has performed research stays in the Danish Institute for Food and Veterinary Research (Denmark), the University of Prague (Czech Republic), the Food and Environmental Research Agency (UK), and the University of Bristol (UK). He was a Leverhulme visiting professor in the Institute of Advanced Sciences in the University of Bristol during the years 2004 and 2005 and Marie Curie research fellow in the faculty of medical and veterinary sciences in the University of Bristol (UK) until 2007.

His research interest is focused on the establishment of reliable, quantitative molecular strategies for detection of important food-borne pathogens from environmental sources and various types of foodstuffs, the characterization of the prevalence of the main foodborne pathogens in food and food-related environments, and the development of emergent food preservation processes and their effects in the microbial virulence. He has participated in a number of coordinated EU-funded projects such as PROMISE, BASELINE, VITAL, FOOD-PCR, SACROHN, and MONI-QA, having established active links with the leading European research groups working in food safety.

He has published more than 100 international scientific papers and book or book chapters regarding food safety. He is currently a member of the editorial board of *Applied and Environmental Microbiology*, *International Journal of Food Microbiology*, *Food and Environmental Virology*, and *International Journal of Food Contamination* and the editor-in-chief of the journal *Food Analytical Methods*. He was awarded with the XV Jaime Ferrán Award in 2013 by the Spanish Society for Microbiology for his promising scientific career in microbiology.

Turid Rustad is a professor and the head of the food science group at the Department of Biotechnology, Norwegian University of Science and Technology. The main research focuses on the biochemistry of marine raw materials, the relationship between biochemistry and quality, and changes in raw material properties during processing. Studies of enzymatic activities in different raw materials have been linked to studies of changes in the biochemistry of these raw materials. She has worked with characterization of composition and enzymatic processes in a wide range of different raw materials, such as fish, fish by-products, and zooplankton in relation to different storage and processing methods such as chilling, heating, superchilling, and frozen storage.

Noel W. Solomons has lived and worked in Guatemala for 40 years. He was born and educated in Massachusetts in the United States. As a young child, he became an amateur naturalist and was a nature counselor at various summer camps; this would guide him to a career in science. In his young adulthood, he would participate in the civil rights and anti-war movements, only to become disillusioned by the intractable nature of the injustice elements in the fabric of American society. As a physician by graduate training, he performed his university studies at Harvard College and Harvard Medical School; it was during overseas electives in his medical training that he visited Peru and Colombia and committed to an expatriate life trajectory outside of his homeland. Clinical training included a residency in internal medicine and infectious diseases at the Hospital of the University of Pennsylvania and specialization in gastroenterology and clinical nutrition at the University of Chicago. He became a resident of Guatemala in 1974 as an affiliated investigator at the Institute of Nutrition of Central America and Panama. He would later commute for eight years to a faculty position in the Department of Nutrition and Food Science of the Massachusetts Institute of Technology. Assuming a full-time Guatemala commitment in 1985, he co-founded the Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM) where he remains its scientific director. Over 40 local university theses have been completed by Central American students in that institution as well as an equal number of master's degree research projects from international students from Europe, and North and South America. He has supervised doctoral dissertations for 12 PhD candidates from the United States, Canada, Germany, Spain, and the Netherlands through CeSSIAM.

Dr. Solomons has 332 publications indexed on Medline. In addition, he has edited two books and contributed over 100 articles, reviews, editorials, and commentaries in nonindexed venues and over 50 book chapters. These are dedicated to the scientific and academic interests of his career including: clinical nutrition; human growth and body composition; lactose maldigestion; dietary intake, nutritional status, intestinal absorption, and food fortification related to various micronutrients (vitamins, trace elements, and essential fatty acids); complementary feeding; nutrition in aging and chronic disease; and the interaction of malnutrition and infection.

Among the honors bestowed upon Dr. Solomons are the International Nutrition Prize of the International Union of Nutritional Sciences and the Kellogg Prize of the Society for International Nutrition Research. He is a fellow of the American Society of Nutrition. He is an academic member of the Guatemalan Academy of Medical, Physical and Natural Sciences and the Spanish Academy of Nutrition and Food Science. He was the awardee of the 2010 National Medal for Science and Technology for Guatemala.

He has been a visiting professor in university courses in Mexico, Peru, Brazil, Indonesia, and Spain. He currently holds adjunct professorial appointments at the Boston University School of Public Health, and the Friedman School of Nutrition Science and Policy and the Department of Community Medicine and Public Health, both at Tufts University. He is a founding board of directors, member of the Hildegard Grunow Foundation in Munich and the Essential Nutrient Foundation of Singapore. Finally, Dr. Solomons is a coordinator for Central America of the Nevin Scrimshaw International Nutrition Foundation in Boston, and an associate editor for the Foundation's *Food and Nutrition Bulletin*. He serves on editorial boards for ten scientific journals.

Maria Tsimidou is a professor of food chemistry and the head of the Laboratory of Chemistry and Technology in the School of Chemistry at the Aristotle University of Thessaloniki (AUTh), Greece. Her teaching is food chemistry, analysis, quality control, and food legislation. Research interests are related to virgin olive oil chemistry, quality and authenticity, saffron chemistry, authenticity and quality, antioxidant activity of plant extracts and constituents, new sources of targeted bioactive compounds (squalene, carotenoids, and phenols), and analytical procedures for their determination. She has published many research papers, review articles, and contributions to scientific books and encyclopedias on the above-mentioned topics. Currently, she is an associate editor in the *European Journal of Lipid Science and Technology* and chairs the COST ACTION FA1101 "Saffronomics."

Jorge Welti-Chanes earned his degree in biochemical engineering (1976) and master of science in food engineering (1978) at Tecnológico de Monterrey (ITESM, Mexico), later he moved to Spain to perform his doctoral studies in chemistry, in the area of food technology, obtaining his degree at the University of Valencia. He is currently the national director of graduate studies at School of Engineering and Sciences at Tecnológico de Monterrey also is professor and researcher in the areas of biotechnology and food at the same institution. He started his academic activity in 1976 as a university professor of ITESM, has additionally been a full professor at the National Polytechnic Institute (IPN, Mexico) and the University of the Americas, Puebla, Mexico (UDLA). He has an experience of 37 years as a teacher and university researcher, 20 of which were spent in combination with the development of administration work in education, science and technology. In the UDLA, he was teaching in the Departments of Chemistry and Biology and Chemical Engineering and Food, in the latter was responsible for the leadership for a period of a year and subsequently became dean of the School of Engineering (1986–1988). From January 1989 to June 2002, he was an academic vice chancellor at UDLA. He has published 14 books and has over 200 scientific publications in refereed journals and books, has given more than 250 presentations at international conferences. He is an associate editor of the journals *Food Engineering Reviews* and *Journal of Food Science* and participates as a member of the editorial boards of *Journal of Food Engineering* and *Current Opinion in Food Science*. In May 2011, he received the Life Achievement Award by the International Association for Engineering and Food (IAEF), for his career as a researcher and academic worldwide, and in January 2014, the Romulo Garza Award from the Tecnológico de Monterrey for the impact of their research work and as recognition for being one of the most productive researchers in the life of Tecnológico de Monterrey. He has been the president of ISOPOW and IAEF and is the currently president of the International Society of Food Engineering (ISFE).

Peter J. Wilde graduated in biophysics at the University of East Anglia in 1985 and has been researching the colloidal and interfacial properties of food systems at the Institute of Food Research (IFR) for over 25 years. IFR is the only publicly funded UK research institute that focuses on the underlying science of food and health to address the global challenges of food security, diet, and health, healthy aging, and food waste. IFR is the one of eight institutes that receives strategic funding from the Biotechnology and Biological Sciences Research Council (BBSRC). It also receives funding from government agencies and departments, the EU, charities, and industry, from the UK and overseas.

Pete's research expertise is the interfacial behavior of proteins and other surface active components in food relevant systems. The aim is to determine how the molecular and interfacial processes control the functionality of foams and emulsions. Currently, the functional aspects of his research have focused on improving the dietary impact of emulsified foods. These include fundamental studies on how interfacial layers control emulsion rheology to develop novel fat reduction strategies; the design of interfacial structures to control lipid digestion to promote satiety or the delivery of fat-soluble nutrients and drugs; and to determine the physico-chemical role played by the salivary film in perceiving fat content in emulsions. The impact of this research will be to aid the rational design of foods with enhanced nutritional benefits to address the global challenges of obesity, type 2 diabetes, and other major diet-related conditions.

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HOW TO USE THE ENCYCLOPEDIA

All articles in the encyclopedia are arranged alphabetically as a series of entries.

1. Contents

Your first point of reference will likely be the contents. The complete contents list appears at the front of each volume providing volume and page numbers of the entry. We also display the article title in the running headers on each page so you are able to identify your location and browse the work in this manner.

2. Cross-references

The majority of articles within the encyclopedia have an extensive list of cross-references that appear at the end of each article, for example:

See also: Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Iron: Biosynthesis and Significance of Heme; Iron: Physiology of Iron.

3. Index

The index provides the volume and page number for where the material is located, and the index entries differentiate between material that is a whole article; is part of an article, part of a table, or in a figure.

4. Contributors

A full list of contributors appears at the end of volume 5.

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INTRODUCTION

Until a few decades ago, virtually all known health effects of foods were related to their content of essential nutrients. The clinical description of most diet-related illnesses mirrored the signs of essential nutrient deficiencies, such as pellagra, beriberi, and others. Consequently, the key public health concern regarding diet was ensuring that everyone consumed enough food. It was only in the past 50 years that large-scale epidemiological observations began to associate chronic diseases like diabetes and cardiovascular disease with nonessential diet constituents such as saturated fat, fiber, and cholesterol. Taking advantage of the emergence of digital informatics, these studies were able to manipulate increasingly large sets of data and provide, for the first time, a picture of the secular changes in the health of large populations and its association with what they ate regularly. These findings progressively shifted the concern from eating enough to avoiding excessive consumption of certain foods. Eating enough was replaced by eating well.

But it turned out that defining how to eat well is far more complex than defining minimum needs of essential nutrients. First, there is no single paradigm to study those relationships, given the wide variety of biological mechanisms and the long exposures involved. Second, many of the experimental models used to define essential nutrient needs are not applicable to the study of long-term effects of diets in free-living populations. And it is now clear that experiments with isolated dietary compounds do not reflect the actual effects of the complex food matrix we consume daily. Finally, while the discovery of essential nutrients and their role in health was the domain of a few specialties speaking a common language (primarily biochemists and physiologists), the study of the long-term effects of whole diets in humans must of necessity involve epidemiologists, social and behavioral scientists, food scientists, clinicians, policy experts, etc., making far more difficult the development of consensus and foundational concepts.

It is thus not surprising that today we have still not achieved a stable consensus on how to eat 'well.' Furthermore, while few nonscientists would care about the minimum requirement of a vitamin to sustain life, there are plenty of opinions among nonscientists on how to eat 'well.'

Our goal in preparing this encyclopedia has been to contribute to the understanding of that complex diet–health relationship by providing a multidisciplinary, integrative and accurate source of information. We aim to serve the needs not only of established and in-training scientists, but also of the increasingly important group of professionals who are key to disseminate and sustain the practice of science: journalists, science writers, science administrators, fund raisers, donors, and policymakers. In preparing this work, we had the enormous advantage of working with one of the publishers with the most extensive expertise in major reference works, Elsevier. This first edition builds on the impressive breadth of knowledge of over 922 authors and on the tireless work of our editorial advisory board. We are very grateful to all of them.

Benjamin Caballero
Paul Finglas
Fidel Toldrá

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Smoked Foods: Principles and Production

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Introduction

Smoking belongs to the oldest methods of food preservation. Meat or fish hung by the bonfire lost part of its water and was penetrated by wood smoke. Thus, spoiling of the food was retarded and the sensory properties were changed, especially when the raw material had been presalted. Gradually, most suitable procedures of smoking in local conditions and various types of equipment were developed to treat the different available raw materials. The smoke used in the process is actually mostly produced in separate generators and may be also converted to liquid, powdered, or gel-like smoke preparations by precipitation, distillation, and further refinement. The preservative effect of smoking has lost much of its importance due to developments in refrigeration and canning, while the sensory and safety aspects have became dominant. Nowadays, different smoking procedures are applied to process a large variety of foods, including meats, fish, cheese, and plums.

Smoking involves penetration of the food by a large variety of smoke components, partial loss of moisture and often fat, and enzymatic and/or heat-induced modifications of proteins. The extent of the changes caused by the process depends on the preparation of the raw material, that is, skinning, gutting, filleting, salting, curing, cutting, chopping, mincing, and stuffing into casings; on the duration of the exposure to smoke of controlled density, composition, temperature, humidity, and flow rate; and on the conditions of final handling and packaging of the products.

Wood Smoke Composition

Wood smoke results from smoldering logs, chips, or sawdust. Most often, hardwood is used, predominantly oak, beech, and hickory, although wood of coniferous trees is also applied. Heather, juniper, and coconut husks may serve for imparting special sensory properties to the products.

Smoke occurs due to thermal degradation of the main constituents of the woody material and to partial oxidation of the products of pyrolysis. Thus, its composition is affected by the contents of hemicellulose, cellulose, and lignin of wood, which degrade, respectively, at 180–300 °C, 260–350 °C, and 300–500 °C, and the conditions of oxidation of the liquid and gaseous degradation products, that is, the temperature in the smoldering zone and the concentration and flow rate of oxygen. The main components of wood smoke include air, water vapor, CO_2, CO, nitrogen oxides, phenols, carbonyls, carboxylic acids, alcohols, esters, and hydrocarbons, as well as minute amounts of N-heterocyclic and O-heterocyclic compounds. The number of components of smoke is in the hundreds; about 400 individual chemicals have been unequivocally identified.

The concentration of water vapor in the smokehouse depends on the moisture content of the wood, on the characteristics of the smoked goods, and on the phase of processing, as well as on the temperature and rate of circulation of the smoke. It affects the relative humidity of the environment and thus the evaporation/sorption phenomena, as well as the rate of heat transfer and thermal changes in the products.

At least 250 phenolics, including, among others, polyhydroxy compounds, components containing additional functional groups, and those with long side chains have been separated and at least 85 identified. The phenolic components present in smoke in the highest concentrations include syringol and its derivatives, especially methyl and ethyl syringol and syringaldehyde, 280 mg $(100\ g)^{-1}$ of oak wood used for smoldering; guaiacol and derivatives, predominantly methyl, ethyl, and vinylguaiacol, 240 mg$(100\ g)^{-1}$; pyrocatechin and its derivatives, including metoxypyrocatechin and methylpyrocatechin, 215 mg$(100\ g)^{-1}$; and phenol and its derivatives, about 160 mg$(100\ g)^{-1}$.

The highest yield of different phenols, especially of guaiacol and syringol and their derivatives, compounds that are essential for the sensory and preservative action of smoking, is at 400–600 °C, since they are formed due to thermal degradation of lignin. In the fraction of carbonyls, about 110 aldehydes and ketones have been identified. The group of compounds present in the highest concentrations include acetaldehyde, about 1200 mg$(100\ g)^{-1}$ of wood; furfural, 1000 mg$(100\ g)^{-1}$; acetone, 750 mg$(100\ g)^{-1}$; furanone; formaldehyde; 5-methylfurfural; 3-methyl-2-cyclopenten-1-one; and cyclotene (2-hydroxy-3-methyl-2-cyclopenten-1-one). Other numerous aldehydes and ketones are present in wood smoke in very much lower concentrations, usually in the range of 1–50 mg $(100\ g)^{-1}$ of wood.

The fraction of carboxylic acids is composed of about 65 chemicals, including mono- and dicarboxylic acids as well as ketoacids. Acetic and formic acids are predominant, about 3700 and 800 mg$(100\ g)^{-1}$ of wood, respectively, while the contents of propionic, valeric, 4-oxovaleric, butyric, oxalic, malonic, maleic, fumaric, and succinic acids are about 100 times lower. Among the identified alcohols are methanol, ethanol, allyl alcohol, *n*-amyl alcohol, 2-pentanol, 3-methyl-3-buten-1-ol, 3-metyl-1-butanol, 2-hexanol, 2,4-pentadiol, and 1-heptanol, while the methyl esters of formic, acetic, butyric, acrylic, propionic, 4-oxovaleric, heptanoic, and pelargonic acids are in the ester fraction. Also, ethyl and butyl acetates, ethyl butyrate, and ethyl valerate have been identified.

Wood smoke is rich in hydrocarbons, containing some 20 aliphatic and 100 aromatic compounds. Among the aliphatic components are mainly methane and ethane. The fraction of polycyclic aromatic hydrocarbons (PAHs) comprises at least 61 identified species. They are formed mainly when the temperature of thermal decomposition of wood is higher than 400 °C. Their molecular weight ranges from 116 Da in the case of indene to 302 Da for dibenzopyrenes.

Interactions of Smoke and Meat/Fish

The wood smoke constituents settle on the sausage or fish by absorption in the surface layer. The mixture of hundreds of chemical compounds of the smoke forms an aerosol. Since the boiling point of most of them is higher than the temperature in the smoking chamber, about 90% of their total mass is present mainly as small liquid droplets or solid particles, 0.08–0.15 μm in diameter, dispersed in the gases and vapors. The proportion of specific smoke components in both phases depends on their boiling point and temperature – heating increases the concentration of smoke constituents in the gaseous phase. Coalescence of the droplets and particles due to Brown's motion, as well as deposition caused by gravitational and centrifugal forces in the smoke ducts, on the walls of the kiln, and on the smoked food, regards mainly the dispersed components. The rate of deposition and sorption depends on the wetness/dryness of the surface and the humidity and temperature of the smoke. The settling of smoke components on the surface of smoked goods may be enormously accelerated due to high-voltage electrostatic field, to take only a few minutes instead of hours.

Depending on their physical state and chemical properties, the smoke components settle on the surface and diffuse to different depths of the smoked goods. The rate and extent of diffusion depend also on the barrier properties and reactivity of the surface and deeper layers. A thick skin of fish, for example, eel, efficiently inhibits the movement of smoke components, including PAHs, into the flesh. The concentration gradients of different compounds across the products diameter are not equal. Phenolic and carbonyl compounds may polymerize and interact with thiol groups in proteins, peptides, and amino acids. In model experiments, such reactions were shown to decrease significantly the number of available –SH groups. However, they do not cause any significant loss in nutritive value of smoked foods, since the concentration of phenols and carbonyls in the edible tissues is very low. On the other hand, even in minute quantities, these compounds affect the sensory properties of the products and have antimicrobial and antioxidant activity.

Sensory Effects of Smoking

Smoking is usually just one of several unit processes involved in manufacturing a food product. Generally, the raw material is also salted or cured, often chopped, minced, and mixed with various ingredients. Thus, the sensory characteristics of smoked meats, fish, and cheese result from simultaneous or concerted action of various factors. The smoke components affect mainly the color and flavor of the products, while the drying and heating induce textural changes.

The color of the surface of smoked meat and fish depends on the original pigmentation of the raw material and on the deposited smoke components. The color added by smoking ranges from light yellow to gold to deep brown and increases in intensity with the density and temperature of the smoke and duration of the process. Raising the smoke temperature and the wetness of the product's surface leads to darker color of sausages.

Fish treated with beech, maple, ash, sycamore, or lime tree smoke turn into goldish yellow; oak, nut, and alder smokes impart yellow brownish coloration, while acacia smoke lemon-like coloration. The color is caused primarily by oxidation and polymerization of the deposited smoke components, mainly phenols, but partially also due to the Maillard reaction with the participation of carbonyl compounds from the smoke and amino groups of the food proteins and amino acids. The predominant direct role of smoke components can be shown by exposing a metal sheet to smoking in an electrostatic field – in 2–3 min, the inert metal surface turns yellow or brown due to deposition of a viscous thin film.

The aroma of smoked products comes from the odor molecules present in the smoke and the odoriferous substances generated in various biochemical and chemical reactions in the food matrix. The group of phenols responsible for the typical, desirable smoky aroma comprises mainly syringol, 4-methylsyringol, 4-allylsyringol, guaiacol, 4-methylguaiacol, *cis*-isoeugenol, *trans*-isoeugenol, *o*-cresol, *p*-cresol, and 2,6-dimethoxyphenol. Pyrocatechol adds a burned, phenolic note to the aroma. Various aldehydes and ketones contribute a sweet, caramel-like smell positively modifying the rather harsh phenolic aroma. Cyclotene contributes to the smoky odor of foods. The variations of the smoky flavor result from the additional action of carboxylic acids, alcohols, and esters, as well as of numerous other components of wood smoke, which, although present in minute concentrations, can modify the perceivable aroma of various products. Thus, the attempts to generate a smoky flavor typical for that of, for example, smoked sausage of a particular assortment or fish of a certain species by mixing in different proportions pure wood smoke components have been unsuccessful.

Various phenols, carbonyl compounds, acids, and the products of their reactions with the components of the food matrix are also responsible for the typical taste of smoked foods.

The Preservative Effect of Smoking

The shelf life of smoked sausages and fish depends on the initial characteristics of the raw material, the conditions of smoking, the packaging, and the temperature and humidity during storage. It changes gradually through the high-quality life to the practical storage time and ends at the stage of unfit for consumption. Smoking prolongs the shelf life only if the whole treatment lowers significantly the water activity in the products due to both added salt and partial drying, decreases the pH as the result of lactic acid fermentation, reduces the viable population of spoilage microflora by heating, and impregnates the meat or fish with wood smoke components of antimicrobial and antioxidant properties. Cured and heavy smoke-dried sausages, produced with a yield of 55–60% in relation to the weight of the cured meat, as well as smoke-dried fish with a water content of about 15% keep well up to a few months at room temperature and low relative humidity. On the other hand, mild smoked frankfurters or hot-smoked mackerel have a shelf life of about 2 weeks under refrigeration.

Hot smoking of sausages may decrease the total number of aerobic bacteria by 2 log cycles mainly due to the heating effect. In manufacturing semidry and dry-fermented products, the

important factor increasing the shelf life is the low pH resulting from the activity of lactic acid bacteria, the decrease in water activity, and impregnation with smoke components. Among the compounds mostly responsible for the antibacterial activity are phenols, especially guaiacol and pyrocatechol and their derivatives, as well as some carbonyls. An aldehyde group in a phenolic compound increases the antimicrobial activity. Different smoke components may reduce the number of various species of bacteria on heavy smoked foods by 2–3 log cycles, but generally, smoking is not an effective way of inhibiting the formation of *Cl. botulinum* toxin or elimination of *Listeria monocytogenes* in products stored in conditions favorable for the growth of food poisoning microflora. Thus, in order to control the proliferation of *Cl. botulinum* in hot-smoked fish, the products should be immediately chilled below 4 °C and kept under refrigeration.

The shelf life of foods, especially those containing fats rich in polyenoic fatty acid residues, is also limited by oxidative rancidity. Smoking has an antioxidant effect due to the activity of phenols, especially pyrogallol, resorcinol, and guaiacol and syringol and their derivatives. Many smoke phenols have an antioxidant activity equal to or higher than that of the well-known antioxidants butylhydroxyanisole and butylhydroxytoluene. Phenols, being true antioxidants, inhibit lipid oxidation even in low concentrations by inactivating the free radicals, which initiate the chain reaction of lipid autoxidation, as well as the secondary radicals, and thus are able to terminate the reaction.

Food Safety Aspects

Smoked foods are perfectly safe for the consumer provided that they have been produced according to good manufacturing practice from fresh raw material free of natural toxins, chemical contaminants, pathogens, and parasites and that the storage conditions do not lead to microbial proliferation or production of toxins.

Health hazards for the consumer resulting from smoking may be caused by carcinogenic PAHs, *N*-nitroso compounds, and heterocyclic aromatic amines contained in the products in too high concentration. It is practically not possible to totally eliminate some harmful compounds from our foods to below the concentration detectable by contemporary analytic techniques, which may be as low as even pg/g. However, national and international regulations limit the residues of these compounds to levels that do not cause health hazards.

PAHs in wood smoke and smoked foods are a reason for concern, since many heavy species, of a molecular weight higher than 216 Da, have been recognized as mutagenic and/or carcinogenic. Most of the PAHs contained in wood smoke, however, belong to the low-molecular-weight species. The group of PAHs recognized as potentially genotoxic and carcinogenic for humans consists of 15 compounds. In the human organism, PAHs are metabolized by various enzymes to form promutagenic and carcinogenic DNA adducts. These metabolic changes are affected by the chemical structure of the aromatics; thus, the carcinogenicity of various PAHs is different. Initially, benzo(a)pyrene (BaP) was used as an indicator of carcinogenic PAHs in wood smoke and smoked products. Earlier data regarding the contamination of foods with carcinogenic PAHs refer to the concentration of this hydrocarbon. However, it was found later that in many smoked commodities, the content of BaP was negligible, while higher levels of other carcinogenic PAHs were detected. Thus, the European Food Safety Authority decided in 2008 that a more realistic indicator should be the sum of the concentration of BaP, benz(a)anthracene, benzo(b)fluoranthene, and chrysene (PAH4) or even better the sum of PAH4 plus the concentration of benzo(k)fluoranthene, benzo(g,h,i)perylene, dibenzo(a,h)anthracene, and indeno(1,2,3-cd)pyrene (PAH8). Therefore, in the Commission Regulation (EU) of 2011, a maximum level of BaP in smoked meat and meat products has been maintained ($5\ ng\ g^{-1}$ until 31 08 2014 and $2\ ng\ g^{-1}$ later) to make possible future comparisons with earlier experimental data, and additionally, the upper limit for PAH4 has been introduced ($30\ ng\ g^{-1}$ from 1 09 2012 to 31 08 2014 and later $12\ ng\ g^{-1}$). These limits can be easily observed if the foods are smoked in modern kilns supplied with smoke generated at not too high temperatures. Hot-smoked sausages produced in such conditions contain usually not more than 1 ng BaP per g. On the other hand, some products smoked traditionally above a pile of smoldering sawdust in the kiln may contain even 50 times higher amounts of BaP. Furthermore, in many products, the concentration of PAHs in the outer layers is up to ten times higher than in the inner parts, for example, in the skin of smoked eel.

Smoked foods are also contaminated with minute amounts of the environmental pollutants nitro-PAHs, ubiquitous in urban air and in many agricultural commodities. Among them are 1-nitropyrene, 2-nitronaphthalene, and 2-nitrofluorene. The concentration of the sum of various detected nitro-PAHs in different foods, in $ng\ g^{-1}$, is about 1.5 in olive oil, 3 in grilled meat, 15 in smoked meats, 30 in smoked sausages, and 35 in roasted coffee beans. No nitro-PAHs were detected in the inner parts of smoked cheese. In tea leaves of different brands, the nitro-PAHs were found in amounts from about 10 to several hundred $ng\ g^{-1}$.

Cured meats and bacon, as well as smoked meat and fish products, contain also different *N*-nitroso compounds, since the reactions of aldehydes with cysteamine and cysteine in the tissues lead to thiazolidine precursors, which can be nitrosated by the nitrogen oxide of the smoke. Among these compounds, *N*-nitrosothiazolidine, *N*-nitrosothiazolidine-4-carboxylic acid, 2-(hydroxymethyl)-*N*-nitrosothiazolidine, and 2-(hydroxymethyl)-*N*-nitrosothiazolidine-4-carboxylic acid were found. In smoked fish, *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosodibutylamine, *N*-nitrosopiperidine, and *N*-nitrosopyrrolidine were determined in amounts up to about $5\ ng\ g^{-1}$. In various smoked meat products, different *N*-nitroso compounds occur in concentrations reaching several hundred $ng\ g^{-1}$. Smoking traditionally in kilns with logs of wood or sawdust smoldering below the hanging rows of meats or fish leads to heavier contamination than processing in a modern smokehouse.

In different smoked meats, fish, and cheese, various heterocyclic aromatic amines have been found, although generally in very small concentrations of about $1\ ng\ g^{-1}$ or lower. Smoking, especially at low temperature, does not favor the pyrolytic reactions of amino acids and proteins and nonenzymatic browning, which lead to the formation of these compounds.

Smoking Procedures

Smoking is either the main unit process in manufacturing a typical smoked product, for example, smoked eel or smoked cod, or just one of several links in an often long chain of treatments applied to produce, for example, canned smoked sprats in oil, smoked sturgeon balyks, or smoked bacon. Thus, different conditions of smoking are applied in various processes, that is, cold, when the smoke temperature is 12–25 °C, warm at 25–45 °C, and hot up to 90 °C.

Cold smoking lasts several hours or days in manufacturing various assortments of raw sausages made from cured meats, for example, salami and mettwurst, or of cured cuts of bacon. In producing raw, cured, fermented sausages, several phases of slow drying and cold smoking contribute to developing the sensory properties typical for such assortments and bring the intended decrease in water activity. The smoke components do not inhibit the lactobacilli, which by producing lactic acid increase the shelf life by controlling the growth of putrefactive and pathogenic organisms.

Drying and cold smoking in different stages for several hours or up to days are applied in producing smoked fish like Finnan haddocks, salmon fillets, or sturgeon balyks. Red herring smoking may last even up to 3 weeks. The preparation of the fish prior to smoking differs depending on the raw material and assortment of the product but includes always brining or other way of salt curing. In proper conditions, brining may lead to enzymatic changes in the flesh. The shelf life of these products depends on the loss of moisture during the whole process and on the contents of salt.

Cold smoking at about 20 °C for 2 h up to about 2 days is used for various assortments of cheese in order to add the desirable flavor and increase the shelf life by forming a dry surface and impregnating it with smoke components.

Hot smoking for 1–1.5 h in dense smoke is one of the processing steps in manufacturing frankfurters. The sausages, after reaching a core temperature of 60–68 °C and the desirable smoky color and flavor, are cooked under a hot-water spray or by steam. In producing jagdwurst, the procedure is more complicated. The sausage batter consisting of a mixture of cured pork, beef meat, and spices, in natural casings, has first to settle up to 12 h at 2–6 °C, or 2–3 h at room temperature. This is followed by 80–90 min of smoking at 80 °C, dry heating at 85 °C for 25 min, the second phase of smoking at 24–32 °C for 12 h, drying for 6–8 days at 14–18 °C and relative humidity of 75–80% to a water content of 55–57%, final smoking at 24–32 °C for 2–3 h, and cooling.

Kabanos, a delicatessen-type, spicy cured pork sausage in natural casings of small diameter, is made by settling for 12 h at 2–6 °C, hot smoking for 50–60 min, followed by 20 min dry heating to a core temperature 69–70 °C and dark reddish brown color, cooling, and drying at 12–18 °C for 5–7 days.

Among the hot-smoked fish products, very popular are smoked sprats. When prepared for canning, they are smoked for about 1 h to attain light color and lose 20–25% of water. Whole sprats prepared for the market, Kieler Sprotten, are smoked to golden brown and to a higher yield in order to make the product succulent. Hot smoking of whole herring, very popular in Northern Europe under the name of buckling, lasts about 2.5 h. Smoking of gutted mackerel takes about 3 h. Fish like large herring or middle-sized cod, threaded on speats through the gills and mouth or through the eyes, may tear under their own weight. Such droppers may cause considerable financial loss to the producer. This happens when at high temperature and humidity, the thermal changes in the collagenous elements of the fish head precede the strengthening of the tissues due to drying and impregnation with smoke components. In smoking of gutted eels, the temperature in the first stage of processing should be high enough to cause thermal shrinkage and curling of the collagen of the belly flaps to expose the abdominal cavity to the smoke. Otherwise, the penetration of the smoke into the flesh only through the thick skin would be very slow. The same can also be attained by dipping the fish in hot water before smoking. Curling is also characteristic for the German specialty Schillerlocken made by hot smoking of the belly flaps of dogfish.

Equipment

A large variety of equipment can be used for smoking of foods, generally classified as traditional kilns and mechanical smokehouses. Numerous types of kilns and tunnels are available both for industrial use and for home smoking.

In traditional kilns, the smoke and heat are generated by burning/smoldering the woody material on the floor below the sausages or fish hanging on speats or rods or placed on wire meshes. The temperature, humidity, and density of the smoke depend significantly on the weather conditions and must be skillfully controlled manually by the operator. He may change the moistness of the wood chips or sawdust and open or close the air inlets to the kiln and the shutter on the chimney. In contemporary traditional-type kilns, the material to be smoked is placed on trolleys. Because of differences in temperature and draft in various parts of the kiln, the smoked goods must in some cases be shifted and rearranged during smoking.

Mechanical smokehouses are usually heated by steam or electricity and are supplied by smoke from external generators. They may be also equipped for vaporization or nebulization of liquid smoke preparations. The distribution, the flow rate, and the parameters of the smoke, as well as the process time, are controlled automatically according to programs for the different assortments of food. The meats or fish to be smoked are transported on trolleys or conveyers into the kilns or tunnels. In an electrostatic smoking unit, the fish or sausages are carried on a grounded metal transporter between two flat high-voltage electrodes at about −40 kV. Due to corona discharges, the ionized smoke particles and droplets, as well as the components of the dispersing phase carried by the 'electrical wind,' are forced towards the material on the grounded conveyor. In the smokehouses used in manufacturing cooked sausages, the cooking can be carried out under hot-water showers or by steam.

Smoke generators with a grated fire bed or heated plate are supplied with chips or sawdust of standardized water content and mesh size. The parameters of smoldering are controlled so as to favor the formation of desirable smoke components and prevent generation of PAHs. In a friction-type machine, the smoke develops due to heat generated in the thin layer of a wood log pressed against a rotating drum or disk.

The temperature in the friction zone is controlled by the rotation rate of the rotor and the pressure exerted on the log, while the access of air depends on the construction of the apparatus. Several other types of smoke generators have been proposed, one of them being that using overheated steam at 250–390 °C for heating the sawdust or chips.

The smoke may be filtered or conditioned under a water spray to control its temperature and humidity and to separate undesirable tar fractions and soot. The exhaust gases from smokehouses should be neutralized in order not to pollute the atmosphere. This can be accomplished by using electrofilters, fibrous filters, or activated charcoal. Afterburners may also be used to oxidize the organic components of the spent smoke at 800–1500 °C or at about 600 °C in the presence of catalysts.

See also: Fish: Processing; Preservation of Foods.

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Snack Foods: Role in Diet

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Introduction

The human body needs the right food and regular maintenance to achieve its health potential. Healthy eating plays a major role in keeping fit for day-to-day activities. The body cannot deliver its full potential if nonnutritional foods are consumed. Various advantages of eating balanced diet are that it (a) promotes energy, (b) maintains and improves the body weight, (c) enhances the immune system, (d) keeps oneself active and fit and helps overcome tiredness, and (e) reduces serious health diseases like diabetes and hypertension and other cardiovascular disorders. Table 1 indicates advances in food and nutrition research in the last 100 years.

Food and Nutrition

Food by definition consists of macronutrients like protein, carbohydrate, and fat and micronutrients like vitamins and minerals, which sustain and support vital processes in a human body. Table 2 indicates the various macro- and micronutrients and their function in a human body. Two decades ago, the US Department of Agriculture (USDA) created Food Guide Pyramid to convey the elements of healthy diet (Figure 1). This food pyramid helps a person to make healthy food choices from a recommended variety of foods, suggested serving sizes that can help you control the amount of calories, trans fat, saturated fat, cholesterol, sugar, or sodium. According to nutritionists, foods are considered healthy if they have fewer fats contained in fried foods, butter, cream, margarine, and certain oils (e.g., saturated and trans), but at the same time, some fats are essential for health and are recommended in prescribed daily amounts (monounsaturated fat, polyunsaturated fat, and omega-3 fatty acids). Foods with lower unhealthy fat content can reduce heart-related diseases. Fiber is the indigestible part of the food and is considered to be very good for the health. It helps to lower cholesterol, prevents gallstones and bowel cancer, and helps to maintain the body weight. Some of the examples of the fiber-rich foods are whole meal, grain breads, brown rice, barley, lentils, beans, and vegetables. Vegetables, fruits, and grains carry an abundance of vitamins, minerals, and numerous other natural substances like phytochemicals. These phytochemicals act as antioxidants and help to prevent the free radical formation and damage in cells, membranes, and DNA. Many scientific studies indicated that vegetables and fruits help lower heart disease and cancer. Sugar adds flavor; however, excessive amounts of sugar can lead to diseases. Higher amount of salts in the food can result in heart-related problems. Most of our salts come from everyday commercial foods (including bread, biscuits, cereals, butter, deli meats, and snack foods), and it is recommended to buy foods that are lower in salt. Water is an important component for keeping the body hydrated and at least 2 l is recommended for efficient functioning of the kidneys.

Processing can help improve the nutritional value of food. Refining and separation of the antinutritional components increase the vegetables nutritional quality, and processing of fresh tomatoes into ketchup increases the bioavailability of the carotenoid lycopene. If the foods are overprocessed, they lose their nutritive value. During processing both micro- and macronutrients can be added to increase its nutritional value. For example, many products are fortified or enriched with vitamins and minerals (e.g., orange juice fortified with calcium for bone health and margarine enriched with plant stanols and sterols for heart health).

Role of Snacks in Diet

By definition, a snack is a small portion of food that is smaller than a regular meal, generally consumed between meals. Snacks are defined as smaller, less structured meal that are not eaten during regular meal times, such as breakfast (morning), lunch (midday), and dinner (evening). Snacks come in a variety of forms including fresh ingredients and packaged and processed foods. Some of the common snack products include tortilla chips, corn chips, potato chips, extruded products (direct expansion and pellets), pork rinds, pretzels, popcorn, nuts, and seeds. Beverages are considered a snack if they have substantive amounts of fruits like strawberries, banana, and kiwis. Processed snack foods are designed to be more satisfying, less perishable, and more durable and portable than prepared foods. Snacking is more common today as snacks and fast food are more readily available and are quick substitutes for regular meals. Consumption of fast food in the United States has increased significantly starting from 1977–78 to 2005–08 (Figure 2). Currently, snacks are eaten as part of main meals and this trend is expected to grow in popularity by 5% by 2018. A typical consumer in the United States takes about 2.12 snacks per day, and children in the United States consume snacks on an average of six times a day, approximately twice as often as in the 1970s. According to Mintel Group report, in 2013, the dollar value of the salty snack category was estimated at $21.8 billion. The report also concluded that potato chips are the largest snack purchased with the maximum sales of about $7.6 billion with an estimated market share of about 34.8%. Figure 3 indicates the revenues of various salty snacks in the United States from 2011 to 2015. The figure clearly indicated chips and crisps are the most consumed compared to others. Recent marketing research data indicated that 47% of Americans purchase salty snacks and dips for snacking throughout the day and 35% are buying more snacks while eating fewer regular meals. Increased snacking frequency and higher revenues from snack products indicate that the role of snacks in the diet has increased significantly.

Snacks are generally categorized as healthy or unhealthy. Snack foods with less or little nutritional value are considered as junk food and do not contribute to general health and

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Table 1 Evolution of nutrition research in the past 100 years

Year	*Name*	*Discovery*
1925	Edwin B. Hart	Trace amounts of copper are essential for iron absorption
1927	Adolf Otto Reinhold Windaus	Synthesized vitamin D and won the Nobel Prize in Chemistry
1928	Albert Szent-Györgyi	Isolated ascorbic acid (vitamin C). In 1932, he proved that vitamin C prevents scurvy. In 1937, he synthesized vitamin C and won the Nobel Prize
1930	William Cumming Rose	Identified essential amino acids that the body cannot synthesize, but are necessary protein components
1935	Eric John Underwood and Hedley Marston	Discovered the necessity of cobalt
1936	Eugene Floyd Dubois	Linked school and work performances with caloric intake
1938	Erhard Fernholz	Discovered the structure of vitamin E, synthesized later by Paul Karrer
1940	Elsie Widdowson	Developed nutritional principles for rationing in the United Kingdom during and after World War II. Coauthored book with Robert McCance in 1940 titled 'The Chemical Composition of Foods.' This book is the basis for modern nutritional thinking
1941	The National Research Council (the United States)	First Recommended Dietary Allowances (RDAs)
1968	Linus Pauling	Introduced the term orthomolecular nutrition. Proposed that the body should get right molecules in right concentration. This work eventually led to large intravenous doses of vitamin C to improve survival times and quality of life of some terminal cancer patients. Awarded with the Nobel Prize in Chemistry
1992	The Department of Agriculture (the United States)	Set up the Food Guide Pyramid
2002	Natural Justice Study (the United States)	Revealed the link between violent behavior and nutrition
2005		Researchers identified adenovirus is the cause for obesity, and bad nutrition

Source: Medical News Today-Knowledge Center.

Table 2 Micro- and micronutrients, minerals, and vitamins and their importance

Macronutrients
- Carbohydrates: Monosaccharides, disaccharides, and polysaccharides. The molecules consist of carbon, hydrogen, and oxygen atoms. Nutritionally, polysaccharides are more favored because of complex molecular sugar chains. Due to its complexity, it takes the body more time to break down and absorb into the bloodstream
- Proteins: Nitrogen, carbon, hydrogen, and oxygen atoms. Simple proteins like monomers are used to create complicated proteins. Nitrogen in protein is eliminated by the kidneys
- Fats: Carbon, hydrogen, and oxygen atoms. Fats are triglycerides – three molecules of fatty acid combined with a molecule of the alcohol glycerol. Fatty acids are simple compounds (monomers), while triglycerides are complex molecules (polymers)
- Fiber: Consists of carbohydrates. Less sugars and starches get into the bloodstream due to less absorption
- Water: About 70% of the nonfat mass of the human body is water. It is unknown how much water the human body needs – approximately 1 and 7 l per day is recommended to avoid dehydration

Micronutrients

Minerals
- Sodium and potassium: systemic electrolyte, required in coregulating ATP
- Chloride: produces hydrochloric acid in the stomach
- Calcium: builds bones and is important for muscle, heart, and digestive health
- Phosphorus: bones and energy processing
- Magnesium: healthy bones
- Zinc: necessary for several enzymes
- Iron: required for proteins and enzymes, especially hemoglobin
- Manganese: cofactor in enzyme functions
- Copper: part of several redox enzymes
- Iodine: biosynthesis of thyroxin
- Selenium: required for activity of antioxidant enzymes
- Molybdenum: uric acid formation and iron utilization, carbohydrate metabolism, and sulfite detoxification

Nutrients
- Vitamins – A, B1, B2, B5, B6, B7, B9, B12, C, D, E, and K
- Vitamins are called supplements, because the human body cannot produce enough of them and should be obtained from the foods consumed
- Vitamins are classified as water-soluble (dissolve in water) or fat-soluble (dissolve in fat). There are 4 fat-soluble (A, D, E, and K) and 9 water-soluble (8 B vitamins and vitamin C) vitamins
- Water-soluble vitamins should be consumed more regularly because they are removed quickly from human body and are not readily stored.
- Fat-soluble vitamins are absorbed through the intestines with the help of fats (lipids). They accumulate in the human body and are harder to eliminate quickly

Source: Medical News Today-Knowledge Center.

nutrition. Typically unhealthy snacks have a high content of refined sugars, saturated fat, and salt, whereas healthy snacks are lower in fat, sugar, sodium, and calories. The increasing chronic illness and health-related problems in developed countries can be partially attributed to the consumption of processed foods especially snack-based products, which are high in sodium, sugar, and fat. Most of the Western countries have obesity problem and it is more severe in the United States. The journal of the American Medical Association has reported that 65% of the adults $\geq$20 years in the United States are either overweight or obese and about 280 184 deaths are attributed to obesity. According to the American Heart Association, more than 64 million Americans have cardiovascular disease, which is a major case of mortality (about 38.5% of all deaths). The other prominent health-related problems in the United States include hypertension (50 million), type II diabetes (11 million), and high-risk total cholesterol concentrations (37 million). One of the reasons for the increased diseases in

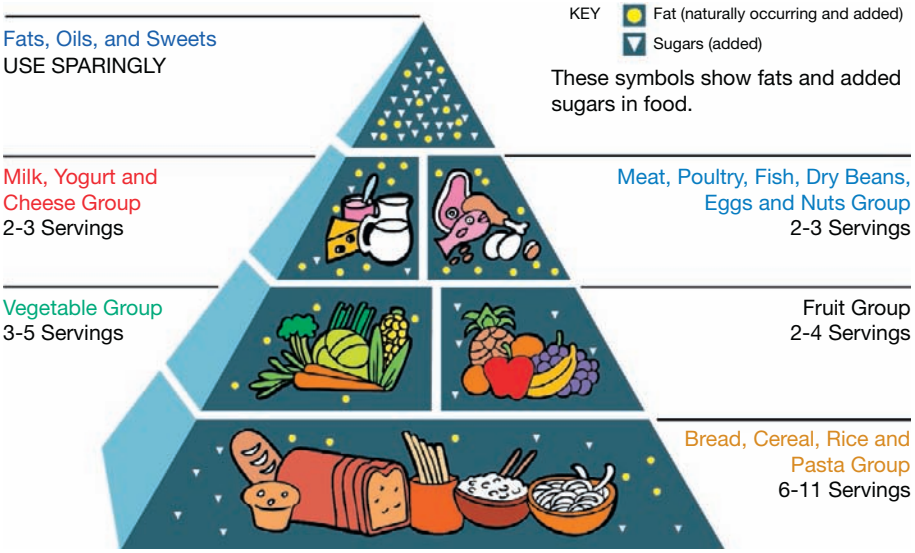
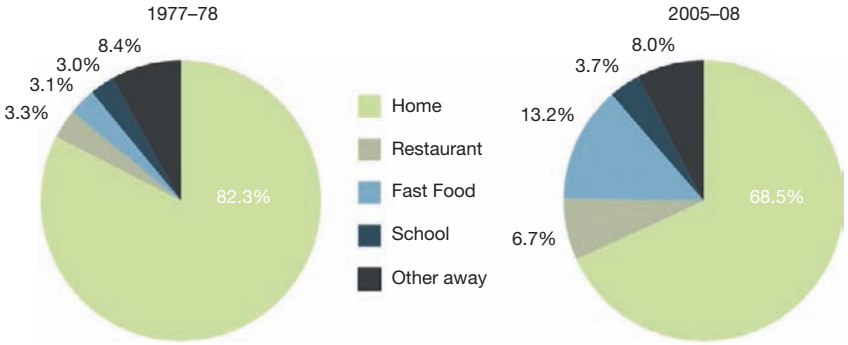


Figure 1 Food pyramid developed by the US Department of Agriculture in 1992.



Note: Totals do not sum to 100 due to rounding.

Source: USDA, Economic Research Service analysis using date from 1977–78 Nationwide Food Consumption Survey and the 2005–08 National Health and Nutrition Examination Survey.

Figure 2 Processed food consumption in the United States.

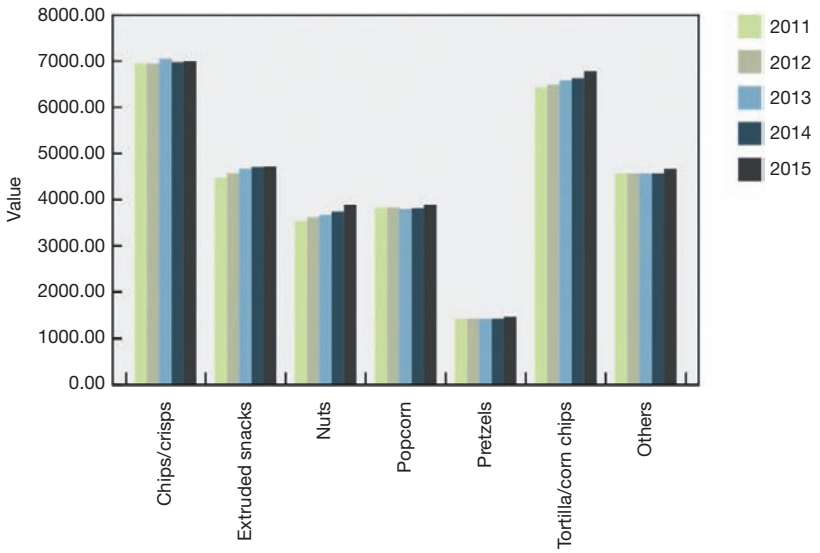


Figure 3 Consumer trends: Salty snack food in the United States (value: US millions). (Source: Agriculture and Agri Food Canada.).

the United States can be due to the exponential increase in commercial food outlets, which started selling processed foods high in sugar, fat, and sodium. Also, the food outlets started selling bigger portion sizes (supersizing) for salty snacks, desserts, soft drinks, and hamburgers. For example, potato fries sold by fast food outlets used to provide about 200 calories in 1960, whereas in 2002 they provided about 600 calories. Research indicated that this trend is similar in most of the Western countries. The increase in consumption of processed food and snacks as a replacement for regular meals resulted in increased carbohydrate consumption in the form of sugar and fat in the form of saturated and trans fat, whereas protein and healthy fat (mono- and poly-saturated and omega-3 fatty acids) intakes have lowered. Figures 4 and 5 indicate the increase in consumption of sugar in the United States and United Kingdom and macronutrients (protein fat and carbohydrates) in the United States. This increased consumption of carbohydrates, harmful fats, and sugars majorly contributed to obesity-related diseases like hypertension, diabetes, and cardiovascular diseases. Growing health-related problems have alerted the consumers, as they are now more conscious of the nutritional value of the snacks they consume. Also, many health organizations are advising consumers to be more vigilant on the calorie count of the snack they are consuming. Cutting the unhealthy snack and replacing them with healthy snack can have a positive impact on consumer's health. Research has indicated that replacing an unhealthy snack with a healthy snack every day can help to reduce nearly 4 g of

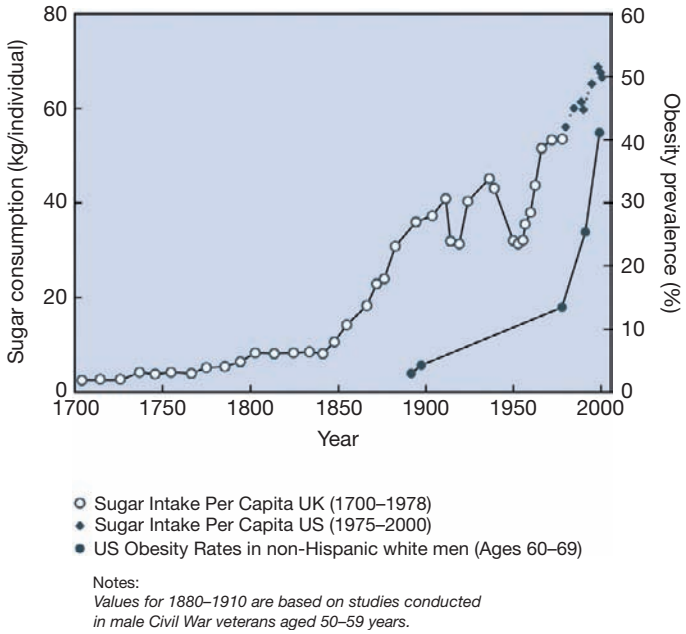


Figure 4 Increase in sugar consumption with time in United Kingdom and United States. (Source: Johnson et al., 2007.).

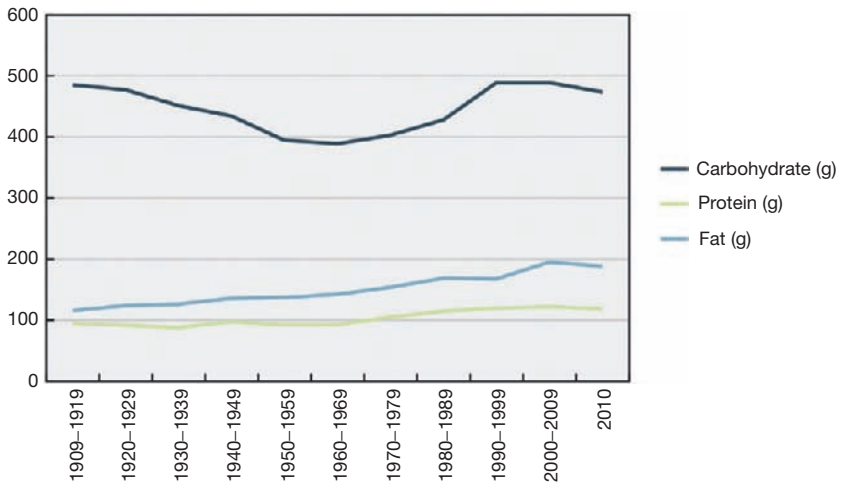


Figure 5 Nutrient content of the U.S. Food Supply: macronutrients per captia per day, 1909–2010. (Source: USDA.).

saturated fat and 0.5 g of sodium consumption. Both sodium and saturated fats are major contributors for obesity-related diseases. At present, potato chip manufacturers have started using healthier fats (olive or avocado oil) for frying. These oils that are rich in antioxidants and monounsaturated fats are good for heart health. Right now, there is an urgent need to reduce the role of unhealthy snacks in the current diet. The diseases caused by unhealthy snacks can lead to poor health for aging individuals and require higher costs in medical care, which leads to economic issues for the nation. Snack food manufacturers should focus on developing processes, technologies, and nutrient-rich ingredients to produce healthy snacks. This can reduce the obesity-related diseases in developed countries and malnutrition issues in developing and underdeveloped countries.

Extrusion Technology for Healthy Snack Food Processing

Snack industry is one of the fastest-growing division of the food industry, and extrusion technology is commonly used to produce new and creative products. The use of extrusion technology to produce novel snack food products holds great promise for future snack industry. Extrusion is one of the most versatile techniques available to the food processing industries for transforming ingredients into intermediate or finished products. In extrusion process, the material is exposed to temperature, pressure, and shear forces and is pushed through a constricted hole to give a desired shape and texture. During extrusion cooking, the material undergoes mixing, cooking, and shearing. Major advantages of extrusion cooking of foods are it (a) reduces the antinutritional factors and (b) converts insoluble dietary fiber to soluble dietary fiber. This technology helps to develop new products using both traditional and novel ingredients. Some examples of extruded snack foods are corn curls, chips, crackers, chewing gum, chocolate, and soft/chewy candy. Snacks with a wide variety of shapes (pillows, tubes, bars, triangles, and stripped products), structure and texture, color, and flavor can also be produced. A recent advance in extrusion cooking is supercritical fluid injection, coupled with the continuous twin-screw extrusion, which helps to create new engineered products. This process resulted in new product developments in cereals, confectioneries, pastas, flavorings, and snacks. The major advantages of this new process are (a) little or no loss of micro- and macronutrients and (b) expanded structure of the extruded products that are free of oxygen, which prevents oxidative rancidity during storage and increases snack product shelf stability.

Third-generation snacks

The snack food industries are looking for technologies that can help to produce healthier snacks to attract the consumer market. Extrusion technology is widely used to produce multidimensional third-generation snacks or pellets. The preparation of these snacks includes blending of ingredients and passing through a cooking extruder to form a dense pellet. The extruded dense pellets or extrudates are dried to a stable moisture content to increase the shelf stability. Extrudates can be further sold as is or puffed by immersion in oil or heating in microwave. They can also be seasoned with salt and/or various spices, packaged, and sold to the consumer as a ready-to-eat snack. This type of snack adds a new dimension to its marketing potential resulting in a cost-competitive product because of its shelf stability and high bulk density, making transportation to longer distances economical. A major advantage of multidimensional third-generation snacks is that they can be prepared using traditional, novel, low-cost locally available nutritional ingredients and nutrient-rich by-products from food processing industries, which can be blended to make excellent recipes. These recipes can significantly reduce the cost of the snacks without comprising the quality and nutrition. Different types of proteins and protein supplements such as meats (whole fresh shrimp, chicken, and beef), dairy products (cheese, yogurt, and milk solids), and legume proteins (soy, pea, and bean) can be added to third generation snack recipes. Further, it is important to consider the ingredients available locally because ingredient cost is a major expense in snacks production. For example, in the United States, corn and wheat are major starch sources, while in Europe, potato is a major source, and Asia uses tapioca or rice as a major starch source. Major cereal grain starch options for extruded third-generation snack production include rice, wheat, corn, barley, and oats. The bland flavor of these cereal flours can be overcome by extruding with other ingredients. Rice and wheat flour when extruded with meat and fish gives nutritional products with good textural and sensory properties. Similar nutritional products can be produced by extruding corn, oat, and barley with animal, fish, or dairy proteins. These types of low-moisture third-generation extruded products that can be prepared using locally available ingredients can significantly reduce the cost of the snack products and are particularly advantageous in food-insecure and low-income countries. Also, these products have high shelf stability and amenable to easy handling and storage at room temperature.

Reduced Salty Snacks

Currently, sodium reduction in snacks is gaining importance. Researchers from Deakin University, Burwood, Australia, found that children who eat salty foods will have an increased craving to drink sugary beverages. The combination of salty foods and sugar-sweetened beverages has potential to increase weight and lead to obesity in consumers. But reducing sodium in the snacks impacts the flavor of the products. Currently, ingredient manufacturers offer sodium replacements to reduce the total sodium content of the snack. Cargill's salt products division, Minneapolis, introduced FlakeSelect in 2011, which is produced by combining sodium chloride and potassium chloride using a roller. According to the company, the flat crystal produced using this process provides better flavor utilization than ordinary salt. FlakeSelect, and other products like Soda-Lo from Tate & Lyle, Hoffman Estates, Ill., depend on physical modification of salt crystal shapes. Sensient Flavors LLC, Hoffman Estates, Ill., developed natural flavor for healthier salty products. The natural flavor products which are made from yeast extracts can help the snack manufacturers to reduce sodium content in their products.

Enrichment and Fortification of Snacks

Enrichment or fortification of snacks with micro- and macronutrients can produce healthier snacks. In the developing world, feeding the vast population is a major challenge, and protein malnutrition remains to be a major issue. Public health problems related to deficiency in vitamins and iron are real issues. Enrichment and fortification are two ways that food processing can restore or raise nutrient levels of the snacks. Enrichment is a process where nutritional value is increased by mixing low nutritional value ingredients with high-quality and nutritional-rich ingredients to make new products. For example, wheat and other legume flour can be mixed with meat-based products to make them rich in protein. These products make up the nutritional deficiencies that regular snacks have. Currently, many foods are fortified (e.g., cereals, snack bars, and orange juice). The process of fortification is adding nutrients during processing. According to the FDA, the addition of a nutrient to a food may be appropriate if it corrects a dietary insufficiency and restores the level of the nutrient lost during processing or storing. Fortified snack foods can help children achieve a desired healthier intake of key nutrient. Studies indicate that children have lower levels of calcium and vitamin D even after taking supplements. Kids between ages 2 and 18 get only 40–45 mg of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), while the recommendations range from 100 to 250 mg. The other limitations of taking fortified snacks can be overdosing the nutrients. According to an Environmental Working Group, a lot of cereals and snack bars are fortified at levels that are considered very high for young children.

Healthy Snacks

With the growing concerns on snack food influence on general health, government bodies like Health Canada and the US FDA suggest people make a conscious effort to eat healthier, natural snacks like fruit, vegetables, nuts, and cereal grains while avoiding high-calorie, low-nutrient junk food. A healthy snack is high in nutritional value (protein, vitamins, minerals, and fiber) and relatively low in calories, total fat, saturated fat, sugar, and sodium. Increase in nut consumption led manufacturers, such as Blue Diamond Growers and Roll International, experiencing significant growth in their sales, which can be attributed to high nutritional content in the nuts and trail mix recipes. Nuts have more protein, fiber, fat, and nutrients and are considered as a healthy snack. For the period of 2008–13, Mintel's report recorded 53% growth in better-for-you snacks such as nuts and trail mix. Blue Diamond Growers, Sacramento, California, sells almonds with flavors like wasabi soy, caramel macchiato, and raspberry. Fresh fruits and vegetables also make a very healthy snack. Additionally, crackers made from whole grains are considered healthy. Kashi Original 7 Grain Sea Salt Pita Crisps, from Kellogg, is considered a healthy cracker as it provides 5 g of fiber from the Kashi seven-grain flour and 3 g of fat and 180 mg of sodium. **Table 3** indicates healthy fruit, cereal and nut based snacks recommended to grab and go.

Table 3 Recommended snacks to grab on the go

Fruits, Vegetables, and Cereal based products
Fresh or individually packed fruit containers
Raw vegetables (carrots, peppers, zucchini, and tomatoes) and fruits (cherry and grapes)
Baby carrots and whole wheat pita triangles with hummus
Pumpernickel bagel with peanut butter and banana
Sweet peppers (red, yellow or green) and bread sticks with salad dressing or low fat dip
Whole wheat tortilla wrap with salmon or tuna, salad dressing, onions, celery and green peppers
English muffin (with melted cheese) and apple slices
Dry mixed cereal and container of milk
Plain popcorn
Nuts and Seeds
Almonds, orange slices with dark green leafy salad
Nuts, pumpkin or sunflower seeds
Fruit Juices and Dairy Products
Fresh, frozen or canned fruit with low fat yogurt or smoothie
100% fruit juice popsicles or yogurt
Fortified soy beverage, 100% fruit juice, milk, and water

Source: Health Canada.

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See also: Biscuits, Cookies and Crackers: Nature of the Products; Cashew Nuts; Cereals: Dietary Importance; Cherries (*Prunus* spp.): The Fruit and Its Importance; Convenience Food; Date Palm: A Wealth of Healthy Food; Extrusion Cooking: Chemical and Nutritional Changes; Extrusion Cooking: Principles and Practice; Fatty Acids: Determination and Requirements; Fatty Acids: Essential Fatty Acids; Fatty Acids: *Trans* Fatty Acids; Fish: Dietary Importance and Health Effects; Low-fat Foods: Types and Manufacture; Low-salt Foods: Types and Manufacture; Novel Foods; Nuts: Health Effects; Oats; Obesity: The Role of Diet; Olive Oil: Its Role in the Diet; Peanuts; Protein: Requirements; Raspberries and Related Fruits; Rice: Role in Diet; Snack Foods: Types and Composition; Soy Beans: Dietary Importance; Strawberries; Sucrose: Dietary Importance; Traditional Foods; Vitamins: Overview.

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Snack Foods: Types and Composition

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Introduction

Snacks are convenient items commonly high in oil and flavored with salt or salty flavorings. Snacks can be manufactured by a wide array of processes. The simpler snacks are those produced from popcorn, dehydrated fruits, peanuts, and nuts and the most complex are those obtained by thermoplastic extrusion.

Snack foods have always been an important part of life, and these products represent an important segment of the food industry worldwide, especially in developed countries. Snacking is increasing from factors such as increases in one-person households, higher proportion of working mothers, and more school age children. On a worldwide basis, the United States is by far the leading snack food-consuming country followed by England, Germany, and France.

Classification of Snacks

Snacks are classified according to the manufacturing process (Table 1). The simplest category, also named first-generation snacks, is the easiest to produce and consists of natural products such as popcorn, nuts, peanuts, and dried fruits. Most of the snacks consumed today fall into the second-generation snack category. These include simple formed products mainly obtained after direct extrusion processes (such as corn chips, puffed or expanded corn products, balls, and curls) or by cutting/sheeting/forming (such as both regular and fabricated potato chips, tortilla chips, and pretzels). The most elaborated category in terms of ingredients and processing is third-generation snacks produced by extrusion processes aimed toward the production of pellets or half products. Extrusion commonly consists of two sequential steps: cooking and forming. The forming extruder yields dense pellets that require drying and further processing to reach the consumer. Frying the pellets produces most third-generation snacks although conventional baking or microwaving fabricates a number of low-calorie items.

First-Generation Snacks

Popcorn

Popcorn is one of the oldest and most popular snacks worldwide obtained after popping a special kind of flinty or vitreous maize, which contains an endosperm with tightly packed starch granules and test weight at least 10–12 kg higher compared to regular corn. Popcorn is merchandized in three kernel types: white, small yellow, and large yellow. Color differences in popcorn kernels result from genetic differences in the pericarp, aleurone, and endosperm. Kernels of white popcorn are usually rice-shaped, while small and large yellow popcorns are pearl-shaped. The integrity and soundness of the bran is critically important to optimize popping volume because it acts as the lid of a pressure cooker.

In order to produce the best-quality popcorn, farmers should harvest kernels when they reach full maturity and contain approximately 17% moisture. The harvesting operation is critical and should be aimed to minimize kernel damage. After harvesting, kernels are cleaned and dried to lower the moisture to around 13–13.5%. Kernels must be dried slowly to prevent the formation of stress cracks but fast enough to prevent mold development. Kernels should not be dried below 11% moisture; otherwise, the popping expansion will not be realized.

Expansion volume is the most critical quality factor for popcorn. The popped volume is important because the commercial buyer purchases the popcorn by weight and sells it by volume. Most commercial popcorns have 30–40-fold expansion.

Popping occurs at about 177 °C, which is equivalent to a steam pressure of 2.5 ton cm^{-2} inside the kernel. The water in the grain is superheated and at the moment of popping converts to steam, which provides the driving force for expanding the endosperm after the kernel ruptures. Popping occurs when the internal vapor pressure exceeds the sum of the burst pressure of the pericarp and atmospheric pressure. Hybrids or varieties with high proportions of translucent, flinty, or vitreous endosperm attain higher expansion rates.

Basically, there are two types of popcorn configurations: mushroom or butterfly. Popped kernels with a spherical shape are called mushroom or ball type. Due to its configuration, it is preferred in the confection industry because it is less susceptible to breakage, more resistant to handling, and more efficiently coated with flavorings and confectionery syrups. The butterfly type has a higher expansion or lower apparent bulk density and better mouthfeel; it is preferred for on-premises popping where it is sold by volume. For production of mushroom or ball popcorn, more heat should be applied during popping. Temperatures of 215 and 235 °C are recommended to favor the production of butterfly- and mushroom-shaped flakes, respectively. The highly expanded or butterfly-shaped popcorn is more tender and contains fewer partially popped kernels that are hard to chew.

There are basically two types of popping methods: wet and dry. Most processors employ wet popping because it produces better flavor. However, dry popping is ideally suited for production of low-fat products. Normally, popcorn, oil, and salt are placed into a heated pan for production of wet-popped product. At the moment of expansion, the oil is absorbed and the salt sticks to the flakes. The most frequent popcorn/oil ratio is 3:1 and the popping cycle varies from 2.5 to 3 min.

Dry poppers come in two basic types. The older method consists of a rotary wire drum and the modern method of a steel auger. The rotary type heats popcorn kernels over an open flame, whereas in the steel auger, kernels are thermally treated

Table 1 Nutritional and chemical composition of selected snack foods (100 g)

	Popcorn				*Tortilla chips*			*Extruded snacks*		*Wheat-based*		
Nutrient	*Air-popped*	*Oil-popped*	*Potato chips*	*Corn chips, plain*	*Plain*	*Regular ranch-flavor™*	*Light*	*Puffs, cheese-flavor*	*Cones, nacho-flavor*	*Pretzels, hard plain*	*Bread sticks, sesame*	*Sesame sticks*
Water (g)	4.1	2.8	1.4	1.0	1.8	1.7	1.3	1.5	1.9	3.3	6.1	2.0
Energy (kcal)	382	500	558	539	501	490	465	554	536	381	412	541
Protein (g)	12.0	9.0	5.9	6.6	7.0	7.6	8.7	7.6	6.5	9.1	12.0	10.9
Lipids (g)	4.20	28.1	38.4	33.4	26.2	23.8	15.6	34.4	31.7	3.5	9.5	36.7
Saturated (g)	0.57	4.89	9.45	4.55	5.02	4.56	2.84	6.59	26.70	0.75	1.41	6.48
Monounsaturated (g)	1.10	8.17	7.27	9.66	15.45	14.05	6.34	20.28	2.14	1.36	3.57	10.91
Polyunsaturated (g)	1.90	13.42	19.98	16.48	3.63	3.30	5.02	4.76	0.84	1.22	2.09	17.42
Carbohydrates (g)	77.9	57.2	51.0	56.9	62.9	64.6	73.4	53.8	57.3	79.2	68.4	46.5
Dietary Fiber (g)	15.1	10.0	3.6	4.9	6.5	3.9	5.7	1.1	1.0	2.8	3.0	2.8
Calcium (mg)	10	10	24	127	154	141	159	58	36	36	22	170
Iron (mg)	2.66	2.78	1.5	1.32	1.52	1.46	1.63	1.10	1.27	4.32	4.28	0.74
Sodium (mg)	4	884	450	630	528	612	1003	1050	952	289	657	1488
Thiamine (mg)	0.20	0.13	0.21	0.03	0.08	0.11	0.22	0.26	0.21	0.46	0.59	0.12
Riboflavin (mg)	0.28	0.14	0.12	0.14	0.18	0.24	0.28	0.20	0.12	0.62	0.55	0.06
Niacin (mg)	1.94	1.55	3.15	1.18	1.28	1.46	0.42	1.18	1.41	5.25	5.28	1.55
Folate (mcg)	23	17	7.0	20	10	17	16	23	5	171	162	22
Vitamin E (mg)	0.29	5.01	2.2	1.36	3.53	–	3.53	–	–	0.35	1.01	3.85

	Almonds			*Cashews*		*Hazelnuts*	*Macadamia*	*Pecans*		*Pistachio*
Nutrient	*Dry roasted, salted*	*Honey roasted, unblanched*	*Oil roasted, salted*	*Dry roasted, salted*	*Oil roasted, salted*	*Dry roasted, unsalted*	*Dry roasted, salted*	*Dry roasted, salted*	*Oil roasted, salted*	*Dry roasted, salted*
Water (g)	2.6	1.7	2.8	1.7	2.3	2.5	1.6	1.1	1.1	2.0
Energy (kcal)	597	594	607	574	581	646	716	710	715	568
Protein (g)	22.1	18.2	21.2	15.3	16.8	15.0	7.8	9.5	9.2	21.4
Lipids (g)	52.8	49.9	55.2	46.4	47.8	62.4	76.1	74.3	75.2	46.0
Saturated (g)	4.05	4.73	4.21	9.16	8.48	4.51	11.95	6.24	7.24	5.56
Monounsaturated (g)	33.66	32.40	34.79	27.32	25.92	46.61	59.28	43.96	40.97	24.22
Polyunsaturated (g)	12.65	10.47	13.52	7.84	8.55	8.46	1.50	20.57	23.58	13.90
Carbohydrates (g)	19.3	27.9	17.7	32.7	30.2	17.6	12.8	13.6	13.0	26.8
Dietary fiber (g)	11.8	13.7	10.5	3.0	3.3	9.4	8.0	9.4	9.5	10.3
Calcium (mg)	266	263	291	45	43	123	70	72	67	110
Iron (mg)	4.51	2.83	3.68	6.00	6.05	4.38	2.65	2.80	2.47	4.20
Sodium (mg)	339	130	339	640	308	0	265	383	393	405
Thiamine (mg)	0.074	0.114	0.092	0.200	0.363	0.338	0.710	0.450	0.473	0.840
Riboflavin (mg)	0.859	0.953	0.781	0.200	0.218	0.123	0.087	0.107	0.110	0.158
Niacin (mg)	3.850	2.819	3.665	1.400	1.736	2.050	2.274	1.167	1.200	1.425
Folate (mcg)	33	32	27	69	25	88	10	16	15	50
Vitamin E (mg)	26	–	25.97	0.92	0.92	15.28	0.57	1.30	2.53	1.93

USDA (2014).

while traveling along the screw. Oil, salt, flavorings, or sugar-based flavorings are directly applied or sprayed onto the product.

Microwavable popcorn has increased in popularity for its convenience for in-home popping. Most microwave bags contain 75 g popcorn mixed with 25.5 g partially hydrogenated vegetable oil and 1.5 g salt. The mixture is placed in pillow-style microwave bags with a 15 cm susceptor plate and a heat seal band on the inside of the lip. The expansion volume of microwave popcorn should be in the range of 34.2–43.9 ml g^{-1}, the percentage of unpopped kernels from 2.1 to 6.8, and flake size from 4.6 to 7.1 ml.

Peanuts and Other Nuts

Peanuts, almonds, and various sorts of nuts (pecans, walnuts, hazelnuts, and macadamia) are widely used to produce snacks. These snacks are rich in fat and are considered as one of the most nutritious snacks because of their fat-soluble vitamin content, dietary fiber content, high antioxidant activity, and protein quantity and quality. These are considered minimally processed snacks because kernels are roasted or heat-treated and sometimes fried prior to flavoring and packaging. Roasting and/or frying often intensifies and improves the organoleptic properties of the nuts.

Among nuts, peanuts are by far the most important snack. There are three major types of peanuts: Spanish, Valencia, and Virginia. The Spanish varieties produce thin-shelled pods with one or two elliptical flesh-colored kernels. The Valencia and Virginia types produce thick-shelled pods and are the preferred by the snack food industry. The three types have similar chemical and nutrient composition. The timing of the peanut harvest is critical since it can greatly affect quality. If peanuts are harvested too early, many of the pods will be immature, yielding shriveled kernels, whereas if harvesting is too late, many of the pods may be contaminated with molds. Peanuts are usually picked at an average kernel moisture content of 18–30% and dehydrated to lower it to about 5–10%. For marketing, the desired moisture content of shelled peanuts is about 7–8%.

Dry roasting and oil roasting are the two general processes to obtain peanuts. Regardless of the process, selected and mycotoxin-free peanuts are blanched. Blanching is considered one of the most important operations because it affects color, flavor, and overall acceptability.

Second-Generation Snacks

Potato Chips

The traditional potato chip processing method involves deep fat frying thin slices from raw potatoes followed by seasoning. However, there are many variations to this process; some include the use of different processing equipment to achieve a different shape (such as sticks) or additional unit operations to obtain a crispier product with a lower fat content. In recent years, other potato snacks have been introduced to the market; some are made from expanded pellets produced by extrusion. Other potato snacks of commercial relevance are those that imitate potato chips also named fabricated chips. Their processing method consists essentially of making potato dough from dehydrated potatoes mixed with other ingredients; the slices are then mechanically formed and fried.

Starch is the major component of the potato tuber and comprises 65–80% of its dry weight and is the main responsible for the desirable crispiness of potato chips. A direct correlation between starch and dry matter content in potatoes is well documented. Medium to large tubers with specific gravities above 1.085 (~22% dry matter) are optimum for chip manufacture.

Potato cultivars are constantly being improved for specific gravity, low reducing sugar content, and storability. Among the quality tests commonly performed to evaluate a particular cultivar for potato chip processing are slicing, frying, moisture, yield, oil absorption, defects such as blisters, hash, and bulking. High-gravity potatoes absorb lower oil during frying. Better yields are obtained with increasing specific gravity.

In commercial operations, tubers with specific gravities above 1.085 produce chips with oil contents in the range of 35–40% and with yields of finished product ranging between 28% and 36%. The characteristic flavor and yellow color of potato chips are a consequence of the Maillard reaction during frying. The main potato reducing sugars are glucose and fructose, which are used by the processing industry as quality indicators. Upon harvest, the amount of reducing sugars should be below an upper limit range of 2875–3300 ppm fresh weight (equivalent to 0.29–0.33% w/w); above these levels, potato slices will fry too dark for acceptable quality. Tubers with elevated free amine content are also more susceptible to Maillard browning and bitterness development during frying due to pyrazine formation.

Potato chip manufacturing process

The basic flow diagram for the original potato chip process contains nine major operations: peeling, trimming, slicing, washing, draining, frying, inspecting, seasoning, and packaging.

Peeling and trimming

The most widely used method for peel removal is abrasive peeling consisting of rotating drums coated with carborundum. Approximately 10% of the initial potato weight is lost by this peeling method.

Slicing

Crucial to the characteristic crispiness of potato chips is the slice thickness, which allows for rapid moisture loss and crust formation during frying. Raw potatoes are generally sliced to a thickness that varies in the range of 1.27–1.78 mm for straight-cut slices and between 2.54 and 3.05 mm for crinkle-cut chips. Slicing can be performed in continuous or batch slicers.

Slice washing

During slicing, the potato tissues are disrupted, releasing cell wall contents such as starch and internal water. If unwashed slices are placed directly into the fryer; they tend to stick together retaining moisture and oil between the slices. To prevent these defects, potato slices are washed in mesh tumblers by immersion and/or with high-pressure water sprayers. The washing step produces the second largest dry matter losses after peeling. It is estimated that between 10% and 20% of the initial potato weight is lost during this operation.

Preheating

Rinsing the potato slices in warm water at a temperature below potato starch gelatinization (55–60 °C) is an optional unit operation referred to as 'preheating,' and it can be applied to lower reducing sugars or to improve the texture of stored potatoes. Warm water increases the solubility of glucose and fructose, thus preventing browning defects. The mechanism for texture improvement is different; it involves increasing the activity of the endogenous potato enzyme pectin methylesterase (PME). Temperatures of 50–70 °C have been reported to increase PME activity, which strengthens the cell wall–middle lamella complex.

Slice draining

In order to minimize oil uptake during frying, excess washing water should be removed usually by continuous motion that in some instances also includes air injection or vibrating mesh conveyor belt with vacuum suction. Centrifugal devices are also very effective and may also include hot air injection in order to dry the slice surface and reduce frying time and oil uptake.

Frying

After the previous processing operations, raw potato slices, either peeled or unpeeled, washed or not, are ready for deep fat frying. The key for manufacturing a crispy potato chip is rapid moisture loss; the slice goes from a moisture content of 78–1.5% in ~2 min. The characteristic crispiness can be achieved if the drop in temperature caused by the cold product entering the fryer (ΔT) can be maintained below 5 °C. A typical initial temperature for frying oil would be 177 °C. Any hydrogenated or nonhydrogenated fat can be used and may include one or a mixture of oils such as corn, olive, soybean, palm, cottonseed, canola, rapeseed, sunflower, and peanut oils, among others. With the emerging changes in food labeling, which includes the declaration of trans fatty acid content, the snack industry has been moving to the use of nonhydrogenated oil to eliminate trans fats in their products as a health-related marketing strategy.

Seasoning

Most potato chips are salted or seasoned before packaging. The salt and/or seasoning is generally applied when the product is still hot in order to help them adhere to the surface. Commercial equipment for the application of seasonings consists of a unit that applies the seasoning by sprinkling it over the potato chips as they pass below in a conveyor belt or while tumbling in a rotating drum. The salt is generally added in very fine crystals and usually constitutes 1–2% of the finished product weight. Seasonings are generally spice mixtures with other ingredients and additives. Many flavors can be found in the market, with barbecue, cheddar cheese, sour cream and onion, and vinegar, among others, being the most common ones. The flavoring usage levels vary but may comprise from 5% to 10% of the finished product weight.

Packaging

Packaging is the last step of the potato chip manufacturing process. Modern potato chip packages are complex laminates of synthetic polymeric materials. The most commonly used is metalized polypropylene manufactured by the aspersion of aluminum to a polypropylene ply. Each packaging component has a different functionality; the polypropylene contributes mechanical strength, good sealing, good moisture barrier properties, low oxygen permeability, and protection against light, which accelerates oxidative reactions. Potato chips are packaged at a moisture content of 1.5%, and when the moisture increases to 4%, they become soft and stale.

Corn and Tortilla Chips

Corn and tortilla chips are the fastest-growing market within the salted snack category. Extruded corn chips are produced from coarsely ground lime cooked *masa* that is formed and deep-fat-fried, whereas tortilla chips are produced from pieces of baked tortillas that are deep-fat-fried. Baking of tortillas prior to frying greatly affects organoleptic properties and caloric density of these snacks. Tortilla chips have a stronger alkaline-cooked flavor and a crispier texture than corn chips. In addition, tortilla chips are less energy dense because they absorb about 12% units less oil during frying.

Maize kernels, lime, oil, salt, and flavorings are the basic ingredients for the production of these snacks. Product quality and processing parameters greatly depend on the kernel characteristics. However, the oil, salt, and seasonings used for the preparation of maize snacks have a profound effect on flavor, mouthfeel, and overall acceptability.

The nixtamalized snack food process involves several unit operations such as grain cleaning, lime cooking and steeping, stone grinding, kneading, sheeting/molding, cutting, baking, frying, and seasoning. Extruded corn chips are made from coarsely ground *masa* from either fresh *nixtamal* or dry *masa* flour, which is extruded and cut into *masa* chips with different configurations, deep-fat-fried and salted/seasoned. Key operations are the extrusion of the *masa* into different configurations and the deep fat frying. During the 60–90 s frying, the *masa* exchanges water for oil and the product exits the fryer at moisture and oil contents of about 1.5% and 36%, respectively. Resulting chips are usually seasoned with 2% salt and various types of cheese, citric acid, or chili flavorings.

Tortilla chips are generally manufactured from coarsely ground *masa* from either fresh *masa* or dry flour that is sheeted, formed, and cut in preparation for the subsequent steps of baking, equilibrating, frying, and salting/seasoning. Tortilla chips are usually formed by two rotating smooth Teflon-coated rolls that automatically press the *masa* into a thin sheet. The newly formed *masa* disks are baked into tortilla pieces commonly in a three-tier gas-fired oven at temperatures ranging from 350 to 480 °C for 40–60 s. The resulting baked tortilla pieces are cooled for 3–30 min through a series of open tiers that discharge into the fryer. Frying of tortilla chips is characterized by further starch gelatinization and formation of a yellow crust and the characteristic flavor as a result of Maillard reactions. Immediately after frying, the hot chips are salted and seasoned in rotating tumblers equipped with liquid sprayers and/or auger or electrostatic powder dispensers. After seasoning, fried products are cooled and immediately packaged in moisture-proof or aluminized bags to avoid rancidity and loss of crispiness. There are basically two types of low-fat tortilla

chips: fried and toasted. The first type usually contains 1/3 less oil compared with regular counterparts, and the second type does not contain any added oil although some products are sprayed with cooking oil to increase fat content to 7–8% to enhance flavor and texture. For production of reduced oil snacks, moisture is removed from the baked tortilla chip by further baking or toasting or baking/flash frying to yield a crispy product.

Extruded Snacks

Most second- and third-generation snacks are produced by extrusion cooking. Production of snacks with cooking and forming extruders saves space in the processing plant, offers more versatility, adapts to highly productive continuous plants, and saves labor and energy when compared with expenditures of traditional processes. There are basically two types of snack items obtained with thermoplastic extruders: the direct-expanded snacks and the third-generation snacks produced from pellets or half products obtained after sequential cooking and forming extrusion steps. The direct extruded types are subdivided into bake puffs and fried puffs.

Production of direct-expanded baked products

The raw material for collets should be highly refined and properly conditioned. In most instances, refined grits conditioned to moisture contents from 14% to 19% produced the best-quality products. The conditioned grits enter single or twin extruders set at high temperature (90–120 °C) and a screw design that favors compression. The combination of heat and pressure converts the grits into plastic dough that melts and becomes a viscous fluid. At the very instant that the viscous fluid exits the die orifice, it will instantaneously equilibrate with the external atmospheric pressure causing the expansion and setting of the collet structure. During this time, most of the moisture instantaneously vaporizes, so the extrudate drops its moisture to about 5–8%. The puffed collet is cut and sized to the proper length with a rotating knife. The collet expansion rate is generally between 6 to 8 and the bulk density of 50–65 $g l^{-1}$. Resulting puffs are conveyed into a rotary dryer or oven to drop their moisture to around 1–2%. The driers, set to 130–150 °C, dehydrate the low-density collets in only 4–10 min. The baked expanded collets are finally flavored in a rotary tumbler generally with salt–oil–cheese slurry that is sprayed onto the extrudates. The amount of coating applied ranges from 34% to 42% based on the final puff weight. The product is finally cooled and immediately packaged to avoid loss of texture or crispiness. The puffs generally contain from 27% to 36% oil and about 2% salt. Fried expanded curls are still widely accepted among snack consumers because they possess a different flavor and configuration. These snacks have been produced for more than 60 years and are manufactured using a special type of extruder that generates most of the heat by friction. Technically, this extruder falls into the medium shear category and works similarly as the direct-expanded high-shear extruders. The major difference is that the fry-type snack extruder does not extrude the product through a typical die. Instead, it uses a set of plates: one rotating and the other stationary. These plates shape and expel the extrudate that is internally cut by rotor fingers.

Tempered refined cereal grits are introduced and transported into the barrel section of the stator, and the shear applied by the screw movement increases both the pressure and internal temperature so the starch gelatinizes, melts, and plasticizes. The rotor fingers cut the extruding material, which are then immediately extruded radially between the two plates. As the material exits from between the plates, the pressure drops from 48 Atm to atmospheric pressure and the water superheats and vaporizes expanding the extrudate. The resulting extrudates are generally fried to reduce the moisture level from around 8% to 1.5% for texture and stability, and the fried curls pick up 20%–25% oil. After frying, the collets are seasoned with a two-stage seasoning system where an oil-based slurry is first applied followed by dry seasoning generally consisting of cheddar cheese. Ten to fifteen percent of seasonings containing 10–15% salt are usually dosaged onto the extrudates. This brings the total fat content to a level of around 30–35%.

Third-Generation Snacks

Third-generation snacks are produced from half products or pellets. Pellets are obtained after extrusion cooking and forming and dehydration to yield a shelf-stable product that could be stored for long periods of time. For snacks, the key additional process is frying and less frequently conventional or microwave baking. During these heat treatment operations, pellets expand and lose moisture so a crisp snack is produced. Pellets are cooked in an extruder and forced through a die at temperatures below 100 °C. The lower temperature prevents expansion and favors the production of a high-density pellet that is further dried to yield a shelf-stable intermediate product. Pellets, in contrast to the directly expanded puffs, offer the advantage of being distributed with high weight and low volume. The basis for the wide variety of these snacks is their shape imparted by the die design and configuration. Most common shapes are flat, tube, shell, ring, screw, and wheels.

Most pellets are manufactured from mixtures of starch-rich raw materials that include dehydrated potato products and/or potato starch, modified starches, wheat flour, and refined cereal grits. The starch content of the blends approximates 90% of the dry matter. Key operations for the production of third-generation snacks are extrusion, pellet drying, and frying. These snacks are usually flavored with powdered seasonings generally applied with a belt-type flavor depositor or in a revolving tumbler. After seasoning, fried products are cooled and immediately packaged in moisture-proof bags to avoid rancidity and loss of crispiness due to moisture uptake.

Wheat-Based Snacks

Wheat flour is extensively utilized to produce snacks such as crispbreads and pretzels. The industrial manufacturing process to obtain toasted bread is practically identical to production of fresh bread with the additional operation of bread toasting or drying. There are many bread formulations generally produced to form refined wheat flour, whole wheat flour, or composite flours produced from mixtures of wheat flour with oat, rye, or various crushed grains. Sesame is frequently used as topping. Toasted bread or crispbreads have prolonged microbial shelf life because they generally contain approximately 6% water

equivalent or an a_w of less than 0.5 and a pH below 5. The dehydration toasting process enhances staling and the crispy and brittle texture that characterize crispbreads. Fresh bread normally contains 32%–35% moisture, and this moisture should be carefully removed to produce top-quality crispbreads. The rate of product dehydration depends on the oven settings and product volume or dimensions. Toasting is different from baking because the baked product is dehydrated at lower temperature. Alternatively, crispbreads are produced with extrusion technology into the traditional rectangular-shaped crisps, but new filled forms such as U-shaped and hollow tubes are becoming more important.

Production of Pretzels

A pretzel is a wheat-based snack food with 800 years of history. The typical pretzel configuration clearly shows a cross in the middle representing the crossed arms of a Christian prayer. Pretzels are very popular in the European community and the United States. Pretzels are manufactured from fermented wheat flour dough that is treated with a covering of a sodium hydroxide or other alkali salt solutions prior to baking. Pretzels are unique because of its characteristic flavor and color due to the sodium hydroxide wash. The typical flavor profile is also affected by the products yielded during fermentation, nondiastatic malt and salt. Wheat flour is mixed with about 42% water, 0.25% oil, 0.25% sugar, and 0.027% yeast to form a coarse dough that is rested for 20 min before mechanical rolling and forming into cylinders that are molded into traditional configuration. Pretzels are fermented with yeast for a short time (30 min), formed, and washed for 25–30 s with a 1% sodium hydroxide solution heated to approximately 93 °C. Next, they are immediately salted, flavored, and baked. Baking is performed in three sequential steps: the first step lasts approximately 10 min at high temperature and is aimed toward the formation of the typical dark brownish crust. The other two baking stages are to dehydrate the product and produce the typical crisp texture. Hard pretzels are usually packaged in laminated or coextruded materials with an inert gas to protect the flavor and decrease the rate of product breakage during handling.

Nutritional Value of Snack Foods

Snack foods are one of the most concentrated forms of food energy because the majority contain large quantities of oil and starch and are practically free of water (Table 1). The dietary fiber, vitamin, mineral, and protein content and quality of most snacks are low because they are generally manufactured to form refined cereal grits and/or starches. Furthermore, the various thermal operations decrease the availability of vitamins. All these reasons explain why snacks are considered as 'empty foods' and have bad image among dietitians. Most snacks are salted or flavored with salt-based flavorings and therefore contain large quantities of sodium.

Today, food scientists are including in their research and product development equations for production of snacks with minimal fat calories and fat decomposition products known to have adverse effects on human health and production of snacks with similar sensory properties (flavor, texture, and overall acceptability) compared with their regular counterparts. A healthful balance of fatty acids and the production of snacks free of *trans* fatty acids are taken into consideration.

See also: Dietary Fiber: Determination; Extrusion Cooking: Principles and Practice; Fatty Acids: Determination and Requirements; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Maillard Reaction; Maize; Oats; Peanuts; Potatoes and Related Crops; Sodium: Properties and Determination; Starch: Structure, Property, and Determination; Tortillas.

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Sodium: Properties and Determination

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Properties and Determination

Sodium, prepared for the first time by the British chemist Humphry Davy in 1807, is the eleventh element of the periodic table (atomic number 11). Its symbol is Na, and it is included in group 1 (or IA), thus corresponding to the alkali metals. It is an extremely soft silvery metallic element and even can be cut with a knife (hardness of 0.4). It is a very reactive element that tends to be used extensively in the chemical industry. It rusts very quickly when exposed to air and reacts violently with water, forming sodium hydroxide and hydrogen and producing a yellowish flame. It has a fusion point of 98 °C, a boiling point of 883 °C, an atomic weight of 22.98977, and a relative density of 0.97 g cm^{-3}.

Sodium has an electronic structure of [Ne] $3s^1$, and consequently, it always acts with valence 1. It has only one external electron, in orbital 3s, and so the degree of attraction that it suffers with respect to the nucleus rivals the repulsion of the lower orbitals, making it very easy for this electron to disappear, forming the Na^+ cation, its most common state.

Two percent of the Earth's crust comprises sodium, making it the sixth most abundant element in nature, where it is always found in a combined state. Sodium is currently manufactured by electrolytic procedures on melted sodium chloride. Its combining facility yields numerous compounds; with oxygen, it forms Na_2O, Na_2O_2, and even NaO_2, an important whiting and rusting agent. However, the most abundant form and one of the main sources for human use is in combination with chlorine in the form of sodium chloride (NaCl), which is usually extracted from the sea and salt lakes, or in mineral form as rock salt. Sodium can also be found in the form of sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), and sodium sulfate (Na_2SO_4). Also worth mentioning is sodium hydroxide, known as caustic soda, which is used throughout industry, from the manufacture of soap to the manufacture of rayon, paper, rubber, and oilskin, in petrol refineries, or in the textile industry. Sodium fluoride (NaF) is used as an antiseptic, as a poison for rats and cockroaches, and in the manufacture of ceramics. Nitrate from Chile (sodium nitrate) is used as a fertilizer. Sodium thiosulfate ($Na_2S_2O_35H_2O$) is used in photography as a fixing agent and is even used in the nuclear power industry as a cooling agent.

All these specific uses of sodium are only a few examples, since this cation is one of the most common companions of different molecules, both in nature and when created by man for different purposes.

In terms of the nutritional characteristics of sodium, 90% of sodium provided in the diet is obtained from the sodium chloride consumed. The rest of the sodium is from other sources such as bicarbonate and sodium glutamate. Of the total sodium intake, it has been shown that only 10% originates naturally from foods, around 15% from salt added to cooking or to season food once cooked, and the remaining 75% from the processing and manufacturing stages of foods.

Given the physiological need to supply the human body with sodium in order to maintain extracellular osmotic pressure, and as a result of the obligatory losses through urine and sweat, a regular intake of sodium is required. In ancient times, this meant a dependence on salt deposits, and a huge value was placed on what is now common salt, so much so that this gave rise to the Latin word salarium, corresponding to the payment in the form of salt made to soldiers in ancient Rome, and it was also used as money in Tibet and Ethiopia, as a tax in Asian countries, and as a ritual element in Greek, Roman, Hebrew, and Christian religions.

This physiological need for sodium may explain why the tongue contains specific receptors for the taste of salt. For this reason, in addition to the tradition of preserving certain foods in salt, although culturally and gastronomically unnoticeable, salt is consumed in developed societies.

Over the last few years, there has been a growing trend in some international organizations in collaboration with food industry to recommend a reduction of sodium levels in foods as a consequence of processing (e.g., in bread), due to the high incidence of some pathologies such as hypertension and coronary diseases. The World Health Organization (WHO) has estimated that reducing dietary sodium intake by 50 mmol $\cdot day^{-1}$ (2.9 g salt) would lead to a 22% reduction in the number of deaths from strokes and a 16% reduction in deaths from coronary heart disease. However, fulfillment of this objective is not easily achieved since sodium chloride has several important technological properties such as flavor improvement, structuring, preservative, and modulating water activities. The replacement of salt with potassium chloride does not result in any significant production disadvantages but has an adverse impact on the flavor, due to the production of metallic taste, when it rises above a certain percentage.

Preparation of Samples for Sodium Analysis

The determination of sodium in foods may be achieved using a variety of sample preparations, ranging from noninvasive methods, such as those used for determining sodium by selective electrodes, to complete incineration of the sample and subsequent solubilization of the ash in acid solutions. Each analytic technique requires specific preparation of the sample, since the instrumental requirements in each are very different.

As sodium is usually found in soluble form in foods, the simplest method of preparing samples involves washing and then analyzing the sodium from the solution sample; for this purpose, the food often only has to be crushed, solvent-added, homogenized, and filtered or centrifuged. Solvents to be used may be simple water or slightly acidic or alkaline solutions.

Some methods even propose the direct use of food (particularly liquids) in the detection equipment. Although these may yield good analytic results, other food components such as fats, sugars, or proteins can also deteriorate or stain the instruments.

Given the stable character of sodium, the most aggressive techniques usually yield very good results in terms of the recovery of analyses; hence, the use of incineration at around 500 °C overnight and subsequent recovery in weak acid solutions can be used as a standard sample preparation method for a wide range of foods, modifying temperature and time according to the food in order to obtain soft ash, evidence of good interaction.

Other methods for destroying organic matter, such as acid hydrolysis, can also be used without significantly affecting the detection of sodium content, and a wide range of acids may be used in variable concentrations.

The use of incineration or acid hydrolysis is particularly recommended, due to the possibility of obtaining solutions in which other cations of interest for food can be analyzed, such as K, Ca, Fe, Cu, and Zn.

Methods of Analysis

Although sodium may be determined by a wide variety of instrumental techniques, the recommended and most often used technique is atomic spectroscopy, either by emission or by absorption. This technique is based on the atomic properties of the elements and more specifically on their capacity to react in response to specific stimuli, modifying their orbital distribution, for which purpose they absorb light at a specific wavelength for each element, passing on to a state of higher energy, or lower energy if passing from the previous stage, emitting light at the same wavelength. In the case of sodium, the recommended specific wavelengths are 589.6 and 589.0 nm.

In order to achieve atoms and the passage of electrons to a state of higher energy, it may be necessary to increase the temperature, usually by means of a flame, and the incidence of a beam of light of a specific wavelength. The latter technique enables the determination of the analytic concentration by determining the difference between the energy supplied to the sample and that that remains after crossing – this is generically known as flame atomic absorption spectroscopy (FAAS).

In contrast, if the energy of the element is increased by another method (flame), what is usually measured in order to determine the concentration of the analyte is the emission of characteristic wavelength light when returning to the state of lower energy; this technique is known as atomic emission spectroscopy (AES).

Atomic Absorption Spectroscopy

Although the basis of the technique is the same as that for atomic emission and, in many cases, the same equipment is used for both analyses, this technique is only favored by fewer analysts, due to partly the concentrations of sodium in foods that may be calculated for emissions with limited need of dilutions and to the added cost of using a hollow cathode lamp for analysis, without evident analytic improvement. However, the existence of multielement lamps can reduce further costs. This technique is more suitable for low concentrations as a lower detection limit may be obtained. Several official methods for sodium by FAAS exist, such as AOAC Official Methods 985.35 for infant formula, enteral products, and pet foods and ISO 8070:2007 method for milk and milk products. Standard working conditions for analyzing sodium by atomic absorption spectroscopy are listed in Table 1.

Table 1 Standard conditions for sodium analysis by atomic absorption spectrometry

Spectrometer's setting	*Sodium*
Wavelength/slit (nm)	589.0/0.2
Nebulizer	Spoiler
Oxidant	Air
Fuel	C_2H_2
Flame condition	Oxidizing
Optimum concentration range in solution (μg ml^{-1})	1–20
Interferences	Ionization

Atomic Emission Spectroscopy

The determination of sodium in biological and food samples, as in the case of potassium, may be performed using flame photometers and atomic absorption spectrophotometers (with the option of working in emission conditions). In both cases, a sample in a liquid state is introduced into a fuel carrier (acetylene or propane) and combined with atomized or nebulized N_2O_2 air or oxygen. This dispersion is led to a burner where the fuel combusts. The heat of the flame prompts an increase in the energy of the sodium atoms, which, upon returning to their fundamental state, emit a light beam of characteristic wavelength that is quantified by a detection system. The amount of light emitted is proportional to the concentration of the analyte in the sample.

With this technique, the optimum gain is adjusted to the highest standard of sodium used, and an attempt is made to keep the burner free of sodium impurities emitted by the actual samples and standards analyzed, since these impurities may introduce undesirable emissions or obstruct the passage of fluids through the burner; when the flow decreases, the signal obtained will also decrease. Avoiding samples with high concentrations of sodium (such as undiluted seawater) is considered good practice.

A further potential problem lies in small fluctuations that may occur in the flame or in the carried mixture; these may lead to inaccurate and variable results. To correct this problem, some devices are equipped with a second detector that enables the emission of a second element (usually lithium) to be estimated, with a different wavelength to that of sodium. This second element must be added to solutions of both samples and patterns in a stable and known concentration. When there is a variation caused by the flame or the flow, both detectors must reflect this simultaneously, distinguishing this from variations in the analyte.

Another precautionary step involves the adjustment of the calibration line since, in most cases, this is not linear; instead, it displays a decrease in the slope when concentrations increase due to the fact that the area of linearity between the analytic concentration and the atomic emission is exceeded. The simplest solution involves diluting the samples until the standards

Table 2 Accuracy and precision of the sodium analysis by flame emission

Accuracy	*Certified ($g\ kg^{-1}$)*	*Found ($g\ kg^{-1}$)*	*IC (95%)*[a]	*Rec (RSD)*[b]
Citrus leaves SRM-1572	0.16 ± 0.02	0.17 ± 0.03	0.11–0.24	108 (18)
Nonfat milk powder NIST-1549	4.97 ± 0.10	5.17 ± 0.14	4.90–5.46	104 (2.8)
Precision	Relative standard deviation			
Plant food	1.15			
Dairy food	1.20			

[a]Intervals of confidence (95%).
[b]Recovery percentages and relative standard deviation (RSD).

that include them present a straight adjustment; however, it is also feasible to make a logarithmic adjustment between the emission and the concentration of the type $E = a^{*}\ln[\mathrm{Na}] + b$, by a simple logarithmic transformation of the concentrations prior to the calculation of the regression line, and the subsequent retrotransformation when applying the linear equation to calculate concentrations. This type of adjustment is usually extremely useful when the sample values are high but not very different. As a result of this restriction, determination of the detection or quantification limit cannot be performed reliably on the standards used, although the high concentrations used guarantee that the readings can never be confused with the fluctuation of the baseline of the device.

The accuracy and precision of sodium analysis by flame emission spectroscopy are shown in Table 2. In recent years, some manufacturers have named AES instruments as 'optical emission spectrometers' (OES) because they measure the emitted light when excited atoms return to the ground state. Since this latter term is more widely used, we will use OES rather than AES onward in the article, but both of them are practically interchangeable.

Inductively Coupled Plasma Optical Emission Spectrometry

Another way to produce the excitation of the atoms in food analysis is the use of electrically generated plasmas (highly ionized gases) resulting on inductively coupled plasma optical emission spectroscopy (ICP-OES). It allows to analyze samples with a high degree of variability and minimum interferences that can be prepared either by wet oxidation or by dry ashing. It has been applied to a wide variety of food matrices such as fish, mushroom species, and chocolate-flavored beverages and has been used for sodium analysis in the 234 foods of the US Total Diet Study. AOAC Official Method 984.27 for infant formula uses this technique. A wide range of concentrations can be used without the need for dilution or concentration, and as a multielemental technique, many of the mineral elements can be simultaneously quantified, thus reducing analysis time. These advantages, together with the high speed and excellent instrument stability, make the ICP-OES method highly attractive in view of its analytic applications.

ICP can also be used coupled with a mass spectrometer leading to inductively coupled plasma mass spectrometer (ICP-MS). Mass spectrometers are instruments that separate ions according to their mass to charge ratio (m/z) and then accurately count these ions as they strike a detector. ICP-MS has the potential to yield accurate results for sodium when analyzing a wide range of foods with low sodium concentrations. However, due to the high cost of the equipment, it has been used less frequently when sodium concentrations are higher.

Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) comprises the spectral region ranging from wavelength 12 800 to 4000 cm^{-1} (or 780–2500 nm). In spite of the early discovery of infrared radiation by William Herschel about 200 years ago, the interaction between NIR radiation and matter has been extensively studied by researchers only over the last few years, essentially due to NIR spectral complexity and weak absorptions when compared, for example, with the absorptions in the mid-infrared region. NIR spectra are the result from overtones and combination bands generated by different functional groups such as C—H, N—H, and O—H. Classical statistical methods, such as univariate analysis, are not considered valid to obtain this information. The establishment of chemometrics, a distinctive discipline in chemistry, comprising many specialized mathematical and statistical tools, has greatly promoted the development of NIRS.

As already mentioned, NIR spectrum of an organic material gives a global signature of composition based on the assessment of the organic chemical structures containing C—H, N—H, and O—H bonds. However, the determination of inorganic compounds as sodium is more challenging compared to organic compounds owing to the lack of a direct relation between the element involved and the spectra obtained. In this case, NIR spectroscopy could be used for determining mineral concentration, due to the association between minerals and organic functional groups in the food matrix and the effect on O—H bonding. The determination of sodium and other mineral elements by NIR is therefore generally dependent on the presence of those elements in mixtures of organic or hydrated compounds and salts (cations and anions).

NIR spectroscopy is being widely adopted for sodium analysis of many food products such as wine and cheeses and human diets, among others. NIR technology is rapid, accurate, low cost (does not require sample processing), nondestructive, and more environmental respectful when compared with other analytic techniques. Table 3 summarizes NIR methods for sodium and sodium chloride in food and biological samples.

Titrimetric Methods

The spectroscopic methods described previously may assume a significant financial barrier for smaller laboratories due to the maintenance of gas supplies and fume extraction equipment. As an analytical chemistry technique, potentiometric titration method for chloride ion (AOAC Official Method 971.27) represents a very quick, sensitive, and cost-effective alternative for the quantification of sodium chloride in foods. The analysis

Table 3 Analytic methodologies with NIR spectroscopy for sodium analysis

Study	*Reference*
Determination of the sodium chloride content of sausages by near infrared spectroscopy	Ellekjær, M. R., Hildrum, K. I., Næs, T., Isaksson, T. (1993). *Journal of Near Infrared Spectroscopy* 1, 65–75
Mineral analysis (Fe, Zn, Ca, Na, K) of fresh Iberian pork loin by near infrared reflectance spectrometry: Determination of Fe, Na, and K with a remote fiber-optic reflectance probe	González-Martin, I., González-Pérez, C., Hernández-Méndez, J., Álvarez-García, N. (2002). *Analitica Chimica Acta* 468, 293–301
Nondestructive prediction of moisture and sodium chloride in cold smoked Atlantic salmon (*Salmo salar*)	Huang, Y., Cavinato, A. G., Mayes, D. S., Bledsoe, G. E., Rasco, B. A. (2002). *Journal of Food Science* 67, 2543–2547
Nondestructive determination of moisture and sodium chloride in cured Atlantic salmon (*Salmo salar*) (Teijin) using short-wavelength near-infrared spectroscopy (SW-NIR)	Huang, Y., Cavinato, A. G., Mayes, D. S., Kangas, L. J., Bledsoe, G. E., Rasco, B. A. (2003). *Journal of Food Science* 68, 482–486
Predicting sodium chloride content in commercial king (*Oncorhynchus tshawytscha*) and chum (*O. keta*) hot smoked salmon fillet portions by short wavelength near-infrared (SW-NIR) spectroscopy	Lin, M., Cavinato, A. G., Huang, Y., Rasco, B. A. (2003). *Food Research International* 36, 761–766
The mineral composition (Ca, P, Mg, K, Na) in cheeses (cow's, ewe's, and goat's) with different ripening times using near infrared spectroscopy with a fiber-optic probe	González-Martín, I., Hernández–Hierro, J. M., Revilla, I., Vivar–Quintana, A., Lobos-Ortega, I. (2011). *Food Chemistry* 127, 141–152
Trace metal studies of selected white wines and alternative approach	Sauvage, L., Frank, D., Stearne, J., Milikan, M. B. (2002). *Analytica Chimica Acta* 458, 223–230
Quantitative analysis of minerals and electric conductivity of red grape homogenates by near infrared spectroscopy	Cozzolino, D., Cynkar, W., Shah, N., Smith, P. (2011). *Computers and Electronics in Agriculture* 77, 81–85
Assessment of internal beet quality by NIRS (near infrared spectroscopy) and FTIRS (Fourier transform mid infrared spectroscopy)	Huijbregts, A. W. M., Heijuen, C. J., Moulin, B., Noé, B. (2006). *Sugar Industry Zuckerindustrie* 131, 16–20
Direct determination of minerals in human diets by infrared spectroscopy and x-ray fluorescence	Mir-Marqués, A., Garrigues, S., Cervera, M. L., de la Guardia, M. (2014). *Microchemical Journal* 117, 156–163

involves the determination of chloride ion content by a potentiometric precipitation titration using a silver nitrate solution as titrant to subsequently infer the sodium content from the stoichiometry of sodium chloride. The titrations are performed using a Ag Titrode electrode and the equivalence point is determined automatically by the instrument's software. The main disadvantage is the accuracy relies on that sodium chloride is by far the major contributor to the overall sodium and chloride content. However, other many types of sodium sources are used in food industry as preservatives, stabilizers, emulsifiers, humectants, and flavor enhancers such as sodium phosphates, sodium nitrate, sodium ascorbate, and sodium glutamate, among others, and as a result, the sodium content could be underestimated. Likewise, other chloride sources are present in foods such as potassium chloride. A novel trend is to substitute a portion of the sodium chloride additive with potassium chloride in order to maintain a level of saltiness. Finally, the measurement of chloride by silver nitrate titration is known to undergo interference from halogens and other anions, but fluoride, bromide, and iodide contents are normally negligible in foods unless specific technological treatments of the raw materials have been applied.

Titrimetric methods for the determination of sodium ion itself based on the insolubility of sodium zinc uranyl acetate or a complexometric procedure using CyDTA (*trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid) have been developed but not widely accepted.

Sodium-Selective Electrodes

Together with the optical techniques of absorption and atomic emission, this is one of the most common techniques for testing sodium content in foods due to the low cost of the instruments, ease of use, speed, noninvasive nature, and even the at-line analysis in food production lines.

The technique is based on the passage of ions through a glass membrane, being specific for an ion or small group of ions. In the case of sodium, the probe can detect this activity once the membrane has been crossed; this activity is proportional to concentration. Possibilities of interference with other ions exist, that is, specificity is not as high as with spectromorphometric methods, and the formation of complexes with other substances reduces sodium activity and therefore its capacity for determination. However, it is useful for high levels of sodium like in salted products such as ham and cheese, in which controlling salt penetration may be recommended.

Other Techniques

A large number of additional techniques may be used to determine sodium content in foods, from classical gravimetric methods, now virtually obsolete, to techniques that involve the use of instruments that are expensive, sophisticated, and requiring highly skilled personnel, which can limit their effective implementation. The most relevant techniques include the following: nuclear magnetic resonance (NMR) spectroscopy, ion chromatography, x-ray fluorescence, neutron activation analysis, capillary ion electrophoresis analysis, electron-probe microanalysis, helium glow photometry, ion-microprobe analysis, laser probe analysis, ion-scattering spectrometry, Auger electron spectroscopy, impact radiation analysis, enzymatic determination, and α-particle backscattering analysis.

NMR spectroscopy has been used increasingly in recent years for determining sodium content, mainly in living tissue

or biological samples. Therefore, it can be used for foods, particularly for establishing sodium distribution in such samples. NMR detects signals from nuclei in atoms and molecules when they are excited in a strong homogeneous magnetic field. In this technique, Na^+ has a nuclear magnetic moment of 3/2, which allows it to undergo NMR; ^{23}Na is extremely easy to detect in biological samples. However, the results must be analyzed carefully as discrepancies in the quantitative results have been observed, detecting only 60% of sodium.

See also: Hypertension and Diet; Infrared Spectroscopy: Applications; Potassium: Properties and Determination; Spectroscopy: Types.

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Solanaceous Fruits Including Tomato, Eggplant, and Peppers

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Introduction

The tomato (*Solanum lycopersicum*) is one of the most commercially important vegetable cultivated worldwide. Tomato is a member of the nightshade family or Solanaceae that includes more than 3000 species. This family also includes other popular species, such as potato, tobacco, peppers, and eggplant (aubergine). Tomato is the only domesticated species of the section *Lycopersicon* of the genus *Solanum*, which consists of 13 species or subspecies. The tomato originated in the western coastal plain of South America. It was first domesticated in Mexico and the fruit was introduced to Europe by the Spanish explorers in the mid-1500s. For many years, tomatoes were considered as poisonous and unsafe to eat as they are members of the nightshade family. Tomatoes were introduced to the United States in 1710 but only became popular as a food item later in that century. Today, tomato is the second most consumed and widely grown nonstarchy vegetable in the world after potato. The average world production of tomato is estimated to be around 159 million tons. In the last few years, tomato consumption has increased considerably as they became an important part of the human diet. Therefore, the area of tomato cultivation has also expanded globally in the last few years. The leading tomato producers are China, India, the United States, Turkey, and Egypt. According to the USDA's estimate, 35% of raw tomatoes are processed into sauces, 18% into tomato paste, 17% into canned tomatoes, 15% into juices, and 15% into catsup.

Pattern of Consumption

Tomatoes form a vital part of a variety of dishes and food products including curries, soups, and pasta. Tomatoes are eaten fresh and commonly consumed as part of salads and sandwiches. They are also served roasted, broiled, and stewed. Ripe tomatoes are processed into various products including tomato paste, ketchup, sauces, sorbets, juice, and puree. Ripe tomatoes are also canned and sold as diced or whole tomatoes. They are also sundried and used in many products. Wines are also made from tomatoes. Unripe green tomatoes are also eaten deep-fried, pickled, or used in salsa. Tomatoes form an essential part of Indian cuisines especially in curries. They are also extensively used in Middle Eastern, Mediterranean, Italian and Spanish cuisines and are used to make pizzas, spaghetti, and pastas.

Botany and Plant Description

The tomato is a perennial plant typically growing to 1–3 m tall, but it is grown as an annual plant. It is a branching herbaceous diploid plant ($2n = 24$), and the growth habit ranges between erect and prostrate. Stem is green and covered with glandular trichomes. Leaves are spirally arranged and the leaflets are ovate to oblong covered with hairs. Inflorescence is a cyme consisting of clusters of 6–12 flowers known as 'truss.' Bright yellow colored flowers are bisexual, pentamerous, and pendulous. Flowers are mostly self-pollinated and also partly cross-pollinated. Bees and bumblebees are the principal pollinators. The fruit is an edible berry and the shape is variable with cultivars. Tomato fruit may be round, oblate, flattened at the top and bottom, or pear-shaped. Immature berries are pale green turning to red when ripe. Botanically, tomato is a berry, but it is nutritionally classified as a vegetable. Berries are fleshy and juicy, which can be bi-or multilocular. Fruit is composed of pericarp, placental tissue, and seeds. Pericarp or the ovary wall can be divided into exocarp, mesocarp, and endocarp. The exocarp consists of a cuticle layer that thickens with fruit maturity and the skin, which includes an epidermal cell layer and three to four layers of collenchymatous tissues. Numerous flattened straw-colored seeds are attached to the placenta, a parenchymatous tissue that becomes gelatinous and softens as the fruit matures.

Tomato Production

Tomato is a frost-sensitive tender warm season crop. It requires a relatively cool, dry climate for optimum growth and high yield with good quality. However, it can survive a wide range of climatic conditions from temperate to hot and humid tropical. An average daily mean temperature of 20–24 °C is considered optimum for growth, yield, and fruit quality. Plants can survive a range of temperatures, but the plant tissues are damaged below 10 °C and above 38 °C. Fruit set and quality are poor at temperatures below 12 °C and 35 °C. Significantly higher or lower temperatures can have negative effects on fruit set and quality. The tomato is a self-pollinating plant and outdoors, can be effectively pollinated by wind currents. Tomato prefers deep, well-drained, sandy loam soils that are rich in organic matter and plant nutrients, with a pH value of 6–7. Soils with very high organic matter content like peat soils, are less suitable due to their high water holding capacity and nutrient deficiencies. Tomatoes are generally transplanted to the field from greenhouse-grown plants. Seeding tomatoes directly into the field is not recommended due to the high cost of hybrid seed and the specific conditions required for adequate germination.

Tomato plants can be distinguished into the determinate or the bush type and the indeterminate or the tall type. Plants are erect and bushy with restricted flowering and fruiting in determinate types and plant growth stops with the commencement of reproductive stage. They have a short and concentrated fruit set that lasts only 2 or 3 weeks, and the fruits ripen much faster than those of indeterminate types. The commonly cultivated commercial varieties are mostly determinate types. The

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indeterminate or the tall varieties continue to grow after flowering and are suitable for a long season cropping and harvest period. Most heirloom garden varieties and greenhouse tomatoes are indeterminate, and they produce flowers and fruit throughout the life of the plant. Most cultivated tomatoes require around 75 days from transplanting to first harvest and can be harvested for several weeks before production declines.

Most commercial determinate tomatoes are grown using short-stake culture for trellising. This type of culture produces fruits that are higher in quality and easier to harvest and enhances spray coverage. Staking or trellising tomato plants with bamboo poles, wood stakes, or other sturdy materials provides support and keeps the fruit and foliage off the ground. Staking will increase fruit yield and size, reduce fruit rot, and make spraying and harvesting easier. Determinate tomatoes often require some level of pruning or the removal of suckers (axillary shoots). The need for pruning varies with the variety used but can significantly impact fruit quality and yield. Indeterminate varieties should be staked to facilitate pruning, pinching, harvesting, and other cultivation practices.

Greenhouse Production System

Greenhouse production of tomato has many advantages over the field production such as careful monitoring of the growth conditions and proper maintenance techniques, which allows for off-season year-round production. Greenhouses can produce $\sim$15 times more yield per acre than under field conditions. Also, $>$90% of the yields are marketable fruits in greenhouse compared to 40–60% marketable fruit under field production. Selection of suitable tomato cultivars is a prerequisite for greenhouse tomato production. Generally, most greenhouse tomato cultivars are indeterminate in growth habit. Because of high production costs, the number of fruit per plant is very important, so the chosen cultivar or variety should also set a high number of fruit per plant.

Cluster tomatoes are grown under greenhouse production system. Two systems commonly used for greenhouse tomato production are the soil or the soilless culture. In soil culture, tomatoes are grown under a greenhouse in a plot of soil using similar techniques that are used in the field. In soilless culture (also called hydroponics), the necessary nutrients are delivered to the plant root system in the irrigation water through a network of pipes. The commonly used substrates for plant growth include rock wool, coco fiber (coir pith), perlite, peat, sawdust, and pine bark. Greenhouse tomatoes are started from high-quality transplants to ensure uniform crop establishment.

Fruit Development

After fertilization, tomato fruit development proceeds through several stages. In the first stage, rapid cell division follows immediately after fertilization, leading to a progressive increase in pericarp cell number. The end of this stage is marked by a sharp fall in the rate of cell division, which occurs after 2 weeks of pollination. During the second stage, an increase in fruit weight occurs as a consequence of cell expansion. The fruit turns into the mature green (MG) stage during the third phase. After reaching the MG stage, elaborate metabolic changes occur in the tomato fruit, which marks the beginning of the fruit ripening process. The breaking (BR) and the ripening (RR) stages are the two phases of ripening.

Skin color is the most widely used external index of tomato maturity, and distinct changes in external color can be used to determine harvest maturity. Seed development and locular gel formation are important internal indexes of maturity. MG fruit has fully developed straw-colored seeds, which will not be cut when the fruit is sliced with a sharp knife. The locules of MG fruit are completely filled with jelly at maturity, and the immature green fruits have one or more locules without jelly. Skin color remains green during fruit development on the plant. On reaching maturity, the blossom end of the tomato fruit changes to a light green or whitish color followed by the appearance of one or more distinct white streaks usually at the blossom end. This stage is referred to as 'mature green.'

Ripening

Tomato is considered as a climacteric fruit. Fruit ripening involves a series of biochemical events that convert the green unripe hard acid tasting tomato fruit into a soft fruit with sweet taste and aroma. An increase in respiration, increase in ethylene production, fruit acidity changes, and changes in starch and sugar content are some major biochemical changes that occur during ripening. Tomato fruit develops coloration following a typical sequence with ripening. In most red-colored cultivars, after the MG stage, the tip of the blossom end changes to a pinkish-yellow color, which is referred to as the 'breaker stage.' The breaker stage usually occurs within a day after the MG stage. Later, the entire fruit turns to pink, followed by light red and finally deep red. The ripening stages of mature tomato fruit are classified as green, breaker, turning, and pink. Color change in tomato from green to red during ripening is due to chlorophyll degradation and carotenoid accumulation. Red color is caused by high concentration of lycopene. After red ripe, the last stage is the process of senescence, which involves degradation of cellular tissue eventually leading to the death of the cells.

Tomato flavor involves perception of the tastes and aromas of many chemical compounds and is achieved by a balanced interaction and combination between sugars and organic acids in tomato fruit. The ratio of soluble solids to organic acids is important to sweetness and sourness of tomatoes. Sourness of tomatoes is due to organic acid content, mostly malic and citric acids. Citric acid was found to be the principal organic acid in ripe tomatoes and during maturation its level rises. High acidity and low soluble solid contents will produce a tart tomato, while high soluble solids and low acidity will result in a bland taste. When both soluble solids and organic acids are low, the result is a tasteless, insipid tomato. More than 400 volatile compounds have been identified in tomato, and these volatiles are usually concentrated in the pulp and locular gel of tomatoes, but not in the skin and seeds. Sugars and their influence on taste are gauged in several ways. Total soluble solids (TSS), the titratable acid (TA) ratio, and the total sweetness index (TSI) are three common measures of tomato fruit quality. TSS reflects dry matter content and is inversely proportional to

fruit size. TSS in beefsteak tomatoes ranges from 3% to 5%, in medium-sized fruit from 5% to 7% and in cherry tomato fruit from 9% to 15%. A minimum TSS and TA of 5 and a minimum TA of 0.4, respectively (TSS:TA of 12.5), are considered desirable to produce a good-tasting table tomato.

Postharvest Handling and Storage

Tomatoes picked at the MG stage have a longer postharvest shelf life and can withstand the stresses of handling and transport much better than the fully ripe fruits, which are easily bruised and damaged. Properly handled MG tomatoes can ripen normally and develop flavor to the same extent as vine ripened fruit. However, if immature green stage fruit is inadvertently picked, eating quality is reduced. Immature green tomatoes will not ripen properly and be of low quality. Tomato fruits are very delicate with a very short postharvest shelf life and are easily susceptible to damage and bruises. Appropriate postharvest handling and storage techniques are essential for maintaining acceptable quality and extending the postharvest shelf life of tomatoes. After harvesting, tomatoes are cooled to reduce field heat, washed, and sorted by size, color, and variety. Fresh tomatoes can be stored after they have been harvested and sorted or they can first be packaged before storing. Cooling before and during storage is important. Tomatoes will deteriorate if they are kept at temperatures below 10 °C for longer than 2 weeks or if kept at 5 °C for longer than 6–8 days. Tomatoes are sensitive to chilling. Tomatoes that suffer chilling injury fail to ripen, their color development is irregular, and they are likely to suffer premature softening, browning of seeds, and increased decay.

Chemical Composition of Fresh Tomato

The chemical composition of tomatoes is greatly dependent on factors such as cultivars, maturity, light, temperature, weather, and cultural practices. The chemical composition of tomato fruit is important in the development of various fruit quality attributes: color, texture, appearance, nutrient value, taste, flavor, and aroma. Generally, sugar content of tomato is a function of stage of maturity. Sugars contribute to the soluble solids portion of the fruit that in turn contribute to the tomato flavor. Free sugars constitute about 60% of the tomatoes and are mainly D-glucose and D-fructose with traces of sucrose and raffinose. Starch content declines with fruit maturity. It varies from 1–1.22% in immature fruit to 0.1–0.15% in red ripe fruit. The texture of fruit is greatly controlled by the pectin content. Organic acid content and acidity influence the taste and flavor of tomato fruit. Citric acid and malic acid are the principal organic acids in tomato fruit. As the fruit matures, acidity increases to a maximum and then declines. Acidity is maximum at breaker and pink stages.

Tomatoes represent a low-energy nutrient-dense food with unique composition of several phytochemicals that may positively affect health. Tomato and tomato-derived products are excellent sources of folate and vitamins A, C, and E in the form of α-tocopherol (Table 1). Alpha-tocopherol can improve endothelial functions by preventing platelet aggregation and adhesion. Among the regularly consumed vegetables, only carrots are a better dietary source of vitamin A than the tomato-based foods. Tomatoes and tomato products also contain appreciable amounts of fiber. The tomato paste contains about 11.8 g fiber per cup. Vitamins C and E have very potent antioxidant properties. Tomato fruits are also an excellent source of ascorbic acid and are the major source of vitamin C next to citrus. The major phytochemicals in tomato are the carotenoids comprising 60–64% lycopene, 10–12% phytoene, 7–9% neurosporene, and 10–15% carotenes. Tomato contains significant amounts of α-, β-, γ-, δ-carotene ranging in concentrations from 0.6 to 2.0 mg kg^{-1}, significantly contributing to the provitamin A and vitamin A content. Tomatoes are excellent dietary sources of potassium. Dietary potassium helps lower blood pressure, reduces the adverse effect of excess sodium intake on blood pressure, reduces the risk of kidney stones, and possibly reduces age-related bone loss. Tomatoes can supply at least twice the potassium per 100 kcal compared with other common sources. Increasing potassium intake

Table 1 Nutrient composition of solanaceous fruits (tomatoes, eggplants, and pepper)

Nutrients	*Units*	*Tomatoes (red, ripe, and raw)*	*Eggplants*	*Peppers (sweet, green, and raw)*
Water	g	94.52	92.30	93.89
Energy	kcal	18	25	20
Protein	g	0.88	0.98	0.86
Total lipid	g	0.20	0.18	0.17
Carbohydrate	g	3.89	5.88	4.64
Fiber	g	1.2	3.0	1.7
Sugars	g	2.63	3.53	2.40
Calcium	mg	10	9	10
Iron	mg	0.27	0.23	0.34
Magnesium	mg	11	14	10
Phosphorus	mg	24	24	20
Potassium	mg	237	229	175
Sodium	mg	5	2	3
Zinc	mg	0.17	0.16	0.13
Vitamin C	mg	13.7	2.2	80.4
Thiamin	mg	0.037	0.039	0.057
Riboflavin	mg	0.019	0.037	0.028
Niacin	mg	0.594	0.649	0.48
Vitamin B_6	mg	0.080	0.084	0.224
Folate	μg	15	22	10
Vitamin B_{12}	μg	0	0	0
Vitamin A	μg	42	1	18
Vitamin E	mg	0.54	0.30	0.37
Vitamin D	μg	0	0	0
Vitamin K	μg	7.9	3.5	7.4
Fatty acids, total saturated	g	0.028	0.034	0.058
Fatty acids, total monounsaturated	g	0.031	0.016	0.008
Fatty acids, total polyunsaturated	g	0.083	0.076	0.062
Cholesterol	g	0	0	0

Nutrient values are for 100 g edible portion.
Source: The USDA National Nutrient Database for Standard Reference.

through increased tomato consumption is a healthful choice to increase the content of potassium in human diet.

Tomato Bioactives

Flavonoids constitute the largest group of phenolics in tomatoes and influence aroma, fragrance, and color characteristics of fruit. Flavonols (such as quercetin and kaempferol), flavanols (such as catechins), flavanones (such as naringenin), and stilbenes (such as resveratrol) constitute the main classes of tomato flavonoids. Up to 98% of flavonoids are usually concentrated in the tomato skin, and relatively, small quantities occur in the other parts of the fruit. According to the USDA flavonoid database, red tomatoes contain on an average 15 mg flavonoids as aglycones per kg FW. Naringenin (45%) is reported as the principal flavonoid followed by quercetin (39%), myricetin (10%), and kaempferol (5%). Besides flavonoids, chlorogenic acids and the related compounds form the main phenolic components in tomato. Other studies also detected rutin as another major flavonoid in tomato. Occurrence of ferulic, caffeic, chlorogenic, and p-coumaric acid was also identified in tomato fruit and skin. Many of these phytochemicals have potent antioxidant properties and in combination with lycopene may contribute to the numerous health benefits of tomatoes. The content of phenolic compounds of processed products was lower than that of fresh tomatoes. Tomato fruit usually does not contain anthocyanins, and the red color in tomato is from the carotenoid pigment, lycopene. Recently, purple-fruited tomatoes were developed through breeding programs in Oregon and Canada and also through genetic engineering. Purple tomatoes have the added health benefits from the presence of anthocyanins besides the other health benefiting components in them.

Lycopene

Lycopene content of fresh tomatoes often varies with cultivars and cultivation practices. On a fresh weight (FW) basis, tomato (on average) contains about 35 mg kg^{-1} of lycopene, with red cultivars containing an average 90 mg kg^{-1} of lycopene and yellow ones only 5 mg kg^{-1}. Processed tomatoes (sauce, paste, juice, and ketchup) contain two- to 40-fold higher lycopene content than fresh tomatoes. Nearly 85% of the average daily intake of lycopene in the human diet (about 25 mg day^{-1}) is obtained from fresh and processed tomato products. According to some studies, field-grown tomatoes appear to contain higher levels of lycopene ranging from 5.2 to 23.6 mg per 100 g FW than greenhouse-grown tomatoes (0.1 and 10.8 mg per 100 g FW).

Lycopene is the most abundant carotenoid in tomatoes and tomato-based foods, which is responsible for the characteristic red color of ripe tomato fruits. Unlike some carotenoids, lycopene lacks the provitamin A activity. Lycopene is about 2 times greater than β-carotene and 10 times greater than vitamin E in its potency to quench singlet oxygen and is one of the most potent antioxidants in food. Lycopene occurs in all-*trans*-configuration (95%) in fresh tomatoes, while processing and cooking can convert trans-form into the more bioavailable cis-form. Lycopene is the principal phytochemical in tomatoes accounting for its strong antioxidative activity associated with its ability to act as free radical scavenger. Generally, tomato and tomato-based food products can supply at least 85% of lycopene in human diet. Lycopene is a key intermediate in the biosynthesis of many important carotenoids, such as beta-carotene, and xanthophylls.

β-Carotene

β-Carotene is a strong antioxidant and also a scavenger of singlet oxygen. It is also a provitamin A as it can be converted into retinol. In commercial cherry tomato varieties, β-carotene content reached 1.2 mg per 100 g FW. Bioaccessibility of β-carotene from raw tomatoes was reported to be about 0.1%. Studies demonstrated the photoprotective effects of β-carotene on photooxidative damage and sunburn in humans. A significant reduction in erythema formation was also noticed when β-carotene was applied on human skin or with a dietary intervention alone or in combination with α-tocopherol for 12 weeks. Low concentrations of serum β-carotene have also been associated with an increased risk of congestive heart failure and cardiac death in men. However, some studies do not favor the absolute supplementation of high doses of β-carotene because the initial antioxidant activity of β-carotene is followed by a prooxidant action at high oxygen tension, which may be related to its adverse effects. Furthermore, intervention studies have shown that β-carotene supplementation had no protective effect on total mortality of diabetic male smokers and also in subjects with lethal prostate cancer with the use of β-carotene during radiation therapy compared with that of placebo.

Lutein

Average lutein content of raw tomato was reported as 32 μg per 100 g FW, while cherry tomatoes were reported to contain up to 800 μg per 100 g FW. These carotenoids have also attracted attention for their role in preserving eye health and vision in association with zeaxanthin. It has also been reported to be used in atrophic age-related macular degeneration. The association between the consumption of lutein and the maintenance of visual function and eye health has not been clearly demonstrated so far.

Health Benefits

Antioxidant Activity

Several studies have investigated on the antioxidant effects of lycopene and tomato supplementation in human diet. Data from studies that examined the antioxidant effects of lycopene and tomatoes consistently show improved antioxidant status with tomato/tomato product consumption and after lycopene supplementation. However, two clinical trials of lycopene supplementation reported neutral effects on oxidative stress markers. One study conducted in relatively healthy individuals ($n=60$) and two studies ($n=40$ and $n=57$) in individuals with type 2 diabetes showed decreased lipid peroxidation rates and decreased susceptibility of low-density lipoproteins

(LDLs) to oxidation after daily consumption of tomatoes or tomato juice. Others have reported less susceptibility to oxidation of DNA and LDL after tomato product consumption. Studies also reported that the plasma antioxidant capacity decreases when tomatoes and tomato products are removed from the diet and increased when they are added back.

Cardiovascular Diseases (CVD)

Oxidative modification of LDL is a key step in the development of atherosclerotic lesions. Consuming antioxidant-rich plant foods such as tomatoes may be one way to reduce the risk of cardiovascular atherosclerotic disease through inhibition of the oxidative process of LDL. High levels of tomato-based product intake were associated with a reduced risk of cardiovascular disease (CVD) and myocardial infarction in women. Furthermore, some clinical trials have also supported a relationship between CVD and lycopene intake. Studies have also shown that lycopene may reduce cholesterol synthesis and increase LDL degradation. Decreased triglyceride levels and LDL cholesterol and increased high-density lipoprotein cholesterol levels were noticed in subjects who consumed fresh tomato and tomato juice. Consumption of tomato products daily for 2–4 weeks has been shown to reduce plasma lipid peroxides and susceptibility of LDL to oxidation and also to increase antioxidant enzymes. An inverse association between greater consumption of tomato-derived product intakes (>7 servings per week) and CVD risk was observed in women, and such an association was not observed with lycopene intake alone. Epidemiological data and results from *in vitro* and animal experiments partly showed promising role of lycopene and tomato products in preventing CVD. However, there is still a lack of more well-organized human clinical trials and dietary intervention studies using well-defined subjects to provide clear evidence for the protective mechanism of lycopene in the prevention of CVDs.

Cancer

Consumption of tomatoes and tomato-based products had been found to be associated with a decreased risk of chronic diseases, such as cancer. Epidemiological studies and various *in vitro* and *in vivo* studies also support the protective effects of high intake of tomato and tomato products containing lycopene on some types of cancer including prostate cancer. The Health Professionals Follow-Up Study, a prospective epidemiological study of about 47 000 men, is of particular importance since it provided the strongest epidemiological evidence to support an association between tomato product consumption and a reduced incidence of prostate cancer. This study showed that consumption of 2–4 servings of raw tomatoes per week was associated with a significant 26% reduced risk of prostate cancer when compared with no servings per week.

Some clinical trials also observed a reduction in prostate-specific antigen (PSA) levels as a surrogate marker for prostate cancer status, while other studies showed a weak effect or no effect of tomato consumption or lycopene supplementation on prostate cancer risk. Preliminary cell culture studies conducted using human prostate and mouse hepatic cancer cell lines indicated that tomato aglycone polyphenols, including quercetin, kaempferol, and naringenin, inhibited cancer cell proliferation in both types of cell lines in a dose-dependent manner, without having cytotoxic effects. Lycopene administration (10 mg) for 3 months decreased PSA level, tumor grade, bone pain, and urinary tract symptoms in patients with metastatic prostate cancer. However, lycopene is not potent enough to be used clinically as a drug for prostate cancer because high concentrations are needed to achieve significant response in humans.

In addition, lycopene can inhibit other cancer types (breast, colorectal, endometrial, lung, oral, and pancreatic). Mixed results have been noticed in the epidemiological studies examining the association between lycopene and colorectal cancer risk. Some clinical trials have also reported beneficial effects of lycopene-containing supplements on colorectal and breast cancer biomarkers. Several possible mechanisms of the action of tomato phytochemicals responsible for the anticancer effects have been suggested including antioxidant activities, alteration of xenobiotic metabolism, modulation of the IGF-1, inhibition of cell-cycle progression, and increase in the formation of gap junctions.

Metabolic Diseases, Obesity, and Other Conditions

Evidence to support the benefits of tomatoes in terms of protection against diseases associated with metabolic syndrome and obesity is available. In particular, lycopene reduces transcript levels of proinflammatory cytokines with the downregulation of interleukin-6 expression. The effect of tomato-derived lycopene consumption on markers of inflammation and oxidative stress was monitored in a group of patients with extreme obesity. Abnormally higher markers of inflammation and oxidation products and lower plasma carotenoids were noticed in obese patients compared to control subjects. Studies also showed that anti-inflammatory effects derived through consumption of tomato products were superior to that of lycopene ingested as a single compound. Supplementation of a low-tomato diet with tomato products resulted in changes in inflammatory markers such as C-reactive protein (CRP), IL-6 and tumor necrosis factor-α (TNF-α). A decline in CRP and cholesterol level was noticed after a 2-week dietary tomato juice supplementation (containing ~21 mg lycopene and 45.5 mg and 435 mg each vitamin C), and this effect was stronger with higher dose of vitamin C addition. It has been demonstrated that lycopene can stimulate the production of anti-inflammatory cytokines such as IL-10 to control inflammation and can inhibit the activation of the transcription nuclear factor kappa B (NFkB) and the generation of the proinflammatory cytokines including TNF-α, IL-6, and IL-8 in macrophages and adipocytes.

Two studies reported reduced blood pressure after dietary lycopene supplementation. Studies also demonstrated an inverse association between serum lycopene and markers of oxidative stress and bone resorption in postmenopausal women aged 50–60 years. Also, significant increase (20.6%) in the bone resorption marker NTx was noticed after 30 days of dietary lycopene restriction, which could lead to a long-term increased risk of fracture. Health beneficial effects of the dietary intake of α- and β-carotene and of lycopene on glucose metabolism in men have been noticed. Studies also reported a linear decrease in β-carotene and lycopene content in persons with impaired glucose tolerance and in persons with newly

diagnosed diabetes compared to persons with normal glucose concentrations.

UV Protection

The protective effect of tomato products and lycopene against UV-induced sunburn has been reported. In these studies, subjects who consumed synthetic lycopene alone, a softgel encapsulated tomato extract, or a tomato drink showed a decrease in solar simulator-induced sunburn indicating the skin-protective effects of tomato and tomato products.

Bioavailability

Lycopene bioavailability is influenced by a number of factors including food processing and dietary composition. In plant foods, lycopene can occur in a number of forms such as carotenoid–protein complexes in chloroplasts or in crystalline form inside the chromoplasts. Lycopene bioavailability can be greatly enhanced by various processing methods that break down the cell walls, making lycopene more accessible, and also through enhanced *cis*-isomerization. Bioavailability of lycopene is higher in processed tomato products than in unprocessed fresh tomatoes. Furthermore, lycopene bioavailability is also affected by processing methods and storage. Improper processing methods, handling, and storage (i.e., exposure to light and oxygen) can degrade lycopene or alter the ratio of lycopene isomers.

In fact, thermal processing greatly improves lycopene bioavailability by disrupting cellular membranes releasing lycopene from the tissue matrix. Multiple studies have demonstrated that lycopene from thermally processed tomato products is more bioavailable than lycopene from fresh tomatoes. Lycopene bioavailability is greatly affected by dietary composition and food matrix. Consuming lycopene-containing food with fat increases its bioavailability as lycopene is a lipid-soluble compound. For instance, a study found that the consumption of tomato salsa with avocado (as lipid source) led to a 4.4-fold increase in lycopene absorption as compared with salsa without avocado.

On ingestion, lycopene is released from the food matrix, and once inside the enterocyte, lycopene is packaged with other dietary lipids into chylomicrons. Then, it is incorporated into mixed micelles. Micelles contain bile salts, cholesterol, and fatty acids from the meal, and the amphiphilic nature of the micelle structure helps to keep the lipophilic nutrients soluble in the aqueous phase. Heat processing of tomatoes converts the all-*trans* lycopene to various *cis*-isomers. Cis-lycopene isomers are regarded as more bioavailable because they are more soluble and better absorbed from the intestinal lumen than the all-*trans* isomer. Isomerization may also take place *in vivo* and the gastrointestinal lumen, liver, and enterocytes were identified as potential sites of lycopene isomerization *in vivo*. Lycopene passively diffuses across the apical membrane of the intestinal cells. Studies have shown that lycopene absorption can also be facilitated by a cholesterol membrane transporter known as scavenger receptor class B type I (SR-BI). It has been suggested that lycopene absorption may be facilitated by other transporters, but this has not yet been confirmed. Chylomicrons are then transported across the basolateral membrane and make their way into the lymphatic system, which eventually releases chylomicrons into the blood. Competition by other carotenoids or cholesterol may also influence lycopene absorption.

Solanum melongena L. (Eggplant)

Solanum melongena L. or eggplant is also known as aubergine in England or brinjal in India. Eggplant is believed to have originated in India. *Solanum melongena* belongs to the tribe Solaneae and is a member of the subgenus *Leptostemonum*, the largest subgenus of the genus *Solanum* which includes almost one-third of the species within the genus *Solanum*. Eggplant is a diploid species ($2n = 24$) with a basic chromosome number of 12. Eggplant is a perennial plant but cultivated as an annual for its edible fruit. The plant has an erect woody branching stem with or without spines. Purple flowers have 5 calyx lobes and occur singly or in clusters of 2 or more flowers. Fruit are borne singly or in clusters. Eggplant fruit is a pendant berry and shows high variability in their shape (round, ovoid, cylindrical, or elongated), size, and fruit color (green, white, violet, purple, black, or orange with or without patches or stripes). The presence or absence of chlorophylls and anthocyanins as well as their distribution pattern determines the fruit color diversity in eggplants. Two other *Solanum* species *S. aethiopicum* and *S. macrocarpon* L. are also commonly known as 'eggplants' and cultivated for their edible fruits. *Solanum aethiopicum* also known as the scarlet eggplant is a native of Africa. It differs from *S. melongena* in its small white corollas and usually bright scarlet fruit that often resemble *Capsicum* peppers. This species is widely grown in Africa and South America for its edible fruits. *Solanum macrocarpon* L., the gboma eggplant, is also a native to central Africa and is grown for its edible fruits and leaves. The presence of deeply lobed leaves and very large calyxes usually distinguishes this plant from *S. melongena*.

Economic Importance, Production, and Uses

Today, eggplant is cultivated globally for its edible fruits. In 2011, total eggplant production accounted to about 46.8 million tons. China and India lead the world production of eggplant followed by Turkey, Egypt, Japan, and Italy. Eggplant is grown in many countries throughout the tropics, subtropics, and Mediterranean area, since it requires a relatively long season of warm weather to give good yields. It grows best between 20 and 35 °C, while temperatures below 20 °C and above 40 °C retard growth. Growth is halted at temperatures below 10–12 °C and the crop is frost-sensitive. It thrives well in fertile well-drained soils with a pH of 5.5–6.8. A growing season of about 120 days is required for its successful production. Eggplant is transplanted rather than directly seeded in the field to establish uniform plants. Eggplant is commonly grown in open field, but it is also commercially grown in greenhouses. Grafting is mostly used in conditions of intensive production. Eggplant is grafted mostly onto tomato or tomato interspecific hybrids (*L. esculentum* × *L. hirsutum*), which are resistant to several soil pathogens and also tolerant to low soil temperatures. Eggplant cultivar diversity differs from country to country. Most European and American commercial cultivars are

characterized by fruits that are globose, intermediate, or long, with an almost black and shiny epidermis and weighing 200–400 g. Asian countries such as Thailand, China, and India are also characterized by astonishing diversity in eggplant fruit sizes, shapes, and colors. Eggplants are used in a variety of ways such as grilled, stewed, and fried. It is also added to various curry dishes in India and Thailand.

Nutritional Composition

Eggplant is low in calories and fats and contains mostly water (Table 1). It also has some protein, fiber, and carbohydrate. Eggplant fruit is a good source of various vitamins like B_1, B_2, B_6, C, K, thiamine, niacin, and pantothenic acid and minerals like magnesium, potassium, manganese, and copper. In general, the purple varieties showed more protein content than the white and green varieties. Purple eggplant also contained significant amounts of essential amino acids, such as leucine, valine, lysine, and isoleucine. Additionally, eggplant fruit contains various antioxidant bioactive compounds including phenolic compounds, such as caffeic, p-coumaric and chlorogenic acid, and flavonoids, such as nasunin and delphinidin in the peel of colored varieties. Nasunin is an anthocyanin purple pigment (delphinidin-3-coumaroylrutinoside-5-glucoside), which is the predominant phytochemical in eggplant. Nasunin is mainly confined to the peel of eggplant and it represents between 70% and 90% of the total anthocyanins in the peel. Nasunin is a potent antioxidant that effectively scavenges reactive oxygen species, such as hydrogen peroxide, hydroxyl, and superoxides. Chlorogenic acid is another predominant phenolic compound found in eggplant, which is also a powerful free radical scavenger found in plant tissues. Some of the health benefits associated with chlorogenic acid include antimutagenic (anticancer), lipid-lowering, antimicrobial, and antiviral activities. Besides chlorogenic acid, 13 other phenolic acids have been identified at varying levels in different commercial eggplant cultivars, although chlorogenic acid was the predominant phenolic compound in all of them. The phenolic acids are responsible for eggplants' bitter taste and the browning that result when the flesh is cut. Eggplant is ranked as a vegetable with high antioxidant activity that is attributed to the anthocyanins and phenolic compounds contained in eggplants. Phenolic composition of skin and pulp of eggplant fruit differs. Generally, eggplant peels contain more phenolic compounds than the pulp. Eggplant fruits also contain other antioxidants including the carotenoids lycopene, lutein, and α-carotene, as well as the flavonoids myricetin and kaempferol.

The presence of two kinds of steroidal saponosides imparts bitter taste to eggplant. They are glycoalkaloids and melongosides. The principal glycoalkaloid specific to eggplant is solasonine and is the eggplant analogous of the potato solanine (aglycone solanidine) and the tomato tomatine (aglycone tomatidine). The glycoalkaloids solamargine and solasonine and their common aglycone solasodine were also found in eggplant. High concentration of solasonine and melongosides in the fruit can cause bitterness, making the fruit unpalatable. Most cultivated species of eggplant normally do not contain high levels of alkaloids. Eggplant fruit bitterness is influenced by cultivars, fruit maturity, cultivation and harvest methods. These steroidal saponosides are characterized by their antimicrobial, insecticidal, fungicidal, hypocholesterolemic, anticancer, and hypotensive activities. Eggplants also have nicotine than any other edible vegetable ($100\ ng\ g^{-1}$). The nicotine content in eggplant or any other food is negligible compared to passive smoking. Eggplant also contains measurable amounts of oxalates, which at high concentration can crystallize and cause health problems such as kidney calcium oxalate stones.

Health Benefits

Numerous health benefits are associated with the consumption of eggplants. Besides having potent antioxidant potential, eggplant has been used to lower blood cholesterol. A human trial showed only a modest and transitory effect of the consumption of eggplant powder on total cholesterol and its fractions, triglycerides, and apolipoproteins in blood. Some *in vivo* studies also have demonstrated that eggplant fruit phenolic compounds had a significant hypolipidemic property. Another study found no effects at all on serum cholesterol levels and does not recommend eggplant as a replacement to statins. Among the eight commonly consumed vegetables, eggplant showed the lowest bile acid binding capability, severalfold less than okra and beets. Circulation of bile acids in the body is prevented by binding bile acids resulting in reduced fat absorption and the utilization of cholesterol from the bloodstream to synthesize more bile acids, thereby lowering cholesterol levels. Also, eggplant extracts have been found to inhibit protein-activated receptor-2 inflammation associated with atherosclerosis. Eggplant phenolics are touted as having anticancer properties. Nasunin, the major anthocyanin in eggplant skins has antioxidant activity, and it has been shown to suppress the development of blood vessels required for tumor growth and metastasis in an *ex vivo* animal study and an *in vitro* human cell assay. Nasunin is bestowed with numerous health beneficial biological properties such as protecting blood cholesterol from peroxidation, preventing cellular damage, and lessening free radical damage in joints, which is a primary factor in rheumatoid arthritis. The eggplant glycoalkaloids solamargine and solasonine and their common aglycone solasodine showed antiproliferative activities against human colon (HT29) and liver (HepG2) cancer cells.

Capsicum sp. (Peppers)

Peppers are members of the genus *Capsicum*, which is composed of more than 200 varieties and is native to the tropics of Central and South America. The genus name *Capsicum* is believed to be originated from the Greek word *capsicon* via the Latin *kaptein*, which mean 'to bite.' The five principal domesticated species of *Capsicum* are *Capsicum annuum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens*, *and Capsicum pubescens*. *Capsicum annuum* is, perhaps, the most economically important widely cultivated species today and includes sweet, bell, hot, and jalapeño peppers. The species *C. baccatum* include aji amarillo, a chili that is an integral part of Peruvian cuisine, Bishop's crown, peppadew, and aji limon. Habanero, bhut jolokia, and Scotch bonnet peppers are some *C. chinense* varieties known for their heat and pungency.

Capsicum chinense is the most commonly cultivated and distributed species of northern South America and West Indies. *Capsicum frutescens* known indigenously as the 'bird pepper' are usually very small, pungent and include the famous tabasco pepper. *Capsicum pubescens* is primarily found in Central and South America and is characterized by hairiness of leaves and black seeds, and some varieties like the rocoto and manzano can thrive in higher elevations.

Capsicum fruits show great variations in their pod color, shape, size, pungency, and flavor. Bell pepper is a cultivar group of *Capsicum annum*. All five species yield pungent fruits commonly called red pepper or simply capsicum. Varieties of *C. annuum* produce mild fruits commonly known as paprika, bell pepper, sweet pepper, or green pepper. Bell peppers are grown for their edible bell-shaped colored fruits with three or four lobes. The color of bell peppers can be red, yellow, orange, green, purple, brown, white, and black. Green peppers are unripe, less sweet and slightly bitter than the colored varieties. Purple varieties also tend to be less sweet and bitter than the orange, red, or yellow varieties. Plants are branched, mostly erect, annual or perennial herbaceous shrub that grows to 30–80 cm tall. Small, off-white, pendent flowers are borne singly or in clusters of one to two flowers. Corolla is five-lobed with five stamens and with bluish/purplish anthers. Fruit is a berry with a fleshy, firm, and hollow pericarp. Usually, fruits of the purple and white varieties do not have a green stage.

Method of Consumption

Capsicum annuum and *C. frutescens* are the most consumed spices in the world. Peppers form a significant part of the human diet in many parts of the world. Fruits are consumed in fresh, cooked, or processed forms. Fresh peppers are often used in salads. Chilies are also sold as frozen, canned, dried (whole or crushed pieces), and pickled fruit. Fruit is also processed into various fermented products, powders, sauces, curry pastes, and salsas. Dried chilies are often ground into powders and added into various culinary preparations and other dishes. Fresh or dried chilies are also used to make hot sauce. Chili in the form of powder or paste is a vital ingredient of Indian curries. Fresh market types, such as bell, Cuban, and squash, are usually nonpungent and eaten raw or stir-fried. Leaves of *Capsicum* are edible and are eaten or added to soup and stews in many parts of Southeast Asia. Carotenoid extracts (oleoresins) are used as a coloring agent by the food, cosmetics, and pharmaceutical industries.

Nutritional Value

Peppers are good sources of vitamins C and A. Significant cultivar differences exist in the content of vitamin C. On a FW basis, vitamin C content has been shown to increase with fruit maturity. A serving (100 g) of raw green sweet pepper can provide more than the recommended dietary allowance (60 mg) of this vitamin. Peppers also contain vitamins B_2 (riboflavin), B_3 (niacin), E, K, and B_6 (Table 1). Vitamin A occurs in pepper as β-carotene and provitamin A. Vitamin A content and its accumulation increase with fruit maturity. Carotenoids serve as powerful antioxidants and have provitamin A activities. More than 30 different carotenoid pigments have been identified in pepper fruit. These include β-carotene and a number of xanthophylls including lutein, zeaxanthin, violaxanthin, cucurbitaxanthin A, and β-cryptoxanthin. Among them, only β-carotene and β-cryptoxanthin possess provitamin A activity and the oxidative cleavage of these compounds yields retinal. Beta-carotene contents vary within varieties of peppers. Some cultivars of hot pepper have as much as 12 mg kg^{-1} total carotenoids, while in others, its level is undetectable. The main carotenoids responsible for the red color of pepper fruits are the three xanthophylls unique to *Capsicum*, that is, capsanthin, capsorubin, and capsanthin 5,6-epoxide. Though these carotenoids lack provitamin A activity, they are effective antioxidants and a source of red pigments.

Carotenoid content is often an indicator of nutritional value and quality in pepper. The predominant carotenoids in unripe fruit are lutein, β-carotene, and violaxanthin. During maturation, lutein levels decline and levels of β-carotene, β-cryptoxanthin, and zeaxanthin increase. At the breaking stage of fruit development, the red carotenoids, capsanthin and capsorubin, are produced. Capsanthin levels continue to increase as fruit ripen composing between 37% and 80% of total carotenoid content. Carotenoids accumulate as the fruit ripens and the red color of some peppers results from the carotenoid accumulation. Antioxidant phytochemicals, carotenoids, and anthocyanins are responsible for the pigmentation of peppers. The principal red pigments are capsanthin and capsorubin, while lutein, beta-carotene, zeaxanthin, violaxanthin, and antheraxanthin are the yellow and orange pigments. Red peppers also contain lycopene, which has many health beneficial attributes. Besides being rich in phytochemicals, peppers provide dietary fiber.

Though the pericarp, flesh, and seed of pepper contain flavonoids, pepper is not considered to be a rich source of flavonoids. Flavones and flavonol aglycones constitute the most prevalent flavonoid groups in pepper. Quercetin and luteolin are the prevalent flavonoids in peppers. Luteolin and its derivatives form the primary flavones, while quercetin and its derivatives are the primary flavonol aglycones. Quercetin is the major flavonoid in chili pepper. Quercetin derivatives also dominate in green pepper. Other flavones identified in pepper include apigenin, O-glycosyl flavones, and C-glycosyl flavone. Several studies also reported that total flavonoid content and the levels of individual flavonoids decline with fruit maturity. Studies also found that colored peppers generally had higher quercetin and luteolin contents (red peppers had higher contents than yellow and orange peppers).

The major phytochemicals in hot peppers are capsaicinoids. They are a group of phenolic alkaloids specific to the genus *Capsicum*. More than 20 capsaicinoids have been identified in pepper. Capsaicin is the predominant component of the pungent flavor in hot pepper, followed by its analog dihydrocapsaicin and these two compounds together contribute more than 80% of the total capsaicinoid content. Capsaicin is the primary component of pepper spray. Capsaicinoids are synthesized in the fruit placenta. A sensation of heat and pain is caused by the binding of the capsaicinoids to receptors in mouth and throat resulting in sensing 'heat' and serves as a natural defense against herbivory. Birds are not affected by these compounds. Domestication and breeding efforts have reduced pungency and capsaicinoid content of some peppers

especially the bell peppers. Capsaicinoid content is a major quality factor in spice peppers (chili, tabasco, and paprika). There is a direct correlation between total capsaicinoid levels and pungency (as measured in Scoville heat units, which is a subjective organoleptic measure, and chili hotness is now determined by high-performance liquid chromatography). Capsaicin and dihydrocapsaicin are undetectable in sweet pepper varieties, but significant amounts of these compounds are present in chili and paprika types. Significant variations in the profile of capsaicinoids are found between and within pepper species.

Health Benefits and Medicinal Uses

Capsicum is used internally to treat diarrhea, cramps, colic, and toothache, and it acts as stomachic, carminative, and stimulant. It can clear the lungs and sinuses, protect the stomach by increasing the flow of digestive juices, trigger the brain to release endorphins, and help protect the body against cancer through antioxidant activity. Peppers have anti-inflammatory activity and shown to have both antihyperglycemia and antihypertension potential. Pepper occupies a top place among various vegetables and ranks high for antioxidant activity. Antioxidant vitamins such as A and C help to prevent inflammation, cell damage, cancer, and age-related ailments and help to boost immunity. Vitamin K helps protect cells from oxidative damage, promotes proper blood clotting, and strengthens bones. Beta-cryptoxanthin, a carotenoid in red peppers, has shown promise for helping to prevent lung cancer related to smoking and passive smoke.

Capsaicin, the pungent compound in chili peppers, is an ingredient of several commercial medicinal formulations for the treatment of muscle pains, toothaches, burning-mouth syndrome, gastric ulceration, painful diabetic neuropathy, and arthritis. Various topical and injectable formulations containing capsaicin have been developed for the treatment of pain. The capsaicin in hot peppers has been shown to decrease blood cholesterol and triglycerides and reduce the risk of stomach ulcers. Although capsaicinoids demonstrated gastroprotective effects in several animal studies, it can also have detrimental effects on the gastrointestinal system at very high doses. Capsaicin also has analgesic, antibacterial, and antidiabetic properties. It is also used to treat bladder hypersensitivity, vasomotor rhinitis, and hyperreflexia of spinal origin. Capsaicin has been reported to have substantial anticarcinogenic effects in several *in vitro* and *in vivo* studies suggesting its use for chemoprevention. Capsaicin showed its antitumorigenic activities in breast cancer and prostate cancer cells. Capsaicin exerted a number anticarcinogenic and tumor-preventive mechanisms such as prevention of cell proliferation and migration and induction of apoptosis. Capsanthin and related carotenoids from fruits of *C. annuum* were found to have *in vitro* antitumor activity in some animal and *in vitro* studies. Epidemiological studies also have revealed that consumption of chili peppers in large quantities was at higher risk of developing gastric cancer than nonconsumers, thus demonstrating the carcinogenic properties of capsaicin. Evidence from animal experiments and clinical studies has suggested that capsaicinoids possess antiobesity properties by enhancing energy expenditure and reducing body fat accumulation.

See also: Phenolic Compounds: Bioavailability and Health Effects; Phenolic Compounds: Occurrence, Classes, and Analysis.

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Sorghum: A Novel and Healthy Food

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Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) ranks the fifth most important cereal crop in the world in terms of total production and constitutes a major source of proteins, calories, and minerals for millions of people (Figure 1). Total world annual sorghum production is about 60 million tons from cultivated area of 46 million hectares. The most important producers of sorghum are the United States, Sudan, Nigeria, Mexico, India, China, Burkina Faso, Brazil, Australia, and Argentina (Figure 2). More than 35% of sorghum is grown directly for human consumption. The rest is used primarily for animal feed, alcohol production, and industrial products.

With increasing world population and decreasing water supplies, sorghum represents an important crop for future human use. While sorghum is a vital food crop for millions of people in parts of Africa and Asia, it is an underutilized resource in most developed countries, with sorghum being primarily used as animal feed. The use of sorghum not only provides farmers with a market for their products but also saves money, which would otherwise be required to import cereals, which can be very costly. The demand for gluten-free foods for people with celiac disease and other intolerances to wheat is growing particularly in the developed countries. The development of white, tan plant, the so-called food-grade sorghum lines (Figure 3), has enabled white, bland-tasting flour to be produced from sorghum grain. This flour is useful in food products because it does not impart unusual colors or strong flavors and it may be desired over maize flour for these reasons. The most common foods prepared with sorghum are tortillas; thin porridge, for example, 'bouillie'; stiff porridge, for example, tô; couscous; injera; nasha and kisra; traditional beers, for example, dolo, tchapallo, pito, and burukutu; ogi; and baked products, for example, cakes, cookies, muffins, bread, and pizza. The purpose of this article is to summarize the characteristics of sorghum as a plant species and as human food and to discuss the relevance of experimental results obtained with food-grade sorghum cultivars grown in field trials in San Bartolomeo in Galdo, southern Italy, since 2006. These results demonstrate that sorghum cultivars grow well in Mediterranean environment and that it is possible to obtain white flour for the production of gluten-free cookies of high quality and palatability as food suitable for both celiac patients and healthy population. These studies also provided evidence that DNA sequences encoding toxic proteins for celiac patients are absent in the sorghum genome as also shown by biochemical and immunochemical analyses.

Botanical Nomenclature

Sorghum is a plant belonging to the tribe Andropogoneae and the family Poaceae. Linnaeus described three species of cultivated sorghum in 1753: *Holcus sorghum*, *Holcus saccaratus*, and *Holcus tricolor*. Later, Moench distinguished the genus *Sorghum* from the genus *Holcus*, and Person suggested the name *Sorghum vulgare* for *Holcus sorghum* (L.). In 1961, Clayton proposed the name *Sorghum bicolor* (L.) Moench as the correct name for cultivated sorghum and this is currently the accepted nomenclature. Like most angiosperm (flowering plant) lineages, sorghum is thought to be ~200 Ma old. Sorghum, maize, rice, and wheat diverged from a common ancestor only 50–70 Ma.

Sorghum is a C4 crop particularly adapted to drought-prone areas: hot, semiarid tropical environments with 400–600 mm rainfall – areas that are too dry for other cereals (except for the millets). Sorghum is also found in temperate regions and at altitudes of up to 2300 m in the tropics. It is well suited to heavy soils commonly found in the tropics, where tolerance to water logging is often required.

The main races of cultivated sorghum are bicolor, vulgare, caudatum, kafir, guinea, and durra. Common names of sorghum vary from continent to country levels.

Sorghum is a vigorous grass that varies between 0 and 6 m in height. It has deep and spreading roots with a solid stem. Sorghum leaves are long (0.3–1.4 m) and wide (1–13 cm), with flat or wavy margins. The flower is a panicle, usually erect, but sometimes recurved to form a goose neck. The sorghum kernel is a naked caryopsis and consists of three main anatomical parts – seed coat (testa or pericarp), endosperm (storage tissue), and germ (embryo), which generally account for 6%, 84%, and 10% of the seed mass, respectively (Figure 4). Sorghum is the only cereal grain known to have starch in the mesocarp layer of the pericarp. The endosperm, which is composed of an aleurone layer and peripheral, corneous, and floury areas, is the main storage tissue. The grain or caryopsis is usually partially covered by glumes. Glumes are the maternal plant tissues in the panicle that holds the developing caryopses after pollination. The caryopsis is round and bluntly pointed, from 4 to 8 mm in diameter, and varying in size, shape, and color with variety. Caryopsis color is an important trait that affects grain quality in sorghum.

In some sorghum genotypes, the testa is highly pigmented. The presence of pigment and the color are genetic characters controlled by the R and Y genes. The thickness of the testa layer is not uniform and is governed by the Z gene. In some genotypes, there is a partial testa, while in others, it is not apparent or is absent. Sorghum does not develop significant levels of aflatoxins and fumonisins in the field prior to harvest.

Life Cycle of White Food-Grade Sorghum in Mediterranean Environments

The life cycle of sorghum is very similar to that of corn. The ages of attainment of various stages of development are

Figure 1 *Sorghum bicolor* (L.) Moench.

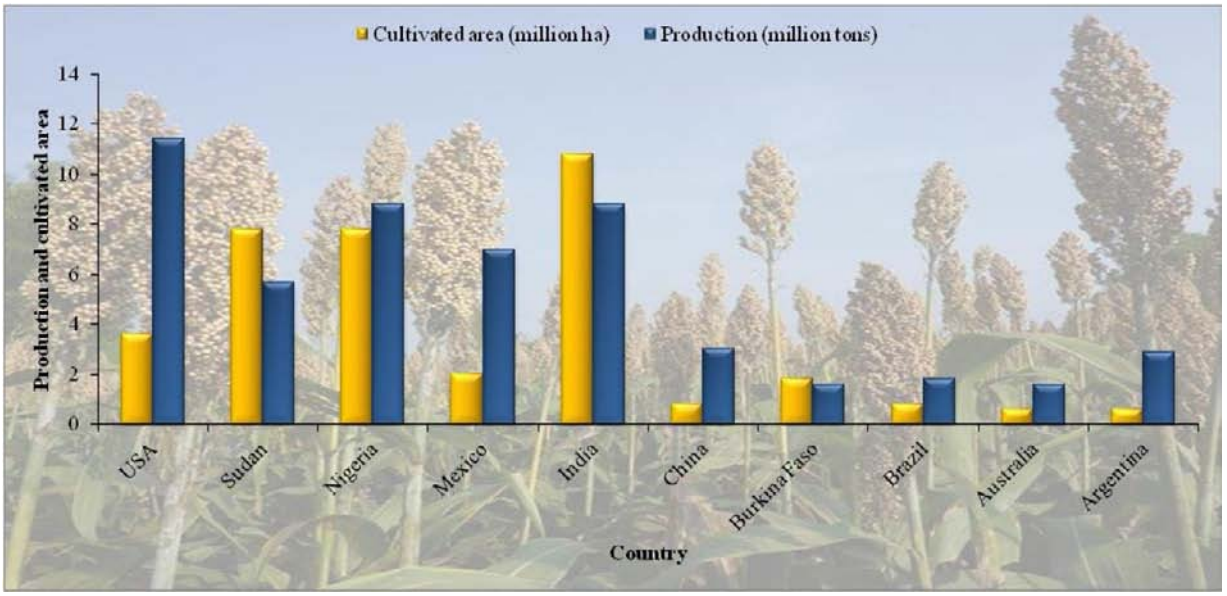

Figure 2 World annual sorghum production and cultivated area. Data are from Food and Agricultural Organization (FAO) (2005). FAOSTAT. http://faostat.fao.org/faostat/.

Figure 3 White food-grade sorghum line.

naturally highly variable depending on the time of sowing, the environment, and the precocity of cultivars. After sowing, seed germination and seedling emergence is completed in 2–3 weeks. The early stages of plant growth are relatively slow: About a month after emergence, the plants have 7–8 leaves and the differentiation in the vegetative reproduction (color change) begins. Immediately after the color change starts, rapid development of the main culm takes place. The plant growth lasts 40–70 days, depending on the variety and the environment, and ends with the formation of the panicle (earring). Immediately after the formation of the panicle, flowering begins and continues along the panicle from top to bottom. After pollination and fertilization, the embryo is formed and the kernels begin to gain weight. Proteins, starches, and lipids accumulate with an increase in the dry weight of the caryopsis and a decrease in water content. The following are the typical stages of maturation of the caryopsis: milk ripeness, dough stage, and physiological maturity. The latter, which marks the end of the accumulation of reserve substances in the seed, is marked by the formation of a layer of suberized cells, called black layer, that closes the phloem and xylem vessels of the stalk of the fruit, effectively cutting them off from the plant. At physiological maturity, the grain has a moisture content of around 35%. After the formation of black layer, the grain begins to dry rapidly, which, in the early varieties grown in Italy, takes place in a comprehensive manner, so that the grain moisture at harvest is almost always

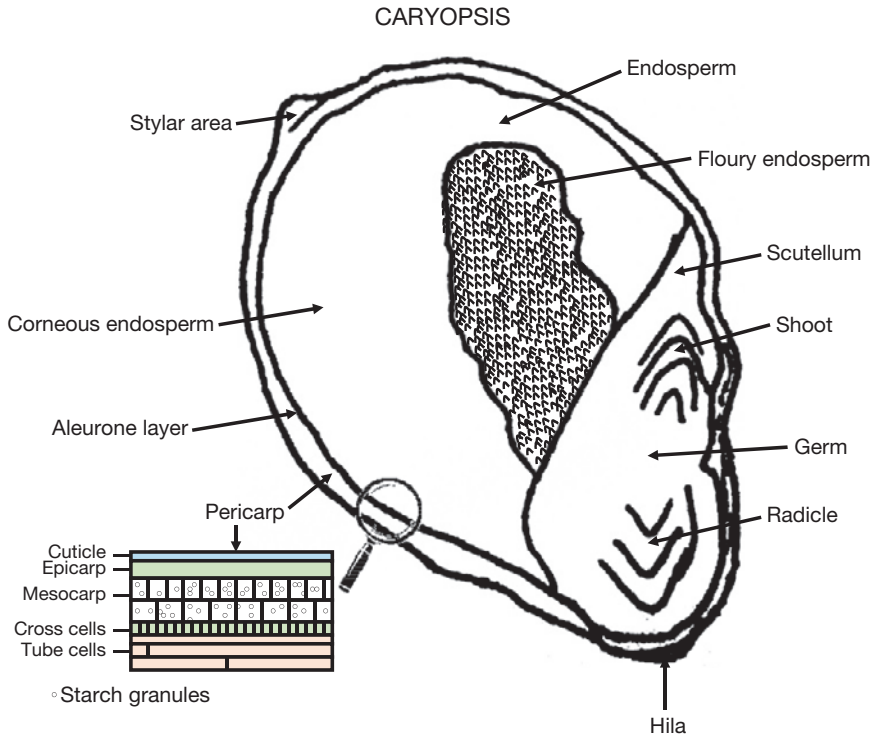

Figure 4 Drawing structure of sorghum grain.

lower than the standard value of 14%. At grain maturation, the sorghum plant remains green, except in cases where extreme environmental conditions (drought and high temperatures) or diseases (such as diseases caused by *Macrophomina* and *Fusarium*) cause premature death of the plant. The complete growth cycle of sorghum in Italy is highly variable in duration ranging between 90 and 150 days. Compared to corn, sorghum has a shortened interval from flowering to physiological maturity: on average, sorghum matures 20–25 days before corn.

Nutritional Components

With the exception of lower oil content, the composition of grain sorghum is quite similar to that of corn. On average, the endosperm makes up 82% of the grain; the germ, 10%; and the pericarp, 8%. The whole grain tends to be relatively low in fiber (2.7%) and contains 7–15% proteins and up to 75% starch. As previously mentioned, the germ is a rich source of oil and protein (28% and 18%, respectively), and the majority of the endosperm is composed of starch and protein. The composition of sorghum grain may fluctuate between different sources due to the genetic and environmental conditions and crop management.

Starch

Sorghum and corn starch have similar properties. The granules have the same shape, but sorghum starch granules are slightly larger. There are minor differences in the rheological characteristics of the starch, including swelling capacity and paste viscosity. The gelatinization temperature of sorghum starch ranges from 71 to 80 °C, which is higher than that of corn, which gelatinizes from 62 to 72 °C. Therefore, sorghum requires a longer cooking time and more thermal energy during processing. It was reported that most sorghum starch contains 20–30% amylose and 70–80% amylopectin; however, waxy varieties (< 5% amylose) have been developed.

Proteins

The majority of sorghum proteins are found within the endosperm of the kernel, distributed within both protein bodies and the endosperm's protein matrix. The major sorghum protein fractions are prolamins and glutelins, with prolamins predominating. The prolamin in sorghum is kafirin, a protein similar to maize zein in molecular weight, structure, solubility, and amino acid composition. Kafirin is found to occur primarily in three forms: α-, β-, and γ-kafirin, with α-kafirin being the major form. These sorghum prolamins are located within the protein bodies in the starchy endosperm. Like many cereals, the limiting amino acid in sorghum is lysine. Sorghum proteins lack the ability to form a gas-holding network in a viscoelastic dough. Therefore, sorghum's use in baked products has been limited thus far.

Fibers

Sorghum contains 6.5–7.9% fiber, of which 86.2% is insoluble. This fiber functions as a structural and protective feature. In cereal grains, dietary fiber (made up of soluble and insoluble fractions) is generally located in the pericarp. As such, the fiber composition in sorghum products, such as flour, depends on the degree of pericarp removal or extraction

level. The insoluble fiber components in sorghum are rich in glucuronoarabinoxylans. In comparison, barley is rich in soluble β-glucans, while wheat has both soluble and insoluble arabinoxylans. These differences in fiber composition may have a significant impact when sorghum is used in value-added products.

Lipids

The majority of lipids found in sorghum are located in the germ. The fatty acid composition of these lipids is quite similar to those found in corn oil. The sorghum oil is primarily made up of the following fatty acids, in order of decreasing concentration: linoleic, oleic, palmitic, stearic, myristic, and hexadecanoic.

Ash

Ash is considered to be the mineral component of the plant and its content could range from 0.99% to 3.40% depending by a number of factors including both the genotype and the environment.

Sorghum for Human Food

Sorghum has traditionally been used for livestock feed in the western hemisphere with a few exceptions, while it has been used as food in both Africa and Asia. Feed sorghums usually are red-seeded with purple or red plant color that causes significant off-color and, often, off-flavors in products used for food. Thus, genetic improvement of this crop with delivery of desirable genes for food processing is a major goal of a number of R&D programs in the United States as well as in other countries.

Previous work has suggested that sorghum possesses characteristics that have both nutritional and functional properties that lend itself to the development of healthy, nutritious foods. Sorghum grain is nutritionally comparable and even superior to major cereals with respect to protein, energy, vitamins, and minerals. It is also a rich source of dietary fiber, phytochemicals, and micronutrients. Traditionally, sorghum has been used in unfermented and fermented breads, porridges, couscous, rice-like products, snacks, and malted alcoholic and nonalcoholic beverages. Recently, interest in sorghum for high-end food products, especially in health food markets, has begun in regions of the world that have traditionally not used this grain in human food systems. Japan has taken the lead in developing snack food products using sorghum, and interest in sorghum's gluten-free properties has developed a demand for high-quality food sorghums within the United States.

Recently, interest has spread in the Mediterranean area for both the cultivation of white sorghum plant species and the production of white sorghum-based foods for healthy gluten-free nourishment, both for celiac populations and for non-celiac populations, as an alternative food to wheat, as part of the program on the development of 'a sorghum chain in the Italian Campania region.' Extensive research information is available on the processing and product development of sorghum. The basic conclusion from that research is that sorghum can be used in a similar fashion, with slight modifications, as other grains, in the production of food products through processing technologies that are currently available. Utilization of sorghum can be increased by various processing treatments including blanching, malting, dry heating, acid treatment, and popping. All these treatments decrease the level of antinutrients and improve digestibility. Sorghum can be utilized for the development of various food products. These include traditional products (porridges, flatbreads, chips, bhakri, suhali, khichri, dalia, shakkerpara, etc.), baked products, extruded products, health products, and weaning and supplementary foods.

Traditional Sorghum Food Products

The main foods prepared with sorghum are tortillas (Latino America); thin porridge, for example, 'bouillie' (Africa and Asia); stiff porridge, for example, tô (West Africa); couscous (Africa); injera (Ethiopia); nasha and kisra (Sudan); traditional beers, for example, dolo, tchapallo, pito, and burukutu (Africa); ogi (Nigeria); and baked products (the United States, Japan, and Africa). Tortillas are a kind of chips prepared from sorghum alone or by mixing sorghum with maize and cassava. Nasha is a traditional weaning food (infant porridge) prepared by fermentation of sorghum flour. Ogi is an example of traditional fermented sorghum food used as weaning food, which has been upgraded to a semi-industrial scale. Injera is a fermented pancake-like bread prepared in Ethiopia from sorghum. Kisra is traditional bread prepared from fermented dough of sorghum. Tô is prepared by cooking a slurry of sorghum flour. Thin porridges (usually used as weaning food) are also prepared in the same manner with less flour to obtain a fluid end product. Porridges prepared with malted sorghums have several order of magnitude lower viscosities than those of nonmalted sorghums. These porridges are particularly useful for the formulation of weaning foods for infants because of their high energy density. Furthermore, the exogenous sources of α-amylase from higher West African plants are useful in reducing the viscosity of cereal porridges, including sorghum ones. Couscous is a steamed and granulated traditional African food originating from North Africa. The traditional method of preparing couscous is a steam-cook process in a special pot called 'couscoussière.' Couscous is prepared by mixing flour with water to obtain agglomerated flour–water mixtures. The agglomerates are then put on top of the 'couscoussière.' The stew cooks in the bottom pot while the granules are steamed on top. White sorghum varieties from tan plants yield the best couscous product. Couscous quality criteria include size uniformity, color, stickiness, and mouthfeel. Dolo is a reddish, cloudy, or opaque local beer prepared essentially from red sorghum malt. The sorghum malting process starts by immersing the grain in water to activate hydrolytic enzymes. Traditional germination involves seedling growth in warm water-saturated air for 3–5 days. The germinated grain is then dried to moisture content of 10–12%. The malt obtained is used to prepare dolo. Briefly, dolo preparation starts by mixing malted sorghum flour with water (1:10, w/v). The mixture is decanted and the supernatant (containing hydrolytic enzymes) is separated from the precipitate (containing

starch). Water is added to the precipitate and the mixture is boiled to gelatinize the starch, but the supernatant is not boiled. It is interesting to note that people, who originally prepare dolo ('dolotières'), empirically know that the supernatant contains 'some things,' for example, enzymes that are thermolabile so they should not be boiled. After cooling, the precipitate is filtered to separate soluble components (starch, sugars, proteins, etc.) and the spent (used as animal feed). The mashing step (incubation of hydrolytic enzymes with their substrates) consists of combining the previous supernatant and the filtrate at 50–60 °C for 12–16 h, to obtain the wort. The wort is cooked and then recooled overnight at room temperature (35–40 °C). The cooled wort is a sweet nonfermented beverage highly appreciated by children. It is traditionally called 'soft dolo.' The fermentation (1–2 days) is initiated by the addition of dried yeasts to the wort. The final product, dolo, is separated from yeasts by filtration. Characteristics of dolo are alcohol content (2–4%, v/v), pH 4–5, stability at room temperature (12–16 h), red color, and opaque appearance.

Sorghum for Baking Products

Sorghum flour does not contain proteins that produce viscoelastic properties similar to that of gluten found in wheat; therefore, acceptable yeast-leavened products from 100% sorghum flour are difficult to obtain. However, sorghum and wheat flour blends have been used to produce many non-gluten-free baked products, including yeast-leavened pan, hearth and flatbreads, cakes, muffins, cookies, biscuits, and flour tortillas. The level of sorghum substituted for wheat flour depends on the strength and quality of gluten in the wheat flour; the baking procedure; the definition of acceptable bread quality; the color, particle size, and aroma of the sorghum flour; and the use of gums, emulsifiers, and other additives.

From 5% to 50% sorghum flour can be substituted for wheat flour. The particle size and shape of sorghum flour often increase the grittiness of the baked products. However, tempering, attrition milling, and other modifications during processing can modify the sandiness of the flour.

White food-grade sorghum has a white kernel with tan plant and glume characteristics (white/tan/tan). The tan plant and light-colored glumes reduce the amount of anthocyanin pigments that cause off-colors in otherwise similar white grains that have purple or red glumes. Tan plants with straw-colored glumes provide grain with excellent properties for processing into whole-grain foods or milling into meal, flour, and grits. White food sorghums do not contain genetically modified organisms, are gluten-free (as are all sorghums), are bland in flavor and light-colored, and have excellent processing properties.

Sorghum Is a Suitable Gluten-Free Food for Celiac Patients

Celiac disease (CD) is a syndrome characterized by damage to the mucosa of the small intestine caused by ingestion of certain wheat proteins and related proteins in rye and barley. CD is a reaction to gluten proteins. Gluten is a complex mixture composed primarily of two classes of the wheat storage proteins, gliadins and glutenins. Gliadins are generally recognized as the primary agent responsible for the development of CD in celiac people. Most of the research into the toxic effects of gliadins has been done with a fraction of α-gliadins, called A-gliadin either intact or digested into small peptides. However, both classes of gliadins and glutenins are reported as toxic. CD is a malabsorptive disorder, present in genetically predisposed individuals, which is the result of ingestion of gluten proteins in wheat or related proteins from barley, rye, and possibly in oats. Symptoms include diarrhea pale stools, enlarged abdomen, physical wasting, anorexia, vomiting, anemia, loss of appetite, fatigue, infertility, anxiety, depression, osteoporosis, and weakness. Modern screening studies show that CD is much more prevalent than previously thought. The average worldwide prevalence has been estimated as high as 1:266. Estimates place the number of persons with CD in the United States at roughly 3 million. The cornerstone treatment for celiac disease is the total lifelong avoidance of gluten ingestion. This means that wheat, rye, and barley have to be avoided, including durum wheat, spelt wheat, kamut, einkorn, and triticale. Sorghum is often recommended as a safe food for celiac patients, because it is only distantly related to the Triticeae tribe cereals wheat, rye, and barley, being a member of the Panicoideae subfamily that also includes maize. Sorghum therefore provides a good basis for gluten-free breads and other baked products like cakes and cookies (biscuits) and in snacks and pasta. In the United States, white food sorghum is currently used in the production of high-quality, gluten-free sorghum flour utilized in a variety of gluten-free foods. Besides, as reported in a later section recently, molecular evidence demonstrating the absence of toxic gliadin-like peptides in sorghum was published, confirming that sorghum can be considered safe for consumption by people with CD.

The mild flavor, light color, pleasing texture, and healthy nutrients in sorghum – a gluten-free grain – make it ideal for gluten-free cooking. As the celiac population reaches three million in the United States and the market for gluten-free foods reaches $700 million, sorghum's versatility makes it appropriate in recipes for gluten-free breads, desserts, pizza, breakfast dishes, and main dishes. Sorghum is an excellent choice for gluten-free foods because it has a similar profile to wheat on the basis of the following characteristics:

1. *Color*: White food-grade sorghum flour is very light in color and will not affect the color of baked goods. This means that it can be used in virtually any type of dish, ranging from white cakes, to sugar cookies, to white bread (Figure 5).
2. *Flavor*: Some people think that sorghum tastes more like wheat than any other gluten-free grain. It is generally described as a pleasant flavor that does not interfere with or dominate the flavor of the food in which it is used. This means that it can be used in any gluten-free food, making it extremely versatile.
3. *Texture*: Unlike rice flour – which is the most commonly used gluten-free flour – sorghum flour has a smooth, non-gritty texture due to processing modifications comprising tempering and attrition milling, as mentioned earlier in the text. A perceived grittiness in rice-based, gluten-free foods is one of the chief objections of newly diagnosed celiac patients.

Figure 5 Sorghum in baking: (a) sandwich breads; (b) pizza; (c) quick breads; (d) cakes. Reprinted with permission from Fenster, C. (2014). *Gluten-free 101: the essential beginner's guide to easy gluten-free cooking*. Houghton Mifflin Harcourt.

4. *Nutrients*: Sorghum flour comes fairly close in nutrient content to unbleached wheat flour, the type used most often in American baking. It is not as nutritious as wheat flour, however.

As mentioned previously, sorghum is gluten-free, and this is particularly important since the incidence of food allergies is growing and it is not unusual for some people to be unable to eat not only wheat but also corn, rice, and soy, which are the most common replacements for wheat. Sorghum provides yet another alternative at least until continued exposure to this grain over time possibly leads to an allergy or intolerance to it.

Sorghum in Nutrition and Health

Sorghum could find a significant niche in the nutritional foods market in developed countries. Unique health foods that have high levels of insoluble dietary fiber, phytates, and phytochemicals could be made from sorghums with high levels of catechins, flavonoids, and phytates and harder to digest proteins. Tannin sorghums contain high levels of antioxidants and may have anticancer properties. Besides, sorghum can contain substantial levels of a wide range of phenolic compounds. The concentration of phenols in some sorghum varieties is comparable to or higher than that of blueberries. Phenolic compounds, especially flavonoids, have been found to inhibit tumor development. The phenolic compounds, flavonoids and tannins, are concentrated in the pericarp, which can be abrasively milled to produce ingredients providing health benefits. Sorghum bran fractions contain high levels of phenols with good antioxidant values and could be exploited as a source of phytonutrients in foods. Their health-promoting properties, in particular their antioxidant activity, and their use as nutraceuticals and functional foods have been reported.

Nutraceuticals are the fastest-growing segment of the food industry. Clearly, with the increasing demand for antioxidant-rich foods and products, new sources are bound to play an important role in this market. Specialty sorghum brans are a promising source of these compounds, given their excellent coloring ability and antioxidant activity. In addition to the potential health benefits of sorghum phenolics, sorghum wax may also have unique health properties. Sorghum wax, which is concentrated on the surface of the pericarp of the grain, is composed of fatty aldehydes (46%), fatty acids (7.5%), fatty alcohols (41%), hydrocarbons (0.7%), wax and sterol esters (1.4%), and triacylglycerols (1%).

The fatty alcohols in sorghum wax can be classified as policosanols, which are primary long-chained alcohols. Since sorghum wax contains only a small amount of wax esters, it was suggested that the term 'long-chained lipids' might be more appropriate than 'wax.' The latter study reported long-chained lipids from different sorghums in the range 37–44% for policosanols, 44 (48)–55% for aldehydes, and 4–5% for acids and that long-chained lipids constituted 0.2–0.3% of sorghum kernels. Therefore, the policosanol content of sorghum kernels was approximately 800 ppm. Sorghum–dried distiller's grains (DDGs), in which nonstarch components are concentrated, contained approximately 2500 ppm policosanols. These amounts are high relative to other sources, for example, brown rice, rice bran, rice germ, and wheat germ. Octacosanol (28:0) and triacontanol (30:0) comprised more than 80% of the policosanols in sorghum kernels and DDG. It has been suggested that mixed C24–C34 alcohols including octacosanol and triacontanol lower the amount of low-density lipoprotein (LDL) cholesterol and raise the amount of high-density lipoprotein (HDL) cholesterol, thus improving the LDL/HDL ratio. It was also indicated that long-chain fatty alcohols, aldehydes, and acids are interconverted in cellular metabolism, so that all three classes might lower cholesterol. It was reported that policosanols are a promising resource for the prevention and therapy of cardiovascular disease. It was found that a crude lipid extract from whole kernel sorghum, which

comprised a wide range of lipid substances including plant sterols and policosanols, lowered cholesterol absorption and plasma non-HDL cholesterol in hamsters.

From the point of view of health, starches in sorghum are considered beneficial to diabetic, since they are released more slowly than those in other cereals. Considering the proteins of sorghum, we must make a separate discussion, since they occupy an important position in bringing benefits in the diet of celiac patients who are intolerant to gluten.

Safety of White 'Food-Grade' Sorghum for Celiac Patients: Clinical, Genomic, Biochemical, and Immunochemical Evidences

In 2007, it was demonstrated by clinical trials that sorghum did not contain toxic peptide sequences with regard to celiac disease and could be considered 'gluten-free' (Figure 6). Until 2011, the white 'food-grade' sorghum was used as food for celiac patients, particularly in the United States. However, experimental evidences at the genomic, biochemical, and immunochemical levels about the lack of gluten in sorghum had not yet been reported. Therefore, research activity was aimed at demonstrating the absence of gliadin in sorghum. In this study, *in silico* genomic approaches and biochemical experiments (Figure 7) were used to characterize protein profiles of storage proteins in different sorghum varieties. These analyses provided molecular evidence for the absence of toxic gliadin-like peptides in sorghum. This finding was supported by immunochemical experiments using the monoclonal antibody R5, which reacts with the gliadin fractions from wheat and corresponding prolamins from rye and barley. It recognizes among others the toxic sequence QQPFP, which occurs repeatedly in the prolamin molecules (Figure 8). Thus, sorghum, similarly to maize and rice, should be considered safe for gluten-intolerant people.

Introduction of White 'Food-Grade' Sorghum in the Mediterranean Area

Since 2006, sorghum grain produced from the white food-grade sorghum cultivars coming from both the United States and Africa was grown in field trials in southern Italy, as shown in Figure 9, and analyzed.

The average values of the results of analyses on white food-grade sorghum grain, grown in a Mediterranean environment, concern the chemical composition reported in Table 1, lipid content reported in Table 2, and the composition of the amino acids reported in Table 3. Those results show that the characteristics of the sorghum cultivars grown in southern Italy are comparable with those of the same sorghum cultivar grown in different environments, namely, the United States and Africa.

In addition, the flour produced from the grain of white food-grade sorghum grown in southern Italy was used to produce, by formulations with other ingredients, pleasing and palatable *gluten-free* cookies shown in Figure 10.

Concluding Remarks

White food-grade sorghum varieties have huge potential for wider use: it can be used in baked, extruded, and other cereal-based products (bread, cookies, expanded snacks, pasta, breakfast cereals, etc.) as partial or complete substitutes for other cereals. The sorghum brans can be used to fortify bread, cookies, and other snacks and to improve the phytonutrient content, as well as dietary fiber and sensory properties. With increasing world population and decreasing water supplies, sorghum represents an important crop for future human use. In fact, while climate change and drought will reduce, especially in the Mediterranean area, the production of cereals, such as wheat, less suitable to those environmental changes, sorghum for human use can be considered a viable alternative to them. Therefore, the aim would be to spread in Italy and in the European countries the use of white sorghum flour mixed with other natural ingredients, such as flour and other gluten-free food components, in order to produce healthy energy-producing foods as an alternative to food based on wheat, where the presence of gluten is the main cause of gastrointestinal intolerance.

Acknowledgments

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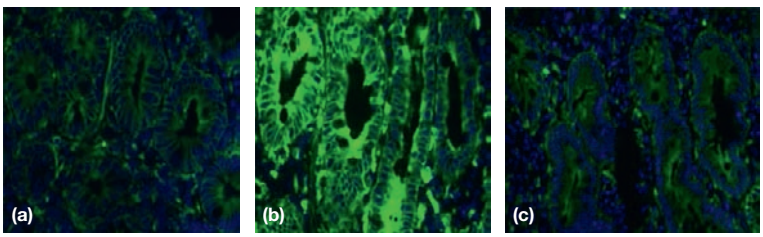

Figure 6 Human leukocyte antigen D-related (HLA-DR) expression is a specific, gluten-related marker of mucosal inflammation of the intestine of celiac patients exposed to gluten or analogous toxic proteins. (a) HLA-DR expression in intestinal biopsy cultured with medium (negative control). No staining is noted. (b) HLA-DR expression in intestinal biopsy cultured with medium added with gliadin digest (positive control). The picture shows intense crypt staining, sign of mucosal inflammation. (c) HLA-DR expression in intestinal biopsy cultured with medium added with sorghum digest. No staining is noted, as in (a); thus, no inflammation is elicited by sorghum digest in the celiac mucosa. Reprinted from Ciacci, C., Maiuri, L., Caporaso, N., et al. (2007). Celiac disease: in vitro and in vivo safety and palatability of wheat-free sorghum food products. *Clinical Nutrition* **26**, 799–805. Copyright 2007, with permission from Elsevier.

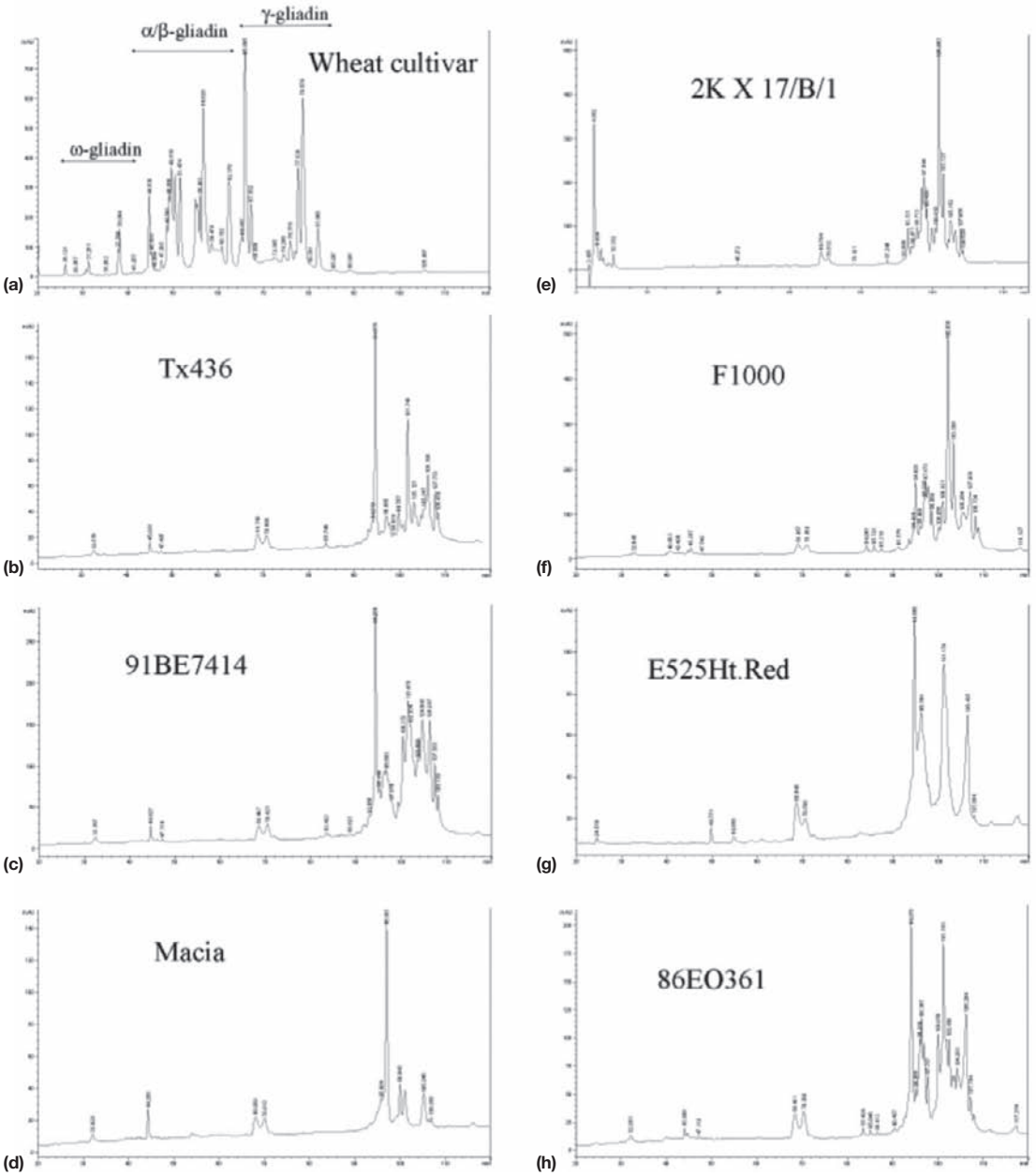

Figure 7 RP-HPLC chromatograms of kafirins extracted from sorghum varieties ((b)–(h)) compared to gliadin extracted from subclasses with improved resolution, on the basis of surface hydrophobicity. The HPLC of gliadin (a) showed a heterogeneous protein profile that could be grouped, on the basis of retention time, in ω-, α/β-, and γ-gliadins, as previously reported. On the contrary, completely different UV chromatograms were observed with kafirins ((b)–(h)). The results provided evidence that proteins with characteristics similar to those of wheat gliadins are absent in all sorghum varieties analyzed. Reprinted with permission from Pontieri, P., Mamone, G., De Caro, S., et al. (2013). Sorghum, a healthy and gluten-free food for celiac patients as demonstrated by genome, biochemical and immunochemical analyses. *Journal of Agricultural and Food Chemistry* **61**, 2565–2571. Copyright 2013 American Chemical Society.

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See also: Allergies: Public health; Antioxidants: Role on Health and Prevention; Bioactive Peptides in Foods; Biscuits, Cookies and Crackers: Nature of the Products; Cereals: Dietary Importance; Cereals: Storage; Cereals: Types and Composition; Dietary Surveys: National Food Intake; Food Allergies; Food and Agriculture Organization of the United Nations; Maize; Novel Foods; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Rice: Role in Diet; Tannins; Wheat: Grain Structure of Wheat and Wheat-based Products; Wheat: The Crop.

(a)

- Wells from H to C: ppm Gliadin Standard: 0,5,10,20,40,80, respectively
- Wells from B to A: Protein samples extracted from Sorghum flours

It is observed in the H to C wells an increasing coloration, according to the gliadin standard concentration , due to the antigen-antibody immunochemical reaction, while no staining is observed in both the B and A wells containing the protein samples extract from sorghum flours.

(b)

Cultivar	Type	Content*
Macia	Sorghum	<5
86EO361	Sorghum	<5
91BE7414	Sorghum	<5
T x 436	Sorghum	<5
F1000	Sorghum	<5
Epuripur	Sorghum	<5
Sekodo	Sorghum	<5
Gliadin Standard	Wheat	56

Figure 8 (a) Sandwich enzyme-linked immunosorbent assay microtiter plate. (b) The RIDASCREEN® standard test kit (RIDASCREEN® Gliadin (Art. No R7001), R-Biopharm AG) sandwich ELISA-based method was used to identify gliadins in grain flour samples according to the manufacturer's instructions. Determinations were carried out according to the instruction leaflets sent with the kit; the measurements were made photometrically at 450 mn. The results indicated that the gluten levels in sorghum flours were <5 ppm. These values are well below the 20 ppm threshold that has been proposed to be safe for celiac patients.

Figure 9 Phase of growth of white food-grade sorghum cultivar in San Bartolomeo in Galdo (BN), southern Italy. (a) Emergency sprouts; (b) raising plant; (c) flowering panicle; (d) ripening grain.

Table 1 Chemical composition of whole sorghums grown in field trials in southern Italy

	Average value
Humidity (%)	12.66
Proteins (%)	9.95
Carbohydrates (%)	83.24
Ashes (%)	1.41
Fats (%)	2.93
Fiber (%)	1.06
Energy value (kcal 100 g^{-1})	363.91

Table 2 Fatty acid content (%) of total lipids from sorghums grown in field trials in southern Italy

		Average value
Palmitic	$C_{16:0}$	13.86
Palmitoleic	$C_{16:1}$	0.57
Margaric	$C_{17:0}$	0.08
Margaoleic	$C_{17:1}$	0.13
Stearic	$C_{18:0}$	1.36
Oleic	$C_{18:1}$	39.98
Linoleic	$C_{18:2}$	45.66
Arachidic	$C_{20:0}$	0.21
Linolenic	$C_{18:3}$	1.84
Eicosenoic	$C_{20:1}$	0.34
Behenic	$C_{22:0}$	0.15

Table 3 Amino acid compositions in percent of whole sorghums grown in field trials in southern Italy

Amino acid	*Average value*
Asx	8.86 ± 0.44
Thr	3.34 ± 0.12
Ser	0.69 ± 0.27
Glx	14.05 ± 1.14
Gly	10.32 ± 0.24
Ala	19.63 ± 0.72
Val	3.61 ± 0.16
Cys	1.27 ± 0.25
Met	1.81 ± 0.22
Ile	2.40 ± 0.41
Leu	10.88 ± 0.45
Tyr	3.92 ± 0.44
Phe	6.36 ± 0.25
Lys	2.68 ± 0.14
His	2.44 ± 0.15
Arg	2.76 ± 0.48

Data are presented as average value ± SD.

Figure 10 Sorghum flour-based cookies produced in Italy by Celi.net s.r.l.

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Soy Beans: Dietary Importance

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Introduction

Soybeans and their constituent isoflavones have been studied extensively in relation to many aspects of human health. The hypothesis that soy isoflavones could serve as an alternative to hormone replacement therapy has prompted research into menopause-related symptoms and breast cancer and osteoporosis. In addition, cardiovascular disease (CVD) and conditions relevant to men, such as prostate cancer, have also been examined with the common rationale of estrogenic-related etiologies. The dramatic increase in soybean-related peer-reviewed literature over the past 20 years and the history of numerous international symposia on the role of soybeans in health promotion and chronic disease prevention and potential treatments document the magnitude of research activity in these areas. This article will focus on the isoflavones in soybeans and summarize background information on their classification, foods sources, and intake and current literature in specific areas of health including menopausal symptoms, osteoporosis, breast cancer prevention, CVD, and prostate cancer.

Soy Isoflavone Classification, Food Sources, and Dietary Intake

Isoflavones are classified as phytoestrogens, which are defined as a phenolic, nonsteroidal compound structurally similar to estrogen and naturally occurring in plants. The structural similarity of isoflavones to endogenous estrogen allows them to bind to estrogen receptor-α and estrogen receptor-β with a preference for estrogen receptor-β.

Isoflavones have limited distribution in the plant kingdom, its most predominant presence being in soybeans, although they are found in smaller amounts in some nonsoybean foods. Isoflavones are associated with the protein content of the soybean and range from 3 to 5 mg isoflavones per gram soy protein with precise levels dependent on not only processing of the soybean but also geographic location of cultivation, variety, and harvest year. To contribute to a better understanding of isoflavone content of foods and allow for more accurate quantifications of isoflavone intake, the US Department of Agriculture and Iowa State University created a comprehensive and publicly available database of the isoflavone quantity of foods (http://www.ars.usda.gov/Services/docs.htm?docid=24953).

Intake of soy isoflavones is very low in Western populations and much higher in Asian populations. To summarize the current knowledge of adult Asian soy isoflavone intakes, Messina and colleagues in 2006 examined a total of 24 dietary surveys from Japan, China, Hong Kong, and Singapore. The results indicated a typical Japanese intake of 25–50 mg aglycone equivalent isoflavones per day, with lower intakes in Hong Kong and Singapore, and significant regional intake differences in China. Upper intakes were documented at 100 mg day^{-1} in Japan, although only in a minority (5%) of the population.

Soy Isoflavone Metabolism and Pharmacokinetics

Isoflavones are present in soybeans and soy foods in four different chemical forms, namely, aglycone (as is the case in fermented foods) and, more commonly, β-glycoside, malonlyl-β-glycoside, and acetyl-β-glycoside (conjugated to a glycoside unit). Although there is some evidence that bioavailability of the aglycone is greater than the conjugated form, arising from comparisons of maximal circulating concentrations, both forms are absorbed and have comparable half-lives. The major isoflavones (glycoside/aglycone) present in soybeans and soy foods include genistin/genistein, daidzin/daidzein, and glycitin/glycitein at ~50%, 40%, and 10%, respectively.

Following consumption of soy foods, intestinal bacterial enzymes and/or mucosal β-glucosidases cleave the isoflavone glycosidic conjugate to yield the aglycone form. The aglycone isoflavone can be absorbed or may interact further with intestinal bacteria to produce isoflavone metabolites, such as equol (from daidzein), *O*-desmethylangolensin (from daidzein), and p-ethylphenol (from genistein), all of which can also be absorbed. Following absorption, isoflavones undergo extensive first-pass metabolism and then are conjugated to glucuronic acid or sulfate by hepatic phase II enzymes, after which they undergo enterohepatic circulation. The maximum circulating concentrations of isoflavones occur 6–8 h postconsumption, although a biphasic pattern with peaks at 1–2 h and 4–8 h has also been observed. The half-life of isoflavones is ~8 h with most excreted within 24 h. Serum and urinary concentrations of isoflavones have been demonstrated to reflect intake, providing end points for compliance in dietary soy intervention studies.

Isoflavone absorption and metabolism are highly variable among individuals. Isoflavone bioavailability can be influenced by many factors, such as chemical form, dose, source of isoflavone, frequency of consumption, sex, age, microflora, gastrointestinal transit time, food matrix, and background diet. Among the many factors that can influence isoflavone exposure, there is particular interest in the large interindividual variation found in urinary excretion of the isoflavone metabolite equol. Equol is produced by microflora bacteria from daidzein and has unique qualities among the isoflavone and isoflavone metabolites, the most obvious of which is that equol is produced by only 30–40% of the population. Furthermore, equol is one of the most potent isoflavones, with a higher affinity for the estrogen receptor than its precursor daidzein and a longer half-life than both genistein and daidzein, suggesting that the ability to produce equol may increase soy isoflavone exposure. The relevance of equol continues to be an active area of research with the working hypothesis that

equol production status is a major confounding factor in clarifying the health effects of soy isoflavone consumption.

Soy Isoflavones and Health

It has been documented by Messina that, since the early 1990s when the US National Cancer Institute announced their first research program to explore the potential cancer-protective effects of isoflavones, there have been more than 10 000 peer-reviewed articles published. Indeed, as summarized in the following sections, there is an abundance of literature from which to examine the role of soy and its constituent isoflavones in human health.

Menopausal Symptoms

The potential for a dietary alternative to alleviate menopausal symptoms is a major rationale for the study of soy isoflavones, and the intensity of these efforts is evident by the large number of published reviews and meta-analyses. These reviews have focussed primarily on reduction of frequency and/or severity of hot flashes/flushes by soy isoflavones and have concluded either a beneficial role or no apparent benefit over placebo.

In a comprehensive 2003 review, Messina and Hughes concluded that data from 13 soy (34–100 mg isoflavones per day) intervention studies suggested that those with frequent hot flashes should consider soy foods or isoflavone supplements for the alleviation of symptoms. This was confirmed in a subsequent meta-analysis, by Howes in 2006, of 17 isoflavone (48–160 mg day^{-1}) intervention studies that showed overall a significant reduction in hot flashes and the effect was related to the number of baseline hot flashes. The effect of isoflavones on hot flashes was further summarized by Williamson-Hughes et al., but their review of 11 studies identified isoflavone composition as an important consideration: hot flashes were decreased significantly in studies that provided 15 mg genistein per day or more, but not those providing <15 mg genistein per day.

In contrast to the beneficial effects reported in these analyzes, others have concluded that soy or soy isoflavones have no apparent beneficial effects on menopausal symptoms, including a 2004 report by Krebs et al. that summarized 25 studies using a systematic review and subsequent reviews from the Cochrane database in 2007 and 2013. Overall, the repetition of studies and reviews of studies in this area attests to the worthiness of the idea and the fact that some studies conclude that a beneficial effect in the face of methodological challenges provides credence to the role of soy isoflavones in the alleviation of menopausal symptoms.

Osteoporosis

Osteoporosis is a major health risk faced by postmenopausal women due to reduced estrogen production. Soy isoflavones are of relevance for many reasons, including their estrogenic nature, reports of lower incidence of fractures in Asian women (who consume more soy), and associations between isoflavone intake and reduced bone resorption, improved bone mineral density (BMD), and reduced risk of fracture. This evidence has provided a rationale for the completion of multiple intervention studies that have, in turn, progressed to multiple systematic review and meta-analyses aiming to summarize the role of soy isoflavones in the prevention of osteoporosis.

A 2008 review article by Atmaca et al. examined epidemiological and intervention studies on soy isoflavones and bone health in postmenopausal women and suggested beneficial effects on bone turnover markers and BMD. In the same year, Ma and colleagues published two meta-analyses of randomized controlled trials summarizing evidence for effects of soy isoflavone intake on markers of bone turnover and BMD. For their meta-analysis of bone turnover markers, the authors selected nine studies using soy products or isoflavones (37.3–118 mg day^{-1}) for at least 4 weeks, included healthy pre- and postmenopausal women, and included the bone resorption marker urinary deoxypyridinoline and/or the bone formation marker bone alkaline phosphatase. Results suggested significant favorable effects of isoflavone consumption, including decreased urinary deoxypyridinoline and increased bone alkaline phosphatase, with stronger effects observed in participants who were Asian and/or postmenopausal. For their BMD meta-analysis, 10 randomized controlled trials were included that intervened with soy products or isoflavones (4.4–150 mg day^{-1}) for at least 3 months, which included healthy pre- and postmenopausal women, and BMD was an outcome measure. Results suggested a significant favorable increase in spine BMD due to isoflavone consumption. Unlike their bone turnover analysis results, which did not depend on dose or duration, Ma and colleagues found enhanced effects on BMD with isoflavone intake >90 mg day^{-1} for at least 6 months. In contrast to the positive results from the meta-analyses by Ma et al., a meta-analysis by Liu and colleagues in 2009 examining ten randomized controlled trials involving 896 women concluded that the average dose of 87 mg soy isoflavones for at least 1 year did not significantly affect BMD. Further, a meta-analysis of randomized controlled trials by Taku and colleagues in 2010, which included 1240 menopausal women, concluded that soy isoflavone extract supplements (average dose 82 mg isoflavone aglycone equivalents/day for 6–12 months) significantly increased lumbar spine BMD but not femoral neck, hip total, or trochanter BMD. The same investigators published a meta-analysis in the same year that focussed on bone turnover markers and found that an average of 56 mg aglycone equivalent soy isoflavones for 10 weeks to 12 months significantly decreased urinary deoxypyridinoline by 14%, but this was not accompanied by significant improvements in serum bone alkaline phosphatase or osteocalcin. Most recently, however, a systematic review by Wei and colleagues in 2014 concluded that there was a significant effect of soy isoflavones on BMD and urinary deoxypyridinoline, relative to menopausal status, supplement type, isoflavone dose, and intervention duration.

Overall, studies of soy isoflavones in relation to bone health have produced inconsistent results with earlier studies summarizing favorable effects on BMD and bone turnover, while others have found inconsistent or no significant benefit of isoflavones on bone health. These discrepancies will prompt

continued investigation to further clarify the relevance of soy isoflavones in the prevention of osteoporosis.

Breast Cancer Prevention

The link between soy isoflavones and breast cancer prevention was first documented by Lee et al. in 1991 when the diets of 200 Singaporean Chinese women with breast cancer and 420 matched controls were compared; it was found that those with higher intakes of soy protein and total soy products had significantly decreased breast cancer risk. Numerous studies followed and, although inconsistent, meta-analyses suggest a significant protective association. For example, Qin et al. in 2006 pooled 14 case–control and seven cohort studies to conclude an overall inverse association between breast cancer risk and intake of soy foods (RR = 0.75) and soy isoflavones (RR = 0.81). In the same year, Trock et al. reported a meta-analysis of 12 case–control and six cohort studies that also concluded an inverse association between breast cancer risk and intake of soy (RR = 0.86). However, the association was not significant among women living in Asian countries and was stronger in premenopausal women. Wu et al. in 2008 extended the process by selecting only high-quality studies best designed to address the relationship between soy consumption and breast cancer by ensuring that they had a (relatively) complete assessment of dietary soy exposure and appropriate control of potential confounding factors. They also addressed the issue of isoflavone exposure by separating analyses into high-soy-consuming Asians and low-soy-consuming Western individuals. Results from one cohort and seven case–control studies with high-soy-consuming Asians showed a dose-dependent inverse association between breast cancer risk and soy isoflavone intake with a 14% reduction in risk for an isoflavone intake of 10 mg day^{-1} and a 29% reduction for $\geq$20 mg day^{-1}. In contrast, results from four cohort/nested case–control and seven case–control studies with low-soy consumers concluded that there was no significant association between breast cancer risk and soy isoflavone intake in the narrow intake range 0.15–0.8 mg day^{-1}. This is similar to more recent research results from 35 studies meta-analyzed by Chen and colleagues in 2014, which generated a significant inverse association (OR = 0.59) for Asian but not Western women. Overall, although these meta-analyses provide support for a role of soy isoflavones in breast cancer prevention, there is heterogeneity among studies, and there remain inconsistent results with some studies reporting no significant relationship between soy and breast cancer risk regardless of intake.

The relationship between soy and breast cancer prevention has evolved to consider the age of exposure, an idea that suggests that intervention is most effective during the period of most active change, as would be the case for breast development during late childhood/adolescence/puberty. Lamartiniere and colleagues tested and confirmed this hypothesis in several studies using rat models. Furthermore, multiple epidemiological studies have supported the idea by reporting consistently that soy and soy isoflavone exposure during adolescence is related to a significantly reduced adult breast cancer risk. Thus, the concept of timing of exposure is supported and adds a dimension to current understanding of the relationship between soy isoflavones and breast cancer prevention.

Overall, the area of soy and breast cancer prevention continues to be studied, with intervention studies focussing on breast cancer biomarkers, mechanistic studies to explore both estrogen-dependent and estrogen-independent actions, and studies investigating the relevance of individual response, including equol production and nutrigenomics.

There has also been concern regarding safety of soy in relation to breast cancer. These concerns stem from the possibility that a compound with estrogenic properties could cause increased growth of an estrogen-dependent tumor. This is a growing research area, but epidemiological studies that have examined the association of breast cancer recurrence or mortality in relation to soy isoflavone exposure have found either a protective or no significant association.

Cardiovascular Disease

CVD is one of the most frequently studied areas in relation to soy and health with relevance for both women and men. This soy–health relationship also has the greatest public awareness following the 1999 approval of a food health claim by the Food and Drug Association (FDA) in the United States. This health claim allows food manufacturers to label products that meet the criteria of at least 6.25 g soy protein per serving. The claim reads, 'Diets low in saturated fat and cholesterol that include 25 g of soy protein a day may reduce the risk of heart disease. One serving of (this food) provides (this amount) of soy protein.' This health claim was approved based on the totality of evidence at that time and, in particular, a meta-analysis published in 1995 in the *New England Journal of Medicine*, which examined 38 human clinical studies evaluating the effects of soy protein on circulating lipids and reported overall reductions in total cholesterol by 9.3%, LDL cholesterol by 12.9%, and triglycerides by 10.5% following an average soy protein intake of 47 g day^{-1}. This also led the Nutrition Committee of the American Heart Association (AHA) to release a science advisory on soy protein in 2000, which recommended the inclusion of soy protein foods in a diet low in saturated fat and cholesterol.

It is noteworthy that, in the approval of the health claim, the FDA indicated that available evidence did not support a significant role for soy isoflavones in the cholesterol-lowering effects of soy protein. This idea has been corroborated by subsequent meta-analyses of studies intervening with isoflavone extracts, which have not demonstrated significant effects on circulating lipids, compared with soy protein, which has shown significant effects. However, although these soy protein meta-analyses found significant effects, the magnitudes of change in LDL and/or total cholesterol observed were more modest (5%) than the Anderson et al. meta-analysis on which the health claim is based.

The large numbers of studies examining soy and CVD since the approval of the health claim prompted the Nutrition Committee of the AHA to reevaluate the evidence and update their 2000 science advisory. In 2005, they published a revised statement in relation to soy protein, isoflavones, and cardiovascular health that indicated isoflavone supplements are not recommended and earlier research demonstrating clinically favorable effects of soy protein has not been confirmed subsequently.

Although the science advisory noted that soy products could still be beneficial to cardiovascular and overall health, because of their high content of polyunsaturated fat, fiber, vitamins, and minerals and low content of saturated fat, their discouraging statement generated activity among the scientific community, including an announcement in December 2007 from the US FDA that they also intended to reevaluate scientific justification for the soy and coronary heart disease (CHD) food health claim. However, the US health claim remains, and in 2014, Health Canada reached out to stakeholders in their jurisdiction for comments on a proposed health claim relating soy protein and cholesterol-lowering effects. Research activity in the area of soy and CVD continues to clarify the role of soy protein and soy isoflavones by exploring effects of soy on other CVD biomarkers, and better understanding the molecular mechanisms involved using, for example, systems biology.

Prostate Cancer

The hypothesis that soy isoflavones could reduce prostate cancer risk was generated from epidemiological data documenting that prostate cancer rates are low in Asian countries and these rates increase when Asians migrate to the United States. The potential relevance of soy isoflavones to these differences was also supported by higher isoflavone concentrations in the blood, urine, and prostatic fluid of Japanese men relative to their Western counterparts.

Numerous epidemiological studies have focussed on the association between soy intake and prostate cancer risk, which has culminated in meta-analyses. The first, published by Yan and Spitznagel in 2005, included two cohort and six case–control studies and concluded that soy consumption was associated with a 30% reduction in prostate cancer risk. Four years later, the same authors published an updated meta-analysis that included epidemiological studies that related prostate cancer to consumption of soy (15 studies) or isoflavones (9 studies). Results revealed combined significant RR/ORs of 0.74 for soy intake and 0.88 for isoflavone intake. Another meta-analysis by Hwang and colleagues in 2009 combined five cohort and eight case–control studies and reported that both total soy (OR = 0.69) and nonfermented soy (OR = 0.75) foods were significantly associated with reduced prostate cancer risk and, among individual foods assessed (tofu, soybean milk, miso, and natto), tofu was the only food significantly associated with reduced prostate cancer risk (OR = 0.73). The most recent meta-analysis in 2014, by vanDie and colleagues, focussed on men at high risk of prostate cancer and observed a significant inverse association between soy isoflavones and prostate cancer (OR = 0.49), although it only includes two studies.

Given the inaccuracies inherent in dietary assessment, other studies have related prostate cancer risk to circulating and urinary isoflavones. This also allows consideration of the ability to produce equol, the focus of two case–control studies by Akaza and colleagues (2002 and 2004), the first of which showed that those able to produce equol were significantly higher among controls relative to prostate cancer cases. The second case–control study showed not only that equol producers were more frequent among Japanese and Korean men, compared to American men, but also that the proportion of equol producers among Japanese and Korean men was significantly higher in the control group (46% and 59%, respectively) compared with prostate cancer cases (29% and 30%, respectively). The role of soy isoflavones in prostate cancer risk is well studied and includes associations with dietary, circulating, and urinary isoflavones. Nutrigenomics or systems biology more widely continues to advance knowledge and understanding in relation to soy isoflavones and prostate cancer risk.

Summary

The relationship between soy and health has been studied extensively with the focus not only on menopause (menopausal symptoms, osteoporosis, and breast cancer) but also conditions relevant to men (prostate cancer) or both sexes (CVD). Soy has also been studied in respect of other health aspects including cognitive function, diabetes, thyroid health, infant health, and male fertility. Future human soy isoflavone interventions should take into consideration recommendations from the 2009 National Institutes of Health scientific workshop, 'Soy Protein/Isoflavone Research: Challenges in Designing and Evaluating Intervention Studies,' published in the *Journal of Nutrition* (http://jn.nutrition.org/content/140/6/1192S.long).

See also: Soy Beans: Processing; Soy Beans: Properties and Analysis; Soy Beans: The Crop; Soybean Oil.

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Soy Beans: Processing

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Soybeans and soybean products have been used extensively in the Orient as a source of protein and energy in human diets for at least 4000 years in one form or another. Traditional soy foods such as tofu, soy milk, miso, and tempeh are popular with Asian consumers, whereas modern soy ingredients such as soy flour and other protein ingredient are used by the food industries in the United States and Europe. Awareness of soybeans' properties and the use of soy ingredients in foods have increased dramatically during the last few years. As consumer demands grow for healthy foods, the use of soybean as a key ingredient is expected to expand in the food industry. The author has tried to explain different forms of soy ingredients that are available in the market for a variety of foods; working with all these soy ingredients into food products requires some practical and technical consideration. Food product developers can find a host of different soy ingredients with an array of nutritional benefits with different functionalities associated with each ingredient. There are a variety of specially modified soy concentrates and isolates available for specific application. These soy concentrates and isolates will have different solubility, emulsification, and texturization properties. A number of enzyme-modified concentrates and isolates are also available for food application. These special soy ingredients can boast foaming and aeration properties that allow replacing egg white in some products. Some of these soy ingredients may not be stable in highly acid conditions. Most of the soy protein typically exhibits very good freeze/thaw stability.

Soybean occupies a unique position among legumes. Traditional food legumes are rich in protein but contain limited amount of oil (energy). Soybean contains more protein than the other legumes and also a much higher content of edible oil. However, raw soybeans cannot be used successfully in human foods unless processed properly. There are several antinutritional factors that have been identified in raw soybeans, that is, trypsin and chymotrypsin inhibitors; phytohemagglutinins (lectin); and allergenic factors: glycinin and beta-conglycinin. All of these antinutritional factors can be deactivated, modified, or reduced through proper heat treatment to minimize or eliminate their adverse effects. Since all these inhibitors are proteins, caution should be taken to assure that no destruction of the oilseed protein occurs. This can be accomplished only through optimum processing and good quality control measure.

Processing of Soybeans

Today's modern soybean processing plant will process between 3 and 4 million pounds of soybeans daily or roughly 75 000 bushels or 2500 acres of soybean each day. Furthermore, it is not uncommon to have in excess of one million bushels of bean stored at the processing plant.

Whole soybeans can be processed into several different soy ingredients based on their application. Presently, soybean ingredients that are available in the market for food application are (1) roasted soy nuts; (2) enzyme-active full-fat soy flour and grits; (3) enzyme-inactive full-fat soy flour and grits, also called toasted full-fat soy flour and grits or heat-treated full-fat soy flour; (4) extruder-processed full-fat soy flour and grits (enzyme-inactive): (5) enzyme-inactive low-fat soy flour or grits; (6) enzyme-active defatted flake/soy flour (90 PDI); (7) defatted soy flake/flour with 70 PDI; (8) defatted soy flake/flour with 20 PDI; (9) lecithinated soy flour; (10) textured soy flour; (11) refatted soy flour; (12) soy concentrates; (13) textured soy concentrates; (14) soy isolates; (15) soy germ; (16) soy isoflavones (mechanically extracted); (17) soy isoflavones chemically extracted; (18) fiber from hulls; (19) soy fiber from cotyledon; and (20) organic soy flour and concentrates.

Roasted Soy Nuts

To process soy nuts, soybeans go through cleaning, sizing, and color sorting plant and are then washed prior to being soaked. The beans are evenly distributed into soaking tanks that have monitored water timers. The beans are soaked for a predetermined time based upon the ambient temperature, moisture levels, etc., and then drained. The cleaned, soaked, and drained beans are dumped on a conveyor that carries the beans from the processing room to the roasting line. The beans are placed in a large hopper and then distributed on the stainless steel roasting belt by means of a vibratory feeder. The beans are spread evenly over the roasting belt with a second belt resting on top of them. This keeps the beans completely submerged in the soy oil used for roasting. The beans move through the roaster at a prescribed time based upon the size, temperature, moisture level, etc.

After the beans have been roasted, they enter a second stainless steel conveyor that cools them. They move slowly through the cool down conveyor while large fans pull the heat from them. The beans exit onto a shaker table that eliminates any loosened hulls or pieces. They are then conveyed into a flavoring vessel. Once the flavoring vessel has reached its prescribed volume, the required amount of flavoring is added. The vessel is then sealed and put into the roller/tumbler for mixing. After the flavoring is finished, the vessel is opened and placed in the dumper. The dumper lifts the vessel and dumps the flavored, roasted soy nuts onto a conveyor that transports them to holding hoppers. The selected flavor can then be directly packaged into either bulk or individual bags at the appropriate time. Soy nuts are ideal for healthy eating and dieting. These nuts are low in sodium and fat and at the same time rich in protein and fiber. These nuts can be mixed with other nuts or sold on their own.

Enzyme-Active Full-Fat Soy Flour and Grit Processing

To make enzyme-active full-fat soy flour and grits, the first step is cleaning.

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Cleaning

Cleaning is important for producing high-quality end products as well as protection of equipment. Cleaning of soybeans is done on sieves under air aspiration so that dust, plant tissue, pebbles, and light contaminating material, as well as bigger impurities (stones, stems, nails, etc.), are separated. The larger impurities are mostly separated step by step in a destoner and a magnetic iron separator. After cleaning the soybeans, the next step is drying the soybeans.

Drying

Soybeans are dried in grain driers to a moisture level of 10%, which is needed prior to dehulling. The temperature of the dryer should be in between 70 and 76 °C to achieve the desired moisture level. Uniform drying of the soybeans is very important for removal of hulls, since we need to remove the hulls from every individual bean and not an average. To do a good job of hull removal, it is essential to subject the beans to some kind of thermal impact. This is necessary because the hulls are attached to the bean with a proteinaceous material, which, when exposed to heat, affects the release of the hulls. After drying the beans, they are stored for tempering approximately for 72 h to stabilize the moisture contents. Dried beans are usually cleaned to remove as many loose hulls, pods, sticks, and other foreign material as possible. This step will increase the efficiency of the cracking rolls and aspirator. After drying, the next step is cracking and dehulling the soybeans.

Cracking and dehulling of soybeans

The primary reason for dehulling is to produce high-protein end products. Hulls amount to around 7–8% by volume of the total of bean processed, and removal of these hulls prior to processing will increase the protein content of the desired end products. Sometimes, fibers are not required in food application where this end product will be used. Clean and dry soybeans are cracked into suitable pieces for dehulling using cracking mill. There should be a minimum of fines and no mashed beans. After cracking, the hulls are separated under air flow and the lighter hulls are withdrawn. It is important at this point to understand exactly how air separation works, since it is presently the basis for all soybean dehulling. With properly dried and cracked soybeans, the cotyledons should separate easily from the hulls. As a practical matter, it is not possible to get an absolute separation of hulls from the cotyledons. After removing the hulls, the next step is milling.

Milling

These dehulled soybeans are ground by hammer or impact pin mill into flours with desired particle sizes. Full-fat products are not easy to pulverize or sieve. Coarse material can be separated from fine by air classification. To make grits, different sizes of screens are used in the hammer mill. These types of flour or grits are used in bread for bleaching wheat flour and conditioning the dough. Usually, enzyme-active soy flour is used in the range of 0.5%.

Enzyme-Inactive Full-Fat Soy Flour and Grit Processing

This type of flour is also called 'toasted full-fat soy flour' or heat-treated full-fat soy flour. In this process, soybeans are cleaned as described earlier. The soybeans are steamed under slight pressure for 20–30 min; cooled, dried, and cracked to remove the hulls; and then milled with sieving as described earlier. These types of flour have very low lipoxygenase activity, so there is less beany flavor. In this process, the undesirable enzymes have been destroyed by heating, and the product will have low PDI (20–30). There are different methods that can be used to heat the soybeans, like infrared radiation. This type of full-fat soy flour is used in bakery products, soy breads, cakes and doughnuts, sweet goods, chocolates and confectionaries, spreads, and wafer filling.

Extruder-Processed Full-Fat Soy Flour and Grits (Enzyme-Inactive)

Extruder-processed full-fat soy flour is processed using wet or dry single-screw extruders. After cleaning, drying, and dehulling, soybeans are coarse ground to be processed in the extruder (as discussed earlier). In this process, a single-screw extruder (wet or dry) is used to cook the soybeans. Extruded soybeans are then ground with a grinding mill to make full-fat flour. This type of flour is used in soup or gravy and some other foods in Brazil and Argentina.

Enzyme-Inactive Low-Fat Soy Flour or Grits

This type of flour is also called 'mechanically processed soybean flour.' In this process, soybeans are cleaned, dried, dehulled, and cracked (as discussed in the preceding text) and then fed to a dry extruder. The extrudate exiting the extruder is in a semifluid and frothy state, while the oil is free within the matrix. The soybean extrudate is fed immediately into a screw press, where the oil is pressed out. The protein-rich meal that comes out of the press in the form of press cake is delumped by using a roller mill and is passed through a meal cooler. After cooling, this material is ground into flour using pulverizer. This type of flour will have 6–8% oil depending upon the processing condition. This flour can be used in bread and bakery and in making textured soy protein.

Enzyme-Active Flake/Defatted Soy Flour or Grits (90 PDI)

This type of soy flake/flour is produced using solvent extraction process. After cleaning, drying, and dehulling (as discussed earlier), the next steps are conditioning and flaking.

Conditioning

Cracked and dehulled soybeans are conditioned in cookers to approximately 70 °C with steam. Small amounts of water can be added to adjust the moisture to approximately 11%, which makes the beans ideal for flaking. A correct heat treatment and adjustment of the moisture will assist in disrupting the cell membranes during flaking so that the oil can be extracted more easily. The main function of the conditioning is to facilitate the flaking step.

Flaking

In order to extract the oil from the beans, it is important to destroy the cell structure of the beans. To achieve this, beans are flaked in the flaking machines, since solvent can flow much more readily through a bed of flakes than through a bed of soy meats or fine particles. The ideal thickness of a flake is in between 0.25 and 0.35 mm. The thickness of these flakes depends upon the size of the cracked beans, conditioning, and the adjustment of the flaking rolls.

Expanding of flakes

Some soy processing facilities started using an expander–extruder after the flaking step and before the extraction. The use of expanders was originally developed in Brazil and was introduced in the United States in early 1980. According to Watkins et al., the use of expander offers several advantages in processing soybeans for extraction:

- The seed is finely homogenized in the expander, freeing the oil for rapid extraction.
- Dense collets are formed (weigh more per cubic foot).
- The collets are porous and do not restrict percolation of solvent through the extractor bed, as it may occur with fragile flakes.
- More oil is recovered by solvent from collets as compared to flakes.
- The solvent drains more completely from the extracted collets, resulting in more complete removal of oil.
- Less energy is required for desolventizing the collets compared to flakes.

Extraction of flakes

Soy flakes are fed into a flash desolventizer or vapor desolventizer. In the flash desolventizer, solvent wet flakes go directly from the extractor into superheated vapor blown at high velocity through a long tube. Solvent is heated under pressure to 115–138 °C. As this vapor is blown through the tube, flakes are conveyed in and picked up by the vapor stream. In short seconds, the superheated solvent will flash off the residual liquid solvent leaving desolventized flakes to be collected in the cyclone. High-PDI soy ingredients are more soluble, but at the same time, they also have highly active enzymes and antinutritional factors. These flakes will have 95 PDI. Defatted flakes can be milled using a hammer mill to produce soy flour. At least 97% of the flour must pass through a US Standard No. 100 sieve. This flour contains 52–54% protein as is. This type of flour is used in bakery application as crumb whitener or dough conditioner. The use of this flour is very limited (0.5%) because of enzymes and beany flavor.

Enzyme-Inactive Defatted Soy Flake/Flour or Grits (70 PDI)

This type of soy flake/flour is produced by using the same method as described in the preceding text (enzyme-active), except that solvent extraction is done at higher temperature. This temperature deactivates the lipoxygenase enzymes and produces defatted soy flake/flour, which is enzyme-inactive and had a PDI of 70. Typical applications of this flake/flour are waffle, pancake mixes, bread, doughnuts, tortilla, and bagels. This flake/flour can be used in bakery products up to 5% of the dry ingredients.

Enzyme-Inactive Defatted Soy Flakes/Flour or Grits (20 PDI)

This type of soy flour is produced by using the same method as described earlier in 70 PDI, except this flake/flour is further toasted after the solvent extraction to reduce the PDI to 20. Typical application for these types of flakes/flours is various bakery applications like biscuit and milk replacer up to 5% of the dry ingredients.

Textured Soy Flour

Defatted soy flour of 70 PDI is processed through a thermoplastic extrusion process to produce textured soy flour. This type of flour is used in processed meats, pizza topping, hamburger patties, tacos, etc. The size of the textured flour will depend upon the application and desired texture. After texturizing the flour, it can be processed through the Comitrol mill to produce different sizes.

Lecithinated Soy Flour

This type of flour is produced by spraying lecithin/vegetable oil onto 70 and 20 PDI soy flour. The most common ratios used in this type of flour are 3%, 6%, and 15% lecithin by weight. This type of flour is a good emulsifier and can be used for partial or total replacement of egg. It improves dispersion of the flour and other admixed ingredients in confection and cold beverage products. It also can increase the water retention in the bakery good and extend the shelf life. Also, this is a good animal protein substitute for use in vegetarian cookies.

Refatted Soy Flour

This flour is produced by adding 1–15% fat back into flour for certain application, mainly to reduce the dustiness and provide fat for a product formula.

Production of Soy Protein Concentrates

Soy protein concentrates are processed selectively by removing the soluble carbohydrates from soy protein flour by either aqueous alcohol or isoelectric leaching. Soy protein concentrates contain at least 65% protein. Defatted flakes or soy flour is used as starting material. Soy protein concentrates contain at least 65% protein. During processing of soy concentrates, objectives are to immobilize the protein while leaching away the soluble, removing the strong flavor components and the flatulence sugars (stachyose and raffinose). In turn, both protein and dietary fiber contents are increased.

There are several different methods to produce soy concentrates:

Extraction of flakes with aqueous 20–80% ethyl alcohol
Acid leaching of flakes or flour
Denaturing the protein with moist heat and extraction with water

Alcohol extraction is considered to produce the blandest products. Mild heat drying conditions are used in an acidic water extraction process to retain high PDIs. Isoflavone content in soy concentrate depends on whether it has been water-washed or alcohol-washed, as isoflavones are soluble in alcohol. Because of its blander flavor, soy concentrate is often preferred over soy flour. Typically, soy concentrates are used in meat patties, pizza topping, bakery products, and meat sauces.

Textured Soy Protein Concentrates

Soy protein concentrates are texturized by using a wet extruder. The size of the textured concentrates depends on its application. After, extrusion-textured soy protein concentrates can be converted into different sizes using size reduction equipment like Comitrol. Typical application of textured soy protein concentrate is in meat products, like patties, sausages, and pizza topping.

Production of Soy Protein Isolates

There are several ways that have been described for manufacturing isolated soy protein. However, the only commercial procedure currently being used is extraction of defatted soy flakes or soy flour with water, followed by centrifugation. In this process, the protein is solubilized at pH 6.8–10 at 27–66 °C by using sodium hydroxide and other alkaline agents approved for food uses. The protein solution is then separated from the flakes or flour by centrifugation. The solids are recovered as a by-product, containing 16–36% protein, 9–13% crude fiber, and 45–75% total dietary fiber when dried to 6–7% moisture content and have been marketed for food use. The solution is then acidified to pH 4.5 by using hydrochloric or phosphoric acid, and the protein is precipitated as a curd. The curd is then washed with water and concentrated by centrifugation and can be neutralized to pH 6.5–7.0 or spray-dried in its acidic form.

Soy isolates, the most concentrated form of soy protein (90% dry bases) available, generally contain about half as many isoflavones as soy flour as a result of losses during processing. Functionally, isolated soy protein has some of the same attributes as soy flour and concentrates but normally is utilized for their higher solubility, whippability, foaming, and binding functionality for a number of food products including meat.

Soy Germ

Soy germs are mechanically separated from the soybeans during the process of dehulling and cracking. Several companies use their patented technology to separate the germ. These germs are then roasted, milled, and converted into different forms, like flour and granules of different sizes. These germs are considered a very rich source of isoflavones and other nutrients. On an average, these germs will have 40% protein, essential fatty acids, omega-3 and omega-6, lecithin, vitamin E, and saponin. Soy germ is by far the most nutrient-dense soy ingredient one can consume to obtain all of the proved health benefits of the soybean.

Isoflavones

Isoflavones currently receive much attention from the scientific world, so consequently, the industry takes up the opportunity to develop unique products that offer consumers an alternative to consuming traditional amounts of soy. Isoflavones are found in high amounts in soybeans. Genistein and daidzein are the major isoflavones found in soybeans. In whole soybeans, the isoflavones are present in a concentration of 0.2–3.0 mg g^{-1}. Soy experts recommend between 30 and 50 mg of isoflavones per day. One would need to consume 20–150 g of soy foods, 200 g or almost 7 ounces of tofu, or 3 cups of soy milk daily to get the recommended amount of isoflavones. Isoflavones are available in several different forms, crunchy granules, flour, and fine powder, can be labeled as 'soy isoflavone concentrate,' which can be incorporated in cereals, snacks, cookies, breads, health bars, drinks, and meat replacements.

Processing of Isoflavones

The concentration of isoflavones in soy foods and soy ingredients depends upon the (1) genetics of soybeans, (2) environment where soybeans are grown, and (3) processing technologies. The level of isoflavones in soybeans, even from the same field, grown all in the same year, may vary in concentration by a factor of five. There are two methods to extract isoflavones from soybeans: (1) chemical extraction and (2) mechanical extraction.

Chemical Extraction of Isoflavones

Material from the solvent extraction process (discussed earlier), when converted into soy concentrates and isolates (discussed earlier), generates a by-product called soy molasses. These molasses are a rich source of isoflavones. In conventional process for the production of a soy protein concentrate in which soy flakes or flours are extracted with an aqueous acid or an aqueous alcohol to remove water-soluble materials from the soy flakes or flour, a large portion of the isoflavones is solubilized in the extract. The extract of water-soluble material including the isoflavones is soy molasses. These isoflavones are chemically extracted by using patented processes by different food companies. The soy molasses is a by-product material in the production of soy protein concentrate that is typically discarded.

Mechanical Extraction of Isoflavones

In this process, soybean germs are mechanically separated from the cotyledons as discussed in the preceding text. These soy germs have five to six times more isoflavones than the rest of the seed. On an average, 400 pounds (181 kg) of soybeans will produce 1 pound (0.45 kg) of germ. Some companies sell these soy germs as a source of mechanically extracted isoflavones to be used in food application, like cereal, bars, supplements, and beverages.

Soy Fiber

Two types of edible fiber are produced from soybean processing operations: hulls and soy cotyledon fiber.

Soy Hull as a Source of Fiber

The soybean hull is also known as the seed coat. On a dry weight basis, hulls constitute about 8% of the total seed, depending on variety and the seed size. In general, the larger the seed, the lower the proportion of hulls. The hull of a dry mature soybean contains about 7–8% moisture. Dry soy hulls contain approximately 85.7% carbohydrates, 9% protein, 4.3% ash, and 1% lipids. Kikuchi et al. studied the chemical and physical properties of soybean cell wall polysaccharides and their changes during cooking. They found that the soy cell wall contains about 30% pectin, 50% hemicellulose, and 20% cellulose. Therefore, most soybean carbohydrates fall into a general category known as dietary fiber. The outer hull is an excellent source of dietary fiber (6 g fiber per 1 cup cooked).

Soy fiber (also called soy bran) is produced from the hull of the soybeans. Until recently, soybean hulls were considered a by-product of soy processing. After separation from seeds during rolling or flaking, they are toasted and ground and then blended back into defatted soy meal to make a meal containing 44% protein. Recently, several new uses of soy hulls have been explored, with an emphasis on use in human food.

During soybean processing operation, the hulls are removed, washed, sterilized, dried, toasted, and ground to different sizes depending upon the applications, such as breads, cereal, and snacks. The natural grittiness of the product typically requires fine grinding. Soy fiber contains approximately 38% crude fiber and 76% total dietary fiber. The fiber components in soybean hulls and in the cotyledon are distinct and should be considered separately.

Soy Cotyledon Fiber

During processing, beans are carefully selected for color and size. These beans are then cleaned and conditioned (to make removal of hulls easier), cracked and dehulled, and rolled into flakes. The flakes are subjected to a solvent bath that removes the oil. The solvent is removed and the flakes are dried, creating the defatted soy flakes that are the basis of all soy protein products.

The production of soy protein isolate involves the removal of soluble material from defatted flakes by aqueous leaching. In the process, part or all of the protein fraction is solubilized, usually at alkaline pH, and the insoluble protein separated by centrifugation. These solids are analogous to the okara product from making soy milk or tofu, but they differ in that they do not contain hulls and have been defatted and treated with mild alkali. The insoluble residue obtained during protein isolation is made up mainly of various hemicellulose materials. After appropriate purification, this material is dried and sold as soy fiber, in competition with other fibers, such as wheat and oat bran. This soy fiber contains 80% total dietary fiber. The dietary fiber content of soy fiber varies greatly, depending on the method used for analysis.

Several of these products are made during processing of soy fiber, after dehulling and oil extraction, and after the soluble sugars and proteins have been removed. By proprietary processing techniques, the water adsorption/binding can be controlled to make products useful in products like beverages, puddings, and retorted soups or in food systems with less water like baked bread, muffins, crackers, cookies, or breakfast bars. Different soy fibers can be produced and are available for commercial application. But most of the published research is on polysaccharides, a mixture of cellulosic and noncellulosic structural components of the cell wall. Generally, it is thought that soluble fiber is associated with cholesterol lowering and improved diabetic control, whereas insoluble fiber is associated with enhanced bowel function. Soy polysaccharides contain mostly insoluble dietary fiber, and clinical studies have shown it to have properties of both insoluble and soluble fibers.

Soy protein concentrates contain both insoluble and soluble fibers. Therefore, foods made with soy protein concentrates can be good sources of dietary fiber. Unlike meat, milk, fish, and egg, soybeans combine their protein bounty with a good source of dietary fiber in products such as soy flour and textured vegetable protein.

Organic Soy Flour and Concentrates

Recently, organic soy flour and concentrates are available for food application. These soy ingredients are made using solvent-free proprietary and patented methods. There is not enough information available for these processes.

Conclusion

Soybeans can be processed into different ingredients based on their application. In general, there are more than 20 different soy-based ingredients, which are available in the market to be used in food and beverages. Each ingredient processing requires a specific procedure and equipment. Processing will affect the functional properties that are important for the food system. The soy processing industry can make one ingredient with different functionalities and characteristics for different food applications. For example, there are more than ten different soy concentrates available in the market. Each of these concentrates is for special application (dairy, meat, beverage, bakery, meat, pet food, nutritional drink, etc.). The same is true for soy isolates. Food industries can buy soy isolates to make baby milk, for meat emulsion, for breakfast cereal, for energy bars, and for nutritional beverages.

See also: Soy Beans: Dietary Importance; Soy Beans: The Crop; Soy Beans: Properties and Analysis.

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Soy Beans: Properties and Analysis

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Soyfoods Made from Whole Soybeans

Soyfoods (e.g., edamame, natto, and miso) made from whole soybeans (seeds) of the soybean plant (botanical name *Glycine max* (*L.*) Merrill) contain 0.25% calcium, 0.57% phosphorous, 0.01% sodium, 1.50% potassium, 0.02% chlorine, 0.29% magnesium, 0.3% sulfur, 90 ppm iron, 16 ppm boron, 15 ppm copper, 40 ppm zinc, 25 ppm manganese, 1.4 ppm fluorine, 0.5 ppm selenium, 0.05 ppm iodine, 2.5 ppm molybdenum, 6.0 ppm nickel, 6.5 ppm thiamine, 1.0 ppm riboflavin, 18–20% soybean oil, 40–45% soy protein (see in the succeeding text for amino acid profile of the protein), and 35% carbohydrates (see in the succeeding text for sugar profile of the carbohydrate). They also contain ~0.04–0.24% isoflavones (i.e., produced initially to ward off infection challenges by soybean plant diseases during a growing season such as *Phytophthora sojae*). Research indicates significant daily human consumption of soybean isoflavones can increase bone density, thereby lowering risk of osteoporosis; help to lower blood content of low-density lipoproteins; help to prevent prostate enlargement; and reduce the risk of certain types of cancer (e.g., breast cancer, colon cancer, lung cancer, prostate cancer, and uterine cancer). Isoflavones also exhibit antioxidant properties, when consumed.

In 2014, the Food and Drug Administration decided that US consumers' major dietary shortfall of potassium is a public health problem that urgently needs addressing. Because soyfoods are a good source of dietary potassium, an increase in soyfood consumption would help reduce this particular public health concern. An example of the health impact on one US population segment is that only 2.8% of postmenopausal women, ages 50–79, achieve the USDA recommendation that women eat at least 4700 mg of potassium daily. A 2014 American Heart Association study of 90 137 women showed that those who consume >4700 mg of potassium daily are significantly less likely to have strokes and die than women who eat smaller daily amounts.

Other whole-soybean-source soyfoods such as tofu have a variety of additional ingredients added during their manufacture (e.g., to facilitate coagulation of the protein), so they would have even more nutrients/amounts than listed earlier.

Soy Protein (Extracted from the Soybean)

Soy protein is an edible protein (after heat processing) that is produced within soybeans. When removed from soybeans via crushing or extrusion involving adequate heat treatment, soy protein is (historical average) composed of the following amino acids: 2.5% cysteine, 3.4% histidine, 5.2% isoleucine, 8.2% leucine, 6.8% lysine, 1.1% methionine, 5.6% phenylalanine, 4.2% threonine, 1.3% tryptophan, 4.2% tyrosine, 5.4% valine, 4% alanine, 7.7% arginine, 6.9% aspartic acid, 19% glutamic acid, 3.7% glycine, 0.1% 4-hydroxyproline, 5.3% proline, and 5.4% serine.

In its initial form (i.e., following crushing/extrusion from soybeans as described earlier), soy protein is known as soybean meal and contains a bit less than half protein by weight. The carbohydrate portions are 2.5–8.2% sucrose, 0.1–0.9% raffinose, 1.4–4.1% stachyose, 0.6% rhamnose, 0.1% fucose, 0.1% ribose, 2.4% arabinose, 1.0% xylose, 0.9% pinitol, 0.9% mannose, 7.6% galactose, and 8.1% glucose.

Following a sifting process, it is known as soy flour. The most common use of soy flour is for the addition to baked goods to increase their protein content and extend their shelf life. Other uses are in nutrition bars and textured vegetable protein meat extender.

If the soy protein is washed with water (following crushing/extrusion) to remove soluble polysaccharides (e.g., the carbohydrates known as stachyose and raffinose), the resultant soy protein is known as soy protein concentrate and contains at least 65% protein by weight. It is commonly utilized for making textured soy protein concentrate meat substitute added to sausage and comminuted meat products and for making functional soy protein concentrate emulsification-aid ingredient used to stabilize high-fat soups and sauces.

If the soy is washed with water-and-alkali solution, followed by isoelectric precipitation of the soluble protein, the result is 'isolated soy protein.' It is often also called soy protein isolate or soy isolate and contains a minimum of 90% protein by weight. It is most commonly used in the production of high-protein beverages, infant formula, ground meat, meatballs, frankfurters, and bolognas to improve texture and in brines for injected-meat products.

Applicable to Human Nutrition

Soy protein concentrate and soy protein isolate each are a complete (i.e., 'ideal') protein (i.e., one that provides all essential amino acids in diet) for humans. They are also a good dietary source of calcium, with an absorption rate approximately equal to milk.

In 1999, the US FDA approved a (label) health claim that associates the consumption of a minimum of 25 g soy protein per day with reduced blood cholesterol content and with reduced coronary heart disease (CHD) in humans.

Applicable to Animal Nutrition

For animals, the ideal protein concept refers to the amino acid profile of the feed ration (total food) eaten by livestock, poultry, and/or farmed fish. Feed rations that contain ideal protein are those in which the protein – when digested – yields all of the essential amino acids, in proper proportions, for the

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growth and/or bodily tissue maintenance needs of that animal. That can be achieved by mixing soy protein with corn (*Zea mays* L.) in specific varying proportions to match the nutritional needs of the animal by species, age, etc.

Ideal protein varies for different species (e.g., pigs require different protein amino acid profile than chickens do). Ideal protein varies for different stages in the life of a given animal (e.g., poultry require more sulfur-containing amino acids such as methionine during life stages when feather growth is at a comparatively high rate, and dairy cattle require additional methionine during peak milk production period). During those brief time periods, the (extraordinary) amino acid needs of the animals can be met via the addition of small amounts of applicable synthetic amino acids to the daily feed ration.

Research indicates significant daily consumption of soybean isoflavones will increase the lean muscle growth rate of swine.

Animal Feeding Environmental Impact

Feed rations formulated to contain 'ideal protein' have been shown to reduce the amount of nitrogen (nitrates) excreted by livestock and poultry, by as much as 50%. That is because nonideal protein rations result in the removal of amino groups from molecules (e.g., in ration) via the energy-consuming metabolism of 'excess' amino acids eaten by that animal. For example, when livestock are fed with more lysine (amino acid) than their body needs in a given day (i.e., the animal's body can only utilize the essential amino acids in precise amounts/ratios of their daily diet), that excess lysine is metabolized to nitrogen-laden urea and then excreted in the animal's urine.

Conventional Soybean Oil

Soybean oil is an edible oil that is produced within its beans (seeds) by the soybean plant (botanical name *Glycine max* (*L.*) Merrill). When removed from soybeans via crushing and refining processes, soybean oil is (historical average) composed of 60.8% polyunsaturated fatty acids, 24.5% monounsaturated fatty acids, and 15.1% saturated fatty acids. However, soybean varieties have recently been created that possess as little as 7% saturated fatty acids. Soybean oil's individual fatty acid constituents are linoleic (octadecadienoic) acid 53.2%, oleic (octadecenoic) acid 24%, palmitic (hexadecanoic) acid 11.0%, alpha-linolenic (octadecatrienoic) acid 7.8%, stearic (octadecanoic) acid 4.0%, eicosenoic acid 1.0%, behenic (docosanoic) acid 0.5%, arachidic (eicosanoic) acid 0.3%, myristic (tetradecanoic) acid 0.1%, palmitoleic ((hexadecenoic)) acid 0.1%, and margaric (heptadecanoic) acid 0.1%.

In 2014, the US FDA allowed a (label) health claim that associates the consumption of a product serving containing a minimum of 160 mg ALA (alpha-linolenic acid) with being a 'good' source of linolenic acid per the ALA RACC (reference amount customarily consumed) ($\geq$10% of RACC 1.6 g day^{-1}).

Soybean oil also contains 9–12 mg g^{-1} of alpha-tocopherol, 74–102 mg g^{-1} of tau-tocopherol, and 24–30 mg g^{-1} of delta-tocopherol.

Value-Added Soybean Oils

Commercially grown in 2014 were new soybean varieties whose oil contains more than 70% oleic acid, instead of the typical 24% oleic acid content of soybean oil produced from traditional varieties of soybeans. That elevated oleic acid content and the resultant reduced saturated fat content provide an oil that is more heart-healthy to consume and possesses greater heat resistance for longer effective oil product life during frying.

Coming after 2014 were new soybean varieties whose oil contains more than 18% stearidonic acid, instead of the typical 0% stearidonic acid content of conventional soybean oil. Stearidonic acid is a fatty acid naturally produced in the seeds of some plants (e.g., *Nasa carunculata*, *Nasa hornii*, *Nasa* cf. *magnifica*, and *Borago officinalis L.*). When consumed by people, stearidonic acid is partially converted within the human body into the long chain $n-3$ (i.e., 'fish oil-type' omega-3) fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

When fed to applicable farmed fish (grown in aquaculture production), stearidonic acid is partially utilized by the animal for energy and partially converted into EPA and DHA, which are subsequently deposited in the fish meat. In 2004, the US Food and Drug Administration granted a label 'qualified health claim' status to EPA and DHA, for the product label to state 'supportive but not conclusive research shows that consumption of EPA and DHA fatty acids may reduce the risk of CHD.' Research also indicates that human consumption of adequate amounts of EPA and DHA helps to prevent postpartum depression.

The coming stearidonic acid-containing soybean oil will thus be a source of beneficial omega-3 fatty acids without the risk of mercury contamination commonly present in fish meal made from wild-caught ocean fish. This land-source omega-3 dietary alternative will also ease pressure to overfish the wild species of omega-3-containing fish.

See also: Dietary Fiber: Determination; Protein: Food Sources; Soy Beans: Processing; Soy Beans: The Crop; Soybean Oil.

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Soy Beans: The Crop

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Soybean Plant

Soybean (*Glycine max* (L.) Merrill.), a green, bushy legume, is the world's single largest provider of protein for mankind's use. That protein can be consumed directly in the form of soy foods such as tofu, edamame, nattō, miso, and soy flour-fortified baked goods or indirectly in the form of meat, milk, and eggs resulting from the feeding of soy protein to livestock, poultry, or farmed fish. Soybeans are also a major source of edible soybean oil.

This summer annual plant varies in height from less than a foot (0.3 m) to more than 3 ft (1 m) tall. The seeds (soybeans) are borne in pods and, historically, contain 13–26% oil and 38–45% protein (on a moisture-free basis). Its leaves contain some carotenoids (i.e., isoprenic plant pigments utilized by higher animals in the formation of vitamin A), which may be consumed by livestock in the form of soybean hay.

A massive increase in the production and international trade of soybeans during the past 60 years has been a major cause of the world's average human life span increasing from 46.6 to 69.3 years during that time period. Inclusion of adequate dietary protein has had an especially beneficial impact on the average life span in developing countries, because their diets had been universally too low in protein.

Although 45 countries commercially grow at least some soybeans, for a world total of 284 million metric tons (MMT) in 2013, only seven countries produce enough soybeans to consistently have a significant annual exportable soybean surplus. They are Brazil (55 MMT), the United States (48.7 MMT), Argentina (40.7 MMT), Paraguay (7.3 MMT), Uruguay (4.4 MMT), India (4 MMT), and Canada (4 MMT). Those seven countries export soybeans and soybean meal to more than 100 countries.

Soybean Physiology and Production

The soybean plant has approximately 80 000 genes. It is a self-pollinating plant (i.e., the male and female reproductive structures are located within each flower, so monoecious pollination occurs prior to the flower opening). Research indicates that post-opening pollination by bees can help increase yield by reducing the number of aborted flowers, that is, those that bloom but subsequently fail to become soybean pods.

Soybeans are planted in the spring after topsoil warms up to consistently greater than 50 °F, or they can be planted in midsummer after a wheat/barley or vegetable crop has been harvested from the field (in the southern half of the United States or northern half of South America). Shortly after planting, the soybean germinates and its roots produce isoflavone compounds to signal and attract *Rhizobium japonicum* bacteria to live symbiotically within the nodules on the soybean plant's roots where the *Rhizobium* 'fix' nitrogen from the air, thereby providing natural nitrogen-source fertilizer needed by the crop. In return, the soybean plant manufactures sugars needed by the *Rhizobium* bacteria and provides those sugars to the nodules in its roots. Soybean plants produce flowers 6–8 weeks after planting and can continue adding flowers (each capable of becoming a soybean pod) for up to an additional 6 weeks.

Conventional breeding of soybean varieties had slowly increased the genetic yield potential of the US soybean crop during 1945–95. As a result of US farmers' post-1995 adoption of new soybean production technologies such as biotechnology-derived varieties (now >94% of US-planted area), precision agriculture (also known as site-specific crop management), 'no tillage,' and other conservation tillage practices (in which herbicides are utilized for weed control instead of mechanical tillage/disturbance of soil), the US national soybean crop average yield reached a record-high 47 bushels per acre (3 MT ha^{-1}) in 2014. Other nations that have adopted fewer of these new technologies have proportionally lower yields on comparable soil/climate fields.

The biotech herbicide-tolerant varieties enable greater weed control compared to conventional soybean production. These new soybeans enable crop rows to be planted closer – as close as 7 in. instead of the conventional 30 in. – because the farmer no longer needs room between rows for the mechanical tools of weed control. That closer crop row spacing also conserves field soil moisture and provides additional natural weed suppression, by shading the ground between plants.

In precision agriculture, the required application amounts of inputs such as seed, fertilizer, and pesticides are minimized via satellite-guided steering and automatic nozzle shutoff avoiding double planting/spraying of field headlands where the equipment is turned around in order to head back down the field each pass. According to the 2008 research, the average input-reduction savings achieved by adopters of precision agriculture at that time were $19 per acre for corn, $18.50 per acre for soybeans, and $39 per acre for cotton. In addition, for soybeans, avoiding the double planting of field headlands results in an *increase in yield per acre* (versus double-planted field areas). The 2009 research by Ohio State University compared a nonprecision self-propelled sprayer using foam markers to a satellite RTK-guided sprayer possessing satellite shutoff individually controlled sprayer nozzles. On fields with no obstructions such as waterways, it found the former to waste two or three field passes versus the latter. That equated to eight wasted (i.e., overlapped) acres on a 100 acre field. The wastage, and thus the concomitant savings achieved via precision agriculture, was even greater on fields with waterways or other natural obstacles.

Farmers using the no-till method of production plant soybean seeds directly into the soil, through the previous crop's dead plant residue. This reduces the need for fuel-consuming deep plowing and multiple cultivation tillage operations with

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heavy equipment, which reduces field greenhouse gas emissions by approximately 88%. The reduction in plowing/cultivation greatly diminishes field soil erosion and soil moisture loss during the growing season. Because the plant residue from the previous crop is left on the field surface, it also captures drifting snow during the winter months in northern latitudes and holds it so that snowmelt soaks into the field's soil when it melts in the spring. The plant residue is eventually consumed by the field's subterranean earthworms and microbes, so it eventually passes into the soil in the form of organic (humus) soil material. Another result of the soil moisture conservation and snow-capture impacts of no-till soybean production has been a slight expansion of the soybean-planted areas into adjacent regions that were previously considered too arid for soybeans. Soybeans now reliably produce an economically viable yield of one metric ton (37 bushels) per acre in dry US regions where total annual precipitation (rainfall plus snowmelt) is only 18 in. These developments have, in their aggregate, resulted in US soybean production now being sustainable (i.e., able to produce at this level essentially forever without any degradation of fields).

Coming in the future will be new drought-tolerant soybean varieties that hold the potential to significantly expand the world's geographic areas on which soybeans are produced. Some of the strategies being pursued to create these new soybean varieties are

- insertion into soybean plant of the HAHB4 gene from the sunflower (*Helianthus annuus* L.) plant, resulting in the soybean plant tolerating drought stress longer before yield loss occurs;
- insertion into soybean plant of the flavodoxin gene, resulting in the soybean plant tolerating drought stress longer;
- insertion into soybean plant of a promoter to increase expression of the gene for aldehyde dehydrogenase, an enzyme that detoxifies (metabolites incurred via drought stress);
- breeding for enhanced leaf survival so that the crop can survive intermittent severe drought stress and still produce a harvestable yield (this has involved selecting for those with extreme low epidermal (leaf) water conductance, moderate osmotic adjustment, and low critical relative water content);
- breeding for the *slow-wilting* trait (in which higher ascorbic acid content of leaf stomata cells results in them closing faster in times of water stress to minimize leaf water loss);
- breeding for increased pinitol (a cyclic-molecule carbohydrate) content in leaves, which enables photosynthetic activity to continue longer during times of drought stress.

The likely regions for significant overseas expansion of soybean-planted area, following commercialization of the coming drought-tolerant soybean varieties, will include northern Australia, Ethiopia, Kazakhstan, Mozambique, northwest Russia, Sudan, and Tanzania.

Following harvest, soybeans are sold by grade. In the United States, soybean grade criteria are overseen by the US Department of Agriculture (USDA). After cleaning, US grade No. 1 soybeans are processed directly into soy foods such as tofu, miso, nattō, and edamame. They cannot contain more than 1% foreign material (e.g., weed seeds and dust), more than 2% damaged kernels, or greater than 10% split kernels. That is because those could potentially result in the production of undesirable flavors in the resultant soy foods.

US grade No. 2 soybeans cannot contain more than 2% foreign material, more than 3% damaged kernels, or greater than 20% split kernels. US grade No. 3 soybeans cannot contain more than 3% foreign material, more than 5% damaged kernels, or greater than 30% split kernels.

US grade Nos. 2–4 soybeans are typically crushed under high pressure at an elevated temperature to separately yield soybean oil and soybean meal/flour. The heat inactivates antinutritional factors (e.g., trypsin inhibitors) that are present within raw soybeans. Heating of soybeans to a temperature of 212 °F (100 °C) for 15 min causes these trypsin inhibitors to be rendered inactive in the resultant soybean meal/flour, so digestion by human/animal is unimpeded.

See also: Cellulose; Chlorophyll; Colors: Properties and Determination of Natural Pigments; Fatty Acids: Fatty Acids; Oxidation of Food Components; Phospholipids: Properties and Occurrence; Phytic Acid: Properties, Uses, and Determination; Proteins: Chemistry, Characterization, and Quality; Soy Beans: Processing; Soy Beans: Properties and Analysis; Soybean Oil.

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Soybean Oil

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Historical

The soybean (*Glycine max*) was domesticated in China and has spread from there, first to neighboring countries and later all over the world. The bean was used to produce soya sauce, tempeh, and soymilk, which is also used to make tofu. Soybean oil production did not start until solvent extractors became available. In a patent disclosing the production of lecithin (German Patent 382912) that was granted to Hermann Bollmann in 1923, the inventor describes how he extracts soybeans with a mixture of 75 parts of benzene and 25 parts of alcohol. The extraction is continued for about an hour and he then removes the solvent from the miscella. He warms the evaporation residue to 103 °C and introduces steam for a quarter of an hour. This causes lecithin to precipitate.

The interesting thing about this patent is that Bollmann used 1000 kg of soybeans. They were apparently available when he applied for the patent in Germany in 1921. In the United States, Francis Turner also applied for a patent (US Patent 1,448,581) for a water-degumming process of crude vegetable oils "such, for instance, as cottonseed, cocoanut, sesame and peanut oils." Turner did not mention soybean oil, but in 1923, the first soybean extraction plant was opened in the United States by the Monticello Cooperative Soybean Products Co. in Illinois. It used benzene and was the first solvent extraction plant in the United States.

In 1935, the main soybean producer was Manchuria; it produced five times the amount grown in the United States and exported it to Europe. In Belgium, the oil mill Vandemoortele that originally crushed flaxseed started to use soybeans from Manchuria when moving into the realm of edible oils in the 1930s but had to stop production during WWII due to the lack of raw materials. Soybean extraction was resumed after the war but another oil mill, De Smet, was less lucky. Its extractor did not survive the war so the owner designed and built a new one himself, which was the start of the current engineering company Desmet Ballestra.

After WWII, the United States became the major producer of soybeans, and soybean oil became the most important oil, not only in the United States but also worldwide. In the 1970s, soybeans started to be grown in Brazil, Paraguay, and later Argentina so that at the time of writing, South America produces more soybeans than the United States – 157 million tons versus 108 million tons for the United States of a global production of just over 300 million tons. In 2006, soybean oil production was overtaken by the production of palm oil.

Because the soybean is such an important crop in the United States, it has been and continues to be the subject of intensive research and development. This R&D covers plant breeding, genetic modification, oil extraction, refining and modification processes, oil meal utilization, oil properties and use in food products, and so forth.

Oil Production

Soybeans contain about 18–21% oil and 38–44% protein on a moisture-free basis. This explains why soybeans are primarily grown for the meal rather than for the oil. In this respect, soybeans differ from all other oilseeds. Sunflower seed and rapeseed are grown to provide oil. They also provide a meal, but the value of the oil produced from these latter seeds is much higher than the value of the coproduct meal. For soybeans, the meal represents 60–70% of the total returns.

It is therefore the demand for meal that determines how many soybeans are planted. This determines in its turn how much soybean oil will be available. This amount of oil is less than global demand for edible oil, so other crops will have to meet this demand and this is the role played by palm oil since this oil does not have meal as a coproduct. Of course, there are other oils beside soybean oil and palm oil, and the production of most of these oils also entails the production of meal, but several of these oils are local like cottonseed oil, sesame seed oil, and groundnut oil.

Producing palm oil costs a certain amount of money, and except when there is a shortage of oil, this cost price sets the price for other vegetable oils including soybean oil. Producing soybeans also costs a certain amount of money, and given the price of soybean oil, the value of meal can be calculated as a difference. In other words, world demand for meal determines the amount of soy, and together with the world demand for oil, it also determines the amount of palm oil produced. The cost price of palm oil determines the cost of the (soybean) meal.

The situation is more complex since the oil palm goes on producing once it has started growing fruit bunches, and palm oil also has a meal coproduct in the form of palm kernel meal. There are also other oilseeds and vegetable oils. One of these oils is olive oil, an oil that leads a life of its own. It contributes to the global availability of vegetable oils, but it is expensive to produce. So, the amount produced reflects what some people are willing to pay for a specialty or gourmet oil. Another oilseed is cottonseed. This is a by-product of growing cotton. Given the prices of oil and meal, it is profitable to mill this by-product and sell the resulting oil and meal, but that only affects the amounts of soybean and palm oil produced but not their interdependence. It is this interdependence that causes more palm oil to be needed when a European government decides to subsidize the use of rapeseed oil for the production of biodiesel.

Because its use in poultry farming requires a high (>48%) protein content and a low (<3.5%) fiber content, soybeans are often dehulled before being extracted. In the classical dehulling process, the beans are dried and tempered in intermediate storage. This makes the beans shrink away from the hulls so that after cracking, the hulls can be separated from the cracked cotyledons by winnowing. In the so-called 'hot dehulling' process, the beans are conditioned to a temperature of

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65–70 °C in a tower in which tubes that are heated with low-pressure steam heat the beans quite quickly. This and the subsequent superficial heating in a fluid bed dryer cause the hull to shrink away from the cotyledon. Cracking leads to a mixture of separate hull fragments and cracked cotyledons that are separated in cascade aspirators.

For the cracking process, two or three sets of superimposed fluted rolls are used wherein opposing rolls run at different speeds to grip the beans. Cracking should aim at breaking the bean into four to six smaller pieces and avoiding fines and mashed beans. Subsequently, the cracked cotyledons are flattened into flakes of some 0.25–0.30 mm thickness between polished flaking rolls.

Processing the flakes in an expander is optional. The expander is a kind of extruder in which the flakes are compressed until on leaving the die, the compacted mass expands, causing further cell rupture. The resulting collets have the advantage that they have a higher bulk density than the flakes, so expanders are often used to increase the capacity of an existing oil mill. Collets also have the advantage of a reduced liquid holdup, which saves energy in the desolventizer.

The extraction solvent used is invariably hexane. Industrial hexane is a mixture of alkanes with a boiling range of 66–69 °C comprising *n*-hexane (~62%), methylpentanes (~23%), and methylcyclopentane. It is highly inflammable, and its explosive range in air is 1–8% by volume. Consequently, extractors are operated at a slightly subatmospheric pressure to prevent the hexane from escaping.

Solvent extractors operate countercurrently to minimize the oil content of the solvent-wet extraction residue, or marc, leaving the extractor, and maximize the oil content of the miscella, the solution of oil in hexane. Two types of extractor are in industrial use: percolation and immersion. In the immersion type, the screen under the material being extracted has so little open area that it constitutes the greatest flow restriction. Immersion extractors provide more contact time between solvent and seed material but require more draining time than percolation extractors. Both types ensure a miscella strength of around 30% oil and a residual oil content in the meal of <1 wt%.

The marc leaving the extractor is then treated to remove the solvent, first by indirect heating and then by heating with live steam. This raises the temperature of the meal to 105–100 °C in the toasting section where antinutritional enzymes like urease and trypsin inhibitor are denatured. Since the live steam causes the moisture content of the meal to increase, the meal has to be dried. Air is used to dry the meal to a residual water content of 13–14 wt% and cool it down at the same time.

The hexane-laden vapors leaving the desolventizer are at atmospheric pressure. Using their latent heat to evaporate the hexane from the miscella requires the latter to be under a reduced pressure; in industrial practice, this pressure is about 500 hPa absolute. The latent heat content of the vapors leaving the desolventizer upon evaporation is <90% of the hexane present in the miscella so that the composition of the liquid leaving the first evaporator is around 80 wt% oil. It is further concentrated using steam and finally treated in a steam stripper operated at 200–400 hPa absolute to remove the last traces of hexane.

If the oil has to be shipped, it must be degummed with water to prevent a deposit being formed during transport and storage. In this water-degumming process, an amount of water that is about the same as the gum content of the oil is mixed with the hot oil, allowed to hydrate the mucilaginous matter, and then separated by a centrifuge. Drying these gums yields lecithin.

However, this lecithin may originate from soybeans that have resulted from genetic modification, and some food manufacturers have cashed in on the irrational and emotional fear among some consumers in Europe of anything to do with GMOs. They substituted soy lecithin by sunflower seed oil lecithin and charge more for their products, which they can now promote as GMO-free. This has affected the demand for soy lecithin.

Oil Refining

Crude soybean oil, the evaporation residue of the miscella, has a phosphatide content of 2–4 wt%. Water degumming can reduce this content to 0.5% or less but the lecithin market is too small to absorb all the dried gums. This means that they have to be disposed of in another way. One way is to mix the gums with the meal and sell them at meal value, but a more profitable way may be the enzymatic hydrolysis of the gums. Figure 1 shows the molecular structure of the phosphatides and which bonds are susceptible to which phospholipase.

If phospholipase C is used to catalyze this hydrolysis, the reaction products are diglycerides and phosphate esters of choline, ethanolamine, and inositol. The first phospholipase C that was commercially available was specific for phosphatidylcholine (PC) and phosphatidylethanolamine (PE), but since then, a phospholipase C has been developed that catalyzes the hydrolysis of phosphatidylinositol (PI) into a diglyceride and inositol phosphate. These hydrolysis products do not retain any oil, and the diglycerides contribute to the neutral oil yield. If the enzyme is a phospholipase A_1 or A_2, the reaction products are lysophosphatides or ultimately glycerol phosphates and free fatty acids. These enzymes catalyze the hydrolysis of all vegetable phosphatides and also reduce oil retention by the gums.

If crude oil is used as the substrate for this enzymatic hydrolysis, the resulting oil will have a residual phosphorus content that is close to the phosphorus content that would have resulted from water degumming. This means that the oil has to be neutralized by alkali refining. If the enzymatic hydrolysis is preceded by an acid treatment to decompose the nonhydratable phosphatides, the residual phosphorus content could be sufficiently low to permit physical refining. However, the yield advantage of physical refining over alkali refining is small for soybean oil because of its low free fatty acid content.

The alkali refining process that is used in the United States is the so-called long-mix process. In this process, dilute lye (14–18 °Bé or 130–180 g NaOH per l of water) is added and mixed into cold oil. Then, the oil is heated until a clear separation between oil and soapstock is obtained. The soapstock is removed by centrifugal separator, and to lower the residual soap content, the oil is either washed with water or treated with silica hydrogel. The soapstock can be acidulated by

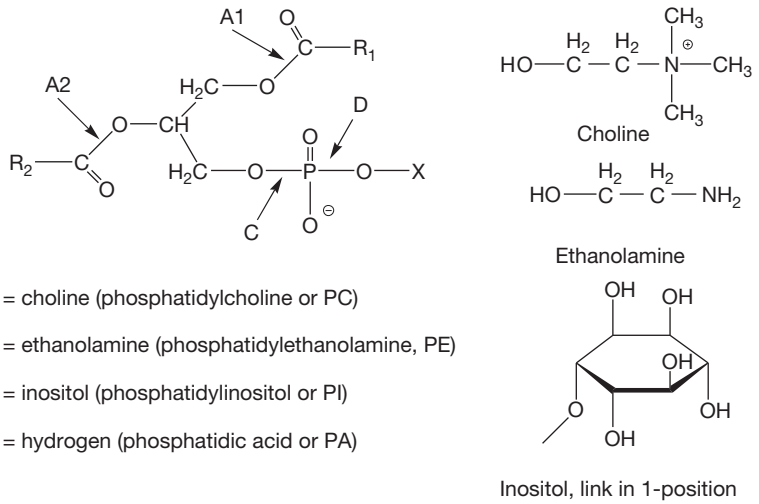

Figure 1 Molecular structures of phosphatides and their substituents.

adding sulfuric acid and collecting the supernatant layer of acid oils or sold as a feed ingredient. The acid oils can be sold as a feed ingredient or as raw material to the oleochemical industry or biodiesel industry.

After having been neutralized or degummed to <10 ppm phosphorus, the oil has to be bleached. The bleaching process achieves more than just reducing the oil color. It catalyzes the decomposition of peroxides, removes residual soaps and residual phosphatides by adsorption, and also adsorbs colored compounds like chlorophyll. The bleaching process is simple. If the oil temperature is below 100 °C, the oil is heated to this temperature; an amount of, for instance, 0.5 wt% of bleaching earth is added and dispersed in the oil that is kept under a mild vacuum. After some 30 min, the spent earth is removed by filtration, and to reduce oil loss via the spent bleaching earth, the filter cake is blown with steam.

In vertically integrated oil mills that also comprise a refinery, the spent earth can be sent to the extractor. This has the advantage that the hexane-extractable oil is recuperated from the spent earth. Another advantage is that the extracted spent earth can then be sold at meal value. To facilitate transport of the spent earth to the extractor, it can be mixed with some crude oil. Blowing the filter cake with steam can be omitted.

Next, the bleached oil is stripped under vacuum with steam. This process is called either deodorization when the oil is almost neutral by having been alkali-refined or physical refining when the oil has only been degummed. In both processes, malodorous compounds present in the crude oil are removed by volatilization together with other volatile compounds. Examples of these other volatile compounds are free fatty acids and tocopherols.

Although some batch deodorizers are still in use for specialty oils, soybean oil is invariably deodorized in large continuous deodorizers comprising a number of superimposed trays. The oil to be deodorized is first of all deaerated to prevent dissolved oxygen reacting with the oil; then, it is heated by an extensive system of heat recuperation comprising plate heat exchangers where heat is exchanged between outgoing and incoming oil and one or two thermosiphon systems. In the trays, the oil is agitated to ensure heat transfer and removal of volatiles by injecting stripping steam. The amount of steam in weight is small (around 1 wt%), but the volume of this steam is very large because of the low molecular weight of water, the low pressure, and the high temperature. 1 wt% water at vacuum stripping conditions of some 220–250 °C and 2–4 mbar absolute pressure occupies 5000 times the volume of oil.

A very efficient system of volatile removal is the use of a packed column through which the oil trickles downward, while stripping steam moves upward. This counter-current system makes more efficient use of the stripping steam than the cross--current trays. So, less steam is needed, a smaller vacuum pump suffices, and less energy is required to maintain the vacuum.

The vapors leaving the deodorizer pass through a scrubber where they are washed with cooled condensate or they are condensed onto one of two ice condensers operating in tandem. In the case of soybean oil, it may be advantageous to condense the deodorizer vapors in two stages. The least volatile components of this vapor will be condensed in the first, high-temperature stage and provide a condensate that is relatively rich in tocopherols that have a high market value (vitamin E and antioxidants). The second low-temperature scrubber or condenser will take care of the remaining condensables. After the oil has been cooled, it has to be stored and protected from oxidation. A low temperature and nitrogen blanketing help in this respect.

Oil Modification and Food Uses

Three modification processes are commonly used to alter the physical properties of an oil or oil blend: hydrogenation, interesterification, and fractionation. In the case of soybean oil, the hydrogenation process is the most important, but because this process produces *trans* isomers and their content has to be mentioned on the labels of consumer packages, this process has become less prevalent. Formerly, soybean oil (IV = 131) would be hydrogenated to an IV of 110, and the resulting product would be fractionated (winterized) to remove high-melting triglycerides so that the resulting olein

fraction would have a good cold stability at refrigerator temperature and improved oxidative stability making it an ideal salad oil. Now, non-modified oil is used instead.

Formerly, the oil used for margarine production would be hydrogenated in a rather nonselective manner to a melting point close to 40 °C and used for margarine as such. Now, it is more common to blend a hardstock with liquid oil whereby attempts are made to reduce the *trans* isomer content of this hardstock. If the soybean oil to be used in this hardstock has to be *trans*-free, this can only be achieved by full hydrogenation (IV = 0) followed by interesterification of the fully hydrogenated fat with liquid oil. However, the solid fat content profile of the interesterification product differs from what can be achieved by partial hydrogenation. Its crystallization behavior differs and final product properties are different.

Better products are obtained by using hardstocks based on fats that are more saturated like palm oil and lauric oils like coconut oil and palm kernel oil and, especially, their interesterification products. Soybean oil can then be used as the liquid oil in the margarine fat blend.

Composition of Soybean Oil

Crude soybean oil as obtained by miscella evaporation contains many different components. The main component is triglyceride oil or triacylglycerol (TAG). The composition of this oil is determined by its fatty acid composition or, to be more precise, by the fatty acid compositions of the *sn*-1-, 2-, and 3-positions.

Table 1 shows that saturated fatty acids have a strong preference for the outer 1,3-positions and that linoleic acid (18:2) has a certain preference for the middle 2-position. This is a common phenomenon for vegetable oils. In animal fats like lard, the saturated palmitic acid has a preference for the 2-position, and there is a difference between the 1- and 3-positions.

According to Table 1, soybean oil contains 7% linolenic acid. At one stage, this acid was blamed for the flavor reversion and short shelf life of soybean oil. This led to research aiming at selective hydrogenation of linolenic acid and also to plant breeding efforts to reduce the linolenic acid level. Now, we have the impression that yes, linolenic acid is more prone to degradation but not so much more than linoleic acid, which also contains bis-allylic hydrogen atoms in the methylene group in between two double bonds. So, the 54% of linoleic acid contributes much more to the lack of stability of soybean oil than its 7% linolenic acid.

Another reason to change the fatty acid composition of soybean oil by plant breeding and genetic modification is the current *trans* isomer scare. Soybean oil is the main oil in the United States, and it is used in margarines and shortenings after partial hydrogenation. This process leads to the formation of *trans* isomers, especially elaidic acid and its isomers. They provide the oil with the solid fat content profile that is required for these consumer products. In order to produce fats with a similar solid fat profile that contain less or no *trans* isomers, more saturated fatty acids are required; this has been another target in soybean oil modification. Table 2 gives some examples of what has been achieved in this respect.

Another genetically modified soybean could be quite interesting from a nutritional point of view. Its oil contains 28% of stearidonic acid and 8% of gamma-linolenic acid (GLA). Stearidonic acid (18:3 or (6*Z*,9*Z*,12*Z*,15*Z*)-octadeca-6,9,12,15-tetraenoic acid) is the Δ6-desaturase product of linolenic acid, a metabolic step that is difficult in humans and a step in the direction of EPA (eicosapentaenoic acid). The other nutritionally significant acid, GLA (18:3 or (6*Z*,9*Z*,12*Z*)-octadeca-6,9,12-trienoic acid), also bypasses a difficult desaturase step on the way to arachidonic acid, 20:4$\omega-6$.

In addition to TAG molecules, soybean oil also contains some diacylglycerol (DAG) molecules. These have been formed by partial hydrolysis of the TAG. This hydrolysis also generates a free fatty acid. Since the FFA content of crude soybean oil is low in comparison with most other major vegetable oils, the DAG content of soybean oil is also lower.

The next group of compounds present in crude soybean oil consists of the phosphatides. The three main phosphatides are PC, PE, and PI. Their concentrations depend on climatic conditions during seed fill. In addition, lecithin contains some phosphatidic acid (PA) or rather its potassium salt and some acetylated PE. Water-degummed soybean oil contains variable amounts of calcium and magnesium salts of PA and some PE (Table 3).

Table 1 Fatty acid composition of soybean oil by position

	16:0	*18:0*	*18:1*	*18:2*	*18:3*
sn-1 position	16.7	5.4	24.3	46.4	6.4
sn-2 position	2.2	0.3	23.4	68.1	5.7
sn-3 position	16.1	4.6	24.6	47.0	7.0

Source: Table 2.28 in Gunstone, F. D., Harwood, J. L. and Dijkstra, A. J. (2007) (eds.) *The lipid handbook* (3rd ed.). Boca Raton, FL: CRC Press.

Table 2 Fatty acid compositions of modified soybean oils

	16:0	*18:0*	*18:1*	*18:2*	*18:3*
Normal line	11.2	3.4	21.5	55.8	8.0
Low linolenic	10.1	5.3	41.1	41.2	2.2
Low palmitic	5.9	3.7	40.4	43.4	6.6
Low saturates	3.0	1.0	31.0	57.0	9.0
High palmitic	26.3	4.5	15.0	44.4	9.8
High stearic	8.6	28.7	16.2	41.6	4.9
High saturates	23.3	20.0	10.5	39.7	6.5
High oleic	6.4	3.3	85.6	1.6	2.2

Source: Table 2.33 in Gunstone, F. D., Harwood, J. L. and Dijkstra, A. J. (2007) (eds.) *The lipid handbook* (3rd ed.). Boca Raton, FL: CRC Press.

Table 3 Ranges (in percentage of total) of concentrations and fatty acid composition of soybean phosphatides

	PC	*PE*	*PI*
Concentration	35–46	25–27	13–18
16:0 Content	14.9–20.5	15.8–31.6	25.6–47.7
18:0 Content	3.2–6.3	3.2–4.4	8.2–11.7
18:1 Content	7.5–13.7	8.4–8.7	4.9–9.1
18:2 Content	58.8–64.8	53.2–64.7	36.2–44.4
18:3 Content	2.0–6.0	3.2–63	2.7–6.4

The observation that the fatty acids in PI tend to be more saturated than in the other phosphatides is also shown in the stereospecific fatty acid compositions of the triglycerides and phospholipids listed in Table 4.

Because the literature values in the tables in the preceding text refer to different samples, there are some differences between these values. However, they clearly show that the phosphatides are more saturated than the triglycerides and that PI is the most saturated phosphatide.

Like other vegetable oils, soybean oil also contains phytosterols that can be free or esterified with a fatty acid. Table 5 lists their concentrations in the oil.

The data in Table 5 originate from three different sources. The range is from one source, the next column from another, and the last four columns from the third source. They do not contradict each other, and together, they provide a clear picture of the phytosterol content of crude soybean oil. When the oil is refined, the sterol content decreases. It does not decrease during degumming operations, but decreases somewhat when the oil is alkali-refined. Bleaching hardly affects the sterol content, but the vacuum steam stripping process can lead to substantial loss of sterols.

Table 4 Measured values of fatty acid compositions as percentage of total

Lipid	*Position*	*16:0*	*18:0*	*18:1*	*18:2*	*18:3*
TAG	*sn*-1	19	8	25	40	8
	sn-2	3	1	28	61	7
	sn-3	15	6	35	39	5
	Total	12	5	29	47	7
PC	*sn*-1	26	23	7	38	6
	sn-2	4	4	10	71	11
	Total	11	12	9	59	10
PE	*sn*-1	29	17	5	42	7
	sn-2	4	2	9	74	12
	Total	16	8	7	57	12
PI	*sn*-1	45	36	5	17	2
	sn-2	5	3	6	71	15
	Total	22	19	6	43	9

Source: Fig 7.4 in Johnson, L. A., White, P. J. and Galloway, R. (2008) (eds.) *Soybeans, chemistry, production, processing, and utilization*. Urbana, IL: AOCS Press.

Table 5 Phytosterol content of crude soybean oil in ppm

Phytosterol	*Range*		*Ester*	*Free*	*Total*	*% Free*
β-Sitosterol	650–2360	1730	401	1371	1772	77
Campesterol	248–1310	571	56	627	683	92
Stigmasterol	219–770	577	47	549	596	92
Δ5-Avenasterol	27–135	135	86		86	0
Δ5-Stigmasterol	28–150					
Δ7-Avenasterol	20–40					
Total	1210–4050		590	2547	3137	

Source: Table 7.5 in Johnson, L. A., White, P. J. and Galloway, R. (2008) (eds.) *Soybeans, chemistry, production, processing, and utilization*. Urbana IL: AOCS Press; Table 2.47 in Gunstone, F. D., Harwood, J. L. and Dijkstra, A. J. (2007) (eds.) *The lipid handbook* (3rd ed.). Boca Raton: CRC Press; Table 7.5 in Verleijen, T. (2002) *Stability of minor components during vegetable oil refining*, PhD Thesis, Ghent University.

Soybean oil also contains tocopherols but no tocotrienols, which are only found in palm oil and coconut oil. The tocopherol content of soybean oil is given in Table 6.

Again, the table has been compiled using data from different sources. The columns headed Range and Mean refer to the first source and the two columns headed Single refer to two other sources. The tables are internally reasonably consistent and therefore provide a reliable picture of the tocopherol content and composition in soybean oil.

Like most vegetable oils, soybean oil also contains some β-carotene and lutein (xanthophyll), but their concentrations are quite low at only a few ppm. This is very much lower than the carotenoid content of palm oil, which is in the range of 500–700 ppm. Chlorophyll content of soybean oil is also quite low at <500 ppb. This is very much lower than the chlorophyll content of rapeseed oil, which ranges from 5 to 25 ppm, or olive oil, where the range is 10–30 ppm.

Squalene (6*E*,10*E*,14*E*,18*E*)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene, a symmetric triterpene, is a precursor for phytosterols. Soybean oil contains about 7–17 ppm, and because of its volatility, the squalene content of deodorizer distillate may reach a few percent, which may be sufficiently high to warrant its isolation. In comparison with some other oils, the squalene content of soybean oil is on the low side as illustrated by Table 7.

Sphingolipids are a group of nonglyceride lipid components that have stimulated interest in soy foods from a human health perspective because sphingolipid consumption has been associated with decreased colon cancer and skin cancer in rats. Sphingolipids have three characteristic components, one of which is a sphingosine (2-amino-4-octadecene-1,3-diol). The amino group of the sphingosine forms an amide linkage with an α-hydroxy fatty acid such as α-hydroxypalmitic

Table 6 Tocopherol contents of soybean oil (ppm)

Tocopherol	*Range*	*Mean*	*Single*	*Single*
α-Tocopherol	44–158	96	100	143
β-Tocopherol	20–29	11		16
γ-Tocopherol	926–1559	1048	590	870
δ-Tocopherol	254–477	372	260	341
Total	1363–2195	1527	960	1370

Source: Table 7.3 in Johnson, L. A., White, P. J. and Galloway, R. (2008) (eds.) *Soybeans, chemistry, production, processing, and utilization*. Urbana IL: AOCS Press; Table 2.48 in Gunstone, F. D., Harwood, J. L. and Dijkstra, A. J. (2007) (eds.) *The lipid handbook* (3rd ed.). Boca Raton: CRC Press.

Table 7 Squalene content of some vegetable oils (ppm)

Oil	*Squalene content (ppm)*
Olive oil	136–708
Groundnut oil	13–49
Corn oil	19–36
Sunflower seed oil	8–19
Soybean oil	7–17
Cottonseed oil	4–12

Source: Table 3.80 in Gunstone, F. D., Harwood, J. L. and Paley, F.B. (1994) (eds.) *The lipid handbook* (2nd ed.). London: Chapman & Hall.

acid, and the primary hydroxyl group of the sphingosine forms an ether linkage with glucose. So, the resulting glucosylceramide has a strongly polar group, which is why it remains in the defatted soy flakes after extraction.

See also: Choline: Physiology; Enzymes: Analysis and Food Processing; Fatty Acids: Essential Fatty Acids; Fatty Acids: Fatty Acids; Fatty Acids: *Trans* Fatty Acids; Genetically Modified Foods; Genetically Modified Microorganisms; Heat Treatment: Principles and Techniques; Mayonnaise; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Phospholipids: Properties and Occurrence; Rapeseed Oil/Canola; Soy Beans: Processing; Sunflower Oil; Tocopherols: Properties and Determination; Triacylglycerols: Characterization and Determination; Triacylglycerols: Structures and Properties; Vegetable Oils: Dietary Importance.

Further Reading

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Spectroscopy: Types

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Introduction

'The Light of Knowledge' is used often as a phrase, but it is appropriate with reference to spectroscopy. All of us know the structure of atoms and molecules comes from studying their interaction with light. Spectroscopy deals with the study of interaction between matter and radiated energy as a function of wavelength. It deals with the use of visible light dispersed according to wavelength historically, but later on, the concept was expanded to the measurement of the quantity as a function of either wavelength or frequency. It is one of the most important tools in a scientist's toolkit. Astronomers use spectroscopy to study light from different objects in the universe.

Latin: 'spectrum' – ghost or spirit

Greek: 'σκοπειν' – to look carefully

This article gives a brief introduction about spectroscopy and its types. It mainly focuses on the various types of spectroscopic techniques that are commonly used in analytic purposes in diverse fields, especially in food and health sciences. These spectroscopic techniques have been successfully applied in food and health sciences since decades to ensure safety, quality, and traceability of food and provide healthy lifestyle to mankind. The objective of this article is to provide healthy information about the types of spectroscopic techniques to the readers.

Electromagnetic Radiations

Electromagnetic radiation is a type of energy that is commonly known as light. Generally, light travels in waves, and all electromagnetic radiation travels at the same speed, which is about 3.0×10^8 ms^{-1} through a vacuum. Electromagnetic waves are produced by motion of electrically charged particles. The energy propagates as a wave such that the crest and troughs of wave move in a vacuum at a speed of 299 792 458 ms^{-1}. The many forms of these radiations appear different to the observer; light is visible to the human eye, but x-rays or radio waves are not. Some important characteristics of electromagnetic radiations are as follows:

1. These are produced by the oscillation of electric charge and magnetic field. The electric and magnetic components are mutually perpendicular to each other and are coplanar.
2. These are characterized by their frequencies or wavelengths or wave number.
3. All types of these radiations travel with the same velocity and require no medium for their propagation.
4. The energy of electromagnetic radiation is directly proportional to its frequency. The phenomenon of it was given by Planck known as Planck's law, $E = h\nu$.
5. When visible light (a region of electromagnetic radiations) is passed through a prism, it split up into seven colors. This phenomenon is known as dispersion (Figure 1).

Terms related to electromagnetic radiations:

1. *Wavelength* (λ): The distance between two adjacent crests or troughs in a particular wave. It can be expressed in angstrom unit or nanometer ($1\ \text{Å} = 10^{-10}$ m and $1\ \text{nm} = 10^{-9}$ m).
2. *Wave number* ($\bar{\upsilon}$): It is the reciprocal of wavelength and is expressed in per centimeter. It is defined as the total number of waves that can pass through a space in 1 cm.
3. *Frequency* (ν): It is defined as the number of waves that can pass through a point in 1 s. It is expressed in cycles per second or in hertz ($1\ \text{Hz} = 1\ \text{cycle s}^{-1}$).
4. *Energy* (E): Energy of the particular radiation can be calculated by applying the relation $E = h\nu = hc/\lambda$, where h = Planck's constant = 6.626×10^{-34} Js.

Table 1 shows the relationship among various parameters associated with electromagnetic radiations.

Electromagnetic Spectrum

Different regions of electromagnetic spectrum (Figure 2) provide different kinds of information as a result of such interactions. Electromagnetic spectrum comprises all the possible range of electromagnetic radiations. Radiations can be considered as a form of radiant energy that is propagated as a transverse wave. It vibrates perpendicular to the direction of propagation and imparts a wave motion and described in terms of wavelength or frequency. The reciprocal of the

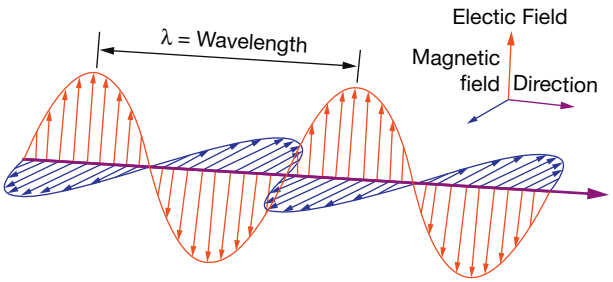

Figure 1 Electromagnetic radiation.

Table 1 Relation among various parameters associated with electromagnetic radiations

Quantity	*Relationship*	*Units*
Wavelength	$\lambda = c/\nu$	m, nm
Wave number	$\bar{\upsilon} = 1/\lambda$	m^{-1}, cm^{-1}
Frequency	$\nu = c/\lambda$	s^{-1}
Velocity	$c = \nu/\lambda$	ms^{-1}
Energy	$E = hc/\lambda$	erg, joules

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00643-7

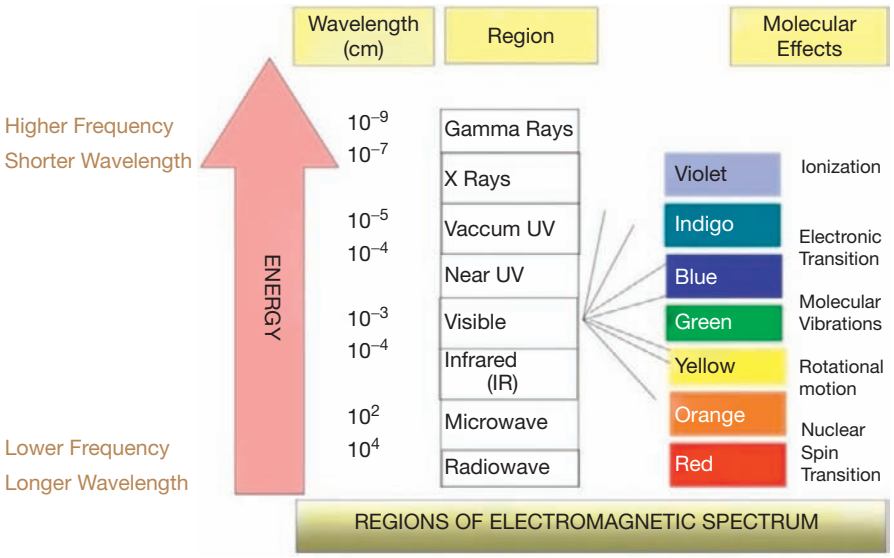

Figure 2 Regions of electromagnetic spectrum.

wavelength is termed as wave number and is the number of waves in unit length.

Spectroscopic Types

1. UV–vis spectroscopy
2. Vibrational spectroscopy
   2.1 Infrared spectroscopy
   2.1 Raman spectroscopy
3. Nuclear magnetic resonance (NMR)
4. Electron spin resonance (ESR)
5. Mass spectrometry

Spectroscopic techniques have been applied in all the technical fields of science and technology. Techniques of spectroscopy are so much sensitive that the single atom or different isotopes of atoms can easily be identified among 1020 atoms or among different species. Trace amounts of pollutants are often detected by these spectroscopic techniques.

UV–Vis Spectroscopy

It involves the promotion of electrons from the ground state to higher energy state, so the alternate name for this is electronic spectroscopy:

- It is useful to measure the aromatic conjugation and the number of conjugated double bonds.
- It also distinguishes between homoannular and heteroannular conjugated dienes.
- Electronic transition occurs in the range of 200–800 nm.

This rests heavily on empiricism, particularly in the study of aromatic and heteroaromatic systems, but it can provide information completely unobtainable from spectrophotometer of any other spectroscopic technique.

Theory of electronic transitions

After absorbing the UV light of a particular energy, electron is promoted to higher energy and the other electrons are unaffected. The excited state is produced in a very short time, and consequently during electronic excitation, the atoms of molecule do not move. Not all the transitions from filled to unfilled orbitals are allowed; some are forbidden. For the forbidden transitions, the probability of the transition to occur is low and the intensity of associated absorption band is also low.

Types of electronic transitions

- $\sigma-\sigma^*$: UV photon, high energy required, and methane absorbing at 125 nm
- $\pi-\pi^*$: Organic compounds, wavelengths 200–700 nm, and requiring unsaturated groups
- $n-\sigma^*$: Saturated compounds with heteroatom containing unshared pair of electrons
- $n-\pi^*$: Unsaturated groups, low epsilon (10–100), and having shorter wavelengths

The $\sigma-\sigma^*$ transition requires an absorption of a photon with a wavelength that does not fall in the UV–vis range. Thus, only $\pi-\pi^*$ and $n-\pi^*$ transitions occur in the UV–vis region (Figure 3).

The absorption law (the Lambert–Beer law)

When a beam of monochromatic radiation passes through a homogeneous absorbing medium, the rate of decrease of intensity of radiation with thickness of absorbing medium is proportional to the intensity of incident radiation as well as the concentration of the solution.

Mathematically,

$$-\frac{dI}{dx} = k'Ic$$

where I = intensity of radiation after passing through thickness 'x', dI = small decrease in intensity of radiation, k = proportionality constant or absorption coefficient, and c = concentration of the solution in moles per liter.

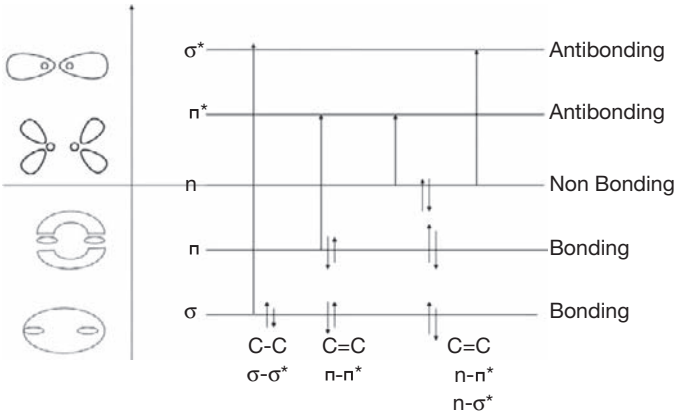

Figure 3 Types of electronic transitions.

The absorbance of radiation can be calculated as

$$A = \varepsilon cl$$

Components of spectrophotometer

A spectrophotometer measures the amount of light that a sample absorbs. The instrument operates by passing a beam of light through a sample and measuring the intensity of light reaching a detector:

- *Source of UV radiation*: It is important that the power of the radiation source does not change abruptly over its wavelength range. The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum. The mechanism for this involves the formation of an excited molecular species, which breaks up to give two atomic species and an ultraviolet photon. This can be shown as

$$D_2 + \text{electrical energy} \rightarrow D_2^* \rightarrow D' + D'' + h\nu$$

- *Sources of visible radiation*: The tungsten filament lamp is commonly employed as a source of visible light. This type of lamp is used in the wavelength range of 350–2500 nm. The energy emitted by a tungsten filament lamp is proportional to the fourth power of the operating voltage. Tungsten lamps contain a small amount of iodine in a quartz 'envelope' that also contains the tungsten filament. The iodine reacts with gaseous tungsten, formed by sublimation, producing the volatile compound WI_2.
- *Cuvettes*: The containers for the sample and reference solution must be transparent to the radiation, which will pass through them. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. These cells are also transparent in the visible region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000 nm.
- *Detectors*: The photomultiplier tube is a commonly used detector in UV–vis spectroscopy. It consists of a photoemissive cathode (a cathode that emits electrons when struck by photons of radiation), several dynodes (which emit several electrons for each electron striking them), and an anode. Photomultipliers are very sensitive to UV and visible radiation. They have fast response times. Intense light damages photomultipliers; they are limited to measuring low-power radiation.

When the sample absorbs light, its intensity is lowered. The photoelectric cells (P1 and P2 in Figure 4) will receive an intense beam from the reference cell and weak beam from the sample cell. It generates alternating current that flows from the photoelectric cell to the electronic amplifier. The amplifier is coupled to small motor that in turn is coupled to pen recorder. Thus, it records the absorption bands automatically. The amplifier coupled with motor drives an optical wedge into the reference beam until photoelectric cell receives light of equal intensities from the sample as well as the reference beam.

UV spectroscopy and food and health

Food is a complex system comprising water, proteins, carbohydrates, and fats, together with many other minor components. Appearance, color, flavor, and texture are important aspects of sensory quality of food. The demand for good quality of food and safety in food production requires appropriate analytic tools to investigate food. UV–visible spectroscopy has been useful in the analysis of food additives, food preservatives, flavors, fat content, enzymes, glucose, flavoring minerals, vitamins, etc. Food additives and preservatives help to keep the product's life span longer and make the product healthier for consumer. The UV–visible spectroscopy has been mainly used to examine the quality of edible oils. It has been used in the determination of anisidine value, peroxide value, and color of the edible oils. In most of the analytic methods, UV–visible spectrophotometer is used as a detector coupled with either GC or HPLC systems.

Vibrational Spectroscopy

Infrared spectroscopy

The 'infrared' covers the range of the electromagnetic spectrum between 0.78 and 1000 mm. Unlike ultraviolet spectrum that consists of relatively few peaks, the IR spectrum contains a large number of absorption bands. These bands provide a wealth of information about the structure of an organic compound. IR radiation causes the excitation of the vibrations of covalent bonds within the molecule. These vibrations include

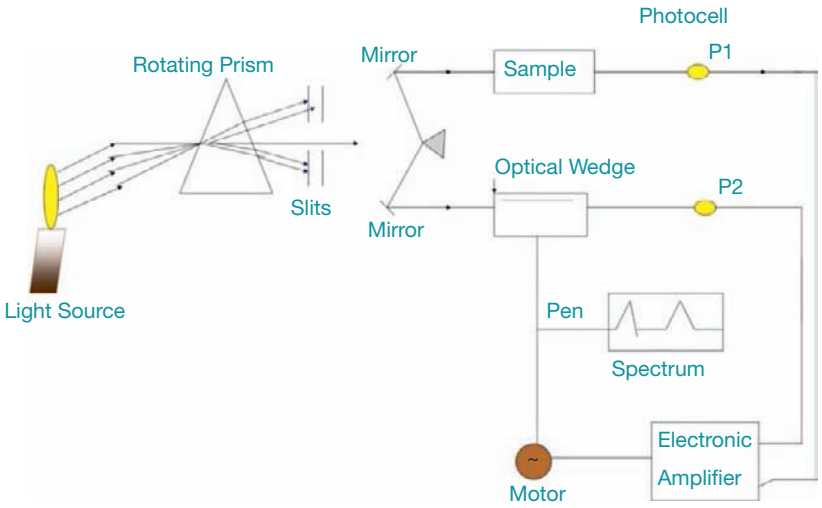

Figure 4 Block diagram of ultraviolet spectrophotometer.

the stretching and bending modes. An infrared spectrum is commonly obtained by passing infrared radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. IR absorption positions are generally presented as either wave numbers ($\bar{\upsilon}$) or wavelengths (λ). For a molecule to show IR absorptions, it must possess a specific feature, that is, an electric dipole moment of the molecule must change during the vibration. This is the selection rule for infrared spectroscopy.

Region	*Wavelength range (mm)*	*Wave number range (cm^{-1})*
Near	0.78–2.5	12 800–4000
Middle	2.5–50	4000–200
Far	50–1000	200–10

Principle

The molecules have to fulfill certain requirements to absorb IR radiations:

- The natural frequency of vibrations of some part of a molecule should be the same as the frequency of incident radiation.
- Its absorption should cause a change in the molecule electric dipole (dipole moment).

Molecular vibrations

Each atom has three degrees of freedom, corresponding to motions along any of the three Cartesian coordinate axes (x, y, and z). Linear molecules possess $3N–5$ fundamental vibrational modes, while nonlinear molecules have $3N–6$ vibrational modes. Among the $3N–6$ or $3N–5$ fundamental vibrations (normal modes of vibration), those that produce a net change in the dipole moment may result in an IR activity, and those that give polarizability changes may give rise to Raman activity.

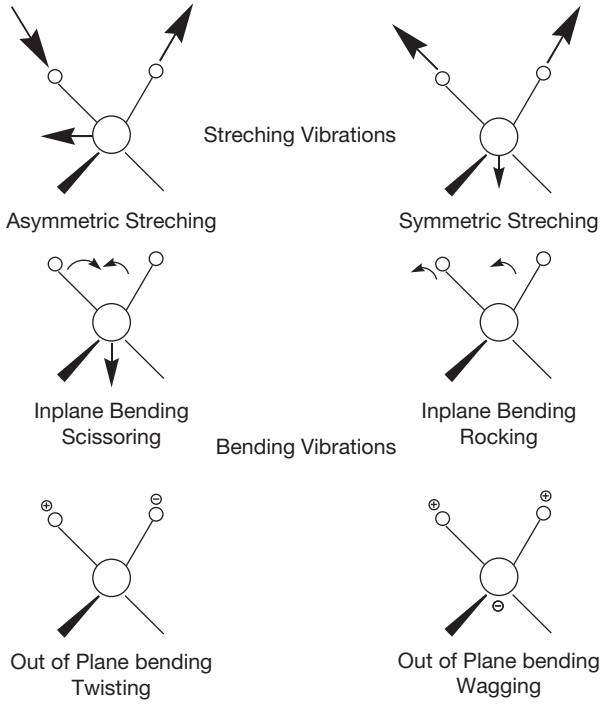

Figure 5 Different types of vibrational modes for a nonlinear CH_2 group.

Naturally, some vibrations can be both IR-active and Raman-active. The major types of molecular vibrations are stretching and bending. The various types of vibrations are illustrated in Figure 5.

Infrared radiation is absorbed, and the associated energy is converted into these types of motions. The absorption involves discrete, quantized energy levels. However, the individual vibrational motion is usually accompanied by other rotational motions. These combinations lead to the absorption bands, not the discrete lines, commonly observed in the mid-IR region. Each of the vibration modes may give rise to a different

absorption band, so that CH_2 group gives rise to two C–H stretching bands at ν_{sym} and ν_{anti}. Some of them have the same frequency and they may overlap. It is also necessary that a particular vibration should produce a fluctuating dipole; otherwise, it cannot interact with electric field of the infrared light.

Vibrational frequency

The stretching vibrational frequency of a bond is calculated accurately by applying 'Hooke's law,' which is represented as follows:

$$\overline{\upsilon} = 1/2\pi c(k/\mu)^{1/2}$$

where 'μ' is the reduced mass, m_1 and m_2 are masses of the atoms concerned in grams, k is the force constant of the bond and relates to the strength of the bond, and c is the velocity of radiation.

IR spectroscopy and food and health

IR spectroscopy is mainly used to distinguish the organic compounds on the basis of carbonyl group stretching frequency. As far as food science is concerned, the use of near IR (NIR) region is of significant importance. NIR uses the range from 780 to 2500 nm. The record of NIR region involves the response of molecular bonds O–H, C–H, C–O, and N–H. There is an extensive use of NIR technique in the field of milk and dairy products. IR spectroscopy is useful in the identification of a variety of adulteration problems using experimental and statistical methods. NIR is another rapid, reliable, and nondestructive technique widely used for quality and processing control for the characterization of various food products without using reagents or solvents. Spectroscopic methods act as state of art to test various food supplements.

Raman spectroscopy

The phenomenon of Raman scattering was discovered in 1928 by Indian physicist C. V. Raman, who received the Nobel Prize in Physics in 1930 for his work. Raman spectroscopy provides information about the molecular vibrations that can be used for sample identification and quantification. Raman spectroscopy can be used to study the solid, liquid, and gaseous samples. This technique involves shining a monochromatic region source on a sample and detection of the scattered light. Typically, in Raman spectroscopy, high-intensity laser radiation with wavelengths in either the visible or NIR regions of the spectrum is passed through a sample. The major portion of the scattered light has the same frequency as the excitation source; this is known as Rayleigh or elastic scattering. A very small portion of the scattered light has different energy from the laser frequency due to the interaction of light with the vibrational energy levels of the molecule in the sample. The shift in energy (wavelength) depends upon the chemical structure of the molecules. Plotting of intensity of this shifted light versus frequency results in a Raman spectrum of the sample. Generally, Raman spectra are plotted with respect to the laser frequency such that the Rayleigh band lies 0 cm^{-1} on the scale. On the scale, the band positions will lie at frequencies that correspond to the energy levels of different functional group vibrations. Thus, the Raman spectrum can be interpreted similar to the IR absorption spectrum.

Basic principle and its origin

The Raman spectrum is based upon molecular deformations in the electric field, E, determined by molecular polarizability, α. The high-intensity laser beam can be considered as an oscillating electromagnetic wave with electric field. Upon the interaction of electric field, E, with the sample, it induces electric dipole moment, $P = \alpha E$, which deforms the molecule. Because of periodical deformations, molecule starts vibrating with characteristic frequency. Therefore, monochromatic light with particular frequency excites molecules and transforms them into oscillating dipoles. Such oscillating dipoles emit light of three different frequencies (energies). A simplified diagram (Figure 6) illustrates these concepts. Thus, three types of phenomena can occur: (1) when the light is incident on the molecule, it interacts with the molecule, but the net energy change is zero. So, the frequency of the scattered light is the same as that of the incident light. This process is known as Rayleigh scattering (2) When the light is incident and the material absorbs energy and the emitted light photon has a lower energy than the absorbed photon, this outcome is labeled as Stokes Raman scattering (3) When the light is incident on the molecule and the molecule loses energy and the emitted photon has a higher energy than the absorbed photon, this process is known as anti-Stokes Raman scattering.

Raman shift

Raman shifts are typically reported in wave numbers, as this value is directly related to energy. The following formula is used:

$$\Delta\omega = \left(\frac{1}{\lambda_0(\text{nm})} - \frac{1}{\lambda_1(\text{nm})}\right)$$

where '$\Delta\omega$' is the Raman shift expressed in wave number, λ_0 is the excitation wavelength, and λ_1 is the Raman spectrum wavelength. Most commonly, the unit used for expressing wave number in Raman spectra is cm^{-1}. Since wavelength is often expressed in units of nanometers (nm), the aforementioned formula can be expressed as

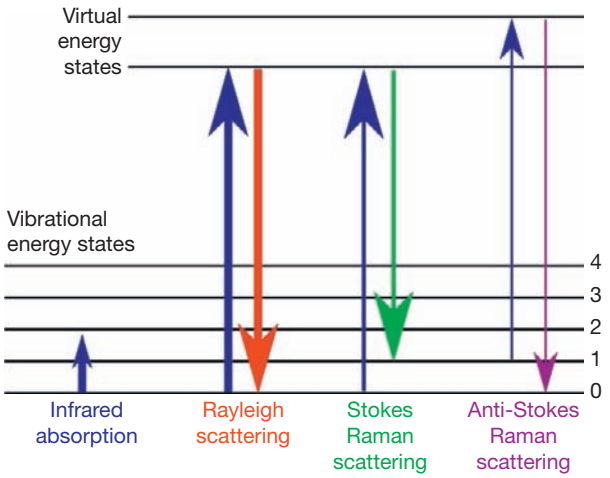

Figure 6 Mechanism of Raman scattering.

$$\Delta\omega\,(\mathrm{cm}^{-1}) = \left(\frac{1}{\lambda_0(\mathrm{nm})} - \frac{1}{\lambda_1(\mathrm{nm})}\right) \times \frac{10^7\,\mathrm{nm}}{\mathrm{cm}}$$

Instrumentation

A Raman spectrometer consists of an excitation source, a monochromator, a sample holder, and a detector. Two major types of techniques are used to collect the Raman spectra: dispersive Raman spectroscopy and Fourier transform Raman spectroscopy. These differ from one another in terms of the laser source used and the way by which the Raman scattering is detected and analyzed. Several types of lasers can be used as excitation sources, like argon ion (488.0 and 514.5 nm), krypton ion (530.9 and 647.1 nm), He:Ne (632.8 nm), and diode laser (630 and 780 nm). Nowadays, more and more often researchers use multichannel detectors like photodiode detector or more commonly charged coupled devices to detect the Raman scattering light.

It was found that Raman signals are quite weak and people are constantly trying to improve Raman spectroscopy techniques. Surface-enhanced Raman spectroscopy (SERS), confocal Raman spectroscopy, coherent anti-Stokes Raman scattering, resonance Raman spectroscopy, and Raman sensing are new advancements in the field of Raman spectroscopy, which increase the sensitivity of this technique.

Raman spectroscopy and food and health

The study of biomolecules with Raman spectroscopy helps in the evaluation of quality of natural food products as it provides information about the change in the structure of proteins, lipids, and water of muscle food, which occurs during deterioration. SERS with gold-coated substrates was used to analyze phosmet, a pesticide in apples. SERS can be used to target specific DNA and RNA sequences using a combination of gold and silver nanoparticles and Raman-active dyes. The gold nanoparticles facilitate the formation of a silver coating on the dye-labeled regions of DNA or RNA, thus allowing SERS to be performed. Raman gas analyzers have many practical applications. For instance, they are used in medicine for real-time monitoring of anesthetic and respiratory gas mixtures during surgery. Raman spectra were used to differentiate between the normal and diseased tissues and different chemical stages of cells and tissues. Raman techniques can be used as an alternative tool for the detection of cancer as they have been used for the determination of the nature of tumor.

Electron Paramagnetic Resonance Spectroscopy

Electron paramagnetic resonance (EPR) spectroscopy or ESR is a nondestructive analytic technique with a variety of applications that include oxidation and reduction processes, biradicals and triplet-state molecules, and reaction kinetics, as well as numerous additional applications in medicine, biology, and physics. Its basic principle is similar to NMR, but it is electron spins that are excited instead of spins of atomic nuclei. ESR technique is less widely used as compared to NMR as all stable molecules have all their spins paired.

Principle

The basis of EPR lies in the spin of electron and its associated magnetic moment. Every electron has magnetic moment and spin quantum number, $s=1/2$, with magnetic components, $ms=+1/2$ and $ms=-1/2$. When the electron is placed in applied magnetic field, B_0, the two possible spin states of the electron have different energies. The lower energy state occurs when the magnetic moment of the electron, μ, is aligned with the magnetic field and a higher energy state occurs when μ is aligned against the magnetic field. The two energy states are labeled by the projection of electron spin, ms, on the direction of magnetic field, where $ms=-1/2$ is the parallel state and $ms=+1/2$ is the antiparallel state (Figure 7). So for a molecular species with one unpaired electron, the energy state of the electron can be defined as $E=g\mu B_0 ms=\pm 1/2 g\mu B_0$:

$$\Delta E = g\mu B0$$

where 'g' is electron's so-called g-factor, $g=2.0023$ for the free electron, and 'μ' is the Bohr magneton.

An unpaired electron can move between the two energy levels by either absorbing or emitting a photon of energy $h\nu$ such that the resonance condition, $h\nu=\Delta E$, is obeyed. This leads to the fundamental equation of EPR spectroscopy, $\Delta E=g\mu B_0$.

The EPR measurements are made with microwaves in the 9000–10 000 MHz (9–10 GHz) region. Furthermore, EPR spectra can be generated by either varying the photon frequency incident on a sample while keeping the magnetic field constant or doing the reverse. In practice, it is usually the frequency that is kept fixed. A collection of paramagnetic centers, such as free radicals, is exposed to microwaves at a fixed frequency. When the strength of external magnetic field is increased, the gap between $ms=+1/2$ and $ms=-1/2$ energy states is widened until it matches the energy (frequency) of the microwaves. At this point, the unpaired electrons can move between their two spin states. Since there typically are more electrons in the lower state, due to the Maxwell–Boltzmann distribution, there is a net absorption of energy, and thus, the absorption observed is converted into a spectrum.

Hyperfine coupling

Since an EPR spectrum is produced as a result of a change in an electron's spin state, it might be thought that all EPR spectra for a single electron spin would consist of a single line. However, in the actual practice, an unpaired electron, by way of its

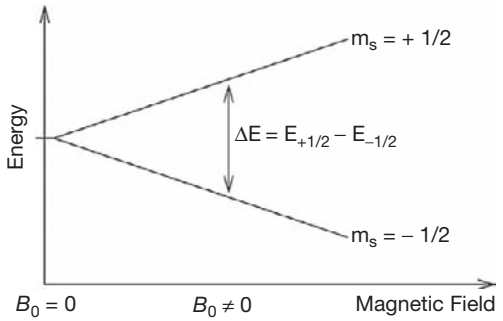

Figure 7 Splitting pattern of energy states of electron as a function of magnetic field.

magnetic moment, interacts with nearby nuclear spins. The resulting interaction between the electron and nuclei is called hyperfine splitting. As a result of hyperfine splitting, a multilined spectrum is obtained. Hyperfine interactions provide a great deal of information about the sample including providing information about the number and identity of nuclei in a complex as well as their distance from the unpaired electron. Thus, the previous equation gets modified to $E = g\mu B_0 ms + amsmi$.

where 'a' is the hyperfine coupling constant and mi is the nuclear spin quantum number for the neighboring nucleus. The coupling patterns that are observed in EPR spectra are determined by the same rules that apply to NMR. So the number of lines that result from the coupling can be determined by the formula $2nI+1$, where n is the number of equivalent nuclei and 'I' is the spin. This formula gives only the number of lines and not their relative intensities. Coupling to n equivalent nuclei, each with spin ½, gives '$n+1$' lines. But since there are multiple nuclei interacting, the relative intensities of the lines follow the binomial distribution as shown in the succeeding text.

Number of equivalent nuclei	*Relative intensities*
1	1:1
2	1:2:1
3	1:3:3:1
4	1:4:6:4:1
5	1:5:10:10:5:1
6	1:6:15:20:15:6:1

For example, the EPR spectrum of the radical anion of benzene $(C_6H_6)^-$ gives seven lines with relative intensity, 1:6:15:20:15:6:1, as the electron is delocalized over six equivalent hydrogen atoms.

If an electron couples with several sets of nuclei, then the overall pattern is determined by first applying the coupling to the nearest nuclei and then further splitting of each line by the coupling to the next nearest nuclei and so on, for example, radical anion of pyrazine, where coupling to two nearest equivalent N^{14} ($I=1$) nuclei gives a quintet with relative intensities: 1:2:3:2:1.

ESR spectroscopy and food and health

1. Food irradiation is used to destroy microorganisms, parasites, molds, yeasts, and insects, which are responsible for deterioration and spoilage reduction of the quality of food. In this process, the radiations inhibit the division of microorganisms and create radiolytic products and free radicals. Free radicals in wet samples decay in a fraction of seconds with the formation of neutral species. These radicals are quite stable in a dry environment. Their concentration in rigid matrices is quite low but high enough to be detected by EPR spectroscopy. The direct detection of persistent radicals by ESR is usually possible in dry foods. In the ESR spectrum of dry foods, generally, a single line with a g-value ranging between 2.003 and 2.006 and a peak-to-peak line width between 4 and 12 G are observed. However, in many cases, a sextet of lines due to the presence of Mn^{2+} is obtained, which is frequently observed in the ESR spectra of biological systems.
2. ESR spectroscopy can be used to study the oxidative processes in food and beverages. Oxidative reactions in the food cause the spoilage of food and affect the shelf life and quality of food. The oxidation of lipids causes rancidity, the oxidation of proteins results in off-flavors, the oxidation of vitamins and polyunsaturated fatty acids reduces the nutritional value, while the oxidation of food pigments leads to discoloration. Radicals are intermediates in many of these reactions. Therefore, ESR can be used to study the early stages of these reactions and for designing protective measures against oxidation of food.

NMR Spectroscopy

Over the last 50 years, NMR has become the prominent technique for the determination of organic molecules. NMR was first described and measured in molecular beams by Isidor Rabi in 1938. In 1944, Rabi was awarded the Nobel Prize in Physics for this work. In 1946, Felix Bloch and Edward Mills Purcell expanded the technique for use on liquids and solids, for which they shared Nobel Prize in Physics in 1952. It is an analytical chemistry technique that is used in quality control and research for determining the content and purity of sample as well as its molecular structure. It has become one of the most powerful techniques for the determination of molecular structure, now extending to species as large as proteins. It is also routinely used in advanced medical imaging techniques, such as in magnetic resonance imaging (MRI).

Basic theory and principle

NMR is based on the absorption of electromagnetic radiation in the radiofrequency region of the spectrum resulting in changes in the orientation of spinning nuclei in a magnetic field. This technique is exclusively applicable for nuclei with spin, $I \neq 0$. In the presence of magnetic field, these nuclei become nondegenerate due to differences in the spin. The application of electromagnetic radiation can excite the nuclei into higher energy level. The frequency that causes the excitation is determined by the difference in energy between energy levels.

Commonly used solvents in NMR are carbon tetrachloride (CCl_4), deuterochloroform ($CDCl_3$), deuterodimethyl sulfoxide (DMSO-d6), deutero water (D_20), deuterobenzene (C_6D_6), and trifluoroacetic acid, and trimethylsilane (TMS) is used as internal standard in NMR.

Chemical shift

It expresses the difference in the resonance frequency of a given proton compared with that of the methyl protons of TMS. Chemical shift is usually expressed in parts per million:

$$\text{Chemical shift}\,(\delta) = \frac{\text{Diiference between a resonance frequency and that of reference substance}}{\text{Operating frequency of the spectrometer}}\,(\text{ppm})$$

Chemical shift is affected by the shielding and deshielding of the concerned nucleus.

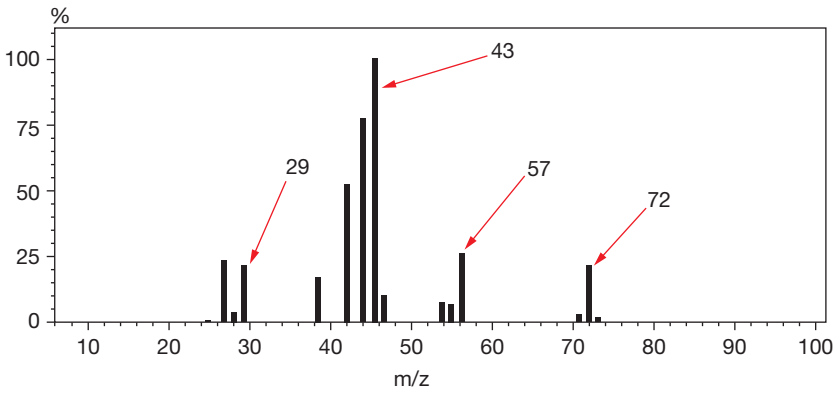

Figure 8 Mass spectrum of pentane.

NMR and food and health

The major application of NMR is MRI, which is an important tool in the diagnosis and evaluation of diseases. It is a test that uses the strong magnetic field and pulses of radio wave to make detailed images of organs and structures inside the body. MRI scan can be used to examine almost any part of the body including the brain and spinal cord, bones and joints, breasts, heart and blood vessels, and liver, uterus, and prostate gland. The results of an MRI scan are used to help diagnose conditions, plan treatments, and assess how effective previous treatment has been. MRI scan is the best way to see inside the human body without cutting it out:

1. Due to the fact that many foods are proton-rich due to the presence of water, carbohydrate, and proteins, proton NMR becomes the most common type of NMR to determine these abundant food components. These components influence the intrinsic properties of food during processing, storage, and transportation. NMR is used in fat analysis and fatty acid composition.

Mass Spectrometry

Mass spectrometry is a powerful analytic laboratory technique used to quantify the known material, to identify unknown compounds present in a sample, and to elucidate the chemical structure of different molecules. The complete process involves the conversion of sample into gaseous ions, with or without fragmentation, followed by the characterization by their mass-to-charge ratio (m/z) and relative abundances. It is based upon slightly different principles to other spectroscopic principles.

Basic principle

The mass spectrometer generates multiple ions from the sample under investigation; it then sorts and separates according to their specific mass-to-charge ratio and then records the relative abundance of each ion type. The first step in the mass spectrometric analysis is vaporization and ionization basically by electron ionization. These molecular ions undergo fragmentation. The ions are then accelerated through a potential difference and focused into a beam. The charged particles passing through a magnetic field are deflected along a circular path on a radius that is proportional to the mass-to-charge ratio, m/z. The ions are separated according to their mass-to-charge ratio and are detected in proportion to their abundance. Thus, the mass spectrum of a molecule is produced. It displays the result in the form of a plot of ion abundance versus mass-to-charge ratio.

The ionization process may be represented as

$$\underset{\text{Molecule}}{\text{M}} : +e^- \rightarrow \underset{\text{Molecule ions}}{2e^- + \text{M}^+} \rightarrow \underset{\text{Fragment ions}}{m_1^+ + m_2}$$

Components of instruments

The instrument consists of three major components:

1. *Ion source*: For producing gaseous ions from the sample being studied
2. *Mass analyzer*: For resolving the ions into their characteristic mass components according to m/z.
3. *Detector*: For detecting the ions and recording the relative abundance of each of the resolved ionic species

The mass spectrum

A mass spectrum is usually presented as a vertical bar graph, in which each bar represents an ion having a particular m/z ratio and the length of bar represents the relative abundance of that ion in the sample. The most intense ion is assigned an abundance of 100, and it is referred as the base peak. Most of the ions formed have a single charge so the m/z value is equal to the mass itself. A few compounds have mass spectra that do not contain molecular ion peak, because all the molecular ions break into fragments. A typical mass spectrum of pentane (C_5H_{12}) is shown in Figure 8 in which $m/z = 43$ is the base peak.

Mass spectroscopy in food and health

As far as health science is concerned, the sample used for MS study may include body fluid such as urine, blood, serum, and saliva or tissue samples such as blood cells. MS is based on the breakdown of sample into charged ions. MS has widespread applications and is used in the identification of proteins, peptides, and oligonucleotides.

See also: Infrared Spectroscopy: Applications; Mass Spectrometry: Applications; Mass Spectrometry: Principles and Instrumentation.

Further Reading

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Spices and Flavoring Crops: Fruits and Seeds

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Introduction

There are about 30 types of fruits and seeds that are commonly used as spices and for flavoring purposes throughout the world. Their frequency of use varies in different parts of the world. In this article, some of the most commonly used spices and flavoring crops are discussed in detail, and the remaining equally important ones are summarized in Table 1: each section deals with an individual spice in terms of its common name, botanical name, family, and parts used, along with diagnostic, morphological, and anatomical characters; details of handling, drying, and storage have also been given wherever available. Additionally, information about antioxidant content and antifungal or bacterial properties has been given where applicable.

Allspice or Pimento

Allspice consists of the dried fruits of *Pimenta officinalis* Lindl. (Myrtaceae). Dried berries are globular, 4–7 mm in diameter, hard, and dark reddish brown; the surface of the berry is rough with protuberances, and the apex bears the remains of the calyx and the style; there is a short stalk, or its scar at the base. The berries are bilocular each with hard, dark brown, reniform seeds (Figure 1(a) and 1(b)).

The epidermis of the exocarp bears stomata and nonglandular hairs up to 150 μm in length. The mesocarp, toward the outside, consists of numerous oval or rounded oil cavities up to 210 μm in diameter; numerous scattered, variable, pitted stone cells occur singly or in groups; beneath, there is a zone of fibrovascular bundles; ground tissue is parenchymatous and contains 'rosette' calcium oxalate crystals. A group of stone cells, 30–60 μm in diameter, are present toward the inner side. The endocarp consists of a few layers of compressed parenchyma (Figure 1(c)).

Allspice is aromatic and pungent and possesses the flavor and aroma of clove, nutmeg, cinnamon, and black pepper, hence the name 'allspice.' Mature berries are removed from the twig when still green by hand or flail, sorted, and dried in the sun for 6–8 days; when dull reddish brown, the berries are packed in jute bags. At present, sun-drying of berries is done on a concrete barbeque. Because of frequent shifting of the berries in and out of the sheds during rainy days, many berries break. Hence, mechanical drying is preferable, and it is also more economical than sun-drying.

Anise

Anise is the dried fruit of *Pimpinella anisum* L. (Apiaceae). The schizocarps are 2–5 mm, ovoid–conical, grayish brown, and pubescent, with a pedicel attached; they are crowned with a short, bifurcate stylopod. The mericarps, attached to the carpophore, are broadly ovoid; they are flat or concave on the commissural side and convex and pubescent on the dorsal side (Figure 1(d) and 1(c)).

The exocarp has an outer striated cuticle with stomata and rectangular epidermal cells bearing surface hairs up to 150 μm in length. The mesocarp consists of five fibrovascular bundles underlying ridges, 2–4 vittae on the commissural side and 20–40 vittae on the dorsal side. The endosperm of the seed is composed of thick-walled parenchymatous cells containing oil and aleurone grains and one or two small 'rosette' calcium oxalate crystals (Figure 1(f) and 1(g)).

Anise has a characteristic and agreeable aromatic odor and a pleasant taste. Fruits are harvested when their color changes from green to gray; shattering is minimized by avoiding overripening and harvesting during the morning with scythes or reaping machines. The crop is piled into small stacks and allowed to wither for 2–3 days; it is then threshed and dried in partial shade. After winnowing, sieving, and cleaning, the crop is dried naturally to 18% moisture followed by artificial drying at 80–90 °C; it is then packed and stored in a cool, dry room. For keeping spices belonging to family Apiaceae, the maximum tolerance limit of moisture is 10%. Reducing the moisture below 8% can generally have a bad effect on the quality due to loss of volatile oils. Crushed or ground spice is stored in airtight containers.

Anise extract functions as an antimicrobial agent for *Proteus mirabilis*, *Citrobacter koseri*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterobacter aerogenes*, *Micrococcus luteus*, *Staphylococcus epidermidis*, and *Candida albicans*. Additionally, anise was shown to contain antioxidants powerful enough to pass the same tests as common synthetic antioxidants such as BHA and BHT.

Black Pepper

Black pepper consists of the dried, unripe fruit of *Piper nigrum* L. (Piperaceae). It is the oldest and most important spice and commonly known as the 'king of spices.' There are about a hundred cultivars of black pepper under cultivation. There is considerable variability among the accessions with regard to various morphological as well as floral characters like leaf shape, size, spike length, density, composition of male, female, and bisexual flowers in the spike, fruit set, shape, weight and volume of the fruit, and time taken for maturity. The fruit, though known as a berry, is a sessile globose drupe with a pulpy pericarp. The berry is globular, 6 mm or less in diameter, brownish black, with a coarsely reticulate surface, and single-seeded. The seed is almost white hollow and adheres to the pericarp (Figure 1(h) and 1(i)).

Table 1 Some other commonly used spices from fruits and seeds

Botanical name	*Common name*	*Family*	*Part used*	*Characters*
Aframomum korarima (Pereira) Engler	Korarima cardamom	Zingiberaceae	Fruit and seed	Fruits ovate, about 4 cm by 2 cm pointed seeds reddish brown, striated
Aframomum melegueta (Rosc.) K. Schum.	Grains of paradise	Zingiberaceae	Fruit and seed	Fruits ovoid 10 cm long, pointed. Seeds inverted subpyramidal, somewhat four-sided, 2–3 mm by 2.5 mm, testa rich reddish brown, papillose
Amomum subulatum Roxb.	Greater or Nepal cardamom	Zingiberaceae	Fruit and seed	Fruit is dark red-brown, globose capsule, 2.5 cm long; several cardamom seeds, similar to true cardamom, embedded in a vicid pulp
Bunium persicum (Boiss.) Fedts	Black caraway	Apiaceae	Fruit	Mericarp oblong (3–4 mm by 0.7–1.0 mm), yellowish dark brown with ridges
Dipteryx odorata Willd.	Tonka beans	Fabaceae	Fruit	Fruit egg-shaped with hard shell and pulpy flesh surrounding a single seed with a black wrinkled surface
Garcinia indica Choisy	Kokam	Clusiaceae	Fruit	Fruit globose or spherical 2.0–3.75 cm in diameter, dark purple when ripe
Juniperus communis L.	Juniper	Pinaceae	Fruit	Fruit subglobose, 5–9 mm in diameter externally smooth, shining, purplish black to dusky red purple internally yellowish brown to dusky yellow flesh, containing many large, schizogenous cavities; ripe fruits are collected, and dried flavor diminishes after storing fruits for more than a year
Mangifera indica L.	Amchoor	Anacardiaceae	Fruit	Fruit a drupe, variable in form and size, leathery, dotted with glands; mesocarp of unripe fruit hard, acidic; ripe fruits juicy; endocarp stony; skin of unripe fruit removed, mesocarp sliced and dried in sun; the dried product is packed in wooden casks and may also be seasoned before drying
Nigella sativa L.	Black cumin	Ranunculaceae	Seed	Seeds trigonous, black, rugulose, and tuberculate; seeds are collected mostly from wild plants
Piper cubeba Linn. f.	Cubeb	Piperaceae	Fruit	Fruit globular, 3–6 mm in diameter with a stem-like portion about 7 mm long, apex with four remains of stigma; dusky red, brown or, rarely, grayish; coarsely reticulate; nearly full grown fruits are collected and dried in sun
Piper longum L.	Long pepper	Piperaceae	Fruit	Catkin is subcylindrical, up to 40 mm by 5–7 mm; grayish black and composed of numerous small immature drupes and bracts embedded spirally in the axis; immature catkins are harvested and immediately dried in the sun or oven to prevent formation of mold
Pistacia vera L.	Pistachio	Anacardiaceae	Fruit	Drupe oblong, linear to globose, laterally compressed, 10–20 mm by 6–12 mm; outer husk variously colored, readily separating from the dehiscent or indehiscent grayish white, bony keeled nutshell (endocarp) enclosing yellow to green kernel
Punica granatum L.	Anardana (pomegranate)	Punicaceae	Seed	Seeds hard, angular, covered with a juicy red, pink or yellowish white pulp; seeds of ripe fruits are taken out and sun-dried or dehydrated
Sisymbrium irio L.	London rocket (khub-kalan)	Brassicaceae	Seed	Seeds ovoid (1.3 mm long), reddish or yellowish brown, mucilaginous
Trachyspermum ammi (L.) Sprague	Bishop's weed (Ajwain)	Apiaceae	Fruit	Cremocarp ovoid (2–3 mm long), muricate, grayish brown; mericarps compressed with distinct ridges and tuberculate surface harvested when fruits turn brown; plants are pulled out and dried completely on threshing floor; fruits are separated by rubbing
Tamarindus indica L.	Tamarind	Fabaceae	Fruit	Fruits 5–20 cm by 2 cm in size; epicarp surface rough, brownish hard and brittle; mesocarp pulpy light brown changing with age to dark brown or black; containing some branched fibers and numerous seeds
Vanilla pompona Schneider	Pompon or West Indian vanilla	Orchidaceae	Fruit	Pods are 10–17.5 cm long and 2.5–3.3 cm in diameter
Vanilla tahitensis J.W. Moore	Tahitian vanilla	Orchidaceae	Fruit	Pods 12–14 cm long and about 9–10 mm wide with tapering ends; pods are harvested when tips of the pods turn brown
Xylopia aethiopica (Dunal) A. Richard	Xylopia, Netawu	Annonaceae	Fruit	Mature carpels cylindrical, incurved, about 5 cm long, embedded in the center of the receptacle; seeds glabrous, ellipsoid (about 0.5 mm by 0.25 mm), brown

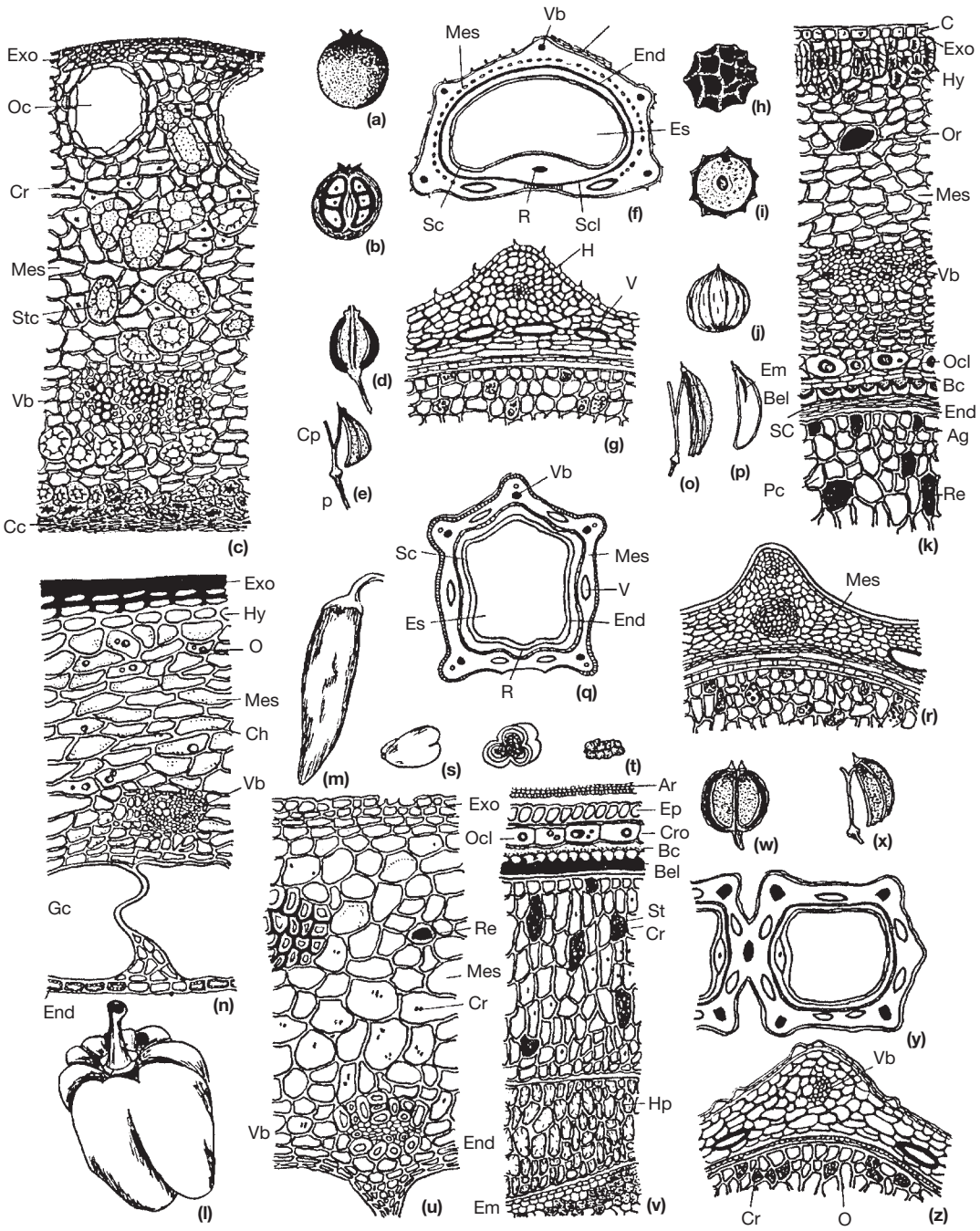

Figure 1 Spices from fruits. Allspice: (a) fruit; (b) longitudinal section of fruit; (c) cross section of pericarp. Anise: (d) schizocarp; (e) mericarp; (f) cross section of mericarp; (g) cross section through ridge of pericarp. Black pepper: (h) fruit; (i) cross section of fruit; (j) pericarp, seed coat and portion of perisperm; (k) White pepper. Capsicum: (l) *Capsicum annum* fruit; (m) *C. frutescens* fruit; (n) cross section of pericarp of *C. frutescens*. Caraway: (o, p) mericarp; (q) cross section of mericarp; (r) cross section through ridge of pericarp. Cardamom: (s) fruit; (t) seeds; (u) cross section of pericarp; (v) cross section of seed. Celery: (w) schizocarp; (x) mericarp; (y) cross section of mericarp; (z) cross section through ridge of pericarp. Abbreviations: Ad, amylodextrin granules; Ag, aleurone grains; Ar, aril; Bc, beaded cells; Bel, beaker cells; C, cuticle; Cc, compressed cells; Ch, chloroplast, Col, collenchyma; Cot, cotyledon; Cp, carpophore; Cr, crystal; Crc, crystal cells; Cro, cross cells; Dl, dehiscence line; Em, embryo; End, endocarp; Ep, epidermis; Es, endosperm; Exo, exocarp; Fl, fiber layer; Fo, fixed oil; Gc, giant cell; H, hair; Hc, hourglass cells; Hp, horny protein; Hy, hypodermis; Li, light line; Mc, mucilage cells; Mes, mesocarp; Mp, mesophyll; O, oil; Oc, oil cavity; Ocl, oil cells; Or, oleoresin; P, pedicel; Pa, parenchyma; Pal, palisade; Pe, perisperm; Pl, placenta; R, raphe; Rc, reticulated cells; Re, resin; Rub, rudimentary vascular bundle; Sc, seed coat; Scl, sclerenchyma; Sd, seed; Se, secretory cells; St, starch; Stc, stone cells; V, vittae; Vb, vascular bundle. Reproduced from Macrae, R., Robinson, R. K. and Sadler. M. J. (eds.) (1993). Spices and flavoring (flavoring) crops: fruits and seeds. *Encyclopedia of food science, food technology and nutrition*. London: Academic Press.

The exocarp is cutinized and darkly pigmented, with rectangular cells beneath containing calcium oxalate crystals. The hypodermis consists of stone cells interspersed with parenchyma. The mesocarp is comparatively broad: The outer seven to eight layers are parenchymatous with scattered oleoresin cells, and beneath, there is a zone of fibrovascular bundles, a region of compressed cells, a layer of large oil cells, and two layers of small parenchyma cells. The endocarp consists of a layer of 'beaker cells,' the radial and inner walls of which are strongly lignified. The seed coat is differentiated into the distinct layers; whereas the outer perisperm cells contain aleurone grains, the inner cells contain starch grains (Figure 1(k)).

The fruits are aromatic and pungent. The quality of black pepper depends upon piperine and essential oil present in the oleoresin. Piperine contributes to the pungency, and essential oil is responsible for the characteristic flavor. The harvested green berried spikes are heaped for a day and spread on a mat or floor. The berries are separated from spikes by rubbing. After separation, the berries are cleaned and put out for drying. The fresh harvested berries will have a moisture content of 80%, and this has to be dried to 8% moisture content for safe storage. A blanching treatment by dipping the berries for 1 min in boiling water gives a glossy appearance to the dried berries and reduces the microbial load on the product. Due to enzymic oxidation of color compounds present in the skin, the color of pepper turns black during drying, which effectively masks the green color that is left in the berries after drying. The berries are dried under the sun for 4–7 days until the outer skin of the berries becomes black in color with a wrinkled appearance. Small and light berries are removed from the dried produce. Berries can be dried in a solar cabinet dryer in 4 days with a spreading density of 18 kg m^{-2} and resulting in a black dried produce. By contrast, sun-dried berries take 5 days to dry with a spreading density of 5 kg m^{-2} and resulting in a dull black color. Dark black berries obtained from solar cabinet drying are preferred in trade over the dull black berries obtained from open sun-drying. Sixty kilograms of fresh pepper can be dried in the indirect heating type of dryers in about 30 h. After drying, the pepper has to be garbled. Ungarbled pepper contains fractions, dust, stalk, pinheads, and immature, overmature, and large berries. The grading is done by a combination of size sieving and weight classification by air blast. The major grade is the average-sized black pepper known as 'Malabar Garbled,' which constitutes 95% of India's export. The recovery of black pepper from fresh berries is 35–36%. For black pepper powdering, a normal pin type of hammer mill is used so that there is no temperature rise during grinding. The ground pepper is only used as a raw material for oleoresin and in the oil industry. White pepper (Figure 1(j)) is usually prepared from overripe berries. In this state, the skin comes off easily, and the resultant product is dried. White pepper fetches double the price of black pepper. The piperine content also is found to be apparently higher in white pepper due to its concentration in the endosperm of the berries. In India, the berries are dipped in water and the skin removed by rubbing with hand. The skinless berries are dried in open sun for 7 days to obtain white pepper. The skin is also removed in a pulping machine after steam cooking. At the National Research Centre for Spices in Calicut, a process of steaming and boiling the despiked pepper for 13 min and rolling it in a container in running water to remove outer skin has been developed. In some less developed countries, the berries are soaked in river water prior to skin removal and drying.

After sun-drying, the berries are cleaned and polished with a piece of cloth. The recovery of white pepper is about 28% of the fresh ripe berries. In European countries, the green or fresh flavor of pepper is desired in certain food preparations. Green pepper is made in canned and bottled forms. In the canned form, it is prepared by heat-sterilizing green berries and packing them in 2% brine solution for protection. However, the rupturing of berries and making the covering liquid turbid are problems associated with this method. These problems have been overcome with proper canning methods (commercial sterilization or 'bot cook') and the introduction of a trace of pepper oil into the can before sealing. In bottled green pepper, heat processing is avoided, but a higher salt strength of 15–20% of the covering liquid is essential for good preservation. At CFRI, undermature berries are dried by arresting the enzyme action responsible for oxidative blackening and drying by mechanical means at low temperatures. The product has an excellent green flavor.

Extract from the black pepper has been found to be an effective antimicrobial agent for *Bacillus subtilis*, *Bacillus sphaericus*, *Staphylococcus aureus*, *Klebsiella aerogenes*, and *Chromobacterium violaceum*. During the drying process of the black peppers, the antioxidants are lost, but they are available predrying and are comparable to those of anise.

Capsicum

Capsicum is composed of dried fruits of the genus *Capsicum* L. (Solanaceae). Cayenne pepper and red pepper are two of the various synonyms of *C. frutescens* L., whereas paprika is *C. annuum* L. The fruits of *C. frutescens* are 6–150 mm long, laterally compressed, musky brownish to deep red, smooth or wrinkled, and bi- to tetralocular with central placentae united at the base-apical placentae with longitudinal ridges; the seeds are numerous, discoid, flat, hard, smooth, yellow, and about 2–5 mm long (Figure 1(l) and 1(m)).

The exocarp consists of five to seven rows of subrectangular cells, 25–60 μm by 15–20 μm; the outer epidermis is cuticularized and the mesocarp is parenchymatous, 30–85 μm by 15–20 μm. Many cells contain oil droplets and chromatophores, or sandy crystals. The innermost layer consists of larger cells, 0.8–1.7 mm by 0.2–0.4 mm. The endocarp is single-layered, consisting of parenchymatous and sclerenchymatous cells, the latter directly beneath a layer of giant cells possessing thick, lignified, porous walls. The dissepiment consists of a zone of tangentially elongated, collapsed cells (some containing microcrystals) and epidermal cells on either side, radially elongated, containing capsaicin (Figure 1(n)).

The odor of the spice is characteristic, sternutatory but agreeable, and mildly to intensely pungent. The stem of the entire plant is cut, hung, and exposed to the sun to remove 80% moisture; it is then dried further in a dryer to a critical moisture level; pricking the skin longitudinally reduces the drying time. Completely dried fruits are removed by a dressing machine and packed in polythene bags. The dried peppers can then be

ground and packaged, or they can be extracted to obtain the essential oil for food and nonfood applications.

Several variations of *Capsicum* have been shown to contain antimicrobial properties in respect to *B. cereus*, *B. subtilis*, *C. albicans*, *C. sporogenes*, *C. tetani*, *E. aerogenes*, *E. Cloacae*, *E. coli*, *K. pneumonia*, *P. vulgaris*, *P. aeruginosa*, *S. typhimurium*, *S. marcescens*, *S. aureus*, *S. epidermidis*, and *S. pyogenes*. *Capsicum annuum* has been shown to contain a large amount of antioxidants.

Caraway

Caraway is the dried fruit of *Carum carvi* L. (Apiaceae). The mericarps are separated, crescent-shaped, 3–7 mm by 1–2.3 mm, and brown, with five lighter-colored, filiform primary ribs, between which, on the dorsal surface, there is a secondary rib (Figure 1(o) and 1(p)).

In the transverse section, the epicarp consists of slightly tangentially elongated epidermal cells, and its outer surface is cuticularized; the mesocarp consists of a zone of several rows of collapsed, tangentially elongated parenchymatous cells, with fibrovascular bundles located in the rib portion; large vittae, two and four in number, are present on the commissural and dorsal side, respectively; the endocarp contains inner epidermal 'cross cells' that are closely adherent to the seed coat (Figure 1(q) and 1(r)).

The odor and taste of caraway are characteristically aromatic. The endosperm structure of the seeds and handling, drying, and storage of the spice are the same as for anise.

Caraway has been proved to be an effective antibacterial agent for *Escherichia coli*, *Pseudomonas syringae* pv. *phaseolicola*, *P. syringae* pv. *Pisi*, *P. syringae* pv. *aptata*, *P. syringae* pv. *apii*, *P. syringae* pv. *atrofaciens*, *P. syringae* pv. *lachrymans*, *P. syringae* pv. *maculicola*, *P. syringae* pv. *tomato*, *P. syringae* pv. *glycinea*, *P. cichorii*, *P. viridiflava*, *P. corrugata*, *P. tolaasii*, *P. reactans*, *P. agarici*, *Erwinia carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, *E. herbicola*, *Agrobacterium tumefaciens*, *Burkholderia gladioli* pv. *agaricicola*, *Ralstonia* solanacearum, *Xanthomonas campestris* pv. *phaseoli*, *X. campestris* pv. *phaseoli* var. *fuscans*, *X. campestris* pv. *vesicatoria*, *X. campestris* pv. *campestris*, *Bacillus megaterium*, *Clavibacter michiganensis* subsp. *michiganensis*, *C. flaccumfaciens* pv. *betae*, *and Rhodococcus fascians*. Caraway has also been shown to contain a moderate amount of antioxidants.

Cardamom

Cardamom is the dried fruit of *Elettaria cardamomum* Maton (Zingiberaceae). It is popularly known as the 'queen of spices.' There are many varieties, which are classified on the basis of their capsule size and color. Some of the important varieties are Alleppey, Mysore, Malabar, and Mangalore cardamoms. The capsules are trilocular, containing seeds on an axile placenta; they are 1–2 cm long, oblong–ovoid, three-sided, and greenish to pale buff or yellowish in color; each locule contains five to eight seeds, 3 mm by 2–3 mm, and dark reddish brown, adhering together with a membranous aril to form a single mass (Figure 1(s) and 1(t)). The pericarp in the transverse section shows an outer exocarp with cells up to 30 μm long. Parenchymatous cells of the mesocarp are up to 180 μm long, containing one or more calcium oxalate crystals. The inner mesocarp contains fibrovascular bundles and resin cells. Endocarp cells are compressed and invaginate into locular partitions (Figure 1(u)). Seeds, in the transverse section, show a three cell-layered aril containing a pale-colored substance and calcium oxalate crystals. It has an outer epidermis followed by a layer of pigmented, large, clear cells containing volatile oil, a one- to two-layered parenchyma, and a layer of sclerenchymatous, thick-walled, dark brown 'beaker cells,' each with a nodule of silica; the perisperm consists of starch-containing polygonal cells, the endosperm cells containing proteinaceous substance having a waxy or horny nature, and the embryo cells containing aleurone grains and fixed oil (Figure 1(u) and 1(v)).

The seeds are much more aromatic than the pericarp and possess a characteristically warm and slightly pungent taste. Fruits on the same raceme ripen at different times, and ripe fruits are collected before splitting. A green curing method is common in commerce. Fully mature green capsules, not having excessive shrinkage and splitting, which are not fully ripe yet, retain a good color after drying. The temperature and relative humidity of drying air inside the drying chamber are the two important factors to be controlled in the cardamom drying system. The recommended temperature for cardamom is 45–55 °C. Cardamoms are dried by different systems by kiln, driers, electric driers, or infrared driers. Cardamom drying has two phases; in the first phase, the surface moisture and moisture in fleshy parts of the mesocarp have to be removed, and in the second phase, the moisture in the seeds inside has to be removed. Controlled temperature, quick removal of the moisture, and quick flushing out of moisture-laden air from inside the drying chamber to prevent condensation are basic criteria in the retention of a green color. The dried cardamom capsules are placed into a wire mesh cylinder. The wire mesh cylinder is swung to separate the flower stalks, and the capsules are cleaned by winnowing. The cleaned capsules are packed in gunny bags lined with black polythene sheet for better retention of green color during storage. Grading of green cardamom is done according to the standard specifications at the exporter level. Extra Bold and Bold are the superior qualities.

Cardamom extract has been proved to be an effective growth inhibitor for *Staphylococcus aureus*. Additionally, cardamom has been shown to contain moderate levels of antioxidants.

Bleached Cardamom or White Cardamom

Bleached or white cardamom refers to varieties that do not retain the green color during drying or lose the color during storage. This product fetches lower prices. Freshly harvested ripe capsules are pretreated by soaking in a 1% solution of bleaching powder for 30–60 min followed by dipping the lot in dilute HCl solution (0.2–0.5%) for 2–3 min. The treated capsules are washed and sun-dried to 8% moisture level. But the sulfur dioxide content was found more in the capsule treated by the previous method; a simple treatment with hydrogen peroxide (solution of 0.5% strength) has been standardized to overcome this problem. Complete drying using a solar cabinet dryer is obtained in 3 days in comparison with 5

days under open sun-drying, and the color of the capsules is white, the required bleaching obtained without sulfur bleaching. The volatile oil in cardamom is responsible for cardamom's aroma and therapeutic properties. Green cardamom yields more oil than bleached cardamom.

Celery

Dried celery seeds are the dried fruit of *Apium graveolens* L. (Apiaceae). The schizocarps are ovoid and laterally compressed and occasionally possess a pedicel. The mericarps, usually separated, are ovoid, slightly curved, 1–2 mm by 0.5–1 mm, and light to olive brown; the commissural surface is flat and the dorsal surface convex and smooth, with five longitudinal ridges (Figure 1(w) and 1(x)).

In the transverse section, the mericarps are equilaterally pentagonal; the exocarp has a striated cuticle, and the epidermis has stomata – some of the cells are papillose; the mericarp is parenchymatous and has 4–15 vittae, of which two are on the commissural side, and fibrovascular bundles occur in each primary rib; the endocarp consists of a layer of tangentially elongated cells with yellowish orange to greenish yellow walls, and the spermoderm consists of collapsed cells. The structure of the endosperm is similar to that of anise (Figure 1(y) and 1(z)).

The fruit bears a characteristic celery aroma and taste. Celery yields 2–3% of pale yellow volatile oil, which is valued both as a fixative and as an ingredient of perfumes. Handling, drying, and storage of the spice are also similar to those of anise. The quality of celery depends upon its volatile oil content, and hence, the higher the oil content, the better the quality. The seed yield shows a significant and positive correlation between plant height, total umbels per plant, number of umbellets per umbel, weight of the main umbel, and number of seeds per umbel. All these characters have a positive effect on celery seed yield.

Coriander

Coriander spice is derived from the dried, ripe fruits of *Coriandrum sativum* L. (Apiaceae). It was one of the earliest spices used by mankind. The schizocarp is globular, yellowish brown, and 2–5 mm in diameter, with five calyx teeth and a short, conical stylopodium at the apex. Each mericarp has five prominent, straight, longitudinal primary ribs and four undulate, secondary ribs, which are easily separated, being deeply concave on the commissural side and convex on the dorsal side (Figure 2(a) and 2(b)). In the transverse section, the exocarp consists of a layer of small, thick-walled epidermal cells, and the mesocarp consists of several rows of thin-walled, somewhat collapsed parenchymatous cells, separated from a broad continuous zone of thick-walled, strongly lignified sclerenchyma fibers. The commissural side has two large elliptical vittae. The endocarp is a layer of tabular cells, and the cells of the seed coat are tangentially elongated and brownish. The structure of the endosperm is similar to that of anise (Figure 2(c) and 2(c)).

The spice has a characteristically pleasant and fragrant odor and an aromatic taste. Significant variations for oil content in coriander have been observed, ranging from 0.17% to 0.26%. The preferred cultivars of the plant for essential oil production are the small fruit varieties with a variable oil content of 4% or more. For spice use, however, it is the larger fruits from Morocco and India that are more commonly preferred. It has been reported that large thick-skinned fruits are less aromatic and have a lower oil content than the smaller thin-skinned fruits. There is no correlation between the number of fruits in the main umbel and yield. The flowers on the branches in the middle of the stalk are the most productive. It has been found that the number of umbels per plant is the chief contributing character and that the plant height determines the yield. Handling, drying, and storage are similar to those for anise.

Coriander has been proved to be an effective antibacterial agent for *B. subtilis* and *E. coli*. Additionally, there have been moderate levels of antioxidants discovered to be present in coriander.

Cumin

Cumin consists of the dried, ripe fruit of *Cuminum cyminum* L. (Apiaceae). The schizocarps are about 6 mm long, attached to short pedicels, and separated into mericarps. The fruit resembles caraway but can be distinguished by its straighter mericarps and bristly hairs along the ribs (Figure 2(e)–2(h)).

In the transverse section, the exocarp has an outer epidermis bearing stomata and a thin and striated cuticle; the hairs on the secondary ridges are larger than those on the primary ridges, being up to 2 mm long, and multicellular with broad bases and rounded apices; the mesocarp is parenchymatous, consisting of five fibrovascular bundles beneath the primary ridges, two vittae on the commissural side, and four vittae on the dorsal side. The parenchymatous cells of the endocarp have pale yellowish walls, and the seed coat consists of a layer of epidermal cells, each containing a prismatic crystal of calcium oxalate. The endosperm is similar to that of anise fruits (Figure 2(h) and 2(i)).

The spice has a peculiar, strong odor, and the flavor is warm and slightly bitter. The volatile oil content varies from 3% to 6%. The crop is piled into small stacks and allowed to wither for 2–3 days; it is then threshed and dried in partial shade. After winnowing, sieving, and cleaning, the crop is dried naturally to 18% moisture followed by artificial drying at 80–90 °C; it is then packed and stored in a cool, dry room. For keeping spices belonging to family Apiaceae, the maximum tolerance limit of moisture is 10%. Reducing the moisture below 8% can generally have a bad effect on the quality due to loss of volatile oils. Crushed or ground spice is stored in airtight containers.

Cumin has also been proved to be an effective antibacterial agent for *Escherichia coli*, *Pseudomonas syringae* pv. *phaseolicola*, *P. syringae* pv. *pisi*, *P. syringae* pv. *aptata*, *P. syringae* pv. *apii*, *P. syringae* pv. *atrofaciens*, *P. syringae* pv. *lachrymans*, *P. syringae* pv. *maculicola*, *P. syringae* pv. *tomato*, *P. syringae* pv. *glycinea*, *P. cichorii*, *P. viridiflava*, *P. corrugata*, *P. tolaasii*, *P. reactans*, *P. agarici*, *Erwinia carotovora* subsp. *carotovora*, *E. carotovora* subsp.

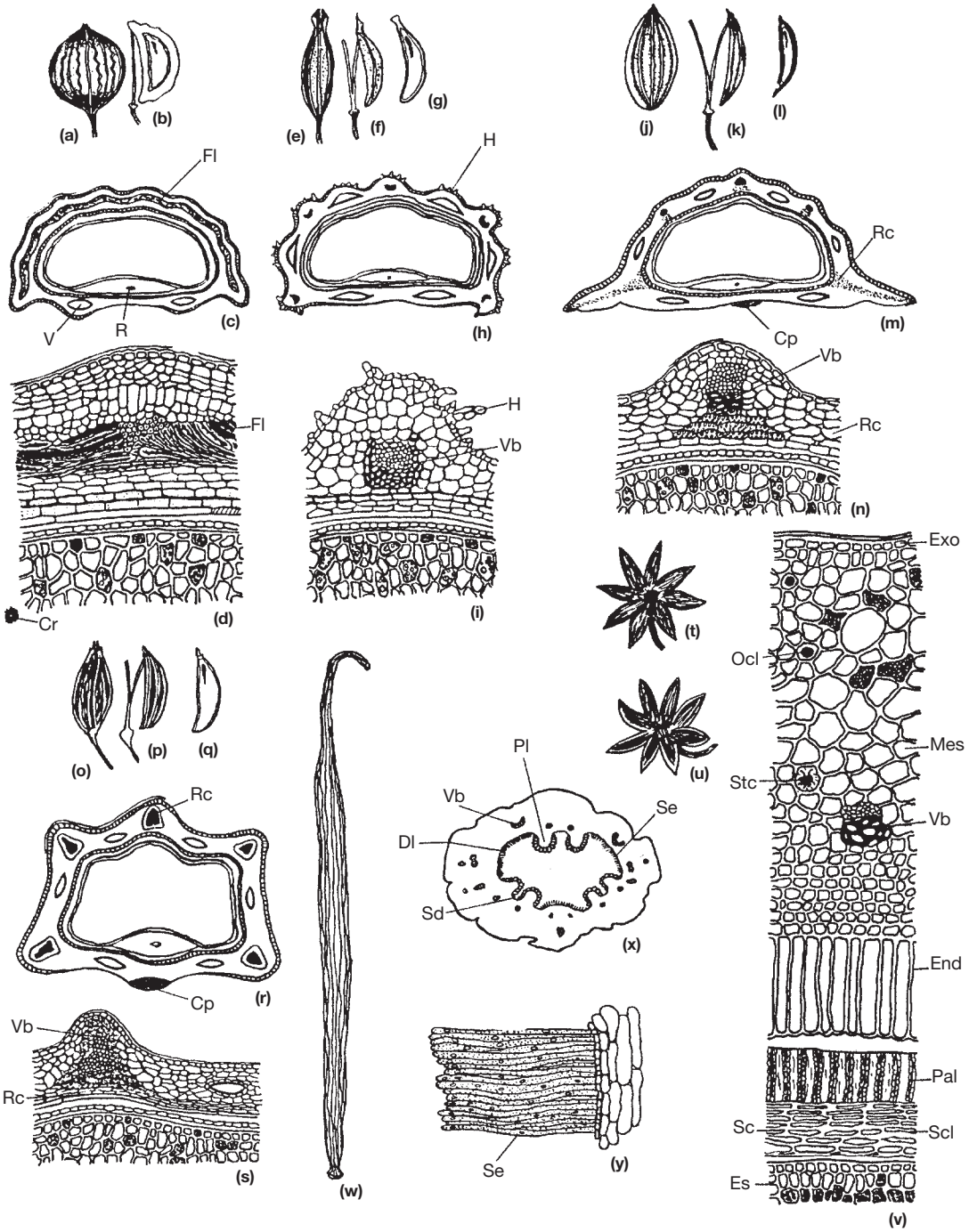

Figure 2 Spices from fruits. Coriander: (a) schizocarp; (b) mericarp; (c) cross section of mericarp; (d) cross section through primary ridge of pericarp. Cumin: (e) schizocarp; (f, g) mericarp; (h) cross section of mericarp; (i) cross section through ridge of pericarp. Dill: (j) dorsal view of mericarp; (k, l) side views of mericarp; (m) cross section of mericarp; (n) cross section through ridge of pericarp. Fennel: (o) schizocarp; (p, q) mericarp; (r) cross section of mericarp; (s) cross section through ridge of pericarp. Star anise: (t, u) fruit; (v) cross section of fruit and seed. Vanilla: (w) fruit; (x) transverse section of fruit; (y) secretory hairs. Abbreviations: Ad, amylodextrin granules; Ag, aleurone grains; Ar, aril; Bc, beaded cells; Bel, beaker cells; C, cuticle; Cc, compressed cells; Ch, chloroplast, Col, collenchyma; Cot, cotyledon; Cp, carpophore; Cr, crystal; Crc, crystal cells; Cro, cross cells; Dl, dehiscence line; Em, embryo; End, endocarp; Ep, epidermis; Es, endosperm; Exo, exocarp; Fl, fiber layer; Fo, fixed oil; Gc, giant cell; H, hair; Hc, hourglass cells; Hp, horny protein; Hy, hypodermis; Li, light line; Mc, mucilage cells; Mes, mesocarp; Mp, mesophyll; O, oil; Oc, oil cavity; Ocl, oil cells; Or, oleoresin; P, pedicel; Pa, parenchyma; Pal, palisade; Pe, perisperm; Pl, placenta; R, raphe; Rc, reticulated cells; Re, resin; Rub, rudimentary vascular bundle; Sc, seed coat; Scl, sclerenchyma; Sd, seed; Se, secretory cells; St, starch; Stc, stone cells; V, vittae; Vb, vascular bundle. Reproduced from Macrae, R., Robinson, R. K. and Sadler. M. J. (eds.) (1993). Spices and flavoring (flavoring) crops: fruits and seeds. *Encyclopedia of food science, food technology and nutrition*. London: Academic Press.

atroseptica, *E. herbicola*, *Agrobacterium tumefaciens*, *Burkholderia gladioli* pv. *agaricicola*, *Ralstonia solanacearum*, *Xanthomonas campestris* pv. *phaseoli*, *X. campestris* pv. *phaseoli* var. *fuscans*, *X. campestris* pv. *vesicatoria*, *X. campestris* pv. *campestris*, *Bacillus megaterium*, *Clavibacter michiganensis* subsp. *michiganensis*, *C. flaccumfaciens* pv. *betae*, *and Rhodococcus fascians*. Additionally, cumin has been show to contain moderate levels of antioxidants.

Dill

Dill consists of the dried and ripe fruits of *Anethum graveolens* L. (Apiaceae), usually composed of separated mericarps, which are brown, glabrous, broadly oval, and compressed (about 4 by 2.5 mm) with five ribs – three short dorsal and two lateral, yellowish, and winglike (Figure 2(j)–2(m)).

The exocarp has a striated cuticle and an epidermal layer of rectangular cells bearing stomata. The parenchymatous mesocarp has three fibrovascular bundles below the dorsal ridges and two in lateral ridges. The inner side of the vascular bundles consists of several tangentially elliptical, sclerenchymatous cells having reticulate thickenings. The mesocarp has two vittae on the commissural side and four vittae on the dorsal side. The seed coat consists of a layer of epidermal cells, and the endosperm is similar to that of the anise fruits (Figure 2(m) and 2(n)).

The aromatic odor and taste of dill are similar to those of caraway. Handling, drying, and storage are as described for anise.

Dill has been proved to be an effective antimicrobial agent for *P. aeruginosa and S. flexneri*.

Fennel

The spice is the dried fruit of *Foeniculum vulgare* Miller (Apiaceae). The schizocarp is oblong–oval or elliptical, usually with long pedicels (4–5 mm by 1–3.5 mm); at the end is a short, conical stylopodium. The mericarps are attached to a divided carpophore and separate easily. Each is broadly elliptical, more or less curved (dorsal surface is convex), and light brown to light olive in color, with five prominent, longitudinal, primary ribs (Figure 2(o)–2(q)). Based on the appearance of the plant and seed and the compositional data of the volatiles from the fruit, fennel is classified into two subspecies and further classified into numerous varieties. The important varieties are the following:

- *F. vulgare* ssp. *piperitum* wild fennel
- *F. vulgare* ssp. *capillaceum* with three varieties
  (1) *F. vulgare* var. *vulgare* – bitter fennel
  (2) *F. vulgare* var. *dulce* – sweet fennels
  (3) *F. vulgare* var. *azoricum* – culinary Florence fennel

In the transverse section, the epidermis of the exocarp shows polygonal tabular cells, having occasional stomata and a smooth cuticle; the mesocarp is parenchymatous with two to four vittae on the commissural side and four vittae on the dorsal side, whereas the endocarp consists of narrow, elongated cells in a parquetry arrangement in a transverse section as long, narrow, rectangular cells with groups of short cells. The endosperm has a structure similar to that of anise (Figure 2(r) and 2(s)).

Fennel has a pleasant, aromatic odor and a taste resembling that of anise. The volatile oil content in fennel ranges from 0.7% to 6.0%. The main constituent of oil is anethole. Handling, drying, and storage of this spice are also similar to those of anise.

Fennel has been proved to be an effective antimicrobial agent for *P. aeruginosa and S. flexneri*.

Star Anise

Star anise is the ripe dried fruit of *Illicium verum* Hook. Filius (Magnoliaceae). The fruit whorl (2.5–4.5 cm in diameter) is formed of six to eight (rarely seven to nine), one-seeded, boat-shaped, woody, wrinkled, ridged, reddish brown follicles that are internally smooth, lustrous, and light brown, about 9–19 mm in length, and surmounted on a curved peduncle. The seed is light brown, compressed-ovoid, shiny, and smooth with a conspicuous hilum and raphe (Figure 2(t) and 2(u)).

In the transverse section, the exocarp consists of a striated cuticle and epidermal cells bearing stomata. The mesocarp consists of parenchymatous cells with brown contents. The cells toward the central zone are larger (up to 220 μm in length), and the inner mesocarp cells have thicker walls, the thickness increasing toward the dehiscent side of the carpel. Resin and stone cells occur scattered throughout the mesocarp, and vascular bundles occur in between the central and inner regions. The endocarp consists of thin-walled, sclerenchymatous palisade cells up to 440 μm in length. The seed coat consists of an outer, thick-walled epidermis of sclerenchymatous, pitted palisade cells up to 200 μm in length, followed by five layers of sclerenchyma and two or more layers of parenchyma containing numerous calcium oxalate crystals (Figure 2(v)). The odor and taste of star anise are anise-like. The plant starts fruiting at the age of 6 years and continues until it is over 100 years old. Fruits are collected before full maturity and dried in the sun.

Vanilla

Vanilla is the cured, fully grown, unripe fruit of *Vanilla planifolia* Andr. (Orchidaceae). The fruits are linear and flattened (120–350 mm by 5–9 mm), and both ends taper; the fruit occasionally splits into three parts in the tip; fruits are externally dusky to moderate brown, longitudinally wrinkled, and covered with minute crystals of vanillin; many minute seeds are embedded in a brownish black, balsamic fluid (Figure 2(w) and 2(x)).

In the transverse section, the outer epidermis of the epicarp shows red to brown bodies, some with calcium oxalate and vanillin crystals. The hypodermis is composed of collenchymatous cells with dark-colored contents, and the sarcocarp consists of loosely arranged, large deeply undulate parenchyma, becoming smaller on the inner side; most cells contain brownish contents and oily globules, and some have raphides of calcium oxalate up to 400 μm long. There is an interrupted circle of leptocentric fibrovascular bundles surrounded by a few spiral or pitted tracheae. The cells of the inner epidermis of the endocarp are elongated into numerous, thin-walled, nearly

straight, glandular hairs containing a gummy, resinous secretion (Figure 2(x) and 2(y)).

At the time of harvest, in the vanilla fruit or pods, the normal aromatic components forming the characteristic vanilla flavor do not exist in their final form, but appear only following a curing (fermentation) process involving enzymatic actions on glucosides, the most important of which is glucovanillin, which produces vanillin (the main aromatic component of vanilla) and sugar as a result of the action of glucosidase. Similarly, aldehydes, protocatechuic acid, benzoic acid, vanillic acids, anisic alcohol, etc. are also formed. These different compounds impart subtlety to the fragrance of natural vanillin.

The different stages of the curing methods are as follows:

(1) Cessation of the vegetative life of the bean to allow the onset of enzymatic reactions
(2) Raising of the temperature to promote this action and to stimulate at first rapid drying, thereby preventing the appearance of harmful fermentation
(3) Slower drying followed by the production of different fragrance components
(4) Conditioning of the product, during which the procedures for commercial presentation and to obtain a good preservation are carried out

The improved method involves sorting or grading, scalding or blanching, autoclaving, loading, and mechanically drying in a tunnel dryer, followed by drying in the shade. After scalding and autoclaving, the vanilla is chopped into 20 or 30 cm pieces. This leads to an acceleration of the oxidative and enzymatic reactions. The cuts are put through the dryer at a temperature of 65 °C each day. As soon as they come out, they are put into an isothermal chest for the next 24 h, with the temperature being maintained at around 50 °C. These operations are repeated for 12 days, after which a product containing 20–25% moisture is obtained, with a yield of about 4.5–1.0. The transfer to the isothermal chests after the tunnel drying is vital, in addition to equilibrate their moisture content. The process requires a series of sheds or buildings for 'sunnings' and 'sweatings' and 'mahogany sweating boxes' or 'aging boxes.' The total process takes about 5–6 months, including aging. Finally, the beans are graded, bundled, and packed in special tins lined with waxed paper.

The best-grade beans are very dark, chocolate brown in color and oily in appearance with a strong vanilla odor. Vanilla should be stored in a cool, dry place in an airtight container to stop the beans becoming brittle.

Fenugreek

Fenugreek is the dried seed of *Trigonella foenum-graecum* L. (Fabaceae). The seeds are yellowish brown, oblong–rhomboidal (2.5–5 mm by 2–3.5 mm) with an oblique furrow on one side, smooth, hard, and mucilaginous. The hilum is in a notch, the raphe short, the endosperm translucent, and the embryo waxy and yellow (Figure 3(a)–3(d)).

In the cross section, the spermoderm consists of an outer cuticle followed by a layer of palisade cells (measuring 65–70 μm by 8–20 μm) with flat bases and pointed apices, a layer of hourglass cells, and four to five layers of parenchyma. The endosperm consists of an outer layer of cells containing aleurone grains, followed by several layers of thin-walled mucilaginous cells; the embryo cells contain aleurone grains and fixed oil (Figure 3(d)).

Fenugreek possesses a strong, pleasant, sweetish odor and tastes mucilaginous, farinaceous, and slightly bitter. The pods split easily and are threshed on the floor with sticks to free the seeds. The seeds are then winnowed, cleaned, and further dried in the sun. Dried fenugreek seeds can be stored for about 2 years.

Mustard

Mustard is composed of the dried, mature seeds of the following *Brassica* L. species (Brassicaceae): *B. nigra* (L.) Koch (black mustard), *B. alba* Boiss. (white or yellow mustard), and *B. juncea* (L.) Czern & Coss. (Rye or Indian mustard).

Black mustard seeds are globular, 1–1.6 mm in diameter, dark brown to nearly black, minutely reticulate, and mucilaginous. The kernel is greenish yellow and oily (Figure 3(e) and 3(f)). White mustard seeds are spherical, about 2 mm in diameter, minutely pitted, and mucilaginous (Figure 3(h)). Indian mustard seeds resemble black mustard but are larger and brown in color.

The testa of black mustard in transverse section consists of an outer epidermis of polygonal tubular cells, 40–50 μm long, containing mucilage; hypodermis consists of empty, polygonal cells, 10–105 μm by 60–90 μm; the inner epidermis of testa consists of slightly lignified, palisade cells (unequal in height), followed by cells of tegmen containing tannin. The endosperm has an outer aleurone layer of thick-walled cells containing fixed oil and aleurone grains and an underlying layer of collapsed cells. Embryo cells are polyhedral and thin-walled, containing fixed oil and aleurone grains (Figure 3(g)). White mustard in the transverse section resembles black mustard but differs in that it has two layers of somewhat collenchymatous cells beneath the epidermis of the testa; the palisade cells have no tannin (Figure 3(i)).

Black mustard is slightly odorous and tastes acrid and pungent when crushed and moistened. White mustard seed is not pungent. The fruits are harvested when they begin to turn yellow. Then, they are threshed by beating with wooden mallets. The threshed seeds are separated from the husks by winnowing and then dried in the sun for a couple of days. Seeds are stored in seed bins or gunny bags. Storage of the whole seed for 12 months reduces the volatile oil content by 54%, but for ground seeds, the loss is 84%.

Nutmeg and Mace

Nutmeg is the dried kernel, and mace is the dried aril of the seed of *Myristica fragrans* Houtt. (Myristicaceae). The kernel constitutes 16–17% and mace constitutes 2.0–2.5% by weight of the fruit. Nutmegs are ovoid (2–3.5 cm by 1.5–3 cm) and the kernels are grayish brown externally and reticulately marked with small furrows. Numerous minute, dark reddish brown points and lines, constituting a thin layer of perisperm,

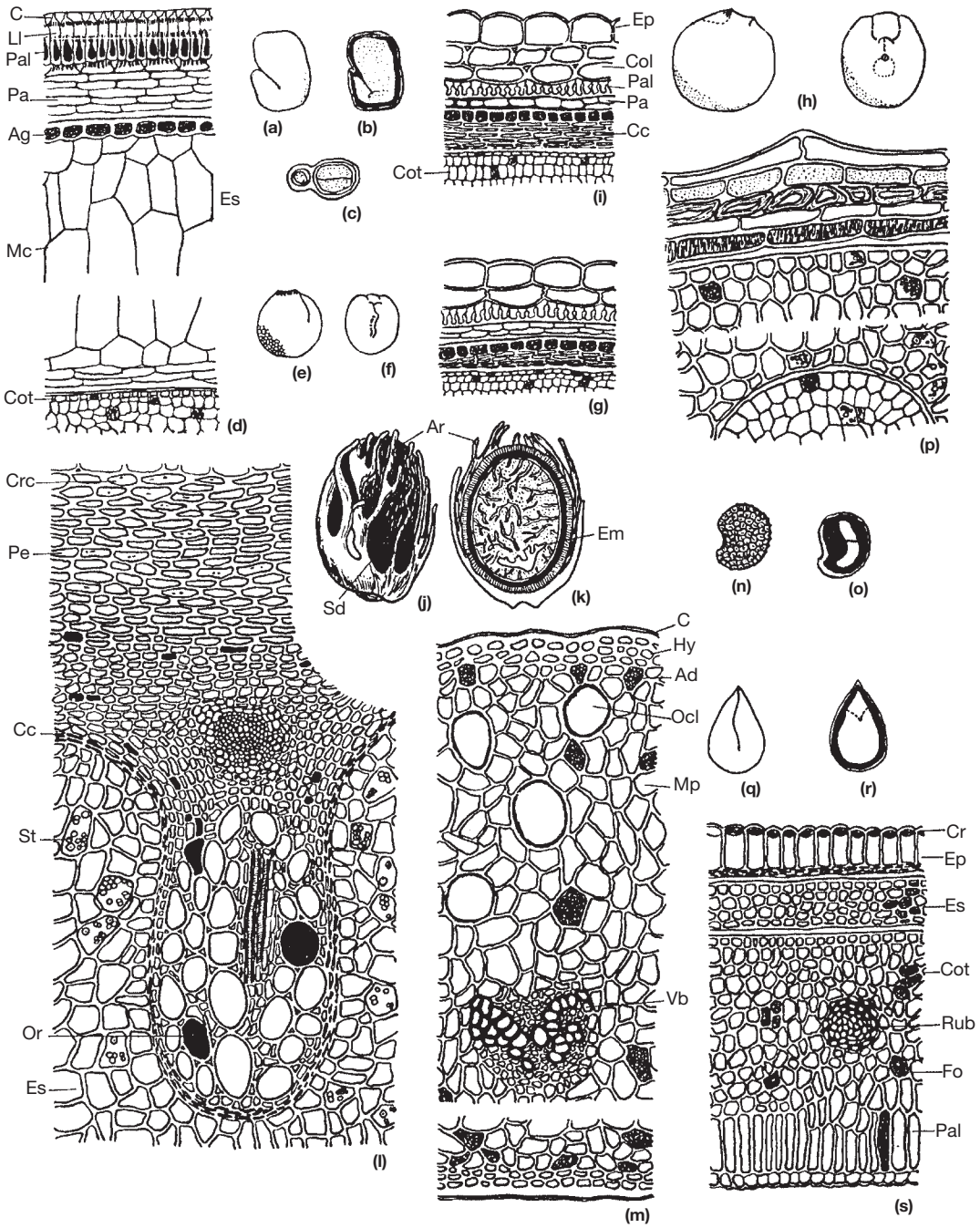

Figure 3 Spices from seeds. Fenugreek: (a) seed; (b) longitudinal section of seed; (c) transverse section of seed; (d) cross section through seed coat, endosperm and part of cotyledon. Mustard black: (e, f) seed; (g) cross section through seed coat, endosperm and part of cotyledon; (h) Mustard, white or yellow; (i) cross section through seed coat, endosperm and part of cotyledon. Nutmeg and mace: (j) nutmeg surrounded by mace; (k) same as (j), cut longitudinally; (l) cross section through perisperm and endosperm of nutmeg; (m) cross section through mace. Poppy: (n, o) seed; (p) cross section through seed coat, endosperm and part of cotyledon. Sesame: (a, r) seed; (s) cross section of seed. Abbreviations: Ad, amylodextrin granules; Ag, aleurone grains; Ar, aril; Bc, beaded cells; Bel, beaker cells; C, cuticle; Cc, compressed cells; Ch, chloroplast, Col, collenchyma; Cot, cotyledon; Cp, carpophore; Cr, crystal; Crc, crystal cells; Cro, cross cells; Dl, dehiscence line; Em, embryo; End, endocarp; Ep, epidermis; Es, endosperm; Exo, exocarp; Fl, fiber layer; Fo, fixed oil; Gc, giant cell; H, hair; Hc, hourglass cells; Hp, horny protein; Hy, hypodermis; Li, light line; Mc, mucilage cells; Mes, mesocarp; Mp, mesophyll; O, oil; Oc, oil cavity; Ocl, oil cells; Or, oleoresin; P, pedicel; Pa, parenchyma; Pal, palisade; Pe, perisperm; Pl, placenta; R, raphe; Rc, reticulated cells; Re, resin; Rub, rudimentary vascular bundle; Sc, seed coat; Scl, sclerenchyma; Sd, seed; Se, secretory cells; St, starch; Stc, stone cells; V, vittae; Vb, vascular bundle. Reproduced from Macrae, R., Robinson, R. K. and Sadler. M. J. (eds.) (1993). Spices and flavoring (flavoring) crops: fruits and seeds. *Encyclopedia of food science, food technology and nutrition*. London: Academic Press.

grow inward into the endosperm at the furrows; the hilum lies in a small, circular depression at one end (Figure 3(j) and 3(k)). Mace consists of flattened lobed pieces about 25 mm or more in length, somewhat less in breadth, 1 mm thick, buff or light brown, translucent, smooth, horny, and brittle (Figure 3(j) and 3(k)).

In the longitudinal section, nutmeg has a perisperm of about 0.12 mm thick. The cells of the peripheral perisperm are flattened and polyhedral; some contain prisms of an unidentified crystalline substance. The cells of the inner perisperm are parenchymatous and grow into endosperm, blunt-ended ruminations, each containing a vascular strand and numerous volatile oil-containing cells. The endosperm contains abundant bunches of needle-shaped, fat crystals; small, irregular aleurone grains with large crystalloid; and rounded (up to 22 μm in diameter) simple or compound starch grains and scattered tannoid cells (Figure 3(l)).

In the transverse section, mace contains the following: an outer epidermis of irregularly shaped, thick-walled cells covered by cuticle; a hypodermis consisting of a layer of thick-walled cells and one or more additional layers of comparatively thin-walled cells; and a parenchymatous mesophyll consisting of densely packed amylodextrin granules, numerous large oleoresin cells, and vascular tissues (Figure 3(m)).

Nutmeg and mace have a characteristic nutlike aroma and taste. The volatile oil content of nutmeg and mace is 15% and 20%, respectively. The fruits are harvested when split on the tree or, more usually, gathered after they have fallen to the ground. Mace is separated from the pericarp by hand or between boards, without breakage (which reduces its quality), and dried in the sun or artificially. Drying of mace and its storage is a delicate process. Mace has to be dried to a 5% moisture level, for which it takes about 16 h under open sun, 24–28 h in the shade, and 5–6 h in mechanical drying. A mild blanching followed by sulfiting helps in the retention of color and quality of mace. Nutmegs are dried in their shells on large wooden trays and turned daily for uniform drying and to avoid fermentation. Artificial drying between 35 and 40 °C prevents cracking of the shells and any melting of fat in the kernels. Various traditional methods are practiced to crack the nuts, which are stored and graded according to size and quality. This involves immersing the nutmegs in water and separating unhealthy or floating ones; the sound ones are drained, air-dried, and stored in bins before packing. Introducing a little carbon disulfide prevents insect attack.

Extract from nutmeg and mace has been found to be an effective antimicrobial agent for *Bacillus subtilis*, *Bacillus sphaericus*, *Staphylococcus aureus*, *Klebsiella aerogenes*, and *Chromobacterium violaceum*.

Poppy Seeds

Poppy seeds are obtained from *Papaver somniferum* L. (Papaveraceae) capsules. The seeds are white, yellow, brown, bluish, or nearly black and subreniform (1–1.25 mm long), with a reticulate surface; the hilum and micropyle are situated in a depression near one end (Figure 3(n) and 3(o)).

In the cross section, the seed consists of an outer epidermis of tangentially elongated, thin-walled cells, a layer of parenchymatous cells containing numerous very small prismatic crystals of calcium oxalate, a layer of thick-walled cells, a layer of parenchymatous cells, and a layer of thick-walled reticulate cells. The cells of the endosperm and embryo are polygonal and contain aleurone grains and globules of fixed oil (Figure 3(p)).

The odor and taste of the seeds are pleasant and nutlike. For the collection of the seeds, the capsules are dried on the plant for about 15 days. After plucking by hand, these are threshed, and the seeds are separated and stored.

Sesame

Sesame is the seed of *Sesamum indicum* L. (Pedaliaceae). The seeds are yellowish white, brown, or gray to black, flat pyriform (2.5–3.5 mm by 1.2–2 mm by 1 mm), and finely punctate, with four delicate, longitudinal ridges at the edges of the flat faces. The raphe extends from the hilum at the pointed end as a line along the center of one flat face to a broader end, and the scanty endosperm surrounds a large embryo. The hulled seeds are cream or pearly white in color (Figure 3(q) and 3(r)).

In the cross section, the seed coat consists of an epidermis of radially elongated cells (30–60 μm on sides and 45–130 μm at ridges), with thin, cutinized outer walls; each cell contains a 'rosette' calcium oxalate crystal; the rest of the testa consists of collapsed cells containing numerous, prismatic calcium oxalate crystals, and the endosperm and cotyledons consist of polygonal parenchymatous cells containing fixed oil and small aleurone grains measuring 2–10 μm (Figure 3(s)).

The seeds have a faint nutty odor and taste and are ripe at the end of about 5 months. The whole plants are then cut down and made into stooks to dry, and the seeds are shaken out from the capsules and stored.

Sesame seeds have been proved to have moderate amounts of antioxidants.

See also: Drying: Physical and Structural Changes; Drying: Principles and Types; Sensory Evaluation: Sensory Rating and Scoring Methods.

Further Reading

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Spices and Flavoring Crops: Leaf and Floral Structures

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Introduction

The aromatic leaves and floral structures of more than 30 plants are commonly used as spices and flavorings. The leaves and floral structures have delicate flavors, and these spices require proper care, particularly during the drying process. Leaf spices are also used fresh. The diagnostic morphological and anatomical characters of the most commonly used spices are dealt with in detail, along with their common names, botanical names, family, and parts used. Handling, drying, and storage details are given wherever available. Additionally, data about antioxidant content and antifungal or bacterial properties have been given where applicable. Other spices that have specific, but equally important, uses are summarized in Table 1.

Chervil

Chervil consists of the dried leaves of *Anthriscus cerefolium* Hoffm. (Apiaceae). The leaves are decompound and oval, and the leaflets are 2–5 × 2–4 cm, alternate, thin pale green, and pinnate; a few hairs occur on the midvein and the margins of leaflets (Figure 1(a)).

In surface view, the lower epidermal cells have more wavy walls than those on the upper epidermis; stomata are numerous on the lower epidermis, but absent on the upper; hairs, usually unicellular and pointed, are on both surfaces of the main vein and the margins, but more arise on the lower surface; the mesophyll consists of a row of palisade cells, 18–75 × 16–35 μm in size, followed by spongy parenchyma containing vascular bundles; an oil duct is found below the midvein (Figure 1(b) and 1(c)).

The odor of chervil somewhat resembles that of caraway and anise. The leaves, after collection, are then dehydrated by circulating hot air (50 °C) and fragmented mechanically and packed in plastic-lined bags.

Laurel or Sweet Bay

The leaves are derived from *Laurus nobilis* L. (Lauraceae). They are lanceolate–acuminate, 10 cm or more long, coriaceous, pellucid-punctate, and with revolute, entire margins; the upper surface is glabrous and shiny and olive green to brown; the lower surface is dull olive to brown with a prominent midrib and veins (Figure 1(d)).

In a cross section, the leaf has a thick cuticle, and the epidermal cells, in surface view, have sinuous, pitted, and thick walls; the lower epidermal walls are more curvilinear and distinctly beaded; the lower epidermis alone bears stomata arranged singly or in pairs; the mesophyll has two layers of palisade parenchyma and a region of spongy parenchyma containing scattered spheroidal oil reservoirs and fibrovascular and collenchyma tissues; the latter occur above and below the vascular bundle of the midrib, but not in the veins (Figure 1(e)–1(h)).

The leaves are characteristically fragrant when crushed but taste bitter.

Laurel has been shown to be an effective antimicrobial agent for *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus*. Laurel has also been shown to have a moderate antioxidant content when cultivated but a higher content when found in the wild.

Marjoram (Sweet)

Marjoram consists of dried leaves, with or without flowering tops, of *Majorana hortensis* (L.) Moench (Lamiaceae). The pinnate leaves are obovate to broadly elliptical, 21 × 11 mm in size with entire margins and short petioles; both surfaces are grayish green, glandular-punctate, and pubescent. The floral parts have light grayish yellow, obovate, thin, and up to 5.5 × 3.5 mm bracts; gamosepalous calyxes, about 3 mm long with five prominent teeth; and gamopetalous, white to pink or pale lilac corolla, about 3.5 mm long (Figure 1(i)).

In a transverse section and/or surface view, it is possible to see an epidermis with a thin cuticle, one to two palisade layers, and upper and lower epidermal cells with slightly wavy, knotty, and vertical walls. Glandular and nonglandular hairs occur on both surfaces, and the latter are uniseriate, with two to five cells in length and more or less bent inward at the apex; some are papillose. Glandular hairs are of two types, one with a one- to two-celled stalk and a one- to two-celled glandular head and the other with no stalk and a large, eight-celled head; the latter is sunk in a depression in the epidermis; stomata occur on the lower epidermis with two 'neighboring' cells (Figure 1(j) and 1(k)).

The odor and taste of sweet marjoram are aromatic, characteristic, and slightly pungent; the plants are harvested as soon as they flower. The plants are cut and tied in bunches and dried in open air or spread on wire trays in ventilated rooms and dried by circulating warm air.

Marjoram was shown to contain a considerable amount of antioxidants that could be most effectively extracted using supercritical fluid extraction.

Mint

Mint consists of the dried leaves and flowering tops of *Mentha piperita* L. (peppermint) and *Mentha spicata* L. (spearmint) belonging to the family Lamiaceae. *Mentha piperita* is a hybrid

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Table 1 Some other commonly used spices from leaves, flowers, and flower parts

Botanical name	*Common name*	*Family*	*Part used*	*Characters*
Allium ascalonicum L.	Shallot	Liliaceae	Bulb	Bulbs about the size of a nut, white, leaves basal; bulbs may be taken up and dried or allowed to remain in the ground and dug up when needed; after lifting, they are skinned, washed, and tied in bunches for marketing
Allium cepa L.	Onion	Liliaceae	Bulb	Bulbs have variable sizes: small bulbs are silvery and large bulbs are red or yellow in color; bulbs are harvested when mature, which is indicated by the tops drooping just above the bulb while the leaves are still green; onions are stored in crates, and before storage, they are thoroughly cured
Allium fistulosum L.	Leek	Liliaceae	Leaf	Leaves terete and hollow, usually clustered
Allium sativum L.	Garlic	Liliaceae	Bulb	Compound bulb, subglobose, has 8–15 bulblets, bulblets ovoid, three- to four-sided, covered with whitish scale leaf that encloses whitish flesh, one or two translucent scales, in the center, two or three yellowish to yellowish green leaves present; harvested when tops turn yellow, exposed to sun for 4–5 days and winnowed; after a fortnight of curing, they are packed in bags and stored
Allium schoenoprasum L.	Chive	Liliaceae	Leaf	Leaves 10–25 cm, terete or grooved above, scape 15–35 cm; the cut leaves are gathered into bunches of about 2.5 cm diameter and secured and packed in layers in trays
Angelica archangelica L.	Angelica	Apiaceae	Leaf and young stem	Leaves ovate, 30–90 cm, two- to three-pinnate, ultimate pinnae toothed; leaves cut for drying and frozen in early summer; the stems should be harvested before the plant flowers
Cinnamomum tamala Nees & Eberm	Tejpat (Indian cassia lignea leaf)	Lauraceae	Leaf	Leaves subcoriaceous, opposite, triple-nerved rarely alternate, elliptic–oblong, acute base, acuminate, glabrous, shining; leaves are collected and dried in sun for 3–4 days and packed in a cylindrical bamboo nets for marketing
Gaultheria fragrantissima Wall.	Wintergreen	Ericaceae	Leaf	Leaves alternate, oblong–lanceolate to elliptic rhomboid, 7–13 × 2–6 cm, serrate, stiffly coriaceous, glands dotted
Hyssopus officinalis L.	Hyssop	Lamiaceae	Leaf	Leaves sessile, 13 × 2–8 mm; opposite, linear, or lanceolate; entirely hairy on both surfaces; harvested just as flowers begin to bloom
Levisticum officinale Koch	Lovage	Apiaceae	Leaf and flowering top	Lower leaves large with long petioles, up to three-pinnate, about 70 cm long; uppermost leaves entire and lanceolate; flowers yellow in stiff umbels; fresh leaves are generally used; drying of thick leaves takes 4–5 days
Marrubium vulgare L.	Farasiyum	Lamiaceae	Leaf	Leaves opposite, petiolate, downy, oval, up to 5 cm long, surface wrinkled, and has protuberances, margin dentate; harvested before full flowering and dried in the shade below 35 °C
Melissa officinalis L.	Balm	Lamiaceae	Leaf	Leaves opposite, ovate, or triangular; up to 3.5 cm long, hairy, margin broadly dentate; usually, fresh leaves are used; for drying, stems are cut and tied in small bunches and hung in airy space
Mentha pulegium L.	Pennyroyal	Lamiaceae	Leaf	Leaves shortly petioled, up to 1.25 cm long, round to oval, entire or slightly crenate and pubescent; leaves are used fresh or in dried form
Murraya koenigii L. Spreng.	Curry leaf	Rutaceae	Leaf	Leaves imparipinnate, leaflets 9–25, ovate–lanceolate, dentate, acuminate, base oblique, glabrous above, pubescent beneath, gland-dotted; fresh or shade-dried leaves are used
Nepeta cataria L.	Catnip	Lamiaceae	Leaf	Leaves ovate, coarsely crenate; harvested when in full bloom
Pimenta racemosa (P. Miller) J. W. Moore	West Indian bay	Myrtaceae	Leaf	Leaves obovate or elliptic, leathery, shining above; during harvesting, either leaves are stripped from the trees or both shoots and leaves are removed and tied in bundles for drying
Ruta graveolens L.	Rue	Rutaceae	Leaf	Leaves alternate, 15 cm long, two to three, pinnate, oblong to spathulate, with many small oil glands; leaves are usually used fresh
Salvia sclarea L.	Clary, clary sage	Lamiaceae	Leaf	Leaves petiolate, opposite, ovate, apex obtuse, base cordate, crenate margin, thinly hairy above
Tanacetum vulgare L.	Tansy	Asteraceae	Leaf and flowering top	Leaves downy, one to two pinnate, margin dentate, capitula in flat umbels, golden yellow, about 1 cm in diameter; collected when in flower and dried in the shade at below 35 °C

between *Mentha spicata* and *Mentha aquatica* since *Mentha spicata* itself is a hybrid. Therefore, *Mentha piperita* is a triple hybrid. It is an allohexaploid, $2n = 6x = 72$. However, this species has a variable chromosome number, which ranges from $2n = 36$ to $2n = 134$ with polyploid and aneuploids. *Mentha spicata* ($2n = 48$) has originated by hybridization between *Mentha longifolia* and *Mentha suaveolens* and doubling of chromosomes. The resultant polyploid behaves as allotetraploid. Peppermint leaves are dark green, about 4.5 × 2.3 cm, petiolate, ovate, and with serrate margins. The apex is acute and the

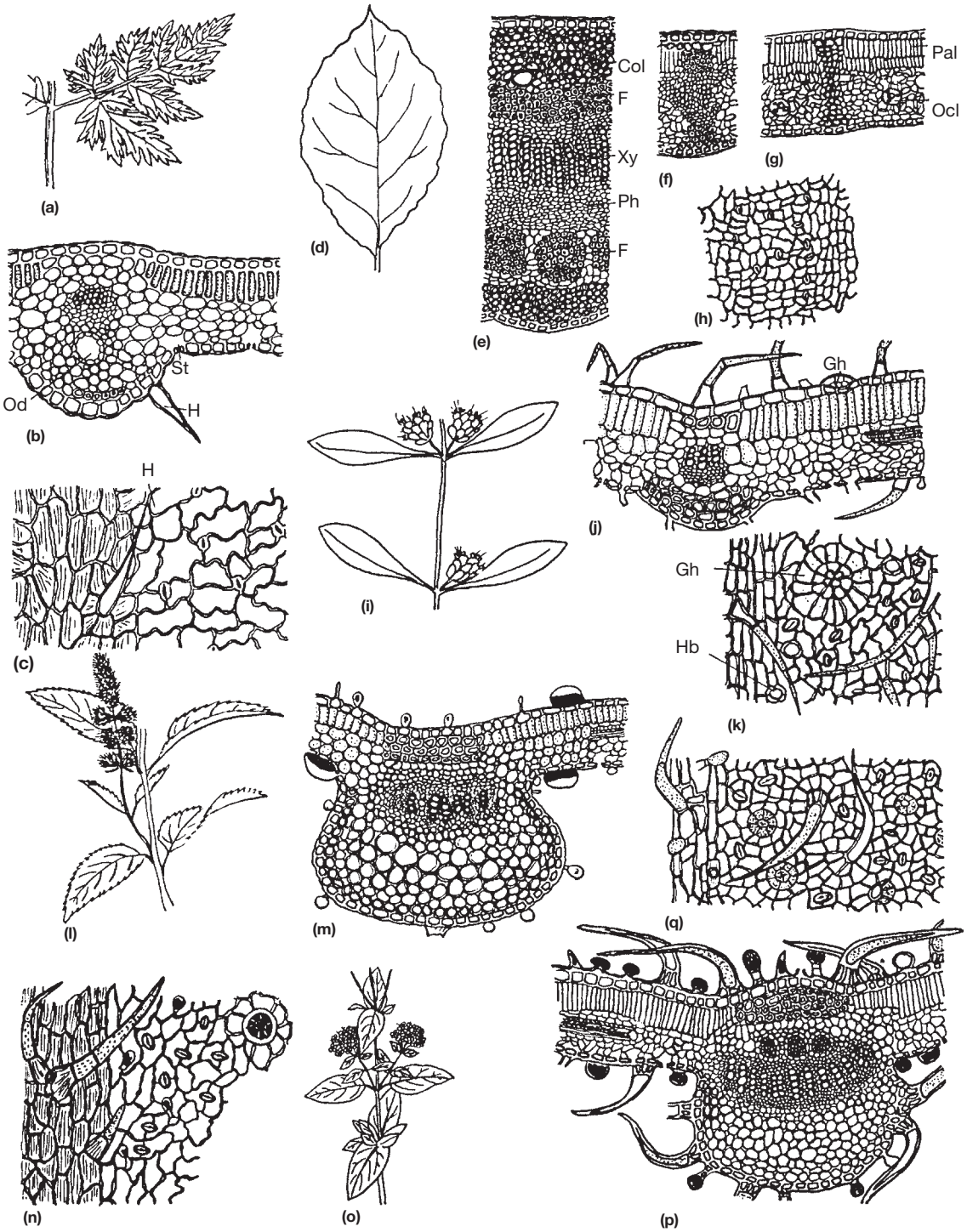

Figure 1 Spices from leaves and flower tops. Chervil: (a) leaf; (b) cross section of leaflet through midrib; (c) lower epidermis in surface view. Laurel: (d) leaf; (e) cross section of leaf through midrib; (f) vein; (g) leaf blade; (h) lower epidermis in surface view. Marjoram: (i) a portion of flowering twig; (j) cross section of leaf through midrib; (k) surface view of lower epidermis. Peppermint: (l) a portion of flowering twig; (m) cross section through midrib of leaf; (n) surface view of the lower epidermis. Origanum: (o) a portion of flowering twig: (p) cross section through midrib of leaf; (q) lower epidermis in surface view. Abbreviations: C, calyx; Col, collenchyma; Cor, corolla; Cp, compressed parenchyma; E, endocarp; F, fiber; Gh, glandular hair; H, hair; Hb, hair base; Hy, hypodermis; Mes, mesophyll; O, ovary; Oc, oil cavity; Ocl, oil cell; Od, oil duct; P, pericarp; Pal, palisade; Pap, papilla; Pg, pollen grain; Ph, phloem; Pp, pigmented parenchyma; R, receptacle; Rc, reticulate cells; S, stigma; Sc, stone cells; St, stomata; Sta, stamen; Sty, style; Vb, vascular bundle; Xy, xylem. Reproduced from Spices and flavoring (flavouring) crops: leaf and floral structures. In: Macrae, R., Robinson, R. K. and Sadler, M. J. (eds.) *Encyclopaedia of food science, food technology and nutrition*. London: Academic Press, 1993.

midrib is prominent, and dot-like oil glands are visible with a hand lens. Nonglandular hairs are present on the lower epidermis over the midrib and veins at the margins, and a few arise on the midrib of the upper epidermis. Verticillaster inflorescences are usually arranged in compact, oblong, or oval terminal spikes: calyxes are gamosepalous, purplish, about 3 mm long, glandular-punctate, and five-toothed; corolla is short, tubular, light purple, and four-cleft; there are four stamens; and style is with bilobed stigma (Figure 1(l)). Spearmint differs from peppermint in having a sharply pointed, long, narrow spike; an absence of nonglandular hairs in the upper epidermis; fewer and shorter hairs on the lower surface over the midrib, with few, if any, over the veins; and the absence of crystals in the glandular hairs.

In a cross section, peppermint leaves show a cuticle covering at both the upper epidermis and lower epidermis, with the upper epidermal cells having moderately thicker outer walls than the lower epidermal cells; glandular hairs containing groups of crystals; a mesophyll consisting of a layer of palisade cells and spongy tissue; and a conspicuous group of collenchyma cells between the upper epidermis and the vascular tissue of the midrib and veins. In the surface view, the cuticle is striated over the midrib, veins, and margins, and the upper epidermal cells are irregular with wavy walls, except in the latter regions; numerous small crystals are present in the cells of the upper epidermis and lower epidermis (Figure 1(m) and 1(n)).

Peppermint has a characteristic aromatic odor and taste, with a cooling sensation on the breath, whereas the odor and taste of spearmint are characteristic and aromatic but not followed by a cooling sensation. Mints are harvested, when in bloom, in bright sunny weather, by cutting with a sickle 2–3 cm above the ground. The harvested herb is allowed to dry partially in the open. Drying is completed by circulating warm air, which avoids overheating and obviates drying for too long. After attaining the desired brittleness, the leaves are stripped from the stem and completely dried. Afterward, they are fragmented mechanically or through wire sieves.

Origanum or Oregano

This consists of the dried leaves and flowering tops of *Origanum vulgare* L. (Lamiaceae). The leaves are light green to greenish brown, with crenate to crenate–serrate margins, and are about 3 cm long, and are glandular-punctate. The flowers are in spikelike clusters: bracts are broadly ovate, $2.5–3 \times 2$ mm with prominent hairs on the outer surface; calyxes are gamosepalous, four-toothed, and deeply clefted on two sides; corolla is 5 mm long and gamopetalous; four stamens are epipetalous; and style is plumose (Figure 1(o)).

In surface view and/or transverse section, the epidermal cells of both surfaces of the leaf are irregular in shape with uneven and thickened walls; both surfaces have numerous curved, pointed, unicellular, nonglandular hairs up to 900 μm long; the surfaces of some hairs are smooth, whereas many have papillose surfaces. Uni- to multicellular glandular hairs are present on both surfaces (Figure 1(p) and 1(q)).

Origanum has a strong camphoraceous odor and a warm, pungent, bitter, camphoraceous taste. The plant is collected when in bloom and dried in the shade at $< 35\ ^\circ C$. Both Turkish oregano and Greek oregano differ from Mexican oregano in aroma and flavor. Oregano has been shown to be an effective antimicrobial agent due to its thymol and carvacrol content.

Parsley

Parsley consists of the dried leaves of *Carum petroselinum* Hoffm. (Apiaceae). The leaves are pinnately compound of three-lobed leaflets; each lobe is divided into smaller, toothlike segments that are usually curly (Figure 2(a)).

The upper and lower epidermal cell walls are, in surface view, sinuate, and stomata are few in the upper and numerous in the lower epidermis. In a cross section, beneath the upper epidermis, the mesophyll consists of a layer of palisade cells about 40–55 μm long followed by a spongy parenchyma; below the vascular bundle of the midrib is an oil duct (Figure 2(b) and 2(c)).

The dried leaves have a characteristic, agreeable, aromatic odor and taste. Fresh leaves are dried rapidly under controlled conditions of heat and air flow to avoid loss of essential oils and to preserve a good, green color.

Rosemary

Rosemary consists of the dried leaves of *Rosmarinus officinalis* L. (Lamiaceae). The leaves are about 3.5 cm long and 2–4 mm wide, numerous, opposite sessile, linear, leathery, entire, and slightly glossy with revolute margins. The upper surface is dark green, while numerous branched trichomes on the lower surface make it gray and wooly and glandular-punctate with a prominent midrib (Figure 2(d)).

In surface view, the cells of the upper epidermis are polygonal with unevenly thickened and beaded walls, while the lower epidermal cells are larger and thin-walled. In a cross section, the collenchyma tissue present below the upper epidermis forms a thick-walled hypodermal layer; collenchymatous cells also surround the vascular bundle of the midrib. One to three layers of palisade cells are up to about 80 μm in length and are followed by a zone of spongy parenchyma. A dense, tangled mass of branched, multicellular, smooth hairs cover the lower epidermis, and the tips of the hairs are pointed and curved; glandular hairs are uni- to multicellular. In the midrib vascular bundle, the vessels are arranged in radial rows (Figure 2(e) and 2(f)).

The odor of rosemary is characteristically aromatic, having a slight camphoraceous note; the taste is pungent, aromatic, bitter, and camphoraceous. Rosemary is harvested when flowering has started. At that time, 30–50 cm of the top along with leaves and flowers is harvested with a sickle or by forage harvester. Care should be taken not to cut the woody portions of the stem. The first harvest is obtained $\sim$6 months after planting, and subsequent harvests can be obtained every 4 months. Harvested leaves are dried in the shade.

Rosemary proves to be an effective antimicrobial agent for *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Candida albicans*. Additionally, rosemary antioxidants have allowed it to be used in meat formulations.

Figure 2 Spices from leaves and flower tops. Parsley: (a) a portion of flowering twig; (b) cross section of leaflet through main vein; (c) surface view of lower epidermis. Rosemary: (d) a portion of flowering twig; (e) cross section through midrib of leaf; (f) lower epidermis in surface view. Sage: (g) a portion of flowering twig; (h) cross section through midrib of leaf; (i) cross section of a portion of leaf blade; (j) lower epidermis in surface view. Savory: (k) a portion of flowering twig; (l) cross section through midrib of leaf; (m) cross section through leaf blade; (n) lower epidermis in surface view. Sweet basil: (o) a portion of flowering twig; (p) cross section of leaf through midrib; (q) cross section through side vein; (r) surface view of upper epidermis. Abbreviations: C, calyx; Col, collenchyma; Cor, corolla; Cp, compressed parenchyma; E, endocarp; F, fiber; Gh, glandular hair; H, hair; Hb, hair base; Hy, hypodermis; Mes, mesophyll; O, ovary; Oc, oil cavity; Ocl, oil cell; Od, oil duct; P, pericarp; Pal, palisade; Pap, papilla; Pg, pollen grain; Ph, phloem; Pp, pigmented parenchyma; R, receptacle; Rc, reticulate cells; S, stigma; Sc, stone cells; St, stomata; Sta, stamen; Sty, style; Vb, vascular bundle; Xy, xylem. Reproduced from Spices and flavoring (flavouring) crops: leaf and floral structures. In: Macrae, R., Robinson, R. K. and Sadler, M. J. (eds.) *Encyclopaedia of food science, food technology and nutrition*. London: Academic Press, 1993.

Sage

Sage consists of the dried leaves of *Salvia officinalis* L. (Lamiaceae). The leaves are grayish green, oblong–lanceolate, about 8.5×2.5 cm, petiolate, and with an acute apex, finely crenate margins, densely pubescent and glandular-punctate having glands obscured by hairs (Figure 2(g)).

The upper epidermal cells, in surface view, have few stomata and straight, beaded walls, whereas the lower epidermis bears numerous stomata, and the cells have wavy, beaded walls; numerous nonglandular and glandular hairs occur on both surfaces. The nonglandular hairs are uni- or multicellular, and the multicellular hairs are more numerous, very long, narrow-pointed, and mostly smooth, but some have a papillose surface; glandular hairs are also uni- or multicellular. The midrib vascular bundle is surrounded by parenchymatous and collenchymatous cells, and the mesophyll consists of one to three layers of palisade cells followed by spongy tissues (Figure 2(h)–2(j)).

Sage is aromatic in odor and somewhat bitter in taste. The leaves are gathered for use while the plants are in bloom and are dried in the shade or indoors by circulating warm air.

Sage proves to be an effective antimicrobial agent for *B. cereus*, *B. megaterium*, *B. subtilis*, *Aeromonas hydrophila*, *Aeromonas sobria*, and *Klebsiella oxytoca*. Sage was extremely effective in small quantities against *Staphylococcus aureus* and *Aeromonas hydrophila*. There is evidence of a moderate antioxidant content in sage that can easily be extracted.

Savory

Savory consists of dried leaves and flowering tops of *Satureja hortensis* L. (Lamiaceae). The leaves are green to grayish green, nearly sessile, entire, linear oblong to spatulate when unfolded, glandular-punctate, acute to rounded at the apex, tapering at the base, $12–35 \times 1–2$ mm, and pubescent. The flowers are tubular–bilabiate, purple, and up to 5 mm long in leaf axils; calyxes are campanulate, hispid on the nerves, and with five subulate teeth; corolla is about 4–5 mm long; stamens are didynamous; style is with bilobed stigma (Figure 2(k)).

In the surface view, epidermal cell walls of the upper and lower surfaces are sinuate and beaded; the stomata are elliptical. The nonglandular hairs are uniserial and numerous, with up to six cells and a pointed, distal cell; the glandular hairs are numerous and have an up to 12-celled head and one-celled stalk (Figure 2(l)–2(n)).

The odor of savory is characteristically aromatic, and the taste is aromatic and warm. The leaves are collected when the plants are in bloom and are dried in the shade or indoors by circulating warm air, before packaging.

Savory has been shown to be an effective antimicrobial agent for *B. amyloliquefaciens*, *B. atrophaeus*, *B. macerans*, *B. megaterium*, *B. pumilus*, *B. sphaericus*, *B. subtilis*, *Escherichia coli*, *Kocuria varians*, *Micrococcus luteus*, and *Pantoea agglomerans*.

Basil (Sweet)

Sweet basil consists of the dried leaves of the *Ocimum basilicum* L. (Lamiaceae). The leaves are ovate, tip acute, petiolate, green, up to 5 cm long, and finely serrate. Hairs arise on the veins, especially on the underside of the leaf, which is also glandular-punctate (Figure 2(o)).

In surface view, the upper epidermis and lower epidermis have wavy cell walls, and the nonglandular hairs are one- or multicelled, uniseriate, and with pointed apices; the surfaces of the hairs are finely warty. Glandular hairs have a two- to four-celled head and occur on both surfaces, but more on the upper surface. In transverse section, a single palisade layer lies beneath the upper epidermis, followed by a zone of spongy parenchyma; the vascular tissue of the midrib and large veins is separated from the upper epidermis and lower epidermis by parenchymatous and collenchymatous tissues (Figure 2(p)–2(r)).

The odor of basil is fragrantly aromatic, and the taste is warmly aromatic and pungent. Fresh leaves can be dried in open air but more efficiently indoors by controlled artificial heat and circulating air. Dried leaves can be ground and graded to produce fine, medium, or coarse particle sizes before packaging.

Basil proves to be an effective antimicrobial agent for *Staphylococcus aureus*, *Escherichia coli*, *B. subtilis*, *Pasteurella multocida*, and some pathogenic fungi. Additionally, basil contains moderate levels of antioxidants.

Tarragon

Tarragon consists of the dried leaves of *Artemisia dracunculus* L. (Asteraceae). The leaves are lanceolate, entire, sessile, glabrous, and bright green. The edges of dried leaves curve toward the lower surface and are finely revolute, 4×3.5 mm in size (Figure 3(a)).

The cuticle on both surfaces is striated over the midrib and veins. The epidermal cell walls of both surfaces are undulate; occasionally, small, inconspicuous glandular hairs of 35–40 μm diameter are present on the epidermis. The mesophyll consists of two rows of palisade cells toward the upper surface and a single row toward the lower. Below the midrib vascular bundle are a few collenchymatous cells, and on the upper side, two secretory ducts are present (Figure 3(b) and 3(c)).

The odor and taste of tarragon are agreeable, aromatic, and like that of anise. The leaves are dried indoors by artificial heat and circulating air to ensure retention of aroma and color. After being crushed by machine into particles of a suitable size, the product is packed in bags or kraft paper or retail packaging (glass, plastic, and metal).

Tarragon proves to be an effective antimicrobial agent for *Alternaria alternata*, *Alternaria solani*, *Aspergillus niger*, *Botrytis* sp., *Dreshlera* sp., *Fusarium acuminatum*, *F. chlamydosporum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. nivale*, *F. oxysporum*, *F. proliferatum*, *F. sambucinum*, *F. scirpi*, *F. semitectum*, *F. verticillioides*, *Monilia* sp., *Penicillium jensenii*, *Phoma* sp., *Pythium ultimum*, *Phytophthora capsici*, *Rhizoctonia solani*, *Sclerotinia* sp., *Trichothecium* sp., *Verticillium dahliae*, and *V. tenerum*.

Thyme

Thyme consists of the dried leaves and flowering tops of *Thymus vulgaris* L. (Lamiaceae). The leaves are linear/

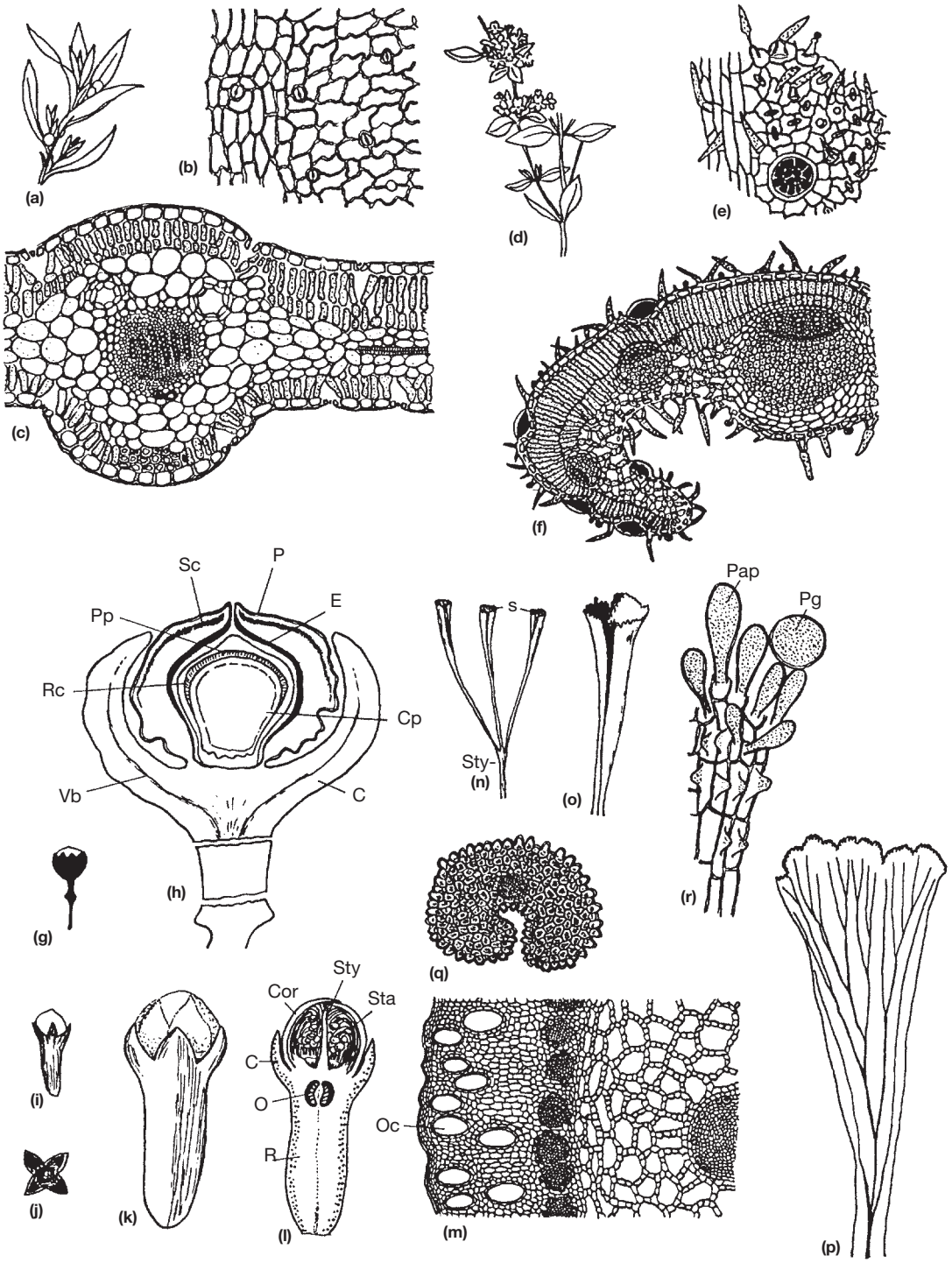

Figure 3 Spices from leaves, flower tops, and flowering parts. Tarragon: (a) a portion of flowering top; (b) surface view of upper epidermis; (c) cross section of leaf through midrib. Thyme: (d) a portion of flowering twig; (e) surface view of lower epidermis; (f) cross section of leaf blade. Cassia bud: (g) bud; (h) longitudinal section through bud. Clove: (i,k) whole clove; (j) same viewed from above; (l) longitudinal section of the same; (m) transverse section through the receptacle. Saffron: (n) style with three stigmas; (o) a stigma; (p) the same showing vascular supply; (q) transverse section through lower portion of stigma; (r) upper end of stigma showing papillae and pollen grain. Abbreviations: C, calyx; Col, collenchyma; Cor, corolla; Cp, compressed parenchyma; E, endocarp; F, fiber; Gh, glandular hair; H, hair; Hb, hair base; Hy, hypodermis; Mes, mesophyll; O, ovary; Oc, oil cavity; Ocl, oil cell; Od, oil duct; P, pericarp; Pal, palisade; Pap, papilla; Pg, pollen grain; Ph, phloem; Pp, pigmented parenchyma; R, receptacle; Rc, reticulate cells; S, stigma; Sc, stone cells; St, stomata; Sta, stamen; Sty, style; Vb, vascular bundle; Xy, xylem. Reproduced from Spices and flavoring (flavouring) crops: leaf and floral structures. In: Macrae, R., Robinson, R. K. and Sadler, M. J. (eds.) *Encyclopaedia of food science, food technology and nutrition*. London: Academic Press, 1993.

linear–lanceolate, ovate or oblong, up to 6 × 0.5–2 mm, and with an acute apex, obtuse base tapering into a petiole, and revolute margins. The upper surface is light gray or light brownish gray to weak olive green with numerous hairs; the lower surface is grayish, pubescent, and glandular-punctuate. About 12 flowers arise in axillary whorls: the calyxes are tubular, bilabiate, about 4 mm in length, pubescent, and 9–12-nerved with the upper lip being three-toothed; the lower lip has two hairy, ascending, attenuate divisions and the throat is hairy; the corolla is about twice as long as the calyx, purplish, and bilabiate; the stamens are didynamous; and style is with bilobed stigma (Figure 3(d)).

The epidermis of both leaf surfaces in the surface view shows wavy walls and numerous nonglandular and glandular hairs; the nonglandular hairs are uni- or multicellular with papillose walls and are stiff and pointed. The glandular hairs are of two types: one with a short stalk and unicellular head and the other with an 8–12-celled head, which measures up to 18 μm in diameter, and with no stalk. In a cross section, the mesophyll consists of one or two layers of palisade cells and is variable in size, followed by spongy tissues; the vascular bundle of the midrib has numerous fibers; the lamina contains two to three small vascular bundles on both sides of the midrib (Figure 3(e) and 3(f)).

Thyme has a fragrant and aromatic odor when crushed, and the taste is aromatic, warm, and pungent. About 15 cm from the tops of the plants is harvested, when in bloom, and the clipped stems are dried in the sun or in a well-ventilated shed or room.

Thyme proves to be an effective antimicrobial agent for *Salmonella enteritidis*, *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, *Y. enterocolitica*, *Shigella flexneri*, *Shigella sonnei*, and *Staphylococcus aureus*.

Cassia Buds

Cassia buds consist of the dried flowers of *Cinnamomum cassia* Blume (Lauraceae) gathered shortly after opening. They are dark brown, are club, have urn- or top-shaped bodies, and are enclosed within a grayish brown, cup-shaped calyx with a dome-like visible top of the pericarp. The calyx tube is rough and hard and frequently has the pedicel attached; the exposed pericarp is smooth and lustrous with the small protuberant base of the style visible (Figure 3(g) and 3(h)).

In longitudinal section, the cortex of the calyx contains parenchymal cells up to 75 μm long, secretion cells up to 66 μm long, and stone cells up to 80 μm long. The pericarp consists of a sclerenchymatous epicarp about 45–58 μm in radial thickness; the parenchymatous mesocarp contains prismatic calcium oxalate crystals, stone cells, secretion cells, and vascular tissues. The endocarp sclerenchymatous cells of the ovary canal are thick-walled and without a lumen, while the cells of the remaining region are rectangular and moderately thick-walled and contain large prismatic crystals of calcium oxalate (Figure 3(h)).

Cassia buds have a cinnamon-like odor and a sweet, warm pungent taste akin to that of cassia bark.

Cassia bud extract has been proved to be an effective antimicrobial agent for *Aeromonas hydrophila*, *Alcaligenes* sp., *Citrobacter* sp., *Enterobacter aerogenes*, *Escherichia coli*, *Flavobacterium* sp., *Klebsiella ozaenae*, *Klebsiella pneumonia*, *Micrococcus roseus*, *Plesiomonas shigelloides*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Staphylococcus aureus*, *Streptococcus anginosus*, *Streptococcus intermedius*, *Streptococcus mitis*, *Streptococcus morbillorum*, *Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus salivarius*, *Streptococcus sanguis*, *and Streptococcus uberis*. Antioxidants have been shown to be present in moderate concentrations from the extracts of the cassia buds.

Cloves

Cloves are dried flower buds of *Syzygium aromaticum* (L.) Merrill & Perry (Myrtaceae). Cloves are like small, round-headed nails, about 10–17.5 mm long, and blackish brown in color. The stalk consists of a cylindrical hypanthium, above which is a bilocular ovary containing numerous ovules on axile placentas; the 'head' consists of four, slightly projecting, calyx teeth, four membranous petals, and numerous incurved stamens surrounding a large style (Figure 3(i)–3(l)).

In transverse section, the hypanthium (receptacle), cut below the ovary, shows an epidermis with a thick cuticle and numerous, scattered, ranunculaceous stomata on the surface. The outer cortex contains two to three rows of large, ovoid to elliptical oil reservoirs (up to 200 μm in length) lined by a secretory epithelium and embedded in the parenchyma; beneath is a zone consisting of an interrupted circle of bicollateral, fibrovascular bundles accompanied by a few sclerenchyma fibers and crystal fibers containing rosette calcium oxalate crystals. The bundles are embedded in parenchymatous cells with sinuate walls, and beneath these is a zone of parenchyma, in which some cells possess thickened angles and wavy walls, whereas others form chains around intercellular air spaces. Next is a zone of small-celled parenchyma followed by a second or inner zone of fibrovascular bundles, along with a few thick-walled, pericyclic fibers (Figure 3(m)).

The sepals have an epidermis similar to the hypanthium, with numerous stomata on the outer surface; a mesophyll with a few slender, vascular strands and numerous, ovoid oil glands; and rosette crystals of calcium oxalate. The petals have straight-walled epidermal cells, an undifferentiated mesophyll containing oil glands, rosette calcium oxalate crystals, and small vascular strands. The filament of the stamen bears oil glands beneath the epidermis, and the connectives bear a large oil gland at the apex.

Cloves have a strong, pungent, and spicy odor and a pungent, aromatic taste. Cloves are propagated by seeds. Hulled washed seeds produce better seedlings than unhulled fruits. The seeds are planted in shaded nurseries, and seedlings are raised. Shade and wind breaks are necessary in the early stages of growth. Trees begin to flower at about 4–5 years and continue to produce until 70 years of age. The crimson buds are picked before the corolla expands and are evenly spread under the sun on a concrete floor or on mats and gently stirred to bring about uniform drying. In good weather, drying is completed in 4–7 days. Quick drying produces the best-quality spice. Some cloves are dried on zinc sheets or in kilns. Cloves are apt to absorb moisture if, while drying, they are left in the open overnight. The average annual yield of dried cloves per

tree is about 3 kg but yields of 18 kg or more per tree are also not uncommon. Finally, they are packed in sacks known as 'mats' made of coconut leaves and stored in a dry room. Prolonged storage may cause a loss of volatile oils.

Cloves have been shown to contain a moderate amount of antioxidants in its extracts.

Saffron

Saffron is made from the dried stigmas of *Crocus sativus* L. (Iridaceae). The three stigmas are attached to the top of the style and are 25 mm long, cornucopia-shaped, dark red, and with fimbriate margins. The styles are about 10 mm long, cylindrical, solid, and yellowish brown to pale yellowish orange (**Figure 3(n)–3(p)**).

The stigma consists of thin-walled parenchyma containing coloring matter and covered by a thin-walled epidermis. The distal end of the stigma shows numerous bladderlike, cylindrical papillae up to 150 μm in length, among which occur a few smooth, spherical, pollen grains from 40 to 120 μm in diameter (**Figure 3(q)–3(r)**).

The odor of saffron is strong and characteristically aromatic, and the taste is bitter/aromatic. The flowers are collected every morning, as they open, and the stigmas are then collected, by either pulling them out or cutting them using fingernails, after which the flowers are discarded. The value of saffron depends heavily on the methods by which the stigmas are processed. The stigmas are placed in sieves in layers 2–3 cm thick. These sieves are placed 15 cm above an almost spent fire for drying. By stacking them and by changing their order or position, the product is carefully dried. Stigmas are protected against dampness and light, because light bleaches saffron to a dull yellow color. Saffron should be preserved in amber-colored bottles or tin boxes.

See also: Drying: Principles and Types; Drying: Physical and Structural Changes; Sensory Evaluation: Sensory Rating and Scoring Methods; Spices and Flavoring Crops: Fruits and Seeds.

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Spices and Flavoring Crops: Tubers and Roots

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Background

The use and cultivation of spice plants date back to the beginning of our history. Most of the spices that we value today were known to the ancient civilizations of China, Egypt, Greece, Rome, the East Indies, and the rest of the Old World. Spices have also been responsible for the rise and fall of empires and the great sea voyages to explore the distant corners of the globe. The search for spices by early European explorers also led to the discovery of new continents and waterways.

Spices are derived from different plant parts, including roots, stem, leaves, flowers, fruits, and seeds. This article, however, deals only with those spices and flavoring crops that are obtained from tubers and roots. These are categorized into two groups, major and minor crops, depending upon their distribution and usage in different parts of the world.

Major Crops

Ginger

Ginger is the most important of all the spices obtained from underground plant parts. It has been in use in India and China since very ancient times. However, it is difficult to trace with certainty the exact place of its origin. Ginger was mentioned by the Chinese philosopher Confucius (551–479 BC) in his *Analects*. Ginger was also one of the first oriental spices to be known to the Europeans. The Greeks and Romans first obtained ginger from the Arab traders. It was known in Germany and France in the ninth century and in England during the tenth century.

Ginger is derived from the rhizomes of *Zingiber officinale* Rosc., a plant belonging to family Zingiberaceae. It is a slender perennial herb, mostly grown as an annual, and is 30–100 cm tall with a robust rhizome borne horizontally just below the soil surface. The fleshy sympodial rhizome is hard and thick, somewhat laterally compressed, often palmately branched, and varying in shape and size in different cultivated types. It is covered with small, distichous scales with an encircling insertion and with fine fibrous roots in the top layers of the soil. It is pale yellow in color externally and greenish yellow within.

The crop is always propagated vegetatively using small fragments of the rhizome known as 'seeds' or 'setts.' It is also grown both as a pure crop and as an intercrop. It may be grown in rotation with paddy rice, samai (*Panicum miliare*). If irrigation facilities are available, ginger may be rotated with vegetables, chili, groundnut, ragi (*Eleusine coracana*), and maize. Ginger is sometimes grown mixed with turmeric, which grows rapidly early on and provides partial shade to the developing ginger. It is harvested at different stages depending upon the requirements of the user. Green ginger is harvested between 5 and 7 months after sowing, and the rhizomes are used for preservation as preserved ginger. Final harvesting is carried out after 8–10 months from planting for the production of dried ginger. Shriveling, yellowing, and withering of the leaves, accompanied by drying and lodging of the aerial shoots, indicate maturity of the rhizomes.

The maturity of the rhizome has a significant influence on both its quality characteristics and its suitability for processing into preserved or dried ginger. The optimum time for harvesting ginger in India is 245–260 days from planting, after which the rhizomes become more fibrous. Harvesting of the mature ginger is mostly done by hand using a spade, hoe, or digging fork. In advanced countries, the late crop is also harvested by mechanical diggers. Extra care is required for the harvesting of healthy rhizomes. The soil, roots, and shoots are subsequently removed, and the rhizomes are carefully washed and dried for storage (see Figures 1 and 2).

Several commercial types of ginger are recognized in cultivation, which are generally named after the localities where they are grown. The types with less fiber, which varies from 1.7% to 9.0%, have a higher demand. Some of the prized types of ginger in international trade include Jamaican ginger, Indian ginger, African ginger, and Chinese ginger, and these vary in taste and aroma.

Since the rhizome is perishable, it is susceptible to attack by the soilborne fungi, insects, and white ants. Rhizome rot or soft rot is the most serious disease found all over the ginger-growing areas of the world. Healthy and disease-free rhizomes are thus selected for storage. The use of specific fungicides for the treatment of rhizomes before storage is recommended on a regional basis. The treated rhizomes are air-dried under shade to remove excess moisture. The optimum conditions for the

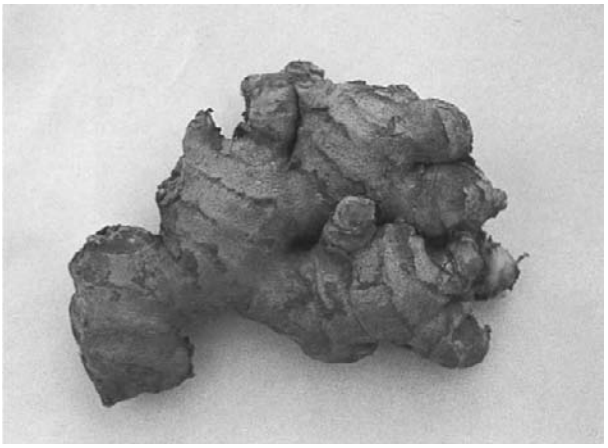

Figure 1 Spices and flavoring crops: rhizome of green ginger (*Zingiber officinale*).

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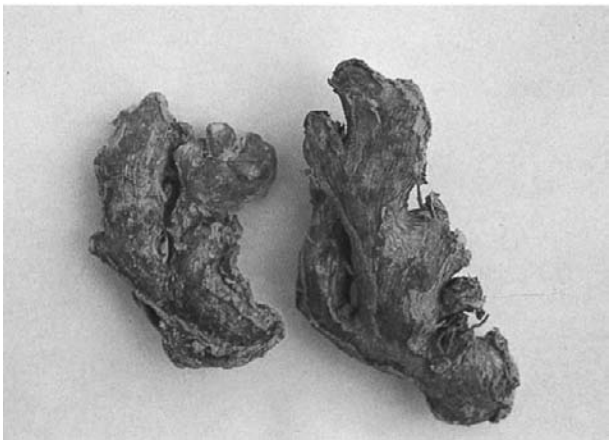

Figure 2 Spices and flavoring crops: rhizome of dried ginger (*Zingiber officinale*).

Figure 3 Spices and flavoring crops: rhizome of turmeric (*Curcuma domestica*).

storage of fresh ginger have been investigated in detail in Hawaii. Harvested rhizomes should be washed immediately and then air-dried in the shade for 1–2 days. The rhizomes can be stored safely for up to 6 months by maintaining the temperature at 12 °C and the relative humidity at 65%.

In India, the ginger is loosely stored in pits, which may be up to 1 m deep. These pits are cool and provide protection against sunlight. A layer of sand or sawdust is spread at the bottom of the pit before storing the rhizomes. The pit is left uncovered or covered with a wooden plank, which in turn is plastered with mud, with a hole in the center for aeration.

As mentioned earlier, there are three primary products of ginger rhizomes: fresh or green ginger, preserved ginger in syrup or brine, and dried ginger. Green ginger is consumed mostly in the areas of production and nearby places. Preserved and dried products are the major forms in which ginger is traded internationally. Dried ginger is used as a spice and also for the preparation of its extracts.

Commercial preparation of preserved ginger is mostly practiced in China, Hong Kong, Australia, and India. Young juicy rhizomes are dried, cleaned, and boiled in water until they become tender. They are then peeled, scraped, and boiled several times in a sugar solution. Sometimes, preserved ginger is also prepared in the dry state by dusting the drying rhizomes with crystalline sugar.

The preparation of dried ginger involves processing the cleaned rhizomes by peeling, splitting them into pieces, immersing them into boiling water for about 10 min, and, finally, drying. The appearance of processed ginger (black or white), the contents of volatile oil and fiber, the pungency level, and an assessment of the aroma and flavor are important in the quality evaluation of dried ginger.

The characteristic pleasant and aromatic odor of ginger is due to essential oil, which can be separated from the dried rhizome by steam distillation. The essential oil, known in the trade as 'oil of ginger,' is greenish to yellow in color; mobile (viscous on aging), with the characteristic warm and aromatic odor; but not the pungent flavor of the spice. The pungent principles of ginger are nonvolatile oleoresin, commonly known in the trade as 'gingerin.' This can be extracted from dried ginger by percolation with a suitable solvent. It is a viscous and dark brown oil and retains the full pungency of the spice. Besides the pungent principles, the oleoresin also contains gingerol, shogaol, and zingerone.

In medicine, ginger is used as a carminative and digestive stimulant. It also dilates the blood vessels in the skin, causing a feeling of warmth. Its preparations are also useful in the treatment of coughs and colds. Ginger is extensively used in culinary preparations such as soups, puddings, pickles, gingerbread, and cookies and is an ingredient of all curries, except those used with fish. Ginger is also popular for flavoring beverages such as ginger ale and ginger beer.

Ginger has been shown to contain high levels of antioxidants, which are easily extracted.

Turmeric

Turmeric, another important spice, is also obtained from the underground plant part, that is, the stem. The plant, a native of Southeast Asia, is widely cultivated in India, Sri Lanka, Indonesia, China, Taiwan, Peru, Haiti, and Jamaica, of which India is by far the largest exporter.

Turmeric is obtained from the rhizomes of *Curcuma domestica* Val. (syn. *C. longa* L.), also a member of the family Zingiberaceae. The plant is a robust perennial herb with a short stem and tufted leaves. The rhizomes, brownish yellow in color, consist of a central bulbous portion bearing a number of fingerlike lateral offshoots. Although the characteristic yellow matter is distributed throughout the plant, it is highly concentrated in the rhizome. There are several varieties of turmeric distinguished by the names of the localities in which they are grown. Turmeric cultivated in the hills is said to be of a better quality than that raised in the plains. Even the same variety that is grown in plains and on the hills shows distinct differences in quality and yield (see **Figure 3**).

Cultivation of turmeric requires a hot and moist climate, a liberal water supply, and a well-drained soil. It can be grown successfully in rice fields on raised beds beyond the reach of stagnant water. It is usually rotated with ragi (a cereal grass cultivated in Africa and Asia), paddy, and sugarcane. It is rarely cultivated as a pure crop and is usually grown mixed with castor bean,

maize, ragi, onions, brinjal, and tomato. The plants thrive well in partially shaded areas, but shade adversely affects the yield.

Turmeric is a good host for many pathogenic microorganisms and insect pests. Rhizome rot and leaf spot caused mainly by *Taphrina maculans* Butler are the important diseases of turmeric. The use of Bordeaux mixture or any other nonmetallic fungicide has been suggested as a remedy.

The crop is ready for harvesting in about 9–10 months, when the lower leaves turn yellow. The rhizomes are carefully dug up with handpicks. The leafy tops are then cutoff, and the roots are removed. The adhering soil particles are removed by brisk shaking or by rubbing, and the rhizomes are then thoroughly washed with water, placed in small heaps, covered with turmeric leaves, and left to dehydrate. The fingers and bulbs (or mother rhizomes) are then separated and cured separately.

The curing of turmeric involves boiling or steaming the fresh rhizomes in water, followed by drying and polishing. The rhizomes are boiled for 45–60 min over a slow fire until they become soft. The rhizomes are then cooled and spread out to dry in the sun. Boiling of the rhizomes in very dilute solutions (0.05–0.1%) of mild alkali modifies the color of the core of the rhizome to the desired yellow orange. The duration of boiling markedly affects the color and aroma of the final product. After boiling, the rhizomes are dried in sunlight for 10–15 days. Sun-drying gives rise to a surface-bleaching effect. After drying, the rhizomes become quite hard and brittle and break with a metallic sound. Drying is followed by polishing, which includes cleaning off the outer skin, rootlets, and adhering particles of soil and making the rhizomes smooth. Polishing is performed either manually or in power-operated drums. The dried turmeric is coated with a slurry of turmeric powder in the course of polishing to impart a better color to the rhizomes.

The cured rhizomes are stored in pits dug at a raised site. The bottom and sides of these pits are lined with *Saccharum spontaneum* (commonly known as Rellu grass) and palmyrah mats. The pits, after being filled with cured turmeric, are covered with mats and grass and, finally, with earth. The produce may be stored for a year. The turmeric is also stored in 'godowns' or warehouses in gunny bags.

The coloring matter of turmeric is yellowish crystalline curcumin, which can be extracted from the powder either by direct solvent extraction or by extraction with alkali and subsequent precipitation with acid.

Turmeric is mainly used for domestic culinary preparations and is an important constituent of curry powder. Turmeric is also used as coloring agent in the textile, food, and pharmaceutical industries. In the Indian system of medicine, turmeric is used to some extent as a stomachic (i.e., promoting the appetite or assisting digestion), tonic, and blood purifier. The juice of the fresh rhizome is used as an antiparasitic for many skin diseases. Oil of turmeric, distilled from the dried rhizome, shows antiseptic properties.

Minor Crops

Spices Obtained from Roots

Angelica

Angelica has been in use as a spice since AD 1500. It is obtained from the roots of *Angelica archangelica* L., a member of the family Apiaceae. It is native to Syria but is known to occur in many parts of Europe and western Asia. In India, it is grown in the Kashmir Valley.

The plant is a stout perennial herb with large, pinnately compound leaves and small, greenish-white flowers. The dry rootstocks yield 0.35–1% of essential oil, the main constituent of which is β-phellandrene. The roots contain several furocoumarins such as angelicin, bergapten, and xanthotoxin in addition to umbelliprenin and several phenols. The dried roots are frequently used for flavoring cakes, candy, and beverages. Angelica root is the main flavoring ingredient of gin. It is also used in perfumery and medicine. In medicine, the roots have been shown to possess stimulant, expectorant, and diaphoretic (i.e., inducing perspiration) properties.

Horseradish

The horseradish (*Armoracia lapathifolia* Gilib.) is a member of the family Brassicaceae and has been in use as a condiment since ancient times. The plant is a native of southeastern Europe and is widely grown in Europe, America, and the hilly regions of India.

The plant is a tall, hardy herb with glossy green, toothed leaves. The roots are fleshy, yellowish white, and cylindrical. Initially, the root is about 2.5 cm in diameter, but it may reach a length of 1 m. The English refer to the plant as 'red cole,' perhaps due to the hot sensation experienced when ingesting even small pieces of root. The roots are dug up and are scraped or grated before use. Both fresh and dried roots are used as a condiment, which aids digestion and prevents scurvy. It is similar to mustard in most of its properties. The smell and flavor of horseradish come from the glycoside sinigrin, which decomposes in the presence of water by enzyme action to form mustard oil.

Asafoetida

Asafoetida is the dried latex obtained mainly from living rootstocks or tap roots of several species of Ferula, namely, *F. foetida* Regel., *F. alliacea* Boiss., *F. rubricaulis* Boiss., *F. asafoetida* Linn., and *F. narthex* Boiss, of the family Apiaceae. The plants are perennial herbs that are mainly distributed from the Mediterranean region to Central Asia. The main regions of asafoetida production are eastern Iran and western Afghanistan. Some species are also found in the Punjab and Kashmir regions of India.

The plants bear large, carrot-shaped roots, 10–15 cm in diameter at the crown after 4–5 years of growth. At the time of flowering, the upper part of the root is laid bare, and the stem is cut off close to the crown. The exposed surface is covered by a dome-shaped structure made up of twigs and mud. The exudate (milky juice) oozing out from the cut surfaces is scraped off after a few days. The collection of the resin and the slicing of the root are repeated until exudation ceases.

Asafoetida occurs in three forms in commercial trade: tears, mass, and paste. The tears make the purest form of resin; it is bitter and acrid in taste and emits a strong and peculiar odor.

Asafoetida contains mainly resin (40–64%), gum (25%), and volatile oil (10–17% on a dry-weight basis). The resin portion consists mainly of asaresinotannol, free or combined with ferulic acid. Umbelliferone seems to be present in combined state. Oil of asafoetida is obtained by steam distillation of the gum resin.

Asafoetida is generally used for flavoring curries, sauces, and pickles. Medicinally, it stimulates the intestinal and respiratory tracts and the nervous system. It is useful in asthma, whooping cough, and chronic bronchitis.

Sarsaparilla

Sarsaparilla is obtained from the dried roots of several tropical species of Smilax, a member of the family Liliaceae. Some of the important spice-yielding species include *S. aristolochiaefolia* Mill. (Mexico), *S. officinalis* H.B. & K. (Honduras), and *S. regelii* Killip & Morton (Jamaica). The plant thrives best in hot and humid climates and on light, well-drained sandy loam rich in humus.

The plants are climbing or trailing vines with prickly stems. The rhizomes are short and thick, with very long, thin roots. The plants are harvested after attaining 2–3 years of age. They are carefully dug up and cut off near the stock, which is then covered up again with surface soil. The harvested roots are then washed well, dried in the sun, and tied up in bundles. The roots contain a bitter principle that is used as a flavoring agent. It is chiefly used as a beverage condiment for the preparation of soft drinks. Sarsaparilla is mostly used in combination with winter green and other aromatic plants.

Medicinally, sarsaparilla has been used to treat syphilis and other skin diseases.

Sarsaparilla contains a moderate amount of antioxidants that can be extracted and helps reduce oxidative damage in the intestinal tract.

Spices Obtained from Rhizomes or Tubers

Zedoary

Zedoary (*Curcuma zedoaria* Rosc. syn. *C. zerumbet* Roxb.) closely resembles turmeric (*C. longa*) in appearance. It is a native of northeast India and is widely cultivated in many parts of China, Sri Lanka, and India.

The plant bears green leaves with brownish-purple veins and grows up to a height of 50 cm. The rhizomes, which are large and fleshy, are cut into small pieces and dried. Dried slices have a bitter and strong, camphoraceous taste and are used for commercial purposes.

Steam distillation of the rhizome yields a light yellow oil. The odoriferous constituent is said to be a sesquiterpene alcohol belonging to the tricyclic group.

Zedoary was an important spice in the past, but these days, it is usually used only for flavoring liqueurs and curries. The rhizomes have aromatic, stimulant, and carminative properties. Besides its use as spices, the rhizomes are also used for the preparation of shoti starch (flour), a substitute for arrowroot and barley. It is highly valued as a dietary item, especially for infants and convalescents. A red powder, 'abir,' is also prepared from powdered rhizomes by treatment with a decoction of sappan wood.

Mango ginger

Mango ginger (*Curcuma amada* Roxb.) is a member of the family Zingiberaceae. The plant is native to India and occurs in the wild state in parts of Bengal, Konkan, and Madras (see Figure 4).

The plants are herbaceous, and the rhizomes have the characteristic odor of green mangoes. At the time of harvesting, entire plants are lifted up with a crowbar, and the rhizomes are collected, cleaned, and dried in the shade. On distillation, the rhizome yields essential oil, which consists mainly of pinene, ocimene, and linalool. The rhizome is mostly used in pickles and is considered stomachic and carminative. It is also applied over contusions and sprains.

Figure 4 Spices and flavoring crops: rhizome of mango ginger (*Curcuma amada*).

Mango ginger has proved as an effective antimicrobial agent for *M. luteus*, *B. cereus*, and *B. subtilis*. Additionally, antioxidants were found to be present in mango ginger and were found to be easily extractable.

Galangal

Galangal is of three types: lesser galangal, greater galangal, and light galangal. The reddish-brown rhizomes are used as condiment and have an aromatic spicy odor and a pungent taste.

The lesser galangal (*Alpinia officinarum* Hance.), a member of the family Zingiberaceae, is a native of southern China. The plant is a perennial herb with a raceme of showy flowers and attractive foliage. The rhizome is smaller and has a stronger odor and taste. The lesser galangal is used as a spice in cooking and also in medicine and for flavoring liqueurs. It is also used to impart a pungent flavor to vinegar.

The greater galangal (*Alpinia galanga* (L.) Willd.) is also a perennial herb with showy flowers and beautiful foliage. It is commonly found in Indonesia and Malaysia and is also cultivated in Bengal and southern parts of India. The green rhizomes contain essential oil consisting mainly of methyl cinnamate and cineol. It gives a pungent taste like a mixture of pepper and ginger. Medicinally, it is useful in respiratory troubles, especially in children. The rhizomes are also carminative and stomachic.

The light galangal (*Alpinia speciosa* (Wendl.) K. Schum) is a native of the Eastern Archipelago, off the Coromandel Coast of southeast India. Its rhizome is much larger and is generally used as substitute for greater galanga, and even as a substitute for ginger in many preparations.

Galangal has proved to be an effective antimicrobial agent for *Staphylococcus aureus*, but not for *Escherichia coli*. In addition, antioxidants have been found in a moderate concentration that can function as a lipoxygenase inhibitor.

See also: Spices and Flavoring Crops: Fruits and Seeds.

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Spices and Flavoring Crops: Uses and Health Effects

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Herbs and Spices

Herbs such as basil, mint, and rosemary are grown and harvested fresh for cut leaves and used for cooking, garnish, or medicinal purposes. Spices are parts of herbal plants such as aromatic seed, berries, bark, root parts, and even the stigma of a flower. Herbs and spices have a long history of both culinary and medicinal uses. Spices are traditionally consumed as food adjuncts to enhance sensory quality of foods, the quantity and variety consumed in tropical countries being particularly extensive. Besides contributing to flavor, color, and aroma to our everyday diet, spices have also been long recognized to possess physiological effects supposed to be beneficial to human health. They act as stimulus to the digestive system and relieve digestive disorders, and some spices are of antiseptic value. Their attributes, such as tonic, carminative, stomachic, diuretic, and antispasmodic – largely empirical nevertheless efficacious, have found pharmacological applications in the indigenous systems of medicine. In India, the indigenous Ayurvedic system of medicine uses herbs and spices for health including tumeric for jaundice, basil to protect the heart, mace for stomach infections, cinnamon to stimulate circulation, and ginger as the universal medicine, in particular for relieving nausea and indigestion. Traditionally, the Chinese have integrated food and health including herbs and spices in specially prepared soups, dishes, or beverages for both sustenance and purported health benefits. Ginseng and ginkgo biloba are used to improve stamina and cognitive performance, respectively. Other medicinal uses include galangal for abdominal pain, nutmeg for the management of diarrhea, and cinnamon for colds and flu (Figures 1 and 2).

With the long history of the use of spices dating back to 5000 years BC, and spices significantly contributing to human health by providing bioactives, these may be considered as the first ever recorded functional foods. Spices make foods palatable without salt and hence assist in the desirable reduced daily intake of sodium. Similarly, they make foods palatable without fat, thus enabling to meet healthy fat intake. The use of spices also encourages variety in food intake, thus supporting nutrient diversity by encouraging number of food choices.

Spices do not contribute anything significant to human nutrition. Due to low levels of consumption of spices, their impact on nutrient makeup may not be as dramatic as other food ingredients. In the last four decades, several health beneficial effects of spices have been experimentally documented that suggest that the use of these food adjuncts extends beyond taste and flavor. The emerging research literature suggests that specific spices may confer unique health benefits. Although human studies are limited, considerable attention to this has been drawn because of the positive results from *in vitro* and *in vivo* animal studies.

Proven Health Effects of Herbs and Spices

Digestive Stimulant Action of Spices

Digestive stimulant action of spices is the most common experience of many. Spices such as ginger (*Zingiber officinale*), mint (*Mentha spicata*), ajowan (*Trachyspermum ammi*), cumin (*Cuminum cyminum*), fennel (*Foeniculum vulgare*), coriander (*Coriandrum sativum*), and garlic (*Allium sativum*) are common remedies used in traditional medicines or ingredients of preparations to cure digestive disorders. The mechanism of digestive stimulant action of spices has been understood by extensive animal studies. Many commonly consumed spices – turmeric (*Curcuma longa*), red pepper (*Capsicum annuum*), ginger, fenugreek (*Trigonella foenum-graecum*), mustard (*Brassica nigra*), cumin, coriander, ajowan, tamarind (*Tamarindus indica*), and onion (*Allium cepa*) – stimulate bile acid production by the liver and its secretion into bile. Bile acids play a major role in fat digestion and absorption. Several spices are also evidenced to stimulate the activity of digestive enzymes of pancreas, particularly lipase and terminal digestive enzymes of small intestinal mucosa (Figure 3).

Antidiabetic Potential of Spices

Among spices that have been evaluated for antidiabetic attribute, fenugreek seeds, garlic and onion, and turmeric have been found to be effective in improving the glycemic status and glucose tolerance in diabetic animals/type 2 diabetic patients. Animal studies and clinical trials on antidiabetic properties of fenugreek and onion have been particularly extensive, while human studies are limited in the case of garlic and turmeric. Studies have unequivocally demonstrated the antidiabetic potential of fenugreek in both type 1 diabetes and type 2 diabetes. The addition of fenugreek seeds to the diets of diabetic patients or animals results in a fall in blood glucose and improvement in glucose tolerance. The hypoglycemic effect is attributed to the fiber and gum, which constitute as much as 52% of fenugreek seeds. The fiber-rich fenugreek is believed to delay gastric emptying by direct interference with glucose absorption.

Garlic and onion are two other spices, which have been widely tested for their antidiabetic potential. Both these spices have shown hypoglycemic potential in different diabetic animal models and in limited human trials. The hypoglycemic potency of garlic and onion is attributed to the disulfide compounds present in them, di(2-propenyl) disulfide and 2-propenylpropyl disulfide, respectively, which cause direct or indirect stimulation of insulin secretion by the pancreas. In addition, they may also have insulin-sparing action by protecting from sulfhydryl inactivation by reacting with endogenous thiol-containing molecules such as cysteine, glutathione, and serum albumins (Figure 4).

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Figure 1 Spices used for enhancing taste and flavor of foods.

Turmeric is also claimed to possess beneficial hypoglycemic effect and to improve glucose tolerance in a limited number of studies. Nephropathy is a common complication in chronic diabetes. High blood cholesterol is an added risk factor that determines the rate of decline of kidney function in diabetic situation. Dietary curcumin (of turmeric) and onion have been found to have a promising ameliorating influence on the severity of renal lesions in streptozotocin-diabetic rats. Hypocholesterolemic effect of these spices as well as the ability to lower lipid peroxidation under diabetic condition is implicated in the amelioration of renal lesions. Capsaicin of red pepper has been shown to be useful in diabetic neuropathy. Topical application of 0.075% capsaicin cream was effective in pain management. These antidiabetic spices may be used in conjunction with antidiabetic drugs to minimize drug dosage.

Cardioprotective Effect of Spices

(a) *Hypolipidemic influence*: Several common spices have been evaluated for cholesterol-lowering effect in a variety of experimental situations in both animals and humans. Garlic, onion, fenugreek, turmeric, and red pepper are found to be effective as hypocholesterolemic agents under various conditions of experimentally induced hypercholesterolemia/hyperlipidemia. Garlic, fenugreek, and onion are effective in humans with hyperlipidemic condition. Consumption of garlic has been associated with a reduction in total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels. Many clinical studies have indicated that consuming one clove of garlic (or equivalent) daily will have at least 10% cholesterol-lowering effect. With the availability of dehydrated garlic powder containing a standardized level of the parent sulfur compound – alliin – effective clinical work could be undertaken with a relatively low and acceptable daily dosage of 300–900 mg (equivalent to one clove of garlic). Fenugreek seeds were hypocholesterolemic in rats with hyperlipidemia induced by either a high-fat or high-cholesterol diet. Defatted fenugreek seed was effective in diabetic hypercholesterolemia in dogs and in humans. The hypolipidemic effectiveness of turmeric/curcumin, of red pepper/capsaicin, and of onion and garlic has been periodically reviewed. Spice compounds curcumin and capsaicin have been associated with a decrease in LDL cholesterol and an increase in HDL cholesterol levels in animal studies.

(b) *Antithrombotic property*: Besides the beneficial effect on serum lipid profile (lowering of LDL cholesterol and triglyceride levels), the antiplatelet aggregation and the antiplatelet adhesion properties of several spices also contribute to cardiovascular protection. The spices/spice compounds documented to have inhibitory effect on platelet aggregation are garlic, onion, curcumin, cuminaldehyde, eugenol, and zingerone. Garlic in particular exhibits antithrombotic and hypotensive properties, both of which also contribute to cardiovascular protection besides the hypolipidemic properties.

(c) *Suppression of LDL oxidation*: Antioxidant properties of spices are of particular interest in view of the impact of oxidative modification of LDL cholesterol in the development of atherosclerosis. Recent evidences have indicated that free radicals contribute to cardiovascular disease. Oxidative modification of LDL is hypothesized to play a key role during the development of atherosclerosis. Since spices have high antioxidant concentrations that have the potential to inhibit the oxidation of LDL, the use of antioxidant spices is a promising proposition.

(d) *Thermogenic influence*: Obesity-related insulin resistance has emerged as a potent risk factor for cardiovascular disease. Dietary factors that affect satiety and thermogenesis could play an important role in determining the prevalence and severity of this problem. Among spices that may have a role to play in this regard, red pepper (or its pungent principle capsaicin) and garlic are promising and more data are required to substantiate this benefit. The use of spices to displace fats and salt in the diet may reduce cardiovascular risk.

Gallstone Preventive Effect

Persistent high-cholesterol diet leads to cholesterol saturation in bile resulting in the formation of cholesterol crystals – gallstones in gall bladder. Studies on experimental induction of cholesterol gallstones in mice and hamsters by feeding a lithogenic diet have revealed that the incidence of gallstones is 40–50% lower when the animals are maintained on 0.5% curcumin or 0.015% capsaicin-containing diets. Animal studies have also revealed significant regression of preformed cholesterol gallstones by these spice principles in a 10-week feeding trial. The gallstone preventive potential of other known hypocholesterolemic spices – garlic, onion, and fenugreek seeds – has also been recently evidenced in animal

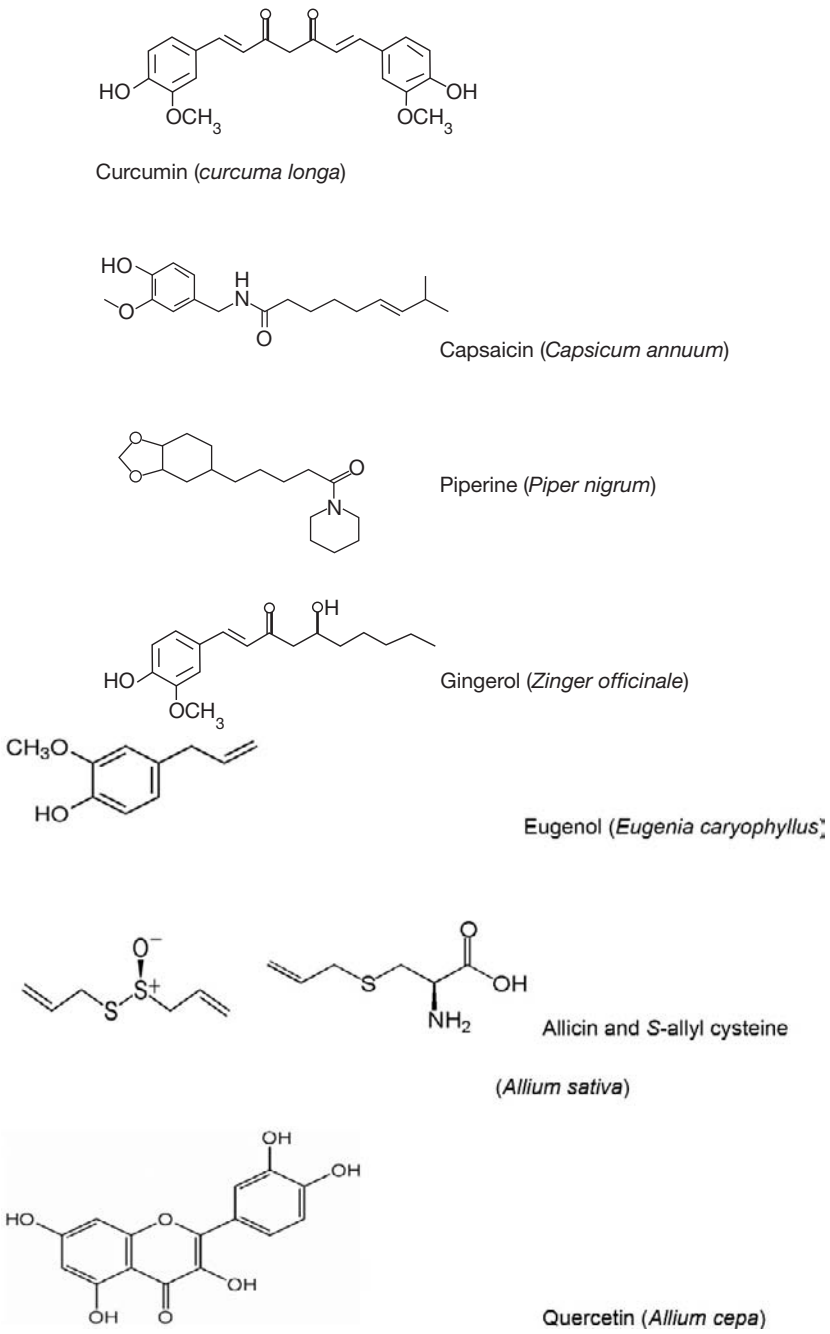

Figure 2 Formulas of compounds present in spices with health effects attributable to their bioactive compounds.

studies. The antilithogenicity of these spices is attributable to the lowering of cholesterol concentration and enhancing the bile acid concentration, both of which contribute to the lowering of cholesterol saturation index and hence its crystallization. In addition to their ability to lower cholesterol saturation index, the antilithogenicity of these spices may also be due to their influence on biliary proteins.

Anti-Inflammatory Property

Lipid peroxides play a crucial role in arthritis and other inflammatory diseases. Turmeric happens to be the earliest anti-inflammatory drug known in the indigenous system of medicine in India. Turmeric extract, curcuminoids, and volatile oil of turmeric have been found to be effective as anti-inflammatory in several animal studies. Both *in vitro* and *in vivo* animal studies have documented the anti-inflammatory potential of spice principles curcumin (of turmeric), capsaicin (of red pepper), and eugenol (of clove). Anti-inflammatory effect of curcumin in patients who have undergone surgery for hernia was found comparable to that of phenylbutazone. In rheumatoid arthritis patients, administration of curcumin produced significant improvement. Capsaicin has received considerable attention as a pain reliever. In patients with

osteoarthritis and rheumatoid arthritis, topical application of creams containing 0.025% or 0.075% capsaicin was an effective and safe alternative to analgesics employed in systemic medications. Capsaicin has also been suggested for the initial management of neuralgia consequent to herpes infection. There is also evidence for the benefit of ginger in ameliorating arthritic knee pain. Ginger doses of 0.5–1.0 g per day were efficacious in osteoarthritis and rheumatoid arthritis.

Antimutagenicity and Anticancer Effect

Phenolic substances present in spices have been reported to possess substantial anticarcinogenic activities. The majority of these naturally occurring phenolics possess antioxidative and anti-inflammatory properties, which appear to contribute to their chemopreventive or chemoprotective activity. Certain phytochemicals from spices have been shown to inhibit one or more of the stages of the cancer process (initiation, promotion, growth, and metastasis). Inhibition of phase I metabolic enzymes (involved in procarcinogen activation) and induction of phase II metabolic enzymes (involved in carcinogen deactivation) may account for the chemopreventive effects of spices. Spices may also protect against oxidative stress and inflammation, both of which are a risk factor for cancer initiation and promotion (as well as other pathological conditions).

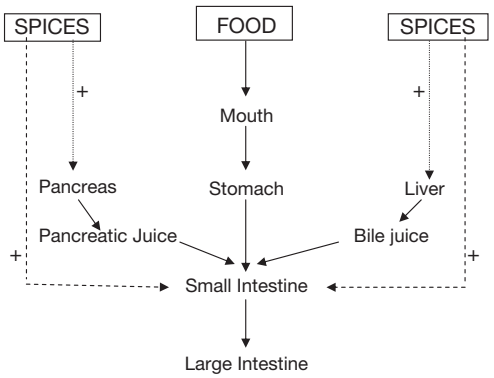

Figure 3 Digestive stimulant action of spices.

Spices that have antioxidant property can function as antimutagens. Since mutagenesis has a direct bearing on cancer initiation, antimutagenic spices can probably be anticarcinogenic too. Turmeric/curcumin and garlic have been shown to be antimutagenic in several experimental systems. *In vivo* studies in experimental animals suggest that turmeric and curcumin inhibit the formation of mutagens. Spices with known anticarcinogenic effects in animal models of cancer include turmeric, garlic, and ginger. Turmeric has been found to have chemopreventive effects against cancers of the skin, forestomach, liver, colon, and mouth in mice. Animal studies involving experimental induction of tumor of specific tissues with potent chemical carcinogens have revealed significant reduction of the incidence of tumor in curcumin treatment. Several studies indicate that curcumin can suppress both tumor initiation and tumor promotion. Curcumin is a powerful inhibitor of the proliferation of several tumor cells. With many evidences suggesting that curcumin can suppress tumor initiation, promotion, and metastasis, and with proven safety of its consumption (up to 10 g per day), curcumin offers enormous potential in the prevention and therapy of cancer.

Cancer-preventive ability of garlic has been indicated by etiological studies wherein higher intake of Allium products is associated with reduced risk of several types of cancers, especially stomach and colorectal. Garlic is effective in the detoxification of carcinogens through its effects on phase I and phase II enzymes. Several mechanisms have been proposed to explain the cancer-preventive effects of garlic and its organosulfur compounds. These include inhibition of mutagenesis, modulation of enzyme activities thus suppressing bioactivation of carcinogen molecules, inhibition of carcinogen-DNA adduct formation, free radical scavenging, inhibitory effects on cell proliferation and tumor growth, and induction of apoptosis. Pungent vanilloids especially [6]-gingerol present in ginger (*Z. officinale*) have been found to possess potential chemopreventive activities.

Antioxidant Activity

Oxidative damage has been implicated in the etiology of disease processes such as cardiovascular disease, inflammatory

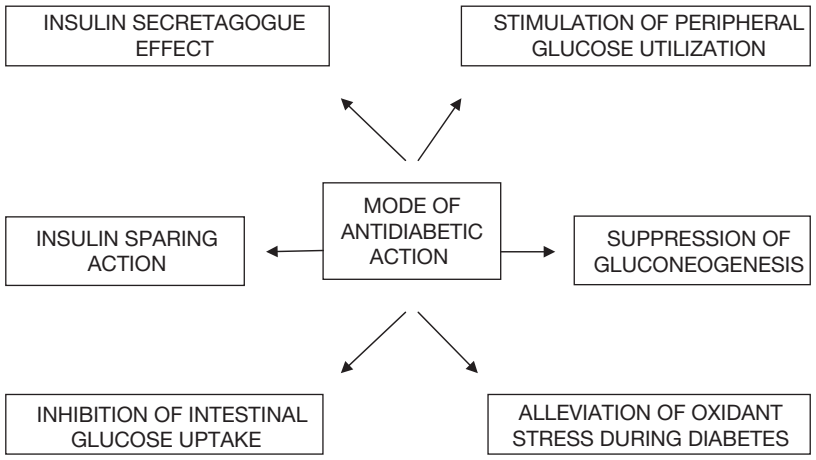

Figure 4 Possible mechanisms of antidiabetic action of spices.

diseases, cancer, neurodegenerative diseases, and other degenerative diseases. Excessive free radical generation overbalancing the rate of their removal leads to oxidative stress. Antioxidants are compounds that hinder the oxidative processes and thereby delay or suppress oxidative stress. The bioactive compounds present in spices that possess potent antiatherogenic, anti-inflammatory, antimutagenic, and cancer-preventive activities are in fact antioxidants that are experimentally evidenced to control cellular oxidative stress and thereby exert a beneficial role in preventing oxidative stress-mediated diseases.

Most of the health effects of spices on cancer, cardiovascular disease, inflammatory diseases, and neurodegenerative diseases may be mediated through their potent antioxidant effects. Suppression of oxidative stress and inflammation by spices is important in their cancer-preventive role, since both oxidative stress and inflammation are a risk factor for cancer initiation and promotion. The antioxidative effects of spice compounds – curcumin, eugenol, capsaicin, piperine, gingerol, and spices such as garlic, onion, and fenugreek – have been experimentally evidenced. The studies to this effect are exhaustive and experimental evidences are plenty in the case of curcumin of turmeric and eugenol of clove. Studies with several *in vitro* systems as well as *in vivo* animal studies have revealed that spice principles curcumin, eugenol, and capsaicin have beneficial antioxidant property by quenching oxygen free radicals, by inhibiting the production of reactive oxygen radicals, and by enhancing the antioxidant enzyme activities.

The antioxidant activity of the spice compounds in mammalian system involves one or more of the following: (1) free radical scavenging, (2) suppressing of lipid peroxidation, (3) enhancing the antioxidant molecules in tissues, (4) stimulating the activities of endogenous antioxidant enzymes, (5) inhibiting the activity of inducible nitric oxide synthase, (6) inhibiting LDL oxidation, and (7) inhibiting enzymes of arachidonate metabolism. By virtue of antioxidant activity, curcumin has been documented to be anti-inflammatory, antimutagenic and cancer-preventive, antiatherogenic and cardioprotective, hepatoprotective, neuroprotective, anti-cataractogenic, effective wound healant, etc. (Figure 5).

Beneficial Influences of Pungent Spices on Gastrointestinal System

Influence on small intestinal ultrastructure and absorptive function

Piperine of black pepper also causes an increase in intestinal brush border membrane fluidity and stimulated the activities of membrane bound enzymes due to the alteration in enzyme kinetics. Ultrastructural studies with piperine showed an increase in microvilli length. Thus, piperine may induce alterations in membrane dynamics and permeation characteristics,

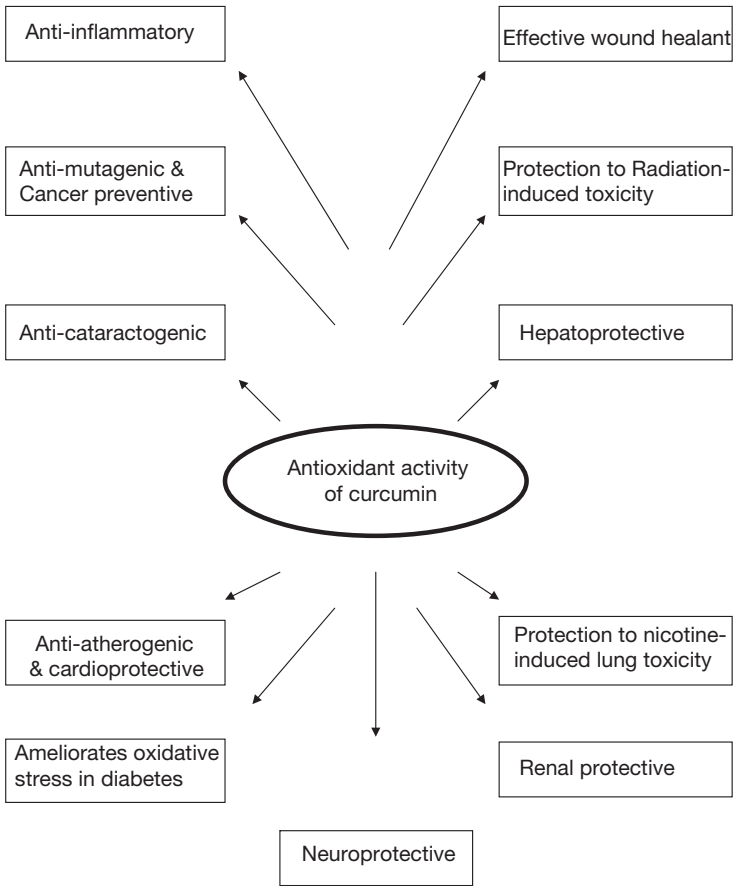

Figure 5 Health implications of antioxidant property of spices.

along with induction of the synthesis of proteins associated with cytoskeletal function, resulting in an increase in the absorptive surface, thus assisting efficient permeation through the epithelial barrier. Dietary pungent spices – black pepper/piperine, red pepper/capsaicin, and ginger – were evidenced to induce alteration in brush border membrane fluidity and passive permeability property, associated with the induction in the increased microvilli length, resulting in increased absorptive surface of the small intestine.

In view of dietary black pepper/piperine, red pepper/capsaicin, and ginger specifically altering the ultrastructure and permeability characteristics of small intestines, these spices have been examined for their possible influence on intestinal absorption of minerals. Higher absorption of iron, zinc, calcium, and β-carotene from duodenum, jejunum, and ileum portions of small intestines isolated from rats prefed these spices has been observed. These pungent spices alter permeation characteristics presumably by increasing absorptive surface and thereby enhance intestinal absorption of micronutrients.

Effect on gastric mucosa

Pungent spices have long been implicated to cause gastric mucosal injury. On the other hand, protective action of piperine against experimental gastric ulcer has been evidenced in rats and mice. Piperine protected animals from gastric ulceration in a dose-dependent manner as indicated by inhibition of the volume of gastric juice, gastric acidity, and pepsin activity. Protective effect of dietary black pepper/piperine, red pepper/capsaicin, and ginger with respect to activities of antioxidant enzymes in gastric and intestinal mucosa has been evidenced with significantly enhanced activities of antioxidant enzymes in both gastric and intestinal mucosae, suggesting a gastrointestinal protective role. These pungent spices were also found to alleviate the diminished activities of antioxidant enzymes in gastric and intestinal mucosa under conditions of ethanol-induced oxidative stress. The gastroprotective effect was also reflected in their positive effect on mucosal glycoproteins, thereby lowering mucosal injury. The amelioration of the ethanol-induced decrease in the activities of antioxidant enzymes in gastric and intestinal mucosa by dietary pungent spices suggests their beneficial gastrointestinal protective role.

Enhancing Effect of Piperine of Black Pepper on Bioavailability of Drugs/Phytochemicals

Piperine of black pepper is now established as a bioavailability enhancer of various structurally and therapeutically diverse drugs and other substances. Potential of piperine to increase the bioavailability of drugs in humans is of great clinical significance. Most of the clinical trials have shown that piperine increases levels of medications. This effect is due to the inhibitory interaction of piperine with cytochrome P_{450} enzymes of the liver and small intestine that are involved in drug metabolism.

Antimicrobial Activity

Antimicrobial activity has been demonstrated *in vitro* for several herb and spice extracts and essential oils from thyme, oregano, parsley, cilantro, and cinnamon. Growth of several bacteria strains has been shown to be inhibited by various concentrations of these culinary herb and spice extracts in the culture medium. Antimicrobial phytochemicals fall into several categories: phenolics/polyphenols, terpenoids and essential oils, alkaloids, and lectins and polypeptides. Phenolic and polyphenols possess multiple antimicrobial mechanisms of action when compared to other phytochemical categories. They are capable of forming an irreversible complex with nucleophilic amino acids in protein, leading to their inactivation and therefore loss of function in bacteria. Phenolic and polyphenols have been reported to disrupt microbial membranes, inactivate microbial enzymes, and activate immune response by stimulating macrophages.

Other Flavoring Crops

Vanilla (*Vanilla planifolia*)

Vanilla is a flavor derived from pods of the genus Vanilla (*Vanilla planifolia*). Among many compounds present in the extracts of vanilla, the compound vanillin (4-hydroxy-3-methoxybenzaldehyde) is primarily responsible for the characteristic flavor and smell of vanilla. A major use of vanilla is in flavoring ice cream. Vanilla contains high levels of antioxidants. It has sedative property and hence alleviates anxiety. Vanilla is also used as an aromatherapy remedy being considered a popular scent for conferring aphrodisiac effects (Figure 6).

Cocoa (*Theobroma cacao*)

Cocoa bean is the dried and fully fermented fatty bean of *Theobroma cacao*, from which cocoa solids and cocoa butter are extracted. They are the basis of chocolate. A cocoa pod has a rough and leathery rind about 3 cm thick, which is filled with sweet, mucilaginous pulp. In general, chocolate and cocoa are considered to be a rich source of antioxidants such as procyanidins and flavonoids, which may impart antiaging properties. Chocolate and cocoa also contain good amount of flavonoids, specifically epicatechin, which may have beneficial cardiovascular effects on health. The stimulant activity of cocoa is attributable to the compound theobromine. Prolonged intake of flavanol-rich cocoa has been linked to cardiovascular health benefits. It is believed that the improved blood flow after consumption of flavanol-rich cocoa may help to achieve health benefits in the heart and brain in terms of learning and memory. Foods rich in cocoa are understood to reduce blood pressure.

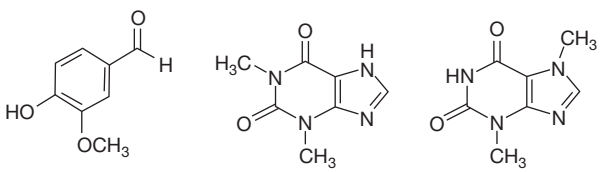

Figure 6 Formulas of bioactive compounds present in other flavor crops.

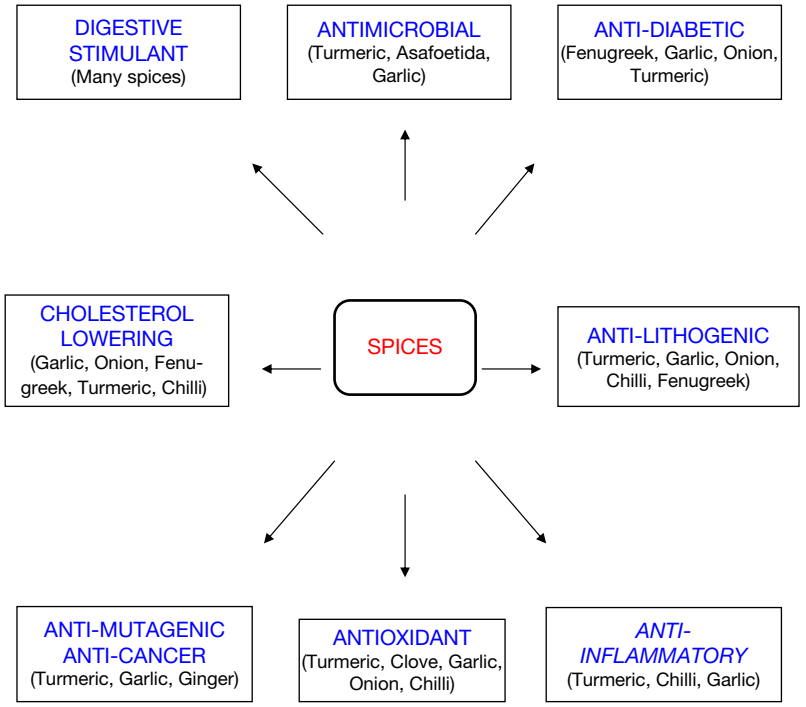

Figure 7 Summary of multiple health effects of spices.

Tea Leaves (*Camellia sinensis*)

Tea is the most widely consumed aromatic beverage in the world. Tea contains a large number of bioactive chemicals, including flavonoids, vitamins, caffeine, and several polysaccharides. It has been suggested that green and black tea may protect against cancer; catechins found in green tea are thought to be more effective in preventing liver cancer and colorectal cancer, while both green and black teas may protect against cardiovascular disease. Epidemiological studies have investigated the effects of green tea consumption on the incidence of human cancers and suggest significant protective effects of green tea against oral, pharyngeal, esophageal, prostate, digestive, urinary tract, pancreatic, bladder, skin, lung, colon, breast, and liver cancers and lower risk for cancer metastasis and recurrence.

Conclusions

By making the food attractive and palatable through flavor, aroma, and color, spices can reduce the need to use less healthy ingredients such as salt, fat, or sugar. Many health beneficial attributes of these common food adjuncts have been experimentally evidenced in the past few decades that include digestive stimulant action, antiatherogenic and cardioprotective potential, antilithogenic property, antidiabetic influence, anti-inflammatory property, and cancer-preventive potential. The antioxidant and hypolipidemic properties of spices have far-reaching nutraceutical value. The antioxidant properties of bioactive compounds present in spices are of particular interest in view of the impact of suppression of oxidative stress in the development of degenerative diseases such as cardiovascular disease, neurodegenerative disease, inflammatory disease, and cancer. Spices thus deserve to be considered as nutraceuticals beyond their role in imparting taste and flavor to our food (Figure 7).

See also: Cocoa: Composition and Health Effects; Tea: Health Effects.

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Spoilage: Bacterial Spoilage

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Introduction

Although food spoilage cannot be considered a health issue, it deals with food safety and has important economic and ecological consequences. In fact, about one-third of all the food that is produced for human consumption worldwide (i.e., dairy, fish and meat products, fruits and vegetables, cereals, and roots) is lost or wasted. A significant proportion of the loss is due to spoilage by microorganisms, resulting in final products with an inadequate shape or appearance. Consequently, spoiled food is lost because it does not fit the quality criteria, and will thus be removed from the market before commercialization, or it is wasted at the end of the food supply chain, because it will be rejected by the consumer. Various bacteria can spoil food, and whether or not they are harmful for consumer health, they cause a change in color or texture, an undesirable taste, or an unpleasant odor. The list of bacterial species involved in food spoilage is long and encompasses both Gram-positive and Gram-negative species. Some can develop in a wide range of products and are responsible for the spoilage of different foodstuffs, whereas others are specific to a product or a process. Moreover, worldwide, for a given type of food, the bacterial spoilage may be different, depending on the various processes or storage conditions that may be in use in different countries. As an example, low temperature during the storage and transportation of meat or milk, used mostly in industrialized countries, selects psychrotrophic bacteria. This is not the case in some developing countries, which in addition may have a warm and humid climate.

A variety of methods have been used to ensure microbial food safety, like fermentation, smoking, drying, and addition of various curing agents and subsequent storage at low temperatures or under different oxygen concentrations. Fermentation is quite an efficient process, so that spoilage of fermented products is relatively rare. Salts like nitrite/nitrate and NaCl also contribute to diminishing bacterial growth, although some bacterial species can resist salt addition. The use of cold storage considerably reduces spoilage as it limits bacterial development. However, as mentioned earlier, it may select psychrotrophic or psychrophilic bacteria, and some of these may cause spoilage. The use of atmospheres poor in oxygen or of vacuum packaging limits spoilage by aerobic bacteria but may result in the development of other types of spoilage like blown pack spoilage of vacuum-packed meat caused by the bacterial production of gas.

Clearly, the bacterial spoilage of food is diverse, results from many different mechanisms, and can be caused by many different bacteria. We will present the main bacterial spoilage for dairy, meat, and seafood products and products of vegetable origin. We will also briefly discuss new information that is being gained through high-throughput DNA sequencing methods about the bacterial species that may be responsible for the spoilage of food products and through metabolomics studies that can reveal the bacterial metabolic activities responsible for spoilage.

Dairy Products

Milk production exceeds 700 million tons annually, but about 10–20% of the initial milk and dairy production is lost worldwide. Europe is the main producer of dairy products. A large proportion of them found on the market are fermented, like cheese (20 million tons) or yogurt. Starter cultures, often used in the industrial production of fermented products, usually overgrow indigenous microbiota, lead to a pH drop, and thus limit the possibility of spoilage bacteria having a negative effect on the quality of the products. The addition of salt in cheese making also contributes to reducing spoilage. Nevertheless, dairy product spoilage is still reported, and some examples are listed in the succeeding text.

In fresh cheese, like cottage cheese, the relatively high pH may not be sufficient to inhibit the development of psychrotrophic species of *Pseudomonas* and coliforms. The spectacular 'blue mozzarella cheese' spoilage has been reported in Italy and Germany. It is caused by *Pseudomonas fluorescens* strains producing a blue pigment (i.e., pyocyanin) after mozzarella cheese packs are opened, changing the milky white balls typical of this product into inky blue balls. *Pseudomonas fluorescens* and other *Pseudomonas* species, such as *Pseudomonas putida* and *Pseudomonas fragi*, may also be responsible for the rancid flavors and odors caused by their lipid and fatty acid metabolism. In addition, several *Pseudomonas* and *Bacillus* species can produce proteases that may degrade caseins causing the bitterness of cheese and influencing milk coagulation. Extracellular lipase and protease activities produced by *Pseudomonas* can be heat-stable and therefore affect milk quality even after heat treatment, such as pasteurization or ultra-high-temperature treatment. Slime production by some lactic acid bacteria has also been reported. This property is considered beneficial for fermented yogurts since it contributes to their texture but undesirable in many other products. Slime production by *Alcaligenes viscolactis* in cottage cheese and by *Pseudomonas putida* or *Pseudomonas fragi* on cheese surfaces has been reported as spoilage. Moreover, the butter smell due to the diacetyl molecule participates in the quality of some dairy products but is undesirable in others. Diacetyl is produced by various bacteria, in particular lactic acid bacteria, but degraded by other species. Therefore, some lactic acid bacteria may be considered spoilers through diacetyl production in some products, whereas diacetyl degradation by some species may result in spoilage in other products requiring a buttery flavor. *Propionibacterium freudenreichii* contributes to the Swiss-type cheese taste and to the 'eyes' or holes typical of these cheeses by producing gas. This phenomenon is well controlled by cheese makers, but occasionally, lactic acid bacteria or Clostridia may produce a gassy defect in an uncontrolled

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manner, leading to holes of unwanted size. *Clostridium tyrobutyricum* may cause this spoilage and, by producing butyric acid, may also provoke a rancid taste. Early blowing in fresh cheese in the early ripening stage and late blowing occurring well into the ripening stage of hard and semihard cheeses are also important causes of spoilage. It results in considerable losses as large-volume productions can be spoiled. Gas (CO_2) and butyric acid production by bacterial metabolism lead to balloon-like expansion of cheeses and to rancidity, respectively. Several gas-producing bacterial species belonging to Enterobacteriaceae can be responsible for early blowing, while *Clostridia* spp. are the main causes of late blowing. Clostridial spores, initially present in raw milk, can germinate and then grow during the cheese making process. *Clostridium tyrobutyricum* is the main species causing late blowing, but *Clostridium butyricum* and *Clostridium sporogenes* have also been reported as involved in this defect.

These examples show that bacterial spoilage of dairy products may affect the color, texture, odor, or appearance of the product and can be caused by various Gram-positive and Gram-negative species.

Seafood Products

Seafood consumption is constantly increasing worldwide, with industrial Asia being the main producer. Seafood products constitute notably the main source of protein, lipid, vitamins, and mineral nutrients in many countries. This significant role in consumer food choices is strongly linked to the healthy diet image and the nutritional properties associated with seafood product consumption. At the same time, losses and waste of seafood products can reach a high level, due to their high sensitivity to spoilage. For example, 30–50% of the initial catch is discarded, lost, or wasted, with more than half at the production, distribution, and consumer stages, thus largely because of spoilage. In the first steps after catching fish or seafood, spoilage is the result of either autolytic activities by free enzymes, naturally present in the flesh (lipases, proteases, decarboxylases, etc.), or lipid autooxidation, leading to secondary metabolites (esters, alcohols, aldehydes, ketones, etc.) associated with strong off-odors. In the subsequent processing steps, most spoilage is due to the development of specific spoilage bacteria. These microorganisms can grow easily on seafood products due to their ideal physiological conditions, such as a neutral pH, a high water activity, and a high content of low-molecular-weight compounds like free amino acids and nucleotides.

The microbiota present in seafood and fish products arise from an initial contamination by the environment (i.e., seawater) and fish microbiota (skin and gastrointestinal tract), and subsequently by contact with different surfaces during the processing steps, or through the addition of various ingredients. By growing on seafood, spoilage bacteria can implement metabolic pathways resulting in the production of a variety of molecules. In fact, bacterial spoilers can produce volatile compounds, organic acids, biogenic amines, or exopolysaccharides responsible for slime production. However, the main agents in this microbial spoilage are not always clearly identified, as there is no correlation between a total number of bacteria and spoilage, and only a fraction of the total flora is involved. Nevertheless, many studies have been conducted to elucidate this spoiling fraction in seafood products, and, among them, several have shown that the nature of the spoilage microbiota is partly selected by the production process (thermal treatment, preservation process, atmosphere used for packaging, storage temperature, etc.). For example, spoilage of temperate and tropical raw fresh fish, such as halibut, sea bass, mullet, swordfish, salmon, haddock, and sea bream, stored on ice under aerobic storage, is mainly provoked not only by Gram-negative bacteria belonging to the *Shewanella* genus, especially the group '*Shewanella putrefaciens*-like' including *Shewanella putrefaciens* and *Shewanella baltica*, but also by different *Pseudomonas* species, such as *Pseudomonas fragi*, *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas lundensis*. Species from the '*Shewanella putrefaciens*-like' group are notably able to produce sulfur compounds like hydrogen sulfide (H_2S) from the sulfur-containing amino acid L-cysteine. H_2S is responsible for the strong, putrid, and sulfur off-odor of spoiled seafood. These bacteria can also produce hypoxanthine from inosine (Ino) or inosine monophosphate (IMP). Ino and IMP may themselves arise from the autolytic processing of adenosine triphosphate, present in large amounts in fish muscles, which is degraded into adenosine diphosphate and adenosine monophosphate. Hypoxanthine is responsible for the bitterness and acrid taste of spoiled fresh fish. Some *Pseudomonas* species are also able to produce spoilage metabolites like esters, ketones, and aldehydes resulting from amino acid (notably glycine, serine, and leucine) catabolism and sulfur compounds such as methyl mercaptan (CH_3SH) and dimethyl sulfide [$(CH_3)_2S$], both formed from methionine breakdown. Another example of a raw seafood product in which the spoilage microbiota is dominated by Gram-negative bacteria is farmed freshwater prawn (*Macrobrachium rosenbergii*). After long-term storage on ice, its spoilage microbiota is dominated by more than 80% of Gram-negative bacteria such as *Pseudomonas*, *Aeromonas hydrophila*, *Aeromonas veronii*, and *Shewanella putrefaciens*. Another significant case is the king scallop (*Pecten maximus*) stored at cold temperature under aerobic conditions, which can be spoiled not only by a microbial flora dominated by *Pseudomonas* and *Shewanella* but also by the psychrotrophic Gram-positive species *Brochothrix thermosphacta*.

One way to reduce the impact of these spoilage bacteria is to change the atmosphere surrounding the seafood products by using either vacuum or CO_2-enriched modified atmosphere packaging (MAP), the latter being particularly suitable for inhibiting the growth of *Shewanella* and *Pseudomonas*. Nevertheless, CO_2 does not inhibit all Gram-negative bacteria. For example, *Photobacterium phosphoreum*, a Gram-negative bacterium regularly described as a seafood product spoiler, exhibits a very low sensitivity to CO_2. Consequently, *Photobacterium phosphoreum* is now recognized as the main spoilage bacterial species of several chilled marine fish, like cod, garfish, halibut, and salmon, packaged under modified atmosphere conditions. The main characteristic of *Photobacterium phosphoreum* spoilage is the production of trimethylamine [$N(CH_3)_3$] due to the use of trimethylamine *N*-oxide [$(CH_3)_3NO$] as a terminal electron acceptor during anaerobic respiration. Trimethylamine is responsible for the typical strong fishy, urine, and ammonia-like off-odors of spoiled fish and seafood products.

Another way to increase the protection of fish and seafood products against bacterial spoilage is to apply a thermal treatment during processing, followed by a specific packaging or various processes such as smoking, salting, and/or drying. These treatments are generally sufficient to stabilize or lightly preserve seafood by reducing the load of the majority of spoilage bacteria. However, seafood products can then undergo bacterial recontamination, mainly by Gram-positive bacteria, from the processing equipment, the environment in the production plant, and human handling. Their growth on seafood products can again result in the production of spoilage compounds. This is the case for cold-smoked salmon, where lactic acid bacteria play an important role in the spoilage process. In fact, experimental inoculation of sterile cold-smoked salmon with species from the *Lactobacillus* genus can provoke various spoilage defects. Notably, some *Lactobacillus sakei* strains can produce sulfurous (H_2S), floor cloth, and amine off-odors, *Lactobacillus alimentarius* can produce pungent and sour off-odors, and *Lactobacillus farciminis* can produce sour, acid, milky, and cabbage off-odors. Other lactic acid bacterial species participate in the spoilage of cold-smoked salmon, like *Carnobacterium maltaromaticum*, which produces butter and sour off-odors correlated with the production of large amounts of 2,3-butanedione and 2,3-pentanedione. *Brochothrix thermosphacta* also strongly affects the quality of cold-smoked salmon by producing large amounts of 2-heptanone and 2-hexanone, which are probably responsible for the blue cheese- and butyric acid-like off-odors.

Cooked shrimps constitute an interesting case of microbiological spoilage. Their shelf life depends on several parameters, such as the initial contamination, the efficiency of the heat treatment, the characteristics of the product (Nordic or tropical shrimps, peeled or whole, and brined or not with various brine solutions), and the packaging conditions (atmosphere and temperature of storage), which can all create hurdle effects regarding the growth of spoilage microbiota. The shelf life of Nordic and tropical shrimps packed under modified atmosphere, stored at different temperatures, and brined with various NaCl concentrations has been studied. After storage at 5 °C, shelf life ranged from 3 months when the brine solution was 2.3% NaCl to 6 months with 3.3% NaCl. Lactic acid bacteria were shown to dominate the bacterial population when products reached spoilage, with notably *Carnobacterium divergens* and *Lactobacillus curvatus* when the storage temperature was lower than 8 °C and *Enterococcus faecalis* for a storage temperature higher than 15 °C. The organic acid composition of the brine solution can also influence the spoilage microbiota. For example, a study on brined Nordic shrimps showed that *Enterococcus malodoratus*, *Carnobacterium maltaromaticum*, coagulase-negative *Staphylococcus* spp., *Lactobacillus sakei*, and *Pseudomonas fluorescens* could be detected when the brine solution was composed of benzoic, citric, and sorbic acids. However, when the brine solution contained acetic, citric, and lactic acids, the spoilage microbiota was constituted exclusively by yeast and *Lactobacillus sakei*, which was responsible for the production of sour and butter off-odors. Brine pH has also a significant effect on microorganisms. Indeed, a recent study performed by Danish scientists examined the effect of brine pH on the survival and growth of a spoilage species (*Lactobacillus sakei* isolated from brined and drained MAP shrimp and cold-smoked Greenland halibut) and on several pathogenic bacteria, previously isolated from various seafood products. Enumeration of bacteria after inoculation in brine containing sodium benzoate, citric acid, potassium sorbate, and sodium chloride, at three different pH values (i.e., 4.0, 4.4, and 4.8), showed significant growth reduction at pH 4.0 and 4.4.

Regarding the shelf life of unbrined, cooked, and peeled shrimps (Nordic or tropical), packed under modified atmosphere, the main bacteria involved in their spoilage are *Brochothrix thermosphacta* and *Carnobacterium maltaromaticum*, with the production of strong cheese/feet, sour/fermented, and butter off-odors, related to the release of chemical compounds such as 3-methyl-1-butanal, 2,3-butanedione, 2,3-heptanedione, and acetaldehyde. Interestingly, a study reported that when co-inoculated on cooked and peeled shrimps, *Brochothrix thermosphacta* and *Carnobacterium maltaromaticum* can form a typical wet dog off-flavor, whereas they do not produce this off-flavor when inoculated separately. No new metabolite was detected in *Brochothrix thermosphacta–Carnobacterium maltaromaticum* cocultures by comparison with those produced by both species inoculated alone. The authors suggested that this phenomenon cannot be considered as metabiosis, where one bacterium uses a product from another bacterium as a substrate to synthesize one or several new metabolites, since no new metabolite was produced by the coculture. It can therefore be hypothesized that the wet dog off-flavor is most probably resulting from an interaction between metabolites formed by *Carnobacterium maltaromaticum* and *Brochothrix thermosphacta*.

In conclusion, these examples show that fish and seafood bacterial spoilage mainly affects the odor of the products. More rarely, an undesirable taste (bitterness) or appearance (slime production) results from the spoilage of these food products.

Meat Products

The consumption of meat products is a source of protein for the human diet. According to the Food and Agriculture Organization of the United Nations, there is a need to increase drastically (60%) meat production in order to feed the human population by the year 2050. Worldwide, ~20–25% of meat production is wasted or lost. In industrialized countries like North America, Europe, and industrialized Asia, losses arise mostly at the end of the food supply chain. This might be explained by a large proportion of waste by retailers and consumers, suggesting that spoilage may be the main cause of such waste in this part of the world (which also has the highest meat consumption per capita). Reducing meat spoilage will therefore be an important challenge not only to feed the world but also to ensure the sustainable production of meat products.

Spoilage of meat varies depending on the type of product. As in fish and seafood products, early contamination in the slaughterhouse originates from animal microbiota (gastrointestinal tract, hide, skin, and feather). Consequently, this initial contamination is slightly different when the meat comes from different animals, for instance, birds or ruminants. As for other foodstuffs, subsequent contamination results from contact with equipment and material, during manipulations throughout the processing chain, and from the addition of various ingredients used in meat transformation.

As mentioned earlier for dairy products, fermented or dry-cured meats are usually stable products, and their spoilage by bacteria is not very common. In fact, putative spoiler species belonging to pseudomonads and enterobacteria initially present in the meat decrease during the fermentation and ripening steps. However, proteolytic bacteria like *Serratia*, associated with *Aeromonas hydrophila* and *Burkholderia cepacia*, have been reported to cause spoilage of Iberian dry-cured ham. Cured meat products are also sensitive to lipid oxidation, which can be directly or indirectly linked to bacterial metabolism and provokes undesired odors. The typical color of dry-cured meat products, resulting from bacterial, enzymatic, and chemical reactions, may be affected by the presence of some bacterial species. During fermentation, nitrite reacts with myoglobin, leading to nitrosomyoglobin that contributes to the typical color of cured meat products. Nitrate, added for fermentation, can be reduced to nitrite by bacterial metabolism, and therefore, bacteria can interfere with the desired color of cured meat.

Nonfermented but processed meat products are more sensitive to bacterial spoilage because of the increased number of processing steps and potential contaminations. The bacterial spoilage of various types of nonfermented sausage has been reported, involving some lactic acid bacteria (*Carnobacterium*, *Leuconostoc*, or *Weissella* species), Enterobacteriaceae, *Pseudomonas* species, and *Brochothrix thermosphacta*. Unacceptability due to this spoilage is mainly characterized by off-odors resulting from bacterial metabolism. Various bacterial species may also cause discoloration, especially for red meat products in which a greenish or grayish color is unacceptable.

In northern Europe, marinades are used to preserve and flavor raw meat. They consist of brine with salt, phosphate, sugar, organic acids, spices, and herbs to make up a sauce to flavor meat cuts. This may result in spoilage as sugar addition may contribute to bacterial development, particularly that of lactic acid bacteria.

The use of vacuum packaging has been largely developed for red meats like beef and venison, or lamb, to prolong their shelf life. The absence of oxygen in this type of packaging impairs the development of aerobic bacterial species, like *Pseudomonas* spp. However, the association of vacuum packaging with a cold or chilled storage temperature may result in the development of psychrophilic or psychrotrophic bacteria that produce gas, resulting in blown pack spoilage. The gas provokes swelling of the packs, which are then not acceptable for consumption, and, depending on the gas-producing bacterium, this spoilage may be accompanied by the production of off-odors. Several Clostridia have been reported or suspected to be responsible for blown pack including *Clostridium algidicarnis*, *Clostridium estertheticum*, *Clostridium gasigenes*, and *Clostridium putrefaciens* and psychrotolerant Enterobacteriaceae strains belonging to *Enterobacter*, *Serratia*, *Hafnia*, and *Rahnella* genera. Several lactic acid bacteria (*Lactobacillus sakei*, *Lactococcus piscium*, *Carnobacterium divergens*, and *Carnobacterium maltaromaticum*) have also been isolated from blown packs of beef meat. CO_2 is generally the main gas produced by bacteria responsible for blown pack, but several volatile organic compounds are sometimes associated, too. Consequently, the detection of butanoic acid, 1-butanol, dimethyl disulfide, acetic acid, and 3-methyl-1-butanol associated with CO_2 production has been proposed as a marker to detect the presence of bacterial species responsible for blown pack.

The use of oxygen-enriched atmospheres is also common to ensure red meat storage and enhance the typical bright red coloration of meat due to myoglobin. The high oxygen concentration (up to 70%) may also contribute to inhibiting the growth of strict anaerobic bacteria. However, *Brochothrix thermosphacta* and species of *Leuconostoc*, *Lactobacillus*, *Carnobacterium*, and *Clostridium* genera and of the family Enterobacteriaceae can resist these storage conditions and have been commonly associated with spoilage of modified atmosphere-packed meat products. Although lactococci have long been considered only in dairy products, the species *Lactococcus piscium* has been found in meat products stored under MAP and shown to be responsible for spoilage.

Various meat products, such as pâtés, hams, and sausages, are cooked but remain sensitive to recontamination after cooking. In fact, a single contaminant can develop without any competition on the product because it is not impaired by the presence of an indigenous flora. This is illustrated by a 'special spoilage problem in vacuum-packaged cooked meat products' that was reported in Finland. These products were contaminated by ropy slime-producing *Lactobacillus sakei* strains, which caused a ropy slime defect for a while in a large range of cooked meat products before being eradicated.

From the list of examples cited earlier, it is clear that bacterial spoilage of meat has multiple consequences: the color, odor, texture, and appearance, including that of the packaging, may be affected by a large number of bacterial species. Among them, *Brochothrix thermosphacta* has been the most frequently reported since it is the most versatile species, adapted to the vast diversity of meat products. As a facultative anaerobe, it can resist the various atmospheres used for meat packaging (vacuum, air, and modified atmosphere), although under vacuum packaging, it can be overgrown by other species. It can also cope with high salt concentrations, low pH, and low temperatures. This may explain why it is often incriminated in the spoilage of not only a large variety of meat products but also seafood and fish products, which have some common characteristics with meats from mammals or birds.

Vegetables

The consumption of fruit and vegetable products has increased by 20–30% in Western countries in recent decades. Several sources estimate global losses and waste at around 30% of the initial production intended for human consumption on the basis of literature and FAO food balance sheets. The microbiota present in vegetables partly reflects that of the soil and depends on culture methods (irrigation and fertilization). Compared with the other foodstuffs discussed in this article, one additional reason for bacterial spoilage is due to bacteria that are pathogenic to the plants. Contamination of fruits and vegetables is often related to the sanitary quality during the transformation process, with a bacterial population of the raw vegetable that can reach about 5–7 log CFU g^{-1} and is reduced to 3–4 log after blanching. This flora then increases after the packaging stage to reach 5–7 log CFU g^{-1}. The microbial limit

for consumer consumption of fresh processed vegetables is 8 log CFU g^{-1} of mesophilic aerobic bacteria.

Bacteria can enter through natural openings (stomata and lenticels) or areas injured by different types of plant damage (physical and biological), contaminating and spoiling these vegetables. Vegetables are rich in water (74–94%) but contain low amounts of sugars (8.6%), proteins (2%), and fat (0.3%). They also contain vitamins and minerals. This nutrient-rich environment is favorable for the growth of microorganisms. Unlike fruits, the pH of most vegetables is close to neutral and allows the development of plant spoilage agents (fungi, yeasts, and bacteria). The relatively high redox potential explains the greater presence of aerobic or anaerobic optional bacterial flora. The cell wall of plants is composed of polysaccharides including cellulose, pectin, and hemicellulose. Some spoilage bacteria can produce enzymes, such as xylanases, polygalacturonases, cellulases, proteases, and lipases, which degrade plant cell walls. These phenomena cause cell necrosis and tissue maceration (soft rot). The bacteria most involved in this type of spoilage are *Pseudomonas marginalis* and *Pectobacterium carotovorum*. Recent studies have shown that the aerobic flora of plants increases in the spoiled products but that bacterial diversity is reduced. From a study of 1000 isolates from different vegetables (lettuce, broccoli, onion, spinach, potato, carrot, etc.), 14 different genera and 20 different species were identified as the major ones. Most isolates were Gram-negative, but some Gram-positive ones (two *Bacillus* and one *Clavibacter*) were also present. Seven genera belonged to the family Enterobacteriaceae and several species of *Pseudomonas* were also identified, the majority belonging to the species *Pseudomonas fluorescens* and also *Pseudomonas chlororaphis*, *Pseudomonas corrugata*, and *Pseudomonas putida*. *Pectobacterium carotovorum* and *Dickeya zeae* can lead to maceration of plants through the production of extracellular enzymes such as cellulase and pectate lyase. *Pectobacterium carotovorum* also produces polygalacturonase, which alters the cell wall. *Pseudomonas chlororaphis* produces pectinase, which is the origin of the alteration of plants, and *Burkholderia* strains produce a pectate lyase that can macerate different kinds of plants. This suggests that pectate lyase is the major hydrolytic enzyme involved in the spoilage of vegetables. In processed vegetable products that are packaged under modified atmosphere (at concentrations below 2% O_2 and above 10% CO_2), several Gram-positive bacteria, mostly lactic acid bacteria, have been associated with spoilage of fresh-cut vegetables. It has also been reported that increasing the CO_2 concentration or reducing that of O_2 seems to favor the growth of lactic acid bacteria in fresh processed vegetables. These bacteria can increase from 3 to 6 log CFU g^{-1} after 7 days of storage. *Leuconostoc mesenteroides* has been reported to cause exudation and loss of texture in shredded carrots packed under modified atmosphere and stored at 10 °C for 10 days.

For fruits, bacterial contamination can occur at various stages of agricultural production and processing when the outer surface of the fruit comes into contact with soil, water, the production plant environment, surfaces of conveyor belts, and hands of packing workers. Since spoilage microorganisms may be harbored in the rough outer rind of the fruit, it is difficult to wash and clean fruits effectively. Bacteria are transferred from the external fruit surfaces to the edible portions during cutting, thus contaminating fresh-cut products. Moreover, fresh-cut products may be recontaminated through cross-contamination and improper handling during distribution.

The average water content of fruits is 85% and the carbohydrate content is about 13%. Like vegetables, fruits have a low protein (0.9%) and lipid (0.5%) content and provide a substrate for yeast, fungi, and bacteria growth. However, because of their low pH (3.8–4.6), fruits are not altered by bacteria, but mostly by yeasts and molds. Nevertheless, spoilage bacteria can cause defects in some fruit juices.

In fruit juices, *Alicyclobacillus acidoterrestris* is a thermoacidophilic spore-forming microorganism able to grow at pH values between 2.5 and 6.0. *Alicyclobacillus acidoterrestris* spores can survive conventional juice pasteurization procedures and then germinate, grow, and cause spoilage. The germination of spores and subsequent vegetative cell growth can occur in apple (pH 2.5), tomato (pH 4.0), white grape (pH 2.8), orange (pH 3.6), and pineapple (pH 3.3) juices. Spoilage is characterized by medicinal or phenolic off-flavors or off-odors, whereas the juice may appear normal or have a slight sediment. The compounds responsible for spoilage have been identified as mainly 2-methoxyphenol (guaiacol) and 2,6-dichlorophenol and 2,6-dibromophenol that occur at lower concentrations. The taste thresholds for guaiacol in orange and noncarbonated fruit juices were reported to be around 2 $\mu g\,l^{-1}$ (ppb) and 2.32 ppb in apple juice, while the concentrations of 2,6-dichlorophenol and 2,6-dibromophenol in spoiled mixed fruit drinks were reported to be 16–20 $ng\,l^{-1}$ (ppt) and 2–4 ppt, respectively. A cell concentration of 10^5–10^6 CFU ml^{-1} of *Alicyclobacillus acidoterrestris* is enough to spoil fruit beverages by producing 2 ppb guaiacol.

In low-acid canned food, spoilage resulting from microbial growth is characterized by gas production, abnormal odors/colors, or pH variation. Industrial canning processes are designed to reach sterilization values that ensure 'commercial sterility.' Thermophilic spore-forming bacteria may impair the commercial viability of products stored at ambient temperatures. The species *Moorella thermoacetica/thermoautoautrophica* and *Geobacillus stearothermophilus* have been identified from spoiled green peas and green peas with carrots, with *Moorella* representing more than two-thirds of spoilage bacteria. However, *G. stearothermophilus* accounts for more than 40% of spoilage cases of green beans and more than 80% of those of sweet corn. An *Anoxybacillus* contaminant has been identified exclusively in soups. *Thermoanaerobacterium* (formerly *Clostridium thermosaccharolyticum* spp.) and *Bacillus* sp. have also been identified in 8% and 9% of spoiled canned food samples, respectively. *Bacillus coagulans* is the most common species involved in the spoilage of tomato products. Other genera represent $< 5\%$ of spoilage cases: *Caldanaerobius* sp., *Anoxybacillus* sp., *Paenibacillus* sp., *Thermoanaerobacter* sp., *Clostridium thermopalmarium/thermobutyricum*, *Thermoactinomyces* sp., and several species of *Geobacillus* are the less reported bacteria.

Future Prospects for Studying Bacterial Spoilage with Next-Generation Sequencing Methods

Most of the studies aimed at understanding bacterial spoilage of food are based on the isolation of bacteria from spoiled

products. It is generally considered that the dominant species found in spoiled products is the one responsible for spoilage. Many studies have proved the role of these species in causing spoilage by inoculating food matrices with such isolates to reproduce spoilage. However, the cooperation of species to produce spoilage has also been reported. For instance, the enhancement of the production of cadaverine by *Escherichia coli* when cocultured with *Brochothrix thermosphacta* (which does not produce cadaverine) has been reported. In addition, putrescine production by *Serratia liquefaciens* or *Hafnia alvei* grown alone or with *Carnobacterium divergens* or *Lactobacillus sakei* was enhanced in cocultures. This phenomenon, named metabiosis, reveals that spoilage may result from the combined action of several species.

The recent development of genomics and metagenomics is now used for the description of microbial communities from food, without prior plating on agar media. These technologies detect 'uncultured' bacterial species, sometimes dominant, revealing the limitations of the previous methods used to characterize food bacterial communities. One can thus expect that, in the near future, new species or genera potentially involved in food spoilage will be discovered. Although food microbial communities are generally well known, there is no doubt that, as for many other bacterial ecosystems (soil, gastrointestinal tract, etc.), metagenomics will reveal unexpected bacteria. In addition, the recent deluge of bacterial genome sequences has demonstrated significant intraspecies genomic diversity; within a bacterial species, different strains may have a genome up to 20% longer than other strains. As bacterial genes have an average size of 1 kb, this means that, within a species, several hundreds of additional genes may be present in strains harboring the longest genomes. For example, ~3500 proteins are predicted from the smallest *Escherichia coli* genome, whereas ~5500 are predicted from the largest. These discrepancies reflect varying abilities in the expression of different functions. This also applies to bacterial species responsible for food spoilage; within one species, not all the strains may harbor the same spoilage potential. The genome analysis of bacteria responsible for spoilage will certainly help understand the functions involved in spoilage development, in their regulation, and in the diversity of spoilage.

Metabolomics to Characterize Further the Bacterial Spoilage of Food

The production of malodorous molecules is often involved in the development of food spoilage. In many cases, the presence of bacterial spoilage flora can be correlated to the presence of volatile organic compounds. The detection of aldehydes, acids, alcohols, and/or ketones has often been correlated to spoilage, and there are some examples proving the ability of spoilage bacteria to produce such molecules either in laboratory conditions or in food models. Yet, the difficulty of identifying the bacterial metabolic pathways involved in the production of such molecules persists. This might be because only the end products are analyzed, and information about intermediate metabolites is lacking. Some compounds may also result from spontaneous chemical reactions of precursor molecules and therefore cannot always be attributed to a bacterial enzymatic reaction. Complementary analyses, using various methods, should provide a better description of bacterial metabolism leading to food spoilage. Volatile organic compounds are often analyzed, because methods are available to detect them and because it is simple to link such compounds to undesirable odors. Precursor molecules, such as amino acids, sugars, and fatty acids, should also be measured to decipher metabolic pathways leading to molecules responsible for spoilage. These molecules should then be correlated to bacterial enzymatic functions (and therefore genes) for a better description of the bacterial pathways leading to spoilage.

See also: *Bacillus*: Occurrence; Beef; Biogenic Amines: Toxicology and Health Effect; Cheese: Chemistry and Microbiology; *Clostridium*: Occurrence and Detection of *Clostridium perfringens*; Controlled Atmosphere Storage: Applications for Bulk Storage of Foodstuffs; Controlled Atmosphere Storage: Effect on Fruit and Vegetables; Fermented Foods: Fermented Meat Products; Fish: Processing; Fruit Juices; Heat Treatment: Effect on Microbiological Changes and Shelf Life; Lactic Acid Bacteria; Meat: Eating Quality and Preservation; Milk: Processing of Milk; Preservation of Foods; Quality Control in Food Processing; Smoked Foods: Principles and Production; Spoilage: Yeast Spoilage of Food and Beverages; Wastage of Food.

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Spoilage: Yeast Spoilage of Food and Beverages

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Introduction

This article reviews spoilage of foods and beverages by yeasts. Here, yeasts are defined as unicellular eukaryotic microorganisms, and typical species in food substrates include the well-known fermentative yeast *Saccharomyces cerevisiae*, ubiquitous *Candida* and *Hanseniaspora* spp., and problematic species *Zygosaccharomyces* and *Dekkera*. Yeasts are a common microbial partner in foods and beverages (raw, processed, and fermented), and their metabolic activity, if noticeable, generally assists in the creation of a product (e.g., wine, beer, bread, and cheese). Yeasts are used to start the fermentation to create these distinctive foods and beverages, but the same yeasts may also cause spoilage. Contaminant yeasts may grow and adversely affect foods where they are not normally associated, which is of great concern to the food processing and manufacturing industries. In general, spoilage of a food or beverage occurs when the product no longer appeals to the consumer due to a change in texture, color, flavor, shape, or odor and can lead to large economic losses through wastage. Food spoilage will occur when yeasts are in an unbalanced, chemically unstable, or contaminated food or beverage. Control measures for yeast spoilage typically address environmental control points. Here, typical spoilage in economically important foods such as dairy, meat, fruit juices, and wine will be discussed, with a focus on the risk factors associated with foods and beverages and the underlying biochemistry that leads to spoilage characteristics.

Implications of Spoilage and Food Safety

When a food or beverage is spoiled by yeast growth, the consumer will reject the product, leading to wastage, loss of reputation by the food brand or company, and cost of plant and industry cleanup.

Fortunately, despite the range of yeast species associated with food spoilage, yeasts are rarely if ever associated with loss of human life due to food contamination and infections. Often, yeasts pathogenic to humans (e.g., *Candida albicans* and *Cryptococcus neoformans*) are not associated with food and beverage ecosystems. However, there is evidence that the presence of yeasts in food can lead to human allergic and hypersensitive reactions like migraines, respiratory problems, asthma, and gut and bowel syndromes. The literature in this area is growing and may result from human metabolic reactions to intrinsic yeast cellular components, such as cell wall polysaccharides, metabolites, and cellular proteins.

There is an increasing body of evidence that points to an increase in human infections by opportunistic pathogens, such as *Saccharomyces cerevisiae*, *Candida krusei*, *Yarrowia lipolytica*, and *Rhodotorula* spp. Although these outbreaks are not linked to consumption of spoiled foods and beverages, these yeast species are commonly found in food and beverage ecosystems. Under these conditions, healthy individuals are not at risk of illness due to consumption of foods spoiled with yeasts; however, patients with weakened health or immunocompromised status may be susceptible to infection. Trends in this area have raised questions about food safety and yeasts and are relevant in a discussion regarding spoilage of food and beverages by unwanted yeasts.

Factors Affecting Growth and Survival of Yeasts

Yeasts are commonly found in the environment and may be a normal part of the microbiota of a particular food. This can mean that the yeasts may be carried over into the finished food product, for example, in the production of fermented beverages from fruit juice. Control of the growth and proliferation of yeasts is therefore closely associated with the inherent properties of the food or beverage and includes factors such as temperature, water activity, pH and acidity, and the presence of antimicrobials. The stress imposed by each of these factors may limit the survival and growth of yeasts, but the exact limit required to prevent growth is difficult to define as the impact of one factor may affect the impact of another factor. For example, the antimicrobial effect of sulfur dioxide used in fruit and beverage preservation is dependent on the natural acidity and pH, which may influence the growth characteristics of yeasts directly. This also points to the impact of combined factors being synergistic or additive to the overall viability of yeasts. Additionally, the growth state of the yeast cell can influence its susceptibility to an environmental stress, where stationary phase cells are significantly more resistant to changes in temperature, pH, and antimicrobials than cells in the exponential growth phase. Sublethal levels of the factors that affect yeast growth may induce adaptive responses in the yeast population and can lead to inheritable resistance in the spoilage yeast population. Prevention of yeast spoilage requires fundamental understanding of these growth factors and the ability to adjust them to prevent unwanted yeast growth and thus spoilage.

Temperature

The majority of yeasts exhibit optimal growth in the range of 20–40 °C, but there is much variability among yeast species. Although few yeasts grow at temperatures above 40 °C, many yeasts can grow quite rapidly at lower temperatures of 2–10 °C. This is problematic for the stability of refrigerated or low-temperature foods and beverages. Additionally, some yeasts of the species *Candida*, *Cryptococcus*, and *Rhodotorula* can grow at temperatures < 0 °C and can spoil frozen foods, although this is quite uncommon. Yeasts can spoil foods that have been subjected to heat processing, such as soft drinks, syrups, and fruit juices and concentrates. The rate of destruction of yeasts at high temperatures has been calculated in some cases, and it is well

known that *Saccharomyces cerevisiae* is particularly heat-resistant in its ascospore state when compared to the budding cell.

Temperature significantly affects the growth rate of yeasts, and movement outside of the temperature optimum of particular yeasts can reduce generation time and thus proliferation. Another effect of temperature is to influence resistance to antimicrobials (e.g., ethanol), biochemical transformation of sugars and salt, and production of spoilage metabolites.

Water Activity

Water activity (A_W) is an effective way to reduce the growth and viability of bacteria, but less so for yeasts. Many yeast species are tolerant of low A_w and exhibit significant growth. The activity of water is associated with the presence of high solute concentrations, and there is wide variability of yeast ability to grow that often depends on the solute in question. Some yeasts are able to grow in media or substrates with high sugar or salt concentrations, for example, *Debaryomyces hansenii* is very salt-tolerant, and some strains can tolerate up to 24% (w/v) NaCl. *Saccharomyces cerevisiae* also exhibits significant salt tolerance, but this is strain-dependent and is also influenced by pH of the food or beverage in question. *Zygosaccharomyces rouxii* is exceptionally tolerant to high sugar concentrations (up to 70% w/v sucrose).

pH and Acidity

Yeasts exhibit best growth in the range of pH 4.5–7.0. The lower end of the pH scale is preferred by many yeast species, and many species readily grow at pH 2.0–2.5. More rarely, *Hanseniaspora uvarum* may grow at pH 1.5. The pH range of food and beverages encompasses a wide range as well, but it is worth noting that many fruits and vegetables, and juices made from these products, are low in pH, typically pH 2.0–4.0. Fermented dairy products such as cheese and yogurts are stabilized at pH 3.0–4.0, after acidification of the raw milk by bacterial (or other) fermentation. Stronger growth of yeasts at low pH means that they are successful at competing with and dominating other microbial partners, including bacteria. At the other end of the pH scale, some yeasts can grow with reduced vigor at pH 7.0–7.5; however, very few yeast species can grow at pH 9.0. Importantly, as the pH scale is related to acidity in the food product, this factor may work with other factors such as temperature, solute, and water activity to stabilize a product and prevent yeast spoilage.

Antimicrobials

Antimicrobials are a diverse class of chemical compounds, which work to inhibit or minimize growth of microorganisms. Endogenous compounds or exogenous addition of antimicrobials can stabilize foods and beverages to prevent yeast growth.

A commonly used group of antimicrobials used as preservatives in foods and beverages are weak organic acids such as propionic, acetic, benzoic, and sorbic acids. Yeasts have a wide range of resistances to these compounds, but in general, effectiveness depends on the chemical composition of the material. For example, these weak organic acids are only effective at low pH (4.0 and below) as the acids are undissociated and are able to traverse the yeast cell membrane to have their antimicrobial effect. Many yeast species have evolved resistance to these compounds and include *Zygosaccharomyces*, along with some strains of *Candida krusei* and *Pichia membranifaciens*. However, weak organic acids remain a common way to reduce the possibility of yeast spoilage in the food and beverage manufacturing industries.

Ethanol is a potent antimicrobial and is also produced by fermentative yeasts on sugar substrates. For example, the principal wine yeast *Saccharomyces cerevisiae* produces ethanol during the fermentation of grape sugars in wine making. It has been hypothesized that the effect of the ethanol is to inhibit the growth of competing yeast species and allows *Saccharomyces cerevisiae* to numerically and metabolically dominate the ecosystem. However, ethanol does inhibit the growth and metabolism of *Saccharomyces cerevisiae* itself, leading to the undesirable situation of incomplete sugar utilization in grape must and juice with high sugar concentration. There are some yeast species that are tolerant to ethanol at high concentrations, although their metabolism may be affected, and these include *Zygosaccharomyces*, *Dekkera*, *Pichia*, and *Saccharomycodes*. These yeast species are implicated in the spoilage of high ethanol beverages such as wine. The complementary metabolic end product to ethanol, carbon dioxide, may also have an inhibitory growth effect on potential spoilage yeasts. Yeasts that are tolerant to high ethanol are often tolerant of high carbon dioxide and include the species mentioned earlier.

Dimethyl dicarbonate is a preservative compound that has been approved for use in the food industry to control the growth and metabolism of spoilage yeasts. It is effective in the range of 200–250 mg l^{-1}. Natural antimicrobials that work to inhibit growth of spoilage yeasts include essential oils of garlic and onion and vanillin. As these compounds are potent flavoring compounds in food, their use is somewhat limited.

Biochemistry of Spoilage

Food constituents are the substrates for yeast growth, with consequent spoilage, so a good understanding of the biochemical processes of spoilage is important. Transformation of the available compounds gives a wide range of end products that affect the food's sensory, chemical, and physical properties. The range of possible compounds produced is dependent on not only the genetic capability of the yeast in question but also the environmental parameters that can vastly change biochemical pathways, including temperature, pH/ acidity, and antimicrobial concentration.

Carbohydrates

Sugars are the major substrate of spoilage yeasts, and they are widely found in foods and beverages. The availability of sugars such as glucose, fructose, sucrose, and maltose allows central metabolism to occur via glycolysis in some yeasts, for example, *Saccharomyces cerevisiae*, and gives rise to a wide variety of end products such as CO_2 and ethanol that lead to spoilage. Minor compounds like acids, esters, ketones, aldehydes, alcohols, and sulfur compounds are also produced, which can drastically change the aroma and flavor of a spoiled substrate.

Polysaccharides are complex carbohydrates found in fruits and vegetables, which may be extracted and be present in prepared foods and beverages. Polysaccharides such as cellulose and xylan are not commonly affected by yeast growth but can cause problems in a few cases (e.g., growth of *Aureobasidium* and *Galactomyces* on fresh fruit and vegetables). More commonly, spoilage yeasts can degrade pectin, which provides structural stability to a plant cell, and are an integral part to the functionality of foods such as fruits and vegetables. Degradation of starch by yeasts has not been reported in cereal-containing foods, or those that have flour or other cereals as ingredients, although common spoilage yeasts contain amylolytic enzymes.

Nitrogen Compounds

Nitrogen in either free amino acids or inorganic ammonium compounds is required for the growth of spoilage yeasts. Assimilation and metabolism of these compounds affect the production of spoilage-specific sensory compounds, including alcohols, organic acids, and esters. Decarboxylation of free amino acids can lead to the production of flavorsome amines and has been implicated in adverse reactions in sensitive consumers. Although amine production varies with yeast species, in some cases, a carcinogen can be produced, as with the production of urea from arginine and citrulline to produce ethyl carbamate.

Meat products are rarely degraded by proteolysis by spoilage yeasts, as most do not produce extracellular proteases. There are some notable exceptions, for example, *Y. lipolytica*, *Rhodotorula*, and *Cryptococcus* spp. In meat products cured with nitrates, spoilage with *Pichia* and *Cryptococcus* spp. can lead to metabolism of sodium nitrate and sodium nitrite.

Organic Acids

Organic acids are important regulators of acidity and pH in food systems. Changing the acidity and pH can have implications for the flavor and aroma of the product. Organic acids are by-products of central sugar metabolism, with acetic and succinic acids having a large sensory impact. Acetic acid also has a low sensory impact threshold, with several yeasts such as *H. uvarum*, *Candida krusei*, and *Dekkera* spp. being able to produce significant amounts during spoilage of beverages, particularly wine. Yeasts are also able to oxidize organic acids, which can decrease the acidity and increase the pH of the food substrate. As the sensory profile of the food or beverage changes under these conditions, the susceptibility to bacterial contamination may change. Of particular importance is the growth of *Debaryomyces hansenii* and *Y. lipolytica* in fermented dairy products with the removal of lactic acid. The growth of *Schizosaccharomyces pombe* and *Z. bailii* removes malic acid from fruit juices, which increases the pH and dramatically affects the taste and stability of the juice.

Sulfur Compounds

Yeasts commonly produce volatile sulfur compounds as by-products of central metabolism, and this class of compounds is important, as the sensory threshold of these compounds is low. In general, sulfur compounds have negative impacts on the sensory profile of foods, with rotten, smelly, and eggy descriptors given to compounds such as hydrogen sulfide, dimethyl sulfide, mercaptans, and thioesters. *Saccharomyces cerevisiae* differs by strain in the production of sulfur compounds, but yeasts such as *P. membranifaciens*, *Dekkera* spp., and *Wickerhamomyces anomalus* can also produce significant amounts. Volatile sulfur production by *Y. lipolytica* is considered important for distinctive aroma production during the maturation of cheeses; however, this is not considered to be a spoilage process.

Lipids

Lipids are a group of compounds in foods and beverages that confer textural, functional, and sensorially important factors. Lipids can be degraded enzymatically to produce free fatty acids that have rancid and unpleasant off-aromas. Breakdown of lipids is important in meat, dairy, and olive oil systems, where growth of *Candida*, *Cryptococcus*, and *Rhodotorula* can produce lipolytic enzymes that can spoil these foods.

Important Yeasts in Food and Beverage Spoilage

Foods and beverages can support yeast growth, but the spoilage of the product results from secondary metabolism, fermentative, or oxidative processes. The following are examples of yeasts that spoil food using each of these representative mechanisms.

Dekkera/Brettanomyces

Dekkera bruxellensis are spoilage yeasts associated with wine and to a lesser extent other beverages. Its teleomorph is *Brettanomyces*, and these yeasts are commonly known as the 'Brett' yeasts in the wine industry, where they cause an undesirable spoilage of maturing wine. *Dekkera bruxellensis* is slow growing and ethanol- and carbon dioxide-tolerant, which allow it to form significant populations in stable wine that is maturing in cellars. After the primary ethanol fermentation, red wine is stabilized by the addition of sulfur dioxide and stored in oak casks or tanks to mature before blending and bottling for sale. As this maturation time is often for years, *Dekkera* has ample time to grow in the affected wines. *Dekkera* produces distinctive off-taints with secondary metabolism, where phenolic and mousy flavors can spoil the aroma and desirability of the wine. Once a wine is tainted with these aromas, it cannot be recovered and leads to significant economic losses.

Understanding the causes and control of *Dekkera* spoilage in the wine industry has been the focus of vigorous research in recent years, with some interesting findings. Although the yeasts can be isolated from grapes in the vineyard and may persist during alcoholic fermentation, it is thought that contamination may occur via oak barrels and stainless steel wine processing equipment such as pumps, presses, transfer lines, and bottling lines. As sanitation of oak barrels is difficult and often incomplete, quarantine and rigorous record keeping is necessary to stop cross contamination. It is thought that the yeasts may be able to persist in oak barrels by utilizing the wood-derived sugar cellobiose. Additionally, it is thought that

a nonmetabolically active form of the yeast can exist as a viable but nonculturable state, which makes detection and control difficult.

Prevention of *Dekkera* contamination in wines is mostly about good winery sanitation to prevent occurrence in the first place. Intrinsic properties of the wine may affect *Dekkera* growth, as some strains are affected by ethanol >9%, and strains may show adaptation to ethanol concentrations >12%. *Dekkera* is a facultative anaerobe, which suits the wine storage environment where microaeration through the oak barrels can assist with maturation of wine. Small amounts of oxygen stimulate growth and production of off-aromas by secondary metabolism. Although the optimal growth temperature for this yeast has been reported to be between 25 and 28 °C, spoilage has been demonstrated at 15 and 20 °C. Wine cellars are typically kept at low temperatures, which may reduce the risk of growth. The effect of pH has not been investigated to a large degree in *Dekkera*, although some strains can grow at pH 2.0. Application of sulfur dioxide to wine appears to be the best way to reduce growth of *Dekkera*, where 0.2–0.5 mg l^{-1} of molecular SO_2 inhibits growth of most strains. As the presence of the molecular form of sulfur dioxide is dependent on pH, maintaining good control of acidity levels in the wines, especially following secondary (bacterial) malolactic fermentation, is essential. The effect of molecular SO_2 is additive with ethanol concentration, where increasing sensitivity to sulfur dioxide is seen with increasing ethanol.

Contamination by *Dekkera* gives rise to what is commonly called the 'Brett' character in wine. The unique sensory array of aromas is almost diagnostic of *Dekkera* contamination in the winery and can include aromas such as spice, smoky, medicinal, 'band-aid,' horsey, and animal descriptors. The responsible compounds are volatile phenols, with some contribution from acetic and fatty acids. The volatile phenols 4-ethylphenol and 4-ethylguaiacol are sensorially active above 700 mg l^{-1} and are metabolized by secondary metabolism. The starting compound hydroxycinnamic acid is decarboxylated to 4-vinylphenol and 4-vinylguaiacol intermediates before reduction to the aromatic compounds. Other microorganisms in the wine ecosystem are able to produce these compounds, but *Dekkera* is the only yeast able of producing them in high enough quantities to cause a sensory impact on the wine.

Zygosaccharomyces spp.

The *Zygosaccharomyces* genus consists of six species, of which *Z. bailii*, *Z. bisporus*, and *Z. rouxii* are the most relevant to the spoilage of foods and beverages. These yeasts are characterized by fermentative spoilage of products that are high in sugar and include fruit juices, fruit concentrates, syrups, sauces, alcoholic beverages, honey, jams, and confectionary. *Zygosaccharomyces* spp. are found naturally associated with the raw fruit product and can be introduced into processing and manufacturing plants via the fruit surface and be carried through to the final packaged product. Temperature predicts which species is present, as it is more likely *Z. bailii* is present at lower temperatures than *Z. rouxii*.

The fermentative nature of *Zygosaccharomyces* metabolism produces carbon dioxide that is of concern if the food product is in a sealed container, as the increase in gas can cause the container to leak or, in extreme cases, explode. Haze or sediment can occur in beverages as cellular biomass accumulates, and in some cases, surface biofilms can form. Taste modification can occur by the production of secondary metabolites with sensorial impacts on the food or beverage and include acetic acid, esters, and higher alcohols.

In addition to tolerance to osmotic stress caused by high concentrations of sugar solutes, *Z. bailii* is a fructophilic yeast, which is a preference for utilizing fructose during fermentation. Fructose is a fruit-derived sugar, which can be used for energy-yielding metabolism. *Zygosaccharomyces* spp. have a separate high-capacity transport system for fructose and can ferment additional sugars, sugar alcohols, and ethanol. For example, *Z. rouxii* is able to grow in high concentrations of sugar, at 72% glucose and after adaptation 90% glucose. This range of capabilities makes fruit and fruit-derived produces an ideal substrate for growth and fermentation of *Zygosaccharomyces* spp. Antimicrobials found in the processing of fruit products such as wine may not confer any resistance to *Zygosaccharomyces* spoilage as the genus. The genus is resistant to ethanol and thus is a possible spoilage agent of alcoholic beverages. *Z. bailii* can grow in ethanol up to 20%, although this is highly strain-dependent. Fruit substrates are also characterized by low pH, where *Zygosaccharomyces* spp. are adept at growth.

Preventing *Zygosaccharomyces* contamination is the major control point in food industries, so extensive cleaning, sanitation, and quarantine measures for primary processing equipment are paramount. Antimicrobials to be used as biocides have some relevance for this species; however, there is much strain variability in resistance. In particular, *Z. bailii* is particularly resistant to benzoates and sorbates used as preservatives, minimum concentrations to prevent growth often being much higher than the maximum limits allowable by food laws. *Z. rouxii* has a similar resistance profile to *Z. bailii*, but is quite susceptible to benzoic acid, while *Z. bisporus* has intermediate resistance.

Y. lipolytica

Y. lipolytica (previously *Candida lipolytica*) is a dimorphic fungus that is associated with the detrimental spoilage of meat and dairy products and is able to grow in hydrophobic substrate environments. This yeast is characterized by strong extracellular proteolytic and lipolytic properties, which are able to modify the surrounding environment, leading to enhanced growth. These extracellular enzymatic activities can drastically change the aroma, flavor, and texture of the food substrate and result in distinctive spoilage markers.

Y. lipolytica has been associated with the spoilage of cheese, dairy products, meat, and poultry. *Y. lipolytica* is adept at growth at low temperatures, where food products such as these are stored or matured. There are also reports of this yeast growing on and spoiling frozen meats. As this yeast has strong growth in oxidative environments, surface growth is common. Surface discoloration of cheese is common with the growth of *Y. lipolytica*, where catabolism of the amino acid tyrosine gives the pigmented compound melanin. Surface spoilage of uncooked and cooked meats (such as chicken or

turkey) has been observed, where the extracellular proteolytic and lipolytic activities result in spoilage aromas.

Growth of *Y. lipolytica* can be controlled by preservatives, as this yeast shows sensitivity to benzoates and sorbates at reasonable concentrations. Surface application of lactic acid, as well as novel inhibitors such as herb extracts, essential oils, and natamycin, is effective in controlling *Y. lipolytica* growth.

It is worth noting that *Y. lipolytica* is often found in mixed communities of microorganisms in spoiled foods, presumably as the lytic activities release nutrients that can be used by other yeasts or bacteria. In these cases, initial spoilage by *Y. lipolytica* can allow potentially pathogenic organisms to grow and cause disease and other health problems in humans.

On a positive note, the presence of *Y. lipolytica* is necessary and required for the characteristic flavor and aroma of some dried meats and cheeses. For example, the lipolytic and proteolytic activities of this yeast are essential for the maturation and distinctive flavors and aromas of salami, via breakdown of fatty tissues and proteins. Its ability to grow at low A_w and tolerate salt concentrations makes its growth possible. Texture and aroma development in cheese is made possible by growth of *Y. lipolytica*. Lipases, proteases, and esterases release characteristic compounds that contribute to flavor of blue cheese (Roquefort), soft fresh artisanal cheeses, and Pecorino, Cantalet, and Livarot cheeses.

See also: Spoilage: Bacterial Spoilage.

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Sports Nutrition

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Introduction

The science of sports nutrition is an evolving field offering some exciting new knowledge that can be translated to practical recommendations and may improve performance, but there are also examples of practices and dietary advice that are no longer considered effective or completely safe. Therefore, exercisers and athletes are advised to work with sports nutrition professionals. International Olympic Committee consensus statement on sports nutrition from 2010 states that "athletes will benefit from the guidance of qualified sports nutrition professionals who can advise on their individual energy, nutrient and fluid needs and help develop sport-specific nutritional strategies for training, competition and recovery." The role of sports nutrition professionals is to ensure support for best performance and set foundations for an athlete's long-term health.

Athletes' body weight and height, body composition, training, and daily energy expenditure are immensely diverse across sports disciplines, so individual approach when planning diet is essential, even for team sports because teammates may have quite different nutrition goals. Also, diet should be periodized and adjusted to training. During training, adherence to sports nutrition guidelines is needed on a daily basis. Periods when it is not necessary to achieve sport-specific nutrient goals are opportunity to emphasize nutrient-dense foods. A prudent and balanced diet should be planned for a period of few days, up to 1 week.

Sports nutrition recommendations are primarily for athletes, some recommendations are meaningful only for top-level athletes, but sports nutrition recommendations can be also useful for recreational exercisers. Generally, if weekly volume of exercise is 5 h or less, this can be considered as a desired level of physical activity in a healthy lifestyle, and such individuals can follow guidelines aimed for a general population. If we ought to simplify differences between sports and general nutrition, athletes have higher energy requirements, although some female athletes with low body weight can have very low requirements, and water requirements are higher due to water loss through sweating. Energy balance and water homeostasis are regulated by many long- and short-term mechanisms, but hunger and thirst are principal. Exercise, especially if long in duration and high in intensity, disrupts those regulators, meaning that planning is necessary, but when creating an athlete's diet and hydration plan, dictates of hunger and thirst should be acknowledged. Taste preferences are another important nutritional factor that should not be neglected in individualized sports nutrition, and an athlete's cooking skills can affect compliance and comprehension.

The science of nutrition is complementing reductionist approach with the concept of food synergy. Reductionist approach enabled to detect which nutrients are essential, but we do not consume nutrients but foods, and food synergy considers whole foods and dietary patterns as the most important. Food synergy accredits health effects of, for example, tomato or kiwi fruit to the whole food rather than exclusively to lycopene or vitamin C, respectively. Health effects of whole foods are not explained just by the sum of food components, but additional benefit is provided through synergy, that is, $1+1>2$. In sports nutrition, there are several examples of whole foods that can be considered 'natural sports foods': milk or chocolate milk can be a sports drink or a recovery drink, cherry juice can aid in recovery after exercise and possibly regulate sleep, and highly publicized beetroot juice reduces oxygen cost of exercise and can be regarded as an effective ergogenic aid with small but meaningful effect for endurance exercise. There are studies documenting usefulness of whole foods like bananas or raisins as carbohydrate sources during intense exercise or superior results of meat versus supplements in improving exercise-induced compromised iron status. Studies involving whole foods are valuable contribution to efforts of improving performance, diet, and health of both recreational exercisers and athletes.

Energy Availability

Total daily energy expenditure (TEE) has three components: basal, that is, resting energy expenditure; thermic effect of food (~10% of energy intake on a mixed diet); and physical activity energy expenditure (PAEE). The fourth component is present in a case of a disease or injury. The most variable component of TEE is PAEE and can be as low as 100 kcal for sedentary or higher than resting energy expenditure for very active individuals. PAEE can be divided into nonexercise expenditure and exercise expenditure. Nonexercise activities include occupation, leisure, and all other daily activities. When planning an athlete's diet, PAEE, that is, exercise expenditure, can be estimated using metabolic equivalents (MET). 1 MET = 1 kcal kg^{-1} h^{-1} what is assumed standard resting metabolic rate, and expenditure for a physical activity is expressed as a multiplier. For example, 12 min of vigorous freestyle swimming (MET value of 9.8, code 18230 in 2011 Compendium of Physical Activities) for a person that weighs 75 kg equals 147 kcal (9.8 kcal kg^{-1} h^{-1} × 75 kg × 0.2 h).

For athletes, the concept of energy availability (EA) (energy intake minus exercise expenditure) is preferred over concept of energy balance (energy intake minus TEE). If an athlete achieves energy balance, this does not necessary imply a state that supports optimal health, performance, and desired body weight. Available energy signifies energy that is available for all physiological functions other than exercise. Low EA (<30 kcal kg^{-1} FFM) is the underlying cause of female athlete triad. Adequate level of EA is at least 45 kcal kg^{-1} FFM. Higher levels should be chosen for weight gain and 30–45 kcal kg^{-1} FFM for weight or body fat loss. For the latter, it is important to apply relative reduction of 15–25% rather than absolute

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reduction of energy intake and if possible to conduct slow weight loss of 0.7–1% per week for the reason not to compromise body composition.

Application of EA concept requires information of body composition. Athletes can choose among dual-energy x-ray absorptiometry, air displacement plethysmography, skinfolds, or other available methods. Data should be interpreted with great caution, and repeated measurements should be performed in similar condition regarding previous meals, hydration, and exercise and with emptied bowel and bladder. Preferable methods are those that do not rely heavily on prediction equations that are valid for the general population since athletes can be extremely lean, heavy, or tall.

Practical and adequately accurate way to estimate daily energy intake is application of dietary assessment methods. Dietary assessment methods that are used among athletes are no different than those used among other population groups; however, several specificities exist. For estimation of energy intake, several 24 h recalls or 3 to preferably 7 day food records should be substantial. It is important to capture days that are representative with regard to training and to pay attention to items that are often (inadvertently) underreported, like occasional snacks or liquids. Unlike solid foods, liquids are not always perceived as a meal or as energy source, regardless of actual energy content. In an athlete's diet, such underreported liquids can be sports drinks. During keeping of food records, method of choice for estimation of portion size is to weigh food, but for athletes, because of possible very high number of eating occasions and training obligations and if most of meals are eaten away from home, this can be impractical. Acceptable alternative is description using kitchen utensil or food photography atlases.

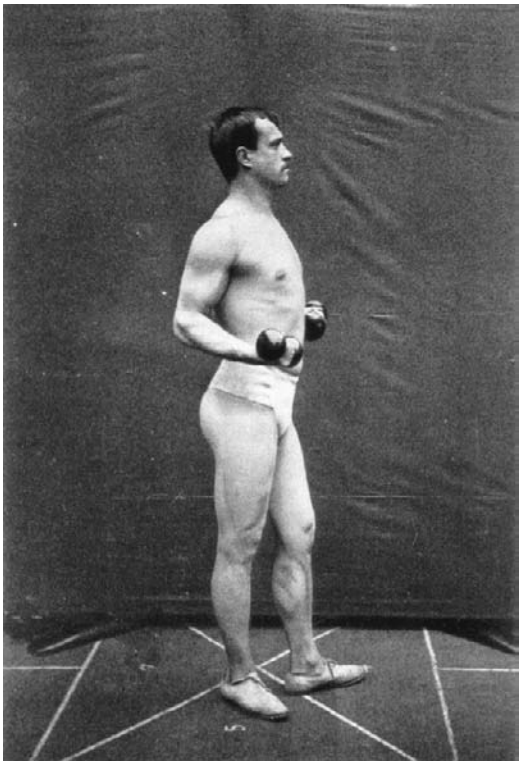

Figure 1 Photography of Charles J. Fritz, US soldier, aged 28 years, taken at the end of his participation in Chittenden's experiment from 1 November 1903 to 8 April 1904. His initial body weight was 76.0 and 72.6 kg at the end of experiment. During experiment, Chittenden varied nitrogen and energy intake and measured nitrogen balance. Reproduced from Carpenter, K. J. (2003). A short history of nutritional science: part 2 (1885–1912). *Journal of Nutrition* **133**, 975–984.

Protein Intake: Quantity, Timing, and Sources

Many exercisers and athletes are focussed only on adequacy of protein intake and thereby neglect food groups that are low in protein like vegetables and fruit. Proteins were once thought the only nutritive food component and have always received special attention. In 2005, Simpson and Raubenheimer proposed protein leverage hypothesis that ad libitum food, energy, and possibly fat and carbohydrate intakes are regulated according to protein intake or diet protein density, meaning that low protein intake leads to overeating and vice versa.

Justus von Liebig (1803–73), German organic chemist, great authority, and considered by some as the father of nutritional science (in Europe), promoted a hypothesis that degradation of muscles is the energy source for contractions. He and other contemporary authorities recommended daily protein intake of 100–135 g for a person of 70 kg and 145–150 g for hard work. Perhaps, these are the roots of protein myth that persists today among some exercisers and athletes having protein intake of 3–4 g kg^{-1}. Early high protein recommendations were challenged by a series of experiments done by Chittenden in 1904 who defined minimal protein requirements as 0.6–0.64 g kg^{-1} (Figure 1).

Recommended dietary allowance (RDA) for adults is 0.8 g kg^{-1} of good-quality protein, and estimated average requirement (EAR) is 0.66 g kg^{-1}. RDA level is sufficient for nearly all (97–98%) and EAR for 50% of healthy individuals. For an individual diet planning, RDA level should be used.

Current RDA and EAR values are based on nitrogen balance studies. This approach has been criticized, and indicator amino acid oxidation method has been proposed for defining protein requirements. The method is based on reasoning that deficiency of an essential (limiting) amino acid increases oxidation of all other amino acids because protein synthesis is impaired. Oxidation of an indicator amino acid is followed, while limiting amino acid intake is increased. When oxidation of indicator amino acid stops decreasing and reaches constant values, this is an indication that limiting amino acid, that is, protein intake level, meets requirements. Proposed RDA and EAR protein requirements based on indicator amino acid oxidation method are estimated to be 1.2 and 0.93 g kg^{-1}, respectively.

Managing an athlete's diet can start with setting protein requirements. It is important to observe protein intake within the context of a whole diet, and adequate protein intake should be accompanied by sufficient energy and carbohydrate intake to prevent protein, both somatic and dietary, being used as an energy source beyond normal levels. Athletes should have protein intake higher than RDA level of 0.8 g kg^{-1} and recommended range for strength and endurance athletes is 1.2–1.7 and 1.2–1.4 g kg^{-1}, respectively. There is an increase in protein

oxidation during endurance exercise and muscle hypertrophy, especially for novices, maintenance of larger muscle mass, and muscle repair during recovery, which explain increased protein needs. There is no evidence to justify protein intake above 2 g kg^{-1} for muscle hypertrophy at adequate EA, but higher protein intake (1.5–2.3 or even 1.8–2.7 g kg^{-1}) is recommended for the reduction of body weight and optimization of body composition. There are some health risks associated with enormous protein intake, but they are not so accented as before, and it is now recognized that both low protein intake and high protein intake have detrimental effect on bone health and protein intake that is optimal for bone health is 1–1.5 g kg^{-1}. Higher protein intake (<2.8 g kg^{-1}) will not impair renal function, but protein intake should be restricted for preexisting or confirmed renal disease.

Blood aminoacidemia before, during, and after exercise and protein intake before sleep improve recovery and muscle protein synthesis, but it is most important to ensure adequate amount of proteins as soon as possible, preferably within <1–5 h after resistance exercise. Adequate amount is usually defined as 20–25 g of high-quality protein, that is, 8–10 g of essential amino acids, depending on the body weight (~0.3 g kg^{-1}). There is some new research showing that the same level of muscle protein synthesis stimulated with recommended dose (20–25 g) can be achieved with formulations that provide suboptimal protein intake but are enriched with leucine, the key amino acid regulator of muscle protein synthesis. When matching the timing of protein intake with desired timing and duration of aminoacidemia, it is useful to account characteristics of fast (whey) and slow (casein) proteins. Whey protein ingestion causes sharper increase in blood amino acids, and return to resting levels occurs within 2–3 h, while casein ingestion results in moderate but prolonged aminoacidemia (~6 h).

There is a shift of protein recommendations for athletes toward meal-based. Daily protein intake should be spread evenly through daily eating occasions, and skewed protein intake, for example, 10% at breakfast and >50% for evening meal, should be avoided. If an athlete's goal is, for example, 80 g day^{-1}, this amount should preferably be ingested as 20 g trough each of 4 daily eating occasions, and it was shown that this pattern is more anabolic than 8×10 g or 2×40 g.

The concept of functional amino acids unifies essential, nonessential, and conditionally essential amino acids. All amino acids are required for tissue protein synthesis, tissue maintenance, gene expression, immunity, energy metabolism, growth, etc. Therefore, both essential and nonessential amino acids are functional as regulators of metabolism, and there is a suggestion that definition of an 'ideal protein' should be extended to include nonessential amino acids. Current definition of high-quality protein is that it provides all essential amino acids in recommended amounts, provides nitrogen for synthesis of nonessential amino acids, and is easily digestible. By this definition, all foods of animal origin (except for gelatin) provide high-quality proteins, while for plant proteins, usually lysine (grains) and methionine (legumes) are limiting amino acids. Lower digestibility and amino acid profile of plant proteins are not major issues in a varied diet with adequate energy and adequate amount of proteins of both plant and animal origins. A variety of protein sources ensure complementation of proteins deficient in one or more essential amino acids and result in completed amino acid pattern. Few plant proteins, including soy, quinoa, and amaranth, are considered high-quality proteins; however, milk proteins (especially whey) are considered superior in enhancing muscle protein synthesis than soy proteins.

When choosing protein source, whole foods should come first, but of course, if this is highly impractical; protein supplements are much better option than not consuming proteins at all. For example, 100 g (raw weight) of meat or fish provides ~20 g of high-quality proteins and additionally ~2 mg of highly bioavailable iron. One egg provides 6.3 g of proteins (3.6 g is in 1 egg white and 2.7 g in 1 yolk) and additionally highly bioavailable lutein and zeaxanthin that accumulate specifically in macular pigment of the retina with potential to improve visual performance of athletes through the reduction of glare discomfort, the reduction of photostress recovery time, enhancement of chromatic contrast, and increase of visual range. Some authors describe lutein and zeaxanthin as 'internal sunglasses.' It was shown that daily consumption of 1 egg through several weeks increases serum lutein and zeaxanthin without compromising serum lipid profile. There has been a paradigm shift regarding dietary cholesterol (and therefore egg consumption) and cardiovascular disease.

Vegetarian Athlete

The latest position of the Academy of Nutrition and Dietetics on vegetarian diets from 2009 states that "well-planned vegetarian diets are appropriate for individuals during all stages of the life cycle, including pregnancy, lactation, infancy, childhood, and adolescence, and for athletes." Adequacy of protein intake in vegetarian diets is not a major issue provided that energy intake is adequate and a variety of plant protein sources are included. Because of quality and digestibility of proteins in plant-based diets, vegetarian athletes should have 10% higher protein intake, that is, 1.3–1.8 g kg^{-1}.

Many characteristics of plant-based diets are beneficial for athletes, like higher intake of carbohydrates or antioxidants, but intake of carninutrients, the term proposed by McCarty in 2004, that are almost exclusively present in foods of animal origin is negligible with vegan diet. Taurine, carnitine, creatine, and carnosine (β-alanyl-L-histidine) are carninutrients with potential effect on performance, and according to current evidence, vegetarian athletes are most likely to benefit from supplementation with creatine and β-alanine.

Carbohydrates

Carbohydrates are ergogenic for all phases of exercise, and failing to achieve adequate intake can adversely affect not only performance but also recovery, mood, or immunity. Strategies that are proved to be effective should be applied appropriately; for example, glycogen supercompensation is not needed for exercise duration <90 min and carbohydrate intake during exercise at low to moderate intensity is counterproductive for recreational exercisers because exogenous carbohydrates provoke insulin secretion and depress fat

oxidation. Strategy that requires additional research is training with low carbohydrate availability to enhance mitochondrial biogenesis. Concomitant protein supplementation or caffeine intake (3 mg kg^{-1}) might counteract disadvantages of 'train low' strategy affecting muscle protein balance and volume and intensity of exercise.

Generally, the recommended range for carbohydrate intake is 6–10 g kg^{-1}, but 5–7 g kg^{-1} is adequate intake for bodybuilders, 3–5 g kg is adequate for lighter training, and 8–12 g kg^{-1} may be needed when duration of daily training is several hours. When using food composition tables in planning diet according to targeted carbohydrate intake, it is important to use data for available or digestible carbohydrates or to subtract dietary fiber from total carbohydrates. Dietary fiber can have energy density of ~0–2 kcal g^{-1} depending on their colonic microbiota fermentability and formation of short-chain fatty acid. When choosing a carbohydrate source, glycemic index (GI) can be orientation, and low GI (<55) is preferable before, moderate to high GI (56–69) during, and high GI (>70) after exercise. The concept of GI has been upgraded with glycemic load (GL) that combines GI and amount of carbohydrates in one serving, and low and high GL is <10 and >20, respectively. The form of carbohydrate source, liquid, gel, or solid food, is not critical for performance or recovery so can be chosen according to individual preferences.

Pre-exercise meal is consumed 1–4 h before exercise and should provide 1–4 g kg^{-1} of carbohydrates. Individuals prone to reactive hypoglycemia should ingest carbohydrates few min or >90 min before exercise.

Exercise should start with adequate muscle and liver glycogen stores. During exercise (>1 h), carbohydrate intake of 30–80 g h^{-1} is ergogenic. For activities that are too short for ingested carbohydrates to pass through the stomach and reach active muscles, novel strategy of rinsing the mouth with carbohydrate solution for a few seconds (swallowing is not necessary) can be useful. Probable explanation for ergogenic effect of this strategy is brain response and perception of fatigue after sensing carbohydrates, both sweet and nonsweet, by receptors in the oral cavity. Perhaps, a factor of anticipated energy flux is included, and perhaps, there are mechanisms connecting central fatigue, energy and glucose homeostasis, and recently discovered sweet taste receptors in extraoral tissues including the gut, brain, and adipose tissues. Mouth rinsing with carbohydrate solution can be useful for individuals experiencing gastrointestinal discomfort due to carbohydrate intake; effect is expected to be greater in a state of depleted carbohydrate reserves, but mouth rinse can also have an additive effect in a state of optimal carbohydrate availability; periodic mouth rinse can be useful during high-intensity exercise when fluid ingestion is not well tolerated or during, for example, soccer match where intermittent high-intensity activity (sprints) slows gastric emptying. There are interesting recent studies showing benefits for sprint performance of mouth rinsing with solutions containing caffeine and quinine.

For exercise duration >2–3 h, recommended carbohydrate intake is 60–90 g h^{-1}. Exogenous carbohydrates oxidation rate can be increased above 1 g min^{-1} if multiple transportable carbohydrates (glucose and fructose) are used. Higher than expected intestinal absorption rate occurs because glucose and fructose utilize different sugar transport proteins, sodium-dependent glucose transporter (SGLT1) and fructose transporter (GLUT5), respectively. A. Jeukendrup introduced the term 'nutritional training' for efforts of adaptation to carbohydrates intake >1 g min^{-1} as a part of training program since very high carbohydrate intake can cause gastrointestinal disturbances. Gastrointestinal disturbances among athletes are primarily consequence of gastrointestinal ischemia, but there are also nutritional factors that include high fructose intake.

Sports drinks that contain 6–8% of carbohydrates are usual carbohydrate source for exercise that lasts <1 h, but for longer exercise, additional carbohydrate sources can be included, and carbohydrate concentration and volume of a sports drink can be manipulated to achieve energy and hydration goals.

Carbohydrates are a limiting factor for prolonged low- to moderate-intensity exercise and a major fuel for high-intensity exercise (>80% VO_2max); therefore, all athletes should restore muscle glycogen during recovery. Optimal amount is 1.2 g kg^{-1} h^{-1} preferably ingested periodically every 15–30 min. The most important factor for adequate glycogen replenishment is to provide 10 g kg^{-1} of carbohydrates during 24 h recovery period. During recovery, endurance athletes are focussed on carbohydrates and strength athletes on proteins, but recovery should address both nutrients. Combination of 0.8 g kg^{-1} h^{-1} of carbohydrates and 0.2–0.4 g kg^{-1} h^{-1} of proteins results in adequate glycogen synthesis, and both carbohydrates and proteins have insulinotropic effect and promote insulin secretion. Elevated insulin has a positive effect on net muscle protein balance (insulin decreases protein breakdown and has no significant effect on protein synthesis).

Fat Intake

General recommendation for fat intake is 20–35% of total daily energy intake. Fat intake under 20% kcal compromises diet palatability, energy intake, essential fatty acids intake, and absorption of fat-soluble vitamins and phytochemicals. Higher than recommended fat intake (>60% kcal) can result in useful 'supercompensation' of intramuscular fat (analogous to glycogen supercompensation), but this is not recommended. To improve often inadequate ω-3 fatty acids intake, especially intake of long-chain eicosapentaenoic and docosahexaenoic acid and ω-3 and ω-6 intake ratio, 2–4 140 g portions of fish per week including at least one portion of fatty fish and also to use olive oil (>70% oleic acid) for cooking instead of other vegetable oils where ω-6 predominate (>50–60%), like sunflower, are recommended. Fish oil supplementation is being studied for its potential to counteract anabolic resistance to diet and exercise among the elderly (~2 g per day through several weeks), but anabolic effect of supplementation with long-chain ω-3 fatty acids was also shown among 25–45-year-old healthy subjects.

Vitamins, Minerals, and Antioxidants

Diet that provides adequate energy and macronutrient intake through a variety of whole foods is the most likely to be also adequate regarding intake of micronutrients and beneficial nonnutritive food components, but it is a challenge to plan

nutrient-dense diet for low energy intake. Micronutrient supplements will have performance-enhancing effect only if deficiency is present. Athletes, especially endurance athletes and females, should periodically check iron status since reversing anemia can take several months and inadequate stores can adversely affect performance before onset of anemia, but routine supplementation without proof of its necessity is strongly discouraged. It might be that magnesium supplements are ergogenic even if deficiency is not present. Indiscriminate use of various vitamin and mineral supplements is no longer considered completely risk-free.

High doses of antioxidant supplements can block beneficial metabolic adaptations to physical activity like increase in insulin sensitivity and mitochondrial biogenesis. Preferable way of ensuring adequate exogenous antioxidants is whole foods that provide additional benefits through synergy of food components. It was shown with a mice model that bioavailability of vitamin C is superior from kiwi than from solution of sodium ascorbate and possible explanation is 'vitamin C_2,' a phytochemical present in kiwi that enhances vitamin C absorption and tissue uptake or perhaps spares vitamin C from oxidation.

Some authors consider endogenous antioxidants more important than exogenous, but there is no doubt that abundance of foods high in antioxidants is important for our health, and it is important to note that food components with antioxidant properties have other roles and, for example, act as enzyme cofactors. Vitamin C is antioxidant and enzyme cofactor for collagen and carnitine biosynthesis, and this is probably why muscles are major body pool of vitamin C. It was shown that ~70 mg of vitamin C saturates muscle tissue and this can easily be achieved by supplementing diet with 0.5–2 kiwi fruits.

Vitamin that is getting significant attention is vitamin D. The roles of vitamin D go far beyond skeletal, and it is now known that muscles contain significant amount of vitamin D vitamers. Vitamin D is needed for skeletal muscle development and improves muscle strength. There are studies showing high prevalence of vitamin D inadequacy among athletes and benefits of supplementation. Deficiency is more likely among athletes who train indoors. Athletes should include reasonable sun exposure in their lifestyle and periodically monitor vitamin D status (serum $25(OH)D_3$ of 75–150 nmol l^{-1} indicates sufficiency) because of potential effects on performance and to reduce risk of chronic diseases. More research is needed on this topic. Also, it will be interesting to see if there is interplay between vitamin D and recently discovered myokine irisin involved in browning of white adipose tissue. Exercise can depress vitamin D levels and induce irisin production, but both affect muscle function and glucose homeostasis.

Hydration

Water balance is affected by both water intake and losses. The most common recommendation for adults is to consume 1 ml of water per 1 kcal of TEE, and it is likely that future recommendations will additionally include reference to urine volume. Water homeostasis among athletes can be heavily disturbed through sweat losses. Sweat evaporation is the most important way of thermoregulation, with the exception of aquatic sports where heat is dissipated through conduction and convection. Loss of hypotonic sweat results in hypertonic hypovolemia, that is, dehydration that affects many physiological functions. Sweat rate for different sports can be <0.5 to $>2\ l\ h^{-1}$.

Many athletes start with exercise in a state of hypohydration, what can easily be avoided unless there is insufficient time between exercise sessions. Water is an adequate choice and can be included in a meal before exercise (~500 ml) or ingested early enough to avoid urge to urinate during exercise. Important advice for recreational athletes is to start the exercise in a state of euhydration since commencing exercise in a state of hypohydration adversely affects the perception of efforts and therefore can reduce the probability of continuation of exercising. Urine color chart validated by Armstrong in 1994 is a practical way to estimate hydration status.

Dehydration defined as loss of $\sim>2\%$ of body weight impairs performance and cognition, and this level of dehydration coincides with occurrence of thirst. It is possible that this level of body weight loss occurs during exercise without hypohydration because of factors affecting both sides of water balance like oxidation of macronutrients or release of water associated with glycogen.

Water intake during exercise should be adjusted to exercise duration, environmental conditions, and carbohydrate intake goals. Ending exercise with increased body weight is a risk factor for hyponatremia. Water intake during exercise should be planned according to previously estimated sweat rate, but dictates of thirst should be highly respected. Sweat losses of vitamins, calcium, copper, iron, magnesium, and zinc do not alter daily requirements, but sweat sodium losses are substantial with great interindividual variability, and sports drinks with 0.5–0.7 $g\ l^{-1}$ of sodium are needed for exercise >2 h. Sodium-containing beverages for shorter events can increase palatability and therefore ingested volume and contribute to water retention. Additional factors that contribute to hedonic ratings of a fluid are flavoring and temperature. Preferred temperature is 10–20 °C, and colder drinks may be appealing but result in earlier satiation of thirst and reduce ingested volume.

Common recommendation for water intake during recovery is to ingest up to 150% of weight loss.

There are many benefits associated with euhydration, including prevention of chronic diseases, and expanded muscle cell volume favors anabolism, but the rule 'more is better' should not be followed.

Ergogenic Aids

To quote R. Maughan: "Most supplements that are effective are against the rules of sport. Most substances that are not banned are not effective. There are, however, some exceptions." These few exceptions include caffeine, blood- and muscle-buffering agents, and creatine. Many athletes strongly believe that use of one or several ergogenic aids is necessity for top-level performance; therefore, because of considerable positive and negative (nocebo) effects of placebo, an athlete should continue with supplementation if it is completely risk-free. Decision about supplementation should be based on best available evidence regarding effective protocol and dose, long-term

safety, and information about quality of the product (actual amount of active component and contamination with toxins and prohibited substances).

See also: Amaranth; Anemia: Prevention and Dietary Strategies; Fish: Fish in the Human Diet; Mediterranean Diet; Obesity Management; Protein: Requirements; Vegetarian Diets.

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Stabilizers: Types and Function

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'Stabilizers' is a generic term for additives that are used for avoiding or retarding physical changes in foods. They typically prevent spatial food parts of different composition from changing in size. Examples of such uses could be the diminishing of whey exudation from a drinkable fermented milk or the maintenance of uniform distribution of suspended vegetable pieces in a salad dressing. The phenomena that are mitigated in these examples involve what chemists may refer to as phase separation and what generally can be named, for example, as flocculation, coalescence, lumping, creaming, demixing, and sedimentation.

There is, on the other hand, no unambiguous rule for when to consider a food material a stabilizer. Additives may also be termed stabilizers when they provide and maintain specific rheological properties; in this role, the same materials could have been called 'thickeners' or 'gelling agents.' When a stabilized food is an emulsion, the stabilizer might be considered an emulsifier, but it is not common to use both designations for the same material. The classification into the one or the other category in practice follows the molecular size, so polymeric materials are considered stabilizers. Arguably, stabilizers are normally thought of in context with prevention of phase separation, but the stability that is provided may also be with respect to other phenomena, for example, the preservation of the brightness of a color.

Chemically, almost all of those stabilizers that can mitigate phase separation are polymers of biological origin, and they can under appropriate circumstances either dissolve or form a gel or a gelatinous suspension with water. They can be classified as gums. The list of stabilizers provided by the Food and Agriculture Organization (FAO) of the United Nations does, on the other hand, include some nonpolymeric materials and some water-insoluble materials.

In a stabilized food system, a polymeric stabilizer normally modifies the contact between an aqueous liquid and some other material. In the absence of stabilizer, the surface of contact between these immiscible parts would 'strive at' shrinking. In the (typical) case that the one material is drops or pieces dispersed in a continuum of the other material, these dispersed bodies would be attracted to each other, and they would 'attempt to' merge, minimizing their combined surface. The various hypothetical working mechanisms for how individual stabilizers can reduce this stickiness involve either adsorption of the stabilizer at the surfaces, or thickening of the food, or a combination of both.

If the stabilizer adsorbs, it can by virtue of its water solubility connect between the aqueous phase and the material to which it adsorbs, and being polymeric, it can do so over a relatively large distance. While still being attached to the surface, it can embrace water and retain it at the surface that thereby becomes less sticky. This mechanism is commonly referred to as steric stabilization (Figure 1).

If the stabilizer thickens the food and we assume the typical case that the aqueous phase is continuous, then the thickening reduces the mobility of pieces of dispersed material and, in turn, their possibility of contacting each other or distributing unevenly – as they would do in the absence of stabilizer due to gravity and buoyancy effects. The reduction of mobility will obviously be particularly effective if the stabilizer turns the system into a gel. On the other hand, for products with which a fluid and drinkable texture is preferred, it may suffice that the stabilizer provides a pseudoplastic viscosity, that is, a viscosity that is high at the low-shear condition of product storage, but low at the higher-shear condition of drinking.

Sources and Production

Some widely used polymeric stabilizers and ingredients that can be used as stabilizers have been collected in Table 1.

Agar, alginate, and carrageenan are isolated from marine plants. Gelatin is isolated from animal parts, typically skin and bones. Xanthan and gellan are produced by bacteria and commercially manufactured by fermentation. Cellulose derivatives (cellulose gum, CMC), guar gum, gum arabic, locust bean gum, pectin, and starch (modified starch) are isolated from terrestrial plants.

The final stabilizers that are sold to food manufacturing companies are ideally and typically powders that have been purified from unwanted parts of the raw material and that (in spite of possible variations in raw material properties) are batch-to-batch uniform. The commercial manufacturing processes typically involve an aqueous extraction that leaches the stabilizer out from the originally solid raw material, followed by operations that separate the ensuing solution from the parts that did not dissolve, in turn followed by operations that separate the stabilizer from the solution, in turn followed by milling of the dried stabilizer so that it becomes a powder. This roomy description fits the aforementioned stabilizers except cellulose derivatives, starch, and traditional qualities of seed gums (guar gum and locust bean gum) and gum arabic.

Extraction is usually done at elevated temperatures, and it may involve the addition of either acid or alkali in order to break linkages that attach the stabilizer to the insoluble raw material parts or linkages that make the precursor of the stabilizer insoluble. The operations that separate the ensuing stabilizer solution from insoluble materials may typically be filtration and centrifugation.

The operations that isolate the stabilizer from the solution include evaporation of water, gel pressing, precipitation, and drying. In some cases, for example, with agar, cooling the hot extract induces gelling. The gel can then be handled so that the held water becomes released while the gel shrinks – a phenomenon known as syneresis. Addition of di- or trivalent salts may

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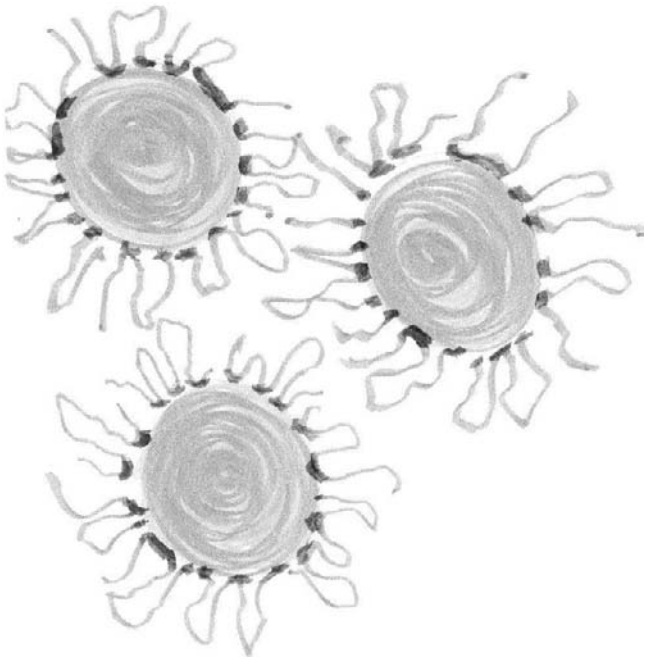

Figure 1 *Steric stabilization*, the pieces of dispersed material (drawn as spheres) are coated by stabilizer molecules (drawn as loops and tails) that on the one hand adsorb to the surfaces of the dispersed pieces (adsorbing stabilizer parts drawn as dark segments) but on the other hand protrude from the surfaces and reach into the surrounding continuous phase.

facilitate the syneresis or precipitate the stabilizer as an insoluble salt. Pressing of gels or subjecting gels to freeze–thaw cycles (traditional Japanese process originally used for agar) may also facilitate syneresis. A different technique is to pour the stabilizer solution into an alcohol whereby the stabilizer precipitates. This is commonly used for manufacturing pectin, xanthan, gellan, clarified versions of LBG and guar, and some types of carrageenan. The disadvantage is that the alcohol needs to be recovered by costly distillation, but the advantage over precipitation with salts is that the alcohol can be removed from the stabilizer by drying, and the stabilizer thereby can regain its water solubility.

The manufacturing of some stabilizers involves modification of functional groups. CMC is produced by reacting cellulose with sodium hydroxide followed by reacting with monochloroacetic acid. The new functional groups contain ionizable carboxyl acid groups that make the product soluble in cold water. Modified starch is a generic term that includes starch that has only been physically modified, but it also includes types modified with adipic anhydride or phosphorous oxychloride for creating starch adipates or starch phosphates, respectively. A certain class of pectin, known as amidated pectin, is made by exposing pectin to ammonia whereupon some of its methyl ester groups are converted into amide groups.

In addition to the stabilizers mentioned in Table 1, a list provided by the FAO of the United Nations also includes the polymeric materials curdlan (CASRN 54724-00-4), dammar gum (9000-16-2), gum ghatti (9000-28-6), karaya gum (9000-36-6), konjac flour (37220-17-0), polydextroses (68424-04-4), propylene glycol alginate (9005-37-2), tara gum (39300-88-4), tragacanth gum (9000-65-1), and a number of cellulose derivatives other than CMC. Further, some nonpolymeric materials are included, for example, sodium, potassium, and calcium salts of acetic, citric, phosphoric, and tartaric acids. In addition to the materials currently listed by the FAO, there are polymeric stabilizers with oriental tradition, for example, soluble soybean polysaccharide and pullulan (9057-02-7). Inulin (9005-80-5) is also often mentioned alongside with stabilizers.

Patterns of Consumption

Commercial stabilizers and their food uses are regulated by national legislation, which in many cases refers to internationally acknowledged standards like the CODEX Alimentarius based on the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Often, the national legislation sets forth the conditions under which permitted food additives may be used in all categories of foods.

JECFA has evaluated that most of the gums that comply with the given specifications/monographs have an acceptable daily intake 'not specified.' This goes for all of the stabilizers listed in Table 1. This means that most stabilizers can be used in many foods in levels that the EU legislation describes as 'quantum satis' (QS) and CODEX JECFA describes as good manufacturing practice (GMP). That is, there is no numerical maximum level, but the use must comply with good manufacturing practice including that the dosage should not exceed what is necessary for achieving the intended effect, and the use should not conceal food of inferior quality or

Table 1 Commonly used stabilizers

Stabilizer	*Raw material*	*Chemical properties*	*Manufacturing*
Agar CASRN 9002-18-0 E 406	*Gelidium* and *Gracilaria* sp. (seaweeds)	Mixture of uncharged agarose and agaropectin, a sulfate-containing, thus anionic, polymer	Pretreatment with either acid or alkali, then hot extraction, filtration, and gel pressing
Alginates CASRN 9005-32-7 (alginic acid) E 400 through E 405	*Laminaria* sp., *Ascophyllum* sp., and other brown seaweed types	Linear polymer of β-D-mannuronic acid and α-L-guluronic acid that can follow in diverse sequences along the molecules; anionic	Alkali treatment of the peel followed by aqueous extraction, then filtrations, and precipitation with calcium salts or acid. Conversion of the precipitate to sodium alginate
Carrageenan CASRN 9000-07-1 E 407 PES E 407a	Rhodophyceae (red seaweeds), wild types and types cultivated in marine fields	Galactose polymer with sulfate ester groups and 3,6 anhydrogalactose varying in amount and location; anionic	Hot extraction at high pH, filtration, gel pressing, or alcohol precipitation. Special types (include PES) that essentially are alkali-treated seaweeds that have not been extracted also exist
CMC, Cellulose gum CASRN 9004-32-4 E 466	Wood or cotton	β-linked glucopyranose (polymer that can be hydrolyzed to glucose) with which some hydroxyl groups are replaced with carboxymethyl groups. Anionic.	Reaction with NaOH followed by $CH_2ClCOOH$
Gelatine CASRN 9000-70-8	Animal skin and bones, typically from pig or cow	Mixture of protein and peptide; amphoteric; hot water-soluble gels upon cooling	Extraction at either low pH (type A gelatin) or high pH (type B gelatin); filtration; drying
Gellan CASRN 71010-52-1 E 418	*Sphingomonas elodea* (microorganism)	Linear polymer with carboxyl, acetyl, and glycerate groups; anionic; soluble in hot water	Fermentation, filtration of broth, and alcohol precipitation. Low-acyl versions by alkali treatment
Guar gum CASRN 9000-30-0 E 412	Endosperm of seeds of *Cyamopsis tetragonolobus* (annual crop)	Galactomannan (polymer of galactose and mannose); mostly uncharged; highly effective thickener	Mechanical processing of seeds for isolating the endosperm; milling; clarified versions have further been water-dissolved, filtered, and precipitated
Gum Arabic CASRN 9000-01-5 E 414	Exudate from the trunks of *Acacia senegal* or *Acacia seyal* trees	Complex branched polymer of neutral saccharides and acidic saccharides; contains glycoproteins; very soluble in water (>30%)	Traditional GA is only mechanically purified (from dust, bark pieces, etc.). Versions that have been purified by dissolution in water, filtration, and drying are commercially available
LBG (locust bean gum and carob gum) CASRN 9000-40-2 E 410	Kernels of St. John's bread – seeds of the carob tree, *Ceratonia siliqua*	Galactomannan (polymer that can be hydrolyzed to galactose and mannose); mostly uncharged; thickener	The hull of the fruit is removed by a combination of charring and mechanical ways. The seed endosperm is then milled. Clarified versions have further been water-dissolved, filtered and precipitated
Pectin CASRN 9000-69-5 E 440	Edible plants. In particular citrus peel, apple solids, sugar beet	Polydisperse, complex structure. Main component mostly linear with carboxyl groups some of which are methyl-esterified; anionic. Special versions contain amide groups	Extraction at low pH, filtration, and alcohol precipitation. Methyl ester content can be varied though acidity of extraction or with enzymes. Amide groups can be introduced by exposure to ammonia (E440 ii)
Starch and modified starch E 1410 through E 1450	Edible plants, for example, corn, tapioca, wheat, and potato	α-Linked glucopyranose (polymer that can be hydrolyzed to glucose). Native types are uncharged	
Xanthan CASRN-11138-66-2 E 415	*Xanthomonas campestris* (microorganism)	Long backbone of cellulose (β-linked glucopyranose), with every second hexose unit having a trisaccharide side chain that contains a carboxyl acid group; anionic	Fermentation, filtration of broth, and alcohol precipitation

adulterated food, and it should not create a nutritional imbalance. The original background for these positive assessments is in some cases that the stabilizers are isolated from materials that have a long record of being eaten as food (e.g., agar, pectin, and seed gums), while in other cases the materials have a long tradition as stabilizers (e.g., gum arabic).

Stabilizers are consumed with the stabilized foods. Stabilized foods are, for example,

- cheese spreads (0.3% carrageenan),
- cocoa milk and milk shake (0.03% carrageenan),
- confectionery jellies (1–2% pectin or carrageenan),
- cultured (acidified) drinkable milk products (0.3% pectin or 0.6% CMC),
- flan and pudding (0.1–0.6% carrageenan),
- hard/chewy candies, for example, liquorice pastilles (30% gum arabic or ≈10% modified starch),
- ice cream (0.1–0.5% alginates, 0.01–0.03% carrageenan, 0.2–0.3% guar, 0.2–0.4% CMC, or 0.2–0.3% LBG – often in combination),
- jam, fruit toppings and ripples, and fruit-containing or fruit-flavored gels (0.3–1% pectin or 0.1–0.3% LBG or combinations),
- marinated or brine-injected meat (≈0.5% carrageenan),
- marshmallow (5–8% gelatin),
- olive fillings (≈1% alginate),
- salad dressing (0.2–0.5% xanthan),
- soft drinks and sodas (gum arabic, LBG),
- tortillas and similar baked products (0.1–0.5% CMC),
- water dessert gels (0.4–0.6% agar or ≈0.4–1.2% carrageenan),
- wine gum (6–10% gelatin or modified starch),
- yogurt (≈1–2% modified starch or 0.3–0.5% gelatin or 0.2% pectin).

Absorption and Mechanism

Owing to their polymeric nature and their resilience to degradation in the upper parts of the human digestive tract, stabilizer additives (those stabilizers that are not legally considered ingredients) pass almost intact through until the large intestine in which they are digested into short-chained fatty acids. This qualifies them for being considered dietary fibers and – since they feed the gut microorganisms – prebiotics. On the other hand, the amounts that would be acquired from normal consumption of stabilized foods are modest in comparison with the dosages that have proved a significant effect in feeding studies.

In particular, pectin has been thoroughly studied because it is a soluble dietary fiber that occurs abundantly in vegetables and fruit and thus in the normal human diet. Administration of pectin in adequate amounts, that is, at least 6 g day^{-1}, has been demonstrated to have the beneficial effect of contributing to the maintenance of normal blood cholesterol level, and the European Food Safety Agency has approved this as a health claim under its Article 13.1 program. The mechanism seems to be rather general and shared with other soluble dietary fibers, like beta-glucan, konjac (glucomannan), hydroxypropyl methylcellulose, and guar gum, all of which have acceptance for similar claims. The dietary fibers influence the uptake of materials from the mid or lower parts of the digestive tract, and they increase the excretion of some materials (among these are bile acids) with the stools. The human body produces bile acids from cholesterol in the liver and secretes them in the duodenum (the first section of the small intestine). After having served their role as a part of the digestive system, the major part of the bile acids is reabsorbed in the ileum (the last section of the small intestine) and returned to the liver, while a minor part leaves with the stools. Pectin and other soluble dietary fibers (and thus most stabilizers) reduce the reabsorption and thus increase the loss of bile acids. This makes the liver synthesize more bile acids for replenishing the stock; and cholesterol is used and removed from the bloodstream in this process.

Health Effects

The stabilizers that are legally considered additives (rather than ingredients) are used in dosages that normally (with exceptions) constitute much less than 1% of the stabilized foods. So, their contribution to the overall diet, its energy content and its nutritional properties, is unimportant. Their safety has been evaluated by expert committees of the major international legislations and should under all circumstances be unproblematic when following the legally stipulated guidelines for use. Some stabilizers have been isolated from edible plants, and their health effects are typical of dietary fibers although the normal human ingestion of them through stabilized foods is so low that their principally beneficial effect must be considered modest.

See also: Emulsifiers: Types and Uses; Food Colloids and Emulsions; Gums: Properties and Uses; Mayonnaise.

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Staphylococcus: Detection

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Organism and Sources

The *Staphylococcus* genus includes Gram-positive, nonmotile, and catalase-positive bacteria. Staphylococci are ubiquitous and widely distributed in nature, with some species inhabiting specific ecological niches. Staphylococci live as commensals on the skin and mucous membranes of warm-blooded animals and humans but can also be isolated from environmental sources and from a wide range of foodstuffs such as meat, cheese, and milk and from the surfaces of cookware, utensils, furniture, and clothing, among other items. Foods frequently implicated in staphylococcal food poisoning (SFP) include meat and meat products; poultry and egg products; salads, such as egg, tuna, chicken, potato, and macaroni; bakery products, such as cream-filled pastries, cream pies, and chocolate éclairs; sandwich fillings; and milk and dairy products. Foods that require considerable handling during preparation and that are kept at slightly elevated temperatures ($>7.2\ ^{\circ}C$) after preparation are frequently involved in SFP.

The genus *Staphylococcus* includes more than 70 species and subspecies, which can be divided into two groups according to their capacity to coagulate rabbit plasma: coagulase-positive staphylococci (CPS) and coagulase-negative staphylococci (CNS). The main CPS is *Staphylococcus aureus*. Other CPS include the *Staphylococcus intermedius* group (*Staphylococcus intermedius*, *Staphylococcus pseudintermedius*, and *Staphylococcus delphini*), *Staphylococcus schleiferi* subsp. *coagulans*, *Staphylococcus lutrae*, and some strains of *Staphylococcus hyicus*. In particular, members of SIG are opportunistic pathogens in many animal hosts and occasionally in humans.

Some CNS (particularly *Staphylococcus xylosus*, *Staphylococcus carnosus*, and *Staphylococcus equorum*) have a positive impact on fermentation processes and sensory characteristics of food products; they are frequently used as a component of meat and cheese starter cultures. In other circumstances, some CNS species can present health risks to humans and/or animals. For example, *Staphylococcus saprophyticus*, which may contribute to sausage aroma formation and help to prevent off-flavor during sausage ripening, is a ubiquitous species and is also involved in acute urinary tract infections in young adult women.

S. aureus is the most pathogenic species within the genus and is endowed with a variety of virulence factors, some of which (such as exfoliative toxins A and B; enterotoxins; hemolysins α, β, δ, and γ; leukocidins; and antibiotic resistance determinants) were also detected in genomes of CNS. Both CPS and CNS virulent species or strains can produce a variety of infections in humans and in companion and farm animals. Another relevant phenotypic feature of some *S. aureus* strains is the capacity to form biofilms, a trait that enhances tolerance to antimicrobials and persistence.

S. aureus is the etiologic agent predominantly associated with SFP. In Europe, 346 outbreaks (representing 6.4% of total outbreaks) were caused by staphylococcal toxins in 2012 according to the EFSA EU Summary Report 2012 on zoonoses, zoonotic agents, and foodborne outbreaks. SFP results from ingestion of foods containing preformed staphylococcal toxins that behave as superantigens (SAgs). The International Nomenclature Committee for Staphylococcal Superantigens distinguishes three types of SAg toxins in staphylococci: (i) staphylococcal enterotoxins (SEs) with proved emetic activity in primates, (ii) SAgs whose emetic properties remain unconfirmed (SE-like toxins; SEls), and (iii) toxic shock syndrome toxin-1 (TSST-1). At least 23 different SAgs have been described: the SEs A–E, G–J, and R–T (SEA–SEE, SEG–SEJ, and SER–SET), the SEls K–Q and U–X (SElK–SElQ and SElU–SElX), and TSST-1. Most SAgs have a dose-dependent capacity to cross the intestinal wall and produce a local and systemic action on the immune system. SEs do not seem to be an exclusive trait of *S. aureus*, as they have also been described in CNS from foods and in the genomes of CNS from different sources. SEs are globular single-chain proteins with molecular weights ranging from 22 to 29 kDa, resistant to heat treatments and other environmental conditions (such as freezing, drying, and low pH) that inactivate enterotoxin-producing strains. Unless heat processes are applied, staphylococci are expected to exist in any and all foods that are handled directly by humans or are of animal origin.

One additional concern on staphylococci is their capacity to acquire antimicrobial resistance and particularly the spread of methicillin-resistant strains in health care institutions, the community, and livestock herds. In 2008, the European Centre for Disease Prevention and Control estimated that a total number of 171 200 nosocomial methicillin-resistant *S. aureus* (MRSA) infections are acquired annually in the member states of the European Union (EU), and in Iceland and Norway, resulting in 5400 attributable excess deaths, more than 1 million excess days of hospitalization, and EUR 380 million excess in hospital costs. In 2008, the proportion of MRSA in *S. aureus* blood culture isolates was $<5\%$ in northern Europe, including the Netherlands, and $<10\%$ in Austria, Luxembourg, and Slovenia; between 10% and 24% for isolates in Belgium, the Czech Republic, France, Germany, Hungary, Latvia, Poland, and Switzerland; and $\geq 25\%$ in Bulgaria, Croatia, Cyprus, Greece, Israel, Italy, Malta, Portugal, Ireland, Romania, Spain, Turkey, and the United Kingdom. Bacteremias caused by MRSA are now decreasing (4.8% per year), whereas the number attributable to methicillin-sensitive *S. aureus* increased continuously (3.4% per year). Methicillin resistance results from the acquisition of the *mecA* gene, which codes for an alternative penicillin binding protein (PBP29 or PBP2a) and is associated with the staphylococcal cassette chromosome (SCC) *mec* (SCC*mec*) elements, found in both CPS and CNS. The recently described gene homologue *mecC* is also not restricted to *S. aureus* but found in several staphylococcal species including *Staphylococcus sciuri*, *Staphylococcus stepanovicii*, and *S. xylosus*.

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Methicillin-resistant *S. aureus* strains have been detected in companion and food animals and in raw meat (pork, poultry, and beef) and milk and may also be present in a variety of other foods. The primary route of zoonotic transmission of MRSA is considered to be the direct or indirect occupational contact of livestock professionals with colonized animals, while the role of food as a source of human colonization or infection is presently considered to be low. If meat and other foods contaminated with MRSA are cooked properly, MRSA cells will be inactivated. However, as with enterotoxigenic methicillin-sensitive *S. aureus* strains, under conditions of temperature abuse, MRSA cells could grow in foods, produce heat-stable enterotoxins, and cause foodborne intoxication. In some individuals, whose normal flora has been depleted by antibiotic treatment, MRSA cells that could potentially be present on contaminated ready-to-eat foods, including processed meats, cheeses, and fresh produce, could cause staphylococcal enterocolitis.

This article focuses on the identification of staphylococci, particularly *S. aureus* (including MRSA), and the detection of SEs in foods.

Detection of Staphylococci in Foods

Given the risk of SFP and the heat stability of SEs, it is required to enumerate CPS at the steps of the production process where the number of staphylococci is expected to be the highest (process hygiene criteria) and to screen samples for the presence of SEs above well-specified M values (food safety criteria) to assess the compliance of the product with the food regulatory requirements (such as EC regulations no. 2073/2005 and 1441/2007).

Although CNS are not routinely screened, they should also be taken into consideration according to the conclusions of several studies:

- The importance of the pathogenic potential of CNS as a source of SEs has been highlighted in human and animal infections as well as in food poisoning.
- Multiple CNS species can serve as reservoirs for *mecA*. In addition to the clonal transmission of methicillin-resistant CNS in meat, horizontal occurrence of SCC*mec* is observed in staphylococcal species.
- The need of monitoring antimicrobial resistance in CNS not only in the meat of slaughtered animals but also in fish products at retail.
- The risk to public health due to consuming raw or undercooked shellfish containing enterotoxigenic plus methicillin-resistant CNS.

Contamination of food processing factories by *Staphylococcus* spp. is screened by culture-based microbiological methods, but characterization of the isolates is usually limited to determination of coagulase positivity. A variety of culture-dependent and culture-independent methods have been developed to detect *S. aureus* in foods. Depending on the suspected level of contamination and the purpose of the analysis, early steps may include nonselective enrichment, selective enrichment, or even direct detection on selective and differential media. In Europe, the analytical reference method for CPS is EN/ISO 6888-1 or ISO 6888-2 (EC regulations no. 2073/2005 and 1441/2007). Several commercial identification systems based on phenotypic tests allow discrimination of CPS and CNS at species levels. RAPIDEC Staph, API Staph, VITEK 2, *MicroScan Pos ID* and Rapid *Pos ID panel* systems, Crystal Rapid Gram-Positive Identification System, Biolog MicroStation System, MIDI Sherlock Microbial Identification System, and RiboPrinter Microbial Characterization System are a few examples. Complementary agglutination tests arranged on different supports (such as latex beads) are often used to confirm the presence of coagulase and staphylococcal protein A. One emerging technology for bacterial detection is based on aptamers. Aptamers are small RNA/DNA molecules that can form secondary and tertiary structures capable of specifically binding proteins or other cellular targets; they are essentially a chemical equivalent of antibodies. Based on the light scattering capacity of gold nanoparticles, aptamer-conjugated gold nanoparticles could be used for rapid single-cell detection of *S. aureus*.

Rapid detection of *S. aureus* can be made using the Accu-Probe culture identification test, which is based on a highly specific DNA probe directed against rRNA. Polymerase chain reaction (PCR) analysis of the 16S–23S rRNA intergenic spacer region (singly or combined with restriction analysis) can discriminate among several *Staphylococcus* species and allows for the identification of pure culture staphylococci within 24–48 h. Furthermore, a variety of identification methods based on amplification (by conventional or multiplex PCR and real-time PCR) of target genes such as *rpoB* (RNA polymerase B subunit), *nuc* (thermostable *S. aureus* nuclease), *coa* (coagulase enzyme), and *sodA* (manganese-dependent superoxide dismutase) among others have been proposed in recent years for identification at species level of both CPS and CNS. Specific detection of *S. pseudintermedius* by PCR reactions targeting the *nuc* gene or PCR-restriction fragment length polymorphism (RFLP) targeting the phosphoacetyltransferase (*pta*) gene has also been described. An oligonucleotide array has also been developed, which targets the *sodA* gene. It is a valuable tool that distinguishes the validated staphylococcal species in one shot and allows rapid, accurate identification of staphylococcal strains from clinical and food origins. DNA microarrays for simultaneous detection of *S. aureus* and other foodborne pathogens have also been developed.

Detection of MRSA

The spread of MRSA outside of clinical settings is a matter of concern. Recent reports of MRSA in livestock, particularly pigs, and in individuals with contact with livestock provided the first evidence of the existence of a livestock-associated (LA-MRSA) reservoir throughout Europe. Since then, LA-MRSA has been identified in many other countries. LA-MRSA strains have important genotypic and phenotypic characteristics: (i) They are nontypeable by pulsed field gel electrophoresis (PFGE) using *Sma*I; (ii) many of them belong to CC398 by multilocus sequence typing (MLST); (iii) SCC*mec* elements are different from those carried by other MRSA genotypes commonly found in the community and health care settings; (iv) they often exhibit coresistance to many non-β-lactam antimicrobials (e.g., antibiotics and metals), including those

commonly used in animal production; (v) the majority of isolates lack toxins such as PVL and other enterotoxins. The EFSA recommends sampling in the environment of pig stables to determine the risks of herds being positive for MRSA.

Conventional methods for the laboratory detection of MRSA include antimicrobial susceptibility testing, detection of PBP2a by latex agglutination tests, and the molecular detection of the *mecA* or *mecC* genes. The EFSA has provided a detailed flowchart for isolation, identification, and typing of MRSA and recommends a harmonized set of antimicrobial substances to be used for testing of susceptibility in *S. aureus*/MRSA. Disk diffusion is recommended for antibiotic sensitivity testing by the National Committee for Clinical Laboratory Standards. Other methods, such as oxacillin agar, have also been demonstrated to have good accuracies. Recently, cefoxitin disks have been reported to be highly accurate for predicting methicillin resistance. Routine diagnostic tests do not, however, provide a mechanism for the identification of *mecA* or *mecC*, which still requires confirmation using PCR assays. However, an oxacillin-sensitive/cefoxitin-resistant profile on antibiotic susceptibility tests is indicative of a *mecC* MRSA genotype. Microelectromechanical systems aiming at determination of antibiotic susceptibility and detection of key genes or proteins are gaining increasing attention for rapid detection of MRSA, especially in the medical field.

When testing antibiotic resistance, care should be taken since MRSA strains can be heterogeneously resistant to methicillin, so only a small proportion of the *S. aureus* colonies that grow from a given sample may show resistance in standard laboratory tests. Consequently, if too few cells appear resistant, an oxacillin-resistant strain may go undetected. Also, in *mec*-negative strains of *S. aureus*, methicillin resistance can be due to hyperproduction of β-lactamase, the production of normal PBP with altered binding capacities and/or other factors that have not been identified to date. These isolates usually have MICs at or just above susceptibility break zones. The isolates are sometimes referred to as borderline-resistant *S. aureus*. And last, the phenotypic expression of resistance may also vary despite genetic homogeneity depending on culture conditions that include temperature and the osmolarity of the medium.

Detection of SEs

European regulations (EC 2073/2005 and 1441/2007) require screening of SEs if CPS counts in milk products during the manufacturing process at the processing step exceed 10^5 CFU g^{-1}. Commercial toxin detection kits (such as Vidas® SET2 kit or Ridascreen® SET Total kit) have been recommended for this purpose by the National and European Union Reference Laboratories for Coagulase Positive Staphylococci. The different types of methods available for detection and investigation of bacterial toxins in food are based on bioassays, immunologic techniques, molecular biology, and mass spectrometry-based methods.

- Bioassays are based on the capacity of an extract of the suspected food to induce symptoms such as vomiting, gastrointestinal symptoms in animals, and/or superantigenic action in cell cultures. Historically, SEs have been detected based on their emetic activity in monkey-feeding and kitten-intraperitoneal tests and, more recently, using animal models such as house musk shrews (*Suncus murinus*). Others (SEls) have not been tested or gave negative results on emetic activity tests.
- Immunologic methods. The most commonly used method for detecting SEs in food is based on the use of anti-enterotoxin polyclonal or monoclonal antibodies. Commercially available kits have been developed according to two different principles: (1) enzyme immunoassay comprising ELISA and enzyme-linked fluorescence assay and (2) reversed passive latex agglutination. Whatever the detection method used and owing to the low amount of SEs present in food, it is crucial to concentrate the extract before performing detection assays. One limitation can be interference from the food matrix, leading to false-positive results. Recent studies have improved these methods in order to make them more sensitive and specific.

Recent advances in nanomaterials, microelectromechanical systems, and microfluidics resulted in the so-called lab-on-a-chip systems for immunodetection of bacteria and their toxins. Some of these innovations can be applied for immunodetection of SEs. For example, antibodies coupled to fluorescent magnetic nanoparticles have been used for a novel sandwich immunoassay on a 3-D fiber solid phase for the detection of SEA and TSST in milk samples. Also, antibodies coupled to fluorescent magnetic microspheres allowed the multiplex toxin detection of SEB and other toxins in a suspension assay. Quantum dots (semiconductor nanocrystals) coated with antibodies were used for the simultaneous detection of SEB and other toxins based on excitation and fluorescence emission. An eight-channel chip and single-walled carbon nanotube-based immunoassay were combined for the detection of SEB, enabling the detection of 0.1 ng ml^{-1} of SEB in a 10-μl sample. Later, this microfluidic platform was improved for the label-free detection of SEB and termed 'biological semiconductor.'

- Molecular biology methods often involve the PCR. These methods usually detect genes encoding enterotoxins in strains of *S. aureus* isolated from contaminated foods. However, these methods have two major limitations: First, staphylococcal strains must be isolated from food, and second, the results inform as to the presence or absence of genes encoding SEs but do not provide any information on the expression of these genes in food. This is important to be considered, since enterotoxin genes may show different patterns of expression in relation to bacterial growth and therefore may be influenced by factors affecting growth and survival such as pH, temperature, water activity, redox potential, and nutritional factors. Very recent efforts have been directed to determining directly which *SE* genes are found in suspected foods. Following the huge SFP event, which occurred in Japan in July 2000 (more than 13 000 people were intoxicated by powdered or liquid milk), a PCR-based methodology was developed whereby *SEA*, *SEG*, *SEH*, and *SEI* genes could be detected in the incriminated powdered skim milk, although cultivable *S. aureus* were not recovered from the sample. Moreover, to evaluate the toxic potential of strains isolated from SFP outbreaks, various authors have recently designed primers to perform PCR and reverse transcription PCR for *SE* genes.

Regarding CNS, failure of SE gene detection in CNS might sometimes be explained by the use of inappropriate methods of DNA preparation, in which cell wall destabilization using lysostaphin was not applied. Another potential cause of false-negative results is that primers for CNS enterotoxin detection were based on known *S. aureus* sequences, whereas CNS sequences, despite close phylogenetic vicinity of organisms, are likely to be divergent. Therefore, polynucleotide DNA microarrays able to detect sequence with similarities down to 70% in contrast to oligonucleotide-based ones seem to be more useful in the detection of CNS enterotoxins.

- Mass spectrometry (MS)-based methods. Owing to the drawbacks with currently available detection methods and the lack of available antibodies against the newly described SEs, other strategies based on physicochemical techniques have been developed recently. Among these, MS has newly emerged as a very promising and suitable technique for analyzing protein and peptide mixtures.

In the case of food analysis, the situation is complex because the matrix can contain many proteins, lipids, and many other molecular species that interfere with the detection of the targeted toxin and may distort quantification. Sample preparation remains the critical step of the analysis. It could be improved by, for example, optimizing digestion parameters or by adding a purification step. The strategy of incorporating an isotopically labeled internal standard into the samples has also been developed.

Thus, an overall approach combining classical microbiology to enumerate CPS strains with immunologic techniques, molecular biology, and MS-based methods offers an interesting alternative for attributing outbreaks to SEs.

Strain Typing

Investigation of the genetic diversity of staphylococcal populations is important in order to distinguish subpopulations or clones with specific features such as enhanced virulence or antibiotic resistance. Subspecies genotypic information on microbial contaminants in food processing factories may be of practical importance, as it may facilitate identification of transient versus persistent strains. While metabolic fingerprinting has limited discriminatory power and is prone to errors due to differences in gene expression, molecular typing methods are widely applied to characterize *S. aureus* subpopulations and hopefully will also be used for typing of other CPS and for CNS. However, the parameters of throughput, speed, simplicity, and cost are very important for routine application in the food industry.

- Random amplification of polymorphic DNA (RAPD-PCR). The simplest and cheapest random PCR-based methods such as RAPD-PCR lack sufficient reproducibility but still can be used for approximation of strain diversity within a given food system or for lab-scale comparison of diversity within a collection of strains.
- Amplified fragment length polymorphism (AFLP) is based on selective amplification of genomic restriction fragments. Interestingly, AFLP has been used for typing of CNS.
- RFLP analysis is based on PCR amplification of variable regions within the bacterial chromosome such as the 16S–23S rRNA gene spacer or the coagulase (*coa*) or protein A (*spa*) genes. The products from PCR amplification are then digested with suitable restriction enzymes and the resulting fragments analyzed by gel electrophoresis. PCR-RFLP can also be used for lab-scale comparison of strain diversity.
- PFGE allows separation of large DNA fragments, such as those generated after chromosome digestion with restriction enzymes. Whole bacterial cell DNA is incubated with (usually one) restriction enzyme(s) (usually *Sma*I for *S. aureus*), resulting in a band pattern that depends on the number of enzyme recognition sites on the bacterial DNA. Changes in the bacterial DNA (such as point mutations, insertions, deletions, gene duplications, and inversion of DNA fragments) may affect the number of restriction sites and the sizes and numbers of the generated DNA fragments. Comparison of band patterns with suitable computer programs allows the calculation of similarity coefficients and the detection of specific clones, such as MRSA clones. One limitation of PFGE is interlaboratory variations.
- Multiple-locus variable-number tandem repeat analysis (MLVA) is based on heterogeneity of loci carrying a variable number of tandem repeats (VNTR). In MLVA, the variability in the number of short tandem repeat sequences is utilized to create DNA profiles. MLVA was initially based on six tandem repeat loci (*spa*, *clfA*, *clfB*, *sdr*, *sspa*, and *coa*). Bioinformatic analysis of *S. aureus* sequenced genomes has allowed the identification of additional VNTR loci. Within these, the variable number of same-sized inserts is reflected by a well-defined variability in length of the loci. The use of single multiplex amplification and flow-through gel electrophoresis by an automated analyzer improved the precision of molecular weight determination and practical applicability of the method in terms of reduction in time and labor requirements.
- MLST was developed as a universal typing method that avoids the biases introduced by experimental conditions. Selected internal fragments of seven housekeeping genes are sequenced after PCR amplification: *arc* (carbamate kinase), *aro* (shikimate dehydrogenase), *glp* (glycerol kinase), *gmk* (guanylate kinase), *pta* (phosphate acetyltransferase), *tpi* (triosephosphate isomerase), *yqi* (acetyl coenzyme A acetyltransferase), and the sequences compared to an MLST database. Mutations in the housekeeping genes yield different allele types, and the combinations of allele types of the different housekeeping genes define each particular sequence type (ST). After analysis with suitable bioinformatic programs (such as eBURST), each strain is assigned to an ST (either existing in the database or new). Cluster analysis of STs allows definition of clonal complexes (CCs), which can be related with pathogenicity or antibiotic resistance. For example, the MRSA ST398 of CC398 is an emerging ST that is massively colonizing livestock and food products and causing human infections.
- Single-locus sequence typing is based on the polymorphic repeat region of the *spa* gene. Spa sequences can be compared to databases (Ridom SpaServer) and used for reliable, accurate, and discriminatory typing of MRSA.
- Typing of the SCC*mec* is widely used for typing of MRSA strains. SCC*mec* mobile elements contain the methicillin

resistance (*mec*) gene complex, the recombinase (*ccr*) gene complex, and the joining region. According to diversity and gene orientation, strains can be assigned to SCC*mec* allotypes.

- DNA microarrays. This technique utilizes multiplex linear DNA amplification where labeled amplicons are hybridized to probes on a slide. Microarrays allow not only strain typing but also multiple detection of other relevant genes such as those coding for virulence determinants or antibiotic resistance.
- Whole genome comparison. The development of technologies for massive DNA sequencing and analysis has introduced the possibility of doing phylogenomic analysis for bacterial strains. The core genome of *S. aureus* contains a set of highly conserved genes present in all strains, while the accessory genome may include mobile genetic elements and encode antimicrobial resistance determinants, toxins, SAgs, or other factors that promote infection. Genome comparisons based on single-nucleotide polymorphisms (SNPs) of the core genome and/or variations in the accessory genome structure and function can provide novel insights into the population structure and biology of this bacterium.

Conclusions

SFP remains one of the most common foodborne diseases in the world following the ingestion of SEs that are produced by enterotoxigenic strains of CPS, mainly *S. aureus*. Prevention of SFP is based on hygiene measures to avoid or reduce contamination of food by *S. aureus*. These procedures must include control of raw materials, proper handling, cleaning, and disinfection of equipment from farm to fork. Thus, an overall approach combining classical microbiology to enumerate CPS strains with immunologic techniques, molecular biology, and MS-based methods offers an interesting alternative for attributing outbreaks to staphylococci. In any case, subspecies genotypic, enterotoxigenic, and resistance information on staphylococci strains, in food processing factories, is of great importance as it may help to properly manage the process hygiene standards, to identify a possible need for improvements in the efficiency of sanitation, or to improve the control of materials entering the food processing facility.

Summarizing remarks: (i) Investigation of both CPS and CNS is important for food safety; (ii) CNS may be growing in relevance as pathogens and carriers of antibiotic resistance (including resistance to methicillin and also to other not less important antimicrobials); (iii) there is a need to clearly establish the enterotoxigenic potential of CNS; (iv) in addition to classical detection methods, the introduction of miniaturized systems and nanomaterials may speed up detection of staphylococci, strain typing, and enterotoxin production.

See also: Food Poisoning: Classification; Food Poisoning: Epidemiology; *Staphylococcus*: Food Poisoning; *Staphylococcus*: Occurrence and Properties.

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Staphylococcus: Food Poisoning

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Introduction

Staphylococci, and their most prominent food poisoning representative *Staphylococcus aureus*, are probably among the most established foodborne pathogens. It was in 1871 when Recklinghausen, a German scientist, observed cocci in a diseased kidney and called them 'micrococci,' incriminating staphylococci as human pathogens. Later, after several changes in their nomenclature, Sir Alexander Ogston coined the name *Staphylococcus*. The foodborne disease involving staphylococci was for the first time investigated in Michigan (the United States) in 1884 by Vaughan and Sternberg, as a response to the food intoxication involving cheese probably contaminated by staphylococci. Proof of the involvement of staphylococci in food poisoning was first brought in 1914 by Barber who discovered that a toxin substance produced by staphylococci was responsible for staphylococcal food poisoning. The capacity of some strains of *S. aureus* to produce food poisoning was conclusively demonstrated in 1930 by Dack et al. who showed that the symptoms could be produced by feeding culture filtrates of *S. aureus*. Today, it is well established that food poisoning caused by staphylococci is a foodborne intoxication, with staphylococcal enterotoxins (SEs) as etiologic agents. SEs are produced in food during exponential and stationary growth phase of enterotoxigenic strains causing foodborne intoxication after the consumption of contaminated food. *S. aureus* is the species almost invariably involved, although enterotoxin production by several other species has been reported.

Species of Concern in Food

Genus *Staphylococcus* contains 47 species and 24 subspecies as reported in the List of Prokaryotic Names with Standing in Nomenclature as of the full update in 6 August 2013 (http://www.bacterio.net/s/staphylococcus.html). The genus *Staphylococcus* comprises Gram-positive, catalase-positive cocci. Metabolism of carbohydrates may be either oxidative or fermentative. Cells are small (~1 mm diameter) and often form characteristic clumps resembling bunches of grapes. Their classification distinguishes between coagulase-producing strains, designated as coagulase-positive staphylococci (CPSs), and noncoagulase-producing strains, called coagulase-negative staphylococci (CNSs). Staphylococci are predominantly of animal origin, although some species were isolated from the environmental sources. They may be present as part of the normal microflora of humans and animals. *S. aureus* is carried on skin and nasal cavities of about 30% of the healthy human population. Among those, enterotoxigenic species are of potential interest in food safety, although not all of them have been documented in foods. Table 1 shows an overview of reported enterotoxigenic *Staphylococcus* spp. The most important member of genus *Staphylococcus* in relation to food safety is *S. aureus*, followed by *S. hyicus* and *S. intermedius*. Next to the well-established role of CPS in food poisoning, several CNSs have also been potentially involved in foodborne outbreaks. Among CNS isolates reported to produce detectable enterotoxins are those belonging to *S. saprophyticus*, *S. epidermidis*, *S. cohnii*, and *S. warneri*. Recently, following consumers' complaint, a coagulase-negative *S. saprophyticus* was found in an oyster sauce (Rapid Alert System for Food and Feed, RASFF, alert 2012.0605). It is also important to note that CNS isolates play an important role in mastitis etiology and that milk from subclinically infected cows can be hazardous to human health not only because of the risk of pathogen transmission but also because of contamination with enterotoxins.

Regarding the percentage of strains that are enterotoxigenic, widely different percentages have been found depending on the source of isolates. The numbers vary from 10% (of 236 raw milk isolates) to ~60% (200 food isolates). The relative incidence of specific enterotoxins among strains recovered from various sources varies widely, too. The existing data should be critically interpreted as the methods used to determine enterotoxigenic potential differ among studies, including types of SE that were screened for. It is however important to note that one strain can produce multiple enterotoxins.

S. aureus in Foodborne Intoxications

Symptoms

The incubation period and severity of symptoms depend on the amount of enterotoxins ingested and the susceptibility of each individual. However, it is generally understood that all people are susceptible to staphylococcal food intoxication. *Symptoms* are of rapid onset and are commonly described by nausea and violent vomiting. Early symptoms comprise nausea followed by incoercible characteristic vomiting (in spurts) and appear within 30 min to 8 h (about 3 h on average) after ingestion of contaminated food. Other commonly described symptoms include abdominal pain, diarrhea, dizziness, shivering, and general weakness sometimes associated with a moderate fever. In the most severe cases, headache, prostration, and low blood pressure have been reported. In the majority of cases, recovery occurs within 24–48 h without specific treatment, while diarrhea and general weakness can last 24 h or longer. Death is rare, occurring primarily in those susceptible to dehydration (infants and elderly people) and in those affected by an underlying illness. The vomiting symptoms of staphylococcal intoxication are identical to that of *Bacillus cereus* emetic intoxication, caused by cereulide. It is interesting to mention that the symptoms of *B. cereus* diarrheal toxicoinfection are identical to that of *Clostridium perfringens* toxicoinfection. An overview of the symptoms of these toxigenic species is provided in Table 2.

Table 1 Overview of reported enterotoxigenic *Staphylococcus* spp.

Staphylococcus *spp.*	*Coagulase*	*Enterotoxins produced (some of the SE types produced/or* se *encoding genes detected)*
S. aureus	+	+ (All known SE types)[a]
S. caprae	−	+
S. carnosus	−	+ (SEA, SEE, SHE)
S. cohnii		+ (SEA, SEB)
S. chromogenes	−	+
S. delphini	+	+ (SEA, SEE)
S. epidermidis	−	+ (SEA, SEC, SED)
S. equorum	−	+ (SED, SEH)
S. haemolyticus	−	+
S. hyicus	(+)	+ (SEA, SEC, SED)[b]
S. intermedius	+	+ (SEA, SEB, SEC, SED, SEE)[c]
S. lentus	−	+
S. piscifermentans		+ (SEH)
S. saprophyticus	−	+ (SEA)
S. schleiferi subsp. *schleiferi*	−	+
S. sciuri	−	+
S. warneri	−	+
S. xylosus		+ (SEH)

[a]Staphylococcal enterotoxins are members of a family of more than 20 different staphylococcal and streptococcal exotoxins that are functionally related and share sequence homology. Those with established emetic activity, involved in food intoxications, are SEA, SEB, SEC, SED, SEE, SEF, SEG, SHE, and SEI.
[b]Until recently, it has been thought that *S. hyicus* produced SE other than classical SEA–SEE.
[c]Even though *S. intermedius* is known to produce SE (next to *S. aureus* the most often cited as enterotoxigenic staphylococci), only one outbreak has been associated with *S. intermedius* since the identification of this species.

Reservoirs

The staphylococci are ubiquitous in nature, with humans and animals as the primary reservoirs. They are present in the nasal passages and throat, in the hair, and on the skin of many healthy individuals. About 40–45% of healthy individuals are nasal carriers of staphylococci, and 8–22% have them on their skin. In fact, estimates range from 20% to 30% for persistent and 60% for intermittent colonization. Staphylococci can be isolated from animals, with the bovine being the most important because of the involvement of staphylococci in mastitis. The prevalence of subclinical and clinical bovine mastitis caused by *S. aureus* ranges from 5% to 50% in different countries.

Much uncertainty remains about the origin and public health implications of livestock-associated methicillin-resistant *S. aureus* (LA-MRSA), but it is known that it is capable of causing zoonotic infections. Recent reports of MRSA in livestock, particularly pigs, and in humans with contact to livestock provided the first evidence of the existence of a true LA-MRSA reservoir throughout Europe. Since then, LA-MRSA has been identified in Canada, the United States, China, Malaysia, Korea, Thailand, etc.

Although animals and humans are the major source, staphylococci also can be found in the air, dust, water, and human and animal wastes. From these sources, they can be transferred to different types of foods.

Implicated Foods and Outbreaks

Foods that have been frequently incriminated in staphylococcal intoxication include meat and meat products; poultry and egg products; milk and dairy products; salads; bakery products, particularly cream-filled pastries and cakes; and sandwich fillings. In the European Union, the largest proportion of 345 outbreaks caused by staphylococcal toxins in 2011 were attributed to mixed food (40.0%). The second most frequently single food category reported was dairy products (14.3%), followed by bakery products (11.4%). Broiler meat and products thereof, cereal products, eggs and egg products, meat and product thereof, vegetables and juices and other products thereof, and other foods were also implicated. In the United States, pork – particularly baked ham – is the food most frequently involved in outbreaks; poultry, salads (meat, potato, etc.), and cream-filled bakery goods are responsible for many of the outbreaks. Table 3 shows an overview of foods involved in staphylococcal outbreaks in the United States in the period 1998–2008. Salted food products, such as ham, have also been implicated, resulting from the capacity of *S. aureus* to grow at relatively low water activity ($a_w = 0.83$). For a number of dried meat products, *S. aureus* remains one of the main microbial safety issues. In RASFF database, about 83 different notifications were issued on the presence of staphylococci or their enterotoxin in many different foods of different geographic origin. Perhaps, surprising to the common knowledge is that more than 20 of these alerts were related to fish and fishery products and crustaceans and products thereof.

Outbreaks of staphylococcal food poisoning are numerous. *S. aureus*, *B. cereus*, *and C. perfringens* create the group of three most important causative agents of bacterial exotoxin-related foodborne diseases. It is estimated that of 9.4 million foodborne illnesses caused by a known pathogen annually in the United States, 1.3 million are caused by *B. cereus*, *C. perfringens*, or *S. aureus*. Meat or poultry dishes were commonly implicated in *C. perfringens* (63%) and *S. aureus* (55%) outbreaks, and rice dishes were commonly implicated in *B. cereus* outbreaks (50%). Errors in food processing and preparation were commonly

Table 2 Comparison of syndromes caused by toxins of *B. cereus*, *S. aureus*, and *C. perfringens*

		B. cereus		S. aureus	C. perfringens
Bacteria/syndrome/toxin		*Diarrheal syndrome (hemolysin BL (HBL), nonhemolytic enterotoxin (NHE),* B. cereus *enterotoxin T (bceT), and cytK)*	*Emetic syndrome (cereulide)*	*Enterotoxins*	*Enterotoxin (CPE)*
Symptoms		Abdominal pain, cramps, watery diarrhea (secretory type), and occasionally nausea	Nausea, vomiting, malaise, and ultimately a fatal liver failure	Nausea, vomiting, sometimes diarrhea	Intense abdominal cramps, diarrhea, and flatulence
Usual incubation time (h)		8–24 (or longer depending on the dose and host susceptibility)	0.5–5	1–8	6–24
Usual resolution time (h)		12–24 (up to several days in severe cases)	6–24	24–48	Within 24
Intoxication/infection dose		Ingestion of more than 10^5 CFU of diarrheal toxin-producing *B. cereus* strains	$\sim$10 μg kg^{-1} bw, 0.01 μg g^{-1} of food (*B. cereus* concentration more than 10^5 CFU g^{-1} food, depending on the strain, food, and conditions)	100 ng of ingested toxin, 0.05 ng ml^{-1} of food (produced when *S. aureus* counts reach $\sim10^5$ CFU ml^{-1} (g^{-1}))	10^6–10^7 CFU g^{-1} of food (ingested vegetative cells produce CPE during intestinal sporulation possibly under impact of bile and stomach acids)
Toxin produced		In the small intestine of the host	Preformed in the food	Preformed in the food	In the small intestine of the host
Food involved		Milk- and meat-containing products, soups, vegetables, puddings	Rice, pasta, potato puree, noodles, sauces, paella, meat, polenta	Meat- and milk-containing products, creams, chickens salads, canned mushrooms	Meats, meat products, gravy, cooked beans, stews, soups
Toxin stability	Heat	5 min at 56 °C	90 min at 121 °C	SEA 3 min at 80 °C, 1 min at 100 °C; SEB 87 min at 99 °C	5 min at 60 °C
	pH	4–8	2–11	Wide range, resistant to gastric pH	5–10
	Proteinases	Nonresistant	Resistant	Resistant	Increased activity

reported (93%), regardless of etiology; contamination by a food handler was only common in *S. aureus* outbreaks (55%). The incidents involving *S. aureus* are related to different establishments but are very frequent in catering services where food is premade in larger volumes so that cooling is not as fast as required. Moreover, the food is maintained at convenient temperatures for fast serving, and those are temperatures that can support growth and enterotoxin formation. Other studies confirm that the food handler is the principal source of food contamination. In a recent outbreak in Germany, an *S. aureus* isolate from the stool sample was matched to the food isolate and isolate coming from the food handler. Reporting of outbreaks is mandatory and the confirmation of *S. aureus* outbreak, based on the guideline of Centers for Disease Control and Prevention, consists of the isolation of organism of same phage type from stool or vomitus of 2 or more ill persons, or the detection of enterotoxin in epidemiologically implicated food, or the isolation of 10^5 organisms per gram from epidemiologically implicated food. There is an internationally agreed basic set of phages used for typing strains of *S. aureus* of human origin, which is updated regularly to reflect changes in the typability of human clinical isolates. There are problems of nontypability, since 15–20% of isolates may be untypable. Today, more robust, reliable, and convenient molecular typing methods exist, such as pulsed field gel electrophoresis, which is the method of choice in most of the EU member states and is also used worldwide. Also, CDC had published recently a unified pulsed-field gel electrophoresis (PFGE) protocol for Gram-positive bacteria. However, the typing of *S. aureus* is not the common practice in the world. In its report, the European Food Safety Agency states that 19 member states (MSs) and 3 non-MSs currently perform molecular typing for *S. aureus* isolates from animals, food, and feed. Six MSs and one non-MS reported that they neither perform or purchase molecular typing of *S. aureus* isolates.

Control and prevention

As mentioned earlier, humans and animals are usual carrier of *S. aureus*. Consequently, the contamination of foods often happens via the factory or kitchen staff by the hand contact or their respiratory system. Therefore, the good personal hygiene is essential as a preventive measure for *S. aureus*

Table 3 Foods implicated in outbreaks caused by *Staphylococcus aureus*, in the United States, 1998–2008 (for details, see Bennett et al., 2013)

	Staphylococcus aureus *outbreaks*		
Food type	*Confirmed*	*Suspected*	*All Outbreaks*
Outbreak food ethnicity	$n=150$	$n=242$	$n=1074$
Any ethnic	18 (12)	34 (14)	241 (22)
Mexican style	7 (5)	13 (5)	132 (12)
Asian	9 (6)	10 (4)	81 (8)
Single food groupings	$n=106$	$n=199$	$n=820$
Meat or poultry	58 (55)	53 (27)	336 (41)
Beef	6 (10)	8 (15)	116 (35)
Poultry	8 (14)	26 (49)	115 (34)
Pork	39 (67)	14 (26)	92 (27)
Unspecified origin	4 (7)	4 (8)	11 (3)
Other	1 (2)	1 (2)	2 (1)
Rice dishes	5 (5)	5 (3)	85 (10)
Sandwiches	8 (8)	45 (23)	68 (8)
Pasta dishes	9 (8)	10 (5)	43 (5)
Burritos and tacos	4 (4)	5 (3)	39 (5)
Soups and stews	3 (3)	3 (2)	36 (4)
Bean dishes	0 (0)	0 (0)	32 (4)
Sauces	2 (2)	4 (2)	32 (4)
Salads	4 (4)	14 (7)	31 (4)
Pizza	1 (1)	25 (13)	30 (4)
Seafood	3 (3)	11 (6)	21 (3)
Other produce	1 (1)	6 (3)	19 (2)
Pocket foods	2 (2)	1 (1)	16 (2)
Dairy	1 (1)	5 (3)	9 (1)
Baked goods	2 (2)	3 (2)	8 (1)
Other foods	3 (3)	9 (5)	15 (2)

Data represent number of outbreaks and (%).

contamination. Nevertheless, *S. aureus* is also able to nest itself on the surfaces of process equipment due to inadequate and insufficient cleaning and disinfection. CPSs, which serve as analytic indicator of the presence of toxin-producing *S. aureus*, not only are determined because of their pathogenic nature but also are often detected as an indication of the level of personal hygiene and good manufacturing practice (GMP) performance. Low numbers (100–1000 CFU g^{-1}) can be tolerated, depending on the inherent properties of the food and envisaged storage conditions and length of the shelf life.

It is generally understood and accepted that *S. aureus* as a food intoxicant presents a risk only when the growth to high numbers occurs ($>10^5$ CFU g^{-1}). Since *S. aureus* cannot grow at temperatures below 7 °C and that multiplication of *S. aureus* in raw products is inhibited or slowed down by competing accompanying microflora, these high numbers are normally not reached under the conditions of maintained cold chain. However, the mere presence (and not the growth) of *S. aureus* is associated with the number of cold stored foods and even with ice creams. Nevertheless, problems with *S. aureus* are most often an indication of temperature abuse or postcontamination. With respect to growth temperature, SEB production in ham has been recorded already at 10 °C, as well as lower amounts of SEA, SEB, SEC, and SED in cooked ground beef, ham, and bologna-type sausage.

A simplified scenario in *S. aureus* food poisoning encompasses foods in which production involves a number of manipulations through which the intrinsic factors (especially a_w – given the high salt tolerance of *S. aureus*) have been amended so that the competitive flora becomes inhibited and *S. aureus* is free to multiply at insufficient cooling and produces one or more of its enterotoxins (Figure 1). Similar scenario is when postpasteurization contamination occurs. If in the analyzed food *S. aureus* is found in numbers of 10^5 CFU g^{-1} and higher, then also the presence of SE should to be suspected. However, the SE production is largely strain- and food-dependent, and evidence of SE production at lower numbers and the lack of production at counts even higher than 10^5 CFU g^{-1} exists. Due to the heat stability of enterotoxins, it is possible that *S. aureus* is eliminated in the food, which was heated for the consumption, but that its toxin(s) is still present and active (Table 2). *S. aureus* cells are considerably more sensitive to heat than the SEs, with *D* values ranging from ~2 to 15 at 60 °C. The actual heat stability of each of SE is largely dependent on the environment in which heat treatment occurs.

Knowing that the main source of food contamination with *S. aureus* comes from food handlers, food contact materials, or contaminated raw materials, the main preventive measure is strict application of good hygienic practices and good manufacturing practices. The factors that contribute to staphylococcal food poisoning are inadequate refrigeration, inadequate cooking or heating processing, and poor personal hygiene of infected handlers that results in cross contamination.

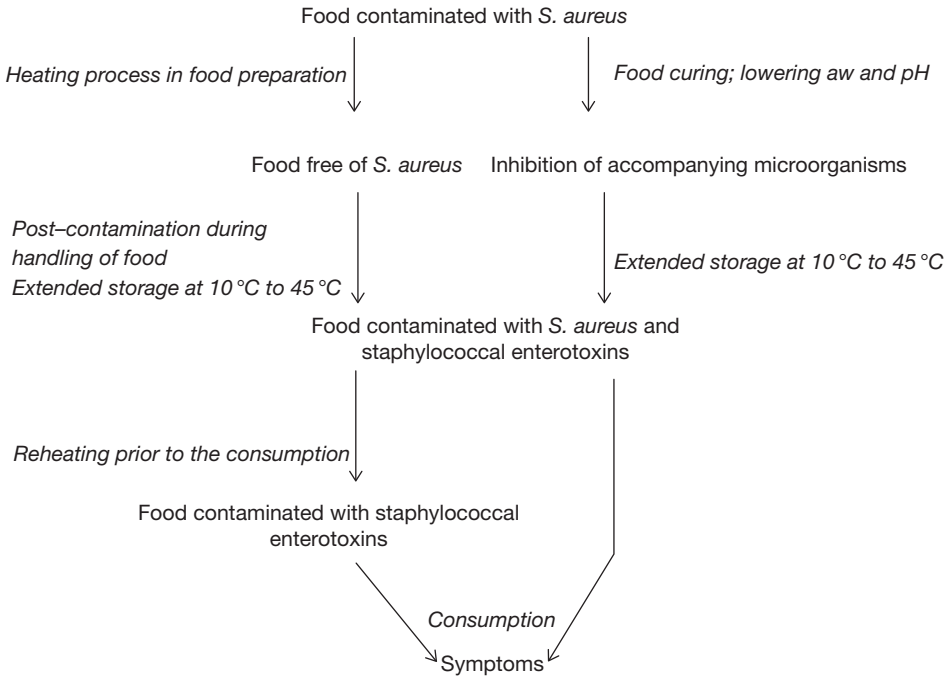

Figure 1 Simplified scholastic representation of usual scenarios leading to staphylococcal foodborne intoxication.

Keeping food below 5 °C and above 50 °C will not allow SE production, nor *S. aureus* multiplication.

Enterotoxins

SEs are the most notable virulence factors associated with *S. aureus*. SEs belong to a great family of staphylococcal and streptococcal pyrogenic exotoxins (PT), characterized with common phylogenetic relationships, structure, function, and sequence homology. SEs function not only as potent gastrointestinal toxins causing emesis but also as superantigens that stimulate nonspecific T-cell proliferation. Although these are two separate functions localized on separate domains of the proteins, certain evidence exists that these two activities can exist independent from each other.

To date, 21 SEs and enterotoxin-like (SEl) types have been described: enterotoxins A (SEA), B (SEB), C1 (SEC1), C2 (SEC2), C3 (SEC3), D (SED), E (SEE), G (SEG), H (SEH), I (SEI), J (SElJ), K (SElK), L (SElL), M (SElM), N (SElN), O (SElO), P (SElP), Q (SElQ), R (SER)[13], S (SES), T (SET) [14], U (SElU), and U2 and V, as reviewed in detail by Hennekinne et al. in 2010 in 'Toxins' (see 'Further Reading' for more information). The incidence of enterotoxin producers among human isolates is reported to be much higher than among nonhuman isolates.

Next to the SEs with demonstrated emetic activity (SEA to SEE, SEG to SEI, and SER to SET), there are also staphylococcal-like (SEl) proteins, which are not emetic in a primate model (SElL and SElQ) or have yet to be tested (SElJ, SElK, SElM to SElP, SElU, SElU2, and SElV). SEs and SEls have been traditionally subdivided into classical (SEA to SEE) and new (SEG to SElU2) types. Several studies attempted to characterize the emetic potential of SEl. In one of them, an emetic potential of SElK, SElL, SElM, SElN, SElO, SElP, and SElQ was assessed using a monkey-feeding assay. All the SEls that were tested induced emetic reactions in monkeys at a dose of 100 μg kg^{-1}. Although a number of affected monkeys were significantly smaller than a number that were affected after consuming SEA (six out of seven) or SEB (four out of four) at the same concentration and the latency periods of the classical SEs seems to be shorter than those of the SEls, these results indicate that SEl may nevertheless play certain role in staphylococcal food poisoning. However, the role of SEls in human food poisoning currently remains unclear, but is it well known that not all of the SEs have equal emetic potential (Table 4). The enterotoxin gene that is most frequently detected in clinical isolates of foodborne staphylococcal intoxications is *sea*. Of note, strains of human origin are highly prevalent in the strains isolated from SFP outbreaks, and *sea* is predominantly carried by human strains. This information does not necessarily correspond to incidence of specific SE genes in food isolates and cow mastitis isolates.

The currently known SEs form a group of serologically distinct, extracellular proteins that share important properties. Among these are the abilities to cause emesis and gastroenteritis in primates, exhibit superantigenicity through an unspecific activation of T lymphocytes followed by cytokine release and systemic shock, and resist heat treatments and digestion by pepsin and structural similarities. SEs are resistant to proteolytic enzymes, hence retaining their activity in the digestive tract after ingestion. Their known heat stability is not the same for all SEs and also depends on the food matrix and toxin concentration. So, for example, *SEA* is more *heat*-sensitive than *enterotoxin* SEB, and SEB is more sensitive than SEC. The biological activity of SEB was retained after heating for 16 h at 60 °C and pH 7.3. Heating of one preparation of SEC for 30 min at 60 °C resulted in no change in serological reactions. The heating of SEA at 80 °C for 3 min or at 100 °C for 1 min

Table 4 Overview of reported SE doses required for intoxication in different hosts

Organism	*Toxin*	*Minimal dose to induce toxic effect or (ED_{50} per kg body weight)/host*
S. aureus	SEA	(25 μg)/rhesus monkey
		(10 μg)/rhesus monkey
		(∼10 mg)/rhesus monkey
		(144 ± 50 ng)/humans
		0.60 ng ml^{-1} of chocolate milk
	SEB	(0.4 μg)/humans
		(0.9 μg)/rhesus monkey
	SEC1	(1.0 μg)/pigtail monkey
	SEG	∼80 μg/rhesus monkey
	SEI	>150 μg/rhesus monkey
	SET	0.05–1.0 ng ml^{-1} milk/humans
		0.1–1.0 μg/humans

resulted in the loss of serological reaction. When evaluating heat inactivation of SE, one should be aware that SE may lose serological activity in detection immunologic assay, but remain biologically active. Crude toxin preparations have been found to be more resistant than purified toxins.

Enterotoxin and enterotoxin-like proteins are globular, single polypeptides with molecular weights ranging from 22 to 29 kDa. They can be encoded in prophages, plasmids, or chromosomal pathogenicity islands. The *seb* gene is carried on the *S. aureus* pathogenicity island, SaPI3, 59 while enterotoxin C (SEC) exists in multiple variants, C1, C3, and Cbov, which are situated on SaPI4, SaPIn1/m1, and SaPIbov, respectively. The *sed* gene is situated on a pIB485 plasmid, likewise *sej*. The expression of *seb*, *sec*, and *sed* genes is induced during the transition from the exponential to the stationary phase, an expression pattern characteristic of proteins encoded by genes regulated by the Agr regulatory system. *Seb* and *sec* undergo a much stronger induction than the plasmid-encoded *sed*. The *sea* gene is carried by a polymorphic family of temperate bacteriophages, and the transcription of sea is linked to some extent to the life cycle of the SEA-encoding prophage. The polymorphic nature of the prophages has been found to affect the amount of SEA produced by the bacterial strain carrying the prophage. Sequence analysis of the *sea* gene and its neighboring genomic regions has further indicated that SEA-producing strains can be grouped into two major groups, SEA_1 and SEA_2. SEA is produced throughout the growth phases. The amount of SEA produced has been related to the group and to the expression level of *sea*. The *see* gene is situated on a (defective) prophage and its expression appears to be unaffected by bacterial growth. It seems that virulence expression of *se* genes is upregulated in iron-depleted environment and in the conditions of the host environment. In other words, *in vivo* expression seems to be higher than *in vitro* expression.

The regulation of production of SE is SE-dependent, as well as strain- and environment-dependent. Under the same conditions, different strains may produce different amounts of SE. Different SEs are synthesized by *S. aureus* in different growth phases. This reflects also in the amounts of different SEs produced by *S. aureus* growing under optimal conditions in laboratory media. For SEB and SEC, the amounts may exceed 100 μg ml^{-1}, compared with 1–10 μg ml^{-1} for SEA and SED. Some indications exist that low amounts of SEB are produced already in early exponential growth phase. Reports exist that show that SEB appears in cultures as early as 4–6 h. However, SEA and SED are produced in foods under a wider range of pH, Eh, and aw than are the other SEs, which explain why SEA and SED are principal toxins involved in staphylococcal food poisoning.

Based on large-scale outbreak data, the intoxication dose of SEs (SEA) can be as low as 94–184 ng, corresponding to about 0.3 ng SEA per ml of chocolate milk.

There are different ways to detect SE in foods, each with its own characteristics. Described assays include those based on animal testing; immunologic, molecular, and biological assays; biosensors; and lately also chromatography-based methods. There are several commercial kits/systems available for SE detection, which are in routine use. ELISA-based kits for enterotoxin detection are commercially available from various manufacturers like 3 M™ Tecra™ (Staph Enterotoxins Visual Immunoassay; screening of the presence of SEA–SEE using AOAC International official method 3 M™ Tecra™ SET VIA and/or specific identification of present SEA–SEE using 3 M™ Tecra™ SET ID VIA; both methods have limit of detection at about 1 ng ml^{-1} of sample extract according to the manufacturer), bioMérieux (VIDAS SET2, which is a reference method for the European screening method of the Committee Reference Laboratory for milk and milk products and is also recommended as the primary screening method for suspect food samples and culture isolates in FDA BAM Chapter 13A and an official method on a variety of foods by AOAC International), Diffchamb AB (Transia Plate SE official methods recommended by Ministere de l'Agriculture, France, Transia Tube SE), R-Biopharm (RIDASCREEN A, B, C, D, and E with sensitivity of below 0.031 ng ml^{-1} in buffer for SEA, SEB, SEC, and SEE and 0.062 for SED and performance equal or better than VIDAS SET2 in cheese), and Toxin Technology (SET-EIA). In the latest years, some very prominent research groups in the field are working on proteomics-based methods, mainly using different formats of LC–MS, to provide new sensitive, robust, and instrumental detection and quantification of SE. Since as little as 100–200 ng of the toxin can cause symptoms of staphylococcal intoxication, the methods are required to have very high sensitivity.

European Legislation

In its latest Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs, the European Commission provided two kinds of microbiological criteria, the process hygiene criteria and food safety criteria. The rationale behind is that the safety of foodstuffs is mainly ensured by a preventive approach, such as implementation of good hygiene practice and application of procedures based on hazard analysis and critical control point (HACCP) principles. Process hygiene criteria that can be used in validation and verification of HACCP procedures and other hygiene control measures were set to define the acceptability of the processes. The food safety microbiological criteria set a limit above which a foodstuff should be considered unacceptably contaminated with the microorganisms for which the criteria are set. The food safety criteria were not set for *S. aureus*, but for SEs. This

was done only for cheeses, milk powder, and whey powder, with safety criteria defined as follows: $n=5$, $c=0$, and $m=$absence in 25 g. The criteria are valid for these products placed on market during their shelf life. As for process hygiene criteria, CPSs were defined for cheeses made from raw milk ($n=5$, $c=2$, $m=10^4$ cfu g^{-1}, and $M=10^5$ cfu g^{-1}), cheeses made from milk that has undergone a lower heat treatment than pasteurization and ripened cheeses made from milk or whey that has undergone pasteurization or a stronger heat treatment ($n=5$, $c=2$, $m=100$ cfu g^{-1}, and $M=1000$ cfu g^{-1}), unripened soft cheeses (fresh cheeses) made from milk or whey that has undergone pasteurization or a stronger heat treatment ($n=5$, $c=2$, $m=100$ cfu g^{-1}, and $M=1000$ cfu g^{-1}), milk powder and whey powder ($n=5$, $c=2$, $m=10$ cfu g^{-1}, and $M=100$ cfu g^{-1}), and shelled and shucked products of cooked crustaceans and molluscan shellfish ($n=5$, $c=2$, $m=100$ cfu g^{-1}, and $M=1000$ cfu g^{-1}).

See also: *Bacillus cereus* and Other *Bacillus* sp. Causing Foodborne Poisonings, Detection of; *Clostridium*: Food Poisoning by *Clostridium perfringens*; *Clostridium*: Occurrence and Detection of *Clostridium botulinum* and Botulinum Neurotoxin; Cured Foods: Health Effects; Diarrheal Diseases; *Staphylococcus*: Detection; *Staphylococcus*: Occurrence and Properties; Toxins in Food: Naturally Occurring.

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Staphylococcus: Occurrence and Properties

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Introduction

Staphylococci are gram-positive spherical bacteria belonging to the Firmicutes phylum. They are mostly facultative anaerobic and catalase-positive bacteria that display a high salt tolerance. To date, the *Staphylococcus* genus comprises 48 species and 21 subspecies. It is divided into two distinct groups: the coagulase-positive staphylococci (CPS), such as *Staphylococcus aureus* and six other species, and the coagulase-negative staphylococci (CNS). Staphylococci are widespread in nature. They are commonly found on human and animal skin and on mucous membranes and are generally considered as commensal microbiota. They can be associated with opportunistic infections. Some species are recognized as human and animal pathogens, in particular *S. aureus*, which can express various virulence factors including enterotoxins that are implicated in food poisoning. The CNS are a vast and heterogeneous group. Some species, such as *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, and *Staphylococcus saprophyticus*, produce virulence factors and may be implicated in clinical cases and nosocomial infections. Nevertheless, CNS are not associated with pathogenic risk after food consumption and are considered mostly as food-associated beneficial microbiota. They can be commonly found in foods of animal origin, especially in fermented foods such as cheese and dry fermented sausage where they can be present at high levels (more than 10^6 CFU g^{-1}). Moreover, some species are exploited in the food industry and currently used as meat or dairy starter cultures, as is the case for selected strains of *Staphylococcus carnosus* and *Staphylococcus xylosus*. They contribute to enhance color and flavor formation and to limit the rancidity of meat products. They can also be used as bioprotective cultures and thus contribute to the control of spoilage and pathogenic bacteria. The object of this article is firstly to discuss safety risks associated with the presence of staphylococci in food and secondly to analyze the benefits of staphylococcal ecosystems in foods and of specific species with technological interest.

Risks Associated with Staphylococci in Food

Among all the staphylococcal species, only *S. aureus* belonging to the CPS is considered as a foodborne pathogen. Although CNS isolated from food are generally recognized as safe bacteria, they can nevertheless be sometimes associated with safety hazards such as the synthesis of toxins or biogenic amines and/or the presence of transmissible genes coding antibiotic resistances (Table 1).

S. aureus Involvement in Food Risks

Strains of *S. aureus* that produce enterotoxins can cause staphylococcal food poisoning (SFP) that is one of the leading causes of foodborne illness worldwide. The characteristic symptoms of SPF are severe nausea, projectile vomiting, abdominal cramps, and exhaustion between 2 and 8 h after consumption of contaminated food by preformed enterotoxins. The main reservoirs of *S. aureus* are humans and animals. More than 30% of the humans are carriers of *S. aureus* in their anterior nares and on their hands. *S. aureus* is also commonly found in the nostrils and on the skin of livestock, where it can, for instance, lead to mastitis in dairy cows. It can also colonize food contact surfaces and become a persistent organism due to its capacity to form biofilm. *S. aureus* can contaminate a variety of food products during preparation and processing and has the ability to grow and to produce enterotoxins in a wide range of temperatures, pH, water activities, and sodium chloride concentrations. *S. aureus* can be destroyed by suitable heat treatments commonly used in food processing, but the enterotoxins are extremely resistant to heat and can remain active. Most SFP are caused by unhygienic practices and inadequate refrigeration of foods, allowing the growth of *S. aureus* and production of enterotoxins.

Staphylococcal enterotoxins (SEs) are emetic toxins. They belong to the pyrogenic toxin family because of their superantigenic activities, structural relatedness, and sequence similarities. To date, 11 antigenic types of SE have been reported: SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SER, SES, and SET. The detection of genes encoding enterotoxin homologues has further expanded the list of the SE family. These related enterotoxins are designated as enterotoxin-like (SEl) to indicate that their emetic activity has not yet been demonstrated. So far, the majority of SFP involved the five enterotoxins SEA to SEE. Among them, SEA is the most commonly implicated enterotoxin. The role of other SE and SEl could be underestimated because of the difficulty of detecting them in foods. Moreover, the genes encoding SE and SEl are located on mobile genetic elements that can spread among *S. aureus* strains or other staphylococcal species and could contribute to the evolution of SFP.

In addition to the concerns caused by the production of enterotoxins, *S. aureus* can exhibit resistance to antibiotics often encoded on mobile genetic elements. The emergence of antibiotic-resistant *S. aureus* strains constitutes an additional risk due to the potential genetic transfer of these mobile elements including plasmids, transposons, pathogenicity islands, and the staphylococcal cassette chromosomes to bacteria present in foods or in the digestive tract.

CNS Involvement in Food Risks

No clinical cases have been reported to be associated with the consumption of foods contaminated by CNS. Nevertheless, contamination by *S. epidermidis* could be of importance for public health owing to its incidence in nosocomial infections. Moreover, some strains of CNS isolated from food and

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Table 1 List of staphylococcal species and subspecies and their implication in food risks

Species and subspecies	*Coagulase*	*Species group*	*Food risks*[a]
S. arlettae	−	*S. saprophyticus*	+/− (AR)
S. agnetis	−	*S. hyicus* and *S. intermedius*	
S. aureus	+	*S. aureus*	+ (SE), +/− (AR)
S. aureus subsp. *aureus*			
S. aureus subsp. *anaerobius*			
S. auricularis	−	*S. auricularis*	
S. capitis	−	*S. epidermidis*	+/− (AR, SE)
S. capitis subsp. *capitis*			
S. capitis subsp. *urealyticus*			
S. caprae	−	*S. epidermidis*	
S. carnosus[b]	−	*S. simulans*	+/− (BA, SE)
S. carnosus subsp. *carnosus*			
S. carnosus subsp. *utilis*			
S. chromogenes	−	*S. hyicus* and *S. intermedius*	
S. cohnii	−	*S. saprophyticus*	+/− (SE)
S. cohnii subsp. *cohnii*			
S. cohnii subsp. *urealyticus*			
S. condimenti	−	*S. simulans*	+/− (BA)
S. delphini	+	*S. hyicus* and *S. intermedius*	
S. devriesei	−	*S. haemolyticus*	
S. epidermidis	−	*S. epidermidis*	+/− (AR, BA, SE)
S. equorum[b]	−	*S. saprophyticus*	+/− (AR, BA, SE)
S. equorum subsp. *equorum*			
S. equorum subsp. *linens*			
S. felis	−	*S. hyicus* and *S. intermedius*	
S. fleurettii	−	*S. sciuri*	
S. gallinarum	−	*S. saprophyticus*	+/− (SE)
S. haemolyticus	−	*S. haemolyticus*	
S. hominis	−	*S. haemolyticus*	
S. hominis subsp. *hominis*			
S. hominis subsp. *novobiosepticus*			
S. hyicus	+	*S. hyicus* and *S. intermedius*	
S. intermedius	+	*S. hyicus* and *S. intermedius*	
S. kloosii	−	*S. saprophyticus*	
S. leei	−	*S. saprophyticus*	
S. lentus	−	*S. sciuri*	+/− (AR, SE)
S. lugdunensis	−	*S. lugdunensis*	
S. lutrae	+	*S. hyicus* and *S. intermedius*	
S. massiliensis	−	*S. saprophyticus*	
S. microti	−	*S. hyicus* and *S. intermedius*	
S. muscae	−	*S. hyicus* and *S. intermedius*	
S. nepalensis	−	*S. saprophyticus*	+/− (AR)
S. pasteuri	−	*S. warneri*	+/− (AR)
S. pettenkoferi	−	*S. saprophyticus*	
S. piscifermentans	−	*S. simulans*	+/− (BA, SE)
S. pseudintermedius	+	*S. hyicus* and *S. intermedius*	
S. pseudolugdunensis	−	*S. lugdunensis*	
S. pulvereri	−	*S. sciuri*	
S. rostri	−	*S. hyicus* and *S. intermedius*	
S. saccharolyticus	−	*S. epidermidis*	
S. saprophyticus	−	*S. saprophyticus*	+/− (AR, BA)
S. saprophyticus subsp. *bovis*			
S. saprophyticus subsp. *saprophyticus*			
S. schleiferi	+	*S. hyicus* and *S. intermedius*	
S. schleiferi subsp. *coagulans*	−		
S. schleiferi subsp. *schleiferi*			
S. sciuri	−	*S. sciuri*	
S. sciuri subsp. *carnaticus*			
S. sciuri subsp. *rodentium*			
S. sciuri subsp. *sciuri*			

(*Continued*)

Table 1 (Continued)

Species and subspecies	*Coagulase*	*Species group*	*Food risks*[a]
S. simiae	–	*S. aureus*	
S. simulans	–	*S. simulans*	+/– (BA, SE)
S. stepanovicii	–	*S. sciuri*	
S. succinus[b]	–	*S. saprophyticus*	
S. succinus subsp. *casei*			
S. succinus subsp. *succinus*			
S. vitulinus[b]	–	*S. sciuri*	
S. warneri	–	*S. warneri*	+/– (AR, BA)
S. xylosus[b]	–	*S. saprophyticus*	+/– (AR, BA, SE)

(+), established food risks; (+/–), potential food risks.

AR, potentially transferable antibiotic resistance gene; BA, biogenic amine production; SE, staphylococcal enterotoxin.

[a]Note that the food risks are strain-dependent.

[b]Strains of these species are commonly used as starter cultures or have been tested as potential starter cultures.

belonging in particular to *Staphylococcus capitis*, *S. carnosus*, *Staphylococcus cohnii*, *S. epidermidis*, *Staphylococcus equorum*, *Staphylococcus lentus*, *Staphylococcus gallinarum*, *Staphylococcus piscifermentans*, *Staphylococcus simulans*, and *S. xylosus* have been described as being able to produce enterotoxins, although no case of SFP involving these species has ever been reported. However, recent studies have revealed that the genes coding SE are not present in the majority of CNS strains isolated from food. The low sensitivity and specificity of the methods used for the detection of enterotoxins could explain the discordant results between the ability to produce these toxins and the lack of the corresponding genes. Despite such data, the potential risk due to enterotoxin production by CNS should not be ignored and should be monitored as the genes encoding enterotoxins in *S. aureus* are carried by mobile transferable elements that could be acquired by the CNS.

Like for *S. aureus*, another risk is the increase of antibiotic-resistant strains within CNS. In fermented foods, CNS can be present at high levels and may act as reservoirs of transferable antibiotic resistance genes. The prevalence of CNS resistant to antibiotics is high but varies widely between the different species. The most frequent resistances identified in foodborne CNS are those to ampicillin, erythromycin, penicillin, lincomycin, and tetracycline, and some strains have been described as resistant to multiple antibiotics. CNS can survive the passage through the gastrointestinal tract and exchange mobile genetic elements with gut bacteria. Therefore, it is crucial that the strains of CNS used as starter cultures do not carry transferable antibiotic resistance genes. With respect to the two species currently used as meat starter cultures, most strains of *S. xylosus* exhibit potentially transferable genes encoding resistance to tetracycline and/or erythromycin, whereas these genes are generally absent in the strains of *S. carnosus*.

Among the safety hazards associated with foodborne CNS, the potential production of biogenic amines should also be considered. Biogenic amines are natural compounds of many fermented food products. They are produced mainly by decarboxylation of precursor amino acids by enzymes of bacterial origin. It has been shown that some CNS can be associated with weak productions of biogenic amines such as tyramine, phenylethylamine, putrescine, and cadaverine. The production of biogenic amines is strain-dependent and was detected in some strains of *S. carnosus*, *S. epidermidis*, *S. xylosus*, and *Staphylococcus warneri* isolated from meat products.

Given these data, the selection of strains of *S. carnosus* and *S. xylosus* used as starter cultures should include safety criteria, such as the lack of production of enterotoxins and biogenic amines and of transferable antibiotic resistance genes.

Benefits of CNS in Food

CNS are often present in fermented foods as a part of the indigenous food microbiota. They originate from raw materials such as meat and milk or from the food environment, themselves contaminated by CNS as many staphylococcal species colonize skin and mucous membranes of livestock. Moreover, due to their salt-tolerant characteristics, they are frequently isolated from cured or salted foods. Among all CNS species, only few species are used as starter cultures. *S. xylosus* and *S. carnosus* have a GRAS (generally recognized as safe) status and are currently used as commercial starter cultures in meat fermentation. They have also some applications in dairy or fish fermentation. *S. equorum* not only has been recently introduced in the market as surface starter culture for cheese but also has been used in meat or fish fermentation processes. Some other species such as *Staphylococcus succinus* and *Staphylococcus vitulinus* have been tested as potential starter cultures in dairy or meat fermentation. However, a high species and infraspecies staphylococcal diversity can be found in particular in traditional fermented products. CNS are recognized to play an important role in the flavor of end products, particularly in meat and dairy products.

Biodiversity of CNS in Foods

The development of appropriate molecular methods has allowed reliable identification of the different species of staphylococci isolated from various foods and the characterization of strains within a species.

Meat products

CNS are one of the two main groups of bacteria involved in fermentation and ripening of cured meat products. CNS are recognized to play an important role in flavor and color of meat products such as low-acid fermented sausages. In traditional fermented sausages produced without the addition of starter cultures, many CNS species can cohabit. Species diversity obviously depends on the types of products and the manufacturing process. *S. equorum*, *S. saprophyticus*, and *S. xylosus* are commonly isolated from traditional fermented sausages and are considered as the three most prevalent species. A codominance of these three species has been reported in different types of Italian fermented sausages. *S. xylosus* has been reported as the predominant species in Spanish, German, and certain Italian fermented sausages, while *S. saprophyticus* has been described as the predominant one in some Greek, Argentinean, and Belgian fermented sausages. *S. equorum* was the dominant species in traditional French sausages and in some Spanish chorizo. However, a high diversity of species can be found in some fermented sausages with more than ten species identified in one product. As a nonexhaustive summary, the species *Staphylococcus arlettae*, *S. carnosus*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *Staphylococcus hominis*, *S. lentus*, *Staphylococcus pasteuri*, *Staphylococcus pulvereri*, *Staphylococcus sciuri*, *S. simulans*, *S. succinus*, *S. vitulinus*, and *S. warneri* have been reported in low-acid traditional fermented sausages.

In addition to the cohabitation of various species, infraspecies diversity can be revealed. The characterization by pulsed field gel electrophoresis (PFGE) analysis of isolates of the main species isolated from meat products, *S. equorum*, *S. saprophyticus*, and *S. xylosus*, has shown a genetic diversity within each species. In particular, high infraspecific diversity has been reported for *S. equorum* showing that different strains can survive throughout the manufacturing process and in the final products.

Dairy products

Many species of CNS can be found on surfaces of various types of cheeses such as hard, semihard, soft, and smear cheeses. *S. equorum*, *S. saprophyticus*, *S. simulans*, *S. succinus*, and *S. xylosus* are commonly isolated from cow and goat cheeses of various origins. In addition, many other species such as *S. arlettae*, *Staphylococcus auricularis*, *S. capitis*, *S. carnosus*, *Staphylococcus chromogenes*, *S. epidermidis*, *Staphylococcus fleurettii*, *S. gallinarum*, S. *haemolyticus*, *S. hominis*, *S. lentus*, *Staphylococcus rostri*, *S. sciuri*, *S. vitulinus*, and *S. warneri* can be found in milk and various French and Italian cheeses. This wide diversity of species identified in these products can be attributed to the large variety of cheeses, all manufactured with different processes.

Characterization by PFGE analysis of isolates of the main species isolated from French cheeses, that is, *S. equorum*, *S. xylosus*, and *S. saprophyticus*, has also revealed infraspecies diversity. The comparison of strains isolated from cheeses and dry fermented sausages has revealed that there was no clustering of the strains by type of food sample or geographic origin.

Fish products

CNS of the fish products are less documented as staphylococci do not belong to the usual fish microbiota. Some studies have reported the identification of *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus*, *S. simulans*, and *S. warneri* in salted cod fish. Moreover, the species *S. cohnii*, *S. epidermidis*, *S. lentus*, *Staphylococcus nepalensis*, *S. piscifermentans*, *S. saprophyticus*, *S. sciuri*, and *S. xylosus* have been found in fermented fish products. Staphylococci are currently isolated from fish sauce, a fermented fish product. In the process of fish paste fermentation, staphylococci become dominant among halophilic and halotolerant bacteria.

Functional Properties of CNS

CNS have a relevant role in the manufacture of fermented products of animal origin. The functional properties of CNS are particularly well characterized for fermented and cured meat products, where they contribute to the development and stability of the color through their nitrate reductase activity and to the development of flavor (Figure 1). Hence, the role of CNS is fundamental in the final sensorial characteristics of fermented meat products. As opposed to the more unpredictable traditional spontaneous fermentation processes, staphylococcal starter cultures are used in industrial meat fermentations to ensure repeatable and uniform sensory qualities and safety of the end products. They are inoculated at a rate of 10^6 viable germs/g of mixture. *S. carnosus* and *S. xylosus* are the two species commonly used as starter cultures but other species, such *S. equorum* or *S. succinus*, may also be excellent candidates.

Nitrate reduction

Nitrite and/or nitrate is added in meat curing for the development of the characteristic color and unique flavor of cured meat products, for the control of lipids oxidation, and for the inhibition of spoilage and pathogenic bacteria. Nitrite is a reactive compound that is chemically converted in nitric oxide (NO) in meat products. NO reacts with the iron atom of the heme group of myoglobin and metmyoglobin to form nitrosylmyoglobin, a rather stable bright red pigment responsible for the characteristic color (Figure 1). Nitrate is only effective if it is first reduced to nitrite. CNS naturally present in meat or originating from the starter culture play a fundamental role in this reduction. The nitrate reductase activity is widespread among CNS and has been reported in species such as *S. carnosus*, *S. equorum*, *S. lentus*, *S. pulvereri*, *S. saprophyticus*, *S. simulans*, and *S. xylosus*. The genetic determinants of nitrate reductase have been well characterized in *S. carnosus* and are conserved in other staphylococcal species such as *S. xylosus*.

Catalase and antioxidant activities

The formation of hydrogen peroxide due to the growth of lactic acid bacteria (LAB) in meat products can lead to discoloration of nitrosylmyoglobin and lipid oxidation, which can result in the development of rancid off-flavors. Peroxidation corresponds to the chemical reaction between fatty acids and the reactive forms of oxygen, such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). *S. xylosus* and *S. carnosus* are well equipped with genes encoding enzymes such as several catalases, one superoxide dismutase, and diverse peroxidases, contributing in synergy to limit the formation of oxygen reactive species (Figure 1). The catalase and superoxide dismutase activities have been notably studied in *S. xylosus*.

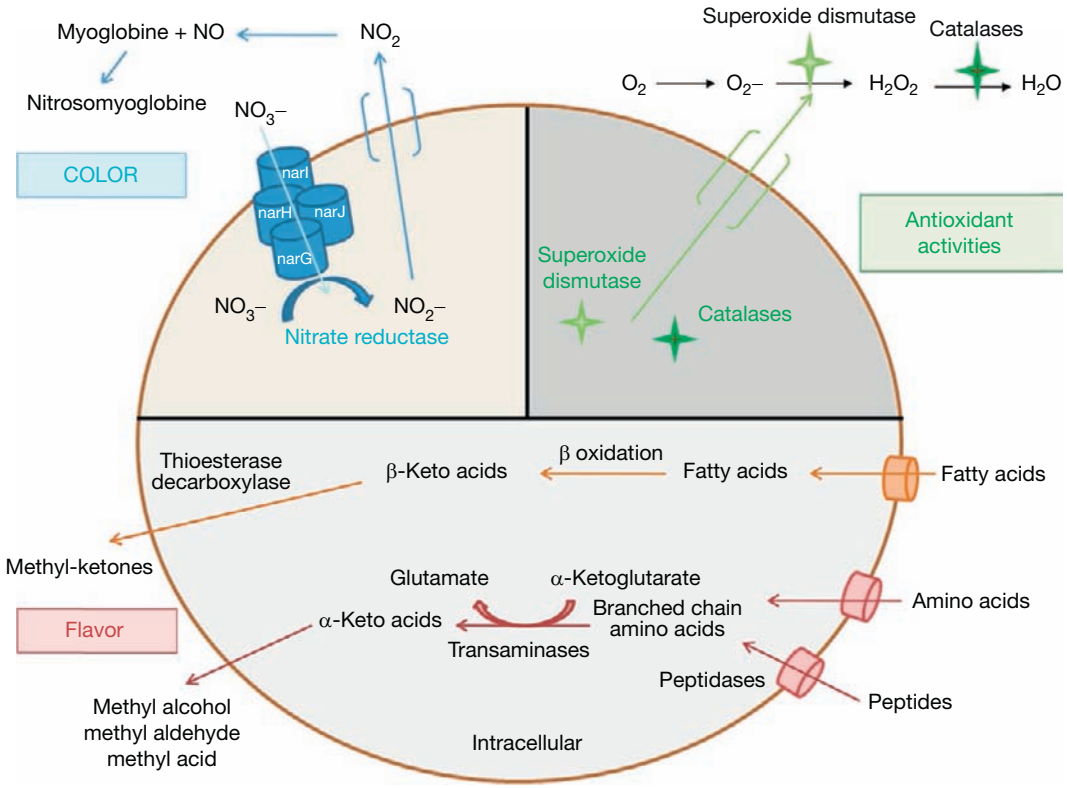

Figure 1 Main functional properties of coagulase-negative staphylococci (CNS) in meat product.

Flavor development

CNS contribute to flavor development through their amino and fatty acid catabolism, leading to the production of volatile compounds such as amino acid catabolites, pyruvate metabolites, and methyl ketones (Figure 1). Catabolism of amino acids is one of the key determinants of flavor development not only in fermented meat products but also in cheeses. The conversion of branched chain amino acids (leucine, isoleucine, and valine) or phenylalanine results in aldehydes, alcohols, and acids with aromatic qualities. The first step of the involved amino acid metabolisms is a transaminase reaction in which the amino group is transferred to an α-ketoacid. *S. carnosus* and *S. xylosus* have, for instance, been associated to the production of volatile compounds such as 3-methyl-1-butanol, 3-methylbutanal, and 3-methylbutanoic acid from leucine. These species thus contribute to the sensorial characteristics of low-acid fermented sausages typical of southern European countries. Moreover, the production of volatile compounds differs per type of CNS, for instance, depending on the use of strains of *S. carnosus* or *S. xylosus* as starter cultures. Fatty acid degradation also plays a role in flavor development. The incomplete β-oxidation of fatty acids leads to the production of methyl ketones. The β-oxidation process usually converts fatty acids into acetic acid by the successive removal of acetyl-CoA groups. However, intermediate CoA esters can be successively converted into β-ketoacids via thioesterase activity and then into methyl ketones via a decarboxylase reaction. In particular, it has been shown that *S. carnosus* has thioesterase and decarboxylase activities and is associated with the production of methyl ketones in fermented sausages.

Competitive growth

Most CNS are good competitors with endogenous bacteria of meat and dairy products due to their ability to grow anaerobically at low water activity, in high concentration of NaCl, and at low temperature. Strains of *S. xylosus* and *S. carnosus* that are used as starter or bioprotective cultures have been selected for their competiveness allowing them to dominate the indigenous microbiota present in the meat during the fermentation process and to survive for long periods in the final products. The use of staphylococcal starter or bioprotective cultures thus contributes to food safety and shelf life of cured meat products because they can prevent the growth of pathogenic and/or spoilage bacteria. However, CNS are poorly competitive in the presence of fast acidifying strains of LAB even if *S. xylosus* and *S. carnosus* starter cultures are also selected for their pH tolerance. Within these two species, the strains of *S. carnosus* are always more resistant to low pH and can survive rather well in these unfavorable conditions. Strains of *S. carnosus* and *S. xylosus* in combination with strains of LAB are currently commercialized as bioprotective cultures for application in meat products to control growth of spoilage and pathogenic bacteria such as indigenous LAB and *Listeria monocytogenes*.

Control of biogenic amines

Strains of *S. xylosus* and *S. carnosus* used as meat starter cultures must be selected based on their inability to produce biogenic amines. When applied, they can help to prevent the formation of biogenic amines by limiting the growth of potential biogenic amine producer bacteria. For instance, a *S. xylosus* starter culture was able to inhibit the formation of histamine and to a lesser extent of tyramine in a salted and fermented anchovy and the use of a strain of *S. carnosus* reduced the accumulation of histamine during fish sauce fermentation. Moreover, it has been shown that some strains of *S. xylosus* and *S. carnosus* can even reduce the level of tyramine, cadaverine, and/or putrescine in fermented sausages. The ability to reduce cadaverine in fermented sausages has also been shown for *S. equorum* strains.

Potential effect in gastrointestinal tract

CNS can be present at levels higher than 10^6 CFU g^{-1} in fermented foods such as fermented sausages or cheeses. These bacteria have the ability to resist to the food processes and to survive in the final products under different environmental conditions. The food matrix can support the survival of bacteria through the gastrointestinal tract. The potential impact of CNS on human health should be considered as some of them could survive gastric conditions and remain alive in the intestinal tract.

Conclusion

Staphylococci are widely spread and are present in various environments and in fermented foods. Among the 48 species belonging to the *Staphylococcus* genus, some species are associated with diseases in humans or animals and others are part of food microbiota and have technological interest, some strains even being used as starter cultures. The yin and yang of staphylococci underline the importance of the accurate identification and characterization of these bacteria at the species and strain levels.

See also: Biogenic Amines; Cheese: Processing and Sensory Properties; Cured Foods: Health Effects; Fermented Foods: Fermented Meat Products; Fermented Foods: Use of Starter Cultures; Sausages and Comminuted Products: Dry Fermented Products; *Staphylococcus*: Detection; *Staphylococcus*: Food Poisoning.

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Starch

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Starch Polymers

Starch consists of a mix of amylose and amylopectin. Starch is about 70% amylopectin by weight, though the amount varies depending on the source (higher in medium-grain rice to 100% in glutinous rice, waxy potato starch, and waxy corn and lower in long-grain rice, amylomaize, and several varieties of potato such as russet potato).

Amylopectin

Amylopectin is highly branched, being formed of 2000 to 2 million glucose units. Its inner chains are formed by 20–24 glucose subunits. Glucose (D-glucopyranose) units are linked in a linear way with α-(1→4) glycosidic bonds. Carbon 1 is the aldehyde containing carbon linked to the number 4 carbon in the next glucose. Branching takes place with α-(1→6) bonds occurring every 24–30 glucose units, resulting in a soluble molecule that can be quickly degraded.

Amylose

Amylose is made up of α-(1→4) joined glucose molecules. The number of repeated glucose units is usually in the range of 300–5000 but can be many more. Amylose contains very few α-(1→6) bonds. It reduces the degree of crystal structure of amylopectin and reduces the access of water (and enzyme). There are many more smaller amylose molecules in starch. Amylose can form either a single L-handed helix (and the chains can align) or a double L-handed helix that has extensive hydrogen bonding, which excludes water and is resistant to amylase.

Amylase

In humans, both the salivary and pancreatic amylases are α-amylases. The α-amylases (EC 3.2.1.1) (alternative names: 1,4-α-D-glucan glucanohydrolase and glycogenase) are completely unable to function in the absence of calcium. By acting at random locations along the starch chain, α-amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose (three glucoses) and maltose (two glucoses) from amylose or maltose, glucose, and dextrins (mixtures of polymers of D-glucose units linked by α-(1→4) or α-(1→6) bonds) from amylopectin.

Significance of the Amylose/Amylopectin Ratio in Human Health

The proportion of amylose in the starch is important for humans as it makes the starch less soluble and less easily penetrated by water and amylase. In addition with fewer branch points, there are fewer places for amylase to bind and hydrolyze so digestion is slower. Starch that cannot be digested at all is called resistant starch and may have implications for colonic health and this will be discussed separately. Starch that has been cooked and cooled (particularly potato starch) can become resistant to amylase by virtue of its crystalline structure. The rate at which starch is digested and glucose is absorbed has long-term health implications as rapid absorption and high glucose peaks appear to be associated with chronic diseases. A measure of how rapidly glucose is absorbed is called the glycemic index (GI) that calculates the area of the glucose curve above baseline over 2 h (Figure 1) compared with a standard material (usually glucose but can be white bread). A 50 g bioavailable dose is used (or 25 g if the food has a low carbohydrate amount and the standard is given a value of 100). The method was first developed by David Jenkins in Canada.

The amylose/amylopectin ratio is not the only determinant of GI. How finely milled the food is determines the degree of access of water and amylase to the starch granule so refined starches such as in white bread have easily accessible granules and a high GI, while coarsely ground breads such as pumpernickel have a low GI. In addition, the presence of fat and protein in the food will slow gastric emptying and lower the GI even when white refined starch is used. Sucrose by comprising 50% glucose intrinsically has a low GI so foods with a high amount of sugar combined with refined starch may not have high GI. Thus, in terms of using GI for health promotion, the amount of sugar and fat in the food needs to be limited to make the low-GI symbol reflect the health attributes of the food. Glycemic load is defined as the amount of carbohydrate consumed multiplied by the GI of each of the component foods and this has also been related to disease outcomes.

GI and Health

Heart attack and stroke

Several meta-analyses have looked at the association between GI/GL and heart disease. The most recent one from David Jenkins' group examined ten prospective cohorts. Pooled analyses showed a nonsignificant increase in coronary heart disease (CHD) risk for the highest glycemic load (GI) quantile compared with the lowest, with RR = 1.11 (95% confidence interval (CI) 0.99–1.24) and a significant association for GL, RR = 1.27 (95% CI 1.09–1.49), both with evidence of some heterogeneity. Female cohorts showed significance for GI (RR = 1.26, 95% CI 1.12–1.41) and for GL (RR = 1.55, 95% CI 1.18–2.03), whereas there was no effect in men at all. A more extensive meta-analysis using 15 prospective studies by Fan et al. produced a similar result with a slightly higher RR in women (RR = 1.49, 95% CI 1.27–1.73). Among overweight and obese subjects, dietary glycemic load level was associated with increased risk of CHD (RR = 1.49, 95% CI 1.27–1.76; P for interaction = 0.003). Higher dietary GL, but not GI, was positively associated with stroke (RR = 1.19, 95% CI

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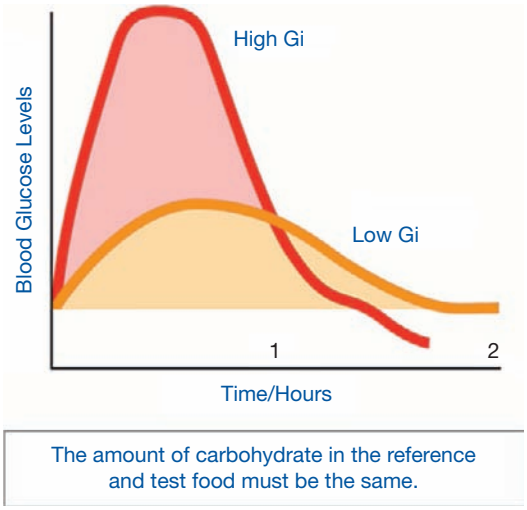

Figure 1 GI index from GI Foundation (http://www.gisymbol.com/).

1.00–1.43). There was a linear dose-response relationship between dietary GL and increased risk of CHD, with pooled RR of 1.05 (95% CI 1.02–1.08) per 50-unit increment in GL level. An earlier meta-analysis of 37 prospective studies showed an overall weak association between GI and CHD (GI RR = 1.25, 95% CI: 1.00, 1.56), but gender was not examined.

Type 2 diabetes

An updated meta-analysis from the Harvard group of their three pooled cohorts showed those in the highest quintile of energy-adjusted GI had a 33% higher risk (95% CI: 26%, 41%) of type 2 diabetes (T2D) than those in the lowest quintile. Participants in the highest quintile of energy-adjusted GL had a 10% higher risk (95% CI: 2%, 18%) of T2D. Participants who consumed a combination diet that was high in GI or GL and low in cereal fiber had an ~50% higher risk of T2D. In the updated meta-analysis including other cohort studies, the summary RRs (95% CIs) comparing the highest with the lowest categories of GI and GL were 1.19 (1.14, 1.24) and 1.13 (1.08, 1.17), respectively. Another meta-analysis of 21 cohorts showed the summary RR was 1.08 per 5 GI units (95% CI 1.02–1.15; $P = 0.01$) and 1.03 per 20 GL units (95% CI 1.00–1.05; $P = 0.02$). In an earlier meta-analysis, the GL was positively associated with RR of T2D of 1.45 (95% CI: 1.31, 1.61) for a 100 g increment in GL ($P < 0.001$; $n = 24$ studies) that is much larger than the estimates in later studies.

Resistant Starch and Health

Although resistant starch contributes to fecal bulking, the evidence for its direct association with disease prevention is lacking; however, total starch intakes are inversely related to the incidence of colorectal cancer, and these cross-country studies are heavily confounded by other lifestyle attributes. Resistant starch increases colonic butyrate that is believed to influence colonic tumor growth by inhibiting histone deacetylase. A randomized trial in young people with familial adenomatous polyps showed no effect of 30 g day^{-1} of resistant starch in polyp growth over 1–12 years.

Whole Grains and Health

There is a very large epidemiological literature relating whole grains (all the components of the grain in the final product regardless of milled particle size) to cardiovascular disease (CVD) and T2D. The most recent meta-analysis showed from seven prospective cohorts that 2.5 servings of whole grain versus 0.21 servings of whole grain were associated with a 21% reduction in CVD events. Intake of refined grain was not significantly associated with CVD events. A whole grain intake of three servings per day was associated with a 32% reduction in T2D in ten cohort studies.

Major Starch Crops

In 2010, world production of cereals was 844 million tons for maize, much for animal production; 672 million tons for rice; and 651 million tons for wheat. Wheat is the leading source of vegetable protein in human food, having a higher protein content than other major cereals, maize (corn) or rice. In terms of total production tonnages used for food, it is currently second to rice as the main human food crop.

In this article, we will not discuss the use of starch for alcohol production or for industrial conversion to glucose and fructose for human consumption nor for use as an animal feed.

We will discuss the various food sources of starch in terms of nutritional value, particularly amount and quality of protein and micronutrients. Most starch crops contain polyphenolic compounds as protection against insects and diseases, but I will not discuss the health implications of the phenolics other than to say that flavonoid intake has been related to protection from CVD. These will be well covered elsewhere in this book. All data on nutritional content are taken from the USDA and crop size data are taken from the Food and Agriculture Organization (FAO).

Rice

Rice is consumed by more than 7 billion people worldwide with an Asian consumption of 103 kg per head with a global consumption of 65 kg per head. Virtually all of this rice is refined polished white rice.

One cup of cooked white rice (210 g) contains 242 cal, 4.4 g of protein, 53.2 g of carbohydrate and 0.4 g of fat, and 0.6 g of fiber, while one cup of brown rice contains 218 cal, 4.5 g of protein, 45.8 g of carbohydrate and 1.6 g of fat, and 3.5 g of fiber. The bran and germ contain much of the fiber in brown rice.

Health Effects of White Rice

When consumed at subsistence levels, white rice is not likely to be associated with adverse outcomes other than nutritional deficiencies. However, as populations become overweight and obese, it is possible that the highly available starch in white rice might contribute to chronic diseases. In an epidemiological study from Shanghai in 117 000 men and women in

whom 70% of total carbohydrates came from white rice and 17% from refined wheat products, the risk of heart disease was nearly three times higher in the highest quartile of intake of carbohydrate after full adjustment ($P < 0.001$). Carbohydrate intake accounted 68% of energy intake. Rice also accounted for the risk of T2D in middle-aged Chinese women by 80% when comparing top to bottom quintiles. Both GI (20% increase) and glycemic load (34% increase) were important. A meta-analysis examined the relationship between rice intake and T2D in seven prospective cohorts. The risk was 55% higher in the highest category of intake in Asian populations but was only 12% higher and not significant in Western populations. The average intake was three to four servings per day in Asian populations and one to two servings per week in Western population. A study in Singapore (the Singapore Chinese Health Study) showed no relationship between total carbohydrate intake and ischemic heart disease mortality in 53 469 participants. However, total starch was weakly related in both men and women with a 3–8% increase in the highest quantile, while fiber intake was protective with a 6–29% reduction in risk with the effect being much stronger in women. Replacement of one serving of rice per day with vegetables, fruit, and whole wheat bread was associated with a 24%, 12%, and 19% lower risk of death, respectively. In contrast, in Japan, rice intake was not associated with heart disease and stroke incidence or total CVD deaths in a cohort of 91 000 men and women aged 40–69 years. In a different Japanese cohort, rice intake was inversely correlated with cardiovascular deaths in men (17–30% reduction in the highest intake) but not in women. It is not clear why there are differences between Chinese and Japanese, but the Chinese body weight increases have been greater than those of the Japanese.

Cancer

Rice takes up inorganic arsenic from soil and water and cooking water and arsenic is a known carcinogen, so in certain high arsenic areas (such as West Bengal and Bangladesh), rice intake may be associated with cancer. It is estimated in China that inorganic arsenic adds 100 cases per 100 000 when the median population cancer incidence is 177 per 100 000.

Eating brown rice once a week cuts the risk of colorectal cancer by 40% in the Adventist Health Study 2. A Japanese study (the Japan Public Health Center-based Prospective Study) found no association of rice or noodles or bread with colorectal cancer risk.

Prostate cancer in Jamaican men is related to a high-GI diet (rice, pasta, sugar sweetened beverages, and sweet baked foods) with a two- to threefold increase comparing tertiles of intake. Similar observations were made in Swedish men with the high-risk foods being low fiber biscuits, cakes, rice, and pasta. Renal cell carcinoma in Italians is associated with high-GI foods bread (94% increase) and pasta and rice (29% increase) comparing quintiles although inverse associations were shown for esophageal cancer (30% reduction) for pasta and rice.

Wheat

Raw wheat can be ground into white flour or, using hard durum wheat only, can be ground into a coarse yellow semolina that is used to make pasta, breakfast cereals, puddings, and couscous. Wheat can be germinated, which produces glucose, maltose (disaccharide) and maltotriose, and maltodextrins (short chains with side chains from amylopectin) from amylase secreted from the aleurone layer, and dried creating malt that is used for drinks, biscuits, and breads; crushed or cut into cracked wheat; and parboiled (or steamed), dried, crushed, and debranned into bulgur (groats). Wheat is a major ingredient in major Western foods as bread, biscuits, pies, cakes, cookies, and breakfast cereals. If the bran (43% fiber) and germ (14% fiber) are retained with the flour from the endosperm, then whole wheat flour is produced.

One hundred grams of whole grain hard red winter wheat contains about 12.6 g of protein, while the same weight of hard red spring wheat contains about 15.4 g of protein.

Much of the carbohydrate fraction of wheat is starch but the protein (gluten) is also important economically. In wheat, phenolic compounds are mainly found in the form of insoluble-bound ferulic acid.

Most of the associations in Western countries of high GI and load and chronic disease are due to refined wheat flour that has only 20% of the fiber level of whole grain wheat. Bread made with 100% whole wheat flour has a GI of about 50, while bread made with white wheat flour has a GI of about 70. The vitamin levels are much the same in both flour types as white flour is fortified. The particle size of whole grain wheat is up to twice that of white refined flour, and this fact plus the fiber that slows gastric emptying accounts for the difference in GI.

Maize (*Zea mays*)

Maize and cornmeal (ground dried maize) constitute a staple food in many regions of the world. Cornstarch (maize flour) is a major ingredient in home cooking and in many industrialized food products.

Virtually, every dish in Mexican cuisine uses maize. Maize is Africa's most important staple food crop. Maize meal is made into a thick porridge in many cultures: for example, from the polenta of Italy to the mealie pap in South Africa. Maize meal is also used as a replacement for wheat flour, to make cornbread and other baked products. In the West, popcorn and cornflakes are the major use of maize. Sweet corn, a genetic variety that is high in sugars and low in starch, is usually consumed in the unripe state. The cooked, unripe kernels may also be shaved off the cob and served as a vegetable in side dishes.

Normal maize starch can be waxy (100% amylopectin) to containing 20% amylase. Special high amylase varieties are available with 50–70% amylase that are usually, but not necessarily, more resistant to digestion, have lower glycemic profile, and offer fecal bulking.

Potato

Potato is a starchy, tuberous crop from the perennial nightshade *Solanum tuberosum* L. It is the world's fourth-largest food crop, following maize, wheat, and rice.

The average annual consumption is about 33 kg of potato. It is an important crop in Europe but increasingly in China and India.

The United Nations FAO reports that the world production of potatoes in 2010 was about 324 million tons.

Potatoes and Health

They contain about 26 g of starch for a medium potato. They are a valuable source of nutrients such as vitamin C, potassium, and fiber. They are relatively low in energy when eaten boiled and unpeeled (276 kJ/100) and low in sodium. However, energy value increases dramatically when potatoes are fried to 800 kJ/100 for homemade chips and 2160 kJ/100 for potato crisps. Salt is often added to potato crisps increasing the sodium content to 620 mg/100 g. Increasing globalization of the food supply and the introduction of fast-food chains mean that potatoes especially eaten as chips are included in the diet of traditionally rice-eating societies such as China and India.

Data from the Framingham Heart Study Offspring Cohort show that over 17 years of follow-up, the percentage of energy from total carbohydrates decreased with the number of weekly servings of carbohydrate foods including potato chips decreasing during that time. Data from the US National Health and Nutrition Examination Survey (NHANES) 2009–10 show that mean total energy intake for the population was 2080 kcal day^{-1}, with white potatoes and French-fried potatoes providing ~4% and ~2% of total energy, respectively. In consumers of white potatoes, they provided ~23% of dietary fiber and ~20% of potassium but only ~11% of total energy in the diet.

The GI of potatoes varies according to type and method of preparation. Boiled white potatoes have a GI of 82 while potato chips have a GI of 51. A new potato cultivar, Carisma with a GI of 53, has recently become available. In a study of the NHANES data, total carbohydrate intakes were not associated with HbA1c, plasma glucose, or serum insulin concentrations. However, diets higher in carbohydrate from potato have been associated with higher levels of HbA1c in people with type 1 diabetes.

Potatoes are included as a separate food group in some but not all epidemiological studies of diet and disease. In a study by Mozaffarian, weight change over 4 years was strongly associated with the intake of potato chips (0.8 kg) and potatoes (0.6 kg). Central obesity was associated with a higher frequency of eating boiled potatoes in a study by Mostad. The authors suggest that a higher frequency of potato consumption that occurred in the presence of lower frequency of vegetable intake might indicate a higher energy density of the main (dinner) meal. In contrast in a study in Chinese participants, a dietary pattern rich in soybeans, grains, potatoes, and flour was associated with reduced triglycerides, fasting glucose, waist circumference, and waist–hip ratio. An increased risk of prostate cancer has been reported in a small study of patients with prostate cancer whose diet was defined as a 'Western diet' and was high in sweets and desserts, organ meat, snacks, tea and coffee, French fries, salt, carbonated drinks, and red or processed meat.

In a prospective cohort study in the Netherlands, a Nutrient-Rich Food (NRF) Index was used to examine associations between the index an incidence of CVD events and all-cause mortality. Food groups that contributed most to the NRF index score were vegetables, milk and milk products, fruit, bread, and potatoes. A high NRF score was inversely associated with all-cause mortality but the score was not associated with incidence of CVD.

Potatoes contain resistant starch that varies depending on the variety and cooking method. Normal cooked potato starch contains about 7% resistant starch that doubles on cooling.

In the United Kingdom, potatoes are not considered by the NHS as counting toward the five portions of fruit and vegetables diet. Chlorogenic acid constitutes up to 90% of the potato tuber natural phenols but its health effects are not clear.

Cassava

Cassava (*Manihot esculenta*), also called manioc or tapioca root, is cultivated as an annual crop in tropical and subtropical regions for its edible starchy, tuberous root. Cassava, when dried to a powdery extract, is called tapioca.

Cassava is the third largest source of food carbohydrates in the tropics, after rice and maize. Cassava is a major staple food in the developing world, providing a basic diet for over half a billion people. It is one of the most drought-tolerant crops, capable of growing on marginal soils.

Like other roots and tubers, both bitter and sweet varieties of cassava contain antinutritional factors and toxins. It must be properly prepared before consumption. Improper preparation of cassava can leave enough residual cyanide to cause acute cyanide intoxication and goiters and may even cause ataxia or partial paralysis.

World production of cassava root was estimated to be 184 million tons in 2002, rising to 230 million tons in 2008.

Cassava is a high-GI crop, but in Congo, consumption of cassava is not related to body mass index (BMI) or insulin sensitivity and β-cell function in people with type 2 diabetes mellitus (DM). Diabetes does not appear to be related to cyanide toxicity from cassava consumption.

Soybean

The soybean or soya bean (*Glycine max*) is a species of legume native to East Asia, widely grown for its edible bean that has numerous uses. The plant is classed as an oilseed rather than a pulse by the UN FAO even though it contains 11% carbohydrate and 13% protein versus 6.8% fat.

Sweet Potato

The sweet potato (*Ipomoea batatas*) has large, starchy, and sweet-tasting tubers. The sweet potato is only distantly related to the potato (*Solanum tuberosum*) and is botanically very distinct from a genuine yam (*Dioscorea*), which is native to Africa and Asia.

Besides simple starches, raw sweet potatoes are rich in dietary fiber and have moderate contents of other micronutrients, including vitamin C and potassium. It is ranked highest in nutritional value per kJ.

Sweet potato varieties with dark orange flesh have more β-carotene than those with light-colored flesh. A study of 10 000 households in Uganda found that 50% of children who ate normal sweet potatoes suffered from vitamin A

deficiency compared with only 10% of those on the high β-carotene variety.

Crop size: World production in 2004 was 127 million tons. Most comes from China and half of this is fed to livestock.

Sorghum

Sorghum bicolor, a grain-bearing grass, is widely cultivated in tropical and subtropical regions and is important in arid regions. In many parts of Asia and Africa, its grains are used to make flatbreads and the grains can also be popped in a similar fashion to popcorn. In India, sorghum is one of the staple sources of nutrition. It is used to make such foods as couscous, sorghum flour, porridge, and molasses.

Crop size: The world harvested 55.6 million tons of sorghum in 2010.

Plantains or Bananas (Musa)

There is no botanical difference between the two and plantains are typically eaten cooked and are usually large, angular, and starchy, in contrast to dessert bananas, which are typically eaten raw and are usually smaller, more rounded, and sugary. *Musa* are indigenous to the tropical regions of Southeast Asia and Oceania.

Plantains are a major food staple in West and Central Africa, Central America, the Caribbean islands, and northern, coastal parts of South America.

Plantain is a carbohydrate source. Its utilizable protein content as percentage of calorie ingestion is higher than sago and cassava, but is much lower than other staples such as maize, rice, and wheat. On per gram consumed basis, plantain's essential amino acid concentrations are very low, even lower than cassava. Cooked green plantain (and cooked green banana) has a low GI, unlike potatoes and many grains. Plantain contains very little β-carotene. The vitamin C content of plantain is very similar to those of sweet potato, cassava, and potato.

In Ghana, a traditional dietary pattern containing fruits, plantains, green leafy vegetables, fish, fermented maize products, and palm oil was associated with T2D with a 54% increase per SD.

Crop size: World production of bananas was 145 M tons.

Yam

Yam is the common name for some plant species in the genus *Dioscorea* (family Dioscoreaceae) that form edible tubers and are cultivated in Africa, Asia, Latin America, the Caribbean, and Oceania.

The protein content and quality of roots and tubers are lower than other food staples. Of all roots and tubers, the protein content of yam and potato is the highest, being ~2% on a fresh weight basis. Yams, with cassava, provide a much greater proportion of the protein intake in Africa, ranging from 6% in East and southern Africa to about 16% in humid West Africa.

Yam, like other root crops, is not a good source of essential amino acids. It is rich in phenylalanine and threonine but limiting in the sulfur amino acids, cysteine, and methionine and in tryptophan. Yam-consuming areas of Africa have a high incidence of kwashiorkor. Except for potassium, vitamin B_6, and vitamin C, yam is a food with moderate nutrient density. Yam generally has a lower GI compared to potato products.

In 2010, the world harvested 48.7 million tons of yam, 95% of which was produced in Africa.

Barley

Barley (*Hordeum vulgare* L.) is a major cereal grain. Barley grain is a staple in Tibetan cuisine and is widely used as whole grain barley meal in the Middle East in soups and stews, porridges, and gruels and in barley bread and biscuits. It is also eaten in Eastern and Central Europe and Africa. Barley grains are commonly made into malt for alcoholic and nonalcoholic beverages. Pearled barley has been steam-treated to remove the bran but still contains the bran.

Barley contains eight of the nine essential amino acids. Whole grain barley eaten at the evening meal can reduce the glycemic response to a standard breakfast compared to white bread or spaghetti and spaghetti plus bran. Fermentation of indigestible carbohydrates was greater with barley with higher breath hydrogen and blood propionate.

Like wheat and rye, barley contains gluten, which makes it an unsuitable grain for consumption by those with celiac disease that is a cell-mediated immunity to gluten that damages the intestinal mucosa and can cause malabsorption.

In a 2007 ranking of cereal crops in the world, barley was fourth in terms of quantity produced (136 million tons).

Oat

The common oat (*Avena sativa*) is a cereal grain. Oats are rolled or crushed into oatmeal or ground into fine oat flour. Oatmeal is chiefly eaten as porridge but may also be used oatcakes, oatmeal cookies, and oat bread and muesli and granola.

Oat β-glucan is a soluble fiber with good cholesterol-lowering properties with at least 3 g day^{-1} reducing low density lipoprotein (LDL) cholesterol by 5–10%. The US Food and Drug Administration (FDA) approved a health claim for β-glucan soluble fiber from oats for reducing plasma cholesterol levels and risk of heart disease in 1997. Similarly, in 2004, the UK Joint Health Claims Initiative (JHCI) allowed a cholesterol-lowering health claim for oat β-glucan for hypercholesterolemic subjects. Reducing the molecular weight of oat β-glucan to 210 000 from 500 000 reduces its cholesterol-lowering ability by 50%.

20 M metric tons is produced each year.

Rye

Rye (*Secale cereale*) is a member of the wheat tribe (Triticeae) and is closely related to barley (*Hordeum*) and wheat (*Triticum*). Rye grain is used for flour, rye bread, rye beer, and crispbread. It can also be eaten whole, as either boiled rye berries or rolled, similar to rolled oats.

Rye bread, including pumpernickel, is a widely eaten food in Northern and Eastern Europe. Rye is also used to make

crispbread. Rye flour is high in gliadin but low in glutenin and has a lower gluten content than wheat flour and contains a higher proportion of soluble fiber.

13.3 M metric tons is produced each year.

Dietary Pulses (Beans, Chickpeas, Lentils, and Peas)

FAO recognizes 11 kinds of pulses: 9 dry beans, 3 dry broad beans, 2 dry peas, and a variety of peas, vetches, lupins plus chickpeas, and lentils.

Pulses are 20–25% protein by weight, which is double the protein content of wheat and three times that of rice. While pulses are generally high in protein, and the digestibility of that protein is also high, they are often relatively poor in methionine, an essential amino acid. Grains are usually deficient in lysine so the two food groups complement each other.

A recent meta-analysis of 26 pulse interventions found that at a median intake of 130 g/LDL, cholesterol was lowered by 0.17 mmol l^{-1}. A meta-analysis of eight trials showed that pulses, exchanged isocalorically for other foods, significantly lowered systolic (MD = −2.25 mmHg (95% CI, −4.22 to −0.28) $P = 0.03$) and mean arterial BP (MD = −0.75 mmHg (95% CI, −1.44 to −0.06), $P = 0.03$).

Chickpeas

Dried chickpeas have 61% carbohydrate (11% sugar and 17% fiber), 6% fat, and 19% protein by weight.

Mature chickpeas can be cooked (or eaten raw) and eaten cold in salads, cooked in stews, ground into a flour (*besan* in India), ground and shaped in balls and fried as falafel, stirred into a batter, and baked to make farinata or paneled or a flatbread.

Chickpeas are widely used in the Mediterranean and the Middle East and Burma. Hummus is the Arabic word for chickpea that is often cooked and ground into a paste and mixed with tahini (sesame seed paste). In India, chickpeas are used in vegetarian curries.

11 M metric ton per year is grown.

Lentils

With about 30% of their calories from protein, lentils have the third-highest level of protein, after soybeans and hemp. Proteins include the essential amino acids isoleucine and lysine, and lentils are important in Asia and India. Lentils are deficient in two essential amino acids, methionine and cysteine.

Lentils are high in amylose, and thus, low-GI red lentils have a GI of 21 and green lentils 22–37. Canned lentils have a higher GI of 42–52. Lentils also have some antinutritional factors, such as trypsin inhibitors and relatively high phytate content. Lentils are a good source of iron, having over half of a person's daily iron allowance in a one cup serving.

The FAO reported that the world production of lentils for calendar year 2009 was 3.917 M metric tons.

See also: Barley; Biscuits, Cookies and Crackers: Nature of the Products; Bread: Breadmaking Processes; Bread: Chemistry of Baking; Carbohydrate: Digestion, Absorption and Metabolism; Cereals: Dietary Importance; Cereals: Storage; Cereals: Types and Composition; Chapatis and Related Products; Legumes in the Diet; Maize; Oats; Pasta: Manufacture and Composition; Pasta: Role in Diet; Rhum–Ron–Rum: Technology and Tradition; Rice: Role in Diet; Rice: Types and Composition; Risk Assessment of Foods and Chemicals in Foods; Soy Beans: Dietary Importance; Soy Beans: Processing; Soy Beans: Properties and Analysis; Soy Beans: The Crop; Starch: Modified Starches; Starch: Sources and Processing; Starch: Structure, Property, and Determination; Wheat: Grain Structure of Wheat and Wheat-based Products; Wheat: The Crop; Whey and Whey Powders: Fermentation of Whey; Whey and Whey Powders, Principles and Applications of Dialysis; Whey and Whey Powders: Production and Uses; Whey and Whey Powders: Protein Concentrates and Fractions; Whisky, Whiskey and Bourbon: Composition and Analysis of Whisky; Whisky, Whiskey, and Bourbon: Products and Manufacture; Wines: Champagne and Sparkling Wines – Production and Effervescence; Wines: Madeira, Port and Sherry Fortified Wines – The *Sui Generis* and Notable Peculiarities. Major Differences and Chemical Patterns; Wines: Types of Table Wines; Wines: Wine and Health; Wines: Wine Production; Wines: Wine Tasting.

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Relevant Websites

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http://en.wikipedia.org/wiki/Starch – Wikipedia-Starch.

http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancorRetrieved – 'FAOSTAT' – Food and Agricultural Organisation statistics.

http://www.gisymbol.com/ – GI index.

http://www1.lsbu.ac.uk/water/hysta.html – London South Bank University.

http://polysac3db.cermav.cnrs.fr/discover_starch.html – Database of polysaccharide structures.

Starch: Modified Starches

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Introduction

Starch contributes greatly to the textural properties of many foods and is widely used in many industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent, and water retention agent. However, its inability to withstand processing conditions (low shear resistance, thermal resistance, and thermal decomposition) and low storage stability (high tendency toward retrogradation) limits its use in some industrial food applications. Starch modification, which involves the alteration of the physical and chemical characteristics of native starch to improve its functional and nutritional characteristics, can be used to tailor starch to specific food applications. Starch modification is generally achieved through derivatization such as etherification, esterification, cross-linking, and grafting of starch; decomposition (acid or enzymatic hydrolysis and oxidization of starch); or physical treatment of starch using heat or moisture (Table 1). The properties of some modified starches and their applications are presented in Table 2. The effects of modifications on thermal, morphological, and pasting/rheological behavior of starches may be quantified using instrumentation such as differential scanning calorimetry, scanning electron microscopy (SEM), Visco-Amylo-Graph/Rapid Visco Analyser, and dynamic rheometer, respectively.

Common Types of Modified Starches

Physically Modified Starches

Physical modification of starch is the preferred form of modification as it does not involve any chemical treatment. Drum drying, extrusion cooking, spray drying, radiation, sonication, annealing (ANN), and heat–moisture treatment (HMT), as well as high hydrostatic pressure (HHP) treatment, are the most commonly employed methods for the physical modification of starch.

Pregelatinization

Pregelatinized starches are the 'instant' starches that provide a desirable texture when dispersed in water at temperatures below the gelatinization temperatures of the parent starch. These starches find applications in convenience foods for a palatable texture and for holding other food components in a uniform suspension. Most of the pregelatinized starches are made by applying starch slurry to a hot drum followed by grinding the thin sheet to a fine powder. The native starch granule structure is completely lost during this process, and no intact granules are generally present in the final product. The limitation of drum-cooked unmodified starch is that upon rehydration, it gives a paste of reduced consistency with a dull, grainy appearance and gels of reduced strength, which is the reason that sometimes chemically modified starches are used as a raw material for drum drying.

Cold water-dispersible starches

Granulated cold water-dispersible or cold water-swellable starches give greater viscosity and smoother texture and have more processing tolerance than traditional pregelatinized starches. The methods used to prepare these starches include heating of starches in aqueous alcohol (sufficient water for gelatinization and sufficient alcohol to maintain granule integrity), high-temperature and high-pressure treatments, and alcoholic-alkaline treatment. The modified starches prepared by these methods differ in their cold water solubility/swellability. These types of starches swell rapidly when added to cold water and are commonly used in cold water-dispersible foods, such as infant foods, ice creams, and dry baking premixes.

Alcoholic-alkaline treatment

Alcoholic-alkaline treatment involves treatment of starch with a mixture of sufficient ethanol and sodium hydroxide to swell starch granules, followed by neutralization with hydrochloric acid, washing with alcohol, and finally drying. The starch granules retain their granular form (depending on starch type and extent of modification, Figure 1) but lose their crystallinity during the process. Solubility has been reported to range between 11.7% and 93.3% for waxy and high-amylose maize starches prepared by different alcoholic-alkaline treatments. However, the effectiveness of the method depends mainly on the starch cultivar, concentration of ethanol and sodium hydroxide, and reaction temperature.

Hydrothermal and pressure treatments

Hydrothermal treatments used for starch modification include extrusion cooking, sonication, and radiation. The modification conditions such as water content, pressure, temperature, and shear forces vary considerably between the different processes. Starch is partially gelatinized during these processes, which results in decreased hydrogen bonding. Extrusion cooking is a popular method of starch modification because of its low cost and continuous mode of processing. The extrusion-modified starch exhibits lower hot paste viscosity and higher water solubility.

HHP treatment offers starch gelatinization at room temperature or even lower. It restricts the swelling of starch granules, resulting in reduced starch paste viscosity. Reduced paste viscosity allows transferring, mixing, and pumping of these starches even at concentrations of 25–30%. Also, HHP-treated starches (at concentrations $>15\%$) produce paste with creamy texture, which can find applications in dietary foods instead of oil.

Annealing and heat–moisture treatments

Two of the other hydrothermal treatments commonly used to modify starch are ANN and HMTs. These treatments modify the physicochemical properties of starch without destroying granular structure. Starch is heated in excess water/intermediate ($>40\%$) water content in case of ANN or at low moisture ($<35\%$) levels for a certain time period, at a temperature above the starch glass transition temperature (T_g) but below its

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Table 1 Different starch modification types and preparation techniques

Modification	*Types*	*Preparation*
Physical	Heat–moisture treatment	Heat–moisture treatment – heating starch at a temperature above its gelatinization point with insufficient moisture to cause gelatinization
		Annealing – heating a slurry of granular starch at a temperature below its gelatinization point for prolonged periods of time
	Pregelatinization	Pregel/instant/cold water-swelling starches prepared using drum drying/spray cooking/extrusion/solvent-based processing
Conversion	Partial acid hydrolysis	Treatment with hydrochloric acid or orthophosphoric acid or sulfuric acid
	Partial enzymatic hydrolysis	Treatment in an aqueous solution at a temperature below the gelatinization point with one or more food-grade amylolytic enzymes
	Alkali treatment	Treatment with sodium hydroxide or potassium hydroxide
	Oxidation/bleaching	Treatment with peracetic acid and/or hydrogen peroxide, or sodium hypochlorite or sodium chlorite, or sulfur dioxide, or potassium permanganate or ammonium persulfate
	Pyroconversion (dextrinization)	Pyrodextrins – prepared by dry roasting acidified starch
Derivatization	Etherification	Hydroxypropyl starch – esterification with propylene oxide
	Esterification	Starch acetate – esterification with acetic anhydride or vinyl acetate
		Acetylated distarch adipate – esterification with acetic anhydride and adipic anhydride
		Starch sodium octenylsuccinate – esterification by octenylsuccinic anhydride
	Cross-linking	Monostarch phosphate – esterification with orthophosphoric acid, or sodium or potassium orthophosphate, or sodium tripolyphosphate
		Distarch phosphate – esterification with sodium trimetaphosphate or phosphorus oxychloride
		Phosphated distarch phosphate – combination of treatments for monostarch phosphate and distarch phosphate
	Dual modification	Acetylated distarch phosphate – esterification by sodium trimetaphosphate or phosphorus oxychloride combined with esterification by acetic anhydride or vinyl acetate
		Hydroxypropyl distarch phosphate – esterification by sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide

Source: Singh, J., Kaur, L. and McCarthy, O. J. (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications – a review. *Food Hydrocolloids* **21**, 1–22.

Table 2 Some properties and applications of modified starches

Types	*Properties*	*Applications*
Pregelatinization	Cold water dispersibility	Useful in instant convenience foods
Partial acid or enzymatic hydrolysis	Reduced molecular weight polymers, reduced viscosity, increased retrogradation, and setback	Useful in confectionery, batters, and food coatings
Oxidation/bleaching	Low viscosity, high clarity, and low temperature stability	Used in batters and breading for coating various foodstuffs, in confectionery as binders and film formers, in dairy as texturizers
Pyroconversion (dextrinization)	Low to high solubility depending on conversion, low viscosity, high reducing sugar content	Used as coating materials for various foods, good film-forming ability, and as fat replacers in bakery and dairy products
Etherification	Improved clarity of starch paste, greater viscosity, reduced syneresis, and freeze-thaw stability	Used in wide range of food applications such as gravies, dips, sauces, fruit pie fillings, and puddings
Esterification	Lower gelatinization temperature and retrogradation, lower tendency to form gels, and higher paste clarity	Used in refrigerated and frozen foods, as emulsion stabilizers and for encapsulation
Cross-linking	Higher stability of granules toward swelling, high temperature, high shear, and acidic conditions	Used as viscosifiers and texturizers in soups, sauces, gravies, bakery, and dairy products
Dual modification	Stability against acid, thermal, and mechanical degradation and delayed retrogradation during storage	Used in canned foods, refrigerated and frozen foods, salad dressings, puddings, and gravies

Source: Singh, J., Kaur, L. and McCarthy, O. J. (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications – a review. *Food Hydrocolloids* **21**, 1–22, with permission from Elsevier.

gelatinization temperature. However, the temperature–time combinations required to achieve desired functionality vary among different starch sources. Although no complete gelatinization of starch takes place, these treatments cause considerable changes in its physicochemical, functional, and digestion characteristics.

Chemically Modified Starches

Chemical modification involves the introduction of functional groups into the starch molecule, resulting in markedly altered physicochemical properties. Such modification of native granular starches profoundly alters their gelatinization, pasting, and

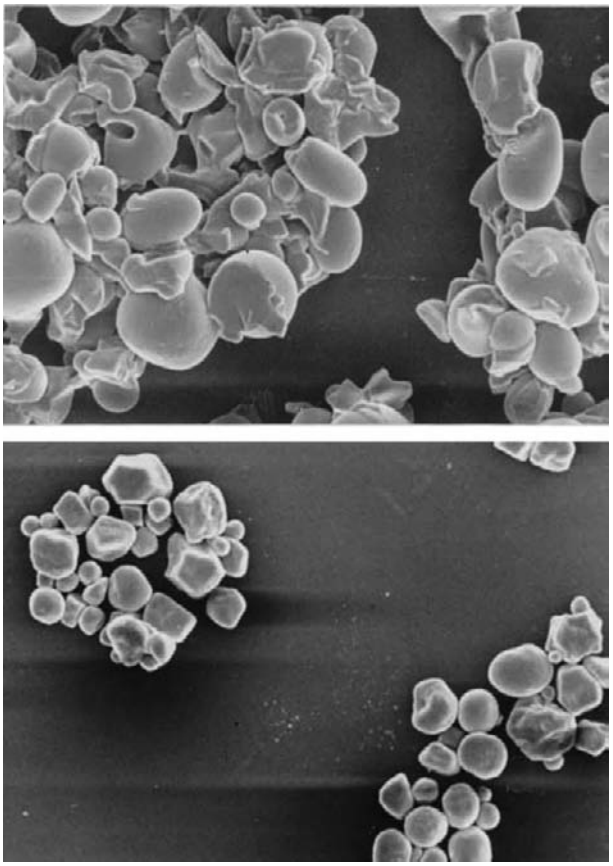

Figure 1 Scanning electron micrographs of granular cold water-soluble potato (top) and corn (bottom) starches prepared using alcoholic-alkaline treatment. Reproduced from Singh, J. and Singh, N. (2003). *Food Hydrocolloids* **17**, 63–72, with permission from Elsevier.

retrogradation behavior. Food-grade starches are chemically modified mainly to increase paste consistency, smoothness, and clarity and to impart freeze-thaw and cold storage stabilities. Some chemical modification reactions of starch are presented in Table 3.

Substitution (stabilization)

The chemical and functional properties achieved when modifying starch by chemical substitution depend, inter alia, on starch source, reaction conditions (reactant concentration, reaction time, pH, and the presence of catalyst), type of substituent, extent of substitution (degree of substitution, DS, or molar substitution, MS), and the distribution of the substituent in the starch molecule. Acetylated starch with a low DS is commonly obtained by the esterification of native starch with acetic anhydride in the presence of an alkaline catalyst, whereas the food-grade hydroxypropylated starches are generally prepared by etherification of native starch with propylene oxide in the presence of an alkaline catalyst. The introduction of (bulky) acetyl groups into starch molecules by acetylation leads to structural reorganization owing to steric hindrance; this results in repulsion between starch molecules, thus facilitating an increase in water percolation within the amorphous regions of granules and a consequent increase in swelling capacity. The hydroxypropyl groups introduced into the starch

Table 3 Some common starch chemical modification reactions

Modification type
Etherification
Hydroxyalkyl starch (with alkylene oxide)
Esterification
Starch acetate (with vinyl acetate)
Starch acetate (with acetic anhydride)
Starch phosphate (with orthophosphates)
Carboxymethyl starch (with mono chloroacetic acid)
Cross-linking
With $POCl_3$
With STMP
With EPI

St, starch; $POCl_3$, phosphorus oxychloride; STMP, sodium trimetaphosphate.
Source: Singh, J., Kaur, L. and McCarthy, O. J. (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications – a review. *Food Hydrocolloids* **21**, 1–22, with permission from Elsevier.

chains are capable of disrupting the inter- and intramolecular hydrogen bonds, thereby weakening the granular structure of starch, leading to an increase in motional freedom of starch chains in amorphous regions. The decrease in associative forces within the starch granule due to hydroxypropylation may result in an increased penetration of water during heating that leads to the greater swelling.

Cross-linking

Cross-linking treatment stabilizes and strengthens the starch granule by adding intra- and intermolecular bonds at random locations in the granule. Starch pastes from cross-linked starches are less likely to break down with extended cooking times, increased acidity, or severe shear. Cross-linking minimizes granule rupture, loss of viscosity, and the formation of stringy paste during cooking, yielding starch that is suitable for canned foods and other food applications. Nutritional benefits of cross-linked starch as a new source of dietary fiber have also been reported. Cross-linking is generally performed by the treatment of granular starch with multifunctional reagents capable of forming either ether or ester intermolecular linkages between hydroxyl groups on starch molecules. Sodium trimetaphosphate, monosodium phosphate, sodium tripolyphosphate, epichlorohydrin (EPI), phosphoryl chloride ($POCl_3$), a mixture of adipic acid and acetic anhydride, and vinyl chloride are the main agents used to cross-link food-grade starches. Cross-linking strengthens the bonding between the starch chains, causing an increase in the resistance of the granules to swelling with increasing degree of cross-linking. Higher concentrations of fast-acting cross-linking reagents such as $POCl_3$ result in greater reductions in the swelling potential as compared with slower-acting agents such as EPI. A large concentration of $POCl_3$ cross-links at the surface of the granule causes the formation of a hard outer crust that restricts granule swelling.

Starch phosphates, which are conventionally prepared, have been reported to give clear pastes of high consistency with good freeze-thaw stability and emulsifying properties and may be grouped into two classes: monostarch phosphates and distarch phosphates (cross-linked starches). In general, monostarch

Table 4 DS and MS of some modified starches from different botanical sources

Starch source	*DS (acetylated)*	*MS (hydroxypropylated)*	*DS (cross-linked)*
Normal potato	0.115–0.238	0.098–0.122	0.07–0.26
Normal maize	0.104–0.184	0.091–0.092	0.09–0.25
Normal maize	ND	0.061–0.094	ND
Waxy maize	0.081	0.067–0.127	ND
High-amylose maize	ND	0.078–0.119	ND
Hybrid normal maize	0.030–0.040	ND	ND
Normal wheat	0.035–0.131	0.117–0.123	0.004–0.020
Normal rice	0.087–0.118	>0.03	0.025–0.035
Normal rice	0.018	ND	ND
Waxy rice	0.016	ND	ND

DS, degree of substitution; MS, molar substitution; ND, not detected.
Source: Singh, J., Kaur, L. and McCarthy, O. J. (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications – a review. *Food Hydrocolloids* **21**, 1–22, with permission from Elsevier.

phosphates (monoesters) can have a higher DS than distarch phosphates (diesters) because even a very few cross-links (in the case of diesters) can drastically alter the paste and gel properties of the starch. Starch phosphates are conventionally prepared through the reaction of starch with salts of ortho-, meta-, pyro-, and tripolyphosphoric acids and $POCl_3$.

Dual modification

Dual modification, a combination of substitution and cross-linking, has been demonstrated to provide stability against acid and thermal and mechanical degradation of starch and to delay retrogradation during storage. Dual-modified starches are used widely in salad dressings, canned foods, frozen foods, and puddings. The control of reaction conditions is important during preparation of dual-modified cross-linked/hydroxypropylated starches using different cross-linking reagents with different starch bases such as maize, tapioca, wheat, waxy maize, waxy barley, rice, and sago. The quantity of cross-linking reagent required to prepare a dual-modified starch (with desirable properties) varies with the source of starch, the type of cross-linking reagent, the efficiency of the cross-linking reaction, the DS required, and the specified range of final modified-starch properties. The effects of different reaction conditions such as starch base concentration, temperature, pH, and the concentration of the catalyst salt play an important role during preparation of duly modified hydroxypropylated-cross-linked starch.

Extent of chemical modification

The rate and efficiency of the chemical modification process depend on the reagent type, the botanical origin of the starch, and the size and structure of its granules. This also includes the surface structure of the starch granules, which encompasses the outer and inner surfaces, depending on the pores and channels, and leads to the development of the so-called specific surface. Channels that open to the granule exterior provide a much larger surface area accessible by chemical reagents and provide easier access by the reagents to the granule interior. However, the reagent may diffuse through the external surface to granule matrix in the absence of channels. Although starches from various sources exhibit fundamental structural similarities, they differ in the specific details of their microstructure and ultrastructure. These structural differences have the potential to affect the chemical modification process. The DS and MS of some chemically modified starches prepared from different sources are presented in Table 4.

Morphology of chemically modified starches

Microscopy (light and SEM) has played an important role in increasing the understanding of granular structure of modified starches. It has been used to detect structural changes caused by chemical modifications and the most substituted regions in starch granules. Most of the structural changes upon hydroxypropylation take place at the relatively less organized central core region of the starch granule, that is, where the hydroxypropyl groups are most densely deposited. The 'pushing apart effect' exerted by the bulky hydroxypropyl groups, especially in the central region of the granule, might lead to an alteration in granule morphology upon hydroxypropylation. Another possible explanation is that the starch granule itself is not structurally homogeneous from a physical and chemical point of view, since it has different physical natures (amorphous and crystalline regions) as well as different chemical compositions in each region. The treatment of potato starch granules with propylene oxide (10%, dwb) alters granule morphology (Figure 2(a) and 2(b)). Many of the less affected modified granules develop a depression that later leads to slight fragmentation, indentation, and the formation of a deep groove in the central core region along the longitudinal axis in highly affected granules (Figure 3). These granules appear as folded structures with their outer sides drawn inward, giving the appearance of a doughnut (Figure 3(a) and 3(b)). Moreover, these altered regions have been observed to be apparently larger in large granules compared with small granules in all the potato starches examined; this may be attributed to differences in the native granule architecture and fragility. The peripheral regions and also the outer layer of the less affected starch granules remain unaltered, and the changes remain confined to the central core regions. By contrast, highly affected starch granules develop blister-like appearances, cracks, and small protuberances on their surfaces and a deep groove in the central core region, which suggests that the granule peripheral regions may be the last to be modified (Figure 3(c)).

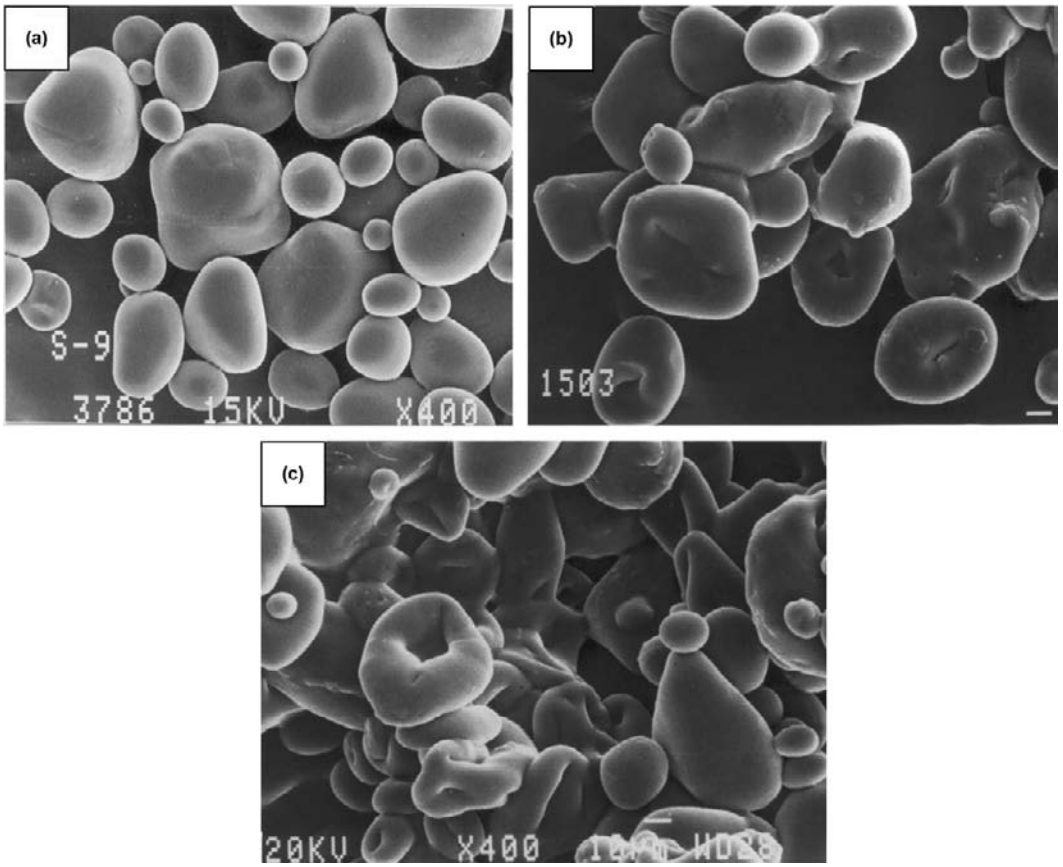

Figure 2 Effects of hydroxypropylation on the granule morphology of potato starches. (a) Native potato starch granules, (b) potato starch granules after hydroxypropylation (at 10% propylene oxide concentration), (c) effect of increased concentration of propylene oxide (15%) on the starch granule structure. Reproduced from Kaur, L., Singh, N. and Singh, J. (2004). Factors influencing the properties of hydroxypropylated potato starches. *Carbohydrate Polymers* **55**, 211–223, with permission from Elsevier.

The granule structure is more substantially altered when the reaction is carried out with a higher concentration of propylene oxide (15% compared with 10%). Highly affected granules appear as if gelatinized, having lost their boundaries and fuse together to form a gelatinized mass (Figure 2(c)). The material within the inner regions of potato starch granules has been reported to be more susceptible to reaction (with propylene oxide) than that in the outer granule layers. Also, as potato starch granules do not possess channels, the reagent diffuses inward through the exterior granule surface. Propylene oxide, being less reactive than other modification reagents, may diffuse into the granule matrix prior to reacting. However, one study on chemically modified pea and waxy maize starches reported that hydroxypropylation occurred uniformly throughout the starch granules. The structural changes in hydroxypropylated starch granules become more evident with increasing MS. Hydroxypropylation leads to alteration in the structure of the gelatinized potato starch gels also, which may be attributed to a weakened granule structure, leading to increased granule disruption during gelatinization. Hydroxypropylated starch gels maintain well the planar structure during storage at 4 °C for 10 days, whereas stored gels of native starches show extensive aggregation of granule remnants. Numerous rod-shaped fuzzy clustered microfibrils appear in hydroxypropylated starch gels (after 30 days' storage at 4 °C) that are easily distinguished from the other starchy materials (Figure 4). The extensive phase separation occurring during long-term storage at 4 °C may be responsible for the formation of these rod-shaped microfibrils. Slow cooling has also been reported to enhance the formation of non-spherulitic morphologies in starch gels.

Acetylation treatment has also been found to alter the granule morphology, although to a lesser extent. It has been observed to cause granule fusion in both maize and potato starches (Figure 5). The granule fusion is more pronounced in potato starches with small size granules. Maize starch granules show a higher resistance toward acetylation treatment than potato starches. The addition of 4% acetic anhydride results in the fusion of granules in potato starches, while maize starch granules tend to fuse at concentrations of acetic anhydride of 8% or higher. The granule surface of maize and potato starch granules has been observed to be slightly rough upon acetylation. The granule surface of rice starches also becomes rough, and the granules tend to form aggregates upon acetylation. However, some granule fusion and deformation and the rough appearance of the granule surface in the acetylated starches could also be the result of surface gelatinization upon the addition of NaOH to maintain alkaline conditions during acetic anhydride addition.

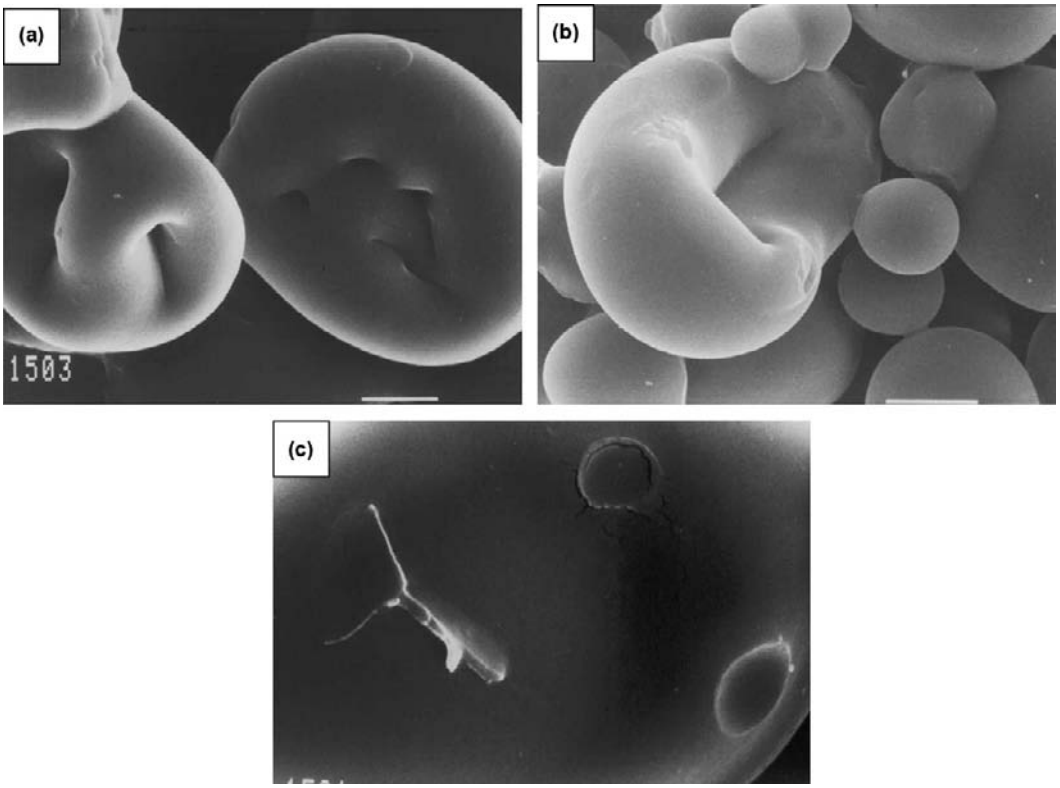

Figure 3 Effects of hydroxypropylation on the granule morphology of potato starches. (a) Formation of deep groove in the central core region, (b) folding of starch granules and formation of doughnutlike appearance, (c) formation of blister-like appearance and cracks on the starch granules. Reproduced from Kaur L, Singh N, and Singh J (2004) Factors influencing the properties of hydroxypropylated potato starches. *Carbohydrate Polymers* **55**, 211–223, with permission from Elsevier.

Starch granule size is very responsive to the changes in the DS by phosphate groups; the higher the DS, the larger the granule size. The average increase in granule size has been observed to be higher for rice and maize starches than for potato starch for a given DS. This increase could be due to the substitution by phosphate groups inside the modified granules, building certain repulsive forces that increase the sizes of inter- and intramolecular spaces, allowing more water molecules to be absorbed. Phosphorylation does not cause any detectable change in the general granule shape. After being cross-linked using EPI and $POCl_3$, potato starch granules remain smooth and similar to native starch granules in morphology when viewed under SEM, suggesting that the modification does not cause any detectable morphological change. Some chemical modifications of starch have been reported to increase granule surface area and granule porosity.

Enzymatically Modified/Acid-Modified Starches

Acid modification changes the physicochemical properties of starch without destroying its granule structure. It increases solubility and gel strength and decreases paste viscosity of starches. These starches find applications in the confectionary industry to prepare thin boiling starches. The modification procedure generally involves treating a concentrated starch suspension (36–40%) with acid at a temperature below the gelatinization temperature of the starch for several hours or days (depending on the treatment temperature). In acid modification, the hydroxonium ion attacks the glycosidic oxygen atom and hydrolyzes the glucosidic linkage. Its action proceeds from surface toward the center of the starch granule. The degree of acid hydrolysis increases with increasing amylopectin content in starches from the same species. Acid has been reported to preferentially attack the branched molecules more readily than the linear molecules within the granule. It has also been suggested that acid modification of starches in long-chain alcohols assists in reducing the degree of polymerization of amylopectin more effectively than short-chain alcohols. Alcohols have been reported to be capable of converting the crystalline regions into more amorphous regions that are prone to acid hydrolysis. Different shortcomings of the method include random attack, high yield of glucose, and removal of acid later on, which is the reason for a shift from the acid hydrolysis of starch to the use of starch-converting enzymes in the production of maltodextrin, modified starches, or glucose and fructose syrups.

Enzyme-modified starches are also considered among 'clean label' food products as there is no chemical treatment involved in the process. In enzymatic modification, selective hydrolysis of gelatinized/granular starch is carried out with starch-degrading enzymes, such as α-amylase, isoamylase, and pullulanase. Saccharification and liquefaction are the two major events taking place during starch amylolysis, and the major enzyme-modified products are linear dextrins of varying DE, high-fructose syrups, glucose syrups, dextrose, maltodextrins, and cyclodextrins.

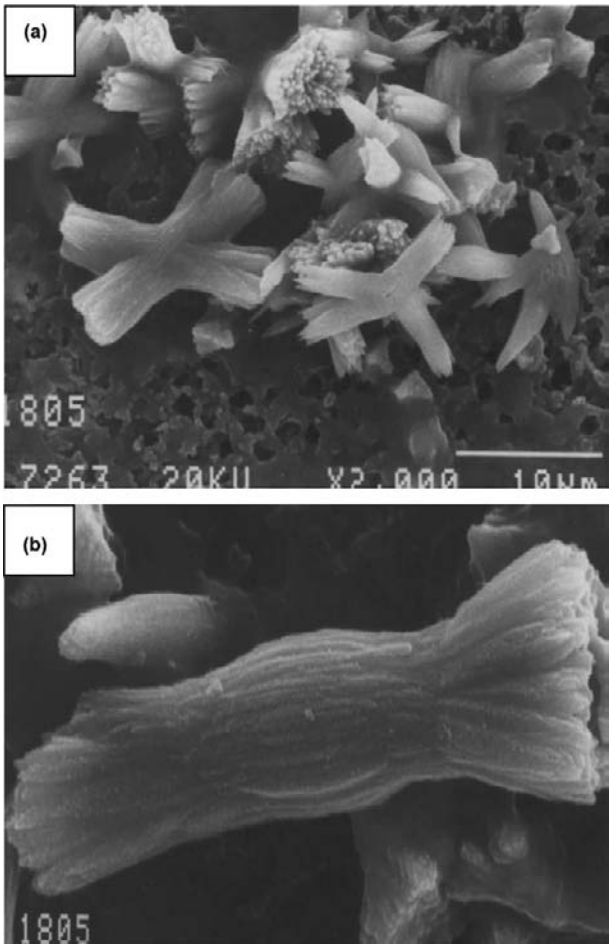

Figure 4 Rod-shaped microfibrils (showing longitudinal arrangement of crystalline structures) formed during long-term storage of hydroxypropylated gelatinized starch pastes. Reproduced from Kaur, L., Singh, N. and Singh, J. (2004). Factors influencing the properties of hydroxypropylated potato starches. *Carbohydrate Polymers* **55**, 211–223, with permission from Elsevier.

High-fructose syrups are considered high-value products in the food industry because of reduced calorie content and good tolerance by diabetics. They can be used as fat mimetics and calorie reducers in flour-based dry mixes, baking systems, fillings, etc. Cyclodextrins are well-known cyclic maltooligosaccharides produced by the enzymatic hydrolysis of starch using cyclodextrin glycosyltransferases from *B. circulans* and *B. macerans*. Cyclodextrins form inclusion complexes with some hydrophobic molecules and find application in food processing, pharmaceutical, and agrochemical industries for preparation, separation, purification, and protection of pharmaceuticals, fragrance, flavors, and steroids.

Modified Starches and Starch Digestibility

Many studies have suggested that the physiological effects of chemically modified starches are affected by the type of modification. There are many reports available that modifications of starch such as hydroxypropylation, octenylsuccinylation, and combinations of cross-linking and substitution reduce the extent of their enzyme-catalyzed hydrolysis. Substitution and oxidation have been suggested to increase the resistant starch content of the maize starches, whereas cross-linking does not affect starch digestibility to a considerable extent (Figure 6). However, some studies on the *in vitro* digestibility of hydroxypropylated and cross-linked waxy and nonwaxy starches have shown no significant changes in resistant starch content compared to control unmodified starch samples. It is important to point out that the general resistant starch assays may not be appropriate to measure the resistant starch content of chemically modified starches. It has been suggested that the pancreatin-gravimetric method involves exhaustive digestion and is therefore more appropriate for the determination of resistant starch in chemically modified starches. The chemical modification of starch by acetylation also improves the satiating, glycemic, and insulinemic properties of the meal. Phosphorylated/cross-linked starches are slowly digestible and are thought to provide nutritional benefits for humans. Also, the slowly digesting modified starches could be used for the treatment of certain medical modalities (e.g., glycogen storage disease and diabetes mellitus). Several studies have highlighted various methods and technologies (other than chemical modification) to modify the starch for the formation of slowly digestible starch (SDS). The prominent methods to produce SDS include ANN and HMT.

Nutritional and Toxicological Aspects

Many modified starches made for food use contain only small amounts of substituent groups and have been used as safe food ingredients. During acetylation and hydroxypropylation of food-grade starches, the level of monosubstitution groups introduced is relatively low. The maximum permitted levels of substitution for starch acetates, starch phosphates, and hydroxypropylated starches are 2.5%, 0.4%, and 10%, respectively. Similarly, cross-linked food starches containing one substituent cross-linking group per 1000 or more anhydroglucose units are considered safe. The legislative approval for the use of novel starch derivatives in processed food formulations is still under debate, but several tailor-made starch derivatives with multiple modifications are being prepared and characterized. Some of the starch derivatives are being increasingly used as fat replacers or fat substitutes. These derivatives are either partially or totally undigested and therefore contribute zero calories to the food on consumption. Starch-based fat replacers find applications in high-moisture food systems, for example, salad dressings, meat emulsions, and some bakery products.

Acknowledgments

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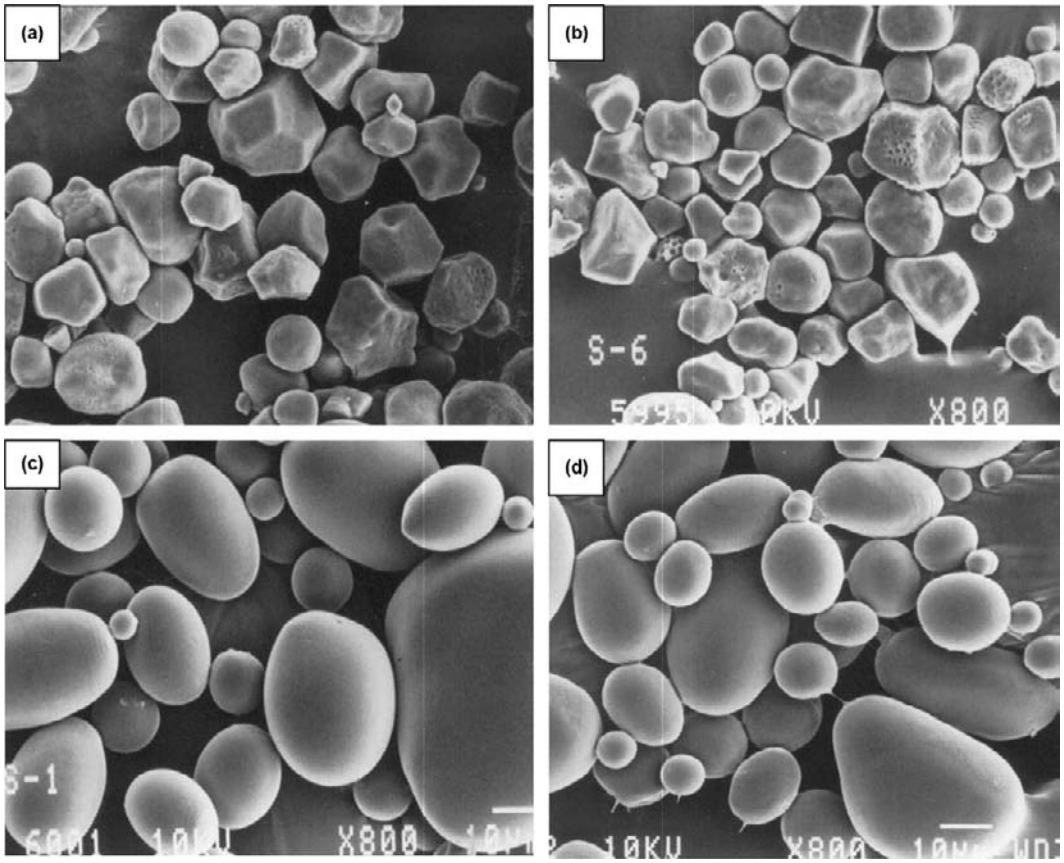

Figure 5 Effects of acetylation on the granule morphology of maize and potato starches. (a) Native maize starch granules, (b) acetylated maize starch granules, (c) native potato starch granules, (d) acetylated potato starch granules. Reproduced from Singh, N., Chawla, D. and Singh, J. (2004). *Food Chemistry* **86**, 601–608, with permission from Elsevier.

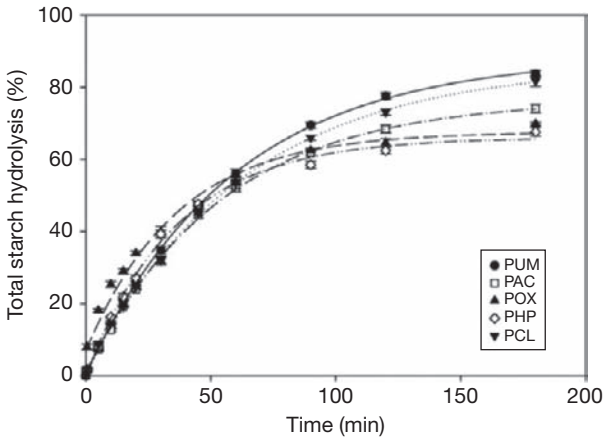

Figure 6 Starch hydrolysis patterns of the chemically modified maize starches in the prime (granular) state with porcine pancreatic α-amylase for 3 h at 37 °C. PUM, unmodified starch in the prime state; AC, acetylated starch; OX, oxidized starch; HP, hydroxypropylated starch; CL, cross-linked starch. Reproduced from Chung et al. (2008). *Food Research International* **41**, 579–585, with permission from Elsevier.

See also: Cereals: Types and Composition; Potatoes and Related Crops; Rheological Properties of Food Materials; Starch; Starch: Structure, Property, and Determination.

Further Reading

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Starch: Sources and Processing

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Background

Starch is found in green plants. The primary function of starch is to serve as an energy reserve and as a carbon source for *de novo* biosynthesis of macromolecules. As plants mature, starch often accumulates in storage organs such as endosperm or cotyledons of seeds or in fruit, stems, roots, and tubers. Starch is synthesized as discrete granules in amyloplast found in cells of storage organs. Simple starch granules occur when only one granule forms per amyloplast. Compound starch granules occur when many small starch granules form an aggregate structure in an amyloplast.

Starch is composed of two polysaccharides, amylose and amylopectin. Both amylose and amylopectin are long-chain α-1,4 glucans with α-1,6 branches. Amylopectin forms the principal structure of the granule. Amylopectin is a large, highly branched molecule with a degree of polymerization of 10^4–10^7 with 4–5% of the linkages involved with branch points. Amylose is smaller than amylopectin with a typical degree of polymerization of 10^3–10^4 and is nearly nonbranched with only 0.3–0.6% of the linkages involved with branch points. In general, starch granules are composed of 75% amylopectin and 25% amylose.

Starch granules differ in their molecular packing density, which affects their crystalline nature as determined by x-ray diffraction. Starch can be classified based on its x-ray diffraction pattern. Starch found in cereal grains has an A-crystalline type, which indicates densely packed crystals with few water molecules associated with the starch; B-crystalline type is common with starch granules found in tubers and roots and indicates less densely packed crystals with more water associated with the starch. C-crystalline-type starches are often found with legume starches and indicate that the granule contains both A-crystalline and B-crystalline types, where the B-crystalline type is found in the center of the granule, while the A-crystalline type forms the outer layer. The x-ray crystalline pattern, ratio of amylopectin to amylose, and granule size and shape depend on botanical source and can affect the functionality of starch and their subsequent use in food and industrial applications.

Industrial Starch Production

Worldwide, corn, wheat, rice, cassava, and potato are important plant sources for commercial starch production. Together, they represent over 99% of the starch produced. Other plant sources, such as sago palm, sorghum, and legumes, are important depending on geographic area and unique properties of their starch. Starch industry is constantly searching for new starch sources with unique functional properties that can fit niche markets.

Starch Extraction

For grains, starch extraction can begin with the intact grain or with its ground flour. Starch extraction from intact grain begins with steeping, followed by coarse milling to create a coarse suspension or slurry, which undergoes degerming to remove germ and associated lipids, followed by fine milling, screening, and centrifugal separation of starch from protein, fiber, and water-soluble material. Alternatively, grain can be dry-milled and the flour is discharged into a stream of water to form a slurry. The slurry then undergoes similar process steps as described earlier. Very small seeds are often dry-milled first and then defatted since removing the germ during wet milling is difficult due to their small size.

During steeping, the grains are soaked in a warm (40–55 °C), aqueous solution of sulfur dioxide for 24–48 h. Sulfur dioxide enhances the growth of favorable microorganisms, for example, lactobacillus, and inhibits the growth of undesirable microorganisms. Steeping softens the grain, which allows the germ and seed coat to be removed after cracking the seed using a coarse milling process. In cereal grain, starch stored in the endosperm is often embedded in a protein matrix. Metabisulfite, 0.2%; lactic acid, 0.5%; and sodium hydroxide, 0.2% w/w, are added to the steepwater to help disperse the protein matrix that surrounds the starch granules, thus facilitating their release. The germ, which is rich in lipid, weighs less than the other ground seed parts and is removed from the slurry by a hydrocyclone separator. Large pieces of fiber or seed hull are removed by screens. The remaining slurry is generally finely milled and the protein is separated from starch using their differences in density. Germ, oil, fiber, and protein are further refined to add value to 'waste' products associated with starch extraction.

Similarly, starch extraction from vegetative organs such as stems, tubers, and roots involves washing and peeling followed by a grinding or shredding step to form a slurry. Starch in vegetative organs is not embedded in a protein matrix. The slurry is passed through screens and is centrifuged to separate the starch from fiber, protein, and water-soluble material.

Industrial starch extraction processes often use countercurrent washing to conserve water and reduce the amount of wastewater. Countercurrent washing is the process where clean water is introduced to the system at the last washing step. The water contains 0.05% sulfur dioxide in order to control microorganisms. The subsequent wash water flows backward through the extraction system beginning with starch washing and ending as steepwater.

Factors That Affect Starch Extraction

Starch recovery from grain is dependent on starch content, kernel size, and kernel weight. Starch recovery tends to decrease with age of the grain. Separation of starch from protein becomes more difficult with age, increased harvest moisture, high grain drying temperature, and increased levels of starch damage.

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Cereal Starches

Corn and sorghum

Corn (*Zea mays*) originated in Central America and is widely adapted to many environments, although it is considered a warm season crop. Corn is monoecious, which means that staminate and pistillate flowers are borne on separate inflorescences on the same plant. Pistillate flowers are located on spikes called ears. An ear of corn contains 8–28 rows of grains with each row having 20–70 grains. There are several types of corn: dent corn, flint corn, flour corn, popcorn, and sweet corn. Dent corn is the most widely cultivated type and is used in corn starch production.

Dent corn has a 1000-kernel weight of 300–360 g and contains about 65–75% starch, 8–15% protein, 3–6% oil, and 10–15% fiber (dry basis). Dent corn consists of an embryo (12%) and endosperm (82%), both of which are surrounded by the pericarp or hull layer (5%). Endosperm cells contain amyloplasts with simple starch granules that contain about 25% amylose. Starch granules range in size from 5 to 25 μm and have round and polygonal shape. The endosperm has two regions, horny endosperm and floury endosperm. The horny endosperm is found near the embryo and along the sides of the kernel. The floury endosperm is found in the middle and top of the kernel. The floury region is characterized by larger cells, large round starch granules, and a relatively thin protein matrix. Horny endosperm consists of a protein matrix surrounding starch granule making the granules angular in shape. Horny endosperm contains only about 1.5–2% more protein than does the floury endosperm. The floury endosperm is opaque, friable, and easily crushed. Starch granules in the horny endosperm are smaller than those in the floury endosperm region.

Sorghum (*Sorghum bicolor*) is a native of Africa, south of the Sahara desert. It is a staple food crop in semiarid tropics. There are seven agronomic groups of grain sorghum based on where they originated. Sorghum inflorescence is a loose to dense panicle having many spikelets. Each spikelet contains two flowers; only one is fertile and sets a seed.

Sorghum has a 1000-kernel weight of 25–35 g and contains 60–80% starch, 9–15% protein, 2–6% fat, and 1–13% fiber (dry basis), all of which depend on cultivar and environment. Sorghum kernel has the same gross morphology as corn. Sorghum has a very hard kernel consisting of bran or hull (6%), endosperm (84%), and germ (10%). Endosperm differs from that of corn only in the relative proportions of floury and horny endosperms. All sorghums have a horny endosperm that surrounds the floury region. The dense horny endosperm layer comprises a larger proportion of the kernel than it does in corn, which results in much greater problems during starch purification. Starch is deposited as simple starch granules in amyloplasts found in endosperm cells. Sorghum starch granules contain about 20–30% amylose and range in size from 3 to 27 μm and have a smooth surface but angular and polygonal shape.

Commercial starch production normally utilizes low-grade or sample grade corn or sorghum. Starch is extracted from corn and sorghum using similar wet milling procedures. The grain is cleaned and steeped for 30–48 h in warm water (45–50 °C) that contains sulfur dioxide. The prolonged steeping is necessary for effective separation of the whole intact germ from the endosperm. The protein matrix that surrounds the starch granules is composed of glutelin protein. Sulfur dioxide weakens the glutelin matrix by breaking inter- and intramolecular disulfide bonds. Zein proteins occur in spherical bodies that are embedded in the glutelin matrix. During steeping, soluble materials including albumins and globulins are extracted (6–6.5%, db) and the kernel softens as the protein matrix begins to loosen and release starch granules.

Steepwater is drained from the kernels and condensed on a multistage evaporator. Softened kernels are coarsely ground using an attrition mill. The cracking of the kernel loosens the hull and breaks the bonds between germ and endosperm. Proper steeping ensures that the germ is freely released intact from kernel by gentle milling with minimal expelling of oil from the germ into the steepwater. The free germ and associated oil that float on the water surface are removed from the steepwater by centrifugal force.

The remaining kernel particles are finely ground, using an impact or attrition mill, to release starch and protein from the endosperm cell walls. A hydrocyclone separator separates the starch from the protein based on their differential weight (density). Protein weighs less and is removed from the top and the heavier starch is removed from the bottom of the separator. The protein is recovered and the starch is washed and centrifuged repeatedly until contaminants have been removed. The refined starch is dewatered, dried, sifted, and packaged.

Wheat

Wheat (*Triticum* spp.) originated in the eastern Mediterranean, Near East, and Middle East areas. Wheat is adapted to cool, dry, and temperate climate. There are five classes of common wheat (*Triticum aestivum*), each with different milling and end use qualities. Wheat inflorescence is a dense spike consisting of multiple spikelets. Each spikelet typically has three florets of which two generally become pollinated and produce a seed.

Wheat has a 1000-kernel weight of 31–35 g and contains about 80% starch, 15% protein, 2.5% fat, and 3.5% fiber, dry basis. Wheat kernel is composed of bran (15%), germ (3%), and endosperm (82%). In the endosperm, starch granules formed in amyloplasts are embedded in a protein matrix. Wheat starch granules contain about 25–28% amylose. There is a bimodal distribution of starch granules based on size, which are designated as A-starch and B-starch granules. A-starch granules are large (20–45 μm) and are lenticular in shape, while B-starch granules are small (2–8 μm) and are spherical in shape.

Starch can be extracted from the wheat kernel. However, wet milling for extracting starch and gluten from wheat kernels is hindered by tendency of gliadin and glutenin storage proteins to agglomerate. Wheat storage proteins tend to entangle with bran particles for which the proteins can no longer be separated. Thus, even though starch yield is reduced by the loss of starch to milling streams and to starch damage, wheat flour is the most common starting material for isolating starch from wheat. Wheat starch generally is isolated from the flour of poor quality wheat using either the dough process (often called the Martin process) or the batter process (often called the Scandinavian process).

The dough process is the traditional method for obtaining gluten and starch from refined wheat flour. In this method, wheat flour is hydrated to 40–60% and mixed to form a dough. The dough is formed as the hydrated storage proteins interact to form a gluten matrix. The resulting dough is continuously kneaded as it is washed with water. Starch and water-soluble compounds are removed from the dough's gluten matrix, creating a starch slurry. Dough strength is reduced by the addition of more water, which causes it to produce suspended curds of gluten particles that contain low levels of residual starch. Gluten curds are recovered on gyrating sieves. The remaining slurry containing starch and water-extractable material is centrifuged, which separates the water-extractable fraction from the starch. The yield of prime grade starch, which has low protein and lipid contents, is about 60% of the weight of flour and contains a high percentage of the large A-starch granules. The A-starch granules are heavier than the small B-starch granules and so precipitate out of the slurry quicker during centrifugation. About 15% low-grade starch is collected, which tends to be rich in pentosans that originate from the cell walls. The starch fractions are separately dried to 12% moisture in a flash dryer using hot air.

In the batter process, wheat flour is continuously discharged into a stream of warm water forming a slurry. The wheat flour slurry is homogenized in a high-speed in-line disintegrator. The homogenized slurry is separated into three fractions using hydrocyclone separators, which takes advantage of the higher density of starch granules compared to gluten particles. The first fraction is the heavy phase that contains A-starch. The second intermediate density fraction contains gluten and B-starch. Subsequently, the B-starch is washed out of the gluten by further centrifugation with hydrocyclones. The third fraction is the light phase that contains water-soluble compounds including proteins and pentosans.

The A-starch is further refined by washing with fresh clean water. Hydrocyclone separators are used to reduce fiber and soluble protein contents to low levels. Purified A-starch slurry is discharged to a centrifuge for dewatering. The dewatered starch is discharged by gravity to a moist starch hopper where it is conveyed into a flash dryer and dried to 12–13% in hot air. Dried starch is ready for storage or for bagging.

Rice

Rice (*Oryza sativa*) probably originated in India or southeastern Asia. Rice can be categorized into two subspecies, *O. sativa indica* and *O. sativa japonica*. Rice thrives in hot humid conditions, with 90% of world production grown in Asia. Rice is able to germinate and thrive in water. The rice plant is able to transport oxygen to the submerged roots from the leaves where oxygen is released during photosynthesis. The rice inflorescence is a loose terminal panicle of perfect flowers. At maturity, the panicle in different varieties may be enclosed in the sheath, partly enclosed in the sheath or fully exposed. Each panicle branch bears a number of spikelets. Rice has one flower spikelet laterally compressed and two short glumes. The rice grain is enclosed by the lemma and palea, which form the hull.

Rice has a 1000-kernel weight of 15–20 g. Rice, after the hull and bran have been removed, contains about 90% starch, 7–8% protein, 0.3–0.5% fat, and 0.2–0.5% fiber. Rice grain consists of a hull composed of lemma and palea (20%), bran layer (10), germ (2%), and endosperm (68%). Brown rice is rice where the hull has been removed but still has the bran layer. White or polished rice is rice where the bran layer has been removed. Endosperm cells contain very little protein matrix but are filled with amyloplasts that contain compound starch granules. Individual rice starch granules contain about 20% amylose and range in size from 3 to 8 μm and have smooth surface but angular and polygonal shape.

Starch is extracted from low-quality rice flour and from broken polished rice grains. Broken kernels are steeped in a warm alkali solution (0.3–0.5% sodium hydroxide) for 24 h and then wet-milled on a pin mill or hammer mill. The resulting ground material is soaked in an alkali solution for 10–24 h. The steeping temperature can range from room temperature to 50 °C. The long steeping time is necessary to remove protein from the starch. Rice protein in the endosperm is tightly associated to the surface of the starch granules and is difficult to remove. Sodium hydroxide is used because at least 80% of the protein in rice is glutelin, which is soluble in alkali solutions. Sometimes, *Bacillus megaterium* is added. *B. megaterium* produces proteases that break down rice protein. After storing the starch suspension for 10–24 h, the fiber is removed by passing the suspension through screens and starch is collected by centrifugation. The extracted starch is washed several times with water to remove impurities and residual sodium chloride. The purified starch is then dried, sifted, and packaged. Solubilized protein can be recovered by neutralization with hydrochloric acid or sulfur dioxide. The precipitate is allowed to settle and is recovered in filter presses or by centrifugation. Isolated protein is mostly used as an animal feed component.

Rice flour can be used in starch production. Flour is added to the alkali solution for 10–24 h, sieved to remove fiber, and centrifuged to collect starch, as described earlier. Starch is dried, reground, and packaged. Wet grinding is preferred to dry as starch damage is more extensive in dry milling. Damaged starch can dissolve in alkali solution.

Rice starch production is limited because of its relatively high product cost associated with long steeping/soaking time and intensive washing procedures needed to remove residual sodium chloride. Alkaline steeping step generates large quantities of alkali and salt, which increases cost for wastewater treatment.

Legume Starches

Pulses

Pulses are dried seeds from plants in the legume family that have relatively low oil content. Dry edible beans (*Phaseolus vulgaris*), dry peas (*Pisum sativum*), lentils (*Lentilla lens*), and chickpeas (*Cicer arietinum*) are the most common pulses. Pulses are cool season crops. Pulse seeds are borne in pods. Each pod contains several seeds. Pods are botanically a fruit, since they contain seeds developed from the ovary of a flower. Pulses can be a good starch source and provide unique functionality due in part on their crystalline structure and relatively high level of amylose (35–40%). Pulse starches generally have B-type x-ray diffraction pattern.

Dry pea is a common source of starch. Dry pea has a 1000-kernel weight of 125–300 g. Seed consists of a seed coat (8–10%), two cotyledons (88–90%), and embryo (1–2%). Dry pea contains about 40–45% starch, 20–27% protein,

1–2% fat, and 19–22% fiber. Cotyledon cells are densely packed with protein bodies and amyloplasts that contain simple starch granules. The total amylose content ranges from 33% to 48%. Starch granules range in size from 8 to 35 μm and are round to elliptical with smooth surfaces.

Dry pea starch can be extracted by dry milling or by wet milling process. Dry fractionation process involves removing the seed coat using a dehuller, followed by milling using a pin mill. The starch-rich and protein-rich fractions are separated based on particle size using an air classifier. Small particles are rich in protein, while large particles are rich in starch (about 65%). Dehulling is easier with peas and lentils than with dry beans.

Wet milling involves dehulling the dry peas, which are then ground using a hammer mill. Pea flour is mixed with a warm alkaline (sodium hydroxide, pH 9–10) solution for 1 h, during which the proteins and lipids are solubilized. The starch is removed by a decanter or ultrafiltration. The solubilized protein can then be precipitated by adjusting the solution pH to 4.5. The protein and starch are then dried in a drum drier. Starch purity is about 98%. Isolation of highly pure starch is difficult due to lipids and proteins associated with the starch and to the presence of highly hydrated fine fiber fraction, which is very difficult to remove from the starch granules.

Tuber and Root Starches

Root crops contain about two-thirds of their weight as water. This affects cost of transportation and shelf life before starch extraction.

Potato

Potato (*Solanum tuberosum*) originated in the Andes highlands of Peru and Bolivia. Potato is a cool season plant and is grown in cool climates found in northern Europe and northern United States. Potatoes form stolons. Stolons start to grow within 10 days of the plant emerging aboveground. Tubers form on the end of the stolon. Thus, a potato tuber is a modified stem, with branches forming from eyes or buds located on the tuber. The interior of the tuber has a pithy central core with branches leading to each bud or eye. Surrounding the pith are parenchyma cells that contain the amyloplasts where simple starch granules are formed. Potato starch has a smooth oval shape and granule size of 5–100 μm with median size of 30 μm. Potato starch granule contains about 20% amylose. The vascular cambium is located toward the outer edge of the tuber, followed by the cortex, suberin epidermis, and periderm. About 20% of a large sound tuber consists of the peel, which is composed of the periderm and part of the cortex. A whole potato consists of about 76% water, 20% starch, 2% protein, 0.2% fat, and 1% fiber. Starch content decreases with storage time.

Cull potatoes, which are those that are too small or large or misshapen or damaged, are used in potato starch production. First, soil and dirt are removed from the potatoes. Next, they are conveyed to a washing drum, which has a low amount of water so that the potatoes do not float. The potato peel is removed by abrasive action of the potatoes rubbing against each other while in the drum. The potatoes are rasped with a rotary saw blade or are sent through a hammer mill where the macerated potatoes are forced against a screen and the pulp passes through the holes. Sulfur dioxide is added to the extraction water to prevent discoloration that occurs once cells are exposed to air. The pulp is screened through a rotary sieve to separate free starch from the pulp. The pulp is often reground in a hammer mill using fine screen opening. Fiber and pulp are removed from starch by the centrifugal force inside a hydrocyclone. The fractionated starch is diluted with freshwater to remove soluble impurities and then concentrated in a continuous hydrocyclone separator. There may be several washings of the starch. The final washing is with freshwater and the starch is concentrated using rotating vacuum filters. The extracted starch is dried to 17–18% moisture in a flash dryer using moderately hot air. The dried starch is sifted and packaged.

Cassava

Cassava (*Manihot esculenta*) originated from tropical regions of Central and South America. Today, cassava is an important staple food crop in sub-Saharan Africa and is an important starch source in Thailand, Brazil, and Malaysia. Cassava typically grows as a perennial woody shrub well adapted to tropical regions and grows well under marginal conditions. Cassava produces a taproot and fibrous roots. Several of the fibrous roots and the taproot will become storage roots. Thus, cassava root is a true root. A cassava plant can produce 3–14 storage roots per plant. Storage roots are composed of periderm, cortex, storage parenchyma, and central xylem bundle. The phloem is located in the cortex. Periderm and cortex make up the peel. About 11–20% of fresh weight of storage root is peel. Storage parenchyma cells are filled with amyloplasts that contain simple starch granules that range from 5 to 25 μm and are truncated in shape. Cassava starch granules contain about 18–25% amylose. Typical composition of the storage root is 70% moisture, 24% starch, 2.2% protein, and 4.5% fiber. Starch in the storage roots can range in color from white to yellow depending on carotene content.

Processing facilities must be close to where the cassava is harvested. Cassava roots need to be processed within 24–48 h of harvest to prevent loss of starch from enzymatic oxidation and rot. Cassava begins to rot within 2 days of harvest. The fresh roots are washed, peeled, chopped, and pulverized in an industrial blender. After disintegration of the pulp, the slurry is washed on screens where the fiber remains and the starch slurry passes through. The fiber can be used as a fertilizer or pressed and dried for cattle feed.

After removing the fiber, crude starch slurry passes through a degritting screen to remove small nonstarch material. The starch slurry is separated into a heavy fraction and a light fraction by a hydrocyclone separator. The heavy fraction is starch and the light fraction contains fine fiber and soluble material. The starch is reslurried and recentrifuged for further purification. Purified starch slurry is dewatered in a continuous vacuum filter or batch-operated basket-type centrifuge. Moisture content of starch after dewatering step ranges from 32% to 45%. The dewatered starch is conveyed to hot air dryers and dried to 12–14%, cooled, and packaged.

Stem Starches

Sago palm

Sago palm (*Metroxylon sagu*) originated in the area extending from Moluccas of Indonesia to New Guinea. Sago palm is a tropical plant grown in Southeast Asia and Oceania where it is able to survive in swampy peat environment. Sago palm

produces an erect trunk about 10 m tall and 75 cm thick. Its vegetative phase lasts 7–15 years during which starch is stored in the trunk. At maturity, an enormous branched inflorescence develops at the top of the trunk. Starch reserves are at their maximum just before flowering and fruiting deplete these reserves. Sago palm dies after mature fruit fall from the tree. The trunk is composed of epidermis, cortex, and pith or conjunctive tissue. Pith contains vascular bundles and storage parenchyma cells. The storage parenchyma cells are packed with amyloplasts that contain simple starch granules that range from 20 to 60 μm and have oval or polygonal shape. Sago palm starch granules contain about 27% amylose. Pith contains 6–12% soluble solids and 79–88% starch and sugars.

Commercially, trees are harvested. The trunks are stripped of leaves and cut into 1 m segments for handling. The segments are split lengthwise and fed into slicers that slice the pith from the bark. Alternatively, the bark is removed from sections of the logs that are then fed into a mechanical rasper that rasps the pith into small pieces. The pith is fed into a hammer mill and discharged into water where a starch slurry is formed. Starch slurry is passed through a series of centrifugal sieves to separate the coarse fibers. The starch can be washed several times to improve purity. The purified starch is dewatered in a rotary vacuum drum dryer and dried with hot air. One palm trunk can yield 180–250 kg dw of sago starch.

Sago starch is known to vary in color from gray to white. It can be bleached with bisulfite but the starch still tends to discolor with prolonged storage. Mature palms are needed for the production of high-quality starch. Immature pith contains impurities, and starch made from it is of inferior quality and has a greater tendency for browning. Starch quality varies with its location in the trunk and growth conditions.

Starch Quality

Starch quality is based on its physical and functional properties. Physical properties include its purity, for example, amount of residual protein, lipid, and fiber and whiteness. Extraction conditions such as holding time and pH and temperature of slurry affect purity and discoloration of starch. Functional properties are generally associated with its pasting properties such as gelatinization temperature and its retrogradation characteristics. Functional properties can be affected by growth environment, genotype, and processing conditions. Steeping additives, for example, sulfur dioxide, lactic acid, and sodium hydroxide, can affect pasting properties by affecting the amount of starch damage and starch purity.

Physical and Chemical Modifications

Starch functionality in industrial and food systems is primarily related to its gelatinization, retrogradation, and pasting properties. Functionality of native starch varies with botanical source, amylopectin fine structure, and amylose–amylopectin ratio and granule size. Starch functionality can be changed through physical and chemical modifications. Physical modifications include pre-gelatinization, annealing, and heat–water treatment. Annealing is a physical reorganization of starch granules when heated in excess water at or below its glass transition temperature. Annealed starch retains its granular structure. Annealing optimizes crystalline order and increases rigidity of amorphous regions. Annealing results in reduced starch swelling, elevated starch gelatinization temperature, diminished gelatinization temperature range, and restricted amylose leaching. Heat–water treatment involves exposing starch with low moisture content (18–27%) to high temperature. The low moisture content limits molecular mobility and gelatinization during treatment. Starch that has been heat–water-treated will have an increased gelatinization temperature. Common chemical modifications involve anionic substitution (carboxymethyl, phosphate, and acetate), cationic substitution (quaternary ammonium), hydroxypropylation, oxidation, cross-linking chains, and depolymerization of chains.

See also: Cassava: The Nature and Uses; Cereals: Types and Composition; Drying: Physical and Structural Changes; Maize; Peas and Lentils; Potatoes and Related Crops; Sorghum: A Novel and Healthy Food; Starch: Modified Starches; Starch: Structure, Property, and Determination; Wheat: Grain Structure of Wheat and Wheat-based Products.

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Starch: Structure, Property, and Determination

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Introduction

Starch is the second largest biomass produced on Earth, next to cellulose, and is synthesized in amyloplasts of higher plants for energy storage. Starch is a major energy source for human and animal diets and an important raw material for industrial applications. Starch granules consist of highly branched glucan, amylopectin, and, a primarily linear glucan, amylose. The amylopectin and amylose molecules are aligned radially in the semicrystalline starch granule. Native starch granules have specific densities around 1.5 g cm^{-3}. Because of the large density, the granular starch can be readily isolated from grains, tubers, roots, and stems by gravity using a wet milling process. The granular structure of starch also facilitates its chemical modifications, which can be washed to remove residues and by-products. Consequently, starch is produced as an economical commodity for food and industrial applications. Major markets of starch are in the corn syrup, paper, textile, food, and bioethanol industries.

The semicrystalline starch granules are not dispersible in water at ambient temperatures. After starch is cooked above its gelatinization temperature, the starch loses its crystalline structure and can then be dispersed to a paste in excess water with continuous heating and stirring. Depending on the composition and structure of starch, such as amylopectin branch chain length, and contents of amylose, lipids, and phosphate monoester derivatives, starch displays different properties. Chemical and physical structures of starch in food determine the enzymatic digestive rate of the starch after the food is ingested, which is reflected as the glycemic response. Prolonged consumption of starchy food with large glycemic index is known to cause insulin resistance, type-2 diabetes, obesity, and other metabolic syndrome. Ingesting of resistant starch (RS) that cannot be hydrolyzed by enzymes in the small intestine and is then passed to the colon, however, reduces the glycemic response and facilitates the growth of microbiota in the colon. Fermentation of the RS in the colon by microbiota can produce short-chain fatty acids to maintain colon health and prevent colon cancer.

This article aims to provide an overview on the composition and structures of starch molecules, morphology and internal structures of starch granules, functional properties of starch, and methods used to determine these characteristics. Other relevant publications, which provide in-depth discussions of the subjects covered in this article, are listed in section 'Further Reading' for information on starch structures and properties.

Composition, Structures, and Analysis of Starch Molecules

Normal starch consists of about 70–80% amylopectin and 20–30% amylose, waxy starch consists of mostly amylopectin and 0–8% amylose, and high-amylose starch consists of 40–90% amylose. In addition to amylose and amylopectin, most cereal normal starches also contain lipids, including phospholipids, which have significant impacts on the pasting, gelling, and digestive properties of the starch. Many tuber and root starches and some cereal starches have phosphate monoester derivatives. Some starches, such as high-amylose maize starch and *sugary-2* starch, contain intermediate component that has branched molecules, however, with smaller molecular weights and longer branch chain lengths than amylopectin. *Sugary-1* starch consists of phytoglycogen, a water-soluble glucan with a highly branched structure and substantially shorter branch chains. Structures of these components are discussed in the following sections.

Amylose

Amylose is a primarily linear molecule, consisting of mainly α-1–4 linked D-glucopyranose with a few α-1–6 branches. The molecular weight of amylose varies from degree of polymerization (DP) 500 in high-amylose maize starch to more than DP 6000 in potato starch. The number of branches increases with the molecular size of amylose.

The α-1–4 glycosidic bonds of amylose chains render a flexible random-coil conformation to the molecule. The presence of hydrophobic hydrocarbon groups and hydrophilic hydroxyl groups on the anhydroglucose units (AGU) prompts two adjacent amylose chains in the aqueous dispersion to fold together and form double-helical crystallites, resulting in the hydrocarbon groups of the AGU folded inside of the double helix, away from the aqueous medium to reach a lower energy state. This crystallization process of dispersed starch molecules taking place during storage of cooked starch is known as retrogradation. The retrograded amylose displays the B-type x-ray diffraction pattern (polymorph), having the amylose double helices packed in a hexagonal unit cell.

Amylose can also form single-helical complexes with many chemicals (complexing agents) that consist of a hydrophobic moiety, such as alcohols and fatty acids. The amylose single-helical complex, also known as an inclusion complex, has the hydrophobic moiety of the complexing agent located inside the hydrophobic cavity of the helix. Depending on the cross-sectional diameter of the complexing agent, amylose can form single helices of different sizes, including six, seven, and eight AGU/turn, to accommodate the diameters linear complexing agents (e.g., *n*-butyl alcohol), branched complexing agents, (e.g., isobutyl alcohol), and bulky ones (e.g., α-naphthol), respectively. The x-ray diffraction pattern of the amylose single-helical complexes is known as the V pattern.

Amylopectin

Amylopectin consists of α-1–4 linked D-glucopyranose linear chains that are connected by α-1–6 branch linkages, about 5%.

The molecule is highly branched and has a molecular size about DP 10^6. Branch chains of amylopectin molecules can be categorized into three groups: the A chain, with its reducing end attached to a B or C chain but not carrying any other chains; the B chain, not only having the reducing end attached to another B or C chain but also carrying other A or B chains; and the C chain, being the only chain of the molecule carrying a reducing end (Figure 1). The A chains are generally short and extend within one cluster. The B chains have different chain lengths, which can extend through one cluster (B1 chain), two clusters (B2 chain), three clusters (B3 chain), or more clusters. The branch chain-length distribution of amylopectin varies with the botanical origin of the starch, which determines the polymorphic form of the crystalline structure and the gelatinization, pasting, and retrogradation property of the starch. Specific properties of starch will be discussed in section 'Properties of Starch.'

Branch chains of amylopectin are arranged in clusters, which are evidenced by wormlike cluster structures remaining after exhaustive acid hydrolysis of granular waxy maize starch at room temperature, as viewed using transmission electron microscopy. The wormlike cluster is attributed to the crystalline structure of outer chains of amylopectin molecules. The amorphous structure of the waxy maize starch, mainly consisting of branch linkages, is hydrolyzed during the acid hydrolysis.

Starches that consist of amylopectin with larger proportions of long branch chains (e.g., B2 and B3 chains), such as potato, high-amylose maize, and *ae* waxy maize starches, display the B-type x-ray pattern. Starches that consist of amylopectin with more short branch chains of A and B1 chains, such as maize, wheat, taro, barley, and rice starches, display the A-type x-ray diffraction pattern, having a monoclinic unit-cell packing.

Extra-long branch chains are present in amylopectin of many cereal normal starches, such as normal wheat, rice, barley, and maize starches, but are not found in cereal waxy starch and potato starch. The extra-long branch chains have amylose-like structures. For example, the extra-long chains of wheat amylopectin have DP ~ 720, which contribute to larger blue values and iodine affinities of the amylopectin of normal wheat starch than that of the waxy wheat starch. The concentration of the extra-long branch chains of wheat amylopectin is positively correlated to the dosage of the waxy gene encoding granular-bound starch synthase that is primarily responsible for amylose biosynthesis.

Intermediate Component and Phytoglycogen

Intermediate component is found in starch of many mutants, including maize *ae* mutant and *sugary-2* mutant. The intermediate component has a branched structure, but its molecular weight is similar to amylose and its branch chain length is longer than amylopectin. After the starch is fractionated using a complexing agent, such as *n*-butyl alcohol, to complex and precipitate the amylose, the intermediate component remains in the supernatant with amylopectin. Gel permeation chromatograms of the mixture collected from the supernatant show that amylopectin is eluted first at the void volume and the intermediate component is eluted later. High-amylose maize starch consists of up to 52% intermediate component. The intermediate component together with amylose of the high-amylose starch forms double-helical crystallites, resulting in a conclusion gelatinization temperature (T_c) up to 130.0 °C. Consequently, the starch remains semicrystalline after cooking and has a large RS content (up to 40%).

Phytoglycogen is another water-soluble glucan, which is found in rice and maize *sugary-1* mutants that are deficient in the starch-debranching enzyme. Phytoglycogen has a smaller molecular weight and shorter branch chain length than amylopectin. The branch chains of the phytoglycogen display a

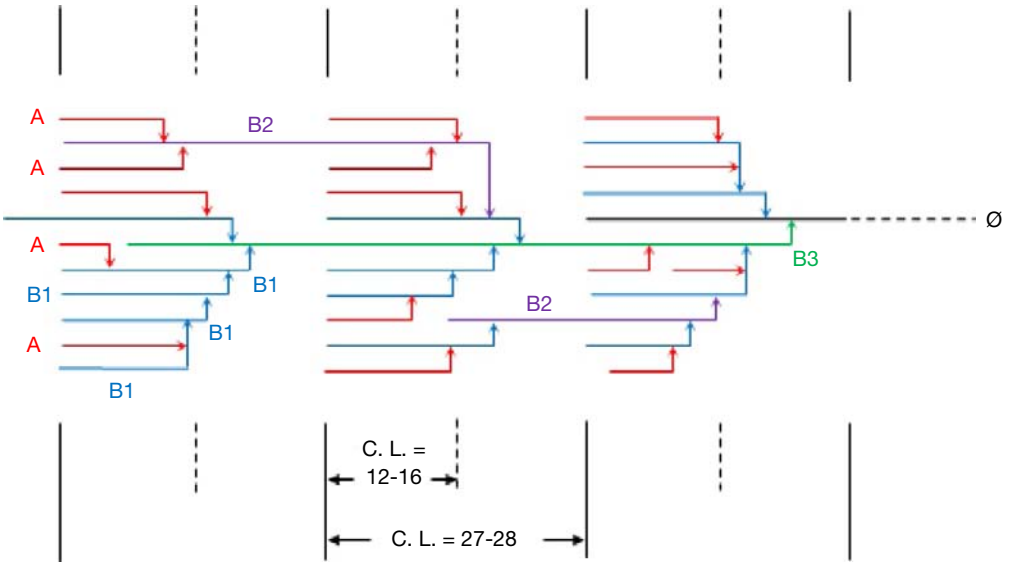

Figure 1 A proposed cluster model of amylopectin with A(–), B1(–), B2(–), and B3(–) chains. The chain carrying the reducing end (Ø) is the C chain. –, α-1–4 glucan chain; →, α-1–6 linkage; C.L., chain length. Reprinted from Hizukuri, S. (1986). Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydrate Research* **147**(2), 342–347.

single-modal chain-length distribution, which is different from the bimodal distribution of amylopectin.

Lipids and Phospholipids

Lipids and phospholipids are mainly found in cereal starches. Cereal normal starch contains up to 1% lipids, and the lipid content is positively correlated with the amylose content of starch. Starches of different botanical origins consist of different species of lipids. For example, normal maize starch consists of mainly free fatty acids, triglycerides, and little phospholipids; normal rice starch contains substantial amount of phospholipids and some free fatty acids; and wheat, barley, rye, and triticale starches consist of exclusively phospholipids. Substantial amount of amylose in normal starch is found in helical complexes with lipids, which were revealed by using solid ^{13}C cross polarization/magic angle spinning NMR. Cereal waxy starches contain little lipids, whereas high-amylose starches contain substantially more lipids. Root and tuber starches contain very little lipids and no detectable phospholipids.

Free fatty acids, monoglycerides, diglycerides, and phospholipids are good complexing agents with amylose and long branch chains of amylopectin. Differential scanning calorimetric (DSC) thermograms show dissociation peaks of the amylose–lipid complex (ALC) at 90 °C or higher. Unlike the retrogradation of starch, which is a slow process and normally takes days for the double helices to develop, the ALC formation is an instant reaction. The dissociation peak of the ALC reappears upon an immediate rescan of the starch sample using a DSC. The ALC present in native starch is amorphous (type 1), which does not give a V-type x-ray diffraction pattern and displays a dissociation peak at a lower temperature (90–100 °C). After cooking, the ALC is reorganized to a lamellar, semicrystalline structure (type 2), which displays a V-type x-ray diffraction pattern and shows a higher dissociation temperature (100–125 °C).

Phosphate Monoester Derivatives

Phosphate monoester derivatives are covalently attached to the amylopectin of potato starch and are also found in many other tuber and root starches. The phosphate groups carry negative charges, which repel one another and, thus, reduce the gelatinization and pasting temperatures and increase the viscosity and clarity of the starch paste. In the presence of salt, the viscosity of the potato starch paste reduces significantly because the negative charges of the phosphate are masked by cations of the salt.

Traditionally, phospholipid and phosphate monoester derivative contents of starch are analyzed by measuring the total phosphorus content after dry ashing of the starch. The method, however, relies on a complete removal of phospholipids by solvent extraction, which is difficult to achieve except by using a 90% dimethyl sulfoxide solution. ^{31}P-NMR spectroscopy has been demonstrated to give unambiguous results for qualitative and quantitative analyses of phospholipid and phosphate monoester contents of starch. Phospholipids, being phosphate diesters, have chemical shifts at 0–1 ppm, whereas phosphate monoesters have chemical shifts at 4–5 ppm at pH 8. Quantitative results can be obtained when adequate acquisition times are programmed to allow spin relaxation.

Structures of Starch Granules

Morphology of Starch Granules

Morphologies of starch granules isolated from different botanical sources and different organs of the plant are different. Images of some starch granules viewed using scanning electron microscopy are shown in Figure 2. Shapes of starch granules include spherical, oval, disk, polygonal, elongated, kidney, and lobe shapes. Starch isolated from leaves has flat and small granules. Starch from wheat, barley, and rye kernels in the triticale family displays bimodal granule-size distributions, the large disk-shaped A-granules, and the small spherical B-granules (Figure 2(g)). Rice and oat have compound starch, resulting from multiple granules being synthesized within a single amyloplast. High-amylose starch from maize and other sources consists of elongated starch granules, and some granule displays a rod shape that is 3 μm in diameter and 30 μm in length (Figure 3(A)). Diameters of common starch granules vary from submicrons to more than 100 μm. For example, the diameters of potato starch are 15–75 μm, wheat starch A-granules 18–33 μm and B-granules 2–5 μm, maize starch 5–20 μm, rice starch 3–8 μm, and amaranth starch 0.5–2 μm.

When viewed under a polarized light microscope, starch granules display birefringence, known as Maltese cross, which reflects the radial arrangement of starch molecules in the starch granule (Figure 2). The center of the Maltese cross, the hilum, is where the biosynthesis of the starch granule initiates. The hilum is not necessarily at the geometric center of the granule; some can be located close to one end of the granule, reflecting the initiating site of the starch biosynthesis (Figure 2(c), 2(f), and 2(h)). Elongated starch granules of high-amylose maize starch (Figure 3) display multiple hila and several Maltese crosses overlapping in a single granule (Figure 3(C) and 3(D)). Confocal laser scanning micrographs also show multiple granules in a single elongated granule (Figure 3(B)). Transmission electron micrographs of developing endosperm tissue reveal that the biosynthesis of the elongated granules is a result of an interaction between amylose molecules of adjacent starch granules initiated in the same amyloplast. The small granules are fused together and continuously synthesized to one elongated granule.

Internal Structures of Starch Granules

Starch of the A-type polymorph consists of amylopectin with large proportions of short branch chains, whereas that of the B-type polymorph consists of amylopectin with more long branch chains that extend through two or more clusters. Starch of the C-type polymorph has amylopectin with a combination of the A- and B-type structures. Because of the large proportion of short branch chains of the A-type polymorphic starch, the short double helices of the branch chains are arranged to a closely packed monoclinic unit cell, resulting in a porous internal structure (Figure 4(a1) and 4(b1)). On the contrary, the B-type starch granules, consisting of more B2 and B3 chains that extend through multiple clusters (Figure 1), maintain a

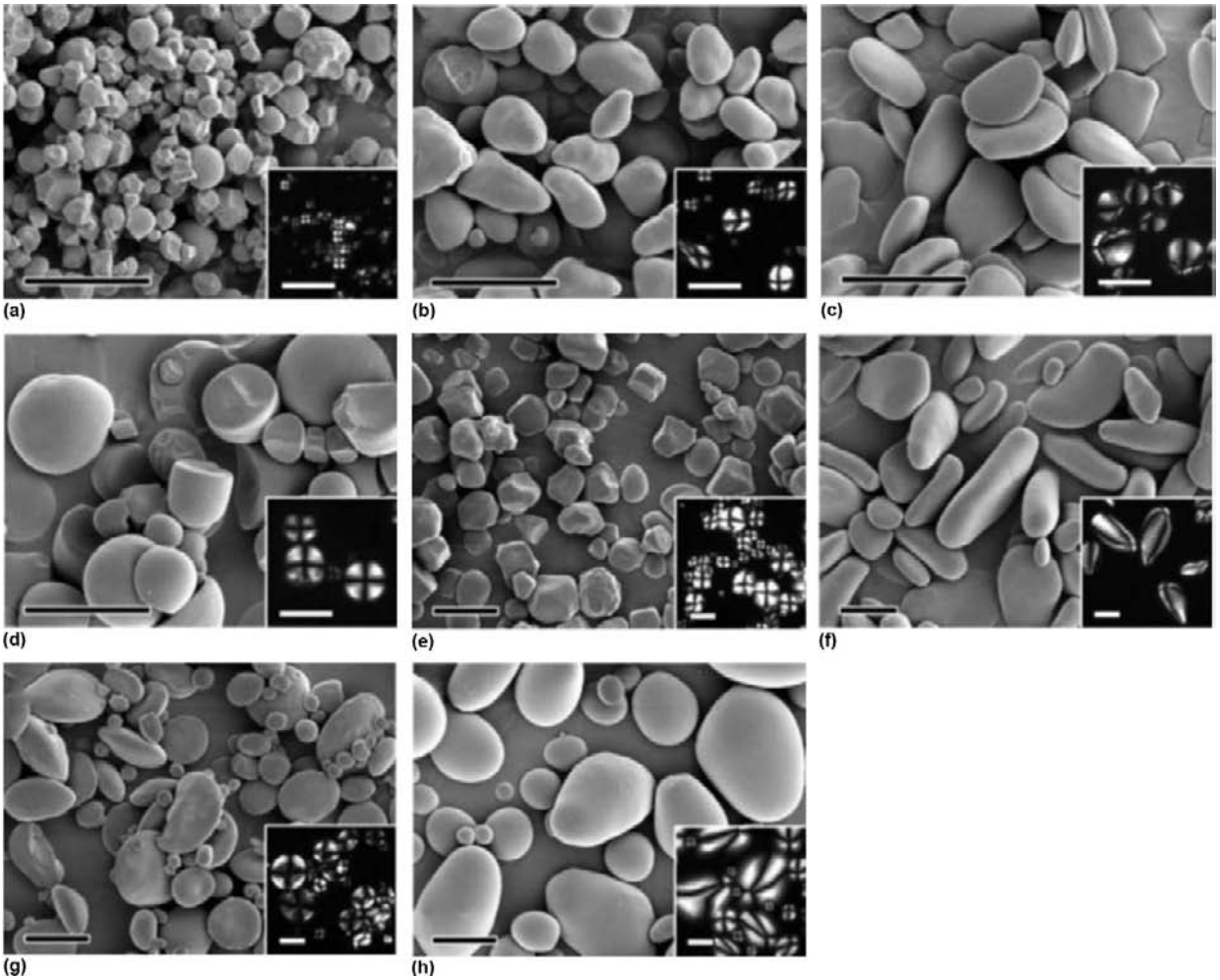

Figure 2 Scanning electron microscopy (SEM) and polarized light microscopy (insets) images of native starch granules from various botanical sources: (a) taro; (b) chestnut; (c) ginger; (d) manioc; (e) maize; (f) green banana; (g) wheat; (h) potato. Scale bars in all images: 20 μm. Reprinted from Pérez, S. and Bertoft, E. (2010). The molecular structures of starch components and their contribution to the architecture of starch granules: a comprehensive review. *Starch-Starke* **62**, 389–420.

homogeneous internal structure (Figure 4(a2) and 4(b2)). The porous structure of the A-type polymorphic maize starch granules was first revealed using a nitrogen gas surface adsorption method, which has later been confirmed using a surface-gelatinized technique, and confocal laser scanning microscopy (Figure 4). The porous structure of the A-type starch granules prompts greater susceptibility of the granule to chemical penetration and enzymatic hydrolysis. The A-type starches, such as sorghum and maize starch, display pinholes on the surface of the starch granule, which are open ends of serpentine-like channels likely hydrolyzed by amylases and penetrating into the granule.

Relative locations of amylose and amylopectin molecules in the starch granule have been studied using chemical cross-linking reactions. Results of the cross-linked starch granules show that amylose molecules are primarily cross-linked to amylopectin molecules, an evidence that amylose is interspersed among amylopectin molecules. Amylose is more concentrated at the periphery of the granule than at the hilum as revealed by using the surface gelatinization of starch granules with saturated neutral salt solutions, such as LiCl and $CaCl_2$. This result is consistent with the fact that the amylose content of starch increases with increased size of the starch granule.

The large concentration of amylose at the periphery of starch granules renders a hard shell on the surface of normal starch granules, making it difficult for enzymatic hydrolysis. The hard shell is sometimes misinterpreted as a membrane. But it is known that starch chains on the surface of the starch granule can be elongated with the addition of ADP–glucose, which excludes the presence of a membrane on the surface of the granule. The apparent shell on the surface of starch granules, therefore, can be attributed to tightly associated amylose and amylopectin molecules. It is well known that amylose produces tough films.

Properties of Starch

Starch has versatile properties and applications. It can be used in a granular form for an anticaking agent or facial/baby

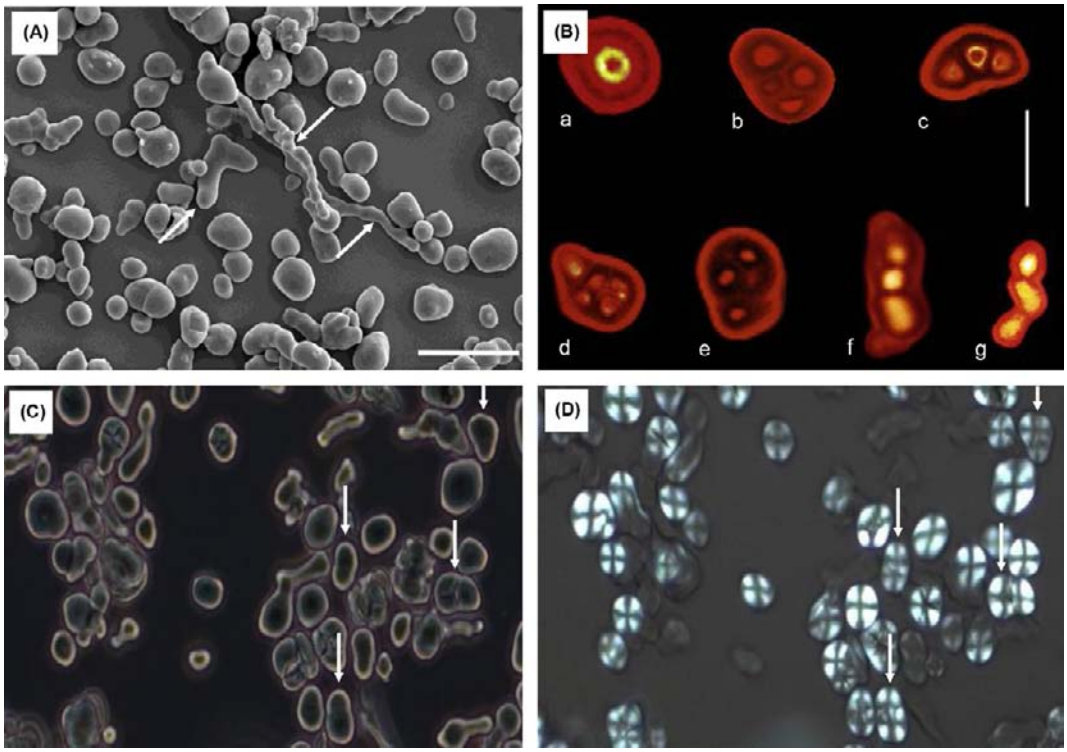

Figure 3 (A) Scanning electron microscopy (SEM) image of high-amylose maize starch. Arrows indicate elongated starch granules. Scale bar: 20 μm. (B) Confocal laser scanning microscopy images of high-amylose maize starch stained by using 8-aminopyrene-1, 3, 6-trisulfonic acid (APTS): (a) spherical granule with bright color around one hilum; (b–g) elongated granules with multiple hila differing in intensity of fluorescence. Scale bar: 10 μm. Phase contrast (C) and polarized (D) light microscopy images of high-amylose maize starch. Arrows indicate multiple Maltese crosses overlapping in one granule. (B) Reprinted from Jiang, H., et al. (2010). Formation of elongated starch granules in high-amylose maize. *Carbohydrate Polymers* **80**(2), 533–538. (C) and (D) Reprinted from Jiang, H., et al. (2010). Variations in starch physicochemical properties from a generation-means analysis study using amylomaize V and VII parents. *Journal of Agricultural and Food Chemistry* **58**(9), 5633–5639.

powder, a dispersed paste for thickening or sizing agents, a hydrogel structure for puddings or bread, and a crystalline structure (retrograded amylose) for RS (type 3) to improve health. These broad applications are the results of the transformation of starch from semicrystalline granules, to amorphous structures by losing the native crystalline order (gelatinization), to a paste after absorbing water and developing viscosity, to a gel after network formation, and to RS after recrystallization (Figure 5). The transformation and properties of starch are discussed in the following sections.

Starch Gelatinization

Native starch granules can absorb about 30% (w/w) moisture, when steeped in water at an ambient temperature. The water is distributed in the amorphous region of starch granules. When starch is heated to a specific temperature in the presence of water (two times or more), it loses the crystalline structure and the Maltese cross and becomes transparent. This thermal transition is known as gelatinization, and the temperature at which starch loses the crystalline structure is the gelatinization temperature. Gelatinization temperatures of selected A-, B-, and C-type polymorphic starch varieties are shown in Table 1. The gelatinization temperature is a characteristic property of each starch, and it is, in general, positively correlated with the branch chain length of the amylopectin. Phosphate monoester derivatives of starch, however, reduce the gelatinization temperature. For example, potato starch displays a much lower onset gelatinization temperature ($T_o = 58.2\,^{\circ}C$) than the normal maize and rice starches (64.1 °C and 70.3 °C, respectively), despite the fact that potato amylopectin has much longer branch chains than the normal maize and rice starches. This is a result of a high concentration (0.08%) of the phosphate derivatives of the potato starch. The repulsion between negative charges of the phosphate groups destabilizes the double-helical structure of potato starch and decreases the gelatinization temperature.

Starch gelatinization temperature can be determined by observing the loss of Maltese cross using a light microscope equipped with a polarizer and a hot stage, measuring the transparency of a starch suspension during heating, or using a DSC. The DSC can measure both the temperature and enthalpy change of starch gelatinization, which is an endothermic reaction. To obtain a consistent gelatinization temperature of starch, it needs to have at least two times (w/w) water. Without sufficient water, starch is gelatinized at a higher temperature with a broader temperature range. Without the presence of water or other plasticizers, starch will not gelatinize by heat but will be thermally decomposed at a temperature above 250 °C.

DSC thermograms of cereal normal starch show an additional thermal transition peak at temperatures above 90 °C, corresponding to the dissociation of ALC. Dissociation

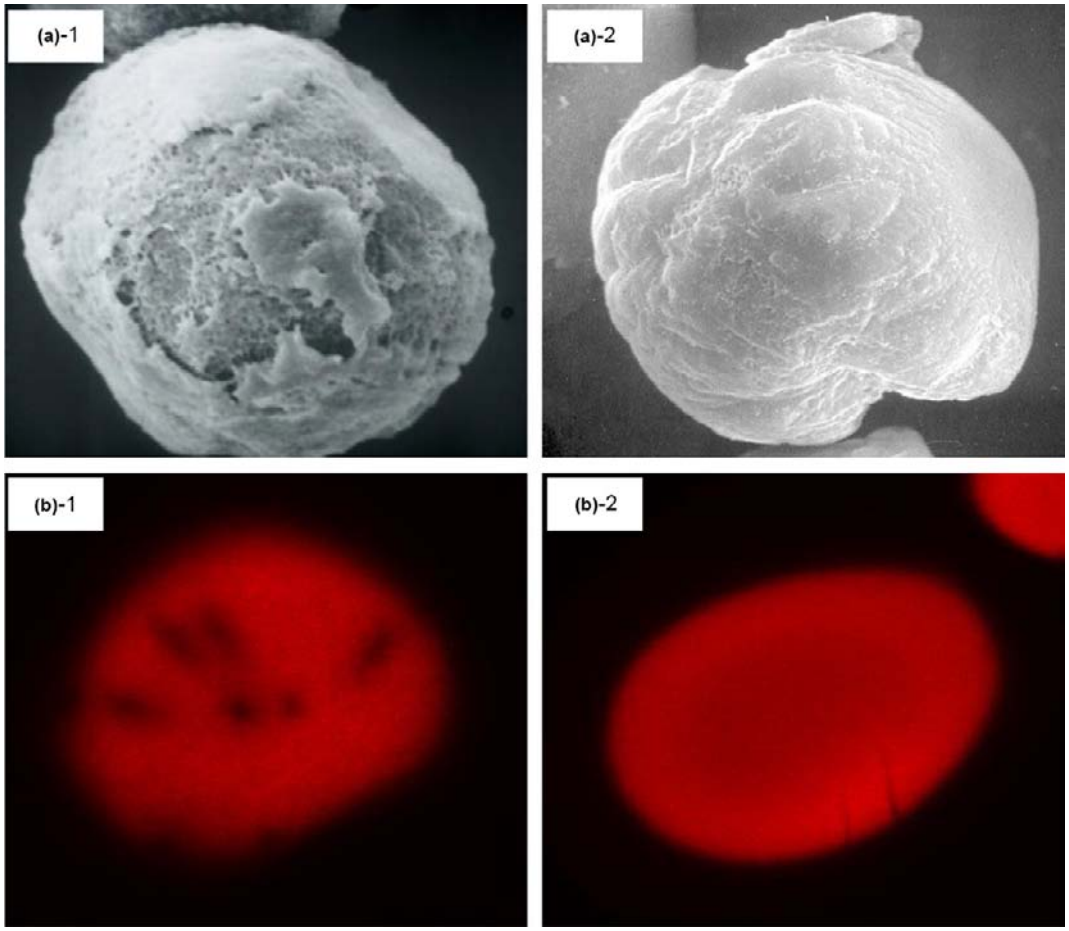

Figure 4 (a) Scanning electron microscopy (SEM) image of surface-gelatinized normal maize starch (1) and potato starch (2). The granules have about 65% surface starch removed. (b) Confocal laser scanning microscopy images of normal maize starch (1) and potato starch (2). Reprinted from Jane, J. (2006). Current understanding on starch granule structures. *Journal of Applied Glycoscience* **53**, 205–213.

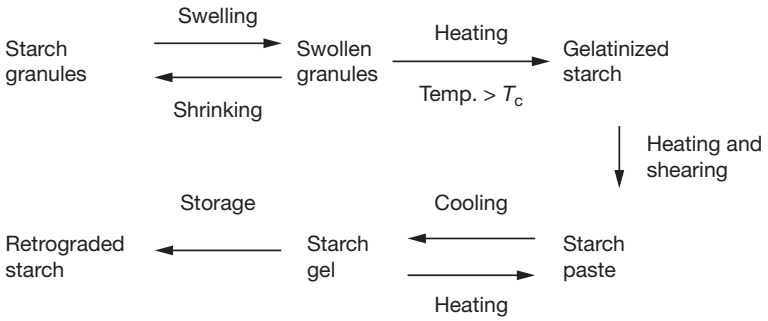

Figure 5 Transformation of starch structures.

temperature of the ALC, in general, increases with increasing chain length of the fatty acid and decreases with an increasing number of double bonds. Among the unsaturated fatty acids, the fatty acids with *trans*-double bonds form ALC with a higher dissociation temperature than those with *cis*-double bonds.

Starch gelatinization can also be achieved by using chemical solutions, such as aqueous alkaline solutions (e.g., NaOH and KOH), dimethyl sulfoxide, and various neutral salt solutions (e.g., KI, KSCN, $CaCl_2$, and LiCl). Aqueous solutions of NaOH and KOH (0.5 M or higher) and dimethyl sulfoxide (90%) are commonly used to gelatinize and disperse starch. A concentrated sodium sulfate solution, however, can be used to increase the gelatinization temperature of starch.

Pasting Property

When gelatinized starch is continuously heated in excess water with shearing, starch granules swell to develop viscosity and some starch molecules are dispersed in the aqueous medium (Figure 5). The viscosity development in this process is known

Table 1 Gelatinization properties of starches with different polymorphic structures analyzed using differential scanning calorimetry

Type	T_o (°C)[a]	T_p (°C)	T_c (°C)	*Range (°C)*	ΔH (J g^{-1})
A-type starch					
Normal maize	64.1 ± 0.2	69.4 ± 0.1	74.9 ± 0.6	10.8	12.3 ± 0.0
Waxy maize	64.2 ± 0.2	69.2 ± 0.0	74.6 ± 0.4	10.4	15.4 ± 0.0
du Waxy maize	66.1 ± 0.5	74.2 ± 0.4	80.5 ± 0.2	14.4	15.6 ± 0.2
Normal rice	70.3 ± 0.2	76.2 ± 0.0	80.2 ± 0.0	9.9	13.2 ± 0.6
Waxy rice	56.9 ± 0.3	63.2 ± 0.3	70.3 ± 0.7	13.4	15.4 ± 0.2
Sweet rice	58.6 ± 0.2	64.7 ± 0.0	71.4 ± 0.5	12.8	13.4 ± 0.6
Wheat	57.1 ± 0.3	61.6 ± 0.2	66.2 ± 0.3	9.1	10.7 ± 0.2
Barley	56.3 ± 0.0	59.5 ± 0.0	62.9 ± 0.1	6.6	10.0 ± 0.3
Waxy amaranth	66.7 ± 0.2	70.2 ± 0.2	75.2 ± 0.4	8.5	16.3 ± 0.2
Cattail millet	67.1 ± 0.0	71.7 ± 0.0	75.6 ± 0.0	8.5	14.4 ± 0.3
Mung bean	60.0 ± 0.4	65.3 ± 0.4	71.5 ± 0.4	11.5	11.4 ± 0.5
Chinese taro	67.3 ± 0.1	72.9 ± 0.1	79.8 ± 0.2	12.5	15.0 ± 0.5
Tapioca	64.3 ± 0.1	68.3 ± 0.2	74.4 ± 0.1	10.1	14.7 ± 0.7
B-type starch					
ae Waxy maize	71.5 ± 0.2	81.0 ± 1.7	97.2 ± 0.8	25.7	22.0 ± 0.3
Amylomaize V	71.0 ± 0.4	81.3 ± 0.4	112.6 ± 1.2	41.6	19.5 ± 1.5
Amylomaize VII	70.6 ± 0.3	N.D.[b]	129.4 ± 2.0	58.8	16.2 ± 0.8
Potato	58.2 ± 0.1	62.6 ± 0.1	67.7 ± 0.1	9.5	15.8 ± 1.2
Green leaf canna	59.3 ± 0.3	65.4 ± 0.4	80.3 ± 0.3	21.0	15.5 ± 0.4
C-type starch					
Lotus root	60.6 ± 0.0	66.2 ± 0.0	71.1 ± 0.2	10.5	13.5 ± 0.1
Green banana	68.6 ± 0.2	72.0 ± 0.2	76.1 ± 0.4	7.5	17.2 ± 0.1
Water chestnut	58.7 ± 0.5	70.1 ± 0.1	82.8 ± 0.2	24.1	13.6 ± 0.5

[a] T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; $T_c - T_o$, range of gelatinization; ΔH, enthalpy change.
[b] Not detectable.
Source: Jane, J., et al. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry* **76**(5), 629–637.

Table 2 Pasting properties of starches measured using a Rapid Visco Analyzer with 8% (dry starch basis) starch content

		Viscosity (RVU)			
Starch	*Pasting temperature (°C)*	*Peak*	*Hot paste*	*Final*	*Setback*
Normal maize	82.0	152	95	169	74
Waxy maize	69.5	205	84	100	16
du Waxy maize	75.7	109	77	99	22
ae Waxy maize	83.2	162	150	190	40
Waxy rice	64.1	205	84	100	16
Sweet rice	64.6	219	100	128	28
Normal rice	79.9	113	96	160	64
Wheat	88.6	104	75	154	79
Barley	91.2	88	58	116	58
Waxy amaranth	70.2	125	75	86	11
Cattail millet	74.2	201	80	208	128
Chinese taro	73.1	171	88	161	73
Tapioca	67.6	173	61	107	46
Lotus root	67.4	307	84	138	54
Potato	63.5	702	165	231	66
Mung bean	73.8	186	161	363	202
Green banana	74.0	250	194	272	78
Water chestnut	74.3	61	16	27	11

RVU, rapid visco unit.
Source: Jane, J., et al. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry* **76**(5), 629–637.

as the pasting property. Pasting property of starch is very important for many applications of starch, such as for thickening agents and sizing agents, and different starch varieties display different viscosities and pasting temperatures. Pasting property of starch is commonly measured using an amylograph, such as Brabender amylograph, and Rapid Visco Analyzer (RVA).

Pasting properties of common starches analyzed using an RVA are summarized in Table 2. Amylopectin is primarily responsible for the swelling power of starch granules and

viscosity. Amylose molecules, particularly with the presence of lipids, tend to restrict the swelling of granules. Consequently, normal maize starch that has 29.4% amylose and around 1% lipids shows a higher pasting temperature (82.0 °C) and a lower peak viscosity (152 rapid visco units, RVU) than its waxy counterpart (0% amylose, 69.5 °C and 205 RVU, respectively). Amylose molecules, however, are responsible for increasing the setback viscosity upon cooling of the starch paste, resulting from the network formation between amylose and amylopectin molecules. For example, normal maize starch has a setback viscosity of 74 RVU, which is larger than the waxy maize starch, 16 RVU.

ALC formed in starch granules renders entanglements between amylose and amylopectin molecules and restricts the swelling of granules during cooking. Consequently, it increases the pasting temperatures, decreases the pasting viscosities, and results in an opaque appearance of the starch paste. Among all the cereal normal starches, wheat and barley starches display the highest pasting temperatures and the lowest peak viscosities (Table 2) because both have high concentrations of phospholipids, which are readily available for the complex formation. After removal of endogenous lipids using detergents (e.g., sodium dodecyl sulfate), wheat starch shows a pasting temperature and peak viscosity similar to tapioca starch. In contrast, potato starch has a remarkably low pasting temperature (63.5 °C) and great peak viscosity (702 RVU) (Table 2), resulting from its large content of phosphate monoester derivatives. Repulsion between the negative charges of the phosphate monoester groups enhances the swelling of starch granules and increases the peak viscosity and clarity of the paste. The large granule size of the potato starch with a diameter up to 75 μm (Figure 2) also contributes to the great peak viscosity.

Gel Formation

After cooling and storing a paste of 5–15% starch, swollen starch granules in the paste fill up the volume, deform one another, adhered by amylose leached out from the swollen granules, and hold the shape of the mold to form a gel (Figure 5). To maintain the volume of swollen starch granules, amylose and amylopectin molecules must interact with one another to form a network to hold water in the swollen granules. Cereal normal starches, such as normal maize, wheat, and rice starches, are known to retain rigid swollen granules and form strong gels. Waxy starch, normal potato starch, and tapioca starch tend to freely swell and disperse after cooking because of the lack of amylose or lipids to facilitate stable interactions between amylose and amylopectin in the granules. Consequently, those starches cannot form gels. After being thoroughly mixed with lipids, including soy lecithin, oleic acid, and linoleic acid, before cooking, the mixtures of tapioca starch and lipid can develop gels. This result suggests that the formation of ALC facilitates molecular interactions in the starch granules, which restrict the swelling and maintain the integrity of swollen granules for the gel development. Potato starch granules with a prior heat-and-moisture treatment or chemical cross-linking can also develop a gel. Starch gels are formed by storing starch pastes at a refrigeration temperature for a selected period of time, and gel strength can be measured using a texture analyzer.

Starch Retrogradation/Syneresis

After prolonged storage, amorphous starch molecules in the aqueous medium form double-helical crystalline structures and lose their water-binding capacity (Figure 5). This process is known as starch retrogradation, as shown in staled bread and syneresis of gravy. The rate of starch retrogradation can be increased by increasing the concentration of starch in the paste, storing the starch paste at 0–5 °C, increasing amylose (linear molecule) content, having chain length of DP 80–100, and increasing the branch chain length of amylopectin. Cereal normal starch in a paste or a gel tends to retrograde faster than tuber and root starches because of the limited swelling of starch granules. Molecules of cereal normal starch are located in close proximity within the swollen granules, which facilitate the retrogradation.

Chemical derivatives of starch, such as acetyl and hydroxypropyl groups, retard starch retrogradation. When the starch paste or gel is stored below the glass-transition temperature (T_g), the starch retrogradation is retarded because of the lack of molecular mobility. Addition of complexing agents, such as monoglycerides, to bread making can reduce the retrogradation rate of the bread because of the single-helical complex formed with the monoglycerides. When the hydrophobic sides of starch molecules are bound with the complexing agent, it retards the double-helical formation.

Starch Digestibility

Starch, a major energy source, can be hydrolyzed by amylolytic enzymes to generate glucose to support lives of plants, animals, and humans. Starch granules have semicrystalline structures and are less readily hydrolyzed by enzymes. After starch is gelatinized and becomes amorphous, starch molecules can be hydrolyzed quickly.

The enzymatic digestive rate of granular starch is determined by factors including polymorphism/amylopectin structure, amylose content, and size of starch granules. Starch granules with the A-type polymorphs (e.g., normal wheat, rice, and maize starches) are hydrolyzed much faster than those of the B-type (e.g., potato and high-amylose maize starches). Starch with the C-type polymorph (e.g., green banana starch) displays a hydrolysis rate in between (Figure 6). The greater hydrolysis rate of the A-type starch is attributed to the large proportions of short branch chains of the amylopectin (DP 6–12) and the porous structure of the granule (Figure 4). For starch granules with the same type of polymorph, the enzymatic hydrolysis rate decreases with increased amylose content; for example, waxy starches are hydrolyzed fast, whereas high-amylose barley starch and mung bean starch are hydrolyzed more slowly (Figure 6). This could be the result of amylose restricting the swelling of the starch granules and concentrating at the periphery to form a hard shell, which reduces the accessibility of starch granules to enzymatic hydrolysis. Small-granule starches, such as amaranth and rice starches, are more susceptible to enzymatic hydrolysis (Figure 6) because they have a larger relative surface area.

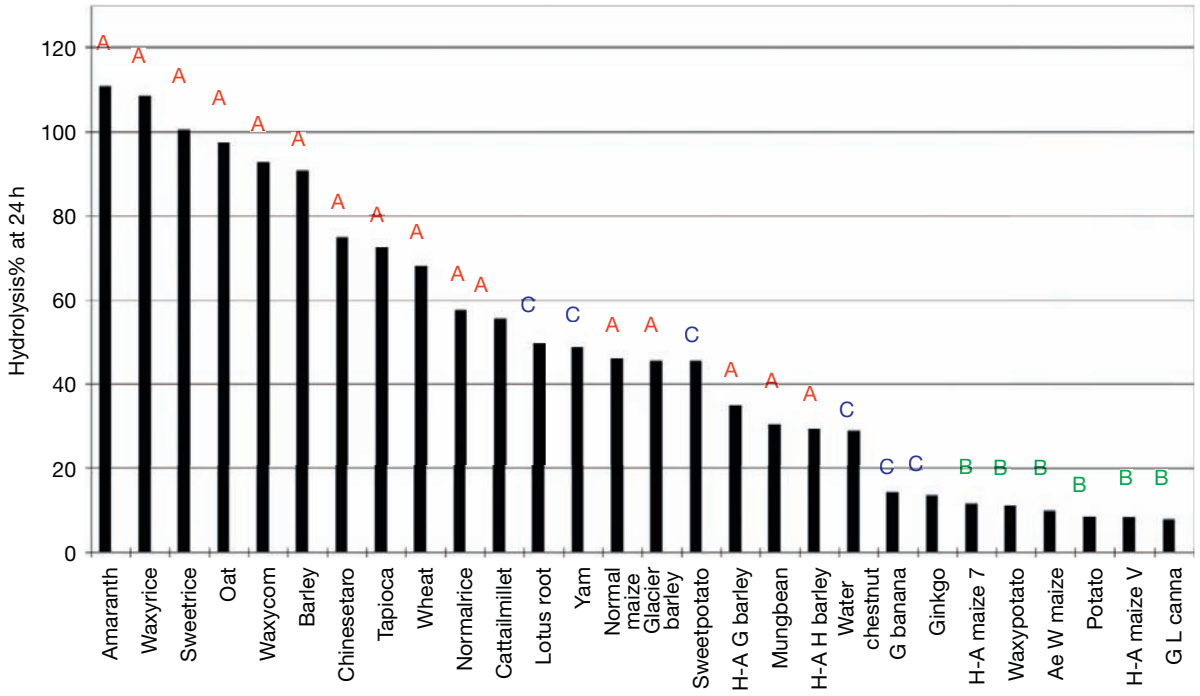

Figure 6 Percentages enzymatic hydrolysis of starches with different polymorphic structures. Porcine pancreatic α-amylase (120 U/20 mg starch in 9 ml phosphate buffer, pH 6.9) was used to hydrolyze starch at 37 °C for 24 h. The percentage enzymatic hydrolysis was determined by measuring the total carbohydrate content in the supernatant using a phenol–sulfuric acid method. The letter above each bar indicates the x-ray diffraction pattern of the starch. Reprinted from Jane, J., Aol, Z., Duvick, S. A., Wiklund, M., Yoo, S. -H., Wong, K. -S., and Gardner C. (2003). Structures of amylopectin and starch granules: how are they synthesized? *Journal of Applied Glycoscience* **50**, 167–172.

The digestive rate of starch in a diet is measured as the glycemic response, which determines the blood glucose concentration. A consistently high blood glucose concentration can cause insulin resistance and type-2 diabetes. The enzymatic hydrolysis rate of starch in a diet is determined by factors including the presence of retrograded starch, chemical derivatives of the starch, formation of ALC, and presence of cellulosic materials and the protein matrix. Retrograded starch is resistant to enzymatic hydrolysis. Chemically modified starch (e.g., cross-linked and octenyl succinic starch) is less hydrolyzable. Lamellar crystallites of ALC are protected from enzymatic hydrolysis. The ALC can also restrict the swelling of gelatinized starch and reduce the enzymatic hydrolysis rate of the starch. Type 5 resistant starch (RS5) is made by complexing high-amylose maize starch with free fatty acids. After human subjects were fed with bread made with 60% of the RS5, it significantly reduced postprandial glucose concentration (55%) and the insulin response (43%) compared with 100% for human subjects fed with white bread.

DSC thermograms and x-ray diffraction studies show no complex formation between amylose and triglycerides, a major component of lipids in foods. Enzymatic hydrolyses of normal- and high-amylose starch cooked with corn oil (triglycerides), however, show substantial decreases in the hydrolysis rate of the starch. The effect is not observed with waxy maize starch. ^{13}C-NMR spectra confirm the formation of the amylose helical complex between amylose and corn oil. Cooking rice with corn oil to prepare fried rice and rice pilaf decreases the starch hydrolysis rate. Surrounding of starch by cellulosic materials and protein matrix also reduces its susceptibility to enzymatic hydrolysis.

See also: Amaranth; Bananas and Plantains; Barley; Beer: Fermentation; Biofilms; Biscuits, Cookies, and Crackers: Chemistry and Manufacture; Bread: Chemistry of Baking; Browning: Non-enzymatic browning; Carbohydrate: Digestion, Absorption and Metabolism; Cassava: The Nature and Uses; Cereals: Dietary Importance; Cereals: Types and Composition; Chapatis and Related Products; Chilled Foods: Effects on Shelf-life and Sensory Quality; Cooking: Domestic Techniques; Fatty Acids: Fatty Acids; Fructose and High-Fructose Corn Syrup; Glucose: Metabolism and Regulation; Legumes in the Diet; Maize; Microscopy: Light Microscopy and Histochemical Methods; Millets; Pasta: Manufacture and Composition; Phospholipids: Properties and Occurrence; Potatoes and Related Crops; Pulsed Electric Fields; Rice: Types and Composition; Sorghum: A Novel and Healthy Food; Starch: Modified Starches; Starch: Sources and Processing; Starch; Tortillas; Triacylglycerols: Structures and Properties; Wheat: Grain Structure of Wheat and Wheat-based Products.

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Sterilization of Foods

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Nomenclature

A	Area, m^2
B	Thermal processing heating time, min
b'	Slope parameter. Weibull kinetic model, $(min^{-1})^n$
C_P	Heat capacity at constant pressure, J mol^{-1} K^{-1}
CUT	Come-up time, time needed to reach the operating heat-processing temperature, min
D_T	Decimal reduction time describing lethality at a constant temperature, min
D_{Tref}	Decimal reduction time describing lethality at a reference temperature, min
F_0	Lethal heat-processing time at a reference temperature typically set at 121.1 °C (250 °F), min
F^{zT}_{Tref}	Lethal heat-processing time for unsteady-state food-processing time, min
f	Heating rate constant on the log T versus processing time plot
h	Convection heat transfer coefficient, W m^{-2} K^{-1}
K	Kinetic rate constant, min^{-1}
L	Lethality given by a thermal food process
m	Mass, kg
N	Microbial population, cfu ml^{-1}, cfu g^{-1}
N_0	Microbial population prior to thermal treatments, cfu ml^{-1}, cfu g^{-1}
n	Exponent. Weibull kinetic parameter
SV	Number of decimal reductions achieved by thermal treatments
T	Temperature, °C, °F
T_0	Initial food product temperature, °C, °F
T_{0e}	Extrapolated food product temperature in the linear approximation of temperature as a function of heat-processing time, °C, °F
T_R	Retort temperature, °C, °F
T_{ref}	Reference temperature, °C, °F
t	Time, min
t_C	Thermal processing cooling time, min
z_T	Thermal resistance parameter, °C, °F

Introduction

Nicolas Appert and Louis Pasteur provided the basis for thermal food processing in the nineteenth century, and more than 200 years later, heat processing remains as a reference for food preservation. According to the FDA 'Guidance for Industry: Acidified Foods,' the definition of 'commercial sterility' stands for the condition achieved by "the application of heat which renders the food free of microorganisms capable of reproducing in the food under normal nonrefrigerated conditions of storage and distribution and free of viable microorganisms (including spores) of public health significance." Nonthermal food-processing technologies have become popular during the past 25 years, but most of them need further improvements to be considered at an industrial scale. Even widely accepted novel food technologies such as high-pressure processing lack the broad kinetic databases or standardized procedures developed for thermal food processing, particularly for the canning industry. Most of the theoretical basis for current research and industrial thermal processing design of 'sterile' food products was proposed in the beginning of the twentieth century, remaining as an important guideline for any food engineer.

The first step in the thermal process design is to define the objective. Process objectives are set by prioritizing the effects of a pathogen on human health and the possibility of causing a food-borne disease outbreak. If pathogens are not likely to be present as in the case of most acid foods (pH < 4.5), the processing objective is now defined to control the growth of spoilage microorganisms and enzyme activity or to avoid nutrient degradation or sensorial quality losses. Usually, the objective is defined in terms of the desired number of microbial, enzyme, nutrient, or quality factor decimal reductions (SV). Once the processing objective is defined, kinetic mathematical models are developed to determine the degradation rate of the microorganism, enzyme, nutrient, or quality factor at different temperatures. Temperature evolution of the sample and the heating media must be also analyzed, since variations occur due to the diversity of intrinsic thermodynamic and geometric features of food products and containers. Finally, all the kinetic model and temperature evolution information is analyzed using standardized charts, tabulated data, and/or mathematical procedures developed during the past 100 years to establish the necessary operating conditions to achieve commercial food sterilization. Mastering such an important task requires an in-depth analysis of kinetics, thermodynamics, and transport phenomena, and only the most fundamental aspects to analyze and develop commercial food sterilization processes are briefly presented in this contribution.

Microbial Inactivation Kinetic Models

First-Order Kinetics

The first-order microbial inactivation kinetics states that the change of the microbial population (N) as a function of time (t) at constant temperature is directly proportional to a kinetic rate constant k (eqn [1]). The first-order kinetic model is obtained by integrating from $t=0$ to treatment time t and from $N(t=0)=N_0$ to the microbial population at $N(t)$. The first-order kinetic model can be further modified by changing the Napierian logarithm to a $\log_{10}$ base to report the decimal reductions of a microorganism (eqn [2]). This way, the decimal

reduction time (D_T) is defined as the time required to reduce one-tenth of the microbial population at a constant lethal temperature (eqn [3]). The magnitude of the decimal reduction time determines the thermoresistance of a particular microorganism – the higher the value of D_T, the longer the duration of the heat treatment required to inactivate the microbial population:

$$\frac{dN}{dt} = -kN \quad [1]$$

$$\log_{10}\frac{N}{N_0} = -\frac{k}{2.303}t \quad [2]$$

$$D_T = \frac{2.303}{k} \quad [3]$$

The parameter D_T widely varies among different media, microorganism properties (physiological state, strain, etc.), and the selected processing temperature. Parameters k and D_T are not exclusive for microbial inactivation kinetics and may also be used to describe enzyme inactivation or chemical compound degradation kinetics. Therefore, the processing time (F_T) at a constant temperature required to achieve a defined objective can be found by multiplying the desired number of decimal reductions (SV) by the decimal reduction time (D_T) at a constant temperature (eqn [4]). Twelve decimal reductions (SV = 12D) of *Clostridium botulinum* spores are typically required by agencies validating the food sterilization processes. The main concern about *C. botulinum* is the production of the botulinum toxin that causes severe nerve paralysis that may result in death. *C. botulinum* is commonly found in soil and is capable of germinating in low-acid foods (pH > 4.6). These 12D reductions were set by defining the survival probability of *C. botulinum spores* as 1 spore in 1000 billion containers (10^{12}) and have remained a standard for the canning industry:

$$F_T = SV \cdot D_T \quad [4]$$

Temperature Dependence of Microbial Inactivation Kinetic Parameters

Bigelow established the term 'thermal death time' (D) to describe the amount of time to inactivate the microbial population at a constant temperature and pH. The author was also the first to observe the linear dependence of the thermal death time logarithm with the process temperature (Figure 1). The negative inverse slope of this plot, parameter z_T, is the temperature change required to increase or decrease the decimal reduction time by 10 (eqn [5]):

$$\log_{10}\frac{D}{D_{ref}} = -\frac{T - T_{ref}}{z_T} \quad [5]$$

The reference temperature (T_{ref}) for heat sterilization of low-acid foods is usually set as 121.1 °C (250 °F), since it is the minimum temperature level to eliminate *C. botulinum* spores in most media. These reference conditions usually prevail even if *C. botulinum* spores are not present in the media, reflecting the concern on the possible severe adverse health effects caused by the pathogen. The subscript of both the decimal reduction time and thermal processing time is set as

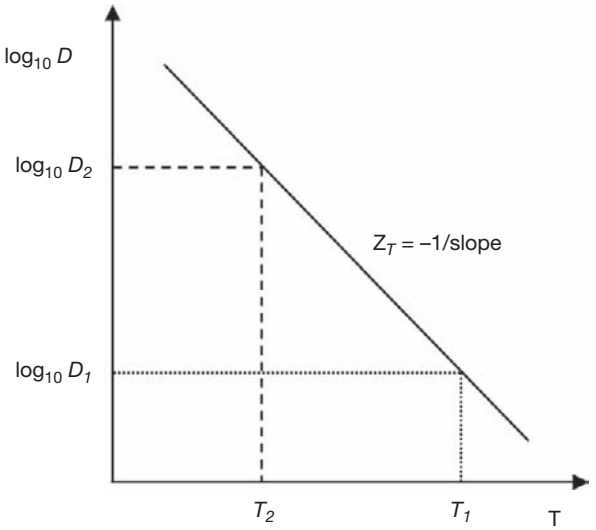

Figure 1 Graphical representation of the thermal resistance constant (z_T).

subscript zero (*0*), to denote that the kinetic parameters were obtained at the reference temperature level (D_0, F_0). Nevertheless, not all food products are able to withstand a thermal process at such high temperature levels, but regulations stood that *C. botulinum* spore destruction should remain as a reference. As a result, food scientists defined the lethality of a thermal process (L, eqn [6]) as the processing time at a temperature level T required to yield the same lethality (microbial decimal reductions) as a 1 min heat treatment at 121.1 °C (250 °F) as shown in Table 1. The *C. botulinum* spores are not capable of surviving at pH < 4.6, for which the biggest concern of thermal processing of medium- or high-acidity foods focuses on microorganisms that are known for food spoilage:

$$L = \frac{F_T}{F_0} = \frac{SV \cdot D_T}{SV \cdot D_0} = 10^{\left(-\frac{T - T_0}{z_T}\right)} \quad [6]$$

Industrial Food Design

General Method

The desired temperature level is not instantaneously reached during heating nor immediately brought back to room temperature on the subsequent cooling cycle. Thus, the complete temperature–time history must be considered when addressing microbial inactivation kinetics to avoid under- or over-processing of foods. Bigelow figured out that the area below the curve of the lethality versus process time plot corresponded to the F value considering the heating and cooling cycles (Figure 2), which can alternatively be represented as the integral shown in eqn [8]. In this case, F values are reported with a superscript related to the thermal resistance (z_T) of the target microorganism, enzyme, or chemical compound and a subscript indicating the temperature level used as a reference to yield a process with equivalent lethality. This procedure was later known as the 'general method' since the calculation applies for any type or shape of either the food product or

Table 1 FDA thermal-resistant data for bacteria in the heat processing of foods

Class of food	*Type of microorganism*	*Microorganism*	*Reference temperature (°F)*	D_T *(min)*	z_T *(°F)*
Low-acid food (pH > 4.6)	Thermophiles	Flat-sour group (*Bacillus stearothermophilus*)	250	4.0–5.0	14–22
	Mesophiles (spores)	Putrefactive anaerobes (*C. botulinum* types A and B)	250	0.10–0.20	14–18
		Clostridium sporogenes group (including P.A. 3679)	250	0.10–1.50	14–18
Acid food and acidified food (pH 4.0–4.6)	Thermophiles (spores)	*Bacillus coagulans* (facultative mesophilic)	250	0.01–0.07	14–18
	Mesophiles (spores)	*Bacillus polymyxa* and *Bacillus macerans*	212	0.10–0.50	12–16
		Butyric anaerobes (*Clostridium pasteurianum*)	212	0.10–0.50	12–16
		Bacillus licheniformis	200	4.5	27
Acid food and acidified food (pH < 4.0)	Yeasts, molds, and mesophilic nonspore-bearing bacteria	*Lactobacillus* species, *Leuconostoc* species	150	0.50–1.00	8–10

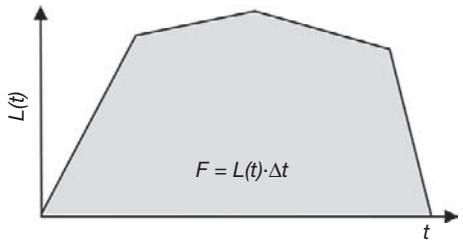

Figure 2 Calculating a heat process lethality with the general method.

container. The only requirements for the 'general method' consist in the knowledge of the temperature profile of the food product and the kinetic values (D_T and z_T) for the microbial, enzyme, nutrient, or quality factor used to design the thermal process. The area under the curve is integrated, and the temperature–time history is modified until it meets the specified F, increasing or reducing the process time. Nevertheless, this main advantage also results in an important drawback, since the predictions will only be available for the currently used temperature profile. If the heat treatment conditions or food or container properties are changed, a new temperature–time profile of the food product must be obtained, and the integration optimized to meet the new processing objectives. Heat processing times vary depending on the type of food and the container size shown for F_0 values ranging from 2 to 15 min (Table 2):

$$F_0^{z_T} = \int_0^t 10^{\frac{T(t)-T_0}{z_T}} dt \qquad [7]$$

Formula Method

The lack of computational tools during the development of the 'general method' made the application of this procedure a laborious, unpractical task for thermal food process design. Colin O. Ball and collaborators partially solved the issue by providing a set of formulas based on experimental data of the unsteady-state heat transfer between the can and the water vapor heating fluid. The formula method is useful in determining a thermal process effectivity using heat penetration data or by defining the correct temperature–time conditions to achieve a certain lethal value F_0.

Table 2 Calculated sterilizing values (F_0) for some current commercial processes according to the American Can Company

Product	*Can sizes*	*Approximated calculated sterilizing value,* F_0 *(min)*
Asparagus	All	2–4
Green beans, brine-packed	No. 2	3.5
	No. 10	6
Chicken-boned	All	6–8
Corn, whole kernel, brine-packed	No. 2	9
	No. 10	15
Cream-style corn	No. 2	5-6
	No. 10	2
Dog food	No. 2	6
	No. 10	6
Mackerel in brine	No. 10	2.9–3.6
Meat loaf	No. 2	6
Peas, brine-packed	No. 2	7
	No. 10	11
Sausage, Vienna, in brine	Various	5
Chili con carne	Various	6

The heating process is first split into the heating and cooling cycles, and the F_i value given in eqn [9] is expressed as in eqns [9] and [10], going from $t = 0$ to the time in which the heating step is finished ($t = B$) and subsequently cooled down until the food product is at room temperature for $t = t_c$:

$$F_i = F_H + F_C \qquad [8]$$

$$F_i = \int_0^B 10^{[T(t)-T_0]/z_T} + \int_B^{t_C} 10^{[T(t)-T_0]/z_T} \qquad [9]$$

Ball's formula method states that microorganisms have a greater chance of surviving at the region in which the food product has the lowest temperature, which is frequently specified to be located at the center of the food product. Ball assumed that the temperature difference between the retort or heating media temperature (T_R) and the center of the container decays exponentially as a function of time. However, the

assumption where the coldest spot is located in the center of the food product is only true for solid foods with a high thermal conductivity for which heat is only transferred by conduction. In the case of liquid foods, heat is mostly transferred through convection mechanisms that generate fluid flow within the container. Thus, the coldest spot of the food product is not necessarily constant, but may be found near the bottom of the can container.

The semilog plot of the temperature profile in the coldest spot against time will yield fairly straight curves that can be approximated with a linear model (eqn [10]; **Figure 3**). Most parameters of eqn [10] can be obtained with the temperature profile shown in **Figure 3**. The plot shown in **Figure 3** may be constructed with the recorded logarithm of the can temperature on the left y-axis (T_{can}; °F or °C), the logarithm difference of the retort heating medium temperature, and the temperature of the coldest point on the right y-axis ($\log_{10}(T_{retort} - T_{can})$), against the heating time on the x-axis (t_h). The temperature profile is not linear at the beginning of the heating curve, but in most cases, a straight line can be drawn across the majority of the experimental data (**Figure 3**). Parameter (f_h) is of significant importance, since it establishes the time required to cover one log cycle of the straight line drawn across the $\log_{10}(T_{retort} - T_{can})$ data (**Figure 3**). Parameter I_h is defined as the difference between the heating medium and the initial product temperature (T_i; eqn [9]), whereas g_h (eqn [10]) corresponds to the temperature difference between the heating medium and the maximum temperature recorded in the coldest point of the can during the heating cycle (T_{max}). The last parameter of Ball's formula method (j_h) corresponds to a correction factor for the initial nonlinear part of the heating curve. The food product temperature does not instantaneously meet the heating media temperature of the retort, and the time interval in which the heating medium temperature rises from ambient temperature to the desired retort operation condition is known as the come-up time (CUT). In practice, it is considered that only 40% of the value of CUT has a lethality, equivalent to the one it would have if the heating medium temperature process instantaneously reaches lethal temperature levels. If CUT = 3 min, only the heat penetration data beyond 60% of the CUT (0.6 (3 min) = 1.8 min) is considered to be lethal. Therefore, the pseudo-initial temperature (T_{ie}) used to calculate j_h (eqn [12]) can be found by extrapolating the linear portion of the $\log_{10} T_{can}$ curve to $t = 1.8$ min (**Figure 3**), the time at which the heating process is considered to begin its lethality:

$$t_h = f_h \log\left(\frac{j_h I_h}{g}\right) \quad [10]$$

$$I_h = T_R - T_i \quad [11]$$

$$g_h = T_R - T_{can} \quad [12]$$

$$j_h = \frac{T_R - T_{ie}}{T_R - T_i} \quad [13]$$

The lethality factor F_i for the current sterilization process is compared with the lethality factor established under the previously defined temperature and time reference conditions (F_0), to determine its equivalent microbial inactivation through parameter U (eqn [14]). Therefore, parameter U corresponds to the F value at the heating media temperature of the current heat sterilization process:

$$U = F_0 \cdot F_i = F_0 10^{(T_{ref} - T_R)/z_T} \quad [14]$$

Improvements on Ball's formula method. All parameters of Ball's formula method may be calculated from the heat penetration curves except for g_h, since it may be difficult to determine at first glance the maximum temperature the food product must meet to achieve the desired sterilization level. Stumbo and Hayakawa generated charts and tables reporting with different f_h/U, j_h combinations that have been created to quickly calculate $\log_{10} g_h$. Stumbo overcame some of the limitations of Ball's formula method by covering a wider range of microbial thermal resistance parameter (z_T) and heat penetration data (parameter j_h) to determine the g_h value while

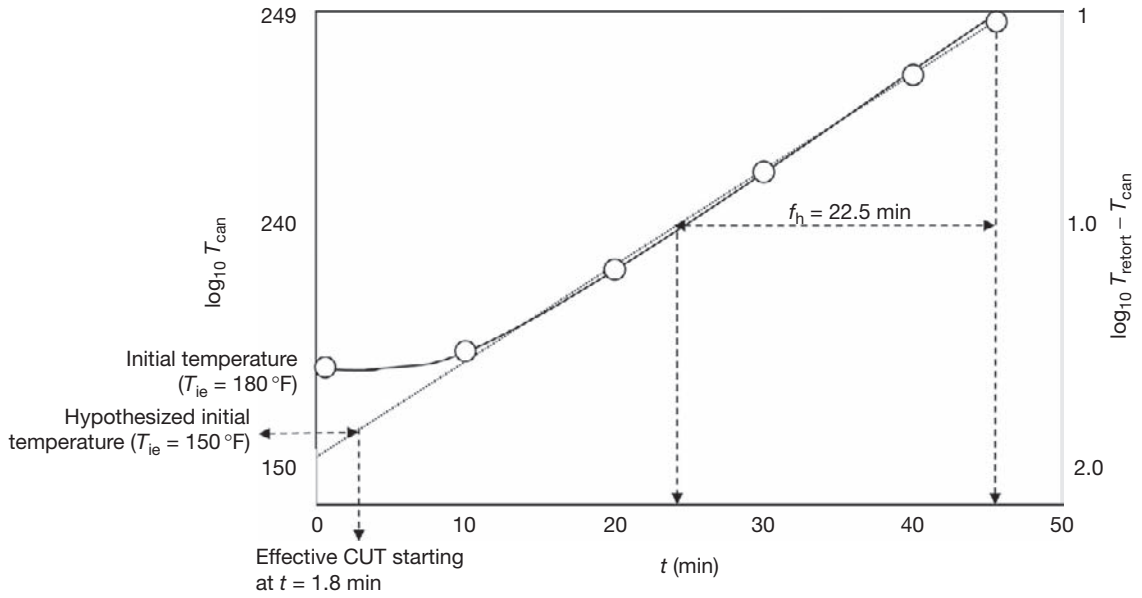

Figure 3 Heat penetration curve and Ball's formula method parameters.

Table 3 Tabulated values of f_h/U for some thermal process calculations generated with Stumbo's procedure

	$z_T = 14\ °F$		$z_T = 18\ °F$		$z_T = 22\ °F$	
f_h/U	g	$\triangle g/\triangle j$	g	$\triangle g/\triangle j$	g	$\triangle g/\triangle j$
0.2	0.0000910	0.0000118	0.0000509	0.0000168	0.0000616	0.0000226
0.5	0.0396	0.0111	0.0506	0.0159	0.065	0.0197
1	0.438	0.009	0.523	0.192	0.685	0.196
5	4.02	1.32	5.40	1.59	6.51	2.39
10	6.55	2.22	8.78	2.69	10.67	3.77
25	10.7	3.18	13.60	4.80	16.9	6.10
50	14.2	4.00	17.7	6.40	21.9	8.3
100	17.6	5.2	22.3	7.80	27.0	10.4
200	20.8	6.7	27.1	9.10	32.7	12.1

assuming that the heating and cooling curves remain equivalent. Stumbo calculated the lethality of heat processing for 57 tables containing 18 513 datasets, some of which are exemplified in Table 3. Linear interpolation is a common practice to determine a process lethality not listed in similar tabular data, although error might be introduced in the results due to the rounding of numbers during the calculations. Similarly, parameter g_h can be estimated through plots of factors f_h/U for different j_h values (data not shown).

The original formula method considered the lethality effects of the heating process equal to those of the cooling stage. Furthermore, the formula method was improved by calculating the lethality of the heating and cooling stages separately. Thus, a similar temperature history plot is developed for the cooling cycle, where T_c stands for the cooling media temperature, and the rest of Ball's formula method calculations remain almost the same, with the subindex changing from 'h' to 'c' as in cooling. Once more, a straight line is drawn trying to match most of the $\log_{10}(T - T_c)$ data. The main differences in Ball's formula parameter estimation relate to the initial nonlinear portion of the $\log_{10}(T - T_c)$, since the initial temperature of the cooling cycle corresponds to T_{max}, when the sterilization process has its maximum lethality. Consequently, the hypothesized cooling cycle initial temperature (T_{ie}) is now associated to the intercept of the straight line with the y-axis at $t_c = 0$. After the analysis of the heating and cooling cycles, their corresponding lethal times (F_H and F_C, respectively) are added to calculate the total operating time (F_i; eqn [8] and [9]). Hayakawa made a similar contribution as Stumbo, but tabulated the lethality factors for the heating and cooling cycles separately. Therefore, according to the method proposed by Hayakawa, $f_h \neq f_c$. An additional contribution made by Hayakawa is the simplification of temperature profiles by fragmenting the heating and cooling curves into several linear portions for which the lethality is more easily calculated. Canning industry experts suggest that the general method is more accurate, but Ball's formula method remains as the most popular choice mainly because of its simplicity and quickness.

Concerns of Current Practices for Industrial Food Process Design

Historically, Bigelow's kinetic model based on the D_T and z_T may be the most extensively recognized model in food microbiology. It continues to be used for industrial food process design and remains as a *must* cover topic in conferences and food science courses since it was proposed in the 1920s. However, the first-order kinetic model (eqn [2]) assumes a homogenous microbial population that proportionally reduced for equal time intervals, which is rarely true.

Several nonlinear models have been proposed, among which the cumulative form of the Weibull distribution is one of the most popular, reliable, and easy to use ($G(t)$; eqn [15]). The Weibull model has been employed in other engineering areas to predict the failure time of mechanical or electrical systems. In the case of microbial inactivation kinetics, the Weibull model adapts and determines the time at which the microorganism cells will fail to survive as a result of the applied stress conditions (eqn [16]). Parameter b' determines the rate at which the microorganisms decrease as in the case of the inactivation rate constant of the first-order kinetic model (k). The exponent (n; eqn [16]) reflects the resistance of the microbial population; for $n < 1$, the microbial survival curve shows 'tail formation' (downward concavity) indicating that the microorganisms are resistant to the current processing conditions. Conversely, when $n > 1$, the inactivation curve shows upward concavity suggesting that the accumulated damage makes the microorganisms susceptible to the treatment. If $n = 1$, eqn [16] transforms into the first-order kinetic model indicating a homogeneous inactivation for the microbial population. As in the case of the first-order kinetic model, an equivalent of the decimal reduction time (δ) can be developed as in eqn [17]:

$$G(t) = -\left(\frac{t}{\beta}\right)^{\alpha} \qquad [15]$$

$$\log_{10}\frac{N}{N_0} = -\frac{1}{2.303}\left(\frac{t}{b}\right)^{n};$$

$$b' = \frac{1}{2.303}\left(\frac{1}{b}\right)^{n}; \quad \log_{10}\frac{N}{N_0} = -b' t^{n} \qquad [16]$$

$$\log_{10}\frac{0.1N_0}{N_0} = -1 = -b' \cdot t^{n} \qquad [17]$$

$$\delta = \left(\frac{1}{b'}\right)^{n} = D \qquad [18]$$

Additionally, the linear temperature dependence of $\log_{10} D_T$ is usually seen for narrow temperature intervals. The 12D reductions were set by defining the survival probability of *C. botulinum spores* as 1 spore in 100 billion cans (10^{-12}), but food products

often show traces of spoilage at much lower concentration ($\approx 10^3 - 10^6$ cfu ml^{-1}) of any type of microorganism. The former results in data extrapolation of microbial kinetics that are also assumed to be 'linear' and the incorrect prediction of operating times. Consequently, food scientists and engineers opt to overprocess the food products by indiscriminately elevating the temperature or extending the duration of the treatment, severely affecting the sensorial and nutrimental properties.

Final Remarks

Preservation technologies will continue to play an important role in preserving food as the world population continues to grow. Despite the emergence of alternative nonthermal food technologies, thermal food processing will continue to remain as a standard for industrial needs. The challenge related to thermal food processing include the use of alternative, more environmentally friendly packages. The availability of scientific computational software and the advances in computer science also facilitate the use of more intensive calculation algorithms to enhance the extents of current kinetic, thermophysical, and transport phenomena modeling and to better comprehend these phenomena on foods.

See also: Canning: Process of Canning; Codex Alimentarius Commission: Role in International Food Standards Setting; Heat Treatment: Effect on Microbiological Changes and Shelf Life; Heat Treatment: Principles and Techniques; Pasteurization: Principles and Applications; Preservation of Foods.

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Storage Stability: Mechanisms of Degradation

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Background

Food products start to degrade as soon as they enter into the food chain, with the exception of certain dry seeds. The undesirable degradation of foods renders them unacceptable to the consumers due to safety and quality issues. The stability of foods during storage, either fresh or processed, is determined by their degradation rate, which is highly associated with the underlying physical, biological, and chemical causes. Unraveling the corresponding degradation mechanisms could help the food industry in finding effective means to protect food products and to extend their shelf life. Generally, this task is complicated by the large variety of existing food products and their different constituents. As an example, the storage stability of cake is affected by numerous factors, such as microbial spoilage (e.g., development of molds), crumb staling (dryness and other sensory defects), rancidity and other off-flavor development, crystallization of sugars (e.g., grittiness in cream fillings), syneresis of jams and jellies, chocolate bloom, structural weakness, color fade and change, and moisture migration and loss. Such factors are, in their turn, a function of the prevailing process parameters, determining the rate and intensity of the physical, biological, and chemical degradation mechanisms, which are described in this article.

Mechanisms of Physical Degradation

Physical changes include, for instance, softening or toughening, loss of solubility, loss of water holding capacity, wetting, lumpiness, or agglomeration, all of which mainly correspond to water migration and physical damage. Physical change itself results in undesirable textural or appearance changes of food products.

Water Migration and Loss

Water migration and loss, either appearing within the foods (such as staling) or via exchange with the environment (influx or drying), is the major physical mechanism affecting food quality and storage stability. It may result in hardness or in the development of a rubbery soft, spongy, or flabby character of foods, which have a negative effect on consumer acceptance.

Fresh foods, such as leafy vegetables, wither and soften during storage because of the changes in tissue tension upon water loss and turgor pressure, which leads to an increase in the ease of cell separation in many nonlignified tissues. The softening of acidic fruit tissues during heating has been ascribed to acid hydrolysis of cell wall polysaccharides. High-temperature heating of fruit and vegetable tissues causes an initial loss of instrumental firmness due to the fatal disruption of the cell membrane and an associated loss of turgor pressure. Such thermally induced disruption of the cell membrane in fruits and vegetable is linked to the β-eliminative degradation of pectin polysaccharides involved in cell adhesion. The latter degradation process is strongly influenced by pH and is enhanced considerably under increasing alkaline conditions. It is also strongly influenced by ions and organic acids, for example, Ca^{2+}, Mg^{2+}, K^{+} ions, citrate, malate, and phytate. Besides, thermal treatment of fruit and vegetable tissue is often accompanied by wall swelling. This may be due to the thermal degradation of polymers that laterally stabilize the wall, facilitating disruption of the cell membrane, and may also be affected by changes in the ionic composition of the cell wall. The ability of calcium to cross-link pectin, and thereby reduce its solubilization, provides a dual role for this ion as a promoter of pectin degradation through eliminative degradation while enhancing texture through cross-linking.

Water loss will also affect the storage stability of processed foods, predominantly in foods where low water activity is a major conservation hurdle. Crispness or crunchy texture is essential to the quality of a number of cereal and snack products. If the moisture content increases due to the absorption of water vapor or the mass transport of water from other domains, crispness is lost. Dry foods in a package that allows the influx of water vapor can show the transition from glass to rubber as indicated in Figure 1. Crispness is lost if water is gained to reach a water activity (aw) above 0.35–0.50 for crisp snack foods like chips, saltine crackers, and popped popcorn. Similarly, bread crumb, which initially is soft and resilient, gradually loses both of these essential quality attributes, which involves intermoisture migration from crumb to crust and water redistribution between bread components.

Control of moisture migration and loss is thus critical to the storage stability of foods. A wide range of film-forming compounds is now available and facilitates tailoring moisture barriers with optimized functional properties.

Physical Damage

Physical damage, mainly due to physical forces or changes, is another important cause of substantial degradation in food products. Physical forces include the shock, vibration, and compression forces experienced by the food products. Surface deterioration then provides an ideal entry point for many food pathogens and microorganisms, proliferating on leaked nutrients from damaged tissues. Damage also allows water loss, which compromises the quality of the foods and promotes an increase in respiratory rate and an accumulation of secondary metabolites. As a result, ethylene production may be stimulated in plant tissues, which can lead to premature yellowing or ripening of commodities.

For fresh foods like fruits and vegetables, physical damage can arise at any stage. In the earliest stage, when the produce is severed from the mother plant, many fungi invade the produce through the stem end. Bruising can occur, for instance, due to

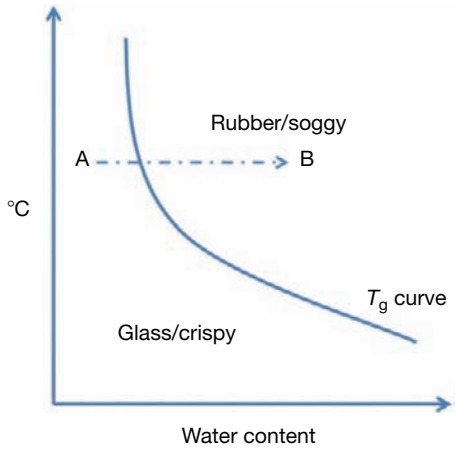

Figure 1 Texture changes as water migration and loss.

dropping or compression, when produce is stacked too high or packs are overfilled. Significant levels of wastage occur in the potato industry due to internal bruising of potato tubers during storage, handling, and transportation. Some fruits and vegetables, such as carrots, will encounter frozen storage, which will then cause the formation of ice crystals. The size of ice crystals may change due to fluctuations in temperature during frozen storage. The number of ice crystals tends to decrease, while their size increases, resulting in progressive physical damage of the vegetable tissue. The technique of osmotic dehydrofreezing has recently been recognized to maintain the texture of fruits and vegetables after freeze-thawing, particularly for carrot and broccoli, with minimal damage to cellular integrity. Moreover, at the retail level, the stability of many fresh food products is considerably reduced by physical damage caused by rough handling, where the produce is loose and can be picked over by the potential customer.

Physical damage will also affect the stability of processed food during storage. Poor packaging of processed food can create problems. Such issues can be related to the lack of padding or underfilling of cartons, allowing movement of processed food within the pack during transport and handling. The processed food in the broken packaging will be exposed into the atmosphere without any protection, thus causing degradation.

Mechanisms of Biological Degradation

Enzymatic effects and microbial metabolism are the two leading biological degradation mechanisms for foods during storage. Fresh foods with natural origin, like fruits, vegetables, cereal grains, fish, and meat, proceed to ripen after harvesting and eventually decay through a process of senescence, which is the normal aging process related to biological degradation. These processes, resulting in reduced storage stability, are usually mediated by several enzymes naturally presented in the food or by enzymes produced by certain microorganisms. Such enzymes, for example, polyphenol oxidase (PPO), peroxidase (POD), and lipoxygenase (LOX), are active during the storage of foods without the control of the synthesizing unit, as is the case before harvesting.

During storage, fresh vegetables and fruits continue respiration through the consumption of oxygen and the generation of carbon dioxide and water vapor until the depletion of energy required for metabolic process. The oxygen level is a key external factor to determine the respiration rate, which controls the rate of senescence. The reduction in respiration rate in response to low oxygen levels is not the result of cytochrome oxidase activity, but rather due to the decreased activity of PPO, ascorbic acid oxidase, and glycolic acid oxidase. In the meantime, the biosynthesis of ethylene will be accelerated, which initiates the ripening of fresh fruits. This ripening process is coded into the genetic makeup of the produce. It can be partially controlled by temperature and composition of the environment (controlled or modified atmosphere). Bananas, for example, are typically harvested green, ripen to a yellow fruit, and finally overripen first to a speckled brown, then to primarily brown, and ultimately to a black appearance. The speed of this process is dependent on the production of ethylene. In addition, the accumulation of ethylene will markedly enhance both PPO and POD activities. Induction of these enzymes can increase browning susceptibility and browning development (Figure 2), which is related to a loss in storage stability. Tissue browning is mainly due to the oxidation of phenolic constituents and degradation of red pigments by PPO, whereas POD may be responsible for deterioration of flavor, color, and nutritional value. Relationships between POD activity and off-flavor development due to oxidation of lipids and phenolic constituents have been established. POD can rapidly oxidize 4-methylcatechol in the presence of H_2O_2 and, thereby, form brown polymeric pigments. Loss of vitamin C is related to ascorbic acid peroxidase, but it can also be linked to the co-oxidation of ascorbic acid with phenoxyl radicals generated by POD. Complementary localization pattern and synergistic roles of POD and PPO in browning reactions have been suggested. Also, an involvement of anthocyanase renders major phenolic constituents (anthocyanin) accessible to POD or PPO.

Another major enzyme group participating in food degradation is constituted by the LOXs. LOXs are a diverse group of enzymes widely spread throughout both the plant and animal kingdoms. They are present in legumes, cereals, fruits, and potato. These enzymes act mainly on polyunsaturated fatty acids containing *cis*, *cis*-1,4-pentadiene systems, and they catalyze the formation of hydroperoxides intermediates similar to those formed by nonenzymatic oxidation. However, LOXs are specific with respect to the substrate and to the positional and stereoisomeric hydroperoxides they produce. The different LOXs present in different plants, or isolipoxygenases in the same plant, vary in their substrate and product specificities, as well as in their optimum parameters (e.g., pH). It is believed that iron, a constituent of LOX, plays a key role in the mechanism by which the enzyme catalyzes hydroperoxides formation. The breakdown of hydroperoxides leading to the formation of volatile and nonvolatile products may also be catalyzed by enzymes (i.e., hydroperoxidases). Obviously, due to the greater specificity in enzyme-catalyzed formation and decomposition of fatty acid hydroperoxides, fewer and more specific oxidation end products are encountered.

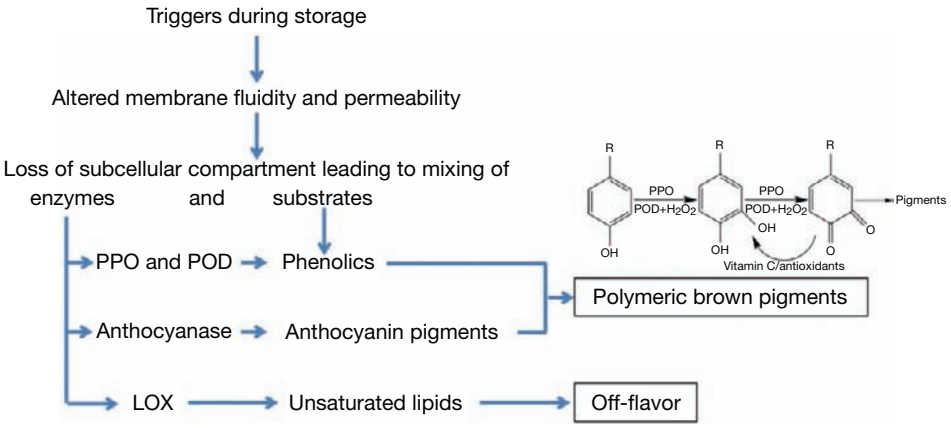

Figure 2 Enzymatic degradation in fresh vegetables and fruits.

Similar to the fresh fruits and vegetables, animal tissue and fish products undergo changes, such as proteolysis, from the moment of slaughter. During chilled storage of meat or fish products, biological changes derived from enzyme action are known to take place, such as changes in the protein, nucleotide, and lipid fractions and the formation of peptides, free amino acids and free fatty acids, and hypoxanthine. Meat that is frozen and then thawed will normally deteriorate rapidly, even at refrigeration temperatures. This deterioration may be due to the physical breakage by ice crystals. Since membranes throughout the tissue will be broken, other compartmentalized proteases may also be released. In general, peptidases active in postmortem muscle have the ability to catalyze the degradation of proteins, such as titin, nebulin, filamin, desmin, and troponin-T, into many of the same degradation products produced in myofibrils during storage. They will cause the meat to become tender but sometimes mushy because of extensive proteolysis, and the resultant low-molecular-weight substrates will facilitate microbiological growth. Fish quality deteriorates after rigor mortis, with proteolytic activity being a causal factor. Proteolytic deterioration of fish meat continues even in the frozen state if the storage temperature is not low enough due to the release of proteinase. Additionally, LOX, found in platelets, microsomal membranes, and other specialized tissues of animals, will also oxidize the polyunsaturated fatty acids. The consequences of these events are the deterioration in sensory quality, a loss of nutritional value, and negative modifications of the physical properties of muscle. These changes are temperature- and atmosphere-dependent and are also influenced by the types of enzymes in the animals and fish.

The action of enzymes could also lead to a decrease in stabilization and consumer acceptability in processed food during storage. In orange juice, the cloud particles, a complex mixture of protein, pectin, lipids, hemicellulose, cellulose, and other minor components, impart the characteristic flavor, color, and mouthfeel. The enzyme pectin methylesterase (PME) in the juice could cause the loss and precipitation of cloud in orange juice through the de-esterification of the methyl groups on the galacturonic acid backbone of pectin and the creation of charged regions that complex with Ca^{2+}, forming gels, which precipitate and clarify the juice. At least three isozymes of PME, which is a heat-resistant enzyme, are known: PME I, PME II, and TS-PME. Elevated pasteurization temperatures are commonly used to inactivate PME and to prevent juice cloud precipitation in the juice industry.

In addition to the actions within the biological system of fruits, vegetables, grains, meat, and fish products, food losses are experienced through the actions of microorganisms. This is universal for both fresh and processed food products during storage. For instance, *Serratia marcescens* isolated from raw milk is found to produce extracellular lipase. This microorganism and its lipase demonstrate lipolytic and proteolytic activity even at 6 °C after 12 h, which could contribute to flavor defects in milk and dairy products. Lactic acid bacteria (LAB) produce lactic acid as acidification to inhibit the growth of spoilage agents. But some LAB species, such as *Lactobacillus sake* and *Lactobacillus curvatus*, have been detected on various types of vacuum-packaged cooked sausages and could deteriorate the storage stability of sausage and sausage slices by forming the undesirable sour aroma sensory attributes and taste.

Traditionally, successful strategies to combat biological degradation include thermal processing (e.g., blanching and pasteurization) and storage at a reduced temperature and under modified packaging (MAP), for inactivating enzymes and controlling microbial growth in fresh foods. Some novel nonthermal technology has been recently introduced as a mild alternative for some conventional pasteurization processes, such as high-pressure and dense-phase CO_2.

Mechanisms of Chemical Degradation

In addition to the physical and biological degradation stated earlier, chemical forces also degrade food. Even in a class of products made commercially sterile and storable at ambient temperature by thermal processing, there can be a wide range of shelf lives because of chemical degradation during storage. Chemical degradation includes thermal degradation, oxidative degradation, nonenzymatic hydrolytic degradation, photodegradation catalyzed by light, and radiation degradation.

Thermal Degradation

Thermal degradation happens for a large variety of food products when applying heat processing. Caramelization is such reaction associated with thermal degradation and can result in complex mixtures of molecules. During storage, thermal degradation is unusual except for foods or constituents that are extremely sensitive to temperature fluctuation or that are distributed in the tropical area. The breakdown of glycosidic bonds in a wide range of carbohydrates is a typical thermal degradation, which will produce mainly acids and other specific compounds depending on the structure and conformation of carbohydrates and processing conditions. Some thermolabile colorants, like anthocyanins, and nutrients, like ascorbic acid, may be vulnerable to such degradation.

Anthocyanins are popular ingredients responsible for the attractive red colors of many fruits and fruit products. Due to thermal instability, anthocyanins readily degrade and form a colorless compound or an undesirable brown color. The storage temperature will determine the thermal degradation rate of anthocyanins in food products and the rate increases during storage as the temperature rises. Many studies have been carried out to investigate the storage stability of anthocyanins in different juices. A recent study suggested a first-order reaction for the degradation of blood orange anthocyanins with half-lives of 55.7, 9.7, and 2.1 days for 45 °Brix samples at storage temperatures of 5, 20, and 37 °C, respectively. The degradation of blackberry juice anthocyanins followed similar first-order reaction kinetics, resulting in half-lives of 330, 32.1, and 11.7 h for concentrated juice (65 °Brix) at storage temperatures of 5, 25, and 37 °C, respectively. The difference of half-lives between juices is primarily attributed to the thermal sensitivity of individual anthocyanin molecules.

Thermal degradation of anthocyanins is primarily caused by oxidation and cleavage of covalent bonds, resulting in a variety of species depending upon the severity and nature of storage temperature. Figure 3 shows the degradation of a typical anthocyanin, that is, pelargonidin, and the formation of various intermediate compounds. The initial degradation step in such degradation mechanism is the hydrolysis of the sugar moiety and aglycone formation due to the formation of cyclic adducts. However, others believe the opening of the pyrylium ring and chalcone formation is the first degradation step for anthocyanins.

Maillard Reaction

The Maillard reaction is of importance for the storage stability of foods, chiefly in commercially available potato chips, potato fries, cereals, coffee, and bread. The Maillard reaction, which induces browning and attractive flavor formation of foods, occurs under both heating processing and storage at room temperature. However, it also has an adverse effect on nutritive value and toxicological implications such as the formation of acrylamide during storage.

The sequences of reactions that make up nonenzymatic browning or Maillard reactions have been extensively studied. In fact, these reactions consist of thousands of subreactions, so that at least 2500 volatile compounds are formed. The Maillard reaction is usually divided into three stages: (i) the condensation between an amino group and a reducing sugar, leading to an *N*-glycosylamine in the case of an aldose sugar that rearranges into the so-called Amadori/Heyns product; (ii) the formation of Amadori/Heyns products, leading to sugar fragmentation products and release of the amino group; and (iii) the generation of all kinds of dehydration, fragmentation, cyclization, and polymerization reactions in which amino groups participate again. Especially in relation to flavor formation, the so-called Strecker degradation is of utmost importance, in which amino acids are degraded by dicarbonyls formed in the Maillard reaction, leading to deamination and decarboxylation of the amino acid.

The degradation caused by Maillard reactions during storage, which involve amino groups, can result in decreased nutritional quality, especially to the extent that the ε-group of lysine is coupled. More importantly, Maillard reaction products are responsible for the appearance of acrylamide, a compound that has been classified as probably carcinogenic in humans. The participants in this reaction are an amino acid

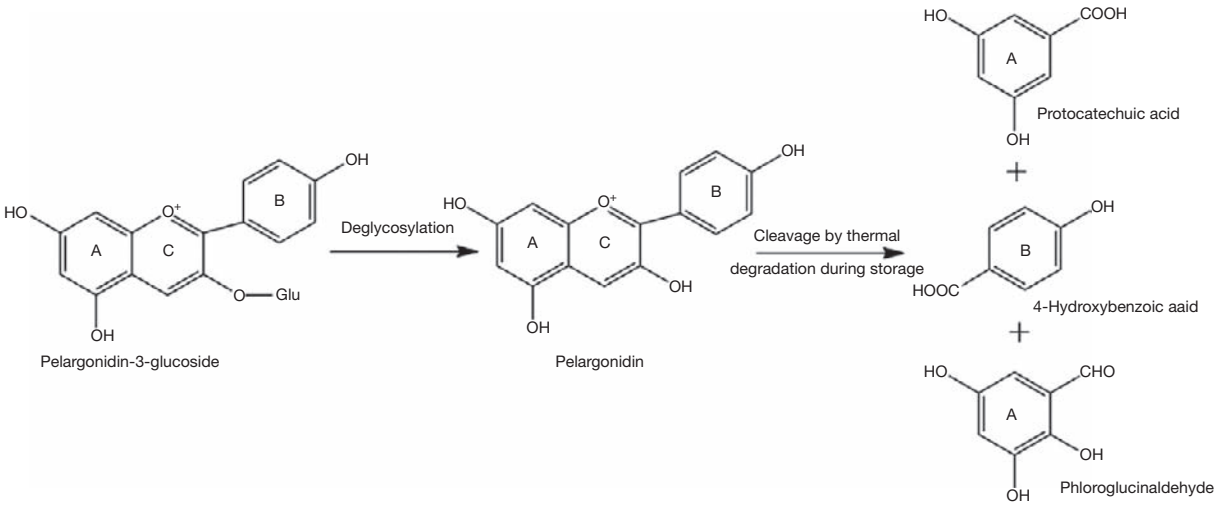

Figure 3 Possible thermal degradation mechanism of pelargonidin.

(asparagine) and reducing sugars (fructose and glucose). In some foods, acrylamide concentrations are reported to increase during storage, probably because of Maillard reactions although the reaction rate may be comparatively slow.

Among the factors that affect browning reactions during storage are temperature, moisture content and water activity, direct reactions of the reactants (either carbohydrates or proteins), and indirect reactions that could affect the pH.

Photochemical Degradation

Basically, a photochemical degradation is a chemical reaction that is induced by light or radiation. Photoexcitation is the first step in a photochemical degradation process, which is initiated by the photosensitizer (Sen). The photon can be absorbed rapidly by the photosensitizer after the exposure to ultraviolet or visible radiation, after which it elevates to a state of higher-energy, unstable, excited, singlet molecule (^{1}Sen*). The excited singlet photosensitizer (^{1}Sen*) loses its energy by internal conversion, emission of light, and intersystem crossing (Figure 4). Internal conversion involves the transformation from a high-energy to a lower-energy state by releasing energy as heat. Emission of fluorescence converts the excited singlet sensitizer to a ground-state singlet sensitizer (^{1}Sen). The excited sensitizer may also undergo an intersystem crossing from the excited singlet molecule to an excited triplet molecule (^{3}Sen*). The ^{3}Sen* may degrade foods through type I or type II pathways. In type I pathway, ^{3}Sen* may react directly with a compound such as linoleic acid or with phenol compounds by donating and accepting hydrogen or electrons and producing free radicals or free-radical ions. In type II pathway, ^{3}Sen* reacts with triplet oxygen ($^3O_2$) to form singlet oxygen ($^1O_2$) and ^{1}Sen by a triplet–triplet annihilation mechanism. The sensitizer returns to ground state (^{1}Sen) and may begin the cycle again to generate singlet oxygen. Both triplet oxygen and singlet oxygen can react with other components, such as lipids, proteins, polysaccharides, colorants, and vitamins, in the food to finally destabilize food during storage. These reactions, as fast as 10^{-9} s, are several orders of magnitude faster than thermal degradation.

Food constituents such as chlorophyll, pheophytins, porphyrins, riboflavin, myoglobin, and synthetic colorants are the common photosensitizers that can absorb energy from light and transfer it to triplet oxygen to form singlet oxygen. The photochemical degradation can thus happen in the foods containing these photosensitizers during storage. Milk, for instance, is susceptible to photochemical degradation due mainly to implication of riboflavin and exposure to fluorescent light to enhance consumer attraction. Therefore, not only the nutritional value but also the storage stability of milk could decrease under such conditions. Similar implications are valid for other dairy products, such as cheese and cream. Packaging materials can effectively protect against photochemical degradation of milk by blocking or reducing the transmission of light. As another example, meat will suffer photochemical degradation because of the presence of myoglobin and other hemoproteins, which are the primary photosensitizers. Both myoglobin and oxymyoglobin have the ability to absorb light energy, resulting in the formation of grayish metmyoglobin.

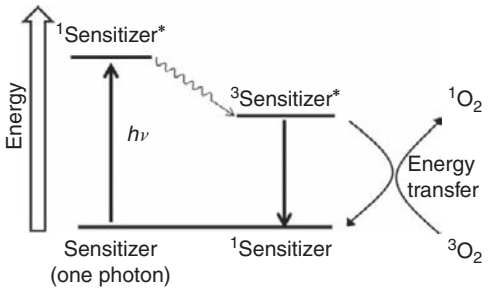

Figure 4 The formation of singlet oxygen in the presence of photosensitizer.

Oxidative Degradation

Oxidative degradation is one of the paramount causes of quality deterioration in natural and processed foods. It is a large economic concern in the food industry because it impairs many quality characteristics such as flavor (rancidity), color, texture, and the nutritive value of foods. In addition, it produces potentially toxic compounds. Thus, oxidative degradation is one of the major processes that limit the shelf life and storage stability of foods. The previously stated chemical degradation mechanisms, for example, heat treatment and singlet oxygen, could initiate oxidative degradation. Oxidative degradation is a general term used to describe a complex sequence of chemical interactions between substitutes, such as lipids, protein, and carbohydrates, in foods with active oxygen species. Each of the substitutes has different degradation mechanisms. Free radicals, however, can participate in all the oxidative degradations as summarized in Figure 5.

Theoretically, the mechanism of lipid oxidation is a free-radical chain reaction between unsaturated fats and oxygen that can occur in an autocatalytic manner. However, lipid oxidation in many food systems is accelerated by prooxidants such as transition metal ions, photosensitizers, UV light, and certain enzymes. The overall mechanism of lipid oxidation involves three stages: (1) initiation, the formation of free radicals; (2) propagation, the free-radical chain reactions; and (3) termination, the formation of nonradical products. A simple scheme for the free-radical mechanism can be summarized as follows:

Initiation:

$$In^{\cdot} + LH \rightarrow In\ H + L^{\cdot} \quad [1]$$

Propagation:

$$L^{\cdot} + O_2 \rightarrow LOO^{\cdot} \quad [2]$$

$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot} \quad [3]$$

Termination:

$$LOO^{\cdot} + LOO^{\cdot} \rightarrow LOOL + O_2 \quad [4]$$

$$L^{\cdot} + LOO^{\cdot} \rightarrow LOOL \quad [5]$$

$$L^{\cdot} + L^{\cdot} \rightarrow LL \quad [6]$$

In the initiation step, a fatty acid radical known as the alkyl radical ($L^{\cdot}$) is formed by abstraction of a hydrogen from a fatty

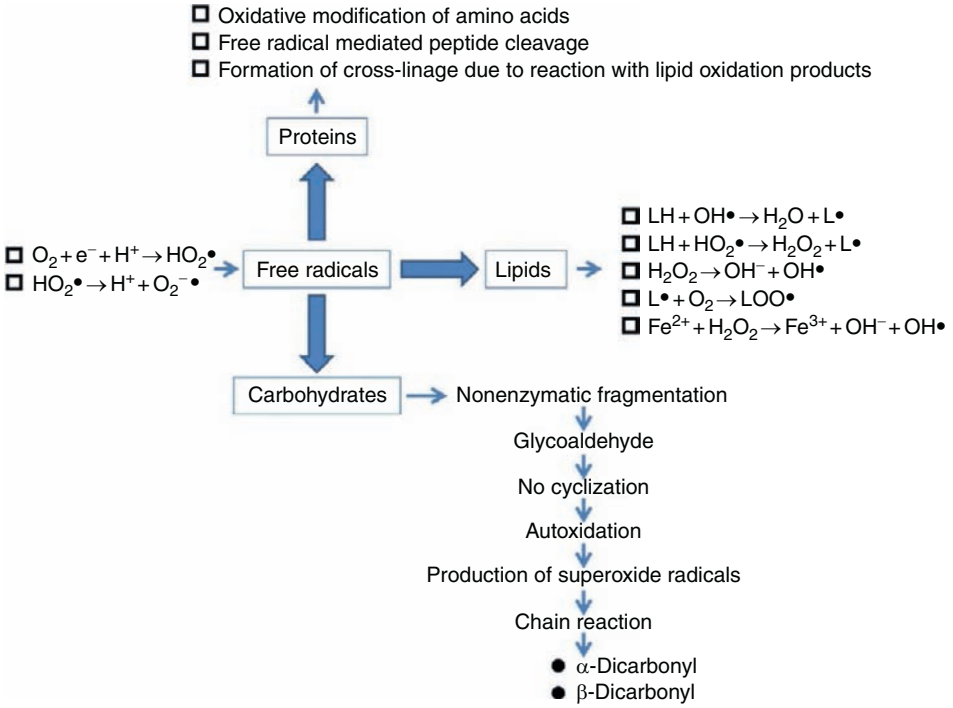

Figure 5 Free radical-initiated oxidative degradation in major food components.

acid in the presence of an initiator ($In^{\cdot}$) (reaction [1]). Once the alkyl radical forms, the free radical can delocalize over the double bond system resulting in double bond shifting and, in the case of polyunsaturated fatty acids, formation of conjugated double bonds. In bulk oils, the ease of formation of alkyl radicals in fatty acids increases with increasing degrees of unsaturation. The first step of propagation involves the addition of oxygen to the alkyl radical, resulting in the formation of peroxyl radical ($LOO^{\cdot}$), which has higher energy than the alkyl radical (reaction [2]). Thus, the peroxyl radical can abstract hydrogen from another unsaturated fatty acid and produce a lipid hydroperoxide (LOOH) and a new alkyl radical (reaction [3]). The interaction of two free radicals to form nonradical species will terminate the process (reactions [4]–[6]). This step is usually less relevant during storage, since the food is already rancid before significant termination reactions take place. An exception is in the low-oxygen environment of frying oils, where the termination reaction can occur between alkyl radicals to form fatty acid dimers.

Rancidity in food occurs when unsaturated fatty acids decompose into volatile compounds. These volatile oxidation products are produced from the decomposition of fatty acid hydroperoxides. The homolytic cleavage of hydroperoxides (LOOH) between these two oxygen molecules is the most likely hydroperoxide decomposition pathway. This reaction yields an alkoxyl radical ($LO^{\cdot}$) and a hydroxyl radical ($^{\cdot}OH$). The alkoxyl radical ($LO^{\cdot}$), which is more energetic than either the alkyl ($L^{\cdot}$) or the peroxyl radical ($LOO^{\cdot}$), can enter into a number of different reaction pathways. Alkoxyl radicals can attack another unsaturated fatty acid, a pentadiene group within the same fatty acid or the covalent bonds adjacent to the alkoxyl radical. This last reaction is known as β-scission reaction and is important to food quality as it causes fatty acids to decompose into low-molecular-weight volatile compounds that cause rancidity.

In the β-scission reaction, the highly energetic alkoxyl radical ($LO^{\cdot}$) is able to abstract a hydrogen from the carbon–carbon bond on either side of the oxygen radical. The decomposition product on the carboxylic acid end of the fatty acid is usually esterified to the glycerol of a triacylglycerol or phospholipids; thus, it would not be volatile and therefore would not contribute to rancidity unless it undergoes further decomposition reactions to form low-molecular-weight compounds. Cleavage of the hydrocarbon chain by alkoxyl radicals on the methyl end of the fatty acid will produce volatile compounds. Upon cleavage of the fatty acid chain, the resulting radicals will interact with a variety of compounds to produce secondary lipid oxidation products such as aldehydes, ketones, alcohols, furans, hydrocarbons, and acids.

The oxidative degradation of protein is common in meat and fish products during storage. In meat, protein oxidation may lead to decreased eating quality such as reduced tenderness and juiciness, flavor deterioration, and discoloration. There are three distinct ways that protein can be oxidatively modified: (1) oxidative modification of a specific amino acid, (2) free radical-mediated peptide cleavage, and (3) the formation of protein cross-linkage due to reaction with lipid peroxidation products.

Physical and chemical changes in oxidized proteins include amino acid destruction, decreases in protein solubility due to protein polymerization, loss of enzyme activity, and formation of amino acid derivatives including carbonyls. One of the consequences of protein oxidation is the reduction of the tenderness of meat by the formation of protein aggregates through both noncovalent and covalent intermolecular bonds. Additionally, intermolecular cross-links formed in

highly oxidative conditions can make proteins less susceptible to enzymatic proteolysis, further reducing development of tenderness by proteolysis in meat. Color deterioration of meat products is another important consequence of protein oxidation that may influence consumer acceptance.

Carbohydrates are quite stable to oxidative degradation, and other components like lipids and proteins are expected to be oxidized before carbohydrates. However, radicals formed in other components are able to react with carbohydrates, producing the formation of carbonyl compounds. Regarding oxidative degradation of carbohydrates, the formation of free radicals could contribute to glycoxidative degradation. During the initial stages of nonenzymatic glycoxidation, the C—H bond of the secondary alcoholic group is cleaved to generate a carbon-centered radical. This radical adds oxygen instantly to produce a peroxyl radical. This radical can either release the hydrogen peroxyl radical to produce oxo-derivatives of sugars or suffer an electronic rearrangement to fractionate the carbohydrate carbon chain and produce a glycolaldehyde and abstract a hydrogen radical to produce an acid and oxygen peroxides after hydrolysis. Different monocarbonyl and dicarbonyl compounds are produced in these reactions, depending on the site of attachment by the lipid peroxyl radical, the carbohydrate involved, and the fractionation or not of the carbohydrate molecule. In fact, 1,2-dicarbonyl compounds are common constituents in many food products that have been described as cytotoxic, mutagenic, carcinogenic, and prooxidative reagents.

Many important factors, such as antioxidants, chemical structure of lipids, oxygen concentration, and temperature, have been found to influence the oxidative stability of lipids, protein, and carbohydrates. A better understanding of these factors could lead to the development of practical strategies for retarding oxidative degradation in food systems.

Conclusions

The quality of fresh and processed products held in storage will degrade over time. Understanding degradation mechanisms is a prerequisite for maximizing nutritional and sensory qualities. Assuming that throughout the storage duration the appropriate conservation temperature is respected, a point in time will be reached when the product quality degrades, perceptively or unperceptively, to a level taken as a cutoff limit. That point in time corresponds to the product shelf life. The quality cutoff limit and the shelf life under specific storage conditions vary over a wide range and depend on the nature of the product, the predominant degradation process, and the mode of storage. Roughly speaking, the degradation processes can be classified as physical, biological, and/or chemical. Major concerns in fresh or minimally processed products are the growth of spoilage microorganisms, enzymatic browning, color deterioration, and rancidity development. Physical degradation, including moisture migration and loss, starch crystallization, and physical damage, some of which significantly affect texture, can determine the shelf life of products in which microbial growth is not a factor. Chemical degradation, primarily the oxidation of constituents in foods initiated by variable factors, such as free radicals, singlet oxygen, and transition metals, generates rancidity and off-flavors perceptively and can determine the shelf life of products when any of the other two is not a major factor. Depending on the susceptibility of the product to one or more of these degradation mechanisms, it would be stored under freezing, chilling, or ambient temperatures or in the presence of certain food-grade additives or types of packaging to maintain a marketable quality for as long as is needed to move throughout the food chain from producer to consumer.

See also: Acrylamide; Antioxidants: Role on Health and Prevention; Browning: Enzymatic Browning; Foodborne Pathogens; Maillard Reaction; Spoilage: Bacterial Spoilage; Spoilage: Yeast Spoilage of Food and Beverages.

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Storage Stability: Shelf Life Testing

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Introduction

The quality of most foods and beverages decreases from the moment of the packaging until its consumption due to complex reactions based on biological, chemical, or physical mechanisms. There is a finite length of time after which the product becomes unacceptable. This length of time in which a foodstuff material is stored under specified packaging and environmental conditions is referred to as shelf life. A useful definition of shelf life of food has been available in IFST Guidelines:

Shelf life is defined as the time during which the food product will

- remain safe;
- be certain to retain desired sensory, chemical, physical, and microbiological characteristics;
- comply under the recommended conditions.

Safety and quality are the two main aspects of shelf life of food. While food safety is a legal requirement, the food quality is a choice of the manufacture to respond to consumer requirement. In fact, the words quality, acceptability, and desired characteristics can have several interpretations, depending on the product and on the market. Thus, a proper determination of the shelf life of a product is a critical point for a factory, mainly if one considers that inadequate shelf life will often lead to consumer dissatisfaction and complaints.

The critical factors affecting the shelf life of a food are

- product characteristics (formulation and processing),
- packaging,
- storage conditions.

All the previously mentioned factors are critical, but their relative importance depends on the specific food. In general, for most perishable foods, such as fruits and vegetables, fresh meat, and fish, factors related to initial quality of the product and to packaging and storage conditions are critical for the shelf life, whereas for thermally stabilized foods, such as some beverages, sauce, or cooked ready meal, process parameters, such as pasteurized temperature–time couple, are critical for the ending of their shelf life.

The possible interplay among critical factors must be taken into consideration because they can lead to different storage conditions as a function of the product and package property.

Appropriate shelf life testing is normally required to take into account the different scenarios brought about by this interplay. Once the scenarios are defined, an understanding of the key factors affecting the shelf life of a commodity is the starting point to shelf life prediction.

This article focuses on the methods used to assess shelf life and in particular will focus on the description of the main steps involving shelf life determination by simulation method, in particular

1. identification of the deterioration mechanisms,
2. identification of critical factors for the identified deterioration mechanism,
3. identification of a quality index high related to the deterioration mechanism and its critical level related to the unacceptability of the product,
4. quantification of the evolution of the quality index as function of the time.

Identification of Deterioration Mechanisms

Knowledge on deterioration reactions, which affect food quality, is the first step to determine the shelf life of a specify commodity. Fresh and processed foods deteriorate by a variety of mechanisms including oxidation, browning (enzymatic and nonenzymatic), moisture content changes, enzymatic reactions, microbial attack, structural changes, photodegradation, senescence, loss of carbonation, recrystallization of sugars, and caking. The major modes of food degradation are senescence, microbiological decay, chemical deterioration, and physical degradation. The relation between deterioration mechanisms and quality attributes that change during storage is reported in Table 1.

Although several deterioration modes may occur simultaneously, it is the most sensitive one that limits shelf life. A good understanding of the interaction between the product, the package, and the environmental system can lead to a right identification of the limiting deterioration mechanism.

Factors Influencing Deterioration Process

Many factors can influence the deterioration process and therefore the shelf life of foods. They can be classified as intrinsic factors, related to the final property of the product, and extrinsic factors, related the environment encountered by the product through the food chain (temperature, atmosphere composition, relative humidity (RH), and light). Food deteriorations dependent on environmental factors can be controlled by the package or package technologies.

All these factors are important for the stability of the product. In this section, a brief description of the most important activation factors for foods stability in terms of shelf life is given. In particular, among the intrinsic factor, water activity will be examined, while among the extrinsic factors, temperature and gas composition will be considered. It should be emphasized that loss of quality often occurs by a combination of these factors and, when such interactions are indicated or suspected, consideration must be given to mixed effects. For instance, nonenzymatic browning is a function of food composition, moisture content, and temperature.

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Table 1 Relationship between deterioration mechanisms and quality attributes that change during storage

Deterioration process	*Mechanism*	*Quality attribute changes*	*Environmental critical factors*
Senescence	Catabolism of carbohydrates	Color, flavor, nutrients, texture, tissue damage	Temperature, oxygen, relative humidity
Microbiological decay	Growth of pathogen or spoilage organism	Off-flavor, visual appearance, unsafe food	Temperature, relative humidity, oxygen
Chemical reaction	Enzymatic or nonenzymatic reaction, lipid oxidation	Color, aroma, taste, off-flavor loss of vitamins, degradation of proteins	Temperature, oxygen, light
Physical degradation	Physical crushing, moisture loss/gain, staling	Texture, sedimentation, creaming	Temperature, relative humidity

Environmental critical factors are also reported for each deterioration process.

Water Activity

Water is the most important diluent of water-soluble food components and plasticizer (softener) of various water-miscible polymeric compounds as well as often the main food components. Chemical reaction, enzymatic changes, and microbial growth may occur readily in foods with high water content, while foods with low water content undergo loss of quality by water absorption. Water has several effects on food stability, palatability, and overall quality, and they are a function of the physicochemical state of water, which is related to water activity.

The parameter water activity is defined as the ratio of vapor pressure above a sample, p_w, and that of pure water (p_{wo}) at the same temperature. Mathematically,

$$a_w = \frac{p_w}{p_{wo}} \quad [1]$$

It describes the degree to which water is free or bound to other components. Water activity depends on the composition, temperature, and physical state of the compounds.

When a food is placed in an environment at a constant temperature and RH, it will eventually come to equilibrium with that environment. The corresponding moisture content at steady state is expressed as the equilibrium moisture content.

The difference between the RH of the surrounding environment and water activity of the food determines whether a food gains or loses moisture during storage.

For the main food product, steady-state relationships between a_w and water content at a constant temperature are described by a sigmoid-shaped curve. This curve is known as sorption/desorption isotherm. Such plots are very useful in assessing the stability of foods and in selecting effective packaging.

Temperature

Temperature is one of the most important factors affecting the shelf life of foods. Raising the storage temperature will accelerate many aging processes, as well as inactivate enzymatic reaction and/or microbiological spoilage. The relationships among temperature, respiration, transpiration, and other deteriorative changes are well documented. The models used to represent the dependence of reaction rate on temperature include the Arrhenius model, the temperature quotient, and Williams–Landel–Ferry (WLF) model.

The most common and generally valid relationship used to describe the influence of a constant storage temperature on the reaction rate is the Arrhenius equation:

$$K = A \exp\left(-\frac{E_a}{RT}\right) \quad [2]$$

where A is the so-called pre-exponential factor, E_a is the activation energy, and R and T are the gas constant and absolute temperature, respectively. The value of E_a is a measure of the temperature sensitivity of the reaction. High activation energy implies that the reaction is strongly temperature-dependent, that is, accelerates greatly with increase in temperature.

An alternative way of expressing temperature dependence of a reaction is to use the concept of Q_{10}:

$$Q_{10} = \frac{K_{(T+10)}}{K_T} \quad [3]$$

It indicates how fast a reaction will occur if the temperature is raised by 10 °C and can be used to predict expected product shelf life.

The last relationship generally used to describe the dependence of food stability from temperature derives from the application of polymeric science principle and theory to food science, which has led to the glass transition temperature (t_g) being put forward as an alternative, potentially preferable parameter, for defining food stability, where the rate of degradation processes is critically dependent on $T-T_g$, particularly at temperature close to T_g. If a change in a quality factor is diffusion-controlled and dependent on viscosity, the observed rate constant may become a function of the true rate constant and diffusivity. In similar studies, the observed rate constant of diffusion-controlled reactions and quality changes in amorphous food matrices have been assumed to be proportional to $1/\eta$, which has led to the direct application of the WLF equation. Mathematically,

$$\log \frac{k_s}{k} = \frac{-C_1 \times T - T_s}{C_2 + T - T_s} \quad [4]$$

where k is the observed rate constant and k_s is the observed rate constant at the reference temperature T_s. C_1 and C_2 are constants for which values of −14,44 and 51,6 are often used, although they are not always applicable. In general, because it is difficult to measure the mechanical parameter at T_g, it is more accurate to use T_g +50 °C as a reference temperature (in place of T_g) and to determine constant C_1 and C_2 graphically

from experimental data. Nelson and Labuza had discussed methods of determining WLF coefficients for assessing the applicability of the WLF equation to the temperature dependence of chemical reactions in food.

Gas Atmosphere

The composition of the atmosphere that surrounds the product affects its deteriorative process. When the food product is packed, the gas composition of the package head space is a function of the packaging properties (gas permeability) and product properties (respiration rate or chemical reaction kinetics). Oxygen is an active reactant in many chemical reactions (oxidation, enzymatic browning, and physiological metabolism). Its availability has detrimental effects on the nutritive quality of foods. Thus, in many cases, removing oxygen from the surrounding atmosphere of a product leads to positive effects on the shelf life of the product. Exceptions are all the respiring products, which, after harvest, keep on respiring, consuming oxygen and sugar, and producing carbon dioxide, water, and energy. If the product has less oxygen available for the metabolic process, it reduces the respiration velocity to preserve itself. Low oxygen content also suppresses the production of ethylene, therefore delaying the onset of ripening. Moreover, concentrations of oxygen lower than the threshold value below which anaerobic condition establishes can be even detrimental for the shelf life of the product. At the same time, it is reported that high concentrations of CO_2 inhibit the respiration rate, and as for oxygen, high levels of CO_2 may affect negatively the quality of the product.

Carbon dioxide and carbon monoxide improve the stability of the foods. In fact, high levels of carbon dioxide lead to a reduction in microbiological activity, especially mold growth, denaturation of some protein by acidification, inhibition of pectin hydrolysis, and a reduction in the cold damage of the vegetable tissue. However, different commodities have different optimum gas composition requirements for maximum shelf life.

Quality Index and Acceptability Level

Once the major deterioration modes are known, and the critical factors for the identified deterioration mechanism have been identified, the following step is to identify quality indexes to quantify the extent of deterioration. Any quality factor should possess three characteristics: measurable, reproducible, and relevant.

Moreover, the quality index has to be correlated to the acceptability of the product and must describe well the kinetic of the degradation process. An index could be a sensory, chemical, or physical parameter, depending on the product and on the degradation process. In Table 2, the main quality indexes used for measuring shelf life are reported.

As a function of the food product, each quality index has a different relative importance. Thus, for bakery product, the water content and mechanical properties are the most important quality indexes, whereas for fresh red meat, microbial count, color, and odor attributes have the main role in defining product acceptability, and for fresh fish, color, odor attribute, and oxidation index are critical for shelf life evaluation.

In order to establish the product shelf life, it is also necessary to define the critical level of each attribute that describes the quality evolution: the critical level must represent the level of the quality under which the product is not anymore acceptable by the consumers. Apart from microbiological quality aspect, for which the legislation provides clear indication (EU regulation (EC) No. 852/2004; 2073/2005), for the other aspects of quality, the critical level must be arbitrarily defined.

Nevertheless, considering that the shelf life of food depends on the interaction of the food with the consumer, a possible approach to define the critical level is to perform a consumer acceptability test. Consumer acceptability test gives a direct measure of liking that can be used more directly to estimate the shelf life. The most common procedure is to ask consumer representative of the target population to scale acceptability on a 9-point category scale, anchored from like extremely to dislike extremely acceptability test. For this kind of test, a large number of consumers are required (minimum of 50 but preferably over 100 consumers), and suitable experimental designs should be used, in conjunction with appropriate statistical analysis.

An alternative method to take consumer response into account is to estimate the probability of a consumer accepting a product beyond a time t using the survival analysis. In this method, samples with different storage times are presented to consumers. Consumers are asked a question such as 'Would you normally consume this product? Yes or No?,' and a survival

Table 2 Main quality indexes used to measure the principal quality change of food during storage

Deterioration process	*Quality index*	*Quality changes*
Senescence	Respiration rate, ethylene, water content, sensory attributes; appearance, flavor, taste/texture, electronic noses	Color, flavor, nutrients, texture, tissue damage
Microbiological decay	Microbial count, pH, acidity, metabolite production, electronic noses	Off-flavor, visual appearance, unsafe food
Chemical reaction	Vitamin concentration, acidity, peroxide value (PV), free fatty acid content (FFA), thiobarbituric acid value (TBA), malondialdehyde content (MDA), hexanal, fatty acid composition, volatile compounds, sensory attributes, color	Color, texture, aroma, taste, off-flavor loss of vitamins, degradation of proteins, loss of essential fatty acids
Physical degradation	Water content, water activity, weight loss, mechanical parameters, sensory attributes, rheological properties, glass transition temperature (T_g)	Texture, sedimentation, creaming

function is defined as the probability of consumers accepting a product beyond a certain storage time. In general a 50% probability can be fixed as level of acceptability. This method has been applied to determine the shelf life of fresh-cut apple, yogurt, pan bread, UHT milk, minced beef, and 'Fuji' apple.

Both methods described are useful to establish in specific package and storage condition the acceptability level in terms of storage days. Then, in order to define the critical level of each quality attribute, it is necessary to know the value of the attribute at the specific time (critical storage days) in the specific condition. For example, if, from the survival analysis, results highlight that after 7 days, the probability that a food product packed in a specific package and a fixed environmental condition can be not anymore acceptable by 50% of the consumers, the corresponding critical level of each quality attribute will be the value of the attribute at 7 days in the same storage conditions. Then, that value will be a reference value for whatever storage conditions, that is, the critical acceptability value of quality index of the specific product.

Quantification of the Evolution of the Quality Index: Shelf Life Test

The knowledge of the main deterioration processes and of the factors affecting food stability is the starting point to measure the shelf life of a given product. With the expression 'simulation of the shelf life,' one refers to a classic approach consisting in representing in laboratory the real storage condition in order to determine at which time the food product loses its commercial quality. This approach is useful when exact knowledge on the deterioration process is not available and the property of a new package is unknown or when their interaction is too complex to be predicted. Moreover, this approach gives the most realistic answer, but data obtained with this method cannot be generalized and are not applicable to different conditions of storage or to different product typologies.

The determination of shelf life by simulation of real storage condition is costly and time-consuming. Efficient design of experiments is important. Commonly, a single batch of product (or replicate batches) is stored under controlled condition and sampled at time zero and at regular time intervals on the bases of the expected shelf life. This type of design has the clear advantage that data related to shelf life are generated at intervals and provide a moving picture of deteriorative change taking place.

An ideal design for evaluating sensory and instrumental properties at different times of storage requires the availability of a large number of samples to be tested all together. In principle, this can be done in three different ways: A possibility is to use samples from successive production batches so that samples with a given shelf life are available on a wide range of time. This approach may suffer if there is fluctuation in production quality and is convenient only in situations in which production consistency can be assured. A variant of this design consists in the use of a single large batch, which is held in conditions that assure that quality changes are effectively zero, for example, frozen storage. Samples are removed from the frozen chamber at appropriate intervals and stored under the desired conditions. The last possibility is that a large batch of the product is sampled and samples are picked up after appropriate time intervals and kept under nonchanging conditions until the required storage has been reached. The main difficulty of the last two experimental designs is to identify appropriate nonchanging storage conditions, since a few foods can be stored at freezing temperature without changes in some important quality attributes.

Once the most appropriate design for shelf life experimental is chosen, the storage conditions need to be chosen carefully, and a good understanding of the storage, handling, and climacteric conditions of the markets in which the product will be sold is essential to the accuracy of shelf life tests. Generally, for chilled foods and frozen foods, temperatures are typically set between 0 and 10 °C and around −18 °C, respectively. For shelf-stable food, shelf life test temperature must be chosen based on climatic conditions: 38–40 °C/80–90 RH for tropical climates and 20–25 °C/50–70% RH for temperate conditions.

Once the storage condition is defined, the quality over time should be monitored. This is done by measuring attributes correlated to the deterioration processes. Chemical, physical, and microbiological analyses are performed according to the product characteristics and the deterioration processes in act (Table 2).

As a function of the expected shelf life, one must decide the time interval of test and the number of samples for each test. Time interval mainly depends on quality degradation kinetics. In the majority of the cases, a constant time interval can be convenient in order to describe the quality changes during time, even if in the same situation, it can be useful to have a high frequency at the end of the shelf life. For example, for microbial growth, the first part of the growth curve is stationary, whereas in the exponential phase, the quality changes are faster.

Then, one must decide the duration of the test. Usually, for highly perishable products, shelf life tests last maximum to 15 days, whereas for more stable product, the duration of a test can range from months to years. In this last case, it can be convenient to follow a different shelf life testing procedure based on shelf life prediction.

See also: Emerging Foodborne Enteric Bacterial Pathogens; Food Poisoning: Classification; Food Poisoning: Tracing Origins and Testing; Foodborne Pathogens; Heavy Metal Toxicology; Meat: Eating Quality and Preservation; Mycotoxins: Occurrence and Determination; Preservation of Foods; Spoilage: Bacterial Spoilage; Spoilage: Yeast Spoilage of Food and Beverages.

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Strawberries

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Introduction

Strawberry is one of the most economically important berry fruits consumed for its nutrient content and pleasant flavor. Strawberry is a member of the Rosaceae family and one of the most popularly consumed berries in the world. It is commercially cultivated worldwide for its highly appreciated sweet, aromatic, and juicy fruit. The United States is the world's leading producer of strawberries producing over 36 billion pounds in 2012 and accounting for 29% of the total world production. Among the fruits consumed in the United States, strawberries ranks fifth in consumption behind bananas, apples, oranges, and grapes. In the United States, California accounts for the highest commercial production of this berry crop, followed by Florida and Oregon. California's 12-month growing season contributes to higher strawberry yields per acre than any other growing region. Turkey, Spain, Egypt, and Mexico are the other leading strawberry-producing countries. Most strawberries produced are consumed fresh.

Sources and Production

The dessert strawberry, *Fragaria* X *ananassa* L. Duchesne, is the most popular cultivated strawberry and it dominates strawberry cultivation. The cultivated strawberry is a chance hybrid between *Fragaria chiloensis* Duch. and *Fragaria virginiana* Duch. *Fragaria* X *ananassa* is a herbaceous perennial plant that grows from a dense, compressed, central crown. The crown consists of a central core surrounded by a vascular ring. Leaves, stolons, inflorescences, and roots emerge from the crown. In strawberry plants, leaves are trifoliate and arranged spirally around the central crown. The root system of the strawberry plant is relatively shallow. Strawberry flowers typically have five white petals and pink flowers are seen in some varieties. Flowers are borne in clusters known as a cyme and the first flower to appear in the center is the king or the terminal flower. It also produces the largest berry. Honeybees are the primary pollinators in the open field and bumblebee is commonly used as a pollinator in protected cropping.

Botanically, strawberry is an aggregate fruit, originating from the expanded receptacle of the strawberry flower. Receptacle is the flower base that develops into the edible part of the strawberry. A number of ovaries occur on a common receptacle that develops into one-seeded fruits or achenes, attached to the outer surface of the receptacle in spirally arranged rows. The true fruit are the seedlike achenes borne on the surface of the receptacle. Fruit have a central cylinder of pith tissue surrounded by the cortical tissue containing parenchymal and epidermal cells. Horticulturally, the enlarged receptacle with achenes is considered as a berry. The achenes originate at the base of each pistil and are a combination of seed and ovary tissue. The achenes are embedded in the epidermal layer of the receptacle and each berry can have 20–500 achenes depending on the cultivar and growing conditions. Achenes are ovate and each achene contains one ovary. The mature achene is composed of a thick and hard pericarp, a thin testa, an endosperm consisting of one cell layer, and a small embryo. Immature fruits are green in color.

Strawberry plants propagate vegetatively through the production of runners or stolons, which are trailing aboveground stems that can take root at their nodal regions to develop into new clonal daughter plants. Strawberry cultivars are divided into three types based on their photoperiodic response for flower initiation: short-day, long-day, and day-neutral. Short-day types, also called June bearers in the northern hemisphere (single-cropping or non-everbearing strawberries), and day-neutral types are the two main types of strawberries cultivated worldwide. June bearers are suitable for commercial production in colder regions because of the availability of many cold-hardy cultivars that can be overwintered for perennial systems. Short-day cultivars initiate flower buds when the photoperiod is less than 14 h and day-neutral cultivars initiate buds continuously throughout the growing season. Regardless of the photoperiod, most cultivars typically initiate buds at low temperatures (about 10–15 °C). Long-day cultivars are also called everbearing or double-cropping strawberries and often bear fruits more than once. These cultivars never gained popularity and were superseded by day-neutrals.

Production Systems

There are two main management systems in strawberry production. They are matted row and the annual hill system. Strawberries are cultivated under annual or perennial production systems in the field or grown in tunnels or greenhouses. Two methods of cultivation are generally followed: annual plasticulture and a perennial system of matted rows or mounds.

Matted Row System

The matted row system is a perennial system suitable for producing June-bearing strawberries in colder climates. This system is also suited for outdoor field production and not well suited for high tunnel production. In the matted row system, the mother plants are spaced at planting and then allowed to runner freely to form a dense row of plants and trimmed to a specific width. For commercial cultivation, plants are propagated from runners and are distributed as bare root plants or plugs. This system is best suited for June bearers and requires the least amount of labor.

Annual Hill Plasticulture

The majority of strawberries grown worldwide are produced in plasticulture system. In annual hill plasticulture, strawberries are grown as annuals in a high-density system with raised beds

and removed after the fruiting cycle. Each year, raised beds are prepared, fumigated, and covered with plastic to prevent weed growth and to maintain bed shape. Plants are planted through holes punched in the plastic cover and runners are removed from the plants to encourage more and larger crowns resulting in larger berries and higher yields. Single- or double-trickle irrigation lines are laid on the surface of the raised beds to provide water and nutrients. At the end of the harvest, the plastic cover is removed and the plants are ploughed into the ground. The same procedure is repeated for the next production cycle. Initially, plantings are established with fresh-dug plants, partially or fully chilled dormant plants, or plug plants. Dormant plants are commonly used for off-season production systems. These plants can be stored at 0–1.1 °C for 1–9 months prior to planting. Plasticulture has some advantages over the other production systems. In this system, plants are unaffected by summer diseases, drought, or weeds and have an early harvest, large berry size, and shorter time interval between planting and first harvest. Higher initial cost and requirement for excellent management skills are some of the disadvantages of this system. The annual strawberry cultivation system is rather complex. Hill system is the ideal production system for everbearing and day-neutral cultivars because it offers maximum productivity and is well suited to the plant's growth habit.

Strawberry Fruit Development and Ripening

Strawberry fruit development is influenced by a number of factors including the number of achenes and distribution of achenes on the surface of the receptacle and the area of receptacle tissue around the achenes. Growth of the receptacle is primarily controlled by auxin synthesized in the achenes. Several factors including poor pollination and damage to achenes by frost, insects, or diseases that prevent the synthesis of auxins by achenes will result in malformed and distorted fruit development. The nonclimacteric, auxin-dependent strawberry fruit development can be divided into three stages: division, expansion, and ripening during which color shifts from green to white to dark red in about 40 days following anthesis. Growth of the fruit is mainly by cell enlargement with cell size increasing towards the inner part of the fruit. Cells in the cortex primarily contribute to fruit size, while cells in the cortex and pith are responsible for the growth of receptacle. Cell division occurs mostly before anthesis accounting for only 15–20% of the total fruit growth.

Although auxin stimulates the swelling of receptacle, it inhibits the overall ripening process. An important triggering factor for fruit ripening is probably the decline in auxin content that occurs during achene maturation. Green, white, pink, and red are the four consecutive stages of strawberry fruit development. During ripening, a series of physiological and biochemical changes occur that transform the inedible fruit into an edible fruit with favorable organoleptic characteristics. The most notable events associated with ripening involve alterations to fruit size, shape, texture, and pigmentation and the production of aroma and flavor compounds. As ripening progresses, an increase in anthocyanin content is accompanied by a decrease in fruit firmness. The other changes include increase in soluble solids, total sugars, ascorbic acid, reducing sugars, and water-soluble pectins and a reduction in acidity, total phenols, and cellulose. Sucrose is continuously imported from photosynthetic tissue during strawberry fruit development. Sucrose is hydrolyzed into glucose and fructose, the major sugars accumulated in ripe strawberries. The physiological changes associated with ripening result from changes in gene expression and enzyme activities.

Strawberry Consumption and Products

Strawberries are highly perishable and are easily susceptible to mechanical injury, water loss, and microbial decay. So it has a very demanding postharvest handling requirement. For fresh market use, the vast majority of strawberries are hand-harvested. Mechanically harvested berries are only suitable for processing because of extensive bruising during harvesting. Strawberry is a nonclimacteric fruit and has a short shelf life. Strawberries are primarily consumed as fresh but some strawberries are also frozen or processed into other products. Strawberries are often processed into puree, juice, and concentrates and used to make such products as jams, jellies, ice creams, and yogurts. The production of jams and jellies requires heating the fruit puree and the addition of pectin and sugar. Strawberries are also processed into spreads, preserves, syrups, juice blends, nectars, drinks, milk shakes, and fillings for bakery products such as pies, pastries, doughnuts, and cheesecakes.

Generally, strawberries are frozen individually or in block after the removal of cap and stems. Prior to quick-freezing, harvested strawberries are precooled to maintain color, texture, and flavor and graded and quickly rinsed with chlorinated water to remove field debris. Individually quick-frozen strawberries maintain their color and shape and so they are preferred as raw materials for manufacturing value-added products such as freeze-dried or infused dried strawberries. In some cases, sugar is also added to strawberries and the product is stored frozen. These products are added to yogurts and ice creams after pasteurization. Infused frozen strawberries are made by infusing and pasteurizing whole or sliced strawberries in sugar syrup or in other sweeteners. These products are pasteurized and are used in ice creams, sorbets, and bakery products. Infused dried strawberries are made by infusing strawberries to different sweetness levels prior to drying.

Nutritional Profiles of Strawberries

Soluble sugars, organic acid, amino acids, and other secondary metabolites contribute towards the fruit quality and nutritional value of strawberry. Being low in total calories, with a 100 g providing only 32 kcal, strawberry represents a healthy food choice. According to the available data (Table 1), they are a significant source of B vitamins, vitamins C and E, potassium, folic acid, carotenoids, and flavonoids such as pelargonidin, quercetin, and catechin. This fruit is also rich in manganese. Strawberries have also been considered as a moderate source of potassium, iodine, magnesium, copper, iron, and phosphorus.

Phytochemicals

Besides the common vitamin and mineral components, strawberries are exceptionally high in health-supporting

Table 1 Nutrient composition of strawberries. Value per 100 g

Nutrient	*Unit*	*Raw strawberry*	*Frozen, unsweetened*	*Sweetened whole*	*Sweetened, sliced*
Water	g	90.95	89.97	78.05	73.18
Energy	kcal	32	35	78	96
Protein	g	0.67	0.43	0.52	0.53
Total lipid	g	0.3	0.11	0.14	0.13
Carbohydrate	g	7.68	9.13	21	25.92
Fiber	g	2	2.1	1.9	1.9
Total sugars	g	4.89	4.56	18.61	24.01
Calcium	mg	16	16	11	11
Iron	mg	0.41	0.75	0.47	0.59
Magnesium	mg	13	11	6	7
Phosphorus	mg	24	13	12	13
Potassium	mg	153	148	98	98
Sodium	mg	1	2	1	3
Zinc	mg	0.14	0.13	0.05	0.06
Vitamin C	mg	58.8	41.2	39.5	41.4
Thiamin	mg	0.024	0.022	0.015	0.016
Riboflavin	mg	0.022	0.037	0.077	0.051
Niacin	mg	0.386	0.462	0.293	0.401
Vitamin B_6	mg	0.047	0.028	0.028	0.03
Folate	μg	24	17	4	15
Vitamin B_{12}	μg	0	0	0	0
Vitamin A, RAE	μg	1	2	1	1
Vitamin A, IU	IU	12	45	27	24
Vitamin E	mg	0.29	0.29	0.24	0.23
Vitamin D	μg	0	0	0	0
Vitamin K	μg	2.2	2.2	1.9	1.7
Total saturated fatty acids	g	0.015	0.006	0.007	0.007
Total monounsaturated	g	0.043	0.015	0.019	0.018
Total polyunsaturated	g	0.155	0.054	0.068	0.064
Cholesterol	mg	0	0	0	0

Source: National nutrient database for standard reference Release 27.

phytochemicals such as polyphenolics. Several research groups have examined the phytochemical composition of strawberry and found them highly variable. The chemical composition of strawberries can be significantly affected by genotype and other factors such as cultivation practices, environment, maturity stage, and postharvest storage. Processing of fresh strawberry fruit into products such as jams, juices, and purees is also associated with some loss of bioactive components.

Phenolic Compounds

The main phenolic compounds in strawberries are anthocyanins, flavonols, flavanols, followed by hydrolyzable tannins (ellagitannins and gallotannins), phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), and condensed tannins (proanthocyanidins). The polyphenols account for most of the health benefits of strawberries. Anthocyanins are responsible for the red color of strawberries. Prior to maturity, the level of anthocyanins is undetectable in strawberries. As fruits enter the white stage, anthocyanins begin to accumulate and continue until full ripening. Anthocyanins are the best-known polyphenolic compound in strawberries, and more than 25 anthocyanin pigments have been reported in different varieties of strawberries by various research groups. Many studies reported anthocyanin contents ranging from 150 to 800 mg kg^{-1} fresh weight. Cyanidin-3-glucoside, pelargonidin-3-glucoside, 3-rutinoside, and 3-malonyl glucoside are the major anthocyanins in strawberries. Many previous studies reported pelargonidin-3-glucoside as the prominent anthocyanin component in many cultivars. In strawberries, flavonols occur in monomeric (catechins) and polymeric forms called condensed tannins or procyanidins. The most common flavonols in strawberries are quercetin and kaempferol derivatives. Acylated flavonols such as quercetin- and kaempferol-3-malonylglucoside have also been detected in strawberries. Several studies reported that procyanidins possess antioxidant, antimicrobial, antiallergic, and antihypertensive properties.

Most of the ellagic acid in strawberries is in the form of ellagitannins, which are a different class of phenolics. Strawberry ellagitannins are complex and diverse involving different combinations of gallic acid and hydroxydiphenic acid with glucose. The total content of ellagitannins ranges between 7.7 and 18.2 mg 100 g^{-1} FW. Ellagic acid is reported as the main phenolic acid in strawberry. Among the berries, raspberries, and strawberries contain the highest content of ellagitannins. Ellagic acid and ellagitannins also contribute significantly to the total antioxidant capacity and anticarcinogenic property of strawberries. Other phenolic acids have also been noticed in strawberries in small amounts. The derivatives of *p*-coumaric acid and hydroxybenzoic acid have also been detected in

strawberry. Ferulic acid derivatives were also identified in some cultivars. The predominant phenolic acids in strawberries are *p*-hydroxybenzoic acid and *p*-coumaric acid.

Strawberry Volatiles

Strawberries are enjoyed and appreciated for their vibrant color, distinct aroma, sweetness, and characteristic flavor. Volatile compounds are responsible for the unique aroma of fresh strawberries and are also an important quality attribute influencing consumer acceptability. The production of volatiles is one of the noticeable changes that occurs during ripening. Several studies have determined the aroma components of various cultivars of strawberry. Strawberry volatiles are highly complex consisting of several hundred compounds. Although these aromatic compounds are present in very low concentrations of about 0.01–0.001% on a fresh fruit weight basis, individual compounds may have a significant impact on the overall aroma of the strawberry. The volatiles and flavor compounds in strawberry have been studied exhaustively in many genotypes and cultivars. Various studies have detected more than 360 compounds in strawberry. Strawberry aroma compounds can be grouped into several chemical classes, including acids, aldehydes, ketones, alcohols, esters, lactones, sulfur compounds, acetals, furans, phenols, epoxides, and terpenes. However, esters are the main contributors to strawberry aroma. Esters constitute 25–90% of the total volatiles in ripe strawberry. Among the esters, butanoates comprised the predominant proportion. The esters methyl butanoate, ethyl butanoate, methyl hexanoate, ethyl hexanoate, ethyl 2-methylbutanoate, hexyl acetate, and hexenyl acetate are considered as flavor components that contribute to the fruity and green aroma. Normally, terpenes and sulfur compounds comprise less than 10 and 2% of total volatiles produced, respectively. The terpene alcohol, linalool, has been attributed to the floral, fruity, sweet, and berrylike aroma notes of strawberries. Another lactone compound, gamma decalactone, which imparts spicy, floral, and fruity characteristics, has been found in high concentrations in some cultivars. Mesifurane and furaneol give the characteristic caramel-like sweet, floral, and fruity aroma. Hexenal is thought to be responsible for the green or fresh fruit aroma. Strawberry aroma is determined by a complex series of compounds that can be affected by many factors including cultivar or varietal differences, stage of ripeness, and growing conditions.

Strawberry Consumption and Health Effects

The consumption of strawberries has been linked to several health benefits including prevention of inflammation, oxidative damage, cardiovascular disease (CVD), cancers, type 2 diabetes, obesity, and neurodegeneration.

Antioxidant Capacity

Antioxidants could serve a crucial role in disease prevention by reducing the formation of free radicals through free radical scavenging and stimulating the defense system. The antioxidative properties and potentials of polyphenols have been studied including their capacity for free radical scavenging, inhibition of lipid oxidation, and enhancement of the activity of endogenous antioxidative enzyme systems such as superoxide dismutase, glutathione reductase, and catalase. The antioxidant potential of polyphenols is dependent on their chemical structure and is mostly correlated to their polyphenol and vitamin contents. In many studies comparing the antioxidant capacity of fruits, strawberry consistently received higher ranking compared to other fruits and vegetables. Strawberries were ranked fourth among common fruits in total antioxidant and antiproliferative activities on human liver cancer cells. In a study conducted in various strawberry cultivars, vitamin C was shown to be the greatest contributor (>30%) of the total antioxidant capacity of strawberry extracts followed by anthocyanins (contributing 25–40%), ellagitannins, and flavonols. Human intervention studies conducted with fresh, frozen, or freeze-dried strawberries have demonstrated the antioxidant capacity of strawberries to reduce oxidant stress associated with high-fat meals, hyperlipidemia, or metabolic syndrome. Flavonoids of strawberries have been shown to exert its antioxidant action mainly by scavenging free radicals, inducing phase 2 enzymes of antioxidant defense and detoxification, and modulating antioxidant gene expression. An increase in postprandial plasma antioxidant capacity was noticed in healthy subjects or hyperlipidemic volunteers after consuming strawberry or mixed berry. A number of *in vitro* studies have demonstrated the potent antioxidant activities and free radical scavenging ability of phenolic compounds.

Cardiovascular Diseases

Obesity, hyperglycemia, hyperlipidemia, hypertension, inflammation, and oxidative stress are some of the known risk factors for the development of CVD. The oxidation of low-density lipoprotein (LDL) was suggested to be an early event in the development of atherosclerosis. Evidence on the positive effects of the consumption of berries and berry flavonoids on cardiovascular health is available. Only limited epidemiological studies provide evidence for the protective and beneficial effects of strawberry on cardiovascular health particularly in women. A significant reduction in CVD mortality associated with strawberry intake (a mean anthocyanin intake of 0.2 mg per day) was noticed in a study conducted in 34 489 overweight postmenopausal women during a 16-year follow-up period. In another women's health study conducted in female US health professionals ($n = 38\,176$), strawberry intake has also been associated with lower levels of C-reactive protein, which is a stable inflammatory biomarker associated with inflammation and is a high risk factor of CVD. A decreasing CVD incidence was also observed in subjects consuming higher amounts of strawberries during a follow-up study period of 11 years.

Improvement in serum lipid profile through a reduction of total cholesterol, LDL-C, and triglyceride levels was noticed in a study conducted in healthy young volunteers who consumed 500 g of strawberries for 1 month. The nutrients and phytochemicals in the fruit might have favorably affected the plasma lipid profiles. Some human

intervention studies provide evidence for the efficacy of freeze-dried strawberry in the nutritional management of elevated lipid profiles. A few epidemiological studies have shown a positive correlation between flavonoid consumption and reduced risk of CVD.

Anticancer and Cytotoxicity

Evidence is available that supports the anticarcinogenic effects exerted by extracts or purified strawberry phytochemicals mediated via detoxification of carcinogens, protection against DNA damage, antiproliferative effects on cancer cells via apoptosis, and increase in serum antioxidant capacity. Strawberry tannins have also demonstrated anticancer properties through antiproliferative effects on human breast, cervix, and colon carcinoma cells. Purified ellagic acid from strawberries has also shown potent anticarcinogenic benefits in several human cancer cell models. Strawberries treated with thymol, menthol, and eugenol showed stronger inhibition of human HT29 colon cancer cell growth than the untreated berries. The antiproliferative effects of organically and conventionally grown strawberries were evaluated and the organically grown strawberries showed greater antiproliferative activity than the conventionally grown strawberries owing to the higher content of bioactive substances present.

Protection against UV Radiation and DNA Damage

Some *in vitro* studies have suggested that the phytochemicals in strawberry could be helpful in skin protection. Recently, the photoprotective effect of anthocyanin-rich strawberry extracts on human dermal fibroblast exposed to UV-A radiation was studied. Incubation of human dermal fibroblasts in strawberry extract has resulted in an increase in cellular viability and a decrease in DNA damage in a dose-responsive manner when compared to control cells. Consumption of fresh strawberries by young and middle-aged subjects also resulted in significant increase in serum total antioxidant capacity and vitamin C concentrations. A decrease in oxidative biomarkers in urine and blood plasma was also noticed after strawberry consumption for a month.

Bioavailability and Metabolism of Strawberry Phytochemicals

Factors affecting the absorption and metabolism of strawberry phytochemicals (dietary polyphenols) are complex. Bioavailability and metabolism are crucial to the potential health-promoting effect of polyphenols. The bioavailability of phenolic compounds in foods is a complex process influenced by their chemical structure, size, solubility, degree of glycosylation, and conjugation with other compounds. Polyphenols are released from the food matrix during digestion and the amounts of consumed polyphenols available for absorption may undergo modifications before reaching the target tissue. Bioavailability of polyphenols is generally low and in the range of 0.5–1.5% of the amount ingested. Interaction of polyphenols with other structural components such as proteins, carbohydrates, and fibers occurring in food matrices is one of the factors determining the bioavailability of polyphenols.

It was noticed that most polyphenol glucosides in the small intestine are deglycosylated by glucosidases or transported by the lactase–phlorizin hydrolase through the epithelial barrier. After reaching the small intestine, flavonoids in the form of glycoside conjugates are hydrolyzed by lactase–phlorizin hydrolase leading to the release of aglycone. These aglycones pass into the epithelial cells by passive diffusion as a result of its increased lipophilicity. Those flavonoids that are not taken up in the small intestine pass to the colon where they are processed by colonic microflora that hydrolyze glucosides into aglycones and extensively metabolize these aglycones into various aromatic acids. The resultant aglycones are transformed into phenolic acids and hydrocinnamates that are assimilated. In the liver, they are metabolized and excreted in the urine. Flavonoid glucosides that enter the colon from the small intestine are exposed to a microbial population that is capable of modifying the molecular structure of the phenolic compounds through hydrolysis, dehydroxylation, demethylation, ring cleavage, and decarboxylation. Flavonoids undergo glucuronidation in the small intestine by UDP-glucuronyl transferase. But their absorption is relatively low. Some of the ingested flavonoids are possibly absorbed in the stomach.

The absorption and excretion of anthocyanins are influenced by the anthocyanidin structure and the sugar moiety. Anthocyanins are maintained as the stable flavylium cation form under the acidic pH of the stomach, and so only a limited quantity of anthocyanins is quickly assimilated from the stomach. Some of the anthocyanins are absorbed as intact glycosides. The principal anthocyanin in strawberry is pelargonidin-3-glucoside, and it undergoes gastric conversion to pelargonidin glucuronide followed by its conversion to 4-hydroxybenzoic acid. Urinary excretion of pelargonidin-3-glucoside and its metabolites such as pelargonidin and monoglucuronides was observed in human subjects with increased consumption of fresh strawberries. On reaching the small intestine, anthocyanins are converted into some neutral pH forms such as hemiketals, chalcones, and quinonoids. These compounds are partially assimilated in the jejunum and undergo conjugation reactions mostly in the liver, kidney, and also in the intestine. *In vitro* digestion using fecal microbiota has shown that the bacterial metabolism of anthocyanin glucosides involves the cleavage of the anthocyanin heterocyclic rings. The resulting aglycone is further transformed into low-molecular-weight compounds such as phenolic acids. The compound 4-hydroxybenzoic acid has been suggested as a major metabolite of pelargonidin-3-gluciside from strawberry. Protocatechuic acid has been proposed as a potential metabolite from the microbial conversion of cyanidin-based anthocyanidin. Also, coumaric acids, 4-hydroxybenzoic acid, and protocatechuic acid were retrieved from the plasma 8 h after strawberry consumption.

See also: Berries and Related Fruits; Phenolic Compounds: Bioavailability and Health Effects; Raspberries and Related Fruits.

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Sucrose: Dietary Importance

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Definition

Carbohydrates are the world's primary source of food energy. The Food and Agriculture Organization of the United Nations (FAO) and, more recently, the European Food Safety Authority (EFSA) have advised that carbohydrate intake should range from 45% to 60% for both adults and healthy children older than 1 year of age.

Dietary carbohydrates can be in the form of complex molecules (polymers or polysaccharides) or more simple molecules, monomers (monosaccharides) or dimers (disaccharides), commonly referred to as sugars. Sucrose is a disaccharide composed of the monosaccharides glucose and fructose. Sucrose is the organic compound commonly known as table sugar, cane sugar, or beet sugar. Sucrose is an odorless, white, crystalline powder with a sweet taste. It is a nonreducing sugar, which is soluble in water and crystallizes easily. It is used to sweeten foods, improve the sour and bitter taste of many foods, and preserve foods by increasing osmotic pressure, which prevents the growth of many microorganisms.

Sucrose is hydrolyzed in the small intestine via the action of the sucrase–isomaltase enzyme complex, and the resulting monosaccharides are later metabolized, rendering energy or serving as a carbon skeleton for other biological compounds, primarily glycogen, fatty acids, and cholesterol.

Chemical and Physical Characteristics

Chemical Characteristics

Sucrose (α-D-glucopyranosyl-β-D-fructofuranoside) is a disaccharide with the general molecular formula $C_{12}H_{22}O_{11}$, and it has a molecular weight of 342.30. In solution, it is present primarily in the cyclic form, which can be represented in various ways (e.g., a Fischer projection or as a Haworth-type structure; Figure 1). All sugars are fairly reactive but, in sucrose, the reactive sites of glucose and fructose are bonded together. Sucrose is a nonreducing sugar and is stable when heated. Fructose will decompose at temperatures above 103 °C, and both glucose and fructose are unstable in alkaline solutions. In acidic solutions, sucrose will invert or break down to its component monosaccharides. This reaction is accelerated with increasing acidity or temperature. In solution (including in human metabolism), most sucrose reactions begin with the inversion reaction.

Physical Properties

Sucrose is normally used as a solid because it is extremely stable in its crystalline form. Pure sucrose is a colorless, odorless crystalline powder with a pleasing sweet taste without any other flavor or aftertaste; it is the standard reference for sweetness. If sucrose is given a sweetness rating of 100, the relative sweetness of fructose is between 105 and 125, and that of glucose between 65 and 75, depending on the conditions (e.g., acidity, pH, and temperature). The specific gravity of a single sucrose crystal is 1.5879 g ml^{-1}.

Sucrose melts and decomposes at 186–188 °C to form caramel and, when combusted, it produces carbon, carbon dioxide, and water. The heat of combustion is 1351.3 kcal mol^{-1} or 3.95 kcal g^{-1}. Sucrose is, therefore, less caloric than fat (9.3 kcal g^{-1}) and protein (4.1 kcal g^{-1}).

The International Commission for Uniform Methods of Sugar Analysis (ICUMSA) is an international standards body, founded in 1897, which publishes detailed laboratory procedures for the analysis of sugar. Sucrose is extremely soluble in water and alcohol and other polar solvents. Solubility is one of the most studied properties of sucrose because it is such an important factor in production and use. Numerous tables are available to determine the saturation point of sucrose (maximum solubility) under various conditions.

Because the amount of sucrose dissolved in water is important industrially, a series of measuring indexes have been developed to indicate the relative proportions of the two materials. The most important of these is the Brix scale, which provides the percentage of sucrose (by weight) in a water solution. For example, 65° Brix would represent a solution that is 65% sucrose and 35% water. Thus, a Brix reading can be used to obtain the corresponding specific gravity or refractive index of a solution that, in practice, corresponds to a solution of pure sucrose at a set reference temperature, usually 20 °C. This index is very useful for estimating the sucrose content in many foods containing relatively high amounts of sucrose, namely, fruit juices.

Other properties that are commonly measured include viscosity, osmotic pressure, boiling point elevation, and equilibrium relative humidity, because these physical properties are very useful in the food technology field.

Sources and Production

Table sugar (sucrose) comes from plant sources. Two important sugar crops predominate: sugarcane (*Saccharum officinarum* L.) and sugar beet (*Beta vulgaris* L. var. *rapa*). Sugar can account for between 12% and 20% of dry weight. Minor commercial sugar crops include the date palm (*Phoenix dactylifera*), sorghum (*Sorghum vulgare*), and the sugar maple (*Acer saccharum*).

Sucrose is present naturally in many fruits and fruit juices, but it can also be found as added sugar in baby foods, baked products, beverages, breakfast cereals, cereal grains and pasta, dairy and egg products, ethnic foods, fast foods, fat and oil products, finfish and shellfish products, legumes and legume products, meals, entrées and side dishes, nut and seed products, poultry products, pork products, sauces, gravies and soups, sausages and luncheon meats, snacks, spices and herbs, sweets, and vegetables and vegetable products (see Table 1).

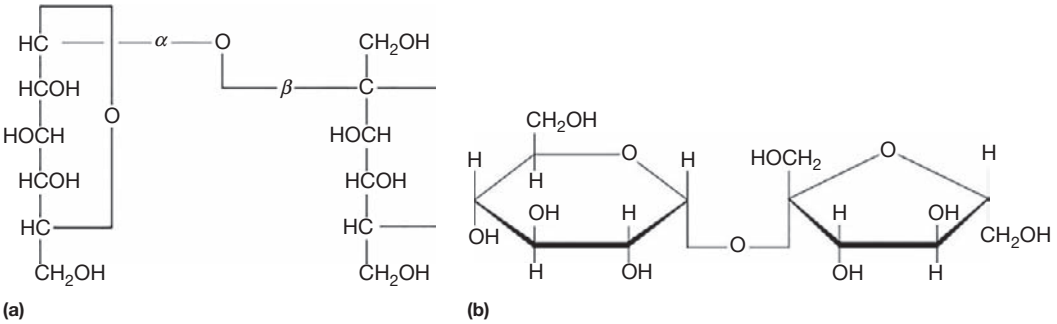

Figure 1 The chemical structure of sucrose. Macrae, R., Robinson, R. K., and Sadler, M. J. (eds) (1993). *Encyclopaedia of food science, food technology and nutrition*. Academic Press.

Table 1 The sucrose content of foods[a]

	Range in mg per 100 g serving
Baby foods	40–9340
Baked products	160–44 528
Beverages	50–59 896
Breakfast cereals	30–44 357
Cereal grains and pasta	20–1700
Dairy and egg products	10–10 000
Ethnic foods	10–20 081
Fast foods	10–32 098
Fat and oil products	290–6399
Finfish and shellfish products	30–2950
Legumes and legume products	340–8680
Meals, entrées, and side dishes	320–3501
Nut and seed products	340–51 503
Poultry products	20–600
Pork products	40–780
Sauces, gravies, and soups	240–1100
Sausages and luncheon meats	80–1300
Snacks	120–16 508
Spices and herbs	20–4089
Sweets	700–94 546
Vegetables and vegetable products	10–5250

[a]Detailed information for specific products can be found at http://nutritiondata.self.com/foods-00000900000000000000-w.html.

Sugarcane

Most sugarcane comes from countries with warm climates because sugarcane does not tolerate frost. Approximately 80% of the world's sucrose is derived from sugarcane; the remainder is almost entirely from sugar beets.

In 2013, Brazil, India, China, Thailand, and the United States were the major sugarcane-producing countries in the world. Brazil produced approximately 739 million tons of sugarcane in 2013, whereas India produced 341 million tons, China 125 million tons, Thailand more than 100 million tons, and the United States more than 27 million tons. Viewed by region, South America predominates in sugarcane production, providing 44.7% of the global production in 2013. Asia comes in the second place with 39.7% of the global production; Africa and Central America produce 11.2% and Oceania produces 1.5%.

Since the sixth century BC, sugarcane producers have crushed the harvested vegetable material from sugarcane to collect and filter the juice containing the sugar. Sugarcane processing requires two stages: mills extract the raw sugar from the freshly harvested cane and 'mill-white' sugar, which is intended for local consumption, is sometimes produced immediately afterward. Sugar crystals appear naturally white in color during the crystallization process. Sulfur dioxide is added to inhibit the formation of color-inducing molecules and to stabilize the sugar juices during evaporation.

Sugar Beets

Sugar beets come from regions with cooler climates, including northwest and eastern Europe and America; the Russian Federation produced more than 39 million tons in 2013, France 33 million tons, Germany 22 million tons, and the United States over 29 million tons.

White beet sugar is made from beets, which are harvested in the autumn and early winter by digging them out of the ground. The processing begins by slicing the beets into thin chips, which increases the surface area, making it easier to extract the sugar.

The extraction takes place in a diffuser, where the beet is kept in contact with hot water for about an hour before being squeezed in screw presses to extract as much juice as possible. The juice is cleaned up before it can be used for sugar production by carbonatation, where small clumps of chalk are grown in the juice. As the clumps form, they collect many nonsugars; filtering out the chalk also removes any nonsugar molecules.

The juice is evaporated in a multistage evaporator, and the syrup placed in a large pan, typically holding 60 tons or more of sugar syrup. In the pan, water is boiled off until the conditions are right for sugar crystals to grow. Once the crystals have grown, the resulting mixture of crystals and mother liquor is spun in a centrifuge to separate the two, similar to how laundry is spin-dried. The crystals are dried with hot air before being packed and/or stored for dispatch.

Patterns of Consumption

Global Scenario

At the present time, cane sugar and beet sugar are produced in more than 130 countries. As far as the consumption per country is concerned, India, China, Brazil, the United States, and Russia stand out, as they represent 45.2% of global consumption for the 2010–11 sugar cycle. India and Russia showed the

greatest increases in demand at 8.5% and 3.3%, respectively, for the same cycle.

There are six sugar-consuming regions in the world. The first is found in Asia (composed of 36 countries) with a deficit (between production and consumption) of 6.3 million tons and an annual consumption of 14.9 kg per capita. The region with the second highest consumption is composed of the 12 countries of the former Soviet Union, with a deficit of 4.8 million tons. In the third place is North Africa with a sugar consumption deficit of 3.9 million tons. In the fourth and fifth places are North America and Europe with a deficit of 2.8 and 2.4 million tons, respectively. In North America, the deficit region is composed of two countries, Mexico and the United States, which have a population of 422 million inhabitants who maintain a high sugar intake compared with production levels; in per capita terms, the sugar consumption deficit in this region is 2.8 million tons.

Sucrose Intake by Country

Although overall energy intake has steadily increased globally, worldwide trend data do not support the widely held view that refined sugar, as available for consumption, has increased dramatically over the last 40–50 years.

The most comprehensive trend analysis for the National Health and Nutrition Examination Survey (NHANES) 1999–2000 through 2007–08 revealed a decrease in total energy intake (from 2145 to 2070 kcal day^{-1}) for the US population. The absolute intake of added sugars decreased from 100 g in 1999–2000 NHANES to 77 g in 2007–08 (−24%). In addition, the percentage of total energy, via added sugar intake, fell from 18.1% to 14.6%.

In the United Kingdom, there were significant reductions for male children in total sugars (≈9%) and nonmilk extrinsic sugars (NMES) (≈22%) between 1997 and 2008–09, but there was no reduction in the overall total food energy. In female children, the only significant difference was a reduction in the percentage of food energy from NMES, which fell from 16.8% to 14.6%, with no other differences.

In France (INCA surveys), sugar intake only increased significantly in younger men aged 18–34 years (≈10 g day^{-1}, +9.8%) and in middle-aged women aged 35–54 years (≈10 g day^{-1}, +12.7%) between 1998–99 and 2006–07.

In Denmark, between 1999 and 2007, added sugar intake fell by 47% in 2-year-old children and by 62% in 12-month-old children.

In Sweden, the levels of sucrose intake between reports (1989 and 1997–98) increased by 6 g day^{-1} in men but were unchanged in women. The percentage of energy from sucrose between the reports increased marginally in men but was essentially unchanged in women.

In Spain, the daily sugar intake per capita is 111.2 g, according to the 2011 ENRICA study. China's per capita daily sugar consumption is less than 31 g and lower still in rural areas (5.5 g day^{-1}), which is much lower than levels consumed in medium- and high-income countries. In India, the per capita daily sugar consumption is greater than 58 g.

In Australia, the total sugar intake increased significantly between 1983 and 1995 by 14 and 5 g in men and women, respectively. In terms of the percentage of energy contribution, in men, the proportion of energy from total sugars increased between 1983 and 1995 from ≈18% to 20%. In women, it stayed relatively constant at 21% Figure 2 shows the sugar daily energy intake (kcal day^{-1}) in the world.

Digestion, Absorption, and Metabolism of Sucrose

The human body is able to capture some of the chemical energy from carbohydrates through cellular metabolism, resulting in the generation of an intermediate chemical form, adenosine triphosphate, which acts as an energy source for cellular processes. Adenosine triphosphate is regenerated from adenosine diphosphate using energy from food. The brain, nervous system, and red blood cells have an obligatory requirement for glucose as an energy source.

The intestinal digestion of sucrose requires hydrolysis of its α(1,2′)β-linkage, which involves the anomeric carbon of both hexoses, to yield fructose and glucose. In mammalian species, the hydrolysis of sucrose is performed via the sucrase activity of the sucrase–isomaltase complex in the enterocytes of the small intestine.

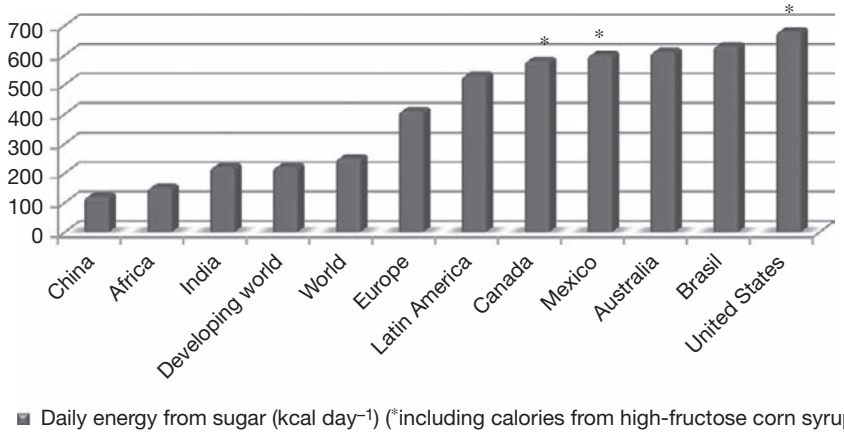

Figure 2 The world caloric intake of sugar. Adapted from USDA-ERS, Conadesuca, OECD, Credit Suisse Research, 2013.

Following absorption, glucose and fructose are transported to the liver by specific transporters, SLGT1 and GLUT-5, respectively. Glucose is phosphorylated to glucose-6-phosphate in the liver by glucokinase, which functions in glycogen synthesis or lipogenesis via the glycolytic pathway, or it reaches the systemic circulation. The plasma concentration of insulin increases immediately after the ingestion of glycemic carbohydrates. A family of glucose transporters mediates the cellular uptake of glucose, while in some tissues (e.g., adipose tissue and skeletal muscle), it is insulin-dependent (GLUT-4).

Fructose is metabolized by conversion to glycolytic pathway intermediates. In the liver, it is phosphorylated to produce fructose-1-phosphate in a reaction catalyzed by ketohexokinase or fructokinase. Fructose-1-phosphate is cleaved by the action of aldolase B to form dihydroxy-acetaldehyde-phosphate (DHAP) and glyceraldehyde. To metabolize glyceraldehyde, it has to be phosphorylated by triose kinase, creating glyceraldehyde-3-phosphate, which enters the glycolytic pathway, along with DHAP, at the triose-phosphate level. Under postprandial conditions, fructose is primarily converted to acetyl-coenzyme A and later to fatty acids and cholesterol.

Glucose is made available to body tissues via the bloodstream, and it is the primary carbohydrate used for energy. The balance between oxidation, biosynthesis, and glucose storage depends on the hormonal and nutritional state of the cells, tissues, and organism. The predominant metabolic pathways of glucose vary indifferent cells depending on the physiological demand. Thus, the liver has a fundamental role in glucose homeostasis in the body.

Glucose can be completely oxidized by hepatocytes to obtain energy, and it can be stored in the form of glycogen, or it can be used to supply carbons for fatty acid and amino acid synthesis. Heart and skeletal muscles can completely oxidize glucose or store it in the form of glycogen. Glucose can be partially broken down in fatty tissues to provide glycerol, which is necessary for triacylglycerol synthesis, or oxidized completely to supply two units of carbon (acetyl-CoA) for fatty acid synthesis.

The brain depends on the continuous supply of glucose, which can oxidize completely to form CO_2 and water. Conversely, erythrocytes, which have a limited capacity for oxidizing glucose because they do not have mitochondria, depend entirely on metabolic fuels for energy production, partially oxidizing glucose to lactate via glycolysis. Other specialized cells such as corneal cells, lens and retina cells, leukocytes, testicular cells, and renal medulla cells are predominantly glycolytic.

Nutritional Aspects of Sucrose

Although at first glance, the sole function of carbohydrates appears to be an energy source for human and animal metabolism, sugars have other significant structural functions in living organisms, such as important components of membrane antigens and proteins that are secreted by many cells.

The release of insulin, in response to sucrose ingestion, maintains blood glucose within a closely regulated range, which is essential for normal functioning of the central nervous system. Sugar does not cause abnormal insulin and blood glucose responses.

Sucrose as a Sweetener

Sweeteners can be categorized as nutritive and nonnutritive. Nutritive sweeteners (NSs) contain carbohydrates and provide energy (4 kcal g^{-1} and an average of 2 kcal g^{-1} for sugar alcohols). Nonnutritive sweeteners (NNSs) offer little or no energy when ingested. They are referred to as high-intensity sweeteners because, as sweetening ingredients, they are many times sweeter than sucrose. NNSs can replace the sweetness of sugar or energy-containing sweeteners. However, they do not have the same functional properties, such as browning, crystallization, and microbial inhibition.

Enjoying a sweet taste is innate, but the perception of sweetness and the preferred level of sweetness vary among individuals. Taste perception begins on the tongue and soft palate, where taste receptors interact with food or beverages. Taste receptor cells are organized into taste buds, which are distributed throughout the tongue and on specialized structures called papillae. A sweet taste is elicited by interaction with a sweet receptor, which is a dimeric G protein-coupled receptor composed of T1R2 and T1R3 subunits with multiple active sites. T1R2 and T1R3 receptors respond to sugars (e.g., sucrose, fructose, galactose, glucose, lactose, and maltose) and NNSs (e.g., acesulfame K, aspartame, cyclamate, dulcin, neotame, saccharin, and sucralose), although their specific preferential binding sites may vary.

Sucrose is an important part of both groups of sweeteners. In the NS group, which includes sucrose, glucose, and fructose, the carbohydrate adds texture, flavor, and color to baked goods; supports the growth of yeast for leavening or fermentation; contributes to volume in ice cream, baked goods, and jams; enhances the creamy consistency of frozen desserts; enhances the crystallization of confectionary products; balances acidity in salad dressings, sauces, and condiments; and helps to maintain the natural color, texture, and shape of preserved fruits. In the NNS group, the carbohydrate is present in a modified form, such as sucralose.

The chlorination of three hydroxyl groups on sucrose produces a modified sucrose that is approximately 600 times sweeter than sucrose (sucralose). Sucralose is used as a sugar substitute in numerous food products, including beverages, baked goods, dairy products, canned fruits, syrups, and condiments, and it is sold direct to consumers as an NS sugar replacement (e.g., Splenda™).

Health Effects

In the last several decades, numerous epidemiological studies have associated a high intake of sugar with higher prevalence of noncommunicable chronic diseases, primarily dental caries, being overweight or obese, diabetes, and cardiovascular disease (CVD). However, recent reviews and meta-analyses do not always support this hypothesis, particularly when the results are adjusted to the energy intake.

Dental Caries

There is consistent evidence of moderate quality supporting a relationship between the amount of total sugars consumed and

the development of dental caries. Dental caries progress with age, and the effects of sugar on dentition are lifelong. Even low levels of caries in childhood are significant to the amount of caries throughout an individual's lifetime. Nevertheless, dental caries, regardless of sugar consumption, has decreased dramatically in developed countries due to increased buccodental hygiene and the use of fluoridated toothpaste and/or water supplies.

Population studies support the dose–response effect between sugar intake and dental caries with 18 of 20 studies showing a positive association, one showing a neutral association and one showing a negative association between sugar intake and the presence of dental caries. Additionally, nine population studies provide evidence of positive correlations between sugar intake and caries levels. There is also evidence of moderate quality showing that dental caries are lower when free sugar intake equates to less than 10% of the total food energy. Indeed, countries with estimated sugar intakes are less than 10% – or even 5% – of the total food energy that do not have lower prevalence of caries. Changes in the sugar supply do not reliably predict the magnitude or trends in the prevalence of caries. Most developed countries have a lower prevalence of caries than many countries with lower sugar intake, despite intakes being greater than 10% of the total food energy. However, the majority of data are for children meaning the situation in regard to adult dental health is unclear.

Obesity

Obesity occurs when energy intake from food and drink consumption is greater than energy expenditure through the body's metabolism and physical activities over a prolonged period, resulting in the accumulation of body fat. Changes in body weight would be expected from any change in macronutrient intake that leads to an overall excess or deficit in food energy compared with the requirements for weight stability. An earlier World Health Organization (WHO)-sponsored expert review stated, "excess energy in any form will promote body fat accumulation."

A recent review and meta-analysis reported that isocaloric substitution of sugars for other carbohydrates does not lead to weight gain. Therefore, there is no consistent or reliable evidence that sugars affect obesity more than any other macronutrient.

Currently, there is no clear evidence of a dose–response gradient for the amount of sugar consumed and body weight. There are a number of short-term intervention studies that focus on increasing or decreasing intakes of sugars and sugar-containing foods, but the majority are unreliable as evidence of the effect of 'free sugars' on obesity because of confounding information on the caloric intake from other macronutrients.

Conversely, there are no randomized controlled trials in children to evaluate the potential effects of sugar on body weight. Therefore, some authoritative reviews have concluded that there is no consistent or reliable body of evidence to support any specific sugar intake level for the avoidance of obesity in childhood.

The overconsumption of food energy, whatever its macronutrient composition, will lead to weight gain. However, there is no convincing evidence to support the widely held view that sugar, including sugars in drinks, is specifically responsible for obesity.

In addition, there is no convincing evidence that sugar is addictive or stimulates overconsumption. Until scientists develop valid and reliable methods for measuring energy intake in individuals and can balance this against energy expenditure, more precise causes of weight gain will remain elusive. Although obesity may be related to morbidity and mortality rates, whether this is predominately a reflection of inadequate physical activity and low physical fitness beyond high energy and dietary sugar intake requires further examination.

Diabetes

Diabetes mellitus (DM) is one of the most prevalent endocrine diseases in the general population. A number of expert reports have considered the possible role of sugar in the causation of DM. In the 1980s, the US Food and Drug Administration Sugar Task Force and UK Committee on the Medical Aspects of Food (and Nutrition) both dismissed the notion that sugar causes DM directly. The EFSA has indicated, "available evidence is insufficient to set an upper limit for sugars based on their effects on type 2 DM risk."

Likewise, the UK recommendations do not consider it appropriate to make specific recommendations for single nutrients, including added sugars. Moreover, the American Diabetes Association suggests minimizing the substitution of sucrose-containing foods for isocaloric amounts of other carbohydrates. Nevertheless, there is moderate evidence that high consumption of sugar-sweetened beverages (SSBs) contributes to an increased risk for type 2 DM. The direction of the association indicates that greater consumption of SSBs is detrimental to health and the association is biologically relevant. However, recent recommendations to prevent DM are focussed on the restriction of total carbohydrate and energy intake and increasing physical activity to allow for weight control, particularly in those who are obese.

Cardiovascular Diseases

There is some concern that increased consumption of carbohydrates (particularly sugars and especially fructose), as may occur on a low-fat high-carbohydrate (LFHC) diet, could result in increased plasma triacylglycerols and reduced High density lipoproteins (HDLs) cholesterol (HDL-c), which are both independent risk factors for CVD. A number of in-depth reviews have examined the effects of carbohydrates on triglycerides and factors that may influence any effect on CVD.

These reviews have shown that in controlled isocaloric feeding studies, LFHC diets are associated with marginally reduced HDL-c (although it often results in an improved total cholesterol: HDL ratio) and slightly increased triacylglycerols. The authors found no adverse effects on cardiovascular risk factors (i.e., blood lipids, glucose, and insulin) when sucrose replaced starchy foods at the levels of at least 25% of total energy intake.

Hypocaloric diets (not ad libitum), which can be low or high in sucrose, have been shown to reduce serum triacylglycerol levels. Consumption of fructose within the range

observed across the US population is not associated with hyperlipidemia in normal, overweight, or obese individuals, although high consumption may result in nonalcoholic fatty liver disease.

A low-fat (particularly low in saturated fat) high-carbohydrate diet, adequate physical activity/cardiorespiratory fitness, and abstinence from tobacco use are the current recommendations to reduce the risk of CVD. Although changing to a high-carbohydrate diet may increase blood triacylglycerols, the implications for CVD risk are unknown, and outcomes are moderated by many factors that require further study. Thus, currently, there is insufficient evidence to conclude that sugar directly or indirectly increases the risk of CVD.

See also: Candies and Sweets: Sugar and Chocolate Confectionery; Carbohydrate: Digestion, Absorption and Metabolism; Obesity: Causes and Prevalence; Sweeteners: Classification, Sensory and Health Effects.

Further Reading

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http://nutritiondata.self.com/foods-00000900000000000000-w.html – SELFNutritionData Foods highest in sucrose.

http://www.wsro.org/ – The World Sugar Research Organisation.

Sucrose: Properties and Determination

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Introduction

Sucrose occurs in many foodstuffs. This disaccharide forms a major element in confectionery and desserts. Cooks also use it for sweetening – its fructose component, which has almost double the sweetness of glucose, makes sucrose distinctively sweet in comparison to other food carbohydrates. Furthermore, it can act as a food preservative when utilized in sufficient concentrations. Sucrose is important to the structure of many foods, including biscuits and cookies, cakes and pies, candy, and ice cream and sorbets. Finally, it is a common ingredient in many processed foods and the so-called junk foods, which include salted snack foods, gum, candy, sweet desserts, fried fast-food, and sugary carbonated beverages. That is why knowledge of its quantitative distribution in those foodstuffs and in dairy products, meat and meat derivatives, and other natural matrices is essential, since they are involved in very important characteristics, such as flavor, maturity, quality, authenticity, and storage conditions.

Definition and Properties

Sucrose is a carbohydrate (sugar). A white, odorless, crystalline powder with a sweet taste, it is best known for its role in food. The molecule (molecular weight 342.30) is a disaccharide composed of the monosaccharides glucose and fructose (both are $C_6H_{12}O_6$) with the molecular formula $C_{12}H_{22}O_{11}$. Sucrose is a molecule with nine stereocenters and many sites that are reactive or can be reactive. The molecule exists as a single isomer.

In sucrose, the components glucose and fructose are linked via an ether bond between C1 on the glucosyl subunit and C2 on the fructosyl unit. The bond is called a glycosidic linkage. Glucose exists predominantly as two isomeric 'pyranoses' (α and β), but only one of these forms links to the fructose. Fructose itself exists as a mixture of 'furanoses,' each of which having α and β isomers, but only one particular isomer links to the glucosyl unit. What is notable about sucrose is that, unlike most disaccharides, the glycosidic bond is formed between the reducing ends of both glucose and fructose and not between the reducing end of one and the nonreducing end of the other. This linkage inhibits further bonding to other saccharide units.

Physical Properties

Sweetness is probably the main characteristic of sucrose. In fact, sucrose is the standard sweetener to which all other sweeteners are compared. For this purpose, the relative sweetness of sucrose is set to 1. A growing number of alternative sweeteners exist on the market, all of them with somewhat different sweetness compared to sucrose. In this sense, sweeteners are divided into two main groups: bulk sweeteners (sorbitol, glucose, fructose, etc.), with a relative sweetness lower or slightly higher than sucrose, and high-intensity sweeteners (such as saccharin, aspartame, and acesulfame K), with a relative sweetness considerably above 1.

Sucrose crystallizes in the monoclinic space group $P2_1$ with the following room-temperature lattice parameters: $a = 1.08631$ nm, $b = 0.87044$ nm, $c = 0.77624$ nm, and $\beta = 102.938°$. It should also be remarked that the specific rotation at 20 °C using yellow 'sodium D' light (589 nm) is +66.47°.

The solubility of sucrose in water increases with increasing temperature. This relatively high solubility is an important parameter for its bulking effect in many foods and beverages. The dissolved sugar increases the viscosity of water-based solutions or mixtures, resulting in enhanced mouthfeel. On the other hand, dissolved sucrose lowers the freezing point of ice cream by preventing the water molecules from combining to form ice crystals, which slows down the freezing process. The frozen water crystals no longer in solution increase the sucrose concentration in the remaining solution and lower the freezing point even further.

Finally, sucrose does not deteriorate at ambient conditions. It does not melt at high temperatures. Instead, it decomposes – at 186 °C – to form caramel.

Chemical Properties

When dissolved in water, sucrose ($pK_a = 12.51$ at 25 °C) is chemically incapable of altering the amount of protons or hydroxide ions in solution. Therefore, the solution will exhibit no change in pH.

Like other carbohydrates, it combusts to carbon dioxide and water. Mixing sucrose with the oxidizer potassium nitrate produces the fuel known as rocket candy. The simplified reaction is

$$C_{12}H_{22}O_{11} + 6\,KNO_3 \rightarrow 9\,CO + 3N_2 + 11H_2O + 3K_2CO_3$$

Sucrose burns with chloric acid, formed by the reaction of hydrochloric acid and potassium chlorate:

$$8HClO_3 + C_{12}H_{22}O_{11} \rightarrow 11H_2O + 12CO_2 + 8HCl$$

On the other hand, sucrose can be dehydrated with sulfuric acid to form a black, carbon-rich solid, as indicated in the following idealized equation:

$$H_2SO_4(\text{catalyst}) + C_{12}H_{22}O_{11} \rightarrow 12\,C + 11\,H_2O + \text{Heat (and some } H_2O + SO_3 \text{ as a result of the heat).}$$

Since the molecule of sucrose contains no anomeric hydroxyl groups, it is classified as a nonreducing sugar. This absence of redox properties plays a capital role in the fundamentals of the different chemical methods proposed for the determination of this disaccharide.

Hydrolysis breaks the glycosidic bond converting sucrose into glucose and fructose. This process is also called inversion,

since sucrose is dextrorotatory, whereas the resulting mixture of glucose and fructose (a.k.a. inverted sugar) is slightly levorotatory. Hydrolysis is, nevertheless, extremely slow, but, if the enzymes sucrase and invertase are added, the reaction will proceed rapidly. Hydrolysis can also be accelerated with some acids, such as diluted HCl or monochloroacetic acid. Likewise, gastric acidity converts sucrose to glucose and fructose during digestion.

Determination

Sample Preparation

Regardless of the food product – and prior to the analysis – sucrose in the food sample must be dissolved in water (extraction) and separation or fractionation of food components may be necessary to remove interfering substances (cleanup).

For a general purpose, perhaps the most widely utilized method consists of using clarifying agents, whose function is to eliminate colored compounds (which might interfere with the passage of light through the solution), the rest of optically active substances, and other potentially interfering constituents (proteins, fatty acids, etc.). These clarifying agents should not affect the chemical nature of sucrose in the final solution. Moreover, any excess of clarifying agent must be removed to avoid undesirable interferences. The clarifying agents most frequently used in the analysis of sucrose in foodstuffs are lead acetate, zinc ferrocyanide, activated charcoal (for qualitative analysis), ion-exchange resins (used to remove potential acidic or basic interferents), and phosphotungstic acid.

Another alternative, solvent precipitation, is often used when the extraction solvent chosen has been water alone, since in this case, polar compounds such as salts and amino acids may contaminate the extract. Precipitation with an appropriate solvent, for example, a mixture of acetate buffer/acetonitrile, is then required.

In the case of chromatographic analyses of sucrose, the presence of some high- and low-molecular-weight ionic species in the food material (and hence in its extract) may seriously interfere. For example, the risk of overlapping peaks increases when salts and/or amino acids occur in the sample, since some of them under certain chromatographic conditions may elute very near to sucrose. In most of these cases, the use of a mixed-bed ion-exchange resin permits the complete removal of all these interfering species with no loss of analyte.

Apart from ion exchange, the use of chemically modified silica (e.g., C_{18}) has also become a common chromatographic procedure for extract cleanup, either in an open column or – as usually preferred – in the form of a Sep-Pak cartridge attached to the end of a syringe. In this last way, once the aqueous extract is forced through the cartridge, any nonhydrophilic molecules will be retained and sucrose will pass straight through with no decrease in its concentration.

Methods of Analysis

Traditional methods

Physical methods of analysis

Refractometry Refractive index of a solution depends on the concentration of solute. Therefore, it can be used to determine the concentration of sucrose solutions and the corresponding instruments (refractometers) may be calibrated with refractive index or directly with sugar concentration scales. For example, they are often calibrated in Brix, a parameter that is numerically equivalent to %sucrose w/w; in this case, these instruments are utilized only with sucrose solutions. When only refractive index scales are available, tables are required to obtain sucrose concentration. Unlike older instruments, modern refractometers are electronically controlled and (theoretically) free from operator error. Measurements are usually made at 20 °C using the sodium D line as the light source. Sucrose is most frequently utilized as the calibration medium for the instruments and the readings expressed as %sugar w/w 'as sucrose' in the food. Obviously, such instruments are only accurate for pure sucrose solutions, although they are also widely used for foods containing other sugars such as glucose syrups or invert sugar, since the reading obtained may be corrected if required; usually, the reading is used as a reference value to the sugar content (total soluble solids) of the solution. On the other hand, refractive index is heavily influenced by changes of temperature and wavelength of the light source; thus, control is necessary. Nevertheless, this drawback has been overcome by modern instruments, since their reading is temperature-compensated.

Polarimetry Polarimetric determinations are one of the common methods utilized in sucrose analysis, especially in the case of milk and dairy products. In this sense, special equipment (saccharimeter) exists that quotes the optical rotation in international sugar degrees (°S). 100°S (34.620°) is defined as the polarization of a sucrose solution of 26.000 g/100 ml in a 200 mm tube at 20 °C with white light filtered by potassium dichromate. Alternatively, yellow sodium light can be used.

Temperature is a major factor to be controlled since it may influence optical and specific rotations. For example, with an invert sugar solution (glucose + fructose) using sodium light, specific rotation is 0 at 90 °C and −20 at 20 °C.

In the case of the determination of sucrose in food samples, high-molecular-weight compounds such as proteins and polysaccharides must be removed, as well as reducing sugars.

When other sugars such as common monosaccharides occur in the food sample, the optical rotation must be recorded before and after hydrolysis, which is carried out with either diluted HCl or the invertase enzyme. From the difference in the optical rotation before and after hydrolysis, the concentration of sucrose can be determined, following the well-known Clerget method.

Isotope dilution Despite its widespread use in the analysis of sucrose in foods, polarimetry is known to be unreliable in the presence of optically active nonsucrose constituents. Instead, the isotope dilution technique is not affected by interferences from other species in solution. This technique measures the yield of a nonquantitative process. A small amount of radioactive sucrose is added to a sucrose solution (it is then an internal standardization method). After a nonquantitative purification of sucrose (regardless of its origin, various procedures have been developed for this purpose), the radioactivity is

measured. The extent of dilution of the radioactive tracer indicates the amount of sucrose originally present.

Liquid scintillation counting techniques have been applied in isotope dilution studies with no loss of accuracy over gas-flow proportional counters, but with an increase in the number of samples that can be analyzed.

Chemical methods of analysis

Classical methods What we know as 'classical methods' are related to the determination of reducing sugars, but sucrose – although a nonreducing carbohydrate – may also be determined by some of these procedures using modified versions of them. For example, according to the Lane and Eynon method, boiling Soxhlet reagent (Fehling A+Fehling B) is titrated with the sample using methylene blue as an indicator. Although this titration only determines reducing sugars, a simple modification enables it to measure sucrose. After the initial determination of reducing sugars, a sample of the carbohydrate solution is hydrolyzed using either hydrochloric acid or invertase. This converts sucrose into a mixture of glucose and fructose (invert sugar), and by remeasuring reducing sugars, the percentage of sucrose can be calculated.

The basic method has also slightly been changed by making use of alkaline copper citrate rather than tartrate, with Na_2CO_3 providing the alkaline environment. It is then known as the Luff–Schoorl method: An excess of the reagent is added to the carbohydrate solution, and after the reduction reaction is complete, the excess copper citrate is reacted with excess KI and the liberated iodine is titrated with $Na_2S_2O_3$ using starch as indicator. By subtraction of a blank, the amount of copper solution reacted with the sugar solution can be obtained.

Colorimetric methods According to the anthrone method, sucrose reacts with anthrone (9,10-dihydro-9-oxoanthracene) under acidic conditions to produce a blue/green color. The reaction takes place between the dehydration products of the sugar – furfural and hydroxymethylfurfural – forming condensation products with the anthrone. A modification to the standard technique, known as the Clegg's anthrone method, is based on the previous digestion of the food with $HClO_4$. Thereafter, sucrose will be hydrolyzed to their component reducing sugars and these react with the anthrone reagent.

Another typical colorimetric assay, the phenol–sulfuric acid method, involves two stages: dehydration of sucrose to furfural and hydroxymethylfurfural with concentrated H_2SO_4 and condensation of these substances with phenol to produce a yellow color. This method has a high sensitivity and is therefore utilized when microgram quantities of sucrose occur.

An alternative procedure is based on the addition of 3,5-dinitrosalicylate in alkaline solution. This compound reacts with reducing sugars, the resulting product being measured colorimetrically at 540 nm; in this case, two measurements are required: before and after acid hydrolysis of sucrose. Its percent is then calculated from the difference in absorbance before and after the inversion.

Finally, sucrose also reacts with picric acid (2,4,6-trinitro-1-phenol) in alkaline solution to produce red picramates, which can also be monitored spectrophotometrically at 490 nm. This procedure is known as the picric acid method.

Modern methods

Near-infrared (NIR) reflectance

Spectroscopic measurements in the near-infrared region of the electromagnetic spectrum have often been used in quantitative analysis of sucrose in a wide range of foodstuffs including soybean, chocolate, sugar cane, and beet. This technique (which relies effectively upon chemometrics, since it develops statistical models for the spectral data to predict the sucrose content in the food samples analyzed) is straightforward with a great potential, given its advantages in terms of minimal sample preparation. Nevertheless, further improvement in the sensing configuration and calibration methods is needed in some cases.

Chromatography

In the determination of sucrose in foods, high-performance liquid chromatography (HPLC) and gas chromatography (GC) provide rapid analyses with greater precision and specificity. These features are notably enhanced if these techniques are coupled to others, mainly mass spectrometry. Several factors are involved in the comparison between both methods; the general conclusion – according to the bibliography – is that GC gives better selectivity, higher sensitivity, and a wider range of compounds analyzed but that, in simpler (in fact more frequent) cases, HPLC of the underivatized samples is more recommendable. In global terms, HPLC methods are preferred for the analysis of syrups, honeys, and foodstuffs containing major amounts of sucrose, for example, jams, biscuits, and confectionery products, whereas the greater sensitivity of GC methods can be used to advantage for the determination of sucrose where it may be present in only minor or trace amounts.

Both techniques need a careful sample treatment, as mentioned earlier in the text. In particular, GC requires the use of dual solid-phase columns, hexamethyldisilazane and o-trifluoroacetyl, and other procedures for extraction purposes, as well as derivatization of sucrose (preparation of a volatile derivative in quantitative yield). In this last case, o-trimethylsilyl (TMS) derivatives are predominantly used for the analysis of this disaccharide, although both acetates and trifluoroacetates have occasionally been utilized.

High-Performance Liquid Chromatography

HPLC is one of the most commonly employed methods in the determination of sucrose in foods, especially in fruits and fruit juices, vegetables, yogurt, beverages, and cereals. Partition systems (partition chromatography) employing polar-bonded phases are now the cornerstone of HPLC analysis of sucrose in foodstuffs. Amine-bonded silica gels are the most frequently utilized systems for sucrose separations, silica being usually bonded by a primary amine. Nevertheless, care must be taken to avoid or minimize the formation of Schiff bases. Additionally, the lifetime of these amino-bonded phases remains quite short because of a certain degree of phase hydrolysis, although this problem may be overcome by using trifunctionalsilane (e.g., 3-aminopropyltriethoxysilane) or, alternatively, an eluent recycling system to extend the column life.

Theoretically, a wide range of polar mobile phases could be used with these polar amino-bonded phases, water being a

major component to ensure adequate solubility of sucrose. Nonetheless, the eluent is usually an acetonitrile/water mixture, the relative proportions of which can alter the retention times (the less complex the carbohydrate molecule, the more percentage of acetonitrile is required).

As far as detection is concerned, the refractive index detector (RID) is still the most widely used detection method for sucrose, in spite of its lack of sensitivity (although this does not offer a big problem in most foodstuffs, since sucrose often occur as a major component). However, RID is nonspecific and its response may be altered by changes in environmental conditions as well as any dissolved solute (e.g., salt) that will produce a peak irrespective of its nature, which may appear near sucrose. Furthermore, its principle of operation with a reference cell for the mobile phase rules out any possibility of making use of gradient elution. All in all, some steps must be taken when using RID, namely, (a) solvent delivery must be degassed and pulse-free, (b) isocratic elution has to be employed, (c) the detector must be carefully thermostated, and (d) the temperature of the column must also be kept constant to ensure the equilibrium of the solvent components between both phases and therefore the composition of the eluate.

The evaporative light-scattering detector (ELSD, 'the mass detector') is also gaining popularity; it is an evaporative analyzer in which the mobile phase is removed by nebulization and evaporation prior to the determination of nonvolatile carbohydrates (such as sucrose) by light scattering. ELSD offers sensitive detection and allows gradient elution (eluent is removed before detection). Another important advantage is its great flexibility of use given the absence of baseline drift and its rapid equilibration. And last but not least, it is not sensitive to temperature changes nor to variations in the mobile-phase flow.

The use of a pulsed amperometric detector (PAD) should also be remarked; it allows specific electrochemical detection without derivatization, by direct oxidation on a gold electrode in a highly alkaline medium. It is based on an extremely fast three-step potential cycle; the −OH groups can be oxidized at the first potential and the gold electrode surface is then oxidized to remove reaction products and then finally reduced to its initial state by the application of the third voltage.

This detection system is coupled with high-performance anion-exchange chromatography (HPAEC), which has the same alkaline pH requirements. The sensitivity of PAD is considerably higher than that yielded using RID. Moreover, this technique requires no sample derivatization and is compatible with gradient elution; these features make anion-exchange chromatography with PAD especially suitable for the analysis of sucrose in foodstuffs.

Finally, other detectors have been proposed for the HPLC determination of sucrose in foodstuffs, such as Fourier transform infrared spectroscopy, Raman spectroscopy, and charge aerosol detector (CAD). The latter is based on the nebulization of the solution leaving the column and the evaporation of the mobile phase as the aerosol goes through a heated tube, but unlike the ELSD, it measures charged particle flux.

Gas chromatography

GC methods have been applied to a wide range of determinations of both naturally occurring sucrose and that added during food manufacture, for example, in honey, bun mix, or soybean. The first essential step is the preparation of a volatile derivative in quantitative yield. A second requirement is that a mixture of derivatives, for example, mono- and disaccharides, be completely resolved upon analysis. Anyway, there is no universally applicable procedure for the preparation of sucrose for GC, which is suited to all types of sample and all possible carbohydrate mixtures.

As regards detection systems, in most sucrose analyses, the flame ionization detector (FID) has been used, although mass spectrometry (MS) has also found interesting applications. Both detectors have a high sensitivity. In many cases, even the sensitivity of thermal conductivity detectors (0.01–0.001 times the sensitivity of a FID) would be adequate. In this sense, sucrose in several beverages has successfully been determined as its TMS derivatives using thermal conductivity detection. The determination of sucrose as acetate or TFA derivative presents no detection problems, but combustion of TMS derivative produces silica that accumulates on the ion collector of the FID and gives rise to a gradual decrease in detector response.

In the analysis of sucrose in foodstuffs, it is unlikely that greater sensitivity than that obtained by the use of the FID will be required. However, in some cases, much greater sensitivity can be achieved by the use of selective detectors, such as nitrogen-selective alkali FID or the combination of a glass capillary column and an electron capture detector.

Enzymatic methods of analysis

They are in theory very specific, but in practice, their specificity depends on the purity of the enzymes used. On the other hand, enzymatic determinations require very small quantities of material and sampling is therefore important. Anyway, and unlike many chemical and physical methods, enzymatic methods represent a sensitive, rapid, and reproducible means of measuring sucrose in foods, although costs may be higher than for conventional techniques. Nevertheless, the use of immobilization techniques contributes to cheapen this type of analysis. Furthermore, since enzymes are so specific, there is no need for elaborate cleaning up procedures to remove interfering compounds, although trace metals may cause some problems.

The main enzymatic method for the determination of sucrose in foodstuffs is based on the following reactions:

$$\text{Sucrose} \xrightarrow{\text{Invertase}} \text{Glucose} + \text{Fructose}$$

$$\text{Glucose} + 2\ \text{ATP} + \text{Fructose} \xrightarrow{\text{Hexokinase}} \text{G6P} + \text{F6P} + 2\ \text{ADP}$$

$$\text{G6P} + \text{NAD} \xrightarrow{\text{G6PDH}} \text{NADH} + 6 - \text{Phosphogluconate}$$

where ATP = adenosine triphosphate, G6P = glucose-6-phosphate, F6P = fructose-6-phosphate, ADP = adenosine diphosphate, NAD = nicotinamide adenine dinucleotide, G6PDH = reduced form of G6P, and NADH (measured spectrophotometrically) = reduced form of NAD.

Test kits are also commercially available for the enzymatic determination of sucrose in foodstuffs such as bread and bakery goods, cereal products, dietetic food, meat products (sausages and hamburger), and potatoes.

Finally, combined instrumental–enzymatic methods have also been developed. In particular, a spectrophotometric method for determining sucrose in green and roasted coffee beans has been proposed. Sucrose is hydrolyzed by invertase and the resulting glucose is oxidized by means of glucose oxidase; then, the produced H_2O_2 reacts with phenol-4-sulfonic acid sodium salt and 4-aminoantipyrine in the presence of peroxidase, yielding a pink dye with an absorption maximum at 505 nm. This method has recently been enhanced by adapting it to enzyme-linked immunosorbent assay (ELISA) plates, which enables large-scale, low-cost analyses to be carried out.

Flow injection analysis

Flow injection analysis (FIA) is an automated method in which a sample (analyte) is injected into a continuous flow of a carrier solution, which mixes with other flowing solutions (reagents) before reaching a detector. This technique is straightforward with very low sample and reagent consumption and high sampling frequencies. In the case of sucrose, each food sample is analyzed in duplicate for its content in reducing sugars: before and after inversion of sucrose. The difference between both results yields the content of sucrose.

Three automatic FIA methods have been proposed for the determination of reducing sugars in beverages based on classical reactions (the picric acid method, the Cu(II)–neocuproine method, and the Nelson–Somogyi method). The first one presents a dual-channel configuration, with pH being adjusted with NaOH at a confluence point. The same manifold is utilized for the Cu(II)–neocuproine method, whereas the automation of the Nelson–Somogyi method makes use of a reversed FIA system, in which the sample flows continuously and then is mixed with the NaOH stream. In all cases, the reactor in which the chemical reaction takes place is immersed in a thermostatic bath at a suitably high temperature. Additionally, and in the case of colored samples, the use of a dialysis unit (inserted between the injection valve and the reactor) permits to avoid tedious decolorizations with charcoal.

Different scientists have also been working on the flow injection automation of the previously mentioned enzymatic determinations of sucrose. In this sense, several biosensors have also been designed on this basis. For instance, a specific sucrose electrode has been developed for the determination of this disaccharide (in fruit juices) in a FIA system without interference from glucose, fructose, or any other carbohydrate present in the food sample. This osmium-based biosensor is based on an enzymatic cascade coupled to electrocatalytic detection of NADH; it is incorporated into a FIA system with piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) as a carrier.

Capillary electrophoresis

Capillary electrophoresis (CE) is straightforward with low sample consumption, high speed of analysis, high resolution, and efficiency; nevertheless, in the case of sucrose analysis, it is still limited by the lack of functional groups containing chromophores and charges.

High electric fields are used in CE to force ionic solutes to migrate through a buffer- or gel-filled capillary. The injected species are separated along the tube on the basis of charge, size, or both and are subsequently detected near the capillary end.

The separation of sucrose (a 'neutral' carbohydrate) by CE requires the *in situ* conversion of this polyhydroxy compound into charged species via (a) complex formation with other ions such as borate and metal cations and (b) ionization of the hydroxyl groups at extremely high pH using appropriate electrolyte solutions. Besides, several detection strategies have been developed including indirect UV spectroscopy, laser-based refractive index measurement, laser-induced fluorescence (LIF) detection, electrochemical detection, dynamic labeling via complexation with absorbing or fluorescing ions, and precolumn derivatization with a suitable chromophore and/or fluorophore. More recently, other detection modes such as capacitively coupled contactless conductivity (CCD) have also been used for the CE analysis of sucrose, for example, in the detection of adulterations in instant coffee by the addition of coffee husks and corn or the analysis of sucrose in soft drinks, isotonic beverages, and fruit juices. On the other hand, it should also be noticed that several applications utilize micellar electrokinetic capillary chromatography as separation mode. Finally, in the case of determining sucrose in honey, a suitable alternative has turned out to be the amperometric detection at constant potential after a strong dilution of the sample with an appropriate buffer.

Sensors

A first example could be a prototype of amperometric biosensor for sucrose determination based on electrochemical polymerization of m-phenylenediamine onto a platinum electrode. Enzymes invertase, mutarotase, and glucose oxidase are employed for biomembrane formation and entrapped into the polymer film during the electropolymerization process. An important advantage of this sensor is the reduction in interference from ascorbic acid due to the mechanism of selective partitioning and diffusion within the polymer film.

Sucrose sensors based on pH alteration generated by a series of boronic acid derivatives have also been proposed. In this sense, a recent development configured a pH indicator and boronic acid ensemble array with extended diversity of boronic acids and applied the probe selection process to optimize the system for quantitative dose–responses sucrose from soft drinks.

Another type of electrochemical sensors for sucrose analysis has been prepared by using graphene, due to its excellent electrical conductivity and electrocatalytic activity. For example, a recent graphene–copper nanoparticle composite paste electrode was successfully applied for the determination of this disaccharide in milk, peach, and banana. The advantages of this type of sensors include higher sensitivity, satisfactory stability, surface renewability, bulk modification, and low expense of fabrication.

Finally, a selective electrochemical sensor has been developed for the determination of sucrose in sugar beet juices by integrating electropolymerization of molecularly imprinted polymer with multiwall carbon nanotubes. The sensor was prepared by electropolymerization of o-phenylenediamine in the presence of template, sucrose, on a multiwall carbon nanotube-modified glassy carbon electrode. Hexacyanoferrate (II) was used as a probe to characterize the sensor using electrochemical impedance spectroscopy, cyclic voltammetry, and differential pulse voltammetry. Capturing sucrose by the

modified electrode causes a decrease in the response of the electrode to $Fe(CN)_6^{4-}$. This sensor provides an efficient way for eliminating interferences from compounds with similar structures to sucrose.

Other techniques

Apart from the earlier-mentioned leading analysis techniques of sucrose in foods, other developments are also noteworthy. For example, a simple and fast procedure was developed for the direct determination of sucrose in beetroot samples through attenuated total reflectance Fourier transform infrared (ATR-FTIR) absorbance measurements. The method only requires a previous crushing or liquefaction of samples and it is free from matrix effects and from the interference of minority of carbohydrates.

A promising approach is also a relatively rapid and inexpensive enzymatic–fluorometric microtiter plate assay for sucrose quantification in sugar beetroot dry matter. As expected, refractometric analysis generally overestimated sucrose content, whereas enzymatic–fluorometric analyses reasonably correlated with HPLC results.

Some work is in progress concerning sonic-spray ionization (SSI), a liquid chromatography–mass spectrometry interfacing technique in which a liquid flow is sprayed from the tip of a capillary under atmospheric pressure with a gas flow coaxial to the capillary. In contrast to the electrospray or atmospheric pressure chemical ionization techniques, SSI forms charged droplets without heating the capillary or applying an electric field to the tip of the sprayer. Therefore, it is ideally suited for the analysis of thermally unstable compounds such as sucrose.

Finally, surface plasmon resonance (SPR) has also been used in the detection of food carbohydrates. It monitors biospecific interactions that occur on a surface of a metal layer between the immobilized ligand and the free analyte, by measuring changes in resonance angle due to an increase of the mass concentration of the analyte in the vicinity of the surface. Recent improvements in the instrumentation have enabled the detection of smaller carbohydrates, including sucrose, in foodstuffs.

Future developments

The outstanding features of the many modes of CE analysis – including capillary electrochromatography (CEC) – are driving many chemists to further explore its unique capabilities in the field of sucrose analysis in foods.

Considerable progress has been made in HPLC in such a way that it is now routinely used for quantitative analysis of sucrose in foodstuffs. Some separations still remain to be improved, but the development of new bonded phases with various organic loadings (mainly from different amino–alkyl silanes) together with the establishment of ELSD as an alternative to RID will offer much scope in enhancing the resolution of sucrose in the presence of other carbohydrates.

A promising approach for the indirect determination of sucrose in carbohydrate-rich foods such as caramel syrup or mint syrup is the coupling of FIA systems to an HPLC. The method is based on postcolumn derivatization of reducing sugars with *p*-aminobenzoic acidhydrazide in an alkaline medium following elution from the chromatographic column with an acetonitrile/water gradient as mobile phase. The manifold also allows nonreducing sugars (such as sucrose) to be hydrolyzed prior to insertion into the chromatograph, thus making them compatible with the derivatization reaction and with continuous decolorization of the sample on an activated carbon column.

Finally, many highly purified enzymes are now commercially available for the specific detection and quantification of sucrose. As foods become more complex and new sugars and their derivatives are employed in their formulation, there is no doubt that enzymatic methods will be increasingly used to determine specific compounds, such as sucrose. The widespread use of immobilized enzymes, including their use in enzyme electrodes, should allow the continuous assay of this essential component of some foods, not only for the purpose of classical on-line analysis but also with a view to on-line and in-line monitoring of food production processes. The latter is already a well-established analytic procedure and the growing availability of biosensors will open a promising path. Additionally, the increasing stability of many enzymes that can be achieved by cross-linking and immobilization techniques will extend the range of such determinations.

See also: Candies and Sweets: Sugar and Chocolate Confectionery; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Condensed Milk; Food Composition Databases; Fruit Juices; Glucose: Properties and Analysis; Sucrose: Dietary Importance; Sugar Alcohols; Sweeteners: Classification, Sensory and Health Effects; Yogurt: The Product and its Manufacture.

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Sugar Alcohols

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Introduction

Sugar alcohols are white crystalline, non- to slightly hygroscopic powders and clear syrups. They are generally stable to heat and do not take part in Maillard-type browning reactions. Chemically, sugar alcohols are hydrogenated mono-, di-, and oligosaccharides. The more important compounds in these groups have the structures shown in Figure 1, and their main physical properties are summarized in Table 1. Erythritol is described separately further in the text.

Sensory and Nutritional Properties

Compared with sucrose, sugar alcohols have slightly reduced sweetness values, as shown in Table 2. Sugar alcohols produce a characteristic mouth-cooling effect that is directly related to their heats of solution and their solubility in water (Table 1). Sugar alcohols, also known as polyols, may give particular advantages with regard to diet control and dental health (Table 3). The benefits that polyols can give in reducing caloric intake are due to their different metabolic behaviors after oral ingestion compared with other carbohydrates. Because polyols may have a mono-, di-, or oligosaccharide carbohydrate structure, small differences can occur with regard to their metabolic fate in the human body.

Except for maltitol-containing syrups (55–75% maltitol), in which a small fraction of oligomeric structures occur, polyols are not degraded by saliva enzymes (amylases) upon oral ingestion. In the stomach, slight acid hydrolysis of the di- and oligosaccharide polyols may occur. However, once the small intestine is reached, extensive hydrolysis by α-amylases of the

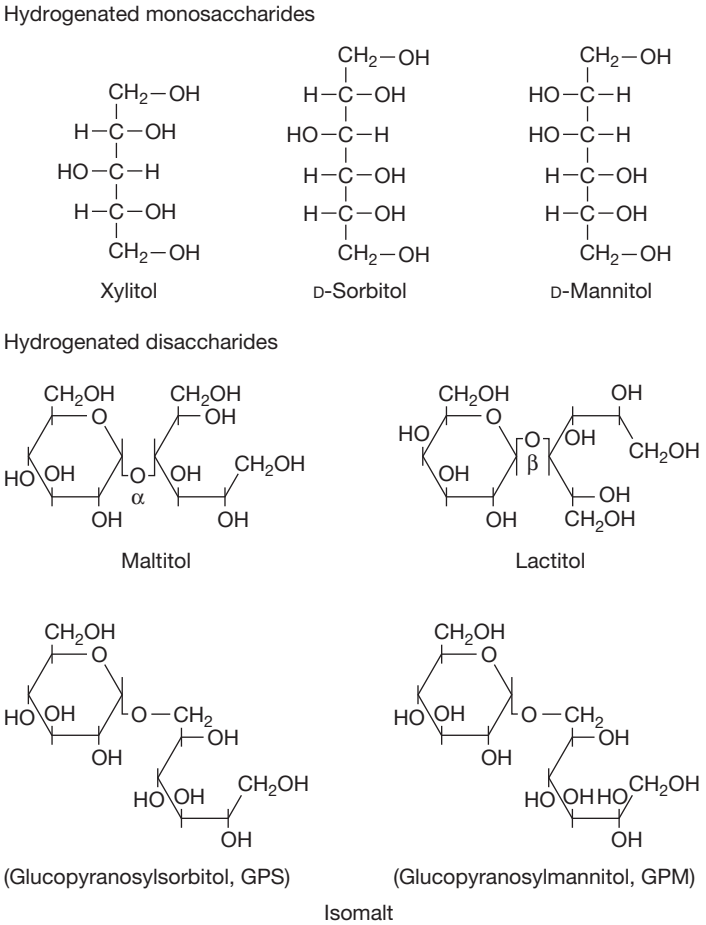


Figure 1 Structures of the most important sugar alcohols. Reproduced from Sugar alcohols. In: Macrae, R., Robinson, R. K. and Sadler, M. J. (eds.) (1993) *Encyclopaedia of Food Science, Food Technology and Nutrition*. Academic Press.

Table 1 Physical properties of sugar alcohols

	Xylitol	*Sorbitol*	*Mannitol*	*Maltitol*	*Lactitol*	*Isomalt*
M	152	182	182	344	344	344
$[\alpha]b^{20}$ (°) [$c = 0.1$; H_2O]	0[a]	−2	−0.49	+106.5	+11.5	−[b]
Melting point (°C)	92–96	93–97	165–168	148–152	94–97	145–150
Solubility (g in 100 g water at 20 °C)	169	235	18	160	125	34
Heat of solution (kJ g^{-1})	−153	−111	−121	−69	−53	−39
Viscosity (mPa, 20 °C)	60%, 21	70%, 180	–	75%, 2500	60%, 68	30%, 4

[a]Meso compound.
[b]Varying composition.

Table 2 Sweetness values of sugar alcohols

Compound	*Relative sweetness*
Polyol[a]	
Xylitol	80–100
Sorbitol	50–60
Mannitol	50–60
Maltitol	80–90
Lactitol	30–40
Isomalt	40–50
Erythritol	60–70
Sucrose[a]	100

[a]10% in H_2O: 20 °C.

mucosa occurs, and up to 60–80% of the maltitol and isomalt are hydrolyzed. A 50% glucose/50% sorbitol or a 50% glucose/25% sorbitol/25% mannitol mixture, respectively, is obtained. The other disaccharide polyol lactitol is almost unchanged because the required β-galactosidase is only present in trace amounts. Sorbitol, mannitol, and xylitol reach the small intestine unchanged.

In the small intestine, the monosaccharide polyols are partly absorbed into the bloodstream by a passive diffusion process at rates depending upon their molecular weight (Table 3). Also, the applied dose and the actual intestinal transit time affect the rate of absorption. The absorbed fraction of sorbitol and xylitol is channeled into the normal liver metabolism and contributes energy to the same extent as glucose.

The unabsorbed polyols finally reach the large intestine where the microflora ferments them almost completely to volatile fatty acids (VFAs). From the energy contained in the fermented fraction, 20% is used by the bacteria for growth. The remainder is absorbed from the gut as VFAs, which are then transported in the blood to be metabolized by the liver into fat constituents. In total, about 50% of the energy of the fermented polyols is available to the human body.

Based on the earlier metabolic behavior, the calorific value of polyols can approximately be calculated (Table 3). However, depending upon the methodologies used, values vary over a wide range. Therefore, and because many polyols are used in combination with each other, a European Union Directive has laid down an average calorific value of 2.4 kcal g^{-4} for all polyols.

Because of the reduced digestibility of polyols, their intake level is recommended to be restricted to 40–50 g day^{-1} (adults) and 30 g day^{-1} for children in order to avoid gastrointestinal discomfort. Higher doses may cause osmotic diarrhea or increased flatulence due to slow absorption and extensive fermentation, respectively, but tolerance levels improve upon adaptation. On the other hand, polyols are very suitable for diabetics, because rapid glycemic or insulinemic response is avoided. For the same reason, sorbitol and xylitol are often applied in parenteral nutrition to provide a more controllable carbohydrate energy source compared with glucose or fructose.

Concerning dental health, polyols have long been known to have no adverse effect on teeth. This is due to the fact that mouth bacteria are unable to ferment polyols, thereby avoiding the production of enamel solubilizing acids. With the intraplaque pH telemetry test, it was shown that eating polyol-based sweets did not decrease the plaque pH below the critical value of 5.7. Additionally, for xylitol, there is ample proof of a cariostatic effect. In several studies, of which the Turku study (Finland) is most famous, the incorporation of xylitol in a normal diet was proved to reduce the occurrence of dental caries. Most probably, xylitol selectively discriminates against the growth of *Streptococcus mutans*, the major organism involved in dental caries.

Occurrence and Methods of Production

Polyols like sorbitol, mannitol, and xylitol have been well known over the last 30 years due to their occurrence in a number of fruits, vegetables, and cereals. Although they occur in nature, their concentration in plant material is too low for a simple, economic commercial extraction, as practiced with sucrose. A more complicated process is therefore needed for the production of sugar alcohols.

Several techniques have been described to produce polyols, but only a few of them are of industrial importance. These are mainly based on catalytic hydrogenation of easily accessible carbohydrates, for example, dextrose, glucose syrups, maltose syrups, invert sugar, isomaltulose, xylose, and lactose. These carbohydrates are obtained by either hydrolysis or isomerization of natural raw materials like starch, sucrose, milk (whey), and straw (Figure 2).

The carbohydrate raw material is hydrogenated in an aqueous solution at elevated temperatures (120–190 °C) and hydrogen pressure (20–200 bar) using an activated nickel catalyst. Hydrogenation can be performed in either a batch process or a continuous process and results in the addition of hydrogen to the carbohydrate precursor. Hydrogen pressure, temperature, pH value, and type of catalyst are the most

Table 3 Absorption characteristics of sugar alcohols

Polyol	Absorbed from the small intestine (%)	Excreted (%)		Fermented (%)	Calorific value (kcal g^{-1})
		Urine	Feces		
Xylitol	25–40	0	<1	60–75	2.9
Sorbitol	15–20	0	<1	80–85	2.6
Mannitol	15–20	15–20	<1	80–85	1.9
Maltitol	45–60	0	<1	40–55	3.2
Lactitol	<1	0	<1	99	2.3
Isomalt	35–45	0	<1	55–65	3.0

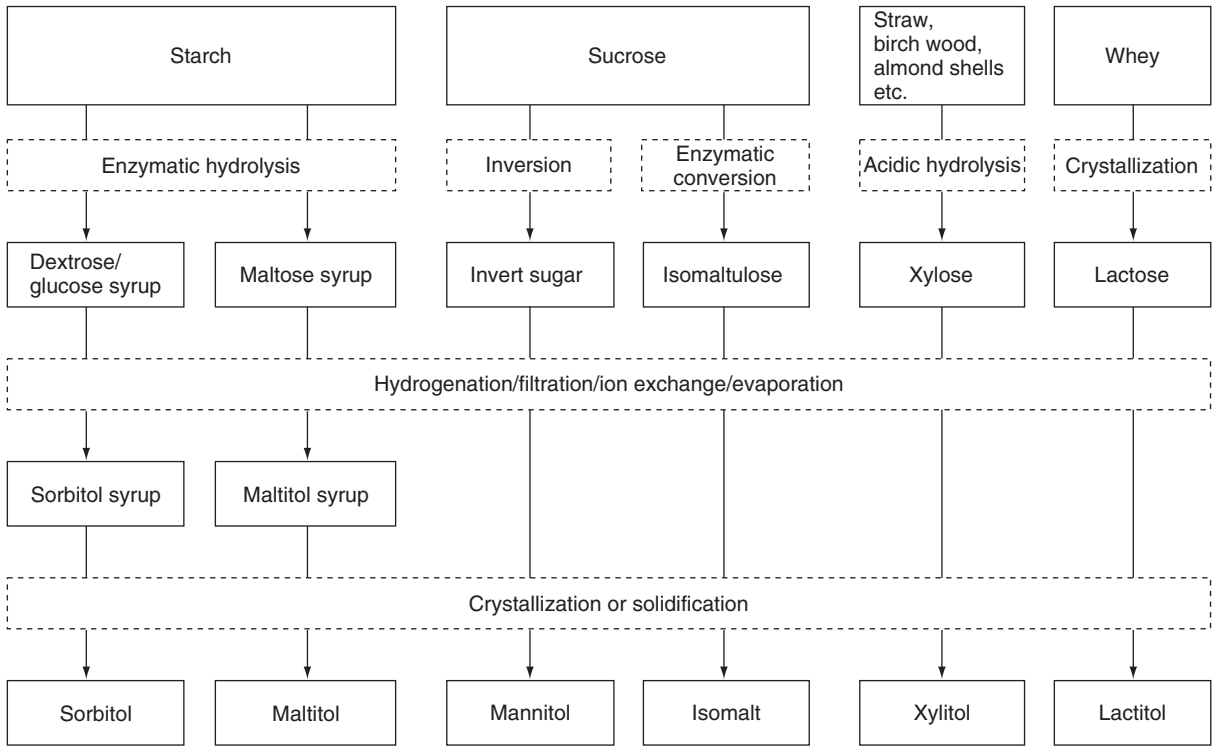


Figure 2 Commercial production of sugar alcohols. Reproduced from Sugar alcohols. In: Macrae, R., Robinson, R. K. and Sadler, M. J. (eds.) (1993) *Encyclopaedia of Food Science, Food Technology and Nutrition*. Academic Press.

important parameters influencing reaction time and selectivity. They have to be well adjusted to obtain a high-purity product in high yield. Purity of the raw material is also of importance in this respect. The hydrogenated carbohydrate (polyol-containing) solution is subsequently refined by filtration, carbon, and/or ion exchange resin treatment to remove salts and colored by-products formed during hydrogenation.

Sorbitol and maltitol are available as aqueous solutions and also as crystalline material. Mannitol, xylitol, lactitol, and isomalt are available as crystalline material. All crystalline polyols are produced by crystallization, solidification, or spray drying of an aqueous solution at an appropriate concentration.

Uses in Foods

Substitution of sugars in food to achieve specific nutritional objectives is not a new practice. Initially, polyols like sorbitol and mannitol were used to sweeten food for diabetics. To cope with sensorial and technological requirements for noncariogenic food, the range of polyols has been extended to include products such as maltitol, xylitol, isomalt, and lactitol.

For the production of polyol-based foods, most conventional types of food manufacturing equipment are used. However, manufacturing conditions (temperature, vacuum, etc.) very often need to be adapted. As some polyols are moderately hygroscopic, the relative humidity of air during production and storage conditions require careful control. It is also necessary to assure good protection by careful wrapping and/or packaging. When necessary, the sweetness of polyol-based foods can be enhanced by adding the necessary amounts of intense sweeteners like aspartame, acesulfame K, cyclamate, and saccharin.

In addition to the production of sugarless foods, polyols, for example, sorbitol, can also be used in conventionally sweetened food products in order to give specific sensorial effects

such as texture and reduced color. There are also technological advantages such as plasticity, workability, and shelf life. In particular, sorbitol is widely used for its humectancy power to extend food products shelf life.

The main uses of polyols in food products are outlined in the succeeding text.

Sugar-Free Confectionery

This is the most important application field for polyols.

Hard Confectionery

Hard confectionery is produced by boiling polyol solutions or syrups to a very high dry matter content (98–99.4%), followed by cooling, molding, and wrapping. The boiling temperature for such polyol confectionery varies between 155 and 170 °C, depending on the type of polyol. Severe vacuum conditions are needed. With the exception of mannitol, all the polyols can be used alone. However, for technical and economic reasons, very often, combinations of polyols are applied. It should be kept in mind that the solubility of polyols strongly influences crystallization and graining.

Soft and Chewable Confectionery

The main component in this application is a polyol or a polyol blend (65–70% dry matter) that is presolubilized and cooked together with vegetable fat (about 6%), emulsifier (about 0.8%), gelatin (about 1.4%), and other minor components, for example, food-grade acid, flavor, and color. After cooling, the mass is formed and wrapped. Chewability of the sweets can be obtained by acration of the cooked mass after partial cooling using conventional acration techniques such as pulling and continuous foaming.

Gums and Pastilles

Maltitol syrups, alone or in combinations with either lactitol or isomalt, are very suitable for the production of gelatin gums. For some compositions, it is necessary to adapt the gelatin type and level and the cooking conditions in order to obtain gum properties equivalent to those obtained with conventional sweetened gums.

In pastilles, a gum arabic/polyol ratio of 50/50 on a dry-weight basis is advised. This composition is cooked to 70–72% dry matter content, molded in starch, and dried to 90% dry matter. After demolding, the gums are waxed. Maltitol syrups give good pastilles with optimal shelf life properties.

Tableting

Although all powdered polyols can be compressed on rotary machines to give tablets, sorbitol is the most common polyol used in this field.

Powdered sorbitol has excellent flow properties for tableting. Good flowability is very important to provide a uniform tablet weight. Compared with other directly compressible materials, powdered sorbitol gives a high increase of tensile strength as a function of compression force and is therefore very suitable for direct compression. Due to their rather high hygroscopicity, sorbitol-based tablets should be protected or stored under relatively dry conditions in order to avoid softening due to moisture pickup. The priability of sorbitol tablets, even those compressed at low pressure, is extremely low and is improved further by storage at close to equilibrium relative humidity. The overall performance of sorbitol powder in tableting depends upon different factors, such as particle size and distribution, crystal morphology, and compression conditions.

Chewing Gum

The main components of sugar-free chewing gums are gum base (25–30%), polyol powder as solid phase (45–63%), and sorbitol or maltitol syrups as liquid phase (10–30%). The solid phase can be sorbitol powder, but more often, a combination of sorbitol and xylitol is used. The main advantages of xylitol are its excellent cariostatic properties, high sweetness, and a pleasant cool taste due to its negative heat of solution. The addition of a small quantity of glycerol improves the plastifying properties; mannitol addition prevents stickiness.

Chocolate

Total replacement of sucrose by polyol powder in both bitter chocolate and milk chocolate is possible provided the powder has an optimum purity, crystal morphology, and granulometry. A bitter chocolate consists of 40–50% cocoa mass, 40–50% polyol powder, and 5–10% cocoa butter.

Originally, only sorbitol was used for sugar-free chocolate. Recent trends are toward the use of maltitol, isomalt, and lactitol.

Dragée Coating

Coating consists in covering cores, for example, chewing gum with a syrup that is close to its saturation point, and then inducing crystallization by evaporation. Generally, a lot of layers (40–50) are needed. The temperature of polyol syrups, drying air, centers, and core bed are very important processing parameters. Sorbitol is the most common sweetener for sugarless coating, but maltitol, xylitol, isomalt, and lactitol can also be used.

Bakery Products

Polyols can be used in bakery products due to their specific properties, which are lacking in many other bulk sweeteners. The sugar alcohols contribute to softness and storage capacity. They also regulate moisture, taste, and sweetness. A combination of polyols or a polyol combined with another sweetener is preferred. If browning is too light, the baking temperature can be raised or a small amount of fructose can be added.

The baking industry uses crystalline sorbitol and sorbitol solutions in products for special dietary use. It is also employed as a humectant to extend the shelf life of baked goods. The stability of baked products can be improved by partial substitution of sucrose by sorbitol, for example, in cakes. Sorbitol is

used in sponges and cakes at between 5% and 8% of flour weight. In biscuits and cream fillings, it is used up to 10%. The freshness of gingerbread is improved by adding only 5% sorbitol. It is also used to substitute sugar in classical formulations for low-sugar products.

The use of the other polyols in bakery is rapidly developing. The level of sugar replacement with these sweeteners depends on the type of polyol and the application objective. While, for some polyols, rather high usage levels can be achieved, for example, for maltitol, lactitol, and isomalt, for others, for example, xylitol and mannitol, usage levels are generally low. These differences are also related to the specific sensory and physicochemical properties of the polyols used. An important contribution of the other polyols is, as for sorbitol, the humectant properties that control the moisture level in bakery products when stored for long periods. In some cases, combinations of polyols can give interesting complementary effects. The main applications in the bakery field are cakes, biscuits, pastry, fillings, and icings. In the last two products, xylitol and mannitol can be an asset due to their perceptible cooling effect.

Other Areas of Application

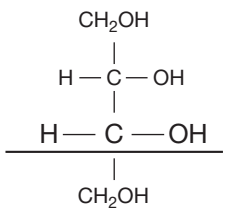
Polyols can also be used in sugarless jams and ice creams. Sorbitol-containing jams and preserves have a clear taste and good texture. A sufficiently high solids content can be obtained in order to inhibit microbiological activity, thereby protecting the jams against mold development. In order to avoid crystallization during storage, the solubility of the polyol(s) should be checked before formulating the food.

Erythritol

Chemical and Physical Properties

Erythritol is a linear carbohydrate molecule of four carbon atoms, each carrying one hydroxyl group. The molecular structure is shown in Figure 3.

Chemically, erythritol therefore belongs to the class of monosaccharide polyols like sorbitol, mannitol, xylitol, and glycerol. Erythritol is also a symmetrical molecule and therefore, it exists in only one form, the mesoform. It forms anhydrous crystals with a moderately sweet taste without off-tastes or off-odors. The powder has a transparent white brilliant appearance and dissolves in water to give a colorless nonviscous solution. Crystals melt at 122 °C to form a colorless and brilliant nonviscous melt.



1,2,3,4-butanetetrol

Figure 3 Molecular structure and chemical formula of erythritol.

Erythritol's chemical properties are similar to those of other polyols in that it has no reducing end groups and thus has excellent heat and acid stability. It differs in having a low solubility (37% ww at 25 °C), a property that among these simple polyols it shares with mannitol and isomalt. The heat of solution is very low ($-181\ J\ g^{-1}$), a property shared with xylitol. However, compared with the group of polyols presently used as sugar replacers, erythritol has the lowest molecular weight (122), which of course provides different properties, such as higher osmotic pressure and lower water activity in solution. It has also a high freezing point depression and boiling point elevation.

Compared with sucrose, erythritol has a sweetness value of 60–70%. It has a clean, sweet taste, similar to sucrose, and a strong cooling effect and shows many synergies with intense sweeteners.

Occurrence and Methods of Production

Erythritol occurs in several foods at levels up to 0.13%. It is also present in human and animal tissues and body fluids.

Briefly, erythritol is produced from wheat or cornstarch by enzymatic hydrolysis yielding glucose, which is fermented by a safe and suitable yeast-like fungus, *Moniliella pollinis*. Once erythritol is separated from the sterilized fermentation broth, it is purified by ion exchange resin, active carbon, ultrafiltration, and crystallization. The final crystalline product, which looks very similar to sugar, is more than 99.5% pure.

Uses in Foods

The main uses of erythritol in food products are outlined in the succeeding text.

Tabletop Sweeteners

In tabletop applications, erythritol will be used at levels up to 99.9% as a noncaloric, noncariogenic carrier for intense sweeteners. In these applications, the sensorial profile-modifying properties of erythritol are of great importance, resulting in sweetness synergy, improved mouthfeel, and masking of off-flavors. In addition, due to erythritol's crystalline structure and nonhygroscopic property, it offers excellent flowability and stability as carrier.

Beverages

The synergies that erythritol shows when used in combination with intense sweeteners are also very useful in low-calorie beverages. Also, at use levels of 3.5% or less, erythritol can mask certain off-flavors in beverages like coffee, tea, and grapefruit juice.

Chewing Gum

Good-quality noncaloric and noncariogenic chewing gum can be formulated by using solely erythritol (use level up to 60%).

Most of the sugar-free chewing gum produced today has a tendency to become brittle and harder during aging. This can be avoided, and a sugar-free chewing gum with softer texture, greater flexibility, and improved shelf life can be obtained by partial replacement of the other sugar alcohols using erythritol.

Chocolate

The use of erythritol in chocolate compositions allows a dry conching process at high temperatures. Due to the good heat stability and low-moisture pickup of erythritol, it is even possible to work at higher temperatures than traditionally used. This results in enhanced flavor development.

Erythritol-based chocolate is not only easy to produce but also considered a true 'reduced-calorie' product.

Candies

Sugar-free fudge with texture and shelf life properties equivalent to conventionally sweetened fudge can be produced using erythritol (up to 40%) in combination with maltitol syrup (75% maltitol) to control crystallization.

Depending on the cooking temperature and seeding level with erythritol, the texture can be varied from soft to hard. In hard candies, erythritol in its crystalline form is used up to 50% for its cooling effect as sherbet filling in die-formed hard candies or 'sandwiched' in between two layers in deposited hard candies.

Fondant

Using erythritol, it is possible to obtain sugar-free low-calorie noncariogenic fondant with identical technical properties to classical products. Previously, this was impossible with the other low-calorie bulking agents currently available. Pure erythritol induces too high a crystallization, but 60% erythritol in combination with maltitol syrup as a liquid phase helps to control this phenomenon.

Lozenges

Research has shown that lactitol, crystalline maltitol, and erythritol are the only suitable materials to produce a lozenge with a lower calorie content than the conventional sugar-based lozenges, but are otherwise identical. In addition to the lower calorie content and pronounced cooling effect, erythritol has the added advantage over lactitol and crystalline maltitol of requiring a shorter drying time to obtain the required texture at low residual moisture content. Lozenges based on solely erythritol (up to 99%) have excellent shelf life properties, even when stored under high-humidity conditions.

Bakery (Pastry) Products

In fat cream, a very good texture is obtained with erythritol at a use level of 60% and a particle size distribution below 300 μm. The manufacturing process and shelf life properties of the erythritol-based fat cream are similar to those for conventional fat/sucrose compositions. Erythritol has an effect of masking the fatty mouthfeel, giving a more refreshing and attractive product. In addition, the calorie content of the composition is also reduced.

Reduction of calories in bakery products is particularly difficult if only the sugar moiety is replaced. Erythritol can reduce the caloric content by more than 30% (dependent on the application) without introducing undesirable side effects. Additionally, erythritol improves the shelf life of bakery products. Erythritol can be applied successfully in cookies, biscuits, and cakes, where it improves baking stability and shelf life at an addition level of about 7%.

See also: Biscuits, Cookies and Crackers: Nature of the Products; Cakes: Types of Cakes; Sweeteners: Classification, Sensory and Health Effects.

Further Reading

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Sunflower Oil

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Abbreviations

EFA	Essential fatty acid
HDL	High-density lipoproteins
HOSO	High-oleic-acid sunflower oil
HOHSSO	High-oleic-acid–high-stearic-acid sunflower oil
HSSO	High-stearic-acid sunflower oil
LDL	Low-density lipoproteins
SFA	Saturated fatty acids
SFA/MUFA/PUFA	Saturated fatty acids/monounsaturated fatty acids/polyunsaturated fatty acids ratio
U-AMDR	Upper value of acceptable macronutrient distribution range

Origin and Systematics

The cultivated sunflower (*Helianthus annuus* L.) is a plant of the family Asteraceae (Compositae). The term *Helianthus* is derived from the Greek *Helios*, meaning sun, and *anthos*, meaning flower, and from Latin *annuus*, meaning annual. The property of this flower 'turning with the sun' is clearly expressed by the French and Spanish languages when naming this plant. The flower is appreciated through the world (e.g., it is the national flower of Ukraine and the state flower of Kansas; it is used as a symbol by green ideologies, Spiritualist church, and also vegans). The genus *Helianthus* comprises 51 species, most of them perennial. The genus is distinguished technically by the fact that the ray flowers, when present, are sterile and by the presence of a pappus on the disk flowers that is of two awn-like scales that are deciduous (i.e., easily detached and falling at maturity). Some species also have additional shorter scales in the pappus or the pappus is not even present.

The domestic sunflower possesses a single large inflorescence (flower head) at the top of an unbranched stem. It is an annual species, which has an erect rough hairy stem, reaching heights of 2–3 m. The flowers in the center of the head are called disk flowers. The disk flowers are arranged spirally, following a Fibonacci number arrangement. This pattern produces the most efficient packing of seeds mathematically possible within the flower head. These disk flowers mature into fruits, containing the sunflower 'seeds.' *H. annuus* grows best in fertile, moist, well-drained soil with heavy mulch and needs full sun. In commercial planting, seeds are planted 45 cm apart and 2.5 cm deep. The *H. annuus* is diploid with a base chromosome number of 17 and an estimated genome size of 2871–3189 million base pairs (Mbp), although some references suggest that its true size is around 3500 Mbp.

Archeological evidence indicates the occurrence of heads and achenes of domesticated types of sunflower as early as the first millennium BC, and it has been suggested that cultivation began as early as 2600 BC probably in Tabasco, Mexico. Many indigenous American peoples such as Aztecs and Otomi in Mexico and Incas in South America employed sunflower as a solar deity symbol. In 1510, Spanish explorers encountered the sunflower and carried and introduced its seeds to Europe. It was grown initially as an ornamental plant and later for food and medical purposes. The sunflower was developed as an important source of oil during the early 1800s in Russia. More recently, wild species have been used in breeding programs. They have been found useful in improving disease and insect resistance of cultivars. The success of breeders, most from the former Soviet Union, in improving the oil content of seeds from less than 30% to over 50% was a major factor in the development of the sunflower as a major world oilseed crop.

Oils and fats present great importance in the field of health and industry. *H. annuus* is mostly grown as a crop for its edible oil and seeds, but new promising applications have made sunflower a premium seed. 'New' sunflower oils have appeared in the market as a consequence of researches and methodology applications. Some of these oils can be used for general food preparation and dressing, but some of them are specifically designed for manufacturing margarines, pastries, and confectionaries due to their high plasticity and also in deep frying due to the high thermal stability of some types (see succeeding text).

World Sunflower Crop

Sunflower ranks among the most important annual field crop grown in world for the production of edible oil. During the last few decades, the total production has increased a lot. Thus, in 1989–91, it was estimated at about 22.3 million metric tons. In 1998–99, world production was about 26.7 million tons, and at the 2013, it was about 44.7 million tons (Figure 1(a)). The increase in efficiency production and the new applications of this oil seed (e.g., biodiesel production) seem related.

Although sunflower is grown primarily for its 'conventional oil production,' there is limited cultivar production for 'new' sunflower seeds or non-oilseed or confection purposes. Sunflower grows well in most regions of the world, with significant production occurring in just few countries (Table 1 and Figure 1(b)). Europe and America are the leading producers of sunflower seed, accounting for about 50% of the world's total production. Recent data show that the ex-USSR (Russia plus Ukraine), the EU-27, and Argentina are the largest producers (Table 1 and Figure 1(b)). Other leading producers are also shown. However, because production depends on cultivars and in many countries, efficiency is determined by the weather, the leading order can change. In some countries, a

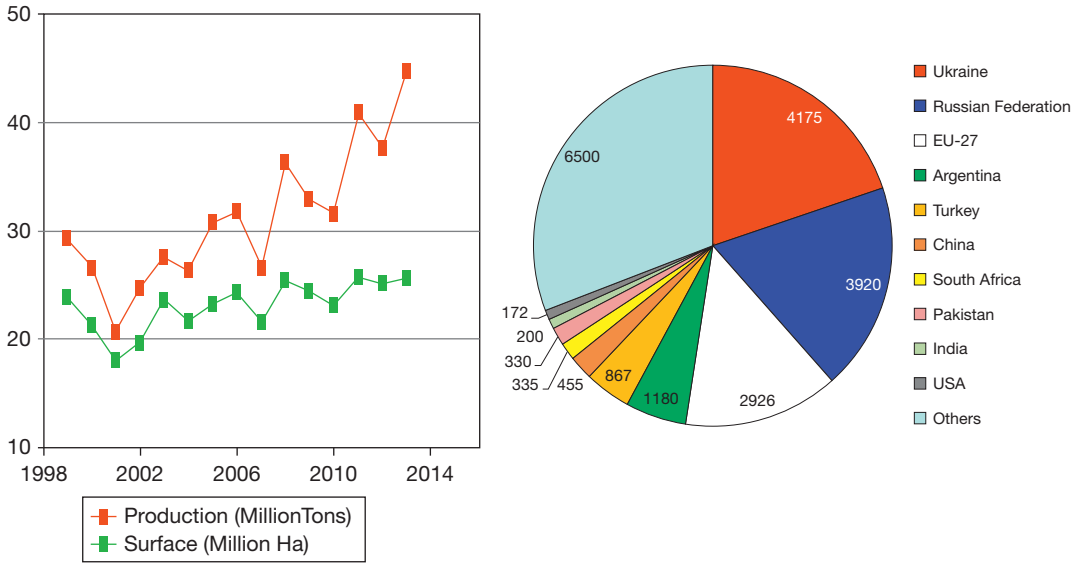

Figure 1 (a) Cultivated surface (million hectares/year) and world production (million metric tons/year) of sunflower oil seed. (b) Sunflower oil price change (US$/metric ton). The average price of each year is given.

Table 1 World sunflower crop and sunflower oil production (million tons)

Country	*Year*						
	2001	*2004*	*2009*	*2010*	*2011*	*2013*	*2014*[a]
Ex-USSR	5.4	7.3					
Central Europe	1.9						
EU-15	2.9	2.8					
Ukraine		3.05	6.36	6.77	8.67	11.05	10.0
Russia		4.80	6.45	5.34	9.70	10.53	9.8
EU-27/28			6.80	7.01	8.30	8.9	8.55
Argentina	3.18	3.16	2.48	2.22	3.67	3.10	2.81
The United States	1.55	0.93	1.38	1.24	0.92	0.92	0.41
South Africa	0.69	0.65	0.80	0.49	0.86	0.56	0.80
China	1.47	1.55	1.96	3.30	2.31	2.38	1.08
India	0.68	1.20	0.85	0.65	0.52	0.60	0.48
Turkey	0.65	0.90	1.06	1.32	1.35	1.52	2.06
Oil production[b]	8.91	10.0	13.7	13.2	16.6	18.8	21.0

Food and Agriculture Organization of the United Nations. FAOSTAT. http://faostat3.fao.org/home/index.html#DOWNLOAD.
[a]Estimated according to different Web sources (accessed on September 2014).
[b]Calculated as 42% of yield production.

high percentage of sunflower comes from dry-ground cultivars; thus, sunflower seed production may change dramatically from a wet year to a dry year (by about half a million tons).

Figure 1(a) clearly shows that the cultivar surface has increased during the last decade; however, the trend increase was much lower than that observed for the seed production, clearly suggesting the efficiency improvement of cultivars all over the world.

World Sunflower Oil Production

Sunflower oil is the fifth in production among edible vegetable oils in the world. World edible oil and fat production has increased in the last few years with an estimated total seed production of about 400 million metric tons in the year 2014. Oil production is highly dependent on the crop and, thus, is changeable from season to season. The total world sunflower oil production since 2001 is presented in Table 1. This was set considering the accepted approach that 42 g sunflower oil can be obtained from 100 g sunflower seed. World production of sunflower seed oil in the 2014 season is currently forecast at about 21 million metric tons.

Conventional vegetable oils, such as sunflower, are main raw materials to elaborate biodiesel. The use of sunflower oil for biodiesel production was initiated in South Africa in 1979 and presently is opened all over the world, contributing to the increase of seed prices all over the world and that of sunflower oil in particular (Figure 2).

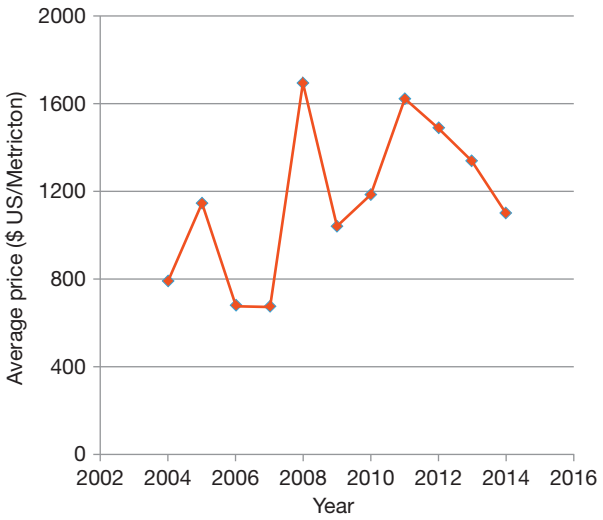

Figure 2 Top ten producer countries of sunflower oil. Estimated productions in million tons for 2014 are given.

Although sunflower oil is considered premium oil, with a correct equilibrium between linoleic acid and tocopherols, the benefits of monounsaturated fatty acids to health have encouraged the production of new sunflower oils. Thus, recently, several new variants of sunflower oils have appeared with the aim of catering to the demand for oils richer in oleic acid, such as the high-oleic-acid sunflower oil (HOSO), and with much lower price than olive oils. Moreover, because it has greater oxidative stability than the conventional sunflower oil (see succeeding text), HOSO is expected to significantly expand food and industrial uses of sunflower oil all over the world. Moreover, due to both the 'neutrality' under the lipoprotein metabolism point of view and the stability at high temperature of stearic acid, new sunflower oils have appeared in the market, such as high-stearic-acid sunflower oil (HSSO) and high-oleic-acid–high-stearic-acid sunflower oil (HOHSSO), with very promising properties.

Market and Prices

Sunflower derives most of its economic value from the oil extracted from the seeds; the remaining value is from the meal. The oil is considered to be of very high quality and generally sells for a premium in world markets, over soybean, palm, and rapeseed oils. In the period of 1999–2001, sunflower oil price ranged between US$ 400 and 600. The year 2002 prices for crude and refined sunflower oil were 700 € per metric ton and 733 € per metric ton, respectively. After 2004, the sunflower oil price has greatly changed with top pick at 2008 and 2011, where the average yearly price was about US$ 1600 per metric ton (Figure 2).

Oil Extraction

Before oil extraction, sunflower seed (c.30% hulls) is usually dehulled to 8–12% because of the high wax content in the hulls. An outline of the extraction procedure is shown in Figure 3. Before pressing, other manipulations are necessary, such as delinting, cracking, breaking, conditioning, and structuring. Nowadays, the presses used on an industrial scale are screw presses or expellers. The pressure applied in pressing can be as high as 3000 kPa but is commonly around 150 000 kPa. Capacities of expellers used for pressing are usually 100–200 t per day, but equipment exists that can handle up to 500 t per day. The solvent extraction is mainly carried out by percolation through the platelets of prepared seed using nontoxic solvents that are powerful solvents for oils and nonsoluble in water, low in price, and also nonflammable and nonexplosive.

Treatment of Crude Oil

In some countries, the oil from the first pressing of seed is used directly. However, refining the oil to change its color, odor, flavor, and physical properties has become a common procedure to satisfy consumer demands.

Refining

Fats and oil contain minor compounds that must be removed because they confer taste and smell that can diminish consumer acceptance. Steps in the chemical and physical refining are, in general terms, similar to the ones processed in other vegetable oils (Figure 3).

Composition

Conventional and Wild Sunflower Oils

Triacylglycerol species and fatty acid composition

The chemistry and properties of natural fats and oils are mainly dependent on their overall composition and especially on the relative composition of the fatty acid constituents. Furthermore, the structures of the fats, in terms of the relative proportions of different molecular species or of the stereospecific distribution of fatty acids on the glycerol moieties, have a bearing on their properties.

Table 2 shows the characteristics that conventional sunflower oil has to have in order to be an acceptable edible oil according to international regulations. In addition, specific aspects for other sunflower oils are also given. The different fatty acid fractions contained in the conventional oil may be quite variable. Nevertheless, polyunsaturated fatty acids predominate. The percentage of oleic acid (C18:1, $n-9$) plus linoleic acid (C18:2, $n-6$) is rather constant (85–87%) because both fatty acids originated in the same biosynthesis chain. The linoleic acid is produced from oleic acid by desaturation carried out by the seed Δ^9 desaturase enzyme. The major $n-6$ fatty acid is linoleic acid, ranking 48–74%. This edible oil also contains about 11–12% of saturated fatty acids (SFA) (mainly palmitic and stearic acids). Oleic acid represents about 14–39.4% and only minor amounts of linolenic acid are present. Sunflower oils from cold climates are poor in oleic acid and very rich in linoleic acid (about 15% and 75%, respectively) with an iodine value of 125–136 and do not require winterization. Southern US oils have been found to

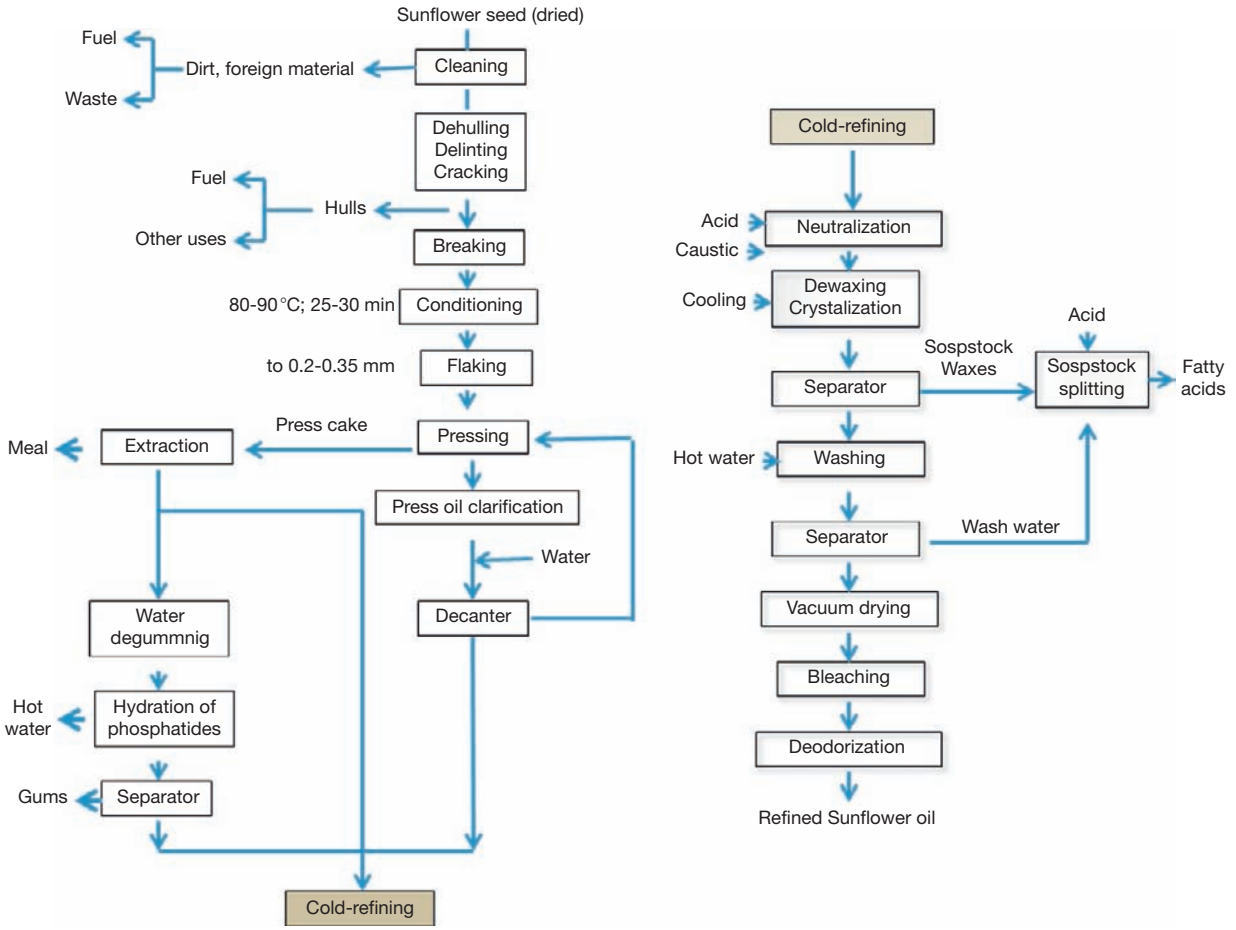

Figure 3 Sunflower oil extraction and refining procedure scheme. General lines have been set in the scheme. Modified from Sánchez-Muniz, F. J., Cuesta, C. (2003). Sunflower oil. In: Caballero, B., Trugo, L., and Fligas, P. (eds.) *Encyclopedia of food science and nutrition* (2nd ed.), pp. 5672–5680. Amsterdam: Elsevier Science Ltd. and completed following general accepted procedures.

contain about over 50% of oleic acid and as little as 35% of linoleic acid with an iodine value of ~106.

Wild *H. annuus* has been defined also as a potential source of palmitic and stearic acids. Compared with many edible vegetable oils, the SFA concentration of 12 g 100 g^{-1} in sunflower (*H. annuus* L.) oil is considered moderate, with the principal SFA being palmitic and stearic acids. A reduction of SFA in sunflower oil to the 6.0–8.0 g 100 g^{-1} level would enhance the acceptability of sunflower oil when looking for sunflower oils low in saturated fatty acids. Reports have indicated that the wild *H. annuus* populations from North Dakota tend to have the lowest average stearic and palmitic acid concentrations, while the highest stearic and palmitic concentrations were observed in wild populations from Kansas.

The stability of the reduced SFA trait for a selected population of wild *H. annuus* has been confirmed by growing the progeny of the original population in a habitual greenhouse environment (22–25 °C and 16 h of daylight). Interspecific F1 hybrids were produced in the field using a nuclear male sterile analog as the female parent and the wild *H. annuus* population as the male parent.

New Sunflower Oils

As previously mentioned, the industry requires an ample range of oils and fats, some of them solid or semisolid at room temperature, to prepare some foods. These 'new oils' can be a suitable and healthier alternative to animal fats and some tropical fats such as palm oil, palm kernel, and coconut oil and hydrogenated vegetable oils. Similarly to the techniques used to produce HOSO, mid-oleic-acid sunflower oil and other sunflower oils such as the HSSO can be obtained. Hybridization is the main mechanism to obtain high-quality HOSO and HSSO seed producers.

Biotechnology has taken advantages of the knowledge of biochemistry reactions and pathways occurring in the sunflower seed. To get linoleic acid (the major fatty acid of conventional sunflower oil) from other shorter and less unsaturated fatty acids, a cascade of enzyme reactions take place in an organelle of the seed called 'plastidial.' Thus, from acetyl CoA after its conversion in acetyl-acyl carrier protein (acetyl-ACP) and malonyl-ACP, through successive condensation of two carbon units, it can obtain palmitoyl-ACP by the

Table 2 Different sunflower oil characteristics

	Conventional sunflower oils	*High-oleic-acid sunflower oil*	*Mid-oleic-acid sunflower oil*	*High-oleic-acid–high-stearic-acid sunflower oil*[a]
Minimum quality characteristics				
Aspect	Clear, transparent after 24 h at 20 ± 2 °C			
Odor and taste	Normal, no rancidity, no alteration			
Color	≤25 UA and 2.5 UR			
Moisture and volatile compounds	≤0.1%			
Insoluble impurities in petroleum ether	≤0.05%			
Free acidity (% oleic acid)	≤0.2%			
Peroxide index ($mmol^{-1}$ active O kg^{-1} oil)	≤10			
Soap residues	Negative			
Cold test, for all oils submitted to winterization	Negative			
Purity test				
Vizern test	Negative			
Saponification index	188–194	182–194	190–191	
Halphen reaction	Negative	Negative	Negative	Negative
Iodine index (Hanus method)	118–141	78–90	94–122	55–72
Refraction index (at 25 °C)	1.461–1.468	1.467–1.471	1.461–1.471	1.462–1.467
Nonglyceric esters	Negative			
Saturated fatty acids (triglyceride β-position)	≤0.1%			≤1.5%
Fatty acids composition				
Lauric acid (12:0)	≤0.1%	ND	ND	≤0.1%
Myristic acid (14:0)	≤0.1%	ND	ND	≤0.1%
Palmitic acid (16:0)	5.0–7.6%	2.6–5.0	4.0–5.5	3.5–6.5%
Palmitoleic acid (16:1)	≤0.3%	≤0.1%	≤0.05%	≤0.2% (all isomers)
Heptadecanoic acid	≤0.1%	≤0.1%	≤0.06%	≤0.1%
Stearic acid (18:0)	2.7–6.5%	2.9–6.2%	2.1–5.0%	15.0–36.5%
Oleic acid (18:1)	14.0–39.4%	75.0–90.7%	43.1–71.8%	>50%
Linoleic acid (18:2)	48.3–74.0%	2.1–17.0%	18.7–45.3%	4.0–7.0%
Linolenic acid (18:3)	≤0.3%	≤0.3%	≤0.5%	≤0.5%
Arachidic acid (20:0)	0.1–0.5%	0.2–0.5%	0.2–0.4%	≤2.3%
Gadoleic acid (20:1)	≤0.3%	0.1–0.5%	0.2–0.3%	≤0.5%
Behenic acid (22:0)	0.3–1.5%	0.5–1.6%	0.6–1.1%	≤2.7%
Tetracosanoic acid (24:0)	≤0.5%	≤0.5%	0.3–0.4%	≤0.5%
Unsaponifiable (g kg^{-1})	≤15	≤15	≤15	≤15
Sterol composition				
Total sterols (mg kg^{-1} oil)	2400–5200	1700–5200		1700–5200
Cholesterol	≤0.7%	≤0.5%	0.1–0.2%	≤0.5%
Brassicasterol	≤0.2%	≤0.3%	≤0.1%	≤0.2%
Campesterol	6.5–13.0%	5.0–13.0%	9.1–9.6%	5.0–13.0%
Stigmasterol	6.0–13.0%	4.5–13.0%	9.0–9.3%	4.5–13.0%
ß-Sitosterol	50.0–70.0%	42.0–70.0%	56.0–58%	42.0–70.0%
Δ5-Avenasterol	≤6.9%	1.5–6.9%	4.8–5.3%	1.5–6.9%
Δ7-Stigmastenol	6.5–24.0%	6.5–24.0%	7.7–7.9%	6.5–24.0%
Δ7-Avenasterol	3.0–7.5%	≤9.0%	4.3–4.4%	3.0–9.0
Other sterols	≤5.3	5.3–9.5	5.4–5.8	≤9.5
Total tocopherols (mg · kg^{-1})	440–1520	450–1120	509–741	
Alpha-tocopherol (mg · kg^{-1})	403–935	400–1090	488–668	
Beta-tocopherol (mg · kg^{-1})	≤45	10–35	19–52	

ND, nondetectable, defined as ≤0.05; UA and YA refer to the Lovibond test; YU, yellow units; RU, red units.

[a]BOE (2013). Real Decreto 308/1983 de 25 de *Enero, por el que se aprueba la Reglamentación Técnica Sanitaria de Aceites Vegetales Comestibles*. BOE-A-1983-5543.

Source: Codex Alimentarius Commission. JOINT FAO/WHO FOOD Standards Programme CODEX Alimentarius Commission. ALINORM 03/17. Twenty-Sixth Session. Rome, Italy, 30 June–7 July 2003. *Report of the eighteenth session of the CODEX committee on fats and oils*. London, United Kingdom, 3–7 February 2003.

action of an enzymatic complex entitled fatty acid synthases I and III (FASIII/I). After a new condensation addressed by the FAS II, a new fatty acyl moiety is produced, the stearyl-ACP. This unit is a substrate of the first desaturation that induces the production of oleoyl-ACP. Both stearoyl-ACP and oleoyl-ACP are the main products synthesized into the plastidial. Following that, enzymes called thioesterases hydrolyze and transport outside the plastidial both ACP-bound moieties that are transformed in stearyl-CoA and oleoyl-CoA, now available to esterify hydroxyl groups of the glycerol molecule and to produce specific triacylglycerols. Available cytoplasmic oleic acid can be unsaturated to linoleic acid, after binding to a molecule of phosphatidylcholine.

International research institutions have developed by conventional mutagenesis and hybridization a collection of new oils. Original seeds have been modified by alkylating agents and later selected according to their fatty acid profile. From initial seed lines through crossing and selection, commercial seeds, richer in specific fatty acids, were obtained. In addition, autofecundation and crossing with common sunflower lines have permitted to increase the specific content of oleic or stearic acids. Seeds from which HOSO should be obtained must have the enzyme expression of the oleoyl-desaturase depressed. Several mechanisms have been proposed; however, high temperatures have been shown to highly depress the linoleic acid content of sunflower seed. When talking about high-stearic-acid sunflower oils (HSSO), the key reaction to be partially inhibited should be catalyzed by the thioesterase that transforms the stearic acid to oleic acid.

Although the individual proportion of fatty acids in the 'new' oils changes with respect to that of the conventional one, fortunately, no other fatty acids than those existing in the conventional sunflower oils are produced in partial blocking of the thioesterase and/or desaturase. In addition, the amount of SFA in the β-position of the triglyceride is also modified. According to the available information, commercialized HOSO, HSSO, and HOHSSO should cover all international requisites for conventional sunflower oil (Table 2), except those related to fatty acid composition, the iodine index value, and the SFA content in the β-position of triglycerides, as oleic acid and stearic acid have been deeply modified. At present, several studies have been performed with HOSO showing the suitability of this oil under several health and technological aspects (see succeeding text). With respect to HSSO and HOHSSO, although nonexhaustive toxicity evaluation of these oil types has been performed, the available information suggests that nondeleterious effects can be suspected, as they are being produced through conventional mutagenesis and hybridization techniques. Moreover, the information provided on the qualitative composition of these oils does not demonstrate the presence of new fatty acids deriving from the obtaining process. The content of the rest of the fatty acids is within the approved ranges established for other edible vegetable oils. Despite the Scientific Committee not having performed an exhaustive assessment and the lack of available epidemiological data, no possible adverse effects can be deduced from the information provided.

The HOSO shows a fatty acid composition similar to other monoenoic oils, such as olive oil (Tables 2 and 3). The HOHSSO is enriched in stearic acids. Also, some new variants are enriched in palmitic acid (Table 3). The conventional sunflower oil is rich in trilinolein, while the HOSO is rich in triolein. HOHSSO and other 'new' sunflower oils are richer in tristearin or tripalmitin (data not shown).

Table 3 Major fatty acid composition of different sunflower oil types available at the market

Acid type	*Palmitic*	*Stearic*	*Oleic*	*Linoleic*
Conventional	7	5	30	58
High oleic	5	4	88	3
High oleic–high palmitic	30	7	51	10
High linoleic–high palmitic	33	10	7	48
High stearic I	5	27	15	53
High stearic II	7	35	8	50
Medium stearic	5	14	19	62
High oleic–high stearic	6	24	62	8

Source: Martínez Force, E. and Garcés Mancheño, R. (2004). New sunflower oils: the future for a more healthy food industry. CTC Alimentacion **21**, 49–54.

Due to more and more production and commercialization of those new oils every day, their identification and clear classification to avoid misleading and fraudulent uses are of great importance. Near-infrared reflectance spectroscopy has been proposed to this aim; however, some oils, very similar in composition, do not completely permit their identification.

Minor Components

In the last two decades, certain minor constituents of oils have been extensively studied because of their antioxidant properties and because they can modulate the dietary cholesterol absorption, the fatty acid metabolism, and thus the serum cholesterol levels. Table 2 shows the accepted rank composition of minor compounds of the different main variants of sunflower oils. Large amount of phytosterols and tocopherols is present. It is remarkable that, although conventional sunflower oil has a different fatty acid composition than HOSO, the profile of minor compounds (in absolute and relative amounts) of these two oils is quite similar. The stability in minor components of the different sunflower oils is also evident and can be related with the fact that neither new sterols nor new molecules have been detected in the HSSO and HOHSSO production.

Other Aspects

In the few last years, new regulations and norms have been stated, as the one originated after detecting contamination risk in sunflower oil by mineral oil. Thus, the European Food Safety Authority has set that sunflower oil imported into the community shall not contain more than 50 mg kg^{-1} mineral paraffin. This has to be certified according to EN ISO/IEC 17025 taken in accordance with the provisions of Regulation (EC) No 333/2007 and the international standard ISO 5555:2003.

Food Uses and Applications

Conventional sunflower oil is appreciated among vegetable oils because of its bland flavor, light color, high smoking point, high level of linoleic acid, and very poor linolenic acid content.

Sunflower oil is primarily used as a salad dressing and cooking oil. Furthermore, it is extensively used as a major ingredient in some margarine and shortening products. For some decades, population living near to the Mediterranean coast continues consuming a relatively large quantity of olive oil, mainly for frying purposes. However, other cheaper oils, mostly sunflower oil, have largely substituted olive oil. Economic reasons have promoted the production and consumption of sunflower oil. However, at present, some 'new' variants of sunflower oil are been produced and commercialized, mostly for culinary uses that have to be performed at relatively high temperatures.

Margarines and Spreads

Margarines were originally obtained from lard first and from other fat sources such as seed oils later. However, the association of saturated and *trans*-fatty acids with coronary and other cardiovascular diseases led to the development of new margarines from vegetable origin. In fact, most margarines and spreads are presently obtained by transesterification that permits the specific enrichment of the oil in a given fatty acid.

Sunflower oil is one of the oils employed in the formulation of fat blends to achieve an adequate product. High polyunsaturated margarines should contain no less than 45% of *cis*, *cis* linoleic acid or less than 50% PUFA. Thus, in order to reach 45–50% in PUFA, 70–80% sunflower seed oil was demanded. Nonetheless, as previously mentioned, the generation of different types of sunflower oils, in particular that of HSSO and HOHSSO, has changed the facilities of bakery and margarine industries, permitting obtaining new foodstuffs, due to the plasticity and thermostability conferred by those oils.

Deep Fat Frying

Deep fat frying is one of the quickest cooking elaboration procedures of food with cultural and economic importance. Sunflower oil is being extensively used for deep frying, at home and fast-food restaurants and in the industry.

Frying is a relative complex process, consisting in browning, dehydration, and heat and mass transfer. During frying, the culinary fat is exposed to the action of moisture from the foodstuff, oxygen from the atmosphere, and high temperature at which the operation takes place. Thermally oxidized and hydrolytic compounds are present in different quantities depending on a considerably high number of frying variables. Finally, apart from migration of lipids from the food into the frying oil, the presence of some compounds from the foods can substantially affect the thermal oxidation reactions.

Oil entrance can increase some organoleptic properties of the food (texture, flavor, aroma, taste, and crispiness) and improve or impair the fatty acid profile giving rise to a most balanced saturated fatty acids/monounsaturated fatty acids/polyunsaturated fatty acids (SFA/MUFA/PUFA) ratio. Thus, the level of linoleic acid in the food can be deeply increased by frying with sunflower oil. Moreover, lipophilic compounds (such as tocopherols) can also access the food. This seems very convenient when the oil is used for the very first times when oil is unaltered or slightly altered or in industrial cooking, where there is a high turnover of fresh oil. However, usually, the frying is performed on demand, mostly at home, bars, and small restaurants with non-turnover or very low turnover of fresh oils.

The global alteration of oil (thermal oxidation plus hydrolysis) can be evaluated measuring the percentage of total polar artifacts (as polar content) by column chromatography. Also, polymer determination is extendedly used. The 22–25% polar material or the 10–12% polymers' cutoff points have been accepted for oil discarding. Frying potatoes in sunflower oil following domestic conditions shows that polar content increased in oil and frying potatoes. However, when frying is performed with frequent oil turnover, the amount of polar material in sunflower oil is substantially below the critical level (Figure 4). The amount of polymers (dimers + oligomers) formed in sunflower oil during frying is also lower in the frequent turnover of fresh oil than in the null turnover one (Table 4). The benefit of the high turnover has been found in the frying of frozen foods (potatoes, fish fingers, croquettes, tuna pastries, etc.) under domestic conditions at 180 °C. More recently, we found that the frequent turnover together with lower frying temperature (160 °C) permits to use the oil longer, keeping the thermal oxidation in the sunflower oil used 40 times to fry different foodstuffs reasonably low (Table 4).

HOSO is a rather stable oil. Our studies using HOSO to fry potatoes following a domestic frying model suggest that the frequent replenishment of used HOSO with fresh HOSO permits the frying of sets of fresh potatoes at a very high rate (Figure 4). Moreover, HOSO performs much better than the conventional sunflower oil in the frying of frozen prefried

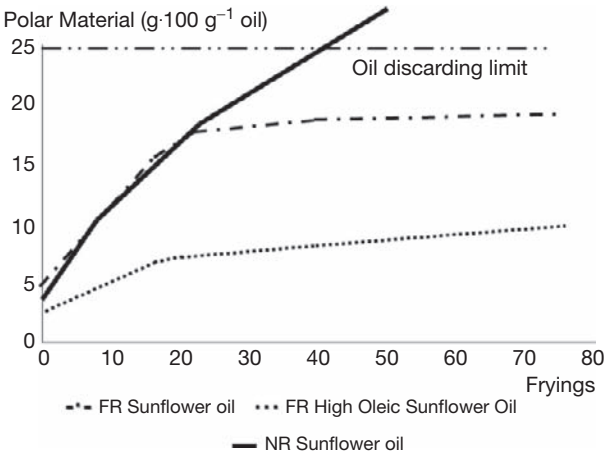

Figure 4 Sunflower oil and high-oleic-acid sunflower oil behavior in potato frying. Influence of frequent or null turnover of fresh oil. Polar fraction (mg 100 mg^{-1} oil) change. NR, null turnover; FR, frequent turnover. Modified from Sánchez-Muniz, F. J., Cuesta, C. (2003). Sunflower oil. In: Caballero, B., Trugo, L., and Fligas, P. (eds.) *Encyclopedia of food science and nutrition* (2nd ed.), pp. 5672–5680. Amsterdam: Elsevier Science Ltd.

Table 4 Total polar content and polymers in different oils before and after frying frozen prefried foods with null or frequent fresh oil addition to the fryer oil at different temperatures

					Polar (mg 100 g^{-1} oil)		Polymers (mg 100 g^{-1} oil)	
	Temperature	*Food*	*Frying number*	*Oil addition*	*Initial*	*Final*	*Initial*	*Final*
Sunflower oil (SO)[a]	180	(1)	20	Yes[b]	3.70	15.9	1.40	8.58
Sunflower oil (SO)[a]	180	(1)	20	No	3.70	20.5	1.40	11.40
High-oleic-acid sunflower oil (HOSO)[a]	180	(1)	20	Yes[b]	3.06	13.3	0.27	5.91
High-oleic-acid sunflower oil (HOSO)[a]	180	(1)	20	No	3.06	15.4	0.27	7.15
Sunflower oil (SO)	160	(2)	40	Yes[c]	5.28	14.37	1.05	6.89
High-oleic-acid sunflower oil (HOSO)[a,d]	160	(2)	40	Yes[c]	2.52	8.06	0.19	3.05

(1) Frozen prefried foods: par-fried potatoes (six times), chicken croquettes (three times), hake fish fingers (once), fritters (once), tuna pastries (twice), breaded ham and cheese (twice), battered squid (twice), breaded hake (twice), and spring rolls (once). (2) Fresh potatoes and frozen prefried foods: fresh potatoes (nine times), par-fried potatoes (five times), chicken nuggets (eight times), chicken croquettes (five times), and hake fish fingers (13 times).

[a]Tocopherol content (~500 ppm).

[b]Oil addition (15–20 ml to 3 l fryer) after each frying to maintain fryer oil volume constant. After each five fryings, 50 ml was taken for analysis and 50 ml fresh oil added.

[c]Oil addition (45–50 ml to 1.5 l fryer) after each five fryings to maintain fryer oil volume constant and oil determinations.

[d]Contained dimethylpolysiloxane as antifoaming, and ascorbyl palmitate and alpha-tocopherol (~700 ppm).

Source: Sánchez-Muniz, F. J., Bastida, S., Márquez-Ruiz, G., Dobarganes, M. C. (2008). *Effects of heating and frying on oil and food fatty acids*. In: Chow, C. K. (ed.), *Fatty acids in foods and their health implications* (3rd ed.), pp. 511–543. Boca Raton, FL, USA: CRC Press, Taylor and Francis Book; Bastida, S., Sánchez-Muniz, F. J., di Lorenzo, L. (2006). *Estudio comparativo de estabilidad frente al comportamiento en fritura de alimwentos frescos y congelados, de los diferentes aceites de más alto consumo por la población española*. EDOCUSA, Madrid.

foods, at both frequent or null turnover techniques. Interesting results were found frying with HOSO at 160 °C. The higher stability (lower polar artifact and polymer formation) of HOSO at high temperature, with respect to conventional sunflower oil (Table 4), seems related to differences in the fatty acid composition (mainly more oleic acid and less linoleic acid).

Among natural sunflower oil antioxidants, tocopherols seem to be more relevant. In this way, it is interesting to note, in continuous frying operations, a regular turnover of fresh oil maintains the level of antioxidant in the oil. However, data from some publications suggest that the addition of the antioxidant exerts little influence in extending frying life of the oil used in frying but it can extend the shelf life of the food reducing the rate of oxidation of the absorbed oil. According to others, peroxidative degradation of frying highly increases when the tocopherol is lacking in the oil. Recent publications suggest that the addition of concentrates of carob fruit fiber (very rich in nonextractable tannins) significantly increases the frying shelf life of sunflower oil and olive oil–sunflower oil blend, reducing the thermal oxidation and polymerization and decreasing the tocopherol content losses.

As previously mentioned, frying performance with HOHSSO offers very promising results as it, first, permits keeping the alteration of the oil low and, second, enriches the food in oleic and stearic acids, with potential benefits under a cardiovascular disease point of view. Nonetheless, further research is demanded.

Other Food Uses

Sunflower 'whole seed' is sold as a snack food, raw or after roasting in ovens, with or without salt, and/or with seasonings added. In Germany, it is popular to make Sonnenblumenkernbrot, sunflower butter mixed with rye flour. It is also sold as food for birds and can be used directly in cooking and salads. American Indians had multiple uses for sunflowers in the past, such as in bread, medical ointments, dyes, and body paints. The sunflower meal, obtained after the oil extraction, displays high protein content (about 42% for the hulled seed meals) and is used primarily in food rations for livestock and poultry. Compared to soybean, sunflower meal contains lower energy and lysine but higher fiber and methionine content. Variants of sunflower seeds with low oil content (non-oilseed sunflower) are primarily used as a snack food; as an ingredient in baked foods, salads, and candies; and for feeding birds and small animal pets. During the last decades, a flowering market has grown round these seeds.

Finally, sunflower hulls present potential uses as litter for livestock or poultry and as a source of roughage in animal feeding. Building or insulation boards and fireplace logs are other potential applications.

Due to its higher price than other seed oils, sunflower oil is not widely employed for industrial purposes. Some paints, varnishes, and plastics take advantage of its semidrying properties without the yellowing problems associated with oils rich in linolenic acid. In some eastern European countries, the oil is used by detergent and soap manufacturers. Like other vegetable oils, it has potential value for uses in the production of adhesives, agrochemicals, surfactants, additional plastics and plastic additives, fabric softeners, synthetic lubricants, coatings, and other products. HOSO has extra value in industrial products where oleic acid and related chemicals are currently utilized. It can be used as a source of oleic acid in the spread and margarine industry, as an ingredient in infant food formulas, which require stability, bland flavor, and specific fatty acid composition. Its fatty acid profile together with its high oxidative stability is valuable for cosmetic and pharmaceutical

manufacture. This should also be more evident in the case of HOHSSO.

Dietary Importance and Health Effects

Sunflower Oil as Energy Source

While carbohydrates and protein provide 17 kJ g^{-1} (4 kcal g^{-1}), long-chain triglycerides provide 38 kJ g^{-1} (9 kcal g^{-1}). The fatty acids are used for energy production in the cell mitochondria and peroxisomes. Present recommendations for fat are between 20 and 35% of total energy (45–78 g day^{-1} in a 2000 kcal diet). Due to a large percentage of total fat (more than 50% in some Mediterranean populations) being used in the form of oils for food cooking and dressing, the fatty acid composition of a diet largely depends on the culinary oil/fat used.

Research data suggest that a unit of energy from fat may be more fattening than a unit of energy from most carbohydrates. This fact can be related to the limited body capacity to convert carbohydrate into fat and the faster adaptation to oxidize excess carbohydrate than excess fat. Moreover, body capacity for storing carbohydrate as glycogen is limited, whereas in adipose tissue, fat store is virtually unlimited. Long-term signals regulating food intake and energy homeostasis suggest that both insulin and leptin act in the central nervous system to inhibit food intake and to increase energy expenditure. Insulin, through increasing oxidative glucose metabolism in adipocytes, stimulates leptin production from adipose tissue. Dietary fat (mostly saturated fat) does not stimulate insulin secretion and therefore does not increase leptin production. This suggests the convenience of decreasing saturated fat consumption and substituting saturated fat for a more unsaturated one.

Sunflower Oil as Essential Fatty Acid Source

Linoleic and linolenic acids are the two parent members of omega-6 ($n-6$) and omega-3 ($n-3$) fatty acid families, respectively. Both are essential and must be supplied by the diet because humans and animals have lost the ability to synthesize them. Moreover, other fatty acids of the $n-6$ family such as γ-linolenic acid (18:3, $n-6$), dihomo-γ-linolenic acid (20:3, $n-6$), and arachidonic acid (20:4, $n-6$) or from the $n-3$ family as eicosapentaenoic acid (20:5, $n-3$) and docosahexaenoic acid (22:6, $n-3$) cannot be formed.

The major $n-6$ fatty acid in the Western diet is by far linoleic acid. Although other oils are richer in linoleic acid, sunflower oil appears to be the primary source of this fatty acid, at least in Europe and particularly in Mediterranean countries. The importance of linoleic acid consumption is detailed elsewhere (see 'Essential Fatty Acids').

Deficiency of this fatty acid is called essential fatty acid (EFA) deficiency; it is well documented in rodents and can be produced in several animals, including humans. The disease is characterized by skin symptoms such as dermatosis. Growth is retarded, reproduction is impaired, and there is degeneration or impairment of functions in many organs of the body. EFA deficiency is characterized by changes in the fatty acid composition of many biological membranes, depending on the profile of the fat consumed. Overt EFA deficiency is only seen when linoleic acid provides less than 1% of dietary energy (about 2 g day^{-1} in 2000 kcal diet). Present recommendations for linoleic acid have been set in adults (2.0–3.0%En for adequate intake (AI); 2.5–9% upper value of acceptable macronutrient distribution range (U-AMDR)). In 6–12- or 12–24-month-old children, AI has been set in 3–4.5%En, while the U-AMDR in <10%En.

Sunflower Oil as Antioxidant Source

Sunflower oil contains valuable amount of minor compounds such as α-tocopherols, γ-tocopherols, and phytosterols (Table 2). The correct equilibrium between PUFA and antioxidants protects lipoprotein membranes from oxidative stress. Among other properties, phytosterols act not only as free radical scavengers but also as antipolymerization agents in frying oils.

Influence of Linoleic Acid on Cholesterolemia and Lipoproteinemia

It is currently recommended that dietary total fat, SFA, and cholesterol should be reduced, while the intake of PUFA and MUFA should be increased. As a result of these considerations, the consumption of unsaturated oils has been recommended, and margarines rich in PUFA and MUFA, called healthy margarines, have been developed. Linoleic acid had been considered for many years to be the unique fatty acid that had hypocholesterolemic effects (at serum and lipoprotein levels). Recent investigations suggest that oleic acid also presents such an effect on low-density lipoprotein (LDL) particles. On the other hand, a large intake of linoleic acid (higher than 10%En) would decrease high-density lipoprotein (HDL) cholesterol and increase the oxidation of LDL and HDL particles. Due to its fatty acid profile, HOSO presents similar cholesterolemic effects as olive oil. However, differences in minor compounds would clearly explain differences observed in some studies with respect to the effects on lipoproteinemia and LDL oxidation of these two oils.

Influence of Polyunsaturated Fatty acids $n-6$ on Other Physiological Processes

$n-6$ fatty acids have been proven to exert important roles in cytokine production and monocyte chemotaxis and in eicosanoid production. Platelet aggregation, inflammation, and cellular immunity are processes modulated by metabolic products derived from $n-6$ fatty acids. Because sunflower oil contains large amounts of linoleic acid (the fatty acid mother of $n-6$ family), the intake of this fatty acid should be adequate, avoiding low and very high intakes that would induce EFA deficiency or $n-6$ biomembrane saturation. In some circumstances, the capacity to transform linoleic acid into arachidonic acid is largely depressed (e.g., small for gestational age neonates, aging, diabetes, high alcohol, saturated fat, and cholesterol intakes) by decreasing the activity of $\Delta-6$ desaturase.

Like other fats, sunflower oil may modulate all these previously cited processes because of its ability to change the fatty

acid composition of membrane phospholipids. As a consequence, membrane fluidity may change and also, when cytokines act on target cells, the amount and types of prostaglandin and leukotriene originated are modified. Dihomo-γ-linolenic acid and arachidonic acid derive from linoleic acid and are precursors of eicosanoids. These fatty acids can be metabolized via different enzymatic pathways, giving rise to thromboxanes, prostacyclins, prostaglandins, leukotrienes, hydroxy and hydroperoxy fatty acids, and lipoxins, which have been proved to modulate cellular growth, angiogenesis, inflammation, cellular immunology, thrombosis, cardiovascular disease, cancer, etc.

According to recent recommendations, linoleic acid consumption must be $<9\%$En (20 g in a 2000 kcal diet). Other scientific societies had previously recommended that linoleic acid should contribute 3–6%En in the diet. However, the effects of linoleic acid on cell properties and health are highly dependent on the $n-6/n-3$ ratio and personal conditions. Thus, the adequate amount for linoleic acid, and sunflower oil, should be individually considered taking into account age, sex, and physiological status together with possible nutrigenetic, nutrigenomic, and epigenetic characteristic interactions.

Future studies should be addressed to avoid potential misleading and fraudulent uses of these new oils. Clearly identifying and defining their composition and properties and regulating their production and uses are demanded. The absence of adverse effects of new sunflower oils consumption must be assessed throughout epidemiological studies. In addition, the negative impact of all these new cultivars on the conventional ones has also to be checked and controlled.

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Supercritical Fluid Extraction

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Introduction

Supercritical fluid extraction (SFE) is a technique based on the use of solvents at temperatures and pressures above their critical points. SFE can be a fast, efficient, and clean method for the extraction of natural products from several matrices. The ease of tuning the operating conditions in order to increase the solvation power makes this technology a good option for the selective recovery of several types of substances.

The discovery of the critical phenomena is attributed to Charles Cagniard de la Tour in 1822. SFE was first introduced in 1879 by Hannay and Hogarth. Despite the advantages associated to the use of supercritical fluids (SCFs) as extracting agents, it was not until around 1960 that this technique started to be thoroughly investigated as an alternative to conventional extraction methods. The field of critical phenomena has thrived and now forms a keystone of modern science, both experimental and theoretical, and its development exemplifies how a topic of purely fundamental research can diversify into initially unanticipated directions.

On the pressure–temperature diagram (see Figure 1), the point at which critical temperature (T_c) and critical pressure (P_c) meet is called the critical point of the substance. As the substance approaches its critical temperature, the properties of both, gas and liquid phases, converge, resulting in only one phase at the critical point: a homogeneous SCF. Physical properties of SCFs are between those of a gas and those of a liquid. Density is close to liquids, and viscosity and surface tension are close to gases. This is one of the key features of SFE since the solvating power (quantified using Hildebrand solubility parameter, δ) of the fluid can be strongly influenced by small changes in pressure and temperature favoring either the extraction of the target compounds or the precipitation of the solutes dissolved in the SCF. Many other physical properties such as relative permittivity and solvent strength, highly related to density, show large gradients with pressure above the critical point. Changes in those properties are crucial when dealing with extraction since they are related to changes in solubility and mass transfer ratios and, therefore, in the selectively of the solvent.

Despite every single substance having a critical point, not all of them are used in SFE, and green solvents (generally recognized as safe, GRAS) with mild critical properties are preferred. Among them, carbon dioxide (critical conditions = 31.2 °C and 7.38 MPa) is undoubtedly the most commonly employed. CO_2 is cheap, environmentally friendly, and GRAS. Another advantage is that CO_2 is gaseous at room temperature and pressure, which makes extract recovery very simple and provides solvent-free extracts. Also important for food and natural products' extraction is the ability of SFE using CO_2 to be operated at low temperatures using a non-oxidant medium, which allows the extraction of thermally labile or easily oxidized compounds. Its main drawback is its low polarity, which can be overcome by adding small amounts of polar cosolvents (methanol, ethanol, and water).

Due to the absence of toxic residues in the final product, among other advantages, SFE has been used in different fields such as food, pharmaceutical, chemical, and fuel industries. The widest application of SCFs is extraction, especially with carbon dioxide. The first patent dealing with SFE was published in 1943, although the first industrial application was developed in 1978.

In the food and health areas, SFE can be employed

- to extract valuable bioactive compounds such as flavors, colorants, and other biomolecules, or
- to remove undesirable compounds such as organic pollutants, toxins, and pesticides.

Basic Instrumentation for SFE

The SFE general extraction procedure might be divided into three phases. At the beginning of the extraction process, the supercritical solvent diffuses into the matrix and decreases the mass transfer resistance; after this step, soluble compounds are dissolved into the SCF and are further transferred by diffusion first into the surface of the solid and later to the bulk of the fluid phase. The extraction process ends with the transport of the solute and the bulk fluid phase and their removal from the extractor.

A basic scheme of the equipment used to perform SFE is shown in Figure 1. It consists of a container of the extraction solvent (A) (usually CO_2), a pump to pressurize the fluid (B), an oven containing the extraction vessel (C), and a restrictor to maintain the high pressure inside the system (D). Due to the action of the oven and the pump, the CO_2 changes from liquid to supercritical conditions. The recovery consists on a trapping vessel (E), where pressure is reduced to a gas equivalent pressure. Optionally, it can include a second trapping vessel (F), a CO_2 recycling system (G), and a modifier pump (H). Nevertheless, from this starting point, the instrumentation employed might be more or less sophisticated.

Parameters of SFE

In order to optimize extractions using SCFs, different parameters should be considered:

- *Raw material*: It is normally imposed by the process, but in general, it is recommended to increase the surface contact among the matrix and the SCF to increase the mass transfer ratio. For solid samples, factors such as particle size, shape, and porosity of the solid material are of crucial importance. Small particles increase extraction rates, but too small particles must be avoided since their use can compact the bed,

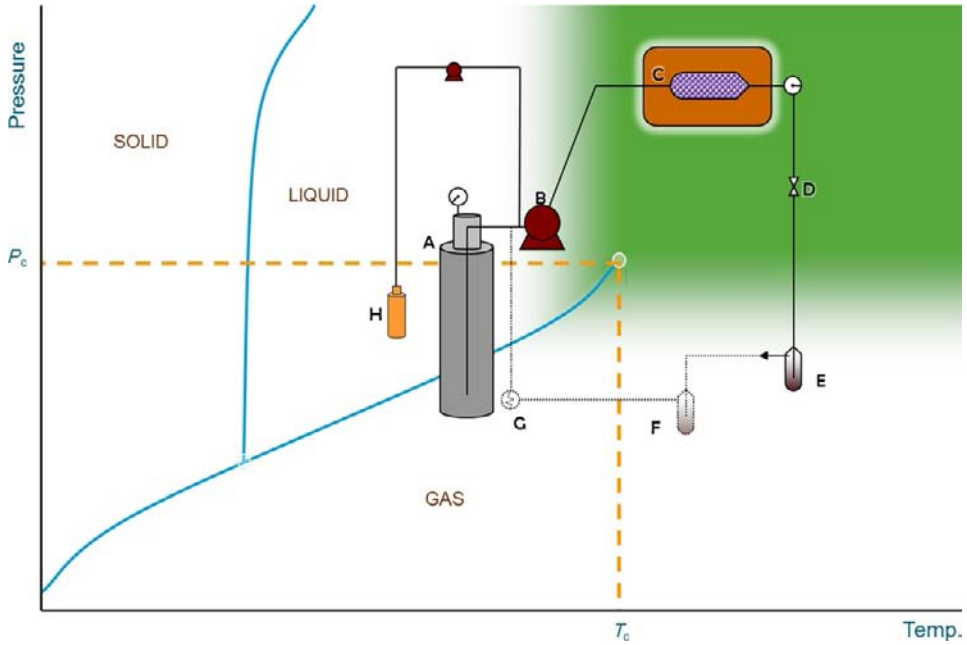

Figure 1 Scheme of the typical supercritical fluid extractor superimposed into the CO_2 phase diagram. The different parts of the SFE are named as liquid CO_2 cylinder (A), CO_2 pump (B), heated extraction cell (C), static/dynamic valve (D), collection vessel 1 (E), collection vessel 2 (F), CO_2 recycling system, and modifier container (H).

increasing the internal mass transfer resistance and causing channeling inside the extraction bed; as a result, the extraction rate may decrease due to a nonhomogeneous extraction. Sometimes, it is necessary to use dispersing agents (e.g., sea sand) to avoid sample clogging or to adsorb/retain interfering compounds present in the matrix, such as water or lipids; some examples are the employment of silica gel or sepiolite for water adsorption or C18 for lipids retention. In the case of liquid samples, countercurrent SFE (CC-SFE) is usually carried out. Extractions under CC conditions are performed using columns (normally packed) where the solvent (supercritical CO_2) is pumped from the bottom and the liquid matrix is pumped from the top; both streams contact each other in opposite directions (countercurrent flow), which increase the number of equilibrium steps and therefore the mass transfer ratio. In this case, factors such as size of the column, type of packing material, and solvent/feed ratios are the main parameters to be considered to increase the extraction rate.

- *Solubility*: One of the main advantages of using SCFs is the possibility of tuning the solubility, which is highly dependent on the pair pressure–temperature and therefore on the density of the fluid. Solubility of most components in SCFs increases with the increase of the SCF density, which can be accomplished by increasing the extraction pressure. Other important aspects influencing solubility of components in the SCF are their polarity and molecular weight and the extraction temperature. In SFE processes using CO_2, the component solubility is lowered as the polarity and/or the molecular weights of the solutes are increased. Increasing the temperature, at constant pressure, promotes two opposite effects: It reduces the solvent power of CO_2 by a decrease of the density, and, on the other hand, it increases the vapor pressure of solutes that can be more easily transferred to the supercritical phase. Depending on the working pressure, different effects can be observed: In fact, near the critical pressure, the fluid density is the predominant effect, and a moderate increase in temperature leads to an important decrease in fluid density and, therefore, to a decrease in solute solubility. At high pressures, the vapor pressure is the most prevalent effect; thus, the solubility increases with the temperature. This phenomenon is known as retrograde behavior.
- *Modifiers* (*cosolvents*): Since CO_2 is the main fluid used in SFE, and due to its low polarity, the addition of modifiers to CO_2 can improve the extraction proficiency by raising the solubility of polar solutes. As a general rule, the amount of modifier used is lower than 10–15%. The most used modifiers are methanol, ethanol, and water. It is important to consider that the critical point of the mixture CO_2: modifier is different from that of the pure compounds (it also depends on the proportions of CO_2 and modifier in the mixture). Since cosolvents are not gases at room conditions, liquid residues are obtained in the extracts and the remaining matrix after SFE.
- *Solvent-to-feed ratio*: It must be high enough to provide a good extraction yield in a short time, but it should also grant enough contact time among solvent and solutes. But it must be taken into account that higher solvent rates increase operational costs. In general, it is common to use solvent-to-feed ratios around 25–100:1 for analytic processes and 5–15:1 for large-scale processes.

Due to the high amount of parameters affecting SFE, the process optimization can be a highly tedious work. In order to

optimize SFE process, two main strategies are followed: phase equilibrium engineering and experimental designs. The first one is based on thermodynamic calculations; it is very useful and accurate, but not easy to carry out, mainly when dealing with complex matrices. The use of experimental designs offers a framework where researchers create an experiment, controlling experiment factors so as to predict or establish a result based on dependent and independent variables. Experimental designs give the possibility to evaluate interactions between factors while limiting the number of experiments. Optimization based on experimental design is based on statistical calculations.

Applications

SFE has been used since the end of 1970s to isolate compounds; however, for a long time, applications were focused only on a few of them. Nowadays, a high amount of research works has been published dealing with the use of SFE as a powerful extraction technique for recovering a wide variety of bioactive compounds from different natural sources such as plants, marine sources, and agricultural and food by-products. Although CO_2 is the main fluid used in SFE, its low polarity limits seriously its ability to extract polar components from natural products. To address this drawback, small amounts of cosolvents are added to CO_2. This fact increases the CO_2 polarity, enabling SFE to be also applied to extract from slightly polar to polar bioactive compounds.

On the other hand, the use of chemometric tools to determine the optimum values of the different parameters involved in the SFE process is of great interest to set up a robust SFE extraction procedure. Thus, Taguchi, Box–Behnken, or central composite experimental designs have been used, among others, for the optimization of variables. Along with these methodologies, response surface methodology has also been employed since it allows not only the choice of the best extraction parameters but also the graphical observation of the influence of these parameters on the studied factors (response variables).

Plants

SFE has been extensively employed as extraction technique for recovering bioactive compounds from plants. A wide variety of solid materials have been employed as natural sources to extract compounds possessing a particular bioactivity. Table 1 gives details of different works for the recovery of valuable compounds, mainly with antioxidant activity. Although optimum extraction conditions obviously depend on the sample, normally, pressures from 9 to 30 MPa and temperatures from 35 to 100 °C have been employed.

As can be observed in Table 1, the bioactive compounds extracted by SFE from plants belong to a wide range of chemical structures covering a broad range of polarities. Considering the low polarity of CO_2, it has been mainly used to extract nonpolar bioactive compounds. For example, neat CO_2 has been used to isolate the less polar bioactives from essential oils. The extraction of low-polar compounds, such as carotenoids, has been performed employing two different strategies: using pure CO_2 at higher pressures (60 MPa) and using small proportion of a modifier (see Table 1). Both strategies enable us to improve the extraction rate of medium to nonpolar compounds. On the other hand, fractions enriched in polar bioactive compounds (phenolic compounds) were also isolated by using SFE with cosolvent (see Table 1). Although the most frequent modifiers are ethanol and methanol, others, such as diethylamine and water, have been also investigated, for instance, for extracting alkaloids.

Marine Products

Marine environments have been pointed out as an extraordinary source of high added value products due to their wide range of chemical structures and biological diversity. In this regard, organisms like algae, microalgae, crustaceans, and fish often possess highest concentration of bioactive compounds suitable for human health. Moreover, the conversion of waste and by-products discharged by fisheries, such as fish heads, viscera, skin, and tails, into valuable materials, through, for instance, extraction of high added value compounds, is a goal for a sustainable and ecological production and can provide enormous benefits from an environmental and economic point of view.

Table 2 gathers different applications of SFE dealing with the extraction of bioactive compounds such as ω-3 polyunsaturated fatty acids (ω-3 PUFAs), carotenoids, and phenolic compounds from marine sources. The possibility of obtaining ω-3 PUFAs, mainly eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), from marine sources has been intensively studied taking into account its important potential biological properties (anti-inflammatory, antithrombotic, antiarrhythmic, etc.). Fish oil and fish by-products usually present higher amounts of PUFA than seed oils or microalgae. This fact makes them the major natural dietary source of ω-3 PUFAs. Due to the characteristic of this kind of compounds, their extraction by SFE using CO_2 is especially appropriate. SFE has demonstrated to be especially useful for fish oils' fractionation, operating under different temperatures and pressures. For instance, it has been recently demonstrated how the probability of fractionating the triacylglycerols with respect to EPA and DHA from fish oil is improved using fish oil with lower ω-3 fatty acids content as the basis. On the other hand, the use of SFE to add value to waste products of the fish industry has been also demonstrated by using different fish by-products and some marine invertebrates as raw materials to obtain bioactive compounds. In this case, the addition of ethanol to improve the extraction yields of, for instance, lipids and astaxanthin from red-spotted shrimp waste compared to the extraction without a cosolvent is sometimes necessary. Increasing the proportion of ethanol also produced an increase in the concentration of the ω-3 fatty acids in the lipids of the obtained extract.

On the other hand, SFE technology has been used to obtain carotenoids, isoflavones, polyphenols, and flavonoids from algae and microalgae as natural sources. As it can be observed in Table 2, carotenoids have been mainly extracted adding ethanol or methanol, although a study has demonstrated the possibility of extracting astaxanthin or canthaxanthin without cosolvent. Besides extraction, another interesting recent application is the purification of carotenoids by supercritical antisolvent precipitation. This technology has been applied, for

Table 1 Details of some works for the recovery of valuable compounds from plants using SFE

Group of compounds	*Compounds of interest*	*Plant material*	*Related bioactivities*	*Extraction conditions*
Lipids	Fatty acids	*Borago officinalis*		CO_2 + methanol, 35 MPa, 65 °C, 10 min static + 10 min dynamic
	Fatty acids and antioxidants	*Camellia sinensis*	Antioxidant activity	CO_2, 32 MPa, 45 °C, 90 min (static)
	Essential oils	*Lamiaceae* plants	Antimicrobial activity	CO_2, 30 MPa, 40 °C, 90 min (dynamic, 2.4 kg h^{-1})
	Essential oil	Spearmint (*Mentha spicata* L.)		CO_2, 9 MPa, 35 °C, 30 min (static)
	Essential oil	Spearmint (*Mentha spicata*)	Antioxidant activity	CO_2, 30 MPa, 50 °C, 180 min (dynamic, 0.3 kg h^{-1})
	Hernandulcin and other sesquiterpenes	*Lippia dulcis*		CO_2, 12 MPa, 35 °C, 60 min (static) + dynamic period
	Oleuropein	Olive leaves	Antioxidant activity	CO_2 + 20% methanol, 30 MPa, 100 °C
	Lutein, zeaxanthin	*Hemerocallis disticha*	Antioxidant activity	CO_2, 60 MPa, 80 °C, 30 + 30 min (static + dynamic, 0.01 l min^{-1})
	Tocopherols, lycopene, and β-carotene	Sea buckthorn	Radical-scavenging activity	CO_2, 40 MPa, 35 °C, 60 min (static)
	Lutein	Spinach	Antioxidant activity	CO_2 + ethanol, 30 MPa, 50 °C, 90 min (static)
Phenolic compounds	Polyphenols, flavonoids	Kale, spinach	Antioxidant activity	CO_2 + 5% methanol, 25.8 MPa, 50 °C, 30 min (static)
	Phenolic compounds	Olive leaves	Cytotoxic activity	CO_2 + 6.6% ethanol, 15 MPa, 40 °C, 120 min (static)
	Phenolic compounds	Rosemary (*Rosmarinus officinalis*)	Antioxidant activity	CO_2, 30 MPa, 40 °C, 300 min (dynamic, 3.6 kg h^{-1})
	Phenolic compounds	Rosemary (*Rosmarinus officinalis*)	Antioxidant activity	CO_2 + 6.6% ethanol, 15 MPa, 40 °C, 120 min (static)
	Phenolic compounds	Rosemary (*Rosmarinus officinalis*)	Anti-inflammatory activity	CO_2, 35 MPa, 80 °C, 30 static + 90 min dynamic
	Total phenols	Strawberry	Antioxidant activity	CO_2 + 19.7% ethanol, 60 MPa, 48 °C, 60 min (dynamic, 15 g min^{-1})
	Phenolic compounds Volatile compounds	Rosemary, oregano, chamomile, marigold	Antioxidant activity	Phenolic: CO_2 + 17–40% ethanol, 10 MPa, 35–40 °C, 20 min (dynamic 0.001 l min^{-1}) Volatile: CO_2 + 0–10% ethanol, 10–17 MPa, 35–40 °C, 10–20 min (dynamic 0.001–0.002 l min^{-1})
	Phenolic compounds	Clove buds (*Syzygium aromaticum*)	Antioxidant and antibacterial activities	CO_2, 10 MPa, 40 °C, 300 min (dynamic, 0.62 kg h^{-1})
Alkaloids	Alkaloids	*Mitragyna speciosa*		CO_2 + 28.8% ethanol, 30 MPa, 65 °C, 45 min (dynamic)
	Alkaloids	*Nelumbo nucifera*	Several bioactivities	CO_2 + 10% diethylamine and 1% water, 30 MPa, 70 °C, 120 min (dynamic, 1.1 kg h^{-1})

example, to the purification of zeaxanthin from the ultrasonic extract of *Nannochloropsis oculata*. Other antioxidant compounds different from carotenoids, such as isoflavones, polyphenols, and flavonoids, have been also extracted by SFE using methanol or ethanol as cosolvents.

Agricultural and Food By-Products

SFE has been widely used to value agricultural and food by-products generated as food waste during food manufacturing. The generation of a large variety of by-product wastes during the industrial activities makes imperative to find new uses for these materials in order to reduce their environmental impact. In this sense, SFE processes are being developed to recover high valuable products from food wastes. As Table 3 shows, SFE has been used to obtain from different agricultural and food by-products a huge variety of bioactive compounds, from fatty acids, essential oils, and tocopherols to carotenoids and polyphenols with antioxidant properties. This fact once again demonstrates the versatility of SFE toward the extraction of lipophilic and hydrophilic compounds when a cosolvent is added to CO_2.

Different SFE methodologies have been developed to extract lycopene, which has the highest antioxidant activity among all dietary antioxidants and represents the most abundant carotenoid in tomatoes, accounting for more than 80% of

Table 2 Examples of SFE procedures for the recovery of valuable compounds from marine sources

Group of compounds	*Compounds of interest*	*Marine source*	*Related bioactivities*	*Extraction conditions*
Lipids	ω-3 PUFAs	Fish oil	Anti-inflammatory, reduce risk of certain heart diseases	CO_2, 20 MPa, 33 or 40 °C (static 30 min + dynamic, 0.8 l min^{-1})
	ω-3 PUFAs Astaxanthin	Brazilian red-spotted shrimp waste (shell and tail)	Antioxidant activity	CO_2 + 15% ethanol, 30 MPa, 50 °C, 100 min (dynamic, CO_2 at 3 l min^{-1} and ethanol at 0.001 l min^{-1})
	ω-3 PUFAs Astaxanthin	Brazilian red-spotted shrimp waste (heads, shell, and tail)	Anti-inflammatory, reduce risk of certain heart diseases Antioxidant activity	CO_2, 30 MPa, 50 °C (static 20 min + dynamic 200 min, 1.5 l min^{-1})
	ω-3 PUFAs	Fish by-products (trout heads, spines, and viscera)	Anti-inflammatory, reduce risk of certain heart diseases	CO_2, 50 MPa, 60 °C (dynamic,0.6 kg h^{-1})
	ω-3 PUFAs	Northern shrimp by-products	Anti-inflammatory, reduce risk of certain heart diseases	CO_2, 35 MPa, 40 °C, 90 min (dynamic, 3–5 l min^{-1})
	ω-3 PUFAs	Shellfish by-products (abalone gonad)	Anti-inflammatory, reduce risk of certain heart diseases	CO_2, 28 MPa, 50 °C, 80 min (dynamic, 0.42 l min^{-1})
	Lipids Zeaxanthin	*Nannochloropsis oculata*	Anti-inflammatory, reduce risk of certain heart diseases Antioxidant activity	CO_2 + 16.7% ethanol, 35 MPa, 50 °C (dynamic, CO_2 at 0.01 l min^{-1})
	Lipids	*Schizochytrium limacinum*	Anti-inflammatory, reduce risk of certain heart diseases	CO_2 + 95% ethanol, 35 MPa, 40 °C, 120 min (static)
	Astaxanthin	*Haematococcus pluvialis*	Antioxidant activity	CO_2 + ethanol (2.3 ml g^{-1} sample), 43.5 MPa, 65 °C (static 60 min + dynamic 180 min, CO_2 at 10 l min^{-1} and ethanol at 0.03 l min^{-1})
	Astaxanthin	*Monoraphidium* sp. *GK12*	Antioxidant activity	CO_2 + ethanol (biomass/ethanol ratio, 1/20), 200 bar, 30 °C, 15 min (static)
	Lutein	*Chlorella vulgaris*	Antioxidant activity	CO_2 + ethanol, 40 MPa, 40 °C, 45 min (dynamic, CO_2 at 0.003 l min^{-1} and ethanol at 3×10^{-4} l min^{-1})
	Canthaxanthin and astaxanthin	*Chlorella vulgaris*	Antioxidant activity	CO_2, 30 MPa, 40 °C, 30 min (dynamic, 0.04 kg h^{-1})
	Astaxanthin, β-cryptoxanthin	Pink shrimp	Antioxidant activity	CO_2, 30 MPa, 60 °C, 25 min (dynamic, 0.798 kg h^{-1})
Phenolic compounds	Polyphenols and flavonoids	*Chlorella vulgaris C–C*	Antioxidant activity	CO_2 + 50% ethanol, 31 MPa, 50 °C, 20 min (static)
	Isoflavones	Sea and freshwater algae and cyanobacteria	Antioxidant activity	CO_2 + 3% methanol/H_2O (9:1 v/v), 35 MPa, 40 °C, 60 min (dynamic, 0.75–0.85 l min^{-1})
	Phlorotannins	*Sargassum muticum*	Antioxidant activity	CO_2 + 10% ethanol, 15.2 MPa, 60 °C, 90 min (static)

the pigments present in fully red ripe fruits. Although its SFE extraction has been mainly carried out from tomato by-product, it can be also extracted, for instance, from guava by-products. Several works have demonstrated an increase in the extraction yield of lycopene when a vegetable oil (roughly crushed hazelnut or tomato seed oil) is added as cosolvent.

As it can be observed in Table 3, the addition of a moderately polar modifier is critical for extracting polyphenols from agricultural and by-products. Usually, the extraction efficiency of SFE extraction of polyphenols is measured by the total phenolic content, although it can also be performed by measuring the levels of a specific compound, such as resveratrol.

Conclusions

In this work, the interest of SCFs to extract bioactive compounds from natural sources such as plants, marine, and food by-products is discussed. Although most of the applications presented have been performed at small scale, they are

Table 3 Details of some SFE works for the recovery of valuable compounds from agricultural and food by-products

Group of compounds	*Compounds of interest*	*Agricultural or food by-product*	*Related bioactivities*	*Extraction conditions*
Lipids	Fatty acids	Broccoli leaves	Prevention of different diseases	CO_2, 30 MPa, 60 °C (static 10 min + dynamic 90 min, 0.003 l min^{-1})
	Fatty acids	Sesame	Antioxidant activity	CO_2 + ethanol, 20 MPa, 35 °C, 210 min (dynamic, 0.15 kg h^{-1})
	Fatty acids, tocopherols	Pomegranate seed oil	Antioxidant activity	CO_2, 15–30 MPa, 35–65 °C, 120 min (static)
	Vitamin E and provitamin A	Piquillo red pepper by-products	Different protective effects	CO_2, 24 MPa, 60 °C, 120 min (dynamic, 0.03 l min^{-1})
	Lycopene	Red ripe tomato cultivars	Antioxidant activity	CO_2, 45 MPa, 65–70 °C, variable ratio tomato matrix/roughly crushed hazelnuts, 180 min (dynamic, 18–20 kg h^{-1})
	Lycopene	Guava by-products (decanter)	Antioxidant activity	CO_2 + 10% ethanol, 30 MPa, 55 °C (static 30 min + dynamic 150 min, 0.0025 l min^{-1})
	Lycopene	Tomato peel by-products	Antioxidant activity	CO_2, 40 MPa, 90 °C, 180 min, ratio tomato peel to seed of 37/63 (dynamic, 0.003 l min^{-1})
	Tocols	Rice germ	Different protective effects	CO_2, 13.8 MPa, 60 °C (dynamic, 5 l min^{-1})
	Fatty acids, α-tocopherol, phenolic compounds	Vinification residues	Different actions	CO_2, 25 MPa, 80 °C, 60 min (dynamic, 4.14 kg h^{-1})
	Essential oil	Rapeseed		CO_2, 30 MPa, 50 °C, 180 min (dynamic)
	Essential oil	Rhizomes of *Cyperus rotundus* Linn.		CO_2, 30 MPa, 35 °C, 120 min (dynamic,0.42 l min^{-1})
	β-Carotene, isomers of β-carotene, lutein, and lutein esters	Pumpkin	Antioxidant activity	CO_2 + 10% ethanol + 10% oil, 25 MPa, 50 °C, 60 min (dynamic, 0.015 l min^{-1})
Phenolic compounds	Phenolic compounds	Carob pulp kibbles	Antioxidant activity	CO_2 + 12.4% ethanol:water (80:20 v/v), 22 MPa, 40 °C (static 15 min + dynamic 450 min, CO_2 at 0.29 kg h^{-1})
	Phenolic compounds	Olive oil mill waste	Antioxidant activity	CO_2, 35 MPa, 40 °C, 60 min (dynamic 0.12 kg h^{-1})
	Polyphenols	Orange pomace	Antioxidant and antimicrobial activities	CO_2 + 8% ethanol, 25 MPa, 50 °C, 300 min (dynamic, 1.02 kg h^{-1})
	Proanthocyanidins	Grape seed	Antioxidant activity	CO_2 + 15–20% ethanol, 25–30 MPa, 30–50 °C, 60 min (dynamic 0.3 kg h^{-1})
	Resveratrol	Grape by-products (seeds, stems, skin, and pomace)	Antioxidant activity	CO_2 + 5% ethanol, 40 MPa, 35 °C, 180 min (dynamic, 0.048 kg h^{-1})
	Phenolic compounds	Brewery waste	Phenolic compounds	CO_2 + 6% ethanol (flow at 0.0002 l min^{-1}), 14 MPa, 40 °C, 30 min (dynamic, 0.18 kg h^{-1})
Alkaloids	Caffeine	Coffee husks		CO_2, 30 MPa, 100 °C, 300 min (dynamic, 197 kg CO_2 kg^{-1} husk)

important from the process development point of view and as a first step toward the possible establishment of the technology, for each particular application, at large scale. To do so, more realistic economic studies are needed to extend the degree of application of the SFE technology at industrial scale. We intend to demonstrate the possibilities of SCFs as an alternative technology to improve sustainability through the development and launch of initiatives dealing with the extraction and recovery of valuable compounds from non-costly sources. By doing so, new products intended for human health improvement will be developed ranging from new foods to new bioactive ingredients and pharmaceuticals mimicking the possibilities offered by nature.

See also: Antioxidants: Role on Health and Prevention; Cancer: Diet in Cancer Prevention; Carotenoids: Occurrence, Properties and Determination; Chromatography: Supercritical Fluid Chromatography; Essential Oils: Isolation, Production and Uses; Fatty Acids: Fatty Acids; Fish Oils: Production and Properties; Functional Foods; Spices and

Flavoring Crops: Uses and Health Effects; Phenolic Compounds: Bioavailability and Health Effects; Tocopherols: Properties and Determination.

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Sweeteners: Classification, Sensory and Health Effects

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Introduction

For most of human history, sweetness has provided a reliable orosensory cue to a food's energy content. Sweet-tasting foods are more calorically dense than less sweet foods, making sweetness a valid predictor of subsequent energy availability. The fondness of humans for sweet foods is inborn: studies have shown a preference for sweet-tasting nutrition in newborns. Therefore, mankind has always added sweet substances to their food. The first recorded sweetener was honey, which was used in the ancient cultures of Greece and China. Honey was later replaced by saccharose, common sugar, which was originally obtained from sugarcane. During the World Wars, sugar beets were the major source of saccharose. Saccharin was the first artificial sweetener, which was synthesized in 1879 by Remsen and Fahlberg in Baltimore, the United States. It was well accepted during World Wars I and II because of its low production costs and the shortcoming of regular sugar. As economies recovered and living standards increased after the wars, sugar became affordable. Artificial sweeteners are currently being used as sugar substitutes in considerable and increasing amounts in food and beverages, especially for those who are diabetic and/or obese. There are numerous known intense sweeteners that provide little or no energy, but only a few of them are approved for use in the food industry. They have also been used in other personal care and pharmaceutical products such as toothpastes. Although, from the beginning of their use, there has been a controversy over their risk as potential carcinogens, these sweetener compounds are generally considered to be safe for use in foodstuffs. Some of the low-calorie sweeteners currently approved by different international authorities as direct food additives include acesulfame, aspartame, cyclamate, saccharin, and sucralose. Over the past 30 years, the incidence of obesity has increased dramatically, which led to consumer awareness of a healthy lifestyle, maintaining normal body weight by consumption of a balanced diet and adequate physical activity. Sucrose, the most well-known carbohydrate, is present in numerous food products in which it affects their taste, texture, and consistency. However, as the main cause of obesity, as well as diabetes and dental cavities, sucrose is often replaced with alternative sweeteners. In order to find adequate replacement for refined white sugar, the food industry is intensively promoting its artificially sweetened products (frequently called 'diet' or 'light'), highlighting their benefits. Intense sweeteners are among the most controversial food additives due to concerns of adverse health effects. On the other hand, there are a wide range of natural, unrefined sweeteners that, aside from providing sweetness to the product, contain various bioactive compounds, such as vitamins, minerals, or polyphenols that are known to exhibit positive health effects and contribute to the concept of functional foods.

Classifications

Consumers have a wide range of choice in sweeteners. One distinguishing characteristic of sweeteners is the provision of energy. Sweeteners can be grouped in various ways. For the purpose of this article, sweeteners will be grouped as nutritive and nonnutritive. Nutritive sweeteners provide a sweet taste and a source of energy; nonnutritive sweeteners (NNS) are sweet without energy. Nutritive sweeteners contain carbohydrates and provide energy; they may be further classified into monosaccharides or disaccharides, which impart 4 kcal g^{-1}, or sugar alcohols (polyols), which provide an average of 2 kcal g^{-1}. Different terms are used to refer to nutritive sweeteners, including *sugars*, *sugar*, *caloric sweeteners*, and *added sugars*. Sugars occur naturally (intrinsic) in all fruits, vegetables, and dairy foods or are added (extrinsic) to foods during processing or in preparation for consumption by an individual. Sugars commonly found in foods include glucose, fructose, galactose, sucrose, maltose, corn-based sweeteners, and agave nectar. *Sugar* often refers to sucrose, which is derived from sugarcane or sugar beet. The US Department of Agriculture uses *added sugars* to refer to sugars and syrups added to food during processing and preparation or before consumption. In addition to imparting a sweet taste, sugars have the following functions that are important to the safety and quality of foods:

- Inhibit microbial growth by binding water in jams and jellies.
- Add texture, flavor, and color to baked goods.
- Support the growth of yeast for leavening or fermentation.
- Contribute volume in ice cream, baked goods, and jams.
- Enhance the creamy consistency of frozen desserts.
- Enhance the crystallization of confectionery products.
- Balance acidity in salad dressings, sauces, and condiments.
- Help to maintain the natural color, texture, and shape of preserved fruits.

Existing evidence does not support the claim that diets high in nutritive sweeteners *by themselves* have caused an increase in obesity rates or other chronic conditions (e.g., hyperlipidemia, diabetes, dental caries, and behavioral disorders). Nonetheless, consumers who want the taste of sweetness without added energy may select NNS to assist in the management of weight, diabetes, and other chronic diseases. NNS offer little to no energy when ingested. They are referred to as high-intensity sweeteners because, as sweetening ingredients, they are many times sweeter than sucrose. NNS can replace the sweetness of sugar or energy-containing sweeteners. However, they do not have the same functional properties such as browning, crystallization, and microbial inhibition. NNS also have the potential to assist in dental health and dietary quality. Scientists have responded to consumer demand by developing, researching, and producing a number of energy-reduced or NNS.

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The use of nutritive and NNS is evaluated by governing bodies throughout the world; these include the Food and Drug Administration (FDA) of the United States and the European Food Safety Authority (EFSA) of the European Union and expert scientific committees such as the Joint Expert Committee on Food Additives (JECFA) of the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO). In the United States, some sweeteners are considered generally recognized as safe (GRAS) ingredients, and others are food additives as defined by the 1958 Food Additives Amendment to the Federal Food, Drug, and Cosmetic Act. GRAS sweeteners have scientific consensus on their safety based on a history of use prior to 1958 or on well-known scientific information (21 CFR, parts 182 and 184). Some, but not all, GRAS substances are listed in 21 CFR 182 and 184. Manufacturers often determine that use of a substance is GRAS and sometimes will notify FDA of their conclusions. Because substances whose use is GRAS are not subject to FDA approval, manufacturers may market on the basis of their own determination, provided that such a determination is correct.

Nutritive Sweeteners

Sucrose and fructose, which are GRAS substances, are primary sugar sweeteners that occur naturally or are added to foods. In addition to their sensory qualities, nutritive sweeteners add functional properties to foods through their effects on the physical (e.g., crystallization and viscosity), microbial (e.g., preservation and fermentation), and chemical (e.g., caramelization and antioxidation) characteristics. Sucrose is a disaccharide composed of glucose and fructose that provides 4 kcal g^{-1} ($\sim$16 kcal per teaspoon). Commercially, sucrose comes by processing sugarcane or sugar beets. Refinement removes the yellow-brown pigments of unrefined sugar to produce the white crystal form of table sugar. Molasses is the least refined form of sucrose. The monosaccharide fructose also provides 4 kcal g^{-1}. Fructose is present in fruit (also known as fruit sugar or levulose) and is added to foods and beverages as high-fructose corn syrup (42–55% fructose) or in the crystalline form. Fructose has replaced sucrose in many foods and beverages because of its sweetening power, lower cost, and functional properties that enhance flavor, color, and product stability. Fructose also synergizes the sweetness potential of sucrose and certain NNS. Polyols or sugar alcohols have been used in food products for many years to decrease the intake of carbohydrates that raise blood glucose levels. Polyols can be used alone but are more often used in combination with other polyols or NNS because of the bulking property of some polyols. Many polyols are found in nature but may be manufactured from monosaccharides or polysaccharides to be used as food ingredients. Foods containing polyols and no added sugars can be labeled as sugar-free. Trehalose and D-tagatose are other sweeteners used in food. Trehalose is found naturally in foods such as honey and unprocessed mushrooms. The enzyme trehalase is present in the brush border membrane of the small intestine and hydrolyzes the α-1,1 bond of trehalose into two glucose molecules. D-Tagatose is similar to fructose in structure other than an inversion of the hydroxyl and hydrogen groups at the fourth carbon and is found in many foods, including dairy products. Thus, consumers should base their selection of nutritive sweeteners on sensory or functional properties and not on misconceptions of differences in nutrient value.

Nonnutritive Sweeteners

Up to nine in ten consumers in the United States buy or use low-calorie products, including sugar-free and reduced-fat foods and beverages. NNS have also seen increased use in European countries (due to the growing interest in health and an aging population) and in developing countries (with interest in making limited diets more palatable). High-intensity sweeteners can offer consumers a way to enjoy the taste of sweetness with little or no energy and/or glycemic response. NNS may assist in weight management, control of blood glucose, and prevention of dental caries. The trend is to blend high-intensity sweeteners with other nonnutritive and nutritive sweeteners to create new sweet taste profiles. Blending can cause sweetness synergy (i.e., the combination is sweeter than the individual components), which can decrease the amount of sweetener needed and can improve the overall sweet taste profile.

Since the discovery of saccharin in the late 1800s, NNS have been used by consumers to achieve a sweet taste, for reasons of economics, blood glucose control, or energy control. NNS approved for use in the United States have been tested and determined to be safe at levels that are within the acceptable daily intake (ADI). Food products may contain a blend of different NNS, which further complicates estimation of intake. Although consumption of NNS in foods and beverages has increased since 1965, only about 15% of the population reported consuming any foods or beverages with NNS in 2003–2004. A systematic review of NNS intake data indicated that average intakes by adults are consistently well below their ADIs.

The FDA has approved five NNS and regulates them as food additives: saccharin, aspartame, acesulfame K, sucralose, and most recently neotame. NNS, like other food ingredients, appear on the food label in the ingredient declaration. Other NNS include alitame, cyclamate, stevia, and thaumatin.

Acesulfame K

Acesulfame K has been developed as a sweetener by Hoechst. This high-intensity sweetener is a potassium salt of 6-methyl-123-axathiazine-4 (3H)-one 2,2-dioxide with molecular formula $C_4H_4KNO_4S$ and molecular weight of 201.24. It is a white crystalline powder and $\sim$120 times sweeter than sucrose and has high water solubility. Acesulfame K is heat-stable so it can be used in cooking and baking. It may have a bitter aftertaste when used alone to sweeten food or beverages. Ace K is often blended with other sweeteners (usually sucralose or aspartame) whereby each sweetener masks the other's aftertaste and exhibits a synergistic effect by which the blend is sweeter than its components. The European Commission's Scientific Committee on Food (SCF) reevaluated this sweetener and supported its safety but recommended an ADI from 0–9 mg kg^{-1} to 0–15 mg kg^{-1}

body weight per day. The amount of acesulfame K added to food products is very small because of its intense sweetening power and because it is often used in combination with other sweeteners. The estimated daily intake is estimated at 20% of the ADI because of its intense sweetening power. Estimated intakes in children are below the ADI (ranges from 3 to 9 mg kg^{-1} body weight per day).

Alitame

Alitame is composed of L-aspartic acid, D-alanine, and a novel C-terminal amide moiety and is 2000 times sweeter than sucrose without the bitter or metallic qualities of high-intensity sweeteners. The JECFA reviewed safety data on alitame in 2002. The committee concluded that there was no evidence that alitame is carcinogenic. An ADI of 0–1 mg kg^{-1} body weight was allocated on the basis of the no-observed-adverse-effect level of 100 mg kg^{-1} body weight per day to an 18-month study in dogs. Alitame has already been approved in Mexico, Colombia, and China and Australia and New Zealand.

Aspartame

Aspartame, a dipeptide (L-aspartyl-L-phenylalanine methyl ester), is 160–220 times sweeter than sucrose. Intestinal esterases hydrolyze aspartame to aspartic acid, methanol, and phenylalanine. These components are found in much greater amounts in the normal diet in fruits, vegetables, meat, and milk. For example, a serving of nonfat milk provides about 6 times more phenylalanine and 13 times more aspartic acid, whereas a serving of tomato juice has about 6 times more methanol than an equal volume beverage sweetened 100% with aspartame. In 1981, the FDA approved aspartame as a sweetener for a number of dry uses (e.g., tabletop sweetener, cold breakfast cereal, gelatins, and puddings) and in chewing gum. This approval was expanded in 1983 to include carbonated beverages. The Council on Scientific Affairs of the American Medical Association in 1985 concluded that "Available evidence suggests that consumption of aspartame by normal humans is safe and is not associated with serious adverse health effects." In EFSA's first ever full risk assessment of the food additive aspartame, the experts concluded that aspartame and its breakdown products are safe for human consumption at current levels of exposure. Aspartame does not have any carcinogenic effect, and there are no possible effects of aspartame on the development of the unborn organism (fetus). The sweetener is authorized in the European Union for use in drinks, desserts, sweets, dairy products, chewing gums, and energy-reduced and weight control products and as a tabletop sweetener. This assessment was carried out as part of the reevaluation of the safety of all previously authorized food additives in the EU. The first safety assessment of aspartame carried out in Europe was published by the SCF in 1984, and an ADI for aspartame of 40 mg kg^{-1} body weight per day was established.

Cyclamate

Cyclamate was discovered in 1937. It was used as a low-calorie sweetener in the United States in the 1950s and 1960s. It is a salt of cyclohexylsulfamic acid. Sodium cyclamate is used as an NNS, and the analogous calcium salt is used especially in low-sodium diets. Cyclamate is 30 times sweeter than sucrose. It has a bitter aftertaste but has good sweetness synergy with saccharin. It is soluble in water and its solubility can be increased by preparing the sodium or calcium salt. It is approved by the JECFA and SCF and is in use by more than 50 countries worldwide. The JECFA set an ADI for cyclamate at 11 mg kg^{-1} body weight per day. Cyclamates were banned by the FDA as a food ingredient in 1969 because the saccharin/cyclamate mixture was shown to cause cancer in experimental laboratory rats. The primary concern was that it could be toxic to some individuals who appear to metabolize cyclamate to cyclohexylamine.

Neotame

Neotame is a derivative of a dipeptide compound of the amino acids – aspartic acid and phenylalanine. Neotame has been developed as a sweetener with a high degree of sweetness and is obtained by N-alkylating aspartame. Its degree of sweetness varies according to the kind of food and blend composition. It is 7000–13 000 times and about 30–60 times sweeter than sugar and aspartame, respectively. Globally, neotame is approved for use in multiple countries in North America, South America, Europe, Africa, Asia, and Australia. In June 2003, the JECFA confirmed the safety of neotame and granted an ADI of 2 mg kg^{-1} body weight per day.

Saccharin

Saccharin was discovered by Remsen and Fahlberg in 1878 at the Johns Hopkins University, Baltimore. It is an NNS of 1,2-benzoisothiazol-3-(2H) on 1,1 dioxide. Saccharin has an unpleasant bitter or metallic off-taste. As the parent compound is only sparingly soluble in water, the sweetener is usually used as the sodium or calcium salt. Both salts are highly water-soluble, 0.67 g ml^{-1} of water at room temperature. It is about 300 times sweeter than sucrose. The FDA has approved saccharin (in the ammonium saccharin, calcium saccharin, and sodium saccharin forms) as a sweetener in beverages in amounts not to exceed 12 mg per fluid ounce, as a sugar substitute packaged in amounts not to exceed the sweetening power of 1 teaspoon of sugar (20 mg) for use in cooking or at the table, and in processed foods in amounts exceeding 30 mg per serving. The label must state saccharin in the ingredient declaration, the amount of saccharin listed per fluid ounce for beverages, milligrams in the dispensing unit for cooking or tabletop use, and milligrams per serving for processed goods. The FDA lists the ADI for saccharin at 5 mg kg^{-1}.

Stevia

Stevia (stevioside), derived from a South American shrub, imparts a sweet taste but cannot be marketed or sold as a sweetener in the United States. It is a natural herb. This zero-calorie sweetener mainly contains steviol glycoside, which is 10–15 times sweeter than sucrose. The human body does not metabolize these sweet glycosides and so obtains no calories from stevia. Unlike artificial sweeteners, the sweet glycoside does not break down in heat, which makes stevia an excellent sweetener for cooking and baking. Studies have indicated that stevia tends to lower elevated blood pressure. The ADI is

4 mg kg^{-1} body weight expressed as steviol equivalents based on GRAS notification. It also significantly improves the nutritional status of diabetic patients.

Sucralose

Sucralose was discovered in 1976. This NNS is made from sucrose by a process that substitutes three chloride atoms for three hydroxyl groups on the sucrose molecule. Sucralose is 450–650 times sweeter than sucrose and has a pleasant sweet taste, and its quality and time–intensity profile are very close to that of sucrose. It has a moderate synergy with other nutritive and NNS. Sucralose was approved in April 1998 by the FDA as a tabletop sweetener and for use in a number of desserts, confections, and nonalcoholic beverages. In 1999, sucralose was approved as a general purpose sweetener. The FDA concluded from a review of more than 110 studies in human beings and animals that this sweetener did not pose carcinogenic, reproductive, or neurologic risk. According to the EFSA, the ADI of sucralose is 40 mg kg^{-1} body weight per day.

Thaumatin

Thaumatin is a sweet-tasting basic protein extracted from the fruits of a tropical plant, *Thaumatococcus daniellii* Benth., found in rain forests of Western Africa from Sierra Leone to Zaire, Sudan, and Uganda. The first description of this plant was made in the mid-nineteenth century. It is a mixture of proteins with tight disulfide bonds, imparts an intensely sweet taste, and acts as a flavor enhancer. In the United States, thaumatin is GRAS as a flavor adjunct for a number of categories. Thaumatin at a recommended use range of 1–5 mg kg^{-1} complete feed is safe for all animal species with a considerable margin of safety. Consequently, thaumatin can be administered simultaneously via feed or water for drinking. Thaumatins are highly digestible proteins and no residues in edible tissues/products are expected. Consequently, there are no concerns for consumer safety.

Sugar Alcohols

Sugar alcohols are neither sugars nor alcohol; it includes lactitol, xylitol, maltitol, polydextrose, mannitol, isomalt, polyols, palatinit, sorbitol, polyol syrups, hydrogenated starch, and hydrolysates. They are used to sweeten foods labeled 'sugar-free' or 'no added sugar.' Small amounts of sugar alcohols are found naturally in fruits and vegetables. They can also be manufactured. They are only partly absorbed by your body, have fewer calories than sugar, and have no major effect on blood glucose. Consumption of more than 10 g of sugar alcohols a day can cause side effects such as gas, bloating, and diarrhea.

Sensory and Health Aspects of Sweeteners

The sense of taste mainly consists of saltiness, sourness, sweetness, bitterness, and umami, the so-called five basic tastes. These basic tastes indicate different biological signals. Saltiness, which is basically caused by salt, is a signal of the electrolyte balance in foods and beverages. Sourness and bitterness are good indicators of rottenness and poisons, respectively. Umami is a signal of proteins and certain amino acids. Sweetness is a signal of nutrient sources, such as sugars and sugar alcohols. In human taste sensing, each basic taste is perceived on taste buds, which are sensory organs on the tongue. Taste buds consist of about 50–150 taste receptor cells. The mechanisms of sweetness and umami taste perception were clarified by the discovery of sweetness receptors and umami receptors, which have broad selectivity to the corresponding tastes and are capable of detecting substances with various chemical structures in spite of only one type of heterodimeric receptor in each cell. In a biological taste system, the signal responses to sweeteners are initially coded in taste buds of the epithelium by action potentials. It is known that all sweeteners are mediated by heterodimeric G protein-coupled receptors in type II cells in the bud, which have a plurality of binding sites to correspondingly identify sweet stimuli of different structures. However, studies have suggested that there are two kinds of sweetness signal transduction models that are different but interdependent to conduct different types of sweet stimuli. One is the cyclic adenosine monophosphate pathway utilized by natural sugars such as sucrose to elicit membrane depolarization, and the other is the inositol triphosphate (IP3) and diacylglycerol pathway used by artificial sweeteners for signal transduction. Many researchers have pointed out that receptor activities toward the artificial sweeteners greatly depend on residues in the amino terminal domains of the sweet taste receptors as ligand binding sites. This means the signal responses of taste receptor cells to natural sugars and artificial sweeteners may be different and can be used to characterize different sweeteners.

The worldwide demand for high-potency sweeteners is expected to increase, especially with the new practice of

Table 1 Properties of some nutritive sweeteners

Sweetener	*Glycemic index*	*Type*	*Source*
Glucose	100	Monosaccharide	Hydrolyzed starch
Fructose	19–23	Monosaccharide	Enzymatically isomerized glucose
Lactose	46	Disaccharide	Milk sugar
Lactulose	0	Disaccharide	Alkaline isomerization of lactose when milk is heated
Maltose	105	Disaccharide	Enzymatic hydrolysis of starch
Mannitol	0	Sugar alcohol	Hydrogenation of invert sugar or fructose
Sorbitol	9	Sugar alcohol	Hydrogenated glucose
Sucrose	61–65	Disaccharide	Cane and beet
Tagatose	0	Galactose isomer	Hydrolyzed lactose, galactose converted by alkali
Xylitol	7–13	Sugar alcohol	Hydrogenated xylose

combining different sweeteners. The properties of some nutritive sweeteners are given in Table 1. Among naturally derived sweeteners, stevia and licorice root are likely to become prevalent sources of high-potency sweeteners for the growing natural food market in the future. Rebaudioside A derived from stevia is the least astringent and the least bitter, has the least persistent aftertaste, and was judged to have the most favorable sensory attributes of the four major steviol glycosides. Both stevioside and rebaudioside A are synergistic in mixtures with other high-potency sweeteners and are, like glycyrrhizin, good candidates for inclusion in various food products. Sweet substances are compounds with diverse chemical structures and sizes, for example, sugars (sucrose), sugar alcohols (xylitol), sulfonyl amides (saccharin), peptides (aspartame), D-amino acids (D-tryptophan), and proteins (thaumatin). Sweetness receptors are heterodimeric proteins that consist of two kinds of G protein-coupled receptors (T1R2 and T1R3). T1R2 + T1R3 heterodimeric receptors are activated by all sweeteners. Sweetener potency is defined as the number of times that a sweetener is sweeter than sucrose. The potency of a sweetener is compared with sucrose mainly in the threshold levels of the sweetener and sucrose. Sugars and sugar alcohols, such as sucrose and xylitol, are low-potency sweeteners, whose sweetener potencies are about 1 and under. On the other hand, sweeteners that have a sweetener potency exceeding 10 are called high-potency sweeteners, such as saccharin and aspartame. Interestingly, low-potency sweeteners, such as sucrose, exhibit higher sweetness intensity than high-potency sweeteners at very high concentrations. That is why low-potency sweeteners are also called high-intensity sweeteners. Some approved NNS are given in Table 2.

Sweetness is not the only relevant sensory characteristic when evaluating sucrose replacement by low-calorie sweeteners. Other sensory characteristics, such as bitterness, aftertaste (metallic, sour, etc.), texture attributes (viscosity and mouthfeel), and freshness, should also be taken into account. Bitterness and metallic off-flavors have been one of the most common problems of low-calorie sweeteners. The dynamic of these sensory characteristics throughout consumption, particularly onset and linger, is also relevant for the performance of these ingredients. Temporal dominance of sensations (TDS) is a new dynamic methodology that consists of presenting a list of attributes to the assessors, who are asked to continuously select the attribute that is perceived as dominant at each time of the evaluation. Considering that TDS is focused on dominant attributes, instead of quantifying attribute intensity, results from this methodology could better explain consumer's perception and more accurately identify the sensations that determine their hedonic perceptions.

In sensory evaluations of foods, beverages, or pharmaceutical products, well-trained sensory panelists actually taste samples to evaluate them; therefore, sensory tests have some problems such as low objectivity and reproducibility and the stress possibly imposed on panelists. In addition, it is very costly to select and train panelists. In particular, in the medical and pharmaceutical industries, it is difficult to perform sensory tests because of the potential for side effects. Though quantitative analysis is conducted, it cannot estimate the strength of each basic taste. Thus, taste evaluation methods without using human sensory systems have attracted attention. The development of taste evaluation methods should contribute greatly to the palatability of drug products and the quality of foods and beverages. To solve the problem, electronic tongues (e-tongues) have been introduced and used successfully to evaluate the taste. e-Tongues primarily use metal and ion-selective electrodes in a potentiometric multisensor system; therefore, principal component analysis and partial least squares are generally used to analyze taste information obtained by sensor outputs with low selectivity.

Over the years, the effects of nutritive and NNS use on human health have been a concern among health professionals and the public for a variety of reasons. One area involves the safety of sweeteners for use by children, when sweetener intakes are high relative to body weight, and pregnant women, when the goal of the diet is to support maternal and fetal health. The use of nutritive sweeteners is acceptable during pregnancy. Recommendations for NNS use during pregnancy must be based on well-designed and approved clinical investigations to ensure healthy pregnancy outcomes. Women who consumed one or more NNS-sweetened soft drinks per day were significantly more likely to deliver preterm. The association was stronger for carbonated than for noncarbonated drinks. At the time of the study, aspartame and acesulfame K were used most often for carbonated drinks, and saccharin and cyclamates were more often used for noncarbonated drinks. From the survey, it was concluded that daily intake of beverages containing NNS may be associated with an increased risk of preterm delivery. It is important to point out that the incidence of preterm birth was low, and the increased risk was primarily as a result of

Table 2 Some approved nonnutritive sweeteners

Type	*Regulatory status*	*Description*
Acesulfame K	Approved as a general purpose sweetener	200 times sweeter than sucrose, noncariogenic, and produces no glycemic response
Aspartame	Approved as a general purpose sweetener	160–220 times sweeter than sucrose, noncariogenic, and produces limited glycemic response
Neotame	Approved as a general purpose sweetener	8000 times sweeter than sucrose, noncariogenic, and produces no glycemic response; sweetening power is not reduced during heating
Saccharin	Approved as sweetener for beverages and as a tabletop sweetener in food with specific maximum amount allowed	200–700 times sweeter than sucrose, noncariogenic, and produces no glycemic response
Sucralose	Approved as a general purpose sweetener	600 times sweeter than sucrose, noncariogenic, and produces no glycemic response

medically induced preterm birth. This finding has not been confirmed in other studies.

Dental caries are the localized destruction of dental hard tissue by acidic material from bacterial fermentation of dietary carbohydrates. Factors that influence the development of dental caries include microbiological shifts in the biofilm, salivary flow, buffering capacity of saliva, frequency and kind of dietary sugars consumed, length of time the oral bacteria have to ferment the fermentable carbohydrate and make organic acids, tooth susceptibility, and preventive behaviors such as cleaning of teeth. A child who consumes more than three between the meals nutritive sweetener-containing snacks or beverages per day is considered at increased risk for dental caries. Xylitol is considered cariostatic and anticariogenic and aids in the prevention of dental caries. It is very soluble in water and sparingly soluble in ethanol. Human tolerance studies indicate that consumption exceeding 50 g per day leads to diarrhea. Excess body fat arises from the energy imbalance caused by taking in too much energy and expending too little energy. Recent concerns have been expressed regarding high intakes of sweetened foods and beverages and the possible association with the increasing prevalence of overweight and obesity across the population, including children. Of particular interest is consumption of high-sugar, low-nutrient dense foods, specifically sweetened sodas and drinks. There is speculation that high intakes of fructose (particularly in the form of sweetened liquids) increase energy intake and obesity risk through the blunting of circulating insulin and leptin levels. Obesity is another complex problem, and its cause cannot simply be attributed to any one component of the food supply such as sweeteners. Troiano and colleagues found higher intakes of energy from sweetened soft drinks among overweight than nonoverweight youths, yet they suggest that physical inactivity may be more significant to the secular increase in weight within this population. Nutritive sweeteners containing fructose and sucrose are of primary interest related to hyperlipidemia. Diets high in these sweeteners have been shown to increase serum triacylglycerol and low-density lipoprotein cholesterol levels in short-term studies, particularly if the diet is low in fat, with fructose being more hyperlipidemic than sucrose. It should be emphasized that not all studies show a positive association. LDL concentrations have been shown to rise with increases in sugar intake. The effects on high-density lipoprotein levels are inversely related to sugar intake. As part of the FDA approval process, toxicology testing can examine the impact of NNS on behavior. The approved NNS did not show significant effects on behavior, especially when consumed within the ADIs. Attention has been paid to the association between aspartame and a range of central nervous system and behavioral conditions including headaches, seizures, cognitive impairment, and mood disorders; a recent critical review of the scientific literature refutes all such associations.

Conclusion

Nutritive and NNS add to the pleasure of eating. Consumers can enjoy a wide range of sweeteners in a wide variety of foods and beverages. Consumers can incorporate nutritive sweeteners into a healthful eating plan and meet current guidelines for healthful diets. The range of nutritive and NNS allows choice in the type and amount of sweeteners to include in the diet. NNS are safe for use within the approved regulations. They can increase the palatability of fruits, vegetables, and whole grain breads/cereals and thus have the potential to increase the nutrient density of the diet while promoting lower energy intakes. The trend in sweetener blending will maximize the sweetening potential and support intakes of NNS well within the acceptable levels.

See also: Beverage: Health Effects; Fructose: Sources, Metabolism, and Health; Pregnancy: Metabolic Adaptations and Nutritional Requirements; Saccharin – How Sweet It Is; Sucrose: Dietary Importance.

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Taints: Analysis and Identification

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Introduction

Taints and Off-Flavors

The presence of unacceptable flavors in food products is the utmost motive for its rejection. In this regard, every year the food industry receives a lot of complaints from consumers concerning taints or off-flavors in fresh, processed, and packaged foods. Basically, a taint is defined as an unpleasant odor or flavor conferred to food through external sources (e.g., packaging or storage). An off-flavor is a typical odor or taste resulting from a compound formed by internal food deterioration, such as microbiological spoilage or lipid oxidation. Even though they have different origins, both are frequently caused by trace amounts of volatile organic compounds not usually present in that food, giving to the consumer the impression of poor food quality. Occurrence of taint and off-flavor affects the quality, causing financial loss to food industries and thus damaging their reputation because the product could be even rejected by the consumer.

The description of a taint by the consumer is in general notoriously variable, partly for an absence of any training in analytical descriptive methods but mainly for unfamiliarity with chemical species responsible for taint. The most frequently unacceptable taints and off-flavors detected by the consumer are described as disinfectant, moldy, musty, earthy, fecal, metallic, fishy, painty, and plastic-like between others. The occurrence of these unpleasant aromas in products of individual industries is highly variable and is related to low concentration levels of different analytes. However, the monitoring of compounds is important as an integral component of the product quality control previously done to its introduction to the market.

Taints and off-flavors can originate from several sources, including microbiological degradation, migration from packaging, contaminated process water, or an unsuitable storage environment of ingredients or finished products. The origins of some chemicals responsible for taints and off-flavors and the detail of several specific events during their formation have been described and are out of the scope of this article. A compilation of compounds causing typical organoleptic defects with their taint descriptors, odor thresholds, and the possible origin is presented in Table 1. In general, the perception threshold for the majority of compounds is at the level of low nanogram per liter emphasizing the necessity of reliable analytical methods for its analysis and identification.

Cork taint: a particular case

An organoleptic defect that has captured the attention in recent years and is one of the most well-known food taints refers to the cork taint. It is considered as a key organoleptic defect in wine, producing a moldy and musty aroma. The main compounds responsible for this defective aroma are haloanisoles, although there are other analytes causing the same fault. Haloanisoles are also known to cause off-flavor in food and beverages, but they are specially associated with defective wines.

The manufacturing of cork stoppers for wine bottles involves several stages including cork ripening. In this step, the cork can suffer deterioration because it is exposed to the environment that is susceptible to the proliferation of fungi and bacteria. To overcome this problem, wood preservatives are added to the cork in order to minimize its trouble. The most efficient wood preservatives are based on halophenols, particularly chlorophenols. However, these compounds are biomethylated by fungi present in the cork, yielding their respective haloanisoles that are mainly responsible for cork taint in wines. Furthermore, the chlorination of lignin degradation products in the presence of free chlorine or anionic species containing chlorine also produces different chlorophenols, suffering the same microbiological degradation. Besides, wood pollution in winemaking procedures such as washing of oak barrels (hypochlorite bleaching) or transportation, storage, and handling of wine or packing materials could also increase the cork taint concentration in the final product.

The incidence of cork taint in wines of individual wineries is highly variable. However, the monitoring of haloanisoles in wines is important as an integral component of the strategies of product quality control prior to the introduction of the wine to the market.

Analytical Methods

Compounds causing taint in foods are commonly present at trace levels (ng l^{-1} or ng g^{-1}). Nevertheless, the appearance of these defects raises the question if there is any risk to human health based on risk assessment studies. Generally, a food with taint or off-flavor does not represent a safety risk for the consumer, although the perception of low quality, brand damage, and adverse publicity can be exceptionally costly to the food industry. Consequently, the selection of the most suitable

Table 1 Compilation of some compounds responsible for taints and off-flavors, odor thresholds, and taint description

Compound	*Taint aroma*	*Odor thresholds (ng l^{-1})*
2,4,6-Trichloroanisole	Mold	0.03–50
2,4,6-Tribromoanisole	Mold	3.4–7.9
2,3,4,6-Tetrachloroanisole	Mold–dust	5–15
Pentachloroanisole	Dust	10 000
2,4,6-Trichlorophenol	Phenolic	n.a.
Pentachlorophenol	Phenolic	n.a.
2,4-Dichloroanisole	Musty, sweet, fruity, scented	400
2,6-Dichloroanisole	Musty, medicinal, phenolic	40
2-Methoxy-3,5-dimethylpyrazine	Fungal must	2
Geosmin	Muddy–earthy	80–90
2-Methylisoborneol	Muddy–earthy	30
1-Octen-3-one	Fungus	70
1-Octen-3-ol	Fungus	40 000
Guaiacol	Smoky	50 000
4-Cresol	Phenolic, horse manure	200 000
2-Bromophenol	Disinfectant, phenolic	100
2-Chlorophenol	Disinfectant, medicinal	3000
2,6-Dichlorophenol	Phenolic	3000
Hexanal	Rancid	0.19–30[a]
Indole	Fecal	300[a]
Methyl methacrylate	Plastic	200[a]
Cis-oct-2-enal	Sour, rancid	3000
Skatole	Fecal, animal, nauseating	10 000
2-Pentylfuran	Beany, rancid–greasy	6000

[a]Concentrations expressed as ng g^{-1}.

methodology for the identification and quantification of taints and its occurrence in foods is crucial.

The analysis and identification of compounds causing taints and off-flavors are similar to those of volatile compounds contributing positively to the flavor of food. Due to the low concentration levels of analytes and considering the food samples, complexity, the determination of these compounds represents a challenge for the analytical chemists being necessary the application of sample preparation tools prior to the instrumental analysis. Also, highly selective and sensitive analytical techniques are required for the unequivocal identification and determination of taint compounds. In this sense, considering that most of compounds causing taints in food are volatile, gas chromatography (GC)-based techniques have been the choice for its analysis. Mass spectrometry (MS) is the selected analytical technique for taint investigations when identification and quantification of trace amounts of unknown contaminants are required. Currently, modern MS detectors can achieve suitable identification of nontargeted analytes, increasing the spectrum of identified compounds and the applicability of methods. Besides, other detectors including electron capture (ECD), atomic emission (AED), and olfactometry (O) were reported for the analysis of the compounds of interest. During recent years, the instrumental techniques used for the identification of food volatiles have been efficiently shared with sensory methods to determine the relative odor influence of unknown components in food. The sensory descriptor of a taint can often be the key point to performing targeted chemical analysis. In this way, sensory panels trained in give objective assessments and descriptions of taints can provide an insight when a consumer has complained that the foodstuff tastes spoiled.

Since the concentration of taints is usually low, it is necessary to count on highly efficient extraction/preconcentration techniques for its evaluation. Thus, with the developing interest in miniaturization in analytical chemistry for solvents and sample savings, innovative miniaturized techniques have been also reported and successfully applied for odor analysis.

Techniques for Chemical Analysis

Sample Preparation Strategies: Isolation and Preconcentration

Sample preparation is a critical step in the identification and quantification of analytes. The majority of separation and detection techniques require different extraction and cleanup procedures to minimize matrix effects, mainly when complex food samples are analyzed. The techniques used for sample preparation of volatile compounds, particularly taints and off-flavors, have been changing during the last 20 years. Liquid–liquid extraction (LLE) has been progressively replaced, and efforts were placed on the miniaturization of LLE by significantly reducing the required organic solvent amount, increasing the sustainability of the analytical process. The proposed techniques include simultaneous steam distillation solvent extraction, static headspace, dynamic headspace, solid-phase microextraction (SPME), headspace sorptive extraction (HSSE), and stir bar sorptive extraction (SBSE). The most recently developed techniques include liquid-phase microextraction approaches such as dispersive liquid–liquid microextraction, ultrasound-assisted emulsification microextraction, and single-drop microextraction, as well as the novel QuEChERS (quick, easy, cheap, effective, rugged, and safe) method.

Liquid–liquid extraction

Some methods have been reported for taints and off-flavors in foods that use direct solvent extraction. High concentrations of androstenone and/or skatole in pig carcasses are associated with unpleasant odors in meat, the so-called boar taint. Most of the described methods to quantify boar taint compounds employ LLE to extract the compounds from adipose tissue. The muscle is heated prior to extraction, and liquid fat is mixed with the organic solvent. The extracts are often frozen and centrifuged or filtered to remove insoluble material. Different organic solvents, such as methanol, ethanol, hexane, ethyl acetate, or petroleum ether, and solvent mixtures have been used to extract androstenone and/or indolic compounds from these matrices with satisfactory recoveries (98.9% and 93.8% recovery of skatole and indole).

Due to the differences in polarity of steroids and indole compounds, it is a difficult challenge to select an extraction

solvent that can be used to simultaneously extract both groups with high extraction yields. Methanol has shown to be suitable for parallel extraction of androstenone and indole compounds, achieving recoveries closer to 100%. However, most laboratories perform extraction of steroids and indole compounds separately.

Solvent extraction methods have the drawback that the complexity of obtained extracts makes necessary the application of some additional sample preparation step such as preconcentration and purification in order to isolate compounds of interest previous to instrumental analysis. The preconcentration step simplifies the collection of chromatograms that allow a correct identification and quantification of analytes. The purification allows the removal of potential interfering compounds present in complex matrices being a crucial step of any method. Solid-phase extraction (SPE) is the most commonly used technique for the cleanup of food samples during taint and off-flavor analysis. Different authors have employed C_{18} or HLB sorbents for cleaning up samples and for preconcentrating compounds such as haloanisoles and halophenols with satisfactory results. The advantage of SPE is that for liquid samples such as wine, high extraction efficiencies could be achieved by using small sample and elution solvent volumes.

Indole compounds can be retained by both polar and nonpolar extraction mechanisms. So, a strongly polar extraction column, like Bond Elut 20H Diol, has been used, whereas the hydrophobic stationary C_{18} phases show good efficiency to remove lipids and fatty acids in meat samples.

Supercritical fluid extraction

An alternative to traditionally used LLE is supercritical fluid extraction (SFE) that has been applied for the extraction of boar taint compounds in porcine samples. SFE employs the unique properties of supercritical fluids to facilitate the extraction of organic analytes from solid samples. A supercritical fluid is a substance above its critical temperature and pressure that has a good solvating power (like liquid), high diffusivity, low viscosity, and marginal surface tension. Due to these characteristics, SFE permits a rapid mass transfer in the supercritical phase and an improved ability to penetrate the pores in the sample matrix, achieving a fast and efficient extraction. SFE may be an environmentally sustainable alternative to the conventional organic solvent extraction because it avoids the use of large amounts of toxic solvents, being also rapid, automatable, and selective.

SFE using supercritical carbon dioxide has been used to isolate androstenone from fat, achieving extraction efficiencies as high as 97%, although the efficiency obtained for skatole was 65%. This fact could be related to the polarity difference between both compounds. In order to improve the extraction of a wide spectrum of analytes, the addition of a cosolvent could be a suitable alternative for future developments.

Solid-phase microextraction

The most common sample preparation technique reported for extraction/preconcentration of taint and off-flavor compounds is SPME. It has the advantage, when compared with other techniques, that it is solvent-free and can be easily automated.

The application of SPME in the field of food taints and off-flavors is mostly explored for compounds that are important for the food industry and frequently befall those that cause musty–earthy off-flavors (haloanisoles, halophenols, geosmin (GEO), and methylisoborneol (MIB)), those that cause medicinal off-flavors (phenolic compounds and sulfur-containing compounds), and carbonyl compounds that produce food spoilage due to oxidative changes in lipids.

There are various SPME approaches combined with different detectors for the determination of the compounds of interest. The HS-SPME is the most extensively used extraction design for these compounds. Direct extraction of halophenols from water provides higher peak areas than HS extraction. On the other hand, in the case of wine, HS-SPME yields an approximate fourfold increase in peak areas compared with direct immersion (DI). Additionally to the reduced sensitivity reported by using the DI mode of SPME fibers applied into wines, increased contamination of the injector system and shorten lifetime of the SPME fibers and analytical GC column are observed. When the equilibrium is reached, the amount of analyte extracted on the fiber should be the same, either the fiber is immersed in liquid matrix or extraction is performed from the HS. Consequently, differences in the quantity of extracted analytes in DI and HS extraction can be a result of the different phenomena to reach equilibrium for both extraction methods. During HS, the analytes have to diffuse from liquid to gaseous phase and then from gaseous phase to the fiber coating. In the case of DI, they have to transport from liquid phase to the fiber coating. In addition, the DI extracts a lot of matrix coextractives that could affect selectivity and sensitivity of the methodology; and also, the fiber has a reduced lifetime.

The extraction temperature is also an important factor during SPME method development because it affects the extraction efficiency. An increase of temperature causes an augment of extraction yield because of the enhancement of mass transfer; however, high temperature decreases the efficiency of extraction due to a decrease of the distribution constant. Furthermore, the application of stirring could increase the extraction efficiency for haloanisoles. Stirring accelerates the transfer from liquid matrix to HS, and the equilibrium is achieved much faster in the stirred system than in nonstirred ones.

In view that the fiber coating is the 'heart' of the extraction, it is very important to select the right fibers for the desired application. The nonpolar polydimethylsiloxane (PDMS) and the combined adsorbent capacity of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) were the most used coating for cork taint compound extraction from wine samples. Additionally, polyacrylate fibers were also used with good performance. The DVB/CAR/PDMS fibers were more utilized in the analysis of a wide range of cork taint compounds, including haloanisoles, halophenols, MDMP, MIB, 1-octen-3-one, 1-octen-3-ol, geosmin, and guaiacol; meanwhile, PDMS was more used for the analysis of haloanisoles. Based on the reported methods, DVB/CAR/PDMS-based SPME techniques showed an increased extraction efficiency (between 50% and 100%) related to PDMS. However, some authors reported low reproducibility of DVB/CAR/PDMS fibers, justifying the selection of PDMS as coating.

The lipophilic boar taint compounds interact strongly with the interfering fat matrix, and thus, the sample preparation

must overcome such fat-associated matrix effects. HS-SPME using PDMS/DVB fiber and an extraction temperature of 100 °C after cross validation revealed comparable results to previously reported methods using the same separation and detection technique.

Taking this into account, the development of a successful SPME method needs a reproducible agitation, suitable control of sample temperature and headspace volume, and the extraction time. The variability of ionic strength and levels of polymeric materials (e.g., polysaccharides and tannins) in wines significantly affect the headspace concentration of volatiles. Thus, the quantitation by external calibration method is generally not suitable, and the use of a labeled internal standard (IS) or the method of standard additions may be required for an accurate quantitation of analytes.

The most accurate, precise, and robust method to determine the levels of important compounds in problematic matrixes and eliminate matrix effects is the application of stable isotope dilution analysis (SIDA). In any analysis, the applied IS should be as similar as possible to the measured analyte, without being present originally in the matrix. The isotopically labeled ISs are ideal because they have almost identical physicochemical properties to their unlabeled analogs and nevertheless can be easily discriminated from the analyte of interest by MS. In this sense, the accuracy of analysis is not reduced by inefficiency in isolation or by analyte decomposition because the labeled IS is virtually chemically identical to the substrate being assayed. This fact avoids the necessity of an exhaustive extraction of the analyte of interest from the matrix, improving the applicability for equilibrium extraction techniques such as SPME or SBSE, extensively used for taint and off-flavor analysis. Another advantage is that methods can be simplified, being possible the determination of several analytes (even though compounds with different chemical properties) in a single extraction and GC–MS run.

Hence, SIDA is the method of choice, especially when the quantitation of low concentrations of odorants is desired. In this sense, it has been reported for quantitation of boar and cork taint compounds using SPME-GC–MS.

Stir bar sorptive extraction

During SBSE, analytes are extracted from a liquid phase of an aqueous matrix into a magnetic stir bar covered with a polymeric phase. As for SPME, HSSE can also be used for specific analytes. In contrast to extraction with adsorbents, whereby the analytes interact with active sites on a surface, in SBSE, the solutes migrate into the sorbent phase. In comparison with SPME, SBSE has much higher amount of sorbent available resulting in higher sample capacity. Also, SBSE leads to more rapid methods with a sensitivity improvement, being able the determination of taint compounds with extremely low sensory thresholds (ng l^{-1}), even in the presence of large matrix components. However, taking into account the nonselective sorption capability of sorbents, a coconcentration of undesirable matrix components from the sample could be possible. In this sense, SBSE could produce analyte preconcentration in the sorbent, but, sometimes, the achieved cleanup is not satisfactory for complex samples.

The SBSE technique has been applied for the extraction of taint compounds in different samples, principally cork and wines achieving similar or better analytical performance than SPME.

As SPME, SBSE is not an exhaustive extraction technique, and likewise for all equilibrium-based techniques, it is necessary the utilization of ISs or standard additions for quantitation.

Comparison of taint extraction/preconcentration techniques

Taking into account the principles and reported applications for taint analysis using the mentioned techniques, some comparative comments can be given. For the analysis of volatile compounds, the most efficient and convenient approaches refer to SPME and SBSE. These approaches have the advantages of proved extraction of a wide scope of taint and off-flavor compounds, reduced sample extraction time, and excellent analytical figures of merit. Also, the use of organic solvents is practically avoided being also sustainable alternatives to LLE methods. An indisputable advantage of SPME is its possibility of complete automation of analytical process, increasing the reproducibility and accuracy of the obtained results. It also allows the reduction of the analysis time during routine analysis and method development, which increases the sample throughput of the technique, a requirement for methods to be applied in the industry.

Recently, numerous LLE miniaturizations have been reported particularly for the analysis of cork taint compounds. These methods appear as suitable alternatives, especially the methods determining simultaneously haloanisole and halophenol families, by coupling novel one-step extraction and in situ derivatization strategies. In general, microextraction techniques are faster than SPME; however, the last has the advantage of allowing the full automation of methodology. In addition, SPME has found a wide application for taint analysis being very simple and the only real solventless sample preparation technique reported for these compounds.

Derivatization

Due to the polar nature of some taint compounds such as halophenols, some chemical modification of the analytes is necessary prior to instrumental analysis in order to enhance its detection. Derivatization is commonly used for the determination of chlorophenols due to the poor GC response and peak tailing generally obtained for these compounds. Also, the derivatization step allows the simultaneous determination of different groups of compounds (i.e., haloanisoles and halophenols), improving the applicability of the technique. There are different strategies to achieve satisfactory derivatization of polar analytes. Some authors proposed the in situ derivatization reaction, providing a one-step derivatization and extraction/preconcentration technique, particularly for polar chlorophenols that simplify the operability and shorten the analysis time. These approaches were successfully applied in microextraction techniques using acetic anhydride as derivatization reagent. In SPME techniques, on-fiber derivatization of halophenols using *N*-methyl-*N*-trimethylsilyltrifluoroacetamide after a preconcentration on a SPME fiber has been proposed.

Instrumental Techniques

Taking into account that the majority of taint and off-flavor compounds are volatile or semivolatile, the selected analytical instrumentation for its analysis is GC-based techniques. However, there are other compounds such as those causing boar taint (skatole, indole, and androstenone) where the utilized technique is high-performance liquid chromatography.

GC is coupled with different detectors including MS, MS/MS, high-resolution (HR) MS, ECD, AED, and O. GC–MS has the advantage of achieving a practically unequivocal identification, particularly when MS/MS or HRMS is used. In the specific case of cork taint compounds in wine samples, some authors reported difficulties to produce reliable data in certain trace levels (low ng L^{-1}) due to severe coelution problems of haloanisoles and halophenols. Thus, the selection of MS/MS methods unique for each analyte combined with SIDA quantitation strategies permitted to avoid ambiguous identification of compounds. Besides, MS/MS methods could evade some matrix interferences remaining after sample preparation step, increasing the selectivity and sensitivity of the analytical methodology. In fact, the reported MS/MS methods for cork taint compounds showed lower Limits of detection (LODs) than MS methods. GC-HRMS was reported for the determination of 2,4,6-TCA and 2,4,6-TBA with higher selectivity. This technique showed similar sensitivity than MS/MS methods, giving the exact mass of analytes but having the disadvantage of a high purchase and maintenance cost.

The use of multidimensional GC (MDGC) coupled with ECD was recently reported, allowing the reliable quantification of different groups of cork taint compounds at sub-ng L^{-1} level avoiding coelution problems and without necessity of extensive sample preparation previous to instrumental analysis. This strategy gives benefits from the additional separation achieved using MDGC and using SIDA deprived of the utilization of MS detectors. In this way, some authors explained the selection of ECD because of its relatively low purchase and maintenance costs, combined with its relatively good sensitivity. Nevertheless, ECD is only suitable for halogenated taint compounds being the principal disadvantage compared to the most used GC–MS detectors. In this sense, the variety of detected compounds is reduced, and the determination of other minor or less common analytes and the nontargeted analysis are not possible. So, future developments using MDGC combined with MS detectors appear as appropriate options to reach the detection of a wide spectrum of analytes.

A valuable analytical instrument for the detection and identification of new aroma compounds in wine or other foods is GC-O detection. It is based on coupling to GC a human nose as a detector for sensory evaluation of the eluate from the chromatographic column with the aim to discerning the active odor compounds present in a particular sample. This allows establishing whether a particular compound is sensory active at a given concentration (i.e., whether it appears in the sample at a higher level than the sensory detection threshold). The presence of a special attachment, named olfactometric port, connected in parallel to conventional detectors such as MS, due possible the determination of the analyte's odor. GC-O can be useful in correlating odors to compounds and can be used as an initial screening of volatile compounds or to confirm the presence of a specific taint compound. The flow of the eluate is split in such a way that the analytes reach both detectors at the same time, so both signals could be compared. The combination of the O detector with MS is particularly useful, allowing not only the evaluation of the odor compounds but also its identification with mass spectral information. Taking into account that aroma thresholds and descriptors vary from person to person, each sample should ideally be run more than once and evaluated by more than one sniffer to avoid nonobjective results. GC-O could be a useful tool to establish the importance of each aroma component. However, one of the main limitations of GC-O is that some compounds that are detected at the heated sniffing cup of the O detector are not normally detected by the human nose. In GC-O detection, many compounds that are not essentially volatile in the matrices in which they occur are normally vaporized in the injector of the GC. In this sense, this detection strategy can overestimate the significance of an aroma compound and change the perceived aroma character in a real-life situation. The use of reference standards with GC-O is important for both matching retention times and odor characteristics; quantification can be performed subsequently using instrumental techniques such as GC–MS.

Other separation/detection techniques have been applied for taint and off-flavor identification. Ion mobility spectrometry (IMS) is a technique that characterizes molecules by their gas-phase mobility, offering high sensitivity, instrumental simplicity, low cost, analytical flexibility, and real-time monitoring capability. Due to these advantages, IMS is a suitable possibility for the detection of volatile compounds. However, it experiences a limited selectivity, which has been overcome by the separation of the analytes using gas capillary chromatographic columns or multicapillary columns (MCCs). This approach has been proposed for the determination of 2,4,6-TCA, and the use of MCC allowed an increase of selectivity of IMS detection.

Other promising detection techniques are the immunoanalytical technologies, which involve a specific antibody raised against the analyte. These analytical tests are based on the specific interaction between the antibody and the antigen. Because of their high sensitivity and selectivity, immunoassays have been successfully used for both qualitative analysis and quantitative analysis of several compounds at trace levels. The disadvantages of these techniques are the low sensitivity and selectivity and the unfeasibility of determination of more than one analyte at the same time. This new generation of analytical tools appears as suitable alternatives for future developments in this matter.

Conclusions and Future Trends

The avoidance of taints and off-flavors is crucial to ensure food quality and will be achieved by controlling processes, packaging, and storage conditions. The application of different analytical methodologies for the analysis and identification of taint compounds varies between laboratories and the needs of specific quality control of each industry. The selected analytical method depends on many factors such as

instrument accessibility and analyst experience. Likewise, the sensitive and selective detectors required for taints, sample preparation as extraction, and preconcentration play a critical role in the development of methods for taint and off-flavor analysis. SPME poses substantial advantages, which prompts this technique for this type of analysis. It is sensitive and selective being useful for screening and targeted analysis of compounds. Besides, the possibility of total automation of analytical process, promises successful developments for the future. The use of conventional techniques such as those based on LLE is time-consuming and requires high volumes of organic solvents and solvent concentration steps. These facts emphasize the utilization of sustainable alternatives for this type of analysis.

Although these are well-established techniques that can be effectively applied for the analysis of taint and off-flavor compounds at concentrations lower as their human detection threshold, there are only a small number in which two or more families of analytes have been determined at the same time. Consequently, the challenge for analytical chemists will be the developments of consistent methods that can thoroughly cover the variety of taint compounds present in foods, achieving their simultaneous determination through more sustainable sample preparation approaches.

See also: Chromatography: Focus on Multidimensional GC; Chromatography: Combined Chromatography and Mass Spectrometry; Mass Spectrometry: Principles and Instrumentation; Mass Spectrometry: Applications; Quality Control in Food Processing; Wines: Wine Tasting.

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Tannins

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Introduction

Phenolic compounds or polyphenols are the most abundant and ubiquitous secondary metabolites present in the plant kingdom with more than 8000 phenolic structures currently known with highly diverse structures and molecular masses ranging from <100 Da for simple phenolic compounds to >30 000 Da for highly polymerized structures. They possess a common structure comprising an aromatic benzene ring with one or more hydroxyl substituents. They represent a large and diverse group of molecules including two main families: the flavonoids based on common C_6–C_3–C_6 skeleton and the non-flavonoids. Phenolic compounds play an important role in plant growth and reproduction, providing protection against biotic and abiotic stress such as pathogen and insect attack, UV radiation, and wounding. Polyphenols are widely distributed in the human diet mainly in plant-derived food and beverages (fruits, vegetables, nuts, seeds, herbs, spices, tea, and red wine) and can influence multiple sensorial properties such as flavor and color and contribute to the aroma and taste, for example, astringency and bitterness.

In this article, phenolic compounds, particularly tannins and more particularly the proanthocyanidins and hydrolyzable tannins, will be emphasized. Their structures, biosynthesis, and occurrences will be described. The term 'tannins' is etymologically derived from the ancient keltic lexical root 'tann-' meaning oak and was first used in 1976 in tanneries. This word has also been used to designate the substance of vegetable origin capable of transforming fresh hide into leather. In 1962, tannins were defined by Bate-Smith and Swain as "water soluble phenolic compounds having molecular weights between 500 and 3000, and besides giving the usual phenolic reactions, and they have special properties such as the ability to precipitate alkaloids, gelatin and other proteins." Indeed, in tannin-rich food and beverages, this latter capacity is easily recognized as an astringent character once tannins precipitate with salivary proteins. The definition was later expanded by Edwin Haslam at the molecular level by adding structural and polyphenolic character. Besides the water-soluble properties and molecular weights already defined, tannins also possess some 12–16 phenolic groups and 5–7 aromatic rings per 1000 Da of relative molecular mass. Depending on the monomer structure, tannins are classically divided into four groups: condensed tannins or the proanthocyanidins (i.e., polymers of flavanol unit), the hydrolyzable tannins (i.e., gallotannins and ellagitannins), the complex tannins (tannins composed of hydrolyzable tannin moieties and flavonoid moieties), and the phlorotannins found in algae (oligomers of phloroglucinol unit).

Proanthocyanidins

Nomenclature and Structures

Proanthocyanidins or condensed tannin compounds are derived from the oligomerization of flavan-3-ol units such as catechin and/or epicatechin and epigallocatechin. Apart from lignin, they represent the most abundant class of natural phenolic compounds and the most common flavonoid. Indeed, flavonoids are the major constituents with more than 4000 compounds having been characterized. They share a common flavan core consisting of fifteen carbons with two aromatic rings (A and B), bounded by three carbon atoms, which form an oxygenated heterocycle (ring C) (Figure 1(a)). The flavonoid skeleton C_6–C_3–C_6 can have numerous substituents and occurs naturally as glycosides rather than aglycones. Due to differences in the oxidation state and substitution on ring C, flavonoids can be further be divided into different subgroups comprising flavones, flavonols, flavan-3-ols, isoflavones, flavanones, and anthocyanins (Figure 1(b)).

Unlike flavones, flavonols, isoflavones, and anthocyanidins, which are planar molecules, flavan-3-ols, proanthocyanidins, and flavanones have a saturated C_3 element in the heterocyclic C-ring and are thus nonplanar. The two chiral centers at C_2 and C_3 of the monomeric flavan-3-ol produce four isomers for each level of B-ring hydroxylation. (+)-Catechin and (−)-epicatechin are widespread in nature, whereas (−)-catechin and (+)-epicatechin are comparatively rare (Figure 2).

An important feature of the flavan-3-ols is their ability to form polymers, which are often referred to as proanthocyanidins or even condensed tannins as opposed to hydrolyzable tannins (gallotannins and ellagitannins). Although usage of the term 'oligomeric' varies somewhat, dimers, trimers, and up to heptamers are generally assigned to as oligomeric ($n = 2$–5), whereas longer chains are generally attributed to as polymeric procyanidins or tannins ($n = 6$–∞). Oligomeric and polymeric proanthocyanidins have an additional chiral center at C_4 in the upper and lower units, and their structures vary in function of the nature of their constitutive subunits, mean degree of polymerization (mDP), and linkage position. Proanthocyanidins composed mainly of (epi)catechin units are called procyanidins and these are the most abundant type of proanthocyanidins in plants. Other oligomeric tannins contain (epi)afzelechin and (epi)gallocatechin subunits and are called propelargonidins and prodelphinidins, respectively (Figure 2).

The current valid nomenclature for oligomeric procyanidins was defined by Hemingway et al. in 1982 after being first introduced by Weinges et al. in 1968 and later extended

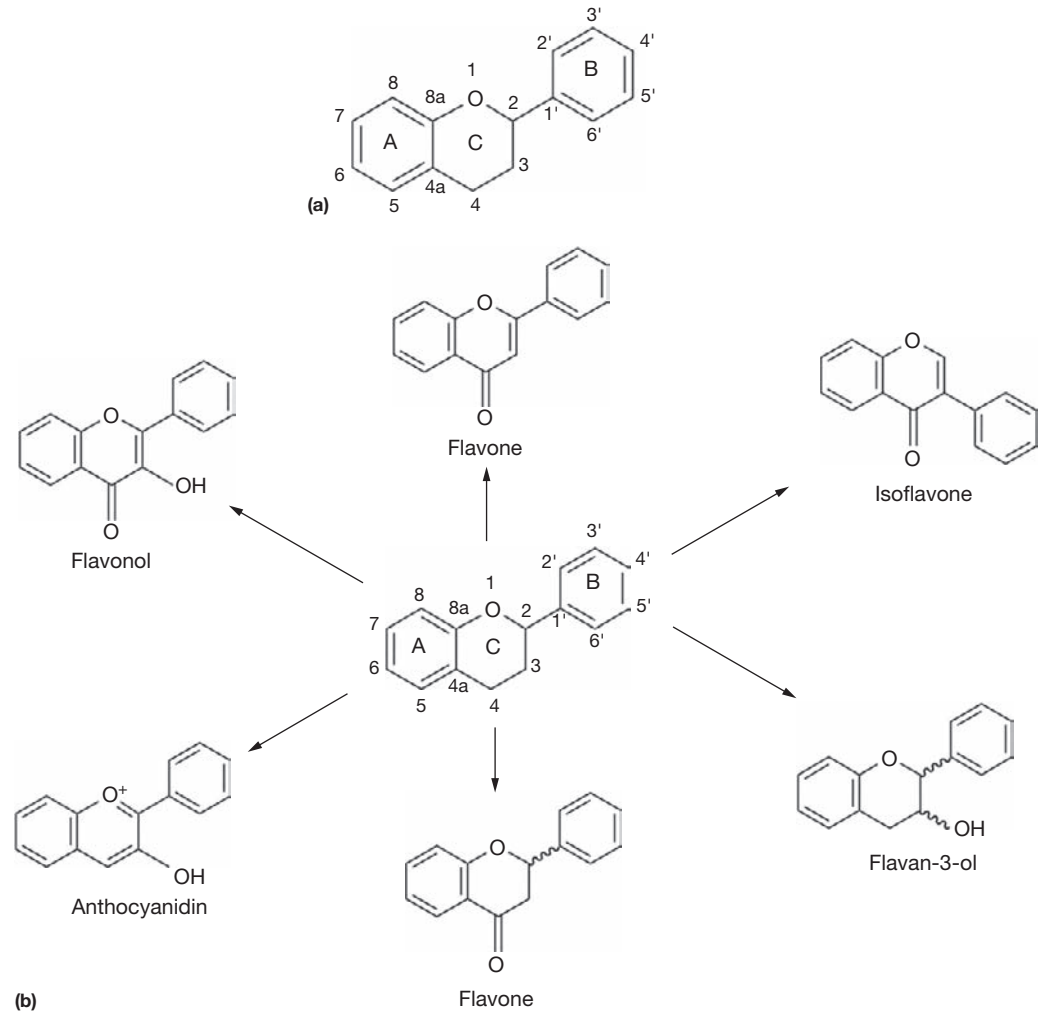
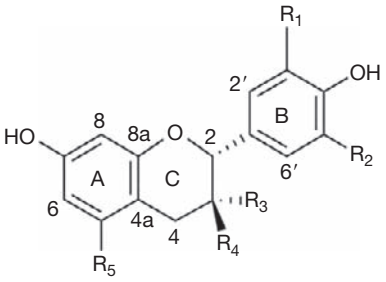


Figure 1 (a) Flavonoid ring structure and numbering system and (b) structure of the flavonoid skeleton.



2*R* Flavan-3-ol monomers	R_1	R_2	R_3	R_4	R_5
(+)-Afzelechin	H	H	H	OH	OH
(–)-Epiafzelechin	H	H	OH	H	OH
(+)-Catechin	H	OH	H	OH	OH
(–)-Epicatechin	H	OH	OH	H	OH
(+)-Gallocatechin	OH	OH	H	OH	OH
(–)-Epigallocatechin	OH	OH	OH	H	OH

Figure 2 2*R* isomer flavan-3-ols structure.

by Thompson et al. in 1972. The IUPAC system also works in naming proanthocyanidins and proves to be useful in cases that absolute stereochemistry at all chiral centers have to be described. Proanthocyanidins have high structural diversity based on four possible monomer units, (2*R*, 3*S*)-catechin, (2*S*, 3*R*)-ent-catechin, (2*R*, 3*R*) -epicatechin, and (2*S*, 3*S*)-ent-epicatechin, with the diastereomeric monomers (2*R*, 3*S*)-catechin and (2*R*, 3*R*)-epicatechin being predominant (Figure 2). Regarding the stereochemistry of the C_2–C_3 bond, they exhibit *cis* [(2*R*, 3*R*) (−)-epicatechin] or *trans* [(2*R*, 3*S*) (+)-catechin] configuration. Because most common flavan-3-ols exist as 2*R* isomers, the prefix 'ent' is used to label less common 2*S* isomers. The interflavanol linkage and its direction are indicated in parentheses with an arrow (4→) and its configuration at C_4 is described as α or β. Moreover, proanthocyanidins possessing a glycone are given a qualifying ending of '-in,' while those lacking a glycone are qualified with an ending of 'idin.'

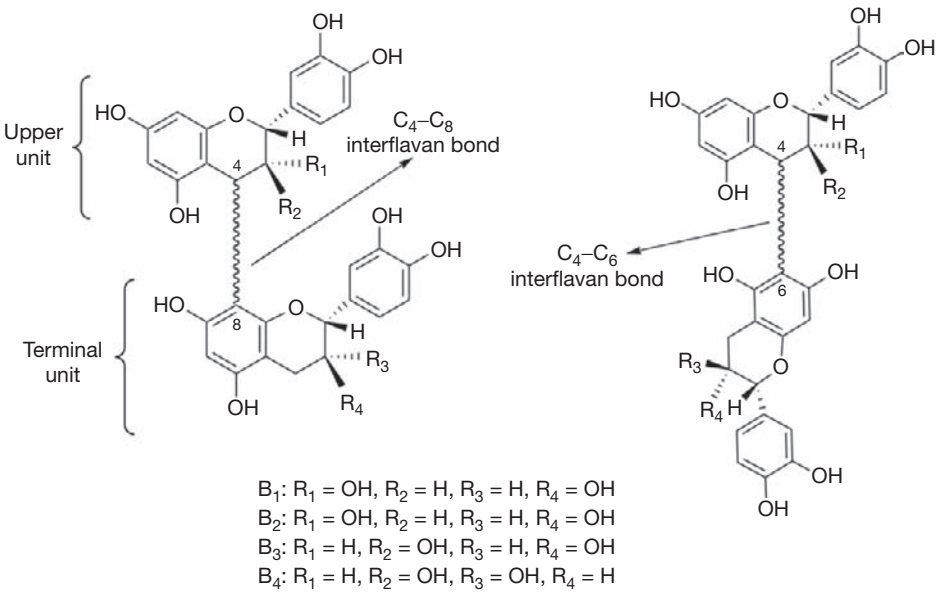


Figure 3 Type B procyanidin structures and C_4–C_8/C_4–C_6 interflavan bonds.

Stereochemistry at C_4 is strongly influenced by the hydroxylation on the A-ring. Procyanidins and prodelphinidins with 2,3-*cis* stereochemistry frequently adopt 3,4-*trans* stereochemistry during interflavanoid bond formation with $C_4 \rightarrow C_8$ or $C_4 \rightarrow C_6$ interflavanoid linkages occurring in relative proportion of 3:1. Type B proanthocyanidins are dimers resulting from these types of linkage between two units of flavan-3-ols (Figure 3). The four most common B-type proanthocyanidin dimers are the B_1, B_2, B_3, and B_4 (Figure 3). A-type proanthocyanidins are dimers that, in addition to the C–C interflavan linkage, that is, $C_4 \rightarrow C_8$ or $C_4 \rightarrow C_6$ interflavan bond, also have a C–O bond between the C_5 and C_7 carbons of the upper units. This subclass lacks two hydrogens compared to the B-type linkages. Trimers or C-type proanthocyanidins, such as C_1, consist of three flavan-3-ol units linked by two $C_4 \rightarrow C_8$ interflavan bonds. Table 1 illustrates the most common proanthocyanidins.

Besides, proanthocyanidins can also be distinguished depending on the nature of the liberated anthocyanidins in acidic conditions. Proanthocyanidins that are composed of (+)-catechin and (−)-epicatechin release cyanidins and prodelphinidins and those composed of (+)-gallocatechin and (−)-epigallocatechin liberate delphinidins.

Table 1 Common proanthocyanidins and their structures

Proanthocyanidins common name	*Structures*
Procyanidin B_1	(−)-Epicatechin-(4β→8)-(+)-catechin
Procyanidin B_2	(−)-Epicatechin-(4β→8)-(−)-epicatechin
Procyanidin B_3	(+)-Catechin-(4α→8)-(+)-catechin
Procyanidin B_4	(+)-Catechin-(4α→8)-(−)-epicatechin
Procyanidin B_5	(−)-Epicatechin-(4β→6)-(−)-epicatechin
Procyanidin B_6	(+)-Catechin-(4α→6)-(+)-catechin
Procyanidin B_7	(−)-Epicatechin-(4β→6)-(+)-catechin
Procyanidin B_8	(+)-Catechin-(4α→6)-(−)-epicatechin
Procyanidin A_1	(−)-Epicatechin-(4β→8,2β→O→7)-(+)-catechin
Procyanidin A_2	(−)-Epicatechin-(4β→8,2β→O→7)-(−)-epicatechin
Procyanidin C_1	(−)-Epicatechin-(4β→8)-(−)-epicatechin-(4α→8)-(−)-epicatechin
Procyanidin C_2	(+)-Catechin-(4α→8)-(+)-catechin-(4β→8)-(+)-catechin

Biosynthesis of Flavan-3-ol and Proanthocyanidin

The flavonoid biosynthetic pathway has largely been characterized, especially not only in *Arabidopsis thaliana* but also in *V. vinifera*. Proanthocyanidins are synthesized along the phenylpropanoid pathway by the activity of a cytosolic multienzyme complex. The upstream pathway consists of the formation of the core, the basic skeleton of all flavonoids, starting from three molecules of malonyl-CoA and one of 4-coumaroyl-CoA. Chalcone synthase (CHS) and chalcone isomerase (CHI) are the enzymes involved in the two-step condensation, producing a colorless flavanone named naringenin. The latter is a central intermediate as from this point onward, the flavonoid biosynthetic pathway diverges into several side branches each resulting in the production of different classes of flavonoids including isoflavones, flavanones, flavones, flavonols, flavan-3-ols, and anthocyanins. The oxidation of the latter compound by flavanone 3-hydroxylase (F3H) yields the dihydrokaempferol that subsequently can be hydroxylated on the 3′ by flavonol 3′-hydroxylase (F3′H) producing dihydroquercetin. Dihydroflavonol reductase (DFR) then converts dihydroflavonols to leucoanthocyanidins with the synthesis of leucocyanidin from dihydroquercetin. Indeed, leucoanthocyanidins are key intermediates in the formation of flavan-3-ols, proanthocyanidins, and anthocyanidins. The enzyme leucocyanidin 4-reductase (LAR) is responsible for the conversion of leucocyanidin to the flavan-3-ol (+)-catechin, whereas leucocyanidin

deoxygenase (LDOX) catalyzes the synthesis of cyanidin, which anthocyanidin reductase (ANR) converts to (−)-epicatechin. Figure 4 shows the schematic pathway and enzymes involved in the production of flavan-3-ols and proanthocyanidins.

Occurrences in Grapes and Wines

It is well known that grapes and red wines contain oligomeric procyanidins and prodelphinidins. The content of proanthocyanidins in grape berries depends on genetics, stages of ripeness, climatic geographic factors, and cultural practices. These compounds are mainly localized in the solid part of grape bunches (skins, seeds, or stems).

In the berry skins, they are found as a free form in the vacuolar sap or bound to proteins and polysaccharides in the internal face of the tonoplast and to the cell wall, respectively. Some authors have described a decrease in the content of flavan-3-ol monomers and proanthocyanidins after veraison and a concomitant increase or decrease of proanthocyanidin mDP in grape skins. Grape seeds are composed by an outer integument including a soft outer parenchymatous layer covered by a cuticle and an inner layer of hard, lignified, brown cells (hard, brown 'hull'). The endosperm constitutes the white soft tissue inside the inner integument. The majority of both monomeric and polymeric flavan-3-ols are localized in the outer seed coat with two to five times higher concentrations than the endosperm, which is mainly composed of monomeric flavan-3-ols. A considerable amount of proanthocyanidins can also be found in seed hull. The accumulation of flavan-3-ols in seeds can be delayed when compared to skin, and maximal concentration is reached a few weeks after veraison. The concentration of flavanol monomers then decreases sharply, while slight accumulation of procyanidin oligomers is observed.

The major flavan-3-ol monomers in grapes are (+)-catechin and its isomer (−)-epicatechin and, to lesser extent, (+)-gallocatechin, and (−)-epigallocatechin (Figure 2). Moreover, their galic acid ester (−)-epicatechin-3-*O*-gallate and (+)-gallocatechin-3-*O*-gallate have also been identified. Grape seed proanthocyanidins comprise only procyanidins, whereas grape skin proanthocyanidins include both procyanidins and prodelphinidins. Skin proanthocyanidins have a higher mDP and a lower proportion of galloylated subunits than seed ones.

During red wine making, grape proanthocyanidins are extracted from seeds and skins and concentration depends not only on the original grape profile but also on the extraction and winemaking techniques employed. Extraction kinetic depends on the solubility of the compounds and on their accessibility within the berry tissues, which can be modulated by physiological factors such as the maturation stage. It is further influenced by other technological factors, including the concentration of alcohol and of sulfur dioxide in the liquid phase, the temperature, and the extent of must homogenization. Consequently, the wine flavonoid composition is also influenced by the duration of pre- and postfermentation maceration phases and by treatments enhancing cell wall or berry degradation.

Condensed tannins can be found in grapes with different degrees of polymerization with two or more units of flavan-3-ols, reaching as high as 80 units of flavanol. Degrees of polymerization between 3–83 and 2–16 have been reported in grape skins and grape seeds, respectively. In red wine, their degree of polymerization changes continuously during aging as a result of the chemical transformations. The concentration of flavan-3-ols and proanthocyanidin in red wine ranges from ~500 to 5000 mg l^{-1} and in white wines between 100 and 300 mg l^{-1}. Proanthocyanidins are of great importance to sensory quality of red wine due to their astringent and bitter properties and their role in the long-term color stability via some chemical reactions with anthocyanins (copigmentation and/or condensation).

Molecular size of proanthocyanidins reflected in the mDP affects the mouthfeel properties, especially their relative bitterness and astringency. Indeed, monomers are more bitter than astringent, whereas the reverse is true for large-molecular-weight derivatives.

Hydrolyzable Tannins

Classification and Structures

Hydrolyzable tannins are synthesized by a wide variety of plants and trees where they occur in wood, bark, leaves, and galls. Some species produce either gallotannins or ellagitannins, while others produce complex mixtures containing gallo-, ellagi-, and condensed tannins, for example, *Acacia*, *Acer*, and *Fagaceae*. The designation 'hydrolyzable tannins' for gallotannins and ellagitannins is due to the fact that these compounds can be hydrolyzed, releasing gallic acid and/or ellagic acid, by treatment with dilute acid, whereas condensed tannins are not. Indeed, hydrolyzable tannins are esters of gallic acids and a polyol, mainly D-glucose, but fructose, xylose, saccharose, and structures like hamamelose are also found. Gallic acid is the base unit of gallotannins, whereas gallic acid and hexahydroxydiphenoyl (HHDP) moieties are both subunits of the ellagitannins. Regarding these facts, a large variety of gallotannin and ellagitannin structures derive from these two basic moieties (i.e., glucose unit and gallic acid).

A classification was first introduced by Haslam and coworkers and the two known subclasses of hydrolyzable tannins were subdivided into three groups A, B, and C. However, because of the great abundance of newly identified structures, a new classification with four groups was proposed by Okuda and coworkers in which hydrolyzable tannins are classified on the basis of the oxidation level of their galloyl ester groups and modeled on a plausible progressive biogenetic elaboration of hydrolyzable tannins, first hypothesized by Schmidt and Mayer. Gallotannins are defined as type I hydrolyzable tannins. Type II gathers the HHDP-bearing ellagitannins such as the monomeric tellimagrandins II and I, and type III those featuring the dehydrohexahydroxydiphenoyl (DHHDP) unit (i.e., dehydroellagitannins), as exemplified by geraniin (Figure 5). Ellagitannins in which the DHHDP unit has suffered additional transformations, such as the aforementioned chebulagic acid and ascorgeraniin, constitute the type IV group (Figure 6).

Besides this classification, many monomeric ellagitannins that result from chemical transformation need to be taken into account such as important structural modifications involve the opening of the D-glucopyranose core, the formation of *C*-aryl

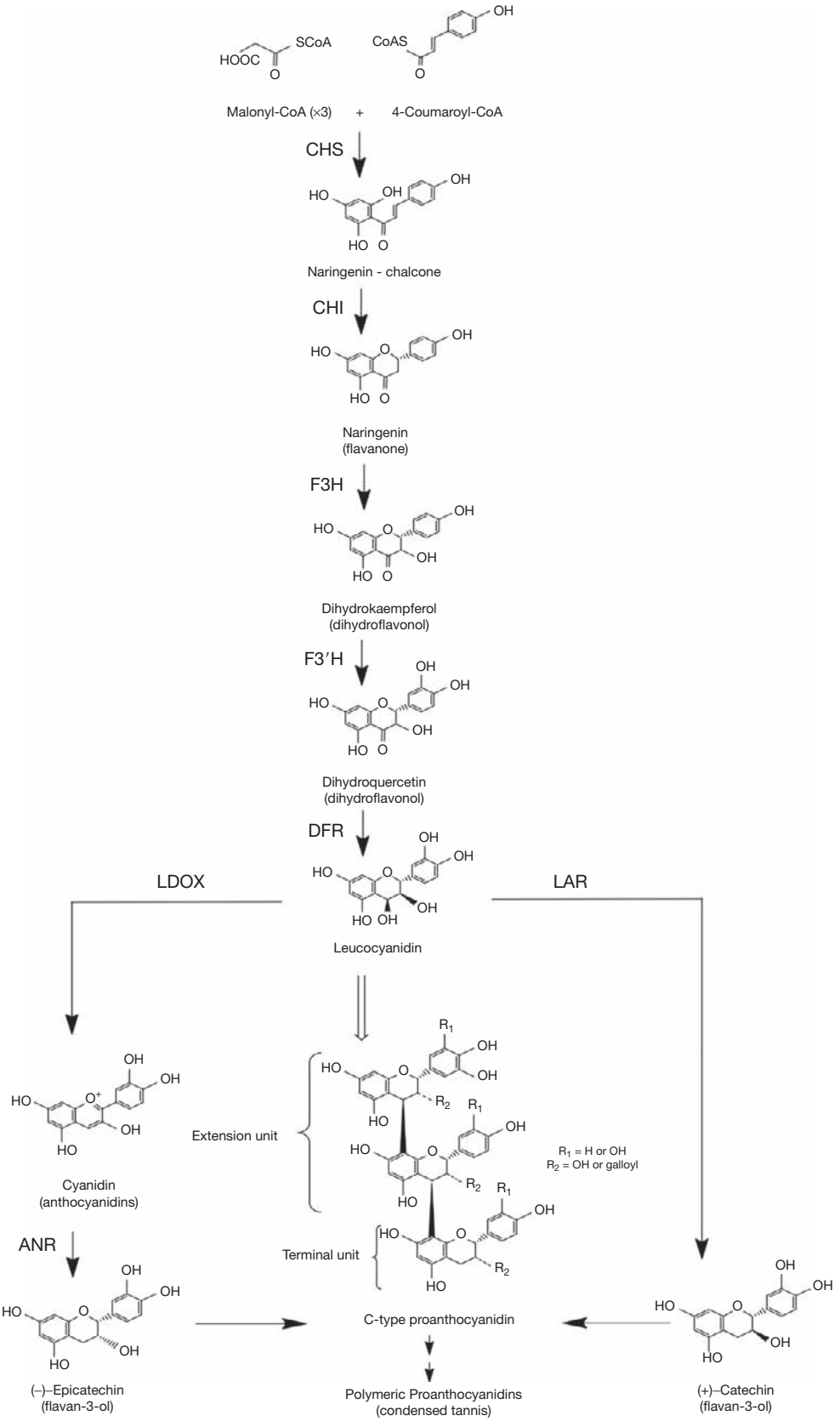


Figure 4 Schematic pathway and enzymes involved in the production of flavan-3-ols and proanthocyanidins. Enzyme abbreviations: CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3′H, flavonol 3′-hydroxylase; DFR, dihydroflavonol 4-reductase; LAR, leucocyanidin 4-reductase; LDOX, leucocyanidin deoxygenase; ANR, anthocyanidin reductase. Adapted from Crozier, A., Jaganath, I. and Clifford, M. N. (2006). Phenols, polyphenols and tannins: an overview. In: Crozier, A., Clifford, M. N. and Hiroshi, A. (eds.) *Plant secondary metabolites: occurrence, structure and role in the human diet*. Oxford: Blackwell Publishing.

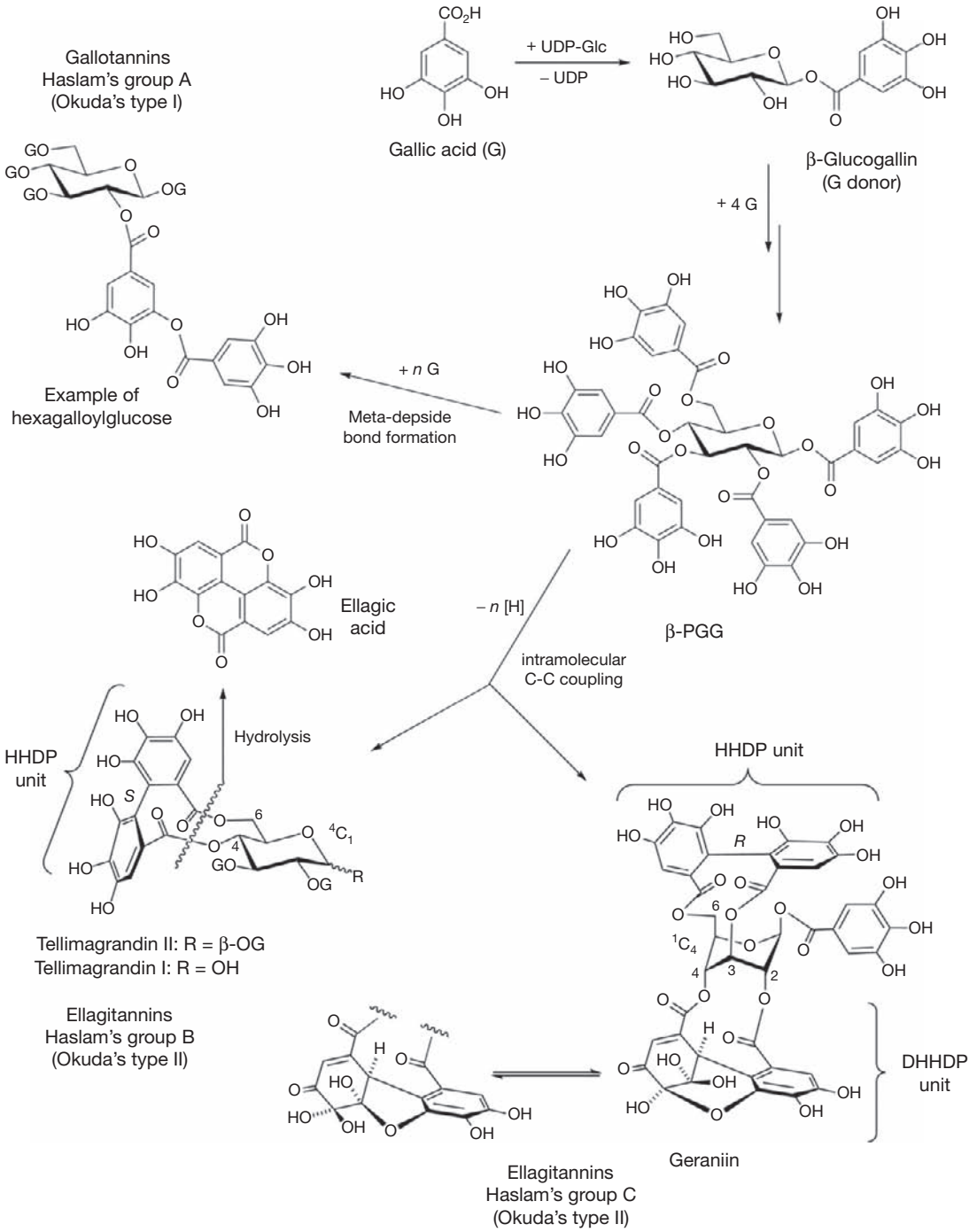


Figure 5 General biosynthetic pathway of hydrolyzable tannins (i.e., gallotannins and ellagitannins) as well as Okuda's and Haslam's classification. Adapted from Jourdes, M., Pouységu, L., Deffieux, D., Teissedre, P.-L. and Quideau, S. (2013). Hydrolyzable tannins: gallotannins and ellagitannins. In: Ramawat, K. G. and Mérillon, J.-M. (eds.) *Natural products*. Berlin, Heidelberg: Springer.

glucosidic bonds, and the condensation reactions taking place at the glucose C_1 locus. Since these ellagitannins still mostly display the characteristic structural features of compounds belonging to primary types II–IV, further classification takes into account these structures as II to IV+ types.

As ellagitannins of essentially types II and II+ oligomerize via various modes based on oxidative coupling reactions between free and C–C-coupled galloyl groups (i.e., HHDP units) on different glucopyranosic ellagitannins, as well as on condensation reactions at the C_1 center of *C*-glucosidic ellagitannins, classification becomes further complicated. After a first proposal based on the structures of the constituting monomers, Okuda and coworkers instead classified oligomers into five types according to the nature of the interunit linkage

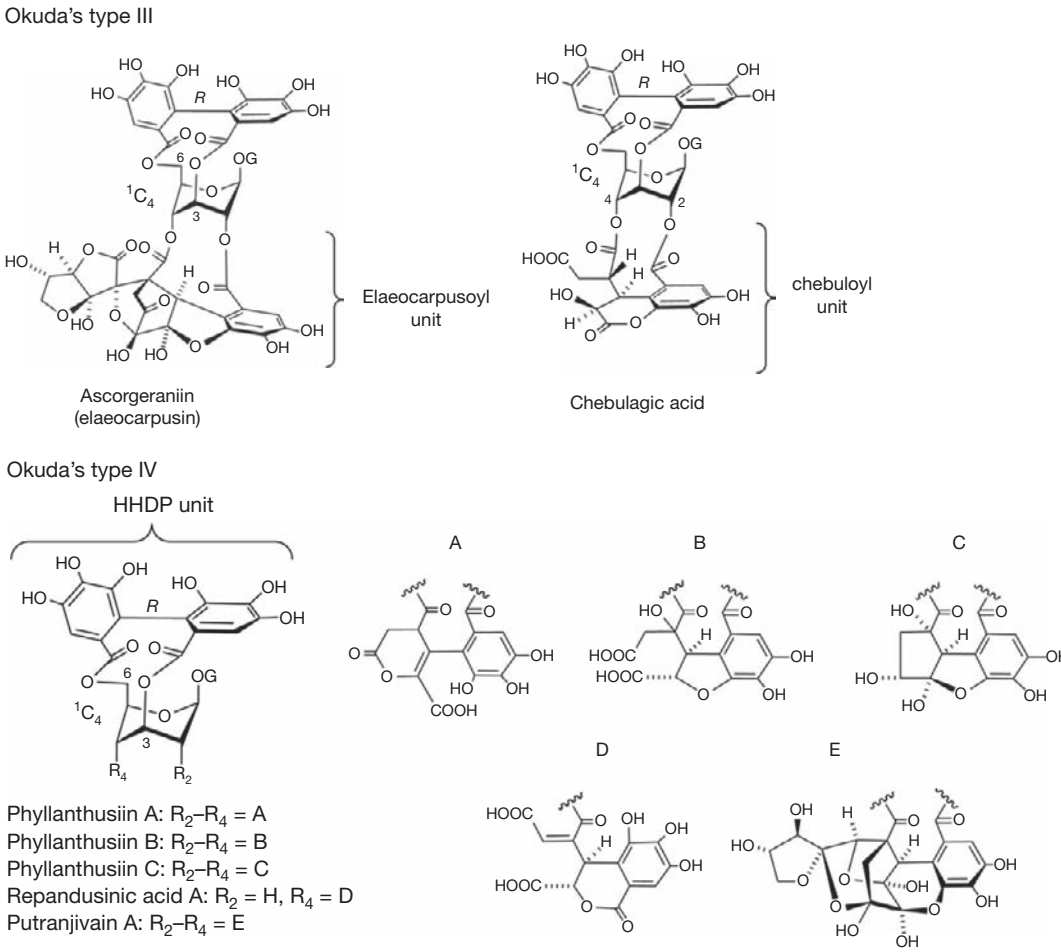


Figure 6 Examples of Okuda's type III and type IV ellagitannins. Adapted from Jourdes, M., Pouységu, L., Deffieux, D., Teissedre, P.-L. and Quideau, S. (2013). Hydrolyzable tannins: gallotannins and ellagitannins. In: Ramawat, K. G. and Mérillon, J.-M. (eds.) *Natural products*. Berlin, Heidelberg: Springer.

between monomers: (1) GOG (and GOGOG), (2) DOG, (3) GOD, (4) D(OG)2, and (5) C-glucosidic type, for which G = galloyl, O = oxygen, and D = HHDP. A more comprehensive and extensive information concerning the structures, descriptions, and examples of these oligomeric hydrolyzable tannins can be found in different reviews.

Biosynthesis

Gallic acid appears to be formed primarily via the shikimic acid pathway from 3-dehydroshikimic acid. Enzyme studies with extracts from oak leaves have shown that gallic acid is converted to β-glucogallin, which, in turn, is converted via a series of position-specific galloylation steps to penta-*O*-galloyl-glucose (β-PGG). Penta-*O*-galloyl-glucose is a pivotal intermediate that leads to the synthesis of gallotannins and ellagitannins. Figure 5 illustrates the biosynthetic pathway of hydrolyzable tannins.

Gallotannins are the result of further galloylations of β-PGG and are characterized by the presence of one or more meta-depsidic digalloyl moieties, as exemplified by hexagalloyl-glucose 2-*O*-digalloyl-1,3,4,6-tetra-*O*-galloyl-β-D-glucopyranose (Figure 5). On the other way, β-PGG can evolve through intra- and intermolecular phenolic coupling processes leading to the formation of covalent bond such as C–C biaryl and C–O diaryl ether bonds between two spatially adjacent galloyl groups. The intramolecular coupling leading to the formation of HHDP biaryl unit is the characteristic structural motif that defines ellagitannins since during acidic hydrolysis, it will lead to the formation of ellagic acid. Moreover, the ellagitannins harboring this characteristic HHDP biaryl unit belong to the hydrolyzable tannins classified as Okuda's type II. The nature of the atropisomeric form of these chiral biaryl motifs, such as the (*S*)-HHDP unit of the tellimagrandins or the (*R*)-HHDP unit of geraniin, is determined by the position of the galloyl motifs on the glucopyranose core in either its $^{4}C_{1}$- or its $^{1}C_{4}$-conformation. Moreover, the HHDP motif can go through additional oxidation transformation leading to the DHHDP unit that featured hydrolyzable tannins classified as Okuda's type III (i.e., dehydroellagitannin) such as geraniin. Furthermore, the DHHDP unit can also undergo many additional transformations leading to the formation of the chebuloyl and elaeocarpusoyl ester groups, which characterized hydrolyzable tannins classified as Okuda's type IV.

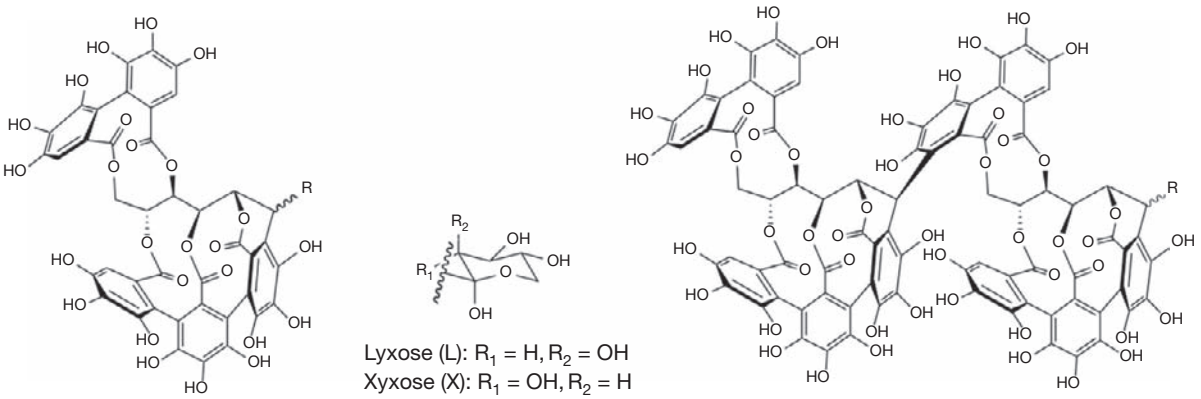


Figure 7 Structure of main monomeric ellagitannins vescalagin, castalagin, as well as the grandinin and roburin A–E isolated from *Castanea* (chestnut) and *Quercus* (oak) species.

Occurrences in Wines

As noted earlier, the primary source of wine tannins is grape berry seeds and skins, which contain proanthocyanidins in their vacuoles and cell walls. Another important source of tannins is the oak heartwood used to make barrels in which red wines are stored or aged.

The main *C*-glycosidic ellagitannins extracted by wine during its aging in oak barrels are the two monomers vescalagin and its C_1 epimer castalagin (Figure 7) that have been first isolated and characterized from *Castanea* (chestnut) and *Quercus* (oak) woody species by Mayer and coworkers in 1967. Indeed, these two molecules represent between 40% and 60% by weight of the ellagitannins in *Quercus petraea* and *Q. robur* heartwoods, which are the two main oak species used to make barrels along with the American species *Q. alba*. Furthermore, six other *C*-glycosidic ellagitannins, isolated from fagaceous *Quercus* and *Castanea* hardwood species, that is, the lyxose/xylose-bearing monomers grandinin and roburin E, the dimers roburins A and D, and the lyxose-/xylose-bearing dimers roburins B and C were also found in wines. Once in the wine, these ellagitannins slowly but continuously transform through condensation, hydrolysis, and oxidation reactions.

Moreover, it has also been observed that ellagitannins can affect red wine organoleptic properties such as color stability, perception, and sensory profile and also protect red wine against oxidation. Implications of ellagitannins on red wine are further described in a review.

Conclusion

Actually, some tannins have been identified and quantified in different vegetable origins including grapes and beverages, but more investigations need to be done to elucidate more structures and to explore their occurrence and concentrations in food and beverages as well as their biodisponibility and functional activity after ingestion. This family of compounds possesses several potent beneficial health effects and could be used as daily diet additive with a defined oral daily requirement. Another aspect of study concerns structural elucidation of tannin evolution during food processing (chocolate, wine, teas, fruits, etc.) and their implication in color evolution and sensory aspects of foods and beverages.

See also: Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Beverage: Health Effects; Grapes; Phenolic Compounds: Occurrence, Classes, and Analysis; Wines: Types of Table Wines; Wines: Wine Production; Wines: Champagne and Sparkling Wines – Production and Effervescence; Wines: Wine and Health; Wines: Wine Tasting; Wines: Madeira, Port and Sherry Fortified Wines – The *Sui Generis* and Notable Peculiarities. Major Differences and Chemical Patterns.

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Tea: Analysis and Tasting

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The Flavor of Tea

Types of Made Teas

Next to water, tea is the second most popular beverage in the world. According to processing procedures, tea that is currently made in the world can be classified into six main types including black, green, white, yellow, oolong, and reprocessed teas. Green tea is a nonfermented tea as the first step in its processing is to inactivate enzymes by dry heating or steaming. Most of the chlorophyll is preserved, and oxidation of catechins is limited. In contrast, black tea is a full-fermented tea because catechins are oxidized to theaflavins (TFs) and thearubigins (TRs), which are responsible for the typical color and taste of black tea. Oolong tea is a semifermented tea. During manufacturing of oolong tea, young shoots are allowed to wither for a short time (<8 h) before parching to inhibit the enzyme activity. Oxidation of catechins occurs during processing but to a much less extent than for black tea (Table 1). The yellow tea processing is very similar to that of green tea except with an additional step known as 'men huang' or literally 'heaping until it turns yellow,' while white tea is produced with only withering and firing. Additionally, reprocessed teas (e.g., scented tea and compressed tea) are usually processed with a series of postprocessing, such as scenting and compacting. The principal types of tea produced and consumed in the world are black and green teas, with small amounts of other types.

Theoretically, fresh young shoots of tea plants can be processed into any types of tea. However, in practice, young shoots from a specific cultivar may only be suited for processing into one of the six types of tea depending on physical, chemical, and biological characteristics of the young shoots. For example, tea cultivars suitable for black tea (generally belonging to *Camellia sinensis* cv. *assamica*) are not considered a good choice for green and oolong teas because of the high polyphenol contents. The latter two types of tea are usually produced from *C. sinensis* cv. *sinensis*. Good quality oolong tea requires cultivars with thick leaves, medium to dark leaf color, and medium polyphenol contents.

General Concept of Tea Flavor

The quality of tea is determined by its appearance, its color, and most importantly by its flavor. The flavor of tea comprises taste and aroma. Taste is characterized by astringency, bitterness, mellowness, and slight sweetness. Aroma is often described as fresh, fragrant, flowery, or dull and grassy. However, the factors that determine quality differ somewhat between black and green teas (Table 1). TFs, TRs, catechins, and caffeine are responsible for black tea quality. In contrast, green tea quality depends more on the content of amino acids, catechins, and caffeine. In addition, the appearance of the made tea and the color of the brew play major roles in the evaluation of green tea; young leaves and a clear brew with a pale green-yellowish tint indicate high quality. The quality of a green tea declines with signs of cloudiness and brown-reddish pigments.

Chemical Composition Related to Tea Flavor

The flavor of tea is principally determined by the chemical components it contains. Volatile compounds contribute to the aroma and nonvolatile compounds to the taste. These compounds are either direct metabolites that have accumulated in young shoots during growth or products of various reactions using these metabolites as substrates during processing. Thus, the flavor of made tea is largely affected by the abundance of chemical constituents and their relative composition in young shoots. Table 2 presents the most important constituents related to tea flavor and their typical concentrations in fresh young tea shoots. The chemical composition of tea depends on the following factors: genetic strain, climatic conditions, soil, growth altitude and horticultural practices, the plucking season, sorting (grading) of the leaves, the processing, storage, etc. Some parameters are more important than others. For example, the best green teas are usually plucked during the first flush in April or May (there are three main plucking times: spring, summer, and autumn).

Relish Origin Compounds in Teas

Amino acids and amides

Concentrations of total free amino acid (including amides) in tea range from 1% to 5%. The most abundant components are theanine (Thea), glutamine (Gln), glutamic acid (Glu), and arginine (Arg). Each free amino acid (or amide) has its own taste that may be sweet, salty, sour, bitter, and umami (Table 3). Free amino acids infused in a green tea brew have been recognized as the principal contributor to the taste of mellowness. Some volatile compounds found in made tea are derived from free amino acids. Amino acids can be transformed to aldehydes by decarboxylation and deamination. In the presence of flavonoids and carbohydrates, amino acids can undergo Strecker degradation to form aldehydes, alcohols, and carboxylic acids. Under heated conditions during the manufacturing process, free amino acids and especially L-arginine and L-theanine may react with sugars to form furan, pyrazine, and pyrrole (Maillard reaction), which contribute to the roast aroma of green tea.

Polyphenols

Flavonoids, which were previously called tea tannins or tea polyphenols, are the main phenolic compounds comprising 20–40% of dry matter in young shoots of tea plants. The characteristics of made tea, including color, taste, and aroma, are directly or indirectly associated with these phenolic compounds.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00687-5

Table 1 Main chemical compounds in made tea and their typical concentrations (mg g^{-1}, dry weight base)

		Green tea	*Black tea*	*Oolong tea*
Workflow	Withering	No	Yes	Yes
	Fixing	Yes	No	Yes
	Rolling/CTC	Yes	Yes	Yes
	Fermentation	No	Yes	No
	Firing	Yes	Yes	Yes
Principal components	Catechins	60–150	10–20	50–70
	Theaflavins (TFs)	~1	5–15	3–10
	Thearubigins (TRs)	~10	60–150	40–110
	Theabrownin	ND	40–90	~20
	Theanine	15–20	8–14	10–20
	Amino acids	30–40	25–35	30–35
	Peptides/protein	130–170	130–160	130–160
	Organic acids	10–15	10–20	18–25
	Sugars	20–30	20–40	20–40
	Other carbohydrates	180–270	150–260	170–270
	Lipids	35–75	40–80	40–80
	Caffeine	30–40	30–40	30–40
	Other methylxanthines	2–2.5	2–2.5	2–2.5
	Minerals/ash	~60	~60	~60
	Chlorophyll	1–5	0.1–0.2	0.3–2
	Volatile compounds	0.2–0.5	0.1–0.3	0.2–0.5
Sense characteristics	Color	Greenish or yellowish-green without any trace of red or brown	Bright reddish and brown	Reddish-brown (moderate to heavy fermented); dark greenish (light fermented)
	Taste	Strong astringency and bitterness, median umami and sweetness, and slight sourness	Brisk and strong taste	Mellowness and sweetness, lower astringency, and stronger sweetness than green tea
	Aroma	Fresh flavor	Sweet and rich flavor	Fruity and floral

Table 2 Chemical compounds in young tea shoots and their typical concentrations (dry weight base)

Compound	*Concentration in young shoots*
Polyphenols	20–40%
Flavan-3-ols (catechins)	10–30%
Flavonols and glycosides	~1%
Flavones	1–2%
Anthocyanins	~1%
Phenolic acids	2–3%
Free amino acids	1–5%
Theanine	0.5–2.5%
Alkaloids	2–5%
Caffeine	2–5%
Theobromine	~0.2%
Theophylline	~0.05%
Carbohydrates	20–30%
Soluble reduced sugars	0.8–4%
Polysaccharide	20–26%
Proteins	14–17%
Lipids	4–9%
Pigments (chlorophyll, carotenoids, and xanthophyll)	~1%
Minerals (P, K, Ca, Mg, Al, Mn, etc.)	~6%
Volatile compounds	0.01–0.02%

Table 3 The flavor and thresholds of selected chemical components in teas

Taste and Odor	*Chemical component*	*Threshold (μmol l^{-1})*	*Other tastes (in different concentrations)*
Astringent	Flavonoids		
Astringency and drying	Catechin	410	
	Epicatechin-3-gallate	260	Bitter
	Epigallocatechin-3-gallate	190	Bitter
	Epigallocatechin	520	Bitter
	Gallocatechin	540	
	Epicatechin	930	Bitter, umami, salty
	Catechin-3-gallate	250	
	Gallocatechin-3-gallate	390	
	Theogallin	90	
	Gallic acid	200	
Rough and mouth-coating	Theaflavin	16	
	Theaflavin-3,3′-digallate	13	
	Theaflavin-3-gallate	15	
	Theaflavin-3′-gallate	15	
	Theaflavic acid	24	
Mouth-drying and velvety-like astringency	Quercetin-3-O-[α-L-rhamnopyranosyl-(1–6)-β-D-glucopyranoside]	0.00115	
	Kaempferol-3-O-[R-L-rhamnopyranosyl-(1–6)-β-D-glucopyranoside]	0.25	
	Quercetin-3-O-β-D-galactopyranoside	0.43	
	Quercetin-3-O-β-D-glucopyranoside	0.65	
	Kaempferol-3-O-β-D-glucopyranoside	0.67	
	Myricetin-3-O-β-D-glucopyranoside	2.1	
	Quercetin-3-O-[β-D-glucopyranosyl-(1–3)-O-α-L-rhamnopyranosyl-(1–6)-O-β-D-galactopyranoside]	1.36	
	Myricetin-3-O-β-D-galactopyranoside	2.7	
	Kaempferol-3-O-β-D-galactopyranoside	6.7	
	Kaempferol-3-O-[β-D-glucopyranosyl- (1–3)-O-α-L-rhamnopyranosyl-(1–6)-O-β-D-glucopyranoside]	19.8	
	Quercetin-3-O-[β-D-glucopyranosyl-(1–3)-O-α-L-rhamnopyranosyl-(1–6)-O-β-D-glucopyranoside]	18.4	
	Apigenin-8-C-[α-L-rhamnopyranosyl-(1–2)-β-D-glucopyranoside]	2.8	
	Myricetin-3-O-[α-L-rhamnopyranosyl-(1–6)-β-D-glucopyranoside]	10.5	
	Kaempferol-3-O-[β-D-glucopyranosyl-(1–3)-O-α-L-rhamnopyranosyl-(1–6)-O-β-D-galactopyranoside]	5.8	
	γ-Aminobutyric acid	20	
Bitter	Caffeine	500	
	Tyrosine	5000	
	Citric acid	2600	Sweet
	Isoleucine	11 000	
	Leucine	12 000	
	Phenylalanine	58 000	
	Valine	21 000	Sweet
	Arginine	0.1 g l^{-1}	
	Arginine HCl	0.3 g l^{-1}	Sweet
	Ornithine	3500	Sweet
	Methionine	0.3 g l^{-1}	Umami
	Histidine	0.2 g l^{-1}	
	Tryptophan	0.9 g l^{-1}	
Sour	Histidine HCl	0.05 g l^{-1}	Salty, bitter
	Succinic acid	900	Umami
	Oxalic acid	5600	
	Malic acid	3700	
	Ascorbic acid	700	
	Aspartic acid	7500	Umami
	Glutamate	200	Umami
	Asparagine	0.03 g l^{-1}	Bitter
Sweet	Glucose	90 000	
	Saccharose	24 000	
	Fructose	52 000	

(*Continued*)

Table 3 (Continued)

Taste and Odor	*Chemical component*	*Threshold (μmol l^{-1})*	*Other tastes (in different concentrations)*
	Serine	30 000	Umami
	Alanine	8000	Umami
	Glycine	30 000	
	Proline	26 000	Intensively bitter
	Threonine	40 000	
	Hydroxyproline	0.5 g l^{-1}	Bitter
	Lysine HCl	0.5 g l^{-1}	Bitter
	Glutamine	0.05 g l^{-1}	Umami
Umami-like	Aspartate/Na	4000	
	Glutamate/Na	3000	
	Adenosine monophosphate (AMP)	2000	
	Guanosine monophosphate (GMP)	300	
	Theanine	6000	Sweet, astringent
Salty	Sodium	7500	
	Potassium	15 000	
	Magnesium	4000	
Odor	Hexanol	92–97 μg l^{-1}	Green, grassy
	Hexanal	10 μg l^{-1}	Green, grassy, metallic
	Trans-2-hexenal	190 μg l^{-1}	Green, fruity
	Cis-3-hexenol	13 μg l^{-1}	Fresh, fruity green
	Methyl jasmonate	–	Sweet, floral
	Linalool	0.6 μg l^{-1}	Floral, citrus-like
	Linalooloxides	–	Sweet floral, citrus, and fruity
	Geraniol	3.2 μg l^{-1}	Floral, roselike
	2-Phenylethanol	1000 μg l^{-1}	Flowery, roselike, honey-like
	Benzyl alcohol	–	Sweet, fruity
	Phenylacetaldehyde	–	Honey-like
	Coumarin	0.02 ppb	Sweet, camphoraceous
	β-Ionone	0.2 μg l^{-1}	Woody, violet-like
	Damascenone	0.004 μg l^{-1}	Honey-like, fruity

Source: Reproduced from Scharbert and Hofmann, 2005; Kaneko et al., 2006; Yang et al., 2013, http://www.ajinomoto.co.jp/amino/e_aminoscience/bc/b-7.html

The predominant flavonoids in tea are catechins (flavan-3-ol) and their gallates, which include the following and generally account for up to 30% of dry weight: (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG).

For black tea production, catechins are oxidized and catalyzed by polyphenol oxidase to form pigments TFs and TRs, which provide the typical color and flavor of black tea. This characteristic structure produces yellowish and bright reddish colors and provides a bright and yellowish appearance to the beverage. The content of total TFs in black tea is usually less than 2.5%. Pure TFs have a very fierce astringency, inducing a mouth-drying and rough-astringent oral sensation. The taste thresholds of TFs (between 13 and 26 μmol l^{-1}) are much lower than those of their precursor catechins (Table 3).

Catechins and their oxidation derivatives are the principal contributors to astringent and bitter tastes of tea beverages, especially of green teas. The taste thresholds of individual catechins range from 18.1 to 51.0 mg/100 ml or from 190 to 930 μmol l^{-1} (Table 3). Galloyl esters show lower taste thresholds than nongalloylated catechins. Catechins are responsible for the astringent taste and strength of green tea infusion. Quality green tea is characterized by high contents of free amino acids with appropriate concentrations of catechins and caffeine.

While not influencing the color of tea, other polyphenols, such as flavonols, gallic acid, and chlorogenic acid, and their glycosides, have been linked to the taste of astringency. Some flavon-3-ol glycosides, which usually do not undergo significant changes during processing, induce a silky, mouth-drying, and mouth-coating sensation with extremely low taste thresholds spanning from 0.001 to 19.8 μmol l^{-1}. The sensory activity of these flavon-3-ol glycosides is influenced by the structure of aglycon, sugar moiety, and sequence of the individual monosaccharides in glycosidic chain.

Caffeine

Caffeine (1, 3, 7-trimethylxanthine) is one of the major alkaloids in tea. Its content in young tea shoots ranges from 2% to 5% (Table 2). Other important alkaloids in tea are theobromine (3, 7-dimethylxanthine) and theophylline (1,3-dimethylxanthine). Generally, alkaloids undergo minimal changes during processing. Pure caffeine tastes unpleasantly bitter (threshold is approximately 3 mg l^{-1} in water), but its bitterness diminishes in the presence of catechins and TFs. This is because in tea brew, a portion of caffeine can form complex

with catechins, TFs and TRs, and alleviates astringency of the latter compounds. The quality of tea positively correlates with the concentration of caffeine, which contributes to briskness of black tea infusion. In cooled black tea brew, cream is formed as a complex of caffeine, catechins, theaflavin-3′-gallates, polysaccharides, protein, and some other compounds. The creaming property is an important indication of black tea quality.

Carbohydrates

Free sugars in young tea shoots consist of mainly glucose, fructose, sucrose, raffinose, and stachyose. Starch is low in young shoots but occurs in a high concentration serving as the main reserve polysaccharide in roots. Pectin substances consist of galactose, arabinose, galacturonic acid, rhamnose, and ribose. With heating during the green tea processing, sugars can react with free amino acids to produce compounds with roast aroma. The slight sweetness of tea brew may be partly accounted for by the existence of sugars.

Tea polysaccharides (TPSs) were found to be mostly glycoconjugates in which a protein carries one or more carbohydrate chains covalently attached to a polypeptide backbone, usually via N- or O-linkages. The contents of TPSs are usually higher in old than in young leaves and varied greatly among tea varieties, during harvest seasons and during processing.

Flavor Origin Compounds in Tea

Volatile compounds

The aromatic property of teas is being intensively investigated. Compounds contributing to tea aroma are primary products present in fresh leaves, which are biosynthesized by plants and do not undergo changes, or are secondary products formed during the manufacturing process from substrates (such as lipids, amino acids, and carotenoids) through enzymatic and nonenzymatic reactions. More than 600 volatile compounds associated with tea aroma have been identified, and the number is still increasing as new compounds are continuously reported. These compounds are often classified into groups including hydrocarbons, alcohols, ketones, aldehydes, lactones, phenolic compounds, acids, esters, nitrogenous and sulfur compounds, and miscellaneous oxygenated compounds. Alcoholic compounds such as geraniol, linalool, and its oxides are the main sources of the floral flavor of teas. In fresh tea shoots, only a limited proportion of these alcoholic compounds exist in free states, while most of them are present as glycosides, which can be hydrolyzed either enzymatically or nonenzymatically. During processing of oolong tea and black tea, the enzymatic hydrolysis occurs strongly and the liberated volatile compounds contribute greatly to the floral aroma of these teas.

The aromatic property of a specific tea is determined by the manufacturing process, tea variety, developmental stage of the fresh young shoots, climatic conditions, and agronomical management. The three major types of tea, that is, green tea, black tea, and oolong tea, have different aromatic profiles. The green tea flavor includes freshness, flower, and roast. The fresh and greenish odors are characteristics of green tea and are mainly due to (Z)-3-hexenol, its esters, and (E)-2-hexenal. The distinct roast aroma of pan-firing green tea (such as most Chinese green tea) is generated by pyrroles, pyrazines, and furans. In contrast, black tea often has large contents of linalool and its oxides, geraniol, methylsalicylate, (E)-2-hexenal, (Z)-3-hexenol, and (E)-2-hexenyl formate. Oolong tea has a great proportion of (Z)-jasmone, jasmine lactone, methyl jasmonate, and indole but comparatively little hexenol and hexenol esters. Linalool and geraniol are considered as the principal compounds contributing to flowery odor of all three types of teas.

Carotenoids

Carotenoids are the substrates of many of the volatile compounds present in made tea. During processing, carotenoids degrade via oxidation during withering and fermentation or paralytic reaction during firing. In green tea, a change of structure occurs through the conversion of violaxanthin to auroxanthin. Volatile compounds of ionine series are formed as primary oxidation products of carotenoids. These compounds smell sweet floral aroma and are believed to have a major effect on the aroma of tea.

Lipids

Glycolipids are the most abundant constituent accounting for more than half of the total lipid content, followed by neutral lipids (c. 35%) and phospholipids (c. 15%). Linolenic, linoleic, and palmitic acids are the major fatty acids. Linolenic acid is rich in glycolipids, while a high proportion of linoleic and palmitic acids are present in phospholipids. Neutral lipids contain high contents of lauric, myristic, palmitic, stearic, oleic, and linoleic acids. The lipid content increases from bud to third leaf and is low in the stem. This variation is related to the maturity of chloroplast. Compounds such as (Z)-3-hexenol, its esters, and (E)-2-hexenal, which principally contribute to the fresh and greenish odor of green tea, are oxidation products of free fatty acids formed during manufacturing.

Color Origin Compounds in Tea

Chlorophyll epimers and pyropheophytins are not in young shoots. Most of the chlorophyll is preserved during manufacturing of green tea, thereby creating a green color. In black tea, chlorophyll degrades and transforms either to pheophytin or first to chlorophyllide, catalyzed by chlorophyllase, and finally to pheophorbide. Pheophytin is a darker pigment with 10% higher specific absorbance at 665 nm compared to pheophorbide. Pheophytin is black and pheophorbide is brown; therefore, their relative proportion determines the blackness, an important criterion in the evaluation of made black tea. Nevertheless, some chlorophyll remains unchanged, and the residual chlorophyll has been linked to dull infusion and grassy taste of black tea.

However, some studies have suggested that the influence of chlorophyll and breakdown products on black tea quality might be dependent on the levels of other chemical compounds, such as TRs and TFs. At high concentrations, TFs can alleviate or mask the negative influence of chlorophyll on the appearance of black tea infusions. Oolong tea, a semioxidation tea, contains pheophytin b and high concentrations of pyropheophytin a but low concentrations of chlorophyll a and chlorophyll b.

Chemical Analysis of the Flavor-Related Compositions

Determination of Nitrogen-Containing Compounds

Amino acids

Amino acids form a purple complex with ninhydrin (pH = 8.0) under heating/derivatization conditions. The total content of amino acids is then determined by spectrophotometer at 570 nm. The components of amino acids are analyzed both quantitatively and qualitatively after preseparation with paper chromatography, thin-layer chromatography, or high-performance cation exchange chromatography. Another popular method is pre/postcolumn derivatization with reversed-phase high-performance liquid chromatography (RP-HPLC). Different sensitivities have been obtained by various precolumn derivatizations such as o-phthalaldehyde and the AccQ●Tag technology developed by Waters Corporation. In AccQ●Tag derivatization, primary and secondary amines are transformed into highly stable fluorescent derivatives, which can be completed within 10 min, and the dramatized products can be stored at room temperature for a week. The detection limit of fluorescence and ultraviolet detector (UV) absorption were 50–300 fmol and 0.1–0.30 pmol, respectively (Table 4). The AccQ●Tag technology has also been used in conjunction with ultra high-performance liquid chromatography or MS. Gas chromatography–mass spectrometry (GC–MS), capillary electrophoresis (CE), and anion exchange chromatography with pulsed amperometric detection are also used for amino acids analysis. In GC–MS analysis, tallow fatty acid, pentafluorophenol, n-propanol, and heptafluorobutyric acid silanization reagents are commonly used as derivatization reagents. The advantages of these methods are short separation time and high column efficiency. The disadvantage is that the derivative reaction can be easily interfered.

Alkaloids

The alkaloids (caffeine, theobromine, and theophylline) in tea have a conjugate structure, which can absorb strong UV. Therefore, alkaloids can be simply and rapidly determined by spectrophotometry. High-performance liquid chromatography (HPLC) coupled with a UV detector is popularly adopted (Table 4). The alkaloids can be separated and detected synchronously with catechins in C_{18} column by gradient elution with acetonitrile and water. Caffeine can also be extracted, preconcentrated, and analyzed by dispersive liquid–liquid microextraction and gas chromatography/nitrogen–phosphorus detection as the simple, fast, and green method. The limit of detection (LOD) and limit of quantity are 0.02 and 0.05 μg ml^{-1}, respectively.

Determination of Phenolic Compounds

The first step for measurement of phenolic compounds in teas is extracting them completely without chemical modifications. Boiling water and aqueous organic solvents (aqueous methanol, acetone, ethanol, and dimethylformamide) are among the most frequently used agents. For example, in ISO 14502, the ground tea is extracted twice with 70% aqueous methanol at 70 °C for measurement of total polyphenol and catechins. The total tea polyphenol content in extracts is detected by spectrophotometry. For example, in ISO 14502-1, the determination of total polyphenol content is based on the Folin–Ciocalteu assay, in which Folin–Ciocalteu reagent is added to the extract after dilution along with a Na_2CO_3 solution and the blue color is evaluated photometrically at 765 nm.

The catechins (flavan-3-ols) and flavonols in extracts are detected by HPLC, GC, and CE (capillary zone electrophoresis and micellar electrokinetic capillary chromatography). HPLC with UV or diode array detector (DAD) is the most frequently used method (Table 4). The chromatographic columns are mainly C_{18} and OD column, and the most common mobile phases are methanol and acetonitrile. A small amount of acid (acetic, formic, or phosphoric acid) is added in the mobile phase to prevent catechins from oxidation and complexion, to adjust the retention time, and to improve the peak shape of some compounds. Recently, the near-infrared spectroscopy (NIRS) was used to determine polyphenols/catechins and showed advantages in speed, efficiency, nondestructiveness, and environmental friendliness. However, low sensitivity, big relative error, and a requisite correction model (calibration) limit its widespread application. In the case of the flavonol and flavone glycosides, hydrolysis is performed followed by independent determinations of the aglycones and individual glycosides after a suitable cleanup. Analysis of flavonol and flavone glycosides in teas has always been a challenge due to the number components and the lack of commercial standards. Mass spectrometry especially high-resolution mass spectrometry coupled with liquid chromatography has been increasingly adopted for qualitative and quantitative determinations of flavonoids in teas.

The separation of major and minor TFs is accomplished by isocratic or gradient RP-HPLC. A stabilization solution consisting of EDTA and ascorbic acid is added to the samples to maintain stability of analytes. TRs are the most abundant but complicated group of phenols in black tea. Isolation of compound and purification and structural elucidation of TRs have long been the challenges, and direct quantification of TRs is currently not possible. There are two approaches to isolate TR fractions. One employs liquid–liquid extraction with ethyl acetate and *n*-butanol to separate the TRs in three fractions, the SI, SII, and SIa. Another approach to isolate TR fractions is precipitation by the addition of caffeine, yielding the formation of a TR–caffeine complex, which precipitates. Some recent researches employed matrix-assisted laser desorption/ionization-time of flight to get molecular mass distribution and structural information of TR fractions.

Analysis of Sugars

The total sugar content can be easily determined by anthrone chemical methods. However, careful operation is required due to the use of concentrated sulfuric acid. Sugar components such as fructose, glucose, and sucrose can be separated by HPLC, high-performance anion exchange chromatography, and GC–MS (Table 4). Special HPLC columns, for example, aminopropyl silica- or potassium-bonded, are necessary for the separation of sugars as their chemical and physical properties vary only slightly. The UV absorption of sugars is low. Therefore, evaporative light-scattering detector and MS are frequently used as detectors.

Qualitative and quantitative determination of polysaccharides in teas is currently rather hard due to their complex

Table 4 Brief introduction to representative analysis methods of chemical compounds in teas

Compounds	*Separating technology*	*Detecting technology*	*Pretreatment (extraction, derivatization, etc.)*	*Detecting condition*	*Validity and LOD*
Amino acids	TLC	Spectrophotometry	Ninhydrin (pH = 8.0)	570 nm	
	GC	MS	Tallow fatty acid, pentafluorophenol, and *n*-propanol		
	CE	UV/MS			μmol level
	UHPLC	MS		Coupled with postcolumn derivatization using ninhydrin	
	HAEC	PAD	Without derivatization		pmol–fmol level, interfered by carbohydrates
	HPCEC	Spectrophotometry	Postcolumn derivatization with ninhydrin	440 nm and 570 nm, 1 h	Automatic amino acid analyzer
	RP-HPLC	Fluorescence, UV	Dansyl-Cl	35 min	1×10^{-12} mol
			6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate	10 min	1×10^{-13} mol
		Fluorescence	o-Phthalaldehyde	1 min	fmol 1×10^{-15} mol
			9-Fluorenylmethyl chloroformate	1 min	fmol, 1×10^{-15} mol
		UV	Phenylisothiocyanate	20 min, 254 nm	1 pmol, 1×10^{-12} mol
			2,4-Dinitrofluorobenzene	365 nm, 60 min	1×10^{-12} mol
Alkaloids	HPLC	UV/MS	C18 column	220–280 nm	
	TLC	Fluorescence	Extraction with methanol or water	GF_{254}, thin-layer chromatogram scanner	
	CE	UV		200–210 nm	
	GC	NPD	GC × GC also used		
	–	Spectrophotometry	Removal of the protein and polyphenols first	274 nm	
	–	NIRS	Without pretreatment		Fast
Phenolics	GC	FID/MS	Derivatization with tetramethylsilane		
	TLC, PC	TLC scanner	Extraction with methanol or water		
	UPC[2]	MS	Extraction with methanol or water		Analysis of heterogeneous polymers
	UHPLC	Q-TOF-MS	Extraction with methanol or water		High resolution and high throughput
	CE	UV	Containing SDS and a phosphate–borate buffer (pH = 7)	200 nm, 10 min	With high separation efficiency
	–	AAS	Reaction with metal ion, such as Cu $(AC)_2$		
	–	NIRS		1000–2500 nm, 7432.3–6155.7 nm, 5484.6–4192.5 nm	
	–	NMR		^1H, ^{13}C	
	–	Spectrophotometry	Ammonium molybdate or ferrous sulfate	280/210 nm, 500–520 nm, 341 nm	
	HPLC	Fluorescence detector			10^{-13} g
		MS or MS–MS	Extraction with methanol or water		High sensitivity
		Chemiluminescence	Extraction with methanol or water		More sensitive than UV
		Electrochemistry	Extraction with methanol or water		High selectivity
		UV and DAD	Hydrolysis of flavonoid glycosides	210 nm, 270–285 nm, 35 min	0.2 ng
Chlorophyll and carotenoids	HPLC	UV/DAD	Extraction with dimethylformamide or acetone, etc.	440 nm	
	–	Spectrophotometry	Extraction with dimethylformamide or acetone, etc.	663 nm, 645 nm, 470 nm, 440 nm	
	HPTLC	TLC scanner	Mineral ether and acetone (7:3)	450 nm	

(*Continued*)

Table 4 (Continued)

Compounds	*Separating technology*	*Detecting technology*	*Pretreatment (extraction, derivatization, etc.)*	*Detecting condition*	*Validity and LOD*
Sugar	–	Spectrophotometry	Anthrone chemical or enzyme	470 nm	0.04 μg ml^{-1}
	HPLC	ELS			2.5 ng–0.20 μg
		MS		ESI	
		Refractive index detector		Zorbax carbohydrate, NH2, or Hypersil NH2 columns	5 mg g^{-1}
		UV	Edaravone, 2-aminopyridine, 2-anthranilic acid	245 nm	pmol
	HPAEC	PAD		0.25 M sodium hydroxide and 1.0 M sodium acetate	
	IEC	PAD			μg l^{-1}
	GPC			μ-Bondagel column, TSK column	Analysis of macromolecular compound
	GC	MS	Alkylation and acetylation		By-products also can hinder separation
	HPCE		2-Aminopyridine, 9-fluorenylmethyl chloroformate, and edaravone	200 nm	nl level
Nutrient elements	ICP-OES or ICP-MS		High-temperature (dry) or concentrated-acid (wet) digestion		
	AAS		High-temperature (dry) or concentrated-acid (wet) digestion		
Volatile constituents	GC	MS/flame ionization detector	Solid-phase extraction, etc.		
Thearubigins and theaflavins	HPLC	DAD/MS		280 nm, C18, Hypesil ODS	0.1–1.0 μg ml^{-1}
	–	Spectrophotometry	Derivatization with AlCl3/flavognost	625 nm/525 nm	
	CE	UV	Acetonitrile, methanol, and acetic acid (71:25:4) and 90 mmol l^{-1} ammonium acetate	380 nm, 10 min	20 μg ml^{-1}
Organic acids	HPLC	UV	Mobile phase requiring strict pH	210 nm	
		AAS	Calcium oxalate		
	GC	TOF-MS	Derivatization required		
	IEC			Anion exchange column	0.48–1.34 mg l^{-1}
	IEC	Conductometric detection/UV		TSK gel OA pack-A, Ion-30 column	
	HPCE			30 kV	

TLC, thin-layer chromatography; GC, gas chromatography; MS, mass spectrometry; UV, ultraviolet detector; UHPLC, ultrahigh-performance liquid chromatography; HPLC, high-performance liquid chromatography; DAD, diode array detector; ICP-OES, inductively coupled plasma optical emission spectrometry; CE, capillary electrophoresis; HAEC, high-performance anion exchange chromatography; HPCEC, high-performance cation exchange chromatography; RP-HPLC, reversed-phase high-performance liquid chromatography; UPC2, ultraperformance convergence chromatography; PC, paper chromatography; HPTLC, high-performance thin-layer chromatogram; HPCE, high-performance capillary electrophoresis; AAS, atomic absorption spectrophotometry; IEC, ion exchange chromatography; NIRS, near-infrared spectroscopy; FLD, fluorescence detector; NMR, nuclear magnetic resonance spectroscopy; PAD, pulsed amperometric detector; FID, flame ionization detector; NPD, nitrogen–phosphorus detection; ELSD, evaporative light-scattering detector; Q-TOF-MS, quadrupole time-of-flight mass spectrometry; GPC, gel permeation chromatography.

structures. There are two categories of methods to determine polysaccharides in teas. One is the so-called direct detection by HPLC, gel chromatography, density gradient centrifugation, and electrophoresis (usually detected by DAD or electrochemical detector after being separated by capillary zone electrophoresis with 50 mmol l^{-1} sodium tetraborate buffer (pH 9.3), alkalescence and precolumn derivatization also being required). Direct measurement is limited because of difficulties in preparing TPSs of high purity. Most commonly, only polysaccharides of homogeneous groups within certain molecular weight ranges can be separated and purified. Another method is the indirect one based on the condensation reaction by monosaccharide such as the anthrone sulfuric acid method or phenol sulfuric acid method after polysaccharides are digested to monosaccharides.

Analysis of Volatile Constituents

The first step in the analysis of volatile compounds in teas is extraction, and there are a number of extraction methods to

collect volatile compounds. The key point in the selecting extraction method is to obtain the extract sufficiently or really represent the original composition in the sample being analyzed. Simultaneous distillation and extraction (SDE) is widely used because it is a one-step isolation–concentration and enhances the yield of extract. The main drawback in SDE is the alteration of some compounds from degradation and hydrolysis or the formation of new products by thermal reactions during the extraction process. To minimize changes in volatile compounds and save time in preparation, solid-phase microextraction (SPME) that involves the use of fused silica fibers coated with different types of polar or nonpolar adsorbents is increasingly used in preparation for analysis of volatile compounds from dry tea samples and tea infusion. Adsorption of target compounds onto the fiber can be done either from the headspace of solid or liquid samples or by directly dipping the fiber into an aqueous solution using a variety of mixing techniques. The effectiveness of SPME extraction is influenced by sampling time, temperature, and sample volume, although it is a fast, effective, and simple method for collecting volatiles. Maintaining consistency in these factors is crucial for obtaining comparable results. In addition, other preparations include microwave-assisted extraction, accelerated solvent extraction, supercritical fluid extraction, subcritical water extraction, full evaporation dynamic headspace, column chromatography extraction, and drop-to-drop solvent microextraction.

GC–MS is the most efficient technique for the separation, identification, and quantification of volatile compounds. Polar or midpolar GC columns are generally used to investigate most tea volatile compounds. When investigating the stereochemistry of volatile compounds, a CHIRAMIX column may be used. Separation of target components from complex mixtures justifies the use of a multidimensional GC × GC–MS. Peaks are identified through comparison with authentic standards, searching the commercial NIST library and the use of retention indices. GC coupled with olfactometry methods is effective to determine the odor-active compounds and their contribution to the aroma quality. Extracts of volatile compounds are stepwise diluted with an organic solvent and injected into GC–olfactometer. Each volatile compound separated by GC is simultaneously evaluated by a panel of experts at the sniffing port. The contribution of a specified component to tea aroma is indicated by its dilution factor.

In recent years, faster volatile analytic systems such as E-nose and transportable zNose have been used. The E-nose consists of a sampling apparatus, a trap tube, a detector unit containing the array of sensors, and a computer system for data recording and analysis. The sensors mimic the human olfactory receptors. E-noses have been successfully used to identify tea grades, to predict tea quality, and to monitor the black tea fermentation process. The zNose is an ultrafast portable GC-based volatile compound analyzer with high sensitivity. The volatile compounds are separated by fast GC and detected by a highly sensitive surface acoustic wave quartz microbalance detector.

Analysis of Chlorophyll and Carotenoids

As with other botanical samples, the principal components of pigment in tea are chlorophyll, xanthophyll, and carotene, a natural pigment. Spectrophotometry is often used for the determination of chlorophyll content because it absorbs different spectra at different wavelengths. And the LC method is applied for analyzing pigment components, with the importance of comparison and selection of solvent extraction. *N*,*N*-Dimethylformamide (DMF) and acetone are commonly used for pigment extraction, while the DAD detector is a good way for identifying the spectral characteristics of chlorophyll and carotenoids.

Determination of Elements in Teas

The total nitrogen is commonly determined by the Kjeldahl method. A more convenient method for nitrogen and additionally carbon, sulfur, and hydrogen is by Dumas combustion on an elemental analyzer. For other elements, dry ashing and wet digestion are the common strategies for sample preparation. At present, closed vessel wet digestions facilitated by the microwave energy are increasingly used. In general, atomic and mass spectrometries are used for the elemental analysis in teas. These include flame-and-graphite furnace atomic absorption spectrometry (F-AAS and GF-AAS), inductively coupled plasma optical emission spectrometry, and inductively coupled plasma mass spectrometry (ICP-MS). The two latter ICP-based techniques are the most effective in the multielement analysis of tea samples. In the case of ICP-MS, information on isotopic distribution is obtained. Hydride generation combined with spectrometric detectors, that is, atomic fluorescence spectrometry, is preferred for the measurements of hydride-forming elements, for example, As, Bi, Se, and Te.

Taste Perception and Analysis of Tea Infusion and Beverage

To bridge the gap between pure structural chemistry and human taste perception, many studies try to establish a reliable analysis system that identifies those tea components responsible of the taste and aroma. The electronic nose, NIRS, aroma sensors, taste sensor system, human psychophysical experiments, and olfactory neural network have been more or less successfully applied.

Taste omission experiments have been performed to narrow the key taste compounds in tea infusion. Based on this, a reduced recombinant containing the bitter-tasting caffeine, nine velvety astringent flavonol-3-glycosides, and the puckering astringent catechin as well as the astringent and bitter-tasting epigallocatechin-3-gallate were prepared. The taste profile of this reduced recombinant did not differ significantly from that of the complete taste recombinant, thus confirming these 12 compounds as the key taste compounds of the tea infusion.

The aqueous taste model was also used to confirm the quantitative results. For example, 15 amino acids, 14 flavonol-glycosides, eight flavan-3-ols, five TFs, five organic acids, three sugars, and caffeine were blended as aqueous solutions in their 'natural' concentrations, and the sensory analyses revealed that the taste profile of this artificial cocktail did not differ significantly from the taste profile of the authentic tea infusion.

Classification of Tea and Discrimination of Geographic Origin

Classification

Due to the distinct fermentation degrees during processing, components in different tea types varied greatly. Common technology (such as HPLC) has been demonstrated to be a useful method to discriminate tea categories (Table 5). For example, based on the content of free amino acids obtained by HPLC and back-propagation in multilayer linear perceptron, five main tea types (green, black, oolong, white, and Pu-erh teas) were successfully classified. Asn, Ala, Leu, Ile, and Glu were the most important discriminating parameters. Compared to HPLC-UV/DAD, UPLC–MS provides larger numbers of differentiating markers. For example, the analysis by nontargeted method utilizing UPLC and high-resolution mass spectrometry showed greater numbers of flavan-3-ols, flavonol, and flavonoid glycoside, which distinguished fermented teas from nonfermented teas. The electronic tongue technique has also been successfully applied in the classification of green and black teas. Based on the different concentrations in phenolic compounds, 43 samples of green and black teas were analyzed by cyclic voltammetry and well separated with the support vector machine (SVM) for pattern recognition method.

Table 5 Examples of classification and geographic tracing in tea products

Classification/ tracing	*Samples*	*Analysis method*	*Data processing*	*Main differentiating markers*
Processing technology	Green, white, black, oolong, and Pu'er teas	HPLC–MS	PCA, k-nearest neighbors, LDA, and artificial neural networks	Polyphenols (catechin, theaflavin, quercetin and kaempferol triglycosides, and quinic acid esters), amino acids (glutamic acid, asparagine, serine, alanine, leucine, and isoleucine), and caffeine
	Green and black teas	Electronic tongue technique	PCA and SVM analysis	–
	Green, oolong, and black commercial tea samples from around the world	UHPLC and high-resolution mass spectrometry	PCA PLS-DA	Caffoyl quinic acid, kaempferol 3-O-rutinoside, kaempferol 3-O-acyl glycosides, gallocatechin, and quercetin-3-O-glucoside
	Oolong tea of five similar varieties	HSPM with GC–MS	HCA, PCA, and stepwise LDA	18 volatiles with the best discriminating capacity selected
Meteorologic and seasonal variations	Spring tea Longjing 43	HPLC and spectroscopy	SVM technology	Tea polyphenol, amino acid, caffeine, and total catechins
	Australia-grown tea	HPLC	Analysis of variance	Epigallocatechin gallate, epicatechin gallate, and epigallocatechin
	Chinese green tea	HPLC	K-nearest neighbor method and Bayesian discriminant method	Free amino acids, catechins, and caffeine
Geographic origin (country of origin)	Teas from various Asian and African countries	ICP-AES and ICP-MS	PCA, CA, LDA, and soft independent modeling of class analogy	Al, Ba, Ca, Co, Cr, Cs, Cu, Fe, Mg, and Mn
	191 green teas from different countries	NMR	PCA and CA	Some minor sugar-containing, theobromine, theogallin, and 2-O-(α-L-arabinopyranosyl)-myoinositol
	15 types of tea from 10 different countries	ICP-AES and ICP-MS	Chemometrics approach	Ca, Sr, Al, Fe, and Mg
	Black tea from nations and different Chinese provinces	FT-NIRS	PLS	Caffeine, water extract, total polyphenols, and free amino acids
Geographic origin (protected producing areas)	Xihu Longjing tea Non-Longjing flatten-shaped tea	HP LC	PCA and LDA	Caffeine, epigallocatechin gallate, and epicatechin gallate
	Oolong tea from protected or unprotected producing areas in China	NIRS	PLS-DA	–

HPLC, high-performance liquid chromatography; MS, mass spectrometry; ICP, inductively coupled plasma; AES, atomic emission spectrometry; HSPM, headspace solid-phase microextraction; GC, gas chromatography; NMR, nuclear magnetic resonance spectroscopy; NIRS, near-infrared spectroscopy; UHPLC, ultrahigh-pressure liquid chromatography; FT, Fourier transform; PLS, partial least squares; DA, discriminant analysis; HCA, hierarchical cluster analysis; PCA, principal component analysis; LDA, linear discriminant analysis; CA, cluster analysis; SVM, support vector machine analysis.

Within the same tea category, differences caused by the varieties and processing technologies were relatively small. Consequently, more purposeful methods are needed. For example, 75 oolong tea samples of five similar varieties (Tieguanyin, Benshan, Maoxie, Huangjingui, and Jinguanyin) were analyzed by headspace solid-phase microextraction coupled with GC–MS. The relative content of 26 major volatile compounds varied significantly among the varieties, and 4 discriminant functions enabled simultaneous discrimination of the five oolong varieties with 100% correct rate.

Free amino acid, catechin, and caffeine levels in green tea leaves also show considerable variations among seasons. Such variations are useful information to identify the production seasons of commercial green teas. HPLC-UV and spectroscopy have been used to quantify levels of these quality related compounds (Table 5). By using the SVM technology, the relationships between meteorologic factors and the tea biochemical components have been discovered. The results showed that caffeine and the ratio of tea polyphenols and amino acid of the spring tea Longjing 43 significantly changed according to the daily mean temperature (3–5 days before harvest) and average precipitation (1–19 days before harvest). In addition, for the HPLC method, quantitative data were compared using analysis of variance. EGCG and ECG could be used as quality descriptors for monitoring the seasonal variations of phenols in Australia-grown tea leaves, and the ratio (EGCG + ECG)/EGC has been suggested as a quality index for measuring the differences in flavanol levels in fresh tea shoots across the growing seasons. The K-nearest neighbor (KNN) method in combination with chemical analysis is also recommended for the discrimination of the production seasons of Chinese green tea. The optimal accuracy of the KNN method was <=97.61 and <=94.80% as validated by resubstitution and cross validation tests, respectively, and that of linear discriminant analysis (LDA) was <=95.22 and <=93.54%, respectively.

Geographic Tracing

Geographic origin of teas has been of concern for customers. Recent studies have shown that teas from different locations (countries or provinces of the same country) were well discriminated by analysis of elements and chemical components (Table 5).

Trace element and metal concentrations were always considered to be the main differentiating markers of the geographic origin. ICP-AES/MS coupled with chemometrics analysis can easily identify teas from different countries. With statistical analysis of 17 elements (Al, Ba, Ca, Cd, Co, Cr, Cu, Cs, Mg, Mn, Ni, Pb, Rb, Sr, Ti, V, and Zn), the LDA gave correct assignation percentages of 100.0% and 94.4% for the African and Asian teas, respectively.

NIRS combined with chemometrics tools was utilized as a rapid analysis method to assess quality and to differentiate geographic origins of black teas from different nations (e.g., China, India, Kenya, Sri Lanka, and Burma) and different provinces (e.g., Anhui, Hubei, and Yunnan) in China. The identification accuracy for black tea from different geographic origins reached 94.3%.

Some compounds in connection with quality were also marked as the main difference in compounds of Chinese and non-Chinese teas by nuclear magnetic resonance spectroscopy (^{1}H NMR). With ^{1}H NMR analysis, a total of 191 green teas from different countries, the principal component analysis showed Chinese tea (Longjing) contain higher levels of theanine, gallic acid, caffeine, epigallocatechin gallate, and epicatechin gallate and lower levels of epigallocatechin when compared with other teas.

The appearance and main quality components were similar between the teas from protected or unprotected producing areas, and most studies only provide an identification model without diversity of special compounds. For example, a combination of chromatographic chemical fingerprints with LDA provides a simple and rapid approach to identify Xihu Longjing (highest quality Chinese tea) from other teas (other types of flatten-shaped teas like Longjing and some inauthentic Longjing from other tea-producing areas). High total accuracy rates of 94.8% and 87.6% for the training and test set, respectively, were achieved for distinguishing Xihu Longjing from the other types of tea by stepwise discriminant analysis. The combination of NIRS and statistical model selection also provides an effective and rapid method to discriminate the geographic producing area of Anxi Tieguanyin (a special oolong tea of high quality). A total of 570 representative samples (450 from the original producing area and 120 from unprotected producing areas in China) were measured by NIR. Partial least squares discriminant analysis was performed to develop a classification model and predict the authenticity of unknown objects. As the best model, the sensitivity and specificity reached 0.931 and 1.000, respectively.

Safety Evaluation of Tea Product

The food quality and safety have attracted an ever-increasing attention. Pesticide residues, mycotoxins, and heavy metals are the major contaminants found in teas.

Detection of Pesticide Residues

Pesticide residues are the major potential chemical contaminants in teas for widespread use of pesticides in plantations. Strict regulations have been established for the maximum residue levels in many countries (e.g., in European Community, EC No. 42/2000 and EC No. 1881/2006). Direct determination of pesticide in tea is not easy because of low concentration and matrix complexity. For accurate measurements of pesticide residues, samples must be properly pretreated prior to quantitative determination and a separation/preconcentration step is required. Recently, a number of preparation methods have been developed for extraction, preconcentration, and isolation of pesticides from tea because it is not a well-resolved issue yet. Tea is highly complex, and it has many interference compounds that need to be eliminated prior to determination. GC, HPLC, MS, and immunoassay (ELISA) are usually used in determining pesticide residues. A reliable and highly sensitive determination of multipesticide residues can be achieved by chromatography techniques coupled with MS, tandem MS (MS/MS), triple quadrupole MS, or high-resolution MS (Q-TOF).

Hygiene Tests and Determination of Toxin and Microbe

Unclean process may lead to harmful microorganisms contaminated in tea, especially in Pu'er and dark teas. *E. coli* or mycotoxins can contaminate agricultural products and pose a serious threat to human health. Mycotoxins are toxic metabolites naturally produced by fungi, including the genera *Aspergillus*, *Fusarium*, and *Penicillium* that are the natural fungal flora usually found in the food chain. The major mycotoxins concerned are aflatoxins, deoxynivalenol, zearalenone, ochratoxin, T-2 toxin, and fumonisin. They have been recognized as a serious threat to human health because of their carcinogenic and teratogenic effects. Nevertheless, few reports have been published on the mycotoxin detection in tea samples through HPLC, MS, ELISA, and immunochromatography.

Polycyclic aromatic hydrocarbons (PAHs) also receive increasingly more attention, especially in the smoked black teas. According to the few studies focused on them, GC × GC-TOF-MS can be a good way for the determination of PAHs.

Determination of Fluoride and Heavy Metals in Tea

There has been an urgent desire for detecting heavy metals in tea. Pb, Cd, Hg, and Sn have been considered contaminants by commission regulation of the European Community. Techniques used for heavy metal determination are the same as those used for determining trace elements to establish geographic origin. It is noteworthy that *Camellia sinensis* plants can tolerate and accumulate quite elevated quantities of Al and F. The older leaves contain higher levels of Al and fluoride than young leaves. Dark teas (e.g., brick teas) made with mature leaves are exposed to high fluoride concentrations. Fluoride can be easily determined by the use of fluoride ion-selective electrode or ion chromatography.

Conclusions

The sensory quality and health functions of tea depend on the chemical compositions in tea plant such as flavonoids (or phenolic compounds), theanine, and alkaloids. Recently, with the development and improvement of detection technologies and chemometrics methods, the identification and quantitation of these compounds become more accurate and easily. And these works will fill the gap between the exact chemical components that contribute to the tea flavor and human taste perception. However, more technologies should be developed to evaluate the quality of tea in the specific region or process (e.g., different geographic, ecological environment, and cultural practices, and processing technologies) that produced the inherent distinctive characteristics, which reflect the sensory quality, economic value, and health function. Otherwise, the increasing number of pesticides and other potentially dangerous toxins in tea products also pose special analytic challenges to detection technologies.

See also: Alkaloids: Properties and Determination; Amino Acids: Determination; Pesticides and Herbicides: Residue Determination; Phenolic Compounds: Bioavailability and Health Effects; Phenolic Compounds: Occurrence, Classes, and Analysis; Tea: Chemistry and Processing; Tea: Health Effects; Tea: Types, Production, and Trade.

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Tea: Chemistry and Processing

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Introduction

Tea, produced from the leaves of the plant *Camellia sinensis*, is one of the most widely consumed beverages in the world. It has been used as a daily beverage and crude medicine in China and Japan for thousands of years. The leaves can be manipulated to generate over 300 different kinds of tea. Generally, they are divided into three types: nonfermented green tea, semifermented oolong tea, and fully fermented black tea. To make green tea, fresh tea leaves are steamed or pan-fried, which inactivates the enzymes and prevents the oxidation of tea polyphenols. Black tea manufacture involves crushing the tea leaves to promote enzymatic oxidation and subsequent condensation of tea polyphenols in a process known as fermentation. Oolong tea is semifermented to permit a moderate level of enzymatic oxidation during processing and then dried. About 78% of the tea production worldwide is black tea, whereas green tea and oolong tea constitute about 20% and 2%, respectively. Green tea and oolong tea are more popular in China, Japan, Korea, and some African countries, whereas black tea is preferred in India and the Western countries. The tea plant is considered native to south China and is now cultivated in many other countries. The major tea-producing countries are China, India, Japan, Sri Lanka, Indonesia, and Central African countries.

Epidemiological studies associate the drinking of tea with reduction in the risk of chronic diseases, such as cardiovascular diseases and cancer. There are also laboratory studies suggesting beneficial health effects of tea consumption. These effects are attributed to the polyphenol compounds in tea. Catechins are the most abundant polyphenols in green tea. The main pigments in black tea are theaflavins and thearubigins, which are formed by the oxidation and polymerization of catechins during fermentation. Although thearubigins account for up to 60% of the dry weight of black tea extract, the chemistry of thearubigins is still unclear. In this article, we will discuss the current knowledge on the chemistry of tea and the impact of processing on the profile of tea components.

The Major Components in Tea

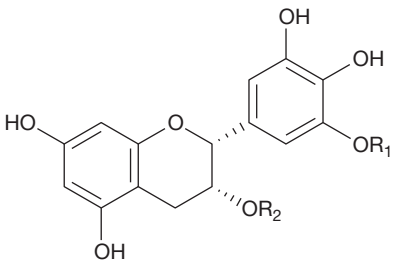
Catechins

A typical tea beverage, with 2.5 g tea leaves in 250 ml hot water for a 3 min brew, usually contains 620–880 mg of water-extractable solids. Tea polyphenols, known as catechins, usually account for 30–42% of the dry weight of the solids in brewed green tea. Catechins are members of a more general class of flavonoid, the flavan-3-ols. They are characterized by di- or tri-hydroxyl group substitution of the B ring and the meta-5,7-dihydroxy substitution of the A ring.

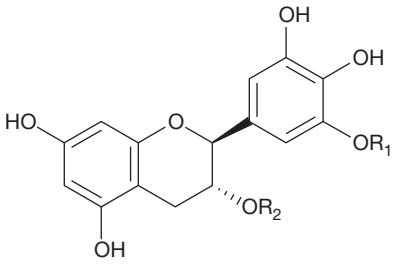
The major catechins in tea are (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epicatechin (EC) (Figure 1). Gallocatechin gallate (GCG), gallocatechin (GC), catechin gallate (CG), catechin (C), epigallocatechin digallates, epicatechin digallate, 3-*O*-methyl EC, 3-*O*-methyl EGC, and afzelechin are present in smaller quantities. 3″-*O*-methyl-EGCG and 4″-*O*-methyl-EGCG have been identified in different tea samples. EGCG is the most abundant catechin in green, oolong, and black teas. Green and oolong teas typically contain 30–130 mg of EGCG per cup (237 ml), whereas black tea may have up to 70 mg of EGCG per cup.

Flavonols and Flavones

Both flavonols and flavones have been reported from tea. Quercetin, kaempferol, myricetin, and their glycosides (mono-, di-, and tri-) (Figure 2) are the major flavonols present in tea. They make up about 0.5–2.5% wt/wt extract as aglycones in tea infusions. Flavones, such as apigenin, the only flavone identified in tea, and its glycosides, have also been detected in tea but represent a very small fraction of the tea polyphenols. More recently, 19 *O*-glycosylated flavonols, 7 *C*-glycosylated flavones, 28 acylated glycosylated flavonols, and 3 flavonols were identified from green teas and fermented



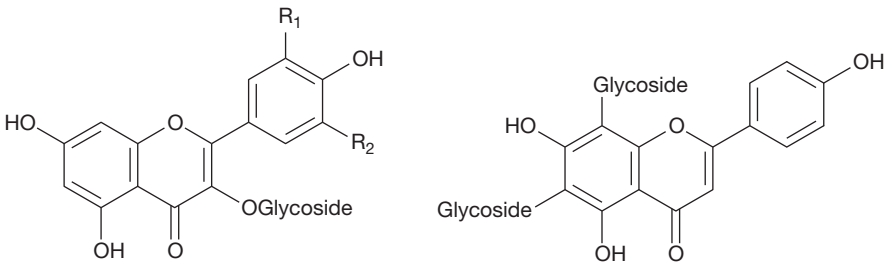
Epicatechin: $R_1 = R_2 = H$
Epigallocatechin: $R_1 = OH$, $R_2 = H$
Epicatechin 3-gallate: $R_1 = H$,R_2 = Galloyl
Epigallocatechin 3-gallate: $R_1 = OH$, R_2 = Galloyl



Catechin: $R_1 = R_2 = H$
Gallocatechin: $R_1 = OH$, $R_2 = H$
Catechin 3-gallate: $R_1 = H$, R_2 = Galloyl
Gallocatechin 3-gallate: $R_1 = OH$, R_2 = Galloyl

Figure 1 Structures of the major catechins in tea.

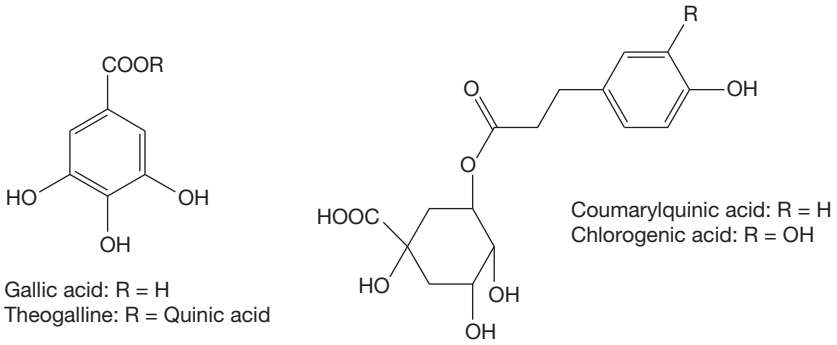
 http://dx.doi.org/10.1016/B978-0-12-384947-2.00685-1



Kaempferol glycoside: $R_1 = R_2 = H$
Quercetin glycoside: $R_1 = OH$, $R_2 = H$
Myricetin glycoside: $R_1 = R_2 = OH$

Apigenin glycoside

Figure 2 Structures of the major flavonol glycosides and flavone glycoside.



Gallic acid: R = H
Theogalline: R = Quinic acid

Coumarylquinic acid: R = H
Chlorogenic acid: R = OH

Figure 3 Structures of simple polyphenols in tea.

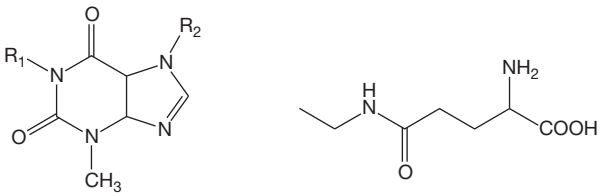
teas using liquid chromatography with diode array and electrospray ionization mass spectrometric detection (LC-DAD-ESI/MS).

Simple Polyphenols

The major simple polyphenols found in tea are gallic acid and its quinic acid ester, theogallin (Figure 3). The amount of gallic acid in black tea is significantly increased due to the de-esterification of the 3-galloyl-substituted catechins by either native esterase or oxidative degallation during fermentation, while the amount of theogallin is decreased during the fermentation process due to the formation of a new theaflavin-type compound, theagallinin, which is the condensation product between EC and theagallin. In addition, cinnamic acid derivatives of quinic acid, the coumaryl and caffeoylquinic acids (Figure 3), have also been identified from tea.

Caffeine and Theanine

The amount of caffeine in tea beverages is determined by the leaf size, the brewing time, and the temperature. It has been reported that there is no difference regarding the caffeine content between green and black teas indicating that caffeine is very stable during the fermentation process. Water-extractable tea solids contain about 2–5% caffeine and much smaller quantities of theobromine and theophylline (Figure 4). Tea contains around 17% nitrogenous materials as protein (~6%)



Caffeine: $R_1 = R_2 = Me$
Theobromine: $R_1 = H$, $R_2 = Me$
Theophylline: $R_1 = Me$, $R_2 = H$

Theanine

Figure 4 Structures of caffeine and its derivatives and theanine.

as well as amino and nucleic acids (~8%). Among the 19 amino acids found in tea, theanine (γ-*N*-ethyl glutamine) (Figure 4) is unique to tea. It is a significant component of both green tea and black tea, comprising about 3% wt/wt of extract solids. Theanine has been associated with improved flavor and antihypertensive effect. It can also be made synthetically on a commercial scale with good yield.

Potassium, calcium, magnesium, and aluminum are the predominant minerals (10–15%) found in the ash of the water-soluble solids of tea. Tea beverage is also a significant source of fluoride at ~1 mg per serving.

Impact of Processing on the Profile of Tea Components

There are two major enzymes involved in the fermentation process in making oolong and black tea. One is polyphenol

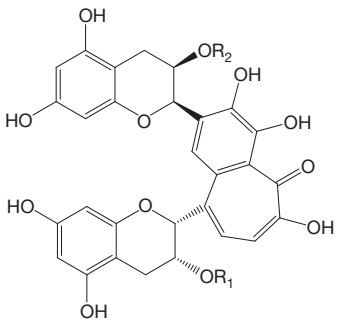
oxidase (PPO), which plays a key role in the oxidation of flavanols to black tea components such as theaflavins and thearubigins. This enzyme is compartmentalized in the leaf microsomes and is released by the rolling and crushing of tea leaves after the withering process, which allows the enzyme to react with the cytoplasmic catechins in tea leaves. It is mainly the action of this enzyme that produces theaflavins. Many studies have been carried out on the PPO-catalyzed formation of black tea oxidation products. Peroxidase (POD) can also catalyze the oxidation of *o*-diphenols to their quinones using peroxide, such as hydrogen peroxide, that were formed by the action of PPO on certain flavanols. In fresh tea leaves, the specific POD activity is more than five times higher than that of PPO and was found to increase during black tea manufacturing. During the firing step of black tea processing, PPO is inhibited thermally, whereas POD remains active to a certain extent. Model oxidation systems have also been used to compare the oxidation products obtained with tea PPO and with horseradish POD. However, the contribution of POD to the formation of black tea pigment in tea fermentation is still not clear. The major polyphenols in black tea are theaflavins and thearubigins, which are formed by the oxidation and polymerization of catechins during fermentation. Theasinensins are the major oxidized catechins found in oolong tea.

Theaflavins

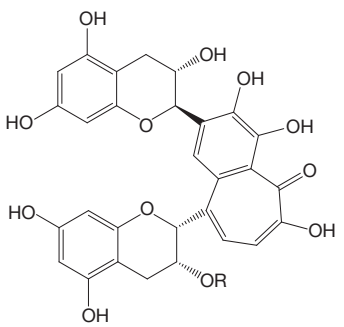
Theaflavins are responsible for the bright red color of black tea and possess a benzotropolone skeleton that is formed from the co-oxidation of selected pairs of catechins, one with a *vic*-trihydroxyphenyl moiety and the other with an *ortho*-dihydroxyphenyl structure. The reaction involves the oxidation of B ring of the catechin molecule to the quinone, which will react with the quinone of a gallocatechin through a Michael addition followed by carbonyl addition across the ring and subsequent decarboxylation to form theaflavins. It is known that theaflavins make important contributions to the properties of black tea, such as color, 'mouthfeel,' and extent of tea cream formation. Their structures are well studied. There are four major theaflavins in black tea, these include theaflavin, theaflavin 3-gallate, theaflavin 3′-gallate, and theaflavin 3,3′-digallate (Figure 5), which are produced by oxidative coupling between EC and EGC, EC and EGCG, ECG and EGC, and ECG and EGCG, respectively. Stereoisomers of theaflavins and a number of theaflavin derivatives, including theaflavic acids and theaflavates, have also been reported from black tea. Our recent study showed that horseradish POD can oxidize tea catechins to form theaflavin-type compounds in the presence of H_2O_2. Eighteen benzotropolone derivatives, which include all the major theaflavins, theaflavates, and theaflavic acids reported in black tea as well as several new benzotropolone derivatives, were synthesized by the reaction of selected pairs of compounds, one with a *vic*-trihydroxyphenyl moiety and the other with an *ortho*-dihydroxyphenyl structure, using horseradish POD in the presence of H_2O_2.

Thearubigins

Thearubigins, the brown pigment in black tea, are heterogeneous polymers of tea catechins. They account for up to 60% of the water-extractable material of black tea by dry weight. Information about their formation, structures, and contribution to black tea quality is still very limited. A partial structure of thearubigins from black tea was elucidated using chemical degradation, which indicated that these compounds were heterogeneous polymers of flavan-3-ols and flavan-3-ol gallates having bonds at C-4, C-6, C-8, C-2′, C-5′, and C-6′ in the flavan-3-ol units. In addition, the possible participation of theaflavins in the formation of thearubigins has been suggested. It was shown that horseradish POD would oxidize theaflavins into thearubigin-like compounds in the presence of H_2O_2. We found that the galloyl ester group of theaflavin 3-gallate is as reactive as the B ring (*vic*-trihydroxy) of EGCG or EGC and the galloyl ester group of ECG and can further react with EC to form the new theaflavin-type tea catechin trimer, theadibenzotropolone A, which was characterized from black tea extract by LC/ESI-MS/MS. Two additional peaks corresponding to the same molecular weight as that of theadibenzotropolone A were also found in black tea extract. To identify these unknown peaks, two new dibenzotropolones, theadibenzotropolones B and C, together with one new tribenzotropolone, theatribenzotropolone A (Figure 6), were synthesized by the reaction of theaflavins

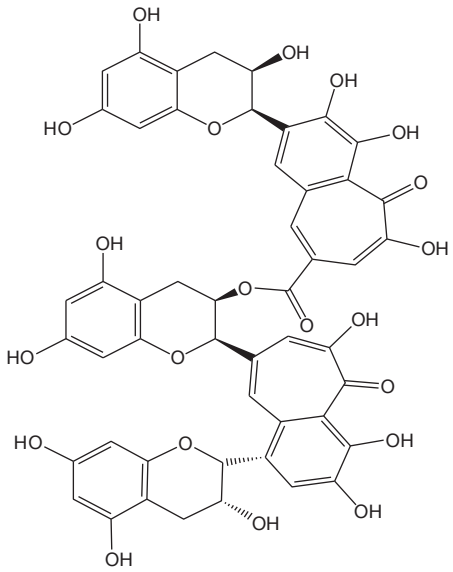


Theaflavin: $R_1 = R_2 = H$
Theaflavin 3-gallate: R_1 = Galloyl, $R_2 = H$
Theaflavin 3′-gallate: $R_1 = H$, R_2 = Galloyl
Theaflavin 3,3′-digallate: $R_1 = R_2$ = Galloyl

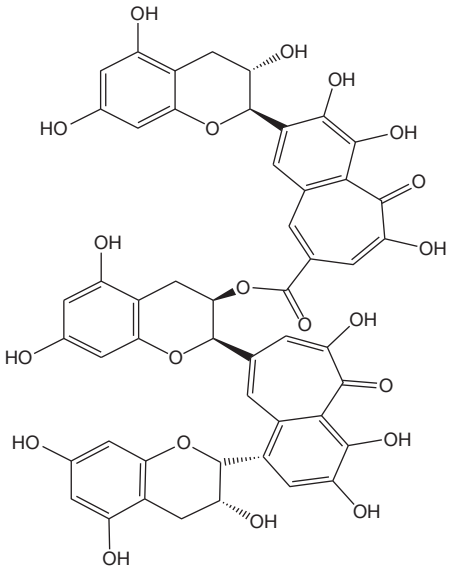


NeoTheaflavin: R = H
NeoTheaflavin: 3-gallate: R = Galloyl

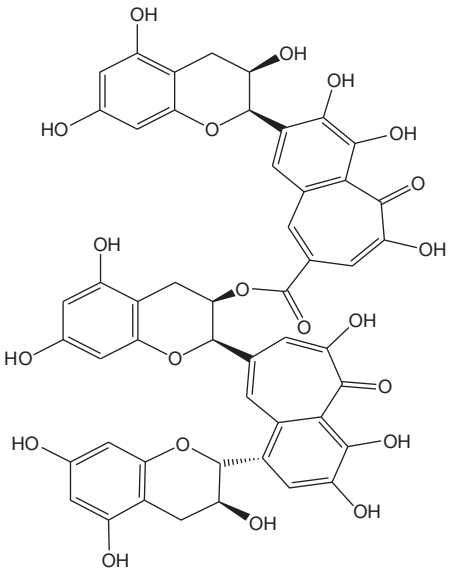
Figure 5 Structures of major theaflavins in tea.



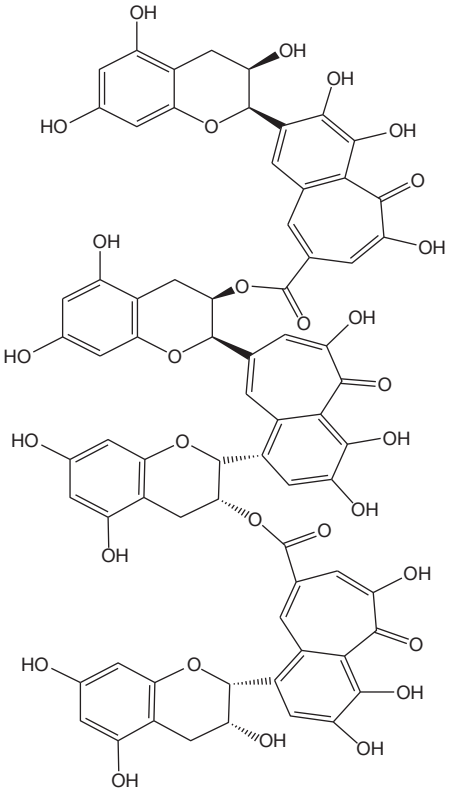
Theadibenzotropolone A



Theadibenzotropolone B



Theadibenzotropolone C



Theatribenzotropolone A

Figure 6 Structures of theadibenzotropolones A, B, and C and theatribenzotropolone A.

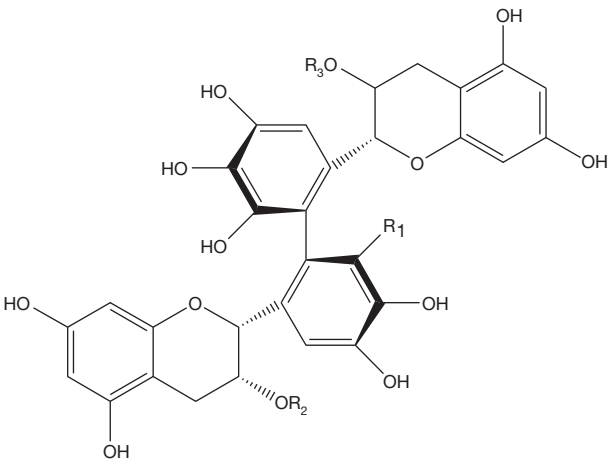
and tea catechins catalyzed by horseradish POD in the presence of H_2O_2. Theaflavin 3-gallate reacts with not only EC to form theadibenzotropolone A but also catechins to form the isomer of theadibenzotropolone A, theadibenzotropolone B, whereas the isomer of theaflavin 3-gallate, neotheaflavin 3-gallate, reacts with EC to form theadibenzotropolone C. Interestingly, theatribenzotropolone A, instead of the two isomers of theadibenzotropolone expected, was obtained through the reaction between theaflavin 3,3′-digallate and EC. We failed to obtain theaflavin 3′-gallate-related theadibenzotropolone derivatives. We further

confirmed the existence of theadibenzotropolone B in the black tea extract prepared from Yunnan black tea using tandem mass spectrometry.

Our results clearly show that theaflavins can further react with tea catechins to form di- or tribenzotropolone-type compounds. The observation that the galloyl ester group of theaflavins can be oxidized to form di- or tribenzotropolone skeletons strongly implies that this type of oxidation is an important pathway to extend the molecular size of thearubigins. Theadibenzotropolones A and B are the first theaflavin-type trimers of catechins discovered in black tea. Using delayed pulsed ion extraction of ions generated via the matrix-assisted laser desorption ionization (MALDI) technique, on line with a linear time-of-flight (TOF) mass spectrometer, we found that theasinensins and/or procyanidins, which are dimers or oligomers of catechins, also react with catechins to generate benzotropolone-type polymers further indicating that this type of oxidation is one of the major pathways for the formation of thearubigins. More recently, Kuhnert and coworkers reported that thearubigins are solely composed from low-molecular-weight compounds with a mass below 2100 g mol^{-1} based on results generated from MALDI-TOF-MS. Whether this conclusion is due to the limitation of current analytic tools to detect larger-molecular-weight components in thearubigins is a topic for further study in order to completely understand the chemistry of thearubigins.

Theasinensins

Theasinensins are compounds formed by the coupling of quinone with catechol or the pyrogallol ring of catechins. Seven theasinensins have been isolated and identified from oolong tea with theasinensins A (EGCG dimer), B (EGCG and EGC dimers), C (EGC dimer), and F (EGCG and ECG dimers) being the most abundant ones (Figure 7). They occur only in very small quantities in black tea probably due to the instability of these compounds during further oxidation and polymerization.



Theasinensin A: R_1 = OH, R_2 = R_3 = Galloyl
Theasinensin B: R_1 = OH, R_2 = Galloyl, R_3 = H
Theasinensin C: R_1 = OH, R_2 = R_3 = H
Theasinensin F: R_1 = H, R_2 = R_3 = Galloyl

Figure 7 Structures of theasinensins A, B, C, and F.

Conclusions

The chemistry of green tea has been well studied. However, information on black tea and oolong tea polyphenols is still lacking. The chemistry and the formation mechanism of thearubigins are almost totally unknown. These large-molecular-weight black tea polyphenols, even though not absorbed, may exert effects in the oral–digestive tract through direct contact or through their microbial metabolites. Further study on the chemistry of thearubigins using advanced analytic techniques is warranted. The formation mechanism of thearubigins under different processing conditions is another topic of future research.

See also: Tea: Types, Production, and Trade; Tea: Health Effects.

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Tea: Health Effects

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Types, Sources, and Production of Tea

Tea, prepared by infusion of the dried leaves of *Camellia sinensis*, is a popular beverage in many parts of the world. Archaeological evidence indicates that tea was first consumed during the early Paleolithic era, but the first documented references to tea appeared in China in the third century CE, when it was used as a medicine. Tea was imported into England in 1657, regarded at that time as a medicinal herb, and later grew in popularity as a beverage.

Several types of tea are known, differing in the method of treatment of the leaves after harvesting. The most popular type is black tea, which accounts for some 80% of the market. This is prepared by crushing the leaves, followed by a period of fermentation before drying. The preparation of green tea, which is consumed mainly in Japan and China, involves no crushing or fermentation. Leaves for the preparation of oolong tea, produced mainly in south eastern China, are gently rolled and subjected to a brief period of fermentation.

India is the largest tea producer, followed by China, Sri Lanka, and Kenya. On a per capita basis, the highest consumers of tea are in Qatar, Ireland, the United Kingdom, Iraq, Turkey, and Kuwait.

Dried aqueous or alcoholic extracts of tea leaves are also available on the market, where they are sold as dietary supplements with putative benefits to human health.

Tea Composition

The leaves of *C. sinensis* contain a number of polyphenols, of which (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epigallocatechin gallate (EGCG) (Figure 1) predominate.

These compounds are preserved in green tea, but the crushing and fermentation processes involved in the manufacture of black tea result in the oxidation of a large proportion of these substances to theaflavins, thearubigins, and other oligomers. The theaflavins (Figure 2) are responsible for the characteristic color and taste of black tea. The degree of oxidation is lower in oolong tea, and this contains a lower proportion of theaflavins than black tea.

As discussed in the succeeding text, these substances are believed to be responsible for the biological effects of tea.

A 200 ml cup of tea contains 150–210 mg of total polyphenols, of which the majority is catechins in green tea and theaflavins and thearubigins in black tea.

Bioavailability of Tea Components

As expected for relatively high molecular weight compounds bearing numerous hydroxyl groups, the absorption of tea polyphenols from the small intestine of animals and of humans is low. Significant amounts pass into the large intestine, where the polyphenols are degraded by gut bacteria to 5-(3′4′,5′-trihydroxyphenyl)-γ-valerolactone, hydroferulic acid, 3,4-dihydroxyphenylacetic acid, and hydrocaffeic acid. In mice, plasma levels of ECGC were only 1.3–1.5 μM even after a very high dose (1.6 g kg^{-1}), and low absorption has also been seen in rats. In these animals, plasma levels of EGCG increased to a maximum after 14 days of continuous administration in drinking water, but then rapidly declined by day 28, possibly reflecting an increased rate of conjugation and excretion. Absorption in dogs appears to be much higher than that in rodents. In fed animals dosed each day with EGCG at 500 mg kg^{-1}, plasma levels were 16.5 and 12.5 μM at day 1 of the experiment in male and female dogs, respectively, and plasma levels were similar after 78 days of feeding. In fasted dogs given the same dose of EGCG, plasma levels were much higher, with

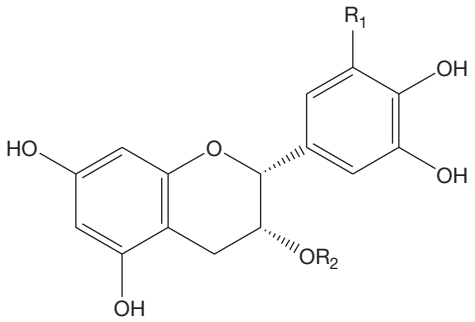

Figure 1 (−)-Epicatechin (EC): $R_1 = R_2 = H$; (−)-epigallocatechin (EGC): $R_1 = OH$, $R_2 = H$; (−)-epicatechin gallate (ECG): $R_1 = H$, $R_2 =$ galloyl; (−)-epigallocatechin gallate (EGCG): $R_1 = OH$, $R_2 =$ galloyl.

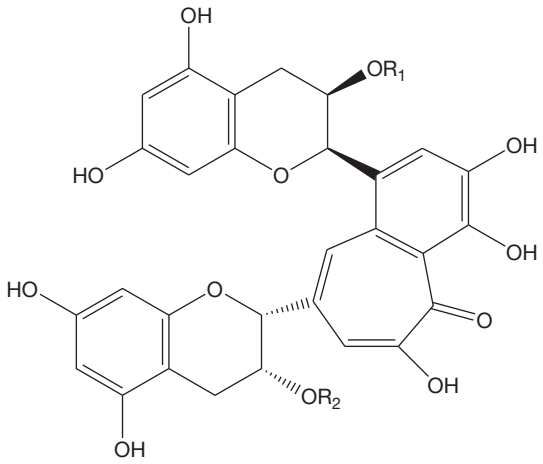

Figure 2 Theaflavin, $R_1 = R_2 = H$; theaflavin-3-gallate, $R_1 = H$, $R_2 =$ galloyl; theaflavin 3′-gallate, $R_1 =$ galloyl, $R_2 = H$; theaflavin-3,3′-digallate, $R_1 = R_2 =$ galloyl.

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concentrations of 50.9 and 30.9 μM being recorded in male and female animals at day 1 of the experiment and levels increased with prolonged administration, being approximately double the day 1 concentration after 81 days.

In humans, it was estimated that < 0.1% of EGCG is absorbed from green tea, and doses of up to 800 mg of this substance gave plasma levels of 1 μM or less when given with food. As in the case of dogs, absorption of EGCG in fasted individuals was shown to be higher than that in fed, and a plasma level of 8.7 μM was observed after a dose of EGCG of 1200 mg in fasted volunteers. A dose of 700 mg of theaflavins in humans, equivalent to 30 cups of tea, gave a maximum plasma theaflavin concentration of only 1 $\mu g\, l^{-1}$. Most of the absorbed polyphenols from both black tea and green tea is rapidly metabolized in the liver, and the major plasma constituents are the glucuronic acid conjugates and methyl ethers of the polyphenols. These metabolites are excreted in the urine, with only traces of the unchanged polyphenols. The 2′- and 2″-cysteinyl conjugates of EGCG were found in the urine of mice given high doses of this substance. These are derived from the o-quinone of EGCG, indicating that oxidation of EGCG occurs *in vivo*, as it does *in vitro* (see in the succeeding text).

Potential Health Benefits of Tea and Its Components

Antioxidant and Pro-oxidant Effects

Because of the presence of the catechol or pyrogallol moieties in the constituent polyphenols and theaflavins, both green and black teas are strong antioxidants, with similar activity *in vitro*. They have been shown to scavenge many oxidizing species, including superoxide radical, hydrogen peroxide, hydroxyl radical, peroxyl radicals, hypochlorous acid, and peroxynitrous acid. Among the green tea constituents, the order of antioxidant activity is EGCG = ECG > EGC > EC. The antioxidant action of phenols depends upon their ability to form a phenoxyl radical, and the potency of the antioxidant effect is proportional to the stability of this radical. The gallate moiety in green tea polyphenols is particularly effective in stabilizing the phenoxyl radical, which would account for the relatively high antioxidant activity of these compounds.

While there is clear evidence of an antioxidant effect of both black and green polyphenols *in vitro*, there is no convincing evidence of an antioxidant effect of tea or tea polyphenols *in vivo*. This is as expected from the low bioavailability of these substances, since the low plasma levels that are achieved *in vivo* are unlikely to compete with endogenous antioxidants such as ascorbate, tocopherols, and urate. Furthermore, *in vivo* metabolism by methylation and glucuronidation will decrease antioxidant activity. These conjugates are less effective than the parent compounds, due to blocking of the phenolic hydroxyl groups required for antioxidant activity.

Like other antioxidants, tea polyphenols also act as pro-oxidants through their ability to undergo autoxidation, forming superoxide radical, hydrogen peroxide, and hydroxyl radical, collectively known as 'reactive oxygen species' (ROS). Brewed tea contains hydrogen peroxide, resulting from polyphenol oxidation, and urinary levels of hydrogen peroxide are increased after tea consumption.

The autoxidation process of tea polyphenols is initiated by ionization to the phenolate anion (reaction 1), which reacts with molecular oxygen either directly (reaction 2) or through the participation of transition metals, yielding superoxide radical. Superoxide is also formed in the reaction of the semiquinone with molecular oxygen (reaction 3), initiating a superoxide-driven chain reaction (reaction 5). The quinone formed in reaction 3 undergoes comproportionation with the catechol, yielding 2 molar equivalents of the semiquinone (reaction 4). The ultimate products of the autoxidation are the *o*-quinone and hydrogen peroxide. The quinone has been shown to undergo reduction by NADH and thiols, establishing a redox cycle between the quinone and catechol. In the presence of a transition metal, hydroxyl radical is formed from hydrogen peroxide.

Autoxidation of tea polyphenols occurs at neutral pH, although the rate increases with increasing pH, reflecting higher concentrations of the phenolate anion. Tea polyphenols also readily undergo autoxidation in tissue culture media, and ROS production in this situation may be of significance in interpreting the toxic effects of these substances in cancer cells *in vitro*, as discussed in the succeeding text. The rates of autoxidation differ among the various tissue culture media. It is particularly high in Dulbecco's modified Eagle's medium, which contains added iron salts, effective in catalyzing the autoxidation reaction.

The relative rates of autoxidation of green tea polyphenols at neutral pH correlate with their antioxidant activity, with EGCG and EGC being stronger pro-oxidants than ECG and EC. In the gallate derivatives, the reaction proceeds via semiquinone formation on the B ring, with formation of the radical on the central hydroxyl group of the gallyl ring.

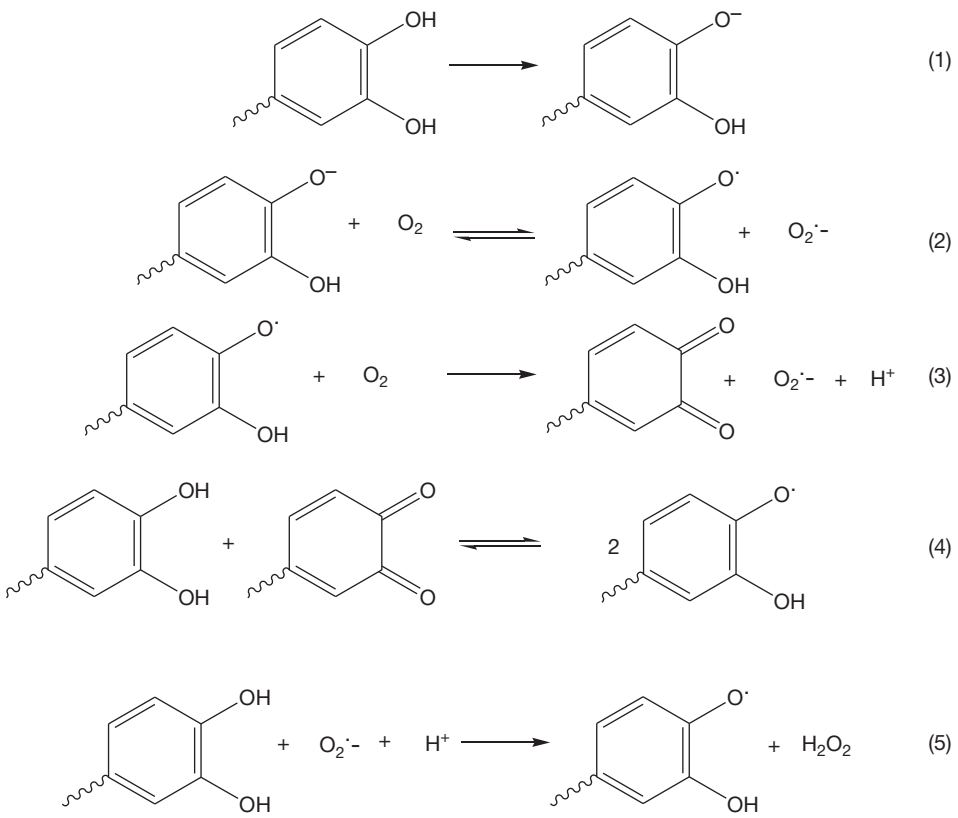
In the presence of salts of copper or iron, tea and tea polyphenols cause DNA cleavage, mediated by ROS. The effect on DNA is inhibited by catalase or by metal chelators.

The antioxidant activity of tea and tea polyphenols has attracted considerable interest, in view of the widely held public perception that ROS generation *in vivo* leads to degenerative diseases and that the initiation or progression of such diseases could be prevented by antioxidants. It was also recognized that the pro-oxidant activity of tea and its components could be valuable, particularly against cancer. Many of the drugs currently used in cancer therapy are pro-oxidants, and the effect of tea polyphenols on cancer cells *in vitro* and on cancer xenografts *in vivo* has been extensively studied. Preliminary experiments on the possible use of tea polyphenols in cancer therapy have also been conducted, as discussed in the succeeding text. The ability of tea constituents to protect against chemically induced cancer has also been extensively studied.

Tea and Cancer

Effect of Tea and Tea Constituents on Cancer Cells *In Vitro*

Green tea and black tea and their constituent polyphenols have been shown to be toxic to many human and animal cell lines *in vitro*, including those derived from the brain, pancreas, prostate, liver, urinary bladder, oral cavity, breast, larynx, colorectum, bone marrow, skin, ovary, tongue, cervix, lung, and miku stomach, to leukemia, sarcoma, and melanoma cells. The toxic changes comprise inhibition of cell growth, cell cycle arrest, and cell death. In most cases, the mechanism of cell death has been identified as apoptosis via the intrinsic



pathway, involving decreased mitochondrial membrane potential, release of cytochrome *c*, and activation of caspase 9 and caspase 3. For the expression of these cytotoxic effects, polyphenol concentrations $>50\,\mu M$ are generally required, and some dose–response studies have shown an increase in cell proliferation at lower concentrations. Comparative studies have shown that normal (nontransformed) cells are relatively resistant to the toxic effects of tea polyphenols.

Among the green tea polyphenols, cytotoxicity to cancer cells has generally been found to decrease in the order EGCG > EGC > ECG > EC. Among the black tea constituents, theaflavin 3,3′-digallate is more cytotoxic than theaflavin 3-gallate, theaflavin 3′-gallate, and theaflavin.

In many cases, ROS have been detected in cells incubated with tea or tea polyphenols, and these substances have been held responsible for the observed toxic effects. Complete or partial protection has been observed by the destruction of extracellular hydrogen peroxide by the addition of catalase to the culture medium. Pyruvate, which similarly detoxifies hydrogen peroxide, has also been shown to protect against the cytotoxic effects.

Addition to the medium of *N*-acetylcysteine, which is known to increase intracellular levels of reduced glutathione and thus potentiate antioxidant defense, has been shown to protect against the cytotoxicity of tea polyphenols, as has superoxide dismutase and metal chelating agents.

While there is good evidence for the involvement of ROS in cytotoxicity *in vitro*, the site of production of these species is uncertain. Tea polyphenols are taken up by cells *in vitro*, where they may undergo autoxidation to form ROS. But, as mentioned previously, the polyphenols readily undergo autoxidation in cell culture media, and hydrogen peroxide formed extracellularly may cross the cell membrane and exert toxic effects within the cells. The inhibitory effect of extracellular catalase is consistent with either intracellular or extracellular generation of hydrogen peroxide, since this substance readily crosses cell membranes, so that intracellularly generated hydrogen peroxide could be detoxified by extracellular catalase. The inhibitory effect of superoxide dismutase, however, definitely indicates extracellular production of ROS, since the superoxide radical does not cross cell membranes, and SOD may protect by decreasing the rate of polyphenol autoxidation in the medium. The effect of metal chelators would also be consistent with extracellular formation of ROS.

Effect of Tea and Tea Constituents on Cancer Xenografts *In Vivo*

The toxic effects of tea polyphenols seen in cancer cells *in vitro* stimulated research on the possible destruction of cancer xenografts in animals. Decreased tumor growth of PC-3, 22Rv1, LNCaP, or LAPC4 prostate cancer cells injected subcutaneously into athymic or severe combined immunodeficient mice was observed with green tea polyphenols. EGCG was more effective than EGC, ECG, or EC. Green tea polyphenols decreased the growth of breast cancer cells 4T1, MCF-7, and MDA-MB-231

implanted in mice, and survival time was increased. EGCG decreased the growth of H1299 lung cancer cells, and apoptosis and oxidative DNA damage, as indicated by increased levels of 8-hydroxydeoxyguanosine, were observed in the cancer cells but not in the host tissue. Evidence for oxidative damage to DNA was also observed in LPPC4 xenografts, along with oxidative damage to proteins. Again, no such effects were seen in normal tissue. Apoptosis in tumor tissue was observed in lung, prostate, and breast cancer xenografts, indicating irreversible damage to the cells. In the experiment with PC-3 cells, however, the tumors were found to undergo rapid growth after the administration of EGCG ceased.

Clinical Trials with Tea Polyphenols

The effects of tea polyphenols on cancer cells *in vitro* and in xenografts *in vivo* stimulated interest in the possible use of these materials as cancer chemotherapeutics, and these substances have been tested in phase I and phase II clinical trials in cancer patients. In a phase I trial, a new treatment is tested in a small group of people in order to determine a safe dosage range and to identify side effects. A phase II trial involves a larger group of people to test efficacy and to further evaluate the safety of the test material.

In two studies without a placebo group, a decrease in plasma markers associated with prostate cancer was observed in men with prostate cancer, but a later double-blind placebo-controlled trial showed no effect, as did a study on hormone-refractory prostate cancer. In a small proof-of-principle study, green tea polyphenols or a placebo was dosed to men with high-grade intraepithelial prostatic neoplasia. After 1 year, only 1 tumor was detected among the 30 treated men, while nine cancers were seen among the 30 men who received the placebo. A follow-up study of available participants after 2 years showed a persistent effect, with only 1 prostate cancer in 13 treated men, compared to 2 out of 9 controls. It is reported that larger trials are under way. No significant effect on markers of cell proliferation was seen in the cancer tissue of women given green tea polyphenols for an average of 35 days before surgery for breast cancer. No clinical benefit was seen in a double-blind placebo-controlled study on cervical intraepithelial neoplasia or in individuals with advanced lung cancer, but a phase II trial with high doses of tea polyphenols in patients with asymptomatic chronic lymphocytic leukemia showed a decline in absolute lymphocyte count and palpable adenopathy in the lymph nodes of 31% of the participants. Liver damage, as indicated by increased plasma levels of alanine aminotransferase and aspartate aminotransferase, was observed in some participants in this trial.

Protection against Chemical Carcinogens by Tea and Tea Constituents in Animals

The possible protective effect of tea and tea polyphenols against a variety of chemical carcinogens, including nitrosamines, heterocyclic amines, polycyclic aromatic hydrocarbons, azoxymethane, 1,2-dimethylhydrazine, and *N*,*N*-dimethylaminoazobenzene, has been investigated in rodents. Various test protocols have been employed, involving the administration of the test materials before, after, or before, during, and after exposure of the animals to the carcinogen. Results have been variable. Some studies have shown a decreased incidence of cancer in the treated animals, although most positive studies have shown no effect on cancer incidence but a decrease in tumor multiplicity and/or tumor size and an increase in latency time to first tumor. Other experiments have shown no effect on cancer incidence or progression, while increases in tumor multiplicity and tumor size have occasionally been reported. In general, green tea has been found to be more effective than black tea, and protection was seen after administration of the test material both before and after the carcinogen, indicating effects on both initiation and progression of cancer.

The effect of tea polyphenols on adenomatous polyposis coli (APC^{min+}) mice has been extensively studied, with most experiments showing a decrease in the spontaneous development of intestinal tumors in these animals. However, EGCG in combination with genistein, which increases the bioavailability of the polyphenol, was surprisingly found to increase the multiplicity of intestinal tumors in APC^{min+} mice. Green tea polyphenols have been shown to inhibit the incidence of prostate cancer in the transgenic adenocarcinoma of mouse prostate mouse model and to decrease spontaneous lung, mammary, and hepatic tumors in certain mouse strains.

Epidemiological Studies on Tea and Cancer

The possible effect of tea consumption on cancer incidence at various sites, including the brain, breast, cervix, colorectum, esophagus, head and neck, kidneys, larynx, lungs, mouth, nasopharynx, ovaries, pancreas, prostate, renal pelvis and ureter, skin, stomach, and urinary bladder, has been investigated. Results are inconclusive, with some studies showing a decrease in cancer incidence in individuals consuming large amounts of black or green tea, others showing no effect, and some indicating an increase in cancer susceptibility. One consistent observation is the increase in esophageal cancer in individuals consuming very hot tea, presumably reflecting repetitive thermal damage to the esophageal epithelium. Similar effects have been recorded in individuals consuming other hot liquids and are likely attributable to the temperature of the liquid rather than its composition.

Tea and Cardiovascular Disease, Stroke, and Diabetes

There is evidence that tea polyphenols decrease fat absorption, as reflected in an increased level of fecal lipids, and green tea has been shown to decrease plasma lipid levels in rats. The effect of tea on blood cholesterol levels has been extensively investigated. Most studies have shown a significant decrease in total cholesterol and LDL cholesterol ('bad cholesterol'), with no significant effect on HDL cholesterol ('good cholesterol'). Other clinical trials with green tea and black tea indicated small, but statistically significant, decreases in systolic and/or diastolic blood pressure.

Several cohort studies have shown an inverse association between tea consumption and risk of cardiovascular disease, although others have indicated no such effect, and one study in Welsh men indicated an increased risk of heart disease with tea consumption. Case control studies gave similar results. Many of the positive studies have been conducted in China and

Japan, where green tea consumption is high. Black tea has shown no effect in several studies, although a European study did show a positive effect in continental Europe, but not in the United Kingdom.

Several epidemiological studies have shown a decreased risk of stroke with tea consumption, while others have shown no effect, and one study suggested an increased risk. An inverse association between consumption of green tea, but not black tea or oolong tea, and risk of type 2 diabetes has been reported.

Induction of Phase 2 Enzymes by Tea and Its Constituents

Increases in the activity of phase 2 enzymes such as glutathione *S*-transferase (GST), NAD(P)H quinone oxidoreductase 1 (NQO1), and uridine 5′-diphosphoglucuronosyltransferase (UDPGT) have been demonstrated in animals and in humans dosed with tea and tea polyphenols. These enzymes detoxify harmful chemicals and facilitate their elimination from the body, and their induction has been shown to protect against many toxic chemicals, including carcinogens. GST was induced in the stomach, small intestine, liver, and lungs of mice; in the mammary gland and liver of rats; and in human lymphocytes. Increased levels of NQO1 were observed in the mammary gland of rats; in the stomach, small intestine, liver, and lungs of mice; and in the liver of hamsters, and UDPGT was increased in the liver and lung of rats. In animals dosed with green tea, black tea, or green tea polyphenols, increased amounts of the detoxification products of aflatoxin B_1 in humans and of 2-amino-3-methylimidazo[4,5-*f*]quinoline in rats have been reported, which may reflect increased activity of phase 2 enzymes.

Toxicity of Tea Constituents to Animals

Single high doses (up to 2000 mg kg^{-1}) of EGCG given by gavage to fasted mice caused severe hepatic necrosis, accompanied by increased malondialdehyde levels in the liver, indicative of oxidative stress. Repeated administration of green tea polyphenols (up to 1000 mg kg^{-1} day^{-1}) by gavage to rats and mice over a period of weeks also induced hepatic necrosis. No evidence of liver damage was observed, however, when similar or higher doses were administered in food or drinking water. Severe toxicity has been observed in fasted dogs given daily doses of EGCG or green tea polyphenols in capsules at relatively low levels (150–200 mg kg^{-1} day^{-1}), including death, centrilobular hepatic necrosis, necrosis of the renal proximal tubules, thymic atrophy, and hemolytic anemia. In contrast, no effect was seen in fed dogs dosed with EGCG at 500 mg kg^{-1} day^{-1}, presumably reflecting the lower degree of absorption of this substance in fed animals.

Toxicity of Tea Constituents to Humans

As noted earlier, evidence of hepatotoxicity was observed in a clinical study with high doses of tea polyphenols. Such compounds are now readily available as 'dietary supplements,' which are marketed primarily as antioxidants and inducers of weight loss. Uncontrolled use of such supplements is not, however, without risk, since liver damage, involving hepatocellular necrosis and cholestasis, has been observed in users of such supplements. While this is an idiosyncratic reaction, seen only in a small minority of consumers, it is a serious problem. In view of such observations, the safety of green tea extracts was reviewed by the US Pharmacopeia Dietary Supplement Information Expert Committee, who classified them in class 2, requiring a warning statement on the product label.

Conclusions

Tea is a popular drink worldwide, with a history of putative beneficial effects on human health. Such history has led to many investigations into the possible beneficial effects of tea and its constituents, with a major focus on green tea and its major constituent EGCG, and on potential effects on cancer. The antioxidant effects of tea have long been discussed, though it is unlikely that tea constituents, or other chemical antioxidants, can compete with the enzymatic antioxidants *in vivo* and thereby augment antioxidant defenses.

There is good evidence for the generation of ROS by tea and tea constituents in isolated cells *in vitro*, and it appears that such species are largely responsible for the cytotoxic effects observed. It is difficult to extrapolate from *in vitro* effects to *in vivo* benefits, however, since much higher concentrations were employed *in vitro* than those that could be achieved *in vivo*. Furthermore, oxygen partial pressure in cell culture media (~150 mmHg) is much higher than that in blood or tissues (<40 mmHg), and the higher oxygen levels will enhance ROS production, and cell systems often do not replicate metabolism *in vivo*. The ability of tea polyphenols to undergo autoxidation, with the generation of ROS, in culture media leads to the conclusion that it is likely that extracellular autoxidation of the tea polyphenols is responsible, at least in part, for the cytotoxic effects of these substances *in vitro*. It is interesting to note that low levels of tea polyphenols have been reported to increase cell proliferation *in vitro* while causing growth inhibition or cell death at high levels. Similar effects have been seen with other ROS producers *in vitro*.

There is comparatively little information on the generation of ROS from tea polyphenols *in vivo*, although some studies have shown evidence of oxidative damage in xenografts after administration of tea polyphenols.

Effects of tea and its constituents on chemically induced cancer are interesting and may be related to the ability of these compounds to increase tissue activities of phase 2 enzymes. Effects on relatively few tissues of rodents have been investigated so far, and a detailed study of organ specificity would be valuable.

Tea constituents have been shown to cause toxic effects in laboratory animals, with fasted animals being more susceptible than fed. It has also been shown that administration via dietary feeding leads to fewer toxic effects than when equivalent amounts are given by bolus delivery, an important point with regard to the relative safety of normal tea consumption compared to the use of tea constituents as therapeutics or dietary supplements.

The bioavailability of tea constituents is low, and efforts have been made to increase bioavailability by use of specific vehicles or by chemical modification. Of particular interest is the work on polyacetylated derivatives, which are well absorbed and are subsequently hydrolyzed to the parent phenols intracellularly. The potential of such compounds in therapy deserves further investigation, although the possibility of toxic effects must be kept in mind.

Reports of potential beneficial effects of tea, particularly green tea, stimulated a market for green tea extracts for use as dietary supplements, and the market is predicted to grow to US $368 million by the year 2020. While clinical trials have generally shown good tolerance in volunteers (although individuals in one experiment given very high doses showed evidence of liver damage), personal use is different from that in a clinical setting in that dosage levels may not be accurately followed. Furthermore, it is possible that individuals using such products for weight loss may be fasting, which will increase the absorption of these substances and thus enhance toxicity. Regulators in the United States have advised that such supplements should be taken with food, not in the fasting state, and that the liver function of users should regularly be checked.

The results of epidemiological studies on tea are presently inconclusive, particularly with regard to cancer incidence. Large prospective cohort studies are required to provide a definitive conclusion with regard to the possible health benefits of tea.

With the green tea polyphenols, there is a correlation between autoxidation rate and pro-oxidant activity, cytotoxicity, and effects on xenografts, suggesting a common role of ROS in all these processes. Similarly, ROS are often involved in the induction of phase 2 enzymes. Other natural products that generate ROS are currently under investigation as cancer preventative agents and therapeutics, and it is possible that given appropriate doses and specific therapeutic targets, some such compounds will in the future prove valuable in the promotion of human health.

See also: Antioxidants: Role on Health and Prevention; Beverage: Health Effects; Cancer: Diet in Cancer Prevention; Tea: Types, Production, and Trade; Tea: Chemistry and Processing; Phenolic Compounds: Bioavailability and Health Effects.

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Tea: Types, Production, and Trade

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Tea infusions, which are consumed by a large number of the world's population, are obtained from the leaves of *Camellia sinensis* plant. Tea leaves are primarily manufactured as green, black, or oolong, with black tea representing ~80% of tea products consumed. Regular intake of tea is associated with an improved antioxidant status that may contribute to lowering the risk of heart disease and reduced mutagenicity. Tea possesses significant antioxidative, anti-inflammatory, antimicrobial, anticarcinogenic, antihypertensive, neuroprotective, cholesterol-lowering, and thermogenic properties. Tea has attracted considerable attention for its potential cardioprotective effects, and the primary chemical components of tea are thought to have a beneficial effect by reducing arterial stiffness. Tea polyphenol, caffeine, as well as theanine, are considered as three major bioactive secondary metabolites in *C. sinensis*. The ratio of total polyphenols content to theanine content could be used as a featured parameter for differentiating teas, and theanine in teas could be a reliable quality parameter that is complementary to total polyphenols. In this article, origin, history, types of tea introduced, as well as tea production and consumption are discussed.

Origin and History of Tea

Tea plants are native to East and South Asia and probably originated around the meeting points of the lands of north Burma and southwest China. The tea plant – *C. sinensis* has been cultivated by the Chinese people for more than 2700 years. The Chinese certainly knew of the tea plant and its value to them long ago and had discovered that the infusion of leaves produced a very pleasing drink, if carefully processed. The leaves of tea plant were considered as for medicinal purposes at first, then later as a beverage.

Tea was discovered around 28 BC, the Chinese Shennong time. The Herbal Lection by Shennong quotes: I tasted many herbs and was poisoned, tea healed me. This was the first time that tea had been recorded in history. In Chunqiu times, the person named Ying Xiang Qi Jing Gong (547–490 BC) wrote in his book *Yan Zi Chun Qiu*: Using corn, eggs and tea leaves to prepare dinner. This showed that the tea leaves became a cooking seasoning at that time. It was a common drink during the Qin Dynasty and became widely popular during the Tang Dynasty, when it was spread to Korea, Japan, and Vietnam. In India, it has been used for medicinal purposes for a long period, but apart from the Himalayan region seems not to have been used as a beverage until the British introduced Chinese tea there. Tea was first introduced to Portuguese priests and merchants in China during the sixteenth century, at which time it was termed *chá*. The first European to successfully transplant tea to the Himalayas, Robert Fortune, was sent by the East India Company on a mission to China in 1848 to bring the tea plant back to Great Britain.

As tea is the second most consumed beverage in the world after water, in many cultures it is also consumed at elevated social events, such as afternoon tea and the tea party. Tea ceremonies have arisen in different cultures, such as the Chinese and Japanese tea ceremonies, which employ traditional techniques and sophisticated protocol of brewing and serving tea for enjoyment in a refined setting. One form of Chinese tea ceremony is the *Gongfu tea ceremony*, which typically uses small Yixing clay teapots and black tea.

Types of Tea

In *C. sinensis*, two varieties are found – var. *sinensis* and var. *assamica*. In regard to tea processing suitability, var. *sinensis* has a small leaf and a bush-type habit, which green made in China and Japan is produced from; var. *assamica* has a large leaf and a tree-type habit, which black tea is made from. Tea plants are propagated from seed and cuttings; about 4–12 years are needed for a plant to bear seed and about 3 years before a new plant is ready for harvesting.

Tea is classified into three different varieties or manufacturing types – nonfermented tea, semifermented tea, and completely fermented tea, according to its fermentation level. Green tea, oolong tea, and black tea belong to those three varieties, respectively. Furthermore, there are another three specific tea varieties in China – yellow tea, white tea (lightly fermented tea), and dark tea (postfermented tea), as shown in Figure 1.

The tea fermentation is totally different from the microbial alcoholic fermentations and is defined as the hydrolysis and oxidation of leaf constituents by the endogenous enzymes. As green tea is produced by steaming or pan-firing leaves immediately after plucking, enzyme action is inhibited, and endogenous components in the leaves are retained in the product mainly unchanged; the taste is primarily determined by the choice of clone, time of plucking, shoot maturity, and the cultivation method. In the manufacture of oolong tea, fresh leaves are withered under sunlight for a short period and further indoors for several hours with a gentle rolling. As black tea, the most important physical functions are water removal and particle size determination, whereas the most important chemical aspects occur in withering, fermentation, and drying. The formation of the flavor of black tea is thus initiated during these processes.

'Instant tea,' both hot and cold, is a popular alternative to the brewed products. Similar to freeze-dried instant coffee, but not requiring boiling water, instant tea was developed in the 1930s. The process of instant tea consists of the following operations: selection of raw materials, extraction, aroma stripping, cream processing, concentration, and drying. Every operation will be considered individually. Canned tea is sold prepared and ready to drink. It was introduced in 1981 in

Figure 1 Photographs of six different types of tea products (a, green tea; b, white tea; c, black tea; d, yellow tea; e, oolong tea; f, dark tea).

Japan. In 1983, Swiss-based Bischofszell Food Ltd., was the first company to bottle iced tea on an industrial scale.

Tea Production

In 2003, world tea production was 3.21 million tons annually but by 2010 had reached over 4.52 million tons. The largest producers of tea are the People's Republic of China, India, Kenya, Sri Lanka, and Turkey. Cultivation of the tea plant can be found in more than 60 countries, and tea consumption occurs in more than 160 countries; 2 billion people have the habit of drinking tea in the world. Asia is the main tea-producing area in the world, producing about 80% of the tea. The total tea output of Asian countries reaches 80% in the world. In China, the tea output rose from 676 thousand tons in 2000 to 1552 thousand tons in 2011. The output value rose from 9 billion RMB (Renminbi) in 2000 to 72.8 billion RMB in 2011.

China is the largest producer of tea in the world. A Chinese person discovered the method of plucking tea and tea processing. One-tenth of tea production in China is exported to other district in recent years, and the rest of the tea is for the domestic market. China was not only the largest tea producer, but also the largest exporter of tea in the world in the late nineteenth and early twentieth centuries. The output of tea was 1915 million kg in China in 2012, a leading number for the entire world. Currently 322 million kg of tea is exported, which this year is ranked in second place.

In 2014, China has 41.12 million mu of land for tea plantation area, of which 31.62 million can be plucked. The output of tea was 2092 million kg in China in 2014, and the production of Zhejiang province, Fujian Province, as well as Hunan Province were leading areas.

Sri Lanka is the largest tea exporter in the world. Sri Lanka is an island country in the Indian Ocean (formerly known as Ceylon). The black tea made from Sri Lanka in world market has become more and more popular in recent years. The output of tea was 330 million kg in Sri Lanka in 2012, third in the world after China and India, and 300 million kg of tea were exported, with exports ranked in second place in 2012.

India is one of the largest countries of tea cultivation and export. More than 90% of tea for exporting is black tea in India, whose production is much different from China. A large number of places are suitable for tea plant cultivation, with the area

of Darjeeling and Assam very famous. Approximately 80% tea product is from the Assam area, and the black tea output in the Darjeeling area accounts for 2% of the gross amount.

Japan is a famous area for the cultivation and production of steamed, unshaded green tea (sen-cha). The tea plant was brought from China to Japan by Buddhist monks in the twelfth century, and this led to the production of a steamed brick tea that was used only as a medicine or in upper-class social affairs. The output of tea was 90 million kg in Japan in recent years, and the consumption amount of tea per year according to the number of person is relatively high, reaching 1500 g.

Tea Trade

There have been few changes in three centuries of tea trading. London became the center since drinking tea was first imported to Britain in the seventeenth century. Tea was considered a man's drink to be enjoyed with colleagues at London coffee shops. These were popular meeting places for many walks of life – politicians, lawyers, poets, actors, and writers. By the eighteenth century the ladies of the more affluent classes were going 'Chinese Mad,' using tea as an excuse for displaying their extravagant purchases of Chinese porcelain and Dresden tea sets. A comprehensive tea tray would consist of a teapot and stand, teacups and saucers, a sugar bowl, a milk jug, and a basin. Tea was still expensive and kept in locked tea-caddies. As the nineteenth century progressed, changes in commerce and working hours resulted in the main meal of the day being taken much later in the evening. The prospect of lasting from breakfast until evening did not appeal to the Duchess of Bedford, who was usually credited with being the first to alleviate late-afternoon hunger pangs by introducing a small 4 o'clock meal served with tea.

According to the Food and Agriculture Organization in 2007, the largest importer of tea, by weight, was the Russian Federation, followed by the United Kingdom, Pakistan, and the United States. Kenya, China, India, and Sri Lanka were the largest exporters of tea in 2007 (with exports of 374 229, 292 199, 193 459, and 190 203 tons, respectively). And at that time, the largest exporter of black tea in the world was Kenya, whereas the largest producer (and consumer) of black tea in the world was India.

The tea business, once limited by national borders, has now become more independent than ever. Regional organizations are gaining their own prestige and legal clout. At the global level, the World Trade Organization is now the ultimate authority, with decision-making power that already influences nations tea tariffs, import/export rules, subsidies, and quotas. These regional and global systems are creating new opportunities – and new conflicts – for the entire tea trade. The global process for bringing buyers and sellers more directly together, in this case without auction houses, is termed disintermediation. Disintermediation has already produced cost-effectiveness and shareholder profit for modernized business, with catalog sales of premium tea as a perfect example. Advanced Internet systems, combined with disintermediation, can support a price-stabilizing tea futures market, assuming the market is financially transparent, ethically instituted, and justly enforced.

America entered the tea trade with three American millionaires, T. H. Perkins of Boston, Stephen Girard of Philadelphia, and John Jacob Astor of New York, who all made their fortunes in the China trade. America began direct trade with China soon after the Revolution was over in 1879.

Russian tea tradition included imperial Russia attempting to engage China and Japan in trade at the same time as the East Indian Company. The Russian interest in tea began as early as 1618 when the Chinese embassy in Moscow presented several chests of tea to Czar Alexis.

Tea Consumption

In 1706 the first coffeehouse that offered tea was Tom's Coffee House in London. The House realized that they needed to introduce an added attraction to compete with the many other coffeehouses, and tea was rare, exotic, and extremely expensive. The popularity of tea continued to spread, but it was not until 1839 that the first shipment of Assam India tea was landed in Britain. A healthy trade with India was soon established, and tea clippers were reaching the peak of their sailing days. In 1879 the first limited shipments of Ceylon tea began to arrive, and by 1880 this had been firmly established alongside India and China teas, giving the broad range of teas that are available today.

In recent years, consumers tend to spend proportionally less on tea and more on coffee, as household expenditure on beverages increases. Prices and incomes have a relatively smaller impact on the consumption of tea. Marketing efforts should focus on demographic differences among consumers, increasing accessibility through improved visibility and retailing, and supporting innovative technologies, especially at the consumer level, as demonstrated by the coffee industry.

Most tea consumed outside East Asia is produced on large plantations in the hilly regions of India and Sri Lanka and is destined to be sold to large businesses. Opposite this large-scale industrial production are many small 'gardens,' sometimes minuscule plantations, which produce highly sought-after teas prized by gourmets. These teas are both rare and expensive and can be compared to some of the most expensive wines in this respect.

India is the world's largest tea-drinking nation, although the per capita consumption of tea remains a modest 750 g per person every year. The average tea consumption per person per year was 3157 g in Turkey in 2011, which is the greatest per capita tea consumer all over the world, followed by Ireland (2191 g) and England (1942 g). This data revealed that there is no direct relationship between tea production and tea consumption.

See also: Tannins; Tea: Analysis and Tasting; Tea: Chemistry and Processing; Tea: Health Effects.

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Tequila: Raw Material, Classification, Process, and Quality Parameters

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Introduction

Tequila is a Mexican spirit obtained from the distillation of fermented agave juice. In pre-Columbian times, it was called *mezcal*, a drink made from fermenting the juice obtained from the hearts of the agave plant, of which many species occur in Mexico. In colonial times, Pedro Sánchez de Tagle (1530) added distillation to the indigenous technology, developing the tequila known today. The distilled drink is obtained from the fermented juice of the blue agave (*Agave tequilana* Weber var. azul). It is cultivated in the western central region of Mexico, in the state of Jalisco, and some municipalities of the states of Tamaulipas, Nayarit, Michoacán, and Guanajuato.

The elaboration of tequila and its quality are governed by Mexican legislation 'NORMA Oficial Mexicana NOM-006-SCFI-2012, *Bebidas alcohólicas-Tequila-Especificaciones*.' According to this norm, tequila can be classified into two categories: tequila 100% and tequila. The differentiation depends on the percentage of sugar derived from the agave plant used in tequila production.

In tequila 100%, the sugars must come exclusively from *A. tequilana*, grown in the territory specified in the declaration of the denomination of origin. For this reason, this product is labeled '100% agave tequila.' It must also be bottled in a distillery that is located within the territory specified in the declaration of the denomination of origin, and under the supervision of the Consejo Regulador del Tequila.

Tequila refers to a product made with at least 51% agave juice, enriched up to a maximum of 49% of the total reducing sugars, from a source other than the agave juice. This alternative source of sugar must not come from another variety of agave.

In addition, depending on the characteristics acquired in postdistillation, tequila is classified into five subclasses:

White or silver tequila: a transparent, light-colored product obtained from distillation. Dilution with water is used only in appropriate cases to set the required commercial graduation. If matured in oak barrels, this lasts <2 months.

Tequila young or gold: the product of a mixture of rested and aged (or extra-aged) tequila. It can also be called young or gold tequila if additives, allowed by official rules, are incorporated (often, colors or flavors).

Rested tequila (*reposado*): the product has been matured for at least 2 months in oak barrels. It can still be called *reposado* if the *reposado* is blended with aged and extra-aged tequilas.

Tequila *añejo* (aged tequila): this version has been subject to an aging process that is achieved by maturing white tequila for at least 1 year in oak barrels (maximum capacity 600 l). It may also be called *añejo* tequila if it is a blend of aged tequila and extra-aged tequila.

Extra-aged tequila (extra *añejo*): this version has been subjected to maturation for at least 3 years in oak cooperage (maximum capacity 600 l).

Tequila production has evolved into the modern version mainly due to distillation, which modified the ancient *mezcal* produced by Indians before the arrival of Spanish colonizers. However, the basic unit operations have not changed: cultivation and harvest (termed *jima*) of the raw material (derived from the stems of *Agave tequilana* rich in fructooligosaccharides), cooking (to obtain fermentable sugars from the hydrolysis of the agave fructans), juice extraction, fermentation, distillation, filtration and aging, and bottling according to the type of tequila being produced.

Raw Material: *A. tequilana* Weber var. azul

The genus *Agave* (Asparagaceae, formerly Agavaceae) is a monocotyledon, a major subgroup of angiosperms (flowering plants). The genus occurs natively from the southern United States to northern South America, and throughout the Caribbean. Mexico is its most important center of diversity and possesses about 200 recognized species. Since ancient times, many of its species have been of great economic importance in Mexico, used as a source of fibers, medicine, and food, and in the manufacture of alcoholic beverages such as *mezcal* and tequila.

Agaves are rosetteous, perennial, monocarpic plants that live for many years and die after fruiting. The genus is divided into two subgenera: Agave and Littaea. Those in the subgenus Agave have tall inflorescences or scapes (flowering stems arranged in panicles, with the flowers produced in subpanicles). On the other hand, members of the subgenus Littaea have racemose scapes (the flowers being borne in clusters on lateral peduncles).

A. tequilana Weber var. azul is the source of the raw material used for tequila production. It is cultivated in the state of Jalisco, and part of the states of Michoacán, Nayarit, Guanajuato, and Tamaulipas. *A. tequilana* is a species in the Rigidae (a grouping within the Agave subgenus) that possesses rigid lanceolate. It achieves maturity within 7–8 years. Its scape reaches 7–8 m high (Figure 1(a)) and possesses hermaphrodite flowers (Figure 1(b)) that are borne in sub-panicles. The blooming season occurs from May to July, with the fruit and seeds developing from August to September. *A. tequilana* can also reproduce vegetatively by bulbils produced on the subpanicles (Figure 1(c)) and from basal rhizomatous shoots produced around the mother plant. Commercial plantations are started from the rhizomatous shoots (Figure 1(d)) to avoid the high genetic variability inherent in seed produced by cross-pollination.

The part of the plant used as a source of fermentable carbohydrates is the "*piña*". It comprises the true stem and the small basal segment of leaves that remain attached to the stem after harvesting, termed *jima* by agave farmers (Figure 1(e)).

Figure 1 The raw material: *Agave tequilana* Weber var. azul. (a) Mature commercial plantation with some scapes or floral stalks. (b) Hermaphrodite flowers borne in sub-panicles. (c) Vegetative reproduction by means of bulbils produced on sub-panicles. (d) Rhizomatous shoot used to start a new plantation. (e) Plant stems or piñas after harvesting.

These *piñas* take diverse shapes, from ellipsoid to ovoid, depending on environmental conditions and crop management. Finally, the *piñas* are cut in half, or smaller portions, prior to cooking.

Cooking and Carbohydrate Extraction

The ancient process of agave cooking hydrolyzes agave fructans under acidic conditions (pH 3.5–4.5) at temperatures between 80 and 110 °C. This liberates fermentable fructose and glucose molecules. For centuries, brick ovens were used for cooking. However, stainless steel ovens have replaced the former brick ovens to shorten the cooking time and enhance control. Cooking is also an important step because it produces classic sensory notes (e.g., caramel, smoked and wet straw) from compounds such as furans (furfural, 2-acetylfuran, methyl-2-furoate, 5-methylfurfural, 5-hydroxymethylfurfural, and 2-methyl-tetrahydrofuran). Most of the methanol found in tequila is derived from the action of pectolytic enzymes on pectin present in the raw material reacting or via thermal degradation. Methanol formation from pectins has been studied in the production of other spirits, such as *orujo* and the Greek spirits *tsipouro* and *tsikoudia*.

Once cooked, the agave stems are conveyed to steel rolling mills, similar to those used in the sugar cane industry. The mills press the shredded agave. Water is sprayed over the agave bed to leach the sugars from agave fibers. Juice with a solid content of 12–14 g/100 ml is obtained during this step.

Today, equipment called 'diffusers' are also used to extract water-soluble carbohydrates. A diffuser consists of a large steel belt (~2 m wide, and 20–23 m long) that conveys a layer of shredded raw agave across a countercurrent flow of hot water (80 °C). The carbohydrates obtained are hydrolyzed under acidic conditions at temperatures between 80 and 85 °C for 4–6 h. Phosporic acid and hydrochloric acid may be used to achieve the desired pH (1.8–3.0).

Fermentation Process

Fermentation, as in all alcoholic beverages, is a key stage in the elaboration of tequila. During this step, agave sugars are metabolized to ethanol, carbon dioxide, and other flavor-active compounds by the action of different microorganisms, particularly yeasts.

As mentioned earlier, Mexican regulation permits the use of only *A. tequilana* as the main carbon source. This species is characterized by the synthesis and accumulation of a particular reserve carbohydrate, a highly branched fructan that has been dubbed by some authors as *agavina*. In the agave juice, obtained after milling, these polysaccharides are hydrolyzed in order to permit fermentation. Sugars presents in the juice are mainly fructose, with a small percentage of glucose molecules (~10%). In the case of 100% tequila, the only source of the sugars comes from the hydrolyzed fructans obtained in the cooking step. In the case of the product simply designated as tequila, the law authorizes the addition of other sugars, in amounts of up to 49% by weight in the must formulation. Sugars currently employed are cane sugar; *piloncillo* (obtained from the crystallization of whole cane juice); cane molasses; and more recently, hydrolyzed corn syrup.

For the majority of the tequila industry, fermentation is performed using just the agave juice (must). However, some companies still employ a rustic process where the cooked agave is ground in an archaic mill named a *tahona*. In this process, the juice is not extracted and fermentation occurs in the presence of agave fibers. These tend to rise to the surface of the fermentation tanks and cover the fermenting must. Such fermentations are generally longer (several days to more than a week), and the conversion of sugars to ethanol is lower than in the case of liquid must fermentations. Under standard fermentation conditions (without fibers), the initial sugar concentration can range from 4% to 10% w/v (100% tequila), and 8% to 16% w/v (tequila). In some distilleries, in the formulation of

the must, nitrogen or other nutrients are added, such as urea, ammonium sulfate, ammonium phosphate, magnesium sulfate, and in some cases complex commercial nutrients. The amount added depends on the producer.

In general, there are two main types of tequila fermentations: spontaneous and induced (with the addition of selected yeasts). Some companies still prefer spontaneous fermentation as a traditional process because the greater diversity of microorganisms generates diversity of flavor compounds, despite producing less ethanol. For most companies, though, the must is inoculated with a particular strain of *Saccharomyces cerevisiae*. This may come from packaged bakers' yeast, or a commercial dry yeast (normally used for the production of wine, beer, or rum). Another interesting practice is the use of a proprietary, native, isolated yeast strain. Some companies develop their own specific inoculum in tanks on agave juice, using agitation and air incorporation, to produce the desired biomass and suppress ethanol generation. Inoculation of the fermentation tanks is done at 5–10% by volume.

Most producers use stainless steel fermentation tanks, possessing volumes of 2000 to 120 000 l. Some companies still use rustic wooden tanks, with volumes from 2000 to 10 000 l, especially when fermenting in the presence of agave fibers.

A detailed work characterizing the mycobiota has been conducted by a firm that uses traditional spontaneous fermentation. Several yeast species were detected at the beginning. These included *Dekkera bruxellensis*, *Hanseniaspora guillermondii*, *H. vineae*, *Kluyveromyces marxianus*, *Pichia membranifaciens*, *Torulaspora delbrueckii*, and *S. cerevisiae*. During fermentation, the populations of non-*Saccharomyces* yeasts tended to decrease, with *S. cerevisiae* becoming dominant by the end of fermentation. It is important to mention that bacteria were also detected. These could also play an important role in the formation of volatile compounds, or may become a serious contamination problem. Thus, they could affect the conversion of sugar to ethanol.

With must formulated using only juice, a typical inoculated fermentation may be rapid (about 20 h), or up to 3 days in a slower cases. Yeast populations range between 100 and 200 - million cell ml^{-1} by the end of the fermentation. If an inoculum is not added, or fermentation occurs in the presence of fibers, fermentation may take more than 7 days, reaching lower yeast populations in the tank. The temperature reached during fermentation is relatively high, ranging between 30 and 35 °C, and sometimes rising to 40 °C. The rate of fermentation depends on different factors, such as the type of fermentation (with or without fibers), the yeast strain used, medium composition, and operating conditions. At the end of fermentation, the alcohol content ranges between 4% and 9% (v/v), depending mainly on the initial sugar concentration.

Besides ethanol production, fermentation contributes significantly to the generation of other compounds, particularly flavor-active chemicals. Generally, the content of these flavor-active compounds is lower in faster rather than slower fermentations. In a study carried out on different 100% tequila samples, the most important compounds (by concentration), after ethanol, were higher alcohols, methanol, ethyl acetate, and acetaldehyde. The generation of these compounds (except for methanol) occurs during fermentation due to yeast metabolism. Additionally, 24 different esters have been identified, for example, ethyl decanoate, ethyl dodecanoate, ethyl lactate, and ethyl octanoate. In this case, the main yeasts responsible for their production were non-*Saccharomyces* yeasts. *S. cerevisiae* donated few esters. Thus, to obtain these aromatic products, the presence of non-*Saccharomyces* yeasts is desirable. Another interesting function of some non-*Saccharomyces* species is the production of enzymes, such as β-glucosidase, β-cellobiosidase, and β-xylosidase. These are able to hydrolyze complex polysaccharides present in the raw material, generating aromatic compounds such as terpenes.

Distillation

Alembic distillation is an ancient separation process that has been used worldwide by different cultures to obtain spirits. At the beginning of the tequila industry, in the eighteenth century, the only distillation equipment used was the alembic. At the time, it was made out of copper and heated with wood. Without analytical methods, the distillation process was loosely controlled. The distillate was of inconsistent quality and under the empirical control of the distiller. Since the mid-twentieth century, the tequila industry has introduced technological advancements that include steam as the heat source, both for agave cooking and distilling, and the use of stainless steel instead of traditional copper distillation equipment. Recent changes also include the use of continuous leaching processes to extract water-soluble carbohydrates from agave, and continuous distillation columns to derive more distillate.

Traditional tequila distillation consists of two steps. In the first (stripping), fermented must possessing an ethanol content between 4% and 9% V/V is distilled using differential (alembic) distillation. This technique is characterized by the separation of ethanol, together with additional volatiles called congeners. The objective of stripping is to eliminate residual sugars, yeast, minerals, and fibers. This mixture is known as *vinasses*, and the distillate obtained is called *ordinario* (25–30% ethanol volume).

The second step enriches and rectifies the distillate. It involves three fractions: the first (heads), middle (heart), and last (tails). Heads volume usually is around 1% of the batch in the pot of the alembic, and contains mainly ethanol, water, acetaldehyde, isobutyl and isoamyl alcohols, and ethyl acetate. The middle volume, possessing an alcohol content of 55–60%, contains ethanol, esters, terpenes, isobutyl and isoamyl alcohols, organic acids, and other chemical compounds that form the particular and unique sensory profiles of tequila. The size of the heart fraction depends on the ethanol degree in the *ordinario* and contains 80–90% of the ethanol of the initial *ordinario*. The tails fraction contains water, methanol, phenethyl alcohol, furfural, acetic acid, and other larger chain organic acids such as hexanoic and octanoic acids. When continuous columns are used, the obtained distillate usually is blended with alembic distillate to meet regulation dictates and to achieve the desired sensory profile.

To obtain the finished product, the heart fraction is transferred into oak casks in the aging area, or diluted and filtered to obtain silver and gold tequilas. Distillate aging permits the production of tequila *reposado* and tequila *añejo*, with at least 2 months aging for the first, and 1 year for the second. An extra-aged tequila, with at least 3 years in-oak maturation, may also be produced.

Quality of Tequila

The quality of a product for human consumption is defined by the needs and expectations of the consumer. Regarding distilled beverages, a consumer commonly wants and expects a product that is both pleasant to taste and smell, as well as safe. In this regard, tequila quality control is oriented toward understanding, characterizing, and controlling its volatile composition. This involves maintaining methanol and higher alcohols within safe limits and investigating compounds, such as esters and aldehydes (Table 1), and ketones, that donate the bouquet. Quality control analysis also include determining dry matter, ash, alcohol, and furfural.

The identification and quantification of volatile compounds is carried out by gas chromatography, combined with flame ionization or gas chromatography, coupled with mass spectrometry. Solid-phase microextraction is also often used with polydimethylsiloxane or polyethylenglycole as the stationary phase. For general assessment detection, a few microliters of tequila can be directly injected into a gas chromatograph. It permits to know the proportions of the principal functional volatile groups – aldehydes, ketones, esters, and alcohols – and can differentiate among compounds such as isoamyl alcohol, butanol, and methanol. For more detailed analysis, concentration of the volatile compounds before injection allows the identification and quantification of volatiles in microgram quantities. The detailed volatile profile obtained permits correlating tequila's sensory attributes with its chemical origin.

Origin of Tequila's Sensory Attributes

As with other spirits, the distinctive sensory character of tequila is principally provided by the flavor compounds that come from the raw material, cooking, and fermentation. The most abundant components are ethanol and water, with tequila's sensory identity being provided by diverse volatiles in small quantities. Additional volatiles may come from maturation in wood cooperage or occasionally other flavorings. The age of the agave and the region in which it is grown bring diversity to the flavor of tequila. Even though agave must be grown and harvested only in specific appellation of origin regions, microclimates and soil differences within regions bring diversity to the bouquet of tequila.

In fermentation, the use of the local microbial flora will also have an impact on the final character of the product, contributing to distinctive variations among different brands, regions, factories, and spirits. Distillation performed either in copper or stainless steel, and the way it is carried out, affects volatile recovery and restricts the presence of undesirable compounds.

Tequila Flavor Origin

Tequila flavor volatiles have been extensively studied since the last decade of the twentieth century, when this spirit became widely distributed and increasingly popular. About 300 flavor volatiles have been identified. In addition, olfactometry has shown the importance of components such as vanilla, eugenol, esters, and phenyl ethanol. Much of the work has been summarized in a review by Gschaedler. However, as in other foods or beverages, tequila flavor depends on the interaction of multiple volatile compounds, and not solely on components with low thresholds, although they have great impact. Tequila flavor develops progressively, with every step in its production contributing specific sensory attributes to the final product.

Flavor Contribution of Raw Material and Its Elaboration

As mentioned earlier, cooking of the mature agave stem or heart not only releases fermentable sugars, but also promotes the formation of compounds derived by Maillard and caramelizing reactions. The latter provide mainly furanic volatiles. The agave plant is rich in terpenic compounds, and contains both vanillin and syringaldehyde in its juice. The latter two are normally derived from wood cooperage in other spirits. Other compounds with spicy notes, such as eugenol, are also present in the agave.

Table 1 Physicochemical specifications of tequila

	Tequila										
	White		*Young or Gold*		*Reposado*		*Añejo*		*Extra añejo*		*Método de Ensayo (Prueba)*[a]
	Min	*Max*	*Min*	*Max*	*Min*	*Max*	*Min*	*Max*	*Min*	*Max*	
Alcoholic degree 293 K (20 °C) (% Alcohol Vol.)	35	55	35	55	35	55	35	55	35	55	NMX-V-013-NORMEX
Extracto Seco (g l^{-1})	0	0,30	0	5	0	5	0	5	0	5	NMX-V-017-NORMEX
Content in mg/100 ml of ethyl alcohol, anhydride											
Higher alcohols (alcohols MW > ethanol or fusel oil as isoamyl alcohol)	20	500	20	500	20	500	20	500	20	500	NMX-V-005-NORMEX
Methanol[b]	30	300	30	300	30	300	30	300	30	300	NMX-V-005-NORMEX
Aldehydes (as acetaldehyde)	0	40	0	40	0	40	0	40	0	40	NMX-V-005-NORMEX
Esters (as ethyl acetate)	2	200	2	200	2	250	2	250	2	250	NMX-V-005-NORMEX
Furfural	0	4	0	4	0	4	0	4	0	4	NMX-V-004-NORMEX

[a]Valid Official Mexican Standards and Mexican Standards.

[b]Minimum setting may decrease if the producer of Tequila demonstrates to the Conformity Assessment Body, that has a viable method to reduce the content of methanol.

Some agave compounds are transformed during cooking; however, others survive and are present in the final product. There is little published research on the effect of using different juice extraction conditions on volatile composition, especially the use of diffuser technology during cooking. These steps of juice extraction and cooking are very important to tequila's sensory character, as they impact the way volatiles from agave juice are produced, modified, and recovered before fermentation.

Methanol is an important volatile compound produced early in tequila production, coming from the demethylation of the pectin, which occurs abundantly in agave. At high concentrations it is a toxic compound, but at lower concentrations it is a flavor-impact compound (and, as such, a potential authentication marker). In practice, tequila authentication has commonly been directed at the detection of a distinctive agave profile, in particular the presence of terpenes. Authentication has been explored with the use of isotopic ratios, notably the carbon, hydrogen, and oxygen in ethanol and other primary alcohols. This approach appears to be the most accurate.

Ethanol and higher alcohols are mainly produced by yeasts during fermentation. Yeast fermentation also produces esters, aldehydes, and other volatile organics. Moreover, specific yeast strains may enhance ester production, as well as favor the release of terpenes, which provide herbal and floral notes. Yeasts can also affect the presence of vanillin, depending on the conditions, by metabolizing the compound.

Distillation is important in recovering and selectively separating volatile constituents. Nevertheless, chemical transformations of volatiles can also take place, especially when copper is used. A paired comparison test showed significant differences between tequila obtained from stainless steel alembic versus copper pots, the latter being more fruity and flowery.

Including maturation in wood in the production process has a special impact on the spirit's character, but in contrast with some other spirits, such as cask-aged whiskies, unoaked tequila has a pleasant character, especially with 100% tequila. Agave's raw material volatiles blend with those derived from fermentation, after careful distillation. This is illustrated in the sensory profiles obtained for silver tequila (Figure 2).

Rested (*reposado*) tequila remains in wooden barrels for at least 2 months and up to a year. The character of the final product depends on the type of wood used for barrel manufacture, and whether new or reused barrels are used. Wooden barrels provide lignin-derived compounds, such as vanillin, syringaldehyde, coniferaldehyde, and cinnamaldehyde, which are extracted by the spirit, as are oak lactones. Extracted phenolic nonvolatile compounds provide color and induce structural changes in the alcoholic matrix that can modify the volatile release of some compounds. Tequila classified as aged has to be matured in cask for more than 1 year. For this class of tequila, the oaky character is stronger. This results from more compounds having been extracted from the barrels, as well as from phenolic polymerization and longer oxidation. Aged tequila composition may resemble other long-aged, cask-matured spirits, such as whiskies and cognac.

Flavor Attributes of Tequila

Flavor descriptors relate to the raw material, fermentation aromas, and the bouquet obtained during maturation. Cooked agave has odor-flavor notes of browning reactions that are reminiscent of caramel, molasses, or raw brown sugar. They are also similar to cooked pumpkin and overripe or fermented fruits. Agave also can donate spice flavors, due to the presence of eugenol and wood-related compounds (vanillin and syringaldehyde), as well as terpenes with floral and herbal notes. Fermentation provides a vinous character due to the production of higher alcohols, similar to how its generation of esters donates fruity flavors. Terpene transformation and release, carried out by yeasts, also contribute to herbal-floral character. Among impact compounds,

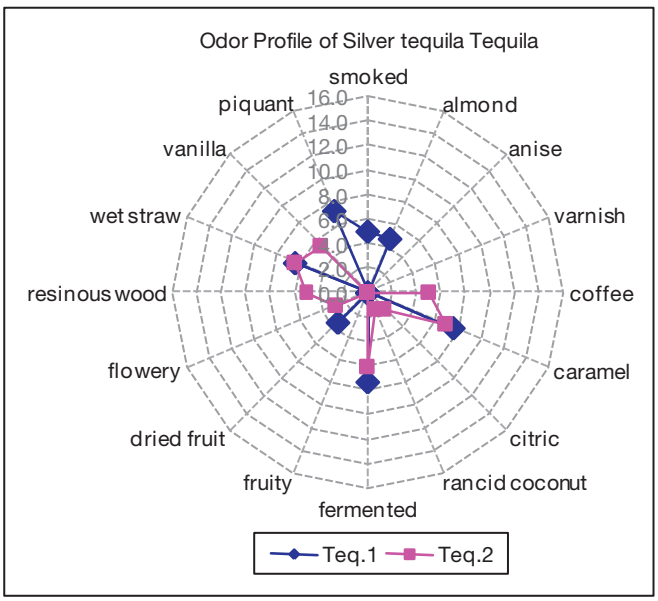

Figure 2 Odor profile of silver tequila from Tequila.

phenylethanol has been reported as odor-active, with a floral odor, with β-damascenone contributing to a fruity-winey smell. Lopez reported these compounds as odor-active components, and indicated that 3-methyl butanol, phenylethyl acetate generate a fermented fruit note. As indicated in the preceding paragraphs, region, growth, and agave cultivation conditions, as well as the fermentation conditions, can impact tequila's sensory profile. Tequila and Los Altos are the traditionally representative regions for tequila production, as shown by the sensory profiles provided by trained judges (Figure 3).

The source material and size of the barrels used in maturation, as well as its duration, generate different volatile profiles in tequila and can offer a variety of sensory nuances suited to different consumers (Figure 4).

Volatile acids can donate sour attributes, whereas vanilla and other woody notes may appear in white/silver tequilas. Nonetheless, the latter are more distinguished by their agave flavor, and the fruitiness and vinous character are provided by fermentation. Oaky, nutty, and spiced flavors, as well as enhanced vanilla and caramel notes, are found in the rested and aged tequilas. With prolonged aging, the woody bouquet may override the agave notes.

Sensory Evaluation

As the tequila industry has grown and the product's popularity has increased, producers have focussed their attention on diversifying tequila's sensory characteristics and improving methods to maintain these attributes. Some large producers now work with expert tasters and have implemented more structured sensory evaluation methodologies. These involve employing trained assessors for quality control, as well as in research and development.

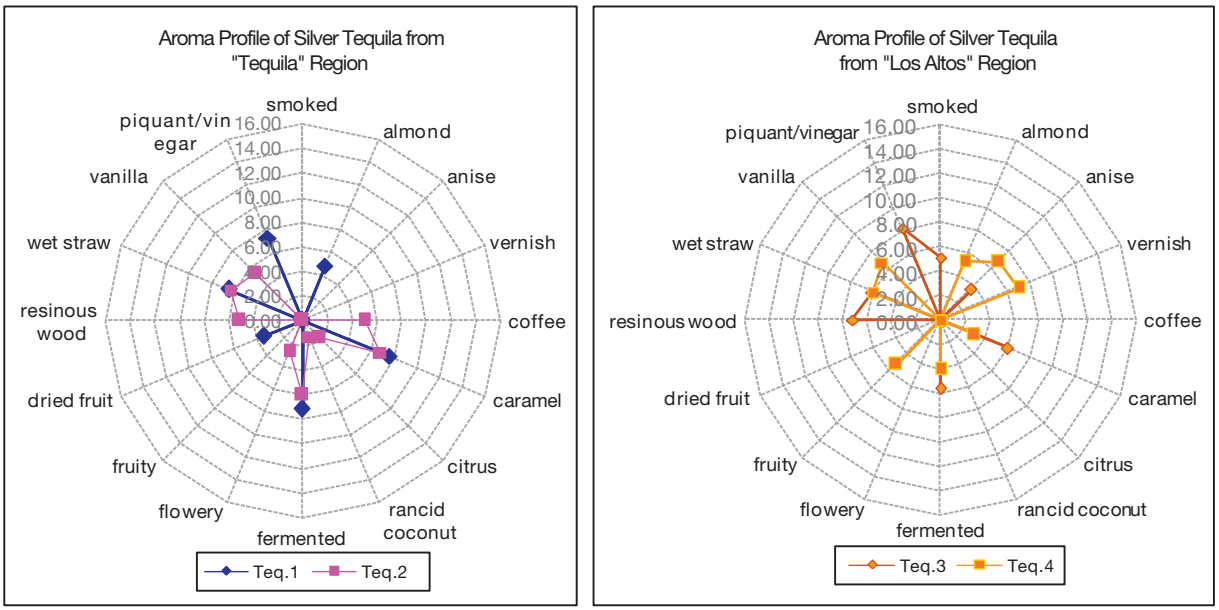

Figure 3 Aroma profile of tequila from Los Altos and tequila from tequila.

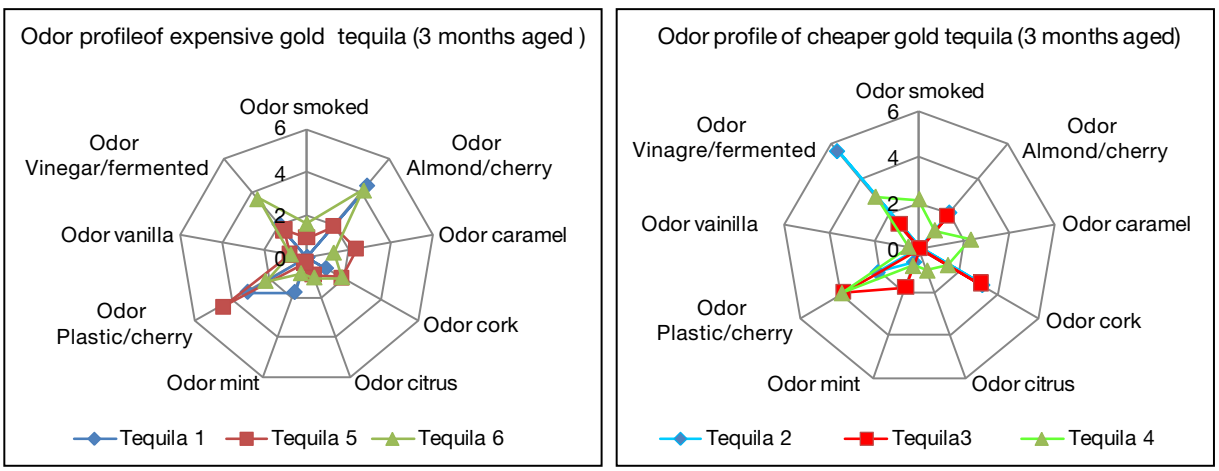

Figure 4 Odor profile of aged tequila.

Other tequila producers have also realized the need for a more structured sensory control protocol. This often follows procedures outlined by the International Standards Organization (ISO). However, few medium or small producers have research and development departments.

Quality Control

Discriminative tests, such as triangle or duo-trio tests, are the most frequently used statistical methods for sensory quality. They are fast and practical, using a typical product as a reference.

Some tequila companies have their own research and development departments. These have facilities for training descriptive assessors who can participate in specific sensory evaluation tests.

Implementing a sensory analysis location on the premises has been important in sensory analysis carried out by research institutions and private consultants.

See also: Alcohol: Properties and Determination; Authenticity of Food; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Fructose: Sources, Metabolism, and Health; Yeasts.

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Thiamin: Physiology

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Background

The discovery of thiamine or vitamin B_1 as an essential nutrient has opened a new area in the field of nutrition research in two respects. First, the nomenclature 'vitamin,' used for the whole class of the later on discovered essential micronutrients of organic origin, was derived from a functional group (NH_2 group) of the molecule. Second, thiamine was the first nutritional factor that could be identified as a curative agent against a deficiency disease in animals.

At the end of the nineteenth century, the Dutch medical officer Ch. Eijkman discovered the beneficial effect of rice bran or unpolished rice, respectively, for the treatment of polyneuritis in chickens resembling beriberi in man, which had been previously induced by feeding polished rice. In 1911, the German-Polish chemist C. Funk isolated a crystalline substance from rice bran and regarded the amino function as the essential principle of the molecule. The exact chemical structure of the thiamine molecule was elucidated by R. Williams and A. Windaus. Because of the sulfur content of the molecule or its thiazole moiety combined with the amine function, the compound was designated as thiamine instead of the former name aneurine. Nevertheless, the classical form of thiamine deficiency, beriberi, mainly affects human beings and has been well known in the rice-eating countries of East Asia for centuries. Even 25 years after the ingenious work of Eijkman, who clarified the cause of this deleterious disease, the mortality rate from beriberi in industrialized countries such as Japan was still more than 25 000 per year.

Physiological Function

Like other water-soluble B vitamins, thiamine is in its primary function coenzymatically active. The thiamine-dependent enzymes are involved in carbohydrate and energy metabolism (Figure 1). Key enzymes in these metabolic pathways are mitochondrial pyruvate dehydrogenase (EC 1.2.4.1) linking glycolysis with the citric acid cycle, α-ketoglutarate dehydrogenase (KDH) (EC 1.2.4.2) within the citric acid cycle, and the cytosolic transketolase (EC 2.2.1.1) in the pentose phosphate shunt. Hence, it follows that the energy turnover of the organism is related to thiamine demand. As dietary carbohydrates are the predominant fuel for the rapid metabolizable energy of the organism, the thiamine requirement is often related merely to the carbohydrates ingested. KDH was identified as the rate-limiting enzyme of the cerebral glucose utilization. A low enzyme activity caused by a reduced intracellular thiamine diphosphate (TDP) concentration results in lactacidosis, cerebral dysfunction of the energy metabolism, and neuronal cell degeneration. Additional thiamine-dependent enzymes are involved in the metabolism of branched-chain amino acids, for example, leucine, isoleucine, and valine. All enzyme reactions are characterized by transfer of an 'active aldehyde' from α-keto acids formed after previous decarboxylation to coenzyme A or an aldopentose.

The coenzymatically active form is TDP, also called thiamine pyrophosphate (TPP) or cocarboxylase, which is formed in the mucosal cell of the intestinal barrier with the aid of thiamine pyrophosphokinase (EC 2.7.6.2) in the presence of ATP. The latter enzyme is also found in mammalian liver and other tissues such as the brain and nerves and in the heart of mammals and birds. Further phosphorylated forms of thiamine, which are found in varying quantities in the tissue, are thiamine monophosphate (TMP) and thiamine triphosphate (TTP). These derivatives are synthesized and metabolized according to the scheme in Figure 2.

Thiamine phosphorylation represents a partitioning process in a deeper compartment. Cell membranes are impermeable for the intracellularly active coenzyme TDP, which is released only after hydrolysis via TMP to thiamine. The total thiamine concentration of the blood cells and of the cerebrospinal fluid (CSF) has a nonlinear relation to the corresponding plasma concentrations. A linear increase in blood cells and CSF has been observed only with thiamine plasma levels above 10 nM (3 $\mu g\,l^{-1}$). Otherwise, with extremely low plasma and CSF concentrations, a disproportionately high TDP content of erythrocytes and TMP content of the CSF are maintained for a longer period. Thus, the intracellular phosphorylation should be seen as a protecting mechanism, inhibiting rapid thiamine depletion by decreasing the membrane permeability.

After TDP, TTP is the next most phosphorylated metabolite, albeit the steady-state concentrations in tissues are rather low. No coenzymatic function has been found for this metabolite. Several investigators have demonstrated in a variety of animals (rats, rabbits, cows, frogs, and pigs) that TTP, together with a distinct part of TDP, is neurophysiologically active in the central and peripheral nervous systems independent of the coenzyme function of the latter. After electrical stimulation of the nerves or treatment with neuroactive drugs, for example, acetylcholine, tetrodotoxin, and lysergic acid diethylamide (LSD), a release of the phosphorylated esters TDP and TTP concomitant with a shift to TMP and free thiamine has been observed. Furthermore, in patients with the deleterious 'subacute necrotizing encephalopathy,' or Leigh's disease, the TTP level in the brain was low and tended toward zero. It has been known for a long time that severe thiamine depletion of the mammalian central nervous system leads to encephalopathies, which are accompanied by regionally selective changes in neurotransmitter function. Though the exact function of TTP is not yet known, it plays an essential role in nerve excitation and transmission, possibly in gating the ion transport. From recent investigations, it has been argued that TDP might also be involved in the inhibition of nonenzymatic glycosylation

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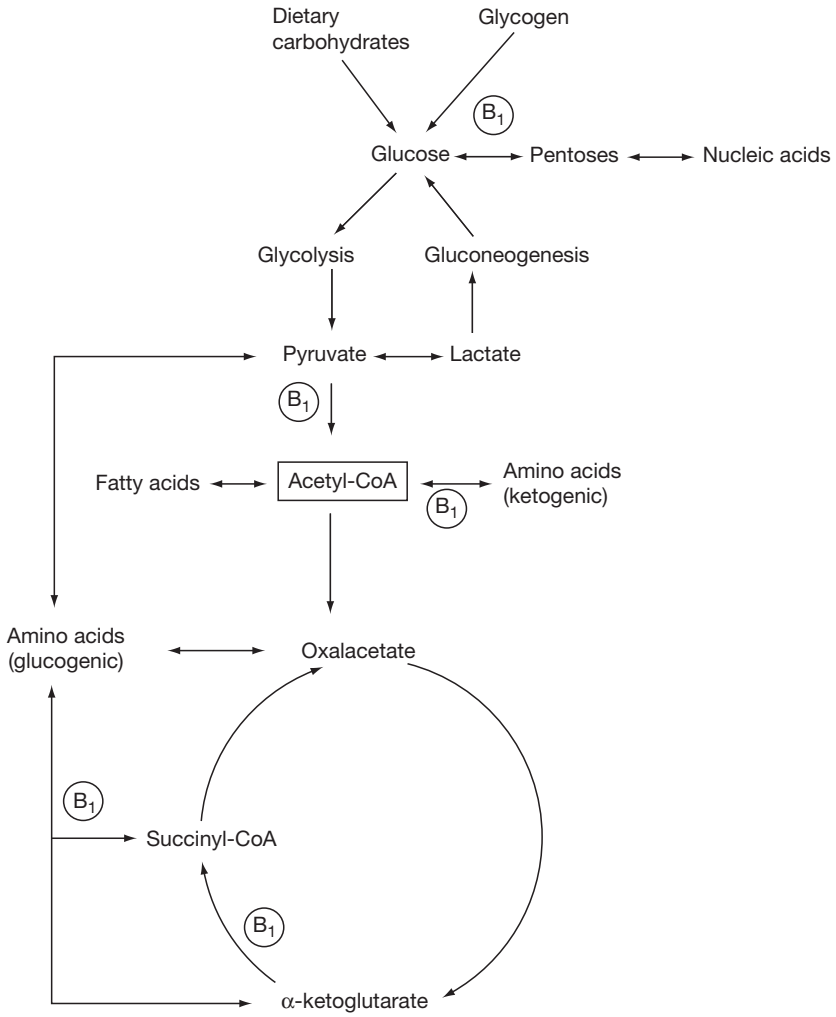


Figure 1 Metabolic pathways involving thiamine.

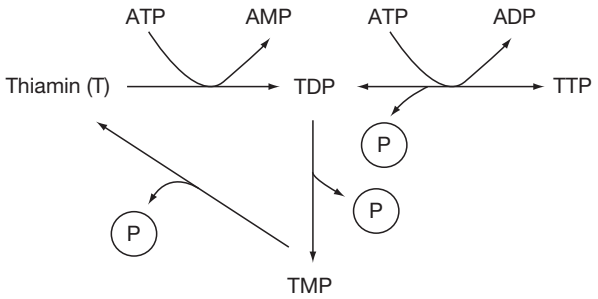


Figure 2 Interconversion of thiamine phosphates (according to Rindi, modified).

processes. It is suggested that the emerging advanced glycosylation end products (AGEs) are the reason for the segmental demyelinization observed in neurodegenerative diseases. Studies on cultivated cells have demonstrated that thiamine and TDP could prevent the AGE formation. In this regard, TDP is more effective than the standard inhibitor aminoguanidine that is normally used.

Absorption, Storage, and Excretion

Thiamine is absorbed from the gastrointestinal tract by a dual system, as has been shown for humans and rats. At low concentrations, it follows an energy-dependent active transport with a saturation kinetics up to a maximal concentration of 2 μM. At higher concentrations, a simple diffusion process is predominant. However, the relative absorption decreases with increasing vitamin doses, so that with gram doses, only about 5% are incorporated.

A close association was assumed between thiamine transport and phosphorylation as well as Na^+ dependency. However, the coupling of thiamine transport with phosphorylation is now doubtful, and there is much evidence of a specific protein carrier being involved in the absorption, but the following intracellular phosphorylation seems to be a rate-limiting step in the active transport of thiamine across the epithelial cells of the intestine. In contrast, in the guinea pig intestine, thiamine seems to cross the brush-border membrane by simple diffusion throughout the concentration range from 0.06 to 10 μM.

The jejunum and ileum have been found to be the main sites of thiamine absorption in rats with a maximal rate in the proximal 22 cm of the small intestine. This specificity seems to resemble that of other animals as well as humans.

After uptake from the intestinal tract, thiamine is distributed rapidly into the organs and tissues according to the requirements. In humans and animals, the heart contains the highest thiamine concentration (3–8 $\mu g\,g^{-1}$ fresh weight), followed by the kidney (2.4–6 $\mu g\,g^{-1}$ fresh weight), liver (2–7.5 $\mu g\,g^{-1}$ fresh weight), and brain (1.4–4.4 $\mu g\,g^{-1}$ fresh weight). Lesser amounts are found in other tissues. Whole blood contains 0.05–0.12 μg of thiamine per milliliter, about 90% of this being found in the corpuscular constituents. The total storage capacity of man is estimated to be 25–30 mg of thiamine, 40% of this being in the muscles. Interestingly, pig muscle has a relatively high thiamine content (8–12 $\mu g\,g^{-1}$) because of feeding conditions, higher than that in muscles of other mammals (1–2 $\mu g\,g^{-1}$) and pig liver (2–4 $\mu g\,g^{-1}$). The thiamine contents of several plant and animal foods are given in Table 1.

As with other water-soluble vitamins, thiamine excretion is dependent on intake. Within the physiological range, most of it is excreted by urine. Besides free thiamine and small amounts of TMP, numerous metabolites can be detected as minor excretion products such as thiazole, pyrimidine, and methylthiazol acetic acid.

After ingestion of therapeutic dosages, the amount of thiamine excreted via bile increases proportionally, as does the unabsorbed part in feces.

Lipid-Soluble Thiamine Derivatives

Under relatively mild conditions (e.g., in the gastrointestinal lumen), thiamine reacts with allicin, the active principle of garlic and onions, to form a lipid-soluble compound, called allithiamine, that is able to penetrate through membranes more easily than the water-soluble salts (Figure 3). Additional *S*-alkyl derivatives have been identified in cruciferous plants, and several lipid-soluble derivatives have been synthesized with a better stability than that of the spontaneously formed allithiamine (*S*-benzoyl- and *S*-tetrahydrofurfuryl analogs being the most important).

Oral administration of these lipid-soluble analogs results in higher thiamine blood levels than with equimolar thiamine doses, because the allithiamines from the gut lumen are preferentially absorbed by passive diffusion and rapidly converted to the active vitamin by reducing SH compounds. These allithiamines are, because of their improved bioavailability, suitable transfer substances with which to establish high thiamine levels in target tissues comparable with drug targeting. The elevated blood level results in an improved vitamin status, as measured by enzymatic criteria.

Table 1 Thiamine content of plant and animal foods (mg per 100 g fresh weight)

Content	*Foods*	
1.0–2.0	Whole wheat and rye	Plant foods
0.9	Para nuts	
0.7–0.8	Soy flour	
0.6–0.7	White bran	
0.3–0.5	Whole cereal products, lentils, oats, unpolished rice, lima beans, whole corn meal, cashew nuts, peanuts, hazelnuts, walnuts, mung beans	
0.1–0.2	Parboiled rice, sorghum, beans and peas cooked, cauliflower, potatoes, fennel, broccoli cooked, asparagus, soy sprouts, mushroom	
<0.1	Fruits, milk products, white flour products	
0.9	Loin pork	Animal foods
0.3–0.5	Liver, kidney (calves, beef, pork), duck	
0.1–0.2	Lean meat (calves, lamb, beef), eel, tuna, salmon, mackerel, plaice and flounder, oysters	

Source: Souci, S. W., Fachmann, W. and Kraut, H. (2000). *Food composition and nutrition tables* (6th revised ed.). Boca Raton, FL: CRC Press/Medpharm Scientific.

Thiamine Antagonists

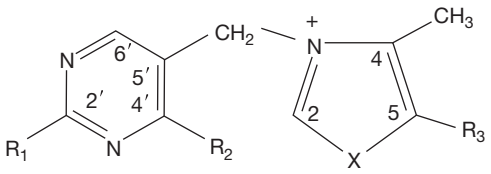
Synthetic Antivitamins

Like most of the B vitamins, thiamine has a rather high structural specificity, that is, minor modifications of the molecule lead to a decrease or a total loss of physiological activity or even to antagonistic effects. Of the numerous analogs synthesized so far, only the 2-ethyl- and the 2-*n*-propyl homologues in the pyrimidine moiety of the molecule show any biological activity comparable with that of thiamine. For example, when the 4-amino group is replaced by a hydroxyl function, oxythiamine, one of the most potent thiamine antagonists, is obtained. Another prominent antivitamin is pyrithiamine, which contains an ethylene group instead of sulfur in the thiazolium ring. Some thiamine antagonists have been found to exhibit antiprotozoal activity, as in the case of amprolium, which is effective as a prophylactic agent for coccidiosis of fowl. Some of the modified thiamine derivatives are shown in Figure 3.

The use of antagonists results in rapid and severe deficiency symptoms in humans and animals. Amprolium is already known to affect the absorption process already, and this has been suggested as the mechanism for its anticoccidial action in fowls. Other antivitamins appear to function at the coenzyme level, that is, they bind to the apoenzyme but inhibit the following enzymatic reaction. Nevertheless, there are several differences in the action on neurological symptoms. Only pyrithiamine (and not oxythiamine) can block the action potential of a single myelinated nerve fiber. It is also well known that only pyrithiamine produces polyneuritis. In addition, differences in neurophysiological action are caused by the different abilities of both antagonists to cross the blood–brain barrier, which has only been verified in the case of pyrithiamine. However, it can be shown that the amino group of the thiamine molecule is essential for the active transport of the vitamin from the rat small intestine and the formation of TPP.

Thiaminases and Other Antithiamine Factors

Two thiamine-cleaving enzymes have been identified, called thiaminase I and thiaminase II.



	R_1	R_2	R_3	X
Thiaminium ion	Methyl	Amino	Ethanol	Sulfur
Ethylthiamin	Ethyl	Amino	Ethanol	Sulfur
N-propythiamin	*N*-propyl	Amino	Ethanol	Sulfur
Oxythiamin	Methyl	Hydroxy	Ethanol	Sulfur
Pyrithiamin	Methyl	Amino	Ethanol	Ethene
Amprolium	*N*-propyl	Amino	Hydrogen	Ethene
Allithiamin	Methyl	Amino	Ethanol	*S*-alkyl (thiazol oxidatively splitted at C_2)

Figure 3 Thiamin derivatives and antagonists.

Thiaminase I is found in shellfish, clams (but not oysters), some freshwater fish viscera, crustacea, and certain ferns, but very few higher plants. Also, certain species of *Bacillus* and *Clostridium*, which are components of the human and animal intestinal flora, have been found to produce this enzyme. The enzyme catalyzes an exchange reaction, in which the thiazole moiety of the molecule is displaced by another N-containing base or a SH compound. The effect of this compound was first observed when silver foxes were fed with raw fish waste, resulting in a deleterious thiamine deficiency disease called Chastek paralysis. Thiaminase I may also be produced by the rumen microflora of ruminants or by plants and, in the presence of suitable cosubstrates, for example, niacin, and pyridoxine, and certain anthelmintics, seems to be responsible for the ruminant CNS disorder, polioencephalomalacia.

Thiaminase II is of bacterial origin (predominantly *Bacillus*, *Candida*, and *Oospora*) and breaks down the free vitamin, but not the TPP, into pyrimidine and thiazole components. More prevalent are thermostable thiamine-inactivating factors of plant origin, for example, polyphenolic substances such as flavonoids and catechol derivatives in fermented tea, ferns, sweet potatoes, and betel nuts and, in small quantities, in other leaves, fruits, and roots. These can decompose the thiazole component of the vitamin, accelerate the oxidation to the disulfide form, or form unabsorbable adducts with thiamine. Rats fed with food high in polyphenolics have been shown to develop deficiency symptoms as a result of a marked decrease in levels of cerebral thiamine and thiamine-dependent enzymes. Likewise in man, high tea consumption leads to a deficient thiamine status, but ascorbic acid, if present, completely inhibits the thiamine-inactivating processes.

Status Assessment

In terms of biochemical status, urinary vitamin excretion and blood enzymes are suitable criteria. Urinary thiamine excretion is, as with most B vitamins, largely dependent on intake and therefore provides an indication of the recent dietary intake but does not adequately reflect the body stores. At intake levels below 0.5 mg of thiamine per 1000 kcal day^{-1} (0.1 mg MJ^{-1}), thiamine excretion varies only slightly, and the proportion of metabolites in the urine exceeds the vitamin content itself. Because urinary excretion follows a diurnal rhythm, 24 h urine or the creatinine excreted is taken as the basis for status assessment. Occasionally, the status can be interpreted more accurately by a thiamine load test. After administration of a 5 mg dose, vitamin excretion is measured for the following 4 h. A deficient status can be assumed if less than 20 μg is excreted during this period.

Erythrocytic transketolase activity (ETKA), the rate-limiting enzyme in the pentose phosphate pathway, and its activation coefficient (AC) α_{ETK} are often used as an indication of functional status as they give a better idea of body stores than urinary excretion. According to the saturation deficit of the apoenzyme, the transketolase activity can be stimulated *in vitro* by the addition of TDP. The ratio of stimulated to basal activity, which increases with the degree of deficiency, indicated by the AC or α_{ETK}, is commonly taken as a prognostic status index. The AC is better as a comparison of status in groups, because of the large interindividual variation in basal enzyme activity. Moreover, it can be assumed that apoenzyme levels are not affected by the vitamin deficiency. Nevertheless, in alcoholics, either the apoenzyme synthesis or the linkage to the coenzyme TDP seems to be impaired, and the transketolase assay may indicate misleading results about the true status. Furthermore, confounding factors in the enzymatic assay, irrespective of the thiamine status, may include certain cancers, uremic neuropathy, diabetes, and treatment with certain anticancer drugs (fluorouracil and cytoxan) or diuretics, such as furosemide.

Recently, in view of improved high-performance liquid chromatography techniques, the TDP content in erythrocytes has been suggested to be a better indicator of the body stores

Table 2 Reference values of thiamine status parameters

Indicator	Adequate	Deficiency	
		Marginal	Severe
Thiamine excretion in urine (μg per gram of creatinine)	>66	27–66	<27
ETKA–AC	<1.15	1.15–1.25	>1.25
TDP content in erythrocytes (ng per milliliter of sediment)	>38.5	29.5–38.5	<29.5

Source: Finglas, P. M. (1994). Thiamin. *International Journal for Vitamin and Nutrition Research* **63**, 270–274; Frank, T., Bitsch, R., Maiwald, J. and Stein, G. (2000). High thiamine diphosphate concentrations in erythrocytes can be achieved in dialysis patients by oral administration of benfotiame. *European Journal of Clinical Pharmacology* **56**, 251–257.

compared with ETKA and independent of confounding factors. The criteria of the thiamine status indices are listed in Table 2.

Thiamine Requirement

Regarding the essential role of thiamine-dependent enzymes in carbohydrate and energy metabolism, the requirement for this vitamin is usually related to energy intake. Irrespective of the catalytic function of enzymes emerging basically unchanged from a biochemical or metabolic reaction, controlled studies in man have demonstrated that static and functional status criteria (transketolase activity and urinary excretion) deteriorate with increasing energy uptake even when the thiamine supply remains unchanged, thus indicating an increased vitamin turnover. The dependence on energy metabolism seems to be particularly evident in a marginal vitamin status.

Previously, dietary fat was postulated to have a 'sparing effect' on thiamine requirements. This effect is, however, comparatively small, even with major increases in fat in the diet. Furthermore, in a starvation or semistarvation status when the vitamin intake is zero and increasing amounts of fatty acids are metabolized to satisfy the body's energy needs, the tissue stores of thiamine are rapidly depleted.

International committees recommend safe levels of intake amounting to 100–130 μg of thiamine per megajoule, equivalent to 0.4–0.54 mg of B_1 per 1000 kcal. A critical intake point appears to be approximately 0.05 mg of B_1 per megajoule (0.2 mg per 1000 kcal), below which urinary excretion is low, and the first clinical signs of deficiency may appear. A minimum intake of 1 mg day^{-1} is recommended to provide an adequate margin, even for those consuming less than 8 MJ (2000 kcal) daily. The additional values for pregnancy and lactation mostly exceed the calculated additional requirement of pregnant and lactating women. These extra values ensure optimal pre- and postnatal development. In the past, clinical deficiency symptoms of infants were sporadically observed in spite of an inconspicuous appearance of the mother (infantile beriberi; see Table 4).

Owing to the low storage capacity, regular thiamine intake is important. Although, within the margin of physiological requirements, the organism attempts to prevent considerable thiamine losses by partial tubular reabsorption and intracellular fixation as TDP, the biological half-life time of this vitamin is assumed to be 9–18 days. After this period, when thiamine uptake is restricted, early nonspecific deficiency symptoms occur.

Table 3 National recommended daily allowances for vitamins

Country	B_1 (mg day^{-1})
Poland	1.9–2.0
Belgium, Denmark, Finland, France, Hungary, Luxembourg, Portugal, Spain	1.4
Greece, Norway	1.1–1.4
Czech Republic, Sweden	1.2
Austria, Germany, Ireland, Switzerland	1.0–1.3
The Netherlands, the United States	1.0–1.2
Italy, the United Kingdom	0.8–1.2

The recommended daily allowances for several European countries together with the US dietary reference intakes are listed in Table 3.

Deficiency Diseases

In thiamine deficiency, primarily, organs and tissues, such as the central and peripheral nervous system and the heart, are affected showing an increased energy turnover or close carbohydrate dependency. Clinical signs described in most animals refer to lesions in these tissues.

Since Eijkman's observations in fowls, the degeneration of the peripheral nerves was the first pathological symptom of thiamine deficiency noted in animals, leading to spastic pareses and paralysis of the legs. Later on, the role of thiamine in the central nervous system, especially in pyruvate metabolism of the brain, could be demonstrated, too.

Neurological signs are also characteristic of the deficiency state in human beings. The classical manifestation of deficiency can be seen in beriberi. This disease is endemic in several regions of East Asia. Depending on how far the disease has progressed and additional nutrients are involved (e.g., protein deficiency), it is characterized by either polyneuropathies and degenerative muscle wasting (dry or atrophic form) or cardiac arrhythmias and edema (wet or exudative form). The development of exudative beriberi predominates in cases of a high carbohydrate intake and increased physical activity. Protein deficiency is probably involved too. The Wernicke–Korsakoff syndrome, the cerebral form of deficiency, is characterized by mental confusion, memory disturbances, ataxia, ophthalmoplegia, and nystagmus. This syndrome can be interpreted as neuropathy of the central nervous system and is most commonly observed in malnourished alcoholics.

Table 4 Symptoms of clinical thiamine deficiency

Deficiency form	*Neurological signs*	*Cardiovascular signs*	*Other signs*	*Nutrient interactions*
Atrophic beriberi (dry or polyneuritic form)	Degenerative polyneuropathy of the lower extremities, paresthesia, reflex disorders, drop foot, muscle waste, convulsions			Caloric restriction, anorexia
Exudative beriberi (edematous or cardiovascular form)		Sinus tachycardia, cardiac arrhythmias, heart dilatation (beriberi heart), pericardial effusion, cardiac insufficiency	Lung and peripheral edemas, ascites, dyspnea, rarely lactic acidosis without edemas	High carbohydrate intake, protein deficiency, intense physical activity
Wernicke–Korsakoff syndrome (cerebral form)	Nystagmus, ophthalmoplegia, cerebellar ataxia, paralysis of the sixth brain nerve, polyneuropathy (burning feet syndrome), reflex disorders		Psychoses, hallucinations, memory loss, irritability	Alcohol abuse
Infantile beriberi (2–6 months)	Convulsions (increased intracranial pressure)	Tachycardia, cardiac insufficiency	Vomiting, diarrhea, abdominal distension, anorexia, cyanosis, dyspnea	

Source: Bitsch, R. (1997). Vitamin B_1 (thiamin). In: Biesalski, H. K., Schrezenmeier, J., Weber, P. and Weiß, H. (eds.) *Vitamine*, *Physiologie*, *Pathophysiologie*, *Therapie*, pp. 67–74. Stuttgart: G. Thieme.

The thiamine-dependent pyruvate dehydrogenase (PDH) and KDH complexes are key enzymes in the metabolic pathways of glucose and other energy-delivery substrates. The activities of both enzymes were found to play a rate-limiting role in mammalian brain mitochondrial preparations. Thus, lactic acid accumulation, as a result of the impaired glucose oxidation and disruption of oxidative phosphorylation utilizing pyruvate, α-ketoglutarate, and succinate as substrates, has been demonstrated in brain mitochondria from deficient rats. There is some evidence that localized lactic acid accumulation may contribute to the brain lesions in the Wernicke–Korsakoff syndrome. Furthermore, it is suggested that neurological symptoms may involve an impairment of the cholinergic neurotransmitter function. Acetylcholine and γ-aminobutyrate are primarily produced through the oxidative metabolism of glucose or via the citric acid cycle with acetyl coenzyme A (acetyl-CoA) and α-ketoglutarate as precursors. Although no change in regional brain acetylcholine levels was found in thiamine-deficient rats, acetylcholine utilization and turnover were reduced, and the central cholinergic mechanisms appear to be depressed in a deficient state. These alterations may be due to a decreased PDH and thereby activity limited acetyl-CoA production.

Infantile beriberi occurring in breast-fed infants between 2 and 6 months has particularly devastating consequences. Symptoms include cyanosis, dyspnea, tachycardia, abdominal distension, and convulsions. Sudden death often occurs as a result of cardiac failure. Nursing mothers, however, often do not show any signs of clinical deficiency. The thiamine deficiency symptoms are summarized in Table 4.

Therapeutic Administration and Toxicity

The importance of thiamine for the metabolism of the central and peripheral nervous systems, as is becoming clear from the deficiency symptoms, implicates its therapeutic application in all neurological system disorders. The recommended doses are 100–300 mg day^{-1}, often combined with pyridoxine and cobalamin. Because of the limited gastrointestinal absorption, the dose should be split up over the day. In progressive polyneuropathy and clinical beriberi, the same dosage may be initially administered i.m. or i.v., for a rapid tissue restoration, followed by oral therapy.

Lipid-soluble allithiamine derivatives can be given in smaller doses, because of the improved absorption. The passage of those derivatives across the blood–brain barrier is alleviated, too, as has been shown in animals. A significant improvement in the neuropathy score by oral application of the lipophilic benfotiamine has been observed in diabetic polyneuropathy patients as well.

As with other water-soluble vitamins, thiamin toxicity is very small when given orally, because of the limited absorption. In rare cases, with parenteral dosages above 100 mg, impaired sensations or even anaphylactic reactions have been observed, but it is not clear as to what extent additional components of the injection solution may interfere.

Thiamine-Dependent Enzymopathies

Thiamine-dependent enzymes are involved in four rare hereditary enzymopathies: leucinosis, Leigh syndrome, congenital lactacidosis, and thiamine-dependent megaloblastic anemia.

In leucinosis or maple syrup disease, the degradation of branched-chain amino acids is inhibited with following the accumulation of ketoanalogs. In the alleviated or intermittent form, this disorder can be improved by an intake of 10–150 mg of B_1 per day under simultaneous protein

Table 5 Congenital thiamine-responsive enzymopathies

Metabolic disorder	*Biochemical defect*	*Clinical symptoms*	*Therapy*
Leucinosis (maple syrup disease)	Keto acid dehydrogenase for the metabolism of branched-chain amino acids	Acidosis, ketosis, cerebral degeneration	Leucine-, isoleucine-, and valine-poor diet, thiamin (orally) 10–150 mg daily
Leigh syndrome (necrotizing encephalomyelopathy)	TTP formation, keto acid dehydrogenase	Lactacidosis, nystagmus, ataxia, convulsions, mental and psychic abnormal development	Thiamine (orally) 2.0 g daily (lipid-soluble derivatives)
Congenital lactacidoses	PDH	Lactacidosis, neurological symptoms, mental retardation	Thiamine lipid-soluble (?)
Thiamine-responsive megaloblastic anemia	Thiamine transport in tissue compartments	Megaloblastic anemia, diabetes, labyrinthine deafness	Thiamine (orally) 20–100 mg daily (lipid-soluble derivatives)

restriction. If untreated, severe neurological complications and physical and mental dysfunctions develop, followed by death.

Leigh syndrome is characterized by a necrotizing encephalomyelopathy and is also observed in ruminants. The exact biochemical defect is poorly understood but is thought to be a hereditary malfunction of TTP transferase, leading to impaired TTP formation, occasionally combined with a decrease in the amount of keto acid dehydrogenase. Neurological symptoms develop, progressing from nystagmus and paralysis of the external eye muscles to convulsions, ataxias, and disorientation resembling the Wernicke disease. Thiamine therapy may be successful, particularly when lipophilic derivatives (fursultiamine) are ingested up to gram dosages. Lactacidosis (which occurs at the same time) can be alleviated by oral intake of hydrogen carbonate combined with a low-carbohydrate diet.

The clinical picture of congenital lactacidosis resembles Leigh syndrome, and it is often impossible to distinguish between the two. The primary cause is a defect of the PDH or of partial enzymes of this complex. Generally, the prognosis is deleterious. The lactacidosis and the concomitant neurological symptoms could only in rare cases be affected by therapeutic thiamine doses.

Thiamine-responsive megaloblastic anemia has until now been very rare. The characteristic symptoms are a very strange combination of anemia, labyrinthine deafness, and insulin-dependent diabetes. The anemia can be improved only by oral doses of 20–100 mg of thiamine. Folate and vitamin B_{12} administration is ineffective. In one case, the diabetes was improved, and insulin therapy was no longer needed.

The primary metabolic defect is unclear. Thiamine-dependent enzymes remain unchanged, and there may be an impairment in thiamine transport in certain tissues. The enzymopathies are summarized in **Table 5**.

See also: Bioavailability of Nutrients; Vitamins: Overview.

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Thiamin: Properties and Determination

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Introduction

Thiamine, also known previously as aneurin or antineuritic factor, is a water-soluble B vitamin, designated B_1. Many human biochemical pathways require thiamine, and this has to come from diet, because humans do not synthesize thiamine. Food rich in thiamine can be sourced from legumes, whole grain cereals, vegetables, meat, milk, and dairy produce. The richest sources are yeasts (e.g., dried brewer's and baker's yeasts), liver (especially pork liver), and pork meat. In plant-based foods, thiamine predominantly occurs in the free form, while in animal-based foods, it is bound to proteins.

Chemically, thiamine is composed of a pyrimidine (2-methyl-4-amino-pyrimidine) moiety and a thiazole (5-(2-hydroxyethyl)-4-methylthiazolium) that are linked by a methylene bridge. The pyrimidine ring has an amino group on C_4 and the thiazole ring contains quaternary nitrogen, which confers free thiamine its instability. Cline and Williams first studied the chemical structure of thiamine in 1936, and they subsequently identified the pyrimidine moiety. The structure was eventually confirmed through synthesis of thiamine hydrochloride, thereby also pioneering the large-scale production of thiamine. The chemical structures of thiamine and its phosphorylated vitamers are shown in Figure 1.

Thiamine occurs in the free form and as an array of interconvertible phosphorylated vitamers, such as thiamine monophosphate (TMP), thiamine diphosphate (also called pyrophosphate, TDP), and thiamine triphosphate (TTP). Adults store approximately 30 mg thiamine that is mainly found in the liver, kidneys, brain, heart, and skeletal muscle. The predominant vitamer (80%) is thiamine diphosphate, which is also biologically active.

Thiamine is an enzyme cofactor, involved in the metabolism of carbohydrate and the branched-chain amino acids (leucine, valine, and isoleucine). It also plays an important role in defense mechanisms, and thiamine triphosphate is essential for neural function. According to the dietary reference intake levels set by the US Institute of Medicine, the recommended dietary allowance for thiamine is 1.0 mg day^{-1} for women (19–50 years) and 0.9 mg day^{-1} for men (19–50 years). The recommended daily intake of thiamine in Australia is 1.2 mg day^{-1} for males aged 19 years and over and 1.1 mg day^{-1} for females. Thiamine deficiency, in its early stages, produces nonspecific symptoms that may be overlooked or easily misinterpreted. As it progresses, the deficiency presents as beri-beri, a disease historically prevalent in countries where polished rice is the staple food. Milling yields rice endosperm with low thiamine content, because the thiamine-rich bran is removed. The clinical symptoms of beri-beri include anorexia, apathy, confusion, irritability, muscle weakness, and cardiovascular effects. Edema develops in wet beri-beri, while dry beri-beri is characterized by muscle wasting. In industrialized countries, thiamine deficiency is associated with chronic alcoholism, which affects the central nervous system, collectively presented as the Wernicke–Korsakoff syndrome. Inadequate dietary intake of thiamine has caused governments to implement public nutrition interventions, such as education, dietary supplementation, and food fortification. Upper limits for thiamine intake are not currently specified, because there have been no reports of adverse effects during pharmaceutical trials where thiamine was administered at high doses.

Properties

In food, thiamine is sensitive to pH. The vitamin is stable between pH 2.0 and 4.0, but not stable under alkaline, slightly alkaline, or strongly acidic conditions. The pyrimidine moiety is more inert than the thiazole. According to Bettendorff, at pH <4, the N-1′ of the pyrimidine ring is protonated, but the N-3′ is only protonated in the presence of concentrated sulfuric acid. In alkaline solutions between pH 8 and 10, the hydroxyl group attaches to C-2′ of the thiazole ring and leads to formation of the so-called 'yellow form' degradation product. Consequently, loss of thiamine occurs during commercial cooking of peas or beans, when sodium bicarbonate is added in order to preserve their color. According to Combs, instability due to the presence of sulfites can also occur. Sulfites react with thiamine to cleave the methylene bridge linking the pyrimidine and thiazole ring; this reaction is slow at high pH but is rapid under neutral and acidic conditions. Sodium disulfite ($Na_2S_2O_5$) and sodium bisulfite ($NaHSO_3$) are widely used food preservatives, and these may be responsible for thiamine degradation in aqueous solution during storage, even at low temperature. Free thiamine is easily oxidized to thiamine disulfide and other derivatives including thiochrome (Figure 2), which is fluorescent and thereby useful for detection and quantitative analysis.

Thiamine is also sensitive to high temperature and UV radiation. Loss of thiamine occurs during domestic and commercial food preparation through leaching of the vitamin into water. Prolonged cooking in vigorously boiling water increases the thermal degradation of thiamine as more oxygen is available and stays in contact with the food. Degradation losses of thiamine vary depending on cooking methods; boiling of vegetables causes a 14–66% thiamine loss, pressure cooking 14–35%, and steaming 11–27%. According to Kimura, boiling of pork meat results in 70% thiamine loss, steaming 40%, parching 35%, and frying 30%. Roasted and deep-fried chicken shows a higher thiamine loss in comparison with braising and microwave cooking. Thermal degradation of thiamine also releases sulfur-containing volatiles, such as 4,5-dihydro-2-methyl-3-furanthiol and 5-(2-hydroxyethyl)-4-methylthiazole.

Whole grain cereals are good sources of thiamine. In wheat, the germ contains around 64% of the total thiamine, the bran 33%, and the endosperm 3%. However, the germ and the bran are removed during milling, which yields flour low in thiamine. During bread making, up to 56% of total thiamine is lost due to baking (compared with the wheat flour from which it was

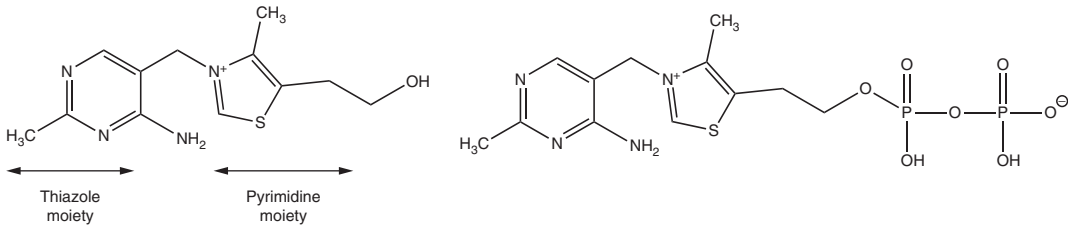

Figure 1 (Left) Free thiamine consists of pyrimidine and thiazole moieties and (right) exists predominantly as thiamine diphosphate.

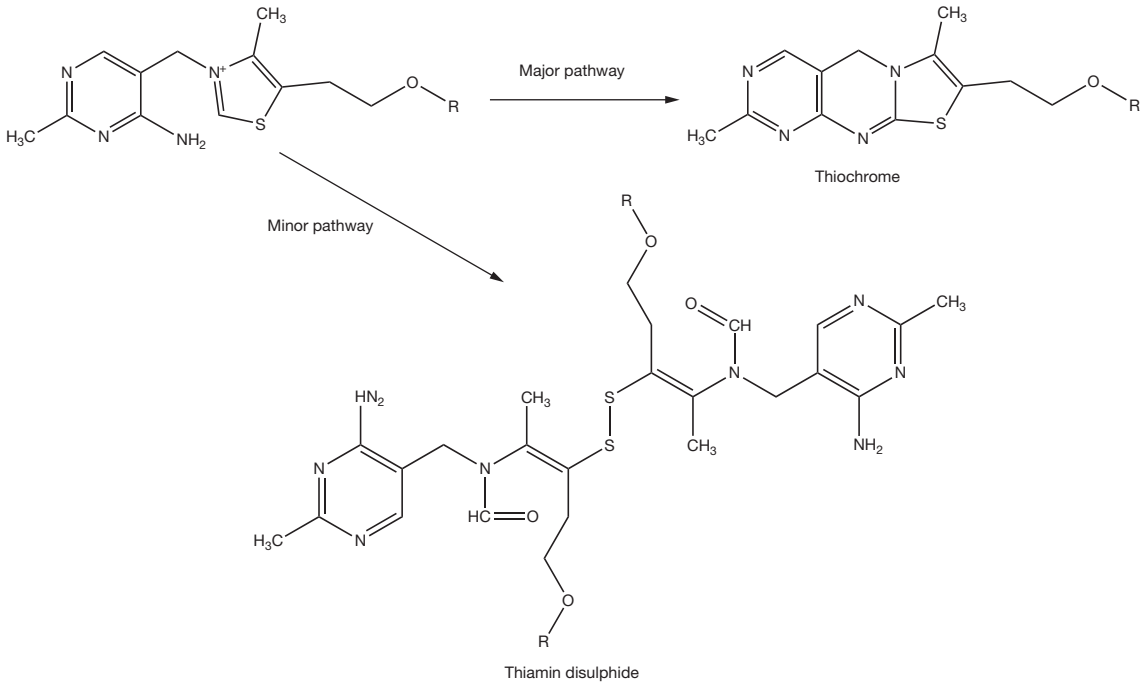

Figure 2 Oxidation of thiamine to fluorescent thiochrome (major pathway) and thiamine disulfide (minor pathway).

made). Extended fermentation during the making of rye sourdough bread also showed a 20–45% thiamine loss.

Anti-thiamine Factors

Several naturally occurring compounds such as o- and p-hydroxy phenols/polyphenols (e.g., caffeic acid, chlorogenic acid, and tannic acid) in ferns, tea, and betel nut behave as thiamine antagonists. Reaction of some phenols/polyphenols with thiamine oxidizes the thiazole ring yielding the non-absorbable (non-fluorescent) thiamine disulfide. Another anti-thiamine factor is the enzyme thiaminase, which is present in microorganisms, ferns, shellfish, and fish. Thiaminase breaks down thiamine by displacing the pyrimidine methylene group with a nitrogenous base or SH compound, thus eliminating the thiazole moiety. Consuming food containing thiaminase, without cooking, consequently leads to thiamine deficiency.

Quantitative Determination of Thiamine Vitamers in Food

To date, several techniques have been used for the identification and quantitative analysis of thiamine vitamers, each with advantages and constraints. High-performance liquid chromatography (HPLC) is the most reported method and is employed for the determination of thiamine and its speciation in various food matrices, dietary supplements, and biological fluids. Advances in mass spectrometry have produced studies, which exploit this technique for thiamine quantitation. Other analytical techniques that have been documented include colorimetric, fluorometric, spectrophotometric, and titrimetric techniques; capillary electrophoresis; microbiological assay; and gas chromatography. The Association of Official Analytical Chemists (AOAC) uses a thiamine assay based on fluorometry that measures thiochrome (AOAC 15th edition 1990, 18th edition 2005), first actioned in 1942. For the purpose of identification and quantitation, thiamine has to be extracted and released from food matrices. This involves sample preparation, which plays an indispensable role in any vitamin analysis.

Extraction of Thiamine

Thiamine in food is bound to protein and occurs naturally in the free form and phosphorylated forms. The initial step prior to any analysis is sample preparation in order to isolate and release thiamine from its binding proteins. Hot acid releases

thiamine from protein and enzymatic treatment(s) may be used to dephosphorylate the vitamers where the objective is measurement of total thiamine rather than speciated quantitation. In plant-based food, acid treatment also hydrolyzes the glycosides. According to the AOAC (Official Method 957.17), thiamine extraction involves hot acid digestion (0.1 M hydrochloric acid) at 95–100 °C for 30 min or autoclaving samples with 0.1 M hydrochloric acid at 121 °C for 30 min. There has been little agreement on the choice of acids; the use of 0.1 M hydrochloric acid, trichloroacetic acid, and sulfuric acid is reported in the literature. Trichloroacetic acid treatment has been suggested to retain the phosphorylated thiamine vitamers, allowing free thiamine to be measured separately from the other vitamers. Digestion of cereal-based food with only 0.1 M hydrochloric acid at 100 °C for 30 min is problematic due to starch gelatinization that prevents the release of thiamine from the food matrix. This potentially leads to inaccurate quantitation of thiamine. Hot acid hydrolysis alone is not sufficient to release protein-bound thiamine from samples of flour, yeast, and pork and needs to be followed by diastase treatment.

Literature has emerged that shows contradictory findings on enzymatic digestion for thiamine extraction. Diastases, such as taka-diastase and clara-diastase, exhibit amylase, protease, and phosphatase activities. Treatment of samples with diastase is performed to measure free thiamine, as its protease function releases thiamine from its binding proteins, amylase hydrolyses starch, and phosphatase digests the phosphate ester. It is suggested that diastase alone is sufficient to extract thiamine from food matrices, and treatment with multiple enzymes (α-amylase, potato acid phosphatase and papain protease) is not necessary. A combination of diastase and acid digestion at 100 °C was shown to be less effective for the extraction of endogenous thiamine, in comparison with treatment with diastase and protease. Extraction of thiamine from yeast, baby food, and cereals shows a high recovery using a combination of taka-diastase and β-amylase after hydrolysis with 0.1 M hydrochloric acid. Digestion with multiple enzymes consisting of papain, amylase, and phosphatase has also been reported for thiamine extraction. The optimized length of enzymatic treatment ranges from 4 to 18 h. Addition of sodium 1-octanesulfonate, during sample processing, produces much cleaner extracts. Sodium 1-octanesulfonate forms an ion pair complex with tetra-ammonium ion and other amino groups found in dried yeast during thiamine extraction, thus minimizing matrix interferences during chromatographic separation. To reduce the incubation time, enzymatic digestion (amylase, papain, and phosphatase) may be performed in an ultrasonic bath for 1 h. Jakobsen compared this approach with taka-diastase digestion at 45 °C for 18 h and found that mean thiamine was not significantly different ($p > 0.05$). The author concluded that varying types of enzymes, pH, and duration of ultrasonication affect the thiamine release causing significant differences in measured thiamine. The AOAC recommends processing of food samples with either taka-diastase or α-amylase treatment (pH 4.0–4.5) at 45–50 °C for 3 h after acid hydrolysis.

Extraction of thiamine from dietary supplements is a simpler and less time-consuming routine due to the high concentrations of synthetic thiamine. Powdered tablets are dissolved in nitric acid (HNO_3) and filtered before oxidation to thiochrome. Milli-Q water is also suitable to dissolve the homogenized powdered tablets followed by centrifugation and filtration. Determination of vitamins in food is often reputed to be lengthy and complex; nonetheless, simple and rapid extractions have been documented. Heat sonication of defatted raw almonds has been reported to simultaneously extract water-soluble vitamins, including thiamine. Non-enzymatic extraction using only 40% methanol (v/v) in 0.1 M aqueous hydrochloric acid was used to measure thiamine in polished rice. The recoveries were 94–101% with incubation at 60 °C for 30 minutes, suggesting that the extraction of thiamine in methanol–hydrochloric acid solution is efficient.

Oxidation of Thiamine

Converting thiamine to thiochrome in the presence of an oxidizing agent is only a prerequisite for fluorometric detection. Oxidation of thiamine in an alkaline solution of potassium ferricyanide (potassium hexacyanoferrate (III) ($K_3Fe(CN)_6$) was first described by Barger et al. in 1935, although the structure was not confirmed (Cline and Williams) until 1936–37. In this study, they found that thiamine hydrochloride was transformed to thiochrome, a pale yellow compound containing sulfur that has an intense blue fluorescence. More recently (2007), hydrogen peroxide derived from horseradish peroxidase was shown to be able to oxidize thiamine to thiochrome and was used prior to fluorometric spectrophotometry.

Solid-Phase Extraction

According to the AOAC method (AOAC 14th edition, 15th edition, 16th edition, 17th edition, and 18th edition), thiochrome is purified using a reusable weak cation exchange column (Bio-Rex 70, Bio-Rad Laboratories) and a weak acidic potassium chloride elution buffer. Afterwards, thiochrome is extracted into isobutanol (2-methyl-1-propanol), prior to fluorometric determination. Solid-phase extraction (SPE) using a Bio-Rex 70 column is an inefficient process in comparison with a reusable reversed-phase C_{18} column. The packing material in a Bio-Rex 70 column needs to be treated and repacked for reuse, unlike the C_{18} column, which can be washed free of sample matrix contaminants. Thiochrome has also been successfully recovered from a C_{18} column, when the elution buffer contains 30% methanol. This suggests that extraction of thiochrome from a potassium chloride elution buffer into isobutanol is no longer essential. The disadvantage of using the reusable cation exchange or the C_{18} column is that the column has to be regenerated and re-equilibrated after each use. In more recent times, disposable SPE columns in the form of cartridges have become commercially available in many different sorbent types. These are usually employed to selectively concentrate thiamine and simultaneously remove sample matrix components from extracts. Ollilainen et al. used radioisotope-labeled (^{14}C) thiamine to assess recovery during sample preparation and found that loss of ^{14}C-labeled thiochrome occurred during oxidation to thiochrome and SPE. The results showed that loading of samples onto SPE columns led to a 14% loss, and using the commercially available C_{18} sorbent,

the extraction efficiency was 85%, which was higher than using phenyl and cyanopropyl sorbents. Surveying the literature reveals little agreement about the merits of performing SPE in the context of thiamine determination. Some researchers perform filtration instead of SPE prior to HPLC analysis.

There are many factors that may influence the extraction of thiamine from food matrices including pH, added amount of enzyme, temperature, and duration of enzymatic incubation. Other considerations, including the length of the thiamine extraction protocol, analysis time, and maximum signal intensities, are also relevant in quantitation. Extraction efficiency is matrix-dependent and needs to be experimentally optimized for each food matrix in order to maintain assay sensitivity across food groups.

Microbiological Assay

In microbiological assay, thiamine is determined based on the growth response of thiamine-dependent microorganisms. Bacteria, such as *Lactobacillus viridescens* or *Lactobacillus fermentum*, are inoculated into the food extracts. After several hours of incubation, the turbidity corresponding to the growth of bacteria is measured using a spectrophotometer at 660 nm. Microbiological assay is useful for measuring total thiamine contents in food, but it has its drawbacks. The composition of thiamine vitamers in food is not ascertainable from bacterial inhibition, because the bacterial growth response does not differentiate between vitamers. Generally, thiamine concentrations determined using microbiological assay are higher than using fluorometry or HPLC.

Fluorometric Assay

Fluorometric spectrophotometry has been the AOAC recommended technique to determine thiamine since 1960. The intensities of thiochrome are proportional to the amount of thiamine present when determined at 365 nm of excitation wavelength and 425 nm of emission wavelength. Thiamine concentrations are then calculated using an external standard calibration to yield measurements of total thiamine.

High-Pressure Liquid Chromatography

HPLC is a separation technique utilizing the differential interaction of analytes with the stationary phase and mobile phase. Chromatographic separation of thiamine vitamers allows free thiamine and individual phosphorylated thiamine forms to be identified and quantified. Using HPLC, it is possible to simultaneously separate all vitamins from the B complex, and this is useful for measuring multivitamins in dietary supplements. Vitamin B complex in various food matrices can also be simultaneously analyzed. Due to varying physicochemical properties and stability of vitamins in food, different extraction protocols have to be performed for some vitamins.

HPLC thiamine analysis is based on ion pair chromatography, a type of reversed-phase chromatography, used for the separation and determination of ionic species. The mobile phase consists of aqueous buffer containing both organic solvent and counterions. As a counter ion combines with thiamine or thiochrome to form a neutral ion pair, this is then retained on a reversed-phase column. Mobile-phase systems suitable for reversed-phased columns include methanol, hexanesulfonate, and/or octanesulfonate. Normal-phase columns, as well as phenyl columns, have also been employed for thiamine analysis, even though they are not widely used.

HPLC can be interfaced with several modes of detection, for example, ultraviolet (UV), UV–Vis (diode array), fluorescence, Fourier transform infrared (FTIR), and electrospray mass spectrometry. Thiamine is detected and identified based on retention time, the wavelength at maximum absorption (UV), UV–Vis spectrum (diode array), and excitation/emission wavelengths (fluorescence). UV detection is useful for measuring high levels of thiamine in fortified food and dietary supplements. Fluorescence detection is suitable for detecting low concentrations of naturally occurring thiamine, as it offers better sensitivity than UV detectors. For fluorometric detection, thiamine is oxidized to fluorescent thiochrome either precolumn or (online) postcolumn.

Pre- and postcolumn oxidation of thiamine

Van de Weerdhof et al. pioneered the use of a fluorescence detector to determine thiochrome after a postcolumn oxidation with potassium hexacyanoferrate [$K_3Fe(CN)_6$]. The composition of the mobile phase was 0.1 M phosphate buffer (pH 6.8) and 10% methanol. Adding methanol as organic modifier in the mobile phase generally increased the formation of thiochrome and produced higher signal intensities. The signal's reproducibility improved with the addition of 12% methanol, while more than 12% methanol led to asymmetrical peaks. A mobile-phase system with pH $>$ 4.0 retained (postcolumn oxidized) thiochrome longer, in contrast to pH between 2.0 and 3.0. A postcolumn oxidation thiamine metabolite, 2-(1-hydroxyethyl)thiamine, was first identified by Ujiie et al., in fish and meat, during 1990. This metabolite is otherwise quantitatively analyzed as thiamine when precolumn oxidation is performed. Animal-based food contains 7–24% 2-(1-hydroxyethyl)thiamine, and in yeast, it makes up 37% of total thiamine. With the widespread use of reversed-phase columns, thiamine oxidation is performed precolumn rather than postcolumn, as it improves the sensitivity. The limitation of precolumn derivatization is that thiochrome has to be analyzed immediately to minimize degradation (due to its inherent instability).

Oxidizing agents for fluorometric detection

Besides potassium hexacyanoferrate ($K_3Fe(CN)_6$), mercuric chloride ($HgCl_2$), copper (II), cyanogen bromide (BrCN), and cobalt (II) have been used as oxidizing agents for fluorometric detection. In fact, thiamine is not stable in the presence of ferrous and cupric salts, thus necessitating the addition of a metal chelation agent to minimize thiamine degradation. Oxidation of thiamine in the presence of potassium hexacyanoferrate yields only 67% thiochrome formation. Fluorescence intensities depend on the pH and the stability of thiochrome. High peak intensities were detected when thiamine was oxidized at pH 7 rather than pH 6, while increasing the pH to 13 reduced the peak intensities. Mercuric chloride ($HgCl_2$) might be an alternative, producing higher fluorescence intensities than potassium hexacyanoferrate; yet, as an oxidizing agent, it is not stable. Oxidation of thiamine leads also to the

formation of non-fluorescent thiamine disulfide as a by-product. The oxidation conditions influence the relative amounts of thiochrome and thiamine disulfide formed. The ratio of thiochrome to thiamine disulfide depends on the pH, type of solvents, and oxidizing agents. Sulfites dissociate the pyrimidine and the thiazole rings causing thiamine degradation, which in turn affects the yield of thiochrome formation.

Liquid Chromatography–Mass Spectrometry

Electrospray (ESI) and atmospheric pressure chemical ionization (APCI) mass spectrometers are often interfaced to liquid chromatographs (HPLC-MS) as 'molecular weight-selective' detectors. In actuality, these systems ionize the compounds eluting from the HPLC by protonation, and the mass-to-charge ratio (m/z) of these 'pseudomolecular ions,' for example, $[M+H]^+$, is measured. It should be noted that these singly charged ions have an m/z ratio that is 1 Da higher than the molecular weight. Quantitation is based on the signal intensity of the individual ion for each analyte. Compounds with chromatographically overlapping peaks are easily distinguished if they have differing molecular weights. They can therefore be independently quantified. Hence, MS detection can offer more specificity than is possible using UV or fluorescence. Ions for all thiamine vitamers entering the MS can be selectively identified and quantified without prior chemical modification. Yamanaka et al. successfully determined thiamine levels in dried yeast using APCI-MS for detection. The thiamine pseudomolecular ion was monitored at m/z 265, along with the structurally related fragment ions (M-CH_2OH) and (M-H_20) at m/z 234 and m/z 247, respectively. APCI is a soft ionization technique that involves the transfer of charges from aerosols within a high electric field. Limited fragmentation of analyte molecules sometimes occurs during the spray ionization process, but the dominant species generated is normally the pseudomolecular ion.

Tandem mass spectrometers (MS/MS) are used as even more specific detectors in HPLC vitamin analysis. MS/MS instruments contain two mass-to-charge ratio analyzers, separated by a collision region where pseudomolecular ions can be deliberately fragmented by energetic interactions with an inert gas. The first analyzer is used as a mass filter to transmit only the selected pseudomolecular ion. This is then fragmented in the collision region, and the second mass analyzer is employed to monitor the fragments generated. When a single pseudomolecular ion and fragment pair are continuously monitored, this is called selected reaction monitoring (SRM). This mode of operation yields the most selective form of detection currently possible, as detected compounds must have a specific molecular weight and form a specific fragment upon dissociation. This selective and highly sensitive technique can be used to quantify trace-level target vitamers in complex matrices, free of interference from co-eluting molecules with similar molecular weights. The most frequently monitored thiamine fragments are m/z 144 (pyrimidine derived) and m/z 122 (thiazole derived), following the cleavage of the methylene bridge during collision-induced dissociation. The sensitivity of tandem MS/MS is also enhanced in SRM mode compared to scanning mode, because the mass analyzers are dedicated to only monitor thiamine-related m/z values and do not spend large parts of their duty cycle scanning m/z values, which are not relevant to thiamine. Positive-ion ESI and SRM mode were used for simultaneous determination of water-soluble vitamin B in pasta and in dietary supplements for regulatory purposes. Riboflavin, niacin, and pyridoxine were extracted from pasta simultaneously with thiamine, while folic acid and pantothenic acid needed to be extracted separately. The accuracy and precision of quantitative analysis using MS/MS are improved by adding known concentrations of stable isotope-labeled internal standards at the start of sample processing. These compounds are 'heavy' thiamine vitamers with molecular weights increased by inclusion of ^{13}C, ^{15}N, or ^{2}H into their structures during synthesis. The exercise then becomes one of relative quantification, dramatically reducing the impact of preparative losses and instrument variables.

Other Analytical Techniques

Thin layer chromatography finds its application for simultaneous separation of thiamine, riboflavin, and niacin. Thiochrome is detected using luminescence, and the method obviates the extraction of thiochrome into isobutanol.

FTIR spectrophotometry interfaced with HPLC was tested for its utility for determination of thiamine, riboflavin, pantothenic acid, and pyridoxine by Li and Brown in 2003. The reproducibility of the method, expressed as a coefficient of variation for thiamine standard solutions, was 6.7% and lower than that obtained using a UV detector.

A method employing capillary zone electrophoresis, coupled with UV detection, was developed and validated for simultaneous determination of thiamine and other water-soluble vitamins in dietary supplements. The separation in this technique is based on the electrophoretic mobility of solvated ions in an electric field. A more recent study showed that the sensitivity of UV detection at 214 nm (default setting) was not sufficient for thiamine quantitation in fortified energy drinks.

Gas chromatography (GC) is a common technique used to separate analytes in the gas phase and requires samples to be volatile. Chemical modification of analytes, to impart volatility prior to analysis, is a common practice for the analysis of biologically relevant molecules with this technique. Partitioning of the analytes occurs as vaporized samples and the inert carrier gas pass through the column. This is a long fused silica capillary tube coated on its inner surface with analyte-retentive polymers. Echols et al. employed a GC equipped with a nitrogen–phosphorus detector for quantitation of thiamine in cereals, meat, and vegetables, because the sensitivity of the more standard flame ionization detector was found to be inadequate for measuring endogenous thiamine in samples. Thiamine could only be detected by GC in the 4-methyl-5-(2-hydroxyethyl)thiazole form, and this approach is therefore of limited utility.

See also: Alcohol: Metabolism and Health Effects; Antinutritional Factors in Legume Seeds: Characteristics and Determination; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: High-Performance Liquid Chromatography; Mass

Spectrometry: Applications; Spectroscopy: Types; Thiamin: Physiology; Toxins in Food: Naturally Occurring; Vitamins: Overview.

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Tocopherols: Physiology and Health Effects

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Introduction

Tocopherols and tocotrienols comprise eight structurally related lipid-soluble compounds synthesized by plants and are widely distributed in vegetable oils and foods. They all behave as lipophilic antioxidants and have been associated with other biological functions in the body mainly revealed in cell studies and animal experiments. Importance of tocopherols for animal life was discovered nearly a hundred years ago when scientists Evans and Bishop reported in 1922 that reproductive failure of female rats kept on a diet containing rancid fat could be restored by the administration of fresh green lettuce or ground whole wheat. In sequence to other newly discovered vitamins, this factor was named vitamin E.

The significance of vitamin E as an essential nutrient has been demonstrated widely in different animal species including rodents, cattle, chicken, fish, and primates. Specific deficiency symptoms in different animal species are variable and include encephalopathy in chickens and muscular dystrophy in swine and cows. In humans, deficiency of vitamin E is very rare; typical neurological disorders are discovered only in individuals who suffer from inadequate fat absorption for any reason or who bear a genetic defect in α-tocopherol transport protein.

The term vitamin E used traditionally covered all tocopherols and tocotrienols, and vitamin E activity has been assessed as α-tocopherol equivalents. Besides α-tocopherol, these equivalents include small amounts of vitamin activity estimated to be provided by other tocopherols and tocotrienols. In 2000, the Food and Nutrition Board, Institute of Medicine, in the United States, considered only α-tocopherol an essential nutrient for humans. Nevertheless, the term vitamin E continues to be used as a generic term for different tocopherol homologues. In this article, the name vitamin E is reserved to α-tocopherol.

Of eight tocopherols and tocotrienols that appear naturally in foods, α-tocopherol is nutritionally the most important, and thus far, the majority of studies on their physiology and health effects are about α-tocopherol.

Dietary Sources

Vegetable oils, vegetable oil-based spreads, nuts, and seeds are good sources of tocopherols. Sunflower oil contains high amount of α-tocopherol, followed by corn, rapeseed, olive, and soybean oils. However, γ-tocopherol is the major tocopherol in corn, soybean, and rapeseed oils. Soybean oil provides also moderately δ-tocopherol. Besides α-tocopherol, palm oil contains also tocotrienols. Through use in food preparation, dietary fats affect importantly the tocopherols of mixed dishes and their contribution to the dietary intake of tocopherols. Only small amounts of α-tocopherol are detected in habitually consumed natural foods (cereals, fresh vegetables, and fruits and berries) and in animal products (meat, milk, butter, eggs, and fish), transferred from the feeds of animals. Despite low α-tocopherol concentrations, these foods make a notable contribution to the vitamin E intake due to their consumed amounts. Tiny amounts of other tocopherols and tocotrienols are also detected in several natural foods.

In order to improve nutritive value of foods, α-tocopherol can be added to foods. Synthetic vitamin E habitually used in food fortification and supplements is an equal mixture of eight stereoisomers of α-tocopherol, whereas tocopherols and tocotrienols appearing naturally in foods are free RRR-stereoisomers. In preparations, both synthetic and natural α-tocopherols are esterified in order to increase their stability.

Tocopherol Intake

Data of tocopherol intake are nearly totally limited to vitamin E. Most available nutrient databases do not present values for all tocopherols, and they often report vitamin E as α-tocopherol equivalents (α-TEs). It has been calculated that in diets typical for the United States and Canada, approximately 80% of total α-TEs from foods are contributed by α-tocopherol.

In developed countries, average dietary intakes of vitamin E range between 6 and 16 mg day^{-1}. Marked differences between countries not only are due to the types of oil consumed but also are affected by other dietary habits and may depend on the dietary survey method used and accuracy of food composition tables. In Europe where sunflower, olive, and rapeseed oils are habitually consumed, vitamin E intake is generally higher than in the United States where soybean oil dominates. Among the participants of the NHANES study in 2003–06, mean daily intake of vitamin E from diet of individuals aged 19 years and older was approximately 8 mg in men and 6 mg in women. Variable but somewhat higher intakes of vitamin E are reported in European populations. Mean daily intake of vitamin E from diet in eight European countries ranged from 8 to 16 mg in men and from 7 to 11 mg in women.

Use of vitamin E supplements can increase total vitamin intake greatly. Approximately one-third of men and 40% of women of the NHANES 2003–06 participants reported the use of supplemental vitamin E and their total vitamin intake was 10–20 times that of the supplement nonusers. In Europe, the use of vitamin supplements differs greatly between countries but is generally less common than in the United States. The effect of food fortification on average vitamin E intake is relatively small although certain fortified foods appear high in the ranking of foods by vitamin E content.

No representative dietary intake data of non-α-tocopherols and tocotrienols are available. In the United States, γ-tocopherol is the major tocopherol with estimated intakes exceeding that of α-tocopherol by a factor of 2–4. In Europe, γ-tocopherol intake is clearly lower but variable between countries predicted from the patterns of vegetable oil consumption. Based on few studies

from the United States and Finland, average daily intakes of other tocopherols and tocotrienols are very low ranging from <0.1 to 2–3 mg.

Physiology

Absorption

In foods, tocopherols are associated with lipid structures, and their absorption, transport, and tissue delivery are closely related with overall lipid and lipoprotein metabolism. The presence of bile salts, pancreatic enzymes, and formation of micelles are necessities for their absorption. Esterified forms used in supplements need to be hydrolyzed before absorption. Protein transporters involved in cholesterol absorption also facilitate the intestinal uptake of α-tocopherol. All forms of tocopherols and tocotrienols are taken up by intestinal cells (Table 1), and shortly after intake, they appear in the circulation in concentration reflecting their ingested amounts.

The presence of dietary fat and food matrix appear key factors in α-tocopherol absorption, and vitamin E supplements should be taken at meals. Without fat, approximately 10% of a single dose of naturally labeled α-tocopherol impregnated in fresh apples was absorbed; from a meal with 11 g fat, absorption of α-tocopherol was 33%. Uptake of vitamin E into circulation from supplements taken alone or with a low-fat meal is marginal. However, knowledge of the efficiency of vitamin E absorption is incomplete. The values for α-tocopherol absorption in healthy adults have been greatly variable ranging between 30% and 80% in experiments carried out by various techniques with different doses of α-tocopherol supplied in meals of variable composition. Large individual differences in the absorption of α-tocopherol indicate that there are important, probably genetically determined factors, which affect the uptake of vitamin E in humans.

Transport

Tocopherols and tocotrienols embedded in chylomicrons are transported mainly via lymph to circulation, though they may also be bound to high density lipoprotein (HDL) and excreted directly into portal circulation. In the circulation, chylomicrons are hydrolyzed by the lipoprotein lipase, and tocopherols are transferred to other lipoproteins and peripheral tissues. Chylomicron remnants are taken up by the liver, and at this stage, no discrimination appears between different tocopherols. However, in the liver, α-tocopherol transport protein (α-TTP) preferentially binds α-tocopherol, which controls the intracellular trafficking of α-tocopherol and is necessary for the transport of the vitamin to the plasma membrane before excretion from the liver. When secreted from the liver, α-tocopherol is associated with very low density lipoprotein (VLDL) in plasma, but uptake by HDL is also possible. The transfer through membranes to circulation is assisted by membrane proteins.

In the plasma, vitamin E is transported nonspecifically by all lipoproteins, and there is continuous exchange of α-tocopherol between different lipoprotein fractions assisted by human plasma phospholipid transfer protein. In fasting, plasma α-tocopherol is mainly bound to low density lipoprotein (LDL) and HDL. Delivery from the circulation to tissues can take place by various mechanisms that are available for lipoproteins.

α-TTP has central role in the distribution of α-tocopherol within the body. It regulates the fate of vitamin E and other tocopherols in the liver. Compared to α-tocopherol, the relative affinity of α-TTP for β-, γ-, and δ-tocopherols and α-tocotrienol was *in vitro* 38%, 9%, 2%, and 12%, respectively. α-TTP is also detected in other vital tissues such as the placenta and the brain. The major importance of α-TTP is verified by human vitamin E deficiency disease, which is caused by rare mutations in the α-TTP coding gene. In addition, other α-tocopherol-binding proteins, which may also bind other tocopherols, tocotrienols, and other ligands, have been detected in cells. Several different membrane proteins that are linked with absorption, uptake, and efflux and biliary secretion of vitamin E recognize all tocopherol isoforms similarly.

Metabolism

The metabolism of α-tocopherol is tightly regulated, and unlike other fat-soluble vitamins, there is no toxic accumulation in the liver. α-Tocopherol that is not released into circulation is excreted into the bile via protein transporters or is metabolized and excreted mainly in urine. All tocopherols and tocotrienols are metabolized by ω-hydroxylation and oxidation by cytochrome P450 enzymes followed by side-chain degradation through β-oxidation, while the chromanol ring is left intact. Cytochrome CYP4F2 is engaged in ω-oxidation of tocopherols in humans. This enzyme's catalytic activity for non-α-tocopherols is clearly greater than for α-tocopherol. The final product of α-tocopherol metabolism, α-carboxyethyl hydroxychroman (α-CEHC), is excreted in urine after conjugation. Feces, which contain unabsorbed vitamin, are however the major route for α-tocopherol excretion.

The turnover of α-tocopherol is slow; the depletion of tissues takes decades rather than weeks. In a recent kinetic study with tracer-marked RRR-α-tocopherol in healthy men and women, α-tocopherol half-life in plasma was 44 days and 96 days in red blood cells during 460-day tracking. In further analysis, it was estimated that half-life of the slowest turning over compartment of α-tocopherol, adipose tissue, was on average 184 days.

Non-α-tocopherols and tocotrienols are aggressively metabolized in the liver. Much greater proportion of γ-tocopherol

Table 1 Summary of tocopherol and tocotrienol physiology

	Alpha-tocopherol	*Non-α-tocopherols and tocotrienols*
Intestine	Absorbed similarly	
Circulation	During chylomicron hydrolysis transfer to other lipoproteins and peripheral tissues	
Liver	Chylomicron remnants are taken to the liver, no restriction at this stage	
Liver	Bound by α-tocopherol transport protein (α-TTP), the excess of α-tocopherol is metabolized and excreted	Metabolized and excreted
Circulation	Transport from the liver in VLDL and delivery to other lipoproteins and peripheral tissues	No transport from the liver to circulation?

than α-tocopherol is excreted in urine when consumed in equimolar amounts. Administration of α-tocopherol causes further increase in urinary excretion of non-α-tocopherols and tocotrienols in relation to the size of vitamin E dose.

Tissue Concentration

α-Tocopherol is detected in different tissues all over the body in concentrations that are always higher than those of other tocopherols. The highest concentrations are found in adrenals and adipose and nerve tissues. Adipose tissue is the major reservoir of vitamin E in the body. More than 90% of the total body α-tocopherol is located in fat droplets of adipose tissue cells. However, vitamin E is not easily removed from this store. Brain tissue appears especially resistant to depletion of vitamin E.

In developed countries, average plasma α-tocopherol concentrations range between 20 and 30 μmol l^{-1}, but two to three times higher concentrations are found among vitamin E supplement users. Plasma γ-tocopherol concentrations are always much lower even when γ-tocopherol is the major tocopherol, and use of vitamin E supplements causes further decrease in plasma γ-tocopherol. Among the nonusers of vitamin supplements in NHANES 1999–2000, plasma γ-tocopherol was on average 6 μmol l^{-1} but only 2 μmol l^{-1} among those who used supplements >400 IU day^{-1}. In European countries, mean plasma γ-tocopherol concentrations habitually range from 1 to 2 μmol l^{-1}.

In fasting state, tocotrienols are undetectable in plasma, and after a single dose, they peak in plasma in few hours and disappear within 24 h. However, during long-term supplementation, tocotrienols in different tissues are significantly increased.

Function

Main biochemical function of α-tocopherol is suggested to be its antioxidant activity. As a chain-breaking antioxidant, α-tocopherol prevents propagation of free radical reactions of polyunsaturated fatty acids (PUFAs) in membranes and aids to maintain membrane integrity. However, direct end products of the α-tocopherol oxidation are hardly detected in the tissues, which may depend on the fact that the tocopheryl radical can be reduced back to tocopherol by vitamin C or other reducing agents. Evidence of decreased oxidative stress with α-tocopherol supplementation in humans is inconsistent. Primarily based on *in vitro* studies, α-tocopherol exerts several other important biological functions including modulation of cell signaling and gene expression.

Several enzymes especially those affecting cell proliferation, membrane trafficking, and metabolism of xenobiotics can be modified by α-tocopherol. Genes involved in the metabolism and excretion of vitamin E are regulated by α-tocopherol. Also, the products of vitamin E metabolism are proposed to have biological activities. Several molecular and cellular functions are suggested for water-soluble α-tocopheryl phosphate, which appears in minute amounts in foods and tissues. Yet, the mechanisms causing specific deficiency symptoms of vitamin E such as female infertility of rodents and neurological defects in humans are not known.

Non-α-tocopherols and tocotrienols behave as efficient lipid-soluble antioxidants *in vitro*; however, their biological activity is greatly limited due to discrimination in the liver metabolism. Various nonantioxidant functions that clearly differ from those of α-tocopherol have been associated with non-α-tocopherols and tocotrienols mainly based on cell studies and animal experiments. γ-Tocopherol appears to have stronger antinitrative and anti-inflammatory effects than α-tocopherol. Tocotrienols are suggested to have neuroprotective effects, to inhibit cholesterol synthesis, and to reduce growth of cancer cells.

Recommendations for Vitamin E

Estimates of required amounts of tocopherols concern only vitamin E. In 2000, the Food and Nutrition Board considered only α-tocopherol an essential nutrient. Previously, vitamin E was estimated in α-tocopherol equivalents, which also included small amounts of vitamin activity assumed to be provided by non-α-tocopherols and tocotrienols. Based on rat fertility experiments, biological activities of different tocopherols and tocotrienols were defined as follows: α-tocopherol x1, β-tocopherol x0.5, γ-tocopherol x0.1, δ-tocopherol x0.03, α-tocotrienol x0.03, and β-tocotrienol x0.05; no biological activity was found for γ-tocotrienol and δ-tocotrienol.

In the absence of any specific metabolic function ascribed to vitamin E, it has been demanding to set the basis for the estimation of vitamin E requirement. The Food and Nutrition Board (2000) based their assessment on the intake of α-tocopherol, which was estimated to maintain plasma α-tocopherol at sufficient level to prevent *in vitro* hemolysis of red blood cells taken from humans who participated in vitamin E deficiency experiment. Accordingly, the estimated average requirement of adults was set at 12 mg day^{-1}, and after addition of safety margin, the recommended daily intake was set at 15 mg day^{-1} for men and women.

The recommendation applies to natural RRR-α-tocopherol and 2R-α-tocopherol stereoisomers detected in synthetic α-tocopherol preparations. Synthetic α-tocopherol, all-rac-α-tocopherol or dl-α-tocopherol, is an equal mixture of eight stereoisomers. Due to the low affinity of α-TTP to 2S-vitamers, the relative bioavailability of the synthetic form of α-tocopherol was estimated to be only half of the natural α-tocopherol. This means that only α-tocopherol found naturally in foods and 2R-α-tocopherols in vitamin E preparations contribute to vitamin E activity. Recommended amounts of vitamin E given as milligrams of RRR-α-tocopherol can be transformed into international units (IU) by the following factors: 1 mg of α-tocopherol is equivalent to 1.49 IU of the natural form and 2.22 IU of the synthetic form of the vitamin.

Another approach to estimate vitamin E requirement is based on the relation between the need for vitamin E and the intake of PUFAs. In Europe, the Scientific Committee on Food considered that 0.4 mg of vitamin E per g of total PUFAs is adequate for adults provided that daily vitamin E does not fall below 4 mg for adult men and 3 mg for adult women. Habitual intake of vitamin E among populations without signs of vitamin deficiency has also been used to guide the assessment of the need of vitamin E. Worldwide, there is considerable variation in the national recommendations for vitamin E, and some authorities have considered the scientific basis too vague thus far to set any specific numeric value for vitamin E requirement.

Toxicity of vitamin E is very low. No adverse effects have been described from vitamin E provided by food sources. The Food and Nutrition Board (2000) set the upper intake limit of supplemental α-tocopherol at 1000 mg day^{-1} for adults. This amount covers both synthetic and natural forms of supplemental vitamin E and includes also 2S-α-tocopherol stereoisomers without vitamin activity, but not vitamin E occurring naturally in foods. In Europe, somewhat lower tolerable upper intake level, 300 mg day^{-1} for adults, was proposed by the Scientific Committee on Food in 2003.

Deficiency of Vitamin E

Vitamin E deficiency due to low dietary intake has not been described in normal, healthy individuals. However, deficiency may arise due to prolonged fat malabsorption for any reason, due to genetic defects in lipoprotein transport or genetic defect in hepatic α-TTP. In humans, the peripheral nervous system is most susceptible to vitamin E deficiency. Neurological disturbances associated with low vitamin E status were first described in children who suffered from deficient lipid transport in plasma due to genetic defect in lipoprotein formation.

Primary vitamin E deficiency is caused by genetic defects in α-TTP formation. Symptoms of AVED (Ataxia with Vitamin E Deficiency) include peripheral neuropathy, ataxia, and skeletal myopathy. Retinitis pigmentosa is often associated with vitamin E deficiency. Several different defects have been identified in the gene coding α-TTP, and severity of the symptoms appears to vary depending on the type of genetic defect. Absorption of α-tocopherol is normal in AVED patients, but due to defective α-TTP, tissue vitamin concentrations are not maintained. Progression of deficiency symptoms can be halted by high oral doses of α-tocopherol, and some symptoms may be even reversed if supplementation is started at an early stage.

Although overt deficiency of vitamin E is very rare, risk of marginal deficiency is possible when food intake and fat intake are greatly restricted. There are several intestinal disorders that affect absorption of nutrients such as gastrectomy, celiac disease, Crohn's disease, and short bowel syndrome in addition to hepatobiliary syndromes and cystic fibrosis where the risk of vitamin E deficiency is generally recognized. Medications used to decrease fat absorption also reduce absorption of vitamin E.

In the absence of more specific measurements for the estimation of adequate vitamin E status, determination of plasma α-tocopherol is commonly used. However, since the plasma lipid level influences the α-tocopherol concentration, correction for plasma lipids may be warranted in subjects with high lipid levels. In an experimental vitamin E depletion study, increased hemolytic tendency was discovered in subjects with plasma α-tocopherol concentration below 12 μmol l^{-1}, corresponding to α-tocopherol/total cholesterol ratio of 2.25 μmol l^{-1}. Plasma α-tocopherol concentration above 16.2 μmol l^{-1} has been suggested as an indicator of acceptable vitamin E status.

Vitamin E and Chronic Diseases

Due to its effects on oxidation, immunologic function, and inflammation, vitamin E has been linked to several chronic diseases such as cardiovascular diseases, cancer, and dementia (Table 2). The associations have been studied in humans in observational and interventional settings having either dietary or supplementary intake or serum levels as exposure. Anti-inflammatory and antioxidative functions are also associated with other tocopherols and tocotrienols. Mainly based on nonhuman studies, their efficiency in the prevention of cancer, cardiovascular, and neurodegenerative diseases could be even superior in comparison with α-tocopherol. Thus far, no large-scale human intervention studies of non-α-tocopherols or tocotrienols have been carried out.

Table 2 Disorders or diseases linked to vitamin E

	Dietary intake or plasma levels of vitamin E	*Supplemental vitamin E*
Cardiovascular diseases	Inverse association	No benefit
Cancer	Inconclusive results	No benefit
Type 2 diabetes	Weak inverse association	No benefit
Cognitive decline	Weak inverse association	No effect
Eye disease	Weak inverse association	No effect
Lung function, asthma	Inconclusive results	Insufficient evidence

Cardiovascular Diseases

Atherosclerosis is the central pathway leading to cardiovascular diseases that is thought to be affected by vitamin E. Atherosclerosis is characterized by excessive deposition of cholesterol in the arterial wall and involves LDL oxidation. Most importantly, vitamin E has antioxidant properties and has reduced LDL oxidation especially in *in vitro* and animal studies. In addition, vitamin E could reduce inflammation and affect foam cell formation, plaque stability, and gene expression related to cell signaling.

In observational studies, high vitamin E intake or better status was associated to reduced risk of cardiovascular diseases. The primary prevention trials have not, however, shown significant benefit from the α-tocopherol supplements in relation to different cardiovascular outcomes including myocardial infarctions and strokes. The daily doses have ranged from 30 mg to 300 mg. Accordingly, it has been concluded that there is adequate evidence showing that supplementation with vitamin E in healthy populations without known nutritional deficiencies does not reduce the risk of cardiovascular disease and supplementation is not recommended.

Even though there is no benefit in healthy population, some subgroups experiencing high levels of oxidative stress have been suggested to have benefit. Firstly, it is suggested that diabetic subjects with haptoglobin genotype 2-2 might have benefitted from tocopherol supplementation. The hypothesis is that the diabetic subjects have more LDL cholesterol oxidation and the haptoglobin 2-2 genotype is less efficient in removing oxidative complexes with hemoglobin. Another subpopulation suggested to benefit from tocopherol supplements is hemodialysis patients.

Cancer

Cancers include heterogeneous nature of different types of cancer, and their risk is affected by multiple environmental and genetic influences. Even though risk factors for cancer are considerably more complex than for cardiovascular diseases, the same pathway, that is, the antioxidant properties of vitamin E, is central. Vitamin E could prevent DNA damage and reduce lipid oxidation and activation of carcinogens such as nitrosamines. In addition to acting as a free radical scavenger, vitamin E enhances the body's immune response, which may play a role in cancer prevention.

Several observational studies have analyzed the associations between tocopherols and risk of site-specific cancer incidence. Regarding risk of prostate cancer, observational studies having serum α-tocopherol or dietary vitamin E intake as exposure have been inconclusive. Suggested reduced risk of prostate cancer by 50 mg daily supplementation with synthetic alpha-tocopheryl acetate in the Finnish ATBC (Alpha-Tocopherol, Beta-Carotene Cancer Prevention) study has not been confirmed in other large-scale trials with higher doses of vitamin E; rather, a nonsignificant increased risk of prostate cancer was suggested in SELECT (Selenium and Vitamin E Cancer Prevention Trial) study. Furthermore, the results of SELECT study suggested that in those who have high serum α-tocopherol concentration, selenium supplements can increase prostate cancer risk. Most studies have focused on α-tocopherol; however, it is hypothesized that γ-tocopherol might have beneficial effects instead of α-tocopherol.

Similarly for breast cancer, vitamin E status estimated from intake or plasma concentrations in observational studies or the supplemental intake in clinical trials has not shown significant association or effect. Furthermore, studies on the risk of colon or lung cancer have not provided evidence supporting the role of vitamin E. On the contrary, dietary intake of vitamin E, but not the serum α-tocopherol concentration, was associated with reduced gastric cancer risk in meta-analysis of observational studies.

Meta-analysis of randomized clinical trials has concluded that vitamin E supplementation does not affect cancer incidence or mortality. Dietary supplements are not recommended for cancer prevention.

Type 2 Diabetes

In addition to antioxidant and anti-inflammatory properties, vitamin E is suggested to have an effect on insulin sensitivity through enhanced adiponectin expression. This effect is similar to the thiazolidinediones, which have structural similarities with tocopherols and have been used in type 2 diabetes treatment.

Cohort studies have provided evidence for reduced risk of type 2 diabetes with higher dietary vitamin E intake. However, beneficial effects of vitamin E supplementation in prevention of type 2 diabetes have not been confirmed in large-scale randomized interventions.

Dementia

Dementia and cognitive impairment with increased aging are associated with increased oxidative stress and are thought to be preventable by antioxidants, most of all, by vitamin E. It is hypothesized that the central nervous system with high lipid and iron content is particularly susceptible to oxidative damage by free reactive oxygen species.

Several, but not all, observational prospective studies have pointed to decreased cognitive impairment or reduced Alzheimer disease risk by higher vitamin E intake or higher plasma α-tocopherol concentration. For the prevention of Alzheimer disease, the studies suggest that vitamin E should come from food not from supplements. Cochrane Review in 2012 concluded that there is no convincing evidence that vitamin E is of benefit in the treatment of Alzheimer disease or mild cognitive impairment. A more recent study has, however, suggested slower functional decline and reduced caregiver burden with α-tocopherol supplementation in subjects with mild to moderate Alzheimer disease.

Eye Diseases

Oxidative damage of lens proteins leads to cataract development, and oxidative stress is hypothesized to be one of the pathogenic mechanisms in age-related macular degeneration. Vitamin E is concentrated in lens fibers and membranes and may inhibit cataract by reducing peroxidation of lens lipids and stabilizing lens cell membranes.

A modest borderline protective effect in primary prevention of age-related macular degeneration has been found for vitamin E in an analysis of prospective cohort studies. However, little or no effect for other dietary antioxidants was shown. In intervention studies aimed to prevent or decrease progression of eye diseases, daily supplementation with vitamin E alone or in combinations with other antioxidants or selenium has not reduced cataract risk; neither was it found to be effective against occurrence of age-related macular degeneration.

Respiratory Tract Effects

The role of vitamin E has been suggested in different conditions including common colds, pneumonia, and asthma. Supplementation with α-tocopherol above the recommended levels is suggested to improve immune function, especially in the aged, and thus could affect, for example, the risk of common colds. As asthma is an inflammatory lung condition, a role for vitamin E as an anti-inflammatory agent has been suggested.

Results from few intervention studies on the effect of α-tocopherol on respiratory tract infections have been inconsistent. Even though higher serum α-tocopherol concentration has been associated with better performance in spirometry in cross-sectional analysis and serum levels of α-tocopherol have been lower in subjects with asthma, the role of α-tocopherol in asthma is unclear. It is of note that γ-tocopherol is suggested to elevate allergic inflammation in asthma contrasting the anti-inflammatory effects of α-tocopherol.

Other Health Effects

Preeclampsia is associated with increased oxidative stress. Several randomized controlled trials have been carried out with a combined daily supplementation of 1000 mg of vitamin C and 400 IU of vitamin E. However, the results have been

disappointing, and meta-analysis of nine trials reported no difference in occurrence of preeclampsia between treatment and placebo groups. Combined supplementation of vitamins C and E does not decrease the risk of preeclampsia and should not be offered for pregnant women for prevention of preeclampsia or other pregnancy-induced hypertensive disorders.

Controversially, experimental evidence has suggested that in addition to its antioxidative effect, α-tocopherol could have a possible direct role in bone remodeling, promoting osteoclast fusion, and thus stimulating bone resorption. Two studies have suggested that low serum α-tocopherol concentrations or low dietary intake are associated with increased risk of hip fracture.

Possible Adverse Effects

In general, antioxidant supplements do not seem to prevent cancer, cardiovascular diseases, or death. Contrary to general belief of antioxidant supplements being of no harm, the systematic reviews have concluded that vitamin E supplements may increase mortality. Dietary intakes have not been associated with increased mortality. The following are suggested reasons for the increased mortality: There is interference of the balance between antioxidants and oxidative stress; endogenously derived antioxidants are more important than diet-derived ones; dietary intake in Western countries meets the requirements; and the synthetic forms of vitamin E have different effects than their dietary forms.

In addition, vitamin E is suggested to interfere vitamin K metabolism. High doses of vitamin E could adversely affect vitamin K status and gamma-carboxylation of proteins in the coagulation cascade. The clinical significance of this effect is, however, uncertain, as it could be either beneficial as a mild anticoagulant or adverse by increasing the risk of bleeding especially in users of oral anticoagulants.

See also: Antioxidants: Role on Health and Prevention; Ascorbic Acid: Physiology and Health Effects; Cancer: Diet in Cancer Prevention; Dietary References: US; Food Composition Databases; Vegetable Oils: Composition and Analysis; Vegetable Oils: Dietary Importance; Vitamins: Overview.

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http://www.serbianfood.info/eurreca/ – Eurreca Micronutrient database. Nutri-RecQuest gives the latest micronutrient recommendations of a country or organization.

Tocopherols: Properties and Determination

NE Craft, Craft Technologies, Inc., Wilson, NC, USA

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The tocochromanols have absorbance maxima near 220 and 300 nm. They also exhibit fluorescence emission near 325 nm when excited near their absorbance maxima. These characteristics are used to measure the tocochromanols after being isolated from the sample matrix. They are insoluble in water and highly soluble in several organic solvents. Free tocochromanols can be directly extracted but complex samples often require saponification to hydrolyze ester linkages of triglycerides and tocopheryl esters.

Tocochromanols can be measured by gas chromatography (GC) with flame ionization detection (FID) or mass spectrometry (MS) detection. When the correct column and conditions are used, the eight major tocopherols and tocotrienols can be separated with or without derivatization. High-performance liquid chromatography (HPLC) has become the most popular mode of analysis during the past 25 years. In combination with HPLC, tocochromanols can be detected using UV detection, fluorescence detection, electrochemical detection (ECD), evaporative light-scattering detection (ELSD), or MS detection. The eight major tocochromanols frequently are separated using normal-phase HPLC with silica, diol, or amino columns. Until recently, it was not possible to separate the eight tocochromanols by reversed-phase (C18 or C8) HPLC. Now, they have been separated using C30, pentafluorophenyl (PFP), and cyclodextrin columns.

Tocopherols have three chiral centers, which result in eight stereoisomers. All of the stereoisomers have not been separated in a single chromatographic separation. The tocopherol methyl ethers can be separated into five peaks using a CHIRALCEL OD-H column. The addition of GC separation is required to separate the 2S-isomers.

Background

The vitamin E family of compounds is biosynthesized in plants, predominantly in the chloroplasts. They are abundant in the oils of seeds such as corn, soy, rice, wheat, sunflower, and safflower. Unlike plants, animals cannot synthesize vitamin E and must obtain it from their diet. Vitamin E is one of four essential fat-soluble vitamins. It was discovered during the 'Renaissance' of Nutrition in the 1920s and 1930s when many of the vitamins were discovered and isolated. In 1922, Evans and Bishop identified a fat-soluble substance, 'factor X,' which was required for normal reproduction in rats. Observations were confirmed by Sure in 1924 and the name 'vitamin E' was proposed.

α-Tocopherol was isolated from wheat germ in 1936 by Evans' group and synthesized by Karrer et al. in 1938. Evans' group also isolated β- and γ-tocopherols from vegetable oil in 1937. In the 1960s, the tocotrienols were isolated from rice bran oil. Tocotrienols were synthesized in 1976 by Scott et al.

Definitions

'Vitamin E' is a generic descriptor for all tocol and tocotrienol derivatives exhibiting qualitatively the biological activity of α-tocopherol.

'Tocopherol' is the generic description for all mono-, di-, and trimethyltocochromanols, irrespective of biological activity, and is not synonymous with the term 'vitamin E.'

'Tocotrienol' is a trivial name for 2-methyl-2-(4′,8′,12′-trimethyltrideca-3′,7′,11′-trienyl)chroman-6-ol, possessing three double bonds in the phytyl tail.

'Tocol' is the trivial name for 2-methyl-2-(4′,8′,12′-trimethyltridecyl)chroman-6-ol, having no methyl substitutions in the chroman ring.

'Tocochromanols' will be used in this article to refer to all tocopherols, tocomonoenols, tocodienols, and tocotrienols.

Vitamin E: Chemical Properties

There are more than eight naturally occurring tocopherol compounds that have been isolated from plant sources and all have a 6-chromanol ring and a phytyl side chain. The most prominent ones include four tocopherols and four tocotrienols. They differ in the number and position of methyl groups on the phenolic ring (Figure 1). Tocotrienols differ from tocopherols in that they contain three double bonds at the 3′, 7′, and 11′ positions in the phytyl tail.

The tocopherol molecule has three chiral centers at positions 2, 4′, and 8′ in the phytyl tail. As such, there are eight possible stereoisomeric configurations (2^3). All naturally occurring tocopherols (α-, β-, γ-, and δ-) have RRR molecular configuration (2d, 4′d, 8′d, x-tocopherol) in their phytyl groups. Since tocotrienols have only one chiral center at position 2, there are only two possible stereoisomers. The naturally occurring stereoisomer of α-tocopherol, formerly known as D-α-tocopherol, is now designated RRR-α-tocopherol to distinguish it from the synthetic version.

In addition to the natural forms of vitamin E, there are many synthetic forms that are often esterified with different functional groups such as acetate, succinate, and nicotinate. The esterified forms of vitamin E are less susceptible to degradation and thus frequently used as sources of vitamin E in foods and pharmaceuticals. The tocopherol esters must be hydrolyzed to serve as antioxidants.

More recently, additional natural analogs of vitamin E have been identified. These include α-tocomonoenol from palm oil, marine-derived tocopherol from salmon egg, δ-tocomonoenol from kiwi, γ-tocomonoenol from pumpkin seeds, tocotrienols in palm oil, and desmethyl and didesmethyl tocotrienols from rice bran.

Tocopherols are viscous oils at room temperature with a light yellow color; however, they are nearly colorless and

	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
δ	H	H	CH_3
γ	H	CH_3	CH_3

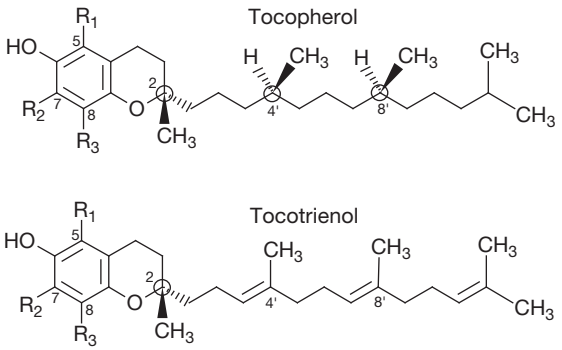

Figure 1 Chemical structures of tocopherol and tocotrienol. α-, β-, δ-, and γ-Homologues exist for both molecules and the R-groups of each are listed in the table. The circles indicate chiral centers, each of which permits stereoisomers. Each tocopherol has eight (2^3) stereoisomers while each tocotrienol has only two.

Table 1 Trivial name, chemical name, chemical formula, molecular weight, wavelengths of absorbance maximum, absorptivity coefficients, and fluorescence emission maximum

Common name	*Systematic name*	*Chemical formula*	*Molecular weight*	*Absorbance maxima (nm)*	$E^{1\%}_{cm}$ *[ε]*	*Fluorescence emission (nm)*
α-Tocopherol	2,5,7,8-Tetramethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol	$C_{29}H_{50}O_2$	430.71	292/*294*	75.8 [3265] *71*	320
β-Tocopherol	2,5,8-Trimethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol	$C_{28}H_{48}O_2$	416.69	296/*297*	89.4 [3725] *87.6*	322
δ-Tocopherol	2,8-Dimethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol	$C_{27}H_{46}O_2$	402.66	298/*298*	87.3 [3515] *91.2*	322
γ-Tocopherol	2,7,8-Trimethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol	$C_{28}H_{48}O_2$	416.69	298/*298*	91.4 [3809] *92.8*	322
α-Tocotrienol	2,5,7,8-Tetramethyl-2-(4′,8′,12′-trimethyltrideca-3,7,11-trienyl)-chroman-6-ol	$C_{29}H_{44}O_2$	424.67	292/*292.5*	86.0 [3652] *91*	323
β-Tocotrienol	2,5,8-Trimethyl-2-(4′,8′,12′-trimethyl-3,7,11-tridecatrien-1-yl)-6-chromanol	$C_{28}H_{42}O_2$	410.64	296/*296*	86.2 [3540] *87*	323
δ-Tocotrienol	2,8-Dimethyl-2-(4′,8′,12′-trimethyl-3,7,11-tridecatrien-1-yl)-6-chromanol	$C_{27}H_{40}O_2$	396.61	297	85.8 [3403] 88.1	324
γ-Tocotrienol	2,7,8-Trimethyl-2-(4′,8′,12′-trimethyl-3,7,11-tridecatrien-1-yl)-6-chromanol	$C_{28}H_{42}O_2$	410.64	297	91.0 [3737]	324
Tocol	2-Methyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol	$C_{26}H_{44}O_2$	388.61	298–298.5	97–98.4	327
α-Tocopherol acetate	2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2H-chromen-6-yl acetate	$C_{31}H_{52}O_3$	472.74	284–286/*285.5*	42–43.6 [2061]	313
α-Tocopherol succinate	2,5,7,8-Tetramethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol acid succinate	$C_{33}H_{54}O_5$	530.78	*286*	*38.5* [2044]	313

Absorbance maximum and $E^{1\%}_{cm}$ in ethanol. Values in brackets [ε] are molar extinction coefficients calculated from $E^{1\%}_{cm}$, in ethanol. Values in *italics* were taken from *The Merck Index*, 13th ed. (2001) where other solvents may be specified.

Source: Eitenmiller, R. and Lee, J. (eds.) (2004). *Vitamin E: food chemistry, composition, and analysis*. New York: Marcel Dekker; Machlin, L. J. (ed.) (1980). *Vitamin E: a comprehensive treatise*. New York: Marcel Dekker.

odorless when highly purified. They are practically insoluble in water but are soluble in oils and organic solvents, such as acetone, alcohols, chloroform, hexane, and tetrahydrofuran (THF). Tocopherols have molecular weights ranging from 396.6 to 439.7 and boil between 200 and 220 °C. They have maximum ultraviolet light absorbance (λ_{max}) at about 220 and 290–300 nm. The absorptivity coefficient ($E^{1\%}_{cm}$) at the higher λ_{max} ranges from 75.8 to 91.4 (Table 1). In addition, tocopherols

exhibit fluorescence emission near 320–325 nm upon UV excitation near the 220 or 290 nm range (Table 1). Vitamin E esters exhibit UV absorbance to a lesser extent and ~6–8 nm hypsochromic to the corresponding tocopherols (286–292 nm). The $E_{cm}^{1\%}$ of the tocopherol esters is about half that of the free tocopherols and the fluorescence is much weaker (Table 1). An example of the UV absorbance spectrum and the fluorescence emission is provided in Figure 2.

Tocopherols can be oxidized by atmospheric oxygen, and the oxidation is accelerated by heat, light, alkali, and metal ions. However, tocopherols are reasonably stable to heat and alkali in the absence of oxygen. The antioxidant property of tocopherols is due to their ability to interrupt free-radical chain reactions by quenching the free radical. The hydrogen from the hydroxyl group in the phenolic ring is donated to the free radical thereby generating a relatively stable radical form of vitamin E, the α-tocopheroxyl radical.

Tocopherols and tocotrienols are effective free-radical chain-breaking antioxidants because they react with peroxyl radicals impairing unsaturated lipid peroxidation.

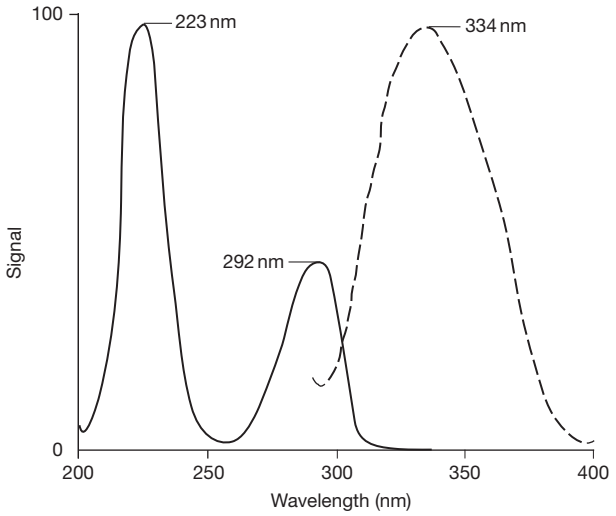

Figure 2 α-Tocopherol UV absorbance spectrum and fluorescence emission spectrum.

Vitamin E: Biological Activity

Biological activity of vitamin E can be expressed in two ways: as α-tocopherol equivalents (α-TEs) and as international units (IUs). According to the National Research Council, one α-TE is equivalent to 1 mg of RRR-α-tocopherol. Historically, biological activities were determined by fetal resorption studies in rats. Other functional indicators have been reported, but recently, the binding of tocochromanols to α-tocopherol transfer protein (α-TTP) has been measured as a potential indicator of biological activity. The biological activity of the various tocochromanols is provided in Table 2.

Tocochromanols are amphipathic molecules consisting of a polar chromanol ring at the head and a lipophilic phytyl tail. These molecules are oriented within biological membranes with the chromanol ring at the polar surface of a membrane and the phytyl tail embedded into the bilayer core. In this manner, they serve as antioxidants to inhibit lipid peroxidation.

Vitamin E is the primary lipid-soluble antioxidant in biological organisms. It is present in membranes throughout the body and transported with lipids in lipoproteins. In biological systems, its role as an antioxidant is the most well-established function. It is likely that ascorbic acid regenerates α-T by reducing the α-tocopheroxyl radical. This recycling cascade is important to spare α-T to provide ongoing protection of membranes (Figure 3).

Table 2 Biological activity of vitamin E

Vitamin E form	*IU (mg)*	*α-TE (mg)*	*α-TPP binding*
Natural (RRR-)			
α-Tocopherol	1.49	1.00	100
β-Tocopherol	0.75	0.50	38
δ-Tocopherol	0.05	0.03	1.5
γ-Tocopherol	0.15	0.10	9
α-Tocotrienol	0.75	0.50	12.5
β-Tocotrienol	0.08	0.05	n.d.
δ-Tocotrienol	n.a.	n.a.	n.d.
γ-Tocotrienol	n.a.	n.a.	n.d.
Synthetic			
All-rac-α-tocopherol	1.10	0.74	
RRR-α-Tocopheryl acetate	1.36	0.91	
All-rac-α-tocopheryl acetate	1.00	0.67	
RRR-α-Tocopheryl succinate	1.21	0.81	
All-rac-α-tocopheryl succinate	0.89	0.60	
RRR-α-tocopheryl nicotinate[a]	1.20	0.80	
All-rac-α-tocopheryl nicotinate[a]	0.88	0.59	

[a]Calculated based on tocopheryl succinate.

Source: DellaPenna, D. and Pogson, B. J. (2006) Vitamin synthesis in plants: tocopherols and carotenoids. *Annual Reviews in Plant Biology* **57**: 711–738; Eitenmiller, R. and Lee, J. (eds.) (2004). *Vitamin E: food chemistry, composition, and analysis*. New York: Marcel Dekker.

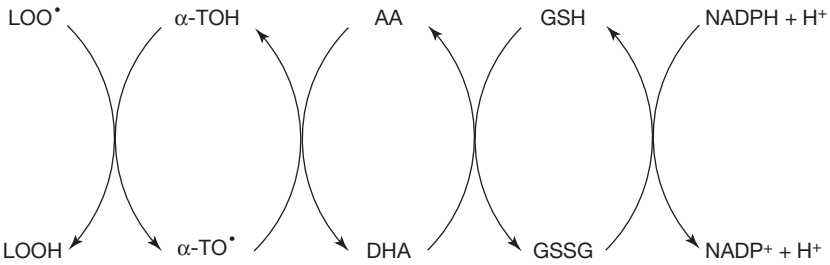

Figure 3 Tocopherol radical recycling.

Due to the presence of a selective α-TTP, α-T is likely to have very specific, non-antioxidant functions. Considerable work is under way to elucidate these functions. It has been reported that α-T regulates gene expression at transcription. Whether these observations are unique, transcriptional functions or indirect responses to oxidative stress and alterations in membrane integrity are still under debate.

Vitamin E: Analysis

The earliest methods to measure vitamin E activity were biological fertility tests. The Emmerie–Engel colorimetric method was the first and long-standing chemical method for the analysis of tocopherols. It is based on the ability of tocopherols to reduce ferric chloride and the colorimetric determination of the ferrous salt with α,α′-dipyridyl or *o*-phenanthroline. In addition, vitamin E has been measured using spectrophotometric and spectrofluorometric assays.

These methods were inaccurate measures of vitamin E activity since they do not separate the various tocochromanols with their differing biological activities. Paper and thin-layer chromatographies soon followed with the necessary selectivity to resolve the various homologues. These early methods have been displaced by GC and (ultra)high-performance liquid chromatography ((U)HPLC). Tocochromanols have been separated using supercritical fluid chromatography, capillary zone electrophoresis, capillary electrochromatography, and micellar electrokinetic chromatography but these techniques are not well suited to lipid-soluble analytes.

Sample Extraction

Tocochromanols are soluble in many organic solvents but insoluble in water. For samples that have relatively high tocol-to-lipid ratios, direct extraction with hexane, petroleum ether, alcohols, THF, chloroform, or ethyl acetate is adequate prior to separation. For many samples that contain high lipid and low tocochromanol content, separation from lipid is necessary prior to chromatography. This may be accomplished by enzymatic or alkaline hydrolysis or solid-phase extraction (SPE).

Tocochromanols are part of the nonsaponifiable fraction of lipids. Saponification is an alkaline hydrolysis that is used often to cleave triglycerides and ester linkages in complex samples. The remaining lipophilic portion of the sample that may be extracted with organic solvents is called the nonsaponifiable fraction. Saponification is used to simplify the sample matrix prior to extracting the tocochromanols. The process typically involves hydroalcohol mixtures of potassium hydroxide or sodium hydroxide. Time, temperature, alkali concentration, presence of antioxidants, and final pH influence the recovery of the various tocochromanols. Saponification may be essential to achieve complete extraction of vitamin E from complex samples, such as grains. There are reports that tocochromanols degrade during saponification. Some methods recommend that vitamin E be hydrolyzed at higher temperatures for shorter times to minimize degradation. For this reason, antioxidants such as ascorbic acid, butylated hydroxytoluene (BHT), and pyrogallol are added to the saponification mix to minimize oxidative losses. BHT and other phenolic antioxidants (butylated hydroxyanisole, hydroquinone, and tert-butylhydroquinone) must be used with caution since they exhibit UV absorbance and fluorescence similar to tocochromanols and may coelute chromatographically. Tocochromanol recovery, particularly the more polar ones, is hampered by alkaline pH during extraction into organic solvents. Tocochromanols will extract efficiently into hexane and similar solvents near neutral pH. The extraction efficiency is affected by the ratio of alcohol and fatty acids relative to hexane. Several good comparisons of extraction/saponification conditions arrive at similar conclusions:

1. Longer solvent exposure with heating and agitation improves extraction.
2. Several organic solvents can successfully solubilize the tocochromanols but the sample matrix will influence which solvent should be used.
3. Many samples require a polar organic solvent to precipitate proteins followed by partitioning into a water-immiscible solvent.
4. It is best to exclude saponification, if possible.
5. Antioxidants should be included and oxygen excluded during saponification.
6. Minimize exposure to alkaline conditions.
7. Alcohol concentration should be diluted to less than ~30% prior to solvent extraction.
8. The addition of saturated saltwater will assist to drive tocochromanols into the organic solvent.

Saponification

An example saponification procedure is described. Samples may be saponified directly or be extracted first and then

saponified. The latter offers greater control over the hydrolysis of oil and vitamin E esters but does not expose the sample matrix to the hydrolysis conditions. For tough samples (seeds), the matrix components may need to be saponified directly to achieve complete extraction of lipids.

Example

Accurately weigh approximately 0.2 g of oil or 2 g of homogenous sample into a 50 ml glass tube with Teflon-lined screw cap. If an internal standard is to be included, it should be added at this time. (Do not use tocopheryl esters as internal standards since they would be converted to free tocopherols.) Mix the sample with 4 ml of ethanol. Add 1 ml of 10% pyrogallol in ethanol. Add 5 ml of 40% KOH in methanol. Displace the air with N_2, cap, and incubate in a shaking water bath at 65 °C for 30 min. Vortex-mix with 10 ml of heptane/10% THF. Add 20 ml of saturated sodium chloride and adjust the pH to ~8 with 1 M HCl. Invert several times and allow phases to separate. Transfer the upper layer to a clean 50 ml tube. Repeat extraction with heptanes/THF two more times. If hexane or petroleum ether alone is used for extraction, it is necessary to dilute the ethanol to less than 30% to obtain efficient extraction of polar tocochromanols. Wash the combined extract with 10 ml of water transferring the lower layer to waste. Repeat until the aqueous layer is no longer hazy. To remove traces of water from the organic extract, add sodium sulfate until crystals do not dissolve. Transfer the organic extract to a clean glass tube and evaporate under a stream N_2 or using a centrifugal evaporator. Dissolve the residue in an appropriate volume of solvent compatible with the chromatographic technique.

Solid-phase extraction

If tocopheryl esters are to be measured, saponification is not applicable. Lipids must be removed via another approach, such as SPE. SPE is a simple, rapid, inexpensive form of chromatography used to isolate a fraction of the sample containing the analytes from other matrix components. It can be performed off-line or on-line to clean up and/or concentrate the sample prior to chromatography. One often uses a packing material (or phase) that is different from the chromatographic column to take advantage of different chemical interactions.

Example

The sample (0.2 g oil or oil from 2 g of solvent-extracted sample) is brought to 10 ml in a volumetric container with hexane. If an internal standard is to be included, it should be added before bringing to volume. A silica SPE cartridge (3 ml volume with 200 mg silica) is conditioned with 3 ml isopropanol followed by 6 ml hexane. A 2 ml aliquot of the diluted oil solution is passed into the column and washed with 6 ml hexane/4% diethyl ether to remove triglycerides. Tocochromanols are eluted into a clean test tube with 3 ml hexane/10% acetone. The hexane/acetone is evaporated under a stream of N_2 or using a centrifugal evaporator. Dissolve the residue in an appropriate volume of solvent compatible with the chromatographic technique.

Chromatographic Separation of Vitamin E

Gas Chromatography

GC has been used to measure α-tocopherol since 1960. Wilson et al. improved the methodology and measured 20 tocochromanols and tocopherol esters using three different columns. Early GC columns packed with stationary phase produced lower resolution and could not separate the β- and γ-homologues. They have been replaced by capillary open-tubular columns with the stationary phase layered on the inner surface of the glass capillary tubing. These longer, narrow columns have the ability to separate all major tocochromanols, typically with increased analysis time. Tocochromanols can be measured underivatized or derivatized using FID or MS. The quantitation limit using capillary GC with FID is ~1 ng but can be reduced to low femtograms using MS detection. The tocochromanols may be derivatized as trimethylsilyl (TMS) ethers or as methyl esters. The derivatized tocochromanols are more stable and volatile and result in better chromatography. The TMS ethers are preferred since they form rapidly at room temperature. Derivatized tocochromanols can be separated using nonpolar, moderately polar, and polar GC stationary phases, for example, DB-1, DB-17, and DB-23, respectively. During GC, cholesterol is a common interference with the α-T and often occurs at much higher concentrations than the tocochromanols.

Until recently, separation of the eight major tocochromanols without derivatization had not been reported, but now, this can be accomplished with or without derivatization. The elution order using GC is influenced by the degree and position of methyl substitutions following the order $\delta < \beta < \gamma < \alpha$.

GC-FID separation of tocochromanols without derivatization

The lipid fraction from samples after SPE or saponification is dissolved appropriately in isooctane. As seen in Figure 4, the eight major tocochromanols and the internal standard, palmityl palmitate, are baseline-resolved during a 12 min chromatogram using the conditions listed in the succeeding text. This method is simple, rapid, and competitive with current LC methods.

Column	Restek Rtx®-20, 30 m, 0.53 mm ID, 0.50 μm
Sample	
Diluent	Isooctane
Conc.	1000 μg ml^{-1} each component
Injection	
Inj. vol.	1 μl split (split ratio 20:1)
Liner	Splitless (4 mm ID)
Inj. temp.	320 °C
Oven	
Oven temp.	270–290 °C at 2 °C min^{-1} to 320 °C at 10 °C min^{-1} (hold 1 min)
Carrier gas	H_2, constant flow
Flow rate	5.2 ml min^{-1}
Detector	FID at 320 °C

Conditions supplied by Restek.

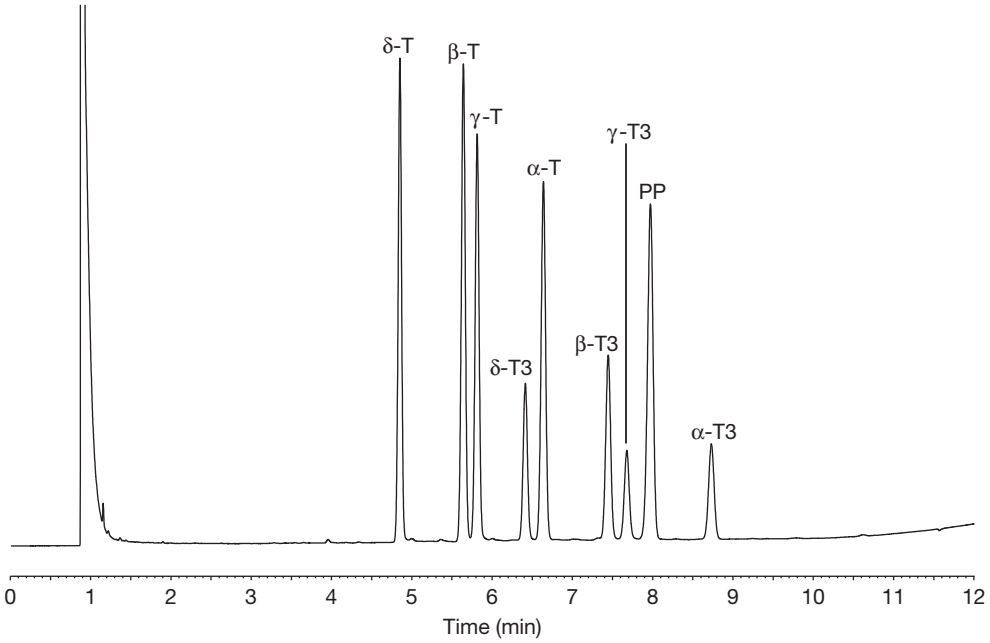

Figure 4 GC-FID separation of underivatized tocopherols and tocotrienols. Reproduced with permission from Restek Corporation.

(Ultra)High-Performance Liquid Chromatography

Vitamin E was first separated by HPLC in 1971 by Schmidt et al. using a Zipax superficially porous packing. A normal-phase separation of both tocopherols and tocotrienols was reported using Corasil II in 1974. HPLC is the technique of choice to measure tocochromanols since the eight homologues can be separated in short analysis times without laborious sample preparation. The abbreviation 'HPLC' is used to represent both high-pressure liquid chromatography and ultrahigh-pressure liquid chromatography. The principal difference is that instruments used for UHPLC will function above 6000 psi permitting the use of <2 μm particle columns at higher linear velocity. UHPLC columns can be used on conventional HPLC below 6000 psi, but the instruments are not optimized for low flow rates and low dead volume. In recent years, solid-core particle columns have been promoted to achieve performance similar to UHPLC columns using conventional HPLC instruments. Separation examples are provided later in the text.

There are two primary forms of HPLC used for tocochromanol separations: 'normal-phase' liquid chromatography (NPLC) and 'reversed-phase' liquid chromatography (RPLC). NPLC incorporates polar stationary phases and nonpolar mobile phases resulting in longer retention of polar compounds. NPLC separations are well suited for tocochromanol separations due to the solubility of oily fractions in the solvents and the ability to resolve all structural isomers, including β- and γ-homologues. RPLC incorporates nonpolar stationary phases and polar mobile phases resulting in longer retention of hydrophobic compounds. RPLC was developed after NPLC and separates in the reverse order – thus the name 'reversed-phase.' Until more recently, the β- and γ-isomers could not be separated easily by RPLC. However, due to its versatility, ruggedness, and reproducibility, RPLC is more commonly used to separate vitamins.

NPLC separation of tocochromanols

There are several types of NPLC packing materials that have been used to separate tocochromanols. They operate by adsorptive mechanisms (hydrogen-bonding and dipole–dipole) interacting with polar functional groups in the molecules. Two key benefits of NPLC are the solubility of lipids and ability to separate structural isomers. Silica was the first NPLC packing material to be reported for tocopherol separations. Other polar NPLC phases include amino, cyano, and diol. Kamal-Eldin et al. compared the separation of tocochromanols using various NPLC columns and mobile phases. They successfully separated the eight major tocochromanols on 11 columns representing silica, amino, and diol columns. The elution order is the same on the columns, α-T < α-T3, < β-T < γ-T < β-T3 < γ-T3 < δ-T < δ-T3. Bonded NP columns are preferable since they reequilibrate faster and are less sensitive to traces of polar solvents than bare silica. An example separation using a diol column is illustrated in the succeeding text.

Diol HPLC separation of tocopherols and tocotrienols

The lipid fraction from samples after dilution or saponification is dissolved appropriately in hexane. In Figure 5, the eight major tocochromanols and the internal standard, tocol, are baseline-resolved during an 18 min chromatogram using the conditions listed later in the text. Both fluorescence detection and UV detection are displayed. Fluorescence is more specific and sensitive than UV; however, UV has a wider linear range (Figure 5).

Column	Chromegabond diol, 150 × 4 mm, 3 μm
Mobile phase	Hexane/5% dioxane
Flow rate	1 ml min^{-1}
Detection	
Absorbance	296 nm
Fluorescence	Ex 296 nm/Em 340 nm

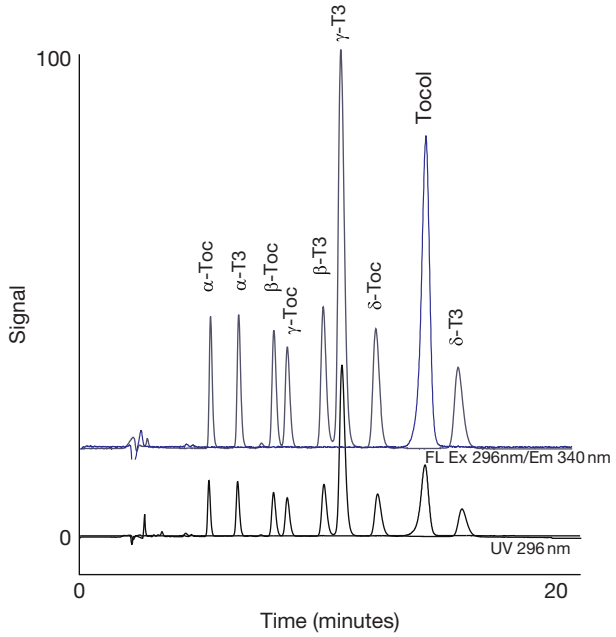

Figure 5 Separation of tocopherols and tocotrienols by NPLC using a diol column.

RPLC separation of tocochromanols

Interestingly, the first published HPLC separation of vitamin E was done using RPLC. There are many types of RPLC packing materials that separate compounds by hydrophobic interactions. These include octyl (C8), octadecyl (C18), triacontyl (C30), phenyl, cyclodextrins, PFP, and polyvinyl alcohol. The strengths of RPLC include ruggedness, rapid gradient equilibration, buffering for pH adjustment, and amenability to electrochemical and MS detection. The weaknesses include poor solubility of lipids, difficulty in separating isomers (β- and γ-homologues), and their poor stability above pH 9–10. α-Tocopherol and its esters are well separated using C18 columns (see Figure 6). Since vitamin E activity is not calculated using the other homologues, C18 separations are the most common. More recently, several RPLC separations have successfully resolved the eight major tocochromanols. Examples of RPLC separations of standard mixtures are illustrated in the succeeding text including UHPLC and core–shell.

C18 HPLC separation of tocopherols and tocotrienols

Column	Thermo Scientific Accucore C18, 150 × 2.1 mm, 2.6 μm (core–shell)
Mobile phase	75 Acetonitrile/15 methanol/10 dioxane
Flow rate	0.3 ml min^{-1}
Detection	Absorbance at 290 nm
Temperature	32 °C

Note the use of conventional HPLC instruments with highly efficient separation and short analysis time (<5 min, **Figure 6**).

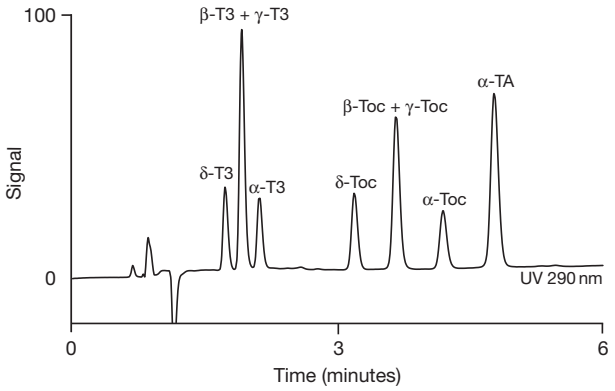

Figure 6 Separation of tocopherols, tocotrienols, and α-tocopheryl acetate using core–shell C18 column.

C30 HPLC separation of tocopherols and tocotrienols

Column	Waters YMC C30, 250 × 4.6 mm, 3 μm
Mobile phase	73 Acetonitrile/19 THF/8 water
Flow rate	1 ml min^{-1}
Detection	Absorbance at 300 nm
Temperature	32 °C

Figure 7 illustrates an RPLC separation of all eight tocochromanols using a C30 column. Analysis time is 22 min but α-TA is still retained. The column is very retentive of hydrophobic compounds so it is necessary to include a gradient at the end of the run to elute strongly retained materials. As of this writing, no <2 μm or core–shell C30 columns with the polymeric synthesis of the YMC C30 column are available. Those currently available do not separate all eight tocochromanols.

PFP HPLC separation of tocochromanols

Column	Thermo Scientific Hypersil Gold PFP, 100 × 2.1 mm, 1.9 μm (UHPLC)
Mobile phase	90 Methanol/10 water
Flow rate	0.25 ml min^{-1}
Detection	Absorbance at 290 nm
Temperature	40 °C

Figure 8 illustrates the RPLC separation of all eight tocochromanols and TA using a PFP column. The elution order for the RPLC columns is δ-T3 < β-T3 < γ-T3 < α-T3 < δ-T < β-T < γ-T < α-T < α-TA. The C30 column is an exception with γ-congeners eluting before β-congeners.

Chiral separation of tocochromanols

There are three chiral carbons in the tocopherols and one chiral carbon in the tocotrienols. Conventional chromatography does not recognize the different stereoisomers unless they are bonded with chiral derivatives. To determine if a product is 2R,4′R,8′R α-tocopherol (the natural form), it is only necessary to demonstrate a single peak using a CHIRALCEL OD-H column that produces two peaks for S- and R-isomers at the position 2. To date, all eight stereoisomers have not been separated using a single chiral chromatographic separation.

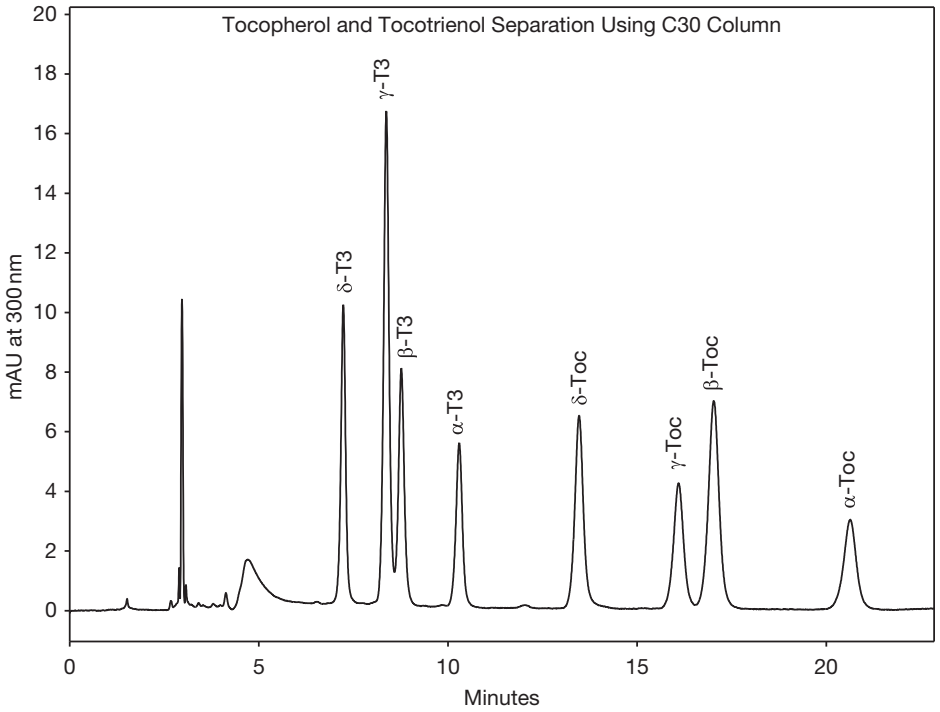

Figure 7 C30 separation of tocopherols and tocotrienols.

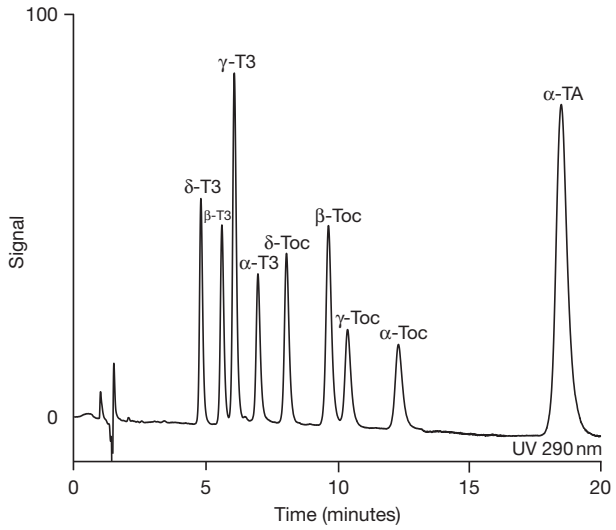

Figure 8 Tocopherols, tocotrienols, and α-tocopheryl acetate using a PFP UHPLC column.

Jensen and Lauridsen resolved five stereoisomeric peaks of the methyl ether derivatives using a CHIRALCEL OD-H column. The isomers elute in the order: peak 1 = four 2S-isomers (SSS-, SSR-, SRR-, and SRS-), peak 2 = RSS-, peak 3 = RRS-, peak 4 = RRR-, and peak 5 = RSR-α-tocopherol.

Quantitative Analysis of Tocochromanols

The most important component of analytic chemistry is standardization. This process was the biggest contributor to methodological error in the Micronutrients Measurement QA Program at NIST. The availability of reliable, high-quality standards and reference materials is limited. For this reason, standards should be qualified before use. Pure tocochromanols are oily materials and problematic to dispense small quantities since they stick to surfaces. It is recommended that a small amount of standard (~10 mg) be transferred to a 10 ml volumetric flask and brought to volume with hexane. The stock standards should be diluted to ~80 ppm in ethanol and scanned spectrophotometrically in a quartz cuvette from 270 to 320 nm. Most $E^{1\%}_{cm}$ for tocochromanols are provided in ethanol. Note that the values in hexane or petroleum ether are about 12% lower than those of ethanol. The absorbance maxima should agree with the values reported in Table 1. Failure to note solvents used in some publications has caused discrepancy regarding λ_{max} and $E^{1\%}_{cm}$. Greater consistency and traceability result from using published values compared to gravimetric standards. The concentration of each standard should be calculated using Beer–Lambert equation and then corrected for its purity measured by HPLC at the λ_{max} of each standard.

Example:

Absorbance at $\lambda_{max}/E^{1\%}_{cm}$ = Concentration g/100 ml
Absorbance at 292 nm α-T/$E^{1\%}_{cm}$ = Concentration α-T g/100 ml × HPLC area purity
=> 0.60 AU/75.8 $E^{1\%}_{cm}$ = 0.007916 g/100 ml × 0.95 purity = 0.007519 g/100 ml
=> 0.007519 g/100 ml × 1000 mg g^{-1} × 1000 μg mg^{-1} = 75.19 μg ml^{-1}

The standards with corrected concentration can be used for method calibrations on any detector type (UV, FL, electrochemical, and MS). If NPLC is being used, several

calibrator solutions from 10 to 200 μg ml^{-1} should be prepared by diluting stock standards in mobile phase. If RPLC is being used, the hexane in the stock standards should be evaporated and then dissolved using mobile phase in the range of 10–200 μg ml^{-1}. Calibrations can be generated at a wavelength slightly away from the λ_{max} but should avoid steep areas of the absorbance spectrum as a slight detector wavelength deviation will cause signal variation. Samples must be measured at the same wavelength and under the same conditions as used for the calibration.

Calibrations can be either external standardization (ESTD) or internal standardization (ISTD).

ESTD is the most common and straightforward form of calibration. Known graduated amounts of the tocochromanols are separated chromatographically and the peak heights or areas are plotted against the standard concentrations, typically creating a linear relationship. When a sample is analyzed, its concentration is estimated by determining where its peak height or area falls on the calibration curve (line). The calculations are easily performed in a spreadsheet or by a data system but the accuracy relies heavily on good laboratory practice, quantitative extraction, and highly reproducible instrumentation. It often involves the use of sample 'spikes' with known amounts of the analytes to determine percentage recovery.

Much of the rigor and uncertainty of ESTD methods can be minimized by incorporating an ISTD. ISTD calibration involves the addition of a known quantity of a compound similar to the analytes at the beginning of the extraction process to adjust for any losses, extraction efficiency, and instrument variability that may occur. The calculations are only slightly modified to make a correction for the 'measured amount of ISTD' relative to the 'added amount of ISTD.' This can be applied to an ESTD calibration as a correction at the end or directly in the ISTD calibration curve by plotting the 'area of analyte'/'area of ISTD' relative to 'concentration analyte'/'concentration of ISTD.' The use of ISTD is preferred over ESTD but the choice of an appropriate ISTD is critical and not always possible. Even with ISTD, one must realize that the ISTD may not be compartmentalized or bound in the same manner as the endogenous tocochromanols and, therefore, not extracted in the same manner.

Internal Standards

Ideally, an ISTD is a chemical that is very similar, physically and chemically, to the analytes of interest. A known amount is added to the sample at the beginning of extraction to correct for any losses that may occur during sample preparation and analysis. As such, it should 'behave' like the compounds being extracted and measured. It is common for a known amount of a homologue of the analytes, not present in the samples, to be added at the beginning of sample processing. The best possible ISTD would be stable isotopes or radiolabeled isotopes of the analytes. This type of ISTD can only be resolved using specific modes of detection, such as MS, and is not universally applicable. For more common methods of analysis, a closely related compound is used, which can be 'seen' by the detector and separated chromatographically. Common ISTDs include tocol, tocopherol acetate, 5,7-dimethyltocol, and tocochromanols not present in most samples, such as δ-tocopherol or γ-tocotrienol. The sample type, mode of separation, mode of detection, and mode of sample preparation all influence the choice of an appropriate ISTD. For example, α-tocopherol acetate would not be a good choice if the sample required saponification or fluorescence detection since α-tocopherol acetate fluoresces poorly and is hydrolyzed to α-tocopherol during saponification. Tocol or 5,7-dimethyltocol would be better choices since they have similar UV absorbance and fluorescence, are not altered by hydrolysis, can be separated chromatographically from the other tocochromanols, and are commercially available (Matreya, LLC).

In GC, ISTDs do not require similar UV or fluorescence properties since FID is the signal response to burning the carbon atoms in a compound. In principal, different molecules produce comparable detector responses so an ISTD needs only exhibit similar retention on the GC column. As such, cholesterol, cholestane, and palmityl palmitate have been used as ISTDs for GC analysis.

Detection of Vitamin E

Ultraviolet detection

Tocopherols and tocotrienols have very similar properties and can be measured using the same types of detection. As noted in Table 1, they have absorbance maxima near 220 and 295 nm. The lower wavelength has approximately fivefold greater absorbance. However, many other compounds exhibit absorbance in the low 200 nm region so it is often less selective and is below the absorbance cutoff for many solvents. Therefore, 290–300 nm is most frequently the region selected for UV detection. UV detection is not highly selective or sensitive (low nanogram) but is very reliable, inexpensive, and the most frequent mode of detection. Photodiode array detection is a form of UV/visible detection that allows the entire UV/visible spectrum to be monitored simultaneously. This offers improved selectivity by verifying that the UV spectrum of sample components matches that of the standards and alternative detection wavelengths should interferences occur.

Fluorescence detection

The tocochromanols fluoresce near 330 nm when excited at 220 or 295 nm. This characteristic produces a selective and sensitive (high pg range) mode of detection. Fluorescence sensitivity is typically 4- to 20-fold greater than UV sensitivity depending on the compound and the HPLC solvents. FL is one of the most common methods to measure tocochromanols, often negating the need to perform saponifications. Note that the tocopherol esters have poor fluorescence and lesser UV absorbance at ~10 nm lower wavelength.

Electrochemical detection

ECD can be used to measure tocochromanols in RPLC when a 'supporting electrolyte' is present in the mobile phase. ECD is the most sensitive mode of detection for tocochromanols due to their low oxidative potential, typically 10- to 20-fold better than FL. The two common forms of ECD are amperometric and coulometric. In amperometric detection, the column eluant passes over a glassy carbon electrode at ~600 mV providing oxidation of ~1% of the sample. In coulometric detection, the column eluant passes through a porous carbon electrode at 350–500 mV providing nearly 100% oxidation of the sample

for sensitivity in the low pg range. The supporting electrolyte is commonly an acetate or perchlorate buffer at 5–50 mM concentration. ECD is a relatively nonselective form of detection, and if other components coelute with the tocochromanols, they could be oxidized simultaneously. However, the tocochromanols are highly electroactive so other components provide minimal interference at the voltage potentials used.

Evaporative light-scattering and corona aerosol detection

ELSD or corona aerosol detection (CAD) are two universal, less-sensitive modes of detection. They may have application when tocochromanols are at higher concentrations. They are amenable to gradient elution in both RPLC and NPLC. In these detectors, the eluant is nebulized and the solvent is evaporated leaving analyte particles to be detected as they are swept through a beam of light. Sensitivity is in the microgram range for ELSD and nanogram range for CAD.

Mass spectrometric detection

MS can be used with either GC or HPLC to measure tocochromanols. It offers high sensitivity (pg range), selectivity, and the ability to identify peaks. Sensitive detection of tocochromanols can be achieved using simpler and inexpensive detectors, such as FL or ECD. It is not a good detector for measuring isomeric compounds (or isobaric) unless they are chromatographically resolved. It has been quite useful when mated with GC since coeluting compounds typically had different masses and fragmentation. The current, common MS interfaces include electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) or atmospheric pressure photoionization (APPI). While the analysis of tocochromanols has been reported using ESI, it is not best suited to measure tocochromanols since they are not readily ionized. APCI or APPI is better suited to lipophilic and poorly ionizing compounds. Szterk et al. recently compared sample preparation conditions and detection sensitivity for the analysis of tocochromanols in meat. The limits of detection of tocochromanols using APCI and APPI were from 0.3 to 2.9 ng ml^{-1}. Other values measured with similar MS methods and ESI are reported in the range of 0.8 ng ml^{-1} to 1 μg ml^{-1}. They concluded that FL was 10- to 1000-fold more sensitive than MS detection.

See also: Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Infrared Spectroscopy: Applications; Palm Oil; Spectroscopy: Types; Tocopherols: Physiology and Health Effects; Vegetable Oils: Composition and Analysis; Vitamins: Overview.

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Tortillas

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Introduction

A tortilla can be defined as a flat, round, unfermented bread produced from lime [$Ca(OH)_2$]-cooked maize (*Zea mays L.*) or wheat flour (*Triticum aestivum L.*). The processes and characteristics of maize and flour tortilla differ considerably. Corn tortillas are manufactured from pregelatinized and cohesive doughs, whereas flour tortillas from gluten-developed doughs high in shortening or fat. Both types of tortillas originated in Mexico, where they are considered the national breads and are generally consumed with other fillings such as beans, meats, eggs, and vegetables.

Tortillas currently represent 30% of all baked product sales in the United States and keep being the most popular food in Mexico and many places in Central America. Approximately 120 million tortillas are consumed yearly in the United States, making these the second most popular baked product, after white bread. An average Mexican consumes more than 80 kg of maize tortillas annually. Today, derived products such as flat tostadas and tortilla chips are extensively sold as snacks and for the preparation of fast foods. Taco shells are the American version of tostadas with the only difference that they are usually fried bent (U form) and rarely colored. Regular, low-fat, and fat-free tortilla chips and related products are available in grocery stores in Mexico and the United States.

Tortillas are the main source of energy, protein, calcium, and other important nutrients in Mexico and Central America. Lime cooking considerably increases calcium and the bioavailability of niacin and significantly diminishes the amount of aflatoxins and other mycotoxins originally present in contaminated maize.

Lime-Cooked Maize Products

Matured maize kernels, lime, and water are the three basic ingredients needed for the production of tortillas. Other ingredients, such as preservatives, acidulants, emulsifiers, and hydrocolloids, are used to improve microbial and textural shelf life of table tortillas. Frying oil, salt, and different seasonings are key additional ingredients for the production of fried snacks.

Currently, nixtamalized products are obtained from three distinctive processes: the traditional and laborious method first practiced by indigenous Mesoamericans, the industrial fresh *masa*, and their conversion from dry *masa* flours. In Mexico, 41%, 37%, and 23% of tortillas are produced by these manufacturing processes, respectively. In this country, more than 4.8 million tons of dry *masa* flours is annually manufactured yielding close to 9 million tons of table tortillas. In the United States and other parts of the world, dry *masa* flour is the preferred method to produce related foods. The dry *masa* flour manufacturing process originated in Mexico in the middle of the twentieth century and has rapidly expanded to many countries around the globe. Precooked dry flours for different applications are rapidly growing worldwide because of their convenience and versatility. Flours are convenient because they have a shelf life up to 1 year in storage and require only water to reconstitute the *masa*. The major advantage of using dry flour is product flexibility. *Masa* flours for the production of white and yellow table tortillas, restaurant-style chips, tortilla chips, corn chips, and tamales are available. Additionally, these can be easily blended with other dry additives (e.g., preservatives, gums, and enrichment mixes) before processing.

Three major types of products are industrially manufactured from lime-cooked maize: table or soft tortillas, corn chips, and tortilla chips (Figure 1). Corn and tortilla chips are primarily produced and consumed in developed countries, where they have an important share within the salted snack food market.

The ancient or traditional process to obtain tortillas is still practiced by indigenous people in Mexico and Central America. Traditional maize is lime-cooked in clay pots over a fire, followed by steeping for 8–16 h (generally overnight). The cooking liquor, called *nejayote*, is discarded and then the *nixtamal* is hand-washed. *Nixtamal* is ground into a fine *masa* with a stone grinder called *metate* and then hand-molded, patted, or pressed into disks, which are baked on both sides on a hot griddle. Traditional tortillas are generally produced on a daily basis and are usually thicker and heavier than their industrially produced counterparts.

The modern lime-cooking processes use the same principles as the ancient traditional procedure first devised by Mesoamerican civilizations, but the equipment and procedures have been mechanized to improve production efficiency. The three major operations for tortilla production are lime cooking, stone grinding, and tortilla baking (Figure 1). Lime cooking is considered the most relevant part of the process because it affects functionality and finished product characteristics. Stone grinding also plays a key role because it disrupts swollen starch granules forming *masa* with a given granulation. The degree of cooking and grinding dictates the type of *masa* produced. Fine grinding produces *masa* suitable for table or soft tortillas, while coarse *masa* is preferred for fried snacks. *Masa* is transformed into tortillas after a baking step that forms the typical tortilla structure. This thermal operation inactivates all microbial loads, affects color and sensory properties via browning reactions, and further gelatinizes starch for a structured tortilla. Approximately 146 kg of table tortillas with 44–46% moisture is obtained from 100 kg of cleaned maize.

The industrial production of maize tortillas is labor-intensive and requires considerable equipment. The quality of end products depends on the nature of the raw material and manufacturing practices. The ideal kernel should be sound, free of fissures, uniform, brightly colored, and with intermediate-to-hard endosperm. In addition, the kernels

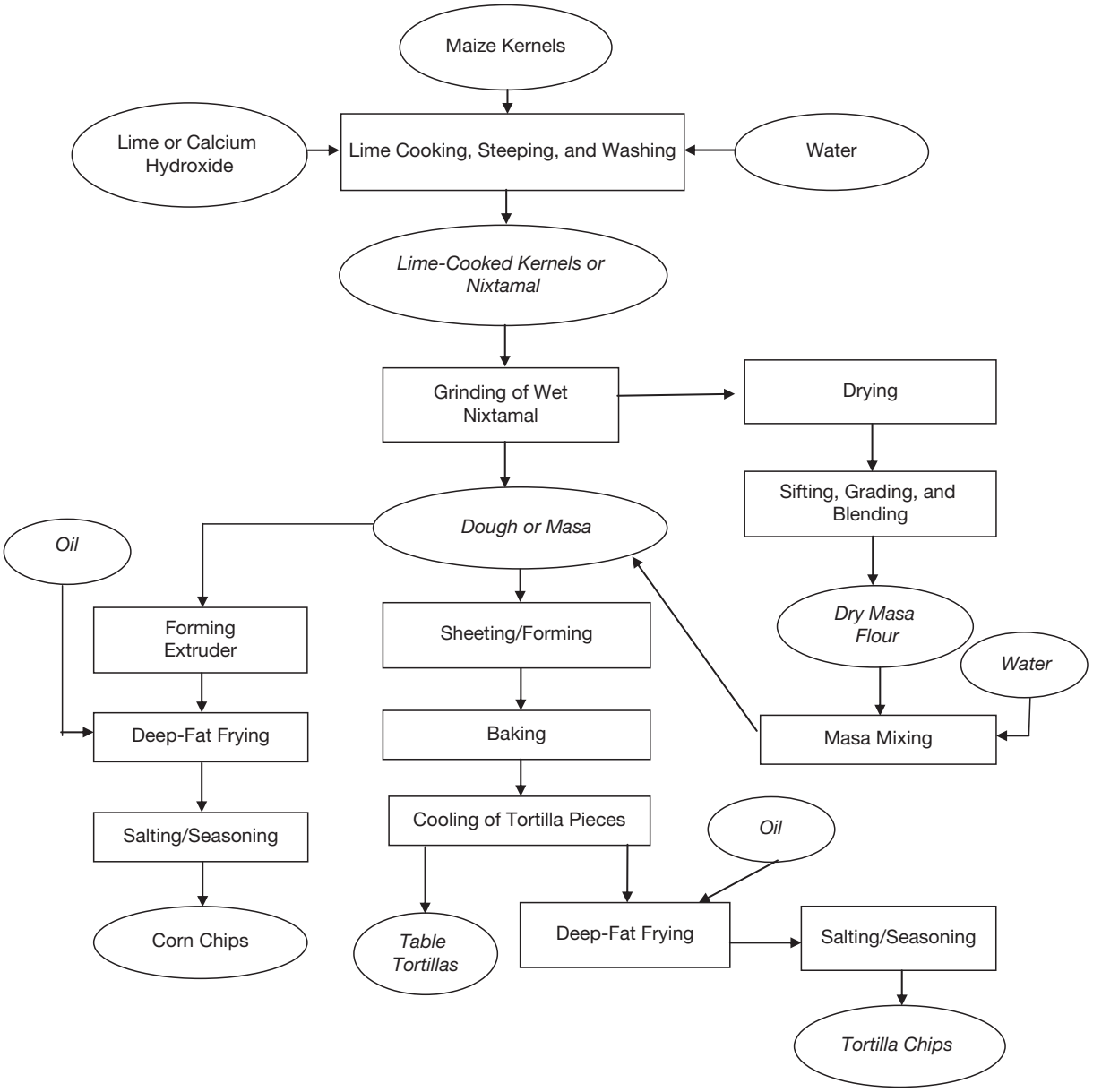

Figure 1 Flowchart of industrial processes for the manufacture of table tortillas, corn chips, tortilla chips, and dry *masa* flour.

should have a rounded crown and a shallow, unwrinkled dent, and the bran or pericarp should be easily removed after lime cooking and steeping. Yellow maize and white maize, or mixtures therefrom, are commercially manufactured into alkaline products. Recently, blue maize and other colored maize have gained market for production of tortillas and related snacks because they are viewed as functional foods.

The industrial tortilla process starts when the cleaned maize is lime-cooked in agitated open baths, vertical cookers, or steam kettles. Kernels are generally mixed with three parts water and 1% lime, based on grain weight, and cooked for 15–45 min at temperatures ranging from 85 to 100 °C. The *nixtamal* is then steeped for 8–16 h in the hot lime solution. After steeping, the lime-cooked grain is pumped with the steep liquor or dropped by gravity to washers. The cooking liquor known as *nejayote* is drained and the resulting *nixtamal* washed with pressurized water. Most of the pericarp and excess lime are removed during this step. Several important changes occur when maize is lime-cooked. Cooking causes partial dissolution of the bran tissue, and the lime penetrates the kernel mainly through the germ and internally transported to the rest of the kernel at a very slow rate. The lime also weakens the endosperm cell walls enhancing the penetration of water and heat that promotes partial swelling and gelatinization of the starch granules.

The cleaned *nixtamal* is discharged into a stone grinder, where *masa* is continuously produced. The grinder consists of two volcanic or synthetic stones carved radially, one stationary

and the other rotating at 500–700 rpm. *Masa* participle size is directly related to the extent of cooking, the gap of pressure between the stones, and the size and depth of the internal grooves. During grinding, the *nixtamal* is disrupted into a plastic and cohesive dough called *masa*. The ground particles are held together by a glue-like mixture of mechanically released partially gelatinized starch granules and sheets of the protein matrix. The attrition during grinding increases the *masa* temperature to near 50 °C and the partially hydrated starch granules are mechanically damaged.

The *masa* is then kneaded by mixers or extruders that feed the forming machine or sheeter rolls. During forming, the *masa* is rolled into a thin sheet (1.5–2 mm thick), which is cut by a rotating cutter positioned underneath the rolls. The formed pieces of *masa* are fed into a three-tier, gas-fired oven for baking. Tortillas are baked at temperatures ranging from 280 to 302 °C for 30–60 s depending on the temperature profile in the different sections of the baking oven. During baking, about 10–12% moisture is lost from the *masa*. This thermal operation also causes further starch gelatinization and protein denaturation, color development due to Maillard browning reactions, and the inactivation of microorganisms. Next, the baked tortillas are cooled on a series of open tiers and packaged when they reach ambient temperature. Tortilla shelf life greatly depends on the effectiveness of cooling. Improper cooling causes microbial problems, sticking of stacked and packaged tortillas, and moisture condensation inside the package.

Tortillas are generally treated with additives such as acidulants, preservatives, emulsifiers, and gums to improve their textural and microbial shelf lives. In fresh *masa* operations, these additives are dosed during grinding or incorporated during the *masa* kneading step before baking.

Fried Products

Frying has stretched the market for nixtamalized foods because the final products have excellent organoleptic properties and a long shelf life. The two most popular snacks, tortilla and corn chips, are usually made from coarsely ground *masa*. Corn chips are produced directly from *masa* and absorb more oil than tortilla chips during frying (Table 2). Tortilla chips are baked similarly to table tortillas before frying. Tortillas chips absorb less oil and have a firmer texture and a stronger flavor compared to corn chips. *Masa* for corn chips is extruded trough a die and cut by rotating knives before frying, whereas *masa* for tortilla chips is formed into triangles, strips, or circles before baking, equilibrating, and frying.

Corn and tortilla chips are often salted and flavored immediately after frying. The hot chips are usually conveyed into an inclined rotating cylinder that delivers onto the product dry seasonings or oil-based slurries. Most popular flavorings include nacho cheese, hot/spicy, barbecue, lemon salt, and jalapeño. Corn and tortilla chips are packaged with aluminized multilayered or plastic bags to prevent texture loss and delay rancidity. A good hot tack and initial seal strength are critical factors to control during packaging of these snacks. Corn and tortilla chips require the typical moisture barrier for most salted snacks.

Dry *Masa* Flour

Production of table tortillas and related snacks from dry *masa* flour has increased significantly during the past 20 years. The major companies that originated in Mexico dominate the industry. The largest company has nearly 30 manufacturing plants in Mexico, the United States, and Central America and keeps constructing new plants in Eastern and Western Europe, Asia, and Australia. Manufacturing tortillas and lime-cooked snacks from dry *masa* flour facilitates the process because processors do not have to concern about selection and storage of the maize kernels, lime cooking, and grinding. Furthermore, the use of dry *masa* flour greatly reduces processing time, labor, capital investment for processing equipment, and wastewater treatment. Additionally, it can be easily blended with other dry ingredients (e.g., preservatives, gums, and enrichment mixes) before processing. Disadvantages include its higher price compared to fresh *masa* and the subtle differences in flavor and texture in favor of fresh *masa* products. The major advantage of using dry *masa* flour is product flexibility. *Masa* flours for the production of white, yellow, and blue table tortillas, restaurant-style chips, tortilla chips, corn chips, and tamales are available.

The industrial process to produce dry *masa* flour is detailed in Figure 1. Dry *masa* producers stress the selection of the best maize hybrids. The ideal white or yellow maize for its production has intermediate endosperm texture, test weight higher than 70 kg hl^{-1}, true density of 1.26 g cm^{-3}, 1000 kernel weight higher than 300 g, and bright colored kernels, which are preferably threshed from white cobs.

In the dry *masa* process, selected maize kernels are heated in a $Ca(OH)_2$ solution and steeped for short periods of time and the resulting *nixtamal* washed with water. Cooking is generally performed in large reactors for 20–30 min at 85–100 °C. The amount of lime usually employed is 1% based on grain weight. The *nixtamal* and cooking solution are separated and the cooked kernels with ~36% moisture are washed with water to remove excess lime and hydrolyzed bran tissue. Then, the nixtamal is ground with a special type of hammer mill into coarse particles in preparation for drying. Drying is commonly performed in drying towers in which warm air flows countercurrent to the pieces of *masa*. The first drying step is flash where *masa* pieces are dehydrated at high temperatures for a short period of time with the aim of removing most free water. Next, particles are dehydrated at lower temperature with the aim of avoiding over gelatinization and attaining the optimum moisture content (around 10%). The particles are then sized or graded in order to formulate different flours for different applications. The coarse particles that do not meet size specifications are reground and then incorporated into final flours. The dry *masa* flour is packaged in 1, 2, or 22.7 kg (50 lb) bags. From 100 kg of cleaned maize kernels, around 93 kg of dry *masa* flour is usually obtained.

At processing plants, dry *masa* flour is simply reconstituted with water to produce *masa*. The correct addition of water and adequate mixing are the most critical steps for obtaining the best *masa* properties. The flour is blended with the water in a slow and gentle manner for 5–10 min. The amount of water commonly required to hydrate flours for table tortillas is 1.2–1.3 l kg^{-1} of flour, whereas for snacks, 1–1.1 kg^{-1} of

flour. Then, the nixtamalized foods are produced similarly as explained before.

Wheat Tortillas

A flour tortilla can be defined as a circular chemically leavened flat bread. Flour tortillas are mainly produced from refined flours although whole wheat and whole grain tortillas are gaining popularity. Generally, tortillas are 2 mm thick and have diameters that vary from 15 to 33 cm. Most wheat tortillas are industrially manufactured by hot-press, die-cut, or hand-stretch procedures (Figure 2). Each operation requires different flour specifications, dough preparation, and baking conditions, which result in various tortilla characteristics.

Wheat flour tortillas are among the fastest-growing baked goods worldwide because they are fundamental in the elaboration of many Mexican dishes widely sold by fast-food and conventional restaurants. In the United States, wheat flour tortillas are considered the fastest-growing market with wholesale sales that surpass 4 billion dollars per year. Tortillas are mainly used as wraps to produce tacos filled with shredded meats, beans, cooked eggs, vegetables, cheeses, and other fillings.

All wheat tortillas contain flour, water, fat, and salt. Table 2 depicts typical formulation for white, whole wheat, and whole grain wheat tortillas. The second major ingredient is shortening that is used to improve dough machinability, flavor, and tortilla texture especially in terms of reheating capacity. In the United States, tortillas contain several other ingredients to improve flavor, softness, rollability, and shelf life. These ingredients include chemical leavening agents, emulsifiers, antimicrobial agents, acidulants, gums or hydrocolloids, and reducing agents.

Wheat flour, the most important ingredient, is preferably enriched and bleached and should have intermediate protein content. Equipment limitations and processing conditions determine the functionality of the flour. Flours for hot-press and hand-stretch tortillas generally require less protein and gluten strength compared to counterparts for die-cut tortillas. Dough mixing time and dough properties are modified by reducing agents (sodium sulfites or cysteine), emulsifiers (lecithin, mono- and diglycerides of fatty acids, and sodium stearoyl-2-lactylate), salt, gums, fat, water content, and dough temperature.

Water (45–55% of flour weight) is needed to form the gluten complex. In some bakeries, water is warmed (35–35 °C) before mixing. Solid or liquid fats (5–15% of flour weight) are added to improve dough properties, retard staling, and produce a softer and more flexible tortilla. Salt (1–2%) is added for taste and to strengthen the gluten complex. Baking powder (1.0–2.5%) gives whiter, less dense, and spongy products. Various natural and modified cellulose gums are added at 0.1–0.5% levels to improve dough machinability and decrease stickiness of baked tortillas. Antimicrobial agents (propionates and sorbates) and acidulants (citric, fumaric, and phosphoric acids) limit fungal growth and extend shelf life.

Wheat tortilla dough is mixed to incorporate the dry ingredients, fat, and water and to form a pliable and viscous dough. The dough is optimally mixed to slightly overmixed and varies in temperature from 26 to 38 °C, depending upon subsequent operations. Then, the dough is divided and rounded into dough balls for hot-press and hand-stretch operations (Figure 2). The dough balls are rested in a warm, moist environment for 5–30 min to relax the gluten complex. Rested dough balls machine easier and form better tortillas. Regardless of the process, the formed tortillas are baked (190–260 °C for 30–50 s) in gas-fired ovens that generally have three tiers. Oven conditions vary depending upon tortilla thickness, type of conveyor (slat or wire), and forming operation. Puffing of tortilla occurs near the end of baking and is more common in hot-press and hand-stretch tortillas. Tortillas are cooled to <32 °C on cooling conveyors before placing into plastic bags for distribution. Improper cooling causes the tortilla to stick together and increase microbial problems. Most processors manufacture both white and different versions of whole wheat flour tortillas.

Hot-press tortillas are slightly off-round, elastic, and resistant to tearing, have a smooth surface texture, and resist moisture absorption from fillings. They are consumed as gourmet table tortillas, *fajitas*, and soft tacos. Die-cut tortillas are perfect circles and have lower moisture content and less resistant to cracking. Most have dusting flour on the surface and are mainly used in *burritos*, frozen Mexican foods, and fried products (e.g., taco salad bowls, taco shells, *chimichangas*, and sweet *buñuelos*). Hand-stretch tortillas are irregular in shape, elastic, and moderately resistant to tearing and usually have leftovers of dusting flour on the surface. Hand-stretch tortillas are consumed as table tortillas, *burritos*, and some fried products.

Hand-stretch operations require more labor, sanitation, and maintenance. The preformed and proofed dough balls are forced to pass through a pair of rolls; the first pair of rolls presses the dough ball into an ellipsoidal tortilla, while the second positioned perpendicularly further compresses into a semiround configuration. The preformed tortillas fall onto a hot surface where operators manually stretch them into round forms. That is the reason this method is named hand-stretch.

In die-cut operations, the dough is shaped into a sheet that is further thinned by a series of cross-rollers on a moving belt. The thin sheet of dough (about 0.5 mm) is cut by a circular die, which forms the shape. The scrap dough is returned to the dough pump and processed. Independent of the process, $\sim$150 kg of wheat flour tortillas with 32% moisture is obtained from 100 kg of flour.

Nutritional Value of Tortillas and Related Snacks

Both maize and wheat tortillas are considered as excellent sources of calories due to their high starch content. Maize tortillas are basically ground maize mixed with small quantities of lime (CaO) that was absorbed during cooking and steeping. Some nutritionists consider maize tortillas as a whole grain item although the alkali treatments break down fiber that is partially lost during the subsequent steps of steeping and *nixtamal* washing. The starch is in practical terms 100% digested and absorbed, and while being digested, glucose is transported to the bloodstream more slowly, thus favoring its value for diabetic people. The consumption of 100 g serving of tortillas (three pieces) with 46% moisture provides about 220 kcal (Table 1). The protein quality of maize and wheat

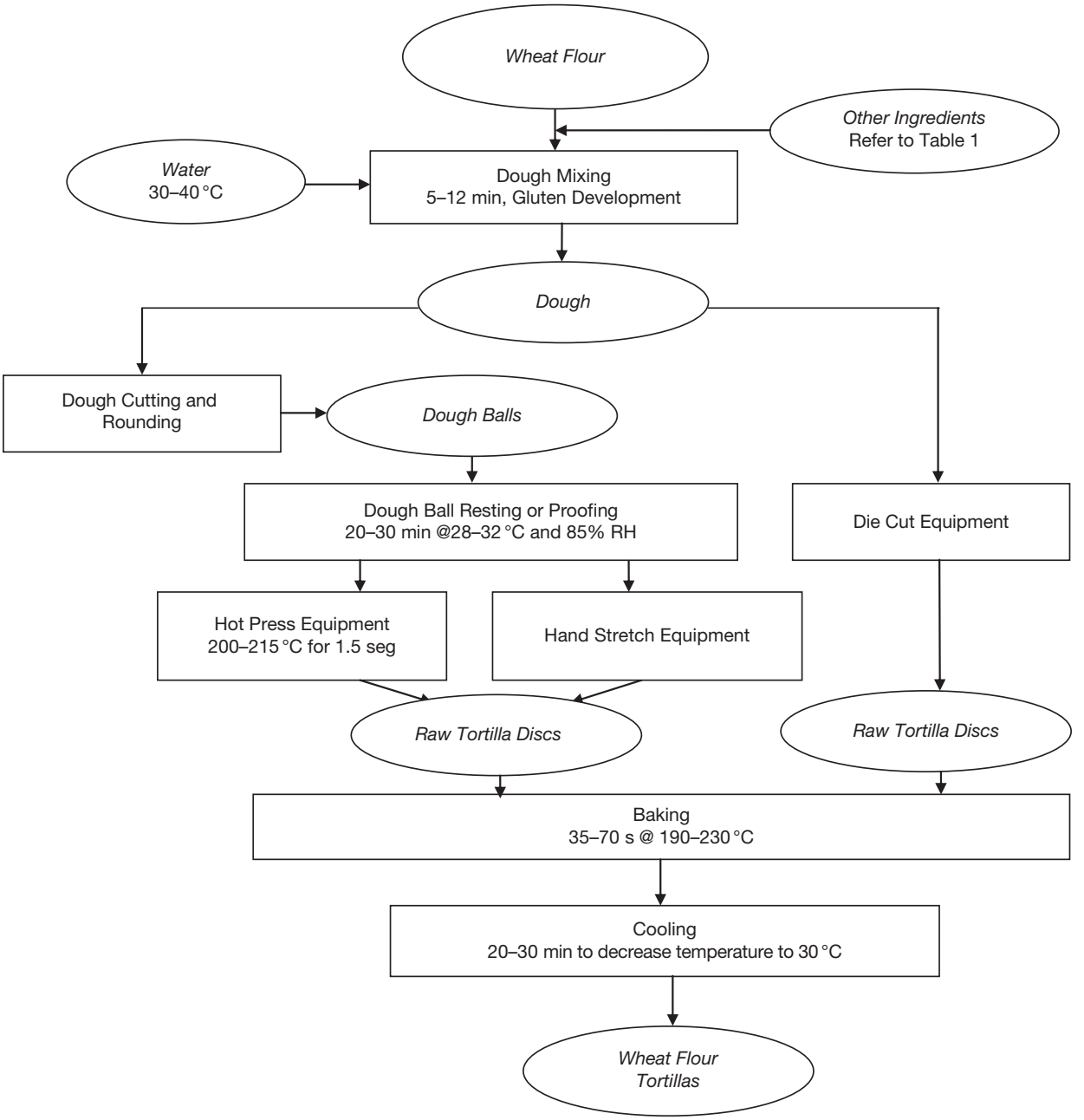

Figure 2 Flowchart of industrial processes for the manufacture of wheat flour tortillas.

tortillas is considered low because these products do not contain enough quantities of lysine to sustain regular growth in infants. This particular amino acid is present in about half of the amount required for optimum growth. Animal and human trials have demonstrated the clear nutritional superiority of opaque-2 and quality protein (QPM) over regular maize tortillas. This is because QPM has higher levels of lysine and tryptophan that result in better nitrogen retention and growth. Lime cooking also increases significantly calcium and most B vitamin levels. The caloric density of corn chips and tortilla chips is more than two times higher compared to table tortillas (Table 2). This is due to oil absorption during frying and their low moisture content. Most snacks are salted or flavored with salt-based flavorings; therefore, these products usually contain large quantities of sodium. This essential electrolyte is considered the single nutrient most abused by modern humans because an average diet usually provides at least ten times of the recommended intake. Hypertension is the main health risk associated with the high intake of this mineral.

Corn and tortilla chips are an important source of energy, protein, dietary fiber, and calcium. Corn chips are higher in calories and oil than tortilla chips. The average oil content of regular corn and tortilla chips is 36% and 24%, respectively (Table 1). Corn and tortilla chips are energy-dense because

Table 1 Nutritional composition of lime-cooked maize products and wheat flour tortillas (per 100 g serving)

	Nixtamalized products			*Wheat flour tortillas*	
Nutrient	*Soft tortillas*	*Corn chips*	*Tortilla chips*	*Wheat flour tortilla*	*Whole wheat flour tortilla*
Moisture (g)	45.89	1.0	1.8	29.3	30.0
Energy (kcal)	218	539	501	322	301
Energy from fat (kcal)	26	301	236	83	69
Protein (g)	5.70	6.6	7	7.2	8.0
Fat (g)	2.85	33.4	26.2	9.2	7.7
Carbohydrates (g)	44.64	56.9	62.9	53.0	49.8
Dietary fiber (g)	6.3	4.9	6.5	1.8	6.9
Minerals					
Calcium (mg)	81	127	154	42	170
Iron (mg)	1.23	1.32	1.5	1.5	2.3
Sodium (mg)	45	630	528	686	512
Zinc (mg)	1.31	1.58	1.46	0.6	0.2
Vitamins					
Thiamin (B1) (mg)	0.094	0.03	0.08	0.57	0.33
Riboflavin (B2) (mg)	0.065	0.14	0.18	0.38	0.06
Niacin (mg)	1.498	1.18	1.28	4.52	3.78
Folate (mcg)	5	20	10	201	138

Source: Serna-Saldivar, S. O. (2010). Role of cereals in human nutrition and health. Chapter 17. In: *Cereal grains: properties, processing and nutritional attributes*. Boca Raton, FL: CRC Press (Taylor & Francis Group); USDA National Nutrient Database for Standard Reference: http://ndb.nal.usda.gov/.

Table 2 Typical formulations for the elaboration of regular, whole wheat, and whole grain wheat flour tortillas

Ingredients	*Regular (%)*	*Whole wheat (%)*	*Whole grain wheat (%)*
Wheat flour[a]	100	50	0
Whole wheat flour	–	50	0
Whole grain wheat	–	–	100
Water[b]	48–52	48–51	48–51
Shortening	8–14	8–14	8–14
Salt	1.5–2.0	1.5–2.0	1.5–2.0
Double acting baking powder	1.5–2.0	1.5–2.0	1.5–2.0
Nonfat dry milk	1.0–1.5	–	–
High fructose syrup/honey/molasses	–	1.0–2.0	1.0–2.0
Gums[c]	0.15–0.25	0.1–0.2	0.1–0.2
Emulsifiers[d]	0.1–0.3	0.1–0.3	0.1–0.3
Preservatives[e]	0.2	0.2	0.2
Fumaric acid[f]	0.2	0.2	0.2
Reducing agent[g]	10 ppm	–	–

[a]Generally, all-purpose flours with 10.5% protein are ideally suited for most applications. A higher protein content for whole wheat and whole grain tortillas is recommended. The whole wheat flour may contain different levels of wheat bran, whereas the whole grain wheat flour consists of the whole milled kernel (includes germ, aleurone, and bran at the levels found in the whole grain).

[b]In most instances, the water is warmed to 40–50 °C before dough mixing.

[c]Most common gums are carboxymethyl cellulose or CMC, xanthan, and guar gums sometimes used alone or in combination.

[d]Most common emulsifiers are lecithin, sodium stearoyl-2-lactylate, and monoglycerides used alone or combined.

[e]Most common antimold agents are calcium propionate and potassium sorbate normally used alone or combined.

[f]Quantities vary according to the desired dough pH. Generally, the pH is lowered to 5.2–5.9.

[g]Most common reducing agents are sodium bisulfite and cysteine.

most of the moisture evaporates during the frying process and part of this moisture is substituted by the frying oil. A 100 g serving of regular corn and tortilla chips provides more than 540 and 500 kcal, respectively (Table 1).

Wheat flour tortillas have a nutritional composition similar to table bread although they are slightly more caloric because their formulation contains higher quantities of shortening. In addition, most wheat tortillas are chemically leavened and not fermented. However, wheat flour tortillas contain ~36% more calories compared to maize tortillas due to their lower moisture and higher fat contents. The consumption of 100 g of flour tortillas provides about 320 kcal (Table 1). Nutrient density of whole wheat flour tortillas decreases by about 7% when compared with refined wheat counterparts. Whole wheat flour

tortillas contain 6.9% dietary fiber (3.2% insoluble and 3.7% soluble) instead of 1.8% (0.7% soluble and 1.1% insoluble) of counterparts produced from refined flour. Fat is the second largest nutrient in flour tortillas (4–10% fat), and therefore, they are high in energy from fat and saturated fatty acids especially when hydrogenated shortenings or lard is utilized. Degree of fat saturation or hydrogenation affects contents of saturated and *trans* fatty acids. Wheat flour tortillas have a high rate of protein digestibility (85%) but a relatively low protein quality because gliadins and glutelins have insufficient levels of lysine and threonine. Lysine is present in about half of the amount required for optimum child growth.

See also: Aflatoxin: A Global Public Health Problem; Emulsifiers: Types and Uses; Fatty Acids: *Trans* Fatty Acids; Functional Foods; Gums: Properties and Uses; Hypertension and Diet; Maize; Mycotoxins: Classification; Mycotoxins: Occurrence and Determination; Mycotoxins: Toxicology; Niacin; Oxidation of Food Components; Snack Foods: Types and Composition; Wheat: Grain Structure of Wheat and Wheat-based Products.

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Toxins in Food: Naturally Occurring

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Introduction

The issue of food safety concerns various factors including bacteria, chemicals, and natural toxins. The most important natural toxins in food have been reviewed here, but some naturally occurring toxins in animal that do not cause food poisoning (e.g., snake toxins and cone shell toxins) are not reviewed. Meanwhile, the polluted toxicants such as pesticides, animal drugs, food additives, processed derivatives, and environmental chemicals also are not described here. This description provides useful information of natural toxins in food.

Classification of Toxic Compounds in Mushrooms and Terrestrial Plants

Cyanogenic Glycosides (Cyanogens)

At least 1000 plant species are reported to contain cyanogens. The cyanide in plants is present as a glycoside. At least 20 kinds of glycosides are identified in less than 50 species. Cyanogen is present in the kernels of apples, cherries, peaches, and related genera. Amygdalin is found in the highest amounts in the seeds of the bitter almond, *Prunus amygdalus* var. *amara*. Amygdalin is also a component of some herbal remedies for cancer that have produced unintentional cyanide poisoning. Cassava is a staple food starch from *Manihot esculenta* grown extensively in some parts of Africa as a major food source. The untreated root contains linamarin, a cyanogenic glucoside. Chronic ingestion of linamarin in cassava has been proposed as the cause of epidemics of konzo, a form of tropical myelopathy with sudden onset of spastic paralysis.

Stimulants and Other Psychoactive Compounds

The most widely used food stimulants are xanthic bases (caffeine in coffee, tea, cocoa, and kola nuts; theophyllin in tea; and theobromine in cocoa). Coffee is probably the most popular beverage all over the world. The xanthic bases, especially caffeine, have stimulant effects on nervous and cardiorespiratory systems.

Psychoactive compounds include myristicin, dioscorine, carotatoxin, psilocybin, and psilocin. Myristicin is present in nutmeg, mace, black pepper, parsley, celery, dill, and carrots. Myristicin may play as a weak monoamine oxidase inhibitor. However, nutmeg and mace induce greater narcotic and psychotomimetic activity than an equivalent amount of myristicin or elemicin, also a component of nutmeg.

Dioscorine is found in a few species of yams of the genus *Dioscorea* including *D. dumetorum*, *D. hirsute*, and *D. hispida*. In humans, dioscorine induces a burning sensation in the mouth and throat, abdominal pain, vomiting, diarrhea, speech disturbances, vertigo, salivation, lachrymation, sensation of heat, exophthalmos, deafness, and delirium. In severe cases, death may occur. Carrots and celery contain an acetylenic alcohol, carotatoxin. This compound resembles the powerful central nervous system (CNS) stimulant cicutoxin, a very poisonous substance from the water hemlock (*Cicuta maculata*). Several species of *Strychnos* contain strychnine and brucine (dimethoxystrychnine). Both indoles cause marked CNS stimulation by blocking glycine-gated chloride channels. Cases of unintentional poisoning from seeds of *S. nux-vomica* have been reported. Seeds of *S. ignatii* in the Philippine islands have caused similar toxic effect.

Psilocybin and psilocin are from the mushroom *Psilocybe mexicana*, and muscarline and bufotenine are from *Amanita muscaria* and other mushrooms. Meanwhile, *A. pantherina* contains the excitatory amino acid, ibotenic acid, and its derivative, muscimol. These toxic effects are somewhat variable, including CNS depression, ataxia, hysteria, hallucinations, myoclonic twitching, and seizures.

Several species of the pea family contains several species that produce excitatory amino acids in the seeds. Willardiine, 1-(2-amino-2-carboxyethyl) pyrimidine-2,4-dione, has been isolated from *Acacia willardiana*, *A. lemmoni*, *A. millefolia*, and *Minosa asperata*. Willardiine acts as an agonist on glutamate receptors. Other important excitatory amino acids are present in *Lathyrus sylvestris* (flat pea). Seeds of *L. sativus* (grass pea) are used as food and contain the excitatory amino acid β-L-ODAP (β-*N*-oxalyl-L-α, β-diamino propionic acid).

Cholinesterase Inhibitors

Cholinesterase inhibitors have been detected in several edible fruits and vegetables, including broccoli, Valencia oranges, sugar beets, rutabagas, cabbage, peppers (*Capsicum frutescence*), pumpkins, squash, carrots, strawberries, tomatoes, Jonathan and Stayman apples, lima beans, eggplant, asparagus, turnips, radishes, and several species of potatoes. The most active inhibitor is found in potatoes. The principal acetylcholinesterase inhibitor in potatoes is solanine, a glycoalkaloid formed from aglycone solanidine and glycoside. Solanine is also found in other poisonous Solanaceas, such as the common nightshade (*Solanum nigrum* and *S. americanum*). Greening in potatoes may increase the solanine content. The sprouts may contain lethal amounts of solanine. The symptoms include hyperesthesia, dyspnea, itchiness in the neck, drowsiness, and gastrointestinal effects such as vomiting and diarrhea. The alkaloids chaconine and tomatine have similar toxicities and are also found in potatoes and similar solanaceous foodstuffs.

Cardioactive Glycosides

The plant *Digitalis purpurea* (foxglove) contains the cardioactive glycoside digoxin. In the lily family, squill (*Scilla maritima*) contains scillaren, and lily of the valley (*Convallaria majalis*) contains convallatoxin in the bulbs. Both glycosides have

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actions resembling *Digitalis*. Milkweeds (*Asclepias* spp.) are noted for the glycosides in the plants. The cardiac glycoside 6′-O-(E-4-hydroxycinnamoyl) desglucouzarin in *A. asperula*, like *Digitalis*, inhibits Na^+, K^+-ATPase. Other species of *Asclepias* also contain cardenolides. *Nerium oleander* (oleander) contains two major glycosides, oleandrin and nerium. *Thevetia peruviana* (yellow oleander) poisoning has been reported in Australia, Melanesia, Thailand, and India. The seeds are the major source of the cardiac glycosides, the most active of which is thevetin A.

Cardiac Neuroactive and Vasoactive Chemicals

These species of *Veratrum* (*V. album* and *V. californicum*) contain veratrum alkaloids, including protoveratrine, veratramine, and jervine. After ingestion, the alkaloids cause nausea, emesis, hypotension, and bradycardia. The bulbs of the wild camas (*Zigadenus paniculatus* and other species of *Zigadenus*) have been mistaken for wild onions producing severe gastrointestinal toxicity.

Aconitum species (*A. napellus*, *A. carmichaeli*, and *A. kusnezoffii*) have caused accidental poisonings. The concentration of the alkaloids (aconitine, mesaconitine, and hypoaconitine) varies depending on species, place of origin, time of harvest, and processing procedures. Grayanotoxins are reported to induce mad honey poisoning and produced by several genera of Ericaceae (*Rhododendron ponticum*, *Kalmia angustifolia*, and *R. macrophyllum)*. The effect of toxins is marked bradycardia, hypotension, oral parenthesis, weakness, and gastrointestinal upset, resembling aconitine poisoning.

Mistletoes, *Phoradendron flavescens* and *V. album*, are parasitic plants on trees and contain polypeptides phoratoxin and viscotoxins, respectively. Phoratoxin and viscotoxins cause similar effects, including hypotension, bradycardia with negative inotropic actions on heart muscle, and vasoconstriction of the vessels of skin and skeletal muscle. Some fungi (e.g., *Claviceps purpurea*, *Aspergillus fumigatus*, and *Acremonium coenophialum*) are parasitic on grains and/or plants to induce food poisoning. They produce ergot alkaloids, including lysergic acid, lysergic acid amide, ergotamine, ergonovine, and other derivatives. Human lathyrism is a neurological disease associated with the consumption of legumes of the genus *Lathyrus*, particularly *L. sativus*, *L. clymenum*, and *L. cicera*. The toxic compounds include diaminobutyric acid (BA), *N*-γ-oxalyl-α-γ-diaminobutyric acid (ODBA), *N*-β-oxalyl-α-β-diaminopropionic acid (ODPA), and γ-glutamyl-β-diaminopropionitrile (GAPN). Among them, three compounds are neurolathyrogens except for GAPN as an osteolathyrogen.

Hepatoactive Chemicals

Senecio species contain significant concentrations of pyrrolizidine alkaloids (Pas) responsible for liver damage. Four genera *Echium* (bugloss), *Cynoglossum* (hound's tongue), *Heliotropium* (heliotrope), and *Symphytum* (comfrey) also contain Pas. *Lantana camara* also contain lantadene A, which induces hepatotoxicity.

Several species of mushrooms are responsible for most fatal poisoning such as *Amanita*, *Galerina*, and *Lepiota*. *A. phalloides* contains two types of toxins, phalloidin and amatoxins. Phalloidin combines with actin in muscle cells. The α, β, and γ-amanitins are bicyclic peptides. The most toxic α-amanitins bind strongly to RNA polymerase II in hepatocytes, thus inhibiting protein synthesis. Various fungi present in food and produce toxins. Most notable toxins are aflatoxins from *Aspergillus* species, ochratoxin from *Aspergillus* and *Penicillium* molds, and zearalenone from *Fusarium* and *Gibberella* species. Aflatoxin from *A. flavus* and *A. parasiticus* has been associated with primary hepatocellular carcinoma in humans. Fumonisins are *Fusarium moniliforme* and *F. proliferatum* growing on corn.

Some Asian people specially believe that consuming animal bile juice from snake, common carp, grass carp, and other animals can improve their visual ability and liver functions. However, the major component in bile juice of common carp and grass carp is cyprinol sulfate that may induce renal failure. Moreover, bile acids (cholic acid, chenodeoxycholic acid, deoxycholic acid, and lithocholic acid) that are major components in other terrestrial animals may induce serious liver dysfunction and then progressively induce renal failure.

Favic Agents

Consumption of faba beans (*Vicia faba*) or inhalation of its pollen has been associated with the disease known as favism or pollinosis. The symptom is characterized by hemolytic anemia, hemoglobinuria, and shock. The toxic compounds are vicine (glucose + divicine) and convicine (glucose + isouramil). *Poa* and *Festuca* species are major contributors to the allergic response. Pollen from pellitory (*Parietaria* species) is an important cause of pollinosis. Pollen from mugwort, *Artemisia vulgaris*, and ragweed, *Ambrosia artemisiifolia*, also contains allergens causing summer rhinitis. The allergen has been identified as a protein.

Photodynamic Substances

The photodynamic compounds include fagopyrin (in buckwheat *Fagopyrum esculentum*), hypericin (in Klamath weed and St. John's wort, *Hypericium perforatum*), photofagopyrin (in buckwheat), isopimpinelin (in citrus fruits, celery, parsley, and other plants), and pyropheophorbide (from chlorophyll of plants and algae). Specially, abalone poisoning is caused by abalone viscera poison and is unusual in that it causes photosensitization. The toxin, pyropheophorbide, is stable to boiling, freezing, and salting. It is found in Japanese abalone, *Haliotis discus* and *H. sieboldii*. Nori (dried alga products) also caused this pyropheophorbide poisoning in Taiwan.

Toxic Phenolic Substances and Alcohols

There are more than 800 phenolic compounds in plants. These compounds may be divided into two major groups. The first group includes phenolic acids such as caffeic acid, ferulic acid, sinapic acid, gallic acid, flavonoids, lignin, hydrolyzable tannins, ellagic acids, and derivatives of these compounds. Some of these compounds have antinutritive properties and the tannins are carcinogens. The second group includes gossypol (in cotton plant), phlorizin (glycoside with aglycone phloretin in apple), coumarins, myristicin, urushiols (in mango, cashew, pistachio, sumac, oak, ivy, and lac tree), phenolic amines, and catecholamines.

Carcinogens

The Pas from certain *Senecio* plants induced liver tumors. Tannic acid also is hepatocarcinogen. Tannic acid derivatives can be divided into hydrolyzable and condensed tannins. The hydrolyzable tannins are gallic, digallic, and ellagic acid esters of glucose or quinic acid. The condensed tannins are flavolans, which are polymers of flavonoids such as catechins or anthocyanidins. Tannins are widely distributed in plants. Mango fruit contains gallotannins abundant in an unripe fruit. Many tropical fruits are rich in tannins, including dates, persimmons, and sapote fruit.

Cycasin and related azoxyglycosides (neocycasins) are found in the plant Cycadaceae, especially for *Cycas circinalis*, *C. revolute*, *Encephalartos barkeri*, and *Macrozamia spiralis*. Cycasin, neocycasins, and related aglycones resemble the nitrososamine in their biochemical action. A number of alkenyl- and alkylbenzenes with a methylenedioxy substituent in 3,4 position have been already proven to be carcinogenic. These include safrole, isosafrole, and their derivatives. Safrole and isosafrole are 3,4-methylenedioxy-1-allybenzene and 3,4-methylenedioxy-1-propenylbenzene, respectively. Safrole is a hepatocarcinogen and a pulmonary carcinogen. Safrole is present endogenously in sassafras tea (*Sassafras albidum*), *Cinnamomum micranthum*, *C. camphora* var. *glaucescens*, *C. cassia*, *C. zeylanicum*, and *C. loureirii*. Safrole is a trace constituent in cocoa, nutmeg, mace, California bay laurel, black pepper, anise, Japanese star anise, and Japanese wild ginger.

Terpenes and other isoprenoid compounds are cyclic or acyclic hydrocarbons whose derivatives are exact multiples of the isoprene unit. These compounds have been shown to be carcinogenic, cocarcinogenic, or tumor-promoting and are found in oranges, lemons, limes, grapefruits, black pepper, and ginger. Black pepper contains carcinogenic alkaloids piperine, chavicine, and piperettine. Polycyclic aromatic hydrocarbons have also been found in several raw green vegetables, cabbage, leeks, lettuce, tomatoes, and spinach. Dimethylnitrosamine was found in wheat flours, grains, and certain plants. The precursors, secondary amines, and nitrates (easily reduced to nitrites in unfresh vegetables) have been found in many plants. Thiourea, which is a goitrogen and a carcinogen, is produced endogenously in the seeds of *Laburnum* species. Coumarin and lactose are minor constituents of certain edible fruits, for example, strawberries, cherries, and apricots, and a major constituent of tonka beans. Coumarin is present in cassie, woodruff, lavender, lovage, citrus oils, and carrot seed oil. *Petasites japonicus* is used in Japan as a food and herbal remedy. A carcinogen petasitenine, a Pas, has been identified in this plant.

Miscellaneous Endogenous Toxicants

Human lathyrism is a neurological disease associated with the consumption of legumes of the genus *Lathyrus* (*L. sativus*, *L. clymenum*, and *L. cicera*). The corresponding toxic compounds include BA, ODBA, ODPA, and GAPN. BA, ODBA, and ODPA are neurolathyrogens and GAPN is an osteolathyrogen.

The functions of plant estrogens are the same as the animal estrogens. Those phytoestrogens are estrone (in palm kernels, *Elaeis guineensis*), coumestrol (in alfalfa, *Medicago sativa*), several bitter acids (coupulon, lupulon, and adlupulon in hops, *Humulus lupulus*), and isoflavones in soybeans and other sources. Several unusual fatty acids exhibit toxicity. These fatty acids include erucic acid (in rapeseeds and mustard seeds), sterculic acid and malvalic acid (cyclopropene fatty acids in cottonseed and kapok seed), cetoleic acid (in herring oil), and phytanic acid.

In avocado, D-mannoheptulose shows adverse effect on both human and animals. Umbellulone can cause skin irritation, headache, and unconsciousness and is isolated from California bay laurel. Glycyrrhizin is obtained from the root of *Licorice* plant. *Licorice* may pose a considerable hazard in those with heart or kidney disease. The oil of the wormwood plant contains the ketoterpene thujone as a major component. Thujone acts as a convulsant. Onions, garlic, and related species are well known to contain active agents (alliin and allicin). Peppermint oil contains about 40% menthol (a terpene). Cathartic compound, dihydroxyphenylisatin, may be the major agent of prunes. Curcumin is a major component of turmeric and a cholagogue and antioxidant. Carpaine is an alkaloid and is present in papaya. It possesses a powerful cardiac and diuretic action. Several acidified starfruit species in Taiwan contain high amounts of oxalic acid, which may induce renal failure. Meanwhile, Chinese herb nephropathy (CHN) is a new type of subacute interstitial fibrosis of the kidney occurring in females. CHN is not limited to China, but has been reported in France, Spain, the United Kingdom, and Japan. The toxin aristolochic acid is found in these medicinal plants of *Aristolochia* spp.

Classification of Toxic Compounds in Oceanic Plants and Animals

Tetrodotoxin

Tetrodotoxin poisoning is caused by consumption of Japan fugu or puffer. However, the toxin has been reported to be present in newts, frogs, octopus, goby fish, starfish, flatworms, xanthid crabs, and various gastropods. Puffers are found in subtropical and tropical marine waters but can live in fresh, brackish, and marine waters. Tetrodotoxin is distributed in all the tissues, especially in the ovaries, roe, liver, intestines, and skin. Those toxic animals are intoxicated through the food chain. The source of tetrodotoxin comes from bacteria, especially *Vibrio* spp.

Tetrodotoxin is a neurotoxin and is water-soluble and stable to boiling except in an alkaline solution. Toxicity is manifested as a tingling or prickly sensation of the fingers and toes; malaise; dizziness; pallor; numbness of the lips, tongue, and extremities; ataxia; nausea, vomiting, and diarrhea; epigastric pain; dryness of the skin; subcutaneous hemorrhage and desquamation; respiratory distress; muscular twitching, tremor, incoordination, and muscular paralysis; and intense cyanosis. Fatality rates are high.

Ciguateric Toxins

The name ciguatera is derived from the Spanish name 'cigua' for the sea snail *Turbo pica*. The toxins include ciguatoxin, maitotoxin, palytoxin, scaritoxin, and gambiertoxin. Among

these toxins, gambiertoxin is produced by dinoflagellate *Gambierdiscus toxicus*. The notorious fish include moray eels, surgeonfish, red snapper, trunkfish, parrotfish, grouper, and barracuda. These toxic fish accumulate toxins from the food chain. Palytoxin is produced by the soft coral *Palythoa* spp. and dinoflagellate *Ostreopsis siamensis*. The fish, crabs, and polychaete worms living in close association with or eating this mass may become contaminated with palytoxin. Palytoxin has been reported in mackerel, sardine, parrotfish, crabs, and trunkfish. The symptoms include bitter and metallic taste from the meat, nausea, vomiting, diarrhea, myoglobinuria, a burning sensation around the mouth and extremities, muscle spasms, dyspnea, and dysphonia. Death may be caused by the result of myocardial injury. Further, palytoxin is a powerful hemolysin and a predominant inhibitor of ouabain-sensitive Na^+, K^+-ATPase.

Paralytic Shellfish Poisons

Paralytic shellfish poisons (PSPs) contain saxitoxins, gonyautoxins, protogonyautoxins, and related derivatives. Those toxins are found in mussels, cockles, clams, soft shell clams, butter clams, scallops, crabs, gastropods, starfish, puffer, and mackerel. Bivalve mussels are the most common vehicles. Saxitoxin is originally isolated from toxic 'Alaskan butter clams' (*Saxidomus giganteus*) and includes neosaxitoxin and gonyautoxins 1–4. Their action is to bind to the surface of the sodium channels. The toxin produces paresthesia and neuromuscular weakness without hypotension and lacks the emetic and hypothermic action of tetrodotoxin. The toxin is an alkaloid and is relatively heat-stable. The toxin is produced by several planktons (*Alexandrium catenella*, *A. tamarensis*, *A. minutum*, *Pyrodinium* spp., *Ptychodiscus brevis*, *Gymnodinium catenatum*, and others).

Neurotoxic Shellfish Poison

In the coast of Florida, *Gymnodinium breve* forms red tide blooms containing polycyclic ether toxins called brevetoxins. Brevetoxins bind to voltage-dependent sodium channels and the strength of binding varies with the specific affinity of the toxin and thus the relative potency. Symptoms of neurotoxic shellfish poisoning include nausea, tingling and numbness of the oral area, loss of motor control, and severe muscular pain. An additional route of entry in humans may be through the inhalation of aerosolized toxin as a result of the relative case of lysis of unarmored *G. breve* organism during the breaking of waves on the shore. Symptoms are seen as irritation of the throat and upper respiratory tract. Human exposure is primarily via consumption of filter-feeding organisms, which may concentrate the toxin.

Amnesic Shellfish Poison

Amnesic shellfish poison (ASP) is found in mussels harvested from the area of Prince Edward Island in 1987. The poisoning was attributed to domoic acid produced by the diatom *Pseudonitzschia multiseries*. Occurrence of domoic acid has also been reported in California shellfish and produced by *P. pseudodelicatissima* and in anchovies produced by *P. australis*. Domoic acid has been reported in shellfish in other places like Canada, Alaska, Washington, and Oregon and may be as frequent as PSP toxins. Domoic acid has also been reported in red seaweed *Chondria armata*. Domoic acid induces gastrointestinal effects and neurological effects, including dizziness, disorientation, lethargy, seizures, and permanent loss of short-term memory. Domoic acid is an analog of glutamine, a neurotransmitter, and of kainic acid; the toxicity of all three are similar as they are excitatory and act on three types of receptors in the CNS with the hippocampus being the most sensitive. Domoic acid may be a more potent activator of kainic acid receptors than kainic acid itself. The stimulatory action may lead to extensive damage of the hippocampus, but less severe injury to the thalamic and forebrain regions.

Diarrheic Shellfish Poisons

Diarrheic shellfish poisons (DSPs) are produced by dinoflagellates *Dinophysis fortii*, *D. acuminate*, *D. acuta*, *D. norvegica*, *D. mitra*, and *Prorocentrum lima*. The toxins include okadaic acid and its derivatives, dinophysistoxins, pectenotoxins, and yessotoxins. These toxins are polyether compounds and are accumulated by fish and shellfish through the food chain. Okadaic acid was the major toxin in mussel specimens from most of the European countries, while dinophysistoxin-1 was the major toxin in mussel in Japan and in Sogndal, Norway. Scallops in Japan show the most complicated toxin profile. Pectenotoxins were detected only in Japanese shellfish. Victims suffer from diarrhea, nausea, and stomach pain but recover within 3 days without serious aftereffects. Furthermore, okadaic acid analogs possess potent tumor-promoting activity. Pectenotoxins cause acute liver injuries, while dinophysistoxins cause severe injuries on the intestinal mucosa.

Microcystins

The main genera responsible for freshwater toxic blooms are *Microcystis*, *Anabaena*, *Aphanizomenon*, and *Oscillatoria*. Toxins produced include (1) anatoxins, alkaloids, and peptides of *Anabaena*; (2) the peptide microcystin and related peptides of *Microcystis*; and (3) PSPs from *Aphanizomenon*. The properties of *Oscillatoria* toxin suggest they are peptides similar to those of *Microcystis*. Microcystins are peptides that contain three invariant D-amino acids, alanine, erythro-β-methyl aspartic, and glutamic acids, and two variant L-amino acids, *N*-methyl dehydroalanine and a β-amino acid. Individual toxic strains have one or more multiples of this peptide toxin, which is a hepatotoxin and a tumor-promoting factor. Indirect evidence indicates that one or all of these toxins are responsible for certain cases of human gastroenteritis and dermatitis from municipal and recreational water supplies.

Other Marine Toxins

Azaspiracids were observed in mussels (*Mytilus edulis*) in 1995. The symptoms observed were nausea, vomiting, severe diarrhea, and stomach cramps. Until now, azaspiracid poisoning has been reported in Ireland, the Netherlands, the United Kingdom, Italy, France, Norway, and Spain. Shellfish species

involved include mussels (*M. edulis and M. galloprovincialis*), scallops (*Pecten maximus*), and crabs (*Cancer pagurus*).

In 1997, *Pfiesteria piscicida* was reportedly implicated in killing more than 30 000 fish and making ill more than 36 people in Maryland. Victims appeared with symptoms characterized by unique skin lesions, respiratory problems, and neurological complications primarily including short-term memory loss. The *Pfiesteria* toxin was a ligated copper compound. Meanwhile, *Karlodinium veneficum* co-occurs with *Pfiesteria* spp. and produces a unique suite of compounds (karlotoxins) with hemolytic, cytotoxic, and ichthyotoxic properties. The activity of karlotoxins is like those of prymnesin, isolated from another dinoflagellate *Prymnesium parvum*, and amphidinols, a family of hemolytic toxins isolated from the dinoflagellate genus *Amphidinium*.

The Indo-Pacific moray eel (*Lycodotis nudivomer*) possesses a mucous skin secretion with hemolytic, toxic, and hemagglutinating properties. The hemolytic properties can be separated from the hemagglutinating properties. The hemolytic property is lost upon treatment with trypsin and is unstable in the presence of heat or acidic or alkaline media. The skin mucous of other species of eels, European eel (*Anguilla anguilla*), pike eel (*Muraenesox cinereus*), and Japanese eel (*A. japonica*), were found to have proteinaceous toxins.

There is a high content of retinol (vitamin A) in the liver of tuna, shark, grouper, polar bear, seal, and whale. The symptoms of hypervitaminosis A are as follows: headache, nausea, vomiting, fever, vertigo, visual disorientation, and peeling. The muscle of some fish contains a laxative. They can be divided into three types, depending on the major component of the muscular lipid. The fish of the first type contain triglyceride in black cod, *Erilepis zonifer*. The second type contains wax ester in castor oil fish *Ruvettus pretiosus* and the escolar *Lepidocybium flavobrunneum*. The fish of the third type contains diacyl glyceryl ethers. Furthermore, several deep-sea gastropods contain tetramine.

Conclusion

As mentioned earlier, there are a number of natural toxins or toxicants, many of which are not confined to a single species and are therefore most likely to be influenced by the environment. However, some natural toxins are specific to a single species or genus. These natural toxins can induce different types of food poisonings depending on their toxic effects. Those potent neurotoxic toxins, inducing severe symptoms, must be recognized to prevent from mistake.

See also: Alkaloids: Properties and Determination; Alkaloids: Toxicology and Health Effects; Antinutritional Factors in Legume Seeds: Characteristics and Determination; Food Poisoning: Classification; Food Poisoning: Tracing Origins and Testing.

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Trace Minerals and Trace Elements

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Introduction

Trace minerals are inorganic substances that, along with vitamins and other micronutrients, belong to those constituents in the human diet that are needed in very small quantities. Some trace minerals are essential components of enzymes and other proteins. Because trace minerals are involved in many tissue-specific cellular functions, severe deficiency of some of these dietary micronutrients may cause debility and disease and may possibly result in death. Whether a mineral is defined as a major mineral, the trace mineral, or even ultratrace mineral, appears to be somewhat arbitrary. Calcium and magnesium are among the major minerals. Iron is treated in separate articles of this encyclopedia. Here, we concern ourselves with a selection of the trace minerals of interest to human nutrition: chromium, copper, manganese, molybdenum, selenium, and zinc, as well as the trace element iodine, which frequently appears in discussion among trace minerals. Arsenic, boron, bromine, cadmium, lead, lithium, nickel, silicon, and vanadium are often labeled as ultratrace elements. Specific sections in this encyclopedia provide further information on arsenic, boron, calcium, cadmium, chromium, lead, lithium, magnesium, and manganese.

The trace mineral-specific recommended dietary allowances (RDA), tolerable upper intake levels (UL), and adequate intake (AI) values, part of the dietary reference intakes (DRI) as used by the Food and Nutrition Board of the Institute of Medicine of the US National Academies, are defined by the average daily intake that meets the basic nutrient requirements of 97.5% of the apparently healthy US population. Slightly different values may be available for European and other populations. The USDA National Nutrient Database for Standard Reference allows convenient calculation of the amounts of trace minerals and other nutrients in our diet.

Chromium

Trivalent chromium, because of its possible involvement in the insulin-regulated metabolism of carbohydrates, lipids, and proteins, is frequently, and more recently controversially, considered an essential mineral for human health. In its hexavalent form, chromium is toxic and even carcinogenic and is not considered a dietary nutrient.

Sources and Production

Chromium is a highly abundant element in the Earth's crust and found in concentrations of 1–300 mg kg^{-1} soil, with the trivalent form, Cr(III), as the dominating species in most cases. Chromium is an important alloying material for steel, and South Africa has maintained the lead in chromium ore production, with ferrochromium and metallic chromium as the two main products of chromium ore refining. Chromates, chromium oxides, and chromium salts have been further used in the pigment and leather tanning industries.

Patterns of Consumption/Human Exposure

In terms of its biological role, considerable controversy exists in terms of chromium's essentiality in human health. Chromium is present in many vegetables (e.g., broccoli, 10 μg/100 g) and fruits and fruit-derived beverages (e.g., grape juice, 28 μg/100 ml, and red wine, 0.7–10 μg/100 ml). Good sources also include beef (55 μg/100 g), poultry, and fish. In the United States, dietary chromium intake ranges from 23–29 μg day^{-1} in women to 39–54 μg day^{-1} in men. Large amounts of trivalent chromium may be consumed through popular dietary supplements, which generally contain 50–200 μg chromium/serving in forms such as chromium chloride, chromium nicotinate, and chromium picolinate, even though the effectiveness of these supplements appears to not have been clinically demonstrated. In the United States, the current dietary guidelines suggest an AI of 25 or 35 μg chromium for adult women and men, respectively. However, in 2014, the European Food Safety Authority (EFSA) published a report stating that the intake of trivalent chromium has no beneficial effect in a healthy population and concluded that no average requirement and no reference intake for chromium could be defined. Subsequently, the EFSA panel suggested that the setting of an AI for chromium was not appropriate, and thus, the AI values that currently exist for the US population have been excluded from Table 1. Excess intake of trivalent chromium from food or dietary supplements has not been observed to result in adverse health effects, and, currently, no UL has been set for dietary trivalent chromium.

Availability, Absorption, and Metabolism

Even though chromium has a fairly low bioavailability, and only 0.4–2.5% of the chromium ingested is absorbed in the intestinal tract, the human body contains about 4–6 mg of this trace mineral. The vast majority of chromium is excreted in the urine and feces, up to 0.4 μg day^{-1}, with increased excretion in those with diets high in simple carbohydrates. Consumption of vitamin C and niacin and intake of insulin and anti-inflammatory drugs appear to increase chromium absorption, whereas intake of antacids and proton pump inhibitors may block chromium absorption or increase excretion.

Health Effects/Physiology

Chromium deficiency is rare, and the recent EFSA report calls into question if a low intake in humans will result in physiological consequences. Those potentially at risk are those that may excrete higher levels of chromium or are unable to absorb chromium from the diet. Although few if any toxic effects of trivalent chromium have been described, exceedingly high quantities of trivalent chromium may inhibit DNA polymerase activity and increase the rate of various mutations in the DNA. Additionally, organ damage, including renal and liver dysfunctions, has been reported in people who have ingested

Table 1 Recommended dietary allowance (RDA), upper limits (UL), and adequate intakes (AI) for copper, fluoride, iodine, selenium, and zinc, as set forth by the Institute of Medicine of the National Academies

		Copper		*Iodine*		*Manganese*		*Molybdenum*		*Selenium*		*Zinc*	
Ages		*RDA (μg day^{-1})*	*UL (μg day^{-1})*	*RDA (μg day^{-1})*	*UL (μg day^{-1})*	*AI (mg day^{-1})*	*UL (mg day^{-1})*	*RDA (μg day^{-1})*	*UL (μg day^{-1})*	*RDA (μg day^{-1})*	*UL (μg day^{-1})*	*RDA (mg day^{-1})*	*UL (mg day^{-1})*
Infants	0–6 months	200	ND	110	ND	0.003	ND	2	ND	15	45	2	4
	6–12 months	220	ND	130	ND	0.6	ND	3	ND	20	60	3	5
Children	1–3 years	340	1000	90	200	1.2	2	17	300	20	90	3	7
	4–8 years	440	3000	90	300	1.5	3	22	600	30	150	5	12
Males	9–13 years	700	5000	120	600	1.9	6	34	1100	40	280	8	23
	14–18 years	890	8000	150	900	2.2	9	43	1700	55	400	11	34
	19–30 years	900	10000	150	1100	2.3	11	45	2000	55	400	11	40
	31–50 years	900	10000	150	1100	2.3	11	45	2000	55	400	11	40
	51–70 years	900	10000	150	1100	2.3	11	45	2000	55	400	11	40
	>70 years	900	10000	150	1100	2.3	11	45	2000	55	400	11	40
Females	9–13 years	700	5000	120	600	1.6	6	34	1100	40	280	8	23
	14–18 years	890	8000	150	900	1.6	9	43	1700	55	400	9	34
	19–30 years	900	10000	150	1100	1.8	11	45	2000	55	400	8	40
	31–50 years	900	10000	150	1100	1.8	11	45	2000	55	400	8	40
	51–70 years	900	10000	150	1100	1.8	11	45	2000	55	400	8	40
	>70 years	900	10000	150	1100	1.8	11	45	2000	55	400	8	40
Pregnancy	14–18 years	1000	8000	220	900	2.0	9	50	1700	60	400	12	34
	19–30 years	1000	10000	220	1100	2.0	11	50	2000	60	400	11	40
	31–50 years	1000	10000	220	1100	2.0	11	50	2000	60	400	11	40
Lactation	14–18 years	1300	8000	290	900	2.6	9	50	1700	70	400	13	34
	19–30 years	1300	10000	290	1100	2.6	11	50	2000	70	400	12	40
	31–50 years	1300	10000	290	1100	2.6	11	50	2000	70	400	12	40

Note: For all trace minerals listed, the values for infants are AI; ND, not determined.

chromium picolinate supplements containing between 0.6 and 2.4 mg of chromium.

Copper

Copper, like many other trace minerals, is toxic in large amounts, but essential in the human diet at trace levels, and necessary for normal iron metabolism, red blood cell formation, blood coagulation, oxidation–reduction reactions, and scavenging of free radicals. The majority of the body's copper is found as Cu^{2+} species, though it is able to shift between the cuprous (Cu^{1+}) and cupric (Cu^{2+}) valence states.

Sources and Production

Copper occurs naturally in the Earth's crust within rocks, coal, clay, and soil, thus spreading naturally through the environment through dust particles, volcanic eruptions, forest fires, decaying vegetation, and sea spray. Copper is also introduced into our immediate environment through copper mining, manufacturing of copper-containing materials, waste dumps, combustion of fossil fuels, wood production, and phosphate fertilizer production. The concentrations of copper in soil, water, and air are increased in areas polluted by smelting and mining operations and combustion sources. Thus, copper is found throughout the environment from both natural and anthropogenic sources and is widely – but unevenly – distributed in our food supply, including copper-containing dietary supplements. The amount of copper within the soil is not indicative of copper content within the plants growing in the soil, because the mineral binds strongly to the surface layer of the soil and is not in a soluble form.

Patterns of Consumption/Human Exposure

Food sources with high copper content typically include organ meats (e.g., beef liver, ~14 mg/100 g), seafood (e.g., mollusks, 0.7–1.4 mg/100 g), nuts and seeds (1–2 mg/100 g), whole grains, and cocoa products (e.g., semisweet chocolate, 0.7 mg/100 g). Chicken, milk, potatoes, and tea have a lower copper content, but consumption of these foods in the general US population and elsewhere is rather large, leading to a relatively high copper intake. Copper is also present in varying amounts in drinking water due to copper plumbing and brass water fixtures. The median dietary copper intake for men and women in the United States is 1.2–1.6 mg day^{-1} and 1.0–1.1 mg day^{-1}, respectively. Similar ranges have been estimated for adults in different European countries. Regular intake of dietary supplements likely leads to increased copper intakes of 1.3–2.2 mg day^{-1} and 1.0–1.7 mg day^{-1} for men and women, respectively. Pregnant and lactating women require increased copper due to the needs of the fetus or breast-fed infant. To determine the UL of copper consumption, liver damage was used as the critical end point. The adult UL for copper is 10 mg day^{-1} (Table 1).

Availability, Absorption, and Metabolism

Copper is primarily absorbed across the mucosal barrier in the small intestine through a highly effective (55–75% absorption), energy-independent, carrier-mediated diffusion. However, excess uptake of other divalent cations, such as iron and zinc, can inhibit the absorption of copper. Transport of copper through the basolateral membrane into the interstitial fluid and blood is through an energy-dependent, rate-limiting mechanism, before absorbed copper can be transported by albumin to the liver. The primary method of copper excretion is via the gastrointestinal tract, specifically through bile, which has the highest concentration of copper in comparison with other secreted fluids. Copper also can be excreted in low concentrations through urine.

Health Effects/Physiology

General malnutrition or malabsorption can result in copper deficiency, and the condition leading to deficiency can be acquired or inherited. Importantly, copper deficiency can also occur as a result of excessive zinc intake. Copper deficiency, though relatively rare, can cause symptoms of anemia, leucopenia, and neutropenia. Menkes syndrome, a genetic disorder of copper transporter proteins, is characterized by a prominent copper deficiency caused by a reduction in the copper transport. The clinical symptoms of this disease include kinky hair, neurological degeneration, and ineffective temperature regulation and can even result in death at a young age.

Copper toxicity, outside accidental exposures in industrial settings, is rare. Moderately excessive doses can cause acute toxicity symptoms such as nausea, diarrhea, lethargy, and anorexia. Chronic or excessively large doses can result in toxic effects of copper including blood in urine, jaundice due to hepatic necrosis, and little to no urine production due to kidney damage. Copper consumed in quantities of about 1000 times the normal dietary intake level is lethal. Genetic conditions such as Wilson's disease and Indian childhood cirrhosis lead to increased risk of copper toxicity because of the inability to incorporate copper in ceruloplasmin synthesis or to excrete copper in bile, thus causing an adverse hepatic copper buildup.

The primary biochemical role of copper is as a cofactor for specific enzymes and electron transport proteins, fundamental to energy and antioxidant metabolism. Copper is incorporated into several key proteins, including ceruloplasmin (oxidative enzyme and transport protein in the blood), cytochrome oxidase (cellular respiration), superoxide dismutase (catalyzes the removal of oxygen radicals), and metallothionein (copper storage). In the presence of inflammation and cancer, plasma copper and ceruloplasmin concentrations are increased, resulting in increased protection against oxygen radicals. How copper contributes to the development and maintenance of the immune system, demonstrated by an increase in white blood cells in malnourished infants receiving copper supplements, still remains unclear. The role of copper in cardiovascular disease, Alzheimer's disease, Parkinson's disease, and the onset of osteoporosis is currently being investigated with limited conclusive findings.

Iodine/Iodide

The essential trace element iodine is best known for its integral part in the synthesis of the thyroid hormones thyroxine and triiodothyronine, which are required for normal growth,

development, and metabolic processes. Iodine is commonly found as iodide, as well as organically bound forms. In humans, up to 80% of systemic iodine is concentrated in the thyroid gland. Although a nonmetal, iodine is frequently included in the discussion of trace minerals and thus included here.

Sources and Production

Iodine is the elemental form of this trace element; however, it is more commonly found in its inorganic, highly water-soluble, salt form, iodide. Iodide is variable and relatively rare in the terrestrial environment but concentrates in saltwater environments and the plants contained therein. Commercially, iodine is produced from iodine-containing brines, including natural brines and those of gas and oil fields, especially in Chile, Japan, Russia, and the United States. In contrast, large-scale recovery of iodine from seaweed is no longer commercially viable.

Patterns of Consumption/Human Exposure

Common dietary sources of iodine are seaweed (16–2984 $\mu g\, g^{-1}$), seafood such as codfish (117 μg/100 g), yogurt (2.1 mg/100 g), and eggs (50 μg/100 g). Fruits and vegetables also contain iodine; however, this varies widely with the iodine content in the regional soil, anywhere from 10 to 1000 $\mu g\, kg^{-1}$. Iodine fortification is common in agricultural animal feed and table salt, which increases the iodine intake of most individuals to an adequate level. Commercially available dietary iodine supplements are most frequently offered in the form of potassium or sodium iodide. Clinical evaluation of iodine status is difficult, until it is so far advanced that a palpable or visible enlargement of the thyroid gland in the neck (goiter) has emerged; however, common assessment approaches for milder states are urinary excretion over time, serum thyroid-stimulating hormone (TSH) levels, and serum thyroxine levels. Despite worldwide implementation of iodine supplementation programs, inadequate iodine consumption remains a public health problem across the United States and Europe.

Availability, Absorption, and Metabolism

When ingested, iodine in the form of potassium iodide is absorbed to an extent of 96.4%. A vast majority of an individual's iodine taken in through the diet or supplements resides within the thyroid gland, with only 20–30% left to circulate through the blood. After absorption, iodide is converted to iodine in the thyroid gland for the later formation of triiodothyronine and thyroxine. Nonincorporated inorganic forms of iodine are readily excreted by the kidney. The half-life of iodine in a healthy adult's plasma is $\sim$10 h, and up to 90% of total ingested iodine is excreted in the urine within 24–48 h.

Health Effects/Physiology

Iodine deficiency can have serious consequences among both adults and children. Common effects of iodine deficiency are inadequate thyroid hormone activity and birth defects such as mental retardation. When iodine intake is below 100 $\mu g\, day^{-1}$, TSH activity will increase to sequester iodine from the bloodstream. In more serious situations, iodine intake below 10–20 $\mu g\, day^{-1}$ induces hypothyroidism and goiter. If this diminished iodine intake is chronic, thyroid cancer may develop. Groups at increased risk for iodine deficiency include those living in iodine-poor soil regions, pregnant women, and individuals who ingest high levels of goitrogens. Goitrogens, which are organic compounds that inhibit iodine, are found in foods such as soy, cruciferous vegetables, and cassava (also known as manioc or tapioca). These goitrogens are often heat-inactivated during cooking or processing; however, pasteurization of dairy products does not effectively eliminate them. As of 2013, up to 30 countries have been reported as having endemic iodine deficiency.

Iodine toxicity from dietary sources is rare but may occur when highly iodide- or iodophor-contaminated foods or excessive amounts of iodine-containing dietary supplements are consumed. Excessive intake of >1–2 mg day^{-1} may cause thyroid gland irregularities, similarly to what is observed in iodine deficiency. Because the only known function for iodine in humans relates to thyroid hormone synthesis, other symptoms for excessive iodine consumption have not been described.

When iodine status is at an adequate level, it can exhibit beneficial antioxidative and anti-inflammatory effects that can prevent or treat many common ailments. Benefits have been seen in human clinical trials with benign prostatic hyperplasia and benign breast disease. Iodine is an essential component in the human diet, and it is vital to balance food and supplement choices to allow iodine, and other beneficial trace elements, for proper physiological function.

Manganese

Manganese is a constituent of three metalloenzymes, including the principal antioxidative enzyme in the mitochondria, manganese superoxide dismutase, and is known to activate a host of other enzymes. Therefore, though frequently considered an ultratrace element, it is a required mineral of high importance to human health.

Sources and Production

Manganese is widely distributed throughout the Earth's crust and in nodules across the seafloor and thus a relatively abundant metal. It has many important industrial metal alloy uses, especially in steel production, because it improves strength, hardenability, and abrasion resistance. For many years, China has been the world's largest manganese alloy-producing country. Manganese ions are used industrially as pigments and as oxidation chemicals.

Patterns of Consumption/Human Exposure

In contrast to manganese-poor meat and dairy products, plant-based foods, including legumes, dried fruits, nuts (1.8–6.5 mg/100 g), and also tea, are rich sources of manganese. Some fruits, such as pineapple, appear to be a particularly good source of manganese (65 mg/100 g). The manganese content of whole grain products varies, as it largely depends on the plant species and the milling process used. Depending on the

diet, adult intake ranges from 2 to 9 mg day^{-1}, with vegetarians having a higher intake. The daily recommended AI for manganese is 2.3 mg for adult men and 1.8 mg for adult women, as, inexplicably, women may be able to absorb greater amounts than men.

Availability, Absorption, and Metabolism

Manganese is thought to be absorbed in its ion form, Mn^{2+}, throughout the small intestine, likely involving apical transport proteins, such as the divalent mineral transporter. Absorption of this mineral is typically $<5\%$, and homeostatic regulatory control mechanisms keep manganese levels in the desired range. Thus, the efficiency of manganese absorption appears to be inversely correlated with dietary intake. However, relatively little is known regarding factors affecting manganese absorption. Upon entering the circulation, manganese is rapidly removed from the blood and transported into cells. The half-life of manganese in blood appears to be about 10–42 days, and it is subsequently excreted, primarily through the bile in feces.

Health Effects/Physiology

Albeit having been described for various animal species, a diet-dependent manganese deficiency is unlikely in humans. Deliberate elimination of manganese from the diet over the course of several weeks has been reported to result in decreased serum manganese levels and occurrence of symptoms such as nausea, dermatitis, and altered metabolism. The UL for manganese has been set at 11 mg day^{-1} for adults. Manganese toxicity due to organ accumulation has been described in individuals with liver failure. Neonates that may be exposed to high levels of manganese through parenteral nutrition also may possibly show signs of manganese toxicity. Furthermore, increased chronic exposure to airborne manganese dust, an environmental or occupational inhalation health hazard in welders and smelters, may cause symptoms such as coughing, reduced lung function, and multiple neurological problems, because inhaled manganese appears to be transported directly to the brain.

Molybdenum

The trace mineral molybdenum is widespread in the human diet and depends on the molybdenum content of soils, which varies geographically. This element has strong redox properties and plays an important role as cofactor for four metalloenzymes.

Sources and Production

Molybdenum is mined and processed worldwide, with major operations in China, the United States, and Chile. Even though molybdenum is contained in various minerals, only molybdenite appears to be suitable for the industrial production of marketable molybdenum products, primarily for metallurgical applications including high-strength alloys. Other commercial applications include its use in fertilizer and in the semiconductor industries. Excessive, long-term exposure to soluble molybdenum compounds can potentially be found in industrial settings, where molybdenum dusts and fumes can be generated by mining or metalworking. Even though this type of exposure can be toxic, it is also relatively rare.

Patterns of Consumption/Human Exposure

Dietary intakes of molybdenum have been reported to vary widely, about 40–460 μg day^{-1}. Legumes (up to 184 μg/100 g) and grains grown in molybdenum-rich soils are good sources of dietary molybdenum. Nuts and vegetables other than legumes usually contain fewer than 50 μg/100 g, whereas meats (beef, pork, poultry, and fish) may provide up to 130 μg/100 g. The mean dietary intake of molybdenum has been estimated to range from 76 to 109 μg day^{-1} in women and men. Molybdenum intake is increased in those consuming nutritional supplements that frequently contain sodium molybdate or ammonium molybdate.

Availability, Absorption, and Metabolism

Molybdenum, usually in the form of the dietary molybdate, is readily absorbed in the small intestine, most of which appears to occur through passive diffusion. Absorption of molybdenum ranges from 50% from foods to over 90% when ingested as a dietary supplement in the form of ammonium molybdate. Ingestion of large quantities of sulfates may inhibit molybdenum absorption. Retention of dietary molybdenum is well regulated, and most of the excess molybdenum is excreted in the urine.

Health Effects/Physiology

In terms of the biological role, molybdenum is frequently found as a metal heteroatom at the active site of at least four known enzymes, which are important oxidoreductases. Even though severe molybdenum deficiency may result in amino acid intolerance, disorientation, and eventually coma, this appears to have only been encountered in a single reported patient maintained on total intravenous nutrition for 18 months. Dietary molybdenum deficiency in healthy populations has not been observed thus far, though very rare inborn metabolic disorders affecting the molybdenum cofactor affect the activity of molybdenum-containing metalloenzymes. Much like dietary molybdenum deficiency, toxicity due to excess molybdenum intake is rare. Accordingly, the UL for molybdenum is set at 2 mg. Of greater concern is the potential interference of high levels of molybdenum with the absorption of copper, potentially resulting in a copper deficiency.

Selenium

Selenium is a mineral that occurs in inorganic (elemental selenium, selenide, selenate, and selenite) and organic (including methylated selenium compounds and selenoamino acids) chemical species. It is toxic in large amounts but essential in the diet at trace levels. Most of its effects in human health are due to its incorporation through selenocysteine, the 21st amino acid, into selenium-containing proteins (selenoproteins).

Sources and Production

Selenium is fairly ubiquitous in the environment, though the amounts in which it is found vary geographically. Except in those areas where the burning of fossil fuels and industrial waste from steel plants contributes significantly, the contribution of aerosolized selenium is considered to be insignificant to human health. The primary bioavailable forms of selenium in water are inorganic selenate and selenite ions, and federal standards allow up to 50 $\mu g\,l^{-1}$ in drinking water, as regulated by the US Environmental Protection Agency (EPA) under the Safe Drinking Water Act. At typical levels, the selenium content in drinking water contributes only slightly to the daily intake, except in instances where selenium levels are elevated, as in areas of the Midwestern United States. In that part of the country, locations are known for their infamously high soil selenium content, and values from 50 to 330 $\mu g\,l^{-1}$ in wells have been reported. In the terrestrial environment, the weathering of phosphatic rocks, coal, and other organic-rich deposits is a major source of selenium, and seleniferous soils are widespread in parts of China, Russia and in parts of North and South America. However, selenium-poor soils are also found within the same regions, indicating strong geographic variation. Overall, the contribution of exposure to inorganic forms in water and soil to human selenium status is generally very minor. More important for the health of the general population are dietary exposures to mainly organic selenium forms in plants and animals. The largest use of selenium, and thus exposure for humans, is pharmaceutical and agricultural supplement use for human and farm animal diets. The addition of sodium selenite to fertilizer and/or animal feed for cattle, hogs, and chickens in areas where the soil is selenium-poor, including areas such as southwestern Oregon, is a common practice.

Patterns of Consumption/Human Exposure

Based on extrapolations from animal studies and repletion studies in selenium-deficient regions of China, the DRI for selenium in the United States was established in 1989, with an RDA of 70 μg for men and 55 μg for women, and then further adjusted in 2000 to 55 $\mu g\,day^{-1}$ for both men and women (Table 1). This amount, which is slightly lower than allowances for many European countries, but substantially higher than the recommendation in countries such as Japan, was found to achieve maximal activity of plasma glutathione peroxidase; at the same time, it is well below the adult UL of 200 μg. It should be noted that the third National Health and Nutrition Examination Survey (NHANES III), which assessed plasma selenium in over 17 000 participants, provided very compelling evidence that over 99% of the participants were selenium-replete and that routine supplementation with selenium does not appear warranted in the United States to meet current RDA requirements.

Our primary source of selenium intake is through food. Animal and human studies have shown that the bioavailability of selenium compounds varies depending on the source and form of selenium. In the United States, wheat products are highly popular and thus are one of the primary sources of dietary selenomethionine, the major available form of selenium found in grains. On average, wheat-based breakfast cereals contain below 3 μg/100 g (e.g., cooked cream of wheat) to over 100 μg/100 g (e.g., puffed wheat). However, highly variable selenium levels in about 50% of all food items exist, and the total selenium content in wheat can vary as much as 500-fold due to geographic differences and fertilization techniques. Fruits and vegetables typically contain only small amounts of selenium, unless grown under selenium-enriched conditions, which can increase their selenium content greater than 50-fold. Other plants that can accumulate selenium include various species of algae, multiple *Brassica* species, garlic, and, notably, Brazil nuts, which have a reported average selenium content of 1500–1900 μg/100 g. Fungi, such as mushrooms and yeast, are also capable of accumulating substantial amounts of organic and inorganic forms of selenium. In the United States, meat, especially organ meats, and fish are other important sources of dietary selenium. Domestic beef, chicken, lamb, and pork have an average selenium content of 10–40 μg selenium/100 g tissue, whereas dairy products contribute 3–15 μg/100 g. The selenium content in fish ranges from about 12 μg/100 g (freshwater catfish) to over 70 μg/100 g (canned tuna). The content of selenium in commercially available dietary supplements (including multimineral supplements) may include any of the organic and inorganic forms of selenium or selenium-enriched yeasts. The supplemented daily dose varies dramatically, and may be 10–200 μg, at the upper end reaching nearly four times the RDA for adults and 50% of the UL.

Availability, Absorption, and Metabolism

Absorption of the various chemical forms of dietary selenium is efficient and occurs primarily in the small intestine. Initial absorption is estimated to be around 80% but depends on the presence or absence of other trace elements and proteins. The transport mechanism of dietary selenium across the intestinal epithelium remains to be elucidated. For extracellular transport, two selenoproteins, selenoprotein P and glutathione peroxidase 3, are known to distribute selenium through the plasma. Subsequent utilization and metabolism of organic and inorganic selenocompounds, and therefore bioavailability and retention of absorbed selenium, appear to depend on the selenium species profile in the diet. The overall bioavailability has been reported to be around 14% of the original selenium content in the food, and 2–20 mg selenium can be retained in an adult human body. Metabolism of dietary selenium depends on the form ingested. Selenium levels are detectable not only in the red blood cells, liver, spleen, and heart but also in nails and hair. Excretion of selenium can occur via the kidneys in urine, the gastrointestinal tract in feces, and the lungs through expired air.

The basic human selenium requirements needed for survival and the amounts sufficient to maintain near-optimal functions of the molecular mechanisms in which selenium is involved are in a delicate balance. There appears to be a U-shaped profile for predicting the risk–benefit consequences of supplementation with dietary selenium, especially considering the differential responses to selenium supplementation due to human gene polymorphisms in selenoprotein genes. Thus, the established level of suggested selenium intake for the general population is still highly controversial. Like with many other

dietary micronutrients, the individual biological response may depend on factors, such as gender, ethnicity, selenium status, and background health.

Health Effects/Physiology

Selenium plays an important role in human development and boosting the immune function. At least two diseases have been linked with severe selenium deficiency, which are generally restricted to children of selenium-poor geographic areas in China, Central Africa, and southeast Siberia, requiring prophylactic oral selenium administration. Keshan disease, which results in congestive cardiomyopathy (heart failure and pulmonary edema), and Kashin–Beck disease, which results in osteoarthropathy and joint necrosis, both respond to dietary selenium supplementation. Myxedematous endemic cretinism, which results in mental retardation, appears to be associated with a combined iodine and selenium deficiency.

Selenium toxicity, or selenosis, is defined by blood selenium levels $>100\ \mu g\ dl^{-1}$ and is rare outside accidental industrial exposures or manufacturing errors of dietary supplements. Selenosis can result in gastrointestinal upsets, hair loss, white blotchy nails, garlic breath odor, fatigue, irritability, and mild nerve damage.

Rather than small-molecular-weight selenocompounds, more attention in recent years has been given to selenoproteins being the primary responsible agents in the health benefits of selenium. The one health benefit that has received the most attention is selenium's role as a cancer-preventive agent, primarily mediated through the action of specific selenoproteins (e.g., thioredoxin reductase 1). However, the evidence is very inconsistent, with at least one human clinical cancer prevention trial using selenium supplementation having shown a decreased incidence in prostate, colon, and lung cancers, whereas other trials, including the recent study SELECT in a selenium-replete population, found no positive health benefits. Considering the negative outcomes of this last major clinical trial, and the recent evidence that certain selenoproteins may not only prevent but also promote cancer, the American Institute for Cancer Research and the National Institutes of Health do not recommend selenium supplementation in cancer prevention.

Zinc

Zinc is an essential dietary trace element found in many natural and synthetic sources and is a constituent in over 150 essential mammalian enzymes. Many of these enzymes are involved in major metabolic processes, including DNA and insulin syntheses.

Sources and Production

Although zinc is ubiquitous in our environment, the levels of zinc vary geographically with soil type and are also influenced by proximity to industrial-based institutions that increase the metal content of soils. Plants and microorganisms take up the zinc that presents in water-soluble forms, and it thus enters our food supply. Commercially, zinc is obtained through zinc ore mining and hydrometallurgical or pyrometallurgical extraction from zinc blende. Because of the industrial processes involved, in some areas of the world, current production and/or previously established zinc dumps increase the environmental concentration of zinc in soil and thus plants.

Patterns of Consumption/Human Exposure

Seafood, red meats, and poultry are major sources of dietary zinc with examples such as cooked oysters (89 mg/100 g), beef (7.9 mg/100 g), and Alaskan king crabs (7.5 mg/100 g). Chicken, pork, or meats with high fat content have moderate zinc contents (between 1 and 2.5 mg/100 g). Other adequate sources of dietary zinc are beans, nuts, dairy products, and fortified breakfast cereals, whereas green leafy vegetables and fruits are only modest sources of zinc (<1 mg/100 g). Dietary supplements are another main source of zinc, mainly in the forms of zinc gluconate, zinc acetate, or zinc sulfate, only 23% of which is elemental zinc. Zinc lozenges and denture creams are also sources contributing to zinc status. The median zinc intake values in adult males and females are only 14 mg day^{-1} and 9 mg day^{-1}, respectively. Accordingly, clinical studies have showed that between 35% and 45% of adults over the age of 60 years fail to meet the RDA (Table 1). In contrast, excessive intake of zinc can also be detrimental. Above 150–450 mg day^{-1}, chronic effects such as immune function reduction, low levels of high-density lipoproteins, and impaired iron and copper statuses occur.

Availability, Absorption, and Metabolism

Although zinc is found in many parts of a standard diet, the zinc bioavailability from these dietary sources is not equal. Zinc is more bioavailable from animal-based foods than plant- or grain-based products, exacerbated by the fact that phytates, which are found in whole grains and legumes, bind to zinc and inhibit its absorption from dietary sources. Individuals sustaining a vegetarian diet may require up to 50% more dietary zinc than nonvegetarians due to the large amount of dietary phytates consumed.

The cellular transport of zinc is predominantly accomplished through two major solute-linked carrier gene families, the zinc transporter families, which promote cellular zinc efflux, and ZIP, which supply zinc to metalloproteins. Zinc is distributed in many tissues throughout the body, and only 10–20% of total zinc is found within the blood plasma. Upon consumption, zinc is absorbed primarily in the duodenum for later transport to the liver for binding to albumin. Extraneous zinc is secreted by the kidneys, pores in the skin, or male ejaculate fluid.

Health Effects/Physiology

Zinc deficiency can be a serious health concern. However, assessing this deficiency is not easily achieved. Plasma zinc concentration is a common method to assess zinc status; however, this is susceptible to diurnal variability. Minor symptoms of zinc deficiency are nonspecific and include loss of appetite, impaired immune function, and growth retardation. More severe symptoms include hair loss, diarrhea, impotence,

delayed sexual maturity, skin or eye lesions, impaired wound healing, hypogonadism, and lethargy. Individuals at higher risk for zinc deficiency are those whose physiological well-being is already compromised, such as the elderly, those suffering from gastrointestinal diseases, pregnant or lactating women, sickle-cell anemia patients, and alcoholics. Instances of low zinc levels in human milk impairing infant zinc status have been reported.

Zinc toxicity can present different pathologies depending upon the form of zinc consumed. Acute and chronic high intake of salts, such as zinc sulfate, can induce gastrointestinal symptoms such as pain, cramping, diarrhea, nausea, and vomiting. Elemental metallic zinc, however, can induce quite different symptoms such as light-headedness, lethargy, and other neurologically based difficulties.

Zinc has been implicated in the successful therapeutic treatment for multiple common ailments, including severe diarrhea in children, which can cause damage to the intestinal mucosa and affect the absorption of other dietary nutrients. The World Health Organization has suggested 10 mg day^{-1} zinc supplementation for children under 6 months of age upon onset of diarrhea or 20 mg zinc for children above 6 months. China has implemented such a regimen with an ~26% reduction in risk of severe diarrhea in children under 5 years of age.

There has been some evidence in the utilization of zinc supplementation in the treatment of common cold symptoms. Virus-induced common cold cases are commonplace worldwide, and these viral infections induce oxidative stress in the body. This stress elicits the activation of immune cells, such as macrophages, and inflammation. Zinc supplement lozenges, when taken within 24 h of symptom onset, may decrease the severity and length of a viral common cold infection; the suggested mechanisms are zinc's antioxidant properties and the ability to block viral binding sites to host cells. Zinc deficiency also hampers the development of T cells in the body, therefore making a response to infectious disease less effective. Another disease influenced by zinc is age-related macular degeneration. This condition can be worsened by inflammatory damage and oxidative stress, both of which can be alleviated by zinc and other antioxidative nutrients. Various skin conditions, such as dandruff, rosacea, acne, eczema, and warts, have also been treated successfully with zinc-based products.

Although zinc has many physiological benefits, deleterious interactions between dietary zinc and several common prescription medications occur. Antibiotics, such as tetracyclines and quinolones, may interact with zinc and cause diminished activity of the drugs and reduce zinc availability, itself. Similarly, thiazide diuretics can increase the urinary excretion of zinc leading to a zinc deficiency.

See also: Arsenic: Properties and Determination; Arsenic: Toxicology and Health Effects; Boron; Cadmium: Properties and Determination; Cadmium: Toxicology; Calcium: Physiology; Calcium: Properties and Determination; Chromium: Physiology; Chromium: Properties and Determination; Iron: Biosynthesis and Significance of Heme; Iron: Physiology of Iron; Iron: Properties and Determination; Magnesium; Manganese.

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Relevant Websites

www.iom.edu – Institute of Medicine of the National Academies: provides dietary reference intakes.

http://www.nal.usda.gov/ – USDA search tool (based on Release 23 of the USDA National Nutrient Database for Standard Reference): allows calculation of amounts of dietary micronutrients.

http://ods.od.nih.gov – Office of Dietary Supplements (National Institutes of Health): Fact Sheet for Health Professionals.

Traditional Foods

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Introduction

The semantic meaning of 'traditional food' should be clear: a food in which broad-sense knowledge (ingredients, way of preparation, role, etc.) is transmitted from generation to generation. Although not encompassing explicit reference to the way the knowledge is transmitted, common interpretations of this definition are the following:

- 'Traditional' knowledge is the complex of information and skills belonging to communities, as a result of the interaction with the environment in which they live and the available resources.
- The transmission of 'traditional' knowledge generally occurs through informal means, often orally.

Recent debates indicate that people have no difficulty to independently interpret tradition, in connection to food, attaching personal facets to this term, such as old-fashioned, consumed often in daily life, linked to special events, folkloric, belonging to a specific place, homemade, not elaborated, tasty, and natural.

For sure, 'traditional' is not a technical term and does not indicate precisely defined attributes; it is rather routinely used as a broadly agreed concept, with a not surprising share of subjective interpretation. The term 'traditional food' encompasses a series of different, overlapping, sometimes contrasting attributes, with relation to the sociocultural and economic context.

Nevertheless, reaching a so-called objective definition of traditional foods has been central in contemporary discussions, even since traditional foods recently attracted the interest of both industry and consumers, for different and, also in this case, partially contrasting reasons.

These aspects will be shortly treated in this presentation.

What 'Traditional' Foods Really Are

The Intimate Nature of 'Traditional' Foods

The intimate nature of 'traditional' foods cannot be disjointed from the context in which they originate.

The two attributes of 'being known in the local expertise' and 'transmitted through generations' are intrinsic in the term 'traditional.' This situation is typical of the so-called indigenous communities, in which individual foods do not represent merely a recipe, but the final step of food systems, a complex of knowledge and skills through which the available resources are produced or collected and processed into foods. Besides an intrinsic variability, due to geographic and seasonal constraints, some other traits characterize true-sense 'traditional' food systems of local communities:

- Raw materials and ingredients are mostly supplied on-place, from either farming or wild harvesting or a mix of the two. Of course, even in the more remote societies, external inputs to food systems have always occurred (e.g., sugar, salt, spices, and other ingredients exchanged), not affecting, however, the main pattern of supply.
- Food preparation is mostly done in-house, with intrinsic variability due to personal interpretations, likes, and supply constraints.
- In these situations, the knowledge for raw materials production, food preparation, and storage is embedded in local culture.
- Food value is directly perceived by individuals, without the need for third-party mediation.

As a result, in such societies, the concept of a 'traditional food' is substantially ill-defined, since tradition and customs largely coincide. Raw material producers, food processors, and consumers very often coincide in the same subjects. 'Traditional foods' are substantially everyday's food that can be accessed locally.

Contemporary Facts About Traditional Food Nature

Contemporary urban societies are characterized by a complex network of social relations, by the specialization of roles and lifestyles, simply making not possible the adoption of food habits similar to local rural communities:

- When food is prepared at home, the ingredients are almost entirely purchased.
- Food preparation very often is not done in-house: a relevant share of prepared foods is bought from the market.
- The knowledge and skills (and possibility) for raw materials production are almost completely lost; a similar trend is sometimes occurring for food preparation skills.
- The perception of food values increasingly occurs through the communication from third parties.

Very rarely, consumers are also producers of the food they eat. 'Tradition' is not a part of everyday's ways of life and therefore also tends to become a communicable credence character. Food is mainly 'market food' and traditional foods tend to become a market category. Recent opinions convincingly stated that 'tradition' is therefore a product of modernity, rather than an intrinsic attribute of localized food systems.

The current debate for a 'traditional food' definition developed in this context, with the aim of fixing the characterizing traits of this category.

The Trend Toward Traditional Food Definitions

Formal Definitions

The effort to attain a 'traditional food' definition is mainly, and rather typically, west European, for the following reasons:

- In west Europe, a prevailing centralized regulatory approach in setting quality standards, including foods, is adopted.

- West Europeans largely attained a quantitatively satisfactory food intake and the population growth rate is low. Food consumption is therefore not expected to significantly increase in the medium term. In this situation, food processors try to find occasions of expansion with product differentiation. Traditional foods became a tassel of this strategy, generally positively perceived by consumers, but needing some kind of categorization in order to set proprietary standards.

A road toward definitions already started during the setting up of procedures for denominations of origin legislation. Recently, two EU-funded network projects independently attained a definition, adopting different approaches.

The EuroFIR definition is based on a critical re-elaboration of previous approaches, definitions, and regulations, with special focus on the need to (a) agree upon a definition to be used in the research environment and (b) attain a 'standardization of traditional food concept,' in view of their registration, within the EU regulatory systems.

The EuroFIR definition is articulated and deals with three levels of the food production chain:

- Traditional ingredient (raw material or primary product). "Raw material (species and/or varieties) or primary product either alone or as an ingredient, that has been used in identifiable geographical areas and remains in use today (taking into account cases where use was abandoned for a time and then reinstated) and its characteristics are in accordance with current specifications of national and EU legislation."
- Traditional composition. "The uniquely identifiable composition (in terms of ingredients) that was first established prior to the Second World War and passed down through generations by oral or other means (taking into account cases where composition was abandoned for a time and then reinstated) and when necessary is differentiated from the composition defined by the generally recognized characteristics of the wider food group to which the product belongs."
- Traditional type of production and/or processing. "The production and/or processing of a food that: (a) has been transmitted from generation to generation through oral tradition or other means, and (b) has been applied prior to the Second World War and remains in use (taking into account cases where application was abandoned for a time and then reinstated) despite its adjustment to binding rules from national or EU food hygiene regulations or the incorporation of technological progress, under the condition that production and/or processing remains in line with methods used originally and that the food's intrinsic features such as its physical, chemical, microbiological, or organoleptic features are maintained."

The following points of this detailed definition may be the subject of further discussion:

- Setting a time reference to World War II as a divide between traditional and industrial ways of food preparation may fit to some urban areas of Europe, but cannot be generalized, since it tends to exclude all the still alive knowledge in rural areas, even in Europe, and in most parts of the so-called developing world. With respect to time reference, other definitions set different, more flexible time limits, such as 25 or 50 years or 'one generation.'
- The idea of fixing standards may fit to a regulatory or proprietary claim context but is in contrast with what happens in local societies, where recipes are continuously fitted to the available resources and constraints and to the availability of new ingredients and tools.

The EuroFIR definition was therefore substantially mainly aimed at the use in a scientific and regulatory context.

The TRUEFOOD definition was generated by quantitative surveys aimed at investigating consumers' perceptions of the traditional food concept, with special respect to the relations to technological innovation and the potential for industrial exploitation. The definition is

- A product frequently consumed or associated with specific celebrations and/or seasons, normally transmitted from one generation to another, made accurately in a specific way according to the gastronomic heritage, with little or no processing/manipulation, distinguished and known because of its sensory properties and associated with a certain local area, region, or country.

It is clear that, together with some consistent statements, as the association with a certain region, or the reference to reduced manipulations, this definition contains a combination of the different facets that consumers perceive. As an example, the two aspects of being 'frequently consumed' or 'associated to specific events' represent two rather opposite aspects of tradition: the first being part of everyday's life and direct perception and the latter being communicated through religious or other kinds of beliefs.

The Categories in the European Regulatory Context

The steps for food registration at EU level are the following:

- Setting up a pool of promoters (generally associated growers or processors)
- Defining a disciplinary of production, containing all the necessary details
- Submitting the application to the EU, through the competent national authorities and having it approved
- Committing the control and certification to an authorized external body
- Finally, bringing the system to a steady state of functioning

The use of the registered food name, labels, and deposited logo is reserved to the consortium associates who, in turn, must comply with the agreed and certified rules.

Apart from wines, for which specific designations are in force, the European categories of specific foodstuff registration (and protection) are the following:

PDO and PGI: Council Regulation (EC) No. 510/2006 of 20 March 2006

TSG: Council Regulation (EC) No. 509/2006 of 20 March 2006

- Protected designation of origin (PDO). "Designation of origin is linked to the name of a region, a specific place or, in exceptional cases, a country, used to describe an agricultural product or a foodstuff:

  - originating in that region, specific place or country, and
  - the quality or characteristics of which are essentially or exclusively due to a particular geographical environment with its inherent natural and human factors, and
  - the production, processing and preparation of which take place in the defined geographical area."
- Protected geographical indication (PGI). "Geographical Indication is linked to the name of a region, a specific place or, in exceptional cases, a country, used to describe an agricultural product or a foodstuff:
  - originating in that region, specific place or country, and
  - which possesses a specific quality, reputation or other characteristics attributable to that geographical origin, and
  - the production and/or processing and/or preparation of which take place in the defined geographical area."
- Traditional specialty guaranteed (TSG). "Traditional specialty guaranteed designation does not refer to an origin, but highlights the traditional composition or means of production of the product."

Not all the presently registered products belong to traditional foodstuffs (e.g., mineral waters are clearly a different case), but the registration of most of them started from an approach aimed at promoting the values connected to the native local food chain systems.

It is rather evident that a strong relation between the need for standardization and description, on one side, and registration, on the other, does exist. It is also clear that all the effort of registration is addressed at commercial production of the registered foodstuffs, but it often has only a vague connection to the 'protection' of traditional foods in their original contexts and little to do with the food values as locally perceived, which do not need third-party mediation.

Together with official EU categories, many others do exist at national or local levels, in different countries. An interesting example is represented by the Slow Food approach. Although being a private label and a successful market operation, this system embeds the concepts of food as a component of social networks and local production systems.

Some Characteristics of Traditional Foods in On-Site Food Systems

A relatively high amount of research in local tribal communities contributed to a clear vision of their food systems. Recently, investigations have also been carried out to document local food systems in industrialized countries, revealing typical and lesser known aspects, somewhat in line with the food concepts of indigenous communities and contrasting with the contemporary traditional food stereotype. Some traits are hereafter illustrated, with special reference to rural south and east Europe, in which the dietary basis is largely represented by starchy materials.

Food Strategies

Although largely modified by present-day styles of life, the basic strategy of traditional food supply in local contexts is still well present, not only in the memory of the elderly.

The prevailing strategy was that of one-course meal, generally from the combination of an energetic staple (cereal or potatoes) with other ingredients of various nature. The concept of multiple courses within a meal was almost absent, particularly with respect to that of a 'dessert.'

Cereals were primarily used as flour or grits to prepare gruels or porridges, easier and faster to prepare than products requiring the kneading of dough. Porridges can easily be combined with an array of ingredients, allowing the more effective exploitation of what is available: animal fats or meat, eggs, dairy products, vegetables, or all kinds of fruits. Among foods from dough, the unleavened types are more typical of precarious rural situations.

The fats used were those locally available, of either plant or animal origin.

Typical foodstuffs were generated by the need of preservation of perishable materials, from classical drying of meat, to drying or reducing water content of fruits and vegetables, to fermentation. All these preservation methods gave rise to some presently renowned 'typical products' and to many others, still well rooted in local or regional food systems, although not yet well known by the public.

Variability

Variability of composition is a consistent trait of local foods, mainly depending on

- the need of preparing a palatable and nutritional meal using the available ingredients, subject to seasonal and other supply constraints;
- the personal interpretation and likes of each household manager;
- the need of using the more expensive materials, like wheat, as cash crops, replacing them in domestic life with other materials, like corn and potatoes; this motivation does not hold anymore at present times, but generated a series of foods that are still known nowadays.

Because of these reasons, an almost continuous variation of both the kind and amounts of some food ingredients was in many cases generated. Perfect examples are again represented by cereal gruels with greens, in which composition may include any available green, either wild or cultivated, and in which texture may range from a thick porridge to a soup, according to the ratio between the cereal components, water, and the other ingredients. Similar situations do exist for filled pastries or other baked products. Among the latter, another illuminating example is the 'bustrengo,' a typical product of the Romagna and Marche regions (Italy) that is made of a cereal component and dried fruits; originally, it was prepared from leftover bread basis, with the addition of corn flour, mixed with the available dried fruits (apples, pears, figs, cornelian cherries, etc.); it was used as a main course, not as a dessert. During subsequent time and space steps, the cereal basis changed to rice or wheat flour; chocolate or coffee were added, as well as candied fruits and other ingredients. As a result, nowadays, asking for the 'true bustrengo recipe' is simply a nonsense. And this fact would certainly create relevant difficulties and debate in the case that whatever of the currently

prepared samples should be chosen as a reference specimen for a registration proposal as the 'true bustrengo.'

The final message is that the definition of a standard recipe, representing the reference specimen, seems to be more a matter of choice, convenience, and preference, often a mere artifact, than a real possibility for most traditional foods.

Convergence of Ingredients and Preparation

In many cases, similar ingredient utilization strategies, available raw materials, and local conditions determined the occurrence of striking cases of morphological and compositional convergence, also in remote and not communicating regions. The examples are innumerable. The cereal flour and condensed fruit syrup (mainly grape) puddings called 'sughi' in Italy and 'pelamushi' in Georgia are exactly the same. The range of variation of half-moon-shaped pastries filled with seasoned greens is perhaps higher within-type than between an average Italian (Romagna region) 'crescione' and Armenian (Nagorno-Karabakh Republic) 'zhynghial.'

Almost all these convergent foods are, however, perceived as very typical in their origin areas.

Traditional Food Evolutions and Trends

Ingredient Migration, Adoption, and Substitution

Native crops and animals have been always migrating with people. New crops and animals have always been exploited by explorers and colonizers. As a consequence, a progressive trend to the inclusion of new ingredients in local food systems occurred. Migrations are either historical or contemporary, the only difference being in the perception of the moment in which a new ingredient became 'traditional.' The examples are, again, innumerable, the better known of which are perhaps represented by the diffusion of American crops, like tomatoes, potatoes, corn, and chili pepper, to Europe first and worldwide later. And nobody would doubt to consider tomato as a characterizing ingredient of some old world traditional foods, like the Italian pizza or sweet pepper as a staple for Balkan sauces like the 'lyutenitza' or 'ajvar.'

After its introduction to Europe, corn substituted a range of previously used cereals in gruel preparation, because of yield advantages and socioeconomic reasons. Corn was also used to replace the more priced wheat flour in baked foods, losing popularity again when average economic conditions improved but, at the same time, giving rise to a series of products that are nowadays considered as 'traditional.'

Overall, new crops and animals are regularly adopted in local systems, when they do represent an advantage, whatever its nature, provided it is easily and directly perceived in the local economic culture.

Availability of New Tools and Technologies

New 'soft' technologies, compatible with the local skills, enterprise size, and financial resources, in the case of commercial productions, are readily adopted in local food systems.

Sometimes, the availability of new technologies determined substantial shifts in the kind of products and also in the overall resource exploitation strategies. As an example, the availability of refrigeration and freezing technologies tended to bring out of use the practice of fruit and, in some cases, herb drying, although to variable extent in different geographic areas.

In many cases, the availability of new technologies allowed the manufacturing of 'new' foods from native ingredients, as it is the case of the many kinds of puffed grain cakes from a range of local cereals. It is interesting to note that these new products are still perceived as connected to local dimension, provided the main ingredient is clearly individuated as local.

Standardization

As already stressed, variation and diversity are intrinsic and functional attributes of local food systems. However, in a world increasingly characterized by intensive flows of materials, information, and finances, purely local, closed systems are quite rare. A move toward standardization of ingredients and procedures can be connected to (a) convenience of the players in the food chain; (b) safety, hygiene rules, and private standards; and (c) origin or other certification schemes (e.g., organic).

Standardization by convenience of stakeholders

Raw materials. High raw material variability is acceptable for home productions, in which most operations are done manually, or when it does not substantially hamper cultural practices. When production is scaled up to commercial level, even at small scale, a degree for raw material standardization allows attaining consistent flow charts in time and buffering lags in the production flow. The standardization of raw materials may be attained either by means of selection within local plant and animal populations, when feasible and economically sustainable, or by substituting traditional ingredients with more easily available ones (e.g., wild greens with spinach), or by purchasing ingredients on the market.

Processes and products. Despite variability being a characterizing trait of traditional foods, also small-scale processors feel the need of a degree of recipe standardization in order to

- calibrate flow charts and keep them as constants as possible;
- offer a constant product in time to customers;
- be able to attach consistent nutritional information;
- differentiate from competitors.

This latter point is very important: although within-firm standardization is often sought for, the homogenization with potential competitors is mostly avoided. Local-scale processors produce their own variants, with small, but sometimes significant differences in ingredient types and balance and overall sensorial characteristics. With this respect, however, it has been pointed out that a weakness of local food manufacturers is their failure to offer a sufficient amount of product with constant characteristics, to be permanently known and available on the market.

Rules (hygiene, safety, and private standards)

Rather strict national or supernational food safety rules are applied in the Western world, with special respect to Europe, often requiring relevant financial exposure to comply with, often representing an objective barrier for small-scale processors.

In addition, private standards proliferate in Western food distribution systems. It has also been pointed out that private standards may impact small growers and processors, from limited and often contingent integration in global systems to exclusion.

Registration and certification

As explained, designations of origin and other certification forms, like TSG, are a typical European tool of traditional food promotion. In official documents, it is stated that registration can help the promotion of rural economies of the more remote and less favored areas.

Until now, the registered products belong to quite different categories, characterized by extremely different production size: from the worldwide renowned Parmigiano–Reggiano cheese to very small and local products, hardly known outside their native areas.

Some implicit facts of the registration scheme are conflicting with the real nature of traditional food:

- Registration needs the codification of standard procedures, which are hardly applied in traditional food systems management.
- Registration implies the certification of procedures by a third-party body; this is contrast with the fact that in local contexts, knowledge belongs to the community and is internally shared.
- Registration implies that the communication to consumers is also mediated by third parties. This may be unavoidable in present urban societies, characterized by a high level of specialization and low awareness of food origin, but is anyway contrasting with the fact that in local systems, pure consumers, intended as mere buyers of food manufactured by third parties, do not exist, since most people maintain high capability of producing food, and the share of food produced and manufactured at individual or community level is generally high.

While registration can be an effective tool for commercial protection of the better-known registered foods, there is little evidence that this had significant effects for the promotion of very local minor foodstuffs. In general, it seems that the chances of success for a registered product are higher if registration is pursued as a means for the consolidation of already existing agreements among producers and production and processing schemes and if the definition of the area and procedures is as inclusive as possible.

Among the facts connected to registration, the appropriation of a collective, locally consolidated food name by only the registered producers is often perceived as little acceptable.

Finally, there is often a basic skepticism about the efficacy of a centralized approach for the characterization of local products. Small-scale growers and processors are often seeking for different, more flexible, trust-based, self-certified ways of 'branding from below' that, however, lack a clear legislative background.

Trend to Work in Chain

Raw materials processed in local food systems almost never are market-purchased commodities. Small-scale, market-oriented enterprises tend to promote the use of local raw materials, although suppliers and processors do not necessarily coincide in the same persons. Moreover, local raw materials themselves (the so-called typical crops) often represent the starting point for the establishment of small-scale food systems.

The initiative for traditional food production can be generated by growers who also become processors or, in other cases, from processors who establish raw material supply agreements with individual or associated growers. In all cases, the establishment of local production chains may generate overall benefits for the economy of the location. All these examples do not correspond to the concept of a closed, traditional market system, but rather represent the answer of local stakeholders to the challenges of revalorization of small-scale, differentiated agro-food productions that recover knowledge of places, in a well-defined niche or regional-scale market perspective.

Relations of Traditional Foods to Other Specific Categories

- *Local foods*. Localized productions can coincide or not with a traditional food concept. Cases of present-day supply chains, like on-farm marketing, are often based on non-traditional products and ways of production.
- *Street foods*. Street foods are defined as foods sold on the street and for which some steps of final preparation are also carried out on the street. At almost world level, a relevant share of street foods is generated from some kind of traditional foods.
- *Ethnic foods*. Ethnic foods are typical of well-defined communities. Most ethnic foods in reality are traditional foods, outside their original contexts. Therefore, they substantially belong to the traditional food panorama, although lacking the requisite of being processed within their native systems.
- *Fusion foods*. Fusion foods are foods mixing the experiences of different cultures, in terms of ingredients or ways of preparation. Although intrinsically variable, fusion foods often retain multiple links to the concepts of ethnicity and tradition. Some fusion foods, well before the term was invented, became traditional: an example is represented of corn gruels ('polenta') in Italy, generated by the combination of the pre-Roman concept of a cereal gruel with the availability of corn, as a new ingredient.

Concluding Remarks

Despite the apparent semantic straightforwardness, the term 'traditional food' encompasses articulated facets, including tensions between new and traditional, small- or large-scale, and local and global.

Two characterizing points of local food systems are being still governed by substantial local knowledge and dominated by the perception of experience characters. Both conditions cannot be applied to urban societies, where a constant communication pressure, including the traditional food message, is exerted on consumers by third-party players.

The move toward standardization, although clear in its aims and potential economic impact, is again not matching the intrinsic nature of traditional foods. Basically, the main drawback of the current academic ad regulatory approach to traditional food definitions is to consider them as a mere recipe. Traditional foods are, in reality, the final product of a

system, in which variable and evolving conditions determine an almost continuous variation of their composition. The present debate about defining a standard traditional food is therefore rather redundant. A standard recipe of a traditional food, with consistent compositional and sensory properties over time, may represent an excellent and successful achievement, a sample of tradition-type family of foods, but could hardly be considered the 'true' specimen of something that is intrinsically variable.

Contrary to common belief, traditional foods are far from static, and local foods systems are prone to evolution, provided it is compatible with the local management structure and finances.

In the transect from almost closed tribal rural communities to globalized urbanized societies, a continuous range of situations is still present, also within industrialized countries, supporting often viable local economies. The best perspective for its preservation is perhaps represented by avoiding making too narrow categories, enhancing consumers' awareness and capability of self-appreciation of food quality, and accepting and stimulating the coexistence of exploitation schemes at different scales.

See also: Amaranth; Apples; Authenticity of Food; Bananas and Plantains; Barley; Beer: History and Types; Berries and Related Fruits; Brassica: Characteristics and Properties; Bread: Types of Bread; Cassava: The Nature and Uses; Cherries (*Prunus* spp.): The Fruit and Its Importance; Cider (Cyder; Hard Cider): The Product and Its Manufacture; Consumer Protection Legislation; Convenience Food; Drying: Principles and Types; Ethnic Foods; Famine, Hunger, and Undernourishment; Fermented Foods: Fermented Vegetables and Other Products; Fermented Foods: Origins and Applications; Food Classification and Description; Food Systems: Food Miles; Fruits of Tropical Climates: Biodiversity and Dietary Importance; Fruits of Tropical Climates: Dietary Importance and Health Benefits; Garlic; Herbs: Composition and Dietary Importance; Legal Requirements for Food Hygiene; Legumes in the Diet; Mediterranean Diet; Milk: Sources and Composition; Millets; Mustard; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Olives: Types and Consumption; Peas and Lentils; Peppers and Chilies; Pickling; Potatoes and Related Crops; Preservation of Foods; Quinoa; Rice: Types and Composition; Salad Crops: Leaf-Type Crops; Salad Crops: Root, Bulb, and Tuber Crops; Sausages and Comminuted Products: Dry Fermented Products; Solanaceous Fruits Including Tomato, Eggplant, and Peppers; Sorghum: A Novel and Healthy Food; Soy Beans: Dietary Importance; Spices and Flavoring Crops: Fruits and Seeds; Tortillas; Vegetable Oils: Types and Properties; Vegetables of Temperate Climates: Carrot, Parsnip, and Beetroot; Vegetables of Temperate Climates: Leafy Vegetables; Vinegar; Wheat: The Crop.

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http://www.truefood.eu/ – TRUEFOOD – Traditional United Europe Food.

Triacylglycerols: Characterization and Determination

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Abbreviations

FA	Fatty acid
GC	Gas chromatography
GC-MS	Gas chromatography coupled to mass spectrometry
HPLC	High-performance liquid chromatography
HPLC-MS	High-performance liquid chromatography coupled to mass spectrometry
L	Linoleic acid ($C_{18:2}$)
Ln	Linolenic acid ($C_{18:3}$)
O	Oleic acid ($C_{18:1}$)
P	Palmitic acid ($C_{16:0}$)
RP	Reversed phase
S	Stearic acid ($C_{18:0}$)
TAG	Triacylglycerol
TG	Triglyceride
TLC	Thin-layer chromatography
UV	Ultraviolet spectroscopy
XC-MS	Either gas chromatography or high-performance liquid chromatography coupled to mass spectrometry

Introduction

A triglyceride (TG) – also called triacylglycerol (TAG) or triacylglyceride – is a molecule in which all the three –OH positions of glycerol are esterified by fatty acids (FAs) (see Figure 1): since the three fatty acyl moieties R1, R2, and R3 may in general be different, a high number of triglyceridic species are present in food products.

Indeed, TAGs are the main constituents of vegetable oils (where unsaturated FAs are mainly linked to the glycerol backbone) and animal fats (in which saturated FAs are usually predominant). The number of TGs in a given natural oil or fat is a function of the number of FAs present and the specificity of the enzyme systems involved in the particular fat synthesis reactions. Many plant seed fats have the potential to provide 125–1000 different TGs, animal fats contain potentially 1000–64 000 TG species, and butterfat could generate 2 863 288 TGs from 142 different FAs. In general, the chain lengths of the FAs in naturally occurring TGs vary, but most of those that can be found in oils or fats contain an even number of carbon atoms (as a consequence of their biosynthetic pathway, which passes through acetyl-CoA) in the range between 14 and 20. On the other hand, triglycerides containing FAs with an odd number of carbon atoms may be found in the fat of some animals, in particular ruminants, due to the action of specific bacteria that are able to synthesize odd- and branched-chain FAs.

TGs play a key role in both human life and activities. They represent the predominant way of storing energy in plants and animals and, for humans, are both a source of fat for consumption and a raw material for industrial processes.

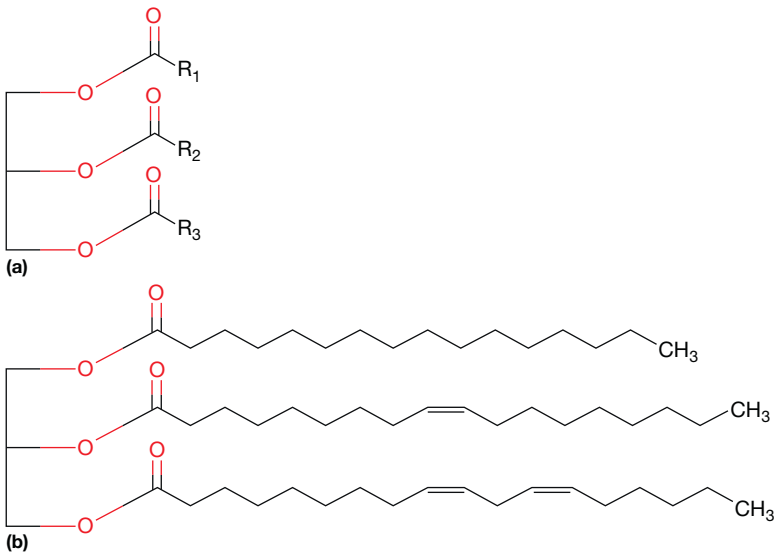

Figure 1 (a) Chemical structure of a generic triacylglycerol. (b) Chemical structure of a specific triglyceride in which R1 is palmitic acid ($C_{16:0}$, P); R2, oleic acid ($C_{18:1}$, O); and R3, linoleic acid ($C_{18:2}$, L). Using the conventional abbreviations for largely occurring fatty acids, such triglyceride would also be indicated as POL.

In this context, the chemical analysis of TAGs and, in particular, the possibility of characterizing and quantifying the individual molecular species play a determinant role in both the biochemical and technological fields and for food quality control. Indeed, the analytical chemistry of TGs allows not only to understand the biosynthetic, metabolic, and technological processes involving these molecules but also to authenticate the genuineness of a particular oil or fat product or assess its nutritional value.

For instance, the determination of serum TG levels is a standard analytic procedure in clinical practice, but it carries very little information on the metabolic status of the individual. On the other hand, specific metabolic disorders may be evidenced through the screening of the distribution of TG molecular species in a lipidomic platform. In the technological field, the possibility of predicting, controlling, or designing the consistency characteristics is very important to the properties of margarines, shortening, and specialty fats. This datum has been often related to the FA composition or with the liquid/solid ratios at various temperatures, but it is mainly related to the crystal structure of TGs, which may exist in different polymorphic forms. Since the predominance of one of these forms within a particular temperature range is related to the distribution of the unsaturations among the three positions of the TG, if not even to the chemical nature of the individual molecular species, chemical profiling of the TAG fraction might allow to define the consistency characteristics of commercial fats and oils in terms of their particular TG distribution.

TG analysis plays an important role also for the investigation of the biosynthetic metabolism of these molecules. Indeed, many studies have reported how the patterns by which nature assembles glycerol and FAs into complex mixtures of TGs should follow only two or three pathways in living organisms. Accordingly, the possibility of relating the total FA composition to the complete triglyceridic profile represents a valid supporting tool for the formulation and validation of biochemical hypotheses. A direct consequence of this is that the distribution of the various TG molecular species reflects the botanical or animal origin of oils and fats. This observation, in turn, means that TG analysis may be used to detect frauds and adulterations in the framework of the quality control of oil and fat products (e.g., fraudulent addition of seed oil to extra virgin olive oil).

From an analytic standpoint, two factors make the analytical chemistry of natural fat TGs particularly difficult: the extremely large number of possible molecular species and the very similar chemical and physical properties of most of these molecules. These two issues result in the fact that chromatography, often hyphenated with mass spectrometry (MS), is almost always used for such purpose. In the remainder of the article, the main approaches for the characterization and quantification of TG molecular species will be presented.

Chemical Analysis of TAGs: General Considerations

TAGs, as many of the existing classes of lipids, occur in nature as a very complex mixture of individual constituents that are often very similar to one another. Indeed, all the members of the family differ only in the kind of FAs that is esterifying the three available hydroxyls of the glycerol moiety. Accordingly, for a complete structural analysis of the triglycerols, it is necessary to separate them into individual molecular species, that is, into single molecules having a specific combination of acyl residues linked to the glycerol backbone. In this respect, considering the high similarity among the FA residues, which are usually found in natural triglycerides, the task can be highly challenging, if not almost impossible, when the separation of enantiomeric forms of the same species is concerned.

Moreover, the aim of the analyst is usually to try to separate these lipids into their individual species in their native form, for example, without any preliminary hydrolysis or similar reaction that may break the molecules into constituent parts: in this way, it is possible to characterize each species in terms of its metabolic function or biosynthesis or, again, physical and chemical properties.

From an analytic standpoint, chromatography, both used alone or in combination with mass spectrometric detection, is the technique of election for the characterization of TAGs. In this respect, the choice of which specific approach to use depends on the kind of information that is sought. Indeed, for instance, reversed-phase liquid chromatography provides a separation of the triglyceridic species, which is based on the total number of carbon atoms in moiety corrected by the number of unsaturations. On the other hand, in silver ion chromatography, the separation along the column is mainly driven by the degree of unsaturation of the acyl moieties. If some of the TAGs contain a polar substituent, such as – OH or NH_2 groups, then absorption chromatography could be used to separate those polar species from the standard ones, which are less polar. In general, none of the methods available are able to produce a complete fractionation of the TGs into their constituent species (even if either gas chromatography or high-performance liquid chromatography coupled to mass spectrometry (XC-MS) techniques is very promising in this respect), so that it is often customary to achieve a better separation by coupling two or more approaches in series.

Chromatographic Techniques

As anticipated, chromatography plays a fundamental role in the characterization and quantification of triglyceridic species, as the large majority of the methods proposed for this purpose involve one or more chromatographic steps. In the succeeding text, the role of the different chromatographic techniques proposed so far for TG profiling will be discussed together with their advantages and disadvantages.

Absorption Chromatography

Absorption chromatography on silica, both in thin-layer chromatography (TLC) and in column, may be successfully used to separate groups of TGs having large differences in polarity, allowing, for instance, the resolution of molecules containing short chains or oxygenated FAs (keto, hydroxy, or epoxy derivatives). As a general principle, smaller molecules (having a lower molecular weight/containing a lower number of carbon atoms) are more retained by the stationary phase than the larger ones, which travel faster. Literature studies report on

the use of such approach for the fractionation of 'acetin fats' (TGs containing both long-chain FAs and acetic acid) according to acetate content or for the characterization of beluga whale TGs, which are characterized by the presence of isovaleric acid and consequently can be separated according to isovalerate content.

Even if polarity and molecular weight are the main driving forces determining the elution order in absorption chromatography of TAGs on silica, the degree of unsaturation can provide a further modulation of the retention properties. Indeed, given the total same number of carbon atoms, the affinity for the stationary phase increases with the degree of unsaturation, so that the more unsaturated species are more retained and elute later than the less unsaturated ones. However, it should be stressed that this characteristic does not lead to a complete separation of the differentially unsaturated species having the same number of carbon atoms, which still remain partly overlapped with one another. On the other hand, absorption chromatography offers rather scarce possibilities of identifying positional isomers (such as ABA or AAB, A and B being two different FAs) unless the acyl moieties have very different chain lengths.

This technique is instead very useful and effective in fractionating oxygenated TAGs, that is, those molecular species containing hydroxy, ketone, aldehyde, ester, epoxy, or ozonide groups on their FA moieties. Since the presence of these groups may significantly alter the polarity of the species, it is possible to use absorption chromatography to separate the TGs on the basis of the number of oxygenated FAs per molecule.

Silver Ion Chromatography

Introduced in the 1960s by de Vries and Morris, silver ion chromatography proved immediately to be one of the most useful techniques for the separation of TGs. It is based on the weak interactions that may form between the Ag^+ ion and the π-electrons of double and triple bonds. Indeed, the nature of these interactions is such that the binding energy of the Ag^+/unsaturated FA complex is low enough to allow that it may be easily formed and dissociated during the time involved in the chromatographic process. Since the exposure to Ag^+ ions does not induce any chemical modification in the TG structure, the separated fractions may be recovered unaltered for further analysis, for example, by reversed-phase high-performance liquid chromatography (RP-HPLC). Moreover, silver ion chromatography can be accomplished both in TLC systems and using HPLC apparatuses, allowing a greater versatility of implementation.

Thin-layer chromatography

Silver ion TLC is usually implemented by impregnating the layer of absorbent with a silver salt (in general, $AgNO_3$), even if some authors employed an alternative approach involving the addition of the silver salt to the mobile phase in conjunction with reversed-phase TLC separation. In order to impregnate the layer, the Ag^+ salt is dissolved in a suitable solvent (water, methanol, ethanol, ammonia, or acetonitrile). For the development, the mobile phase is constituted mainly of two-component systems, the best combination seeming to be chloroform/methanol followed by hexane/diethyl ether or toluene/diethyl ether.

In silver ion chromatography, the fractionation of the TGs occurs mainly according to the total number of unsaturations in the molecule, but the way the double bonds are distributed among the FA moieties plays also an important role: given the total number of unsaturations, a molecule in which all of those are concentrated on an individual FA will be retained more firmly by the stationary phase. Accordingly, by considering TGs made up of FAs, which may contain zero to three double bonds, the following elution order may be expected for the different species:

SSS – SSM – SMM – SSD – MMM – SMD – MMD – SDD – SST – MDD – SMT – MMT – DDD – SDT – MDT – DDT – STT – MTT – DTT – TTT

where S, M, D, and T indicate saturated, monounsaturated, diunsaturated, and triunsaturated fatty acyl moieties, respectively. In the literature, there have been also attempts to resolve TG mixtures containing more highly unsaturated species, but the extent of separation has not been satisfactory.

In general, to improve the resolution of the separation, to repeat the development of the plates more than once has been proposed, changing the polarity of the solvent: in particular, it is suggested to start with the most polar mobile phase for the first development and, successively, proceed with other phases of decreasing polarity.

Another key characteristic of silver ion chromatography is that it is able to distinguish between species that contain isomeric FAs: *trans* olefins form weaker π-complexes with silver ions than do *cis* olefins; hence, molecules containing *trans* isomers are less retained than those with *cis* double bonds.

Additionally, it has been shown that this technique allows also the resolution of positional isomers of TAGs. For instance, it has been shown that when only one of the three fatty acyl moieties contains unsaturation, the symmetric species SUS (S and U indicating saturated and unsaturated FAs, respectively), where the olefin esterifies position 2 of the glycerol molecule, are less retained by the stationary phase, probably because of the lower availability of the central unsaturated acid to the Ag^+ ions.

High-performance liquid chromatography

HPLC (especially in its reversed-phase configuration; see also section '**Reversed-Phase High-Performance Liquid Chromatography**') is by far the most widely used technique for the separation of lipids, so, it has been natural, once suitable universal detectors for these class of substances (such as those based on refractive index or evaporative light scattering) have become available, to try to implement silver ion chromatography through the use of such an instrumentation.

Silver ion HPLC may employ two different kinds of packed columns: those containing silica gel impregnated with silver nitrite and the ones who involve the use of silver ions attached to a cation exchanger. The former kind has more disadvantages as there may be leaching of silver ions, plugging of the column, or precipitation of silver nitrite, all phenomena that can significantly limit its working life. Indeed, columns of the second type are the ones that are most often used now, due to their commercial availability (or to the fact that they may be easily

prepared from a commercial ion exchange HPLC column). In this respect, different studies in the literature report cation exchange columns containing benzenesulfonic acid chemically bonded to silica gel to be the most promising stationary phase. As far as the mobile phase is concerned, it is suggested to employ gradients of acetone and acetonitrile or mixtures of the two into chlorinated solvents (usually a dichloromethane/dichloroethane 1:1 mixture).

The use of reversed-phase configurations with mobile phases containing Ag^+ ions has also been reported, but it has not been demonstrated to give significant improvements over the standard RP-HPLC fractionation.

Analogously to what was already discussed, the elution order of the species reflects the degree of unsaturation of the molecules, even if there are slight differences with respect to the one for TLC, and some TGs may coelute. Indeed, the order that is normally observed is (the abbreviations having the same meaning as in section '**Thin-layer chromatography**')

SSS – SSM – SMM – SSD – MMM – SMD – MMD – SDD = SST – SMT = MDD – MMT – SDT = DDD – MDT – STT – DDT – MTT – DTT – TTT

Also in this case, it is possible to achieve further fractionation of geometric and positional isomers, by opportunely tuning the experimental conditions.

Reversed-Phase High-Performance Liquid Chromatography

Reversed-phase liquid chromatography is the most widely used method for the analysis of the TG mixtures. Due to the nature of the used phases, the retention process is influenced by a combination of the total length of the acyl chains (total number of carbon atoms) and of the overall degree of unsaturation. Indeed, to understand and rationalize the elution order of the different TGs in a mixture when RP-HPLC is used, a quantity called 'equivalent carbon number' (ECN) or 'partition number' (PN) has been introduced: it has been defined as the total number of carbon atoms (CN) in the fatty acyl moieties (i.e., without counting the glycerol backbone) reduced by twice the number of double bonds in the molecule (n), according to the formula:

$$\mathrm{ECN} = \mathrm{CN} - 2n$$

Chromatographic elution will therefore follow the order of increasing ECN, the lighter and more unsaturated species having lower retention times than the larger and more saturated ones. At the same time, TGs with the same value of ECN will tend to be partially or completely coeluted. This is what can be expected, for instance, when considering $C_{14:0}$–$C_{16:0}$–$C_{18:0}$, $C_{16:0}$–$C_{16:0}$–$C_{18:1}$, $C_{14:0}$–$C_{18:0}$–$C_{18:1}$, $C_{18:0}$–$C_{18:1}$–$C_{18:2}$, $C_{16:0}$–$C_{18:0}$–$C_{16:1}$, and so on. However, it must be stressed that the resolving power of actual instruments is such that complete coelution never occurs, as the factor for each double bond may not be considered to be constant and equal to 2.0, but its value may become slightly lower and depend on the position of the unsaturations (for instance, accounting for minimum differences between a single diene and two monoenes). A typical RP-HPLC chromatogram of a vegetable oil is shown in **Figure 2**.

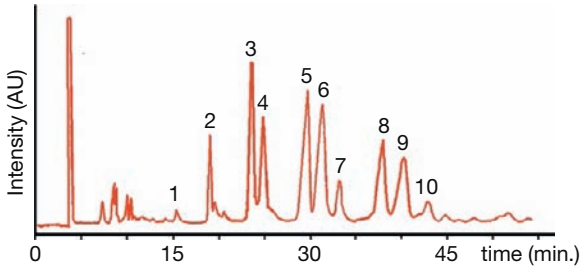

Figure 2 Example of an RP-HPLC chromatogram of a rice bran oil sample showing the elution order of the TG molecular species. Chromatographic conditions: isocratic elution with acetone/acetonitrile (55:45 v/v) on an LC-18 column (250 mm length, 4.6 mm i.d., and 5 μm particle size); differential refractometric detection. Legend: 1, LLLn; 2, LLL; 3, LLO + LnOO; 4, PLL; 5, LOO + SLL; 6, PLO; 7, PPL; 8, OOO; 9, POO + LOS; and 10, PPO (meaning of the abbreviations is described in the list at the beginning of the article).

Although aqueous mobile phases were generally used with alkyl-bonded phase columns, due to the lipophilicity of TGs, water could not be used in the mobile phase for this particular application; therefore, the mobile phases generally employed consisted of mixtures of weak and strong nonaqueous solvents, the former being almost always acetonitrile while the latter being usually chosen among chloroform, dichloromethane, isopropanol, or acetone. Among those, the combination acetone/acetonitrile is by far the mobile phase that is used most often, both because it guarantees a very good partition for almost all the TG species and because it does not interfere with most of the detectors employed (the only exception being ultraviolet spectroscopy (UV) as the cutoff for acetone is significantly higher than the absorption wavelengths of the analytes). The major difficulty in the HPLC of TAGs is that of detection, as many of the available universal detectors possess a poor sensitivity and may suffer from major drawbacks. As already mentioned, one possibility can be the use of UV absorbance as detecting system; in particular, the chromophores that are present in TGs are the ester carbonyl group, and isolated double bonds, which result in a UV absorbance at low wavelengths (190–237 nm). Such a wavelength interval is the limiting factor in the choice of the solvent system to be adopted (it was already stated earlier that acetone cannot be used with such detector and alternative strong solvents, e.g., dichloromethane, should be employed). Moreover, significant baseline drift may be observed when operating in gradient. Another widely used alternative is represented by the measure of the refractive index; however, differential refractometry is much less sensitive to TGs than is UV detection, and it is incompatible with the use of gradient elution. On the other hand, the complexity of natural TG mixtures would require gradient elution to fully utilize the resolution power of liquid–liquid chromatographic systems. These drawbacks have been mostly overcome by the introduction of the commercially available mass detector, which allowed the expansion of the possible conditions for TG separations, including that of gradient elution. The mass detector system, which possesses the characteristics of showing very narrow and symmetrical peaks in the separation of many TG mixtures regardless of their carbon number distribution, is the evaporative light scattering detector (ELSD). ELSD is universal and may be used with almost any solvent, also in gradient,

provided that they are volatile enough to evaporate in the heating chamber, without showing any drift in the baseline.

MS using either atmospheric pressure chemical ionization (APCI) or electrospray (ESI) is for sure an invaluable tool for both identifying and quantifying molecular species of TAGs. This topic will be covered in detail in section '**Hyphenated Techniques (XC-MS)**.'

High-Temperature Gas Chromatography

Due to its desirable characteristics in terms of repeatability, relative inexpensiveness, robustness, and wide range of linearity, many researchers investigated the possibility of using gas chromatography (GC) coupled with flame ionization detection for the analysis of the molecular species of TAGs. However, considering the high molecular weight of the species involved, from an experimental standpoint, the use of GC in the analysis of TGs presents several difficulties. First of all, in order to elute these family of lipids, high temperatures are needed, which are close to the limits of thermal stability both for the stationary phase and for the analytes themselves. This issue reflects in the need of paying great attention to the sample injection device and to select very carefully the nature of the stationary phase. In this respect, there has been a considerable improvement in the temperature stability of GC stationary phases, by the introduction of chemical bonding to the support and cross-linking of polymers. In particular, low-polarity phases (of the methyl silicone kind) are most stable to high temperatures but only permit separation by chain length (even if both the degree of unsaturation and the different chain lengths of the fatty acyl moieties may still provide a partial resolution). On the other hand, higher-polarity or polarizable phases (usually consisting of phenyl methyl silicones) provide an improved separation by both chain length and degree of unsaturation, but they are least stable to high temperatures. Accordingly, it is nowadays more and more customary to shift toward the use of medium-polarity phases, as a compromise, which provides an adequate degree of separation by degree of unsaturation for many practical purposes with acceptable temperature stability.

In general, if the instrumental conditions (flow rate, temperature ramp, and choice of the injection technique) are controlled and set to values that ensure that artifacts are not present and that the sample is not lost selectively during injection or through degradation on the column, high-temperature gas chromatography (HT-GC) analysis of TGs provides accurate recoveries and may attain a high precision.

Hyphenated Techniques (XC-MS)

MS is a powerful analytic tool, as it allows obtaining both structural (qualitative) information about the species and quantitative data relating to mass. Under optimum conditions, it can provide the molecular weight, the empirical formula, and often the complete structure of an unknown compound in addition to giving a measure of the amount present. It is therefore not surprising that in more and more applications, the RP-HPLC and HT-GC approaches described in sections '**Reversed-Phase High-Performance Liquid Chromatography**' and '**High-Temperature Gas Chromatography**' are coupled to mass spectrometric detection, in order to improve the performances of the methods.

In GC-MS, many applications involved the use of electron impact ionization, even if – more recently – both chemical ionization (for instance, using NH_3 as the reagent gas) and ESI have been gaining more and more relevance. In the latter case, it is suggested that the inspection of the fragments at m/z $[M-RCOO]^+$, $[RCO+128]^+$, $[RCOO+74]^+$, or $[RCO]^+$ can be used to identify the molecular species (M being the molecular ion). On the other hand, when chemical ionization is used, limited fragmentation is observed – the base peak being either the quasimolecular ion or the $[MH\text{-}RCOOH]^+$ one, depending on the nature of the reagent gas.

A similar consideration may be done as regards HPLC-MS. In this case, ionization is carried out by means of one of the atmospheric pressure ionization (API) interfaces, most commonly either APCI or ESI. These API sources share the same common characteristics of providing a 'soft' ionization of molecules and resulting usually in a protonated molecule or molecular adduct ion and only a few large fragments. However, as already briefly discussed in the preceding text, in the case of GC-MS, ESI and chemical ionization carry a diverse and often complementary information, as they result in spectra in which the relative proportions of the intact protonated molecule and adduct ions versus the fragments are different.

ESI almost always produces an intact molecule ion $[M+H]^+$ or adduct ion $[M+X]^+$ (X = Li, Na, NH_4, etc.) and only small abundances of fragments, unless tandem MS is applied. However, in order for TAGs to be amenable to be analyzed by ESI-MS, the addition of an electrolyte to produce adduct ions (e.g., ammonium formate or ammonium acetate to form $[M+NH_4]^+$) is often necessary. In this context, the possibility of using lithium adducts $[M+Li]^+$ for the analysis of TAGs by ESI-MS appears as a valid option, as it allows the identification of regioisomers and the localization of double bonds in a relatively easy fashion.

On the other hand, APCI results in a high quantity of diacylglycerol-like fragment ions $[M-RCOO]^+$, while the amount of protonated molecule formed is determined by the degree of unsaturation in the molecule. Indeed, in the case of polyunsaturated TAGs, the molecule ion $[M+H]^+$ represents the base peak, while it is almost absent in the case of saturated TAGs. Moreover, APCI-MS allows also the characterization of the regioisomeric composition of TGs, as the ratio of the $[M-RCOO]^+$ fragments produced is related to such an information.

See also: Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Fats: Classification and Analysis; Triacylglycerols: Structures and Properties; Vegetable Oils: Composition and Analysis.

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Triacylglycerols: Structures and Properties

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Introduction

Lipids are a large and diverse group of naturally occurring substances that are characterized for being mostly soluble in nonpolar organic solvents such as hydrocarbons, chloroform, benzene, ethers, and alcohols and usually insoluble in water. This group of substances includes fatty acids, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides. Triacylglycerols belong to the glycerolipid class. This class comprises all compounds containing fatty acids esterified to the glycerol with the exception of glycerophospholipids. These last compounds are considered a separate class because of their abundance.

Most of all commercially important fats and oils of animal and plant origins are composed almost exclusively of triacylglycerols. They can be solid or liquid at room temperature depending on the nature of the fatty acid composition. They also represent the majority of dietary lipids, playing a major role in nutrition. This article will be dedicated to describing their structure and properties. The following article will describe their characterization and determination.

Triacylglycerol Structure

Triacylglycerols are composed of a glycerol molecule that has been esterified with three molecules of fatty acids (Figure 1). If the glycerol is only esterified with two molecules of fatty acids, the resulting glycerolipid is named diacylglycerol. Analogously, when the molecule of glycerol is esterified with one molecule of fatty acid, the resulting glycerolipid is named monoacylglycerol. Triacylglycerols can be converted into di- and monoacylglycerols by the action of lipases, and these hydrolyzed glycerolipids can also be further esterified into triacylglycerols.

Because more than one thousand fatty acids have been described and they can be distributed among the three positions of the glycerol skeleton, the number of possible combinations is very high. However, the number of common triacylglycerols is much reduced.

Although glycerol is not a chiral molecule, when substituents at positions 1 and 3 are different, a chiral carbon is created at carbon 2 of the glycerol backbone and each triacylglycerol can exist in two enantiomeric forms. To avoid confusions between the two terminal carbons of the glycerol molecule, the 'stereospecific numbering' (*sn*) system is employed.

In a Fischer projection, as the one shown in Figure 1, the secondary hydroxyl group is on the left of the carbon 2 of glycerol. Using this projection, the carbon atom above C-2 is numbered as C-1, and the carbon atom below C-2 is numbered as C-3. The triacylglycerol is termed as triacyl-*sn*-glycerol when stereochemistry is considered. When the detailed stereochemistry is not specified, the terminal carbons of glycerol are termed as α- and α′-positions, and the central carbon as the β-position. This nomenclature is also employed for mono- and diacyl-*sn*-glycerols (Figure 1). Thus, three isomers exist for each diacylglycerol (1,2-, 2,3-, and 1,3-diacyl-*sn*-glycerol) and for each monoacylglycerol (1-, 2-, and 3-monoacyl-*sn*-glycerol). When the detailed stereochemistry is not specified, only two diacylglycerols (1,2- and 1,3-diacylglycerol) and two monoacylglycerols (1- and 2-monoacylglycerol) are considered.

As an abbreviated nomenclature, triacylglycerols are commonly described by a combination of three letters representing the composing fatty acids. Thus, for example, the tripalmitin or tripalmitoylglycerol is represented as PPP, and POS would represent a triacylglycerol composed of palmitic, oleic, and stearic acids.

In general, triacylglycerols are a heterogeneous mixture of fatty acid combinations that will depend on the fatty acid composition of the source. However, fatty acids are not usually distributed at random among the carbons of the glycerol molecule. Thus, for example, in vegetable oils, saturated fatty acids are predominantly esterified at positions *sn*-1 and *sn*-3 of the glycerol, and there is not too much difference between both positions. Polyunsaturated fatty acids (mainly linoleic and linolenic acids) are esterified primarily at position *sn*-2. Monoenoic acids are relatively evenly distributed. However, less-common fatty acids tend to be concentrated at position *sn*-3.

For most species, in animal depot fats, saturated fatty acids are found predominantly at position *sn*-1; position *sn*-2 tends to contain mainly unsaturated fatty acids, although shorter-chain fatty acids can also accumulate at this position in some cases, and longer-chain fatty acids exhibit some preference for accumulating at position *sn*-3. On the other hand, pig and related species accumulate palmitic acid at position *sn*-2, stearic acid is at position *sn*-1, and position *sn*-3 is preferred by oleic and linoleic acids.

Triacylglycerol compositions of milk fat are different for each species and depend on both the diet and the nature of the fatty acids synthesized in the mammary gland. Among them, triacylglycerols of cows' milk are especially asymmetrical containing all the butyric acid and most of the hexanoic acid at position *sn*-3.

In fish oils, myristic, palmitic, and palmitoleic acids are mainly esterified at positions *sn*-1 and *sn*-3; oleic acid and long-chain monoenoic acids are mainly located at positions *sn*-1 and *sn*-3 with a higher proportion at position *sn*-3 when the chain length increases; polyunsaturated fatty acids are concentrated at position *sn*-2 and there are also substantial amounts at position *sn*-3.

Triacylglycerols are also fairly extended in eukaryotic organisms such as yeasts, molds, and fungi. Among prokaryotic organisms, only species from the actinomycetes group accumulate triacylglycerols to a significant extent.

Triacylglycerol Properties

Triacylglycerols are hydrophobic molecules that are the result of the triple esterification of the hydroxyl groups of glycerol by fatty acids. This structure is going to play a major role in their physical

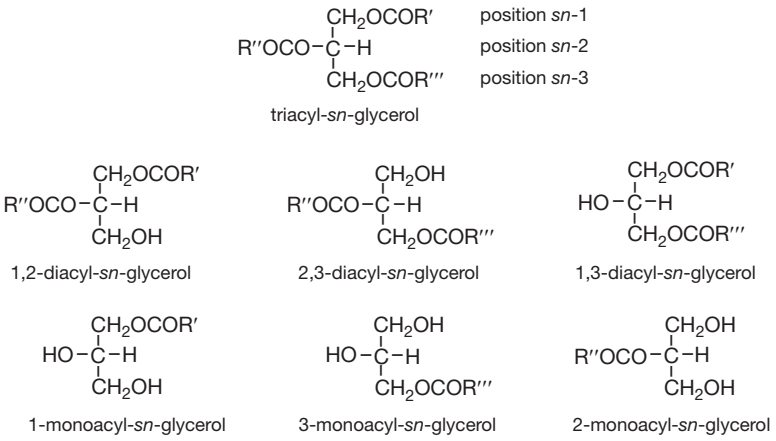

Figure 1 Chemical structures and numbering for triacyl-*sn*-glycerols, diacyl-*sn*-glycerols, and monoacyl-*sn*-glycerols.

properties, which will be determined by the organization of the hydrocarbon chains of the present fatty acids; their chemical properties, in which both the ester groups and other functional groups of the fatty acyl chains will be the groups susceptible to be modified; and their biological properties, because of the importance of essential fatty acids in the daily diet.

Physical Properties

The physical properties of triacylglycerols are of great practical importance because many of their technical applications, including their uses in edible products, depend on the oiliness, surface activity, solubility, melting behavior, or other properties peculiar to long-chain compounds.

Crystallization and polymorphism

Triacylglycerols exhibit a monotropic polymorphism. This means that each triacylglycerol has several crystalline varieties, which can be converted from one variety to other. All these varieties can be obtained from the liquid-state and solid-state transitions are very rare. Usually, the most stable form of triacylglycerols has a triclinic subcell with parallel hydrocarbon chain planes, which is noted as β. A second common subcell is hexagonal with no specific chain plane conformation, which is noted as α. Other varieties are noted as β′. For pure triacylglycerols, the most stable variety is generally β, but for mixtures of triacylglycerols, it is most frequently β′. Some triacylglycerols exhibit several β′ and/or β varieties. In such cases, the notation of the different varieties is done by ascending order starting from the most stable to the least stable. Thus, for example, β_1 is more stable than β_2. Therefore, the stability of the different polymorphic varieties in pure triacylglycerols increases in the order $\alpha < \beta'_2 < \beta'_1 < \beta_2 < \beta_1$. The melting points increase in the same order.

Among the different fats, the polymorphic behavior of cocoa butter is especially complex and five different polymorphs (or six according to other authors) are recognized (γ, α, β′, β_2, and β_1). The desirable physical properties of cocoa butter and chocolate – snap, gloss, melting in the mouth, and flavor release – depend on the formation of the polymorph β_2, which has to be obtained under controlled temperature conditions. Unfavorable storage conditions can produce the change of polymorph β_2 to the most stable polymorph β_1, which can make the product unsuitable for consumption.

Melting

Each individual triacylglycerol has a specific melting point for each polymorphic variety. It depends on the average chain length of the fatty acids, the position of the fatty acids on the glycerol molecule, the relative proportion of saturated to unsaturated fatty acids, and the processing techniques employed. However, fats are mixtures of mixed triacylglycerols that crystallize in several crystal forms, and all these forms have specific melting points. Therefore, fats do not have sharp melting points, but do have a melting range. If the melting range is $< 5\,^{\circ}C$, the fat is considered to be nonplastic. If the melting range is large (sometimes it may exceed $40\,^{\circ}C$), the fat is called plastic.

Plasticity

Plasticity is a consequence of the semisolid state of fats at room temperature, in which a certain proportion of triacylglycerols are solids and others are liquids. Within this temperature range, fats can be molded and they will maintain the produced deformation after the removal of the stress. One of the most important properties of fats is their solid fat content. Fats may retain their solid character with solid fat contents as low as 12–15%. The relationship between hardness and solid fat content indicates that there is a range of solids in which fats are plastics (this is the plastic range). Shortening is an example of a fat with an extended plastic range.

Viscosity

Triacylglycerols are more viscous than water as a consequence of the attractions among their long fatty acyl chains. This is one of the reasons why oils have the ability to form oily or lubricating films. Viscosity of triacylglycerols increases with oxidation and polymerization. For this reason, viscosity has been used to determine the condition of a fat employed in deep frying.

Vapor pressure

Triacylglycerols of long-chain fatty acids have very low vapor pressure. For this reason, they can only be distilled by molecular or short-path distillation.

Refractive index

Each triacylglycerol has a specific refractive index that depends on triacylglycerol structure, temperature, and density. Refractive index normally increases with hydrocarbon chain length, with the number of double bonds, and with conjugation and decreases when temperature increases.

Ultraviolet spectroscopy

Triacylglycerols containing saturated, monounsaturated, or methylene-interrupted polyunsaturated fatty acyl chains absorb ultraviolet light at wavelengths too low for convenient study. On the other hand, ultraviolet spectroscopy is a valuable tool for detecting triacylglycerols containing fatty acyl chains with conjugated double bonds.

Infrared spectroscopy

The most frequent use of infrared spectroscopy is in the recognition of *trans* isomers. The *trans* double bond of a fatty acid chain produces a characteristic absorption at 968 cm^{-1} that does not change for additional double bonds unless these are conjugated. In addition, triacylglycerols have a strong absorption band around 1740 cm^{-1} that may be of diagnostic value.

Nuclear magnetic resonance spectroscopy

Low-resolution instruments are employed to determine the proportion of solid and liquid in a fat (or mixtures of triacylglycerols) and the content of oil in a seed. High-resolution instruments provide a great amount of information about triacylglycerol structure.

Chemical Properties

Reactions of fatty acyl chains at double bonds, at allylic carbons, or at carboxylic groups of triacylglycerols are important both in biology and for industry. Thus, oxidation reactions can produce food spoilage and can cause problems within living tissues. In contrast, chemical reactions on fatty acyl residues are widely employed to develop products for industry (from cosmetics to detergents).

Oxidation

Oxidation of triacylglycerols is responsible for their deterioration. When present in foods, oxygen causes rancidity accompanied by odors that make foods difficult to accept. On the other hand, triacylglycerol oxidation products can be desirable for certain foods or processes such as some deep-fried flavors. These reactions also play a major role in tissues, although oxidation usually takes place enzymatically after hydrolysis of the triacylglycerol.

Triacylglycerol oxidation is a consequence of the existence of unsaturated or polyunsaturated fatty acyl chains. Thus, oleic acid is generally more susceptible to lipid oxidation than any saturated fatty acid because of the lower bond energy of hydrogen atoms adjacent to a double bond (allylic protons) that allows an easier abstraction. Furthermore, hydrogen atoms in the center of the 1,4-pentadiene system of polyunsaturated fatty acyl chains (doubly allylic protons) are around 20 times easier to remove than allylic protons of oleic acid. Moreover, the higher the number of 1,4-pentadiene units, the higher its susceptibility to oxidation. Therefore, saturated triacylglycerols should be expected to be relatively stable to oxidation, but triacylglycerols containing highly polyunsaturated fatty acids are easily oxidized unless lipid oxidation is inhibited in some way.

The mechanism of autoxidation of unsaturated triacylglycerols is a catalytic process that proceeds via free radicals and involves the reaction of oxygen with the unsaturated fatty acyl moieties of triacylglycerols according to Figure 2. It includes three general steps: initiation, propagation, and termination.

The formation of a radical (TAG•) from an unsaturated fatty acyl chain is the key event in the initiation step. This can occur by thermal or photochemical cleavage of the allylic CH bond or by hydrogen atom abstraction by a free radical initiator.

The first step of propagation normally begins with the addition of molecular oxygen to the TAG• to form a peroxyl radical (TAGOO•). This step is produced easily. However, the rate-limiting step of the reaction is the abstraction of a hydrogen atom from a second molecule of triacylglycerol (TAG) to produce a radical in this new molecule of triacylglycerol to continue the radical chain. Radical scavengers can also produce the hydroperoxide at the same time that they break the free radical propagation.

Once formed, the hydroperoxide can suffer a homolytic cleavage to produce alkoxyl and hydroxyl radicals. Termination reactions are those reactions that produce nonradical products from the radicals derived from triacylglycerols. This includes monomeric compounds with molecular weights similar to the initial triacylglycerols but containing oxygenated groups such as hydroxyl, hydroperoxy, keto, or epoxy; polymeric compounds that can also contain oxygenated groups; and volatile products, including hydrocarbons, aldehydes, and ketones, among others. Alkoxyl radicals can also be neutralized by radical scavengers.

Hydrolysis and saponification

Triacylglycerols can be hydrolyzed in basic, acidic, and even neutral media. The reaction is irreversible in basic medium (saponification) and is reversible under acidic or neutral conditions. It produces fatty acids (or their salts) and glycerol, and it is the best procedure to obtain the fatty acids required by the industry. Additionally, this reaction is also produced in the presence of lipases.

Interesterification

Interesterification is employed to change fatty acid composition or fatty acid distribution in the triacylglycerol molecule (Figure 3). It can be carried out in a single triacylglycerol or between several triacylglycerols. If it is carried out within a single triacylglycerol, fatty acids are moved to the different positions of the triacylglycerol. As a consequence, the triacylglycerol is randomized and changes in the physical properties of the triacylglycerol are produced. If it is carried out between two triacylglycerols, fatty acid chains are interchanged among the three positions of both triacylglycerols. This reaction can be

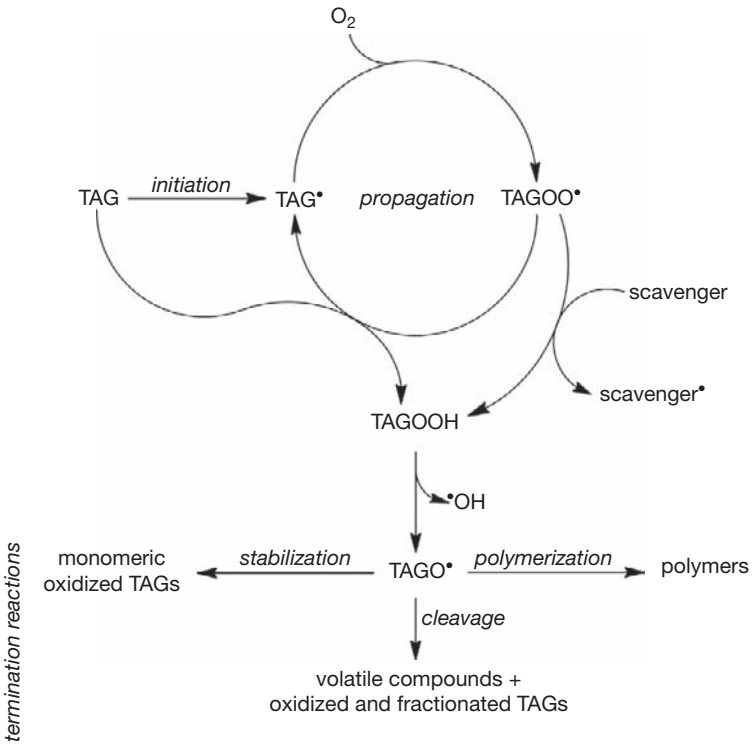

Figure 2 Triacylglycerol oxidation. Abbreviations: TAG, triacylglycerol; TAG•, triacylglycerol radical; TAGO•, alkoxyl radical from triacylglycerol; TAGOO•, peroxyl radical from triacylglycerol; TAGOOH, hydroperoxide from triacylglycerol.

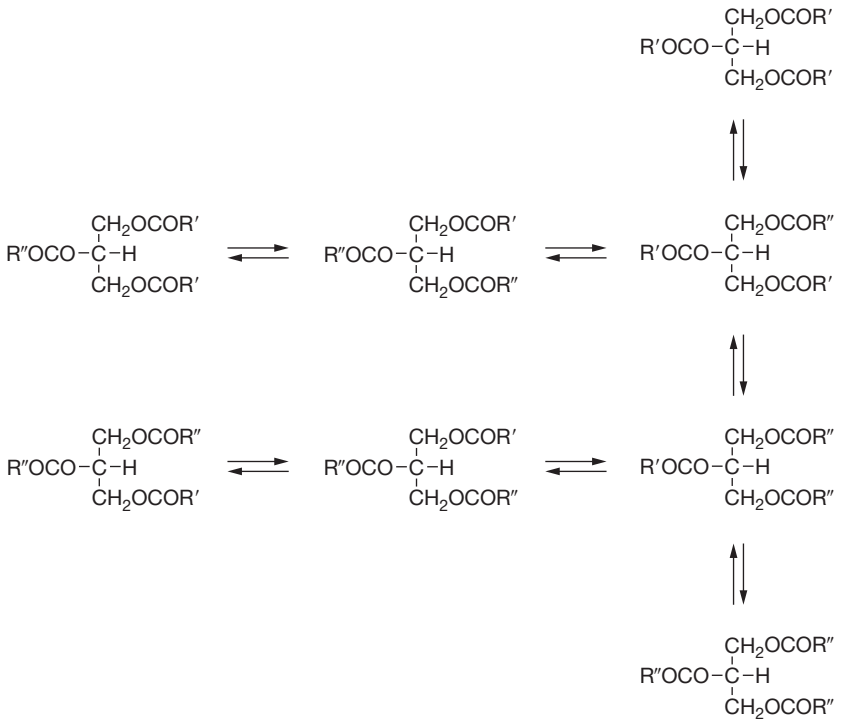

Figure 3 Interesterification reaction of triacylglycerols.

carried out either chemically or enzymatically. Enzymatic interesterification has the advantage that it can be directed to certain positions, which avoids randomization.

Alcoholysis

Alcoholysis is the esterification reaction between a triacylglycerol and an alcohol in the presence of sodium methoxide or lipases. If the reaction is carried out with glycerol, the products are mono- and diacylglycerols, which are widely employed as emulsifiers. This last reaction is usually performed using nonspecific lipases. If reaction is carried out below the temperature of crystallization of monoacylglycerols, the equilibrium of the reaction is shifted to the production of monoacylglycerols.

Hydrogenation

Hydrogenation of triacylglycerols is carried out either to increase its melting point (transform a liquid triacylglycerol into a solid or semisolid one) or to increase its oxidative stability during storage. Reactions are carried out at relatively high temperatures in the presence of catalyzers. Isomerizations can be produced, including the formation of *trans* double bonds.

Formation of polyols

The triacylglycerol containing ricinoleic acid at the three positions of the glycerol molecule is a natural polyol that is widely employed in making a variety of polyurethane products ranging from coatings to foams. Unsaturated triacylglycerols can also be converted into polyols by cleavage of ethylenic chains, which can be carried out by ozonolysis. This reaction is produced by addition of ozone to a carbon–carbon double bond with the formation of an ozonide, which later breaks down to produce aldehydes, carboxylic acids, or – under reductive conditions – alcohols. Under these last conditions, the produced compounds are polyols with terminal primary hydroxyl groups. Additionally, formation of secondary hydroxyl groups in fatty acyl chains can be achieved by epoxidation and ring opening.

Functional Properties

Triacylglycerol properties are very important in food design. Triacylglycerols are incorporated into processed foods for a variety of reasons, including their action as lubricants, enhancers of eating properties, moisture barriers, heat conductors, and nutrition providers, among others. The functional ability of triacylglycerols depends on their structure and a mixture of triacylglycerols is usually employed. In this way, plasticity and melting can be adapted to the specific food product. Thus, for example, a shortening will make tender biscuits only if the employed mixture of triacylglycerols has the proper ratio of solids to liquids because solids provide structure and creaming and the liquid oil provides lubricity.

Nutritional Properties

Triacylglycerols are a major source of storage energy and are important precursors both in the body's metabolic processes and in the essential components of cell membranes and other biological structures. Triacylglycerols also play an important role in the absorption of fat-soluble nutrients, including fat-soluble vitamins and other dietary and medical supplements.

Triacylglycerol metabolism

Triacylglycerols cannot be absorbed as such by the intestine. In addition, they are not soluble in water. To absorb these molecules, the dispersion of triacylglycerols into small particles with sufficiently exposed surface area for rapid attack by digestive enzymes is needed. This is achieved by detergent action (bile salts, fatty acid soaps, and monoacylglycerols) and mechanical mixing. Once triacylglycerols are emulsified, they are hydrolyzed by enzymes excreted by the pancreas, being pancreatic lipase the most important enzyme. This enzyme is specific for primary ester linkages, which results in the formation of free fatty acids and 2-monoacylglycerols. These compounds are absorbed from the lumen by intestinal mucosal cells where triacylglycerols are resynthesized, packaged into chylomicrons, and directed to the lymphatic vessels of the abdominal region and finally enter the circulation via the thoracic duct.

Fatty acids can be liberated from triacylglycerols in chylomicrons with lipoprotein lipase. Adipose tissue is especially active for secreting this enzyme into the surrounding blood vessels. This enzyme rapidly hydrolyzes the triacylglycerols at the cell surface, releasing free fatty acids, which are absorbed into the adjacent adipocytes and reutilized for triacylglycerol synthesis within the cell. Triacylglycerols are stored as cytoplasmic lipid droplets enclosed by a monolayer of phospholipids and hydrophobic proteins. Chylomicron remnants reach the liver, where the remaining lipids are hydrolyzed and absorbed. The fatty acids within the liver can be utilized for a variety of purposes, including their conversion into triacylglycerols and their storage as lipid droplets within the cytoplasm of the cells. Most of these newly synthesized triacylglycerols are exported into the plasma in the form of very-low-density lipoproteins (VLDL). These particles are transported to the peripheral tissues, where they are hydrolyzed and the fatty acids absorbed.

Triacylglycerols and health

Triacylglycerols are present in plasma in the form of multiple triacylglycerol-rich lipoproteins, predominantly intestinally synthesized chylomicrons in the postprandial state and hepatically synthesized VLDL in the fasted state. If triacylglycerols are not correctly removed and their concentration increases, hypertriglyceridemia is produced. Patients with hypertriglyceridemia may experience diverse complications (including diabetes, hypertension, and obesity) that confer an increased risk of cardiovascular events. In fact, epidemiological evidence indicates that plasma triacylglycerol concentration is a strong independent risk factor for cardiovascular disease, suggesting that prolonged residence of plasma triacylglycerol-rich lipoproteins, especially in the postprandial state, may contribute to cardiovascular disease susceptibility. For that reason, dietary factors that contribute to raised postprandial triacylglycerols have become a focus of concern. On the other hand, dietary factors that reduce triacylglycerol concentrations can be good for the heart. To this respect, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have shown to reduce cardiovascular mortality at a dose of $\sim$1 g d^{-1} without significant reductions in triacylglycerols, but plasma triacylglycerol reduction can be achieved with higher EPA + DHA doses.

Severe hypertriglyceridemia is also a cause of acute pancreatitis. Although the pathophysiology of hypertriglyceridemic pancreatitis is not well established, it is hypothesized that pancreatic lipase hydrolyzes excess of triacylglycerols into free fatty acids, which are toxic to the acinar cells and the vascular endothelium. Additionally, chylomicrons increase the viscosity of blood and impair blood flow in the pancreas causing ischemia within this organ.

Triacylglycerols are also the main compounds responsible for fatty acid intake in common diet. The metabolic functions of fatty acids depend on the length of the carbon chain, degree of saturation, the stereochemistry and position of the double bonds, and their position in the triacylglycerol molecule. These functions will be discussed in other articles.

See also: Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Fats: Classification and Analysis; Fish Oils: Composition and Health Effects; Fish Oils: Production and Properties; Lipoproteins; Phenolic Compounds: Bioavailability and Health Effects; Phenolic Compounds: Occurrence, Classes, and Analysis; Triacylglycerols: Characterization and Determination; Vegetable Oils: Composition and Analysis; Vegetable Oils: Dietary Importance; Vegetable Oils: Types and Properties.

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V

Vegetable Oils: Composition and Analysis

AJ Dijkstra

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Introduction

Edible oils and fats have been produced for centuries, so it is not surprising that their analysis has a long history. The French chemist Michel Eugène Chevreul, who can be regarded as the first lipid chemist, had a book published in 1823 in which he coined names like stearic acid, oleic acid, and butyric acid, all compounds he discovered and described. He also reported melting points, which he used to assess the purity of compounds obtained by recrystallization – a method he developed – and to define the titer of fats.

One of the fatty substances that Chevreul investigated was cholesterol, which he obtained from human gallstones. He noted that it differed fundamentally from oils and fats in that he could not saponify the cholesterol. Oils and fats were used extensively in soap works and left some 'unsaponifiables.' Accordingly, oils and fats also became characterized by their unsaponifiable content.

Subsequent quantitative analysis made use of what methods were available at the time. Early on, a number of titrimetric methods were developed, the results of which were reported as 'values': acid value, iodine value (IV), etc. Fatty acid compositions started to be determined, but as explained by Gunstone, this determination took a long time (http://lipidlibrary.aocs.org/history/beforechrom/index.htm). This became much easier with the advent of gas–liquid chromatography (GLC), first with packed columns and later with capillary columns. The latter enabled separations between isomers, first *cis–trans* and later also with respect to positional isomers of unsaturated fatty acids. GLC was combined with mass spectrometry; HPLC was used for analysis of the triglyceride composition with silver-ion HPLC providing a means to separate triglycerides according to their degree of unsaturation. Liquid chromatography was combined with mass spectrometry, and more sophisticated analytic techniques were developed for a research environment.

Perhaps, the most useful method to characterize a fat is by its solid fat content (SFC). Its determination makes use of a 'time-domain NMR' technique that is also referred to as 'pulse NMR.' In fact, the equipment used for this determination was specifically developed for fat characterization and replaced the laborious dilatometric method. The same equipment can also be used to determine the oil content of oilseeds.

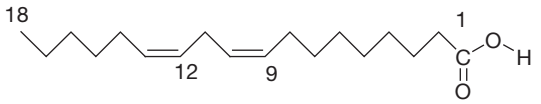
Composition of Vegetable Oils

Saponifiable Fraction

The saponifiable fraction of vegetable oils, usually at least 98% of the oil, is characterized in that it forms soaps (salts of fatty acids), when the oil is treated with an alkali such as sodium hydroxide. The fraction contains several different classes of compounds that will be discussed later. The main constituent of the saponifiable fraction is triglyceride oil, but some of this oil has been hydrolyzed so that this fraction also contains partial glycerides and free fatty acids (FFAs).

Fatty acids

Nearly all naturally occurring fatty acids are carboxylic acids with a straight chain and an even number of carbon atoms. If they contain double bonds, these are mostly in the *cis*-configuration, and in polyunsaturated fatty acids, the double bonds tend to be methylene-interrupted. This is illustrated by the molecular structure of linoleic acid shown below:



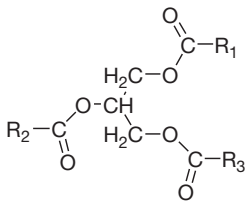
In this molecule, the carbon atom of the carboxyl group has number 1. The first double bond starts at carbon atom number 9 and the second one at number 12. The systematic name of this fatty acid is therefore (9*Z*,12*Z*)-octadeca-9,12-dienoic acid. Because this is rather a mouthful, scientific literature often uses a shorthand notation: 9c12c-18:2. This notation starts with the location and configuration of the double bonds. Then, the number of carbon atoms of the fatty acid chain is given, which is followed by the number of unsaturated bonds. This shorthand notation is explained in http://lipidlibrary.aocs.org/education/names/FAnames1.htm.

When a fatty acid is synthesized in plants, it is elongated at its carboxyl end. Consequently, the position of a double bond does not change with respect to the other, methyl end. So, there are families of fatty acids according to the distance of the last double bond to the methyl end. This distance can be indicated with the prefix *n* or ω. The linoleic acid shown in the preceding text is part of the *n*-6 or ω-6 family of fatty acids.

Table 1 lists some fatty acids that kept their trivial names, their shorthand notations, and sources.

Triglycerides

The official name of a triglyceride molecule is 'triacylglycerol,' indicating that it consists of a glycerol backbone that is esterified by three fatty acids as shown below:



The fatty acid chains R_1, R_2, and R_3 can be the same but seldom are. Only oils with a very high content of a single fatty acid such as oleic acid in 'high-oleic sunflower seed oil' will contain a triglyceride with three identical fatty acids, in this case trioleate. Normally, the fatty acids are different so that a triglyceride oil contains very many different molecular species.

In vegetable oils, the distribution of the fatty acids over the glycerol backbone is not fully random but 1,3-random, 2-random. This is illustrated by the soybean oil data in Table 2. The saturated fatty acids 16:0 and 18:0 predominantly occupy the outer or 1,3-positions in equal amounts: 18:2 mainly occupies the 2-position and 18:1 fills the empty positions. An example of the fatty acid distributions for a few oils is given in Table 2.

Table 1 Trivial names, shorthand notations, and occurrence of several fatty acids

Trivial name	*Notation*	*Source*
Butyric acid	4:0	Butter
Caproic acid	6:0	Butter
Caprylic acid	8:0	Coconut oil, palm kernel oil
Capric acid	10:0	Coconut oil, palm kernel oil
Lauric acid	12:0	Coconut oil, palm kernel oil
Myristic acid	14:0	Coconut oil, palm kernel oil
Palmitic acid	16:0	Palm oil
Stearic acid	18:0	Cocoa butter, shea butter
Oleic acid	18:1(*n*-9)	All vegetable oils
Linoleic acid	18:2(*n*-6)	All vegetable oils
α-Linolenic acid	18:3(*n*-3)	Linseed oil, soybean oil, Canola®
γ-Linolenic acid	18:3(*n*-6)	Evening primrose oil, blackcurrant, seed oil, borage oil
Arachidic acid	20:0	Groundnut oil
Gondoic acid	20:1(*n*-9)	High-erucic acid rapeseed (HEAR) oil
Behenic acid	22:0	
Erucic acid	22:1(*n*-9)	HEAR

Partial glycerides

The hydrolysis of triglycerides leads to the formation of FFAs and di- and monoglycerides: diacylglycerol and acylglycerol. Often, this hydrolysis is catalyzed by a lipase enzyme present in the raw material. This is the reason that palm fruit bunches are sterilized before the oil is extracted from the fruitlets and that rice bran profits from being heated in an expander/extruder as soon as possible. Both partial glycerides have two positional isomers: 1,2- and 1,3-diglyceride and 1- and 2-monoglyceride. The compounds with the fatty acids at the outer position are thermodynamically more stable. So, whereas statistically, the 2-monoglyceride fraction is 1/3, at equilibrium, it is only 10%.

Monoglycerides are so volatile that most will be removed from the oil during vacuum stripping. They can be synthesized by glycerolysis of triglycerides and then concentrated by molecular distillation. They are used as food grade emulsifiers and serve as starting material for various other emulsifiers and oleochemicals. Diglycerides remain in the oil during processing. They do not act as emulsifier but can be used in food as a kind of fat substitute for obese and diabetic patients.

Vegetable waxes

Oils containing waxes comprise sunflower seed oil, rice bran oil, corn germ oil, safflower seed oil, and grape-seed oil. Vegetable waxes are esters of fatty acids and fatty alcohols. Fatty alcohols are monohydric long-chain alcohols. If both the fatty acid and the fatty alcohol are saturated, the resulting wax will have a high (>70 °C) melting point as a result of which oils containing such waxes will throw an unsightly deposit when cooled. When the alcohol is unsaturated, the melting point of the wax is much lower and the wax will remain dissolved when the oil is cooled.

Wax compositions published in the literature are rather contradictory. They also depend on whether they refer to the crystalline wax fraction or the total wax content of the oil. However, there seems to be some kind of consensus that the main fatty acids in the crystalline waxes are 20:0, 22:0, and 24:0 and that the main alcohols are 24:0, 26:0, and 22:0. This

Table 2 Positional distribution of fatty acids in vegetable oils

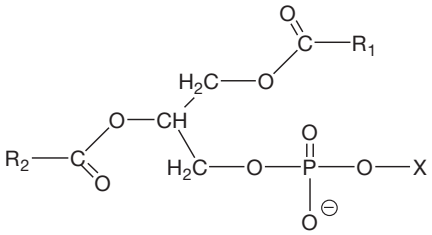
Oil	*Position*	*8:0*	*10:0*	*12:0*	*14:0*	*16:0*	*18:0*	*18:1*	*18:2*	*18:3*
Coconut[a]	1,3	11.4	9.1	32.7	22.2	13.2	4.0	5.6	1.1	
	2	2.9	1.1	78.2	10.2			5.9	2.0	
Palm oil[b]	1,3					62.0	8.0	27.5	2.5	
	2					11.0	2.0	65.0	22.0	
Soybean oil[c]	1					16.7	5.4	24.3	46.2	6.4
	2					2.2	0.3	23.4	68.1	5.7
	3					16.1	4.6	24.6	47.0	7.0

[a]*Source*: Caro, Y. et al. (2004). *European Journal of Lipid Science Technology* **106**, 503–512.
[b]*Source*: Table 3.162 in Gunstone, F. D., Harwood, J. L. and Padley, F. B. (eds.) (1994). *The lipid handbook* (2nd ed.). London: Chapman & Hall.
[c]*Source*: Table 2.28 in Gunstone, F. D., Harwood, J. L. and Dijkstra, A. J. (eds.) (2007). *The lipid handbook* (3rd ed.). Boca Raton, FL: CRC Press.

is to some extent confirmed by the carbon number composition of the waxes themselves that shows a range of 38–52 for the crystalline waxes and a somewhat lower range for the waxes that also contain unsaturated waxes and peaks of around 44. According to some authors, waxes also contain odd-numbered fatty acid and/or fatty alcohol chain lengths.

Phosphatides

Phosphatides are diglycerides that have been esterified with a phosphate group at the *sn*-3 position that itself can again be esterified with the hydroxyl group of compounds indicated as X: choline, ethanolamine, or inositol. The resulting phosphatides are called phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI). The structure in which R_1 and R_2 represent fatty acid chains is given in the succeeding text:



The phosphate group has a free hydroxyl group left that is quite acidic (p*Ka* < 3.5). In crude oil, it may have a metal counterion (potassium, calcium, or magnesium) or hydrogen. This hydrogen is titrated when the acidity of the oil is measured. When the group X is a hydrogen atom, the phosphatide is called phosphatidic acid (PA). It is formed during the drying, conditioning, and extraction of the oilseeds. If PA is present as a free acid or potassium salt, it will be removed from the oil by hydration on water degumming. If PA is present as a calcium or magnesium salt, this salt will remain in the oil on water degumming as a so-called nonhydratable phosphatide.

Besides PC, PE, PI, and PA, there are some minor phosphatides such as phosphatidylserine in which the phosphate group has been esterified with the amino acid serine. There are the lysocompounds in which one of the fatty acids moieties has been eliminated. There is the acetylphosphatidylethanolamine in which the amino group has been acetylated. There is the phosphatidylglycerol in which the phosphate group has been esterified with a glycerol moiety.

Unsaponifiable Fraction

The unsaponifiable fraction of vegetable oils can be isolated by extracting the soaps that result from oil saponification with an organic solvent (diethyl ether).

Tocopherols and tocotrienols

The general structural formula of the tocopherols is given below:

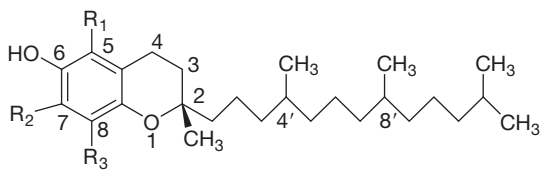
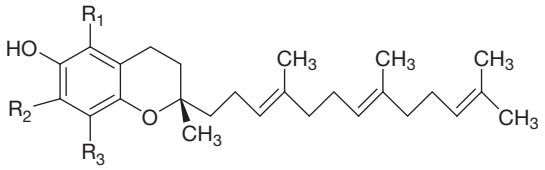


Table 3 Molecular structures of tocopherols and tocotrienols

Type	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

The benzene ring on the left has three substituents named R_1, R_2, and R_3. The extent to which these substituents are methyl groups determines the type in accordance with Table 3.

Accordingly, α-tocopherol, which has the highest vitamin E activity, carries three methyl substituents on its benzene ring and δ-tocopherol only one. The same nomenclature is valid for the tocotrienols illustrated below:



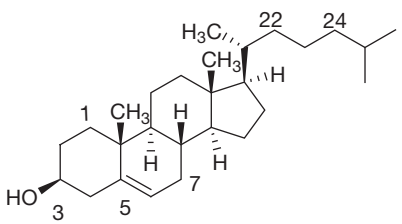
In addition to one or more methyl groups, the benzene ring also carries a hydroxyl group. This group can be esterified, but in vegetable oils, only the free tocopherols are present. Accordingly, they act as antioxidants according to the same mechanism as the synthetic antioxidants butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which also have a stereochemically hindered phenol group.

Vegetable oils vary widely in their tocopherol and tocotrienol contents as illustrated by Table 4.

Given their molecular weight of about 400 Da, which is not that different from that of monoglycerides, it is only to be expected that some tocopherols will be removed from the oil during vacuum steam stripping. The amounts depend of course on the initial concentration of the tocopherol and the stripping process conditions (relative amount of stripping medium, system pressure, and oil temperature). By using a two-stage condensing system, a condensate that is rich in tocopherols can be isolated. It can be used to enrich oils that are poor in tocopherols and thus increase their oxidative stability.

Phytosterols

Phytosterols are the sterols that occur in plants. Their molecular structure is similar to that of cholesterol, the main sterol in animals. Its structure has been represented in the succeeding text together with the position numbers that refer to the double bonds and substituents:



How these various phytosterols differ from cholesterol has been indicated in Table 5.

Table 4 Tocopherol and tocotrienol contents of various vegetable oils

	Tocopherols (mg/100 g)					*Tocotrienols (mg/100 g)*				
Oil	*α*	*β*	*γ*		*Total*	*α*	*β*	*γ*	*δ*	*Total*
Palm	26		32	7	65	14	3	29	7	53
Soybean	10		59	26	96					0
Canola	17		35	1	53					0
Sunflower	49		5	1	55					0
Coconut	0.5		0.6		1	0.5		2	0.6	3
Corn germ	11	5	60	2	78					0
Wheat germ	121	65	24	25	235	2	17			19
Olive	20	1	1		22					0
Rice bran	12	4	5		21	18	2	57		77

Source: Table 2.48 in Gunstone, F. D., Harwood, J. L. and Dijkstra, A. J. (eds) (2007). *The lipid handbook* (3rd ed.), Boca Raton, FL: CRC Press.

Table 5 Molecular structures of various phytosterols

Phytosterol	*Position of additional methyl group*	*Position of double bond in ring system*	*Position of double bond in side chain*	*Substituent in side chain*
Cholesterol	–	5	–	–
Brassicasterol	–	5	22	24-Methyl
Campesterol	–	5	–	24-Methyl
Stigmasterol	–	5	22	24-Ethyl
β-Sitosterol	–	5	–	24-Ethyl
Δ5-Avenasterol	–	5	–	24-Ethylidene
Δ7-Avenasterol	–	7	–	24-Ethylidene
Δ7-Stigmasterol or spinasterol	–	7	22	24-Ethyl
Citrostadienol	4	7	–	24-Ethylidene

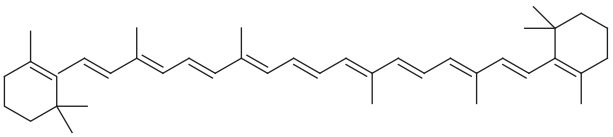
All phytosterols have a hydroxyl group at the 3-position. In vegetable oils, this group can be esterified with a fatty acid but if it is not, it is referred to as a free sterol. Like tocopherols, free phytosterols will also be partially removed from the oil during vacuum stripping; esterified sterols are insufficiently volatile and remain in the oil.

When the double bond in the sterol is saturated, the resulting compound is called a stanol. Cholesterol yields cholestanol on hydrogenation. Phytostanols are used in dietary fat products like margarine because they reduce the cholesterol absorption from food and thus lead to a somewhat reduced blood serum cholesterol content, which some people consider to be desirable.

Coloring compounds

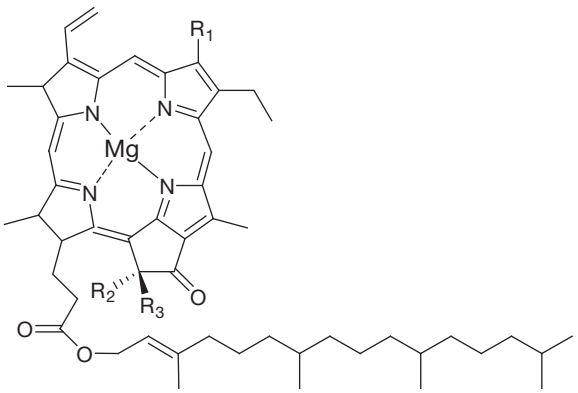
Vegetable oils contain two kinds of coloring compounds: carotenes and chlorophyll. The carotenes give the oils an orange–red color that is very pronounced in crude palm oil. The chlorophylls and their derivatives are responsible for the green color in crude oils.

The structural formula of β, β-carotene is shown below:



Its molecular formula ($C_{40}H_{56}$) and conjugated polyene structure show that it is a polyisoprene-based molecule. The structural formula given above relates to the synthetic, all-*trans* isomer. Crude vegetable oils may also contain isomers with one or more *cis* double bonds. β, β-Carotene is a precursor of vitamin A. In addition to this, vegetable oils can also contain hydroxyl-substituted compounds such as lutein, violaxanthin, and neoxanthin. All of them are thermally unstable. So, when oil containing these compounds is heated, they decompose in the so-called heat bleaching process.

Chlorophyll is an ester of a chlorophyllic acid and a phytol alcohol. Its structure is given in the next column:



The chlorophyllic acid moiety on the left contains a magnesium ion. If this has been replaced by two hydrogen ions,

Table 6 Molecular structures of various chlorophyll-related compounds

Derivative	*Type*	R_1	R_2	R_3
Chlorophyll	α	CH_3	CO_2CH_3	H
	α′	CH_3	H	CO_2CH_3
	β	CHO	CO_2CH_3	H
	β′	CHO	H	CO_2CH_3
Pheophytin	α	CH_3	CO_2CH_3	H
	α′	CH_3	H	CO_2CH_3
	β	CHO	CO_2CH_3	H
	β′	CHO	H	CO_2CH_3
Pyropheophytin	α	CH_3	H	H
	β	CHO	H	H

Source: Zschau, W. (2000). Bleaching. In: O'Brien, R. D., Farr, W. E. and Wan, P. J. (eds). *Introduction to fats and oils technology*. Champaign, IL: AOCS Press.

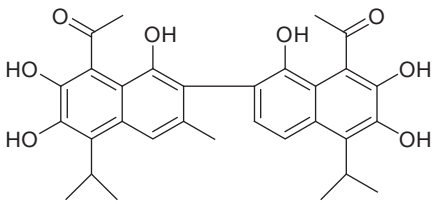
the resulting compound is called pheophytin. The structure contains several groups indicated by R_1, R_2, and R_3. Their nature determines the chlorophyll derivative in accordance with Table 6.

In this table, the α- and β-types differ with respect to the R_1 substituent. In the α-type, this is a methyl group, whereas in the β-type, this is a formaldehyde group. The difference between the types without and with prime is stereochemical with respect to the dimethyl ether group.

Miscellaneous unsaponifiable compounds

The presence of unsaponifiable compounds necessarily precedes their molecular identification, but they could have been given a name when isolated. So shea butter, 'beurre de karité' in French, was found to contain a substantial unsaponifiable fraction that did not dissolve in acetone. It was given the name 'karitene,' but this name does not stand for a compound in true chemical sense; it is the name given to a fraction that is highly unsaturated and probably consists of a mixture of different polyisoprenes.

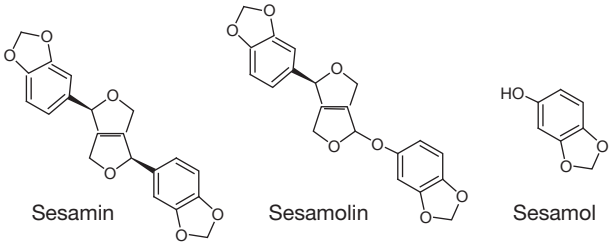
An unsaponifiable compound that is specific for cottonseed oil is gossypol; its molecular structure is shown below:



The systematic name of this compound is 1.1′,6,6′7,7′-hexahydroxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)-[2,2′bi-naphthalene]-8,8′-dicarboxaldehyde. Most of the gossypol remains in the meal, but the (±)-form in cottonseed is toxic to non-ruminants. It is removed from the oil during the neutralization process since the many phenolic hydroxyl groups react with the alkali.

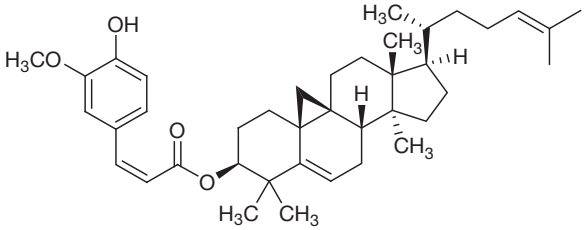
Cottonseed oil also contains some fatty acids (sterculic acid and malvalic acid) that contain a cyclopropene ring. This feature is used for the detection of cottonseed oil by the so-called Halphen test. Kapok seed oil also contains these cyclopropenoid acids and also gives a positive Halphen test.

Sesame oil contains two unsaponifiable compounds that are typical for this oil: sesamin (0.5–1.1%) and sesamolin (0.3–0.6%). The latter compound can release sesamol during oil processing. The molecular structures of these compounds are given in the succeeding text:

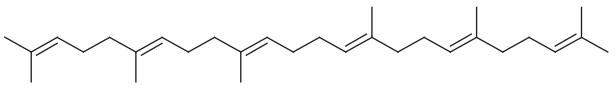


The presence of sesamol can be detected by the reaction of Baudouin in which the oil is treated with hydrochloric acid and an ethanolic solution of furfural; a red color results. For a long time, Belgian margarine had to contain 2% of sesame oil by law to enable the adulteration of butter with margarine to be detected.

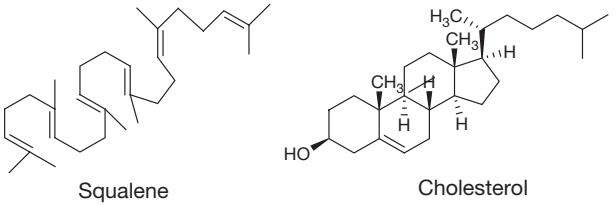
Rice bran oil also contains unsaponifiable compounds that are particular to this oil: oryzanols. They are esters of ferulic acid (4-hydroxy-3-methoxycinnamic acid) and cycloartenol (oryzanol A) or 24-methylenecycloartanol (oryzanol B). The structure of oryzanol A is given in the succeeding text:



Hydrocarbons cannot be saponified. Vegetable oils can contain various hydrocarbons not all of which have been identified. A hydrocarbon of which the structure has been determined is squalene:



Its systematic name is (6*E*,10*E*,14*E*,18*E*)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene. Squalene is found in several vegetable oils in variable amounts. Olive oil contains 700–1200 ppm of squalene, which is about half the total unsaponifiable. Squalene is the precursor of phytosterols, which becomes clear when the molecular structure is represented in a different manner next to cholesterol, as shown below:



Analysis of Vegetable Oils

Because oils and fats are traded and often have to meet specifications, it is necessary to use standard analytic methods for these

specification parameters. Consequently, what is now the American Oil Chemists' Society (AOCS) started as the Cotton Products Analysts. The AOCS therefore publishes 'AOCS Official Methods and Recommended Practices' after having tested proposed methods or changes, by having them evaluated by several laboratories; other national societies like the DGF (Deutsche Gesellschaft für Fettforschung) and countries also publish their own methods.

Points and Values

When early analytic methods became established, their results were expressed as 'points' or 'values.' Points refer to temperatures and values have other units.

Slip melting point

Oils and fats are mixtures of a large number of different triglycerides. Therefore, they do not have a sharp melting point but a melting range. Fats are also polymorphic, and different polymorphs have different melting points. Consequently, the temperature at which the fat is completely molten depends on how this melting point is determined. The agreed method to determine the 'slip melting point' measures the temperature at which a column of fat of specified length starts to rise in an open capillary tube under the influence of a hydrostatic pressure by placing the capillary in a water bath that is heated.

Cloud point

The cloud point of an oil is the temperature at which the oil becomes hazy when it is cooled down. This temperature is especially important for oils that could solidify during transport, and it is also used when estimating the wax content of oil to be winterized. Strict adherence to the method is essential for attaining a reasonable repeatability.

Smoke point

The smoke point is the temperature at which smoking is first detected when the oil is heated in a special cabinet with special illumination. The smoke point decreases from normal values $>200^{\circ}C$ when the FFA content of the oil increases as during deep fat frying.

Acid value

The acid value of an oil is determined by titrating a solution of the oil in diethyl ether with an alcoholic solution of sodium or potassium hydroxide. It is expressed as the amount of KOH (in mg) to neutralize 1 g of oil.

Often, the acid value is converted to an FFA content by multiplying the acid value with a factor that equals the molecular weight of the fatty acid concerned (usually oleic acid, MW = 282.4) divided by ten times the molecular weight of the potassium hydroxide (56.1). This factor ten stems from the fact that the acid value is expressed as mg/g, whereas the FFA content is expressed as a percentage. When the FFA content is expressed as 'wt% oleic acid,' this factor therefore equals 0.50.

Saponification value

Whereas the acid value only takes FFAs into account, the saponification value (SV) refers to the FFA plus any ester bonds present in the sample. It is determined by saponifying a known amount of sample with a known amount of ethanolic potassium hydroxide and titrating the excess of potassium hydroxide with hydrochloric acid. Like the acid value, the SV is also expressed as mg KOH per gram of oil.

The SV is an indication of the average molecular weight of the oil. HEAR with relatively few ester bonds per unit of weight has therefore a low SV of 174–176. Oils with predominantly fatty acids with 18 carbon atoms like sunflower seed oil or soybean oil have an SV of 191–194, whereas lauric oils like coconut oil and palm kernel oil have SVs of 255–270 mg KOH per gram of oil. Because of these differences, it is quite understandable that the SV determination has been an essential tool in vegetable oil characterization.

Ester value

The ester value of an oil is not determined directly. It is calculated as the difference between the SV and the acid value. It is not much used.

Hydroxyl value

The hydroxyl value (HV) of an oil is an indication of its hydroxyl group content. The hydroxyl groups can be part of fatty acid moieties such as ricinoleic acid (9*Z*,12*R*)-12-hydroxyoctadecenoic acid, but they can also be part of the glycerol moiety when this is not esterified as in partial glycerides. So, in oils without fatty acids with hydroxyl groups, the HV is an indication of the partial glyceride content.

The HV is determined by acetylating the free hydroxyl groups with an excess of acetic acid anhydride, hydrolyzing the excess, and determining the acetic acid content of the final reaction mixture. As usual in this kind of determination, a blank is used to avoid systematic errors. The HV is also expressed as mg KOH per gram of oil.

Iodine value

The IV is perhaps the most often quoted value of an oil. It is a measure of the degree of unsaturation. It is determined by weighing an amount of sample, the mass of which depends on the expected IV. When this value is low (0–5 g I_2/100 g oil), a sample mass of about 3 g is appropriate, whereas when the IV > 130, a smaller amount of 0.10 g suffices. The sample is dissolved in 25 ml tetrachloromethane, and then, 25 ml of Wijs solution is added. This solution of iodine monochloride (ICl) in glacial acetic acid is commercially available as such. After mixing, the reaction mixture is allowed to stand in the dark for 1 or 2 h for the addition of the ICl to the double bonds to take place.

After standing, an amount of 20 ml of an aqueous 10% potassium iodide solution is added with some (150 ml) additional water after which the iodine content of the mixture is determined by titration with sodium thiosulfate. By carrying out a blank determination with the same amounts of reagents, the amount of iodine added to the double bonds in the sample can be calculated by difference.

The IV is actively used in the hydrogenation of vegetable oils. Its value describes the extent to which an oil has been hydrogenated. Fully hydrogenated oils specify an IV of <1. The

IV is also used to characterize an oil so as to have an idea about its likely stability.

Peroxide value

When oil is stored, it can deteriorate by oxidation. Since one of the early oxidation products is a peroxide, the peroxide value (PV) of an oil provides information how fresh the oil is. It is determined by allowing the peroxides in the oil to liberate molecular iodine from a saturated solution of potassium iodide. The liberated iodine is then titrated with sodium thiosulfate. The PV is expressed as mmol of active oxygen per 2 kg of oil (Wheeler method) and of per 1 kg of oil (Sully method).

Anisidine value

When primary oxidation products decompose, they can form aldehydes and ketones. The former, especially 2,4-dienals and 2-alkenals, can be determined by allowing them to react with *p*-anisidine. The reaction product is yellow, so its amount is determined by measuring its absorbance at 350 nm against a blank in the reference cell (E_b). The absorbance of the oil itself is also measured against pure isooctane in the reference cell (E_a). The anisidine value is then calculated according to

$$p\text{-AV} = \frac{25 \times (1.2 \times E_b - E_a)}{W}$$

wherein W stands for the mass of the sample in grams. To distinguish the anisidine value from the acid value, the former is often written as *p*-AV so that the AV refers to the acid value.

Totox value

The Totox value is defined as the sum of the anisidine value and two times the PV (p-AV + 2 PV). It is an arbitrary value to indicate the oxidative load an oil has had to suffer.

Miscellaneous values

When it was not yet possible to determine a fatty acid composition by GLC, other methods were used to get an idea about this composition. The Reichert–Meissl value indicates the number of ml 0.1 N lye that is required to neutralize the water-soluble fatty acids that have been volatilized with water vapor from a 5 g fat sample. The Polenske value is similar in that it also indicates how many ml 0.1 N lye are required to neutralize certain fatty acids from a 5 g sample, but the difference is that the Reichert–Meissl value is concerned with the water-soluble fatty acids (butyric acid and caproic acid), whereas the Polenske value titrates the volatile fatty acids that do not dissolve in water (caprylic acid and capric acid).

The determinations of these values are difficult to repeat, and the outcomes are not an accurate representation of the fatty acids concerned. In fact, these values are well and truly obsolete, but in the past, they have been useful to establish the adulteration of butter with margarine.

The Crismer value measures the solubility of an oil in a standard solvent mixture of *t*-amyl alcohol, ethanol, and water (5:5:0.27 by volume). Values are characteristic for each oil since this solubility depends on degree of unsaturation and fatty acid chain length. It was used in international trade, mostly in Europe.

Instrumental Analytic Methods

Some instrumental analytic methods have been used for a long time. The refractive index of an oil can be measured with the Abbe refractometer to indicate the degree of unsaturation of a vegetable oil. The higher its IV, the higher the index. It can be used to follow the progress of a hydrogenation batch.

When UV spectrophotometers became available, they started to be used in the determination of the fatty acid composition. Conjugated dienes and trienes absorb at 232 and 268 nm, respectively. So, after conjugation of the polyunsaturated fatty acids, their content could be quantified. The spectrophotometric method can also be used to assess oil deterioration.

Chromatographic Methods

The advent of GLC greatly facilitated lipid analysis. It is fast, reproducible, and quantitative. In the early days of GLC, packed columns were used and their performance only allowed a fatty acid analysis. Consequently, research in many field focused on the fatty acid composition of lipids rather than on their triglyceride composition. The hydrogenation process is a typical example of this focusing. This focus on fatty acids rather than on triglycerides also affected nutritional studies in which insufficient attention was given to the fact that in the body, fatty acids on the outer position of a triglyceride are digested through a different mechanism as those on the middle position.

Later, with capillary columns, GLC could also tackle triglyceride analysis and distinguish between fatty acid isomers and *trans* fatty acids. Progress was made in the analysis of triglycerides by GLC for the determination of the carbon number composition, and this complemented the analysis HPLC determination of the partition number composition. These and other chromatographic methods are available from *The Lipid Library* (http://lipidlibrary.aocs.org/analysis.html).

Nuclear Magnetic Resonance

Two routine measurements make use of nuclear magnetic resonance methods. One is the low-resolution pulse NMR method to determine the SFC of a mixture of solid, crystalline fat, and liquid oil. It is used intensively to follow the partial hydrogenation of vegetable oils and for specifying fat blends to be used in margarines and shortenings, and it also plays a vital role in confectionery fats.

The method involves melting the fat sample at 80 °C in a special glass tube that fits the NMR spectrometer, tempering the sample at 60 °C for at least 5 min, cooling it for a set period of 60 min at 0 °C, and transferring it to the measuring temperature and keeping it there for 30–35 min before transferring it to the NMR instrument. This sounds very labor-intensive, but the transfers of the glass tubes to the various thermostatic baths and the measuring instrument can be fully automated by laboratory robotics.

For cocoa butter and other fats like cocoa butter equivalent having a high content of symmetrical monounsaturated triglycerides, the thermal treatment of the sample is slightly

different in that it also comprises a tempering stage. After having been placed in the 0 °C bath for 90 min, the samples are tempered at 26 °C for 40 h. Then, they are cooled again at 0 °C for another 90 min before being brought to the measuring temperature where they are kept for 60 min before being transferred to the NMR instrument.

Another NMR method used in lipid chemistry uses the ^{31}P NMR spectrometer for the determination of phosphatide compositions. It uses triphenyl phosphate as internal standard and thus arrives at absolute values for the contents of the various phosphatides. Often, these contents do not account for the total phosphorus content of the sample as determined by inductively coupled plasma emission spectroscopy (ICP-ES) (see later). The reason for this discrepancy is not known, but the presence of phytates has been suggested and after all, you do not need much phytates to introduce a lot of phosphorus.

Inductively Coupled Plasma Emission Spectrometry

Originally, the analytic method using ICP was used to determine the trace element contents of aqueous samples, for instance, effluents. In 1982, the use of this ICP equipment to measure the concentration of these elements in oils was published, and now, the method has become a standard tool for the control of the degumming process, and it has been incorporated in AOCS compendium of analytic methods that can be used when specifying oil in trading contracts.

Oil Stability Index

The oil stability index (OSI) of an oil is determined with a piece of equipment called Rancimat®. In the equipment, samples of oil are kept at a set temperature, and air is bubbled through the sample at a set rate. The air leaving the sample is bubbled through distilled water, the conductivity of which is monitored. In the early stages of the measurement, this conductivity hardly changes, but after a certain period, called the 'induction period,' it increases because the air has stripped volatile acids like formic acid from the oil sample. The conductivity curve shows an inflection and the point in time where this extrapolated inflection commences is called the 'OSI'; it is expressed in hours, and the temperature at which the experiment was carried out should be mentioned as well.

The OSI has been found to be determined by the fatty acid composition of the sample and its antioxidant content. Experiments have been carried out in which oil was stored under different conditions and its OSI was measured at set intervals. Sometimes, the OSI did not alter from one interval to the other. This allows the conclusion to be drawn that the OSI does not predict shelf life. All it can do is reveal batches that deviate from normal.

See also: Carotenoids: Occurrence, Properties and Determination; Choline: Physiology; Chromatography: Focus on Multidimensional GC; Chromatography: High-Performance Liquid Chromatography; Colors: Properties and Determination of Natural Pigments; Fats: Classification and Analysis; Fatty Acids: Fatty Acids; Fatty Acids: *Trans* Fatty Acids; Oxidation of Food Components; Phospholipids: Properties and Occurrence; Quality Control in Food Processing; Rapeseed Oil/Canola; Sunflower Oil; Tocopherols: Properties and Determination; Triacylglycerols: Structures and Properties; Triacylglycerols: Characterization and Determination.

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Vegetable Oils: Dietary Importance

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Introduction

Vegetable oils are obtained from plants, such as seeds, nuts, fruits, and vegetables, and are the major source of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) in the diet. Three of them are rich sources of saturated fatty acids: coconut oil, cocoa oil, and palm oil. Cocoa oil is the first ingredient in ice cream produced by the industry, and palm oil is the usual oil in fast-food preparation. They are also a dietary source of fat-soluble vitamins and antioxidants. Oils made from walnuts, almonds, olives, sesame seeds, and flax seeds have been used for 5000–6000 years for several purposes. Olive oil is known as the oldest vegetable oil as this is concluded from residues of olive oil found in huge clay pots in the Minoan palaces in Crete and from excavations in Tunis.

The worldwide per capita total supply of vegetable oils and certain specific ones during the past 50 years is shown in Figure 1. This figure clearly shows that the worldwide supply of vegetable oils (kcal capita^{-1} day^{-1}) has more than doubled during this period, and this is mainly due to the increased supply of sunflower oil, soybean oil, palm oil, and rape and mustard oil. On the contrary, the per capita consumption of olive oil has remained relatively stable at the international level over this period. Figure 2 presents the food supply (kcal capita^{-1} day^{-1}) of the five most commonly used vegetable oils in 2011 in different parts of the world, expressed as percentage of total per capita daily supply of vegetable oils. Obviously, the supply of vegetable oils varies substantially in different parts of the world; the supply of soybean oil predominates in the Americas, in Northern and Southern Africa, and in Eastern Asia. The supply of sunflower oil predominates in Southern Africa, Central and Western Asia, and Eastern Europe. Rape and mustard oil is mainly used in Northern Europe and Oceania. Palm oil is mainly used in Eastern, Central, and Western Africa and Southeastern Asia. Olive oil remains the most widely used vegetable oil in Southern Europe, whereas it is also supplied in Northern Africa and Western Asia in lower amounts.

Vegetable oils are extracted from oilseeds by pressing using an expeller or cold press, centrifugation, or solvent extraction. Some oils, such as virgin olive oil, are extracted and used with mechanical pressure under controlled temperatures without any further treatment. Most vegetable oils, however, are refined to some extent in order to remove undesirable contents such as free acids and oxidized materials and alter their properties such as color and odor. Refinement enables the production of edible oils improved in terms of taste, smell, appearance, and stability enabling a longer shelf life. Refinement, however, alters the fatty acid composition of vegetable oils. Cold-pressed olive oil and other vegetable oils such as sunflower have been reported to have a lower linoleic acid (LA)/alpha-linolenic acid ratio (ALA) as compared with refined vegetable oils and a higher amount of oleic acid. Similarly, β-carotene was present in cold-pressed vegetable oils but is absent in the refined ones.

Hydrogenation of vegetable oils is a process used in order to produce semisolid or solid fats from vegetable oils. During the process of partial hydrogenation, *trans*-fatty acids (TFAs) are produced that have adverse health effects, and therefore, alternative methods of converting vegetable oils to solid fats have been introduced. Interesterification, that is, rearrangement of fatty acids within a triacylglycerol, is a process now widely used, although there are conflicting data regarding possible adverse health effects on lipoprotein and serum glucose metabolism and hemostasis parameters. More recently, concerns have been raised, as it has been shown that the substitution of interesterified fat for partially hydrogenated fat in the maternal diet of mice poses an increased risk for offspring in adult life. There is also evidence that interesterification may affect obese and nonobese adults' metabolic state in a different way.

Vegetable oils should be stored in dark, cool, and dry places since they are sensitive to heat, light, and exposure to oxygen. Vitamin E is particularly sensitive to sunlight. Storing edible oils in refrigerator may cause them to thicken or become cloudy, although this is reversed when left in room temperature.

One important property of vegetable oils is the smoke point, that is, the temperature that a given oil begins to smoke. In general, olive oil – similarly to butter and lard – has a lower smoke point. The smoke point of vegetable oils is affected by the degree of refinement; more refined vegetable oils have a higher smoke point. On the other hand, the longer a vegetable oil has been exposed to heat, the lower its smoke point, and therefore, the use of the same vegetable oil repeatedly should be avoided in cooking.

Nutrient Composition of Vegetable Oils

Vegetable oils are mainly composed of triacylglycerols, that is, esters of fatty acids with glycerol, but they also contain free fatty acids, monoacylglycerols, diacylglycerols, and nonglyceridic nutrients. The fatty acid composition of vegetable oils varies, and most edible oils are high in MUFA or PUFA, whereas three of them are high in SFA. Nevertheless, the rapid increase in vegetable oils consumption observed during the past 100–150 years represents the most important factor that contributed to the elevation of dietary omega-6-to-omega-3 ratio to its current value of 20–30 compared with a ratio of 2 in the hunter-gatherer diets.

The composition of vegetable oils in fatty acids varies depending on several factors including climate, soil, fruit, seed or plant maturity, genetic variation of the plant, and degree of refinement. It should also be stressed that processed vegetable oils, particularly the partially hydrogenated ones,

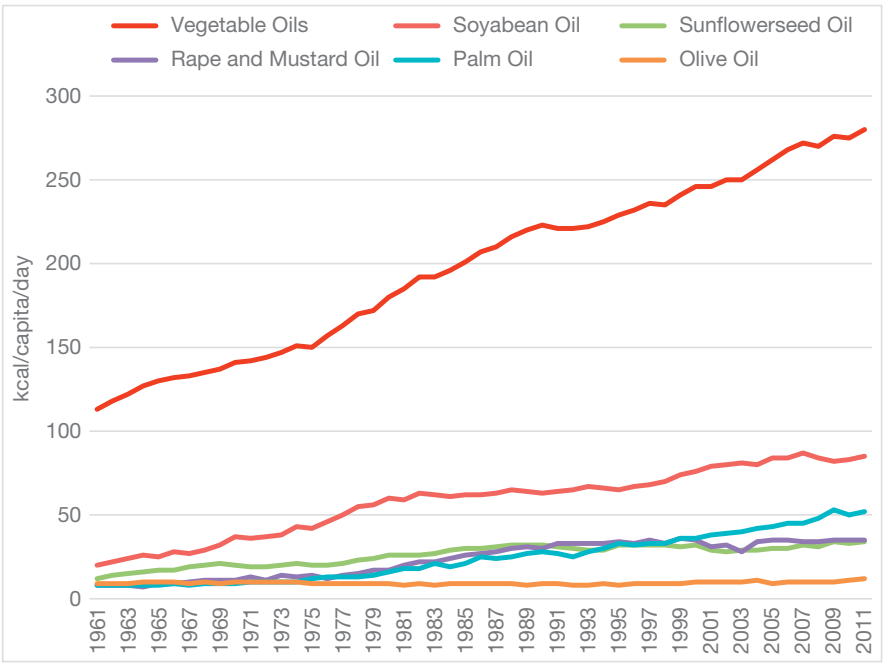

Figure 1 Food supply (kcal capita^{-1} day^{-1}) of vegetable oils in the world between the years 1961 and 2011. Food and Agriculture Organization of the United Nations. Statistics Division (FAOSTAT). http://faostat3.fao.org/home/E. Accessed on 20/12/2014.

also contain considerable amounts of TFA. In Table 1, the composition of certain vegetable oils in total saturated fatty acids (SFAs), PUFA and MUFA, and the essential LA and ALA is presented. The USDA National Nutrient Database for Standard Reference was used as a reference for this table. Blends of vegetable oils and those processed for industrial use were not included in this table. It is obvious from Table 1 that SFAs are the predominant fatty acids in certain vegetable oils (coconut, palm, babassu, and cupu assu) ranging from 49–86%, which is higher than the SFAs in lard (pork fat) and beef tallow. Olive oil, avocado oil, and hazelnut oil are richer in MUFA along with the high-oleic sunflower and safflower oils. The composition of vegetable oils in PUFA varies considerably, but the more favorable ones in terms of LA/ALA ratio are cold-pressed flaxseed oil (LA/ALA ratio of 0.3, with ALA content of 53.4 g/100 g), canola oil (LA/ALA ratio of 2.1), and mustard oil (LA/ALA ratio of 2.6).

Other minor constituents present in vegetable oils include pigments, flavor compounds, hydrocarbons, sterols, phenols, and vitamins. Some of these constituents are present in crude oils, whereas in refined ones, constituents such as phenols may be removed. Table 2 presents the nonglyceridic nutrients most consistently present in the selected vegetable oils derived from the USDA National Nutrient Database for Standard Reference. It should be underlined that minor differences exist between both the glyceridic and nonglyceridic nutrients presented here from USDA database and other locally produced vegetable oils.

Nutritional Value of Vegetable Oils

Dietary fat contributes to the texture, flavor, and aroma of a wide variety of foods. Foods high in fat content are considered the most palatable ones. Dietary fat has a direct impact on taste perception activating a large network of brain areas including the somatosensory cortices and thalamus that control taste.

Although most fatty acids may be synthesized in the body, two are unable to synthesize and are thus called essential fatty acids, which are the omega-6 LA and the omega-3 ALA polyunsaturated fatty acids. A balanced diet is necessary in order to achieve sufficient intake of energy and nutrients for optimal growth, health, and well-being. Fatty acids are important nutrients because they represent a major source of energy; after absorption, fatty acids are converted to energy via β-oxidation or are stored in adipose tissue for later use. Fats and oils enable food digestion and facilitate the absorption of vitamins and phenols.

Fatty acids are important structural molecules in cell membranes, and neural tissues such as the brain are particularly rich in long-chain PUFA (LCPUFA). LCPUFA are also precursors of eicosanoids, that is, inflammatory mediators, mediating platelet aggregation, leukocyte migration, vasoconstriction, vasodilation, blood pressure, bronchial constriction, apoptosis, and oxidative damage. Fatty acids play an important role in cholesterol metabolism, even from an early age, and thus may be associated with cardiovascular disease later in life. Certain fatty acids and drugs affecting lipid metabolism activate peroxisome proliferator-activated receptors (PPARs), which in turn control the expression of genes implicated in intra- and extracellular lipid metabolism, implicating, thus, a role of these fatty acids in the regulation of energy partitioning, insulin sensitivity, adipocyte development, and neural function throughout the life span.

It is now widely accepted that dietary SFAs increase serum LDL-cholesterol levels and thus increase the risk for CHD. Controlled trials and observational studies provide robust

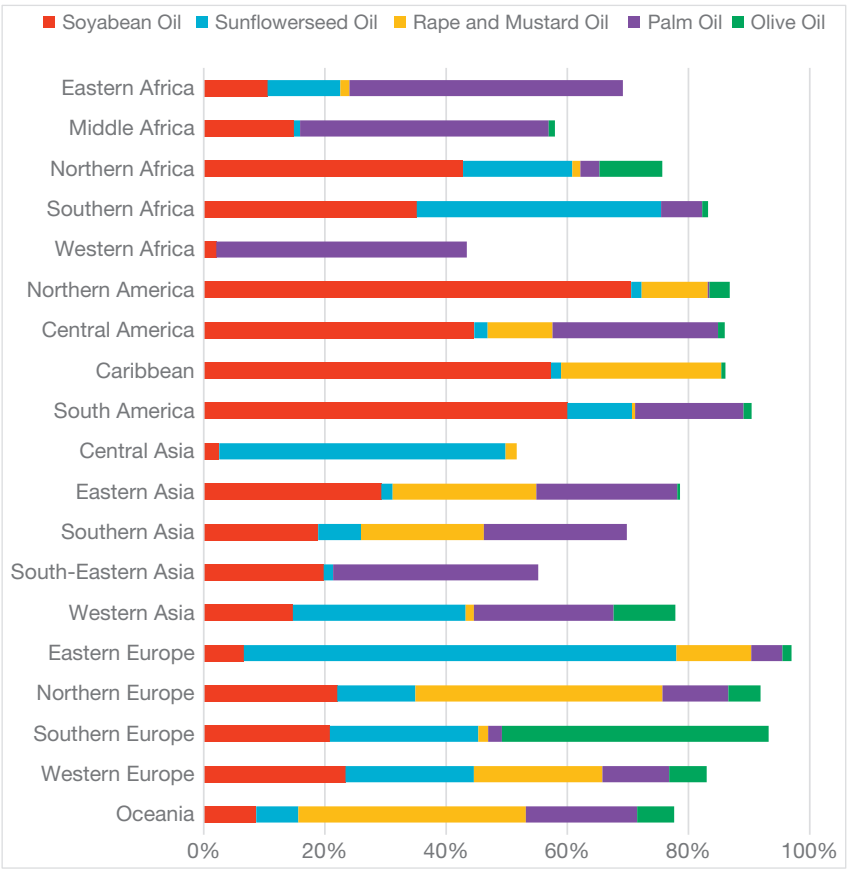

Figure 2 Food supply (kcal capita^{-1} day^{-1}) of five most commonly used vegetable oils expressed as percentage of total daily supply of vegetable oils in different parts of the world in 2011. Food and Agriculture Organization of the United Nations. Statistics Division (FAOSTAT). http://faostat3.fao.org/home/E. Accessed on 20/12/2014.

evidence that consumption of TFA from partially hydrogenated oils adversely affects cardiovascular disease risk factors and increases the risk for cardiovascular disease, perhaps because TFAs act as proinflammatory mediators and induce endothelial dysfunction. A substantial body of evidence suggests that the consumption of diets rich in the long-chain omega-3 PUFA (eicosapentaenoic acid or EPA and docosahexaenoic acid or DHA) or supplements containing these fatty acids is beneficial in inflammatory diseases, cardiovascular disease, and autoimmune diseases. A recent meta-analysis has shown an inverse association between marine omega-3 LCPUFA intake and risk for breast cancer. Particular attention is given upon the intake of LA and the ratio of LA/ALA. It has been shown that sunflower oil rich in LA and high in LA/ALA ratio may predispose to metabolic syndrome. Substantial debate exists in relation to LA intake and cardiovascular disease risk. Nevertheless, it appears that the benefit of LA intake is with substituting LA for SFA in the diet in significantly reducing the risk for CVD. Experimentally, it has been shown that in Wistar rats, a diet supplemented with garden cress oil and its blended oils enriched with ALA significantly modulated fatty acid profile in various tissues increasing content in EPA, DHA, and ALA and decreasing LA and arachidonic acid (AA). At the same time, this diet significantly decreased serum total cholesterol, LDL cholesterol, and triglycerides. However, other studies in humans failed to show any association of the LA/ALA ratio in predicting cardiovascular disease morbidity and mortality.

Sterols comprise an important fraction of the nonglyceridic part of vegetable oils, and they are essential components of cell membranes. When present in foods, sterols efficiently reduce the absorption of dietary and biliary cholesterol, thus enabling reducing serum LDL-cholesterol levels by up to 10%. It should be noted, however, that oil refinement may substantially result in sterol loss by 10–70% depending on the oil and refinement method. Interestingly, beta-sitosterol is thought to have some anticarcinogenic properties, an issue that is still unclear.

Vegetable oils are also a rich source of vitamin E, with known antioxidant properties. The human diet contains eight different vitamin E-related molecules synthesized by plants, but vitamin E is usually expressed as alpha-tocopherol, which is the most active form. Again, processing of vegetable oils, particularly heat-treated oils, results in reduction of tocopherol content. Alpha-tocopherol serves as a peroxyl radical scavenger that protects polyunsaturated fatty acids in membranes and lipoproteins.

Phenols are important natural antioxidants, which are mainly present in olive oil. They bind free radicals, forming phenol radicals, thus preventing lipid tissues from oxidation. More specifically, phenols prevent LDL-cholesterol oxidation and thus prevent atherosclerotic plaque formation. Thus,

Table 1 Fatty acid composition of vegetable oils (g/100 g of vegetable oils)

Oil	*Total SFA*	*Total MUFA*	*Total PUFA*	*LA*	*ALA*
Grams/100 g					
Almond oil	8.2	69.9	17.4	17.4	0.0
Apricot kernel oil	6.3	60.0	29.3	29.3	0.0
Avocado oil	11.6	70.6	13.5	12.5	1.0
Babassu oil	81.2	11.4	1.6	1.6	0.0
Canola oil	7.4	63.3	28.1	19.0	9.1
Coconut oil	86.5	5.8	1.8	1.8	0.0
Cottonseed oil	25.9	17.8	51.9	51.5	0.2
Cupu assu oil	53.2	38.7	3.8	3.8	0.0
Flaxseed oil	9.0	18.4	67.8	14.3	53.4
Grapeseed oil	9.6	16.1	69.9	69.6	0.1
Hazelnut oil	7.4	78.0	10.2	10.1	0.0
Mustard oil	11.6	59.2	21.2	15.3	5.9
Oat oil	19.6	35.1	40.9	39.1	1.8
Olive oil	13.8	73.0	10.5	9.8	0.8
Palm oil	49.3	37.0	9.3	9.1	0.2
Peanut oil	16.9	46.2	32.0	32.0	0.0
Poppyseed oil	13.5	19.7	62.4	62.4	0.0
Rice bran oil	19.7	39.3	35.0	33.4	1.6
Safflower oil, high oleic	7.5	75.2	12.8	12.7	0.1
Safflower oil, linoleic over 70%	6.2	14.3	74.6	74.6	0.0
Sesame oil	14.2	39.7	41.7	41.3	0.3
Shea-nut oil	46.6	44.0	5.2	4.9	0.3
Soybean oil	15.7	22.8	57.7	51.0	6.8
Sunflower oil, high oleic	9.9	83.7	3.8	3.6	0.2
Sunflower oil, linoleic less than 60%	10.1	45.4	40.1	39.8	0.2
Tomato-seed oil	19.7	22.8	53.1	50.8	2.3
Walnut oil	9.1	22.8	63.3	52.9	10.4
Wheat germ oil	18.8	15.1	61.7	54.8	6.9

Note: Blends of vegetable oils and those processed for industrial use were not included in this table.

US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 27. Version Current: August 2014. (Available from http://www.ars.usda.gov/Services/docs.htm?docid=24912.)

phenols have been acknowledged for their benefits in cardiovascular health and aging and their anti-inflammatory and anticancer activities. Vitamin K, an important component of four coagulation factors, is present in some vegetable oils in varying amounts.

The Role of Selected Edible Vegetable Oils in the Prevention of Chronic Diseases

Olive Oil

Olive oil as already mentioned is the oldest known edible oil. Extra virgin olive oil has been routinely used in the traditional Mediterranean diet and is mainly produced and used in Mediterranean countries of Europe and Northern Africa, although recently, it is increasingly used in Northern Europe and North America. It is mainly used as a salad dressing and in cooking. The pioneer Seven Countries Study concluded that populations consuming this traditional Mediterranean diet experienced low coronary heart disease (CHD) and cancer mortality compared with populations consuming a "Western diet." The Mediterranean diet has been shown to reduce the secondary complications, recurrence, and even death after first myocardial infection. The typical Mediterranean diet was rich in fruits, vegetables, legumes, whole-wheat bread, nuts, fish, and extra virgin olive oil as the main source of dietary fat. The consumption of olive oil in the traditional diet of the island of Crete was over 90 g per person per day, which was around one-third of the total daily energy intake.

The fatty acid composition of olive oil depends on several factors such as latitude of production, climate, variety, and maturity of the fruit. It has been shown, for instance, that olive oil from Tunisia has a lower content in MUFA and higher content in palmitic acid compared with olive oil from Mediterranean countries of Europe. The fatty acid composition of olive oil is SFA 14 g/100 g, MUFA 73 g/100 g, and PUFA 11 g/100 g mainly as LA. Olive oil has also important nonglyceridic content. Olive oil is the only edible oil with squalene, which is the precursor of sterols. It also contains sterols, tocopherols, and carotenoids. It should be noted that processing of olive oil may reduce tocopherol content. The high concentration of oleic acid in olive oil renders it resistant to lipid peroxidation. Moreover, the high content of alpha-tocopherol along with the low PUFA content preserves oxidation of olive oil.

The unique composition of olive oil, which is rich in MUFA and antioxidants, leads to a reduction of serum LDL cholesterol and the preservation or even increase of HDL-cholesterol levels. A recent meta-analysis documented the beneficial health effect of MUFA and particularly oleic acid on health status. This meta-analysis showed an overall risk reduction of all-cause mortality (11%), cardiovascular mortality (12%), cardiovascular events (9%), and stroke (17%) in the highest compared with the lowest third in MUFA, oleic acid, and olive oil consumption.

Minor constituents of olive oil are also suggested to play an important role in cardiovascular disease prevention independent of olive oil's fatty acid content. It has been shown that phenolic-enriched virgin olive oil helps to decrease LDL cholesterol and, moreover, olive oil phenolic compounds appear to protect LDL against peroxyl radical-dependent and metal-induced oxidation *in vitro*, an effect that is more pronounced in virgin olive oil than refined olive oil. The higher phenolic content of extra virgin oil appears to reduce postprandial inflammatory markers such as TXB(2) and LTB(4) 2 and 6 h after the meal compared with refined olive oil.

It is argued that a substantial proportion of cancers such as colorectal, breast, pancreas, prostate, and endometrial cancers may be prevented with the adoption the Mediterranean diet. A relatively recent meta-analysis of 19 case–control studies concluded that higher intake of olive oil is associated with lower odds of having any type of cancer irrespective of the country of origin. On the other hand, an older meta-analysis examining the association of breast cancer risk with biomarkers of fatty acids intake showed a positive association of breast cancer risk with oleic acid in cohort studies but failed to show any association in case–control studies although a protective role of ALA for breast cancer risk was identified. It should be stressed that the latter meta-analysis was based on

Table 2 Nonglyceridic composition of vegetable oils

Oil	*Choline, total (mg/100 g)*	*Phytosterols (mg/100 g)*	*Vitamin E (alpha-tocopherol) (mg/100 g)*	*Vitamin K (phylloquinone) (μg/100 g)*
Almond oil	0.4	266	39.2	7.0
Apricot kernel oil		266	4.0	
Avocado oil				
Babassu oil		95	19.1	
Canola oil	0.2		17.5	71.3
Coconut oil	0.3	86	0.1	0.5
Cottonseed oil	0.2	324	35.3	24.7
Cupu assu oil		95	0.6	
Flaxseed oil	0.2		0.5	9.3
Grapeseed oil		180	28.8	
Hazelnut oil		120	47.2	
Mustard oil				
Oat oil			14.4	24.7
Olive oil	0.3	221	14.4	60.2
Palm oil	0.3		15.9	8.0
Peanut oil	0.1	207	15.7	0.7
Poppyseed oil		276	11.4	
Rice bran oil		1190	32.3	24.7
Safflower oil, high oleic	0.2	444	34.1	7.1
Safflower oil, linoleic over 70%		444	34.1	7.1
Sesame oil	0.2	865	1.4	13.6
Shea-nut oil		357		
Soybean oil	0.2		8.2	183.9
Sunflower oil, high oleic	0.2		41.1	5.4
Sunflower oil, linoleic less than 60%		100	41.1	5.4
Tomato-seed oil		100	3.8	
Walnut oil	0.4	176	0.4	15.0
Wheat germ oil	20.0	553	149.4	24.7

US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 27. Version Current: August 2014. (Available from http://www.ars.usda.gov/Services/docs.htm?docid=24912.)

biomarkers of fatty acids intake and, therefore, dietary sources of MUFA other than olive oil may account for this association. These data raise the question of whether olive oil's monounsaturated fatty acid content or its antioxidant components are responsible for its beneficial effects. Many studies have shown that vitamin E protects against a number of cancers, which may be a result of its antioxidant action, stimulation of the immune system, and other actions.

Canola Oil/Rapeseed Oil

Canola oil is produced from rapeseed using plant breeding techniques. These techniques introduced in the early 1970s assured the production of canola oil safe for human consumption because it made it possible to reduce erucic acid to less than 5% from previously 50%, which is potentially harmful for health. The breeding techniques also meant that levels of glucosinolates were also reduced.

Canola oil has lower contents of SFA (7.4 g/100 g), whereas it is high in MUFA content (63.3 g/100 g) and has a balanced ratio of LA/ALA (19.0/9.1 g/100 g). Among the nonglyceridic content, canola oil contains vitamin E (as alpha- and gamma-tocopherol), vitamin K, and beta-sitosterol.

Canola oil is currently thought of as one of the healthiest edible vegetable oils. A recent review has concluded that canola oil decreases serum total cholesterol and LDL cholesterol and, thus, the risk for CHD, although the modulation of HDL cholesterol and triacylglycerols remains controversial. There was also evidence that canola oil-based diets have a positive effect in glucose homeostasis compared with SFA-based diets. This review, also, concluded that canola oil may offer some anticancer activity for some forms of cancer.

Flaxseed Oil

Flaxseed (or linseed) oil is produced from flaxseed and is now increasingly used in nutrition. It contains 9.0 g SFA/100 g, 18.4 g MUFA/100 g, and 67.8 g PUFA/100 g of which 53.4 g is ALA. This unusually high content of ALA makes this oil oxidize rapidly, and therefore, this oil is not supplied as a pure oil, but it is supplied with a suitable antioxidant preparation. Flaxseed oil is also rich in alpha- and gamma-tocopherol, vitamin K, and phytosterols.

The presumed health benefits associated with omega-3 PUFA has made flaxseed oil a good dietary source of achieving the nutritional adequacy in ALA. This is supported by a recent review that collectively shows that increased ALA consumption

is associated with a lower risk for coronary heart disease morbidity and mortality, lower odds for carotid plaques, and thinner segment-specific carotid intimal/media thickness, whereas increased phospholipid ALA content is associated with a lower risk for stroke. Although most studies have reported modest effects of flaxseed oil on blood total cholesterol, LDL cholesterol, and HDL cholesterol, ALA from flaxseed oil tends to increase the concentrations of the large, less atherogenic LDL-cholesterol subfractions. The modulation of serum triglyceride levels by ALA and flaxseed oil consumption remains obscure. Evidence also suggests that dietary ALA is associated with lower blood pressure, but the association of flaxseed oil intake with blood pressure levels is inconclusive. Ingestion of flaxseed oil or ALA may help in preventing or treating a variety of diabetic complications and modulates coagulation in diabetic patients but not in healthy individuals.

Sunflower Oil

Sunflower oil is used in cooking, salad dressings, and manufacture of fat spreads. Plant breeding produced varieties with improved fatty acid profile, such as the high-oleic sunflower oil, and at the same time resistant to insects. The traditional sunflower oil is high in PUFA content (mainly LA) and low in SFA. Currently, however, there are varieties with high oleic and mid oleic content. Sunflower has also nonglyceridic content, consisting of tocopherols and plant sterols.

The demand for the sunflower oil has declined since 2000, and most research regarding health effects was done much earlier. Studies had shown that substituting sunflower oil for SFA in the diet may result in decreasing total cholesterol and LDL cholesterol, which is thought to be favorable with respect to decreasing risk of cardiovascular disease. However, those studies raised concerns because of a parallel decrease in HDL cholesterol. A more recent study comparing intake of mid-oleic sunflower oil with the typical American diet has shown a decrease in both total cholesterol and LDL cholesterol with no effect of the experimental diets on triglyceride levels, rate of oxidation, or lipid hydroperoxides. A newer study, however, has shown that sunflower oil rich in LA and high in LA/ALA ratio may predispose to metabolic syndrome.

Corn Oil

Corn oil is mainly produced and consumed in the United States where it is mainly traded in blends of vegetable oils and also in China and Brazil. Most corn plants in the United States are genetically modified to yield more hardy crops. In the United States, it has been shown that corn oil is mainly used in fast-food restaurants, whereas meat used in fast foods, such as beef and chicken, is from animals fed with corn. It should be noted that US corn agriculture has been criticized as environmentally unsustainable. Refined corn oil is composed of PUFA 59%, MUFA 24%, and SFA 13% with a ratio of LA/ALA of 83. Corn oil contains also gamma- and alpha-tocopherols and beta-sitosterol.

There is only scarce research in the last two decades regarding consumption of corn oil and health. A study that examined the effect of high-fat diet rich in PUFA and low in SFA or TFA compared with a diet high in SFA or TFA leads to improvements in lipid profiles including serum total cholesterol and LDL cholesterol and triglyceride levels, thus reducing cardiovascular disease risk. A diet prepared with corn oil (or palm olein) reduces not only total cholesterol and LDL cholesterol but also HDL cholesterol compared with a diet prepared with coconut oil. The corn oil diet also reduced the serum triglyceride levels.

Soybean Oil

Soybean is mainly used for cooking, but it is also used in margarine and spread production and mayonnaise. According to the Food and Agriculture Organization of the United Nations (FAOSTAT), soybean oil is the most widely used vegetable oil worldwide, which experienced a more than fourfold increase in food supply between 1961 and 2011. Most of the soybeans used today in producing vegetable oils are genetically modified crops, which have been used to modify the fatty acid composition.

The composition of soybean oil is mainly of PUFA (LA 51.0 g/100 g and ALA 6.8 g/100 g), with MUFAs at 22.8 g/100 g and SFA 15.7 g/100 g. Soybean oil is the richest vegetable oil in vitamin K and contains tocopherols (mainly gamma and delta and less alpha). Soybean oil essentially lacks phytosterols but has a considerable amount of beta-sitosterol.

Palm Oil

Palm oil is an edible oil derived from the flesh of the fruit of the palm tree, which should be distinguished from palm kernel oil obtained from the kernel of the fruit, which has limited food interest. Palm oil is separated into liquid (olein) and solid (stearin) fractions, from which several types of palm oil are produced for use as a cooking oil, for manufacture of margarines and shortenings, and for bakery use such as the production of biscuits, cookies, and ice creams. Palm oil is the least expensive among the most common edible oils, and the low price justifies the wider use of palm oil in less developed parts of the world (see **Figure 2**). Equally important, the oxidative stability of the oil because of its fatty acid composition enables its use in high temperatures and ensures a prolonged shelf life of products made of palm oil, thus making it a popular edible oil.

Palm oil has a balanced fatty acid composition between SFAs (approximately 50%) and unsaturated fatty acids (MUFA 39% and PUFA 10% mostly as LA). The major fraction of SFA consists of palmitic acid (44%). Palm oil contains nonglyceridic contents including carotenes, tocopherols, tocotrienols, sterols, and squalene, which diminish after refinement.

Most of the studies on health effects of palm oil focussed on its effects on serum lipid levels. Despite the relatively high SFA content of the oil, its structure is thought to account for the beneficial effects described in numerous nutritional studies; oleic acid is predominantly attached at the sn-2 position in triacylglycerols, whereas SFAs are attached on the sn-1 and sn-3 positions. Dietary palm oil was found to reduce serum total cholesterol and LDL cholesterol and triglycerides and increases HDL cholesterol. Improved lipoprotein(a) and apo-A1 levels were also demonstrated from palm oil diets. Virgin palm oil also provides carotenes apart from tocotrienols and

tocopherols that have been shown to be powerful antioxidants and potential mediators of cellular functions. These compounds can be antithrombotic, cause an increase of the prostacyclin/thromboxane ratio, reduce restenosis, and inhibit HMG-CoA reductase (thus reducing cholesterol biosynthesis).

Coconut Oil

Coconut oil is extracted from the kernel of matured coconuts harvested from the coconut palm and is high in saturated fats. Coconut oil is a common edible oil in certain countries, and there is a controversy regarding its effects on lipid profiles and cardiovascular disease risk. The high content of SFA raised concerns that it could lead to more artherogenic lipid profiles, and thus, health professionals in the 1980s recommended that coconut oil should not be used. This has changed however, as it was noted that countries with high intake of tropical oils had some of the lowest rates of cardiovascular heart disease, and recently, the use of coconut oil has become more popular because of the potential cardiovascular benefits.

SFAs in coconut oil increase serum HDL cholesterol more than LDL cholesterol to give a more favorable lipid profile relative to dietary carbohydrates. Recently, it has been shown that replacing high-PUFA vegetable oils by 75% with coconut oil in the diet of broilers is associated with a reduced fat deposition and increases lipoprotein lipase, hepatic lipase, and total lipase activities, thus affecting favorably lipid profile. In pigs, replacement of standard diet with coconut oil modulates the adipose tissue gene expression and fatty acid composition, with minimal effect on serum lipid profile. Interestingly, in mice with stress-induced injury, coconut oil was recently shown to improve lipid profile and restore oxidative stress.

Other potential outcomes have also been described with use of coconut oil. In breast cancer patients, virgin coconut oil consumption during chemotherapy helped improve the quality of life and reduced symptoms related to side effects of chemotherapy.

Conclusions and Recommendations

The balance of fatty acids in humans' diet is one much debated issue. Research has mainly focussed on certain components of the vegetable oils rather than employing supplementation of whole oil to the diet and estimate a number of specific end points. Moreover, there is mostly a lack of studies comparing health end points between diets consisting of different vegetable oils. Therefore, studies have concluded that many individual vegetable oils may be part of a balanced diet, but we are able to recommend the use of virgin olive oil as the only edible oil with ideal fatty acid composition and many micronutrients with antioxidant properties reducing the risk for all chronic diseases.

Current dietary recommendations propose that SFAs should be lowered to less than 10% of total energy intake. A relatively recent meta-analysis showed that substituting PUFA for SFA helps to reduce CHD events. An interesting study showed that in diabetic patients, a healthy diet, rich in vegetables and a vegetable oil with a considerable amount of PUFA and antioxidants, replacing SFA for 8 weeks, decreases DNA oxidation expressed as DNA strand breaks.

Current dietary recommendations suggest replacing SFA in the diet with MUFA or PUFA. For example, solid fats in home-prepared food such as butter and lard could be replaced with vegetable oils that are rich in MUFA and PUFA. All vegetable oils, however, are more than 99% fat, and therefore, the consumption of vegetable oils or products prepared from vegetable oils should be used in moderation. Thus, modern dietary recommendations suggest the use of a small amount of vegetable oils – 30–45 ml each day – in cooking, salad dressings, margarine, and mayonnaise. PREDIMED study has recently demonstrated that the daily use of 45 g of olive oil resulted in a 30% reduction in the risk of major cardiovascular events among high-risk persons.

The nutritional value of edible oils is based not only on the fatty acid composition but also on the minor components with antioxidant activity. Processing and storage of edible oils may alter the composition of oils in nonglyceridic contents; refinement and exposure to sunlight may destroy the vitamin E and other nonglyceridic contents in vegetable oils. Partial hydrogenation of edible oils, which may be responsible for TFA formation, is now less commonly used, and other methods are utilized in order to provide edible oils with functional and desired organoleptic properties. Interesterification is now one of the methods used. The use of interesterified fat produced from vegetable oils needs further research to determine if its use is safe to human health. As further research is still warranted in order to evaluate currently used vegetable oils, it is likely that a wider variety of edible oils will be available through conventional plant breeding techniques or plant breeding using genetically modified technology. It is therefore safer to use only olive oil, which has been used for thousands of years mainly by the Mediterranean populations having health-promoting effects and disease prevention.

The lack of studies comparing health effects of different vegetable oils is further complicated by the fact that in most societies, a different combination of vegetable oils is currently consumed. Before the introduction of most vegetable oils in modern diets, olive oil was the main vegetable oil used in nutrition for many millennia. Olive oil has been an important constituent of the Mediterranean diet, which proved beneficial in preventing disease and prolonging life expectancy. The Mediterranean diet has been even proved as cost-effective compared to a prudent Western diet in patients with previous CHD. Therefore, the use of olive oil as an unsaturated edible oil should be encouraged when and where possible. However, the current international epidemic of obesity in both children and adults should lead to a restriction of fat and oil intakes specifically for those who are overweight or obese. Oils are high-energy dense foods providing 9 kcal g^{-1} as compared to carbohydrates and proteins providing 4 kcal g^{-1}. A balanced diet, selecting appropriate portions from all five food groups, and daily moderate to vigorous physical activity almost daily are internationally accepted recommendations.

See also: Antioxidants: Role on Health and Prevention; Fats: Classification and Analysis; Olive Oil: Its Role in the Diet; Vegetable Oils: Composition and Analysis; Vegetable Oils: Oil Production and Processing; Vegetable Oils: Types and Properties; Vitamins: Overview.

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Vegetable Oils: Oil Production and Processing

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Introduction

Oils and fats are mixtures of different organic molecules called lipids. Triglycerides are by far the most common component of oils and fats; these biological molecules are water-insoluble. Oils and fats can be extracted from plant or animal sources. The difference between a fat and an oil is that a fat is solid or semisolid at normal room temperature (20 °C in Europe and the United States), while an oil remains liquid.

Early vegetable oil production in Europe was mainly concerned with olive oil. For climatic reasons, it was limited to the Mediterranean; more northern countries used lard or butter as the fat source in their diet. This distinction still exists. Countries used to olive oil now use vegetable oil for cooking, the shortage of butter led to the invention of margarine, and lard has been replaced by vegetable shortening in countries that traditionally used lard.

Oil production has evolved from the early process comprising a grinding step to open the cells in the oleaginous raw material and an expelling step in which the oil is squeezed out of the ground fruit or seed paste to a process that may also comprise a solvent extraction step.

Oils and fats have been used since ancient times for food preparation and in nonfood applications like lamp oil, lubricant, soap manufacturing, and skin care. This article will only deal with the production of oils and fats for use in food preparation or as a food ingredient. Oils and fats in food provide functional and nutritional benefits. They serve as a heat transfer medium at elevated temperatures (frying), provide lubrication (spreads), improve taste (dressings), and give texture to a wide range of foodstuffs. On top of this, they supply a concentrated source of energy, deliver critical building blocks for the body, and act as a carrier for essential minor components like vitamins A and D. A balanced intake of oils and fats is essential for human health.

The Oil and Fat Supply Chains

The supply chains of vegetable oils and fats consist of the following:

- The growing and harvesting of the oil crop.
- Oil extraction to recover the oil; the by-product meal is mostly used as animal feed.
- Purification to reduce the level of unwanted minor components.
- Modification of the melting characteristics as required for particular products.
- All transport and storage from grower to end user.

The main origins of supply for the world vegetable oil and fat market fall into three main categories (more details will be given in other articles):

1. Annually planted oilseed crops like soybean, rapeseed, and sunflower seed
2. Fruit oils like palm and olive oil
3. Kernel oils like palm kernel oil, coconut, and other nut oils

Oilseed crops are generally not grown in the regions where the oils are consumed. Oilseeds are transported and stored as seeds, and the separation into oil and meal is mostly performed close to the consumer markets. Under controlled conditions, oilseeds can easily be stored for a long time, at least until the next harvest arrives. Overseas shipping is mainly in the form of seeds.

Like other fruits, fruit oils have a short shelf life. After a few days, they start to deteriorate, resulting in a poor oil quality. The extraction of oil from fruits is done close to the crop growing area, and the extracted oil is then stored and transported to the consumer markets. Overseas shipping is only as oil.

In contrast, kernels can be stored after drying and transported over long distances, although extraction is generally performed where the fruits are grown. Overseas shipping is mainly as oil.

Vegetable Oil Extraction

Olive Oil

The annual world production of olive oil varies around 2.8 million tonnes. Spain is the main producer with an average annual production of 1.2 million tonnes, followed by Italy and Greece. After harvesting, the olives are first of all cleaned and washed with water. Then, they are crushed in a hammer mill from where the resulting paste is fed to a malaxer in which the paste is slowly agitated to allow the dispersed oil droplets to coalesce. Talc and/or enzymes can be added to improve the oil yield.

In old olive oil mills, the paste is then spread onto circular filter cloths that are stacked inside a hydraulic press. Modern mills use a decanter to separate the oil from the waste. If a two-phase decanter is used, this will separate the paste into some 80 wt.% wet pomace and 20 wt.% turbid oil. This oil is then washed with 10–20% water to yield clear oil and not too dirty washing water. The amount of water used affects the residual polyphenol content of the oil and is therefore quite critical.

When a three-phase decanter is used, only 50 wt.% dry solids (pomace) are produced, but the addition of further washing water leads to a large effluent stream (black water) that has to be treated before it can be discharged. When the pomace is extracted with, for example, hexane, the oil thus obtained can no longer be called 'virgin.' It has to be refined to yield a bland olive oil called 'pomace oil.'

Oils from Seeds with a High Oil Content

Although the production of oil from seeds with a high oil content (around 40%) also leads to the meal by-product, the

value of this by-product is low in comparison with the oil. These seeds are therefore grown for their oil. Unlike fruit oils, seeds can be stored so that an oil mill processing oilseeds can operate the whole year round. After harvesting, the seeds are dried to a water content of less than 16% of the dry, oil-free matter to prevent microbial spoilage during storage.

At the oil mill, the seeds are first cleaned to remove any stones and other extraneous matter that might damage the flaking rolls. Then, they are conditioned to a temperature of 60–70 °C to soften the cell so that the subsequent flaking requires less electrical energy. In the flaking process, two perfectly cylindrical rolls flatten the seeds so that cells within the seed are opened and oil can be squeezed out of the flakes without having to pass through a cell wall.

The flakes are passed to a screw press that is fitted with a decreasing-volume Archimedean screw that builds up pressure along its shaft. This shaft rotates inside a slotted barrel that allows the oil to pass through. This oil contains many meal particles that have to be removed. In a first clarification process, the oil is screened or allowed to settle, and then further clarification is realized by filtration or the use of a decanter centrifuge. The solids removed contain a substantial amount of oil and are therefore returned to the screw press.

The press cake leaving the screw press is too hot to enter the solvent extraction plant. It therefore has to be cooled and broken into pieces to permit proper percolation during solvent extraction. A very effective way of treating the press cake is by pelletizing, since this treatment also ruptures cells that survived the flaking and pressing processes. When the cake has reached a temperature of no more than 60 °C, it is ready for solvent extraction, which will be discussed in the next section.

Because the hull of a sunflower seed accounts for up to 30% of its weight, it can be advantageous to dehull the seeds prior to the expelling step. This increases the capacity of the screw presses and the extraction plant; it lowers the wax content of the oil and increases the protein content of the meal. The dehulling process comprises the opening of the seeds by impact and a separation by winnowing. Some hulls have to be included in the feed of the screw press to give it some body. The hulls are usually used on-site as a boiler fuel.

Oils from Seeds with a Low Oil Content

The prime example of an oilseed with a low oil content is the soybean that contains around 18–21% oil. Soybeans are primarily grown for the protein content of the meal that is used in the animal feed industry on a large scale; the oil can be regarded as a by-product. Because its use in poultry farming requires a high (>48%) protein content and a low (<3.5%) fiber content, the beans are often dehulled before being extracted.

In the classical dehulling process, the beans are dried and held for tempering. This makes the beans shrink away from the hulls so that after cracking, the hulls can be separated from the cracked cotyledons by aspiration. In the so-called 'hot dehulling' process, the beans are conditioned to a temperature of 65–70 °C in a tower in which tubes, which are heated with low-pressure steam, heat the beans quite quickly. This and the subsequent superficial heating in a fluid bed dryer cause the hull to shrink away from the cotyledon. Cracking leads to a mixture of separate hull fragments and cracked cotyledons that are separated in cascade aspirators.

For the cracking process, two or three sets of superimposed corrugated rolls are used wherein opposing rolls run at different speeds to grip the beans. Cracking should aim at breaking the bean into four to six smaller pieces and avoiding fines and mashed beans. Subsequently, the cracked cotyledons are flattened into flakes of some 0.25–0.30 mm thickness in between flaking rolls that are similar to those used for high oil content seeds.

Processing the flakes in an expander is optional. The expander is a kind of extruder in which the flakes are compressed until on leaving the die, the compacted mass expands causing further cell rupture. The resulting collets have the advantage that they have a higher bulk density than the flakes, so expanders are often used to increase the capacity of an existing oil mill. Collets also have the advantage of a reduced liquid holdup, which saves energy in the desolventizer.

The extraction solvent used is invariably hexane. Industrial hexane is a mixture of alkanes with a boiling range of 66–69 °C comprising *n*-hexane (~62%), methylpentanes (~23%), and methylcyclopentane. It is highly inflammable and its explosive range in air is 1–8% by volume. Consequently, extractors are operated at a slightly subatmospheric pressure to prevent the hexane from escaping.

Solvent extractors operate countercurrently to minimize the oil content of the extraction residue leaving the extractor and maximize the oil content of the miscella. Two types of extractor are in industrial use: percolation and immersion. In the immersion type, the screen under the material being extracted has so little open area that it constitutes the greatest flow restriction. Immersion extractors provide more contact time between solvent and seed material but require more draining time than percolation-type extractors. Both types ensure a miscella strength of around 30% oil and a residual oil content in the meal of < 1 wt.%.

The solvent–wet extraction residue or 'marc' leaving the extractor is then treated to remove the solvent, first by indirect heating and then by heating with live steam. This raises the temperature of the meal to 105–100 °C in the toasting section where antinutritional enzymes are denatured. Since the live steam causes the moisture content of the meal to increase, it has to be dried. Air is used to dry the meal to a residual water content of 13–14 wt.% and cool it down at the same time.

The hexane-laden vapors leaving the desolventizer are at atmospheric pressure. Using their latent heat to evaporate the hexane from the miscella requires the latter to be under a reduced pressure; in industrial practice, this pressure is about 500 hPa absolute. The latent heat content of the vapors leaving the desolventizer suffices to evaporate < 90% of the hexane present in the miscella so that the composition of the liquid leaving the first evaporator is around 80 wt.% oil. It is further concentrated using steam and finally treated in a steam stripper operated at 200–400 hPa absolute.

If the oil has to be shipped, it must be degummed with water to prevent a deposit being formed during transport and storage. In this water degumming process, an amount of water that is about the same as the gum content of the oil is mixed with the hot oil, allowed to hydrate the mucilaginous matter. and then separated by a centrifuge. Drying these gums yields lecithin.

Vegetable Oil Refining

Process Sequence

The vegetable oil refining process was introduced at the start of the twentieth century to produce good-quality oils and fats for use in margarine production and as frying oil. Before 1900, oils were cleaned by removing water and solids by decanting. The refining process has been optimized to reduce the natural taste and color and to remove most of the free fatty acids present in the extracted oil. Later, it was discovered that the process also removes many of the contaminants present in the extracted oils, like solvent residues, metals, polyaromatic hydrocarbons, and pesticide residues, bringing these contaminants below the legal limits.

The refining process is a combination of the following process steps; more details are given in the following sections:

- Degumming: A pretreatment process mainly applied to seed oils to reduce their phospholipid contents.
- Neutralization: The purpose of neutralization is to reduce the concentration of free fatty acids by reaction with a diluted alkali solution, typically sodium hydroxide. The reaction product (soap) is removed by decanting or by centrifugation.
- Bleaching: The main purpose is to remove residual soap, color pigments, and oxidized components. In this process, bleaching earth (natural or activated clay and/or silica) is added to the oil as an adsorbent. The earth and adsorbed impurities are subsequently removed by filtration.
- Dewaxing: Removal of natural waxes present in some vegetable oils by cooling and filtration.
- Deodorization: Under high vacuum, the oil is heated to 180–270 °C, while steam is blown through the oil. The objective is to remove volatile components and to create an odorless oil with a bland taste and increased storage stability.

The process sequence of combined degumming/neutralization followed by bleaching and deodorization is called chemical refining, referring to the reduction of free fatty acids by a chemical reaction. The process sequence of degumming followed by bleaching and deodorization is called physical refining, referring to the reduction of free fatty acids by the physical process of vacuum stripping. An overview of the possible process sequences is given in Figure 1.

Degumming Processes

As indicated in the refining process flow sheet (Figure 1), many different routes lead from crude oil to fully refined oil, and the number of steps can differ.

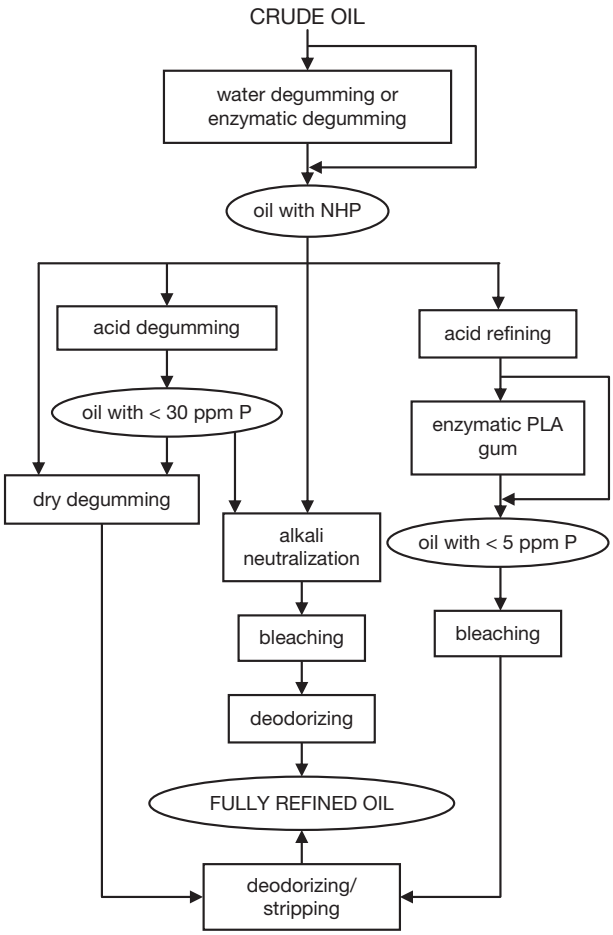

Figure 1 A block diagram of possible vegetable oil refining process routes.

The route with only two steps consists of the dry degumming process, followed by physical refining. In the dry degumming process, some phosphoric acid is mixed into the oil and allowed to react with the phosphatides. Then, bleaching earth is added, and this adsorbs the phosphatides, the excess phosphoric acid, and also the coloring matter present in the oil. This bleaching earth is removed by filtration, and the bleached oil is ready for physical or steam refining. Since the bleaching earth requirement in the dry degumming process is proportional to the phosphatide content of the crude oil, this two-step route is only economical for oils and fats with a low phosphatide content. Examples are palm oil, lauric oils, lard, and tallow.

In the acid degumming step, the crude or water-degummed oil is treated with a strong acid that is subsequently diluted with water to form a gum phase that is removed by centrifuge. However, this dilution raises the pH insufficiently to pull all the gums into the gum phase. Consequently, the acid-degummed oil requires a further degumming treatment before the oil can be physically refined.

In the acid refining process, the crude or water-degummed oil is also treated with a strong acid, but instead of diluting the acid with water, it is neutralized with caustic soda. This raises the pH to a value where still no soaps are formed but where all the phosphatidic acid that has been liberated by the acid treatment is dissociated. It therefore moves completely into the aqueous phase and can be separated from the oil as sodium phosphatidate. The acid refining process produces an oil that can be physically refined after it has been bleached.

The water degumming process and the acid refining process produce a gum phase as by-product. This gum phase also contains oil, and if the gums are disposed of by mixing with the meal, the oil is sold at meal value that is less than if it were to be sold as oil. To recover at least some of this oil, enzymatic gum treatment processes using a phospholipase have been developed. As shown in Figure 1, phospholipase C (PLC) can be used in the water degumming step or on the gums obtained by water degumming. This enzyme catalyzes the hydrolysis of the bond between the phosphate moiety and the glycerol moiety in phosphatides as indicated in Figure 2. This hydrolysis generates diglycerides that move into the oil phase and thus contribute to oil yield. It also produces phosphate esters of choline, which are water-soluble and do not retain any oil.

Another phospholipase that can be used to treat the gums and thereby increase the oil yield is phospholipase A (PLA). Figure 2 shows that there are two enzymes that catalyze the hydrolysis of a fatty acid ester bond: PLA_1 and PLA_2. Both enzymes are commercially available. They form lysophosphatides that retain less triglyceride oil and free fatty acids that are removed during a subsequent neutralization process. They can be added to oil that has already been acid-refined or to the gums resulting from the acid refining treatment. Because the oil savings resulting from the various enzyme treatments are proportional to the amount of gums, these treatments only make sense when applied to oils with a high phosphatide content.

Neutralization Processes

In the neutralization process, oil containing FFAs is treated with lye, a solution of sodium hydroxide or caustic soda in water. This reacts with the FFA to form sodium soaps that are insoluble in the oil and therefore form a separate soap phase. This soap phase also contains phosphatides, when present in the crude oil, and triglyceride oil. After this soap phase has been separated from the oil, it is called soap stock. Formerly, it was used in a soap works adjacent to the refinery. It can be used as an animal feed ingredient, but in Europe, it is more common to react the soap stock with sulfuric acid at elevated temperatures and recuperate its fatty matter as 'acid oils,' a mixture of fatty acids and triglyceride oil. This process also generates an acid-aqueous effluent stream that contains phosphates and sulfates and has a high BOD. It has to be neutralized, its phosphate may have to be removed by a lime treatment, the resulting precipitate will then have to be separated, and then what is left has to be treated in an effluent plant to lower the BOD. All this makes soap stock splitting a cumbersome process that is preferably avoided. It has been a powerful driving force for the shift towards physical refining.

In alkali refining, there are two routes. The oldest route that has been developed and is still in use in the United States is the

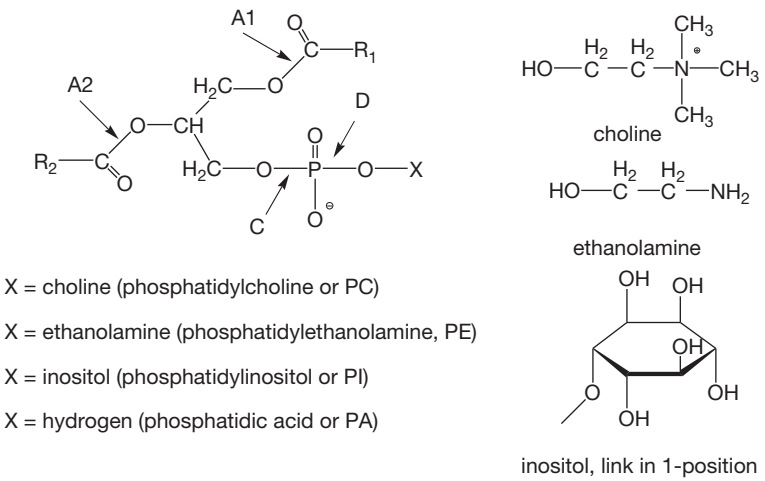

Figure 2 Chemical structure of phosphatides and indications which phospholipase enzyme catalyzes the hydrolysis of which bond.

so-called 'long mix' process. Dilute lye (14–18°Bé or 130–180 g NaOH/L of water) is added and mixed into cold oil, and then the oil is heated until a clear separation between oil and soap stock is obtained. The soap stock is removed by centrifugal separator, and to lower the residual soap content, the oil is either washed with water or treated with silica hydrogel. In Europe, the 'short mix' process is almost universally used. In this process, a degumming acid (phosphoric acid) is finely dispersed in the hot (90–100 °C) oil, it is allowed to react in a holding vessel, and then less dilute lye (20–28 °Bé or 145–215 g NaOH per liter of water) is dispersed in the oil. Separation between oil and soap stock is almost instantaneous, allowing the mixture to be sent to the centrifuge without much holding time. Again, the oil phase can be washed or treated with silica hydrogel, but in Europe, water washing is more common.

Bleaching of Vegetable Oils

Crude and degummed/neutralized vegetable oils differ widely in color. Palm oil is deep red, rapeseed oil is greenish, and sunflower seed oil has not much color. These colors are caused by two groups of compounds. The red color stems from carotenes, with β-carotene being the most common, and the green color originates from chlorophyll. Carotenes are thermally unstable so they break down when the oil is deodorized or physically refined. Because palm oil also contains the thermally more stable chlorophyll, which is removed by bleaching earth, palm oil has to be treated with this adsorbent to yield an almost colorless fully refined oil.

The bleaching process itself is quite simple. Depending on the color of the oil, an amount of 0.5–4.0 wt.% of bleaching earth is mixed into hot (80–110 °C) oil, the suspension is given some time to approach the adsorption equilibrium, and the earth is then removed by filtration. The filters used for this step are invariably pressure leaf filters that are precoated by circulating oil with bleaching earth over the filters. Filter aids like diatomaceous earth can also be used.

Bleaching earth is a pretty universal adsorbent, but it insufficiently adsorbs certain compounds that are considered to be undesirable. Among these compounds are the polyaromatic hydrocarbons (PAHs). Low-molecular-weight polyaromatic hydrocarbons are sufficiently volatile to be removed by vacuum stripping, but high-molecular-weight polyaromatic hydrocarbons require another means of removal. This means is provided by treating the oil with activated carbon, a highly effective adsorbent for this kind of compounds. Bleaching clay and activated carbon can be used simultaneously.

Dewaxing of Vegetable Oils

Some vegetable oils like sunflower seed oil, rice bran oil, and corn germ oil contain waxes. These waxes are esters of fatty acids and fatty alcohols. When both of them are saturated, the wax has a melting point > 70 °C, which causes the waxes to crystallize at refrigeration temperatures and form an unsightly deposit. To prevent this from happening, the oil has to be dewaxed. In the dewaxing process, the oil is cooled down to cause the waxes to crystallize. They can then be removed by filtration with a pressure leaf filter and filter aid. Since the amount of filter aid is proportional to the wax content and oil is also retained in the filter cake, filtration becomes quite expensive when the oil has a high wax content.

Then, it is more economical to remove the bulk of the waxes in a wet dewaxing process using a centrifugal separator and remove residual waxes by filtration. This wet dewaxing process employs water with a detergent, which can be soap, to concentrate the wax crystals in the aqueous phase. It can therefore be combined with the neutralization step.

The ideal cooling profile comprises a slow cooling from above the cloud point of the oil to below this cloud point to limit the number of crystal nuclei. Cooling from below this cloud point can be fast and maturation is not necessary.

Deodorization

The deodorization process is carried out at a high temperature (180–270 °C). Because of this high temperature, residual peroxides and carotenes will decompose, and when the temperature is too high, *trans* isomers can be formed. The more double bonds a fatty acid contains, the more prone it is to this kind of isomerization. This is the reason why refined oil specifications contain an upper limit of 1.5 wt.% for oils containing linolenic acid (soybean oil and canola oil) and an upper limit of 1.0 wt.% for sunflower seed oil and corn oil, which do not contain this fatty acid.

Apart from these chemical reactions, vacuum stripping is a physical process that can be described by the laws of Raoult and Dalton. The reduction of volatiles in the oil depends on the stripping steam flow, the vacuum pressure, the oil temperature, the oil volume, the vapor pressures of the volatiles, and the equipment design.

The deodorization process can be carried out in batch, continuous, or semicontinuous installations. Batch deodorizers have one single compartment in which the process steps of deaeration, heating, stripping, and cooling are sequential in time. In continuous deodorizers, the process steps are simultaneous in different compartments (trays) of the deodorizer, and both input and output are constant over time. In semicontinuous deodorizers, the process steps are simultaneous in the different trays, but tray filling and emptying is discontinuous. Filling stops after a certain level has been reached in the first tray. After allowing sufficient time to perform the process step for that tray, the bottom valve is opened and the content drops into the next tray for the next process step. Figure 3 shows a basic layout of a semicontinuous deodorizer. Oil can also be fed to the top of a packed column and trickled down through this column, while the stripping flows countercurrently. This type of column has a lower stripping steam usage than the crossflow system to reach the same final concentration of volatiles.

Obtaining stable oil is more than just getting rid of the volatiles present in the oil to be deodorized or physically refined. When triglyceride oils are heated for prolonged periods, strong off-flavors are developed. Deep fat frying in coconut oil smells different from frying in sunflower oil, let alone tallow. The chemical nature of these smells is not known, and since they are not released by freshly deodorized oil, they must be formed during deep fat frying from flavor precursors. Accordingly, the deodorization process should

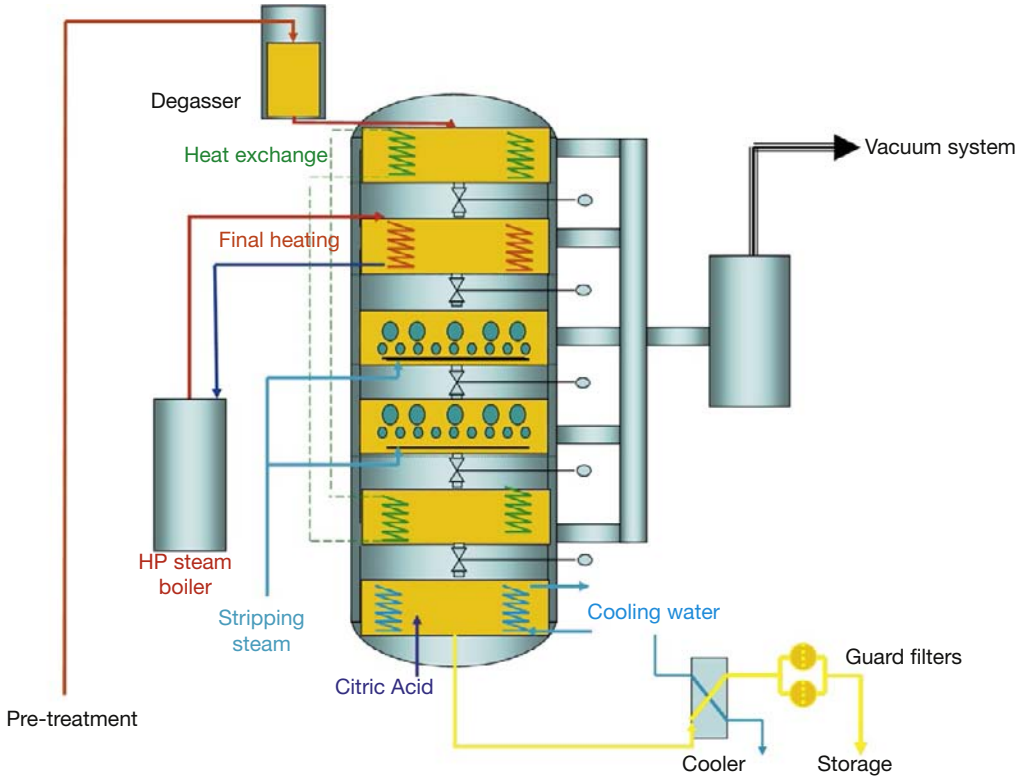

Figure 3 The layout of a semicontinuous deodorizer. From G. van Duijn, lecture material.

provide some holding time at a high temperature to enable the thermal breakdown of these flavor precursors.

There are two reasons why the deodorization process is the most energy-intensive step of the refining process. Firstly, it is carried out at temperatures of up to 270 °C, and secondly, it is carried out under a vacuum of 2–10 hPa, which has traditionally been maintained by steam ejectors. Accordingly, heat exchange between ingoing and outgoing material either directly in a plate heat exchanger or indirectly by using one or even two thermosiphons has led to energy savings. The use of a mechanical vacuum pump in combination with a surface condenser for the distillate has limited the steam usage to the stripping steam but increased the consumption of electrical energy.

Vegetable Oil Modification Processes

The Purpose of Oil Modification

When Mège-Mouriès invented margarine in 1869, he used tallow olein as the fat phase, but when more and more margarine was produced, a shortage of solid fat emerged. Accordingly, the invention of the hydrogenation process in 1903 led to an almost immediate industrialization despite the fact that this also involved building plants for elemental hydrogen. Mège-Mouriès also described a dry fractionation process to obtain his tallow olein, but that process only began to be applied on a large scale with the increased production of palm oil in the last quarter of the twentieth century.

Networks of solid fat crystals play an important role in many food products. For example, margarines consist of an emulsion of small droplets of an aqueous phase dispersed in liquid oil stabilized by a structure of solid fat crystals. The fat crystals supply structure and texture to the product, they prevent the oil from leaking out of the product, and they avoid coalescence of the dispersed aqueous phase droplets. Solid fat crystal networks have similar functions in whipped cream and ice cream, where they stabilize a foam of air bubbles dispersed in an emulsion of fat droplets in water; they also play an important role in bakery products. Most of the seed oils are too low in solids at the temperature at which the product is used (generally ambient temperature) to fulfill this role. Tropical oils, such as palm oil, palm kernel oil, and coconut oil, have a higher solid fat content, but for many applications, they are still too soft. Modification of the melting performance of naturally occurring oils and fats is required to produce fats with optimal properties for the aforementioned applications. The modification technologies that can be applied are the following:

1. Hydrogenation
2. Interesterification
3. Fractionation

Hydrogenation

Hydrogenation of oils and fats has been applied on a large scale since the start of the past century to produce hard fats from a wide variety of oils and fats (mainly liquid oils and fish oil). Hydrogenation involves the addition of two hydrogen atoms to unsaturated double bonds (C═C) in the fatty acid moieties of the triglycerides. The more saturated fat resulting

from this reaction has a higher melting point than the starting material.

In the hydrogenation process, hydrogen gas reacts with liquid oil at elevated temperatures and pressures in the presence of a solid catalyst. The hydrogenation of edible oils is always carried out as a batch process. Oil is preheated by the previous batch, the catalyst (nickel on an inert support) is added, and hydrogen gas is dispersed in the oil. Hydrogenation is an exothermic reaction so the temperature of the batch starts to rise and has to be controlled by cooling. The reaction is continued until the product properties aimed for have been reached (partial hydrogenation). The catalyst is removed by filtration and residual nickel is removed in a post-bleaching step.

As well as saturation, the isomerization of double bonds also takes place during the hydrogenation reaction. Two types of isomerization reaction will occur: geometric isomerization (*cis*–*trans*) and positional isomerization (shift of double bond along the fatty acid chain). The complete reaction mechanism is quite complicated because of the many steps involved, and it is still not yet fully understood and kinetically quantified.

In response to the findings of adverse effects of *trans* fatty acids on blood lipids, the food industry has practically eliminated components containing *trans* fatty acids from their fat phase compositions. This has drastically reduced the application of partial hydrogenation as a method of producing solid fats. Full hydrogenation of almost all double bonds reduces the *trans* isomer content to an acceptable level but results in a product with a very high melting temperature. Such a product can only be used after interesterification with a liquid oil.

Interesterification

Interesterification involves a rearrangement or reshuffling of the fatty acids on the glycerol backbone of the triglyceride molecule. Interesterification is catalyzed by an alkaline catalyst or by a lipase enzyme. The most commonly used alkaline catalysts are sodium methanolate and sodium ethanolate. The early interesterification process was used to extend the plastic range of lard used as shortening. Later blends of different raw materials were used to change their physical properties so that they could be used as hard fat in margarine and as shortening.

For a long time, the interesterification reaction has been assumed to proceed with a glycerolate anion as the catalytic intermediate. This anion would then attack the slightly positive carbon atom of a carboxyl group. If this group left as a hydroxyl anion, ester interchange would have been the overall result. An alternative reaction mechanism has been proposed recently that assumes that the initial catalyst (sodium methanolate) abstracts an α-hydrogen from a fatty acid moiety, thereby forming an enolate anion. This enolate anion then acts as the catalytic intermediate. When it reacts with water, it will form a free fatty acid, the formation of which could not be explained by the glycerolate mechanism.

The alkali (sodium methanolate or ethanolate)-catalyzed reaction takes place in prerefined oil (low in water and in free fatty acids) at elevated temperatures (100–110 °C). The reaction is very fast; full randomization results within a few minutes even in factory-scale vessels (10–40 t oil content). After the reaction, the catalyst is inactivated by the addition of water. This addition of water leads to the formation of free fatty acids and sodium hydroxide that will combine to form soaps. The reaction of sodium methanolate with a fatty acid moiety leads to the formation of methanol, and when this reacts with an enolate anion, methyl esters will be formed. The soap is removed by water washing and decanting. Methyl esters are more volatile than triglyceride molecules and are removed during high-temperature deodorization. Both soap and esters constitute a yield loss.

In addition to the basic catalysts used, lipase enzymes have also been introduced for the interesterification process. Interest in the use of lipase originates from the attempts to produce cocoa butter equivalents (CBEs), symmetrical monounsaturated triglycerides that are compatible with cocoa butter. This isomeric requirement ties in with the *sn*-1,3 specificity of the lipase enzyme. Interesterifying a triglyceride that consists almost entirely of trioleate (high-oleic sunflower seed oil, for instance) with stearic acid or methyl stearate should generate a CBE.

Lipase enzymes are also used in a nonstereospecific interesterification process. To overcome the fundamental problem that enzymes need water to be active but use this water to hydrolyze fats, this process uses a large amount of rather dry, immobilized enzymes. It is a continuous process, using four reactors that are filled with enzymes. The first of these, which is filled with an enzyme that has lost most of its activity, acts as a kind of guard for downstream reactors. It is emptied when the product leaving the last reactor is insufficiently randomized, filled with fresh enzymes, and relocated as the last reactor. What impurities in the oil cause the enzyme to lose its activity is still not quite clear, so as a precaution, the oil fed to the first reactor has been neutralized, bleached, and deodorized. The enzymatically interesterified oil needs to be deodorized before it can be used in food products.

Fractionation

Fractionation is the controlled crystallization of the more saturated and/or longer-chain triglycerides, followed by separation of the solid phase (stearin) and liquid phase (olein). By far, the most important oil, fractionated worldwide, is palm oil, the main reason being the demand for clear liquid oil (palm olein). More recently, there has been a growing interest in the solid product of palm oil fractionation (palm stearin) for the production of cocoa butter equivalents, cocoa butter replacers, and margarine hard stocks. Besides palm oil, palm kernel oil, partly hydrogenated liquid oils, cottonseed oil, and milk fat are also fractionated.

Processes for Producing Virtually *Trans*-Free Hard Stocks

The combination of full hydrogenation, interesterification, and fractionation and the availability of a variety of different oils and fats can be used to produce virtually *trans*-free hard stocks with various melting ranges.

Liquid seed oils, low in solid fats, are first fully hydrogenated to generate solids combined with a very low *trans* level (<1.25%). These fully hydrogenated oils may subsequently be interesterified with nonhydrogenated liquid oil to reduce the solid fat content at high temperature (>40 °C). This solid fat content can be further reduced by fractionation (see **Figure 4**).

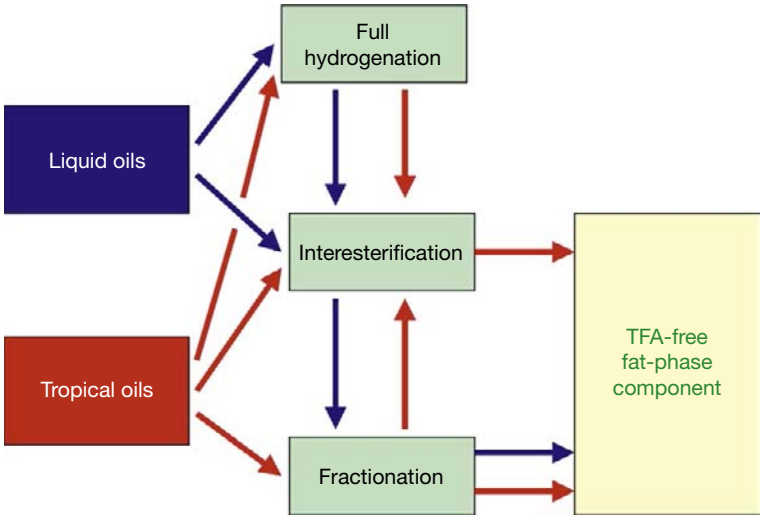

Figure 4 Process routes to produce *trans*-free hard fat components.

The presence of relatively high solid contents in tropical oils creates more flexibility in oil modification routes. Fractionation alone will produce a relatively soft stearin, which is not optimal for structuring margarine. Fractionation followed by interesterification with other (fractionated) components is used to produce 'nonhydrogenated' hard fats. Full hydrogenation followed by interesterification is an alternative way to obtain a hard fat high in solids with a steep melting line without fractionation (see **Figure 4**).

See also: Carotenoids: Occurrence, Properties and Determination; Drying: Principles and Types; Enzymes: Analysis and Food Processing; Fatty Acids: Fatty Acids; Fatty Acids: *Trans* Fatty Acids; Heat Treatment: Principles and Techniques; Oxidation of Food Components; Phospholipids: Properties and Occurrence; Quality Control in Food Processing; Rapeseed Oil/Canola; Sunflower Oil; Tocopherols: Properties and Determination; Triacylglycerols: Characterization and Determination; Triacylglycerols: Structures and Properties.

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Vegetable Oils: Types and Properties

AJ Dijkstra

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Introduction

Vegetable oils can be classified in different ways. One way would be according to the amount of produced oil. Then the list of oils should start with palm oil followed by soybean oil, then rapeseed oil, sunflower seed oil, and so forth. However, this way has the disadvantage that the order may change like in 2006, when for the first time, more palm oil was produced than soybean oil. Another way could be according to the most important fatty acids. Then, the lauric acid oils like coconut oil, palm kernel oil, and babassu oil would be grouped together. This has the disadvantage that it is not clear what determines the importance of a fatty acid. Is it the concentration of the acid or its uniqueness? Should borage seed oil, which contains 23% γ-linolenic acid (GLA), be grouped with other oils that contain this dietary important acid like evening primrose oil and black currant seed oil? Or should it be grouped with oils like soybean oil and corn oil because these oils have in common that linoleic acid is their major acid?

In an encyclopedia, it would be logical to list the vegetable oils in alphabetical order but this also has disadvantages. It would lead to undesirable duplication because the health effects of GLA would have to be repeated for each subsequent oil that also contains this acid. Another aspect to be considered is that several vegetable oils have an entry of their own.

Again, undesirable duplication should be avoided. Therefore, oils not having an entry of their own will be grouped together according to a specific trait, for instance, GLA content. The names of the groups will be in alphabetical order, and within a group, the oils will be listed alphabetically.

What properties to list? Handbooks like the *Physical and Chemical Characteristics of Oils, Fats, and Waxes* list properties such as specific gravity, refractive index, iodine and saponification value, titer, and fatty acid, tocopherol, tocotrienol, sterol, and triglyceride composition. However, many fats have not been analyzed in such detail. Often, only the fatty acid composition has been determined, and after the other properties, nothing has been filled in. So *The Lipid Handbook* does not mention the properties for which no data are available but limits itself to what is known. In addition, it provides some production figures and also some data on international trade.

Confectionery Fats

Because the production of milk chocolate requires additional cocoa butter and the demand for cocoa powder in chocolate-flavored drinks and confectionery is less than amount corresponding to the additional cocoa butter requirement, there is a structural shortage of cocoa butter. This is reflected in the relative prices of cocoa powder and butter. Accordingly, cocoa butter equivalents (CBEs) have been developed. These are vegetable fats that are compatible with cocoa butter; which means that when they are blended with cocoa butter, the blend has the same physical and especially crystallization properties as pure cocoa butter.

This can only be achieved when these CBEs are chemically almost the same as cocoa butter, that is, they have a similarly high content of monounsaturated triglycerides in which the oleic acid is esterified to the middle hydroxyl group (the 2-position) in the glycerol. Because of this positional requirement, 1,3-specific lipase enzymes have been used to develop these CBEs. By using a starting material with a high oleic acid content at the middle position and allowing this to react with stearic acid or its methyl or ethyl ester by the presence of this specific lipase, oleic acid moieties at the outer positions were replaced by stearic acid moieties. Suitable starting materials are high oleic oils such as a sunflower seed variety bred for deep-fat-frying applications and olein fractions resulting from the production of CBEs, such as shea olein.

However, the interesterification reaction is an equilibrium reaction so a large excess of stearic acid moieties is required to ensure that both outer positions are occupied by stearic acid. Lipase enzymes also have the drawback that they need some water to be active and that this water leads to hydrolysis that causes free fatty acids and partial glycerides to be formed. These partial glycerides isomerize, which causes a lack of 1,3-specificity. Consequently, the reaction product of the enzymatic interesterification contains triglycerides with stearic acid on the middle position and also triglycerides that still have an oleic acid at one of the outer positions. Consequently, this reaction product requires extensive purification before a CBE with acceptable quality results.

Moreover, Directive 2000/36/EC of the European Parliament and of the Council of 23 June 2000 relating to cocoa and chocolate products intended for human consumption allows 5% vegetable fat in chocolate and specifies that they are obtained only by the processes of refining and/or fractionation, which excludes enzymatic modification of the triglyceride structure. The vegetable fats allowed in the EU as raw material for producing CBEs are Borneo tallow (illipe butter), palm oil, sal fat, shea butter, kokum gurgi, and mango kernel fat. Table 1 lists some properties of these and similar fats.

In Table 1, the major fatty acids of the butters (16:0, palmitic acid (P); 18:0, stearic acid (St); 18:1, oleic acid (O)) have been listed as well as the contents of the monounsaturated triglycerides. When the butters themselves had to be processed, their stearin fractions have been listed as well.

Allanblackia (*Allanblackia floribunda*)

Allanblackia nuts are grown in West Africa (Ghana). The butter contains > 50% stearic acid (St = 18:0) and > 40% oleic acid (O = 18:1). Consequently, it has a high StOSt content and can be used as a raw material for cocoa butter equivalents. The butter has not been listed among the vegetable fats that can be used in the EU in chocolate.

Table 1 Fatty acid and triglyceride compositions of some raw materials for confectionery fats

Butter/oil	*16:0*	*18:0*	*18:1*	*POP*	*POSt*	*StOSt*	*Total*	*IV*
Cocoa butter	25	35	34	15	39	27	81	
Allanblackia	2–3	52–58	39–45			69		35–39
Borneo tallow	18	46	35	9	29	42	80	
Chinese tallow	64	1	28	70			72	37
Kokum gurgi	4	53	40	1	6	72	77	
Mango kernel fat	6	47	41	6	13	18	37	
Mango kernel	4	59	32	2	13	55	70	
Fat stearin								
Mowrah butter	20	26	38					
Palm oil	45	4	41	36	4	1	36	52
Palm oil midfraction	57	4	34	66	11	2	79	35
Phulwara butter	57	4	36					
Sal fat	6	45	42	5	16	36	57	
Sal stearin	4	48	39	3	10	63	76	
Shea butter	3	44	46	1	6	30	37	
Shea stearin	5	57	32	1	7	74	82	

Source: *The lipid handbook*, 3rd ed., Gunstone FD, Harwood JL, Dijkstra AJ (eds) (2007), Boca Raton, CRC Press.

*Borneo tallow (*Shorea stenoptera*)*

Borneo tallow is also called illipe butter. It is obtained from a tropical tree 6–15 m high with 5 cm nuts. The kernels are enclosed in a thin, brittle case set in an acorn-like cup with winglike attachments that enable the nut to fall clear of the parent tree. The nuts are collected from the ground and dried in the sun until the shells are sufficiently brittle to be separated from the kernels by pounding in rice mortars. The seed is usually solvent-extracted. Similar meals are made from other Shorea species. Illipe butter is one of the fats that are allowed in chocolate in the EU as cocoa butter equivalent.

The triglyceride composition of illipe butter makes it eminently suitable as a cocoa butter equivalent. Its major triglycerides are POP (9%), POSt (29%), and StOSt (42%). It has therefore a higher stearic acid content than cocoa butter itself and thus also a higher StOSt content, which can be used to 'offset' the high POP (P = 16:0) content of the much cheaper palm oil midfraction that is also allowed in chocolate.

*Chinese tallow tree (*Sapium sebiferum*)*

The Chinese tallow tree produces fruits that contain two kinds of oil/fat. The kernel contains a highly unsaturated oil that contains hydroxy acids that may be esterified with a fourth fatty acid. This oil is not for food purposes but can be used in varnishes. The wax on the outer shell is the tallow listed in the table. It contains about 20–25% tripalmitin that has to be removed by fractionation if a fat with a high monounsaturated triglyceride content is to be produced.

*Kokum gurgi (*Garcinia indica*)*

The kokum gurgi tree grows along the western coast of India. Its seeds contain 23–26% fat that after refining can be used as CBE without having to be fractionated. Its use is permitted in chocolate in the EU.

*Mango kernel fat (*Mangifera indica*)*

The annual production of mango fruits approaches 30 million tonnes with about half of this in India. Because of mango juice production and mango fruit canning, the mango kernels are available as a waste product. They contain 7–12% fat. The stearin fraction of this fat has a high StOSt content so it can be combined with the cheaper POP palm oil midfraction to form a CBE. The ratio can be varied to compensate variations in the cocoa butter properties.

*Mowrah butter (*Madhuca latifolia*)*

The mowrah or mahua tree grows in Central India, both wild and cultivated. Mowrah seeds are about 2 cm long and contain some 50% fat, which contains 16% linoleic acid. Fractionating the fat to get rid of triglycerides with linoleic acid is difficult. These triglycerides will be concentrated in the olein, and since the filter cake will also contain some olein, the stearin will contain some of them. Accordingly, mowrah butter is not an ideal raw material for CBEs, but it can be used for filling fats in blends with palm oil midfraction.

*Phulwara butter (*Madhuca butyracea*)*

The phulwara tree is native to Nepal. Its fruits contain three seeds that make up 20% of the fruits. The seeds contain 42–47% oil and the kernels some 60–66%. This oil has a very high palmitic acid content implying that it also contains tripalmitin. When this is removed in a first fractionation step, the first olein can be refractionated to yield a midfraction that will have a high content of monounsaturated triglycerides. It will be similar to palm oil midfraction.

*Sal fat (*Shorea robusta*)*

The sal tree is native to the Indian subcontinent ranging from Myanmar in the east to Nepal and northern and Central India. The kernel content of the seed is about 50% and the oil content of the kernel is some 30%. Besides the palmitic, stearic, and oleic acids listed in the table, sal fat also contains 6–11% arachidic acid (20:0). Its use in chocolate is allowed in the EU, but to increase the monounsaturated triglyceride content of the fat, it has to be fractionated. It is the stearin that is used as CBE.

Shea butter (Vitellaria paradoxa)

The shea tree was formerly also denoted as *Butyrospermum parkii* but this name is superseded. The tree grows across Africa from Cameroon to Uganda, but in the east, the subspecies is mainly *Vitellaria nilotica* and in Cameroon, Mali, and Burkina Faso, it is *Vitellaria paradoxa*. These subspecies differ in that the subspecies *V. nilotica* contains less unsaponifiable and shows a higher oleic acid content than the fruits grown in the west, which show a wide range of fatty acid compositions. The composition given in the table is representative of the shea butter used for CBE.

In many African countries, the women collect the nuts of the wild-growing shea tree, shell them, and boil the kernels with water from which they scoop the butter after it has solidified. The butter can also be produced by screw pressing. Refining involves the removal of the polyterpene constituent of the unsaponifiable. Subsequent fractionation leads to a stearin fraction that is permitted as CBE in chocolate in the EU.

GLA-Containing Oils

From a health point of view, GLA-containing oils are quite interesting since GLA or (6*Z*,9*Z*,12*Z*)-octadeca-6,9,12-trienoic acid is an ω-6 polyunsaturated acid that has been formed by dehydrogenation of linoleic acid. For some people, that can be a slow process so they may therefore benefit from ingesting GLA instead. Once ingested, the acid can be converted into dihomo-γ-linolenic acid (20:3ω-6) by chain elongation, in which the acid can be converted by dehydrogenation into arachidonic acid (20:4ω-6), an essential hormone (prostaglandins, thromboxane, and leukotriene) precursor.

Table 2 shows the fatty acid compositions of a number of oils that contain GLA.

Table 2 shows that the genetically modified rapeseed oil has the highest content of GLA. Moreover, rapeseed is a fully domesticated crop with a low production cost. It is therefore by far the cheapest source of GLA, but purely emotional fear is likely to prevent it from being utilized, at least in Europe. Soybeans have also been genetically modified to increase their stearidonic acid content and that may suffer a similar fate.

Table 2 Fatty acid compositions of various oils containing GLA

Oil	*16:0*	*18:0*	*18:1*	*18:2*	*γ-18:3*	*18-4*	*Other*
Evening primrose oil	6	2	9	72	10		1
Borage seed oil	10	4	16	38	23		9[a]
Black currant seed oil	7	2	11	47	17	3	13[b]
Echium seed oil	6	3	14	13	12	17	35[c]
Arnebia seed oil	7		14	23	3	4	49[d]
GM canola[e]	5	3	23	27	36		

[a]Including 4.5% 20:1; 2.5% 22:1; 1.5% 24:1.
[b]Including 13% α-18:3.
[c]Including 33% α-18:3.
[d]Including 45% α-18:3.
[e]Genetically modified rapeseed oil.
Source: *The lipid handbook*, 3rd ed., Gunstone FD, Harwood JL, Dijkstra AJ (eds) (2007). Boca Raton, CRC Press.

Black currant seed oil (Ribes niger)

Since black currants are used industrially to make fruit juice and jellies and there are no seeds in these products, the processing plant will have the seeds as by-product, making it relatively easy to use them for oil production. Their oil content is only 16% so the oil has to be produced by solvent extraction. The extracted oil is preferably refined.

The oil also contains some stearidonic acid (18:4). This is an ω-3 acid with the systematic name (6*Z*,9*Z*,12*Z*,15*Z*)-octadeca-6,9,12,15-tetraenoic acid. This acid is the first metabolite in the conversion of α-linolenic acid to the longer-chain ω-3 fatty acid eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Borage seed oil (Borago officinalis)

Like black currant seed oil, borage seed oil has an appreciable content of GLA (18:3 23%), and the seed also contains more oil than black currant seeds: 26–38% against 16% for black currant seeds. Borage is cultivated on a small scale for its seed and seed oil in Europe. Borage seed oil also contains 38% linoleic acid (18:2), 16% oleic acid (18:1), and palmitic and stearic acids.

Evening primrose oil (Oenothera biennis)

Evening primrose is grown commercially for its seeds in at least 15 countries, and in 1988, the United States and Canada produced 300–400 t of seed. A cultivar was developed in which GLA content has been increased to a range of 10.4 to 12.1% while total oil content increased to a range of 16.4–26.7%.

Echium *seed oil* (Echium plantagineum)

A common name for *Echium plantagineum* is Paterson's curse, since the plant is toxic to nonruminants like horses because of a high concentration of pyrrolizidine alkaloids. Its seeds contain 27% oil. The plant is native to southern Europe, but it has been introduced in Australia, South Africa, and the United States.

Gourmet Oils

In this section, a number of oils will be grouped that are sold at high prices because of their taste or other presumed, often cosmetic benefits. To maintain their taste, they have in all likelihood not been refined, resulting in quite short shelf life. Some of these gourmet oils have a long history like the local pumpkin seed oil in Styria, one of the federal states in southeast Austria, or walnut oil in southwest France; others are just fashionable, often for short times.

The prime example of gourmet oils is olive oil, but this oil will not be discussed here because it has an entry of its own (502). Accordingly, the discussion will be limited to well-established food oils with a history of their own, whereas the mentioning of other gourmet oils will be limited to their fatty acid composition in the table later in the text. In Table 3 whose data have been assembled from various sources, the totals may not add up to 100% as a result of rounding

Table 3 Fatty acid compositions of various gourmet oils

Oil	*16:0*	*18:0*	*18:1*	*18:2*	*18:3*	*Others*
Almond oil	7	2	62	29		
Apricot kernel oil	6	3	72	18		
Argan oil	12	6	43	37	<1	
Arnebia seed oil	7		14	23	45	
Avocado oil	12	<1	71	12	<1	4% 16:1
Barley germ oil	20	1	19	50	10	
Basil seed oil	8	3	10	20	59	
Brazil nut oil	14	12	30	43		
Candlenut oil	4	1	29	42	24	
Cashew nut oil	12	9	58	21		
Chestnut oil	15		54	25		
Cupuaçu butter	7	31	44	5		11% 20:0
Grapeseed oil	7	3	18	70	<2	
Hazelnut oil	6	3	80	11		
Hempseed oil	6	2	12	55	21	4% γ-18:3
Macadamia nut oil	9	3	65	3		20% 16:1
Melon seed oil	12	7	10	71		
Mustard seed oil	<2	<1	22	14	7	47% 22:1
Niger seed oil	17	7	11	63		
Onion seed oil	7	3	25	65		
Parsley seed oil	3	4	81	11		
Peach stone oil	5	1	67	27		
Pecan nut oil	8	3	50	38	1	
Pine nut oil	5	3	29	44		17% Δ-5 acids
Pistachio oil	8	1	58	30		
Plum kernel oil	6	2	70	20		
Poppy-seed oil	10	2	11	72	5	
Pumpkin seed oil	6	6	35	52		
Tea seed oil	18	3	60	18		
Tobacco seed oil	10	2	12	76		
Tomato seed oil	14	6	22	54		
Walnut oil	7	2	21	58	12	
Watermelon seed oil	11	10	15	63		
Wheat germ oil	16	1	16	58	7	2% 20:1

Source: Various. See 'Further Reading.'

off literature data or because some minor fatty acids have not been included in the table.

*Avocado oil (*Persea americana *and* Persea gratissima*)*

Avocado oil is a fruit oil. The avocado tree is grown in frost-free areas and in particular in the United States (FL and CA), New Zealand, Israel, and South Africa. Most of the oil is in the flesh of the fruit; the seed in the fruit contains only 2% oil. The edible flesh of the fruit represents 50–80% of the fruit weight and its oil content increases with maturity while the fruit remains on the tree. It can reach some 30% late in the season. The oil can be produced by cold pressing but the use of a decanter after the flesh has been homogenized and given some malaxation time is more efficient.

Avocado oil has an IV of 85–90 because it has a high (up to 74%) oleic acid (18:1) content and a low (10–15%) linoleic acid (18:2) content. In fact, its fatty acid composition is quite similar to that of olive oil. Avocado oil is an expensive oil that is used in cosmetics rather than food.

*Grapeseed oil (*Vitis vinifera*)*

The annual world production of grapes is almost 70 million tonnes. Most of these grapes are used for wines, some for jellies and jams, and some are eaten as such. Vintners press the grapes to release the juice and filter off the skins and the pips. Wine filtration depends on the type of wine. For white wine, they filter early, but for red wine, they allow the wine to extract color from the skins so they filter later. This means that the liquid retained in the filter cake contains alcohol so they may distill the filter cake to obtain a liquor (grappa) and thereby char the pips. This is reflected in the quality of the ensuing oil that may be difficult to bleach.

Like jelly manufacturers, wineries collect the pips that can serve as raw material for grapeseed oil. The filter cake must be dried to avoid mold formation. The oil content of the pips is only 6–13% so the oil can only be produced by solvent extraction. Hexane is the most convenient solvent. Grapeseed oil contains waxes so its refining process should comprise a dewaxing step. Well-refined grapeseed oil is almost colorless and has a bland taste. In Belgium, it is sold especially for fondue, which, given its high linoleic acid content, is rather surprising.

*Hempseed oil (*Cannabis sativa*)*

Global hempseed oil production is around 10 000 tonnes *per annum*. Most seed is grown in China and Canada In the US, the cultivation of industrial hemp crops was indiscriminately lumped into the same legal category as drug - *Cannabis* but nowadays, more and more states allow hemp and cannabis to be grown for industrial, medicinal, and recreational purposes. The oil content of the seed is about 35%.

When discussing hempseed oil processing, Callaway and Pate stressed the necessity to maintain an inert atmosphere throughout the processing, and when discussing hempseed oil stability, they ascribed the poor stability of hempseed oil to its high content of polyunsaturated fatty acids. However, when listing typical nutritional values for vitamins and minerals in hempseed, they mentioned an iron content of 14 mg per 100 g or 140 ppm and a copper content of 2 mg per 100 g or 20 ppm. Even if a small fraction of these metals ended up in the oil, for instance, as phosphatidate, that would already explain the poor shelf life of the oil. The statement that 'defatted meal resulting from solvent extraction is not suitable for human food because residual solvents (typically hexanes) contaminate the final product' will most certainly be disputed by those skilled in the art.

Nut oils

Table 3 lists several nut oils: almonds, Brazil nuts, cashew nuts, hazelnuts, macadamia nuts, pine nuts, pecan nuts, pistachios, and walnuts. Nuts have a woody shell that can constitute an important mass fraction in comparison with the kernel inside the nut. Since the residual oil content of a screw press cake is lost when this cake is not extracted with a solvent, it usually pays to shell the nuts before the pressing step and only feed the screw press with the kernels. This is especially true when shelling can be mechanical. If shelling is manual as with walnuts, this is reflected in the price of the oil.

The nuts themselves can be roasted before being extracted or the paste that results from grinding the shelled nuts can be roasted. This makes the taste of the oil more pronounced and characteristic. Nut oil production is usually small scale and limited to hydraulic or screw pressing of the paste. Purification of the resulting cold-pressed oil is limited to a filtration step. Extracting the press cake with a solvent increases the oil yield but the resulting crude oil needs refining.

Pumpkin seed oil (Cucurbita pepo)

This oil originated from southeast Austria and the surrounding regions of Slovenia, Croatia, and Hungary where seed production is about 8000 tonnes. The pumpkins ripen in the autumn, and then, the seeds are harvested manually and dried before being processed. The oil content of the dried seeds ranges from 38% to 49%. It takes 30–40 pumpkins to obtain 1 liter of oil and the pumpkin flesh is discarded.

According to Murkovic, the seeds are processed in small batches of 50–100 kg. They are milled into a paste, and then, a small amount of water is added prior to roasting. During the heating, the water evaporates so that the temperature can rise to about 115 °C, which causes the oil to emerge from the subcellular structures. Many chemical compounds are formed during roasting including the typical aroma that is associated with the nutty flavor. The oil also dissolves the green color that is present in the thin seed coats. The roasted paste is expelled hydraulically and a dark green oil with a nutty flavor results. It can be sent to a settling tank or be filtered to get rid of meal particles. The press cake contains some 14% oil and is used as animal feed.

Major Vegetable Oils without Entry of Their Own

Corn germ oil (Zea mays)

Corn germ oil is a by-product of the corn oil milling process. Most corn that is harvested is used as feed but the proportion of the corn that is milled is increasing because of bioethanol production. During the wet milling process, the germ is isolated from the starch using cyclone separators, washed, and dried. The dried germ contains about 50% oil, in which the oil constitutes about 85% of the total amount of oil present in the corn. The oil is produced by first expelling the germ and then extracting the expeller cake with *n*-hexane. Global annual corn production is 700 million tonnes but corn oil production is only 400 000 tonnes.

Crude corn oil can be dark reddish in color. It is refined either by alkali neutralization followed by bleaching, dewaxing, and deodorization or by degumming the oil to a residual phosphorus content below 5 ppm P, bleaching dewaxing and steam refining. The dewaxing step that comprises cooling the oil to cause the high-melting wax components to crystallize is only necessary when the corn oil is to be sold as salad oil. When the corn oil is to be used as a liquid component in margarine, the dewaxing step can be omitted.

Corn oil contains 13–17% saturated fatty acids and hardly any linolenic acid (18:3). Its main fatty acid is linoleic acid (18:2) at 55–62% followed by oleic acid (18:1) at 22–28%. Its unsaponifiable content is high at 1.3–2.3%, which is in line with its high tocopherol content. This provides the oil with a longer shelf life than would be expected given its high content of polyunsaturated fatty acids.

Cottonseed oil (Gossypium hirsutum)

Cottonseed oil is a by-product of the cotton industry and its production forms the cradle of the modern oil refining processes and many of its analyses. Cottonseed oil is exceptional in that its alkali refining process is carried out on the miscella. The miscella resulting from the solvent extraction is partially evaporated and mixed with the press oil so that a miscella strength of 60–70% results. Caustic of 14–20% strength is used in 10–40% excess and mixed with the miscella at a temperature of 50–55 °C. After a holding time of 10–12 min, the soapy and oily phases are separated. The soapy phase is sent to the meal desolventizer and the oily phase is further evaporated to yield neutral oil. A fully refined oil results after bleaching and deodorization.

Cottonseed oil contains around 55% linoleic acid (18:2), 22–26% palmitic acid (16:0), 19% oleic acid (18:1), and a few minor acids. Because of the rather high palmitic acid content of the oil, it throws a deposit on cooling that consists of dipalmitoyl glycerol and other high-melting triglycerides. Cooling happened in winter in large storage tanks and the cold-stable supernatant had therefore been 'winterized.' Nowadays, the oil is winterized when intended for salad oil by refrigerating the oil and removing the precipitate by filtration.

Rice bran oil (Oryza sativa)

When brown rice is polished to produce white rice, a rice bran fraction results that contains about 15–20% oil that can be obtained by solvent extraction. However, most bran emerging from rice mills is not extracted. It may be used in aquaculture or as fertilizer. Consequently, of the potential 4–7 million tonnes of rice bran oil *per annum*, less than 2 million tonnes is actually produced by bran extraction. Often, the oil is of poor quality and ends up in soap-making plants.

Fresh bran contains a lipase enzyme that causes the oil to hydrolyze very fast. To prevent this hydrolysis, the enzyme must be inactivated by heat or acid. A heat treatment can be applied by passing the bran through an expander, which has the additional advantage that it produces collets that facilitate percolation during the extraction process.

Crude rice bran oil often shows a high wax content of 4% or even more. This is much higher than the wax contents encountered in sunflower seed oil or corn germ oil. Whereas these latter oils are usually dewaxed during a cold neutralization step or dewaxed after the oils have been alkali-refined and bleached, dewaxing tends to be the first step in refining rice bran oil. It can be carried out by storing the oil in settling tanks and allowing it to cool so that a wax deposit is formed and a clear supernatant oil is obtained by decantation. A better way is to dewax the oil at the oil mill while as miscella.

That also holds true for the alkali refining. When this process is carried out on the miscella, very little oil is lost with the soapstock and the neutralized miscella hardly contains any residual soap. This option is, however, not open to stand-alone refineries. They have to neutralize the oil either by mixing an excess caustic into the oil and suffer neutral oil loss or by physical refining, also called steam refining because steam is invariably used as stripping agent during this process.

However, physical refining demands that the oil has a low phosphatide content ($P < 10$ ppm) and that the oils have been bleached. Various degumming processes are available that ensure this low residual phosphorus content.

Rice bran oil contains 22% palmitic acid, 2% stearic acid, almost 40% oleic acid, some 35% linoleic acid, and hardly any linolenic acid. Consequently, and also because of the presence of ferulic acid esters of phytosterols, the so-called oryzanols, which are powerful antioxidants, the oil is very stable. In addition, the oil has been proclaimed to be healthy on the grounds of epidemiological comparisons and that it lowers blood serum cholesterol levels.

*Sesame seed oil (*Sesamum indicum*)*

Sesame seeds are grown in China, India, Myanmar, and Sudan. There is relatively little international trade. The main fatty acids in sesame oil are 9% palmitic acid, 6% stearic acid, 41% oleic acid, and 42% oleic acid, and the oil also contains small amounts of palmitoleic acid (16:1), linolenic acid (18:3), arachidic acid (20:0), gadoleic acid (20:1), behenic acid (22:0), and lignoceric acid (24:0).

Small operators limit their oil production to pressing the seeds that contain 50–60% oil, but larger operators extract the cake and degum, neutralize, bleach, and deodorize the crude oil. The oil is remarkably stable because during bleaching, the sesamolin present in the oil is converted into several phenolic compounds that have antioxidant properties. The hull of the sesame seed also contains antioxidants since the oil that was extracted from whole seeds was found to be more stable than the oil resulting from dehulled seeds.

See also: Cocoa: Production, Chemistry, and Use; Fats: Classification and Analysis; Fatty Acids: Fatty Acids; Fruits of Tropical Climates: Biodiversity and Dietary Importance; Genetically Modified Foods; Genetically Modified Microorganisms; Grapes; Lauric Oils; Maize; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Nuts: Brazil Nuts; Olive Oil: Its Role in the Diet; Palm Oil; Phospholipids: Properties and Occurrence; Rapeseed Oil/Canola; Soybean Oil; Sunflower Oil; Tocopherols: Properties and Determination; Triacylglycerols: Characterization and Determination; Triacylglycerols: Structures and Properties.

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Vegetables of Temperate Climates: Carrot, Parsnip, and Beetroot

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Sources and Production

Carrots (*Daucus carota* L.), parsnips (*Pastinaca sativa* L.), and beets (*Beta vulgaris* L.) are horticultural crops that are grown on a relatively small scale compared with staple food crops, such as rice, maize, and wheat. Unlike cereal grains and dry legumes, many horticultural crops have a relatively short shelf life, requiring ready access to cold storage, which limits their long-distance distribution, especially in less-developed countries. Nonetheless, because all of these vegetables are root crops, their shelf life is long when kept in cold storage, unlike leafy green vegetables, such as spinach and lettuce, although the leaves of carrots and beets can also be consumed.

Plants contain vascular systems that allow them to move water (xylem) and nutrients (phloem) throughout the plant. Besides orange, carrots are found in other colors. In unusually pigmented carrots and beets, the xylem and phloem are readily identifiable (Figure 1). Carrots and beets with more than one color are sometimes referred to as functional foods or designer vegetables. Purple color in carrots is usually darker in the outer phloem of the root and sometimes only on the root surface.

Carrots are available around the world (Table 1) and do well in cooler climates. The first definite records of carrots being used as a root crop are in Afghanistan and surrounding regions of Central Asia around 1100 years ago, when purple and yellow carrots were described. There are some indications that carrot may have been a root crop in the Roman Empire, but records are ambiguous. The color of carrots is an important part of the history of this crop. Orange carrots were first recorded in Europe in the 1500s and are the predominant color of the crop in all growing regions of the world today. Red carrots are primarily of Asian origin and are still common today. Purple carrots predate orange carrots and were one of the first types to be consumed by humans in Central Asia and the Middle East. Although no longer widely grown, white carrots, which lack pigment, were developed as a fodder crop for feeding livestock in Europe.

Biofortification is the process of breeding crops for enhanced nutritional value. Biofortification of orange carrots has resulted in carrots with high levels of α- and β-carotene (Figure 2) with an emphasis on breeding for deeper uniform orange color. Studies have shown that these carrots provide a little extra vitamin A when fed at similar levels in animals and humans, but substantial extra amounts of α- and β-carotene, which both have antioxidant activity, are made available to tissues. Other color combinations have been bred and include purple-white, purple-yellow, purple-orange, purple-red, purple-orange-red, red-orange, and orange-yellow.

Parsnip is a vegetable plant in the family *Apiaceae*. The plant was domesticated in the Mediterranean and was consumed in Roman times. Some confusion exists in historical accounts of this vegetable due to its similarity with carrot, to which it is related. White carrots exist but are not the same vegetable as parsnips. Parsnip is biennial and produces a rosette of leaves and a swollen taproot during the first season of growth. It is well adapted to cool growing regions and substantial production occurs in Canada.

Table beet was domesticated in the Mediterranean and originally used as a leaf crop. In its earliest forms, the crop did not have a swollen root and was only consumed for its leaves and petioles. Swiss chard most likely resembles this early form of the crop. As beet moved throughout the Mediterranean region and spread north, selection occurred for a swollen root that could be stored throughout the winter. This process of selection resulted in swollen rooted forms that became the modern, pigmented table beet. Selection among the swollen rooted forms also resulted in two additional crops: fodder beet and sugar beet; the former is used for livestock and the latter as an industrial source of sucrose. Sugar beet was developed in the eighteenth century from white fodder beets. A sugar beet is about 30 cm (12 in.) long and weighs 1–2 kg (2–5 pounds) at harvest.

Patterns of Consumption

Carrots, parsnips, and beets, like other root vegetables, store well and can be eaten throughout the winter when many other vegetables are out of season. They will keep best when stored in the refrigerator, in the crisper drawer, or in plastic bags and will last for 1 month or more.

Carrots are a popular vegetable, especially in Europe and the United States, and fresh-market carrot consumption in the United States has increased recently due in part to the introduction of 'cut-and-peel' carrots. Cut-and-peeled carrots offer a convenience to consumers in their ready-to-eat form that comes in a variety of package sizes. In the United States, ~4 kg (9 pounds) are available per capita each year (Figure 3 (a)). Orange carrots are likely the highest source of α- and β-carotene in the diet of many people in the United States, and thus, modern orange carrots are a significant source of vitamin A.

Carrot leaves are consumed in parts of Asia, used either fresh in a salad or cooked in other dishes. The young tender leaves are occasionally used as a stir-fried herb in China and Japan. Carrot leaves have a similar appearance to some of its wild relatives that are not carrots, and some of these wild relatives, such as hogweed or cow parsnip, have toxic compounds in their leaves. Carrot top consumers should avoid leaves from these wild plants. In the leaves, the carotenoids are located in the photosystems of the inner chloroplast membrane usually associated with lipids. Carrot leaves have a different carotenoid profile than the roots.

Parsnips can be eaten raw but are usually cooked. Taproots of parsnips have a mild and very pleasant flavor and are prized as an ingredient in soups and stews because of their rich flavor.

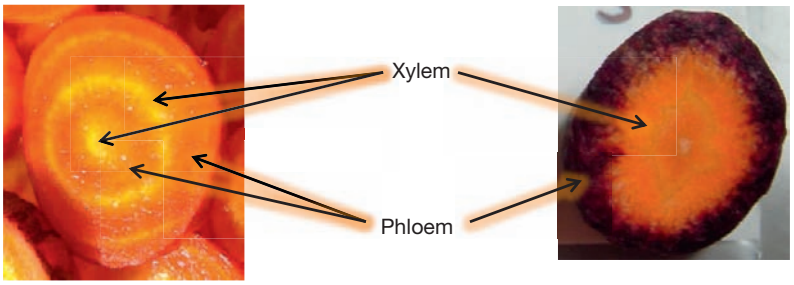

Table beet with red and yellow cambial rings

Carrot with purple phloem and orange-red/yellow xylem

Figure 1 The accumulation of pigments in beet and carrot storage roots often varies across root tissues, and as a consequence, nutritional value varies. Beetroots have multiple cambia and, with that, concentric rings of xylem and phloem tissue, while carrot roots have one cambium that separates the xylem (core) and phloem (cortex).

Table 1 World carrot production and availability, 1965–2011

Continent	*Variable*	*1965*	*1975*	*1985*	*1995*	*2005*	*2011*
Africa	Area (% world)	8	5	6	8	8	9
	Yield (ton ha^{-1})	11	12	12	12	14	16
Asia	Area (% world)	8	27	29	34	53	58
	Yield (ton ha^{-1})	28	17	21	22	23	32
Europe	Area (% world)	54	53	50	43	28	23
	Yield (ton ha^{-1})	24	24	28	20	27	31
Americas	Area (% world)	21	14	14	14	10	10
	Yield (ton ha^{-1})	22	25	24	29	29	31
Oceania	Area (% world)	2	1	1	1	1	1
	Yield (ton ha^{-1})	27	32	32	31	42	49
World	Area (1000 ha)	196	459	558	756	1151	1174
	Yield (ton ha^{-1})	22	19	22	21	24	30
	Availability (kg per capita)	1.3	2.2	2.5	2.8	4.3	5.1

Average of 3 years (e.g., 1975 = average of 1974–76).
Source: FAO yearbook production statistics.

They contain more sugar and starch than other vegetable members of the *Apiaceae* family, such as carrot, and appear to have been used as a source of both of these compounds prior to the introduction of the potato. Parsnips are sweeter than typical carrots, especially when cooked. Parsnips have sugar content comparable to fruits, such as bananas and grapes. They can be baked, boiled, pureed, and roasted and are sometimes fried to make chips. It is interesting to note that the starch in parsnips converts to sugar when exposed to cold and thus winter parsnips are sweeter than autumn parsnips. Refrigeration will also cause them to develop more sweetness.

The table beet is consumed in a variety of ways. Compared with carrots, however, availability per capita in the United States has tended to decrease over the past few decades (Figure 3(b)). Leaves, particularly small leaves known as 'baby leaves,' are consumed as a salad crop. Roots are eaten in salads, placed on sandwiches, pickled, roasted, boiled, or used in soup. A substantial portion of the United States crop is canned. Beet root can be eaten raw and will maintain more of its folate content if not cooked. When table beet roots are consumed, the hypocotyl tissue is also consumed as it is a transitional zone between the root and stem. The root/hypocotyl is actually composed of supernumerary cambia, or zones of xylem and phloem tissue, that increase in size during growth. The root/hypocotyl region also contains storage parenchyma cells, where sucrose is stored. Table beet has long been known as a sweetener and was likely used in Roman times for this purpose.

The sugar beet was developed following the Napoleonic wars when Europe needed a domestic source of sugar that did not depend on the West Indies sugar trade. Sugar beet is a natural source of sucrose, which is not different from cane sugar. Sugar beets contain about 18% sucrose, which is about two to three times higher than table beets. Sugar beet must be processed quickly because it is perishable. Beet sugar represents about 50% of domestically produced sugar in the United States.

Availability, Absorption, and Metabolism

In general, phytochemicals found in vegetables have putative mechanisms of action in the body including antioxidant effects, modulation of detoxifying enzymes, stimulation of the immune system, modulation of hormone metabolism, and antibacterial and antiviral effects. Brightly colored

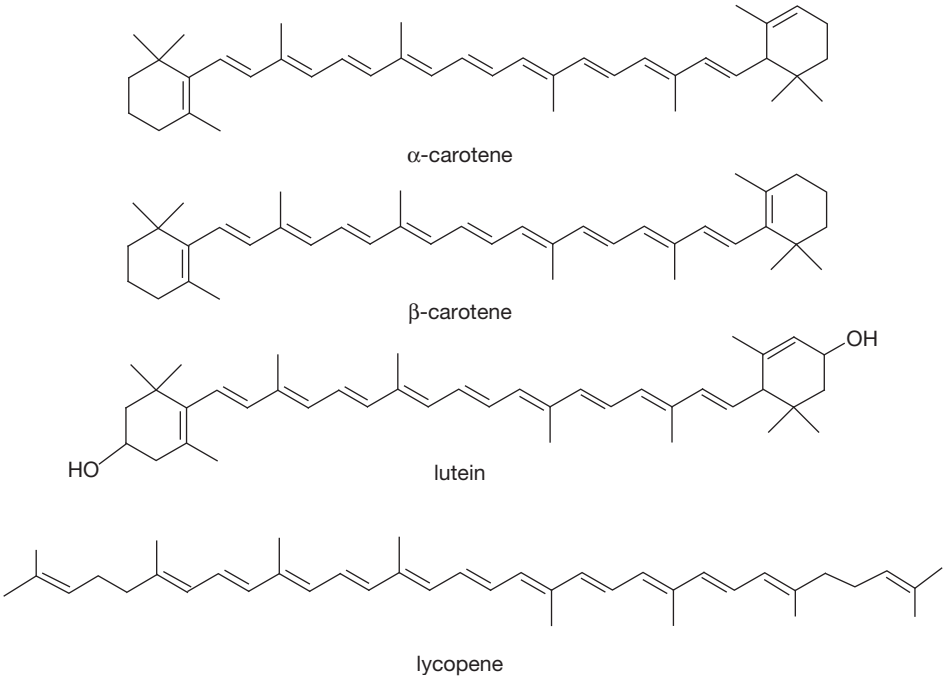

Figure 2 The four main carotenoids that are found in carrots. α- and β-carotene predominate in orange carrots. Lutein is relatively high in yellow carrots compared with β-carotene. Lycopene is high in red carrots in addition to high levels of β-carotene.

vegetables, such as carrots and beets, generally contain high amounts of phytochemicals and nutrients.

In addition to being a significant source of β-carotene, orange carrots are likely the highest source of α-carotene among all vegetables. Both of these orange compounds from carrots provide vitamin A upon central cleavage. Most mammals, including humans, are able to cleave α- and β-carotene to vitamin A. α-Carotene provides one molecule of vitamin A and one molecule of a compound commonly called α-retinol, while β-carotene can provide two molecules of vitamin A due to its symmetrical structure (Figure 2). Thus, orange, red, and some purple carrots are great sources of vitamin A. Vitamin A is essential for healthy eyes, cell growth, and reproduction. It is also important in strengthening the immune system by keeping the skin, lungs, and intestinal tract mucosa healthy. When carrots are eaten, not only do they provide vitamin A, but also the excess carotenoids can be absorbed and circulated intact in the human body. In circulation, α-carotene and β-carotene are predominantly associated with low-density lipoproteins. Therefore, some animals that have higher-density lipoproteins, such as gerbils and chickens, may not circulate much β-carotene in the blood.

Carrots of other colors have a different carotenoid profile. Yellow carrots contain lutein, which is bioavailable to humans, in addition to small amounts of β-carotene. Lutein is a xanthophyll carotenoid, which means it is partially oxygenated. Because it contains hydroxyl groups on both sides of the molecule (Figure 2), lutein cannot be cleaved by mammals to form molecules of vitamin A. A human study demonstrated that lutein from yellow carrots was 65% as bioavailable as that from a lutein supplement dissolved in oil.

Red carrots accumulate lycopene, a common pigment in red tomatoes, in addition to high amounts of β-carotene. Like α- and β-carotene, lycopene is a hydrocarbon carotenoid, but it does not have any cyclic rings, making it quite different structurally from the other well-known carotenoids in carrots (Figure 2). Lycopene from red carrots is about 40% as bioavailable as that from tomato paste. For consumers who do not like tomatoes, red carrots are another food source of lycopene. Consumers in India actually prefer red-pigmented carrots to orange ones.

Other phytochemicals present in purple carrots include flavonoids, such as red or blue anthocyanins (Figure 4), and purple carrots may, or may not, contain carotenoids. Anthocyanin pigmentation develops independently of carotenoid colors, so some purple carrots contain α- and β-carotene. Purple color may mask the appearance of carotenoids. The purple anthocyanins found in carrots are sometimes used as food colorants. Some of the carrot anthocyanins have an acyl chemical group, while some do not. The acylated anthocyanins of carrot are more stable when used as a food colorant, but the nonacylated anthocyanins are more bioavailable. Some strains of purple carrot are sometimes referred to as 'black' carrot because the anthocyanins are so concentrated that they have deep purple color in the phloem and xylem. Efforts to breed carrots of several colors have been on the rise, and within the past decade, purple-orange-red carrots have been bred for increased red color. These particular carrots would have anthocyanins, α- and β-carotene, in addition to lycopene (Figure 1). A common name for these types of carrots is 'rainbow carrots.' Through outreach efforts, rainbow carrot seeds have been distributed to community and youth gardeners to build awareness and to use the harvested carrots to demonstrate the diversity of pigments in vegetables.

Lastly, carrots also contain phenolic acids that have a single aromatic ring. The main phenolic acids are chlorogenic acids

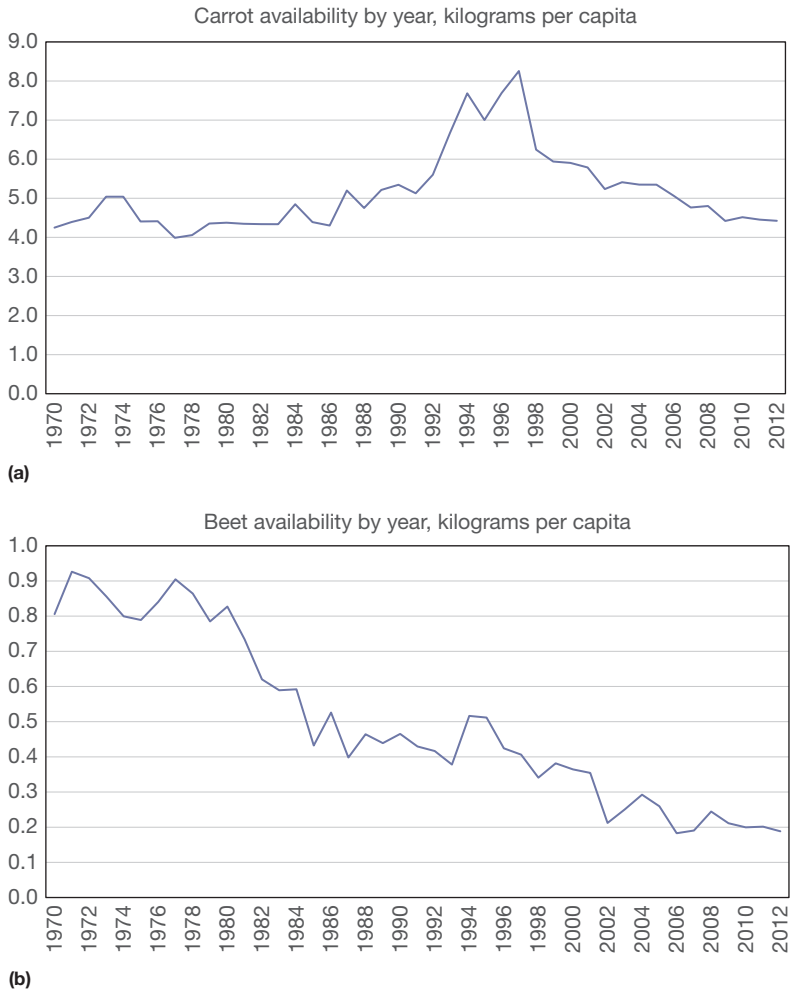

Figure 3 Carrot and beet availability in the United States over the past few decades expressed in kilograms.

(Figure 4), which are hydroxycinnamic acid derivatives formed by the esterification of cinnamic acids with quinic acid. These compounds contribute to the organoleptic properties of fresh and processed carrots. Approximately 30% of chlorogenic acids are absorbed by the intestine and the remainder is extensively metabolized by colonic microflora.

Although typical parsnips are white, they contain compounds known as polyacetylenes, which may impart beneficial properties to this vegetable. Plants synthesize polyacetylenes to protect them against pathogens and pests. They are derived from unsaturated fatty acids. One type of polyacetylene called falcarinol is highly bioavailable in humans, being rapidly absorbed after ingestion (Figure 4). Interestingly, falcarinol was viewed as a potentially undesirable component of food in the 1980s, and now, it is viewed by some as desirable.

Table beet is colored by betalain pigments (Figure 5). The betalains are a unique class of alkaloid pigments found in plant species in the order *Caryophyllales* and in some fungi. Tyrosine is converted to L-DOPA via hydroxylation and subsequently to betalamic acids via the extradiol ring cleavage reaction. Betalains are ultimately formed via spontaneous chemical conversion. Recently, the *R* locus that controls betalain pigment production in table beet was identified as a novel cytochrome P450. This protein provides the cyclo-DOPA moiety that is required for all red betacyanin pigments. Selection has been effective at increasing betalain concentration in table beet. Betalains are water-soluble, fairly easy to extract, and a commonly used source of natural colorants. After ingestion, betalains are thought to be degraded in the stomach and absorbed in the gut, possibly through passive diffusion. An estimated 50% of betalain is lost during digestion, and urinary betalain ranges between 0.3% and 1% after consumption of beet juice or extract, which may reflect low absorption. Food processing also affects betalain absorption, with lower bioavailability from the whole beet matrix compared with purified beet juice or extract.

Health Effects

Root vegetables provide energy through their carbohydrate content and are naturally low in fat. Root vegetables are a good source of fiber, ranging from 3 g fiber in 100 g of raw carrots and beets to 5 g in 100 g of parsnips. Cooking tends to lower the fiber content in beets and parsnips. Carrot fiber is highly regarded and has become of interest to food processers

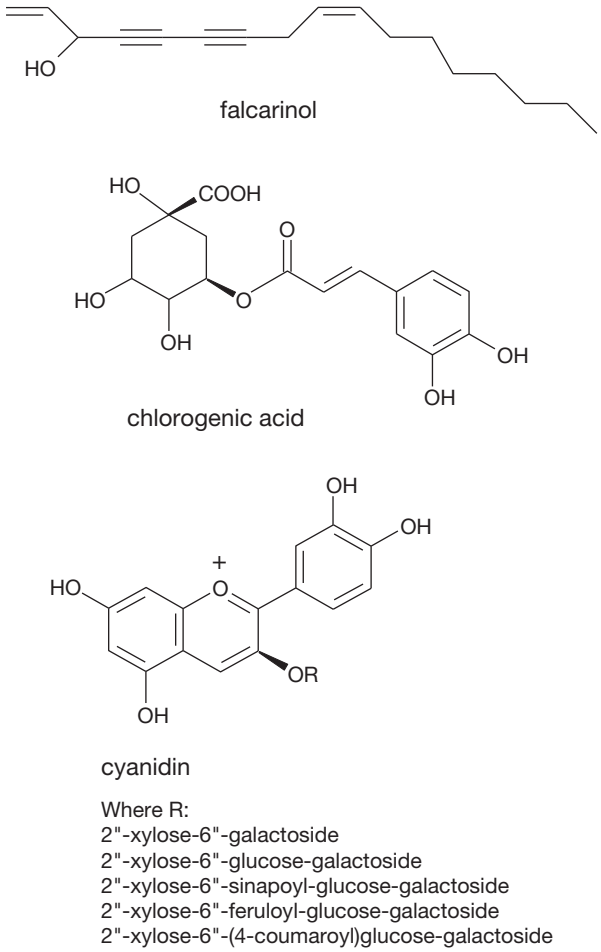

Figure 4 Carrots contain many phytochemicals. Falcarinol contributes to carrot sensory characteristics and is also found in parsnips. Chlorogenic acid is a common phenolic acid found in carrots. Anthocyanins are the red and blue compounds that give purple carrots their hue and contribute to their high antioxidant capacity.

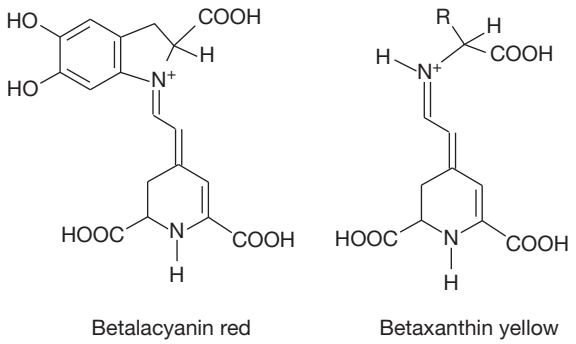

Figure 5 Betalains are the primary pigments in red and yellow beets. These water-soluble pigments are unique to the order *Caryophyllales*, of which the *Amaranthaceae* family, and the table beet in particular, is a member. The betalains are used as a natural alternative to synthetic food dyes in certain food products. They show considerable antioxidant activity and may have potential in improving the health functionality of foods.

due to the large quantities of carrot waste, known as pomace, created in the cut-and-peel and juice industries. The insoluble fibers, primarily cellulose and hemicellulose, constitute the greatest portion of the total dietary fiber with small amounts of lignin. The soluble fibers include fermentable hemicellulose and pectin. The fiber composition of dried carrot pomace differs from whole carrot and depends on the processing method.

Raw root vegetables are also a good source of water ranging from 80% in parsnips to 88% in carrots and beets. Boiling the vegetables does not appreciably change the water content (<2%) when based on a 100 g serving. While cooking breaks down some nutrients, both carotenoids and anthocyanins of carrot are quite stable with light cooking. Root vegetables are also a good source of potassium providing ~350 mg in 100 g.

Research has focused on the numerous health benefits of phytochemicals, including those that are found in the root vegetables. Carrots, in particular, have been studied for nutritive benefits and color components and are widely considered a 'functional food.' The pigments in carrots serve an important role because they have been associated with reduced risks of atherosclerosis, cancer, and inflammation. Carrots have antioxidant capacity because both α-carotene and β-carotene are antioxidants, which are important to trap free radicals and fight against heart disease. Both carotenoids have been reported to have anticancer potential. Yellow carrots contain lutein, which has been implicated in improved eye health. Specifically, lutein and zeaxanthin are concentrated in the macula of the eye and may be important in preventing macular degeneration. Lutein has also been implemented in preventing some forms of cancers and reducing the risk of atherosclerosis. Intake of dietary lycopene, as found in red and some purple carrots, has been associated with a reduced incidence of some forms of cancer. Lycopene, which has much higher antioxidant potential than β-carotene, is also associated with a reduced risk of serum lipid oxidation and heart disease.

When carrots or carrot juice are consumed at a high level in the diet, a condition known as hypercarotenemia can occur. This is due in part to the downregulation of bioconversion of the provitamin A carotenoids to vitamin A as a way to protect the human body from hypervitaminosis A due to high vegetable intake. The carotenoids will accumulate in the adipose tissue and the yellow pigmentation of the palms is usually first noticeable. This is thought to be a benign condition.

Eleven different phenolic acids in orange, purple, yellow, and white carrots have been identified. Total concentration of all identified phenolic acids is greatest in the purple carrots followed by orange, white, and yellow. In particular, chlorogenic acid consumption has been associated with reductions in several chronic diseases.

Anthocyanins in purple and black carrots act as powerful antioxidants to sequester harmful free radicals in the body; however, human data are lacking. Anthocyanins may prevent heart disease by acting as anti-inflammatory agents and reducing lipid oxidation. Solid-colored purple carrots contain the most phenolic compounds and therefore may have higher antioxidant capacity in the human body.

Parsnips are a better source of vitamin C than carrots and beets. Parsnips and beets provide more folate than carrots. Falcarinol may be involved in carrot and parsnip beneficial

health effects including anti-inflammatory, antiplatelet aggregation, and potential anticancer activity. While falcarinol can cause allergic skin reactions, contact dermatitis from *Apiaceae* family vegetables is rare, likely due to the relatively low concentrations of falcarinol in the species commonly consumed or sensitization from oral intake.

Parsnip leaves also contain furanocoumarins, which can cause photodermatitis if they are touched or brush up against the skin in the presence of light. Burns caused by these compounds can be serious and can leave scars that do not easily disappear. Parsnips are therefore difficult to handle and individuals handling the crop require substantial protection during production. Parsnip root contains photocarcinogenic psoralens, which are not destroyed by cooking. However, the effect and potential toxicity of these chemicals from normal human consumption are not known.

Beet greens by weight have even more micronutrients than beetroot, including vitamin A as β-carotene, vitamin C, vitamin K, calcium, iron, magnesium, potassium, copper, and manganese. Beet leaves also contain a substantial amount of lutein and zeaxanthin and thus have health benefits associated with those carotenoids as detailed earlier for carrots.

Beets are among the vegetables with the highest antioxidant content. Betalain pigments are among the antioxidants in beets and may be associated with cancer prevention. Betalains in beet extract reduced tumors associated with skin and lung cancer in mice, but no human studies have been done to date that demonstrate substantial health benefits. Betalains have a large nonpolar surface area that allows them to interact with lipid membranes and low-density lipoprotein particles in the blood reducing oxidation.

Oxalic acid is related to the formation of kidney stones and has other antinutritive properties. Table beet is considered a high oxalate food, and therefore, breeding to decrease oxalic acid levels is an area of interest. In a collection of table beet cultivars, mean values were from 722 to 1909 mg in 100 g of leaf tissue and 553 to 1679 mg in 100 g leaf tissue for total and soluble oxalate levels, respectively. Root-soluble oxalate values ranged from 103 to 171 mg in 100 g of root tissue, and total values ranged from 95 to 142 mg in 100 g of root tissue. These findings confirm relatively high levels of oxalate in table beet.

Many people are familiar with 'beeturia,' or the excretion of red urine following beet consumption. This occurs in 10–14% of the population and seems to be a harmless idiosyncratic reaction relating to digestion of the beet pigments. The occurrence of beeturia is probably related more to an individual's physiological conditions (such as stomach acidity), nutritional status, type of beet, and amount consumed, rather than genetics as previously thought.

Summary

Root vegetables are a source of energy in the form of carbohydrates and a good fiber source. They contain essential nutrients as well as a variety of classes of phytochemicals associated with optimal health.

See also: Antioxidants: Role on Health and Prevention; Bioavailability of Nutrients; Carotenoids: Occurrence, Properties and Determination; Colors: Properties and Determination of Natural Pigments; Dietary Fiber: Determination; Functional Foods; Phenolic Compounds: Bioavailability and Health Effects; Phenolic Compounds: Occurrence, Classes, and Analysis; Salad Crops: Root, Bulb, and Tuber Crops; Sugar Alcohols; Tannins.

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Vegetables of Temperate Climates: Leafy Vegetables

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Definition of Vegetables

The term vegetable, appearing in the fifteenth century, recorded for the first time the adjective use of the word familiar to us as 'having to do with plants.' Vegetable comes from the Medieval Latin term 'vegetabilis' meaning vegetative; the Latin word 'vegetare' means grow.

Taso and Lo define a vegetable as "an edible, usually succulent plant or portion of it eaten with staples as main course or as supplementary food in cooked or raw form."

Vegetables are commonly grouped according to the edible part: root, stem, leaf, immature flower bud, fruit, and sprout. As a general rule, the edible parts of leafy vegetables are the leaves and the immature flower buds for cauliflower, broccoli, and artichoke.

Among all vegetable species, the leafy vegetable groups show the highest rate of respiration, which has the function of releasing energy from stored chemical compounds in the plant. The depletion of energy reserves leads to the reduction of shelf life of the product. During the shelf life, the fresh product loses water continuously (transpiration), which causes a moisture reduction of tissues and thus an irreversible loss of turgor. Water loss is the major cause of postharvest losses in leafy vegetables. Leafy vegetables are perishable because senescence starts very soon in detached vegetables. Senescence is characterized by the appearance of undesirable symptoms: reduction of greenness and appearance of yellowed leaf areas and loss of flavor and aroma. With ongoing senescence, the nutrition value of the vegetable is corrupted. For this reason, the quality of leafy vegetables is strongly dependent on freshness, such as the time elapsed from cutting (in the field or in a controlled environment) and the cold storage chain (from field to shelf).

Types of Leafy Vegetables

Leafy vegetables are a wide group of horticultural plants that roughly can be defined as "vegetables cultivated for the edible part constituted of foliar structures, comprising lamina, petiole, midrib and veins." A list of the most common species of temperate climate vegetables is presented in Table 1.

Leafy vegetables may be cool-season or warm-season crops and thrive in areas where the mean temperatures range between 15 and 18 °C. These vegetables can be grown as annuals or as biennials or perennials. Leafy vegetables such as lettuce and spinach are most tolerant of shade.

Many leafy vegetables have shallow root systems and cultivation should be done carefully: sandy loam soils are best for growing these vegetables. All leafy vegetables grow best in soils with a pH of 6.0–6.8 except lettuce which grows best at a pH of 6.5–7.0.

Leafy vegetables are defined as 'green' when cooked before consumption (mature leaves of many Brassicaceae and Chenopodiaceae), and 'raw' when consumed fresh (e.g., salads). This latter group includes different lettuces of the botanical genus Lactuca and chicory, escarole, endive, and radicchio are classified as *Cichorium intybus* L. and include minor species such as valerian or lamb's lettuce (*Valerianella locusta* (L.) Laterr.), rocket or arugula (*Eruca vesicaria* (L.) Cav., Diplotaxis spp.), cress (*Lepidium sativum* (L.)), etc.

Consumption of salads is particularly high in the Mediterranean countries where the cultivation of these species has ancient traditions linked to the hot weather and refreshing, thirst quenching, digestive, and diuretic properties of these products. The salad leaves can be eaten by themselves or, according to tradition, served with salt, oil, and different types of vinegar or lemon. Today, they are offered as a side dish or, in the low-calorie diets, as an appetizer or as a main dish topped with a variety of ingredients.

Fresh-Cut or Minimally Processed Leaf Vegetables

The consumption of vegetables with a high content of service – such as fresh salads ready for use – has undergone significant increases in recent times; and high prices, compared to the corresponding unprocessed products, do not seem to be an obstacle to the development of the sector. The high request for the service and the good quality and availability are causing the demand for pre-made salads to grow, even in countries with a strong culture of fresh and loose products, which is still considered safer and more natural.

The most important requirements for fresh-cut or minimally processed products are qualities related to consumer preferences, such as color, brightness, and maintenance over time; high resistance to oxidation processes after cutting; the absence of soil or other solid material, residues of pesticides, and nitrates within the limits of tolerance; limited microbial load; high dry-matter content; and long shelf life.

This category includes all those vegetable crops cultivated with very high plant densities (number of plants per square meter), which are eaten as fresh salad leaves, harvested young – the so-called 'baby leaf vegetables,' The maturity of the plants is defined by their height, which, at harvest, may range from 50–70 mm up to 80–120 mm, depending on the species and the customer demands. At harvest, which is defined as marketable maturation, smart growers apply a cutting height that excludes most of the petiole and includes the major part of lamina.

This group of salad-leaf plants includes chard, chicory, lettuce, rocket, spinach, lamb's lettuce, and other species that are of lesser importance, but which are often used in order to vary the appearance and taste, especially in mixtures (e.g.,

Table 1 The most common cultivated species of temperate climate vegetables

Common name	*Edible part*	*Botanical name*	*Family*
Endive, Escarole	Leaf	*Cichorium endivia* L.	Asteraceae
Chicory, Witloof chicory, Radicchio (Italian chicory)	Leaf	*Cichorium intybus* L. var. foliosum Bisch.	Asteraceae
Butterhead lettuce, head lettuce	Leaf	*Lactuca sativa* L. (var. *capitata* L. *nidus tenerrima* Helm)	Asteraceae
Cos lettuce, Romaine lettuce, leaf lettuce	Leaf	*Lactuca sativa* L. (var. *longifolia* Lam., var. *romana* Hort. in Bailey)	Asteraceae
Crisphead lettuce	Leaf	*Lactuca sativa* L (var. *capitata* L. *nidus jäggeri* Helm)	Asteraceae
Cutting lettuce	Leaf	*Lactuca sativa* L (var. *acephala* Alef., syn. var. *secalina* Alef., syn. var. *crispa* L.)	Asteraceae
Abyssian mustard	Leaf	*Brassica carinata* A. Braun	Brassicaceae
Mustard	Leaf	*Brassica juncea* (L.) Czernj. & Coss.	Brassicaceae
Black mustard	Leaf	*Brassica nigra* L. Koch.	Brassicaceae
Kale, collards	Leaf	*Brassica oleracea* L. Acephala group DC.	Brassicaceae
Cabbage	Leaf	*Brassica oleracea* L. Capitata group L.	Brassicaceae
Savoy cabbage	Leaf	*Brassica oleracea* L. Sabauda group L.	Brassicaceae
Pak choi, Chinese mustard	Leaf	*Brassica rapa* L. Chinensis group (Rupr.) Olsson	Brassicaceae
Chinese cabbage, petsay	Leaf	*Brassica rapa* L. Pekinensis group (Lour.) Olsson	Brassicaceae
Rocket salad, arugula	Leaf	*Eruca sativa* Miller syn. E. vesicaria subsp. sativa (Miller) Thell., *Brassica eruca* L.	Brassicaceae
Wild rocket	Leaf	*Diplotaxis tenuifolia* (L.) DC. syn. *Sisymbrium tenuifolium* L	Brassicaceae
Garden cress	Leaf	*Lepidium sativum* L.	Brassicaceae
Watercress	Leaf	*Nasturtium officinale* R.Br.	Brassicaceae
Chard, Swiss chard	Leaf	*Beta vulgaris* L. cicla group	Chenopodiaceae
Spinach	Leaf	*Spinacia oleracea* L.	Chenopodiaceae
Lamb's lettuce, Common cornsalad	Leaf	*Valerianella locusta* (L.) Laterr.	Valerianaceae
Sorrel	Leaf	*Rumex* spp.	Polygonaceae
Borage	Leaf	*Borrago officinalis* L.	Boraginaceae
Asparagus lettuce, stalk lettuce, stem lettuce, celtuce	Stem	*Lactuca sativa* L. (var. *angustana* Irish ex Bremer, syn. var. *sparagina* Bailey, syn. *L. angustana* Hort. in Vilm.)	Asteraceae
Cardoon	Main nervation	*Cynara cardunculus* L.	Asteraceae
Celery	Prevalently petiole	*Apium graveolens* L. Dulce group (Mill.) Pers.	Apiaceae
Fennel	Thickened base of the petiole	*Foeniculum vulgare* Mill.	Apiaceae
Purslane	Leaf and young shoot	*Portulaca oleracea* L.	Portulacaceae
Rhubarb, pieplant	Petiole	*Rheum rhabarbarum* L.	Polygonaceae
Vegetable rape	Leaf and young flower stalk	*Brassica napus* L. Napus group	Brassicaceae
Parsley	Leaf (culinary herb)	*Petroselinum crispum* (Mill.) Nym. Crispum group	Apiaceae
Italian Parsley	Leaf (culinary herb)	*Petroselinum crispum* (Mill.) Nym. Neapolitanum group	Apiaceae
Chervil	Leaf (culinary herb)	*Anthriscus cerefolium* (L.) Hoffm.	Apiaceae
Spearmint	Leaf and inflorescence (culinary herb)	*Mentha spicata* L. EM. Harley	Lamiaceae
Common basil, sweet basil	Leaf (culinary herb)	*Ocimum basilicum* L.	Lamiaceae
Marjoram	Leaf (culinary herb)	*Origanum majorana* L.	Lamiaceae
Oregano	Leaf (culinary herb)	*Origanum vulgare* L.	Lamiaceae
Perilla	Leaf and seed	*Perilla frutescens* (L.) Britt. Crispa group (Thunb.) Deane	Lamiaceae
Rosemary	Leaf (culinary herb)	*Rosmarinus officinalis* L.	Lamiaceae
Sage	Leaf (culinary herb)	*Salvia officinalis* L.	Lamiaceae

watercress, dandelion, mizuna, purslane, tatsoi, mustard, russian kale, pac choi, etc.).

Quality of Leafy Vegetables

The concept of vegetable quality is dynamic, always evolving and strongly influenced by lifestyle, tastes, religion, culture, and tradition. At the time of the 'green revolution,' science was mainly oriented toward solving problems linked to malnutrition and starvation; genetic studies were performed essentially to enhance productivity and to primarily safeguard the commercial (size and uniformity of size, weight, texture) and organoleptic (taste, crunchiness, color) qualities of the product. At the end of the 1960s, and in the following decade, due to the chemistry boom that had spread the use of synthetic

fertilizers and pesticides in agriculture, the public started to have serious doubts about the indiscriminate use of these substances, especially on horticultural products for fresh consumption, and the problem of the accumulation of nitrates had already begun to be felt.

In those years, the legislator increasingly issued rules regarding safety times, residues of the active ingredients, doses, and sale and use of synthetic chemicals in agriculture. The insufficient dissemination of the culture of hygiene and the use of wastewater (a bad habit not entirely lost even today in many countries) caused salads to be at the center of scandals about the spread of epidemic diseases transmitted via the fecal–oral route, such as hepatitis A, cholera (*Vibrio cholera*), typhoid (*Salmonella typhi*), salmonellosis (*Salmonella* spp.), and bacterial enteritis (*Campylobacter jejuni*).

In the 1960s and 1970s, early alarms about dietary excesses were sounded because of the prevailing presence of high-energy foods in the diet. Therefore, the need to define the criteria for a healthy and balanced diet was born (dietetics science emerged), and vegetables for fresh consumption were highly valued for their contributions of nutritional minerals, vitamins, fiber, and low energy supply. Thus, although it started with traditional commercial and organoleptic characteristics, the concept of the quality of leafy vegetables has been enriched with new attributes related to nutritional and hygienic characteristics. In subsequent years, the rapid development of large and multinational food companies, the increase in the standard of living, and the consequent greater purchasing power have increased the importance of quality, in a broad sense, as a deciding factor in consumer choices.

The cultural level, sensibility, and income growth of communities put consumers in a position to appreciate the quality parameters of the vegetables as they would the health properties, which went beyond the traditional and widely accepted approach to determining product value. These properties are essentially linked to the capabilities of specific substances contained in vegetables, such as vitamins (A, B, C, E, etc.), polyphenols, carotenoids, and other substances, to prevent the onset of serious human pathologies.

Moreover, additional services related to preparing leafy vegetables for fresh consumption (washing, cutting, mixing, and packaging) are increasingly appreciated, perfectly matching the new ways of living and the changes in modern society. Recently, there has been a tendency to broaden the concept of food quality and safety beyond the simple intrinsic characteristics of the products, increasing the quality of the production process. In terms of consumer preferences, the sense of taste and nutritional requirements are combined with and compared to consumer expectations regarding the environment, the biosphere, and perceptions of the guarantees offered by the manufacturers. These new aspects of quality fundamentally linked to the production process call to mind subjects of popular concern, such as environmental pollution, the efficient use of natural resources, and the consequent sustainability of agricultural and job activities, as well as the traceability of food chain and product.

Biologically Active Constituents and Health Properties

Although energy poor, vegetables are rich sources of minerals, fiber, vitamins, and, most of all, many phytochemicals belonging to four main classes: polyphenols, terpenoids, sulfur compounds, and alkaloids. Polyphenols and, to a certain extent, carotenoids and sulfur-containing compounds have been shown through epidemiological cohort studies or through mechanistic *in vitro* or animal studies to prevent coronary heart diseases, chronic inflammatory diseases, obesity, diabetes, neurodegenerative diseases, cancer, macular degeneration, and many others. Owing to their chemical structures, these phytochemicals by-and-large display strong antioxidant capacity *in vitro*.

These horticultural products add diversity, stimulate our senses by having organoleptic properties such as color, flavor, and texture, and contribute to our appetite. Fruits and vegetables have long been recognized for their nutritive value. They are excellent sources of minerals, essential fatty acids, and fibers, but also unique sources of vitamins (C, E, B, and folic acid). Most of all, they are rich sources of bioactive phytochemicals. They contribute, through their high content in non-digestible fibers, to the feeling of satiety. For these reasons, fruits and vegetables, and plants in general, are the base of most of food pyramids.

Where Beneficial Compounds Come From

Desjardins provides an exhaustive essay on the origin of secondary metabolites produced by plants to cope with stress. Because plants cannot move away from stress or danger, they produce molecules to palliate stress. In particular, vascular plants accumulate several thousand secondary metabolites in response to a variety of biotic stresses, such as insects and pathogens, and abiotic stresses, such as phosphorus deficiency, cold temperatures, high light or ultraviolet radiation, and low nitrogen. These secondary metabolites can be grouped in four main classes: polyphenols, terpenoids, sulfur-containing compounds, and alkaloids. Unexpectedly, even if plant secondary metabolites are toxic against pathogens and herbivores and protect against abiotic stress, many of these compounds also show health benefits against many human chronic diseases.

Astorg reports that almost 600 carotenoids from natural sources have been characterized. Green leafy vegetables are considered to be rich in carotenoids. Flavonoids are one of the largest groups of secondary plant metabolites: over 4000 compounds have been identified.

The values shown in Table 2 reveal that the leafy vegetables are generally characterized by a good number of vitamins and minerals, low dry-matter content that at most reaches 10% (spinach and lamb's lettuce), and reduced contents of proteins, carbohydrates, and fats. Consequently, these foods have modest caloric power between 15 and 31 cal per 100 g of fresh product. A summary of the contents of Table 2 follows.

- Amaranth leaves have very low values for thiamine and pantothenic acid. Many values are ranked medium and high, and the leaves have very high contents of Ca, Zn, Mg, and vitamin K.
- Arugula is very low in α-carotene and β-cryptoxanthin, low in Se and β-carotene, and medium-high in lipids, Ca, and fat poly, while no parameter is classified very high.

Table 2 Nutritional parameters in 100 g (raw) of several commercial leafy-vegetable species

Raw material (values per 100 g)	Energy (Kcal)	Protein (g)	Lipid Tot (g)	Ash (g)	Carbohy drt (g)	Fiber TD (g)	Sugar Tot (g)	Ca (mg)	Fe (mg)	Mg (mg)	P (mg)	K (mg)	Na (mg)	Zn (mg)	Cu (mg)	Mg (mg)	Se (µg)	Vit_C (mg)	Thiamin (mg)	Riboflav in (mg)	Niacin (mg)	Panto_Ac id (mg)	Vit B6 (mg)	Folate_ Tot (µg)	Vit A (IU/100 g)	α-Carot (µg)	β Carot (µg)	β Crypt (µg)	Lut+Zea_ (µg)	Vit_E (mg)	Vit_K (µg)	Fa Sat (g)	FAT Mono (g)	Fat Poly (g)
AMARANTH leaves	23	2.46	0.33	1.5	4.02			215	2.32	55	50	611	20	0.9	0.162	0.885	0.9	43.3	0.027	0.158	0.658	0.064	0.192	85	2917						1140	0.091	0.076	0.147
ARUGULA	25	2.58	0.66	1.4	3.65	1.6	2.05	160	1.46	47	52	369	27	0.47	0.076	0.321	0.3	15	0.044	0.086	0.305	0.437	0.073	97	2373	0	1424	0	3555	0.43	108.6	0.086	0.049	0.319
BEETS	43	1.61	0.17	1.08	9.56	2.8	6.76	16	0.8	23	40	325	78	0.35	0.075	0.329	0.7	4.9	0.031	0.04	0.334	0.155	0.067	109	33	0	20	0	0	0.04	0.2	0.027	0.032	0.06
BEET greens	22	2.2	0.13	2.33	4.33	3.7	0.5	117	2.57	70	41	762	226	0.38	0.191	0.391	0.9	30	0.1	0.22	0.4	0.25	0.106	15	6326	3	3794	0	1503	1.5	400	0.02	0.026	0.046
BORAGE	21	1.8	0.7	1.44	3.06			93	3.3	52	53	470	80	0.2	0.13	0.349	0.9	35	0.06	0.15	0.9	0.041	0.084	13	4200							0.17	0.211	0.109
BROCCOLI	34	2.82	0.37	0.87	6.64	2.6	1.7	47	0.73	21	66	316	33	0.41	0.049	0.21	2.5	89.2	0.071	0.117	0.639	0.573	0.175	63	623	25	361	1	1403	0.78	101.6	0.039	0.011	0.038
CHARD (Swiss)	19	1.8	0.2	1.6	3.74	1.6	1.1	51	1.8	81	46	379	213	0.36	0.179	0.366	0.9	30	0.04	0.09	0.4	0.172	0.099	14	6116	45	3647	0	11000	1.89	830	0.03	0.04	0.07
CHICORY (Witloof)	17	0.9	0.1	0.47	4	3.1		19	0.24	10	26	211	2	0.16	0.051	0.1	0.2	2.8	0.062	0.027	0.16	0.145	0.042	37	29							0.024	0.002	0.044
CHICORY greens	23	1.7	0.3	1.3	4.7	4	0.7	100	0.9	30	47	420	45	0.42	0.295	0.429	0.3	24	0.06	0.1	0.5	1.159	0.105	110	5717	0	3430	0	10300	2.26	297.6	0.073	0.006	0.131
CHIVES	30	3.27	0.73	1	4.35	2.5	1.85	92	1.6	42	58	296	3	0.56	0.157	0.373	0.9	58.1	0.078	0.115	0.647	0.324	0.138	105	4353	0	2612	0	323	0.21	212.7	0.146	0.095	0.267
COLLARDS	32	3.02	0.61	1.32	5.42	4	0.46	232	0.47	27	25	213	17	0.21	0.046	0.658	1.3	35.3	0.054	0.13	0.742	0.267	0.165	129	5019	14	2991	28	4323	2.26	437.1	0.055	0.03	0.201
CORIANDER (Cilantro) leaves	23	2.13	0.52	1.47	3.67	2.8	0.87	67	1.77	26	48	521	46	0.5	0.225	0.426	0.9	27	0.067	0.162	1.114	0.57	0.149	62	6748	36	3930	202	865	2.5	310	0.014	0.275	0.04
DANDELION greens	45	2.7	0.7	1.8	9.2	3.5	0.71	187	3.1	36	66	397	76	0.41	0.171	0.342	0.5	35	0.19	0.26	0.806	0.084	0.251	27	10161	363	5854	121	13610	3.44	778.4	0.17	0.014	0.306
ENDIVE	17	1.25	0.2	1.41	3.35	3.1	0.25	52	0.83	15	28	314	22	0.79	0.099	0.42	0.2	6.5	0.08	0.075	0.4	0.9	0.02	142	2167	0	1300	0	0	0.44	231	0.048	0.004	0.087
KALE	49	4.28	0.93	2.01	8.75	3.6	2.26	150	1.47	47	92	491	38	0.56	1.50	0.659	0.9	120	0.11	0.13	1	0.091	0.271	141	9990	54	5927	81	8198	1.54	704.8	0.091	0.052	0.338
KALE, Scotch	42	2.8	0.6	1.28	8.32	1.7		205	3	88	62	450	70	0.37	0.243	0.648	0.9	130	0.07	0.06	1.3	0.076	0.227	28	3100							0.078	0.045	0.289
LETTUCE (Butterhead + Boston & bibbTypes)	13	1.35	0.22	0.57	2.23	1.1	0.94	35	1.24	13	33	238	5	0.2	0.016	0.179	0.6	3.7	0.057	0.062	0.357	0.15	0.082	73	3312	0	1987	0	1223	0.18	102.3	0.029	0.008	0.117
LETTUCE (Cos or Romaine)	17	1.23	0.3	0.58	3.29	2.1	1.19	33	0.97	14	30	247	8	0.23	0.048	0.155	0.4	4	0.072	0.067	0.313	0.142	0.074	136	8710	0	5226	0	2312	0.13	102.5	0.039	0.012	0.16
LETTUCE (Iceberg, incl. Crisphead types)	14	0.9	0.14	0.36	2.97	1.2	1.97	18	0.41	7	20	141	10	0.15	0.025	0.125	0.1	2.8	0.041	0.025	0.123	0.091	0.042	29	502	4	299	0	277	0.18	24.1	0.018	0.006	0.074
LETTUCE, Green leaf	15	1.36	0.15	0.62	2.87	1.3	0.78	36	0.86	13	29	194	28	0.18	0.029	0.25	0.6	9.2	0.07	0.08	0.375	0.134	0.09	38	7405	0	4443	0	1730	0.22	126.3	0.02	0.006	0.082
LETTUCE, Red leaf	16	1.33	0.22	0.55	2.26	0.9	0.48	33	1.2	12	28	187	25	0.2	0.028	0.203	1.5	3.7	0.064	0.077	0.321	0.144	0.1	36	7492	0	4495	0	1724	0.15	140.3	0.017	0.005	0.072
SPINACH	23	2.86	0.39	1.72	3.63	2.2	0.42	99	2.71	79	49	558	79	0.53	0.13	0.897	1	28.1	0.078	0.189	0.724	0.065	0.195	194	9377	0	5626	0	12198	2.03	482.9	0.063	0.01	0.165
RADICCHIO	23	1.43	0.25	0.7	4.48	0.9	0.6	19	0.57	13	40	302	22	0.62	0.341	0.138	0.9	8	0.016	0.028	0.255	0.269	0.057	60	27	0	16	0	8832	2.26	255.2	0.06	0.01	0.11
WATERCRESS	11	2.3	0.1	1.2	1.29	0.5	0.2	120	0.2	21	60	330	41	0.11	0.077	0.244	0.9	43	0.09	0.12	0.2	0.31	0.129	9	3191	0	1914	0	5767	1	250	0.027	0.008	0.035

Within each parameter, values are presented according to a scale of ten colors, with a gradient from dark red (the lowest value) to dark green (the highest value).
Source: USDA National Nutrient Database for Standard References, Release 27, 2014. Cholesterol is not reported since all species show null values.

- Beets are low in vitamin C, Ca, vitamin A, α-carotene, β-carotene, β-cryptoxanthin, lutein + zeazanthin, vitamin E, and vitamin K.
- Beet greens show values higher than those for beet roots.
- For broccoli, most parameters are classified medium–high; selenium is very high, and vitamin A, β-carotene, and fat poly are low.
- Chicory (witloof) has very low contents for most substances, and only its fiber levels are appreciable.
- Chicory greens show values higher than witloof. It is rich in fiber and pantothenic acid.
- Chives are low in Na, α-carotene, β-cryptoxanthin, lutein + zeazanthin, and vitamin E.
- Collards show many medium and medium-high parameters. No parameters are very low.
- Coriander leaves score medium-high for the majority of parameters, but their low levels of fats, both polyunsaturated and total, make them favorable for human health.
- For dandelion greens, many parameters are ranked high: energy, protein, lipids, carbohydrates, fiber, Fe, thiamin, riboflavin, vitamin a, α-carotene, β-carotene, lutein + zeazanthin, vitamin e, and total fats.
- For endive, many parameters are classified medium-low; vitamin B, α-carotene, β-cryptoxanthin, lutein + zeazanthin, vitamin C, selenium, and sugar are very low.
- Kale shows all values high or very high, with pantothenic acid excluded.
- Lettuce (butterhead) has many parameters classified as very low and low. Thiamin and sugar are medium. Other lettuces (red leaf, green leaf, and cos) show high values for vitamin A. Cos and romaine show many very low values for substances such as Cu, Mg, Se, riboflavin, and β-cryptoxanthin. Iceberg lettuce, including crisp head types, shows low values for all parameters (sugar excluded); the levels of copper, vitamin c, α-carotene, and β-cryptoxanthin are very low.
- Spinach shows many parameters ranked as medium-high, while Mg, folate, β-carotene, and lutein + zeazanthin are very high.
- For radicchio, many parameters are ranked medium and low; Ca, vitamin A, α-carotene, β -carotene, and β-cryptoxanthin are very low. Only a few substances are classified as high and very high, such as Mg, folate, vitamin A, lutein + zeazanthin, and vitamin K.
- Watercress is very low in energy, lipids, carbohydrates, fiber, sugar, Fe, Zn, P, vitamin A, α-carotene, β-cryptoxanthin, and polyunsaturated fats. Very few substances in watercress are medium-high.

The leafy vegetables in salads, as with most foods of plant origin, are an important source of minerals, such as potassium, calcium, and iron, even if the absorption of these substances is, in some cases, lower than that resulting from the meat). Leafy vegetables are also excellent sources of vitamins. The fiber in them helps to regulate intestinal peristalsis and to control the levels of sugar and cholesterol in the blood, and studies have shown a relationship between a lack of fiber in the diet and a high incidence of some intestinal diseases with a chronic course, including constipation, diverticulosis, and colon cancer.

In addition to these aspects more traditionally known, nutritionists are focusing on the specific health properties of vegetables, that is, their health-protective properties. A number of epidemiological studies have shown that a diet rich in fruits and vegetables is associated with a reduced incidence of many chronic diseases widespread in advanced countries, in particular various forms of cancer and cardiovascular diseases. These beneficial effects have been attributed to different antioxidant components whose protective effects are associated with the inhibition of free radicals.

Among the different classes of antioxidants present in leafy fresh vegetables, the most common are ascorbic acid carotenoids, phenolic compounds, and tocopherols. A range of factors influences the content of these molecules in vegetables: soil and climate conditions, genotype, cultivation technique, stage of maturity at harvest, and methods of storage and transportation of fresh produce. Thus, defining the pre- and postharvest conditions that maximize the accumulation and storage of these components can help to enhance these products.

During their evolution, plants have developed strategic solutions that allow them to balance growth (mainly due to primary metabolism involving sugars, proteins, and lipids) with needs related to survival (defense against parasites, herbivores, and toxic agents; attraction of pollinators; stress resistance) that are connected to the so-called secondary metabolism. Plants experiencing suboptimal environments tend to strengthen the secondary metabolism and produce food with higher nutritional value. This has significant implications for the production of vegetables in relation to the use, development, and management of agronomic factors of production.

Ultimately, therefore, climatic factors such as temperature, light radiation, relative humidity, soil factors, and water shortages, which characterize the environments of cultivation of leafy vegetables, play crucial roles in the stimulation of secondary metabolism and thus, in the last analysis, the biosynthesis of all biologically active substances.

In recent studies, total phenolic content (TP), a complex of plant secondary metabolites known to possess a wide range of therapeutic uses, ranged from 21.7 to 82.3 mg gallic-acid equivalents per 100 g of dry weight, with the highest value recorded in red lettuce harvested at high light intensity. Among species studied, the red-fleshed leafy vegetable was richer in phenolic content than green leafy vegetables due to the presence of anthocyanins. TP content fluctuated over the two sampling dates, and the variations were highly correlated to the changes in light intensity, with the effect being species-dependent. In fact, the TP content in chicory, green lettuce, lamb's lettuce, mizuna, red chard, and red lettuce were significantly higher by 48.8%, 46.9%, 96.3%, 12.7%, 100.5%, and 68%, respectively, when plants were harvested at high light intensity, whereas no significant differences among treatments were recorded in rocket, spinach, Swiss chard, and tatsoi.

Mineral Content

The increase in the amount of bioavailable micronutrients in vegetables is a particularly important challenge for developing countries, but it could also represent an added value for the same vegetables in the more economically developed societies.

The theme should, therefore, carefully be explored as a function of the possibility of producing vegetables that satisfy specific requirements for chemical composition and nutritional characteristics. This possibility appears to be important for building popular interest, because plant products with tailored compositions and nutritional values could be marketed to categories of consumers who express special dietary needs. For example, baby foods require ingredients with very low nitrate levels and or with high vitamin and mineral contents, and people with special metabolic diseases also require foods with certain compositions. The pursuit of these objectives seems particularly connected to breeding, biotechnology, and cultivation techniques. The use of nutrient solutions at known concentrations and the control of the quantity and quality of the water supply make the soilless system of cultivation potentially interesting for the mineral enrichment of leafy vegetables.

Accumulation of Nitrates

The nitrate content of leafy vegetables was discussed extensively in recent reviews. Here it is worth recalling, briefly, that the intake of water and vegetables with a high concentrations of nitrates is always considered potentially dangerous to human health. From a toxicological point of view, the nitrate has an extremely low acute toxicity, but the product of its reduction, nitrite, and the N-nitrous compounds formed by the reaction between nitrite and other substances are responsible for methemoglobinemia in children and stomach cancer in adults, respectively. Some epidemiological researchers have critically reviewed the potential risks to health caused by the absorption of nitrate in the diet. In addition, the acidic conditions in the stomach lead to the formation of nitric oxide, which is considered essential in the human body's defense against bacterial pathogens.

Leafy vegetables are among the vegetable species most sensitive to nitrate accumulation, thus contributing greatly to the total intake of nitrates in the diet. A high level of nitrates in leafy vegetables is considered undesirable because of the possible implications for health; however, due to the lack of substantial epidemiological studies, many researches have not found a direct correlation between nitrate concentration in food and incidence of cancer. Despite the debate about the positive or negative effects of nitrates on human health, the production and commercialization of leafy vegetables (spinach, lettuce, and rocket) in Europe is subjected to rigorous limitations (Table 3). This is because some groups of the human population could be at higher risk of developing cancer when subjected to elevated levels of dietary nitrate intakes. Therefore, the World Health Organization (WHO) has set an acceptable daily intake for nitrates of 3.7 mg kg^{-1} body weight.

Several factors can significantly influence the level of nitrates in various raw leafy vegetables, including genetic, agronomic (e.g., amount, timing, and form of nitrogen fertilizer), and environmental factors (e.g., temperature, light exposure, photoperiod, and carbon-dioxide concentration). Beyond that, within species and cultivars, the nitrate content also varies in relation to different parts of the plant. According to several authors, the nitrate concentration, although it is generally higher in older tissues, is generally distributed in plants as follows: petioles > leaves > stems > roots > inflorescences > tubers > bulbs > fruits and seeds. This shows that the organs used for the transport of nutrients, unprocessed and therefore richer in xylem, are those with the highest concentrations.

Genetic background, nitrate supply, harvesting time, and light intensity are predominant. For instance, the relationship between light levels and nitrate concentration has been demonstrated in a number of studies. Recently, research was carried out, in the field and in growth chambers, on changes in nitrate concentrations over a 24 h period for spinach, sweet basil, and scallions. The nitrate content fluctuated over the 24 h period, and these species-dependent variations were strongly correlated to changes in light intensity over the same period: the reduction of nitrate content could be possible by harvesting the vegetables at the right time during the day.

Low levels of light radiation on spinach, in addition to promoting the accumulation of nitrate in the leaves, lead to an increase in the content of oxalic acid (antinutritional factor that limits the bioavailability of Ca in the human body) and a decrease in vitamin C, resulting in the further deterioration of the nutritional quality of the product.

There is a lack of information on the effect of the quality of light radiation on the qualitative characteristics of the product,

Table 3 Maximum levels for nitrates in foodstuffs

Foodstuffs	*Maximum levels (mg NO_3 kg^{-1})*	
Fresh spinach (*Spinacia oleracea* L.)		3500
Preserved, deep-frozen, or frozen spinach		2000
Fresh Lettuce (*Lactuca sativa* L.)	Harvested 1 October to 31 March:	
Protected and open-grown lettuce excluding iceburg lettuce	Lettuce grown under cover	4000
	Lettuce grown in the open air	5000
	Harvested 1 April to 30 September:	
	Lettuce grown under cover	4000
	Lettuce grown in the open air	3000
Iceburg lettuce	Lettuce grown under cover	2500
	Lettuce grown in the open air	2000
Rucola (*Eruca sativa*, *Diplotaxis* sp., *Brassica tenuifolia*, *Sisymbrium tenuifolium*)	Harvested 1 October to 31 March	7000
	Harvested 1 April to 30 September	6000
Processed, cereal-based foods and baby foods for infants and young children		200

Commission Regulations (EU) No 1258/2011 of 2 December 2011. Data come from https://www.fsai.ie/uploadedFiles/Reg1258_2011.pdf. COMMISSION REGULATION (EU) No 1258/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for nitrates in foodstuffs.

with particular reference to its nutraceutical value. In rocket, altering the ratio between red and far-red wavelengths increases the content of nitrates and antioxidants in the leaves. Moreover, recent studies have shown a positive role of blue light on the biosynthesis of compounds with antioxidant action in lettuce. With respect to nitrates, high temperatures induce a greater accumulation of such compounds in lettuce.

Cultivation Environment: Greenhouse Versus Open Field

In general, the quality of vegetables is favored by conditions of cultivation in the greenhouse compared to those in the open field. In a protected environment, it is possible to more accurately control microclimatic parameters (light, temperature, humidity, and CO_2) and to employ radical environments (e.g., soilless cultivation). However, the greenhouse environment may negatively affect the quality of vegetables. Some declines are related to the light radiation, both from the point of view of quantity and quality. The reduction of light intensity due to the covering material can, in fact, contribute to, in some areas and times of the year, a higher concentration of nitrate in leaves and a resulting deterioration in the nutritional quality, as observed in lettuce. In addition, the interiors of glasshouses have an almost complete absence of UVB radiation (280–320 nm), with negative effects on the biosynthesis of polyphenols. Similarly, the content of flavonoids in the leafy vegetable *Gynura bicolor* DC has been much higher when the plants were grown in an open field as opposed to a greenhouse. In addition, the content of glucosinolates in different *Brassicaceae* species was lower in plants grown in the greenhouse compared to those grown in an open field, with a consequent decay in the quality and nutritional alteration of the organoleptic quality of the product. Moreover, some greenhouse vegetables are more sensitive to the appearance of physiological disorders that involve a strong deterioration of the commercial quality of the product. For example, the high growth rates in lettuce grown in the greenhouse predispose the plants to the appearance of marginal necrosis of the leaves (tipburn).

Soilless Cultivation Systems

Soilless cultivation systems are the best means of intensive vegetable production. The use of solutions with known concentrations and composition allows the precise control of the water and mineral nutrition of plants, with important consequences on the raw quality. Several studies have shown the possibility of enhancing the nutritional value of the product by changing the content of specific compounds beneficial for human health in the nutrient solution, in order to obtain functional vegetables. For example, in lettuce and spinach, it was found that increasing the concentration of iron in the nutrient solution near harvest time increases the content of this nutrient in the leaves. The systems of soilless cultivation allow for the improvement of product quality, even through the reduction of compounds potentially dangerous to human health. In this regard, the replacement of the nutrient solution with water, a few days before the harvest, has reduced to one-third the concentration of nitrate in the leaves of endive and celery, and up to one-third in lettuce.

Irrigation and Water Quality

Most vegetable crops are localized in ho, arid environments (e.g., Mediterranean countries), where the scarcity of good quality water severely limits productivity and causes a deterioration of some qualitative characteristics of the product.

Irrigation with saline water often determines a yield reduction and an improved product quality. The use of water with high electrical conductivity leads to an increase in the dry mass and a relative increase in nutraceutical substances. That is a consequence of the water-content reduction of organs and the activation of the secondary metabolism in an accumulation of antioxidant compounds. However, the stimulus on the biosynthesis of antioxidant compounds is often found only in conditions of moderate salt stress. The salinity level beyond which the biosynthesis is reduced varies depending on the cultivar, the growing conditions, and the type of bioactive molecule. Salinity also changes the mineral composition of plant tissue. For example, increasing the salinity through the addition of NaCl reduces the concentrations of P, K, Mg, and Zn, due to antagonism by Na and Cl, with a consequent decline in the nutritional quality of the product.

Differently from other vegetable species, leafy vegetables show the most sensitive period for water deficit during the the vegetative period of cultivation. A water deficit during this period has a high effect on the yield. Because their root systems are shallow (max 30 cm), frequent and light irrigations are necessary when about 20% of available water has been depleted by the crop. In general, excellent irrigation systems have to be properly managed to avoid under- and over-irrigation. Overirrigation may cause disease-spreading.

Anyway, incorrect irrigation-scheduling may negatively affect vegetable performance, with yield quality being impaired. All leafy vegetables are irrigated with localized systems. Heavy rainfall and heavy irrigation may cause severe damage to the leaf surface, with a sensitive reduction of the commercial value of product.

Fertilization

The effects of fertilization on the quality of the vegetables may vary depending on the element and its chemical form, genotype, environmental conditions, and cultivation techniques. The form of nitrogen affects the nutritional quality of the vegetables through variation in the mineral composition. The use of nitrogen as ammonium may cause the acidification of the rhizosphere, with increased absorption of Fe, Zn, and Ca; the opposite effects are induced by the use of nitrogen in the nitrate form. The increase in phosphorus availability often impacts phytochemicals such as ascorbic acid, anthocyanins, and flavonoids. Regarding the method of application for fertilizers, the foliar fertilization appears to be more effective in some cases than applying fertilizer to the substrate, especially in the presence of factors limiting the functionality of the root system (e.g., salinity).

Although there is much debate about the quality of organic vegetables in comparison to conventional ones, the availability of a limited number of rigorous studies on the subject does not

allow an objective comparison between the two types of product. Among the distinctive features of organic farming, there is certainly fertilization. In this respect, research has shown a direct influence of organic fertilization on the content of nitrates in a number of leafy vegetables, with levels almost always lower in organic products than in conventional products; this would seem to result from the gradual availability of nitrogen during the crop cycle. However, repeated organic fertilization may provide abundant levels of N, resulting in nitrate concentrations in the leaves of chard and spinach, especially in hot weather.

The relationship between organic fertilization and the content of vitamins, minerals, and secondary metabolites does not always appear clear. Although the supply of fertilizers in organic form has increased the content of phenolic compounds in tomato fruits (but not in pepper fruits), there were no observed differences in the content of phenolic compounds in lettuce raised using conventional and organic systems. The variability of the results may be related to the lack of a direct relationship of cause and effect between organic fertilization and quality of vegetables. However, excessive fertilizer application, especially with N fertilizer, can result in undesirable changes, such as increases in nitrate, titratable acidity, and acid:sugar ratio, while decreasing the concentration of vitamin C, soluble sugar, soluble solids, and Mg and Ca in some crops.

Conventional and Organic Farming

Given that organic farming has the potential to produce healthy foods and to protect the agroecosystem, its use is growing rapidly in a wide range of climate and soil types. The perception among consumers is that organically produced crops possess higher nutritional quality, because organic farmers are committed to excluding synthetic fertilizers, pesticides, herbicides, plant growth regulators, livestock additives, and genetically modified organisms, and they follow a natural way of farming.

Nevertheless, variability in cultivation factors (soil fertility, effectiveness of different types of amendments, planting and harvest dates, irrigation and tillage intensity in each crop cycle, etc.) and unpredictable and uncontrollable production variables (year-to-year weather variation, plant disease, etc.) can produce data variability that can lead to contradictory results. Some detractors claim that organic farming could be unsafe because of the risk of exposure to biological contaminants and foodborne diseases coming from manure, which is known for carrying human and animal pathogens and mycotoxins from molds. Therefore, the large number of variables involved in the vegetable cultivation make it very difficult to draw any general conclusions on the effect of agricultural practices on these parameters of food quality. Our point of view is that differences among cropping systems, presented here as conventional versus organic, may be only evaluated on a case-by-case basis.

Conclusions

There is sound scientific evidence suggesting that the regular consumption of fruits and vegetables is negatively associated with a reduced risk of developing chronic diseases. Unfortunately, the specific contributions of individual nutrients and phytochemicals to health are yet to be fully explained. Further studies are needed to identify the specific combinations of foods, within and across nutrient categories, that exhibit positive synergetic interactions. Consumers should obtain nutrients and bioactive phytochemicals from a wide variety of whole foods for optimal nutrition and healthy well-being.

See also: Antioxidants: Role on Health and Prevention; Energy: Intake and Energy Requirements; Folic acid and Folates: Physiology and Health Effects; Food Composition Databases; Functional Foods; Mediterranean Diet; Nitrites and Nitrates; Trace Minerals and Trace Elements; Traditional Foods; Vitamins: Overview.

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Vegetarian Diets

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Introduction: History, Description, and Trends

Historical Context

The term 'vegetarian' was established by the Vegetarian Society of the United Kingdom in 1847. The origins of vegetarianism, however, date back to at least 600 BC in ancient India and Greece. Religious groups and philosophers promoted a vegetarian diet based on the idea of nonviolence toward animals. Vegetarianism was almost nonexistent in Europe after Christianity reached the Roman Empire and then reemerged to some extent during the Renaissance. Until the last century, vegetarianism was primarily limited to religious units, but in recent decades, the health benefits of such a dietary pattern have become widely recognized.

Definitions of Vegetarian Diets and Rationale for Vegetarianism

There is no consensus concerning the definition of a vegetarian diet. The Vegetarian Society defines a vegetarian as "someone who lives on a diet of grains, pulses, nuts, seeds, vegetables, and fruits with, or without, the use of dairy products and eggs. A vegetarian does not eat any meat, poultry, game, fish, shellfish, or by-products of slaughter." Vegetarian diets encompass all meatless diets and are usually further categorized as lacto vegetarian (i.e., milk and dairy included), ovo vegetarian (i.e., eggs included), or lacto-ovo vegetarian (LOV) (i.e., dairy and/or eggs included). About 90–95% of North American vegetarians include dairy and/or eggs. Strict vegetarian or vegans exclude all meat, poultry, fish, seafood, and animal products, and some vegans also exclude honey. Semivegetarians are occasional meat eaters who predominantly practice vegetarianism, qualified as pescovegetarians (i.e., those who include fish) and pollovegetarians (i.e., those who include chicken). Typical reasons for vegetarianism include health concerns, religious or ethical beliefs, and political, metaphysical, and ecological reasons.

Trends in Vegetarian Dietary Practices: Shift in Logic behind Vegetarian Diets

About 7% of the US and UK population is vegetarian, and approximately 1% of US adults are vegan. The vegetarian population in Asia is large and ranges from approximately 25 to 40%. More females are vegetarian than males, and vegetarians tend to be slightly more educated than the general US population; however, no differences in socioeconomic status have been observed. A large proportion of the vegetarian population is over 40 years of age, but an even greater percentage is composed of young families.

The concentration of vegetarian nutrition research has changed over time. While earlier research focused mainly on nutritional adequacy of vegetarian diets, present knowledge supports vegetarianism as a viable choice for optimum health and the environment. The main theme in 40% of all vegetarian nutrition articles published in 1986–95 was preventive and therapeutic applications of vegetarian diets. The shift in coverage indicates the growing awareness that vegetarian diets, despite marginal intake of a few nutrients (e.g., iron and B_{12}), can provide ample health benefits to humans. The new paradigm suggests that nutrients present in plant foods may prevent chronic disease (e.g., cardiovascular disease (CVD) and some cancers) and promote longevity. Additionally, there is an increased awareness of the environmental impact of meat consumption, which may further promote a vegetarian lifestyle.

Vegetarian Consumption Patterns

Results from the National Health and Nutrition Examination Survey (NHANES) show that vegetarians consume more total grain, legumes, fruit, soy, and dairy compared with nonvegetarians. Vegetarians consume fewer discretionary calories from fat than nonvegetarians. According to the Continuing Survey of Food Intake by Individuals (CSFII) from 1994 to 1996 and 1998, there were 0.9% self-defined vegetarians who reported no meat intake within the nationally represented sample of US adults. According to 24 h recalls, not all self-reported vegetarians avoid red meat, poultry, or fish. However, the dietary intake of self-defined vegetarians, regardless of whether meat intake was reported or not, was lower in fat, saturated fat, and cholesterol and higher in fiber compared with nonvegetarians who ate meat. Self-defined vegetarians who ate no meat had greater intake of grains, legumes, green leafy and yellow vegetables, fruit, and wine than nonvegetarians. Intake of soy among vegetarians is higher than nonvegetarians, but the amount and type of soy consumed are variable. It is estimated that Western vegetarians consume about 5–6 g and vegans consume about 10–12 g of soy per day. Nuts are commonly consumed by vegetarians as snacks or part of a meal and are eaten whole, in spreads, or as oils or contained in commercial products.

Nutritional Status of Vegetarians

NHANES results from 1999 to 2004 revealed that for vegetarian adults over 19 years of age, adjusted means for fiber; vitamins E, A, and C; thiamin; riboflavin; folate; calcium; magnesium; iron; potassium; and phytochemicals were greater than for nonvegetarians. Protein, total fat, cholesterol, sodium, niacin, vitamin B_{12}, and zinc intake was reduced in vegetarians; however, only zinc status was below the recommended dietary allowance (RDA). A study on older adult vegetarians in Taiwan demonstrated that vegetarians consumed less total calories and cholesterol and consumed a greater proportion of fat as polyunsaturated, higher calcium, and more fiber than omnivores. Compared with vegan, ovo or lacto vegetarian diets contain more vitamin K, calcium, magnesium, and fiber. Recent literature has revealed that vegetarians consume a more nutrient-

dense diet, lower in protein and total fat, and higher carbohydrate and fiber intake compared with nonvegetarians. Additionally, data on Flemish vegetarians from show that nutritional intake of lifelong vegetarians is close to the Belgian RDA.

Health Benefits of Vegetarian Diets

It is apparent that vegetarians worldwide display low rates of a variety of health problems, including overweight and obesity, CVD, hypertension, certain cancers, T2D, kidney stones, constipation, gallstones, and diverticular disease, as well as increased longevity. The observed health benefits result from the independent influence of the absence of meat and the presence of a variety of plant foods. A vegetarian diet pattern, with lower intakes of total calories, animal protein, cholesterol, and saturated fat but greater intakes of dietary fiber, complex carbohydrates, vitamins C and E, carotenoids, magnesium, folic acid, and other phytochemicals, has been associated with reduced risk of numerous chronic diseases and overall mortality. Although uncertainty remains regarding whether the observed benefits of a vegetarian diet are due to the presence of plant foods or the absence of meat, it is important to note that epidemiological studies of diet and health among vegetarian and omnivorous populations have recorded more correlations between the protective effect of plant foods and the detrimental impact of animal foods (e.g., meat and dairy). A balanced plant-based diet that includes fish, dairy, and small amounts of red meat has also produced positive health improvements.

Longevity

Cohort studies indicate that those who follow a diet devoid of or very low in meat experience longevity, whereas high meat intake has been associated with increases in total mortality. In contrast, a large European study showed no significant differences in longevity of vegetarian compared with nonvegetarians. The inconsistent findings may be due to the minimal differences in nutrient intake between British vegetarians and nonvegetarians in the European study. The most recent report on vegetarian diets and longevity examined health records of 73 308 people for an average of almost 6 years, and researchers uncovered significant reductions in total mortality, cardiovascular mortality, noncardiovascular noncancer mortality, renal mortality, and endocrine mortality for vegetarians; associations were greater and more significant in men compared with women (Table 1).

Cardiovascular Health

Established risk factors for coronary heart disease (CHD) include lipids and lipoproteins, hypertension, hemostatic factors, glucose intolerance, obesity, smoking, alcohol, and exercise, as well as possibly homocysteine, antioxidant, and $n-3$ fatty acid levels. Numerous studies have demonstrated consistent evidence for the benefit of a vegetarian diet on reducing risk of CHD. A recent meta-analysis concluded that vegetarians have 29% lower heart disease mortality than nonvegetarians. Vegetarians usually have a more favorable lipid profile than nonvegetarians, including lower total and low-density lipoprotein (LDL) cholesterol, reduced susceptibility to LDL oxidation, lower body weight, and reduced incidence of diabetes and hypertension. Research has linked red meat intake to CHD risk, particularly processed meat intake. Many studies have shown that a primarily plant-based diet decreases risk of CHD and reduces rate of ischemic heart disease mortality, possibly due to vegetarians' higher antioxidant intake, reduced total serum cholesterol, and lower prevalence of obesity.

Table 1 Observational studies on vegetarian diets and mortality, 2009–13

Observational studies			
Author	*Methods*	*Outcome*	*Key findings*
Key et al.	Prospective study. $N = 64\,234$ UK adults	Mortality of vegetarian and nonvegetarians	Mortality from circulatory disease and all-cause mortality are not significantly different between vegetarians and omnivores. Differences may exist for specific causes of death
Orlich et al.	Prospective cohort study. $N = 73\,308$ AHS-2	Relationship between vegetarian diets and all-cause and cause-specific mortality	Vegetarian diets are associated with the following: Reduced all-cause mortality Lower mortality due to CVD, noncardiovascular noncancer mortality, renal mortality, and endocrine mortality
Sinha et al.	Cohort study. $N = 500\,000$ adults aged 50–65 years. NIH-AARP Diet and Health Study	Total mortality and deaths due to cancer, CVD, injuries, and other causes compared to meat intake estimated by FFQ	Red and processed meat consumption was associated with small increases in total, cancer, and CVD mortality

AHS-2, Adventist Health Study 2; EPIC-Oxford, European Prospective Investigation into Cancer and Nutrition-Oxford; NIH-AARP, National Institutes of Health-American Association of Retired Persons; FFQ, food frequency questionnaire; CVD, cardiovascular disease

Key, T. J., Appleby, P. N., Spencer, E. A., Travis, R. C., Roddam, A. W., Allen, N. E. (2009). Mortality in British vegetarians: results from the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford). *American Journal of Clinical Nutrition* 89(5), 1613S–1619S

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Overall, vegetarian diets appear to have a protective effect against CHD and are considered a strategy for primary prevention of heart disease. The observed cardiovascular benefits of a vegetarian diet are probably due to specific aspects of a vegetarian diet, particularly low intake of saturated fat, high intake of soluble fiber, and higher intake of whole grains, soy protein, legumes, and nuts. Whole grain intake and reduced CVD risk have been consistently supported by research. A recent meta-analysis concluded that intake of at least 2.5 versus 0.2 servings per day of whole grains reduced risk of CVD events by 21%. There is also evidence that the dietary fiber in whole grains reduces CHD risk through lipid reduction, blood pressure control, body weight regulation, and decreased chronic inflammation. The Japanese Collaborative Cohort Study found an inverse relationship between bean intake, including soy, and risk of CVD. Reductions in LDL attributable to intake of 25 g of soy protein per day have been observed in a recent meta-analysis of 30 studies. Both epidemiological studies and clinical trials have shown that nuts may protect against CHD, possibly through action on inflammation, vascular reactivity, lipids, and lipid oxidation. While individual components of a vegetarian diet may reduce CVD risk, the synergistic effect of these plant foods will probably deliver the maximum effect. As an example, a vegetarian diet that includes soluble fiber, soy protein, almonds, and plant sterols has been shown to reduce LDL to a similar extent as 20 g of lovastatin, a cholesterol-reducing statin drug (Table 2).

Hypertension

There is evidence that a vegetarian diet may prevent and reduce hypertension. The Adventist Health Study-2 (AHS-2) showed that vegetarians, particularly vegans, with otherwise diverse characteristics, have lower systolic and diastolic blood pressure and less hypertension than meat eaters. This is only partly due to their lower body mass index (BMI). A significant ranked relationship between meat intake and blood pressure was also observed. Compared with nonvegetarians, vegans were 75%, LOV 65%, pescovegetarians 38%, and semivegetarians 23% less likely to be treated for hypertension. In AHS-2, differences in BMI between groups only explained part of the observed relationship. The European Prospective Investigation into Cancer and Nutrition (EPIC) – Oxford study showed that those who do not eat meat had a lower prevalence of hypertension and lower systolic and diastolic blood pressures than meat eaters, but this was largely a result of differences in BMI. After adjustment for age, self-reported hypertension prevalence was 15% in male meat eaters, 5.8% in male vegans, 12.1% in female meat eaters, and 7.7% in female vegans. Fish eaters and vegetarians had a comparable intermediate prevalence of hypertension.

Randomized clinical trials have shown that components of a vegetarian diet can lower blood pressure in both normotensive and hypertensive individuals. Although it is well accepted that both weight loss and reducing sodium intake can lower blood pressure, the effect of a vegetarian diet on blood pressure appears to be independent of these, suggesting that other components of the diet are responsible. These results are consistent with previous findings that concluded that diets high in fruit, vegetables, whole grains, and low-fat dairy significantly reduced blood pressure in those with mild hypertension or normal blood pressure (Table 3).

Diabetes

Vegetarian diets are associated with a decreased risk of type II diabetes (T2D). Data from large prospective cohort studies (e.g., Women's Health Study, Nurses' Health Study, Health Professionals Follow-Up Study, and Multi-Ethnic Study of Atherosclerosis) found that men and women with the highest meat consumption had the highest risk of T2D. Intakes of red meat and processed meat were associated with a 21% and a 41% increased risk of T2D, respectively. In AHS-2, vegans were one-third less likely to have diabetes compared with nonvegetarians, with prevalence of 2.9% and 7.6%, respectively. LOV, pescovegetarians, and semivegetarians had an intermediate diabetes prevalence of 3.2%, 4.8%, and 6.1%, respectively. After adjusting for confounding factors, vegans had an almost 50% reduction in the risk of developing T2D, compared with nonvegetarians. A graded risk reduction was observed based on meat consumption, where LOV had a reduction of 46%, pescovegetarians 30%, and semivegetarians 24% in T2D risk (Table 4).

Cancer

Vegetarians tend to have reduced risk of overall cancer when compared with nonvegetarians of the same demographic. A recent meta-analysis showed that overall cancer incidence was 18% lower in vegetarians compared with nonvegetarians, where males possess a stronger degree of protection than females, perhaps due to lifestyle habits.

For specific cancers, results are inconclusive. Despite convincing evidence that red meat and processed meat consumption increases colorectal cancer risk, AHS-2 studies determined that vegetarians have a reduced risk of colon cancer, whereas results from EPIC-Oxford demonstrated that vegetarians and nonvegetarians had similar rates of colon cancer. When comparing vegetarians in different countries, it is important to recognize that the definition of vegetarian may differ between studies; some self-defining vegetarians still consume white meat or seafood regularly. Research suggests that lifelong vegetarianism may be associated with decreased risk of breast cancer due to a higher intake of vegetables, fruit, soy, and legumes and the avoidance of red meat. The lower incidence of breast cancer observed in vegetarians might be attributable to their reduced exposure to estrogen caused by lower blood estrogen levels, longer menstrual cycles, and a later onset of menstruation. Furthermore, red and processed meat intake has been positively associated with certain cancers. Additional research has not supported these findings, and further studies on the prevention of breast cancer by a plant-based diet are necessary (Table 5).

Osteoporosis

Recent cross-sectional and longitudinal population-based studies suggest little or no difference in bone mineral density (BMD) between omnivores and vegetarians. Current research demonstrates that BMD is not statistically different between

Table 2 Observational studies and intervention trials on vegetarian diets and cardiovascular disease, 2002–12

Author	*Methods*	*Outcome*	*Key findings*
Observational studies			
Bernstein et al.	Prospective cohort. N = 84136 women in the Nurses' Health Study	Incident nonfatal infarctions and CHD deaths	Greater intakes of high-fat dairy, red meat, and red meat excluding processed meat were significantly associated with elevated risk of CHD One serving of nuts per day was associated with a 30% decreased risk of CHD compared with one serving of red meat Shifting protein sources in the US diet may help reduce risk of CHD
Ferdowsian et al.	Systematic review of 27 RCTs and observational studies	Modification of plasma lipid concentrations by plant-based diets	An intervention of a vegetarian or vegan diet combined with nuts, soy, and/or fiber reduced plasma LDL-C by up to 35% Dietary interventions including small amounts of meat yielded less improvement of TC and LDL-C
Ginter	Meta-analysis of prospective studies	Ischemic heart disease mortality	Reduced mortality from ischemic heart disease observed in vegetarians
Harland et al.	Systematic review of 30 RCTs	Reductions in mean LDL-C, TC, blood triglycerides	Significant minor reductions in both TC and LDL-C observed with the consumption of 25 g of soy protein per day
Huang et al.	Meta-analysis of cohort studies published from inception to 1022 from the United Kingdom, Germany, California, the Netherlands, and Japan	Mortality from circulatory diseases and ischemic heart disease	Vegetarians have significantly lower mortality from ischemic heart disease compared with nonvegetarians (RR = 0.71; 95% CI, 0.66–1.16) and lower mortality from circulatory diseases (RR = 0.88; 95% CI, 0.70–1.06)
Mellen et al.	Meta-analysis of seven prospective cohort studies	Event estimates adjusted for CVD risk factors	Higher intake of whole grains was associated with a 21% reduced risk of CVD events (OR = 0.79, 95% CI, 0.73–0.85)
Micha et al.	Systematic review and meta-analysis of 17 cohort and three case–control studies included	Risk of CHD	Consumption of processed but not red meat is predictive of CHD risk Processed meat intake was associated with a 42% greater CHD risk
Intervention trials			
Jenkins et al.	Intervention trial	LDL-C, ratio of LDL-C to HDL-C	A vegetarian diet that includes soluble fiber, soy protein, almonds, and plant sterols has been shown to reduce LDL-C to a similar extent as does 20 g of lovastatin

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol

Bernstein, A. M., Sun, Q., Hu, F. B., Stampfer, M. J., Manson, J. E., and Willett, W. C. (2010). Major dietary protein sources and risk of coronary heart disease in women. *Circulation,* 122(9), 876–883

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Mellen, P. B., Walsh, T. F., and Herrington, D. M. (2008). Whole grain intake and cardiovascular disease: a meta-analysis. *Nutrition, Metabolism and Cardiovascular* 18(4), 283–290

Micha, R., Wallace, S. K., and Mozaffarian, D. (2010). Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus: a systematic review and meta-analysis. *Circulation* 121(21), 2271–2283

Jenkins, D. J., Kendall, C. W., Faulkner, D., Vidgen, E., Trautwein, E. A., Parker, T. L., Connelly, P. W. (2002). A dietary portfolio approach to cholesterol reduction: combined effects of plant sterols, vegetable proteins, and viscous fibers in hypercholesterolemia. *Metabolism* 51(12), 1596–1604.

vegetarian and nonvegetarian women. The EPIC-Oxford study found that the fracture risk was similar in vegetarians and in meat and fish eaters, but there was an increased risk for vegans. However, the increased risk of fracture among vegans did not remain in those consuming at least 525 mg per day of calcium, which implies that the lower calcium intake of vegan subjects was the plausible cause for the increased fracture risk. Cross-sectional studies have shown that increased intake of major micronutrients in fruits and vegetables such as potassium, magnesium, vitamin C, vitamin K, and carotenoids is associated with higher BMD, reduced bone loss with aging, and decreased risk of fracture. Plausible mechanisms of this association include the reduced dietary acid load of plant-based diets and the promotion of positive calcium balance due to high potassium and magnesium intake. In contrast, quality and quantity of protein in animal products have been suggested

Table 3 Observational studies and intervention trials on vegetarian diets and hypertension, 1997–2012

Author	*Method*	*Outcome*	*Key findings*
Observational studies			
Key et al.	Prospective study. $N = 64\,234$ adults. EPIC-Oxford	Self-reported prevalence of HTN and SBP and DBP in non-meat eaters and meat eaters	Vegetarians had a lower prevalence of HTN and lower SBP and DBP than meat eaters; this was partly due to differences in BMI
Pettersen et al.	Prospective cohort. $N = 500$. AHS-2	Systolic and diastolic blood pressure of vegans, LOV, partial vegetarians, and omnivores	Vegetarians and vegans have significantly lower blood pressure and presence of HTN than omnivores, in part due to lower body mass of vegetarians
Intervention trials			
Appel et al.	Intervention. $N = 459$	SBP and DBP between control diet rich in fruits and vegetables and combination diet high in fruits and vegetables and low-fat dairy with reduced saturated and total fat	A diet high in fruits and vegetables and low-fat dairy products combined with low saturated and total fat can significantly reduce blood pressure
Whelton et al.	Meta-analysis of 25 RCTs	SBP and DBP	Dietary fiber intake was associated with a significant decrease in DBP and a nonsignificant reduction in SBP Both DBP and SBP were reduced in trials with HTN patients and in interventions lasting at least eight weeks

HTN, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; LOV, lacto-ovo vegetarian; BMI, body mass index; RCT, randomized controlled trial

Key, T. J., Appleby, P. N., Spencer, E. A., Travis, R. C., Roddam, A. W., Allen, N. E. (2009). Mortality in British vegetarians: results from the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford). *American Journal of Clinical Nutrition* 89(5), 1613S–1619S

Pettersen, B. J., Anousheh, R., Fan, J., Jaceldo-Siegl, K., Fraser, G. E. (2012). Vegetarian diets and blood pressure among white subjects: results from the Adventist Health Study-2 (AHS-2). *Public Health Nutrition* 15(10), 1909–1916

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Table 4 Observational studies on vegetarian diets and diabetes, 2008–2012

Observational studies			
Author	*Methods*	*Outcomes*	*Key findings*
McEvoy et al.	Systematic review of epidemiological studies	Risk of T2D	Vegetarian diets and prudent diets reduced risk of T2D. There is evidence that red meat and processed meat intake is associated with increased risk of T2D
Tonstad et al.	Cohort. $N = 15\,200$ men and 26 187 women. AHS-2	Odds ratios for prevalence of T2D in vegetarian versus nonvegetarians	After adjustment for confounders, vegans (OR = 0.381), LOV (OR = 0.618), and semivegetarians (OR = 0.487) had significantly lower risk of T2D than nonvegetarians
Trapp	Systematic review of observational and intervention studies	Weight, glycemia, cardiovascular risk; efficacy, adequacy, acceptability, and sustainability of vegetarian and vegan diets	A low-fat, plant-based diet improves glycemic control, body weight, and cardiovascular risk. Well-planned vegan diets have similar acceptability of other therapeutic diets
Vang et al.	Prospective cohort. $N = 8401$. Adventist Mortality Study and AHS	Development of T2D over a 17-year period	Intake of meat, especially processed meat, is a risk factor for T2D

T2D, type II diabetes; LOV, lacto-ovo vegetarian; AHS, Adventist Health Study

McEvoy, C. T., Temple, N., Woodside, J. V. (2012). Vegetarian diets, low-meat diets and health: a review. *Public Health Nutrition* 15(12), 2287–94

Tonstad, S., Stewart, K., Oda, K., Batech, M., Herring, R. P., Fraser, G. E. (2013). Vegetarian diets and incidence of diabetes in the Adventist Health Study-2. *Nutrition, Metabolism and Cardiovascular Diseases* 23, 292–299

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Table 5 Observational studies on vegetarian diets and cancer, 2007–12

Observational studies			
Author	*Methods*	*Outcome*	*Key findings*
Butler et al.	Prospective study. Singapore Chinese	Incident breast cancer cases by December 2005 for dietary patterns characterized by meat, starch, and dim sum items or a vegetable–fruit–soy dietary pattern	A significant dose-dependent trend for intake of soy and reduced breast cancer risk was observed among postmenopausal women
Fraser	Review of US and UK vegetarian cohorts	Overall and specific cancer rates	Overall cancer rates appear moderately reduced in vegetarians compared with nonvegetarians. Evidence suggests that colorectal cancer risk is lower in vegetarians
Huang et al.	Meta-analysis of cohort studies	Mortality and overall cancer incidence	Vegetarians have an 18% lower incidence of overall cancer (RR = 0.82; 95% CI, 0.67–0.97) compared with nonvegetarians
Key et al.	Prospective study. *N* = 64 234. EPIC-Oxford	Self-reported prevalence of hypertension and systolic and diastolic blood pressure in non-meat eaters and meat eaters	Cancer mortality was not significantly different in British nonvegetarians versus vegetarians
Taylor et al.	Cohort. *N* = 35 372 in UK Women's Cohort Study	Hazard ratio of breast cancer risk based on meat consumption and meat type	Pre- and postmenopausal women with the highest meat intake had the greatest risk of breast cancer. Significant associations were found with total, processed, and red meat consumption

EPIC-Oxford, European Prospective Investigation into Cancer and Nutrition; FFQ, food frequency questionnaire

Butler, L. M. W. A., Wang, R., Koh, W.P., Yuan, J.M., and Yu, M.C. (2010). A vegetable-fruit-soy dietary pattern protects against breast cancer among postmenopausal Singapore Chinese women. *American Journal of Clinical Nutrition* 91, 1013–1019

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Huang, T., Yang, B., Zheng, J., Li, G., Wahlqvist, M. L., and Li, D. (2012). Cardiovascular disease mortality and cancer incidence in vegetarians: a meta-analysis and systematic review. *Annals of Nutrition and Metabolism* 60(4), 233–240

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to increase acid load, which is suggested to have detrimental health effects (Table 6).

Obesity

Obesity is less prevalent among vegetarians compared with omnivores, and meat consumption is positively associated with obesity. This may be attributed to a lower caloric intake in a vegetarian (i.e., about 460 kcal) than a nonvegetarian diet pattern. Vegans weigh about 10 kg less than nonvegetarians; the mean weight was 7.6 kg less and 3.3 kg less in male and female vegetarians, respectively. The AHS revealed 21% of meat eaters versus 10% of vegetarians among men and 13% versus 8% among women were overweight or obese. Data from the CSFII 1994–96 and EPIC-Oxford studies showed that vegetarian men and women had lower BMI than nonvegetarians. A vegetarian diet may be a reasonable strategy for weight management without diminishing diet quality, as a vegetarian diet is higher in vitamins and minerals, overall fiber, and complex carbohydrates, yet lower in total calories, protein, and fat. Nuts and legumes contain slowly digestible carbohydrates, high fiber and protein contents, and moderate energy density, features known to promote weight control. Additionally, vegetarian diets are typically low in saturated fat and high in *n*-6 polyunsaturated fatty acids, and a vegetarian diet has been shown to increase levels of phospholipid linoleic acid (Table 7).

Aspects of Vegetarian Diet Responsible for Health Benefits

Lifestyle factors associated with vegetarians

It is important to note that vegetarianism is associated with nondietary lifestyle factors known to benefit health. Vegetarians tend to exercise more, smoke less, and consume less alcohol and are generally thinner compared with omnivores. It appears that vegetarians are inclined to be more health-conscious than nonvegetarians; however, observed health benefits persist even when these characteristics are controlled.

Presence of plants

There is a protective effect of plant-based dietary patterns that include whole grains, legumes and soy, nuts, fruits, and vegetables. Plant foods are rich in antioxidant nutrients including vitamins A, C, and E and the minerals zinc and copper and also contain many phytochemicals such as carotenoids, phytosterols, tocotrienols, organosulfur compounds, phenolic compounds (e.g., flavonoids, phytoestrogens, and phenolic acids), and nondigestible carbohydrates (e.g., prebiotics and dietary fiber). Phytochemicals are biologically active nonnutrients found almost exclusively in plant foods and are thought to carry antioxidant, antimutagenic, and antiproliferative properties. These plant-derived components have the ability to inhibit synthesis and activities of proinflammatory mediators (e.g., eicosanoids, cytokines, adhesion molecules, and C-reactive protein); decrease blood cholesterol, LDL oxidation, and blood pressure; and

Table 6 Observational studies on vegetarian diets and osteoporosis 2008–11

Observational studies			
Author	*Methods*	*Outcome*	*Key findings*
Ho-Pham et al.	Review of nine studies. $N = 2749$	BMD of vegans, vegetarians, and omnivores	Bone density was lower in vegan and vegetarians compared with omnivores, but at a level unlikely to be clinically significant
Ho-Pham et al.	Prospective longitudinal study. $N = 210$	BMD of femoral neck, fracture incidence, serum βCTX and P1NP, 25 (OH)D, PTH	Vegan diet did not have adverse effect on bone loss and fracture Corticosteroid use and high intakes of animal protein and animal lipid were negatively associated with bone loss
Thorpe et al.	Cohort study. $N = 1865$	Wrist fracture risk	Higher consumption frequencies of foods rich in protein were associated with reduced wrist fracture Similar risk reduction by vegetable protein foods compared with meat intake; sufficient protein intake from plant sources is possible
Wang et al.	Cohort study. $N = 1865$ adults	BMD, prevalence of osteopenia, or osteoporosis in vegetarians and nonvegetarians	No statistical difference in BMD, osteopenia, or osteoporosis prevalence was observed between vegetarians and nonvegetarians of either sex

BMD, bone mineral density; FFQ, food frequency questionnaire; DXA, dual-energy x-ray absorptiometry; βCTX, C-terminal telopeptide of type I collagen; P1NP, procollagen type I N-terminal propeptide; 25(OH)D, serum 25-hydroxyvitamin D; PTH, parathyroid hormone

Ho-Pham, L. T, Nguyen, N. D, and Nguyen, T. V. (2009). Effect of vegetarian diets on bone mineral density: a Bayesian meta-analysis. *The American Journal of Clinical Nutrition* 90(4), 943–950

Ho-Pham, L. T., Vu, B. Q., Lai, T. Q., Nguyen, N. D., and Nguyen, T. V. (2011). Vegetarianism, bone loss, fracture and vitamin D: a longitudinal study in Asian vegans and non-vegans. *European Journal of Clinical Nutrition* 66, 75–82

Thorpe, D. L., Knutsen, S. F., Lawrence Beeson, W., Rajaram, S. and Fraser, G. E. (2008). Effects of meat consumption and vegetarian diet on risk of wrist fracture over 25 years in a cohort of peri- and postmenopausal women. *Public Health Nutrition* 11(06), 564–572

Wang, Y., Chiu, J., Chuang, M., Chiu, J., and Lin, C. (2008). Bone mineral density of vegetarian and non-vegetarian adults in Taiwan. *Asia Pacific Journal of Clinical Nutrition* 17(1), 101–106.

can act as antioxidants to protect against some cancers, CVD, and T2D.

Fruits and vegetables are known to have antioxidant effects through phytochemicals lycopene, carotenoids, anthocyanins, catechins, and flavonols and vitamins A, C, and E. These components may have a synergistic effect on cells to protect from free radicals and lower chronic inflammatory signals that can increase risk of CVD, select cancers, and insulin resistance. Anticancer activity has been observed with intake of carotenoids, flavonoids, isoprenoids, phenolics, and isothiocyanates found in fruits and vegetables. Carotenoids in green leafy vegetables and fruits, vitamin C found in citrus fruits and vegetables, and lycopene abundant in tomatoes can protect against CVD by inhibiting LDL oxidation. The fiber found in fruits and vegetables aids in lowing blood cholesterol. Grape polyphenols, quercetin found in onions and garlic, and vitamin C in citrus fruits have been shown to decrease blood pressure. Grape polyphenols may also improve endothelial function and platelet aggregation and have anti-inflammatory activity.

Isoflavones in soy may inhibit cancer metastasis, have effects on lipid levels, and reduce bone loss after menopause. Soy intake is associated with significant reduction in CVD risk as it provides protein and has favorable fatty acid content with primarily linoleic and oleic acid and a smaller proportion of α-linolenic acid. Genistein and daidzein from soy appear to be protective against CVD by reducing blood pressure and lowering LDL-C. Legumes (e.g., dry beans, peas, chickpeas, and lentils) have nutritional qualities that are known to aid in weight control, including high fiber and protein contents, slowly digestible carbohydrates, and moderate energy density. Randomized controlled trials show a general benefit of legume consumption combined with calorie restriction on weight loss. Observational studies demonstrate a consistent inverse relationship between legume intake and BMI or obesity risk. Results from a recent meta-analysis show that a diet high in legumes other than soy reduces total and LDL cholesterol. The fiber and polyphenols in legumes protect against CVD by lowering blood cholesterol.

The greater fiber intakes of vegetarians may have significant effects in reducing colon cancer risk. The bulking ability of fiber may reduce the transit time of carcinogens through the bowel and decrease the surface contact exposure of the bowel wall to carcinogenic compounds. Additionally, fiber intake is independently associated with reduced weight gain and obesity risk. Fiber forms in whole grains increase intraluminal viscosity, which is associated with reduced obesity risk, but the physiological mechanism behind protective effects is unclear. Whole grain consumption is associated with reduced risk CVD, likely due to the action of fiber and phytochemicals in lowering blood cholesterol, reducing blood homocysteine, and decreasing blood pressure. Benefits of fibers found in whole grains have been demonstrated in glucose metabolism, but results are variable.

Tree nuts and peanuts contain ample nutrients, including unsaturated fatty acids, fiber, high-quality vegetable protein, tocopherols, minerals, phytosterols, and phenolic compounds. Epidemiological studies have consistently shown an association between nut consumption and a reduced risk of CVD, possibly due to the presence of unsaturated fatty acids, tocopherols, and fiber. Recent evidence suggests health-protective benefits of nuts beyond CVD. It is likely that regular consumption of nuts reduces risk of diabetes and cancer in women, and protective

Table 7 Observational studies and intervention trials on vegetarian diets and obesity, 2001–12

Author	*Methods*	*Outcome*	*Key findings*
Observational studies			
Haddad et al.	Cross-sectional analysis. $N = 13313$. CSFII 1994–96, 1998	BMI, nutrient intake from two nonconsecutive 24 h dietary recalls	Self-reported vegetarians had lower BMI, regardless of whether they abstained from or consumed some meat
Kennedy et al.	Review of over 200 studies. $N = 10014$ adults. 1994–96 CSFII	BMI, consumption patterns, HEI	Energy intakes were lowest in those consuming a vegetarian diet. BMI was lowest for men and women consuming a vegetarian diet and women eating a high-carbohydrate, low-fat diet
Newby et al.	Review of epidemiological studies		Protective effect of ready-to-eat cereal on obesity risk was observed Research on fruits and vegetables; beans, legumes, and soy; noncereal grains; fiber; and plant-based diets is inconsistent
Newby et al.	Cross-sectional analysis. $N = 55459$ women. Swedish Mammography Cohort	Prevalence of overweight or obesity (BMI ≥ 25)	A significantly lower risk of obesity was observed in vegans (OR = 0.35; 95% CI 0.18, 0.69), LOV (OR = 0.54; 95% CI 0.35, 0.85), and semivegetarians (OR = 0.52; 95% CI 0.43, 0.62) compared with omnivores
Spencer et al.	Cross-sectional analysis. $N = 37875$ adults. EPIC-Oxford	BMI in vegans, vegetarians, fish eaters, and meat eaters	Meat eaters had the highest BMI Fish eaters and vegetarians had comparable moderate mean BMI Vegans had the lowest BMI Greater protein and reduced fiber intakes were strongly related to increased BMI
Wang et al.	Cross-sectional analysis. 1999–2004 NHANES	Obesity, measured by BMI ≥ 30 or waist circumference ≥ 102 cm in men or ≥ 88 cm in women	There is a positive relationship between meat consumption and risk for obesity
Intervention trials			
Foster et al.	RCT. $N = 123$ adults	Anthropometric and metabolic measurements at baseline, six months, and 18 months	Almond-enriched diet and hypocaloric nut-free diet resulted in similar weight loss at 18 months Those consuming almonds experienced more substantial improved lipid levels

BMI, body mass index; HEI, healthy eating index; CSFII, Continuing Survey of Food Intake by Individuals; NHANES, National Health and Nutrition Examination Survey; RCT, randomized controlled trial

Haddad, E. H., and Tanzman, J. S. (2003). What do vegetarians in the United States eat? *American Journal of Clinical Nutrition* 78(3 Suppl.), 626S–632S

Kennedy, E. T., Bowman, S. A., Spence, J. T., Freedman, M., and King, J. (2001). Popular diets: correlation to health, nutrition, and obesity. *Journal of the American Dietetic Association* 101(4), 411–420

Newby, P. K. (2009). Plant foods and plant-based diets: protective against childhood obesity? *American Journal of Clinical Nutrition* 89(5), 1572S–1587S

Newby, P. K., Tucker, K. L., and Wolk, A. (2005). Risk of overweight and obesity among semivegetarian, lactovegetarian, and vegan women. *American Journal of Clinical Nutrition* 81 (6), 1267–1274

Spencer, E. A., Appleby, P. N., Davey, G. K., and Key, T. J. (2003). Diet and body mass index in 38000 EPIC-Oxford meat-eaters, fish-eaters, vegetarians and vegans. *International Journal of Obesity and Related Metabolic Disorders* 27(6), 728–734

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benefits of frequent nut consumption on gallstone diseases have been observed in both sexes. Additionally, long-term nut consumption is linked with lower body weight and lower risk of obesity and weight gain.

Absence of meat

Dietary meat contains several components that may increase the risk of developing cancer and CVD. These food components include saturated fat, protein, iron, phosphorus, exogenous hormones, antibiotics, feed composition, preservatives and bacterial toxins, and by-products of cooking. There are several studies that relate low meat intake to longevity, including the EPIC-Oxford Health Food Shoppers, OXCHECK, German Vegetarians, and AHS. There is general agreement that red meat consumption increases the risk of colon and bladder cancer. This was the only food association with cancer labeled 'convincing' in the recent report from the World Cancer Research Fund and American Institute for Cancer Research.

Meat is twice as calorically dense as plant-based protein foods. Caloric restriction is linked to increased life span and higher cognitive function in a variety of species possibly due to reduced production of free radicals and oxidative stress. A meat-containing diet is higher in saturated fat and cholesterol and lower in essential fatty acids leading to a low polyunsaturated–saturated fatty acid ratio, which increases oxidative stress and inflammation and contributes to insulin

resistance and increased risk of T2D, CHD, and some cancers. Protein, though necessary for proper physiological function, can be detrimental in excess. Disproportionate intake of protein increases fecal nitrates, and colonic flora forms carcinogenic N-nitroso compounds, the heterocyclic amines formed during cooking of meat and fish associated with the risk of colon cancer and CVD. Excess iron from the highly absorbable heme iron in meat promotes oxidation, resulting in an increase in free radicals known to promote CVD. Furthermore, unabsorbed iron increases fecal concentration, which has a hyperproliferative effect on the colonic epithelium. Meat is also abundant in phosphorous; high phosphorus intake in the context of low calcium intake contributes to secondary hyperparathyroidism causing loss of bone mass and increased risk of osteoporosis.

Red meat, particularly high-fat processed meats, may lead to development of carcinogens (i.e., precursors to cancer) when cooked at a high temperature. Additionally, there are components of meat formed or added during processing or storage that may have detrimental health effects, including growth hormones, antibiotics, and contaminated feed from rendered scraps that can transmit various diseases to humans (e.g., mad cow). Preservatives added to meat, such as nitrates and nitrites, are implicated in colon and gastric cancer (i.e., endogenous formation of N-nitroso compounds), and salting and curing of meats irritate gastric epithelium and may increase risk of gastric cancer. Finally, improper handling of uncooked ground meat can lead to outbreaks of *E. coli* and *Salmonella*.

Potential Concerns of Vegetarian Diets

Protein

Vegetarians generally consume less protein than omnivores; however, they typically have adequate protein intake, assuming a variety of plant protein sources with adequate calories are eaten. Plant sources of protein include soy products (e.g., soy milk, soy yogurt, tofu, and tempeh), nuts, and legumes; small amounts of protein also exist in vegetables and grain products. In general, plant protein sources contain limited amounts of one or more essential amino acids; however, the protein found in soy has a protein digestibility-corrected amino acid score that is analogous to meat. It is currently recognized that strict protein combining is not necessary, assuming a variety of plant foods are eaten daily.

Vitamin B_{12}

Vegetarians consume few and vegans do not consume any vitamin B_{12} food sources. Vitamin B_{12} is essential for homocysteine metabolism, and low intake is associated with elevated plasma homocysteine. Although hyper-homocysteinemia has been observed in vegetarians of various races and genders, the total cardiovascular risk profile of vegetarians remains favorable compared with omnivores, and the role of homocysteine in CVD is inconclusive. Additionally, B_{12} supplements are readily available for vegans and vegetarians who do not consume adequate amounts of this nutrient from foods. For LOV, dairy products and eggs can supply significant amounts of B_{12}; vegans should obtain dietary B_{12} from fortified foods, such as cereals and fortified yeast, soy, or meat analogues.

Calcium and vitamin D

Research demonstrates that calcium intake is similar between vegetarians and omnivores, with vegans usually having lower intake. Some studies have shown that vegetarians, and predominantly vegans, have lower intakes of vitamin D compared with omnivores. However, data from the AHS-2 found no relationship between serum vitamin D concentrations and dietary pattern, suggesting that factors other than diet (e.g., sun exposure) have a greater influence on vitamin D status.

A vegetarian diet can easily meet the daily requirements assuming adequate calorie intake and regular consumption of dairy or soy fortified with vitamin D and calcium. Dairy products provide adequate calcium for LOV, and vegans consume calcium in fortified soy products and other calcium-rich plants. Vegans and vegetarians should select an appropriate milk or milk alternative with 5–7 g protein per serving, calcium fortified with 250–300 mg per serving, and added vitamins A and D. Calcium bioavailability in plant foods varies, but low-oxalate greens have a high bioavailability of 50–60%, and fortified soymilk, sesame seeds, and almonds are approximately 21–24% bioavailable; in comparison, cow's milk is 32% bioavailable. Additionally, a diet rich in fruit and vegetables that is typical of a vegan diet has a positive effect on calcium status and markers of bone metabolism in men and women, due to the effect of high magnesium and potassium intake.

Zinc

Zinc is present in plant foods, but is less bioavailable from plants compared to animal sources. The low absorbability is in part due to phytate content in plants, which binds minerals and hinders their absorption. Vegetarians frequently consume nuts, seeds, and whole grains, which are high in zinc and likely contribute to the observed satisfactory status in vegetarians.

Iron

Despite the lower bioavailability of nonheme iron found in plant foods, iron deficiency anemia occurrence is similar in vegetarians and nonvegetarians. Vegetarian diets are typically high in vitamin C, which enhances iron absorption. Vegetarians tend to have reduced iron stores and serum ferritin levels; however, research indicates that hemoglobin levels of vegetarians are similar to nonvegetarians. Plant sources of iron include cashews, raisins, spinach, soybeans, kidney beans, black beans, cabbage, and tomato juice.

Essential fatty acids

Although intakes of the essential *n*-3 α-linoleic acid (ALA) are similar in vegetarians and meat eaters, intakes of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are reduced in vegetarians and nonexistent in vegans. Due to a lack of direct plant sources of *n*-3 fatty acids and limited efficiency of conversion of ALA to EPA and DHA, experts advise vegetarians to consume twice the current adequate intake of ALA. A recent study demonstrated small differences in plasma *n*-3 PUFA status in fish eaters compared with non-fish eaters; an explanation for this result may be greater conversion of ALA to EPA and DHA in non-fish eaters. To promote optimal *n*-3 fatty acid status in vegetarians, it is recommended that intake of saturated and *trans* fats be minimized and monounsaturated rather

than *n*-6 fats be consumed. Rich sources of ALA include walnuts, soy products, flaxseed, and chia seed; smaller amounts exist in green leafy vegetables; *n*-3-enriched eggs or foods fortified with DHA from algae are direct sources of DHA for vegetarians.

Practical Applications

Dietary Guidelines for Vegetarians

When counseling vegetarians, guidelines similar to the US Dietary Guidelines for Americans are applicable. These guidelines encourage whole, plant-based foods including grains, legumes, vegetables, fruits, nuts, and seeds and discourage meats, full-fat dairy, and eggs as well as all refined and processed foods. It is also recommended that vegetarians include a range of healthy fats (e.g., nuts, seeds, and avocado), consume 25–30 g of fiber per day, and possibly incorporate vitamin and mineral supplements (e.g., B_{12}, vitamin D, and calcium). Additionally, adequate calories and protein are needed to spare lean body tissue and meet nutrient needs. The vegetarian dietary guide specifies that a one ounce-equivalent serving of protein is one egg, 0.25 cup cooked dry beans or tofu, 1 tablespoon peanut butter, or 0.5 oz nuts or seeds as acceptable alternatives to meat products. Numerous foods that usually contain meat or poultry can be made vegetarian (e.g., veggie burgers, soy-based sausage, wheat gluten, and texturized vegetable protein).

Vegetarian Food Guides

A vegetarian food guide pyramid developed by Loma Linda University includes recommended servings of relevant food groups based on energy needs for both vegans and LOV. USDA's MyPlate provides resources for vegetarians, including alternative protein sources, tips for vegetarians, and the Vegetarian Nutrition Resource List. Additionally, the General Conference of Seventh-Day Adventists established the vegetarian MyPlate, a vegetarian version of the USDA MyPyramid; a vegan MyPlate is also available from the Vegetarian Resource Group. A Japanese vegetarian food guide has also been created for both the general public and the health professionals to promote adequate intakes of all nutrients (Figure 1).

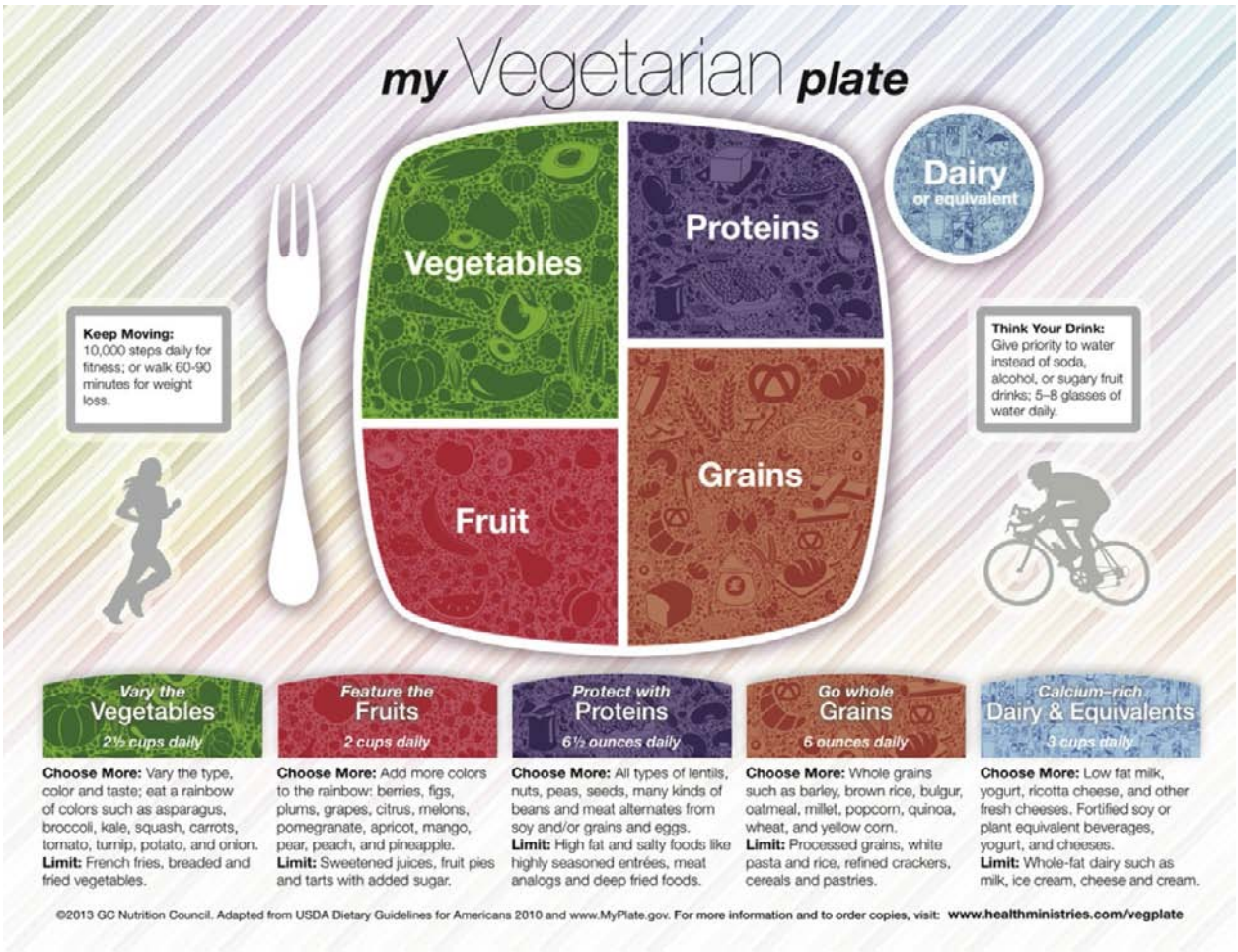
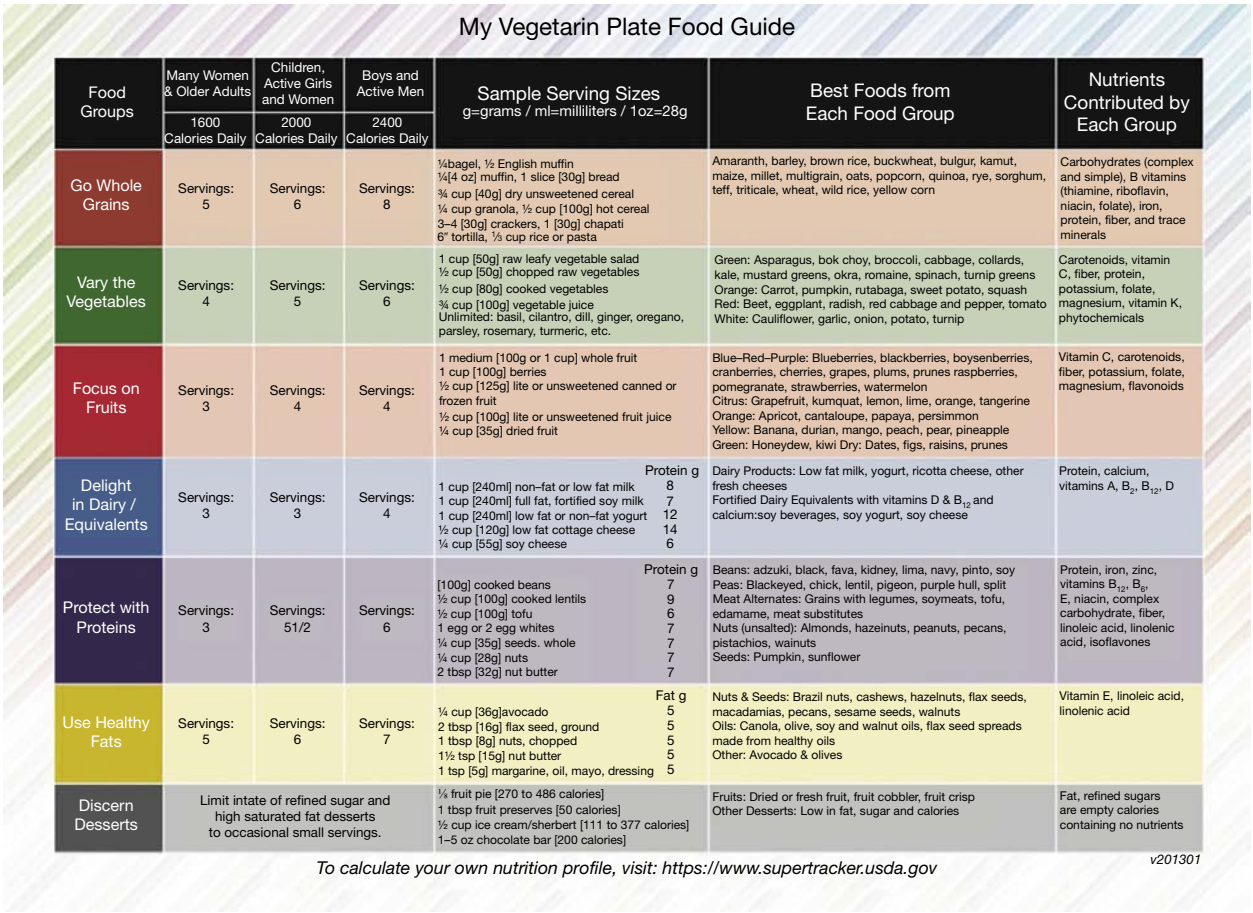


Figure 1



My Vegetarin Plate Food Guide

Food Groups	Many Women & Older Adults 1600 Calories Daily	Children, Active Girls and Women 2000 Calories Daily	Boys and Active Men 2400 Calories Daily	Sample Serving Sizes g=grams / ml=milliliters / 1oz=28g	Best Foods from Each Food Group	Nutrients Contributed by Each Group
Go Whole Grains	Servings: 5	Servings: 6	Servings: 8	¼bagel, ½ English muffin ¼[4 oz] muffin, 1 slice [30g] bread ¾ cup [40g] dry unsweetened cereal ¼ cup granola, ½ cup [100g] hot cereal 3–4 [30g] crackers, 1 [30g] chapati 6" tortilla, ⅓ cup rice or pasta	Amaranth, barley, brown rice, buckwheat, bulgur, kamut, maize, millet, multigrain, oats, popcorn, quinoa, rye, sorghum, teff, triticale, wheat, wild rice, yellow corn	Carbohydrates (complex and simple), B vitamins (thiamine, riboflavin, niacin, folate), iron, protein, fiber, and trace minerals
Vary the Vegetables	Servings: 4	Servings: 5	Servings: 6	1 cup [50g] raw leafy vegetable salad ½ cup [50g] chopped raw vegetables ½ cup [80g] cooked vegetables ¾ cup [100g] vegetable juice Unlimited: basil, cilantro, dill, ginger, oregano, parsley, rosemary, turmeric, etc.	Green: Asparagus, bok choy, broccoli, cabbage, collards, kale, mustard greens, okra, romaine, spinach, turnip greens Orange: Carrot, pumpkin, rutabaga, sweet potato, squash Red: Beet, eggplant, radish, red cabbage and pepper, tomato White: Cauliflower, garlic, onion, potato, turnip	Carotenoids, vitamin C, fiber, protein, potassium, folate, magnesium, vitamin K, phytochemicals
Focus on Fruits	Servings: 3	Servings: 4	Servings: 4	1 medium [100g or 1 cup] whole fruit 1 cup [100g] berries ½ cup [125g] lite or unsweetened canned or frozen fruit ½ cup [100g] lite or unsweetened fruit juice ¼ cup [35g] dried fruit	Blue–Red–Purple: Blueberries, blackberries, boysenberries, cranberries, cherries, grapes, plums, prunes raspberries, pomegranate, strawberries, watermelon Citrus: Grapefruit, kumquat, lemon, lime, orange, tangerine Orange: Apricot, cantaloupe, papaya, persimmon Yellow: Banana, durian, mango, peach, pear, pineapple Green: Honeydew, kiwi Dry: Dates, figs, raisins, prunes	Vitamin C, carotenoids, fiber, potassium, folate, magnesium, flavonoids
Delight in Dairy / Equivalents	Servings: 3	Servings: 3	Servings: 4	Protein g 1 cup [240ml] non–fat or low fat milk 8 1 cup [240ml] full fat, fortified soy milk 7 1 cup [240ml] low fat or non–fat yogurt 12 ½ cup [120g] low fat cottage cheese 14 ¼ cup [55g] soy cheese 6	Dairy Products: Low fat milk, yogurt, ricotta cheese, other fresh cheeses Fortified Dairy Equivalents with vitamins D & B_{12} and calcium:soy beverages, soy yogurt, soy cheese	Protein, calcium, vitamins A, B_2, B_{12}, D
Protect with Proteins	Servings: 3	Servings: 51/2	Servings: 6	Protein g [100g] cooked beans 7 ½ cup [100g] cooked lentils 9 ½ cup [100g] tofu 6 1 egg or 2 egg whites 7 ¼ cup [35g] seeds. whole 7 ¼ cup [28g] nuts 7 2 tbsp [32g] nut butter 7	Beans: adzuki, black, fava, kidney, lima, navy, pinto, soy Peas: Blackeyed, chick, lentil, pigeon, purple hull, split Meat Alternates: Grains with legumes, soymeats, tofu, edamame, meat substitutes Nuts (unsalted): Almonds, hazeinuts, peanuts, pecans, pistachios, wainuts Seeds: Pumpkin, sunflower	Protein, iron, zinc, vitamins B_{12}, B_6, E, niacin, complex carbohydrate, fiber, linoleic acid, linolenic acid, isoflavones
Use Healthy Fats	Servings: 5	Servings: 6	Servings: 7	Fat g ¼ cup [36g]avocado 5 2 tbsp [16g] flax seed, ground 5 1 tbsp [8g] nuts, chopped 5 1½ tsp [15g] nut butter 5 1 tsp [5g] margarine, oil, mayo, dressing 5	Nuts & Seeds: Brazil nuts, cashews, hazelnuts, flax seeds, macadamias, pecans, sesame seeds, walnuts Oils: Canola, olive, soy and walnut oils, flax seed spreads made from healthy oils Other: Avocado & olives	Vitamin E, linoleic acid, linolenic acid
Discern Desserts	Limit intate of refined sugar and high saturated fat desserts to occasional small servings.			⅛ fruit pie [270 to 486 calories] 1 tbsp fruit preserves [50 calories] ½ cup ice cream/sherbert [111 to 377 calories] 1–5 oz chocolate bar [200 calories]	Fruits: Dried or fresh fruit, fruit cobbler, fruit crisp Other Desserts: Low in fat, sugar and calories	Fat, refined sugars are empty calories containing no nutrients

To calculate your own nutrition profile, visit: https://www.supertracker.usda.gov

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Figure 1 (Continued) Vegetarian MyPlate.

Conclusion

A vegetarian diet could present a marked advantage over meat-based diets, and ample research has demonstrated increased longevity and several chronic diseases in vegetarians. A vegetarian diet is lower in total fat, saturated fat, cholesterol, and animal protein but higher in complex carbohydrates, fiber, antioxidants, and unsaturated fats and thus provides a potential dietary option for preventing diabetes, CVD, obesity, and select cancers. Despite lower intakes of certain nutrients for strict vegetarians, careful dietary planning can ensure that current recommended intakes for essential nutrients are met. Additionally, a plant-based diet is abundant protective food components that benefit health. The reported health benefits of a vegetarian diet as well as the economic and environmental ramifications of meat consumption should be considered when assessing the health effects of a vegetarian diet.

See also: Cancer: Diet in Cancer Prevention; Carcinogenic: Carcinogenic Substances in Food; Carotenoids: Occurrence, Properties and Determination; Cholesterol: Factors Determining Blood Cholesterol Levels; Dietary Fiber: Determination; Fatty Acids: Essential Fatty Acids; Hypertension and Diet; Iron: Properties and Determination; Meat: Role in the Diet; Obesity: The Role of Diet; Osteoporosis; Phenolic Compounds: Occurrence, Classes, and Analysis; Phytic Acid: Properties, Uses, and Determination; Protein Quality and Amino Acids in Maternal and Child Nutrition and Health; Pulsed Electric Fields; Riboflavin: Properties and Determination; *Salmonella*: Salmonellosis; Soy Beans: Dietary Importance; Zinc: Properties and Determination.

Further Reading

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Relevant Websites

http://www.vegetariannutrition.org – The International Congress on Vegetarian Nutrition.
http://www.vrg.org – The Vegetarian Resource Group.

Vibrio: Types, Properties, and Determination

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Vibrio Types and Properties

Vibrio Genus

The genus *Vibrio* belongs to the Vibrionaceae family and includes bacteria autochthonous of the marine environment. To date, the genus includes more than 90 species and its taxonomy is continuously updated due to the addition of new species. Morphologically, vibrios are Gram-negative straight or curved rods, with a size between 0.5 and 0.8 μm in width and 2–3 μm in length; they are motile by means of a single polar flagellum and display both respiratory metabolism and fermentative metabolism. Vibrios are nonsporogenous microorganisms, but survival in the environment is facilitated by resistance forms as the viable but nonculturable state (VBNC), a dormant state characterized by a reduction of cells size, and a shift to alternative metabolic pathways. The genus *Vibrio* includes both halotolerant and halophilic bacteria: halotolerant vibrios are capable of growth in the absence of salt (NaCl), whereas halophilic vibrios are dependent on the presence of NaCl for multiplication. Due to their flexible physiology, vibrios are one of the most common microorganisms in marine and estuarine waters throughout the world, and they can persist in a variety of aquatic ecosystems, including freshwater habitats (*V. cholerae* and *V. mimicus*) and seawater, both as free-living cells and in association with aquatic animals and plants.

Several *Vibrio* species are known to be associated with outbreaks and sporadic cases due to the consumption of contaminated water, food manipulated in inadequate hygiene conditions, seafood (raw fish and shellfish), or the exposure of skin lesion (cuts, open wounds, or abrasions) to aquatic environments and marine animals (Table 1). Pathogenicity toward humans is commonly associated with the presence of specific genetic features (e.g. toxin-coding genes and virulence factors) whose variable distribution among the strains often results in the coexistence of pathogenic and nonpathogenic populations in the same species. The most commonly known pathogen is *V. cholerae*, the etiologic agent of cholera, one of the few foodborne illnesses with pandemic potential. Other relevant species that may cause disease, mainly by seafood ingestion, are *V. parahaemolyticus* and *V. vulnificus*, which are normal inhabitants of marine environments and are isolated from seawater and seafood with frequencies varying, according to the studies, from less than 10% up to 36% for finfish samples and 100% for shellfish. Variations in *Vibrio* prevalence and levels are indeed expected, in relation to the food type (with higher presence in filter-feeding animals as shellfish), the geographic origin of the seafood product, and the sampling period. *Vibrio* levels in the aquatic environment, in fact, are known to correlate with water temperatures, and detection is therefore more likely in warm months than in winter periods. Unfortunately, because vibrios are part of the normal marine microflora and, with the exception of *V. cholerae*, are not associated with fecal pollution or anthropic impact on seafood production or harvesting areas, control measures can only rely on adequate manipulation of the products (rapid refrigeration, uninterrupted cold chain, and proper cooking) and on the monitoring of the environmental conditions that may favor the increase of *Vibrio* levels.

Further to the three major human pathogens (*V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*) and to the other species (*V. alginolyticus*, *V. fluvialis*, *V. furnissi*, *V. metschnikovii*, etc.) that are occasionally responsible for disease in susceptible individuals, several *Vibrio* species (e.g., *V. anguillarum*, *V. harveyi*, and *V. salmonicida*) express pathogenicity toward marine animals (fish, shellfish, or mammals) with considerable economic loss in the aquaculture sector due to large mortality episodes or to prolonged decrease of production. Significantly, some *Vibrio* species display potential for both severe diseases in humans and in aquatic animals, as is the case of *V. vulnificus*, which causes mortality in eels and deadly wound infections in aquaculture professionals (fishermen, processors, etc.), or of *V. parahaemolyticus*, causative agent of gastroenteritis in seafood consumers and responsible for the early mortality syndrome in shrimps.

Vibrio cholerae

V. cholerae is the etiologic agent of cholera, an infectious disease responsible, in areas endemic for this microorganism, for large epidemics and for a high number of deaths, especially in the presence of inadequate hygiene standards and improper treatment of wastewaters. The first description of a '*Vibrio*-like' microorganism from patients affected by cholera dates back to 1854 with Filippo Pacini and first isolation of *V. cholerae* was achieved in 1884 by Robert Koch. The modern history of cholera is marked by seven pandemics, during which the disease spread at a global level with reiterated and geographically shifting epidemic peaks.

V. cholerae includes more than 200 hundred serogroups according to somatic antigen O, a thermostable polysaccharide of the cellular wall. Serotypes O1 and O139 are associated with cholera epidemics, whereas the other serotypes, generally defined as '*V. cholerae* non-O1/non-O139,' are occasionally responsible for sporadic cases of gastroenteritis, mild forms of disease similar to cholera, or extraintestinal infections. Serogroup O1 can be further divided in two serotypes (Ogawa and Inaba) and two distinct biotypes: the biotype Classic, deemed responsible for the first six pandemics (1817–1923), and the biotype El Tor, so called by the name of the quarantine center where it was first isolated, responsible for the seventh ongoing pandemic (1961). Biotype differentiation was based initially on the characteristic hemolytic effect of El Tor variant on sheep erythrocytes, but this feature became progressively less discriminating during the seventh pandemics as the nonhemolytic

Table 1 Most relevant *Vibrio* species associated with human diseases

Species	*Source*	*Disease*
V. alginolyticus	Seafood, seawater	Wound infection, ear infections, gastroenteritis
V. cholerae O1-O139	Water or food	Gastroenteritis
V. cholerae non-O1/non-O139	Water or food	Gastroenteritis, wound infection, ear infection
V. fluvialis	Seafood	Gastroenteritis
V. furnissii	Seafood	Gastroenteritis
V. hollisae	Shellfish	Gastroenteritis, ear infection
V. metschnikovii	Shellfish, seawater	Primary septicemia, gastroenteritis
V. mimicus	Shellfish, seawater	Gastroenteritis, ear infection, wound infection
V. parahaemolyticus	Shellfish	Gastroenteritis
V. vulnificus	Shellfish, seawater	Primary septicemia, wound infection, bacteremia, gastroenteritis

strains overcame the hemolytic ones. The distinction, however, maintained an epidemiological convenience, as the different biotypes seem to correlate with the clinical severity of the disease, being the El Tor type less aggressive (only 2% of patients develop severe disease, compared to 11% of the classical infection). Until 1992, the distinction between *V. cholerae* O1 and non-O1 (also described as NAG, nonagglutinable *V. cholerae*) was considered pivotal to differentiate between strains with potential for epidemic cholera and nonpathogenic *V. cholerae* isolates. In early 1993, however, reports appeared in India and Bangladesh of a severe cholera-like disease associated with a new serogroup, O139, sometimes referred to as '*V. cholerae* Bengal,' for the origin of the strain. Since its first appearance, this serogroup, characterized by hybrid features of O1 and non-O1 strains, has spread to other countries (China, Thailand, Pakistan, Nepal, and Malaysia) and, although remaining geographically confined to Southeast Asia, has compelled a global revision of serological testing.

As other *Vibrio* species, *V. cholerae* is part of the autochthonous bacterial flora of estuarine areas, and its growth may occur in variable temperature conditions (from 10 to 43 °C) and in NaCl concentrations ranging from 0.1% to 4.0%. Halotolerance of *V. cholerae* is a key feature of its transmission, especially in developing countries, where ingestion of contaminated water is probably the most important vehicle. Free in the drinkable water, *V. cholerae* can, in fact, survive for several days (up to 13) and can contribute to persistence of the infection also via food prepared with contaminated water, as in the case of some outbreaks generated by rice dishes or fruit juices, or with the use of contaminated water for the irrigation of vegetables. It is also demonstrated that *V. cholerae* can persist in the environment in the absence of human disease, possibly through the colonization of the surface of zooplankton (e.g., copepods) and aquatic plants. Another relevant feature for *V. cholerae* transmission is related to its pH tolerance. *V. cholerae* may grow in a pH range between 5.0 and 9.6 with pH 7.6 being the optimum pH and displays sensitivity to acidic environment (which, in the form of gastric acids, provides the major human defense barrier to *Vibrio* infection). Foods involved in *V. cholerae* transmission (crustaceans, shellfish, cooked meat and rice dishes, nonalcoholic and noncarbonated drinks, and raw vegetables and fruits) usually display neutral or nearly neutral pH, and thus, this aspect can be taken into account to orientate the investigations in outbreak settings.

The main virulence factor of *V. cholerae* is represented by the cholera toxin (CT), a typical AB subunit toxin encoded by the *ctx* gene (*ctxA* and *ctxB*), which is present on a horizontally transmissible filamentous bacteriophage (CTXΦ). CT is responsible for the osmotic disequilibrium in intestinal lumen at the origin of the massive water flows ('rice water feces') characteristic of cholera disease. CT, however, is not the only pathogenicity determinant of *V. cholerae*, as multiple systems are involved in regulation of virulence. Among these, the ToxR regulon controls the expression of several (at least 17) genes involved in the pathogenic mechanism of *V. cholerae*, and therefore, *toxR* gene is often used as a molecular marker in *V. cholerae* analysis. Further to CT, other factors are expressed in virulent strains: the zonula occludens toxin (*zot*), which affects the structure of tight junction and increases the permeability of the intestinal mucosa; the accessory CT (*ace*), which acts on fluid accumulation; and the toxin-coregulated pilus (TCP), long filaments on the surface of the microorganism with an important role in adherence and colonization of intestinal mucosa. Also, non-O1/non-O139 *V. cholerae* strains may occasionally encode for some of these genes, as well as for other specific virulence factors as a heat-stable toxin (codified by the *st* gene), thermostable direct hemolysins, or shigella-like toxins.

Vibrio parahaemolyticus

V. parahaemolyticus was first described as the etiologic agent of the 'shirasu food poisoning' in Japan. Late in 1950, a severe foodborne outbreak (272 cases and 20 fatalities) was reported in Osaka, with patients suffering from acute gastroenteritis, severe abdominal pain, vomiting, and diarrhea. Epidemiological investigation led to identify the source of the outbreak to a small, semidried fish ('shirasu') and to the isolation by Dr. Tsunesaburo Fujino of a new microorganism, *Pasteurella parahaemolytica*, later renamed *Vibrio parahaemolyticus*.

Since then, *V. parahaemolyticus* outbreaks have been repeatedly reported in the world, and at present, *V. parahaemolyticus* is considered the first cause of the nonviral infections related to shellfish consumption in the United States, Japan, and Southeast Asia. This microorganism, in fact, displays the features common to *Vibrio* genus, as the natural occurrence in the marine environment and the correlation with water temperature and salinity, and is therefore commonly present in seafood products, with densities usually lower than 10^3 cfu g^{-1}, although such levels may be exceeded in products harvested from warmer seawaters. In addition, *V. parahaemolyticus* is also characterized by a remarkable replication time (as short as 8–9 min in optimal conditions and in the range of 12–18 min in seafood) that allows multiplication of the microorganism and rapid reaching of the estimated infectious dose (10^5–10^7 cfu for pathogenic strains) in raw, undercooked, or cross contaminated seafood products that are incorrectly stored.

As for *V. cholerae*, not all *V. parahaemolyticus* strains are pathogenic. A strong correlation between human disease and

production of two toxins (the thermostable direct hemolysin (TDH) and the TDH-related hemolysin (TRH)) has been demonstrated. TDH production, which induces beta-hemolytic activity in human erythrocytes on Wagatsuma agar (a process designated as Kanagawa phenomenon), is dependent upon the presence and expression of one or more nonidentical copies of the *tdh* gene (five variants have been reported), while TRH is encoded by the *trh* gene, in which at least two major subgroups (*trh1* and *trh2*) can be defined. Besides these hemolysins, other putative virulence factors are currently under investigation, as it has been shown that about 10% of clinical strains contain neither *tdh* nor *trh*. In particular, the role of the type III secretion systems (T3SSs) detected in *V. parahaemolyticus* (T3SS2-α and T3SS2-β) and of its genomic islands (*Vibrio* pathogenicity islands (VPaIs)) has been scrutinized since the emergence in early 1996 of a O3:K6 strain, whose diffusion, first in Asia (India, Bangladesh, Indonesia, Thailand, Laos, Taiwan, Korea, and Japan) and then in Europe, Mozambique, the United States, Mexico, and South American countries, has given rise to the first *V. parahaemolyticus* pandemic. Serotyping of *V. parahaemolyticus* is based on both somatic O (13 antigens) and capsular polysaccharide K antigens (65), but up to the description of the pandemic strain, there was no correlation between serotype and virulence and, until now, factors providing advantage to O3:K6 over other strains remain unclear.

Vibrio vulnificus

V. vulnificus is the most clinically serious *Vibrio* infection in developed countries, accounting in the United States for almost the totality (95%) of the deaths associated with seafood consumption. *V. vulnificus* is responsible, in fact, for severe infections in susceptible individuals, with two distinct disease syndromes, primary septicemia (with a case-fatality rate of approximately 50%) and necrotizing wound infections.

Based upon the variation of their biochemical profiles, three *V. vulnificus* biotypes can be distinguished. Strains belonging to biotype 1 are most commonly associated with human disease and can be isolated from seafood products (finfish and bivalve mollusks), biotype 2 is usually considered as an eel pathogen, while strains belonging to biotype 3, isolated in Israel in 1996, affect humans through wound infection and bacteremia. *V. vulnificus* is a widespread inhabitant of estuarine environments; its presence has been reported in seawater, shellfish, crabs, etc., and a strong correlation has been detected between water temperature and its isolation.

Infections are usually sporadic, and in almost all of them, the patient has an underlying chronic disease (e.g., cirrhosis or liver- or blood-related disorders) leading to elevated serum iron levels; other cases are reported in the presence of pathologies as chronic renal or gastric diseases, immunosuppression, and diabetes. Many studies have attempted discrimination of virulent and avirulent strains by comparison of clinical and environmental strains, but due to the high degree of genetic diversity, a definitive indicator has yet to be identified. *V. vulnificus* invasivity is related to the high variety of virulence factors expressed by this species: the polysaccharide capsule, which is produced by nearly all strains and confers an opaque aspect to *V. vulnificus* colonies, is essential for infection as it protects the microorganism from phagocytosis and from the activity of the complement system; the endotoxic lipopolysaccharides is responsible for the typical symptoms (fever, hemorrhage, hypotension, etc.) of *V. vulnificus* septicemia; siderophore production allows iron uptake from host serum; and a large number of extracellular enzymes, including proteases, elastase, and a hemolysin/cytotoxin with strong cytolytic activity, support the tissue disruption activity during the infection.

Other *Vibrio* Species

Besides the three species previously described, other vibrios have been recognized as potential pathogens for humans. *V. alginolyticus* is one of the *Vibrio* species most commonly detected in seawater and in seafood products; infections (otitis and wound infections) are mostly related to contact with the marine environment and few reports of its implication as a foodborne pathogen are available, even though severe cases have been described in patients with underlying medical conditions. *V. mimicus*, formerly known as 'sucrose-negative *V. cholerae* non-O1' for its similarity to the more renowned species, is causative agent of sporadic cases of gastroenteritis, mainly in relation to consumption of raw shellfish and crustaceans. Most of the virulence factors expressed by *V. mimicus* are toxins described in other species, including CT, TDH, zot, and st of non-O1/non-O139 *V. cholerae* strains. Finally, two closely related species, *V. fluvialis* and *V. furnissi*, have also been implicated in gastroenteritis episodes or outbreaks; *V. fluvialis*, in particular, has been reported in a large number of pediatric patients in India, and in surveillance data from the Center for Disease Control and Prevention, it represents approximately 5% of the vibriosis in the United States.

Vibrio Detection

Cultural Methods

In routine analysis of food products, the detection of pathogenic vibrios mostly relies on conventional cultural methods, followed by biochemical and, for *V. cholerae*, serological testing. Such approach, applied in internationally recognized methods as ISO/TS 21872 and the FDA Bacteriological Analytical Manual, includes a preenrichment step in a nonselective medium (usually alkaline peptone water with an appropriate concentration of NaCl) and provides resuscitation of bacteria, including cells in a VBNC state and cells stressed due to product refrigeration or freezing. Enrichment is then followed by isolation of colonies on selective agar media, with thiosulfate citrate bile salt agar (TCBS) being the most commonly used. Random selection of colonies with characteristic features represents a critical point of the procedure, as species of interest in food analysis (*V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*) may be overgrown both during enrichment and on agar plates by other vibrios as *V. alginolyticus* or by other genera, therefore making isolation of relevant colonies less efficient. In this context, the formulation of new selective and differential media (e.g., chromogenic media) to use in association with TCBS has progressively improved the chance to differentiate the species targeted by the analysis. The isolation of colonies is critical also under another prospective: as pathogenic strains of *Vibrio* species often represent a small minority of the microbial flora, the number and the proportion of characteristic colonies

selected for further testing may significantly affect the probability of detecting the pathogenic strains of a species (e.g., isolates belonging to the serogroups O1/O139 of *V. cholerae* or *tdh*-positive/*trh*-positive *V. parahaemolyticus* strains). Further to the isolation step, the total performance of the cultural methods may be challenged also by the identification of the isolates; the variability of the biochemical profiles of *Vibrio* environmental strains, in fact, may negatively affect the accuracy of the biochemical identification by conventional tests, dichotomous keys, or miniaturized tests, leading to inconclusive results or to misidentifications.

Conventional cultural methods for *Vibrio* analysis show, overall, important characteristics that encourage their routine adoption, including technical easiness, relatively low costs, and integration with laboratory's workflows. On the other hand, however, the cultural analytic process is time-consuming (4–5 days to obtain the identification of the microorganisms), labor-intensive, and, lastly, unable to provide a characterization of the virulence factors associated with human illness. To this aim, a progressive integration of the cultural and molecular analytic methods for *Vibrio* analysis has been pursued, as testified by the introduction, also in the aforementioned methods for official controls, of polymerase chain reaction (PCR) protocols for sample screening, improvement of species identification, and characterization of pathogenicity factors.

Molecular Methods

During the past years, nucleic acid detection and amplification methods have significantly broadened the spectrum of diagnostic tools for the analysis of *Vibrio* species, and to date, a large number of both conventional and real-time PCR protocols, isothermal amplification (LAMP) procedures, and some hybridization methods have been published for the detection, identification, characterization, or enumeration of potentially pathogenic species. One of the major advantages accorded by nucleic acid-based techniques is related to the fact that, by targeting directly genetic sequences responsible for or associated with virulence of defined *Vibrio* species, they provide more reliable information on the risk associated with the presence of pathogenic vibrios in a product. A second advantage is given by the substantial reduction of the time required for the analysis, with detection of potentially pathogenic species completed, for certain protocols, in 24 h or less. In this sense, the molecular techniques represent the most suitable analytic approach for industries and food producers to prevent release on the market of products containing pathogenic *Vibrio* species, as well as for control authorities to provide timely results, therefore ensuring food safety and preventing outbreaks.

Molecular detection methods for *Vibrio* have been directed, depending on the scope of the analysis, to either conserved region of the genome (species detection or isolates identification) or genes encoding or associated with virulence (detection of potentially pathogenic strains). PCR targeting total *V. cholerae*, for example, has largely relied on the detection of conserved and widespread genes as *toxR*, which is involved in the regulation cascade of virulence-associated genes; *ompW* gene, which encodes an outer membrane protein and is conserved in both clinical and environmental isolates; and *hlyA* gene, a nonclassical fragment of the hemolysin gene present in O1, O139, and non-O1/non-O139 *V. cholerae* strains. Identification of pathogenic strains has, on the other hand, mainly targeted genes associated with the CT production, including *ctxA* and *ctxB*, or codifying for other virulence factors as *zot*, *ace*, *tcp*, or *st* genes. PCR protocols, however, have also been successfully developed for rapid characterization of strains, for example, with the identification of *V. cholerae* O1 epidemic strains through *ctxB* allelic discrimination or by using *rfb* gene for the molecular differentiation of O1 and O139 isolates.

Compared to *V. cholerae*, a multiplicity of sequences has been proposed in the last years for the detection of *V. parahaemolyticus* in food samples and seawater or for the identification of isolates. The 16S ribosomal RNA gene, for example, was repeatedly evaluated as a possible target for molecular analysis, but high sequence homology with other species (e.g., 99.7% with *V. alginolyticus*) and the presence of intrastrain polymorphisms have hampered its use. *GyrB* gene, encoding the B subunit of DNA gyrase, and fragments of the *hsp* (heat shock protein) gene, being characterized by a lower sequence homology with other *Vibrio* species, were also suggested as possible targets for differentiation of *V. parahaemolyticus*. Three other targets, however, have been extensively used and compared, especially for the application in food analysis: the *tlh* gene, codifying for a thermolabile hemolysin (TLH) in *V. parahaemolyticus* and, at present, widely used in the United States and Japan; the *toxR* gene, homologous to the regulator gene of *V. cholerae* and conserved with low sequence identity in the whole Vibrionaceae family, whose application is common in Southeast Asia and Europe; and the pR72H sequence, a restriction fragment generated by HindIII enzyme that includes a coding (phosphatidylserine synthase gene) region and a noncoding region and that appears to be conserved in *V. parahaemolyticus* strains. In contrast to methods for *V. parahaemolyticus* identification, protocols aiming at the detection of potentially enteropathogenic strains have unequivocally targeted the *tdh* and *trh* genes, often in multiplex format with each other or with one of the aforementioned sequences for species identification.

For *V. vulnificus*, identification has been mainly based on the cytotoxin–hemolysin gene (*vvh*), thought to be common to all biotypes, although potential problems of gene loss associated with the use of this target have been reported. Alternative genomic loci, as the 16S rRNA gene, have been therefore investigated, both for identification purposes and to achieve differentiation between clinical and environmental strains; a 17-base sequence variation in the 16S rRNA gene, in fact, was shown to have a statistically significant correlation with clinical isolates. Other genes and sequences, including the virulence-correlated gene (*vcg*), a siderophore-encoding gene (*viuB*), and the capsular polysaccharide operon, have demonstrated, however, comparable or higher discriminative power and have been widely applied for *V. vulnificus* characterization.

In the analysis of foods in which *Vibrio* species may be present, nucleic acid amplification-based methods usually provide results with high specificity and information relevant for the characterization of pathogenicity potential of microorganisms. Nonetheless, a major drawback of these methods is that, due to the low concentration of the targets and the high inhibitory effect of food matrices on PCR amplification, sensitivity levels compatible with food analysis can be achieved only on

preenriched samples. As a consequence, even though several real-time qPCR protocols have been developed for analysis of vibrios in foods, quantification by molecular methods has in the end settled for the use of MPN-PCR formats that, although sensitive, call for a considerable increase of analytic work and costs. An alternative approach for *Vibrio* enumeration is granted by colony hybridization techniques, which combine growth on nonselective agar media with molecular identification/characterization using specific probes, and a few hybridization protocols have been described, especially for quantitative analysis of *V. parahaemolyticus* and *V. vulnificus*.

See also: Codex Alimentarius.

Further Reading

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Vinegar

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Sources and Production

Despite some small local differences, in general, food regulations consider vinegar to be the result of a double fermentation (alcoholic and acetic fermentation or acetification) of any sugary substrate. European countries have specific rules for vinegars sold in different regions. In the European Union, the established limits for acidity and residual ethanol content are strictly set. Therefore, the acidity of wine vinegar (acetification obtained exclusively from wine) must be at least 6% (m/v) of acetic acid, and the maximum residual ethanol allowed is 1.5% (v/v). However, the variety of raw materials used in the production of vinegar is very broad, ranging from by-products and agricultural surpluses to high-quality substrates for the most unique and prized vinegars, such as sherry vinegar (Spain) and Aceto Balsamico Tradizionale (ABT) (Italy). Chemically, the main component of vinegar is acetic acid. Although most of the acetic acid produced in the world is the result of chemical synthesis, only the acetic acid obtained from double fermentation is accepted for the food industry in general.

Vinegars can be classified by their substrates of origin or by their systems of production (Figure 1). According to the origin-derived classification, vinegars can be fruit vinegars (the most common types, especially from grapes, mostly called wine vinegars), grain vinegars (originating from several cereals, such as malt or rice vinegars), alcohol vinegars (mostly derived from pure alcohol obtained by distilling different by-products of the beverage industry: grape or other fruit pomace, corn, etc.), or even vinegars from products of animal origin (such as honey or milk serum). It is necessary to differentiate between those products derived from the double fermentation of a single fruit (most often grapes or apples) and those that are 'flavored' vinegars, that is, vinegars of various origin with fragrances or concentrates of different fruits, flowers, or spices. Although these 'flavored' vinegars are not considered vinegars in some countries, lax regulations in other countries allow these products or condiments to be sold as 'vinegars.'

The first fermentation is an alcoholic fermentation and transforms sugars or processed starches into ethanol. This process is performed by yeast, mostly from the species *Saccharomyces cerevisiae*, although some other species can also perform the alcoholic fermentation in part or in total. The final result is considered the substrate of the second transformation, that is, from ethanol to acetic acid. Although this second process is often referred as 'acetic' or 'oxidative' fermentation, it is not biochemically a fermentation but an oxidation. The proper term is thus 'acetification.' This process involves a two-step oxidation, from ethanol to acetaldehyde and further from acetaldehyde to acetic acid, whereby the end of this process requires an electron acceptor, with molecular oxygen being the most common. The microorganisms responsible for this process are acetic acid bacteria.

Acetic acid bacteria are Gram-negative or Gram-variable, either ellipsoidal or cylindrical, and can be observed under a microscope alone, in pairs, or in aggregates and chains. They have aerobic respiratory metabolism because oxygen is the final electron acceptor, although other compounds may occasionally act as final electron acceptors. As a result, acetic acid bacteria can survive under nearly anaerobic conditions albeit by displaying severely limited growth. These bacteria are found on substrates containing sugars and/or alcohol, such as fruit juice, wine, cider, and beer. On these substrates, the sugars and alcohols are incompletely oxidized, leading to the accumulation of organic acids, such as the production of acetic acid from ethanol or gluconic acid from glucose. Some of the transformations performed by acetic acid bacteria are of great interest to the biotechnology industry. During acetification, these bacteria can also produce ethyl acetate and other esters that are characteristic of their metabolism. Although more than 60 species have been described as acetic acid bacteria, only a limited number of them are involved in the production of vinegar. Most of the species involved in vinegar production were initially classified within the genus *Acetobacter*, but some of them have been reclassified to the genus *Gluconacetobacter* and, more recently, to *Komagataeibacter*. For instance, *Komagataeibacter europaeus*, a common species in high-acetic acid content vinegars, can be found in the literature described as *Gluconacetobacter europaeus* or *Acetobacter europaeus*. The species most commonly found in the production of vinegar are *Acetobacter pasteurianus*, *Komagataeibacter europaeus*, and *Komagataeibacter xylinus*.

Apart from their different substrates, vinegars can also be differentiated by their production systems. In traditional vinegars, the transformation of ethanol into acetic acid is performed by a static culture of acetic acid bacteria at the interface between the liquid and air. Barrels are filled to 2/3 of their capacity, as to leave an air chamber that is kept in contact with the outside air using one opening or various types of openings. This production system, which is considered to be the traditional method, is called 'surface culture.' A more standardized version of this method, the 'Orleans method,' includes side holes for air circulation and adds a funnel with an extension to the base of the barrel to allow wine to be added at the bottom of the barrel, preventing the alteration of the 'mother of vinegar.' This mother of vinegar is the biofilm formed by the transforming microorganisms, that is, the acetic acid bacteria, which develops on the surface due to the need for oxygen (Figures 2 and 3). The vinegars produced by this traditional system are generally considered of high quality because of their organoleptic complexity, which is mainly due to the metabolism of the acetic acid bacteria and the overlapping vinegar production with aging. However, this process is very slow, and the production of vinegar can take from months to years.

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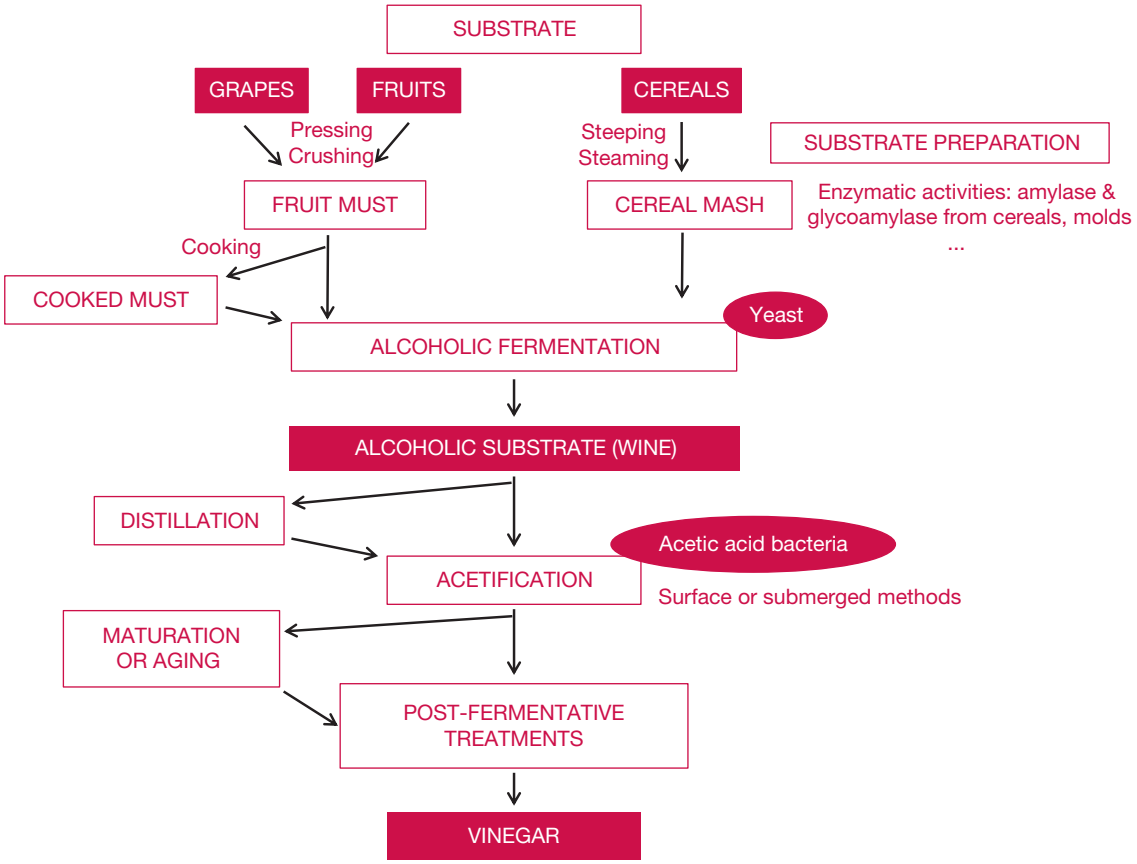

Figure 1 Schematic representation of vinegar production from different substrates using different elaboration systems.

Figure 2 'Mother of vinegar' formed at the liquid–gas interface of a wooden barrel in traditional vinegar production.

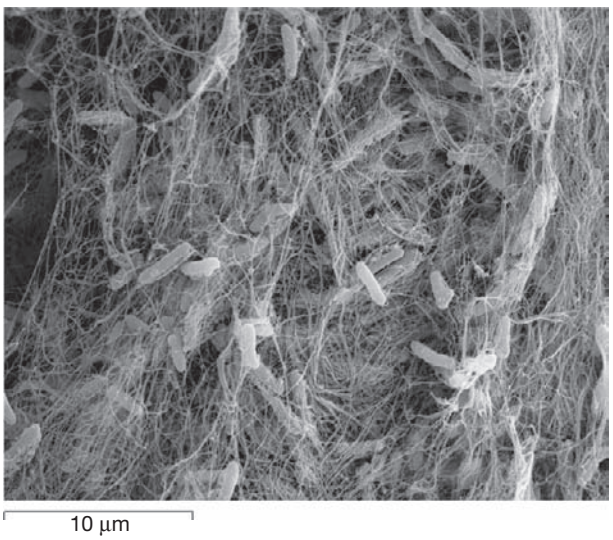

Figure 3 Electron microscopy image of acetic acid bacteria cells embedded in a biofilm that constitutes the 'mother of vinegar.'

To reduce the acetification time, other methods, such as the Schützenbach system or systems with submerged cultures, have been developed. In the former, bacteria are immobilized on wood chips or charcoal, forming a solid bed on which the vinegar spreads. After passing through the bed of chips, the vinegar is collected in a container at the bottom and pumped back to the same fixed bed. The acidity successively increases, and it is possible to obtain vinegar of reasonable quality within a week.

Submerged culture systems provide a much faster alternative. These systems rely on suitable turbines to generate a flow of air bubbles into the wine or alcoholic solution. The oxidative process occurs in the air–liquid interfaces of the air bubbles. Improvements to this process generally involve engineering (improving the maintenance and persistence of the bubbles in the liquid, the uniformity of the bubble size, the recovery of lost aromas, etc.). In this type of vinegar produced

at a significantly lower cost, the bacteria act as bioreactors for the transformation of alcohol into acetic acid, with only very limited production of other metabolites. The airflow also contributes to a considerable loss of the volatile compounds present in the original alcoholic solution, resulting in a less complex product from a sensory point of view. Although early containers for submerged culture processing were made of wood, the usual containers are stainless steel, which is more hygienic and resistant to acidic conditions. Despite the fact that wood containers were meant to provide some organoleptic complexity, there was hardly any transfer of flavor compounds from the wood to the vinegar because of the imbalance between the contact surface and volume and the speed of the process. This limitation can be compensated by subsequent aging in barrels or by submerging wood fragments or wood chips, which may contribute to the recovery of some missing organoleptic character. Despite the loss in product quality, this methodology has two important advantages: speed (the vinegar can be produced in cycles of 24 h or even shorter) and acidity (the product can reach concentrations of acetic acid of up to 23–25%, compared with the 6–13% achieved with other systems). Higher acidity helps to reduce transportation costs by reducing water transport.

Patterns of Consumption

Vinegar consumption dates at least from 5000 BC, when Babylonians used it as a condiment and as a preservative. Its acidic character (until the description of sulfuric acid, it was the strongest known acid) facilitated its use as a preservative due to its antimicrobial activity. This antimicrobial activity was used to heal wounds and as a general antiseptic by 2000 BC, and vinegar was even used for treating wounds during World War I. This preserving use initiated the development of the pickling industry that became extensive with the Greeks. However, in the production of different fermented beverages (such as wine and beer), vinegar became a by-product. The Romans also used it as a drink, as some wines were partially converted into vinegar. In fact, the name 'vinegar' derives from the term 'sour wine' in French ('vin aigre').

As for fermented foods and beverages in general, the consumption of vinegar is a cultural trait. Although the use of some type of acid in the food industry is widespread in every culture, this acidity is achieved by acetic acid or vinegar in some cultures, whereas in others, citric acid (e.g., lemon juice) is used. The use of some types of high-quality vinegars is very common in Europe and Asia and has become fashionable in North America. Wine vinegar is the most common type in Mediterranean countries, although the latest gastronomic trends have led to a considerable expansion of the varieties available in recent years. In Asia, rice vinegar is the most common type, although other types are also found, many of them following very traditional systems of production. In Northern Europe and in the United States, most of the vinegar produced is 'white' vinegar, that is, vinegar produced directly from diluted alcohol. These differences also determine their pattern of consumption, which in turn affects the concept of 'quality.' In Mediterranean countries, most vinegar is used directly or added to salads or to raw or cooked vegetables; thus, the appreciation of the organoleptic characteristics is straightforward. Therefore, 'quality' vinegars are closely associated with these patterns of consumption. In contrast, in other countries, most vinegar is used for pickling or as part of sauces, and the impact of the organoleptic quality, although possibly relevant for the final product, is less evident. However, this trend of consumption is changing, and some premium vinegars are being commercialized worldwide. A typical example of this trend is the increased consumption and trade of Balsamic Vinegar of Modena (Aceto Balsamico di Modena, ABM). In fact, Italy is the country that exports the most vinegar, providing twice the quantities of the other main exporters, Germany, Spain, and France. Moreover, in terms of revenues, Italian vinegars are exported at much higher values than Spanish or German vinegars. The situation in Germany is different, considering that most German vinegar is sold for the pickling or sauce industry, whereas the Spanish exports include also some premium vinegars such as sherry vinegars.

Sherry vinegars that are included in the European Union's protected designation of origin (PDO) framework derive from sherry wines and are necessarily aged in wood barrels for at least 6 months. This aging can be performed by a dynamic system (i.e., passage through different barrels known as 'solera' and 'criaderas' containing vinegar from different vintages or different ages) or a static system (i.e., always in the same barrel). A more complex example is aceto balsamico, which is either ABT, regulated by two different PDO labels (ABT di Modena or ABT di Reggio Emilia), or ABM, which has a protected geographical indication status. The production of ABT is a long process that starts with the cooking of the grape must, which increases the sugar concentration to 400–500 g l^{-1}. Next, a partial alcoholic fermentation, which is initiated by osmophilic yeasts, produces a 'sweet wine' of approximately 7% (v/v) ethanol concentration and over 200 g l^{-1} of residual sugars. Then, some mother of vinegar is added to this 'sweet wine,' and it is left to be acetified. Once acetified, the vinegar is placed in a 'bateria' formed by five to seven barrels of different woods (oak, mulberry, chestnut, cherry, juniper, ash, and acacia) and decreasing volumes (from 60 to 15 l), which are filled up to 2/3 of their total volume. This 'bateria' is kept for at least 12 years with a yearly refilling from the previous barrel in a dynamic aging process. During this aging process, two phenomena occur: the transfer of components from the wood to the ABT and, more importantly, the concentration of the product and the integration of its components. The final product can have up to 500 g of sugar per kg of product, 7% acetic acid (v/v), and 20 g of gluconic acid per kg. The oxidation of glucose by acetic acid bacteria yields gluconic acid. The result is a dark, concentrated, and thick product sold in 100 ml bottles and with a market value that can easily reach 100 euros. In contrast, ABM is a mixture of vinegar, caramel, and cooked or concentrated must that is aged at least 2 months, not necessarily in barrels. However, some of these ABM can be aged for more than three years and are labeled 'invecchiato' (aged). Overall, ABM is a cheaper version of ABT that has been popularized all over the world.

Some Asian vinegars, such as black vinegars from China or 'kurosu' from Japan, are produced from rice and other cereals (including sorghum, wheat, and others) with a very important aging process in which concentration and thickening occur in a similar manner to ABT. These vinegars can even be consumed

as a beverage in small volumes, as is sometimes claimed for ABT (used as a dessert wine). However, the direct use of vinegar as a drink is rare. In fact, despite some exceptions, the individual consumption of vinegar is very limited, whether directly (as an addition to salads or raw vegetables) or indirectly (through its presence in dressings, of which salad dressings as vinaigrette, mayonnaise, or mustard sauce are the most popular). This limitation is mostly due to the pungency of the main component of vinegar, acetic acid. The direct use of vinegar as a drink can produce throat and esophagus wounds due to the caustic character of acetic acid. However, this aspect can be reduced by the composition of the vinegars, especially due to the complexity of different compounds present that, although in low concentrations, can compensate for the acidity. In particular, the sour taste and pungency are mitigated well by the quality of the substrate (wine, for instance) and by combination with some tastes, such as sweetness or umami. This mitigation is the main reason why the direct consumption of small quantities of beverages containing up to 8% (v/v) of acetic acid is possible, while consuming the same amount of acetic acid diluted in water would be impossible.

Composition

Depending on the type of vinegar, its composition may be the result of (i) the raw material (wine or other substrate), (ii) the metabolism of the acetic acid bacteria, which accomplish some additional transformations (mostly oxidations and also other reactions, such as ester formation) in addition to the basic transformation (ethanol to acetic acid), (iii) the interaction between the vinegar and the wood from the barrels, and (iv) the aging process, which integrates all the previously mentioned characteristics.

The main and characteristic compound in vinegars is acetic acid, which is found in concentrations higher than $50\,g\,l^{-1}$, usually expressed as a volume percentage (5% (v/v) of acetic acid or 5 acetic degrees). Acetic acid is essentially the only component in vinegars produced from distilled alcohol. Normally, most vinegars contain residual ethanol in their compositions, mainly to prevent the complete oxidation (or respiration) of acetic acid to oxygen dioxide and water by the acetic acid bacteria, a reaction that is strongly inhibited by the presence of small amounts of ethanol.

Fruit vinegars, especially wine vinegars, are considered to be more complex due to the presence of polyphenols, aromas, etc. that are characteristic of the raw material. In general, it is relatively easy to appreciate the sensory differences between vinegars made by traditional methods and those manufactured at the industrial scale, as submerged culture acetification results in a substantial loss of volatile compounds. In fact, the vinegar aroma is of complex composition, containing many compounds with a wide range of volatilities and polarities and with concentrations ranging from several $mg\,l^{-1}$ to $ng\,l^{-1}$. To date, more than 100 different chemical compounds have been identified in the aroma of wine vinegar, including carbonyl compounds, ethers, acetals, lactones, acids, alcohols, phenols, and volatile esters, all of which are involved to different extents in the final flavor. However, not all of the volatile compounds are responsible for the aroma of the product. These volatile components must not only reach odorant receptors but also interact with them in the olfactory epithelium, and not all volatile compounds are active odorants. The use of techniques based on gas chromatography coupled with olfactometry has allowed to pinpoint the contribution of each volatile compound to the final vinegar flavor. For example, it has been determined that the characteristic aroma of sherry vinegar involves several volatile compounds, such as diacetyl, isoamyl acetate, isovaleric acid, ethyl acetate, and sotolon.

Esters are a significant part of the vinegar aroma and a main element of the quality of vinegars. Of special interest is the production of ethyl acetate, which as a pure compound would smell like glue or nail polish remover but adds some sweet character to vinegar. Interestingly, noticeable concentrations of this ester (in the order of grams per liter) are considered to be defects in wine (as is the presence of acetic acid), yet in vinegar, ethyl acetate compensates for the pungency of acetic acid, and it can thus be appreciated in moderate amounts. Traditional methods of production favor the formation of esters due to not only the metabolism of the bacteria but also the process duration, which allows condensation between acids and the residual alcohol.

In some premium vinegars, residual sugars are also a main component, and they particularly help to reduce the pungency of acetic acid by their sweet taste. In ABT, kurosu, and black rice vinegar, these residual sugars, together with amino acids or peptides, can undergo Maillard reactions due to heating and/or aging, which results in the formation of some melanoidins and their polymerization, yielding a dark color. Furthermore, glucose, which is one of the most characteristic residual sugars in vinegars, can be partially converted to gluconic acid by acetic acid bacteria. Due to its molecular weight and structure, gluconic acid is much less pungent than acetic acid, and it provides a mouth-filling sensation. Other organic acids that are often found in vinegars include citric, malic, and lactic acids; these acids can add some sour flavor, yet they are less pungent than acetic acid.

Polyphenolic compounds, which are ubiquitous in plant products, are of great interest as quality determinants because, in addition to their antioxidant activity, they are responsible for the color and astringency of vinegar and are a useful tool for the purposes of determining authenticity. The reactivity of phenolic compounds with oxygen is specifically analyzed in wine making for its relationship to the browning of white wines and with respect to the reactions of anthocyanins in red wines. These anthocyanin reactions dramatically decrease after the submerged acetification process because the anthocyanins are extensively oxidized. It should be emphasized that submerged systems use excess oxygen to secure and accelerate the process, whereas the oxygen availability is limited in superficial cultures. Additionally, oxygen affects different classes of polyphenolic compounds to different degrees. For example, the flavonol content of vinegars is largely influenced by the oxygen availability during submerged fermentation. Aging in wood strongly determines the phenolic composition, as phenolic aldehydes are released from wooden barrels into the product. The substances provided by the wood depend on the type of wood and roasting, the ratio of the contact surface to the liquid volume, and the aging time. Specifically, oak releases mainly vanillin, syringaldehyde, coniferyl aldehyde,

and cinnamic acid after 90 days of aging. Furthermore, certain flavonoids are chemical markers of the wood that the vinegar has been in contact with; (+)-dihydrorobinetin is a characteristic compound released from nontoasted acacia wood, while (−)-taxifolin is typically released from cherry wood. Chestnut provides remarkable concentrations of gallic acid. Wood toasting can degrade thermolabile compounds such as (+)-dihydrorobinetin or increase the rupture of lignin into phenolic aldehydes of small molecular weight. Therefore, aging is a fundamental aspect of the integration of the different compounds in vinegars. The increase in organoleptic quality after aging is remarkable; in addition to interactions with the wood, a series of chemical reactions, such as the production of esters and reactions between acids and residual alcohols; evaporation; and other processes result in better integration of aromas and metabolites and a reduction in the pungency of acetic acid. The aging process involves the reaction of compounds over time, including both the polymerization and release of compounds from the wood and the losses through evaporation.

Thorough characterization and quality evaluation require both the determination of the content of a number of compounds and sensory analysis. In recent years, there have been significant advances in the elucidation of the compounds responsible for the sensory quality of the products, and production methods have changed to obtain vinegars with high acceptance at very competitive prices. The consumer perception of the product is the most important factor. Vinegar is a difficult product to taste due to the intense sensations it provokes. The pungency of the high acetic acid content masks other flavors, and some familiarity with the product is required to proceed with a tasting. In fact, there is no consensus on how vinegar should be tasted. A vinegar sensory analysis panel requires well-trained tasters, and specific attributes that are useful for differentiating among samples must be chosen. To train a vinegar panel, solutions used are prepared with different concentrations of the compounds most typically found in vinegar, such as acetic acid, ethyl acetate, and wood extract obtained by maceration. The last two should also be prepared in 7% acetic acid to provoke a sensation similar to that of vinegar. The aggressiveness of acetic acid determines the number of samples that can be examined in each session, and each sample is tasted several times. These replicate samples should be tasted on different days to avoid sensorial saturation among the tasters. A descriptive analysis of the samples is prepared based on previously selected attributes that can be evaluated by the panel. For instance, the main attributes used to describe wine vinegar samples are color, aromatic intensity, woody scent, herbaceous smell, fruity odor of ethyl acetate, wine smell, and pungent feeling. As expected, higher sensory thresholds for most compounds are obtained in an acetic acid matrix compared with water solutions. Conversely, high-quality vinegars contain a large number of these compounds at concentrations higher than their threshold limits, including vanillin, eugenol, and benzaldehyde, which could be selected as attributes of high-quality vinegars. Additionally, adequate training and a standardized tasting protocol contribute to the reliability of the descriptive sensory analyses of vinegar and of ascertaining the aging period and wood used in its development.

Bioavailability, Absorption, and Metabolism

The main component of vinegar, acetic acid, is rarely absorbed in the stomach; most of it is absorbed in the small intestine. After its absorption, acetic acid is mostly transferred to the liver through the portal vein. Once in the liver, it is transformed into acetyl coenzyme A and used for lipogenesis. Some amounts of acetic acid that might be left in the circulation are taken up by the peripheral tissues, activated to acetyl coenzyme A, and metabolized through the Krebs cycle. However, not all of the acetic acid in the body is derived from the ingestion of vinegar (whether directly or as an ingredient in sauces): a considerable amount is produced in the large intestine as a main by-product of the fermentation of nondigested saccharides (disaccharides or oligosaccharides) by the gut microbiota, next to lactic acid. Other acids, such as propionic and butyric acids, are also formed, yet they are formed in lower concentrations and depend on the fermented saccharide and the individual microbiota. The acetic acid is absorbed in the large intestine, transferred to portal circulation, and further metabolized as explained in the preceding text.

Health Effects

Although many health effects have been claimed as a result of vinegar consumption, only a few of these effects are based on clear evidence. Certain studies show reductions in hyperglycemia, hyperinsulinemia, hyperlipidemia, and obesity. Therefore, the consumption of acetic acid from vinegar has been related to an antiglycemic effect because acetic acid has an effect on enterocytes, reducing the activity of the disaccharidases and thus decreasing the digestion of di- and oligosaccharides. Indeed, if vinegar is consumed with a high-carbohydrate meal, the glycemic index of that meal decreases. This effect has been observed in both patients with type 2 diabetes and in healthy people, with a notable reduction of insulin resistance in the diabetic patients. This reduction could be one of the reasons for claims that the direct consumption of vinegar can result in weight loss. However, it is important to emphasize the high risk of the direct consumption of vinegar as a drink due to the caustic effects on the pharynx and esophagus and the inflammation of the intestines.

Another health effect is the increased absorption of calcium ions. It is well known that the reduction of calcium absorption can be a problem, especially in the elderly, and many components of the diet result in a reduced absorption. The combination of calcium ions with acetate in the intestine is facilitated by the change of pH between the stomach and intestine, and thus, the formed compound, calcium acetate, is easily absorbed, resulting in an increased absorption of calcium.

Moreover, vinegar has antioxidant activity related to its phenolic composition described in the preceding text. However, the consumption of vinegar may be too low to expect a significant effect on health.

Other claimed activities of acetic acid (or vinegar) on health effects have been less clearly demonstrated, as for its antitumor or hypocholesterolemic activities, among others. However, further research in this field is required. Some of these effects are considered to be related to other components of vinegar. To

that end, it is important to consider the reduced individual consumption of vinegar and the low concentration of those compounds in vinegar.

Conclusion

Vinegar is a food condiment and preservative that has been used for centuries but is still widely used today. Several qualities are available, yet the indirect use through sauces and pickles remains the most common one. The health effects are still under investigation, yet some observations indicate positive effects that could help to increase the overall consumption of vinegar.

See also: Alcohol: Metabolism and Health Effects; Biofilms; Browning: Non-enzymatic browning; Cellulose; Cereals: Types and Composition; Cider (Cyder; Hard Cider): The Product and Its Manufacture; Ethnic Foods; Fermented Foods: Composition and Health Effects; Fermented Foods: Origins and Applications; Fermented Foods: Use of Starter Cultures; Grapes; Maillard Reaction; Phenolic Compounds: Bioavailability and Health Effects; Phenolic Compounds: Occurrence, Classes, and Analysis; Rice: Types and Composition; Sensory Evaluation: Sensory Rating and Scoring Methods; Whey and Whey Powders: Fermentation of Whey; Wines: Types of Table Wines; Wines: Wine and Health; Wines: Wine Production.

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Vitamin K: Physiology

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Background

From the 1930s until the 1970s, the only known role of vitamin K was as an essential antihemorrhagic factor, which initially had been shown to promote the synthesis of active prothrombin (factor II), but later also of factors VII, IX, and X. Collectively, these four factors, all synthesized in the liver and then released into the circulation, became known as the classical vitamin K-dependent coagulation factors. In 1974, it was shown that vitamin K was required as a cofactor for the enzymatic conversion of specific glutamate residues in the prothrombin molecule to γ-carboxyglutamate (Gla) residues. The elucidation of this biochemical pathway leading to the synthesis of a new amino acid was the impetus that led to the discovery of many more vitamin K-dependent proteins. These proteins, also known as Gla proteins, are now known to have a widespread tissue distribution. They include further coagulation proteins (proteins C, S, and Z) and others with putative roles in the regulation of bone turnover (osteocalcin) and bone calcification (matrix Gla protein (MGP)). New evidence suggests that MGP is also a powerful inhibitor of vascular calcification. Other suspected functions of Gla proteins are in vascular repair processes (protein S), cell cycle regulation, cell–cell adhesion (Gas6), and signal transduction (Gas6 and proline-rich Gla proteins). For the most part, the precise physiological function of these new Gla proteins is poorly understood. However, nutritional and epidemiological studies in humans suggest that optimal intakes of vitamin K are necessary to maintain skeletal and cardiovascular health. Despite the possibility of these new health roles, the only clearly defined deficiency syndrome is bleeding due to a lack of functional molecules of the vitamin K-dependent coagulation factors II, VII, IX, and X. Bleeding due to vitamin K deficiency is rare in adults but is more common in infants during the first 6 months of life. The potential risk of serious life-threatening bleeding in infancy means that most countries recommend that all newborns should receive vitamin K prophylaxis.

Intestinal Absorption and Bioavailability

Dietary vitamin K, mainly as phylloquinone, is absorbed chemically unchanged from the proximal intestine after solubilization into mixed micelles composed of bile salts and the products of pancreatic lipolysis. In healthy adults, the efficiency of absorption of phylloquinone in its free-form is about 80%. The bioavailability of vitamin K from foods varies according to food type and the associated matrix. Thus, the efficiency of absorption of phylloquinone may be as low as 10% from green leafy vegetables (e.g., spinach) in which the vitamin is tightly associated with chloroplast membranes. Less is known about the absorption of dietary menaquinones (MKs), but the efficiency of absorption decreases with increasing length of the side chain.

MKs are also synthesized by the intestinal microflora, predominately in the colon. Major forms are MK-10 and MK-11 synthesized by *Bacteroides* species, MK-8 by *Enterobacteria*, MK-7 by *Veillonella*, and MK-6 by *Eubacterium lentum*. It is commonly held that microfloral synthesis provides an important nutritional source of vitamin K for humans, but direct evidence is lacking. A microfloral contribution of vitamin K is supported by the findings that very-long-chain MKs 10–13 are rare in foods but make up the majority of hepatic stores. However, all experimental evidence suggests that the absorption of MKs from the large intestine is an extremely inefficient process.

Transport in Plasma and Tissue Distribution

After intestinal absorption, vitamin K is incorporated into chylomicrons, is secreted into the lymph, and enters the blood via the lacteals. Once in the circulation, phylloquinone is rapidly cleared at a rate consistent with its continuing association with chylomicrons and the chylomicron remnants that are produced by lipoprotein lipase hydrolysis at the surface of capillary endothelial cells. After an overnight fast, more than half of the circulating phylloquinone is still associated with triglyceride-rich lipoproteins, with the remainder being equally distributed between low-density and high-density lipoproteins. Phylloquinone is the major circulating form of vitamin K, but MKs MK-7 and MK-8 have been detected at lower concentrations.

Dietary vitamin K is delivered to the liver and possibly other tissues, including bone marrow, in the form of chylomicron remnants. The cellular uptake of vitamin K is mediated by apolipoprotein E in the chylomicron remnants and heparin sulfate proteoglycans on the cell surface. The liver, the site of synthesis of the vitamin K-dependent coagulation proteins, has a limited capacity for storing phylloquinone. Thus, the hepatic reserves of phylloquinone are several thousandfold lower than those of vitamin A, despite the fact that dietary intakes of phylloquinone are only about tenfold lower. The distribution of molecular forms of vitamin K in the liver is quite different from that in plasma in that the major transport form, phylloquinone, represents only about 10% of total stores; the remainder comprise bacterial MKs, mainly MKs 7–13. Long-chain MKs are also present in other nonhepatic tissues but at lower concentrations than the liver. Some tissues such as the brain and pancreas seem to have the capacity to selectively convert phylloquinone to MK-4, but little is known about the biochemistry or physiological significance of this enrichment.

Catabolism and Turnover

The metabolic transformations leading to the elimination of vitamin K from the body occur exclusively in the liver.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00729-7

Phylloquinone is rapidly degraded with about 20% of a single dose being excreted in the urine within 3 days and about 40–50% excreted in the feces via the bile. The hepatic reserves of individuals on a low-phylloquinone diet decline rapidly. The overall picture is that phylloquinone has a fast turnover time, suggesting that the body stores are being constantly replenished.

The route of hepatic catabolism leading to the urinary excretion of vitamin K proceeds by oxidative degradation of the phytyl side chain, probably involving the same enzymes used for the ω-methyl and β-oxidation of fatty acids, steroids, and prostaglandins. The end products of this pathway are shown in Figure 1. Two major aglycone metabolites have been identified; they are carboxylic acids with five- and seven-carbon atom side chains that are excreted in the urine as glucuronide conjugates. The biliary metabolites have not been clearly identified but are initially excreted as water-soluble conjugates and become lipid-soluble during their passage through the gut, probably through deconjugation by the intestinal microflora. There is no evidence that body stores of vitamin K can be conserved by an enterohepatic circulation, since vitamin K itself is not excreted in bile and the side chain-shortened carboxylic acid metabolites are not biologically active.

Cofactor Role of Vitamin K

Vitamin K-Dependent Carboxylation of Glutamate Residues

The only unequivocal biochemical role for vitamin K is as a cofactor for a unique posttranslational chemical modification confined to a small group of unusual calcium-binding proteins. The vitamin K-dependent step is a carboxylation reaction that transforms selective glutamic (Glu) residues in the protein precursor into Gla. The reaction shown in Figure 2 is catalyzed by a microsomal enzyme called vitamin K-dependent (γ-glutamyl) carboxylase and requires molecular oxygen and carbon dioxide. The form of vitamin K required as the cofactor is not the stable quinone structure found in the diet but the reduced quinol (or hydroquinone) structure (KH_2). The subsequent oxidation of vitamin KH_2 to vitamin K 2,3 epoxide is coupled to the abstraction of a proton from the γ-carbon of the Glu residue to generate a carbanion that reacts with CO_2 to yield the final Gla product. The biological relevance of the Gla structure is that it forms a cage structure to which divalent metal ions such as calcium may be bound. In the case of the vitamin K-dependent procoagulants, this induces the conformational change and membrane-binding properties that are essential for their biological activity.

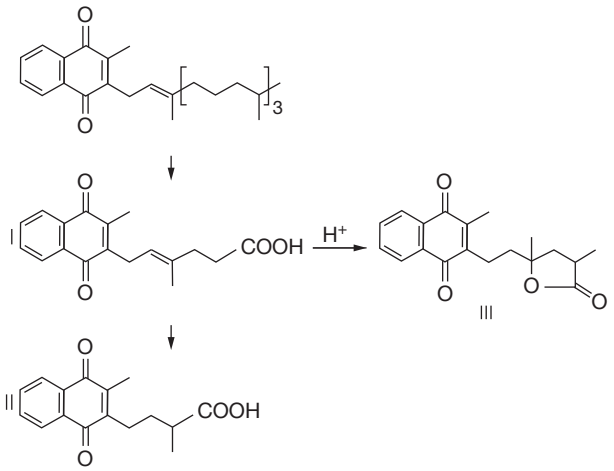

Figure 1 Simplified pathway and chemical structures of the two major urinary metabolites of phylloquinone in their aglycone forms (structures I and II). In acidic conditions, metabolite I may cyclize to produce phylloquinone γ-lactone (structure III). (I) 2-Methyl-3-(5′-carboxy-3′-methyl-2′-pentenyl)-1,4-naphthoquinone; (II) 2-methyl-3-(3′-carboxy-3′-methylpropyl)-1,4-naphthoquinone; (III) 2-methyl-3-(5′-carboxy-3′-hydroxy-3′-methylpentyl)-1,4-naphthoquinone lactone.

Vitamin K Epoxide Cycle

In all tissues and cells that synthesize Gla proteins, vitamin K-dependent carboxylation is intimately linked to a metabolic sequence known as the vitamin K epoxide cycle. This cycle and the associated enzyme activities are shown in Figure 3. During γ-glutamyl carboxylation, vitamin KH_2 is transformed to

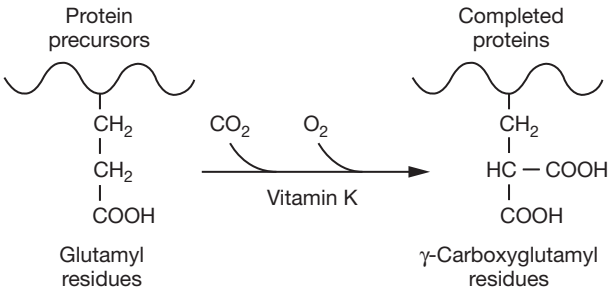

Figure 2 Posttranslational vitamin K-dependent carboxylation reaction showing the conversion of protein-bound glutamyl residues (Glu) to γ-carboxyglutamate (Gla) residues.

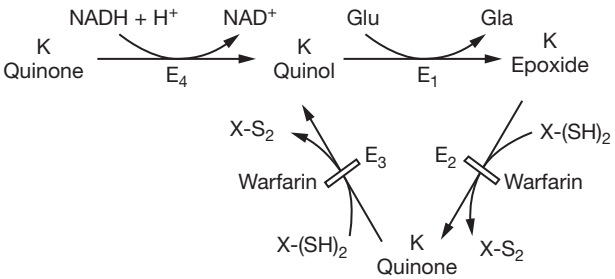

Figure 3 Scheme showing the reactions and enzyme activities (denoted by E_1, E_2, E_3, and E_4) of the vitamin K epoxide cycle. The active form of vitamin K needed for the conversion of glutamate residues (Glu) to γ-carboxyglutamate (Gla) residues is the reduced form, vitamin K quinol (K quinol). The carboxylation reaction is driven by a vitamin K-dependent carboxylase activity (E_1), which simultaneously converts K quinol to vitamin K 2,3-epoxide (K epoxide). K epoxide is reduced back to the quinone (K quinone) by vitamin K epoxide reductase (E_2). The cycle is completed by the reduction of recycled K quinone by a vitamin K reductase activity (E_3). Both E_2 and E_3 reductase activities are dithiol-dependent (X-(SH_2) and X-S_2 denote reduced and oxidized dithiols) and are inhibited by coumarin anticoagulants such as warfarin. Dietary vitamin K (K quinone) may enter the cycle via an NAD(P)H-dependent vitamin K reductase activity (E_4), which is not inhibited by warfarin.

vitamin K epoxide (enzyme activity E_1). The epoxide product is recycled in two steps: first by a vitamin K epoxide reductase activity (E_2) to produce vitamin K quinone and second by a quinone reductase activity (E_3) to produce the coenzyme vitamin KH_2. Both these activities are thiol-dependent and are probably effected by the same enzyme.

An important property of the dithiol-dependent epoxide and quinone reductase activities is their sensitivity to certain antagonists, especially those based on 4-hydroxycoumarin (e.g., warfarin) or indandione structures. Drugs based on these structures have long been used as oral anticoagulants, and it is now clear that their anticoagulant action is based on their ability to inhibit the epoxide reductase activity (E_2) and block the recycling of the vitamin. The dithiol-dependent quinone reductase (E_3) is also sensitive to warfarin, but there is a second quinone reductase activity catalyzed by an NAD(P)H-dependent enzyme activity (E_4) that is less sensitive to warfarin inhibition, and this provides an alternative pathway for the reduction of vitamin K to KH_2 in the presence of warfarin or other oral anticoagulant drugs. Therapeutic anticoagulation is dependent on a balance being achieved between the inhibition of the recycling enzymes and the amount of dietary vitamin K that can enter the cycle to support carboxylation at a reduced efficiency.

Vitamin K-Dependent (Gla) Proteins

Distribution and Structure

γ-Glutamyl carboxylase activity is present in a wide variety of extrahepatic tissues such as bone, kidney, placenta, pancreas, skin, spleen, lung, testis, and vessel wall tissues. New Gla proteins continue to be discovered; the present list together with their tissue distribution is shown in Table 1. Some of these Gla proteins are found in only one major tissue (e.g., procoagulant factors in liver and osteocalcin in bone), while others (e.g., MGP and protein S) are more widely distributed.

All Gla-containing proteins are secretory proteins and are characterized by an intracellular precursor form possessing a leader sequence that facilitates specific posttranslational events and is then normally cleaved before secretion. Within the leader sequence, a hydrophobic presequence facilitates the translocation of the precursor Gla proteins across the endoplasmic reticulum, while a second prosequence serves as a recognition signal for the vitamin K-dependent carboxylase. With the exception of matrix Gla protein, the prosequence of currently characterized Gla proteins is located immediately before the N-terminal of the mature protein. In matrix Gla protein, the carboxylation recognition site is internal and is retained in the mature protein. The number of Gla residues varies: the four classical vitamin K-dependent clotting factors contain 10–12 Gla residues, whereas the much smaller MGP and osteocalcin contain five and three Gla residues, respectively.

Role of Gla Proteins

Hemostasis (procoagulants and anticoagulants)

The vitamin K-dependent coagulation proteins comprise four plasma procoagulants (factors II, VII, IX, and X) and two feedback anticoagulants (proteins C and S). The physiological function of a seventh plasma protein (protein Z) is less established but seems to play a role in dampening coagulation by acting as a cofactor for the inhibition of factor Xa. The four vitamin K-dependent procoagulants have long been known to play a central role in the blood-clotting cascade leading to the formation of a fibrin clot. Circulating as inactive forms (or zymogens) of serine proteases, their biological activity is dependent on their ability to bind to anionic phospholipid surfaces where cleavage of the zymogens yields the active protease clotting factors. The Gla residues of these clotting factors provide an efficient chelating site for calcium ions that enables ion bridges to be made between the factor and the surface phospholipids of platelets and endothelial cells where, together with other cofactors, they form membrane-bound enzyme complexes. The intricate control of coagulation is illustrated by the fact that another Gla protein (protein C), once activated, performs an anticoagulant role by specifically degrading phospholipid-bound activated factors V and VIII in the presence of calcium. This anticoagulant activity of activated protein C is dependent on yet another Gla protein (protein S) that acts as a synergistic cofactor by enhancing the binding of activated protein C to negatively charged phospholipids.

Bone metabolism

The discovery in 1975 that bone tissue contains a Gla protein, which is also one of the most abundant proteins in the body, has revolutionized earlier thinking that vitamin K is only needed for coagulation. This protein, called osteocalcin or bone Gla protein, accounts for about 15% of the

Table 1 Distribution and roles of vitamin K-dependent (Gla) proteins

Gla protein	*Tissue*	*Role*
Prothrombin (factor II), factors VII, IX, and X	Liver (then plasma)	Blood clotting (procoagulants)
Protein C	Liver (then plasma)	Blood clotting (anticoagulant)
Protein S	Liver (then plasma), endothelium, bone	Anticoagulant role (cofactor for protein C); role in bone unknown
Osteocalcin (or bone Gla protein)	Bone	Implicated in bone remodeling
Matrix Gla protein	Most tissues	Inhibits calcification of arteries and cartilage
Gas 6	Most tissues	Implicated in cell cycle regulation, cell survival, cell proliferation, cell adhesion, platelet signaling
Proline-rich Gla proteins	Most tissues	Unknown, but protein structures suggest roles as cell-surface receptors

noncollagenous proteins in the bone of most vertebrate species, although this proportion is less in humans. The three Gla residues of osteocalcin project away from the Gla helix, and their periodic spacing complements the interatomic spacing of the calcium ions of the hydroxyapatite lattice; this is thought to account for the strong mineral-binding characteristics of the fully carboxylated form of osteocalcin. The adsorption affinity of osteocalcin for hydroxyapatite and other features such as its high degree of conservation throughout evolution, its regulation by vitamin D, and its chemoattractant properties suggest an important role for osteocalcin in bone metabolism, although the nature of this role still remains unknown. Osteocalcin is specifically synthesized by osteoblasts, and since its concentration in blood reflects osteoblastic activity, measurements of total osteocalcin have become clinically important in the diagnosis of bone disease.

Besides osteocalcin, bone tissue contains lower concentrations of other Gla proteins: MGP, protein S, and Gas6 (Table 1). MGP is a 10 kDa secretory protein, with five residues of Gla. It was first isolated from demineralized extracts of bone by Price in 1983 but is expressed by a wide variety of tissues and cell types. Its function in bone is unclear but is probably related to its capacity to inhibit localized calcification as recently shown for arteries. Transgenic MGP-deficient mice show inappropriate calcification of the cartilage, including the growth plate, and a phenotype characterized by short stature, osteopenia, and fractures. This function is consistent with the effects of the vitamin K antagonist warfarin, which, when given *in utero* or to young animals, results in excessive mineralization and premature closure of the epiphyseal growth plate. An important role for Gla proteins during human fetal bone development is suggested by the bone defects often seen in infants born to mothers who had received vitamin K antagonists such as warfarin during the first trimester of pregnancy. Evidence that warfarin embryopathy is mediated by a dysfunctional MGP comes from the demonstration that mutations encoding MGP are responsible for a rare inherited disorder called Keutal syndrome, which is characterized by a similar abnormal calcification of cartilage. The functions of protein S and Gas6 in bone are presently unknown.

Vascular calcification

A series of remarkable studies since 1997 have firmly established MGP as a potent inhibitor of calcification of arteries and cartilage. Thus, targeted deletion of the *MGP* gene in mice resulted in rapid focal calcification of the elastic lamellae of the arterial media but not of the arterioles, capillaries, or veins. This process begins at birth and by 3–6 weeks is sufficiently severe to cause the arteries to become rigid tubes that fracture, causing death from bleeding. Most of the phenotypic features of arterial calcification seen in MGP-deficient mice can be mimicked, albeit to a somewhat muted degree, by the vitamin K antagonist warfarin. This effect of warfarin points to the need for Gla residues, and hence vitamin K, to the function of MGP. Overall, the dramatic features of both genetic and biochemical models of MGP depletion strongly suggest that MGP is central to the process by which calcification of arteries is normally inhibited *in vivo* and that there is no effective alternative inhibitor at aortal sites.

Assessment of Vitamin K Status

The traditional criterion for vitamin K sufficiency is the maintenance of normal plasma concentrations of fully γ-carboxylated vitamin K-dependent procoagulants II, VII, IX, and X. The presence of a clinically significant vitamin K-deficient state is usually investigated by global coagulation assays such as the one-stage prothrombin time. Such assays are insensitive to small changes in γ-carboxylation of the vitamin K-dependent coagulants and, while appropriate for detecting overt vitamin K deficiency, are not useful for picking up suboptimal states of vitamin K deficiency.

Modern assessments of vitamin K status need to take into account both coagulation and noncoagulation roles of Gla proteins and their different sites of synthesis. When vitamin K is in short supply, or its action is blocked by an oral anticoagulant such as warfarin, undercarboxylated (des-γ-carboxy) species of Gla proteins are produced at the tissue site of synthesis and, in some species, including humans, are secreted into the circulation. Collectively, these undercarboxylated species are known as proteins induced by vitamin K absence or antagonism (PIVKA). In the case of the coagulation protein prothrombin, specific and sensitive immunoassays for undercarboxylated prothrombin (PIVKA-II) provide a powerful marker of the coagulation status of vitamin K because they can detect small decreases in the Gla content of prothrombin well before any changes occur in conventional coagulation tests. Similarly, the assay of circulating undercarboxylated species of osteocalcin provides a specific functional marker to assess the sufficiency of vitamin K for the carboxylation of bone Gla proteins. Since prothrombin and osteocalcin are synthesized in the liver and bone, respectively, the detection of their respective undercarboxylated species allows the vitamin K status of these tissues to be assessed independently. Such studies have shown that the carboxylation of osteocalcin in bone is more susceptible to reduced dietary intakes of vitamin K than the carboxylation of the classical coagulation proteins. Measurements of undercarboxylated osteocalcin are being increasingly used to assess the importance of optimal vitamin K intakes with respect to bone health. The excretion of free Gla in urine represents the final catabolic product of all Gla proteins, and its measurement has also been shown to reflect dietary intakes and status. Another method for assessing vitamin K status is from direct serum assays of phylloquinone, the predominant transport form of vitamin K. A low serum phylloquinone is a good indicator of a poor overall vitamin K status.

Vitamin K and Health

Role in Hemostasis

Overt vitamin K deficiency in adults, resulting in clinical bleeding, is almost unknown except as a consequence of underlying disease, most commonly resulting from hepatointestinal disorders that disturb the absorption or utilization of the vitamin. However, oral coumarin anticoagulant drugs, such as warfarin, are widely used in the prevention and management of thromboembolic disease. These vitamin K antagonists are used to induce an artificial but controlled state of vitamin K deficiency,

the aim being to reduce the circulating levels of the vitamin K-dependent clotting factors to within a predetermined target range depending on the clinical condition.

In contrast to adults, bleeding due to vitamin K deficiency may occur spontaneously in the first few months of life. The resulting bleeding syndrome is traditionally known as hemorrhagic disease of the newborn, in recognition of the Boston physician Townsend, who is credited with the first description of the syndrome in 1894. The modern name is vitamin K deficiency bleeding (VKDB) of early infancy. Until the late 1960s, VKDB was considered to be solely a problem of the first week of life (classical VKDB), but two other forms (early and late VKDB) have now been recognized (Table 2). Late VKDB, peaking between the third and sixth weeks of life, is the most common syndrome and is still a significant worldwide cause of infant morbidity and mortality. In different parts of the world, some 2–100 cases per 100 000 births of late VKDB have been reported, with a particularly high incidence in the Far East. Unlike the classical form, late VKDB has a high incidence of intracranial hemorrhage, resulting in death or severe and permanent brain damage in around 50% of cases. One major risk factor, exclusive breast-feeding, is common to both classical and late syndromes and is probably related to the relatively low concentrations of vitamin K in breast milk (1–2 $\mu g\,l^{-1}$) compared to about 50 $\mu g\,l^{-1}$ in formula feeds. Another probable etiologic factor is the precarious hepatic stores of vitamin K in newborns because of poor placental transfer of phylloquinone and the lack of neonatal stores of bacterial MKs, which only build up slowly after bacterial colonization of the intestine. Some infants who develop late VKDB have abnormalities of liver function, leading to cholestasis, and an impaired absorption efficiency of vitamin K.

By the early 1980s, reports in the literature were suggesting a worldwide rise in the incidence of late VKDB. This resurgence may have been related to both a decline in routine vitamin K prophylaxis and the promotion of exclusive breast-feeding. In recent years, many countries have reemphasized the need to give all babies extra vitamin K as a public health measure. Often, this was given as a single intramuscular dose at birth, but since 1992, concerns about an epidemiological association between the intramuscular route and later childhood cancer have seen a shift in some countries towards oral prophylaxis. However, there is evidence that even multidose oral regimens fail to give full protection against late VKDB and that this is because there is an underlying reservoir of infants who have a reduced efficiency of absorption due to underlying and unrecognized cholestasis. Several epidemiological studies conducted since 1992 have not substantiated the link between vitamin K prophylaxis and cancer.

Role in Bone Health

Despite mechanistic gaps in our knowledge, a picture is emerging that a suboptimal vitamin K status may play a role in the pathogenesis of osteoporosis. Much of this evidence is still circumstantial, being based on associations of fracture risk and bone mineral density with indices of poor vitamin K status and low dietary intakes. Of particular note is that undercarboxylated osteocalcin is an independent risk factor of both hip fracture risk and bone mineral density. However, osteoporosis is a complex disease, and without a fuller understanding of the biological function of osteocalcin, the interpretation of these associations with vitamin K status remains uncertain.

In the near future, the results from a number of long-term intervention studies on the effects of vitamin K supplementation on bone health will become available. These may help to answer the question of whether extra intakes of vitamin K can influence bone mineral density and bone turnover. Most of these studies have used phylloquinone because this is the major dietary form, but in Japan, the effects of very large doses of MK-4 (menatetrenone) on bone health are being studied. Both phylloquinone and MK-4 are known to promote the γ-carboxylation of osteocalcin, but these forms differ in other important respects. Thus, there is evidence that tissues can convert phylloquinone into MK-4 *in vivo* (but not vice versa) and that MK-4 may have a direct inhibitory effect on bone resorption. In an intervention study in which Japanese women with osteoporosis were treated with a high-dose MK-4 regimen, the incidence of fractures was significantly reduced, and the rate of bone loss was decreased.

Dietary Intakes and Requirements

The major dietary source of vitamin K is phylloquinone. In Western societies, the majority of phylloquinone intakes (up to 50%) come from green leafy vegetables followed by certain vegetable oils such as soybean, canola (rapeseed), and olive oils. Mixed dishes and meals contribute about 15% of total phylloquinone intake, largely because of their oil content. The wide range of the vitamin K content of different food items means that individual daily intakes may vary widely but average adult intakes mostly range from 60 to 120 μg per day. Intakes of this order are more than sufficient to maintain

Table 2 Classification of vitamin K deficiency in the newborn (VKDB)

VKDB syndrome	*Time of presentation*	*Common bleeding sites*	*Etiologic factors*
Early	0–24 h	Cephalohematoma, intracranial, intrathoracic, intraabdominal	Maternal drugs (e.g., warfarin and anticonvulsants)
Classical	1–7 days	Gastrointestinal, skin, nasal, circumcision	Mainly idiopathic, breast-feeding (may be related to low milk intakes)
Late	2–26 weeks	Intracranial, skin, gastrointestinal	Mainly idiopathic, breast-feeding, some degree of cholestasis often present; may reflect underlying disease (e.g., biliary atresia, α-1-antitrypsin deficiency, cystic fibrosis)

normal hemostasis, and even intakes as low as 10 μg per day for a few weeks have no effect on global coagulation assays. However, more sensitive measures of vitamin K status (e.g., undercarboxylation of Gla proteins, urinary Gla excretion, and serum phylloquinone) respond readily to moderate changes in dietary intakes. One conclusion from nutritional depletion/repletion studies is that the daily intakes of vitamin K needed to sustain the posttranslational γ-carboxylation of extrahepatic Gla proteins such as osteocalcin are higher than those needed for the coagulation Gla proteins synthesized in the liver. Therefore, previous recommendations of a daily intake of 1 μg kg^{-1} of body weight, which have been based on the coagulation role of vitamin K, may need to be revised upward if evidence continues to harden on other potential health benefits such as the amelioration of osteoporosis and arterial calcification. Studies in the United Kingdom and the United States already suggest that a substantial proportion of their populations have intakes below 1 μg kg^{-1} of body weight. What this optimal intake should be must await further research on the physiological function of Gla proteins, the further delineation of functional markers of vitamin K status, and studies of the health consequences of different intakes.

See also: Calcium: Physiology; Dietary Surveys: National Food Intake; Infants: Nutritional Requirements; Lipoproteins; Osteoporosis; Vitamins: Overview.

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Vitamin K: Properties and Determination

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Background

Vitamin K is a fat-soluble vitamin widely distributed in nature and comprises several molecular forms. It was discovered as an essential antihemorrhagic factor in the 1930s by the Danish scientist Henrik Dam, who named it *koagulations vitamin*. This article reviews the properties and analysis of vitamin K, with special reference to its measurement in foods. Such measurements have proved difficult, and reliable methods have only become available since the 1980s with the development of physicochemical assays based on high-performance liquid chromatography (HPLC). According to recent evidence, in addition to its well-defined role in blood coagulation, vitamin K is needed for a variety of physiological processes related to a number of extrahepatic vitamin K-dependent proteins.

Chemical Structures and Nomenclature

Naturally occurring compounds with vitamin K activity possess a common 2-methyl-1,4-naphthoquinone nucleus but differ in the structures of the side chain at the 3 position (Figure 1).

In traditional nomenclature (Table 1), the vitamin K found in plants (of which there is only one major chemical form) is known as vitamin K_1 and the multiple forms synthesized by bacteria are known as vitamin K_2, where the number of carbon atoms (n) in the side chain is indicated as vitamin $K_{2(n)}$. This nomenclature was revised by the International Union of Pure and Applied Chemistry–International Union of Biochemistry (IUPAC–IUB) Subcommittee on Nomenclature of Quinones, who recommended that vitamins K_1 and K_2 be called phylloquinone and menaquinones, respectively. Recommended current abbreviations are K_1 for phylloquinone and MK-n for menaquinones. Table 1 also shows a nomenclature that the International Union of Nutritional Sciences recommended should replace the IUPAC system. This new system, however, has not found favor among most workers in this field. Consequently, the IUPAC–IUB nomenclature is still the one most generally accepted at the present time and will be used throughout this article.

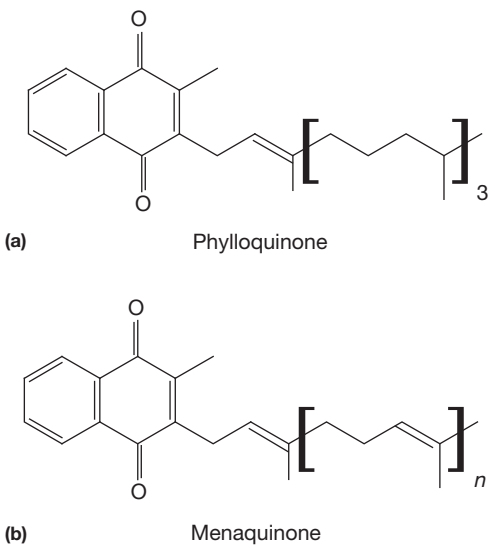

Figure 1 Chemical structures of naturally occurring K vitamins: (a) phylloquinone (vitamin K_1) and (b) menaquinones (vitamin K_2).

The parent compound of the vitamin K group of vitamers is 2-methyl-1,4-naphthoquinone. This structure is not found in nature but, because of its historical use as a synthetic derivative, was originally designated vitamin K_3. Under the IUPAC system, this artificial vitamin K is called menadione.

Phylloquinone, which has the same phytyl side chain as chlorophyll, is found throughout the plant kingdom and in cyanobacteria (blue-green algae). In higher plants, the synthesis of phylloquinone is associated with the chloroplast, while the unusual finding of phylloquinone in cyanobacteria has been cited as supportive evidence for the possible bacterial origin of the chloroplast in evolution.

The menaquinones represent a highly diverse family of compounds all synthesized by bacteria. The side chain of menaquinones is based on a number of repeating unsaturated five-carbon (prenyl) units. The major forms are designated menaquinone-n (MK-n), with n denoting the number of prenyl units. Some bacteria also synthesize menaquinones in which one or more of the multiprenyl side-chain double bonds are saturated. In such cases, the additional hydrogen atoms are indicated by the prefixes dihydro, tetrahydro, hexahydro, etc., and these may be abbreviated to MK-$n(H_2)$, MK-$n(H_4)$, MK-$n(H_6)$, etc. The division between the plant-form phylloquinone and the bacterial menaquinones is somewhat artificial, since phylloquinone may be regarded as a partially saturated form of menaquinone-4, namely, MK-4(H_6), but the distinction serves to emphasize their different origins.

Carbon atoms in the side chain are designated C1′, etc., and the presence of double bonds at C2′ in phylloquinone and at C2′, 6′, 10′, etc., in menaquinones indicates that *cis–trans* isomerism is possible. Natural forms of phylloquinone and menaquinones exist as *trans* isomers, but synthetic forms contain both *trans* and *cis* isomers.

Biological Activities

The biological activity of different vitamin K compounds has been assessed in whole animal systems (*in vivo*) or as a cofactor to the

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Table 1 Nomenclature of K vitamins

Chemical name	*Old name*	*IUPAC–IUB (abbreviation)*	*IUNS (abbreviation)*
2-Methyl-1,4-naphthoquinone	Vitamin K_3	Menadione	Menaquinone
2-Methyl-3-phytyl-1,4-naphthoquinone	Vitamin K_1	Phylloquinone (K)	Phytylmenaquinone (PMQ)
2-Methyl-3-multiprenyl-1,4-naphthoquinone (class)	Vitamin K_2	Menaquinone-*n* (MK-*n*)	Prenylmenaquinone-*n* (MQ-*n*)
2-Methyl-3-farnesyl-farnesyl-1,4-naphthoquinone	Vitamin $K_{2(30)}$	Menaquinone-6 (MK-6)	Prenylmenaquinone-6 (MQ-7)

vitamin K-dependent γ-glutamyl carboxylase in isolated enzyme systems (*in vitro*). γ-Glutamyl carboxylase is an integral membrane glycoprotein that requires vitamin K to activate posttranslational modification of glutamate (Glu) to γ-carboxyl glutamate (Gla) in specific vitamin K-dependent proteins. The *in vivo* systems are normally based on the correction of blood clotting time in chicks induced by vitamin K deficiency or through measurement of undercarboxylated vitamin K-dependent proteins, and their interpretation needs to take account of the route of administration and other metabolic considerations, such as absorption and interconversion to other chemical forms.

It has been established that besides the 1,4-naphthoquinone nucleus, a methyl group at the C2 position is essential for both *in vivo* and *in vitro* activities. The activity of menadione *in vivo* is due to the presence of tissue alkylating enzymes that can transform this compound to MK-4. Recent evidence is accumulating that suggests an alternative *in vivo* route to MK-4 via tissue-mediated conversion of phylloquinone. The essential requirement for *in vitro* activity is for a 2-methyl-3-phytyl (or polyisoprenyl)-1,4-naphthoquinone structure, which can be reduced to the corresponding hydroquinone. The biological activity of K_1 is reduced by saturation of the phytyl double bond (2′,3′-dihydrophylloquinone), whereas the desmethyl or *cis* isomers have no appreciable activity *in vitro*, although some conversion of the *cis* form to the active *trans* form may occur *in vivo*.

Physicochemical Properties

Appearance, Solubility, and Stability

Phylloquinone and menaquinones are golden yellow oils at ambient temperature, though the higher menaquinones may also be obtained as fine, yellow crystals.

All K vitamins are insoluble in water but are soluble in normal lipid solvents such as hexane, chloroform, diethyl ether, and acetone, but are less soluble in ethanol. They are only sparingly soluble in methanol, and the degree of solubility depends on their hydrophobicity (i.e., menaquinones with long side chains are less soluble than those with short side chains).

Vitamin K compounds are reasonably stable towards heat, oxygen, and mild acidic conditions but are degraded by UV light, alkalis, and strong acids.

Spectroscopy

The spectroscopic properties of K vitamins are important for their detection, identification, and quantification in assays. All vitamin K compounds possess the same characteristic ultraviolet spectrum, which is a summation of the benzenoid and 1,4-quinone components. Naturally occurring phylloquinone and menaquinones exhibit typical benzenoid bands with maxima at 242, 248, and 238 nm (shoulder), whereas the quinone contributions appear as distinct bands with absorption maxima at 260 and 269 nm and a higher-wavelength maximum at 326 nm. Ultraviolet spectroscopy is particularly useful for the quantification of standard solutions; the molar absorption coefficient (ϵ) is the same for both phylloquinone and menaquinones and, at 248 nm (the wavelength of highest absorption), has a value of 18 900.

Vitamin K compounds, in their stable quinone forms, do not possess native fluorescence, but they can be readily reduced to the corresponding quinols, whose fluorescent properties have been exploited for the assay of K vitamins in foods and other biological tissues.

The K vitamers also exhibit infrared and nuclear magnetic resonance absorption spectra distinctive of the naphthoquinone nucleus and may be further characterized by mass spectroscopy.

Occurrence and Forms in Foods

Vitamin K is largely found in the membranous fractions of cells. Thus, in plants, phylloquinone is concentrated in the chloroplast lamellae, and in bacteria, menaquinones are located in the plasma membrane. In animals, the vitamin is found in various cellular membranes, especially those of the microsomes and mitochondria.

Recent progress in analytic techniques for this vitamin, as well as increased emphasis on its nutritional significance, is responsible for the current development of a comprehensive database of the vitamin K content of foods. Whereas, before 1980, most published values for the vitamin K content of foods originated from bioassays, contemporary data derive from the highly specific and accurate HPLC methods. These techniques have focussed predominantly on phylloquinone ubiquitously distributed in foods, although more recently, increasing amounts of data are becoming available for the menaquinones.

Table 2 lists some values of the phylloquinone content of various foods arranged into four concentration ranges to illustrate the wide distribution of the vitamin in the diet.

In general, the relative values confirm the known association of phylloquinone with photosynthetic tissues, with the highest values (400–600 μg per 100 g) being found in green, leafy vegetables. Nutritionally significant amounts of phylloquinone are, however, present in many nonleafy vegetables, as well as in fruits, oils, dairy products, eggs, and some meat products. With modern HPLC techniques, naturally occurring phylloquinone can be detected in most foods. Vegetable oils show a high degree of variability, ranging from <5 μg per 100 g in coconut, corn, groundnut, and safflower oils to 100–200 μg per 100 g in soybean and rapeseed oils. This leads to a similar

Table 2 Vitamin K_1 content of common foods determined by HPLC (μg per 100 g)[a]

0.1–1.0	*1–10*	*10–100*	*100–1000*
Bananas	Apples	Avocado	Broccoli tops
Beef, steak	Aubergines	Beans, runner	Brussels sprouts
Bread, white	Baked beans	Beans, French	Cabbage, green
Chicken, thigh	Barley	Beans, broad	Kale
Coconut oil	Beef, minced (1–30)	Cabbage, red	Lettuce
Cod, fillet (0.1–2)	Bilberries	Cauliflower	Margarine
Cornflakes	Bran, wheat	Chick peas	Parsley
Flour, white	Bread, wholemeal	Cucumber	Rapeseed oil
Grapefruit	Butter	Greengages	Soybean oil
Ham, tinned	Carrots	Kiwi fruit	Spinach
Ice cream	Celery	Margarine	Water cress
Maize	Cheeses (1–80)	Mustard cress	
Mangoes	Chocolate, plain	Natto (900–1200)	
Melon, yellow	Corn oil	Olive oil, ex. virgin	
Melon, water	Courgettes	Peas	
Milk, cows	Cranberries		
Mushrooms	Cream, double		
Onion	Dates, fresh		
Oranges	Eggs (10–25)		
Parsnips	Figs, fresh		
Peanuts, roast	Grapes, black		
Pilchards, brine (0.1–2)	Grapes, green		
Pineapple	Infant formula		
Pork chop, lean	Leeks		
Potatoes	Liver, lamb		
Rice white	Liver, ox (10–20)		
Rice brown	Nectarines		
Salmon, brine	Oats		
Sausages, pork/beef	Palm oil		
Spaghetti	Peaches, fresh		
Trout, rainbow (0.1–3)	Pears		
Tuna, brine	Peppers, green		
Turnips	Peppers, red		
Yogurt (0.1–0.4)	Plums, red		
	Raisins		
	Rhubarb		

[a]MK-*n* ranges are shown in parentheses.

variation in the phylloquinone content of margarines according to the oil base. The phylloquinone content of lean meat and fish is generally low (<1 μg per 100 g), but higher amounts are found in animal livers; this organ acts as a storage site of the vitamin. Please refer to individual foods.

There is less information on the menaquinone content of foods. It is known that animal liver contains substantial concentrations of menaquinones, which normally greatly exceed the phylloquinone content. The livers of ruminants contain particularly large concentrations of long-chain menaquinones (MK-6–13), which derive from bacterial fermentation in the rumen. The only other known sources of significant amounts of menaquinones in the Western diet are fermented foods, such as cheese and yogurt. All cheeses contain relatively high concentrations (≤20 μg per 100 g) of MK-8 and MK-9, with the latter predominating. Natto, the Japanese fermented soybean food, contains substantial amounts of MK-7. Whereas menaquinones are detectable in cow's milk at much lower concentrations than phylloquinone, MK-4 has been confirmed to be present at comparable, or higher, levels than phylloquinone in the milk of several mammalian species.

Foods containing hydrogenated vitamin K-rich oils will also include a contribution from 2′,3′-dihydrophylloquinone. The biological activity of this K vitamer is currently under investigation, and hence, its abundance in the diet is of uncertain nutritional significance.

The processing of foods can have a variable effect on retention of vitamin K, with conventional cooking, canning, γ-irradiation, and freezing practices having a relatively minor influence, whereas significant and rapid loss occurs on exposure to UV light.

Use in Food Fortification

Since the requirements for vitamin K are low, fortification of foods with vitamin K is not common, with the exception of infant formula feeds to which synthetic preparations of

phylloquinone are invariably added. Depending on the method of chemical synthesis, the ratio of the natural *trans* to the inactive *cis* form will vary but is typically *c.*7:1.

Although national guidelines for the fortification of infant formulas differ, their common aim is to protect against rare, but life-threatening, bleeding due to deficiency of vitamin K in early life. It is now known that the typical fortification levels of *c.* 50 μg l^{-1} (as fed) are several times higher than human breast milk concentrations of phylloquinone, which average *c.* 2 μg l^{-1}. Although the reasons for adopting such high levels of fortification are somewhat empirical and based on the erroneously high estimates of vitamin K in breast milk by early bioassays, there has been little impetus for change because the low concentrations in breast milk are an established risk factor for neonatal vitamin K deficiency.

The synthetic vitamin K derivative menadione is no longer used to fortify infant formulae because of its association with neonatal hemolytic anemia and liver damage. In fact, the vitamin K activity of menadione is dependent on its *in vivo* conversion to biologically active MK-4. Nevertheless, because menadione is much cheaper to manufacture than phylloquinone, menadione derivatives are widely used in animal husbandry, particularly in poultry feeds, since poultry are very prone to bleeding due to dietary deficiency of vitamin K. In the poultry industry, the birds' dietary requirements may be further increased by the use of antibiotics, the effect of which may be either to inhibit the growth of intestinal microorganisms that synthesize menaquinones or to interfere directly with vitamin K metabolism. Consequently, stabilized water-soluble derivatives of menadione, such as crystalline menadione sodium bisulfite complex, are used universally as poultry feed supplements.

Measurement of Vitamin K in Food

In the 1930s, shortly after the discovery of vitamin K, Dam and colleagues developed a curative bioassay that involved the feeding of substances to vitamin K-deficient chicks and measuring the response on blood clotting. Although values that have been derived from this early work are often given in textbooks and handbooks of nutrition, a closer evaluation of the methodology shows that attempts to use these results as the basis for absolute food values are unjustified. Although such bioassays were greatly improved in the 1960s, little further work has emerged on their routine use and validation for food analysis.

The introduction of modern HPLC techniques has revolutionized the measurement of vitamin K in tissues. This powerful technique has met the criteria of high selectivity and sensitivity, which are also necessary for the measurement of the low concentrations in most foods. Although several diverse protocols have been developed for phylloquinone, only one procedure has been validated by collaborative study. A range of sample extract purification strategies may be implemented prior to the final chromatographic analysis, which is usually accomplished by analytic reversed-phase HPLC. Due to the instability of vitamin K at high pH, saponification techniques commonly utilized for other fat-soluble vitamins are inappropriate for vitamin K analysis. The need for preliminary enhancement techniques is dependent principally on food type, analyte level, and final detection mode and may incorporate one or more techniques including (1) lipid extraction, (2) enzymatic lipid digestion, (3) solid-phase extraction (SPE), and (4) semipreparative normal-phase HPLC.

Extraction and Cleanup

No universal extraction and purification procedure exists for vitamin K analysis in all foods. Nonpolar lipid solvents including hexane, isopropanol, dichloromethane, acetone, diethyl ether, and methanol, used either singly or in combination, have been used for initial total lipid extraction depending on the food type. For certain high-fat foods (fats, oils, and milk), bulk lipid may be effectively removed with enzymatic digestion prior to hexane extraction of triglyceride-depleted lipid. For low-fat foods (vegetables, fruit, cereals, meats, and fish), acetone is an effective solvent, whereby, after the addition of water and hexane, the K vitamins partition entirely in the upper hexane phase, leaving polar lipids in the acetone–water phase. Alternatively, hexane/isopropanol or chloroform/methanol binary solvents are commonly used.

For protocols relying on a preliminary total lipid extraction, further purification is required in view of the overwhelming proportion of triglycerides and their incompatibility with reversed-phase HPLC. Various combinations of lipase digestion, SPE, and semipreparative normal-phase HPLC techniques are commonly recommended.

The extraction stage may also incorporate the addition of an internal standard, which, in general terms, compensates for procedural losses of the target analyte. This technique is essential when multidimensional protocols for vitamin K are implemented. The internal standard is chosen on the basis of several criteria that include its similar physical, chemical, and chromatographic properties to the analyte. Depending on the sample matrix, several vitamin K compounds and analogs (including radiolabeled compounds) have been found to be suitable internal standards for vitamin K assays.

A general scheme outlining commonly applied extraction and cleanup options is illustrated in Figure 2. Purification techniques usually comprise two successive stages based on the principles of adsorption chromatography, which facilitate the separation of K vitamers from most other potentially interfering lipid classes (e.g., hydrocarbons, sterols, and fatty acids). If required, semipreparative normal-phase HPLC can also be used to isolate both K_1 and MK fractions and to separate the *cis* and *trans* isomers of phylloquinone.

The fraction(s) collected at the semipreparative HPLC stage may then be analyzed by reversed-phase HPLC using a variety of detection methods. For certain foods, a lipase digestion and hexane extraction scheme has been found to be sufficient to facilitate a direct analytic HPLC quantitation, without the need for additional purification steps.

Detection and Quantification

The final analytic stage of HPLC is almost exclusively based on the principles of partition chromatography in the reversed-phase mode. This robust mode of liquid chromatography resolves the individual K vitamins from each other while supporting the mobile-phase additives necessary for the most

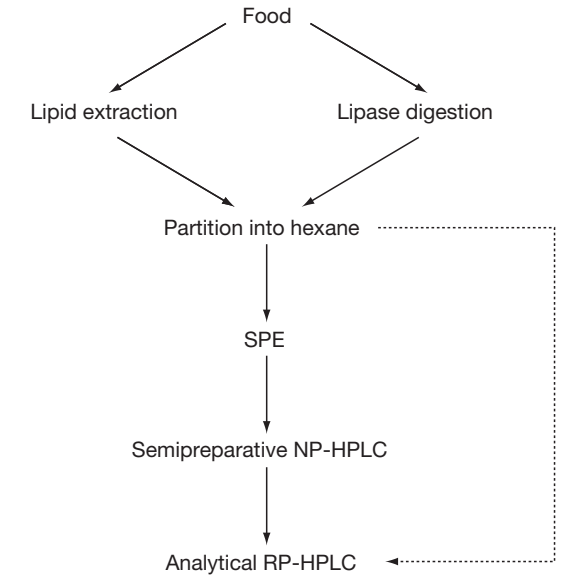

Figure 2 Flowchart summarizing extraction and purification of vitamin K from foods.

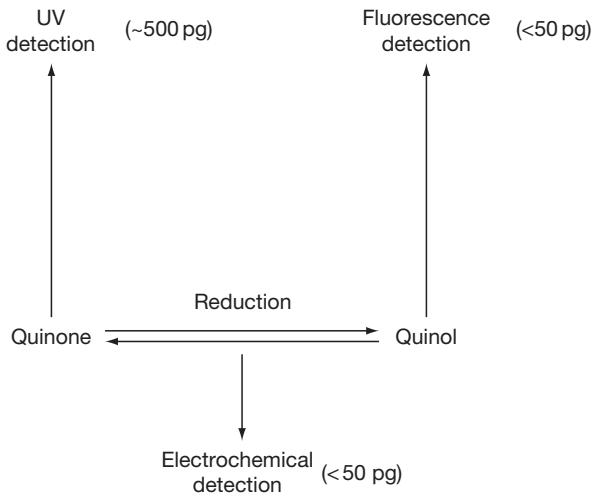

Figure 3 Detection options for vitamin K.

sensitive detection techniques currently utilized. Whereas isocratic systems are generally used for the analysis of vitamin K_1, gradient elution is preferable when profiling the menaquinones, in view of their very wide hydrophobic range. The C18 functionality has most commonly been employed, although the recently developed C30 phase has been shown to allow resolution of the *cis* and *trans* isomers of phylloquinone, previously only accomplished under normal-phase conditions.

The properties of K vitamins offer several avenues of approach to their detection by HPLC, and the most common options are summarized in Figure 3. The parent quinone form, in which the vitamin is isolated, may be detected with reasonable sensitivity (*c.* 500 pg) by ultraviolet absorbance detection, but this lacks selectivity and may be subject to interferences from other lipids. This detection method does, however, give reliable results for green vegetables but is less satisfactory for animal tissues.

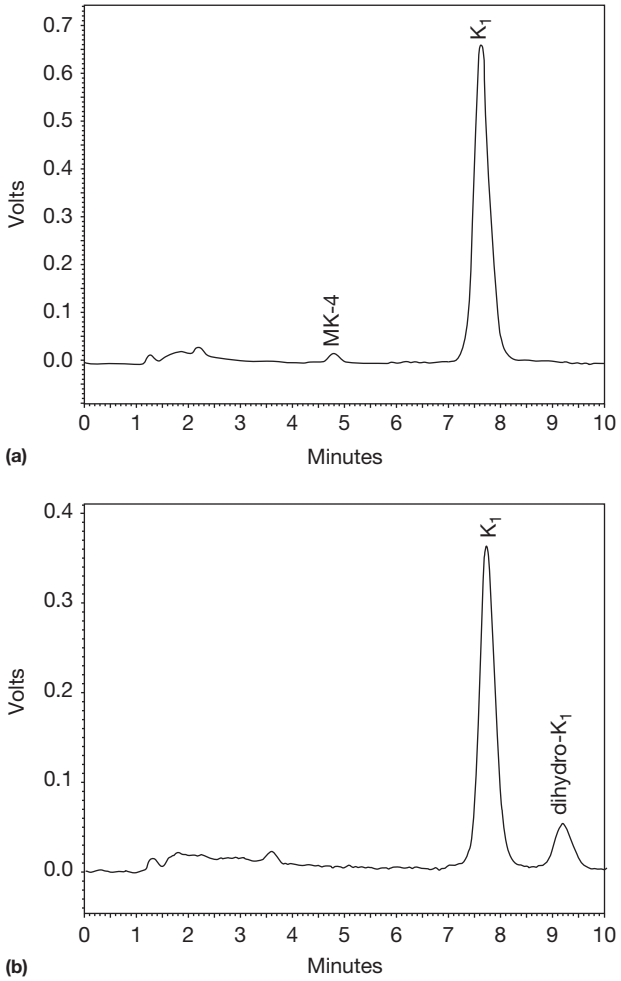

Figure 4 Liquid chromatography obtained for (a) infant formula and (b) margarine. C18 column, isocratic elution, postcolumn reduction, and fluorescence detection.

Alternatively, the more recently developed strategies exploit the reversible reduction of the quinone to the quinol, which may then be detected by either electrochemical or fluorescent techniques. For electrochemical detection, the greatest sensitivity and selectivity are obtained by employing a redox technique in which the detector has a dual-electrode cell with the electrodes arranged in series. Vitamin K is first reduced at the upstream (or generator) electrode to the quinol, which is then reoxidized at the downstream (or detector) electrode: the current generated in the second reoxidation step is measured by the detector and is proportional to the amount of vitamin K passing through the electrochemical cell. This method is capable of detecting 50 pg or less of phylloquinone. Fluorescence detection again requires the postcolumn generation of the quinols after the chromatographic separation of their respective quinones. Reduction can be achieved by chemical, photochemical, or electrochemical techniques, with fluorescence detection providing similar sensitivity to redox electrochemical detection (*c.* 25–50 pg). Currently, the most commonly employed reduction technique involves a short dry-packed zinc or platinum oxide column, configured between the separation column and detector. Examples of chromatography achieved for a representative infant formula and

margarine are illustrated in Figure 4, utilizing a C18 column and fluorescence detection.

Further theoretical and practical details are provided in the methodological references listed in 'Further Reading' section.

See also: Chromatography: High-Performance Liquid Chromatography; Coenzymes and Cofactors; Food Fortification: Rationale and Methods; Infrared Spectroscopy: Applications; Mass Spectrometry: Applications; Mass Spectrometry: Principles and Instrumentation; Spectroscopy: Types; Vitamins: Overview.

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Vitamins: Overview

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Overview

This article covers the vitamins' definition, brief historical account of the discovery, classification and nutritional role, forms found in food, and effects of processing on vitamin stability.

Vitamins are defined as a group of complex organic micronutrients present in small amounts in food or nutritional supplements that the body requires for its normal metabolism and function. They cannot be synthesized by humans in sufficient quantity for normal metabolic requirements. Certain substances that are considered vitamins can be synthesized in small amounts by gastrointestinal tract bacteria (e.g., B-groups). Each vitamin consists of a mixed group of compounds, which may not be related to each other chemically and are therefore classified by function.

History

It was not until the early 1900s that the existence of the 'accessory food factors' was recognized. Indeed, the term 'vitamine' was first used by a Polish biochemist named Funk to describe an 'accessory food factor.' However, deficiency diseases were well known many centuries earlier, the earliest documented disease being beriberi, which was recorded in China around 2600 BC. Yet, it was not until the end of the eighteenth century that the nutritional etiology of the disease was established. In 1885, a Japanese physician named Takaki succeeded in reducing the effect of beriberi among Japanese sailors by feeding them a diet containing more meat and vegetables and less rice. Then, in 1890, a Dutch doctor named Eijkman reproduced symptoms of the disease in chickens fed exclusively with shelled rice. His collaborator, Grijns, showed that these symptoms disappeared by adding rice husks to the chicken diet. Finally, in 1912, Funk isolated the antiberiberi factor from the cuticle of the rice and named it 'vitamine,' the 'life-essential amine.' This term was adopted to name all the nutritional factors responsible for deficiency diseases.

Similarly, scurvy and night blindness were described in ancient Egypt around 1500 BC. During the period AD 1400–1600, the explorers Vasco da Gama and Magellan described in detail the scurvy and night blindness that had devastated their ships' crew. A century earlier, Jacques Cartier had succeeded in saving a good many sailors thanks to an Indian medicine based on pine needles. Finally, in 1747, James Lind found the preventive effect of citrus fruit using controlled experiments at sea, and this was shortly followed by the demonstration, during the voyages of Captain Cook in the period 1768–71, that prolonged sea voyages were possible without the ravages of scurvy.

Thirteen vitamins were isolated, identified, and synthesized between 1929 and 1975. The last vitamin to be discovered was vitamin B_{12} in 1948. Many researchers who contributed to the fundamental discoveries have been honored by the Nobel Prizes Committee, such as Szent-Gyorgyi and King for the simultaneous discovery of ascorbic acid in 1928, Karrer for his work on vitamin A and β-carotene between 1928 and 1933, Dam and Doisy for the discovery and chemical nature of vitamin K in 1943, and Hodgkin for the structural determination of vitamin B_{12} in 1964.

Nowadays, vitamin research is still very active. The understanding of the precise, complex role of vitamins has made remarkable progress owing to the study of vitamin-dependent metabolite diseases of genetic origin. More recently, because of their antioxidant properties, some fat-soluble vitamins, notably vitamins A, E, and C and provitamin A compounds (carotenoids; including β-carotene, lutein, and lycopene), are also considered to play an important role in the prevention against chronic degenerative diseases and cancers. Also, it has been shown that folic acid can reduce significantly the incidence of neural tube defects (such as spina bifida) and folates, in general, may protect against vascular diseases and some cancers, notably colonic cancer.

Classification and Properties

A number of factors can make the identification and classification of vitamins difficult: (1) A vitamin can exist in a number of different structures with similar, or different, biological activities; (2) the biological activity is organism-dependent; (3) the physiologically or metabolically active form of a vitamin *in vivo* may be different from that found in food (e.g., B vitamins are active in coenzyme forms; 1,25-dihydroxycholecalciferol is the active form of vitamin D_3); (4) several forms of the same vitamin can interconvert during extraction (e.g., pyridoxine, pyridoxamine, pyridoxal, and their phosphorylated derivatives); and (5) synthetic forms of a vitamin are often available commercially in a more stable form, for example, α-tocopherol acetate and retinol acetate are more stable compared with the free vitamins and are converted to the letter in the body.

Vitamins are generally classified by their solubility characteristics into fat-soluble and water-soluble groups. A full list of vitamins, forms, functions, and health benefits is given in Table 1. The fat-soluble vitamins (A, E, D_3, and K and carotenoids) are derived from the isoprenoid pathway, whereas the synthesis of the water-soluble vitamins (B_1, B_2, B_6, B_{12}, C, biotin, niacin, and pantothenic acid) is much less connected, and several of them have multiple biosynthetic pathways. Other 'vitamin-like' compounds are sometimes associated with the classical vitamin compounds. These include vitamins F (essential fatty acids), L (*o*-aminobenzoic acid), P (bioflavonoids), U (*S*-methylmethionine sulfonium chloride), and B_{13} (orotic acid); choline; α-lipoic acid; myoinositol; plastoquinone; pyrroloquinoline quinone; and ubiquinones.

 http://dx.doi.org/10.1016/B978-0-12-384947-2.00730-3

Table 1 Fat- and water-soluble vitamins: forms, function, and potential health benefits

Vitamin	*Vitamers and related forms*	*Functions*	*Potential health benefits*
Fat-soluble vitamins			
A	Retinal, retinoic acid, retinol *cis*-isomers, β-carotene, other carotenoids	Needed for normal vision, reproduction, and various tissues; immune function	Protection against various cancers
D	Cholecalciferol (D_3), ergocalciferol (D_2), 1-α-25-dihydroxycholecalciferol	Enhances calcium absorption; regulates calcium/phosphate metabolism; promotes bone mineralization	Reduces risk of osteoporosis
E	All-rac-α-tocopherol, β-tocopherol, δ-tocopherol, γ-tocopherol, various tocotrienols	Prevents lipid oxidation of membranes and PUFAs from autoxidation; essential for normal immune function and healthy tissues	Reduces risk of cardiovascular disease, precancerous lesions, and cancer
K	Phylloquinone (K_1), menaquinone (K_2), menadione (K_3)	Essential for normal blood clotting, formation/maintenance of healthy bones	Reduces risk of blood clotting and osteoporosis
Water-soluble vitamins			
B_1	Thiamine, thiamine monophosphate (TMP), thiamine pyrophosphate (cocarboxylase, TTP) complexed to proteins	Helps convert carbohydrates into energy metabolism; required by the brain, nervous system, and heart	Important role in energy metabolism
B_2	Flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD)	Involved in carbohydrate, protein, and fat metabolism; helps release energy into cells	Important role in energy metabolism; healthy skin
Niacin	Nicotinic acid, niacinamide (nicotinamide), nicotinamide adenine dinucleotide (NAD), and nicotinamide adenine dinucleotide diphosphate (NADP)	Involved in carbohydrate, protein, and fat metabolism	Reduces risk of arteriosclerosis
B_6	Pyridoxine, pyridoxal, pyridoxamine, and related phosphorylated forms	Essential for proper protein utilization; involved in homocysteine metabolism	Reduces risk of cardiovascular disease (with folate and B_{12}) and osteoporosis
B_{12}	Cyanocobalamin, methyl- and hydroxycobalamins, adenosylcobalamin (coenzyme B_{12})	Needed for red blood cell formation and DNA and RNA syntheses; involved in homocysteine metabolism	Prevents certain anemias and reduces risk of cardiovascular disease
Folic acid (folacin)	Pteroylmonoglutamic acid, 5-methyl-tetrahydrofolic acid and 10-formyltetrahydrofolic acid, tetrahydrofolic acid as both mono- and polyglutamates	Involved in amino acid interconversions and methylation reactions, needed for red blood cell formation and DNA and RNA syntheses, involved in homocysteine metabolism and protection against neural tube defects	Prevents certain anemias and birth defects, reduces risk of cardiovascular disease (with B_6 and B_{12}) and certain cancers
Pantothenic acid	Coenzyme A, pantetheine, and acyl carrier protein (ACP)	Active in protein, carbohydrate, and fat metabolism, formation for certain nerve regulators	General health and well-being
Biotin	Biotin, desthiobiotin	Active in protein, carbohydrate, and fat metabolism	Helps develop healthy nails
C	Ascorbic and dehydroascorbic acids, isoascorbic acid	Helps form/maintain collagen; important for healthy tissues and wound healing; enhances iron absorption; protects against free-radical damage and infection	Protects against cancer and cardiovascular and eye diseases; reduces symptoms of colds and flu and risks of osteoporosis

Source: Francis, F. J. (ed.) (2000). Vitamins: survey. In: *Encyclopedia of food science and technology* (2nd ed.), vol. 4, pp. 2440–2449. New York: Wiley.

During the early years of vitamin discovery, their chemical composition was largely unknown and vitamins were each assigned a letter of the alphabet for convenience. This was further complicated when it was found that the activity attributed to a single vitamin was, in some instances, the result of a combination of several vitamins, for example, vitamin B complex. Once a vitamin had been isolated from a food and its chemical structure determined, variations in the structure were found within compounds having the same vitamin activity but in different species. A system of numerical subscripts was therefore adopted to overcome this problem, for example, D_2 and D_3.

In order to simplify the system, letters were sometimes replaced with names, for example, thiamine (vitamin B_1), riboflavin (B_2), pantothenic acid (B_5), and biotin (vitamin H), based on the chemical structure of a specific function or source. 'Thiamine' contains the prefix 'thi,' which is derived from the Greek word for sulfur and refers to its sulfur content. 'Riboflavin' indicates the chemical structure and is derived from the chemical names 'ribose' (a pentose sugar) and 'flavin' (a heterocyclic ketone). 'Pantothenic acid' is derived from the Greek word *pantos*, meaning 'found everywhere.' 'Biotin' originates from the German word *haut*, meaning skin, as this vitamin protects the skin.

Occurrence in Food

One of the main differences between fat- and water-soluble vitamins is their distribution in biological tissues.

The water-soluble vitamins are found in all living tissues, whereas the fat-soluble vitamins are completely absent from some tissues. Vitamins A and D occur widely in plant tissues in the form of a provitamin. Similarly, the amino acid tryptophan can be converted into nicotinic acid in the body. Although it is generally assumed that sufficient amounts of vitamins can be absorbed from a balanced diet for normal requirements, the use of vitamin supplements, either as single compounds or in multivitamin products, is widely consumed. The major vitamin forms found in foods, some commercial forms, and some examples of rich food sources are given in Table 2.

Nutritional Aspects

Vitamins are required in trace amounts in the diet for the maintenance of optimal health, growth, and reproduction. Deficiency symptoms will occur if a single vitamin is omitted from a diet of a species that requires it. Many of the vitamins act as coenzymes, whereas others have no single role but perform certain essential functions. A list of known functions for each vitamin is given in Table 1. Vitamin requirements based on metabolic needs are similar for animals and humans, but dietary needs vary widely among species.

The recommended intakes of nutrients, including vitamins, are intended as daily intake guidelines for preventing deficiencies and maintaining body reserves among healthy individuals. The values are derived from studies on volunteers on nutrient balance studies, measures of tissue saturation, normal vitamin intakes in the general population, and extrapolation from animal studies and are generally revised every 5–10 years by panels of expert scientists. The recommended intakes can vary from country to country owing to a number of factors such as different interpretations of investigations into the determination of requirements, the level of the safety margin needed, and the amounts needed to allow for individual variation in the general, healthy population. The recommended dietary allowances (RDAs) and recommended nutrient intakes used in the United States and the United Kingdom, respectively, are given in Table 3 for adult males and females.

It is also becoming evident that some vitamins have additional health benefits when given, via diet or by supplementation, at levels above those required for eliminating classical deficiency states. Although, for most vitamins, the optimal levels are not yet established, for some vitamins such as folic

Table 2 Vitamins: commercial forms, food forms, and rich dietary sources

Vitamin	*Major commercial forms*	*Main food forms*	*Rich dietary sources*
Fat-soluble vitamins			
A	Retinol acetate and palmitate, β-carotene	Retinyl esters, carotenes (provitamin A compounds)	Fatty fish, butter, margarines, and liver
D	Cholecalciferol, ergocalciferol	Cholecalciferol	Fatty fish, eggs, and margarines
E	(*RRR*)-α-tocopherol, (*RRR*)-α-tocopherol acetate, all-*rac*-α-tocopherol, all-*rac*-α-tocopherol succinate	Tocopherols (α-, β-, and γ-forms) and tocotrienols (α- and β-forms)	Vegetable oils
K	2-(*R*,*S*-)-vitamin K_1 (*cis*/*trans* mixture), menadione sodium bisulfite	Phylloquinone (K_1), menaquinones (K_2)	Green leafy vegetables, beef, and liver
Water-soluble vitamins			
B_1	Thiamine chloride HCl, thiamine mononitrate, and thiamine diphosphate	Thiamine; thiamine monophosphate and thiamine pyrophosphate complexed to proteins	Cereals, potatoes, liver, and yeast extract
B_2	Riboflavin, riboflavin sodium phosphate	Riboflavin; riboflavin mononucleotide, flavin adenine dinucleotide complexed to proteins	Liver, milk, eggs, and green vegetables
Niacin	Niacin, niacinamide	Nicotinic acid; nicotinamide adenine dinucleotide (NAD) and its phosphate form (NADPH) complexed to proteins	Meat and meat products, cereals
B_6	Pyridoxine hydrochloride, pyridoxal-5′-phosphate	Pyridoxine, pyridoxal, and pyridoxamine and their phosphorylated forms bound to proteins	Meat and meat products, eggs, cereals
B_{12}	Cyanocobalamin, hydroxycobalamin	Methyl-, adenosyl-, hydroxy-, and sulfitocobalamins	Kidneys, eggs, milk, and cheese
Folic acid (folacin)	Folic acid	Folic acid; 5-methyltetrahydrofolate, 10-formyltetrahydrofolate, tetrahydrofolate as mono- and polyglutamates	Green leafy vegetables, cereals, yeast, and vegetable extracts
Pantothenic acid	Calcium pantothenate, d-panthenol	Pantothenic acid, coenzyme A, and acyl carrier protein (ACP)	Liver, kidney, yeast, wheat germ, and egg yolk
Biotin	D-biotin	Biotin and biotin bound to lysine and proteins	Widely distributed especially liver, kidney, yeast, and eggs
C	Ascorbic acid, ascorbyl palmitate, and sodium ascorbate	Ascorbic acid, dehydroascorbic acid, and isoascorbic acid	Citrus fruits and vegetables

Source: Francis, F. J. (ed.) (2000). Vitamins: survey. In: *Encyclopedia of food science and technology* (2nd ed.), vol. 4, pp. 2440–2449. New York: John Wiley; also Food Standards Agency (2002). *McCance & Widdowson's The composition of foods* (6th summary ed.). Cambridge: Royal Society of Chemistry.

Table 3 Recommended dietary allowances (RDAs) and reference nutrient intakes (RNIs) for vitamins in adult males and females

Category	Age (years)	A (μg of RE per day)[a]	D (μg of D_3 per day)	E (mg of α-TE per day)[b]	K (μg day^{-1})	B_1 (mg day^{-1})	B_2 (mg day^{-1})	Niacin (mg day^{-1})[c]	B_6 (mg day^{-1})	B_{12} (μg day^{-1})	Folate (μg day^{-1})[d]	C (mg day^{-1})
RDA												
Males	25–50	1000	5	10	80	1.5	1.7	19	2.0	2.0	200 (400)	60
Females	25–50	800	5	8	65	1.1	1.3	15	1.6	2.0	180 (400)	60
RNI												
Males	19–50	700				1.0	1.3	17	1.4	1.5	200	40
Females	19–50	600				0.8	1.1	13	1.2	1.5	200	40

[a]RE, retinol equivalents.

[b]TE, α-tocopherol equivalents.

[c]Nicotinic acid equivalents.

[d]Dietary folate equivalent (DFE) for adults in the United States, rising to 600 μg day^{-1} during pregnancy and 500 μg day^{-1} during lactation. Taken from Food and Nutrition Board, Institute of Medicine. National Academy of Sciences, Subcommittee on Folate, other B-Vitamins and Choline (1998). *Dietary reference intakes*: *thiamine*, *riboflavin*, *niacin*, *vitamin B-6*, *folate*, *vitamin B-12*, *pantothenic acid*, *biotin*, *and choline*. Washington, DC: National Academy Press.

Source: Food and Nutrition Board, National Research Council (1989). *Recommended dietary allowances* (10th ed.). Washington, DC: National Academy Press; Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy (1991). *Dietary reference values for food energy and nutrients for the UK*. London: HMSO.

acid, the health benefits have been clearly demonstrated for taking higher intakes to protect against spina bifida or other neural tube defects.

Availability

The way in which vitamins are absorbed and utilized in the body can be affected by a number of factors that can be broadly divided into dietary-related factors and physiological factors related to the host. The vitamin may not be in a form that can be readily absorbed by the body. For example, nicotinic acid occurs in cereals in a form that is not absorbed from the gut. Other components in the diet may also interact with the vitamin, causing an enhancement, or depression, of absorption. Thus, the nutritive value of a diet for a given vitamin may be somewhat different from the amount actually analyzed chemically. Thiamine requirements are higher when the diet contains large amounts of carbohydrate or alcohol. Similarly, when the intake of polyunsaturated fat is high, vitamin E requirements are also raised.

In general, for food tables, allowances are normally made for reduced biological activities of different forms of three vitamins: 13-*cis*-retinol and retinaldehyde (vitamin A), carotenes other than β-carotene (provitamin A compounds), and tocopherols and tocotrienols other than α-tocopherol (vitamin E).

Adverse Effects

Along with increasing evidence of health benefits from consumption of diets containing vitamins at levels much higher than the RDAs, there is concern over potential adverse effects and even toxicity. Vitamins can be generally divided into two groups: first, those vitamins with a safety level of 100 times or more the RDA for that vitamin for which there is no clear indication of any adverse effects, including most of the water-soluble vitamins and the tocopherols, and, second, those vitamins with a safety level of about ten times the RDA, above which serious adverse reactions can occur. These vitamins include retinol, calciferols, phylloquinone, and pyridoxine.

In particular, chronic intakes of vitamins A and D are to be avoided. For example, an excessive intake of vitamin D can lead to vitamin D intoxication, which is characterized by an increase in serum 25-OH-D and is associated with hypercalciuria and hypercalcemia. The safe upper limit for vitamin D is 25–50 μg day^{-1} (1000–2000 IU day^{-1}). Pregnant women should avoid large doses of vitamin A, either by avoiding foods, such as liver, that contain high concentrations of vitamin A or by avoiding multivitamin supplements that contain doses of vitamin A > 5000 IU.

As for the water-soluble vitamins, there has been some concern about excessive intakes for nicotinic acid and pyridoxine. Vitamin B_6 intakes as low as 50 mg day^{-1} can be associated with possible neurological damage in patients. Similarly, the potential benefits of lipid-lowering effects of high nicotinic acid intakes (2–6 g day^{-1}) have to be considered alongside the possible toxic effects, particularly for the liver. One of the main concerns of folic acid fortification is its potential to mask the diagnosis of pernicious anemia, which is caused by vitamin B_{12} malabsorption, at intakes of ≥ 1 mg day^{-1}, causing progressive and irreversible nerve damage.

Effects of Processing on Vitamin Stability

Vitamin stability varies greatly and depends on a number of factors such as temperature, oxygen, and acid or alkali strength. Table 4 provides general information on stability (S) or instability (U) under several conditions. Several processes have been developed to produce stabilized forms of the vitamin, including spray drying in a suitable matrix (e.g., gelatin) and encapsulation.

Consumer interest over recent years in the nutritional labeling of foods, and the increased awareness in health benefits of vitamins, has focussed attention on the effects of food processing on vitamin stability. It should be remembered also that one of the major benefits of processing is that it has a marked

Table 4 Vitamin stability under different conditions

Vitamin	*Oxygen*	*Light*	*Heat*	*Acid (pH < 7)*[a]	*Alkali (pH > 7)*[a]	*Metals*[b]	*Reducing agents*
A	U	U	U	U	S		
D	U	U	U	U	U		
E	U	U	S	S	S		
K	S	U	S	S	U		
B_1	U	S	U	S	U	U	U
B_2	S	U	U	S	U	U	U
Niacin	S	S	S	S	S	U	S
B_6	S	U	U	S	S	S	U
B_{12}	U	U	U	S	S		
Folic acid	U	U	U	U	S	S	S
Pantothenic acid	S	S	U	U	U		
Biotin	S	S	U	S	S	S	
C	U	U	U	S	U	U	S

[a]In the absence of oxygen.

[b]Information from van der Meer, M. A. (1972). *Voeding* **33**, 277. S, stable; U, unstable.

Source: Chichester, C. O. (1973). Nutrition in food processing. In: Rechcigi, M. (ed.) *Food nutrition and health*. *World review of nutrition and dietetics*, vol. 16, pp. 319–326. Washington, DC: Karger-Basel.

effect on preserving vitamin concentrations by inactivating food enzymes despite any immediate loss.

The content of vitamins in processed foods is determined initially by the content of the raw materials used. There is a wide variability in vitamin concentrations of commercially grown foods. For example, carrots may vary in their carotene concentration 100-fold, and vitamin C can vary by as much as 35-fold. This can be due to a number of factors, including variety differences, climate and growing conditions, maturity at harvest, and postharvest storage conditions. Thus, the vitamin content of raw materials for processing can vary enormously, in many cases exceeding that found in normal processed food.

The different chemical and physical properties of the vitamins mean that they vary widely in their degree of stability. In the pure crystalline form, they retain their activity for long periods of time. However, when foods are processed, vitamins are subjected to a range of conditions that can be detrimental to their stability such as light, moisture, oxygen, heat, and pH, these being typical environmental factors to which the food will be subjected in the food chain (see Table 4). The most labile vitamins are retinol, ascorbate, folate, and riboflavin. Niacin and biotin are the most stable, but information on some of the vitamins, especially vitamin D, is incomplete.

Domestic Cooking

Typical percentage losses of vitamins on cooking for selected food groups are given in Table 5.

Vitamin C is one of the most labile of all the vitamins and is readily leached into water on boiling, particularly when the surface of the food has been cut or damaged. Vegetables are an important source of vitamins in the diet, and typical losses on boiling vegetables are quite variable. The use of microwaving can reduce vitamin losses for cooked vegetables (see in the succeeding text).

Meat is also an important source of several vitamins. A considerable amount of B-group vitamins, especially thiamine, can be lost when meat is grilled or fried (up to 40%). Losses are less when pot roasting and braising are used. Some vitamins that leach into the meat juices during cooking will not be lost if the sauce or the gravy is eaten as part of the actual dish. This means that average losses in meat dishes are not higher than for grilled or fried meat, even though the cooking times are longer. Similarly, when bread is baked, 30% of the thiamine content can be destroyed, and this can increase to as much as 80% if alkaline baking powders are employed.

Microwave cooking can result in retention of vitamins equal to, or greater than, conventionally cooked vegetables. However, a number of studies have shown that cooking time, water volume used, etc. affect considerably vitamin retention with microwave cooking, as with other cooking procedures. For meat and meat products, the effect of microwave versus conventional cooking on vitamin retention seems to be less pronounced. A higher degree of riboflavin retention and a lower degree of thiamine retention have been found, but there is no difference in niacin concentrations.

Other Processing Methods

There has been only limited work reported on the effects of extrusion cooking on vitamin retention. In one of the few studies, the retention of B-group vitamins was investigated during crispbread production. The destruction of the more labile vitamins (thiamine, folate, B_6, and B_{12}) was directly proportional to the energy input, although other factors, such as moisture content, mass temperature, screw geometry, and rate of throughput, are also thought to influence retention. It appears that retention can be improved by increasing both the rate of throughout and moisture content. However, carotenoids in corn starch are fairly stable to extrusion, although some of the forms can be oxidized further. Losses of vitamin C during extrusion of 25–40% have been found.

Radiation-induced changes in vitamin concentrations in foods during irradiation have been reviewed extensively in the literature. At low irradiation doses (<1 kGy) used for

Table 5 Typical percentage losses of vitamins on cooking for selected food groups

	Cereals		*Milk*		*Meats*	*Fish*	*Vegetables*	*Fruits*
Vitamin	*Boiling*	*Baking*	*Boiling*[a]	*Baked dishes*	*Meat, grilled, or fried*	*Baking*	*Boiling*	*Stewing*
A					0	0	0	0
D						0		
E			20		20	0	0	
B_1	40	25[b]	10	25	20	30	35	25
B_2	40	15	10	15	20	20	20	25
Niacin	40	5	0	5	20	20	30	25
B_6	40	25	10	25	20	10	40	20
B_{12}			5	5	20	10		
Folate	50	50	20	50		20	40	80
Pantothenate	40	25	10	25	20	20		25
Biotin	40	0			10	10		25
C			50	50			45	25

[a]In milk-based drinks and custards.

[b]15% in bread-making and toasting.

Source: Food Standards Agency (2002). *McCance & Widdowson's the composition of foods* (6th summary ed.). Cambridge: Royal Society of Chemistry.

disinfectant treatment, losses are negligible compared with the larger variations in the vitamin content of the raw materials. The most thermolabile vitamins are thiamine, tocopherols, and ascorbate, but, with the medium-dose range (1–10 kGy) used to control microbial contamination, losses can be minimized.

It is well known that fermentation can increase the B-vitamin content (e.g., folates) of some foods, such as cheese, milks, yogurts, and other dairy foods, and this is a potential area for production of foods with enhanced health benefits. There does appear to be some potential for producing significant amounts of vitamins with various fermentation conditions and organisms.

See also: Antioxidants: Characterization and Analysis; Antioxidants: Role on Health and Prevention; Bioavailability of Nutrients; Cancer: Diet in Cancer Prevention; Carotenoids: Physiology; Cobalamin (Vitamin B12): Metabolism and Disorders; Coenzymes and Cofactors; Cooking: Domestic Techniques; Dietary References: US; Dietary Surveys: National Food Intake; Extrusion Cooking: Chemical and Nutritional Changes; Folic acid and Folates: Physiology and Health Effects; Food Composition Databases; Food Fortification: Rationale and Methods; Functional Foods; Hypovitaminosis A; Meat: Role in the Diet.

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Vodka

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Vodka is a distilled alcoholic beverage. The word vodka comes from the Slavic word for water (voda). Vodka was first produced in Russia and Poland, but it is now produced globally.

Vodka is made from neutral spirits; high-strength alcohol is fermented from a carbohydrate source and concentrated by distillation so that the organoleptic characteristics of the raw materials and by-products of fermentation are selectively reduced. Neutral spirit is very pure alcohol with low concentrations of associated compounds (known in the industry as congeners). This is then subject to further processing and reduction in alcoholic strength with water to give vodka (Figure 1). Unlike whisky, brandy, and other 'brown spirits,' vodka does not undergo maturation in wooden barrels.

Neutral Spirit Production

'Neutral spirits' or 'neutral alcohols' as defined in the US Code of Federal Regulations (Title 27, Part 5.22) are distilled spirits produced from any material at or above 190° proof (95 vol.%). The equivalent term in European Union Regulation 110/2008 (Annex I) is 'ethyl alcohol of agricultural origin.' This regulation requires distillation at or above 96 vol.% alcoholic strength from a material of agricultural origin and defines specific analytic parameters (Table 1). Elsewhere, the term 'rectified alcohol' may be used along with 'ENA' (for 'extra neutral alcohol' in India) and 'GNS' (for 'grain neutral spirits,' as appropriate).

The production of neutral spirits (like all alcoholic beverages) starts with fermentation of sugars derived from a carbohydrate feedstock into ethanol by the action of yeast. The choice of carbohydrate feedstock is normally based on cost and availability. Neutral spirits may be made from various raw materials such as potatoes, cereals, molasses, and grapes and perishable materials such as sugar cane juice or cheese whey. The start of the process depends upon the ease by which the fermentable sugar, glucose, is extracted from the raw material. For example, cereals are first ground down to small particle sizes in a mill, mixed with water, and cooked under pressure. This process creates a 'mash' by breaking down the granular structure of the starch and causing it to gelatinize (i.e., absorb water and become a viscous liquid gel). Malt or added enzymes then hydrolyze the starch into dextrins (oligosaccharides), which are further broken down into a solution of fermentable sugars known as 'wort.' On the other hand, fermentation from molasses is much simpler as the carbohydrate is present as sugars that are ready for fermentation.

A distiller's yeast (such as *Saccharomyces cerevisiae*) is added to the 'wort' in vats and batch fermentation begins. The process typically takes about 40 h and produces a 'beer' (or 'fermented wash') with an alcoholic strength between 6 and 10 vol.% and a range of trace congeners that include trace methanol, higher alcohols, aldehydes, acids, and esters. The amount of congeners formed is dependent upon the composition of the raw material.

The 'beer' is converted into neutral spirits by distillation in a still that comprises a series of columns that operate continuously and is capable of concentrating alcohol from <10% up to over 95% alcoholic strength. Distillation is the process by which the components of a mixture are separated by differences in their boiling points. Temperatures are highest at the base of the still and decrease to the top of the columns. Vapor rises up the columns through internal components known as 'trays' and liquid flows down. The trays increase contact between vapor and liquid. The various congeners in the liquid boil and recondense according to the temperature at different points on the still. Although there is diversity in the designs of distillation columns found throughout the industry, the objective is to recover pure alcohol and eliminate water and congeners.

Steam is introduced into the first column (known as the 'beer stripper' or 'analyzer') and this separates alcohol and other volatile congeners from residual fermentation solids. Solids are usually recovered as by-products and used as animal feeds or biomass; some producers remove protein and fiber from the initial raw materials as useful by-products.

Subsequent distillation columns concentrate and purify the alcohol through a process of rectification so that the neutral spirit collected at the top of the last distillation column is very pure, has no discernible organoleptic character, and contains only trace congener concentrations. Trace congeners can be further removed using the technique of 'extractive distillation' (otherwise known as 'hydroselection'), a process that separates congeners of different volatility. Some raw materials such as potatoes and grapes yield relatively more methanol than molasses and cereals. Methanol concentrations can then be further reduced on a 'demethylizing' column.

Vodka Production

Vodka, along with other distilled spirits, is subject to definition in the regulations of most countries. The United States defines vodka and flavored vodka in the Code of Federal Regulations (Title 27, Part 5.22), European Union Regulation 110/2008 defines vodka and flavored vodka processes, while Russia (GOST R51355-99), China (GB/T 11858-2008), and India (IS 5286: 2005) each have very detailed and analytical vodka definitions. Neutral spirits/ethyl alcohol of agricultural origin provides the alcohol base from which not only vodka but also wide ranges of other distilled spirits, such as gin, pastis, ouzo, and aquavit, are made.

Although neutral spirits for vodka can be fermented from any carbohydrate source, cereals or potatoes are required raw materials for vodka producers in Baltic countries. Producers in other countries also use molasses (from cane and sugar beet), grapes, and rice depending on cost and market requirements. Indeed, the Canadian regulations (B.02.080.) require vodka to be made from cereals or potatoes, while European Union

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Table 1 Characteristics of ethyl alcohol of agricultural origin from Annex I of EU Regulation 110/2008. Ethyl alcohol of agricultural origin used in the manufacture of vodka and other spirit drinks in the EU must meet the following sensory and analytical standards.

Organoleptic characteristics	*No. detectable taste other than that of the raw material*
Minimum alcoholic strength by volume	96.0 % vol.
Maximum level of residues (measured as g/100 litres alcohol at 100 % vol.)	
Acidity: acetic acid	1.5
Esters: ethyl acetate	1.3
Aldehydes: acetaldehyde	0.5
Higher alcohols: 2-methyl 1-propanol	0.5
Methanol	30
Dry extract	1.5
Volatile nitrogen bases: nitrogen	0.1
Furfural	Not detectable

Source: Regulation (EC) No 110/2008 of the European Parliament and of the Council of 15 January 2008 on the definition, description, presentation, labelling and the protection of geographical indications of spirit drinks and repealing Council Regulation (EEC) No 1576/89.

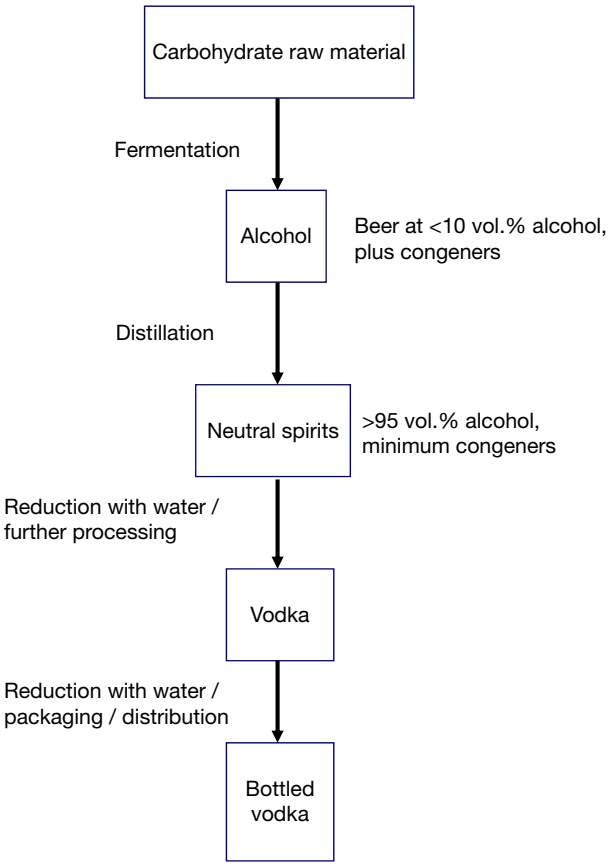

Figure 1 Key stages in vodka production process.

regulations require vodkas that are made from materials other than cereals and potatoes to be labeled as such.

The vodka process utilizes neutral spirits that are normally first reduced in alcoholic strength with pure water before undergoing various processes such as filtration through activated carbon and other media (to further reduce trace congeners). The quality of the reducing water is very important. This water is usually made using a town water supply but further treated by demineralization to reduce trace anions and cations and then passed through activated carbon and ultraviolet light treatment processes to eliminate the risk of foreign off-odors.

Some producers pass their vodka through a series of large columns containing activated charcoal, while others employ simpler systems. Trace additives may be used including sugars, glycerol, propylene glycol, and fermentation products depending on style and local regulations. Some additives were first introduced to create a smoother taste and mouthfeel for a particular brand, while others were designed to make their brand more distinctive. Some government excise agencies once required the use of additives as 'obscurants' to demonstrate that the neutral spirits had undergone further treatments to create vodka.

Finally, more pure water is added to reduce the alcoholic strength down to that required for bottling. If the additives give the product a predominant flavor, then the product is designated as 'flavored vodka' with the name of the flavor included. Minimum bottling strengths are set in different markets ranging from 37.5 vol.% in the EU to 40 vol.% (80° proof) in the United States.

Packaging and Distribution

Vodka is most commonly packaged in 70 and 75 cl glass bottles; lightweight PET (polyethylene terephthalate) bottles are often used in the duty-free trade. Bottle closures are made from aluminum and plastics; some bottle closures are designed to include one-way valves for added packaging security and thus reduce the risk of refilling.

Although alcoholic beverages are generally exempt from nutrition labeling, consumers may require specific information. The energy value of vodka is derived from its alcohol component at 91 kJ/222 kcal per 100 ml; there is zero carbohydrate, zero fat, and zero protein present unless additives are present in specific brands, in which case the energy associated with the additives must be taken into account.

The European Union's allergen labeling regulations include ingredients described as 'cereals that contain gluten' (Annex IIIa in Directive 2003/89/EC, 2003). However, distilled spirits made from cereals do not contain allergenic material as it is involatile and eliminated during distillation. Therefore, cereals used for making distillates (among others) are exempt from allergen labeling (EU COMMISSION DIRECTIVE 2007/68/EC, 2007).

Vodka is generally available for purchase in all countries where alcohol is consumed. It is also manufactured in many countries; the choice of raw material is usually dependent upon its availability and cost in the place of production. Some global brands are produced in one country and then exported, while other brands may be produced and bottled at numerous locations. Global brands usually have consistent compositions in all their markets, the only exception being for those markets where additives are not permitted (such as Canada). Regional vodka brands tend to be consumed in their country of production. Labeling regulations require that the product's label should define its producer, its country of origin, and, if exported, the name and address of the local distributor.

Producers often apply 'track and trace' markings to their bottles and labels. These codes can be interpreted to provide considerable detail on the product's supply chain. 'Track and trace' markings are designed to be compliant with food traceability requirements of modern food law such as Article 18 of European Union Regulation 178/2002. The markings are usually in the form of letters and numbers and are printed behind the front label, on the rear of a label, and on the bottle itself or are applied by a laser that marks the glass. Information such as brand name, batch number, production date, line number, and even details of the first customer can be determined from the code (working in conjunction with the manufacturer's database).

Quality Assurance

Bottled vodka, essentially being 40% ethanol and 60% water with only negligible congeners, is very stable and requires no shelf life/expiry date on the package. It is normally a clear liquid, although some brands may contain additives and other materials that make the liquid opaque. Flavored vodkas on the other hand may be sweetened, blended, flavored, matured, and colored. Check the product's label for more information.

The trace congeners found in vodka are normally those compounds with a similar volatility to ethanol that are not completely removed during distillation. It is normal to detect very low levels of methanol (typically <10 g/100 l absolute alcohol) and occasionally trace acetaldehyde, ethyl acetate, *n*-propanol, and fusel oils. Such congeners are normal in vodka at trace concentrations and have negligible impact on sensory properties.

Sensory assessment and alcoholic strength measurement are applied during the production process. Producers have skilled panelists to conduct sensory tests. Alcoholic strength is defined as the volume of ethyl alcohol at a set temperature and is expressed as a percentage or degrees proof where 100 vol.% is equal to 200° US proof. Its measurement is normally based on the density of the test liquid and various methods and instruments are available.

In addition, gas chromatographic (GC) techniques are used to determine trace congeners. This analysis can give useful information on the efficiency of the distillation/rectification process, on the raw materials employed (as different plants lead to different methanol concentrations), and on contaminants or adulterants. Such analyses usually employ a capillary column GC with flame ionization or mass spectrometric detection. Other chromatographic separations may be used to detect additives.

Patterns of Consumption

Vodka is the most popular global distilled spirit category by volume produced, considerably ahead of rum, Scotch whisky, gin, and tequila. Total global consumption in 2012 was reported by The Economist to be nearly 4.44 billion liters. Russia consumed nearly 2 billion liters corresponding to

Table 2 Major vodka brands in 2012 and their ranking order in the top 100 global and regional distilled spirits

Ranking order	*Brand*	*Brand owner*	*Sales in 2012 (million 9 l cases)*	*Status*
3	Smirnoff	Diageo	25.8	Global
17	Absolute	Pernod Ricard	11.4	Global
24	Khlibniy Dar	Bayadera Group	8.0	Regional
27	Khortytsa	Khortytsa Distillery	7.7	Regional
28	Belenkaya	Synergy	7.3	Regional
30	Pyat Ozer	Alcohol Siberian Group	7.0	Regional
36	Kristall	Minsk Kristall RUP	5.5	Regional
39	Medoff	Eastern Beverage Company	5.4	Regional
43	Krupnik	Belvédère	5.1	Regional
54	Czysta de Luxe (ZG)	Stock Spirits Group	4.3	Regional
66	Svedka	Constellation Brands	3.9	Regional
67	Grey Goose	Bacardi	3.8	Global
69	Skyy	Gruppo Campari	3.7	Global
73	Stolichnaya	SPI Group	3.4	Global
79	Finlandia	Brown-Forman	3.1	Global
82	Russian Standard	Roust	2.9	Global
96	Magic Moments vodka	Radico Khaitan	2.5	Regional
100	Sobieski	Belvédère	2.3	Global

Source: Drinks International (2013). The Millionaires' Club, May, pp. 8–11.

annual per capita consumption at nearly 14 liters. Other major vodka markets, although well behind Russia, were Ukraine, the United States, Poland, Uzbekistan, Belarus, Kazakhstan, Britain, Brazil, and Germany.

Vodka brands are numerous and tend to be either global or regional in their sales profile (**Table 2**). The high vodka consumption in Russia and associated countries is reflected in the number of regional vodka brands that sell in large volumes in these areas.

Vodka is consumed in various ways depending on the drinking culture of the country: on its own neat in Russia and in 'cocktails' and with mixers in the West. As vodka is essentially alcohol, without the sensory impact of congeners present in other spirits, it is readily consumed. It is absorbed and metabolized like other alcoholic beverages. Excess consumption can lead to the same health issues as other alcoholic beverages.

The counterfeiting of alcoholic beverages is an issue in some markets as prices are relatively high with heavy tax duties applied to distilled spirits. Vodka is an easy target as it is relatively easy to imitate, by counterfeiters. Sometimes, empty bottles from genuine brands are refilled with illicit liquor in bars and sold as the real liquid; sometimes, the entire package is counterfeited resulting in consumers being deceived; tax revenue lost and brand owners losing business.

Health issues have been reported when counterfeiters use potentially dangerous liquids such as industrial alcohol and methanol. Owners of genuine brands often have the capabilities to confirm the authenticity of their packaging and their liquids. The additives present in certain brands can act as useful authenticity indicators when analyzing suspect samples.

See also: Alcohol: Metabolism and Health Effects; Alcohol: Properties and Determination; Beverage: Health Effects; Beverage: Patterns of Consumption; Brandy and Cognac: Consumption, Sensory and Health Effects; Brandy and Cognac: Manufacture and Chemical Composition; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: Focus on Multidimensional GC; Fermented Foods: Composition and Health Effects; Gin; Rhum–Ron–Rum: Technology and Tradition; Tequila: Raw Material, Classification, Process, and Quality Parameters; Whisky, Whiskey and Bourbon: Composition and Analysis of Whisky; Whisky, Whiskey, and Bourbon: Products and Manufacture; Yeasts.

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Wastage of Food

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Introduction

The Food and Agriculture Organization of the United Nations (FAO) definition of food loss and waste considers that it is food intended for human consumption but not consumed as such. This definition encompasses landfilled food fit for human consumption (food waste), anaerobic digestion (AD) incineration, and any edible food mass used as animal feed, plowed back, or sent to be composted (food loss).

With global population recently reaching 7 billion, there are growing concerns with regard to medium-term food sustainability. With such risk being introduced into the food supply chain (FSC), fundamental food security questions are being raised in addition to the need to feed an ever-growing number of mouths; estimated to increase to 9 billion by 2050, such population growth suggests that food demand will increase by 50% and that the agricultural and power generation demand for water will outstrip supply by 2030. A 2011 study by the FAO estimated that global food wastage stood at around 1.3 billion tonnes (1.3×10^{12} kg) p.a. In terms of feeding a growing population, it is important to consider this in terms of calories and can crudely be calculated at 6.825×10^{12} kcal. It is also pertinent to draw attention to the additional losses that occur alongside all wasted food, which is the sum total of the resource and energy input to produce that food throughout its life.

The issue of food waste is not however new, and while postwar food shortages were the primary concern of the FAO at its inception in 1945, by 1954, there had been a significant shift in its focus with the Consultative Subcommittee on Surplus Disposal being established within the 1949 established Committee on Commodity Problems. This was necessary as not only had food supply been reestablished in the war-affected areas, but also many surpluses were now being seen.

More recent efforts have been driven by nongovernment organizations (NGOs) such as the Waste & Resources Action Programme (WRAP). Notable activity by WRAP in the United Kingdom has seen the introduction of the Courtauld Commitment in 2005, now in its 3rd phase, through which retailers representing 92% of the UK grocery market, brand owners, and manufacturers publically committed to a series of targets to reduce food waste, packaging waste, and carbon dioxide emissions.

To date, the Courtauld Commitment has demonstrated significant success in the delivery of effective food waste reduction strategies with the first phase reporting reductions of 670 000 tonnes of food waste between 2005 and 2009 and the second phase boasting a reduction of 270 000 tonnes per year between 2010 and 2012, saving the UK consumer an estimated £700m a year.

Societal Contribution

Sociologically, it is disappointing that such external action is required to reduce the ever-growing issue of food wastage, with estimates that up to 1/3 of all food produced goes to waste. What's more, it is estimated that a large proportion of this is avoidable waste driven by consumer preference trends, legislative restrictions, and a fear over perceived health risks.

What is apparent however is that while globally 1/3 of produced food goes to waste and is fairly static worldwide, the categorization and origin of that waste vary significantly. Such disparity across the FSC reflects the maturity of the retail market, the controlled supply chain it demands, the overall wealth of the nations, and the length of supply chain.

The European and North American markets show that of the 900 kg of human food produced per capita, up to 20% is wasted in the FSC and a further 10–15% is wasted by the consumer. A similar pattern is apparent in industrial Asia. However, consideration of developing territories shows a slightly different picture; the actual per capita loss is less (<150 kg per capita in South and Southeast Asia), yet it still represents 1/3 of the total food production. However, only 5–10% of that wasted is at the consumer level. This clearly demonstrates the impact that societal wealth, a mature retail environment, and heavily controlled supply chain can have. The impact of household waste is exacerbated by the fact that for a product to have been a consumer product, it should have achieved its maximum cost and value through its consumption (depletion) of the resources required to create it.

It is also worth taking into consideration that the more developed nations are producing much more edible human food per capita and, in some cases, double; this is resulting in considerably more consumer wastage in societies that are increasingly obese and suffering from many more dietary related diseases such as diabetes and heart disease. For example, in the period between 1993 and 2013, obesity increased in the United Kingdom from around 15% of the population to nearer 25% in a society that disposes of almost 100 kg of edible food per capita per year.

The end of postwar rationing period saw a generation rewarded by better food quality, innovative food processing, and improved supply chains and a generation wanting their

progeny to have everything they did not – and food was an important element of that. This is shift was seen, and perhaps predicted, by the FAO in their 1954 restructure as previously discussed. As highlighted by Helena Semedo, FAO Deputy Director-General at the 2014 Green Week: "*Food production has tripled since 1945 and average food availability per person has risen by 40%.*" This has resulted around 1.5 billion of the global population being overweight or obese with a further 2.8 billion suffering the effect of malnutrition and hunger. This disparity in distribution and availability of excess underpin the root cause of avoidable food wastage. It has been suggested that the US obesity epidemic is in fact a push effect resulting from the availability and oversupply of cheap calories; therefore, actually addressing this surplus would reduce food waste.

It is also important to consider that a number of conflicts exist that also contribute to food wastage that is 'mega trends.' These may include the demand for 'fresh' fruit and vegetables throughout the year, and the demand for more natural and additive free products was in fact counter to efforts to reduce waste by increasing product shelf life.

Increase in Food Waste

By comparison of Europe and North America to less developed economies, it suggests that the hallmark of a developing economy is an increase in waste. This can be seen further in consideration of the US food waste trends, which has seen an increase of around 50% per capita between 1970 and 2005. This growth has been widely acknowledged and resulted in many countries initiating nationwide schemes to engage with manufacturers, retailers, and consumers, such as WRAP in the United Kingdom, in order to ultimately change behavior. The catastrophic impact on the rising population in terms of calorie distribution and potentially food security that continued food wastage growth would have should not be underestimated.

Supply Chain Waste

Within the FAO definition of 'food wastage,' food loss incorporates all agricultural and processing loss and food waste is focussed on consumer and household waste. However, it is distinguished from the EU's Waste Framework Directive (2008/98/EC) 'waste' definition by virtue of the fact that it only includes edible food mass suitable for human consumption and excludes inedible crop by-products, residues, and animal parts. This definition does have limitations due to cultural differences of what may or may not be considered edible.

The FSC can be long, complex, and varied, and from its agricultural origins, whether it is livestock or crops, there are numerous interconnecting component elements of selecting, processing, and packing to name few. As we have seen already, the supply chain element waste is a significant contributory factor in the overall wastage picture.

At the earliest stages, good farming practices should be in place to minimize this. However, efforts to drive quality and homogeneity saw the introduction of EC 730/1999 EC 85/2004 and (EEC) 1677/88, which controlled the sale and classification of fruit and vegetable based largely on their aesthetic appearance rather than their nutritive value. Such control measures will have undoubtedly contributed to the increase in food wastage seen in recent decades. These were largely repealed in 2009, but EU Regulation 543/2011 makes thorough specifications regarding fruit and vegetable quality and classification. Such controls will undoubtedly result in unwarranted wastage of food fit for human consumption based on appearance. It is also unlikely that such losses are quantifiable since products may be selectively harvested, a process by which produce failing to meet the exacting standards are simply left and replowed to enrich the soil rather than incurring the costs of downstream preparation and ultimately a low market value.

Processing Waste

The majority of food materials are processed once harvested, whether that is a simple washing and freezing process as would be seen for vegetable products or a more complex multilayered industrial process such as refined sugar production from sugar beet. During such processes, there are inevitable losses and clear waste minimization strategies need to be employed within a manufacturing environment. The joint food wastage declaration 'Every Crumb Counts' laid out a clear framework and philosophy to be adopted by its signatories to in order to change behavior throughout the supply chain.

The first point of note is the headline 'Every Crumb Counts,' clearly demonstrating the starting philosophy to always 'not waste anything.' In a manufacturing or processing environment, an aggregation of such marginal gains can certainly prove to be of commercial importance. The principle is that macro gains are almost always catered for through efficiency data, preventative maintenance and output data, etc. However, to gain the incremental advantage that takes the process efficiency to a higher level, attention to the minutiae is required as is the need to leave no stone unturned; therefore, each slight improvement builds into a quantifiable gain. It is this approach that many manufacturers, even those who consider themselves efficient, need to take to achieve even further waste reduction benefits. While there are many continuous improvement models and lean manufacturing techniques being used to great effect, the next generation of waste reduction is only likely to be achieved by an even more critical look at the process.

Further part of the toolkit recommendations include partnerships where waste streams could be integrated and utilized; such collaborative and open innovation could be highly beneficial and create value for both parties. This approach is not a new one; after all, yeast extracts are essentially by-products of other processes, but it is a potential avenue that may have been overlooked during times when, as a society, we considered ourselves 'resource-rich.' An analytic (chemical, nutritional, and functional) screen of waste materials is an essential step to understand the nutritional value of what is being discarded, and with knowledge of the nutritive waste, collaborative partners could be more easily identified.

A third key element is the need to drive 'innovation' and develop new products from food and ingredients that would normally be thrown away. Within the confectionery industry,

for example, the idea of reworking or inventing new products from waste is not a new one especially since many well-known products have origins as a by-product, for example, Cadbury Flake. There are many processes that produce what is considered to be unusable waste that may not be reworked into an existing product, but could be valorized through development of something new. However, the success of a new product may result in there being insufficient waste from the primary process to fulfill demand; however, this would be a 'nice' problem to have. More radical waste reduction innovation may be to increase the by-product of a process in order to enhance its value.

A key part of sustainability is to facilitate the move from a linear economy in which waste is an output to a more circular paradigm, where waste and recyclables become an input of design and manufacture; essentially, waste streams become a value to themselves. To achieve this, process waste reviews will look at all of the components of waste material from a manufacturing process from a quality angle and then assess them based on ingredient value and manufacturing input. Systematic analysis of these elements can then identify functionality and start to build a picture of potential utilization of the material as an input into alternative products, either as an internal reformulation or through external partnerships. Open innovation is a paradigm for identification and deployment of new technology, and this provides the perfect model to valorize waste streams through external partnerships. An evocative way of thinking about this is by using a 'farm-to-farm' concept, so that the provenance no longer ends at the fork but considers every element of waste that a product creates and how it will be recycled or reprocessed in such a way that it contributes to long-term ecological stability.

Sustainable innovation is becoming increasingly essential in product and packaging development projects. In many ways, the focus that this brings is resulting in a step change in some of the key thought processes in the innovation life cycle.

Each of these elements is going to require a 'step back' approach, but if waste reduction targets are to be achieved, serious reflection of what was previously considered acceptable and efficient is required. This process will also require a greater degree of collaborative and open innovation of food and drink products to achieve an even more sustainable food chain.

Retail Waste

In the developed nations with mature retail markets, the supply management system tightly controls many aspects such as production planning, distribution, temperature control, and delivery schedules in order to achieve an as efficient as possible integrated system. Such mechanisms are increasingly important in densely populated urban environments, where on-site and local retail storage may be at a premium. The success of such systems, and the overall impact that they have on waste, is apparent as supply chain waste becomes a less significant factor in territories where developed logistics exists, namely, Europe and North America, although it could be equally argued that this significance is somewhat distorted by the excessive consumer waste of such developed societies.

It is estimated that 3.5 billion people live in urban environment and this is expected to increase to 6.3 billion by 2050 due to a combination of population growth and social change. This shift will only further impact on the need for retail-driven supply chains.

In the developed world, retail giants have driven quality and safety across the supply chain and, supported by strong legislation, have without doubt enhanced the standard of food on the shelves and consumer confidence. However, they have also been a contributory factor in the increase in avoidable food wastage. Retailer standards are generally more exacting and less forgiving than legislative requirements. In addition to such high expectations and unforgiving relationships with suppliers, food wastage and its impact were placed on a much lower standing than ensuring continuous supply of homogenous product to the consumer. In more recent years, however, through engagement with organizations such as WRAP, the awareness of their role in the production of waste has been acknowledged. Among the criticism levied at the retail trade and areas that could be addressed to reduce food wastage were inconsistent forecasting and promotion management. Such promotional activity, in particular 'buy one, get one free (BOGOFs),' is considered to be counterproductive in the war on waste as they encourage overpurchase of short shelf life products that then often are disposed of as they exceed their product life.

Household Waste

In industrialized societies, food waste in households is increasingly an issue. The WRAP estimates that in 2012, 7 million tonnes of household waste was produced in the United Kingdom. This does include 1.6 million tonnes as food waste classed as unavoidable, so for consistency within this text, this represents 5.4 million tonnes of food wastage composed of 4.2 m tonnes of avoidable waste and 1.2 million tonnes of possibly avoidable waste such as peeling. This was a considerable reduction when compared to 2007, which showed an avoidable wastage level of 5.3 million tonnes. Almost half of this was cited as 'not used in time.' This links strongly to consumption trends and parallels can be drawn to an earlier suggestion that obesity was a result of availability of excess.

Contributory Factors

There are many deep-seated reasons behind the increase in food waste, and the real action to address it on a global level has occurred since 2000. While these efforts are starting to pay dividend, the changing nature of the growing population provides an ongoing challenge. Among the key activities has been the recognition and challenge to many of the contributory factors.

Quality Standards

The introduction of safety, quality, and legal standards such as the British Retail Consortium Global Standard for Food Safety within the context of the Global Food Safety Initiative has been a positive step. These have been applied rigorously by the major retail chains, in order to deliver consistent, reliable, and transparent supply chains in efforts to instill confidence

in their consumers. They have however introduced exacting standards, as discussed previously that have contributed to food wastage issue. Not only has fruit and vegetable classification and marketing standards led to the devaluing of the so-called ugly fruit, forked carrots, and curved cucumbers for purely aesthetic reasons, it has also driven a general expectation for homogeneity. This has also driven scenarios in industrialized nations where it is not economically viable to harvest lower-class produce and edible food mass is diverted to animal feed, fertilizer, or compost so as not to incur further processing time and expense. Many such cases including the rejection of up to four slices of bread per loaf during pre-prepared sandwich manufacture have been exposed, as it is suggested that the consumer was not accepting of crusts and misshapen slices. The expectation of homogenous food products is then perpetuated by consumers in their selection and/or rejection of product at point of purchase.

An artifact of the drive to improve the overall integrity of food products is the need for tight product specifications and comprehensive traceability of ingredients used. As such, the use of manufacturing by-products, that is, the concept of rework, is not widely supported in standard and premium range products, thus creating a market for 'value' tier products, ultimately devaluing the input of the edible food mass, which has already absorbed the full cost of processing. Such costs are unlikely to be fully recovered, and unless waste reuse is culturally significant within the organization, they will be balanced on a purely financial basis against the alternative of landfill or other nonfood chain waste stream outlets such as AD or incineration.

Legislation

Alongside thorough quality and safety standards, legislative restrictions are in place that have a direct impact on the production of and handling of waste. The discovery of bovine spongiform encephalopathy (BSE) in cattle led to the introduction and subsequent evolution of various controls over the handling of animal by-products, namely, EC 1069/2009 and 142/2011. Much of the components affected by such legislation are not considered to be food wastage since it is most likely by-products not considered fit for human consumption, although the lowest-risk categories refer to by-products fit for, but not intended for human consumption, which should by rights be considered as food wastage.

In 2001, the foot-and-mouth disease in pigs outbreak, in Northumberland, the United Kingdom, was traced to the mishandling and contamination of the pig 'swill' feed; this led to the introduction of the ban on pig swill feeding across the United Kingdom, which was subsequently adopted across Europe, in some US states, and in Australia. This has resulted in food manufacturing and catering waste in particular diverted away from the food chain to AD or incineration. Such waste would not be considered as food wastage since it would not be considered fit for human consumption.

Value

Much has already been discussed regarding the differing split of food wastage across the world and the primary sources of waste. The developing world clearly demonstrates though minimal consumer waste that the food on the plate is valued. The industrialized nations however are failing to recognize this and place less value to their meals. Conversely, the supply chain wastage of wealthier nations demonstrates that wastage can be controlled and to some degree minimized. Regions that rely on local supply chain and subsistence farming however are comparatively more wasteful at the front end of the chain. This is likely to be a result of less well-developed farming techniques; less use of fertilizers, pesticides, antibiotics, etc.; and less controlled or robust transportation networks.

Urbanized societies therefore lose the connection with food and agriculture and hence fail to recognize its wider value, aside from the nutrition and retail cost.

Of particular importance is consideration of Engel's law, introduced by German statistician Ernst Engel in 1857 who observed proportion of income spent or food purchases decreased as income rises and likewise lower-income cohorts spent a greater percentage, which is compounded as food prices increase. This is of particular interest as is widely reported food prices have been exhibiting a general upward trend for many years, influenced by a number of external factors such as fuel prices, crop yields, and political events. Aside from specific spikes, such as in 2008, they have in fact more than doubled since 2000. The corresponding aggregated global wealth is also seen to exhibit similar growth over the same period.

This trend is significant when correlated with the increase in food waste and the shift to a greater proportion of household waste in the so-called richer states. It is therefore suggestive that a lower value is generally assigned to food normalizing household food wastage. In economies where wealth is less and food constitutes a higher proportion of income, household waste is significantly reduced.

Shelf Life

The role of durability coding has been considered a key factor in household food wastage behavior.

In Europe, according to Article 24 and Annex X to Regulation (EU) 1169/2011, the date code is in the form of either a date of minimum durability ('best before' date) or a 'use by' date, when a product is, from a microbiological point of view, highly perishable. In this context exceeding by a products' 'use by' date, a food shall be deemed to be unsafe in accordance with Article 14(2) to (5) of Regulation (EC) No 178/2002. Best before dates are applied when a product simply drops below an acceptable organoleptic standard (i.e., it does not taste very nice or the mouthfeel is not what is expected) after a period of time.

Durability coding is essential in assuring the quality and safety of food and is fundamental in communication to the consumer. They do however contribute to food wastage. The household food and drink waste in the UK 2012 report indicated that almost half (2.0 m tonnes) were wasted as they were 'not used in time,' half of which had not been opened. This results from a disconnection with food to such a degree that consumers are failing to interact with the food and have no confidence in their own judgment; their ability to taste and smell 'off' food; and an overreliance on the retailer, packaging

format, integrity, and a general devaluing of purchased food products. 'Use by' dates are in fact underestimated by manufacturers in order to protect brand values. This would therefore suggest that much of the products disposed off by the consumers were in fact safe and fit for human consumption.

In addition to such legal requirements, there had been an increasing trend in retailers applying 'display until' and 'sell by' dates, which had no legal standing but were in fact stock rotation tools. These were found to be counterproductive and contributed to waste-level increases through confusion of the meaning of dual-coded products. This is now actively discouraged in the United Kingdom through clear government-led guidance.

Significant dietary changes also occur within wealthier nations. Bennett's law shows that as a nation increases its overall wealth, there is a corresponding shift to less starch-based staples and an increase in shorter shelf life products such as meat, fish, and dairy. Such products are more susceptible to storage deterioration and in societies where less value is placed on food household waste increases. Thus, Bennett's law and Engel's law must be considered in parallel.

Solutions

There are no immediate solutions to food wastage; however, increasing awareness of the impact throughout the supply chain is essential. Food wastage has become normalized with developed worlds, and this needs to be challenged. Consumer engagement models exist throughout the world with campaigns such as Love Food Hate Waste (the United Kingdom), Stop Spild Af Mad (Stop Wasting Food) (*Denmark*), *Every Crumb Counts* (*Europe*), Save Food from the Fridge (*the Netherlands*), Food Use for Social Innovation by Optimising Waste Prevention Strategies (FUSIONS) (*European Union*), *and the* Food Waste Reduction Alliance Project (the *United States*). *Such mechanisms are successful and are essential in engaging with the consumers and ultimately changing behavior.*

A key basic philosophy to reduce waste is outlined in EU Waste Framework Directive, which showcases the common waste prioritization pyramid and encourages a hierarchical approach to waste reduction strategies. In effect, this directive made the application of the hierarchy a legal requirement (Figure 1).

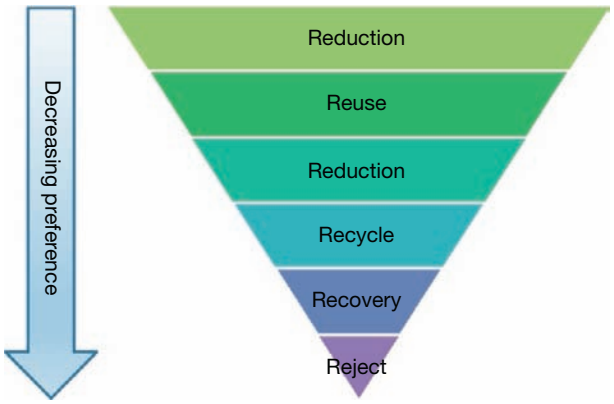

Figure 1 Waste prioritization/hierarchy pyramid.

A key component of this is to ensure that no waste is sent to landfill, and while this does not prevent waste per se, it does as a bare minimum encourage composting of food waste or incineration from which some energy is recovered.

Likely Future Trends

With growing populations, food wastage is going to remain high on the agenda of all nations, and with the impact of food wastage on food supply, it is hoped that the seriousness of the issue will be acknowledged by the comparatively more affluent consumer. As FSC systems develop in poorer regions, it will then be important to ensure that food wastage and surplus is not seen as an aspirational mark of affluence. The potential exists to combine the ideals of both industrial and subsistence regions, so that while some food wastage is inevitable, it is valued throughout the supply chain. The primary ideal in this scenario is to achieve a developing world supply chain waste of less than 20% of total food production and educate the developed world consumer to achieve household waste levels of nearer 5%. This will then go hand in hand with developing an overall healthier dietary landscape as the current levels of excess and overconsumption will be realized.

Sources of Further Information and Advice

With the public need for action on waste, numerous resources are available that provide data, trends, and action. The Waste & Resource Action Programme in the United Kingdom are very much leading the way in engaging with the consumer and industry to address the wasteful cultures that exist. Also in the United Kingdom, the Institute of Grocery Distribution (IGD) started working on a waste initiative in late 2014 to engage with food and grocery companies across the United Kingdom. On a wider European base, FoodDrinkEurope's 'Every Crumb Counts' campaign is designed to engage industry and develop toolkits to target a more sustainable manufacturing base. Further key initiatives aim to drive change of behavior and legislation through more specific activities such as 'The Pig Idea,' a campaign group trying to reverse legislation preventing food waste being returned to the food chain through pig swill and being diverted to nonfood chain routes. The zero waste to landfill agenda is championed in the United Kingdom by Vision 2020.

See also: Appetite Control in Humans: A Psychobiological Approach; Chilled Foods: Effects on Shelf-life and Sensory Quality; Codex Alimentarius; Consumer Protection Legislation; Convenience Food; Dietary Practices; Dietary References: US; Dietary Surveys: National Food Intake; Energy: Intake and Energy Requirements; Food Allergies: Occurrence and Analysis; Hunger; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Obesity: Causes and Prevalence; Obesity: Epidemiology of; Obesity: The Role of Diet; Preservation of Foods; Quality Control in Food Processing; Spoilage: Bacterial Spoilage; Spoilage: Yeast Spoilage of Food and Beverages; World Health Organization.

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Water Management, Treatment and Environmental Impact

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Water Use in Food

Food industries use water in several different functions. There are food industries that add water to the food or drink in order to produce their final product. Other food industries sell water or associated products. Most food industries use water for cooling and cleaning.

During the Production of Raw Materials

The production of food raw material requires the use of water. Vegetables and animals require watering. Depending on the cultivation practices and the laws for irrigation water and animal consumption water, there are different requirements for both the quantity and quality of water used. Fish and seafood do not need watering, but they live inside freshwater, sometimes even springwater, or saltwater.

Inside the Food Final Product

Some processed foods such as canned soups require water to be prepared. In this case, water is used to prepare the food, and this water is also included in the final product. If water is used untreated from a spring, from a well, or from the municipality supply, it is thought to provide a special character to the food taste. If it is undesirable for water to add any taste to the final product, then the local water is treated. For example, water hardness (that occurs mainly due to the presence of calcium and magnesium ions) or conductivity (that occurs mainly due to the presence of dissolved ions in water) can add taste (e.g., salty) to the final product. Water pH, that is, a measure of acid or base in water, can also make the final product taste sour (low pH) or bitter (high pH). Some minerals such as calcium can interact with some of the ingredients found in food, such as proteins, phosphate, and enzymes, with both desirable (stable) and undesirable (less bioavailable) results.

Dilution Water

Some drinks require the addition of water in order to achieve the required alcohol degrees appropriate for consumption or in order to meet legislation rules. Again, based on taste requirements, producers treat (or not) the water available. For example, expensive vodka production involves the mixing of distilled alcohol with ultrapure most commonly deionized water. Ouzo (the Greek distilled anise drink) producers advertise the use of water originating from a local river or mountain spring in order to emphasize the local identity of their product. Beer and whiskey producers achieve the special local flavor of their products by using local water.

For example, in beer, there are four anions found in water that are particularly significant. Calcium is the most important and has three major effects. It stabilizes the enzyme α-amylase and helps the breakdown of starch. It precipitates phosphate and thus increases the acidity that affects the fermentation and the microbiological stability of the enzyme processes. It also promotes flocculation of the yeast during fermentation. Magnesium produces a sour to bitter taste and reduces acidity. Sodium in small amounts gives a salty to sour taste. Potassium also gives a salty flavor and can be particularly laxative above a certain concentration. Modern breweries treat the water to remove the native minerals and then reconstitute it to a given composition for a particular brew. This practice ensures the continuity of the supply quality.

Bottled Water

The bottled water industry uses water as their final product either by bottling the water as it comes from its source or by treating it according to certain regulations in order to be suitable for human consumption. 'Mineral water' comes from a mineral spring when it is bottled at the source and has undergone no or minimal treatment. 'Table water' is a mixture of drinking water and mineral water and can contain added mineral salts and carbonic acid. 'Table water' may be produced and bottled anywhere.

Ice Industry

The ice industry also uses water after it is turned into ice cubes for human consumption or for preservation of sensitive food such as fresh fish.

Wash Water

Many food industries use water in order to wash the raw materials before using them for the production of the final product.

Cooling Water

Some food industries use water to cool their engines and return the water back to its source without having it involved in the food production but having increased its temperature by several degrees.

Deicing Water

Food industries that receive their raw materials as frozen use water to deice them so that they can use them in the production phase undistracted.

Cleaning Water

All food industries use water for cleaning purposes. For example, water can be used to clean the dishes and pots used for

food production and clean and maintain production equipment that sometimes come into direct contact with food products, but it can also be used to clean the floor. This water does not require any special quality, but it has to be free of pathogen microorganisms.

Personnel Water

Food industry personnel use water for drinking and for their personal hygiene. They consume drinking water while they are working their shift at the industry. They also use water to clean their hands while they are working or when they are using the industry toilets or to clean their dishes. Some industries have certain rules for toilet use or lunch breaks and thus of the water consumption of the personnel.

Water Management in Food Industries

There are at least four questions that each food industry manager has to ask himself or herself. Where should the water come from? How much water is needed for each application of the industry? How much water is needed every day, every season, and each year? What kind of water is needed for each application?

Water Supplies

Depending on the location of the food industry, the available water sources of the industry, and the legislation of each country, water in food can originate from several different sources. They can be divided into two main categories: (1) surface water and (2) groundwater. Surface water includes river, lake, dam, and seawater. Groundwater includes springwater and well water.

Water Quantities

Depending on the use of water in each production step, water requirements in terms of quantity have to be determined and recorded along the operation life of the farm or industry. In most cases, the water needed to cultivate the food of an individual is several times more than the water needed by the same individual as drinking water or for any other domestic use. So one cannot compare the water quantities needed for farming or meat production with water needed for food manufacturing. Nowadays, globally, irrigation consumes 70%, industry 22%, and domestic use 8% of freshwater. The increasing use of water due to increasing meat consumption can be demonstrated by the following examples: 1 kg of rice requires 1000–3000 l of water, whereas 1 kg of grain-fed beef requires 13 000–15 000 l of water.

Two terms have emerged in order to describe the water needed for the production of food and the actual water in the food: water footprint and virtual water. The water footprint of a product is the volume of freshwater appropriated to produce the product, taking into account the volumes of water consumed and polluted in the different steps of the supply chain. For example, the water footprint of a potato is 25 l of water, whereas the footprint for a bag of potato chips is 184 l of water. For the production of one glass of milk, 200 l of water is spent. The 'virtual water' contained in a product is the volume of water consumed or polluted for producing the product, measured over its full production chain, and it is considered that if one country exports a water-intensive product to another country, it exports water in virtual form. For some countries, it could be attractive to import water-intensive products instead of producing all water-demanding products themselves. The highest contributions to virtual water trade are from oil and oil crops (28%), meat and animal (26%), cereal (20%), and fish and seafood (14%).

Water Quality

There are certain laws that regulate the water quality depending on each use. There are certain requirements for water to be used for the irrigation of food and different ones for human consumption. According to the European Drinking Water Directive, water intended for human consumption includes

> (a) all water either in its original state or after treatment, intended for drinking, cooking, food preparation or other domestic purposes, regardless of its origin and whether it is supplied from a distribution network, from a tanker, or in bottles or containers.
>
> (b) all water used in any food-production undertaking for the manufacture, processing, preservation or marketing of products or substances intended for human consumption unless the competent national authorities are satisfied that the quality of the water cannot affect the wholesomeness of the foodstuff in its finished form.

The parametric values set in accordance with this regulation shall be complied with not only in the case of water supplied from a distribution network but also in the case of water used in a food-production undertaking, at the point where the water is used in the undertaking.

Generally, water has to be as pure as possible with low calcium and magnesium content (low hardness), low metal content, low nutrient content, and no pollutants. It should also be clear without having any color or any particulate matter. For example, in the preparation of spirit drinks, the addition of water shall be authorized, provided that the quality of the water is in conformity with EU directives and that the water added does not change the nature of the product. This water may be distilled, demineralized, permuted, or softened.

Impact on Water Supplies from Water Usage by Food Producers

Regular Consumption

The establishment of a new farm or new food industry in an area has to take into consideration the availability of water resources. Such activities could highly impact the water resources of a small community with limited access to clean water for regular consumption.

Water Mining

In some cases that the farm or industry has its own water resource, it may divert from sustainable use of water. It may decide to use the available water based on its needs until the

available resource is depleted. This practice is called water mining and it is not advisable since it destroys a nonrenewable source. Some countries that do not have high rainfall allow this practice.

Water Reuse

During the last years, in order to save water, water reuse has been proposed as a promising practice. Some of the activities related to food production do not contaminate the water as much as others. Water involved in such activities could be saved and used again in other activities that do not necessarily require the use of ultraclean water (e.g., for flushing toilets). Even the treated effluent of a wastewater treatment plant can be used for watering plants, if the necessary precautions set by the regulations are met. This is very critical because there are several episodes reported in the newspapers throughout the world that resulted in the death of the consumers of raw products. If there are pathogens in the effluent and irrigation is not performed appropriately, it is possible that the produce is contaminated with pathogens. However, if the appropriate microbe concentration levels have been reached and the appropriate irrigation technique is followed, then the products are considered safe. The consumers are not necessarily positive to such produce, but considering that in arid areas wastewater recycling is a common practice for many years (in some cases and in space exploration, it is even used as a drinking water source), this attitude should eventually change.

Another idea that is very popular is to save water from the different processes. Some industries monitor the water needed for each production step and then test the possibility of reducing the amount of water used. This way, less water is consumed, less wastewater is produced, and less water and wastewater treatment are needed.

Water Treatment

Common Practice

Water treatment is performed in order to improve water quality. The processes employed for water treatment depend on the quality of the water supply. In all cases, water has to be disinfected in order to deactivate any existing microorganisms present in water. So far, this technique was proved to be the most important for the protection of human life. It is commonly done using chlorine or chlorine dioxide, and in many cases, other processes are also used such as ozonation and ultraviolet irradiation. If the water originates from a surface water supply such as a river, lake, or dam, then the suspended particles are the most important problem. Different techniques to remove suspended particles include the addition of coagulants and the use of membranes. If the water originates from groundwater through mountain springs, usually, the water quality is good, and in most cases, only disinfection is required. However, if the water originates from groundwater wells, it may be rich in metals that need to be removed through chemical precipitation. Softening is another technique commonly used to remove hardness in case water is hard, which is performed by the addition of lime and subsequent precipitation of calcium as calcium carbonate and magnesium as magnesium hydroxide.

A common water treatment plant involves the following processes: (1) pretreatment to remove big objects that can be found in the pipelines that transport water from the supply to the treatment plant, (2) softening and/or coagulation for the removal of hardness and/or suspended particles, (3) filtering through sand beds to remove any fine particles that are left in the water stream, (4) activated carbon filter or air/water jet to sorb or oxidize any dissolved organic matter, (5) disinfection for the removal of any pathogens left, and (6) storage of the disinfected water before distribution to the consumers.

Wastewater from Food Production

Production

The regular everyday cycle of water includes clean water acquisition, water use, wastewater production, wastewater treatment, and treated wastewater discharge to an aqueous system.

Characteristics

The main difference between municipal wastewater and food industry wastewater is the high organic loading. The organic loading is measured with different indirect methods that commonly measure the oxygen required by the wastewater to oxidize the organic compounds either by chemical or by biochemical methods. So, there are two wastewater characteristics related to the oxygen consumption of the wastewater during organic compound degradation: chemical oxygen demand (COD) and biochemical oxygen demand (BOD). The higher the values of the COD and BOD are, the higher the difficulty to treat the wastewater. Also, the higher the difference between COD and BOD is, the more toxic the wastewater is, suggesting that a big fraction of the organic compounds is not biodegradable, and thus, it is persistent. The average municipal wastewater demonstrates values of COD from 300 to 500 mg l^{-1} and BOD of 200–300 mg l^{-1}, whereas food industry wastewater demonstrates COD and BOD values from 1000 to several 100 000 mg l^{-1}. The meat, cheese, olive oil, and fish industries produce wastewater with really high organic loadings. In some cases, these wastewaters are nutrient-deficient, and in other cases, they contain toxic compounds that are not biodegradable. For example, whey wastewater demonstrates a deficiency in nitrogen that is a nutrient required for the biological treatment of wastewater. Olive oil wastewater contains a high amount of polyphenols that are disinfectants and thus prohibit the biological treatment of wastewater.

Treatment

The most common method to treat wastewater and lower its organic loading is biological treatment. Wastewater comes in contact with microorganisms and oxygen at the same time. The presence of nutrients is essential for the successful degradation of organic compounds. This combination can successfully remove more than 90% of BOD in most wastewaters. Some food industry effluents are deficient in nutrients, but some others have high amounts of nutrients that require special

Figure 1 A typical municipal wastewater treatment plant. The main components include (1) primary sedimentation tanks, (2) aeration tanks, and (3) secondary sedimentation tanks.

attention. In this case, tertiary treatment is required employing both aerobic and anaerobic biological treatment of wastewater.

A typical wastewater treatment plant (Figure 1) includes the following processes:

(1) Pretreatment that is a mechanical process and is used to remove any big objects that can end up in the wastewater pipes.
(2) Primary sedimentation that is a physical or physicochemical process; the water passes through or stays in a tank and through gravity (sometimes after the addition of coagulants); the suspended solids are separated from wastewater resulting in the primary sludge and the wastewater stream flowing to the next tank.
(3) Secondary or biological treatment that is a biological process taking place in an aeration tank as described in the preceding text; activated sludge that is a mixture of microorganisms is suspended in an aerated tank and degrades the dissolved organic matter that is left in the wastewater stream after the primary treatment.
(4) Secondary sedimentation that separates the microbial mass that overflows from the previous tank from the treated wastewater; the sludge can be recycled by sending it back to the aeration tank or can be removed and further treated.
(5) Disinfection (if it is required by the regulation) to remove any remaining microbes left in the treated wastewater.

Other advanced technologies that can be used in addition to traditional wastewater treatment include the use of activated carbon filters to remove micropollutants or oils, the use of advanced oxidation techniques such as Fenton or catalysis or ultrasound to remove organic micropollutants, ultrafiltration and nanofiltration for further removal of colloids or microbes or decrease of conductivity, and chemical precipitation for the removal of heavy metals.

Facilities

Most of the food industries have their own wastewater treatment facilities that are specifically designed for their specific needs (Figure 2). As mentioned in the preceding text, most of the food industry wastewater facilities do not require all the steps that have been described in the previous section. It is common to use an aeration tank and either add nutrients for the biodegradation to take place (Figure 3) or add another tank that will facilitate the removal of nutrients. However, there are cases where some of the industries lower their BOD to a certain level, and then, they send their wastewater to a central wastewater treatment facility.

Figure 2 Removal of fats from wastewater in a cheese factory. Air blowers in the base of the tank help recover the fat as a froth, and mechanical skimmers on the top collect this froth in this stainless steel device that throws them on the cement ground. The fat is given away as feedstock.

Figure 3 Addition of urea in the biological treatment tank in a cheese wastewater treatment plant.

Impact on Water Quality from Food Industry Wastewater

Legislation

In most countries, legislation exists that regulates the characteristics of wastewater treatment facility effluents. These

characteristics are basically the BOD, COD, and suspended solids (SS). Each wastewater plant needs an environmental impact assessment report that determines the site of the plant, the additional regulations that are needed based on the specific site, and the receiver of the treated effluent.

Receivers

The receiver of the treatment plant effluent is either a nearby river or stream or the nearest sea. Extra treatment, such as disinfection, is required in case that the river or stream is used downstream as a drinking water source. Also, if there is a swimming beach at the sea where the effluent will be discharged, then the pipeline has to be far and deep and the effluent also requires disinfection. If the receiver is a closed gulf or a sensitive wetland, then the effluent is additionally regulated for nutrient concentrations to avoid the occurrence of eutrophication. This is a phenomenon that can be observed once high amounts of nutrients are added to an aquatic system in equilibrium. If these nutrients were the regulating factors for this system to reach equilibrium, the excessive addition results in an enormously high increase of algae production. Then, there is a cycle of events that results in the increased degradation of the aquatic systems. Due to the increased algae production, the light cannot penetrate into the lower levels of the water column, creating the death of organisms living there. Due to the death of organisms, there is an increase in the demand for oxygen needed for their biodegradation and especially during the night as oxygen levels become lower and lower. Anaerobic conditions result in the death of higher organisms, the occurrence of anaerobic microbes, and the production of methane, hydrogen sulfide, and ammonia.

Lack of Adequate Technologies and Improper Discharge

Although legislation does not allow untreated wastewater to be discharged to the environment, there are certain cases that this is happening. As mentioned in the preceding text, some of the food industry wastewater contains toxic compounds that are difficult to treat. In such cases, there is not any available effective technology. Some industries keep their waste in constructed wetlands and discharge them periodically and illegally to nearby streams, or they illegally discharge them to the municipal sewage pipes. This illegal discharge to nearby streams can result in gradual degradation of the environment and the alteration of the stream ecosystem.

Accidents

The occurrence of an accidental release of untreated wastewater to the environment is also possible. In this case, the concentration of the toxic compounds will be too high, and the effects will be visible. Initially, the higher organisms of the aquatic systems die, the drinking water supplies can be affected, bad smell will occur, and a lot of money needs to be spent in order to clean up the mess. Even if such a system is cleaned up, the residual concentrations of the toxic can remain in the system for several years resulting in chronic effects on the organisms of the aquatic system.

See also: Mineral Water: Sources and Storage; Mineral Water: Types of Mineral Water; Water Supplies: Microbiological Analysis; Water Supplies: Water Analysis.

Further Reading

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Water Supplies: Microbiological Analysis

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Background and History

The first documented record that related contaminated drinking water supply to a disease dates back to mid-nineteenth-century London, when Dr. John Snow linked the spread of the cholera epidemic to drinking water polluted by human sewage. In 1883, Robert Koch introduced the concept of testing source water as well as drinking water at the point of use for all microorganisms, which became a precursor to heterotrophic plate counts, widely used today. In 1914, the US Public Health Service (USPHS) recommended the use of total coliforms as the first standard for drinking water quality and safety, beginning the era of using coliform bacteria as indicators of human fecal pollution.

The legislative acts in the United States that recognized the need for monitoring and improving water quality started with the passage of the Clean Water Act (CWA) by the Congress in 1972. The intention of the CWA was to regulate pollutant discharges to surface waters, with the broader scope of attempting to restore the nation's waterways to their designated use (e.g., fishing and primary body contact recreational use). In 1974, the Safe Drinking Water Act (SDWA) was passed with the intent to protect public health by regulating the nation's drinking water supply.

The Revised Total Coliform Rule and Long Term 2 Enhanced Surface Water Treatment Rule and the EU Drinking Water Directive are guidance documents in the United States and European Union, respectively, that govern the quality of finished drinking water (please see 'Further Reading' and 'Relevant Websites' for additional material). These documents specify two general types of testing practices. For drinking water systems that do not use surface water and are not under the direct influence of the surface water, guidelines recommend the use of various bacterial indicator organisms (e.g., total coliforms, fecal coliforms, *Escherichia coli*, and enterococci). For drinking water systems that utilize surface water and/or are directly influenced by it, testing for bacterial indicator organisms is augmented by tests for various pathogens and/or additional indicator organisms (e.g., *Cryptosporidium* spp., *Giardia* spp., enteric viruses, and *Clostridium perfringens* and heterotrophic plate counts). The purpose of this article is to provide an overview of the methodological approaches employed to enumerate indicator organisms, which are discussed in more detail in the section in the succeeding text.

Bacterial Indicator Organisms

Human and animal waste, particularly feces, is fertile ground for human pathogens. These pathogens are numerous and highly diverse, comprising thousands of bacteria, viruses, protozoa, and worms. However, because only a fraction of any given population is generally infected, pathogen levels in water tend to be low. The great diversity of potential pathogens that can be found in water, coupled with their low levels, makes direct testing for pathogens impractical. Ubiquitous fecal microorganisms known as indicator organisms are therefore used as warnings of increased risk of pathogen presence. Ideally, indicator organisms should be confined to gastrointestinal (GI) tracts of humans and animals that carry human pathogens. They should have similar survival times and transport characteristics in the environment compared to those of pathogens, whose behavior they are intended to mimic. Furthermore, their response to treatment strategies such as disinfection should be similar to that of pathogens. The most important criterion for the use of indicator organisms to assess drinking water is that their concentrations are correlated with the presence of pathogens, whether those pathogens are viruses, bacteria, protozoa, or other eukaryotic parasites such as worms.

Indicator organisms are generally commensal inhabitants of human and animal GI tracts and assays used to detect them are, by practical necessity, relatively simple and inexpensive. These idealized criteria described earlier are deceptively simplistic; decades of research have suggested that the ideal indicator organism is an unattainable goal, as none of the organisms used for that purpose to date can meet all of the criteria. For example, in addition to being found in the human GI tracts, bacterial indicator organisms are also commensal inhabitants of the GI tract of other mammals, birds, and various insects. Because the fecal flora of various animal species is more or less likely to include specific waterborne pathogens, the broad distribution of indicator organisms across animal phyla contributes to great uncertainty about the risk associated with the presence of indicator organisms in drinking water. The imperfect fecal source association is further confounded by close association of indicator organisms with sediments, soils, and aquatic vegetation, which not only extends their persistence in the environment but, in some instances, also facilitates extraintestinal growth. Lastly, human pathogens are not necessarily of fecal origin (e.g., *Legionella* spp. and *Vibrio* spp.), span a broad phylogenetic range (e.g., viruses, bacteria, and protozoa), and have equally variable physiology and susceptibility to environmental stressors, making it exceedingly difficult to select a single organism (or groups of organisms) that are likely to reflect the fate of all waterborne pathogens.

Coliform bacteria (comprised of facultatively anaerobic, Gram-negative, non-spore-forming, rod-shaped, lactose-fermenting organisms) have a long history of employment as indices of drinking water quality. Members of the total

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coliform group are further defined as organisms that produce gas from lactose fermentation at 35 °C and include the genera *Escherichia*, *Klebsiella*, *Enterobacter*, and *Citrobacter* spp.

Fecal coliforms are a thermotolerant subgroup of the coliforms, growing well at 44.5 °C. Fecal coliforms include most *E. coli* strains and certain strains of *Klebsiella* spp. and *Citrobacter* spp. The ability of *E. coli* to hydrolyze 4-methylumbelliferyl-β-D-glucuronide (MUG) to yield a fluorogenic product via the enzyme β-glucuronidase is an important phenotypic trait used to differentiate it from *Klebsiella* spp. and other thermotolerant coliforms and is frequently used to identify *E. coli* in enzyme substrate tests (see succeeding text).

Enterococci are Gram-positive cocci arranged in pairs or chains that are catalase-negative, strictly fermentative organisms capable of growth over wide pH (4.5–10) and temperature (10–45 °C) ranges and elevated salt concentrations (10% NaCl). Enterococci were formerly classified as Lancefield group D streptococci based on the serology of cell wall antigens, until phylogenetic analysis placed these organisms into a separate genus. The ability to grow in 6.5% NaCl and the hydrolysis of esculin in the presence of bile are two important phenotypic characteristics that are used to distinguish *Enterococcus* spp. from *Streptococcus* spp.

Analytic Methods

The International Organization for Standardization (ISO) and a publication by the American Public Health Association, American Water Works Association, and Water Environment Federation, 'Standard Methods for the Examination of Water and Wastewater,' describe testing procedures for the analysis of microbiological quality of drinking water. From the methodological standpoint, the analysis can be accomplished in three ways: (1) multiple-tube fermentation method, (2) membrane filtration method, and (3) enzyme substrate test.

Multiple-Tube Fermentation

The multiple-tube fermentation method consists of three phases, presumptive, confirmed, and complete, and is used to enumerate total coliforms, fecal coliforms, and *E. coli*. Presumptive and confirmatory stages involve successive inoculation of broth tubes in order to detect the acid and or/gas production that is characteristic of total coliforms. The third phase of testing, which constitutes a 'completed' test, determines the presence of fecal coliforms and/or *E. coli* and is performed on at least 10% of positive samples from the confirmed phase. The multiple-tube fermentation method requires setting up the testing for the most probable number (MPN) technique where analysts need to use either (1) ten replicate tubes each containing 10 ml of sample or (2) five replicate tubes with 20 ml of sample each containing several dilutions of the sample or (3) a single bottle containing 100 ml of sample (for the presence/absence coliform test when qualitative information is satisfactory). Based on the combination of positive/negative tubes for each dilution, MPN tables provide an estimate of bacterial concentrations, reported as MPN per 100 ml. It is noteworthy that the precision of MPN is rather low and caution should be exercised when examining the results.

Membrane Filtration

The membrane filtration method is less labor-intensive and requires fewer days to complete than multiple-tube fermentation. Rather than inoculating broth tubes, drinking water samples are passed through a membrane filter with pore sizes sufficiently small to trap bacteria on the surface (e.g., 0.45 μm). The membrane filter is aseptically transferred to a petri dish containing selective-differential media for incubation. This method does not involve MPN to enumerate the bacteria; instead, colony-forming units (CFU) are counted and reported per 100 ml of sample filtered. Of note, this particular method is also used in the assessment of sanitary quality of surface waters used for recreation and fishing/shellfishing, although growth media and the incubation temperatures are different than those described in the succeeding text.

Enzyme Substrate

This technique can be used for the simultaneous detection of total coliforms and *E. coli*, and it capitalizes on the ability of each to hydrolyze different compounds (i.e., the entire coliform group possesses β-D-galactosidase, which hydrolyzes lactose, while only *E. coli* has β-glucuronidase). Furthermore, the enzyme substrate test can also be used for the detection of enterococci whereby 4-methylumbelliferyl-β-D-glucoside becomes hydrolyzed by enterococcus β-glucosidase releasing a fluorescent intermediate. The enzyme substrate test is similar to the multiple-tube fermentation technique as it also relies on enumeration by MPN, rather than on counting CFU, and commercially available tests can considerably simplify the procedure. Testing can be performed as a multiple-tube or multiwell setup, where various amounts or dilutions of the sample are prepared and incubated as specified by the manufacturer. In addition, enzyme substrate tests are also commercially available in a plate format, thus allowing the data to be gathered in the CFU format as well. Similar to the membrane filtration method, enzyme substrate is not limited to the assessment of the drinking water samples but is also utilized for testing the sanitary quality of recreational waters.

Specific Analytes

Sections in the succeeding text detail the application of multiple-tube fermentation, membrane filtration, and enzyme substrate methods for detection of total and fecal coliforms, *E. coli*, and enterococci, which are summarized in Table 1.

Total Coliforms

All three methods described earlier can be used to enumerate total coliforms. In the multiple-tube fermentation method, the presumptive phase tests for the ability of organisms to ferment lactose with gas production after incubation at 35 °C for 24 h in lauryl tryptose broth. Testing for gas production necessitates addition of inverted (Durham) tube, while testing for acid production calls for addition of bromocresol purple, a pH indicator. Production of either acidic reaction (manifested by the presence of yellow color) or gas (in the form of bubble trapped in the inverted tube) constitutes a presumptive

Table 1 Characteristics of methods used for enumeration of indicator organisms

Indicator organism	*Medium*	*Incubation temperature*	*Selective components*	*Differential components*
Total coliform				
	Lauryl tryptose broth	35 °C	Growth with development of yellow color and/or gas production; sodium lauryl sulfate is inhibitory to many Gram-positive and Gram-negative organisms	Lactose, bromocresol purple
	Brilliant green lactose broth	35 °C	Growth with gas production; bile and brilliant green are inhibitory to many Gram-positive and Gram-negative organisms	Lactose
	Endo-type agar	35 °C	Red colonies with metallic sheen; sodium deoxycholate and sodium lauryl sulfate are inhibitory to many Gram-positive and Gram-negative organisms	Lactose, basic fuchsin
	Lactose TTC agar with oxidase test	35 °C	Yellow colonies; sodium heptadecyl sulfate is inhibitory to many Gram-positive and some Gram-negative organisms	Lactose, bromothymol blue, TTC, cytochrome oxidase
	Enzyme substrate	36 °C	Presence of β-D-galactosidase and development of red or yellow color	Ortho-nitrophenyl-β-D-galactopyranoside (ONPG, yellow), chlorophenol-red-β-D-galactopyranoside
Fecal coliform				
	EC broth	35 °C	Growth with gas production; bile are inhibitory to many Gram-positive and Gram-negative organisms	Lactose
	MacConkey agar	44.5 °C	Temperature, red/pink colonies; bile salts and crystal violet are inhibitory to many Gram-positive and Gram-negative organisms	Lactose, neutral red
E. coli	Enzyme substrate (also EC MUG)	44.5 °C	Temperature, the presence of β-glucuronidase	4-Methylumbelliferyl-D-glucuronide (MUG)/blue fluorescence
	Lactose TTC agar with indole test	35 °C, 44.5 °C	Temperature, yellow colonies; sodium heptadecyl sulfate is inhibitory to many Gram-positive and some Gram-negative organisms	Lactose, bromothymol blue, TTC, tryptophan deamination/indole production
Enterococci	Slanetz–Bartley TTC	36 °C	Red/maroon/pink colonies; sodium azide inhibits many Gram-negative organisms	TTC
	Bile esculin azide agar	44 °C	Temperature, tan/black colonies; bile salts inhibit many Gram-positive organisms and sodium azide inhibits many Gram-negative organisms	Esculin, ferric citrate
	Enzyme substrate	41 °C	Temperature, the presence of β-glucosidase	4-Methylumbelliferyl-β-D-glucoside/blue halo

positive result for total coliforms. Those tubes that show growth but no gas or acid production require additional 48 h incubation at the same temperature. Samples with presumptive positive reactions are subjected to the confirmatory test in the second phase. The growth medium utilized is brilliant green lactose bile broth containing lactose, oxgall, and brilliant green. Growth with any amount of gas production after up to 48 h incubation at 35 °C is considered to be a confirmed positive result. The completed phase of the multiple-tube fermentation test requires identification of the organisms from confirmed phase as either fecal coliforms or *E. coli* using methods described in the sections in the succeeding text.

The membrane filtration method for the detection of total coliforms utilizes either endo-type medium or lactose 2,3,5-triphenyltetrazolium chloride (TTC) agar, followed by 24 h incubation at 35–36 °C depending on the specific methodology used. Endo-type medium (e.g., LES Endo or M-Endo) contains lactose and basic fuchsin with typical total coliform colonies being red with a metallic or golden sheen. Typical total coliform colonies on lactose TTC agar produce yellow color in the medium under the membrane. Irrespective of the media used, membrane filtration methods for total coliforms necessitate verification of the negative cytochrome oxidase test.

Enzyme substrate test does not require additional confirmatory steps, and a positive result for total coliforms is the development of either yellow or red color, depending whether test uses ortho-nitrophenyl-β-D-galactopyranoside (ONPG, yellow) or chlorophenol-red-β-D-galactopyranoside (CPRG, red) following 24 h incubation at 36 °C or a temperature recommended by the manufacturer.

Fecal Coliforms

Testing for fecal coliforms directly is not a part of methods for the assessment of drinking water quality, but fecal coliform (or *E. coli*) identification of confirmed total coliforms is necessary as a part of the completed phase of multiple-tube fermentation method. Testing for fecal coliforms involves inoculation of

either EC broth containing an inverted tube or MacConkey agar followed by incubation at 35 °C and 44.5 °C for 24 h, respectively. In addition to lactose and bile salts, which are common to both MacConkey agar and EC broth, the former also contains a pH indicator, neutral red. Growth accompanied by gas production and development of red colonies are considered to be positive results for the EC broth and MacConkey agar, respectively.

Escherichia coli

Testing for *E. coli* is a part of the completed phase of the multiple-tube fermentation method, as well as independent test of the drinking water quality via enzyme substrate and membrane filtration methods. Both completed phases of the multiple-tube fermentation method and the enzyme substrate utilize the same test, inoculation into EC-4-MUG broth containing lactose and bile salts. Following 24 h incubation at 44.5 °C, all tubes fluorescing bright blue after exposure to long wavelength UV source are considered to be positive for *E. coli*.

Testing for *E. coli* in the drinking water via membrane filtration method is a two-step procedure, and it initially requires incubation on lactose TTC agar under the conditions described earlier for total coliforms. The second step involves testing either all or at least 10% of colonies for indole production. This involves inoculation of L-tryptophan broth and incubation at 44.5 °C for 24 h, followed by the addition of Kovac's reagent. The development of red color is considered to be a positive result.

Enterococci

Testing for enterococci in drinking water is accomplished by a two-step membrane filtration procedure. The culture medium used in presumptive phase is TTC amended Slanetz and Bartley agar also containing sodium azide. Agar plates are incubated at 36 °C for up to 48 h, and typical positive colonies are red, maroon, or pink. Confirmatory test is accomplished by transferring the membrane onto bile esculin azide agar preheated to 44 °C, followed by 2 h incubation at 44 °C. Bile esculin azide agar, as the name implies, contains dehydrated ox-bile, esculin, ammonium iron (III) citrate, and sodium azide, and confirmed enterococci colonies are those that develop tan to black color in the surrounding medium. Another possibility for the enumeration of enterococci is the use of a commercially available enzyme substrate test, although at the present time, this particular technique is more commonly used for the assessment of the sanitary quality of recreational waters. Briefly, this particular test involves inoculation into media containing 4-methylumbelliferyl-β-D-glucoside followed by 24 h incubation at 41 °C. All tubes/wells fluorescing blue after exposure to UV light are considered to be positive result for enterococci.

Regulatory Uses and Limits

Since 1990, federal regulations for drinking water quality in the United States have been mandated by the Total Coliform Rule. This rule was revised in 2013 (Revised Total Coliform Rule), and compliance is mandated by 2016. The distribution system of public water systems (PWS) must be tested for total coliforms at a frequency that is proportional to the size of the population served, for example, PWS serving at least 50 000 individuals must test a minimum of 60 times per month, while those serving at least 2 500 000 must test a minimum of 420 times per month. The maximum contaminant level (MCL) for total coliforms specifies that the indicator organisms cannot be detected in more than 5% of the 100 ml samples analyzed. A positive total coliform test requires repeat sampling within 24 h, and all positive tests are further analyzed for fecal coliforms or *E. coli*. The Revised Total Coliform Rule removes the MCL for total coliforms and replaces it with an MCL for *E. coli* in recognition of the many possible nonfecal sources of total coliforms. In addition to testing for *E. coli* (and optionally for total coliforms), the EU Drinking Water Directive specifies testing the drinking water for enterococci. The frequency of monitoring is dependent on the volume of water distributed or produced within the supply zone (in m^3 per day). For instance, if the volume of water produced is between 100 and 1000 m^3 per day, the directive recommends testing four times a year, whereas volumes between 1000 and 10 000 m^3 per day call for three additional samples for each 1000 m^3. For volumes below 100 m^3, the frequency of sampling is determined by the individual member states. The allowable limit is zero CFU and any positive samples trigger immediate action to identify and remove the contamination source.

Summary

Routinely testing drinking water directly for pathogens is not practical due to the broad diversity of potential pathogens encompassing viruses, bacteria, and protozoa. Instead, the quality of potable water is assessed by testing for bacterial indicator organisms, including total coliforms, *E. coli*, and enterococci. Methods used to detect bacterial indicator organisms can be broadly divided into multiple-tube fermentation, membrane filtration, and enzyme substrate test, and appropriate media and incubation conditions are used to select for and differentiate specific groups of indicators. Microbial analysis of drinking water is an evolving science, as evidenced by recent changes to the Total Coliform Rule in the United States.

See also: Water Management, Treatment and Environmental Impact; Water Supplies: Water Analysis.

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Water Supplies: Water Analysis

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Water Supplies for Food Industries

Water supply systems obtain water from a variety of sources, mainly including groundwater (aquifers) and surface water (lakes and rivers). In certain cases, seawater can also be used through the process of desalination. Freshwater is not evenly distributed across the world. The availability of freshwater varies both spatially (geographic region) and temporally (seasonally and annually). The renewable fraction of the Earth water is usually found in the form of surface water (rivers, lakes, streams, etc.) and displays an uneven distribution. Although groundwater is more evenly distributed in comparison with surface water, much of it is nonrenewable (fossil water).

The water use in a particular region is determined by a number of factors. Among them, not only the natural groundwater availability but also the population, the land use (agricultural, residential, or industrial) of an area, and economical factors play a key role. The wealth of a community determines attitudes and funding toward water development and treatment as well as environmental issues. For example, in the developed countries, high-quality water is supplied by the municipality to the consumers' tap, and this is required by law. In the developing countries, safe water supply by the municipality is not necessarily provided. Some of the food industries treat the supplied water anyway to make sure that the industrial water meets their needs. The local climate also plays a significant role due to influences on evaporation rates as well as practices such as lawn watering and cooling requirements. Additionally, cultural values, actions, policies, and laws of national governments also have an impact on water use. Finally, the issue of ownership of the resource that is linked to government influences can be an important factor.

Tap Water: Municipal Water Supply

Freshwater

Approximately 97% of the global water supply comprises the saline water of the oceans. On the other hand, a very limited amount of saline water is also located onshore in saline lakes. The remaining 3% of water is fresh. Out of this percentage of the total freshwater supply, nearly 69% is captured in glaciers, accounting for about 2% of the total global resources. Freshwater is also found beneath the Earth's surface as groundwater (approximately 30% of the total freshwater supply) and in surface water reservoirs such as lakes, streams, swamps, and marshes. Finally, limited amounts of freshwater are also stored in the soil, the atmosphere, and biological organisms.

It is widely accepted that water is the basic substance of life on earth and is increasingly in short supply. Water scarcity affects 88 developing countries that are home to half the world's population.

Groundwater

Groundwater is hosted in aquifers. An aquifer is called any saturated (in water) underground layer of rock or sediment that can store and transmit water. Formations that do not conduct water (are well-cemented, unfractured, etc.) are called confining or impermeable layers.

Although globally groundwater is not very significant in volume, it is a critical source of water that can cover the human needs, because it is part of the limited budget of freshwater. From the perspective of long-term sustainability, it is the renewable resource that is most critical. However, it can also be viewed as a nonrenewable resource because the rate that may be withdrawn can be higher than the rate that it replenishes.

Springs

The physical outlets of an aquifer are known as springs. Springs occur mainly in mountainous or hilly terrains, in locations where the water table meets the ground surface (Figure 1). In the past, the community water supplies were often based on springs, and they still comprise a source of water, because springwater usually has a high natural quality and intake arrangements are relatively easy. Springs can be classified in artesian, gravity, perennial, intermittent, tubular, seepage, and thermal springs.

Description of sources of groundwater

The only naturally renewable source of freshwater globally is precipitation (about 110 000 km^3 per year). It occurs in different forms, including rain, snow, and hail. The first rain wets vegetation and other surfaces and then infiltrates into the ground. Out of the precipitation occurring over land, a large fraction (70 000 km^3 per year) moves back to the atmosphere through evaporation and transpiration from plants. Infiltration rates vary depending on land use, the character, and moisture content of the soil, as well as the intensity and duration of precipitation.

In case that the rate of precipitation exceeds the rate of infiltration, overland flow occurs. About 26% of this part of the cycle (18 000 km^3 per year) can be used by humans, for example, for agriculture.

Worldwide, the total annual runoff (including soil infiltration and groundwater replenishment) is estimated to be 41 000 km^3 per year. Geographic remoteness and seasonal issues (e.g., floods) limit the accessibility of water and drop the total annual accessible runoff to 12 500 km^3 per year.

Therefore, it is estimated that about 54% of the accessible runoff and 23% of the total renewable resource (precipitation over land) are currently appropriated for human use in some form.

Characteristics of groundwater

Groundwater within an unconsolidated rock moves only a few centimeters a day, that is, about 10 m per year. Its velocity

Figure 1 Springs.

depends largely on the steepness of the aquifer slopes and the permeability of the rocks. However, in consolidated rocks, the water velocity can be many times higher. A typical example is the karst formations.

Groundwater occurs in aquifers under two different conditions, the unconfined and the confined. In an unconfined aquifer, the water only fills the aquifer partly and its upper surface of the saturated zone may rise and decline. On the other hand, in a confined aquifer, the water completely fills the aquifer that is overlain by a confining bed. The recharge of the saturated zone occurs by percolation of water from the land surface through the unsaturated zone.

Groundwater can be obtained from an aquifer by drilling a well or a borehole below the water table. The water level in wells drilled into unconfined aquifers stands in the same position as the water table in the surrounding aquifer.

The water level in wells drilled into confined aquifers stands at some height above the top of the aquifer but not necessarily above the land surface. If the water level in an artesian well stands above the land surface, the well displays artesian flow, where water rises to the surface without pumping. Pumping water out of an aquifer lowers the water level near the well.

The 'safe yield' of an aquifer is the volume of water that can be withdrawn without depleting the aquifer, that is, the water that is renewable. In case that a higher volume is withdrawn, a number of undesirable effects can occur. In addition to the risk of completely draining an aquifer, seawater intrusion or land subsidence may commonly occur.

The chemical composition of groundwater reflects the geology of the area that has been in contact. If the groundwater is in contact with limestone, then it can be considered as hard water since it is expected to contain high concentrations of calcium and carbonates. In general though, the water quality is not differentiated significantly enough to be a concern to humans, wildlife, aquatic organisms, or vegetation. The human impact is mainly responsible for groundwater quality degradation. There are cases though where pollution sources are absent, while groundwater does not lie within the potable regulations. These waters are usually found in areas with recent volcanic activity. Groundwater displays higher temperatures than the groundwater that is hosted in the surrounding watershed area; it has higher residence time in the aquifer and displays higher salt content than freshwater. Moreover, natural seawater intrusion into the aquifers may exist in many coastal areas worldwide and particularly in karst areas.

Groundwater pollution most often results from overuse of fertilizers and pesticides, improper disposal of wastes on land, industrial and household chemicals and garbage landfills, industrial waste ponds, tailings and processed wastewater from mines, oil field brine pits, leaking underground oil storage tanks and pipelines, leaky underground gas tanks, sewage sludge and septic systems, etc. Groundwater can be also contaminated by overpumping in coastal regions that triggers seawater intrusion. Seawater intrusion in coastal aquifers is a widespread and serious problem in many parts of the world, especially in the Mediterranean region.

Deterioration in water quality may result from either changes in the quality of water in the aquifer or changes in the well. These changes may affect the biological, the chemical, or the physical quality. Both the biological quality and the chemical quality of water from new public-supply wells must be analyzed before the wells are placed in use to determine if the water meets water-supply standards and, if it does not, what treatment is required. A good practice is to monitor the biological and chemical quality of water from all wells, especially those that supply domestic needs, in order to determine if changes in quality occur. Deterioration in biological quality, associated with human or animal wastes, leads to the appearance of bacteria and/or viruses in water.

The most vulnerable water supplies are those hosted in unconfined and karst aquifers, where pollutants can readily diffuse into groundwater supplies. Moreover, in case of water supply through springs, the variation of their water temperature during the day and coloration of their water shortly after rain are indications that the water-bearing soil layer is not deep enough or that there is rapid infiltration of surface water through the topsoil.

Common treatment of groundwater

The most common treatment for groundwater (well water) is a combination of softening, aeration, and disinfection. The only treatment that is used for springwater is disinfection. Sometimes, this is also not necessary but is done anyway to keep the water safe throughout the supply pipelines. Softening is used for the removal of hardness, and most industries do it in order to avoid scaling in the pipelines or the machines that heat water. Aeration is used for oxidation and thus precipitation of iron and manganese. It is also possible to use an oxidation medium such as potassium manganate (VII) or chlorine to help metal precipitation. Disinfection is used to deactivate microorganisms in water and keep the water supply safe throughout the pipelines of the municipality. The most common disinfection method is chlorination that requires the mixing of water with chlorine gas or with sodium hypochlorite. Chlorine is effective for the disinfection from bacteria and is also persistent in the supply pipeline without requiring the addition of another disinfectant. On the contrary, ozone and UV, which are also effective disinfectants, require the addition of chlorine before water supply through pipelines. The use of chlorine as a disinfectant could be a problem for food industries since chlorine has strong taste and odor. Most food

industries use activated carbon filters to remove the taste and odor of chlorine.

Other methods to get water free from salts are distillation, ion exchange, and membrane filtration. Conventional distillation requires high amounts of power and a lot of water for cooling. Solar distillation is friendlier to the environment but requires space and good weather. Ion exchange requires the use of specific resins that remove both anions and cations, but they still require water softening beforehand to avoid excessive cost by the fast consumption of the resins by hardness. Membrane filtration requires high pressure to push water through the membrane.

Contaminated water may require additional treatment before it can be consumed. Toxic metals present in groundwater due to pollution or to natural minerals can be removed by sorbent materials that will strongly bind them. However, in many countries, it is considered preferable to change water supply than try to treat a contaminated one. In places where water supplies are scarce, in-house water filters are commonly used employing ceramic pots and sorbent filtering media (e.g., activated carbon, zeolites, biosorbents, and bone chars).

Surface water

Water in aquifers is brought to the surface naturally through a spring or can be discharged into lakes and streams. Water in the saturated zone moves downward and laterally to sites of groundwater discharge such as springs on hillsides or seeps in the bottoms of streams and lakes or beneath the ocean.

Lakes

Lakes have numerous features, such as catchment area, inflow and outflow, nutrient content, dissolved oxygen, pollutants, pH, sedimentation, and type. Most lakes have at least one natural outflow in the form of a river or stream, which maintains the lake average level by allowing the excess water to be drained. However, there are lakes that do not have any natural outflow and lose water solely by evaporation and/or underground seepage. There are also many artificial lakes that are constructed for power generation, aesthetic or recreational purposes, or agricultural use (e.g., see Figure 2).

Description of sources of lake water

The total land area that contributes surface runoff to a lake or river is called a catchment area. The volume of water depends not only mostly on the amount of rainfall but also on the size of the watershed, the slope of the ground, the soil type and vegetation, and the land use. Any changes in the water level of a lake are controlled by the difference between the input and the output compared to the total volume of the lake. The most significant inputs are precipitation onto the lake surface, the runoff carried by streams from the lake catchment area, the aquifers, and artificial sources from outside the catchment area. The most significant outputs are evaporation from the lake surface, surface and groundwater flows, and any water extraction for human activities. The water level of a lake displays fluctuations related to climate and water abstraction variations.

Figure 2 Lake water.

Characteristics of lake water

The majority of lakes globally contain freshwater. The lake water composition varies depending on many factors. One of the most important is the lake type. Lakes can be classified into tectonic, landslide, saline, volcanic, glacial, and others such as karst lakes.

Based on their nutrients content, lakes can be also classified into oligotrophic, mesotrophic, eutrophic, and hypertrophic. The first category is characterized by clear waters that display low concentrations of plant life, while the last one is characterized by waters excessively enriched with nutrients and poor clarity and is subject to devastating algal blooms caused by human activities, such as heavy use of fertilizers in its catchment area. Such lakes are of little use to humans and have a poor ecosystem due to decreased dissolved oxygen. Water in lakes, bogs, and swamps may gain color, taste, and odor from decaying vegetation and other natural organic matter.

Water in lakes form layers called thermoclines, due to differences in its temperature and its density. When the water at the surface of a lake reaches the same temperature as deeper water, the water in the lake can be mixed, bringing oxygen-depleted water up from the depths and bringing oxygen down to decomposing sediments.

Lake water can be polluted by (i) runoff of soils and farm chemicals from agricultural lands, (ii) waste from cities, (iii) discharges from industrial areas, (iv) leachate from disposal sites, and (v) direct atmospheric pollutants such as rain, snow, and dust.

Common treatment of lake water

The most common treatment for lake or dam water is a combination of coagulation, flocculation, sedimentation, sand filtration, and disinfection (e.g., see Figure 3). Coagulation, flocculation, and sedimentation are needed for the removal of colloid particles that will take a long time to settle due to gravity only. However, in case these suspended solids are microalgae, then more sophisticated treatment is necessary, and then, expensive membranes are employed. Coagulation requires the addition of chemicals called coagulants that are used to neutralize the surface of colloids and allow their flocculation. Colloids repulse each other due to common surface charge. Once their surface charge is neutralized then, they flocculate due to gravity forces. Heavier particles settle much faster by gravity. Sand filtration follows the sedimentation tank and removes the finer flocs that did not settle. Disinfection is used to deactivate microorganisms in water and keep the water supply safe throughout the pipelines of the municipality. In some countries for surface water, sand filtration is mandatory

Figure 3 An aerial view of a surface water treatment plant.

Figure 4 River water.

before disinfection since attached microbes to suspended particles are more persistent than suspended microbes, and thus, disinfection is not as effective.

Some industries use pressure filters, ion exchange, and ultraviolet radiation to remove solids and alkalinity and to disinfect. This way they avoid the addition of any chemicals that could create a taste and odor problem.

Rivers and streams

Rivers are part of the hydrologic cycle. Water generally assembles in a river from precipitation through a catchment area from surface runoff and other sources such as groundwater recharge and springs and in some cases due to the release of stored water, for example, snow and glaciers (Figure 4). The water that reaches the rivers or streams, both by overland flow and from groundwater discharge, moves to the sea, where it evaporates again to perpetuate the cycle.

Description of sources of river water

A river begins at a spring's outlet or at a springs' front and ends at a mouth. The water in a river is usually flowing within a channel formed between its banks. In larger rivers, there is also a wider floodplain shaped by floodwaters, which may be far wider than the size of the river channel.

The discharge of a river varies with time. Higher flow rates typically occur in the spring, and lower flow rates occur in the winter. When the average discharge of a river cannot serve the water supply throughout the year, then a dam is built to block the flow of water. In this place behind the dam, an artificial lake is formed.

Characteristics of river water

Flow in rivers is a function of many factors including precipitation, runoff, interflow, groundwater flow, and pumped inflow and outflow. These factors vary seasonally highly affecting the river discharge and thus the pollutants concentrations.

The chemical composition of the river water is complex and depends on the inputs from the atmosphere, the geology of the catchment area, and the human activities. It has a large effect on the ecology (both plants and animals), and it also affects the uses of the river water. In order to understand and characterize the river water chemistry, a well-designed sampling and analysis is required.

Rivers in higher topographical areas can greatly reflect the local geology. In the absence of any pollution, river water is similar to the rainwater and springwater quality. In case that the local geology is deleterious, the water quality can be poor. The volume of the water is relatively smaller and its chemical composition is affected by fewer rock types. However, as a river flows to lower topographical areas, its water gathers fine soil particles, microbes, organic material, and soluble minerals. Surface runoff acquires usually less dissolved minerals than groundwater, because of its longer direct contact with soil and rock. In populated areas, the quality of surface water as well as groundwater is directly influenced by land use and by human activities. Finally, there are rivers that generate brackish water by having their river mouth in the ocean or sea.

Common treatment of river water

River water follows similar treatment schemes as lake water for the removal of suspended solids and for disinfection.

Seawater

From the perspective of growing the global supply, the most practical way of increasing the renewable water supply is to build new dams and reservoirs. The process of desalination can also have an impact on the increase of the local water supplies. Globally, there are more than 12 000 desalination plants, mainly located in wealthy and energy-rich nations. Unfortunately, many at-risk developing nations do not possess the wealth or energy resources required to install and operate large desalination plants.

Description of sources

Both surface water and groundwater eventually return to the ocean, where evaporation replenishes the supply of atmospheric water vapor.

Characteristics

Seawater cannot be used for drinking or irrigation purposes unless it is first treated.

It displays high salt content ($\sim$35 g l^{-1} of dissolved solids), while its salinity can vary spatially, due to many factors such as

incoming freshwater from rivers, precipitation, evaporation, and in some cases the formation and melting of sea ice. The dissolved substances in ocean water come both from inflowing river water and from the ocean floor.

The sea temperature varies depending on the solar radiation enhancing the continuous circulation of water through the sea. Globally, deep seawater has a temperature varying between −2 and 5 °C. The major ions of seawater are sodium and chloride, which constitute 85% of its total ion composition. The remaining 15% comprises magnesium, calcium, sulfate, bromides, etc.

Besides the natural composition of seawater, in many areas, there are substances derived by human activities that enter the seawater not only through river, aquifer, and spring underflow discharge but also through air and rain. Agricultural, industrial, and sewage outflows contribute heavy metals (such as copper, lead, mercury, cadmium, and zinc), pesticides, PCBs, disinfectants, and other synthetic products such as cleaning products. The same applies with the combustion products (ash) transported through the air and deposited through precipitation. Nuclear facilities and nuclear accidents sometimes cause radioactive material to seep into the sea.

The excess of nutrients (e.g., nitrogen), due to fertilizer or sewage discharge into the sea, can lead to excessive plant growth and in some cases in algal blooms, which lead to the depletion of oxygen in the water and have a severe impact on the ecosystems.

Common treatment

The most common treatment for seawater is a combination of coagulation, filtration, reverse osmosis, and disinfection. Desalination is a process that requires high amounts of energy to create pressure that will facilitate reverse osmosis through expensive membranes. Water molecules are pushed through a semipermeable membrane that separates seawater from clean water.

Private Wells or Springs

Many food industries try to valorize their private water resources and use them in their production. This is done either for saving money or for commercial purposes. If water resources are considered of high quality, they are used to promote the products. Usually, this is done for springwater (e.g., soft drinks made with crystal clear water originating from a famous mountain). If the industry has its own water resource, usually, it needs to provide local agencies with the water analysis requested by law and then obtain a permission to use this water supply in the food manufacturing. Then, there needs to be a periodic monitoring of the water supply to assure that the water is safe for human consumption or the necessary treatment needs to be applied.

Water Analysis

Water analysis is necessary to assure that water used is of the desired quality and does not affect food quality. Some of water characteristics could affect the quality of the final product. If water contains undesired taste, color, or odor, then the final product will be affected. However, if water contains contaminants such as pathogens, heavy metals, pesticides, or other toxic micropollutants, then the final product could be characterized as unsuitable for human consumption and then it has to be returned back to the producer.

Legislation

There are certain laws governing water quality indented for human consumption. Usually, drinking water quality standards are stricter than water quality standards set for industrial water use. However, according to the EU legislation, if the water is in contact with food, the water quality has to be the same as that of drinking water. There are certain recommendations provided by World Health Organization (WHO) and USEPA or EU also set legislation that is then followed by each government or state laws. Legislation sets standards based on epidemiological studies related to compounds that can be found in water and affect human health. Both microbiological and physicochemical water characteristics are regulated. Aesthetics related to the appearance of water are also mentioned. For microbiological parameters, EU laws provide lists of parameters, their desired values, their limits, and corresponding exact methods for their analysis. For chemical parameters, the specified performance characteristics is that the method of analysis used must, as a minimum, be capable of measuring concentrations equal to the parametric value with a trueness, precision, and limit of detection specified. Whatever the sensitivity of the method of analysis used, the result must be expressed using at least the same number of decimals as for the parametric value provided by the regulation. For example, drinking water iron should be lower than 200 $\mu g\, l^{-1}$, and the method of analysis used must be capable of measuring concentrations equal to the parametric value with trueness, precision, and limit of detection equal to 10% of the parametric value.

Water Sampling

Water sampling is very important for the correct outcome of water analysis. Using the correct technique and the appropriate glassware or plasticware is essential for the correct outcome. For example, metals react with glass and can be adsorbed on the glass; for this reason, water sampling for metal analysis is performed in plastic (usually polyethylene) containers. Another important factor is the correct lifetime of the parameters to be used and the preservation method. Some parameters such as metals require immediate pretreatment such as the addition of acid in the sample, and then, storage and other parameters require immediate analysis (e.g., nutrients).

Common Practice

There are standard methods for water analysis and most of the water analysis laboratories use these methods. Commonly, the standard methods are aiming to measure one parameter at the time.

In Situ Monitoring Methods

The unstable parameters of groundwater and surface water are determined on site during the sampling run. More specifically, the water temperature, pH, dissolved oxygen, electric conductivity, and redox potential are measured *in situ* using different electrodes or a multiparameter portable equipment. The color can also be determined on site. Additionally, alkalinity and CO_2 are measured using the titration method. In cases that H_2S is present, for example, when thermal water is present in a watershed, then H_2S is also measured on site using either an electrode or a portable spectrophotometer. The water samples for chemical analyses are collected in acid-washed, well-rinsed polyethylene bottles after pumping when the quality of a borehole is checked. The bottles must be made of materials with minimal reactivity with substances to be measured.

Normally, two to three bottles are collected for subsequent analysis for each sampling site. One bottle of bulk water (1 l) is intended for the anion concentration. One of 0.1 l volume water sample filtered through a 0.45 μm membrane filter and acidified with 0.5 ml ultrapure HNO_3 for the determination of metals. The third bottle of bulk water is used for biological oxygen demand (BOD) and chemical oxygen demand (COD) analyses. Nitrate, NO_2^-, and NH_3 concentrations are easily determined on site or soon after the sampling by a spectrophotometer. Additional water samples are collected in case that biological analyses will be carried out. For microbe analyses, glass bottles that are firstly sterilized are required.

Advanced Methods

Advanced methods require the use of analytic instruments that can measure several parameters at the same time such as gas (GC) and liquid (LC) chromatographies with different detectors such as flame ionization detector (FID), electron capture detector (ECD), mass spectrometry (MS), and ultraviolet detector (UV). They are used to analyze water for specialized chemicals such as pesticides, perfluorinated organic compounds (PFCs), and polychlorinated biphenyls (PCBs). These chemicals are usually present at low concentrations in water and they are usually toxic. Special pretreatment is usually needed for concentrating these chemicals in an organic solvent or on a solid phase that can be used to thermally release the chemicals in the injection port of the analytic instrument. In case the analysis is performed to identify unknown organic compounds in water, more specialized techniques are needed such as GC MS/MS and LC MS/MS. There are also advanced techniques that allow simultaneous analysis of several metals (inductively coupled plasma mass spectrometry, ICP-MS, and inductively coupled plasma optical emission spectrometry, ICP-OES) or ions (ion chromatography, IC) at rather low concentrations.

Certified Laboratories

There are certified laboratories that can undertake water analysis and provide the necessary paperwork for food industries to get permission to use their own water supply. It is possible to find laboratories that are specialized in water analysis and perform both microbiological and chemical analyses or to find specialized only microbiological or only chemical analysis laboratories. Usually, such laboratories attain certification for the analyses of the parameters regulated by law (laboratory that performs certified analysis). However, it is possible to certify the whole laboratory (certified laboratory).

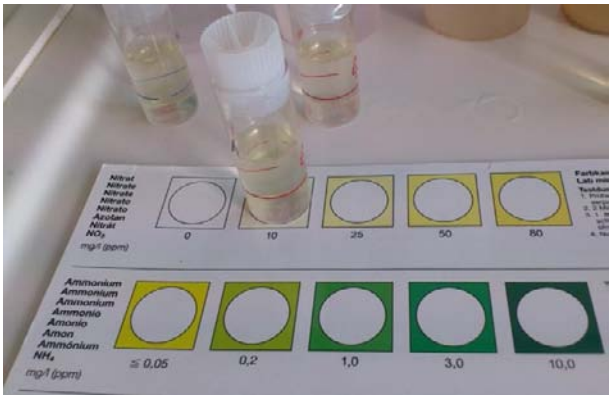

Figure 5 Standardized water analysis kit.

Food Industry: In-House Laboratories

Some food industries have their own laboratories for quality control, and at the same time, they check water parameters. This is most commonly done using automated kits (**Figure 5**) based on standard methods that are routinely used to check the quality of incoming water.

See also: Heavy Metal Toxicology; Mass Spectrometry: Principles and Instrumentation; Mineral Water: Sources and Storage; Mineral Water: Types of Mineral Water; pH: Principles and Measurement; Spectroscopy: Types; Water Management, Treatment and Environmental Impact; Water Supplies: Microbiological Analysis.

Further Reading

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Wheat: Grain Structure of Wheat and Wheat-based Products

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Wheat

Wheat, barley, rye, and triticale are part of the grass family (Poaceae) in the *Triticeae* group. The cultivation of *Triticeae* was evident 10 000 years ago in Egypt, the Middle East, and Turkey. The genus *Triticum* includes common wheat (*Triticum aestivum* L.), club wheat (*Triticum compactum* Host), spelt (*Triticum spelta* L.), and pasta wheat (*Triticum durum* Desf.). According to the FAO, world cereal production in 2014 reached a new record of nearly 2534 million tonnes, which included 725 million tonnes of wheat, with a similar amount predicted for 2015. World cereal production in 2014 reached a new record of nearly 2534 million tonnes, which included 725 million tonnes of wheat, with a similar amount predicted for 2015.

Grains and grain foods have been a staple since ancient times with Hippocrates 400 years BC recommending whole wheat meal or flour for bowel health. In relation to human nutrition, wheat-based foods provide about 20% of the world's energy intake. Wheat-based foods are a good source of essential nutrients such as carbohydrates and proteins; however, most food applications use milled wheat in the form of white or refined flour and some of the most beneficial components of the grain are often lost. While carbohydrates should be the body's major source of energy, diets rich in whole grain are recommended for the prevention of cardiovascular disease and type 2 diabetes. This protective aspect of whole grain and the importance of whole grain food must be highlighted to minimize loss of essential components from the diet. Dietary guidelines around the world recommend that about 30% of food consumption should be grain-based foods and that the consumption of grain (cereal) foods should be mostly whole grain. Whole grain cereals are rich in B vitamins and fiber.

There is an increased demand by consumers for healthy convenient food but consumers also expect food to taste and look good. In these respects, whole meal wheat products meet the requirements of a large range of attractive, tasty, and nutritious convenience foods. However, a limitation is in the use of higher extraction flour streams, which may detract from product appearance, particularly color, and processing ability of the higher fiber and ash flours. Color is particularly important for some Asian products, including steamed buns, noodles, and dumplings. Flours used in the manufacture of these products must meet stringent standards in flour ash levels, to reflect low levels of bran contamination that would otherwise lead to a lack of brightness and the appearance of dark specks in the final product. Nevertheless, there is growing interest in research to raise fiber levels in some of these products by incorporating nutritious high fiber-containing raw materials of plant origin that would cause minimal change in appearance and processing ability.

The Structure and Composition of Wheat Grains

The nature of wheat grain in its native state is important in the understanding of the changes that occur due to processing wheat-based products and health aspects of wheat-based products. A whole grain product would include all components of the grain including the outer pericarp (Figure 1).

Starchy Endosperm and Aleurone

Cereal grains are mainly starchy endosperm (around 80%), which contains the protein component (between 7% and 15%) with associated vitamins and minerals and a diverse range of lipids. The endosperm consists of cells packed with starch granules embedded in a protein matrix. The protein, vitamin, and mineral contents are highest in the outer layers of the endosperm but decrease toward the center. Wheat starch is made up of amylose and amylopectin. Starch is uniquely organized and contains mainly branched amylopectin ($1\rightarrow4$-α-D-glucan with frequent $1\rightarrow6$ branches) and varying amounts of mostly straight-chained amylose ($1\rightarrow4$-α-D-glucan with few $1\rightarrow6$ branches). Waxy grain contains almost all amylopectin. The starchy endosperm also contains nonstarch polysaccharides and phenolic compounds or pigments, but most of the pigments are contained in the bran.

The ratio of amylose/amylopectin has a critical effect on the texture of starchy foods, such as noodles, and this is largely due to the higher swelling ability or water uptake of the amylopectin component upon gelatinization. The ratio is closely linked

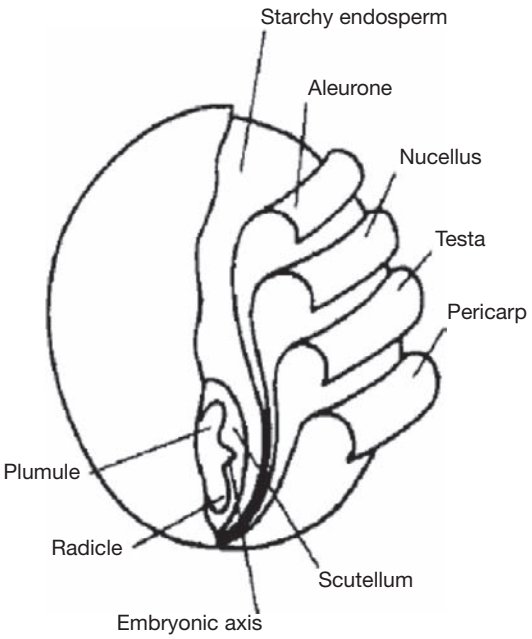

Figure 1 The generalized structure of whole grain wheat caryopsis. Source: Evers, T. and Millar, S. (2002). Cereal grain structure and development: some implications for quality. *Journal of Cereal Science* **36**, 261–284, with permission.

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to the biosynthesis of amylose, which is controlled by granule-bound starch synthase (GBSSI). GBSSI is normally present in three isoforms or isozymes – these are referred to as Wx-A1, Wx-B1, and Wx-D1. These isozymes can be present in eight possible combinations, allowing wheat genotypes to be classified as wild-type (all three isozymes present), single-null (any one isozyme absent), double-null (any two isozymes absent), and triple-null (all three isozymes absent). The lowest degree of swelling occurs with wild types, while highest swelling occurs with triple-null types, also known as full waxy wheats. Wheat varieties developed in Western Australia for the manufacture of Japanese udon noodles are single-null types or partial waxy wheats, which have been determined to give the preferred texture required by the Japanese market.

The single layer of square, thick-walled aleurone cells is part of the endosperm and lies next to the bran layers. The aleurone is not crushed during milling and hence is treated by the miller as part of the bran. The aleurone is a concentrated source of vitamins and minerals, which are often lost during milling (with the bran), unless included as whole grain or whole meal (e.g., whole grain bread). The vitamins and minerals are contained in an indigestible fibrous layer, which is difficult to access from a nutritional aspect.

The major storage proteins gliadin and glutenin make up 80% of the total grain protein. The combination of gliadin and glutenin when mixed with water results in a viscoelastic structure called gluten. Glutenins are multichain linear polymeric proteins and include low-molecular-weight (<90 000) and high-molecular-weight glutenins (>90 000). Gliadins (α, β, γ, and ω) are single-chain globular proteins with low molecular weight (30 000–80 000). Structural and metabolic proteins such as the amylases are also present in wheat.

Bran and Germ

The outer bran layer includes aleurone (because most of the aleurone is removed during milling) and constitutes 14% of the grain. The germ (3–4%) makes up the remainder of the grain. The bran layer is a good source of fiber, mainly arabinoxylans (up to 3% of flour and up to 7% of the whole grain), minerals, and phenolic compounds, such as alkylresorcinols and phenolic acids.

Bran consists of several distinct layers that surround the endosperm, including pericarp and testa: It constitutes the main fiber component of grains, and it is relatively high in lipid.

The outer pericarp and inner pericarp (cross cells and tube cells) lie outside the seed coat. The testa is the seed coat. The nucellus or nucellar epidermis surrounds the endosperm and embryo.

The germ or embryo includes the embryonic axis or sprouting section of the seed and the scutellum. At germination, the scutellum becomes the organ for the plant to digest starch and protein from the endosperm and to transfer it to the growing plant. The plumule becomes the rudimentary shoot and the radicle the rudimentary root. The germ is high in ferulic acid, phenolic compounds, and lipids. Wheat germ has a high proportion of unsaturated fatty acids and is a rich source of vitamin E (α-tocopherol).

Wheat-Based Products

Figure 2 indicates the protein content and grain hardness requirements of wheat-based products. Wheat is labeled hard or soft according to its grain hardness and this is genetically

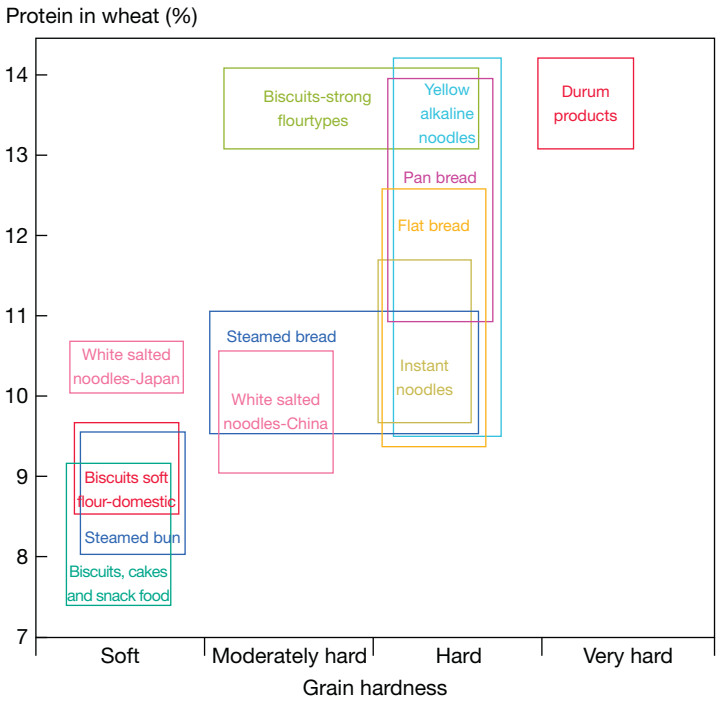

Figure 2 Grain hardness and protein content requirements of wheat-based products. Source: Blakeney, A. B., Cracknell, R. L., Crosbie, G. B., et al. (2009). *Understanding Australian wheat quality*. Kingston, ACT: Wheat Quality Objectives Group and Grain Research and Development Corporation.

controlled. Gluten strength is also largely genetically controlled, and hard wheats are generally bred with stronger gluten, while soft wheats are bred to have weaker gluten – again, this is to conform to the requirements of various end products and markets. Hard wheat is usually grown in higher protein situations, so it is 'fit for purpose,' for example, to bake bread, while soft wheat is grown in lower protein situations for products such as udon noodles, biscuits, and cakes. Protein content is influenced mainly by environmental conditions, particularly rainfall, and also soil type and soil fertility, fertilizer use, and cropping history.

Wheat Protein in Food

The wheat gluten proteins are the main storage proteins found in the endosperm cells and form a continuous viscoelastic network when flour is mixed with water to form dough. Gluten proteins form heat-set gels. When combined with starch, as in noodles, the protein concentration will affect firmness/hardness of the gel. Flour proteins as gluten, the hydrated form of the gliadins and glutenins, provides the structure of bread and noodles. Protein content in the flour and gluten strength affects noodle elasticity and hardness.

The protein content of bread wheat is on average 11–14% and the performance of that wheat in bread making relates mainly to the properties of the gliadin and glutenin proteins. Protein strongly influences the texture of wheat-based food, but it can also influence the color of foods, due to difference in inherent color or contribution to enzymatic reactions or its association with lipids. The Maillard reaction is important for the formation of the brown bread crust color and delicious aroma of the bread. Nonenzymatic chemical reactions between the reducing sugars from the carbohydrate and amino acids, amines, and small peptides from the proteins are involved in the Maillard reaction.

Wheat Starch in Food

In grain-based foods, the starch contributes in complex ways to the final food characteristics. The major component in wheat starch is amylopectin but amylose varies from 0% to 28%. Amylopectin usually makes up over 75% and amylose less than 25% of the starch granule. When amylose increases to 28% or more, the grain is described as high amylose, even though the starch is still mainly amylopectin.

When foods such as noodles are made, it is the swelling and gelatinization of starch and the amount and quality of the protein and subsequent gel formation that determine appearance and texture. The type of gel formed depends on amylose to amylopectin ratio, granular size, branch chain length, and crystallinity. The higher the amylopectin content, the more a starch will swell and the softer the starch gel.

Caramelization is also important for the formation of the bread crust color and delicious aroma of the bread. The high temperatures used in baking result in caramelization of sugars in the bread.

Whole Grain Wheat

In order to meet the whole grain definition for AACCI, the whole grain product must be an intact grain kernel or 'ground, cracked or flaked caryopsis, whose principal anatomical components (the starchy endosperm, germ, and bran) are present in the same relative proportions as they exist in the intact caryopsis.' The HEALTHGRAIN project defines whole grain as having the starchy endosperm, germ, and bran present in the same relative proportions as they exist in the intact kernel. Polishing or milling resulting in small losses of less than 2% of the grain and less than 10% of the bran still allows a whole grain label. Examples of whole grain include wheat flakes, wheat flake biscuits, and bulgur. Whole grain foods include breakfast cereals, pasta, and breads made with whole grains and whole grain flours. Whole grain foods must have a whole grain content of 27% or more to meet the AACCI definition.

Regular consumption of whole grain reduces the rate of coronary heart disease, cancer, and type 2 diabetes. The reduction in the risk of cardiovascular disease provided by a diet rich in whole grain is a result of the high dietary fiber and bioactive compounds, especially those in the bran and germ. Arabinoxylan, a viscous dietary fiber found in cell wall material, provides health benefits such as greater satiety or a feeling of fullness after eating. Whole grain wheat food provides satiety as a result of the high viscous fiber content, resistant starch, energy density, macronutrient composition, particle size, and palatability of food. The larger particle size and resistant starch result in slower digestion of the grain resulting in a greater satiety or feeling of fullness over a longer period. Raw whole grains are not eaten unless they are processed by at least heating because of the lack of palatability and digestibility.

Whole Grain and Whole Grain Foods

Products that meet whole grain definition include wheat flakes, wheat flake biscuits, bulgur, freekeh (firik), and chapatti. Whole grains have a dietary fiber content of 9–12%, but if grain has been processed to remove additional bran, this dietary fiber content will be lower. Whole grain foods include breakfast cereal, pasta, noodles, and breads made with whole grains and whole grain flours.

Wheat Flakes

Wheat flakes are a toasted breakfast cereal made preferably from hard-grained wheat.

The main quality characteristic of wheat for flaking is consistency for size, moisture content, and protein content (11–12%).

Whole wheat grains, often malted, are used for the manufacture of wheat flakes. The grain is tempered; bumped (slightly flattened between rollers); cooked under pressure with water, sugar, malt, and salt; and then passed through flaking rolls and toasted and dried to a very low moisture content of 2–4%, to ensure a long shelf life and a reasonable time before they go soggy after milk addition. Wheat flakes are a good source of dietary fiber (~12%).

Wheat Flake Biscuits

Wheat flake biscuits are eaten as a breakfast cereal, with milk, or as dry biscuits with a topping.

The main quality characteristic for wheat for flaked biscuits is consistency for size, moisture content, and protein (11–12%).

Flaked Wheat biscuits (photo V.Solah)

As with wheat flakes, the whole wheat grain is tempered, bumped, and then cooked under pressure with water, sugar, malt, and salt and sometimes vitamins and minerals. After passing through flaking rolls, the flakes are layered in a mold and baked and then decanted and dried to a very low moisture content of 2–4% to ensure eating quality. They soften on the addition of milk but keep their shape.

Wheat flakes biscuits are a good source of dietary fiber (~12%).

Bulgur (Burghul)

Bulgur is a minimally processed whole grain wheat product and the final product is harder than pasta.

Bulgur (photo V.Solah)

Durum wheat (*Triticum durum*) is the preferred raw material for making bulgur, due to its natural properties of yellow color, hardness, low-swelling (high-amylose) starch, and high protein content (>13%), but bulgur can be made from hard wheat and soft wheat.

Bulgur (bulgor, boulgur, burgul, broughoul, and burghoul) has been a staple whole grain cereal in the Middle Eastern diet for many centuries, and bulgur is popular as a health food in Western Europe especially Germany. Bulgur was referred to as 'arisah' in the Old Testament. Bulgur is extremely popular in Turkey where it is seen as a traditional health food and is used in many dishes including tabouleh, kofta, or kibbeh.

Bulgur is made from intact whole grain wheat and processed by the following basic, traditional steps of soaking or tempering to 20–24% moisture, cooking by boiling for 1–3 h until tender, drying, and polishing. Wheat is then milled into coarse to fine particles and classified by size (3.5–2.0 mm for coarse, 2.0–1.6 mm for middle, 1.6–0.5 mm for fine, and <0.5 mm for flour). Bulgur is traditionally sun-dried in the open air, or commercially, it is hot air-dried.

Quality bulgur should be yellow and hard and have high protein content. The high protein, high amylose, and hardness of the bulgur, due to the boiling and drying process, all contribute to the high resistant starch and high satiety characteristics. The polishing step removes about 6–10% bran so it is considered a whole grain food. Bulgur is high in dietary fiber (~12%) and is a good source of vitamins and minerals.

Freekeh

Freekeh (firik) is a whole grain product produced from green immature durum wheat. The green wheat plants are cut and roasted on flames and then threshed to provide grain that is processed in a similar way to bulgur. Freekeh is a good source of dietary fiber (~12%) and minerals.

Chapatti

Chapatti, chapati, or chapathi is a type of flat unleavened bread, which is a traditional and staple food for the people of India and the Indian subcontinent. Chapatti is made from whole wheat flour called atta and water. Atta is traditionally stone-milled whole grain wheat flour. Initially, there can be a pearling stage prior to milling to remove up to 10% of the bran or the atta is sieved afterward to remove coarse bran particles.

Chapatti (photo H.Fenton)

Quality characteristics of atta for good chapatti making include medium-hard grain with a protein content of 10–12% and medium dough strength, which aids in having a dough that is easy to knead and sheet. Atta with high damaged starch and high water absorption is also important and helps

contribute toward a softer textured product and chapattis that puff easily.

Traditionally, the process to make chapatti involves mixing atta and water to form a dough, resting and then sheeting the dough to a thickness of 1.5–3.0 mm that is typically circular in shape and 150 mm in diameter. A cast iron griddle pan or gas-heated griddle, referred to as a tawa or tava, is used to cook the chapatti. The chapatti is cooked for a short time on one side and flipped and baked on the other side. The water remaining in the chapatti converts to steam and temporarily puffs the chapatti to create two layers. Chapattis are traditionally prepared fresh at meal times as they are preferred to be consumed while still warm.

Quality chapattis are ideally creamy in color with a scattering of light brown spots over the surface. Full puffing of chapatti is important so that two distinct layers are formed. The texture of chapatti is soft and pliable, and it is important that chapattis can tear and fold easily. A slight soft chewy texture in the mouth, a sweet wheatish taste, and a pleasant wheaty aroma are also characteristics of quality chapatti. Chapatti are a good source of dietary fiber (~9%).

Whole Grain or Whole Meal Pan Bread and Hearth Bread

Pan and hearth bread are made from wheat flour (11–14% protein), water, yeast, and salt but often include fat, gluten-modifying chemicals or enzymes, and sugar. Pan bread is oven-baked in a baking tin or pan, using hot air, and direct heat from contact with a hot surface or using steam. Hearth bread is leavened bread that is baked without a tin, directly on the hearth of the oven widely used in small bakeries around the world. The main flour type used for bread is from hard-grained, medium- to high-protein wheat (Figure 2). Pan bread should have a soft crumb, even cell size, high volume, and a brown crust. Hearth breads should have a crisp crust, white soft crumb, and a coarse open texture and is usually eaten on the day of baking.

Hearth bread – Pan de sal (photo Blanca Villarino)

Whole grain or whole meal bread can be found in every country of the world. Whole meal flour is called 100% extraction flour meaning 100% of the grain has been utilized in the flour. More often, whole meal flour is white flour with the bran fraction (which has been removed during milling) added back to the flour. Stone-milled whole meal flour is 100% extraction flour as the flour components are not separated, and it includes the bran and the germ. Bread made with whole grain or whole meal flour or mixed grain bread includes whole grains mixed into the dough and baked or pressed into the surface and baked. Bulgur and kibbled wheat (whole wheat that has been milled into smaller particles and steamed and dried) are added to mixed grain bread with other grain types such as kibbled rye and triticale. Spelt (*Triticum spelta* L.) is high protein (>14%) and used in spelt bread and pasta.

The quality for whole grain, whole meal, or multigrain pan bread is the same as for white bread. Quality requirements are more difficult to achieve with the additional fiber (bran and germ) interfering with gluten development, therefore impacting on bread loaf volume and density. The protein content is usually higher due to added gluten (14.0%). The fat content is about 1.8%. The process for making whole grain or whole meal bread is similar to white bread, but there is a higher water requirement and dough mixing time and resting times are longer. Whole meal and mixed grain bread are an important source of dietary fiber (9–12%).

Refined Wheat Foods

Traditional products from refined white flour or semolina such as noodles, pasta, bread, and couscous have a place in a healthy diet. Four to six serves of grain-based foods, including bread, pasta, and noodles, should be consumed each day depending on age and gender and should be processed and eaten without fats and sugars.

Dietary and functional fibers include naturally occurring edible carbohydrate polymers, nonstarch polysaccharides (arabinoxylan and β-glucan), and resistant starch and have a physiological effect that plays a key a role in weight regulation and the reduced risk of disease. Satiety is the sensation of fullness after eating that deters the desire to eat more. Foods that contain carbohydrates that digest more slowly and release glucose gradually provide a prolonged feeling of fullness (satiety) and may lead to increased time between eating occasions and reduced food intake. Slower metabolism of the carbohydrate, lower glycemic index, and higher satiety are a result of higher dietary fiber, higher amylose to amylopectin ratio, larger particle size, and increased hardness, and they are also influenced by the cooking method. High amylose content, in the food consumed, is correlated with increased resistant starch. The high-protein and hard, vitreous, coarse character of the semolina, which remains intact in pasta, makes pasta slower to digest and more satiating compared to noodles. In uncooked pasta, the protein is not denatured and the starch is ungelatinized. In addition, cooked noodles and pasta contain water (75%) and this contributes to a lower energy density.

Bread

Bread is only briefly covered in this article because there are articles on bread specifically by Cauvain, Collar, and Rosell in this encyclopedia. Bread is the most common wheat-based staple food consumed in many forms worldwide. There are four main types of bread: pan bread (baked in tins), hearth bread, flat bread, and Chinese steamed bread. There are numerous differences in bread types and quality around the world, and consumers prefer the bread they are used to. Baguette, also known as French bread or Vienna bread, has a thick hard crust due to steam injection into the oven during

baking. Pan de sal is a small traditional Philippines bread roll with a crisp thin crust, soft white crumb, and sweet taste due to added sugar. Sliced pan bread has a thin, soft crust. All leavened bread types undergo four steps: mixing of the dry ingredients with water, fermentation, proofing, and baking. Important parts of bread making are the formation of the gluten network and the formation of air and carbon dioxide bubbles during fermentation, which allows dough expansion and the setting of this matrix during cooking.

Flat bread

In addition to whole grain chapatti, there are flat breads that are popular in the Middle East, North Africa, India, Turkey, and Central and South America and around the world. Diets of the world's population are now more diverse due to immigrants bringing their traditional foods to their new homes. Flat breads vary in size and thickness and are made from whole meal flour or white flour (9.5–12.5% protein) and sometimes a mix of whole meal and white flour, water, salt, yeast, and soda or baking powder. Leavened breads include lavash, naan, and English muffins, and unleavened bread includes wheat tortilla or chapatti and paratha and puri that is made from whole meal atta. In single-layered flat breads, the dough is baked immediately after sheeting, and for Arabic and Turkish flat breads, the two layers that form during baking are a result of docking of the dough (perforating the surface). Flat breads are usually baked at very high temperatures 300–600 °C (572–1112 °F) for a very short time (seconds) in clay ovens (such as tandoors) or hot presses.

Turkish bread (photo V.Solah)

Flat bread must be soft and pliable and are traditionally usually eaten the day they are made.

Steamed bread or steamed bun

Steamed bread is a staple food of China and there are many types, but these can be broadly classified into three styles northern-style, southern-style, and Guangdong-style. Steamed bread is made from flour with low to medium protein (9.5–11%) in northern China and low protein (8–10%) in southern China. Part of the flour is mixed with water, dry yeast, and lactic acid to start the sour-dough fermentation, which is used in the production of most Chinese steamed bread, which can be unfilled or filled. Mantou is a type of fermented bread with no filling and has smooth white skin and a compact crumb structure. Bao or pao has a sweet or savory filling. The northern-style steamed bread is made from hard wheat flour with high protein level and medium to strong dough strength and has a firm, cohesive, and chewy texture. The southern-style steamed bread is softer and less chewy and made from wheat flour with medium dough strength and protein content. Guangdong-style steamed breads/buns such as char siew bao are typically made from soft wheat with low protein (7.5–8%) and weak dough strength, water, dry yeast, lactic acid, and sugar (20%). Important quality attributes of steamed bread include a white-colored skin and crumb, a smooth, glossy thin skin, and a fine uniform cohesive crumb structure.

Steamed buns (photo V.Solah)

Noodles

There is a vast range of types of wheat-based Asian noodles that vary with respect to formulation, size and shape, and processing method. White salted noodles, prepared from a simple mixture of flour, water, and salt, are popular in China, Japan, and Korea and are growing in popularity worldwide. In Japan, popular types include very thin somen, thin hiya-mugi, and flat wide kishi-men or hira-min and thick and square (sharp-edged) udon. In China, there is very thin long xu mian, thin xi mian, and flat wide dai mian or cu mian. Another major category of Asian noodles is broadly referred to as yellow alkaline noodles, and these incorporate alkaline salts, particularly sodium and potassium carbonates. Instant noodles, which are popular throughout Asia, also fall into the category of alkaline noodles because of the incorporation of a small addition of alkali in their formulation.

Noodle appearance, texture, and taste are strongly influenced by the characteristics of the wheat flour, other ingredients, and how the noodles are processed. Protein, fat, and salt contents and energy density and how they are eaten (in soup or stir-fried) are important considerations in determining the role of noodles in the diet.

White salted noodles: udon (Japan)

Japanese white salted noodles are known as udon. Udon noodles are made from flour, water, and salt, and

sometimes starch, such as tapioca, is added. Ingredients are made into a crumbly dough and sheeted through sheeting rolls and then made into strands by passing through cutting rolls.

Soft wheat flour is recommended with protein 8.0–10.0% with high-swelling starch (Figure 2), but medium-hard wheat with similar protein content and high-swelling starch is also suitable. Low extraction flour (60%) is needed to provide the low ash and bright creamy white color, speck-free high-quality noodles. Fresh or dried udon is boiled in water and eaten in soup or mixed with vegetables, seafood, or meat. Udon noodles should be soft but not excessively soft, elastic, or springy and have a smooth mouthfeel, with slight stickiness.

White salted noodles (China)

Chinese white salted noodles are made from hard wheat flour, water, and salt. The ingredients are usually made into crumbly dough and sheeted through sheeting rolls and then cut into strands. The flour protein content ranges from 9.5% to 11.0%. Fresh or dried noodles are boiled in water and often eaten in soup. The noodle color should be bright white and the surface visually smooth, and when eaten, noodles should be medium-firm, elastic, and smooth, with no stickiness, and have a fragrant wheat smell.

Dried Chines noodles (photo V.Solah)

Yellow alkaline noodles

Yellow alkaline noodles are consumed in most Asian countries. The ingredients typically consist of flour, water, salt, and kansui (alkaline solution), which are mixed to form a crumbly dough sheeted through sheeting rolls and then cut into strands. The flour protein content (Figure 2) and kansui used vary for the many various types of noodles. Ramen (Japan) is 10.5–12% protein and uses sodium and potassium carbonate as the alkali, and Hokkien (Southeast Asia) is 10.0–11.5% protein and uses sodium hydroxide as the alkali. Sometimes, additives such as eggs or yellow coloring are added.

Cooked noodles colored with β-carotene (photo V.Solah)

The noodles should be bright, yellow, and glossy with no specks and the texture should be firm and elastic.

Durum

Durum wheat, *Triticum durum*, is harder than other wheat and grown at a higher protein content (13–14%; Figure 2). Semolina is coarsely reduced endosperm. Semolina is used for traditional pasta, couscous, and semolina pudding such as halva.

Pasta

Pasta is produced from a mixture of durum semolina at 13–14% protein and water, sometimes with the addition of eggs. Pasta is mixed into a slightly dry dough and sheeted through sheeting rolls and then cut into sheets as for lasagna or strands as in fettuccine or extruded under pressure through a die into vermicelli, spaghetti, penne, or macaroni or stamped into shapes such as farfalloni (bow tie) or rotelle (wagon wheel).

Cooked pasta should be firm (al dente), with a creamy yellow color, and have a rough surface due to the effect of coarse semolina. Pasta can be made from a mix of durum and hard wheat semolina or hard wheat semolina alone but the eating and nutritional quality would decrease.

Pasta can be made with whole meal flour but this has a large impact on texture, especially in thin pasta. Pasta is digested more slowly than bread and other foods, and it is more satiating due to the hard, vitreous, coarse character of the semolina, which remains essentially intact in the final product.

Couscous

Couscous is rolled granules of durum semolina (10–12% protein) originating from northern Africa. Couscous is popular in Morocco, Algeria, Libya, France, and Egypt and around the world. Couscous is produced by mixing durum semolina and water into a crumbly dough. The dough is then rolled by hand or rotated in a drum or sieved to form small round particles. The raw couscous is then steamed and dried in the sun or using

a commercial rotary drier. Couscous should have an even creamy yellow color, and the granules should have an even size and should remain in their granule form and not be sticky when cooked. The hard, vitreous, coarse character of the semolina, which remains partially intact in cooked couscous, makes it slower to digest and more satiating.

Limitation

Instant noodles, cakes, and biscuits are allowed as 'sometimes food,' that is, eaten only sometimes, that are not recommended to be consumed every day and therefore are not included in this article.

Conclusion

The composition, structure, and special properties of the wheat grain have allowed the evolution of many unique food products worldwide. The understanding of the development of the gluten and starch gel matrix has grown in recent times but much more research is needed into wheat quality for specific end uses. In ancient times, methods were developed to process wheat into bulgur, chapatti, bread, noodles, pasta, and couscous. In modern times, industrialization and mechanization have meant new equipment and methods to mix, sheet, cut, shape, and bake. The consumer interest in healthier wheat products that taste great will mean new methods and equipment in the future.

See also: Bread: Breadmaking Processes; Bread: Chemistry of Baking; Bread: Dough Mixing and Testing Operations; Bread: Types of Bread; Wheat: The Crop.

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Wheat: The Crop

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Wheat Production and Consumption

Wheat is one of the world's major staple food grains with over 700 million tonnes (Mt) produced annually. It is the primary food grain for human consumption and in terms of total grain production is ranked 2nd only to corn, with >975 Mt produced annually, much of which is used for animal feeding purposes, and well ahead of rice, wheat is the other major human food grain (~475 Mt annually).

Table 1 lists the countries with an annual wheat production of over 8 million tonnes. The European Union (EU-27) is now the world's largest wheat-producing region, with France, Germany, the United Kingdom, Poland, Spain, and Italy being the leading contributors. Table 1 also lists the total cereal grain production for some countries, highlighting the relative importance of wheat compared with the other grains, in particular rice and corn. The reasons for this are twofold, the adaptability of the wheat plant and the unique nature of wheat storage proteins enabling the production of a plethora of food types. These will be covered in more detail later in this article.

Some countries both consume and produce substantial quantities of wheat so that they are approaching self-sufficiency. These include China, the European Union, India, Iran, and Pakistan. However, depending upon seasonal production and other conditions, countries such as these might be a net importer or exporter. In contrast, other countries with large wheat consumption do not produce wheat sufficient for their needs, so are regular major importers such as Algeria, Brazil, Egypt, Indonesia, and Japan. Finally, there are other countries like Argentina, Australia, and Kazakhstan that are large producers of wheat but are relatively minor consumers, resulting in significant surpluses that they routinely export.

Table 1 Average world production of wheat and all cereal grains for the 10-year period 2004/2013 by countries and regions in millions of tonnes (metric tons)

Country/region	*Wheat production (Mt)*	*Production of all cereal grains (Mt)*
The European Union	137	304
China	111	480
RUK	83	
India	80	240
The United States	59	436
Russia	50	88
France	38	
Canada	27	59
Australia	24	37
Germany	23	
Pakistan	22	34
Ukraine	19	60
Turkey	20	32
Kazakhstan		
Iran	14	
The United Kingdom	18	
Argentina	9	47
Egypt	8	
World	706	2432

Source: The Food and Agriculture Organization of the United Nations (FAO).

Wheat-Growing Conditions

The wheat plant is extraordinarily adaptable, and as exemplified by the countries listed in Tables 1 and 2, it can be grown in a wide range of environments stretching from the dryland conditions of Australia and Mexico where yields of as little as 1 tonne ha^{-1} can be experienced due to poor soil types or limited rainfall, to the ideal wheat-growing conditions of the southern United Kingdom and northeastern France where 10 tonnes ha^{-1} of crops is commonplace, to the extreme winter temperatures experienced in the northern parts of the United States, Canada, and Russia.

In order to survive and flourish in these vastly disparate climates, the wheat plant has adapted remarkably. Much of the Northern Hemisphere wheat crop has a 'winter' habit, being sown in autumn or 'fall,' laying relatively dormant under the snow that provides both protection from freezing air temperatures and water for ongoing development once warmer spring temperatures arrive and the snow melts. This process is known as vernalization and is the trigger for the plant to continue its development through to grain development and maturity. In some temperate climates, this can result in a very long growing period between sowing and harvest, putting additional pressure on the farming practices being employed.

Conversely, the extreme winter conditions that are typical of the Prairie Provinces of Canada and the Great Plains of the Northern United States are way too severe for winter wheat to survive. In their place, 'spring' wheat are typically grown, sown in spring once the land has thawed and can be worked, and these tend to be fast-growing and gradually overhaul the 'winter' types grown further south to be ready for harvesting before winter returns. This provides the additional challenge of falling temperatures, which can prevent the grain from drying down to a level where it can be stripped from the stem and safely stored. This has required the development of a process called 'swathing' where the crop is cut and pushed up into 'windrows' where the grain dries down to a manageable moisture level prior to threshing to separate the grain from the straw, rachis, and glumes. There is an added complication that as this final drying stage extends into winter, there is an

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Table 2 Global wheat dynamics – consumption, production, imports, and exports

Country	5-Year average consumption (thousand tonnes)[a]	Per capita supply (kg year^{-1})[b]	FAO ranking	5-Year average production (thousand tonnes)[a]	FAS ranking	5-Year average imports (thousand tonnes)[a]	FAS ranking	5-Year average exports (thousand tonnes)[a]	FAS ranking
Algeria	9410	184.9	4	2555[b]		6877	4		
Brazil	11 320	53.5	78	5536	16	7081	2		
Canada	9465	69.6	54	28 525	6			19 618	4
China	120 700	63	63	120 307	2	Varies seasonally			
Egypt	18 280	146.1	13	8110	14	10 144	1		
The European Union	121 916	101.8	36	141 447	1	5248	6	24 683	2
India	87 035	58.9	70	90 394		Varies seasonally		3560	10
Indonesia	6793	23.5	117	–[b]		7060	3		
Iran	16 680	152.3	11	13 440	12	4160	11		
Iraq	6194[b]	139.6	16	2800[b]		3522	15		
Japan	7355[b]	48.6	84	750[b]		6189	5		
Mexico	6390[b]	33.6	106	3628[b]		4297	8	1032	11
Morocco	8570	177.3	5	6018[b]		3681	13		
Pakistan	23 840	113.8	28	24 240	8	Varies seasonally			
Russia	35 850	130.8	20	49 312	5			15 487	5
Turkey	17 530	173.5	6	16 860	10	4185	10	3660	9
Ukraine	12 290	106	33	20 341	9			7397	7
The United States	33 284	79.5	48	57 529	3	3706	12	29 795	1
Uzbekistan	8120	170.6	7	6690	15	2061	21		
Others	138 975			12 275		51 607		11 670	12
Countries of note making up part of 'others' grouping									
Argentina		103.2	35	12 900	13			6949	8
Australia		69.8	53	26 236	7			19 819	3
Bangladesh		17.3	124			3121	17		
Kazakhstan		94.4	42	13 730	11			7438	6
South Korea		51.8	82			4695	7		
Nigeria		24.5	116			4285	9		
The Philippines		14.7	129			3605	14		
Saudi Arabia		90.6	43			2649	18		
Sudan		35.8	103			2202	20		
Thailand		11.8	133			2025	22		
Vietnam		8.8	142			2220	19		
Yemen		114	27			3135	16		

[a]5-Year average 2010/11 to 2014/15 World Wheat Production, Consumption, and Stocks and World Wheat Flour and Products Trade reports from the US Department of Agriculture, Foreign Agricultural Service.
[b]2011 wheat and product food balance sheet from the FAO for 149 countries.

increasing chance of early snowfall, further compromising grain moisture levels.

In Southern Hemisphere environments on the other hand, the problems are the complete reverse; with, depending on the latitude and altitude of the region, and without the fear of extreme winter conditions, sowing normally takes place in autumn from April to June and the plant develops through the southern winter months of July, August, September, and October and then matures with the onset of summer. This usually concludes with hot, dry, harvesting conditions that produce grain of less than 12% moisture content, making it ideal for long-term storage. There are however still danger periods, as a result of either early flowering where an untimely frost can kill developing florets or, at the other end of the spectrum, late flowering where high temperatures can have an adverse effect on protein development leading to weak dough properties. In really extreme arid climates such as those experienced in Saudi Arabia where wheat is grown under irrigation, grain moisture levels as low as 6% can result. This then provides further but potentially profitable challenges for the miller when the grain has to be 'conditioned' by water addition to toughen the outer bran layers and bring it up to the

optimum moisture content of from, say, 13% to 16.0% before being milled into flour.

Another interesting phenomenon is seen in temperate climates where the so-called 'dual-purpose' wheat can be employed. They are sown very early as soon as there is sufficient moisture to do so, and they can then be used for grazing livestock, following which the plants recover and go on to produce a grain crop in the conventional manner. Even then, the farmer has the flexibility of cutting and baling the entire crop for feed purposes, or if the grain is of marketable quality, it can be harvested and marketed in a conventional manner. These are usually very late-maturing, long-season varieties that can take up to 11 months from the time of sowing to harvest.

The Green Revolution

Wheat varieties developed for broad-acre agriculture were traditionally tall plants with a long straw that was also used extensively for animal feeding or thatching. However, with that came the downside of the plants being unstable, particularly in dry, sandy soils, and subject to 'lodging' where in windy conditions, whole sections of the crop would lay down, making it difficult to harvest. The problem was largely solved by the introduction of semidwarfing genes into wheat genomes. A Japanese dwarf wheat cultivar 'Norin 10' was the primary source and the resultant short-strawed and much more stable varieties contributed to improved wheat yields worldwide. These have been credited with being one of the main factors in the 'Green Revolution,' the phenomenon whereby cereal production more than doubled in many developing countries between the years 1961 and 1985. These production increases can be attributed roughly equally to irrigation, fertilizer, and seed improvements, and the overall impact of the Green Revolution on global food security is the subject of much debate.

The world population has grown by about 4 billion since the beginning of the Green Revolution, and India saw an annual wheat production rise from 10 million tonnes in the 1960s to 73 million in 2006. Between 1950 and 1984, as the Green Revolution transformed agriculture around the globe, world grain production increased by over 250%, and these production increases are often credited with having helped to avoid widespread famine and feeding billions of people.

Conversely, there are also claims that as well as saving many lives, the Green Revolution has decreased food security in many developing countries by encouraging a shift from subsistence agriculture to cropping aimed at producing grain for export or stockfeed.

Origins of Wheat and Its Products

Most authorities agree that the cultivation of wild grass species first took place around 10 000 BC in the area now bounded by parts of Israel, Palestine, Jordan, western Syria, southeast Turkey, and northern Iraq, commonly referred to as the Fertile Crescent, and the early forbearers of modern wheat can still be found in this region, providing valuable sources of new genes for wheat breeders (Figure 1).

The bread, durum, and spelt wheat that we know today are all the result of natural hybridization between wild grass species. These are mostly diploids containing one set of seven pairs of chromosomes. Two diploid grasses with different genomes (chromosome complements) naturally hybridized to produce new plant types with 14 pairs of chromosomes. Tetraploids like the wild emmer wheat (*Triticum turgidum*) were the origins of modern durum or pasta wheat (*Triticum turgidum* ssp. *durum*). Further natural hybridization events occurred between domesticated emmer and another diploid grass (*Aegilops tauschii*) to produce plants with 21 pairs of chromosomes (hexaploids), giving rise to the bread (*Triticum aestivum*) and spelt (*Triticum aestivum* ssp. *spelta*) wheat.

This cultivation of wild grasses for food purposes and the associated establishment of semipermanent settlements of individuals who sowed, tended, harvested, and stored these early food crops are now linked to what is loosely termed 'civilization.' The cultivation and utilization of temperate cereal grains as a staple source of food then spread throughout the Mediterranean and parts of what is now Europe and Central Asia, and while the chronology of these movements is uncertain, there is archaeological evidence of emmer being cultivated in Iraq as early as 10 000 BC.

The Etruscans developed handmade pasta, and this manufacture is depicted in tombs near Rome built in the fourth century BC and was eventually handed down to the Romans and still exists today. The Etruscans seemed to have sourced their grain locally, but the Romans brought their wheat mostly from Egypt and Sicily where durum wheat appears to have been cultivated. Drawings from Egyptian tombs from about 2500 BC show farm activities indicating that emmer was grown and harvested in ancient Egypt.

By 1700 BC, wheat had a role in the Chinese diet. The ancient Chinese ground wheat in a stone mortar to make flour. There is evidence that noodles were made during the Han dynasty, 206 BC to AD 220, and were extremely popular in the Ming dynasty, AD 1368–1644, when they were first called *miàn*. Noodles have continued to evolve since that time and many types exist today. The Chinese also developed steamed breads, steamed buns, and dumplings (Figures 2 and 3).

The origins of bread follow closely on those of the organized cultivation of grains. Initially, grains would have been consumed fresh from harvesting; however, it was discovered that the caryopses of cereal species are extremely robust and can be stored for lengthy periods without any significant loss of functionality or palatability. This was a massive breakthrough in early food technology and provided these early civilizations with the ability to store a staple food and in so doing provide insurance against future periods of acute food shortage.

Grains would have been ground into a rough wholemeal between heavy stones, mixed with water to form a dough and baked on hot stones either around or dragged from within an open fire, into primitive flatbreads.

Some of these doughs would have been left exposed to natural yeast and allowed to ferment under the high ambient temperature conditions common to the region, prior to the final baking stage. The bread produced in this way would have been immediately recognized for its more appealing flavor, texture, and appearance, and this simple and most likely

Figure 1 The Fertile Crescent.

Figure 2 Various types of dried noodles.

Figure 3 Fried and steamed Chinese dumplings.

accidental process would have been the precursor for the art of baking as we know it today.

Over the following millennia, with the constant migration of conquering armies and civilizations, the cultivation of cereals and the consumption of bread as a staple food spread throughout what we now know as Western and Eastern Europe, with the common bread wheat, *Triticum aestivum*, largely replacing the primitive einkorn (*T. monococcum*) and emmer (*T. dicoccum*) species that had gone before.

Bread was initially baked in the home using simple home-ground wholemeal flours, and the styles of bread and the methods used to make them evolved to satisfy local tastes and eating habits. Flour milling emerged as a local business where, for a fee, farmers could have their grain milled into flour for

Figure 4 Traditional-style Arabic pocket bread.

Figure 5 Traditional Arabic flatbread.

their own use or to sell in the marketplace. Subsequently, bread making on a commercial scale emerged as a vital component of local society, and as will be outlined later in this article, wheat-based foods evolved into the myriad styles that we know today.

Today, in the Middle East, traditional flatbreads are fast-baked in very hot ovens; however, they were originally baked on hot stones around open fires. Similar techniques were used in Central Asia and the Indian subcontinent (Figures 4 and 5).

In Europe, the use of yeast-fermented loaf-type breads has predominated, resulting in the myriad of styles seen today throughout the entire region (Figure 6).

In Eastern Europe and Scandinavia, many types of bread, with or without the inclusion of rye flour in the formulation, are produced by the sourdough process where the leavening agents are naturally occurring wild yeast and bacteria. The bread has a characteristic 'sour' flavor and is as old as the history of leavened bread itself. Most of the bread is produced as small loaves or rolls, which all have extended shelf lives. In Italy, rather robust and chewy breads, *pane di casa*, are baked in wood-fired ovens from flour often milled from a grist including durum wheat.

The development of pan breads occurred in Britain. Yeasted doughs were baked in an open metal tin, which determined the shape of the loaf. This eventually led to the development of lidded tins and the popular 'sandwich loaf' that we know today.

Figure 6 Various types of hearth or artisan bread.

European Colonization

From the fifteenth to the eighteenth centuries, European explorers, and in particular the Portuguese, Spanish, British, and French, sailed the globe in search of land to claim for their own. The early colonizers took their staple foods and domestic animals with them, and in so doing, wheat, wheat flour, and bread spread to the Americas, the Indian subcontinent, Southern and Western Africa, and Australasia, becoming the staple food of the then New World, now known as the Western world.

Much more recently, these 'Western-style' foods have become popular in many regions around the world, in particular Central, Southeast, and North Asia. This is in much the same way that Asian foods are now an integral component of the menu in most Western countries.

Interestingly, however, the Western foods that have gained popularity throughout Asia have often been modified to suit local tastes. In particular, the volume, appearance, and sugar content of popular bread types have emerged into styles that are more akin to confectionery than plain bread, reflecting the higher status that bread often holds in these societies than in the West.

Why Wheat Is So Different

The processing quality of wheat is arguably more complex than that of any of the popular food grains, with numerous genetically determined quality traits interacting with each other, in ways that can have a profound influence on the quality of the finished product and the profitability of commercial milling and baking operations. The situation can be further influenced by environmental factors – the conditions under which the crop was grown – which can also have a significant impact on the processing performance, product quality, and hence market value of the grain.

When wheat flour and water are mixed, the inherent water-insoluble proteins known as 'gluten' combine to form a continuous matrix referred to as a 'dough,' and of all of the many quality traits of wheat, it is the unique viscoelastic properties of wheat flour dough that set it apart from the other food grains.

The inherent strength, resistance to extension, and extensibility of wheat flour doughs can vary enormously between

wheat varieties. By careful management of the variety and the environmental conditions under which it is grown, protein content and quality can be controlled to produce dough properties that suit particular food products and manufacturing processes. It is this unique capability to produce viscoelastic doughs of vastly differing physical characters that provides the key to the extraordinary range of wheat-based foods consumed worldwide.

As a consequence, wheat tends to command a premium in the marketplace above prices obtained for most other food grains, and the protein content and inherent protein quality of wheat have a significant impact on its market value.

Dough Processing

Once one has produced a wheat flour dough of the desired quality, it may be manipulated in a variety of ways to generate foods of vastly different appearances, tastes, and characters.

Doughs may be leavened, the process by which gas is introduced into the dough by yeast fermentation or chemical addition, to produce an attractive light open-textured structure in the finished baked product.

Before baking, dough pieces may be shaped into an endless array of styles of breads, buns, and rolls, which are often a characteristic of the cultures that have developed them.

Doughs may be rolled into sheets and baked in that form into flatbreads and tortillas, which for convenience are then used to wrap other foods prior to eating. Dough sheets may be cut into shapes and baked into biscuits and are used to encase fillings such as with Chinese dim sum and Italian-style filled pasta, prior to cooking.

After sheeting, doughs may be cut into strips before further processing, as is the case with the seemingly limitless styles of noodles produced and consumed throughout Asia and now spreading to the rest of the world.

Doughs may also be extruded through dies, which is the popular means of producing pasta products in an endless array of shapes, lengths, and thicknesses and most snack foods.

Food Preparation

Once a dough piece has been produced and leavened, sheeted, cut, stamped, or extruded, it may be dried for preservation as it happens in dry pasta and then cooked, or it can be cooked straight away by a variety of means.

The earliest and arguably most common form of wheat-based food preparation is baking to produce foods such as pan and hearth breads, buns, cookies, biscuits and cakes, and pastries.

However, processed doughs may be cooked in many other ways in order to produce foods with different flavors, textures, and appearances, with the most common being steaming, boiling, frying, or freezing.

Steaming is a very common practice in many parts of North Asia where steamed bread in a range of forms; steamed buns, both plain and filled with sweet or savory fillings; and steamed dumplings are an integral part of the daily diet.

Boiling is the standard method of preparation for most noodle types and for all forms of pasta products, whether they be short (e.g., penne and macaroni) or long (spaghetti and fettuccine) extruded goods or filled types like ravioli and cannelloni.

Frying has been the traditional form of processing for most types of instant noodles. The finished product is deep-fried prior to packaging in order to pregelatinize the starch and introduce flavor and texture, which may then be reconstituted into a ready-made meal simply by adding boiling water. However, rising concerns about the deleterious effects of fried food consumption have seen a swing to steaming for the final processing stage prior to packaging.

Finally, freezing is now a very common form of wheat-based food processing, where the product is taken to a very late stage in its preparation and then frozen prior to packaging, distribution, and marketing. The product, which can be safely stored for extended periods, is thawed prior to the final step in its preparation whether it is by steaming, boiling, or baking.

Similarity of Products Around the World

As a result of the unique properties of wheat flour doughs, distant populations independently developed similar wheat-based foods.

Dumplings that use thin dough sheets to enclose fillings are quite popular in China. Similar types of dumplings are also consumed in Japan and Korea.

Other types of dumplings are also popular in many Eastern European countries. Various types (*pelmeni*, *pierogi*, *knödel*, and *kalduny*) exist in Russia and countries from the former Soviet Union, Hungary, Poland, and Germany. *Kreplach* are the Jewish version of these Eastern European dumplings.

The Italians use sheeted doughs to make *lasagna*, *ravioli*, *and cannelloni*.

In the north of China, thin-sheeted flatbreads called *bing* are popular. Peking duck pancakes are a type of bing, and *shaobing* is a thin-pocketed bread that can be stuffed with savory fillings. These breads are similar to some Middle Eastern flatbreads and Mexican tortillas.

Chinese steamed breads (*mantou*) and filled steamed buns (*bao*) are quite similar to the boiled dumplings made in some European countries.

Some European pasta products bear a striking similarity to the texture and appearance of white salted noodles.

White- Versus Red-Grained Wheat

Wheat is categorized in the international grain trade by its seed coat color. There are two distinct types, red-grained wheat and white-grained wheat, and they are genetically very similar.

Both red and white wheat have advantages and disadvantages. Red wheat are in widespread production in the Northern Hemisphere where the harvest period is in late autumn and early winter. Rain events often interrupt harvest, so grain dormancy becomes an important attribute conferring sprouting tolerance. White wheat do not possess the same tolerance

to preharvest sprouting and tend to be grown in regions where the likelihood of rain at harvest is lower.

Australia is unique among international wheat exporters in that it only produces white-grained wheat for milling purposes. As a result, Australian wheat is preferred in certain markets ahead of the red wheat mostly grown by the other major wheat exporters. This strong customer preference has seen Australia's major competitors like Canada and the United States attempt to develop sufficient quantities of white wheat to compete in the highly demanding markets of Asia. However, to date, North American hard white wheat varieties have failed to show any yield advantage over comparable red-grained varieties, which, together with reduced sprout tolerance and limited storage options, has resulted in much of the hard white crop being absorbed by the US domestic market for products like pizza bases.

Red wheat produce dark and quite bitter bran that limits their use for whole wheat products and for products using flour that requires a bright and white appearance, like noodles, dumplings, and steamed breads.

The bran from white wheat does not impart a bitter flavor to grain products, and any bran specks that get into the flour are less noticeable. Thus, white wheat can be milled to higher flour extractions without the deleterious effects of bran contamination. Flours produced from Australian white wheat are ideal for producing Asian products such as noodles, dumplings, and steamed breads where a bright, clean external appearance of the final product is paramount.

Wheat and Health Issues

As outlined earlier, wheat is one of the major sources of nutrition worldwide, and in many countries and cultures, it is a major source of energy, protein, essential amino acids, essential fatty acids, vitamins, minerals, and fiber. Traditionally, wheat and other cereal grains have been one of the key components in all scientifically based healthy food diets, but for a number of reasons, those recommendations are now being called into question.

In the past decade, the cereal industry has witnessed the emergence of a new food phenomenon variously described as wheat allergies and gluten intolerance. In the popular press, this has been sensationalized, raising concerns among consumers, many of whom, without any formal diagnosis, declare themselves to be intolerant to wheat-based foods and seek a gluten-free diet. The gluten-free food explosion is massive with anecdotal evidence in many Western countries, suggesting an annual increase of around 20%. This is providing a challenge to the wheat-based food industries with concerns about reduced consumption rates and the need to produce alternative food products that address the demand for 'gluten-free' products, whether it is clinically based or not.

For a small proportion of individuals in many countries, gluten intolerance is very real with potentially very serious consequences. For around 1% of the population, wheat and other cereals including rye, barley, oats, and triticale are poisons, not foods.

Celiac disease (CD) is not an allergy but is an immune-mediated condition triggered in genetically susceptible individuals by the ingestion of grains containing gluten. Once expressed, the condition is lifelong, causing damage to the small intestine, which prevents efficient absorption of nutrients, bowel upset, and an increase in the risk of intestinal cancers, osteoporosis, chronic fatigue, and infertility and a doubling of the overall risk of death. There is no known cure and the only treatment is lifelong avoidance of gluten. In Australia, only about 20% of cases are currently diagnosed. The percentage is significantly lower in most other countries of the world.

Many patients presenting with symptoms of allergy (including asthma, eczema, migraine, Ménière's disease, irritable bowel syndrome, brain fog, nasal polyps, and some mental disorders such as depression) are found to have elevated antibodies to gluten, yet they are negative for other markers of CD such as tissue transglutaminase and are normal on small intestinal biopsy. Removal of all gluten-containing foods from the diet has been shown to have significant beneficial effects. At this time, it is unclear what role, if any, gliadin-specific IgA and IgG play in this condition.

Gluten intolerance (nonceliac gluten sensitivity) is more prevalent than CD worldwide, with up to 12% of the population affected. In a recently published book, *The Gluten Syndrome*, Dr. Rodney Ford, a pediatric allergist in New Zealand, provides compelling evidence from a 1000-patient cohort that 10% of the population may be sensitive to gluten but are not celiac. Typically, these patients experienced a range of different symptoms including gut disorders, behavior problems, allergy/immune issues (e.g., eczema), and growth problems.

Clearly, there is growing clinical evidence that health problems associated with dietary gluten aside from CD are real and far more prevalent than might be expected and are going to lead to major threats and opportunities for the food industry.

The growing number of wheat-related food disorders draws attention to this serious health concern. In addition to the obvious detrimental effects of health and daily diet, there are potentially long-term medical, psychological, and social effects. Correctly diagnosing wheat- and gluten-related disorders is often challenging, especially given the limitations of current diagnostic testing. However, it is important to prevent children from unnecessary invasive treatments and potentially unhealthy dietary restrictions. Therefore, research is needed to determine the precise prevalence of different wheat- and gluten-related disorders, to discover more accurate and less invasive procedures for the identification of the mechanisms that lead to adverse reactions against wheat and related cereals and find suitable source materials with low or no allergenic effects.

See also: Bread: Types of Bread; Cereals: Dietary Importance; Cereals: Storage; Wheat: Grain Structure of Wheat and Wheat-based Products.

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Whey and Whey Powders: Fermentation of Whey

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Whey as a Fermentative Source

Whey is a by-product of cheese; ~85% of the total milk used for manufacturing cheese is discarded as whey. Most milk plants do not have proper treatment systems for dealing with this amount of whey; it is completely prohibitive to divert whey in waterways or even in municipal sewage systems. Thus, disposal of whey becomes a big problem – not only because of the environmental, economic, and human health problems, but also for the significant loss of food and energy potential. Whey retains about 55% of milk nutrients. During the last 50 years, to overcome this problem, the research community has made efforts to develop new biotechnological processes in order to suggest ways of exploiting cheese whey to decrease the whole production cost. Those new processes could be based on the application of valorization technologies – recover proteins and lactose or produce subproducts obtained by hydrolyses – and another choice could be the application of biological and/or physicochemical treatments without valorization, aiming for the legal discarding of whey. Nevertheless, the richness of whey composition leads us to consider it as a resource and not only as a waste effluent, in view of the large potential of new added-value products production, which may contribute wholly or partially to the disposal costs.

Even so, nowadays, only 50% of world cheese whey is treated, from which 50% is used in liquid form, 30% as whey powder, 15% as lactose and its by-products, and the remaining 5% in the form of whey protein concentrates. The costs associated with valorization technologies are normally intolerable by medium and small producers, so they are forced to choose the cheapest solution.

As previously referenced, there are two options regarding the enhancement of whey. The first one – more commonly used when whey powder is produced in excess – is based on the application of technology in order to recover the added-value compounds (proteins by membrane filtration systems and/or chromatography techniques and lactose by crystallization). The second one is applying the fermentation process to obtain whey by-products. Sometimes there is a combination of both, using whey permeate obtained from the whey protein concentrate production as the fermentation medium, which is rich in lactose (~80%). When it is desirable to concentrate this solution, nanofiltration and reverse osmosis processes can be used.

Lactose is the carbohydrate reservoir of whey (70% of total whey solids), being largely responsible for the whey-polluting load. Per year in the European Union, there is a surplus of ~620 000 tons of lactose, which is not used in the production of lactose and its by-products, thus representing a serious environmental problem.

This carbohydrate is a disaccharide formed by galactose and glucose. Its solubility and sweetness is low when compared with other sugars, even its hydrolysis products (glucose, galactose, fructose, and sucrose). Lactose can be used as a food ingredient in infant formula, the confectionary industry, baker's yeast, meat products, and kefir-like whey drinks. It can also be applied in the pharmaceutical industry as an excipient for most table drugs because of its characteristics (innocuous, nonhygroscopic, inert). When submitted to fermentation coupled with other essential nutrients for microbial growth, lactose presents a wide range of possibilities, making the whey permeate a suitable raw material for the production of several by-products, such as organic acids (e.g., lactic, succinic, and propionic); single cell protein (SCP) and single cell oils (SCO); biopolymers (enzymes, bioplastics, and exopolysaccharides (EPS)); bacteriocins; and biofuels (potable or bioethanol, biogas, biohydrogen), with lactose providing the carbon source.

Lactose-Consuming Microorganisms

There are different types of microorganisms that use lactose as a carbon source and as energy, among which are bacteria, yeast, and filamentous fungi.

Bacteria have different strategies in order to hydrolyze lactose. The most common involves phosphorylation and sugar transport into the cell, which can be done through two mechanisms: lactose-proton symporter and lactose–galactose antiporter. Once inside the cell, if phosphorylated, lactose is broken by phospho-β-galactosidase if not by β-galactosidase.

The lactic acid bacteria (LAB) are Gram-positive, usually catalase-negative, anaerobic facultative, nonmotile, nonspore forming, devoid of cytochromes, fastidiously acid tolerant, and strictly fermentative. They comprise several genera; the most usual are *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*. Due to their low toxicity, LAB are usually known as GRAS (generally regarded as safe) bacteria, so they are commonly used in the food industry, being the most significant lactose-consuming microorganisms with an important role in lactic acid production. The essential feature of LAB metabolism is the efficient carbohydrate fermentation, coupled with substrate level phosphorylation. Basically, there are two major pathways for hexose fermentation in LAB: homolactic and heterolactic (facultative or obligate). Homolactic fermentation is the process where lactic acid is the unique product of glucose fermentation. Through the Embden–Meyerhof glycolytic pathway, homolactic bacteria can extract twice the energy from a defined amount of glucose than heterolactic fermentation. Homofermentative behavior is observed when the glucose is metabolized, but not necessarily when pentoses or gluconate are, because some homolactic bacteria produce acetic and lactic acid when using pentoses. Theoretically, through homolactic fermentation, one molecule of glucose produces two molecules of lactic acid and a net gain of two ATP molecules per molecule of glucose.

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The homofermentative character of some strains can be changed by changing the growth conditions (glucose concentration, pH, and nutrient limitation).

In the heterolactic fermentation process, the production of lactate, carbon dioxide, acetic acid, and ethanol from hexose occurs. In the facultatively heterolactic fermentative process, hexoses are metabolized through the Embden–Meyerhof glycolytic pathway, but pentoses and some other substances are metabolized via a phosphoketolase-dependent pathway to produce lactic acid and other products (typically acetic acid and ethanol). On the other hand, the obligate heterolactic fermentative species use only the phosphoketolase-dependent pathway for sugar metabolism. So in addition to lactic acid, they produce significant quantities of acetic acid and/or ethanol with the generation of carbon dioxide. The presence of oxygen may also significantly affect the metabolism. The heterolactic bacteria are more important than the homolactic in the production of aroma and flavor components such as acetaldehyde and diacetyl. The process of the formation of diacetyl from citrate in the food industry is essential for the formation of odor, for example in the manufacture of butter.

Different factors affect the growth of LAB in fermentation media, such as temperature, hydrogen ion concentration, and pH.

The second most commonly used microorganisms in the lactose fermentation process are yeasts; the ones that assimilate lactose aerobically are widespread, whereas the ones that ferment lactose are rare (*Kluyveromyces lactis*, *K. marxianus*, and *Candida pseudotropicalis*).

Filamentous fungi utilize lactose, but at a very low rate. In fungi, the lactose break can be done in two ways: one by an extracellular hydrolysis and a catchment of lactose monomers (*Hypocrea jecorina* and *Aspergillus niger*), and the other by lactose molecule uptake as a disaccharide and then hydrolysis inside the cell (*Aspegillus nidulans*, *Neuspora crassa*, and *Fusarium graminearum*).

Whey Fermentation Products

Organic Acids

Although acetic, propionic, lactic, lactobionic, citric, gluconic, and itaconic acids can be obtained from lactose/whey fermentation, from an economical point of view, lactic acid is the most important.

Lactic acid

Lactic acid or α-hydroxypropionic acid is widely used in the food, chemical, textile, and pharmaceutical industries. As previously mentioned, a process for the recovery of whey lactose is through LAB fermentation, in order to produce lactic acid as the major metabolic end-product of sugar fermentation. Ultrafiltration is one of the processes used to get raw lactose from cheese whey. The resulting permeate of this method presents a low protein content but is high in lactose and minerals; these factors are favorable for lactic acid production.

The industrial demand for lactic acid by fermentative production (~90%, ~72 000 tons worldwide per year) has been increasing considerably, owing to its use in an expanding range. For example, in the food industry, besides being used as a natural preservative and acidulant, it is used in novel foods and products designed to have specific nutritional or other health-enhancing benefits (nutraceuticals, prebiotics, probiotics, etc.). However, it has been mainly used as a raw material in the promising applications in food packaging, and one of the widest fields of the plastics market.

In recent years, several studies aimed at optimizing the production of the lactic acid process have been performed. Thus, among LAB, *Lactobacillus helveticus* appears to be the most effective, followed by *Streptococcus*, which also shows satisfactory results with respect to lactic acid productivity. The search for satisfactory and efficient LAB strains for cheese whey fermentation has been carried out by genetic engineering approaches.

When cheese whey or its permeate is used to obtain lactic acid through LAB, the addition of supplements is very important. Otherwise, at the industrial scale, productivity is drastically affected, as nutrient supplementation is a key factor limiting process efficiency. Accordingly, supplementation of manganese, yeast extract, peptones, molasses, corn-steep molasses, malt combing nuts, soya flour, lactose, glucose medium, vitamins, minerals, amino acids, hydrolyzed whey, and inorganic supplements ($MgSO_4$, $MnSO_4$, and $(NH_4)_2SO_4$) to the whey is useful for increasing the lactic acid yield. This production could double if proteolytic enzymes or proteolytic microorganisms are used instead of these supplements. In the same way that supplementation with nutrients is important for good growth of the microorganisms with the concomitant high production of lactic acid, also the temperature or use of mixed cultures affects the production of lactic acid.

Immobilization technology applied to whey fermentation processes (bio-film, membrane-based cell recycle reactors, fibrous-bed) is identified as a good solution to improve the economics of the process, allowing cells reutilization, higher cell densities in bioreactors, long-term stability, and easier purification and productivity of lactic acid. However, it also presents some limitations. The continuous fermentation process presents the main problem, with the biggest difficulty being the complete conversion of sugar, exhibiting limited production of lactic acid due to the lack of pH stability, clogging of the column reactor, destabilization of the gel used for immobilization/entrapment by chelating calcium lactate, diffusion limitations of substrate and product, and loss of stability of the biocatalyst activity. LAB have been immobilized by several methods on different supports (calcium alginate, k-carrageenan, agar, and polyacrylamide gels); however, more studies must be undertaken to try to eliminate the less positive aspects and improve the performance of the reactors to achieve high stability, high productivity, and low operating and capital costs.

Biofuels

Nowadays, biofuels can be produced by fermentation of sugars, followed by distillation and drying. The most widely produced are bioethanol and biogas (methane); however, biohydrogen can also be produced. The ability to produce biofuels from raw material, such as whey permeate disposal, shows low

environmental effects. That will be the key to making it competitive with other fuels, such as gasoline.

Biogas

Nowadays, the world markets for biogas are booming. Biogas is produced by anaerobic microorganisms and can be used to produce thermal energy (heating); electricity; or if compressed, fuel vehicles. It is a gas comprising methane (CH_4 – 50–70%), responsible for determine the biogas properties, and carbon dioxide (CO_2 – 50–30%), being produced by a wide variety of available waste organic materials through anaerobic fermentation, leading to a safer disposal of effluents. One cubic meter of biogas has an average calorific value of about 6.5 kWh. Advances in biotechnology, high tech, molecular science, and microbiology have contributed to enhancements in biogas yield production. As a result, biogas competes with petroleum-based fuels in terms of performance, cost, and additional benefits such as reducing greenhouse gas emissions. Innovations are still necessary to support research and development in the field of renewable energy. In that order, renewable biomass feedstock is needed.

Although microorganisms involved in anaerobic digestion are not fully identified, at least four groups of microorganisms are involved in this process. Hydrolytic bacteria degrade organic molecules into simpler compounds (CO_2, hydrogen, alcohols, and organic acids); acetogenic bacteria convert organic acids and alcohols into acetate and hydrogen; homoacetogenic bacteria form only acetate from hydrogen, CO_2, organic acids, alcohols, and carbohydrates; and methanogenic bacteria produce methane from acetate, CO_2, and hydrogen. All of these microorganisms play an equally important role in methane production. The accumulation of intermediate compounds led to a methanogen inhibition and concomitant microorganism imbalance, decrease in methane production, or process failure. The two main steps (acidogenesis and methanogenesis) are normally out of balance even at low digester feed rates. The usual fermentative bacteria are *Lactobacillus*, *Eubacterium*, *Clostridium*, *Escherichia coli*, *Fusobacterium*, *Bacteroides*, *Leuconostoc*, and *Klebsiella*. Examples of acetogens are *Acetobacterium*, *Clostridium*, and *Desulfovibrio*. Methane-producing organisms are classified under domain Archaea, phylum AII, Euryarchaeota. Archaea is a group of prokaryotes that differ from bacteria.

Concerning biogas production by anaerobic digestion of whey, some studies have shown a decrease of chemical oxygen demand, which is intensified with the use of two reactors in series. Increasing methane production results when an anaerobic sludge blanket reactor is used and with anaerobic treatment of diluted and undiluted cheese whey supplemented with trace metal, in contrast with the presence of copper chloride, zinc chloride, or nickel chloride presenting a significant inhibition of methane production, which could be reverted with the addition of sulfide.

When two-phase processes are used, such as anaerobic digestion with acidogenic and methanogenic reactors coupled to a membrane filtration system, a thermophilic process with partial acidification followed by a methanation step and a continuous process enables the removal of the soluble effluent while retaining solids, an increase in methane production, and an increase in hydrogen and methane production, respectively. A mathematical model was applied to determine the optimum physiological conditions during acidogenesis of cheese-whey wastewater.

Bioethanol

Bioethanol is an alcohol made by fermentation, mostly from carbohydrates produced in sugar or starch crops such as corn, sugarcane, cereal, sugar beet, or sweet sorghum. It has a number of advantages over conventional fuels, being biodegradable and far less toxic than fossil fuels. It is generally CO_2 neutral, and it can help reduce the amount of CO_2 as well as other poisonous gases produced during fuel combustion. Bioethanol can be used as a fuel for vehicles in its pure form, but it is usually used as a gasoline additive to increase octane and improve vehicle emissions. Nowadays, the bioethanol market continues to grow rapidly.

Whey could be a suitable and less expensive substrate for bioethanol production. All over the world, there are several industrial-scale whey-ethanol plants. Fermentation could be done in batch cultures or in a semicontinuous process, for which a kinetic model was developed.

Yeasts are usually the microorganisms used in the fermentation of lactose from ultrafiltered whey permeate to bioethanol production. The potential applications of yeast strains are focussed on *Kluyveromyces* sp. Though it has a great advantage because of the use of lactose as a direct substrate, it is very sensitive to ethanol concentration (inhibiting the process at very low concentrations). In addition, it presents a low conversion yield (~40%). Thus, *Saccharomyces cerevisiae* can be used, presenting a fermentation yield of 50% and a fourfold ethanol tolerance. Notwithstanding, the fermentation performed by this yeast is an indirect one, not having the ability to hydrolyze lactose directly. Although the yeasts that assimilate lactose aerobically are widespread, those that ferment lactose are rare. In the last decades, researchers have been studying ways to overcome this problem. Genetic engineering is used in order to achieve the optimal yeast strain. Other efforts have been made, aiming to facilitate the process with a simultaneous lactose hydrolysis, for example by coimmobilization of yeast cells with the enzyme.

The development of yeasts' biotechnology processes and engineering techniques leads to the application of different stimulation processes that improve the biological activity of yeasts, achieving new ways to produce higher ethanol amounts. It has been seen that proper ultrasonic stimulation has the function of promoting the activity of enzymes, cell growth, and cell membrane permeability.

Biohydrogen

Biohydrogen is commonly used for heating, lighting, and as a motor fuel. Its production has grown over the years; it has been increasingly used as alternative energy, with the great advantage of the absence of polluting emissions, where the only product of combustion is pure water. Nowadays, most hydrogen is obtained from fossil fuels that generate gases, contributing to global warming due to the greenhouse effect.

On a laboratorial scale, there are different ways to obtain biohydrogen through fermentation (photofermentation, dark fermentation, and both combined); however, information given on an industrial scale is rare.

Fermentative hydrogen production could be coupled with the anaerobic process of acidogenic bacteria of biogas production. This fermentation is usually called dark fermentation and leads to the formation of organic acids from organic material degradation. A two-stage fermentation process, in which acidogenesis and methanogenesis occur in the separate reactors, may offer several advantages, such as improved total wastewater degradation and enhanced biohydrogen and methane production. Studies pertaining to the use of carbohydrate-rich wastewaters as fermentation substrate in order to obtain biofuel on an industrial scale are still very embryonic. Although there are preliminary studies that indicate great potential for the use of raw materials in biohydrogen production, details with respect to the optimization of process and operating parameters are needed, whether on an industrial or laboratory level in some cases. The scale-up of industrial production is a key point.

Single Cell Protein and Single Cell Oil

The production of SCP or SCO can be performed by algae, actinomycetes, bacteria, yeast, molds, or higher fungi, and its dried cells can be obtained by large-scale culture systems, which can be used as a protein or lipid source. In the case of whey fermentation, lactose is usually converted in biomass by yeasts, usually GRAS. Lactose can be directly used by yeasts, hydrolyzed into glucose and galactose, or fermented by LAB into lactic acid and galactose. The use of whey derived or even lactose as a carbon source for producing yeast biomass leads to increased harnessing of industrial by-products, normally used as feed or baker's yeast.

Depending on the type of yeast, it is not always necessary to add vitamin supplements to achieve a high fermentation yield. However, the specific growth rate usually increases with the addition of yeast extract and corn-steep liquor. It is also necessary to add a nitrogen source; in this case, ammonium salts are enough. If the pH and temperature optima of microorganisms cannot match the optimum conditions for production of single cells, there must be a compromise.

The most common processes applied on SCP are the Vienna process, which entails only a single species *Candida intermedia*, with an exclusive oxidative metabolism; and the Bel process, which presents a balance between three species: *Kluyveromyces marxianus* var. *marxianus* (metabolizes lactose), *K. marxianus* var. *lactis* (metabolizes lactic acid), and *Candida pintolopepsii* (metabolizes ethanol). The Bel is a continuous aerobic process, where deproteinated whey is used as substrate and cannot be supplemented with nitrogen, phosphorus, and trace elements, depending on the type of whey.

When biomass is produced from whey with a high amount of lactose hydrolyzed, it is possible to choose strains that do not metabolize lactose, so the first sugar to be metabolized is glucose. The process for the production of baker's yeast involves whey as substrate and an immobilized enzyme (lactase) followed by glucose–galactose fermentation.

Kluyveromyces and Candida are the most commonly used strains in SCP production from whey. Some studies have been done in order to achieve the optimal conditions of the processes. Thus, different fermentation modes, temperatures, substrates, supplements, and concentrations have been tested:

- *K. fragilis* is used in bath production using whey as substrate.
- Yeast biomass with an improved nutritional profile is produced by methionine-enriched *K. lactis* mutants in whey permeate.
- Production of SCP uses *K. marxianus* CBS6556; in deproteinized sweet whey supplemented with trace elements, ammonium and calcium, and sour cheese whey concentrates supplemented with ammonium, trace elements and vitamins.
- Considering the economic factor, studies with several batch reactors in series were done, having seen a drastic decline in energy costs.
- Mathematical models have been developed so that experimental data is described.

Other yeasts and microorganisms are also been explored:

- Increased SCP production of *Wingea robertsii* using cheese whey as substrate supplemented with minerals and yeast extract
- Development of a fed-batch system for scale-up kefir biomass production using whey
- Use of whey or buttermilk supplemented with yeast extract for the growth of thermophilic LAB
- Probiotic biomass from deproteinized and non-supplemented milk whey
- Batch, continuous, and fed-batch *Lactobacillus casei* in deproteinized goat milk whey

For SCO production, oleaginous microorganisms (*Zygomycetes*, *Mortierella isabellina*, *Thamnidium elegans*, and *Mucor* sp.) were tested with the aim of discovering their ability to produce biomass with specialty lipids, lipid-containing g-linolenic acid (GLA), and polyunsaturated fatty acids from whey. An appreciable microbial growth and a variation of GLA concentration depending on strains and fermentation time can be seen.

Biopolymers

Enzymes

Whey or whey derivates in the form of lactose have also been used as substrates for the production of different enzymes, with β-galactosidase (β-D-galactoside galactohydrolase), commonly known as lactase, as one of the most important enzymes produced due to its important role in the lactose enzymatic hydrolysis, catalyzing the break of the β1–4′ linkage in lactose to yield glucose and galactose. This enzyme plays a key role in food processing.

β-galactosidase can be isolated and purified from a wide range of microorganisms. Notwithstanding the fact that bacteria *Streptococcus thermophilus* and *Bacillus stearothermophilus* can be used to produce lactase, yeasts and fungal sources are mainly responsible for the production of this enzyme. *Kluyveromyces* spp. *K. marxianus*, *K. lactis*, and *K. fragilis* are the strains normally responsible for production in a lactose-rich medium. In most cases, this medium requires the addition of supplements in order to optimize production of the enzyme.

The optimum conditions for enzyme production and extraction vary according to yeast strains. However, the improvement

of new technologies can help optimize the feeding rate in fed-batch cultures and overcome the extraction problem. Genetic engineering can also improve β-galactosidase enzymatic activity at different temperatures, fostering its use in the hydrolysis of lactose in milk and other dairy products.

Lactose-rich products have been also employed on the production of other enzymes, such as protease, amylase, and polygalacturonase, though different microorganisms viz. fungus, bacteria, and yeast, respectively, although for supplementation with different nutrients such as yeast extract, gelatin, peptone, aspartic acid, casein, or acetamide, dried sweet whey is required in all cases.

Bioplastic synthesis

Polylactic acid

As previously mentioned, lactic acid could be used as a raw material in the plastics market. Namely lactic acid polymer, the polylactic acid (PLA), is an environmentally friendly biodegradable plastic (if disposed in landfills, it will last in the environment for years). Not only is it used as a specialty medical material, but it also has other industrial applications as an alternative to plastics derived from petrochemicals. Also, it has applications as a biodegradable plastic component (polylactide, polymers, polyhydroxybutyrate).

The physical properties of PLA depend on the enantiomeric composition of lactic acid, where an optically pure lactic acid (one enantiomer) is desirable and extremely important. Once the type of fermentation and the optical configuration of lactic acid are dependent on the genera of LAB, a fermentative means of lactic acid production has an advantage over chemical synthesis. Thus, it is possible to choose the specific LAB strain that will degrade cheese whey, in order to produce the desired lactic acid.

Polyhydroxyalcanoate

Polyhydroxyalkanoates (PHAs) are aerobic bacterial polyesters that have received interest in recent years, as they can replace common plastic materials. As a surplus product with environmental impact, whey lactose can be applied for the production of these future-oriented biopolyesters, thus providing a promising approach to solving prevailing economic problems.

Among biodegradable plastics, PHAs have been considered good substitutes for petroleum-derived synthetic plastics (polyethylene and polypropylene). Though they possess similar chemical, physical, and mechanical properties, PHAs are distinguished by their degradability after disposal, UV resistance, oxygen impermeability (fundamental property for food packaging), and biocompatibility (essential property for medical and surgery applications). PHA biosynthesis occurs via condensation of acetyl-CoA units. They are synthesized using expensive synthetic substrates, so it is necessary to search for a cheap alternative. Usually when a nutritional factor is limited (e.g., nitrogen, phosphorus, potassium, oxygen, sulfur, or magnesium), in the presence of an excess carbon source, several microorganisms redirect the carbon flux from biomass formation toward accumulation of PHAs. The optimum strategy of applying whey for PHA production depends mainly on the selected production strain. Bacteria that have a GRAS status for PHA production, such as LAB and bacilli belonging to probiotic species, might constitute an added value to these biotechnological processes.

Several studies were related to the production of PHAs from whey and its derivates using pure cultures of wild-type microorganisms or recombinant ones. In several of these works, whey lactose was hydrolyzed by lactase.

There are different ways to convert whey lactose to PHAs. Only a restricted number of microorganisms directly convert lactose to PHAs, for example the wild-type stains of *Hydrogenophaga pseudoflava* DSM 1034 and *Sinorhibium melitoti*. This can be overcome when whey lactose is hydrolyzed enzymatically or chemically to glucose and galactose; these monosaccharides are converted by a broader number of organisms. Poly-β-hydroxybutyrate (PHB) was produced with *Ralstonia eutropha* DSM545 on whey permeate and inverted sugars. *Pseudomonas hydrogenovora* DSM 1749, *Hydrogenophaga pseudoflava*, and *Haloferax mediterranei* are able to cometabolize glucose and galactose from lactose-hydrolyzed whey permeate supplied with sodium valerate to produce PHB. A pH-stat fed-batch fermentation and supplemented whey powder has been used for PHB production using a recombinant *E. coli* strain GCSC 6576 and strain K24K harboring the PHA biosynthesis genes of other species. PHB production was also described in LAB belonging to *Lactococcus*, *Lactobacillus*, *Pediococcus*, and *Streptococcus* genera. PHB was also produced on whey and lactose by *Methylobacterium* sp. ZP24.

A third possibility arises from the anaerobic conversion of lactose in a first-step fermentation by LAB such as *Lactobacillus lactis*, *Propionibacterium*, *Lactobacillus delbrueckii* toward lactic acid with high yields; lactic acid can serve as substrate for PHA production. These strains could act as a coculture fermentation system, where lactic acid produced by LAB is taken up by *Cupriavidus necator* to produce PHAs, resulting in an increased yield, as well as the possibility of utilizing substrates cheaper than glucose for production of PHAs. Thermus species have been found to utilize disaccharides such as lactose.

Exopolysaccharides

These molecules are polysaccharides with diverse high molecular weights (10–1000 kDa). They may be homopolymeric, when composed of either D-glucose (glucans) or D-fructose (fructans) residues, with different types of linkage and branching degree; or heteropolymeric, constructed from multiple copies of an oligosaccharide (the most common) and showing little structural similarity to one another: glucose, galactose, xylose, mannose, arabinose, and rhamnose.

The interest in EPS has increased in recent years because of their potential properties, having revolutionized the industrial and medical sectors. They can be added to food products (ensuring safety qualification) or produced in situ during dairy product fermentation, acting as natural thickeners, giving the product a suitable consistency, and acting as emulsifiers and gelling agents, improving viscosity and reducing syneresis. Some of them may have beneficial effects on human health, being related with microbial cell protection from toxic compounds, phagocytosis, osmotic stress, antibiotics, prebiotics, anticoagulants, antithrombotics, immunomodulation, and anticancer effects. That way, LAB-synthesized EPS have been implicated as antitumor agents, immunostimulators, and blood cholesterol lowering agents. The increasing demand by consumers for novel dairy products requires a better understanding of the effect of EPS on existing products and, at the same time, a search for new EPS-producing strains with

desirable properties. The use of genetically modified organisms capable of producing high levels of EPS or newly designed biopolymers is still very limited.

The ability to produce EPS is widespread among LAB, although they can be produced by microalgae, too. The GRAS and probiotic status of some lactobacilli give them more preference for consumable EPS production; the best known are *L. casei*, *L. acidophilus*, *L. brevis*, *L. curvatus*, *L. delbrueckii bulgaricus*, *L. helveticus*, *L. rhamnosus*, and *L. plantarum*. Nevertheless, *Lactobacillus* sp. are not the best polysaccharide producers; there are other bacteria offering a great yield. For EPS production, there are Gram-negative bacteria *Xanthomonas campestris*, *Acetobacter xylinum*, and *Sphingomonas paucimovilis*; for xanthan, acetan, and gellan production, respectively, or even for dextran production by strains of the LAB *Leuconostoc mesenteroides*.

As expected, due to high lactose content, whey and its derivates are great substrates for EPS production by fermentation; some practical examples are presented in Table 1. All of the studies related to EPS production and optimization point to a strict relationship among the effects of temperature, whey permeate concentration, type and level of supplementation, dilution rate, and interactions on biomass with the yield. Furthermore, the functional properties of the EPS could be modified by the influence of growth conditions. Studies pertaining to immobilized cell technology for production of EPS as a functional food ingredient clearly show its high potential. Immobilization and culture time-induced cell aggregation could be used to produce new symbiotic products with very high viable cell and EPS concentrations.

Bacteriocins

Bacteriocins have been claimed to be valuable tools in improving food safety, namely in dairy, bakery, meat, and fishing products, as well as in fruits, vegetables, and beverages. Besides the food industry, they can be applied in medical, pharmaceutical, and veterinary fields. The term bacteriocin means a protein/peptide antibiotic of relative high molecular weight, which is active mainly against the same or closely related species via adsorption to receptors on the target cells. Bacteriocins are synthesized, at the ribosomal level, through fermentation by various Gram-positive and Gram-negative bacteria, acting toward strictly correlated bacteria (growth inhibition or kill) – they are an antibacterial substance. LABs are able to synthesize different classes of bacteriocins, including antagonistic molecules. Generally, bacteriocins are produced at the end of the exponential growth phase, and their spectrum of action can vary depending on the producing species. Nysin is the only bacteriocin that is industrially gaining widespread permission and authorization in commercial food application. Several studies have been done in the last few decades in order to achieve potential production and application of bacteriocins, once they present important features inhibiting the growth of foodborne bacterial pathogens and demonstrating biopreservative action in food.

Bacteriocins are divided into three classes: antibiotics, small hydrophobic heat-stable peptides, and large heat-labile proteins. Such compounds can be incorporated in food in a variety of ways: using a preparation of semipurified or purified bacteriocins as an ingredient (e.g., nisin); incorporating a previously fermented culture containing bacteriocin; using a substitute for the bacterial culture; or as part of the culture itself, which can release bacteriocin in situ (which is the approach closest to the concept of bioconservation).

Although bacteriocins can be produced in the food matrix during food fermentation (in situ), bacteriocins by LAB can be produced in much higher amounts during fermentations under optimal physical and chemical conditions. Bacteriocin production depends on pH, temperature, media composition, agitation, and aeration.

Bacteriocin production by whey or its derivates' fermentation is summarized in Table 2. Nisin can be produced in batch cultures during the bacteria exponential growth phase, using filtered or unfiltered skim milk, whey, sweet whey, or pasteurized whey as substrate. Higher nisin concentration obtained in whey filtered, or in some cases diluted, was probably related to insoluble proteins released into the media; higher nisin production was observed in diluted whey than in concentrated whey.

Table 1 Exopolysaccharide production by whey or its derivates' fermentation

Microorganism	*Fermentation mode*	*Substrate*	*Supplement*
Lactobacillus bulgaricus LB3 and LBs	Batch fermentation	Whey	1% peptone
Lactobacillus rhamnosus RW-9595-M	Batch fermentation	Whey permeate	Yeast nitrogen base
Lactobacillus rhamnosus RW-9595-M	Repeated fermentation with immobilized cells	Whey permeate	$MgSO_4{\cdot}7H_2O$, $MnSO_4{\cdot}7H_2O$, Tween 80 and yeast extract
Lactobacillus rhamnosus RW-9595-M	Continuous fermentation with free and immobilized cell chemostat culture	Whey permeate	Yeast extract
Lactobacillus delbrueckii subsp. *bulgaricus*	Continuous fermentation	Deproteinized whey	Peptone, $MgSO_4{\cdot}7H_2O$, $MnSO_4{\cdot}7H_2O$, Tween 80 and yeast extract
Aureobasidium pullulans P56	Batch fermentation	Deproteinized whey + enzyme-hydrolyzed whey	K_2HPO_4, L-glutamic acid, olive oil, and Tween 80
Xanthomonas cucurbitae PCSIR B-52	Batch fermentation	Partially deproteinized whey	Corn-steep liquor and penicillin waste mycelium
X. campestris pv mangiferaeindicae 1230 and pv manihotis 1182	Batch fermentation	Whey	$MgSO_4{\cdot}7H_2O$ and K_2HPO_4
Streptococcus thermophilus ST 111	Batch fermentation	Milk + whey protein hydrolyzate	Nitrogen source

Table 2 Bacteriocin production by whey or its derivates' fermentation

Bacteriocin	*Microorganism*	*Fermentation mode*	*Substrate*	*Supplement*
Nisin	*Lactococcus lactis* UQ2	Bioreactor (controlled pH 6.5)	Sweet whey	Soybean peptone
	Lactococcus lactis subsp. *lactis*	Batch fermentation (extremely low-frequency magnetic field)	Whey permeate	
	Lactococcus lactis subsp. *lactis* CECT 539	Batch fermentation	Diluted whey	
	Lactococcus lactis	Batch fermentation	Skim milk or whey	
	Lactococcus lactis ATCC 11454	Batch fermentation	Pasteurized whey (filtered)	
	Lactococcus lactis subsp. *lactis* ATCC 11454	Continuous fermentation with immobilized cells (packed-bed bioreactor)	Whey permeate	Casein-hydrolyzate
Nisin-Z	*Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* UL719	Repeated fermentation with immobilized cells (controlled pH 6.5)	Whey permeate	
Pediocin	*Pediococcus acidilactici* NRRL B-5627	Fed-batch fermentation (re-alkalized)	Diluted whey	2% yeast extract
	Recombinant strains *Lactococcus lactis* ssp. *lactis*, *Streptococcus thermophilus*, and *Enterococcus faecalis*	Batch fermentation	Skim milk or whey	0.5% yeast extract
Enterocin AS-48	*Enterococcus faecalis* A-48-32	Batch fermentation	Whey derived – Esprion-300	1% glucose

The same result could be achieved for pediocin production obtained in diluted whey supplemented with yeast extract.

Studies encompassing the immobilization of strains in the presence of supplemented supplemented media showed an increase in production, emphasizing the case of nisin continuous production through *Lactococcus lactis* subsp. *lactis* ATCC 11454 in a whey permeate supplemented with casein hydrolyzate, where the bioreactor was operated continuously for 6 months and submitted to the optimal conditions, without encountering any clogging, degeneration, or contamination problems.

In recent years, special attention has been given to the study of bacteriocins derived from *Bacillus*, because they present a broader spectrum of inhibition compared to LAB, leading to an increase in potential applications.

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See also: Cheese: Composition and Health Effects; Dairy Products: Dietary and Medical Importance; Milk: Sources and Composition; Whey and Whey Powders, Principles and Applications of Dialysis; Whey and Whey Powders: Production and Uses; Whey and Whey Powders: Protein Concentrates and Fractions.

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Whey and Whey Powders, Principles and Applications of Dialysis

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Membrane Protein Separation

One booming market is that of health-promoting foods – foods that are able to promote health and reduce the risk of diseases if regularly included in balanced diets. Over the years, whey has gained a strong position as a valuable source for dairy-based ingredients with applications within a wide range of physiological roles (namely related to the immune, cardiovascular, nervous, and gastrointestinal systems), being able to respond to demand by specific consumer groups. Thus, the dairy industry has been searching for nonconventional conversion of effluents into added-value products, following economically feasible routes. Consequently, whey begins to no longer be a disposal problem; it is now a profit center. The value of whey products and their individual components increases significantly when the components are separated and purified. Because of this, a great number of techniques have been developed, namely centrifugation and membrane technologies.

Membrane separation processes are used to fractionate and/or concentrate a liquid by passing it through a semipermeable membrane, which allows differential concentration of a liquid provided that the solute at stake is larger in diameter than the membrane pore (cannot cross membrane – retentate or concentrate). The liquid that percolates the membrane (permeate or filtrate) has in its composition components smaller than the membrane pore diameter. Thus, the separation process is based on the selective permeability of the initial different liquid compounds. The selective permeation is the result of a driving force; the application of hydrostatic pressure; or in a few cases, electric potential, depending on the type of separation. The mechanisms that mediate the mass transport in different membrane processes vary, depending on the type of membrane, process conditions, and equipment configuration. Usually, the different types of membranes are described with reference to pore size and the cutting point regarding molecular weight.

During the classic filtration process, the suspension to be filtered is poured perpendicular to the filter media. Under these conditions, it produces an accumulation of material, forming a layer that decreases the porosity and, consequently, the flow rate of filtration. So, this kind of filtration does not allow an acceptable duration of time when applied to the industrial scale. The frontal filtration processes are improved by introducing a third component, in the form of a speed gradient parallel to the membrane surface. This new process vector acts at the side of fluid to concentrate. Thus, the tangential filtration is a membrane process under the action of a pressure gradient in which the membrane is filled by a liquid-containing substance, which will be separated. This liquid must be pushed by enough gradient pressure in order to allow passage of filtrate or permeate through the membrane, created by a pump or an electric current. The concentrate or retentate corresponds to the part of the liquid that does not pass through the membrane, in which one or more compounds present in the initial feed liquid are concentrated. The filtration flow rate corresponds to the flow rate of permeate that passes through a given membrane surface and is usually expressed in $l\ h^{-1}\ m^{-2}$.

In the last 40 years, the development of membrane techniques for food industry applications has been intrinsically related to the membrane evolution. From the earliest membranes made of cellulose acetate to the third generation of mineral membranes, important advances have been made. Changes that allow the expansion of filtration technologies include the improvement of their selective permeability, high resistance to temperature and extreme pH values, greater mechanical strength, dead volume decrease, replacement facility, and reduction of cost per square meters. Market membranes can be classified according to different criteria, such as selectivity, chemical nature, structure, mode of operation, and geometric configuration.

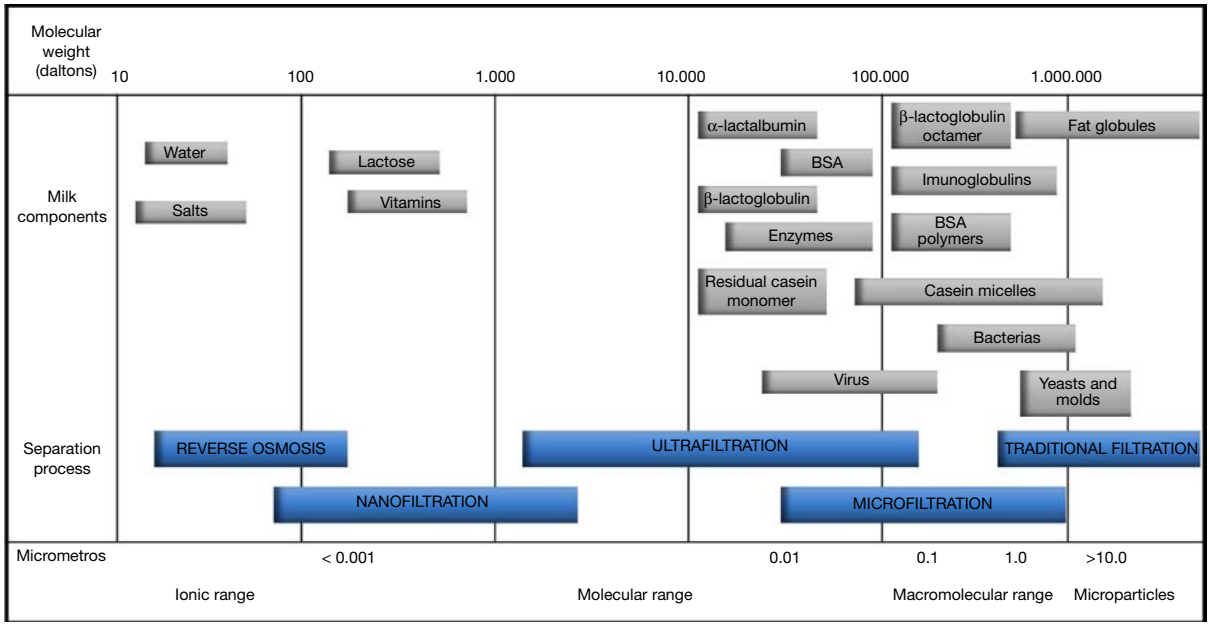
So, membrane technology presents important features such as its straightforward scale-up and relatively low cost when compared with alternative separation/concentration methods, as well as elimination of heat treatment that allows the maintenance of nutritional and physiological properties of whey components.

Membrane Selectivity and Process Type

The food industry, especially the dairy field, has been a pioneer in developing equipment and techniques of membrane filtration. Whey proteins can be recovered in a nondenatured state through filtration processes already implemented on an industrial scale, as well as fractionation of whey proteins that allows studies of the biological properties of some high-purity whey components, with the prospect of possible industrial and biomedical applications. With water content in the raw material of about 94%, there are numerous possibilities for water recovery in whey processing.

One of the most relevant barriers to the widespread use of whey for food is its high relative ash, two times higher than for milk. The mineral salts affect the flavor, functionality, quality, and value of whey products. This problem is solved by whey demineralization, aimed at removing divalent metals and reducing active acidity, leading to expand the scope of whey use in the food industry. In the same way, the large-scale reduction of microbial loads could be performed.

According to their selectivity, one can distinguish membranes and processes that can be applied to whey. Figure 1 shows the spectrum of separation of milk components that can be obtained by different membrane processes, according to the size of the molecules and the pore diameter of the used membranes. Typically, the procedures include basic membrane



Process:	Reverse osmosis (RO)	Nanofiltration (NF)	Ultrafiltration (UF)	Microfiltration (MF)
Applied pression:	200 – 1.200 psig	150 – 400 psig	30 – 150 psig	20 – 100 psig
Retentate:	Total solids	Total solids except monovalent ions	Proteins and fats	fats, large proteins and particles
Filtrate:	only water	monovalent ions	salts, non-protein nitrogen and lactose	Lactose and salts
Separation method:	ability organization of the compounds into tetrahedral structures determine the permeation capacity	diffusion and flow through the pores controlled by mass transfer, characteristics of diffusion and charges	shape, charge, flexibility, MW determines the ability to cross the membrane	size determines the ability to cross the membrane

Figure 1 Relationship between membrane filtration techniques (membrane pore size range) and relative size of various components of milk retained. Adapted from Alfa Laval/Tetra Pak, 1995.

separation, for example, reverse osmosis (RO), ultrafiltration (UF), and diafiltration (DF), which permit fractionation of proteins, as well as concentration and purification thereof; nanofiltration (NF) and electrodialysis (ED), which allow removal of salts or low-molecular weight contaminants; and microfiltration (MF) to remove suspended solid particles or microorganisms. Sometimes it is necessary to use a combination of membranes in order to get the desired effect.

MF is a process that uses membranes with internal pore diameter of 0.1–20 μm separating particles bigger than 200 kDa molecular weight. Depending on the pore size, one may separate whey components with remains of caseins, phosphoproteins, or skimmed milk components such as somatic cells, bacteria, and spores. The working pressures used are less than 0.2 MPa.

UF is a membrane process that separates components based on a molecular filter. Membranes have a pore size of between 0.015 and 0.1 μm, allowing the separation of molecules within a molecular weight of 1–200 kDa. In the case of cheese whey UF, lactose, nonprotein nitrogen, water-soluble vitamins, and salts pass through the membrane, though protein and fat are retained. The choice of the pore diameter depends on the molecular mass and conformation of molecules that are intended to be separated (more specifically, its Stokes radius). The working pressures used are smaller than 1 MPa.

DF is employed to increase the recovery of solutes across the permeable membrane during the UF or MF processes. The DF process consists in diluting the concentrate, usually with water, and then continuing the separation until a satisfactory degree of solute is removed. It allows, for example, the obtainment of a protein concentrate with low lactose and minerals content.

NF is a membrane process under high pressure, which is able (as RO) to reverse the effects of osmotic liquids. The NF is processed by mass transfer phenomena, consisting of diffusion and flow through the pores; it can also use membranes that are electrically charged, so that they can select which ions can pass through. In addition, it can be used to concentrate. NF membranes are partially permeable to ions, minerals, and other low molecular weight components (they let in compounds with a molecular weight cutoff between 200 and 1000 Da); being employed to demineralize. The monovalent ions normally pass through the permeate while the divalent ions are concentrated in the retentate. The diameters of the pores are between 0.001 and 0.015 μm, to working pressures of 0.7–3.5 MPa. This process began to be applied in the dairy industry in order to obtain concentrates with low concentrations of salts, although in some cases it also reduces the lactose content.

RO is a process in which the membrane separates the solvent (usually water) and solutes with molecular weights below 150 Da of the remaining components of the solution. This separation is achieved by applying external pressure that exceeds the osmotic pressure, so it gets water flow through a membrane, contrary to what would be produced naturally. It is a process of solute concentration. The pore diameter is less

than 0.001 μm, and the working pressures used are between 4 and 8 MPa. One of their applications is in the concentration of whey by eliminating ionized water and minerals.

Electrodialysis

ED principle

ED belongs to the group of membrane-based separation processes. It is a combination of two phenomena: a membrane phenomenon, which implies multimembrane cells responsible for the dilution/concentration and water dissociation applications (dialysis method); and an electrode phenomenon with electrolysis cells for redox reactions (electrolysis method). So, ED is an electrochemical separation process where small electrically charged ions are transported through one or more selective permeable membranes (electrically charged) from a desalted stream (permeate) to a stream with a higher concentration of salts (concentrate), under the influence of an electrical potential (Figure 2). This phenomenon leads to the separation of salts from the electrically uncharged compounds. Therefore, it is a method based on the selective movement of ions in solution, contrary to other membrane technologies that separate particles based on their molecular weight.

ED takes place in an electrodialyzer, an equipment with a stack that consists of a series of anion and cation exchange membranes (AEM and CEM) arranged in an alternating pattern between two electrodes (anode and cathode) to form individual cells and spacers. Worldwide, ED dominates as a desalting process, exceeding RO, and distillation applications. It is used in the pharmaceutical, chemical, and food industries, namely in the separation and purification of inorganic and organic solutions, water treatment (ultrapure, waste, sea, brackish, salty mine, and industrial waters), recycling of chemical substances, wine stabilization, desalination of fruit juices, whey demineralization, and others.

Ion-exchange membrane

Ion-exchange membranes are made of molecular material containing fixed ions immobilized in the membrane. These could be positively charged with alkyl ammonium groups in the case of anionic membranes, or negatively charged with sulfonic, carboxylic, arsenic, or phosphoric groups in the case of cationic membranes. Both of these membranes are monopolar, being permeable to only one type of ion. Therefore, the transport of an ionic species through the membrane depends on the nature of the ion. Membranes are electronically neutralized by counterions (mobile ions with opposite sign charges), which carry current in the membrane, exchanging by diffusion between membranes and the surrounding solution.

ED membranes present selectivity in the result of an electrostatic repulsion. That way, they may be permeable to cations (in the case of a CEM), where anions are repulsed, or anions (in the case of an AEM), where cations are repulsed, and do not allow the passage of water back into whey because of the chemistry of the porous membranes used. Thus, the process leads to separation of electrolytes from nonelectrolytes, concentration or reduction of electrolytes in solution, and the exchange of ions between solutions.

ED types and applications in the dairy industry

There are two types of ED, the most common use being monopolar membranes. The principle of the method has already been described. In the case of dairy products, it is responsible for demineralization of milk whey, protein separation and purification, acid caseinate production, and organic acid. The main application of this technique in food technology is whey demineralization, with the aims of producing whey concentrate with low ash content. This is necessary for further whey use as a raw material for other valuable products such as infant formula and animal feed, or for the recovery of proteins. These applications use the sole property of dilution-concentration of the monopolar ion-exchange membrane.

In addition to this conventional method, there is a newer one that is performed with bipolar membranes, presenting the same support principle as monopolar ED, although carrying out the dissociation of water molecules. Besides AEM and CEM, bipolar membranes possess a hydrophilic transition layer at their junctions. It is not yet well known the explication

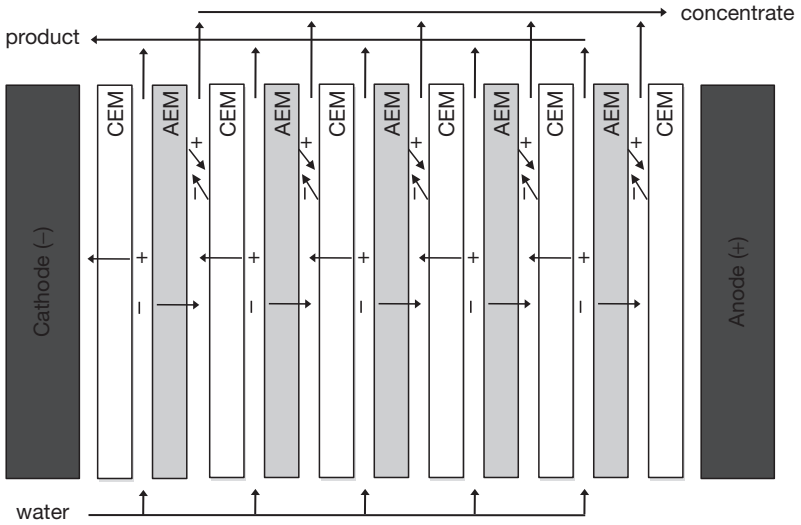


Figure 2 Principles of electrodialysis.

of the magnitude of the electric current and changes of pH value observed near the membrane with this method. In the case of the dairy industry, it is applied in the purification of fermented solution – lactic acid production from whey, production of caseinates, and fractionation of whey proteins.

Whey ED

Nowadays in the dairy industry, the ED is successfully applied, in order to obtain a whey raw material without mineral ions (ca. 6–15% in a solid base), keeping the microbiological quality, the native protein content, and key properties.

Whey and water are pumped through the track. The first is pumped in the interspace of adjacent sheets of membrane, while the second is pumped through the flat spaces between those interspaces (see Figure 2). The applied voltage encourages cations to move through the porous membranes from the whey into the water toward the cathode. Negatively charged, cation-selective membranes reject ions with the same charge, letting positively charged ions pass. The same happens with anions moving to the anode. Ions can be removed from whey by placing multiple membranes in a row, which alternately allow positively or negatively charged ions to flow through. In some columns concentration of ions will take place, and in other columns ions will be removed. The concentrated saltwater flow is circulated until it has reached a value that enables precipitation. At this point, the flow is discharged. Particles that do not carry an electrical charge are not removed.

All types of whey can be demineralized by ED. In order to minimize fouling and microbial contamination, some care must be taken. Operation conditions (flow rate, type of spacer, spacer thickness, etc.) and membrane type should be appropriated; in some cases, pretreatment is necessary before the ED can take place and do regular regeneration cycles. Suspended large particles (e.g., casein) and fat need to be removed, or else they will plug the membrane pores. For industrial applications, pretreatment methods should be performed in order to concentrate whey, leading to an increase of the efficiency of ED. Evaporation, NF, or RO are usually used. These complementary methods, namely the membrane ones, simultaneously demineralize whey at the time of concentration, helping in terms of cost, time, and water disposal. Whey preconcentration leads to a conductivity increase, with a large-scale migration of ions resulting in low electrical resistance, which is desirable in order to reduce energy losses and consequently intensify the process efficiency. Although when these complementary methods are used alone, they are suitable only for partial demineralization of whey, which is the same as what happens with DF. Beyond ED, high levels of desalting degrees could be achieved with an ion-exchange column. This technology presents some limitations, the major one being the need for lots of water and chemicals for the regeneration of resins. When ion-exchange and ED methods are compared, it can be verified that the ion-exchange column produces three times more waste water and chemical oxygen demand, four times more salt (expressed in mass), and higher cumulative energy demand than ED does.

Whey ED parameters

ED can be performed in continuous, feed-and-bleed (continuous with recycle), and batch recirculation processes; however, the last one is the most efficient and used mode for obtaining desalted whey because it leads to high levels of demineralization (90–95%). To obtain the same yield level in a feed-and-bleed system, several ED stacks in series are needed; in continuous mode, low demineralization levels are achieved during one pass through an ED unit.

Several actual studies deal with ED for whey demineralization parameters, among which can be highlighted ash, protein and lactose content, pH, titratable acidity, type of membranes and spacers, operating conditions (voltage, linear velocity, temperature), and others. Only pH can be continuously monitored during the process, and the maximum demineralization rate is obtained near the whey protein isoelectric point at pH 4.6, where the neutral global net charge is not involved in ion membrane cross. Conductivity is an important parameter to control during the process because it is intrinsically related to ash content. It can be measured online but requires calibration for different kinds of whey.

When referring to the ED unit, it can be seen that spacer thickness does not affect total demineralization time and energy consumption. The selectivity of transport of inorganic ions is observed; monovalent ions are transported preferably. It is unlikely that the voltage has a strong influence on demineralization time and energy consumption, with the application of higher voltage resulting in shorter demineralization time and higher energy consumption.

Whey ED advantages

The main advantages of membrane-based processes are feasibility of a substance separation without phase changes and additional chemicals, and energetically advantageous separation (low energy consumption), allowing high capacity of industrial units. Other advantages include easy operation of ED equipment, presenting a modular design, usually processed at ambient temperature, and also the chance for automation and continuing of these processes. Finally, membrane-based processes enable the demineralization of biological solutions with minimal losses of valuable compounds (proteins, lactose, etc.), without affecting their quality.

In the dairy industry, ED has great potential as an economical and efficient method.

See also: Cheese: Composition and Health Effects; Dairy Products: Dietary and Medical Importance; Milk: Sources and Composition; Whey and Whey Powders: Fermentation of Whey; Whey and Whey Powders: Production and Uses; Whey and Whey Powders: Protein Concentrates and Fractions.

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Whey and Whey Powders: Production and Uses

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Whey

Whey is the liquid remaining from the precipitation and curd removal during cheese manufacture. Like milk, whey may have different origins (e.g., goat, sheep, and buffalo), but the most relevant in terms of production volume and economical value is that obtained from cow milk processing; this will be the main focus of this text. Whey generally represents a volume fraction of 90% in milk and is classified in two different types: sweet whey, resulting from rennet-coagulated cheese with a pH of at least 5.6; and acid whey, obtained from acid-coagulated cheese with a pH below 5.1.

Up until very recently, whey resulting from the curd during manufacture of cheese was regarded as a polluting effluent from the dairy industry, being commonly discarded in rivers or lands (without any treatment), thus resulting in a serious environmental problem due to its high production volumes and organic content. This paradigm has been changed due to the combination of three critical driving forces: (i) legislation, (ii) potential of whey proteins, and (iii) evolution of technology. In short, legislative regulations, together with the growing consumer concern regarding environmental problems and incentives to recycle whenever possible, forced the dairy industry to explore other approaches to managing whey as a waste. Since Ancient Greece, whey has been claimed to be a cure for many illnesses, used as a medicine or as skin balm. Nowadays, the potential of a vast range of whey proteins and their peptides with great potential health benefits is well known. Despite these, application of whey or whey powder in human food products was always very limited due to the uneven composition of whey solids. However, the developments of separation technologies relying on selective porous membranes are allowing superior isolation and fractionation of whey components, which are undoubtedly becoming one of the most widespread additives in food. Consequently, over the last 40 years, the number of commercial and technological applications using whey has increased considerably. Digestion processes were applied as an important step to minimize the pollution problem caused by the deposition of this by-product through the use of enzymes and microorganisms, resulting in the production of commodities for other industries, such as monosaccharides and amino acids. Recently, whey gained interest as a food ingredient, coming into use as a technological agent particularly through whey proteins, achieving a unique blend between nutritional and functional properties with applications both in food and health.

Whey is a turbid yellowish liquid, consisting in its majority (90–94%) of water, while also having a high and diverse organic content. Its composition and characteristics may vary according to origin and producing method. Lactose is the major organic fraction present in whey, followed by proteins, minerals, lactic acid, and lipids. The general whey composition and its major applications are summarized in Table 1. Other minor components encompass citric acid, nonprotein nitrogen compounds (urea and uric acid), and vitamins of the B-complex.

Whey Proteins

Whey proteins are the most economically and technologically interesting fraction in whey. These proteins are globular molecules with a substantial content of α-helix motifs, in which the acidic/basic and hydrophobic/hydrophilic amino acids are distributed in a fairly balanced way along their polypeptide chains.

Whey protein fraction is mainly constituted by β-lactoglobulin (β-Lg), α-lactalbumin (α-La), immunoglobulins (IGs), bovine serum albumin (BSA), lactoferrin (LF), and lactoperoxidase (LP), representing at least 50%, 20%, 10%, 10%, 3%, and 0.3%, respectively. The general physiochemical properties of whey proteins are listed in Table 2.

Whey Protein System

Since the 1920s, efforts have been made to concentrate whey, essentially using heat-drying methods. However, these efforts never resulted in commercial and technological interest once the characteristics of the obtained product (i.e., yellow-brownish colour) and their composition (i.e., high content of lactose and denatured protein), coupled with the high costs of the process, limited their use. Later, with the implementation of an innovative process (i.e., spray drying), the thermal degradation of whey components, as well as the cost associated to its concentration reduced significantly. Nevertheless, not until the 1970s, with the utilization of membrane filtration, was it possible to achieve an efficient separation and concentration of whey protein fractions while preserving their integrity. This technique employs semipermeable surfaces (membranes) with specific pore sizes, where the permeate flows though while the retentate is blocked based on size/molecular weight. Throughout the combination of successive filtrations steps, it is possible to produce protein fractions with different compositions and degrees of purity. These fractions can then result in a dry product if followed by a spray-drying process. This technology leads to a selective concentration of proteins, which after drying is called whey protein concentrate (WPC). This whey product contains different protein concentrations (usually ranging from 35% to 80%) and appreciable amounts of lactose and minerals, thus fitting several applications. Further purification, often conjugating other techniques as

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ion-exchange chromatography, allows the achievement of higher degrees of purity with residual or no lactose content and higher desalination, resulting in whey protein isolate (WPI) containing at least 90% protein. With the application of more advanced techniques, such as chromatography, partial hydrolyses, and selective precipitation combined with centrifugation and dialysis, it is yet possible to obtain pure whey protein fractions. Figure 1 shows the main whey protein processing approaches to obtain different whey protein subproducts and products, and purer fractions.

Nowadays, whey protein fractions have a vast range of applications in several areas. Nutrition is by far the most important, although there is growing interest in the pharmaceutical field, particularly regarding enriched whey protein ingredients, essentially due to their biological activities.

Concentrated whey is typically incorporated in animal feed or used as a component in growing media for bacteria, yeast, and algae. Small quantities may also be incorporated in human food, although its high salt and lactose contents, together with low protein composition (~10%), do not make it attractive for this purpose.

Among concentrated whey products, WPI followed by WPC gather suitable characteristics for food applications such as higher protein and amino acid contents; low calorie, fat, and sodium contents; absence of pathogens and toxic compounds; biocompatibility and generally recognized as safe status; ready availability; and inexpensive products. Some of these potential food applications will be discussed below in a critical manner.

Table 1 Whey composition profile, their relative concentration, and main applications

Whey component	*Concentration ($g\ l^{-1}$)*	*Applications*
Lactose	45–60	Browning agent, sweet syrups, fermentation substrate
Proteins	6–11	Nutrition, technological agents, bioactive compounds
Minerals	8–10	Nutrition, fertilizers, growing medium
Lactic acid	0.5–9	Food preservative, acidulant, biodegradable plastic component
Lipids	0.6–5	Nutrition

Table 2 Composition of major proteins in bovine whey, their relative concentration, molecular weight (Mw), and number of amino acid residues

Whey protein	*Concentration ($g\ l^{-1}$)*	*Mw (kDa)*	*Amino acid residues*
Total	7.0	–	–
β-Lactoglobulin	3.5	18.3	162
α-Lactalbumin	1.2	14.2	123
Immunoglobulins	0.7	150–900	[a]
Bovine serum albumin	0.4	66.4	583
Lactoferrin	0.02–0.35	80.0	700
Lactoperoxidase	0.01–0.03	78.5	612

[a]Variable values.

Whey Protein Biological Properties

The development of new processing technologies and more sophisticated analytical methods has allowed better insight into the nutritional and biological properties of whey proteins and whey-derived products and their role in human health, including prevention of diseases and health conditions. Whey proteins exhibit a range of biological activities that influence digestion, metabolic responses to absorbed nutrients, growth and development of specific organs, and resistance to disease. Other biological activities of the individual whey proteins, and

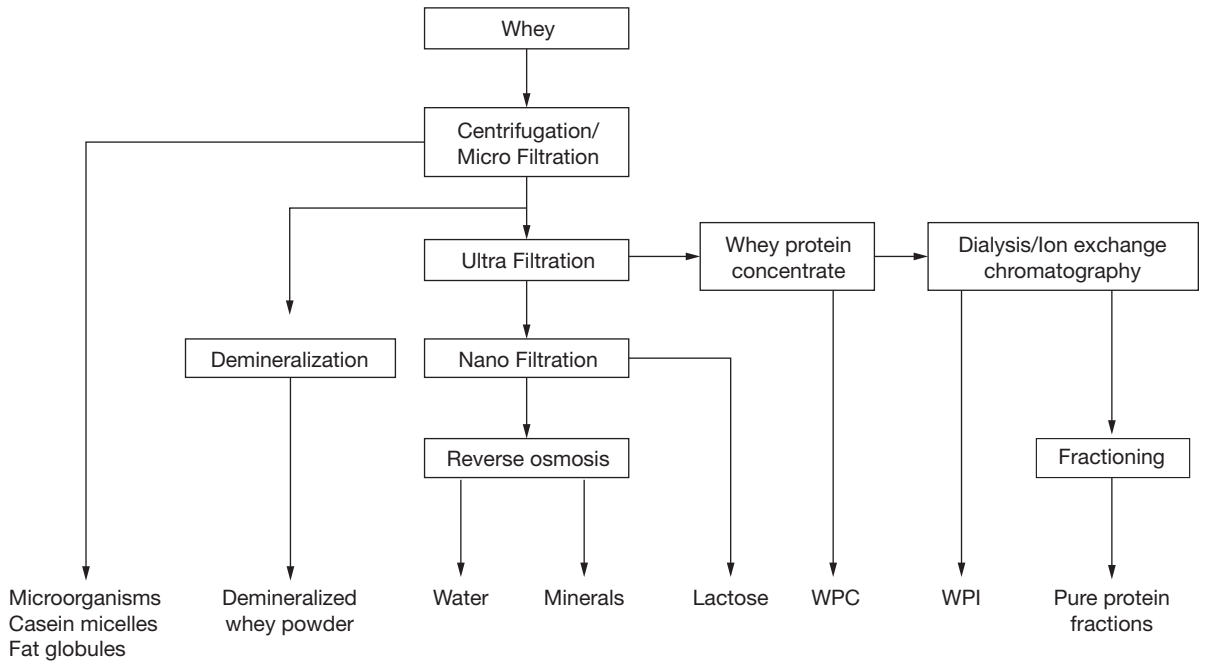

Figure 1 Liquid whey processing strategies to obtain protein-based ingredients.

of their fragments obtained by hydrolysis (termed bioactive peptides), include antihypertensive, antimicrobial, opioid, antioxidant, antiviral, immunomodulant, anticarcinogenic, or mineral binding. The main biological activities of whey proteins are summarized in Table 3.

The physiological effect of whey proteins can be attributed to their fast digestion, which leads to a rapid increase in serum amino acids after ingestion. After exercising, people enter in a form of metabolic stress that requires immediate availability of usable energy, which can be gathered by the consumption of essential amino acids (e.g., leucine, isoleucine, and valine). Therefore, whey proteins, via their composite amino acid residues and/or associated compounds, can provide substrates and bioactive components that permit extending the overall benefits of regular physical activity. Thus, whey proteins have been the protein source choice by elite athletes, in addition to those people whose health is compromised. Moreover, these proteins can contribute to improving muscle strength and reducing the tendency for bone breaking. These features are once again directly related to the unique amino acid sequences. Furthermore, whey protein dairy consumption has been shown to play an important role as a metabolic regulator in protein and glucose homoeostasis and in lipid metabolism; this allows better weight control, lower plasma and liver cholesterol, and lower plasma triacylglycerol levels, and helps to reduce fat deposition and improve insulin sensitivity.

Table 3 Biological activities and potential health benefits of whey protein consumption

Protein	*Biological activity*
Whole whey protein	Prevention of cancer (e.g., breast and intestinal cancer) Increment of gluthatione levels Increase of tumor cell vulnerability HIV treatment Antimicrobial activities Increment of satiety response Increment in plasma amino acids, cholecystokinin, and glucagon-like peptide
β-Lactoglobulin	Transporter of retinol, palmitate, vitamin D, cholesterol, fatty acids, and triglycerides Protection during passage through the stomach conditions Development and transfer of passive immunity Regulation of mammary gland metabolism Anticarcinogenic activity Immunomodulatory activity
α-Lactalbumin	Anticarcinogenic activity Lactose synthesis Treatment of chronic stress-induced disease
Bovine serum albumin	Synthesis of lipids Antimutagenic activity Antioxidant activity Anticarcinogenic activity
Immunoglobulins	Immunomodulatory activity Growth and development Disease protection through passive immunity Antibacterial activity Antifungal activity Opioid activity HIV treatment
Lactoferrin	Antimicrobial activity Antifungal activity Antiviral activity Antithrombotic activity Immunomodulatory activity Antiproliferative activity
Lactoperoxidase	Antimicrobial activity Antifungal activity Immunomodulatory activity

Source: Hernández-Ledesma, B., Ramos, M. and Gómez-Ruiz, J. Á. (2011). Bioactive components of ovine and caprine cheese whey. *Small Ruminant Research* **101**(1–3), 196–204. doi: http://dx.doi.org/10.1016/j.smallrumres.2011.09.040; Madureira, A. R., Tavares, T., Gomes, A. M. P., Pintado, M. E. and Malcata, F. X. (2010). Invited review: physiological properties of bioactive peptides obtained from whey proteins. *Journal of Dairy Science* **93**(2), 437–455. doi: http://dx.doi.org/10.3168/jds.2009-2566.

β-Lactoglobumin

β-Lg is a small, soluble, and globular protein. Its actual biological functions are still unclear; however, it can bind to small hydrophobic ligands acting as carrier systems of these compounds, for example, retinol, fatty acids, triacylglycerols, aromatic compounds, vitamin D, cholesterol, palmitic acid, and calcium. Reported biological activities for β-Lg are summarized in Table 3. The homology of β-Lg tertiary structure with the plasma retinol-binding protein, as well as its high stability to proteolytic activity of digestive enzymes, has suggested that this protein can act as a resistant carrier of retinol. Moreover, its high stability at low pH protects hydrophobic molecules during passage through the stomach, in order to permit a specified delivery of such compounds to receptors located in the intestine, thus facilitating the digestion of milk lipids.

β-Lg plays also an important role in developing passive immunity with immunoglobulin G and in its transference to the newborn, and in regulation of phosphorus metabolism at the mammary gland. The amino acid content of this protein is rather important because, besides stimulating muscle growth, it is a rich source of cysteine, which is essential for synthesis of glutathione, an anticarcinogenic tripeptide produced by the liver for protection against intestinal tumors.

The high nutritional and functional value of β-Lg is now widely recognized and has made of this protein an ingredient of high quality in the formulation of modern foods and beverages.

α-Lactalbumin

α-La is a small and globular protein rich in cysteine, essential as a coenzyme for the biosynthesis of lactose – an important source of energy. Some important bioactivities have been reported for α-La (see Table 3).

α-La contributes to reducing the risk of incidence of some cancers (e.g., breast and colon cancer), as it constrains cell division when present in intestinal cell lines. This whey protein has also been associated with the treatment of chronic stress-induced cognitive decline due to its high tryptophan content (~6%); tryptophan (as a precursor of serotonin) is important for maintaining the essential levels of serotonin in the brain for good cognitive performance.

Bovine serum albumin

BSA is not synthesized in the mammary gland; it appears in milk through passage from the blood stream. Due to its size and structure, BSA can bind to fatty acids, which are stored in the human body as fat, or to free fatty acids and flavor compounds, hence participating in synthesis of lipids (which are a part of all outer and inner cell membranes) and in energy production. Moreover, this whey protein has revealed antioxidant activity, thus protecting lipids against phenolic oxidation, and potent inhibitory activity against breast cancer cell proliferation through modulation of activities of the autocrine growth regulatory factors (see Table 3).

Immunoglobulins

IGs represent the most abundant of the established bioactive proteins found in whey. IGs constitute a complex group of globular proteins produced by B-lymphocytes, making a significant contribution to the whey protein content, besides exerting an important immunological role. IGs are present in the serum and physiological fluids of all mammals; some of them attach to surfaces where they behave as receptors, whereas others function as antibodies that are released in the blood and lymph. These whey proteins protect the gut mucosa against pathogenic microorganisms; impart passive immunity; combat infections, providing protection against diseases, particularly in a neonate until its own immune system is developed; improve physical performance and recovery times in athletes; and allow faster recuperation of immunocompromised persons. Important biological properties of IGs are summarized in Table 3.

Lactoferrin

Lactoferrin, also known as lactotransferrin, is a globular multifunctional protein that binds, transports, and supplies the organism with iron. It is present in the human body as a secretor protein and is synthesized by glandular epithelial cells and mature neutrophils. LF possesses antimicrobial activity attributed to the aforementioned iron-binding property, as it makes such cation unavailable to the (otherwise active) microorganisms, or by direct binding to their microbial membrane. The antimicrobial activity of this protein is not restricted to bacteria, but is available also to a wide range of other microorganisms, including fungi and some viruses (Table 3).

LF plays a relevant role upon stimulation of the immune system, owing to the increase of macrophage activity mediated thereby, as well as upon induction of inflammatory cytokines, stimulation of proliferation of lymphocytes, and activation of monocyte (natural killer) and neutrophil cells. This whey protein also stimulates humoral immune response to sheep red blood cells, promotes appropriate intestinal and peripheral specific antibody response, and modulates production of lymphokines. Moreover, LF has been shown to potentiate bone growth enhancement properties manifested through stimulation of growth of osteoblasts and inhibition of osteoclasts (Table 3).

Lactoperoxidase

LP is a member of the family of mammalian peroxidases, and is an important part of the natural host defense system in mammals, which provides protection against invading microorganisms. This protein, combined with LF, displays suppressive effects on mitogen-activated lymphocyte proliferation. LP also regulates expression of genes involved in the metabolism, immunity, apoptosis, and cell cycle of epithelial intestinal cells, while also displaying an important role in controlling microorganism growth in milk from lactating animals, and in cell-mediated pathogen killing. Additionally, this whey protein plays a significant function in oral health care, promoting the inhibition of harmful microorganisms associated with gingivitis and oral irritation, and combating both the causes and effects of halitosis. Biological activities for LP are summarized in Table 3.

Other Bioactive Components in Whey

Lactose and oligosaccharides

Lactose is the major carbohydrate in whey and is a valuable nutrient because it favors the intestinal absorption of essential minerals such as calcium (Ca), magnesium (Mg), and phosphorous (P), and the use of vitamin C. On the other hand, whey oligosaccharides possess prebiotic and anti-infective properties. Many of these oligosaccharides contain sialic acid; this compound present in whey seems to promote several positive effects in the human body, with particular relevance during the development of the infant's brain.

Minerals

Many major and trace minerals play an important role in the physiology and metabolism of the human body. The most abundant elements are Ca, sodium (Na), P, Mg, and potassium (K), while those present in minor amounts encompass zinc (Zn), iron (Fe), copper (Cu), and manganese (Mn). The levels of Ca, P, Mg, Zn, Fe, and Cu are higher in sheep than in cow whey; the opposite appears to be the case for K and Na. Goat whey contains about 134 mg of Ca and 121 mg of P per 100 g. Na and K are almost entirely soluble and fully available in whey, whereas Ca, Mg, and P are associated in different proportions to the colloidal suspension of casein micelles and, therefore, are partly retained in the curd during cheese-making. The presence and concentration of these minerals, essential for the proper functioning of the human body, varies depending on the element and on the needs of each person.

Vitamins

Vitamins are physiological, biochemical, and metabolical bioactive molecules of small dimension and low molecular weight, being divided into liposoluble and water-soluble vitamins according to their solubility. They are essential for the growth, development, and normal maintenance of human organisms. However, humans do not have the capacity to synthesize vitamins, except vitamins D and B_3, meaning that they must obtain them through external sources. Therefore, whey appears as a suitable source of these compounds, as it is extremely rich in vitamins. However, part of the liposoluble vitamins remain in the curd during cheese-making, whereas water-soluble vitamins go into the whey. In the literature, it is described that sheep whey is richer than cow whey for most of the vitamins. For instance, goat and sheep whey have higher amounts of vitamin A than do their cow counterparts. However, goat whey has a significant dearth of folic acid and vitamin B_{12} compared to cow whey.

Applications

Industrial Applications – Overview

Whey ingredients are an excellent way to increase the overall nutritional value of foods. For example, whey ingredients with low protein content, such as WPC, are frequently used in low-value products such as dairy and bakery. More purified products such as WPI are used in high-value products such as meat, seafood, confectionaries, health foods, infant formula, and sports supplements. Besides nutritional aspects, these ingredients have found several applications as technological agents regarding gelation, thermal stability, foam formation, or emulsification. Additionally, pure fractions of whey proteins have the potential to enable the development of novel food products with improved functionalities, as well as new applications in nutrition, cosmetic, and pharmaceutical industries.

Figure 2 summarizes some of the most relevant industrial applications of whey components in food. In particular, functional properties of WPC and WPI are very interesting and render these two whey protein ingredients extremely versatile. They support the development of food products, as follows:

- Improve aeration in bakery and confectionary products.
- Improve color and taste by interaction between proteins and lactose during thermal processing (Maillard reactions) in candy products (e.g., toffees, caramels, and cooked syrups).
- Replace skim milk powder in dairy product formulations (e.g., yogurt, ice cream, and milk chocolate drinks).
- Improve quality of meat and fish due to emulsion, gelation, and hydrophilic attributes.
- Develop infant formula with nutritional benefits by adapting infant formula to that of human milk.
- Increase bioavailability of salts such as Ca and Zn in elderly foods.
- Develop dietetic foods with high satiety value – low fat and high protein content, with an excellent amino acid composition.
- Develop nutraceuticals due to the presence of important bioactive proteins (e.g., LF and LP).

Together with the applications listed above, whey proteins are being successfully used in the production of edible films and coatings, with the purpose of improving the shelf life of perishable food products. In addition, the by-products of whey protein concentration (i.e., lactose and Ca-rich milk) are also valuable, as previously shown. They are becoming increasingly useful in pharmaceutical and dietary supplement food markets. Lactose is mainly used as an excipient in tablet processing, and the milk minerals are used for the fortification of various foods and beverages with Ca. In the following subsections, the use of whey components for the development of both bioactive packaging systems and functional foods will be pointed out.

Bioactive Packaging

Food packaging has evolved considerably during the last decades to have new functionalities and to assist in meeting the demands of extremely stringent markets that constantly trigger the development of stable and fresh food products. Depending on the type of food, packaging materials need to fulfill different requirements in terms of light, moisture, water vapor, and gas (i.e., oxygen and carbon dioxide) barriers in order to avoid color or taste deterioration, loss of nutritional value, and eventually growth of potential pathogens. To fulfill these requirements, there is an ongoing interest in sustainable packaging materials throughout the use of renewable/biodegradable and edible biopolymers (e.g., proteins, polysaccharides, and lipids), in an attempt to replace environmentally unfriendly petroleum-based plastic films, which are still widely used in the packaging industry. Whey is one of the most promising biopolymers in the field of packaging. Currently, the best potential applications of whey protein-based edible films lie in protective coatings for foods. When applied on surfaces of food as a coating, these films can protect food from chemical or microbial degradation, thus lengthening product shelf life and maintaining high product quality. Further, edible films and coatings manufactured from whey proteins have demonstrated mechanical and barrier properties better than competitive protein-based (e.g., corn zein, wheat

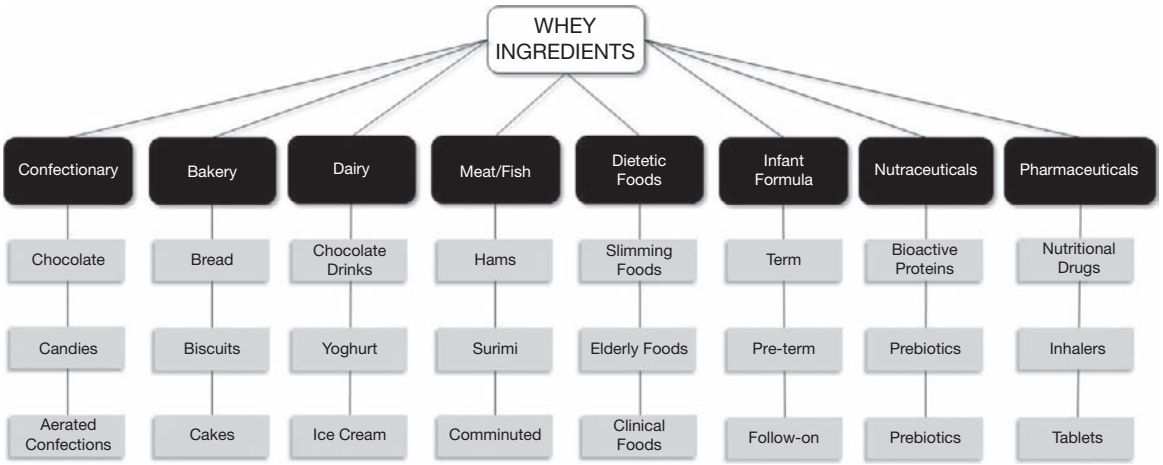

Figure 2 Various industrial applications of whey ingredients. Adapted from de Wit, J. N. (2001). *Lecturer's handbook on whey and whey products*. Brussels: European Whey Products Association.

gluten, and soy protein isolate) or polysaccharide-based (e.g., starch, cellulose, carrageenan, and pectin) films.

Whey protein films and coatings have already been applied in several food products such as peanuts, walnuts, frozen salmon, fruits, and breakfast cereals, aiming for good aroma, fat, moisture, and gas oxygen barriers. They also have the potential to improve the appearance of food products by adding gloss. Several examples of whey protein-based coating and film applications are described. In particular, these have the potential to increase the shelf life of roasted peanuts by reducing lipid oxidation and avoiding the appearance of rancid off-flavors. Fresh-cut fruits and vegetables can also be better preserved due to the minimization of both moisture loss and oxidative browning. These coatings also provide an efficient protection of frozen salmon against lipid oxidation and water loss. In addition to the aforementioned features, those edible films can be the vectors of various bioactive agents, such as antimicrobials, antioxidants, nutraceuticals, or even flavors and colorants, enhancing not only food quality and safety, but also functionality while providing some health benefits for the consumer. Films or coatings carrying antioxidants in their composition have shown promise for minimizing rancidity in meat products. Active antimicrobials approved for use in contact with foods (e.g., nisin, sorbic acid, and potassium sorbate) can be incorporated in whey protein film matrices protecting food from microbial spoilage. Current research on antimicrobial packaging and coatings is being directed toward the surface protection of cheese, meat, and fruit products. In fact, antimicrobial food packaging using edible whey protein films is starting to be envisioned as a part of the hurdle technology, thus reducing the risk of pathogen contamination and extending the shelf life of perishable food products, when in combination with other processes. In addition, whey protein edible films can be employed to carry flavor compounds, thus providing a way to minimize the deleterious effects of aroma loss. Flavor and aroma can be incorporated into a coating for delayed release in order to improve the flavor profile of the food. This release can occur during the intermediate steps of food preparation (heating or rehydration), or even in the mouth by mastication.

Development of Functional Foods

Food and pharmaceutical industries are constantly triggering the development of novel products with a strong nutraceutical function using whey or whey-derived ingredients (e.g., WPC and WPI) as a valuable source. The list of nutraceutical compounds associated to whey-derived products includes vitamins, probiotic cultures, and bioactive peptides, and the scientific evidence to support health benefits, either by prevention or treatment of some diseases, is steadily growing. As previously shown, a diverse group of bioactive proteins with antimicrobial activity can be obtained from whey, such as in the case of LP, lysozyme, and LF.

However, whey proteins and their properties have been extensively studied not only due to their importance as food ingredients, but also due to their ability to serve as vehicles for bioactive compounds. Gelation properties of globular proteins such as β-Lg allow the development of micro- and nanostructures such as microgels, nanohydrogels, nanofibrils, and nanotubes. Figure 3 illustrates the wide range of structures (macro, micro, and nano) that can be developed for the

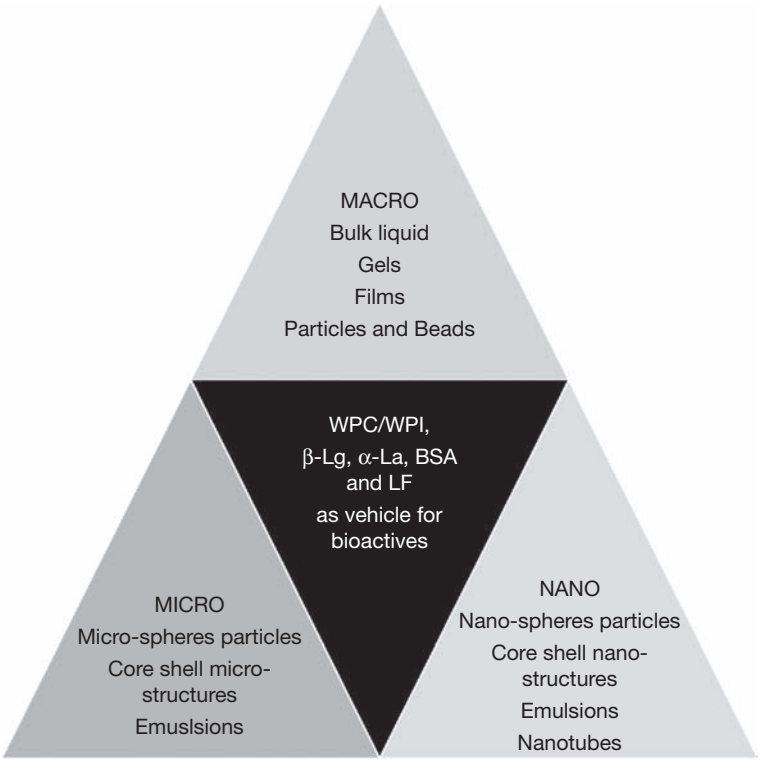

Figure 3 Globular whey protein systems used as vehicles of bioactive compounds in foods.

encapsulation and controlled release of bioactive compounds using whey proteins as a carrier system. Microgels have been specifically targeted for two main functions: (1) as a means to tune more precisely the texture and mouthfeel of food, especially as a replacement for fat; and (2) to encapsulate functional food ingredients or other nutraceuticals that have potential health benefits (e.g., vitamins, flavor volatiles, antioxidants, omega-3 fatty acids, phytosterols, flavonoids, and folic acid). Regarding nanostructures, the swelling capability is the most important characteristic feature and is attributed to the presence of hydrophilic moieties such as hydroxyl, amino, and carboxyl groups in the protein structure. These structures, known as nanohydrogels, can absorb or expel water in response to several stimuli as small changes in pH or temperature. This capability is responsible for their soft and elastic properties, but more important, it makes protein nanohydrogels an ideal structure for use in the encapsulation and controlled release of bioactive compounds in specified sites of the gastrointestinal (GI) tract. Further, the ability of globular whey proteins to form gel networks under cold conditions is also attracting attention due to the possibility of applicating these structures in novel food and nonfood areas. The formation of cold-set gels opens interesting opportunities for food proteins as carriers of heat-sensitive nutraceutical compounds.

Whey proteins can be used in clinical foods aiming at providing supplemented nutritional support to persons/patients who are incapable of digesting adequate amounts of conventional food products or who have special nutritional needs. After a starting stage of digestion and absorption in the stomach (under acidic conditions and pepsin), dietary whey proteins can be further hydrolyzed by pancreatic enzymes (chymosin and chymotrypsin), resulting in a combination of unique amino acids and small peptides with remarkable physiological functions (e.g., stimulation of growth factor and blood flood regulation). *In vitro* hydrolyzed whey proteins can also be used on patients with deficient enzymatic digestion or with specific diseases, having the need of parenteral diets (i.e., small amino acids and peptides injected directly into the blood stream) and enteral diets (i.e., oral diets containing larger peptides that can be further digested in the GI tract). However, in this case, food formulae demand high levels of purity and low allergenicity, thus entering in the category of hypoallergenic foods. Lactose, also a major component of whey, can be used as an excipient in the formulation of pharmaceuticals such as tablets, capsules, and inhalers. Lactose has a wide range of derivatives, including lactobionic acid (produced by oxidation), lactulose (produced by isomerization), lactitol (produced by hydrogenation), galactooligosacccharides (produced by hydrolysis and chain extension), galactose, and glucose (produced by hydrolysis). This range of derivatives has great versatility acting as nutritional supplements, as carriers of antibiotics and protection against diseases (e.g., colon cancer).

Concluding Remarks

There is now increasing evidence that many whey components, particularly proteins and their factions (peptides), are effective in reducing the risk of developing some diet-related chronic diseases. As a result, there is growing interest by the dairy, food, and pharmaceutical industries to design and formulate products (e.g., functional foods) that incorporate bioactive components of whey proteins, nutraceuticals, or active medicinal agents in an effort to meet consumer trends for health and well-being. In fact, the dairy industry has achieved a leading role in the development of functional foods and has already commercialized several products that boost the immune system, kill pathogenic microorganisms, or reduce blood pressure. Also, fractionation and marketing of bioactive whey ingredients has emerged as a lucrative new sector for dairy industries and specialized bio-industries. In leading dairy countries, this industrial sector is moving from commodity marketing to the ingredient marketing business, which entails good prospects for adding more value to the products. Current challenges in the exploitation of bioactive components are their maximal recovery from milk, colostrum, whey, and milk fat; their stability in different food matrices; and their optimal bioavailability in the body in order to deliver expected health effects. To meet these challenges, it is crucial to develop appropriate technologies and product concepts, as well as to verify their bioavailability not only upon ingestion, but also through the digestive tract, and after absorption through the intestinal walls into the blood circulation system using *in vivo* tests.

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See also: Dairy Products: Dietary and Medical Importance; Milk: Sources and Composition; Whey and Whey Powders: Fermentation of Whey; Whey and Whey Powders, Principles and Applications of Dialysis.

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Whey and Whey Powders: Protein Concentrates and Fractions

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Cheese Whey

Health is one of the major reasons that determine consumer's choices of food; following and maintaining a healthy diet is crucial. However, the difficulty in having consumers change radically their eating habits has led to the emergence of a number of foods that have the same appearance as the conventional ones but that contain certain functional ingredients able to bring health benefits in addition to responding to the nutrition issue.

The liquid portion obtained during the coagulation and syneresis process in a cheese production is a by-product called whey (the water-soluble fragment). That can be found in different sources, depending on which milk it arises, although the majority is derived from cows. The worldwide production of bovine whey is estimated as ca. 10^8 tons per year. Unfortunately, all over the world, a great part of whey does not undergo those downstream processing routes, so a significant portion is (1) sprayed onto fields while the resulting smell and salt levels often prove troublesome; (2) discharged into rivers, lakes, or seas, although the very high polluting power usually precludes other species to survive therein; (3) or discharged into municipal sewage systems. These discharges cause serious environmental problems, because whey is a strong organic pollutant – with particularly high biological oxygen demand (40–60 g l^{-1}) and chemical oxygen demand (50–80 g l^{-1}).

An increased interest in whey protein products, owing to their nutritional role and their role upon human health, has been seen in the last decades. There are two categories of food uses for whey: as raw material for fermentation (due to its high lactose content) aiming at the production of bioproducts or through its protein components as whey protein isolates, whey protein concentrates, whey fractions, and heat-stable whey proteins to bioactive peptide production derived therefrom. These science-based nutritional solutions are perfectly formulated for the production of functional ingredients showing a variety of functional, physiological, and nutritional properties that make them potentially useful in a wide range of applications, such as healthy beverages, nutrition bars and supplements, and pharmaceutical preparations – thus providing a promising alternative for whey upgrade.

Whey Protein Composition

Whey retains c.50% of the total milk nutrients; the most important components of whey are soluble proteins (0.6–0.8%, w/v) that represent 20% of total milk proteins, namely, β-lactoglobulin (β-Lg), α-lactalbumin (α-La), immunoglobulins (Igs), bovine serum albumin (BSA), lactoferrin and lactoperoxidase enzymes and caseinomacropeptide, lactose (4.5–5%, w/v), lipids (0.4–0.5%, w/v), and mineral salts (8–10%, w/w using a dry extract basis). The concentration of whey protein could vary depending on the source of milk, time of the year, type of feed, stage of lactation, quality of the cheese manufacture process, and the type of whey (acid or sweet). Whey proteins have unique characteristics. Beyond their great importance in nutrition (supply energy and essential amino acids), they exhibit chemical, physical (gel formation, foam stability, and water retention), physiological (useful in therapies, including antimicrobial and antiviral actions, immune system stimulation, and anticarcinogenic activity), functional (growth and cellular repair), and technological features. Based on these properties, individual proteins and protein concentrates of whey have been incorporated in food formulation by industry. The most relevant whey proteins are briefly characterized in Table 1.

Key components of whey

What makes the whey proteins being so high quality in what concerns to biological activities? The amino acid content of each protein influences whey protein products' bioactivities, being one of the reasons for such success. Whey proteins are rich in sulfur-containing amino acids (i.e., cysteine and methionine); therefore, they are susceptible to intramolecular bond formation via disulfide bridges.

The high content of cysteine leads to achieve whey as an important and cheap container of dietary L-cysteine. Although this amino acid is not an essential one (can be synthesized from methionine), it appears to have some manner of deficiency syndrome associated with it, at least indirectly via glutathione. Glutathione, tripeptide in which cysteine is the limiting substrate, is an antioxidant molecule responsible for several functions, including immune-modulation mechanism, cellular defense against oxidative stress, and detoxification. Aging, cancer, and HIV patients are supplemented with whey protein in order to increase glutathione levels. A lack in glutathione is a common problem in HIV-infected individuals. Cysteine could be itself an adjuvant in diabetes type II therapy. As mentioned before, theoretically, an increase in cysteine leads to an increase of glutathione production that can relieve the risk of diabetes when this is caused by oxidative stress. Otherwise healthy persons can also benefit when taking a whey protein supplement. The increase of glutathione levels leads to the increase of resistance training.

In addition to the glutathione production and its role in the defense against oxidative stress and detoxification, cysteine might also have an important role on hydrogen sulfide (H_2S) cardioprotective control. H_2S is produced and transported by the enzyme action, acting as a signaling molecule, promoting vasorelaxation of blood vessels. It is also characterized by antioxidative capabilities. Low levels of H_2S increase homocysteine levels, which is an indicative of heart and neurovascular diseases. H_2S is derived from a circulating cysteine pool that remains in storage in the body and fit to buffer a pool of H_2S that preserves physiological functions.

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Table 1 Whey protein characterization

Proteins	*Concentration (% whey dry bases)*	*MW (kDa)*	*Solubility*	*Structure*	*Source of*	*Properties*
β-Lactoglobulin	50	18.4	Solubility depends on pH and ionic charge pI: 5.2 Temp. >65 °C, pH 6.5 Denature and aggregate formation Temp. < 65 °C pH 5.2–7.2 – stable dimmer pH 3.2–5.2 – octamer formation pH 3.0 and 8.0 – monomer Do not precipitate during milk acidification	Globular protein with rigid spatial structure Composed mainly of β-sheet motifs Monomer: 1. Free thiol group 2. Disulfide bridges	BCAAs (25.1%) Leucine (13.5%) Cysteine	Gelling capacity Structuring and stabilizer agent Resistant to gastric digestion Binds small hydrophobic molecules assuming their transport Stimulates glutathione synthesis (Cys content) High nutritional value (high sulfur content) – sport supplements
α-Lactalbumin	20–25	14.2	pI: 4.7–5.1 Low pH, high temperature, or moderate concentrations of denaturants – 'molten globule' Neutral pH the 'apo' state (partially unfolded state) pH > 6.7 Irreversible denaturation/ aggregation Temp. <75 °C *R*eversible denaturation	Compact globular protein – highly heterogeneous, 26% α-helix, 14% β-sheet (significantly unfolded), and 60% of other motifs 4 Disulfide bonds make this protein relatively heat-stable The 'molten globule' presents a balance between native and unfolded states Ca^{2+} stabilizes the conformation of chiefly the β-sheet domain	Essential amino acids BCAAs Leucine (10.6%) Isoleucine Valine Aspartic acid (10.6%) Cysteine	Structure and composition with 72% homology to human α-lactalbumin – supplements for infant formula High nutritional value (amino acid content) – sport supplements Lactose biosynthesis – acting as a coenzyme

(*Continued*)

Table 1 (Continued)

Proteins	*Concentration (% whey dry bases)*	*MW (kDa)*	*Solubility*	*Structure*	*Source of*	*Properties*
					(6.5%) Tryptophan	
Immunoglobulins	10–15	150–1000	pI: 5.5–8.3	Small Y-shaped proteins (4 polypeptide chains) 2 Light 2 Heavy Bond by disulfide bonds	Cysteine	Not inactivated by gastric acids Partially resistant to proteolytic enzymes
Caseinomacropeptide	10–15	8	pI: 4.0–5.0 pH 1–10 – soluble Temp. 80–95 °C, pH 4 and 7, 15 min – soluble pH 1–5 – minimum solubility (88%) Emulsifying activity – minimum near pI Decrease in pH – decrease in CMP volume	Glycosylates through *O*-glycoside bridge Phosphorylates by phosphorylation of a Ser residue (conditions without post-transcriptional modifications)	BCAAs Acidic and hydroxyl amino acids	Increases absorption of Zn^{2+} Interactions with cellular components, for example enterotoxins, bacteria, and viruses Ingredient in diets, patients with liver diseases and phenylketonuria (amino acid content) Interactions with hormone cholecystokinin Sialic acid – regular functioning of cell membranes and membrane receptors Lactose synthesis
Bovine serum albumin	5–10	69	pI: 4.7–4.9 Heat-induced gelation at pH 6.5 pH 4.5–8.0 – forms a heart-shaped molecule	Globular protein 17 Intrachain disulfide bonds 1 Free sulfhydryl group	Essential amino acids	Binds small hydrophobic molecules – assuming their transport
Lactoferrin	1–2	78	pI: 8.0–8.5	Monomeric glycoprotein 1–4 Glycans (depending on the species)	BCAAs Leucine (9.58%) Alanine (9.72%)	Iron-binding glycoprotein Iron transport and absorption in the gut
Lactoperoxidase	0.5	89	pI: 9.6 Acidic pH – more active			Catalyzes oxidation of thiocyanate (SCN^-)

They also present a high concentration of branched-chain amino acids (BCAAs) (in some cases higher than 26%) – leucine, isoleucine, and valine, being important factors in tissue growth and repair. Leucine has been identified as the key amino acid in protein metabolism during the translation–initiation pathway of protein synthesis. In that order, leucine-rich proteins are efficient in improving muscle protein synthesis. It has been speculated that the quality of a particular protein for enhancing muscle hypertrophy and stretch is related with the content of leucine. Thus, nutritional supplements containing these proteins may be efficient in preventing sarcopenia in the elderly and would represent a safe and convenient nutritional strategy. In addition, a high leucine concentration leads to enhance the immune function through intracellular conversion to glutathione.

Tryptophan is the precursor of niacin, serotonin neurotransmitter, and melatonin hormone; the last two regulate many neurobehavioral effects, for example, appetite, satiety, mood, airway sensory perception, pain sensation, and rhythm of sleep and wake.

Zinc is an essential mineral involved in bone, hormone, and enzyme metabolism.

Variants of Whey

The emergence of new processing technologies and more sophisticated analytical methods has permitted the production of a number of whey protein components already available in the market and used as additives. Those products comprise whey protein isolate (90–95% protein), almost pure protein; whey protein concentrate (ranging from 25 to 89% protein), most commonly available as 80%, with reduced lactose, fat, and mineral contents (as protein concentration increases, fat, lactose, and mineral contents decrease); hydrolyzed whey protein, presenting a variable protein concentration in which fat, lactose, and mineral contents also vary with protein concentration (hydrolysis used to obtain smaller peptide fractions, with an increase of bioactive peptides, reduces allergic potential and a theoretical improvement of solubility and digestibility when compared with nonhydrolyzed whey protein); and, finally, undenatured whey concentrate, presenting a variable protein concentration that usually ranges from 25 to 89%, processed to preserve native protein structures, and typically has higher amounts of immunoglobulins and lactoferrin. This variability is important when choosing whey fractions for specific nutritional applications.

Whey and Whey Protein Features

Whey protein is classified as high-quality protein based on human amino acid requirements, digestibility, and their bioavailability. Besides being susceptible to catalysis, they can be hydrolyzed via gastric, pancreatic, and microbial proteases, thus generating peptides that may present biological activity. Nevertheless, advances in nutritional biochemistry and biomedical research have undergone a notable intensification in search of peptides obtained via controlled *in vitro* hydrolyses in order to better understand the complex relationship between nutrition and prevention and/or treatment of diseases. Accordingly, scientific evidences have shown a wide range of peptides playing crucial physiological functions and modulating some regulatory processes. However, only some of such health effects, found *in vitro*, have eventually been confirmed in studies encompassing human volunteers.

Studies showed that whey proteins appear to be resistant to gastric digestion and pass quickly to the intestinal tract. Due to the lack of hydrolysis in the stomach, there is no apparent difference between the time of digestion and absorption and subsequent plasma amino acid profile of a whey isolate and a whey hydrolyzate. It was also found that whey protein takes ca. 40–60 min to reach the blood plasma after ingestion.

A number of favorable health effects have indeed been claimed for some whey protein concentrate, isolate, or its hydrolyzate. They truly open up a wide range of possibilities, within the market, for functional foods. They are able to affect the cardiovascular, nervous, digestive, or immune systems, including antimicrobial properties, and being implicated in diseases such as cancer, diabetes, osteoporosis, stress, obesity, or cardiovascular complications. The main biological activities of whey proteins related with specific diseases and its respective studies are listed in Table 2.

In terms of whey powders, biological function of their proteins is not fully known. Nevertheless, there has been an effort to enhance and justify the market product claims.

Whey proteins have been shown to lower plasma and liver cholesterol, as well as plasma triacylglycerol levels in model animals fed with cholesterol-containing diets. High-protein dairy products, and in particular those that contain whey proteins, may reduce fat deposition and improve insulin sensitivity.

Whey hydrolyzates are characterized by a low degree of allergenicity and very rarely cause adverse reactions. Currently, a hypoallergenic, hydrolyzed formula is recommended to children nonbreast-fed or partially breast-fed with a high allergy risk. The risk for atopic dermatitis in high-risk infants can be reduced with certain whey milk hydrolyzates when breastfeeding is insufficient.

β-Lactoglobulin animal studies suggest reduction of blood pressure by inhibition of angiotensin-converting enzyme and exhibition of immune-enhancing activity. *In vitro* studies reveal that β-lactoglobulin, chemically modified by 3-hydroxyphthalic anhydride, shows antiviral activity against human herpes simplex virus type 1 (HSV-1) and its hydrolyzate antimicrobial activity against several gram-positive bacteria and bactericidal activity.

α-Lactalbumin contributes to reduce the incidence risk of some cancers, and its hydrolyzate reduces the blood pressure. This whey protein has also been associated with treatment of chronic stress-induced cognitive decline. It presents bactericidal activity against *Streptococcus pneumonia* and its hydrolyzate antimicrobial activity against several gram-positive bacteria.

BSA has been shown *in vitro* to protect lipids against phenolic-induced oxidation. One important property that has been associated with this protein is the ability to inhibit breast cancer growth *in vitro*. β-lactoglobulin and α-lactalbumin show antiviral activity against HSV-1, when chemically modified.

Lactoferrin is a bioactive protein with an ability to bind iron. In opposition to anticancer feature, the antibacterial activity has been attributed to the iron-binding property.

Table 2 Specific diseases/whey protein biological functions

Clinical diagnosis	*Type of study*	*Sources*	*Results/advantages*
Cardiovascular disease	*In vitro*	Hydrolyzed: whey protein concentrate, α-lactalbumin, and bovine serum albumin	Angiotensin-converting enzyme (ACE) inhibition
		Caseinomacropeptide	ACE-inhibition activity
	Animal	Hydrolyzed whey protein (peptide concentrate), β-lactoglobulin, and α-lactalbumin	Reduced blood pressure (ACE-inhibiting peptides)
	Human trial	Whey protein concentrate	Hypertensive and obese persons (with resistance exercise):
			Increase of HDL and total antioxidant capacity
			Decrease of triglycerides, systolic blood pressure, and total cholesterol
			Atherosclerosis patients:
			Decrease in CCL5 (atherosclerosis biomarker) levels
Immunologic disease	*In vitro*	α-Lactalbumin	Macrophages enhance function via a tripeptide of glycine–leucine–phenylalanine
		Caseinomacropeptide	
		β-Lactoglobulin	Macrophages enhance function by low concentrations of caseinomacropeptide (CMP)
		Lactoperoxidase and lactoferrin	
		Whey protein isolated	Enhanced production of spleen and lamina propria lymphocytes
			Suppressive actions on lymphocyte proliferation
			Stimulation in the proliferation of murine spleen lymphocytes
	Animal	Caseinomacropeptide	Exertion of anti-inflammatory effects in a rat model of colitis
	Clinical trial	Whey protein concentrate	Increased lymphocyte and glutathione levels in patients with lung inflammation and cystic fibrosis
		Protectamin®	
			Increased glutathione levels (human immunodeficiency virus (HIV))
Gastrointestinal disease	Animal	Whey protein concentrate	41% Reduction in ulcerative lesions and 73% with repeated doses
		Hydrolyzed whey protein (peptide concentrate)	Reduction of gastric injuries to 68.5% (sulfhydryl-containing moieties)
		α-Lactalbumin	Reduction in ulcerative lesions (prostaglandin production)
	Clinical trial	Lactoferrin (infant formula)	Increased bifidobacteria proportion
		Hydrolyzed whey protein (infant formula)	Increased bifidobacteria proportion improving gastrointestinal immunity
			Decreased potential for developing atopic diseases
		Hydrolyzed whey protein (infant formula)	Decreased incidence of infantile colic
		Hydrolyzed whey protein	Bowel resection and protective effect on the gastric mucosa (sulfhydryl component – glutathione production)
Neurological disease	Animal	α-Lactalbumin	Protective effects and better seizure control as assessed by myoclonus (involuntary muscle twitch)
	Clinical trial	α-Lactalbumin	Increased plasma tryptophan (a precursor of serotonin – key for the treatment of stress-induced cognitive decline)
			Improved seizure control in persons with drug-resistant epilepsy; antidepressive effects and sleep quality
Microbial disease	*In vitro*	Whey protein concentrate (enriched with *Helicobacter pylori* – antibodies)	Prevention of infections
		Hydrolyzed β-lactoglobulin	Antimicrobial against several gram-positive bacteria and bactericidal activity
		α-Lactalbumin	Bactericidal activity (*S. pneumonia*)
		Hydrolyzed α-lactalbumin	Antimicrobial against several gram-positive bacteria
		Caseinomacropeptide	Antimicrobial activity
		β-Lactoglobulin, α-lactalbumin, and bovine serum albumin (chemically modified by 3-hydroxyphthalic anhydride)	Exhibition of antiviral activity against human herpes simplex virus type 1 (HSV-1)
	Clinical trial	Lactoferrin	Bacteriostatic and bactericidal activity against *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Listeria monocytogenes*, *Bacillus stearothermophilus*, *B. subtilis*, and *Micrococcus luteus*
			Antifungal activity against *Candida albicans*
			In combination with lysozyme, immunoglobulins, or antibodies – more potent bacteriostatic agent against *Pseudomonas aeruginosa*, *L. monocytogenes*, and *E. coli*
			Decreased intracellular group A streptococci in chronic pharyngitis patients
			100% Eradication of *Helicobacter pylori*

Table 2 (Continued)

Clinical diagnosis	*Type of study*	*Sources*	*Results/advantages*
Cancer	*In vitro*	Whey protein concentrate	Increment of glutathione levels; stimulation of immunity; detoxification of potential carcinogens
			Mutagenic agent causing oxidative damage to tissues (iron-binding capacity)
		Whey protein isolate	Increased glutathione levels; protection of human prostate cells against oxidant-induced cell death
		Whey protein isolate (with coadjuvant baicalein)	Protection of human hepatoma cell line
		α-Lactalbumin	Antiproliferative effects in mammalian, colon adenocarcinoma, and intestinal cell lines; delaying initiation of cell apoptosis
		Bovine serum albumin	Inhibition of growth in human breast cancer cells
		Lactoferrin	Induced cell death in tumor cells and exertion of cancer-protective mechanisms in melanoma cells, breast cancer cells, stomach cancer cells, lung cancer cells, lymphoma cells, colorectal cancer cells, and polyps
		Immunocal®	Enhanced cytotoxicity – high rate of apoptosis
	Animal	Whey protein concentrate	Lower incidence of colon and mammary tumors and fewer aberrant crypts (colon cancer)
			Prevention of breast and intestinal cancers in female rats
			Increased glutathione concentration, leading to stimulation of immunity
			Depletion of tumor cells (glutathione concentration higher than normal cells)
		Hydrolyzed whey protein	Prevention and treatment of 5-fluorouracil chemotherapy-induced oral mucositis
		β-Lactoglobulin	Immune-enhancing
		Lactoferrin	Inhibition of metastasis of primary tumors
	Clinical trial	Nondenatured whey protein concentrate	16/20 Survivors at 6 months
			Increased NK cell function, glutathione levels, hemoglobin, and hematocrit
			Improved quality of live
		Lactoferrin	Reduced risk of colon carcinogenesis
		Immunocal® (with coadjuvant baicalein)	2/7 Patients had tumor regression after 6 months
			Increase in glutathione levels in healthy cells and decrease in glutathione levels in cancer cells
Diabetes	Animal	Whey protein concentrate and α-Lactalbumin	Improved postprandial glucose tolerance and long-term glucose control
			Anti-inflammatory effect
			Protection against endothelial dysfunction, lowering of HbA1c, and insulin resistance
			Cellular glucose toxicity
			Reduction in parameters of oxidative stress
	Clinical trial	Whey protein concentrate	Decrease in triglycerides and the exposure to glucose (AUC) by up to 44%
			Increased insulin secretion and reduced postprandial (after meal) exposure to glucose by 21%
Hepatitis	*In vitro*	Lactoferrin	Prevention of hepatitis C virus infection in a human hepatocyte line
		Whey protein concentrate	Reduced inflammatory markers and improved liver enzymes (hepatitis C patients)
	Clinical trial	Immunocal®	Decreased serum lipid peroxidase levels and serum alanine transferase activity (hepatitis B patients)
			Increased IL-2, NK activity, and plasma glutathione levels
Osteoporosis	*In vitro*	Milk basic protein (lactoferrin, lactoperoxidase, and minor components)	Stimulation in the proliferation and differentiation of osteoblastic cells and suppression of bone resorption
	Animal	Whey protein concentrate and hydrolyzed whey protein	Increased femoral bone strength in young ovariectomized rats
	Clinical trial	Milk basic protein	Increased bone formation (osteocalcin and procollagen I carboxy-terminal propeptide levels increase), bone mineral, and radial density
			Inhibition of osteoclast-mediated bone resorption

(*Continued*)

Table 2 (Continued)

Clinical diagnosis	*Type of study*	*Sources*	*Results/advantages*
Exercise	Clinical trial	Nondenatured whey protein concentrate	Increase in body mass composition and improved quality of life on HIV-positive women
		Whey protein concentrate	Improvement in one of the four muscles, lean tissues mass, and strength
			Significant improvements in peak power and increase of 30 s work capacity
			Increased lymphocyte glutathione levels
			Decrease in percent body fat improving body mass index
Obesity	*In vitro*	Whey protein concentrate	Prevention and/or attenuation
	Clinical trial	Whey protein concentrate	Reduced fat buildup in obese women with high liver fat content and reduced triglycerides and total cholesterol

Lactoferrin develops a direct role in defending the body against pathogens. This protein possesses bactericidal power against a wide range of microorganisms. The antimicrobial effect can be taken advantage of in the treatment of enteric infections. Recent studies have demonstrated that iron-unsaturated lactoferrin is active against the fungi – which are responsible for dermatophytosis. Lactoferrin possesses antiviral effects too and plays an important role upon stimulation of the immune system.

Whey Proteins, Interaction with Different Organs

Whey proteins are beneficial to the intestinal lining, especially in clinical situations where gut function is impaired. A study in rats showed that whey promotes neutrophil chemokines – GCP-2 activity in the intestines and subsequent intestinal cell growth. In a clinical study, it was found that the outcome provided by a whey protein concentrate administered to a person with Crohn's disease was very close to the behavior of glutamine, reducing intestinal permeability.

In the liver, whey proteins exhibited an important role in studies with nonalcoholic fatty liver, showing the ability to reduce liver enzyme (ALT, alanine transaminase; AST, aspartate aminotransferase; and GGT, gamma-glutamyl transpeptidase) levels and fat deposits, enhancing glutathione (due to cysteine). A reduction in weight and waist circumference has also been verified. Another study showed that high-dose supplementation in obese women with high fat content in the liver was able to reduce the accumulation of fat in the liver, reducing triglycerides and total cholesterol.

Whey Powder and Exercise

After performing exercise, subjects enter into metabolic stress, requiring consumption of essential amino acids, whey proteins, minerals, or other serum-derived compounds (usable energy). From the mechanistic point of view, due to their composition in amino acids, whey proteins can provide substrates and bioactive components that are capable of extending the overall benefits of regular physical activity. Accordingly, they have been chosen by bodybuilders and elite athletes, in addition to people whose health is compromised. Whey protein can help to improve muscle strength and reduce the likelihood of bone fracture; these characteristics are directly related to their unique amino acid composition, as mentioned earlier, which are quite similar to those of skeletal muscle proteins. However, the improvement seen in increased skeletal muscle protein occurs through mechanisms that are definitely beyond those assigned to the said content of essential amino acids. When it comes to a moderate exercise, there is an improvement in immunity. On the other hand, when the training is heavy, there is stress in the immune system, leading to the production of free radicals and increased inflammatory activity. It is thought that those factors can harm the immunobiological activity of athletes who train heavily, in which muscle performance and recovery may be affected by oxidative stress. The availability of glutathione can be used to combat this problem. Other studies have shown that whey proteins have helped to achieve more easily the desirable weight and shape body, being more effective than caseins in what concerns to satiety.

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See also: Cheese: Composition and Health Effects; Dairy Products: Dietary and Medical Importance; Milk: Sources and Composition; Whey and Whey Powders: Fermentation of Whey; Whey and Whey Powders, Principles and Applications of Dialysis; Whey and Whey Powders: Production and Uses.

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Whisky, Whiskey and Bourbon: Composition and Analysis of Whisky

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Sensory Assessment

Whisky has traditionally been assessed by expert blenders with many years of experience and training within the industry. An experienced blender knows what flavors of distillate a still can produce, which are desirable, and how a whisky is likely to develop during maturation. The blender's task is then to identify faults and deviations from the expected path of maturation and to select a specific maturation point at which a whisky can contribute to the blend. Blenders use a system of flavor description to assist their work, but their method of working is largely by comparison of samples with experience or a reference. Each sample is evaluated in terms of its similarity to an expected or acceptable product.

Scotch grain whiskies are light-bodied continuous still spirits, largely regarded as providing a background for a blend. Malt whiskies provide the majority of the character of a particular blend and, though the distilleries all produce slightly different whiskies, for convenience, they are divided into groups sharing common characteristics. The Highland malts, the most numerous, are further divided into first, second, and third classes. These are not intended to be quality evaluations, but are simply descriptive classes. The Lowland malts are not regarded as having a distinctive character and thus can be used in relatively large quantities in blends. There are three remaining Campbeltown distilleries and this group has, as a result, declined in importance. The Islay whiskies tend to be full-flavored and are usually heavily peated and thus have a significant impact on a blend even when used in small proportions. The Highland group contains most of the great whiskies that have established reputations as single malts and for their value in blending. A wide range of flavors is available and a skilled blender can make use of them to create a distinctive blend.

Formal methods of laboratory sensory analysis have also been used. Many companies use difference tests for quality control monitoring, and the whisky industry seems to have been one of the first to adopt the duo–trio and triangle tests for such purposes. Over recent years, there has also been an increasing interest in the assessment of whiskies by profiling or descriptive methods, largely for research into the origins and development of flavor in whiskies. Several broadly similar vocabularies of descriptive terms have been used. These vary mainly in the number of terms they include and therefore in the detail with which they attempt to describe flavor. They have often been presented in the form of a 'flavor wheel,' with varying numbers of concentric tiers, which while having no particular theoretical significance provide a framework for communication and training (Figure 1).

The Chemistry of Flavor Compounds

With the advancement in chromatographic techniques and, in particular, their coupling with mass spectrometry, the number of compounds identified from whisky has increased dramatically. Particular use is made of gas chromatography with a number of detectors for the measurement of volatile organic compounds. Nonvolatile constituents may be measured as a whole spectrophotometrically or individually by high-performance liquid chromatography. Problems arise as compounds of sensory importance may be present at very low concentrations (nanograms per gram). Major volatile congeners in a range of whiskies are shown in Table 1, and the major categories of compounds thought to be important in the development of flavor are discussed in the succeeding text.

Alcohols, Carboxylic Acids, and Esters

Aliphatic alcohols, acids, and esters are produced by the yeast (*Saccharomyces cerevisiae*) during fermentation, though ester formation may also occur during distillation and maturation. Saturated and unsaturated chains are found, ranging from 1 to 16 carbon atoms. Short-chain alcohols such as propanols, butanols, and pentanols are the most abundant. Nonaliphatic alcohols include 2-phenylethanol and 2,3-butanediol. The principal organic acid is acetic acid, accounting for between 50% and 90% of the total content of volatile acids. Octanoic, decanoic, and dodecanoic acids are quantitatively important, though lower-molecular-weight acids such as butanoic and pentanoic acids may have more sensory impact. The most abundant esters are the ethyl esters of the acids named, their concentrations reflecting the relative abundance of the acid. Any other combination of the acids and alcohols present may occur. After ethyl esters, the most abundant are acetate esters such as isoamyl acetate and phenylethyl acetate. The esters of hydroxy acids, such as lactic acid, and of dicarboxylic acids, such as succinic acid, can be distilled along with alcohol and steam and so occur in whisky.

Aliphatic Carbonyl Compounds

The majority of carbonyl compounds are produced by the metabolic actions of the yeast during the fermentation. However, their formation by oxidation of unsaturated fatty acids or Strecker degradations has been shown to occur at almost any stage of production. Acetaldehyde is the most common and is often used to determine total aldehydic content. Many other aldehydes, ketones, and acetals have also been identified. For aldehydes and ketones, aliphatic chain lengths, both saturated and unsaturated, range from 2 to 14 carbon atoms. Due to the high alcoholic strengths of the distillate, aldehydes exist in equilibrium with the corresponding acetal and hemiacetal. The most frequently reported acetal is 1,1-diethoxyethane, though a range of aldehydes up to six carbon atoms have been shown to form acetals.

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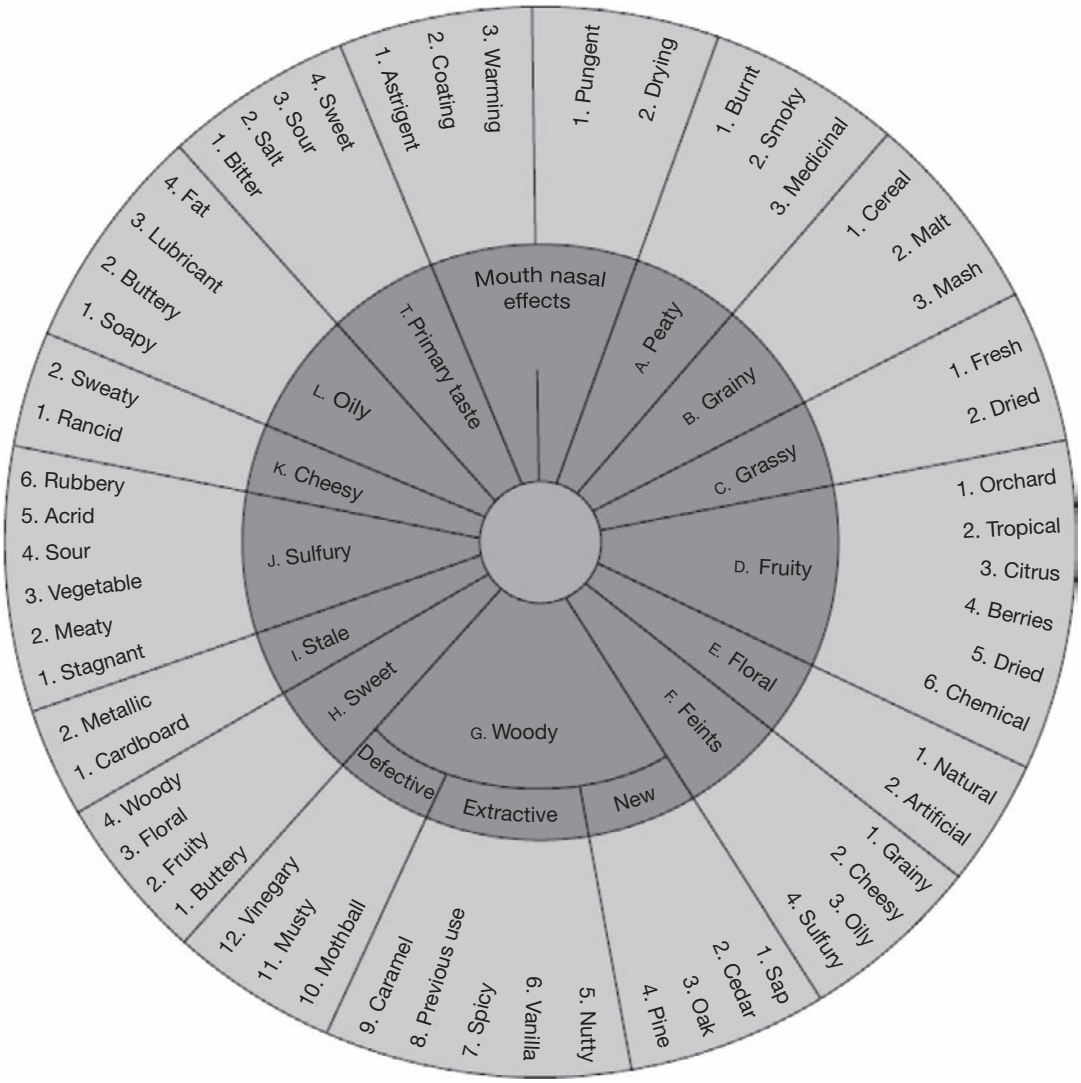

Figure 1 Whisky flavor wheel. *Note*: 'Feints,' also tails or after shots, is the final fraction of distillate, redistilled with the next batch of low wines. Reproduced with permission from Lee, K. Y. M., Paterson, A., Piggott, J. R. and Richardson, G. D. (2001). Origins of flavour in whiskies and a revised flavour wheel: a review. *The Journal of the Institute of Brewing* **107**, 287–313. Copyright 2001 The Institute of Brewing & Distilling.

Table 1 Major volatile congener concentrations (g/100 l abs. alc.) in single samples of whiskies

Whisky type	*Acetaldehyde*	*Methanol*	*Ethyl acetate*	n-*Propanol*	*Isobutanol*	*2-Methylbutanol*	*3-Methylbutanol*	*2- and 3-Methylbutanol*	*Ratio 1*	*Ratio 2*
Scotch malt whisky	17	6.3	45	41	80	62	114	176	2.2	2.8
Scotch grain whisky	12	8.5	18	72	68	8	15	23	0.3	2.8
Scotch blended whisky	5.4	8.9	23	55	62	26	46	72	1.2	2.8
Irish whiskey	4.1	10	13	28	15	18	32	49	3.1	2.8
Kentucky bourbon whiskey	15	17	89	28	160	143	242	385	2.4	2.7
Canadian whisky	3.3	7.9	7.1	6.2	6.9	7	9	16	2.3	2.2

Ratio R1 = 2- and 3-methyl butanol/isobutanol; ratio R2 = 3-methylbutanol/2-methylbutanol.

Source: Reprinted from *Whisky technology, production and marketing*. Aylott, R., Whisky analysis, pp. 275–306. Copyright (2003), with permission from Elsevier.

Table 2 Concentrations (micrograms per liter at 100% alcohol) of sulfur compounds in malt whiskies of various ages

Compounds	*Age (years)*				
	0	*1*	*2*	*3–5*	*6–10*
Dimethyl disulfide	359	345	272	149	91
Dimethyl trisulfide	12	16	15	19	14
3-(Methylthio)propanal	7	t	0	0	0
Dihydro-2-methyl-3(2H)-thiophenone	77	53	3	0	0
Ethyl 3-(methylthio)propionate	21	11	t	0	0
3-(Methylthio)propyl acetate	274	139	3	0	0
2-Thiophenecarboxaldehyde	66	83	49	60	54
3-(Methylthio)propanal	8	0	0	0	0
5-Methyl-2-thiophenecarboxaldehyde	15	15	10	16	16
Benzothiophene	39	27	27	31	38
Benzothiazole	18	16	14	15	32

t, trace.

Source: Masuda, M. and Nishimura, K. (1981). Changes in volatile sulfur compounds of whisky during aging. *Journal of Food Science* **47**, 101–105. Copyright © 2006, with permission from John Wiley and Sons.

Sulfur and Nitrogen Compounds

Yeasts have the ability to utilize inorganic sulfur, sulfur-containing amino acids and vitamins, and from these sources, volatile sulfur-containing compounds are produced. The range of compounds detected include heterocycles, such as thiazoles and thiophenes, and aliphatic mono-, di-, and trisulfides. The formation of sulfur compounds has also been shown to occur during distillation and concentrations are highest in new spirit. Cask char, wood extractives, and traces of elemental copper are important in the degradation of such compounds during maturation (Table 2). Heterocyclic nitrogen compounds such as pyrazines, pyrroles, and pyridines are formed via the Maillard reaction during cooking, mashing, distillation, and barrel charring. As they have relatively low odor thresholds, they may well make a contribution to the aroma of whisky.

Phenols

A wide range of phenolic compounds are found in whisky. Simple phenols such as phenol, the isomeric cresols, xylenols, ethyl phenols, and guaiacols arise through the thermal degradation of benzoic acid derivatives from malt and from peat smoke. Phenolic aldehydes such as vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde are formed from the breakdown of wood lignin during cask charring and maturation. Their corresponding acids and ethyl esters have also been detected. Polyphenols extracted from the wood include ethanol-soluble lignin and tannins derived from both gallic and ellagic acids.

Heterocyclic Oxygen Compounds

The main types of these compounds found in whisky are furaldehydes and lactones. Furfural (2-furaldehyde) is formed from pentoses and 5-hydroxymethyl-2-furaldehyde from hexoses via the Maillard reaction during mashing, distillation, and cask charring. Lactones are formed by the dehydration of aliphatic hydroxy acids and most are derivatives of 2(3H)-furanones. The most abundant of these are the cis and trans isomers of 5-butyl-4-methyldihydro-2(3H)-furanone, the oak or whisky lactones, which are formed and extracted from oak casks during maturation.

Variations with Processing

Though there are only four basic processes in whisky production, local, regional, and national variations in these processes are responsible for the large number of whiskies available. Each stage of production, from the production of a fermentable extract, to fermentation, to distillation, to maturation, can affect the character of the final product. However, each stage is interlinked and identifying one, as the most important for a particular type of whisky, is often impossible.

The Production of a Fermentable Extract

The cereal grains in common use are wheat, corn (maize), rye, and barley, though the choice of cereal is often legally restricted. Flavor differences between cereals arise from two quite separate sources: the composition of the extract and the levels of precursors present. The choice of cereal can also indirectly affect the flavor of the final product. The composition of the fermentable extract varies with cereal used and can influence the metabolism of the yeast. Factors such as pH, amino acid concentration, and levels of insoluble material are known to affect the levels of fusel alcohols, acids, and esters produced, which may influence the flavor of the final product. Between cereals, differences also exist in the levels of precursors for a number of classes of flavor compounds. Examples are compounds formed by the chemical and enzymatic oxidation of fatty acids, the reaction of free amino acids and reducing sugars (Maillard reaction), and the thermal degradation of biosynthesized precursors like *S*-methylmethionine and benzoic acid derivatives. However, the breakdown of these compounds frequently occurs during fermentation and distillation and is affected by variations in these processes. Consequently, no flavors in the final product have been unequivocally linked to components of the original cereal.

Fermentation

Fermentation is responsible for the primary production of a large number of flavor compounds in whiskies. Factors that are known to alter the production of these compounds are the strain or mix of yeasts used, design and level of aeration in the fermenter, the duration of the fermentation, and the presence of contaminating bacteria. These factors alter according to distillery design and the raw materials employed and so may be important in producing regional and national differences in whiskies.

Distillation

In batch distillation using pot stills, the selection of the cut points in the still run has a marked influence on the flavor of the product. Generally, aldehyde and short-chain ester concentrations are determined by the primary cut from foreshots to spirit, while the concentration of fusel alcohols and acids is determined by the cut from spirit to feints. Other factors, which affect the physical process of separation during distillation, are still design parameters such as the still height, the angle, and length of the lyne arm and the type of condenser used. In addition, a number of chemical reactions can occur when the wash or low wines are heated for distillation. This is particularly true for batch distillation in pot stills where the heat impact is greater. Examples include the formation of sulfur compounds from sulfur-containing amino acids, the breakdown of unsaturated fatty acids to carbonyl compounds, and the dehydration of β-hydroxypropionaldehyde to acrolein. Copper catalyzes many of these reactions.

In continuous stills, congener concentrations are determined by the design, operation, and efficiency of the still. The range of reactions described for pot stills can still occur but become progressively less important as distillate strength increases. Collection of spirit at high ethanol concentrations (>90%) results in low levels of congeners in the spirit.

Maturation

During the maturation period, the new distillate becomes highly modified as a result of its contact with the cask (Figure 2). The composition of the cask wood has a major effect on the extent of this modification and varies with the species of oak used, the pretreatment applied to the wood, and the previous use of the cask. In general, American oak (*Quercus alba*) yields higher concentrations of vanillin and *cis* oak lactone, while Spanish oak (*Quercus robur*) gives higher concentrations of tannins. Charring increases the levels of lignin breakdown products and lactones extracted during maturation, whereas previous use of a cask greatly reduces the amount of extractable lignin breakdown products and lactones. The use of sherry casks results in raised levels of tannins and sugars. The cask is the major determinant of mature quality, but other factors such as filling proof, climate, and warehouse conditions also affect the course of maturation. Higher temperatures produce greater evaporation rates and also increase extract levels and some reaction rates, but do not necessarily produce a more mature product.

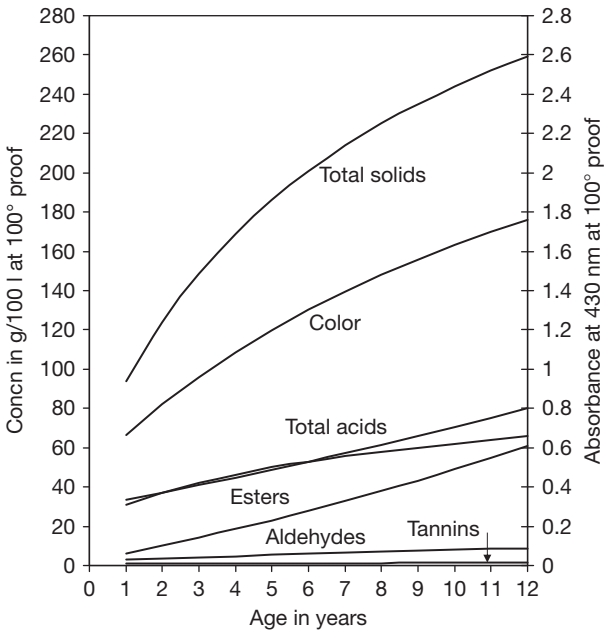

Figure 2 Congener changes during maturation of whisky. Reprinted from Baldwin, S. and Andreasen, A. A. (1974). Congener development in Bourbon whisky matured at various proofs for twelve years. *Journal of the AOAC* **57**, 940–950. Copyright 1974 by AOAC INTERNATIONAL. Permission is granted for a reprint of Figure 8.

Correlation of Chemical Composition with Sensory Data

The flavor of whisky is dependent upon a complex mixture of components that originate from and are modified by different stages of production. While improved analytical methods have revealed much about the chemistry of whisky, this has not resulted in a better understanding of the chemical basis of whisky flavor and quality. Frequently, chemical differences can be related to variation in production processes, but identifying the actual compounds responsible for the flavor differences has been much more elusive.

The extent to which volatile compounds from cereals survive fermentation, distillation, and maturation is the hardest to establish. Whisky prepared from heavily peated malt retains a characteristic medicinal, smoky flavor that is strongly correlated with the total phenols content of the malt and of the whisky. However, the range and concentration of phenols present are greatly altered by mashing, fermentation, and distillation and whether the phenols fully explain the 'peated' flavor is still uncertain. Likewise, some whiskies (especially Canadian) have a characteristic 'spicy' or 'minty' flavor, which is possibly related to the use of rye in the mash, but no compound has been identified as responsible.

Various flavors in new distillates have been correlated with sulfur compounds. Polysulfides are present in whisky at levels around and above their sensory thresholds (3–6 ng g^{-1} for dimethyl trisulfide), and the data available suggest that these compounds are correlated with 'sulfury' and 'heavy' characters in fresh distillates. Other sulfur-containing compounds are thought to be important contributors to flavor, and their formation is dependent on the levels of precursors present in different cereals/cultivars. However, such compounds are

frequently formed during fermentation and distillation and then degraded during maturation. Each stage has its own impact on the concentration in the final product making it harder to establish their role in whisky flavor.

The products of fermentation and distillation make a substantial contribution to whisky flavor. However, these compounds, except when present in excess, appear to form what has been described as the 'integrated whisky complex,' and their individual contributions have not been determined. It does appear that relatively constant proportions of some of these compounds must be present for a recognizable 'whisky' character, though substantial changes can occur with only minor changes in flavor.

Compounds considered to be important in maturation are vanillin, eugenol, and *cis* oak lactone. These have been correlated to 'vanilla' and 'clove-like' flavors in some whiskies matured in new casks. However, cask reuse causes a general decrease in wood extractives and their importance in whisky matured in refill casks is still uncertain.

Attempts to create a model of whisky flavor have been largely unsuccessful. Attempts were based on compositional analyses of the liquid. Research has since shown that there are complex relationships governing the release of flavor compounds from whiskies. Major factors identified include alcoholic strength and the nonvolatile content of the whisky matrix. Dilution of spirits for sensory assessment, to reduce the pungency of ethanol, can greatly alter the release of aroma compounds more soluble in ethanol than in water. Dilution also increases the capacity for matrix effects that suppress the release of ethyl esters and other aroma compounds. Matrix effects may occur through competition for release at the liquid surface or changes in solubility of flavor components. Changes in solubility can be related to the physical structuring of the ethanol–water mixture. Differential scanning calorimetry, light scattering, and mass spectrometry have all demonstrated that physical changes occur during maturation, adding another layer of complexity to whisky flavor and reinforcing the uniqueness of each individual product.

Health Effects

Apart from the well-known benefits of light to moderate alcohol consumption, whisky, in common with some wines and other wood-matured spirits, contains phytochemicals with potentially beneficial effects. Most attention has been given to antioxidants, but wood and spirit matured in it contain a range of other components with possible effects, such as amelioration of allergic reactions. Phytophenols may also reduce the rate of alcohol metabolism, thereby reducing blood acetaldehyde level and prolonging exposure to alcohol. In animal models, whisky has also been found to have a less damaging effect on the stomach compared with aqueous ethanol. The highest antioxidant activity has been found in red wines, followed by Armagnac, Cognac, whiskies, and some wood-aged rums, with white spirits showing no activity. The extent of the activity varies with the maturation time, older spirits showing greater activity, and with the wood and its heat treatment before use. Oak, the species used most commonly for spirit maturation, is among the most active. Activity is correlated with the total phenolic content and with ellagic acid, a major component of the wood-derived materials present in matured spirits. In some studies, flavonoids have been found to be most important for the antioxidative capacity of spirits, whereas in other cases, nonflavonoids appear to be important. Consumption of alcoholic beverages containing phenolic compounds transiently raises plasma phenol concentrations and antioxidant capacity in humans, but as yet, there is little evidence of any real benefit in terms of chronic disease risk.

See also: Antioxidants: Role on Health and Prevention; Browning: Non-enzymatic browning; Chromatography: Focus on Multidimensional GC; Chromatography: Combined Chromatography and Mass Spectrometry; Chromatography: High-Performance Liquid Chromatography; Mass Spectrometry: Principles and Instrumentation; Mass Spectrometry: Applications; Phenolic Compounds: Occurrence, Classes, and Analysis; Sensory Evaluation: Sensory Rating and Scoring Methods.

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Whisky, Whiskey, and Bourbon: Products and Manufacture

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Types of Product

Whiskeys are distilled beverages that have been produced by the distillation of fermented cereals and have been matured in oak casks. They can be classified on the basis of the nature of the cereal; the manner in which the product is blended, if at all; and the country of origin. Although there is some evidence for a distillation-type process in ancient Egypt, the first reports of whiskey production arise in Ireland in the twelfth century, and the first description of a whiskey production process is found in Scottish records dating from 1494. The term 'whiskey' was derived from the Gaelic *uisge beathe*, which means 'water of life.' Major countries of production are Scotland, the United States, Ireland, Canada, and Japan, while similar products are produced in many other countries.

Regulations in the European Union (EU) define whiskey as a spirit produced by the distillation of a mash of cereals and matured for at least 3 years in wooden casks. There are limitations on the enzymes that can be used to hydrolyze starch to fermentable sugars and on the distillation strength, and yeast must be used for the fermentation. Within this definition, Scotch and Irish whiskeys are further restricted. In practice, this provides for three distinct products: malt whiskey, produced from 100% malted barley; grain whiskey, produced from unmalted cereal grains; and the bulk of the whiskey produced in Scotland, which is blended from 60 to 70% grain whiskey and 30 to 40% malt whiskeys. This blended whiskey usually contains up to 40 individual malts, which are blended to produce a consistent brand flavor, and every component of the blend must be matured for the minimum period or the age specified on the bottle. Malt whiskeys may contain either a blend of malt whiskeys of a given minimum age or malt whiskey from a single distillery. Scottish malts have a different character depending upon whether they are from the west coast, such as Campbeltown and the Islay region, which produce highly peated malts, or from the highlands or the lowlands. Irish whiskeys have a similar flavor to Scotch but do not have a peaty character.

Whiskey produced in the United States has evolved in a different legislative framework, allowing for a greater variety of distinctive products. The basic definition of US whiskey is that it is distilled from a fermented grain mash at less than 95% alcohol (190° US proof). Within this broad definition, there are two special categories that are bottled as such or provide flavoring whiskeys for blending. In the first group, the cereal content of the mash is restricted to not less than 51% of the named cereal (e.g., rye for rye whiskey and maize for bourbon), the distillation must be at less than 80% alcohol, and maturation must be in new charred oak. Bourbon takes its name from Bourbon County in Kentucky. Corn whiskey must be from a mash of 80% maize and matured in new uncharred or used oak. If matured for 2 or more years, these are 'straight whiskeys.' They are to the blender the equivalent of the Scotch malts and provide the majority of the flavor in a blend with lighter flavored grain spirits. US blenders are also permitted to use 'blending materials,' such as sherry or blending wine, at up to 2.5%.

Canadian whiskey production follows the same general practice as in Scotland and the United States. Canadian blends are light column still whiskeys with normally 10–15% of heavier-bodied flavoring whiskeys. Up to 9.09% (on the basis of absolute alcohol content) of 'flavorings' is permitted, which may include wines and sherries, rum, brandy, bourbon, or malt whiskey. Maturation must be for not less than 3 years, and oak casks of 182 l (40 imperial gallons) are commonly used. The range in flavor required by the blender cannot be achieved by using the products of many different distilleries, but techniques such as variation of the composition of the mash (corn, wheat, rye, or barley), cooking procedures, yeast strain, distillation procedure, barrel type, and maturation period can be used. Traditionally, pot stills were used but these have largely been phased out.

Similar products are produced in many other countries and are subject to local regulations, which, while generally similar in principle to the EU definition, allow different extents of variation. In Brazil, regulations permit the addition of a proportion of agricultural alcohol to blended whiskey, and in Japan, the locally produced spirit is frequently blended with imported Scottish malts. Spain is a substantial producer of whiskey, which must meet the EU requirements. EU and US regulations also include provision for grain spirits or grain brandies, which are essentially similar to whiskeys but not matured. In other parts of Europe (e.g., Poland), 'whiskeys' are produced that are essentially vodkas, matured briefly, and flavored with wood extracts or other materials and that may or may not meet regulations commonly accepted for whiskeys.

Raw Materials

The major grains used in whiskey production are maize (*Zea mays*), barley (*Hordeum vulgare*), wheat (*Triticum vulgare*), and rye (*Secale montanum*). These are the grains traditionally used for whiskey, on the basis of a high fermentable starch content providing a good yield of alcohol. A typical composition of these grains is shown in Table 1. In Scotland and Ireland, all the amylase activity required for starch breakdown is obtained from malted barley, in the form of either a kilned malt, which is stored at a moisture content of 5%, or a green malt, which is prepared specifically on-site as a source of enzyme activity for the production of grain whiskey. Some regulations also permit the use of other (normally microbial) enzymes during wort production. Cultivars are normally selected on the basis of extract and fermentability, corresponding to spirit yield, or enzyme activity in the case of malt for grain distilling. Enzyme activity should be considered under process conditions, as this

Table 1 Composition of cereals used in the production of whiskey worts

	Corn	*Rye*	*Barley*	*Wheat*
Percentage of total				
Endosperm	82	87	84	85
Germ	12	3	3	3
Bran	6	10	13	12
Chemical composition (%, *dry-weight basis*)				
Nitrogen-free extract	69.2	70.9	66.6	69.9
Starch	72	68	63–65	69
Sugars	2.6	0	2–3	0
Protein	8	12.6	12	13.2
Soluble nitrogen (% of total)	4.7	0	11	0
Crude fiber	2	2.4	5.4	2.6
Fat	3.9	1.7	1.9	1.9
Ash	1.2	1.1	2	1.9

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will not be the same as activity measured under optimum laboratory conditions. Ethyl carbamate is an undesirable trace component in distilled beverages, and its content in malt whiskey is largely determined by the cyanogenic precursor, epiheterodendrin, in malt. Barley cultivars that do not contain the precursor are used. The gene responsible for its production has been identified, and differences in the DNA sequence between producers and nonproducers (i.e., different alleles) have been used to develop a simple diagnostic assay that discriminates between them. This can be used as a screening test to eliminate experimental varieties, which are producers.

Water is required for mashing, cooling during distillation, dilution of the spirit before maturation, and blending. The quality of the product may be influenced by the nature of the water supplied, and many distilleries take their water supply from individual water courses or special wells. Soft water is normally used for malt distilleries, but some degree of hardness is preferred in grain distilleries since this has a stabilizing effect on the amylase enzymes. The water should be monitored for chemical and microbiological contamination and be free of decaying vegetable matter. The water used for dilution of the spirit must have a low calcium and iron content and is normally demineralized, to minimize the risk of discoloration or precipitation occurring in the final product.

The yeast used for fermentation is normally a specially grown distilling yeast, which has good physiological and microbiological properties for producing alcohol. However, some recycled brewer's yeast may be used in Scotland, and yeasts from previous distillery fermentations may be used in some bourbon processes; both influence the range of flavor products in the wash. The main criterion for yeast strain selection is normally efficiency in converting fermentable sugars to ethanol. To a limited extent, strains may be selected to provide the required composition and flavor characteristics in the distillate, particularly the ester content, and yeast cultivation and storage conditions also affect its performance. Yeasts expressing glucoamylase activity have been developed.

Barrels used in whiskey production are produced from either American white oak, *Quercus alba*, and related species or the Spanish oak, *Quercus robur*. Scotch whiskey is traditionally matured in barrels that have previously been used for bourbon maturation or sherry production. Spanish sherry production is carried out in barrels constructed from either Spanish or American white oak. Except where the regulations provide otherwise, barrels are commonly reused until they fail to provide a satisfactory maturation. Such barrels may be regenerated by scraping out the inner surface and charring the inside of the barrel, but this does not produce performance equivalent to a new barrel. During periods when sherry casks have been in short supply, barrels have also been wine-treated or wine-seasoned (filled with sherry for up to 6 months), and Madeira, port, or ale casks are also occasionally used.

Method of Manufacture

Malting and Mashing

The traditional method of production involves the production of wort from cereal grains using amylases from barley. These α- and β-amylases are produced by the barley during the malting process. Malting of barley is carried out by steeping the grain in water for 2–3 days and permitting the grain to germinate until the root is approximately one-third the length of the grain. When this stage is reached, the grain is dried down to a moisture level of 5% by kilning. The grain is then stored until immediately prior to use when it is milled before being transferred to a mash tun. In Scotch whiskey production, peated malt is prepared by drying the malt in the presence of smoke from a peat fire and is an important source of flavor compounds, normally assessed on the basis of the total phenols content of the malt (up to 50 ppm), although other components may contribute to flavor. Nitrosamine contamination of malt could arise from the use of natural gas in directly fired kilns but is controlled by the use of burners designed to reduce nitrogen oxides, by burning sulfur to add sulfur dioxide to the air stream, or by indirect firing. Most of the breakdown of the starch occurs during the process of mashing, which is carried out in a mash tun. Barley grist can be mashed directly since it has a high-amylase activity, whereas worts produced from maize and other cereals require the addition of malted barley as a source of enzymes. The process of mashing may be a simple batch process similar to the infusion mashing techniques used in brewing beer, in which the grist is mixed with water at 63–68 °C for 0.5–1.5 h before the wort is filtered off. However, in the production of grain whiskey, an initial cooking stage, to gelatinize the starch of the nonbarley cereals, is required. The cooking may be carried out as a batch process at 120 °C for 1.5 h or, as is more usual in North America, as a continuous process. In either case, the mash is cooled to 60–65 °C before the addition of the malted barley. Careful control is required at this stage to prevent the solidification of the gelatinized starch. When a cooking stage is used, amylase activity is normally provided by adding 10–15% freshly malted barley (green malt) after the cooking stage (Figure 1).

During the malting stage, β-glucanase enzymes are also produced; these facilitate the breakdown of the endosperm cell walls and the release of starch and proteases. The latter are involved in the breakdown of some of the barley grain

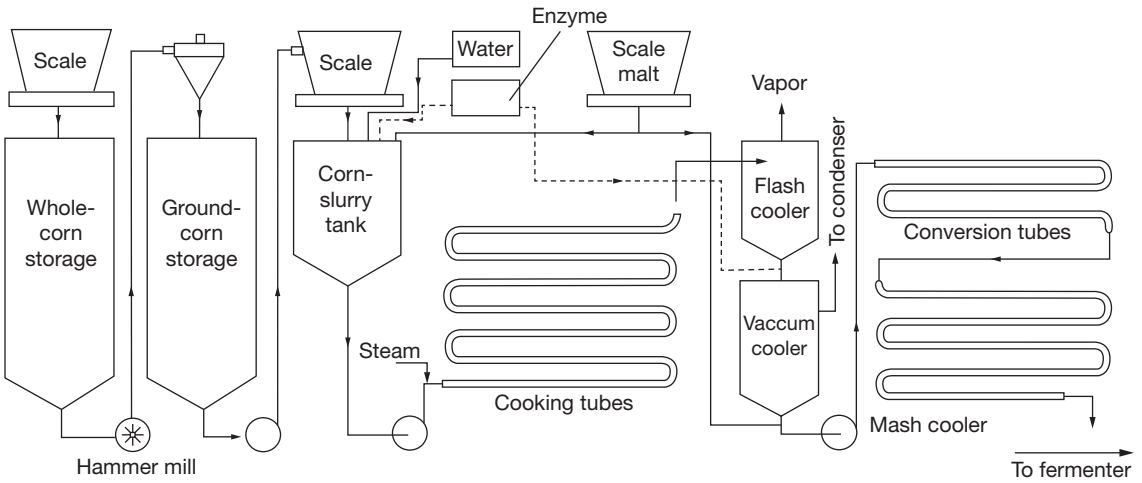

Figure 1 Maize process for whiskey. Reproduced with permission from Simpson, A. C., 1985. Advances in the spirits industry. In: Birch, G. G. and Lindley, M. G. (Eds.), *Alcoholic beverages*. London: Elsevier Applied Science. © Elsevier Applied Science Publishers Ltd. 1985.

Table 2 Chemical composition of a typical Scotch grain wort

	Content
Total soluble carbohydrate (as glucose) (%)	9.00
Insoluble solids (%)	2.20
Sugars (%)	
Fructose	0.13
Glucose	0.29
Sucrose	0.28
Maltose	4.65
Maltotriose	0.96
Maltotetraose	0.15
Dextrin	2.54
Amino nitrogen (as leucine) (%)	0.09
Ash (%)	0.27
Containing P_2O_5	0.09
Containing K_2O	0.09
Containing MgO	0.02
Vitamins ($\mu g\ ml^{-1}$)	
Thiamin	0.46
Pyridoxine	0.61
Biotin	0.01
Inositol	236
Nicotinic acid	11.1
Pantothenate	0.71

Reproduced with permission from Pyke, M., 1965. Manufacture of scotch grain whisky. *Journal of the Institute of Brewing* 71, 209–218. Copyright 1965 The Institute of Brewing & Distilling.

proteins to amino acids and peptides. These amino acids are an essential nitrogen source for the growth of the yeast.

The mash is usually filtered prior to the fermentation to give a clear wort, but in some processes, a higher yield is obtained by fermenting the whole mash directly. The composition of a typical Scotch grain whiskey wort is shown in Table 2.

Fermentation

The wort, which is produced when the contents of the mash tun have been filtered, is then transferred to a fermentation vessel and fermented using one or more strains of the yeast *Saccharomyces cerevisiae*. When the wort is not boiled (as is the case in malt whiskey production), it contains microorganisms and active amylases. It is cooled to 20–25 °C before inoculation with a strain of distilling yeast and, in some processes, secondary yeasts. Yeast growth occurs rapidly and is completed in the first 8–12 h. Ethanol production follows growth initially and then continues in a linear manner until the metabolizable sugars have been consumed. The resulting solution contains approximately 7% ethanol and a large number of flavor compounds. During the growth period, wort sugars are rapidly metabolized and the temperature may rise to 33 °C if it is not controlled. The pH drops from an initial value of 5.0–5.5 to 4.2–4.5 as a result of the production of organic acids, such as acetate, succinate, and pyruvate. A wide range of flavor compounds, such as higher alcohols, fatty acids, esters, and carbonyl compounds, are also produced throughout the fermentation; they pass through into the distillate and constitute an important part of the product. In contrast, glycerol, which is also produced, is nonvolatile and does not pass into the distillate; this represents an important loss of yield. At the end of the fermentation, usually between 48 and 72 h, death and autolysis of the yeast may occur. The fermentation is not a sterile process, so bacterial contamination can occur. Most of the bacteria that are introduced from the raw materials die during the early stages of the fermentation. However, lactobacilli can survive and grow with a concurrent formation of lactic acid during the later stages of the fermentation. Limited growth of lactic acid bacteria may be beneficial in flavor terms, but to prevent excessive growth and resulting yield losses, fermentation is normally completed in 40–48 h, rather than the longer times traditionally used. Heavy contamination by lactic acid bacteria can also result in a loss of ethanol yield and the production of acrid off-flavors.

Distillation

The fermented wash (wort after fermentation) is then distilled either in a series of two or three pot stills (Figure 2) or in a

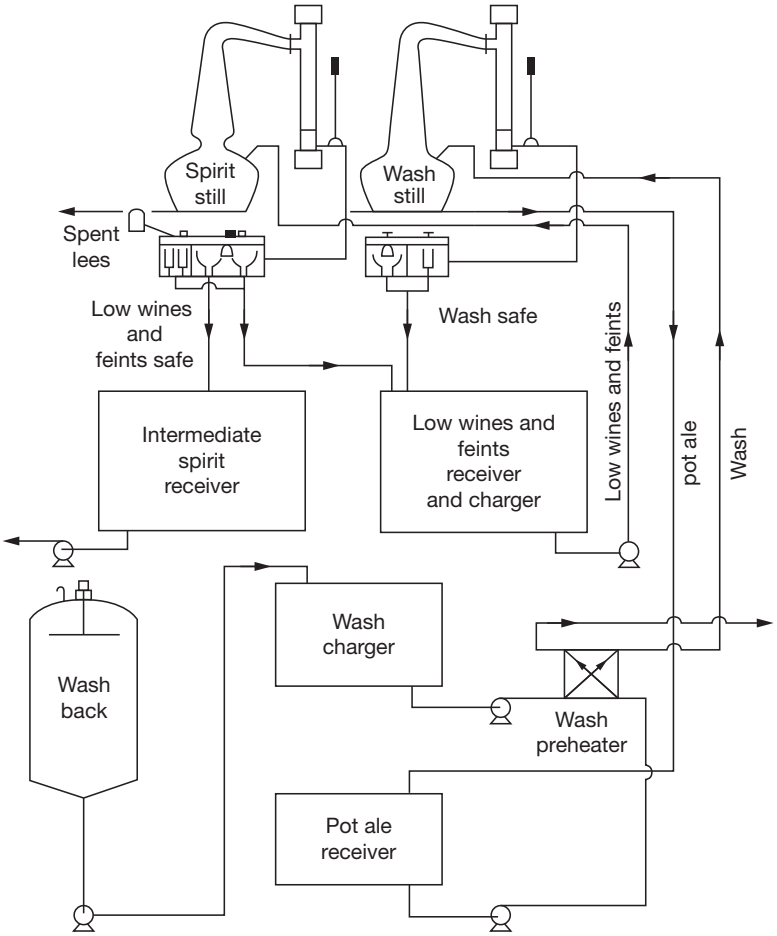

Figure 2 Typical malt distillery flow diagram. Reproduced with permission from Piggott, J. R., Sharpe, R., and Duncan, R. E. B. (Eds.), 1989. *The science and technology of whiskies*, Pearson Education Limited, © Longman Group UK Ltd 1989.

series of column stills. The distillation process varies with the type of product and the country of origin. Historically, the first whiskey processes used copper pot stills and this type of process is still used for the production of malt whiskey in Scotland, where two stills – the wash still and the spirit still – are used. In Ireland, a three-still process is typical. Pot stills have also been used in the United States and Canada, but grain spirit is normally produced by continuous distillation in column stills. The first continuous process was associated with Scotch whiskey production and the development of the Coffey or patent still by Aeneas Coffey in 1830. The ethanol concentration of the distillate determines the extent of impurities (congeners) present, a higher strength distillate being purer aqueous ethanol with a less intense flavor.

In batch distillation processes, all the contents of the fermenter are transferred to the large wash still and boiled for 5–6 h until all the ethanol has been recovered, to produce a distillate, known as low wines, which contains 20–25% (v/v) ethanol. This is transferred to the low wines or spirit still where it is distilled to give a spirit containing around 70% (v/v) ethanol (Figure 3). In Scotch malt whiskey production, the first fraction of the distillate and the third fraction (after collection of the spirit) are recycled for redistillation with a subsequent batch of low wines. The Coffey still consists of two columns, referred to as the analyzer or beer column and the rectifier column. Wash is fed toward the top of the first column and alcohol-free stillage is withdrawn at the bottom of the column; vapors are fed from the top to the base of the rectifying column (Figure 4). It produces a 190° US proof spirit and is still used for the production of grain spirit in Scotland. In the United States, whiskey production employs more complex distillation processes involving the use of up to five columns. The heat for most distillation processes is supplied as steam at the present time, although pot stills directly fired by solid fuel or gas are also in use. In addition to producing spirit, distillation processes produce a large quantity of stillage, which is processed into various by-products, such as dark grains and distiller's solubles, for animal feed.

Maturation

All whiskeys are matured and most legal definitions of whiskey incorporate a minimum maturation period. The legal minimum varies from 1 year for some American grain whiskeys to 3 years for Scotch, Irish, and Canadian whiskeys. In effect, these are the minimum requirements and most spirits are matured for longer periods. Branded products are generally matured for between 4

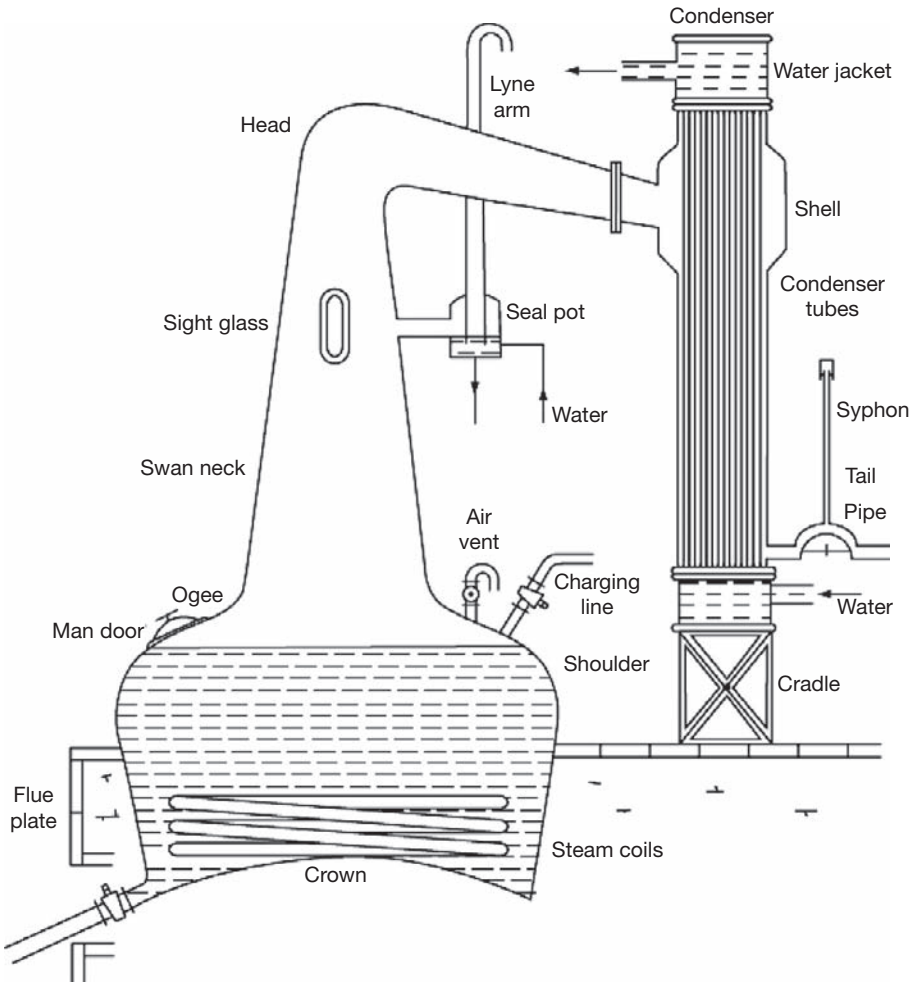

Figure 3 Plain wash still. Reproduced with permission from Piggott, J. R., Sharpe, R., and Duncan, R. E. B. (Eds.), 1989. *The science and technology of whiskies*, Pearson Education Limited, © Longman Group UK Ltd 1989.

and 8 years, while single malts and premium straight whiskeys are matured for 8 to more than 18 years. The process of maturation involves storing the raw spirit from the still, diluted to 50–80% ethanol, in barrels made from a limited range of oak woods. The type of cask used greatly affects the properties of the mature spirit. American straight whiskeys (e.g., straight bourbon whiskey, but excluding straight corn whiskey) are matured in new charred American oak barrels that by law cannot be reused. Maturation in new charred casks is associated with an increase in color, solids, acids, esters, aldehydes, and tannins, together with increases in sugars, such as arabinose, glucose, and xylose. New charred casks give a strong sweet, wood-derived flavor to the final product. The casks used for straight whiskey maturation are American standard barrels (180–200 l) made from American white oak, charred where required by regulation. 'White oak' is a cooperage trade classification used for at least 10 oak species, the principal being *Quercus alba*.

Most other types of whiskey are matured either in barrels previously used for maturation of bourbon or in sherry production. Bourbon barrels may be used 'as is' or reassembled as hogsheads, with additional staves and new ends to increase capacity (250 l). Reuse of casks decreases the levels of wood components extracted during maturation and gives a much less intense wood-derived flavor. Sherry casks used for the maturation of some whiskeys are generally butts or puncheons (480–5201), which have been used for the fermentation and/or aging of various styles of sherry. Sherry casks can be either American or Spanish oak casks (predominantly *Quercus alba* and *Quercus robur*). American oak is generally used for fino and amontillado sherries, while Spanish oak is used for oloroso sherry. For sherry casks, distillers will specify the species of oak, whether the cask is used for fermentation or the contact time with maturing sherry, as all affect the final properties of the mature spirit. In all but bourbon production, casks are reused until they are exhausted, that is, they fail to produce a satisfactory change in the spirit over an economical maturation period. The life of a cask can be extended by the removal of the old char layer and refiring the cask. This regeneration increases the levels of wood polymer breakdown products extracted during subsequent maturation, but does not regenerate all wood constituents and such casks give different sensory properties when compared with a new cask.

Maturing whiskey was traditionally stored in stone-built warehouses located beside the distillery. Casks were stored in

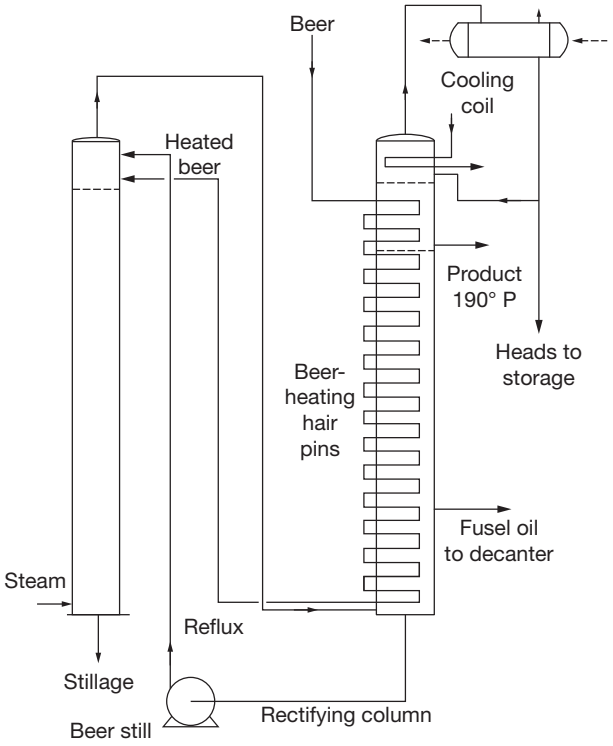

Figure 4 Coffey still. Reproduced with permission from Piggott, J. R., Sharpe, R. and Duncan, R. E. B. (Eds.), 1989. *The science and technology of whiskies*. Pearson Education Limited, © Longman Group UK Ltd 1989.

racks or in 'stows,' two or three high on the earth floor. Expansion in production resulted in much larger warehouses with steel racking, which allowed casks to be stored up to 12 high. The temperature and humidity within warehouses vary between different countries, and this can affect the way in which a spirit matures. In cool damp climates, such as Scotland and Ireland, more ethanol than water evaporates from the cask, and consequently, strength decreases during the maturation period. In warm dry climates, such as the United States, more water than ethanol evaporates and strength increases. During maturation, many of the chemical changes that occur are influenced by the storage conditions, particularly temperature. However, though higher maturation temperatures have been shown to increase the rate of extraction of wood components, this does not generally correlate with improvements in sensory quality.

Blending to Bottling

With the exception of occasional 'single cask' bottlings, all whiskeys are to some extent blended. Even in the most tightly controlled environments, batch-to-batch variations can occur at any stage in the production process. Blending is used to produce a consistent product that has the distinctive flavors of that particular brand. For bourbon and single malt production, variations are evened out to create the recognized brand from different batches of whiskey. True blended whiskeys consist of light-bodied spirits mixed with a number of heavier-bodied spirits in a wide range of proportions. 'Light-bodied spirits' are those distilled to high ethanol concentrations using continuous column stills and include Scotch grain and American light whiskeys. 'Heavier-bodied' whiskeys are either batch pot still products or column still products distilled to lower ethanol concentrations. The nature and components of blends are determined by the traditions and regulations of the country of origin. In Scotland, there are a large number of distilleries and blend recipes are complex with as many as 50 malt whiskeys used. Purchasing or exchanging new whiskeys by producers maintains the complexity of these blends. In North America, where there are fewer distilleries and trading between competitors is uncommon, the variety of whiskeys for blending can be increased by variations in mash bills, fermentation conditions, distillation parameters, maturation periods, and cask types.

The physical process of blending is, however, very similar. Approved whiskeys are drained from casks into passivated steel troughs, which convey the whiskey to a blending vat. Mixing uses mechanical agitators and compressed air. When the blend is correct, deproofing water is added to reduce the alcohol content to bottle strength. In Scotland, blending is sometimes followed by a further maturation or marrying period to allow integration of the individual components into the final product. An additional maturation or finishing period is also used for some single malts. This frequently employs a distinctive wood type, such as port casks, and is used to add complexity to a whiskey or to increase the number of styles available from a single distillery.

Most whiskeys are filtered prior to bottling to reduce the risk of haze formation during storage. In heavier-bodied older whiskeys and whiskeys matured at high strengths, high-molecular-weight lipids and wood components slowly flocculate when the whiskey is reduced to bottling strength. This is controlled by chill filtration, where whiskey is cooled to between -10 and 10 °C for a specified period of time and the problem compounds are removed by physical separation and adsorption by a filter. The conventional filter is a plate and frame filter with preformed pads of cellulose or cellulose impregnated or precoated with diatomaceous earth. Operational parameters depend on batch size, the nature of the product, and the filtration rate required.

Patterns of Consumption

The top ten whiskey markets by volume are India, the United States, France, Japan, the United Kingdom, Spain, Canada, Brazil, South Africa, and Germany. Most of these are also major producing countries, but small amounts of whiskey are distilled in many other countries. Some imported whiskey is sold in India, but the majority is 'Indian-made foreign liquor.' These include whiskey, rum, and brandy, but Indian-made foreign liquors are generally manufactured from molasses and therefore do not meet the standards commonly accepted for whiskeys. The total market for whiskey in the United States in 2012 was 50 million 9 l cases (12 bottles of 75 cl), of which the main varieties were bourbon and Tennessee (17 million cases) and Canadian (16 million cases). After a peak in 1983, Japanese whiskey production steadily declined until a small

rise in 2009 to around 100 million liters (11 million cases). A small but increasing volume is exported. Scotch whiskey production in 2011 was 518 million LPA (liters pure alcohol) (approx. 144 million cases). UK sales have been steadily declining for many years and amounted to 25 million LPA (approx. 6.9 million cases), with exports steadily increasing to 354 million LPA (approx. 98 million cases). Major export markets are France, the United States, and Spain, which together accounted for 114 million LPA (approx. 32 million cases). Small amounts of other whiskeys are also consumed in the United Kingdom, mostly North American. Consumption in Canada in 2012 was 4.7 million cases, of which Canadian whiskey accounted for 72%. The market in Brazil in 2009 was 4.2 million cases, of which 1.3 million cases were locally produced blended whiskey (malt whiskey blended with local agricultural alcohol). The remainder was mostly North American and Scotch.

See also: Barley; Brandy and Cognac: Manufacture and Chemical Composition; Lactic Acid Bacteria; Maize; Phenolic Compounds: Occurrence, Classes, and Analysis; Rhum–Ron–Rum: Technology and Tradition; Sherry Wines: Manufacture, Composition and Analysis; Starch: Structure, Property, and Determination; Tannins; Wheat: Grain Structure of Wheat and Wheat-based Products.

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http://www.acd.ca – Spirits Canada.
www.discus.org – The Distilled Spirits Council of the US.
http://www.ecfr.gov – Code of Federal Regulations Title 27 Part 5.22 Labelling and advertising of distilled spirits; The standards of identity.
www.scotch-whisky.org.uk/ – Scotch Whisky Association.

Wines: Champagne and Sparkling Wines – Production and Effervescence

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Introduction

Since the time of the Benedictine monk Dom Pierre Pérignon, cellar master of the Abbey of Hautvillers from 1668 to his death in 1715, champagne has been a worldwide renowned French sparkling wine. From a strictly physicochemical point of view, champagne is a multicomponent hydroalcoholic systems, with a density close to unity and a viscosity about 50% greater than that of pure water (mainly due to the presence of 12.5% v/v ethanol). Champagne is also supersaturated with dissolved CO_2 formed together with ethanol during the second fermentation process (called *prise de mousse* and induced by adding yeasts and a certain amount of sugar to bottles filled with a base wine and sealed with a cap). Champagne therefore possesses a concentration of dissolved CO_2 proportional to the level of sugar added to permit the second fermentation. A standard 750 ml bottle typically contains about 9 g of dissolved CO_2, which corresponds to a volume close to 5 l of gaseous CO_2 under standard conditions for temperature and pressure. It is no wonder that tasting champagne and sparkling wines differs so much from still wines. The effervescence and dissolved CO_2 impact the terms used in tasting relative to the following: (i) visual perception (the presence of rising bubbles in the glass), (ii) taste and mouthfeel (the fizzy and chemosensory excitation of nociceptors in the oral cavity, as dissolved CO_2 converts to carbonic acid), and (iii) aromatic perception (as bursting bubbles release both gaseous CO_2 and volatile aromatic compounds above the wine's surface).

The modern production of champagne is not far removed from that empirically developed by Dom Pierre Pérignon in the late seventeenth century. The method is also used outside the Champagne region. Sparkling wines produced as such are labeled *méthode traditionnelle* (*méthode champenoise*). Indeed, most American and Australian sparkling winemakers use this method to elaborate their sparkling wines. This method involves several distinct key steps described hereafter. In this article, particular focus is given to the importance of dissolved CO_2 in champagne and sparkling wine tasting. Infrared imaging techniques were recently applied to visualize the cloud of gaseous CO_2 escaping the bottleneck during uncorking, as well as the cloud of gaseous CO_2 desorbing from the wine during pouring.A brief overview of these two steps (short in duration, but highly important in terms of dissolved CO_2 loss) preceding sampling is presented.

From Grape Must to the Bottle: *Méthode Traditionnelle* in a Few Key Steps

A First Alcoholic Fermentation

Three grape cultivars are grown in the 75 000 acres of Champagne vineyards: Chardonnay (a white grape), Pinot Meunier, and Pinot noir (both dark grapes). Usually, around mid-September, the grapes harvested from these vineyards are pressed to release the juice, called 'grape must.' After pressing, the must is transferred into open vats where yeast (*Saccharomyces cerevisiae*) is added. In the Champagne region, the must typically possesses about 150 g l^{-1} of sugars. Generally speaking, the key metabolic process during winemaking is alcoholic fermentation: the conversion of sugars into ethanol and carbon dioxide by yeast. The process of fermentation was first scientifically described by Joseph Louis Gay-Lussac in 1810, when he demonstrated that glucose was the basic starting block for producing ethanol:

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \qquad [1]$$

The manner yeasts contributed to the fermentation process was not clearly understood until 1857, when Louis Pasteur discovered that not only does yeast fermentation not require oxygen, but also alcohol yield is reduced in the presence of oxygen.

The amount of sugar found in grape juice determines the final ethanol level during fermentation. In cool climates, such as Champagne, Alsace, and Germany, where the vines 'struggle' to ripen their grapes, sugar levels are relatively low. Consequently, following eqn [1], such wines often only reach 8–9% (vol/vol) ethanol after the initial alcoholic fermentation. This value is insufficient to produce fine sparkling wines, as those elaborated in the Champagne area (the natural sugar content of the grapes is too low). Winemakers add sugar to the juice in a process called 'chaptalization' (after Jean-Antoine Chaptal (1756–1832) who suggested the process). He contributed significantly to the development of modern winemaking by applying advances in chemistry to agriculture (see **Figure 1**).

Chaptalization involves adding pure cane or beet sugar to the juice extracted from the grapes. Ideally, the first alcoholic fermentation will produce a wine with between 10.5% alcohol and 11.5% alcohol. This percentage must not be exceeded as the law prohibits the sale of champagne with an ethanol content higher than 13% (a subsequent, second, carbon dioxide-trapping fermentation adds between 1.2% and 1.5% of ethanol to the wine). It is important to reach but not exceed levels of ethanol that help the development of important flavors and secondary aromas in the wine. However, during exceptionally sunny years, the grapes reach sugar contents high enough to reach the desired level of ethanol after the first alcoholic fermentation. Under such conditions, chaptalization may be unnecessary.

The Art of Blending

Because it is rare that a single wine from a single vintage, vineyard, and grape variety will provide the perfect balance of flavor, sugar content, and acidity necessary for making a fine champagne or sparkling wine, winemakers often combine several different still (base) wines. This is called the *assemblage* (or

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Figure 1 Jean-Antoine Chaptal (1756–1832) contributed to the development of the modern wine industry by attempting to apply to traditional agriculture, the huge advances in modern chemistry done in the early nineteenth century.

Figure 2 A cellar master (Thierry Gasco, from Champagne Pommery) will blend several different still wines from various grape varieties, vineyards, and vintages to produce one champagne. Alain Cornu/ Collection CIVC.

blending) step. It is carried out shortly after the first alcoholic fermentation is complete. Blending is considered a key step in the art of champagne making. A cellar master will sometimes blend up to 80 different wines from various grape varieties, vineyards, and vintages to produce a single champagne or sparkling wine (see Figure 2). The blending of still wines in champagne can come from three grape cultivars. The blend then undergoes a second fermentation – the key step in producing the 'sparkle' in champagne and other sparkling wines.

The *Prise de Mousse*: A Second Alcoholic Fermentation in a Sealed Bottle

Once the blend of base wines is created, sugar (about 24 g l^{-1}) and yeast are added. The entire concoction is put in thick-walled glass bottles and sealed with caps. The bottles are then placed in a cool cellar (12–14 °C), and the wine is allowed to slowly ferment for a second time, producing alcohol and carbon dioxide again. Following eqn [1], the added sugar promotes a second alcoholic fermentation that produces about 11.8 g of CO_2 and 12.3 g of ethanol (per liter) within each bottle. This gain of ethanol increases the ethanol content to about 12.5%. Moreover, the production of gaseous CO_2 is roughly equivalent to half of the concentration of sugar added into the base wine. Thus, a typical 750 ml bottle will contain about 9 g of gaseous CO_2. Due to the molar mass of CO_2 (44 g mol^{-1}) and the molar volume of an ideal gas (~24 l mol^{-1} at 12 °C), it can be deduced that about 5 l of gaseous CO_2 is trapped into a single 750 ml bottle (i.e., six times its own volume).

During the second fermentation process, the CO_2 released cannot escape and progressively dissolves into the wine. Therefore, CO_2 in the wine and headspace under the cork progressively establish equilibrium – following Henry's law. It states that the partial pressure of a given gas above a solution is proportional to the concentration of the gas dissolved into the solution. This is expressed by the following relation:

$$c = k_H P_{CO_2} \quad [2]$$

where c is the concentration of dissolved CO_2 molecules, P_{CO_2} is the partial pressure of CO_2 in the vapor phase, and k_H is its Henry's law constant (i.e., its solubility in wine).

The solubility of gas in a solution is strongly temperature-dependent (the lower the temperature, the higher the solubility). In champagne and other sparkling wines, Agabaliantz examined the solubility of dissolved CO_2 as a function of both temperature and wine parameters. For a typical sparkling wine, Agabaliantz established the temperature dependence of the Henry's law constant. Thermodynamically speaking, the behavior of the constant as a function of temperature can be conveniently expressed with a van't Hoff-like equation as follows:

$$k_H(T) = k_{298\,K} \exp\left[-\frac{\Delta H_{diss}}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right] \quad [3]$$

where k_{298K} is the Henry's law constant of dissolved CO_2 at 298 K (≈1.21 g l^{-1} bar^{-1}), ΔH_{diss} is the dissolution enthalpy of CO_2 molecules in a liquid medium (in J mol^{-1}), R is the ideal gas constant (8.31 J K^{-1} mol^{-1}), and T is the absolute temperature (in K). By fitting Agabaliantz data with the latter equation, the dissolution enthalpy of CO_2 in champagne was estimated at $\Delta H_{diss} \approx 24\,800$ J mol^{-1} (see Figure 3).

Disgorging and Cork Insertion

The second fermentation is followed by a minimum aging period of 15 months in contact with dead yeast cells (termed *sur lie* aging). The yeasts undergo autolysis during this aging period. During autolysis, the yeasts release a range of compounds that modify the wine's organoleptic properties, donating roundness and its characteristic aroma and flavor. More details about chemical and biochemical features involved in sparkling wine production (from a traditional to an improved winemaking technology) can be found in the 'Further Reading' section at the end of the article.

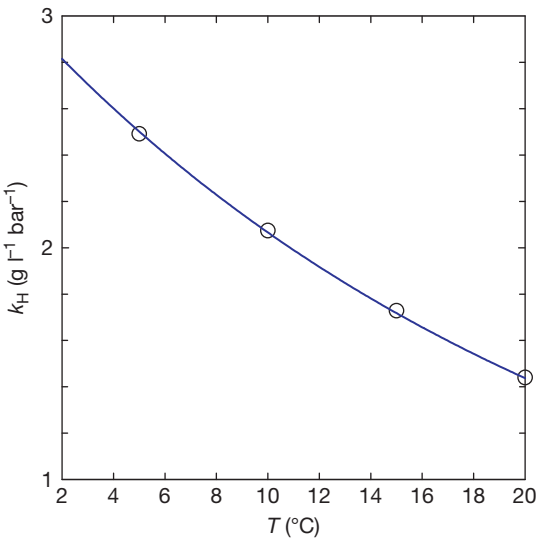

Figure 3 Temperature dependence of dissolved CO_2 solubility in wine (i.e., Henry's law constant) (○), redrawn from Agabaliantz data; the solid line is the best fit to Agabaliantz data, drawn with the van't Hoff-like eqn [2] and with $\Delta H_{diss} \approx 24\,800\,J\,mol^{-1}$.

After aging, the bottles are disgorged. The caps are removed and the plug of dead yeast cells next to the cap is ejected (see Figure 4). A specific dosage, consisting of a mixture of sugar and aged wine, is then added to replace the wine lost during disgorging. A small amount of dissolved CO_2 is also inevitably lost at this step, but rapid resealing with a traditional or technical cork prevents a significant loss of CO_2. After corking, dissolved and gaseous CO_2 in the wine quickly reestablish their equilibrium. Dissolved CO_2 progressively desorb from the liquid raising the gas pressure under the cork. Nevertheless, because the solubility of CO_2 is strongly influenced by temperature, the pressure under the cork also strongly depends on the wine's temperature. As noted earlier, this equilibrium is designated by both Henry's law (for dissolved CO_2) and the ideal gas law (for gaseous CO_2 in the headspace under the cork). Moreover, the total mass of CO_2 is maintained as the wine in the bottle is sealed. Therefore, by combining the two aforementioned laws with mass conservation, the following relationship can easily be determined, linking the pressure P of gaseous CO_2 under the cork (in bars) with both temperature and bottle's parameters as

$$P \approx \frac{mRT}{4.4 \times 10^3 v + (k_H RT)V} \qquad [4]$$

where m is the total mass of CO_2 within the bottle (in grams); k_H is the Henry's law constant, given in eqn [3] (in g l^{-1} bar^{-1}); V is volume of wine within the bottle (in liter); and v is the volume of gas under the cork (in liter).

Typically, champagne and other sparkling wines are available in three bottle sizes, namely, the standard (750 ml), the magnum (1500 ml), and the half bottle (375 ml). Despite their different capacities, each has the same headspace volume ($v \approx 25$ ml), as well as similar cork stoppers (in terms of length and cross section).

The effect of temperature on pressure under the cork in the three bottle sizes is demonstrated in Figure 5. At a temperature typical for tasting champagne (8–10 °C), the pressure within the bottle is close to 5 bars (i.e., $5 \times 10^5\,N\,m^{-2}$), independent of bottle volume. Thus, bottle size has little influence on the internal pressure.

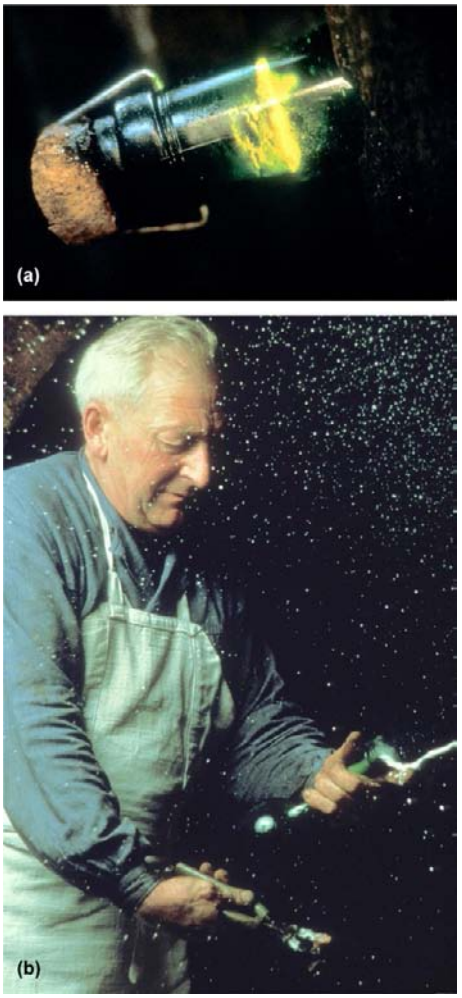

Figure 4 During disgorging, the necks of the bottles are frozen, creating a small ice plug that traps the sediment of dead yeast cells in the bottleneck (a). Traditional cork (illustrated) or bottle cap is removed, and the CO_2 pressure that is released shoots the ice out of the bottleneck, along with all the yeast sediment. Bottles are then corked with traditional cork stoppers (b). Kumesawaga/Collection CIVC.

Moreover, since dissolved and gaseous CO_2 exist in equilibrium in the bottle, the concentration (c) of CO_2 dissolved in sparkling wine can be expressed as follows:

$$c = k_H P \approx \frac{k_H mRT}{4.4 \times 10^3 v + (k_H RT)V} \qquad [5]$$

The relationship between temperature and CO_2 concentration for the three standard bottle sizes is demonstrated in Figure 6. It shows that the smaller the volume, the lower the dissolved CO_2 concentration. Note also that the concentration of dissolved CO_2 is only moderately temperature-dependent (despite the pressure within the bottle being strongly temperature-dependent; see Figure 5).

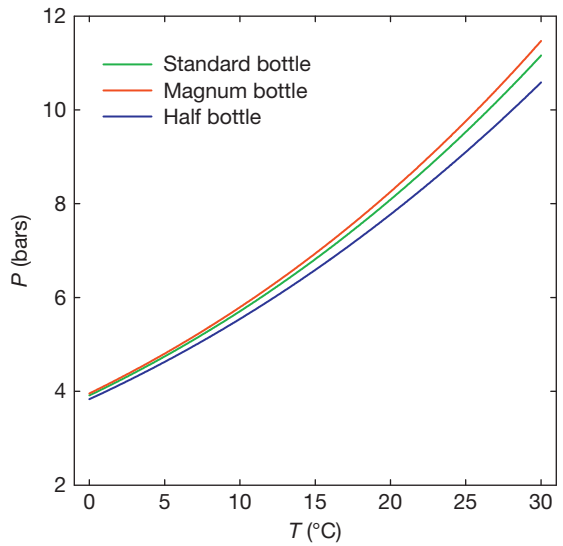

Figure 5 Temperature dependence of the pressure P under the cork, within the three most common champagne bottle types, namely, the standard bottle, the magnum bottle, and the half bottle.

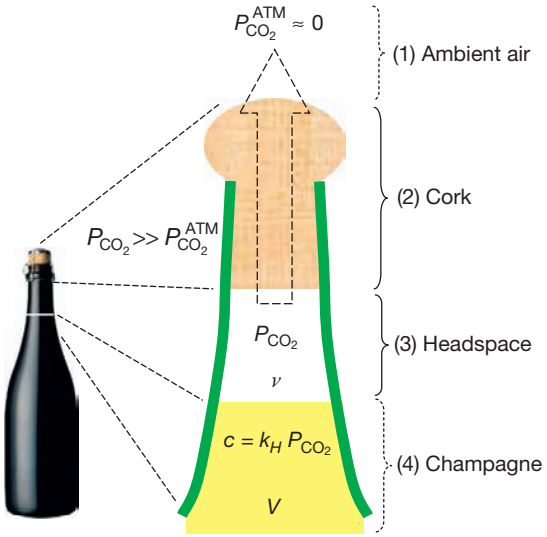

Figure 7 Scheme of the bottleneck and compilation of the various cork, bottle, and wine parameters.

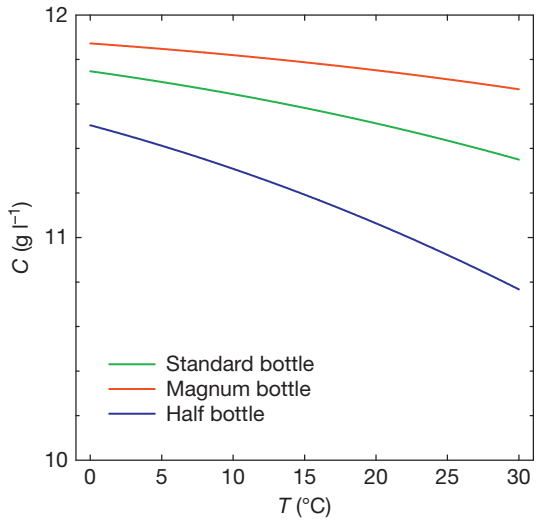

Figure 6 Temperature dependence of the dissolved CO_2 concentration within champagne, within the three most common champagne bottle types.

Concentrations of dissolved CO_2 are easily accessed using carbonic anhydrase, the official method recommended by the OIV (Office Internationale de la Vigne et du Vin) for measuring CO_2 content in champagne and sparkling wines. Numerous experiments with early disgorged champagne samples demonstrated that dissolved CO_2 contents inside freshly opened 750 ml bottles are in the order of 11.5 g l^{-1}, in good accordance with eqn [5].

The Impact of Aging

Cork stoppers used to seal bottles of sparkling wine are far from completely hermetic in terms of gas exchange. Cork is a porous material. Thus, gas species are able to slowly diffuse through the cork, along their associated inverse partial pressure gradients (see Figure 7). In case of corked champagne bottles, the huge difference in CO_2 partial pressure between the headspace (close to 6 bars at 12 °C) and the ambient air (in the order of 0.0004 bar) forces CO_2 molecules to slowly diffuse through the cork. Therefore, during storage, a corked bottle progressively loses both its gaseous and dissolved CO_2 contents.

As noted earlier, early disgorged champagnes possess about $c \approx 11.5\,g\,l^{-1}$ CO_2. Recently, the concentration of dissolved CO_2 in older champagne (bottled in the late 1900s and subsequently stored in a cool cellar) was measured. As expected, the approximately 10-year-old champagne contained substantially less dissolved CO_2 ($c \approx 8\,g\,l^{-1}$) than young versions. This significant loss is interpreted as a consequence of the continuous diffusion of gaseous CO_2 through the pore structure of the cork. By combining diffusion through a porous medium with Henry's law, a multiparameter model was built that shows the dissolved CO_2 content found in champagne across the whole aging period. Both champagne temperature and bottle volume are key parameters concerning the kinetics of CO_2 loss through cork. Nevertheless, additional investigations concerning how cork properties alter over time in the bottle are needed to better understand how their porosity and permeability affect gas transfer.

Chemical Composition

From the chemical point of view, all sparkling wines can be viewed as multicomponent hydroalcoholic solutions, supersaturated with dissolved CO_2. The chemical composition of a typical champagne is given in Table 1.

Typically, gases like CO_2 undergo specific reactions with water. Equilibrium develops between the dissolved $(CO_2)_{aq}$ and H_2CO_3 (carbonic acid):

$$(CO_2)_{aq} + H_2O \leftrightarrow H_2CO_3 \quad [6]$$

Table 1 Average composition of a typical champagne

Compound	*Quantity*
Ethanol	≈12.5% v/v
Dissolved CO_2	11–12 g l^{-1}
Glycerol	≈5 g l^{-1}
Tartaric acid	≈2.5–4 g l^{-1}
Lactic acid	≈4 g l^{-1}
Sugars	10–50 g l^{-1}
Proteins	5–10 mg l^{-1}
Polysaccharides	≈200 mg l^{-1}
Polyphenols	≈100 mg l^{-1}
Amino acids	0.8–2 mg l^{-1}
Volatile organic compounds (VOCs)	≈700 mg l^{-1}
Lipids	≈10 mg l^{-1}
K^+	200–450 mg l^{-1}
Ca^{2+}	60–120 mg l^{-1}
Mg^{2+}	50–90 mg l^{-1}
SO_4^{2-}	≈200 mg l^{-1}
Cl^-	≈10 mg l^{-1}

Typically, pH ≈ 3.2 and the ionic strength is 0.02 M.
Source: Dussaud, A. (1993). *Etude des propriétés de surface statiques et dynamiques de solutions alcooliques de protéines: Application à la stabilité des mousses de boissons alcoolisées*. PhD Thesis, ENSIAA, Massy, France.

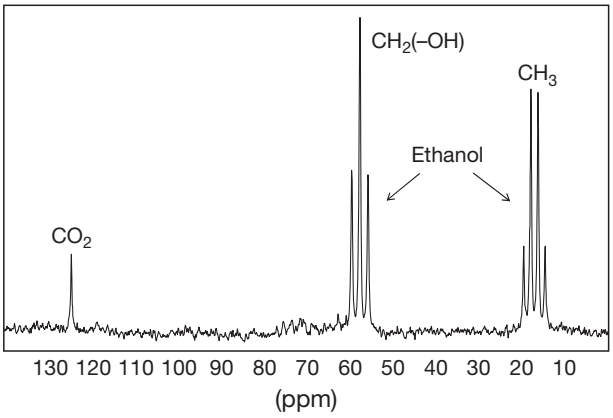

Figure 8 ^{13}C spectrum recorded to measure the CO_2 concentration in champagne; it is clear that no carbonated species (CO_3^{2-} and HCO_3^-) coexist with dissolved CO_2.

Moreover, carbonic acid is a weak acid that dissociates in two steps:

$$H_2CO_3 + H_2O \leftrightarrow H_3O^+ + HCO_3^- \quad pK_{a1}\ (\text{at } 25\,^\circ\text{C}) = 6.37 \qquad [7]$$

$$HCO_3^- + H_2O \leftrightarrow H_3O^+ + CO_3^{2-} \quad pK_{a2}\ (\text{at } 25\,^\circ\text{C}) = 10.25 \qquad [8]$$

However, as the pH of most sparkling wines is relatively low (about 3.2), no carbonated species (CO_3^{2-} and HCO_3^-) should coexist. ^{13}C Magnetic resonance spectroscopy is a nonintrusive, nondestructive method able to determine the amount of CO_2 dissolved in closed bottles of champagne and other sparkling wines. Different well-separated peaks in the ^{13}C spectrum can be seen in the Figure 8: (i) The quadruplet of CH_3 group of ethanol appears at 17.9 ppm, (ii) the triplet of $CH_2(-OH)$ group of ethanol appears at 57.3 ppm, and (iii) the singlet of CO_2 appears at 124.4 ppm, thus confirming the absence of other carbonated species (CO_3^{2-} and HCO_3^-) in the liquid matrix (contrary to fizzy waters, e.g., where pH values near neutrality enable the aforementioned carbonated species to cohabit with CO_2 dissolved gas molecules).

Uncorking and Pouring

Uncorking the Bottle

In order to visualize the cloud of gaseous CO_2 escaping and expanding when the cork is removed from a bottle (invisible in the visible light spectrum), a technique based on infrared imaging has been used. Time sequences displayed in Figure 9 illustrate the uncorking of two champagne bottles, stored at 4 and 18 °C.

As seen in Figure 9, the volume of gaseous CO_2 under pressure in the headspace suddenly expands and gushes out of the bottle. It varies considerably depending on the wine's temperature (mainly due to temperature's influence on headspace gas pressure before uncorking).

After uncorking, the pressure experienced by gaseous CO_2 inside the bottle suddenly falls. Consequently, the thermodynamic equilibrium shifts, and dissolved CO_2 in the wine must inevitably escape to reestablish its equilibrium (fulfilling Henry's law). After removing the cork, the bottle is generally inclined to pour champagne into a flute or coupe. The following paragraph closely examines how dissolved CO_2 progressively escapes from the wine into the headspace of the flute or coupe.

Pouring

Until recently, the impact of pouring on the loss of dissolved CO_2 has remained purely speculation. During pouring, the free air/champagne interface considerably increases. Moreover, pouring causes considerable turbulence, including entrapping air bubbles inside the wine. This also contributes to the diffusion of dissolved CO_2 from the wine to the ambient air. Besides mixing the wine during pouring, eddies of various sizes form in the liquid matrix. These also undoubtedly contribute to a significant loss of dissolved CO_2. The impact of pouring on CO_2 loss was studied at three temperatures (4, 12, and 18 °C) and whether it was poured vertically or on a tilt into a flute (see Figure 10).

Concentrations of dissolved CO_2 (c_L), measured immediately after pouring, are displayed in Figure 11. It clearly shows a correlation between higher temperatures and lower dissolved CO_2 concentrations after pouring. Moreover, it also demonstrates that pouring on a tilt (as beer tends to be poured) has less impact on the concentration of dissolved CO_2 in champagne after pouring.

Visualization, based on infrared imaging, has also been used to film the gaseous CO_2 fluxes outgassing from champagne. Figure 12 illustrates how outgassing is affected as pouring progresses at both 4 and 18 °C. The higher the temperature, the more easily dissolved CO_2 escapes during pouring, confirming the tendency noted in chemical measurements. Note

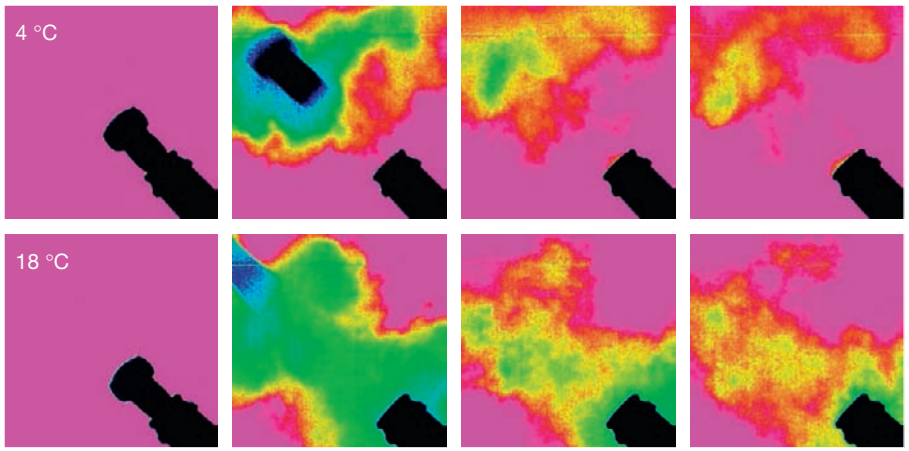

Figure 9 Time sequence illustrating the uncorking of a champagne bottle as seen through high-speed infrared imaging; the time interval between successive frames is 100 ms. The color is an indirect estimation of gaseous CO_2 content (CO_2 molecules absorb some IR wavelengths). High levels show dark blue, while lower levels are in pink/red.

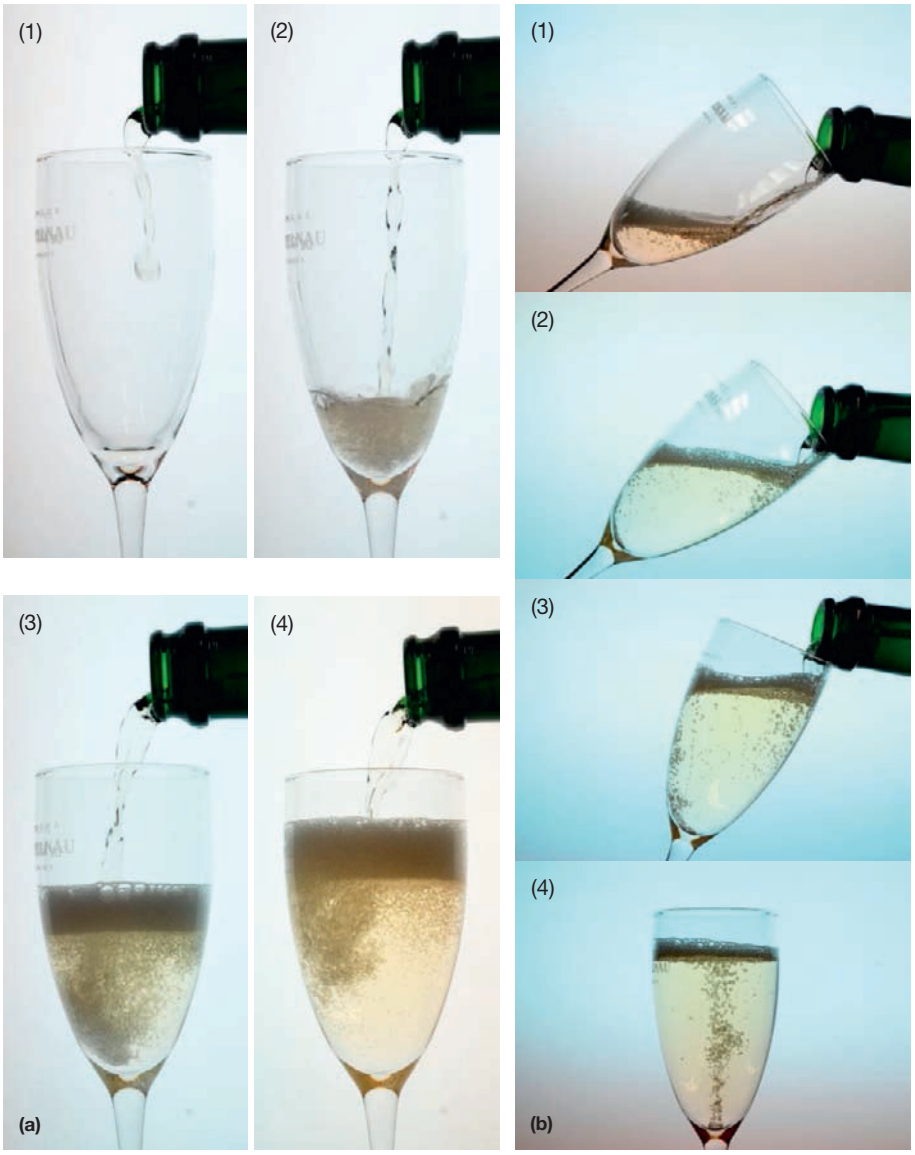

Figure 10 Champagne can be poured straight down the middle of a vertically oriented flute (a) or down the side of a tilted flute (b), with significant differences in the concentration of dissolved CO_2 found within champagne after serving it.

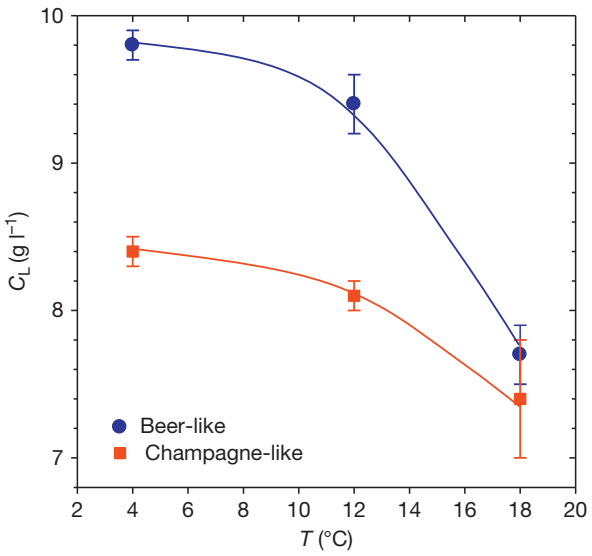

Figure 11 Concentrations of dissolved CO_2, as chemically measured once champagne was poured into the flute, for both ways of serving champagne and for each champagne temperature; each dot is the arithmetic average of four successive values provided from four successive pouring (for both ways of serving champagne); standard deviations correspond to the root-mean-square deviations of the values provided by the four successive data recordings.

that the flow of escaping CO_2 during champagne pouring appears to flow down from the opening of the flute, as if 'licking' the glass walls, rather than diffusing isotropically around it. This observation demonstrates the fact that gaseous CO_2 is approximately 1.5 times more dense ($\rho_{CO_2} = 1.87\,g\,l^{-1}$ at 20 °C) than dry air ($\rho_{air} = 1.20\,g\,l^{-1}$ at 20 °C). Thus, it tends to flow down.

It is worth noting that the surrounding air acts like a huge thermal blanket, quickly warming the outgassing CO_2 during pouring. By considering the gaseous flux of CO_2 discharging from champagne as an ideal gas, the volume of gaseous CO_2 discharging during pouring, denoted $V_{20°C}$, can be expressed as follows:

$$V_{20\,°C} = \frac{\Delta c v_f RT}{M_{CO_2} P_0} \qquad [9]$$

with Δc being the loss in dissolved-CO_2 concentration during pouring (i.e., $\Delta c = c_i - c_L$, expressed in g l^{-1}), v_f being the volume of champagne poured into the flute (in liters, namely, 0.1 l in the present case), T being the ambient temperature (namely, 293 K in the present case), M_{CO_2} being the molar mass of CO_2 (44 g mol^{-1}), and P_0 being the atmospheric pressure (close to 10^5 Pa).

Table 2 compares the losses of dissolved CO_2 (with regard to the initial concentration c_i before pouring of $c_i \approx 11.5\,g\,l^{-1}$)

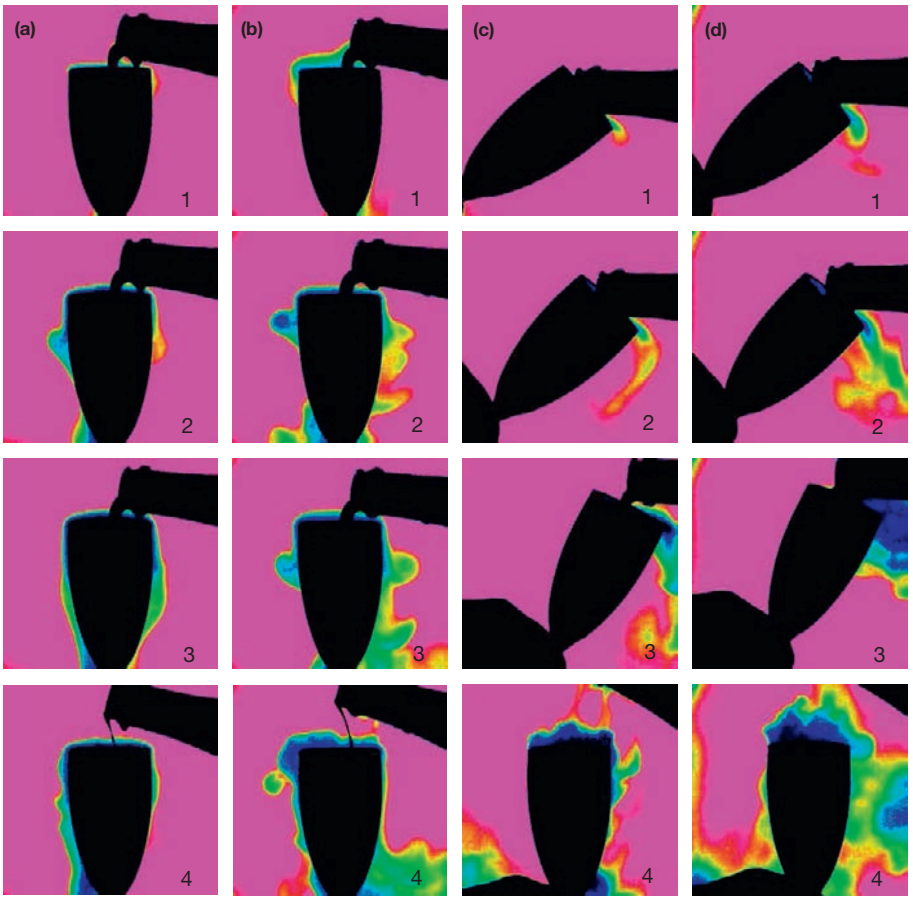

Figure 12 Snapshots illustrating both ways of serving champagne, as seen through infrared imaging – for a bottle stored at 4 °C (a) and (b) and for a bottle stored at 18 °C (c) and (d). The color is an indirect estimation of gaseous CO_2 content (CO_2 molecules absorb some IR wavelengths). High levels show dark blue, while lower levels are in pink/red.

Table 2 Losses of dissolved CO_2 concentrations in champagne during pouring (in g l^{-1}) and corresponding volumes of gaseous CO_2 discharged from champagne (converted in cm^3), for both ways of serving and at three champagne temperatures

	Champagne-like		*Beer-like*	
Temperature (°C)	$\Delta c \left(g\,l^{-1}\right)$	V_{CO_2} (cm^3)	$\Delta c \left(g\,l^{-1}\right)$	V_{CO_2} (cm^3)
4	3.0 ± 0.2	166 ± 11	1.6 ± 0.2	88 ± 11
12	3.3 ± 0.2	182 ± 11	2.0 ± 0.3	110 ± 17
18	4.0 ± 0.5	221 ± 28	3.7 ± 0.3	204 ± 17

Source: Liger-Belair, G., Bourget, M., Villaume, S., Jeandet, P., Pron, H. and Polidori, G. (2010). On the losses of dissolved CO_2 during champagne serving. *Journal of Agricultural and Food Chemistry* **58**, 8768–8775.

and the corresponding volume of gaseous CO_2 escaping during pouring. During the few second it takes to pour champagne into a glass vertically (as typical for sparkling wines), champagne loses approximately as much dissolved CO_2 as during the subsequent 10 min in the glass. To prolong the wine's coolness and to help champagne retain its dissolved CO_2 (and effervescence), the champagne-like manner of serving it is certainly not the most appropriate.

Acknowledgments

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Wines: Madeira, Port and Sherry Fortified Wines – The *Sui Generis* and Notable Peculiarities. Major Differences and Chemical Patterns

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Part I. Madeira, Port, and Sherry Fortified Wines: The *Sui Generis* and Notable Peculiarities, the History, Grapes Used, and Winemaking Process

Introduction

Wine is an alcoholic beverage consumed all over the world. Over the centuries, its production has grown both in popularity and in sophistication. Using a wide range of grape varieties, fortified wines derive their distinctive attributes from the specific and unique winemaking and aging processes employed in their production.

The most well-known fortified wines include *madeira* and *port*, both produced in Portugal, and *sherry* produced in Spain. All three involve the addition of alcohol (fortification with brandy or spirits), with the aim of increasing the total alcohol content up to 18–20%. This procedure may be used to stop fermentation, generating wines with different degrees of sweetness.

Traditional fortified wines are generally associated with areas where the climate and soil conditions do not favor the production of high-quality grapes. In fact, the warm and hotter climates of the *sherry* wine region produce bland, white table wines, the red wines from Douro region are astringent, and the cool, damp summers of the island of Madeira produce wines with acidic characters. However, the application of fortification, maturation, and blending techniques produces wines of great typicity and style. In the succeeding text, we will discuss the technologies used to produce *madeira*, port, and *sherry* wines. In addition to describing historical and traditional practices, their evolution and the chemical characteristics of wines are highlighted.

History

Each wine type has its own history and winemaking characteristics. *Madeira* wine is produced in a variety of styles, ranging from dry (consumed as an aperitif) to sweet (usually consumed with desserts). Historical records show that only 25 years after colonization had begun, the island was already exporting a *madeira*-produced wine. By the middle of the seventeenth century, most producers already fortified their wines. At that time, the West Indies were the main market for *Madeira* wines. Their transportation in the hold of ships exposed the wines to very high temperatures during their passage through the tropics. Eventually, part of the wine returned to Europe, where it was discovered that its quality was greatly enhanced. This induced the intentional transport of barrels of *madeiras* to the West Indies to improve its quality. The eighteenth century was particularly important to *Madeira* wine improvement, which benefited from the general adoption of fortification and *estufagem* (heating process) in its production. *Madeira* wine notoriety in North America is illustrated by its use in toasting of the America Declaration of Independence, in 1776. The commitment to improving the quality of *madeira* continues to be an important concern of producers and trade companies. IVBAM (Madeira Wine, Embroidery and Handicraft Institute) is the organization that regulates *madeira* production and commercialization.

Port has a long history dating back to 1756, when the DDR (Douro Demarcated Region) was declared the third protected wine region in the world, after Chianti (1716) and Tokaj-Hegyalja (1730). Its name comes from the name of O*porto* (*Porto*), the second largest city in Portugal. *Port* is produced in the Douro valley of northern Portugal, in wineries called *quintas*. The vineyards line terraced hills rising up from the Douro river to the east of Oporto, almost reaching the Spanish border. This fortified wine became popular in England during the peninsular campaigns against France. Following the conflict, French wines were banned from England, benefiting Portuguese wines, and *port* in particular.

Port production is tightly regulated by the IVDP (Port and Douro Wines Institute) that limits wine production in the DDR. This region incorporates the subregions of the Lower Corgo, Upper Corgo, and Upper Douro. The first is the oldest and most westerly viticultural area, being simultaneously the least mountainous and most cultivated region (37%). The Lower Corgo favors higher production, generating less concentrated, complex wines. The central subregion, the Upper Corgo, occurs upstream from the previous, covering steep slopes along the Douro. *Port* wines of higher quality are produced in this region that features schistose soils and a more hostile climate. These conditions produce vine stress that lowers yield but enhances grape quality. The last of the three DDR subregions, the Upper Douro, is positioned further eastward and is the largest region (47% of the surface area) but paradoxically is the least productive (7.5% of total production).

Sherry is the most famous fortified Spanish wine. It comes from the southern regions of Jerez de la Frontera and Montilla-Moriles. These include vineyards located within the municipal

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boundaries of Jerez de la Frontera, El Puerto de Santa María, Sanlúcar de Barrameda, Trebujena, Chipiona, Rota, Puerto Real, Chiclana de la Frontera, and Lebrija.

Sherry wine is produced in a variety of dry styles, made primarily from the Palomino grape. These styles range from light versions, similar to white table wines (Manzanilla and fino), to darker versions aged in barrels (amontillado and oloroso). In Spain, *sherry* is referred to as *vino de Jerez*. Its earliest records date back to the eighth century, when Moorish Muslims controlled Spain. Over a thousand years earlier, the Phoenicians occupied this area and are believed to have exported wine. It is unknown if the Phoenicians produced wines similar to *sherry*. By the sixteenth century, the wines of Jerez began to resemble contemporary *sherry* and were regularly exported throughout Europe, particularly to England. At this time, *sherry* was widely regarded as one of the finest wines in the world.

Terroir

Grapevines, like other plants, show differences in morphological and physiological phenotype. The relationship between varietal instability and environmental conditions is regulated by hereditary traits influencing phenotypic malleability and selection. The vineyard environment influences all aspects of vine metabolism, growth, and development. Many studies have investigated these relationships, which define the *terroir*, and their effects on wine quality. Overall, the *terroir* involves not only the climate, geology, topography, and soil but also the viticultural ecosystem, grape handling, vinification, and cultural and socioeconomic factors (Figure 1).

Madeira is a volcanic island in the Atlantic Ocean, ~970 km southwest of Lisbon and 600 km northwest of the West African coast. Its total surface area is about 73 200 ha, but its <2% grows *Vitis vinifera* L. grapes. The main viticulture districts are Câmara de Lobos, São Vicente, and Santana (Figure 2).

Madeira has a climate with some tropical influences, with high rainfall and an average mean temperature of 19 °C. Threats from fungal diseases, such as *Botrytis cinerea*, are a constant viticultural hazard as they often inhibit fermentation, resulting in a lower alcohol production. To combat these threats, vineyards are often trained on low trellises, known as *latada* (Figure 3(b)), to raise the canopy off the ground.

The mountainous terrains of the island are difficult to cultivate, so vineyards are planted on man-made terraces of red and basaltic bedrock. These terraces, known as *poios* (Figure 3(a)), are very similar to the DDR terraces where *port* wine grapes are cultivated. The topography does not allow the use of mechanical harvesting or vineyard equipment, making grape growing a costly endeavor.

Regarding *port*, its distinctive qualities are certainly influenced by the unique climatic conditions, topography, and soils of the DDR. The climatic conditions are characterized by a mean annual precipitation between 400 and 900 mm but with only about 30% falling from April to September. The monthly average temperatures range from 5–8 °C to 21–24 °C. The topography involves severe landscape orientations with reliefs unsuitable to mechanization. The soils are schistose, with slatelike metamorphic rock rich in nutrients. They are low in organic content (0.6–1.6%), acidic pH (4.6–5.5), and potassium and phosphorus levels. The surface rock formation has excellent viticultural properties, such as water retention. This enables the roots to penetrate deeply into the subsoil, as well as resisting erosion. In addition, the soil retains heat during the day, transmitting it back to the vines at night. One of the most important factors is the region's

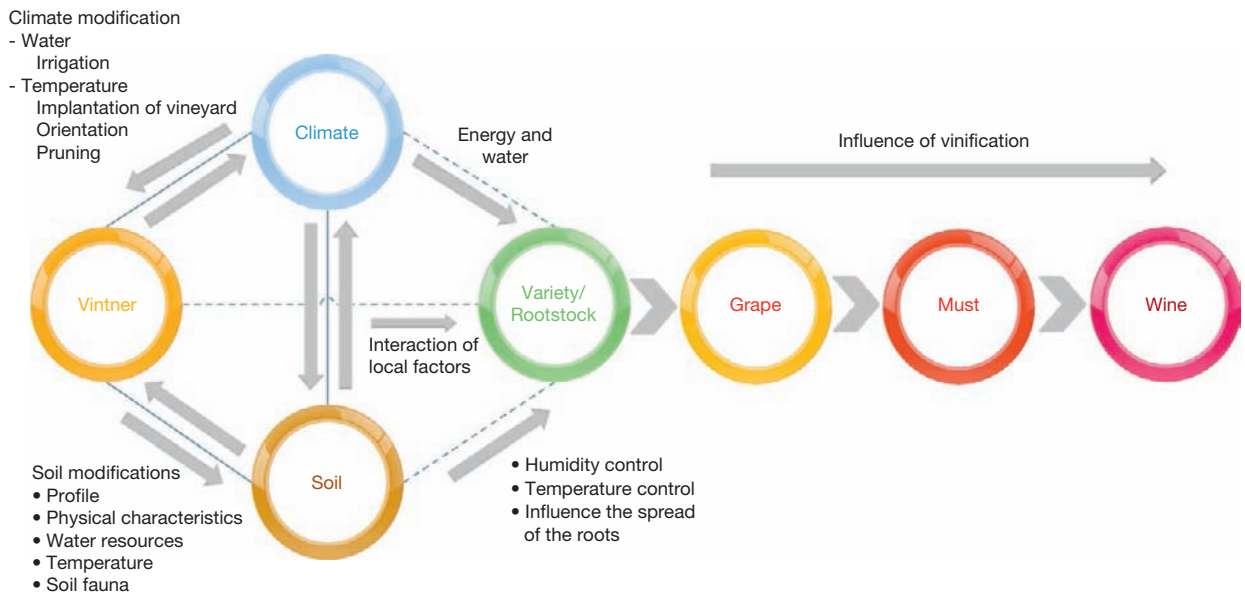

Figure 1 Factors related to *terroir* definition. Adapted from Circle, V. t. W. (2013). http://www.vinetowinecircle.com/en/terroir-2/terroir-and-viticulture (retrieved October 2013).

Figure 2 Main viticultural regions of Madeira island.

(a) (b)

Figure 3 Vineyards traditional systems called *poios* (a) and *latada* (b).

natural climatic variability along its length. However, current accelerated human-induced climate changes are already affecting *port* production. This is mainly because highly variable climatic fluctuations result in marked influences on vineyard management and varietal adaptation. Therefore, much effort will be needed to keep viticulture and winemaking viable and profitable in the region. Possibly, the climate variations will drive shifts in suitable viticultural zones, with some traditional regions becoming too hot while others becoming more viable.

In Jerez, where *sherry* wines are produced, the climate is relatively stable, with a yearly average temperature of 18 °C. The soils are very absorbent and compact, retaining and optimizing the limited rainfall that the region receives. Nevertheless, *albariza*, *barros*, and *arenas* typologies can be distinguished. The *albariza* is a light soil, blending limestone, clay, and sand, and rich in calcium carbonate, being more suitable for Palomino grapes growing. Additionally, these soils reflect sunlight up into the vineyard, thus increasing vine photosynthesis. In turn, *barros* is a dark soil with high clay content and *arenas* is a yellowish soil with high sand content. Both of them are more appropriate for Pedro Ximénez and Moscatel (Muscat) grapes.

Vitis Vinifera L. Grapes

Grapes Used in *Madeira* Production

The *V. vinifera* L. grapes used to produce *madeira* are mainly Bual, Malvasia, Sercial, Verdelho (white varieties, termed 'noble' varieties), and Tinta Negra (a red variety). Unlike the first varieties, Tinta Negra is very resistant to disease (namely, odium and phylloxera). For this reason, it is considered the 'workhorse' variety for *madeira* production. Terrantez and Bastardo are rarely used, although they produce wines of excellent and unique quality. Bastardo, Tinta, and Verdelho Tinto are still recommended, whereas Complexa, Deliciosa, and Listrão can be used, but are not recommended.

Grapes Used *in Port* Production

The number of grape varieties existent in Douro is remarkable, with more than 150 varieties described, most of them of autochthonous origin. This diversity was potentially problematic to *port* wine commercialization and guarantee of authenticity. Thus, since 1985, a list of varieties and respective representativeness in *port* production has been drawn up.

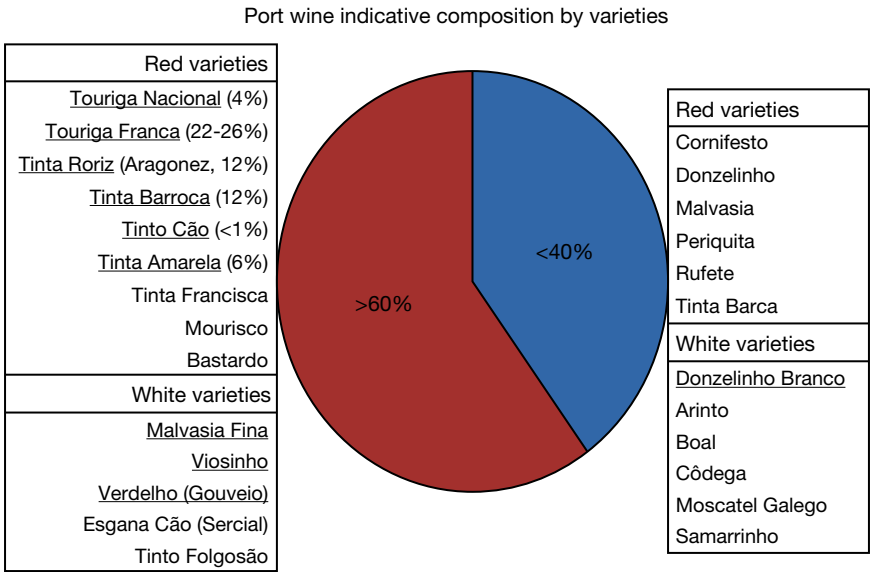

Figure 4 Most common grape varieties used in port production. The underlined grape varieties are the most popular and important varieties used. The vine coverage data available are indicated in brackets.

Table 1 Main grape varieties used in port wine

Grape varieties	*Vineyard coverage (%)*	*Characterization*
Touriga Nacional	4	Its blue-black grapes are small, deep in color, and rich in tannic compounds, being one of the most aromatic of the region (De Pinho et al., 2007). These grapes confer a deep coloration (ruby to violaceous) and a complex and intense aroma (containing essences of red fruits (raspberry and cherry), jammy black fruits (plum), berries (blackberry), raisin, and floral due to its high terpenoid content (α-terpineol, nerol, geraniol, and β-ionone)). In the mouth, these wines are well balanced and structured, with rounded tannins (De Pinho et al., 2007)
Touriga Franca	22–26	Confers to wine an intense color (ruby to violaceous) and a complex, fine, and fruity aroma, containing red fruits (such as raspberry and cherry), berries (blackberry), and floral notes, with high terpenoid concentration, although lower than Touriga Nacional wines (Oliveira et al., 2006). The wines also possess a light herbaceous taste combined with a full-bodied structure. These wines have fine sensory qualities, being a very important component in Port blends
Tinta Roriz	12	It is very resistant to hot weather, giving two to three times higher yields than Touriga Nacional, although intolerant to high mineral content soils and sensitive to mildew and fungal diseases. Produces wines well structured, possessing flavors and tannins of similar quality to Touriga Nacional, mainly when the vine carries few grapes and they are harvested with no rain (Guichard et al., 2003)
Tinta Barroca	11.6	Originates a robust wine with a flowery aroma that contrasts with the fruity aroma of other varieties. In fact, it is often mixed with other varieties to improve the quality of grapes ripened at low elevations (Moreira and de Pinho 2011)
Tinto Cão	<1	Produces small and loose grapes with high sugar contents, being its high natural acidity very important to the quality of port blends with extended aging
Tinta Amarela	6	Its utilization, however, is decreasing due to the high susceptibility of its thin skin to *Botrytis cinerea* vine disease (Moreira and de Pinho 2011)

Nevertheless, the number of grape varieties permitted (>20) is still high. Figure 4 indicates the most important varieties and their representativeness in *port* production, with Table 1 noting their organoleptic contribution. Despite *ports* are normally considered to be red wines, white grape varieties are widely grown, constituting about 34% of vineyard hectarage.

Grapes Used in Sherry Production

For sherry production, only the three white grape varieties, Palomino, Pedro Ximénez, and Moscatel are permitted. These are also the grape varieties best adapted to the region's climate and soil. The Palomino Fino clone is the main grape variety used in sherry wine production. In contrast, Moscatel represents only 2% of the planting area, being mainly used to flavor wines, whereas Pedro Ximénez is used to sweeten wines and can be acquired from outside the demarcated area.

L. Grape Chemical Composition

Defining the desired grape maturity is a difficult task, as several physical and biochemical changes occur simultaneously

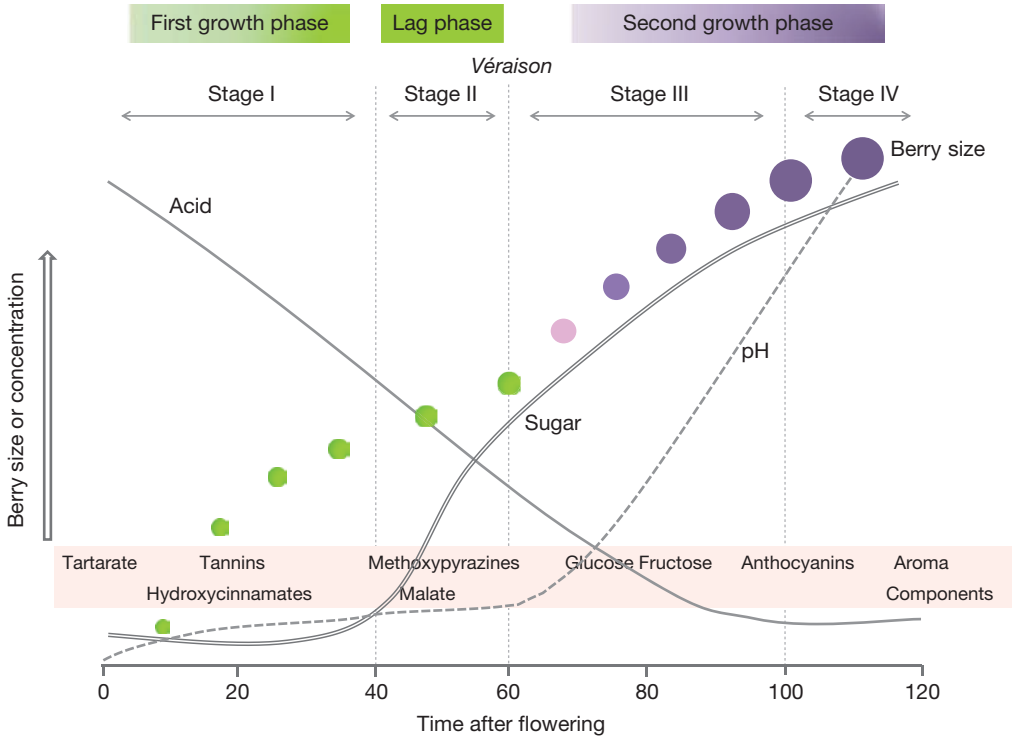

Figure 5 Schematic representation of grape berries composition change during development and ripening.

during grape ripening, affecting weight, sugar content, acidity, pigment development, and metabolic activity, among others. Grape berries exhibit two distinct growth phases (Figure 5), involving four stages.

Stage I begins after fruit set and can last about 40 days. It is characterized by pericarp growth (flesh and skin), increase in seed size, high tartaric and malic acid contents in the mesocarp (flesh), and limited sugar accumulation. The berries are green and hard. In most grape varieties, the first growth phase is followed by a lag phase (stage II), in which cell expansion slows, whereas seed maturation commences. The end of the first growth phase corresponds to the final grape herbaceous phase. Its duration depends on the variety, environment conditions, and agricultural practices. The onset of grape ripening (*véraison*) precedes stage III and interrupts the period of growth absence. It is characterized by an increase in sugar content and pH and decrease in acidity (Figure 5). Moreover, during *Véraison*, chlorophyll degradation and phenolic accumulation commence a color change, for example, yellow gold (flavonols) and pink/red (anthocyanins). Finally, stage IV, also known as engustment, is characterized by full maturity, aroma, and flavor components accumulation. In addition, the berries reach their maximum size and sugar accumulation slowly. The duration of each stage depends on the grape variety, environment, and agricultural practices.

Grape quality depends mainly on its primary and secondary metabolites. Secondary metabolites may not be crucial to plant survival, but they affect grape chemical diversity. They comprise a large number of phytochemical groups, such as alkaloids, terpenoids, tannins, sterols, saponins, and phenolics. Several of them have proved to be of great value in the pharmaceutical, agrochemical, food, and fragrance industries. Their levels can be regarded as a response of biological systems to genetic and environmental conditions. These metabolites occur in both free and glycosylated forms. The latter are aroma precursors, susceptible to transformation into volatile components during various stages of wine production and aging. The free forms, present mainly in grape skins, contribute directly to odor. These include terpenoids (e.g., mono-, di-, and sesquiterpenoids), C_{13} norisoprenoids, methoxypyrazines, thiols, and aromatic alcohols. The monoterpenoids have been associated positively with grape and wine complexity and quality due to their low odor threshold ($\mu g\, l^{-1}$) and pleasant odor descriptors (tea, violet, exotic flowers, and fruit). Similarly, β-ionone (violet-like) and β-damascenone (honey and flower notes) are examples of important C_{13} norisoprenoids. Nonvolatile, glycosylated odorless components are susceptible to transformation into volatile compounds during wine production and aging. Their contribution to wine quality depends on multiple factors, including their content (cultivar- and maturity-dependent) and release from glycosidic bonding by acid and/or enzymatic hydrolysis. Acid hydrolysis occurs slowly during wine storage, although heating can accelerate the process. Enzymatic hydrolysis occurs primarily via glycosidases that present a greater potential to release the aglycone than acid hydrolysis. This reaction starts with the cleavage of the terminal sugars by α-L-arabinosidase, α-D-apiosidase, and α-L-rhamnosidase, followed by the action of α-D-glucosidase (Figure 6). A recent study identified almost 100 odor-active components using enzymatic hydrolysis. However, the issue of which odorants play an active role in wine aroma development has not been systematically addressed.

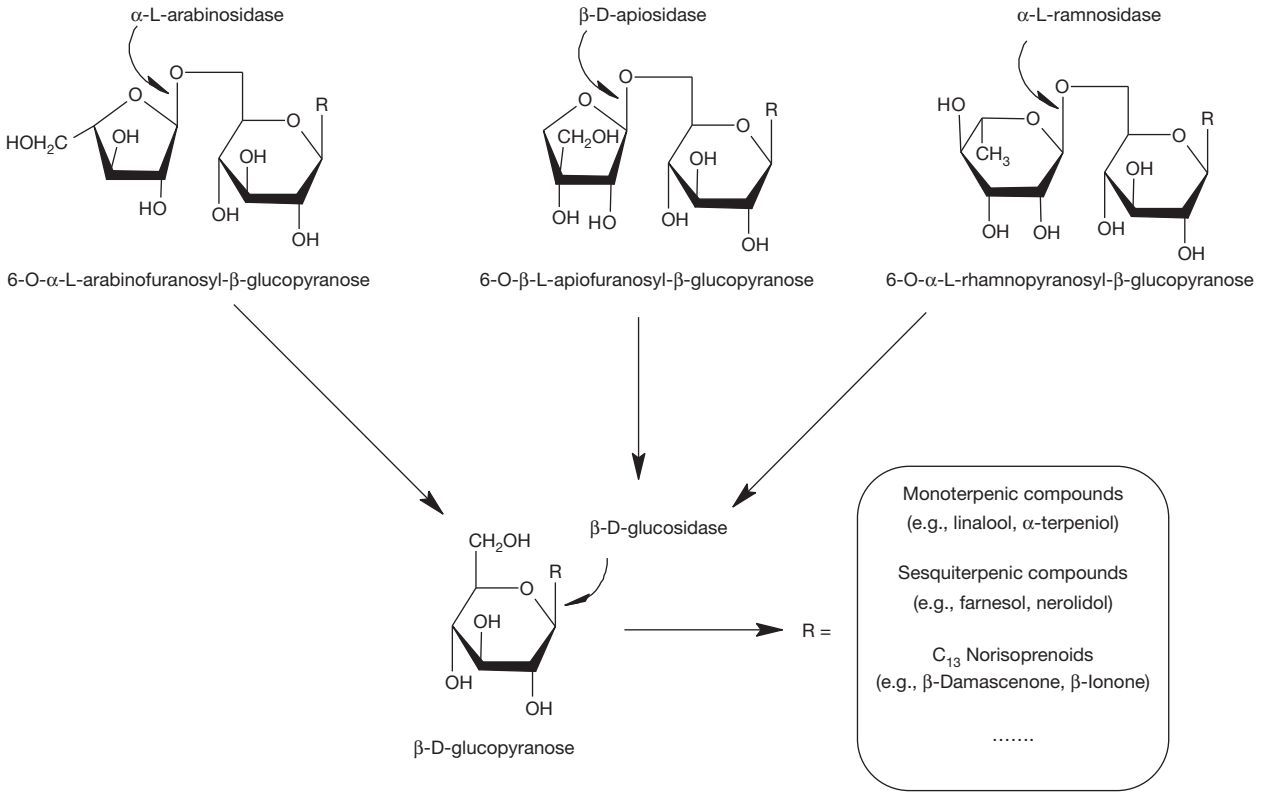

Figure 6 General glycoconjugate structures and hydrolytically released chemical groups occurring in *V. vinifera* L. grapes.

Winemaking Process

Madeira Wines

The grapes are collected and selected, crushed, and fermented using regular procedures. Depending on the style, fermentation is interrupted at different stages by the addition of neutral grape spirits (fortification to 18–19%). Figure 7 shows a schematic illustration of *Madeira* winemaking and aging.

The fortification procedure determines the sweetness level of the four main *madeira* styles (see Figure 8). Traditionally, Sercial is used to produce dry Madeira, Verdelho for medium dry wines, Bual for medium sweet wines, and Malvasia for sweet *Madeira* wines. A heating process, termed *estufagem*, is used to slowly heat the wine (at about 5 °C per day). This is achieved by immersing rods, containing hot water, into the wine. Once having reached 45–50 °C, the temperature is maintained within this range for a minimum of 3 months. During heating, wine organoleptic characteristics change due to the occurrence of several chemical reactions, mainly Maillard reactions and oxidation. After this step, the wines are transferred to oak casks and matured for a minimum of 3 years. The highest-quality *madeiras*, however, are not submitted to *estufagem*. Instead, they are aged in oak casks placed on supports (*canteiros*) in the top floors of cellars. Here, they are exposed to natural temperature and humidity fluctuations (up to 35 °C and 75% humidity during the summer) for up to 20 years or more.

Despite the process chosen, wine quality and production is regulated and controlled by IVBAM (Madeira Wine, Embroidery and Handicraft Institute). Based on their criteria, *madeira* can be characterized on four main parameters: grape variety, aging process, color variation, and wine structure (see Figure 8). It should be also noted that, although not considered in the chart, the heating process used during maturation markedly influences wine quality. The colors shown in the Figure 8 illustrate the color variation during aging. Top-quality *madeiras* typically state the variety used, the *canteiro* aging process, and the duration of maturation (20 years minimum for *Frasqueira*, for instance). Indication of age or harvest year is reserved for *madeiras* exclusively produced with noble cultivars and aged for a minimum of 7 years (for Sercial) and 5 years for the other varieties. Other *madeiras* fall within a broad range of qualities, depending on the duration of aging and blending procedures, *canteiro* being the simplest. In this case, the wine ages for a minimum of 2 years in wood, is then bottled, and is released to the market not before 3 years after harvest. At the other extreme are *solera madeiras*. Starting with good quality wines, 10% is bottled yearly and replaced by an equal amount of another wine of equivalent quality. This procedure can be repeated up to ten times, after which all the *solera* is bottled.

In *madeiras*, heating induces a thermoconversion of some constituents, leading to the production of multiple new aromatic compounds. These include furanic derivatives and volatile phenols. Additionally, wine oxidation is favored by the diffusion of oxygen through the pores of the cooperage (used in the top-quality wines) or controlled exogenously when stainless steel maturation tanks are used. These oxidation reactions are responsible for changes in the wine profile that

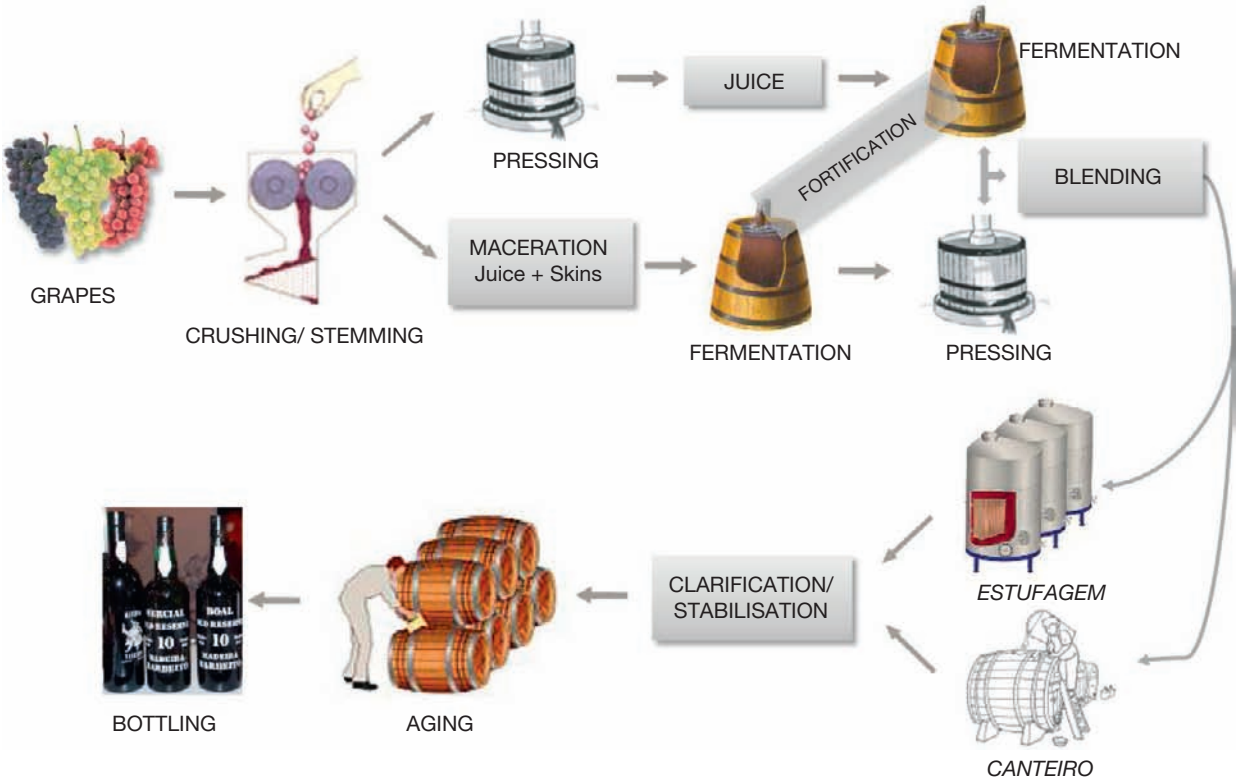

Figure 7 Schematic of winemaking and aging processes to obtain Madeira wine.

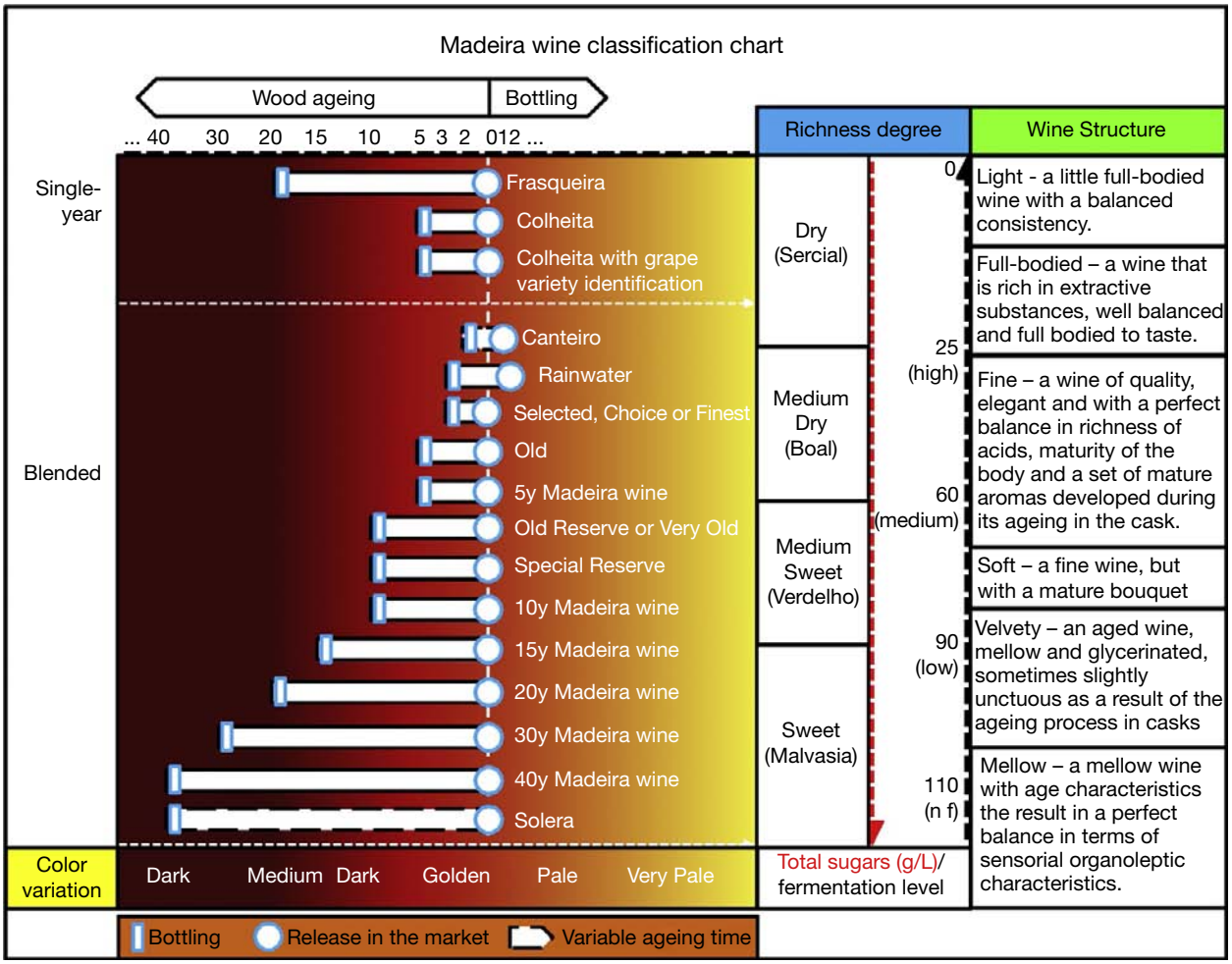

Figure 8 Madeira wine classification chart. The data used in this figure was obtained in the review from Perestrelo et al. (2011).

generate a separate organoleptic profile – usually described as oxidized in dry wines, rancio in sweet fortified red wines, and maderized in sweet fortified white wines. Nevertheless, the most elaborated *canteiro*-aged *madeiras* have a rich bouquet, containing more complex aromas and intense flavors than *madeiras* aged using the *estufagem* process. In fact, for these older *madeiras*, the bouquet is more dependent of the aging process than the grape variety.

Port Wine Production

Port winemaking still employs ancient and traditional procedures, differing only in mechanical improvements. These have been required to cope with the high labor costs associated with grape harvest and crushing (Figure 9). Wine production involves interrupting must fermentation by adding grape spirits (locally designated by *benefício*). This is followed by the preparation of different lots of wines, which are then matured in bottle or wood cooperage.

The grapes selected for vinification should have an alcohol potential between 12° and 14° Baumé. For red *ports*, the stalks are removed (destemmed) and grapes crushed, followed by extensive maceration to increase extraction from the grape skin (particularly the anthocyanins and tannins that confer the deep red color to the wine). This is performed in stainless steel tanks (similar to those used for table wines) to facilitate the control of several parameters, such as fermentation temperature and mixing during maceration.

Fermentation occurs most often spontaneously, using natural yeasts present on the grapes (and winery equipment) at a temperature between 25 and 30 °C. Fermentation continues until the desired residual sugar content is reached, usually within 2–3 days. At this point, fermentation is stopped by discarding the pomace and adding grape spirits (77.0 ± 0.5% ethanol). In the case of white *ports*, both maceration and fermentation with grape skins are virtually absent. An increasingly smaller portion of *Port* wine production involves manual labor, being confined to stalk removal and grape crushing. This is done by treading the fruit in granite tanks (*lagares*). This manual process is much more expensive and, therefore, reserved for selected grapes with the potential to produce a *port* of superior quality.

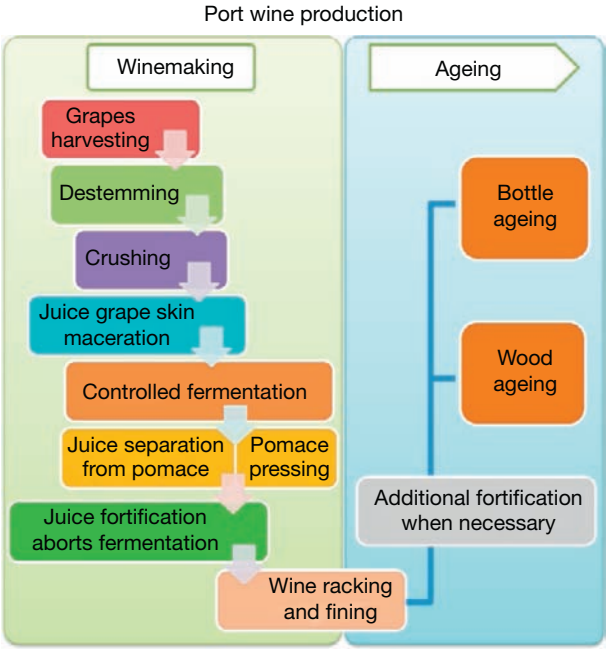

Figure 9 Schematic of winemaking and aging process used to produce port wine. The data used in this figure was obtained in the review from Moreira and de Pinho (2011).

Types of port *wines*

After production, the young wines may remain in the Douro or be transferred to cellars for maturation. Transport was once done in small boats (*rabelos*) along the Douro river but this tradition currently functions only as a trademark for Oporto and *port* wines. As soon as the wines arrive at the cellars in Gaia, they are racked and fined, the alcohol content raised if necessary and the quality assessed by an IVDP (Port and Douro Wines Institute) tasting panel. At this point, the wines follow different maturation processes, depending on their quality and the style preferred. As shown in Figure 10, red *port* maturation may follow two stylistic directions – ruby or tawny. Ruby wines are usually bottled young (after wood maturation for up to 3 years) and aged in bottle for several years, depending on their quality. Correspondingly, oxidation is limited and reductive aging occurs. This constitutes the simplest *port* style, retaining its deep red color and producing a full-bodied wine, rich in tannins and fruity aromas. In contrast, tawny wines are usually matured in small oak cooperage, with no in-bottle aging. Thus, the degree of oxidation is higher, although controlled. As oxidative maturation is prolonged, there is a concomitant loss in color, progressing from tawny, to medium tawny, to finally light tawny. Coincidently, the bouquet resembles dried fruits and develops wood flavors. White *port* wines are far less common. Several variations in the type and duration of maturation, as well as sweetness level, are of recent development. The simplest white *ports* are aged in stainless steel or neutral oak for 3 years, blended, bottled, and released. These *ports* are crisp, with an intense bouquet that combines aromas of melons and peach, with hints of citrus fruit, chamomile, and lemon. In contrast, white *ports* aged in oak for many years acquire a golden color, resembling an old tawny *port*, and show a complex aroma and nutty character.

Grape quality at harvest is the major factor determining the style chosen. As such, a small portion of the best wines is submitted to longer aging to produce the finest *ports*, whether in the ruby or tawny categories. As shown in Figure 10, ruby *ports* include single vintage-based wines: vintage and late-bottled vintage styles. *Colheita* ports are another single-vintage port but can mature in either a ruby or a tawny style. Single-quinta vintage ports are at the pinnacle of the port quality scale, having an additional requisite of being obtained from a single wine estate. All other wines are typically used for blending the great majority of ports, both ruby and tawny. Blending may involve wines from different *quintas*, as well as vintages, except for ports with a stated date of origin. For example, tawny ports are a blend of different lots of wines, matured in casks or vats for different periods, producing a cascade of quality: tawny, tawny reserve, and tawny with an indication of age (10, 20, 30, and 40 years old). Similarly, blended ruby ports may be designated as ruby, ruby reserve, and *crusted* (a blend of

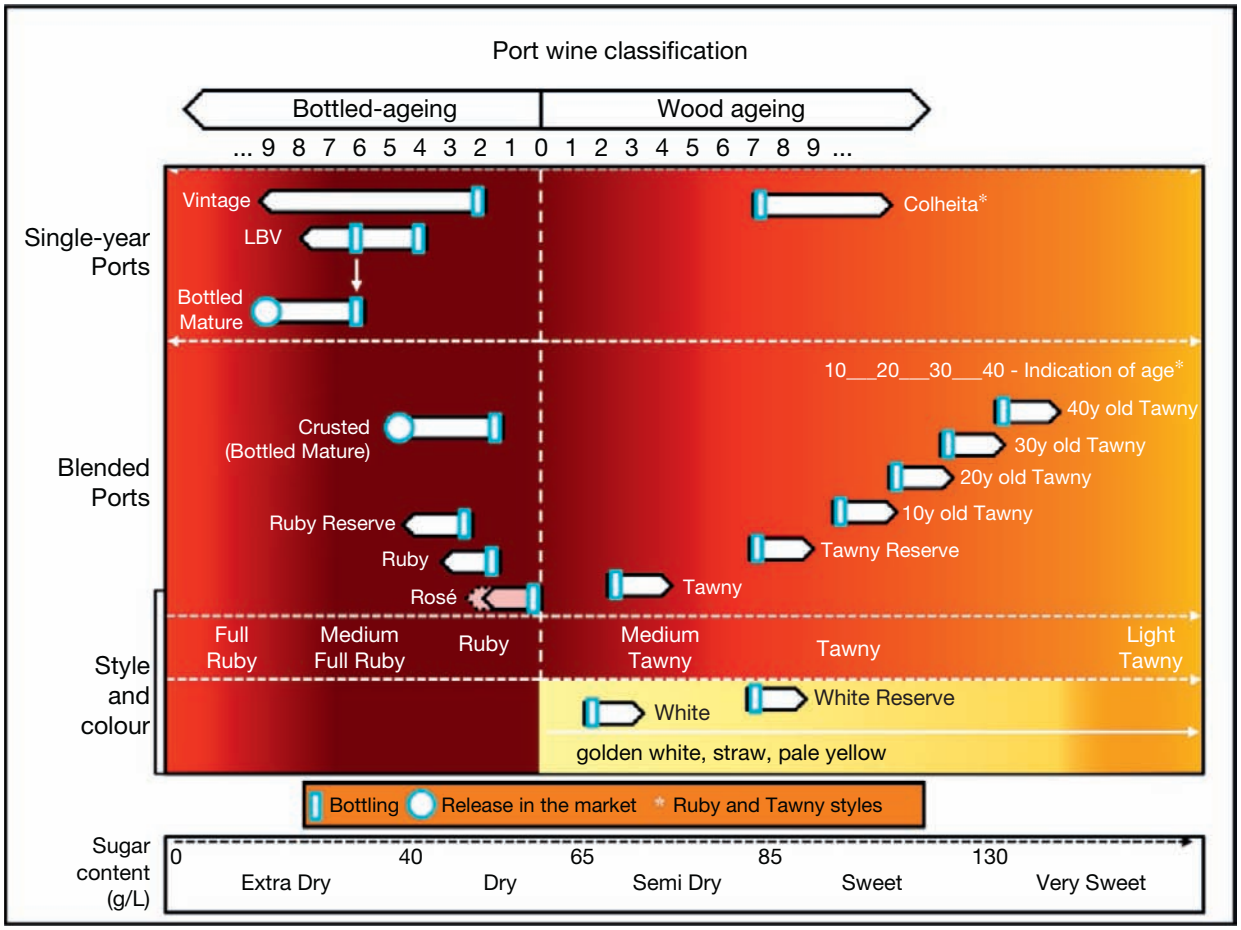

Figure 10 Port wine classification chart. There are several forms of port wine that can be differentiated by its style (ruby, tawny, white, or rosé), aging time and process (in wood, in bottle, or both), grape variety and wine composition (single-year or blended ports), and sweetness (sugar content in g l^{-1}). The colors used in the figure illustrate the variation that occurs in the different port wines during their production and aging and are just an approximation of the real color that the respective wines present. The data used in this figure was obtained in the review from Moreira and de Pinho (2011).

several vintages). In organoleptic terms, all these wines are distinct and become richer and more complex as maturation is prolonged. Generically, ruby-style ports are deep in color and present a full body with exuberant aromas of red fruits and wild berries, balanced with strong tannins. In contrast, tawny ports may possess a wide range of colors (from golden red to tawny) and have a rich bouquet dominated by aromas of dried fruits, roasting, wood, and spices.

Sherry Wines

Vinification starts with grapes harvesting and crushing (stalks included), followed by a rapid juice draining and pressing to limit fermentation in contact with the stems and grape skins. Figure 11 represents schematically the process of sherry wine-making and maturation.

Sherry production limits total phenol content to <200 mg l^{-1} in the free-run juice. Pressed fractions, containing a phenolic content up to 500 mg l^{-1}, can still be used, but heavily pressed juices, containing more than 850 mg l^{-1} total phenols, are not permitted. Wine derived from the first fraction undergoes biological aging (fino style), while that from the first press fraction is usually destined for oxidative aging (oloroso-type style). These fractions cannot exceed 70% of the total wine production each year. Harvesting must, therefore, be done quickly and carefully to avoid grape damage, leading to the release of polyphenol oxidases and juice browning. Similarly, grape crushing and juice separation are performed shortly after harvesting (up to 4 h). An additional requirement demands that juice solids constitute $<1\%$ the juice volume, before fermentation starts. For this, the juice is usually cooled to 12–15 °C, promoting solids precipitation (8–18 h rest period), followed by centrifugation. Fermentation usually involves yeasts naturally present on the grapes. Traditionally, it occurred in oak casks (500–600 l) but nowadays is performed in large, open, temperature-controlled (25 °C), cylindrical tanks (500–1000 hl). Fermentation is usually complete by the end of autumn. Cool temperatures at this time favor yeast and solids settling, which are eliminated by racking. The resultant pale dry wine, containing 11–12.5% alcohol, presents a delicate and slightly fruity flavor and low acidity. The finest wines undergo biological aging, yielding a fino *sherry*. In contrast, wines of more structure (known as *gordura*) are used for oloroso production, which involves oxidative aging. The stylistic direction of maturation is assisted by fortifying fino-style wines to 15.5% alcohol and oloroso *sherries* to

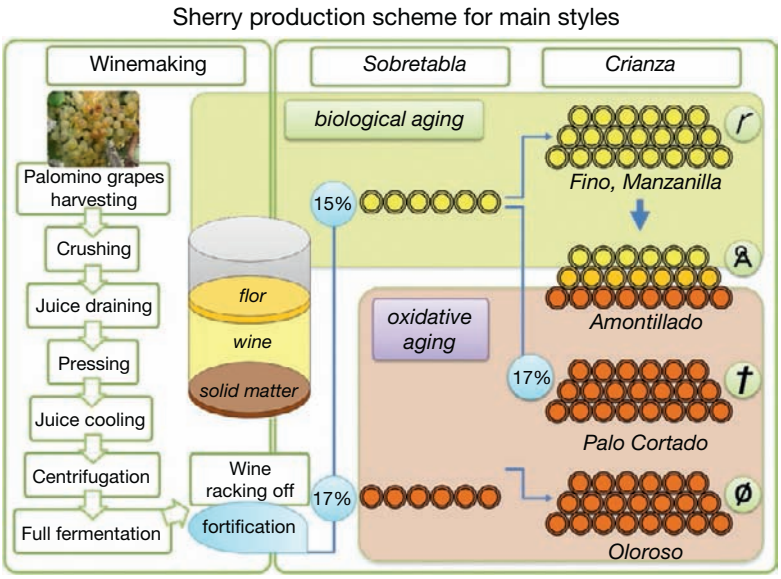

Figure 11 Schematic of winemaking and aging process used to produce sherry. The data used in this figure was obtained in the review from Pozo-Bayón and Moreno-Arribas (2011).

18–20%. These fortified wines, known as *encabezado*, are subsequently transferred to smaller oak barrels where they mature. In fino *sherries*, yeasts still present switch from fermentative to oxidative (respiratory) metabolism, using ethanol as a carbon source. This is associated with the formation of a surface yeast biofilm (velum) called *flor*. It produces a barrier limiting oxygen exchange and wine oxidation. Important metabolic consequences include the production of acetaldehyde and a reduction in glycerol and acetic acid contents. At this stage (*sobretablas*), the winemaker makes a final selection relative to stylistic direction (fino or oloroso). This is possible because additional fortification prevents yeast growth, essential to fino production. Following this decision, the wines are clarified by sedimentation, fortified (if necessary), and placed in oak butts, undergoing further maturation and blending (known as the *solera* and *criadera* system). Fino *sherries* produced in Sanlúcar de Barrameda are called *Manzanilla*, being the driest and most pungent *sherries*.

Oloroso-style aging begins under a *flor* velum, like a fino, but due to their higher polyphenol content and consequent organoleptic differences, wines are subjected to fractional blending involving only oxidative maturation. This starts with fortification to 18–20%, preventing any subsequent *flor* activity, leaving the wine in direct contact with air. Oxidation activates important changes in chemical composition, namely, polyphenol oxidation and a gradual darkening in color. This change is encouraged by putting the wine outside to heat under the high summer temperatures of southern Spain. When brown, the dry wine is known as an oloroso. A proportion may be sweetened with *arrope* and termed a *cream sherry*. The selection between fino or oloroso styles is also affected by climatic conditions during the growing season. In hot years, with drying easterly winds, the vintage is early, and more oloroso is produced, while in cooler, more humid years, the vintage is late, and more fino is produced.

Amontillado is an intermediate, highly appreciated sherry style. It starts out with biological aging (like a fino) but subsequently shifts to a slow oxidative aging (following fortification to 18–20%). Maturation usually lasts 8 years, producing an aromatic wine containing the complexities of both a fino and an oloroso. *Palo cortado* is a special oloroso sherry. The wine is fortified to 15% alcohol and undergoes maturation under *flor* until the *sobretabla* stage. If the winemaker detects special organoleptic characteristics, may select the wine for *palo cortado* production. This involves oxidative maturation and alcohol volume up to 17%. This creates an amontillado-like sherry with an oloroso character, but without losing the elegance of an amontillado. *Palo cortado* is one of the most exclusive *sherries*. Finally, Moscatel and Pedro Ximénez *sherries* are made from late-harvested or dried grapes (dried under the sun or artificially). Fermentation is stopped early to preserve the wine's sweetness and the wine left to oxidize like an oloroso. Figure 12 presents an overview of the different sherry styles and parameters that allow their distinction. As noted earlier, some styles are differentiated by their residual sweetness (based on the duration or absence of fermentation). The degree of oxidation (biological vs. oxidative aging) affects the wine color (from pale to dark).

The solera *and* criadera system*: a never-ending aging–blending system*

The *solera* and *criadera* system is one most distinguishing features of Spanish sherry production. Casks are stacked in rows (*criaderas*) so that all the wine in a single row is of the same type and age. The oldest wines are located at the bottom (the *solera*), followed by progressively younger wine rows. Each cask is filled to about 80% full, if *flor* yeast development is desired. Periodically, up to three to four times per year, about 40% of the *solera* wine is withdrawn for bottling and released to the market. This amount is replaced by wine from the first *criadera* (second row in the system), which in turn is replaced by wine from the second *criadera* (third row of the system). This continues until the uppermost *criadera* is reached and the volume of wine replaced with *sobretabla* wine. The number of *criaderas*

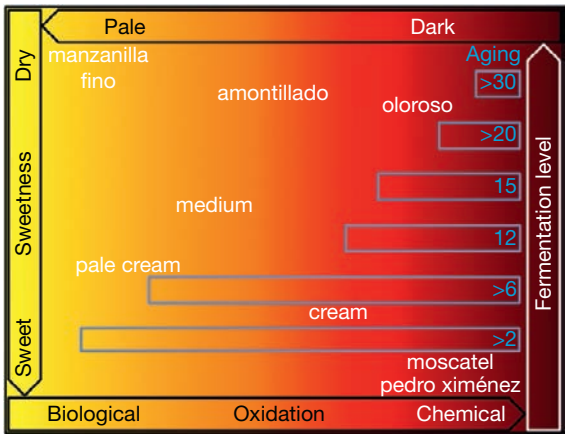

Figure 12 Sherry wine classification chart.

is positively correlated with the quality of the final blend, being typically between 4 and 6. This dynamic system facilitates a reliable, progressive, and fractional blending of the wines. Therefore, most *sherries* on the market are a blend of many vintages, maintaining specific proprietary characteristics. Although most *sherries* are *solera*-aged, there are vintage versions. These are produced via a static aging system known as *añadas*. They are a recent development established to differentiate special vintages, designated as *sherries* of certified age. Accordingly, *sherries* matured for over 20 and 30 years use the initials VOS and VORS, respectively.

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Part II. Madeira, Port, and Sherry Fortified Wines: The *Sui Generis* and Notable Peculiarities, Major Differences and Chemical Patterns

Main Style Comparison: Differences and Similarities

Madeiras, *ports*, and *sherries* share several similarities, as well as significant differences (Table 1).

They are exclusively produced in delimitated or demarcated regions, using specific grape varieties (mainly five for *madeiras*, more than a dozen for *ports*, and three for *sherries*) and according to certain vinification specifications. They are fortified wines, meaning that grape spirits have been added during production, although their addition occurs in different stages in vinification and in different concentrations. *Madeiras* and *ports* usually possess an alcohol concentration between 19% and 22%, being lower for *sherries*, about 15% to fino-style versions, and 18% for the *olorosos*. Moreover, in *madeiras* and *ports*, fortification is used to stop fermentation at different stages, allowing a full range of wine sweetness, from dry to sweet. In contrast, in *sherries*, fortification occurs only after fermentation is complete. Therefore, all *sherries* are initially dry, except for the sweet Moscatel and Pedro Ximénez wines (which are not fermented at all). Regarding aging, most *sherries* undergo a unique biological aging process that occurs under a yeast layer (*flor*). This avoids the oxidative aging that occurs in some *sherry* styles. For *ports*, aging may be oxidative (in casks) or reductive (in bottle), while *madeiras* undergo a heating process, in which they are submitted to high temperatures for 3 months before oxidative aging. These particularities during fermentation and aging produce wines with distinctive characters that permit a clear differentiation among them. *Ports* and *madeiras*, for example, present richer, sweeter, and heavier textures than *sherries*, since fortification occurs halfway through the fermentation process. Regardless of these differences, longer oxidative aging produces wines that are more exclusive, possessing higher quality, and consequently are more expensive.

Finally, it is worth noting the differences in the blending procedures used in these wines. *Sherry* winemakers developed a sophisticated system (*solera* and *criaderas*) that allows an accurate and reliable blending from year to year. Thus, *sherry* vintages are infrequent. In contrast, vintage designation is common in superior years for *ports* and *madeiras*, although blended versions are the most common. Interestingly, there is a *madeira* style, *solera*, which is obtained using blending procedures resembling those of *solera* system *sherries*.

Chemical Profile of Fortified Wines

Wine aroma is one of the most important intrinsic factors that influences perceived wine quality and consumer acceptance. Aroma is the cumulative effect of a diverse group of volatile compounds typically detected at very low concentrations (between 10^{-4} and 10^{-12} g l^{-1}). These compounds include mainly alcohols, esters, aldehydes, ketones, fatty acids, terpenes, phenols, and sulfur compounds. Wide differences in their proportions and characteristics result from interactions among five factors: vineyard geographic location, soil, climate, grape variety, and winemaking practices. Several hundred volatile compounds have been identified in fortified wines. However, only a few are present at concentrations above their odor threshold (OT) and thus likely to be responsible for

Table 1 Comparison between fortified wines

	Madeira wine	*Port wine*	*Sherry wine*
Protected designation of origin (PDO)/demarcated region (DR)	Madeira wine/DR of Madeira, Madeira island, Portugal	Port wine/DR of Douro and Porto, Portugal	'Jerez-Xeres-Sherry' and 'Manzanilla of Sanlúcar de Barrameda'/Jerez de la Frontera and Montilla-Moriles, Spain
Alcohol content	19–22% vol	19–22% vol	15% (fino styles), 18% (oloroso styles)
Grape varieties	Bual, Malvasia, Sercial, Verdelho (white grapes), and Tinta Negra (red grape)	High number of varieties allowed, with minimum regulated proportions of ten specific red and white varieties	Palomino Fino, Pedro Ximénez, and Moscatel
Vinification and aging processes	Variable fermentation levels and fortification followed by *estufagem* or *canteiro* oxidation; final wood aging	Variable fermentation levels and fortification followed by wood (oxidative) and bottle (reductive) aging	Mostly full fermentation and fortification followed by biological (*flor*) or oxidative (wood) aging
Styles	Single-year (*Frasqueira* and *Colheita*) and several blends of aged wines	Single-year rubies (vintage, LBV, and *Colheita*) and tawny (*Colheita*) and several blends of aged rubies and tawnies (reserve and indication of age); rosé and white ports	Sophisticated blending system (*solera* and *criaderas*) used in several styles (fino, manzanilla, amontillado, oloroso, palo cortado, etc.). Vintages are not very frequent
Texture	Several textures from light, full-bodied, fine to soft, velvety, or mellow	Rich, heavy, and complex textures, with different degrees of sweetness	Mostly full dry wines, except for the sweet Moscatel and Pedro Ximénez sherries

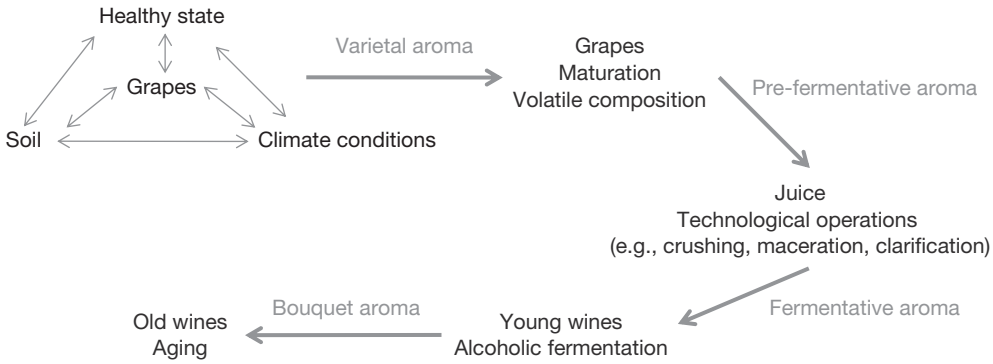

Figure 1 Technological sequence of winemaking process and its relation with different aroma types.

aromatic distinctiveness. These volatile compounds originate from grapes (varietal origin), technological procedures (e.g., crushing and pressing), fermentation, and reactions during maturation (e.g., wood extractives) (Figure 1).

Volatile Profile of Young Fortified Wines

Volatile profile plays an important role in wine quality. By generating odor and occasionally taste sensations, volatiles influence consumer acceptance or rejection. Theoretically, any compound at above its odor threshold (OT) may generate a characteristic aroma (impact odorant). However, impact odors often consist of combinations of distinct odorants. Many of these are present at only trace amounts ($\mu g\,l^{-1}$ or $ng\,l^{-1}$) and at concentrations below their individual OTs. Nonetheless, they may contribute to the overall aroma through interactions with other wine constituents, therefore impacting the wine's aroma.

The aroma profile of *madeiras* has been studied by several techniques. In all analyzed samples, the predominant volatiles identified have been ethyl octanoate, ethyl decanoate, and diethyl succinate. Traces of monoterpenoids (e.g., linalool, nerol oxide, and α-terpineol), C_{13} norisoprenoids (e.g., β-damascenone, β-ionone), and sesquiterpenoids (e.g., γ-eudesmol, α-cadinol, and ι-muurolol) were detected in *madeiras*, which are important from a sensorial point of view. Terpenoids can be employed to differentiate the must and *madeiras* based on the *V. vinifera* L. varieties used. Linalool, α-terpineol, citronellol, and farnesol are the predominant varietal terpenes in the grape varieties used. However, terpenes appear not to contribute significantly to the wine's aroma.

Malvasia musts show the highest terpenoid concentrations, whereas Verdelho the lowest. Malvasia wines are characterized by the highest monoterpenoid concentrations, whereas Bual wines are distinguished by C_{13} norisoprenoids, higher alcohols, and carbonyl compounds; Sercial wines by their acetate, acid, ethyl ester, and volatile phenolic contents; and Verdelho wines by the highest contents of ethyl esters and furans.

The aroma profile of young *ports* differs remarkably from that of oak- and bottle-aged *ports*. Young wines are characterized by floral, bergamot-like, violet, and jasmine descriptors, due to the presence of some terpenoids and norisoprenoids. Figure 2 demonstrates a comparison of volatile pattern of young *ports* and *madeiras*.

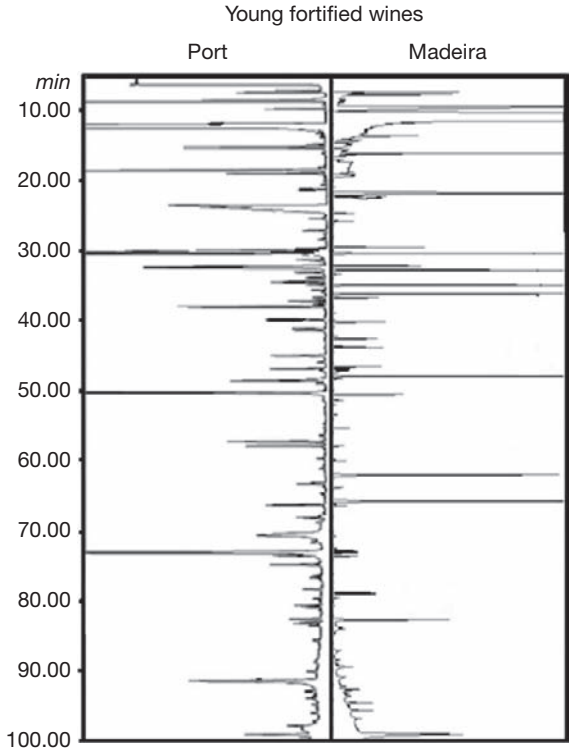

Figure 2 Typical GC–qMS chromatograms of a dichloromethane extract from young ports and madeiras.

The presence of nerol, linalool, citronellol, α-terpineol, geraniol, 1,2-dihydro-1,1,6-trimethylnaphthalene, (*E*)-β-damascenone, (*Z*)-β-damascenone, α-ionone, geranyl acetone, and some esters, like hexyl acetate, ethyl acetate, isovalerate, and ethyl 3-hydroxyhexanoate could explain the citrus and floral odors of young fortified wines. All these varietal and fermentative components, except ethyl acetate, possess low odor thresholds (Table 2).

More than 80 volatiles have been identified in Tinta Negra (used in producing *madeiras*). These include higher alcohols, ethyl esters, volatile acids, and lactones. 3-Methylbutan-1-ol, 2-phenylethanol, isoamyl acetate, diethyl succinate, 2-phenylethyl acetate, phenylacetaldehyde, γ-nonalactone,

Table 2 Volatiles related to young fortified wines, their odor threshold (OT), and odor descriptors

Chemical groups	*OT ($\mu g\ l^{-1}$)*	*Odor descriptors*
Terpenoids		
α-Pinene	6	Camphoraceous, citrus, fruity, green, pine, sweet, wood
α-Phellandrene	200	Black pepper, citrus, fruity, slightly green, terpenic
1,8-Cineole	12	Camphoraceous, eucalyptus, menthol, pine, sweet
p-Cymene	6	Citrus, fruity
Limonene	10	Citrus, mint, wood
Terpinolene	200	Citrus, fresh, pine, sweet
γ-Terpinene	10	Citrus, sweet, terpenic, tropical
(*E*)-Linalool oxide	190	Fatty, floral, rose, sweet
Linalool	6	Citrus, lavender, floral, fruity, green, muscat, sweet
Borneol	2	Camphor, earth, pine, wood
Menthol	920	Cool, fresh, green, herbaceous
Myrtenol	7	Berry, medicinal, mint, vanilla, wood
4-Terpineol	340	Metallic, must, nutmeg, rancid
p-Cymen-8-ol	22 500	Camphor, coumarin, cherry, floral, fruity, sweet
α-Terpineol	250	Anise, floral, fruity, mint, oil, toothpaste
Nerol	290	Citrus, floral, marine, rose
Citronellol	30	Citrus, clove, floral, fresh, green, rose, sour, sweet
Carvone	7	Herbaceous, mint, peppermint, spearmint, warm
Geraniol	40	Citrus, floral
Perilla alcohol	1660	Dried orange peel, floral, green, spice, sweet, waxy, wood
Norisoprenoids		
2,2,6-Trimethylcyclohexanone	100	Citrus, honey, pungent
Naphthalene	6	Medicinal
β-Cyclocitral	5	Floral, sweet
1,2-Dihydro-1,1,6-trimethylnaphthalene	2.5	Floral, fruity, pleasant, wine
(*Z*)-β-Damascenone	0.05	Floral, fruity, honey, sweet, tobacco
(*E*)-β-Damascenone	10	Baked fruit, floral, fruity, honey, ripe fruit, sweet, tobacco
α-Ionone	0.6	Floral, violet
Geranyl acetone	60	Floral, fruity, green, waxy, wood
β-Ionone	1.5	Floral, violet
Sesquiterpenoids		
β-Caryophyllene	64	Berry fruit, infusion, wood
α-Bisabolol	0.08	Floral, pepper, wood
Esters		
Ethyl acetate	7500	Acidic, buttery, fruity, nail polish, pungent, solvent, sweet
Ethyl propanoate	10	Ethereal, fruity, rummy, sweet, wine
Ethyl isobutyrate	15	Alcoholic, ethereal, fruity with pungent, fusel, rummy, sweet
Ethyl butyrate	20	Acetone, bubblegum, caramel, fruity
Ethyl lactate	14 000	Acidic, ethereal, fruity, sweet
Ethyl 2-butenoate	130	Cognac, floral, fruity, meaty, pungent, rum, sharp
Ethyl isovalerate	3	Fruity, green, sharp, sweet
Isoamyl acetate	30	Banana, fresh, fruity, sweet
Ethyl pentanoate	10	Fruity, grass, green, mint
Ethyl 3-hydroxybutanoate	20 000	Coconut, grape, nutty
3-Hexenyl acetate	210	Fruity, fresh green, sweet
Ethyl 3-hexanoate	1.9	Anise, fruity, sweet, wine
Hexyl acetate	10	Acidic, citrus, fruity, green, herbal, rubber, spice, sweet wine, tobacco
Methyl benzoate	0.52	Floral, fruity, herbal, honey, lettuce
Ethyl heptanoate	2	Berry, cognac, fruity, sweet
Ethyl 3-hydroxyhexanoate	265	Citrus, fruity, green, sweet
Ethyl benzoate	575	Celery, chamomile, fruity, must, tea
Diethyl succinate	200 000	Fabric, floral, fruity, lavender, potato, sweat
Ethyl octanoate	24	Fruity, must, soap, sweet, waxy
Methyl salicylate	40	Berry, sweet, warm, wine
2-Phenethyl acetate	250	Floral
Ethyl salicylate	84	Balsamic, cooling, spice, sweet, tutti-frutti, wintergreen
Ethyl nonanoate	377	Cognac, fruity, tropical, waxy, wine
Ethyl decanoate	200	Fruity, pleasant, soap, sweet, waxy
Isoamyl octanoate	125	Fatty, fruity, green, sweet, waxy
Ethyl cinnamate	165	Balsamic, cinnamic, floral, fruity, spice, sweet
Ethyl dodecanoate	1500	Soap, sweet
Ethyl hexadecanoate	>2000	Cream, fruity, milk, rancid, sweet, waxy

ethyl hexanoate, ethyl octanoate, hexanoic acid, and octanoic acid seem to be the most sensory important odorants.

The volatile profile of fino *sherries* appears to be characterized by the presence of ethyl acetate, 2-methyl-1-butanol, isoamyl alcohol, 2,3-butanediol, ethyl lactate, 3-hydroxy-2-butanone, acetic acid, 2-phenylethanol, γ-butyrolactone, hexanoic acid, and octanoic acid.

Evolution of Volatile Compounds During Fortified Wine Aging

Aging promotes several modifications in fortified wine composition. These may involve baking, oxidative conditions, and, to a lesser extent, phenomena common to all wine, which are physical, chemical, and microbiological processes. The compounds released from the wood include aromatic aldehydes (e.g., vanillin) and γ-lactones (e.g., *cis*- and *trans*-oak lactones). These can have a great influence on the wine aroma, whereas 2-furfural and 5-methyl-2-furfural, also derived from oak, have little influence (Table 3).

Oxidative conditions can increase the aldehyde content, mainly acetaldehyde and acetals. At wine pH values, acetalization between acetaldehyde and glycerol is highly favored (Figure 3).

The heterocyclic acetals noted in Figure 3 have been shown to form during the aging of both Madeira and Port wines. The content of *cis*-dioxane and *cis*-dioxolane is directly correlated with wine age (Figure 4). The *cis*-dioxane isomer occurs at higher concentrations than its *trans*-isomer.

The predominant changes in *madeiras* during aging involve significant decreases in the concentration of fatty acid ethyl esters, acetates, and terpenoids that are responsible for fresh fruity odors. In contrast, the concentration of ethyl esters of diprotic acids, fatty acids, lactones, volatile phenols, and furanic compounds increases markedly during aging. Figure 5 shows the comparative profile of the volatile compounds of aged *ports* and *madeira*s.

Of more than 120 volatile compounds found in aged *madeiras*, only sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone), 2-furfural, 5-methyl-2-furfural, 5-(hydroxymethyl)-2-furfural (HMF), 5-(ethoxymethyl)-2-furfural, *cis*-dioxane, and *cis*-dioxolane were directly correlated with age. The establishment of potential aging markers is important in fraud detection and to ensure wine authenticity. Techniques such as a combination of two dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC–ToFMS) with solid-phase microextraction are aiding in the establishment of potential marker for different grape varieties, wine types, and ages. Considering the chemical groups furans, lactones, volatile phenols, and acetals, 103 volatiles have been tentatively identified. Diethoxymethane, 1,1-diethoxyethane, 1,1-diethoxy-2-methyl-propane, 1-(1-ethoxyethoxy)-pentane, *trans*-dioxane, 2-propyl-1,3-dioxolane, 5-methylfurfural, and *cis*-oak lactone are potential aging markers (Figure 6), independent of variety and wine type.

The impact of oxygen (O_2), temperature, pH, and free sulfur dioxide (SO_2) on the content of dimethyl sulfide (DMS), 2-mercaptoethanol, dimethyl sulfone, methional, and methionol in *ports* has been evaluated. Based on the results obtained, temperature and oxygen are the two main extrinsic factors that contribute to white wine deterioration, since methional level is greatly favored by elevated O_2 concentrations and higher temperatures. However, other studies have shown that methionol decreases significantly in the presence of O_2. Nonetheless, this decrease is not related with a conversion to methional. 2-Mercapethanol decreased in the presence of O_2, whereas the respective disulfide was formed. In summation, oxidative reactions, those occurring during the aging of *ports* in barrel, increase the aldehyde, methyl ketone, acetal, and lactone levels. Other studies have evaluated the influence of O_2, pH, free SO_2, temperature, and time on norisoprenoid content. Bottle-aged *ports* develop floral and violet notes that could be associated to the presence of some norisoprenoids, such as β-ionone, β-damascenone, vitispirane, 2,2,6-trimethylcyclohexanone (TCH), and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN). However, in traditional barrel aging, vitispirane, TDN, and TCH increased, whereas the levels of β-ionone and β-damascenone decreased. Aged *ports* had more than 15, 5, and 3 times higher level of TDN, vitispirane, and TCH than young versions.

The influence of barrels on the volatile composition of aged wines was studied relative to either French or American oak and at the end of their use cycle (5–6 years). Aging in American oak donates higher concentrations of *cis*-oak lactone (above its odor threshold) but lower concentrations of vanillin, syringaldehyde, and acetovanillone than French oak. For fino *sherries*, sotolon, 1,1-diethoxyethane, and *cis*-whisky lactone were the volatiles most associated with aging and odor impact among the 63 volatile compounds identified. Moreover, fino, amontillado, and oloroso *sherries* can be distinguished chemically relative to their biological or oxidative aging processes.

Impact Odorant in Fortified Wines

Volatile profile plays an important role in wine quality. They can promote several sensations during consumption: odors (due to molecules that can bind olfactory receptors) and tastes (due to molecules that simulate taste bud receptors), which can lead to either consumer acceptance or rejection. In general, a presence of a single molecule, at a concentration above its OT, is enough to provide a characteristic product aroma (impact odorant). Nevertheless, impact odorants often consist of a combination of several hundred of different odorants (molecules). Many of these are present in only trace amounts (usually at $\mu g\, l^{-1}$ or $ng\, l^{-1}$ level). However, even when present at concentrations below their OT, they may contribute to the overall aroma as a result of interactions with other molecules, thereby having an impact on wine aroma perception.

Information related to younger (3–5 years old) and older (10–40 years old) *madeira* aromas is shown in Table 4. It was obtained using a trained panel and is also supported by previous studies. As observed in Table 4, younger *madeiras* are characterized by their freshness and fruitiness descriptors (e.g., citrus, floral, and fruity), whereas old *madeiras* by caramel, dried fruits, spice, toast, and wood notes.

Figure 7 shows the aroma map network of *madeiras*, where each color represents an aroma note, normalized by the mean of OT of their respective chemical group. The line width corresponds to the number of shared aroma compounds. It indicates that grape variety seems to have a great impact on the sensorial properties of young *madeiras*, along with other parameters (e.g.,

Table 3 Volatiles related to fortified wines aged in oaks, their odor threshold (OT), and odor descriptors

Chemical groups	*OT ($\mu g\, l^{-1}$)*	*Odor descriptors*
Sulfur compounds		
2-(Methylthio)ethanol	250	French bean, meat, sulfurous
Methional	0.2	Baked potato, grass, soup
Methionol	1000	Boiled potato, cooked cabbage, rubber, soup
Furans		
2-Furfural	14 100	Almond, caramel, sweet, wood
2-Furanmethanol	15	Honey, medicinal, sweet, toast
2-Acetylfuran	10 000	Balsamic-cinnamic, cereal, sweet, toast, tobacco
5-Methyl-2-furfural	20 000	Acidic, almond, caramel, coffee, spice, toast
2-Pentylfuran	6	Buttery, green bean, mushroom
Ethyl 2-furoate	16 000	Balsamic, scorched tone, vanilla
Furaneol	5	Baked, caramel, cotton candy, toast
Maltol	35 000	Caramel, cotton candy, jammy fruity, sweet, toast
5-Ethoxymethyl-2-furfural	–	Curry, spice
5-Hydroxymethylfurfural	100 000	Almond, cardboard, nutty
Lactones		
γ-Butyrolactone	35 000	Caramel, coconut, cream, peach
Pantolactone	50	Caramel, licorices, sweet, toast
γ-Hexalactone	260	Apricot, peach
γ-Heptalactone	400	Caramel, cheese
γ-Octalactone	7	Coconut, cream, fatty, herbaceous, nutty
trans-oak-lactone	790	Coconut, nutty, toast, wood
cis-oak-lactone	67	Coconut, nutty, toast, wood
γ-Nonalactone	30	Butter, coconut, sweet
γ-Decalactone	10	Fruit, sweet
γ-Dodecalactone	7	Coconut, fruity, musk
δ-Dodecalactone	160	Coconut, fruity, musk, sweet
Sotolon		
Volatile phenols		
Phenol	5900	Bland, medicinal, phenolic, sweet
o-Cresol	31	Leather, spice
p-Cresol	55	Animal, phenolic, smoke
o-Guaiacol	10	Burnt, chemical, clove, phenolic, smoke
p-Ethylphenol	440	Animal, musty, shoe polish, urine
p-Methylguaiacol	65	Candy, eugenol, leather, smoke, spice, sweet, vanilla
p-Ethylguaiacol	33	Clove, leather, phenolic, smoke
p-Vinylguaiacol	40	Almond shell
Eugenol	5	Balsamic, camphoraceous, herbaceous, honey, spice
p-Propylguaiacol	10	Animal, leather
Methyl eugenol	820	Clove, spice
Vanillin	60	Sweet, vanilla
Ethyl vanillate	990	Honey, vanilla
Acetals		
Diethoxymethane	–	Acrid
1,1-Diethoxyethane	719	Green, licorices, nutty, pungent, wood
1,1-Diethoxy-2-methyl-propane	–	Fruity
cis-Dioxane	–	Old port, sweet
1,1-Diethoxybutane	–	Mild
cis-Dioxolane	–	Old port, sweet
trans-Dioxolane	–	Old port, sweet
1,1-Diethoxy-3-methylbutane	–	Fatty
trans-Dioxane	–	Old port, sweet
1,1-Diethoxyhexane	–	Apple, cognac, fruity, hyacinth, pear
1-(1-Ethoxyethoxy)-hexane	–	Earth, floral, fungal, green
1,1-Diethoxyoctane	–	Fatty, fruity, green, oil, spice, wood
1,1-Diethoxynonane	–	Aldehydic, wood
1,1-Diethoxydecane	–	Aldehydic, coconut, green, orange with cognac, waxy

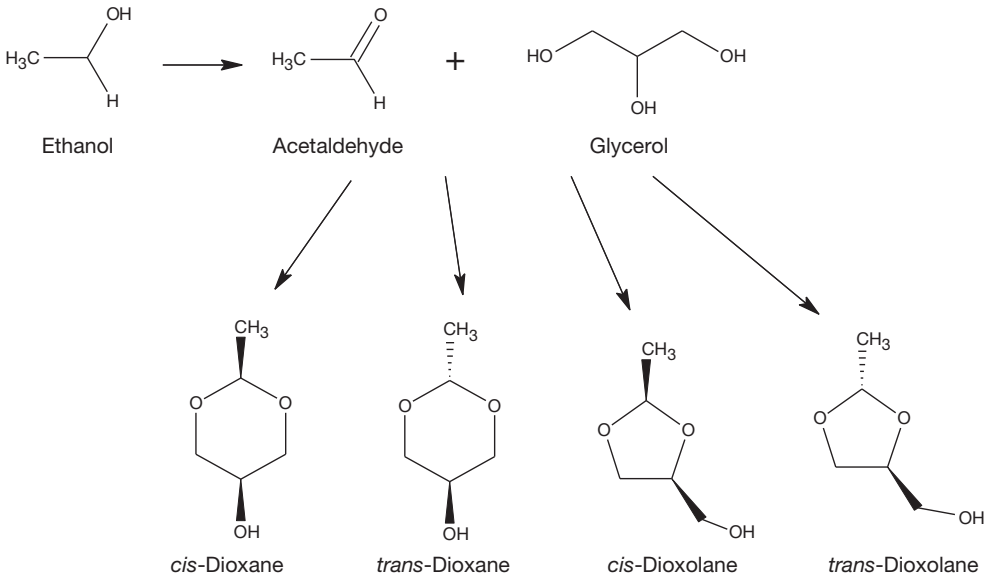

Figure 3 Formation of heterocyclic acetals by the acetalization reaction between acetaldehyde and glycerol.

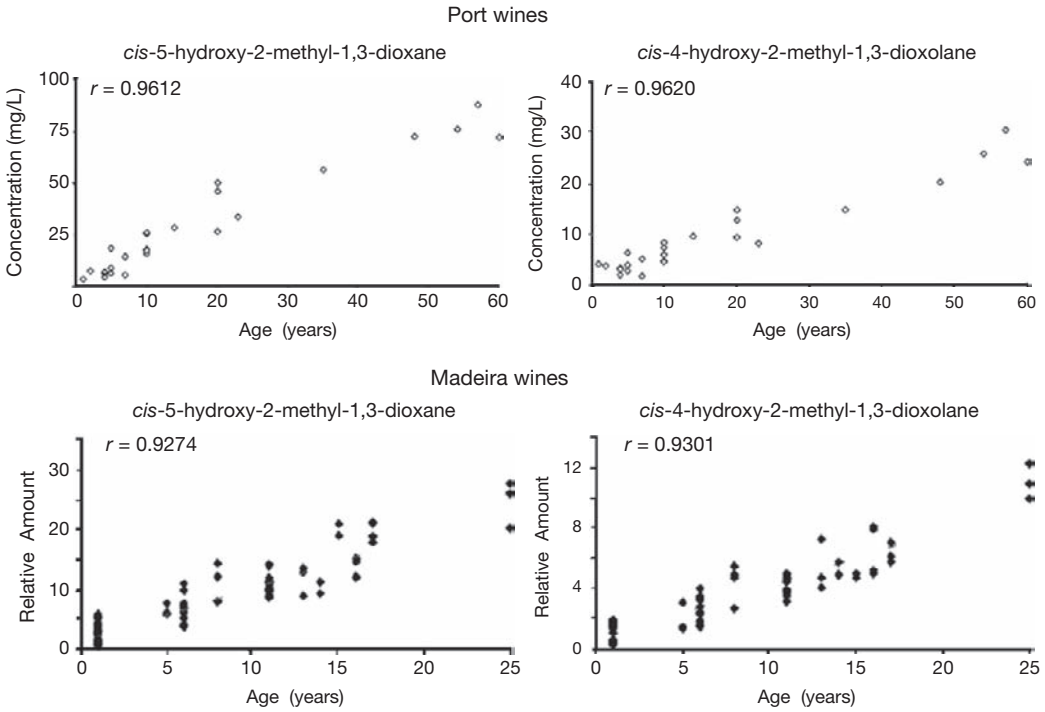

Figure 4 Evolution of *cis*-dioxane and *cis*-dioxolane with age for Port and Madeira wines.

vinification procedures). For example, Malvasia and Bual grapes, used to produce sweet and medium sweet *madeiras*, are characterized by almond and cocoa odors, whereas dry and medium dry versions produced from Sercial and Verdelho grapes are characterized by mushroom and honey notes.

Several studies have focused on potential impact odorants that could contribute to the overall aroma of *madeiras*. Gas chromatography–olfactometry (GC–O) and gas chromatography–mass spectrometry (GC–MS) have been used to establish the aroma profile of four *madeiras*. The GC–O profile of *madeiras* lacks important varietal odorants, such as linalool, 3-mercaptohexyl acetate, and methoxypyrazines, but is rich in sotolon, phenylacetaldehyde, and wood extractable aromas. It also contains a large number of unidentified, intense odorants not present in young *madeiras*. Moreover, furanic compounds, such as 2-furfural, 5-methyl-2-furfural, HMF, and 5-(ethoxymethyl)-2-furfural, are quantitatively predominant, but were not detected by GC–O. This suggests that these

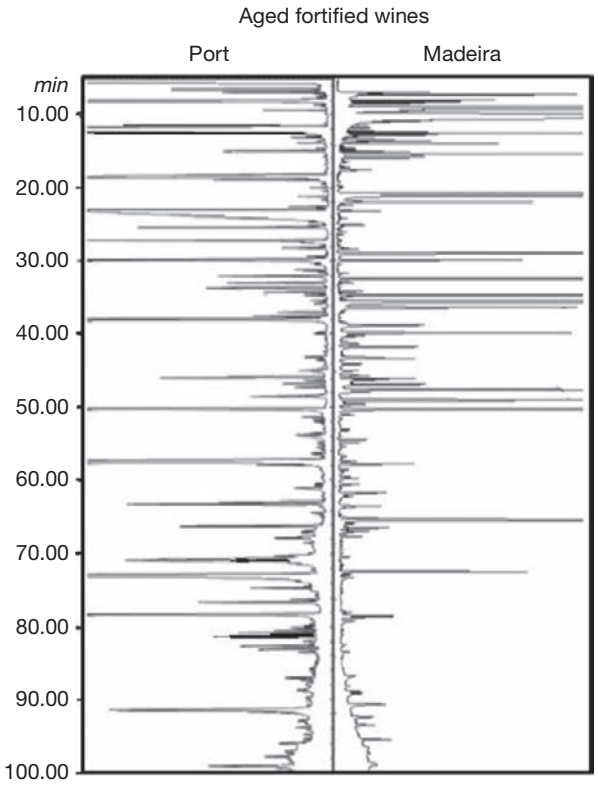

Figure 5 Typical GC–qMS chromatograms of a dichloromethane extract from aged port and madeira.

furanic compounds, although very abundant, are not relevant to a *madeira* aroma. The main reason for this is the high OT that these compounds present (Table 3). In addition, the lowest scores (OT) are related to spicy and lacquer descriptors, whereas the highest OT scores are related to candy, toasty, maderized, and dried fruits notes. However, these contributions depend on the grape variety, winemaking process, and aging. The impact of aging on *madeira* flavor was also studied by GC–O. For this purpose, an expert panel was used to analyze seven descriptors, including dried fruit, nutty, musty, baked, oak, mushroom, and brown sugar. On the basis of the aroma extract dilution analysis, several Maillard by-products such as sotolon, 2-furfural, 5-methyl-2-furfural, 5-(ethoxymethyl)-2-furfural, methional, and phenylacetaldehyde were identified. This may explain the baked, brown sugar, and nutty odor notes, characteristic of aged *madeiras*.

Sotolon is produced from an aldolization between α-ketobutyric acid (from the deamination of L-threonine) and acetaldehyde. Due to its low odor threshold (10 $\mu g\,l^{-1}$), sotolon is recognized as a key constituent in the typical aroma of barrel-aged *ports* and *sherries*. This donates a degree of similarity to old *madeiras*. Generally, the sotolon concentration in *sherries* is above 200 $\mu g\,l^{-1}$ but depends of the duration of aging. Thus, there is good correlation between wine age and sotolon content. The presence of sotolon is also dependent on O_2 regimes during aging, having a major impact on 'age perception' in barrel-aged *ports*. Other lactones extracted from oak constitute important odorants in barrel-aged fortified wines. Example includes α-butyrolactone, pantolactone, and *cis*- and *trans*-whisky lactone. Besides the duration of aging, lactone concentration is closely linked to yeast strain. Other compounds derived from precursors extracted by ethanol from the casks (Figure 8) contribute with clove-like spicy notes to fortified wines aroma, notably 4-ethylphenol, 4-ethylguaiacol, and eugenol. The concentration of these compounds increases with aging time.

From a sensorial point of view, using the calculated odor activity values (OAVs) and OT, sensorial differences were observed between the three types of Sherry wines. Fino *sherries* are marked by floral and fruity (e.g., farnesol, β-citronellol, and β-ionone), cheesy and rancid (e.g., butanoic acid), and pungent (e.g., acetaldehyde) notes. Oloroso *sherries* exhibit smoky and ethereal notes associated with 4-ethylguaiacol and ethyl acetate, respectively. Amontillado *sherries* exhibit flavor notes from both aging processes, resulting in the more complex aroma of the three styles analyzed. Acetaldehyde, ethyl acetate, ethyl lactate, β-citronellol, and β-ionone showed intermediate concentrations between fino and oloroso *sherries*, revealing the participation of both aging types. Recently, GC–O has been used to evaluate the evolution of the odor-active compounds in amontillado *sherries* during aging. Twenty-five odor-active compounds changed in during the first few years of oxidative aging. Ethyl octanoate, ethyl butanoate, eugenol, ethyl isobutanoate, and sotolon are the powerful odorants. Most analyzed odorants exhibited increases in concentration during aging, leading to the more intense flavor of the wines.

Phenolic Profile

Phenolic compounds are unstable, with their composition beginning to change as soon as crushing or pressing is complete and continues throughout winemaking and aging. These reactions may involve self-interaction and binding with aroma compounds. The latter can influence aroma release and thereby perception. The different winemaking procedures used in producing fortified wines result in remarkable differences in phenolic composition. Moreover, phenolic concentration increases due to extraction from wood cooperage. Wood type, storage time, and temperature can all influence phenolic structure and composition.

Gallic acid is the predominant phenolic in *madeiras*, followed by *p*-coumaric, caffeic acids, *trans*-resveratrol, and epicatechin. The antioxidant potential and color of the wines increase during *estufagem*, whereas phenolic content decreases, with the exception of caffeic, ferulic, *p*-coumaric, gallic, and syringic acids. Some of these time-related changes in phenolic content have been investigated as a means of verifying the stated ages of *madeiras* and *ports* wines. Particularly valuable in this regard are changes in the structure of anthocyanins. For example, acylation on the sugar moiety appears to decrease steadily with time. The rate of structural changes is also a function of maturation in oak, stainless steel, or glass interaction as well as oxygen exposure.

Changes in the phenolic content of *sherries* also reflect the production style (fino, amontillado, or oloroso) – greater oxidative aging enhancing the phenolic content. In fino *sherries*, phenolic aldehydes were low to almost absent, whereas benzoic acid contents increased. In oloroso *sherries*, benzoic and cinnamic acid contents remained constant, whereas phenolic

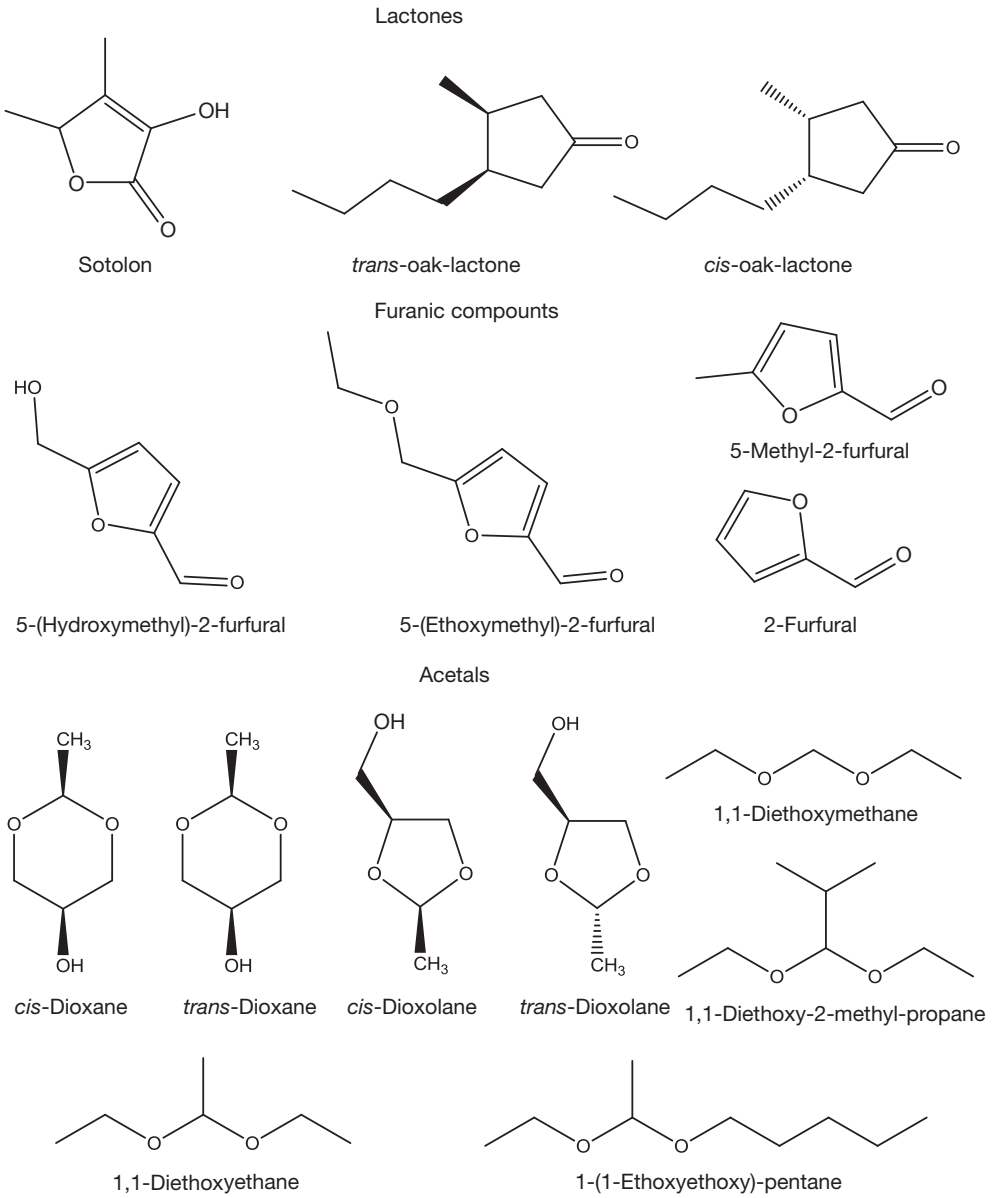

Figure 6 Chemical structure of potential aging markers of fortified wines.

Table 4 Madeira wine aroma notes obtained from different grape varieties, types, and ages

	Madeira wine aroma notes[a]	
Grape variety	Younger wines (3–5 years old)	Older wines (10–40 years old)
Malvasia	Almond, banana, cocoa, floral, orange, tobacco, wood	Almond, caramel, dried fruits, dried orange peel, spice, tobacco, toast, vanilla, wood
Bual	Almond, banana, cocoa, floral, tea	Almond, caramel, dried fruits, spice, tea, toast, wood
Sercial	Honey, mushroom, orange, waxy	Dried fruits, dried orange peel, honey, spice, toast, vanilla, wood
Verdelho	Banana, floral, honey, mushroom, spice	Dried fruits, ethereal, honey, spice, toast, wood
Tinta Negra	Orange, ripe fruit, tea, wood	Caramel, dried fruits, dried orange peel, spice, tea, toast, wood

[a]Information obtained from H. M. Borges Company trained panel.

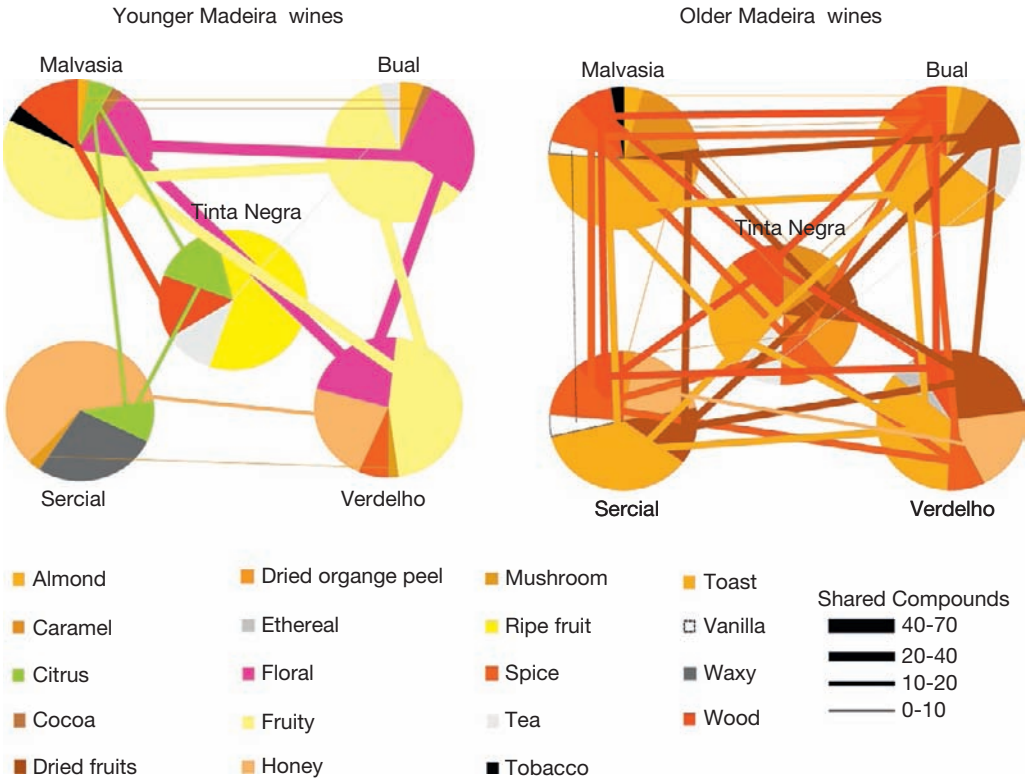

Figure 7 Aroma map network. Each color represents an aroma note, normalized by the mean of OT of chemical group respective, and the width line corresponds to the number of shared aroma compounds.

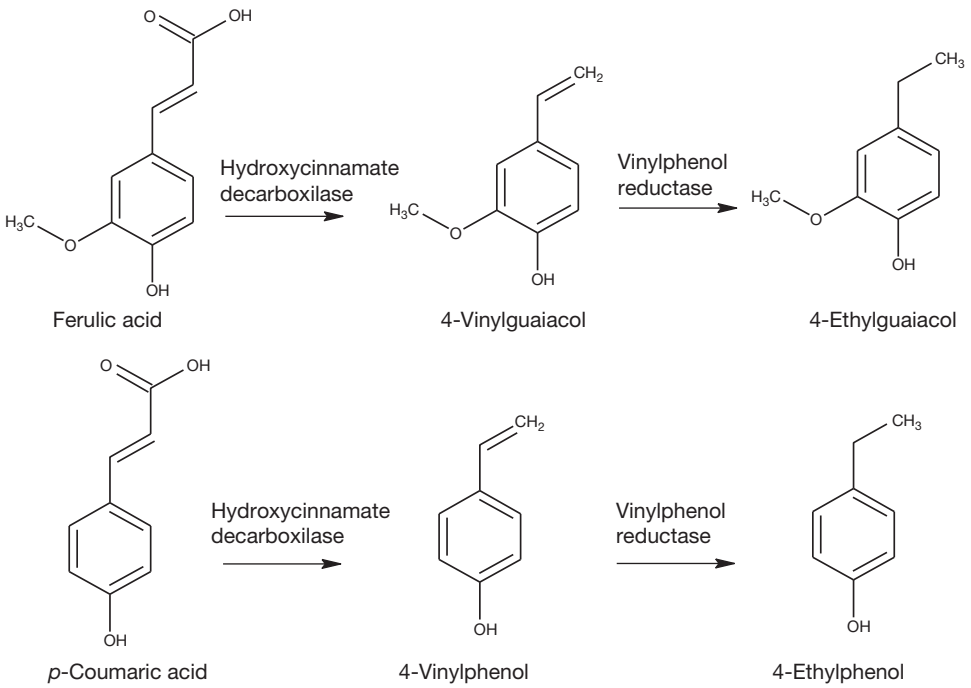

Figure 8 Formation of ethylphenols from hydroxycinnamic precursors by yeasts transformation (e.g., *Brettanomyces* and *Dekkera*).

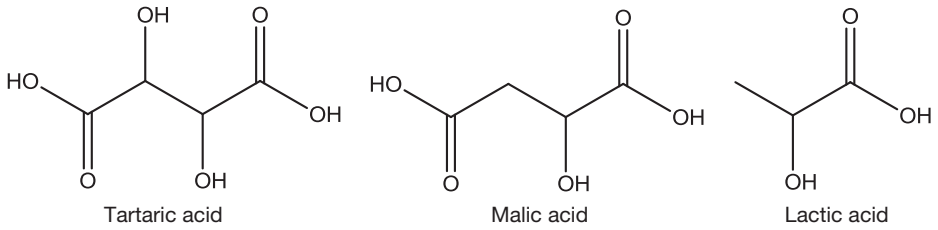

Figure 9 Chemical structure of predominant organic acids in Madeira wines.

acids, such as gallic, syringic, and caffeic acids, changed significantly. Not surprisingly, amontillado *sherries* were characterized by a phenolic composition between fino and oloroso styles. Some of these differences appear to be useful in chemically distinguishing *sherry* styles.

Organic Acids

The organic acid profile provides information relative to fermentation, wine stability, sensory attributes, microbial state, and geographic authenticity. Tartaric, malic, and citric acids (Figure 9) are grape in origin and the most common organic acids in wines, whereas succinic, lactic, and acetic acids result from alcoholic and malolactic fermentations.

In *madeiras*, with the exception of acetic acid, volatile acids usually occur below their odor threshold. In contrast, acetic acid occurs at low concentrations in *sherries*. This results from it being metabolized by *flor* yeast. Glucuronic acid levels can be used as a measure of the amount of rot in harvested grape. Only concentrations below 1 g l^{-1} are considered acceptable for *sherry* production.

Nitrogen-Containing Compounds

Proteins, peptides, and amino acids constitute the must's nitrogen pool. These are important as yeast nutrients, deficiencies often leading to sluggish or stuck fermentations. Occasionally, some amino acids can produce undesirable compounds, such as ethyl carbamate, biogenic amines, ochratoxin A, and carbolines. Factors influencing their occurrence include the grape variety, fertilization, season, ripeness, and enological practices.

The most abundant amino acids in *madeiras* are phenylalanine, arginine, and γ-aminobutyric acid, whereas biogenic amines occur at below their detection limit. This is not surprising as most biogenic amines are a by-product of malolactic fermentation, a process that does not occur during *madeira* production.

Sherry biological aging reduces the amino acid content and other nitrogenous constituents, such as ammonium and urea in the wine. Thus, proline remains the main nitrogen source for *flor* yeast growth during fino maturation. The consumption of several other amino acids is also accelerated during maturation.

Mineral Composition

Assessing mineral content is important as those such as potassium, calcium, iron, and copper can produce precipitates and cloudiness. Maximum contents are also set by the international community due to potential toxic effects. Mineral content can also affect a wine's geographic authentication or negatively influence its organoleptic properties. Content depends on features such as soil, viticulture practices, aging, environmental contamination, and adulteration. The mineral content of *madeiras* is in agreement with most values reported in the literature, except for sodium. Mineral content may also assist in validating a wine's geographic origin.

Only Fe and Mn contents appear to likely influence the browning of fino *sherries*. *Sherries* tend to have significantly higher levels of Ca and Mg ($p < 0.005$) than white and red table wines but lower Cu contents. Ca and Mg contents appear to be the best markers for wine region classification.

Recent Developments

Fortified wines, in particular *madeiras*, *ports*, and *sherries*, are primarily produced using ancient vinification procedures that have been empirically improved over time. Thus, they are not prone to innovation. Correspondingly, modifications to *madeira* production are uncommon. However, a new rosé style of *port* has been introduced, as well as dry versions of white *ports* with a lower alcohol content created. Rosé *port* derives its color from using a short maceration of red grapes and avoiding oxidation during it maturation. These wines are designed to be drunk young, being characterized by red fruits notes (cherry, raspberry, and strawberry). They are soft and pleasant on the palate and should be drunk chilled, with ice, alone, or in a cocktail. Light dry white *port* was developed to fulfill the demand for less alcoholic *ports*. This new variant of white *port* is characterized by a floral and complex aroma and an alcohol content about 16.5%. Finally, in the early 2000s, *sherry* age dating has been permitted to promote the sale of more expensive *sherries*. This initiative started with the *Vinum Optimum Signatum* and *Vinum Optimum Rare Signatum* designations for *sherries* of exceptional quality and aged for 20 or 30 years, respectively. Only amontillado, oloroso, palo cortado, and Pedro Ximénez *sherries* are suitable styles to receive either of these designations. Following the success of these rare sherries, another two age-dated series have been created, 12- and 15-year-old *sherries*. In this instance, only individual *soleras* of a certain age and quality are suitable for this classification. Again, they are not applicable to fino or manzanilla styles. Finally, there is a very rare style, *añada*, reserved for vintage-dated *sherries*. Considering the complexity of *sherries* production, much research has been conducted to improve some technological aspects, namely, those involving the biological aging. *Flor* is a very fragile structure, highly susceptible to

environment and climate conditions. Nonetheless, it has to be maintained on the top of the wine for at least 4 years. It has been proposed that microoxygenation in stainless-steel containers, followed by aging in oak casks under cellar conditions, could shorten the biological aging period without compromising wine quality. Another improvement related to the production of sweet *sherries* that has been already adopted is the use of driers to blow hot air over harvested grapes, thus facilitating rapid water loss. Rapid partial dehydration is crucial in concentrating the grape's sugar content and overcoming problems associated with the unfavorable climate conditions.

Acknowledgments

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Wines: Types of Table Wines

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Classification is a common human trait. Without it, precise communication would be impossible. Unfortunately, the natural desire to group wines into logical categories is fraught with difficulties. Table wines are an eclectic mix of grape-based beverages. Their production reflects local soil and climatic conditions, indigenous grape varieties, technological developments, and cultural and stylistic preferences. To complicate matters further and to distinguish their wines from their neighbors, winemakers are turning to age-old, neglected techniques to provide that *je ne sais quoi*. Consequently, there is little rational or evolutionary affiliation among these diverse products, other than their typical consumption with food. In the absence of a fully useful basis for organizing wines, they have generally been grouped according to simplistic features that tend to be relatively stable and easily detected (e.g., color, effervescence, and alcohol content) or denoted (e.g., provenance or variety). Regrettably, most of these are of limited sensory value.

Despite its limitations, classification based on these features gives consumers the felling that they indicate their sensory attributes. For example, red wines typically are more flavorful, jammy, drier, and astringent than white wines, which generally have milder flavors, are more floral/fruity, and more acidic. White wines may also come in semisweet and sweet styles, something red wines seldom do. Other stylistic variations, not discussed here, include those with an obvious effervescence (see Sparkling Wines) and the various forms of fortified wines (see Fortified Wines). In contrast to table wines, sparkling wines are typically associated with celebration (but can also pair well with food). Fortified wines are typically taken alone, before eating as an aperitif (if dry), or after the meal (if sweet). The mention of a particular grape cultivar or region can suggest, to those possessing sufficient experience, the likely presence of a particular set of flavor and taste attributes. Provenance seldom does this, despite the common belief that it does. This view is one of the rationales for the common organization of wines by country and region. The division of wines into table, sparkling, and fortified categories is also often used by governments – the latter two being taxed more highly.

The problem with any of these empirical groupings is that whatever sensory predictive value they possess depends heavily on experience, a property most consumers lack. Although people can readily distinguish color categories (if in bottled in clear glass) and may recognize some geographic and stylistic names, they seldom have a clear concept of their range of sensory attributes, which can be enormous. Despite the value of a clear, useful, classification of table wines, the diversity of table wines is beyond any fully rational, readily understandable, coherent categorization. Ideally, organization should be based on those features that generate the diversity found in table wines (Figure 1). Although it would be more intellectually satisfying, it still fails to provide the neophyte with data sufficient to knowledgeably direct their purchases.

The most critical element influencing the sensory features of a wine is the winemaker. Although little is noted on wine back-labels or winery publicity, their significance is often considered pivotal to profitability. This significance is partially reflected in the increasing popularity of 'flying winemakers' – those who have developed a reputation for superior winemaking acumen. Winemaker input is so marked that, starting with red grapes, an almost infinite spectrum of wines can be produced – from almost colorless to yellow, pink, light red, and dark red purple, with equivalent variations in flavor intensity and aging potential. Fewer options are available employing white grapes, except where sweet table wines are concerned. In addition, the winemaker may direct production toward sparkling versions of any of the former styles or choose to produce sherry-, port-, or madeira-like wines. Wines may also be distilled to produce brandy. Possibly because the influence of the winemaker can be so marked, and unpredictable, it is typically neglected or submerged under regional or stylistic categories. This is possible because tradition, if not legal constraints, may severely limit winemaker freedom in the style(s) produced.

In Europe, most regions tend to specialize in one or very few wine styles, made from one or a few, typically local, grape varieties. This feature permitted the association of particular flavor traits with regionally produced wines. However, grape cultivars generally generate wine with similar flavor attributes elsewhere. For example, 'Cabernet Sauvignon' and 'Chardonnay' can produce wines equivalent to those produced in their respective homelands, Bordeaux and Burgundy. Sensory attributes are often more dependent of yearly climatic variations, and winemaker decisions, than soil characteristics. Thus, the predictive value of varietal origin depends more on the skill of the winemaker, and the maturity and health of the grapes, than geographic origin. Although winemaker skill is difficult to quantify, annual variations in grape and wine quality are often semiquantified in the now-ubiquitous vintage ranking found in the popular press.

Varietal Origin

Ideally, wine should be classified by the cultivar or cultivars used in its production. Varietal origin sets outer limits on the wine's sensory attributes. Unfortunately, most grape cultivars do not, or possibly more correctly are not known to, possess a characteristic or readily distinguishable varietal aroma. Thus, varietal origin is of significance only for those cultivars that possess, or develop, unique aromatic fragrances in their wines. In addition, its effective application can be severely compromised by the various synonyms under which cultivars may go. This applies not only among countries but also between regions. For example, *Syrah* in France is *Shiraz* in Australia; *Cannonau* in Sardinia is *Garnacha* in Spain and *Grenache*, *Alicante*, or *Carignane* in France; *Zinfandel* in California is

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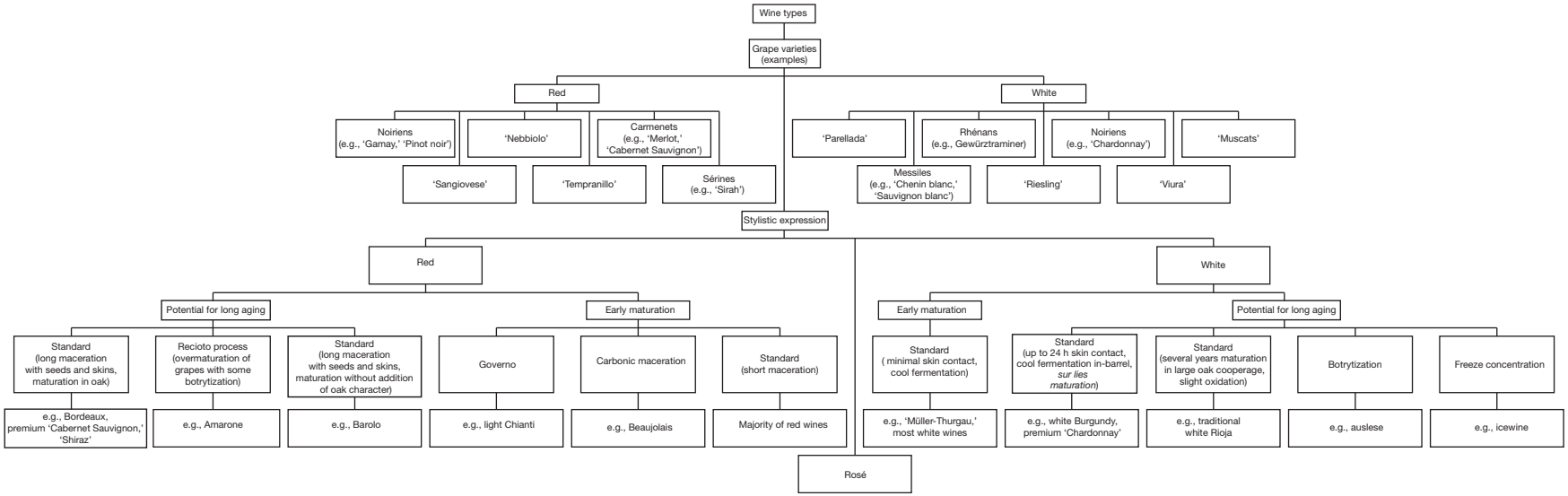

Figure 1 Categorization of table wines based on general sensory attributes, with some common examples.

Primitivo in southern Italy and *Crljenak kastelanski* in Croatia; whereas *Tempranillo* in much of Spain is called *Ull de Llebre* in Catalonia. The issue can be even more confusing when the same varietal name is applied to different cultivars. For example, the *Torrontés* in Argentina is equivalent to none of the cultivars so named in Spain. Finally, many European wines do not designate on the bottle label the cultivar or cultivars used. Furthermore, the blend of varieties used in regions such as Bordeaux and Châteauneuf-du-Pape can vary from year to year, depending on vintage conditions. Historically, varietal designation was not considered necessary because single regions, grew only one or a select few cultivars, produced in a relatively consistent style, and were often the only wines locally available. Labeling has not caught up to the realities of modern commerce. Thus, even with varietal or regional designation, the consumer must have considerable prior experience with these to be of benefit.

Most European wines carry geographic appellations that have become familiar over time. Nonetheless, consistency of style is more an illusion than reality. As noted in the preceding text, traditionally produced regional wines may be derived from a variable number of cultivars. In addition, the proportional mix can change from producer to producer, as well as vary from year to year, depending on vintage conditions and the views of the winemaker. Finally, with the spread and adoption of winemaking techniques around the globe, regional distinctiveness has blurred even further.

Several of the better-known cultivars, with the potential for donating a distinctive varietal character, are briefly noted in the succeeding text.

Red Cultivars

'Cabernet Sauvignon' is the best-known member of the Carmenet family of grape cultivars. Its renown comes from its involvement in most Bordeaux wines (France) and their equivalents elsewhere. Other members of the family include 'Merlot,' 'Malbec,' and 'Cabernet Franc.' Their inclusion in most Bordeaux blends moderates the tannin content donated by 'Cabernet Sauvignon' and adds complexity to the wine's fragrance. The tendency of 'Merlot' to mature more quickly has made it a useful addition and a popular substitute for 'Cabernet Sauvignon' on its own. It appears to be the result of 'Cabernet Franc' × 'Magdeleine Noire des Charentes' parentage. 'Cabernet Sauvignon' itself appears to be the result of a crossing between 'Cabernet Franc' and 'Sauvignon blanc.' Under optimal conditions, 'Cabernet Sauvignon' yields a fragrant wine possessing a blackcurrant aroma. Under less favorable conditions, it donates a marked bell pepper aroma.

'Gamay Noir à jus blanc' is the primary, white-juiced, Gamay cultivar. It is likely a crossing between 'Pinot noir' and 'Gouais blanc.' Its reputation has risen in association with the popularity of Beaujolais wines. Crushed and fermented by standard procedures, it produces a light-red wine with few distinctive aromatic characteristics. However, when the grapes undergo a crude form of carbonic maceration, it yields a distinctively fruity wine that can be drunk a few weeks after its production. Most of its unique fragrance comes from the initial grape-berry fermentation phase of carbonic maceration.

'Nebbiolo' is generally considered to produce the most highly regarded red wine in northwestern Italy. With traditional vinification, it produces a wine high in acidity and tannin content, requiring years to mellow. The color has a tendency to oxidize somewhat early. Common aroma descriptors include tar, violets, and truffles. Its parentage remains unknown but is suspected to have arisen indigenously in Italy's Piedmont region.

'Pinot noir' is the most famous Noirien grape variety. It is particularly environmentally sensitive, producing its unique fragrance (beets, peppermint, or cherries) only occasionally. The cultivar exists as a varied collection of distinctive clones. Usually, the more prostrate, lower-yielding clones produce more flavorful, varietally distinctive wines. The upright, higher-yielding clones are more suited to the production of rosé and sparkling wines. The origin of 'Pinot noir' is still a mystery. Due to its feral vegetative traits, and unusual anthocyanin content, it is presumed to be of ancient origin. It may be a fortuitous selection from local wild *V. vinifera* vines that once grew in the Burgundy.

'Sangiovese' is an ancient cultivar consisting of many distinctive clones, grown extensively throughout central Italy. It is most well known for the light- to full-bodied wines of Chianti, but it also produces many fine red wines elsewhere in Italy. Under optimal conditions, it yields a wine possessing an aroma said to be reminiscent of cherries, violets, and licorice. 'Sangiovese' is also grown under an extensive range of local synonyms, such as 'Brunello' and 'Prugnolo,' used in producing Brunello di Montalcino and Vino Nobile di Montepulciano wines, respectively.

'Shiraz' ('Syrah' in France) has become famous for yielding deep-red flavorful wine, with long aging potential in Australia. This has helped 'Syrah' regain the prominence it once held in the Rhône Valley and has promoted its cultivation in many parts of the world. Its wines have a distinctive peppery flavor, combined with aspects suggestive of violets, raspberries, and currants.

'Tempranillo' is arguably the finest Spanish red cultivar. It appears to be the progeny of two indigenous cultivars 'Albillo Mayor' and 'Benedicto.' Under favorable conditions, it yields a deliciously fragrant wine that ages well. It is the most important red cultivar in Rioja, used either alone or blended with other indigenous cultivars. Outside Spain, it is primarily grown in Argentina. In California, it usually goes under the name 'Valdepeñas.' 'Tempranillo' is considered to generate an aroma distinguished by a complex berry jam fragrance, with nuances of citrus and incense.

'Zinfandel' is extensively grown in California. Its European counterpart in southern Italy is 'Primitivo' and 'Crljenak Kaštelanski' in Croatia. The latter is thought to be its original provenance. Under any name, it is used to produce a wide range of wines, from ports to light blush wines, depending on the country. In rosé versions, it shows a raspberry fragrance, whereas full-bodied, red versions possess rich berry flavors.

White Cultivars

'Chardonnay' is the most famous white member of the Noirien cultivar family. It appears to be the progeny of a cross between 'Pinot noir' and 'Gouais blanc.' Not only does it produce wines with an appealing fruity fragrance, but also it tends to do well

worldwide, not just Burgundy. In addition to producing superb table wines, it is typically found alone, or in a blend, to produce champagne. Under optimal conditions, the wine develops aspects reminiscent of various fruits, notably apple, peach, and melon.

'Müller-Thurgau' is possibly the most well-known modern *V. vinifera* cultivar, being derived from a 'Riesling' and 'Madeleine Royale' crossing. It constitutes about 30% of German hectarage. Its mild acidity, subtle fruity fragrance, and early maturity are ideal for producing light wines in cool climates.

'Muscat blanc' is one of many members of an interrelated, ancient family of cultivars. Their aroma is so distinctive that it is described in terms of itself – Muscaty. Because of the intense flavor, slight bitterness, and tendency to oxidize, Muscat grapes have been used most commonly in producing dessert wines. The reduced bitterness and lower oxidation susceptibility of the modern Muscat cultivar, 'Symphony,' permit the production of dryer wines with better aging potential. 'Moscato bianco' is the primary variety used in the flourishing sparkling wine industry in Asti, Italy.

'Parellada' is a variety distinctive to the Catalonian region of Spain. It produces an aroma that is apple- to citrus-like, occasionally showing hints of licorice or cinnamon.

'Pinot gris' and 'Pinot blanc' are gray and colorless skin mutants, respectively, of the red 'Pinot noir.' They are both cultivated throughout the cooler climatic regions of Europe for the production of dry wines. 'Pinot gris' typically yields subtly fragrant wines with aspects of passion fruit, whereas 'Pinot blanc' is somewhat fruity, supposedly with suggestions of hard cheese.

'Riesling' is without doubt Germany's most highly respected grape variety. It appears to be an ancient cultivar possessing 'Traminer' in its lineage. It produces fresh, aromatic, well-aged wines, which can vary from dry to sweet, depending on grape ripeness and winemaker preferences. Its complex floral aroma, commonly reminiscent of roses and pine, has made it acclaimed throughout central Europe and much of the world. Outside Germany, its largest plantings are in California and Australia.

'Sauvignon blanc' is the main white Carmenet variety in Bordeaux and the principal white cultivar in the upper Loire Valley. It has become popular in California and New Zealand in the recent years. Often, its aroma shows elements of green peppers, as well as a herbaceous aspect, especially in cooler climates. Better clones possess a subtly floral character.

'Traminer' is a distinctively aromatic cultivar grown throughout the cooler regions of Europe and much of the world. Like 'Pinot noir,' it seems to be of ancient origin, being a selection from indigenous, wild grapevines. It consists of a wide variety of clones, the grapes varying from white to rosé colored. 'Traminer' is fermented to produce both dry and sweet styles, depending on regional preferences. Intensely fragrant ('Gewürztraminer') clones are generally distinguished by a lychee fruit aroma.

'Viura' is the main white variety cultivated in Rioja. In cooler areas, it produces a fresh wine possessing a subtle floral aroma with aspects of citron. After extended aging in large wood cooperage, it develops a golden color and rich butterscotch to banana fragrances. The latter characterizes the traditional white wines of Rioja.

Stylistic Origin

Although varietal attributes set the outer limits of a wine's sensory characteristics, its expression is often significantly influenced by enological procedures as well as regional, microclimatic, and annual variations in growing conditions. Thus, the way varietal attributes develop depends on both vine health and grape health and berry maturity, how the grapes are transformed into wine, and how the wine is subsequently matured and aged. Thus, after varietal origin, vintage and production conditions are the most important factors establishing the features that characterize wine. Production-derived attributes are organized in the succeeding text, based on whether these distinctive features developed arise before, during, or after grape fermentation.

Prefermentation Conditions

Carbonic maceration is probably one of the oldest production techniques known. If whole grapes are piled together in sufficient quantity, their respiration soon consumes all the available oxygen, and the fruit shifts to fermentative metabolism. This phase produces little alcohol (~2%) but generates a unique set of aromatic compounds. These grape-derived flavors give the wine a distinct raspberry–kirsch fragrance. They also tend to mask any grape varietal aromas. After berry fermentation ceases (the cells die), the fruit is crushed and yeasts consume the remaining grape sugars. The process produces a wine that is ready to drink a few weeks after production. In contrast, most wines are drinkable, at the earliest, a year or more after fermentation. Because the grape-fermentation odors fade rapidly, the wines often do not age well. This is particularly so with Beaujolais nouveau, where consumption is recommended within 6 months to 1 year post bottling.

Another technique that can fundamentally modify a wine's sensory attributes is grape overmaturation. It generally involves leaving the grapes on the vine for several weeks past normal maturity (late harvest). It often produces more a flavorful but more alcoholic wine. If fermentation does not go to completion, the wine has a sweet finish. Its most classic white versions, the grapes often undergo a 'noble' rot, caused by *Botrytis cinerea*. The infection not only concentrates the juice but also donates a unique apricot, honey-like fragrance to the wine. Examples are the Auslesen of Germany, the Tokaji wines of Hungary, and the Sauternes of France. Alternatively, juice concentration can be achieved by partial dehydration, involving leaving the harvested grapes under the sun on mats or stored on trays under cool, dry conditions. The latter process is commonly referred to as *appassimento*. Another form of juice concentration involves leaving the grapes on the vine until they freeze in late fall or early winter (>-7 to $-8\,^{\circ}C$). The grapes are then harvested and pressed while still frozen (most of the water remaining as ice in the press). The concentrated juice is very high in sugar content. Fermentation typically terminates before little more than half the sugars have been fermented, leaving the ice wine very sweet.

Although more commonly used in the production of white wines, overmaturation is occasionally used in the production of red wines. For example, in the production of Chianti, a portion of the harvest may be set aside to slowly dry and overmature,

while the majority of the crop is processed into wine. A month or so later, the overmatured portion is crushed, begins fermentation, and is added to the previously fermented wine. The effect of the second fermentation is to produce a wine that is fruitier and matures earlier than otherwise. The process is termed the *governo* process. It currently is less popular than in the past, possibly because it does not promote aging potential – the 'gold standard' of quality for many connoisseurs.

Appassimento is also used in Veneto to produce Recioto della Valpolicella Amarone. In this instance, specially selected mature grape clusters are laid thinly on trays stacked in well-ventilated, but unheated, storage sheds. After several months of partially drying, the grapes are crushed and fermented under cool conditions. During the drying process, a portion of the grapes may undergo a slow transformation under the action of *Botrytis cinerea*. This results in a marked increase in the concentration of glycerol and sugars, as well as the partial oxidation of grape phenolics. These appear to donate the distinctive tulip/daffodil pungency that characterizes the best amarones.

Prefermentative Skin Contact

For white wine production, the juice is separated from the seeds and skins immediately upon, to several hours after crushing, and prior to the commencement of fermentation. This limits the extraction of bitter/astringent phenolics from the seeds and skins but equally skin-derived aromatics. The result is a pale-colored to yellowish wine, unless red grapes are used. In the latter case, the wine will often possess a slightly pink to rosé color. For red wines, the juice is left in contact with the seeds and skins throughout much of the fermentation period. The duration of the maceration period with the seeds and skins significantly influences the flavor attributes derived from these parts of the grape. In contrast, skin contact has little effect on flavorants and other constituents found in the juice (e.g., sugars, acids, and non-flavonoid phenolics).

Fermentation Conditions

With red grapes, early (<24 h) separation of the fermenting juice from the seeds and skins yields a rosé. In contrast, for standard red wines, the fermenting juice is left in contact with the seeds and skins (pomace) for about 4–5 days. The duration and method of agitating the mixture during fermentation affect both color depth and stability, as well as tannin uptake. Red (anthocyanin) pigments tend to be extracted first, whereas tannins are dissolved more slowly. Traditionally, red wines designed for long aging have been left on the seeds and skins for more than 2–3 weeks. Although it generated more flavorful wines, it demanded prolonged aging before the high tannin content 'softened.' Modern techniques can now optimize both flavor and pigment uptake, without the extraction of excessive amounts of bitter, astringent tannins. Most varietal flavors are dissolved from the skins within 3–5 days. For grapes low or unstable in pigmentation, the crushed grapes may be given cold maceration prior to fermentation. Other techniques include thermovinification, the addition of the press material (seeds and skins) from additional grapes, or the addition of juice from white grapes possessing phenolics that enhance color stability.

Another condition that fundamentally affects wine characteristics is fermentation temperature. White wines are typically fermented at cooler temperatures than red wines. Cool temperatures (about 15 °C) enhance the production and retention of yeast-synthesized esters, giving the wine a fruity fragrance. This is particularly important for white wines produced from cultivars with little varietal aroma. Wines from aromatically distinctive varieties may be fermented at somewhat higher temperatures, to accentuate their varietal aroma. Red wines are typically fermented at about 22–25 °C. This facilitates yeast growth in the presence of grape phenolics and favors pigment and flavorant extraction.

Other fermentation conditions influence the stylistic expression of a wine, but their use is so variable as to negate their value in characterizing wine types. Examples are the use of different yeast strains, the employment of malolactic fermentation, and the use of in-barrel versus tank fermentation.

Maturation Conditions

Wines designed for early consumption are usually maturated in stainless steel or other inert cooperage. This avoids the expense and uptake of flavorants from the cooperage that could compromise the simpler or mild varietal characters of the wine. In contrast, wines benefitting from extended aging, or possessing a distinct varietal aroma, often merit from being matured for several months to years in oak cooperage prior to bottling. It adds an additional complexity often appreciated in such wines. For white wines, the oak exposure rarely lasts more than 6–12 months. This may or may not be combined with *sur lies* maturation (3–6 months' contact with the dead and dying yeast cells at the end of fermentation). The latter is often considered to enhance the wine's flavor. For red wines, maturation often occurs in new or used oak barrels (~250 l) or some combination thereof. Maturation in large (1000–10 000 l) oak tanks is now uncommon. These supplied less oak character while providing slower, but enhanced, oxidation. Where some oxidation is desired, without the uptake of oak flavors, several microoxidation procedures are available. It is thought, like oak maturation, to encourage earlier wine development (and drinkability).

Although these features can markedly influence the wine's sensory attributes, labels rarely, if ever, mention technical details. The only effect of wine production, typically denoted on the label, is terms relating to coloration (white, rosé, and red).

Geographic Expression

Although it is often implied that the sensory attributes of a wine are clearly impacted by its geographic origin, this is rarely the case. Even professionals often have difficulty identifying the varietal origin of wines, let alone more subtle aspects such as regional origin. Identification is, not surprisingly, more likely when the origin and selection are limited. When wines from a single region, derived from the same cultivar and produced similarly, are tasted together, it may be possible to differentiate regional expressions. However, if the wines come from different countries, it is unlikely that their individual geographic origins will be identified consistently. The critical word here is consistently. Yearly variations in weather

conditions, grape maturity, production decisions, and aging can generate greater fluctuations in sensory attributes than climatic differences among regions.

The prominence given geographic origin in most wine classifications comes from the familiarity that can be developed with their names. Nonetheless, even this can be a stumbling block to the neophyte. Typically, regional names bear no logical interrelationship. For example, Pommard and Pauillac do not obviously suggest that the first is from Burgundy and the second from Bordeaux, whereas Chianti and Valpolicella are noted without any indication of their Tuscan and Veneto provenances. Learning these appellations is often considered a rite of passage to connoisseurship. Because provenance is one of the simplest attributes of a wine, along with the wine's color, both features are predominantly employed in organizing wines. It also facilitates consumer purchases, typically based on geography, color, and price. That their associated sensory features may not be recognizable without the bottle seems unsuspected and immaterial.

Appellation Control Laws

The frequent grouping of wine by geographic origin and the economic value of clear distinction have combined to promote the establishment of AC laws. Although their dictates often vary from country to country, and even from region to region, they act as a certification of geographic origin. As such, they tend to prohibit blending from different regions. They may also regulate permitted varietal composition, conditions of grape culture, and fermentation and wine-processing procedures. Although important to protecting the use of regional names, they also denote cultural views on the origins of wine quality.

In most established European regions, it is often assumed that historically accepted practices and geographic features define and preserve wine quality. However, they tend to petrify production methods, limiting improvement. This is so significant that some producers selectively defy the requirements of the local AC laws. This leaves them free to experiment, often leading to superior wines. Furthermore, weather conditions can be so variable as to nullify any attempt or implication that the law guarantees wine quality. This is particularly so in the absence of effective yearly assessments of wine quality.

In Europe, AC laws fall into two philosophically distinct categories. One group ascribes greater importance to geographic features (e.g., France, Italy, and Spain), whereas the other accentuates the significance of grape maturity (e.g., Germany and Austria).

For example, in the French version, regions are classed according to historic percepts relative to their quality – premium (AOC – appellation d'origine contrôlée), superior (VDQS–vins délimités de qualité supérieure), good (vins de pays), and basic (vins de table). AOC regions are typically subdivided, with progressively smaller zones viewed as producing more distinctive (better) wines than those from larger regions. This subdivision may be taken down to single vineyards. In most cases, though, it goes down only to single parishes or townships. As the area becomes smaller, production regulations often become more precise and restrictive.

In addition to the classification of regions, individual vineyards (or wineries) may be hierarchically categorized. The most well-known example is the 1855 grouping of some Bordeaux vineyards. This arrangement is called the *cru classé* system. As in AC designations, the *cru classé* is based on historic potential to produce fine wine. Thus, yearly sensory assessments are deemed unnecessary.

In Italy, most wine regions are organized hierarchically into DOCG (Denominazione di origine Controllata e Garantita), DOC (*Denominazione di origine Controllata*), IGP (*Indicazione Geografica Tipica*), and Vino da Tavola categories. One of the unique features of the Italian system is its potential pyramidal interconnections. Wine production exceeding the quantities/vineyard surface area stipulated in the regulations is progressively demoted too lower and lower categories. The use of the highest (DOCG) designation requires annual, governmental, and sensory evaluation tests.

In the Germanic AC expression, the greatest emphasis is placed on grape maturity. Thus, it is prominently displayed, along with geographic origin, on the label. Examples are the *Prädikat*, *Spätlese*, *Auslese*, *Beerenauslese*, and *Trockenbeerenauslese* categories. In addition, annual achievement of a minimum score on a governmental sensory evaluation test is required before permission is granted to use any of the QbA (*Qualitätswein bestimmter Anbaugebietes*) or QmP (*Qualitätswein mit Prädikat*) designations. Geographic indications note only the source of the grapes used in producing the wine. Historical reputation has no influence on how the quality or geographic designations are denoted in the German system.

Most New World countries are developing AC regulations. In most instances, they relate primarily to geographic and varietal origin. This results from the diversity of wine styles produced within the designated regions. This achieves the primary purpose of geographic differentiation in the marketplace. Cultivar designation, typically denoted on the label, is there primarily as a guide to the general aromatic characteristics that might be expected. They usually do not require that only the cultivar(s) noted were used. Depending on the region, the minimum proportion of the named cultivars can vary. This flexibility permits the winemaker to blend to some particular flavor profile while still retaining the predominate attributes of the variety or varieties stated on the label.

See also: Alcohol: Properties and Determination; Alcohol: Metabolism and Health Effects; Wines: Wine Production; Wines: Champagne and Sparkling Wines – Production and Effervescence; Wines: Madeira, Port and Sherry Fortified Wines – The *Sui Generis* and Notable Peculiarities. Major Differences and Chemical Patterns.

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Wines: Wine and Health

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Introduction

Wine consumed moderately (considered to be about 100–200 ml day^{-1}) is estimated to marginally extend life expectancy. This effect can be visualized graphically as a J-shaped curve (Figure 1). It illustrates that both abstainers and those consuming alcohol in excess have greater risk of earlier mortality than those consuming wine moderately. Although encouraging, the benefits of reduced morbidity (from a multitude of age-related chronic ailments) are even more heartening, but, regrettably, more difficult to quantify and, thereby, illustrate graphically. In addition, for those who appreciate wine, its sensory attributes and association with food and companionship are as important as, or more important than, its health benefits. Nonetheless, despite the benefits of moderate wine consumption in reducing overall health costs for wine consumers, most governments prefer to consider wine as a taxable luxury and consequently a desirable revenue source.

The positive contribution of moderate wine consumption to both physical and psychological health appears to be independent of factors such as educational level, wealth, food choice, and other environmental and cultural factors. However, correlation does not causation make. Establishing direct links demands a complete understanding of the chain of events from consumption, uptake via the alimentary tract, transport and survival in the blood, incorporation by tissue cells, and the molecular means by which wine-derived products have their beneficial effects. Studying the latter often involves tissue cultures in the laboratory, where the effects of individual chemicals can be more easily assessed. Many potentially beneficial effects, such as protection from the undesirable consequences of oxygen radicals, can often be detected within minutes. Such investigations provide plausible explanations for the observed long-term benefits of moderate wine consumption observed in population studies. Nonetheless, several steps in the sequence from consumption to cellular action still require elucidation. These include demonstrating that the chemicals found in wine, and demonstrated to have beneficial effects at the cellular level in laboratory studies, are absorbed via the intestinal tract and transported to and subsequently incorporated by cells in sufficient quantities to have the effects observed under laboratory conditions. Not uncommonly, physiologically active wine constituents are degraded in the intestinal tract, subsequently modified by plasma, liver, and cytoplasmic enzymes, and rapidly metabolized or eliminated in urine or feces (see Figure 2). All these factors potentially reduce or obviate *in vivo* effects. For example, the antioxidant activities of phenolics are often diminished as the active moiety (OH groups) reacts with other compounds in the body. Examples are reactions with sugars (glycosylation), sulfate (sulfation), methyl (methylation), or acyl (acylation) groups. These conjugation reactions, and other liver-induced changes, facilitate their rapid elimination in the bile and urine. Although glycosylation reduces the antioxidant potential of phenolics, it increases their solubility and, thereby, potential availability for cellular uptake. The presence in the plasma or lymph does not necessarily mean cellular uptake. The latter is often a function of appropriate transport systems in the cell membrane. This is particularly pronounced with access to the brain. The brain is protected from direct exposure to many compounds transported in blood plasma by a special layer of cells lining brain capillaries, termed the blood–brain barrier. It selectively regulates the passage of molecules into the cerebral fluids that surround brain tissue. Nonetheless, small amounts of anthocyanins and several flavonol monomers appear to gain rapid access to brain tissue. This feature may be associated with headache induction in some people, by activating prostaglandin production and its associated dilation of cerebral blood vessels in the meninges, increasing intracranial pressure. Finally, there are subtle, and not-so-subtle, differences in human physiology between individuals and ethnic groups. In addition, differences in intestinal flora influence the structural characteristics and concentrations of wine phenolics that become available for potential uptake in the blood. Thus, as with many medications, individual responses to wine constituents may differ markedly, making any blanket pronouncements on health benefits fraught with potential and unknown risk.

Establishing the dynamics of *in vivo* effects in humans is difficult, not only because of wine's chemical complexity and their various pleiotropic effects but also because of ethical issues surrounding clinical investigations, alcohol consumption, and epidemiological uncertainties. The latter include accuracy in the self-reporting of the quantity, type, consistency, and duration of consumption, as well as the difficulty of differentiating between the effects of wine, dietary factors, and lifestyle, such as exercise over the extended time frame required for such studies. In addition, most ingredients absorbed from the intestinal tract are quickly metabolized or eliminated. Consequently, any effects induced by wine constituents are likely to be of short duration, requiring prolonged and repeated intake to have long-term benefits. Thus, population studies need to be continued for decades. Nonetheless, there is currently sufficient consistency between population and laboratory findings to warrant the tentative conclusion that moderate wine consumption has several lifelong health benefits.

That wine has consistently been shown to have health benefits, in contrast to other alcohol-containing beverages, probably relates to the fortuitous combination of wine's moderate ethanol and relatively high phenolic contents. Both have independent and synergistic health benefits in moderate amounts. In addition, wine is frequently consumed with food. This both prolongs and delays ethanol uptake via the intestinal tract, thereby diminishing its maximal concentration while extending its duration in the plasma and, thereby, maintaining alcohol's beneficial cellular effects. Also, the likelihood of any toxic influences, such as oxygen-radical production, acetaldehyde accumulation, and inebriation associated with a

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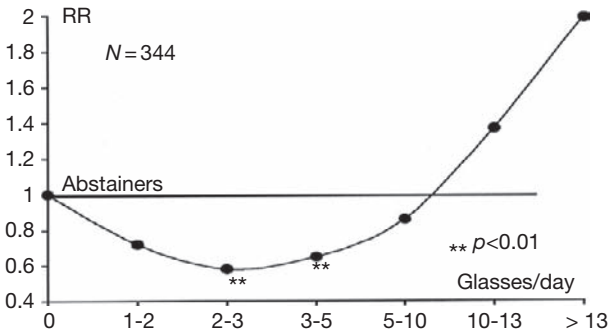

Figure 1 Relative risk (RR) of death from all causes in relation to the intake of wine compared to abstainers, adjusted for age, smoking, body mass index (BAI), and education. Other causes were the sum of the causes of death with the exception of cancer, cardiovascular causes, cirrhosis, and violent death. Reproduced with permission from Renaud, S., Lanzmann-Petithory, D., Gueguen, R., and Conard, P. (2004) Alcohol and mortality from all causes. *Biological Research* 37, 183–187.

rapid and excessive rise in blood-alcohol level, is reduced, if not totally obviated.

During fermentation, yeast action produces ethanol as its main by-product. Although ethanol assists other wine constituents in inactivating pathogenic bacteria and viruses, its most significant benefit derives from enhanced high-density lipoprotein (HDL) concentrations, resulting in reduced low-density lipoprotein (LDL) levels in the blood and vessel walls. Ethanol also reduces platelet aggregation, as well as accelerates clot dissolution. In addition, low levels of blood alcohol have a mild diuretic action, aid calcium retention in the bone, diminish the likelihood of age-related macular degeneration, appear to limit the stomach irritation associated with certain medications, act as a mild muscle relaxant and social activator (by suppressing certain areas in the brain), and may counteract insomnia. In the latter instance, elevated blood-alcohol levels are counterproductive as they provoke early arousal. Other yeast-synthesized alcohols are found in wine, but in very small amounts. Their significance relates only to their involvement in generating important aromatic wine flavorants.

Although wine's ethanol content has health benefits, additional benefits arise from the grape phenolics extracted during fermentation. They are produced as by-products of a complex secondary metabolic pathway in the fruit, commencing with the amino acid phenylalanine. The major branch of the pathway leads to the synthesis of flavonoids, notably flavones (e.g., quercetin), flavan-3-ols (e.g., catechins and their tannin polymers), and pigments (anthocyanins). Although the latter constitute the principal colorants in red wines, they contribute little to the health benefits of wine. Another metabolic branch of the pathway gives rise to stilbenes (e.g., resveratrol). Various flavonoid phenolics have antimicrobial and antioxidant actions, appear to retard platelet aggregation, delay neurodegeneration, possess phytoestrogenic effects, slow the onset of osteoporosis (by increasing Ca^{2+} uptake), and detoxify nitrites in food. Stilbene phenolics also possess antioxidant and anti-inflammatory properties, diminish platelet aggregation, and may reduce arthritic pain.

Although some phenolic-derived benefits are correlated with the presence of large polymers (tannins) in wine, their poor solubility restricts their direct absorption and transport to tissues in the body. Thus, *in vivo* activity of polymeric phenolics is indirect and depends first on their degradation by intestinal and colonic microbial enzymes to their constituent monomers. The uptake of these degradation products into and transport via the blood depends on their respective water and lipid solubilities and any selective transport proteins associated with the colon lining. Nonetheless, some monomeric anthocyanins and nonflavonoids appear to be directly and rapidly absorbed via the stomach. Exposure to stomach acids appears to have little, if any, effect on phenolic structure.

Beneficial Effects

Antimicrobial Activities

Possibly the first health benefit to be associated with wine consumption was its salubrious influence in reducing the incidence of gastrointestinal infections. In Roman times, wine was often added to drinking water before consumption. Although the microbial origin of many diseases was unknown until the middle of the 1800s, the effect of wine in making most water for drinking potable was empirically discovered millennia ago. Not only is wine's alcohol content mildly antimicrobial, but also this effect is enhanced by other wine constituents, notably its phenolic content, its acidity, and several fatty acids. The most beneficial wine phenolics are thankfully those found in the highest amounts – flavonoids and stilbenes. A secondary health benefit arose from wine's solvent properties. The combination of the fat- and water-soluble properties of wine's aqueous ethanol mixture helped solubilize the antimicrobial and other potential medicinal properties of many ancient herbal concoctions.

The antimicrobial effects of wine have been studied relative to their effects occurring before consumption (as in a marinade), during consumption (oral microbiology), shortly after consumption (in the stomach and small intestine), or later on the colon microbial flora and in the blood. Wine's antimicrobial influences have also been investigated relative to selected effects on specific types of bacteria and viruses. Another approach has been to consider how the antimicrobial effects are expressed – by direct inactivation or indirectly by preventing adhesion to cell surfaces and/or the formation of a biofilm, chelating essential nutrients, or by synergy with stomach acids or stimulating gastric acid secretion. Finally, the various effects of individual constituents, or their joint actions, have been investigated.

Due to ethical and economic reasons, most antimicrobial investigations have been time/inactivation studies conducted using solid or liquid bacterial cultures. Only a few investigations approximating *in vivo* conditions have been conducted, usually involving a simple food equivalent at pH levels similar to those found in the stomach. In either situation, wine often reduces the viable population of common food-/water-borne pathogens within several minutes to hours. Used as a marinade, wine's efficacy can be enhanced by combination with certain herbs and spices, notably oregano and thyme.

Although the antimicrobial efficacy of wine (and beer) was once of significance in Europe, improvements in food

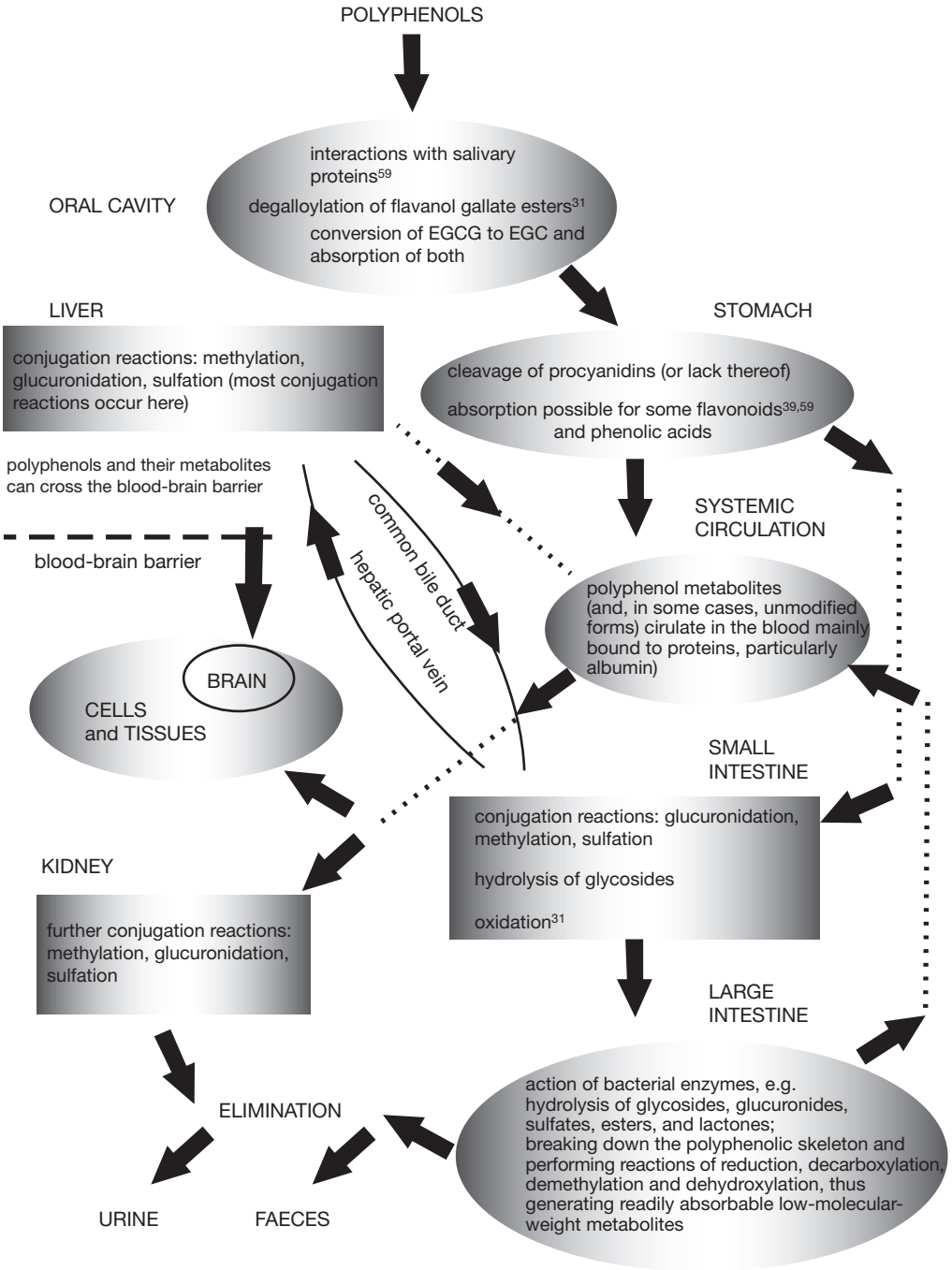

Figure 2 Schematic illustration of the metabolism and absorption of dietary polyphenols in humans. Reproduced with permission from Lewandowska, U., Szewczyk, K., Hrabec, E., Janecka, A., and Gorlach, S. (2013) Overview of metabolism and bioavailability enhancement of polyphenols. *Journal of Agricultural and Food Chemistry* 61, 12183–12199.

handling and water treatment have largely obviated its value. However, wine's antimicrobial action could still be efficacious in many parts of the world, but wine is seldom available, affordable, or permitted where its benefits would be of greatest value.

Because wine remains in the mouth for only a short period, any antimicrobial effect is probably limited to suppressing the adhesion and biofilm-forming potential of bacteria involved in tooth decay, notably *Streptococcus mutans*. However, in the stomach, wine enhances the effects of gastric juices (pH ~1–2) in inactivating many pathogenic bacteria (e.g., *Campylobacter jejuni*, *Helicobacter pylori*, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, and *Vibrio cholerae*). Regrettably, wine appears to be relatively ineffective against toxigenic strains of *E. coli*. While microbial inactivation occurs slowly, the time frame falls within the range that food tends to remain in the stomach. Most studies also indicate that the collective effect of all of a wine's constituents is more effective

than any of its individual constituents. For example, ethanol is one of wine's least effective antibiotic constituents (being most antiseptic at about 72–78% v/v). In addition, wine acids, pH value, phenolics, and fatty acids have been variously shown to have limited individual antibiotic effects, being dependent on the pathogenic agent and location within the gastrointestinal tract. The sulfur dioxide content of wine is too low to have any influence alone. Because wine composition is highly variable, changes during aging, as well as passage through the intestinal tract and presence in the plasma and cytoplasm, it is impossible to provide specific recommendations for particular wine types against individual enteric pathogens.

Far fewer studies have investigated wine's antimicrobial action within the small or large intestine. Nonetheless, wine appears to be capable of inactivating the toxin produced by *Vibrio cholerae*. Other studies have shown wine's potential to reduce the adhesion of enteric pathogens to the colon wall and inactivate *Bacillus cereus*. Wine phenolics are also known to variously aid or limit, depending on the compound, the attachment of beneficial probiotic lactobacilli to the intestinal wall. Studies of wine's activity against parasitic protozoa or as a vermifuge seem not to have been conducted. Even studies on wine's effectiveness against viruses are rare. Nonetheless, epidemiological data suggest a protective influence against viruses inducing the common cold and hepatitis A. Laboratory studies have also demonstrated wines' ability to inactivate several enteric viruses.

Even less is known about wine's effectiveness against internal disease organisms. Resveratrol, other phenolics, and ethanol are known to have anti-inflammatory and other immunologic effects. This could be beneficial in some situations, but detrimental in others. The same applies to wine's antioxidant effects. In most situations, wine's antioxidant action is beneficial, limiting the damage caused by free-oxygen radicals. Nonetheless, oxygen radicals are generated by lymphocytes as part of their arsenal in destroying pathogens. Thus, wine's medicinal properties seem similar to those of more traditional medications – potentially a double-edged sword, depending on the concentration and duration of their physiologically active presence.

Antioxidant Action

The principal antioxidant actions associated with wine consumption relate to its flavonoid and stilbene contents. These influences were first thought to be the principal beneficial effects of moderate wine consumption. Wine phenolics reduce the oxidative stresses associated with certain respiratory and other oxidative reactions. These are unavoidable consequences of normal cellular metabolism. In addition, free-oxygen radicals are produced as a consequence of inflammatory responses, following the return of oxygen to tissues occluded by blood clots (ischemia-associated reperfusion) or during the destruction of pathogens engulfed by phagocytic lymphocytes. Free oxygen radicals generated directly include the superoxide anion [$O_2^{\bullet -}$], the hydroxyl radical [$OH^{\bullet}$], hydrogen peroxide [H_2O_2], and hydrochlorous acid [HOCl] and, indirectly, lipid peroxide radicals [$LOO^{\bullet}$]. Although tissue cells produce several enzymes, notably superoxide dismutase, glutathione peroxidase, and catalase, that rapidly inactivate most oxygen radicals and have access to antioxidants such as glutathione, vitamin C (ascorbic acid), vitamin E (α-tocopherol), and carotenoids, these activities do not permanently protect cells against oxidative damage. Thus, over a normal life span, oxygen radicals slowly disrupt essential macromolecules and organelles, causing impairment in their structure and function. These changes can lead to the development of a range of age-related, chronic degenerative diseases. Wine (and other dietary) phenolics can reduce and/or retard this development by a variety of influences, such as inhibiting enzymes involved in oxygen-radical production, by chelating metals (e.g., iron and copper ions) essential in oxygen-radical production, by directly quenching oxygen radicals (forming more stable, less disruptive phenoxyl radicals), by enhancing the production of endogenous antioxidants such as glutathione, or by functioning as signaling molecules (stimulating the activation of endogenous oxygen stress-related pathways and antioxidant enzymes, such as catalase and superoxide dismutase). As signaling molecules, the trace amounts of phenolics typically found in blood, and their short half-lives, could still have marked beneficial effects. Synergism between the diverse phenolics found in wine, combined with daily consumption (maintaining low but effective levels of phenolics in the blood), could contribute to the observed long-term benefits of moderate wine consumption.

On a shorter term basis, wine phenolics help limit not only LDL peroxidation, an early stage in atherosclerosis, but also undesirable actions of lipoxygenases throughout the body. Particularly active in this regard are the tannin monomers (flavan-3-ols–catechins and epicatechins) and flavonols (such as quercetin). Resveratrol also has similar effects but often occurs at lower concentrations than do many flavonoid phenolics. The respective concentrations of the various types of phenolics in wine vary considerably, depending on the grape cultivar, grape maturity at harvest, the duration of skin contact before or during fermentation, the type of fining, duration of aging, and other factors. It is the popular impression that red wines are more beneficial than white wines, due to their higher flavonoid content. Nonetheless, it is a belief based largely on supposition, not comparative experimental data.

The alcohol content in wine is not itself an antioxidant. Nonetheless, ethanol can induce the production and enhance the concentration of hydroxytyrosol in blood. This influence is apparently more marked than achieved by consuming olive oil (hydroxytyrosol is one of the most important antioxidants in olive oil).

Cardiovascular Issues

The strongest association between alcohol consumption and health is found in data relating deaths due to heart disease (**Figure 3**). Similar correlations have been found with other cardiovascular issues, notably hypertension, peripheral vascular disease, and ischemic stroke. These diverse problems primarily arise from structural/functional changes in the vascular system (atherosclerosis) and an increased risk of thrombosis (blood clot formation). Atherosclerosis appears to develop as a response to chronic inflammation in artery walls. This, in turn, results from the slow oxidation of LDL deposits in vessel walls, inciting the formation of cytotoxins. These provoke immune and smooth muscle cells to accumulate around oxidized LDLs.

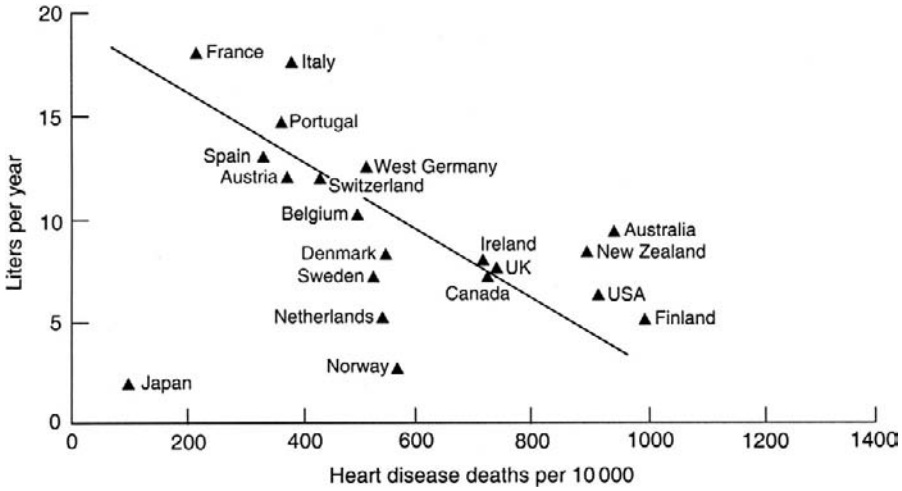

Figure 3 Relationship of per capita alcohol consumption with 1972 heart disease death rates in men aged 55–64 in 20 countries. Reproduced with permission from LaPorte, R. E., Cresanta, J. L. and Kuller, L. H. (1980) The relationship of alcohol consumption to arteriosclerotic heart disease. *Preventive Medicine* **9**, 22–40.

This, in turn, causes the formation and enlargement of swellings, termed plaques. If these bulge out into the vessel lumen, plaques induce progressive vessel occlusion (blockage). Reduced blood flow can aggravate hypertension and peripheral vascular disease. However, whether plaques bulge inward into vessel lumen or outward into surround tissue, if they rupture, thrombosis rapidly occurs, blocking the vessel. Depending on the nature of the size and location of the rupture, it may provoke a heart attack, stroke, or some less serious vascular problem.

Ethanol appears to reduce the incidence of cardiovascular disease by enhancing the production of a particular subclass of HDLs (HDL_3). They favor the removal of cholesterol (deposited within LDLS in vessel walls), thereby limiting cytotoxin production induced by cholesterol oxidation. Alcohol also increases the production of prostacyclin (which directly interferes with clot formation) and a plasminogen activator (a clot-dissolving factor). Moderate alcohol consumption (with meals) also has beneficial effects on blood glucose and insulin levels. The result is the maintenance of lower plasma triglyceride and LDL levels. These are both correlated with reduced cardiovascular risk. Procyanidins (small polymers of flavan-3-ols) also appear to counter cardiovascular problems by suppressing the production of endothelin-1 (a vasodilator) (Figure 4). Endothelin-1 is one of the activators of an early stage in plaque development and the localized movement and accumulation of immune cells in the artery wall. Wine phenolics can also bond with LDLs, limiting oxidation of their lipid constituents. Resveratrol has similar effects. It also favors the production of nitric oxide by endothelial cells (those lining the inner surface of blood vessels), as does chlorogenic acid (a nonflavonoid found in wine). Nitric oxide both induces vasodilation, resulting in reduced blood pressure, and limits platelet adhesion (stickiness) to vessel walls (part of clot formation). Low blood-alcohol contents also reduce the likelihood of thrombosis in a similar fashion.

Phenolics can also reduce some of the severe consequences of infarction size (the area with blocked blood flow), cardiomyocyte apoptosis (heart muscle cell death), and reperfusion following myocardial ischemia (the return of oxygen-laden blood to starved heart tissues) and improve postischemic ventricular performance (the two large chambers that pump blood away from the heart).

Neurodegeneration-Related Diseases

There is much concern about the rapidly rising incidence of Alzheimer's and other neurodegenerative diseases. This partially relates to increasing life span, providing more time for the effects of chronic nerve degeneration to express themselves. Correspondingly, there has been enhanced research into their causes and control options. Because oxidation of cellular structures and associated low-grade inflammation appear to be involved in so many chronic diseases, studies have focussed on the effects of dietary antioxidants and mild inflammation suppressors. In this regard, long-term moderate wine consumption has been correlated with delayed onset and occurrence of Alzheimer's and other forms of dementia. This is consistent with the established effects of phenolics and, to a lesser extent, small amounts of ethanol at a molecular level. For example, ethanol intake is correlated with enhanced omega-3 fatty acid content of the plasma. Omega-3 fatty acid is in turn correlated with improved neurological function as well as red blood cell concentration (better cerebral oxygen supply).

Wine phenolics have also been shown to have specific beneficial effects on what is currently thought to induce Alzheimer's disease. For example, tannins tend to inhibit the formation of and destabilize β-amyloid fibrils, whereas resveratrol promotes the degradation of β-amyloid peptides. Although the known effects are insufficient to induce one to begin to consume wine, its potential in retarding neurodegeneration may be a serendipitous side effect of enjoying wine.

Other Assorted Health Benefits

Among the lagniappes associated with moderate wine consumption is the reduced incidence and severity of osteoporosis

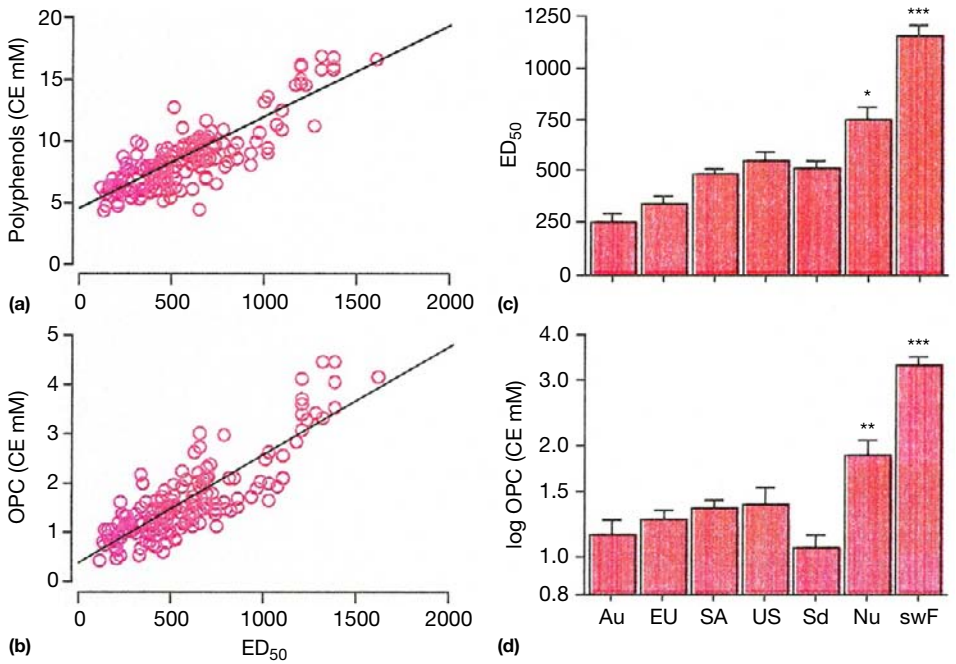

Figure 4 Relationship between procyanidin content and vasoactive properties of red wine. Total polyphenol (a) and oligomeric procyanidin (OPC) (b) content correlate with the inhibition of synthesis of endothelin-1. Comparison of inhibition of endothelin-1 synthesis (c) with OPC concentration (d) of wines from different geographic regions. Au, Australia; EU, France, Greece, Italy, or Spain; SA, South America; US, the United States; Sd, Sardinia; Nu, Nuoro province, Sardinia; swF, southwest France; CE, catechin equivalents. Reproduced with permission from Corder, R., Mullen, W., Khan, N. Q., Marks, S. C., Wood, E. G., Carrier, M. J., and Crozier, A. (2006) Red wine procyanidins and vascular health. *Nature* **444**, 566.

(the slow loss of calcium from bone). How wine has this effect is uncertain, but it may be connected with alcohol's enhancing the uptake and retention of calcium. However, since wine appears to have a greater protective influence than other alcoholic beverages, other wine constituents presumably must be involved.

Alcohol's well-known activity as a mild diuretic may be the origin of wine's correlation with reduced severity of some symptoms of arthritis and decreased incidence of kidney stone development – arthritis being partially connected with retention of fluid in joints and kidney stones with the accumulation of calcium oxalate, presumably due to insufficiently frequent urination. Alcohol's positive influence in retarding glucose uptake and reduced blood-insulin levels may partially explain wine's salubrious effect in diminishing the risk of developing type 2 diabetes. For example, ethanol increases the circulating levels of adiponectin and adipokine, thereby enhancing cell insulin sensitivity. Nonetheless, part of wine's effect may be related to the hypoglycemic effects of selected anthocyanins and the combined hypoglycemic and hypolipidemic effects of resveratrol.

Another J-shaped connection associated with moderate wine consumption is the reduced likelihood of age-related macular degeneration (separation of the retina from the back of the eye). Macular degeneration is correlated with a localized manifestation of atherosclerosis reducing oxygen and nutrient supply to the macula of the eye. A similar interrelationship has been found with cataract development. In contrast, periodic high alcohol intake, and its pro-oxidant influences, probably explains why the benefits associated with wine consumption are related only with long-term, moderate usage.

Ambivalent Effects

Digestion

For most consumers, wine is consumed with food, often being considered its foil. Correspondingly, one might expect wine's association with dining to be universally positive. Although largely so, it may not always be so. Part of the benefit derived with wine's consumption with food relates to its fragrance. It can activate the release of gastrin and gastric juices in the stomach, preparing it for digestion. The succinic acid content of wine also favors gastric juice release in the stomach. Wine also delays gastric emptying, favoring food digestion and its partial microbial sterilization. Delayed gastric emptying appears to be due to phenolics activating STC-1 cells in the stomach lining. These cells release a peptide hormone, cholecystokinin, that reduces gut peristalsis.

The moderate levels of ethanol in wine, as well as wine's acidity and some aromatic constituents, also activate the release of bile from the gall bladder into the small intestine. This facilitates fat emulsification, easing their breakdown, essential for their uptake and metabolism by the body. In contrast, high ethanol contents, typical of fortified spirits, can have the reverse effect, as well as potentially activate unpleasant stomach spasms.

Despite beneficial effects, high tannin levels in wine can interfere with the activity of some digestive enzymes, notably α-amylase, lipase, and trypsin. Tannins can also bind with and structurally modify some food proteins, complicating their digestion. Some protein–tannin polymers are readily reversible in the small intestine, whereas others are relatively stable until they reach the colon. Saliva proteins and some ionic carbohydrates in food tend to limit such polymer formation. In contrast to the tendency of tannins to retard protein digestion in the small intestine, several monomeric tannin components activate pepsin-induced protein digestion. Thus, as with many aspects of wine's association with health, the issue is complicated and will undoubtedly take decades to unravel.

Although wine influences food digestion, food conversely slows gastric emptying and, thereby, the rate at which alcohol enters the blood. About 80% of alcohol consumed is absorbed via the small intestine. Thus, retarding gastric emptying supplies additional time for liver metabolism to keep blood-alcohol content within safe limits. Rates of alcohol metabolism vary from person to person, and between men and women, but generally fall within the range of 90–130 mg $kg^{-1} h^{-1}$.

Cancer

The association between wine consumption and cancer is even more ambivalent than that with digestion, reducing the likelihood of some cancers but increasing the risk of others. This may not be as surprising as one might initially think. Cancer is a complex of syndromes of diverse etiology, linked semantically because they express relatively uncontrolled cell growth and a potential to become metastatic.

The strongest direct link between wine consumption and human cancers is that of the throat and gastrointestinal tract. The association is strongest with more than 'moderate' wine consumption. The other relatively clear link between wine consumption and cancer involves breast cancer. The link seems more marked with possession of the alcohol dehydrogenase gene variant (ADH1C*1). The gene facilitates fast ethanol metabolism, generating a rapid peak in acetaldehyde in the blood (the first by-product of ethanol metabolism in the liver). Acetaldehyde is a known carcinogen, reacting and binding with many proteins and DNA, as well inducing an immune reaction against these adducts. Ethanol by itself appears to not have these effects. Nonetheless, ethanol can enhance the transforming effect of some carcinogens. In contrast, at low concentrations, ethanol can reduce the carcinogenicity of others. Thus, as with so much in science, the issues are far more complex than we might prefer.

Another chemical with carcinogenic potential, once found in wine that had been heated, is ethyl carbamate. However, with its origin well understood, and limits set on its occurrence in wine, its involvement in any possible wine-associated cancer is unlikely.

Some phenolics in wine, normally viewed as beneficial, have been demonstrated to be mildly carcinogenic in standard laboratories mutagenicity tests (mutagenicity is often associated with carcinogenicity). However, as an indicator of the complexities of correlating laboratory results with *in vivo* situations, many of the phenolics associated with mutagenicity are only carcinogenic at concentrations significantly above those found in blood or tissue. Furthermore, quercetin, which has shown anticarcinogenic effects in whole-animal studies (where free-oxygen levels are low), can be carcinogenic at the much higher oxygen concentrations found in laboratory tissue cultures. In addition, most phenolics in the blood are quickly metabolized to glycosides. Glycosidic phenolics tend to lose any carcinogenic tendencies they might have shown as aglycones (not associated with sugar).

On the positive side, wine phenolics can detoxify the traces of nitrites commonly found in some foods. Thus, by reducing the concentration of free nitrites, marinating can reduce the production of carcinogenic heterocyclic amines and diazo phenols potentially generated during smoking and broiling/barbecuing food. Of even greater significance may be the laboratory-demonstrated anticancer effects of several wine phenolics. These include encouraging DNA repair (reversing mutations), direct carcinogen detoxification, enhanced apoptosis (programmed cell death, e.g., cancer cells), disruption of proliferative cell growth, and enhanced immunostimulation (favoring cancer cell recognition and destruction). A specific example is the effect of resveratrol in the redistribution of a cell membrane factor called Fas. It is the attachment site for an immune factor called tumor necrosis factor (TNF). TNF favors the destruction (apoptosis) of cells to which it is attached. Resveratrol is also an inhibitor of angiogenesis – the development of blood vessels essential for the nourishment of cancerous tumor growths. Furthermore, resveratrol has an inhibitory action on cyclooxygenase-2 and P450 1A1. Both appear to play a role in converting several environmental toxicants into carcinogens.

Negative Effects

For those who love wine, it would be pleasing if there were no negative side effects to even moderate wine consumption. Regrettably, for some people, or at times in their lives, even moderate wine consumption is contraindicated or needs to be strictly limited. This especially applies to individuals allergic and intolerant to certain wine additives. The most well-known potential allergen is sulfite, a by-product of the addition of sulfur dioxide to wine. The overzealous use of sulfites in salad bars provoked legislation requiring the indication 'may contain sulfites' on any food or beverage containing more than 10 ppm sulfites. The highest concentration of sulfites in wine occurs shortly after bottling, due to the addition of sulfur dioxide as a protection against wine oxidation and microbial spoilage. This value slowly decreases to undetectable as the wine ages. Sulfur is more commonly added to white wine than red wine because oxidation results in an obvious browning of white wines. Many in the wine industry consider browning to be sales-deterrent. Less obvious, but more significant, are oxidative changes in wine flavor. The antimicrobial action of sulfur dioxide also limits the likelihood of postbottling fermentation that could result in slight bubbling on bottle opening. In addition, it protects against far rarer wine 'diseases' that can provoke visual and flavor faults. While the wine industry is seriously searching for a substitute to sulfur dioxide, none has yet been found with its range of benefits, efficacy, and overall safety record. Nonetheless, the use of sulfur dioxide by the wine industry is considerably less than in the past. Despite

this, and even without the addition of sulfur dioxide, sulfites can accumulate in wine. Sulfite is a metabolic by-product of yeast degradation of sulfur-containing amino acids.

Sulfites in wine are quickly absorbed by blood via the stomach lining. For those sensitive to its presence, it induces contraction of smooth muscle around the bronchus, restricting breathing. The rapidity of the reaction suggests one or more of the following: reduced conversion of sulfites to sulfates by sulfite oxidase in the blood, a malfunction of glutathione *S*-transferase (reducing sulfite to glutathione *S*-sulfonate), and inadequate amounts of glutathione in the lung and bronchial lining. One of the conundrums associated with sulfite hypersensitivity is its episodic occurrence and more frequent connection with red wine. The concentration of sulfur dioxide is consistently lower in red wine, partially due to sulfite's binding with a multitude of wine constituents, but even more because the addition of sulfur dioxide to red wine is less necessary. Red wines, being less sensitive to oxidative browning and microbial spoilage, do not need the protection considered necessary for white wines. Thus, there appear to be additional, unknown factors involved in sulfite sensitivity than just sulfite levels.

The histamine content of wine was once thought to provoke or enhance the severity of allergic reactions. Nonetheless, the histamine content of wines is generally low, and the contents found in wine appear insufficient to provoke histamine-associated allergic reactions. Despite this, the administration of antihistamines has been found to reduce the rhinitis occasionally associated with drinking red wines. Whether this is due to a placebo effect or the administration of antihistamines is unknown.

Some people, more frequently of Asian ethnicity, have an allergy-like reaction to alcohol-containing beverages. It results in rapid facial and neck flushing (cutaneous erythema) and potentially other unnerving symptoms such as elevated heart rate, nausea, abdominal discomfort, and bronchoconstriction. This reaction has been correlated with a malfunctional variant of the gene (*ALDH2*2*) coding for mitochondrial acetaldehyde dehydrogenase (ALDH). The reaction is particularly marked in individuals homozygous for two copies of the malfunctional gene. ALDH is the principal enzyme involved in metabolizing acetaldehyde, the first breakdown product of ethanol metabolism, to acetic acid. Malfunction of one gene, and notably both genes, results in a rapid increase in blood acetaldehyde concentration in association with alcohol intake. It is acetaldehyde that is thought to provoke the diverse set of symptoms noted earlier. Some of the symptoms may also be provoked by the release of cellularly generated histamines by acetaldehyde. These reactions can be partially curtailed by the administration of antihistamines and acetylsalicylic acid (aspirin), prior to the consumption of wine or other alcohol-containing beverages.

Various idiopathic allergic or immunologic sequelae, such as nausea, vomiting, headache, and general malaise, have been reported in the medical literature in relation to wine consumption. In most instances, their cause(s) is unknown. However, in a few instances, they have been linked to the presence of trace amounts of pathogenesis-related proteins (often found in grapes). Other potential sources of allergic-type reactions include egg white or fish proteins (trace amounts may remain after their use in wine fining). Headache sequelae have also occasionally been associated with wine phenolics. In some instances, this can be prevented by taking a prostaglandin-synthesis inhibitor (e.g., acetylsalicylic acid, acetaminophen, or ibuprofen) shortly before wine consumption. This has been hypothesized to be associated with limiting prostaglandin production in the brain, in response to phenols traversing the blood–brain barrier. Prostaglandins can induce blood vessel dilation and thereby pain receptors in the meninges that cover the brain. Sensitivity to this type of wine-induced headache may be related to reduced activity of a plasma-bound enzyme, phenol sulfotransferase. It detoxifies phenols in the blood.

Some people associate one of the most severe headache sequelae, a migraine, with wine consumption, usually red wine. Because histamine and tyramine are often implicated in migraine inception, it has been natural to suspect wine's histamine content as the culprit. Nonetheless, wine histamine contents are usually lower than considered adequate to provoke a migraine attack. In addition, double-blind investigations appear to exonerate wine histamine content as a causal agent, at least by itself. The influence of ethanol in suppressing the action of diamine oxidase, an enzyme that inactivates histamine in the intestinal tract, may contribute to increasing histamine uptake by the blood. Another contributing factor may be the phenolic content of red wines. They are known to prolong the action of hormones and nerve transmitters such as histamine, dopamine, and serotonin. The latter is known to enhance blood vessel dilation. Clearly, more needs to be known before a more definitive answer can be given.

Whether fetal alcohol syndrome (FAS) or its milder version, fetal alcohol spectrum disorder, is implicated with moderate wine consumption is highly controversial. Due to the apparent complex etiology of these syndromes, and the first association of FAS in children born to women of poor diet, who also consumed illicit drugs, were alcoholics, and/or were heavy smokers, any association with moderate wine consumption has been difficult to prove or disprove. Thus, as a precaution, women who wish to become, or are, pregnant are typically counseled to avoid consuming any alcohol-containing beverage during these stages of their life.

Although most people would not associate the simple act of tasting wine with any unpleasant side effects, one of the potential occupational hazards of professional wine tasters is a unique form of dental erosion. It is most frequently expressed as cupping, a depression in the enamel, exposing the underling dentine on molar cusps. In extreme cases, it can lead to severe root abrasion at the gumline. These effects are thought to arise from extended dental contact with wine acids, dissolving calcium essential enamel's hardness. The softened enamel becomes prone to the abrasive action of mastication and tooth cleaning. To avoid this, it is now recommended that food consumption or toothbrushing be delayed for at least one hour after an extended tasting session. This period permits the reincorporation of the lost mineral content, reestablishing the surface enamel's original hardness.

Finally, but not least, there are a set of medical conditions for which wine (or any other alcohol-containing beverage) is contraindicated. Certainly, this applies to anyone with a past history of alcoholism and is a judicious precaution for those with a family history of alcohol abuse. Other situations where abstinence is required involve treatments for ulcers

and cancers of the oral and intestinal tract, liver and kidney diseases, and urogenital infections. Consumption of alcoholic beverages is also ill-advised several days prior to surgery as it reduces clotting time. Alcohol-containing beverages may also increase the severity or incidence of acid reflux, epileptic convulsions, and some consequences of cardiovascular disease, notably hypertension and atrial fibrillation. Even consuming some types of mushrooms with wine or other alcohol-containing beverages can lead to a set of very unsettling symptoms. These can include flushing, sweating, vertigo, nausea, chest pain, palpitations, and tachycardia (rapid heart rate). The mushrooms to avoid are inky cap (*Coprinus atramentarius*) and the lurid bolete (*Boletus luridus*). Similar reactions have occasionally been linked with taking certain antibiotics (e.g., cephalosporins, griseofulvin, chloramphenicol, sulfonylurea, and metronidazole).

Thankfully, the vast majority of wine consumers have none of these aforementioned problems and can enjoy wine without any ill effects (in moderation). In addition, it is pleasing to consider that what gives one sensory pleasure also is in no small measure reducing the risk of several chronic diseases usually associated with aging. Thus, Mark Twain's comment that

> The only way to keep your health is to eat what you don't want, drink what you don't like, and do what you'd rather not.

is not always the case.

Acknowledgment

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See also: Alcohol: Metabolism and Health Effects; Canning: Process of Canning; Wines: Champagne and Sparkling Wines – Production and Effervescence; Wines: Wine Production.

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Wines: Wine Production

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Introduction

The key to the production of good wine begins in the vineyard. This article on wine production is based on the premise that grapes suitable for the intended wine style are available. The style may be commercial or lower price category where harvest yield is more significant than specific flavor development. On the other hand, grapes destined for wines at the top price bracket (<5% of total sales) have well-defined harvest criteria. The harvest yield, unless regulated, tends to reflect the intended wine category. This may range from 30 kg per vine for commercial wines to 0.5 kg per vine for premium wines.

An overview of the production of white wine and red wine following grape receival at the winery is presented. The object is not to provide a recipe, but rather to describe decisions that confront a winemaker during the vintage period. The production steps assume that sulfur dioxide will be used, as its use is still the mainstay in protection of wine from spoilage. Options for minimal sulfur dioxide use and natural wine production are mentioned.

White Wine Production

Figure 1 sets out the general processes for white wine production. There are a large number of options available, many of which relate to the final wine style that can range from delicate and aromatic, to robust and full-bodied, to dry and acidic, to high residual sugar.

Minimizing oxidation and the onset of fermentation from yeasts on the grape skins following harvesting is a challenge faced by all winemakers. Lowering the temperature and the addition of sulfur dioxide are approaches to preserving grape characters.

Grape Processing

The wine world is polarized as to whether grape handling prior to fermentation should be oxidative or reductive. Reductive handling assists in preserving grape aromas and flavors through to the finished wine. The downside of this approach is the retention of phenolic compounds that may be unattractive in more delicate wine styles. Oxidative handling makes no attempt to minimize oxygen access to grape juice. The deliberate addition of oxygen (hyperoxidation) will lead to the loss of phenolic compounds and increase the stability against oxidation in the finished wine. This in turn can lead to the requirement of lower SO_2 levels. Generally, juice oxidation will result in lower varietal aromas.

Destemming and crushing

It is now fairly common practice to remove the stems to minimize uptake of coarse phenolic compounds. The degree of crushing tends to be gentle, reducing the damage to grape skins and seeds with less chance of releasing oxidative enzymes. Modern destemmer/crusher units have considerable flexibility allowing the winemaker to target the operation to the desired wine style.

The must (crushed grapes and juice) should fall from the crusher to a hopper from where it can be pumped direct to the press or draining vessel. In warmer climates, the must may be passed through a heat exchanger to lower the temperature to 10–12 °C.

Draining and pressing

The juice from crushed white grapes flows relatively freely. Larger operations may use a vertical or horizontal drainer. While efficient in terms of juice yield, many drainers lead to high phenolic concentration that requires subsequent removal.

The alternative is to pump the must direct to the press, allowing the free-run juice to be collected first and then increasing the pressure and collecting the pressings until the phenolic content reaches an unacceptable taste level. The free run and pressings may be combined or kept separate.

Press types vary from the traditional basket press to the modern tank press in which the must is totally enclosed in the press tank. The modern presses give higher yields of good quality juice and minimize the juice/air contact, thereby reducing oxidation.

Some robust styles leave the juice from crushing in contact with the skins to extract some flavor and phenolic compounds. Timing of this contact is critical and care must be taken to ensure that fermentation does not commence.

Whole bunch pressing

Grape bunches are added direct to the press and under pressure burst to release juice. There is minimal contact with stalks, skins, and seeds, so the juice obtained by this method is lower in phenolic compounds and grape solids.

Clarification

The extent of juice clarification remains the subject of debate. Relatively clear juice will give a fruitier wine, while fermentation on grape solids can give rise to baggy or wet cardboard aromas. However, highly clarified juice may reduce the amount of nutrients available to the yeast leading to slow or even stuck ferments, whereas the inclusion of some solids may increase flavor complexity.

Clarification has been traditionally achieved by settling and the clear juice then removed from the lees. Poor settling can lead to considerable losses. Larger operations may have access to centrifugation, flotation, rotary drum or earth filtration, and membrane static or cross flow filtration. Pectin from the grape pulp can cause difficulty in clarification, so it is common practice to add pectolytic enzymes as an aid.

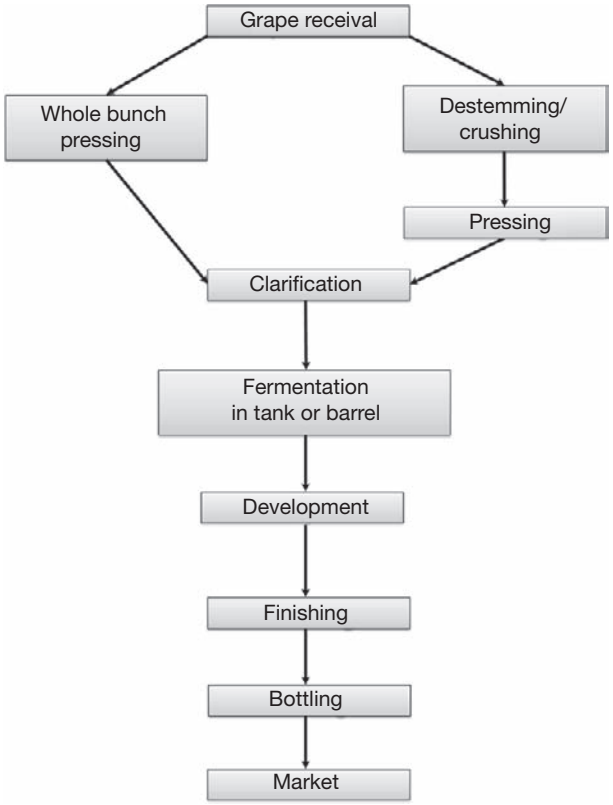

Figure 1 Major steps in white wine production.

Additions to juice

Some countries allow the addition of sugar (chaptalization). The amount of sucrose that can be added is usually regulated.

Acid adjustment may also be required. In warmer climates, tartaric acid may be added to increase the acidity. Ion exchange for pH adjustment is now not permitted in many countries. In colder climates, a high concentration of wine acids, especially malic acid, may require deacidification by the addition of calcium carbonate. Deacidification may be performed on the juice or after fermentation.

Fermentation Strategies

The two main options for fermentation vessels are stainless steel (inox) or oak vessels, although some concrete tanks are still in use. The choice is based on many factors including tradition, cost, efficiency of fermentation control, hygiene, final wine style, and scale of operation.

Temperature control

Temperature control is a critical issue in white wine fermentation as a high temperature can encourage oxidation and microbiological spoilage and the loss of desirable volatile and flavor compounds. Some yeasts will not function above 38 °C. On the other hand, if the fermentation temperature is too cold, the yeast may suffer stress causing the ferment to slow down, or even stop, with the consequent production of acetic acid. Many winemakers would argue for temperature control that allows about 1% alcohol increase per day.

Stainless steel tanks can be surface-cooled with cold water or circulating brine at −5 °C. Insulation can be used on tanks that are outside. Temperature control of barrel ferments is more difficult to achieve and an air-conditioned room is probably the best option. Refrigeration costs may total 50% or more of winery electricity consumption.

Fermentation vessels

Stainless steel tanks are available in various sizes to suit small or large operations. Generally, stainless steel tanks can be used for both fermentation and storage. Cleaning the tanks is a relatively easy task.

Oak barrels used for white wine fermentation tend to be barriques (225–228 l), puncheons (318 l), and hogsheads (238 l). If the purpose is to extract oak aromas and phenolic compounds, new oak barrels are required.

Oxygen

Early in the fermentation, there is sufficient carbon dioxide released to generate a protective cover over the wine. However, toward the end of fermentation, the carbon dioxide cover is less effective and exclusion of oxygen is essential. The use of an air trap is one effective means of stopping oxygen gaining access to the wine.

Yeast selection

There are a range of options available including the following:

- Natural yeasts from the surface of the grape (this is sometimes referred to as 'uninoculated' or 'wild' fermentations)
- A starter culture where a portion of an actively fermenting yeast culture with high cell numbers is added to the juice
- Inoculation with a selected (active) dried yeast after rehydration
- A combination of selected and natural yeasts

There is considerable argument as to the best option, much of which relates to tradition, wine style, and market positioning. The use of selected yeast cultures does allow for a more predictable fermentation.

Fermentation and wine composition

The critical role of the yeast is the conversion of glucose and fructose to ethanol. If insufficient ethanol is produced, the wine may be insipid, whereas a high concentration can lead to a hot taste sensation.

Glycerol is an important fermentation output. It is thought to contribute to mouthfeel, although the sensory aspects remain the subject of debate. There have been attempts to modify wine yeasts to increase glycerol production at the expense of ethanol production. These modified strains are not used for commercial wine production.

Several commercially available yeasts release fermentation esters that add to the aroma of the wine for some months after the fermentation. Other yeasts add little fermentation character and are labeled 'neutral.'

Many aroma compounds originating from the grape exist as nonvolatile glycoconjugates of volatile norisoprenoids, monoterpenes, and other volatile compounds. Commercial enzyme preparations are now available that contain

β-glucosidase activity, as well as α-arabinosidase, α-rhamnosidase, and pectolytic activities, that lead to enhanced wine aroma. As enzyme activity may be inhibited by glucose, they are commonly added toward the end of the fermentation.

Yeast health

Yeasts require nutrients, especially nitrogen, to maintain a healthy fermentation. If the yeast becomes stressed, off-odors can be produced. Winemakers may measure the amount of yeast available nitrogen or the yeast available amino nitrogen prior to commencing a fermentation and may supplement with diammonium phosphate (DAP) or other propriety nitrogen supplements. The release of hydrogen sulfide from yeast catabolism of sulfur-containing amino acids is an argument used to justify nitrogen supplementation.

Development Postfermentation

At the end of the fermentation, the wine is highly susceptible to oxidative spoilage. Sulfur dioxide is commonly added at this point as it has both antioxidant activity and antimicrobial activity. The amount of sulfur dioxide added is dependent on wine style, although 20–30 mg l^{-1} free sulfur dioxide is a common working rule. During this period up to bottling, the wine may be stored in stainless steel tanks or oak barrels or casks.

Malolactic fermentation

Malolactic fermentation (MLF) is an option that some winemakers choose, especially for the more robust wine styles. MLF is the conversion of malic acid to lactic acid leading to a reduction in acidity and the production of aroma and flavor compounds. The wine may be seeded with the required bacteria or use a natural source of the bacteria (uninoculated). MLF may be performed by inoculation during yeast fermentation. It is essential that the wine is checked regularly for the growth of unwanted yeast and bacteria especially oxidative film yeasts that lead to acetaldehyde production. Sulfur dioxide must be absent when the wine is going through MLF, increasing the potential for spoilage.

Time on lees

While it is common practice to rack the wine from its lees soon after the end of the fermentation, there are certain wine styles, notably Chardonnay and Muscadet, where the wine is left on lees for a considerable period. This *sur lie* practice is generally accompanied with stirring (*bâtonnage*) that distributes the yeast lees throughout the barrel and should be performed in full barrels without air ingress.

Finishing

Protein stabilization

The white wine at this stage will have residual protein that may be denatured, if the wine is not stored properly, resulting in brown haze formation. Treatment with bentonite clay is one way to achieve protein stability. When the bentonite with adsorbed protein has settled, the wine is racked off to a clean vessel. This also aids clarification but is slow and can lead to considerable wine losses. The impact on aroma and flavor is the subject of debate and the search for alternatives is continuing.

Tartrate stabilization

A white, generally crystalline, deposit of potassium hydrogen tartrate (KHT) may form in the bottle when the white wine is stored at cool temperatures. KHT stabilization can be achieved by holding the wine in tank at around −4 °C for several days and inducing crystallization by the addition of seed crystals. This process adds to refrigeration costs.

Fining

The wine may be fined to reduce the content of phenolic compounds and to soften the palate. The fining agents may be inorganic compounds (e.g., silica gel) or proteins. The proteins include gelatin, isinglass, egg white (albumin), and casein or skimmed milk, and each reacts with a specific class of phenolic compounds. The use of these fining agents, while successful, does require allergen labeling in many countries.

Final adjustments

Before bottling, the SO_2 concentration should be checked and adjusted if required. Ascorbic acid may also be added as an additional antioxidant. If acid adjustments are made, a further check on tartrate stability should be performed.

Wines of different varieties and years may be blended, although this is regulated in many countries. All checks should be performed on the blend, even if the individual components are stable before passing to bottling.

Red Wine Production

The major steps are summarized in Figure 2. Strategies are designed to extract the color and phenolic compounds from grape skins. Red wine styles range from light-bodied (soft and fruity), to medium-bodied, to full-bodied. Phenolic levels and astringent taste increase through these styles. Rosé wines have minimal color extraction and production tends to be closer to that for white wine.

Grape Processing

Destemming is normally employed as unripe stalks can contain undesirable phenolic compounds. One obvious exception to this approach is in Burgundy where stalks are routinely included.

Roller-type crushers are now routinely used. The rollers may be set to allow some whole berries to pass through or to achieve complete crushing. Older style 'beater' crushers can cause not only skin tearing and seed rupture that may lead to higher extraction of color and phenolic compounds but also the release of undesirable taste components.

Color and Phenolic Compound Extraction

The skins contain the colored anthocyanin compounds. The skins and seeds also contain various phenolic compounds, some of which are beneficial, while others are not.

Color extraction can be considered a combination of breakdown of grape skin cell membranes and diffusion of anthocyanin pigments from skin cells into the must. Cell breakdown

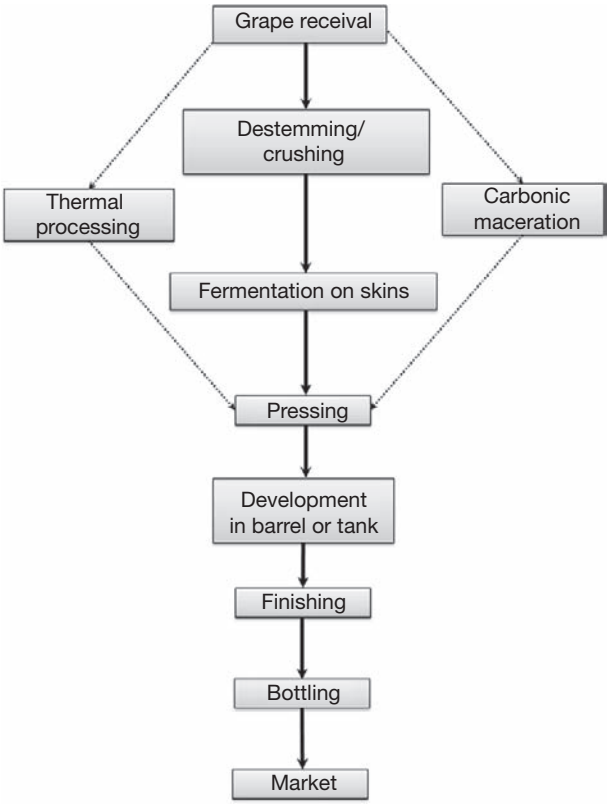

Figure 2 Major steps in red wine production.

can be achieved by exposure to an extracting solution (water and alcohol), higher temperature, and asphyxiation, while diffusion of pigments is best achieved by effective mixing.

There are several ways in which these requirements for color extraction can be met:

- Destemming/crushing followed by fermentation on skins
- Thermal processing of grapes or must
- Asphyxiation of intact berries as in carbonic maceration

Carbonic Maceration

This technique is most closely aligned with Beaujolais, although it is used elsewhere for the production of light-bodied wines. Intact bunches of berries are placed in a closed container and held under carbon dioxide. The berries asphyxiate and an anaerobic fermentation produces about 2% ethanol. This process may continue for about 2 weeks at temperatures up to 32 °C before the liquid is pressed off the skins and seeds and then allowed to ferment to dryness. MLF may follow the alcoholic fermentation.

There are many variations, both regional and personal, in carbonic maceration. The wines show a distinct strawberry or cherry aroma. Generally, the wines do not age well.

Thermal Processing

Extraction of anthocyanins, tannins, and polysaccharides using heat before alcoholic fermentation is useful for the production of lighter, fruitier wines. Two approaches have been adopted:

- Flash heating (flash détente) where ripe and destemmed grapes are rapidly heated to 95 °C and then placed under vacuum causing a breakdown of skin cells
- Thermovinification where berries or crushed grapes are heated to 70–75 °C for around 30 min, followed by pressing and cooling

Clarification by rotary drum vacuum filtration before inoculation is a lengthy and expensive procedure that generates large amounts of waste. Fermentation is normally performed at cooler temperatures. Thermal processing is potentially advantageous for grapes that are low in anthocyanin or are affected by *Botrytis* rot, as the heat removes any enzyme activity that can reduce color.

Fermentation on Skins

Preferment maceration

Before inoculation of the must, a preferment maceration or cold soak may be employed. The concept is to allow aqueous extraction of monomeric anthocyanins that may result in enhanced red color of the finished wine. The must should be kept less than 10 °C to limit the onset of fermentation. Added SO_2 can improve color extraction and minimize the growth of unwanted yeasts.

Yeast inoculation

Yeast selection has as many variables as in white wine production. The need for additional nutrients in a red wine fermentation is usually not an issue as the skins are a source of nitrogen. The measurement of available nitrogen may be performed before inoculation. Elevated temperatures can be an issue leading to hydrogen sulfide production and DAP may be added as a precaution.

Fermentation cap management

Once the fermentation commences, CO_2 evolution coupled with skin buoyancy causes the skins to rise to the surface forming a cap. Good mixing of the cap with the alcoholic fermenting medium is required for effective extraction of skin and seed phenolic compounds. The fermentation rate is faster in the cap, leading to an elevated temperature, and if not regularly mixed, it may dry out with significant production of acetic acid.

Fermenters can be broadly classified as either static or dynamic. Static fermenters can be constructed from stainless steel, oak, or concrete. Manual cap plunging can be achieved with a hand plunger, by foot or by a 'robotic foot.' Heading down boards that keep the cap below the surface can be placed in the ferment once CO_2 evolution has slowed. For larger vessels, the ferment can be pumped over the surface to irrigate the cap. Alternatively, in the *délestage* or rack-and-return approach, the fermenting liquid can be pumped to a separate tank and then pumped back on top of the skins/seeds.

A dynamic rotofermenter is essentially a large drum that mixes by rotation. Construction is expensive, but automation is possible. Fermentation occurs under a CO_2 atmosphere and aeration, if required, is difficult. The wine style obtained from a rotofermenter tends to be less astringent than from a static fermenter. In an autofermenter, pneumatic pistons are placed

in the lid of a tank to plunge the cap automatically. The timing of the piston plunging can be under computer control or activated by pressure from the evolving carbon dioxide.

Fermentation temperature

Temperatures lower than 20 °C retain fruit flavors and are more appropriate for early drinking styles. Temperatures up to 30 °C or even 32 °C result in more robust wines with greater mouthfeel complexity but less obvious fruit character and less yeast-derived fermentation characters.

Aeration during the ferment

The addition of air to the ferment can reduce the formation of H_2S and initiate the formation of polymeric phenolic compounds, which in turn leads to increased color stability.

Pressing the Wine from the Skins and Seeds

The fermentation is commonly allowed to continue until essentially all fermentable sugar is consumed (dryness) or it may be pressed off before completion and allowed to continue to dryness in a separate vessel. This approach may be used when the phenolic compounds being extracted late in the fermentation detract from overall wine quality.

Postferment maceration

Leaving the wine in tank in contact with skins and seeds post the end of the fermentation is a practice that perhaps developed in Bordeaux. The tank must be completely full and sealed during this maceration period. The technique seems to be effective with Cabernet varieties but is usually negative with Pinot Noir. During the maceration, phenolic compound extraction is mainly from the seeds, so it is essential to taste regularly and the maceration stopped if the wine is no longer harmonious.

Pressing options

Most modern-day wineries have elevated fermentation tanks so that the press can be moved into position underneath. When the tank door is opened, the wine, skins, and seeds will flow into the press, aided by a sloping floor in the tank. The traditional basket press may be used in small wineries, although most now use some type of membrane or bag press. The skins and seeds may be collected for further processing or for distillation.

There are significant compositional differences between the free-run wine and the pressed wine with higher color, astringency, and pH in the pressed wine. A membrane press will give about 700 l of wine per ton of grapes processed, while extensive pressing may give an additional 100 or so liters. Tasting determines the appropriate blend of free-run wine and pressed wine. Some of the hard-pressed wine may be blended with other wines that may require additional body.

Development

It is generally advantageous to rack the wine off yeast lees as soon as possible after the end of the fermentation as the lees can adsorb anthocyanins and thereby reduce color. The wine is pumped to a storage vessel for aging before bottling. The storage vessels may be either oak barrels, oak cubes, or stainless steel tanks. More recently, smaller wineries have shown interest in high-density polyethylene tanks.

Sulfur dioxide addition

The addition of SO_2 as soon as practical after racking helps ensure microbiological stability. Caution must be exercised in terms of the amount of SO_2 added as it can bind to anthocyanins, thereby bleaching wine color.

Malolactic fermentation

MLF is a routine operation in red wine production. As the concentration of SO_2 is lower in red wine compared with white wine, the conversion of malic acid to lactic acid removes a major substrate for potential microbiological spoilage.

MLF may be performed prior to or during the alcoholic fermentation, but it is more commonly carried out after the wine is racked off yeast lees. Post-alcoholic fermentation MLF results in an obvious aroma change with a reduction of fruit character and the appearance of diacetyl buttery aroma.

Oenotannin addition

If winemakers regard the wine tannin level as too low, the option to add tannin is a possibility permitted in most countries. There are a range of commercially available products marketed as oenotannins. These include grape tannin, gallotannins, and ellagitannins, the latter two sourced from oak and other plants. The advantages of this approach remain the subject of debate.

Oak treatment

Aroma and palate structural changes result from time in oak. The development of oak aromas requires new barrels, and this introduces a considerable production cost. French and American oak barrels are the most common. More recently, there has been considerable interest in oak sourced, for example, from Hungary or Russia. Oak forest, wood seasoning, and toasting level are some of the factors that impact on the type and amount of compounds that can be extracted from oak.

A cheaper alternative is to use pieces of oak that can be placed in stainless steel or plastic tanks. These include staves, cubes, chips, and powder, the latter three being contained in infusion bags. Wine price point tends to determine the selection of oak material.

Oxygen during development

Contact with oxygen during red wine development can be beneficial for color stabilization. Changes in tannin structure may also occur during this development phase, leading to a softening in palate sensory response. There is a slow permeation of air through the pores between the staves in barrels. Some plastic tanks are now marketed with the claim of defined oxygen permeation rates.

When stored in barrel or plastic tanks, quality control issues include temperature and humidity control, regular topping up, and regular sulfur dioxide checks. If the vessels are not completely full, a film *flor* yeast may grow on the surface and lead to enhanced acetaldehyde concentrations. The so-called 'Brett' character from the growth of *Dekkera* yeasts will result in

off-odors including 4-ethylphenol (band-aid and horse saddle) and 4-ethylguaiacol (smoked bacon).

Micro-oxygenation (MOX) is a means of introducing controlled amounts of oxygen into a wine stored in a stainless steel tank at a rate such that all oxygen is consumed by chemical reactions with wine components. MOX would appear to be effective in smaller tanks, but limited dispersion in large tanks questions its general application.

Clarification

Wine stored in barrel for 12 or more months is often free of particulate and colloidal material and can be directly bottled. In some cases, a settling agent may be added. Egg white is a common choice as it aids settling and softens the palate structure, although its use requires allergen labeling.

Bottling: The Final Step in White and Red Wine Production

There are two major considerations in the bottling process: the stability of the wine and using packaging materials that make the product attractive to consumers in a crowded marketplace.

Microbiological Stability

Sterile filtration is common practice for white, rosé, and lighter red wines but is more difficult to achieve for many red wines that may be bottled without filtration. All equipment used in filling and sealing the bottle should be sterilized before use. Hot water and steam are the best options.

Stability Against Oxidation

SO_2 levels should be adjusted to the desired level before bottling. It is advantageous to check the SO_2 levels for up to 4 weeks after bottling as a significant drop may indicate high potential for oxidative spoilage. Ascorbic acid may also be used in white wines as an antioxidant.

Every attempt should be made to exclude oxygen when filling and sealing the bottle. There is a variety of approaches used to minimize oxygen ingress, including purging with nitrogen or argon or using dry ice to expel air. Stability checks postbottling are always advisable as part of routine quality control.

Bottle Selection

There is a range of bottle colors and shapes. Winemaking issues include the ability to protect wine from light exposure and bottle weight. Clear or lighter-colored glass is preferable from a recycling perspective but offers less protection against light-induced spoilage. Newer lower weight bottles reduce transport costs, thereby lowering the carbon footprint.

Closures

While cork has been the traditional material used for closing the wine in bottle, there are several options available including agglomerated cork, synthetic extruded closure, and screw cap. Market acceptance of alternative closures is a major factor in decision making, as is effectiveness in sealing, potential shelf life, ease of closure removal, and evidence of tampering. The wines stored for protracted periods under screw cap may lead to sulfidic off-odor production. This is the subject of ongoing research.

Once the bottle is filled and sealed, it is washed and labeled. Some wine styles demand bottle aging, whereas others are released almost immediately. It is then time for the marketer to take over.

Summary

Sulfur dioxide has been described as the 'molecular guardian of wine' since it plays a dual role as an antimicrobial agent and as an antioxidant. The allergic reaction suffered by some consumers toward SO_2 has led to a search for alternatives. Ascorbic acid is now widely used as an antioxidant in white wine, although still in combination with SO_2. Glutathione is under investigation as another possible antioxidant. An effective alternative to SO_2's antimicrobial activity has not been found, despite extensive research.

Organic and biodynamic wines relate more to vineyard practices as the grapes, once harvested, tend to be processed by the steps described here. Natural wine is an extension of the organic/biodynamic vineyard practice as it applies to the production of wine with no or only minimal treatments and inputs. In essence, the hand-harvested grapes are fermented without external inoculation and the wine is preferably decanted and bottled without SO_2. The approach presents a major challenge for a successful outcome.

See also: Spoilage: Bacterial Spoilage; Spoilage: Yeast Spoilage of Food and Beverages; Wines: Types of Table Wines; Wines: Champagne and Sparkling Wines – Production and Effervescence; Wines: Wine and Health; Wines: Wine Tasting; Wines: Madeira, Port and Sherry Fortified Wines – The *Sui Generis* and Notable Peculiarities. Major Differences and Chemical Patterns; Yeasts.

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Wines: Wine Tasting

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In the popular mind, wine tasting conjures up images of vintners sampling wine out of barrels in cobwebbed cellars: aficionados clustered around old bottles, intensely swirling and sipping their wine; partygoers jostling one another to get to cheese, crackers, and wine; or some variant thereof. All are real, but the most serious wine tasting occurs in laboratories set up with individual cubicles, each equipped with dentist-style cuspidors, low-intensity lighting, and other features, designed to minimize distractions and maximize concentration, as well as computers for recording and analyzing data. This, the sober side of wine tasting, is typically termed sensory analysis and a far cry from most peoples' perception of wine tasting. Nonetheless, it is the most valuable aspect of tasting, relative to producing fine wines. It permits researchers, grape growers, and winemakers to assess the effects of adjustments to viticultural and/or enological procedures.

Critical sensory analysis is now a fundamental aspect of all university-based training and an integral part of research relating to wine production. In contrast, most consumer-based tasting is designed to enhance the appreciation of the finished product. This is as it should be. Nonetheless, both approaches require a method to detect the finest qualities a wine may possess. Otherwise, wine appreciation can degenerate into snobbism (derived from possession) rather than objective evaluation. Understanding the origin of wines, vintage details, producer renowned, and price can enliven discussion, but focusing attention on important aspect of the wine's relative quality is far more valuable. It is clearly detectable quality that gives (or should) distinguish premium wines from the pack. However, detailed tasting demands more time and concentration than most consumers know or may even be willing to bestow. Consequently, much fine wine is consumed without its full range of attributes ever being detected. For most wine, this may be a good thing – close scrutiny reveals both a wine's best and least desirable attributes.

Detailed assessment is most worthwhile with excellent wines. Regrettably, there are no external indicators that can accurately predict a wine's quality. It only begins to be detectable after being poured. Price, prestige, provenance, producer, and vintage are all inconsistent and, too frequently, poor predictors of wine quality. Interestingly, it is this particular feature (uncertainty) that provides connoisseurs with much of the intrigue that makes wine appealing. Unfortunately, variability also permits many a mediocre, but expensive, wine to masquerade under a premium label. Too often, features such as a poor vintage, bottle-to-bottle variation, or lack of personal acuity are used to explain away nondescript quality in pricey wines. Vinous quality should be obvious, not so subtle as to require an overactive imagination. Tasting should be conducted as a search for the truth – not to justify preconceived ideas or views. Personal perception should not be subjugated to the views of others, regardless of their 'authority,' nor become an act of genuflection to label prestige.

No one tasting approach is appropriate for all contingencies. What is appropriate depends on the situation and intent. For example, what is suitable at the dinner table would be unacceptable for critical appraisal. Detailed analysis of a wine at a meal is inconsistent with, and disruptive to, the principal function of dining with family or friends. However, when the purpose is to compare or analyze wines, every possible sensory attribute should be assessed. For aficionados, these are essential in fairly assessing wine quality, as well as attempting to divine its varietal, stylistic, regional, and aging potential attributes. The latter are often a critical element in connoisseur appreciation. Detailed assessment is also essential to winery and research analyses.

Often, those with little wine experience wonder if all the fuss is necessary or whether they have the sensory acuity to distinguish superior from ordinary wine. Assuming a willingness to focus attention, and the presence of a normal sense of taste and smell, the answer is definitively 'yes.' What cannot be predicted is whether the individual will consider the differences detected worthy of the effort. As in other aspects of life, what one gets out of an activity is usually directly proportional to what one puts in. Experience, habituation, inclination, maturity, and social pressure can all play roles in the evolution of a love for what may initially be disappreciated.

Figure 1 outlines a programmed set of steps designed to focus sequentially on every sensory aspect a wine may possess. It contains aspects that may not be necessary or appropriate under certain circumstances, but the schema provides a palate from which desired attributes can be adequately assessed.

Detailed Tasting Technique

Except when dining, most wine tastings are comparative – that is, several wines are sampled together. This has the advantage of highlighting differences that facilitate the identification of significant features. These include aspects such a varietal composition, wine style, provenance, and age. For this reason, and because all sensory analysis tests are done in a comparative fashion, much of what is described in the succeeding text relates to this situation. Nonetheless, regardless of the situation, the steps involved are identical, except where tasting is only cursory.

Because glass shape subtlety alters the dynamics and concentration of released aromatics, all wines need to be poured into identical vessels. Current practice prefers clear, tulip-shaped, stemmed glasses, with a capacity of about 215 ml. Of those available, the International Standards Organization (ISO) wine tasting glass (Plate 1) is ideal, being specifically designed and assessed for the purpose. The incurved sides facilitate swirling, promoting the release of volatiles, as well as their retention within the bowl. Thin lead crystal versions add at touch of class, but are unnecessary. Less expensive, noncrystal versions, or slight variations in design, are more

readily available and more dishwasher-friendly. An example is the Libbey Citation 8470. The only exception involves sparkling wines. In this instance, elongated, flute-shaped glasses are typical. They assist in recording details of the effervescent and mousse attributes of the wine.

Due to the influence of heat and volume on volatilization, all samples should be presented at the same temperature and in equivalent amounts. Volumes of about 30–50 ml are usually adequate. This permits both vigorous swirling and angling of the glass to assess the wine's countenance.

Each sample should be poured into identical, clear, tulip-shaped, wine glasses. They should each filled (1/4 to 1/3 full) with the same volume of wine.

I. Appearance

1- View each sample at a 30° to 45° angle against a bright, white background.
2- Record separately the wine's:
clarity (absence of haze)
color hue (shade or tint) and depth (intensity or amount of pigment)
viscosity (resistance to flow)
effervescence (notably sparkling wines)

II. Odor "in-glass"

1- Sniff each sample at the mouth of the glass before swirling.
2- Study and record the nature and intensity of the fragrance (see Fig 1.3 and 1.4)
3- Swirl the glass to promote the release of aromatic constituents from the wine.
4- Smell the wine, initially at the mouth and then deeper in the bowl.
5- Study and record the nature and intensity of fragrance.
6- Proceed to other samples.
7- Progress to tasting the wines (III)

III. "In-mouth" sensations

(a) Taste and mouth-feel

1- Take a small (6 to 10 ml) sample into the mouth.
2- Move the wine in the mouth to coat all surfaces of tongue, cheeks and palate.
3- For the various taste sensations (sweet, acid, bitter) note where they are perceived, when first detected, how long they last, and how they change in perception and intensity.
4- Concentrate on the tactile (mouth-feel) sensations of astringency, prickling, body, temperature, and "heat".
5- Record these perceptions and how they combine with one another.

(b) Odor

1- Note the fragrance of the wine at the warmer temperatures of the mouth.
2- Aspirate the wine by drawing air through the wine to enhance the release of its aromatic constituents.
3- Concentrate on the nature, development and duration of the fragrance. Note and record any differences between the "in-mouth" and "in-glass" aspects of the fragrance.

(c) Aftersmell

1- Draw air into the lungs that has been aspirated through the wine for 15 to 30 s.
2- Swallow the wine (or spit into a cuspidor).
3- Breath out the warmed vapors through the nose.
4- Any odor detected in this manner is termed aftersmell; it is usually found only in the finest or most aromatic wines.

[a] Although fragrance is technically divided into the aroma (derived from the grapes) and bouquet (derived from fermentation, processing and aging), descriptive terms are more informative.

Figure 1—cont'd

IV. Finish

1- Concentrate on the olfactory and gustatory sensations that linger in the mouth.
2- Compare these sensations with those previously detected.
3- Note their character and duration.

V. Repetition of assessment

1- Reevaluate of aromatic and sapid sensations of the wines, beginning at II.3– ideally several times over a period of 30 min.
2- Study the duration and development (change in intensity and quality) of each sample.

Finally, make an overall assessment of the pleasurableness, complexity, subtlety, elegance, power, balance, and memorableness of the wine. With experience, you can being to make evaluations of its *potential*– the likelihood of the wine improving in its character with additional aging.

Figure 1 Parts 1 and 2. Wine tasting sequence. Reproduced from Jackson, R. S. (2009). Wine tasting: a professional handbook (2nd ed.). London: Academic Press. Copyright Elsevier, with permission.

Plate 1 ISO International wine tasting glass: Royal Leerdam Wine Taster #9309RL – 229 ml, 7¾ oz. Photo courtesy Libbey Inc., Toledo, OH.

The palate should be cleansed between samples, to avoid residual effects influencing the perception of those subsequently assessed. Current data suggest that a 1% pectin solution is an effective cleanser. Unsalted crackers are a more common, but less ideal palate cleanser but far superior to water. Water is ineffective at removing residual tannic constituents and dilutes the saliva. Cheese should be avoided. It tends to suppress or mask several wine sensations. This effect, characteristic of food in general, is of value only if it is specifically desired. For example, the saltiness of cheese, or the proteins in food, mollifies both bitter and astringent attributes, respectively.

Appearance

The principal features of significance are color and clarity. Of these, color not only is the most obvious but also donates the wine's first esthetic pleasure. Young red wines are characterized by a rich, vivacious countenance, older versions progressively take on subtler brickish shades. White wine, if sweet, often possesses a golden opulence – it being a harbinger of the sapid pleasures to follow. Dry whites, depending on the cultivar, maturity, and duration of skin contact, vary from pale straw to yellow. With age, white wines darken in color, whereas reds lose color depth. Aberrant colors for age, cultivar, or style often foretell the presence of inappropriate fragrances or off-odors. Despite this, such clues should be taken with caution, to avoid unfairly prejudicing subsequent perceptions. Where color bias must be avoided (e.g., when sampling wines of different vintages or cultivars), sampling is often conducted under dim red lights (to disrupt color perception) or tasted in black glasses.

For color assessment, the glass is typically tilted at about a 30–45° angle and viewed against a bright, white background. This permits viewing the hue through varying wine depths. This is of particular value when sampling red wines. They typically show a clear rim and a purplish cast when young. The first sign of aging (or undesirable premature oxidation) appears as a brickish coloration at the rim.

Clarity assessment is also facilitated by tilting. With modern clarification and fining techniques, most wines remain perfectly clear. Nonetheless, some old red wines may develop sediment (precipitated tannins and tartrate salts). These may be resuspended during transport from storage or pouring, generating a haze and bitterish/clayey taste. This can be avoided by due caution during the transfer of old wine from a cellar and judicious decanting just prior to sampling.

Only wines possessing very high sugar, glycerol, and/or alcohol contents may show slight modifications in perceptible viscosity. Thus, this attribute is rarely of sensory or diagnostic value. The only significant exception involves a bacterial spoilage termed ropey.

Tears (legs) are another visual attribute of trivial sensory significance. They form following swirling, as a thin layer of wine adheres to the sides of the glass. The relatively greater volatilization of alcohol results in a progressive increase of the surface tension in the wine film. This, in turn, leads to the

formation of droplets ('tears'). As the droplets enlarge, gravity initiates their decent – *ipso facto*, the formation of 'legs.'

Occasionally, bubbles may develop along the bottom and sides of the glass. This typically results when the wine is bottled before the excess carbon dioxide, dissolved in a newly fermented wine, has had time to escape. Continuous bubble formation is a characteristic only of sparkling wines. In the latter instance, the size, number, and duration of effervescence, and the formation of a ring of bubbles next to the glass (*cordon de mousse*), are considered important and essential quality attributes.

Although a wine's visual features can act as harbingers of other sensory attributes to follow, it is usually more useful for consumers to concentrate on the pleasure so derived.

Aroma and Bouquet

Traditionally, two aromatic attributes have been distinguished – aroma and bouquet. Aroma is differentiated as being derived from the grape, whereas bouquet originates from fermentation or aging processes. While seemingly distinct, the compounds generating each may be identical. Correspondingly, the current trend is to avoid this distinction, choosing a more general term – fragrance.

Assessing a wine's fragrance involves two distinct aspects. The first assesses the fragrance orthonasally (in the glass) and later retronasally (in the mouth). Although essentially assessing the same compounds, their qualitative perceptions can differ markedly. Aromatics appear to be detected (and remembered) as temporal impulse patterns. These arise as aromatic pass over the olfactory patches in the nose. Because the receptors, responding to different aromatics, appear to be arranged in a more or less linear pattern along the olfactory patches, detection is sequential, similar to isolation via gas chromatography. Because the direction of flow is reversed when detection occurs via the back of the throat (retronasally), a different time-sequence pattern is generated. It may be the equivalent of playing a series of notes in reverse order. Depending on the number of different olfactory receptors involved, and their position, the time-sequence pattern can be markedly different. A classic example is the sensory difference between the smell and in-mouth response to Limburger cheese or durian fruit. In addition, the concentration of aromatic compounds entering the nose via the back of the throat is usually less than that received via the nostrils (affecting threshold-sensitive reactions). Selective absorption and enzymatic modification in the mouth can also modify the aromatic composition detected retronasally. The volatile dynamics of the wine's aromatics may be also affected by the temperature difference between wine in the glass and in the mouth.

To facilitate the escape of aromatics, the wine is swirled in the glass. This increases the surface area over which volatilization can occur, favoring concentration in the headspace above the wine. This is far more effective than the now-popular wine 'aerators.' Repeated swirling also creates turbulence that refreshes the concentration of aromatics at the surface of the wine (where volatilization occurs). For vigorous swirling, glasses should be no more than ⅓ full. The inward curve of tulip-shaped wine glasses also facilitates vigorous swirling and the retention of aromatic compounds in the bowl.

It is traditional to initiate fragrance analysis at the rim followed by in the bowl. This permits sensation of the aromatics at different concentrations, with only the most volatile/low-threshold members being initially detected at the rim. This may result in the generation of distinct sensations. Where the fragrance is mild, some professionals place a watch glass over the mouth of the glass. This permits even more vigorous swirling while trapping the aromatics within the bowl. Small petri dish covers or coffee cup tops can be used similarly where of an appropriate size.

Studies have shown that no particular technique need be learned for adequately assessing wine fragrance. Whiffs of about a second duration, separated by 20–30 s, appear fully adequate. This interval relates to the time required for olfactory receptors to regain their intrinsic sensitivity. Nonetheless, different sniffing procedures do seem to affect the qualitative perception of odors. For example, extended inhalation periods can facilitate the analysis of complexly aromatic wines, such as ports. As particular olfactory receptors adapt and no longer respond, the odorant pattern transmitted by those still being detected may generate different qualitative perceptions. This can activate a kaleidoscope of olfactory memories.

Professional tasters are usually trained in odor recognition, using samples specifically designed for the purpose. Reference samples are typically available during tastings. Precise and consistent term use is critical where panels of assessors are used to define and measure the differences among wines. Fragrance and off-odor charts may also be supplied, but these are more commonly used in wine appreciation courses or by wine tasting clubs. Although common, it is more valuable for consumers to concentrate on memorizing the holistic perceptions that distinguish particular wines than to focus on individual aromatic resemblances. This is akin to the greater value of recognizing faces than separately doting on individual facial features. In addition, overattention on designating aromatic descriptors can detract from the actual appreciation of the wine. Focusing on odor descriptors is best left to teaching people to take due notice of the most distinctive and variable attributes of a wine – its fragrance. This is a prerequisite to memorizing the more useful, distinctive odor patterns of different grape varieties, wine styles, aging attributes, and potential regional characteristics.

Both positive and negative impressions should be recorded. Figure 2 provides an example of a table designed to focus attention on a range of potential attributes. Simpler and more appropriate for consumers is graphic representation, such as illustrated in Figure 3. It has the advantage of being quick, succinct, and effective. In addition, it clearly represents the fleeting changes in flavor intensity and character that distinguish fine wines.

One of the principal values of recording impressions is the concentration it demands, leading potentially to enhanced appreciation. It facilitates detecting features that otherwise might be missed. It also encourages the development of a wine lexicon. Except where derived during training, the terms most people use strongly reflect individual familial, cultural, and geographic upbringing. That such a language may not be as precise, consistent, or even appropriate, as required for scientific investigation, is not a serious drawback for the consumer. Its primary purpose is for recording personal impressions and

Sample Number: ___	Wine Category: ___	Exceptional	Very Good	Above Average	Average	Below Average	Poor	Faulty	Comments
Visual	Clarity								
Odor (orthonasal)	Intensity*								
	Duration **								
	Quality***								
Flavor (taste, mouth-feel, retronasal odor)	Intensity								
	Duration								
	Quality								
Finish (after-taste and lingering flavor)	Duration								
	Quality								
Conclusion									

* *Intensity:* the perceived relative strength of sensation- too weak or too strong are equally undesirable.
** *Duration:* the interval over which the wine develops or maintains its sensory impact; long duration is usually a positive feature if not too intense.
*** *Quality:* the degree to which the feature reflects appropriate and desirable varietal, regional or stylistic features of the wine, plus the pleasure these features give the taster.

Figure 2 Wine tasting sheet for quality assessment. Reproduced from Jackson, R. S. (2009). Wine tasting: a professional handbook (2nd ed.). London: Academic Press. Copyright Elsevier, with permission.

focusing attention on the wine's attributes. However, using any olfactory vocabulary, both consistently and accurately, is difficult. Most people have great difficulty even correctly naming familiar odors in the absence of visual clues. Only pronounced odors, such as the smell of fried bacon, toast, or lilacs, are sufficiently intense as to develop an odor memory independent of visual or other clues. The problem of naming most recognizable odors has been aptly dubbed the 'tip-of-the-nose' phenomenon. This probably reflects the dominance of visual information in human sensory perception.

Recognizing the varietal, stylistic, age, or providence attributes of wines typically demands considerable concentration, as well as extensive experience. It often requires repeated sampling, employing both inductive and deductive memory searches. Features, both present and absent, are often required. Nonetheless, as the primary source of a wine's unique character, the study of fragrance warrants the intense scrutiny it demands.

Oral Assessment

After an initial assessing of the wine's orthonasal fragrance several times, attention begins to turn to in-mouth sensations. Small amounts of wine are sampled and rolled around in the mouth to contact all its surfaces. As far as feasible, the volume of each sample should be kept identical. This favors equivalent comparison of the samples.

Technically, taste is limited to sweetness, sourness, saltiness, savoriness (umami), and bitterness. Of these, only sweetness, sourness, and bitterness have relevance to wine tasting. The other major in-mouth sensation is astringency. It is considered a touch rather than a taste, due to its being detected by free-nerve endings of the trigeminal nerve rather by specific receptors localized largely within taste buds on the tongue.

Because many consumers fail to concentrate on the wine's fragrance, taste and touch sensations tend to be more significant to them than wine aficionados and judges. When specifically assessed, the distinctive temporal dynamics and relative location of each sensation help distinguish their relative intensities.

The in-mouth sensation most rapidly detected is sweetness. If present, sensitivity is most noticeable at the tip of the tongue. Occasionally, the illusion of sweetness is detected in dry, but fragrant, white wines. This results from previously learned associations between certain odorants and sweetness. This induces the brain to 'perceive' sweetness, even though the

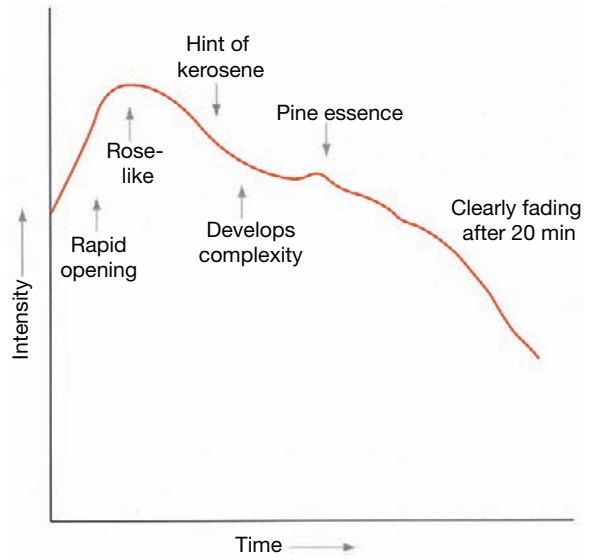

Figure 3 Graphic representation of the development of a wine's fragrance and its relative intensity. Specific observations can be written on the graph to represent when it was detected. Reproduced from Jackson, R. S. (2009). Wine tasting: a professional handbook (2nd ed.). London: Academic Press. Copyright Elsevier, with permission.

wine is dry (the illusion of sweetness disappears by pinching the nose).

In the absence of detectable sweetness, the first taste perception detected is sourness. This is frequently most obvious along the sides of the tongue and cheeks. When marked, it causes a puckery sensation as well as irritation in the mouth.

Detection of bitterness tends to require several seconds to develop. Except for some alkaloids found in vermouths, most wine bitterness is detected primarily at the back of the tongue. Alkaloids are often perceived at the tip of the tongue. Depending on an individual's personal sensitivity, it can take more than 15 s before the intensity of bitterness reaches its peak.

The last, prominent, in-mouth sensation, notably in red wines, is the tactile perception of astringency. Like bitterness, it is induced primarily by wine tannins. Due to their extreme variability in structure and size, they can activate a wide variety of mouthfeel sensations. These can range from dry, dust-in-the-mouth, puckery to velvety sensations. The response to astringency, similar to bitterness, is relatively slow to express itself, often taking 15 s or more to reach its maximum. The fading of the sensation is equal to, or slower than, its development. Unlike bitterness, it is detected throughout the mouth.

Other potentially detected in-mouth sensations include the burning sensation of alcohol (notably concentrations above 14%), the prickling aspect of carbon dioxide (sparkling wines), and temperature. Like all mouthfeel sensations (involving trigeminal nerves), they are sensed throughout the mouth.

Depending on the intention of the tasting, it may be worth noting differences between the first and subsequent sips. Tannins and acids react with salivary and membrane-bound proteins in the mouth, initially diminishing their respective bitter/astringent and sour/astringent sensations. Thus, the initial sample often appears smoother and more balanced than subsequent samplings. The first sample approximates the perception generated when wine is taken with food – proteins reacting with tannins and acids, reducing their initial intensity. If assessing how a wine might balance with food, sampling should progress slowly. This provides time for activated saliva production to compensate for its dilution during tasting. Cleansing the palate between samples reduces tannin and acid residual effects that can distort perception.

Professional tasters and aficionados often aspirate the wine to enhance retronasal odor detection. This involves closing the mouth while leaving the lips slightly ajar or pursing the lips and slowly drawing air through the wine. This has an effect similar to swirling wine in the glass, enhancing odorant release. The volatilized compounds migrate up into the nasal passages, especially during swallowing and breathing out. Slow, deliberate exhalation apparently improves retronasal detection, possibly only due to conscious focusing on the process. The olfactory sensations so induced, combined with gustatory sensations, generate the multimodal sensation termed flavor. Flavor detection, following swallowing or expectoration, is termed the wine's finish. Its duration is considered a quality factor. The importance of retronasal olfaction to flavor detection is immediately apparent by clamping the nose.

Fruity–floral essences, associated with refreshing acidity, epitomize most better white wines, whereas complex berry/jammy fragrances, combined with flavorful, velvety tannins, exemplify the best red wines. Fortified wines, possessing more intense flavors, have very long finishes. These attributes may be supplemented with, or enhanced by, oak-derived flavors. The nature of their contribution depends on the duration of contact, the type of oak used (European vs. American), and the degree of toasting during barrel construction. Additional flavor notes may be derived from malolactic fermentation and *sur lies* maturation. With aging, the initial varietal/fermentation flavors tend to fade, hopefully being replaced by aged fragrances. These tend to be relatively similar, regardless of the wine, at least for red wines.

Although significant in some critical tastings, noting a wine's individual taste/mouthfeel attributes is less important than concentrating on how they integrate to produce holistic perceptions, such as flavor, complexity, balance, and body. The duration and development in flavor over the course of the tasting are highly desired features.

When wines are assessed critically, expectoration is the norm. This helps minimize any increase in blood alcohol content and the associated decline in taster acuity and discriminatory skill. In social situations, expectoration is usually anathema and can be a waste of limited amounts of good wine.

Integration, Interpretation, and Quality Assessment

To experience the full range of a wine's sensory attributes, it is important to sample the wine over a 15–30 min period. Otherwise, features such as duration and development will not be adequately assessed. Duration relates to how long the wine retains a distinctive fragrance, whereas development refers to its qualitative changes. Development is often compared to the opening of a flower or the transformations of sunset. These features not only retain taster interest but also generate one of the rationales justifying the higher cost of premium wines. For these reasons, wine should not be opened or decanted in

advance – it can obviate the full detection of a wine's development.

During any tasting, attention slowly shifts from observing individual attributes to focusing on their integration and interpretation. This may involve assessing aspects such as conformity with, and distinctiveness within regional norms, varietal origin, stylistic characteristics, and an appraisal of the wine's overall (esthetic) quality. Quality, as independent from its historic, geographic, stylistic, or varietal attributes, is typically described in terms used in artistic appreciation. Complexity refers to the presence of many, distinctive, aromatic elements, rather than one or a few easily recognizable odors. Balance (harmony) denotes the perceived equilibrium of all olfactory and oral sensations, where individual attributes do not dominate. This is particularly desirable when the wine is to be sampled by itself. It is less significant when consumed with food, where balance involves how the wine complements and embellishes the appreciation of the food, and vice versa. Occasionally, an aspect of imbalance can be intriguing, donating the element of apparent 'nervousness.' Fine wines should ideally show extended duration, a fascinating development, and a lingering and appealing finish. These aspects both enthrall and sustain interest. Implied, but seldom enunciated, are properties such as power, elegance, and finesse. Without these attributes, attractiveness is shallow. Occasionally, these perceptions are so remarkable that the experience becomes unforgettable – a feature termed memorableness. Although highly desirable, it tends to require an element of surprise. This provides an additional incentive for tasting wines blind, that is, knowing nothing about its origin. This aspect is further enhanced when the wines are served in black glasses.

Other Tasting Situations

The previous section has dealt primarily with serious tasting – typically referred to as sensory analysis. The technique outlined focuses attention on all the sensory delights (and deficits) a wine may possess. Nonetheless, under most situations, its application is either unnecessary (wines of mediocre character), inappropriate (during a meal), or too prolonged (large stand-up tastings). Under such circumstances, the procedure needs to be truncated. Suggestions for such situations are briefly noted in the succeeding text.

Most wine and cheese parties are intended to promote social interaction, not assess either the wine or the cheese. Nonetheless, cheese mollifies the wine's less pleasant attributes and helps to keep the hands of participants occupied. The wines selected are all too frequently as innocuous or inexpensive as possible, to avoid interfering with the true function of the gathering. In addition, few attendees are really interested in the wine or cheese. Under such circumstance, there is little value in attempting a critical analysis, unless it is to confirm, if necessary, the nondescript nature of both the wine and the cheese. Many wine societies are also venues for developing social and business contacts rather than for learning about wine. The meal, usually an integral part of such gatherings, confounds the act of critical analysis but does favor an assessment of food and wine combinations. Although less than ideal for assessing the wine's full range of attributes, such tasting may indirectly act to encourage people to become more critical and thoughtful consumers.

Commercial tastings are usually designed to showcase participant wines and/or raise funds. In both situations, some of the wines may be of a quality worthy of assessment. Regrettably, the setting is usually not designed for critical appraisal – social interactions deflect attention from the wines, the time allotted for each wine tends to be too short, and the cheese/bread/food accompaniments, typically supplied, are inappropriate, albeit appreciated by most participants. Conditions are improved if the number of wines is limited, the tastings sequential and organized, and the participants seated. Despite these limitations, a reasonable evaluation of a select number of wines is possible, if sufficient concentration is applied to counter the all-too-abundant surrounding distractions of such venues. In contrast, large, stand-up tastings are reduced to quick swirls, sniffs, sips, and spits (or swallowing) – there being little opportunity to assess flavor duration or development. Although doing injustice to most quality wines, it favors the positive perception of wines that are ready for immediate consumption. This is not such a problem. These are usually the only wines presented. Because of the large number of wines available, those sampled should be judiciously chosen and limited to those that appear to warrant attention. Beyond a quick evaluation of the wine's immediate impression, attention may also be directed to evaluating the wines' expression of some geographic, stylistic, or varietal expression. Frequently, though, the wines are evaluated only on a personal preference, price/quality basis.

Most wine tastings, though, occur in association with food. This is a long-established European custom, probably reflecting wine's beneficial antimicrobial properties and its ready availability. Only sweeter or fortified wines tend to be balanced for consumption alone. In addition, there is no inherent compatibility between wine and food. The predominant flavors of wine (floral/fruity fragrances and acidic, bitter, astringent, and alcoholic attributes) find little in common with meaty or vegetal flavors. Nonetheless, wines do possess some of the features common to food condiments – adding elements of interest. Wines are also par excellent palate cleansers, refreshening the mouth, allowing one to enjoy anew the attributes of food. Conversely, food cleanses the palate of wine flavors, permitting the wine to be sampled afresh. Wines and food also tend to diminish each other's less pleasant attributes – wine's acidity, bitterness, and astringency are reduced and a food's fatty attributes diminished. The result is a mutual and synergistic enhancement in the appreciation of both. Thus, when attempting to critically assess a wine at the dinner table, it is better to sample the wine(s) in advance of the food's arrival. This avoids any skewing or reduction in the sensory characteristics of the wines. This is especially important for better wines, permitting their attributes to be fully detected. It also provides an opportunity to make comparisons, take notes, and share perceptions with fellow aficionados. Comparisons are more informative (less chance for knowledge-based bias) if the identity of the wines, or at least their order, is withheld during the sampling period. In contrast, if the tasting occurs synchronously with the meal, most young table wines will appear better by the combination and therefore beneficial. It is primarily with older, red table wines, which have developed subtle flavors,

that combination with food is usually a significant mistake. Unless the food is very mild-flavored, it will overpower the wine's ethereal characteristics, obviating a full appreciation of the qualities that may have taken decades to develop.

See also: Alcohol: Metabolism and Health Effects; Alcohol: Properties and Determination; Wines: Champagne and Sparkling Wines – Production and Effervescence; Wines: Madeira, Port and Sherry Fortified Wines – The *Sui Generis* and Notable Peculiarities. Major Differences and Chemical Patterns; Wines: Wine Production.

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World Health Organization

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Introduction

The World Health Organization (WHO) is a specialized agency of the United Nations (UN), which is concerned with international public health. It is the directing and coordinating authority for health within the UN system. It is responsible for providing leadership on global health matters, shaping the health research agenda, setting norms and standards, articulating evidence-based policy options, providing technical support to countries, and monitoring and assessing health trends. It was established on 7 April 1948, headquartered in Geneva, Switzerland. The WHO is a member of the UN Development Group; its predecessor, the Health Organization, was an agency of the League of Nations. The constitution of the WHO had been signed by 61 countries on 22 July 1946, with the first meeting of the assembly (World Health Assembly (WHA)) finishing on 24 July 1948. It incorporated the International Office of Public Hygiene and the League of Nations Health Organization. Since its creation, it has played a leading role in the eradication of smallpox. Its current priorities include communicable diseases, in particular HIV/AIDS, Ebola, malaria, and tuberculosis. Among the remaining foci are mitigation of the effects of noncommunicable diseases; sexual and reproductive health, development, and aging; nutrition, food security, and healthy eating; occupational health; substance abuse; and driving the development of reporting, publications, and networking. The WHO is responsible for the World Health Report, a leading international publication on health, the World Health Survey, and the World Health Day (7th of April every year).

The WHO works directly with and through other agencies of the UN system, nongovernmental organizations, and collaborating centers across the world in numerous disciplines. Due to resource constraints in 1990s, the WHO places emphasis on meeting the most pressing health needs. In seeking to prevent and overcome malnutrition, the WHO promotes the tailoring of approaches to fit situation. While the rapidly increasing threat of noncommunicable diseases accounts for at least 40% of all deaths in developing countries and 75% in industrialized countries, many millions still cannot meet basic needs for energy and protein, are deficient in essential micronutrients, or are severely malnourished. Thus, coordinated action is called for on both fronts. The WHO has played a unique normative, scientific, and advisory role for the last half century. It strives to support all its member states in developing food and nutrition policies that will make healthy choices the easy choices for their populations.

Historical Background

The middle of eighteenth century saw the first international exhibition in London, at which products of technical skill and craftsmanship of many countries were displayed. This was possibly due to the increased speed and facility of transportation. But the same factor was also responsible for rapid and extensive spread of cholera that arrived in Europe and became a prominent reason for the origin of International Sanitary Conference, which opened in Paris on 23 July 1851. Eight years later, the second conference was held in Paris; it lasted for 5 months, during which a new draft was signed. This, in turn, was followed by a conference in Constantinople (1866), which ended in an agreement on a number of technical questions. On 1 July 1869, the fourth conference was held in Vienna, which resulted in a plan for a permanent International Commission on Epidemics followed by conferences in 1885 (Rome), 1892 (Venice), 1893 (Dresden), 1894 (Paris), and 1897 (Venice) with a focus on cholera or plague and removal of bars on trade. In 1903, the 11th conference was held at Paris with a more scientific approach toward epidemics. A proposal for translating scientific knowledge into practical public health measures by establishing the International Public Health Office was favorably received and acted upon on 9 December 1907, with the creation of the International Office of Public Hygiene (OIHP) in Paris. It was the first international office with the intent to disseminate to member state information on general public health, epidemics, and the ways to prevent and remedies for the same. Before the outbreak of the First World War, OIHP was working with an aim of controlling epidemics, opening and managing hospitals, and establishing food hygiene and biological standardization. Its activities were suspended in 1914, due to the outbreak of war, with the exception of the publication of its *Bulletin*. While OIHP was being formed, the Americans came up with International Sanitary Bureau in 1902, which was renamed as Pan American Sanitary Bureau in 1923. Besides these, there were various other regional councils of health in the eighteenth and nineteenth century in Egypt, in the Ottoman Empire, in the Moroccan region, etc.

Following the First World War, in 1920, a need for a larger, better organized, radical, dynamic, and better coordinated international health organization was felt. The world observed a breakdown of the sanitary conditions that caused the outbreak of cholera and typhus, both of which were endangering millions of lives in Poland, Russia, and other parts. The task of reestablishment of sanitary decency was a Herculean one, demanding an organization with a larger-scale, vaster outreach, more solid budget, and greater efficiency and effect. On the request of the Council of the League of Nations, International Health Conference met in London from 13 to 17 April 1920. After a lot of trials and tribulations, the league came up with its own health committee to work in coordination with OIHP. The two worked parallel for many years until after the Second World War, when the globe started making attempts to establish public health as fundamental to attainment of peace and prosperity. The UN Conference on International

Organization in San Francisco was held in 1945, at which an international health organization was established. The constitution of the WHO was approved in 1946 at the International Health Conference in New York. Along with 18 states, the Interim Commission (1946-48) took over the work of OIHP, the Health Organization of the League of Nations, and the Health Division of the UN Relief and Rehabilitation Administration. In 1948, the WHO Constitution obtained enough support to bring it into force and was established on 7 April 1948.

WHO: Structure and Management

The WHO consists of one headquarters (in Geneva) and six regional offices working together for three main bodies: the Health Assembly (HA), the Executive Board, and the Secretariat. Nearly 8000 employees from more than 150 countries work for the organization. The staff includes not only medical doctors, public health specialists, researchers, and epidemiologists but also administrative, financial, and information systems specialists, as well as experts in the fields of health statistics, economics, and emergency relief.

WHO: Headquarters

The WHO headquarters is in Geneva, operated by 1800 staff members. It contains different departments consisting of teams designed to work for specific projects/programmes. Each department has a director responsible for its functioning and reporting it to the higher authorities. The organization is headed by the director general, who is appointed by the HA on the nomination of the executive board.

WHO: Regional Offices

The WHO has six regional offices, namely, the African region, region of the Americas, the Southeast Asia region, the European region, the Eastern Mediterranean region, and the western Pacific region. It should be noted that the region of the Americas is embodied in the Pan American Health Organization (PAHO), which had been established 46 year before the founding of the WHO. All the regional offices are individual functional units with committees constituting the delegates from health ministries of that region's member states. The committee has a regional director that is selected every 5 years. It holds a meeting annually where the agenda for the coming year is decided upon and progress reports regarding earlier agendas are assessed. To ensure maximum coordination and transparency between the regional office and its member states, each state has a WHO representative. The WHO receives such huge amount of statistics, concerning old and new diseases, outbreaks, health indicators, preventive measures and their efficiency, nutrition, etc., from the regional offices.

WHO: Structure and Governance

The supreme governance of the WHO resides in the WHA, which is responsible for ratifying decisions. It generally meets every year in the month of May in Geneva and is attended by delegations from all 194 member states. Its main function is to determine the policies of the organization. The HA appoints the director general and also supervises the financial policies and reviews and approves the budget of organization. It similarly considers reports of the executive board, which instructs it in regard to matters upon which further action, study, investigation, or report may be required.

WHO: Executive Board

Executive board is composed of 34 individuals designated by a member state that the WHO elects. The elected board members are technically qualified in the field of health and are elected for a term of 3 years. Board meets at least twice a year, in January and May. The first meeting agrees upon the agenda for the forthcoming HA and second meeting immediately after the HA, for more administrative approvals. The main functions are to give effect to the decisions and policies of the HA, to advise it, and generally to facilitate its work. The board elects for itself a chairman, four vice-chairmen, and one rapporteur from among its members each year at its first session after the HA. The election follows a principle of rotation among geographic regions. The chairman of the board is not eligible for reelection for the next 2 years. During his course of work, the chairman is responsible for the declaration of the opening and closing of each meeting of the board, directing discussions, putting forth questions, according the right to speak, and ensuring the application of rules.

WHO: Committees of the Board

The board has the power to establish committees as and when necessary for the study of, and reporting on, any item on its agenda. Standing committees are composed of members of the board or their alternates. All committees other than standing committees are open-ended, composed of all interested member states to serve a specific purpose and also under exceptional circumstances. The position of chairman and that of other officers rotate regularly between regions and, wherever applicable, between developed and developing countries and countries in transition within the regions. The board reviews from time to time the need to maintain any committee established under its authority. The director general usually reports the board on the technical, administrative, and financial implications of all the agenda items submitted to the board. Communication of any reports of sessions of the board, containing resolutions, recommendations, summary, and other formal decisions to all member states and associate members of the organization, is the responsibility of the director general. Decisions by the board on important questions shall be made by a two-thirds majority of the members present and voting.

WHO: Ad Hoc Open-Ended Intergovernmental Working Group

The WHA in May 2001 proposed that the executive board should conduct a review of its and other subsidiary bodies' working methods, so that efficiency, transparency, and improved participation by member states can be ensured. Thus, it was decided to establish an ad hoc open-ended intergovernmental working group to make recommendations to the executive board on its working methods. Such a group was

established in the 109th session of executive board and had its first meeting held on 16 January 2002. The objectives decided for the working group were the following:

(1) To ensure that they are effective, efficient, and transparent and that participation of member states in the proceedings of the board, including working groups and drafting committees, is improved. The working group will conduct a review of the working methods of the executive board and those of its subsidiary bodies and include the rules of procedure in light of the functions of the board and interaction between the board and other organs of the WHO.
(2) To make recommendations for improvement and to include relevant cost implications.
(3) To report on its work at every session of the board.
(4) To prepare draft provisions and other measures for implementing its recommendations and to submit them to the board for consideration.

WHO: The Secretariat

The Secretariat is composed of the director general, six regional directors, and technical and administrative staff based in headquarters and regional offices and in specific countries. All countries that are members of the UN may become members of the WHO by accepting its constitution. Other countries may be admitted as members when their application has been approved by a simple majority vote of the WHA. Territories that are not responsible for the conduct of their international relations may be admitted as associate members upon application made on their behalf by the member or other authorities responsible for their international relations.

The Tasks Entrusted to the WHO

The WHO is defined by its constitution as the directing and coordinating authority on international health work. It was constructed with the aim of reducing incidences of disease and death throughout the world. Since the beginning, its functions have been manifold, which include the following:

- International protection against communicable diseases and comparability of the causes of death and disease
- Emergency measures to assist governments, trusts, territories, etc.
- Standardization of biological and pharmaceutical products and diagnostic procedures
- Improved standards of medical teaching
- Public health administrative technique and hospital services
- Scientific research with international coordination
- Statistics pertaining to health
- Central information for counseling on health issues
- Collaboration with governmental health administration
- Cooperation between the WHO and other organizations

The top priorities enlisted in WHO's agenda during its first assembly in June 1948 were malaria, women's and children's health, tuberculosis, venereal diseases, nutrition, and environmental sanitation. The WHO has come a long way since then in terms of both manpower and the vastness of issues it encompasses under its umbrella. The WHO covers health problems that were not even known in 1948, including relatively new communicable diseases such as HIV/AIDS, Ebola, malaria, and tuberculosis. It also is responsible for the mitigation of the effects of noncommunicable diseases; sexual and reproductive health, development, and aging; nutrition, food security, and healthy eating; occupational health; substance abuse; and driving the development of reporting, publications, and networking. The major functions of the WHO are enlisted in the succeeding text:

- To provide leadership on matters critical to health and engage in collaboration in joint ventures
- To shape the research agenda and stimulate the generation, translation, and dissemination of valuable knowledge
- To set norms and standards and to promote and monitor their implementation
- To articulate ethical and evidence-based policy options
- To provide technical support, catalyze change, and build sustainable institutional capacity
- To monitor the health situation and assess health trends

The general programme contains the earlier mentioned elements as the core functions, which provides framework of work, budget, resources, and results.

The WHO is striving ceaselessly to accomplish the tasks it is accountable for through a varied range of outputs available for monitoring and maintaining the global health situations mentioned in the succeeding text:

- An access to WHO projects, initiatives, activities, information products, and contacts related to various health topics
- Exhaustive data files on various communicable and noncommunicable diseases, injuries, health systems, and various millennium development goals (MDGs)
- Maintaining a media center that updates itself on recent disease outbreaks, prevention methods, statistics, factsheets, situation assessments, crises, and emergencies all round the world
- Publishing annual World Health Reports, World Health Statistics, and various journals concerning sanitary, disease, and nutrition topics
- Country-wise data on health profile, mortality and burden of disease, nutrition and child malnutrition data, and strategy briefs on collaboration of the country with the WHO
- Recommendations that can impact upon health policies or clinical interventions

WHO's Work

The work like its structure is also decentralized to six regional offices in Cairo, Copenhagen, Brazzaville, New Delhi, Manila, and Washington. Each regional office has a regional director who is responsible to the director general and the regional committee for carrying out the various programmes of the organization. To these regional offices, the governments bring their requests for assistance, in the form of surveys, and support for eradication programmes, seeking advices on national health planning, trainings, or fellowships for training. The requests are analyzed and approved based on their priority and value and then included in headquarters' programme and budget. Since

its inception, the WHO has worked for the prevention and curing of various diseases and health conditions, such as chickenpox, yellow fever, typhoid, and river blindness, and achieved success in partial control or total eradication of many such global health issues. The efforts taken by the WHO have given significant results; few landmark achievements of WHO campaigns are given in the succeeding text:

Landmark achievements of the WHO or with WHO support

1948: The WHO took over the responsibility for the International Classification of Disease (ICD), which dates back to the 1850s and was first known as the International List of Causes of Death. The ICD is used to classify diseases and other health conditions and has become the international standard used for clinical and epidemiological purposes.

1952–64: Dr. Jonas Salk (the United States) develops the first successful polio vaccine. One of the first diseases to claim WHO's attention was yaws, a crippling and disfiguring disease that afflicted some 50 million people in 1950. The global yaws control programme, fully operational between 1952 and 1964, used long-acting penicillin to treat yaws with one single injection.

1967: South African surgeon, Christiaan Barnard, conducts the first heart transplant.

1970: Apart from disease control, the WHO has also worked in the area of family planning for which it launched its Expanded Programme of Research, Development and Research Training in Human Reproduction, which was to focus on fertility regulation and birth-control methods.

1974: Beginning in this year, the WHO worked for 30 years to eliminate onchocerciasis (also known as 'river blindness') from West Africa. More than 600 000 cases of blindness have been prevented and 18 million children spared from the disease. Thousands of farmers have been able to reclaim 25 million hectares of fertile river land that had been abandoned because of the risk of infection. The WHA adopts a resolution to create the Expanded Programme on Immunization (EPI) to bring basic vaccines to all the world's children and immediately launches its EPI, aiming the vaccination of children worldwide against diphtheria, pertussis, tetanus, measles, poliomyelitis, and tuberculosis. This goal has now been transferred to Global Alliance for vaccines and immunization.

1977: The first essential medicine list appeared in 1977, 2 years after the WHA introduced the concepts of 'essential drugs' and 'national drug policy.' 156 countries today have a national list of essential medicines.

1978: The International Conference on Primary Health Care, in Alma-Ata, Kazakhstan, sets the historic goal of 'Health for All' – to which the WHO continues to aspire. The WHO adopted the Declaration of Alma-Ata, calling on all governments to make high-quality primary health care an essential feature of their national health systems.

1980: The most prominent and celebrated achievement of the WHO has been the eradication of smallpox as a result of an aggressive campaign. The last natural case of infection by smallpox occurred in 1977, and the declaration of world being smallpox-free by Global Commission for Certification of Smallpox Eradication came in 1980.

1983: Institut Pasteur (France) identifies the human immunodeficiency virus (HIV), the etiologic pathogen for the acquired immunodeficiency syndrome (AIDS).

1988: Global Polio Eradication Initiative is established. Since its launch in 1988, the Global Polio Eradication Initiative has reduced the number of cases of polio by more than 99% – from more than 350 000 per year to 1956 in 2006. Spearheaded by the national governments, WHO, Rotary International, US Centers for Disease Control and Prevention, and UNICEF, it has immunized more than two billion children thanks to the mobilization of more than 20 million volunteers and health workers. As a result, five million children are today walking, who would otherwise have been paralyzed, and more than 1.5 million childhood deaths have been averted. Next is the control of polio disease, confining it to Africa and South Asia only. The campaign started in 1988 and the declaration of America being polio-free came in 1994, Western pacific region in 2000, Europe in 2002, and India in 2013.

1990: The WHO launched various programmes against lifestyle diseases such as cancer, cardiovascular disease, and diabetes and promoted healthy and tobacco-free living.

2003: Severe acute respiratory syndrome (SARS) first recognized and the initial outbreak was controlled.

2004: The Global Strategy on Diet, Physical Activity and Health was adopted.

2005–07: The WHA revises the International Health Regulations (IHR). The IHR came into force. This legally binding instrument is the primary means of protecting the world from new and reemerging diseases, microbial shocks, and other threats to public health and global health security. Countries are obligated, by 2016, to establish a series of functions to ensure that they can detect, verify, assess, and respond to public health threats. The core capacities needed to fulfill these functions include national legislation, policy, and financing; coordination and communication through a national focal point; surveillance; response; preparedness; risk communication; human resources; and laboratories. For many countries, implementing all of the IHR's provisions is a challenge. In 2007, the WHO organized work on pandemic influenza vaccine development through c9: initial trials in collaboration with many experts.

2009: A pandemic involving the H1N1 influenza virus was declared in April 2009. A number of vaccines were approved for use 3 months after the pandemic declaration. This response was only possible because of the extensive preparations undertaken in during the last decade.

MDGs of the WHO

The UN MDGs include eight goals that were signed in the UN Millennium Declaration on September 2000 by all 191 UN member states, as goals to be achieved by 2015. The MDGs are interdependent and influence health, and health influences all the MDGs. Each MDG has targets set for 2015 and indicators to monitor progress from 1990 levels. The eight MDGs are summarized as follows, along with comments on the progress toward each:

Note: The registry of the goals is missing.

MDG1: To Eradicate Extreme Poverty and Hunger

The indicator for undernutrition is the proportion of underweight children under the age of 5 in region as undernutrition

includes fetal growth restriction, stunting, wasting, and deficiencies of vitamin A and zinc, along with suboptimal breastfeeding. Undernutrition in children is responsible for about 45% of all deaths under 5 years of age. In developing countries, between 1990 and 2012, a remarkable decline was observed in underweight children from 25% to 15%, which was close to the rate required to meet the MDG target, though being uneven across regions.

MDG2: To Achieve Universal Primary Education

The goal was to ensure that all children, boys and girls alike, can complete a full course of primary schooling. The efforts have encouraged an enrolment of 43 million children worldwide between 1999 and 2009, and the rate of enrolment in sub-Saharan African has increased from 58% to 76%. Some of the challenges before the WHO are that there are still 61 million children left to be enrolled in schooling, and the youth illiteracy rate exceeds more than 120 million people worldwide.

MDG3: To Promote Gender Equality and Empower Women

The MDG 3 indicators track key elements of women's social, economic, and political participation and guide the building of gender-equitable societies. Empowerment of women, including ensuring access to health information and control of resources such as money, is important for achieving gender equality and health equity. However, the ratio of female-to-male earned income is well below parity in all countries for which data are available. Up to one in three women worldwide will experience violence at some point in her life, which can lead to unwanted pregnancy and abortion, among other things. In partnership with member states and others, it will further empower the women, support the prevention of and response to gender-based violence, promote women's participation and leadership, define ways in which men can be engaged to promote gender equality and to contribute more to their own health and that of their families and communities, build the capacity of the WHO and its member states to identify gender equality-related gaps, and provide support for gender-responsive policies and programmes.

MDG4: To Reduce Child Mortality

Across the globe, the progress in this goal was significant with the reduction being 47% (6.6 million) deaths of children under 5 years as compared to 12.6 million in 1990 and the global reduction being 3.9% during 2005–12. But still, the world is unlikely to achieve the MDG target of a two-third reduction in 1990 mortality levels by the year 2015. It has been achieved through increase in levels of immunization coverage, with 66% member states reaching 90% coverage.

MDG5: To Improve Maternal Health

Maternal deaths were significantly reduced to 289 000 in 2013 from an estimated 523 000 in 1990, but this reduction was not able to achieve the target set by MDG, which was only less than half of a three-quarter reduction in the mortality ratio between 1990 and 2015. In three of the six WHO regions, births attended by skilled personnel, which is crucial for reducing perinatal, neonatal, and maternal deaths, were above 90%. In African region, the coverage for improving maternal health needs to be increased as its remains $<50\%$.

MDG6: To Combat HIV/AIDS, Malaria, and Other Diseases

In case of newly HIV-infected people in 2012, as compared to 2001, an estimated reduction of 33% was reported. Scaling-up malaria interventions during the 2000–12 period helped to save about 3.3 million lives worldwide, and mortality rates of population at risk and due to malaria have fallen to about 42% and 29%, respectively. It was observed among neglected tropical diseases (NTDs) that in about 119 countries in which leprosy was endemic and was a great concern for public health, it was totally eradicated. In case of dracunculiasis (a crippling parasitic disease), an incidence of only 148 was reported in 2013. In 2011, worldwide, about 728 million people were treated for at least one NTD through chemotherapy. But still, neglected diseases, which are caused by a variety of pathogens including viruses, bacteria, protozoa, and helminths, affect more than one billion people.

MDG7: To Ensure Environmental Sustainability

As compared to 76% in 1990, the target for access to safe-drinking water has been met by 90% of the world's population using an improved source of drinking water. But the rate of progress regarding basic sanitation is slow, as depicted in the data stating nonaccessibility to improved sanitation facilities to about 2.5 billion people worldwide.

MDG8: To Establish a Global Partnership for Development

Prices of medicines are higher in private sector as compared to public sector, but worldwide, large groups of people are forced to purchase through the private sector due to scarcity of medicines. Per the data of a survey conducted in 2007–12, availability of generic medicines in the public sector was only 57%, in low- and middle-income countries. Some generic medicines have higher costs than comparable prices in the international reference. For some generic preparations, the lowest price in private sector averages from 5 up to 16 times higher than references (public sector) prices.

In developing countries, even treating common disease may be beyond the reach of the low-income households, while the patients suffering from chronic diseases always end up paying the greatest price for generic medicines. Treating global chronic disease remains a great challenge, and the burden to treat them is increasing, as in most of the countries treating chronic disease still remain out of reach.

WHO: Food Safety and Security

Freedom from hunger and malnutrition is among the fundamental rights of every human being. There can be no sound social and economic development without adequate food and nutrition. This is not the same as saying that people in rich

countries necessarily have a better chance of being properly nourished than do people in poor countries. To meet the continuously growing food demand, food production in case of either agricultural or animal/livestock products is intensified or industrialized throughout the world. Food safety has become a great concern and is presenting new opportunities and challenges for food technologists. Food safety is also affected by climatic changes including temperature that can render the food unsafe for human consumption by increasing the risks associated with food production, storage, and distribution. It is estimated that about two million casualties take place every year only from foodborne and waterborne diarrheal disease in which most of them are children.

In general, the WHO is working in multiple areas to ensure safe and ample food supply globally by adopting various measures such as aiding in detection and management of foodborne diseases by generating data on diseases, supporting implementation of infrastructure, promoting safe food handling, educating food handlers and consumers on hygiene, and controlling microbiological risks by providing guidelines on risk assessment, management, and communication of chemical hazards. The WHO also defines the critical exposure levels to chemical hazards to ensure the safety of the public, which forms the base for drafting and setting the national and international food safety standards; it serves two causes, that is, to protect the consumers and also help in implementing fair-trade practices. It also has set limits for usage of antimicrobials in foods to avoid overexposure of drugs in consumers. The WHO also strives continuously in collaboration with Food and Agriculture Organization (FAO) for advancement of food technology and safety, and then, the WHO provides scientific advice on the assessments of food and food products that have been produced by using technological advancements such as genetic modification and nanotechnology to its member states.

With the advancement of food technology and modern manufacturing techniques, chances of contaminations rendering the food unsafe have also increased; in order to cater the needs related to food safety, the WHO has established International Food Safety Authorities Network (INFOSAN), with whom it helps to manage food safety risks to its member states and ensures that the information pertaining to food safety is shred throughout them. During food safety emergencies, INFOSAN along with the WHO helps to identify and stop the supply of contaminated food from one country to another among its member states. Recently, in August 2014, decisions were taken for linking the activities of INFOSAN with other food safety programs in the region and must also be bridged with the IHR (2005). It states that food safety and nutrition are inextricably linked, particularly in places where food supplies are insecure. It is observed that with rise in food scarcity, the chances of manufactures to ignore hygiene, safety, and nutrition increase. Less availability of good food makes people shift to less nutritious diets and consume more unsafe food, and to overcome this problem, the WHO has the Department of Nutrition for Health and Development, which has set global targets 2025 for improving maternal, infant, and young child nutrition. The targets include the Healthy Growth Project, aiming at a 40% reduction in the number of children under age of 5 years who are stunted, and the Iron Deficiency Anemia Project, targeting a 50% reduction of anemia in women of reproductive age. Other goals across the agenda include care of the preterm/low-birth-weight infants: reducing low birth weight by 30%, increasing the rate of exclusive breastfeeding in the first 6 months up to at least 50%, and reducing and maintaining childhood wasting to $<5\%$.

WHO: Ebola Virus

Ebola virus disease is a severe, often fatal illness, with a death rate of up to 90%. Ebola affects humans and nonhuman primates (monkeys, gorillas, and chimpanzees). In humans, infection of Ebola generally occurs from direct contact through broken skin or mucous membranes with the blood or other bodily fluids or secretions (stool, urine, saliva, and semen) of infected people. Infection can also occur if breaks in the skin or mucous membranes of a healthy person come into contact with environments that have become contaminated with an Ebola patient's infectious body fluids such as soiled clothing, bed linen, or used needles. In the current outbreak in West Africa in March 2014, the majority of cases in humans have occurred as a result of human-to-human transmission. In August 2014, the WHO declared an epidemic as the world health emergency.

The UN Mission for Ebola Emergency Response (UNMER) has set the goals of '70–60.' This corresponds to the aim to isolate and treat 70% of cases suffering from Ebola and to safely bury 70% of the deceased, and all these activities will be completed in 60 days from the beginning of October 2014. The WHO is continuously supporting this cause by working in various Ebola-affected countries like Sierra Leone, Liberia, and Guinea by training burial teams in protecting themselves while caring patients; in contact tracing, social mobilization, and case management; and in conducting safe and dignified burials. As of 24 November 2014, the WHO had received US $162 million, with a further $35 million pledged.

The WHO aspires to give in-clinic training to 40 national and international personnel per week over two training sessions, after already training 100 participants in the hot zone (phase 3) in Guinea. In Sierra Leone also, the WHO along with the UK government has trained 4115 health-care workers, hygienists, and trainers in basic personal protective equipment, infection prevention and control, and site layout and plans to other treatment centers in other areas. It is also running community-based programmes in enabling them to recognize symptoms of Ebola early and move their family members to care so they do not infect other family members or their society. In August 2014, the WHO released a roadmap on Ebola to scale up response to the outbreak and stop transmission in affected countries within 6–9 months, prevent international spread, and assist governments and partners in the revision and resourcing of country-specific operational plans for Ebola response and the coordinating international support for their full implementation.

The WHO is also been providing regular epidemiological support on Ebola, such as data situation reports and an assessment of the response measured against the core indicators, wherever available. Updates about the Ebola are generally provided to the countries, which have been reported with widespread and intense transmission of Ebola. The nations

are classified as those with or that have had an initial case or cases or with localized transmission and those countries that neighbor or have strong trade ties with areas of active transmission.

The WHO has also developed a consolidated Ebola virus disease preparedness checklist to ensure countries are ready to respond in cases of outbreak. They are provided with checklist, simulation exercises, and methods to test their level of readiness. The WHO, the UN, and other partners are accelerating the deployment of international preparedness strengthening teams to help countries build upon their existing work and planning. At the end of each mission, technical experts remain in country to support and maximize capacity-building efforts to prepare for public health emergencies, including EVD.

See also: Aflatoxin: A Global Public Health Problem; Alcohol: Metabolism and Health Effects; Allergies: Public health; Anemia: Causes and Prevalence; Anemia: Prevention and Dietary Strategies; Antioxidants: Role on Health and Prevention; Appetite Control in Humans: A Psychobiological Approach; Arsenic: Toxicology and Health Effects; Ascorbic Acid: Physiology and Health Effects; Bioavailability of Nutrients; Caffeine: Consumption and Health Effects; Cancer: Diet in Cancer Prevention; Carcinogenic: Carcinogenic Substances in Food; *Clostridium*: Food Poisoning by *Clostridium perfringens*; Codex Alimentarius Commission: Role in International Food Standards Setting; Codex Alimentarius; Consumer Protection Legislation; Diarrheal Diseases; Eggs: Composition and Health Effects; Emerging Foodborne Enteric Bacterial Pathogens; Fish Oils: Composition and Health Effects; Food Fraud; Food Poisoning: Epidemiology; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; Hunger; Malnutrition: Prevention and Management; Nutrition and Health Claims for Food: Regulatory Controls, Consumer Perception, and Nutrition Labeling; Pesticides and Herbicides.

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Yeasts

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General Consideration and Taxonomy

The association of yeasts with human society was probably initiated many thousands of years ago. Evidence for the production of fermented beverages by yeasts, mostly *Saccharomyces cerevisiae*, dates back as far as 7000 BC, which is documented by several archaeological sites in China, Iran, and Egypt. Ancient humans unwittingly utilized yeasts to produce either alcohol-containing beverages or carbon dioxide for leavening. These practices and their associated yeasts spread throughout the world, inducing a particular selection, that is, 'domestication,' in particular for some foods such as bread, beer, and wine. However, the deliberate addition and commercial use of yeasts started only at the end of the nineteenth century after isolation and identification by Pasteur. Today, many species of yeasts contribute to the production of a myriad of commodities other than bread, beer, and beverages. Food-grade yeasts are now used as sources of high-nutritional-value proteins, enzymes, and vitamins, and processing aids are derived from them. With respect to foods and health foods, yeasts are utilized as nutritional supplement, food additives, conditioners, and flavoring agents and as starter cultures for producing specific foods or for their probiotic activity. Antifungal activity is exhibited by some yeasts that can be considered as biocontrol agents of food spoilage. Unfortunately, there are some drawbacks to yeast activity too, because some can cause spoilage of many commodities and may lead to pathogenic infections in immunocompromised persons or lead to allergic reactions. Table 1 reports the main roles of yeasts in food production and in the health of consumers.

The name yeast derives from the German word 'gift,' which describes the foam formed during beer fermentation. Yeast can be also described using the French word 'levure,' which indicates the role of yeasts inducing bread dough to rise. Yeasts are eukaryotic microorganisms whose vegetative growth results predominantly from budding or fission and that do not form their sexual states within or upon a fruiting body. Yeasts have two valid names: the first referring to the sexual state (teleomorph) and the second one to the asexual state (anamorph). The rules of yeast taxonomy are established in the 'International Code of Nomenclature for algae, fungi, and plants,' which was adopted at the 18 as apex International Botanical Congress in Melbourne, Australia. The 5 as apex edition of 'The Yeasts: A Taxonomic Study' in 2011 describes 1500 yeast species; but this number is increasing. The difference between the number of described species and that of new species is due to the fact that some ecological niches are far from being completely investigated and to the presence of cryptic species, which still have not been carefully characterized. Yeast taxonomy is based on biochemistry and molecular biology and is rapidly evolving. Criteria for differentiating genera have focussed on spore and cell morphology, type of conidiogenesis, life cycles, biochemical features, type of coenzyme Q, and G+C content. However, DNA hybridization or reassociation contributes to the establishment of relationships among yeast species. Two species are considered conspecific if they share more than 70% of DNA sequence homology, but this method does not allow assessing phylogenetic relationships between yeasts. Recently, the application of rapid, easy, and reliable molecular techniques allowed describing new phylogenetic relationships. As a result, the number of separate species groups was reduced and the diversity within them was increased. The most diffused molecular methods used in the identification of yeast species are based on the variability of the ribosomal genes 5.8S, 18S, and 26S because they show a low intraspecific polymorphism and a high interspecific variability. The first marker used for separation of the hemiascomycetes from the filamentous ascomycetes was the 18S rRNA gene. Subsequently, the D1/D2 variable region of the 26S rRNA (600 bp) has been adopted to differentiate basidiomycetes, hemiascomycetes, and filamentous euascomycetes. The availability of whole-genome sequences of yeasts revolutionized the taxonomy leading to the description of new markers for yeast phylogeny. Traditionally, the main genera can be divided into two groups: ascomycetes and basidiomycetes. However, budding and fission ascomycetes can now also be differentiated from each other and from euascomycetes by molecular methods, with the only exception of genus *Eramascus* (which does not form budding cells). A similar approach is applied to separate basidiomycetes from mushrooms and from taxa forming fruiting bodies.

The Yeast Cell and Its Organelles

Yeast cells possess ultrastructural features typical of other eukaryotic cells, with the presence of membrane-bound organelles (Figure 1). Often, the words 'yeast' and '*S. cerevisiae*' are used as synonyms. Saccharomyces means 'sugar fungus' in Greek. Yeast cells exhibit a great diversity with respect to cell

Table 1 Main activities of yeasts in food production and in the human health

Activity	*Yeast species*	*Effect*
Food and beverage fermentation	*Candida milleri*, *Debaryomyces hansenii*, *Galactomyces candidus*, *Hanseniaspora uvarum*, *Kluyveromyces marxianus*, *K. lactis*, *Schizosaccharomyces pombe*, *S. cerevisiae*, *Kazachstania exigua*, *Torulaspora delbrueckii*, *Zygosaccharomyces rouxii*, *Yarrowia lipolytica*	Fermentation, flavor and color development, rheology
Food additive production	*Lindnera jadinii*, *K. marxianus*, *S. cerevisiae*, *Y. lipolytica*, *Rhodotorula glutinis*, *Sporidiobolus salmonicolor*	Release of B vitamins, proteins, peptides, amino acids, minerals, antioxidants, aromas, colorants, glutamic acids, nucleotides, vanillin, citronellol, linalool, geraniol, decalactones, β-glucans
Probiotic	*Saccharomyces cerevisiae* var. *boulardii*	Effect on enteric bacterial pathogen, maintenance of epithelial barrier Integrity, anti-inflammatory effects, effects on immune response, trophic effects on intestinal mucosa, clinical effects on diarrheal diseases
Food spoilage	*Brettanomyces bruxellensis*, *Pichia kudriavzevii*, *C. parapsilosis*, *D. hansenii*, *H. uvarum*, *Pichia membranaefaciens*, *Rhodotorula mucilaginosa*, *Rhodosporidium toruloides*, *S. cerevisiae*, *S. pombe*, *Kazachstania exigua*, *Zygosaccharomyces bailii*, *Z. bisporus*, *and Z. rouxii*	Blown cans of soft drinks, cloudy refermented wine, pink or red slime dripping from refrigerated meat, white yeast colonies on food, tainted fruit juices, off-odors, and off-tastes
Allergenic	Different yeast species	Food intolerance, migraines, respiratory problems, chronic fatigue syndrome, dysfunctional gut syndrome, irritable bowel syndrome
Pathogenic	*Candida* spp., *Filobasidiella neoformans*, *P. kudriavzevii*, *W. anomalus*, *K. marxianus*, *S. cerevisiae*, and *Rhodotorula* spp.	Mucocutaneous, cutaneous, respiratory, central nervous, systemic, and organ infections

Nomenclature is in agreement with the yeasts: a taxonomic study (5 as apex edition).

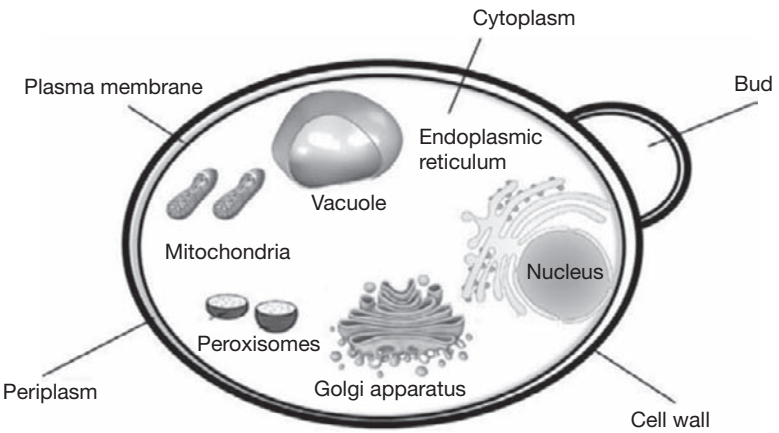

Figure 1 Yeast cell and its main organelles.

size, shape, and color. Cell size may be 2–3 μm in length up to 20–50 μm with a diameter of 1–10 μm. The yeast cell wall is a rigid structure about 100–200 nm thick and constituting about 25% of the total dry mass of the cell. The macromolecules that compose the wall are highly glycosylated glycoproteins (mannoproteins), two types of β-glucans, and chitin. The overall composition can vary considerably in relation with the growth conditions. The surface plasma membrane of yeast is a lipid bilayer, which harbors proteins serving as a cytoskeleton and enzymes for cell wall synthesis, signal transduction, and transport. The lipid components comprise mainly phospholipids and sterols (principally ergosterol and zymosterol). The periplasmic space is a region external to the plasma membrane (35–45 Å) that contains proteins unable to permeate the cell wall, such as invertase and phosphatase.

The yeast cytoplasm is an aqueous, slightly acidic colloidal fluid that contains proteins, glycogen, other soluble macromolecules and larger macromolecular entities such as ribosomes, proteasomes, and lipid particles. Many essential functions for cellular integrity are localized in the cytoplasm. The cytoskeleton of yeast cells comprises microtubules and microfilaments. In the center of the cell or slightly excentrically, the nuclear structure is located, which is surrounded by a double membrane that separates the nucleoplasm from the cytoplasm. The outer nuclear membrane is contiguous with the membrane of the endoplasmic reticulum.

The nucleolus within the nucleus is the specialized subnuclear compartment for ribosome synthesis. The nuclear chromosomes are in the nucleoplasm and packed into a chromatin structure; the genome sizes of yeasts are relatively constant and generally range from 10 to 15 Mb, whereas the number and sizes of the single chromosomes vary among species. Yeast cell contains different organelles surrounded by individual membranes, mostly the endoplasmic reticulum, the Golgi apparatus, and transport vesicles that are necessary for the manufacturing and trafficking of proteins, vacuoles, mitochondria, and microbodies. Structurally, the endoplasmic reticulum is a complex network of membranes, part of which is connected to the nucleus. The endoplasmic reticulum membrane forms a continuous sheet enclosing a single internal space called the endoplasmic reticulum lumen, separating it from the cytosol. It is involved in the selective transfer of secretory proteins between the endoplasmic reticulum and the Golgi apparatus. It also plays a key role in lipid and protein biosynthesis; in fact, it is the site of production of the transmembrane proteins and lipids for most of the cell's organelles (e.g., the endoplasmic reticulum itself, the Golgi apparatus, lysosomes, and endosomes). The Golgi apparatus is composed of stapled stacks of membranes called *cisternae*. Transport vesicles, received from the endoplasmic reticulum, are further processed and sorted for transport, for instance, releasing secretory proteins in the growth medium. Vacuoles are organelles involved in intracellular protein trafficking, receiving proteins from differing routes. In the vacuoles, there are degradative processes catalyzed by different hydrolases, such as phosphatases, phospholipases, lipases, nucleases, endopeptidases, aminopeptidases, and carboxypeptidases. Vacuoles also are involved in other physiological functions, such as the storage of amino acids, polyphosphates, and some metal cations (K^+, Mg^{2+}, and Ca^{2+}). Yeast mitochondria resemble the organelles found in higher eukaryotes, surrounded by an outer membrane and an inner membrane, the two of which embody an intermediate space that contains the mitochondrial matrix. If sufficient oxygen and carbon sources are supplied, few and large mitochondria carry out respiration and synthesis of ATP, whereas under anaerobic conditions, they reduce their size and activity. Other functions are the synthesis and desaturation of fatty acids, biosynthesis of ergosterol, adaptation to stress, mobilization of glycogen, and synthesis of enzymes. Microbodies (peroxisomes and glyoxysomes) consist of a proteinaceous matrix, surrounded by a single membrane that is involved in the primary metabolism of various carbon and organic nitrogen sources, such as ethanol, methanol, primary amines, *n*-alkanes, and fatty acids (oleic acid).

Metabolism

Yeasts are heterotrophic organisms in which the energy and carbon metabolism are interconnected and anabolism is coupled to catabolism. Yeasts preferentially metabolize sugars that are converted principally to ethanol and carbon dioxide, but they can utilize different carbon sources, such as amino and organic acids, polyols, alcohols, fatty acids, and other compounds, depending on the species. According to the process utilized to generate energy (respiration and/or fermentation), they can be classified as:

- nonfermentative yeasts with only a respiratory metabolism;
- obligate-fermentative yeasts, only capable of metabolizing glucose through alcoholic fermentation;
- facultative-fermentative yeasts, possessing either a fully respiratory or a fermentative metabolism or even both in a mixed metabolism depending on the growth conditions, the type and concentration of the carbon source, and/or oxygen availability.

The main nitrogen substrates used by yeasts during growth in food are inorganic ammonium compounds and free amino acids. Decarboxylation of amino acids leads to the production of amines, whereas metabolism of arginine and citrulline gives urea, precursor of the potential carcinogen ethyl carbamate. Whereas yeasts produce organic acids, with succinic and acetic acids being the main acids produced during sugar fermentation, some yeast species can use acetic and lactic acids and weak monocarboxylic acids under starvation conditions. Consumption of organic acids by yeasts may thus create conditions favorable for certain spoilage microorganisms. In cheese, lactate catabolism by some species such as *Debaryomyces hansenii*, *Kluyveromyces lactis*, and *Kluyveromyces marxianus* induces an increase of pH at the cheese surface, having a key role during ripening.

Sulfates are utilized to produce volatile sulfur compounds such as hydrogen sulfide, sulfur dioxide, and at lesser amounts mercaptans, thioesters, and other organic sulfites. Yeasts also show proteolytic and lipolytic activities depending on the species and strain.

Yeasts in Fermented Food and Beverages

Yeasts are undoubtedly one of the most important groups of microorganisms that are exploited for the elaboration of fermented foods. Fermentation is one of the oldest and most economical methods of producing and preserving food, providing also a natural way to reduce the volume of the material to be transported, to destroy undesirable components, to enhance the nutritive value and appearance of the food, to reduce the energy required for cooking, and to make a safer product. In these processes, mixed cultures of yeasts, bacteria, and fungi are active, participating either in parallel or in a sequential manner with a changing dominant microbiota during the course of the fermentation. Some yeast species are included in starter cultures for the production of specific types of fermented foods like cheese, bread, sourdoughs, fermented meats, and fermented vegetable products. Common fermenting yeasts, often selected as starter cultures, belong to the *Saccharomyces* genus, which results in alcoholic fermentation.

Yeasts in Dairy Products

Yeasts play an important role in flavor and texture development during the production of fermented milks, such as kefir and koumiss, and the ripening of cheese. They are used as secondary starter cultures to enhance the aroma or improve

the growth of lactic acid bacteria or brevibacteria. The most important and dominant species, besides *S. cerevisiae*, are *D. hansenii*, *Yarrowia lipolytica*, *K. marxianus*, and *Galactomyces candidus*.

Yeasts in Fermented Meat Products

Studies on the presence and role of yeasts in fermented meat product are limited. The high salt concentration and the low water activity that are typical of fermented sausages favor the growth of *Debaryomyces* species, mostly *D. hansenii*, *Y. lipolytica*, and various *Candida* species, contributing to the development of color (by removing the oxygen) and flavor (by degrading peroxides and via lipolytic and proteolytic activities). Hydrolytic and oxidative changes carried out by yeasts during ripening produce the distinctive flavors of these products.

Fermentation of Cereal Products

Cereals, as a global staple food, are often fermented to improve shelf life and nutritional properties in comparison with the raw material. Fermented cereal products are produced from different cereal substrates and a number of them consist of a combination with legumes, thus improving the overall protein quality of the fermented product. Fermentation improves the nutritional value, sensory properties, and functional qualities. Besides the global importance of (sourdough) bread, a wide variety of different products come from Africa and Asia, as is the case for koko, banku, panjabi waries, papadams, jalebis, idli, dosa, dhokla, kishk, tarhana, ogi, kenkey, pozol, injera, and kisra. The microbiota responsible for the fermentation is in many cases indigenous and includes lactic acid bacteria, yeasts, and fungi. *Saccharomyces cerevisiae* strains are often found in the fermentation of various cereal products. Other yeast species frequently detected are *Kazachstania exigua*, *Candida milleri*, *Torulaspora delbrueckii*, and *Pichia* spp. The yeast species diversity in fermented cereal products, as well as their numbers, is affected by the degree of tolerance to the organic acids produced by the lactic acid bacteria.

Fermented Beverages

As regards the fermented beverages produced by musts obtained from fruits (grape, cider, mixed tropical fruits, etc.), the natural fermentation is started by some non-*Saccharomyces* yeasts, such as species of *Hanseniaspora uvarum*, *Candida stellata*, *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima*, and other yeasts. After 1 or 2 days, species of *Saccharomyces*, in particular *S. cerevisiae*, develop and dominate fermentation by forming high amounts of ethanol. During fermentation, many compounds are produced or metabolized by yeasts contributing to the aroma of beverages. However, during must fermentation, yeasts can produce off-flavors too, as is the case for *Brettanomyces*/*Dekkera* species that are reported to be involved in wine spoilage. Nowadays, selected starter cultures of *S. cerevisiae* are generally used to carry out must fermentation. In beer fermentation, two types of *Saccharomyces* yeasts are involved, that is, ale yeasts (top-fermenting yeasts, *S. cerevisiae*) and lager yeasts (bottom-fermenting yeasts, *S. pastorianus* (synonym *S. carlsbergensis*)). More than a thousand single different starter cultures of brewing yeasts have been described, varying in their technological properties, such as aroma production, rate and degree of attenuation, flocculation, oxygen requirement, and reproduction.

Cash Crops

Cocoa beans and coffee beans must undergo processes that involve the action of yeasts of various species of *Saccharomyces*, *Hanseniaspora*, *Kluyveromyces*, *Saccharomycopsis*, *Candida*, and *Pichia*. Cocoa beans have to be fermented to drain the pulp, stabilize the beans, and generate precursors for chocolate flavor, whereas coffee beans are mostly fermented to remove the pulp and the mucilaginous materials from the seeds.

Yeast for Health

Yeast as Probiotics and Nutraceuticals

The World Health Organization officially defined probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host." Most probiotic microorganisms are bacteria, usually including selected strains of certain genera of *Lactobacillus* spp. or *Bifidobacterium* spp. In recent years, the interest in using yeasts as probiotics increased, not only for human health but also to improve growth and health of animals and birds, in particular in aquaculture or with respect to industry cattle, pigs, and poultry.

Yeasts are able to face the stresses of the gastrointestinal tract, such as enzymes, bile salts, organic acids, and considerable variations of pH and temperature. The first species described as an effective biotherapeutic agent has been *S. cerevisiae* var. *boulardii*, isolated from lychee fruit in Indochina in the 1920s. This yeast is commonly used as a probiotic in different countries, as both a preventive agent and a therapeutic agent for diarrhea and other gastrointestinal disorders caused by the administration of antimicrobial agents. *S. boulardii* has many properties, such as survival transit capacity through the gastrointestinal tract, among other reasons because its temperature optimum is 37 °C, both *in vitro* and *in vivo*. Moreover, the species exerts inhibitory effects toward various enteric pathogens as its cell wall shows binding capacity to enterohemorrhagic *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. Additionally, the yeast inhibits adherence of *Clostridium difficile*. Other probiotic beneficial effects on human health associated to the administration of *S. boulardii* include:

- maintenance of epithelial barrier integrity and permeability,
- restoration of the tight-junction structure,
- reduction of inflammation during bacterial infection by interference with host cell signaling pathways,
- stimulation of the host cell immunity in response to pathogenic infections,
- prevention of reaction to food antigens in particular when mucosal permeability is increased.

Other species, such as *D. hansenii*, *T. delbrueckii*, *K. lactis*, *K. marxianus*, and *Kazachstania lodderae*, have also shown tolerance to passage through the gastrointestinal tract and

inhibition of enteropathogens. Recently, many researchers have focussed on the antioxidant ability and cholesterol assimilation of yeasts. Some reports indicated that *S. cerevisiae*, *K. lactis*, and *Pichia kudriavzevii* can remove cholesterol *in vitro*.

Yeasts can also produce nutraceuticals useful for the development of functional foods and for the protection from cardiovascular disease. The most important compounds seem to be the β-glucans, which act as bioactive polysaccharides, and bioactive peptides that inhibit angiotensin I-converting enzyme activity, reducing peripheral blood pressure and exerting antihypertensive effects *in vivo*. Only some yeast species, that is, *K. marxianus* and *S. cerevisiae*, have been shown to produce these compounds in fermented milks such as koumiss and kefir. Recent studies also indicate that some foodborne yeasts (especially *D. hansenii* and *S. cerevisiae*) are able to reduce the potential genotoxic risk due to mutagenic and genotoxic compounds. A recent study indicated that *S. cerevisiae* and *D. hansenii* show inhibitory activity against two model genotoxins, 4-nitroquinoline-1-oxide and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. The antigenotoxic activity is probably due to the formation of a complex between genotoxins and thermolabile soluble cellular oligosaccharides (i.e., mannans and glucans). Nevertheless, a similar complex may also occur between the genotoxins and other compounds derived from yeast metabolism such as polyamines, mainly spermidine and spermine or the glutathione system.

These healthy effects appear to be promising features for the use of well-characterized yeasts as natural protective agents. In addition, yeast cells are also good sources of vitamins (thiamine, pantothenic acid, riboflavin, biotin, folic acid, B_6, and B_{12}), ergosterol, and coenzyme Q.

Antioxidants

In the past, yeasts and yeast extracts were used as a source of antioxidant compounds to be added to prevent oxidation of food products. Now, natural antioxidants, presumed to be safer for human, can also be used in nutraceutical applications as supplements. Natural antioxidants act by donating hydrogen protons to substrates, rendering them nonreactive to reactive oxygen species. This involves enzymes such as catalases, superoxide dismutases, glutathione (L-γ-glutamyl-L-cysteinylglycine), and NADP-dependent dehydrogenases. Several compounds involved in antioxidant actions are produced by yeasts and can be used to retard oxidative degeneration of fatty substances in food and to improve health and well-being in nutraceutical supplements. They consist of torularhodin, coenzyme Q, glutathione, hydroxymethyl and hydroxyethyl furanone (2H), tocotrienol, α-tocopherols, and other forms of tocopherols, riboflavin (vitamin B_2), and derived flavins. Several oxygenated carotenoids, selenium-enriched yeast cells, the wine component resveratrol, octacosanol, cell wall β-glucans, proteins produced in yeasts under oxidative stress, sulfur-containing amino acids, and cytochrome C are present at the end of fermentation in the products and/or in the yeast biomass.

Single-Cell Proteins

The use of yeasts for single-cell protein (SCP) production is convenient, resulting in nutritional supplements for animal feeds and human foods. In fact, yeast SCP is considered as a potential source of proteins, carbohydrates, fats, vitamins, minerals, and essential amino acids. SCP for human consumption should be free from nucleic acid as purine bases are metabolized to uric acid, a problem for some humans that do not possess the enzyme uricase. SCP production has been obtained by yeasts cultivated in mixtures of sugars and minerals, such as molasses, starch, cassava, whey products, brewing by-products, potato and cellulosic wastes, or other waste streams from agricultural and industrial processes. The yeasts are washed, thermolyzed and dried, and usually spray-dried into a powder. The production of value-added SCP from inexpensive substrates could beneficially contribute to the food supply. Whereas for human applications, *S. cerevisiae* (baker's and brewer's yeast) is the currently the organism of choice, animal feeds rely on *Lindnera jadinii*.

β-Glucans

β-Glucans have several beneficial properties and have found a variety of uses in human and in veterinary medicine, immunopotentiation, pharmaceutical, cosmetic and chemical industries, and food and feed production. Yeast is a good source of β-glucans that are present in the cell walls as branch-on-branch molecules containing linear (1,3)-β-glucosyl chains joined through (1,6)-linkages. Spray-dried yeast β-glucans are useful for food production, as food thickeners, replacers, dietary fibers, emulsifiers, and films. Furthermore, yeast glucans have important properties as water-holding, fat-binding, and oil-binding characteristics, as well as gelling properties. For these reasons, they can also be used in the production of sausages and meat products, mayonnaise, and other food products, such as dressings, frozen desserts, sauces, yogurts, and other fermented milks and soft doughs. They are safe for oral application and have a GRAS (generally recognized as safe) status.

Allergenic or Pathogenic Yeasts

Viable populations of a diversity of yeast species present in fermented foods (dairy products, fermented and cured meats, fruits and fruit salads, table olives, and beer and wine) are unknowingly and inadvertently ingested. Unlike bacteria, viruses, and some filamentous fungi, yeasts are rarely associated with outbreaks of foodborne gastroenteritis or other foodborne infections or intoxications. However, an increase of yeast infections is reported, and some yeasts are now considered as opportunistic pathogens, such as *Candida albicans* and *Filobasidiella neoformans*, which can cause mucocutaneous, cutaneous, respiratory, central nervous, systemic, and organ infections. Moreover, other species that are frequently found in foods, such as *P. kudriavzevii*, *W. anomalus*, *K. marxianus*, *S. cerevisiae*, and various *Rhodotorula* species, can be potential pathogens. Even *S. cerevisiae*, intensively used in industrial processes, has been described as a pathogen in 1958. It displays low virulence against humans, but invasive *S. cerevisiae* infections have been increasingly common in recent decades, having been identified in blood cultures,

including those from immunocompetent subjects. Therefore, vigilance on yeasts and foodborne diseases is required because there is a literature that connects yeast presence in foods to the onset of a broad range of allergic and hypersensitive reaction in humans. Indeed, yeasts may cause or contribute to food intolerances, migraines, respiratory problems, chronic fatigue syndrome, dysfunctional gut syndrome, and irritable bowel syndrome. Generally, these adverse reactions could be associated to the yeast cells themselves, yeast extracts, or associated metabolites such as sulfur dioxide, proteins, and biogenic amines. The latter, especially histamine, tyramine, phenylethylamine, putrescine, and cadaverine, are associated to headaches, hypotension, migraines, and digestive disturbances in humans. Although the quantity produced is not enough to create health concerns, yeasts can thus contribute to the accumulation of biogenic amines in fermented foods and beverages.

Yeasts Technological Features

Aromas

During fermentation, yeasts produce many aromas. For alcoholic beverages, this involves over 400 different chemical compounds, of which fusel alcohols, fatty acids, and their ester represent the main groups. In addition, carbonyl compounds, acetals, phenols, hydrocarbons, nitrogen compounds, sulfur compounds, lactones, sugars, and a variety of other unclassified compounds are present. Besides, yeasts also contribute to the aroma of bread, cheese and other dairy products, fermented meat, cocoa, vanilla, and fermented soy-derived foods.

Colors

The yeasts have been used as sources of a number of carotenoids for use as food colorants such as astaxanthin, β-carotene and γ-carotene, lycopene, lutein, torulene, torularhodin, and zeaxanthin. For instance, *Phaffia rhodozyma* and *Xanthophyllomyces dendrorhous* have been used in industrial-scale production of the carotenoid astaxanthin. This compound can be used for aquafeed as a colorant or as an immunostimulant in food or in poultry, fish, or mammalian feed. Other colorants produced by yeast fermentation are riboflavin and caramel colors produced from the processing of yeast extracts.

Flavors

Flavors produced by yeasts, such as yeast extract, autolysates, and dried preparations, are commonly used in the food industry as a source of savory accents (roasted, nutty, meaty, and other flavors). These flavors can originate from yeast metabolic products including alcohols, amino acids and derivatives, carbohydrates and glycosides, fatty acids and their esters, organic acids, lactones, and aldehydes. Among the yeast-derived products, yeast extract is often produced from spent brewer's yeast and provided as powders or pastes. Yeast extracts are flavor ingredients of which the quality is affected by the specific cell composition. Hydrolyzed edible proteins and peptides from yeasts, which are a good source of the essential amino acid L-lysine, are limited in sulfur-containing amino acids, such as cystine and methionine, which have to be added. Some extracts are also enriched in glutamic acid and nucleotides that function as strong flavor enhancers. Yeast-derived lecithin, inositol, glycerol, and glycolipids, recovered from the cell membrane lipid extracts or from fermentation broth, can be used as flavors in foodstuffs as emulsifiers and surfactants. Moreover, they show additional health benefits so they can also be used as food supplements.

Yeast Spoilage

Food and beverages spoilage by yeasts has been well documented in many recent reviews. Yeasts can cause defects in the final products, and problems with hygiene procedures appear upon failures in the decontamination procedure, leading to significant economic losses. Generally, yeast spoilage occurs in food products characterized by a retarded or inhibited bacterial growth, that is, without bacterial competition. Foods that are usually subjected to yeast spoilage are characterized by low pH, high sugar levels (e.g., more than 10%, w/v), high salt levels (more than 5% NaCl), and low levels of organic acids (e.g., sorbic, benzoic, and acetic). Fruits and derived products (juices, drinks, pulps, concentrates, and syrups), confectionery products, alcoholic beverages, carbonated beverages, vegetable salads with acid dressings, salt- and acid-based sauces, dairy products, and fermented or cured (salted) meat products represent the main candidates for yeast spoilage.

The main spoilage yeast species are *B. bruxellensis*, *P. kudriavzevii*, *Candida parapsilosis*, *D. hansenii*, *H. uvarum*, *Pichia membranaefaciens*, *Rhodotorula mucilaginosa*, *Rhodosporidium toruloides*, *S. cerevisiae*, *Schizosaccharomyces pombe*, *K. exigua*, *Zygosaccharomyces bailii*, *Z. bisporus*, and *Z. rouxii*. However, with the years, some other common food-contaminating yeasts have been described, such as *Y. lipolytica*, *Zygosaccharomyces lentus*, *Z. kombuchaensis*, *Candida wyomingensis*, *C. boidinii*, *Cryptococcus uzbekistanensis*, *Candida natalensis*, and *Pseudozyma antarctica*. The main alterations caused by yeasts in food include the alteration of physical and sensorial properties, discoloration, gas production, cloudiness, sedimentation or pellicle formation, and the formation of off-flavors. For instance, *D. bruxellensis* is responsible of serious economic losses in the wine industry because it produces volatile phenols (4-ethylguaiacol and 4-ethylphenol), which generate off-odors in wines. Exposure of beer to air will induce the growth of oxidative yeast genera such as *Pichia*, *Debaryomyces*, *Candida*, and *Hansenula*, which cause off-flavors and ferment residual dextrans in the beer.

Yeast as Biocontrol Agents

Several yeast species show a strong antagonistic activity against filamentous fungi. For this reason, they can be used for biocontrol of fungi that cause pre- and postharvest spoilage of fruits and vegetables, increasing food quality and hygienic safety. As such, they can the control the development of *Botrytis*, *Penicillium*, *Aspergillus*, and *Rhizopus* spp., reducing the need

for chemical fungicides and, consequently, their potential oncogenic risks. The yeast species showing biocontrol capacity are mostly *M. pulcherrima*, *Candida sake*, *Sporobolomyces roseus*, *W. anomalus*, *Aureobasidium pullulans*, and various *Cryptococcus*. For commercial use, the main species diffused are *C. oleophila* and *Pseudozyma flocculosa*, which are registered as Aspire and Sporodex, respectively. The combination between *C. oleophila* and two bacterial strains is widely used as biocontrol agent of postharvest diseases of citrus and apple fruits. Yeast antagonistic activity is mainly related to the production of killer toxins, inhibitory proteins and peptides, lytic glucanases, and chitinases, which act against fungal cell wall. Moreover, they inhibit fungal development through nutrient competition and the production of toxic metabolites, such as ethanol, acetaldehyde, ethyl acetate, and fatty acids. The use of yeasts as biocontrol agents has several advantages because they are easy to cultivate, are characterized by a fast growth, and can be found in several ecological niches.

Conclusion

In summary, yeasts play an essential role in many food and beverage fermentations, conferring the required stability, safety, and sensory properties to the final product. Moreover, several beneficial health effects are clearly evident such as probiotic (prevention and treatment of intestinal diseases and immunomodulatory action), antigenotoxic, and antioxidant activities. Recent advances in molecular biology (DNA recombination, mutagenesis, etc.) can be applied to obtain specialized yeasts with new properties to improve human life.

See also: Cheese: Chemistry and Microbiology; Cheese: Composition and Health Effects; Fermented Foods: Use of Starter Cultures; Fermented Foods: Fermented Meat Products; Fermented Foods: Fermented Vegetables and Other Products; Fermented Foods: Composition and Health Effects; Fermented Foods: Fermented Milks; Food Additives: Classification, Uses and Regulation; Food Allergies; Foodborne Pathogens; Functional Foods; Probiotics; Spoilage: Yeast Spoilage of Food and Beverages; Wines: Wine Production.

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Yersinia enterocolitica: Detection and Treatment

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Introduction

Yersinia genus comprises 11 species: *Y. aldovae*, *Y. bercovieri*, *Y. enterocolitica*, *Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. mollaretii*, *Y. pestis*, *Y. pseudotuberculosis*, *Y. rohdei*, and *Y. ruckeri* (Table 1). Only three of them are pathogenic to humans: *Y. enterocolitica*, *Y. pestis*, and *Y. pseudotuberculosis*, causing from pneumonic plague to acute gastroenteritis. *Y. enterocolitica* comprises both pathogenic and nonpathogenic genotypes and is regarded as a relevant zoonotic foodborne pathogen, ranking just third among the notifiable bacterial zoonoses in the EU (6471 cases in 2013). *Y. enterocolitica* has been mainly isolated from pig meat and products thereof and in bovine meat and raw cow milk intended for direct human consumption as well as in other animal species, including wildlife animals, cattle, sheep, goats, dogs, cats, and solipeds. More details on *Yersinia enterocolitica* characteristics are provided elsewhere in this encyclopedia.

Detection of *Y. enterocolitica*

The main biochemical characteristics of *Y. enterocolitica* and related species are listed in Table 2.

Classical Microbiological Methods for Detection of *Y. enterocolitica* in Food

There is an international standard developed by the International Organization for Standardization (ISO) for the detection of pathogenic *Y. enterocolitica*: *ISO 10273:2003 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica*. This standard protocol compromises three consecutive steps: enrichment in selective broths, isolation in specific agar plates, and final confirmation. The flowchart is described in Figure 1.

Twenty-five grams or milliliters of food samples is homogenized in 225 ml of peptone–sorbitol–bile salt (PSB) broth, and subsequently, a fraction of this mixture is also 1:10 homogenated in a second selective broth irgasan ticarcillin chlorate (ITC). The sample containing PSB is incubated at 25 °C during 48–72 h with agitation or up to 5 days without agitation, whereas the sample containing ITC is incubated 25 °C during 48 h. Afterward, ITC and PSB broths are streaked on the cefsulodin–irgasan–novobiocin (CIN) agar and on the *Salmonella–Shigella* agar with deoxycholate and calcium chloride (SSDC) agar, respectively, and the plates are incubated at 30 °C for 24–48 h. In parallel, 0.5 ml of the PSB enrichment is transferred into 4.5 ml 0.5% potassium hydroxide solution (KOH) and is mixed gently and is also streaked on the CIN agar, and the plates are also incubated at 30 °C for 24–48 h. The typical *Y. enterocolitica* colony morphology on CIN agar plates is small colonies (1–2 mm diameter) with a deep red center surrounded by a clear, colorless zone with entire edge (a bull's eye aspect). For SSDC agar, the typical *Y. enterocolitica colony* morphology is ≈1 mm diameter small gray colonies with a non-defined border. Those presumptive colonies should be confirmed, and for that, up to five colonies should be taken for further analysis for species confirmation by presumptive assays (urea–indole, Kligler, and oxidase tests) and confirmation biochemical tests (lysine, ornithine, sucrose, rhamnose, trehalose, xylose, citrate, and Tween esterase tests) (Table 3). Once the isolates are confirmed as *Y. enterocolitica*, the biotype must be determined (Table 4) as well as the pathogenic character by presumptive pathogenicity tests (esculin, pyrazinamidase, and Ca^{2+} dependency tests at 37 °C) (Table 3). In addition, it is also advisable to

Table 1 Differentiation of *Yersinia* from other genera

Test	*Yersinia*	*Citrobacter*	*Enterobacter*	*Escherichia*	*Hafnia*	*Klebsiella*	*Proteus*	*Salmonella*
Voges–Proskauer	−	−	+	−	[+]	D	D	−
Citrate	−	+	+	[−]	−	D	D	+
D-Glucose	− or W	+	+	−	+	+	[+]	+
H_2S production	−	D	−	+	−	−	D	+
Phenylalanine	−	−	−	−	−	−	+	−
Lysine	−	−	[−]	[+]	+	[+]	−	+
Motility, 37 °C	−	+	+	[+]	[+]	−	+	+
Motility, 25 °C	+	+	+	[+]	[+]	−	+	+
KCN, growth	−	D	+	−	+	+	+	−
Malonate utilization	−	D	+	D	D	+	−	D
L-Arabinose, acid	−	+	+	+	+	[+]	[+]	+
D-Mannitol	+	+	+	+	+	+	+	+
Mucate	−	+	D	D	−	+	+	+

W, weak; D, divergent; [], most of the isolates.
Source: Bottone, E. J., Bercovier, H. and Mollater, H. H. (2005). Genus *Yersinia*. In: *Bergey's manual of systematic bacteriology* (2nd ed.), vol II.

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Table 2 Biochemical characteristics at 30 °C of *Y. enterocolitica* and related species

Test	Y. enterocolitica	Y. pseudotuberculosis	*Other related species*
Glucose	+	+	+
Gas production	−	−	−
Lactose	−	−	−
ONPG	±	+	±
Adonitol	−	−	−
Cellobiose	+	−	D
Galactitol	−	−	−
Mannitol	+	+	+
Melibiose	−	±	D
Rhamnose	−	+	D
Sucrose	+	−	D
Sorbitol	±	−	D
Trehalose	±	+	+
Xylose	D	+	+
Esculin	D	+	D
Salicine	D	+	D
Urea	+	+	+
Indole	D	−	D
Voges–Proskauer	±[a]	−	D
H_2S	−	−	−
Deaminase	−	−	−
Lysine	−	−	−
Ornithine	±	−	+
Citrate	−	−	D
Lipase (Tween)	D	−	D
Mucate	−	−	D

+, positive; −, negative; ±, most of the strains positive; D, divergent biochemical types.
[a]*Y. enterocolitica* strains are mainly negative at 37 °C.

perform serological tests using the somatic O-antigen for epidemiological purposes.

The US Food and Drug Administration (FDA) has a similar procedure for classic microbiological detection of *Y. enterocolitica*. Twenty-five grams or milliliters of food samples is also homogenized in 225 ml of PSB broth, but the miscut is incubated at 10 °C for 10 days. Afterward, 0.5 ml of the PSB enrichment is transferred into 4.5 ml 0.5% KOH and is mixed gently and streaked on both MacConkey and CIN agars and the plates are incubated at 30 °C for 24–48 h. The typical *Y. enterocolitica* colony morphology on MacConkey agar plates is small (1–2 mm diameter) flat, colorless, or pale pink colonies. Those presumptive colonies from both agar media (MacConkey and CIN) should be confirmed, and for that, up to five colonies should be taken for further confirmatory tests.

Alternative Methods for Detection of *Y. enterocolitica* in Food: Molecular-Based Methods

As described in details earlier, the detection of *Y. enterocolitica* in foods by classical methods becomes a laborious and time-consuming task; it requires at least 7 days for a presumptive result and additional tests and time are required for discrimination between pathogenic and nonpathogenic strains. In addition to this, the commercially available selective media are not completely specific for the *Y. enterocolitica* pathogenic strains hampering the correct selection of the pathogenic strains for confirmation due to the high amount of *Y. enterocolitica*-type colonies present in the agar and that the pathogenic and no pathogenic isolates are virtually indistinguishable.

To overcome those drawbacks, molecular-based methodologies have been developed to reduce the time for final results as well as to increase remarkably the detection sensitivity. Particularly, real-time polymerase chain reaction (PCR)-based methods can facilitate the rapid, sensitive, and specific detection of pathogenic *Y. enterocolitica*. In this sense, the international bodies for standardization ISO and CEN have an ad hoc expert group for molecular detection methods in food (TAG·), which recently has prepared a standard draft for the detection of pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, the *ISO/CD TS 18867 Microbiology of food and animal feed – Polymerase chain reaction (PCR) for the detection of food-borne pathogens – Detection of pathogenic Yersinia enterocolitica and Yersinia pseudotuberculosis*.

This standard method compromises four consecutive steps: microbial enrichment, nucleic acid extraction, amplification, and detection and isolation. The first step, the microbial enrichment, increases the number of pathogenic *Y. enterocolitica* cells by growth in a nonselective or semi-selective liquid nutrient medium. Subsequently, bacterial cells are separated from the nutrient broth and lysed and the nucleic acid extracted for use in a probe-based real-time PCR. After a PCR-positive result, *Y. enterocolitica* can be optionally isolated by using culture methods. The flowchart is described in Figure 2. In more detail, at least 25 g or ml of the sample is properly homogenized with 225 ml of PSB broth and is incubated at 25 °C for 24 h, but this period can be increased up to

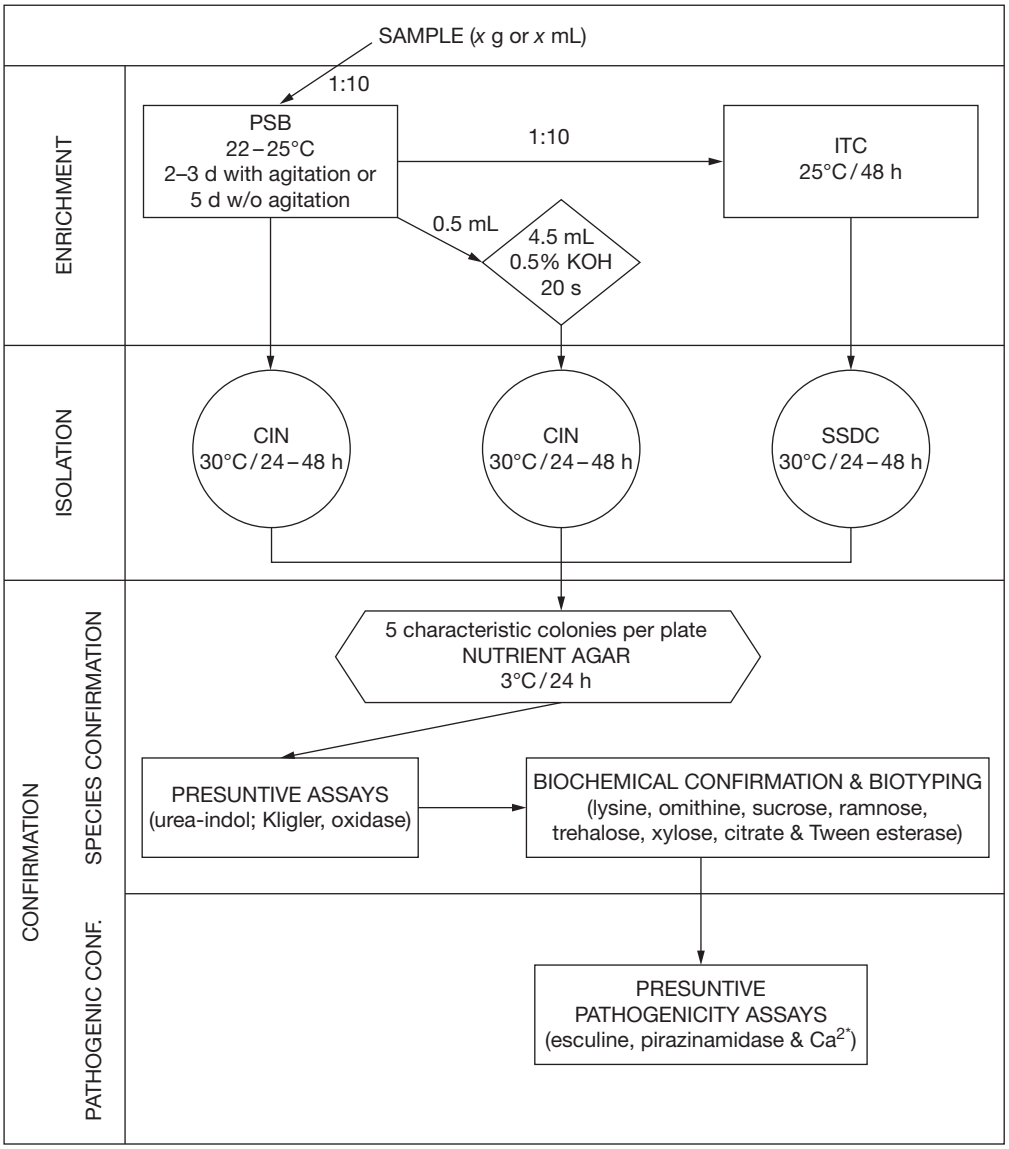

Figure 1

48 h as recommended in the classical standard method, ISO 10273, for certain food matrices. Then, 1 ml of the enriched culture is used for bacterial DNA using a suitable procedure appropriate for Gram-negative bacteria. Finally, a probe-based real-time PCR assay is used for the amplification of the extracted DNA targeting the *Y. enterocolitica* chromosomal virulence *ail* gene. Optionally and in parallel, the isolation of pathogenic *Y. enterocolitica* colonies can be performed. Five hundred milliliters of the PSB enrichment is transferred into 4.5 ml 0.5% KOH and is mixed gently, and 1, 10, and 100 µl of the mixture are inoculated on the surface of three CIN agar plates, which are incubated at 30 °C for 24 h. In parallel, the first 24 h enrichment is also extended for further 24 h and a second plating on CIN agar following KOH treatment is also made. The pathogenic *Y. enterocolitica* in CIN agar plates has a bull's eye appearance and has to be confirmed according to ISO 10273.

Treatment of *Y. enterocolitica*

The optimal treatment strategies for *Yersinia* infections are not evident; antibiotic treatment appears not to impact, in general, uncomplicated acute diarrhea and the value of antibiotic therapy has not been clearly established. Uncomplicated cases usually resolve on their own without antibiotic treatment, and the treatment is primarily supportive, with fluid and electrolyte replacement and good nutrition, avoiding antimotility medications, which could lead to invasion and bacteremia. However, antimicrobial treatment should be prescribed for patients with severe enterocolitis (Box 1), in which antibiotic chemotherapy can reduce the duration of illness, and in invasive infections with bacteremia and extraintestinal manifestations (Box 2).

The antibiotic arsenal available is wide, and *in vitro* susceptibility has been demonstrated to aminoglycosides,

Table 3 Characteristics of pathogenic *Y. enterocolitica* for confirmation tests according to ISO 10273

Type of test	*Assay*	*Result*	
Species confirmation			
Presumptive assays	Urease	+	
	Indole	±[a]	
	Kligler	Glucose fermentation	+[b]
		Lactose fermentation	−[c]
		Gas production	−
		H_2S production	−
	Oxidase	−	
Biochemical confirmation tests	Lysine	−	
	Ornithine	+	
	Sucrose	+	
	Rhamnose	±[d]	
	Trehalose	±[d]	
	Xylose	±[d]	
	Citrate	−	
	Tween esterase	±[d]	
Pathogenicity determination			
Pathogenicity tests[e]	Esculin	−	
	Pyrazinamidase	−	
	Ca^{2+} dependency at 37 °C[f]	+	

+, positive; −, negative; ±, most of the strains positive.
[a]The biotype 1 and some serotypes of biotype 2 are indole +, while biotypes 3, 4, and 5 and some serotypes of biotype 2 are indole −.
[b]Bottom of the tube with yellow color.
[c]Slant part with red color.
[d]Results depending of the *Y. enterocolitica* biotype.
[e]Pathogenic *Y. enterocolitica* strains are negative for esculin and pyrazinamidase tests and positive for the Ca^{2+} dependency at 37 °C test, while nonpathogenic *Y. enterocolitica* strains are positive for esculin and pyrazinamidase tests and negative for the Ca^{2+} dependency test at 37 °C.
[f]The pathogenicity mediated by a virulence plasmid.

Table 4 Biotyping scheme for *Y. enterocolitica*

Biotype	*Associates to human infections*	*Serogroup(s)*	*Esculin*	*Tween esterase*	*Salicine*	*Pyrazinamidase*	*Indole*	*Xylose*	*Trehalose*	*Ornithine*	*Voges–Proskauer*	*Sorbase*	*Inositol*	*Nitrate*
1A[a]	−	O:5, O:6, 30, O:7, 8, O:18, O:46,non-typable	+	+	+	+	+	+	+	+	+	+	+	+
1B	+	O:8, O:4, O:13a, O:13b, O:18; O:20, O:21	−	+	−	−	+	+	+	+	+	+	+	+
2	+	O:9, O:5, 27	−	−	−	−	+[b]	+	+	+	+	+	+	+
3	+	O:1, 2, 3, O:5, 27	−	−	−	−	−	+	+	+	+	+	+	+
4	+	O:3	−	−	−	−	−	−	+	+	+	+	+	+
5	+	O:2,3	−	−	−	−	−	D[b]	−	+(+)	+(+)	−	+	−

+, positive; −, negative; D, divergent biochemical types.
[a]Nonpathogenic.
[b]Usually weak or slow.

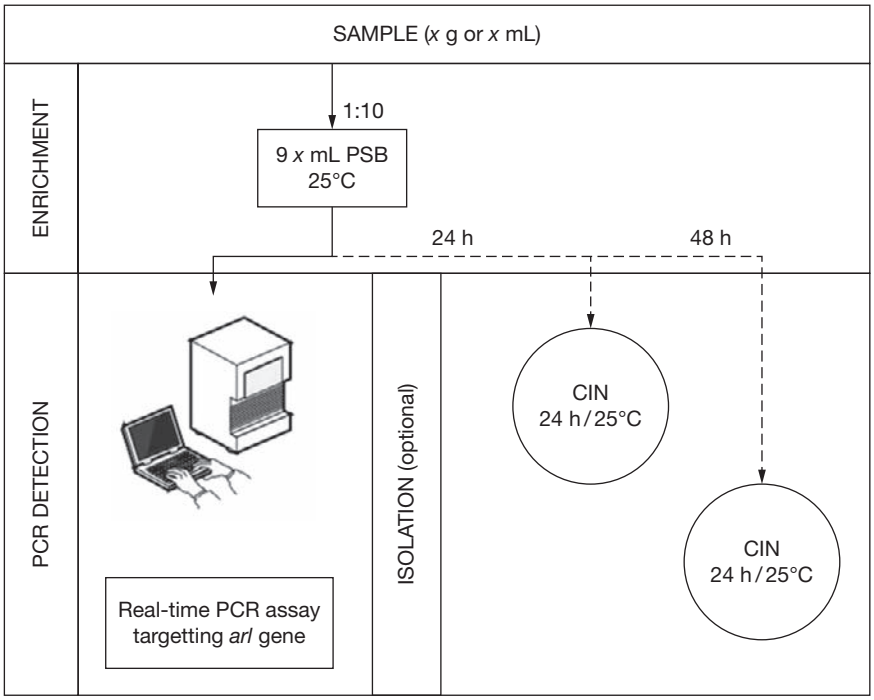

Figure 2

Box 1

Type of Patients in Which Antibiotic Therapy Should Be Considered

Elderly patients
Patients with diabetes
Patients with cirrhosis
Immunocompromised patients
Patients with cancer
Health workers who are at an increased risk of person-to-person spread

Box 2

Primary Extraintestinal Disorders in Patients in Which Antibiotic Therapy Must Be Used

Cellulitis
Ophthalmitis
Endocarditis
Meningitis
Osteomyelitis
Pneumonia
Focal abscesses

trimethoprim-sulfamethoxazole, chloramphenicol, tetracyclines (doxycycline), piperacillin, fluoroquinolones (ciprofloxacin), and third-generation cephalosporins. *Y. enterocolitica*, and particularly the serotype most commonly associated with illness (O:3), is often resistant to penicillin, ampicillin, and first-generation cephalosporins. Resistance to cefotaxime, macrolides, and fluoroquinolones is also sporadically reported.

Clinically, the first drug options are aminoglycosides and trimethoprim–sulfamethoxazole, but also doxycycline, third-generation cephalosporins, and ciprofloxacin can be used (Box 3). However, the use of tetracyclines is not recommended in children <8 years, and fluoroquinolones such as ciprofloxacin are not approved in the United States for use in children <18 years.

See also: Diarrheal Diseases; Emerging Foodborne Enteric Bacterial Pathogens; *Escherichia coli* and Other *Enterobacteriaceae*: Occurrence and Detection; Foodborne Pathogens; HACCP and ISO22000: Risk Assessment in Conjunction with Other Food Safety Tools Such as FMEA, Ishikawa Diagrams and Pareto; *Yersinia enterocolitica*: Properties and Occurrence; Zoonoses.

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Box 3

Type of Antibiotics Used in the Treatment of* Y. enterocolitica *Infections

Antibiotic	Description
Gentamicin	Aminoglycoside It acts by inhibiting bacterial protein synthesis Active against Gram-negative bacteria Not helpful for uncomplicated gastroenteritis
Trimethoprim and sulfamethoxazole or co-trimoxazole	They act by blocking the synthesis of dihydrofolic acid Active against Gram-negative and Gram-positive bacteria and protozoa Not helpful for uncomplicated gastroenteritis
Ciprofloxacin	Second-generation quinolone It acts by interfering with DNA gyrase Active against Gram-negative and Gram-positive bacteria
Ceftriaxone	Third-generation cephalosporin It acts by disrupting the synthesis of the peptidoglycan layer in the bacterial cell wall Active against Gram-negative
Cefotaxime	Third-generation cephalosporin It acts by disrupting the synthesis of the peptidoglycan layer in the bacterial cell wall Active against Gram-negative bacteria Not helpful for uncomplicated gastroenteritis
Doxycycline	Tetracycline It acts by inhibiting bacterial protein synthesis by binding with the 30S and 50S ribosomal subunits Active against Gram-positive and Gram-negative bacteria, as well as against *Mycoplasma*, *Chlamydia*, and *Rickettsia*
Chloramphenicol	It acts by inhibiting bacterial protein synthesis by binding to 50S bacterial-ribosomal subunit Active against Gram-negative and Gram-positive bacteria
Piperacillin and tazobactam sodium	An extended-spectrum beta-lactam antibiotic (ureidopenicillin class) (piperacillin) associated with a beta-lactamase inhibitor (tazobactam) It acts by inhibiting the biosynthesis of cell wall mucopeptide Active against Gram-negative bacteria

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Yersinia enterocolitica: Properties and Occurrence

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Introduction

Yersinia enterocolitica is one of the three human pathogenic species in the genus *Yersinia*. *Y. enterocolitica* and *Yersinia pseudotuberculosis* are enteropathogenic species that have an oral transmission route, while plague-causing *Yersinia pestis* is mainly transmitted by a flea vector. *Y. enterocolitica* is the most common species causing human enteric yersiniosis worldwide. Most of these cases are sporadic and outbreaks are uncommon. *Y. enterocolitica* can cause a variety of symptoms depending on the virulence of the strain and/or the age and the immune status of the infected person. Common symptoms are diarrhea and abdominal pain. Right-sided abdominal pain can be confused with appendicitis. Occasionally, typically among adults, joint pain (reactive arthritis) and skin rash (erythema nodosum) may occur. *Y. enterocolitica* can be transmitted to human via contaminated food, especially via contaminated pork. Pigs are so far the most important reservoir for human pathogenic strains. Because a vast amount of nonpathogenic *Y. enterocolitica*- and *Y. enterocolitica*-like strains are circulating in the environment, it is important to differentiate between pathogenic strains with clinical significance and harmless nonpathogenic strains. Furthermore, *Y. enterocolitica* strains are very heterogeneous in their biochemical and antigenic properties.

Properties

Taxonomy

Y. enterocolitica is one of the three human pathogenic species in the genus *Yersinia*. The two other human pathogenic species *Y. pseudotuberculosis* and *Y. pestis* share only about 50% DNA homology with *Y. enterocolitica*. At the moment, the genus *Yersinia* comprises 17 species including the fish pathogen *Y. ruckeri*. *Yersinia* is a member of the family *Enterobacteriaceae* belonging to *Gammaproteobacteria* class of phylum *Proteobacteria*. *Yersinia* bacteria are a group of gram-negative, oxidase-negative, catalase-positive, lactose-negative, and facultative anaerobic rods or coccobacilli of $0.5–0.8 \times 1–3$ μl in size. They possess neither a capsule nor spores. *Y. enterocolitica* strains are mostly urease-positive, and they ferment sucrose but not rhamnose and melibiose. The chromosome of *Y. enterocolitica* strains is about 4.6 Mb. The G + C content of 47.3% is lower than that of most members of *Enterobacteriaceae*. The 16S rRNA gene sequence similarities of *Yersinia* strains range from 96.9% to 99.8%. *Y. enterocolitica* has been subdivided into two subspecies based on the 16S rRNA sequences and DNA–DNA reassociation values. *Y. enterocolitica* subsp. *enterocolitica* comprises the *Y. enterocolitica*-type strain ATCC 9610^T and strains from high-pathogenic biotype 1B, while *Y. enterocolitica* subsp. *palearctica* comprises the strains from low-pathogenic biotypes 2–5 and nonpathogenic biotype 1A.

Biochemical Properties

Yersinia strains are more active biochemically between 25 and 30 °C than at 37 °C, and thus, temperatures between 25 and 30 °C are typically used for biochemical tests. Identification of *Y. enterocolitica* is still mainly based on biochemical reactions, typically by using commercial kits. However, it may be difficult to differentiate *Y. enterocolitica* from some nonpathogenic *Yersinia* spp. like *Y. massiliensis*, *Y. mollaretii*, and *Y. bercovieri* by biochemical tests. For proper identification and pathogenicity assessment, genotypic methods are often needed.

Y. enterocolitica species is a heterogeneous group of bacteria based on biochemical, antigenic, and virulence properties. Based on biochemical properties, *Y. enterocolitica* strains can be divided into six biotypes. At least five biochemical reactions have to be tested to identify the different biotypes (Table 1). Strains of biotype 1A are considered nonpathogenic, but strains belonging to the biotypes 1B and 2–5 are human and/or animal pathogens. Biotype 1B strains are highly virulent, whereas biotypes 2–5 have a lower pathogenicity. The pyrazinamidase activity test is used to differentiate between potentially pathogenic and nonpathogenic strains.

Antigenic Properties

Serotyping based mainly on the surface O antigenic variation of lipopolysaccharides (LPS) in the cell wall is widely used for *Y. enterocolitica*. Subdivision of *Yersinia* species to serotypes provides useful information concerning their potential pathogenicity and possible reservoir. *Y. enterocolitica* comprises over 30 serotypes. Serotypes O:3, O:5, O:8, and O:9 are associated with human diseases; however, they can also be found in nonpathogenic strains. In Europe, predominating human serotypes are O:3 and O:9. Pigs are worldwide the most important reservoir of the serotype O:3.

Biotype and Serotype Combinations

Only certain bioserotypes of *Y. enterocolitica* have been associated with human and animal diseases. The most common bioserotypes recognized in human yersiniosis are 4/O:3, 2/O:9, 2/O:5,27, 3/O:3, and 1B/O:8. Bioserotype 4/O:3 is the most common through the world followed by 2/O:9 and 2/O:5,27. Bioserotype 3/O:3 is most commonly reported in Japan and China. Highly pathogenic bioserotype 1B/O:8, which has originally been isolated mainly in the United States and Canada, has appeared also in Europe and Japan. An increasing number of human yersiniosis due to clonal 1B/O:8 strains have been reported in different regions in Poland. Strains belonging to bioserotypes 3/O:1,2,3 and 5/O:2,3 have been isolated from chinchillas and hares, respectively. Biotypes 3 and 5, serotype O:2,3 have also been reported in sheep and goats in Australia. Additionally, pathogenic

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Y. enterocolitica of bioserotype 5/O:3 has been isolated from an alpine ibex in Switzerland.

Phage Types

Y. enterocolitica strains can be divided into phage types. Two schemes (Swedish and French) have been used for typing. French scheme recognizes 12 phage types (I–X, including IXa–c) and Swedish scheme seven phage types (A1, A2, B1, B2, C32, C61, and E1). The French scheme has been used more frequently. Bioserotype 4/O:3 strains, which belong to phage type VIII, predominate in Europe and Japan, whereas phage type IXb has been isolated in Canada. Phage typing is available only in few laboratories and thus very rarely used.

Antimicrobial Resistance

Several antimicrobial agents have been used to study the susceptibility of *Y. enterocolitica* strains. *Y. enterocolitica* strains are mostly resistant to penicillins and first-generation cephalosporins due to β-lactamase production. *Y. enterocolitica* strains are typically sensitive to aminoglycosides, third-generation cephalosporins, co-trimoxazole, tetracyclines, chloramphenicol, and fluoroquinolones. Sporadic resistance to amoxicillin/clavulanic acid, streptomycin, tetracycline, and sulfamethoxazole/trimethoprim occurs (Table 2). There are some differences between the resistance patterns among strains of different bioserotypes; biotype 2 strains are more frequently resistant to amoxicillin/clavulanic acid and bioserotype 4/O:3 strains to streptomycin (Table 2). So far, multiresistant *Y. enterocolitica* strains have rarely been reported. Nalidixic acid resistance in *Y. enterocolitica* strains has been linked to the point mutation in the *gyr*A gene.

Table 1 Minimal repertoires of biochemical tests needed to identify different *Yersinia enterocolitica* biotypes

	Reactions for biotype					
Biochemical test	*1A*	*1B*	*2*	*3*	*4*	*5*
Pyrazinamidase activity	+	−	−	−	−	−
Tween-esterase activity	+	+	−	−	−	−
Indole production	+	+	+[a]	−	−	−
Xylose acidification	+	+	+	+	−	+/−
Trehalose acidification	+	+	+	+	+	−

[a]Tryptophan in the test medium and a heavy bacterial suspension are needed for a clear positive reaction.

Virulence

Y. enterocolitica strains are transmitted via the oral route. After passage through the stomach, pathogenic strains colonize the intestinal tract, penetrate the intestinal mucosa, and infect the underlying tissue. Pathogenic *Y. enterocolitica* strains are invasive and have a capacity to resist nonspecific immune responses.

All pathogenic *Yersinia* strains carry an approximately 70-kb virulence plasmid abbreviated as pYV (plasmid for *Yersinia* virulence), which is essential for the virulence (Table 3). The pYV allows *Yersinia* to survive and replicate within lymphoid tissues of the host. It codes for the outer membrane protein YadA (Yersinia adhesion A), a set of secreted proteins called Yops (Yersinia outer membrane protein), and their secretion apparatus called Ysc (Yop secretion), which is a type III secretion system (T3SS). The genes on the pYV are controlled by temperature and calcium concentration and are optimally expressed at 37 °C and in low calcium ion conditions.

Host cell invasion is promoted mainly by the chromosomal-encoded Inv (invasion) and Ail (attachment invasion locus) proteins and the pYV-encoded YadA protein. Inv binds to β1-integrin receptors on the M cells and induces uptake of the bacteria into the epithelial cells during the initial stage of infection. Ail, which is typically found in pathogenic strains, is involved in the attachment and invasion of the host

Table 2 Antimicrobial resistance of pathogenic *Yersinia enterocolitica* strains isolated from humans and pigs

			Resistance (%)					
Source	*No. of strains*	*Bioserotype*	*A*	*AC*	*S*	*T*	*Sxt*	*Reference*
Germany								
Human	60	4/O:3	95	0	5	0	2	Bucher et al.
Pig	140	4/O:3	99	0	8	0	0	
Italy								
Pig	4	2/O:9	100	0	0	0	0	Bonardi et al.
	25	4/O:3	96	0	20	16	8	
Lithuania								
Human	19	4/O:3	100	NS	5	5	NS	Novoslavskij et al.
Pig	41	4/O:3	100	NS	5	5	NS	
Switzerland								
Human	4	2/O:5,27	100	100	0	0	0	Fredriksson-Ahomaa et al.
	22	2/O:9	100	95	0	0	0	
	47	4/O:3	100	15	26	0	6	
Pig	6	2/O:5,27	33	100	0	0	0	Fredriksson-Ahomaa et al.
	2	2/O:9	100	0	0	0	0	
	69	4/O:3	100	0	6	0	1	

A = ampicillin, AC = amoxicillin and clavulanic acid, S = streptomycin, T = tetracycline, Sxt = sulfamethoxazole and trimethoprim.

Table 3 Correlation between biotype, serotype, virulence markers, and the pathogenicity of *Yersinia enterocolitica* strains

			Chromosomal-encoded markers				
Biotype	*Serotypes*[a]	*pYV*[b]	*Ail*	*YstA*	*YstB*	*HPI*[c]	*Pathogenicity*
1A	O:5, O:6, O:8	−	−	−	+	−	None
1B	O:8, O:13, O:20, O:21	+	+	+	−	+	High
2	O:5,27, O:9	+	+	+	−	−	Low to moderate
3	O:1,2,3, O:3	+	+	+	−	−	Low to moderate
4	O:3	+	+	+	−	−	Low to moderate
5	O:2,3	+	+	+	−	−	Low to moderate

[a]The most frequently reported serotypes.
[b]Virulence plasmid of pathogenic *Yersinia* strains.
[c]High-pathogenicity island.

Table 4 Important virulence associated genes of pathogenic *Yersinia enterocolitica*

Genomic origin	*Gene*	*Protein*	*Function*
Plasmid (pYV)	*yad*A	YadA	Attachment, invasion, serum resistance
	yop	Yops	Antiphagocytic action
	ysc	Ysc	Deliver of Yops
Chromosome	*ail*	Ail	Attachment, invasion, serum resistance
	inv	Inv (invasion)	Attachment, invasion
	*yst*A	YstA (enterotoxin)	Diarrhea
	*myf*A	MyfA (fibrilla)	Colonization
	ybt	Yersiniabactin	Iron acquisition
	ure	Urease	Survival in acidic environments

cell. It enables bacterial dissemination to mesenteric lymph nodes by protecting bacteria against host immune system. YadA is a multifunctional virulence factor that promotes binding to collagen, fibronectin, and laminin. It is upregulated at 37 °C when the bacteria are replicating in the Peyer's patches. YadA-promoted adhesion to collagen is crucial to the inflammation of joints in reactive arthritis.

Furthermore, extracellularly located *Y. enterocolitica* strains deliver Yops into the cytosol of the target cell with the Ysc. These Yops destroy the phagocytic and signaling capacities of host macrophages and finally introduce apoptosis and in this way enable *Yersinia* to survive in the host environment.

Serum resistance is critical for the survival of invading bacteria like *Y. enterocolitica* and the establishment of disease. Pathogenic *Y. enterocolitica* strains have the ability to withstand the activity of complement-dependent killing. Two outer membrane proteins of *Y. enterocolitica*, pYV-encoded YadA and chromosomally encoded Ail, are the main factors involved in the serum resistance. They protect the bacteria from complement-mediated lysis and phagocytosis. YadA is the most important serum resistance factor needed for long-term survival. Ail provides a short-term survival and it delays the bacterial killing. In addition, the outer membrane LPS may indirectly be involved in the serum resistance.

The highly pathogenic *Y. enterocolitica* 1B strains harbor the high-pathogenicity island (HPI) in the chromosome. The HPI is a cluster of genes intended for iron uptake and is involved in synthesis of the yersiniabactin, a siderophore responsible for the systemic dissemination of bacteria in the host. Biotype 1B strains are lethal to mice in contrast to strains of biotypes 2–5, which do not kill mice unless iron is made available to bacteria by pretreatment of mice with desferrioxamine.

A heat-stable enterotoxin called Yst is produced by *Y. enterocolitica*. There are three subtypes of Yst: YstA, YstB, and YstC. YstA is mainly produced by pathogenic strains and YstB by nonpathogenic ones (Table 3). The role of Yst in the pathogenesis of *Y. enterocolitica* infection is still unclear; however, the YstA, which is produced by pathogenic strains, may contribute to the pathogenesis of diarrhea.

Presence of Virulence Genes

Y. enterocolitica strains can carry different genes associated with the pathogenicity on the pYV and in the chromosome (Table 4). The occurrence of these genes has been extensively studied. Most of the *Y. enterocolitica* strains carry the urease-encoding gene complex (*ure*) in the chromosome. Urease helps *Yersinia* strains to survive the gastric barrier of the stomach before entering the small intestine. Some *Y. enterocolitica* strains, especially the pathogenic ones, carry the chromosomal *myf*A gene, which encodes the production of mucoid *Yersinia* fimbriae (MyfA) associated with the colonization of intestinal epithelium. The chromosomal *ail* gene is assumed to be detected only in pathogenic *Y. enterocolitica* strains; however, recently, this gene has also sporadically been found in nonpathogenic strains. The *yst*A gene is present mainly in

pathogenic strains, while *yst*B is frequently present in nonpathogenic *Yersinia* species.

Pathogenic strains belonging to biotypes 1B and 2–5 typically carry the virulence genes located on the pYV and the most important chromosomal genes: *ail*, *yst*A, and *myf*A. The highly pathogenic biotype 1B strains carry the *ybt* genes located in the HPI, and these genes are missing in the strains with lower pathogenicity. Biotype 1A strains of *Y. enterocolitica* are considered to be nonpathogenic because they always lack the genes on the pYV and in the HPI. Additionally, they typically also miss the most important chromosomal virulence genes: *ail*, *yst*A, and *myf*A (Tables 3 and 4). The *yst*B gene is usually detected in the biotype 1A strains.

Growth Factors

Y. enterocolitica strains are nutritionally very unfastidious. In food, pH and a_w values are the most important intrinsic factors for the growth of *Yersinia*. The optimum pH is around 7, but *Y. enterocolitica* is able to multiply over a pH range from 4 to 10 and can survive alkaline conditions better than many other gram-negative bacteria. Alkalotolerance of *Yersinia* has been used to separate *Yersinia* strains from background organisms by the treatment of enrichment broth with potassium hydroxide (0.25–0.5%) before streaking onto the agar plates. Survival of *Y. enterocolitica* during the passage through stomach suggests that *Y. enterocolitica* strains are also relatively acid-resistant. Salt (NaCl) tolerance of *Yersinia* is moderate and strongly dependent on storage temperature. *Yersinia* strains can tolerate NaCl at concentrations of up to 5%, while 7% of NaCl has shown to be bacteriostatic. Thus, salt alone will typically not prevent the growth of *Yersinia* and other preservative hurdles like nitrate/nitrite are needed.

The most important extrinsic factors include temperature and gas atmosphere. *Y. enterocolitica* strains are psychrotrophic and have an ability to grow at temperatures between 0 and 44 °C. The optimum growth temperature is around 30 °C. Even though *Yersinia* strains can grow at refrigeration temperatures (0–6 °C), they grow much more slowly below 5 °C. *Yersinia* strains can survive in frozen foods for extended periods even after repeated freezing and thawing. They are susceptible to heat and can be destroyed by pasteurization at 72 °C for 15–20 s. As a facultative anaerobic bacterium, *Y. enterocolitica* can grow in aerobic and anaerobic conditions. *Yersinia* strains can also grow well in modified atmosphere with higher levels of CO_2. Pathogenic *Y. enterocolitica* 4/O:3 strains have shown to grow well on meat when packaged in modified atmosphere with high carbon dioxide (30%) and oxygen (70%) levels and stored at 6 °C even in the presence of high number of lactic acid bacteria.

Occurrence

In Humans

Pathogenic *Y. enterocolitica* strains have been isolated from humans on all continents but the reported incidences differ greatly. Over 7000 cases of yersiniosis were reported in the European Union (EU) in 2011. *Y. enterocolitica* was the most common species reported; it was identified from 98% of the confirmed cases. The number of cases increased by 3.5% compared to 2010. The highest country-specific notification rates were reported in Lithuania and Finland, 11.4 and 10.3 cases per 100 000 inhabitants, respectively. The EU case fatality rate was low (0.02%) in 2011. The incidence of yersiniosis was highest among children under 5 years. However, comparison of the data between different countries is not possible because of the inconsistency of surveillance systems.

Y. enterocolitica bioserotype 4/O:3 is the most common type associated with human disease followed by bioserotype 2/O:9 in Europe. Strains belonging to biotype 1A are usually regarded as nonpathogenic, but discussion has been raised of a potential pathogenicity of some strains of the biotype 1A. However, biotype 1A strains, which were recently isolated from feces of humans with and without diarrhea in Switzerland, were characterized, and no clear difference between *Y. enterocolitica* 1A strains isolated from healthy and symptomatic humans could be demonstrated.

In Animals

Numerous studies have been conducted to isolate *Y. enterocolitica* strains from a variety of animals including farm animals, domestic pets, experimental animals, wild animals, and captive animals. Most of the strains isolated from animal sources, except pigs, are missing the important virulence genes needed for infection. Furthermore, they usually differ both biochemically and serologically from strains isolated from humans with yersiniosis.

Human pathogenic strains have frequently been isolated from pigs. The occurrence of pathogenic *Y. enterocolitica* is dependent on the age of the pigs and sample source: the pathogen can at first be isolated from feces and tonsils before antibodies can be detected by serology. The highest occurrence of pathogenic strains has been obtained in the tonsils of finishing pigs probably due to the high number of this pathogen present in the tonsils. The isolation rates of pathogenic *Y. enterocolitica* strains in pig tonsils at slaughter have shown to be very high in Europe (Table 5). The most frequently isolated bioserotype is 4/O:3, which has a worldwide distribution in the pig population. Serological investigations have shown that 60–90% of the herds are infected and nearly all finishing pigs in infected herds are seropositive. Pathogenic *Y. enterocolitica* strains have typically not been isolated from young breeding pigs but from pigs that have been moved to the fattening pens. More intensive farming and production systems have probably contributed to the high occurrence of this pathogen in fattening units. The occurrence has been shown to be highest in fattening units where pigs have been purchased from various pig producers. Recently, it has been shown in Belgium that there can be a large variation in the within-batch occurrence (between 0% and 83%) among pig farms.

Pathogenic *Y. enterocolitica* strains of different bioserotypes have occasionally also been isolated from cats and especially from dogs. Wild animals, especially wild rats near the pig houses, have shown to excrete pathogenic *Y. enterocolitica* sporadically in the feces. Recently, pathogenic *Y. enterocolitica* 5/O:3 was isolated from an alpine ibex in Switzerland; however, all strains isolated from wild deer were nonpathogenic *Yersinia* spp. indicating that the occurrence of pathogenic

Table 5 Recent isolation rates of pathogenic *Yersinia enterocolitica* in pig tonsils

Country	*Occurrence (%)*	*Bioserotypes*
Belgium	44–46	3/O:9, 4/O:3
Canada	2	2:O5,27, 4/O:3
China	15	2/O:9, 3/O:3, 4/O:3
England	44	2/O:5, 2/O:9, 4/O:3
Estonia	89	4/O:3
Finland	59	4/O:3
Germany	61	4/O:3
Greece	13	4/O:3
Italy	32	2/O:5, 4/O:3
Latvia	35–64	4/O:3
Russia	34	4/O:3
Spain	93	4/O:3
Switzerland	34	2/O:5, 2/O:9, 4/O:3

Source: Reproduced from Laukkanen, R., Fredriksson-Ahomaa, M. and Korkeala, H. (2013). Enteropathogenic *Yersinia* in foods. In: Tham, W. and Danielsson-Tham, M. L. (eds.) *Food associated pathogen*, pp. 313–338. Boca Raton, FL: CRC Press; Liang, J., Wang, X., Xiao, Y., et al. (2012). Prevalence of *Yersinia enterocolitica* in pigs slaughtered in Chinese abattoirs. *Applied and Environmental Microbiology* **78**, 2949; Van Damme, I., Berkvens, D. and De Zutter, L. (2012). Effect of sampling and short isolation methodologies on the recovery of human pathogenic *Yersinia enterocolitica* from pig tonsils. *Foodborne Pathogens and Disease* **9**, 600–606.

Y. enterocolitica is low in wild ruminants. The occurrence in wild boar is clearly higher being around 10%.

In Food

Food, especially raw or undercooked pork, has often been suggested to be the main infection source of human *Y. enterocolitica* infections even though pathogenic strains have seldom been isolated from food samples. Using Polymerase Chain Reaction (PCR), pathogenic *Y. enterocolitica* has been detected more frequently not only especially in pig offal samples (the tongue, lungs, heart, liver, and kidney) but also in samples from the surface of freshly slaughtered pig and poultry carcasses and in pork samples (Table 6). In the United States, the occurrence of pathogenic *Y. enterocolitica* 4/O:3 has shown to be high in chitterlings, a traditional holiday dish made of pig intestines in the south.

In the Environment

Nonpathogenic *Yersinia* strains are common findings in the environment, while pathogenic *Y. enterocolitica* strains are rare. However, strains of biotype 4/O:3 have occasionally been isolated from the environment of slaughterhouses and butchers. Using PCR, pathogenic *Y. enterocolitica* strains have been detected in a variety of environmental sources in pig slaughterhouses (Table 7). In case–control studies, untreated drinking water has also been reported to be a risk factor for sporadic *Y. enterocolitica* infections, and thus, drinking water has relatively widely been investigated. However, only rarely have pathogenic strains been found in water samples. Pathogenic *Y. enterocolitica* strains have occasionally been isolated from sewage water.

Table 6 Occurrence of pathogenic *Yersinia enterocolitica* in raw food samples

Sample		*Occurrence (%)*	
		Culturing	*PCR*
Carcass	Broiler	0	10
	Pig	0	16
Pork	Tongue	26–47	45–83
	Edible offal	35	70
	Ground/minced	0–5	5–38
	Sausages	0	9
	Chitterling	2	79
Chicken	Meat	0	0–1
Game	Meat	0	6–38
Lettuce		0	3–6
Tofu		0	6

Source: Reproduced from Laukkanen-Ninios, R. and Fredriksson-Ahomaa, M. (2012). Epidemiology and genetics of enteropathogenic *Yersinia* species. In: Faruque, S. M. (ed.) *Foodborne and waterborne bacterial pathogens: Epidemiology and molecular biology*, pp. 269–287. Linton, UK: Caister Academic Press.

Table 7 Occurrence of pathogenic *Yersinia enterocolitica* in environmental samples

Sample	*Occurrence (%)*	
Slaughterhouse	*Culturing*	*PCR*
Equipment (brisket saw, hooks, and knives)	14[a]	14
Floors, tables	0	21
Aprons	0	16
Computer keyboard	33[a]	33
Handgrip of the coffee maker	0	33
Untreated water	1	10

[a]*yadA*-positive *Y. enterocolitica* 4/O:3.

Source: Reproduced from Fredriksson-Ahomaa, M., Stolle, A. and Korkeala, H. (2006). Molecular epidemiology of *Yersinia enterocolitica* infections. *FEMS Immunology and Medical Microbiology* 47, 315–329.

Conclusion

Y. enterocolitica is one of the three human pathogenic *Yersinia* species. It is the most frequent cause of yersiniosis, which is one of the most frequently reported bacterial enteric diseases, especially among young children, in Europe. Strains belonging to *Y. enterocolitica* species are very heterogeneous in their biochemical, antigenic, and virulence properties. Pathogenic strains carry the pYV virulence plasmid and several important chromosomal genes associated with the virulence. The most common and widely distributed human pathogenic *Y. enterocolitica* belongs to the bioserotype 4/O:3. This type is frequently isolated from the tonsils of finishing pigs at slaughter. *Y. enterocolitica* strains missing the virulence plasmid and the important chromosomal virulence genes are ubiquitous and frequently isolated from nonhuman sources. Thus, it is important to confirm the pathogenicity of the isolated strains to assess their significance for human and animal health.

See also: Food Poisoning: Epidemiology; Foodborne Pathogens; *Yersinia enterocolitica*: Detection and Treatment; Zoonoses.

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Yogurt: Dietary Importance

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Introduction

Yogurt is a semisolid fermented milk product obtained by the action of specific lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) when added to heat-treated milk. In some countries, the term yogurt is restricted to products made exclusively by the two cultures, while other countries also allow adjunct probiotic cultures to be labeled as yogurt. According to the National Yogurt Association's criteria for 'live and active culture yogurt,' the finished product must contain live bacteria in amounts of $\geq 10^8$ organisms per gram at the time of manufacture, and the cultures must remain active at the end of the stated shelf life.

Regulatory authorities and scientific institutions in most countries acknowledge the importance of yogurt as part of a balanced and healthy diet. Yogurt is often included in the lists of healthy foods due to its high nutritive value. It is an excellent source of proteins, vitamins, and minerals. Yogurt can be prepared specifically to meet particular nutritional requirements of people whose normal processes of metabolism are altered or those who want a particular effect by controlled intake of foods or certain nutrients. Dietary strategies include lowering of calorie content by reducing fat and lactose content and fortification of minerals, vitamins, bioactive substances, or beneficial organisms.

Nutritional Value

Yogurt contributes to diet quality by providing substantial amounts of macronutrients and essential micronutrients. The composition of yogurt is similar to that of the milk from which it is produced; however, the changes in milk constituents that occur during lactic acid fermentation influence the nutritional value of the finished product. The final nutritional composition of yogurt is also affected by the strains of bacteria used, sources and types of milk solids added to milk before fermentation, and the temperature and duration of the fermentation. Moreover, a wide variety of yogurt is available globally, varying in fat and additive content and differing in nutritional status (Table 1).

Milk and milk products are the exclusive source of the lactose in human diets. Before absorption, lactose is hydrolyzed by intestinal β-galactosidase (lactase) into glucose and galactose. These monosaccharides are absorbed and used for energy in the body. However, those with impaired lactose metabolism (lactose-intolerant) cannot utilize lactose. Yogurt bacteria produce functional lactase, the enzyme that hydrolyses 20–30% milk lactose to its more readily absorbed monosaccharides, which may contribute to better tolerance of lactose in yogurt by those who are lactose-intolerant.

Protein in yogurt is more digestible than native milk proteins as bacterial proteolytic enzymes predigest milk proteins. Among the yogurt cultures, *L. delbrueckii* subsp. *bulgaricus* is more proteolytic in nature, which increases the amounts of peptides and free amino acids after milk fermentation. The amino acids liberated also stimulate growth of *S. thermophilus* in a symbiotic association. Heat treatment and acid production during fermentation result in finer coagulation of casein, which may contribute to improved protein digestibility. Both the caseins and whey proteins in yogurt are rich sources of essential amino acids, and the intestinal availability of nitrogen has been reported to be as high as 93%.

Fat undergoes minor changes due to lipase activity, which liberates free fatty acids after fermentation of milk. Yogurt has been shown to have a higher concentrations of conjugated linoleic acid (CLA), a long-chain biohydrogenated derivative of linoleic acid. In addition, yogurt is an excellent source of highly bioavailable calcium and phosphorus. The acidic pH ionizes calcium and magnesium, which facilitates intestinal uptake. One of the major functions of calcium in conjunction with vitamin D is in bone formation and mineralization.

Dairy products in general are an excellent source of B vitamins (riboflavin, niacin, vitamin B6, and vitamin B12). However, during fermentation, some are lost because vitamins are sensitive to changes in environmental factors. Similarly, lactic acid bacterial (LAB) species require B vitamins for growth, but some cultures are capable of synthesizing it. Significant losses can be corrected with the careful use of supplementary LAB cultures that are capable of synthesizing B vitamins. Folate is one of the best examples of a B vitamin that some LAB species synthesize in situ. Depending on the bacterial strains used, folate content of yogurt can vary widely, ranging from 4 to 19 μg/100 g. Inclusion of folate-producing cultures will increase the folate content of the final product.

Dietary Yogurt

Modern consumers are increasingly interested in their personal health and expect the foods they consume to be healthy or support optimal health, reducing the risk or preventing noncommunicable diseases. A number of functional ingredients are being incorporated in yogurt and yogurt-related products to improve market value.

Low-Calorie Yogurt

Over the past decade, attempts to develop low-calorie yogurts to meet consumer needs have been made with some success. Fat and lactose are two major components responsible for increased caloric value of yogurt. For the production of low-calorie yogurt, strategies include either removal of these components from the yogurt base or replacement with low-calorie alternatives. In the case of reduced/

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Table 1 Nutrient content of nonfat milk and nonfat plain, fruit-flavored, and Greek yogurt

Nutrient content	*Nonfat milk with vitamins A and D (100 g)*	*Nonfat milk with vitamins A and D (8 oz RACC)*	*Nonfat yogurt, plain (100 g)*	*Nonfat yogurt, plain (8 oz RACC)*	*Nonfat yogurt, fruit-flavored with vitamin A (100 g)*	*Nonfat yogurt, fruit-flavored with vitamin A (8 oz RACC)*
Water (g)	90.84	226.74	85.23	193.47	75.4	171.16
Energy (kcal)	34	85	56	127	95	216
Protein (g)	3.37	8.41	5.73	13.01	4.4	9.99
Fat (g)	0.08	0.20	0.18	0.41	0.20	0.45
Carbohydrate (g)	5.09	12.70	7.68	17.43	19	43.13
Calcium (mg)	122	305	199	451	152	345
Magnesium (mg)	11	27	19	43	14	32
Phosphorus (mg)	101	252	157	356	119	270
Potassium (mg)	156	389	255	579	194	440
Sodium (mg)	42	105	77	175	58	132
Iron (mg)	0.03	0.07	0.09	0.20	0.07	0.16
Zinc (mg)	0.42	1.05	0.97	2.2	0.74	1.68
Vitamin A (IU)	204	509	7	16	12	27
Vitamin C (mg)	0	0	0.9	2.04	0.7	1.59
Vitamin D (IU)	47	117	0	0	52	118
B6 (mg)	0.037	0.092	0.053	0.12	0.040	0.091
B12 (μg)	0.50	1.248	0.61	1.38	0.47	1.07
Folate (μg)	5	12	12	27	9	20
Niacin (mg)	0.094	0.235	0.124	0.281	0.100	0.227
Riboflavin (mg)	0.182	0.454	0.234	0.531	0.180	0.409
Thiamin (mg)	0.045	0.112	0.048	0.109	0.04	0.091

RACC, reference amount customarily consumed; Oz, unit of liquid (1 oz = 33.81 g ml^{-1}).
Source: Data obtained from the USDA National Nutrient Database (SR 01116).

low-lactose yogurts, three approaches have been used: (a) ultrafiltration of skimmed milk to decrease lactose, (b) addition of lactase and/or prolonged culture to further reduce the lactose content, and (c) artificial sweeteners. The yogurt produced is low in calorie, artificially sweetened with low/very low levels of lactose.

Low-fat and fat-free yogurts have gained popularity because of increasing demand for more healthy options across product categories. In the case of reduced fat yogurt, ingredient selection is important, such as skimmed milk and nonfat dry milk (NFDM). The consequences of adding NFDM, however, can be undesirable since about half of NFDM is lactose, which increases the caloric value of the resulting product. A powdery flavor can also be detected in yogurts fortified with too much of NFDM. Attempts have been made to produce nonfat low-calorie yogurt by adding sugar esters as a fat substitute, aspartame as a sweetener, and a cream-like substance as a flavor enhancer, which provides a better mouthfeel and texture compared with yogurt without fat substitutes. Nonfat yogurt is normally softer and low in total solid (10–12%), and consequently, whey separation is common. To reduce syneresis, thickening agents such as gelatin, starch, cellulose derivatives, alginates, and carrageenan, approved by FDA, can be added. Gelatin and carrageenan promote clustering of casein micelles, while other additives thicken the liquid content, thereby reducing syneresis. Yogurt texture may also be improved by the utilization of ropy strains of yogurt bacteria, that is, *S. thermophilus* and *L. bulgaricus*.

Yogurt with Bioactive Compounds

Almost all milk components may contribute to potential health benefits including proteins, peptides, lipids, carbohydrates, minerals, and vitamins. Some are native to products, while others are produced as a result of bacterial activity or added subsequently to improve functional characteristics (Table 2).

The presence of lactic acid bacteria is thought to be essential for yogurt to exert immune-stimulatory effects, but components of nonbacterial origin, such as whey protein, short peptides, and CLA, are also believed to contribute to beneficial effects as well. Besides the main proteins and naturally occurring bioactive peptides and minor proteins in milk, the activities of bacterial proteases during the fermentation change the physicochemical state(s) of milk proteins leading to the release of free amino acids and bioactive peptides. The most important metabolites in fermented milk may be peptides, which are not present prior to fermentation. Table 3 summarizes the bioactive components found in yogurt and related fermented milk products.

Lactic acid bacteria possess cell envelope-located proteinases, which cause degradation of caseins into oligopeptides. Because

Table 2 Examples of bioactive components in yogurt

Category	*Examples*
Bioactive peptides	α-S1-casecidin, isracidin, casein phosphopeptide, morphiceptin, α-lactorphin
Major milk proteins	α-S1-casein, α-S2-casein, β-casein, κ-casein, α-lactalbumin, β-lactoglobulin, proteose–peptone, glycomacropeptide
Minor proteins and naturally occurring bioactive peptides	Adrenocorticotropic hormone, calcitonin, plasmin enzymes (plasmin, catalase, glutathione peroxidase, and lactoperoxidase), growth factors, immunoglobulins, insulin, lactoferrin, lactoperoxidase, luteinizing hormone-releasing hormone, lysozyme, prolactin, relaxin, somatostatin
Bioactive lipid	Conjugated linoleic acid (CLA)
Vitamins	Vitamin D, vitamin B12, thiamine, riboflavin, niacin, folate
Minerals	Calcium, phosphorus, magnesium, zinc

Source: Ibeagha-Awemu, E. M., Liu, J. R. and Zhao, X. (2009). Bioactive components in yogurt products. *Bioactive Components in Milk and Dairy Products*, 235.

Table 3 Bioactive peptides derived from milk fermentation and/or processing according to their physiological classification

Protein precursor	*Peptide name*	*Physiological classification*
α-S1-casein	α-S1-casecidin	Antimicrobial
	Isracidin	Antimicrobial
	α-S1-casokinin-5	ACE inhibitor
	Casein phosphopeptide	Calcium binding and transport
	α-S1-casein exorphins	Opioid agonist
	Casoxin D	Opioid agonist
	α-S1-casein exorphins	Opioid agonist
α-S2-casein	Casocidin-1	Antimicrobial
	Casein phosphopeptide	Mineral binding
β-Casein	β-Casokinin-7	ACE inhibitor
	β-Casokinin-10	Immunomodulatory and ACE inhibitor
	Antihypertensive	Peptide antihypertensive
	Immunopeptide	Immunostimulatory
	β-Casomorphin-4, -5, -6, -7, and -11	Opioid agonists
	Morphiceptin	Opioid agonist
	Casein phosphopeptide	Mineral binding
κ-Casein	κ-Casecidin	Antimicrobial
	Casoplatelin	Antithrombotic
	Thrombin inhibitory peptide	Antithrombotic
	Casoxin A	Opioid antagonist
	Casoxin B	Opioid antagonist
	Casoxin C	Opioid antagonist
	Casein phosphopeptide	Mineral binding
α-Lactalbumin	α-Lactorphin	ACE inhibitor and opioid agonist
β-Lactoglobulin	β-Lactorphin	ACE inhibitor and opioid agonist
Lactoferrin	Lactoferricin A	Opioid agonist
	Lactoferricin B	Immunomodulatory and antimicrobial
Lactotransferrin	Thrombin inhibitory peptide	Antithrombotic
Bovine serum albumin	Serorphin	Opioid agonist

Source: Data obtained from Fitzgerald and Murray (2006).

microbial proteases hydrolyze milk proteins more randomly than intestinal proteases, fermentation results in significantly more free amino acids and bioactive peptides in yogurt. A large body of scientific research has established that the action of bacterial proteases on milk proteins during yogurt fermentation, or digestion by gut bacteria, results in the release of bioactive peptides, exhibiting different bioactivities, which may be beneficial to health.

Biochemical changes in milk fat also occur during fermentation for yogurt production. Through the activity of bacterial lipase, large amounts of free fatty acids are released. Biohydrogenation during fermentation results in higher CLA concentrations in yogurt than milk. Since low-fat and nonfat varieties of yogurt are preferred, hydrolysis of milk lipids contributes little to the composition of most yogurt products. However, research indicates that the CLA composition can be manipulated/increased while CLA-enriched yogurts have been shown to affect positively energy metabolism and adipose tissue gene expression in healthy subjects.

The beneficial role of dietary fiber in human nutrition has led to growing demand for incorporation of novel fibers in functional foods. Among the various insoluble fibers from soy-, rice-, oat-, corn- and sugar beet-fortified sweetened plain

Table 4 Health Benefits of yogurt and fermented milks

Beneficial effect	*Possible mechanisms*
Improved digestibility	Partial breakdown of proteins, fats, and carbohydrates
Improved nutritional value	Higher levels of B vitamins and certain free amino acids, viz., methionine, lysine, and tryptophan
Improved lactose utilization	Reduced lactose in product and further availability of lactase
Antagonistic action toward enteric pathogens	Disorders such as diarrhea, mucous colitis, ulcerated colitis; prevention of adhesion of pathogens
Anticarcinogenic effect	Reduction of carcinogen-promoting enzymes; inhibitory action toward cancers of the gastrointestinal tract by degradation of precarcinogens; stimulation of the immune system
Hypocholesterolemic action	Production of inhibitors of cholesterol synthesis; use of cholesterol by assimilation and precipitation with deconjugated bile salts
Immune modulation	Enhancement of macrophage formation; stimulation of production of suppressor cells and γ-interferon

yogurt, oat-fortified yogurt resulted in higher apparent viscosity and accelerated acidification rates. Similarly, β-glucan used in low-fat yogurt increased consistency and firmness and decreased syneresis.

Prebiotics are nondigestible complex carbohydrates that selectively stimulate growth and activity of bacteria in the colon and also beneficially affect the host. Examples of prebiotic ingredients being tested for yogurt production are β-glucans, inulin, pectin, gums, and resistant starch.

Yogurt with Health-Promoting Organisms

Most of the health benefits associated with yogurt and fermented milk products are listed in Table 4. A large amount of literature has been accumulated regarding health benefits including improved lactose tolerance, antimicrobial activity, resistance to gastrointestinal infections, anticancer, reduction in serum cholesterol, and immune system stimulation.

Probiotic microorganisms can exert beneficial properties through two mechanisms: (1) direct through live bacteria (probiotics) and (2) indirect via metabolites. Yogurt is seen increasingly as a safe and enjoyable means to deliver probiotics to the gut, where individuals can benefit from both the nutrients and probiotic content. Probiotic species, *L. acidophilus*, *L. rhamnosus* GG, *L. casei*, *Bifidobacterium bifidum*, *B. longum*, etc., are used in yogurt manufacture, and products are reported to enhance resistance to intestinal pathogens, mainly via antimicrobial mechanisms, including competitive colonization and organic acid production (e.g., lactic and acetic acids), bacteriocins, and other primary metabolites.

Conclusion

The market for dietetic foods is increasing tremendously, especially for those containing functional ingredients such as bioactive peptides, CLA, dietary fibers, and probiotic organisms. Consumers are also increasingly health-conscious and demand natural, healthy foods and products with functional aspects. Yogurt is inherently nutritious and provides opportunities for including functionally important compounds and microorganisms.

See also: Bifidobacteria in Foods: Health Effects; Carbohydrate: Digestion, Absorption and Metabolism; Fat Replacer; Fermented Foods: Fermented Milks; Fermented Foods: Use of Starter Cultures; Folic acid and Folates: Physiology and Health Effects; Food Fortification: Rationale and Methods; Food Intolerance: Lactose Intolerance; Glucose: Metabolism and Regulation; Lactose; Low-fat Foods: Types and Manufacture; Milk: Role in the Diet; Prebiotics; Probiotics; Protein: Digestion, Absorption and Metabolism; Vitamins: Overview; Yogurt: The Product and its Manufacture; Yogurt: Yogurt Based Products.

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Yogurt: The Product and its Manufacture

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C Béal, AgroParisTech INRA, Thiverval-Grignon, France

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Introduction

An ancestral version of yogurt probably appeared 9000 or 8000 years BC in Mesopotamia and Egypt and subsequently spread in the northeast of Africa, in the Middle East, in Central Asia, and later in Balkan countries, offering a large variety of 'fermented milks.' Originally, yogurt resulted from a spontaneous, accidental lactic acid fermentation leading to the acidification and coagulation of milk, thus resulting in an efficient way to preserve this raw material otherwise vulnerable to spoilage. Along the following centuries, the knowledge for making homemade yogurts and fermented milks spread all over the world. The first attempts to produce yogurt industrially were performed in Barcelona in 1919 by Isaac Carasso. Since the 1950s, the production of yogurt has grown significantly thanks to the intervention of several powerful agrofood groups setting up international activities. If 'set-type' and 'stirred' yogurts are the basic products, a large number of new recipes and processes have been created to diversify the products and address market demands. These alternations include the modification of the texture, resulting in drinking, frozen, and concentrated yogurts; modification of aroma and taste by addition of fruit, jam, and aroma compounds; modification of nutritional properties by changing the fat and sugar contents; and development of health-related elements with the use of probiotic strains or the inclusion of vitamins or phytostanols. The coverage of these issues has been contributing to a significant expansion of the overall consumption of yogurt and fermented milks worldwide.

As a result, the industrial production of yogurt and fermented milks in the world reached about 32–35 million tons in 2012, for an expected global market in 2015 of about $67 billion. In Europe (27 countries), the annual production was around 9.3 million tons, with Germany, France, and Spain being the three main producers covering 20%, 18%, and 9% of the European production, respectively. For the last 5 years in Europe, the total production increased by 10%, whereas for the last 30 years in France, the increase was of 38%. Besides Europe, the main producers of yogurt are China, Russia, Iran, and the United States, with 4.0, 2.5, 2.2, and 2.0 million tons per year, respectively. Production in South America and Central America and in Africa remains low with 1.5 and 0.4 million tons per year, respectively.

Definition and Main Types of Yogurts

Definition of Yogurt and Probiotic Fermented Milks

In essence, yogurts are fermented dairy products obtained from lactic acid fermentation by two species of lactic acid bacteria, that is, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. This fermentation leads to acidification and milk coagulation, without addition of rennet (as in cheese), and allows an increase of the shelf life as a result of the low pH. The sensory properties of yogurts rely on three main characters: (1) the composition of milk as raw material, which differs according to the milk source (e.g., cow, goat, or sheep; conventional or organic) and the fat content that can be adjusted to obtain full-fat, low-fat, or nonfat products; (2) the addition of ingredients that allow modifying the sensory properties (flavor, color, and texture) of the products, such as sweetening agents (sugar or other sweeteners for low-calorie products), flavoring agents (fruit aromas or vanilla) or fruits (small pieces enriched with sugar or jam), stabilizers (pectin, starch, or gelatin), or emulsifiers; and (3) the technology employed for the manufacture, which may vary depending on the operations during milk pretreatment (fat and nonfat solid standardization, homogenization, or heat treatment) or yogurt post-treatment (stirring, concentration, mixing, cooling, drying, or freezing).

Even if yogurt is the most consumed within the wider family of fermented milks, many probiotic fermented milks are traded around the world. They involve probiotic bacteria, which are defined according to the FAO/WHO in 2011 as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.' As European regulation has stated that these health benefits have to be demonstrated before using the probiotics in foods and supplements, the health claims are not anymore maintained. In other countries, and depending on the country regulation, the following health benefits are recognized: reduction of lactose intolerance, prevention of microbial infections, stimulation of the immune system, and regulation of immune responses.

Main Types of Yogurts and Fermented Milks

Yogurt diversification is reflected by various textures (set-type or firm, stirred, drinking, frozen, concentrated, or powder yogurts), numerous flavors (natural, sweetened, flavored, or with added pieces of fruits or honey), and diverse shelf life and nutritional (fat content and residual lactose content) properties. Figure 1 proposes a synthetic scheme of the several characters that lead to yogurt diversity.

Classification of fermented milks is also based on their potential health properties. They are mainly related to the decrease in lactose content in the product, as a result of lactic acid production. Some strains of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* are able to survive in the gastrointestinal tract, thus being able to play a role in the gut health. Finally, the development of US versions of Greek yogurt is associated to high protein but low carbohydrate contents, thus contributing to the weight management of consumers.

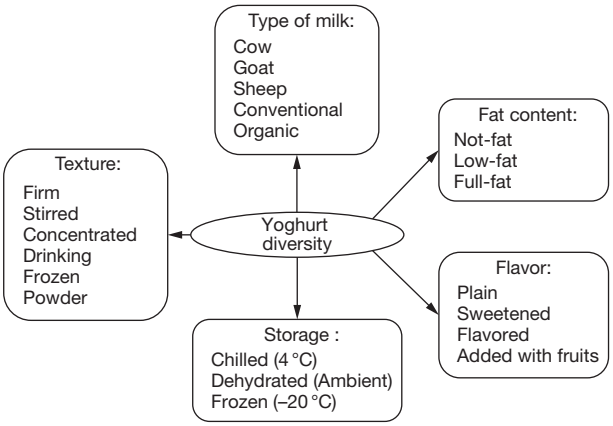

Figure 1 Classification scheme for yogurts and fermented milks.

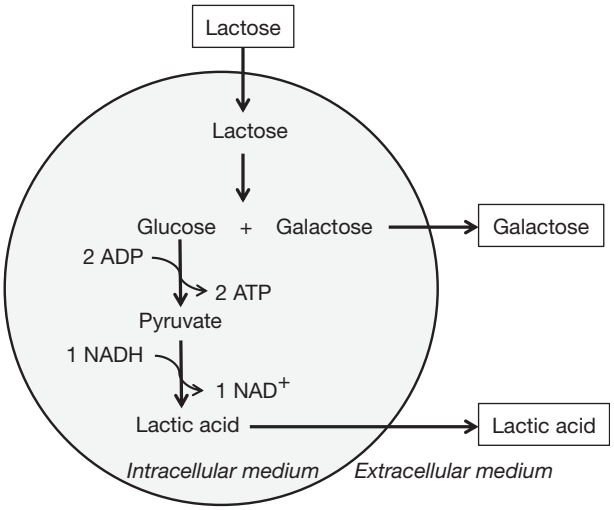

Figure 2 Simplified scheme of metabolic reactions involved in homofermentative metabolism in yogurt bacteria.

Microbiological and Biochemical Mechanisms Involved in Yogurt Manufacture

Microbiological Characteristics of Yogurts

The two thermophilic lactic acid bacteria, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, which trigger yogurt fermentation, are considered as 'Generally Recognized as Safe' in the United States and possess the 'Qualified Presumption of Safety' status in Europe, as a consequence of a long history of safe use in food and an absence of pathogenicity. They are Gram-positive, anaerobic, aerotolerant, and catalase-negative, do not form spores, and have less than 55% G+C content in their DNA. They are able to grow between 42 and 50 °C, but not at 10 °C. *S. thermophilus* forms linear chains of rods, whereas *L. delbrueckii* subsp. *bulgaricus* grows as ovoid cells. They convert lactose into galactose that is not metabolized and glucose that is fermented predominantly to lactic acid, thus corresponding to homofermentative metabolism.

In milk, these two species demonstrate a positive interaction called protocooperation, which is mutually favorable. This phenomenon induces a more rapid growth and acidification, higher production of aroma compounds and exopolysaccharides, and more pronounced proteolysis. An upregulation of biosynthesis pathways for nucleotides and sulfur-containing amino acids is also observed. Growth of *S. thermophilus* is promoted by free amino acids and small peptides that arise from milk proteins by the action of the cell wall protease PrtB of *L. delbrueckii* subsp. *bulgaricus*. In return, *L. delbrueckii* subsp. *bulgaricus* is stimulated by formic acid, folic acid, and CO_2 that are synthesized by *S. thermophilus* in milk. As a consequence of this interaction, growth of *S. thermophilus* starts first by using the nitrogen compounds and stops early as this species is very sensitive to lactic acid inhibition. Growth of *L. delbrueckii* subsp. *bulgaricus* begins later but is prolonged even at low pH, due to the better resistance of this species to acidity.

Probiotic bacteria involved in fermented milk production other than yogurt include different lactobacilli and bifidobacteria. The main bacterial species found in commercial products are *Lactobacillus acidophilus*, *L. casei*, *L. paracasei*, *L. rhamnosus*, *Bifidobacterium animalis* subsp. *lactis*, and *B. breve*. These bacteria demonstrate numerous interactions with the classical yogurt cultures, which depend on specific strain associations.

Biochemical and Physicochemical Changes During Lactic Acid Fermentation

Growth of lactic acid bacteria in milk induces many changes that are desirable in yogurt. These changes include the synthesis of different metabolites (lactic acid, exopolysaccharides, and aroma compounds) and the modification of the texture and the nutritional value of the product.

Lactic acid production

As a result of glycolysis, one mole of lactose is theoretically transformed into one mole of galactose and two moles of lactic acid, together with the production of intracellular energy in the form of two moles of ATP. *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* operate these reactions according to a homofermentative metabolic pathway that is summarized in Figure 2.

Lactose is first internalized into the cell with the help of a lactose permease energized by a proton gradient. Intracellular lactose is then hydrolyzed into glucose and galactose by the enzyme α-galactosidase. Glucose is catabolized to pyruvate via the glycolytic pathway (Embden–Meyerhof–Parnas), whereas galactose is excreted out of the cell. Pyruvate is then reduced into lactic acid through a lactate dehydrogenase, together with the reoxidation of NADH formed earlier. Two different isomers are synthesized: L(+) lactic acid by *S. thermophilus* and D(−) lactic acid by *L. delbrueckii* subsp. *bulgaricus*. Finally, the intracellular lactate is excreted out of the cell via a symport with protons, thus inducing acidification of extracellular medium and progressive inhibition of bacterial growth.

Other lactobacilli and bifidobacteria may use heterofermentative pathways to produce their intracellular energy together with lactic acid. *L. rhamnosus*, *L. casei*, and *L. paracasei* produce one mole of each lactic acid, ethanol, CO_2, and ATP from one mole of glucose. Bifidobacteria synthesize three moles of acetic acid and two moles of (L+) lactic acid and ATP, without generation of CO_2 from two moles of glucose. These different pathways induce different acidification

rates and, consequently, different sensory properties of the final product.

Proteolysis

During milk fermentation, the proteolytic system of lactic acid bacteria degrades caseins into peptides and free amino acids that are essential to bacterial growth and participate in the generation of flavor. *L. delbrueckii* subsp. *bulgaricus* possess the cell surface proteinase PrtB that is highly active and promotes fast growth and rapid acidification of milk. In contrast, even if a few strains of *S. thermophilus* possess the cell wall proteinase PrtS, most strains are protease-negative. Growth of *S. thermophilus* is nevertheless effective in mixed cultures, since available nitrogen compounds are supplied from the proteinase of *L. delbrueckii* subsp. *bulgaricus*.

Flavor compound production

Yogurt taste is mainly characterized by an acid character due to the presence of lactic acid in the product. Yogurt aroma is characterized by about hundred volatile compounds that consist of carbonyl compounds (mainly acids and esters), alcohols, and heterocyclic and sulfur-containing compounds. Among them, acetaldehyde is the major flavor compound of yogurt, where it confers a pleasant fresh and fruity aroma. It is produced by the lactic acid bacteria at a final concentration comprised between 5 and 40 mg kg^{-1}. Most of the acetaldehyde is directly synthesized from pyruvate with the aid of pyruvate decarboxylase or indirectly from acetyl coenzyme A, through the action of pyruvate dehydrogenase and aldehyde dehydrogenase. *L. delbrueckii* subsp. *bulgaricus* is also able to convert threonine into acetaldehyde and glycine, through the action of serine hydroxyl-methyl transferase. In addition, *S. thermophilus* produces α-acetolactate that is partially metabolized into diacetyl or acetoin through the action of α-acetolactate decarboxylase that allows regulating leucine and valine biosynthesis.

Coagulation

Acidification of milk leads to coagulation as a result of destabilization of the casein micelles. The mechanism relies on two concomitant phenomena. During acidification, the net negative charge on casein micelles decreases, thus reducing electrostatic repulsion between charged groups. In the same time, the colloidal calcium–phosphate complex is solubilized, which results in the depletion of calcium in the micelles. Then, electrostatic and casein–casein attractions increase due to enhanced hydrophobic interactions. When the isoelectric point of caseins (pH 4.6) is achieved, coagulation occurs as a result of the formation of a three-dimensional network consisting of clusters and chains of caseins, which leads to the formation of the yogurt gel.

Exopolysaccharide production

Some strains of lactic acid bacteria contribute to the physical properties of stirred fermented milks through biosynthesis of extracellular polysaccharides (EPS), which are either homo- or heteropolysaccharides. These polymers are composed of several hundreds to thousands of repeating units of monosaccharides such as D-glucopyranose, D-fructofuranose, D-glucose, D-galactose, L-rhamnose, *N*-acetyl-D-galactosamine, and *N*-acetyl-D-glucosamine, with molecular masses ranging between 4×10^4 and 6×10^6 Da. *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* produce EPS during growth, to final concentrations comprised between 30 and 600 mg l^{-1} in milk.

Biosynthesis of exopolysaccharides is governed by well-characterized gene clusters. It involves the production of precursors that are formed in the cytoplasm, related to the sequential addition of activated carbohydrates (UDP-glucose, UDP-galactose, and dTDP-rhamnose) by specific glycosyltransferases. These repeating units are coupled to lipid carriers and translocated across the membrane before polymerization.

Changes in nutritional value

Nutritional characteristics of yogurts differ from those of milk by the three main following aspects: (1) as a consequence of lactic acid production, the lactose content is lower in yogurt (30 g l^{-1}) than in milk (50 g l^{-1}), which is important for lactose-intolerant people, by limiting the formation of organic acids, hydrogen, methane, and carbon dioxide in the human gut; (2) the levels of calcium and potassium are higher in yogurt than in milk (200 and 255 mg 100 g^{-1}, respectively), as a consequence of nonfat solid adjustment; and (3) the concentration of folic acid is higher in yogurt than in milk. The latter essential vitamin is biosynthesized by *S. thermophilus* in the range of 20–150 μg l^{-1}, during which it is consumed by *L. delbrueckii* subsp. *bulgaricus*, thus leading to an average net level in yogurt of 80 μg l^{-1}, which exceeds the one in milk (40 μg l^{-1}).

Yogurt Manufacture

General Diagrams of Yogurt Manufacture

The industrial manufacture of yogurts is organized along three main steps: (1) the preparation of the mix and all corresponding physical treatments such as homogenization, heat treatment, cooling, and deaeration; (2) the fermentation process starting after inoculation of the mix; and (3) the yogurt harvesting, post-treatment, and packaging. Depending on the steps performed, at least four types of yogurt can be considered, whose manufacture is presented in Figure 3. One has to notice that each step of the manufacture affects the final quality of the yogurts and that, except for set-type yogurts, the product flavoring and the cup filling are performed after fermentation.

Preparation of the Mix

Milk standardization

In order to obtain the mix to be fermented, milk preparation involves mainly fat and protein content standardization and optional addition of sweeteners and stabilizers. Fat standardization consists of fat removal by centrifugation (at about 55 °C), followed by cream reincorporation to reach the targeted fat content, ranging from nonfat (0.01%), to low- or light-fat (1–2%), to whole-fat yogurts (>3.2%). Protein standardization aims at increasing the protein content of the mix (from 3% to 5–15%) in order to improve the yogurt firmness (texture) and reduce its syneresis. It is mostly done by addition of milk powder, which is the easier and traditional way. The use of milk proteins or milk replacers as caseinates or whey

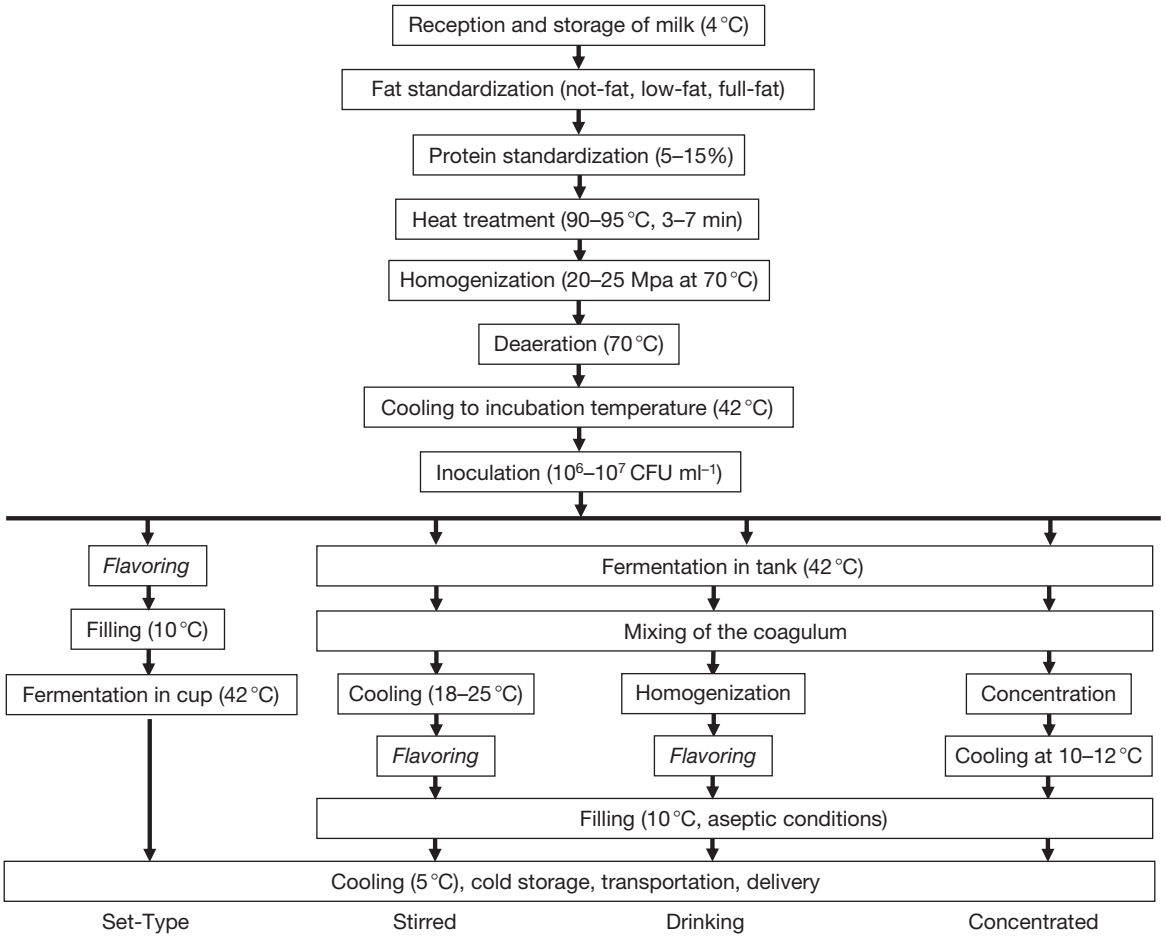

Figure 3 Schematic diagram of the production processes of set-type, stirred, drinking, and concentrated yogurts.

powders is also common. A complete mixing of the dry ingredients without air incorporation is recommended. Concentration of milk by membrane processes (ultrafiltration and reverse osmosis) is an alternative method to increase the protein content of the mix.

For some yogurt recipes, sugars or other sweetening agents are added to the mix, generally after the physical treatments described in the succeeding text. In some countries, the use of thickeners and stabilizers (gelatin, pectin, xanthan gum, carrageenan, starch, etc.) at concentrations varying from 5% to 10% is allowed by FAO/WHO to improve the yogurt texture.

Physical treatments of the mix

Heat treatment is an essential step of the mix preparation. It allows removing spoilage microorganisms, inactivating lactoperoxidases and producing stimulatory compounds in milk. In parallel, heat treatment contributes to improved yogurt texture by allowing whey protein denaturation and interaction with casein, resulting in a decrease of gel syneresis and an increase of gel firmness. During industrial yogurt manufacture, the mixes are generally heated at 90 or 95 °C for 3–7 min before cooling down to fermentation temperature. Plate heat exchangers, with a tubular holding zone, are generally used and are designed in order to cool the mix accurately at the fermentation temperature (between 37 and 43 °C).

Two other physical treatments of the mix, deaeration and homogenization, are closely associated with the heat treatment, and the design of the heat exchangers takes into account the temperature favoring their effect. Homogenization is compulsory for yogurt quality, as it increases the gel texture and reduces syneresis. It provokes a reduction of the size of the fat globules (near 2 μm) and a better link between fat and hydrophilic proteins. Homogenization of the mix is done at high pressure (20 or 25 MPa) and at a temperature close to 70 °C. Associated with the heat treatment of the mix, it takes place just after the holding section of the heat exchanger. Double-stage high-pressure homogenizers are recommended for high-fat yogurts. Vacuum deaeration of the mix is performed at large industrial scale to reduce its oxygen content and consequently shorten the fermentation time, as to improve the yogurt texture and to remove off-flavors. This step is generally performed at 70 °C, before homogenization.

The Fermentation Process

Inoculation of the mix

At industrial scale, yogurts are prepared through inoculation of the mix with concentrated starter cultures of the two yogurt bacteria (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*). The commercial starter cultures are composed of specific

blends of selected and well-defined strains, at a concentration higher than 10^{10} colony-forming units (CFU) g^{-1}, and are preserved as frozen or freeze-dried formulations. The inoculated mix contains generally 10^6–10^7 CFU ml^{-1} of bacteria. After mixing, it is transferred to the fermentation tanks (for stirred, drinking, or concentrated yogurt manufacture) or directly to the packaging machine for fermentation in cups (for set-type yogurt manufacture).

Fermentation step

During the lactic acid fermentation of milk, numerous parameters vary as a function of time, as shown in Figure 4. The growth of *S. thermophilus* occurs first, followed by that of *L. delbrueckii* subsp. *bulgaricus*, reaching final concentrations close to 10^9 CFU g^{-1} (Figure 4(a)). The consumption of lactose and nitrogenous compounds permits the growth of both strains and leads to the accumulation of many relevant metabolites. Lactic acid, galactose, acetaldehyde, and exopolysaccharides are the most important ones, contributing to flavor and texture of the yogurt (Figure 4(b)). The synthesis of extracellular lactic acid provokes an acidification of the mix characterized by a decrease of the pH (Figure 4(a)), the coagulation of proteins, and the subsequent gel formation. Acetaldehyde confers to yogurt its particular aroma, and exopolysaccharides contribute to its texture.

The acidification process is controlled by the final pH of the yogurt and the acidification rate, which are key factors to master quality. The fermentation is stopped (by a fast cooling of the product) when the final pH of the yogurt is reached. The targeted final pH varies from 4.8 to 4.5, as a function of the type of yogurt. A significant postacidification during the yogurt's cooling, harvesting, and storage has to be considered in defining this target. Generally, online measurement of pH of the mix is avoided, because the glass pH probes may break inside the mix and need to be submitted to cumbersome protocols of cleaning and calibration. Consequently, only manual sampling is done during the acidification process to allow offline pH measurements, and the decision to stop the fermentation by cooling requires a good expertise of the process. The acidification rate acts directly on the fermentation time, so that its knowledge and control are very important to properly schedule industrial production. It is influenced by various factors, such as starter composition and activity, mix composition and physical treatments, and fermentation temperature. However, accurate temperature control is quite impossible during yogurt fermentation because of the coagulation phenomenon that occurs at about pH 5.2. As a consequence, the fermentation time can vary in important ranges. For probiotic yogurt, fermentation time can reach 6–8 h, whereas for stirred yogurt, a 3–4 h

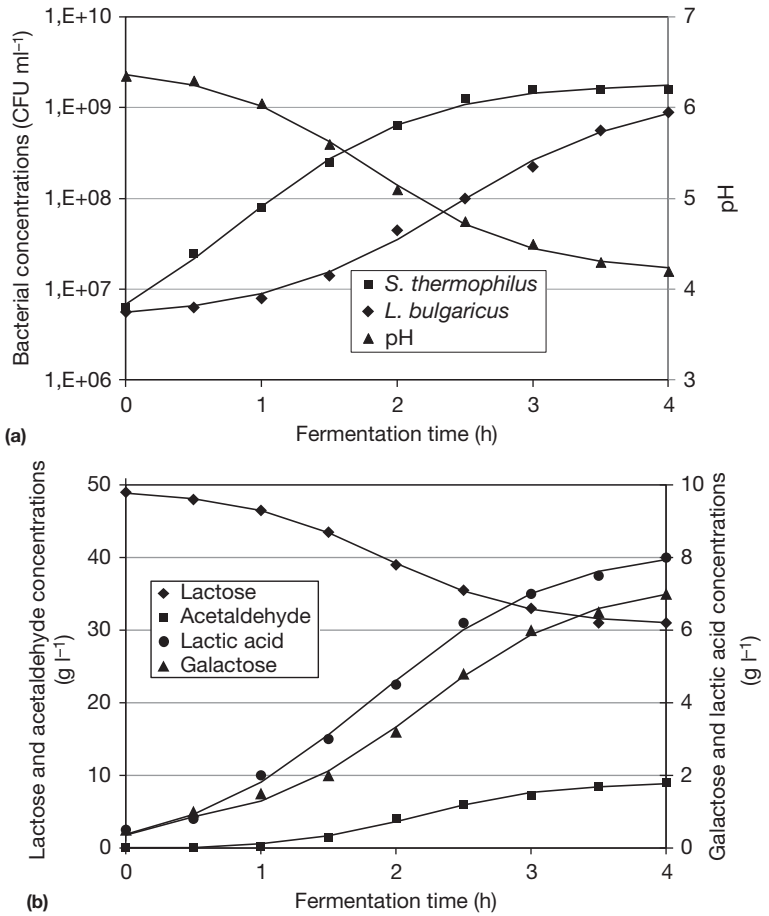

Figure 4 Growth of *S. thermophilus* and *L. delbrueckii* subsp. bulgaricus and milk acidification (a) and evolution of lactose, galactose, lactic acid, and acetaldehyde concentrations (b) as a function of fermentation time.

process is the common target. However, industrial manufacturers have difficulties to master perfectly all the parameters of the process, and longer fermentation times (5 or 8 h) are frequently attained.

The fermentation of set-type yogurts is generally performed in cabinets, incubation rooms, or large tunnels in which the pallets move forward gradually with forced ventilation of warm air. The fermentation of stirred yogurts is performed in large tanks (15–20 m^3 for the largest ones) equipped with mixing devices for mix homogenization, starter mixing, and gel breaking after fermentation.

Yogurt Harvesting and Packaging

Cooling and harvesting of yogurt

The first step in yogurt harvesting corresponds to a fast cooling of the product in order to stop its acidification. It takes place when the required final pH of yogurt is obtained. Set yogurts are cooled within 1 or 2 h to 4 or 5 °C using cold air in ventilated cabinets, cooling rooms, or tunnels, as a function of the size of the manufacturing unit. For stirred yogurt, the cooling is performed in an external heat exchanger reaching an intermediate temperature (between 18 and 25 °C) in less than 1 h (20–60 min for industrial tanks). At this temperature, some additives as aroma compounds, sweeteners, and fruits (jam, pulp, and pieces) can be added to stirred yogurts. In modern large plants, these additions are generally performed online at the level of the packaging machine, using metering pumps and mixers.

The final texture of yogurts, especially stirred ones, is a critical factor for consumer acceptance. As the texture is influenced by many factors (mix composition, strains used, and processing conditions), it is a real challenge to obtain the targeted texture. The mechanical constraints exerted on stirred yogurt by all the harvesting devices (pumps, heat exchangers, pipes, mixers, filling machine, etc.) tend to reduce its texture but can give them some smoothness.

Packaging of yogurt

Yogurt packaging ensures its hygiene and protection during distribution. If plastic and glass cups are always used for set-type yogurts, large up-to-date packaging units use the 'form–fill–seal' technology. The same packaging machine realizes the three main following operations: (1) the thermoformation of the containers at 150–200 °C, using multilayer thermoplastic materials, (2) the filling of the preformed containers under a closed environment and sterile air overpressure, and (3) the thermosealing of the filled containers with an aluminum lid labeled to deliver product information. These high-tech packaging machines allow reaching high security and high capacity (up to 70 000 cups per hour) standards. Consequently, they correspond to the most expensive investment in an industrial manufacturing unit of yogurt.

Overpackaging is then carried out, in the form of multipacks of 2, 4, 8, or 16 cups, with the help of an automatic tray packer. After packaging and overpackaging, the yogurts are stored at low temperature (4 or 5 °C), which is maintained during transportation and commercialization. This low temperature maintenance permits limiting the postacidification in the products and preserving their safety.

Industrial Design of Yogurt Manufacturing Units

All equipments used for milk storage, mix preparation, fermentation and yogurt cooling, and harvesting and packaging are especially designed to allow for the cleaning in place (CIP) procedures commonly used in dairy industry. These procedures assume the existence of a CIP kitchen in the factory to automatically provide the cleaning mixtures at the right temperature and for the right duration.

Yogurt fermentation is a batch process, but some operations such as mix preparation and treatment and yogurt cooling and packaging are designed and managed as continuous or semicontinuous processes. In industrial manufacturing units, automation and process control systems are more and more popular. They encompass (1) sensors that essentially measure physical parameters such as temperature, pressure, level, and weight; (2) programmable logic controllers controlling valves, pumps, and motors that permit the regulation of the main process parameters; and (3) computer supervision that allows traceability. Nevertheless, as an accurate control of the yogurt acidification rate remains limited, optimization of yogurt manufacture is not possible.

Finally, even if the acidity of yogurt contributes to its safety, industrial manufacturers observe good manufacturing practices to control the microbial risk. They act mainly on food contact surfaces that have to be cleaned and sanitized before use and impose clothing and hair covering for the staff.

Control of Yogurt Quality

Yogurt quality requires controls of the raw materials, during the course of the manufacturing process and on the final products.

Quality Controls of Raw Materials

Microbiological controls are carried out on raw materials, in particular fresh milk, powder milk, fruits, sweeteners, and starters. Somatic cell counts are also verified on fresh milk. In addition, many physicochemical properties are checked: (1) temperature, titratable acidity, and fat and protein contents of the fresh milk; (2) the absence of antibiotics, solubility, moisture, and fat content of the milk powder; and (3) pH, viscosity, and Brix of the added fruits. Acidification activity of starter cultures is also assessed, mostly by using the Cinac system that allows determining various quantitative kinetic descriptors such as the absolute value of the maximum acidification rate (Vm, in min^{-1}) and the time (tm, in min) necessary to reach Vm. The lower the value of tm and the higher the value of Vm, the higher the acidification activity will be. Figure 5 shows typical acidification curves and corresponding first derivatives (kinetics) of two yogurt starter cultures growing in milk and displaying low (Vm = −1.33 pH unit h^{-1}; tm = 2.55 h) or high acidification activity (Vm = −1.44 pH unit h^{-1}; tm = 2.05 h).

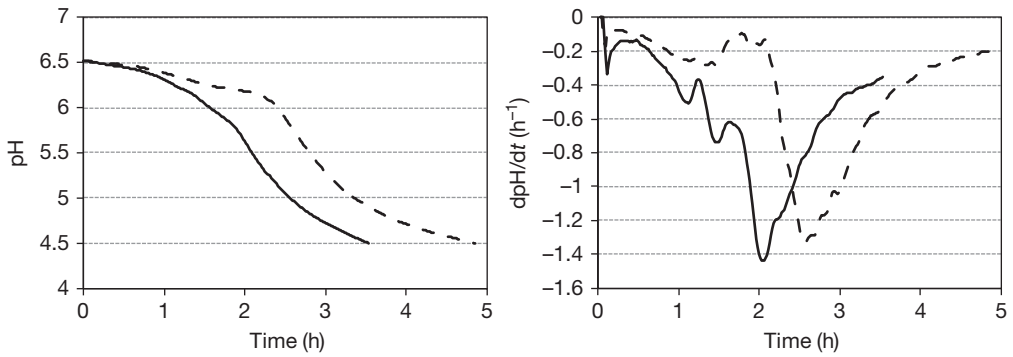

Figure 5 Acidification activity of two yogurt starter cultures, displaying high acidification activity (—) and low acidification activity (—) determined using the Cinac system.

Controls During the Process

During yogurt manufacture, controls are accomplished to ensure repeatability of the productions and maximal levels of quality and food safety of the products. They refer mainly to the control of temperature (in fermentation tanks, heat exchangers, incubation rooms, and cooling systems), pH (by sampling either in the fermentation tanks or directly in cups), and duration of the different steps of manufacture.

In addition to these controls, the use of food safety management systems such as ISO 22000 or International Food Standards is requested to control foodborne safety hazards and guarantee the products' safety. By implementing Hazard Analysis and Critical Control Point systems according to the Codex, three steps are identified as critical control points for microbiological hazards during yogurt manufacture: pasteurization of milk, refrigerated packaging, and cold storage. In addition, packaging is also related to physical hazards.

Quality Controls of the Final Product

Various controls are performed on the final products at the end of their manufacture and during their shelf life. The frequency of sampling is defined by each dairy factory, as stated by its own good hygiene practices.

Counts of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* are controlled to verify that the targeted value of 10^7 CFU g^{-1} at shelf life is achieved. The presence of spoilage and pathogenic microorganisms, including *Listeria monocytogenes*, *Salmonella* spp., coliforms, yeasts, or molds, is also checked.

Physicochemical analyses consist of the verification of the fat and total solid contents, the titratable acidity or the pH of the products, and the assessment of some texture parameters including firmness, consistency, or viscosity, depending on the type of yogurt (set-type, stirred, or drinking yogurt). Sensory evaluations are completed with experienced panelists. The following main sensory attributes are generally employed: appearance (syneresis and color), texture (palatability, firmness, and consistency), aroma and odor, and taste and aftertaste (freshness, acidity, and persistency).

Conclusion

Yogurt is an ancient traditional fermented food that has known, since half a century, a tremendous industrialization of its manufacturing conditions. In more recent years, an intense innovative diversification of recipes and products occurred, which partly explain the increase of their consumption. Although artisan production schemes persist, including homemade yogurt manufacture, the industrial manufacture of yogurt is now well established. Breakthrough innovations are limited and the improvements in process productivity relate mainly to the design and the management of the manufacturing units. Nevertheless, the interesting nutritional properties of yogurts and more generally of fermented milks, their diversity, and the opening and increasing of new markets offer important perspectives for their development at world scale.

See also: Bifidobacteria in Foods: Health Effects; Fermented Foods: Use of Starter Cultures; Lactic Acid Bacteria; Packaging: Aseptic Filling; Probiotics; Rheological Properties of Food Materials; Yogurt: Dietary Importance; Yogurt: Yogurt Based Products.

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Yogurt: Yogurt Based Products

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Introduction

Yoghurt or yogurt is a dairy product made by lactic fermentation of milk, usually using a defined mixed starter culture. The term 'yoghurt' relates to the Turkish word '*Jughurt*' and in particular to *yogurmark* 'to knead' and *yogum* 'dense' or 'thick.' It is believed that Ancient Turkish nomads were the inventor of yoghurt, which they called '*yoghurut*,' after which Turkish immigrants brought it to North-America in the eighteenth century. Isaac Carasso was the person who started industrial production of yoghurt in 1919 under the brand name 'Danone' in Barcelona. The traditional method of yoghurt manufacture employs 'back-slopping,' according to which a part of a previous day's product is added to fresh milk as a starter inoculum. With the advancements in starter culture technology during the last five decades and due to the microbiological problems sometimes associated with the use of back-slopping, which may lead to failed fermentations, yoghurt manufacturers have shown their interest in applying pure forms of starter cultures.

Historical evidence suggests traditional occurrence of yoghurt and yoghurt-based fermented milk products all over the world, although different terminologies are used in different countries. The method of manufacture for these products is nevertheless quite similar to yoghurt production. The only difference is in the use of specific cultures, incorporation of additives, curd treatment, and method of packaging. Generally, commercial yoghurt is produced from cow milk but milk from other animal species like buffalo, goat, camel, yak, sheep, and mare can also be used, in particular in traditional and ethnic products. Recently, plant 'milks' such as soy, coconut, almond, and sesame milk are used to alter the functionality of yoghurt. In order to improve functional and therapeutic importance, yoghurt containing selected probiotic microorganisms has also been developed.

Types of Yoghurt

Yoghurt produced around the world can be categorized on the basis of chemical composition, method of production and texture formation, the use of additives and special cultures, and based on the post-incubation treatments employed (Figure 1). With respect to the chemical composition, the fat content is crucial. As such, yoghurt is classified as skimmed, partially skimmed, and high-fat or full-fat yoghurt. The fat content is adjusted before the heat treatment is given to the milk. On the basis of texture, two main types of 'set' and 'stirred' yoghurt are produced. In addition, a cocktail of set or stirred type product can be made when combining different varieties of yoghurt (flavored/fruit, fortified, low-fat, plain, sweetened, and probiotic yoghurt) (Figure 2). Further, to enhance the shelf life of yoghurt, certain post-incubation processes are employed and the product obtained is termed as heated, frozen, or dried yoghurt. The different global types and varieties of yoghurts are explained below.

Based on Chemical Composition

The Codex Alimentarius has mentioned three types of yoghurt on the basis of the milk fat content. The milk solids other than fat ($\geq$8.25%), the titratable acidity ($\geq$0.9%), and the pH ($\leq$4.5) remain almost similar for full-fat, partially skimmed, and skimmed yoghurt.

Full-fat yoghurt

Full-fat yoghurt is prepared from high-fat milk. As per the standards, milk fat content in full-fat yoghurt should not be less than 3.0%. *Rahmjoghurt* is a type of full-fat creamy yoghurt in Germany consisting of about 10% fat.

Partially skimmed yoghurt

Partially skimmed yoghurt is prepared from milk containing medium fat levels, generally ranging between 0.5 and 3.0%. The fat content in the final product should not be less than 0.5% and not more than 3.0%.

Skimmed yoghurt

Skimmed yoghurt is also called low-fat or reduced-fat yoghurt. The fat content is very low, namely below 0.5%. This form of yoghurt is particularly popular in contemporary food markets and there is growing demand for such products. However, poor flavor, body and texture and more syneresis are the major problems encountered during the production of skimmed yoghurt. These problems can be partially overcome via an increase in the total solid content of milk by addition of skim milk powder, whey protein concentrate, sodium and calcium caseinates or other milk protein-based gradients into the milk before fermentation.

Based on Method of Manufacture/Texture Formation

Set yoghurt

Set yoghurt has gel-like characteristics with particular texture and rheological properties. During its production, the fermentation of milk occurs in cups or containers and the final product remains as such until its distribution and consumption.

Plain yoghurt is a simple type of set yoghurt without use of additives. By virtue of their metabolic activities, yoghurt bacteria convert lactose into lactic acid and produce flavor compounds like acetaldehyde and acetoin during milk fermentation. As a result, the final product exhibits a mild to acidic taste with balanced amounts of natural yoghurt flavor. Plain

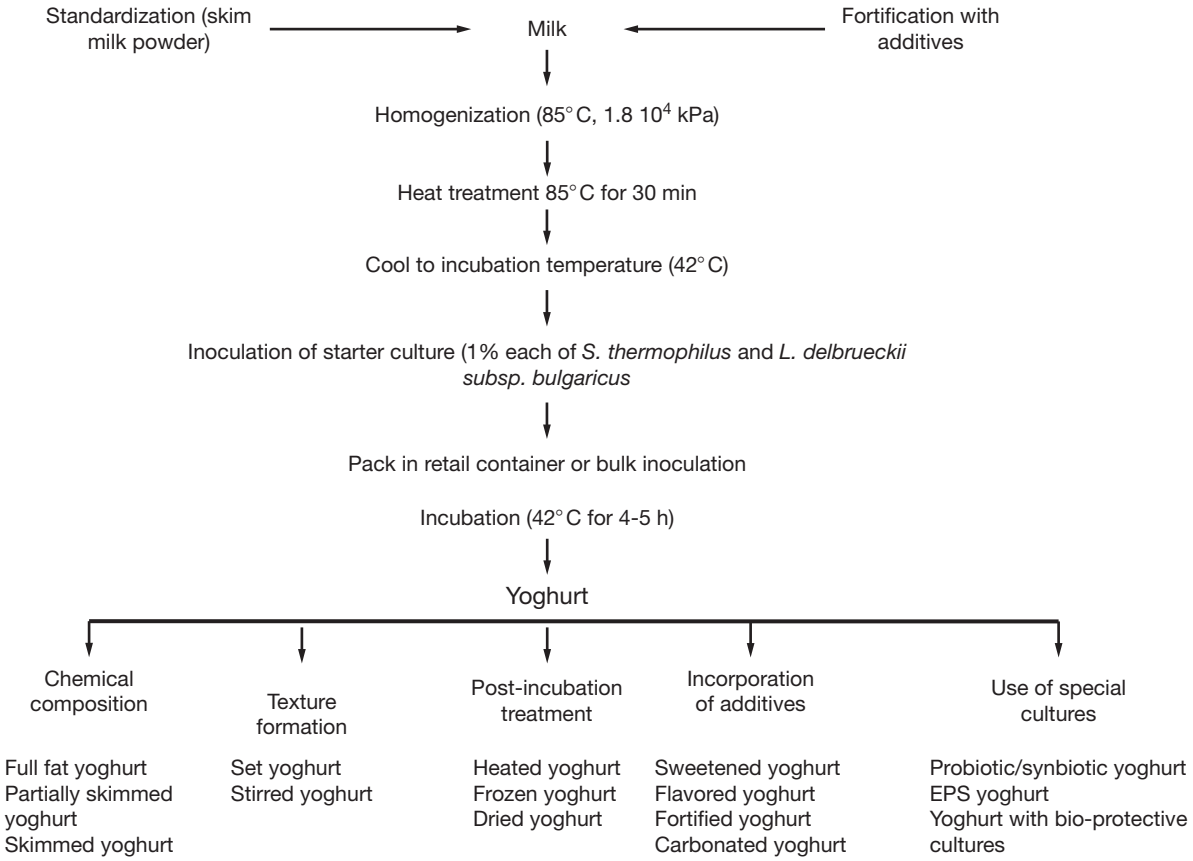

Figure 1 Flow diagrm for manufacture of major types and varieties of yoghurt.

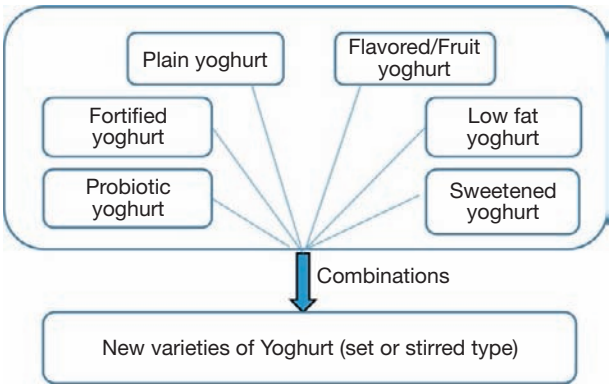

Figure 2 Cocktail of set and stirred yoghurt varieties.

yoghurt is usually consumed with salad or blended with salt or sugar.

Stirred yoghurt

Stirred yoghurt has a semi-solid consistency and is usually prepared by shearing of set yoghurt. The final product does not have gel like texture. Mechanical damage causes whey separation, which is overcome by supplementation of the skim milk powder and addition of stabilizers. Another category of stirred yoghurt is drinking or fluid yoghurt, which has a low viscosity.

Based on Post-Incubation Treatment

Heated yoghurt

Natural yoghurt stored at 4 °C gives a shelf life of one to 3 weeks, depending on the initial quality (based on pH, titratable acidity, and microbial contaminants present). This is due to the increase in acidity by the starter bacteria that continue to grow even at low temperature (post-acidification), resulting in a product that is too sour. Spoilage can also occur due to the growth of fungal contaminants. To increase the shelf life, the yoghurt may be heated at 60–70 °C for 1–5 min. Heated yoghurt is called 'pasteurized'/'UHT'/'heated'/'long life' yoghurt, depending on the time/temperature combination used. Such heating extends the shelf life of yoghurt up to 8 weeks. However, heat treatment also increases whey separation, decreases flavor, and destroys the live (starter) bacteria. As such, it creates legal complications related to regulations based on the FAO/WHO definition, implying that the presence of viable starter organisms in abundance is essential. Use of certain hydrocolloids, such as pectins, alginates, and carboxymethyl cellulose, when added to yoghurt before heat treatment, reduces whey separation and – up to some extent – damage by heat treatment.

Frozen yoghurt

Frozen yoghurt has an ice cream-like texture with a yoghurt flavor. It is generally prepared by mixing plain yoghurt to an ice

Table 1 Varieties of frozen yoghurt

Soft frozen yoghurt	*Hard frozen yoghurt*	*Frozen yoghurt mousse*
Made with a 80% yoghurt base blended with 20% fruit syrup	Made by blending of a 35% fruit syrup and stabilizers/emulsifier into a 65% yoghurt base	Made by mixing of yoghurt with a hot mousse base mixture that consists of skim milk, sugar, and stabilizers/emulsifiers

cream mix, followed by freezing. Different varieties, such as soft, hard, and mousse-frozen yoghurts exist (Table 1). The temperature used during freezing varies from −6 to −25 °C. There are no official standards for frozen yoghurt but some countries have specified that the final product should have more than 3.25% milk fat, 8.25% non-fat milk solids, and 0.15–0.30% of titratable acidity.

Ice cream mixes for frozen yoghurt contain hydrocolloids, emulsifiers, sweeteners, non-fat solids, and fat replacers. From a consumer's health point of view, low-calorie frozen yoghurt has been made by addition of isomalt and soluble dietary fibers. Addition of polydextrose, inulin, and isomalt increase the nutritive value, enhance viscosity, lower the caloric density, and reduce the melting of frozen yoghurt. Addition of a probiotic culture also enhances the perceived health properties as the supplementation of, for instance, *L. acidophilus* and *B. longum* to frozen yoghurt may bestow certain health benefits, such as an improved control of intestinal infections, lactose utilization, and anticancer activity. However, these claims are not always based on solid scientific data. Also, there may be problems associated with the survival of starter and probiotic bacteria due to the frozen food matrix. Addition of sweeteners at high concentration also causes a negative impact on bacterial performance. Other problems encountered with probiotic product varieties are related to the acidic conditions in the final product and the presence of oxygen, that often inhibit *Bifidobacteria*, albeit in a strain-dependent manner. Microentrapment or microencapsulation of probiotic bacteria can partially solve this problem.

Dried yoghurt

Dried yoghurt or powder yoghurt production is practiced in many countries. The main objective for the production of dried yoghurt is to extend shelf life and to facilitate easy handling and transport. Dried yoghurt can be produced by sun drying, air drying, spray drying, freeze drying, or microwave drying. Spray-dried instant yoghurt powder and skim-milk yoghurt powder are prepared commercially. Drying of yoghurt causes low water contents and creates unfavorable conditions for the growth of spoilage-causing microorganisms, especially yeasts and molds. *Kurut* in Turkey is an example of dried yoghurt. However, the nutritive value of yoghurt is degraded during the concentration process and the drying causes a loss of volatile flavor compounds. Powder yoghurt can be used as an ingredient for the manufacture of confectioneries, bakery foods, soup bases, dips, and sauces.

Based on Incorporation of Additives

Sweetened yoghurt

Sweetened yoghurt is prepared by addition of sugar syrup, honey, and cane juice to obtain a sweet taste. *Misti dahi*, *Shrikhand*, and *Kossam* are popular types of sweetened yoghurt in India and Cameroon (see below). Caramelization of sugar may impart a light brown color to the final product as in the case of *Misti dahi*. Yet, high sugar contents in such products also increase the caloric content and may inhibit the growth of starter organisms. Generally, high-sugar tolerating strains are used to produce sweetened fermented milk products. Attempts have been made to reduce calories in these products by replacing sugar with artificial sweeteners like aspartame and acesulfame-K.

Flavored/fruit yoghurt

Flavored yoghurt is very popular among consumers. This form is generally prepared by addition of fruit pieces or cubes, pulp, juice or concentrated juice, paste or flavored syrup, or spices or spice-extracts to milk prior to fermentation. More than 40 types of fruit or their cocktails have been used for the production of fruit yoghurt, often including strawberry, mango, kiwi, peach, apricot, banana, and blue berry fruits.

Fortified yoghurt

A food fortification strategy is used to target specific micronutrient deficiencies among the population. According to the FAO/WHO, fortification refers to 'the practice of deliberately increasing the content of an essential micronutrient, i.e. vitamins and minerals (including trace elements) in a food irrespective of whether the nutrients were originally present in the food before processing or not, so as to improve the nutritional quality of the food supply and to provide a public health benefit with minimal risk to health.' Yoghurt is consumed worldwide and considering the popularity, it has been fortified with micronutrients via the addition of whey powder or specific compounds such as iron, magnesium, or certain vitamins (Table 2).

Carbonated yoghurt/yoghurt soda

Carbonated yoghurt is prepared on the basis of carbon dioxide gas, generally recognized as safe by the U.S. Food and Drug Administration (FDA), after the fermentation of milk has been performed. Carbon dioxide is incorporated in the cooled yoghurt (0.08–0.09 kg cm^{-2}) by the way of a hose. It is made to improve the refreshing ability of yoghurt. Carbonated yoghurt also has a longer shelf life, as carbon dioxide creates an anaerobic environment, inhibiting aerobic microorganisms. Carbon dioxide also forms carbonic acid in an aqueous medium and thus decreases the pH of product, which inhibits the growth of spoilage microorganisms. *Doogh* is an example of a carbonated yoghurt-based beverage. Similarly, carbonated plain yoghurt, flavored yoghurt, and fruit yoghurt can be prepared.

Table 2 Varieties of fortified yoghurt (non-exhaustive list)

Yoghurt type	*Concentration of added compounds*	*Properties*
Mango-based soy yoghurt	14.5% soy milk 7.2% mango pulp	Soy bean provides micronutrients, whereas mango pulp provides easily digestible carbohydrates and improves flavor
Yoghurt with added grapes marc flour	2% marc flour of grapes	Grapes marc provides proteins fibers, phenolic compound, and fructo-oligosaccharides
Yoghurt with added wheat bran and carrots	Carrot 1% Wheat bran 1%	Carrots provide vitamins (ß-carotene), minerals and dietary fiber, whereas wheat bran provides dietary fibers
Inulin-fortified yoghurt	2.5% long-chain inulin	Inulin acts as dietary fiber and prebiotic
Yoghurt with added grape seed extract	5–10 mg gallic acid in 100 g of yogurt	Grape seed extract provides polyphenols and enhances the antioxidative and antiradical activity of yoghurt
(Strawberry) yoghurt fortified with marine fish oil	2% fish oil	Fish oil is source of long-chain $n-3$ fatty acids
β-Glucan fortified set yoghurt	0.3% β-glucan	β-Glucan improves Probiotic viability and stability
Iron-fortified yoghurt	20 mg of iron per kg of milk	Provides iron (a limiting micronutrient in conventional dairy products)
(Lemon-flavored) folic acid-fortified yoghurt	400 μg folic acid in 224 ml of yogurt	Folic acid reduces the risk of colorectal and breast cancers
Yoghurt fortified with vitamins A, D and/or C	10 000 IU of vitamin A and 300 ng of vitamins C and D in a 227 g container of yoghurt	Provides vitamins A and C and vitamin D improves glycemic control in patients
Leutin-fortified (strawberry) yoghurt	Up to 3 mg in 170 g of yogurt	Lutein is a carotenoid that may help to prevent age-related macular degeneration (AMD)

Based on the Incorporation of Special Cultures

Probiotic and synbiotic yoghurt

According to the FAO/WHO standards, yoghurt is 'the coagulated milk product obtained by lactic acid fermentation through the action of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.' In addition, adjunct cultures and probiotic cultures belonging to the genera of *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Propionibacterium*, and *Pediococcus*, as well as yeast and molds are sometimes used in yoghurt-like products. Yoghurt is an often used and suitable carrier for the administration of probiotic bacteria, often in a drinkable form. Probiotics are sometimes used in combination with prebiotics, which is known as a synbiotic approach. Prebiotics are non-digestible food ingredients that selectively stimulate the growth and metabolism of beneficial microorganisms, such as probiotics. Commonly used prebiotics include inuline, fructooligosaccharides, and galactooligosaccharides.

Exopolysaccharide-containing yoghurt

Starter cultures producing exopolysaccharides (EPS) have interesting commercial application. To reduce the amount of added milk solids, improve yoghurt viscosity, enhance texture and mouth feel, and to avoid syneresis during fermentation or upon storage of the fermented milk products, EPS-producing functional starters have received much attention. In the recent past, EPS-producing strains of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* have been used, especially in low-fat/non-fat yoghurts to improve the rheological and sensory properties. Because milk fat contributes to the body, texture, and flavor development of the dairy products, fat reduction may lead to suboptimal product quality. Fermented milk manufacturers have used texture-promoting or ropy starter cultures for many years, particularly when the addition of stabilizers is prohibited or undesired.

Yoghurt with bio-protective cultures

The shelf life of yoghurt is short, i.e., 1 day under ambient condition (25–30 °C) and around 5 days at 7 °C, often due to the growth of yeasts and molds (e.g., *Saccharomyces*, *Candida*, *Debaryomyces*, *Kluyeromyces*, *Rhodotorula*, *Penicillium*, and *Aspergillus* species). Growth of these fungi deteriorates the physicochemical and sensorial characteristics of yoghurt, rendering the product unacceptable for human consumption. Certain *Lactobacillus* species have shown potent antimicrobial activity. For instance, selected *Lactobacillus casei* strains may produce antifungal compounds such as lactic acid, cyclo-(Leu-Pro), 2,6-diphenyl-piperidine, and 5,10-diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a;1′,2′-] pyrazine that can enhance the shelf life of yoghurt up to 14 days at 30 °C. During their growth in milk, protective cultures secrete arrays of antifungal metabolites, composed of organic acids, phenyllactate, antimicrobial peptides, and cyclic dipeptides, that act on the spores, conidia, and cells of fungi and prevent germination. Potent antifungal lactic cultures can thus be used as starter or adjunct or antimicrobial preparations to enhance the shelf life of yoghurt. Commercial antimicrobial preparations of *Propionibacterium shermanii* or specific lactococci, consisting of propionic acid, acetic acid, diacetyl, and other inhibitory metabolites that control the growth of yeasts, molds, and gram-negative psychrophiles can enhance the shelf life of commercial yoghurt up to 82 days at 5 °C. Similarly, a protective culture of *Propionibacterium jensenii* has been used alone and in combination with a *Lactobacillus* culture to enhance shelf life without change in sensory properties.

Yoghurt-Based Products

Dahi

Dahi is a popular fermented milk of India and its neighboring countries. Its therapeutic effects are mentioned in the

Ayurveda, the oldest system of medicine in India. Its body and texture are similar to set yoghurt. Homemade versions are prepared by a traditional method, where a part of old *Dahi* is used as a natural starter culture to prepare a fresh batch. *Dahi* generally contains mixed starter communities, consisting of species of *Lactobacillus*, *Streptococcus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, etc. *Dahi* is also made as an intermediate product in the manufacture of *Desi* butter and ghee or *Shrikhand*. Many commercial players have started manufacturing the product on an industrial scale in India. Value-added *Dahi* with enhanced nutritive and therapeutic properties is now prepared by fortification of fruit pulp, spices, honey, and cereals.

Misti Dahi

Misti Dahi, *Lal Dahi*, or *Payodhi*, a sweetened variety of dahi similar to sweetened yoghurt, is a popular fermented milk product in the Indian subcontinent, especially in eastern India. Metabolic activity of mixed starter cultures of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* converts buffalo and/or cow milk into firm set curd. Traditionally, *Misti Dahi* is prepared from boiling buffalo milk with 6–25% of cane sugar and partially concentrating it by simmering over a low fire during which the milk develops a distinctive light cream to light brown caramel color and flavor. This is then followed by lactic fermentation by using a previous lot of *Misti Dahi* as natural, back-slopping starter culture. It contains a high level of total solids, which give a firm body and develop the brown color and typical caramelized flavor after intense heating. The product is prepared by the Halwais on a small scale. It is a delicacy of choice during religious festivities and is considered an auspicious item to serve before starting a journey or any important work. The product is commonly sold in earthen pots of varying sizes, and served chilled.

Kossam

Kossam is a sweet set fermented milk product of Cameroon prepared by inoculation of yoghurt starter or old *Kossam* in fresh milk. Sugar is added to warm heated milk to minimize the caramelization of sugar.

Shrikhand

Shrikand is a sweetened, semi-solid fermented milk product, popular in the western part of India. It is made from chakka intermediate product and resembles strained yoghurt. Final products have high total solid contents (50–60%). Flavored *Shrikhand* with high nutritive value is prepared by adding cardamom, keser, fruit pulp (apple, mango, banana, papaya, etc.), and flavor (rose). The starter cultures used in the manufacture of *Shrikhand* are similar to the ones that are used in *Dahi* manufacture.

Lassi and *Rob/Roub*

Lassi is a refreshing fermented milk beverage prepared by churning of *Dahi* or yoghurt. It is popular in the northern region of India and mostly consumed during the summer season. Addition of sugar, salt, spices, and fruit pulp enhances its flavor and nutritive value.

Rob/Roub is similar to *Lassi* and popular in Sudan. During its production, churning of yoghurt is done in a goat skin container called Si'in or in a container called Bukhsa. The final product has a low pH of 4.0–4.5 and a pleasant and sour flavor.

Gariss

Gariss is a full-cream, sour, fermented camel milk product popular in Sudan. Milk fermentation occurs in a tanned goat skin bag continuously for months. Mixed cultures of *Streptococcus thermophilus*, *Lactobacillus* spp. and yeasts (*Kluyveromyces marxianus* and *Issatchenkia orientalis*) are involved in the fermentation. At intervals, a portion of the product is withdrawn from the bag and fresh milk is added to make volume.

Zabadi

Zabadi is a fermented milk popular in Egypt. A portion of old *Zabadi*, containing *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, is inoculated into buffalo milk which has been previously concentrated by boiling. The culture-containing milk is then incubated and further concentration of milk solids is achieved by heating the curd and separating whey. The final product shows cooked flavor and a firm texture.

Amasi

Amasi is a fermented milk product of Zimbabwe made by fermentation of bovine raw milk by the natural starter present in milk, container, and air. Fermentation is carried out for 3 days. However, use of raw milk and natural starter culture may hamper the microbiological quality of *Amasi*.

Ergo and *Urubu*

Ergo is a yoghurt-like traditional product of Ethiopia. Spontaneous fermentation of raw milk for 2–4 days results in a final product with a typical sour flavor, thick semisolid consistency and a prolonged shelf life of 15–20 days. *Kibe* (butter) and *Arerra* (buttermilk) are formed after churning of *Ergo*.

The Burundian fermented milk *Urubu* is similar to *Ergo* and is prepared by spontaneous fermentation of milk for 2–3 days. *Amavuta* (butter) and *Amateregwa* (buttermilk) are formed after churning of *Urubu*.

Laban/Leben/Lben

Laban is a set-type, yoghurt-like fermented milk product popular in Lebanon. *Leben* and *Lben* are similar products made in Morocco and Arab countries. Traditional methods of preparation use artisanal, natural starter cultures, whereas for industrial production commercial yoghurt cultures are used. More firm texture is developed using exopolysaccharide-producing strains. *Laban* prepared with undefined natural starters is said to have a more typical flavor.

Gioddu

Gioddu is a Sardinian yoghurt prepared from milk of sheep, cow, or goat. Lactic acid bacteria and yeasts are involved in the fermentation of the milk resulting in an acid-alcoholic product.

Kule naoto

Kule naoto is famous among the Maasai communities in Kenya and Tanzania. It is a yoghurt-like fermented milk product prepared from the raw milk of Zebu cattle. Spontaneous fermentation of the milk is done according to a traditional method with a prolonged incubation period of 5 days. *Lactobacillus*, *Enterococcus*, *Lactococcus*, and *Leuconostoc* species are the predominating microbiota and *L. plantarum* is the dominating bacterium among the lactobacillus species.

Nunu/Nono

Nunu is a Nigerian fermented milk product obtained via an unknown lactic starter fermentation of cow milk for 1–2 days. Fermentation of milk occurs in calabashes or rubber buckets. Final products have a sharp, sour, and bitter flavor.

Nyarmie

Nyarmie is a naturally fermented yoghurt-like milk product of Ghana. Both unpasteurized and pasteurized milk can be used for its production. The final product has a pleasant flavor and displays a prolonged shelf life both at ambient and low temperature. Dominant lactic acid bacteria present are *Leuconostoc mesenteroides* subsp. *mesenteroides*, *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. helveticus*, *L. delbrueckii* subsp. *lactis*, and *Lactococcus lactis*, while predominant yeast include *Saccharomyces cerevisiae*.

Raib/Rayeb

Raib and *Rayeb* are Algerian and Moroccan fermented milk products prepared by spontaneous and uncontrolled fermentation of raw cow or goat milk. Natural lactic starter bacteria are involved in the lactic fermentation. From *Rayeb*, different milk products like *lben*, *zebda beldia* (butter), *Jben*, *Klila* (cheese), and *Smen* (rancid butter) are prepared.

Xueo

Xueo is a yoghurt-like product prepared in Tibet by backslopping of old *Xueo* in boiled and subsequently cooled yak milk. After incubation, a viscous yoghurt-like final product is formed with a shelf-life of 10 days at room temperature. In *Xueo*, *Enterococcus durans* is a dominating bacterium along with *L. fermentum* and *L. paracasei*.

Doogh

Doogh is an Iranian beverage, traditionally prepared by churning of water-diluted yoghurt or fermented milk. Some aqueous extracts of local herbs, spices such as thyme, cucumber, and garlic essence are blended in it to enhance the flavor and functional properties. It differs from *Ayran* in Turkey and *Lassi* in India by dilution ratio, fat content, and rheological and sensory characteristics. For commercial production of *Doogh*, skim milk yoghurt is used. Fortification, carbonation, and addition of gum to overcome phase separation problems are among the new advancements in *Doogh* production.

Ayran

Ayran is considered as a national drink in Turkey and is a cold yoghurt beverage, mixed with salt to enhance flavor. Traditionally, it is made by diluting yoghurt with water at a level of 30–50% and table salt at a maximum level of 1%. In the industrial manufacture of *Ayran*, the milk with adjusted milk solids is fermented by exopolysaccharide-producing strains and the highly viscous curd obtained is diluted with salt and water. The product is then cooled to stop fermentation and further acidity development.

Tarag

Tarag is a Mongolian yoghurt-based product, prepared by lactic fermentation of cow, yak, goat, and/or camel milk. The predominating microbiota of *Tarag* includes *L. delbrueckii* subsp. *bulgaricus*, *L. helveticus*, and *S. thermophilus*.

Kurut

Kurut is a yoghurt-based product, known but prepared differently in two countries, namely Tibet and Turkey. In Tibet, *Kurut* is an acidic and alcoholic fermented milk product made by prolonged natural fermentation (10–15 °C for 6–7 days) of yak milk. *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are the major dominating bacteria. In Turkey, however, *Kurut* is a dried fermented milk product made from cow milk strained yoghurt. Concentrated yoghurt is cut or rolled into small pieces and then air and sun dried for 10–15 days. The drying process enhances its shelf life up to several months.

Suusac

Suusac is a Kenyan yoghurt-based product prepared from camel milk. Unheated or unpasteurized fresh camel milk is spontaneously fermented by an acquired mixed microbiota in smoked Gourd (hollow, dried-out shell of the fruit of the two genera of the calabash tree) or a clay pot at ambient temperature (25–29 °C) for 1–2 days. *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Candida krusei* and *L. plantarum* are predominant microorganisms found in *Suusac*.

Advances in Yoghurt

Plant 'Milk' Yoghurt

Yoghurt can be made from plant 'milks,' such as soy, coconut, almond, sesame, and peanut milk. Plant milks contain reduced content of cholesterol, lactose, and saturated fatty acids and their consumption potentially provides health benefits to humans, in particular with respect to lactose-intolerant

persons. Among the various types, soy milk yoghurt is the most popular variant. Lactic fermentation of soy milk improves typical soy flavor and enhances the nutritional content. In addition, soy is a rich source of antioxidative compounds, isoflavones, and daidzein. Soy yoghurt enriched in glyceollins (a novel class of soybean phytoalexins with potential cancer-preventive and antiestrogenic effects) and probiotic soy yoghurt are manufactured to further enhance nutritive effects. Like soy yoghurt, yoghurt prepared from coconut milk is rich in taste and contains high levels of calcium and other minerals and nutrients of interest.

Yoghurt Bites

Yoghurt bites are ready-to-eat, small-sized frozen yoghurt pieces usually eaten by hand. Yoghurt bites are available in plain, flavored, and fruit-based form. Plain yoghurt bites are small pieces of plain yoghurt, contain total solid contents not less than 22% and are covered with a fat-based coating. Fruit-based yoghurt bites are frozen products that have fruit pieces coated with plain or flavored yoghurt (*e.g.*, strawberry and blueberry-based yoghurt bites).

Conclusion

Yoghurt and related products are very popular in different parts of the world not only because of their nutritional value but also because of their potential health benefits. The recent trend is to manufacture yoghurt with probiotic microorganisms and natural, value-added, often microbial-derived components that provide additional nutritional and functional properties to the end users. Moreover, various milks from animal and plant sources have been applied to manufacture novel yoghurt and have given appreciable results.

See also: Acidophilus Milk; Bifidobacteria in Foods: Health Effects; Dahi; Fat Replacer; Fermented Foods: Use of Starter Cultures; Fermented Foods: Fermented Milks; Food Fortification: Rationale and Methods; Low-fat Foods: Types and Manufacture; Prebiotics; Probiotics; Soy Beans: Properties and Analysis; World Health Organization; Yogurt: Dietary Importance; Yogurt: The Product and its Manufacture.

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Z

Zinc: Physiology and Health Effects

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Physiology

The body content of Zn is second only to iron among the trace elements with approximately 2 g in the adult female and 2.5 g in the adult male. Zn is remarkable for the range of its major biological roles in virtually every cell in the body. At a basic level, this can be attributed to Zn's exceptional ability to form ligands with the side chains of proteins and other organic molecules – ligands that are strong, but flexible and readily exchangeable. Now, prominent among the biological roles of Zn is the Zn finger motif present in the multiple side chains or fingers of an ever-growing number of relatively small, metabolically active proteins. Structurally, the Zn finger motif is a recurring pattern of amino acids with cysteine and histidine at the base, to which Zn binds in a tetrahedral fashion. The number of genes coding for Zn finger domains exceeds 3% and has been reported to be as many as 10% of all identified human genes. Zn fingers vary in their precise conformation. Steroid hormone receptors, for example, have several such domains that are involved in the structure and one each for binding to DNA and RNA polymerases. Many transcription factors contain the Zn finger motif, thus giving the metal a broad role in gene expression. These roles include self-regulation of its own metabolism and induction of metallothionein with which its metabolism is closely associated. Zn binds directly and reversibly to the Zn finger domains of the metal-response element-binding transcription factor-1 (MTF-1), which functions as a cellular Zn sensor. As a result of this binding, MTF-1 assumes a DNA-binding conformation and translocates to the nucleus, where it binds to metal-response elements of the metallothionein gene, thereby initiating transcription. MTF-1 regulates many genes. Polymorphisms suggest that genetic variation in response to MTF-1-regulated genes could be related to dietary Zn intake. Stress hormones are also powerful inducers of metallothionein, suggesting a key role for Zn in the inflammatory response.

Originally identified for their role in initiating transcription by linking with specific sites on the DNA double helix, these proteins have multiple roles in addition to their involvement in gene transcription. These include translation, mRNA trafficking, cytoskeleton stabilization, epithelial development, cell adhesion, protein folding, protein modeling, and Zn sensing.

Zn has multiple roles in addition to those involving Zn finger proteins, including the structure and function of proteins and roles in DNA and RNA metabolism. The number of Zn metalloenzymes with known three-dimensional structures exceeds 200, most notably at the catalytic site. Zn metalloenzymes have been identified in each of the six major enzyme classifications. Zn is also an important regulator of apoptosis, especially via its role in the caspase family of enzymes.

Zn has a major role as a signaling mediator, acting as an intracellular signaling molecule and as a communicator between cells, converting extracellular stimuli to intracellular signals and controlling intracellular events. Zn is involved at all levels of signal transduction. For example, Zn can modulate cellular signal recognition, second messenger metabolism, and protein kinase and phosphatase activities. Early Zn signaling occurs when an extracellular stimulus, including cytokines and growth factors, directly induces elevated 'free' Zn levels within minutes by releasing Zn from a Zn store, as in metallothionein, which then serves as an intracellular signaling molecule or a second messenger. Free Zn itself affects a variety of signaling molecules and also activates ion channels. In the central nervous system, free Zn stored in synaptic vesicles acts as neurotransmitters, being released by exostosis, and then activates postsynaptic cells through transmitter-gated ion channels.

Another major biological role of Zn is that of late Zn signaling, taking several hours to respond, which depends on transcriptional changes to Zn transporters (see succeeding text), MT, and other Zn-related molecules. The Zn transporter, in turn, can alter the Zn intracellular status, thus acting as a second messenger capable of transducing the extracellular stimulus into an intracellular signaling event. Among many examples, there is evidence that Zn has a partially identified role as a novel second messenger that augments insulin activity – which could be pertinent to insulin resistance. Zn has other roles in diabetes, evidence including variants in the Zn transporter, which may have a role in the occurrence of type 2 diabetes.

Though Zn has been termed a relatively safe essential micronutrient, elaborate measures to maintain Zn homeostasis within individual cells and within the mammalian body as a whole, including the human body, developed in early stages of evolution and are very apparent today. These measures are recognized in both protecting against the accumulation of excessive Zn and averting Zn depletion when the availability of Zn for individual cells or/and for the body as a whole is suboptimal. The major role in maintaining Zn homeostasis is attributable to the Zn transporter families of proteins.

Zn delivery to tissues and cells and its intracellular distribution are tightly regulated by a family of proteins that control

uptake, efflux, and compartmentalization of Zn. In mammals, this is accomplished by numerous Zn transporters encoded by at least 27 genes from three families. These are the SLC30 (cation diffusion facilitator/ZnT), the SLC39 (ZIP solute transporters), and the Zn-sensitizing MTs (diffusion facilitator proteins). The metallothioneins (MTs) have a regulatory role in Zn uptake, storage, distribution, and release. The ZIP family facilitates the influx of Zn into the cytosol from the outside of the cells and from the lumen of intracellular vesicles, and the ZnT transporters enable the efflux of Zn out of the cytosol into the lumen of intracellular organelles and to the outside of the cells. ZIP and ZnT transporters work in an opposite but coordinated manner to maintain cellular Zn homeostasis (though elucidation of the mechanism of their coordinated roles remains unclear). The distribution of these transporters is tissue-specific. Each of these Zn transporters has its own subcellular localization and its own physiological roles. A description of these, except for those having a role in Zn homeostasis in the GI tract and in inborn errors of metabolism, is beyond the scope of this article. However, from a clinical perspective, it should be mentioned that many ZnT and ZIP transporters are also regulated by cytokines or hormones. ZIP14, for example, is upregulated by Il-6 in mouse hepatocytes. This may have a role in hypozincemia that accompanies the acute phase response to inflammation and infection.

Whole Body Zinc Homeostasis

The gastrointestinal tract has the major role in maintaining whole body Zn homeostasis. Results of Zn isotope studies are consistent with ingested Zn^{++} entering a common pool in the acid milieu of the stomach but quickly liganding to proteins and their products (peptides and amino acids, notably histidine) and possibly a range of small organic molecules on entering the alkaline medium of the duodenum. Inositol hexaphosphate or phytic acid has received much attention as a major dietary constituent, especially in grains, that influences Zn bioavailability by forming strong ligands with Zn as the chyme passages through the pylorus to the alkaline medium of the upper small intestine. The potentially major impact of these Zn-phytate ligands on Zn absorption in adult humans is widely accepted and is evident in the results of modeling total daily absorption of Zn as a function of dietary zinc and phytate.

ZnT1, ZIP4, and ZIP5 are involved in Zn transport into, across, and out of the enterocyte. ZIP4 is not only localized at the apical surface of the enterocytes, primarily in the duodenum and jejunum, but also recognized in the stomach, cecum, and colon. Abundance of ZIP4 localized to the plasma membrane increases with zinc depletion and decreases with excess Zn availability to this protein. ZIP4-mediated transport and, therefore, Zn uptake into the enterocyte is a saturable process. ZIP4 expression appears to be regulated by transcriptional and posttranscriptional mechanisms in response to Zn availability to ZIP4 at the apical surface of the enterocyte. Adaptation to changes in quantities of available Zn occurs within hours, with Zn repletion resulting in rapid endocytosis into the enterocyte to counterbalance the decrease in the availability of Zn at the apical surface. This may well substantially alter the dissociation constant (thereby, e.g., competing more effectively with phytate for Zn binding). ZnT1 responds to excess Zn in the cytosol of enterocytes (and all other cells) by extrusion of excess Zn (either across the basolateral membrane or into the gut lumen) and Zn vesicular deposition. ZIP5 is localized to the basolateral surface under Zn-replete conditions; it may oppose ZIP4 and be involved in enterocyte sensing of increases in body Zn, thereby increasing serosal to mucosal transport of Zn. ZnT2 and ZnT4–7 are localized to vesicles and the Golgi apparatus and appear to be involved in various phases of membrane trafficking within the enterocytes.

Zn stable isotope techniques are increasingly effective in providing measurements demonstrative of the regulation of Zn homeostasis in the GI tract, including excretion of endogenous Zn that has been secreted primarily via the pancreas. Zn absorption is understood to occur primarily in the duodenum and jejunum though further studies are needed, especially with respect to the reabsorption of endogenous Zn including that secreted in Zn metalloenzymes required for protein digestion.

Zn absorbed into the portal circulation is transported loosely bound in the portal blood to the liver, which has a key role in Zn metabolism, and then into the systemic circulation loosely bound to albumin with a small percentage tightly bound to alpha-2 macroglobulin. The liver is the principal contributor to the exchangeable Zn pool (EZP). Apart from the liver, Zn in all other soft tissue/organs is thought to contribute to this pool, which accounts for approximately 10% of total body Zn and which is regarded as of special metabolic importance. About 60% of total body Zn is in the muscle and 30% in the bone. Excretion of endogenous Zn is primarily via the gut, where it has an important role in regulation of body Zn. The kidneys and integument have secondary excretory roles in normal circumstances.

Significant stores of body Zn have not been identified, though the quantity in intracellular vesicles varies with Zn status, suggesting a substantial readily available store in times of Zn sufficiency for these cells. The liver also contains a modest store 'in times of plenty.' Bone accounts for approximately 30% of body Zn; the availability of this Zn in times of body Zn depletion remains controversial, but the balance of current evidence suggests that at least some of this Zn can be regarded as a store and readily exchanges with plasma Zn, though not so readily as the much smaller liver 'store.' Because of the high percentage of total body Zn in bone, even 20% readily available represents a substantial store.

Health Effects

Though the complex interplay of the impressive number of Zn transporters that have evolved serves to minimize the risks of mammalian Zn depletion or excess, this system lacks perfection. Detectable excess, due primarily to accidental acute or chronic ingestion of large overdoses, is rare but potentially lethal. In contrast, Zn deficiency is common in certain populations and environmental circumstances, though both the incidence and range of adverse effects of acquired Zn deficiency remain to be defined. The difficulty in assessing incidence can be attributed to the meager number and reliability of biomarkers of Zn deficiency, despite extensive efforts to find suitable biomarkers. Furthermore, the symptoms and signs of Zn

deficiency are primarily nonspecific, perhaps reflecting the wide range of the biological roles of Zn in all cells. This has resulted in a number of clinical or public health features of Zn deficiency for which there is some tantalizing evidence but which lack sufficient confirmation. A further confounder is that, judged on plasma/serum Zn data or on the absence of the classical features of Zn deficiency as demonstrated in untreated *acrodermatitis enteropathica* (see succeeding text), even apparently mild Zn deficiency can be associated with or be a primary contributor to a fatal outcome. Is it then correct to consider this a mild Zn deficiency state?

Zn deficiency likely affects every cell in the body. Initially, this may be limited to exporting stored Zn in vesicles. Some cells with rapid turnover are, however, especially vulnerable to Zn deficiency. Notable among these are cells lining the gastrointestinal tract and the central nervous system and cells comprising the immune system. Epiphyseal growth, especially rapid growth in young childhood, is also vulnerable to Zn deficiency. It is the effects on these systems (and possibly others, including the reproductive system in adolescence) that are especially important to the clinical and public health effects of Zn deficiency. The skin and mucosal surfaces are the first tissues to be overtly affected in experimental Zn deficiency. The greatest concern globally for the largest population known to be afflicted by Zn deficiency is for the vast number of poor young children who are dependent virtually entirely on a vegetable-based diet after the early months of breast-feeding. Those who are fortunate enough to also receive a regular supply of animal milk are likely to be at least a partial exception. At the time that our genome developed, including genes vital for Zn homeostasis, our Paleolithic ancestors were hunter-gatherers. Even before they could chew solid meat, a major source of bioavailable Zn, their mothers would premasticate and feed their young from their mouths. Furthermore, they did not cultivate grains, which now provide the bulk of complementary foods and which include substances that impair the bioavailability of Zn. Most notable among these substances is inositol hexaphosphate (phytate) though recent data have suggested that phytate may not play such a prominent role in inhibiting Zn absorption in children as has been observed in adults. The WHO has concluded that Zn deficiency is associated with as many as 300 000 diarrheal-related deaths per year in this population, which typically is bedeviled by an environment characterized by poor sanitation, poor hygiene, dirty water, lack of maternal education, and inadequate nutrition. With a recent notable overall decrease in under-five mortality, this figure requires reevaluation. Meanwhile, heroic, costly, and minimally successful (overall) efforts continue to be directed to the distribution of Zn as an adjunct treatment to oral rehydration fluids (also frequently unavailable). Adequate studies of what it will take to prevent rather than treat the Zn deficiency causally associated with severe diarrhea await attention. Underlying the role of Zn deficiency itself (and its basic poverty-driven causes) are damaged gut mucosa, villous atrophy, and intestinal inflammation. Disruption of the immune system has an intimate relationship to these defects. Zinc deficiency has adverse effects on all aspects of the innate and adaptive immune systems. T-cell functions are especially sensitive to Zn deficiency as is the balance between different T-cell subsets. Other examples of specific defects include the number and function of neutrophils, monocytes, and NK and B cells. Other recognized effects of Zn deficiency that are not readily apparent from specific signs and symptoms include evidence of inflammation, oxidant stress, and DNA damage.

Not all communities, populations, or even entire developing countries have young children who are at high risk of Zn deficiency. The WHO has identified three indicators of populations of young children at high risk of Zn deficiency: prevalence of inadequate dietary Zn, a stunting rate $> 20\%$, and low serum/plasma Zn levels. The challenge of identification is apparent from critical evaluation of each of these indicators; for example, not all stunting can be attributed to Zn deficiency. Though a serum Zn of 70 $\mu g\ dl^{-1}$ is often quoted as a lower limit of normal, this limit depends on age, physiological state, and the conditions, including time of day, under which the sample is collected. Moreover, moderate hypozincemia may be attributable to inflammation. Nevertheless, with due caution in interpretation, some information on Zn status may be gleaned from modest–moderate hypozincemia, and more severe hypozincemia can be used to confirm clinically evident Zn deficiency.

While dietary data are potentially the most useful indicator of the three (a prevalence of $>25\%$ inadequate Zn intake using the cut-point method), there are many pitfalls, especially the uncertainty of the bioavailability of the Zn. Host factors may also be the major factor impairing absorption. This provides one of the persuasive reasons for applying Zn stable isotope techniques combined with Zn analyses of duplicate diets, based on typical daily diets of the population, to a limited number of representative subjects, with data analysis including saturation response modeling. The goal is to determine the quantity of Zn absorbed each day. An example of the latter as well as of impaired bioavailability is illustrated in **Figure 1**, using data from a study of young children in Belgaum, India.

Note the low Zn absorption at low levels of dietary Zn compared with data used for control purposes (presumably despite maximal possible upregulation of ZIP4 in a potentially damaged gut) resulting in a higher level of dietary Zn required to meet normal physiological requirements. Note also that the slope of the curve at Zn intakes higher than that required to meet high physiological requirements is subsequently maintained at a higher level than controls (presumably still at maximal rates of upregulation). This relationship between daily Zn absorption and daily dietary Zn is compatible with the hypothesis, at this stage tentative, that physiological requirements are abnormally (pathologically) high. At a minimum, these data underscore the importance of more extensive studies. Meanwhile, such data potentially provide an improved assessment of dietary Zn requirements for this population.

Though most studies in recent years have focused on young children, the first reports of human Zn deficiency were in relation to the syndrome of adolescent nutritional dwarfism characterized especially by severe stunting and hypogonadism. It is unclear whether this syndrome is still evident in Egypt and Iran, and there has been a notable lack of more recent studies of potential Zn deficiency during the adolescent growth spurt.

Zn deficiency has received primary attention internationally because of current concern about the global extent and adverse effects of inadequate intake and absorption of this prominent micronutrient. However, attention is also merited

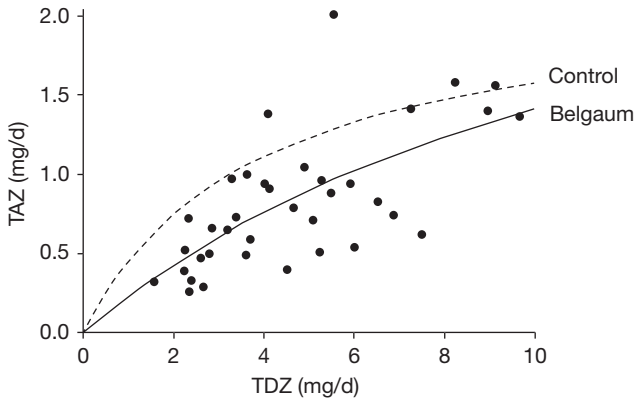

Figure 1 Saturation response model of daily Zn absorption vs daily Zn intake in children ($n = 40$) aged 18–36 months in rural Indian village compared to control subjects (normal fully breast-fed infants aged 4 months). The horizontal line shows the normal physiological requirement for 1–3-year-olds. The vertical lines show the intake needed to meet the physiological requirements.

for acquired Zn deficiency in more affluent countries, especially in recognized subgroups of the population. These include especially premature and low-birth-weight infants; prolonged breast-feeding without adequate Zn from complementary foods; intravenous feeding without adequate Zn in the infusate; disease states, especially of the gastrointestinal tract and probably the pulmonary system; elderly subjects; subjects with more generalized malnutrition; and those on diets low in bioavailable Zn. With respect to more generalized suboptimal nutrition, Zn deficiency has been documented to affect weight gain as well as retard linear growth.

Recent renewed attention has been directed to Zn requirements of premature, especially very low-birth-weight, infants. This has included a randomized controlled study with generous quantities of Zn resulting in favorable clinical outcomes. Current concern is addressed especially to the lack of availability of Zn to add to intravenous nutrition infusates. This concern is not limited to premature infants and is reminiscent of the *acrodermatitis* pattern of skin lesions recognized in the 1970s to result from omission of Zn from the formulas for intravenous feeding.

Breast milk has favorable concentrations of Zn in early lactation. However, there is a notable physiological decline in Zn as lactation progresses to levels that are too low to meet estimated Zn requirements of the infant with breast-feeding alone between 4 and 6 months' lactation. This, not infrequently, becomes a practical health issue during late infancy and the second year with resulting poor growth, poor appetite, and irritability. The feeding difficulties can be especially stressful to mothers and to family dynamics.

The relationships between Zn deficiency and disease of the gastrointestinal tract are likely two-way and complex, having adverse effects on Zn homeostasis and on the progress of gut-related disease of which diarrhea is but one manifestation.

Acquired Zn deficiency or/and favorable effects of Zn therapy have been linked to many other diseases but are beyond the scope of this article.

Inherited diseases of Zn metabolism/homeostasis, though rare, are important for two reasons. First, the clinical manifestations can be treated effectively and, if necessary, for a lifetime with adequate Zn supplements. Second, they have yielded important insight into normal Zn homeostasis. The prototype is *acrodermatitis enteropathica* (AE), which is an autosomal inherited disorder attributable to one of several polymorphisms of the SLC39A4 gene encoding for the ZIP4 zinc transporter. This transporter, when localized to the apical surface of enterocytes primarily in the duodenum and jejunum, has a role in the active, saturable absorption of Zn from the gut lumen. Experimentally, this active saturable pathway has the major role in Zn absorption when available Zn levels in the lumen are not excessive but adequate to meet physiological requirements if the ZIP4 transporter is functioning normally. AE, therefore, provides indisputable evidence of the importance of ZIP4 to the maintenance of normal Zn status and avoidance of severe Zn depletion.

Classically, this disease presents clinically with a severe erythematous rash, primarily in the perineal region and around the body orifices. Other classical signs, that is, alopecia and diarrhea, are inconsistent, though most untreated cases have GI manifestations. Psychological disorders are typical, including loss of hedonic tone. Anorexia also occurs frequently. Growth failure is also apparent in untreated cases. Since 1970, when the empirical value of Zn was first noted, treatment with Zn, sometimes at very high levels, has resulted in correction of the phenotypic abnormalities. However, before Zn therapy was introduced, these infants classically had a progressive downhill clinical course with frequent infections, severe growth failure, and a fatal outcome before the end of infancy. Some AE patients survived into adult life without Zn therapy, contributing factors including the empirical use of dihydro-dioxiquin or less lethal polymorphisms Typically, these subjects had more chronic psoriasiform rashes distributed anywhere on the skin, stunting, depression, and, if pregnant, a high risk of congenital malformations in the offspring. A transient early infancy syndrome with a similar phenotype in the occasional breast-fed infant is attributable to a defect in ZnT2 required for the secretion of Zn into human milk.

Summary

The molecular biology of Zn has been reviewed with special regard to the Zn transporter families, which have a dominant role in Zn absorption and Zn homeostasis in and between all cells, which includes a major contribution of Zn to cell signaling. Clinical effects of Zn deficiency, with emphasis on effects of

global public health relevance, are reviewed. AE is allotted special attention because of the insights it has provided into the clinical effects of severe Zn deficiency and the indisputable evidence it provides of the outstanding role of ZIP4 at the apical surface of the enterocyte in regulating Zn absorption. Both the value and potential (combined with saturation response modeling) of appropriately designed Zn stable isotope studies are emphasized as investigative tools of comparable importance to randomized controlled studies of Zn supplementation. At a basic and human research level, much remains to be learned about Zn, a relative newcomer to that small group of microminerals of major importance for human health.

See also: Bioavailability of Nutrients; Phytic Acid: Properties, Uses, and Determination; Zinc: Properties and Determination.

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Zinc: Properties and Determination

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Zinc Properties

Zinc is the thirtieth element of the periodic table (atomic number 30). Its symbol is Zn, and it is included in group 12 (or IIB), thus corresponding to the transition metals. The element was probably named by the alchemist Paracelsus after the German word *zinke*. Later, Andreas Sigismund Marrggraf, a German chemist, isolated this element for the first time in 1746 by heating calamine and carbon. It has a hexagonal crystal structure, a bluish color, a melting point of 419.6 °C, a boiling point of 907 °C, an atomic weight of 65.39, and a relative density of 7.133 g cm^{-3}. The melting point is the lowest of the transition metals aside from mercury and cadmium.

It has an electronic structure of [Ar] $3d^{10}$ $4s^2$ with two electrons in the first electron shell, eight electrons in the second electron shell, 18 electrons in the third electron shell, and two electrons in the fourth electron shell. Therefore, it is very easy that these two later electrons are lost forming the Zn^{2+} cation, its most common state.

Zinc is a hard, brittle metal and the 24th most abundant element in the earth's crust (0.0075%) with a medium valor of 64 ppm. Seawater contains 30 ppb of zinc. The element has a low affinity for oxides and prefers to bond with sulfides. It is widely distributed in minerals such as sphalerite [(Zn,Fe)S], calamine or hemimorphite ($Zn_4Si_2O_7(OH)_2 \cdot H_2O$), smithsonite [$ZnCO_3$], hydroxincite [$Zn_5(CO_3)_2(OH)_6$], or wurtzite [another zinc sulfide]. This element exists in nature in five isotopes: ^{64}Zn (48.89%), ^{66}Zn (27.81%), ^{67}Zn (4.11%), ^{68}Zn (18.56%), and ^{70}Zn (0.62%). The radioactivity of ^{64}Zn and ^{70}Zn with a half-life of 4.2×10^{18} and 1.3×10^{16} years, respectively, can be ignored.

This element is normally found in association with other metals such as copper and lead in minerals. Long before the discovery of zinc as a metal, zinc ore was already being used to produce the copper–zinc alloys (commonly called brasses in metallurgy). Objects made of brass are known from Babylonia and Assyria from 3000 BC and from Palestine dating from 1400 to 1000 BC. Currently, brasses are used to manufacture wires, weapons, coins, boilers, and several musical instruments such as the saxophone. Until the end of the eighteenth century, zinc was generally imported from India and was considered to be very costly. Other zinc applications include anticorrosive agents with metals, use as an anode with other metals in batteries, automotive industry, luminescent paint, fungicide, protecting wood against fire, and rubber from ultraviolet radiation.

Zinc is a mineral element that is spread throughout the organism with more than 95% located in intracellular space. The highest proportion of body zinc is found in the skeletal muscle (50–60%) and bones (30%) though there are other organs with high levels of zinc such as the liver and kidneys (50–60 μg g^{-1}). The exchange of zinc in these tissues is slow, making it not accessible in deficiency status. Although in short-term zinc deficiencies, small amounts of zinc can be available coming from the hepatic reservoir, generally it is recognized that an organism does not have any specific reservoir of zinc, and therefore, it depends on a regular intake.

It plays an essential role in the genetic expression and regulation of cellular growth and differentiation because it participates as cofactor in more than 120 enzymes (alkaline phosphatase, superoxido dismutase, alcohol dehydrogenase, and peptidases, among others) involved in carbohydrates, proteins, and nucleic acid metabolism. Therefore, it is necessary for DNA, RNA, and protein synthesis, and it plays an important role in the immunologic system and in the masculine reproductive function as it is involved in the mechanism of action of testosterone. In addition, zinc is required for the maintenance of healthy bones, being essential for normal collagen synthesis.

There are several sources of zinc in the diet. Red meats, liver, and mollusks (particularly oysters) are important sources of bioavailable zinc. Presence of zinc in red meat (cow and sheep meat) contributes to meat color; indeed, zinc content in these kinds of products is almost twice as high as in chicken meat. However, in several world regions, most zinc incorporated in human diet comes from cereal and legumes. Among vegetables with high content in zinc could be highlighted beans, lentils, and nuts. Most of zinc content in cereals is in the external part; therefore, zinc content depends on the refinement level of the cereal. Aside from milk, fruits and vegetables are low in zinc. Therefore, zinc bioavailability can be low in vegetarian diets, in particular in those based on integral cereal and vegetables, due to the high content in phytic acid, which is a powerful inhibitor of zinc absorption.

As shown, there are several factors that determine zinc absorption (promoting or impairing its bioavailability). They are classified in physiological or dietary factors. Among dietary factors it has been widely reported that the presence of phytic acid (myoinositol hexaphosphate) may compromise zinc bioavailability from meals because it has strong tendency to form chelates with multivalent metal ions. Similarly, it has been described in the literature several zinc–iron and zinc–calcium interactions. Several studies have found a significant negative interaction between zinc retention and iron content in food. However, this interaction seems to depend on the iron chemical form (heme iron or inorganic iron) and the presence of other dietary components. In contrast, the presence of animal proteins (meat factor) seems to enhance zinc bioavailability. This effect is due to the formed products during the digestion of proteins that bind zinc and facilitate its absorption. Nevertheless, this influence will depend on the digestion level of proteins and its composition of sulfur-containing amino acids such as cysteine because zinc usually binds to proteins via these residues. Finally, several studies have shown that hydrolysis of caseins may have a similar effect. Many of these peptides (called casein phosphopeptides)

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contain highly polar acidic sequences of three phosphoserines followed by two glutamic acid residues, it being partly resistant to the enzymatic hydrolysis in the digestive system. The negative charges of phosphate groups belonging to casein phosphopeptides can form soluble complexes with divalent cations such as zinc producing a gradual release of this element in the intestinal lumen and improving their absorption due to no saturation of determined transport mechanisms.

Zinc Analysis

Sample Treatment

Most of the analytical methods used for zinc determination in food and biological samples require the availability of a sample solution. Only few techniques (such as infrared spectroscopy or x-ray fluorescence) are able to carry out a direct analysis of solid samples. Even these techniques may require in many cases a pretreatment of the sample for its correct use. For this reason, the sample treatment could be defined as the step that involves subjecting the sample to a number of physical and chemical processes to prepare it for analysis. However, it should be noted that the risk of sample contamination and analyte loss at this stage are often high.

In relation to zinc analysis in food samples, this process often involves the destruction of organic matter and other matrix constituents that may interfere with the studied analyte. A common practice is incineration of the sample in a muffle furnace using a time–temperature controlled program (dry mineralization). Therefore the oxidation of organic matter is achieved. Process efficiency and risk of analyte loss primarily depends on the food matrix and the time–temperature program applied. The ash residue is usually dissolved with acids (HCl, HNO_3, etc.) after a blanching step.

The main disadvantages of dry mineralization are that it is a very slow procedure, requires several hours to complete, and is influenced by numerous factors such as time, temperature, and acid mixture. For these reasons, an appropriate alternative method is wet mineralization, which is based on the oxidizing action of strong acids either alone or in acid mixtures that sometimes include hydrogen peroxide. These reagents are very useful for zinc and trace elements determination in a great variety of samples thanks to the availability to release low volatile cations and keep them in solution.

Another drawback of the preceding mineralization methods is that in the case of highly volatile elements such as selenium, mercury, or arsenic, there is a significant risk of analyte partial losses. For zinc determination, this problem is not initially present unless sample preparation is oriented to determine these volatile elements simultaneously. In such cases, performance of wet mineralization in microwave ovens using pressure vessels is recommended as an alternative. These systems have the advantage of using a smaller volume of reagent than traditional ones. The most significant disadvantage is the risk of explosion due to pressure increase caused by the destruction of organic matter. The pressure vessels are placed on a turntable to avoid problems arising from lack of homogeneity in the distribution of radiation within the microwave oven.

Usually, liquid samples treatment is much easier than solid samples. They may require filtration, centrifugation (in the case of suspended particulate matter), and mineralization. Finally, in some cases zinc can be presented in the sample at low concentrations that are below the detection limit of the analytical technique used. To overcome this problem, several zinc preconcentration methods have been studied which are discussed in the following section.

Preconcentration Methods

Determination of zinc in foods and biological samples cannot be easily achieved in many cases because of two main difficulties: the very low concentration of zinc (may be below the detection limit of several analytical techniques) and the interfering effects of the matrix to be analyzed. In these cases, preconcentration and/or separation methods prior to zinc analysis are therefore needed to improve sensitivity and selectivity. Several procedures, such as solid phase extraction, liquid–liquid extraction (LLE), cloud point extraction, and coprecipitation, have been proposed and applied for the enrichment and separation of zinc from a sample matrix. Table 1 summarizes some published papers on zinc preconcentration techniques in foods.

Solid phase extraction (SPE) is a sensitive, inexpensive, and fast sample preparation process that involves partitioning between a liquid phase (sample or solvent with analytes) and a solid phase (sorbent). The analytes, which are dissolved or suspended in a liquid mixture, are separated from the solution by sorption on the solid sorbent according to their physical and chemical properties. The general procedure is to load a solution onto the solid phase, remove undesired components, and later capture the desired analytes with another solvent into a collection tube. The selection of an appropriate SPE extraction sorbent depends on the mechanism of interaction between the sorbent and analyte of interest. Furthermore, control of process parameters such as pH, sample flow rate, and sample volume is highly important. Various solid phase extractants have been used to zinc preconcentration such as alumina, adsorption resin (Amberlite XAD – 16), columns packed with ethyl vinyl acetate, microcrystalline triphenylmethane, multiwalled carbon nanotubes, and modified silica gel, among others. Carbon nanotubes are an allotrope of carbon. They are relatively new materials that have attracted great attention in recent years in analytical chemistry due to a high surface area and strong adsorption ability. A carbon nanotube is a one atom thick sheet of graphite rolled up into a seamless cylinder with diameter of the order of a nanometer.

Liquid–liquid extraction (LLE) is the oldest and the most widely used method in preconcentration procedures for the determination of trace elements due to its simplicity. In this technique, the metal is distributed between two immiscible liquid phases (usually an aqueous and an organic phase). Preconcentration of metals is achieved by the addition of a chelating agent to an aqueous sample and extraction of formed metal complexes into an organic phase. In the case of zinc, ammonium pyrrolidin thiocarbamate and diethylammonium-diethyldithiocarbamate have been used with successful results. In parallel, trichlorotrifluoroethane (Freon TF) or xylene are

Table 1 Preconcentration and separation techniques for zinc analysis

Investigation	*Reference*
Sequential multielement determination of iron and zinc in water samples by high-resolution continuum source graphite furnace atomic absorption spectrometry after column solid-phase extraction onto multiwalled carbon nanotubes	Krawczyk, M., Jeszka-Skowron, M. and Matusienwicz, H. (2014). *Microchemical Journal* **117**, 138–143
Synthesis, characterization, and analytical application of Zn(II)-imprimed polymer as an efficient solid-phase extraction technique for trace determination of zinc ions in food samples	Behbahani, M., Salarian, M., Bagheri, A., Tabani, H., Omidi, F. and Fakhari, A. (2014). *Journal of Food Composition and Analysis* **34**, 81–89
A new sorbent of modified MWCNTs for column preconcentration of ultra trace amounts of zinc in biological and water samples	Tajik, S. and Taher, M. A. (2011). *Desalination* **278**, 57–64
Liquid–liquid extraction in flow analysis: A critical review	Silvestre, C. I. C., Santos, J. L. M., Lima, J. L. F. C. and Zagatto, E. A. G. (2009). *Analytica Chimica Acta* **652**, 54–65
A green and efficient procedure for the preconcentration and determination of cadmium, nickel, and zinc from freshwater, hemodialysis solutions, and tuna fish samples by cloud point extraction and flame atomic absorption spectrometry	Galbeiro, R., Garcia, S. and Gaubeur, I. (2014). *Journal of Trace Elements in Medicine and Biology* **28**, 160–165
Simultaneous preconcentration of copper, zinc, cadmium, and nickel in water samples by cloud point extraction using 4-(2-pyridylazo)-resorcinol and their determination by inductively coupled plasma optic emission spectrometry	Luiz Silva, E., dos Santos Roldan, P. and Giné, M. F. (2009). *Journal of Hazardous Materials* **171**, 1133–1138
Cloud point extraction and spectrofluorometric determination of aluminum and zinc in foodstuffs and water samples	Babili Tabrizi, A. (2007). *Food Chemistry* **100**, 1698–1703
Preconcentration of some metal ions with lanthanum-8-hydroxyquinoline coprecipitation system	Feist, B. and Mikula, B. (2014). *Food Chemistry* **147**, 225–229
Coprecipitation of trace elements with Ni^{2+}/2-Nitroso-1-naphthol-4-sulfonic acid and their determination by flame atomic absorption spectrometry	Dogan Uluozlu, O., Tuzen, M., Mendil, D. and Soylak, M. (2010). *Journal of Hazardous Materials* **176**, 1032–1037

the best choices for extraction phase due to their low solubility of these solvents in water. For the analytical measurement of the extracted zinc ion, a back extraction step to an aqueous medium, usually acid, is recommended. Although LLE has shown excellent results for multielement analysis with a high extraction efficiency, some drawbacks such as the use of large volumes of toxic organic solvents, high sensitivity to operational conditions, and time-consuming process have made LLE a technique not easily automated.

Cloud point extraction (CPE) is related to LLE but has emerged as an alternative for several reasons: (i) its excellent concentration factors, (ii) the smaller required sample size, (iii) the use of a small volume of organic solvent that is generally toxic, (iv) the use of nontoxic surfactants, (v) the reduction of laboratory residues, and (vi) it is recognized as a safer, simpler, and cost-effective procedure. CPE is based on the following phenomenon: in much diluted solutions of nonionic surfactant, the monomers are dispersed in the solvent. But above the critical micellar concentration of the surfactant, these monomers associate spontaneously, forming aggregates of colloidal dimensions termed micelles, due to the diminished solubility of the surfactant in water. The surfactant solution becomes turbid because it attains the cloud point. This clouding phenomenon can be induced by changing the temperature, surfactant concentration, or the choice of a compatible pH buffer, resulting in the separation of a single isotropic micellar phase into two phases, a surfactant-rich phase of small volume and an aqueous phase. Choosing an appropriate chelating agent, able to form with the metal ion a hydrophobic complex that is entrapped by micellar structures, extraction and subsequent preconcentration of zinc in the surfactant rich phase can be achieved. Among the nonionic surfactants commonly used, Triton X – 114 (octylphenoxypolyethoxyethanol) is the most feasible for preconcentration cloud point. Regarding chelating agents, 1-(2-pyridilazo)-2-naphthol, 8-hydroxyquinoline (8-HQ), 4-(2-pyridylazo)-resorcinol (PAR), and 1-(2-thiazolylazo)-2-naphthol have been used in determination of zinc.

Finally, coprecipitation is another technique for zinc preconcentration and separation of food matrices prior to analytical determination. It offers some advantages such as simplicity and the fact that various analyte ions can be preconcentrated and separated simultaneously from the matrix. In the coprecipitation procedure, a precipitate is formed by combination of a carrier element and a suitable inorganic or organic coprecipitant. Further, this precipitate is separated by centrifuging, filtering, or washing. For zinc coprecipitation technique, it has been applied with successful results, several combinations of a carrier element such as cobalt (III), lanthanum (III), molybdenum (IV), or nickel (II) with a suitable organic ligand like diethyldithiocarbamate (DDTC), 8-hydroxyquinoline (8-HQ), 2-nitroso-1-naphthol-4-sulfonic acid (NNSA) or an inorganic ligand like gallium hydroxide.

Atomic Absorption Spectroscopy

The analytical techniques commonly used for the determination of zinc in food samples are the inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled

plasma mass spectrometry (ICP-MS), electrothermal atomic absorption spectrometry (ETAAS), and flame atomic absorption spectrometry (FAAS). Nevertheless, among these techniques, ETAAS and FAAS are extensively employed because of their high sensitivities, wide availability in analytical and clinical laboratories, and low cost. Both techniques fall within atomic absorption spectroscopy (AAS) in which electromagnetic radiation at a specific wavelength is passed through a cell containing gaseous free atoms. Atoms can be produced in two different ways using AAS: flame atomization (FAAS) and electrothermal atomization through a graphite furnace and a L'vov platform (ETAAS). Thus, the determination of zinc using an atomic absorption spectrophotometer requires a light source (wavelength $\lambda = 213.9$ nm) and an atomization source. For the light source, arc discharge lamps may be used for zinc, although hollow cathodes are preferred. Lineal calibration is required to obtain analyte concentrations in the sample. Standard working conditions for analyzing zinc by atomic absorption spectroscopy are listed in Table 2.

The flame atomizer consists of a nebulizer (which is designed to convert the sample solution into an aerosol) and a burner. The sample is aspirated through a capillary into a chamber through which oxidant and fuel are flowing. Air–acetylene and nitrous oxide–acetylene are the most commonly used oxidant–fuel mixtures. When conventional FAAS proves impractical because of zinc values are below or near the detection limit of the instrument, it is recommended to use ETAAS. In ETAAS the atomization is produced by the passage of electrical current through a cylindrical graphite tube (called graphite furnace). This process comprises several steps: preheating, drying, pyrolysis, atomization, and cleaning, which are achieved by a temperature–time interval program (ramps heating). During the drying stage, liquid from the sample is evaporated and driven off. In the pyrolysis stage some of the concomitants in the sample are removed. Finally, the temperature is rapidly increased to quickly vaporize and atomize the sample. During this process, the system is flushed with protective gas (usually argon) to exclude air from the sample compartment and to minimize oxidation of the atomizer material at high temperatures.

To reduce matrix effects when ETAAS is used, samples can be simply diluted with water, or various matrix modifiers are added. To avoid the risk of contamination due to excessive sample handling and/or addition of reagents during the dilution procedure, a flow injection system (FI-ETAAS) has been used to determine zinc in biological samples such as human saliva.

Table 2 Standard conditions for zinc analysis by AAS

Spectrometers setting	*Zinc*
Wavelength (nm)	213.9
Slit	0.2
Nebulizer	Spoiler
Oxidant	Air
Fuel	C_2H_2
Flame condition	Oxidizing
Detection limit ($\mu g\ ml^{-1}$)	0.168
Quantification limit ($\mu g\ ml^{-1}$)	0.560

Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES)

Plasma may be defined as a luminous volume of partially ionized gas. It is generated from radiofrequency magnetic fields induced by a copper coil wound around the top of a glass torch. ICP-OES is a multielemental technique that is able to determine concentrations of multiple elements in a sample with a single aspiration obtaining a high degree of variability and minimum interferences. The sample solution is introduced into the core of an inductively coupled argon plasma that generates a temperature on the order of 10 000 °C where it rapidly undergoes dissolvation, vaporization to molecular level, and dissociation into atoms (some of the atoms are ionized). These atoms are excited in the plasma, and they revert to their ground state emitting light with a characteristic wavelength, which is measured using an optical spectrometer. Therefore, argon plasma is needed to produce atomization and excitation. The nearly complete atomization of the sample minimizes chemical interferences.

Samples can be prepared by dry, wet, or microwave digestion method, and a wide range of concentrations can be used without the need to dilute or concentrate the sample. For zinc determined by ICP-OES, a solution detection limit of 5 $\mu g\ l^{-1}$ and a sample detection limit of 2.5 $\mu g\ g^{-1}$ can be achieved. Also, many of the other mineral elements can be quantified, thus reducing time of analysis. These advantages, together with the high speed and excellent instrument stability, make ICP-OES highly attractive.

ICP-OES has been used for zinc determination in a great variety of food samples such as wines, infant foods, fruits, vegetables, fish, and shellfish, among others. There is also an AOAC Official Method (2011.14) for the determination of zinc in infant and follow-up formula by means of ICP-OES. The analytical lines used for zinc are 213.856 (most used), 206.200, and 202.548 nm, respectively. Other parameters that must be controlled are RF power (W): nebulizer flow ($l\ min^{-1}$), auxiliary gas flow, plasma flow ($l\ min^{-1}$), and sample flow ($l\ min^{-1}$).

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

ICP can also be used coupled with a mass spectrometer leading to inductively coupled plasma-mass spectrometer (ICP-MS). The inductively coupled argon plasma is once again used as an excitation source for the elements of interest. However, in contrast to ICP-OES, the plasma in ICP-MS is used to generate ions that are then introduced to the mass analyzer through a sampler cone followed by a skimmer cone. Mass spectrometers are instruments that separate ions according to their mass to charge ratio (m/z) and then accurately quantify the resulting ions once they pass through a detector.

ICP-MS is also a multielement technique, characterized by low background and offers extremely high sensitivity to a wide range of elements with a lower limit of detection than ICP-OES. For zinc determination by ICP-MS a solution detection limit of 0.1 $\mu g\ l^{-1}$ and a sample detection limit of 0.05 $\mu g\ g^{-1}$

can be achieved. Regarding interferences, spectroscopic interferences are probably the largest ones in ICP-MS and are caused by atomic or molecular ions that have the same m/z as the analytes of interest. Current ICP-MS instrumental software makes a correction for all known atomic 'isobaric interferences,' or those caused by overlapping isotopes of different elements; however, they do not provide corrections for most polyatomic interferences. Such interferences are caused by polyatomic ions that are formed from precursors having numerous sources, such as the sample matrix; reagents used for preparation, plasma gases, and entrained atmospheric gases. Table 3 shows the main polyatomic interferences for zinc determination.

According to Table 3, zinc has five stable isotopes. Different zinc isotopes, including ^{64}Zn, ^{66}Zn, and ^{68}Zn, have usually been attempted for zinc determination in biological samples. The determination of zinc in this type of sample makes difficult by the different degrees of S-containing interferences on the zinc isotopes. It has been demonstrated that apparent zinc concentrations at masses 64, 66, and 67 increase linearly with the concentration of sulfuric acid. Only mass 68 shows no interference due to S-containing polyatomic ions. Therefore, the use of ^{68}Zn is generally recommended for body fluids analysis eliminating the interferences from sulfur with ^{64}Zn and ^{66}Zn.

This technique is also recommended for zinc bioavailability studies. The bioaccessibility of zinc in seafood has been assessed using ICP-MS following a continuous sample leaching procedure with artificial saliva or gastric juice. The methodology was validated using certified reference materials and mass balance calculations by carrying out total digestion of the remaining sample residues. On the other hand, bioimaging studies to quantify zinc and other trace elements within tissue sections have been achieved using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). This technique has been also applied over brain, hypothalamic rodent, or wheat grains cross sections with a spatial resolution ranging about 10–100 μm.

Finally, these multielement techniques (ICP-OES and ICP-MS) have also been used to guarantee the authenticity of some protected designation of origin food commodities. Both techniques provide quick and accurate results when employed as a basis for authentication of products with a certain quality standard, thus refusing possible imitations (see Table 4).

Table 3 Polyatomic interferences for the determination of zinc in ICP-MS

Isotope	*Abundance*	*Interference*
^{64}Zn	48.89	$^{32}S^{16}O_2^+$, $^{48}Ti^{16}O^+$, $^{31}P^{16}O_2^1H^+$, $^{48}Ca^{16}O^+$, $^{32}S^+$, $^{31}P^{16}O^{17}O^+$, $^{34}S^{16}O_2^+$, $^{36}Ar^{14}N_2^+$
^{66}Zn	27.81	$^{50}Ti^{16}O^+$, $^{34}S^{16}O_2^+$, $^{33}S^{16}O_2^1H^+$, $^{32}S^{16}O^{18}O^+$, $^{32}S^{17}O_2^+$, $^{33}S^{16}O^{17}O+$, $^{32}S^{34}S+$, $^{33}S2^+$
^{67}Zn	4.11	$^{35}Cl^{16}O_2^+$, $^{33}S^{34}S^+$, $^{34}S^{16}O_2^1H^+$, $^{32}S^{16}O^{18}O^1H^+$, $^{33}S^{34}S^+$, $^{34}S^{16}O^{17}O^+$, $^{33}S^{16}O^{18}O^+$, $^{32}S^{17}O^{18}O^+$, $^{33}S^{17}O_2^+$, $^{35}Cl^{16}O_2$
^{68}Zn	18.57	$^{36}S^{16}O_2^+$, $^{34}S^{16}O^{18}O^+$, $^{40}Ar_{14}N_2^+$, $^{35}Cl^{16}O^{17}O^+$, $^{34}S_2^+$, $^{36}Ar^{32}S^+$, $^{34}S^{17}O_2^+$, $^{33}S^{17}O^{18}O^+$, $^{32}S^{18}O_2^+$, $^{32}S^{36}S^+$
^{70}Zn	0.62	$^{35}Cl^{35}Cl^+$, $^{40}Ar^{14}N^{16}O^+$, $^{35}Cl^{17}O^{18}O^+$, $^{37}Cl^{16}O^{17}O^+$, $^{34}S^{18}O_2^+$, $^{36}S^{16}O^{18}O^+$, $^{36}S^{17}O_2^+$, $^{34}S^{36}S^+$, $^{36}Ar^{34}S^+$, $^{38}Ar^{32}S^+$

x-Ray Fluorescence Analysis

x-Ray fluorescence (XRF) is based on bombarding a small area of the sample with x-ray photons, which are sufficiently energetic to cause ionization of inner electrons of the atoms. Because of this unstable atomic configuration, this vacancy of an inner electron is filled by an electron from an outer orbital which emitted the difference of energy between the two orbitals as a "secondary" (or fluorescent) x-ray photon. The main difference with other spectrometry techniques (i.e., AAS or ICP-OES) is that in the latter the absorbed or emitted radiation covers the ultraviolet-visible region of the spectrum.

XRF permits a direct and fast analysis of solid samples without any chemical sample preprocessing, and it is also a multielement technique. The main advantage is an easier, cheaper analysis and a green alternative to other methods because of the reduced need for the use of reagents. The main drawback is a low sensitivity and high matrix effects being necessary to use chemometrics methods to extract the information contained in these spectra.

Table 4 Multielement determination to guarantee the authenticity of food commodities

Investigation	*Reference*
Mineral profile of kaki fruits (Diospyros kaki L.)	Mir-Marqués, A., Domingo, A., Luisa Cervera, M. and de la Guardia, M. (2015). *Food Chemistry* **172**, 291–297
Authentication of Alicante's Mountain cherries protected designation of origin by their mineral profile	Matos-Reyes, M. N., Simonot, J., Lopez-Salazar, O., Cervera, M. L. and de la Guardia, M. (2013). *Food Chemistry* **141**, 2191–2197
Influence of manufacturing conditions and discrimination of Northern Spanish cheeses using multielement analysis	Moreno-Rojas, R., Cámara-Martos, F., Sánchez-Segarra, P. J. and Amaro-López, M. A. (2012). *International Journal of Dairy Technology* **65**(4), 594–602
Authentication of the protected designation of origin horchata de Valencia through the chemometric treatment of mineral content	Boeting, K., Aguilera de Benzo, Z., Luisa Cervera, M. and de la Guardia, M. (2010). *Analytical Methods* **2**, 1723–1728
Multivariate analysis techniques as tools for categorization of southern Spanish cheeses: nutritional composition and mineral content	Moreno-Rojas, R., Sánchez-Segarra, P. J., Cámara-Martos, F. and Amaro-López, M. A. (2010). *European Food Research and Technology* **231**, 841–851
Determination of the geographic origin of onions between three main production areas in Japan and other countries by mineral composition	Ariyama, K., Aoyama, Y., Mochizuki, A., Homura, Y., Kadokura, M. and Yasui, A. (2007). *Journal of Agricultural and Food Chemistry* **55**, 347–354
Determination of trace elements in olive oil by ICP-AES and ETA-AAS: a pilot study on the geographical characterization	Zeiner, M., Steffan, I. and Cindric, I. (2005). *Microchemical Journal* **81**, 171–176

Although this technique is not commonly used in zinc analysis, it has been applied to determine zinc and other trace elements of various biological samples such as human diets, rice, human placenta, and blood. The bibliography describes different XRF analysis techniques for measuring zinc, which are summarized in Table 5.

Ion-Selective Electrodes

The potentiometric determination of zinc with ion-selective electrodes is recognized as an accurate, easy-to-implement, and low-cost technique. Moreover, it is applied to online analyses. The potentiometric property of the ion-selective electrode is determined by the ionophore, the key element of membrane composition. Different types of electrodes to determine zinc ions have been described. Those based on ion-exchanger or chelates give poor selectivity and undergo strong interference effects. Zinc solid electrodes based on zinc sulfide, telluride, or selenide (for example, an electrode with a membrane made of a zinc sulfide–silver sulfide mixture applied on a silver wire). Finally, another option is to use a liquid electrode containing zinc dithiozonate as an ionophore. However, because of the insufficiently high sensitivity and selectivity of these potentiometric methods at zinc levels found in food and biological samples, atomic absorption spectroscopy has remained the most used technique.

Currently, zinc-selective electrodes based on crown ethers have been developed. They are cyclic chemical compounds with a ring containing several ether groups. Several factors influence the use of these compounds as an electro active material for zinc-selective electrodes such as structure and cavity size of the cyclicether, stability and selectivity of zinc ion complex, and solubility and ability of the crown ether to extract the zinc ion into the membrane phase. It has been demonstrated that the substitution of some oxygen atoms of crown ethers by nitrogen atoms increases the stability of metal complexes formed with soft transition and heavy metal ions. Based on these considerations, several zinc-selective electrodes have been developed using crown ethers such as *N*,*N*′-bis (acetylacetone)ethylenediamine), dibenzo- 24-crown-8 or 1,4,7,10-tetraoxacyclododecane. They are usually incorporated in a PVC matrix combined with an anion excluder (sodium tetraphenylborate) and a plasticizer.

Together with the aforementioned electrodes, other macrocycle compounds are being employed as the carriers in zinc-selective extraction and membrane transport. Calixarenes are cyclic oligomers of phenol-formaldehyde condensates. The phenolic group of the macrocyclic molecule can also be converted into derivatives containing a wide range of functional groups with ionophoric properties. Another promising material is graphene. The possibility of using noncovalently functionalized graphene layers for potentiometric determination of zinc (taking advantage of adsorption through noncovalent interactions, due to π–π interactions with an aromatic moiety of ligands) has been recently studied with promising results.

Table 5 x-Ray techniques for zinc analysis in food and biological samples

Investigation	*Reference*
Direct determination of minerals in human diets by infrared spectroscopy and x-ray fluorescence	Mir-Marqués, A., Garrigués, S., Luisa Cervera, M. and de la Guardia, M. (2014). *Microchemical Journal* **117**, 156–163
Energy-dispersive x-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain	Paltridge, N. G., Palmer, L. J., Milham, P. J., Guild, G. E. and Stangoulis, J. C. (2012). *Plant Soil* **361**, 251–260
Assessment of trace element concentration distribution in human placenta by wavelength dispersive x-ray fluorescence: effect of neonate weight and maternal age	Ozdemir, Y., Böreczi, B., Levet, A. and Kururdirek, M. (2009). *Applied Radiation and Isotopes* **67**, 1790–1795
Prostatic Zn determination for prostate cancer diagnosis	Shilstein, S.Sh. et al. (2006). *Talanta* **70**, 914–921
Direct analysis of human blood (mothers and newborns) by energy dispersive x-ray fluorescence	Custódio, P. J., Carvalhoa, M. L., Nunes, F., Pedroso, S. and Campos, A. (2005). *Journal of Trace Elements in Medicine and Biology* **19**, 151–158
Total reflection x-ray fluorescence and energy-dispersive x-ray fluorescence analysis of runoff water and vegetation from abandoned mining of Pb–Zn ores	Marqués, A. F., Queralt, I., Carvalho, M. L. and Bordalo, M. (2003). *Spectrochimica Acta Part B* **58**, 2191–2198
Determination of copper, iron and zinc in spirituous beverages by total reflection x-ray fluorescence spectrometry	Capote, T., Marcó, L. M., Alvarado, J. and Greaves, E. D. (1999). *Spectrochimica Acta Part B* **54**, 1463–1468

Other Techniques

There are other techniques less used for zinc analysis. For instance, fluorometry has been applied using fluorescent adducts of zinc-chelating peptides. The main disadvantage of this technique is the interference problems caused by other transition metal cations, mainly with cadmium, which have similar chemical properties than zinc thus producing insufficient selectivity.

On the other hand, UV–visible spectroscopy has been applied with an intensely colorimetric sensor based on hetarylazo derivative. It shows a high selectivity based on a color change from red to faint orange on sensing of zinc ions. The presence of cations such as cadmium does not interfere in the sensing process, although mercury does at high concentrations.

Neutron activation analysis (NAA) has been applied for determination of trace amounts of zinc. The technical basis is that samples are first irradiated with a flood of neutrons generated by a nuclear reactor, creating radioactive isotopes of present elements. The concentration of zinc can be determined through their long-lived radionuclide ^{65}Zn ($t_{1/2} = 244$ days). It is recommended to make an extraction step with dithiocarbamates prior to NAA because the major interfering elements (sodium, chloride, and bromide) do not form complexes with the extracting reagents and are avoided.

Finally, near infrared (NIR) radiation is the electromagnetic spectrum radiation encompassing the interval 780–2500 nm

being situated between the middle infrared and visible radiations. NIR spectroscopy has been used for determining zinc concentration, due to association between minerals and organic functional groups in the sample matrix and the effect on O—H bonding. The main drawback of this technique is the complexity of NIR spectra, with overtones and combination bands generated by different functional groups such as C—H, N—H, and O—H being necessary to use chemometric techniques to extract the information contained therein.

See also: Authenticity of Food; Cobalt: Properties and Determination; Infrared Spectroscopy: Applications; Mass Spectrometry: Applications; Mass Spectrometry: Principles and Instrumentation; Potassium: Properties and Determination; Sodium: Properties and Determination; Spectroscopy: Types; Zinc: Physiology and Health Effects.

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Zoonoses

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Introduction

According to the Joint WHO/FAO Expert Committee on Zoonoses, Second Report, in the year 1959, zoonoses (the expression zoonotic diseases is also used) are "those diseases and infections which are naturally transmitted between vertebrate animals and man." The transmission may take place directly or indirectly by means of vectors. The severity of zoonotic diseases in humans varies from mild symptoms to life-threatening conditions.

Zoonotic diseases are usually transmitted at the human–animal interface through exposure to animals (e.g., rabies), animal products (e.g., brucellosis and salmonellosis), or their contaminated environment (e.g., echinococcosis/hydatidosis). Transmission may occur by a diseased animal or by a clinically normal animal that, nevertheless, is able to transmit pathogens to humans (e.g., campylobacter and verotoxin producing *Escherichia coli* (VTEC)). A distinction is made between foodborne and nonfoodborne diseases. A concise list of zoonoses appears in Table 1.

Emerging and Neglected Zoonotic Diseases

Emerging infectious diseases (EIDs) are diseases appearing in a geographic area or population for the first time. The term reemerging diseases is also used for ancient diseases that are 'forgotten' and thought to be controlled or extinct from a particular area or population and manifest a new appearance. The emergence of EIDs is thought to be driven by socioeconomic, environmental, and ecological factors. They are, mostly, related to free-living wild animals.

The term neglected tropical diseases is used for a certain list of 17 diseases recognized as such by the WHO, including a number of zoonotic diseases appearing in Table 2. Neglected tropical zoonotic diseases are ancient endemic zoonoses in Europe and North America. They are largely controlled and tended to be overlooked, while the attention is shifted to newly emerged zoonoses. They can be, potentially, turned into a pandemic.

Significance of Zoonoses

Although, at the time of the aforementioned WHO report, the number of zoonoses was at the level of 100, they are doubled by then and still counting. Zoonoses may be bacterial, viral, parasitic, or due to unconventional agents (e.g., prions). It is estimated that out of the about 1500 human nosogenic factors, due to biological agents, 60% have multiple hosts and move between species. In addition, 75% of the EIDs, in the last ~30–35 years, are zoonotic; again, 75% of which originate from wild animals.

Further to the significance of zoonoses for human health, one must not underestimate the impact they are having in preventing the efficiency in animal food production and enhancing problems in living animals and animal products international trade.

As an indication of the significance of zoonotic diseases for human health, you can see, in Table 3, the most important zoonoses in terms of human health impact, livestock impact, amenability to agricultural interventions, severity of diseases, and emergence.

Environmental Aspects

Zoonoses present a major threat to human and animal health. These diseases, as already indicated, are multifactorial manifestations and therefore a reflection of the complexities of ecosystems in which animals and humans coexist. They are influenced by multiple interrelated global factors, including ecological evolution, human demographics, and behavior.

Emerging and reemerging diseases represent failures in understanding the socioecological systems we live in and respond to new conditions. What we learn from these failures will largely determine how successful we are in developing sustainable and healthy human communities.

As can be seen in Figure 1, humans, livestock, and wildlife can expose each other and spread, among them, potential pathogenic agents. Any one of these players, therefore, can spill over pathogens to the others. More specifically, changes in human behavior, expressed as population increase and urbanization, and the demand for improved living conditions, requiring in turn proportional increase in food production and related economic activity, in particular agricultural development, are a decisive contributing factor.

For example, water management, associated with still water collections, one way or another, increases mosquito breeding opportunities, which in turn may result in enhancing disease occurrence, like Rift Valley fever. Such conditions are observed after the construction of dams and irrigation networks. Similarly, intensive animal farming promotes disease transmission through untreated or poorly treated waste material spread in the environment or through ventilation system diffusion of contaminated material carrying pathogens.

Due to the problems related to high animal population density, maintained in intensive livestock production (e.g., pigs and poultry), which facilitated disease transmission, efforts were made to combat them with the use of antibiotics, either therapeutically or preventatively. However, these schemes resulted in widespread antibiotic-resistant pathogens, including those of zoonotic significance. These conditions, more specifically, can contaminate animal food products and potentially provide for the production of unsafe foods. It is important in this respect to mention that, according to EU food

Table 1 Concise list of zoonoses[a]

Disease	*Causative organism*
Bacterial diseases	
Anthrax	*Bacillus anthracis* (e–fb)
Bordetellosis	*Bordetella bronchiseptica* (e)
Brucellosis	*Brucella abortus* (e), *Brucella melitensis* (fb–e), *Brucella suis* (e), *Brucella canis* (e)
Campylobacter enteritis	*Campylobacter jejuni* (fb–e), *Campylobacter coli*, *C. fetus*, *C. laridis*
Capnocytophaga infection	*Capnocytophaga canimorsus* (e), *C. cynodegmi* (e)
Cat scratch disease	*Bartonella henselae* (e), *B. quintana* (e)
Clostridial diseases (see also 'Tetanus' in the succeeding text)	*Clostridium perfringens*, type A (e)
Erysipeloid	*Erysipelothrix rhusiopathiae* (e)
Escherichia coli infections (only some infections are considered zoonotic)	Certain strains of *E. coli*, including O157:H7 and others (fb–e)
Glanders	*Burkholderia mallei* (e)
Leptospirosis	*Leptospira interrogans* (e–fb)
Listeriosis	*Listeria monocytogenes* (fb)
Lyme disease (borreliosis)	*Borrelia* species (vb)
Melioidosis (pseudoglanders)	*Pseudomonas pseudomallei* (e)
Mycobacteriosis	Mycobacterium avium–intracellulare complex (fb)
Pasteurellosis	*Pasteurella multocida* and other species (e)
Plague	*Yersinia pestis* (vb–e)
Psittacosis and ornithosis	*Chlamydophila psittaci* (e)
Rat bite fever	*Streptobacillus moniliformis*, *Spirillum minus* (e–fb)
Relapsing fever (borreliosis)	*Borrelia recurrentis* (vb)
Salmonellosis	*Salmonella enterica* (fb–e)
Southern tick-associated rash illness	*Borrelia lonestari* (vb)
Streptococcal infections	*Streptococcus pyogenes*, other streptococci (fb–e)
Tetanus	*Clostridium tetani* (e)
Tuberculosis (see also 'Mycobacteriosis')	*Mycobacterium bovis* (e–fb), *Mycobacterium tuberculosis* (e)
Tularemia	*Francisella tularensis* (e–vb–fb)
Vibriosis	*Vibrio parahaemolyticus* (fb–e), *V. vulnificus*, and other vibrios *Vibrio cholerae* (fb–e)
Yersiniosis	*Yersinia pseudotuberculosis* (fb–e), *Yersinia enterocolitica* (fb–e)
Rickettsial diseases	
Boutonneuse fever, tick bite fever	*Rickettsia conorii*-related *Rickettsia* (vb)
Ehrlichiosis	*Ehrlichia chaffeensis* (vb), *Anaplasma phagocytophilum* (vb), *Ehrlichia sennetsu* (vb), *Ehrlichia ewingii* (vb)
Eperythrozoonosis	*Mycoplasma* (*Eperythrozoon*) species (e–vb)
Murine typhus	*Rickettsia typhi* and related species (vb)
Q fever (query fever)	*Coxiella burnetii* (e–vb–fb)
Queensland tick typhus	*Rickettsia australis* (vb)
Rickettsial pox	*Rickettsia akari* (vb)
Rocky mountain spotted fever	*Rickettsia rickettsii* (vb)
Spotted fever group	*Rickettsia parkeri* (vb)
Scrub typhus	*Orientia tsutsugamushi* and related species (vb)
Typhus	*Rickettsia prowazekii* (vb)
Fungal diseases	
Actinomycosis	*Actinomyces israelii* (e), rarely other *Actinomyces* species (e)
Aspergillosis (allergic bronchopulmonary aspergillosis)	*Aspergillus* species (e)
Blastomycosis	*Blastomyces dermatitidis* (e)
Candidiasis (moniliasis)	*Candida* species (e)
Coccidioidomycosis	*Coccidioides immitis* (e)
Cryptococcosis	*Cryptococcus neoformans* (e)
Dermatophilosis	*Dermatophilus congolensis* (e)
Histoplasmosis	*Histoplasma capsulatum* (e)
Nocardiosis	*Nocardia* species (e)
Pneumocystis pneumonia	*Pneumocystis carinii* (human strain) (e)
Rhinosporidiosis	*Rhinosporidium seeberi* (e)
Ringworm (dermatophytosis)	*Microsporum* (e), *Trichophyton* (e), and *Epidermophyton* species (e)
Sporotrichosis	*Sporothrix schenckii* (e)
Parasitic diseases – protozoans	
Babesiosis	*Babesia microti* (vb), *B. bovis* (vb), *Babesia divergens* (vb)

Table 1 (Continued)

Disease	*Causative organism*
Balantidiasis	*Balantidium coli* (fb)
Chagas' disease (American trypanosomiasis)	*Trypanosoma cruzi* (e)
Cryptosporidiosis	*Cryptosporidium parvum* (e–fb)
Giardiasis	*Giardia lamblia* (fb–e)
Leishmaniasis (kala-azar (visceral))	*Leishmania donovani* and other species (vb)
Malaria of nonhuman primates	Many species of plasmodium (vb)
Microsporidiosis	*Microsporidia* (e–fb), *Enterocytozoon bieneusi* (e–fb), *Encephalitozoon cuniculi* (e–fb), *Encephalitozoon intestinalis* (e–fb), *Encephalitozoon hellem* (e–fb)
Sarcocystis (sarcosporidiosis)	*Sarcocystis suihominis* (fb), *S. hominis* (fb)
Toxoplasmosis	*Toxoplasma gondii* (fb)
Trypanosomiasis (African sleeping sickness)	*Trypanosoma brucei* (vb), *T. brucei* rhodesiense (vb), *T. brucei* gambiense (vb)
Parasitic diseases – trematodes (*flukes*)	
Clonorchiasis	*Clonorchis sinensis* (Chinese liver fluke) (fb)
Dicrocoeliasis	*Dicrocoelium dendriticum* (fb), *D. hospes* (lancet fluke) (fb)
Echinostomiasis	*Echinostoma ilocanum* (fb) and other *Echinostoma* species (fb)
Fascioliasis	*Fasciola hepatica* (fb), *F. gigantica* (fb)
Fasciolopsiasis	*Fasciolopsis buski* (fb)
Gastrodiscoidiasis	*Gastrodiscoides hominis* (vb?–fb?)
Heterophyiasis	*Heterophyes* (fb) and other heterophids (fb)
Metagonimiasis	*Metagonimus yokogawai* (fb)
Opisthorchiasis	*Opisthorchis felineus* (cat liver fluke) (fb), *Amphimerus pseudofelineus*, *Opisthorchis viverrini* (small liver fluke) (fb)
Paragonimiasis (lung fluke disease)	*Paragonimus westermani* (fb), *P. africanus* (fb), *P. mexicanus* (fb), and other species (fb)
Schistosomiasis (bilharziasis)	*Schistosoma japonicum* (vb), *Schistosoma intercalatum* (vb), *Schistosoma mansoni* (vb), *Schistosoma matthei* (vb), *Schistosoma mekongi* (vb)
Swimmer's itch	*Schistosome cercariae* (vb)
Parasitic diseases – cestodes (*tapeworms*)	
Asian taeniasis	*Taenia asiatica* (fb)
Bertielliasis	*Bertiella studeri* (vb?–fb?), *B. mucronata* (vb?–fb?)
Coenurosis	*Taenia multiceps* (fb?), *Taenia serialis* (fb?), *Taenia brauni* (fb?)
Diphyllobothriasis (fish tapeworm infection)	*Diphyllobothrium latum* (fb), *D. pacificum* (fb)
Dipylidiasis (dog tapeworm infection)	*Dipylidium caninum* (fb?)
Echinococcosis	*Echinococcus granulosus* (fb?)
Echinococcosis	*Echinococcus multilocularis* (fb?) *Echinococcus vogeli* (fb?)
Hymenolepiasis (dwarf tapeworm infection)	*Hymenolepis nana* (fb?–vb?)
Inermicapsifer infection	*Inermicapsifer madagascariensis* (fb?)
Mouse or rat tapeworm	*Hymenolepis nana* (vb?), *H. diminuta* (vb?)
Raillietina infection	*Raillietina* species (fb?)
Sparganosis	*Spirometra* species (fb–e?)
Taeniasis (beef tapeworm disease)	*Taenia saginata* (fb)
Taeniasis (pork tapeworm disease), cysticercosis, and neurocysticercosis	*Taenia solium* (fb)
Parasitic diseases – nematodes (*roundworms*)	
Angiostrongyliasis	*Parastrongylus costaricensis* (fb), *Angiostrongylus cantonensis* (fb)
Anisakiasis	Larvae of *Anisakis* (fb) and *Pseudoterranova* species (fb)
Capillariasis (intestinal)	*Capillaria philippinensis* (fb)
Capillariasis (liver)	*Capillaria hepatica* (fb?)
Capillariasis (lung)	*Capillaria aerophila* (fb)
Dioctophymosis (giant kidney worm infection)	*Dioctophyme renale* (fb)
Dracunculiasis (guinea worm infection)	*Dracunculus insignis* (fb)
Dirofilariasis (heartworm infection)	*Dirofilaria immitis* (vb)
Gnathostomiasis	*Gnathostoma spinigerum* (fb)
Gongylonemiasis	*Gongylonema pulchrum* (fb?–vb?)
Larva migrans, skin (see also 'Gnathostomiasis')	*Ancylostoma braziliense* (e), *A. caninum* (e), *Uncinaria stenocephala* (e), *Strongyloides stercoralis* (e)
Larva migrans, visceral (see also 'Angiostrongyliasis' and 'Anisakiasis')	*Toxocara canis* (fb), *T. cati*, *Baylisascaris procyonis* (fb)
Malayan filariasis	*Brugia malayi* (vb)
Strongyloidiasis	*Strongyloides stercoralis* (e), *S. fuelleborni* (e)
Thelaziasis	*Thelazia* species (vb)

(*Continued*)

Table 1 (Continued)

Disease	*Causative organism*
Trichinosis (trichinellosis)	*Trichinella spiralis* and subspecies (fb), *T. nativa* (fb), *T. britovi* (fb), *T. nelsoni* (fb), *T. pseudospiralis* (fb)
Tropical eosinophilia	*Brugia pahangi* (vb)
Parasitic diseases – others	
Hirudiniasis	*Limnatis nilotica* (e) and other leeches (e)
Macracanthorhynchosis	*Macracanthorhynchus hirudinaceus* (fb?) and other species (thorny-headed worms) (fb)
Diseases spread by insects, ticks, or mites	
Acariasis (mange)	Mites of *Sarcoptes* (e), *Cheyletiella* (e), *Dermanyssus* (e), and *Ornithonyssus* species (e)
Myiasis	*Cochliomyia hominivorax* (screwworm), *Chrysomya bezziana* (Old World screwworm), *Cuterebra* species (rodent or rabbit bot fly), *Hypoderma lineatum*, *Gasterophilus* species (equine bot fly), *Oestrus ovis*, *Rhinoestrus purpurensis*, *Hypoderma bovis* (warbles), *Wohlfahrtia* species, *Dermatobia hominis* (human bot fly), *Cordylobia anthropophaga* (tumbu fly)
Nanophyetiasis	*Troglotrema salmincola* (fb)
Pentastomid infections	*Linguatula serrata* (fb), *Armillifer* species (tongue worms) (fb)
Tick paralysis	Envenomation of ticks (vb), *Dermacentor andersoni* (vb), *D. variabilis* (vb), and sometimes *Ixodes*, *Haemaphysalis* (vb), *Rhinocephalus* (vb), and *Argas* species (vb)
Tunga infections	*Tunga penetrans* (sand fleas and jiggers) (e)
Viral diseases	
Argentinean, Bolivian, Brazilian, or Venezuelan hemorrhagic fever	Various arenaviruses (e)
Central European tick-borne encephalitis	Central European encephalitis virus (vb–fb)
Colorado tick fever	Colorado tick fever virus (vb)
Contagious ecthyma (orf)	Orf virus (parapox) (e)
Cowpox	Cowpox virus (e)
Crimean–Congo hemorrhagic fever	Nairovirus (vb)
Eastern equine encephalomyelitis	Eastern equine encephalomyelitis virus (vb)
Ebola hemorrhagic fever, Marburg hemorrhagic fever	Ebola and Marburg viruses (e)
Encephalomyocarditis	Encephalomyocarditis virus (e)
Far Eastern tick-borne encephalitis (Russian spring–summer encephalitis)	Far Eastern (Russian spring–summer encephalitis) virus (vb)
Foot-and-mouth disease	Foot-and-mouth disease virus (e)
Hantavirus pulmonary syndrome	Sin Nombre virus, black creek canal virus (e)
Hemorrhagic fever with renal syndrome	Hantaan virus (e), Dobrava virus (e), Puumala virus (e), Seoul virus (e)
Hendra virus infection	Hendra virus (e)
Hepatitis E	Hepatitis E virus (vb–e)
Herpes B virus disease	Cercopithecine herpesvirus 1 (herpesvirus simiae, B virus) (vb–e)
Influenza type A (swine flu, avian flu, bird flu, and Hong Kong flu)	Influenza virus (myxovirus) (e)
Japanese B encephalitis	Japanese encephalitis virus (vb)
Kyasanur forest disease	Kyasanur forest virus (vb)
La Crosse encephalitis	Bunyavirus species (vb)
Lassa fever	Lassa virus (e)
Louping ill	Louping ill virus (vb)
Lymphocytic choriomeningitis	Lymphocytic choriomeningitis virus (e)
Menangle virus infection	Menangle virus (e)
Milker's nodules (pseudocowpox)	Pseudocowpox virus (e)
Monkeypox	Monkeypox virus (e)
Murray Valley encephalitis	Murray Valley encephalitis virus (vb)
Newcastle disease	Newcastle disease virus (e)
New World hemorrhagic fever	Arenavirus (e)
Nipah virus infection	Nipah virus (e)
Omsk hemorrhagic fever	Omsk hemorrhagic fever virus (vb–e)
Rabies and rabies-related infections	Lyssaviruses (rabies virus, Duvenhage virus, Mokola virus, and Ibadan shrew virus) (e)
Rift Valley fever	Phlebovirus (vb–e)
Ross River fever	Ross River virus (vb)
St. Louis encephalitis	St. Louis encephalitis virus (vb)
Severe acute respiratory syndrome (SARS)	Coronavirus (e)
Sindbis virus disease	Sindbis virus (vb)
Tahyna fever	Bunyavirus species (vb)
Venezuelan equine encephalomyelitis	Venezuelan equine encephalitis virus (vb)

Table 1 (Continued)

Disease	*Causative organism*
Vesicular stomatitis	Vesicular stomatitis virus (e–vb)
Wesselsbron fever	Wesselsbron virus (vb)
West Nile virus infection	West Nile virus (vb–fb?)
Western equine encephalomyelitis	Western equine encephalomyelitis virus (vb)
Yellow fever	Yellow fever virus (vb)
Prion diseases	
Variant Creutzfeldt–Jakob disease	Prion protein (likely from bovine spongiform encephalopathy, also known as mad cow disease) (fb)

vb, vector-borne; e, exposure to infectious material (work-related, contacts, wounds, bites, scratches, 'licks,' and airborne); fb, foodborne; nd, neglected disease; em, emerging–reemerging disease; wb, waterborne.

[a]Many proved zoonoses, including some relatively rare viral infections carried by insects and infections caused by parasitic worms, have been omitted, as well as those diseases caused by fish and reptile toxins.

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Table 2 Zoonoses included in the WHO list of neglected tropical diseases

Dengue/severe dengue (vb)
Rabies
Chagas' disease (vb)
Human African trypanosomiasis (sleeping sickness) (vb)
Leishmaniasis (vb)
Cysticercosis/taeniasis
Dracunculiasis (guinea worm disease)
Echinococcosis
Foodborne trematodiases
Lymphatic filariasis (vb)
Onchocerciasis (river blindness) (vb)

vb, vector-borne.

Extracted from the WHO list of Neglected Tropical Diseases.

legislation (EU Regulations 178/2002 and 852/2004), primary food production premises are considered as food business operations. Consequently, they are obliged to certain responsibilities, related to putting together and effectively implementing and functioning a food safety management system (FSMS), tailor-made for each particular activity.

In Table 4 appears a list of zoonoses emergence linked to agricultural intensification and environmental changes.

Evidently, ecosystems are complicated, and the impact they are having on fauna and thereof on conditions potentially harboring zoonotic agents does not provide for a general interdisciplinary multipurpose approach, with patterns applicable in all cases. On the contrary, principal factors, among others, biological, ecological, economic, and social characteristics, and climatic conditions must be assessed, ad hoc, locally within each ecosystem.

The implementation, therefore, of the concept of One Medicine and One Health, which, as an integrated approach, is very important for facing zoonotic diseases per se because not, only it contributes to animal and human health but also it significantly supports, apart from aspects of economic development, food safety and security.

Foodborne Zoonotic Diseases

Foodborne zoonotic pathogens are transmitted through consumption of contaminated food and drinking water. Infectious agents present in foodstuffs include bacteria (e.g., *Salmonella* and *Campylobacter*), viruses (e.g., norovirus and hepatitis A virus), parasites (e.g., *Trichinella*), and prions (infectious agents of bovine spongiform encephalopathy).

Challenges to food safety continue to increase in unpredictable ways, largely due to changes in food production, processing, distribution, and the environment, which may contaminate food; to emerging germs and toxins of food safety significance; and to new conditions, created by new food technology applications, with an impact on food safety (e.g., minimally processed foods and maintenance of cold chain).

In Table 5, a list of the most important biological hazards responsible for foodborne illnesses appears, along with the relevant most important foods involved in each case.

Nonfoodborne Zoonotic Diseases

Nonfoodborne zoonotic diseases are transmitted through the following:

- *Direct contact or close proximity* with infected animals or through *the environment*. Examples are the following:
  - *Avian influenza*, a viral disease occurring mainly in poultry and other birds but transmissible to other animals or humans.
  - *Q fever*, caused by the *Coxiella burnetii*, affecting animals and humans. Human infection mainly results from the inhalation of contaminated dust from the placenta and birth fluids or feces from infected animals.
  - *Salmonella* infections, which can originate from contact with infected reptiles and amphibians such as pet snakes, iguanas, and frogs or their environment.
  - VTEC, which can be acquired through contact with infected farm animals.

Table 3 The most important zoonoses in terms of human health impact, livestock impact, amenability to agricultural interventions, severity of disease, and emergence

Disease	*Wild life interference*	*Human deaths (annual)*	*Affected humans (annual)*	*Death >1000 people*	*Affected >1 million people*	*Annual impacts high*	*Farm intervention*	*Others (score = 1)*	*Total score*
Gastrointestinal (zoonotic)	Important	1 500 000	2 333 000 000	2	1	1	1	0	5
Leptospirosis	Very important	123 000	1 700 000	2	1	1	1	0	5
Cysticercosis	Some importance			50 000	50 000 000	2	1	1	1
0	5								
Tuberculosis (zoonotic)	Some importance			100 000	554 500	2	0	1	1
0	4								
Rabies	Important	70 000	70 000	1	0	0	1	Severe	4
Leishmaniasis	Important	47 000	2 000 000	2	1	0	1	0	4
Brucellosis	Some importance			25 000	500 000	2	0	1	1
0	4								
Echinococcosis	Important	18 000	300 000	2	0	1	1	0	4
Toxoplasmosis	Important	10 000	2 000 000	1	1	1	1	0	4
Q fever	Important	3000	3 500 000	2	1	0	1	0	4
Trypanosomiasis (zoonotic)	Important	2500	15 000	2	0	1	1	0	4
Anthrax	Some importance			1250	11 000	2	0	1	1
0	4								
Hepatitis E[a]	Some importance			300 000	14 000 000	2	1	0	1
0	4								
Chagas' disease	Important	10 000	8 000 000	2	1	0	0	0	3
Chikungunya	Very important	12 500	500 000	2	0	0	0	Emerge	3
Clostridium difficile disease	Possible importance			3000	300 000	2	0	0	0
Emerge	3								
Dengue fever	Minor	20 000	50 000 000	2	1	0	0	0	3
Ebola	Very important	500	800	2	0	0	0	Severe	3
Hanta disease	Very important	1750	175 000	2	0	0	0	Emerge	3
Avian influenza	Important	77	145	0	0	1	1	Emerge	3
Bovine spongiform encephalopathy	Some importance			182	188	0	0	1	1
Severe	3								
Psittacosis	Important	2250	22 000	2	0	0	1	0	3
Japanese encephalitis	Possibly, bats	11 000	40 000	2	0	0	1	0	3
Buffalopox	Not important	Negligible	Common	0	1	1	1	0	3
Rift Valley fever	Important	45	150	0	0	1	1	Emerge	3

Data from the WHO and authoritative literature: when there are several authoritative estimates, the midpoint is given.

Note: High human mortality gets a double weight as the most important criterion for many stakeholders. Total score = (human death × 2) + (humans affected) + (high livestock impacts) + (farm intervention possible) + (other concerns: severe or emerging disease). The maximum possible score is therefore 6 and the minimum 0.

[a]Importance of zoonotic transmission not fully known. Not a problem in poor countries.

Adapted from, ILRI, 'Mapping of poverty and likely zoonotic hotspots.'

- *Vectors* that can transmit diseases such as malaria, West Nile virus, Lyme disease, tick-borne encephalitis, leishmaniosis, and Crimean–Congo hemorrhagic fever.

Prevention and Control

For zoonoses prevention, the focus is on surveillance, rapid detection, and quick response. Zoonotic infections in animals may produce a distinctive recognizable disease, such as rabies, or they may manifest themselves as a mild illness or the animal may be entirely asymptomatic. However, in this last case, if the pathogen is transmitted to humans, it may result in illness if they lack the specific immunity required.

Anyone, who has contact with animals, can get a zoonotic disease, but some people may be more at risk than others. Risk

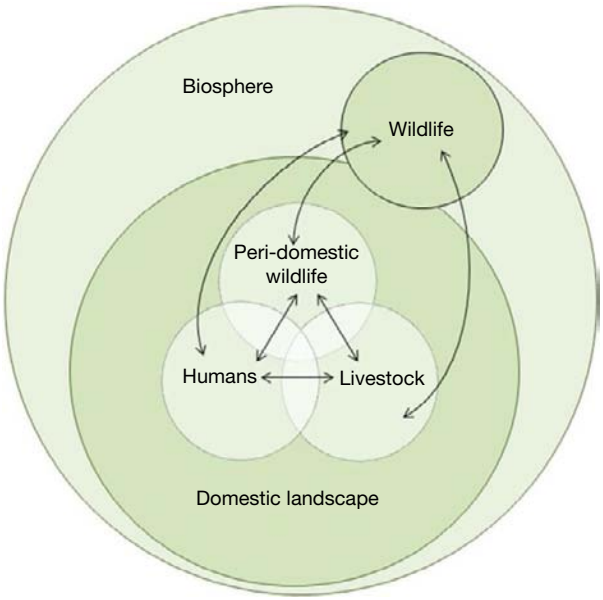

Figure 1 Pathogen flow at the wildlife–livestock–human interface. Arrows indicate direct, indirect, or vector-borne candidate pathogen flow. In each host species, there are a vast array of constantly evolving microorganisms, some of which are pathogenic in the host. These are a source of new organisms for other host species, some of which may be pathogenic in the new host or may evolve in the new host to become pathogenic. If the pathogen is also transmissible in the new host species, then a new transmission cycle may be established. The rate and direction of candidate pathogen flow will depend on the nature and intensity of interaction between wildlife, livestock, and human compartments and the characteristics of the compartments (**Table 2**). Adapted from *Proceedings of the National Academy of Science*, May 21, 2013, Vol. *110*, No 21, p. 8400.

factors for susceptibility to zoonoses, among others, are certain population groups, including professional and occupational groups working in close contact with animals (like livestock attendants, slaughterhouse workers, and veterinarians); homeless and poor people; and in general, people with a weakened immune system, children aged less than five, the elderly, and pregnant women.

The factors promoting zoonotic disease outbreaks in humans, apart from frequent contact with animals, include intensive livestock production, poor animal and personal hygiene, and overlap with wildlife habitat. A common way for vector-borne diseases to spread is through the bite of a mosquito or tick. People can get diseases in most places, where they might have contact with infected animals and insects, including animal displays, farms, petting zoos, county or state fairs, pet stores, child care facilities or schools, nature parks, and wooded and bushy areas.

In order to reduce the risks of transmission of zoonoses from pet animals to humans and also to production animals, the concept of responsible pet ownership (RPO) is advocated. It is recognized that education and awareness promotion of pet owners, for RPO, to prevent zoonoses related to companion animals, is of a paramount importance for eliminating these diseases.

The WHO and EFSA promote activities, of the general public's concern, for preventing zoonoses. They include the following:

- Promotion of awareness to understand the potential risk for human infection from zoonotic diseases, after contact with animals.
- Specific risk communication, as it may be required, through appropriate public actions.

Table 4 Zoonoses emergence linked to agricultural intensification and environmental change

Type of wildlife–livestock–human interface	*Level of biodiversity*	*Characteristics of livestock population*	*Connectedness between populations*	*Examples of zoonotic disease with altered dynamics*
'Pristine' ecosystem with human incursion to harvest wildlife and other resources	High	No livestock	Very low, small populations and limited contact	Ebola, HIV, SARS, and Nipah virus in Bangladesh and India
Ecotones and fragmentation of natural ecosystems: farming edges, human incursion to harvest natural resources	High but decreasing	Few livestock, multiple species, mostly extensive systems	Increasing contact between people, livestock, and wild animals	Kyasanur forest disease, bat rabies, and *E. coli* interspecies transmission in Uganda and Nipah virus in Malaysia
Evolving landscape: rapid intensification of agriculture and livestock, alongside extensive and backyard farming	Low, but increasing peridomestic wildlife	Many livestock, both intensive and genetically homogenous, as well as extensive and genetically diverse	High contacts between intensive and extensive livestock, people, and peridomestic wildlife. Less with endangered wildlife	Avian influenza and Japanese encephalitis virus in Asia
Managed landscape: islands of intensive farming, highly regulated. Farm land converted to recreational and conservancy	Low, but increased number of certain peridomestic wildlife species	Many livestock, mainly intensive, genetically homogeneous, biosecure	Fewer contacts between livestock, and people; increasing contacts with wildlife	Bat-associated viruses in Australia, West Nile virus in the United States, and Lyme disease in the United States

Conceptual framework of types of wildlife–livestock–human interface and their characteristics.
Adapted from *Proceedings of the National Academy of Science*, May 21, 2013, Vol. *110*, No. 21, p. 8402.

Table 5 Biological hazards responsible for most important foodborne illnesses and related foods

Campylobacter (poultry)
E. coli O157:H7 (ground beef, leafy greens, and raw milk)
Listeria (deli meats, unpasteurized soft cheeses, and produce)
Salmonella (eggs, poultry, meat, and produce)
Vibrio (raw oysters)
Norovirus in many foods (e.g., sandwiches and salads)
Toxoplasma (meats)

- Improvement in the level of personal hygiene by
  - acquiring the habit to wash hands thoroughly and frequently, after contact with animals.
  - closely supervising children to ensure they wash their hands properly and avoid hand-to-mouth activities (thumb sucking, eating, and use of pacifiers) after animal contact.
- The use of registered insect repellents and products that contain repellents for use on clothing. Accordingly, treat clothing and gear, such as boots, pants, socks, and tents.
- Inspection for and removal of ticks from the human body, with specific care for children.
- Limitation in the number of places around residential areas for mosquitoes to breed by eliminating places holding water.

For health professionals, recommendations may include the following:

- Responsible services to systematically search for potential sources of human infection from animal sources and the environment
- Joint efforts and coordination among public health authorities and related professionals, both public and private
- Risk communication and information sharing among responsible health services and close coordination to manage risks related to the movement and trade of livestock
- Concerted actions for
  - good practices in the efficient implementation of biosecurity measures in farms and at border or territory crossings;
  - continuously reminding and training people, who work with livestock and in slaughterhouses, for the significant importance of personal hygiene practices;
  - the implementation of the One Health concept

Zoonotic diseases are strongly influenced by social and economic practices. Many of them are thus not only diseases related to climate and environment but also diseases of poverty. In epidemiological terms, conditions of poverty increase the probability of enhanced contact and hence increase the likelihood of epidemics. Combating diseases like tuberculosis and malaria has much more to do with housing, nutrition, and water management than with any advances in biomedical science.

Animal migrations facilitate the global spread of pathogens and increase cross-species transmission. Understanding, predicting, and controlling diseases at the human–animal interface are a huge challenge, for health professionals, in today's world, where international trade and travel globalized diseases. However, even when risks are identified, adequate underlying infrastructure and resources are required to take the measures needed, if outbreaks and emergencies are to be prevented or controlled.

Special consideration needs to be given to vector-borne diseases. Vectors may move to long distances and therefore may introduce disease, in new geographic areas, by means of human traveling, international trade, animal movement (more specifically livestock and migratory birds), the wind, and changes in agricultural practices.

One Health

Health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity. It is the outcome of a complex of several interdependent medical, economic, sociocultural, environmental, and ecological factors. People's health and well-being and equally animal health and welfare are strongly interlinked. Both also influence and are impacted by the health of the environment. Health is a precondition for well-being and respectively welfare. Well-being and welfare reinforce health. The advancement of the health and well-being of people and animals depends on effective and sustained cooperation between varied professions and disciplines, in both the public and private sectors (World Veterinary Association (WVA) position).

Therefore, multisectoral horizontal links at all levels between human and animal health professionals, public and private, are needed to face zoonotic diseases. Reducing relevant risks is impossible to be achieved alone by a particular sector, regardless of its importance. Therefore, there is an increasing convergence to a One Health approach, which incorporates, in an integrated manner, cross-sectoral, multidisciplinary cooperation. One cannot distinguish human, animal, and environmental health. The health of each one of them is the precondition for the health of the others (see again **Figure 1**).

Building sustainable national mechanisms for more effective cross-sectoral cooperation has greatly facilitated risk assessment and management of specific diseases, such as H5N1 influenza and Rift Valley fever. At the international level, strong cooperation among the WHO, OIE, and FAO is improving the efficiency of surveillance, including data collection, risk assessment, and risk management options, allowing for consistent, science-based risk communication on global health threats at the human–animal–ecosystem interface. A strategic agreement outlines the sharing of responsibilities and enhanced coordination of complementary roles and activities between the FAO, OIE, and WHO at national, regional, and global levels. Internally, cooperation takes place across departments, clusters, and regions of the aforementioned organizations. Externally, further cooperation materializes with links and contacts to additional international partners, such as international agencies and networks, NGOs, and academia, and to national agencies, such as institutions and administrative governmental units.

According to statements made by EU officials in support of it, One Health is linked to livelihood and equity and fits with EU objectives to promote global security, social justice, international cooperation, and multilateralism and fight poverty. Further, there should be no resignation vis-à-vis the existence of different health standards across nations.

For the EU, the One Health movement has in many senses grown out of the response to influenza-related crises. The definition of One Health chosen by the European Commission in its external relations and actions reads as follows: the One Health approach consists of (i) improving health and well-being through the prevention of risks and the mitigation of the effects of crises that originate at the interface between humans, animals and their various environments; (ii) for that purpose: (a) promoting a multi (cross) sectoral and collaborative approach; and (b) promoting a 'whole of society' approach to health hazards, as a systemic change of perspective in the management of risk.

In May 2010, a meeting was organized by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, the United States, titled 'Operationalizing "One Health": A Policy Perspective.' 'Critical enabling initiatives' such as 'training,' 'one health global network,' 'information clearing house,' 'needs assessment,' 'capacity building,' 'proof of concept,' and 'business plan' were identified as fundamental to moving forward One Health.

Further, it seems reasonable to think that an improved coordination that includes intersectoral cooperation in surveillance, communications, outbreak response, and sample sharing community-based interventions for the prevention and control of zoonotic diseases is needed. There is further and more specific need for systematic cooperation between strong and autonomous public health services and strong and autonomous veterinary services, in the respect of their specific expertise.

However, a culture of cross-sectoral cooperation does not yet exist all along the chain. Fostering such a culture, stretching from the field level to that of international organizations, is the big challenge for successfully controlling zoonotic diseases in general and more particularly either emerging or neglected ones.

There is a deficit in current university medical training, due to the fact that the whole training concept is geared to treat, rather than prevent, diseases and preserve and promote health. A cultural change putting emphasis on the prevention and appreciation of the importance of the connection, in terms of health and well-being, between humans, animals, and ecosystems, is required. Combating zoonotic diseases is not how to clean up the disease mess after the fact, but on how to prevent the mess from occurring in the first place. It would almost appear that the ideological lenses through which diseases in general are being studied preclude acting on the evidence. This, if nothing else, should raise a warning flag that those who study disease are not necessarily well equipped to promote health.

See also: *Escherichia coli* and Other *Enterobacteriaceae*: Food Poisoning and Health Effects; Milk: Processing of Milk.

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